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(54) RECOMBINANT IMMUNE CELLS,
METHODS OF MAKING, AND METHODS OF
USE

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(57)

ABSTRACT

A recombinant immune cell expresses a heterologous IgG Fc receptor. In some embodiments, the heterologous IgG Fc receptor can be a chimeric IgG Fc receptor. Generally, the chimeric IgG Fc receptor includes an extracellular domain, a transmembrane domain, and an intracellular domain. The extracellular domain generally includes a sufficient portion of CD64 to bind to an IgG Fc region. The intracellular domain of the chimeric IgG Fc receptor includes a sufficient portion of an Fc receptor allowing immunoreceptor tyrosine-based activation motif (ITAM) to initiate cell signaling when an IgG Fc region binds to the extracellular domain.

Specification includes a Sequence Listing.

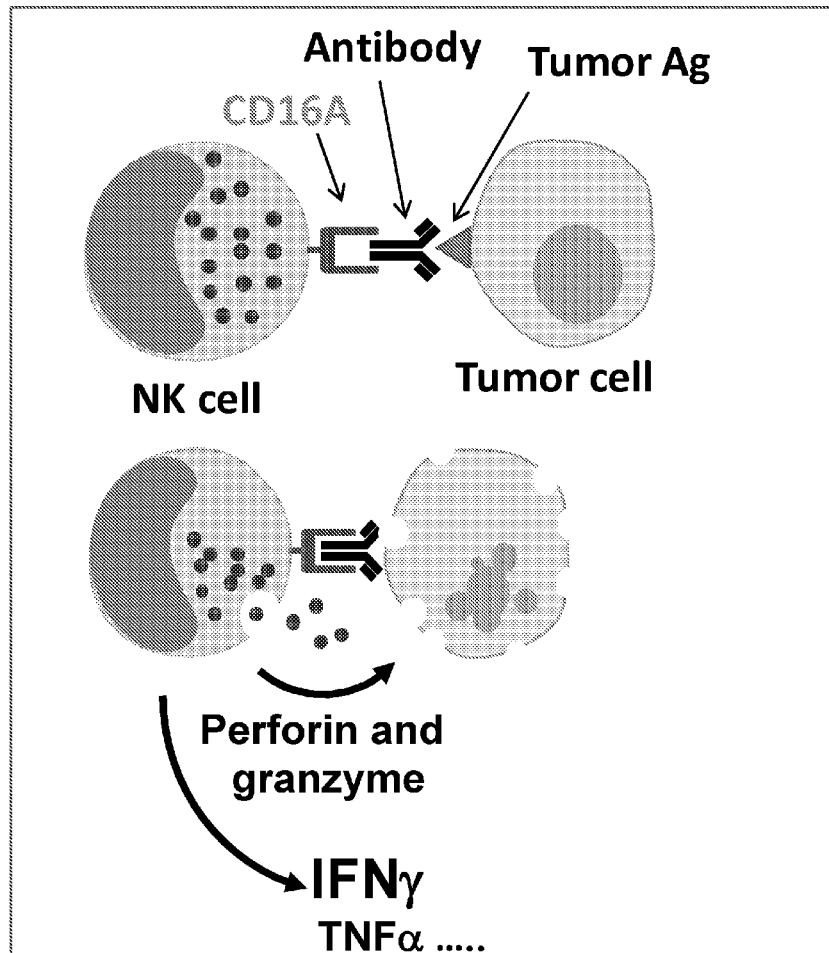


FIG. 1

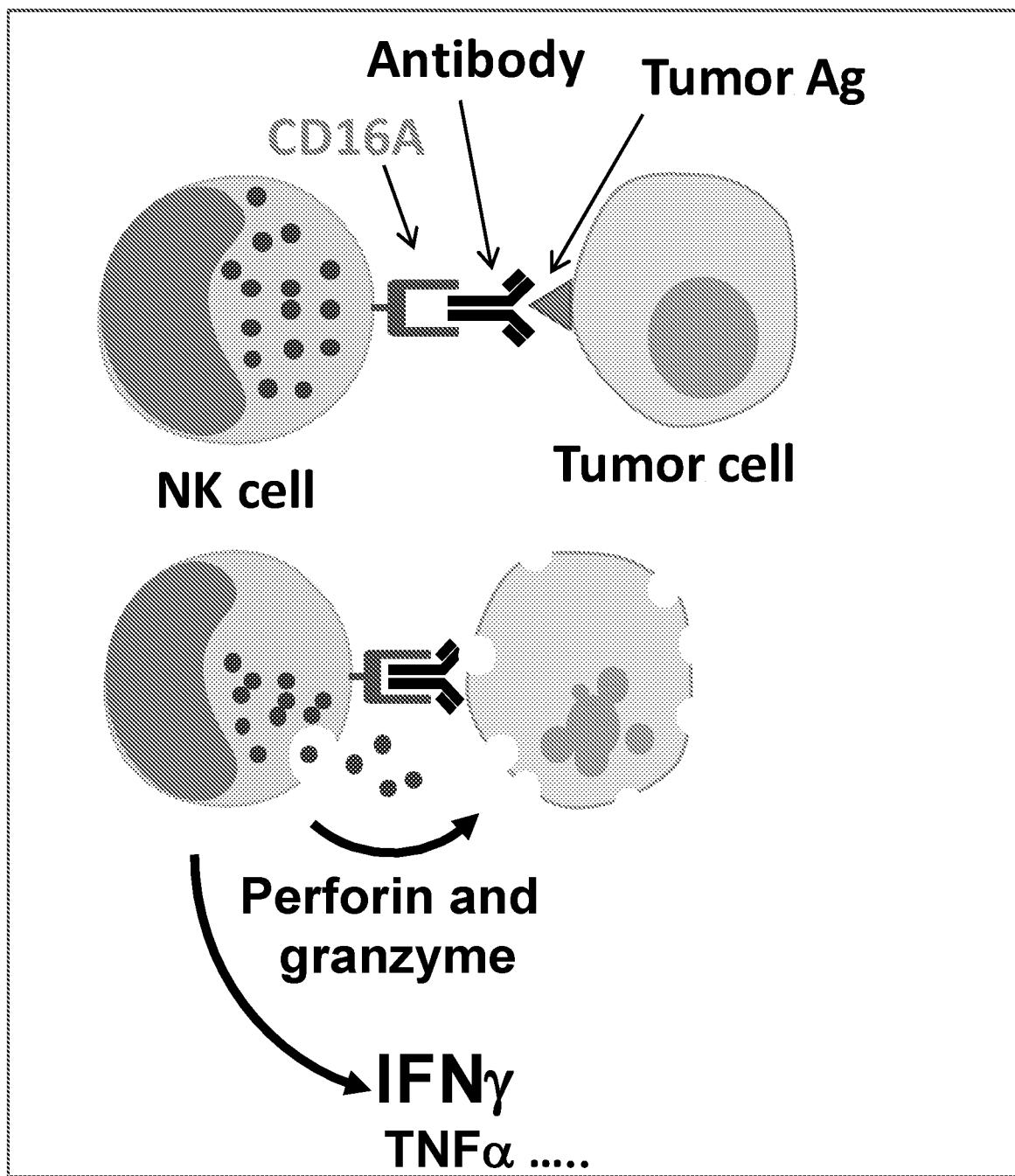


FIG. 2

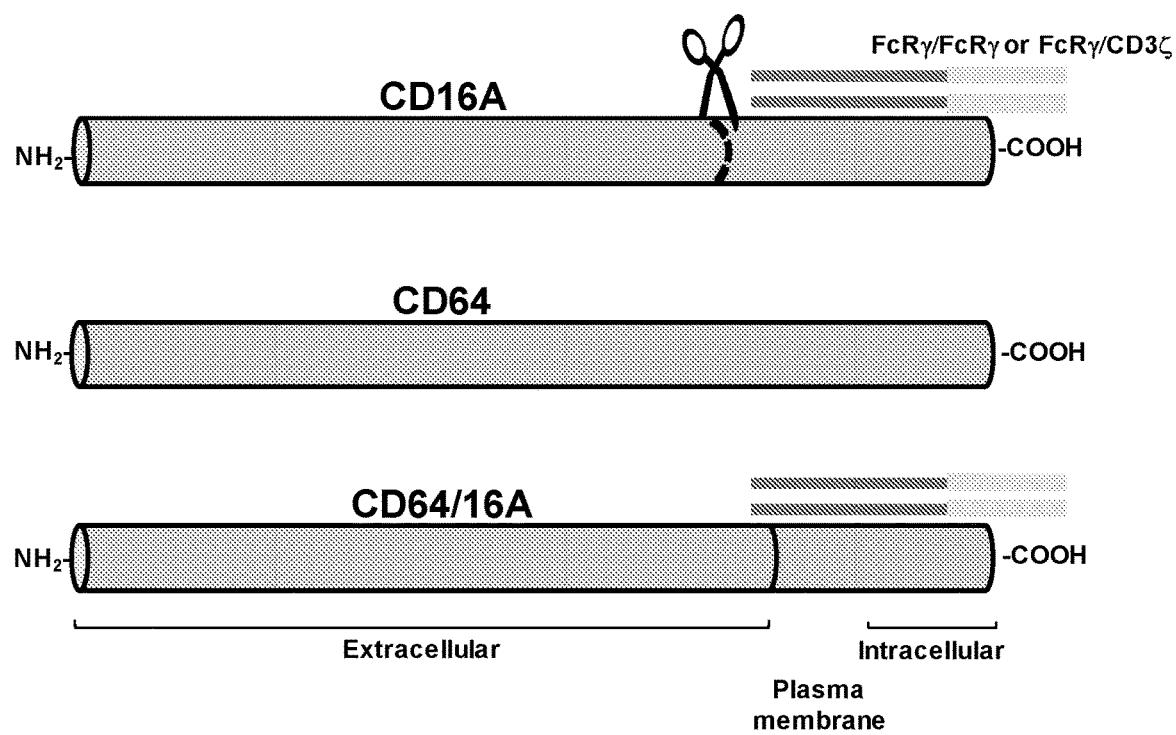


FIG. 3

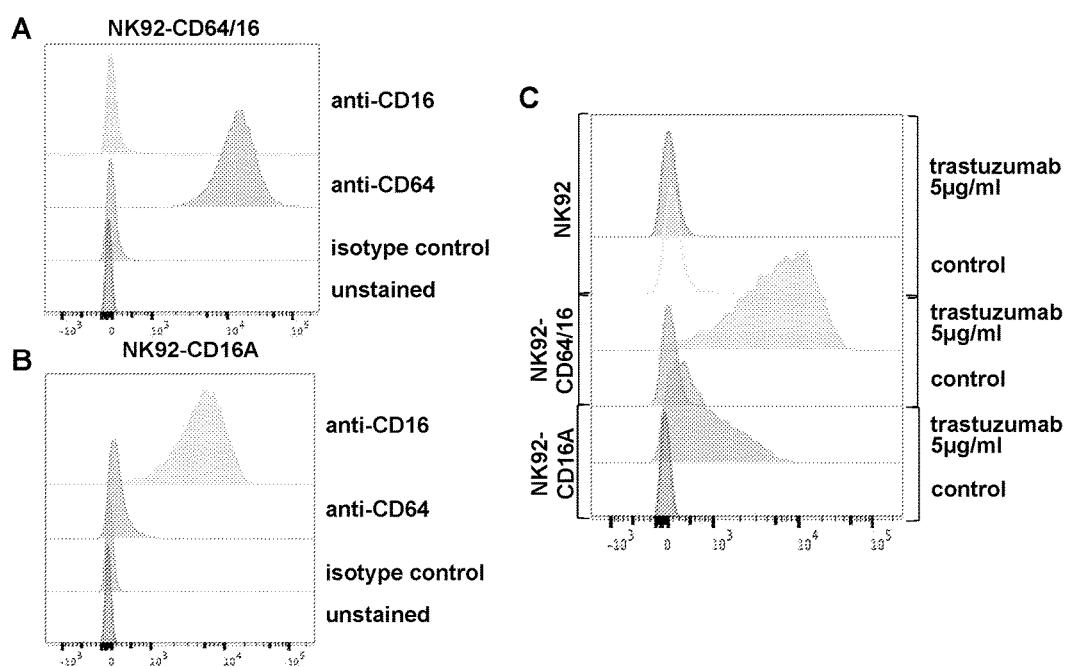
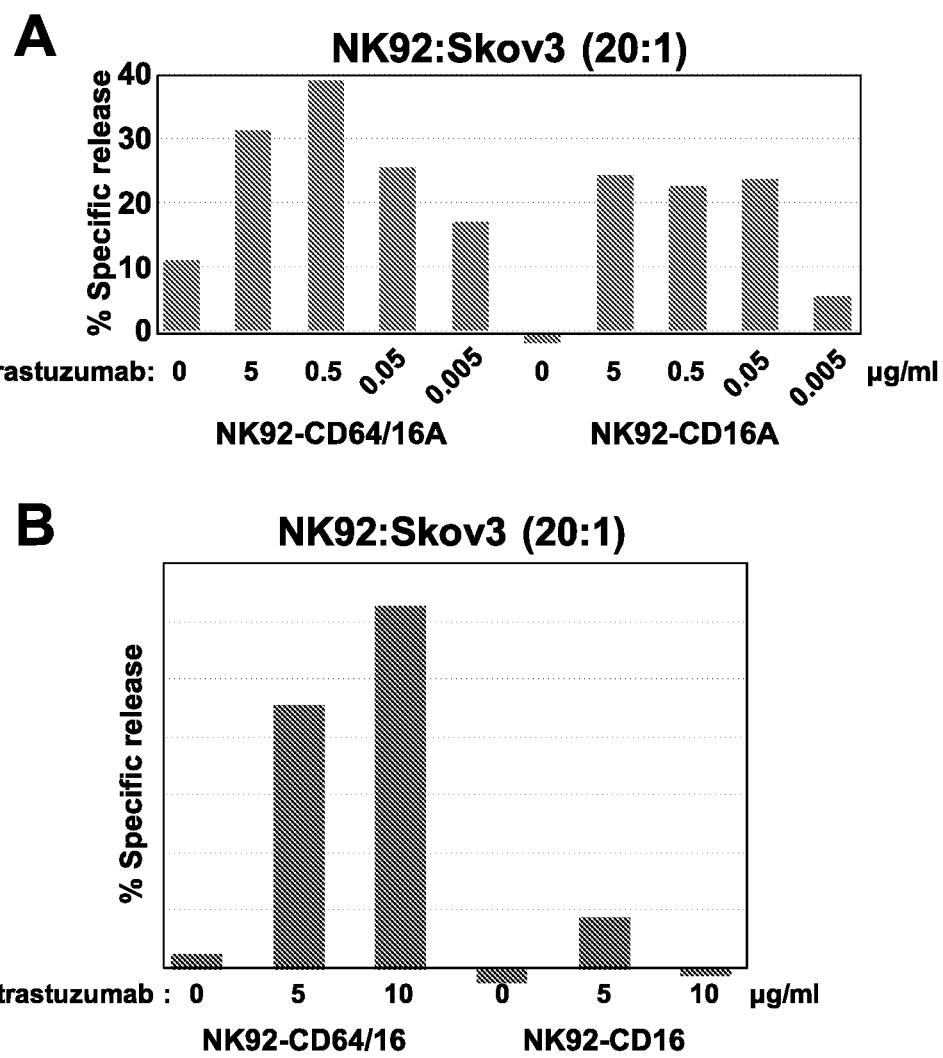
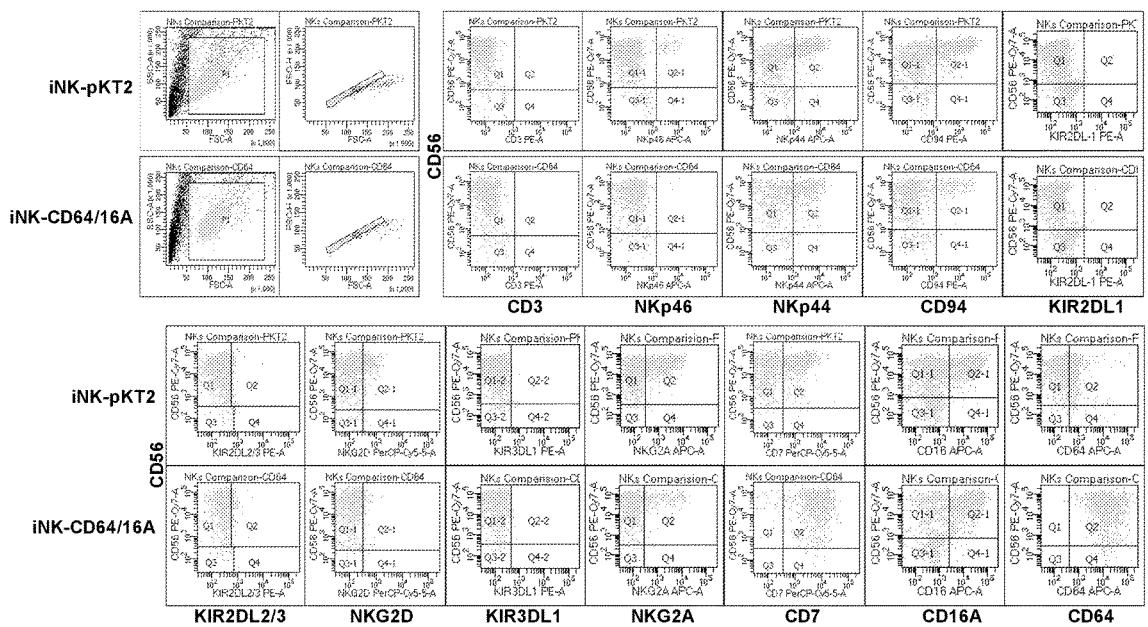


