



US 20250262329A1

(19) **United States**

(12) **Patent Application Publication**
KAPILOFF et al.

(10) **Pub. No.: US 2025/0262329 A1**
(43) **Pub. Date:** **Aug. 21, 2025**

(54) **TREATMENT OF HEART DISEASE BY
DISRUPTION OF THE ANCHORING OF
PP2A**

(71) Applicants: **University of Miami**, Miami, FL (US);
**The Board of Trustees of the Leland
Stanford Junior University**, Stanford,
CA (US)

(72) Inventors: **Michael S. KAPILOFF**, Los Altos, CA
(US); **Jinliang LI**, Palo Alto, CA (US)

(73) Assignees: **University of Miami**, Miami, FL (US);
**The Board of Trustees of the Leland
Stanford Junior University**, Stanford,
CA (US)

(21) Appl. No.: **19/044,158**

(22) Filed: **Feb. 3, 2025**

Related U.S. Application Data

(63) Continuation of application No. 18/420,397, filed on
Jan. 23, 2024, now abandoned, which is a continu-

ation of application No. 16/818,771, filed on Mar. 13,
2020, now Pat. No. 11,938,198.

(60) Provisional application No. 62/848,156, filed on May
15, 2019.

Publication Classification

(51) **Int. Cl.**

A61K 48/00 (2006.01)

A61K 35/761 (2015.01)

A61P 9/04 (2006.01)

(52) **U.S. Cl.**

CPC *A61K 48/0066* (2013.01); *A61K 35/761*
(2013.01); *A61P 9/04* (2018.01)

(57)

ABSTRACT

The present invention provides a method of treating heart failure with reduced ejection fraction, by administering to a patient at risk of such damage, a pharmaceutically effective amount of a composition which inhibits the anchoring of PP2A to mAKAP β . This composition is preferably in the form of a viral based gene therapy vector that encodes a fragment of mAKAP β to which PP2A binds.

Specification includes a Sequence Listing.

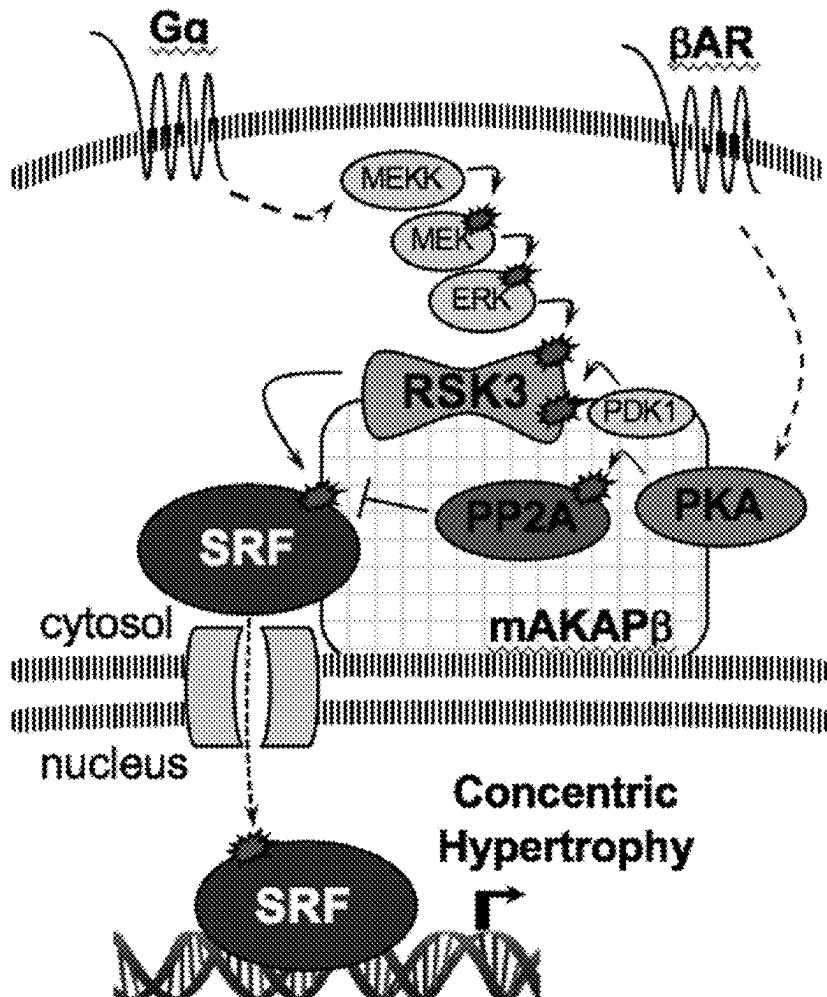


FIGURE 1

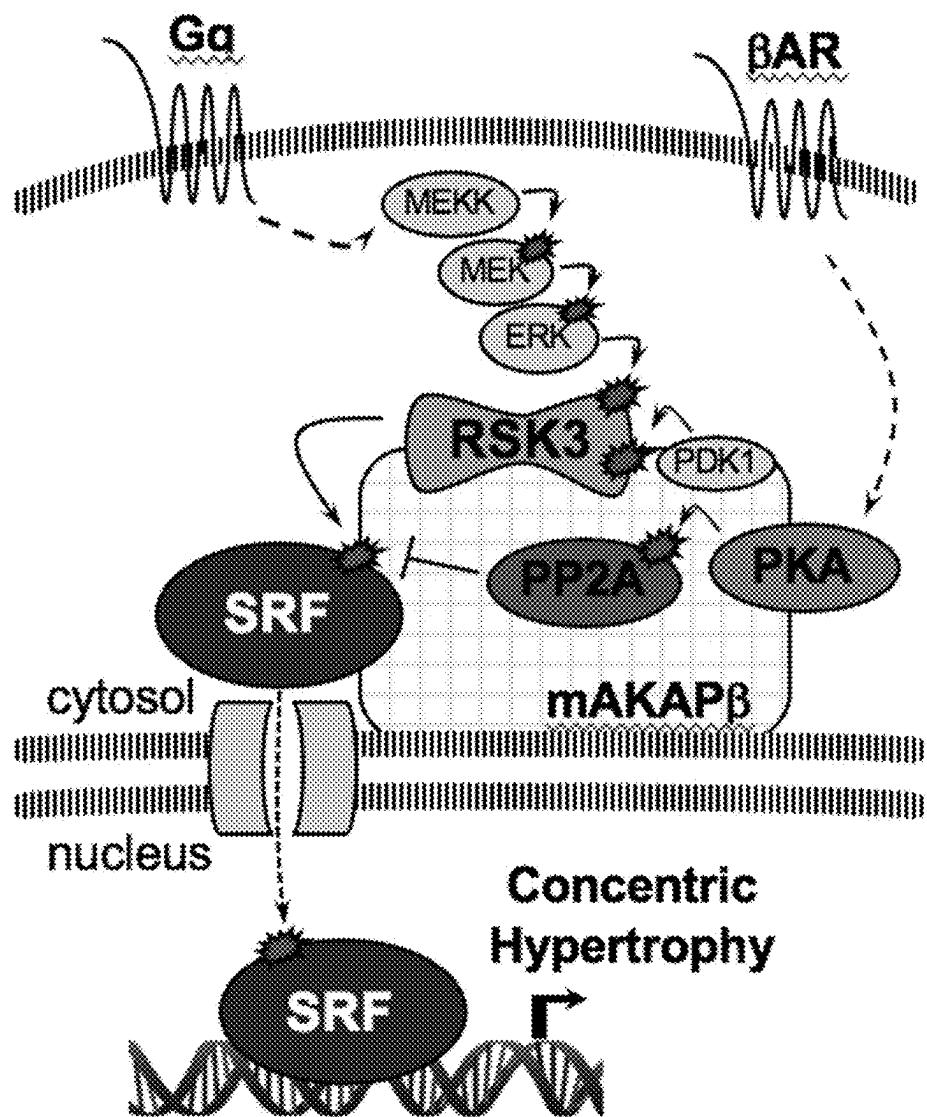


FIGURE 2

```
>h-RSK3 20250821 20250821
MCEKKEFADPPTVYSPGCPSSSSEPEVQVVEVQDISHHVSSECPEKAOPSQFELLEKVLQCGSY
GKVFLVRKVKGSDACQLYANKVLKKATLKVDRVRSKMERDILAEVNHPFIVKLHYAFQTECKLYLILDF
LRGGDLFTPILSKEVMFTEDVKFYLAELALALONLASLGIIYRDLKPMNILLDEZGEHIKITDFGLSKEAI
DHOKRAYSPCGTIEYMAPEVVNRRCGHTQSADNWNSFCVLMFFEMLTGSILPPQGKDRKETMALIILKANLGMPQ
FLSGCRAQSLLRALFKRNPNCNRLGAGIDGVEEIKRHPPFVTIDWNTLYRKEIKPPPFRPAVGKRPEDTFHFDP
EFTARTPTDSPGVEPFSANAHBLFRGFSTVASSLIDEPSQQDLHKVPVHPIVQQLRGNNTRFTDGYEIKED
IGVGSYSVCKRCVHEATDTAYAVKEDKSKHRDPSEEICILLRYCQRFNTITILKDVIDDDGKFVYLNMELMR
GGELLDRIILQRYFSEREASCVLCTITKMDYLHSQGVVBHDLKPGNLLYRDESGGPESIRVCDFGFAKQ
LRAGNGLLMTPCYTANFVAPEVLERQGYAACDIWSLGILLYTMLAGFTFANGPDDTPEEILARIGSGK
YALSGGNWDSISDAAKDVVSKMLSVDFBQRILTAMQVILKHPWVNREYILSPNQLSRQUVHLVKGANAATYF
ALNRTPOAPRLEPVLSNNLAQRRGMKRLTSTEL
```

FIGURE 3**Figure 3. rat mAKAP sequence (PBD highlighted)**

```

1 MLTMSVTLSP LRSQGPDPMA TDASPMAINM TPTVEQEEGE GEEAVKAIDA
51 EQQYGKPPPL HTAADWKIVL HLPEIETWLR MTSERVRDLT YSVQQDADSK
101 HVDVHLVQLK DICEDISDHV EQIHALLETE FSLKLSSY SV NVIVDIHAVQ
151 LLWHQLRVS V LVLRERILQG LQDANGNYTR QTDILQAFSE ETTEGRLDLS
201 TEVDDSGQLT IKCSQDYLSL DCGITAFELS DYSPSEDLLG GLGDMTSQA
251 KTKSFDSWSY SEMEKEFPEL IRSVGLLTVA TEPVPSSCGE ANEDSSQASL
301 SDDHKGEHGE DGAPVPGQQL DSTVGMSSLD GTLANAAEHP SETAKQDSTS
351 SPQLGAKKTQ PGPCEITTPK RSIRDCFNYN EDSPTQPTLP KRGLFLKETQ
401 KNERKGSDRK GQVVDLKPEL SRSTPSLVDP PDRSKLCLVL QSSYPSSPSA
451 ASQSYECLHK VGLGNLENIV RSHIKEISSS LGRLTDCHKE KLRLKKPHKT
501 LAEVSLCRIP KQGGGSGKRS ESTGSSAGPS MVSPGAPKAT MRPETDSAST
551 ASGGLCHQRN RSGQLPVQSK ASSSPCSHS SESSLGSDSI KSPVPLLSKN
601 KSQKSSPPPAP CHATQNGQVV EAWYGSDEYL ALPSHLKQTE VLALKLESLT
651 KLLPQKPRGE TIQDIDDWEL SEMNSDSEIY PTYHIKKHT RLGTVSPSSS
701 SDIASSLGES IESGPLSDL SDEDLCLPLS SVKKFTDEKS ERPSSSEKNE
751 SHSATRSALI QKLMHDIQHQ ENYEAIWERI EGFVNKLDEF IQWLNEAMET
801 TENWTPPKAE TDSLRLYLET HLSFKLNVDS HCALKEAVEE EGHQLLELVV
851 SHKAGLKDTL RMIASQWKE L QRQIKRQH SW ILRALDTIKA EILATDVSV
901 DEEGTGSPKA EVQLCHLETQ RDAVEQMSLK LYSEQYTSGS KRKEEFANMS
951 KAHAEGSNGL LDFDSEYQEL WDWLIDMESL VMDSHDLMMS EEQQQHLYKR
1001 YSVEMISRHL KKSELLSKVE ALKKGGSLP DDILEKVDSI NEKWELLGKT
1051 LREKIQDTIA GHSGSGPRDL LSPESGSLVR QLEVRIKELK RWLRDTELF
1101 FNSCLRQEKE GTSAEKQLQY FKSLCREIKQ RRRGVASILR LCQHLLDDRD
1151 TCNLNADHQ P MQLIIVNLER RWEAIVMQAV QWQTRLQKKM GKESETLNV
1201 DPGLMDLNGM SEDALEWDET DISNKLISVH EESNDLQDP EPMLPAVKLE
1251 ETHHKDSGYE EEAGDCGGSP YTSNITAPSS PHIYQVYSLH NVELHEDSHT
1301 PFLKSSPKFT GTTQPTVLT K SLSKDSSFSS TKSLPDLLGG SGLVRPYSCH
1351 SGDLSQNSGS ESGIVSEGDN EMPTNSDMSL F SVMGDGPSN PETEHPDPQM
1401 GDAANVLEQK FKDNGESIKL SSVSRASVSP VGCVNKGAGD LNSVTKHTAD
1451 CLGEELQGKH DVFTFYDYSY LQGSKLKLPM IMKQPQSEKA HVEDPLLGGF
1501 YFDKKSCAK HQASESQPDA PPHERILASA PHEMGRSAYK SSDIEKTFTG
1551 IQSARQLSLL SRSSSVESLS PGGDLFGLGI FKNGSDSLQR STSLESWLTS
1601 YKSNEDLFSC HSSGDISVSS GSVGELSKRT LDLLNRLENI QSPSEQKIKR
1651 SVSDMTLQSS SQKMPFAGQM SLDVASSINE DSPASLTELS SSDELSLCSE
1701 DIVLHKNKIP ESNASFRKRL NRVADES DV NVSMIVNVSC TSACTDDEDD
1751 SDLLSSSTLT LTEELCLKD EDDDSIATD DEIYEESNL M SG LDYIKNEL
1801 QTWIRPKLSL TREKKRSGVT DEIKVNKG DGG GNEKANPSDT LDIEALLNGS
1851 IRCLSENNGN GKTPPRTHGS GTKGENKKST YDVS KDPHVA DMENGNIEST
1901 PEREREKPQG LPEVSENLAS NVKTISESEL SEYEAVMDGS EDSSVARKEF
1951 CPPNDRHPPQ MGPKLQH PEN QSGDCKPVQN PCPGPLLSEAG VGSRQDSNGL
2001 KSLPNDA PSG ARKPAGCCL EQNETEESAS ISSNASCCNC KPDVFHQKDD

```

FIGURE 3 (cont.)

2051 EDCSVHDFVK EIIDMASTAL KSKSQPESEV AAPTSLTQIK EKVLEHSHRP
2101 IHLRKGDFYS YLSLSSHDS~~D~~ CGEVTNYIDE KSST**TPLPPDA VDSGLDDKED**
2151 **MDCFFEACVE DEPVNEEAGL PGALPNESAI EDGAEQKSEQ KTASSPVLS**D
2201 **KTDLVPLSGL SPQKGADDAK EGDDVSHTSQ GCAESTEPTT PSGKANAEGR**
2251 **SRMQGVSATP EENAASAKPK IQAFSLNAKQ PKGKVAMRYP SPQLTCKEK**
2301 **LVNFHEDRHS NMHR**

FIGURE 4

Sequence for myc-PBD:

1 meqkliseed lsgmiltmsy tisplrsqtp lppdaydsgl ddkedmdcff eacyedepyn
61 eeaglpgalp nesaiedgac qkseqktass pylsdktdly plsglspqkg addakegddy
121 shsqqgaes tepttsgka naegrstmgq vsatpeenaa sakpkigafs lnakqpkgy
181 amryxpsttl tckeklynfh edrhsmhr

FIGURE 5**Figure 5. pscA-TnT-myc-rat mAKAP PBD**

1 ACTCAACCAA GTCATTCTGA GAATAGTGTA TGC GGCGACCC GAGTTGCTCT
 51 TGCCCGGGGT CAATA CGGGTA TAATACCGCG CCACATAGCA GAACTTTAAA
 101 AGTGCTCATC ATTGGAAAAC GTTCTCGGG GCGAAAACTC TCAAGGATCT
 151 TACCGCTGTT GAGATCCAGT TCGATGTAAAC CCACTCGTGC ACCCAACTGA
 201 TCTTCAGCAT CTTTTACTTT CACCAGCGTT TCTGGGTGAG CAAAAACAGG
 251 AAGGCCAAAT GCCGCAAAAAA AGGGAATAAG GGC GACAC CGG AAATGTTGAA
 301 TACTCATACT CTTCCCTTTT CAATATTATT GAAGCATTAA TCAGGGTTAT
 351 TGTCTCATGA CGGGATAACAT ATTTGAATGT ATTAGAAAA ATAAACAAAT
 401 AGGGGTTCCG CGCACATTTC CCCGAAAAGT GCCACCTGAC GTCTAAGAAA
 451 CCATTATTAT CATGACATTA ACCTATAAAA ATAGGCYTAT CACGAGGCC
 501 TTTCGTCTCG CGCGTTTCGG TGATGACGGT GAAAACCTCT GACACATGCA
 551 GCTCCCGGAG ACGGTACAG CTTGTCTGTA AGCGGATGCC GGGAGCAGAC
 601 AAGCCC GTCA GGGCGCGTCA GCGGGTGTG GCGGGTGTG GGGCTGGCTT
 651 AACTATGCGG CATCAGAGCA GATTGTACTG AGAGTGCACC ATATGGACAT
 701 ATTGTCTGTTA GAACGCGGCT ACAATTAAATA CATAACCTTA TGTATCATA
 751 ACATACGATT TAGGTGACAC TATAGAACCTC GAGCCTGCGC GCTCGCTCGC
 801 TCACTGAGGC CGCCCCGGCA AAGCCC GGGC GTCGGGCGAC CTTGGTGC
 851 CCGGCCTCAG TGAGCGAGCG AGCGCGCAGA GAGGGAGTGG CCAACTCCAT
 901 CACTAGGGGT TCCTTGAGT TAATGATTAA CCCGCCATGC TACTTATCTA
 951 CGTAGCCATG CTCTAGAGCA GTCTGGGCTT TCACAAGACA GCATCTGGGG
 1001 CTGCGGCAGA GGGT CGGGTC CGAAGCGCTG CCTTATCAGC GTCCCCAGCC
 1051 CTGGGAGGTG ACAGCTGGCT GGCTTGTC AGCCCCCTCGG GCACCTCACGT
 1101 ATCTCCGTCC GACGGTTTA AAATAGCAAA ACTCTGAGGC CACACAATAG
 1151 CTTGGGCTTA TATGGGCTCC TGTGGGGGAA GGGGGAGCAC GGAGGGGGCC
 1201 GGGGCCGCTG CTGCCAAAT AGCAGCTCAC AAGTGTGCA TTCCCTCTCG
 1251 GGCGCCGGGC ACATTCTGC TGGCTCTGCC CGCCCCGGGG TGGGCCGCG
 1301 GGGGACCTTA AAGCCTCTGC CCCCCAAGGA GCCCTTCCCA GACAGCCGCC
 1351 GGCACCCACC GCTCCGTGGG ACCTAAGCTT GCTAGCGCTA CCGGTGCGCA
 M E Q K L I S E E D L S P G M L
 1401 CCATGGAGCA GAAACTCATC TCTGAAGAGG ATCTGAGGCC GGGGATGTTA
 T M S V T L S P L R S Q T P L P P .
 1451 ACCATGAGCG TGACACTTTC CCCACTGAGG TCACAGACTC CATTGCCACC
 . D A V D S G L D D K E D M D C F F .
 1501 GGACGCTGTG GACTCTGGCT TAGATGACAA GGAAGACATG GACTGCTTCT
 . E A C V E D E P V N E E A G L P
 1551 TTGAAGCTTG TGTTGAGGAT GAGCCTGTCA ATGAGGAAGC TGGTCTCCCC
 G A L P N E S A I E D G A E Q K S .
 1601 GGTGCCCTTC CCAATGAATC AGCCATCGAG GATGGAGCAG AGCAAAAGTC
 . E Q K T A S S P V L S D K T D L V .
 1651 AGAACAAAAG ACAGCCAGCT CTCCTGTGCT CAGTGACAAAG ACAGACCTGG
 . P L S G L S P Q K G A D D A K E

FIGURE 5 (cont.)

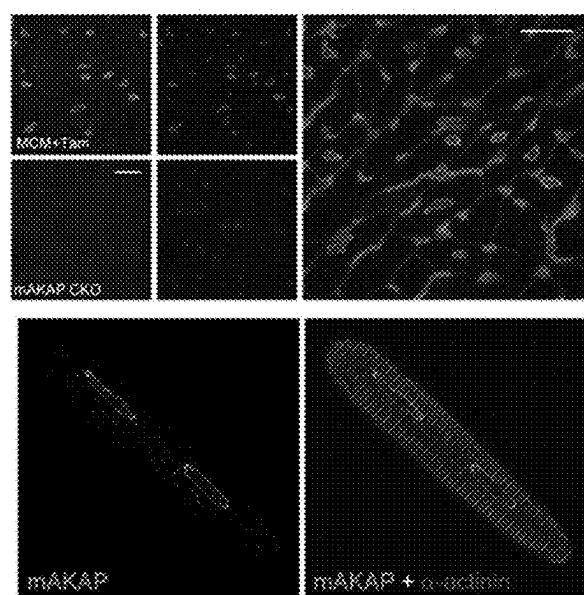
1701 TGCCTCTTTC AGGACTTTCC CCTCAGAAGG GAGCTGATGA TGCAAAGGAA
 G D D V S H T S Q G C A E S T E P ·
 1751 GGAGATGATG TGTCTCACAC TTCCCAGGGC TGTGCAGAGA GCACAGAGCC
 · T T P S G K A N A E G R S R M Q G ·
 1801 TACCACCCCC TCAGGAAAGG CCAATGCAGA GGGGAGGTCA AGAATGCAAG
 · V S A T P E E N A A S A K P K I
 1851 GTGTATCAGC AACGCCAGAA GAAAACGCTG CTTCGGCCAA ACCGAAAATT
 Q A F S L N A K Q P K G K V A M R ·
 1901 CAAGCTTCT CTTTGAATGC AAAACAGCCA AAAGGCAAGG TTGCCATGAG
 · Y P S P Q T L T C K E K L V N F H ·
 1951 GTATCCCAGC CCCCAAACTC TAACCTGTAA AGAGAAGCTC GTAAACTTTC
 · E D R H S N M H R
 2001 ATGAAGATCG ACACAGTAAC ATGCATAGGT AGAGTGTAAAT GCCCCCACGC
 2051 ATGGAAATCA TCTCATTGAA AGATAGCCTG GCTGAAGCTC AGGGCTAGTT
 2101 AAGTTTGATC CAGACATGAT AAGATACATT GATGAGTTG GACAAACCAC
 2151 AACTAGAATG CAGTAAAAAA AATGCTTAT TTGTGAAATT TGTGATGCTA
 2201 TTGCTTTATT TGTAAACCATT ATAAGCTGCA ATAAACAAGT TAACAACAAC
 2251 AATTGCATTC ATTTTATGTT TCAGGTTCAAG GGGGAGGTGT GGGGAGGTTT
 2301 TTAAAGCAAG TAAAACCTCT ACAAAATGTGG TATGGCTGAT TACCACTCCC
 2351 TCTCTGCCG CTCGCTCGCT CACTGAGGCC GGGCGACCAA AGGTCGCCCG
 2401 ACGCCCAGGC TTGCCCCGGG CGGCCTCAGT GAGCGAGCGA GCGCGCCAGC
 2451 TGAAGCTATC AGATCTGCCG GTCTCCCTAT AGTGAGTCGT ATTAAATTG
 2501 ATAAGCCAGG TTAACCTGCA TTAATGAATC GGCCAACGCG CGGGGAGAGG
 2551 CGGTTTGCCT ATTGGGCGCT CTTCCGCTTC CTCGCTCACT GACTCGCTGC
 2601 GCTCGGTCGT TCGGCTGCCG CGAGCGGTAT CAGCTCACTC AAAGGCGGT
 2651 ATACGGTTAT CCACAGAACAC AGGGGATAAC GCAGGAAAGA ACATGTGAGC
 2701 AAAAGGCCAG CAAAAGGCCA GGAACCGTAA AAAGGCCGCG TTGCTGGCGT
 2751 TTTCCATAG GCTCCGCCCT CCTGACGAGC ATCACAAAAAA TCGACGCTCA
 2801 AGTCAGAGGT GGCAGAACCC GACAGGACTA TAAAGATACC AGGCCTTCC
 2851 CCCTGGAAGC TCCCTCGTGC GCTCTCCTGT TCCGACCTG CCGCTTACCG
 2901 GATACCTGTC CGCCTTCTC CCTCAGGGAA GCGTGGCGCT TTCTCATAGC
 2951 TCACGCTGTA GGTATCTCAG TTGGTGTAG GTCGTTCGCT CCAAGCTGG
 3001 CTGTGTGCAC GAACCCCCCG TTCAGCCCGA CCGCTGCGCC TTATCCGGTA
 3051 ACTATCGTCT TGAGTCCAAC CCGGTAAGAC ACGACTTATC GCCACTGGCA
 3101 GCAGCCACTG GTAACAGGAT TAGCAGAGCG AGGTATGTAG GCGGTGCTAC
 3151 AGAGTTCTG AAGTGGTGGC CTAACACTACGG CTACACTAGA AGAACAGTAT
 3201 TTGGTATCTG CGCTCTGCTG AAGCCAGTTA CCTTCGGAAA AAGAGTTGGT
 3251 AGCTCTTGAT CCGGCAAACA AACCAACCGCT GGTAGCGGTG GTTTTTTGT
 3301 TTGCAAGCAG CAGATTACGC GCAGAAAAAA AGGATCTCAA GAAGATCCTT
 3351 TGATCTTTC TACGGGGTCT GACGCTCAGT GGAACGAAAA CTCACGTTAA
 3401 GGGATTGTTGG TCATGAGATT ATCAAAAAGG ATCTTCACCT AGATCCTTT
 3451 AAATTAAGGAA TGAAGTTTA AATCAATCTA AAGTATATAT GAGTAAACCT
 3501 GGTCTGACAG TTACCAATGC TTAATCAGTG AGGCACCTAT CTCAGCGATC
 3551 TGTCTATTTC GTTCATCCAT AGTTGCCTGA CTCCCCGTG TGAGATAAC
 3601 TACGATACGG GAGGGCTTAC CATCTGCCCG CAGTGCTGCA ATGATACCGC

FIGURE 5 (cont.)

3651 GAGACCCACG CTCACCGGCT CCAGATTAT CAGCAATAAA CCAGCCAGCC
3701 GGAAGGGCCG AGCGCAGAAG TGGTCCTGCA ACTTTATCCG CCTCCATCCA
3751 GTCTATTAAT TGTTGCCGGG AAGCTAGAGT AAGTAGTTCG CCAGTTAATA
3801 GTTGCGCAA CGTTGTTGCC ATTGCTACAG GCATCGTGGT GTCACGCTCG
3851 TCGTTGGTA TGGCTTCATT CAGCTCCGGT TCCCAACGAT CAAGGCGAGT
3901 TACATGATCC CCCATGTGT GCAAAAAAGC GGTTAGCTCC TTCGGTCCTC
3951 CGATCGTTGT CAGAAGTAAG TTGGCCGCAG TGTTATCACT CATGGTTATG
4001 GCAGCACTGC ATAATTCTCT TACTGTCATG CCATCCGTAA GATGCTTTTC
4051 TGTGACTGGT GAGT

FIGURE 6

A



B

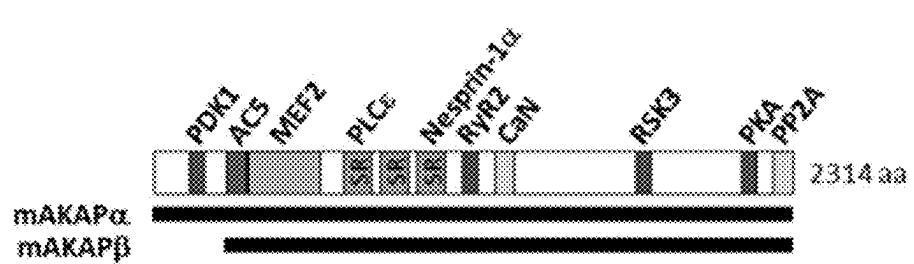


FIGURE 7

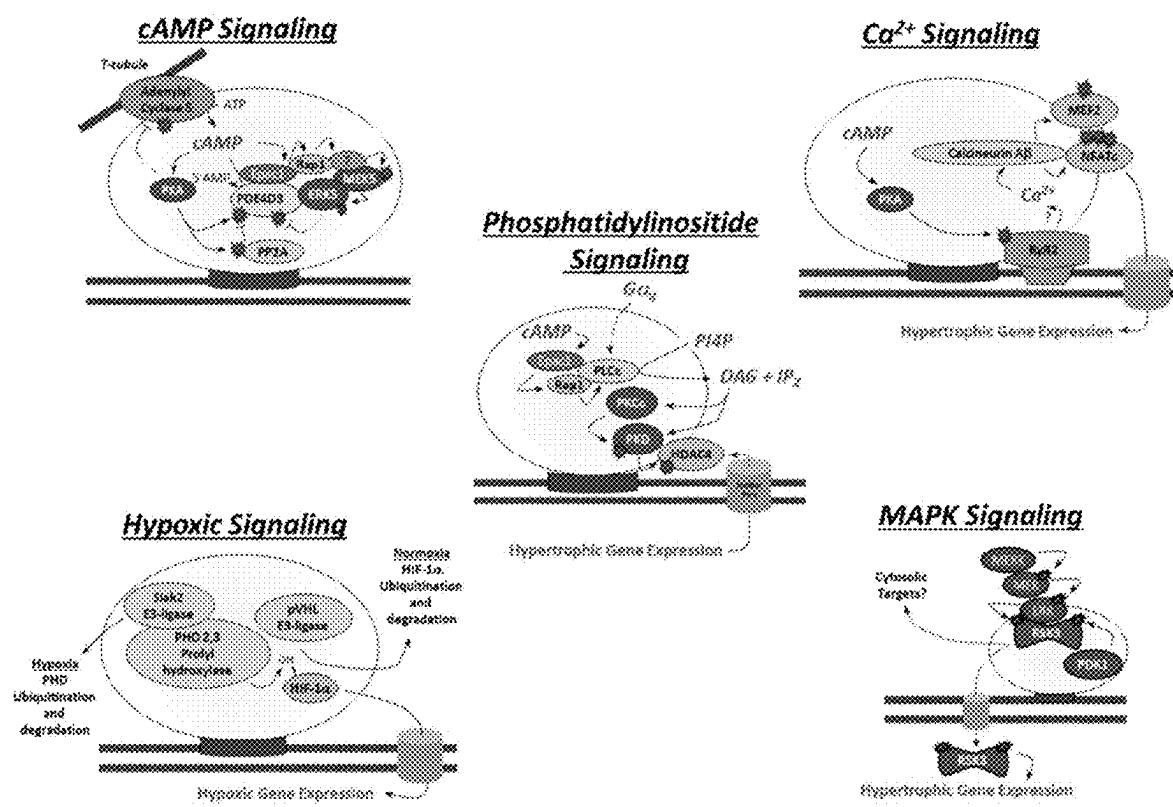


FIGURE 8

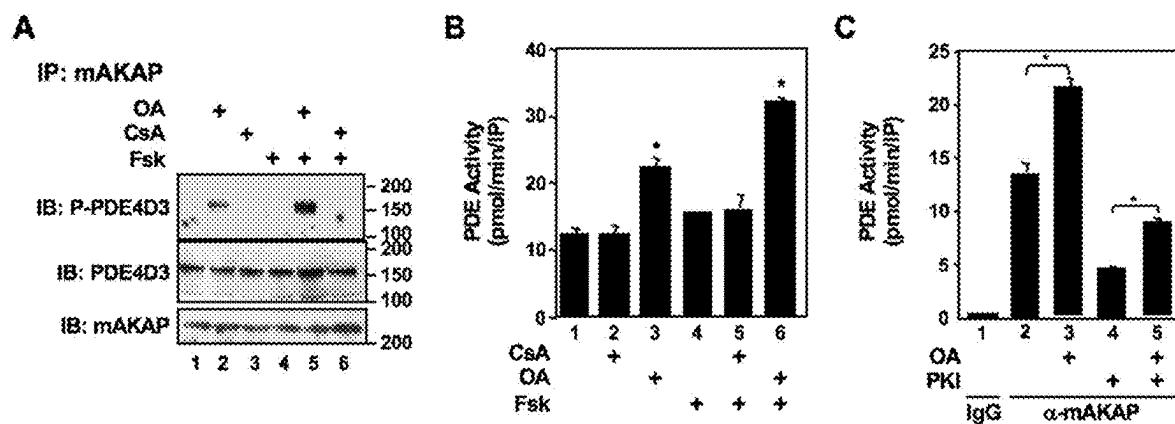


FIGURE 9

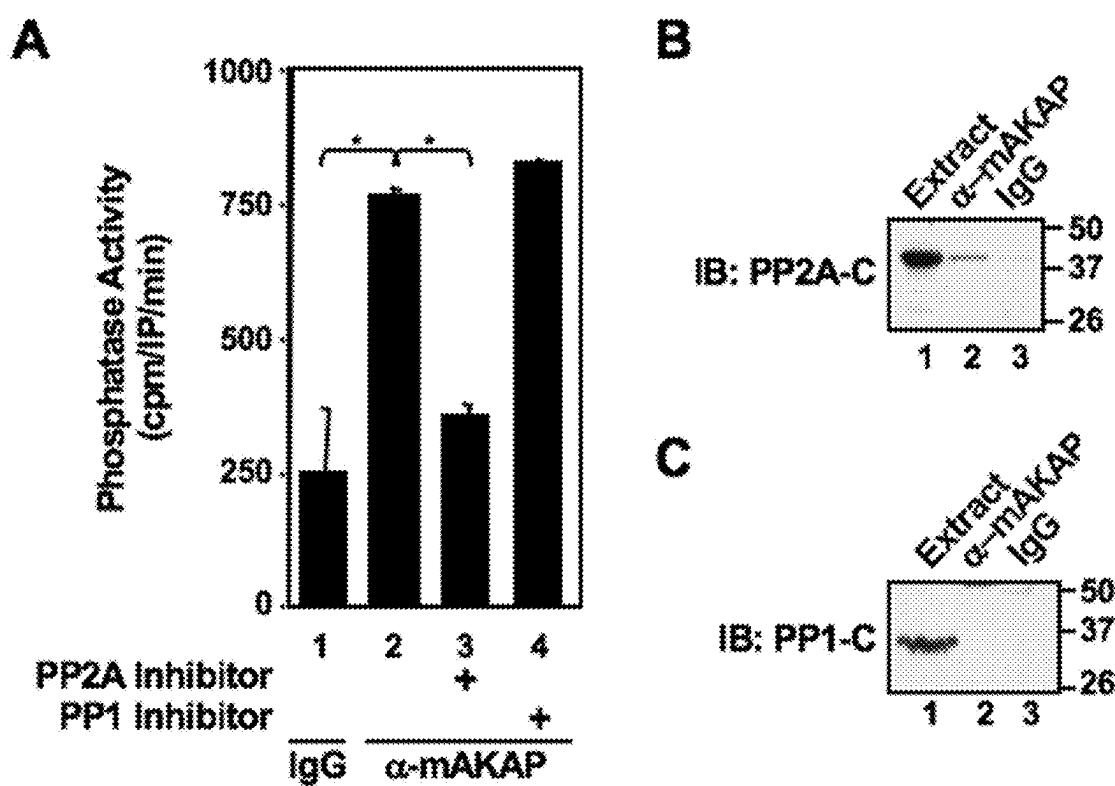


FIGURE 10

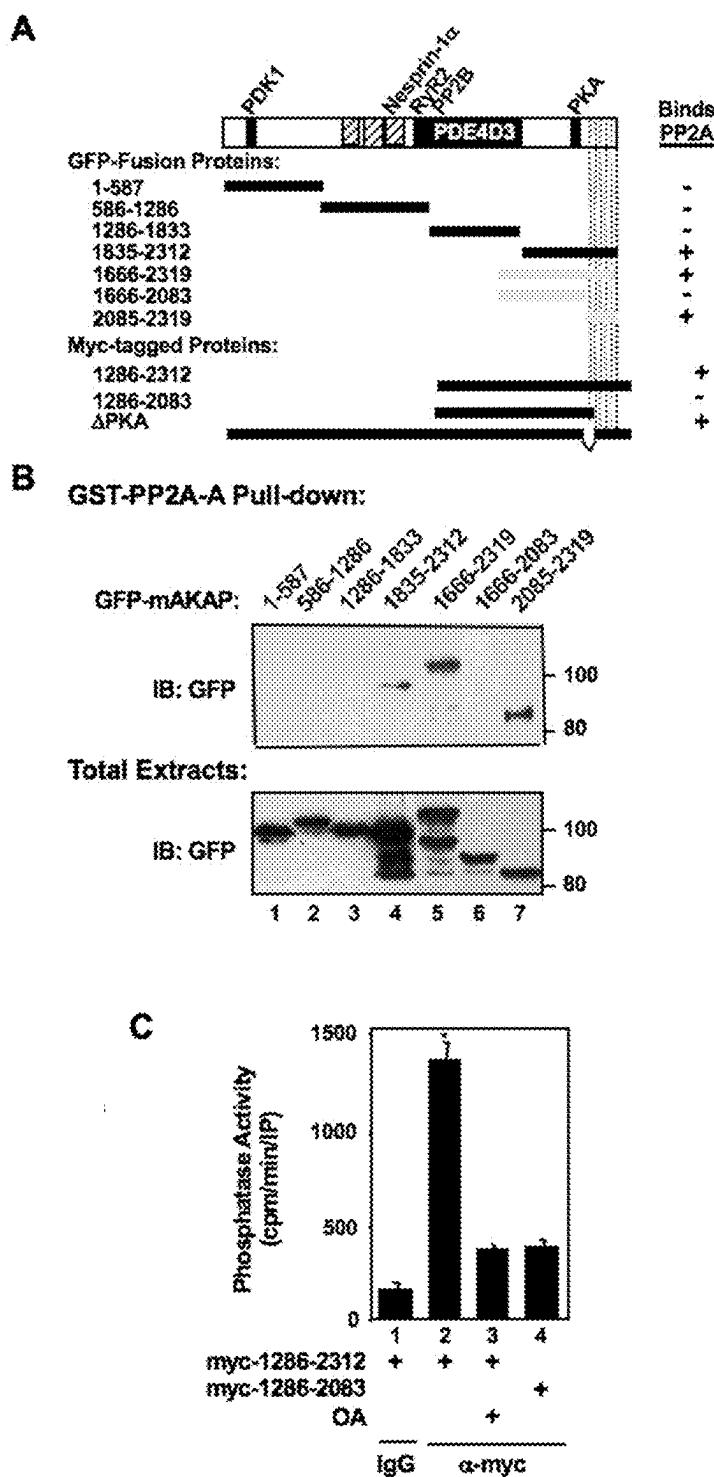


FIGURE 11

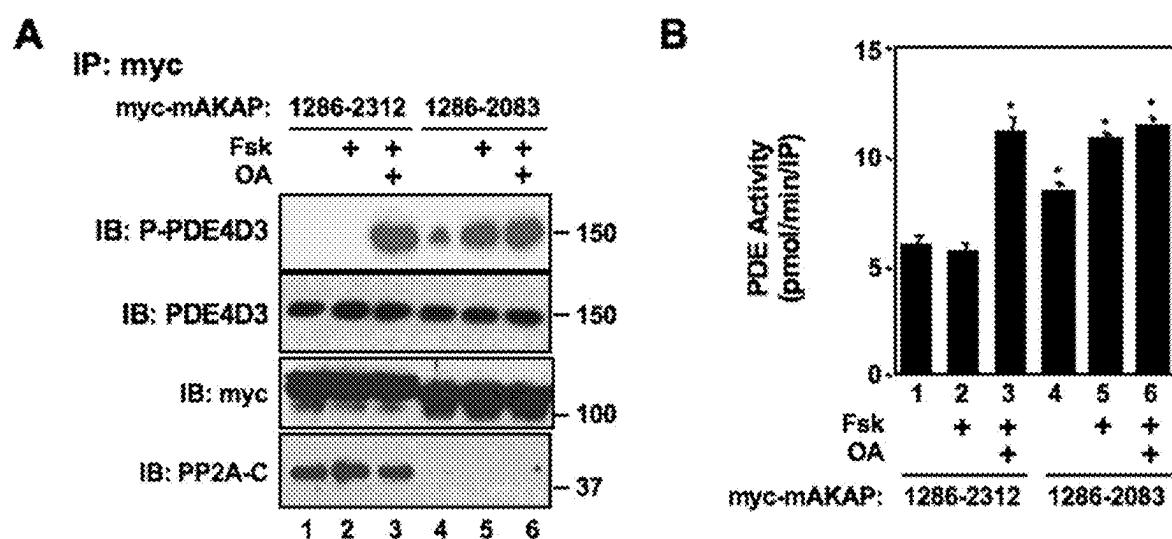


FIGURE 12

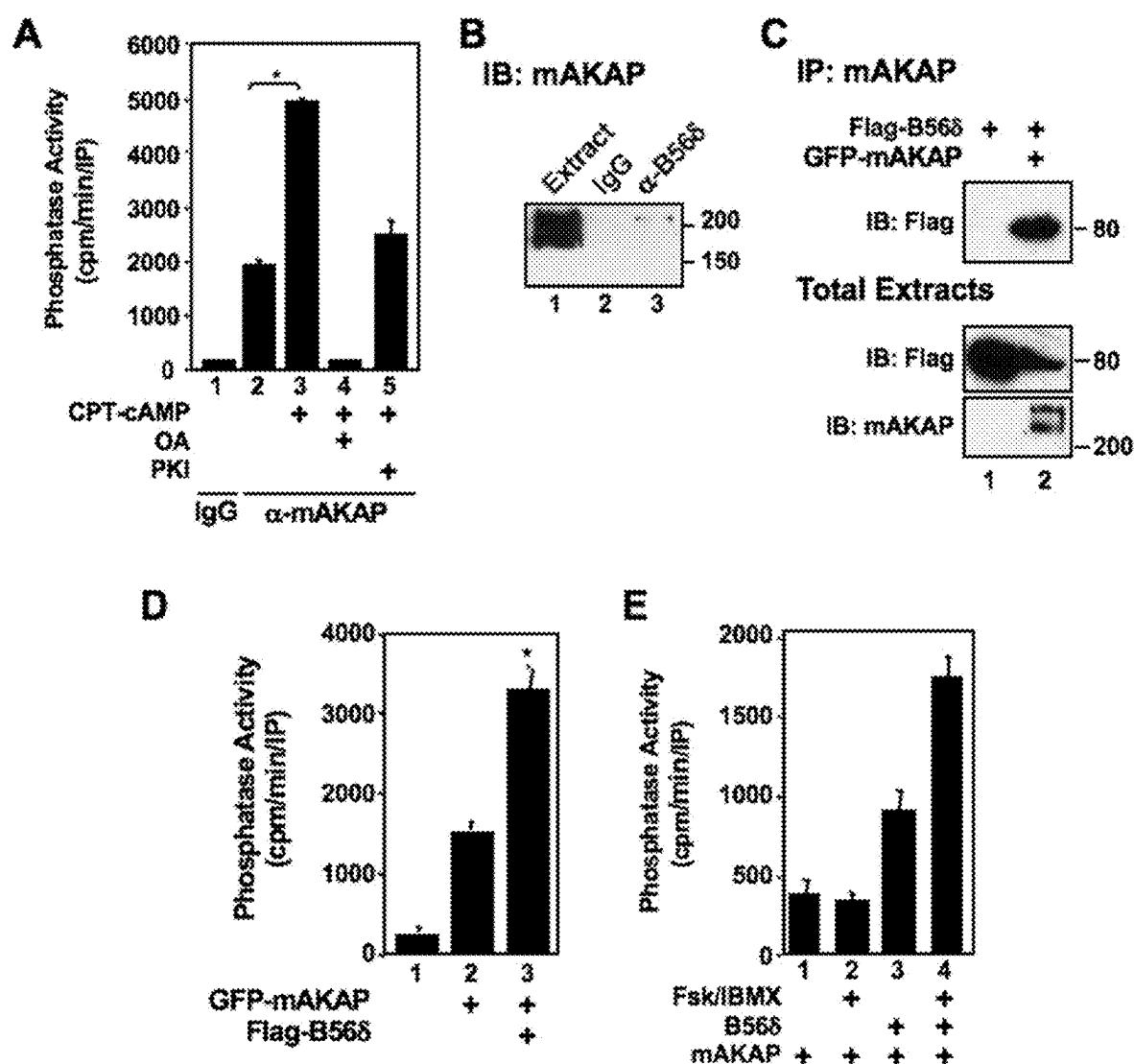


FIGURE 13

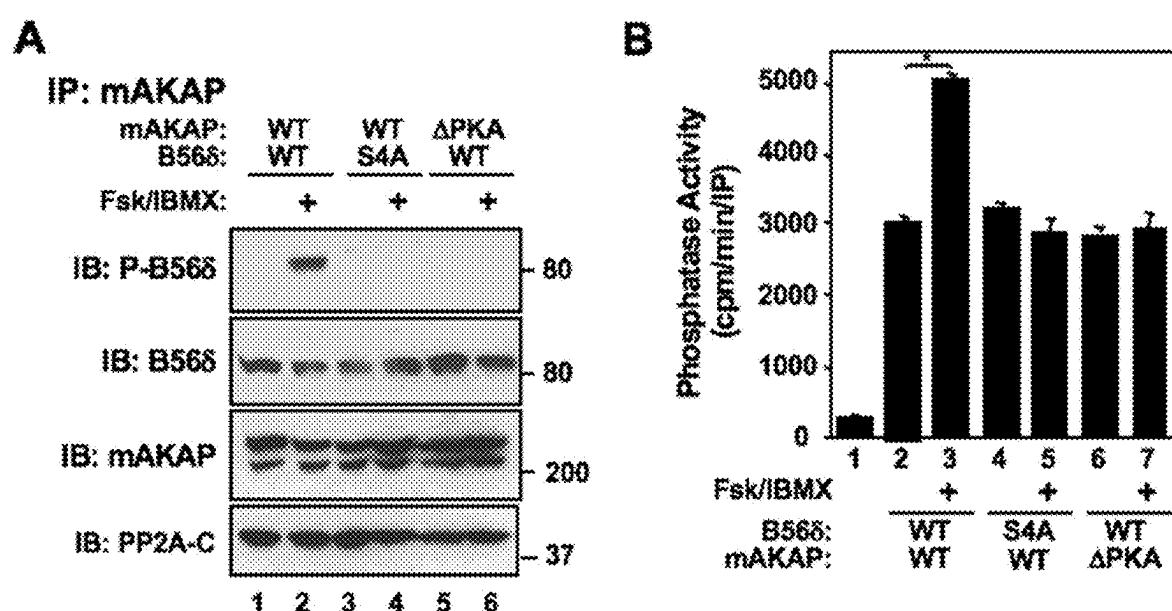


FIGURE 14

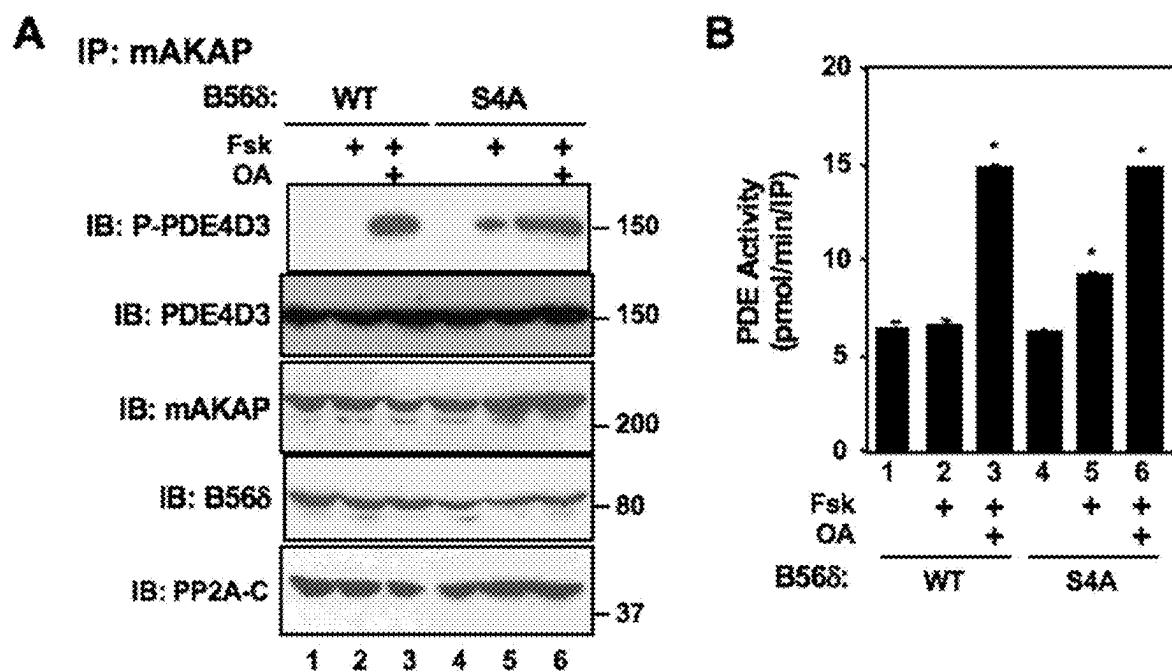


FIGURE 15

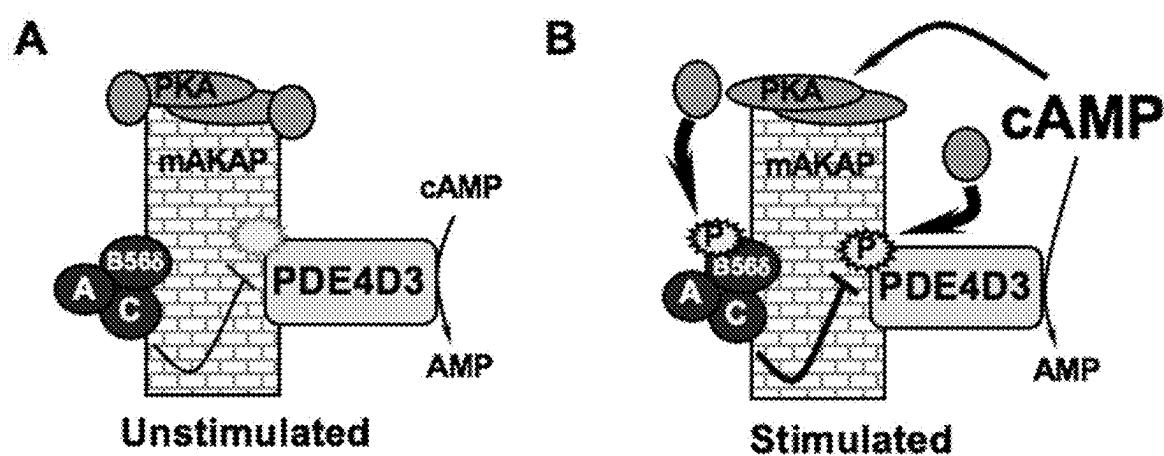


FIGURE 16

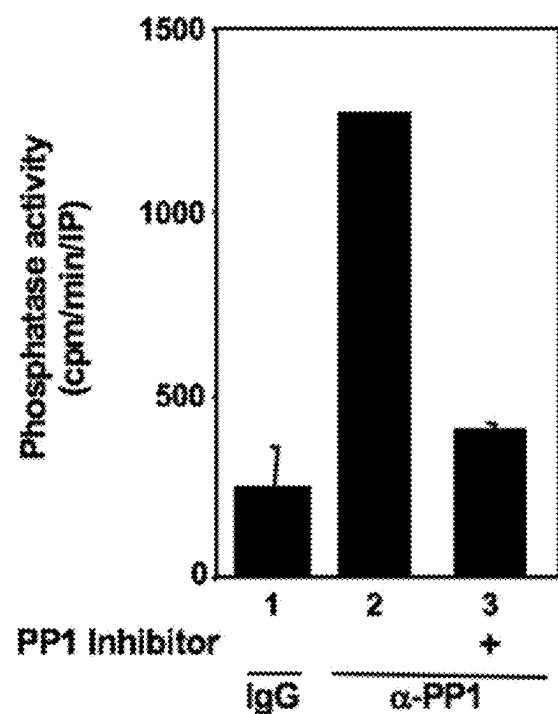


FIGURE 17

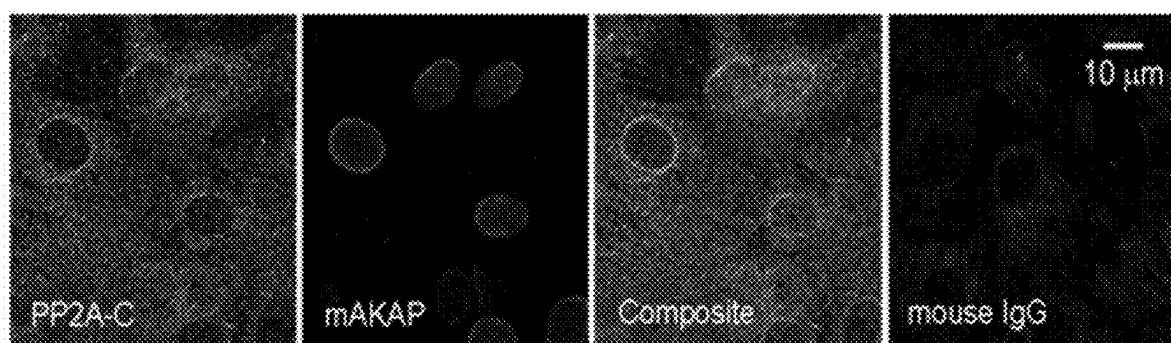
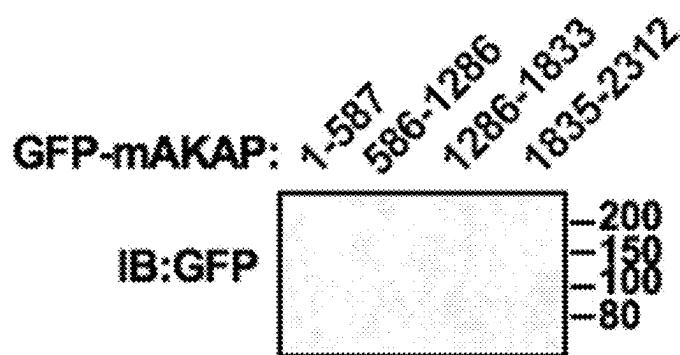


FIGURE 18

IP: PP1



Total Extracts

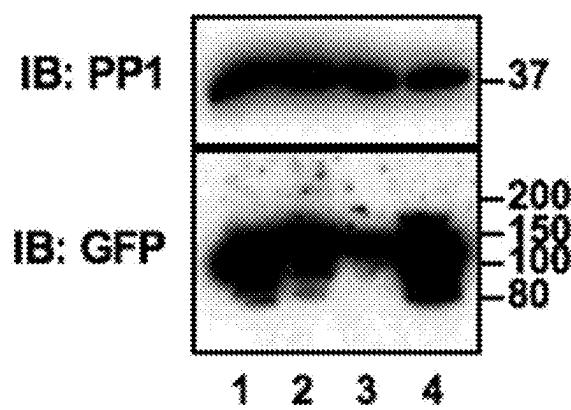


FIGURE 19

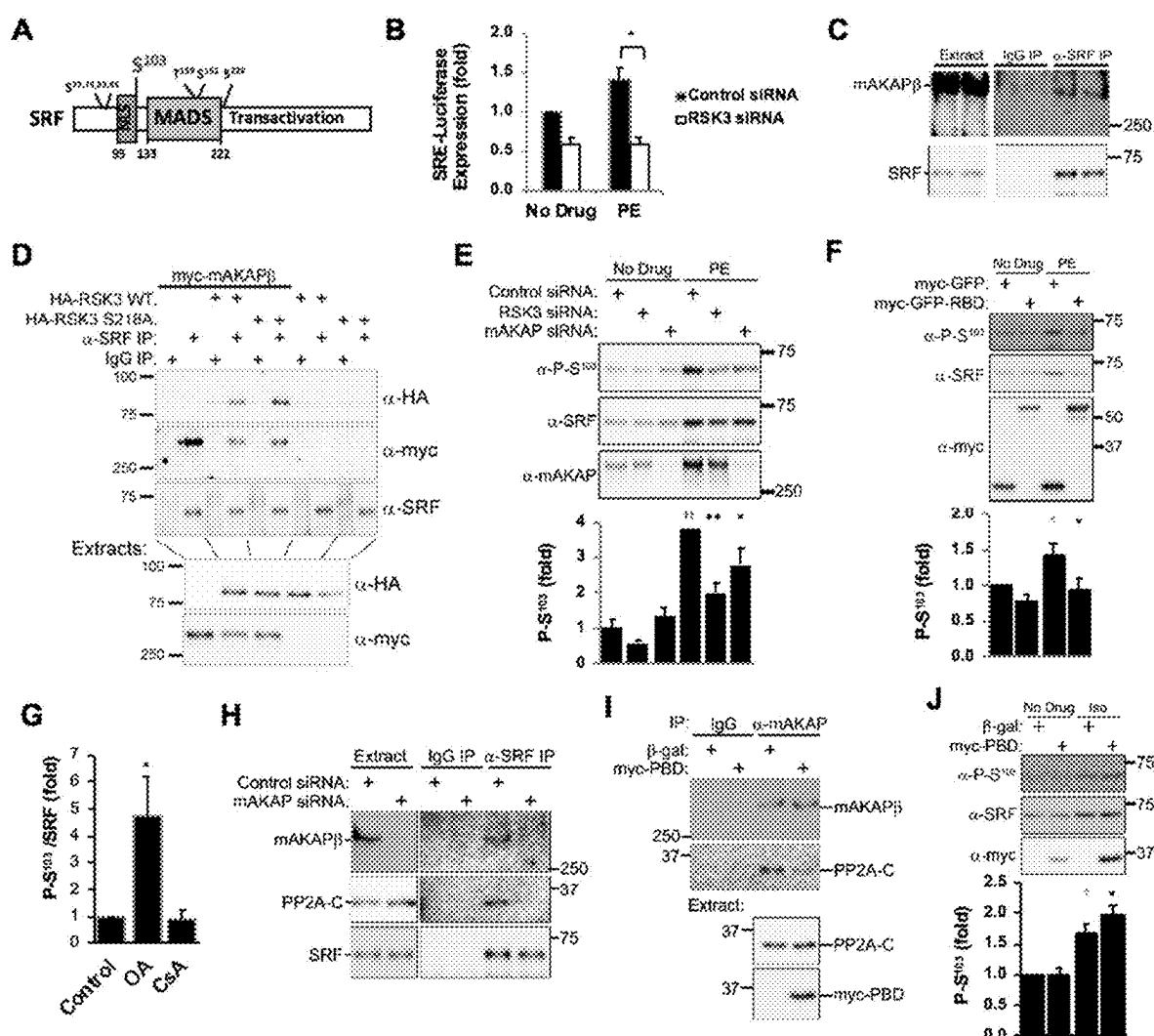


FIGURE 20

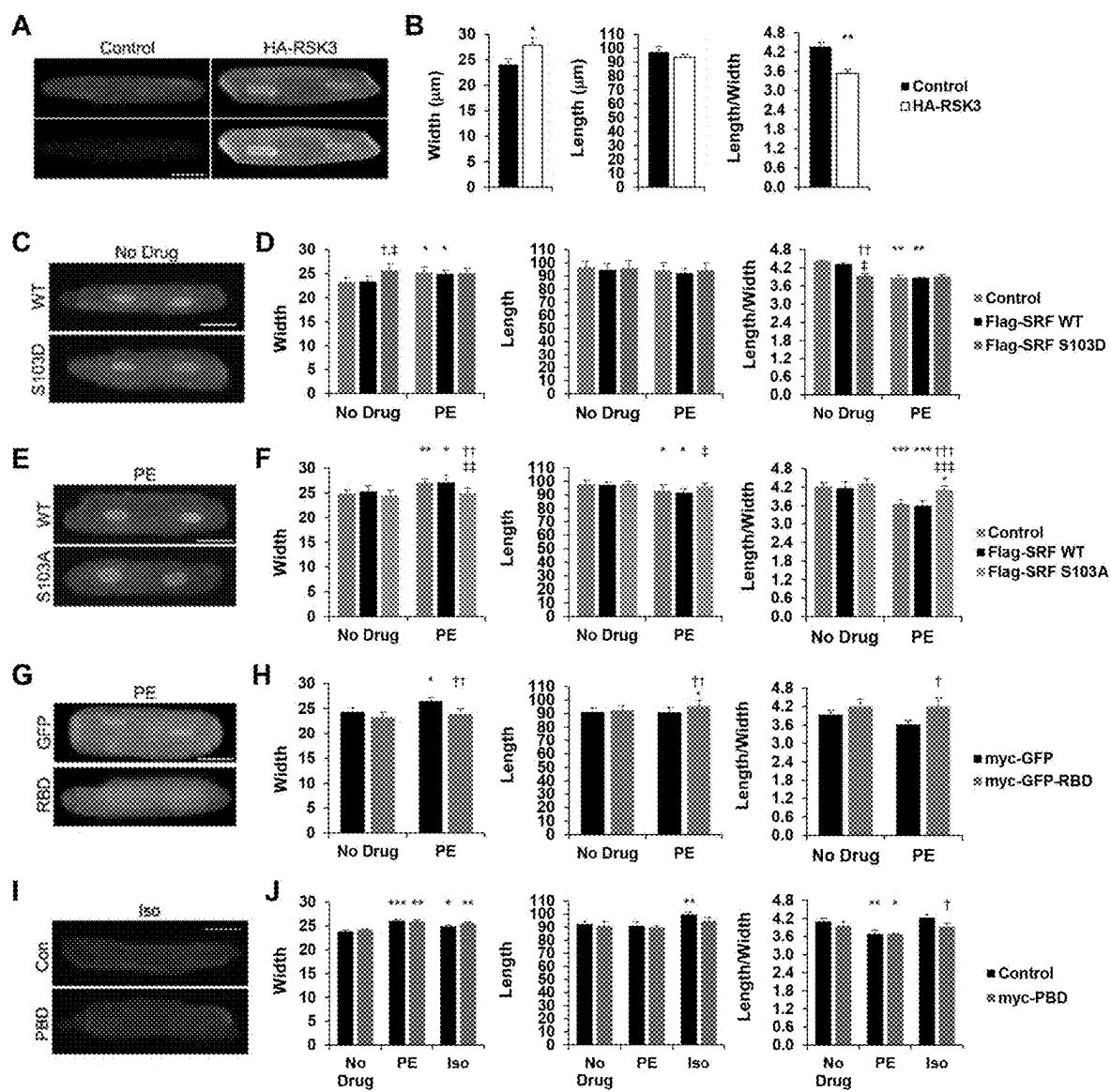


FIGURE 21

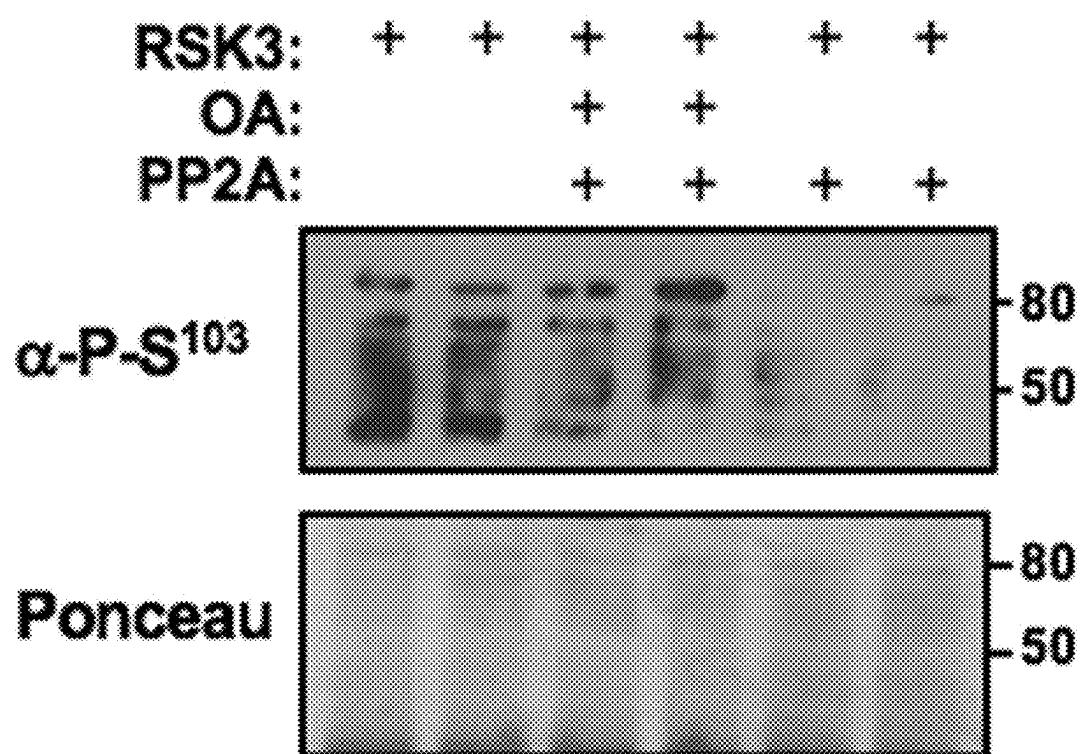


FIGURE 22

A



B

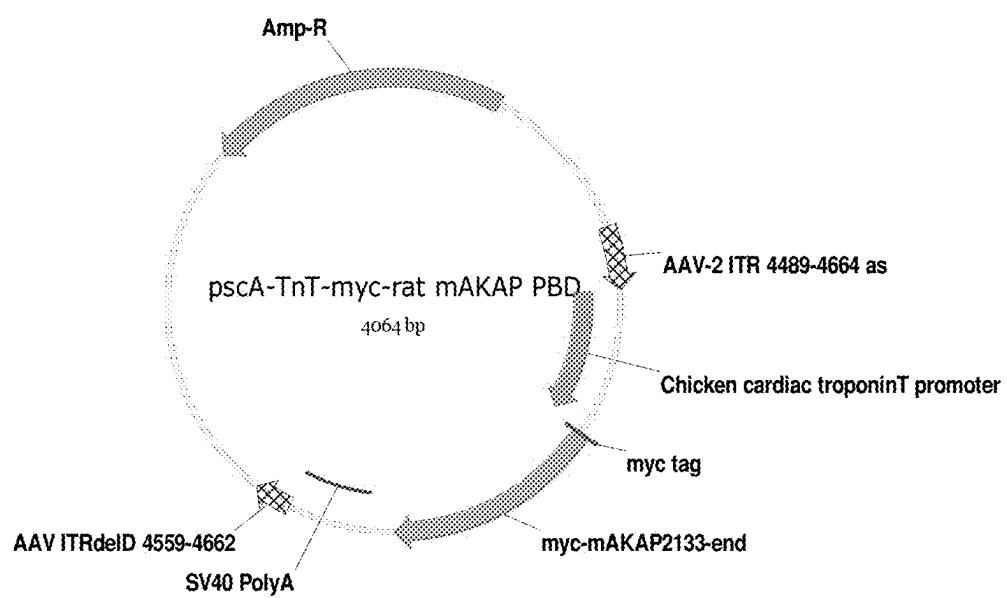


FIGURE 23

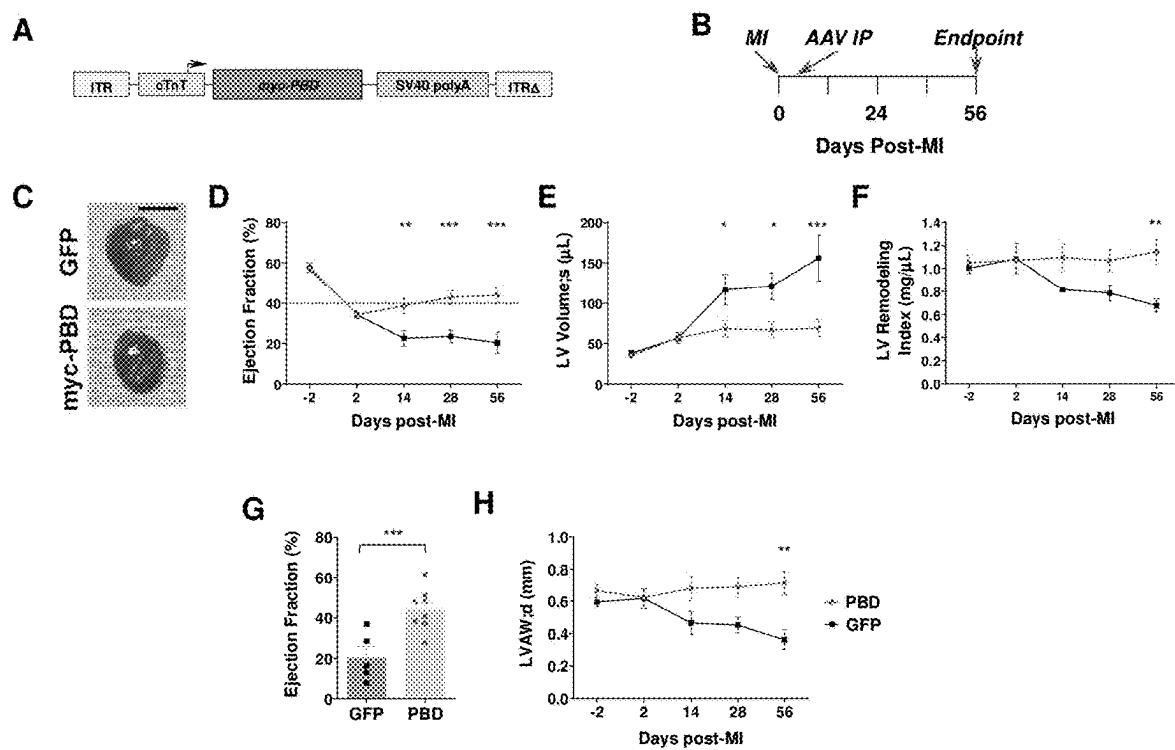


FIGURE 24

LOCUS bRSK3 5817 bp DNA linear PR1 22-JAN-2009
DEFINITION Homo sapiens ribosomal protein S6 kinase, 90kDa, polypeptide 2
(RPS6KA2), transcript variant 1, mRNA.
BASE COUNT 1285 a 1585 c 1591 g 1356 t
ORIGIN

FIGURE 24 (cont.)