FIG. 4





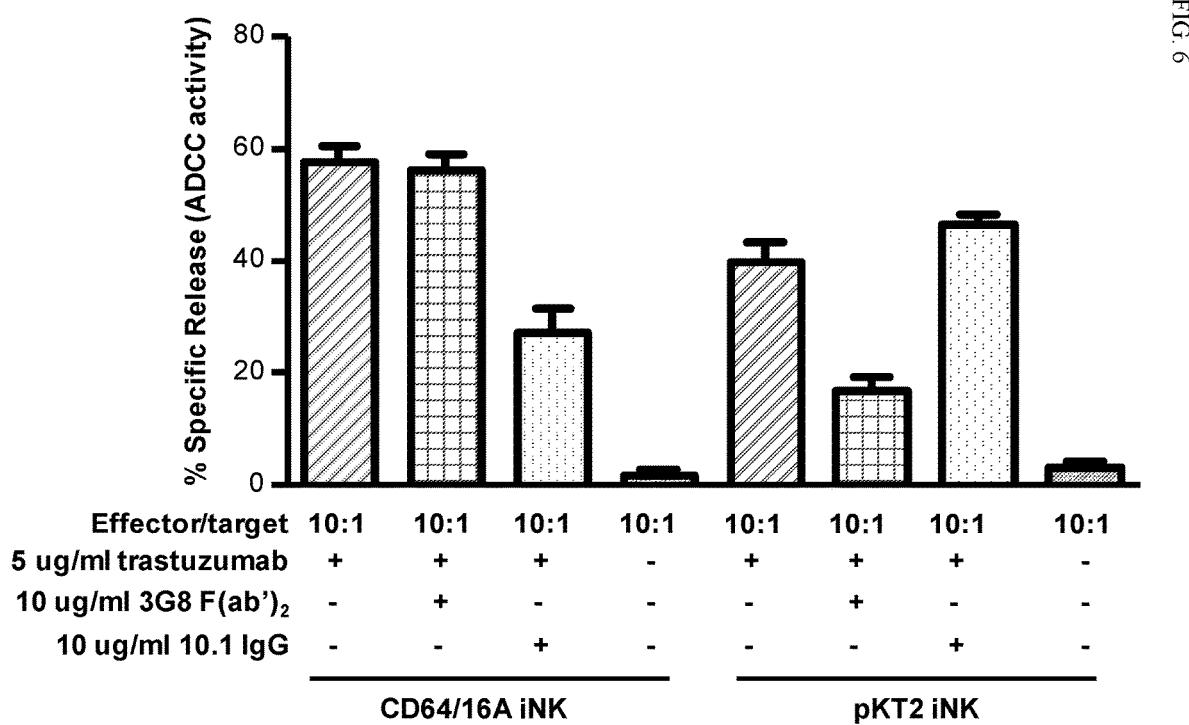


FIG. 7

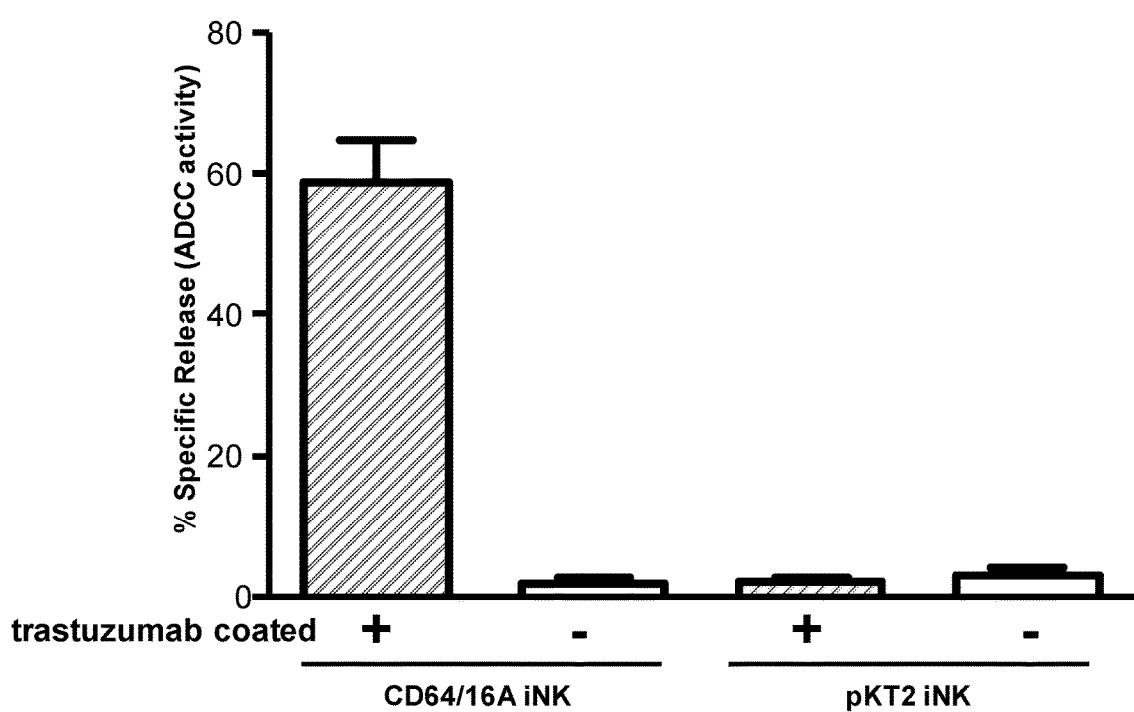


FIG. 8

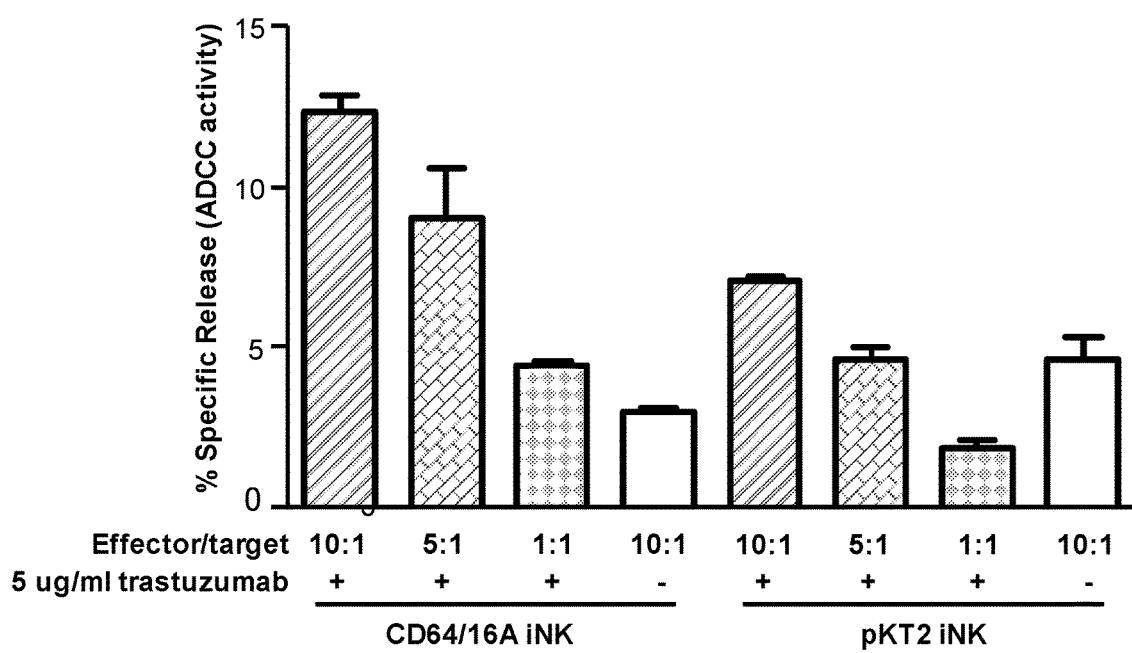


FIG. 9

MWFLTTLLLW VPVDGQVDTT KAVISLQPPW VSVFQEETVT 40
LHCEVLHLPG SSSTQWFLNG TATQTSTPSY RITSASVNDS 80
GEYRCQRGILS GRSDPIQLEI HRGWLILLQVS SRVFTIEGEPL 120
ALRCHAWKDK LVYNVLYYRN GKAKFEHWN SNLTILKTNI 160
SHNGTYHCSSG MGKHRYTSAG ISVTVKELFP APVLNASVTS 200
PILLEGNLVTL SCETKLLLQR PGLQLYFSFY MGSKTILRGRN 240
TSSSEYQITITA REDSGIYWC EAATEDGNVL KRSPPELEIQLV 280
LGFFPPGYQV SFCLVMVLLF AVDTGLYFSV KTNIRSSSTRD 320
WKDHKKFKWRK DPQDK . 336

FIG. 10

MWFLTTLLLW VPVDGQVDTT KAVISLQPPW VSVFQEETVT 40
LIICEVLLHLPG SSSTQWELMG TATQITSTTPSY RITSASVNDS 80
GEYRCQRGLS GRSDPITLEI HRGWLLLQVS SRVETEGEPL 120
ALRCHAWKDK LVYNVLYYRN GKAFFEHWN SNLTILKTNI 160
SHNGTYHCSSG MGKHRYTSAG ISVTVKETLFP APVLNASVTS 200
PLLEGNLVTL SCETKILLQR PGLQLYFSEFY MSGSKTILRGPN 240
TSSEYYQILTA REDSGGLYWC EAATEDGNVL KRSPPELELQV 280
LGLQLPPTPVW FHVLFYLAVG IMFIVNTVILW VTIRKELKRK 320
KKWDLEIISLD SGIEKKVVTSS LQEDRHLEEE LKCQEQQEEQ 360
LQEGVHRKEP QGAT. 375

FIG. 11

MWQLLLPTAL LLIVSAGMRT EDLPKAVVFL EPQWYRVILEK 40
DSVTILKCQGA YSPEDNISTQW FHNESSLISSQ ASSYFIDAAAT 80
VDDSGEYRCQ TNLSTISDPV QIEVHTGWLIL QZAPRWVFKE 120
EDPIHLRCHS WKNTALKVT YLQNGKGKRY FHHNSDFYIP 160
KATLKDSGSY FCRGLEFGSKN VSSETVNITI TQGLAVSTIS 200
SEFPFPGYQVS FCLVMVLLFA VDTGLYFSVK TNIRSSTRDW 240
KDHKEKWKRKD PQDKRSKRSR LHSDYMNMT PRRPGPTRKH 280
YQPYAPPDF AAYRSKRGK KLLYIFKQPF MRPVQTTQEE 320
DGCSCREPEE EGGCELRVK FSRSADAPAY QQGQNQLYNE 360
LNLGREYD VLDKRRGRDP EMGGKPRRKN PQEGLYNELQ 400
KDKMAEAYSE IGMKGERRRG KGHDGLYQGL STATKDTYDA 440
LHMQALPPR. 450

FIG. 12

MWQLLLPTAL LLIVSAGMRT EDLPPKAVVFL EPQWYRVILEK 40
DSVTLLKCQGA YSPEDNINSTQW EHNESSLISSQ ASSYFIDAAAT 80
VDDSGEYRCQ TNILSTLSDPV QLEVHTGWILL LQAPRWVFEKE 120
EDPIHLRCHS WKNWIAIHKVT YLQNNGKGRKY FHHNSDFYIP 160
KATLKDSGSY FCRGLEFGSKN VSSETVNNTT TQGLAVSTIS 200
SFFPPPGYQVS FCLVMVLLFA VDTGLYFSVK TNIRSSTRDW 240
KDHKFKWRKD PQDKKRGRKK LLYIFKQPFM RPVQTTQEEED 280
GCSCREPEE EGGCEIRVKF SRSADAPAYQ QGONQQLYNEI 320
NLGRREEYDV LDKRRGRDPE MGGKPRRKNP QEGLYNELQK 360
DKMAEAYSEI GMKGERRRGK GHDDGLYQQGLS TATKDTYDAL 400
IMQALPPR. 409

FIG. 13

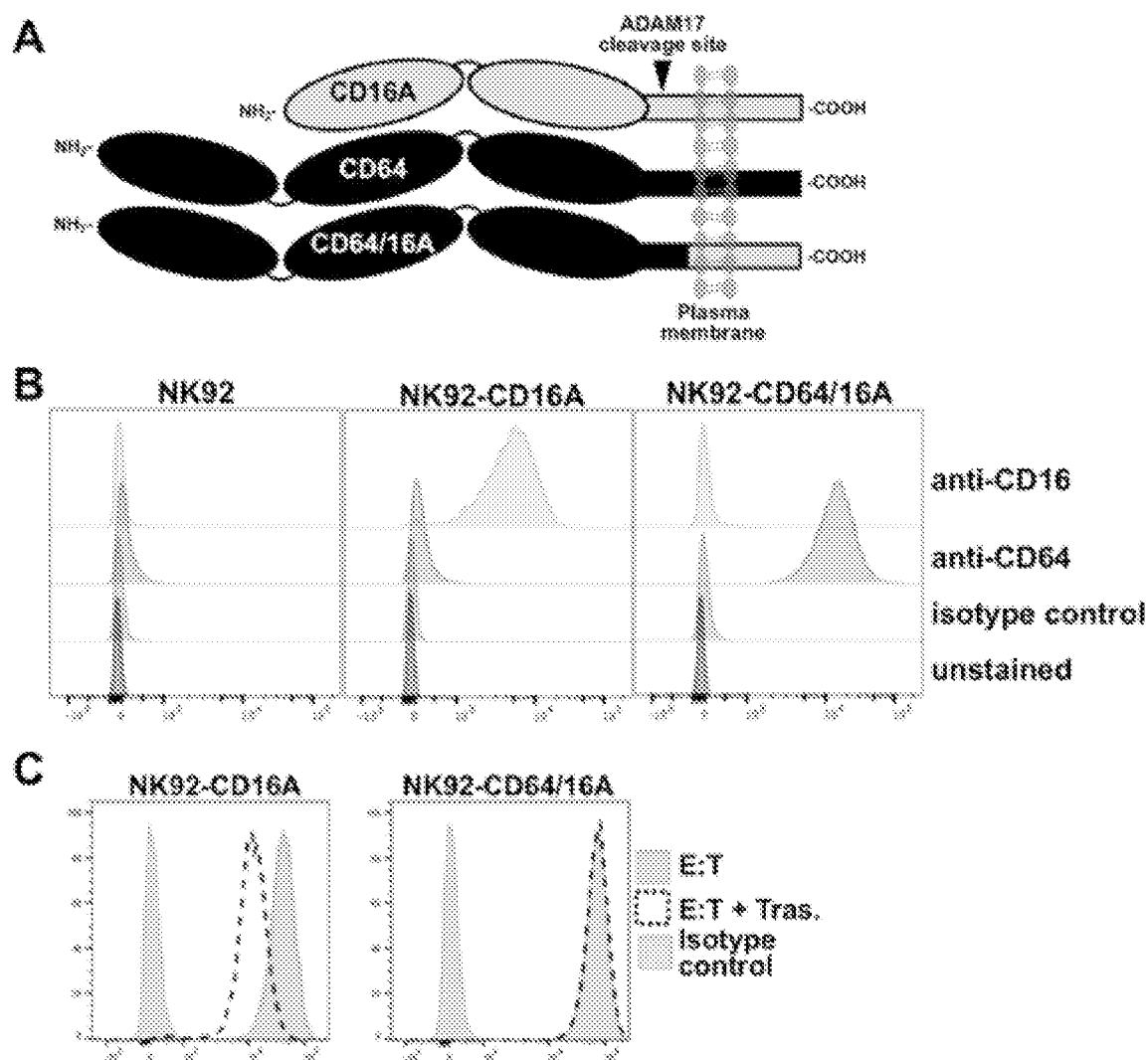


FIG. 14

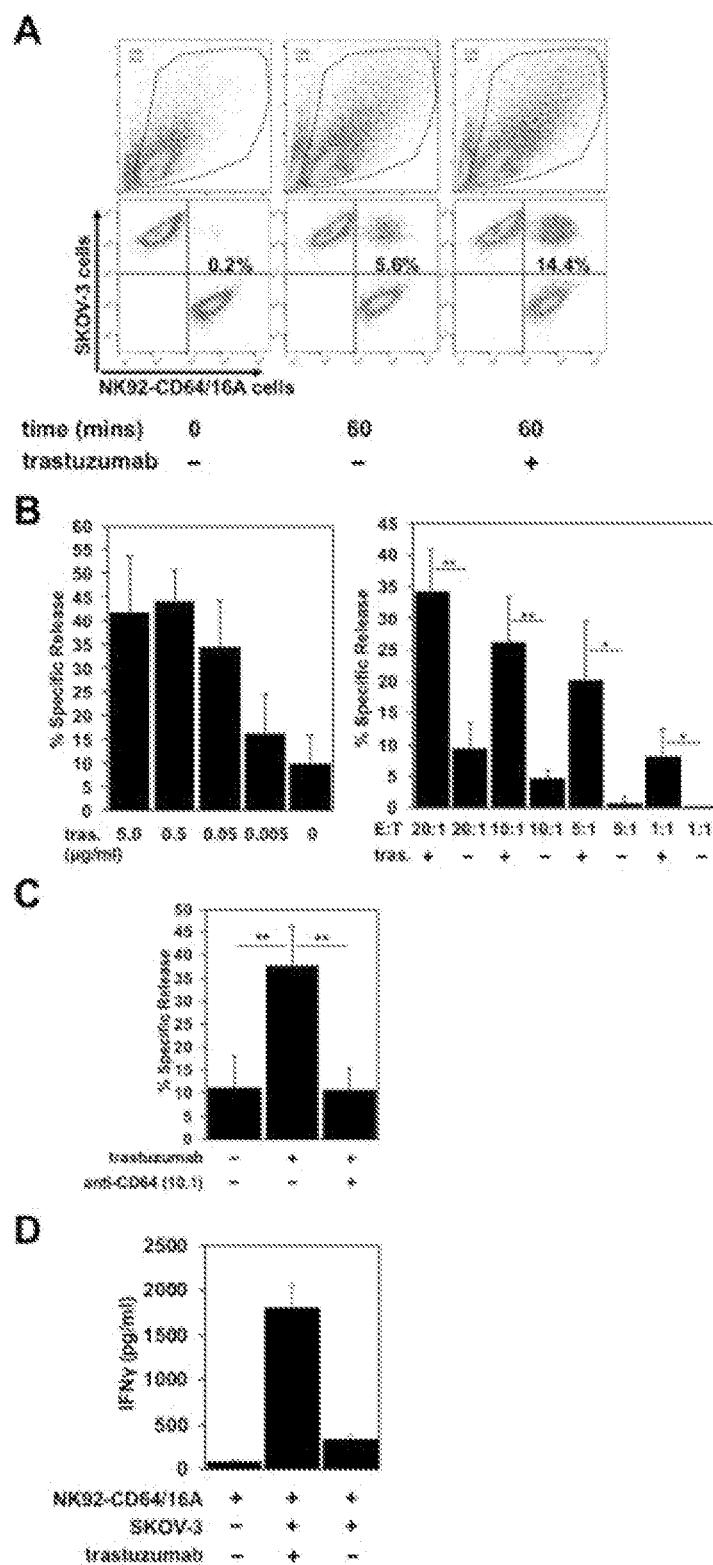
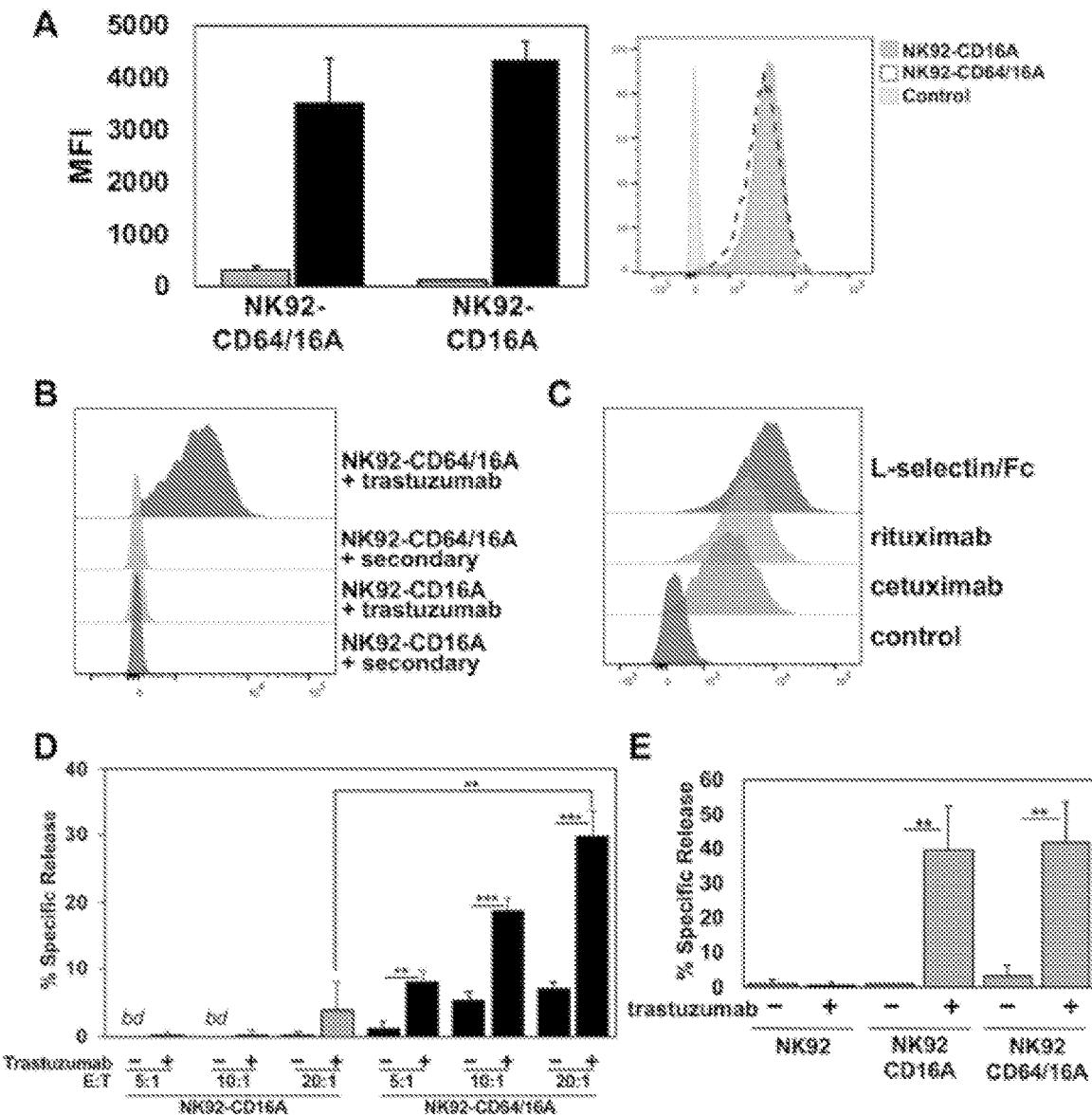


FIG. 15



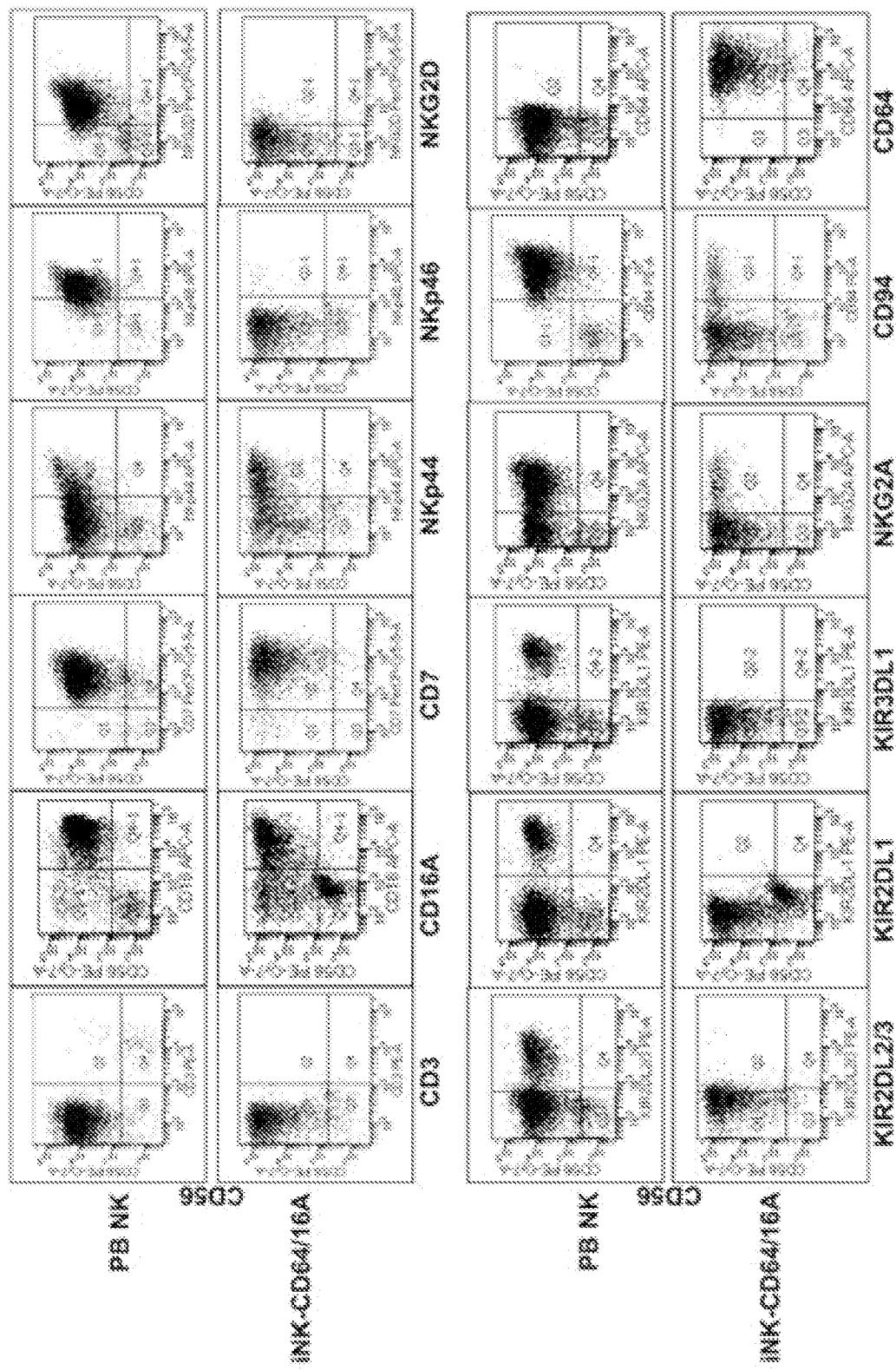


FIG. 16

FIG. 17

A

iNK-pKT2

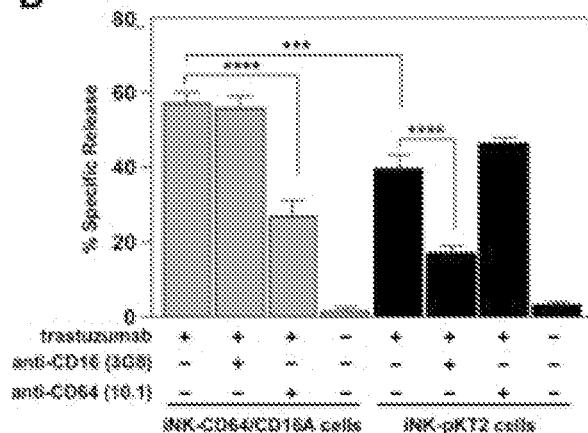
CD56

iNK-CD64/16A

CD16A

CD64

B



C

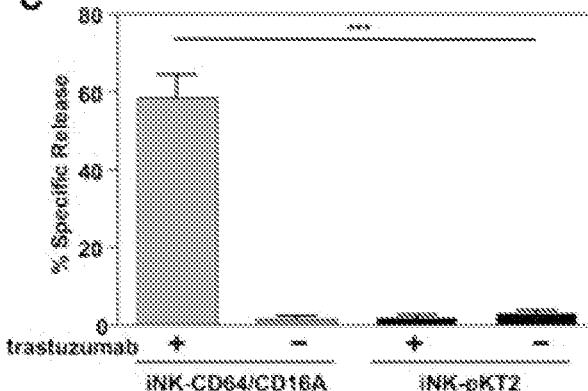


FIG. 18

Majority	<u>MWQLXXXTALLLLLVSAGXXXXDXPKAVVXLEPXWXRVLXXDSVTLNCQGX</u>
	10 20 30 40 50
dogCD16A.pro	MNGLVSS T ALLLLL S ACT-QAEVPKAVV L ESPKNNR I ETD S VT L NCQGX 49
humCD16A.pro	MWQL L PT A LLLLL S AGMRTECL S KAVV P LEPQNYR I LEK D VT L NCQGX 50
Majority	<u>XXXXDNNTKWXHNXXXKISXQXSXYKIXXAXXXXSGEYRCQTXSXLSDPV</u>
	60 70 80 90 100
dogCD16A.pro	HLLRD N YT- W LHNGRP I E N Q I ST V IK N S I K N SGEYRCQ T DQSKL E D N 98
humCD16A.pro	YS P E D N I T O N F H E N S L E S G A S S I F D A T TVDD S GEYRCQ T WNL E D N 100
Majority	<u>QLEVHXGWLLLQXPRXVFXXXXXKIXLXCHSWKNTXXXXVXXYQNGXGKX</u>
	110 120 130 140 150
dogCD16A.pro	QLEVHTGN L L L Q V P R L N Q E G E L T Q H CH N N E N P V R N Y O T F O N G R C K N F 146
humCD16A.pro	QLEVHIC G LLL C AP R W N PK E D P I H R E CH N W N T A L H K T Y L Q N G R Y 150
Majority	<u>FXXNSXXXIFXATXXXXGSYFCRGXXGKXNSSEVNIXIXQGXXXXXS</u>
	160 170 180 190 200
dogCD16A.pro	F Y N I S B Y H I P A T S E H N G N I F C R G X X G K N X S SE V N I X I XQGXXXXX S 197
humCD16A.pro	F H H I S D F I E K G T L K D S G T F C R G L F G S H V E S T N T I T G SLAV S T I 200
Majority	<u>XXXXXXXXQXXFXLVMXLLFAVDTGLYFXVXXXXRSSXXXXXXWXXX</u>
	210 220 230 240 250
dogCD16A.pro	LLL S H W P Q I P F S I N M A L H A N D T G L E S G M G N L E N S I V I S Q G 247
humCD16A.pro	S F F P P G Y Q V S E C L V N V L F A N T I G L E S M K T N I R S E T R D W D H I F K W R K D 250
Majority	<u>XXXX</u>
dogCD16A.pro	S. 249
humCD16A.pro	PQDK 254

Decoration 'Decoration #1': Shade (with deep red at 40% fill) residues that match the Consensus exactly.