2821 gggaaaagtgg cagtgcgagg gcgcagccat tgggtgttc agggccccag agggatgggg
 2881 tgacctggca tcccggggct ccccacggc tggatgacgg ggttggact gtggcgatca
 2941 ggaggagatg ctttgttctg cccaaaataa tccaaagagc cgttccctcc tcgccttca
 3001 gtttttgcct gaggtgtgtgg ttagccatc ttffcccttg tcccagattc aaatgaggag
 3061 taagagccca gacgagagga aggccaggctg gatcttgc tttagagatc cgtgtcacca
 3121 ggatggaaagg ggggtgcctct cggaggagcc tgggtccacc tccagtcgt gtttccccgg
 3181 gggggccaaq cgcactgggc tggcgtctgt cccagatcc cgtggccaca cagctatctg
 3241 gaggcttgc agggagtcgt gggttctgc acctgtctag ccctgtgtcg gtttctgtg
 3301 tgcgtcacata aagctgtgtt ttgtgtgt tcaacttcgt ttttctgtc tggagaaaa
 3361 ctgtgaattt gagaatggg gtcgtgtggc ttcccaaccca aaccccttc a tccagctgg
 3421 aggctggagg gagacacagg ccccacccag cagactgagg ggcagagggca cagggtggag
 3481 ggcagoggag atcagcgtgg acaggagcga tgcacttgtt agatgtgtg gtttgcgtt
 3541 gctgtttgtt tctgtgttc acaagatctgt ttttccacac tgcgtccgtat tccctgggt
 3601 gtgcacacaaag ggcgggtgtg gggcatttag gccatgtgt gttttttttt attgagtaaa
 3661 atcgagtgtgg aggttccggg cagcaggatc gacggccatg ccggccggca gagggaaacac
 3721 acgggtcttt cattgtctgt taaaagggtgtt gaagatgttc cctggcggcc cccaaagcaga
 3781 ctatgtgggaa ggaggcggccg ctcaayccctt caccctgtcat cactgaagaa cggccgtct
 3841 gcaagcaagca gggcttcagg aagggtccgc tggccacago cagggtttcc ctaagaagat
 3901 gtttttttgt tgggttttgt tcccccctca tctcgatct cgttacccaaac taaaaaaaaaaa
 3961 aaaaataaaaga aaaaatgtgc tgegttctgtt aaaaataactc ctttagcttgg tctgtatgtt
 4021 ttcagacatt aaaaatataaa ctgtttcac aagttttaat ccattgtgtat tttttttttt
 4081 ttagagaacc aaaaaacataa aaggagcga gtcggactga ataccgttt coatagtgcc
 4141 cacagggttat tcctcacatt ttctccatag aagatgtttt ttcccaaggc tagaactgaet
 4201 tccaccatgt tgaattttgtt ttttaggtttt taattttttt actttttttt agaaacttag
 4261 gaagaagtgg aataatctgt a ggtcacacaa tctgtttcc cagaaatgaa caaaagtcat
 4321 cactttttct gttgtctaca caggcaacga ttccccccatc agtgcggccg accctttggc
 4381 ctggcttgg tgcaggccot gtcgttttc ttaaagtctag tgggttctgg tgcagggtgt
 4441 gagaagtggg ggaagtgaas gggaaagcat ccgtgagaaa gcccggccacgg tttttccctcc
 4501 ttgtgtggcc atggggcacc agtcatgtt stttttcaat catccccattt tgcacagact
 4561 tagtttctgtt aacttaaaga tgcggaaagg accgacgaga ctccccatca cagcggactc
 4621 tgccttaca tttttttgtt gtcateago ggaggagaac actggcttgg ccctgtcccg
 4681 ctggcttgc tgcggaaatacc tctactttcc ctcacccatc cagaacaaaaa tgataacttga
 4741 catcccttca caaaaatctgttccatc octaaaggaaat tttttttttt atatgttaaa cttaaattttt
 4801 aaaaacccctt aatccatgtt aagtgtactgc ttcaagcag cagtcgcacat gtaaatgtt
 4861 gtgttcttag aatccgtttt ttcacccatc agtcgcaccc cacaacgtt gaaatgttcc
 4921 gtatgtatgtt cacaaatgttccatc atagacccatc cccaaaggta actggcttc ctcccttca
 4981 cagtttccatc taaccccaacc ccccccaccc ggytcatgaa aatccatgttccatc
 5041 tttttttttt aatccatgttccatc gtttccatc tttttttttt atatgttaaa cttaaattttt
 5101 tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt
 5161 tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt
 5221 tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt
 5281 tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt
 5341 tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt
 5401 tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt
 5461 tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt
 5521 tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt
 5581 tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt
 5641 tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt
 5701 tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt
 5761 tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt

FIGURE 25

Figure 25. rat MAKAP α mRNA with open reading frame translated.

1 GCATCATGCA GCAGGTCAAA CAAGGCATCT CCTAGTATTG CATCCTCCAG ATGTGCTGTA AACATCAAA
M L T M S .
71 GGAGACGCTG GGAGCAGGAG ATGCTGTTTG GAAAGAAGT AAGGCTTAGA TTCTCCATG TAAACCATGA
. V T L S P L R S Q G P D P M A T D A S P M A I .
141 GCGTGACACT TTCCCCACTG AGGICACAGG GCCCAGATCC CATGGCGACG GATGCTTCAC CCATGGCAT
. N M T P T V E Q E E G E G E E A V K A I D A E .
211 CAACATGACA CCCACTGTGG AGCAGGAGGA AGGAGAGGGAG GAGGAAAGCCG TGAAGGCCAT AGACGCTGAG
. Q Q Y G K P P P L H T A A D W K I V L H L P E I .
281 CAGCAGTATG GAAAGGCCAC TCCGCTCCAC ACAGCAGCCG ACTGGAAGAT TGTCTGCAC TTACCTGAGA
. E T W L R M T S E R V R D L T Y S V Q Q D A D .
351 TTGAGACCTG GCTCCGGATG ACCTCAGAGA GGGTCCGTGA CCTGACCTAC TCAGTCCAGC AGGATGCCAGA
. S K H V D V H L V Q L K D I C E D I S D H V E .
421 CAGCAAGCAT GTGGAIGTGC ATCTAGTTCA GCTGAAGGAC ATTTGTGAGG ATATTTCTGA CCATGIGGAG
. Q I H A L L E T E F S L K L L S Y S V N V I V D .
491 CAGATCCATG CCCCTCTGAG GACGGAGTTT TCCCTAAAGC TGCTGTCTA CCTGGTCAAC GTCATCGTAG
. I H A V Q L L W H Q L R V S V L V L R E R I L .
561 ACATCCACGC AGTACAGCTG CTCTGGCAC AGCTCCGCGT ATCCGTGCTG GTCTCCGGG AGCGCATCCT
. Q G L Q D A N G N Y T R Q T D I L Q A F S E E .
631 ACAAGGTCTG CAGGACGCCA ATGGCAACTA CACCAGGCAG ACTGACATTC TGCAAGCGTT CTCTGAAGAAA
. T T E G R L D S L T E V D D S G Q L T I K C S Q .
701 ACAACGGAGG GCCGGCTTGA TTCCCTTACA GAACTGGACG ACTCAGGGCA GTAACTATC AAATGTTCAC
. D Y L S L D C G I T A F E L S D Y S P S E D L .
771 AGGATTACTT GTCTCTGGAT TGTGGCATTG CGCATTTGA ACTCTCCGAC TACAGTCCAA GIGAGGAICT
. L G G L G D M T T S Q A K T K S F D S W S Y S .
841 GCTTGGTGGC CTGGGCCACA TGACCACCAAG CCAGGCCAAACTAAATCTT TTGACTCTG GAGCTACAGT
. E M E K E F P E L I R S V G L L T V A T E P V P .
911 GAGATGGAGA AAGAGTCCC TGAGCTTAC CGAACGGTTG GGCTGCTTAC AGTGGCCACC GAGCTGTCC
. S S C G E A N E D S S Q A S L S D D H K G E H .
981 CTTCAGCTG TGGAGAAGCC AATGAGGATT CAITCAAGC GTCCCTTCA GATGATCACA AAGGTAAACA
. G E D G A P V P G Q Q L D S T V G M S S L D G .
1051 CGGGGAAGAC GGTGCICCCG TACCTGGACA GCAGCTGGAC TCAACGGTGG GAATGTCTTC CTIAGACGGC
. T L A N A A E H P S E T A K Q D S T S S P Q L G .
1121 ACCGCTGGCAA ATGCTGCCA ACACCCCTCG GAGACAGCAA ACAAGACTC TACITCCTCC CCACAGCTIG
. A K K T Q P G P C E I T T P K R S I R D C F N .
1191 GTGCGAAGAA AACCCAGCCT GGTCTTGTG AAATTACGAC TCCATCCGGC ATTGCTTAA
. Y N E D S P T Q P T L P K R G L F L K E T Q K .
1261 TTATAACGAG GACTCCCCCA CACAGCCAC ATTACCCAAA AGAGGGCTT TTCTAAAAGA AACTCAAAAG
. N E R K G S D R R K G Q V V D L K P E L S R S T P .
1331 AATGAGCGCA AAGGCAGTGA CAGGAAGGGG CAGGTGGTTG ATTTAAAGCC TGAACGTGAGC AGAACGACCC
. S L V D P P D R S K L C L V L Q S S Y P S S P .
1401 CTICCTGGT GGACCCCCCT GACAGATCGA AGCTCTGCCT AGTGTGCGAG TCCTCCTACC CCAGCAGCCC
. S A A S Q S Y E C L H K V G L G N L E N I V R .
1471 TTCTGCTGCC AGCCAGTCTT ATGAATGTTT GCACAAGGTG GGGCTGGCA ATCTGAAAAA CATACTCAGA
. S H I K E I S S S L G R L T D C H K E K L R L K .
1541 AGTCACATTA AAGAAATTC TTCCAGTCTG GGAAGGCTTA CTGACTGCCA TAAAGAGAAA TTGGACTGA
. K P H K T L A E V S L C R I P K Q G G G S G K .
1611 AAAAGCCACA CAAGACCTG GCCGAAGTGT CTCCTGCGAG ATCCCTAAA CAGGGAGGCC GTTCAGGAAA
. R S E S T G S S A G P S M V S P G A P K A T M .
1681 GCGATCTGAG AGCACCGGGA GCTCAGCAGG GCCCAGCATG GTATCACCTG GAGCTCCCAA AGCCACGATG
. R P E T D S A S T A S G G L C H Q R N R S G Q L .
1751 AGACCAGAAA CAGATTCTGC GTCTACAGCC TCAGGTGGCC TGTGCCACCA GAGAAATCGC AGTGGACAAT
. P V Q S K A S S S P P C S H S S E S S L G S D .
1821 TGCCAGTGCAGTGCAGAAGGCC TOCAGTCAC CCCCTTGCAG TCACAGCAGT GAATCTTCTC TTGGCTCAGA
. S I K S P V P L L S K N K S Q K S S P P A P C .
1891 TAGCTCAAA TCCCCGGITC CTCTCTTCA AAAAACAAA AGCCAAAAAA GCTCCCCACC TGCTCCATGT
. H A T Q N G Q V V E A W Y G S D E Y L A L P S H .
1961 CACGCCACAC AGAACGGTCA GGTGGTGGAG GCTCTGATGA GTACCTAGCG CTGCCCTCTC
. L K O T E V L A L K L E S L T K L L P O K P R .

FIGURE 25 (cont.)

2031 ACCTGAAGCA GACGGAGGTG TTAGCCTCTCA AGCTGGAGAG CCTAACCAAG CTCCCTACCCC AGAAACCCAG
 • G E T I Q D I D D W E L S E M N S D S E I Y P
 2101 AGGAGAGACC ATCCAGGATA TTGATGACTG GGAACGTGCT GAAATGAATT CAGATTCCGA AATCTATCCA
 T Y H I K K K H T R L G T V S P S S S S D I A S .
 2171 ACATACCACA TCAAGAAAAA ACACAGGAGA CTGGGCACAG TGTCTCCAAG CTCATCCAGC GACATAGCCT
 • S L G E S I E S G P L S D I L S D E D L C L P .
 2241 CATCTCTCGG GGAGAGCATT GAATCCGGC CCCTGAGTGA CATTCTTCT GACGAGGACT TATGTCTGCC
 • L S S V K K F T D E K S E R P S S S E K N E S
 2311 CCTCTCCAGC GTGAAAAGT TCACTGACGA GAAATCAGAG AGACCTTCAT CCTCCGAGAA GAACGAGAGC
 H S A T R S A L I Q K L M H D I Q H Q E N Y E A .
 2381 CATTCTGCAA CAAGATCAGC TTTGATTCAAG AAACATATGC ACAGATATTCA GCACCAAGAG AACTATGAAG
 • I W E R I E G F V N K L D E F I Q W L N E A M .
 2451 CCATCTGGGA AAGRATTGAG GGGTTTGTGA ACAAGCTGGA TGAATTCTT CAGTGGCTAA AGCAAGCCAT
 • E T T E N W T P P K A E T D S L R L Y L E T H
 2521 GGAGACCACC GAGAACTGGA CTCCCTCTAA AGCCGAGACC GACAGCCTCC GGCTGTACCT GGAGACACAC
 L S F K L N V D S H C A L K E A V E E E G H Q L .
 2591 TTGAGTTTA AGTTGAACGT AGACAGCCAC TGTGCCCTCA AGGAAGCCGT GGAGGAAGAA GGACACCAAC
 • L E L V V S H K A G L K D T L R M I A S Q W K .
 2661 TTCTTGAGCT CGTTGTATCT CACAAAGCAG GACTGAAGGA CACGCTGAGG ATGATTGCGA GTCAATGGAA
 • E L Q R Q I K R Q H S W I L R A L D T I K A E
 2731 GGAGCTGGAG AGGCAATCA AACGGCAACA CAGCTGGATT CTCAGAGCCC TGGACACCAT CAAAGCCGAG
 I L A T D V S V E D E E G T G S P K A E V Q L C .
 2801 ATACTGGCTA CTGAIGTGTCTG TGTGGAGGAC GAGGAGGGGA CGGGAAGCCC CAAGGCCGAG GTTCAGCTCT
 • H L E T Q R D A V E Q M S L K L Y S E Q Y T S .
 2871 GCCACCTGGA AACACAGAGA GACGCCGTGG AACAGATGTC CCTGAAGCTG TACAGCGAGC AGTACACCAG
 • G S K R K E E F A N M S K A H A E G S N G L L
 2941 CGGGAGCAAG AGGAAGGAAG AGTTTCCCAA CATGTCGAAA GCGCACGCGG AGGGAAGCAA TGGGCTTCTG
 D F D S E Y Q E L W D W L I D M E S L V M D S H .
 3011 GACTTGTATTAGAATATCA GGAGCTCTGG GATTGGCTGA TTGACATGGA GTCCCTCGTG ATGGACAGCC
 • D I M M S E E Q Q Q H L Y K R Y S V E M S I R .
 3081 ACCGACCTGTATGATGTCAGAG GAGCAGCAGC AGCATCTTAA CAAGAGGTAC AGTGTGGAAA TGTCAGCTCAG
 • H L K K S E L L S K V E A L K K G G L S L P D
 3151 GCATCTGAAA AAGTCAGAGC TACTCAGCAA GGTGAAGCT TTGAAGAAAG GTGGCCTTTC ACTACCAGAC
 D I L E K V D S I N E K W E L L G K T L R E K I .
 3221 GATACTCTGG AAAAGTGGG TICAATTAAAT GAAAATGGG AGCTGCTGG GAAAACCTTA AGAGAGAAGA
 • Q D T I A G H S G S G P R D L L S F E S G S L .
 3291 TACAGGACAC AATAGCGGGG CACAGTGGGT CGGGCCCCACG TGACCTGCTA TCTCTGAAA CGGGAAGCCT
 • V R Q L E V R I K E L K R W L R D T E L F I F
 3361 GGTAAGGCAG CTGGAGGTCA GGATCAAAGA GCTGAAAAGG TGGCTAAGAG ATACAGAGCT TTTCATCTTC
 N S C L R Q E K E G T S A E K Q L Q Y F K S L C .
 3431 ATTCTCTGTC TGAGACAAGA GAAGGAAGGA ACAAGCGCCG AGAAACAGCT CCAATACITTA AAGTCGCTCT
 • R E I K Q R R R G V A S I L R L C Q H L L D D .
 3501 GTCTGTAGAT CAAGCAGCGG CGTCGAGGAG TGGCCTCCAT TCTGAGGTG TGCCAGCACC TTCTGGATGA
 • R D T C N L N A D H Q F M Q L I I V N L E R R
 3571 CGGGGACACG TGCAACCTGA ACCGAGATCA CCAGCCCAG CAGCTGATCA TTGTAAACCT CGAGAGGCCGG
 W E A I V M Q A V Q W Q T R L Q K K M G K E S E .
 3641 TGGGAGGCCA TCGTCATGCA AGCTGCTCCAG TGGCAAACAC GGTIACAAA GAAGATGGGG AAGGAATCCG
 • T L N V I D P G L M D L N G M S E D A L E W D .
 3711 AGACTTTGAA TGIGATTGAT CCTGGCTTGA TGGACCTGAA TGGATGAGT GAGGATGCCG TGGAAATGGGA
 • E T D I S N K L I S V H E E S N D L D Q D P E
 3781 TGAAACAGAC ATAAGTAACA AACTCATTAG TGTGCATGAA GAATCAAACG ACCTTGATCA AGACCCAGAG
 P M L P A V K L E E T H H K D S G Y E E E A G D .
 3851 CCTATGCTAC CGCGAGTGAAG GCTTGAAGAG ACACACCACA AGGACTCTGG TTATGAAGAG GAGGCAGGTG
 • C G G S P Y T S N I T A P S S P H I Y Q V Y S .
 3921 ACTGTGGAGG GTCTCCGTAT ACCTCAAATA TCAC TGACCACTTCCAGCC CACATITACC AAGTGTACAG
 • L H N V E L H E D S H T P F L K S S P K F T G
 3991 TCTTCACAAT GTGGAGCTCC ACAGGAGACAG CCACACTCCA TTTCTGAAA GCAGCCTAA GTTCACAGGC
 T T Q P T V L T K S L S K D S S F S S T K S L P .
 4061 ACAACACAGC CTACTGTTT AACTAAGAGC CTCAGCAAGG ACTCTTCCTT TTCACTTACA AAATCGTTAC
 • D L L G G S G L V R P Y S C H S G D L S Q N S .

FIGURE 25 (cont.)

4131 CAGACCTTCT AGGGGGTTCC GGTGTTGGTGA GGCTTACTC GTGTCACAGT GGAGACTTGA GCCAGAAC
 • G S E S G I V S E G D N E M P T N S D M S L F
 4201 AGGCAGTGAG AGTGGAAATTG TCAGCGAAGG AGACAACGAG ATGCCGACCA ACTCTGACAT GAGCTTGT
 • S M V D G S P S N P E T E H P D P Q M G D A A N
 4271 AGTATGGTAG ACGGGTCCCC AAGTAACCCCT GAAACGGAGC ATCCGGACCC ACAAAATGGGA GATGCAGCCA
 • V L E Q K F K D N G E S I K L S S V S R A S V
 4341 ATGTGCTAGA GCAGAAAGTT AAAGACAACG GGGAAAGCAT TAAGCTTCA AGTGTCTCTC GGGCATCCGT
 • S P V G C V N G K A G D L N S V T K H T A D C
 4411 CTCACCAGTG GGTGTTGAAT ATGGAAAAGC AGGGGATTAA AACAGTGTAA CCAAACACAC TGCTGATTTG
 • L G E E L Q G K H D V F T F Y D Y S Y L Q G S K
 4481 TTGGGAGAAG AACTACAAGG AAAACATGAC GTGTTTACAT TTTATGATTA CTCGTACCTC CAAGGCTCAA
 • L K L P M I M K Q P Q S E K A H V E D P L L G
 4551 AACATCAAATT ACCAATGATA ATGAAACAGC CACAGAGTGA AAAGGCACAC GTGGAGGATC CCCITCTIGG
 • G F Y F D K K S C K A K H Q A S E S Q P D A P
 4621 TGGTTTTAT TTGATAAAAA AGTCTGCAA AGCTAAACAT CAGGCTTCAG AGTCACAACC AGATGCGCCT
 • P H E R I L A S A P H E M G R S A Y K S S D I E
 4691 CCCACGAAA GGATTCTGGC AAGCGCGCCC CACGAGATGG GACGCAGCGC ATACAAAAGT AGCGACATAG
 • K T F T G I Q S A R Q L S L L S R S S S V E S
 4761 AGAAGACATT CACGGGCATT CAGAGTGCAGA GACAGCTCTC CCTCTATCT CGTAGCTCAT CTGTAGAGTC
 • L S P G G D L F G L G I F K N G S D S L Q R S
 4831 CCTTCTCCA GGGGGTATT TGTTGGATT GGGAAATCTTT AAAAATGGCA GTGACAGCCT CCAGCGGAGC
 • T S L E S W L T S Y K S N E D L F S C H S S G D
 4901 ACTTCTTATG AAAGTGGTT GACATCCTAT AAGAGCAATG AGGATCTCTT TAGCTGTCAC AGCTCTGGGG
 • I S V S S G S V G E L S K R T L D L L N R L E
 4971 ACATAAGTGT GAGCACTGGC TCAGTGGTG AGCTGAGTAA GAGCACGTAA GACCTCTIGA ATGCCCTGG
 • N I Q S P S E Q K I K R S V S D M T L Q S S S
 5041 GAATATACAG AGCCCCTCGG AGCAAAAGAT CAAGCGGAGT GTTCTGACA TGACTCTACA AAGCAGTTCC
 • Q K M P F A G Q M S L D V A S S I N E D S P A S
 5111 CAAAGATGC CCTTCGCTGG CCAGATGTCA CTGGATGTG CATCCTCCAT CAATGAAGAC TCTCCGGCAT
 • L T E L S S S D E L S L C S E D I V L H K N K
 5181 CTCTTACAGA ACTGAGTAGT AGCGATGAGC TCTCTCTTGT CTGGAGGAGC ATTGTGTTAC ACAAAAACAA
 • I P E S N A S F R K R L N R S V A D E S D V N
 5251 GATCCAGAA TCCAACGCAT CATTAGGAA GCCTCTGAAT CGCTCAGTGG CTGATGAGAG CGACGTCAAT
 • V S M I V N V S C T S A C T D D E D D S D L L S
 5321 GTTACCATGA TTGTCAATGT GTCTCGCACC TCTGCTTGCAT CTGATGATGA AGATGACAGC GACCTCCCT
 • S S T E T L T E E E L C L K D E D D D S S I A
 5391 CCAGCTCCAC TCTCACCTTA ACTGAAGAAG AGCTGTGCCT CAAAGATGAG GATGACGACT CCAGTATTGC
 • T D D E I Y E E S N L M S G L D Y I K N E L Q
 5461 AACAGATGAT GAAATTATG AAGAGAGCAA CCTGATGTCT GGGCTGGACT ACATAAAGAA TGAACGTGAG
 • T W I R P K L S L T R E K K R S G V T D E I K V
 5531 ACTTGGATAA GACCAAAACT TTCCTGACG AGAGAAAAGA AACGGTCCGG TGTCACTGAT GAAATAAAGG
 • N K D G G G N E K A N P S D T L D I E A L L N
 5601 TCAATAAGA TGGGGAGGC AATGAGAAGG CCAATCCCTC GGACACCCCTG GACATCGAGG CCTTCTCAA
 • G S I R C L S E N N G N G K T P P R T H G S G
 5671 TGGCTCCATA AGATGTCTTT CGGAAACAA CGGGAAATGGT AAGACTCCGC CCAGAACTCA TGGCTCAGGA
 • T K G E N K K S T Y D V S K D P H V A D M E N G
 5741 ACCAAAGGTG AAAATAAGAA AAGTACGTAT GAGCTTAGTA AGGATCCGC CGTGGCTGAC ATGGAAAATG
 • N I E S T P E R E R E K P Q G L P E V S E N L
 5811 GCAATATTGA AAGTACCCCA GAAAGAGAAA GGGAGAAGCC ACAAGGGCCT CCAGAGGTGT CAGAGAACCT
 • A S N V K T I S E S E L S E Y E A V M D G S E
 5881 TGCTTCAAAT GTGAAACAGA TTCTGAAATC TGAGCTCAGC GAGTATGAAG CAGTAAATGGA TGCTTCTGAG
 • D S S V A R K E F C P P N D R H P P Q M G P K L
 5951 GATTCAAGTG TTGCCAGAAA GGAATTGTG CCCCCAAATG ACAGACATCC TCCACAGATG GGTCCTAAC
 • Q H P E N Q S G D C K P V Q N P C P G L L S E
 6021 TCCAGCATCC CGAAAATCAA AGTGGCAGT GCAAGCCAGT CCAGAACCT TGCCCCGGGC TACTGTGG
 • A G V G S R Q D S N G L K S L P N D A P S G A
 6091 AGCTGGCGTT GGAAGCAGGC AAGACAGCAA TGGACTAAAA TCCTTGCCTA ACGATGCACC AAGTGGGGCT
 • R K P A G C C L L E Q N E T E E S A S I S S N A
 6161 AGAAAACCTG CGGGTGTG CTCGCTGGAG CAGAATGAGA CAGAGGAAAG TGCTTCTATC AGCAGCAACG
 • S C C N C K P D V F H Q K D D E D C S V H D F

FIGURE 25 (cont.)

6231 CTTCCIGTIG CAACTGCAAG CCAGATGTTT TCCATCAAAA AGATGATGAA GATTGTCAG TACATGACTT
 · V K E I I D M A S T A L K S K S Q P E S E V A
 6301 TGTTAAGGAA ATCAITGACA TGGCATCAC AGCCCTAAAA AGTAAGTCAC AGCCTGAAAG TGAGGTGGCC
 A P T S L T Q I K E K V L E H S H R P I H L R K ·
 6371 GCACCCACAT CACTAACCCA AATTAAGGAG AAGGTGTTAG AGCAITCGCA CCGGCCATA CACCTGAGAA
 · G D F Y S Y L S L S S H D S D C G E V T N Y I ·
 6441 AGGGGGACTT TTACTCCTAC TTATCACTTT CGTCCCACGA CAGTGACTGT GGGGAGGTCA CCAATTACAT
 · D E K S S T P L P P D A V D S G L D D K E D M
 6511 AGATGAGAAG AGCAGTACTC CATTGCCACC GGACGCTGTG GACTCTGGCT TAGATGACAA GGAAGACATG
 D C F F E A C V E D E P V N E E A G L P G A L P ·
 6581 GACTGCCTCT TTGAAGCTTG TGTGAGGGAT GAGCCTGTCA ATGAGGAAGC TGGTCTCCCC GGTGCCCTTC
 · N E S A I E D G A E Q K S E Q K T A S S P V L ·
 6651 CCAATGAATC AGCCATCGAG GATGGAGCAG AGCAAAAGTC AGAACAAAAG ACAGCCAGCT CTCCGTGCT
 · S D K T D L V P L S G L S P Q K G A D D A K E
 6721 CAGTGACAAG ACAGACCTGG TGCCTCTTC AGGACTTTCC CCTCAGAAGG GAGCTGATGA TCAAAGGAA
 G D D V S H T S Q G C A E S T E P T T P S G K A ·
 6791 GGAGATGATG TGTCTCACAC TTCCCAGGGC TGTGAGAGA GCACAGAGCC TACCAACCCC TCAGGAAAGG
 · N A E G R S R M Q G V S A T P E E N A A S A K ·
 6861 CCAATGCAGA GGGGAGGICA AGAATGCAAG GTGTATCAGC AACGCCAGAA GAAAACGCTG CTGGGCCAA
 · P K I Q A F S L N A K Q P K S K V A M R Y P S
 6931 ACCAAAAATT CAAGCTTCT CTTGAAATGC AAAACAGCCA AAAGGCAAGG TTGCCATGAG STATCCCAGC
 P Q T L T C K E K L V N F H E D R H S N M H R
 7001 CCCCAAACTC TAACCTGTAA AGAGAAGCTC GTAAACCTTC ATGAAGATCG ACACAGTAAC ATGCATAGGT
 7071 AGAGTGTAAAT GCCCCCACGC ATGGAAATCA TCTCATTGAA AGATAGCTG GCTGAAGCTC AGGGCTAGCC
 7141 CAATCCACCC TGGGCCGGTC TTGGGCTCCA TCCGTATTC ACTGCCGCT GTCACATIGA CTTCTGAAG
 7211 ACGAACCTTC CTTCCGAATG CAGTCGTCC ACGTGGGCTT CTCGACCTGG ATGTGTGCAT TGCTCTCTT
 7281 AGGTGATCAT CCTAGTCTCA CAAAGCTGCT TGTCTCCCG TGGATTCCTG TCCAAGCTA CCTCTGGCAA
 7351 CCCTGCTCT CCAGCAAGAC TTCGGTTTC CCTCCCCCTC CTCCCCCCCTC TTAAAGTTCC GCGGCTCACCC
 7421 AAATTGATGG TCCATCAAC CCACTGCTG GAATGATACC CCTCCCATCA GTACTGACC AATGTTATGT
 7491 TTTGCTCTGA AAACCTTCGC TGTATTAGAC CAATGTTAT TGAAAGAGAT TTACCTAAAA AGCCCGCCCT
 7561 TGATTTGGTT GCAGTATAGA GGAGACACAT TGATCCTCTT AACAAAATTA AGTGATGCT GAAAGCGCCA
 7631 TTTTAATTAT TTCTTTTAA ATAATGATCT ATGCAGCACT TCAAGAAACA ACTATAACAG TGGTGTATCT
 7701 TATAAARTGG TACATCTAC TATTAAAGTTT GTTTTGGTT TCTATGCTTC TTGAGGTGGT GATGAGAAAA
 7771 ATGGTTTTT TTTAAAACG GTGTCCTTG CTGTATTACT TATAGCATTT ATTAAAAGC TGCTTICATG
 7841 GTAAGATTAC ACTGGTTGA AAGGAGGAAA TAGCAAGGTT AAGATGCGTG CATAATTCT GTATATATGT
 7911 ATAAGCTAGT GCAAACACTG ATGTATGACA GTATAAAATG CTTCATGTT TGTGATGCTC AGTGGTGTGG
 7981 AATATAAGCC TTAAACCGT TCGATTGCT GGTATTAAA ATTCGATTA TAAAATAGC TTATGGGG
 8051 AAAGGAAAT TAATGATCT TTCTACCTGT GTTACCAAT TTCTTCTCATG TGGTCTGGG AAAGAAAAG
 8121 AAACAAACCC CATATATTAG CTTCCAAAAT ATCCATAATG CACAGAAGGC TTAAGTTGCT TAGACTACAG
 8191 ACTGGGCCCTG AAGACTTCAT GATTTCTCA ATTTTCTGT TTCACTATAA ACATCCGAAA TAGCAAAGAT
 8261 TTCTTTCCCC TCCATCAACA GCATTITATT CTGAATGTTT TTATTCATC TTGTTAATGG TTAAAGTTG
 8331 TATTGGAGA TCTCTTACAT GCCCTIAATTI ATTAAATAA TTGAATGGG TTGGTGGAT GGTATAGAAA
 8401 ATTTAATTAT TATTTTATTI AACTACAGA TTTCAGGTGT ATTATTTTG TTAAATATTIC CATTGGTCT
 8471 TTGGTCTTT TTATGACTTG AAAGTTCTAG CTTTTAATTT ATATCATAAC TCCTACTAAA GTGCCTGACA
 8541 CACAGTAGGT ATTCATAGA GTTCCCTGAA TTAGAGTATT GGGTGGTTA TATATATATA TATATATATG
 8611 AGATTCCTGC ATTAAAACCA GAAAAGATG TGCAAAGTGA ACCAGACACA GCATATTATC AGATTTCAA
 8681 AAGGAAAGAG AACATAGCCA CAGAAATGAC AATCAITCAT TCACTAGATT AGCATCTTT GCTGCAAGT
 8751 CACCATCTA GATTCAAGGGA GAGCAGCTAT GACCGATGCA CTGCCTTGG AGGCTCTGT GTAGAGACA
 8821 GAGTGACCTC GTGCCGAATT C

FIGURE 26**Figure 26.** human mAKAP β mRNA with open reading frame translated:

1 GAGCTGGGA GCTGAGTGAC TGAGGGATTG AAACCTTGCT ATAGTITACAG TTCATAACAA GCGTCTAGGC
 71 AGTTAGACCT TAAGTGTCA GGTATGGAAA GAAAGTCATA TGTATGTT TAGATTCTGT TTGTAAGCT
 141 GGTTAACTAG AGACAGCTGA TGAAAAACCA AATGACTTG AGTTACAAGA TCTGGGCTTT CTCTGCTCTG
 211 CTTTCAACCT GTTGGTGGT GTTGGAGTAG CTGACAGAAC CGAATGGCTT GGCTGAGGGAA CATGAAGTGA
 281 CAGCAGCCG TTTAGGACCA CACCACATTG TGGACCTCTT GCTGTGAGT TCAGGACATT TGTGAAGATA
 351 TTCTGTGATCA TGTGAGCAA ATCCATGCC CCCTTGAAAC AGAGTTCTCC CTAAGCTGC TGTCTTACTC
 421 TGTCAACGTG ATAGTGACCA TCCACGCACT GCAGCTCTC TGGCACCCAGC TTGAGTCTC AGTGTGGTT
 491 CTGCGGGAGC GCATTCGCA AGGTCTGCA GACGCCAATG CAACTACAC TAGGCAGACG GACAITCTG
 561 AGCTTCTC TGAGAGACA AAAGAGGGCC GGCITGATIC TCTAACAGAA GTGGATGACT CAGGACATT
 631 AACCATCAAA TGTCTCAAA ATTACTTGTC TCTGGATTGT GGCATTACTG CAITCGAACT GTCTGACTAC
 M T S S Q V K T K P F D ·
 701 AGTCCAAGTG AGGATTGCT CAGTGGCTA GGTGACATGA CCTCTAGCCA AGTCAAAACC AAACCTTGT
 · S W S Y S E M E K E F P E L I R S V G L L T V ·
 771 ACTCTTGGAG CTACAGTGAG ATGGAAAAGS AGTTTCTGAG GCTTATCCGA AGTGTGGTT TACTTACGGT
 · A A D S I S T N G S E A V T E E V S Q V S L S
 841 AGCTGCTGAC TCTATCTCTA CCAATGGCAG TGAAGCAGT ACTGAGGAGG TATCTCAAGT ATCTCTCTCA
 V D D K G G C E E D N A S A V E Q P G I T L G ·
 911 GTAGACGACA AAGGTGGATG TGAGGAAGAC ATGCTTCTG CAGTCGAAGA GCAACCAGGC TTAACACTGG
 · V S S S S G E A L T N A A Q P S S E T V Q Q E ·
 981 GGGTGTATC ATCITCAGGA GAAGCTCTGA CAAATGCTGC TCAACCTCC TCTGAGACTG TGCAGCAAGA
 · S S S S S H H D A K N Q Q P V P C E N A T P K
 1051 ATCCAGTTCC TCCTCCATC ATGATGCAAA GAATCAGCAG CCTGTTCTT GTGAAAATGC ACCCCCCAAA
 R T I R D C F N Y N E D S P T Q P I L P K R G L ·
 1121 CGAACCATCA GAGATTGCTT TAATTATAAC GAGGACTCTC CCACGCAGCC TACATTGCCA AAAAGAGGAC
 · F L K E E T F K N D L K G N G G K R Q M V D L ·
 1191 TTCTCTTAA AGAGGAAACT TTAAGAATG ATCTGAAAGG CAATEGGGA AAGAGGCAAAGG TGTTIGATCT
 · K P E M S R S T P S L V D P P D R S K L C L V
 1261 AAAGCCTGAG ATGAGCAGAA GCACCCCTTC GCTAGTAGAT CCTCTGACA GATCCTAACT TTGCTGGTA
 L Q S S Y P N S P S A A S Q S Y E C L H K V G N ·
 1331 TTGCACTCTT CTTACCCCAA CAGCCCTCT GCTGCCAGCC AGTCTTATGA GTGTTACAC AAGGTGGGAA
 · G N L E N T V K F H I K E I S S S L G R L N D ·
 1401 ATGGGAACCT TGAAAACACA GTCAAATTTC ACATTAAGA AATTTCTCC AGCCTGGAA GGCTTAACGA
 · C Y K E K S R L K K P H K T S E E V P P C R I
 1471 CTGCTATAAA GAGAAATCTC GACTTAAAAA CCCACACAAG ACCTCAGAAG AGGTGCCTCC ATGCCAACAA
 P K R G T G S G K Q A K N T K S S A V P N G E L ·
 1541 CCTAAACGGG GGACTGGTIC AGGCAAACAA CCTAAAAATA CAAAGACCTC AGCAGTGCCTA AATGGAGAGC
 · S Y T S K A I E G P Q T N S A S T S S L E P C ·
 1611 TTCTTATAC TTCCAAGGCC ATAGAGGGGC CACAAACAAA TTCTGCTTCC ACATCCTCAC TTGAGCTTGC
 · N Q R S W N A K L Q L Q S E T S S S P A F T Q
 1681 TAATCAGAGA AGTTGGAATG CCAAATTGCA ATTGCACTGAA GAAACATCCA GTTCAACCAGC TTTTACTCAG
 S S E S S V G S D N I M S P V F L L S K H K S K ·
 1751 AGCAGTGAAT CCTCTGTTGG CTCAGACAAC ATCATGTCTC CGGTGCAACT TCTTCAAAA CACAAAGCA
 · K G Q A S S P S H V I R N G E V V E A W Y G S ·
 1821 AAAAGGTCA AGCCTCTCT CCAAGTCAGC TCACTAGGAA TGGTGGGTT GTGGAGGCCT GGTAIGGCTC
 · D E Y L A L P S H L K Q T E V L A L K L E N L
 1891 TGATGAATAC CTAGCACTGC CCTCTCACCT TAAGCAGACA GAAGTATGG CTTTGAAGTT GGAAAACCTA
 T K L L P Q K P R G E T I Q N I D D W E L S E M ·
 1961 ACAAAAGCTCA TGCCTCAGAA ACCCAGAGGA GAAACCATCC AGAATATGAA TGACTGGAA CTGTCGTGAA
 · N S D S E I Y P T Y H V K K K H T R L G R V S ·
 2031 TGAATTCAAGA TTCTGAAATC TATCCAACCT ATCATGTCAA AAAGAAGCAT ACAAGGCTAG GCAGGGTGTIC
 · P S S S S D I A S S L G E S I E S G P L S D I
 2101 TCCAAGCTCA TCTAGTGCAG TAGCCTCTTC ACTAGGGGAG AGCAATGAAAT CTGGGCCCT GAGTGCACATT
 L S D E E S S M P L A G M K K Y A D E K S E R A ·
 2171 TTCTCTGATG AGGAGTCCAG TATGCCCTCTC CCTGGCATGA AAAAGTATGC TGATGAGAAG TGAGAAAGAG
 · S S S E K N E S H S A T K S A L I Q K L M Q D ·
 2241 CTTCATCCTC TGAGAAAAAT GAGAGCCATT CTGCCACTAA ATCAGCTTA ATCAGAAAC TGATGCAAGA
 · I Q H Q D N Y E A I W E K I E G F V N K L D E

FIGURE 26 (cont.)

2311 TATTCAGCAC CAAGACAAC ATGAAGCCAT ATGGGAAAAA ATAGAGGGT TTGTAAACAA ACTGGATGAA
 F I Q W L N E A M E T T E N W T P P K A E M D D .
 2381 TTCATTCAAT GGTAAATGA AGCCATGGAA ACTACAGAGA ATTGGACTCC CCCTAAAGCA GAGATGGATG
 . L K L Y L E T H L S F K L N V D S H C A L K E .
 2451 ACCTTAACT GTATCTGGAG ACACACTTGA GTTTAAAGTT GAATGTAGAC AGTCATGTG CTCTCAAGGA
 . A V E E E G H Q L L E L I A S H K A G L K D M
 2521 AGCTGTGGAG GAGGAAGGAC ACCAACTTCT TGAGCTATT GCATCTACA AAGCAGGACT GAAGGACATG
 L R M I A S Q W K E L Q R Q I K R Q H S W I L R .
 2591 CTGGGATGA TTGCAAGTCA ATGGAAGGAG CTGCAGAGGC AAATCAAACG GCAGCACAGC TGATTCTCA
 . A L D T I K A E I L A T D V S V E D E E G T G .
 2661 GGGCTCTGGA TACCATCAA GCCGAGATA TGGCTACTGA TGTGTCTGTG GAGGATGAGG AAGGGACTGG
 . S P K A E V Q L C Y L E A Q R D A V E Q M S L
 2731 AAGCCCCAAG GCTGAGGETT AACTTGTCA CCTIGGAAGCA CAAAGAGATG CTGTTGAGCA GATGICCCCIC
 K L Y S E Q Y T S S S K R K E E F A D M S K V H .
 2801 AAGCTGTACA GCGAGCTGA TACCAGCAGC AGCAAGCGAA AGGAAGAGTT TGCTGATATG TCAAAAGTTC
 . S V G S N G L L D F D S E Y Q E L W D C L I D .
 2871 ATTCACTGGG AAGCAATGGG CTTCTGGACT TTGATTCA ATAICAGGAG CTCTGGGATT GCTTGATTGA
 . M E S L V M D S H D L M M S E E Q Q Q H L Y K
 2941 CATGGAGTCC CTTGTGATGG ACAGCCACGA CCTGTGATGT TCAGAGGAGC AGCAGCAGCA TCTTTACAAG
 R Y S V E M S I R H L K K T E L L S K V E A L K .
 3011 CGATACAGTG TGGAAATGTC CATCAGACAC CTGAAAAGA CGGAGCTGCT TAGTAAGTT GAAGCTTTGA
 . K G G V L L P N D L L E K V D S I N E K W E L .
 3081 AGAAAGGTGG CGTTTACIA CCAAATGATC TCCTTGAAAA AGTGGATTCA ATTAATGAAA ATGGGAAACT
 . L G K T L G E K I Q D T M A G H S G S S P R D
 3151 GCTTGGAAA ACCCTAGGAG AGAAGATCCA GGACACAATG GCAGGGCACA GTGGTCGAG TCCACGTGAC
 L L S P E S G S L V R Q L E V R I K E L K G W L .
 3221 CTGCTCTCTC CTGAAAGTGG AAGCCTGGTA AGGCAGCTGG AGGTCAAGGAT CAAAGAACATG AAAGGATGGC
 . R D T E L F I F N S C L R Q E K E G T M N T E .
 3291 TAAGAGATAAC AGAGCTTTTC ATCTTCAATT CCTGTCTGAG ACAAGAAAAG GAAGGAACAA TGAATACTGA
 . K Q L Q Y F K S L C R E I K Q R R G V A S I
 3361 GAAACAAC TG CAATCTTA AGTCCCCCTG TCGTGAATC AAGCAACGAC GTCGAGGAGT TGCCTCCATT
 L R L C Q H L L D D R E T C N L N A D H Q P M Q .
 3431 CTGCACTAT GCCAGCATCTTGGATGAC CGGGAGACTT GCAATCTGAA TGCAGACCAC CAGCCCAGTC
 . L I I V N L E R R W E A I V M Q A V Q W Q T R .
 3501 AGCTGATCAT TGTAAATCTT GAAAGAAGGT GGGAGCCAT TGTCATGCAA GCGCTCCAGT GGCAACACAG
 . L Q K K M G K E S E T L N V I D P G L M D L N
 3571 TCTACAAAAG AAGATGGGAA AGGAATCTGA GACTTGTAAAT GTGATTGATC CTGGCTTGAT GGACCTAAAT
 G M S E D A L E W D E M D I S N K L I S L N E .
 3641 GGGATGAGTG AGGATGCCCT GGAATGGGAT GAAATGGACA TAAGTAACAA GTTAATTAGT TTGAATGAGG
 . S N D L D Q E L Q P V I P S L K L G E T S N E .
 3711 AATCAAATGA CCTTGATCAA GAACCTAAC CTGTTATCCC TTCCCTGAAG CCTGGAGAGA CAAGTAATGA
 . D P G Y D E E A D N H G G S Q Y A S N I T A P
 3781 GGACCTGGT TAIGACGAGG AGGCTGATAA CCATGGGGGA TCTCAGTATG CCTCAAATAT TACTGCCCTT
 S S P H I Y Q V Y S L H N V E L Y E D N H M P F .
 3851 TCTAGTCCAC ACATTTACCA GGTGTACAGC CTCCACAATG TTGAACCTCTA TGAGGACAAC CACATGCCAT
 . L K N N P K V T G M T Q P N V L T K S L S K D .
 3921 TTCTGAAAAAA CAATCCAAAG GTCACCTGGCA TGACACAGCC TAATGTTTA ACTAAAGATC TCACTAAAGA
 . S S F S S T K S L P D L L G G S N L V K P C A
 3991 CTCTCATTT TCATCTACCA AATCTTGGCC AGATCTTCTA GGTGGTTCCA ATTTGGTAAA GCCCTGCGCA
 C H G G D M S Q N S G S E S G I V S E G D T E T .
 4061 TGTCATGGAG GAGACATGAG CCAGAATTCA GGCAGTGAGA GTGGAATTGT CAGTGAAGGA GACACAGAAA
 . T T N S E M C L L N A V D G S P S N L E T E H .
 4131 CCACTACCAA CTCTGAAATG TGCTTGCTCA ATGCAGTGGA TGGGTCCCCA AGTAACCTTG AACTGAACA
 . L D P Q M G D A V N V L K Q K F T D E G E S I
 4201 TCTGGACCA CAAATGGGAG ATGCAGTAA CGTGTAAAG CAAAAATTAA CAGATGAGGG GGAAAGCATT
 K L P N S S Q S S I S P V G C V N G K V G D L N .
 4271 AAGCTTCCAA ATAGCTCTCA GTCGTCATT TCACCACTGG GTTGTGTAAA TGGAAAAGTT GGAGATTAA
 . S I T K H T P D C L G E E L Q G K H D V F T F .
 4341 ACAGTATTAC CAAACATACCC CCTGACTGTT TGGGAGAAGA ATTACAAGGA AAACATGATG TGTTCACATT
 . Y D Y S Y L Q G S K I K L P M I M K Q S Q S E

FIGURE 26 (cont.)

4411 TTATGATTAC TCATACCTCC AAGGCCTCAAA ACTCAAATTA CCAATGATAA TGAAACAGTC ACAAAGCGAA
 K V H V E D P L L R G F Y F D K K S C K S K H Q ·
 4481 AAAGTCATG TGGAGGATCC CCTGCTCGT GGTTTITATT TTGATAAAAA ATCATGCAA TCTAACATC
 · T T E L Q P D V P P H E R I L A S A S H E M D ·
 4551 AGACTACAGA GTTACAACCA GATGTACCTC CCCATGAAAG GATTITGGCA AGTGCATCTC ATGAAATGGA
 · R I S Y K S G N I E K T F T G M Q N A K Q L S
 4621 TCGCATTTCATAAAAGTG GCAATATAGA AAAGACATTC ACTGGCATGC AGAATGCCAA ACAGCTCTCC
 L L S H S S S I E S L S P G G D L F G L G I F K ·
 4691 CTTTATCTC ATAGTCATC TATTGAGTCC CTTCTCCAG GGGGTGATT ATTGATTG GGCATCTTA
 · N G S D S L Q R S T S L E S W L T S Y K S N E ·
 4761 AAAATGGCAG TGACAGCCTC CAGCGAAGCA CTTCTTAGA AAGTTGGTG ACTTCCTATA AAAGCAATGA
 · D L F S C H S S G D I S V S S G S V G E L S K
 4831 AGATCTCTT AGCTGTCAAC GCTCTGGGA TATAAGCGTG AGCAGTGGCT CAGITGGIGA ACTAAGTAAA
 R T L D L L N R L E N I Q S P S E Q K I K R S V ·
 4901 AGAACATTAG ATCTCTGAA TCGTTGGAG AATATCCAGA GCCCCTCAGA GAAAAGATA AACCGAAGTG
 · S D I T L Q S S S Q K M S F T G Q M S L D I A ·
 4971 TTTCTGATAT CACTCTCAA AGCAAGTCCC AAAAGATGTC CTIACTGGC CAGATGTCAT TGGACATAGC
 · S S I N E D S A A S L T E L S S S D E L S L C
 5041 ATCTCTATC AATGAAGACT CAGCGGCATC TCTAACAGAA CTIAGCAGCA GTGACGAGCT CTCTCTTIGC
 S E D I V L H K N K I P E S N A S F R K R L T R ·
 5111 TCAGAGGATA TTGTGTACA CAAGAACAAAG ATCCCGGAAT CGAATGCATC GTTCAGGAAG CGTCTGACTC
 · S V A D E S D V N V S M I V N V S C T S A C T ·
 5181 GTTCAGTGGC TGATGAAAGC GATGTCAATG TCAGCATGAT TGTAAATGTC TCTTGCACCT CTGCTTGAC
 · D D E D D S D L L S S S T L T L T E E E L C I
 5251 TGATGATGAA GATGACAGCG ACCTGCTCTC CAGCTCTACC CTTACCTGTA CTGAAGAAGA GCTGTGCATC
 K D E D D D S S I A T D D E I Y E D C T L M S G ·
 5321 AAAGATGAGG ATGACGACTC CAGTATTGCA ACAGATGATG AAAITTAIGA AGACTGCAAC TTGATGTCAG
 · E D Y I K N E L Q T W I R P K L S L T R D K K ·
 5391 GGCTAGACTA CATAAAGAAT GAATTACAGA CCTGGATTAG GCCAAAATG TCTTGCACAA GAGATAAGAA
 · R C N V S D E M K G S K D I S S S E M T N P S
 5461 AAGGTGCAAT GTCACTGAG AGATGAAGGG CAGTAAAGAT ATAAGTAGCA GTGAGATGAC CAATCCCTCT
 D T L N I E T L L N G S V K R V S E N N G N G K ·
 5531 GATACTCTGA ATATTGAGAC CCTCTAAAT GGCTCTGTAA AACGTGTCTC TGAAAATAAT GGAAATGGTA
 · N S S H T H E L G T K R E N K K T I F K V N K ·
 5601 AGAATTCTC TCATACCCAT GAGTTAGGGA CAAAGCGTGA AAATAAGAAA ACTATTCTA AAGTTAATAA
 · D P Y V A D M E N G N I E G I F E R Q K G K P
 5671 AGATCCATAT GTGGCTGACA TGGAAAATGG CAATATTGAA GGTATTCCAG AAAGGCAAAA GGGCAAACCG
 N V T S K V S E N L G S H G K E I S E S E H C K ·
 5741 AATGTGACTT CAAAGGTATC AGAAAATCTT GGTCACATG GGAAAGAGAT TTCAGAGAGT GAGCATTGTA
 · C K A L M D S L D D S N T A G K E F V S Q D V ·
 5811 AGTGTAAAGC ACTTATGGAT AGTTAGATG ATTCAAATAC TGCTGGCAAG GAATTGTTTCCCAGATGT
 · R H L P K K C P N H H H F E N Q S T A S T P T
 5881 TAGACATCTT CCAAAGAAAT GTCCAATCA CCACCATTT GAAAATCAA GCACTGCTC TACTCCACT
 E K S F S E L A L E T R F N N R Q D S D A L K S ·
 5951 GAGAAGTCTT TCTCAGAACT GGCTTITAGAA ACCAGGTTTA ACAACAGACA AGACTCTGAT GCACTGAAAT
 · S D D A P S M A G K S A G C C L A L E Q N G T ·
 6021 CATCTGATGA TGCACCGAGT ATGGCTGGAA AACCTGCTGG TTGTTGCCCA GCACTGAAAC AAAACGGAAAC
 · E E N A S I S N I S C C N C E P D V F H Q K D
 6091 AGAGGAAAAT GCTCTATCA GCAACATITC CTGTTGCAAC TGTGAGGCCAG ATGTTTCCCA TCAAAAAGAT
 A E D C S V H N F V K E I I D M A S T A L K S K ·
 6161 GCGGAAGATT GTTCAGTACA CAACTITGTT AAGGAAATCA TTGACATGGC TTGACAGACCC CTAAGAAAGTA
 · S Q P E N E V A A P T S L T Q I K E K V L E H ·
 6231 AATCTCAACC TGAAAACCGAG GTGGCTGCTC CTACTCTAAT AACCTCAAATC AAGGAGAAAG TGTGGAGCA
 · S H R P I Q L R K G D F Y S Y L S L S S H D S
 6301 TTCTCACCGG CCCATCCAGC TGAGAAAAGG GGACTTTAT TCGTACTTAT CTCTCTCATC TCATGACAGT
 D C G E V T N Y I E E K S S T P L P L D T T D S ·
 6371 GATGTTGGGG AGGTCAACCA TTACATAGAA GAGAAAAGCA GCACTCCATT GCAACTAGAC ACCACTGACT
 · G L D D K E D I E C F F E A C V E G D S D G E ·
 6441 CGGGCTTACA TGACAAGGAA GATATTGAAT GCTTTTGTGA GGCCTGTGTT GAGGGTACT CTGATGGAGA
 · E P C F S S A P P N E S A V P S E A A M P L Q

FIGURE 26 (cont.)

6511 GGAGCCTTGT TTCTCTAGTG CTCCCTCCAAA TGAATCTGCA GTTCCCACCG AAGCTGCAAT GCCACTACAA
 A T A C S S E F S D S S L S A D D A D T V A L S ·
 6581 GCAACAGCAT GTTCTTCGAA GTTCAGTGAT AGTTCTCTT CAGCTGATGA TGCAAGATA CA GTGGCTCTTIT
 · S P S S Q E R A E V G K E V N G L P Q T S S G ·
 6651 CAAGTCCTTC CTCTCAGGAA AGAGCTGAGG TTGGAAAGGA AGTGAATGGT TTGCCCAAAA CTTCAGTGG
 · C A E N L E F T P S K L D S E K E S S G K P G
 6721 CTGTGCAGAA AACCTAGAGT TTACTCCTTC AAAGCTTGAC AGTGAAGG AAAAGCTCCGG AAAACCAGGT
 E S G M P E E H N A A S A K S K V Q D L S L K A ·
 6791 GAATCTGGAA TGCCAGAAGA ACATAATGCT GCTTCAGCCA AATCTAAAGT TCAAGACCTC TCCTTGAGG
 · N Q P T D K A A L H P S P K T L T C E E N L L ·
 6861 CAAATCAGCC AACAGACAAG GCGCATTGC ATCCCCAGCCC CAAAACTTA ACCTGTGAAG AAAATCTTCT
 · N L H E K R H R N M H R
 6931 AACACTTCAT GAAAAACGAC ATAGAAATAT GCATAGGTAG AATGTACCCC CTCCCCAACG ATGAAAATCA
 7001 TCTCACTGAA AGATAACGCT GGCTGCAACT CAGGGGTGGC CTCATCCTCC CGCCCTGGGC TGGCCTCTGG
 7071 TTCCATCACG TTGTCACTG CCGTTTATTA CATTGACTTC TCCCAGATG AATCTCTT CCAAATGTGT
 7141 TTCTCCACA CAAGCTTGT GATCTGAATG TGTGCGCTGG TTCTCTTGT GTGATCGTCT TTGAAGTCTCA
 7211 GCGAAGCTGC TTGTTCTCCC ATGGATCTCT GTCCTTAAAGCT ACCTCTACCA ACCCTCTCTC TCCAGCTAGA
 7281 CTTTCTCTT GCGCTCTTCC CTTCCCTTCC ACTCTTTAAA GTTCTGCAGT TCACCAACTG GTAGTCCATT
 7351 AAATCTCTCT GTCTAGAATG ACCCCCCCAG CAGTACTGAA CCAATTCAT GTATCAATCT GGATTTTT
 7421 TTAAACGGTA TAATGACTGT GCTTATTGAA AGAGTTTAC CTAAAGGCC AACATTGAA TTGGTTGCAG
 7491 CATAGAGAAG AAACACTGGT CCTTCTTTCA AAATTAAGCA ACTATTTAAA GCGCCATTTC ATTATTTCA
 7561 TTAAAAAAAT AATCTATGCA GCATTCTCAAG AAACAACCAT ATGGCTTGT ATATTTAAA CTGGTGACAT
 7631 TCTACTATTG AATTATGTC AACATTTCA TTTTTATGC TTCTTGAGGT GGTAATGAGA AAAAGTTT
 7701 TTAAAAAAAGT GTGCCCTGCT GTATTCTTA TACCAATTAT TAAAAAGCTG CTTTACGGT AAAATTATGT
 7771 TGGTTGAAA GGAGGAATA GCAAGTTAA GATGTGTGAA TAAATCTGT ATATATGTAT AACCAAGTAC
 7841 AACATCTGAT GTATAATGAC AGTATAAAAT GCTTCTCATGT TTGTGATGTC TAGTGTGTG GAAAATATAA
 7911 GCCTTAAATC CATTAGATG CATGGTAATT AAAATTGGCA TAATAAACAC AGATTATTGG GCGAAAAGGA
 7981 AAATIAGIGA ICTCTTCIAC TATGTTCTTT ACCAAATTTGT TGCATCTGGI TCTGAAAAG TATAGCATGT
 8051 AGCAGCTTCC AAACATATTC ATATTGCTTA AGAGGCTTAA CATTACCTAA ACTAGAGACT AGACGTAAG
 8121 CCTTCAGTT TCAAAATCTT TCTGGTCACT ATAAGAGACT TGGAACACCA ATGATTAAA TGTCACTTCC
 8191 CCTAAACCAA TAAACATTTA TACTAGATT TTTATTCCA CITATCAITA ATGATTAAAT GTGGGATTT
 8261 AGGTACCTTG TATGCTTAA TTTATTAA ATATTTATTT TGAATGAGTT TGATAGAAAG CTAGTAGAAAA
 8331 AGTACAGAAA ATTTGACTAT TATTATAGA TTTCAGGTAT ATTTATATGT GTAAAAAGAAA TTGACAAAGA
 8401 AATATTCTAT CTGGCCTTCA CTGACTCTG TAAATGAG TTTTAAATT ATATCTAAC ACTTACTTAA
 8471 GTCCCTGACA CAGTAGGAT TCAATAAAA TTTACTGAAT TAAAGGATTA AATTAGGTGA CATGGTGACA
 8541 TCTATCCCTT TATTTGACA CTAAAACATG GACACAACCA GAAAGGAGTA CAATGCAATA TAAAGTCACA
 8611 ATAGATAATA TATATCAAT TTCTAAAAGG TAAAGAATGT TGIGGGTTCA TGCAGTCACA GGAATGACAA
 8681 TCATTCAACA GATAGTTCAAG AAACACTTT TATCTGCAAG GCACTATTCT AGATCCAGAA GATGCAATGT
 8751 TGAACAAACA GACAAAGCCC TGCCCTCAGA AGGCTGCTC GCATTAGGAA CAAGTGAACA CGCAAATGAC
 8821 ATGAAGTATT TGTGCAAGAG CTGAGGAACA GAGCAAATGT AGTGTAGAA GCGCAATGAG AGAAGCAGCA
 8891 GTGGGTACAA GGAGGAAGAA AAAGGGCTTG CAGAGAGTGG AAAGTTAGTG GAATATTCTAT GAAACTCTAT
 8961 TGCAGGGGTA ATAGAAGAAA AAGTAAATTG GGAGGACTTA ATGAAAGGTC TTTTAAAGG TTAACTTGG
 9031 GCTCTGTAT GTAAAATGCT AGGTAAATAAG GACACTTGT ACAGGCTGTT TTGCACCTGA TTTTATTAT
 9101 CATTAGTGC ACGCCAAGAT CATTAGACG ATGCTTATCT GTAACTCTAC CACTTAAATA ACTATTTGTA
 9171 TTTTATGCC CCTCTGATC TTTCTCATAT GTATTCTAA ATGATTAAT TATTCTAGGC TTCTTAATAG
 9241 GTAGTAATT GTTCAAAAGC GGTTTAGCC AGACATCTAG TTGCACTGT CAAGAGGATT ATGGGGGAAA
 9311 GAGATTAGAG ATATTGCT AGTTAGGGGG CAGCTGGAGA AAAATAGCTA AGTTGCAAT AACAGACTAC
 9381 ACAAGTATAG TGGCCCAGGA TGTAGTGAAA GAACAAATCC TAGAGTCIT GAAATTCTA AGGGCAITCT
 9451 AGACCTCTGT TGGGATATGG TATTATTCTA CATACTGACA CAACCTAAAT TTCTTGGG TAGTAACTAA
 9521 TGTCAAGTCT ACATCGACTG GTAAAACATT CAAAGAACAA ACTGACAATG ATGTTCTACC TACTTGTAC
 9591 ATGCTCATGG AAGACCGTGC AGTATTGAAA GTATTGTTA ATTAATCTGCT TAGTATTAAAC ACTAAATTG
 9661 TAGAATGACT TTCAGGTITG TTGAAACAATG CCTTTTCAGG TTGGAAGAAG AAAATAGCC TCAATCTCCC
 9731 ACCCCATGTA GGCACACTACCT CCCCAATTAC CCTTAGAAAA TGATCACACC AACTCTGCCT ACACACTTCC
 9801 AGTGATAGTG GCTCATTGTC TGTAAAGGCA AACTGTTCCA CTGTTGGCA TATCTCTTG TTAGAAAGTT
 9871 CTTCTTCTAGG TTGCTAAAAT CTGCCTAGTA CCCCGCTACC CTGTTCTGTC TTATGGAGCA GCCCAGAITA
 9941 TCTTACTCC CTCTTCTCA TGGCAACCTC GAAGATAATC AAGGCCAGTT ACTCATCATC TCCCAACCAC
 10011 TGTTCCTCA ACIGCCCTTC ATATGTCATG GTTTTCAGAT CCATTCACAC CTGACTGAAT GTIAACAGAC
 10081 AGAATTCTC ACATTAAGGA ACTGCTTCA TCACTCATACA TGTAGAAAAG AATCTGAACA TTAAAGTGC
 10151 AAGTTTCTC TAGAAATATA TTCAAGATAT GTTTATTCTA TTATTGTAAA TTCAACACAA TAAATAAAATA
 10221 AGAATCC

FIGURE 27**Figure 27. human mAKAP α mRNA with open reading frame translated:**

```

1 CATCATGCAG CAGGTCAAAC AAGGCATCTC CTAGTATTGC ATCCTACAGA TGTGCTGTAA ACATCAAAAG
   M L T M S .
71 AAGACGGTGG GATCAGGAGA TGCTGTTTG GAAAGAAGTG AGGTTAGAC TCTCCATGT TAACCATGAG
   . V T L S P L R S Q D L D P M A T D A S P M A I
141 CGTGACACTT TCCCCCTGA GGTCACAGGA CCTGGATCCC ATGGCTACTG ATGCTTCACC CATGGCCATC
   N M T P T V E Q G E G E A M K D M D S D Q Q Y .
211 AACATGACAC CCACTGTGGAG GCACGGTGGAG CGAGAAGAGG CAATGAAGGA CATGGACTCT GACCAGGAGT
   . E K P P F L H T G A D W K I V L H L P E I E T .
281 ATGAAAAGCC ACCCCCCACTACACACAGGGG CTGACTGGAA GATTGTCTC CACTTACCTG AAATTGAGAC
   . W L R M T S E R V R D L T Y S V Q Q D S D S K
351 CTGGCTCCGG ATGACCTCAG AGAGGGTCCG AGACCTAACCC TATTCAGTCC AGCAGGATTC GGACAGCAAG
   H V D V H L V Q L K D I C E D I S D H V E Q I R .
421 CATGTGGATG TACATCTAGT TCAACTAAAG GACATTGTG AAGATATTTC TGATCATGTT GAGCAAATCC
   . A L L E T E F S L K L L S Y S V N V I V D I H .
491 ATGCCCTCTG TGAAACAGAG TTCTCCCTAA AGCTGCTGTC TTACTCTGTC AACGTGATAG TGGACATCCA
   . A V Q L L W H Q L R V S V L V L R E R I L Q G
561 CGCAGTGCAAG CTCCTCTGGC ACCAGCTTCG AGTCTCAGTG CTGGTTCTGC GGGAGCGCAT TCTGCAAGGT
   L Q D A N G N Y T R Q T D I L Q A F S E E T K E .
631 CTGCAGGACG CCAATGCCAA CTACACTAGG CAGACGGACA TTCTGCAAGC TTCTCTGAA GAGACAAAAAG
   . G R L D S L T E V D D S G Q L T I K C S Q N Y .
701 AGGGCCGGCT TGAATTCTCTA ACAGAAGTGG ATGACTCAGG ACAAITAACCC ATCAAATGTT CTCAAAATTIA
   . L S L D C G I T A F E L S D Y S P S E D L L S
771 CTGTGCTCTG GATITGIGGCA TTACTGCATT CGAACCTGTC GACTACAGTC CAAGTGAGGA TTGCTCAGT
   G L G D M T S S Q V K T K P F D S W S Y S E M E .
841 GGGCTAGGTG ACATGACCTC TAGCCAAGTC AAAACCAAAC CCTTTGACTC TTGGAGCTAC AGTGAGATGG
   . K E F F E L I R S V G L L T V A A D S I S T N .
911 AAAAGGAGTT TCCTGAGCTT ATCCGAAGTG TTGGTTTACT TACGGTAGCT GCTGACTCTA TCTCTACCAA
   . G S E A V T E E V S Q V L S V D D K G G C E
981 TGGCAGTGAAG CAGCTACTG AGGAGGTATC TCAAGTATCT CTCTCAGTAG ACGACAAAGG TGGAIITGAG
   E D N A S A V E E Q F G L T L G V S S S S G E A .
1051 GAAGACAATG CTTCTCCAGT CGAACAGCAA CCAGGCTTAA CACTGGGGT GTCATCATCT TCAGGAGAAG
   . L T N A A Q P S S E T V Q Q E S S S S S H H D .
1121 CTCTGACAAA TGCTGCTCAA CCCCTCTG AGACTGTGCA GCAAGAATCC AGTTCTCCT CCCATCATGA
   . A K N Q Q P V P C E N A T P K R T I R D C F N
1191 TGCAGAAAT CAGCAGCTG TTCTTGTGA AAATGCAACC CCCAAACGAA CCATCAGAGA TTGCTTTAAT
   Y N E D S P T Q P T L P K R G L F L K E E T F K .
1261 TATAACGAGG ACTCTCCAC GCAGCCTACA TTGCCAAAAA GAGGACTTT TCTTAAAGAG GAAACITTTA
   . N D L K G N G G K R Q M V D L K P E M S R S T .
1331 AGAATGATCT GAAAGGCAAT GGTGAAAGA GGCAAAATGGT TGATCTAAG CCTGAGATGA GCAGAAGCAC
   . P S L V D P P D R S K L C L V L Q S S Y P N S
1401 CCCTTCGCTA GTAGATCTC CTGACAGATC CAAACTTGC CTGGTATTGC AGTCTCTTA CCCAACAGC
   P S A A S Q S Y E C L H K V G N G N L E N T V K .
1471 CCTCTGCTG CCAGCCAGTC TTATGAGTGT TTACACAAGG TGGGGATGG GAACCTGAA AACACAGTCA
   . F H I K E I S S S L G R L N D C Y K E K S R L .
1541 AATTTCACAT TAAAGAAATT TCTTCCAGCC TGGGAAGGCT TAACGACTGC TATAAAGAGA AATCTCGACT
   . K K P H K T S E E V P P C R T P K R G T G S G
1611 TAAAAAGCCA CACAAGACCT CAGAAGAGGT GCCTCCATGC CGAACACCTA AACGGGGGAC TGGTICAGGC
   K Q A K N T K S S A V P N G E L S Y T S K A I E .
1681 AAACAAGCTA AAAATACAAA GAGCTCAGCA GTGCCAAATG GAGAGCTTC TTATACTTCC AAGGCCATAG
   . G P Q T N S A S T S S L E P C N Q R S W N A K .
1751 AGGGGCCACA AACAAATTCT GCTTCCACAT CCTCACTTGA GCCTGTAAAT CAGAGAAGTT GGAATGCCAA
   . L Q L Q S E T S S S P A F T Q S S E S S V G S
1821 ATTGCAATTG CAGTCAGAAA CATCCAGTC ACCAGCTTT ACTCAGAGCA GTGAATCTC TGTGGCTCA
   D N I M S F V P L L S K H K S K K G Q A S S P S .
1891 GACAACATCA TGTCTCGGT GCCACTCTT TCAAAACACA AAAGCAAAA AGGTCAAGCC TCCCTCCCAA
   . H V T R N G E V V E A W Y G S D E Y L A L P S .
1961 GTCACGTAC TAGGAATGGT GAGGTGTGG AGGCCTGGTA TGGCTCTGAT GAATAACCTAG CACTGCCCTC
   . H L K Q T E V L A L K L E N L T K L L P Q K F

```

FIGURE 27 (cont.)