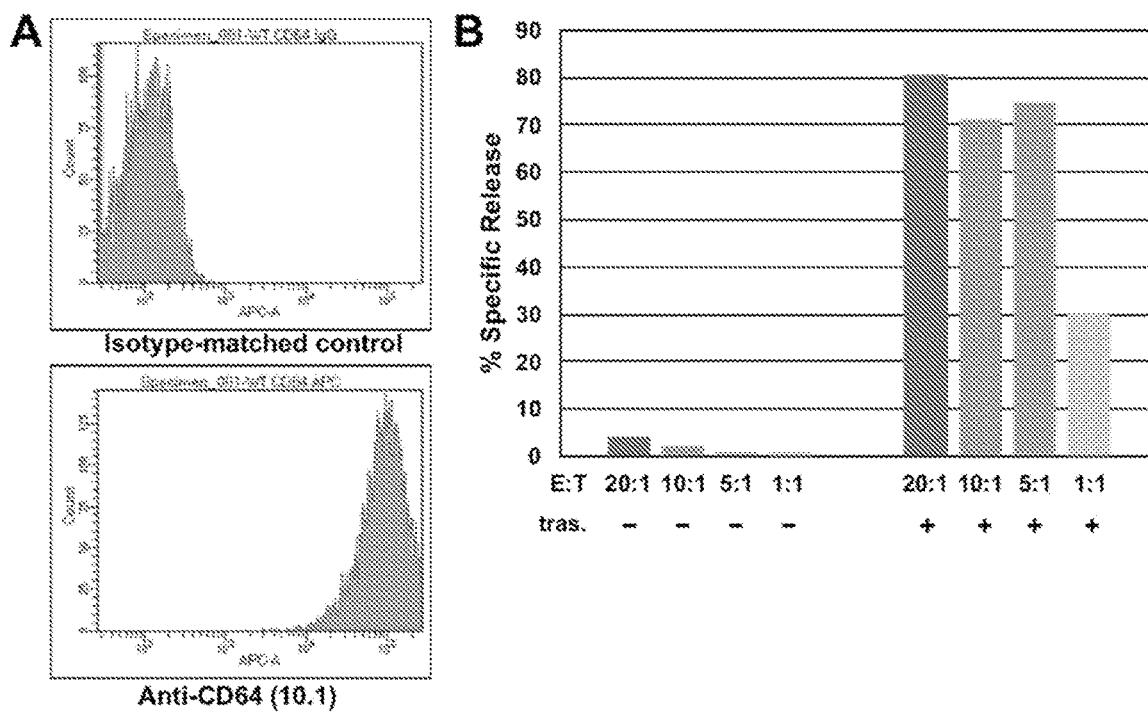
FIG. 19

Majority

	NWLLTVLLLNV[PAGAQTDXXXAVITLQPPNVSVFQEEXVTLXCEXXHLPGXSSTQWPLNG					
	10	20	30	40	50	60
dogCD64sp.pro	[N]LLTVLLLNV[PAGAQTDXXXAVITLQPPNVSVFQEEXVTLXCEXXHLPGXSSTQWPLNG					18
dogCD64.pro	[N]LLTVLLLNV[PAGAQTDXXXAVITLQPPNVSVFQEEXVTLXCEXXHLPGXSSTQWPLNG					60
humCD64.pro	[N]LLTVLLLNV[PAGAQTDXXXAVITLQPPNVSVFQEEXVTLXCEXXHLPGXSSTQWPLNG					60
Majority	TATQXTXPYTRIXXASVNDXGEYRCQXGLSXSDPIQLXIHRIWLILQVSGRVFTBGEPL					
	70	80	90	100	110	120
dogCD64sp.pro	[T]ATQXTXPYTRIXXASVNDXGEYRCQXGLSXSDPIQLXIHRIWLILQVSGRVFTBGEPL					125
dogCD64.pro	[T]ATQXTXPYTRIXXASVNDXGEYRCQXGLSXSDPIQLXIHRIWLILQVSGRVFTBGEPL					120
humCD64.pro	[T]ATQXTXPYTRIXXASVNDXGEYRCQXGLSXSDPIQLXIHRIWLILQVSGRVFTBGEPL					120
Majority	TLRCHGNNNKLVYNVLFYQNGTVLKFS[PQN]SEFTILKTTLH[HN]GTYHCSAMGKHRYESAG					
	130	140	150	160	170	180
dogCD64sp.pro	[T]LRCHGNNNKLVYNVLFYQNGTVLKFS[PQN]SEFTILKTTLH[HN]GTYHCSAMGKHRYESAG					195
dogCD64.pro	[T]LRCHGNNNKLVYNVLFYQNGTVLKFS[PQN]SEFTILKTTLH[HN]GTYHCSAMGKHRYESAG					180
humCD64.pro	[T]LRCHGNNNKLVYNVLFYQNGTVLKFS[PQN]SEFTILKTTLH[HN]GTYHCSAMGKHRYESAG					180
Majority	VISITIKELFPAPVLA[KASL]SSEPILEGH[VNL]S[CETKLL]QRPGLQLYFCFYMGSKTLLSRN					
	190	200	210	220	230	240
dogCD64sp.pro	[V]ISITIKELFPAPVLA[KASL]SSEPILEGH[VNL]S[CETKLL]QRPGLQLYFCFYMGSKTLLSRN					255
dogCD64.pro	[V]ISITIKELFPAPVLA[KASL]SSEPILEGH[VNL]S[CETKLL]QRPGLQLYFCFYMGSKTLLSRN					240
humCD64.pro	[V]ISITIKELFPAPVLA[KASL]SSEPILEGH[VNL]S[CETKLL]QRPGLQLYFCFYMGSKTLLSRN					240
Majority	TSSEYQILTAKKEDGGLYWCEATTEDGNVV[KRSPELELOVVG]PQTLPVWFHVLFVANG					
	250	260	270	280	290	300
dogCD64sp.pro	[T]SSEYQILTAKKEDGGLYWCEATTEDGNVV[KRSPELELOVVG]PQTLPVWFHVLFVANG					315
dogCD64.pro	[T]SSEYQILTAKKEDGGLYWCEATTEDGNVV[KRSPELELOVVG]PQTLPVWFHVLFVANG					300
humCD64.pro	[T]SSEYQILTAKKEDGGLYWCEATTEDGNVV[KRSPELELOVVG]PQTLPVWFHVLFVANG					300
Majority	NIFLVDTIFCNI[HKELQRKE]KKNLEISLYSGLEK[NVD]SYLQ[KERD]LEEP---[Y]QELEQ					
	310	320	330	340	350	360
dogCD64sp.pro	[N]IFLVDTIFCNI[HKELQRKE]KKNLEISLYSGLEK[NVD]SYLQ[KERD]LEEP---[Y]QELEQ					372
dogCD64.pro	[N]IFLVDTIFCNI[HKELQRKE]KKNLEISLYSGLEK[NVD]SYLQ[KERD]LEEP---[Y]QELEQ					357
humCD64.pro	[N]IFLVDTIFCNI[HKELQRKE]KKNLEISLYSGLEK[NVD]SYLQ[KERD]LEEP---[Y]QELEQ					360
Majority	LOEKTPQEPPEGEQQ-					
	370					
dogCD64sp.pro	LOEKTPQEPPEGEQQ-					288
dogCD64.pro	LOEKTPQEPPEGEQQ-					373
humCD64.pro	LOEKTPQEPPEGEQQ-					375

Decoration 'Decoration #1': Shade (with deep red at 40% fill) residues that match the Consensus exactly.

FIG. 20



**RECOMBINANT IMMUNE CELLS,
METHODS OF MAKING, AND METHODS OF
USE**

**CROSS-REFERENCE TO RELATED
APPLICATION**

[0001] This application claims priority to U.S. Provisional Patent Application No. 62/577,425, filed Oct. 26, 2017, which is incorporated herein by reference in its entirety.

GOVERNMENT FUNDING

[0002] This invention was made with government support under CA203348 awarded by National Institutes of Health. The government has certain rights in the invention.

SEQUENCE LISTING

[0003] This application contains a Sequence Listing electronically submitted to the United States Patent and Trademark Office via EFS-Web as an ASCII text file entitled "Sequence-Listing-0589_ST25.txt" having a size of 97 kilobytes and created on Oct. 26, 2018. Due to the electronic filing of the Sequence Listing, the electronically submitted Sequence Listing serves as both the paper copy required by 37 CFR § 1.821(c) and the CRF required by § 1.821(e). The information contained in the Sequence Listing is incorporated by reference herein.

SUMMARY

[0004] This disclosure describes, in one aspect, this disclosure describes an immune cell that expresses a heterologous IgG Fc receptor.

[0005] In some embodiments, the heterologous IgG Fc receptor can be a chimeric IgG Fc receptor. Generally, the chimeric IgG Fc receptor includes an extracellular domain, a transmembrane domain, and an intracellular domain. The extracellular domain generally includes a sufficient portion of CD64 to bind to an IgG Fc region. The intracellular domain of the chimeric IgG Fc receptor includes a sufficient portion of an Fc receptor immunoreceptor tyrosine-based activation motif (ITAM) to initiate cell signaling when an IgG Fc region binds to the extracellular domain.

[0006] In some of these embodiments, the intracellular domain includes at least a portion of the intracellular region of CD16A. In other embodiments, the intracellular domain can include at least a portion of the intracellular region of CD27, CD28, CD134 (OX40), CD137 (4-1BB), FcR γ , or CD3.

[0007] In some embodiments, the chimeric IgG Fc receptor can include the CD16A extracellular cleavage site. In other embodiments, the extracellular domain of the chimeric IgG Fc receptor can lack the CD16A extracellular cleavage site.

[0008] In some embodiments, the heterologous IgG Fc receptor can include an IgG Fc receptor not natively expressed by the immune cell. In some of these embodiments, the immune cell may be a natural killer (NK) cell genetically modified to express CD64.

[0009] In another aspect, this disclosure describes a polynucleotide that encodes any embodiment of the heterologous IgG Fc receptors summarized above.

[0010] In another aspect, this disclosure describes an immune cell that is genetically modified to include the

polynucleotide that encodes an embodiment of the heterologous IgG Fc receptors summarized above.

[0011] In another aspect, this disclosure describes a method of killing a tumor cell. Generally, the method includes contacting the tumor cell with an antibody that specifically binds to the tumor cell and contacting the tumor cell with any embodiment of the recombinant immune cell summarized above under conditions effective for the recombinant immune cell to kill the tumor cell.

[0012] In another aspect, this disclosure describes a method of treating a subject having a tumor. Generally, the method includes administering to the subject an antibody that specifically binds to cells of the tumor and administering to the subject a composition that includes any embodiment of the recombinant immune cell summarized above under conditions effective for the recombinant immune cell to kill cells of the tumor.

[0013] In another aspect, this disclosure describes a composition that includes a complex formed between a therapeutic antibody and any embodiment of the recombinant immune cell summarized above in which the heterologous IgG Fc receptor is bound to the Fc portion of the therapeutic antibody.

[0014] In another aspect, this disclosure describes a method of treating a subject having a tumor. Generally, the method includes administering to the subject any embodiment of the composition summarized immediately wherein the therapeutic antibody specifically binds to cells of the tumor.

[0015] The above summary is not intended to describe each disclosed embodiment or every implementation of the present invention. The description that follows more particularly exemplifies illustrative embodiments. In several places throughout the application, guidance is provided through lists of examples, which examples can be used in various combinations. In each instance, the recited list serves only as a representative group and should not be interpreted as an exclusive list.

BRIEF DESCRIPTION OF THE FIGURES

[0016] 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.

[0017] FIG. 1. Antibody-dependent cell-mediated cytotoxicity (ADCC).

[0018] FIG. 2. Wildtype CD16A, wildtype CD64, and the CD64/16A chimeric construct. The scissors and dashed line shown for CD16A represent the extracellular proteolytic site for ectodomain shedding.

[0019] FIG. 3. NK92 cells expressing either wildtype CD16A or CD64/16A. (A) NK92-CD64/16A cells were stained with anti-CD16, anti-CD64, or control antibodies. (B) NK92-CD16A cells were stained with anti-CD16, anti-CD64, or control antibodies. (C) NK92-CD64/16A, NK92-CD16A, or NK92 parent cells were incubated with trastuzumab then anti-human IgG-APC second stage antibody or anti-human IgG-APC second stage antibody alone (control). All antibody staining levels were determined by flow cytometry.

[0020] FIG. 4. NK92 cells expressing CD64/CD16A induce higher levels of ADCC than wildtype CD16A. (A) A standard ADCC assay was performed in which trastuzumab

(herceptin) was included in the assay. (B) A standard ADCC assay was performed in which the NK92-CD64/CD16A and NK92-CD16A cells were pre-incubated with trastuzumab and then the mAb was washed away prior to the effector cells being incubated with the SKOV-3 target cells.

[0021] FIG. 5. Flow cytometry data comparing phenotypic markers expressed by iNK-CD64/CD16A and iNK-pKT2 cells.

[0022] FIG. 6. Bar graph with data showing iNK-CD64/CD16A cells induce higher levels of ADCC than iNK-CD16A cells using SKOV-3 target cells.

[0023] FIG. 7. Bar graph with data showing iNK-CD64/CD16A, but not iNK-CD16A cells can be pre-loaded with a therapeutic mAb and mediate ADCC.

[0024] FIG. 8. Bar graph with data showing iNK-CD64/CD16A cells induce higher levels of ADCC than iNK-CD16A cells using MA148 target cells.

[0025] FIG. 9. Amino acid sequence of an exemplary CD64/CD16A chimeric IgG Fc receptor (SEQ ID NO:1).

[0026] FIG. 10. Amino acid sequence of CD64 IgG Fc receptor (SEQ ID NO:2).

[0027] FIG. 11. Amino acid sequence of an exemplary CD16A-CD28-BB- ζ chain chimeric IgG Fc receptor (SEQ ID NO:3).

[0028] FIG. 12. Amino acid sequence of an exemplary CD16A-BB- ζ chain chimeric IgG Fc receptor (SEQ ID NO:4).

[0029] FIG. 13. Expression of CD64/16A by NK92 cells. (A) Schematic representation of the cell membrane forms of CD16A, CD64, and CD64/16A. CD16A undergoes ectodomain shedding by ADAM17 at a membrane proximal location, as indicated, which is not present in CD64 and CD64/16A. (B) NK92 parental cells, NK92-CD16A cells, and NK92-CD64/16A cells were stained with an anti-CD16, anti-CD64, or an isotype-matched negative control mAb and examined by flow cytometry. (C) NK92-CD16A and NK92-CD64/16A cells were incubated with SKOV-3 cells with or without trastuzumab (5 μ g/ml) at 37° C. (E:T=1:1) for two hours. The NK92-CD16A and NK92-CD64/16A cells were then stained with an anti-CD16 mAb or an anti-CD64 mAb, respectively, and examined by flow cytometry. Nonspecific antibody labeling was determined using the appropriate isotype-negative control mAb. Data is representative of at least three independent experiments.

[0030] FIG. 14. CD64/16A promotes target cell conjugation, ADCC, and IFN γ production. (A) NK92-CD64/16A cells expressing eGFP and SKOV-3 cells labeled CellTrace Violet were mixed at an E:T ratio of 1:2 with or without trastuzumab (5 μ g/ml), incubated at 37° C. for 60 minutes, fixed, and then analyzed by flow cytometry. Representative data from at least three independent experiments are shown. (B) NK92-CD64/16A cells were incubated with SKOV-3 cells (E:T=20:1) and trastuzumab (tras.) at the indicated concentrations (left panel), or with SKOV-3 cells at the indicated E:T ratios in the presence or absence of trastuzumab (5 μ g/ml) (right panel) for two hours at 37° C. Data are represented as % specific release and the mean \pm SD of three independent experiments is shown. Statistical significance is indicated as * $p<0.05$, ** $p<0.01$. (C) NK92-CD64/16A cells were incubated with SKOV-3 cells (E:T=20:1) in the presence or absence of trastuzumab (5 μ g/ml) and the anti-CD64 mAb 10.1 (10 μ g/ml), as indicated, for two hours at 37° C. Data are represented as % specific release and the mean \pm SD of three independent experiments is shown. Sta-

tistical significance is indicated as ** $p<0.01$. (D) NK92-CD64/16A cells were incubated with SKOV-3 cells (E:T=1:1) with or without trastuzumab (5 μ g/ml) for two hours at 37° C. Secreted IFN γ levels were quantified by ELISA. Data is shown as mean of two independent experiments.

[0031] FIG. 15. CD64/16A attaches to soluble tumor-targeting mAbs and IgG fusion proteins. (A) Relative expression levels of CD16A and CD64/16A on NK92 cells were determined by cell staining with anti-CD16 and anti-CD64 mAbs (black bars), respectively, or an isotype-matched negative control antibody (gray bars). The bar graph shows mean fluorescence intensity (MFI) \pm SD of three independent experiments. Representative flow cytometric data are shown in the histogram overlay. The dashed line histogram shows CD64 staining of NK92-CD64/16A cells, the orange-filled histogram shows CD16A staining of NK92-CD16A cells, and the green-filled histogram shows isotype control antibody staining of the NK92-CD16A cells. (B) NK92-CD16A and NK92-CD64/16A cells were incubated with or without trastuzumab (5 μ g/ml) for two hours at 37° C., washed, stained with a fluorophore-conjugated anti-human secondary antibody, and analyzed by flow cytometry. Data is representative of at least three independent experiments. (C) NK92-CD64/16A cells were incubated with cetuximab or rituximab (5 μ g/ml for each), washed, and then stained with a fluorophore-conjugated anti-human secondary antibody. Control represents cells stained with the anti-human secondary antibody only. NK92-CD64/16A cells were also incubated with L-selectin/Fc (5 μ g/ml), washed, and then stained with a fluorophore-conjugated anti-L-selectin mAb. NK92 cells lack expression of endogenous L-selectin (data not shown). All staining was analyzed by flow cytometry. Data shown are representative of three independent experiments. (D) NK92-CD16A and NK92-CD64/16A cells were incubated in the presence or absence of trastuzumab (5 μ g/ml), washed, and exposed to SKOV-3 cells at the indicated E:T cell ratios for two hours at 37° C. Data is shown as mean \pm SD of three independent experiments. Statistical significance is indicated as * $p<0.01$, ** $p<0.001$. bd=below detection, (i.e., <spontaneous release by negative control cells). (E) NK92-CD16A and NK92-CD64/16A cells were incubated with SKOV-3 cells (E:T=10:1) in the presence or absence of trastuzumab (5 μ g/ml), as indicated, for two hours at 37° C. Data is shown as mean \pm SD of three independent experiments. Statistical significance is indicated as ** $p<0.01$.

[0032] FIG. 16. Generation of iNK cells expressing CD64/CD16A. iPSCs were transduced to stably express CD64/16A, differentiated into NK cells, and then expanded using K562-mbIL21-41BBL feeder cells. iNK-CD64/16A cells and freshly isolated peripheral blood (PB) NK cells enriched from adult peripheral blood were stained for CD56, CD3 and various inhibitory and activating receptors, as indicated. CD64/16A expression was determined by staining the cells with an anti-CD64 mAb. Representative data from at least three independent experiments are shown.