2031 TCACCTTAAG CAGACAGAAAG TATTGGCTTT GAAGTGGAA AACCTAACAA AGCTCTGCC TCAGAAACCC
 R G E T I Q N I D D W E L S E M N S D S E I Y P .
 2101 AGAGGAGAAA CCATCCAGAA TATTGATGAC TGGGAAGTGT CTGAAATGAA TTCAGATTCT GAAATCTATC
 . T Y H V K K K H T R L G R V S P S S S S D I A .
 2171 CAACCIATCA TGICAAAAAG AAGCATACAA GGCTAGGCAG GGTGCTCCA AGCTCATCTA GTGACATAGC
 . S S L G E S I E S G P L S D I L S D E E S S M
 2241 CTCTCACTA GGGGAGAGCA TTGAATCTGG GCCCTGAGT GACATTCTT CTGATGAGGA GTCCAGTATG
 P L A G M K K Y A D E K S E R A S S S E K N E S .
 2311 CCTCTCGCTG CCATGAAAAA GTAIGCTGAT GAGAAGTCAG AAAGAGCTC ATCCTCTGAG AAAATGAGA
 . H S A T K S A L I Q K L M Q D I Q H Q D N Y E .
 2381 GCCATTCTGC CACTAAATCA GCTTTAATTG AGAAACTGAT GCAAGATATT CAGCACCAAG ACAACTATGA
 . A I W E K I E G F V N K L D E F I Q W L N E A
 2451 ACCATATGG GAAAATAG AGGGGTTGAT AAACAAACTG GATGAATCA TTCAATGGTT AAAATGAAGCC
 M E T T E N W T P P K A E M B D L K L Y L E T H .
 2521 ATGAAACTA CAGAGAATTG GACTCCCCCT AAAGCAGAGA TGGATGACCT TAAACTGTAT CTGGAGACAC
 . L S F K L N V D S H C A L K E A V E E G H Q .
 2591 ACTTGAGTT TAAGTGAAT GTAGACAGTC ATITGCTCT CAAGGAAGCT GTGGAGGAGG AAGGACACCA
 . L L E L I A S R K A G L K D M L R M I A S Q W
 2661 ACTTCTTGAG CTTATTGCAT CTCACAAAAGC AGGACTGAAG GACATGCTGC GGATGATTGC AAGTCAATGG
 K E L Q R Q I K R Q H S W I L R A L D T I K A E .
 2731 AAGGAGCTGC AGAGGCAAAT CAAACGGCAG CACAGCTGGA TTCTCAGGGC TCTGGATACC ATCAAAGCCG
 . I L A T D V S V E D E E G T G S P K A E V Q L .
 2801 AGATACTGGC TACTGATGIG TCTGTGGAGG ATGAGGAAGG GACTGGAAGC CCCAAGGCTG AGGTCAACT
 . C Y L E A Q R D A V E Q M S L K L Y S E Q Y T
 2871 ATGCTACCTG GAAGCACAAA GAGATGCTGT TGAGCAGATG TCCCTCAAGC TGTACAGCGA GCAGTATAACC
 S S S K R K E E F A D M S K V H S V G S N G L L .
 2941 AGCAGCAGCA AGCGAAAGGA AGAGTTGCT GATATGTCAA AAGTICATIC AGTGGGAAGC AATGGGCTTC
 . D F D S E Y Q E L W D C L I D M E S L V M D S .
 3011 TGGACTTGA TTCAAAAT CAGGAGCTCT GGGATTGCTT GATTGACATG GAGTCCCTG TGATGGACAG
 . H D L M M S E E Q Q O H L Y K R Y S V E M S I
 3081 CCACGACCTG ATGATGTCAG AGGAGCAGCA GCAGCATCTT TACAAGCGAT ACAGTGTGGA AATGTCCATC
 R H L K K T E L L S K V E A L K K G G V L L P N .
 3151 AGACACCTGA AAAAGACGGA GCTGCTTAGT AAGGTTGAAG CTTIGAAGAA AGGTGGCGTT TTACTACCAA
 . D L L E K V D S I N E K W E L L G K T L G E K .
 3221 ATGATCTCT TGAAAAAGTG GATTCAATT AIGAAAATG CGAACATGCTT GGGAAACCC TAGGAGAGAA
 . I Q D T M A G H S G S S P R D L L S P E S G S
 3291 GATCCAGGAC ACAATGGCAG GGCACAGTGG GTCGAGTCCA CGTGACCTGC TCTCTCTGA AAGTGGAAAGC
 L V R Q L E V R I K E L K G W L R D T E L F I F .
 3361 CTGGTAAGGC AGCTGGAGGT CAGGATCAAA GAACTGAAAG GATGGCTAAG AGATACAGAG CTTTCTATCT
 . N S C L R Q E K E G T M N T E K Q L Q Y F K S .
 3431 TCAATTCTG TCTGAGACAA GAAAAGGAAG GAACAATGAA TACTGAGAAA CAACTGCAAT ACTTTAAGTC
 . L C R E I K Q R R R G V A S I L R L C Q H L L
 3501 CCTCTGTCGT GAAATCAAGC AACGACGTG AGGAGTTGCC TCCATTCTGC GACTATGCCA GCATCTTTG
 D D R E T C N L N A D H Q P M Q L I I V N L E R .
 3571 GATGACCGGG AGACTTGCAA TCTGAATGCA GACCACCGC CCAATGCACT GATCATGTA AATCTTGAAA
 . R W E A I V M Q A V Q W Q T R L Q K M G K E .
 3641 GAAGGTGGGA AGCCATTGTC ATGCAAGCCG TCCAGTGGCA AACACGTCA CAAAAGAAGA TGGGAAAGGA
 . S E T L N V I D P G L M D L N G M S E D A L E
 3711 ATCTGAGACT TTGAATGTA TTGATCTGG CTTGATGGAC CTAAATGGGA TGAGTGGAGA TGCCCTGGAA
 W D E M D I S N K L I S L N E E S N D L D Q E L .
 3781 TGGATGAAA TGGACATAAG TAACAAGTIA ATTAGTTGA ATGAGGAATC AAATGACCTT GATCAAGAAC
 . Q P V I P S L K L G E T S N E D P G Y D E E A .
 3851 TCCAAACCTGT TATCCCTTCC TTGAAGCTG GAGAGACAAG TAATGAGGAC CCTGGTTATG ACGAGGAGGC
 . D N H G G S Q Y A S N I T A P S S P H I Y Q V
 3921 TGATAACCAT GGGGGATCTC AGTATGCCCT AAATATTACT GCCCCCTCTA GTCCACACAT TTACCAAGGTG
 Y S L H N V E L Y E D N H M P F L K N N P K V T .
 3991 TACAGCCCTCC ACAAITGTTGA ACTCTATGAG GACAACCACA TGCCATTCT GAAAACAAT CCAAAGGTCA
 . G M T Q P N V L T K S L S K D S S F S S T K S .
 4061 CTGGCATGAC ACAGCCTAAAT GTTTAACTA AGAGTCTCAG TAAAGACTCT GATTTTCAT CTACCAAATC
 . L P D L L G G S N I V K P C A C H G G D M S Q

FIGURE 27 (cont.)

4131 TTTGCCAGAT CTTCTAGGTG GTTCCAATTG CGTAAAGCCC TCGCGATGTC ATGGAGGAGA CATGAGCCAG
 N S G S E S G I V S E G D T E T T T N S E M C L
 4201 AATTCAGGCCA GTGAGAGTGG AATTGTCAGT GAAGGAGACA CAGAAACCAC TACCAACTCT GAAATGTGCT
 . L N A V D G S P S N L E T E R L D P Q M G D A
 4271 TGCTCAATGC AGTGGATGGG TCCCCAAGTA ACCTTGAAAC TGAACATCTG GACCCACAAA TGGGAGATGC
 . V N V L K Q K F T D E G E S I K L P N S S Q S
 4341 AGTTAACGTG TTAAAGCAAA AATTACAGA TGAGGGGGAA AGCATTAAAGC TTCCAAATAG CTCTCAGTCG
 S I S P V G C V N G K V G D L N S I T K H T P D
 4411 TCCATTTCAC CAGTGGGTG TGTAAATGGA AAAGTTGGAG ATTAAACAG TATTACCAA CATAACCCCTG
 . C L G E E L Q G K H D V F T F Y D Y S Y L Q G
 4481 ACTGTTTGGG AGAAGAATTA CAAGGAAAC ATGATGTGTT TACATTTAT GATTACTCAT ACCTCCAAGG
 . S K L K L P M I M K Q S Q S E K V H V E D P L
 4551 CTCAAARACTC AAATTACCAA TGATAATGAA ACAGTCACAA AGCGAAAAAG TGCATGTGGA GGATCCCCCTG
 L R G F Y F D K K S C K S K H Q T T E L Q P D V
 4621 CTTCTGGTT TTTATTTGA TAAAAAAATCA TGCAAATCTA AACATCAGAC TACAGAGTTA CAACAGATG
 . P P H E R I L A S A S H E M D R I S Y K S G N
 4691 TACCTCCCCA TGAAAGGATT TTGGCAAGTG CATCTCATGA AATGGATCGC ATTCATATA AAAGTGGCAA
 . I E K T F T G M Q N A K Q L S L L S H S S S I
 4761 TATAGAAAAG ACATTCACIG GCATGCCAGA TGCCAAACAG CTCTCCCTT TATCTCATAG ITCATCTATT
 E S L S P G G D L F G L G I F K N G S D S L Q R
 4831 GAGTCCCTT CTCCAGGGGG TGATTIATTI GGATTGGCA TCTTIAAAA TGGCAGTGAC AGCCTCCAGC
 . S T S L E S W L T S Y K S N E D L F S C H S S
 4901 GAAGCACTTC TTTAGAAAAGT TGGTIGACTT CCTATAAAAG CAATGAAGAT CTCTTAGCT GTCACAGCTC
 . G D I S V S S G S V G E L S K R T L D L L N R
 4971 TGGGATATA AGCGTGGCA GTGGCICAGT TGGTGAACTA AGTAAAAGAA CATTAGATCT CCTGAATCGT
 L E N I Q S P S E Q K I K R S V S D I T L Q S S
 5041 TTGGGAGATA TCCAGAGCCC CTCAGAGCAA AAGATAAAAC GAAGTGTTC TGATACTACT CTICAAAGCA
 . S Q K M S F T G Q M S L D I A S S I N E D S A
 5111 GTTCCAAAAA GATGTCCTT ACTGGCCAGA TGTCAATTGGA CATAGCATCT TCTATCAATG AAGACTCAGC
 . A S L T E L S S S D E L S L C S E D I V L H K
 5181 GGCATCTCTA ACAGAACTTA GCAGCACTGA CGAGCTCTCT CTTTGTCTAG AGGATATTGT GTIACACAAG
 N K I P E S N A S F R K R L T R S V A D E S D V
 5251 ACAAGATCC CGGAATCGAA TGCACTGTC AGGAAGCGTC TGACTCGTC AGTGGCTGAT GAAAGCGATG
 . N V S M I V N V S C T S A C T D D E D D S D L
 5321 TCAAATGTCAG CATGATTGTT AATGTCCTT GCACCTCTGC TTGCACTGAT GATGAAGATG ACAGCGACCT
 . L S S S T L T L T E E E L C I K D E D D D S S
 5391 GCTCTCCAGC TCTACCCCTA CCTTGACTGA AGAAGAGCTG TGCACTCAAAG ATGAGGATGA CGACTCCAGT
 I A T D D E I Y E D C T L M S G L D Y I K N E L
 5461 ATTGCAACAG ATGATGAAAT TTATGAAGAC TGCACCTTGTA TGTCAAGGGCT AGACTACATA AAGAATGAAT
 . Q T W I R P K L S L T R D K K R C N V S D E M
 5531 TACAGACCTG GATTAGGCCA AAATTGTCCT TGACAAGAGA TAAGAAAAGG TGCAATGTCA GTGATGAGAT
 . K G S K D I S S S E M T N P S D T L N I E T L
 5601 GAAGGGCAGT AAAGATATAA GTAGCACTGA GATGACCAAT CCCCTCTGATA CTCTGAATAT TGAGACCCCTT
 L N G S V K R V S E N N G N G K N S S H T H E L
 5671 CTAATGGCT CTGTAAAACG TGTCTCTGAA AATAATGGAA ATGGTAAGAA TTCATCTCAT ACCCATGAGT
 . G T K R E N K K T I F K V N K D P Y V A D M E
 5741 TAGGGACAAA GCGTGAACAA AAGAAAAC TTTCAAAGT TAATAAAGAT CCATATGIGG CTGACATGGAA
 . N G N I E G I P E R Q K G K P N V T S K V S E
 5811 AAATGGCAAT ATTGAAGGT TTCCAGAAAG GCAGAAAGGGC AAACCGAAATG TGACTCTAAA GGTATCAGAA
 N L G S H G K E I S E S E H C K C K A L M D S L
 5881 AATCTGGTT CACATGGGA AGAGATTCA GAGAGTGGAC ATTGTAAGTG TAAAGCAGT ATGGATAGIT
 . D D S N T A G K E F V S Q D V R H L P K K C P
 5951 TAGATGATTC AAATACTGCT GGCAAGGAAT TTGTCTCCCA AGATGTTAGA CATCTCTCAA AGAAATGTCC
 . N H H H F E N Q S T A S T P T E K S F S E L A
 6021 AAATCACCAC CATTGAAATC ATCAAAGCAC TGCTCTACT CCCACTGAGA AGTCTCTC AGAACTGGCT
 L E T R F N N R Q D S D A L K S S D D A P S M A
 6091 TTAGAAACCA GGTAAACAA CAGACAAGAC TCTGATGAC TGAAATCATC TGATGATGCA CCGAGTATGG
 . G K S A G C C L A L E Q N G T E E N A S I S N
 6161 CTGGAAAATC TGCTGGTTGT TGCCTAGCAC TTGAACAAAAA CGGAACAGAG GAAAATGCTT CTATCAGCAA
 . I S C C N C E P D V F H Q K D A E D C S V H N

FIGURE 27 (cont.)

6231 CATTCCCTGT TCGCAACTGTG AGCCAGATGT TTTCCATCAA AAAGATGCCG AAGATTGTTC AGTACACAAC
 F V K E I I D M A S T A L K S K S Q P E N E V A .
 6301 TTGTTAAGG AAATCATTGA CATGGCTTCG ACAGCCCTAA AAAGTAAATC TCAACCTGAA AACGAGGTGG
 · A P T S L T Q I K E K V L E H S H R P I Q L R .
 6371 CTGCTCTAC TTCATTAACT CAAATCAAGG AGAAAAGTGT GGAGCATTCT CACCAGGCCA TCCAGCTGAG
 · K G D F Y S Y L S L S S H D S D C G E V T N Y
 6441 AAAAGGGAC TTTTATTCGT ACTTATCTCT CTCATCTCAT GACAGTGTGG GTGGGGAGGT CACCAATTAC
 I E E K S S T P L P L D T T D S G L D D K E D I .
 6511 ATAGAACAGA AAAGCAGCAC TCCATTGCCA CTAGACACCA CTGACTCGGG CITAGATGAC AAGGAAGATA
 · E C F F E A C V E G D S D G E E P C F S S A P .
 6581 TTGAATGCTT TTTTGAGGCC TGTGTGTGAGG GTGACTCTGA TGGAGAGGAG CCTTGTCTCT CTAGTGCTCC
 · P N E S A V P S E A A M P L Q A T A C S S E F
 6651 TCCAATGAA TCTGCAGTTC CCAGCGAACG TCGCAATGCCA CTACAAGCAA CAGCATGTTC TTCTGAGTT
 S D S S L S A D D A D T V A L S S P S S Q E R A .
 6721 AGTATAGTGT CTCCTTCAGC TGATGATGCA GATACAGTGG CTCTTCAAG TCCTTCTCT CAGGAAAGAG
 · E V G K E V N G L P Q T S S G C A E N L E F T .
 6791 CTGAGGTTGG AAAGGAAGTG AATGGTTGC CCCAAACTTC CAGTGGCTGT GCAGAAAAACT TAGAGTTIAC
 · P S K L D S E K E S S G K P G E S S G M P E E H
 6861 TCCTTCAAGG CTTGACAGTG AAAAGGAAAG TTCCGGAAAA CCAGGTGAAT CTGGAATGCC AGAAGAACAT
 N A A S A K S K V Q D L S L K A N Q P T D K A A .
 6931 AATGCTGCTT CAGCCAAATC TAAAGTCAA GACCTCTCT TGAAGGCAAA TCAGCCAACA GACAAGGCCG
 · L H P S P K T L T C E E N L L N L R E K R H R .
 7001 CATGCACTCC CAGCCCCAAA ACTTTAACCT GTGAAGAAAAA TCTCTAAAC CTTCTAGAAA AACGACATAG
 · N M H R
 7071 AAATAATGGCAT AGGTAGAATG TACCCCCCTCC CCAAGCATGA AAATCATCTC ACTGAAAGAT ACGCCTGGCT
 7141 GCAACICAGG GGTGGCCTCA TCCTCCGCC CTGGGCTGGC CTCTGGTTCC ATCACGTTIG TCACTGCCGT
 7211 TTATTAATT GACTTCTCCC AAGATGAATC TTCTTCCAA ATGIGTTITC TCCACACAAG CCTTGTGATC
 7281 TGAATGTGTG CGCTGGTTCT CTTTAGGTGA TCGTCTTGTGA AGTTCAGCAA AGTGTGTGT TCTCCCATGG
 7351 ATTCCCTGTCC CAAGCTACCT CTACCAACCC TCTCTCTCCA GCTAGACTTT TCTCTTGCCTCCTCC
 7421 CCTTCCACTC TTAAAGTTG TGCACTTCAC CAACTGGTAG TCCATTAAT TCTCTGTCT AGAATGACCC
 7491 CCCCACCGT ACTTGACCAA TTICATGTAT CAATCTGGAT TTTCCTTTA ACGGTATAAT GACTGIGCTT
 7561 ATTGAAAGAG TTTCACCTAA AAAGCCAACA TTGAATTGG TTGAGCAGATA GAGAAGAAC ACTGGTCCTT
 7631 CTTCAAAAT TAAGCAACTA TTAAAAGCGC CATTTTATT ATTCAATT AAAATAATC TATGCAGGAT
 7701 TTCAAGAAC ACCATATGG TTGTGTATAT TATAACTGG TGACATCTA CTATGAATT ATGACAAACA
 7771 TTTCATTT TTATGCTCT TGAGGTGTA ATGAGAAAAA AGTTTTIAA AAAAGTGTGC CTIGCTGTAT
 7841 TTCTTATACC ATTATTTAA AAGCTGCTT CACGGTAAAA TTATGTTGGT TTGAAAGGAG GAAATAGCAA
 7911 GGTTAAGATG TGTGAATAAT TTCTGTATAT ATGTATAACC AAGTACAAAC ATTGTGTAT AATGACAGTA
 7981 TAAAATGCTT TCATGTTGT GATGTCTAGT GATGTGGAAA ATATAAGCCT TAAATCCATT AGATTGCATG
 8051 GTAATTAAA TTGGCATAAT AAACACAGAT TATTGGGAA AAAGGAAAAT TAGTGTCTC TTCTACTATG
 8121 TTCTTACCA AATTGTTGCA TCTGGTCTG AAAAAGTATA GCATGTAGCA GCTTCCAAAC ATATTCAAT
 8191 TGCTTAAGAG GCTTAACATT ACCTAAACTA GAGACTAGAC GTAAAGCCTT CAGTTCTAA AATCTTCTG
 8261 GTCACTATAA AGATCTGGC ACAGCAAATG ATTAATGTC AGTTCCCTA AACCAATAAA CATTATAACT
 8331 AGATTTTTA TTCCACTTA ICATTAATGA TTAAATGTTG GATTCAGGT ACCTTGTATG TCTTAATTIA
 8401 TTAAATAT TTATTTGAA TGAGTTGTAG AGAAAGCTAG TAGAAAAGTA CAGAAAATT GACIATTATT
 8471 TATAGATTT AGGTATATT ATATGTTGAA AAGAAATTGA CAAAGAAAATA TTTCATCTGG CCTTTACTGA
 8541 CTCCCTGTAA ATGCAGTTTT AAATTATAT CGTAAACACCT ACTTAAAGTGC CTGACACAGT AGGTATTCAA
 8611 TAAAATTTA CTGAATTAAA GGATTAATT AGGTGACATG GTGACATCTA TCCCTTATT TTGACACTAA
 8681 AACATGGACA CAACTAGAAA GAGGTACAAT GCAATATAAA GTCACAATAG ATAATATATA TCAAATTTCT
 8751 AAAAGTAAA GAATGTTGTG GTTGTATGCA GTCACAGGAA TGACAATCAT TCAACAGATA GTTCAGAAAC
 8821 ACTTTTATC TGCAAGGCAC TATTCTAGAT CCAGAAGATG CAAATGTTGAA CAAACAGACA AAGCCCTGCC
 8891 CTCAGAGGC TGTCTGCAT TAGGACAAAG TGACACCGCA AATGACATGA AGTATTGTT GCAGAGCTGA
 8961 GGAACAGAGC AAATGTAGTG ATAGAACGGC AATGAGAGAA GCAGCAGTGG GTACAAGGAG GAAAGAAAAG
 9031 CGCTTGCAGA GAGTGGAAAG TTAGTGGAT ATTCACTGAA CTTCATTGCA GGGGTAAATAG AAGAAAAAGT
 9101 AAATGGGAG GACTTAATGG AAGGTCTTT AAAAGTAA CTGGAGCTT CTGTATGTAA AATGCTAGGT
 9171 AATAAGGACA CTTTGTACAG GCTGTGTTGC ACCTGATTTT ATTATCATT AGTGCACGC CAAGATCATT
 9241 TAGACGATGC TTATCTGTAA TTCTACCACT TTAATAACTA TTGTGTTTT TATGCCCTT CTGATCTTT
 9311 CCATATGTAT TTCTAAATGG ATAAATTATT CTAGGCTCT TAATAGGTAG TAATTGTT AAAAGCGGTT
 9381 TTAGCCAGAC ATCTAGITGCA AGTGTCAAG AGGATTATGG GGGAAAGAGA TTAGAGATAA TTGTCTAGTT
 9451 AGGGGGCAGC TGGAGAAAAT AAGCTAAGTT TGCAATAACA GAGTACACAA GTATAGTGGC CCAGGATGTA
 9521 GTGAAAGAAC AAATCCTAGA GTCTTGTAAA TTCTAAAGGG CATTCTAGAC CTCTGTTGGG ATATGGTATT

FIGURE 27 (cont.)

9591 ATTTTACATA CTGACACAAC CTAATTTTC TTTGGGTAGT AACTAATGTC AAGTCTACAT CGACTGGTAA
9661 AACAITCAAA GAACAAACTG ACAATGATGT TCTACCTACT TGTTACATGC TCATGGAAAGA CCGIGCAGIA
9731 TTGAAAGTAT TTGTTAATTAA TCTGCTTAGT ATTAACACTA AATITGTAGA ATGACITTCAGA GGTTTGTGAA
9801 ACAATGCCCTT TTCAAGGTGAG AAGAAGAAAA ATAGCCTCAA TCTCCCACCC CATGTAGGCA CTACCTCCCC
9871 AATTACCCCTT AGAAAATGAT CACACCAACT CTGCCTACAC ACTTCCAGTG ATAGTGGCTC ATTGTCTGTT
9941 AAGGCAAACCTT GTTCCACTGT TGGGCATATTC TCTTTGTAG AAAGTCTTT CTTAGGTTGC TAAAATCTGC
10011 CTAGTACCCCC GCTACCCCTGT TCTGCTTTAG GGAGCAGCCC AGATTATCTT TACTCCCTCT TTCTCATGGC
10081 AACCCCTGAAG ATAATCAAGG CCAGTTACTC ATCATCTCCC AACCACTGTT TCCTCAACTG CCCTTCATAT
10151 GTCAATGGTTT TCAGATCCAT TCCAACCTGA CTGAATGTTA ACAGACAGAA TTCTTCACAT TAAGGAACATG
10221 TCTTCATCAT CATACTGTA GAAAAGAATC TGAACATTAA AGTGCAGAGT TTTCTCTAGA AATATATTCA
10291 AGATATGTTT ATTCTATTAT TGTAAATTTC AAACAATAAA TAAATAAGAA TCC

FIGURE 28**Figure 28. human mAKAP sequence (PBD in bold)**

1 MLTMSVTLSP LRSQDLDPM TDASPMAINM TPTVEQGEGE EAMKMDSDQ
 51 QEYKPPPLHT GADWKIVLHL PEIETWLRMT SERVRDLTYS VQQDSDSKHV
 101 DVHLVQLKDI CEDISDHVEQ IHALLETEFS LKLLSYSVNV IVDIHAVQLL
 151 WHQLRVSVLV LRERILQGLQ DANGNYTRQT DILQAFSEET KEGRLDSLITE
 201 VDDSGQLTIK CSQNYLSLDC GITAFELSDY SPSEDLLSGL GDMTSSQVKT
 251 KPFDSWSYSE MEKEFPELIR SVGLLTVAAD SISTNGSEAV TEEVSQVSLS
 301 VDDKGGCEED NASAVEEOPG LTGKVSSSSG EALTNAAQPS SETVQQESSS
 351 SSHDAKNQQ PVPCENATPK RTIRDCFNYN EDSPTQPTLP KRGLFLKEET
 401 FKNDLKGNGG KRQMVDLKPE MSRSTPSLVD PPDRSKLCLV LQSSYPNSPS
 451 AASQSYECLH KVGNGNLENT VKFHIKEISS SLGRLNDCYK EKSRLKKPHK
 501 TSEEVPPCRT PKRGITGSGKQ AKNTKSSAVP NGELSYTSKA IEGPQTNSAS
 551 TSSLEPCNQR SWNAKLQLQS ETSSSPAFTQ SSESSVGSDN IMSPVPLLSK
 601 HKSKKGQASS PSHVTRNGEV VEAWYGSDEY LALPSHLKQT EVLALKLENL
 651 TKLLPQKPRG ETIQNIDDWE LSEMNSDSEI YPTYHVKKKH TRLGRVSPSS
 701 SSDIASSLGE SIESGPLSDI LSDEESSMPL AGMKKYADEK SERASSSEKN
 751 ESHSATKSAL IQKLMQDIQH QDNYEAIWEK IEGFVNKLDE FIQWLNEAME
 801 TTENWTPPKA EMDDLKLYLE THLSFKLNVD SHCALKEAVE EEGHQILLEI
 851 ASHKAGLKD LRMIASQWKE LQRQIKRQHS WILRALDTIK AEILATDVSV
 901 EDEEGTGSPK AEVQLCYLEA QRDAVEQMSL KLYSEQYTSS SKRKEEFADM
 951 SKVHSVGSNG LLDFDSEYQE LWDCLIDMES LVMDSHDLMM SEEQQQHLYK
 1001 RYSVEMSIRH LKKTELLSKV EALKGGVLL PNDLLEKVDS INEKWELLGK
 1051 TLGEKIQDTM AGHSGSSPRD LLSPESGSLV RQLEVRIKEL KGWLRDTELF
 1101 IFNSCLRQEK EGTMNTEKQL QYFKSLCREI KQRERRGVASI LRLCQHLLDD
 1151 RETCNLNADH QPMQLIIVNL ERRWEAIVMQ AVQWQTRLQK KMGKESETLN
 1201 VIDPGLMDLN GMSEDALEWD EMDISNKLIS LNEESNDLQ ELQPVIPLSK
 1251 LGETSNEDPG YDEEADNHGG SQYASNITAP SSPHIYQVYS LHNVELYEDN
 1301 HMPFLKNNPK VTGMTQPNVL TKSLSKDSSF SSTKSLPDLL GGSNLVKPCA
 1351 CHGGDMSQNS GSESGIVSEG DTETTNSEM CLLNNAVDGSP SNLETEHLDP
 1401 QMGDAVNVLK QKFTDEGESI KLPNSSQSSI SPVGCVNGKV GDLNSITKHT
 1451 PDCLGEELQG KHDVFTFYDY SYLQGSKLKL PMIMKQSQSE Kvhvedpllr
 1501 GFYFDKKSCK SKHQTTTELQP DVPPHERILA SASHEMDRIS YKSGNIEKTF
 1551 TGMQNAKQLS LLSHSSSIES LSPGGDLFGL GIFKNGSDSL QRSTSLESWL
 1601 TSYKSNEDLF SCHSSGDISV SSGSVGELSK RTLDDLLNRLE NIQSPSEQKI
 1651 KRSVSDITLQ SSSQKMSFTG QMSLDIASSI NEDSAASLTE LSSSDELSLC
 1701 SEDIVLHKNK IPESNASFRK RLTRSVADES DVNVSMIVNV SCTSACTDDE
 1751 DDSDLLSSST LTLTEEELCI KDEDDDSSIA TDDEIYEDCT LMSGLDYIKN
 1801 ELQTWIRPKL SLTRDKKKRCN VSDEMKGSKD ISSSEMTNPS DTLNIETLIN
 1851 GSVKRVSENN GNGKNSSSH ELGTKRENKK TIFKVNKDPY VADMENGNIE
 1901 GIPERQKGKP NVTSKVSENL GSHGKEISES EHCKCKALMD SLDDSN.TAGK
 1951 EFVSQDVRHL PKKCPNHHHF ENQSTASTPT EKSFSELALE TRFNNRQDSD
 2001 ALKSSDDAPS MAGKSAGCCL ALEQNGTEEN ASISNISCCN CEPDVFHQKD
 2051 AEDCSVHNFV KEIIDMASTA LKSKSQPENE VAAPTSLTQI KEKVLEHSHR

FIGURE 28 (cont.)

2101 PIQLRKGDFY SYLSLSSHDS DCGEVTNYIE EKSSTPLPLD TTDSGLDDKE
2151 DIECFFEACV EGDSDGEEPC FSSAPPNES A VPSEAAMPLQ ATACSSEFSD
2201 SSLSADDADT VALSSPSSQE RAEVGKEVNG LPQTSSGC AE NLEFTPSKLD
2251 SEKESSGKPG ESGMPEEHNA ASA SKVQDL SLKANQPTDK AALHPSPKTL
2301 TCEENLLNLH EKRHRNMHR

FIGURE 29

Figure 29. human PBD sequence:

1	MGKSSTPLPL DTTDSGLDDK EDIECF FEAC VEGDSDGEEP CFSSAPPNES
51	AVPSEAAMPL QATACSSEFS DSSL SADDAD TVALSSPSSQ ERAEVGKEVN
101	GLPQTSSGCA ENLEFTPSKL DSEKESSGKP GESGMPEEHN AASAKSKVQD
151	LSIKANQPTD KAALHPSPKT LTCEENLLNL HEKRHRNMHR

FIGURE 30

Alignment of human and rat PBD sequences

1	-----	-----	-----	-----	-----	50
human mAKAP PBD	(1)	MEQKLISEEDLSPGMLTMSVTLSPISQTELEPDAVDSGLDDKEDDIDCFF				
rat mAKAP PBD	(1)					
Consensus	(1)					
51	-----	-----	-----	-----	-----	
human mAKAP PBD	(28)	EIICV G SDIGE PCFSSA PNEESA PSEAMPLQ TACSSSF DSSPSS				
rat mAKAP PBD	(51)	EACVE D PVNTEAGLPGALNESAAEDGAEQ--K---EQKTTSSPPLLS				
Consensus	(51)	EACVED EE A PNESAIA A TS GCAE E T A S L AD				
101	-----	-----	-----	-----	-----	100
Human mAKAP PBD	(78)	DATIVATSSPSSGERAVVTEVNGIPQTS SGCAENLIEPSKLDPEKISS				
Rat mAKAP PBD	(96)	KTLLPISGLPPKGADGDDASHSQQCAASITPPSGKNAAGR				
Consensus	(101)	D V LS S Q AD AKE L TS GCAE E T A E S L AD				
151	-----	-----	-----	-----	-----	150
Human mAKAP PBD	(128)	GPGESGMPEIHNAAASAKSVDLCKANOPTDIAYHP-SPKTIEEEN				
Rat mAKAP PBD	(146)	SIMQGVSATPENAAASAKPIQAFENAKOPTKGVVARYPSOPTKIK				
Consensus	(151)	K E NAASAK KIQ SL A QP K AL SP TLTC E				
201	-----	-----	-----	-----	-----	200
Human mAKAP PBD	(177)	LLNLLKEKPRMHE				
Rat mAKAP PBD	(196)	LLNLLFEDDLS				
Consensus	(201)	LLN HE RH NMHR				

FIGURE 31

Map of human PBD shuttle plasmid.

Amp-R - mutant - not functional

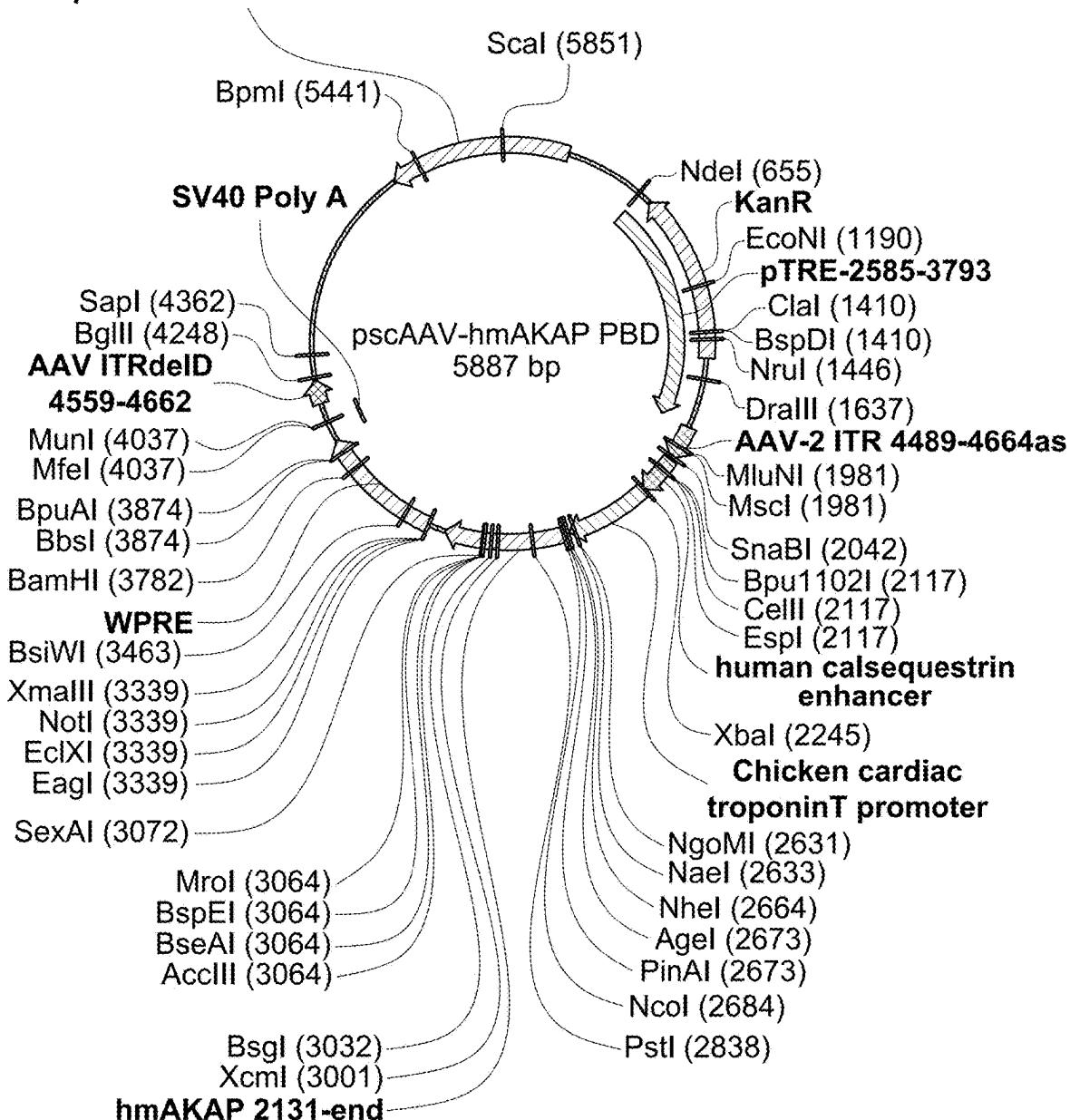


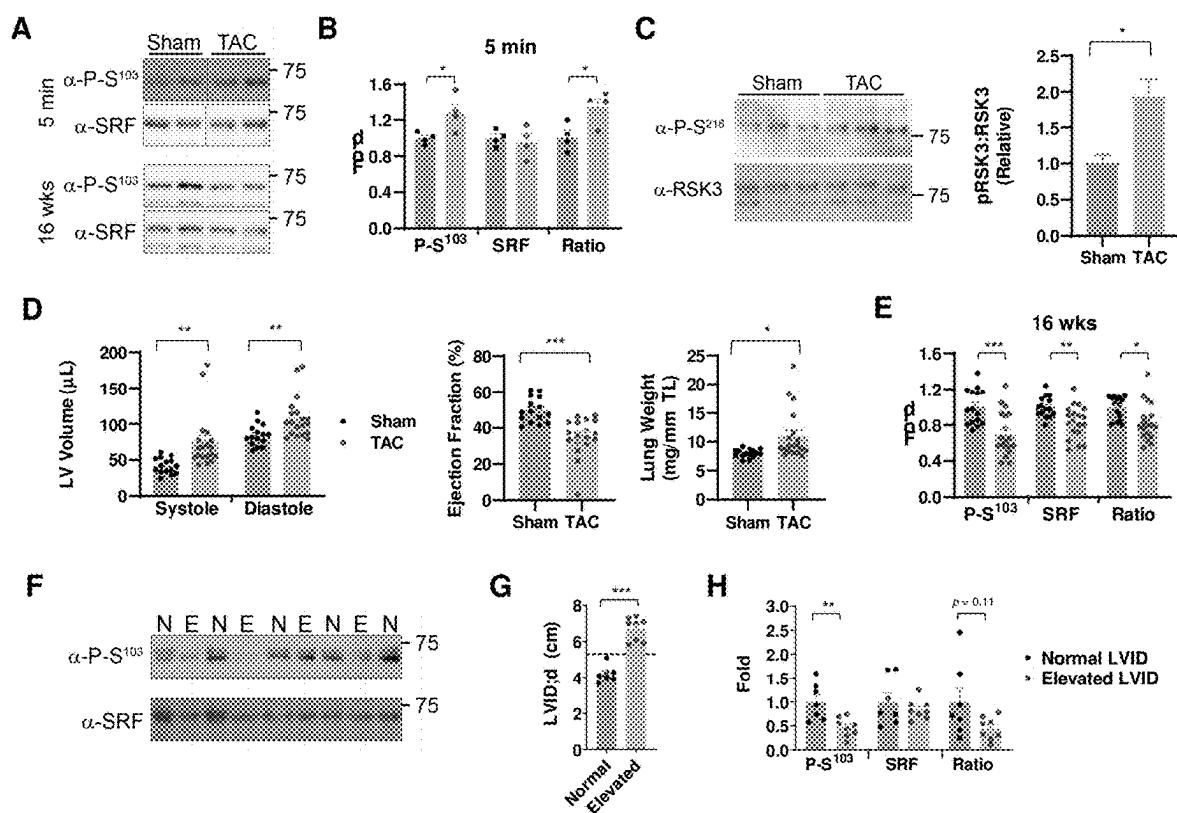
FIGURE 32**Figure 32. Sequence of pscAAV-hmAKAP PBD**

1 ACGAGTTGC TCTCACCCGG CGTCAATAACG GGATAATACCG GCGCCACATA GCAGAACTTT AAAAGTGCTC ATCATTGGAA
 81 AACGTTCTTC CGGGCGAAAA CTCTCAAGGA TCTTACCGCT GTTGAGATCC AGTTCGATGT ACCCAACTCG TGGACCCAAAC
 161 TGATCTTCAG CATCTTTAC TTTCACCCAGC GTTCTGGGT GAGCAAAAAC AGGAAGGCAA ATGCGCGAA AAAAGGGAAAT
 241 AAGGGCGACA CGGAAATGTG CAATTCAACT ACTCTTCCTT TTCAATATT ATTGAAGGAT TTATCAGGGT TAATGCTCTCA
 321 TGAGGGATA CATATTGAA TGTATTGAA AAAATAAACAA ATATGGGGT CCGCGCACAT TTCCCGGAAAG AGTGCACACCT
 401 GACGCTAAG AAACCAATTAT TATCATGACA TTAACCTATA AAAATAGGCC TATCACGAGG CCTTTCGTC TCGCGCGTT
 481 CGGTGATGAC CGTGAACACCG TCTGACATCG GCACGCTCCCG GAGACGCTCA CACCTGTCT GTAGCGGAT GCGGGAGCA
 561 GACAAGCCCG TCAGGGCGCG TCAGCGGGTG TTGGCGGGTG TCGGGCTGG CTIAACTATG CGGCATCAGA GCAGATGTA
 641 CTGAGAGTGC ACCATATGGC CGTCCCCTCA AGTCAGCGTA ATGCTCTGC AGTGTACAA CCAATTAAACC AAATCTGATT
 721 AGAAACACTC ATCGACATC AAATGAAACT GCAATTATTTT CATATCAGGA TTATCAATAC CATATTITIG AAAAAGCCGT
 801 TTCTGTAATG AAGGAGAATCAA CTCAACCCGAG CAGTCCATA GGATGGCAAG ATCTGGTAT CGGTCTGCGA TTCCGACTCG
 881 TCCACATCA ATACAACTCA TTAACTTCCCTCCTGCAAAA ATAAGGTTAT CAAGTGRAGAA ATCAACATGA GTGACGACTG
 961 ATACGGTGA GAATGGAAA AGCTTATGCA TTCTTTCGA GACTTGTCA ACAGGCCAGC CATTACGCTC GTCATCAAA
 1041 TCACTCGCAT CAACCAAACCG GTTATTCTT CCGTGAATGGC CCTGAGCGAG AGGAAATACG CGATCGCTGT TAAAAGGACA
 1121 ATTACAAACAA CGGAATCGAAT GCAACCGCCG CAGGAACACCT GCCAGGGCAT CAACAATATT ITCACTGAA ICAGGATATT
 1201 TTCTCAATAC CTGGAATGCT GTTTTCCCGG CGATCGCAST GGTGACTAAC CATGCTACAG CAGGAGTACG GATAAAATGC
 1281 TTGATGGTGC GAAAGGGCAT AATTCGGCTC AGCGAGTTA CTGCTGACCAT CTCATCTGTA ACATCATGGG CAACGCTAC
 1361 TTGCCATGT TTCAAGAACAA ACTCTGGCGC ATCGGGCTTC CCATACAAATC GATAGATTGT CGCACCTGAT TGGCCGACAT
 1441 TATCGCGAGC CCATTATATAC CCATATAAAAT CAGCATCCAT GTTGAATT AATCGCGGC TCGAGCAAGA CGTTTCCCGT
 1521 TGAATATGGC TCATAACACC CCTTGTATTA CTTGTTATGAA AAGCACAGCAG TTTATGTGTT CATGATGATA TATTTTATC
 1601 TTGTCGAATG TAACATCAGA GATTTGAGA CACRACGTGG CTTTGTTGAA TAAATCGAAC TTGTTGCTGAG TTGAAGGATC
 1681 AGATCACGCA TCTTCCCGAC AACCGAGCGG GTTCTGGCAGG AAAGGAAAGG TTCAAAATCA CCAACTGGTC CACCTACAC
 1761 AAAGCTCTCA TCAACCGTGG CTCCCTCACT TTCTGGCTGG ATGATGGGGC GATTCAGGCC TGGTAGGAGT CAGCAACACC
 1841 TTCTTCACGA GGCAGACCTC AGCGCTACTC GAGECTGCAG GCTGCTCGC TCACTGAGGC CGCCCGSGCA AAGCCGGGC
 1921 GTCGGGCGAC CTTTGGTGC CCGGCGCTAG TGACGAGCG AGCGCCAGA GAGGSAGTGG CCAACTCCAT CACTAGGGGT
 2001 TTCTTGTAGT TAATGATTAA CCGGCCATGC TACTTATCTA CGTAGCCATG CAGTAGAAAC AAGCCAAGG TAGGGAGGCT
 2081 GGGAGGCGAA GCCCCAGATA CCTCTACATCG CTGCTCTAG CCTCTGTCTC ATTAGGAACT CCATTITAG GATSCAGTTS
 2161 TTTCAGGCTA AAAATAAACAT ATGCAATGAA TAAAAAAGTT AGATAAGACA CTGAGGGGG ATTCTGCTGAT ACAGTCTGTC
 2241 CGATCTAGAG CAGTCTGGC TTTCACAAGA CAGCATCTG GGTCTGGCA GAGGGTCGGG TCCGAASCAGC TGCCTTATCA
 2321 GCGTCCCGAC CCCTGGGAGG TGACAGCTGG CTGCTGTGTC TGACGCTCTC GGGCACTCAC GTATCTCGT CCGACGGGTT
 2401 TAAAATAGCA AAACCTGAG CCCACACAAAT AGCTGGCTI TATATGGCTI CCTGTGGGGG AAGGGGGAGG ACGGAGGGGG
 2481 CCGGGCGCGC TTCTGCCAAAT ATAGCAGCTC ACAAGTGTG CCTCTCTC TGGGGCGCGC GCACATTGGT GCTGGCTCTG
 2561 CCCGCCCCGG GGTGGCGCGC GGGGGGACCT TAAAGCTCTI GCCCCCAAG GAGCCCTTCC CAGACAGCCG CGGGCACCCA
 M G K S S T P L P L D T
 2641 CGCTCCGTG GGACCTAAGC TTGCTAGCGC TACCGGTGCG CACCATGGGT AAAASCAACCA CTCCATTGCC ACTAGACACC
 T D S G L D K E D I E C F F E A C V E G D S D G E E .
 2721 ACTGACTCGG CCTTAACTGAA CAAGGAAAGT ATTGAATGCT TTGTTGAGG CTGTTGAG GGTGACTCTG ATGGAGAGGA
 . P C F S S A P P N E S A V P S E A A M P L Q A T A C S .
 2801 GCCTTGTCTC TCTAGTGTCTC CTCCAAATGA ATCTGAGTT CCCAGCGAG CTGCAATGCC ACTACAAGCA ACAGCATGTT
 . S E F S D S S L S A D D A D T V A L S S P S S Q E R .
 2881 TTCTGTAGT CAGTGTAGT TCTCTTICAG CTGATGATGC AGATACAGTG GCTCTTCAA GCCTCTCCGTC TCAAGAAAGA
 A E P V G K E V N G L P Q T S S G C A E N L E F I P S K .
 2961 GCTGAGGTG GAAAGGAAGT GAATGGTTG CCCCCAAACTT CCAGTGGCTG TCGAGAAAAC TTAGAGTTA CTGCTTCAAA
 . L D S E K E S S G K P G E S S G M P E E H N A A S A K S .
 3041 GCTTGACAGT GAAAAGGAAA TTGCGGAAA ACCAGGTGAA TCTGGAATGC CAGAAGAAC TAATGCTGCT ICAGCCAAAT
 . K V Q D L S L K A N Q P T D K A A L H P S P K T L T .
 3121 CTAAAGTCA AGACCTCTC TTGAAGGCAA ATGACCAAC AGACAGGCC GCATTGCAIC CCACCCCAA AACTTTAAC
 C E E N L L N L H E K R H R N M H R
 3201 TGTGAAGAAA ATCTTCTAA CCTTCATGAA AAACGACATA GAAATATGCA TAGGTAGAGT GTATGCC CACGCATGGA
 3281 AATCATCTCA TTGAAAGATA GCCTGGCTGA AGCTCAGGGC TAGTGTAGTT TGATCCGGG CGCCAAATCAA CCTCTGGATT
 3361 ACAAAATTTG TGAAAGATTC ACTGATATTC TTAACTATGT TGCTCTTTT ACGCTGTGTG GATATGCTGC TTAAATACCT
 3441 CTGATCTGTG CTATCTCTC CGCTACGCTT TTCTGTCTC CCTCTCTGTA TAAATCTGG TTCTGTCTC TTAAATAGGA
 3521 GTTGTGGCC GGTGTGGCTG AACGTGGGGT GTTGTGGCTG GTTGTGGCTG AGCGAACCC CACTGGCTGG GGCAATTGCC
 3601 CCACCTGTCA ACTCTCTTCT GGGACTTCTG CTTTCCCTC CCGGATGCC AGGGCAGAAC TCATCGCCGC CTGCTTGC
 3681 CGCTGCTGGA CAGGGGCTAG GTTGTGGCTG ACCTGATAATT CGCGGGTTGTT GTCGGGGAAA TCAATGCTCTT TTGCTTGGCT
 3761 GCTGGCCCTGTG TTGCGCAACT CGGATCTGCG CGGGACGTCC TTCTGCTACG TCCCTTCCGG TCTCAATCCA GGGGACCTCC
 3841 CTTCCCGCGG CCTCTGGCG CCTCTGGCG CTTCTCCGGG CGACTCGGAT CTCCCTTGG

FIGURE 32 (cont.)

3921 GCGCCCTCCC CGCCTGTAGG CCTCACCTGC GATCTCGATG CTTTATTGTC GAAATTGGT AIGCTATTGC TTTATTGTA
4001 ACCATTATAA GCTGCAATAA ACAAGTTAAC AACAAACAATT GCATTCATT TAIGTTICAG GTTCAGGGGG AGGTGTGGGA
4081 GGTTTTIAA AGCAAGTAAA ACCTCTACAA ATIGCTGATG GCTGATIACC ACTCCCTCTC TCGCGCTCG CTCGCTCACT
4161 GAGGCCGGC GACCAAAGGT CGCCCCACGC CGCGGCTTG CCCGGCGGC CTCACTGAGC GACCGAGCGC GCCAGCTGAA
4241 GCTATCAGAT CTGCCGGTCT CCCTATAGTG AGICGTATTAA ATTTGATATA GCCAGGTTAA CCTGCATTAA TGAATGGCC
4321 AACGGCGGG GAGAGGGGT TTGCGTATTG GGCCTCTTC CGCTTCTCG CTCACTGACT CGCTGCGCTC GGTGTTCGG
4401 CTGCGCCGAG CGGTATCAGC TCACTCAAAG GCGGTAATAC GGTATCCAC AGAATCAGGG GATAACGCAAG GAAAGAACAT
4481 GTGACGCAAA GGGCAGCAAA AGGCCAGGAA CGCTAAAAAG GCGCCGTTGC TGCGCTTTT CCATAGGCTC CGCCCCCTG
4561 ACGAGCATCA CAAAAATCGA CGCTCAAGTC AGAGGTGGCG AAACCCGACA GGACTATAAA GATACCAGGC GTTCCCCCT
4641 GGAAGCTCCC TCGTGCCTC TCCGTTCGG ACCCTGCGGC TTACCGATA CCTGTCGGCC TTTCTCCCT CGGGAAAGCGT
4721 GGCCTTCTC CATAGCTCAC GCTGTAGGTA TCTCAGTICG GTGTAAGTCG TTGCTCCAA GCTGGCTGT GTGACGAAAC
4801 CCCCCGTICA GCCCGACCGC TGCGCCTIAT CGCGTAACTA TCGCTTGTAG TCCAACCCGG TAAGACACGA CTTATGCCA
4881 CTGGCAGCAG CCACTGGTAA CAGGATTAGC AGAGCGAGGT ATGTAAGCGG TGCTACAGAG TTCTGAAAGT GGTGGCTAA
4961 CTACGCTAC ACTAGAGAA CAGTATTG GATCTCGCT CTGCTGAAGC CAGTTACCTI CGGAAAAAGA GTTGGTAGCT
5041 CTTGATCCGG CAAACAAACC ACCGCTGGTA GCGGTGGTT TTTCTTGTG AAGCAGCAGA TTACCGCGAG AAAAGGAA
5121 TCTCAAGAAG ATCCTTTGAT CTTTCTACG GGGCTGACG CTCACTGGAA CGAAAATCTCA CGTTAAGGGAA TTTGGTICAT
5201 GAGATTATCA AAAAGGATCT TCACCTAGAT CCTTTAAAT TAAATGAA GTTTAAATC AATCTAAAGT AATATGAGT
5281 AAACCTGGTC TGACAGTAC CAATGCTAA TCAGTGAGGC ACCTATCTCA CGCATCTGTC TATTICGTT ATCCATAGTT
5361 GCCTGACCTCC CCGTCGTGTA GATAACTACG ATACGGGAGG GCTTACCATC TGCGCCAGT GCTGCAATGA TACCGCGAGA
5441 CCCACGCTCA CCGGCTCCAG ATTATCAGC AATAAAATCAG CCAGCGGAA GGGCCGAGCG CAGAAGTGGT CCTGCAACTT
5521 TATCCGCTTC CATCCAGTCT ATTCAITGTT GCCGGGAAGC TAGAGTAAGT AGTTGCCAG TTAATAGTT TCACAACGTT
5601 GTTGGCATTG CTACAGGCAT CGTGGTGTCA CGCTCGTCGTT GGTTATGGC TTCAATTGAGC TCCGGTTCCC AACGATCAAG
5681 GCGAGTTACA TGTCAACCCCA TGTGATGCAA AAAAGCGGTT AGCTCTTCG GTCCCTCOGAT CGTGTGAGA AGTAAGTGG
5761 CCGCAGTGT ATCACTCATG GTTAIGGCAG CACTGCATAA TTCTCTTACT GTCATGCCAT CGCTAAGATG CTTTCTGTG
5841 ACTGGTGAGT ACTCAACCAA GTCATCTGAA GAATAGTGTA TGCGCG

FIGURE 33



TREATMENT OF HEART DISEASE BY DISRUPTION OF THE ANCHORING OF PP2A

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation of U.S. patent application Ser. No. 18/420,397 filed Jan. 23, 2024 which claims priority to U.S. patent application Ser. No. 16/818,771 filed Mar. 13, 2020, which claims priority to U.S. Provisional Patent Application Ser. No. 62/848,156, filed May 15, 2019, which is hereby incorporated by reference in its entirety and this application incorporates by reference in their entireties U.S. patent application Ser. No. 14/821,082, filed Aug. 7, 2015, now U.S. Pat. No. 9,937,228, issued Apr. 10, 2018, U.S. patent application Ser. No. 14/213,583, filed on Mar. 14, 2014, now U.S. Pat. No. 9,132,174, issued on Sep. 15, 2015, U.S. patent application Ser. No. 16/028,004, filed Jul. 5, 2018, U.S. Provisional Application No. 61/798,268, filed Mar. 15, 2013, and U.S. Provisional Application 62/529,224, filed Jul. 6, 2017.

[0002] The instant application contains a Sequence Listing, which has been submitted electronically via EFS-Web in XML file format and is hereby incorporated by reference in its entirety. Said XML copy, created on Jan. 19, 2024, is named 65274_2US02_SL.xml and is 126,514 bytes in size.

STATEMENT OF GOVERNMENTAL SUPPORT

[0003] This invention was made with Government support under contract RO1 HL 075398 and HL126825 awarded by the National Institutes of Health. The Government has certain rights in this invention.

BACKGROUND OF THE INVENTION

[0004] In response to chronic stress, the heart's main compensatory mechanism is myocyte hypertrophy, a non-mitotic increase in volume of the contractile cells (Hill and Olson 2008). The adult mammalian myocyte is roughly cylindrical and can grow either in width or length. Because myocytes contribute the vast majority of the myocardial mass of the heart (Jugdutt 2003), concentric and eccentric hypertrophy of the cardiac myocyte result in thickening of heart chamber walls and dilation of the chambers, respectively. In theory, "concentric" myocyte growth in width involving parallel assembly of sarcomeres reduces ventricular wall stress (Law of LaPlace), while "eccentric" length-wise myocyte growth involving serial assembly of sarcomeres may accommodate greater ventricular volumes without stretching individual sarcomeres beyond the optimum length for contraction (length-tension relationship) (Grossman, Jones, and McLaurin 1975). While the left ventricle will undergo relatively symmetric hypertrophy in response to physiologic stress such as pregnancy or exercise training, concentric ventricular hypertrophy is the predominant initial response to the increased systolic wall stress present in pressure overload diseases such as hypertension or aortic stenosis. Eccentric ventricular hypertrophy predominates during states of volume overload such as occurs following myocardial infarction, as well as during the transition from concentric hypertrophy to the dilated heart in Heart Failure with Reduced Ejection Fraction (HFrEF) in some forms of cardiovascular disease, including diseases mainly characterized by pressure overload. Concentric and eccentric hyper-

trophy are also present in inherited hypertrophic and dilated cardiomyopathies, respectively.

[0005] At the cellular level, cardiac myocyte hypertrophy occurs as the result of an increase in protein synthesis and in the size and organization of sarcomeres within individual myocytes. For a more thorough review of cardiac remodeling and hypertrophy, see Kehat (2010) and Hill (2008), each herein incorporated by reference in their entirety. The prevailing view is that cardiac hypertrophy plays a major role in the development of heart failure. Traditional routes of treating heart failure include afterload reduction, blockage of beta-adrenergic receptors (β -ARs) and use of mechanical support devices in afflicted patients. However, the art is in need of additional mechanisms of preventing or treating pathological cardiac hypertrophy.

[0006] Research suggests that mechanisms that induce "compensatory" concentric hypertrophy early in pressure-overload related heart disease predispose the heart to later systolic dysfunction and eventual failure (Schiaffarella and Hill 2015). In this regard, results show that targeting of RSK3-mAKAP β complexes will attenuate cardiac remodeling due to pressure overload and prevent heart failure (Kritzer et al. 2014; Li, Kritzer, et al. 2013). Accordingly, inhibition of signaling pathways that induce remodeling, including concentric hypertrophy, may be desirable early in pressure overload disease. However, the question remained whether efforts to maintain signals that may promote concentric hypertrophy and oppose eccentric hypertrophy would preserve cardiac volumes and contractility when initiated when the heart is at a stage in the disease process characterized by the eccentric growth and ventricular dilation leading to HFrEF, whether late in pressure overload-related disease or throughout the progression of volume overload-related disease. Further, it is unknown whether the enhancement of concentric myocyte hypertrophy and/or the inhibition of eccentric myocyte hypertrophy in familial dilated cardiomyopathy may be beneficial.

AKAPs and Cardiac Remodeling

[0007] Ventricular myocyte hypertrophy is the primary compensatory mechanism whereby the myocardium reduces ventricular wall tension when submitted to stress because of myocardial infarction, hypertension, and congenital heart disease or neurohumoral activation. It is associated with a nonmitotic growth of cardiomyocytes, increased myofibrillar organization, and upregulation of specific subsets of "fetal" genes that are normally expressed during embryonic life (Frey 2004, Hill 2008). The concomitant aberrant cardiac contractility, Ca^{2+} handling, and myocardial energetics are associated with maladaptive changes that include interstitial fibrosis and cardiomyocyte death and increase the risk of developing heart failure and malignant arrhythmia (Cappola 2008, Hill 2008). Together, these adaptations contribute to both systolic and diastolic dysfunction that are present in different proportions depending upon the underlying disease (Sharma and Kass 2014). Pathological remodeling of the myocyte is regulated by a complex intracellular signaling network that includes mitogen-activated protein kinase (MAPK), cyclic nucleotide, Ca^{2+} , hypoxia, and phosphoinositide-dependent signaling pathways (Heineke and Molkenutin 2006).

[0008] Increased in prevalence by risk factors such as smoking and obesity, in the United States, heart failure affects 6.2 million adults, and each year ~1,000,000 new

adult cases are diagnosed (Benjamin et al. 2019). The prevalence and incidence of heart failure are increasing, mainly because of increasing life span, but also because of the increased prevalence of risk factors (hypertension, diabetes, dyslipidemia, and obesity) and improved survival rates from other types of cardiovascular disease (myocardial infarction [MI] and arrhythmias) (Heidenreich et al. 2013). First-line therapy for patients with heart failure includes angiotensin-converting enzyme (ACE) inhibitors and β -adrenergic receptor blockers (β -blockers) that can improve the survival and quality of life of such patients, as well as reduce mortality for those with left ventricular dysfunction (Group 1987). Subsequent or alternative therapies include aldosterone and angiotensin II receptor blockers, neprilysin inhibitors, loop and thiazide diuretics, vasodilators, and I_f current blockers, as well as device-based therapies (Ponikowski et al. 2016). Nevertheless, the 5-year mortality for symptomatic heart failure remains ~50%, including >40% mortality for those post-MI (Heidenreich et al. 2013; Gerber et al. 2016).

[0009] Cardiac hypertrophy can be induced by a variety of neuro-humoral, paracrine, and autocrine stimuli, which activate several receptor families including G protein-coupled receptors, cytokine receptors, and growth factor tyrosine kinase receptors (Brown 2006, Frey 2004). In this context, it is becoming increasingly clear that A-kinase anchoring proteins (AKAPs) can assemble multiprotein complexes that integrate hypertrophic pathways emanating from these receptors. In particular, recent studies have now identified anchoring proteins including mAKAP, AKAP-Lbc, and D-AKAP1 that serve as scaffold proteins and play a central role in organizing and modulating hypertrophic pathways activated by stress signals.

[0010] As the organizers of “nodes” in the intracellular signaling network, scaffold proteins are of interest as potential therapeutic targets (Negro, Dodge-Kafka, and Kapiloff 2008). In cells, scaffold proteins can organize multimolecular complexes called “signalosomes,” constituting an important mechanism responsible for specificity and efficacy in intracellular signal transduction (Scott and Pawson 2009). Firstly, many signaling enzymes have broad substrate specificity. Scaffold proteins can co-localize these pleiotropic enzymes with individual substrates, selectively enhancing the catalysis of substrates and providing a degree of specificity not intrinsic to the enzyme’s active site (Scott and Pawson 2009). Secondly, some signaling enzymes are low in abundance. Scaffold proteins can co-localize a rare enzyme with its substrate, making signaling kinetically favorable. Thirdly, since many scaffolds are multivalent, scaffold binding can orchestrate the co-regulation by multiple enzymes of individual substrate effectors. Muscle A-kinase anchoring protein (mAKAP, a.k.a. AKAP6) is a large scaffold expressed in cardiac and skeletal myocytes and neurons that binds both signaling enzymes such as protein kinase A (PKA) and the Ca^{2+} /calmodulin-dependent phosphatase Calcineurin (CaN) that have broad substrate specificity and signaling enzymes such as p90 ribosomal S6 kinase 3 (RSK3) that is remarkably low in abundance (FIG. 1) (Wang et al. 2015; Pare, Easlick, et al. 2005; Michel et al. 2005a; Kapiloff et al. 1999b). mAKAP β is the alternatively-spliced isoform expressed in myocytes, in which cells it is localized to the outer nuclear membrane by binding the integral membrane protein nesprin-1 α (Pare, Easlick, et al. 2005).

[0011] Consistent with its role as a scaffold protein for stress-related signaling molecules in the cardiac myocyte, depletion of mAKAP β in rat neonatal ventricular myocytes *in vitro* inhibited hypertrophy induced by α -adrenergic, β -adrenergic, endothelin-1, angiotensin II, and leucine inhibitor factor/gp130 receptor signaling (Zhang et al. 2011; Pare, Bauman, et al. 2005; Dodge-Kafka et al. 2005; Guo et al. 2015). *In vivo*, along with attenuating hypertrophy induced by short-term pressure overload and chronic β -adrenergic stimulation, mAKAP gene targeting in the mouse inhibited the development of heart failure following long-term pressure overload, conferring a survival benefit (Kritzer et al. 2014). Specifically, mAKAP gene deletion in the mAKAP $^{fl/fl}$, Tg(Myh6-cre/Esr1 *), tamoxifen-inducible, conditional knock-out mouse reduced left ventricular hypertrophy, while greatly inhibiting myocyte apoptosis, and interstitial fibrosis, left atrial hypertrophy, and pulmonary edema (wet lung weight) due to transverse aortic constriction for 16 weeks (Kritzer et al. 2014).

[0012] mAKAP gene targeting is also beneficial following myocardial infarction (Kapiloff, unpublished observations). Permanent ligation of the left anterior descending coronary artery (LAD) in the mouse results in myocardial infarction, including extensive myocyte death, scar formation, and subsequent left ventricular (LV) remodeling. Four weeks following LAD ligation, mAKAP conditional knock-out mouse had preserved LV dimensions and function when compared to infarcted control cohorts. mAKAP conditional knock-out mice had preserved LV ejection fraction and indexed atrial weight compared to controls, while displaying a remarkable decrease in infarct size.

Introduction to mAKAP and Cardiac Remodeling

[0013] mAKAP was originally identified in a cDNA library screen for new cAMP-dependent protein kinase (PKA) regulatory-subunit (R-subunit) binding proteins, i.e. A-kinase anchoring proteins or AKAPs (Mccartney et al. 1995). mAKAP was initially named “AKAP100” for the size of the protein encoded by the original cDNA fragment (Mccartney et al. 1995). Subsequently, the full-length mRNA sequence for mAKAP α , the alternatively-spliced isoform of mAKAP expressed in neurons, was defined, revealing that wildtype mAKAP α is a 255 kDa scaffold (Kapiloff et al. 1999b). The sequence for mAKAP β , the 230 kDa alternatively-spliced isoform of mAKAP expressed in striated myocytes, was later obtained, showing that when expressed in heart or skeletal muscle, mAKAP is translated from an internal start site corresponding to mAKAP α residue Met-245 (Michel et al. 2005a).

[0014] mAKAP is localized to the nuclear envelope both in neurons and striated cardiac and skeletal myocytes (FIG. 6), the three cell types in which mAKAP is clearly expressed (Kapiloff et al. 1999b; Pare, Easlick, et al. 2005; Michel et al. 2005a). mAKAP is not a transmembrane domain protein and contains three spectrin-like repeat regions (residues 772-1187) that confer its localization (Kapiloff et al. 1999b). Binding of mAKAP’s third spectrin repeat (residues 1074-1187) by the outer nuclear membrane protein nesprin-1 α is both necessary and sufficient for mAKAP nuclear membrane localization, at least in myocytes and when expressed in heterologous cells (Pare, Easlick, et al. 2005). Nesprin-1 α may also be present on the inner nuclear envelope where it might bind A-type lamins and emerin. Interestingly, mutations in lamin A/C, emerin, and nesprin-1 α have been associated with Emery-Dreyfuss muscular dystrophy, as

well as other forms of cardiomyopathy (Bonne et al. 1999; Fatkin et al. 1999; Muchir et al. 2000; Bione et al. 1994; Zhang et al. 2007). However, no disease-causing mutations have yet been identified in the human mAKAP gene, and mAKAP β knock-out in the mouse heart early in development does not induce cardiomyopathy (Kritzer et al. 2014). Besides binding nesprin-1 α , mAKAP β also binds phospholipase C ϵ (PLC ϵ) through mAKAP's first spectrin repeat, potentially strengthening its association with the nuclear envelope (Zhang et al. 2011). There were early reports of mAKAP β being present on the sarcoplasmic reticulum (Mccartney et al. 1995; Marx et al. 2000; Yang et al. 1998), but these findings have been called into question due to technical issues including antibody specificity (Kapiloff, Jackson, and Airhart 2001; Kapiloff et al. 1999b).

[0015] Besides PKA, PLC ϵ and nesprin-1 α , mAKAP β binds a wide variety of proteins important for myocyte stress responses: adenylyl cyclase type 5 (AC5), exchange protein activated by cAMP-1 (Epac1), cAMP-specific phosphodiesterase type 4D3 (PDE4D3), MEK5 and ERK5 MAP-kinases, 3-phosphoinositide-dependent protein kinase-1 (PDK1), p90 ribosomal S6 kinases 3 (RSK3), protein kinase C ϵ (PKC ϵ), protein kinase D (PKD1, PKC μ), the protein phosphatases calcineurin (CaN) A β and PP2A, the type 2 ryanodine receptor (RyR2), the sodium/calcium exchanger NCX1, ubiquitin E3-ligases involved in HIF1 α regulation, and myopodin (Pare, Bauman, et al. 2005; Pare, Easlick, et al. 2005; Dodge-Kafka et al. 2005; Marx et al. 2000; Kapiloff, Jackson, and Airhart 2001; Michel et al. 2005a; Li et al.; Wong et al. 2008; Zhang et al. 2011; Dodge-Kafka and Kapiloff 2006; Vargas et al. 2012; Faul et al. 2007; Schulze et al. 2003; Kapiloff et al. 2009; Zhang et al. 2013). Bound to mAKAP β , these signaling molecules co-regulate the transcription factors hypoxia-inducible factor 1 α (HIF1 α), myocyte enhancer factor-2 (MEF2), and nuclear factor of activated T-cell (NFATc) transcription factors, as well as type II histone deacetylases (FIG. 7) (Kritzer et al. 2014; Li, Vargas, et al. 2013; Li et al. 2010; Wong et al. 2008; Li et al. 2019; Dodge-Kafka et al. 2018). Some of these molecules are bound directly and some indirectly, some constitutively and some in a regulated manner. Thus, it is likely that the composition of mAKAP β signalosomes depends upon the underlying state of the myocyte. As research continues on mAKAP β , the list of its binding partners grows, confirming its hypothesized role as an important orchestrator of signaling pathways required for remodeling. Most of what is known about mAKAP β is based upon work using cultured neonatal rat ventricular myocytes, in which mAKAP β was early on recognized to be required for the induction of hypertrophy by a variety of upstream receptors, including α - and β -adrenergic and cytokine receptors (Pare, Bauman, et al. 2005; Dodge-Kafka et al. 2005). However, recently, the phenotype of a conditional, cardiac-myocyte specific mAKAP β knock-out mouse has been published confirming the centrality of mAKAP β to remodeling (Kritzer et al. 2014). There are various upstream inputs, downstream effectors (outputs), and integrative circuitry within mAKAP β signalosomes that impact pathological remodeling of the heart.

mAKAPf3—a Prototypical A-Kinase Anchoring Protein

[0016] Like most AKAPs, mAKAP contains an amphipathic helix (residues 2055-2072) responsible for binding PKA (Kapiloff et al. 1999b; Kritzer et al. 2012). PKA is a heterotetramer of two R-subunits and two catalytic C-sub-

units, in the configuration C-R-R-C. Within the holoenzyme, the N-terminal docking and dimerization domains of the PKA R-subunits form a X-type, antiparallel four-helix bundle (Newlon et al. 1999). This bundle contains a hydrophobic groove that accommodates the hydrophobic face of the AKAP amphipathic helix. mAKAP β binds selectively type II PKA (that contains RII subunits) with high affinity ($K_D=119$ nM) (Zakhary et al. 2000). Interestingly, PKA-mAKAP β binding is increased 16-fold following RII α auto-phosphorylation (Zakhary et al. 2000), potentially affecting PKA-mAKAP β binding in states of altered β -adrenergic signaling. Besides mAKAP β , there are over a dozen other AKAPs expressed in the myocyte, each with its own distinct localization and sets of binding partners (Kritzer et al. 2014). Remarkably, mAKAP is one of the rarest AKAPs in the myocyte, such that loss of mAKAP does not even affect the localization of perinuclear PKA (Kapiloff, unpublished observations). Despite the low level of expression of the scaffold, replacement in myocytes of endogenous mAKAP β with a full-length mAKAP β mutant that cannot bind PKA is sufficient to inhibit the induction of myocyte hypertrophy (Pare, Bauman, et al. 2005). Thus, mAKAP β signalosomes serve as an example of both how finely PKA signaling may be compartmentalized even on an individual organelle and how the level of expression of a protein or a protein complex is not necessarily indicative of the functional significance of that protein.

[0017] mAKAP β is remarkable because it binds not only effectors for cAMP signaling, but also enzymes responsible for cAMP synthesis and degradation (Kapiloff et al. 2009; Dodge et al. 2001). The synthesis of cAMP from ATP is catalyzed by adenylyl cyclases (AC), while cAMP metabolism to 5'AMP is catalyzed by phosphodiesterases (PDE). The differential association of ACs and PDEs with AKAPs contributes to cAMP compartmentation in cells, providing both for local activation of cAMP effectors and regulation of local cAMP levels by unique regulatory feedback and feed-forward loops (Scott, Dessauer, and Tasken 2013). mAKAP is capable of binding both AC2 and AC5, but AC5 appears to be the relevant mAKAP β -binding partner in the heart (Kapiloff et al. 2009). The N-terminal, C1 and C2 domains of AC5 bind directly to a unique N-terminal site on mAKAP β (residues 275-340). AC5 activity is inhibited by PKA feedback phosphorylation that in cells is facilitated by mAKAP β complex formation (Kapiloff et al. 2009). This negative feedback appears to be physiologically relevant to the maintenance of basal cAMP signaling. When the tethering of AC5 to mAKAP β is inhibited by a competitive peptide comprising the mAKAP AC5-binding domain, both the cAMP content and size of myocytes were increased in the absence of hypertrophic stimulus (Kapiloff et al. 2009).

[0018] mAKAP was the first AKAP shown to bind a PDE (Dodge et al. 2001). A site within mAKAP 1286-1831 binds the unique N-terminal domain of PDE4D3. Phosphorylation of PDE4D3 serine residues 13 and 54 results in increased binding to the scaffold and increased PDE catalytic activity, respectively (Dodge et al. 2001; Sette and Conti 1996; Carlisle Michel et al. 2004). Because increased PDE4D3 activity accelerates cAMP degradation, PKA and PDE4D3 constitute a negative feedback loop that can modulate local cAMP levels and PKA activity (Dodge et al. 2001). PDE4D3 bound to mAKAP serves not only as a PDE, but also as an adapter protein recruiting the MAPKs MEK5 and ERK5 and the cAMP-dependent, Rap1-guanine nucleotide exchange

factor Epac1 to the scaffold (Dodge-Kafka et al. 2005). Activation of MEK5 and ERK5 by upstream signals results in PDE4D3 phosphorylation on Ser-579, inhibiting the PDE and promoting cAMP accumulation and PKA activation (Dodge-Kafka et al. 2005; Hoffmann et al. 1999; Mackenzie et al. 2008). Epac1 is less sensitive to cAMP than PKA, such that very high cAMP levels results in the additional activation of mAKAP-associated Epac1. Through Rap1, Epac1 can inhibit ERK5 activity, thus preventing PDE4D3 inhibition by MAPK signaling, resulting presumably in maximal PDE4D3 activity due to concomitant PKA phosphorylation (Dodge-Kafka et al. 2005). As a result, Epac1, ERK5, and PDE4D3 constitute a third negative feedback loop that will attenuate cAMP levels in the vicinity of mAKAP complexes opposing cAMP elevation to extremely high levels.

[0019] Additional complexity is afforded by the binding of the serine-threonine phosphatase PP2A to the C-terminus of mAKAP (residues 2083-2319) (Dodge-Kafka et al. 2010). PP2A can catalyze the dephosphorylation of PDE4D3 Ser-54, thereby inhibiting the PDE in the absence of upstream stimulus. PP2A associated with mAKAP complexes contain B56δ B subunits, which are PKA substrates. PKA phosphorylation enhances PP2A catalytic activity (Ahn et al. 2007), such that phosphorylation of B56δ by mAKAP-bound PKA increases PDE4D3 dephosphorylation, inhibiting the PDE. This presumably increases cAMP levels, constituting a positive feedforward loop for the initiation of cAMP signaling. Together with the negative feedback loops based upon AC5 phosphorylation and PDE4D3 regulation by PKA and ERK5, one would predict that cAMP levels at mAKAPβ signalosomes would be tightly controlled by upstream β-adrenergic and MAPK signaling. Signaling upstream of AC5 and ERK5 will promote cAMP signaling that will be initially promoted by PP2A feedforward signaling, while PDE4D3 activation and AC5 inhibition by PKA and Epac1 negative feedback will constrain signaling. Interestingly, Rababa'h et al. demonstrated how mAKAP proteins containing non-synonymous polymorphisms differentially bound PKA and PDE4D3 (Rababa'h et al. 2013). The potential for cAMP signaling to be differentially modulated by crosstalk between upstream signaling pathways or by human polymorphisms makes compelling further work in myocytes to show the relevance of this complicated signaling network.

mAKAPβ and MAP-kinase-RSK3 Signaling

[0020] The recruitment of ERK5 by PDE4D3 to mAKAPβ complexes was initially shown to be relevant to the local regulation of cAMP through the aforementioned feedback loops (Dodge-Kafka et al. 2005). However, ERK5 was also recognized to be an important inducer of myocyte hypertrophy, preferentially inducing the growth in length (eccentric hypertrophy) of cultured myocytes, while also being important for concentric hypertrophy *in vivo* due to pressure overload (transverse aortic constriction in the mouse) (Nicol et al. 2001; Kimura et al. 2010). Notably, inhibition by RNA interference (RNAi) of mAKAPβ expression in cultured myocytes inhibited the eccentric growth induced by the interleukin-6-type cytokine leukemia inhibitory factor (LIF) (Dodge-Kafka et al. 2005). A potential effector for mAKAPβ-bound ERK5 was MEF2 transcription factor, as discussed below. However, in both heart and brain, mAKAP bound PDK1, a kinase that together with ERKs (ERK1, 2 or 5) can activate the MAPK effector p90RSK, a kinase also associated with mAKAP (Ranganathan et al. 2006; Michel et al. 2005a). Importantly, binding of PDK1 to mAKAP

obviated the requirement for membrane association in RSK activation (Michel et al. 2005a). Taken together, these data suggested that mAKAPβ could orchestrate RSK activation in myocytes in response to upstream MAPK signaling.

[0021] p90RSK is a pleiotropic ERK effector that regulates many cellular processes, including cell proliferation, survival, migration, and invasion. RSK activity is increased in myocytes by most hypertrophic stimuli (Anjum and Blenis 2008; Sadoshima et al. 1995). In addition, RSK activity was found to be increased in human end-stage dilated cardiomyopathy heart tissue (Takeishi et al. 2002). RSK family members contain 2 catalytic domains, an N-terminal kinase domain and a C-terminal kinase domain (Anjum and Blenis 2008). The N-terminal kinase domain phosphorylates RSK substrates and is activated by sequential phosphorylation of the C-terminal and N-terminal kinase domain activation loops by ERK and PDK1, respectively, such that PDK1 phosphorylation of the N-terminal domain on Ser-218 is indicative of full activation of the enzyme. There are 4 mammalian RSK family members that are ubiquitously expressed, but only RSK3 binds mAKAPβ (Li, Kritzer, et al. 2013). The unique N-terminal domain of RSK3 (1-30) binds directly mAKAPβ residues 1694-1833, explaining the selective association of that isoform with the scaffold (Li, Kritzer, et al. 2013). Despite the fact that RSK3 is expressed less in myocytes than other RSK family members, neonatal myocyte hypertrophy was found to be attenuated by RSK3 RNAi, inactivation of the RSK3 N-terminal kinase domain, and disruption of RSK3 binding to mAKAP using an anchoring disruptor peptide (Li, Kritzer, et al. 2013). Importantly, RSK3 expression *in vivo* was required for the induction of cardiac hypertrophy by both pressure overload and catecholamine infusion, as well as for the heart failure associated with a mouse model for familial hypertrophic cardiomyopathy (α -tropomyosin Glu180Gly) (Li, Kritzer, et al. 2013; Passariello et al. 2013). In addition, consistent with the reported role of ERK1/2 MAP-Kinase in selectively inducing concentric hypertrophy (Kehat et al. 2011), RSK3 gene deletion inhibited the concentric hypertrophy induced by Raf1^{L613V} mutation in a mouse model for Noonan Syndrome (Passariello et al. 2016). The recognition that this specific RSK isoform is required for cardiac remodeling makes it a compelling candidate for therapeutic targeting.

mAKAPβ and Phosphatidylinositide Signaling

[0022] The cAMP effector Epac1 activates Rap1 at mAKAPβ complexes affecting ERK5 signaling (Dodge-Kafka et al. 2005). In addition, Epac1-Rap1 activates PLC ϵ , a phospholipase whose Ras association domains directly bind the first spectrin repeat-like domain of mAKAPβ (Zhang et al. 2011). Like mAKAPβ, PLC ϵ was required for neonatal myocyte hypertrophy, whether inhibited by RNAi or by displacement from mAKAPβ by expression of competitive binding peptides. In an elegant paper by the Smrcka laboratory, mAKAPβ-bound PLC ϵ has been shown to regulate PKC ϵ and PKD activation through a novel phosphatidylinositol-4-phosphate (PI4P) pathway in which PLC ϵ selectively converts perinuclear PI4P to diacylglycerol and inositol-1,4-bisphosphate (Zhang et al. 2013). PKD phosphorylates type II histone deacetylases (HDACs 4/5/7/9) inducing their nuclear export and de-repressing hypertrophic gene expression (Monovich et al. 2010; Xie and Hill 2013). Smrcka and colleagues found that PLC ϵ was required for pressure overload-induced PKD activation, type II HDAC

phosphorylation and hypertrophy in vivo (Zhang et al. 2013). Subsequently, mAKAP β was also found to be required in vivo for PKD activation and HDAC4 phosphorylation in response to pressure overload (Kritzer et al. 2014). Remarkably, mAKAP β can form a ternary complex with PKD and HDAC4. Together, these results show how local cAMP signaling can affect the regulation of cardiac gene expression.

[0023] Recently it was published that mAKAP β is a scaffold for HDAC5 in cardiac myocytes, forming signalosomes containing HDAC5, PKD, and PKA (Dodge-Kafka et al. 2018). Inhibition of mAKAP β expression attenuated the phosphorylation of HDAC5 by PKD and PKA in response to α - and β -adrenergic receptor stimulation, respectively. Importantly, disruption of mAKAP β -HDAC5 anchoring prevented the induction of HDAC5 nuclear export by α -adrenergic receptor signaling and PKD phosphorylation. In addition, disruption of mAKAP β -PKA anchoring prevented the inhibition by β -adrenergic receptor stimulation of α -adrenergic-induced HDAC5 nuclear export. Together, these data establish that mAKAP β signalosomes serve to bidirectionally regulate the nuclear-cytoplasmic localization of class IIa HDACs. Thus, the mAKAP β scaffold serves as a node in the myocyte regulatory network controlling both the repression and activation of pathological gene expression in health and disease, respectively.

mAKAP β and Calcium Signaling

[0024] Besides cAMP, phosphoinositide and MAP-kinase signaling, mAKAP β contributes to the orchestration of Ca $^{2+}$ -dependent signaling transduction. The second binding partner for mAKAP β identified was the ryanodine receptor Ca $^{2+}$ release channel (RyR2) responsible for Ca $^{2+}$ -induced Ca $^{2+}$ release from intracellular stores (Kapiloff, Jackson, and Airhart 2001; Marx et al. 2000). RyR2 is best known for its role in excitation-contraction coupling, in which bulk Ca $^{2+}$ is released to induce sarcomeric contraction. PKA phosphorylation can potentiate RyR2 currents (Valdivia et al. 1995; Dulhunty et al. 2007; Bers 2006), although the importance of PKA-catalyzed RyR2 phosphorylation to excitation-contraction coupling is highly controversial (Houser 2014; Dobrev and Wehrens 2014). A small fraction of RyR2, presumably located at perinuclear dyads (Escobar et al. 2011), can be immunoprecipitated with mAKAP β and nesprin-1 α antibodies (Pare, Easlick, et al. 2005; Kapiloff, Jackson, and Airhart 2001). mAKAP β appears to bring together elements of the excitation-contraction coupling machinery and signaling molecules important for regulating nuclear events germane to pathological remodeling. Thus, mAKAP β complexes may provide one mechanism for matching contractility to the induction of hypertrophy. β -adrenergic stimulation of primary myocyte cultures results in increased PKA phosphorylation of mAKAP β -associated RyR2 (Pare, Bauman, et al. 2005). PKA-catalyzed RyR2 phosphorylation may potentiate local Ca $^{2+}$ release within the vicinity of mAKAP β signalosomes during states of elevated sympathetic stimulation.

[0025] While it is unlikely that the few mAKAP β -associated RyR2s could affect overall contractility, a potential target for increased perinuclear Ca $^{2+}$ may be the Ca $^{2+}$ /calmodulin-dependent phosphatase calcineurin (CaN) that can bind the scaffold. There are three isoforms of the catalytic subunit for CaN (α, β, γ], but only CaNA β -mAKAP β complexes have been detected in myocytes (Li et al. 2010). Remarkably, CaNA β is the CaNA isoform impor-

tant for the induction of cardiac hypertrophy in vivo, as well as for myocyte survival after ischemia (Bueno et al. 2002; Bueno et al. 2004). CaNA β binds directly to a unique site within mAKAP β (residues 1286-1345) (Pare, Bauman, et al. 2005; Li et al. 2010). CaNA β binding to mAKAP β is enhanced in cells by adrenergic stimulation and directly by Ca $^{2+}$ /calmodulin (Li et al. 2010). Notably, CaNA β -mAKAP β binding was required for α -adrenergic-induced neonatal myocyte hypertrophy in vitro (Li et al. 2010).

mAKAP β and Gene Expression

[0026] Among its many substrates, CaN is responsible for the activation of NFATc and MEF2 transcription factors. The NFATc transcription factor family includes four CaN-dependent isoforms that are all expressed in myocytes and that can contribute to the induction of myocyte hypertrophy (Wilkins et al. 2004). In general, NFATc family members are retained in the cytoplasm when heavily phosphorylated on the multiple serine-rich motifs within the N-terminal regulatory domain. NFATc translocates into the nucleus when these motifs are dephosphorylated by CaN. Multiple NFATc family members can bind mAKAP β , and binding to mAKAP β was required for CaN-dependent dephosphorylation of NFATc3 in myocytes (Li et al. 2010). Accordingly, mAKAP β expression was also required for NFAT nuclear translocation and transcriptional activity in vitro (Li et al. 2010; Pare, Bauman, et al. 2005). These results correlate with recent observations that NFAT-dependent gene expression in vivo was attenuated by mAKAP β cardiac-myocyte specific knock-out following transverse aortic constriction (Kritzer et al. 2014).

[0027] Like NFATc2 and NFATc3, MEF2D is a transcription factor required for cardiac hypertrophy in vivo (Kim et al. 2008; Wilkins et al. 2002; Bourajjaj et al. 2008). MEF2 family members contain a conserved DNA binding domain that includes both a MADS box and a MEF2 homology domain (Potthoff and Olson 2007). The DNA-binding domain of MEF2D binds directly to an N-terminal domain of mAKAP (Vargas et al. 2012; Kim et al. 2008). CaN and MEF2D are important not only in the heart, but also in skeletal muscle (Naya et al. 1999; Naya and Olson 1999; Black and Olson 1998; Friday et al. 2003; Wu et al. 2001). Interference with MEF2-mAKAP β binding blunted MEF2 transcriptional activity and the expression of endogenous MEF2 target genes in C2C12 skeletal myoblasts (Vargas et al. 2012). In addition, disruption of MEF2-mAKAP complexes attenuated the differentiation of C2C12 myoblasts into myotubes, as evidenced by decreased cell fusion and expression of differentiation markers (Vargas et al. 2012). Remarkably, CaN-MEF2 binding is mAKAP β -dependent in cardiac myocytes (Li, Vargas, et al. 2013). Accordingly, disruption of CaN-mAKAP β binding inhibited both MEF2 transcriptional activity in C2C12 cells and cardiac myocyte hypertrophy (Li, Vargas, et al. 2013). Like NFATc2, MEF2D de-phosphorylation in vivo in response to pressure overload was attenuated following mAKAP β conditional knock-out, correlating with the decreased expression MEF2-target genes, including the expression of atrial natriuretic factor (Kritzer et al. 2014).

[0028] The regulation of NFATc, MEF2 and HDAC4 by mAKAP β in vivo during pressure overload shows the importance of mAKAP β to stress-regulated gene expression (Kritzer et al. 2014). Published reports show how, at mAKAP β , NFATc and MEF2 are regulated by CaN, while HDAC4 and HDAC5 are regulated by PKD and PKA (Li,

Vargas, et al. 2013; Zhang et al. 2013; Li et al. 2010; Dodge-Kafka et al. 2018). mAKAP β appears to facilitate the modulation of these gene regulatory proteins by other signaling enzymes. For example, mAKAP β -associated ERK5 may phosphorylate MEF2, activating the transcription factor (Kato et al. 2000). In addition, PKA can phosphorylate MEF2, affecting its DNA-binding affinity (Wang et al. 2005). On the other hand, the Olson group has proposed that PKA phosphorylation of HDAC4 can inhibit MEF2 activity through the generation of a novel HDAC4 proteolytic fragment (Backs et al. 2011). How the activities of the many mAKAP β binding partners are ultimately integrated to control gene expression can be investigated both *in vitro* and *in vivo*.

Other mAKAP β Binding Partners

[0029] There are other binding partners for mAKAP β for whom the significance of docking to the scaffold remains poorly characterized, including myopodin and NCX1 (Faul et al. 2007; Schulze et al. 2003). HIF-1 α , a transcription factor that regulates systemic responses to hypoxia, also binds mAKAP β (Wong et al. 2008). Under normoxic conditions, the abundance of HIF-1 α in the cell is kept low by ubiquitin-mediated proteasomal degradation. HIF-1 α is hydroxylated by a family of oxygen-sensitive dioxygenases called prolyl hydroxylases (PHD1, PHD2, and PHD3) (Ohh et al. 2000). Hydroxylated HIF-1 α is subsequently recognized by the von Hippel-Lindau protein (pVHL), which recruits the Elongin C ubiquitin ligase complex to ubiquitinate HIF-1 α and to promote its proteasome-dependent degradation (Maxwell et al. 1999). Under hypoxic conditions, PHDs are inactivated, HIF-1 α degradation is decreased and HIF-1 α accumulates in the nucleus, where it can dimerize with HIF-1 β to promote the transcription of target genes. mAKAP β can assemble a signaling complex containing HIF-1 α , PHD, pVHL and the E3 ligase Siah2 (seven in absentia homolog 2) in cultured neonatal myocytes (Wong et al. 2008). Under normoxic conditions, mAKAP β -anchored PHD and pVHL favor HIF-1 α ubiquitination and degradation (Wong et al. 2008). Under hypoxic conditions, however, Siah2 activation induces proteasomal degradation of bound PHD, favoring HIF-1 α accumulation (Wong et al. 2008). An mAKAP β knock-out may affect cardiac myocyte survival after ischemia-reperfusion.

mAKAPf3—a Conductor of the Remodeling Symphony

[0030] The above discussion shows how multiple signaling pathways known to be important for cardiac hypertrophy and pathological remodeling are modulated by the binding of key signaling intermediates to the mAKAP β scaffold. Cardiac myocyte-specific, conditional mAKAP knock-out mouse has been characterized, showing the relevance of mAKAP β signalosomes in *vivo* (Kritzer et al. 2014). mAKAP β was required in cardiac myocytes for the induction of cardiac hypertrophy by transverse aortic constriction and isoproterenol infusion. Most remarkable, however, was the prevention of pathological remodeling, including myocardial apoptosis and interstitial fibrosis, and the preservation of cardiac function in the face of long-term pressure overload, together resulting in a significant increase in mouse survival (Kritzer et al. 2014). These results established mAKAP β as the first scaffold whose ablation confers a survival benefit in heart disease. Importantly, mAKAP β did not appear to be necessary for either the development or maintenance of normal adult cardiac function, as the use of a Nkx2-5-directed cre deleter line did not result in an overt

phenotype by six months of age (Kritzer et al. 2014). Although mAKAP β knock-out did attenuate the physiological hypertrophy induced by forced exercise (swimming), the targeting of mAKAP β complexes in disease remains relevant.

[0031] Various strategies for targeting mAKAP β complexes in humans may be envisioned, including siRNA knock-down of the scaffold. However, a relatively detailed understanding of the structure and function of mAKAP β signalosomes provides us with additional approaches to targeting these pathways. For example, the expression of peptides targeting key protein-protein interactions involving mAKAP β has already been shown to be effective *in vitro*, including anchoring disruptor peptides targeting mAKAP β -CaNA β , mAKAP β -MEF2D, mAKAP β -PLC ϵ , and mAKAP β -RSK3 binding (Li, Vargas, et al. 2013; Li, Kritzer, et al. 2013; Vargas et al. 2012; Zhang et al. 2011). A leading cause of death, heart failure is a disease that incurs 50% mortality within 5 years of diagnosis despite modern therapy, at a cost of over \$30 billion/year in the USA alone (Go et al. 2014). Many candidates for potential targeting in cardiac disease are pleiotropic, complicating the development of drugs with sufficient specificity *in vivo*. The specific targeting of mAKAP β signalosomes provides an opportunity to target relatively rare protein-protein interactions that appear to be dedicated to pathological cardiac remodeling and whose ablation may be promoted without significant side-effects. There is a clear need to develop new effective therapies to treat patients with heart failure, as well as to prevent its development in the context of other cardiovascular diseases such as coronary artery disease, hypertension, and valvular disease.

SUMMARY OF THE INVENTION

[0032] The following brief summary is not intended to include all features and aspects of the present invention, nor does it imply that the invention must include all features and aspects discussed in this summary.

[0033] The present inventors have discovered methods of treating cardiac pathological processes by inhibiting the signaling properties of individual mAKAP signaling complexes using drugs that target unique protein-protein interactions. Such a therapeutic strategy offers an advantage over classical therapeutic approaches because it allows the selective inhibition of defined cellular responses.

[0034] In particular, the present inventors have found that disrupting mAKAP-mediated protein-protein interactions can be used to inhibit the ability of mAKAP to coordinate the activation of enzymes that play a central role in activating key transcription factors that initiate cellular processes leading to pathological cardiac remodeling.

[0035] Specifically, the inventors have discovered that inhibiting the binding interaction between PP2A and mAKAP β can protect the heart from damage leading to heart failure, for example, following myocardial infarction.

[0036] Thus, the present invention comprises, in certain aspects a method for protecting the heart from damage, by administering to a patient at risk of such damage, a pharmaceutically effective amount of a composition which inhibits the interaction of PP2A and mAKAP β .

[0037] The invention also relates to a method of treating heart disease, by administering to a patient a pharmaceutically effective amount of a composition which inhibits the interaction of PP2A and mAKAP β .

[0038] The invention also relates to compositions which inhibit the interaction of PP2A and mAKAP β .

[0039] In still other embodiments, the inhibitors include any molecule that inhibits the expression or activity of PP2A and mAKAP β .

[0040] The foregoing and other objects, features and advantages of the invention will be apparent from the following more particular description of preferred embodiments of the invention, as illustrated in the accompanying drawings in which like reference characters refer to the same parts throughout the different views. The drawings are not necessarily to scale, emphasis instead being placed upon illustrating the principles of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

[0041] The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

[0042] FIG. 1. Model for mAKAP β -regulated, SRF-dependent gene expression. Anchored RSK3 is a Gq-protein coupled receptor-ERK effector that phosphorylates SRF associated with perinuclear mAKAP β complexes. mAKAP β -anchored PP2A that can be activated by cAMP-dependent protein kinase A (PKA) opposes SRF phosphorylation. Phosphorylated SRF induces gene expression that promotes concentric hypertrophy.

[0043] FIG. 2. Shows the amino acid sequence of human RSK3 (SEQ ID NO: 1).

[0044] FIG. 3. Shows the amino acid sequence of rat mAKAP (SEQ ID NO: 2).—Note that within this document, references to mAKAP sequences, whether labelled “mAKAP β ” or “mAKAP” are according to the numbering for the mAKAP α alternatively-spliced form which contains within the entirety of mAKAP β and is identical to the originally published mAKAP sequence as shown in this figure (Kapiloff 1999, Michel 2005). “mAKAP” is also referred to as “AKAP6” in reference databases and the literature. mAKAP β starts at residue 245, while mAKAP α starts at residue 1. PP2A binding domain starts at residue 2134.

[0045] FIG. 4. Amino acid sequence of rat mAKAP PBD as expressed in AAV vector. Includes N-terminal myc tag (SEQ ID NO: 12).

[0046] FIG. 5. Sequence for pscA-TnT-myc-rat mAKAP PBD plasmid used to generate AAV9sc.rat PBD (SEQ ID NOs: 13 and 14).

[0047] FIG. 6. mAKAP β —A Perinuclear Scaffold. A. Top montage: Mouse heart sections (left ventricle) stained for with mAKAP antibody (gray scale panels and green), Hoechst nuclear stain (blue), and wheat germ agglutinin (red, shown in enlarged control image only). Lower left panels are from control, mAKAP knock-out mice. Bar=20 μ m. Bottom montage: Adult rat myocyte stained with antibodies to mAKAP (green) and actinin (red). B. mAKAP domain structure. Direct binding partners whose sites have been finely mapped in mAKAP β are shown. mAKAP β starts at residue 245 of mAKAP α . Therefore, all binding sites are numbered per mAKAP α . Images are from Kritzer, et al. (Kritzer et al. 2014).

[0048] FIG. 7. mAKAP β Signaling Modules. mAKAP β binds multiple signaling enzymes and gene regulatory proteins. Modules may be defined that involve cAMP, Ca²⁺,

hypoxic, phosphatidylinositol and MAPK signaling. See above for details. In this figure, the mAKAP β scaffold is presented as a yellow globe sitting on a grey base representing nesprin-1 α , on which are assembled the various signaling molecules. Gold cylinders represent nuclear pore complexes inserted in the nuclear envelope.

[0049] FIG. 8. An okadaic acid-sensitive phosphatase regulates mAKAP-associated PDE4D3. A, transfected HEK293 cells expressing both mAKAP and PDE4D3 were treated with either 300 μ M okadaic Acid (OA) or 500 μ M cyclosporine A (CsA) for 30 min before stimulation with 5 μ M forskolin (Fsk) for 10 min. The phosphorylation state of PDE4D3 present in mAKAP antibody immunoprecipitates was determined using a antibody specific for phosphorylated PDE4D3 Ser-54 (top panel). Total PDE4D3 (middle panel) and mAKAP (bottom panel) present in mAKAP antibody immunoprecipitates were detected using non-phospho-specific antibodies. Note that in these experiments mAKAP was GFP-tagged and PDE4D3 was VSV and GFP-tagged, resulting in increased molecular weights. n=3 B, PDE activity associated with mAKAP antibody immunoprecipitates prepared as in A was assayed using [³H]cAMP substrate. *p<0.05 compared to untreated cells (bar 1). C, endogenous protein complexes were isolated using control (IgG) or mAKAP-specific antibodies from clarified adult rat heart extracts (500 μ g total protein). PDE activity associated with the immunoprecipitates was assayed in the presence of 10 nM OA or 50 nM PKI. n=3; *p<0.05.

[0050] FIG. 9. The protein phosphatase PP2A is associated with the mAKAP scaffold in adult rat heart. A, phosphatase activity associated with protein complexes immunoprecipitated using mAKAP antibody from adult rat heart extracts (500 μ g total protein) was assayed using ³²P-labelled histone substrate in the absence or presence of 30 nM PP2A Inhibitor I (Li, Makkinje, and Damuni 1996) and 100 nM PKA-phosphorylated PP1 Inhibitor-1 (Endo et al. 1996). n=3. *p<0.05. B & C, protein complexes were isolated from adult rat heart extracts (2 mg total protein) using control (IgG) or mAKAP-specific antibody. PP2A (panel B) and PP1 (panel C) catalytic subunits in extracts (80 μ g) and immunoprecipitates (25% loaded) were detected by immunoblotting. n=3.

[0051] FIG. 10. PP2A binds a C-terminal mAKAP domain. A, schematic of mAKAP domains and GFP- and myc-tagged mAKAP proteins used in this paper. mAKAP fragments containing rat and human protein are drawn in black and grey, respectively. Hatched bars indicate the three spectrin repeat domains responsible for nuclear envelope targeting in myocytes (Kapiloff et al. 1999a). Binding sites are indicated for proteins known to bind mAKAP directly, including 3-phosphoinositide-dependent kinase-1 (PDK1, mAKAP residues 227-232) (Michel et al. 2005b), nesprin-1 α (1074-1187) (Pare, Easlick, et al. 2005), ryanodine receptor (RyR2, 1217-1242) (Marx et al. 2000), PP2B (1286-1345) (Li et al. 2009), PDE4D3 (1285-1833) (Dodge et al. 2001), and PKA (2055-2072) (Kapiloff et al. 1999a). The stippled bar marks the PP2A binding site. The first and last residues of each fragment are indicated. B, purified GST-PP2A A subunit fusion protein was incubated with extracts prepared from HEK293 cells expressing the indicated GFP-mAKAP fusion protein and pulled down using glutathione resin. GFP-mAKAP fragments were detected in the pull-downs (25% loaded, top panel) and the extracts (5% loaded, bottom pane) using a GFP antibody. n=3. C, myc-

tagged mAKAP fragments were expressed in HEK293 cells, and phosphatase binding was detected by immunoprecipitation using control (IgG) or myc-tag antibody followed by phosphatase assay using 32 P-labelled histone substrate. n=3.

*p<0.05 compared to the other samples. Note that the C-terminal homologous domain of both rat and human mAKAP binds PP2A.

[0052] FIG. 11. PP2A association with mAKAP-PDE4D3 complexes is required for inhibition of PDE4D3 phosphorylation. A, HEK293 cells expressing (VSV and GFP-tagged) PDE4D3 and myc-tagged mAKAP 1286-2312 or 1286-2083 lacking the PP2A binding site were treated with 300 μ M OA for 30 minutes before stimulation with 5 μ M Fsk for 10 minutes. Protein complexes were immunoprecipitated using myc-tag antibody in the presence of phosphatase inhibitors. The phosphorylation state of co-immunoprecipitated PDE4D3 was determined using an antibody specific for phosphorylated PDE4D3 Ser-54 (P-PDE4D3, top panel). Total PDE4D3, myc-mAKAP, and PP2A C-subunit present in the immunoprecipitates were detected using non-phospho-specific antibodies (lower three panels). n=3. B, PDE activity associated with myc-antibody immunoprecipitates isolated from additional cells treated as in A was assayed using [3 H]cAMP. n=3. *p<0.05 compared to bar 1.

[0053] FIG. 12. mAKAP-bound PP2A contains B56 δ -subunit and is cAMP-activated. A, protein complexes were immunoprecipitated from adult rat heart extracts (500 μ g total protein) using control (IgG) or mAKAP-specific antibody as in FIG. 9B and assayed for associated phosphatase activity. As indicated, the immunoprecipitates were pre-incubated with no addition or with 50 μ M CPT-cAMP, 10 nM OA, or 50 nM PKI for 5 minutes before addition of [32 P]histone substrate. n=3. *p<0.05. B, Endogenous protein complexes were immunoprecipitated from adult heart extract (2 mg total protein) with B56 δ and control (IgG) antibodies. mAKAP in 80 μ g extract and in the immunoprecipitates (25% loaded) was detected by immunoblot. n=3. C, Flag-tagged B56 δ and/or GFP-tagged mAKAP were expressed in HEK293 cells. Protein complexes were immunoprecipitated using a mAKAP antibody. B56 δ in the immunoprecipitates (25% loaded) and total extracts (5% loaded) was detected by immunoblotting with a Flag antibody. n=3. D, phosphatase activity associated with mAKAP-antibody immunoprecipitates prepared as in C was assayed using 32 P-labelled histone substrate. n=3. E, HEK293 cells expressing mAKAP and B56 δ were treated with 5 μ M Fsk and 10 μ M IBMX (Fsk/IBMX) for 10 min before immunoprecipitation of protein complexes with mAKAP antibody. Phosphatase activity associated with the immunoprecipitates was assayed using [32 P]histone substrate. n=3. Note that PP2A B56 δ and C-subunit binding to mAKAP was not affected by Fsk/IBMX (see FIG. 13 below).

[0054] FIG. 13. Phosphorylation of B56 δ by PKA increases mAKAP-associated PP2A activity. A, B56 δ is phosphorylated on serine residues 53, 68, 81, and 566 by PKA (Ahn et al. 2007). B56 δ wildtype or alanine substituted at all four PKA sites (S4A) was co-expressed in HEK293 cells with wildtype mAKAP or a full-length mAKAP mutant lacking the PKA binding site (ΔPKA; FIG. 10A). After stimulation with 5 μ M Fsk and 50 μ M IBMX, protein complexes were immunoprecipitated with mAKAP antibody, and associated proteins were detected by immunoblotting with B56 δ , mAKAP, and PP2A-C antibodies (lower three panels). PKA phosphorylation of B56 δ was detected

by immunoblotting with a B56 δ phospho-Ser-566 specific antibody (P-B56 δ , upper panel). n=3. B, Immunoprecipitates prepared as in B were assayed for associated phosphatase activity. n=3. *p<0.05.

[0055] FIG. 14. Phosphorylation of B56 δ by PKA enhances the dephosphorylation of mAKAP-associated PDE4D3. A, HEK293 cells expressing (GFP-tagged) mAKAP, (VSV- and GFP-tagged) PDE4D3 and either wild-type B56 δ or B56 δ S4A mutant at the PKA phosphorylation sites were treated as indicated with 300 μ M OA for 30 min before stimulation for 10 min with 5 μ M Fsk. Protein complexes were immunoprecipitated with mAKAP antibody in the presence of phosphatase inhibitors. The phosphorylation state of PDE4D3 present in the immunoprecipitates was determined using an antibody specific for phosphorylated PDE4D3 Ser-54 (top panel). Total PDE4D3, mAKAP, B56 δ and PP2A-C protein present in the immunoprecipitates were detected using non-phospho-specific antibodies (lower four panels). n=3. B, PDE activity associated with protein complexes isolated from additional cells treated as in A was assayed using [3 H]cAMP. n=3. *p<0.05 compared to bar 1.

[0056] FIG. 15. PKA and PP2A associated with mAKAP complexes coordinately regulate PDE4D3 activity and cAMP degradation. PKA is composed of two regulatory and two catalytic subunits. mAKAP-bound PP2A contains an A, B56 δ , and C (catalytic) subunits. A, in unstimulated cells, basal PP2A activity maintains PDE4D3 dephosphorylation, presumably allowing for a more rapid rise in cAMP levels in response to subsequent agonist than if PDE4D3 were phosphorylated and activated. At the same time, basal PDE4D3 activity should maintain low local levels of cAMP, preventing spurious signaling. B, G_s-coupled receptor stimulation induces cAMP synthesis, exceeding the rate of cAMP degradation by PDE4D3 and activating mAKAP-bound PKA. PKA phosphorylates and activates both PDE4D3 and PP2A. PDE4D3 activation should limit peak cAMP levels, as well as accelerate the rate of cAMP clearance after GPCR down-regulation. In contrast, PP2A activation opposes PDE4D3 phosphorylation by PKA, attenuating cAMP degradation and contributing to greater, longer lasting cAMP signals.

[0057] FIG. 16. Confirmation that PKA-phosphorylated I-1 inhibits PP1 activity. Protein complexes were immunoprecipitated from rat heart extracts with PP1 or control IgG antibody, and associated phosphatase activity was assayed using [32 P]histone substrate in the absence or presence of 100 nM PKA-phosphorylated PP1 Inhibitor-1 (Endo et al. 1996). n=3.

[0058] FIG. 17. Distribution of mAKAP and PP2A catalytic subunit in rat neonatal cardiac myocytes. Rat neonatal ventricular myocytes were isolated as previously described (Pare, Easlick, et al. 2005). After treatment with 50 μ M phenylephrine for one week to induce myofibrillar organization and mAKAP expression, the cells were fixed and stained with 0.25 μ g/ml mouse anti-PP2A-C(green), 0.1 μ g/ml OR010 rabbit anti-mAKAP (red) affinity purified antibodies and rhodamine phalloidin (blue in composite image) to show actin myofibrils as previously described (Pare, Easlick, et al. 2005). 4-color Images were acquired on a Zeiss LSM510/UV Confocal Microscope at 400x. Separate PP2A C-subunit and mAKAP images are shown for clarity. PP2A-C subunit was present in a diffuse punctuate pattern in the cytosol, while mAKAP was limited to the

location of the nuclear envelope. The presence of PP2A-C subunit staining over the nuclear envelope is consistent with the presence of PP2A-mAKAP complexes (yellow in composite image). Control IgG staining is shown in the right panel. n=3.

[0059] FIG. 18. mAKAP Fragments do not bind PP1 in HEK293 cells. mAKAP-GFP fusion proteins were expressed in HEK293 cells and protein complexes were immunoprecipitated with PP1 antibody. Despite robust expression (bottom panels), no mAKAP fusion proteins were precipitated with the PP1 antibody. n=3.

[0060] FIG. 19. SRF phosphorylation is regulated by mAKAP β signalosomes in cardiac myocytes. (A) SRF Domain Structure. Known phosphorylated residues are indicated (Li et al. 2014; Mack 2011; Janknecht et al. 1992). (B) Neonatal rat ventricular myocytes (NRVM) transiently transfected with siRNA and SRE-luciferase and control renilla luciferase plasmids. Normalized luc:rluc ratios are shown. n=3. (C) Co-immunoprecipitation of endogenous complexes from mouse heart extracts. n=3. (D) HA-tagged RSK3 WT or S218A inactive mutant (Li, Kritzer, et al. 2013) and/or myc-mAKAP β were expressed in COS-7 cells for co-immunoprecipitation assay. n=3. (E) NRVM extracts obtained 2 days after transfection with siRNA+/-10 μ M PE. n=3. * vs. control siRNA+PE; † vs. control siRNA+no drug. (F) Adult rat ventricular myocytes (ARVM) infected with adenovirus expressing myc-GFP or myc-GFP-RBD and treated for 1 day with 20 μ M PE. n=3. * vs. myc-GFP+PE; † vs. myc-GFP+no drug. (G) NRVM in minimal maintenance media were treated for 1 hour with 1 μ M okadaic acid (OA) or 1 μ g/ml cyclosporine A (CsA). n=4. * vs. no drug control. (H) NRVM transfected with control or mAKAP siRNA were used for co-immunoprecipitation assay. PP2A holoenzyme contains an A- and C-subunit homodimer core and a scaffolding B-subunit (Dodge-Kafka et al. 2010). PP2A C-subunit (PP2A-C) was detected by immunoblot. n=3. (I) NRVM infected with adenovirus expressing myc-PBD or β -gal before co-immunoprecipitation assay. n=3. (J) ARVM infected with myc-PBD or β -gal adenoviruses and treated for 1 day with 10 μ M Iso. n=4. * vs. β -gal+Iso; † vs. β -gal+no drug.

[0061] FIG. 20. SRF S¹⁰³ phosphorylation is a determinant of myocyte concentric growth. Adult rat ventricular myocytes (ARVM) were infected with adenovirus and cultured for 24 hours +/-20 μ M PE or 10 μ M Iso before immunocytochemistry and measurement of cell width and length (maximum dimension parallel or perpendicular to striations; bars=25 μ m). (A,B) Myocytes were infected with adenovirus expressing either β -gal (control) or HA-tagged RSK3 and maintained in minimal media. Top: α -actinin—red, nuclei—blue, HA-RSK3—green; bottom HA-RSK3—greyscale. n=4. (C-F) Myocytes were infected with adenovirus expressing SRF WT, S103D, S103A or control virus. Flag-SRF—green, α -actinin—red, nuclei—blue. * vs. no drug for same virus; † vs. control under the same treatment condition; ‡ vs. SRF WT under the same treatment condition. D: n=3; F: n=5. (G,H) Myocytes were infected with adenovirus expressing myc-GFP or myc-GFP-RBD (green). (I,J) Myocytes were infected with adenovirus expressing myc-PBD or j-gal control. (G-J) α -actinin—red, nuclei—blue. * vs. no drug control for same protein; † vs. control protein with same treatment condition. n=4.

[0062] FIG. 21. PP2A dephosphorylates SRF S¹⁰³. GST-SRF fusion protein purified from bacterial extracts and on

glutathione beads was incubated with purified 0.5 μ g RSK3 (Millipore) for 30 minutes before washing twice with PP2A reaction buffer and then incubating for 30 min with 50 ng purified PP2A +/-10 nM okadaic acid.

[0063] FIG. 22. AAV9sc.myc-PBD. A. AAV9sc.myc-PBD includes a minigene that expresses the myc-tagged rat PDB peptide (rat mAKAP aa 2134-2314) and a defective right ITR, conferring self-complementarity and presumably decreasing the latency and increasing the efficacy of expression. (Andino et al., 2007). The AAV has the cardiotrophic serotype 9 capsid protein and directs expression of the encoded protein under the control of the cardiac myocyte-specific, chicken troponin T promoter (cTnT). (Prasad et al., 2011) B. Shuttle plasmid for AAV9sc.myc-PBD.

[0064] FIG. 23. PBD anchoring disruptor therapy. (A) myc-tagged rat mAKAP PBD (AAV9sc.myc-PBD) and myc-GFP (AAV9sc.GFP) were expressed in mice using a self-complementary AAV9 and the cardiac myocyte-specific chicken troponin T promoter. (Prasad et al., 2011) (B) Timeline for AAV9sc.myc-PBD treatment study shown in C-H. Mice were 8 weeks old at initiation of study. (C) Representative whole heart pictures at endpoint. Bar=5 mm. (D-H) Serial M-mode echocardiography. n: AAV9sc.myc-PBD—8 (green); AAV9sc.GFP—5 (black). * p-value for difference in cohorts at given time point. LV Remodeling Index=Mass+End-diastolic volume. LVAW; d—left ventricular anterior wall thickness in diastole.

[0065] FIG. 24. Nucleotide sequence of human RSK3 (SEQ ID NO: 15).

[0066] FIG. 25. Nucleotide sequence of rat mAKAP α mRNA with open reading frame translated (SEQ ID NOs: 2 and 16).

[0067] FIG. 26. Nucleotide sequence of human mAKAP β mRNA with open reading frame translated (SEQ ID NOs: 17 and 18).

[0068] FIG. 27. Nucleotide sequence of human mAKAP α mRNA with open reading frame translated (SEQ ID NOs: 19 and 20).

[0069] FIG. 28. Amino acid sequence of human mAKAP. mAKAP α starts at residue 1, mAKAP β at residue 243. PBD in bold (SEQ ID NO: 8).

[0070] FIG. 29. Amino acid sequence of human PBD as expressed in AAV (SEQ ID NO: 9).

[0071] FIG. 30. Alignment of human and rat PBD amino acid sequences as expressed by AAV species (SEQ ID NOs: 9 and 12). Rat PBD has an N-terminal Myc-tag [EQKLI-SEEDL, (SEQ ID NO: 21), FIG. 4]. The consensus sequence is represented by SEQ ID NO:22 or SEQ ID NO:23.

[0072] FIG. 31. Map of human PBD shuttle plasmid.

[0073] FIG. 32. Nucleotide sequence of pscAAV-hmAKAP PBD plasmid (SEQ ID NOs: 10 and 11).

[0074] FIG. 33. SRF phosphorylation is decreased in dilated hearts. (A-E) Mouse ventricular protein extracts were assayed for phosphorylated and total SRF 5 min (acute pressure overload, n=4,4) or 16 weeks (heart failure, n=15, 19) following TAC or sham survival surgery. (A) Representative western blots. (B) Densitometry of top panel in A. (C) After 5 min of pressure overload, RSK3 was immunoprecipitated using N-16 RSK3 specific antibody and detected using OR43 RSK3 antibody and a phospho-specific antibody for RSK3 S²¹⁸ that indicates RSK3 activation. The immunoprecipitation-western assay was validated using RSK3^{-/-} mice (not shown). n=3 for each condition. (D) 16 weeks of pressure overload induced heart failure. M-mode

echocardiography for left ventricular (LV) volume in diastole and systole and ejection fraction showed that TAC hearts were dilated and had systolic dysfunction. Measurement of wet lung weight (indexed to tibial length) indicating the presence of pulmonary edema showed that TAC mice were in heart failure. (E) Densitometry of bottom panel in A. (F-H) Left ventricular tissue from human patients (including nonischemic and ischemic cardiomyopathies and non-dilated congenital heart disease and controls) were assayed for SRF S¹⁰³ phosphorylation and segregated by normal (<5.3 cm, n=7) or elevated (>5.3 cm, n=8) left ventricular interior diameter in diastole (LVID; d). Equal loading for blots was confirmed using Ponceau S stain for major protein bands (not shown).

DETAILED DESCRIPTION OF THE INVENTION

[0075] As discussed above, AKAP-based signaling complexes play a central role in regulating physiological and pathological cardiac events. As such, the present inventors have examined inhibiting the signaling properties of individual AKAP signaling complexes using drugs that target unique protein-protein interactions as an approach for limiting cardiac pathological processes. Such a therapeutic strategy offers an advantage over classical therapeutic approaches since it allows the selective inhibition of defined cellular responses.

[0076] Anchoring proteins including mAKAP are therapeutic targets for the treatment of cardiac hypertrophy and heart failure. In particular, the present inventors have found that disrupting AKAP-mediated protein-protein interactions can be used to inhibit the ability of mAKAP to coordinate the activation of enzymes that play a central role in activating key transcription factors that initiate the remodeling process leading to cardiac hypertrophy.

[0077] One aspect of the current invention is that improved ventricular geometry, i.e. decreased LV internal diameters due to less elongated myocytes and/or increased LV wall thickness due to wider myocytes, will decrease wall stress (Law of LaPlace) and improve systolic function in the heart prone to HFrEF. Demonstration of the prevention of systolic dysfunction has been obtained for a new gene therapy vector based upon expression of a muscle A-kinase anchoring protein (mAKAP, a.k.a. AKAP6)-derived anchoring disruptor peptide for protein phosphatase 2A (PP2A).

[0078] As discussed below, the inventors have recently discovered that the transcription factor serum response factor (SRF) is Ser¹⁰³ phosphorylated in the cardiac myocyte by RSK3 at mAKAPβ signalosomes where SRF may in turn be dephosphorylated by protein phosphatase 2A (PP2A) bound to the scaffold. Methods to block the eccentric changes in ventricular morphology that typify end-stage disease and HFrEF are the subject of this invention.

[0079] While previously thought to be a constitutive, house-keeping enzyme, it has become apparent that protein phosphatase 2A (PP2A) contributes to the regulation of many phosphorylation events. For example, in the cardiac myocyte, PP2A is involved in the modulation of calcium and MAPK signaling (duBell, Lederer, and Rogers 1996; duBell et al. 2002; Liu and Hofmann 2004). PP2A is a serine/threonine phosphatase that exists as a heterotrimeric complex consisting of a stable, ubiquitously expressed catalytic (PP2A-C) and scaffolding (PP2A-A) subunit heterodimer, and one of 21 known divergent B subunits (Lechward et al.

2001; Wera and Hemmings 1995). PP2A B subunits are grouped into three unrelated families termed B (or PR55), B' (or B56) and B'' (or PR72) and are proposed to regulate both the catalytic activity and the intracellular targeting of the phosphatase (Virshup 2000). The present inventors have previously shown by reconstitution of mAKAP complexes in heterologous cells that protein phosphatase 2A (PP2A) associated with mAKAP complexes can reverse the activation of PDE4D3 by catalyzing the dephosphorylation of PDE4D3 serine residue 54 (Dodge-Kafka et al. 2010). Mapping studies revealed that a C-terminal mAKAP domain (residues 2085-2319) bound PP2A (Dodge-Kafka et al. 2010). Binding to mAKAP was required for PP2A function on PDE4D3, such that deletion of the C-terminal domain enhanced both baseline and forskolin-stimulated PDE4D3 activity. Interestingly, PP2A holoenzyme associated with mAKAP complexes in the heart contains the PP2A targeting subunit B56δ (Dodge-Kafka et al. 2010). Like PDE4D3, B56δ is a PKA substrate, and PKA phosphorylation of mAKAP-bound B56δ enhanced phosphatase activity 2-fold in the complex. Accordingly, expression of a B56δ mutant that could not be phosphorylated by PKA in heterologous cells with mAKAP resulted in increased PDE4D3 phosphorylation. Taken together, these findings demonstrated that PP2A associated with mAKAP complexes may promote PDE4D3 dephosphorylation, serving to both inhibit PDE4D3 in unstimulated cells and also to mediate a cAMP-induced positive feedback loop following adenylyl cyclase activation and B56δ phosphorylation. Thus PKA-PDE4D3-PP2A-mAKAP complexes exemplify how protein kinases and phosphatases may participate in molecular signaling complexes to dynamically regulate localized intracellular signaling. The relevance to cardiac myocyte function and any potential therapeutic significance were not defined in prior studies (Dodge-Kafka et al. 2010).

[0080] The present inventors now disclose a new mechanism of action for mAKAPβ-bound PP2A in the cardiac myocyte and the therapeutic implications of this mechanism. The inventors show that the transcription factor SRF is phosphorylated at Ser¹⁰³ by mAKAPβ-bound RSK3 (FIG. 19) and that SRF phosphorylation at Ser¹⁰³ constitutes an epigenetic switch promoting concentric cardiac myocyte hypertrophy (FIG. 20). Importantly, it is disclosed that SRF Ser¹⁰³ can be dephosphorylated by PP2A bound to the mAKAPβ scaffold (FIGS. 19 and 21). SRF Ser¹⁰³ phosphorylation is shown to induce concentric myocyte hypertrophy (FIG. 20). These findings constitute the discovery of a novel mechanism for the regulation of cardiac myocyte morphology and an unexpected function for mAKAPβ-bound PP2A. In particular, the inventors disclose that consistent with the role of PP2A as a phosphatase for mAKAPβ-bound SRF, displacement of PP2A from mAKAPβ in vitro will promote SRF Ser¹⁰³ phosphorylation in cardiac myocytes (FIG. 19) and concentric cardiac myocyte hypertrophy (FIG. 20) and in vivo will provide protection against the development of systolic dysfunction after myocardial infarction in mice (FIG. 23).

[0081] Inhibition of PP2A binding to mAKAPβ can be achieved by expression of a competing peptide comprising rat mAKAPβ 2134-2314 (FIG. 19) or 2132-2319 of human mAKAPβ, representing a new refinement in the mapping of the PP2A binding site on mAKAPβ and the first demonstration for heart disease in vivo of the inhibition of mAKAP-PP2A binding. Note that the C-terminal domain of human

mAKAP homologous to that in rat mAKAP was also shown to bind PP2A (FIG. 10). Therefore the human sequence (human mAKAP amino acid residues homologous 2132-2319) to rat mAKAP 2134-2314 shown in FIGS. 28-30 is also expected to bind PP2A and constitute a PP2A-mAKAP binding competing peptide.

[0082] Effective delivery of PP2A anchoring disruptor peptides via viral-based gene therapy vectors are demonstrated by efficacy in the mouse infarction model (FIG. 23). Alternatively, delivery of such peptides that might inhibit PP2A-mAKAP β interaction can be enhanced by the use of cell-penetrating sequences such as the transactivator of transcription peptide and polyarginine tails, or conjugation with lipid-derived groups such as stearate. Stability may also be enhanced by the use of peptidomimetics [i.e., peptides with structural modifications in the original sequence giving protection against exo- and endoproteases without affecting the structural and functional properties of the peptide.]

[0083] The inventors have also found that small molecule disruptors can be used to target specific interaction within AKAP-based complexes. Small molecule disruptors can be identified by combining rational design and screening approaches. Such compounds can be designed to target-specific binding surfaces on AKAPs, to disrupt the interaction between AKAPs and PP2A in cardiomyocytes and to enhance the contractility of intact hearts for the treatment of chronic heart failure.

[0084] The present invention relates to methods of treating any cardiac condition which is initiated through the interaction of PP2A and mAKAP β . Such cardiac dysfunction can result in signs and symptoms such as shortness of breath and fatigue, and can have various causes, including, but not limited to hypertension, coronary artery disease, myocardial infarction, valvular disease, primary cardiomyopathy, congenital heart disease, arrhythmia, pulmonary disease, diabetes, anemia, hyperthyroidism and other systemic diseases.

[0085] In accordance with the present invention there may be employed conventional molecular biology, microbiology, and recombinant DNA techniques within the skill of the art. Such techniques are explained fully in the literature. See, e.g., Sambrook et al, "Molecular Cloning: A Laboratory Manual" (4th Ed., 2012); "Current Protocols in Molecular Biology" Volumes I-III [Ausubel, R. M., ed. (1994)]; "Cell Biology: A Laboratory Handbook" Volumes I-III [J. E. Celis, 3rd ed. (2005))]; "Current Protocols in Immunology" Volumes I-III [Coligan, J. E., ed. (2005)]; "Oligonucleotide Synthesis" (M. J. Gait ed. 1984); "Nucleic Acid Hybridization" [B. D. Hames & S. J. Higgins eds. (1985)]; "Transcription And Translation" [B. D. Hames & S. J. Higgins, eds. (1984)]; "Animal Cell Culture" [R. I. Freshney, ed. (1986)]; "Immobilized Cells And Enzymes" [IRL Press, (1986)]; B. Perbal, "A Practical Guide To Molecular Cloning" (1984); C. Machida, "Viral Vectors for Gene Therapy: Methods and Protocols" (2010); J. Reidhaar-Olson and C. Rondinone, "Therapeutic Applications of RNAi: Methods and Protocols" (2009).

[0086] The following definitions and acronyms are used herein:

- [0087] AC5—adenylyl cyclase type 5
- [0088] ACE—angiotensin-converting enzyme
- [0089] ANF atrial natriuretic factor
- [0090] ARVM—adult rat ventricular myocyte
- [0091] CaN—calcineurin
- [0092] CArG box—CC(A/T)₆GG
- [0093] CPT-cAMP—8-(4-chlorophenylthio)adenosine 3',5'-cyclic monophosphate
- [0094] CsA—cyclosporin A
- [0095] CTKD—C-terminal kinase domain
- [0096] ERK—extracellular signal-regulated kinase
- [0097] FBS—fetal bovine serum
- [0098] Fsk—forskolin
- [0099] GFP—green fluorescent protein
- [0100] GPCR—G-protein coupled receptor; HDAC—histone deacetylase
- [0101] Gs—stimulatory G protein
- [0102] GST—glutathione-S-transferase; HIF1 α —hypoxia-inducible factor 1 α
- [0103] HFrEF—heart failure with reduced ejection fraction
- [0104] IBMX—3-isobutyl-1-methylxanthine
- [0105] Iso—isoproterenol
- [0106] LIF—leukemia inhibitory factor
- [0107] MADS—(MCM1, agamous, deficiens, SRF) domain—mediates DNA binding to CArG box CC(A/T)₆GG serum response elements (SRE); the MADS-box gene family got its name later as an acronym referring to the four founding members, ignoring ARG80:
- [0108] MCM1 from the budding yeast, *Saccharomyces cerevisiae*,
- [0109] AGAMOUS from the thale cress *Arabidopsis thaliana*,
- [0110] DEFICIENS from the snapdragon *Antirrhinum majus*, [10]
- [0111] SRF from the human *Homo sapiens*.
- [0112] mAKAP—muscle A—kinase anchoring protein
- [0113] mAKAP α —alternatively spliced isoform expressed in neurons; 255 kDa
- [0114] mAKAP β —alternatively spliced isoform expressed in striated myocytes; 230 kDa
- [0115] MAPK—mitogen-activated protein kinase
- [0116] MEF2—myocyte enhancer factor-2
- [0117] MgAc—magnesium acetate
- [0118] MI—myocardial infarction
- [0119] NCX1—sodium/calcium exchanger
- [0120] NFATc—nuclear factor of activate T-cell
- [0121] NRVM—neonatal rat ventricular myocyte
- [0122] NTKD—N-terminal kinase domain
- [0123] OA—Okadaic acid
- [0124] PBD—“PP2A binding domain” of mAKAP that binds PP2A and that when expressed attenuates eccentric hypertrophy
- [0125] PDE4D3—cAMP-specific phosphodiesterase type 4D3
- [0126] PDK1—3'phosphoinositide-dependent kinase 1
- [0127] PE—phenylephrine
- [0128] PHD—prolyl hydroxylase
- [0129] PI4P—phosphatidylinositol-4-phosphate
- [0130] PKA—protein kinase A
- [0131] PKD—protein kinase D
- [0132] PKI—protein kinase inhibitor
- [0133] PLC ϵ —phospholipase C ϵ
- [0134] PKA—cAMP-dependent protein kinase
- [0135] PP2A—protein (serine-threonine) phosphatase—dephosphorylates SRF Ser¹⁰³
- [0136] PP2B—calcium/calmodulin-dependent protein phosphatase 2B

[0137] RBD—isoform-specific N-terminal RSK3 domain binds a discrete “RSK3-binding domain” within mAKAP β at residues 1694-1833 (RBD)

[0138] RSK—p90 ribosomal S6 kinase

[0139] RyR2—type 2 ryanodine receptor

[0140] siRNA—small interfering RNA oligonucleotide

[0141] shRNA—short hairpin RNA

[0142] SRE—serum response elements

[0143] SRF—serum response factor—transcription factor (SRF Ser¹⁰³ phosphorylation induces concentric myocyte and cardiac hypertrophy)

[0144] siRNA—small interfering RNA

[0145] TAC transverse aortic constriction

[0146] TCA—trichloroacetic acid

[0147] VSV—vesicular stomatitis virus

[0148] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by those of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are described. Generally, nomenclatures utilized in connection with, and techniques of, cell and molecular biology and chemistry are those well known and commonly used in the art. Certain experimental techniques, not specifically defined, are generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification. For purposes of clarity, following terms are defined below.

[0149] The present invention recognizes that the interaction of PP2A and mAKAP β mediates various intracellular signals and pathways which lead to cardiac myocyte hypertrophy and/or dysfunction. As such, the present inventors have discovered various methods of inhibiting that interaction in order to prevent and/or treat cardiac myocyte hypertrophy and/or dysfunction.

[0150] Thus, the present invention includes a method for protecting the heart from damage, by administering to a patient at risk of such damage, a pharmaceutically effective amount of a composition, which inhibits the interaction of PP2A and mAKAP β . It should be appreciated that “a pharmaceutically effective amount” can be empirically determined based upon the method of delivery, and will vary according to the method of delivery.

[0151] The invention also relates to a method of treating heart disease, by administering to a patient a pharmaceutically effective amount of a composition, which inhibits the interaction of PP2A and mAKAP β .

[0152] The invention also relates to compositions which inhibit the interaction of PP2A and mAKAP β . In particular embodiments, these inhibiting compositions or “inhibitors” include peptide inhibitors, which can be administered by any known method, including by gene therapy delivery. In other embodiments, the inhibitors can be small molecule inhibitors.

[0153] Specifically, the present invention is directed to methods and compositions for treating or protecting the heart from damage, by administering to a patient at risk of such damage, a pharmaceutically effective amount of a composition which (1) inhibits the interaction of PP2A and mAKAP β ; (2) inhibits the activity of PP2A and mAKAP β ; or (3) inhibits the expression of PP2A and mAKAP β .

[0154] The invention also relates to methods of treating or protecting the heart from damage, by administering to a patient at risk of such damage, a pharmaceutically effective amount of a composition which inhibits a cellular process mediated by the anchoring of PP2A.

[0155] In one embodiment, the composition includes an mAKAP β peptide. In a preferred embodiment, the mAKAP β peptide is obtained from the carboxy terminus of the mAKAP β amino acid sequence. In a particularly preferred embodiment, the mAKAP β peptide is at least a fragment of amino acids 2083-2319 of the mAKAP β amino acid sequence.

[0156] In one preferred embodiment, the mAKAP β peptide is at least a fragment of amino acids 2132-2319 of the mAKAP β amino acid sequence.

[0157] In another embodiment, the composition includes a small interfering RNA siRNA that inhibits the expression of either or both of PP2A and mAKAP β . In a preferred embodiment, the siRNA that inhibits the expression of mAKAP β is generated in vivo following administration of a short hairpin RNA expression vector or biologic agent (shRNA).

[0158] The composition of the invention can be administered directly or can be administered using a viral vector. In a preferred embodiment, the vector is adeno-associated virus (AAV).

[0159] In another embodiment, the composition includes a small molecule inhibitor. In preferred embodiments, the small molecule is a PP2A inhibitor.

[0160] In another embodiment, the composition includes a molecule that inhibits the binding, expression or activity of mAKAP β . In a preferred embodiment, the molecule is a mAKAP β peptide. The molecule may be expressed using a viral vector, including adeno-associated virus (AAV).

[0161] In yet another embodiment, the composition includes a molecule that interferes with mAKAP β -mediated cellular processes. In preferred embodiments, the molecule interferes with the anchoring of PP2A.

[0162] The invention also relates to diagnostic assays for determining a propensity for heart disease, wherein the binding interaction of PP2A and mAKAP β is measured, either directly, or by measuring a downstream effect of the binding of PP2A and mAKAP β . The invention also provides a test kit for such an assay.

[0163] In still other embodiments, the inhibitors include any molecule that inhibits the expression of PP2A and mAKAP β , including antisense RNA, ribozymes and small interfering RNA (siRNA), including shRNA.

[0164] The invention also includes an assay system for screening of potential drugs effective to inhibit the expression and/or binding of PP2A and mAKAP β . In one instance, the test drug could be administered to a cellular sample with the PP2A and mAKAP β , or an extract containing the PP2A and mAKAP β , to determine its effect upon the binding activity of the PP2A and mAKAP β , by comparison with a control. The invention also provides a test kit for such an assay.

[0165] In preparing the peptide compositions of the invention, all or part of the PP2A or mAKAP (FIG. 3 or FIG. 28) amino acid sequence may be used. In one embodiment, the carboxy-terminal region of the mAKAP β protein is used as an inhibitor. Preferably, at least 10 amino acids of the mAKAP sequence are used. More preferably, at least 25

amino acids of the mAKAP sequence are used. Most preferably, peptide segments from amino acids 2132-2319 of mAKAP are used.

[0166] It should be appreciated that various amino acid substitutions, deletions or insertions may also enhance the ability of the inhibiting peptide to inhibit the interaction of PP2A and mAKAP β . A substitution mutation of this sort can be made to change an amino acid in the resulting protein in a non-conservative manner (i.e., by changing an amino acid belonging to a grouping of amino acids having a particular size or characteristic to an amino acid belonging to another grouping) or in a conservative manner (i.e., by changing an amino acid belonging to a grouping of amino acids having a particular size or characteristic to an amino acid belonging to the same grouping). Such a conservative change generally leads to less change in the structure and function of the resulting protein. A non-conservative change is more likely to alter the structure, activity or function of the resulting protein. The present invention should be considered to include sequences containing conservative changes, which do not significantly alter the activity, or binding characteristics of the resulting protein.

[0167] The following is one example of various groupings of amino acids:

[0168] Amino acids with nonpolar R groups: Alanine, Valine, Leucine, Isoleucine, Proline, Phenylalanine, Tryptophan, Methionine.

[0169] Amino acids with uncharged polar R groups: Glycine, Serine, Threonine, Cysteine, Tyrosine, Asparagine, Glutamine.

[0170] Amino acids with charged polar R groups (negatively charged at pH 6.0): Aspartic acid, Glutamic acid.

[0171] Basic amino acids (positively charged at pH 6.0): Lysine, Arginine, Histidine (at pH 6.0).

[0172] Another grouping may be those amino acids with phenyl groups: Phenylalanine, Tryptophan, Tyrosine.