[0033] FIG. 17. iNK-CD64/16A cells show enhanced ADCC compared to iNK-pKT2 control cells. (A) NK cells derived from empty vector (iNK-pKT2) or CD64/16A (iNK-CD64/16A) transduced iPSCs were stained for CD56, CD64, and CD16A, as indicated. (B) iNK-pKT2 and iNK-CD64/16A cells were incubated with SKOV-3 cells (E:T=10:1) in the presence or absence of trastuzumab (5 μ g/ml), the function blocking anti-CD16 mAb 3G8 (5

$\mu\text{g}/\text{ml}$), and the function blocking anti-CD64 mAb 10.1 ($5 \mu\text{g}/\text{ml}$), as indicated, for two hours at 37°C . Data is shown as mean \pm SD of three independent experiments. Statistical significance is indicated as *** $p<0.001$; **** $p<0.0001$. (C) iNK-pKT2 and iNK-CD64/16A cells were incubated in the presence or absence of trastuzumab ($5 \mu\text{g}/\text{ml}$), washed, and exposed to SKOV-3 cells (E:T=10:1) for two hours at 37°C . Data is shown as mean \pm SD of three independent experiments. Statistical significance is indicated as *** $p<0.001$.

[0034] FIG. 18. Sequence alignment of canine CD16A (SEQ ID NO:5), canine CD64sp (SEQ ID NO:25), and human CD16A (SEQ ID NO:6).

[0035] FIG. 19. Sequence alignment of canine CD64 (SEQ ID NO:7) and human CD64 (SEQ ID NO:8).

[0036] FIG. 20. NK92 cells expressing wildtype human CD64 mediate ADCC. (A) NK92-CD64 cells were stained with an isotype-matched negative control mAb or the anti-CD64 mAb (clone 10.1) and examined by flow cytometry. (B) NK92-CD64 cells were incubated with SKOV-3 cells (at the indicated E:T ratios) in the presence or absence of trastuzumab (tras.) ($5 \mu\text{g}/\text{ml}$) for two hours at 37°C . Representative data from at least three independent experiments are shown.

DETAILED DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

[0037] This disclosure describes recombinant immune cells, methods of making the recombinant immune cells, and methods of using the recombinant immune cells. Generally, the recombinant immune cells are genetically modified to include a heterologous IgG Fc receptor. In some cases, the heterologous IgG Fc receptor can be a chimeric receptor engineered to include domains from two or more receptors. In other embodiments, the heterologous may be an IgG Fc receptor that is not natively expressed by the immune cell. Generally, the recombinant immune cells provide a sustained cytotoxic immune response against a target—for example, a tumor cell—that is targeted for killing by the immune cell because the target binds a therapeutic antibody that is recognized by the IgG Fc receptor.

[0038] A mechanism of cell-mediated immune defense involves the engagement of antibodies attached to target cells by Fc receptors expressed by leukocytes, which results in target cell killing. This process is referred to antibody-dependent cell-mediated cytotoxicity (ADCC). Therapeutic monoclonal antibodies (mAbs) have been generated against a variety of tumor antigens and tested in clinical trials for treating infectious diseases, chronic diseases, and cancers including, for example, AML, breast cancer, ovarian cancer, gastric cancer, neuroblastoma, and lymphoma. Many clinically successful mAbs use ADCC as a mechanism of action. A limitation of antibody therapy, however, is the development of resistance in patients and the non-responsiveness of some malignancies.

[0039] This disclosure describes an approach for augmenting Fc receptor interactions with therapeutic antibodies. The approach involves a chimeric receptor that includes a CD16A domain and a CD64 domain.

[0040] CD16A (Fc γ RIIA) is an IgG Fc receptor expressed by human natural killer (NK) cells, a population of cytotoxic lymphocytes, and is their sole means of recognizing IgG bound to tumor cells or virus-infected cells. CD16A is a potent activating receptor that induces ADCC by NK cells (FIG. 1). The CD16A transmembrane region is responsible

for the association with CD3 and/or FcR γ -chain (Fc γ R) that contain immunoreceptor tyrosine-based activation motifs (FIG. 2; FIG. 13A), and the CD16A cytoplasmic domain interacts with intracellular molecules critical for receptor functions. CD16A is a low affinity Fc γ R with limited capacity to engage therapeutic mAb-coated target cells. CD16A also undergoes a rapid downregulation in expression upon cell activation that markedly reduces its cell surface density and avidity for IgG. CD16A downregulation occurs by a proteolytic event at an extracellular site proximal to the plasma membrane and is referred to as ectodomain shedding. The location of this cleavage site has been reported (Jing et al., 2015. *PLoS One* 10:e0121788) and is shown schematically in FIG. 2 and FIG. 13A.

[0041] CD64 (Fc γ RI) is another IgG Fc receptor, and is expressed by monocytes, macrophages, and activated neutrophils. CD64 is a high affinity IgG receptor. This receptor does not undergo ectodomain shedding upon cell activation and does not naturally transduce signals for ADCC in NK cells.

[0042] This disclosure describes a chimeric Fc γ R that includes a CD16A domain and a CD64 domain. The chimeric receptor includes the extracellular region of human CD64 and the cytoplasmic region of human CD16A, an exemplary embodiment of which is shown schematically in FIG. 2 and FIG. 13A as CD64/16A. In various embodiments, the chimeric CD64/CD16A receptor can include the CD64 transmembrane region or the CD16A transmembrane region. The CD64/16A construct has been engineered so that it lacks the CD16A extracellular cleavage site and thus is not susceptible to ectodomain shedding (FIG. 2; FIG. 13A), but includes at least a portion of the CD16A intracellular region that is involved in intracellular signaling.

[0043] Also, while described herein in the context of an exemplary embodiment in which the CD64 domain and the CD16A contain amino acid sequences of human CD64 and human CD16A, respectively, the chimeric Fc γ R described herein can include an amino acid sequence that is, or is derived from, any suitable CD64 or CD16A natively expressed by any species. FIG. 18 and FIG. 19 provide amino acid sequence alignments of human and canine amino acid sequences for CD16A (FIG. 18) and CD64 (FIG. 19).

[0044] As used herein, the amino acid sequence of a domain is “derived from” the amino acid sequence of a reference polypeptide if the amino acid sequence of the domain possesses a specified amount of sequence similarity and/or sequence identity compared to the amino acid sequence of the reference polypeptide. Sequence similarity of can be determined by aligning the residues of the two polypeptides (for example, the domain amino acid sequence and the amino acid sequence of the reference CD16A or CD64 polypeptide) to optimize the number of identical amino acids along the lengths of their sequences. Gaps in either or both sequences are permitted in making the alignment in order to optimize the number of identical amino acids, although the amino acids in each sequence must nonetheless remain in their proper order.

[0045] A pair-wise comparison analysis of amino acid sequences can be carried out using the BESTFIT algorithm in the GCG package (version 10.2, Madison Wis.). Alternatively, polypeptides may be compared using the Blastp program of the BLAST 2 search algorithm, as described by Tatiana et al., (*FEMS Microbiol Lett*, 174, 247-250 (1999)), and available on the National Center for Biotechnology

Information (NCBI) website. The default values for all BLAST 2 search parameters may be used, including matrix=BLOSUM62; open gap penalty=11, extension gap penalty=1, gap x_dropoff=50, expect=10, wordsize=3, and filter on.

[0046] The amino acid sequence of a domain is “derived from” the amino acid sequence of a reference polypeptide if the amino acid sequence of the domain possesses a specified degree of amino acid sequence “identity” or amino acid sequence “similarity.” Amino acid sequence identity refers to the presence of identical amino acids. Amino acid sequence similarity refers to the presence of not only identical amino acids but also the presence of conservative substitutions. A conservative substitution for an amino acid may be selected from other members of the class to which the substituted amino acid belongs. For example, it is well-known in the art of protein biochemistry that an amino acid belonging to a grouping of amino acids having a particular size or characteristic (such as charge, hydrophobicity and hydrophilicity) can be substituted for another amino acid without altering the activity of a protein, particularly in regions of the protein that are not directly associated with biological activity. For example, nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan, and tyrosine. Polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine and glutamine. The positively charged (basic) amino acids include arginine, lysine and histidine. The negatively charged (acidic) amino acids include aspartic acid and glutamic acid. Conservative substitutions include, for example, Lys for Arg and vice versa to maintain a positive charge; Glu for Asp and vice versa to maintain a negative charge; Ser for Thr so that a free —OH is maintained; and Gln for Asn to maintain a free —NH₂. Likewise, biologically active analogs of a polypeptide containing deletions or additions of one or more contiguous or noncontiguous amino acids that do not eliminate a functional activity of the polypeptide are also contemplated.

[0047] The amino acid sequence of a CD16A domain or a CD64 domain is “derived from” a reference amino acid sequence if the domain amino acid sequence has at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence similarity to the reference amino acid sequence.

[0048] The amino acid sequence of a CD16A domain or a CD64 domain is “derived from” a reference amino acid sequence if the domain amino acid sequence has at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the reference amino acid sequence.

[0049] For the purpose of determining whether a domain amino acid sequence is “derived from” a specified reference amino acid sequence, exemplary suitable reference polypeptides include human CD16A (SEQ ID NO:6), canine CD16A (SEQ ID NO:5), human CD64 (SEQ ID NO:8),

canine CD64 (SEQ ID NO:7), canine CD64sp (SEQ ID NO:25), or the corresponding domains of any of the constructs listed in Table 1.

[0050] Also, while described herein in the context of an exemplary embodiment, illustrated in FIG. 2 and FIG. 13A, the chimeric receptor may be designed to include a different fusion points between CD64 and CD16A than that shown in FIG. 2 and FIG. 13A, to include the CD16A cleavage region, modified functional motifs in regions of CD64 or CD16A, and/or adding an additional signaling domain, such as, for example, a signaling domain of CD27, CD28, CD134 (OX40), CD137 (4-1BB), FcRγ, or CD3. The chimeric receptor may therefore be designed to increase the proliferation of NK cells or other effector cells, increase survival of NK cells or other effector cells, increase potency of NK cells or other effector cells, and/or decrease NK cell exhaustion in vivo.

[0051] In certain embodiments, the chimeric FcγR can be modified to include a cytoplasmic domain or a signaling domain that confers additional functionality to the chimeric receptor. For example, a chimeric FcγR can include a functional portion of CD28, which transduces signals involved in T-cell proliferation, survival, and cytokine production. As another example, a chimeric FcγR can include a functional portion of 4-1BB, which contributes to the clonal expansion, survival, and development of hematopoietic cells. As another example, a chimeric FcγR can include a functional portion of the CD3ζ cytoplasmic domain, which contains three immunoreceptor tyrosine-based activation motifs (ITAMs) that trigger intracellular signal-transduction pathways for ADCC, cytokine production, and cell proliferation and survival. As another example, a chimeric FcγR can include a functional portion of the FcRγ cytoplasmic domain, which contains an ITAM that preferentially recruits Syk kinase to mediate intracellular signals for ADCC, cytokine production, and cell proliferation and survival. As another example, a chimeric FcγR can include a functional portion of the DAP10 cytoplasmic domain, which contains a YxxM motif that specifically activates phosphatidylinositol 3-kinase-dependent signaling pathways for cytotoxicity, cell survival, and proliferation of NK and T cells. As another example, a chimeric FcγR can include a functional portion of the DAP12 cytoplasmic domain, which contains an ITAM that triggers signals for cytotoxicity, survival, and proliferation of NK cells and T cells. As another example, a chimeric FcγR can include a functional portion of the NKG2D (or CD314) transmembrane domain, which specifically associates with DAP10 to mediate signaling pathways for cytotoxicity, cell survival, and proliferation of NK and T cells. As another example, a chimeric FcγR can include a functional portion of the 2B4 (NKR2B4 or CD244) cytoplasmic domain, which transduces signals of cytolytic granule polarization involved in enhanced cytotoxicity of NK cells. As another example, a chimeric FcγR can include a functional portion of the high affinity IgE receptor FcεR transmembrane and cytoplasmic domains, which are constitutively associated with its β-subunit and FcR γ-chain (FcRγ) to mediate the most potent degranulation signals in myeloid cells that initiates the allergic responses, which could be exploited for cancer therapy with the recombinant high affinity IgG Fc receptors.

[0052] Exemplary constructs that include exemplary cytoplasmic domain and/or signaling domain modifications are listed in Table 1.

TABLE 1

Code	Domains (EC/TM/CY/SDs)*	Comments	SEQ ID NO:
rCD64#1	64/64/64/—		9
rCD64#2	64/64/mut64/—	CD64 cytoplasmic domain mutation	10
rCD64#3	64/16A/16A/—		11
rCD64#4	64/16A/16A/28-BB-CD3 ζ		12
rCD64#5	64/16A/16A/28-BB-FcR γ		13
rCD64#6	64/16A/16A/28-BB-Dap10		14
rCD64#7	64/16A/16A/28-BB-Dap12		15
rCD64#8	64/28/28-BB-CD3 ζ		16
rCD64#9	64/Fc ϵ R/Fc ϵ R/—		17
rCD64#10	64/16A/mut16A/—	Mutation on PKC phosphorylation site	18
rCD64#11	64/G2D/2B4/CD3 ζ		19
rCD64#12	64/16A/16A/2B4-CD3 ζ		20
rCD64#13	64/16A/16A/2B4-FcR γ		21
rCD64#14	64/16A/16A/2B4-Dap10		22
rCD64#15	64/16A/16A/2B4-Dap12		23
rCD64#16	64/64/64/2B4-CD3 ζ		24

*EC: Extracellular domain; TM: Transmembrane domain; CY: Cytoplasmic domain; SD: Signaling domain.
64: CD64; High affinity IgG Fc receptor Fc ϵ RI; mutCD64: High affinity IgG Fc receptor Fc ϵ RI with cytoplasmic mutation that results in higher levels of cytokine production and degranulation; 16A: C16A, Low affinity IgG Fc receptor Fc ϵ RIIA; —: no signaling domain; 28: CD28, a co-stimulatory receptor for cell proliferation and activation; BB: 4.1BB or CD137; CD3 ζ : CD3 ζ -chain or CD247; FcR γ : FcR γ -chain; D10: DAP10 signaling adaptor; D12: DAP12 signaling adaptor; Fc ϵ R: High affinity IgE Fc receptor; 16-PKC ζ : CD16A cytoplasmic domain with mutations on PKC phosphorylation site to disrupt cytokine productions mediated by CD16A; G2D: NKG2D or CD314; 2B4: NKR2B4 or CD244.

[0053] The CD64/16A chimeric receptor can be encoded by a cDNA that can be transcribed and translated from an expression vector introduced into a host cell to produce a recombinant cell. The host cell can include a suitable leukocyte-like cell or a primary leukocyte. Suitable leukocyte-like cells include, but are not limited to, a hematopoietic cell line or an induced pluripotent stem cell. Suitable primary leukocytes include, but are not limited to, NK cells, monocytes, macrophages, neutrophils, or T lymphocytes. The expression of CD64/16A by genetically-engineered leukocytes can increase the recombinant cell's effector function in killing target cells—e.g., tumor cells and virus-infected cells—in the presence of natural and therapeutic antibodies compared to the unmodified host cell. Because the CD64/16A chimeric receptor binds to IgG with high affinity, it may also be feasible to attach therapeutic mAbs to effector cells expressing the construct prior to their administration into patients. Hence, CD64/16A with an attached therapeutic mAb would provide the effector cells with a targeting element to direct them to cancer locations.

[0054] NK92 cells are a human NK cell line that lacks expression of endogenous CD16A. NK cells were generated that express in a stable manner wildtype CD16A, wildtype CD64, or the exemplary chimeric receptor CD64/16A. NK92 cells expressing CD64/16A could be stained with an anti-CD64 mAb, but not with an anti-CD16 mAb (FIG. 3A). NK92-CD16A cells could be stained with an anti-CD16 mAb, but not with an anti-CD64 mAb (FIG. 3B). NK92 cells expressing CD64 could be stained with an anti-CD64 mAb, but not by an isotype-matched negative control mAb (FIG. 20A).

[0055] FIG. 3C shows the ability of NK92 cells expressing CD64/16A or CD16A to bind trastuzumab, a therapeutic mAb specific to HER2/EGFR2 overexpressed by certain malignancies. Non-transduced NK92 cells, NK92-CD64/16A cells, and NK92-CD16A cells were incubated with trastuzumab (5 μ g/ml) for two hours at room temperature, washed to remove unbound antibody, incubated with an anti-human IgG second stage antibody conjugated to a

fluorophore, and then examined by flow cytometry. NK92-hCD64/16A cells bound much higher levels of trastuzumab than did NK92-CD16A cells and NK92 cells.

[0056] FIG. 4 presents data that shows the exemplary chimeric receptor CD64/16A conferred NK92 cells with an ADCC effector function. NK92 cells expressing CD64/16A or wildtype CD16A were incubated with the human ovarian cancer cell line SKOV-3 (20:1 ratio) in the presence or absence of trastuzumab at various concentrations (0.005 μ g/ml, 0.05 μ g/ml, 0.5 μ g/ml, or 5 μ g/ml). NK92 cells expressing either CD64/16A or wildtype CD16A demonstrated SKOV-3 cytotoxicity in the presence of trastuzumab. NK92 cells expressing CD64/16A had higher levels of target cell killing than did NK92-CD16A cells at all trastuzumab concentrations examined (FIG. 4A). NK92 cells expressing either CD64 also demonstrated SKOV-3 cytotoxicity in the presence of trastuzumab (FIG. 20B). In addition, NK92-CD64/16A and NK92-CD16A cells were pretreated with trastuzumab at 5 μ g/ml or 10 μ g/ml for two hours, washed to remove unbound antibody, and then incubated with SKOV-3 cells. In this assay, NK92-CD64/16A cells demonstrated a marked enhancement in target cell killing when compared to NK92-CD16A cells (FIG. 4B).

[0057] CD64/16A also was expressed in iPSCs and these cells were then differentiated into NK cells (referred to here as iNK cells). As shown in FIG. 5, iNK cells transduced with either CD64/16A or the empty vector (pKT2) as a control were compared for their expression of several NK cell markers. iNK-CD64/16A and iNK-pKT2 cells are CD56 $^{+}$ and CD3 $^{-}$, indicating that they are indeed NK cells. They also expressed similar levels of various NK cell markers. iNK-CD64/16A and iNK-pKT2 cells were found to express similar levels of CD16A, whereas only iNK-CD64/16A were stained by an anti-CD64 mAb (FIG. 5).

[0058] iNK-CD64/16A and iNK-pKT2 cells were evaluated for ADCC, as described above for the NK92 cells. iNK-CD64/16A and iNK-pKT2 cells were incubated with SKOV-3 cells at 10:1 ratio in the presence or absence of trastuzumab (5 μ g/ml). iNK-CD64/16A cells demonstrated

increased SKOV-3 cytotoxicity in the presence of trastuzumab and higher levels of ADCC when compared to iNK-pKT2 cells (FIG. 6).

[0059] Although the iNK-CD64/16A and iNK-pKT2 cells expressed similar levels of CD16A (FIG. 5), the function blocking anti-CD16A mAb 3G8 only blocked ADCC by the iNK-pKT2 cells (FIG. 6). Conversely, the function blocking anti-CD64 mAb 10.1 only blocked ADCC by the iNK-CD64/16A cells (FIG. 6). iNK-CD64/16A and iNK-pKT2 cells were also pretreated with trastuzumab at 10 µg/ml for two hours, washed to remove unbound antibody, and then incubated with SKOV-3 cells. In this assay, iNK-CD64/16A cells demonstrated a distinct enhancement in target cell killing when compared to iNK-pKT2 cells (FIG. 7). MA148 is a human ovarian cancer cell line that expresses considerably lower levels of HER2 when compared to SKOV-3 cells. ADCC was evaluated in iNK-CD64/16A and iNK-pKT2 cells when exposed to MA148 cells at various ratios in the presence or absence of trastuzumab (5 µg/ml). Again, iNK-CD64/16A cells demonstrated significantly higher levels of tumor cell killing than did iNK-pKT2 cells at all effector to target cell ratios when in the presence of trastuzumab (FIG. 8).

[0060] The chimeric receptor CD64/16A (FIG. 2; FIG. 13A) was stably expressed in the human NK cell line NK92. These cells lack endogenous Fc γ Rs but transduced cells expressing exogenous CD16A can mediate ADCC. As shown in FIG. 13B, an anti-CD64 mAb stained NK92 cells expressing CD64/16A cells, but not parent NK92 cells or NK92 cells expressing CD16A. An anti-CD16 mAb stained NK92 cells expressing CD16A, but not NK92 cells expressing CD64/16A or parent NK92 cells (FIG. 13B). CD16A undergoes ectodomain shedding by ADAM17 upon NK cell activation resulting in its rapid downregulation in expression. CD16A and its isoform CD16B on neutrophils is cleaved by ADAM17, and this occurs at an extracellular region proximal to the cell membrane. The ADAM17 cleavage region of CD16A is not present in CD64 or CD64/16A (FIG. 13A). CD16A underwent a >50% decrease in expression upon NK92 stimulation by ADCC, whereas CD64/16A demonstrated little to no downregulation (FIG. 13C).

[0061] To evaluate the function of CD64/16A, the ability of CD64/16A to initiate E:T conjugation, induce ADCC, and stimulate cytokine production upon NK cell engagement of antibody-bound tumor cells was examined. Prior to the release of its granule contents, an NK cell forms a stable conjugate with a target cell. NK92-CD64/16A cell and SKOV-3 cell conjugation were examined using a two-color flow cytometric approach. SKOV-3 cells are an ovarian cancer cell line that express HER2, and this assay was performed in the absence and presence of the anti-HER2 therapeutic mAb trastuzumab. The bicistronic vector containing CD64/16A also expressed eGFP and its fluorescence was used to identify the NK92 cells. SKOV-3 cells were labeled with the fluorescent dye CellTrace Violet. E:T conjugation resulted in two-color events that were evaluated by flow cytometry. Incubating NK92-CD64/16A cells with SKOV-3 cells resulted in a very low level of conjugation after initial exposure that increased after 60 minutes of exposure (FIG. 14A). However, in the presence of trastuzumab, NK92-CD64/16A cell and SKOV-3 conjugation was appreciably enhanced (FIG. 14A). This increase in conjugation corresponded with higher levels of target cell killing. As shown in FIG. 14B, SKOV-3 cell cytotoxicity by NK92-

CD64/16A cells varied depending on the trastuzumab concentration and E:T ratio. To confirm the role of CD64/16A in the induction of target cell killing, the ADCC assay was performed in the presence and absence of the anti-CD64 mAb 10.1 (FIG. 14C), which blocks IgG binding. Cytokine production is also induced during ADCC and NK cells are major producers of IFN γ . NK92-CD64/16A cells exposed to SKOV-3 cells and trastuzumab produced considerably higher levels of IFN γ than when exposed to SKOV-3 cells alone (FIG. 14D). Taken together, the above findings demonstrate that the CD64 component of the recombinant receptor engages tumor-bound antibody, and that the CD16A component promotes intracellular signaling leading to degranulation and cytokine production.

[0062] CD64 is distinguished from the other Fc γ R members by its unique third extracellular domain, which contributes to its high affinity and stable binding to soluble monomeric IgG. NK92 cells expressing CD64/16A or the CD16A-176V higher affinity variant were compared for their ability to capture soluble therapeutic mAbs. The NK92 cell transductants examined expressed similar levels of CD64/16A and CD16A (FIG. 15A). NK92 cell transductants were incubated with trastuzumab for two hours, excess antibody was washed away, stained with a fluorophore-conjugated anti-human IgG antibody, and then evaluated by flow cytometry. As shown in FIG. 15B, NK92-CD64/16A cells captured considerably higher levels of trastuzumab than did the NK92-CD16A cells (8.1 fold increase \pm 1.3, mean \pm SD of three independent experiments). Moreover, the NK92-CD64/16A cells efficiently captured the tumor-targeting mAbs cetuximab and rituximab, as well as the fusion protein L-selectin/Fc (FIG. 15C).

[0063] NK92-CD64/16A cells with a captured tumor-targeting mAb were tested to determine whether the cells mediated ADCC. For this assay, equal numbers of NK92-CD64/16A and NK92-CD16A cells were incubated with the same concentration of soluble trastuzumab, washed, and exposed to SKOV-3 cells. Target cell killing by NK92-CD64/16A cells with captured trastuzumab was significantly higher than NK92-CD64/16A cells alone, and was superior to NK92-CD16A cells treated with or without trastuzumab at all E:T ratios examined (FIG. 15D). In contrast, SKOV-3 cytotoxicity by NK92-CD16A and NK92-CD64/16A cells was not significantly different when trastuzumab was present and not washed out (FIG. 15E), thus demonstrating equivalent cytotoxicity by both transductants. Taken together, these findings show that NK92 cells expressing CD64/16A can stably bind soluble anti-tumor mAbs and IgG fusion proteins, and that these can serve as targeting elements to kill cancer cells.

Expression and Function of CD64/16A in iPSC-Derived NK Cells

[0064] Undifferentiated iPSCs were transduced to express CD64/16A using a Sleeping Beauty transposon plasmid for nonrandom gene insertion and stable expression. iPSCs were differentiated into hematopoietic cells and then iNK cells as described in the EXAMPLE 2. CD34 $^+$ CD43 $^+$ CD45 $^+$ cells were generated, further differentiated into iNK cells, and these cells were expanded for analysis using recombinant IL-2 and aAPCs. CD56 $^+$ CD3 $^-$ is a hallmark phenotype of human NK cells, and these cells composed the majority of our differentiated cell population (FIG. 16). Expression of activating and inhibitory receptors on the iNK cells also were assessed and compared to expression by peripheral

blood NK cells. Certain receptors, such as CD16A, were expressed by similar proportions of the two NK cell populations. The expanded iNK cells, however, lacked expression of the inhibitory KIR receptors KIR2DL2/3, KIR2DL1, and KIR3DL1 and also certain activating receptors (NKP46 and NKG2D) (FIG. 16). Another difference compared to peripheral blood NK cells was that the iNK cells were stained with an anti-CD64 mAb (FIG. 16), demonstrating the expression of CD64/16A.

[0065] To assess the function of CD64/16A in iNK cells, iNK cells derived from iPSCs transduced with either an pKT2 empty vector or pKT2-CD64/16A were compared. The NK cell markers mentioned above were expressed at similar levels and proportions by two iNK cell populations (data not shown), including CD16A (FIG. 17A), but only iNK-CD64/16A cells were stained by an anti-CD64 mAb (FIG. 17A). Both iNK transductants demonstrated increased SKOV-3 cell killing when in the presence of trastuzumab, yet iNK-CD64/16A cells mediated significantly higher levels of ADCC than did the iNK-pKT2 control cells (FIG. 17B). The anti-CD16 function blocking mAb 3G8, but not the anti-CD64 mAb 10.1, effectively inhibited ADCC by the iNK-pKT2 cells (FIG. 17B). Conversely, 10.1, but not 3G8, blocked ADCC by the iNK-CD64/16A cells (FIG. 17B). These findings show that the iNK cells were cytolytic effectors responsive to CD16A and CD64/16A engagement of antibody-bound tumor cells.

[0066] Also, iNK-CD64/16A and iNK-pKT2 cells were treated with soluble trastuzumab, excess antibody was washed away, and the cells were exposed to SKOV-3 cells. Under these conditions, ADCC by the iNK-CD64/16A cells was strikingly higher than the iNK-pKT2 cells (FIG. 17C), further establishing that CD64/16A can capture soluble anti-tumor mAbs that serve as a targeting element for tumor cell killing.

[0067] Taken together, the data show that CD64/16A binds therapeutic mAbs with higher affinity than CD16A. Moreover, CD64/16A expressed in NK92 cells and iNK cells confers cells with the ability to mediate higher levels of ADCC than NK92 cells and iNK cells expressing wildtype or endogenous CD16A, respectively. NK cells expressing CD64/16A facilitated cell conjugation with antibody-bound tumor cells, cytotoxicity, and IFN γ production, demonstrating function by both components of the recombinant Fc γ R. NK92 cells and iNK cells expressing CD64/16A can be pre-loaded with a therapeutic mAb prior to their exposure to target cells. Cells expressing the chimeric receptor and preloaded with therapeutic antibody may allow one to modify engineered NK cells with different therapeutic mAbs for targeting elements of multiple types of cancer. Finally, CD64/16A lacks the ADAM17 cleavage region found in CD16A and it did not undergo the same level of downregulation in expression during ADCC.

[0068] CD64/16A was shown to be functional in two NK cell platforms, the NK92 cell line and primary NK cells derived from iPSCs. NK92 cells lack inhibitory KIR receptors and show high levels of natural cytotoxicity compared to other NK cell lines derived from patients. NK92 cells have been broadly used to express modified genes to direct their cytolytic effector function, have been evaluated in preclinical studies, and are undergoing clinical testing in cancer patients. iPSCs are also very amendable to genetic engineering and can be differentiated into NK cells expressing various receptors to direct their effector functions. The

iNK cells generated in this study lacked several inhibitory and activating receptors that are indicators of an immature state. In some applications, therapeutic iNK cells lacking inhibitory receptors and certain activating receptors may allow for more directed and/or effective tumor cell killing by engineered receptors.

[0069] The iNK cells expressed endogenous CD16A and mediated ADCC, thus they were cytotoxic effector cells. An individual NK cell can kill multiple tumor cells in different manners. This includes by a process of sequential contacts and degranulations, referred to as serial killing, and by the localized dispersion of its granule contents that kills surrounding tumor cells, referred to as bystander killing. Inhibiting CD16A shedding has been reported to slow NK cell detachment from target cells and reduce serial killing by NK cells in vitro. Due to the CD64 component and its lack of ectodomain shedding, NK cells expressing CD64/16A could be less efficient at serial killing and more efficient at bystander killing.

[0070] NK cells expressing CD64/16A have several potential advantages as a therapeutic in combination with a therapeutic antibody. Modifying NK cells expressing CD64/16A with an antibody can reduce the dosage of therapeutic antibodies administered to patients. Fusion proteins containing a human IgG Fc region, such as L-selectin/Fc, also can be captured by CD64/16A and this may provide further options for directing the tissue and tumor antigen targeting of engineered NK cells. The NK92 and iNK cell platforms for adoptive cell therapies also can be readily genetically modified on a clonal level and expanded into clinical-scalable cell numbers to produce engineered NK cells with improved effector activities as an off-the-shelf therapeutic for cancer immunotherapy.

[0071] In some embodiments, iPSC-derived NK cells that express CD64/16A can exhibit increased in vivo anti-cancer activity in the presence of therapeutic mAbs. For example, NOD/SCID/ γ c $^{-/-}$ (NSG) mice and human cancer cell lines that are stably engineered to express firefly luciferase can be used for bioluminescent imaging to test iNK cell activity against cancer cells. The SKOV-3 and MA148 ovarian cancer cell lines can serve as model in vivo targets. NSG female mice can be subjected to sublethal irradiation, then injected intraperitoneally with tumor cells for bioluminescent imaging to quantify tumor growth or regression. Mice are given IL-2 and/or IL-15 every other day for four weeks to promote in vivo survival of the NK cells. Therapeutic antibody (e.g., trastuzumab) can be administered—e.g., once weekly for four weeks. Tumor growth/regression can be monitored by, for example, bioluminescent imaging and weighing the mice. Mice also can be bled (e.g., weekly) to quantify human NK cell survival, and one can further evaluate the expression/cell surface levels of various effector function markers by FACS. Evidence of metastasis can be determined by, for example, harvesting internal organs and examining the internal organs for metastasis.

[0072] In some embodiments, the number of cells in the therapeutic composition is at least 0.1×10^5 cells, at least 1×10^5 cells, at least 5×10^5 cells, at least 1×10^6 cells, at least 5×10^6 cells, at least 1×10^7 cells, at least 5×10^7 cells, at least 1×10^8 cells, at least 5×10^8 cells, at least 1×10^9 cells, or at least 5×10^9 cells, per dose. In some embodiments, the number of cells in the therapeutic composition is about 0.1×10^5 cells to about 1×10^6 cells, per dose; about 0.5×10^6 cells to about 1×10^7 cells, per dose; about 0.5×10^7 cells to

about 1×10^8 cells, per dose; about 0.5×10^8 cells to about 1×10^9 cells, per dose; about 1×10^9 cells to about 5×10^9 cells, per dose; about 0.5×10^9 cells to about 8×10^9 cells, per dose; about 3×10^9 cells to about 3×10^{10} cells, per dose, or any range in-between. Generally, 1×10^8 cells/dose translates to 1.67×10^6 cells/kg for a 60 kg patient.

[0073] In one embodiment, the number of cells in the therapeutic composition is the number of immune cells in a partial or single cord of blood, or is at least 0.1×10^5 cells/kg of bodyweight, at least 0.5×10^5 cells/kg of bodyweight, at least 1×10^5 cells/kg of bodyweight, at least 5×10^5 cells/kg of bodyweight, at least 10×10^5 cells/kg of bodyweight, at least 0.75×10^6 cells/kg of bodyweight, at least 1.25×10^6 cells/kg of bodyweight, at least 1.5×10^6 cells/kg of bodyweight, at least 1.75×10^6 cells/kg of bodyweight, at least 2×10^6 cells/kg of bodyweight, at least 2.5×10^6 cells/kg of bodyweight, at least 3×10^6 cells/kg of bodyweight, at least 4×10^6 cells/kg of bodyweight, at least 5×10^6 cells/kg of bodyweight, at least 10×10^6 cells/kg of bodyweight, at least 15×10^6 cells/kg of bodyweight, at least 20×10^6 cells/kg of bodyweight, at least 25×10^6 cells/kg of bodyweight, at least 30×10^6 cells/kg of bodyweight, 1×10^8 cells/kg of bodyweight, 5×10^8 cells/kg of bodyweight, or 1×10^9 cells/kg of bodyweight.

[0074] In one embodiment, a dose of cells is delivered to a subject. In one illustrative embodiment, the effective amount of cells provided to a subject is at least 2×10^6 cells/kg, at least 3×10^6 cells/kg, at least 4×10^6 cells/kg, at least 5×10^6 cells/kg, at least 6×10^6 cells/kg, at least 7×10^6 cells/kg, at least 8×10^6 cells/kg, at least 9×10^6 cells/kg, or at least 10×10^6 cells/kg, or more cells/kg, including all intervening doses of cells.

[0075] In another illustrative embodiment, the effective amount of cells provided to a subject is about 2×10^6 cells/kg, about 3×10^6 cells/kg, about 4×10^6 cells/kg, about 5×10^6 cells/kg, about 6×10^6 cells/kg, about 7×10^6 cells/kg, about 8×10^6 cells/kg, about 9×10^6 cells/kg, or about 10×10^6 cells/kg, or more cells/kg, including all intervening doses of cells.

[0076] In another illustrative embodiment, the effective amount of cells provided to a subject is from about 2×10^6 cells/kg to about 10×10^6 cells/kg, about 3×10^6 cells/kg to about 10×10^6 cells/kg, about 4×10^6 cells/kg to about 10×10^6 cells/kg, about 5×10^6 cells/kg to about 10×10^6 cells/kg, 2×10^6 cells/kg to about 6×10^6 cells/kg, 2×10^6 cells/kg to about 7×10^6 cells/kg, 2×10^6 cells/kg to about 8×10^6 cells/kg, 3×10^6 cells/kg to about 6×10^6 cells/kg, 3×10^6 cells/kg to about 7×10^6 cells/kg, 3×10^6 cells/kg to about 8×10^6 cells/kg, 4×10^6 cells/kg to about 6×10^6 cells/kg, 4×10^6 cells/kg to about 7×10^6 cells/kg, 4×10^6 cells/kg to about 8×10^6 cells/kg, 5×10^6 cells/kg to about 6×10^6 cells/kg, 5×10^6 cells/kg to about 7×10^6 cells/kg, 5×10^6 cells/kg to about 8×10^6 cells/kg, or 6×10^6 cells/kg to about 8×10^6 cells/kg, including all intervening doses of cells.

[0077] Some variation in dosage will necessarily occur depending on the condition of the subject being treated. The person responsible for administration will, in any event, determine the appropriate dose for the individual subject.

[0078] In some embodiments, the therapeutic use of cells is a single-dose treatment. In some embodiments, the therapeutic use of derived hematopoietic lineage cells is a multi-dose treatment. In some embodiments, the multi-dose treatment is one dose every day, every 3 days, every 7 days, every 10 days, every 15 days, every 20 days, every 25 days, every 30 days, every 35 days, every 40 days, every 45 days, or every 50 days, or any number of days in-between.

[0079] A compositions that includes a population of cells described herein can be sterile, and can be suitable and ready for administration (i.e., can be administered without any further processing) to human patients. A cell-based composition that is ready for administration means that the composition does not require any further processing or manipulation prior to transplant or administration to a subject. In some embodiments, such a population of cells can include an isolated population of cells that are expanded and/or modulated prior to administration with one or more agents.

[0080] In certain embodiments, the primary stimulatory signal and the co-stimulatory signal for the therapeutic cells can be provided by different protocols. For example, the agents providing each signal can be in solution or coupled to a surface. When coupled to a surface, the agents can be coupled to the same surface (i.e., in “cis” formation) or to separate surfaces (i.e., in “trans” formation). Alternatively, one agent can be coupled to a surface and the other agent in solution. In one embodiment, the agent providing the co-stimulatory signal can be bound to a cell surface and the agent providing the primary activation signal is in solution or coupled to a surface. In certain embodiments, both agents can be in solution. In another embodiment, the agents can be in soluble form, and then cross-linked to a surface, such as a cell expressing Fc receptors or an antibody or other binding agent that will bind to the agents such as disclosed in U.S. Patent Application Publication Nos. 20040101519 and 20060034810 for artificial antigen presenting cells (aAPCs) that are contemplated for use in activating and expanding T lymphocytes.

[0081] The therapeutic compositions suitable for administration to a patient can include one or more pharmaceutically acceptable carriers (additives) and/or diluents (e.g., pharmaceutically acceptable medium, for example, cell culture medium), or other pharmaceutically acceptable components. Pharmaceutically acceptable carriers and/or diluents are determined in part by the particular composition being administered, as well as by the particular method used to administer the therapeutic composition. Accordingly, there is a wide variety of suitable formulations of therapeutic compositions (see, e.g., Remington’s Pharmaceutical Sciences, 17th ed. 1985, the disclosure of which is hereby incorporated by reference in its entirety) well known to those of skill in the art.

[0082] In particular embodiments, therapeutic cell compositions having an isolated population the cells as described herein also have a pharmaceutically acceptable cell culture medium, or pharmaceutically acceptable carriers and/or diluents. A therapeutic composition comprising a population of the cells as disclosed herein can be administered separately by intravenous, intraperitoneal, enteral, or tracheal administration methods or in combination with other suitable compounds to affect the desired treatment goals.

[0083] These pharmaceutically acceptable carriers and/or diluents can be present in amounts sufficient to maintain a pH of the therapeutic composition of between about 3 and about 10. As such, the buffering agent can be as much as about 5% on a weight to weight basis of the total composition. Electrolytes such as, but not limited to, sodium chloride and potassium chloride can also be included in the therapeutic composition. In one aspect, the pH of the therapeutic composition is in the range from about 4 to about 10. Alternatively, the pH of the therapeutic composition is in the range from about 5 to about 9, from about 6 to about 9, or

from about 6.5 to about 8. In another embodiment, the therapeutic composition includes a buffer having a pH in one of said pH ranges. In another embodiment, the therapeutic composition has a pH of about 7. Alternatively, the therapeutic composition has a pH in a range from about 6.8 to about 7.4. In still another embodiment, the therapeutic composition has a pH of about 7.4.

[0084] This disclosure also provides the use of a pharmaceutically acceptable cell culture medium in particular compositions and/or cultures of cells as described herein. Such compositions are suitable for administration to human subjects. Generally speaking, any medium that supports the maintenance, growth, and/or health of the iPSC-derived immune cells are suitable for use as a pharmaceutical cell culture medium. In particular embodiments, the pharmaceutically acceptable cell culture medium is a serum free, and/or feeder-free medium. In various embodiments, the serum-free medium is animal-free, and can optionally be protein-free. Optionally, the medium can contain biopharmaceutically acceptable recombinant proteins. Animal-free medium refers to medium wherein the components are derived from non-animal sources. Recombinant proteins replace native animal proteins in animal-free medium and the nutrients are obtained from synthetic, plant or microbial sources. Protein-free medium, in contrast, is defined as substantially free of protein. One having ordinary skill in the art would appreciate that the above examples of media are illustrative and in no way limit the formulation of suitable media suitable and that there are many alternative suitable media known and available to those in the art.

[0085] In the preceding description and following claims, the term “and/or” means one or all of the listed elements or a combination of any two or more of the listed elements; the terms “comprises,” “comprising,” and variations thereof are to be construed as open ended—i.e., additional elements or steps are optional and may or may not be present; unless otherwise specified, “a,” “an,” “the,” and “at least one” are used interchangeably and mean one or more than one; and the recitations of numerical ranges by endpoints include all numbers subsumed within that range (e.g., 1 to 5 includes 1, 1.5, 2, 2.75, 3, 3.80, 4, 5, etc.).

[0086] In the preceding description, particular embodiments may be described in isolation for clarity. Unless otherwise expressly specified that the features of a particular embodiment are incompatible with the features of another embodiment, certain embodiments can include a combination of compatible features described herein in connection with one or more embodiments.

[0087] For any method disclosed herein that includes discrete steps, the steps may be conducted in any feasible order. And, as appropriate, any combination of two or more steps may be conducted simultaneously.

[0088] The present invention is illustrated by the following examples. It is to be understood that the particular examples, materials, amounts, and procedures are to be interpreted broadly in accordance with the scope and spirit of the invention as set forth herein.

EXAMPLES

Example 1

Generation of Human CD64/16A Expression Constructs

[0089] Total RNA was isolated from peripheral blood leukocytes (PBL) using TRIzol total RNA isolation reagent

(Invitrogen, Thermo Fisher Scientific, Carlsbad, Calif.). Human PBL cDNA was synthesized with the SuperScript Preamplification System (Invitrogen, Thermo Fisher Scientific, Carlsbad, Calif.). The CD64/16A includes a human CD64 extracellular domain (CD64-EC) and CD16A transmembrane and cytoplasmic domains (CD16A-TM-CY). The chimeric construct was generated by overlapping PCR to create EcoR I-flanked RT-PCR products of chimeric cDNA. CD64-EC cDNA fragment (885 bps) was amplified from human PBL cDNA using the forward primer 5'-CGG GAATTC GGA GAC AAC ATG TGG TTC TTG ACA A-3' (SEQ ID NO:28) and reverse primer 5'-TTG GTA CCC AGG TGG AAA GAA GCC AAG CAC TTG AAG CTC CAA-3' (SEQ ID NO:29). CD16A-TM-CY cDNA fragment (195 bps) was amplified from human PBL cDNA using the forward primer 5'-TTG GAG CTT CAA GTG CTT GGC TTC TTT CCA CCT GGG TAC CAA-3' (SEQ ID NO:30) and reverse primer 5'-CCG GAATTC TCA TTT GTC TTG AGG GTC CTT TCT-3' (SEQ ID NO:31). The PCR fragments of CD64-EC cDNA and CD16A-TM-CY cDNA were purified with QIAquick gel extraction kit (Qiagen, Hilden, Germany) and mixed together with the forward primer 5'-CGG GAATTC GGA GAC AAC ATG TGG TTC TTG ACA A-3' (SEQ ID NO:32) and the reverse primer 5'-CCG GAATTC TCA TTT GTC TTG AGG GTC CTT TCT-3' (SEQ ID NO:33). For all primers listed above, underlined nucleotides are the EcoR I cutting site to generate RT-PCR products for the chimeric receptor. RT-PCR was performed with Pfx50 DNA polymerase (Invitrogen, Thermo Fisher Scientific, Carlsbad, Calif.). The resultant CD64/CD16a cDNA was inserted into retrovirus expression vector pBMN-IRES-EGFP (Addgene, Cambridge, Mass.) and pKT2 Sleeping Beauty transposon vector (Jing et al. 2015. *PLoS One* 10:e0121788; Hermanson et al. 2016. *Stem Cells* 34:93-101). The sequences of all cloned constructs were confirmed by direct sequencing from both directions on an ABI 377 sequencer with ABI BigDye terminator cycle sequencing kit (Applied Biosystems, Thermo Fisher Scientific, Foster City, Calif.).

Stable Expression of CD64/16A in NK Cells

[0090] NK92 cells, a human NK cell line that is deficient for endogenous CD16A expression, were stably transduced with pBMN-IRES-EGFP retrovirus expression constructs containing CD64/16A or wildtype CD16A cDNA using retrovirus infection procedures described previously (Jing et al. 2015. *PLoS One* 10:e0121788). Human iPSCs (UCBiPS7, derived from umbilical cord blood CD34 cells) were stably transduced with the CD64/16A-pKT2 expression construct using a Sleeping Beauty transposon system, as previously described (Jing et al. 2015. *PLoS One* 10:e0121788). The transduced iPSC cells were differentiated into hematopoietic cells then mature NK cell as previously described (Jing et al. 2015. *PLoS One* 10:e0121788). eGFP fluorescence and surface expressions of CD64, CD16, and various NK cell phenotypic markers were determined using flow cytometry analysis.