[0173] Another grouping may be according to molecular weight (i.e., size of R groups): Glycine (75), Alanine (89), Serine (105), Proline (115), Valine (117), Threonine (119), Cysteine (121), Leucine (131), Isoleucine (131), Asparagine (132), Aspartic acid (133), Glutamine (146), Lysine (146), Glutamic acid (147), Methionine (149), Histidine (at pH 6.0) (155), Phenylalanine (165), Arginine (174), Tyrosine (181), Tryptophan (204).

[0174] Particularly preferred substitutions are:

[0175] Lys for Arg and vice versa such that a positive charge may be maintained;

[0176] Glu for Asp and vice versa such that a negative charge may be maintained;

[0177] Ser for Thr such that a free —OH can be maintained; and

[0178] Gln for Asn such that a free NH₂ can be maintained.

[0179] Amino acid substitutions may also be introduced to substitute an amino acid with a particularly preferable property. For example, a Cys may be introduced a potential site for disulfide bridges with another Cys. A His may be introduced as a particularly "catalytic" site (i.e., His can act as an acid or base and is the most common amino acid in biochemical catalysis). Pro may be introduced because of its particularly planar structure, which induces β -turns in the protein's structure. Two amino acid sequences are "substantially homologous" when at least about 70% of the amino acid residues (preferably at least about 80%, and most

preferably at least about 90 or 95%) are identical, or represent conservative substitutions.

[0180] Likewise, nucleotide sequences utilized in accordance with the invention can also be subjected to substitution, deletion or insertion. Where codons encoding a particular amino acid are degenerate, any codon which codes for a particular amino acid may be used. In addition, where it is desired to substitute one amino acid for another, one can modify the nucleotide sequence according to the known genetic code.

[0181] Nucleotides and oligonucleotides may also be modified. U.S. Pat. No. 7,807,816, which is incorporated by reference in its entirety, and particularly for its description of modified nucleotides and oligonucleotides, describes exemplary modifications.

[0182] Two nucleotide sequences are "substantially homologous" or "substantially identical" when at least about 70% of the nucleotides (preferably at least about 80%, and most preferably at least about 90 or 95%) are identical.

[0183] Two nucleotide sequences are "substantially complementary" when at least about 70% of the nucleotides (preferably at least about 80%, and most preferably at least about 90 or 95%) are able to hydrogen bond to a target sequence.

[0184] The term "standard hybridization conditions" refers to salt and temperature conditions substantially equivalent to 5 \times SSC and 65°C for both hybridization and wash. However, one skilled in the art will appreciate that such "standard hybridization conditions" are dependent on particular conditions including the concentration of sodium and magnesium in the buffer, nucleotide sequence length and concentration, percent mismatch, percent formamide, and the like. Also important in the determination of "standard hybridization conditions" is whether the two sequences hybridizing are RNA-RNA, DNA-DNA or RNA-DNA. Such standard hybridization conditions are easily determined by one skilled in the art according to well known formulae, wherein hybridization is typically 10-20°C below the predicted or determined T_m with washes of higher stringency, if desired.

[0185] The phrase "pharmaceutically acceptable" refers to molecular entities and compositions that are physiologically tolerable and do not typically produce an allergic or similar untoward reaction, such as gastric upset, dizziness and the like, when administered to a human.

[0186] The phrase "therapeutically effective amount" is used herein to mean an amount sufficient to prevent, and preferably reduce by at least about 30 percent, more preferably by at least 50 percent, most preferably by at least 90 percent, a clinically significant change in a cardiac myocyte feature.

[0187] The preparation of therapeutic compositions which contain polypeptides, analogs or active fragments as active ingredients is well understood in the art. Typically, such compositions are prepared as injectables, either as liquid solutions or suspensions, however, solid forms suitable for solution in, or suspension in, liquid prior to injection can also be prepared. The preparation can also be emulsified. The active therapeutic ingredient is often mixed with excipients which are pharmaceutically acceptable and compatible with the active ingredient. Suitable excipients are, for example, water, saline, dextrose, glycerol, ethanol, or the like and combinations thereof. In addition, if desired, the composition can contain minor amounts of auxiliary sub-

stances such as wetting or emulsifying agents, pH buffering agents which enhance the effectiveness of the active ingredient.

[0188] A polypeptide, analog or active fragment, as well as a small molecule inhibitor, can be formulated into the therapeutic composition as neutralized pharmaceutically acceptable salt forms. Pharmaceutically acceptable salts include the acid addition salts (formed with the free amino groups of the polypeptide or antibody molecule) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed from the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine, procaine, and the like.

[0189] The therapeutic compositions of the invention are conventionally administered intravenously, as by injection of a unit dose, for example. The term "unit dose" when used in reference to a therapeutic composition of the present invention refers to physically discrete units suitable as unitary dosage for humans, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect in association with the required diluent; i.e., carrier, or vehicle.

[0190] The compositions are administered in a manner compatible with the dosage formulation, and in a therapeutically effective amount. The quantity to be administered depends on the subject to be treated, capacity of the subject's immune system to utilize the active ingredient, and degree of inhibition of PP2A-mAKAP β binding desired. Precise amounts of active ingredient required to be administered depend on the judgment of the practitioner and are peculiar to each individual. However, suitable dosages may range from about 0.1 to 20, preferably about 0.5 to about 10, and more preferably one to several, milligrams of active ingredient per kilogram body weight of individual per day and depend on the route of administration. Suitable regimes for initial administration and booster shots are also variable, but are typified by an initial administration followed by repeated doses at one or more hour intervals by a subsequent injection or other administration. Alternatively, continuous intravenous infusion sufficient to maintain concentrations of ten nanomolar to ten micromolar in the blood are contemplated.

[0191] Because of the necessity for the inhibitor to reach the cytosol, a peptide in accordance with the invention may need to be modified in order to allow its transfer across cell membranes, or may need to be expressed by a vector which encodes the peptide inhibitor. Likewise, a nucleic acid inhibitor (including siRNAs, shRNAs and antisense RNAs) can be expressed by a vector. Any vector capable of entering the cells to be targeted may be used in accordance with the invention. In particular, viral vectors are able to "infect" the cell and express the desired RNA or peptide. Any viral vector capable of "infecting" the cell may be used. A particularly preferred viral vector is adeno-associated virus (AAV).

[0192] siRNAs inhibit translation of target mRNAs via a process called RNA interference. When the siRNA is perfectly complementary to the target mRNA, siRNA act by promoting mRNA degradation. shRNAs, as a specialized type of siRNA, have certain advantages over siRNAs that are produced as oligonucleotides. siRNA oligonucleotides

are typically synthesized in the laboratory and are delivered to the cell using delivery systems that deliver the siRNA to the cytoplasm. In contrast, shRNAs are expressed as mini-genes delivered via vectors to the cell nucleus, where following transcription, the shRNA are processed by cellular enzymes such as Drosha and Dicer into mature siRNA species. siRNAs are usually 99% degraded after 48 hours, while shRNAs can be expressed up to 3 years. Moreover, shRNAs can be delivered in much lower copy number than siRNA (5 copies vs. low nM), and are much less likely to produce off-target effects, immune activation, inflammation and toxicity. While siRNAs are suitable for acute disease conditions where high doses are tolerable, shRNAs are suitable for chronic, life threatening diseases or disorders where low doses are desired. (<http://www.benitec.com/technology/sirna-vs-shrna>)

[0193] Guidelines for the design of siRNAs and shRNAs can be found in Elbashir (2001) and at various websites including <https://www.thermofisher.com/us/en/home/references/ambion-tech-support/rnai-sirna/general-articles/sirna-design-guidelines.html> and <http://www.invivogen.com/review-sirna-shrna-design>, all of which are hereby incorporated by reference in their entireties. Preferably, the first nucleotide is an A or a G. siRNAs of 25-29 nucleotides may be more effective than shorter ones, but shRNAs with duplex length 19-21 seem to be as effective as longer ones. siRNAs and shRNAs are preferably 19-29 nucleotides. Loop sequences in shRNAs may be 3-9 nucleotides in length, with 5, 7 or 9 nucleotides preferred.

[0194] With respect to small molecule inhibitors, any small molecule that inhibits the interaction of PP2A and mAKAP β may be used. In addition, any small molecules that inhibit the activity of PP2A and/or mAKAP β may be used.

[0195] Small molecules with similar structures and functionalities can likewise be determined by rational and screening approaches.

[0196] Likewise, any small molecules that inhibit the expression of PP2A and/or mAKAP β may be used.

[0197] In yet more detail, the present invention is described by the following items which represent preferred embodiments thereof:

[0198] 1. A method of treating or preventing heart failure with reduced ejection fraction, comprising administering to cardiac cells of a patient a composition that maintains a level of phosphorylation on serum response factor (SRF).

[0199] 2. The method of Item 1, wherein SRF is phosphorylated on Ser¹⁰³.

[0200] 3. The method of Item 1, wherein dephosphorylation activity of protein (serine-threonine) phosphatase 2A (PP2A) is inhibited.

[0201] 4. The method of Item 3, wherein anchoring of PP2A to muscle A-kinase anchoring protein (mAKAP β) is inhibited.

[0202] 5. The method of Item 4, wherein the composition comprises a fragment of mAKAP β .

[0203] 6. The method of Item 5, wherein the composition comprises an amino acid sequence having at least 90% sequence identity to a fragment of mAKAP β .

[0204] 7. The method of Item 5, wherein the composition comprises a fragment of amino acids 2132-2319 of mAKAP.

- [0205] 8. The method of Item 5, wherein the composition comprises amino acids 2132-2319 of mAKAP.
- [0206] 9. The method of Item 4, wherein the composition comprises a fragment of PP2A.
- [0207] 10. The method of Item 4, wherein said composition comprises a vector that encodes a fragment of mAKAP.
- [0208] 11. The method of Item 4, wherein said composition comprises a vector that encodes an amino acid sequence having at least 90% sequence identity to a fragment of mAKAP.
- [0209] 12. The method of Item 10, wherein the vector encodes a fragment of amino acids 2132-2319 of mAKAP.
- [0210] 13. The method of Item 10, wherein the vector encodes amino acids 2132-2319 of mAKAP.
- [0211] 14. The method of Item 10, wherein the vector is adeno-associated virus (AAV).
- [0212] 15. A composition that encodes a molecule that inhibits the anchoring of PP2A to mAKAP.
- [0213] 16. The composition of Item 15, wherein the molecule comprises a fragment of mAKAP.
- [0214] 17. The composition of Item 15, comprising an amino acid sequence having at least 90% sequence identity to a fragment of mAKAP.
- [0215] 18. The composition of Item 16, comprising a fragment of amino acids 2132-2319 of mAKAP.
- [0216] 19. The composition of Item 16, comprising amino acids 2132-2319 of mAKAP β .
- [0217] 20. The composition of Item 15, comprising a fragment of PP2A.
- [0218] 21. A composition comprising a vector that encodes a molecule that inhibits the anchoring of PP2A to mAKAP.
- [0219] 22. The composition of Item 21, wherein the vector encodes a fragment of mAKAP.
- [0220] 23. The composition of Item 21, wherein the vector encodes an amino acid sequence having at least 90% sequence identity to a fragment of mAKAP.
- [0221] 24. The composition of Item 21, wherein the vector encodes a fragment of amino acids 2132-2319 of mAKAP.
- [0222] 25. The composition of Item 21, wherein the vector encodes amino acids 2132-2319 of mAKAP.
- [0223] 26. The composition of Item 21, wherein the vector encodes a fragment of PP2A.
- [0224] 27. The composition of Item 21, wherein the vector is adeno-associated virus (AAV).
- [0225] The following examples are provided to aid the understanding of the present invention, the true scope of which is set forth in the appended claims. It is understood that modifications can be made in the procedures set forth without departing from the spirit of the invention.

EXAMPLES

[0226] The compositions and processes of the present invention will be better understood in connection with the following examples, which are intended as an illustration only and not limiting of the scope of the invention. Various changes and modifications to the disclosed embodiments will be apparent to those skilled in the art and such changes and modifications including, without limitation, those relating to the processes, formulations and/or methods of the

invention may be made without departing from the spirit of the invention and the scope of the appended claims.

Example 1

SRF Regulation by mAKAP β Signalosomes

Materials and Methods

[0227] Neonatal Rat Ventricular Myocyte Culture: 1-3 day old Sprague-Dawley rats were decapitated, and the excised hearts placed in 1xADS Buffer (116 mM NaCl, 20 mM HEPES, 1 mM NaH₂PO₄, 5.5 mM glucose, 5.4 mM KCl, 0.8 mM MgSO₄, pH 7.35). The atria were carefully removed and the blood washed away. The ventricles were minced and incubated with 15 mL 1xADS Buffer containing 3.3 mg type II collagenase (Worthington, 230 U/mg) and 9 mg Pancreatin (Sigma) at 37° C. with gentle shaking. After 15 minutes, the dissociated cardiac myocytes were separated by centrifugation at 50 g for 1 minute, resuspended in 4 mL horse serum and incubated at 37° C. with occasional agitation. The steps for enzymatic digestion and isolation of myocytes were repeated 10-12 times to maximize yield. The myocytes were pooled and spun down again at 50 g for 2 minutes and resuspended in Maintenance Medium (DMEM:M199, 4:1) supplemented with 10% horse serum and 5% fetal bovine serum. To remove any contaminating fibroblasts, the cells were pre-plated for 1 hour before plating on gelatin-coated tissue culture plastic ware. This procedure yields >90% pure cardiac myocytes. After 1 day culture, the media was changed to maintenance medium containing 0.1 mM bromodeoxyuridine to suppress fibroblast growth.

[0228] Adult rat ventricular myocyte isolation and culture: 2-3 month old rats were anesthetized using Ketamine (80-100 mg/kg) and Xylazine (5-10 mg/kg) IP following 1000 U heparinization for cardiac excision. The heart was transferred immediately into chilled perfusion buffer (NaCl 120 mM, KCl 5.4 mM, Na₂HPO₄·7H₂O 1.2 mM, NaHCO₃ 20.0 mM, MgCl₂·6H₂O 1.6 mM, Taurine 5 mM, Glucose 5.6 mM, 2,3-Butanedione monoxime 10 mM) pre-equilibrated with 95% O₂ and 5% CO₂. After removal of extraneous tissue, the heart was attached via the aorta to a Harvard Langendorff apparatus cannula. Ca²⁺-free perfusion was used to flush out remaining blood with a constant rate of 8-10 mL/min at 37° C. The heart was then digested through circulatory perfusion with 50 mL perfusion buffer containing 125 mg type II collagenase (Worthington, 245 U/mg), 0.1 mg protease (Sigma type XIV) and 0.1% BSA. After perfusion, the atria were removed and the ventricular myocytes dissociated by slicing and repetitive pipetting. The debris was filtered by a 200 μm nylon mesh, and the myocytes collected by one minute centrifugation at 50 g. Ca²⁺ concentration in the buffer was gradually recovered to 1.8 mM and the myocytes were resuspended in ACCT medium (M199 Medium (Invitrogen 11150-059), Creatine 5 mM, L-carnitine 2 mM, Taurine 5 mM, HEPES 25 mM, 2,3-Butanedione monoxime 10 mM, BSA 0.2% and 1× Insulin-Transferrin-Selenium Supplement) and plated on 10 μg/ml laminin pre-coated dishes. Cells were washed with ACCT medium 1.5 hours after plating and subjected to adenoviral infection or siRNA transfection, in which 100-200 Multiplicity of Infection (MOI) of adenovirus and 100 nmol/L siRNA mixed with Dharmafect1 (Dharmacon) were used, respectively. Adrenergic agonists were added the next

day, with biochemical assay and morphological measurement performed after 24 hours of stimulation.

[0229] Other Cell Culture: HEK293 and COS-7 cells were maintained in DMEM with 10% FBS and 1% P/S. These cells were transiently transfected with Lipofectamine 2000 (Invitrogen) or infected with adenovirus and Adeno-X Tet-Off virus (Clontech) as suggested by the manufacturers.

[0230] Luciferase Assays: 225,000 neonatal rat ventricular myocytes in 24 well dishes were transfected with control or RSK3 specific siRNA oligonucleotides (10 nM) and Dharmafect1 reagent (ThermoFisher). The following day, following washing the cells with media, the myocytes were re-transfected with 100 ng SRE-luc (firefly luciferase) and 100 ng -36Prl-rluc (renilla luciferase) reporter plasmids and Transfast reagent for one hour and then cultured in media with 4% horse serum overnight, before washing with media and incubating for one day in the absence or presence of 10 μ M PE. Samples were collected in 100 μ l PLB and assayed using the Promega Dual Luciferase Kit and a Berthold Centro X luminometer.

[0231] Co-Immunoprecipitation: Tissues were homogenized using a Polytron or cells were lysed in IP buffer (50 mM HEPES pH 7.4, 150 mM NaCl, 5 mM EDTA, 10% glycerol, 1% Triton-X 100, 1 mM DTT) with an inhibitor cocktail (1 μ g/ml leupeptin, 1 μ g/ml pepstatin, 1 mM benzamidine, 1 mM AEBSF, 50 mM NaF, 1 mM sodium orthovanadate). Soluble proteins were separated by centrifugation at 3-10,000 g for 10 minutes. Antibodies and protein-G agarose beads (50% slurry, Upstate) were added to extracts and incubated overnight with rocking at 4° C. Beads were washed four times at 4° C. with IP buffer. Bound proteins were size-fractionated on SDS-PAGE gels and developed by immunoblotting as previously described using a Fujifilm LAS-3000 or GE-A1600 imaging system (46). Protein markers were Precision Plus Protein Standards (Bio-Rad, 1610373).

[0232] Immunocytochemistry: Myocytes on coverslips were fixed in 3.7% formaldehyde in PBS for 1 hour, permeabilized with 0.3% Triton X-100, and blocked in PBS containing 0.2% BSA and 1% horse serum. The slides were then sequentially incubated for 1 hour with primary and Alexa fluorescent dye-conjugated specific-secondary antibodies (Invitrogen, 1:1000) diluted in blocking buffer. The slips were washed three times with blocking buffer. 1 μ g/mL Hoechst 33258 was included in the last wash stop to label nuclei. Slides were sealed in SlowFade Gold antifade buffer (Invitrogen, S36938) for fluorescent microscopy. Wide-field images were acquired using a Leica DM4000 Microscope.

[0233] GST-SRF phosphorylation assays: GST-SRF protein was purified using BL21 *E. coli* and glutathione-sepharose as previously described (Vargas et al. 2012). GST-SRF on beads was incubated with 0.5 μ g active recombinant full-length His₆-tagged human RSK3 (Millipore 14-462)+/-50 nM BI-D1870 in ATP-containing kinase buffer for 30 minutes. The GST-SRF beads were then either eluted with Laemmlli buffer or washed with PP2A phosphatase buffer and then incubated for an additional 30 minutes in the presence of 50 ng PP2A +/-10 nM okadaic acid before elution with Laemmlli buffer. Equal loading of GST-SRF protein was determined by Ponceau stain and phosphorylation of SRF was detected using a phospho-SRF S¹⁰³-specific antibody.

Plasmid Constructs

[0234] SRE-luciferase reporter—SRE-luc was constructed by subcloning two copies of a c-fos SRF response element (TCGAC AGG ATG TCC ATA TTA GGA CAT CTG) (SEQ ID NO:3) (Treisman 1985) in an Xho I site upstream of the -36 bp rat prolactin promoter in a firefly luciferase reporter plasmid as previously described (Kapiloff et al. 1991).

[0235] -36 Prl-renilla luciferase—An oligonucleotide containing -36+36 of the rat prolactin promoter with Bgl II and Hind III compatible ends (GATCT CGA AGG TTT ATA AAG TCA ATG TCT GCA GAT GAG AAA GCA GTG GTT CTC TTA GGA CTT CTT GGG GAA GTG TGG TC) (SEQ ID NO:4) was subcloned into pRL-null (Promega) to provide the control renilla luciferase vector.

[0236] mAKAP fragment expression vectors: pS-EGFPC1-mAKAP-1694-1833-mh adenovirus shuttle vector was constructed by subcloning a cDNA encoding a myc, His₆, and GFP-tagged mAKAP aa 1694-1833 fragment (RBD) in pEGFPC1 (Clontech) (Li, Kritzer, et al. 2013) into a pTRE shuttle vector previously modified to contain a CMV immediate early promoter. pS-EGFPC1-mh is similarly designed except lacking the mAKAP sequence. pTRE-myc-mAKAP PBD encoding a myc-tagged mAKAP aa 2134-2314 (PBD) fragment was constructed by digesting pTRE-myc-mAKAP containing a full-length, N-terminally myc-tagged mAKAP cDNA with Apa I-Sca I and ligation. pTRE- β gal encoding β -galactosidase control protein was obtained from Clontech. pAcTnTS-EGFP-mAKAP 1694-1833 mh plasmid that was used to generate AAV-RBD was constructed by subcloning a NheI-BamHI fragment of pEGFPC1-rmAKAP-1694-1833-mh (Li, Kritzer, et al. 2013) into pAcTnTs provided generously by Dr. Brent French of the University of Virginia (Prasad et al. 2011). pAcTnTs-EGFP-mh plasmid to generate AAV-GFP control virus was generated by digesting pAcTnTS-EGFP-mAKAP 1694-1833mh with Acc65I and BsRGI, blunting, and ligation. Other mAKAP plasmids were as previously described (Pare, Bauman, et al. 2005; Kapiloff, Jackson, and Airhart 2001).

[0237] SRF constructs—pFlag-SRF that expressed a Flag-tagged SRF protein was constructed by subcloning a human SRF cDNA from pCGN-SRF (Addgene Plasmid #11977) into the XbaI/EcoRI sites of the pSH160c NFATc1 expression plasmid (Ho et al. 1995). pTRE-Flag-hSRF was constructed by subcloning the Flag-tagged SRF cDNA into pTRE shuttle vector (Clontech). pTRE-3xHA-hSRF was constructed by inserting a custom sequence within the SfiI and SmaI sites of pTRE-Flag-hSRF that replaces the Flag tag with 3 tandem HA tags. S103A and S103D mutations were introduced into the pTRE plasmids by site-directed mutagenesis to introduce the sequences ATCGCTGGCAGAG (SEQ ID NO:5) and GAGCCTG-GATGAA (SEQ ID NO:6) in place of GAGCCT-GAGCGAG (SEQ ID NO:7). pGEX-4T1-FLAG-hSRF for expression of GST-SRF in bacteria was constructed by subcloning a NcoI (blunted)-EcoRI fragment of pTRE-Flag-hSRF into the BamHI (blunted)-EcoRI sites of pGEX-4T1.

[0238] RSK3 expression vectors: Plasmids for HA-tagged RSK3 wildtype and S218A mutant and RSK3 fragments are as previously described (Li, Kritzer, et al. 2013). pS-HA-hRSK3 1-42 adenovirus shuttle vector was constructed by

subcloning a HA-tagged 1-42 cDNA into the BsaBI and NheI sites of pS-EGFPC1-mh replacing the tagged GFP cDNA.

[0239] Adenovirus were prepared using the pTRE shuttle vectors and the Adeno-X Tet-off System (Clontech) via PI-SceI and I-CeuI subcloning and purified after amplification using Vivapure AdenoPACK kits (Sartorius Stedim). These adenovirus conditionally express recombinant protein when co-infected with tetracycline transactivator-expressing virus (adeno-tTA for “tet-off” or reverse tTA for “tet-on”). Some adenovirus were constructed using a modified pTRE shuttle vector (pS) containing a constitutive CMV promoter.

Results

[0240] Given the role of RSK3 and mAKAP β in the determination of concentric myocyte growth, research has focused on the identification of RSK3 cardiac myocyte substrates. The transcription factor serum response factor (SRF) serves important roles in both cardiac development and adult function through the regulation of genes involved in growth and the actin cytoskeleton (Miano 2010). SRF is subject to multiple post-translational modifications (FIG. 19A), including phosphorylation at Ser¹⁰³ (Mack 2011). Because of SRF’s prominent role in myocyte regulation and the previously demonstrated phosphorylation of SRF by other RSK family members (Miano 2010; Rivera et al. 1993; Janknecht et al. 1992; Hanlon, Sturgill, and Sealy 2001), SRF was considered to be an effector for RSK3 in cardiac myocytes. Phosphorylation of SRF Ser¹⁰³ by RSK3 was readily confirmed using purified glutathione-S-transferase (GST)-SRF fusion protein (data not shown). SRF contains a conserved MADS (MCM1, agamous, deficiens, SRF) domain that mediates both DNA binding to CARG box [CC(A/T)₆GG] serum response elements (SREs) and homo- and hetero-dimerization with other transcription factors (FIG. 19A). Using RSK3 small interfering nucleotides (siRNA) to deplete primary neonatal rat ventricular myocytes cultures (NRVM) of SRF by RNA interference (RNAi), it was determined that loss of RSK3 inhibited SRE-dependent transient reporter activity, including that induced by the α -adrenergic agonist phenylephrine (PE, FIG. 19B). As RSK3 binds the scaffold protein mAKAP β (Li, Kritzer, et al. 2013), whether SRF might also be associated with mAKAP β signalosomes, facilitating its phosphorylation was tested. Endogenous mAKAP β was consistently co-immunoprecipitated with SRF from adult mouse heart extracts using SRF antibodies (FIG. 19C). In addition, SRF and RSK3 can associate in the presence of mAKAP β when expressed in heterologous cells, forming ternary complexes (FIG. 19D). Accordingly, inhibition of RSK3 and mAKAP β expression in NRVM inhibited PE-induced SRF Ser¹⁰³ phosphorylation (FIG. 19E). The isoform-specific N-terminal RSK3 domain binds a discrete “RSK3-binding domain” within mAKAP β at residues 1694-1833 (RBD) (Li, Kritzer, et al. 2013). Expression of a myc-tagged, green fluorescent protein (GFP) RBD-fusion protein that can compete mAKAP β -RSK3 binding (Li, Kritzer, et al. 2013) inhibited PE-induced SRF Ser¹⁰³ phosphorylation in both NRVM and primary adult rat ventricular myocyte cultures (ARVM, FIG. 19F and data not shown). Similar results were obtained by anchoring disruption using the N-terminal RSK3 peptide (data not shown). These results were corroborated in vivo. SRF Ser¹⁰³ phosphorylation was decreased in hearts obtained from both RSK3

global and mAKAP β myocyte-specific conditional knock-out mice that were previously described (Kritzer et al. 2014; Li, Kritzer, et al. 2013), as well as in mice expressing RBD in vivo (data not shown). Together these results reveal that SRF is a RSK3 substrate in myocytes whose phosphorylation in response to catecholaminergic stimulation depends upon association with mAKAP β signalosomes.

[0241] mAKAP β binds two phosphatases, the Ca²⁺/calmodulin-dependent phosphatase calcineurin (PP2B, PPP3) and a protein kinase A (PKA)-activated isoenzyme of PP2A that contains B56 δ -subunit (Dodge-Kafka et al. 2010; Li et al. 2010). Treatment of NRVM with the PP1/PP2A inhibitor okadaic acid (OA), but not the calcineurin inhibitor cyclosporin A (CsA) promoted baseline phosphorylation of SRF Ser¹⁰³ (FIG. 19G). Accordingly, purified PP2A readily dephosphorylated SRF Ser¹⁰³ (FIG. 21). Analogous to RSK3, SRF, PP2A, and mAKAP β form ternary complexes in NRVM, as SRF and PP2A could be co-immunoprecipitated only in the presence of mAKAP β (FIG. 19H). PP2A binds a C-terminal domain of mAKAP β (Dodge-Kafka et al. 2010), and expression of the PP2A Binding Domain (myc-PBD, FIG. 4) competed endogenous mAKAP β -PP2A association in myocytes (FIG. 19I). Consistent with a previously published finding that cAMP activates mAKAP β -bound PP2A (Dodge-Kafka et al. 2010), PBD expression potentiated the induction of SRF Ser¹⁰³ phosphorylation in ARVM stimulated with the β -adrenergic isoproterenol (Iso, FIG. 19J). In aggregate, these results show that mAKAP β signalosomes can regulate SRF Ser¹⁰³ phosphorylation in a bidirectional manner in response to different upstream stimuli.

Example 2

SRF Ser¹⁰³ Phosphorylation Promotes Concentric Hypertrophy

[0242] While both neonatal rat ventricular myocytes (NRVM) and adult rat ventricular myocytes (ARVM) are useful for studying molecular signaling pathways, including α -adrenergic and β -adrenergic induced hypertrophy, the two cellular preparations are significantly different in shape, ultrastructure, and in some circumstances cellular regulation (Peter, Bjerke, and Leinwand 2016). Taking advantage of their roughly cylindrical shape, ARVM was developed as an in vitro model for morphologic hypertrophy more relevant to in vivo cardiac remodeling. Characterization of the RSK3 knock-out mouse suggested that RSK3 was important for concentric hypertrophy (Passariello et al. 2016; Li, Kritzer, et al. 2013). RSK3 overexpression selectively increased the width of cultured ARVM, resulting in a significantly decreased length/width ratio (FIG. 20A,B). This result was similar to that obtained following one day of myocyte culture in the presence of the phenylephrine (PE, FIG. 20C,D). PE induced an increase of 8-10% in width and a decrease of 8-14% in length/width ratio in 24 hours, which compares favorably to the increase of 17-21% in width and the decrease of 14-21% in length/width ratio of mouse myocytes in vivo following two weeks of transverse aortic constriction (8, 16). Remarkably, expression of a SRF S103D phosphomimetic mutant also increased ARVM width, inducing concentric hypertrophy to the same degree as PE treatment. Conversely, expression of the SRF S103A mutant did not affect basal myocyte size, but inhibited the PE-induced concentric hypertrophy (FIG. 20E,F). This

result was phenocopied by expression of the RBD RSK3-anchoring disruptor peptide (FIG. 20G,H) that inhibited SRF Ser¹⁰³ phosphorylation (FIG. 19F). In contrast to PE and RSK3 overexpression, chronic stimulation with the β-adrenergic agonist Iso increased both ARVM length and width, resulting in a more symmetric hypertrophy (FIG. 20I,J), similar to the effect of chronic Iso infusion *in vivo* (Li, Kritzer, et al. 2013). Like RBD and SRF S103A expression, displacement of PP2A phosphatase from mAKAPβ signalosomes had no effect on basal ARVM morphology. In addition, like SRF S103D expression, PBD anchoring disruptor expression did not enhance nor diminish PE-induced hypertrophy. In contrast, in the presence of Iso, PDB expression promoted ARVM concentric hypertrophy, with the Iso-induced increase in ARVM width and length tending to be greater and lesser, respectively, in the presence of PP2A displacement. This latter result was consistent with the PDB-dependent potentiation of Iso-induced SRF Ser¹⁰³ phosphorylation (FIG. 19J). Taken together, these results support a model in which mAKAPβ-anchored RSK3 and PP2A regulate SRF Ser¹⁰³ phosphorylation that promotes concentric cardiac myocyte hypertrophy.

Example 3

Regulation of PDE4D3 by mAKAPβ-Bound PP2A

[0243] Antibodies—The following primary antibodies were used for immunoblotting: mouse monoclonal anti-GFP (Santa Cruz; 1:500), mouse monoclonal anti-VSV tag (Sigma; 1:1000), mouse monoclonal anti-mAKAP (Covance, 1:1000), 9E10 mouse anti-myc (Santa Cruz, Inc, 1:500 dilution), polyclonal anti-PP2A-C (Santa Cruz, 1:500), and polyclonal anti-PP1 catalytic subunit (Santa Cruz, Inc, 1:500). A phospho-specific antibody for phospho-PDE4D3 Ser-54 was generated and affinity purified using phospho-ylated and non-phosphorylated human PDE4D3 peptides containing residues 70-81 (21st Century Biochemicals) and was used at a dilution of 1:500. Polyclonal B56δ antibodies, both non-phospho-specific and specific for phospho-Ser-566, are as previously described (Ahn et al. 2007).

[0244] Expression constructs—Expression vectors for Flag-tagged B56δ, Glutathione-S-transferase (GST) PP2A-A fusion protein, and myc- and green fluorescence protein (GFP)-tagged rat and human mAKAP are as previously described (Ahn et al. 2007; Pare, Bauman, et al. 2005; Kapiloff et al. 1999a; Kapiloff, Jackson, and Airhart 2001). The myc-tagged mAKAP construct deficient in PP2A binding was made by subcloning a cDNA fragment encoding rat mAKAP 1286-2083 generate by PCR into pCMV-Myc (Clontech). mAKAPα and mAKAPβ are two alternatively-spliced isoforms of mAKAP expressed in the heart and brain, respectively (Michel et al. 2005b). mAKAPβ is identical to mAKAPα residues 245-2314; all recombinant mAKAP proteins expressed in this paper are based on mAKAPα. The expression vector used for PDE4D3 throughout this paper was constructed by subcloning a cDNA encoding VSV-tagged PDE4D3 (Dodge et al. 2001) into a GFP-expression vector (Clontech), resulting in a double-tagged PDE4D3 protein.

[0245] Immunoprecipitation—HEK293 cells were used in this project as a heterologous system lacking mAKAP in which the various wildtype and mutant proteins could be easily expressed. Cells cultured on 60 mm plates were transfected at 50%-70% confluence by the calcium phosphate method, using 6 µg of each DNA construct per plate.

Cells were harvested 24 hours after transfection in 0.5 ml HSE buffer (HEPES, pH 7.4, 150 mM NaCl, 5 mM EDTA, 1% Triton X-100 and protease inhibitors). Supernatants were incubated with 3 µg antibody and 15 µl prewashed protein A- or G-agarose beads. Following overnight incubation at 4° C., the immunoprecipitates were washed three times with the same buffer. Bound proteins were analyzed by immunoblotting.

[0246] For immunoprecipitation of endogenous, native mAKAP complexes, adult rat hearts (Pel-Freeze) were homogenized in 10 ml HSE buffer. After centrifugation at 15,000×g for 25 minutes, clarified extracts were immunoprecipitated as above.

[0247] PDE assay—PDE activity associated with immunoprecipitated protein complexes was assayed according to the method by Beavo et al. (Beavo, Bechtel, and Krebs 1974). Samples were assayed in 45 µl PDE buffer A (100 mM MOPS, pH 7.5, 4 mM EGTA, 1.0 mg/ml bovine serum albumin) and 50 µl PDE buffer B [100 mM MOPS, pH 7.5, 75 mM MgAc, 1 µM cAMP and 100,000 cpm [³H]cAMP (Dupont, NEN)]. Inhibitors were included as indicated.

[0248] Phosphatase Assay—Phosphatase activity was measured according to the method of Ahn et al. using ³²P-labeled histone as substrate (Ahn et al. 2007). Histone was radiolabeled in reactions containing 250 mM MOPS, pH 7.4, 2.5 mM MgAc, 100 mM P-mercaptoethanol, purified PKA catalytic subunit, 1 µM ATP, 20 µM histone, and 1 mCi [γ -³²P]ATP (6000 Ci/mmol). The reaction was terminated by the addition of 50% TCA, and [³²P]histone was purified from free radionucleotide by centrifugation. The [³²P]histone pellet was washed with 1 ml of ether/ethanol/HCl (4:1:0.1) once and 1 ml of ether/ethanol (4:1) three times. The substrate was then suspended in 200 µl PP2A assay buffer (25 mM Tris, pH 7.4, 1 mM DTT, and 10 mM MgCl₂) before precipitation with 50% TCA. After repeated washing, the [³²P]histone was suspended in 200 µl PP2A buffer.

[0249] To measure phosphatase activity, immunoprecipitated protein complexes were washed twice in HSE buffer and once in PP2A reaction buffer. The immunoprecipitates were incubated for 30 minutes at 30° C. in 20 µl PP2A assay buffer containing 100,000 cpm [³²P]histone in the presence and absence of inhibitors. The PP2A inhibitor (Calbiochem) was used at a concentration of 30 nM. Purified I-1 was phosphorylated by PKA before using as a specific PP1 inhibitor. Reactions were terminated by the addition of 100 µl 20% TCA followed by 10 min centrifugation. TCA supernatants containing released ³²PO₄ were measured by scintillation counting.

[0250] GST-pulldowns—Glutathione resin adsorbed with PP2A-A subunit GST fusion protein or GST control protein were incubated with HEK293 cell extracts. After an overnight incubation, the beads were washed three times. Bound proteins were analyzed by immunoblotting.

[0251] Statistics—Each “n” refers to a completely independent experiment performed using separate cultures or heart preparations. All p-values were calculated using a Student’s t-test.

Results

[0252] Regulation of mAKAP-bound PDE4D3 by an okadaic acid-sensitive phosphatase. A negative feedback loop intrinsic to mAKAP complexes that includes cAMP activation of PKA, PKA phosphorylation and activation of

PDE4D3, and PDE4D3-catalyzed cAMP degradation has previously been described (Dodge et al. 2001). PDE4D3 phosphorylation was dependent upon PKA binding to mAKAP. Symmetrically, a mAKAP-bound phosphatase might be responsible for PDE4D3 dephosphorylation. Both PP2A and the Ca^{2+} /calmodulin-dependent protein phosphatase calcineurin (PP2B) associate with the mAKAP scaffold in cardiac myocytes (Pare, Bauman, et al. 2005; Kapiloff, Jackson, and Airhart 2001; Li et al. 2009). To begin this study, a heterologous system was used to test whether PP2A or PP2B might dephosphorylate PDE4D3 at Ser-54, the residue within the PDE4D3 Upstream Conserved Region required for PKA activation (Sette and Conti 1996). HEK293 cells over-expressing mAKAP and PDE4D3 were treated with 300 μM okadaic acid (OA) to inhibit PP2A (and protein phosphatase 1 [PP1]) activity or 500 μM cyclosporin A (CsA) to inhibit PP2B activity (FIG. 8A). After immunoprecipitation of protein complexes using a mAKAP-specific antibody, PDE4D3 phosphorylation was assayed by immunoblotting with a phospho-specific antibody to residue Ser-54 had been generated. OA treatment resulted in an increase in the baseline phosphorylation of PDE4D3 Ser-54, while inhibition of PP2B had no effect (FIG. 8A, top panel, lane 2). This increased phosphorylation was further enhanced 1.8 fold when PKA was activated by the addition of the adenylyl cyclase agonist forskolin (Fsk, FIG. 8A, top panel, lane 5). Notably, forskolin alone had no significant effect in the absence of phosphatase inhibition (FIG. 8A, lane 4). Immunoblotting using a non-phospho-specific antibody for PDE4D3 and an antibody for mAKAP demonstrated that two proteins were similarly precipitated under each condition (FIG. 8A, lower panels).

[0253] As phosphorylation of PDE4D3 Ser-54 increases phosphodiesterase activity 2 fold (Sette and Conti 1996), whether OA treatment would also increase the activity of mAKAP-bound PDE4D3 was tested. mAKAP complexes were immunoprecipitated from transfected HEK293 cells and assayed for associated phosphodiesterase activity (FIG. 8B). mAKAP-associated phosphodiesterase activity in untreated cells was detected only when mAKAP was co-expressed with PDE4D3 (FIG. 8B, bar 1, and data not shown), consistent with a previous observation that PDE4D3 accounts for all of the phosphodiesterase activity associated with mAKAP in cardiac myocytes (Dodge et al. 2001). In agreement with the results obtained with the phospho-Ser-54 antibody, Fsk treatment alone was unable to significantly stimulate mAKAP-bound PDE4D3 activity in HEK293 cells, while Fsk and OA treatment together synergistically increased PDE4D3 activity (FIG. 8B, bars 3 & 6). CsA had no effect on either basal or stimulated PDE4D3 activity, suggesting that PP2B does not regulate PDE4D3 bound to mAKAP in cells under these conditions. Together, these results show that in this heterologous system, an OA-sensitive phosphatase strongly inhibits both the baseline and Fsk-stimulated phosphorylation and activity of PDE4D3 bound to mAKAP.

[0254] The enhancement of phosphodiesterase activity by OA was seen not only with expression of recombinant proteins in HEK293 cells, but also upon isolation of native mAKAP complexes from adult rat heart extracts (FIG. 8C). Both PDE4D3 and PKA are active in purified mAKAP complexes (Dodge et al. 2001). PKA activity present in endogenous mAKAP complexes is responsible for increasing phosphodiesterase activity 2-fold, as was evident upon

inhibition of mAKAP-bound PKA with the specific PKA inhibitor PKI (FIG. 8C, bars 2 and 4). Importantly, OA inhibition increased mAKAP-associated phosphodiesterase activity 30% (bars 2 and 3) and 60% when PKA was also inhibited (bars 4 and 5). Taken together, these data demonstrate that an OA-sensitive phosphatase associated with the mAKAP complex is responsible for the dephosphorylation of PDE4D3 and the regulation of phosphodiesterase activity.

[0255] PP2A associates with the mAKAP scaffold in the heart. Having established that an OA-sensitive phosphatase was associated with the mAKAP complex, the phosphatase was identified by co-immunoprecipitation experiments. Phosphatase activity associated with mAKAP complexes isolated from heart cell extracts was measured using [^{32}P] histone as a substrate. There was a 3-fold enrichment of phosphatase activity over control IgG immunoprecipitates (FIG. 9A, bars 1 & 2). The mAKAP-associated phosphatase responsible for the immunoprecipitated activity was identified as PP2A, since the phosphatase activity was completely inhibited by 30 nM PP2A Inhibitor I (Li, Makkinje, and Damuni 1996), but not by addition of 100 nM PKA-phosphorylated PP1 Inhibitor-1 (Endo et al. 1996). As a positive control, the PKA-phosphorylated PP1 inhibitor-1 did inhibit PP1 isolated by immunoprecipitation with a PP1 antibody from HEK293 cell extracts (FIG. 16). The mAKAP-associated phosphatase activity was not due to mAKAP-bound PP2B, since no Ca^{2+} /calmodulin was included in the phosphatase assay buffer. Confirmation of these results was obtained by immunoblot analysis of mAKAP immunoprecipitates. PP2A-C subunit, but not PP1 catalytic subunit, was detected in mAKAP-specific immunoprecipitates (FIGS. 9B & C).

[0256] Like PKA, PP2A associates with many cellular substrates and is expected to be present in diverse intracellular compartments (Virshup 2000). Confocal fluorescent microscopy of cultured primary neonatal rat cardiomyocytes revealed that PP2A-C subunit is distributed throughout the cytoplasm in a fine punctate pattern (FIG. 17, green). As found previously, mAKAP was localized primarily to the nuclear envelope (Pare, Easlick, et al. 2005). Consistent with the co-immunoprecipitation of mAKAP and PP2A from adult rat heart extracts, overlap of PP2A and mAKAP staining could be detected at the nuclear envelope (FIG. 17, composite image), supporting the model that a localized signaling complex consisting of discrete pools of PP2A, PKA, and PDE4D3 and the scaffold mAKAP is present in cardiac myocytes.

[0257] mAKAP residues 2083-2319 contain the PP2A binding domain. In order to map the PP2A binding site on mAKAP, a bacterially-expressed PP2A-A subunit GST-fusion protein was used to pull down GFP-tagged fragments of mAKAP expressed in HEK293 cells (FIGS. 10A & B). GST-PP2A-A consistently pulled down only fragments of mAKAP containing a domain C-terminal to residue 2085. Both human and rat mAKAP GFP-fusion proteins bound GST-PP2A-A, including rat mAKAP 1835-2312 and human 2085-2319. As a negative control, the GFP-mAKAP fusion proteins did not bind PP1 in HEK293 cells, consistent with the lack of co-immunoprecipitation of PP1 and mAKAP from heart extracts (FIG. 18). To confirm the mapping of the PP2A binding site on mAKAP, myc-tagged mAKAP fragments expressed in HEK293 cells were immunoprecipitated with a myc-tag antibody and assayed for associated PP2A activity (FIG. 10C). mAKAP 1286-2312, but not mAKAP

1286-2083, co-immunoprecipitated with OA-sensitive phosphatase activity. Together, these data show that PP2A binds a C-terminal site within mAKAP that is separate from the binding sites for PKA, PDE4D3, and other known mAKAP-binding proteins (FIG. 10A).

[0258] mAKAP-anchored PP2A regulates PDE4D3 phosphorylation in the complex. Data obtained using mAKAP complexes isolated from rat heart extracts implied that mAKAP-bound PP2A regulated PDE4D3 in the complex (FIG. 8C). To test whether PP2A anchoring is required for PDE4D3 dephosphorylation, PDE4D3 was expressed in HEK293 cells and a mAKAP construct containing the binding sites for PDE4D3, PKA and PP2A (myc-mAKAP 1286-2312), or a similar mAKAP construct lacking the PP2A binding site (myc-mAKAP 1286-2083). The cells were stimulated with Fsk and OA, and mAKAP complexes were subsequently isolated by immunoprecipitation. Phosphorylation of mAKAP-bound PDE4D3 was assayed by immunoblotting with the Ser-54 phospho-specific antibody. As was found upon expression of full-length mAKAP (FIG. 8A), phosphorylation of PDE4D3 bound to myc-mAKAP 1286-2312 was detected only when phosphatase activity was suppressed by OA (FIG. 11A, lane 3). Notably, upon expression of myc-mAKAP 1286-2083 which lacked significant PP2A binding (FIG. 11A, lanes 4-6), an increase in the baseline phosphorylation of mAKAP-bound PDE4D3 was detected (0.49 ± 0.19 fold of the level obtained with OA; FIG. 11A, lanes 4 vs. 3). Moreover, upon deletion of the PP2A binding domain, Fsk alone increased phosphorylation of the phosphodiesterase to levels equivalent to that associated with PP2A-containing complexes treated with both Fsk and OA (FIG. 11A, lanes 3, 5, & 6). The changes in PDE4D3 Ser-54 phosphorylation were mirrored by changes in phosphodiesterase activity (FIG. 11B). PDE4D3 activity was 30% higher in myc-mAKAP 1286-2083 immunoprecipitates lacking PP2A than in complexes containing the phosphatase (bar 1 and 4). Importantly, no significant difference in PDE4D3 activity was seen between Fsk stimulation and Fsk stimulation in the presence of OA for the complexes lacking PP2A (bars 5 and 6). These data demonstrate the importance of PP2A anchoring for the regulation of PDE4D3 phosphorylation and activity. Furthermore, they demonstrate that PP2A serves not only to attenuate PKA-activated phosphodiesterase activity, but also to maintain a low basal level of PDE4D3 activity in unstimulated cells.

[0259] mAKAP-bound PP2A holoenzyme containing B56δ subunit is regulated by PKA. PP2A holoenzyme is composed of three subunits, including a core A and C subunit heterodimer and a B subunit that may target the holoenzyme to specific intracellular organelles (Virshup 2000). Three closely related B-subunits have been identified that are expressed in the heart and are localized to the nucleus, B56δ, B56γ1 and B56γ3 (Gigena et al. 2005; McCright et al. 1996). Recent work demonstrated PP2A holoenzyme containing B56δ is regulated by PKA phosphorylation (Ahn et al. 2007). Whether PP2A associated with mAKAP complexes might also be regulated by PKA activity was tested. Native mAKAP complexes were immunoprecipitated from adult rat heart extracts and assayed for associated phosphatase activity (FIG. 12A). mAKAP-associated phosphatase activity was increased 2.5-fold by stimulation of bound PKA with the non-hydrolysable cAMP analog CPT-cAMP (lanes 2 & 3). As controls, all immunoprecipitated phosphatase activity was inhibited by 10 nM

OA (lane 4), and the CPT-cAMP-stimulated increase in phosphatase activity was blocked by the addition of the PKA inhibitor PKI (lane 5). Taken together, these data demonstrate that PP2A activity associated with mAKAP complexes in the heart is potentiated by PKA-dependent cAMP signaling.

[0260] Because mAKAP-bound PP2A was regulated by PKA activity, whether mAKAP-bound PP2A holoenzyme contained B56δ subunit was tested. Protein complexes were immunoprecipitated from adult rat heart extracts using B56δ and control (IgG) antibody (FIG. 12B). mAKAP was consistently immunoprecipitated with the B56δ antibody. In addition, Flag-tagged B56δ was expressed in HEK293 cells and showed that B56δ was immunoprecipitated with a mAKAP antibody only when co-expressed with (GFP-tagged) mAKAP (FIG. 12C). Finally, the binding of B56δ to mAKAP was shown to recruit PP2A-C subunit to the complex, because mAKAP complexes immunoprecipitated from HEK293 cell extracts were associated with greater phosphatase activity when GFP-mAKAP was co-expressed with Flag-B56δ (FIG. 12D, lanes 2 & 3). Based upon these results, B56δ recruits the PP2A-A/C core heterodimer to mAKAP complexes in the heart, conferring cAMP-dependent phosphatase activity. Accordingly, elevation of intracellular cAMP with Fsk and the phosphodiesterase inhibitor IBMX increased mAKAP-associated phosphatase activity in HEK293 cells, only when mAKAP was co-expressed with B56δ (FIG. 12E).

[0261] PKA Binding is required for cAMP-dependent PP2A activity in mAKAP complexes. Previous work found that PKA phosphorylates B56δ on four serine residues (53, 68, 81, 566), and Ser-566 is suggested to account for the induction of PP2A activity (Ahn et al. 2007). Since mAKAP complexes include both PKA and PP2A, association of these molecules into a complex appeared to be important for PP2A phosphorylation, just as PP2A binding to mAKAP was required for PDE4D3 de-phosphorylation (FIG. 11). To test this hypothesis, B56δ was expressed in HEK293 cells with wildtype full-length mAKAP or a full-length mAKAP mutant with an internal deletion of residues 2053-2073 comprising the PKA binding site (ΔPKA, FIG. 13A) (Pare, Bauman, et al. 2005). Following stimulation of the cells with Fsk/IBMX to elevate intracellular cAMP, mAKAP complexes were isolated by immunoprecipitation, and the phosphorylation state of B56δ was determined using a phospho-specific antibody to B56δ Ser-566 (FIG. 13A, top panel) (Ahn et al. 2007). B56δ phosphorylation was detected only after FSK/IBMX treatment and only when B56δ was co-expressed with wildtype mAKAP and not the ΔPKA mutant (FIG. 13A, lanes 2 & 6). As a control, equivalent expression of mutant and wildtype mAKAP and B56δ proteins was demonstrated by immunoblotting with non-phospho-specific antibodies (FIG. 13A, middle and bottom panels). Additionally, wildtype mAKAP was co-expressed with a mutant B56δ form containing alanine residues at each of the four PKA substrate sites (S4A). As expected, Fsk/IBMX stimulation did not induce phosphorylation of B56δ S4A (FIG. 13A lane 4). Since B56δ phosphorylation increases PP2A catalytic activity, the mAKAP antibody immunoprecipitates were assayed for phosphatase activity (FIG. 13B). Consistent with the results obtained using the phospho-specific B56δ antibody, cAMP elevation increased phosphatase activity in mAKAP complexes 1.7 fold (FIG. 13B, lanes 2 & 3). This increase required phosphorylation of

B56δ, as complexes containing the S4A mutant showed no augmentation of PP2A activity by increased cAMP (lane 5). Likewise, PKA binding to mAKAP was required to induce PP2A activity, as no increase was obtained when B56δ was co-expressed with the mAKAP ΔPKA mutant scaffold (lane 6). Interestingly, the Fsk/IBMX-induced increase in mAKAP-associated PP2A activity was not due to increased PP2A-C subunit binding to the mAKAP complexes (FIG. 13A, lanes 1 & 2). This result is in accord with an earlier suggestion that B56δ phosphorylation increases PP2A catalytic activity through conformational changes that do not affect holoenzyme formation (Ahn et al. 2007).

[0262] PP2A regulates PDE4D3 phosphorylation in a PKA-dependent manner. The results described above imply that PP2A dephosphorylation of PDE4D3 in B56δ-mAKAP complexes should be enhanced by PKA-catalyzed phosphorylation of the phosphatase. To address the role of B56δ phosphorylation in the regulation of PDE4D3, PDE4D3 and mAKAP were co-expressed with either wild-type B56δ or the B56δ S4A mutant that is not responsive to PKA. Cells were stimulated with Fsk before isolation of mAKAP complexes. As detected by phospho-specific antibody immunoblot and enzymatic assay, Fsk-stimulation of PDE4D3 Ser-54 phosphorylation and phosphodiesterase activity were only observed for mAKAP complexes containing wildtype B56δ when PP2A was inhibited with OA (FIGS. 14A & B, 1-3), consistent with aforementioned data (FIG. 8). In contrast, expression of B56δ S4A resulted in detectable Fsk-stimulated PDE4D3 phosphorylation (0.39 ± 0.15 fold of Fsk/OA-stimulated cells, FIG. 14A, lane 5) and a concomitant increase in phosphodiesterase activity (FIG. 14B, lane 5), albeit not as strongly as when PP2A activity was directly inhibited by OA (FIGS. 14A & B, lanes 3 & 6). Taken together with the results shown in FIGS. 12 & 13, anchoring of a PKA-stimulated PP2A holoenzyme is responsible for the attenuation of both basal and PKA-stimulated PDE4D3 activity in the mAKAP signaling complex.

Discussion

[0263] The results described herein define the biochemical mechanism for the dephosphorylation and inactivation of PKA-phosphorylated PDE4D3 bound by the scaffold protein mAKAP. A PP2A heterotrimer comprised of A-, C-, and B56δ-subunits binds a C-terminal site on mAKAP distinct from the binding sites for other known mAKAP partners (FIG. 10). The association of PP2A with the mAKAP scaffold is of functional significance in two important and novel ways. First, by binding both PP2A and PDE4D3, mAKAP sequesters the phosphatase in close proximity to the phosphodiesterase, allowing for efficient PDE4D3 dephosphorylation and down-regulation (FIG. 11). Second, by binding both PKA and PP2A, mAKAP promotes cAMP-dependent phosphorylation of the PP2A B56δ subunit and induction of PP2A activity (FIG. 13). The relevance of multimolecular signaling complex formation was evident upon expression of mAKAP mutants lacking binding sites for PP2A and PKA.

[0264] The concept of phosphatase targeting to generate substrate specificity was first proposed in the mid-1980's with the identification of the glycogen-particle-associated protein as the first PP1-targeting subunit (Bauman and Scott 2002). Since this initial observation, several other phosphatase targeting motifs have been determined (Virshup 2000). AKAPs represent an important mechanism to link

phosphatases with their appropriate substrates, and several AKAPs bind protein phosphatases. It has been recently published that mAKAP binds PP2B (calcineurin), and that this interaction is important for PP2B-dependent NFATc3 activation in myocytes (Li et al. 2009). However, PP2B binding to mAKAP does not appear to regulate PDE4D3, as inhibition of PP2B did not affect PDE4D3 Ser-54 phosphorylation or phosphodiesterase activity (FIG. 8). The present data support a unique role for PP2A bound to mAKAP in dephosphorylation of the phosphodiesterase and, as a result, in the control of local cAMP levels.

[0265] The overall role of phosphatases in regulating cellular cAMP concentration has yet to be fully explored. In rat adipocytes, PP2A was found to regulate both PDE3B activity and phosphorylation (Resjo et al. 1999). In addition to being phosphorylated by PKA on Ser-54, PDE4D3 is phosphorylated on Ser-579 by MAP kinases, including by ERK5 present in mAKAP complexes (Hoffmann et al. 1999; Dodge-Kafka et al. 2005). Although PP1 does not appear to bind mAKAP (FIG. 9 and FIG. 18), PP1 may dephosphorylate PDE4D3 Ser-579 in other cellular domains, since the addition of purified PP1 to isolated PDE4D3 decreased phosphorylation at this site. Phosphatase(s) are also responsible for the dephosphorylation of mAKAP-bound PDE4D3 at Ser-579, as well as the second PKA site on PDE4D3, Ser-16 (Carlisle Michel et al. 2004).

[0266] The anchoring hypothesis suggests that AKAPs function to target the actions of PKA towards specific substrates by localizing both proteins to the same signaling complex. Herein is demonstrated a new target for PKA in the mAKAP complex, the PP2A B56δ-subunit. Previous work found phosphorylation of B56δ stimulated PP2A activity and enhanced de-phosphorylation of DARPP-32 (Ahn et al. 2007). In accordance with these results, stimulation of cardiac myocytes with β-adrenergic receptor agonists increases PP2A activity (De Arcangelis, Soto, and Xiang 2008). The mAKAP scaffold may facilitate this event, as the association of the anchoring protein with both PKA and PP2A is important for the cAMP-enhanced increase in phosphatase activity (FIGS. 11 & 13). Hence, mAKAP has a role in the regulation of phosphatase activity in the heart.

[0267] Based upon these results, a model is proposed in which PP2A serves a dual role in regulating cAMP levels near mAKAP signaling complexes (FIG. 15). First, PP2A in mAKAP complexes should maintain PDE4D3 in a dephosphorylated, minimally active state in the absence of GPCR stimulation (FIG. 15A), presumably allowing for a more rapid rise in cAMP levels in response to agonist. Second, following induction of activating cAMP levels by GPCR stimulation, PKA will phosphorylate both PDE4D3 and PP2A (FIG. 15B). In contrast to the negative feedback on cAMP levels mediated by enhanced PDE4D3 phosphorylation, PKA phosphorylation of PP2A opposes PDE4D3 activation. By inhibiting PDE4D3 phosphorylation, PP2A presumably potentiates and prolongs the actions of local cAMP as part of a positive feedback loop. Thus, in conjunction with the potential inhibition of PDE4D3 by mAKAP-bound ERK5 that has been previously described (not illustrated) (Dodge-Kafka et al. 2005), the mAKAP signaling complex is poised to finely regulate local cAMP levels both by multiple feedback loops intrinsic to the complex, as well as by crosstalk with upstream MAPK signaling pathways. It has been observed that PP2A expression and intracellular localization are altered in heart failure

(Reiken et al. 2001; Ai and Pogwizd 2005). Whether PP2A-mediated positive feedback or PDE4D3-mediated negative feedback predominately controls cAMP levels local to mAKAP complexes may ultimately depend both on the stoichiometry of PP2A binding to mAKAP and the relative rates of PDE4D3 phosphorylation and dephosphorylation by PKA and PP2A in disease states.

[0268] The present examples demonstrate a novel mechanism by which the scaffold protein mAKAP maintains dynamic regulation of anchored PDE4D3 activity through the association with PDE4D3, PKA and PP2A. Each of the three enzymes plays an important role in the temporal control of cAMP concentration in the vicinity of perinuclear mAKAP complex. This intricate regulation of local cAMP by the mAKAP “signalosome” represents a broader role for AKAPs and phosphatase in the control of cAMP compartmentation.

Example 4

Use of PBD as a treatment for HFrEF

[0269] Heart failure, the common end-stage for cardiac disease, is a syndrome of major public health significance, affecting 6.5 million Americans, including 960,000 new cases each year (Benjamin et al. 2017). Symptomatic heart failure patients can be divided almost evenly into those with reduced (HFrEF) and those with preserved ejection fraction. First-line therapy for heart failure includes angiotensin-converting enzyme (ACE) inhibitors and β -adrenergic receptor blockers (β -blockers) that at least for HFrEF can improve survival and quality of life, as well as reduce mortality (Ponikowski et al. 2016). Despite these and other adjunct therapies, however, 5-year mortality remains about 50% for heart failure (39% in a 2016 post-myocardial infarction study) (Benjamin et al. 2017; Gerber et al. 2016), necessitating the discovery of new therapeutic approaches. Phosphorylation of SRF represents a novel mechanism regulating the transition from compensated hypertrophy to the dilated, failing heart in HFrEF.

[0270] As discussed above, expression of SRF S103D both in vitro and in vivo will promote concentric myocyte hypertrophy. In addition, expression of the PP2A anchoring disruptor PBD attenuated the eccentric hypertrophy induced by Iso-treatment of cultured adult myocytes (FIG. 20). These results suggest that SRF S¹⁰³ phosphorylation drives growth in width, while attenuating any elongation of the cardiac myocyte. Given these results and the association of SRF dephosphorylation with systolic dysfunction induced by long term pressure overload (FIG. 33A, E), restoration of normal or increased SRF phosphorylation will prevent the ventricular dilatation resulting in HFrEF in diseases of chronic pressure overload and ischemic heart disease.

[0271] Mechanisms that induce “compensatory” concentric hypertrophy early in heart disease predispose the heart to later systolic dysfunction and eventual failure (Schiattarella and Hill 2015). In this regard, targeting of RSK3-mAKAP β complexes will attenuate cardiac remodeling due to pressure overload and prevent heart failure (Kritzer et al. 2014; Li, Kritzer, et al. 2013). While inhibition of signaling pathways that induce remodeling, including concentric hypertrophy, may be desirable early in disease, the question remains whether efforts to maintain signals promoting concentric and attenuating eccentric myocyte hypertrophy would preserve cardiac volumes and contractility when initiated when the heart is at a stage in the disease process

characterized by the eccentric growth and ventricular dilatation leading to HFrEF. Accordingly, maintaining SRF phosphorylation is a strategy to block the eccentric changes in ventricular morphology that typify end-stage disease and HFrEF. The fact that maintaining SRF phosphorylation is a strategy to block the eccentric changes in ventricular morphology that typify end-stage disease and HFrEF is further supported by new observations by the present inventors that SRF phosphorylation is increased in mice subjected to acute pressure overload and reduced in mice and humans undergoing ventricular dilation. Phosphorylated SRF was increased 28% in total left ventricular extracts (which includes about one-third myocytes by cell number) within 5 minutes after induction of pressure overload (FIG. 33 A, B), when RSK3 activation, as detected by S²¹⁸ phosphorylation, was increased 1.9-fold (FIG. 33 C). Remarkably, 16 weeks after transverse aortic constriction surgery, when the hearts were dilated and the mice were in heart failure (FIG. 33 D), phosphorylated SRF was suppressed 30% below that present in sham-operated controls (FIG. 33E). These results are consistent with a phosphatase being responsible for dephosphorylating SRF during the induction of eccentric hypertrophy, opposing RSK3-catalyzed phosphorylation. The relevance of these findings to human disease was assessed using patient tissue samples. When compared to SRF Ser¹⁰³ phosphorylation in left ventricular tissue from patients with normal left ventricular interior diameter, SRF Ser¹⁰³ phosphorylation in patients with dilated hearts was reduced 53% (p=0.005, FIG. 33F-H).

[0272] Improved ventricular geometry, i.e., decreased LV internal diameters due to less elongated myocytes and/or increased LV wall thickness due to wider myocytes, will decrease wall stress (Law of LaPlace) and improve systolic function in the heart prone to HFrEF. The prevention of systolic dysfunction has been obtained for a new AAV gene therapy vector based upon expression of the mAKAP β -derived PBD (FIG. 22).

[0273] Treatment of Myocardial Infarction. Coronary heart disease is a leading cause of HFrEF (Writing Group et al. 2016). 8-week old C57BL/6 WT mice were subjected to permanent LAD ligation or sham thoracotomy. Two days post-operatively, heart function was evaluated by echocardiography and the mice were randomized by EF and body weight (FIG. 23B). Two cohorts of mice to be treated with either AAVsc.myc-PBD (n=8) or AAVsc.GFP (n=5) were defined that had average ejection fraction=34% 2-days after LAD ligation (FIG. 23D). Mice were injected via the tail vein 3 days post-operatively with 5 \times 10¹¹ vg. While control GFP mice exhibited progressively decreased ejection fraction (EF to 21%), PBD mice exhibited long term restoration of systolic function (EF at 8 weeks post-operatively=43%; p<0.0001). In addition, AAVsc.myc-PBD treated mice had reduced left ventricular volumes consistent with improved cardiac function (systole—69 μ l for PBD vs 156 μ l for GFP, p<0.001; diastole—118 μ l vs. 192 μ l; p<0.001). At end-point, gravimetrically, ventricular and atrial hypertrophy were reduced (p=0.053 and 0.024, respectively, indexed to tibial length, FIG. 23C), and pulmonary edema, a sign of heart failure, tended to be improved (p=0.078). These results demonstrate that PP2A anchoring disruptor therapy, that displaces PP2A from mAKAP β where it can dephosphorylate SRF, constitutes a novel therapeutic approach for the prevention of heart failure with reduced ejection fraction in ischemic heart disease.

Methods:

[0274] General Method for Ligation of the Left Coronary Artery: The mice were anesthetized with 5% isoflurane for induction and then 2.5-3% for maintenance. Orotracheal intubation was performed using a 16G catheter, and the mouse then ventilated mechanically using a minivent ventilator. The skin over the site of left lateral thoracotomy was prepped and draped in sterile fashion using providone-iodine 10% solution. A heating pad was used to keep mice warm during procedures to prevent heat loss. Surgically sterile non-medicated ophthalmic ointment was applied to the eyes preoperatively to prevent corneal drying. Surgery was performed under microscope view. Once adequate sedation was achieved, the chest was opened via left lateral thoracotomy at the fourth intercostal space. If muscle bleeding was present, hemostasis was achieved by the using a thermal cauterizer (e.g. fine tip Bovie). A 3 mm retractor was used to separate the ribs. Following pericardiotomy, the left coronary artery was ligated with a 7-0 prolene suture to produce an anterior MI. The chest was closed in 3 layers with 5-0 absorbable suture (muscle) and silk 6-0 (for 2 ligatures in the ribs and for the skin). Buprenorphine slow release (Bup-SR-LAB) 0.5-1 mg/kg s.c. was administered in a single dose immediately after surgery to control pain for 72 hr. Fluid replacement was administered immediately after surgery (e.g. Sterile saline solution 0.9%, IP). The mice were allowed to recover until alert and active. Sham-operated mice that experience all but the placement of the coronary artery ligation served as controls.

[0275] Echocardiography: Mice minimally anesthetized with 1-2% isoflurane were studied using a Vevo 2100®, High-Resolution Imaging System (VisualSonics). M-mode images were obtained for mice under anesthesia at various time-points. Posterior wall and anterior wall diastolic and systolic thicknesses and left ventricular cavity end-diastolic (LVEDD) and end-systolic diameters (LVESD) were measured, permitting estimation of LV volumes, fractional shortening and ejection fraction.

[0276] The patent and scientific literature referred to herein establishes the knowledge that is available to those with skill in the art. All United States patents and published or unpublished United States patent applications cited herein are incorporated by reference. All published foreign patents and patent applications cited herein are hereby incorporated by reference. All other published references, documents, manuscripts and scientific literature cited herein are hereby incorporated by reference.

[0277] While this invention has been particularly shown and described with references to preferred embodiments thereof, it will be understood by those skilled in the art that various changes in form and details may be made therein without departing from the scope of the invention encompassed by the appended claims.

REFERENCES

- [0278] Using siRNA for gene silencing is a rapidly evolving tool in molecular biology, ThermoFisher Scientific, retrieved Jun. 16, 2017 <<https://www.thermofisher.com/us/en/home/references/ambion-tech-support/rnai-sirna/general-articles/-sirna-design-guidelines.html>>.
- [0279] Abrenica B, Alshaaban M, Czubryt M P. The A-kinase anchor protein AKAP121 is a negative regulator of cardiomyocyte hypertrophy. *J Mol Cell Cardiol* 46: 674-681, 2009.
- [0280] Ahn J H, McAvoy T, Rakhilin S V, Nishi A, Greengard P, Nairn A C (2007) Protein kinase A activates protein phosphatase 2A by phosphorylation of the B56delta subunit. *Proc Natl Acad Sci USA* 104:2979-2984.
- [0281] Ai X, Pogwizd S M (2005) Connexin 43 down-regulation and dephosphorylation in nonischemic heart failure is associated with enhanced colocalized protein phosphatase type 2A. *Circ Res* 96:54-63.
- [0282] Andino L M, Conlon T J, Porvasnik S L, Boye S L, Hauswirth W W, Lewin A S (2007) Rapid, widespread transduction of the murine myocardium using self-complementary Adeno-associated virus. *Genetic vaccines and therapy* 5:13.
- [0283] Anjum R, Blenis J. The RSK family of kinases: emerging roles in cellular signalling. *Nat Rev Mol Cell Biol*. 2008; 9(10):747-758.
- [0284] Appert-Collin A, Cotecchia S, Nenniger-Tosato M, Pedrazzini T, Diviani D. The A-kinase anchoring protein (AKAP)-Lbc-signaling complex mediates alpha1 adrenergic receptor-induced cardiomyocyte hypertrophy. *Proc Natl Acad Sci USA* 104: 10140-10145, 2007.
- [0285] Avkiran M, Cook A R, Cuello F. Targeting Na+/H+ exchanger regulation for cardiac protection: a RSKy approach? *Curr Opin Pharmacol*. 2008; 8:133-140.
- [0286] Bain J, Plater L, Elliott M, Shpiro N, Hastie C J, McLauchlan H, Klevernic I, Arthur J S, Alessi D R, Cohen P. The selectivity of protein kinase inhibitors: a further update. *Biochem J*. 2007; 408:297-315.
- [0287] Backs J, Worst B C, Lehmann L H, Patrick D M, Jebessa Z, Kreusser M M, Sun Q, Chen L, Heft C, Katus H A, Olson E N (2011) Selective repression of MEF2 activity by PKA-dependent proteolysis of HDAC4. *J Cell Biol* 195:403-415.
- [0288] Bauman A L, Scott J D (2002) Kinase- and phosphatase-anchoring proteins: harnessing the dynamic duo. *Nat Cell Biol* 4:E203-206.
- [0289] Bauman A L, Michel J J, Henson E, Dodge-Kafka K L, Kapiloff M S, "The mAKAP signalosome and cardiac myocyte hypertrophy," *IUBMB Life*. 2007 March; 59(3):163-9. Review.
- [0290] Beavo J A, Bechtel P J, Krebs E G (1974) Preparation of homogeneous cyclic AMP-dependent protein kinase(s) and its subunits from rabbit skeletal muscle. *Methods Enzymol* 38:299-308.
- [0291] Beene D L, Scott J D. A-kinase anchoring proteins take shape. *Curr Opin Cell Biol* 19: 192-198, 2007.
- [0292] Benjamin E J et al. (2017) Heart Disease and Stroke Statistics-2017 Update: A Report From the American Heart Association. *Circulation* 135:e146-e603.
- [0293] Benjamin E J et al. (2019) Heart Disease and Stroke Statistics-2019 Update: A Report From the American Heart Association. *Circulation* 139: e56-e528.
- [0294] Bers D M (2006) Cardiac ryanodine-receptor phosphorylation: target sites and functional consequences. *Biochem J* 396:e1-3.
- [0295] Bers D M. Calcium cycling and signaling in cardiac myocytes. *Annu Rev Physiol* 70: 23-49, 2008.
- [0296] Bione S, Maestrini E, Rivella S, Mancini M, Regis S, Romeo G, Toniolo D (1994) Identification of a novel X-linked gene responsible for Emery-Dreifuss muscular dystrophy. *Nat Genet* 8:323-327.

- [0297] Black B L, Olson E N (1998) Transcriptional control of muscle development by myocyte enhancer factor-2 (MEF2) proteins. *Annu Rev Cell Dev Biol* 14:167-196.
- [0298] Bonne G, Di Barletta M R, Vamous S, Becane H M, Hammouda E H, Merlini L, Muntoni F, Greenberg C R, Gary F, Urtizberea J A, Duboc D, Fardeau M, Toniolo D, Schwartz K (1999) Mutations in the gene encoding lamin N C cause autosomal dominant Emery-Dreifuss muscular dystrophy. *Nat Genet* 21:285-288.
- [0299] Bourajjaj M, Armand A S, da Costa Martins P A, Weijts B, van der Nagel R, Heeneman S, Wehrens X H, De Windt L J (2008) NFATc2 is a necessary mediator of calcineurin-dependent cardiac hypertrophy and heart failure. *J Biol Chem* 283:22295-22303.
- [0300] Brown J H, Del Re D P, Sussman M A. The Rac and Rho hall of fame: a decade of hypertrophic signaling hits. *Circ Res* 98: 730-742, 2006.
- [0301] Burns-Hamuro L L, Ma Y, Kammerer S, Reineke U, Self C, Cook C, Designing isoform-specific peptide disruptors of protein kinase Alocalization. *Proc Natl Acad Sci USA*. 2003 Apr; 100(7):4072-7.
- [0302] Brunton L L, Hayes J S, Mayer S E (1979) Hormonally specific phosphorylation of cardiac troponin I and activation of glycogen phosphorylase. *Nature* 280: 78-80.
- [0303] Buck M, Chojkier M. C/EBPbeta-Thr217 phosphorylation signaling contributes to the development of lung injury and fibrosis in mice. *PLoS One*. 2011; 6(10): e25497.
- [0304] Bueno O F, Wilkins B J, Tymitz K M, Glascock B J, Kimball T F, Lorenz J N, Molkentin J D (2002) Impaired cardiac hypertrophic response in Calcineurin Abeta-deficient mice. *Proc Natl Acad Sci USA* 99:4586-4591.
- [0305] Bueno O F, Lips D J, Kaiser R A Wilkins B J, Dai Y S, Glascock B J, Klevitsky R, Hewett T E, Kimball T R, Aronow B J, Doevidans P A, Molkentin J D (2004) Calcineurin Abeta gene targeting predisposes the myocardium to acute ischemia-induced apoptosis and dysfunction. *Circ Res* 94:91-99.
- [0306] Burchfield J S, Xie M, Hill J A (2013) Pathological ventricular remodeling: mechanisms: part 1 of 2. *Circulation* 128:388-400.
- [0307] Cappola T P. Molecular remodeling in human heart failure. *J Am Coll Cardiol* 51: 137-138, 2008.
- [0308] Cariolato L, Cavin S, Diviani D. A-kinase anchoring protein (AKAP)-Lbc anchors a PKN-based signaling complex involved in alpha1-adrenergic receptor-induced p38 activation. *J Biol Chem* 286: 7925-7937, 2011.
- [0309] Carlisle Michel J J, Dodge K L, Wong W, Mayer N C, Langeberg L K, Scott J D (2004) PKA-phosphorylation of PDE4D3 facilitates recruitment of the mAKAP signalling complex. *Biochem J* 381:587-592.
- [0310] Carlucci A, Lignitto L, Feliciello A. Control of mitochondria dynamics and oxidative metabolism by cAMP, AKAPs and the proteasome. *Trends Cell Biol* 18: 604-613, 2008.
- [0311] Carnegie G K, Smith F D, McConnachie G, Langeberg L K, Scott J D. AKAP-Lbc nucleates a protein kinase D activation scaffold. *Mol Cell* 15: 889-899, 2004.
- [0312] Carnegie G K, Soughayer J, Smith F D, Pedroja B S, Zhang F, Diviani D, Bristow M R, Kunkel M T, Newton A C, Langeberg L K, Scott J D. AKAP-Lbc mobilizes a cardiac hypertrophy signaling pathway. *Mol Cell* 32: 169-179, 2008.
- [0313] Chaturvedi D, Poppleton H M, Stringfield T, Barber A, Patel T B. Subcellular localization and biological actions of activated RSK1 are determined by its interactions with subunits of cyclic AMP-dependent protein kinase. *Mol Cell Biol*. 2006; 26:4586-4600.
- [0314] Chen L, Kurokawa J, Kass R S. Phosphorylation of the A-kinase anchoring protein Yotiao contributes to protein kinase A regulation of a heart potassium channel. *J Biol Chem* 280: 31347-31352, 2005.
- [0315] Chen L, Kurokawa J, Kass R S. Phosphorylation of the A-kinase-anchoring protein Yotiao contributes to protein kinase A regulation of a heart potassium channel. *J Biol Chem* 280: 31347-31352, 2005.
- [0316] Chen L, Marquardt M L, Tester D J, Sampson K J, Ackerman M J, Kass R S. Mutation of an A-kinase-anchoring protein causes long-Q T syndrome. *Proc Natl Acad Sci USA* 104: 20990-20995, 2007.
- [0317] Chen P P, Patel J R, Rybakova I N, Walker J W, Moss R L. Protein kinase A-induced myofilament desensitization to Ca²⁺ as a result of phosphorylation of cardiac myosin-binding protein C. *J Gen Physiol* 136: 615-627, 2010.
- [0318] Christian F, Szaszak M, Friedl S, Drewianka S, Lorenz D, Goncalves A, Furkert J, Vargas C, Schmieder P, Gotz F, Zuhlke K, Moutty M, Gottert H, Joshi M, Reif B, Haase H, Morano I, Grossmann S, Klukovits A, Verli J, Gaspar R, Noack C, Bergmann M, Kass R, Hampel K, Kashin D, Genieser H G, Herberg F W, Willoughby D, Cooper D M, Baillie G S, Houslay M D, von Kries J P, Zimmermann B, Rosenthal W, Klussmann E. Small molecule AKAP-protein kinase A (PKA) interaction disruptors that activate PKA interfere with compartmentalized cAMP signaling in cardiac myocytes. *J Biol Chem* 286: 9079-9096, 2011.
- [0319] Clerk A, Cullingford T E, Fuller S J, Giraldo A, Markou T, Pikkarainen S, Sugden P H (2007) Signaling pathways mediating cardiac myocyte gene expression in physiological and stress responses. *J Cell Physiol* 212: 311-322.
- [0320] Consensus (1987). "Effects of enalapril on mortality in severe congestive heart failure. Results of the Cooperative North Scandinavian Enalapril Survival Study (CONSENSUS)." *N Engl J Med* 316(23): 1429-1435.
- [0321] Cuello F, Snabaitis A K, Cohen M S, Taunton J, Avkiran M. Evidence for direct regulation of myocardial Na+/H⁺ exchanger isoform 1 phosphorylation and activity by 90-kDa ribosomal S6 kinase (RSK): effects of the novel and specific RSK inhibitor fink on responses to alpha1-adrenergic stimulation. *Mol Pharmacol*. 2007; 71:799-806.
- [0322] De Arcangelis V, Soto D, Xiang Y (2008) Phosphodiesterase 4 and phosphatase 2A differentially regulate cAMP/protein kinase a signaling for cardiac myocyte contraction under stimulation of betal adrenergic receptor. *Mol Pharmacol* 74:1453-1462.
- [0323] Diviani D, Abuin L, Cotecchia S, Pansier L. Anchoring of both PKA and 14-3-3 inhibits the Rho-GEF activity of the AKAP-Lbc signaling complex. *EMBO J* 23: 2811-2820, 2004.

- [0324] Diviani D, Dodge-Kafka K L, Li J, Kapiloff M S. A-kinase anchoring proteins: scaffolding proteins in the heart." Am J Physiol Heart Circ Physiol. 2011 November; 301(5):H1742-53.
- [0325] Dobrev D, Wehrens X H (2014) Role of RyR2 phosphorylation in heart failure and arrhythmias: Controversies around ryanodine receptor phosphorylation in cardiac disease. Circ Res 114:1311-1319; discussion 1319.
- [0326] Dodge-Kafka, K. L., M. Gildart, J. Li, H. Thakur, and M. S. Kapiloff. 2018. 'Bidirectional regulation of HDAC5 by mAKAPbeta signalosomes in cardiac myocytes', *Journal of Molecular and Cellular Cardiology*, 118: 13-25.
- [0327] Dodge-Kafka, K. L., A. Bauman, N. Mayer, E. Henson, L. Heredia, J. Ahn, T. McAvoy, A. C. Nairn and M. S. Kapiloff (2010). "cAMP-stimulated protein phosphatase 2A activity associated with muscle A kinase-anchoring protein (mAKAP) signaling complexes inhibits the phosphorylation and activity of the cAMP-specific phosphodiesterase PDE4D3." J Biol Chem 285(15): 11078-11086.
- [0328] Dodge-Kafka, K. L. and M. S. Kapiloff (2006). "The mAKAP signaling complex: integration of cAMP, calcium, and MAP kinase signaling pathways." Eur J Cell Biol 85(7): 593-602.
- [0329] Dodge-Kafka, K. L., J. Soughayer, G. C. Pare, J. J. Carlisle Michel, L. K. Langeberg, M. S. Kapiloff and J. D. Scott (2005). "The protein kinase A anchoring protein mAKAP coordinates two integrated cAMP effector pathways." Nature 437(7058): 574-578.
- [0330] Dodge, K. L., S. Khouangsathe, M. S. Kapiloff, R. Mouton, E. V. Hill, M. D. Houslay, L. K. Langeberg and J. D. Scott (2001). "mAKAP assembles a protein kinase A/PDE4 phosphodiesterase cAMP signaling module." EMBO J 20(8): 1921-1930.
- [0331] Diviani D, Soderling J, Scott J D. AKAP-Lbc anchors protein kinase A and nucleates Galpha 12-selective Rho-mediated stress fiber formation. J Biol Chem 276: 44247-44257, 2001.
- [0332] Dodge K L, Khouangsathe S, Kapiloff M S, Mouton R, Hill E V, Houslay M D, Langeberg L K, Scott J D. mAKAP assembles a protein kinase A/PDE4 phosphodiesterase cAMP signaling module. EMBO J 20: 1921-1930, 2001.
- [0333] Dodge-Kafka K L, Bauman A, Kapiloff M S, A-kinase anchoring proteins as the basis for cAMP signaling." Handb Exp Pharmacol. 2008; (186):3-14.
- [0334] Dodge-Kafka K L, Bauman A, Mayer N, Henson E, Heredia L, Ahn J, McAvoy T, Nairn A C, Kapiloff M S. cAMP-stimulated protein phosphatase 2A activity associated with muscle A kinase-anchoring protein (mAKAP) signaling complexes inhibits the phosphorylation and activity of the cAMP-specific phosphodiesterase PDE4D3. J Biol Chem. 2010; 285:11078-11086.
- [0335] Dodge-Kafka K L, Kapiloff M S, "The mAKAP signaling complex: integration of cAMP, calcium, and MAP kinase signaling pathways." Eur J Cell Biol. 2006 July; 85(7):593-602. Epub 2006 Feb. 7. Review.
- [0336] Dodge-Kafka K L, Langeberg L, Scott J D (2006) Compartmentation of cyclic nucleotide signaling in the heart: the role of A-kinase anchoring proteins. Circ Res 98:993-1001.
- [0337] duBell W H, Lederer W J, Rogers T B (1996) Dynamic modulation of excitation-contraction coupling by protein phosphatases in rat ventricular myocytes. J Physiol 493 (Pt 3):793-800.
- [0338] duBell W H, Gigena M S, Guatimosim S, Long X, Lederer W J, Rogers T B (2002) Effects of PP1/PP2A inhibitor calyculin A on the E-C coupling cascade in murine ventricular myocytes. Am J Physiol Heart Circ Physiol 282:H38-48.
- [0339] Dulhunty A F, Beard N A, Pouliquen P, Casarotto Bond M. AKAP-mediated targeting of protein kinase a regulates contractility in cardiac myocytes. Circ Res 88: 291-297, 2001. Fischmeister R, Castro L R, Abi-Gerges A, Rochais F, Jurevicius J, Leroy J,
- [0340] Dummler B A, Hauge C, Silber J, Yntema H G, Kruse L S, Kofoed B, Hemmings B A, Alessi D R, Frodin M. Functional characterization of human RSK4, a new 90-kDa ribosomal S6 kinase, reveals constitutive activation in most cell types. J Biol Chem. 2005; 280:13304-13314
- [0341] Edgley A J, Krum H, Kelly D J. Targeting fibrosis for the treatment of heart failure: a role for transforming growth factor-beta. Cardiovasc Ther. 2012; 30(1):e30-40.
- [0342] Eide T, Coghan V, Orstavik S, Holsve C, Solberg R, Skalegg B S, Lamb N J, Langeberg L, Fernandez A, Scott J D, Jahnson T, Tasken K. Molecular cloning, chromosomal localization, and cell cycle-dependent subcellular distribution of the A-kinase anchoring protein, AKAP95. Exp Cell Res 238: 305-316, 1998.
- [0343] Elbashir S M, Martinez J, Patkaniowska A, Lendeckel W, Tuschl T, Functional anatomy of SiRNAs for mediating efficient RNAi in *Drosophila melanogaster* embryo lysate, The EMBO Journal, Vol. 20, No. 23, pp. 6877-6888, 2001.
- [0344] Endo S, Zhou X, Connor J, Wang B, Shenolikar S (1996) Multiple structural elements define the specificity of recombinant human inhibitor-1 as a protein phosphatase-1 inhibitor. Biochemistry 35:5220-5228.
- [0345] Escobar M, Cardenas C, Colavita K, Petrenko N B, Franzini-Armstrong C. Structural evidence for peri-nuclear calcium microdomains in cardiac myocytes. J Mol Cell Cardiol 50: 451-459, 2011.
- [0346] Fabiato A. Calcium-induced release of calcium from the cardiac sarco-plasmic reticulum. Am J Physiol Cell Physiol 245: C1-C14, 1983.
- [0347] Farah C S, Reinach F C. The troponin complex and regulation of muscle contraction. FASEB J 9: 755-767, 1995.
- [0348] Fatkin D, MacRae C, Sasaki T, Wolff M R, Porcu M, Frenneaux M, Atherton J, Vidaillet H J, Jr., Spudich S, De Girolami U, Seidman J G, Seidman C, Muntoni F, Muehle G, Johnson W, McDonough B (1999) Missense mutations in the rod domain of the lamin N C gene as causes of dilated cardiomyopathy and conduction-system disease. N Engl J Med 341:1715-1724.
- [0349] Faul C, Dhume A, Scheeter A D, Mundel P. Protein kinase A, Ca²⁺/calmodulin-dependent kinase II, and calcineurin regulate the intracellular trafficking of myopodin between the Z-disc and the nucleus of cardiac myocytes. Mol Cell Biol 27: 8215-8227, 2007.
- [0350] Fink M A, Zakhary D R, Mackey J A, Desnoyer R W, Apperson-Hansen C, Damron D S, Bond M. AKAP-mediated targeting of protein kinase a regulates contractility in cardiac myocytes. Circ Res 88: 291-297, 2001.

- Fischmeister R, Castro L R, Abi-Gerges A, Rochais F, Jurevicius J, Leroy J, Vandecasteele G (2006) Compartmentation of cyclic nucleotide signaling in the heart: the role of cyclic nucleotide phosphodiesterases. *Circ Res* 99:816-828.
- [0351] Fodstad H, Swan H, Laitinen P, Piippo K, Paavonen K, Viitasalo M, Toivonen L, Kontula K. Four potassium channel mutations account for 73% of the genetic spectrum underlying long-QT syndrome (LQTS) and provide evidence for a strong founder effect in Finland. *Ann Med* 36, Suppl 1: 53-63, 2004.
- [0352] Francis S H, Corbin J D. Structure and function of cyclic nucleotide-dependent protein kinases. *Annu Rev Physiol* 56: 237-272, 1994.
- [0353] Fraser I D, Tavalin S J, Lester L B, Langeberg L K, Westphal A M, Dean R A, Marrion N V, Scott J D. A novel lipid-anchored A-kinase anchoring protein facilitates cAMP-responsive membrane events. *EMBO J* 17: 2261-2272, 1998.
- [0354] Frey N, Katus H A, Olson E N, Hill J A. Hypertrophy of the heart: a new therapeutic target? *Circulation* 109: 1580-1589, 2004.
- [0355] Friday B B, Mitchell P O, Kegley K M, Pavlath G K (2003) Calcineurin initiates skeletal muscle differentiation by activating MEF2 and MyoD. *Differentiation* 71:217-227.
- [0356] Fuller M D, Emrick M A, Sadilek M, Scheuer T, Catterall W A. Molecular mechanism of calcium channel regulation in the fight-or-flight response. *Sci Signal* 3: ra70, 2010.
- [0357] Gaffin R D, Pena J R, Alves M S, Dias F A, Chowdhury S A, Heinrich L S, Goldspink P H, Kranias E G, Wieczorek D F, Wolska B M. Long-term rescue of a familial hypertrophic cardiomyopathy caused by a mutation in the thin filament protein, tropomyosin, via modulation of a calcium cycling protein. *J. Mol. Cell. Cardiol.* 2011.
- [0358] Gao T, Yatani A, Dell'Acqua M L, Sako H, Green S A, Dascal N, Scott J D, Hosey M M. cAMP-dependent regulation of cardiac L-type Ca²⁺ channels requires membrane targeting of PKA and phosphorylation of channel subunits. *Neuron* 19: 185-196, 1997.
- [0359] Gao Y, Dickerson J B, Guo F, Zheng J, Zheng Y. Rational design and characterization of a Rac GTPase-specific small molecule inhibitor. *Proc Natl Acad Sci USA* 101: 7618-7623, 2004.
- [0360] Gelb B D, Tartaglia M. RAS signaling pathway mutations and hypertrophic cardiomyopathy: getting into and out of the thick of it. *J Clin Invest.* 2011; 121:844-847.
- [0361] Gentilucci L, Tolomelli A, Squassabia F. Peptides and peptidomimetics in medicine, surgery and biotechnology. *Curr Med Chem* 13: 2449-2466, 2006.
- [0362] Gerber, Y., S. A. Weston, M. Enriquez-Sarano, C. Berardi, A. M. Chamberlain, S. M. Manemann, R. Jiang, S. M. Dunlay and V. L. Roger (2016). "Mortality Associated With Heart Failure After Myocardial Infarction: A Contemporary Community Perspective." *Circ Heart Fail* 9(1): e002460.
- [0363] Gigena M S, Ito A, Nojima H, Rogers T B (2005) A B56 regulatory subunit of protein phosphatase 2A localizes to nuclear speckles in cardiomyocytes. *Am J Physiol Heart Circ Physiol* 289:H285-294.
- [0364] Go A S et al. (2014) Heart disease and stroke statistics—2014 update: a report from the American Heart Association. *Circulation* 129:e28-e292.
- [0365] Gold M G, Lygren B, Dokurno P, Hoshi N, McConnachie G, Tasken K, Carlson C R, Scott J D, Barford D. Molecular basis of AKAP specificity for PKA regulatory subunits. *Mol Cell* 24: 383-395, 2006.
- [0366] Goldschmidt-Clermont P J, Seo D M, Wang L, Beecham G W, Liu Z J, Vazquez-Padron R I, Dong C, Hare J M, Kapiloff M S, Bishopric N H, Pericak-Vance M, Vance J M, Velazquez O C, "Inflammation, stem cells and atherosclerosis genetics," *Curr Opin Mol Ther.* 2010 December; 12(6):712-23. Review.
- [0367] Good M C, Zalatan J G, Lim W A. Scaffold proteins: hubs for controlling the flow of cellular information. *Science.* 2011; 332:680-686.
- [0368] Gould K L, Bretscher A, Esch F S, Hunter T. cDNA cloning and sequencing of the protein-tyrosine kinase substrate, ezrin, reveals homology to band 4.1. *EMBO J* 8: 4133-4142, 1989. Gray P C, Scott J D, Catterall W A. Regulation of ion channels by cAMP-dependent protein kinase and A-kinase anchoring proteins. *Curr Opin Neurobiol* 8: 330-334, 1998.
- [0369] Grossman W, Jones D, McLaurin L P (1975) Wall stress and patterns of hypertrophy in the human left ventricle. *J Clin Invest* 56:56-64.
- [0370] Group, Consensus Trial Study. 1987. 'Effects of enalapril on mortality in severe congestive heart failure. Results of the Cooperative North Scandinavian Enalapril Survival Study (CONSENSUS)', *New England Journal of Medicine*, 316: 1429-35.
- [0371] Guo T, Cornea R L, Huke S, Camors E, Yang Y, Picht E, Fruen B R, Bers D M. Kinetics of FKBP12.6 binding to ryanodine receptors in permeabilized cardiac myocytes and effects on Ca sparks. *Circ Res* 106: 1743-1752, 2010.
- [0372] Guo, H., B. Liu, L. Hou, E. The, G. Li, D. Wang, Q. Jie, W. Che and Y. Wei (2015). "The role of mAKAP-beta in the process of cardiomyocyte hypertrophy induced by angiotensin II." *Int J Mol Med* 35(5): 1159-1168.
- [0373] Hagemann D, Xiao R P. Dual site phospholamban phosphorylation and its physiological relevance in the heart. *Trends Cardiovasc Med* 12: 51-56, 2002.
- [0374] Hanks S K, Quinn A M, Hunter T. The protein kinase family: conserved features and deduced phylogeny of the catalytic domains. *Science.* 1988; 241:42-52.
- [0375] Hanlon M, Sturgill T W, Sealy L (2001) ERK2- and p90(Rsk2)-dependent pathways regulate the CCAAT/enhancer-binding protein-beta interaction with serum response factor. *J Biol Chem* 276:38449-38456.
- [0376] Harada H, Becknell B, Wilm M, Mann M, Huang L J, Taylor S S, Scott J D, Korsmeyer S J. Phosphorylation and inactivation of BAD by mitochondria-anchored protein kinase A. *Mol Cell* 3: 413-422, 1999.
- [0377] Hayes J S, Brunton L L, Mayer S E (1980) Selective activation of particulate cAMP-dependent protein kinase by isoproterenol and prostaglandin El. *J Biol Chem* 255:5113-5119.
- [0378] Heidenreich, P. A., N. M. Albert, L. A. Allen, D. A. Bluemke, J. Butler, G. C. Fonarow, J. S. Ikonomidis, O. Khavjou, M. A. Konstam, T. M. Maddox, G. Nichol, M. Pham, I. L. Pina, J. G. Trogdon, C. American Heart Association Advocacy Coordinating, T. Council on Arteriosclerosis, B. Vascular, R. Council on Cardiovascular,

- Intervention, C. Council on Clinical, E. Council on, Prevention and C. Stroke (2013). "Forecasting the impact of heart failure in the United States: a policy statement from the American Heart Association." *Circ Heart Fail* 6(3): 606-619.
- [0379] Heineke J, Molkentin J D (2006) Regulation of cardiac hypertrophy by intracellular signaling pathways. *Nat Rev Mol Cell Biol* 7:589-600.
- [0380] Hell J W. Beta-adrenergic regulation of the L-type Ca^{2+} channel CaV1.2 by PKA rekindles excitement. *Sci Signal* 3: pe33, 2010.
- [0381] Henn V, Edemir B, Stefan E, Wiesner B, Lorenz D, Theilig F, Schmitt R, Vossebein L, Tamme G, Beyermann M, Krause E, Herberg F W, Valenti G, Bachmann S, Rosenthal W, Klussmann E. Identification of a novel A-kinase anchoring protein 18 isoform and evidence for its role in the vasopressin-induced aquaporin-2 shuttle in renal principal cells. *J Biol Chem* 279: 26654-26665, 2004.
- [0382] Hill J A, Olson E N. Cardiac plasticity. *N Engl J Med* 358: 1370-1380, 2008.
- [0383] Ho S N, Thomas D J, Timmerman L A, Li X, Francke U, Crabtree G R (1995) NFATc3, a lymphoid-specific NFATc family member that is calcium-regulated and exhibits distinct DNA binding specificity. *J Biol Chem* 270:19898-19907.
- [0384] Hoffmann R, Baillie G S, MacKenzie S J, Yarwood S J, Houslay M D (1999) The MAP kinase ERK2 inhibits the cyclic AMP-specific phosphodiesterase HSPDE4D3 by phosphorylating it at Ser579. *EMBO J* 18:893-903.
- [0385] Houser S R (2014) Role of RyR2 phosphorylation in heart failure and arrhythmias: protein kinase A-mediated hyperphosphorylation of the ryanodine receptor at serine 2808 does not alter cardiac contractility or cause heart failure and arrhythmias. *Circ Res* 114:1320-1327; discussion 1327.
- [0386] Huang L J, Durick K, Weiner J A, Chun J, Taylor S S. D-AKAP2, a novel protein kinase A anchoring protein with a putative RGS domain. *Proc Natl Acad Sci USA* 94: 11184-11189, 1997.
- [0387] Huang L J, Durick K, Weiner J A, Chun J, Taylor S S. Identification of a novel dual specificity protein kinase A anchoring protein, D-AKAP1. *J Biol Chem* 272: 8057-8064, 1997.
- [0388] Huang L J, Durick K, Weiner J A, Chun J, Taylor S S. Identification of a novel protein kinase A anchoring protein that binds both type I and type II regulatory subunits. *J Biol Chem*. 1997; 272:8057-8064.
- [0389] Hulme J T, Ahn M, Hauschka S D, Scheuer T, Catterall W A. A novel leucine zipper targets AKAP15 and cyclic AMP-dependent protein kinase to the C terminus of the skeletal muscle Ca^{2+} channel and modulates its function. *J Biol Chem* 277: 4079-4087, 2002.
- [0390] Hulme J T, Lin T W, Westenbroek R E, Scheuer T, Catterall W A. Beta-adrenergic regulation requires direct anchoring of PKA to cardiac CaV1.2 channels via a leucine zipper interaction with A kinase-anchoring protein 15. *Proc Natl Acad Sci USA* 100: 13093-13098, 2003.
- [0391] Hulme J T, Westenbroek R E, Scheuer T, Catterall W A. Phosphorylation of serine 1928 in the distal C-terminal domain of cardiac CaV1.2 channels during betal-adrenergic regulation. *Proc Natl Acad Sci USA* 103: 16574-16579, 2006.
- [0392] Hundsrucker C, Klussmann E. Direct AKAP-mediated protein-protein interactions as potential drug targets. *Hand Exp Pharmacol* 186: 483-503, 2008.
- [0393] Hundsrucker C, Krause G, Beyermann M, Prinz A, Zimmermann B, Diekmann O, Lorenz D, Stefan E, Nedvetzky P, Dathe M, Christian F, McSorley T, Krause E, McConnachie G, Herberg F W, Scott J D, Rosenthal W, Klussmann E. High-affinity AKAP7delta-protein kinase A interaction yields novel protein kinase A-anchoring disruptor peptides. *Biochem J* 396: 297-306, 2006.
- [0394] Jaakkola P, Mole D R, Tian Y M, Wilson M I, Gielbert J, Gaskell S J, Kriegsheim A, Hebestreit H F, Mukherji M, Schofield C J, Maxwell P H, Pugh C W, Ratcliffe P J. Targeting of HIF-alpha to the von Hippel-Lindau ubiquitylation complex by O_2 -regulated prolyl hydroxylation. *Science* 292: 468-472, 2001.
- [0395] Janknecht R, Hipskind R A, Houthaeve T, Nordheim A, Stunnenberg H G (1992) Identification of multiple SRF N-terminal phosphorylation sites affecting DNA binding properties. *EMBO J* 11:1045-1054.
- [0396] Jugdutt B I (2003) Remodeling of the myocardium and potential targets in the collagen degradation and synthesis pathways. *Curr Drug Targets Cardiovasc Haematol Disord* 3:1-30.
- [0397] Kamisago M, Sharma S D, DePalma S R, Solomon S, Sharma P, McDonough B, Smoot L, Mullen M P, Woolf P K, Wigle E D, Seidman J G, Seidman C E. Mutations in sarcomere protein genes as a cause of dilated cardiomyopathy. *N Engl J Med* 343: 1688-1696, 2000.
- [0398] Kammerer S, Burns-Hamuro L L, Ma Y, Hamon S C, Canaves J M, Shi M M, Nelson M R, Sing C F, Cantor C R, Taylor S S, Braun A. Amino acid variant in the kinase binding domain of dual-specific A kinase-anchoring protein 2: a disease susceptibility polymorphism. *Proc Natl Acad Sci USA* 100: 4066-4071, 2003.
- [0399] Kapiloff M S, Chandrasekhar K D, "A-kinase anchoring proteins: temporal and spatial regulation of intracellular signal transduction in the cardiovascular system," *Journal Cardiovasc Pharmacol*. 2011 October; 58(4):337-8.
- [0400] Kapiloff M S, Jackson N, Airhart N. mAKAP and the ryanodine receptor are part of a multi-component signaling complex on the cardiomyocyte nuclear envelope. *J Cell Sci* 114: 3167-3176, 2001.
- [0401] Kapiloff M S, Piggott L A, Sadana R, Li J, Heredia L A, Henson E, Efendiev R, Dessauer C W, "An adenylyl cyclase-mAKAPbeta signaling complex regulates cAMP levels in cardiac myocytes," *J Biol Chem*. 2009 Aug. 28; 284(35):23540-6.
- [0402] Kapiloff M S, Mathis J M, Nelson C A, Lin C R, Rosenfeld M G (1991) Calcium/calmodulin-dependent protein kinase mediates a pathway for transcriptional regulation. *Proc Natl Acad Sci USA* 88:3710-3714.
- [0403] Kapiloff M S, Schillace R V, Westphal A M, Scott J D. mAKAP: an A-kinase anchoring protein targeted to the nuclear membrane of differentiated myocytes. *J Cell Sci* 112: 2725-2736, 1999.
- [0404] Kato Y, Zhao M, Morikawa A, Sugiyama T, Chakravorty D, Koide N, Yoshida T, Tapping R I, Yang Y, Yokochi T, Lee J D (2000) Big mitogen-activated kinase regulates multiple members of the MEF2 protein family. *J Biol Chem* 275:18534-18540.

- [0405] Keely S L (1977) Activation of cAMP-dependent protein kinase without a corresponding increase in phosphorylase activity. *Res Commun Chem Pathol Pharmacol* 18:283-290.
- [0406] Keely S L (1979) Prostaglandin E1 activation of heart cAMP-dependent protein kinase: apparent dissociation of protein kinase activation from increases in phosphorylase activity and contractile force. *Mol Pharmacol* 15:235-245.
- [0407] Kehat I, Davis J, Tiburcy M, Accornero F, Sabae-El-Leil M K, Maillet M, York A J, Lorenz J N, Zimmermann W H, Meloche S, Molkenkin J D. Extracellular signal-regulated kinases 1 and 2 regulate the balance between eccentric and concentric cardiac growth. *Circ Res*. 2011; 108:176-183.
- [0408] Kehat I, Molkenkin J D. Molecular pathways underlying cardiac re-modeling during pathophysiological stimulation. *Circulation*. 2010; 122:2727-2735.
- [0409] Kentish J C, McCloskey D T, Layland J, Palmer S, Leiden J M, Martin A F, Solaro R J. Phosphorylation of troponin I by protein kinase A accelerates relaxation and crossbridge cycle kinetics in mouse ventricular muscle. *Circ Res* 88: 1059-1065, 2001.
- [0410] Kido M, Du L, Sullivan C C, Li X, Deutsch R, Jamieson S W, Thistlethwaite P A. Hypoxia-inducible factor 1-alpha reduces infarction and attenuates progression of cardiac dysfunction after myocardial infarction in the mouse. *J Am Coll Cardiol* 46: 2116-2124, 2005.
- [0411] Kim Y, Phan D, van Rooij E, Wang D Z, McAnally J, Qi X, Richardson J A, Hill J A, Bassel-Duby R, Olson E N (2008) The MEF2D transcription factor mediates stress-dependent cardiac remodeling in mice. *J Clin Invest* 118:124-132.
- [0412] Kimura T E, Jin J, Zi M, Prehar S, Liu W, Oceandy D, Abe J, Neyses L, Weston A H, Cartwright E J, Wang X. Targeted deletion of the extracellular signal-regulated protein kinase 5 attenuates hypertrophic response and promotes pressure overload-induced apoptosis in the heart. *Circ Res*. 2010; 106:961-970.
- [0413] Kinderman F S, Kim C, von Daake S, Ma Y, Pham B Q, Spraggan G, Xuong N H, Jennings P A, Taylor S S. A dynamic mechanism for AKAP binding to RII isoforms of cAMP-dependent protein kinase. *Mol Cell* 24: 397-408, 2006.
- [0414] Klussmann E, Edemir B, Pepperle B, Tamma G, Henn V, Klauschenz E, Hundsucker C, Maric K, Rosenthal W. Ht31: the first protein kinase A anchoring protein to integrate protein kinase A and Rho signaling. *FEBS Lett* 507: 264-268, 2001.
- [0415] Kodama H, Fukuda K, Pan J, Sano M, Takahashi T, Kato T, Makino S, Manabe T, Murata M, Ogawa S. Significance of ERK cascade compared with JAK/STAT and PI3-K pathway in gp130-mediated cardiac hypertrophy. *Am J Physiol Heart Circ Physiol*. 2000; 279(4): H1635-1644.
- [0416] Kontaridis M I, Yang W, Bence K K, Cullen D, Wang B, Bodyak N, Ke Q, Hinek A, Kang P M, Liao R, Neel B G. Deletion of Ptpn11 (Shp2) in cardiomyocytes causes dilated cardiomyopathy via effects on the extracellular signal-regulated kinase/mitogen-activated protein kinase and RhoA signaling pathways. *Circulation*. 2008; 117:1423-1435.
- [0417] Kritzer M D, Li J, Dodge-Kafka K, Kapiloff M S, "AKAPs: the architectural underpinnings of local cAMP signaling," *J Mol Cell Cardiol*. 2012 February; 52(2):351-8.
- [0418] Kritzer, M. D., J. Li, C. L. Passariello, M. Gayanilo, H. Thakur, J. Dayan, K. Dodge-Kafka and M. S. Kapiloff (2014). "The scaffold protein muscle A-kinase anchoring protein beta orchestrates cardiac myocyte hypertrophic signaling required for the development of heart failure." *Circ Heart Fail* 7(4): 663-672.
- [0419] Kumar, D., T. A. Hacker, J. Buck, L. F. Whitesell, E. H. Kaji, P. S. Douglas and T. J. Kamp (2005). "Distinct mouse coronary anatomy and myocardial infarction consequent to ligation." *Coron Artery Dis* 16(1): 41-44.
- [0420] Lacana E, Maceyka M, Milstien S, Spiegel S. Cloning and characterization of a protein kinase A anchoring protein (AKAP)-related protein that interacts with and regulates sphingosine kinase 1 activity. *J Biol Chem* 277: 32947-32953, 2002.
- [0421] Layland J, Solaro R J, Shah A M. Regulation of cardiac contractile function by troponin I phosphorylation. *Cardiovasc Res* 66: 12-21, 2005.
- [0422] Lechward K, Awotunde O S, Swiatek W, Muszynska G (2001) Protein phosphatase 2A: variety of forms and diversity of functions. *Acta Biochim Pol* 48:921-933.
- [0423] Lehnart, S. E., X. H. Wehrens, S. Reiken, S. Warrier, A. E. Belevych, R. D. Harvey, W. Richter, S. L. Jin, M. Conti and A. R. Marks (2005). "Phosphodiesterase 4D deficiency in the ryanodine-receptor complex promotes heart failure and arrhythmias." *Cell* 123(1): 25-35.
- [0424] Lester L B, Langeberg L K, Scott J D. Anchoring of protein kinase A facilitates hormone-mediated insulin secretion. *Proc Natl Acad Sci USA* 94: 14942-14947, 1997.
- [0425] Li C L, Sathyamurthy A, Oldenborg A, Tank D, Ramanan N (2014) SRF phosphorylation by glycogen synthase kinase-3 promotes axon growth in hippocampal neurons. *J Neurosci* 34:4027-4042.
- [0426] Li H, Adamik R, Pacheco-Rodriguez G, Moss J, Vaughan M. Protein kinase A-anchoring (AKAP) domains in brefeldin A-inhibited guanine nucleotide-exchange protein 2 (BIG2). *Proc Natl Acad Sci USA* 100: 1627-1632, 2003.
- [0427] Li J, Kritzer M D, Michel J J, Le A, Thakur H, Gayanilo M, Passariello C L, Negro A, Danial J B, Oskouei B, Sanders M, Hare J M, Hanauer A, Dodge-Kafka K, Kapiloff M S, "Anchored p90 ribosomal S6 kinase 3 is required for cardiac myocyte hypertrophy," *Circ Res*. 2013 Jan. 4; 112(1):128-39.
- [0428] Li J, Negro A, Lopez J, Bauman A L, Henson E, Dodge-Kafka K, Kapiloff M S. The mAKAPbeta scaffold regulates cardiac myocyte hypertrophy via recruitment of activated calcineurin. *J Mol Cell Cardiol* 48: 387-394, 2010.
- [0429] Li J, Negro A, Lopez J, Bauman A L, Henson E, Dodge-Kafka K, Kapiloff M S, "The mAKAPbeta scaffold regulates cardiac myocyte hypertrophy via recruitment of activated calcineurin," *J Mol Cell Cardiol*. 2010 February; 48(2):387-94.
- [0430] Li J, Vargas M A, Kapiloff M S, Dodge-Kafka K L, Regulation of MEF2 transcriptional activity by calcineurin/mAKAP complexes," *Exp Cell Res*. 2013 Feb. 15; 319(4):447-54.

- [0431] Li, J., S. Aponte Paris, H. Thakur, M. S. Kipiloff, and K. L. Dodge-Kafka. 2019. 'Muscle A-kinase-anchoring protein-beta-bound calcineurin toggles active and repressive transcriptional complexes of myocyte enhancer factor 2D', *Journal of Biological Chemistry*, 294: 2543-54.
- [0432] Li M, Makkinje A, Damuni Z (1996) Molecular identification of I1PP2A, a novel potent heat-stable inhibitor protein of protein phosphatase 2A. *Biochemistry* 35:6998-7002.
- [0433] Liu Q, Hofmann P A (2004) Protein phosphatase 2A-mediated cross-talk between p38 MAPK and ERK in apoptosis of cardiac myocytes. *Am J Physiol Heart Circ Physiol* 286:H2204-2212.
- [0434] Lohse M J, Engelhardt S, Eschenhagen T. What is the role of beta-adrenergic signaling in heart failure? *Circ Res* 93: 896-906, 2003.
- [0435] Lu J T, Kass R S. Recent progress in congenital long Q T syndrome. *Curr Opin Cardiol* 25: 216-221, 2010.
- [0436] Lygren B, Carlson C R, Santamaria K, Lissandron V, McSorley T, Lorenz D, Wiesner B, Rosenthal W, Zaccolo M, Tasken K, Klussmann E. AKAP-complex regulates the Ca^{2+} reuptake into heart sarcoplasmic reticulum. *EMBO Rep* 8: 1061-1067, 2007.
- [0437] Lygren B, Tasken K. The potential use of AKAP18delta as a drug target in heart failure patients. *Expert Opin Biol Ther* 8: 1099-1108, 2008.
- [0438] Mack C P (2011) Signaling mechanisms that regulate smooth muscle cell differentiation. *Arterioscler Thromb Vasc Biol* 31:1495-1505.
- [0439] Mackenzie K F, Topping E C, Bugaj-Gaweda B, Deng C, Cheung Y F, Olsen A E, Stockard C R, High Mitchell L, Baillie G S, Grizzle W E, De Vivo M, Houslay M D, Wang D, Bolger G B
- [0440] (2008) Human PDE4A8, a novel brain-expressed PDE4 cAMP-specific phosphodiesterase that has undergone rapid evolutionary change. *Biochem J* 411:361-369.
- [0441] MacKenzie S J, Baillie G S, McPhee I, Bolger G B, Houslay M D (2000) ERK2 mitogen-activated protein kinase binding, phosphorylation, and regulation of the PDE4D cAMP-specific phosphodiesterases. The involvement of COOH-terminal docking sites and NH2-terminal UCR regions. *J Biol Chem* 275:16609-16617.
- [0442] Maloney D J, Hecht S M. Synthesis of a potent and selective inhibitor of p90 Rsk. *Org Lett*. 2005; 7:1097-1099.
- [0443] Maron B J, Maron M S. Hypertrophic cardiomyopathy. *Lancet*. 2013; 381(9862):242-255.
- [0444] Maruyama Y, Nishida M, Sugimoto Y, Tanabe S, Turner J H, Kozasa T, Wada T, Nagao T, Kurose H. Galphai(12/13) mediates alpha(1)-adrenergic receptor-induced cardiac hypertrophy. *Circ Res* 91: 961-969, 2002.
- [0445] Martinez, E. C., C. L. Passariello, J. Li, C. J. Matheson, K. Dodge-Kafka, P. Reigan and M. S. Kipiloff (2015). "RSK3: A regulator of pathological cardiac remodeling." *IUBMB Life* 67(5): 331-337.
- [0446] Marx S O, Kurokawa J, Reiken S, Motoike H, D'Armiento J, Marks A R, Kass R S. Requirement of a macromolecular signaling complex for beta adrenergic receptor modulation of the KCNQ1-KCNE1 potassium channel. *Science* 295: 496-499, 2002.
- [0447] Marx S O, Reiken S, Hisamatsu Y, Gaburjajova M, Gaburjajova J, Yang Y M, Rosemblit N, Marks A R. Phosphorylation-dependent regulation of ryanodine receptors: a novel role for leucine/isoleucine zippers. *J Cell Biol*. 2001; 153:699-708.
- [0448] Marx S O, Reiken S, Hisamatsu Y, Jayaraman T, Burkhoff D, Rosemblit N, Marks A R. PKA phosphorylation dissociates FKBP12.6 from the calcium release channel (ryanodine receptor): defective regulation in failing hearts. *Cell* 101: 365-376, 2000.
- [0449] Maxwell P H, Wiesener M S, Chang G W, Clifford S C, Vaux E C, Cockman M E, Wykoff C C, Pugh C W, Maher E R, Ratcliffe P J. The tumour suppressor protein VHL targets hypoxia-inducible factors for oxygen-dependent proteolysis. *Nature* 399: 271-275, 1999.
- [0450] Mayers C M, Wadell J, McLean K, Venere M, Malik M, Shibata T, Driggers P H, Kino T, Guo X C, Koide H, Gorivodsky M, Grinberg A, Mukhopadhyay M, Abu-Asab M, Westphal H, Segars J H. The Rho guanine nucleotide exchange factor AKAP13 (BRX) is essential for cardiac development in mice. *J Biol Chem* 285: 12344-12354, 2010.
- [0451] Mccartney S, Little B M, Langeberg L K, Scott J D (1995) Cloning and Characterization of a-Kinase Anchor Protein-100 (Akap100)—a Protein That Targets a-Kinase to the Sarcoplasmic-Reticulum. *J Biol Chem* 270: 9327-9333.
- [0452] McConnell B K, Popovic Z, Mal N, Lee K, Bautista J, Forudi F, Schwartzman R, Jin J P, Penn M, Bond M. Disruption of protein kinase A interaction with A-kinase-anchoring proteins in the heart in vivo: effects on cardiac contractility, protein kinase A phosphorylation, and troponin I proteolysis. *J Biol Chem* 284: 1583-1592, 2009.
- [0453] McCright B, Rivers A M, Audlin S, Virshup D M (1996) The B56 family of protein phosphatase 2A (PP2A) regulatory subunits encodes differentiation-induced phosphoproteins that target PP2A to both nucleus and cytoplasm. *J Biol Chem* 271:22081-22089.
- [0454] McKinsey T A, Kass D A. Small-molecule therapies for cardiac hypertrophy: moving beneath the cell surface. *Nat Rev Drug Discov*. 2007; 6:617-635.
- [0455] Miano J M (2010) Role of serum response factor in the pathogenesis of disease. *Lab Invest* 90:1274-1284.
- [0456] Michel J J, Townley I K, Dodge-Kafka K L, Zhang F, Kipiloff M S, Scott J D, "Spatial restriction of PDK1 activation cascades by anchoring to mAKAPalpha," *Mol Cell*. 2005 Dec. 9; 20(5):661-72.
- [0457] Michele D E, Gomez C A, Hong K E, Westfall M V, Metzger J M. Cardiac dysfunction in hypertrophic cardiomyopathy mutant tropomyosin mice is transgene-dependent, hypertrophy-independent, and improved by beta-blockade. *Circ. Res.* 2002; 91(3):255-262.
- [0458] Monovich L, Vega R B, Meredith E, Miranda K, Rao C, Capparelli M, Lemon D D, Phan D, Koch K A, Chapo J A, Hood D B, McKinsey T A (2010) A novel kinase inhibitor establishes a predominant role for protein kinase D as a cardiac class IIa histone deacetylase kinase. *FEBS Lett* 584:631-637.
- [0459] Morissette M R, Sah V P, Glembotski C C, Brown J H. The Rho effector, PKN, regulates ANF gene transcription in cardiomyocytes through a serum response element. *Am J Physiol Heart Circ Physiol* 278: H1769-H1774, 2000.
- [0460] Muchir A, Bonne G, van der Kooi A J, van Meegen M, Baas F, Bolhuis P A, de Visser M, Schwartz K (2000)

- Identification of mutations in the gene encoding lamins A/C in autosomal dominant limb girdle muscular dystrophy with atrioventricular conduction disturbances (LGMD1B). *Hum Mol Genet* 9:1453-1459.
- [0461] Naga Prasad S V, Barak L S, Rapacciuolo A, Caron M G, Rockman H A. Agonist-dependent recruitment of phosphoinositide 3-kinase to the membrane by beta-adrenergic receptor kinase 1. A role in receptor sequestration. *J Biol Chem* 276: 18953-18959, 2001.
- [0462] Naga Prasad S V, Laporte S A, Chamberlain D, Caron M G, Barak L, Rockman H A. Phosphoinositide 3-kinase regulates beta₂-adrenergic receptor endocytosis by AP-2 recruitment to the receptor/beta-arrestin complex. *J Cell Biol* 158: 563-575, 2002.
- [0463] Nakagami H, Kikuchi Y, Katsuya T, Morishita R, Akasaka H, Saitoh S, Rakugi H, Kaneda Y, Shimamoto K, Ogiwara T. Gene polymorphism of myospryn (cardiomyopathy-associated 5) is associated with left ventricular wall thickness in patients with hypertension. *Hypertens Res* 30: 1239-1246, 2007.
- [0464] Nakamura A, Rokosh D G, Paccanaro M, Yee R R, Simpson P C, Grossman W, Foster E. L V systolic performance improves with development of hypertrophy after transverse aortic constriction in mice. *Am J Physiol Heart Circ Physiol*. 2001; 281:H1104-1112
- [0465] Nakayama K, Frew I J, Hagensen M, Skals M, Habelhah H, Bhoumik A, Kadoya T, Erdjument-Bromage H, Tempst P, Frappell P B, Bowtell D D, Ronai Z, Siah2 regulates stability of prolyl-hydroxylases, controls HIF1alpha abundance, and modulates physiological responses to hypoxia. *Cell* 117: 941-952, 2004.
- [0466] Nauert J B, Klauck T M, Langeberg L K, Scott J D. Gravin, an autoantigen recognized by serum from myasthenia gravis patients, is a kinase scaffold protein. *Curr Biol* 7: 52-62., 1997.
- [0467] Naya F J, Olson E (1999) MEF2: a transcriptional target for signaling pathways controlling skeletal muscle growth and differentiation. *Curr Opin Cell Biol* 11:683-688.
- [0468] Naya F J, Wu C, Richardson J A, Overbeek P, Olson E N (1999) Transcriptional activity of MEF2 during mouse embryogenesis monitored with a MEF2-dependent transgene. *Development* 126:2045-2052.
- [0469] Nerbonne J M, Kass R S. Molecular physiology of cardiac repolarization. *Physiol Rev* 85: 1205-1253, 2005.
- [0470] Negro A, Dodge-Kafka K, Kapiloff M S, "Signalosomes as Therapeutic Targets," *Prog Pediatr Cardiol*. 2008 April; 25(1):51-56.
- [0471] Nichols C B, Rossow C F, Navedo M F, Westenbroek R E, Catterall W A, Santana L F, McKnight G S. Sympathetic stimulation of adult cardiomyocytes requires association of AKAPS with a subpopulation of L-type calcium channels. *Circ Res* 107: 747-756, 2010.
- [0472] Newlon M G, Roy M, Morikis D, Hausken Z E, Coghlan V, Scott J D, Jennings P A (1999) The molecular basis for protein kinase A anchoring revealed by solution NMR. *Nat Struct Biol* 6:222-227.
- [0473] Nicol R L, Frey N, Pearson G, Cobb M, Richardson J, Olson E N. Activated MEK5 induces serial assembly of sarcomeres and eccentric cardiac hypertrophy. *EMBO J*. 2001; 20:2757-2767.
- [0474] Niggli E, Lederer W J. Voltage-independent calcium release in heart muscle. *Science* 250: 565-568, 1990. Papa S, Sardanelli A M, Scacco S, Petruzzella V, Technikova-Dobrova Z, Vergari R, Signorile A. The NADH: ubiquinone oxidoreductase (complex I) of the mammalian respiratory chain and the cAMP cascade. *J Bioenerg Biomembr* 34: 1-10, 2002.
- [0475] Ohh M, Park C W, Ivan M, Hoffman M A, Kim T Y, Huang L E, Pavletich N, Chau V, Kaelin W G (2000) Ubiquitination of hypoxia-inducible factor requires direct binding to the beta-domain of the von Hippel-Lindau protein. *Nat Cell Biol* 2:423-427.
- [0476] Oka T, Xu J, Kaiser R A, Melendez J, Hambleton M, Sargent M A, Lorts A, Brunskill E W, Dom G W, 2nd, Conway S J, Aronow B J, Robbins J, Molkentin J D. Genetic manipulation of periostin expression reveals a role in cardiac hypertrophy and ventricular remodeling. *Circ. Res.* 2007; 101(3):313-321.
- [0477] Okumura, S., G. Takagi, J. Kawabe, G. Yang, M. C. Lee, C. Hong, J. Liu, D. E. Vatner, J. Sadoshima, S. F. Vatner and Y. Ishikawa (2003). "Disruption of type 5 adenylyl cyclase gene preserves cardiac function against pressure overload." *Proc Natl Acad Sci USA* 100(17): 9986-9990.
- [0478] Olson G L, Cantor C R, Braun A, Taylor S S. Designing isoform-specific peptide disruptors of protein kinase A localization. *Proc Natl Acad Sci USA* 100: 4072-4077, 2003.
- [0479] Pare G C, Bauman A L, McHenry M, Michel J J, Dodge-Kafka K L, Kapiloff M S. The mAKAP complex participates in the induction of cardiac myocyte hypertrophy by adrenergic receptor signaling. *J Cell Sci* 118: 5637-5646, 2005.
- [0480] Pare G C, Easlick J L, Mislow J M, McNally E M, Kapiloff M S. Nesprin-1alpha contributes to the targeting of mAKAP to the cardiac myocyte nuclear envelope. *Exp Cell Res* 303: 388-399, 2005.
- [0481] Passariello, C. L., J. Li, K. Dodge-Kafka and M. S. Kapiloff (2015). "mAKAP-a master scaffold for cardiac remodeling." *J Cardiovasc Pharmacol* 65(3): 218-225.
- [0482] Passariello C L, Martinez E C, Thakur H, Cesareo M, Li J, Kapiloff M S (2016) RSK3 is required for concentric myocyte hypertrophy in an activated Raf1 model for Noonan syndrome. *J Mol Cell Cardiol* 93:98-105.
- [0483] Passariello C L, Gayanilo M, Kritzer M D, Thakur H, Cozacov Z, Rusconi F, Wieczorek D, Sanders M, Li J, Kapiloff M S (2013) p⁹⁰ ribosomal S6 kinase 3 contributes to cardiac insufficiency in alpha-tropomyosin Glu1 80Gly transgenic mice. *Am J Physiol Heart Circ Physiol* 305:H1010-1019.
- [0484] Patel H H, Hamuro L L, Chun B J, Kawaraguchi Y, Quick A, Rebollo B, Pennypacker J, Thurston J, Rodriguez-Pinto N, Self C, Olson G, Insel P A, Giles W R, Taylor S S, Roth D M. Disruption of protein kinase A localization using a trans-activator of transcription (TAT)-conjugated A-kinase-anchoring peptide reduces cardiac function. *J Biol Chem* 285: 27632-27640, 2010.
- [0485] Pawson C T, Scott J D. Signal integration through blending, bolstering and bifurcating of intracellular information. *Nat Struct Mol Biol* 17: 653-658, 2010.
- [0486] Pawson T, Nash P (2003) Assembly of cell regulatory systems through protein interaction domains. *Science* 300:445-452.
- [0487] Perino A, Ghigo A, Ferrero E, Morello F, Santulli G, Baillie G S, Damilano F, Dunlop A J, Pawson C, Walser R, Levi R, Altruda F, Silengo L, Langeberg L K,

- Neubauer G, S H, Lembo G, Wymann M P, Wetzker R, Houslay M D, Iaccarino G, Scott J D, Hirsch E. Integrating cardiac PIP3 and cAMP signaling through a PKA anchoring function of p110gamma. *Mol Cell* 42: 84-95, 2011.
- [0488] Perrino C, Feliciello A, Schiattarella G G, Esposito G, Guerriero R, Zaccaro L, Del Gatto A, Saviano M, Garbi C, Carangi R, Di Lorenzo E, Donato G, Indolfi C, Avvedimento V E, ChiarIELLO M. AKAP121 downregulation impairs protective cAMP signals, promotes mitochondrial dysfunction, and increases oxidative stress. *Cardiovasc Res* 88: 101-110, 2010.
- [0489] Perrino C, Naga Prasad S V, Mao L, Noma T, Yan Z, Kim H S, Smithies O, Rockman H A. Intermittent pressure overload triggers hypertrophy-independent cardiac dysfunction and vascular rarefaction. *J Clin Invest.* 2006; 116:1547-1560.
- [0490] Peter A K, Bjerke M A, Leinwand L A (2016) Biology of the cardiac myocyte in heart disease. *Mol Biol Cell* 27:2149-2160.
- [0491] Ponikowski, P., A. A. Voors, S. D. Anker, H. Bueno, J. G. Cleland, A. J. Coats, V. Falk, J. R. Gonzalez-Juanatey, V. P. Harjola, E. A. Jankowska, M. Jessup, C. Linde, P. Nihoyannopoulos, J. T. Parissis, B. Pieske, J. P. Riley, G. M. Rosano, L. M. Ruilope, F. Ruschitzka, F. H. Rutten, P. van der Meer and M. Authors/Task Force (2016). "2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure: The Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC) Developed with the special contribution of the Heart Failure Association (HFA) of the ESC." *Eur Heart J* 37(27): 2129-2200.
- [0492] Potthoff M J, Olson E N (2007) MEF2: a central regulator of diverse developmental programs. *Development* 134:4131-4140.
- [0493] Prabhakar R, Boivin G P, Grupp I L, Hoit B, Arteaga G, Solaro J R, Wieczorek D F. A familial hypertrophic cardiomyopathy alpha-tropomyosin mutation causes severe cardiac hypertrophy and death in mice. *J. Mol. Cell. Cardiol.* 2001; 33(10):1815-1828.
- [0494] Prasad, K. M., Y. Xu, Z. Yang, S. T. Acton and B. A. French (2011). "Robust cardiomyocyte-specific gene expression following systemic injection of AAV: in vivo gene delivery follows a Poisson distribution." *Gene Ther* 18(1): 43-52.
- [0495] Rababa'h A, Craft J W, Jr., Wijaya C S, Atrooz F, Fan Q, Singh S, Guillory A N, Katsonis P, Lichtarge O, McConnell B K (2013) Protein kinase A and phosphodiesterase-4D3 binding to coding polymorphisms of cardiac muscle anchoring protein (mAKAP). *J Mol Biol* 425: 3277-3288.
- [0496] Ranganathan A, Pearson G W, Chrestensen C A, Sturgill T W, Cobb M H (2006) The MAP kinase ERK5 binds to and phosphorylates p90 RSK. *Arch Biochem Biophys* 449:8-16.
- [0497] Reiken S, Gaburjakova M, Gaburjakova J, He Ki K L, Prieto A, Becker E, Yi Gh G H, Wang J, Burkhoff D, Marks A R (2001) beta-adrenergic receptor blockers restore cardiac calcium release channel (ryanodine receptor) structure and function in heart failure. *Circulation* 104:2843-2848.
- [0498] Resjo S, Oknianska A, Zolnierowicz S, Manganello V, Degerman E (1999) Phosphorylation and activation of phosphodiesterase type 3B (PDE3B) in adipocytes in response to serine/threonine phosphatase inhibitors: deactivation of PDE3B in vitro by protein phosphatase type 2A. *Biochem J* 341 (Pt 3):839-845.
- [0499] Reynolds J G, McCalmon S A, Tomczyk T, Naya F J. Identification and mapping of protein kinase A binding sites in the costameric protein myospryn. *Biochim Biophys Acta* 1773: 891-902, 2007.
- [0500] Richards S A, Dreisbach V C, Murphy L O, Blenis J. Characterization of regulatory events associated with membrane targeting of p90 ribosomal S6 kinase 1. *Mol Cell Biol.* 2001; 21:7470-7480.
- [0501] Rivera V M, Miranti C K, Misra R P, Ginty D D, Chen R H, Blenis J, Greenberg M E (1993) A growth factor-induced kinase phosphorylates the serum response factor at a site that regulates its DNA-binding activity. *Mol Cell Biol* 13:6260-6273.
- [0502] Rockman H A, Koch W J, Lefkowitz R J. Seven-transmembrane-spanning receptors and heart function. *Nature* 415: 206-212, 2002.
- [0503] Rockman H A, Ross R S, Harris A N, Knowlton K U, Steinheimer M E, Field L J, Ross J Jr, Chien K R. Segregation of atrial-specific and inducible expression of an atrial natriuretic factor transgene in an in vivo murine model of cardiac hypertrophy. *Proc Natl Acad Sci USA.* 1991; 88:8277-8281.
- [0504] Roger V L, Go A S, Lloyd-Jones D M, Adams R J, Berry J D, Brown T M, Carnethon M R, Dai S, de Simone G, Ford E S, Fox C S, Fullerton H J, Gillespie C, Greenlund K J, Hailpern S M, Heit J A, Ho P M, Howard V J, Kissela B M, Kittner S J, Lackland D T, Lichtman J H, Lisabeth L D, Makuc D M, Marcus G M, Marelli A, Matchar D B, McDermott M M, Meigs J B, Moy C S, Mozaffarian D, Mussolini M E, Nichol G, Paynter N P, Rosamond W D, Sorlie P D, Stafford R S, Turan T N, Turner M B, Wong N D, Wyllie-Rosett J; American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Heart disease and stroke statistics—2011 update: a report from the American Heart Association. *Circulation* 123: e18-e209, 2011.
- [0505] Rose B A, Force T, Wang Y. Mitogen-activated protein kinase signaling in the heart: angels versus demons in a heart-breaking tale. *Physiol Rev.* 2010; 90:1507-1546.
- [0506] Russell M A, Lund L M, Haber R, McKeegan K, Cianciola N, Bond M. The intermediate filament protein, synemin, is an AKAP in the heart. *Arch Biochem Biophys* 456: 204-215, 2006.
- [0507] Sadoshima J, Qiu Z, Morgan J P, Izumo S. Angiotensin I I and other hypertrophic stimuli mediated by G protein-coupled receptors activate tyrosine kinase, mitogen-activated protein kinase, and 90-kD S6 kinase in cardiac myocytes. The critical role of Ca(2+)-dependent signaling. *Circ. Res.* 1995; 76(1):1-15.
- [0508] Sapkota G P, Cummings L, Newell F S, Armstrong C, Bain J, Frodin M, Grauert M, Hoffmann M, Schnapp G, Steegmaier M, Cohen P, Alessi D R. B I-D1870 is a specific inhibitor of the p90 RSK (ribosomal S6 kinase) isoforms in vitro and in vivo. *Biochem J.* 2007; 401:29-38.
- [0509] Schiattarella G G, Hill J A (2015) Inhibition of hypertrophy is a good therapeutic strategy in ventricular pressure overload. *Circulation* 131:1435-1447.