Example 2

Antibodies

[0091] All mAbs to human hematopoietic and leukocyte phenotypic markers are described in Table 2. All isotype-

matched negative control mAbs were purchased from BioLegend (San Diego, Calif.). APC-conjugated F(ab')₂ donkey anti-human or goat anti-mouse IgG (H+L) were purchased from Jackson ImmunoResearch Laboratories (West Grove, Pa.). The human IgG1 mAbs trastuzumab/Herceptin and rituximab/Rituxan, manufactured by Genentech (South San Francisco, Calif.), and cetuximab/Erlbitux, manufactured by Bristol-Myers Squibb (Lawrence, N.J.), were purchased through the University of Minnesota Boyston Pharmacy. Recombinant human L-selectin/IgG1 Fc chimera was purchased from R&D Systems (Minneapolis, Minn.).

Cells

[0093] This study was carried out in accordance with and approved by the Institutional Review Board at the University of Minnesota. All subjects gave written informed consent in accordance with the Declaration of Helsinki. Fresh human peripheral blood leukocytes from plateletpheresis were obtained from Innovative Blood Resources (St. Paul, Minn.). Peripheral blood mononuclear cells were further enriched using Ficoll-Paque Plus (GE Healthcare Bio-Sciences AB, Uppsala, Sweden) and NK cells were purified by negative depletion using an EasySep human NK cell kit

TABLE 2

Antibodies			
Antigen	Clone	Fluorophore	Company
CD56	HCD56	PE-CY7	BioLegend, San Diego, CA
CD3	HIT3a	PE	BioLegend
CD16	3G8	APC	BioLegend
CD16	3G8	none	Ancell, Bayport, MN
CD7	CD7-6B7	PE/CY5	BioLegend
CD336/NKp44	P44-8	APC	BioLegend
CD335/NKp46	9E2	APC	BioLegend
CD159a/NKG2A	Z199	APC	Beckman Coulter, Brea, CA
CD314/NKG2D	1D11	PerCP/Cy5.5	BioLegend
CD158a/KIR2DL1	HP-MA4	PE	BioLegend
CD158b1/KIR2DL2/L3	DX27	PE	BioLegend
CD158e1/KIR3DL1	DX9	PE	BioLegend
CD94	DX22	PE	BioLegend
CD64	10.1	APC	BioLegend
CD64	10.1	none	Ancell
CD34	561	PE	BioLegend
CD45	2D1	APC	BioLegend
CD43	CD43-10G7	APC	BioLegend
CD62L/L-selectin	LAM1-116	APC	Ancell

Generation of Human CD64/16A

[0092] Total RNA was isolated from human peripheral blood leukocytes using TRIzol total RNA isolation reagent (Invitrogen, Carlsbad, Calif.) and cDNA was synthesized with the SuperScript preamplification system (Invitrogen, Carlsbad, Calif.). The recombinant CD64/16A is comprised of human CD64 extracellular domain and CD16A transmembrane and cytoplasmic domains. PCR fragments for CD64 (885 bps) and CD16A (195 bps) were amplified from the generated cDNA. The PCR fragments were purified and mixed together with the forward primer 5'-CGG GAATTC GGA GAC AAC ATG TGG TTC TTG ACA A-3' (SEQ ID NO:28), the reverse primer 5'-CCG GAATTC TCA TTT GTC TTG AGG GTC CTT TCT-3' (SEQ ID NO: 31), and Pfx50 DNA polymerase (Invitrogen) to generate the recombinant CD64/16A receptor. For both primers, underlined nucleotides are EcoR I sites. CD64/CD16A and CD16A cDNA (CD16A-176V variant) was inserted into the retroviral expression vector pBMN-IRES-EGFP and virus was generated for NK92 cell transduction, as previously described (Jing et al., 2015. *PLoS One* 10(3):e0121788). Additionally, CD64/CD16A cDNA was inserted into the pKT2 sleeping beauty transposon vector and used along with SB100X transposase for iPSC transduction, as previously described (Jing et al., 2015. *PLoS One* 10(3): e0121788). The nucleotide sequences of all constructs were confirmed by direct sequencing from both directions on an ABI 377 sequencer with ABI BigDye terminator cycle sequencing kit (Applied Biosystems, Foster City, Calif.).

(StemCell Technologies, Cambridge, Mass.), as per the manufacturer's instructions, with >95% viability and 90-95% enrichment of CD56⁺CD3⁻ lymphocytes. Viable cell counting was performed using a Countess II automated cell counter (Life Technologies Corporation, Bothell, Wash.). The human NK cell line NK92 and the ovarian cancer cell line SKOV-3 were obtained from ATCC (Manassas, Va.) and cultured per the manufacturer's directions. The NK92 cells required IL-2 for growth (500 IU/ml), which was obtained from R&D Systems (Minneapolis, Minn.). For all assays described below, cells were used when in log growth phase.

[0094] The iPSCs UCBiPS7, derived from umbilical cord blood CD34 cells, have been previously characterized and were cultured and differentiated into hematopoietic progenitor cells as described with some modifications (Jing et al., 2015. *PLoS One* 10:e0121788; Ni et al., 2013. *Methods in molecular biology* 1029:33-41; Knorr et al., 2013. *Stem Cells Transl Med* 2(4):274-283; Ni et al., 2014. *Stem Cells* 32(4):1021-1031; Hermanson et al., 2016. *Stem Cells* 34(1): 93-101). iPSCs culture and hematopoietic differentiation was performed using TeSR-E8 medium and a STEMdiff Hematopoietic Kit (StemCell Technologies, Cambridge, Mass.), which did not require the use of mouse embryonic fibroblast feeder cells, TrypLE adaption, spin embryoid body formation, or CD34⁺ cell enrichment. iPSC cells during passage were dissociated with Gentle Cell Dissociation Reagent (StemCell Technologies, Cambridge, Mass.), and iPSC aggregates ≥50 μm in diameter were counted with

a hemocytometer and diluted to 20-40 aggregates/ml with TeSR-E8 medium. Each well of a 12-well plate was pre-coated with Matrigel Matrix (Corning Inc., Tewksbury, Mass.) and seeded with 40-80 aggregates in 2 ml of TeSR-E8 medium. Cell aggregates were cultured for 24 hours before differentiation with the STEMdiff Hematopoietic Kit (StemCell Technologies, Cambridge, Mass.) according to the manufacturer's instructions. At day 12 of hematopoietic progenitor cell differentiation, the percentage of hematopoietic progenitor cells was determined using flow cytometric analysis with anti-CD34, anti-CD45, and anti-CD43 mAbs. NK cell differentiation was performed as previously described (Woll et al., 2009. *Blood* 113(24):6094-6101). The iPSC-derived NK cells (referred to here as iNK cells) were expanded for examination using γ -irradiated K562-mbIL21-41BBL feeder cells (1:2 ratio) in cell expansion medium [60% DMEM, 30% Ham's F12, 10% human AB serum (Valley Biomedical, Winchester, Va.), 20 μ M 2-mercaptoethanol, 50 μ M ethanolamine, 20 μ g/ml ascorbic Acid, 5 ng/ml sodium selenite, 10 mM HEPES, and 100-250 IU/ml IL-2 (R&D Systems)], as described previously (Jing et al., 2015. *PLoS One* 10:e0121788; Knorr et al., 2013. *Stem Cells Transl Med* 2(4):274-283; Ni et al., 2014. *Stem Cells* 32(4):1021-1031; Hermanson et al., 2016. *Stem Cells* 34(1):93-101).

Cell Staining, Flow Cytometry, and ELISA

[0095] For cell staining, nonspecific antibody binding sites were blocked and cells were stained with the indicated antibodies and examined by flow cytometry, as previously described (Romee et al., 2013. *Blood* 121(18):3599-3608; Jing et al., 2015. *PLoS One* 10:e0121788; Mishra et al., 2018. *Cancer Immunol Immunother* 67(9):1407-1416). For controls, fluorescence minus one was used as well as appropriate isotype-matched antibodies since the cells of interest expressed FcRs. An FSC-A/SSC-A plot was used to set an electronic gate on leukocyte populations and an FSC-A/F SC-H plot was used to set an electronic gate on single cells. A Zombie viability kit was used to assess live vs. dead cells, as per the manufacturer's instructions (BioLegend, San Diego, Calif.).

[0096] To assess the capture of soluble trastuzumab, rituximab, cetuximab, or L-selectin/Fc chimera, transduced NK cells were incubated with 5 μ g/ml of antibody for two hours at 37° C. in MEM- α basal media (Thermo Fisher Scientific, Waltham, Mass.) supplemented with IL-2 (200 IU/ml), HEPES (10 mM), and 2-mercaptoethanol (0.1 mM), washed with MEM- α basal media, and then stained on ice for 30 minutes with a 1:200 dilution of APC-conjugated F(ab')2 donkey anti-human IgG (H+L). To detect recombinant human L-selectin/Fc binding, cells were stained with the anti-L-selectin mAb APC-conjugated Lam1-116.

[0097] To compare CD16A, CD64, and CD64/16A staining levels on NK92 cells, the respective transductants were stained with a saturating concentration of unconjugated anti-CD16 (3G8) or anti-CD64 (10.1) mAbs (5 μ g/ml), washed extensively with dPBS (USB Corporation, Cleveland, Ohio) containing 2% goat serum and 2 mM sodium azide, and then stained with APC-conjugated F(ab')2 goat anti-mouse IgG (H+L). This approach was used since directly conjugated anti-CD16 and anti-CD64 mAbs can vary in their levels of fluorophore labeling. ELISA was performed by a cytometric bead-based Flex Set assay for human IFN γ (BD Biosciences, San Jose, Calif.) per the

manufacturer's instructions. All flow cytometric analyses were performed on a FACSCelesta instrument (BD Biosciences). Data was analyzed using FACSDIVA v8 (BD Biosciences) and FlowJo v10 (Ashland, Oreg.).

Cell-Cell Conjugation Assay and ADCC

[0098] The pBMN-IRES-EGFP vector used to express CD64/16A in NK92 cells also expresses eGFP. These cells were incubated for two hours at 37° C. in MEM- α basal media (Thermo Fisher Scientific, Waltham, Mass.) supplemented with IL-2 (200 IU/ml), HEPES (10 mM), and 2-mercaptoethanol (0.1 mM). SKOV-3 cells were labeled with CellTrace Violet (Molecular Probes, Eugene, Oreg.) per the manufacturer's instructions, incubated with 5 μ g/ml trastuzumab for 30 minutes and washed with the MEM- α basal media. NK92 cells and SKOV-3 cells were then resuspended in the supplemented MEM- α basal media at 1 \times 10 6 and 2 \times 10 6 /ml, respectively. For a 1:2 E:T ratio, 100 μ l of each cell type was mixed together, centrifuged for one minute at 20 \times g and incubated at 37° C. for the indicated time points. After each time point, the cells were gently vortexed for three seconds and immediately fixed with 4° C. 1% paraformaldehyde in dPBS. Samples were immediately analyzed by flow cytometry. The percentage of conjugated NK cells was calculated by gating on eGFP and CellTrace Violet double-positive events.

[0099] To evaluate ADCC, a DELFIA EuTDA-based cytotoxicity assay was used according to the manufacturer's instructions (PerkinElmer, Waltham, Mass.). Briefly, target cells were labeled with Bis(acetoxyethyl)-2-2:6,2 terpyridine 6,6 dicarboxylate (BATDA) for 30 minutes in their culture medium, washed in culture medium, and pipetted into a 96-well non-tissue culture-treated U-bottom plates at a density of 8 \times 10 4 cells/well. A tumor targeting mAb was added at the indicated concentrations of 5 μ g/mL and NK cells were added at the indicated effector:target (E:T) ratios. The plates were centrifuged at 400 \times g for one minute and then incubated for two hours in a humidified 5% CO₂ atmosphere at 37° C. At the end of the incubation, the plates were centrifuged at 500 \times g for five minutes and supernatants were transferred to a 96-well DELFIA Yellow Plate (PerkinElmer, Waltham, Mass.) and combined with europium. Fluorescence was measured by time-resolved fluorometry using a BMG Labtech CLARIOstar plate reader (Cary, N.C.). BATDA-labeled target cells alone with or without therapeutic antibodies were cultured in parallel to assess spontaneous lysis and in the presence of 2% Triton-X to measure maximum lysis. The level of ADCC for each sample was calculated using the formula: Percent Specific Release=(Experimental release counts-Spontaneous release counts)/(Maximal release-Spontaneous release counts) \times 100%. For each experiment, measurements were conducted in triplicate using three replicate wells.

Statistical Analyses

[0100] Statistical analyses were performed by use of GraphPad Prism (GraphPad Software, La Jolla, Calif., USA). After assessing for approximate normal distribution, all variables were summarized as mean \pm SD. Comparison between two groups was done with Student's t-test, with p<0.05 taken as statistically significant.

[0101] The complete disclosure of all patents, patent applications, and publications, and electronically available

material (including, for instance, nucleotide sequence submissions in, e.g., GenBank and RefSeq, and amino acid sequence submissions in, e.g., SwissProt, PIR, PRF, PDB, and translations from annotated coding regions in GenBank and RefSeq) cited herein are incorporated by reference in their entirety. In the event that any inconsistency exists between the disclosure of the present application and the disclosure(s) of any document incorporated herein by reference, the disclosure of the present application shall govern. The foregoing detailed description and examples have been given for clarity of understanding only. No unnecessary limitations are to be understood therefrom. The invention is not limited to the exact details shown and described, for variations obvious to one skilled in the art will be included within the invention defined by the claims.

[0102] Unless otherwise indicated, all numbers expressing quantities of components, molecular weights, and so forth used in the specification and claims are to be understood as being modified in all instances by the term "about." Accord-

ingly, unless otherwise indicated to the contrary, the numerical parameters set forth in the specification and claims are approximations that may vary depending upon the desired properties sought to be obtained by the present invention. At the very least, and not as an attempt to limit the doctrine of equivalents to the scope of the claims, each numerical parameter should at least be construed in light of the number of reported significant digits and by applying ordinary rounding techniques.

[0103] Notwithstanding that the numerical ranges and parameters setting forth the broad scope of the invention are approximations, the numerical values set forth in the specific examples are reported as precisely as possible. All numerical values, however, inherently contain a range necessarily resulting from the standard deviation found in their respective testing measurements.

[0104] All headings are for the convenience of the reader and should not be used to limit the meaning of the text that follows the heading, unless so specified.

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Asp Lys Leu Val Tyr Asn Val Leu Tyr Tyr Arg Asn Gly Lys Ala Phe
130 135 140

Lys Phe Phe His Trp Asn Ser Asn Leu Thr Ile Leu Lys Thr Asn Ile
145 150 155 160

Ser His Asn Gly Thr Tyr His Cys Ser Gly Met Gly Lys His Arg Tyr
165 170 175

Thr Ser Ala Gly Ile Ser Val Thr Val Lys Glu Leu Phe Pro Ala Pro
180 185 190

Val Leu Asn Ala Ser Val Thr Ser Pro Leu Leu Glu Gly Asn Leu Val
195 200 205

Thr Leu Ser Cys Glu Thr Lys Leu Leu Gln Arg Pro Gly Leu Gln
210 215 220

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Leu Tyr Phe Ser Phe Tyr Met Gly Ser Lys Thr Leu Arg Gly Arg Asn
 225 230 235 240
 Thr Ser Ser Glu Tyr Gln Ile Leu Thr Ala Arg Arg Glu Asp Ser Gly
 245 250 255
 Leu Tyr Trp Cys Glu Ala Ala Thr Glu Asp Gly Asn Val Leu Lys Arg
 260 265 270
 Ser Pro Glu Leu Glu Leu Gln Val Leu Gly Leu Gln Leu Pro Thr Pro
 275 280 285
 Val Trp Phe His Val Leu Phe Tyr Leu Ala Val Gly Ile Met Phe Leu
 290 295 300
 Val Asn Thr Val Leu Trp Val Thr Ile Arg Lys Glu Leu Lys Arg Lys
 305 310 315 320
 Lys Lys Trp Asp Leu Glu Ile Ser Leu Asp Ser Gly His Glu Lys Lys
 325 330 335
 Val Thr Ser Ser Leu Gln Glu Asp Arg His Leu Glu Glu Leu Lys
 340 345 350
 Cys Gln Glu Gln Lys Glu Glu Gln Leu Gln Glu Gly Val His Arg Lys
 355 360 365
 Glu Pro Gln Gly Ala Thr
 370

<210> SEQ_ID NO 3
 <211> LENGTH: 449
 <212> TYPE: PRT
 <213> ORGANISM: Artificial
 <220> FEATURE:
 <223> OTHER INFORMATION: Polypeptide
 <400> SEQUENCE: 3

Met Trp Gln Leu Leu Pro Thr Ala Leu Leu Leu Val Ser Ala
 1 5 10 15
 Gly Met Arg Thr Glu Asp Leu Pro Lys Ala Val Val Phe Leu Glu Pro
 20 25 30
 Gln Trp Tyr Arg Val Leu Glu Lys Asp Ser Val Thr Leu Lys Cys Gln
 35 40 45
 Gly Ala Tyr Ser Pro Glu Asp Asn Ser Thr Gln Trp Phe His Asn Glu
 50 55 60
 Ser Leu Ile Ser Ser Gln Ala Ser Ser Tyr Phe Ile Asp Ala Ala Thr
 65 70 75 80
 Val Asp Asp Ser Gly Glu Tyr Arg Cys Gln Thr Asn Leu Ser Thr Leu
 85 90 95
 Ser Asp Pro Val Gln Leu Glu Val His Ile Gly Trp Leu Leu Gln
 100 105 110
 Ala Pro Arg Trp Val Phe Lys Glu Asp Pro Ile His Leu Arg Cys
 115 120 125
 His Ser Trp Lys Asn Thr Ala Leu His Lys Val Thr Tyr Leu Gln Asn
 130 135 140
 Gly Lys Gly Arg Lys Tyr Phe His His Asn Ser Asp Phe Tyr Ile Pro
 145 150 155 160
 Lys Ala Thr Leu Lys Asp Ser Gly Ser Tyr Phe Cys Arg Gly Leu Phe
 165 170 175
 Gly Ser Lys Asn Val Ser Ser Glu Thr Val Asn Ile Thr Ile Thr Gln
 180 185 190

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Gly Leu Ala Val Ser Thr Ile Ser Ser Phe Phe Pro Pro Gly Tyr Gln
195 200 205

Val Ser Phe Cys Leu Val Met Val Leu Leu Phe Ala Val Asp Thr Gly
210 215 220

Leu Tyr Phe Ser Val Lys Thr Asn Ile Arg Ser Ser Thr Arg Asp Trp
225 230 235 240

Lys Asp His Lys Phe Lys Trp Arg Lys Asp Pro Gln Asp Lys Arg Ser
245 250 255

Lys Arg Ser Arg Leu Leu His Ser Asp Tyr Met Asn Met Thr Pro Arg
260 265 270

Arg Pro Gly Pro Thr Arg Lys His Tyr Gln Pro Tyr Ala Pro Pro Arg
275 280 285

Asp Phe Ala Ala Tyr Arg Ser Lys Arg Gly Arg Lys Lys Leu Leu Tyr
290 295 300

Ile Phe Lys Gln Pro Phe Met Arg Pro Val Gln Thr Thr Gln Glu Glu
305 310 315 320

Asp Gly Cys Ser Cys Arg Phe Pro Glu Glu Glu Gly Cys Glu
325 330 335

Leu Arg Val Lys Phe Ser Arg Ser Ala Asp Ala Pro Ala Tyr Gln Gln
340 345 350

Gly Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu Gly Arg Arg Glu Glu
355 360 365

Tyr Asp Val Leu Asp Lys Arg Arg Gly Arg Asp Pro Glu Met Gly Gly
370 375 380

Lys Pro Arg Arg Lys Asn Pro Gln Glu Gly Leu Tyr Asn Glu Leu Gln
385 390 395 400

Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile Gly Met Lys Gly Glu
405 410 415

Arg Arg Arg Gly Lys Gly His Asp Gly Leu Tyr Gln Gly Leu Ser Thr
420 425 430

Ala Thr Lys Asp Thr Tyr Asp Ala Leu His Met Gln Ala Leu Pro Pro
435 440 445

Arg

<210> SEQ_ID NO 4
<211> LENGTH: 408
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Polypeptide

<400> SEQUENCE: 4

Met Trp Gln Leu Leu Pro Thr Ala Leu Leu Leu Val Ser Ala
1 5 10 15

Gly Met Arg Thr Glu Asp Leu Pro Lys Ala Val Val Phe Leu Glu Pro
20 25 30

Gln Trp Tyr Arg Val Leu Glu Lys Asp Ser Val Thr Leu Lys Cys Gln
35 40 45

Gly Ala Tyr Ser Pro Glu Asp Asn Ser Thr Gln Trp Phe His Asn Glu
50 55 60

Ser Leu Ile Ser Ser Gln Ala Ser Ser Tyr Phe Ile Asp Ala Ala Thr
65 70 75 80

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Val	Asp	Asp	Ser	Gly	Glu	Tyr	Arg	Cys	Gln	Thr	Asn	Leu	Ser	Thr	Leu
85									90						95
<hr/>															
Ser	Asp	Pro	Val	Gln	Leu	Glu	Val	His	Ile	Gly	Trp	Leu	Leu	Leu	Gln
100									105						110
<hr/>															
Ala	Pro	Arg	Trp	Val	Phe	Lys	Glu	Asp	Pro	Ile	His	Leu	Arg	Cys	
115						120								125	
<hr/>															
His	Ser	Trp	Lys	Asn	Thr	Ala	Leu	His	Lys	Val	Thr	Tyr	Leu	Gln	Asn
130						135								140	
<hr/>															
Gly	Lys	Gly	Arg	Lys	Tyr	Phe	His	His	Asn	Ser	Asp	Phe	Tyr	Ile	Pro
145					150				155						160
<hr/>															
Lys	Ala	Thr	Leu	Lys	Asp	Ser	Gly	Ser	Tyr	Phe	Cys	Arg	Gly	Leu	Phe
165						170								175	
<hr/>															
Gly	Ser	Lys	Asn	Val	Ser	Ser	Glu	Thr	Val	Asn	Ile	Thr	Ile	Thr	Gln
180						185								190	
<hr/>															
Gly	Leu	Ala	Val	Ser	Thr	Ile	Ser	Ser	Phe	Phe	Pro	Pro	Gly	Tyr	Gln
195						200								205	
<hr/>															
Val	Ser	Phe	Cys	Leu	Val	Met	Val	Leu	Leu	Phe	Ala	Val	Asp	Thr	Gly
210						215								220	
<hr/>															
Leu	Tyr	Phe	Ser	Val	Lys	Thr	Asn	Ile	Arg	Ser	Ser	Thr	Arg	Asp	Trp
225					230				235						240
<hr/>															
Lys	Asp	His	Lys	Phe	Lys	Trp	Arg	Lys	Asp	Pro	Gln	Asp	Lys	Lys	Arg
245						250								255	
<hr/>															
Gly	Arg	Lys	Lys	Leu	Leu	Tyr	Ile	Phe	Lys	Gln	Pro	Phe	Met	Arg	Pro
260						265								270	
<hr/>															
Val	Gln	Thr	Thr	Gln	Glu	Glu	Asp	Gly	Cys	Ser	Cys	Arg	Phe	Pro	Glu
275						280								285	
<hr/>															
Glu	Glu	Glu	Gly	Cys	Glu	Leu	Arg	Val	Lys	Phe	Ser	Arg	Ser	Ala	
290						295								300	
<hr/>															
Asp	Ala	Pro	Ala	Tyr	Gln	Gln	Gly	Gln	Asn	Gln	Leu	Tyr	Asn	Glu	Leu
305						310								320	
<hr/>															
Asn	Leu	Gly	Arg	Arg	Glu	Glu	Tyr	Asp	Val	Leu	Asp	Lys	Arg	Arg	Gly
325								330						335	
<hr/>															
Arg	Asp	Pro	Glu	Met	Gly	Gly	Lys	Pro	Arg	Arg	Lys	Asn	Pro	Gln	Glu
340								345						350	
<hr/>															
Gly	Leu	Tyr	Asn	Glu	Leu	Gln	Lys	Asp	Lys	Met	Ala	Glu	Ala	Tyr	Ser
355						360								365	
<hr/>															
Glu	Ile	Gly	Met	Lys	Gly	Glu	Arg	Arg	Gly	Lys	Gly	His	Asp	Gly	
370						375								380	
<hr/>															
Leu	Tyr	Gln	Gly	Leu	Ser	Thr	Ala	Thr	Lys	Asp	Thr	Tyr	Asp	Ala	Leu
385						390								400	
<hr/>															
His	Met	Gln	Ala	Leu	Pro	Pro	Arg								
						405									

<210> SEQ ID NO 5

<211> LENGTH: 248

<212> TYPE: PRT

<213> ORGANISM: Canis lupus familiaris

<400> SEQUENCE: 5

Met	Trp	Gln	Leu	Val	Ser	Ser	Thr	Ala	Leu	Leu	Leu	Val	Ser	Ala
1									5					15

Gly	Thr	Gln	Ala	Asp	Val	Pro	Lys	Ala	Val	Val	Val	Leu	Glu	Pro	Lys
									20				25		30

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Trp Asn Arg Val Leu Thr Met Asp Ser Val Thr Leu Lys Cys Gln Gly
35 40 45

Asp His Leu Leu Arg Asp Asn Tyr Thr Trp Leu His Asn Gly Arg Pro
50 55 60

Ile Ser Asn Gln Ile Ser Thr Tyr Ile Ile Lys Asn Ala Ser Ile Lys
65 70 75 80

Asn Ser Gly Glu Tyr Arg Cys Gln Thr Asp Gln Ser Lys Leu Ser Asp
85 90 95

Pro Val Gln Leu Glu Val His Thr Gly Trp Leu Leu Gln Val Pro
100 105 110

Arg Leu Val Phe Gln Glu Gly Glu Leu Ile Gln Leu Lys Cys His Ser
115 120 125

Trp Lys Asn Thr Pro Val Arg Asn Val Gln Tyr Phe Gln Asn Gly Arg
130 135 140

Gly Lys Lys Phe Phe Tyr Asn Asn Ser Glu Tyr His Ile Pro Ala Ala
145 150 155 160

Thr Ser Glu His Asn Gly Ser Tyr Phe Cys Arg Gly Ile Ile Gly Lys
165 170 175

Lys Asn Glu Ser Ser Glu Ala Val Asn Ile Ile Ile Gln Gly Ser Ser
180 185 190

Leu Pro Ser Thr Ser Leu Leu Ser His Trp Pro Gln Ile Pro Phe
195 200 205

Ser Leu Val Met Ala Leu Leu Phe Ala Val Asp Thr Gly Leu Tyr Phe
210 215 220

Ala Val Gln Arg Asp Leu Arg Ser Ser Met Gly Asn Leu Lys Asn Ser
225 230 235 240

Lys Val Ile Trp Ser Gln Gly Ser
245

<210> SEQ_ID NO 6
<211> LENGTH: 254
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 6

Met Trp Gln Leu Leu Leu Pro Thr Ala Leu Leu Leu Val Ser Ala
1 5 10 15

Gly Met Arg Thr Glu Asp Leu Pro Lys Ala Val Val Phe Leu Glu Pro
20 25 30

Gln Trp Tyr Arg Val Leu Glu Lys Asp Ser Val Thr Leu Lys Cys Gln
35 40 45

Gly Ala Tyr Ser Pro Glu Asp Asn Ser Thr Gln Trp Phe His Asn Glu
50 55 60

Ser Leu Ile Ser Ser Gln Ala Ser Ser Tyr Phe Ile Asp Ala Ala Thr
65 70 75 80

Val Asp Asp Ser Gly Glu Tyr Arg Cys Gln Thr Asn Leu Ser Thr Leu
85 90 95

Ser Asp Pro Val Gln Leu Glu Val His Ile Gly Trp Leu Leu Gln
100 105 110

Ala Pro Arg Trp Val Phe Lys Glu Asp Pro Ile His Leu Arg Cys
115 120 125

His Ser Trp Lys Asn Thr Ala Leu His Lys Val Thr Tyr Leu Gln Asn

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130	135	140
Gly Lys Gly Arg Lys Tyr His His Asn Ser Asp Phe Tyr Ile Pro		
145 150 155 160		
Lys Ala Thr Leu Lys Asp Ser Gly Ser Tyr Phe Cys Arg Gly Leu Phe		
165 170 175		
Gly Ser Lys Asn Val Ser Ser Glu Thr Val Asn Ile Thr Ile Thr Gln		
180 185 190		
Gly Leu Ala Val Ser Thr Ile Ser Ser Phe Phe Pro Pro Gly Tyr Gln		
195 200 205		
Val Ser Phe Cys Leu Val Met Val Leu Leu Phe Ala Val Asp Thr Gly		
210 215 220		
Leu Tyr Phe Ser Val Lys Thr Asn Ile Arg Ser Ser Thr Arg Asp Trp		
225 230 235 240		
Lys Asp His Lys Phe Lys Trp Arg Lys Asp Pro Gln Asp Lys		
245 250		
 <210> SEQ_ID NO 7		
<211> LENGTH: 372		
<212> TYPE: PRT		
<213> ORGANISM: Canis lupus familiaris		
 <400> SEQUENCE: 7		
Met Trp Leu Leu Thr Val Leu Leu Trp Val Pro Ala Gly Ala Gln		
1 5 10 15		
Thr Asp Pro Val Lys Ala Val Ile Thr Leu Gln Pro Pro Trp Val Ser		
20 25 30		
Val Phe Gln Glu Glu Ser Val Thr Leu Trp Cys Glu Gly Pro His Leu		
35 40 45		
Pro Gly Asp Ser Ser Thr Gln Trp Phe Leu Asn Gly Thr Ala Thr Gln		
50 55 60		
Thr Leu Thr Pro Arg Tyr Arg Ile Ala Ala Ser Val Asn Asp Asn		
65 70 75 80		
Gly Glu Tyr Arg Cys Gln Thr Gly Leu Ser Val Leu Ser Asp Pro Ile		
85 90 95		
Gln Leu Gly Ile His Arg Asp Trp Leu Ile Leu Gln Val Ser Gly Arg		
100 105 110		
Val Phe Thr Glu Gly Glu Pro Leu Thr Leu Arg Cys His Gly Trp Asn		
115 120 125		
Asn Lys Leu Val Tyr Asn Val Leu Phe Tyr Gln Asn Gly Thr Val Leu		
130 135 140		
Lys Phe Ser Pro Gln Asn Ser Glu Phe Thr Ile Leu Lys Thr Thr Leu		
145 150 155 160		
His His Asn Gly Ile Tyr His Cys Ser Ala Met Gly Lys His Arg Tyr		
165 170 175		
Glu Ser Ala Gly Val Ser Ile Thr Ile Lys Glu Leu Phe Pro Ala Pro		
180 185 190		
Val Leu Lys Ala Ser Leu Ser Ser Pro Ile Leu Glu Gly His Val Val		
195 200 205		
Asn Leu Ser Cys Glu Thr Lys Leu Leu Leu Gln Arg Pro Gly Leu Gln		
210 215 220		
Leu Tyr Phe Ser Phe Tyr Met Gly Ser Lys Thr Leu Leu Ser Arg Asn		
225 230 235 240		

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Thr	Ser	Ser	Glu	Tyr	Gln	Ile	Leu	Thr	Ala	Lys	Lys	Glu	Asp	Ser	Gly
245								250							255
Leu	Tyr	Trp	Cys	Glu	Ala	Thr	Thr	Glu	Asp	Gly	Asn	Val	Val	Lys	Arg
	260							265							270
Ser	Pro	Glu	Leu	Glu	Leu	Gln	Val	Val	Gly	Pro	Gln	Thr	Leu	Thr	Pro
	275						280								285
Val	Trp	Phe	His	Val	Leu	Phe	Tyr	Val	Ala	Met	Gly	Met	Ile	Phe	Leu
	290					295				300					
Val	Asp	Thr	Ile	Phe	Cys	Met	Ile	Ile	His	Lys	Glu	Leu	Gln	Arg	Lys
305					310				315						320
Lys	Lys	Trp	Asn	Leu	Glu	Ile	Ser	Leu	Tyr	Ser	Gly	Leu	Glu	Lys	Arg
	325					330				335					
Val	Asp	Ser	Tyr	Leu	Gln	Lys	Glu	Arg	Asp	Leu	Glu	Glu	Pro	Lys	Tyr
	340					345				350					
Gln	Glu	Leu	Glu	Gln	Leu	Gln	Glu	Lys	Thr	Pro	Gln	Lys	Pro	Pro	Glu
	355					360				365					
Gly	Glu	Gln	Gln												
	370														

<210> SEQ_ID NO 8
<211> LENGTH: 374
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 8

Met	Trp	Phe	Leu	Thr	Thr	Leu	Leu	Leu	Trp	Val	Pro	Val	Asp	Gly	Gln
1						5			10				15		
Val	Asp	Thr	Thr	Lys	Ala	Val	Ile	Thr	Leu	Gln	Pro	Pro	Trp	Val	Ser
				20			25			30					
Val	Phe	Gln	Glu	Glu	Thr	Val	Thr	Leu	His	Cys	Glu	Val	Leu	His	Leu
					35			40			45				
Pro	Gly	Ser	Ser	Ser	Thr	Gln	Trp	Phe	Leu	Asn	Gly	Thr	Ala	Thr	Gln
					50			55			60				
Thr	Ser	Thr	Pro	Ser	Tyr	Arg	Ile	Thr	Ser	Ala	Ser	Val	Asn	Asp	Ser
					65			70			75			80	
Gly	Glu	Tyr	Arg	Cys	Gln	Arg	Gly	Leu	Ser	Gly	Arg	Ser	Asp	Pro	Ile
				85			90				95				
Gln	Leu	Glu	Ile	His	Arg	Gly	Trp	Leu	Leu	Leu	Gln	Val	Ser	Ser	Arg
				100			105				110				
Val	Phe	Thr	Glu	Gly	Glu	Pro	Leu	Ala	Leu	Arg	Cys	His	Ala	Trp	Lys
					115			120			125				
Asp	Lys	Leu	Val	Tyr	Asn	Val	Leu	Tyr	Tyr	Arg	Asn	Gly	Lys	Ala	Phe
					130			135			140				
Lys	Phe	Phe	His	Trp	Asn	Ser	Asn	Leu	Thr	Ile	Leu	Lys	Thr	Asn	Ile
					145			150			155				160
Ser	His	Asn	Gly	Thr	Tyr	His	Cys	Ser	Gly	Met	Gly	Lys	His	Arg	Tyr
					165			170			175				
Thr	Ser	Ala	Gly	Ile	Ser	Val	Thr	Val	Lys	Glu	Leu	Phe	Pro	Ala	Pro
					180			185			190				
Val	Leu	Asn	Ala	Ser	Val	Thr	Ser	Pro	Leu	Leu	Glu	Gly	Asn	Leu	Val
					195			200			205				
Thr	Leu	Ser	Cys	Glu	Thr	Lys	Leu	Leu	Gln	Arg	Pro	Gly	Leu	Gln	
					210			215			220				

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Leu Tyr Phe Ser Phe Tyr Met Gly Ser Lys Thr Leu Arg Gly Arg Asn
 225 230 235 240

 Thr Ser Ser Glu Tyr Gln Ile Leu Thr Ala Arg Arg Glu Asp Ser Gly
 245 250 255

 Leu Tyr Trp Cys Glu Ala Ala Thr Glu Asp Gly Asn Val Leu Lys Arg
 260 265 270

 Ser Pro Glu Leu Glu Leu Gln Val Leu Gly Leu Gln Leu Pro Thr Pro
 275 280 285

 Val Trp Phe His Val Leu Phe Tyr Leu Ala Val Gly Ile Met Phe Leu
 290 295 300

 Val Asn Thr Val Leu Trp Val Thr Ile Arg Lys Glu Leu Lys Arg Lys
 305 310 315 320

 Lys Lys Trp Asp Leu Glu Ile Ser Leu Asp Ser Gly His Glu Lys Lys
 325 330 335

 Val Ile Ser Ser Leu Gln Glu Asp Arg His Leu Glu Glu Leu Lys
 340 345 350

 Cys Gln Glu Gln Lys Glu Glu Gln Leu Gln Glu Gly Val His Arg Lys
 355 360 365

 Glu Pro Gln Gly Ala Thr
 370

<210> SEQ_ID NO 9
 <211> LENGTH: 374
 <212> TYPE: PRT
 <213> ORGANISM: Artificial
 <220> FEATURE:
 <223> OTHER INFORMATION: Polypeptide

<400> SEQUENCE: 9

Met Trp Phe Leu Thr Thr Leu Leu Leu Trp Val Pro Val Asp Gly Gln
 1 5 10 15

Val Asp Thr Thr Lys Ala Val Ile Thr Leu Gln Pro Pro Trp Val Ser
 20 25 30

Val Phe Gln Glu Glu Thr Val Thr Leu His Cys Glu Val Leu His Leu
 35 40 45

Pro Gly Ser Ser Ser Thr Gln Trp Phe Leu Asn Gly Thr Ala Thr Gln
 50 55 60

Thr Ser Thr Pro Ser Tyr Arg Ile Thr Ser Ala Ser Val Asn Asp Ser
 65 70 75 80

Gly Glu Tyr Arg Cys Gln Arg Gly Leu Ser Gly Arg Ser Asp Pro Ile
 85 90 95

Gln Leu Glu Ile His Arg Gly Trp Leu Leu Leu Gln Val Ser Ser Arg
 100 105 110

Val Phe Thr Glu Gly Glu Pro Leu Ala Leu Arg Cys His Ala Trp Lys
 115 120 125

Asp Lys Leu Val Tyr Asn Val Leu Tyr Tyr Arg Asn Gly Lys Ala Phe
 130 135 140

Lys Phe Phe His Trp Asn Ser Asn Leu Thr Ile Leu Lys Thr Asn Ile
 145 150 155 160

Ser His Asn Gly Thr Tyr His Cys Ser Gly Met Gly Lys His Arg Tyr
 165 170 175

Thr Ser Ala Gly Ile Ser Val Thr Val Lys Glu Leu Phe Pro Ala Pro
 180 185 190

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Val Leu Asn Ala Ser Val Thr Ser Pro Leu Leu Glu Gly Asn Leu Val
195 200 205

Thr Leu Ser Cys Glu Thr Lys Leu Leu Leu Gln Arg Pro Gly Leu Gln
210 215 220

Leu Tyr Phe Ser Phe Tyr Met Gly Ser Lys Thr Leu Arg Gly Arg Asn
225 230 235 240

Thr Ser Ser Glu Tyr Gln Ile Leu Thr Ala Arg Arg Glu Asp Ser Gly
245 250 255

Leu Tyr Trp Cys Glu Ala Ala Thr Glu Asp Gly Asn Val Leu Lys Arg
260 265 270

Ser Pro Glu Leu Glu Leu Gln Val Leu Gly Leu Gln Leu Pro Thr Pro
275 280 285

Val Trp Phe His Val Leu Phe Tyr Leu Ala Val Gly Ile Met Phe Leu
290 295 300

Val Asn Thr Val Leu Trp Val Thr Ile Arg Lys Glu Leu Lys Arg Lys
305 310 315 320

Lys Lys Trp Asp Leu Glu Ile Ser Leu Asp Ser Gly His Glu Lys Lys
325 330 335

Val Ile Ser Ser Leu Gln Glu Asp Arg His Leu Glu Glu Leu Lys
340 345 350

Cys Gln Glu Gln Lys Glu Glu Gln Leu Gln Glu Gly Val His Arg Lys
355 360 365

Glu Pro Gln Gly Ala Thr
370

<210> SEQ ID NO 10
<211> LENGTH: 374
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Polypeptide

<400> SEQUENCE: 10

Met Trp Phe Leu Thr Thr Leu Leu Trp Val Pro Val Asp Gly Gln
1 5 10 15

Val Asp Thr Thr Lys Ala Val Ile Thr Leu Gln Pro Pro Trp Val Ser
20 25 30

Val Phe Gln Glu Glu Thr Val Thr Leu His Cys Glu Val Leu His Leu
35 40 45

Pro Gly Ser Ser Ser Thr Gln Trp Phe Leu Asn Gly Thr Ala Thr Gln
50 55 60

Thr Ser Thr Pro Ser Tyr Arg Ile Thr Ser Ala Ser Val Asn Asp Ser
65 70 75 80

Gly Glu Tyr Arg Cys Gln Arg Gly Leu Ser Gly Arg Ser Asp Pro Ile
85 90 95

Gln Leu Glu Ile His Arg Gly Trp Leu Leu Leu Gln Val Ser Ser Arg
100 105 110

Val Phe Thr Glu Gly Glu Pro Leu Ala Leu Arg Cys His Ala Trp Lys
115 120 125

Asp Lys Leu Val Tyr Asn Val Leu Tyr Tyr Arg Asn Gly Lys Ala Phe
130 135 140

Lys Phe Phe His Trp Asn Ser Asn Leu Thr Ile Leu Lys Thr Asn Ile
145 150 155 160

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Ser His Asn Gly Thr Tyr His Cys Ser Gly Met Gly Lys His Arg Tyr
165 170 175

Thr Ser Ala Gly Ile Ser Val Thr Val Lys Glu Leu Phe Pro Ala Pro
180 185 190

Val Leu Asn Ala Ser Val Thr Ser Pro Leu Leu Glu Gly Asn Leu Val
195 200 205

Thr Leu Ser Cys Glu Thr Lys Leu Leu Gln Arg Pro Gly Leu Gln
210 215 220

Leu Tyr Phe Ser Phe Tyr Met Gly Ser Lys Thr Leu Arg Gly Arg Asn
225 230 235 240

Thr Ser Ser Glu Tyr Gln Ile Leu Thr Ala Arg Arg Glu Asp Ser Gly
245 250 255

Leu Tyr Trp Cys Glu Ala Ala Thr Glu Asp Gly Asn Val Leu Lys Arg
260 265 270

Ser Pro Glu Leu Glu Leu Gln Val Leu Gly Leu Gln Leu Pro Thr Pro
275 280 285

Val Trp Phe His Val Leu Phe Tyr Leu Ala Val Gly Ile Met Phe Leu
290 295 300

Val Asn Thr Val Leu Trp Val Thr Ile Arg Lys Glu Leu Lys Arg Lys
305 310 315 320

Lys Lys Trp Asn Leu Glu Ile Ser Leu Asp Ser Gly His Glu Lys Lys
325 330 335

Val Ile Ser Ser Leu Gln Glu Asp Arg His Leu Glu Glu Leu Lys
340 345 350

Cys Gln Glu Gln Lys Glu Glu Gln Leu Gln Glu Gly Val His Arg Lys
355 360 365

Glu Pro Gln Gly Ala Thr
370

<210> SEQ_ID NO 11
<211> LENGTH: 335
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Polypeptide

<400> SEQUENCE: 11

Met Trp Phe Leu Thr Thr Leu Leu Leu Trp Val Pro Val Asp Gly Gln
1 5 10 15

Val Asp Thr Thr Lys Ala Val Ile Thr Leu Gln Pro Pro Trp Val Ser
20 25 30

Val Phe Gln Glu Glu Thr Val Thr Leu His Cys Glu Val Leu His Leu
35 40 45

Pro Gly Ser Ser Ser Thr Gln Trp Phe Leu Asn Gly Thr Ala Thr Gln
50 55 60

Thr Ser Thr Pro Ser Tyr Arg Ile Thr Ser Ala Ser Val Asn Asp Ser
65 70 75 80

Gly Glu Tyr Arg Cys Gln Arg Gly Leu Ser Gly Arg Ser Asp Pro Ile
85 90 95

Gln Leu Glu Ile His Arg Gly Trp Leu Leu Leu Gln Val Ser Ser Arg
100 105 110

Val Phe Thr Glu Gly Glu Pro Leu Ala Leu Arg Cys His Ala Trp Lys
115 120 125

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Asp Lys Leu Val Tyr Asn Val Leu Tyr Tyr Arg Asn Gly Lys Ala Phe
 130 135 140
 Lys Phe Phe His Trp Asn Ser Asn Leu Thr Ile Leu Lys Thr Asn Ile
 145 150 155 160
 Ser His Asn Gly Thr Tyr His Cys Ser Gly Met Gly Lys His Arg Tyr
 165 170 175
 Thr Ser Ala Gly Ile Ser Val Thr Val Lys Glu Leu Phe Pro Ala Pro
 180 185 190
 Val Leu Asn Ala Ser Val Thr Ser Pro Leu Leu Glu Gly Asn Leu Val
 195 200 205
 Thr Leu Ser Cys Glu Thr Lys Leu Leu Gln Arg Pro Gly Leu Gln
 210 215 220
 Leu Tyr Phe Ser Phe Tyr Met Gly Ser Lys Thr Leu Arg Gly Arg Asn
 225 230 235 240
 Thr Ser Ser Glu Tyr Gln Ile Leu Thr Ala Arg Arg Glu Asp Ser Gly
 245 250 255
 Leu Tyr Trp Cys Glu Ala Ala Thr Glu Asp Gly Asn Val Leu Lys Arg
 260 265 270
 Ser Pro Glu Leu Glu Leu Gln Val Leu Gly Phe Phe Pro Pro Gly Tyr
 275 280 285
 Gln Val Ser Phe Cys Leu Val Met Val Leu Leu Phe Ala Val Asp Thr
 290 295 300
 Gly Leu Tyr Phe Ser Val Lys Thr Asn Ile Arg Ser Ser Thr Arg Asp
 305 310 315 320
 Trp Lys Asp His Lys Phe Lys Trp Arg Lys Asp Pro Gln Asp Lys
 325 330 335