- [0510] Scholten A, Poh M K, van Veen T A, van Breukelen B, Vos M A, Heck A J. Analysis of the cGMP/cAMP interactome using a chemical proteomics approach in mammalian heart tissue validates sphingosine kinase type 1-interacting protein as a genuine and highly abundant AKAP. *J Proteome Res* 5: 1435-1447, 2006.
- [0511] Scholten A, van Veen T A, Vos M A, Heck A J. Diversity of cAMP-dependent protein kinase isoforms and their anchoring proteins in mouse ventricular tissue. *J Proteome Res* 6: 1705-1717, 2007.
- [0512] Schulze D H, Mughal M, Lederer W J, Ruknudin A M. Sodium/calcium exchanger (NCX1) macromolecular complex. *J Biol Chem* 278: 28849-28855, 2003.
- [0513] Scott J D, Dessauer C W, Tasken K (2013) Creating order from chaos: cellular regulation by kinase anchoring. *Annu Rev Pharmacol Toxicol* 53:187-210.
- [0514] Scott, J. D. and T. Pawson (2009). "Cell signaling in space and time: where proteins come together and when they're apart." *Science* 326(5957): 1220-1224.
- [0515] Semenza G L. Hypoxia-inducible factor 1 (HIF-1) pathway. *Sci STKE* 2007: cm8, 2007.
- [0516] Semenza G L. Regulation of oxygen homeostasis by hypoxia-inducible factor 1. *Physiology* 24: 97-106, 2009.
- [0517] Sette C, Conti M (1996) Phosphorylation and activation of a cAMP-specific phosphodiesterase by the cAMP-dependent protein kinase. Involvement of serine 54 in the enzyme activation. *J Biol Chem* 271:16526-16534.
- [0518] Sfichi-Duke L, Garcia-Cazarin M L, Sumandea C A, Sievert G A, Balke C W, Zhan D Y,
- [0519] Morimoto S, Sumandea M P. Cardiomyopathy-causing deletion K210 in cardiac troponin T alters phosphorylation propensity of sarcomeric proteins. *J Mol Cell Cardiol* 48: 934-942, 2010.
- [0520] Shan J, Betzenhauser M J, Kushnir A, Reiken S, Meli A C, Wronska A, Dura M, Chen B X, Marks A R. Role of chronic ryanodine receptor phosphorylation in heart failure and beta-adrenergic receptor blockade in mice. *J Clin Invest* 120: 4375-4387, 2010.
- [0521] Shan J, Kushnir A, Betzenhauser M J, Reiken S, Li J, Lehnart S E, Lindegger N, Mongillo M, Mohler P J, Marks A R. Phosphorylation of the ryanodine receptor mediates the cardiac fight or flight response in mice. *J Clin Invest* 120: 4388-4398, 2010.
- [0522] Sharma K, Kass D A (2014) Heart failure with preserved ejection fraction: mechanisms, clinical features, and therapies. *Circ Res* 115:79-96.
- [0523] Shyu K G, Wang M T, Wang B W, Chang C C, Leu J G, Kuan P, Chang H. Intramyocardial injection of naked DNA encoding HIF-1alpha/VP16 hybrid to enhance angiogenesis in an acute myocardial infarction model in the rat. *Cardiovasc Res* 54: 576-583, 2002.
- [0524] Silva, J. M., M. Z. Li, K. Chang, W. Ge, M. C. Golding, R. J. Rickles, D. Siolas, G. Hu, P. J. Paddison, M. R. Schlabach, N. Sheeth, J. Bradshaw, J. Burchard, A. Kulkarni, G. Cavet, R. Sachidanandam, W. R. McCombie, M. A. Cleary, S. J. Elledge and G. J. Hannon (2005). "Second-generation shRNA libraries covering the mouse and human genomes." *Nat Genet* 37(11): 1281-1288.
- [0525] Singh A, Redden J M, Kapiloff M S, Dodge-Kafka K L, "The large isoforms of A-kinase anchoring protein 18 mediate the phosphorylation of inhibitor-1 by protein kinase A and the inhibition of protein phosphatase 1 activity," *Mol Pharmacol*. 2011 March; 79(3):533-40.
- [0526] Skroblin P, Grossmann S, Schafer G, Rosenthal W, Klussmann E. Mechanisms of protein kinase A anchoring. *Int Rev Cell Mol Biol* 283: 235-330, 2010.
- [0527] Smith F D, Langeberg L K, Cellurale C, Pawson T, Morrison D K, Davis R J, Scott J D. AKAP-Lbc enhances cyclic AMP control of the ERK1/2 cascade. *Nat Cell Biol* 12: 1242-1249, 2010.
- [0528] Smith J A, Poteet-Smith C E, Xu Y, Errington T M, Hecht S M, Lanigan D A. Identification of the first specific inhibitor of p90 ribosomal S6 kinase (RSK) reveals an unexpected role for RSK in cancer cell proliferation. *Cancer Res*. 2005; 65:1027-1034.
- [0529] Spinale F G, Janicki J S, Zile M R. Membrane-associated matrix proteolysis and heart failure. *Circ. Res.* 2013; 112(1):195-208.
- [0530] Steinberg S F, Brunton L L (2001) Compartmentation of G protein-coupled signaling pathways in cardiac myocytes. *Annu Rev Pharmacol Toxicol* 41:751-773.
- [0531] Stelzer J E, Patel J R, Walker J W, Moss R L. Differential roles of cardiac myosin-binding protein C and cardiac troponin I in the myofibrillar force responses to protein kinase A phosphorylation. *Circ Res* 101: 503-511, 2007.
- [0532] Sumandea C A, Garcia-Cazarin M L, Bozio C H, Sievert G A, Balke C W, Sumandea M P. Cardiac troponin T, a sarcomeric AKAP, tethers protein kinase A at the myofilaments. *J Biol Chem* 286: 530-541, 2011
- [0533] Takeishi Y, Huang Q, Abe J, Che W, Lee J D, Kawakatsu H, Hoit B D, Berk B C, Walsh R A. Activation of mitogen-activated protein kinases and p90 ribosomal S6 kinase in failing human hearts with dilated cardiomyopathy. *Cardiovasc Res*. 2002; 53:131-137.
- [0534] Terrenoire C, Houslay M D, Baillie G S, Kass R S. The cardiac IKs potassium channel macromolecular complex includes the phosphodies terase PDE4D3. *J Biol Chem* 284: 9140-9146, 2009.
- [0535] Thomas G M, Rumbaugh G R, Harrar D B, Huganir R L. Ribosomal S6 kinase 2 interacts with and phosphorylates PDZ domain-containing proteins and regulates AMPA receptor transmission. *Proc Natl Acad Sci USA*. 2005; 102:15006-15011.
- [0536] Tingley W G, Pawlikowska L, Zaroff J G, Kim T, Nguyen T, Young S G, Vranizan K, Kwok P Y, Whooley M A, Conklin B R. Gene-trapped mouse embryonic stem cell-derived cardiac myocytes and human genetics implicate AKAP10 in heart rhythm regulation. *Proc Natl Acad Sci USA* 104: 8461-8466, 2007.
- [0537] Treisman R (1985) Transient accumulation of c-fos RNA following serum stimulation requires a conserved 5' element and c-fos 3' sequences. *Cell* 42:889-902.
- [0538] Uys G M, Ramburam A, Loos B, Kinnear C J, Korkie L J, Mouton J, Riedemann J, Moolman-Smook J. Myomegalin is a novel A-kinase anchoring protein involved in the phosphorylation of cardiac myosin binding protein C. *BMC Cell Biol* 12: 18, 2011.
- [0539] Valdivia H H, Kaplan J H, Ellis-Davies G C, Lederer W J (1995) Rapid adaptation of cardiac ryanodine receptors: modulation by Mg²⁺ and phosphorylation. *Science* 267:1997-2000.
- [0540] Vargas M A, Tirnauer J S, Glidden N, Kapiloff M S, Dodge-Kafka K L, "Myocyte enhancer factor 2 (MEF2) tethering to muscle selective A-kinase anchoring

- protein (mAKAP) is necessary for myogenic differentiation," *Cell Signal.* 2012 August; 24(8):1496-503.
- [0541] Virshup D M (2000) Protein phosphatase 2A: a panoply of enzymes. *Curr Opin Cell Biol* 12:180-185.
- [0542] Wang X, Tang X, Li M, Marshall J, Mao Z (2005) Regulation of neuroprotective activity of myocyte-enhancer factor 2 by cAMP-protein kinase A signaling pathway in neuronal survival. *J Biol Chem* 280:16705-16713.
- [0543] Wang, Y., E. G. Cameron, J. Li, T. L. Stiles, M. D. Kritzer, R. Lodhavia, J. Hertz, T. Nguyen, M. S. Kapiloff and J. L. Goldberg (2015). "Muscle A-Kinase Anchoring Protein-alpha is an Injury-Specific Signaling Scaffold Required for Neurotrophic- and Cyclic Adenosine Monophosphate-Mediated Survival." *EBioMedicine* 2(12): 1880-1887.
- [0544] Wang, Z., H. I. Ma, J. Li, L. Sun, J. Zhang and X. Xiao (2003). "Rapid and highly efficient transduction by double-stranded adeno-associated virus vectors in vitro and in vivo." *Gene Ther* 10(26): 2105-2111.
- [0545] Wera S, Hemmings B A (1995) Serine/threonine protein phosphatases. *Biochem J* 311 (Pt 1):17-29.
- [0546] Wilkins B J, De Windt L J, Bueno O F, Braz J C, Glascock B J, Kimball T F, Molkentin J D (2002) Targeted disruption of NFATc3, but not NFATc4, reveals an intrinsic defect in calcineurin-mediated cardiac hypertrophic growth. *Mol Cell Biol* 22:7603-7613.
- [0547] Wilkins B J, Dai Y S, Bueno O F, Parsons S A, Xu J, Plank D M, Jones F, Kimball T R, Molkentin J D (2004) Calcineurin/NFAT coupling participates in pathological, but not physiological, cardiac hypertrophy. *Circ Res* 94:110-118.
- [0548] Wong W, Goehring A S, Kapiloff M S, Langeberg L K, Scott J D, "mAKAP compartmentalizes oxygen-dependent control of HIF-1alpha," *Sci Signal.* 2008 Dec. 23; 1(51).
- [0549] Welch E J, Jones B W, Scott J D. Networking with AKAPs: context-dependent regulation of anchored enzymes. *Mol Interv* 10: 86-97, 2010. 114. Wu X, Simpson J, Hong J H, Kim K H, Thavarajah N K, Backx P H, Neel B G, Araki T. MEK-ERK pathway modulation ameliorates disease phenotypes in a mouse model of Noonan syndrome associated with the Raf1(L613V) mutation. *J Clin Invest.* 2011; 121:1009-1025.
- [0550] Wolpert K C, Taga T, Saito M, Narazaki M, Kishimoto T, Glembotski C C, Vernallis A B, Heath J K, Pennica D, Wood W I, Chien K R. Cardiotrophin-1 activates a distinct form of cardiac muscle cell hypertrophy. Assembly of sarcomeric units in series VIA gp130/leukemia inhibitory factor receptor-dependent pathways. *J Biol Chem.* 1996; 271:9535-9545.
- [0551] Writing Group, M., D. Mozaffarian, E. J. Benjamin, A. S. Go, D. K. Arnett, M. J. Blaha, M. Cushman, S. R. Das, S. de Ferranti, J. P. Despres, H. J. Fullerton, V. J. Howard, M. D. Huffman, C. R. Isasi, M. C. Jimenez, S. E. Judd, B. M. Kissela, J. H. Lichtman, L. D. Lisabeth, S. Liu, R. H. Mackey, D. J. Magid, D. K. McGuire, E. R. Mohler, 3rd, C. S. Moy, P. Muntner, M. E. Mussolini, K. Nasir, R. W. Neumar, G. Nichol, L. Palaniappan, D. K. Pandey, M. J. Reeves, C. J. Rodriguez, W. Rosamond, P. D. Sorlie, J. Stein, A. Towfighi, T. N. Turan, S. S. Virani, D. Woo, R. W. Yeh, M. B. Turner, C. American Heart Association Statistics and S. Stroke Statistics (2016). "Heart Disease and Stroke Statistics-2016 Update: A Report From the American Heart Association." *Circulation* 133(4): e38-360.
- [0552] Wu H, Rothermel B, Kanatous S, Rosenberg P, Naya F J, Shelton J M, Hutcheson K A, DiMaio J M, Olson E N, Bassel-Duby R, Williams R S (2001) Activation of MEF2 by muscle activity is mediated through a calcineurin-dependent pathway. *EMBO J* 20:6414-6423.
- [0553] Xie M, Hill J A (2013) HDAC-dependent ventricular remodeling. *Trends Cardiovasc Med* 23:229-235.
- [0554] Xu J, Ismat F A, Wang T, Lu M M, Antonucci N, Epstein J A. Cardiomyocyte-specific loss of neurofibromin promotes cardiac hypertrophy and dysfunction. *Circ Res.* 2009; 105:304-311.
- [0555] Yang J, Drazba J A, Ferguson D G, Bond M (1998) A-kinase anchoring protein 100 (AKAP100) is localized in multiple subcellular compartments in the adult rat heart. *J Cell Biol* 142:511-522.
- [0556] Yang K C, Jay P Y, McMullen J R, Nerbonne J M. Enhanced cardiac PI3Ka signalling mitigates arrhythmogenic electrical remodelling in pathological hypertrophy and heart failure. *Cardiovasc Res.* 2012; 93:252-262.
- [0557] Zakhary D R, Fink M A, Ruehr M L, Bond M (2000) Selectivity and regulation of A-kinase anchoring proteins in the heart. The role of autophosphorylation of the type II regulatory subunit of cAMP-dependent protein kinase. *J Biol Chem* 275:41389-41395.
- [0558] Zhang L, Malik S, Kelley G G, Kapiloff M S, Smrcka A V, "Phospholipase Cepsilon scaffolds to muscle-specific A kinase anchoring protein (mAKAP-beta) and integrates multiple hypertrophic stimuli in cardiac myocytes," *J Biol Chem.* 2011 Jul. 1; 286(26):23012-21.
- [0559] Zhang, L., S. Malik, J. Pang, H. Wang, K. M. Park, D. I. Yule, B. C. Blaxall and A. V. Smrcka (2013). "Phospholipase Cepsilon hydrolyzes perinuclear phosphatidylinositol 4-phosphate to regulate cardiac hypertrophy." *Cell* 153(1): 216-227.
- [0560] Zhang Q, Bethmann C, Worth N F, Davies J D, Wasner C, Feuer A, Ragnauth C D, Yi Q, Mellad J A, Warren D T, Wheeler M A, Ellis J A, Skepper J N, Vorgerd M, Schlotter-Weigel B, Weissberg P L, Roberts R G, Wehnert M, Shanahan C M (2007) Nesprin-1 and -2 are involved in the pathogenesis of Emery Dreifuss muscular dystrophy and are critical for nuclear envelope integrity. *Hum Mol Genet* 16:2816-2833.
- [0561] Zhao Y, Bjorbaek C, Moller D E. Regulation and interaction of pp90(rsk) isoforms with mitogen-activated protein kinases. *J Biol Chem.* 1996; 271:29773-29779.
- [0562] Zhao Y, Bjorbaek C, Weremowicz S, Morton C C, Moller D E. RSK3 encodes a novel pp90rsk isoform with a unique N-terminal sequence: growth factor-stimulated kinase function and nuclear translocation. *Mol Cell Biol.* 1995 August; 15(8): 4353-436.

SEQUENCE LISTING

Sequence total quantity: 23

SEQ ID NO: 1 moltype = AA length = 733
 FEATURE Location/Qualifiers
 source 1..733
 mol_type = protein
 organism = Homo sapiens

SEQUENCE: 1

```
MDSLMMKKFAV RRFFSVYLRR KSRSKSSSSL RLEEEGVVKE IDISHHVKEG FEKADPSQFE 60
LLKVLGQGSY GKVFLVRKVK GSDAGOLYAM KVLKKATLKV RDRVRSKMER DILAEVNHPF 120
IVKLHYAQFT EGKLYLILDF LRGGLDFTRL SKEVMFTEED VKFYLAELAL ALDHLHSLGI 180
IYRDLKPENI LLDEEGHIKI TDFGGLSKEAI DHDKRAYSFIC GTIEYMAPEV VNRRGHTQSA 240
DWWSFGVLMP EMLTGSLPQ GKDRKMPQ ILKAKLGMPQ FLSGEAQSSL RALFKRNPNCN 300
RLGAGIDGVE EIKRHPFFVT IDWNTLYRK EIKPFKPAVG RPEDTFHFDP EFTARTPTDS 360
PGVPPSANAH HLFRGFSFVA SSLIQEPESSQ DLHKVPVHPI VQQLHGNNIH FTDGYEIKED 420
IGVGSYSVCK RCVHKATDTE YAVKIIDKS K RDPEEETIEL RLYGQHPNII TLKDVFDDGK 480
FVYLVMEMLR GGELLEIDLRLR QRYFVMEAREAS DVLCITIKTM DYLHSQGVVH RDLKPSNILY 540
RDESGSPESI RVCDCFGFAKQ LRAGNGLLMT PCYTANFVAP EVLKRCQYDA ACDIWSLGIL 600
LYTMLLAGFTP FANGPDDTPE EILARIGSGK YALSGGNWDS ISDAAKDVVS KMLHVDPHQ 660
LTAMQVLKHP WVNVREYLSP NQLSRQDVHL VKGAMAATYF ALNRTPQAPR LEPVLSSNLA 720
QRGGMKRLTS TRL 733
```

SEQ ID NO: 2 moltype = AA length = 2314
 FEATURE Location/Qualifiers
 source 1..2314
 mol_type = protein
 organism = Rattus rattus

SEQUENCE: 2

```
MUTMSVTLSP LRSQGPDPMA TDASPMAINM TPTVEQEEGE GEEAVKAIDA EQQYGKPPPL 60
HTAADWKIVL HLPETBTWLR MTSERVRLDT YSVQQDADSK HVVDVHLVQLK DICEDISDHV 120
EQIHALLETE FSLKLSSY SV NVIVDIHAVQ LLWHQLRVSV LVLRERILQG LQDANGNYTR 180
QTDLQAFSE ETTEGRLDL TEVDDSGQLT IKCSQDYL SDCGITAFELS DYSPSEDL LG 240
GLGDMTSQA KTKSFDSWSY SEMEKEFP EIRVGLLTVA TEPVPSSCGE ANEDSSQASL 300
SDDHKGEHGE DGAPVPGQQL DSTVGMSL SD TGTANAAEHP SETAKQDSTS SPQLGAKKTQ 360
PGCCEITTPK RSIRDCFN Y EDSPQPTL P KRGFLFLKETQ KNERKGSDRK GQVVDLK PEL 420
SRSTPSLVDP PDRSKLCLV QSSYPSSPSA ASQSYECLHK VGLGNLENIV RSHIKEISS 480
LGRLTDCHE KLRLKKPHKT LAEVSLCRIP KQGGGSGKRS ESTGSSAGPS MVSPGAPKAT 540
MRPETDSAST ASGGGLCHQRN RSGQLPVQSK ASSSPPCSHS SESSLGSNSI KSPVPLLSKN 600
KSQKSSSENE CHATQMGQVU EAWGSDEYL ALPSHLKQTE VIALKLESLT KLLPKPRGE 660
TIQDIDDWEL SEMNSDSEIY PTYHICKKHT RLGTVSPSSS SDIASSLGEIESGPLSDIL 720
SDEBLLCPLS SVKKFTDEF ERPSSEKNE SHSATRSLAI QKLMDHIQHQ ENYEAIWERI 780
EGFVNKLDEF IQWLNEAMET TENWTPPKAE TDSLRLYLET HLSFKLNVD S HCAKEAVE 840
EGHQOLLVELV SHKAGLKDTL RMIAQSQKEL QROIKRQHSW ILRALDTIKA EILATDV SVE 900
DEEGTGSPKA EVQLCHLETQ RDAVEQMSL K LYSEQYTSGS KRKEEFANMS KAHAEGSNGL 960
LDFDSEYQEL WDWLIDMESL VMDSHDLMMS EECQQHLYKR YSVEMSI RHL KKSELLSKVE 1020
ALKGGGLSLP DDILEKVDSI NEKWELLGKT LREKIQDTIA GHSGSGPRDL LSPEGS LVR 1080
OLEVRKELK FWLRDTELF FNSCLRQEKE GTSAEKLOQY FKSLCREIKQ RRRGVASILR 1140
LCQHLLDDRC TCNLNAHDQ MQLIIVNLER RWEAIVMQAV QWQTRLQK KM GKESETLNVI 1200
DPLGLMDLNGM SEDALEWDET DISNKLISVH EESNDLDDP EPMLPAVKLE ETHHHKDSGYE 1260
EEAGDCGGSP YTTSNITAPSS PHIYQVYSLH NVELHEDSHT PFLKSSPKFT GTTQPTVLT K 1320
SLSKDSSFSS TKS LP DLLGG SGLRPYPSCH SGDLSQNSGS ESGIVSEGDN EMPTNSDMSL 1380
FSMVDGSPSN PETEHDPDQM GDAANVLEQK FKDNGESI K SS VSVSRAVSP VGCVNGKAGD 1440
LNSVTKHTAD CLGEELQGH D VFVTFYDYSY LQGSKLKLPM IMKQPQSEKA HVEDPLLG 1500
YFDKKSCAKA HQASESQPD A P PHERILASA PHEMGRSAY SSSDIEKTFG I Q SARQLSLL 1560
SRSSSVESLPS PGGDLFGLGI FKNGSDSLQR STSLESWL TS YKSNEIDL FSC HSSGDIVS 1620
GSVGE LSKRT LDLLNRLENI QSPSEQKIKR SVSDMTLQSS SQKMPFAGQM SL DVASSINE 1680
DSPASLTESLSS D S DELLSLCSE DIVLHKNKP ESNASFRKRL NR SVADES DV NVSMIVNVSC 1740
TSACTDDEDD SDLSSS STL T LEEELCLKD EDDDSIAT DEIYEESNL S GLD YIKNEL 1800
QTWIRPKLSL TREKKRSGVT DEI KV KNDG GNEKANPSDT LDIEALLNGS IRCLSENNGN 1860
GKTPPRTHGS GTKGENKKST YDVSKDPHVA DMENGN IEST PERER EK P QG LPEVSEN LAS 1920
NVTKTISESEL SEYEAVMDG EDSSVARKEF CPPNDRHPPQ MGPKLQH PEN QSGDCKPVQN 1980
PCPGLLSEAG VGSRQDSNGL KSLPNDAPSG ARKPAGCCLL EQNTEESAS ISSNASC C 2040
KPDVFHQKDD EDCSVHDFVK EIIMASTAL KSKSQPESEV AAPTSLTQIK EKVLEHSHRP 2100
IHLRKGDYFS YLSLSSHSDS CGEVNTYIDE KSSTPLPPDA VDSGLDDKED MDCCFEEACVE 2160
DEPVNNEAGL PGALPNESAI EDGAQKSEQ KTASSPVLSD KTDLVPLSGL SPQKGADD AK 2220
EGDDVSHTSQ GCAESTEPTT PSGKANAEGR SRM QGV SATP EENAASAKPK IQAFSLNAKQ 2280
PKGKVAMRYP SPQTLTCKEK LVNFHEDRHS NMHR 2314
```

SEQ ID NO: 3 moltype = DNA length = 29
 FEATURE Location/Qualifiers
 misc_feature 1..29
 note = chemically synthesized
 source 1..29
 mol_type = other DNA
 organism = synthetic construct

SEQUENCE: 3

-continued

tcgacagatgtccatatta ggacatctg	29
SEQ ID NO: 4 moltype = DNA length = 79	
FEATURE Location/Qualifiers	
misc_feature 1..79	
note = chemically synthesized	
source 1..79	
mol_type = other DNA	
organism = synthetic construct	
SEQUENCE: 4 gatctcgaag gttataaaag tcaatgtctg cagatgagaa agcagtgggt ctcttagac 60	
ttttgggg agtgtggc 79	
SEQ ID NO: 5 moltype = DNA length = 13	
FEATURE Location/Qualifiers	
misc_feature 1..13	
note = chemically synthesized	
source 1..13	
mol_type = other DNA	
organism = synthetic construct	
SEQUENCE: 5 atcgctggca gag	13
SEQ ID NO: 6 moltype = DNA length = 13	
FEATURE Location/Qualifiers	
misc_feature 1..13	
note = chemically synthesized	
source 1..13	
mol_type = other DNA	
organism = synthetic construct	
SEQUENCE: 6 gagcctggat gaa	13
SEQ ID NO: 7 moltype = DNA length = 13	
FEATURE Location/Qualifiers	
misc_feature 1..13	
note = chemically synthesized	
source 1..13	
mol_type = other DNA	
organism = synthetic construct	
SEQUENCE: 7 gagcctgagc gag	13
SEQ ID NO: 8 moltype = AA length = 2319	
FEATURE Location/Qualifiers	
source 1..2319	
mol_type = protein	
organism = Homo sapiens	
SEQUENCE: 8 MLTMSVTLSP LRSQDLDPM TDASPMAINM TPTVEQGEQE EAMKDMDSQ QYEKPPLHT 60	
GADWKLLHL PEIETWLRMT SERVRDLTYS VQQDSDSKHV DVHLVQLKDI CEDISDHVEQ 120	
IHALLETEFS KLLLSYSVNV IVDIHAVQLL WHQLRVSVLV LRERILQGLQ DANGNYTRQT 180	
DILQAFSEET KEGRLDLSLT VDDSQLTIK CSQNYLSDLCS GITAFELSDY SPSEDLLSGL 240	
GDMTSSQVKT KPFDSWSYSE MEKEFPELIR SVGLLTVAAD SISTNGSEAV TEEVSQVSL 300	
VDDKGGECEED NASAVEEQPG LTLGGSVSSSG EALTNAQPS SETVQQESSS SSHHDAKNQQ 360	
PVPCEPATPK RTIRDCFYNM EDSPTQPTLP KRGLFLKEET FKNDLKGNGG KRQMVDLKPE 420	
MSRSTPLSVD PPDRSKLCLV LQSSYPNSPS AASQSYECLH KVGNGNLNT VKFHKEIKE 480	
SLGLRLNDCYK EKSRLKKPHK TSEEVPPCRT PKRGTGSGKQ AKNTKSSAVP NGELSUTSKA 540	
IEGPQNTNSAS TSSLPEPCNQ SWNALKLQLOQS ETSSSPAFTQ SSESSVGSDN IMSPVPLLSK 600	
HKSKKKGQASS PSHVTRNGEV WEAWYGSDEY LAPPSHLKQT EVLALKLEN TKLLPKPRG 660	
ETIQNIDDWIE LSEMNSDSEI YPTVHVKKKH TRLGRVSPSS SSDIASSLGE SIESGPLSDI 720	
LSDEESSMPL AGMKKYADEK SERASSSEKN ESHSATKSAL IOKLMQDIQH QDNYEAIWEK 780	
IEGFVNKLDE PIQWLNEAME TTENWTPPKA EMDDLKLYLE THLSFKLNVD SHCALKEAVE 840	
EIGHQNLLELI ASHKAGLKDM LRMIAQSWE LQRQIKRQHS WILRALDTIK AEILATDVSV 900	
EDEEGTGSPK AEVQLCYALE QRDAVEQMSL KLYSEQYTLQSK SKRKEEFADM SKVHSVGNSG 960	
LILDFDSEYQE LWDCCLIDMES LVMDSHDLMM SEBQQQHLYK RYSVEMSIH LKKTELLSKV 1020	
EALKKGGVLL PNDLLEKVDS INEKWELLGK TLGEKIQDMT AGHSGSSPRD LLSPESGLSV 1080	
RQLEVRKIEL KGWLRLTEFL IFNSCLRQEK EGTMNTEKQL QYFKSLCREI KQRRRGVASI 1140	
LRLCQHLLDD RETCNLNADH QPMQLIIVNL ERRWEAIVMQ AVQWQTRLQK KMKGESETLN 1200	
VIDPGLMDLN GMSEDALEWD EMDISNKLIS LNEESNDLQ ELQPVIPSLK LGETSNEDPG 1260	
YDEEADNHGG SQYASNITAP SSPHIYQVYS LHNVELYEDN HMPFLKNNPK VTGTMQPNVL 1320	
TKSLSKDSSF SSTKSLPDLL GGSNLVKPCA CHGGDMSQNS GSESIGIVSEG DTETTTNSEM 1380	
CLLNNAVGDSP SNLETEHLDP QMGDAVNVLK QKFTDEGESI KLPNQSSSSI SPVGCVNGKV 1440	
GDLNSSITKHT PDCLGEELQG KHDVFTFYDY SYLQGSKLKL PMIMKQSQSE KVHVEDPLLR 1500	
GFYFDKKSCK SKHQTTTELQV DVPPHERILA SASHEMDRIS YKSGNIEKTF TGMQNAKQLS 1560	
LLSHSSSIES LSPGGDLFGL GIFTKNGSDSL QRSTSLESWL TSYKSNEDLF SCHSSGDISV 1620	

-continued

SSGSVGELSK	RTLDLLNRLE	NIQSPSEQKI	KRSVSDITLQ	SSSQKMSFTG	QMSLDIASSI	1680
NEDSAASLT	LSSSDLSLC	SEDIVLHKNK	IPESNASFRK	RLTRSVADES	DVNNSMIVNV	1740
SCTSACTDDE	DDSDLLSSST	LTLEELCI	KDEDDDSIA	TDDEIYEDCT	LMSGLDYIKN	1800
ELQTWIRPKL	SLTRDKRKN	VSDEMKGSKD	ISSEEMTNPS	DTLNIELTLN	GSVKRVSENN	1860
GNGKNSSSH	ELGTKRENKK	TIFKVNKDPY	VADMENGNIE	GIPERQKGKP	NVTSKVSEN	1920
GSHGKEISES	EHCKCKALMD	SLDDSNATAGK	EFVFSQDVRLH	PKKCPNHHHP	ENQSTASTPT	1980
EKSFSSELALE	TRFNNNRQDSD	ALKSSDDAPS	MAGKSAGCCL	ALEQNGTEEN	ASISNISCCN	2040
CEPDVFHQKD	AEDCSVHNFV	KEIIDMASTA	LKSKSQPENE	VAAPTSLTQI	KEKVLEHSHR	2100
PIQLRKGDYF	SYSLSLSSHDS	DCGEVTNYIE	EKSSTPLPLD	TTDSGLDDKE	DIECFFEACV	2160
EGDSDFEPPC	FSSAPPNEA	VPSEAAMPLQ	ATACSSSEPFD	SSLSSADDADT	VALSSPSSQE	2220
RAEVGKEVNG	LPQTSSGCAE	NLEFTPSKLD	SEKESSGPKG	ESGMPEEHNA	ASAOKSVQDL	2280
SLKANQPTDK	AALHPSPKTL	TCEENLLNLH	EKRHRNMHR			2319

SEQ ID NO: 9 moltype = AA length = 190
 FEATURE Location/Qualifiers
 source 1..190
 mol_type = protein
 organism = Homo sapiens

SEQUENCE: 9
 MGKSSTPLPL DTTDSGLDDK EDIECFFEAC VEGDSDGEEPF CFSSAPPNEA AVPSEAAMPL 60
 QATACSSEF S DSSLSSADDAD TVALSSPSSQ ERAEVGKEVN GLPQTSSGCA ENLEFTPSKL 120
 DESEKESSGPKG GESGMPEEHNA AASAKSKVQD LSLKANQPTD KAALHPSPKT LTCEENLLNL 180
 HEKRHRNMHR 190

SEQ ID NO: 10 moltype = DNA length = 5887
 FEATURE Location/Qualifiers
 misc_feature 1..5887
 note = chemically synthesized
 source 1..5887
 mol_type = other DNA
 organism = synthetic construct

SEQUENCE: 10
 accggagtgc ttcacccgg cgtcaatacg ggataatacc gcgccacata gcagaacctt 60
 aaaagggtc atcattggaa aacgttcttc gggcgaaaa ctctcaagga tcttaccgct 120
 gttagatcc agttcgatgt aacccactcg tgaccccaac ttagatccatg catctttac 180
 ttccaccagc gtttctgggt gagcaaaaac aggaaggcaa aatggcccaa aaaagggaat 240
 aaggcgacca cggaaatgtt caattcaact actttctt tttcaatatt attgaacat 300
 ttatcagggt tattgtctca tgagcggata catatggaa tgtatttga aaaataaaca 360
 aatagggggtt ccggcacat ttccccggaa agtgccaccc ttagatccaaag aaaccatatt 420
 tattcatgaca ttaacccata aaaaatggcg tattcacggg cccttctgtc tcgcgcgtt 480
 cggtgatgac ggtgaaaaacc tctgacacat gcaagctccc gagacggcaca cagttgtct 540
 gtaagcggat gccggggagca gacaagcccg tcagggcgcg tcagcgggtt ttggcgggt 600
 tcggggctgg cttaaactatcg cggcatcaga gcaatggatcg ctgagatgtc accatattggc 660
 cgtcccgatca agtcagcgta atgtctgtc agtggatccaaatccatcataacc aattctgtt 720
 agaaaaaaactc atcggacatc aatggaaact gcaatttatc catatcaga ttatcaatc 780
 catatttttt aaaaaggccgt ttctgtatg aaggagaaaa ctcaccggg cagttccata 840
 ggatggcaag atccctgtatcg cggatcgatc ttccgactct tccaacatca atacaaccta 900
 ttaatttccc cttcgatcaaaa ataagggtt caatggatggaa atcaccatga gtgacgactg 960
 aatcccggtga gaatggcaaa agtcttgcgta ttctttccca gacttgcata acaggccagc 1020
 cattacgctc gtcatcaaaa tcaactcgat caaccaaacc gttatttatt cgtgattgcg 1080
 cctggcggcg acggaaatacg cgatcgctgt taaaaggacca attacaacaaa ggaatcgaaat 1140
 gcaaccggcg cggaaacact gcaatcgatc caacaaatccatc ttccacatggaa tcaggatatt 1200
 cttctaaatc ttggatgtc gttttcccgq qgatcgcagat ggtgatgtac catgcataat 1260
 caggagtagc gataaaatgc ttgtatgtcg gaagaggcat aaattccgtc agccgatcta 1320
 gtctggccat cttcatgtatc acatccatgg caacgcgtacc ttgcctatgt ttcaaaaaca 1380
 actctggcgc atccgggttc ccataacatc gatagatgtt cgcacccgtat tgcccgacat 1440
 tattcgcgcg ccatttatacc cccatccatc cgcacccatcat gttggaaat aatcgccgccc 1500
 tcgagcaaga cgttcccgatgt tgaatatggc tcataaacacc ctttgtatgtt 1560
 aagcagacacat tttttatgtt catgtatgtatgttgcataatc taacatcaga 1620
 gattttggaa cacaacatgtgg ttgtatgtt gaaaatcgaaat ttttgcgtatgg tttcaaaaaca 1680
 agatcgcgcg ctttcccgac aacgcacccat gttccgtgcg aagcaaaaatc ttcaaaaatca 1740
 ccaactggc accttacacaac aaatcgatc tcaaccatgtgg ctccctact ttctggatgg 1800
 atgtatggggc gattcggcc tggatgtatgtt cggcaacacc ttcttcacgaa ggcagaccc 1860
 agcgctactc gggccgtgc gtcgtcgatc tcaactcgatc cgcccgccgaa aagcccgccc 1920
 gtcggggatc ctttggatc cccggatcgatc tgagcggatcg aegcgcgacaa gagggatgtt 1980
 ccaactccat cactatgggt tccctgtatgtt taatgtatgtt cccgcattgc tacttatata 2040
 cgttagccatc cgtatggatcc acatcgatc tcaaccatgtgg ctccctact ttctggatgg 2100
 ccttacatcg ctctgtatc attaggtatgtt ccattttatgtt gatgcagttt 2160
 ttccatgtatc aaaaatcgatc taaaatcgatc taaaatcgatc taaaatcgatc taaaatcgatc 2220
 attcgtatgtt acatcgatc tcaaccatgtgg tttccatgtatgtt gatgcagttt 2280
 ggttgcgcgc gggatgttgcg tcccaacatgtgg tccctgtatgtt cccgcattgc tacttatata 2340
 tgacatgtgg ctggatgttgcg tcccaacatgtgg tccctgtatgtt cccgcattgc tacttatata 2400
 taaaatcgatc aaaaatcgatc taaaatcgatc taaaatcgatc taaaatcgatc taaaatcgatc 2460
 aaggggggaccc acggggggggg ccggggccgcg tgctgcaaaa atagcagatc acaatgtttt 2520
 catccctctc tggatgttgcg tcccaacatgtgg tccctgtatgtt cccgcattgc tacttatata 2580
 gggggggatc taaaatcgatc taaaatcgatc taaaatcgatc taaaatcgatc taaaatcgatc 2640

-continued

ccgcgtccgtg	ggacctaagc	ttgtctagcgc	taccggcgc	caccatgggt	aaaaggcagca	2700
cttcattggc	actagacacc	actgactcg	gcttagatga	caagggagat	attgaatgt	2760
tttttgggc	ctgtgttgag	ggtgactctg	atggagagga	gccttggttc	tctagtgtc	2820
ctccaatga	atctgcagtt	cccacgcga	ctgcaatgc	actacaagca	acagcatgtt	2880
cttctgagtt	cagtgtatgt	tcttttcag	ctgatgtgc	agatacagtg	gctcttcaa	2940
gttcctccctc	tcaaggaaaga	gctgagggtg	gaaaggaaatg	gaatgggtt	ccccaaactt	3000
caagtggctg	tgcagaaaaac	ttagagttt	ctccatcaaa	gcttgacagt	gaaaaggaaaa	3060
gttccggaaa	accagggtaa	tctggatgc	cagaagaaca	taatgtctgt	tcagccaaat	3120
ctaaagtta	acacctctcc	ttgaaggca	atcagccaa	agacaaggcc	gcattgcac	3180
caagccccaa	aactttaacc	tgtgaagaaaa	atcttctaaa	ccttcatgaa	aaacqacata	3240
gaaatatgca	taggttagt	gtaatgc	cacgcattgaa	aatcatctca	ttgaqagata	3300
gcgtggctg	agctcaggc	tagttaagtt	tgatccgcgg	ccgcaatcaa	cctctggatt	3360
acaaaatttg	tgaaagattt	actgatattt	ttaactatgt	tgctcctttt	acgctgtgt	3420
gatatgtgc	ttaatcacct	ctgtatcg	ctatgttgc	ccgttacggct	ttcggtttct	3480
cctccttgc	taaattctgg	ttgtgtctc	tttataaggaa	gttggcc	gttgcgc	3540
aacgtggcgt	ggtgtgtct	gtgtttgt	acggcaacccc	cactggctgg	ggcattgcca	3600
ccacctgtca	actcctttct	gggactttcg	cttccccctt	cccgatcgcc	acggcagaa	3660
tcatcgccg	ctgccttgc	cgctgctg	caggggctag	gttgcgtggc	actgataatt	3720
cgctgggtt	gtcggggaaa	tcatgtct	ttcccttggct	getcgeotgt	gttgceraact	3780
ggatctgc	ccggacgtcc	ttctgtct	tcccttgc	tctcaatcca	ggggaccc	3840
cttcccgogg	cattctgcgg	gttctgcgg	cttccccgg	tcttcgtt	cgccctccga	3900
cggatcggt	ctcccttgg	ggccgcctcc	cgccctgttgg	cctcacc	gatctcgat	3960
cttattttgt	gaaattttgt	atgtatttgc	tttattttgt	accattataa	gctgcaataa	4020
acaagttaac	aaacaacaa	gtatccatt	ttatgttgc	tttgcgggg	aggtgtggga	4080
ggtttttaa	agcaagtaaa	accttacaa	atgtgttat	gttgcatttt	actccctctc	4140
tgccgcgtcg	ctcgctca	gaggccggd	gacc	acgc	ccgggtttt	4200
ccggggcgc	cctcgatgc	gagcgagcc	gcccgcgt	gttgcgtt	4260	
ccctataatgt	agtctgttta	atttcgat	tttgcatttgc	tttgcattaa	tgaatcgcc	4320
aacgcgcggg	gagaggccgt	tttgcatttgc	tttgcatttgc	tttgcatttgc	tttgcatttgc	4380
cgctgcgtc	ggtcgttgc	cttgcggc	tttgcatttgc	tttgcatttgc	tttgcatttgc	4440
gttgcatttgc	aaatccacgg	gataatcg	tttgcatttgc	tttgcatttgc	tttgcatttgc	4500
aggccaggaa	ggccggggaa	tttgcatttgc	tttgcatttgc	tttgcatttgc	tttgcatttgc	4560
acggatcatca	aaaaatcg	tttgcatttgc	tttgcatttgc	tttgcatttgc	tttgcatttgc	4620
gataccggc	gttccccctt	ggaaagctcc	tttgcatttgc	tttgcatttgc	tttgcatttgc	4680
ttaccggata	cttgcgttgc	tttgcatttgc	tttgcatttgc	tttgcatttgc	tttgcatttgc	4740
gctgttagtta	tctcgttgc	tttgcatttgc	tttgcatttgc	tttgcatttgc	tttgcatttgc	4800
cccccggttca	gcccgcacc	tttgcatttgc	tttgcatttgc	tttgcatttgc	tttgcatttgc	4860
taagacacga	tttgcatttgc	tttgcatttgc	tttgcatttgc	tttgcatttgc	tttgcatttgc	4920
atgttagggc	tttgcatttgc	tttgcatttgc	tttgcatttgc	tttgcatttgc	tttgcatttgc	4980
cagtattttgg	tatctgcgt	tttgcatttgc	tttgcatttgc	tttgcatttgc	tttgcatttgc	5040
tttgcatttgc	aaacaaacc	tttgcatttgc	tttgcatttgc	tttgcatttgc	tttgcatttgc	5100
ttacgcgc	aaaaaaagga	tttgcatttgc	tttgcatttgc	tttgcatttgc	tttgcatttgc	5160
ctcgtggaa	aaaaaaactca	tttgcatttgc	tttgcatttgc	tttgcatttgc	tttgcatttgc	5220
tcaccttagat	cattttat	tttgcatttgc	tttgcatttgc	tttgcatttgc	tttgcatttgc	5280
aaaccttggc	tgcacat	tttgcatttgc	tttgcatttgc	tttgcatttgc	tttgcatttgc	5340
tatttcgttca	atccatagtt	tttgcatttgc	tttgcatttgc	tttgcatttgc	tttgcatttgc	5400
gcttaccatc	tggccccc	tttgcatttgc	tttgcatttgc	tttgcatttgc	tttgcatttgc	5460
atttacatc	aaaaatca	tttgcatttgc	tttgcatttgc	tttgcatttgc	tttgcatttgc	5520
tatccgc	ccatcgatct	tttgcatttgc	tttgcatttgc	tttgcatttgc	tttgcatttgc	5580
ttaatagttt	tcacaacgtt	tttgcatttgc	tttgcatttgc	tttgcatttgc	tttgcatttgc	5640
tttgcatttgc	tttgcatttgc	tttgcatttgc	tttgcatttgc	tttgcatttgc	tttgcatttgc	5700
tgttgtgca	aaaaggcgtt	tttgcatttgc	tttgcatttgc	tttgcatttgc	tttgcatttgc	5760
cccgactgtt	atcactcgat	tttgcatttgc	tttgcatttgc	tttgcatttgc	tttgcatttgc	5820
ccgtaaatgt	tttttgcatttgc	tttgcatttgc	tttgcatttgc	tttgcatttgc	tttgcatttgc	5880
tgccggc						5887

SEQ ID NO: 11 moltype = AA length = 190
 FEATURE Location/Qualifiers
 REGION 1..190
 note = chemically synthesized
 source 1..190
 mol_type = protein
 organism = synthetic construct
 SEQUENCE: 11
 MGKSSTPLPL DTTDSGLDDK EDIECFEEAC VEGDSDGEEPF CFSSAPPNESA VPSEAAMPL 60
 QATACSEFS DSSLADDAD TVALSPSQ ERAEVGKEVN GLPQTSSGCA ENLEPTPSKL 120
 DSEKESSGKP GESGMPEEHN AASAKSKVQD LSLKANQPTD KAALHPSPKT LTCEENLLNL 180
 HEKRHRNMHR 190

SEQ ID NO: 12 moltype = AA length = 209
 FEATURE Location/Qualifiers
 REGION 1..209
 note = myc-tagged rat mAKAP PBD
 source 1..209
 mol_type = protein
 organism = synthetic construct

-continued

```

SEQUENCE: 12
MQQLISEED LSPGMLTMSV TLSPLRSQTP LPPDAVDSGL DDKEDMDCCF EACVEDEPVN 60
EEAGLPGALP NESAIEDGAE QKSEQKTASS PVLSDKTDLV PLSGLSPQKG ADDAKEGDDV 120
SHTSQGCAES TEPPTPSGKA NAEGRSRMCG VSATPEENAA SAKPKIQAFS LNAKQPKGV 180
AMRYPSPQTL TCKEKLVNFH EDRHSNMHR 209

SEQ ID NO: 13      moltype = DNA  length = 4064
FEATURE          Location/Qualifiers
misc_feature     1..4064
source           note = pscA-TnT-myc-rat mAKAP PBD plasmid
                 1..4064
mol_type         mol_type = other DNA
organism          organism = synthetic construct

SEQUENCE: 13
actcaaccaa gtcattctga gaatagtgtc tgccggcacc gagttgcctc tggccggcgt 60
caatacggga taataccggc ccacatagca qaactttaaa aqgtgcctatc attggaaaac 120
gttcttcggg gcgaaaactc tcaaggatct taccgcgtt gagatccagt tcgatgtaaac 180
ccactcgtc acccaactgat tcttcgcgtt ctttacttt caccagcgtt tctgggttag 240
caaaaaacagg aaggcaaaaat gcccggaaaaa agggataaaag ggccgacacgg aaatgttga 300
taactcatact ttccctttt caattattt aqagcattta tcagggttat tgcgtcatga 360
gcggatcacat atttgaatgt atttagaaaaa ataaacaaaat aggggttccg cgccatattc 420
cccgaaaatg gocacatgcgat gtctaaagaaa ccattattat catgacatca acctataaaa 480
ataggcgatc cacggggcc tttcgctcg cgggttccgg tgatgcgtt gaaaacctt 540
gacacatgcgat gtcgggggg acggccacag cttgtctgtt aqccgtatgcg gggggcagac 600
aagccccgtca gggcggtca gccgggttgg ggggtgtcg ggggtggctt aactatgcgg 660
catcagacgat gattgtactg agatgtcacc atatggacat attgtcgatca gaacggcgct 720
acaattaata ctaaacatcta tgcattatcata acataacgtt taggtgacat tatagaactc 780
gagccctcgccg gtcgcgtccg tcaactggggccg cggccggggcc gtcggggccgac 840
cttgggtcgcc cccggccatcg tgacggccggc agggcgccaga gggggggggcc 900
caactgggtt tccctgtatc taatgattaa cccggccatgcg tcaattatcata cgtggccatg 960
ctctagacgat gtcggggctt tcacaaagaca gcatctggggcc ctggccggaga ggggtgggtc 1020
cgaaggcgctg ctttacatcgcc gtcggccggcc ctggggggggcc acatcggtt ggcgggtgc 1080
agccccctggcc gcaactcactg attcgcgtcc gacgggttta aaatagaaaaa actctggggc 1140
cacacaatag ctggggctta tatgggctcc tggggggggaa gggggggggcc 1200
ggggggccgtcg tggccaaaatg aqccgtctca aqgtgttgc ttcctctcg gggccggggcc 1260
acattccgtcc tggctctggcc gccccccccccggggccggggccatca aqccctctgc 1320
ccccccaaggaa gcccctccca gacagccggcc ggacccccccatc gtcggccggggcc 1380
gctagcgatca cccggccatcgcc ccatggggccgca gaaactcattc tctgaagggg atctggggcc 1440
ggggatgttca accatcgatcg tgacacttc cccactggggcc tcaacatgcg cattggccacc 1500
ggacgctgtg gactctgggt tagatgacaa ggaagacatcg gactgttctt ttgaagcttg 1560
tgggtggatg gacccgttca atgaggaatcg tgggtctccccc ggtggcccttc ccaatgaatc 1620
agccatcgatcg gatggggccgatcg agccaaatggcc acagcccgatc tccctgtgc 1680
caatgtggatcg acagactcgatcg tggcccttc tggactttcc ccttcggggcc ggggtgtatcg 1740
tgcaaaaggaa gggatgtatcg tggatccatcg tggccggggcc tggccggggcc gacacggcc 1800
taccacccccc tccggggatcg ccaatgcgatcg gggggggggcc gggggggggcc 1860
aacggccagaa gaaaacgtcg tccggggatcg acggaaaattt caagcttctt ctttgaatgc 1920
aaaacacogaca aaaaacggaaatcg tggccatcgatcg tggatcccgccccaaatcg ttttgc 1980
agagaagatcgatcg tggatccatcg acacatcgatcg atgatcgatcg agatgttaatcg 2040
ggccccccacgcg atggaaatcg tccatcgatcg agatcgatcg tggatccatcg aggggtatcg 2100
aagtttgcgtatcg cagacatcgatcg aagatacattt gatgatcgatcg gacccaaatcg aacttagatcg 2160
caatgtggatcg aatgttgcgtatcg tggatccatcg tggatccatcg tggatccatcg 2220
ataatgtcgatcg aataaaatcg tggatccatcg tggatccatcg tggatccatcg 2280
gggggggggtt gggggggggatcg tggatccatcg tggatccatcg tggatccatcg 2340
taccacccccc tccatcgatcg tggatccatcg tggatccatcg tggatccatcg tggatccatcg 2400
acggccggccgatcg tggatccatcg tggatccatcg tggatccatcg tggatccatcg 2460
agatcgatcgatcg tggatccatcg tggatccatcg tggatccatcg tggatccatcg 2520
ttaatgtcgatcg tggatccatcg tggatccatcg tggatccatcg tggatccatcg 2580
ctcgatcgatcg tggatccatcg tggatccatcg tggatccatcg tggatccatcg 2640
aaaggcgatcgatcg tggatccatcg tggatccatcg tggatccatcg tggatccatcg 2700
aaaaggccatcgatcg tggatccatcg tggatccatcg tggatccatcg tggatccatcg 2760
gtccggccccc ctcggccatcg tggatccatcg tggatccatcg tggatccatcg tggatccatcg 2820
gacaggactatcg tggatccatcg tggatccatcg tggatccatcg tggatccatcg 2880
tccggccatcg tggatccatcg tggatccatcg tggatccatcg tggatccatcg 2940
ttctcgatcgatcg tggatccatcg tggatccatcg tggatccatcg tggatccatcg 3000
ctgtgtgcac gggccatcgatcg tggatccatcg tggatccatcg tggatccatcg 3060
tgatgtccacatcg tggatccatcg tggatccatcg tggatccatcg tggatccatcg 3120
tagcagatcgatcg tggatccatcg tggatccatcg tggatccatcg tggatccatcg 3180
ctacactatcgatcg tggatccatcg tggatccatcg tggatccatcg tggatccatcg 3240
aaagatgtatcgatcg tggatccatcg tggatccatcg tggatccatcg tggatccatcg 3300
ttggcaacatcgatcg tggatccatcg tggatccatcg tggatccatcg tggatccatcg 3360
tacggggatcgatcg tggatccatcg tggatccatcg tggatccatcg tggatccatcg 3420
atcaaaaatcgatcg tggatccatcg tggatccatcg tggatccatcg tggatccatcg 3480
aagtatataatcgatcg tggatccatcg tggatccatcg tggatccatcg tggatccatcg 3540
ctcggccatcgatcg tggatccatcg tggatccatcg tggatccatcg tggatccatcg 3600
tacgatcgatcg tggatccatcg tggatccatcg tggatccatcg tggatccatcg 3660
ctcaccggatcgatcg tggatccatcg tggatccatcg tggatccatcg tggatccatcg 3720

```

-continued

tggtcctgca	actttatccg	cctccatcca	gtcttataat	tgttgccggg	aagctagagt	3780
aaagtatccg	ccagtttgc	gttttgccaa	cgttggttgc	attgtctacag	gcatcgttgt	3840
gtcacgtcg	tcgtttggta	tgggttcatt	cagtcgggt	tcccaacgt	caaggcgagt	3900
tacatgtacc	ccccatgtgt	gaaaaaaago	ggttagtctc	ttcgggtctc	cgatcgtgt	3960
cagaagtaag	ttggccgcag	tgttatcact	catggttatg	gcagcactgc	ataattctct	4020
tactgtatcg	ccatccgtaa	gatgttttc	tgtgactgg	gagt		4064
 SEQ ID NO: 14						
FEATURE						
REGION						
source						
SEQUENCE: 14						
MEQKLISEED	LSPGMLTMSV	TLSPLRSOTP	LPPDAVDSDL	DDKEDMDCCF	EACVEDEPVN	60
EEAGLPGALP	NESAIEDGAE	QKSEQKTASS	PVLSDKTDLV	PLSGLSPQKG	ADDAKEGDDV	120
SHTSQGCAES	TEPTTPSGKA	NAEGRSRMKG	VSATPEENAA	SAKPKIQAFS	LNAKQPKGKV	180
AMRYPSPQTL	TCKEKLVNFH	EDRHNSNMHR				209
 SEQ ID NO: 15						
FEATURE						
source						
SEQUENCE: 15						
ggcgagaagg	aggcgaggagg	agcgattgtg	gccccggccg	cggtggccgg	cgccggccctgc	60
cctttgtgac	cgcagctgc	gccccacgc	ccgcgcggat	ggccgcgcgt	ccgggcctccc	120
tggccacgcg	tgcccgcccc	cggacctgag	ccccgcgcct	ggatgcgcgg	ggatgcgcgt	180
ccccccggccc	tgccgcgtct	ccgggctggg	cgcggggcga	tggacctgag	catgaagaag	240
ttcgcgcgtgc	gcagggttct	ctctgtgtac	ctgcgcggac	agtcgcgc	caagagctcc	300
agcctgagcc	ggctcgagga	agaaggcgctc	gtgaaaggaga	tagacatcag	ccatcatgtg	360
aaggagggct	ttgagaaggc	agatccttc	cagtttgc	tgctgaagg	tttagaccaa	420
ggatcctatg	gaaagggttt	cctgttgagg	aagggtgaagg	ggtccgcacgc	ttggcagctc	480
taacgcgtatg	aggttccat	gaaaggccac	ctaaaatgtt	gggacccagg	gagatcgaaag	540
atggagagag	acatcttggc	aaagatgtat	cacccttc	ttgtgaagct	tcattatgcc	600
tttcagacgg	aaggaaagct	ctacccgtac	ctgacttcc	tgccggggagg	ggaccccttc	660
acccggctct	ccaaagaggt	catgttcacg	gaggaggatg	tcaagtctt	cctggctgag	720
ctggcccttgg	ctttagacca	tctccacac	ctggggatca	tctacagaga	tctgaaggct	780
gagaacatcc	tcctggatga	agaggggcgc	attaagatca	cagatccgg	cctgatgtt	840
gaggccatcg	accacgacaa	gagacgtac	tccttctgc	ggacgtatca	gtatcgccg	900
cccgagggtgg	tgaaccggcg	aggacacacg	cagagtccgc	actgggtgtc	tttcggcggt	960
ctcatgtttg	agatgttcac	ggggcccttg	ccggttccagg	ggaaggacac	gaaggagacc	1020
atggctctca	tccttcacac	caaggtgggg	atgcgcgtat	tcctcgttgg	ggaggccacag	1080
agtttgcgtgc	gagcttctt	caaaccggac	ccctgtcaac	ggctgggtgc	ttggatgtac	1140
ggagtggagg	aaatataagcg	ccatcccttc	ttttgtgacca	tagactggaa	cacgtgtac	1200
cggaaaggaga	tcaaggccac	gttccaaacca	gcatgggggg	ggccctggaga	cacccctcac	1260
tttgaccctgg	agtttgcgtac	gcccggcc	acagacttcc	ttggcgttcc	cccgagtgtca	1320
aacgctcatc	acctgtttag	aggatttgc	ttttgtggct	caagctgtat	ccaggaggccc	1380
tcacagacg	atctgcacaa	agtcccgtt	cacccatc	tcgcacgtt	acacgggaaac	1440
aacatccat	tcacccgtatgg	ctacccatc	aaggaggaca	tcgggggtgg	ctccctactca	1500
gtgtgcgtac	gtatgtgtca	taaaggccaca	gacaccggat	atgcgtgtaa	gtatgttgc	1560
aagagacaa	gagacccttc	ggaagagatt	gatgttcc	tcgggtaccc	ccagcacccg	1620
aacatcatca	ccctcaagga	tgtctatgt	gatggcaagt	ttgtgtactt	ggtatggag	1680
ctgtatgcgt	gtggggagct	cttgacccgc	atcctccggc	agagatctt	ctcgaggcgc	1740
gaagccatgt	acgttctgt	caccatc	aagaccatgt	actacccatca	ttcccgagggg	1800
tttacccctt	ttgcacccatgg	gcccacat	acccctgggt	agatccgtgc	ggggatccgc	1860
gaatccatcc	gagatgtgc	cttcgggtt	gccaaggcgc	tgccgcgggg	gaacgggtgc	1920
ctcatgacac	ctgtatccat	ggggcccttg	aggctcttgc	gctgtcaaggc		1980
tatgtatgcgt	ctgtgtacat	ctgggttttgc	gggatctgt	tgtatccat	gttggcaggaa	2040
tttacccctt	ttgcacccatgg	gcccacat	acccctgggt	agatccgtgc	ggggatccgc	2100
agtggaaagt	atgcctttc	ttggggaaac	tgggacttgc	tatctgtac	gtatgttgc	2160
gtcgtgttca	agatgttca	cgtggaccct	catcagcgc	tgacggcgat	gcaagtgtc	2220
aaacaccggct	gggtgttca	cagagatgt	ctgtccccc	accagcttgc	ccgcacggac	2280
gtgcacccatgg	tgaaggccgc	gatggccgc	acttacttgc	cttccaaacag	aacacccatc	2340
ccccccggcc	ttggcccccgt	gtgttgc	accctggctc	agcgcacagg	catgaagaga	2400
ctcacgttca	cgccgtgt	gccccgtgg	ccctggcc	agcgtccct	gccagcatcc	2460
tcgtgggttc	acagaccccg	gcccggcgc	ccgtctgtca	cccagatgt	ccacaagttcc	2520
agcaggagg	ccgcgcggcc	cctccggctg	tcgtgttttgc	cccgaggagg		2580
gtcctgtac	ggggggatctt	ccaaatgtca	ctgcgcgc	ctccccggcc	getctcttt	2640
ctcccaacat	aaacccatgt	cccccctca	cctccgtgc	ccgtgcgg	ccgggggtt	2700
ctttagatgc	ccgcgggttc	tctcatacat	gggttctgtt	tctgcgc	gatctgttt	2760
ccaattatgt	agccggatcg	tttggtgcaga	ctcccaacat	ccacgttcc	ggttcccggt	2820
ggaaaggatgg	cagtgcgtgg	gcccacat	tttggtgcaga	ccacgttcc	ggttcccggt	2880
tgcacccatgg	ccccccggcc	tggatgcac	tttggtgcaga	ccacgttcc	ggttcccggt	2940
ggaggagatg	ccgttgcgtc	ccccaaat	tccaaagac	ccgttgcgtc	cccccctca	3000

-continued

gtttttgcct	gagggtgtgg	gtagccatc	cttcctctg	tcccagatc	aatgaggag	3060
taagagccca	gacgagagga	aggcaggctg	gatcttgc	ttgagagctc	cgtgtcacca	3120
ggatggagg	gggtgcctct	cgaggaggcc	tgtgtccacc	ccagtcctcg	gtttcccccg	3180
ggggggccaag	cgcaactggc	tgcgtctgt	ccccagctcc	cgtggccaca	cagctatcg	3240
gagggttgc	agggagtcgt	gggttctcg	acctgctca	ccctgtgtcg	gtttctgtg	3300
tgtcaccata	aagctgtgg	tttgcgtgt	tcacttcgt	ttttctgtc	tgtggagaaa	3360
ctgtgaattg	gagaaatgg	getctgtgg	tttccaccca	aaccttc	gtccagctgg	3420
aggctggagg	gagcacacagg	ccccacccag	caagactgagg	ggcagaggca	cagggtggag	3480
ggcagcggag	atcagctgg	acaggagcga	tgcactttgt	agatgtgtg	gtttgtgtt	3540
gegttttgtg	tctctgtgc	acagatctgt	ttttcacac	tgatecgat	tccctgggt	3600
gtgcacacag	ggccgggtgt	gggcatttag	gcacatgtgt	gtctacttc	attgagtaaa	3660
atcgagtgag	agggttcggg	cagcaggatc	gacgcggcagt	ccagcggca	gagggAACAC	3720
acgggtcctt	cattgtctg	taagggtgtt	gaagatgtctc	cctggggccc	cccaagcaga	3780
ctagatggaa	ggaggcgcgg	ctcagccccc	caccctgc	cactgaagag	cgccgcctct	3840
gcagcaagca	gggcttcagg	agggtccccg	tggccacage	caggtttcc	ctaagaagat	3900
gttattttgt	tgggtttgt	tcccccata	tctcgatct	cgtaaaaaac	taaaaaaaaa	3960
aaaataaaaga	aaaaatgtgc	tgcgtctga	aaaataactc	cttagtgg	tctgtatttt	4020
ttcagaccc	aaaataataaa	cttgcattca	aagctttaat	ccatgtgt	ttttttttt	4080
tttagagaacc	aaaaaaacata	aaaggcaca	gtcggactga	ataccgttt	ccatagtgc	4140
cacagggtat	tcctcatt	tttccatag	aaagatgtt	ttcccaaggc	tagaacact	4200
tccaccatga	tgaattgtc	ttttaggtct	taattatttc	acttctttt	agaaaacttag	4260
gaagaagtgg	ataatctgt	ggtcacacaa	tctgtccccc	cagaaatgaa	caaaagtcat	4320
cacccccc	gcttgcata	caggoaacga	tccccccatc	agctggccccc	accctttggc	4380
ctggcttgc	gtgcaggcc	gtctgttgc	ttaaagtca	tgggttttgg	tgcaggaggt	4440
gagaagtggg	ggaagtgaaa	gggaagcat	ccgtgagaa	gcccacccg	ttttccctcc	4500
ttgtgtgcc	atggggcacc	agctcatgt	tttttcagt	catccagg	tgtacagact	4560
tagcttgc	actctaaagaa	tgccaaaggc	acgcgcgcgc	ctcccccata	cagcgacgtc	4620
tgtccttaca	tgtattttgt	gtgcata	ggaggagaac	actggcttgg	ccctgtcccg	4680
ctgagtgct	gtgaaatacc	tctacttcc	ctccatatac	cagaacaaaa	tgatacttg	4740
catcccttca	caaaggatcg	cctaaagaa	ttatggat	atatgttat	ctaagcttc	4800
aaaaaaatgc	atgtacatgc	aaatgtact	tttcaagcag	cagtgcacat	gtaaaatgaa	4860
gtgttcttag	aatttcatt	ttgcgcgtc	agcgcaccc	cacaacgat	gaaatgtcc	4920
gtatgat	tttgcata	ccaaaatgt	atggcttc	tttcttcata	4980	
cagttcatca	taacccaa	cccccccccc	gggtcatgaa	aatcacagaa	tttataaaca	5040
catggaaacc	tagatctcg	gttccctg	ctaccgc	tggcccttgc	ctggccaccc	5100
tataggggtcc	tccttccctg	cgagcccccc	atgtgggaa	aatactgtat	tctcccaatc	5160
tgcagtggtt	gagcttgc	gaattccatc	ccaaaggca	acatggccaa	gagggtggaa	5220
tttcaactt	accctca	ccgatttgc	tgtgatttt	aactaactgt	gtatgtattt	5280
atgtttggaa	gattgttttgc	atttttaaat	gataatgt	cttactgtt	tccacttgc	5340
tttcattaaat	gggtttatcc	ttaaaggcata	cttgcatttgc	ttacacta	ctttactgtat	5400
tctctca	acatggccaa	gtttgatttgc	cactccgttc	atttctgaca	cggttgcgt	5460
cctccctactt	ttctcaagcgt	catgaaatt	cgagaatgg	gaaggacgt	gccggccct	5520
gacccgggttgc	gagggggccgg	aagggtggact	ccagcgcgc	tggggggct	gaggacggag	5580
gctgcacat	ctgtgtcg	ctactgac	cgcttctct	cctcgatct	gactcagcac	5640
ttttgttca	ggctcagcag	ttatgtttac	acatcattt	tatgttctgc	ttttgttatt	5700
catgttttag	atgggtggcc	actgtacaga	tatttattac	gtttccaga	ttttctgaaat	5760
agattttttgc	gaataaacat	ggttttatgc	agtgtatct	ttttctagcc	taacaat	5817

```

SEQ ID NO: 16          moltype = DNA  length = 8841
FEATURE             Location/Qualifiers
misc_feature        1..8841
                      note = rat mAKAPalpha mRNA
source              1..8841
                      mol_type = other DNA
                      organism = synthetic construct

SEQUENCE: 16
gcatcatgca gcagggtcaaa caaggcatct cctagtttgc atctcccaat atgtgtgtat 60
aacatcaaaa ggagacgtg ggaggcaggag atgctgtttt gggaaagaat aaggcttgc 120
tttctccatg ttaaccatg gctgtacatc ttccccacttgc aggtcacagg gcccagatcc 180
catggcgcacg gatgttcac ccatggccat caacatgacca cccatgtgg agcaggagga 240
aggagaggggg gaggagcccg tgaaggccat agacgcgtgg cagcgtatg gaaaggccacc 300
tccgcttccac acagcgcgcg actggaaat tgcctgcac ttacactgaa ttgagactgc 360
gctccggatgc acctcagaga ggggtccgtga cctgacatc tcagtcggc aggatgcaga 420
caagcaatgc gtggatgtgc atctgttgc gctgttgcac attttgcgttgc 480
ccatgtgggg cagatccatc cccttcgttgc gacggggat tccctaaatgc tgctgtctca 540
ctcggttgcac gtcatcgatc acatccacgc agtacactgt ctctggccacc agtccgcgt 600
atccgtgtct gtcctccggg agcgcacatc acatgggtctg caggacgcac atggcaacta 660
caccaggcgc actgcacattc tgcaagcgtt ctctgaagaa acaacgggg gcccggctg 720
ttcccttaca gaatgtggacg actcaggccaa gttaaactatc aaatgttccac aggattactt 780
gtctctggat tggatgttgc cccgcatttgc actctccgc tacatgtccaa gtggggatct 840
gtttgggtgc ctggccgcata tgaccacccat ccaggccaaa actaaatctt ttgactttg 900
gagctacatgt gatgtggaga aagagttcccg tggatgttgc cgaagegttg gggtgtttac 960
agtggccacc gacgcgtgtcc ctccagtcgtg tggagaagcc aatgaggatt catctca 1020
gtccctttca gatgtatcaca aagggtgaaca cggggaaagac ggtgtcccg tacctggaca 1080
gcagctggac tcaacgggtgg gaatgttccctt cttagacgcgc acgtggccaa atgtgcgc 1140
acacccttcg gagacagcaa aacaagactc tacttcctcc ccacagctg tgccgaagaa 1200

```

-continued

aaccgcgtt	ggtccttggt	aaattacgac	tcccaagaga	tccatcccg	attgtttaa	1260	
ttataccgg	gactccccca	cacagccca	attacccaa	agagggttt	ttctaaaaga	1320	
aactcaaag	aatgagcga	aaggcagt	cagaagggg	cagggtgt	attnaaagcc	1380	
tgaactgac	agaagcaccc	cttccctgtt	ggacccccc	gacagatcg	agctctcc	1440	
agtgttgc	tcctcttacc	ccagcagcc	ttctgtgc	agccagtct	atgaatgttt	1500	
gcacaagggt	gggctggca	atcttgaaaa	catacgaga	agtccat	aagaatttc	1560	
ttccagttcg	ggaaggctta	ctgactgca	taaagagaa	ttgcactg	aaaaggccaca	1620	
caagaccc	gocgaatgt	ctctgtcag	aatccctaa	caggaggg	ttcaggaaa	1680	
gcatctgt	agcaccggg	gtcagcagg	gccagatcg	gtatccct	gagctccaa	1740	
agccacatgt	agaccggaa	cagatctgc	gtctacagcc	tcagggtgg	tgtccacca	1800	
gagaatctgc	agtggacaa	ttccagtg	gtcgaaggcc	ttccatgt	cccttgcag	1860	
tcacagcgt	gaatcttc	ttggctcaga	tagcatca	ccccgggt	ctcttcttc	1920	
aaaaaaacaa	agccaaaaaa	gtccccacc	tgctccatgt	cacggccac	agaacggtca	1980	
ggtgtgtgg	gctctgtac	gtctgtat	gtacctagc	ctgccttc	acctgaagca	2040	
gacggaggt	ttatgttca	agctggag	cctaaccaa	ctcttaccc	agaaaccagg	2100	
aggagagacc	atccaggata	ttgtgact	gaaactgtt	gaaatgatt	cagattccg	2160	
aatctatcca	acataccaca	tcaagaaaa	acacacgaga	ctgggcacag	tgtctccaa	2220	
ctcatccagc	gacatggct	catctctgg	ggagagcat	gaatccggc	ccctgagtg	2280	
cattcttct	gacgaggact	tatgttgc	cctctccgg	tgtaaaaaat	tcaactgac	2340	
gaaatcagag	agacccat	cctccggaaa	gaacggag	cattctcgaa	caagatcag	2400	
tttgattcag	aaactaatgc	acgatattca	gcaccaagag	aactatgaag	ccatctggg	2460	
aaqaatttag	gggttgtgt	acaagctgg	tgaatttcatt	cagtggctaa	acgaagccat	2520	
ggagaccacc	gagaactgg	ctcccttcaa	agecgagacc	gacagccccc	ggctgtaccc	2580	
ggagacacac	tttggat	ttgttgcac	tgatggacgt	tgcccttca	aggaaggccgt	2640	
ggaggaagaa	ggacaccaac	ttcttgatct	cggtgtatct	cacaaggag	gactgaagga	2700	
cacgctgagg	atgttgcg	gtcaatggaa	ggagctcg	aggccaaatca	aacggcaaca	2760	
cacgtggatt	ctcagggcc	ttggacaccat	caaaaggccag	atactggcta	ctgtatgtt	2820	
tgtggggag	gggggggg	ggggaaagcc	caaggccgg	tttcagct	gccaccctgg	2880	
aacacagaga	gacgcgtgg	aacagatgtc	cctgaagctg	tacaggggg	agtagacccag	2940	
cgggagcaag	aggaaggaa	agtttccaa	catgtcgaa	gcccacgcgg	agggaaagcaa	3000	
tgggcttctg	gacttttatt	cagaatatac	ggagctctgg	gattggotg	ttgacatgg	3060	
gtccctctgt	atggacggcc	acgacatgt	tgatgtcag	gagcggcag	agcatcttta	3120	
caagagggtac	agtgtgaaa	tgtccatcag	gcatctgaa	aagtcaagac	tactcagca	3180	
gggttgaagct	ttgaagaaag	gtggcctt	actaccagac	gatatccctg	aaaaatgtgg	3240	
tcaattaat	aaaaaaatgg	agctgttgg	gaaaaccctt	agagagaaga	tacaggac	3300	
aatagcgggg	cacagtgggt	cgggccacac	tgacatgt	tctccctgaa	gcccggccct	3360	
ggtaaggcg	ctgggggtca	ggatcaaaga	gctgaaaagg	tggctaaagg	atacagagct	3420	
tttcatctt	aattctgtc	tgagacaaga	gaaggaagg	acaagcccg	agaaacagct	3480	
ccaaatctt	aatgtgtct	gtctgtat	caacgcgg	cggtgggg	tggccctcat	3540	
tctgagggt	ttccggcacc	ttctgtat	ccggggat	tgcacactga	acgcacatca	3600	
ccagccat	cagctgatc	ttgttaccc	cgagggccg	tggggggcc	tcgtcatg	3660	
agctgttcc	ttggcaaacac	ggttacaaaa	gaagatgggg	aaaggatccg	agactttgaa	3720	
tgtgtatgt	cttgcgtt	ttggactgt	ttggatgt	gaggatgccc	ttggatgtgg	3780	
tgaaaacagac	ataagtaaca	aactcattgt	tgtgcatgaa	gaatcaaaac	acccgttca	3840	
agacccagac	ctatgtc	ccgcgtgaa	gttgcggag	acacccacca	aggactctg	3900	
ttatgttgc	gaggcgggt	actgtgggg	gtctccgtat	acctcaata	tcaactgcacc	3960	
ttccggccca	cacatattac	aagtgtat	tcttcataat	gttggagttcc	acggggacac	4020	
ccacactca	tttctggaaa	gcagccctaa	gttcacaggc	acaacacacg	ctactgttt	4080	
aactaaagac	ctcagcaagg	acttcctt	ttcatctaca	aaatcgat	cagacccct	4140	
aggggggtcc	ggtttgggt	ggccttact	gtgtcacat	ggagacttga	gccagaattc	4200	
aggcgtgt	agtggaaat	tcagcgtgg	agacaacgg	atgcggacca	actctgacat	4260	
gagctgttcc	atgtgttag	acgggttcc	aagtaaccct	gaaacccgg	atccggaccc	4320	
acaaatgggg	gtatgtgg	atgtgttca	gcaaaatgtt	aaagacaacg	ggggaaagac	4380	
taagcttca	agtgttctc	gggcattccgt	ctcaccatgt	ttttgtgtaa	atggaaaagc	4440	
agggggat	aacagtgtt	ccaaacacac	tgtgtatgt	ttggggaaag	aactacaagg	4500	
aaaacatgc	tggtttat	tttatgtt	ctcgatctt	caaggttca	aactcaatt	4560	
accaatgata	ataaaacacg	cacagatgt	aaaggcacac	gtggggat	cccttcttgg	4620	
tgggtttat	tttgataaaa	agtcgttca	agtcataat	caggcttca	agtcacaacc	4680	
agatgtcg	ccccccggaa	ggatcttgc	aacgcgttcc	cacggatgg	gacgcacgc	4740	
atacaaaatgt	agcgtgttca	ccaaacacac	tgtgtatgt	ttggggaaag	aactacaagg	4800	
ccttctatct	cgtatgttca	ctgtatgtt	cctttcttca	gggggtgtt	ttttgtgtt	4860	
ggaaatctt	aaaaatggca	gtgacagcc	ccagcgggg	acttctttag	aaatgtgtt	4920	
gacatccat	aagagacat	aggatctt	tagctgtac	agctctgggg	acataatgtt	4980	
gacgtgttcc	tcgtgttgg	agctgtatgt	ttggacgttca	ttgttgcata	ttggatgtgg	5040	
gaatataatgt	agccccctgg	agccaaatgt	ttggcggat	ttttctgaca	tgactctaca	5100	
aagcgttcc	ccaaatgttca	ccttcgttgg	ccagatgtca	ttggatgtcg	catectccat	5160	
caatgttgc	tctccggcat	ctcttacaga	actgtatgt	agcgatgtac	tctcttcttgc	5220	
ctcggggat	atgtgttac	acaaaaacaa	gatcccaga	tccaaacgc	cattcaggaa	5280	
ggccctgtat	cgctcgttgg	ctgtatgtt	ttggatgttca	ttgttgcata	ttggatgtgg	5340	
gtccctgcacc	tctgtgttgc	ctgtatgtt	ttggatgttca	ttttcttctt	ccagctccac	5400	
tctcaccat	actgttgc	ttttcttca	ttggatgttca	ttttcttctt	ccagttatgc	5460	
aacagatgt	gaaatattat	ttttttatgt	ttggatgttca	ttttcttctt	ccagttatgc	5520	
tgaactgtcg	acttggataa	ttttttatgt	ttggatgttca	ttttcttctt	ccagttatgc	5580	
tgtcaactgt	gaaataaaagg	tcaatataa	ttttttatgt	ttggatgttca	ttttcttctt	ccagttatgc	5640
ggacaccctgt	gacatgttca	cccttcttca	ttttttatgt	ttggatgttca	ttttcttctt	ccagttatgc	5700
cgggaaatgtt	aaagactccgc	ccagaactca	ttggatgttca	ttttcttctt	ccagttatgc	5760	

-continued

aagtacgtat	gacgttagta	aggatccgca	cgtggctgac	atggaaaatg	gcaatattga	5820
aagtaccca	gaaagaaaaa	gggagaagcc	acaaggggctt	ccagagggtt	cagagaacct	5880
tgcttcaaat	gtgaaaacga	tttcgtatc	ttagctcage	gagatgttga	cgttatgttga	5940
tggttctgag	gattcaagt	ttgcagaaa	ggaattttgt	cccccaatg	acagacatcc	6000
tccacagatg	ggtccaaac	tccagcatcc	cgaaaatcaa	agtggcact	gcaagccagt	6060
ccagaaccct	tgccccgggc	tactgtcgga	agttggcggt	ggaagcaggc	aagacagcaa	6120
tggactaaaa	tcttgceta	acatgcacc	aagtggggct	agaaaaacccg	ccgggttgcgt	6180
cctgctggag	cagaatgaga	cagaggaaag	tgtttctatc	acgacaaacg	cttctgttt	6240
caactgcaag	ccagatgtt	tccatcaaaa	agatgtatgg	gattgtttag	tacatgactt	6300
tgttaaggaa	atcttgatca	ttggatcaac	agccctaaaa	agtaaqtcac	agcctgaaag	6360
tgagggtggc	gcacccacat	caactaacc	attaaggaa	aagggtttag	agcattcga	6420
ccggccata	cacccgtggaa	agggggactt	ttactcttac	ttatcaccc	cgtccacacg	6480
cagtactgt	ggggaggtca	ccaattacat	agatgagaag	agcagttactc	cattgccacc	6540
ggacgcgtgt	gactctgggt	tagatgcata	ggaagacatc	gactgtttct	ttgaagcttg	6600
tgttggaggat	gagccgtca	atgaggaaac	ttgtctcc	ggtgccttc	ccaatgaaatc	6660
agccatcgag	gatggagcag	agcggaaatc	agacacaaag	acagccagct	ctccctgtgt	6720
cagtgacaag	acagacactgg	tgccttcc	aggactttcc	cctcagaagg	gagctgtatga	6780
tgcaaaggaa	ggagatgtat	tgcttcacac	ttcccaggcc	tgtgcagaga	gcacagagcc	6840
taccacccccc	tcaggaaagg	ccatcgaga	ggggagggtca	agatgcag	gtgtatcagc	6900
aacgcccagaa	gaaaacgcgt	cttcggccaa	acggaaaat	caagctttct	ctttgaatgc	6960
aaaacagccaa	aaaggcaagg	ttgcacatgg	gtatcccagg	ccccaaactc	taacctgtaa	7020
agagaagotc	gtaaaacttc	atgaagatcg	acacagtaac	atgcataatg	agagtgtaat	7080
gccccccacgc	atggaaatca	tctcattgtt	agatagcctc	agggctactc	7140	
caatccaccc	ttggccggtc	tctggcttca	tctgttatac	actgcgcct	gtcacatgt	7200
ctttctgaag	acgaacccctc	cttcggat	cagtctgtcc	acgtggcct	ctcgacactgg	7260
atgtgtgtat	tgctttctt	aggtgtatcc	cctagttcc	caaagctgt	ttgttctcc	7320
tggttgcgt	tcoccaatgt	ccttcggca	ccctgtcttcc	ccgcaagac	ttcggttcc	7380
cctcccccctc	ctccccccccc	ttaaagttcc	gcggctcacc	aaattgtatgg	tccatcaaac	7440
ccactgtctg	gaatgtatcc	cctccatca	gtacttgacc	aatgttatgt	tttgcgtctga	7500
aaacttgcgc	tgttagat	caatgtttat	tgaaagatgt	ttacctaaaa	agccgcct	7560
tgatgggttgc	ggagatgtat	ggagacacat	ttacccctt	aacaaaat	agtgtatgt	7620
gaaaagcgcata	ttttatattat	ttttttttaa	ataatgtatc	atgcacact	tcaagaaaca	7680
actataacac	tggttatct	tataaactgg	tacatttcc	tattaaggat	ttttttgtt	7740
tctatgttc	ttgaggatgg	gatgagaaaa	atgggttttt	ttttaaaacg	gtgtgcctt	7800
ctgttattact	tatagcattt	ataaaaaa	tgctttccat	gtaaggat	actgttttgc	7860
aaggaggaaa	tagcaagggtt	aatgtcggt	cataatttcc	gtatataatgt	ataagctgt	7920
gcaaacactg	atgtatgaca	gtataaaatg	cttcgtatgt	tgtgtatgtt	agtgtgttgg	7980
aatataaagcc	ttaaaccctgt	tcgatttgc	ggtaattaaa	attggcataa	taaaaatagc	8040
ttatgggggg	aaaggaaaaat	taatgtatc	ttcttccctgt	tttacccat	ttctttcatg	8100
ttgttctggg	aaagaaaaaa	aaacaaaccc	catatattag	cttcaccaat	atccatattt	8160
cacagaaggc	ttaagtgtt	tagactacag	actggcctt	aagacttcat	gatttccaa	8220
attttctgt	ttcactataa	acatccgaaa	tagcaaagat	ttctttcccc	tccatcaaca	8280
gcattttatt	ctgatgtttt	tttgcataat	tttgcataat	tttgcataat	tttgcataat	8340
tctcttactat	gccttacattt	attttataat	tttgcataat	tttgcataat	tttgcataat	8400
attnattat	tatttttat	aaactacaga	tttcagggtt	atttttttgc	ttaaatatttgc	8460
catttggct	tttgcgtt	ttatgtatgt	aaagtttgc	ttttttat	atatcataac	8520
tcctactaa	gtgcgttgc	cacatgttgt	atttgcata	tttgcataat	tttgcataat	8580
gggtgggttta	tatataatata	tatataatata	agatgttgc	atttttttgc	tttgcataat	8640
tgcaaagtgt	accagacaca	gcataatatttgc	agatgttgc	tttgcataat	tttgcataat	8700
cagaaatgac	aatcattat	tcagtagatt	agcatcttttgc	tttgcataat	tttgcataat	8760
gattcaggaa	gagcagctat	gaccgtatc	ctgccttgc	tttgcataat	tttgcataat	8820
gagtgcaccc	gtgcgcattt	c				8841

SEQ ID NO: 17 moltype = DNA length = 10227
 FEATURE Location/Qualifiers
 misc_feature 1..10227
 note = human mAKAPbeta
 source 1..10227
 mol_type = other DNA
 organism = synthetic construct

SEQUENCE: 17
 gagtctggga gctgagtgac tgaggatttgc atagttacag ttcataacaa 60
 gcgtcttaggc agtttagaccc taatgtttca ggtatggaaa gaaagtatca tttttatgttt 120
 tagattctgt ttgttgcgtt gtttgcgtt acatgttgc 180
 agtttacaaatg tttttttttt ctcttcaccc ttgttgcgtt 240
 ctgtacacaaatg ggcttgcgtt catgttgcgtt cttttttttt 300
 caccacatcc ttggatccat gtttgcgtt tcaggatccat ttgttgcgtt 360
 ttgttgcgtt atccatgttcc tccttgcataat agatgttgc 420
 ttgttgcgtt atagttgcataat tccttcaccc ttgttgcgtt 480
 agtgcgttgcgtt ctgcgttgcgtt gtttgcgtt 540
 taggcacacgc gacatccatcc aagtttccatcc ttgttgcgtt 600
 tctaacaatggatccat cttttttttt ttgttgcgtt 660
 tctggatccat ccattttccatcc ttgttgcgtt 720
 cttttttttt ggttgcgtt 780
 ctatgttgcgtt atggaaaagg agtttccatcc ttgttgcgtt 840
 agtgcgttgcgtt tctaacaatggatccat cttttttttt ttgttgcgtt 900

-continued

atctctctca	gtagacgaca	aagggtggatg	tgaggaagac	aatgcttcgt	cagtcgaaga	960
gcacccaggc	ttaacactgg	gggtgtcatc	atcttcagga	gaagcttgcg	caaagtgcgc	1020
tcaaccctcc	tctgagactg	tgccgcaaga	atccagttcc	tcctccatc	atgatgc当地	1080
gaatcagcag	ctgttcctt	gtaaaaatgc	aaccccccaa	cgaaccatca	gagattgttt	1140
taattataac	gaggactctc	ccacgcagcc	tacattgcca	aaaagaggac	ttttcttaa	1200
agaggaaact	ttaagaatg	atctggaaagg	caatgggtgg	aaaggccaaa	tggttgatct	1260
aaagcctgag	atgagcagaa	gcaccccttc	gcttagatg	cctctgaca	gatccaaact	1320
ttgcctggtt	ttgcgtctt	cttacccaa	cagcccttc	gtgcgcagcc	agtcttatga	1380
gtgtttcac	aagggtggga	atgggaaacct	tgaaaacaca	gtcaaaattc	acattaaga	1440
aatttctcc	accctggaa	ggcttaacac	ctgctataaa	gagaatctc	gacttaaaa	1500
gccacacaa	acctcagaag	agggtctcc	atgcccaca	cctaaacggg	ggactgggtc	1560
aggcaacaa	gtaaaaaata	caaaagagtc	agcagtgcca	aatggagagc	tttcttatac	1620
ttccaaggcc	atagagggc	cacaaacaaa	ttctgcttc	acatctc	ttgagccctt	1680
taatcagaga	agttgaaatg	ccaaatgtca	atgcgtca	aaacatcca	gttaccacgc	1740
ttttaactcg	agcagtgtat	cctctgttgg	ctcagacaa	atcatgttc	cggtgcact	1800
tctttcaaaa	cacaaaagca	aaaaaggta	acgcttcctt	ccaagtcac	tcactagaa	1860
tggtgaggtt	gtggaggcct	ggtatggctc	tgatgaatac	ctagcactgc	ccttcacact	1920
taagcagaca	gaagtattgg	ctttaaagg	ggaaaaccta	acaaagcttc	tgccctcagaa	1980
acccagagaa	gaaaccatcc	agaatattga	tgactgggg	ctgtctgaaa	tgaattcaga	2040
ttctgaaatc	tatccaac	atcatgtca	aaagaagcat	acaaggctag	gcagggtgtc	2100
tccaagctca	tctagtgaca	tagectctc	actaggggg	agcattgaat	ctggggccct	2160
gagtgcatt	ttttctgtat	aggagtccag	tatgcctc	gttggcatga	aaaagtatgc	2220
tgatgagaag	tcaaaaaag	cttcattc	tgagaaaaat	gagacccatt	ctggccactaa	2280
atcagcttta	atcagaaac	tgatgcaga	tattcagcac	caagaaact	atgaagccat	2340
atggaaaaaa	atagaggggt	ttgttaacaa	actggatgaa	ttcattcaat	ggttaatga	2400
agccatggaa	actacagaga	attggactcc	ccctaaacgca	gagatggatg	accttaaact	2460
gtatctggag	acacacttga	gttttaagg	gaatgttagac	agtcattgt	ctctcaaggaa	2520
agctgtggag	gaggaggac	accaacttc	tgagcttca	gcatctcaca	aagcaggact	2580
gaaggagatc	ctgcggatg	ttcagaatc	atgaaaggag	ctgcagaggc	aaatcaaag	2640
gcagcacacg	tggttctca	gggtcttgg	taccatcaa	gcccggat	tggctactga	2700
tgtgtctgt	gaggatgggg	aaggatctgg	aaggcccaat	gtcgagggtc	aactatgtca	2760
ccttggaa	caaaagatg	ctgttgag	tgatgttcc	aaagtcata	gcggcggat	2820
taccagcgc	agcaacgca	aggaaaggat	tgtgtat	tcaaaatgtc	attcgttggg	2880
aagcaatggg	cttctggact	ttgattcaga	atatcaggag	ctctgggatt	gcttgattga	2940
catggatcc	cttgtatgg	acagccacca	cctgtatgg	tcagaggagc	agcagcagca	3000
tctttagaa	cgatacgtt	ttggaaatgtc	catcagacac	ctggaaaaaa	cggagctgt	3060
tagtaagggtt	gaagcttga	agaaaagg	cgttttacta	ccaaatgtac	tccttggaaa	3120
agtggattca	attaatgaaa	aatgggaaact	gcttggaaa	accctaggag	agaagatcca	3180
ggacacaatg	gcagggcaca	gtgggtcgac	tccacgtgac	ctgcttc	ctgaaatgtt	3240
aagecctgtt	aggcagctgg	ttggatggat	ttggatggat	aaagaaact	aaaggatggc	3300
agagcttttca	atcttcaatt	cctgtctgt	acaagaaatgg	gaaggaaacaa	tgaatactga	3360
gaaacaactg	caatacttta	agtcctctg	tcgtgaaatc	aagcaacgac	gtcgaggagt	3420
tgccttcatt	ctgcgactat	gcccacatc	tttggatgac	cgggagactt	gcaatctgaa	3480
tgcagaccac	cagcccatgc	agctgtat	tgttaatctt	gaaagaagg	gggaatccat	3540
tgtcatcoa	gocgttc	cgatgttca	tctacaaa	aaatggggaa	aggaaatctga	3600
gactttgaat	gtgattgtat	ctgggttgc	ggacctaata	gggatggatg	aggatccct	3660
ggaaatggat	gaaatggaca	taatgaaata	ttttaattgt	ttgatgttgg	aatcaaatga	3720
cettgtatca	gaaacttcaac	ctgttattcc	ttcttggaa	tttggatgg	caagtaatga	3780
ggaccctgtt	tatgtatgg	aggctgtat	ccatgggg	tctcgtat	cctcaatata	3840
tactggcccc	tctatgttcc	acatttacca	ggtgtac	ctccacaat	ttgactctta	3900
tgaggatcaac	cacatgttcc	ttcttggaaa	caatccaa	gtcactggca	tgacacagcc	3960
taatgttata	taatagatgc	tcgtatggaa	ctcttcattt	tcatctaca	aatcttgc	4020
agatcttca	ggtgttcc	attttgtaa	gcccgttca	tgtcatgtt	gagatgttgc	4080
ccagaattca	ggcagtgaga	gttggattgt	cagtgttgc	gacacagaaa	ccactacaa	4140
ctctgaaatgt	tgctgtctca	atgcgttgc	ttgggtccca	agtaaccc	aaactgttca	4200
tctggatcc	caaaatgggg	atgcgttgc	ttgtttaaa	caaaaat	tcagatgttgc	4260
ggaagacatt	aagcttccaa	atgcgttca	gtcgccatt	tcaccagg	tttggatggaa	4320
tggaaaatgtt	ggatatttac	acgttattac	caaacatacc	cctgtacttt	tttggatggaa	4380
attacaagga	aaacatgtat	ttatgttac	ttatgttac	tcatacc	aaggcttcaaa	4440
actcaatgtt	ccatgttata	tttttgttca	tttttgttca	tttttgttca	tttttgttca	4500
cctgcttcgt	gtttttttt	tttgatggaaa	ttatgttata	tttttgttca	tttttgttca	4560
gttacaacca	gtatgttcc	tttttgttca	tttttgttca	tttttgttca	tttttgttca	4620
tcgcatttca	tataaaatgt	gtcaatata	tttttgttca	tttttgttca	tttttgttca	4680
acagcttcc	tttttgttca	ttatgttca	tttttgttca	tttttgttca	tttttgttca	4740
atttttgtt	ggcatgttta	aaaatgttca	tttttgttca	tttttgttca	tttttgttca	4800
aaatgttgc	tttttgttca	aaaatgttca	tttttgttca	tttttgttca	tttttgttca	4860
tataagctgt	tttttgttca	tttttgttca	tttttgttca	tttttgttca	tttttgttca	4920
tcgttttgg	aatatccaga	tttttgttca	tttttgttca	tttttgttca	tttttgttca	4980
cactcttca	tttttgttca	tttttgttca	tttttgttca	tttttgttca	tttttgttca	5040
atcttcttata	aatgttca	tttttgttca	tttttgttca	tttttgttca	tttttgttca	5100
ctcttcttgc	ttatgttca	tttttgttca	tttttgttca	tttttgttca	tttttgttca	5160
gttcaggaa	tttttgttca	tttttgttca	tttttgttca	tttttgttca	tttttgttca	5220
tgttaatgtc	tttttgttca	tttttgttca	tttttgttca	tttttgttca	tttttgttca	5280
cagcttacc	tttacccat	tttttgttca	tttttgttca	tttttgttca	tttttgttca	5340
cagtattgtc	acagatgtat	aaatgttca	tttttgttca	tttttgttca	tttttgttca	5400
cataaagaat	tttttgttca	tttttgttca	tttttgttca	tttttgttca	tttttgttca	5460

-continued

aagggtcaat	gtcagtgtat	agatgaaggg	cagtaaaagat	ataagttagca	gtgagatgac	5520
caatccctct	gatactctg	atatttggat	ccttctaaat	ggctctgtaa	aacgtgtctc	5580
tggaaaataat	ggaaatggta	agaatttcata	tcatacccat	gagttggga	caaagcggtga	5640
aaataaagaaa	actatttca	aagttataa	agatccatat	gtggctgaca	tggaaaatgg	5700
caatattgaa	ggtattccag	aaaggcaaaa	gggcaaaccg	aatgtgactt	caaaggatatac	5760
agaaaatctt	ggttcacatg	ggaaaagagat	ttcagagatg	gagcattgt	agtgtaaagc	5820
acttatggat	agtttagatg	attcaaaatac	tgctggcaag	gaatttgtt	cccagatgt	5880
tagacatctt	ccaaaagaaa	atgtccaaatca	ccaccattt	gaaaatcaa	gcactgcctc	5940
tactcccaact	gagaagtctt	tctcagaact	ggctttagaa	accaggttt	acaacagaca	6000
agactctgtat	gcaactgaaat	catactgtat	tgccccggat	atggctgaa	aatctgtctgg	6060
tttgtgccta	gcaactggac	aaaacggaa	agagggaaat	gcttctatac	gcaacatttc	6120
ctgtgcacat	tgtgagccag	atgtttcca	tcaaaaagat	gcccgaaggatt	gttcagtgaca	6180
caactttgtt	aaggaaatca	ttgacatggc	ttcgacagcc	ctaaaaagta	aatctcaacc	6240
tggaaaacgg	gtggctgtctc	ctacttcat	aactccaaatc	aaggagaag	tgttggagca	6300
ttctcaccgg	cccateccgc	tgagaaaaagg	ggactttat	tctctctatc	6360	
tcatgacage	gattgtgggg	aggtcaccat	ttacataqaa	gagaaaaagca	gcactccat	6420
gccactagac	accactgact	cgggctttaga	tgacaaggaa	gatattgaat	gtcttttta	6480
ggccctgttt	gagggtgtact	ctgtatggaa	ggggctttagt	ttctctatgt	ctccctccaaa	6540
tgaatctgc	gttcccgcg	aagctgtat	gcccactata	gcaacagcat	gttcttctga	6600
gttcagtgat	agttctctt	catactgtat	tgccagatata	tgggcttctt	caagtccctc	6660
ctctcaggaa	agagctgagg	ttggaaagga	agtgaatggt	ttgccccaaa	cttccagttg	6720
ctgtcgacaa	aaccttagatg	tttacttctt	aaagcttgcac	agtggaaaagg	aaagtccgg	6780
aaaaccaggat	gatctggaa	tgccagaaga	acataatgt	gcttcagcata	aatctaaatgt	6840
tcaagaccc	tccttgcagg	ccaaatgcgc	aacagacaag	gcccatttgc	atcccagccc	6900
caaaacttta	acctgtgaag	aaaatcttct	aaaccttcat	gaaaaacgac	atagaaatata	6960
gcatacgat	aatgttacccc	ctcccccaagd	atgaaaatca	tctcactgaa	agataccgcct	7020
gtgtcaact	cagggggtggc	cttacttctt	cgcccttggc	ttggcttotgg	ttccatcag	7080
tttgtcaact	cgggtttata	cattgtactt	tcccaagat	aatcttctt	ccaaaatgtgt	7140
tttctccaca	caaggcttgc	gatctgtat	tgtgcgttgc	ttctctttag	gtgatgtct	7200
ttgaagttca	gcaaaagctgc	ttgttctccc	atggattctt	gtcccaagct	acctcttacca	7260
accctcttc	tccactgtata	cttttcttctt	tgcccttctt	cttcccttcc	actctttaaa	7320
gttctcgat	tcaccaactg	tgatgttccat	aaatttcttct	gtcttagatg	acccccccac	7380
cagtacttgc	ccaaatttcat	gtatcaatct	ggattttttt	tttaacggta	taatgactgt	7440
gcttattgaa	agagttttac	ctaaaaagcc	aacatttgaa	ttgggttgcag	catagagaag	7500
aaacacttgtt	ctttccatca	aaatthaagca	actatttaaa	ggccattttt	atttatttca	7560
ttaaaaaat	aatctatgc	gcatttcag	aaacaacat	atgggttgc	atattataaa	7620
ctgtgtacat	tctacttatt	aatattgtac	aacatttca	ttttttatgc	ttcttgagg	7680
ggtaatgaga	aaaaagtttt	ttaaaaaagt	gtgccttgc	gtattttctta	taccatttt	7740
taaaaaagctg	ctttcacggt	aaaatattgt	ttgtttgaaa	ggagggaaata	gcaagggtta	7800
gatgtgtat	taattttctgt	atatatgtat	aaccaagtac	aaacattgtat	gtataatgac	7860
agtataaaat	gttttcatgt	ttgtgtatgc	tgatgtatgt	gaaaatataa	gccttaat	7920
cattagattg	catgttattt	aaaatttgcac	taataaaacac	agattattgg	ggggaaagga	7980
aaattttagtgc	tcttcttctt	atgttcttct	accaaattgt	tgcatctgt	tctggaaaag	8040
tatagcatgt	agcagcttcc	aaacatattt	atattgttctt	agaggcttta	attttacttac	8100
actagagat	agacgttac	ccttcgttt	tcaaaatctt	tctggtact	attttacttac	8160
tggAACAGCA	aatgttataaa	tgtcgttcc	cctaaacccaa	taaaccattt	tacttagattt	8220
tttattttca	cttatttattt	atgttataa	tttggatttt	aggttacttgc	tatgtcttta	8280
tttattttca	atattttttt	tgtatgtat	tgatgtatgt	tgatgtatgt	tgatgtatgt	8340
atttgactat	tttttttttt	tttcaggat	attttatgt	gtaaaaagaaa	ttgacaaga	8400
aatatatttcat	ctggccctta	ctgactctgt	ttaaaatgc	ttttttttttt	atatctgtac	8460
acctacttac	gtgcgttgc	cagttagat	tcaataaaa	tttactgtat	taaaggattt	8520
atttaggtgc	catgggtgac	tctatccctt	tattttgcac	cttaaaaatgt	gacacaacta	8580
gaaaagggttgc	caatgtatca	taaaatgcac	atagataata	tatataat	ttctaaaagg	8640
taaaaatgt	tgtgggttca	tgccgttcc	ggaatgcacaa	tcattcaaca	gatagttcag	8700
aaacactttt	tatctgtca	gcattttttt	agatccagaa	gatgtttttt	tgaacaaaca	8760
gacaaaggccc	tggcccttgc	agggtgttct	tgatgtatgt	tgatgtatgt	tgatgtatgt	8820
atgttgcattt	tgttgcgtat	ttgcacccat	tttttttttt	tatgttgc	acggccaaat	8880
agaaggcgc	gtgggttacaa	ggggaaatgt	tgatgtatgt	tgatgtatgt	tgatgtatgt	8940
gaatatttcat	tgcaatgttgc	tttttttttt	tttttttttt	tttttttttt	tttttttttt	9000
atgttgcattt	tgttgcgtat	tttttttttt	tttttttttt	tttttttttt	tttttttttt	9060
gacactttttt	acaggcttgc	tttttttttt	tttttttttt	tttttttttt	tttttttttt	9120
catttagatc	atgttgcattt	tttttttttt	tttttttttt	tttttttttt	tttttttttt	9180
ccttctgtatc	tttttttttt	tttttttttt	tttttttttt	tttttttttt	tttttttttt	9240
gttagatattt	gttcaatgttgc	tttttttttt	tttttttttt	tttttttttt	tttttttttt	9300
atggggggat	tttttttttt	tttttttttt	tttttttttt	tttttttttt	tttttttttt	9360
atgttgcattt	aaatgttgc	tttttttttt	tttttttttt	tttttttttt	tttttttttt	9420
tagagtcttt	gaaatttcttca	tttttttttt	tttttttttt	tttttttttt	tttttttttt	9480
cataactgaca	caacctaaat	tttttttttt	tttttttttt	tttttttttt	tttttttttt	9540
gttaaaacatt	caaaggacacaa	tttttttttt	tttttttttt	tttttttttt	tttttttttt	9600
aagaccgtgc	atgttgcattt	tttttttttt	tttttttttt	tttttttttt	tttttttttt	9660
tagaatgtat	ttcagggttt	tttttttttt	tttttttttt	tttttttttt	tttttttttt	9720
tcaatctccc	accccatgtat	tttttttttt	tttttttttt	tttttttttt	tttttttttt	9780
aactctgtat	acacacttcc	tttttttttt	tttttttttt	tttttttttt	tttttttttt	9840
ctgttggca	tatctttttt	tttttttttt	tttttttttt	tttttttttt	tttttttttt	9900
ccccgcattacc	ctgttgcattt	tttttttttt	tttttttttt	tttttttttt	tttttttttt	9960
tggcaaccct	gaagataatc	tttttttttt	tttttttttt	tttttttttt	tttttttttt	10020

-continued

atgcgcccttc atatgtcatg	gtttcagat ccattccaac	ctgactgaat gttaacagac	10080
agaatttcttc acattaagga	actgtcttca tcatacataca	tgttagaaaag aatctgaaca	10140
ttaagtgcg aagtttctc	tagaaatata ttcaagat	gtttattcta ttattgtaaa	10200
ttcaaacaa taaaataata	agaatcc		10227
SEQ ID NO: 18	moltype = AA length = 2077		
FEATURE	Location/Qualifiers		
REGION	1..2077		
	note = human mAKAPbeta		
source	1..2077		
	mol_type = protein		
	organism = synthetic construct		
 SEQUENCE: 18			
MTSSQVKTGP FDSWSYSEME KEFPELIRSV GLLTVAADSI STNGSEAVTE EVSQVLSVD	60		
DKGGCEEDNA SAVEEQPGLT LGVSSSSGEA LTNAAQPSSE TVQQESSSS HHDAKNQQPV	120		
PCECNATPKRT IRDCFNYNED SPTQPTLKR GLFLKEETFK NDLKGNGKGR QMVDLKPEMS	180		
RSTPLVDP DRSKLCLVLQ SSYPNPSAA SQSYECLHKV GNGNLENTVK FHIKEISSL	240		
GRLNDCYKEK SRLKKPHKTS EEVPPCRTPK RGTGSGKQAK NTKSSAVPNG ELSYTSKAIE	300		
GPQTNASSTS SLEPCNQRWSW NAKLQLQSET SSSPAFTQSS ESSVGSDNIM SPVPLLSKHK	360		
SKKGQASSPS HVTRNGEVVE AWYGSDEYLA LPSPHLKQTEV LALKLENLTK LLPQPKPRGET	420		
IQNIDDWELS EMNSDSEIYP TYHVKKKHTR LGRVSPSSSS DIASSLGESI ESGPLSDILS	480		
DEESSMPLAG MKKYADEKSE RASSSEKNEHS HSATKSALIQ KLMQDIQHQD NYEAIWEKIE	540		
GFVNKLDEFI QWLNEADMEN ENWTPPKAEM DDLKLYLETH LSFKLNVDSH CALKEAVEEE	600		
GHLLELIAS HKAGLKDMLR MIASQWKELQ RQIKRQHSWI RLALDTIKAE ILATDVSED	660		
EEGTGSPKAE VQLCYLEAQQR DAVEQMSLKL YSEQYTSSSS RKEEFADMSK VHSGVSNGLL	720		
DFDSEYQELW DCLIDMESLV MDSHDLMMSE EQQQHLYKRY SVEMSIRHLK KTELLSKVEA	780		
LKKGGVLLPN DLLEKVDSN EKWELLGKTL GEKIQDTMAG HSGSSPRDLL SPESGSLVRQ	840		
LEVRIKELKG WLRDTELFIF NSCLRQEKEG TMNTEKQLQY FKSLCRIEKQ RRRGVASILR	900		
LCQHLLDDRE TCNLNADHQP MQLIIVNLER RWEAIVMQAV QWQTRLQKHM GKESETLNVI	960		
DPGLMLDNGM SEDALEWDEM DISNKLISLN EESNDLQEL QPVIPSLKLG ETSNEDPGYD	1020		
EEADNHGGSQ YASNITAPSS PHIYQVYSLH NVELYEDNHM PFLKNNPKV GMTQPNVLT	1080		
SLSKDSSFSS TKSPLDLLGG SNLVKPCACH GGDMSQNSGS ESGIVSEGDT ETTTNSEMCL	1140		
LNAVGDGSPSN LETEHLDPMQ GDADVNLKQK FTDEGESIKL PNSSQSSISP VGCVNGKVDG	1200		
LNSITKHTPD CLGEELQGKH DVFTFYDYSY LQGSKLKLPIM MKQSQSEKV HVEDPLLRF	1260		
YFDKKSCSKM HQTTELQPDV PPHERILASA SHEMDRISYK SGNIKETFTG MQNAKQLSLL	1320		
SHSSSIESLPS PGGDLFGLGI FKNGSDSLQR STSLESWLTS YKSNEDLFSC HSSGDISVSS	1380		
GSGVELSKRT LDLLNRLENI QSPSEQKIKR SVSDITLQSS SQKMSFTGQM SLIDIASSINE	1440		
DSAASLTERE SSDLSELCSE DIVLHKNKIP ESNASFRKRL TRSVADESVD NVSMIVNVSC	1500		
TSACTDDEDL TDLLSSSTLT LTEEELCIKD EDDDSSSIATD DEIYEDCTLM SGLDYIKNEL	1560		
QTWIRPKLSDL TRDKKRCNRVS DEMGKSKDIS SSEMTNPNSDT LNIETLLNGS VKRVSENNGN	1620		
GKNSSSHTHEL GTKRENKKTI FKVNKDPYVA DMENGNIEGI PERQKGKPNV TSKVSENGLS	1680		
HGKEISESEH CKCKALMDSL DDSNTAGKEF VSQDVRHLPK KCPNHHHFEN QSTASTPTEK	1740		
SFSELALTER FNNRQDSDAI KSSSDAPSMA GKSAGCCCLAL EQNGTENAS ISNISCCNCE	1800		
PDVFHQKDAE DCSVHNFKVE IIDMASTPEVNA APTSLQIKE KVLEHSHRPI	1860		
QLRKGDIFYSL LSLSSHSDSC GEVTNYIEEK SSTPLPLDT DSGLDDKEDI ECFFBACVEG	1920		
DSDGEEPFCFS SAPPNESAVP SEAAMPLQAT ACSSEFSDS LSADDADTV A LSSPSSQERA	1980		
EVGKEVNGLP QTSSGCAENL EFTPSKLSE KESSGKPGES GMPEEHNAAS AKSKVQDLSL	2040		
KANQPTDKAA LHPSPKTLTC EENLLNLHEK RHNMHR	2077		
SEQ ID NO: 19	moltype = DNA length = 10343		
FEATURE	Location/Qualifiers		
misc_feature	1..10343		
	note = human mAKAPalpha		
source	1..10343		
	mol_type = other DNA		
	organism = synthetic construct		
 SEQUENCE: 19			
catcatgcgg cagggtcaaac aaggcatctc ctgttattgc atcctacaga tggctgttaa	60		
acatcaaaa aagaacggtg gatcaggaga tgctgttttg gaaagaatgtt aggttttagac	120		
ttctccatgt taaccatgtt cgtgacactt cccccctgtt ggtcacagga cctggatccc	180		
atggctactgt atgcttcacc catggccatc aacatggacac ccactgttgg gcaagggttag	240		
ggagaagagg caatgaagga catggactctt gaccagcgtt atgaaaagcc accccccacta	300		
cacacagggg ctgactggaa gattgtccctc cacttacccg aaatttgagac ctggctccgg	360		
atgacccatcg agagggtcccg agacccataa tattcgttgc agcaggatcc ggacagcaag	420		
catgtggatg tacatctgtt tcaactaaag gatcttggt aagatatttc tgatctgtt	480		
gagcaaatcc atgccttccct tggaaacagag ttccctccaa agctgtgtc ttactctgtc	540		
aacgtgtatg tggacatcca cgcagttccg ctccctctggc accagttcg agtctgtt	600		
ctgggttgc gggagccat tctgtcaaggat ctgcaggacg ccaatggccaa ctacactagg	660		
cajacggaca ttctgtcaaggat ttctctgtt gggacaaaaag aggccggct tgattctca	720		
acagaatggt atgactcagg acaatataacc atcaaaatgtt ctcaaaaatcc ttgtctctg	780		
gattgtggca ttactgtcatt cgaactgttgc gactacgtc caagtggatgat ttgtgtca	840		
gggcttaggtt acatgacactc tagccaaatc aaaaccaaaac cttttgactc ttggagctac	900		
agtggatgtt aaaaggatgtt tcctgtatctt atccgttgc ttggtttact tacgttgc	960		
gtctgtatctt ttcttaccaa tggcagtgtt gcaatgttgc aggaggatctc tcaatgtatct	1020		
ctctcagtagt acgacaaagg tggatgttgc gaagacaaatc ttctgtcaatgttgc	1080		
ccaggcttaa cactgggggtt gtcatcatct tcaggagaag ctctgtcaaa tgctgtcaaa	1140		

-continued

ccctcctctg	agactgtgca	gcaagaatcc	agttcctcct	cccatcatga	tgcaaagaat	1200
cagcagcgtt	ttccttgtt	aaatgcaccc	ccccaaacgaa	ccatcagaga	ttgctttaat	1260
tataacgagg	actctcccac	cgagctaca	ttggccaaaa	gaggacttt	tcttaaagag	1320
gaaaactttt	agaatgatct	gaaaggcaat	ggtgaaaaa	ggcaaatgg	tgatctaag	1380
cctgagatga	gcagaagcac	cccttcgtct	gtagatcctc	ctgacagatc	caaactttgc	1440
ctggtatattgc	agtcttctta	ccccaaacago	ccttctgtct	ccagccagtc	ttatgagggt	1500
ttacacaagg	tgggaaatgg	gaaccttga	aacacagtca	aatttcacat	taaagaatt	1560
tcttccagcc	ttggaaaggct	taacgactgc	tataaagaga	aatctcgact	taaaaaggca	1620
cacaagacct	cagaagaggt	gcctccatgc	cgaacaccta	aacggggac	tggttcaggc	1680
aaacaagact	aaaatacaaa	gagctcagca	gtgccaaatgg	gagactttc	ttatacttcc	1740
aaggccatag	agggggccaca	aacaatctt	gcttccatctt	gccttgcata	gccttgcata	1800
caaaaaagg	ggaatgcac	atttgcattt	caagtgcata	catccagttc	accagcttt	1860
actcagagca	gtgaatcc	tggttgcata	gacaacatca	tgtctccgtt	gccacttctt	1920
taaaaaacaca	aaagcaaaaa	aggtaacagg	tcctctccaa	gtcacgtcac	taggaatgg	1980
gagggttgtt	aggcctggta	tggctctgt	gaataccttag	cactgccttc	tcaccttaag	2040
caaaaaagg	tattggctt	qaatgtgaa	aaccttacaa	agcttgcgc	tcagaaaccc	2100
agaggagaaa	ccatccagaa	tattgtatc	caagtgcata	tgaaaatgg	ttcagattct	2160
gaaaatctat	caacccatca	tgtccaaaaa	aagcatacaca	ggctaggccag	gggtctccca	2220
agtcatctat	gtgacatagc	ctcttcacta	ggggagagac	ttgaatctgg	gcccctgag	2280
gacattctt	ctgtatggaa	gtcctatgt	gtatgcata	gtatgcata	gtatgcata	2340
gagaagtca	aaagagctt	atccctctg	aaaatgaga	gocattctgc	cactaaatca	2400
gtcttaattc	agaaactgt	gcaagatatt	cagcaccaag	acaactatga	agccatatgg	2460
gaaaaaaat	agggggttgg	aaacaaactt	gatgaattca	ttcaatgg	aaatgaagcc	2520
atggaaacta	cagaatatt	gactccccc	aaagcagaga	tggatgactt	taaactgtat	2580
ctggagacac	acttgcattt	taatgttata	gtagacatgc	atttgcctt	caaggaagct	2640
gtggaggagg	aggcacacca	acttttgc	tttatttgc	ctcacaagg	aggactgaag	2700
gacatgtgc	ggatgttgc	aagtgcattt	aaaggcataat	caaaaggccag	2760	
cacagcttgc	ttctcaggcc	tctgtatacc	atcaaaaggcc	agatacttgc	tactgtatgt	2820
tctgtggagg	atgaggaaagg	gacttgcata	ccccaaaggct	aggttcaact	atgttactct	2880
gaagcaca	gagatgtctt	tgagcata	tccctcaacg	tgtatgcga	gcagttatacc	2940
agcagcagca	agcgaaggaa	agatgttgc	gatatgtca	aagtgcattt	agttggaaagc	3000
aatgggttctt	ttgacttgc	ttcagaaat	caggagtc	tttatttgc	tttatttgc	3060
gagtccctt	tgatggacag	ccacgaccc	atgtgtca	aggagcagca	gcagcatctt	3120
tacaagcgat	acagtgttgc	aatgtccatc	agacaccat	aaaagacgg	gctgtttagt	3180
aagggttgc	cttgcata	aaatggatcc	atgatcttct	ttaaaaaaat	ttaaaaaaat	3240
gattcaatta	ataaaaat	ggaactgtt	ggggaaaccc	taggagagaa	gatccaggac	3300
acaatggco	ggcagatgtt	gtcgatcc	cgtgaccc	tctctccgt	aagtgcata	3360
ctggtaaggc	actgtggagg	caggatca	gaacttgc	gatgttgc	gatgttgc	3420
cttttcattt	tcatttc	tctgtatcc	tttatttgc	tttatttgc	tttatttgc	3480
caactgcata	acttgcata	ctcttcgt	gataatcc	tttatttgc	tttatttgc	3540
tccatttc	gactatgc	gtcatttttgc	tttatttgc	tttatttgc	tttatttgc	3600
gaccaccagg	ccatgcagct	gatattgtt	aattttgc	tttatttgc	tttatttgc	3660
atgcacgc	ccactgttgc	aaacatgtt	tttatttgc	tttatttgc	tttatttgc	3720
ttgaatgttgc	tttatttgc	tttatttgc	tttatttgc	tttatttgc	tttatttgc	3780
tggatgat	tttatttgc	tttatttgc	tttatttgc	tttatttgc	tttatttgc	3840
gatcaagaa	tccaaatctt	tatccctt	tttatttgc	tttatttgc	tttatttgc	3900
cctggat	tttatttgc	tttatttgc	tttatttgc	tttatttgc	tttatttgc	3960
gcccccttca	tttatttgc	tttatttgc	tttatttgc	tttatttgc	tttatttgc	4020
gacaaccaca	tttatttgc	tttatttgc	tttatttgc	tttatttgc	tttatttgc	4080
gttttaacta	tttatttgc	tttatttgc	tttatttgc	tttatttgc	tttatttgc	4140
tttatttgc	tttatttgc	tttatttgc	tttatttgc	tttatttgc	tttatttgc	4200
aatttcaggca	tttatttgc	tttatttgc	tttatttgc	tttatttgc	tttatttgc	4260
gaaaatgtct	tttatttgc	tttatttgc	tttatttgc	tttatttgc	tttatttgc	4320
gaccaccacaa	tttatttgc	tttatttgc	tttatttgc	tttatttgc	tttatttgc	4380
agcattaaatgc	tttatttgc	tttatttgc	tttatttgc	tttatttgc	tttatttgc	4440
aaagtttgc	tttatttgc	tttatttgc	tttatttgc	tttatttgc	tttatttgc	4500
caaggaaat	tttatttgc	tttatttgc	tttatttgc	tttatttgc	tttatttgc	4560
aaatttacaa	tttatttgc	tttatttgc	tttatttgc	tttatttgc	tttatttgc	4620
cttcgttgc	tttatttgc	tttatttgc	tttatttgc	tttatttgc	tttatttgc	4680
caaccatgtt	tttatttgc	tttatttgc	tttatttgc	tttatttgc	tttatttgc	4740
atttcataata	aaatggccaa	tttatttgc	tttatttgc	tttatttgc	tttatttgc	4800
cttccttctt	tatctatag	tttatttgc	tttatttgc	tttatttgc	tttatttgc	4860
ggatgtggca	tttttttttt	tttatttgc	tttatttgc	tttatttgc	tttatttgc	4920
tggttgactt	tttatttgc	tttatttgc	tttatttgc	tttatttgc	tttatttgc	4980
agcgttgat	tttatttgc	tttatttgc	tttatttgc	tttatttgc	tttatttgc	5040
tttatttgc	tttatttgc	tttatttgc	tttatttgc	tttatttgc	tttatttgc	5100
cttcaaaatgc	tttatttgc	tttatttgc	tttatttgc	tttatttgc	tttatttgc	5160
tcttatcaatg	tttatttgc	tttatttgc	tttatttgc	tttatttgc	tttatttgc	5220
cttcgttgc	tttatttgc	tttatttgc	tttatttgc	tttatttgc	tttatttgc	5280
aggaagcg	tttatttgc	tttatttgc	tttatttgc	tttatttgc	tttatttgc	5340
aatgtcttgc	tttatttgc	tttatttgc	tttatttgc	tttatttgc	tttatttgc	5400
tctaccctt	tttatttgc	tttatttgc	tttatttgc	tttatttgc	tttatttgc	5460
attgcaacag	tttatttgc	tttatttgc	tttatttgc	tttatttgc	tttatttgc	5520
aagaatgtat	tttatttgc	tttatttgc	tttatttgc	tttatttgc	tttatttgc	5580
tgcaatgtca	tttatttgc	tttatttgc	tttatttgc	tttatttgc	tttatttgc	5640
ccctctgtata	tttatttgc	tttatttgc	tttatttgc	tttatttgc	tttatttgc	5700

-continued

aataatggaa atggtaagaa ttcatctcat acccatgagt tagggacaaa gcgtgaaat 5760
aagaaaaacta ttttcaaagt taataaagat ccatatgtgg ctgacatgga aaatggcaat 5820
atggaggta ttccagaaag gcaaaaggc aaaccgaatg tgacttcaa ggttacgaaa 5880
aatcttgggtt cacatggaa agaggatca gagatgtgc attgttaatg ttaaagcaact 5940
atggatagtt tagatgatc aataactgtg gcaaggaaat ttgtttcca agatgttaga 6000
catcttccaa agaaatgtcc aaatcaccc cattttgaaa atcaaagcac tgcctctact 6060
cccactgta agtctttctc agaactgtg tttagaaacca gggtttaacca cagacaagac 6120
tctgtatgcac tgaaatcatc tgatgtgc ccgagatgtg ctggaaatcc tgcgttgtt 6180
tgctcgtacac ttgaacaaa cggaaacagaa gaaatgtt ctatcagcaa catttcgtt 6240
tgcaactgtg agccagatgt ttccatcatc aaagatgtcc aagattgttc agtacacaac 6300
tttggtaagg aaatcattgt catggcttgc acaggcttaa aaagaataatc tcaacctgaa 6360
aacagggtgg ctgccttact ttccatctaact caaatcaagg agaaatgtt ggagacattt 6420
caccggccca tccagctgaa aaaaggggcattttttactt ctatccatc tccatctcat 6480
gacagtgtt gttggggaggt caccattac atagaagaga aaagcagcac tccattgcca 6540
ctagacacca ctgactcggtt cttagatgc aaggaaatg ttgaatgtt ttttgaggcc 6600
tgggtggagg gtgactctgtt tgagaggagg ctgtttttctt ctatgttcc tccaatgaa 6660
tctgcgttcc ccaggcgaagc tgcaatggca ctcaagaa caccatgttcc tctgtgttc 6720
agtgtatgtt ctctttcagc tgatgtgc gatacagtttgc ctctttcaag tccttctct 6780
caggaaagag ctggagggtt aaaggaaatgtt aatggtttgc cccaaatctc cagtggtctgt 6840
gcaaaaaact tagattttac ttccatcaag ctggacatgtg aaaagaaag ttccggaaaaa 6900
ccagggtgtat ctggaaatggc agaaacatc aatgtctgtt cagccaaatc taaatgtcaa 6960
gaccttccct tgaaggcaaa tcagccaaaca gacaaggccc cattgcatcc cagccccaaa 7020
actttaacct gtgaagaaaaa ttcttctaaac ctccatgaaa aacgcacatag aaatatgcat 7080
aggtagaaatg taccctccccc ccaacgtatc aataatctt actgaagatg acgccttgcgt 7140
gcaactcagg ggtggctca tcccccggc ctgggctggc ctctggttcc atcacgttgc 7200
tcaactgcgtt tatttacatt gacttcccttccaa aatgtatc ttcccttccaa atgttcc 7260
tccacacaaag ctttggatc tgaatgtgtg cgctggtttctttaggtga tcgttcttga 7320
agtgtcaggaa agtctgttgc ttcccatgg atccctgtcc caagtcacctt ctccaaaccc 7380
tctctctcca gctagactt ttctttgc tccccccttcc cttccactc tttaaatgtt 7440
tgcgttccac caactgttagt tccatataat tccctgttcc aatgtatggcc ccccaacccgt 7500
acttgaccaaa ttcatgttat caatctggat ttttttttca aacgttataat gactgtgtt 7560
attgaaaaagat ttttaccaa aaagccaaaca ttgttggatgg ttgcagcata gagaagaaac 7620
actgttgcctt ctccaaatataat taagcaacta taaaagcgc catttttttattt atttcattha 7680
aaaataatc tatgcacatc ttcaaaagaa aaccatattgg tttgttatataataaaacttgc 7740
tgacattcttca ttattgttact atgtacacaa ttccatgttcc tttatgttct tgagggtgtt 7800
atgagaaaaaa agttttttaa aaaatgttgc ctggctgtat ttcttataacc atttattttaa 7860
aaggtgttccatc caccgttataat ttttggatgg ttggaaaggg gaaatgtacaa ggtaatgt 7920
tgtgaataat ttctgtatataatgttataa aatgtacaaac aatgtatgtt aatgtacatc 7980
taaaatgtctt tcatgtttgtt gatgtctgtt gatgtggaaa atataatgtt tttatgttcc 8040
agattgtatc gtaattaaaaa ttggcataat aaacacatg tattggggaa aaagaaaaat 8100
tagtgcatttcc ttcttactat ttccatccaa aatgttgc tctgggttcc aaaatgttgc 8160
gtatgtacca gcttccaaatc atattccat ttttggatgg gtttacatc gtttacatc accttac 8220
gagactagac gtaaaaggcctt cagtttccaa aatcttttgc gtcactataa agatgttgc 8280
acagcaatg atttaatgtc agtccccctt aaccaataaa catttataact agatttttta 8340
tttccactta tcattatgtt tttatgttgc gatttcagggtt accttgcgtat tccatattaa 8400
ttttaatataat ttatgttgc tttatgttgc gtttacatc accttgcgtat tccatattaa 8460
gacttatttataatgttactt atgttgc tttatgttgc gtttacatc accttgcgtat tccatattaa 8520
tttccatctgg ctttactgtt cttctgttta atgcgtttt aatattatataatgttgc 8580
actttaatgtc ctgcacatgtt aggttccatc taaaatgttgc ctgttccatc aatgttgc 8640
agggtacatgtt gtgcacatc tcccttttgc ttttgcactaa aacatgttgc caacttagaaa 8700
gaggatcatat gcaatataatgttgc ttttgcactaa aatgttgc ttttgcactaa aatgttgc 8760
gaatgttgc gtttacatc ttttgcactaa aatgttgc ttttgcactaa aatgttgc 8820
actttttatc tgcaaggcac ttttgcactaa aatgttgc ttttgcactaa aatgttgc 8880
aaggcttgcctt ctcagaaggc ttttgcactaa aatgttgc ttttgcactaa aatgttgc 8940
tttccatctgg ttttgcactaa aatgttgc ttttgcactaa aatgttgc ttttgcactaa aatgttgc 9000
tttccatctgg ttttgcactaa aatgttgc ttttgcactaa aatgttgc ttttgcactaa aatgttgc 9060
tttccatctgg ttttgcactaa aatgttgc ttttgcactaa aatgttgc ttttgcactaa aatgttgc 9120
attcatgttactt aatgttgc ttttgcactaa aatgttgc ttttgcactaa aatgttgc 9180
aaggcttgcctt ctcagaaggc ttttgcactaa aatgttgc ttttgcactaa aatgttgc 9240
tttccatctgg ttttgcactaa aatgttgc ttttgcactaa aatgttgc ttttgcactaa aatgttgc 9300
tttccatctgg ttttgcactaa aatgttgc ttttgcactaa aatgttgc ttttgcactaa aatgttgc 9360
tttccatctgg ttttgcactaa aatgttgc ttttgcactaa aatgttgc ttttgcactaa aatgttgc 9420
tttccatctgg ttttgcactaa aatgttgc ttttgcactaa aatgttgc ttttgcactaa aatgttgc 9480
tttccatctgg ttttgcactaa aatgttgc ttttgcactaa aatgttgc ttttgcactaa aatgttgc 9540
tttccatctgg ttttgcactaa aatgttgc ttttgcactaa aatgttgc ttttgcactaa aatgttgc 9600
tttccatctgg ttttgcactaa aatgttgc ttttgcactaa aatgttgc ttttgcactaa aatgttgc 9660
tttccatctgg ttttgcactaa aatgttgc ttttgcactaa aatgttgc ttttgcactaa aatgttgc 9720
tttccatctgg ttttgcactaa aatgttgc ttttgcactaa aatgttgc ttttgcactaa aatgttgc 9780
tttccatctgg ttttgcactaa aatgttgc ttttgcactaa aatgttgc ttttgcactaa aatgttgc 9840
tttccatctgg ttttgcactaa aatgttgc ttttgcactaa aatgttgc ttttgcactaa aatgttgc 9900
tttccatctgg ttttgcactaa aatgttgc ttttgcactaa aatgttgc ttttgcactaa aatgttgc 9960
tttccatctgg ttttgcactaa aatgttgc ttttgcactaa aatgttgc ttttgcactaa aatgttgc 10020
tttccatctgg ttttgcactaa aatgttgc ttttgcactaa aatgttgc ttttgcactaa aatgttgc 10080
tttccatctgg ttttgcactaa aatgttgc ttttgcactaa aatgttgc ttttgcactaa aatgttgc 10140
tttccatctgg ttttgcactaa aatgttgc ttttgcactaa aatgttgc ttttgcactaa aatgttgc 10200
tttccatctgg ttttgcactaa aatgttgc ttttgcactaa aatgttgc ttttgcactaa aatgttgc 10260

-continued

```
agtgcgaagt tttctctaga aatatattca agatatgttt attctattat tgtaaatcc 10320
aaacaataaa taaataagaa tcc 10343
```

```
SEQ ID NO: 20 moltype = AA length = 2319
FEATURE Location/Qualifiers
REGION 1..2319
note = human mAKAPalpha
source 1..2319
mol_type = protein
organism = synthetic construct
SEQUENCE: 20
MLTMSVTLSP LRSQDLPMA TDASPMAINM TPTVEQGE EAMKDMDSQ QYEKPPPLHT 60
GADWKIVLHL PEIETWLRMT SERVRDLTYS VQQDSDSKHV DVHVLVQLKDI CEDISDHVEQ 120
IHALLETEFS LKLLSYSVNV IVDIHAVQLL WHQLRVSVLV LRERILQGLQ DANGNYTRQT 180
DILQAFSEET KEGRLDSLTE VDDSGQLTCSQNYLSLC GITAFELSDY SPSEDLLSGL 240
GDMTSSQVKT KPFDSWSYSE MEKEFPELIR SISTGLTVAAD SISTNGSEAV TEEVSQVSL 300
VDDKGGEED NASAVEEQPG LTLGVSSSSG EALTNAQPS SETVQQESSS SSHHDAKNQQ 360
PVPCEPATPK RTIRDCFNYM EDSPTQPTLP KRGLFLKBEET FKNDLKGNGG KRQMVDLKPE 420
MSRSTPSLCLV LQSSYPNSPVAASQSYECLVK VKGNGNLENT VKFHIKEISS 480
SLGRLNDCYK EKSRLKKPHI TSEEVPPCRT PKRGTGSGKQ AKNTKSSAVP NGELSYTSKA 540
IEGPQTNSAS TSSLEPCNQR SWNAKLQLQS ETSSSPAFTQ SSESSVGSDN IMSPVPLLSK 600
HISKKKGQASS PSHVTRNGEV VEAWYGSDEY LalPSHLKQET EVLALKLENL TKLLPQKPRG 660
ETIQNIDDWE LSEMNSDTE YPTYHVKKKH TRLGRVSPVSSS SSISSLGE SIESGPLSDI 720
LSDDESSMPL AGMKKYADEK SERASSEKRN ESHSATKSAL IQKLMQDIQH QDNYEAIWEK 780
IEGFVNKLDE FIQWLNEAME TTENWTPPKA EMDDLKLYLE THLSFKLNVD SHCALKEAVE 840
EEGHQLELEI ASHKAGLKDM LRMITASQWKE LQROIKRHOES WILRALDTIK AEILATDVSV 900
EDEEGTGSPK AEVQLCYLEA QRDAVEQMSL KLYSEQYTSS SKRKEEFADM SKVHSVGSSN 960
LILDFTSEYQE LWDCCLIDMES LMVDSHDLMM SEEQQQHLYK RYSVEMSIH LKKTLLSKV 1020
EALKGGVVLL PNDLLEKVDS INEKWELLGK TLGEKIQDMT AGHSGSSPRD LLSPESGLSV 1080
RQLEVRKIEL KGWLDRTELF IFNSCLRQEK EGTMTNEKQL QYFKSLCREEI KQRRRGVASI 1140
LRLCNAVRIKEL RETCNLNADH QPMQLIIVPN ERRWEAIVM AVQWQTRLQK KMGESETLN 1200
VIDPGLMDLN GMSEDALEWD EMDISNLKLS LNEESNDLQ ELQPVIPSLK LGETSNEDPG 1260
YDEEADNHGG SQYASNITAP SSPHYIQVYS LHNVELYEDN HMPFLKNNPV VTGMTQPNVL 1320
TKSLSKDSSF SSTKSLPDLL GGSNLVVKPCA CHGGDMSQNS GSESGIVSEG DTETTTNSEM 1380
CLLNAVGDGSP SNLETEHLDP QMGDAVNVLK QKFTDEGESI KLPNSSQSSI SPVGCVNGKV 1440
GDLNSITKHT PDCLGEELQG KHDVFTFYD SYLGQGSKLKL PMIMKQSQSE KVHVEDPLL 1500
GFYFDKKSCSK SKHQTTELQP DVPPERILA SASHEMDRIS YKSGNIEKTF TGMQNAKQLS 1560
LLSHSSSIES LSPGGDLFGL GIFKNGSDSL QRSTSLESWL TSYKSNEDLF SCHSSGDISV 1620
SSGSVGELSK RTLDLNLNRLE NIQSPSEQNI KRSVSDITLQ SSSQKMSFTG QMSLDIASSI 1680
NEDASAASLTE LSSDSELSC SEDIVLHKNK IPESNASFRK RLTRSAVDES DVNVSMIVNV 1740
SCTSACTDDE DDSDLLSSST LTLTEEECLCI KDEDDEDSIA TDDEIYEDCT LMSGLDYIKN 1800
ELQTWIRPKL SLTRDKKRCN VSDEMKGSKD ISSSEMTNPS DTLNIEFTLLN GSVKRVSENN 1860
GNKGKNSSHTH ELGTRKENK TIFVKVNKDPY VADMENGNI GIPERQKGKP NVTSKVSEN 1920
GSHGKEISES EHCKCKLSDM SLDDNSTAGK EFVSDQVRHL PKKCPNHHHF ENQSTASTPT 1980
EKSFSLEALE TRFNNRQDSD ALKNSDDAPS MAGKSAGCCL ALEQNQTEEN ASISNISCCN 2040
CEPDVFHOKD AEDCSVHNFV KEIIDMASTA LKSKSQPENE VAAPTSLTQI KEKVLEHSHR 2100
P1QLRKDFY SYLSSLSSHDS DCGEVTNYIE EKSTPLPL TTDSGLDDKE DIECFFEACV 2160
EGDSDGEEPK FSSAPPNEA VPSEAMPLQ ATACSSSEFD SSLSADDADT VALSSPSSQE 2220
RAEVGKEVNG LPQTSSGCAE NLETPSKLD SEKESSGKPG ESGMPEEHNA ASAASKVQDL 2280
SLKANQPTDK AALHPSPKTL TCEENLLNLH EKRHRNMHR 2319
```

```
SEQ ID NO: 21 moltype = AA length = 10
FEATURE Location/Qualifiers
REGION 1..10
note = Myc-tag
source 1..10
mol_type = protein
organism = synthetic construct
SEQUENCE: 21
EQKLISEEDL 10
SEQ ID NO: 22 moltype = AA length = 189
FEATURE Location/Qualifiers
REGION 1..189
note = mAKAP PBD
VARIANT 1
note = MISC_FEATURE - Xaa is Lys or Arg
VARIANT 3
note = MISC_FEATURE - Xaa can be any naturally occurring
amino acid
VARIANT 8
note = MISC_FEATURE - Xaa can be any naturally occurring
amino acid
VARIANT 10..11
note = MISC_FEATURE - Xaa can be any naturally occurring
amino acid
```

-continued

VARIANT 21
note = MISC_FEATURE - Xaa is Ile or Met
VARIANT 22
note = MISC_FEATURE - Xaa is Glu or Asp
VARIANT 31
note = MISC_FEATURE - Xaa can be any naturally occurring
amino acid
VARIANT 32
note = MISC_FEATURE - Xaa is Asp or Glu
VARIANT 33..35
note = MISC_FEATURE - Xaa can be any naturally occurring
amino acid
VARIANT 38..42
note = MISC_FEATURE - Xaa can be any naturally occurring
amino acid
VARIANT 44
note = MISC_FEATURE - Xaa can be any naturally occurring
amino acid
VARIANT 50
note = MISC_FEATURE - Xaa is Val or Ile
VARIANT 51..53
note = MISC_FEATURE - Xaa can be any naturally occurring
amino acid
VARIANT 55..56
note = MISC_FEATURE - Xaa can be any naturally occurring
amino acid
VARIANT 57..58
note = MISC_FEATURE - Xaa can be any naturally occurring
amino acid or absent
VARIANT 59
note = MISC_FEATURE - Xaa can be any naturally occurring
amino acid
VARIANT 60
note = MISC_FEATURE - Xaa is Ala or Ser
VARIANT 61..63
note = MISC_FEATURE - Xaa can be any naturally occurring
amino acid or absent
VARIANT 64..67
note = MISC_FEATURE - Xaa can be any naturally occurring
amino acid
VARIANT 68
note = MISC_FEATURE - Xaa is Ser or Ala
VARIANT 69
note = MISC_FEATURE - Xaa can be any naturally occurring
amino acid
VARIANT 71
note = MISC_FEATURE - Xaa can be any naturally occurring
amino acid
VARIANT 72
note = MISC_FEATURE - Xaa is Leu or Val
VARIANT 73
note = MISC_FEATURE - Xaa can be any naturally occurring
amino acid
VARIANT 74
note = MISC_FEATURE - Xaa is Ala or Ser
VARIANT 76..77
note = MISC_FEATURE - Xaa can be any naturally occurring
amino acid
VARIANT 79
note = MISC_FEATURE - Xaa can be any naturally occurring
amino acid
VARIANT 81
note = MISC_FEATURE - Xaa can be any naturally occurring
amino acid
VARIANT 84..85
note = MISC_FEATURE - Xaa can be any naturally occurring
amino acid
VARIANT 87
note = MISC_FEATURE - Xaa can be any naturally occurring
amino acid
VARIANT 89..90
note = MISC_FEATURE - Xaa can be any naturally occurring
amino acid
VARIANT 92
note = MISC_FEATURE - Xaa is Glu or Asp
VARIANT 93

-continued

```
note = MISC_FEATURE - Xaa can be any naturally occurring
amino acid
VARIANT 94 note = MISC_FEATURE - Xaa is Gly or Ala
VARIANT 97..99 note = MISC_FEATURE - Xaa can be any naturally occurring
amino acid
VARIANT 100 note = MISC_FEATURE - Xaa is Leu or Val
VARIANT 101..102 note = MISC_FEATURE - Xaa can be any naturally occurring
amino acid
VARIANT 105 note = MISC_FEATURE - Xaa can be any naturally occurring
amino acid
VARIANT 110..111 note = MISC_FEATURE - Xaa can be any naturally occurring
amino acid
VARIANT 113 note = MISC_FEATURE - Xaa can be any naturally occurring
amino acid
VARIANT 115..119 note = MISC_FEATURE - Xaa can be any naturally occurring
amino acid
VARIANT 120 note = MISC_FEATURE - Xaa is Ser or Ala
VARIANT 121..122 note = MISC_FEATURE - Xaa can be any naturally occurring
amino acid
VARIANT 124..126 note = MISC_FEATURE - Xaa can be any naturally occurring
amino acid
VARIANT 127 note = MISC_FEATURE - Xaa is Lys or Arg
VARIANT 128..135 note = MISC_FEATURE - Xaa can be any naturally occurring
amino acid
VARIANT 137 note = MISC_FEATURE - Xaa can be any naturally occurring
amino acid
VARIANT 144 note = MISC_FEATURE - Xaa can be any naturally occurring
amino acid
VARIANT 146 note = MISC_FEATURE - Xaa is Val or Ile
VARIANT 148..149 note = MISC_FEATURE - Xaa can be any naturally occurring
amino acid
VARIANT 152 note = MISC_FEATURE - Xaa can be any naturally occurring
amino acid
VARIANT 154 note = MISC_FEATURE - Xaa can be any naturally occurring
amino acid
VARIANT 157..158 note = MISC_FEATURE - Xaa can be any naturally occurring
amino acid
VARIANT 160 note = MISC_FEATURE - Xaa can be any naturally occurring
amino acid
VARIANT 162 note = MISC_FEATURE - Xaa is Leu or Met
VARIANT 163..164 note = MISC_FEATURE - Xaa can be any naturally occurring
amino acid
VARIANT 165 note = MISC_FEATURE - Xaa can be any naturally occurring
amino acid absent
VARIANT 168 note = MISC_FEATURE - Xaa can be any naturally occurring
amino acid
VARIANT 173 note = MISC_FEATURE - Xaa can be any naturally occurring
amino acid
VARIANT 175 note = MISC_FEATURE - Xaa can be any naturally occurring
```

-continued

```

VARIANT          amino acid
177
note = MISC_FEATURE - Xaa is Leu or Val
VARIANT          179
note = MISC_FEATURE - Xaa can be any naturally occurring
amino acid
VARIANT          182
note = MISC_FEATURE - Xaa can be any naturally occurring
amino acid
VARIANT          185
note = MISC_FEATURE - Xaa can be any naturally occurring
amino acid
source           1..189
mol_type = protein
organism = synthetic construct
SEQUENCE: 22
XSXTPLPXDX KDSGLDDKED XXCFEACVE XXXXXEEXXX XXAXPNESAX XXXAXXXXXX 60
XXXXXXXXXXS XXXDXXDXV XLSXXSQXX AXXXKEXXXX XXTSXGCAEX XEXTXXXXXX 120
XXEXXXXXXX XXXXXEXNAA SAKXXQXXS LXAXQPXXXX AXXXSPXTL TCXEXLXNXH 180
EXRHXNMHR                                              189

SEQ ID NO: 23      moltype = AA length = 188
FEATURE          Location/Qualifiers
REGION           1..188
note = Protein
VARIANT          2
note = misc_feature - Xaa can be any naturally occurring
amino acid
VARIANT          7
note = misc_feature - Xaa can be any naturally occurring
amino acid
VARIANT          9..10
note = misc_feature - Xaa can be any naturally occurring
amino acid
VARIANT          20..21
note = misc_feature - Xaa can be any naturally occurring
amino acid
VARIANT          30..34
note = misc_feature - Xaa can be any naturally occurring
amino acid
VARIANT          37..41
note = misc_feature - Xaa can be any naturally occurring
amino acid
VARIANT          43
note = misc_feature - Xaa can be any naturally occurring
amino acid
VARIANT          49..52
note = misc_feature - Xaa can be any naturally occurring
amino acid
VARIANT          54..68
note = misc_feature - Xaa can be any naturally occurring
amino acid
VARIANT          70..73
note = misc_feature - Xaa can be any naturally occurring
amino acid
VARIANT          75..76
note = misc_feature - Xaa can be any naturally occurring
amino acid
VARIANT          78
note = misc_feature - Xaa can be any naturally occurring
amino acid
VARIANT          80
note = misc_feature - Xaa can be any naturally occurring
amino acid
VARIANT          83..84
note = misc_feature - Xaa can be any naturally occurring
amino acid
VARIANT          86
note = misc_feature - Xaa can be any naturally occurring
amino acid
VARIANT          88..89
note = misc_feature - Xaa can be any naturally occurring
amino acid
VARIANT          91..93
note = misc_feature - Xaa can be any naturally occurring
amino acid

```


1. A method of treating or preventing heart failure with reduced ejection fraction, comprising administering to cardiac cells of a patient a composition that maintains a level of phosphorylation on serum response factor (SRF).
2. The method of claim 1, wherein SRF is phosphorylated on Ser103.
3. The method of claim 1, wherein dephosphorylation activity of protein (serine-threonine)phosphatase 2A (PP2A) is inhibited.
4. The method of claim 3, wherein anchoring of PP2A to muscle A-kinase anchoring protein (mAKAP β) is inhibited.
5. The method of claim 4, wherein the composition comprises a fragment of mAKAP β .
6. The method of claim 5, wherein the composition comprises an amino acid sequence having at least 90% sequence identity to a fragment of mAKAP.
7. The method of claim 5, wherein the composition comprises a fragment of amino acids 2083-2314 of mAKAP.
8. The method of claim 5, wherein the composition comprises amino acids 2132-2319 of mAKAP.
9. The method of claim 4, wherein the composition comprises a fragment of PP2A.
10. The method of claim 4, wherein said composition comprises a vector that encodes a fragment of PP2A.
11. The method of claim 4, wherein said composition comprises a vector that encodes an amino acid sequence having at least 90% sequence identity to a fragment of mAKAP.
12. The method of claim 4, wherein said composition inhibits the expression of PP2A B56 δ (PPP2R5D).
13. The method of claim 10, wherein the vector encodes a fragment of amino acids 2132-2319 of mAKAP.
14. The method of claim 10, wherein the vector encodes amino acids 2132-2319 of mAKAP.
15. The method of claim 10, wherein the vector is adeno-associated virus (AAV).
16. A composition comprising a vector that encodes a molecule that inhibits the anchoring of PP2A to mAKAP β and maintains a level of phosphorylation on serum response factor (SRF).
17. The composition of claim 16, wherein the molecule comprises a fragment of mAKAP.
18. The composition of claim 16, wherein the molecule has at least 90% sequence identity to a fragment of mAKAP.
19. The composition of claim 17, wherein the molecule is a fragment of amino acids 2132-2319 of mAKAP.
20. The composition of claim 17, wherein the molecule is amino acids 2132-2319 of mAKAP.
21. (canceled)
22. (canceled)
23. (canceled)
24. (canceled)
25. (canceled)
26. (canceled)
27. (canceled)
28. (canceled)

* * * * *