<210> SEQ ID NO 12
 <211> LENGTH: 530
 <212> TYPE: PRT
 <213> ORGANISM: Artificial
 <220> FEATURE:
 <223> OTHER INFORMATION: Polypeptide

<400> SEQUENCE: 12

Met Trp Phe Leu Thr Thr Leu Leu Trp Val Pro Val Asp Gly Gln
 1 5 10 15
 Val Asp Thr Thr Lys Ala Val Ile Ser Leu Gln Pro Pro Trp Val Ser
 20 25 30
 Val Phe Gln Glu Glu Thr Val Thr Leu His Cys Glu Val Leu His Leu
 35 40 45
 Pro Gly Ser Ser Ser Thr Gln Trp Phe Leu Asn Gly Thr Ala Thr Gln
 50 55 60
 Thr Ser Thr Pro Ser Tyr Arg Ile Thr Ser Ala Ser Val Asn Asp Ser
 65 70 75 80
 Gly Glu Tyr Arg Cys Gln Arg Gly Leu Ser Gly Arg Ser Asp Pro Ile
 85 90 95
 Gln Leu Glu Ile His Arg Gly Trp Leu Leu Leu Gln Val Ser Ser Arg
 100 105 110
 Val Phe Thr Glu Gly Glu Pro Leu Ala Leu Arg Cys His Ala Trp Lys
 115 120 125
 Asp Lys Leu Val Tyr Asn Val Leu Tyr Tyr Arg Asn Gly Lys Ala Phe
 130 135 140

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Lys Phe Phe His Trp Asn Ser Asn Leu Thr Ile Leu Lys Thr Asn Ile
145 150 155 160

Ser His Asn Gly Thr Tyr His Cys Ser Gly Met Gly Lys His Arg Tyr
165 170 175

Thr Ser Ala Gly Ile Ser Val Thr Val Lys Glu Leu Phe Pro Ala Pro
180 185 190

Val Leu Asn Ala Ser Val Thr Ser Pro Leu Leu Glu Gly Asn Leu Val
195 200 205

Thr Leu Ser Cys Glu Thr Lys Leu Leu Leu Gln Arg Pro Gly Leu Gln
210 215 220

Leu Tyr Phe Ser Phe Tyr Met Gly Ser Lys Thr Leu Arg Gly Arg Asn
225 230 235 240

Thr Ser Ser Glu Tyr Gln Ile Leu Thr Ala Arg Arg Glu Asp Ser Gly
245 250 255

Leu Tyr Trp Cys Glu Ala Ala Thr Glu Asp Gly Asn Val Leu Lys Arg
260 265 270

Ser Pro Glu Leu Glu Leu Gln Val Leu Gly Phe Phe Pro Pro Gly Tyr
275 280 285

Gln Val Ser Phe Cys Leu Val Met Val Leu Leu Phe Ala Val Asp Thr
290 295 300

Gly Leu Tyr Phe Ser Val Lys Thr Asn Ile Arg Ser Ser Thr Arg Asp
305 310 315 320

Trp Lys Asp His Lys Phe Lys Trp Arg Lys Asp Pro Gln Asp Lys Arg
325 330 335

Ser Lys Arg Ser Arg Leu Leu His Ser Asp Tyr Met Asn Met Thr Pro
340 345 350

Arg Arg Pro Gly Pro Thr Arg Lys His Tyr Gln Pro Tyr Ala Pro Pro
355 360 365

Arg Asp Phe Ala Ala Tyr Arg Ser Lys Arg Gly Arg Lys Lys Leu Leu
370 375 380

Tyr Ile Phe Lys Gln Pro Phe Met Arg Pro Val Gln Thr Thr Gln Glu
385 390 395 400

Glu Asp Gly Cys Ser Cys Arg Phe Pro Glu Glu Glu Gly Gly Cys
405 410 415

Glu Leu Arg Val Lys Phe Ser Arg Ser Ala Asp Ala Pro Ala Tyr Gln
420 425 430

Gln Gly Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu Gly Arg Arg Glu
435 440 445

Glu Tyr Asp Val Leu Asp Lys Arg Arg Gly Arg Asp Pro Glu Met Gly
450 455 460

Gly Lys Pro Arg Arg Lys Asn Pro Gln Glu Gly Leu Tyr Asn Glu Leu
465 470 475 480

Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile Gly Met Lys Gly
485 490 495

Glu Arg Arg Arg Gly Lys Gly His Asp Gly Leu Tyr Gln Gly Leu Ser
500 505 510

Thr Ala Thr Lys Asp Thr Tyr Asp Ala Leu His Met Gln Ala Leu Pro
515 520 525

Pro Arg
530

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<210> SEQ ID NO 13
<211> LENGTH: 460
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Polypeptide

<400> SEQUENCE: 13

Met Trp Phe Leu Thr Thr Leu Leu Leu Trp Val Pro Val Asp Gly Gln
1 5 10 15

Val Asp Thr Thr Lys Ala Val Ile Thr Leu Gln Pro Pro Trp Val Ser
20 25 30

Val Phe Gln Glu Glu Thr Val Thr Leu His Cys Glu Val Leu His Leu
35 40 45

Pro Gly Ser Ser Ser Thr Gln Trp Phe Leu Asn Gly Thr Ala Thr Gln
50 55 60

Thr Ser Thr Pro Ser Tyr Arg Ile Thr Ser Ala Ser Val Asn Asp Ser
65 70 75 80

Gly Glu Tyr Arg Cys Gln Arg Gly Leu Ser Gly Arg Ser Asp Pro Ile
85 90 95

Gln Leu Glu Ile His Arg Gly Trp Leu Leu Leu Gln Val Ser Ser Arg
100 105 110

Val Phe Thr Glu Gly Glu Pro Leu Ala Leu Arg Cys His Ala Trp Lys
115 120 125

Asp Lys Leu Val Tyr Asn Val Leu Tyr Tyr Arg Asn Gly Lys Ala Phe
130 135 140

Lys Phe Phe His Trp Asn Ser Asn Leu Thr Ile Leu Lys Thr Asn Ile
145 150 155 160

Ser His Asn Gly Thr Tyr His Cys Ser Gly Met Gly Lys His Arg Tyr
165 170 175

Thr Ser Ala Gly Ile Ser Val Thr Val Lys Glu Leu Phe Pro Ala Pro
180 185 190

Val Leu Asn Ala Ser Val Thr Ser Pro Leu Leu Glu Gly Asn Leu Val
195 200 205

Thr Leu Ser Cys Glu Thr Lys Leu Leu Leu Gln Arg Pro Gly Leu Gln
210 215 220

Leu Tyr Phe Ser Phe Tyr Met Gly Ser Lys Thr Leu Arg Gly Arg Asn
225 230 235 240

Thr Ser Ser Glu Tyr Gln Ile Leu Thr Ala Arg Arg Glu Asp Ser Gly
245 250 255

Leu Tyr Trp Cys Glu Ala Ala Thr Glu Asp Gly Asn Val Leu Lys Arg
260 265 270

Ser Pro Glu Leu Glu Leu Gln Val Leu Gly Phe Phe Pro Pro Gly Tyr
275 280 285

Gln Val Ser Phe Cys Leu Val Met Val Leu Leu Phe Ala Val Asp Thr
290 295 300

Gly Leu Tyr Phe Ser Val Lys Thr Asn Ile Arg Ser Ser Thr Arg Asp
305 310 315 320

Trp Lys Asp His Lys Phe Lys Trp Arg Lys Asp Pro Gln Asp Lys Arg
325 330 335

Ser Lys Arg Ser Arg Leu Leu His Ser Asp Tyr Met Asn Met Thr Pro
340 345 350

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Arg	Arg	Pro	Gly	Pro	Thr	Arg	Lys	His	Tyr	Gln	Pro	Tyr	Ala	Pro	Pro
355															365
Arg	Asp	Phe	Ala	Ala	Tyr	Arg	Ser	Lys	Arg	Gly	Arg	Lys	Lys	Leu	Leu
370															380
Tyr	Ile	Phe	Lys	Gln	Pro	Phe	Met	Arg	Pro	Val	Gln	Thr	Thr	Gln	Glu
385															400
Glu	Asp	Gly	Cys	Ser	Cys	Arg	Phe	Pro	Glu	Glu	Glu	Gly	Gly	Cys	
	405								410						415
Glu	Leu	Arg	Leu	Lys	Ile	Gln	Val	Arg	Lys	Ala	Ala	Ile	Thr	Ser	Tyr
	420								425						430
Glu	Lys	Ser	Asp	Gly	Val	Tyr	Thr	Gly	Leu	Ser	Thr	Arg	Asn	Gln	Glu
	435								440						445
Thr	Tyr	Glu	Thr	Leu	Lys	His	Glu	Lys	Pro	Pro	Gln				
	450								455						460

<210> SEQ_ID NO 14
<211> LENGTH: 440
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Polypeptide

<400> SEQUENCE: 14

Met	Trp	Phe	Leu	Thr	Thr	Leu	Leu	Trp	Val	Pro	Val	Asp	Gly	Gln	
1															15
Val	Asp	Thr	Thr	Lys	Ala	Val	Ile	Thr	Leu	Gln	Pro	Pro	Trp	Val	Ser
															30
Val	Phe	Gln	Glu	Glu	Thr	Val	Thr	Leu	His	Cys	Glu	Val	Leu	His	Leu
															45
Pro	Gly	Ser	Ser	Ser	Thr	Gln	Trp	Phe	Leu	Asn	Gly	Thr	Ala	Thr	Gln
															60
Thr	Ser	Thr	Pro	Ser	Tyr	Arg	Ile	Thr	Ser	Ala	Ser	Val	Asn	Asp	Ser
															80
Gly	Glu	Tyr	Arg	Cys	Gln	Arg	Gly	Leu	Ser	Gly	Arg	Ser	Asp	Pro	Ile
															95
Gln	Leu	Glu	Ile	His	Arg	Gly	Trp	Leu	Leu	Leu	Gln	Val	Ser	Ser	Arg
															110
Val	Phe	Thr	Glu	Gly	Glu	Pro	Leu	Ala	Leu	Arg	Cys	His	Ala	Trp	Lys
															125
Asp	Lys	Leu	Val	Tyr	Asn	Val	Leu	Tyr	Tyr	Arg	Asn	Gly	Lys	Ala	Phe
															140
Lys	Phe	Phe	His	Trp	Asn	Ser	Asn	Leu	Thr	Ile	Leu	Lys	Thr	Asn	Ile
															160
Ser	His	Asn	Gly	Thr	Tyr	His	Cys	Ser	Gly	Met	Gly	Lys	His	Arg	Tyr
															175
Thr	Ser	Ala	Gly	Ile	Ser	Val	Thr	Val	Lys	Glu	Leu	Phe	Pro	Ala	Pro
															190
Val	Leu	Asn	Ala	Ser	Val	Thr	Ser	Pro	Leu	Leu	Glu	Gly	Asn	Leu	Val
															205
Thr	Leu	Ser	Cys	Glu	Thr	Lys	Leu	Leu	Leu	Gln	Arg	Pro	Gly	Leu	Gln
															220
Leu	Tyr	Phe	Ser	Phe	Tyr	Met	Gly	Ser	Lys	Thr	Leu	Arg	Gly	Arg	Asn
															240
225									230						

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Thr	Ser	Ser	Glu	Tyr	Gln	Ile	Leu	Thr	Ala	Arg	Arg	Glu	Asp	Ser	Gly
245								250							255
Leu Tyr Trp Cys Glu Ala Ala Thr Glu Asp Gly Asn Val Leu Lys Arg															
260								265							270
Ser	Pro	Glu	Leu	Glu	Leu	Gln	Val	Leu	Gly	Phe	Phe	Pro	Pro	Gly	Tyr
275								280							285
Gln	Val	Ser	Phe	Cys	Leu	Val	Met	Val	Leu	Leu	Phe	Ala	Val	Asp	Thr
290							295								300
Gly	Leu	Tyr	Phe	Ser	Val	Lys	Thr	Asn	Ile	Arg	Ser	Ser	Thr	Arg	Asp
305						310			315						320
Trp	Lys	Asp	His	Lys	Phe	Lys	Trp	Arg	Lys	Asp	Pro	Gln	Asp	Lys	Arg
325						330			335						
Ser	Lys	Arg	Ser	Arg	Leu	Leu	His	Ser	Asp	Tyr	Met	Asn	Met	Thr	Pro
340						345			350						
Arg	Arg	Pro	Gly	Pro	Thr	Arg	Lys	His	Tyr	Gln	Pro	Tyr	Ala	Pro	Pro
355						360			365						
Arg	Asp	Phe	Ala	Ala	Tyr	Arg	Ser	Lys	Arg	Gly	Arg	Lys	Lys	Leu	Leu
370						375			380						
Tyr	Ile	Phe	Lys	Gln	Pro	Phe	Met	Arg	Pro	Val	Gln	Thr	Thr	Gln	Glu
385						390			395						400
Glu	Asp	Gly	Cys	Ser	Cys	Arg	Phe	Pro	Glu	Glu	Glu	Gly	Gly	Cys	
405						410			415						
Glu	Leu	Ala	Arg	Pro	Arg	Arg	Ser	Pro	Ala	Gln	Glu	Asp	Gly	Lys	Val
420						425			430						
Tyr	Ile	Asn	Met	Pro	Gly	Arg	Gly								
435					440										

<210> SEQ_ID NO 15
<211> LENGTH: 467
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Polypeptide

<400> SEQUENCE: 15

Met	Trp	Phe	Leu	Thr	Thr	Leu	Leu	Leu	Trp	Val	Pro	Val	Asp	Gly	Gln
1						5			10			15			
Val Asp Thr Thr Lys Ala Val Ile Thr Leu Gln Pro Pro Trp Val Ser															
						20			25			30			
Val Phe Gln Glu Glu Thr Val Thr Leu His Cys Glu Val Leu His Leu															
						35			40			45			
Pro Gly Ser Ser Ser Thr Gln Trp Phe Leu Asn Gly Thr Ala Thr Gln															
						50			55			60			
Thr Ser Thr Pro Ser Tyr Arg Ile Thr Ser Ala Ser Val Asn Asp Ser															
						65			70			75			80
Gly Glu Tyr Arg Cys Gln Arg Gly Leu Ser Gly Arg Ser Asp Pro Ile															
						85			90			95			
Gln Leu Glu Ile His Arg Gly Trp Leu Leu Leu Gln Val Ser Ser Arg															
						100			105			110			
Val Phe Thr Glu Gly Glu Pro Leu Ala Leu Arg Cys His Ala Trp Lys															
						115			120			125			
Asp Lys Leu Val Tyr Asn Val Leu Tyr Tyr Arg Asn Gly Lys Ala Phe															
						130			135			140			

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Lys	Phe	Phe	His	Trp	Asn	Ser	Asn	Leu	Thr	Ile	Leu	Lys	Thr	Asn	Ile
145					150			155				160			
Ser	His	Asn	Gly	Thr	Tyr	His	Cys	Ser	Gly	Met	Gly	Lys	His	Arg	Tyr
	165					170			175						
Thr	Ser	Ala	Gly	Ile	Ser	Val	Thr	Val	Lys	Glu	Leu	Phe	Pro	Ala	Pro
	180					185			190						
Val	Leu	Asn	Ala	Ser	Val	Thr	Ser	Pro	Leu	Leu	Glu	Gly	Asn	Leu	Val
	195				200				205						
Thr	Leu	Ser	Cys	Glu	Thr	Lys	Leu	Leu	Leu	Gln	Arg	Pro	Gly	Leu	Gln
	210					215		220							
Leu	Tyr	Phe	Ser	Phe	Tyr	Met	Gly	Ser	Lys	Thr	Leu	Arg	Gly	Arg	Asn
	225					230		235		240					
Thr	Ser	Ser	Glu	Tyr	Gln	Ile	Leu	Thr	Ala	Arg	Arg	Glu	Asp	Ser	Gly
	245					250		255							
Leu	Tyr	Trp	Cys	Glu	Ala	Ala	Thr	Glu	Asp	Gly	Asn	Val	Leu	Lys	Arg
	260				265			270							
Ser	Pro	Glu	Leu	Glu	Leu	Gln	Val	Leu	Gly	Phe	Phe	Pro	Pro	Gly	Tyr
	275				280			285							
Gln	Val	Ser	Phe	Cys	Leu	Val	Met	Val	Leu	Leu	Phe	Ala	Val	Asp	Thr
	290				295		300								
Gly	Leu	Tyr	Phe	Ser	Val	Lys	Thr	Asn	Ile	Arg	Ser	Ser	Thr	Arg	Asp
	305					310		315		320					
Trp	Lys	Asp	His	Lys	Phe	Lys	Trp	Arg	Lys	Asp	Pro	Gln	Asp	Lys	Arg
	325					330		335							
Ser	Lys	Arg	Ser	Arg	Leu	Leu	His	Ser	Asp	Tyr	Met	Asn	Met	Thr	Pro
	340					345		350							
Arg	Arg	Pro	Gly	Pro	Thr	Arg	Lys	His	Tyr	Gln	Pro	Tyr	Ala	Pro	Pro
	355					360		365							
Arg	Asp	Phe	Ala	Ala	Tyr	Arg	Ser	Lys	Arg	Gly	Arg	Lys	Lys	Leu	Leu
	370					375		380							
Tyr	Ile	Phe	Lys	Gln	Pro	Phe	Met	Arg	Pro	Val	Gln	Thr	Thr	Gln	Glu
	385					390		395		400					
Glu	Asp	Gly	Cys	Ser	Cys	Arg	Phe	Pro	Glu	Glu	Glu	Gly	Gly	Cys	
			405			410			415						
Glu	Leu	Gly	Arg	Leu	Val	Pro	Arg	Gly	Arg	Gly	Ala	Ala	Glu	Ala	Ala
	420					425		430							
Thr	Arg	Lys	Gln	Arg	Ile	Thr	Glu	Thr	Glu	Ser	Pro	Tyr	Gln	Glu	Leu
	435					440		445							
Gln	Gly	Gln	Arg	Ser	Asp	Val	Tyr	Ser	Asp	Leu	Asn	Thr	Gln	Arg	Pro
	450				455			460							
Tyr	Tyr	Lys													
	465														

<210> SEQ ID NO 16
<211> LENGTH: 510
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Polypeptide

<400> SEQUENCE: 16

Met Trp Phe Leu Thr Thr Leu Leu Leu Trp Val Pro Val Asp Gly Gln
1 5 10 15

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Val	Asp	Thr	Thr	Lys	Ala	Val	Ile	Thr	Leu	Gln	Pro	Pro	Trp	Val	Ser	
20															30	
Val	Phe	Gln	Glu	Glu	Thr	Val	Thr	Leu	His	Cys	Glu	Val	Leu	His	Leu	
35															45	
Pro	Gly	Ser	Ser	Ser	Ser	Thr	Gln	Trp	Phe	Leu	Asn	Gly	Thr	Ala	Thr	Gln
50															60	
Thr	Ser	Thr	Pro	Ser	Tyr	Arg	Ile	Thr	Ser	Ala	Ser	Val	Asn	Asp	Ser	
65															80	
Gly	Glu	Tyr	Arg	Cys	Gln	Arg	Gly	Leu	Ser	Gly	Arg	Ser	Asp	Pro	Ile	
85															95	
Gln	Leu	Glu	Ile	His	Arg	Gly	Trp	Leu	Leu	Gln	Val	Ser	Ser	Arg		
100															110	
Val	Phe	Thr	Glu	Gly	Glu	Pro	Leu	Ala	Leu	Arg	Cys	His	Ala	Trp	Lys	
115															125	
Asp	Lys	Leu	Val	Tyr	Asn	Val	Leu	Tyr	Tyr	Arg	Asn	Gly	Lys	Ala	Phe	
130															140	
Lys	Phe	Phe	His	Trp	Asn	Ser	Asn	Leu	Thr	Ile	Leu	Lys	Thr	Asn	Ile	
145															160	
Ser	His	Asn	Gly	Thr	Tyr	His	Cys	Ser	Gly	Met	Gly	Lys	His	Arg	Tyr	
165															175	
Thr	Ser	Ala	Gly	Ile	Ser	Val	Thr	Val	Lys	Glu	Leu	Phe	Pro	Ala	Pro	
180															190	
Val	Leu	Asn	Ala	Ser	Val	Thr	Ser	Pro	Leu	Leu	Glu	Gly	Asn	Leu	Val	
195															205	
Thr	Leu	Ser	Cys	Glu	Thr	Lys	Leu	Leu	Gln	Arg	Pro	Gly	Leu	Gln		
210															220	
Leu	Tyr	Phe	Ser	Phe	Tyr	Met	Gly	Ser	Lys	Thr	Leu	Arg	Gly	Arg	Asn	
225															240	
Thr	Ser	Ser	Glu	Tyr	Gln	Ile	Leu	Thr	Ala	Arg	Arg	Glu	Asp	Ser	Gly	
245															255	
Leu	Tyr	Trp	Cys	Glu	Ala	Ala	Thr	Glu	Asp	Gly	Asn	Val	Leu	Lys	Arg	
260															270	
Ser	Pro	Glu	Leu	Glu	Leu	Gln	Val	Leu	Gly	Leu	Gln	Leu	Pro	Thr	Pro	
275															285	
Phe	Trp	Val	Leu	Val	Val	Gly	Gly	Val	Leu	Ala	Cys	Tyr	Ser	Leu		
290															300	
Leu	Val	Thr	Val	Ala	Phe	Ile	Ile	Phe	Trp	Val	Arg	Ser	Lys	Arg	Ser	
305															320	
Arg	Leu	Leu	His	Ser	Asp	Tyr	Met	Asn	Met	Thr	Pro	Arg	Arg	Pro	Gly	
325															335	
Pro	Thr	Arg	Lys	His	Tyr	Gln	Pro	Tyr	Ala	Pro	Pro	Arg	Asp	Phe	Ala	
340															350	
Ala	Tyr	Arg	Ser	Lys	Arg	Gly	Arg	Lys	Leu	Leu	Tyr	Ile	Phe	Lys		
355															365	
Gln	Pro	Phe	Met	Arg	Pro	Val	Gln	Thr	Thr	Gln	Glu	Glu	Asp	Gly	Cys	
370															380	
Ser	Cys	Arg	Phe	Pro	Glu	Glu	Glu	Gly	Gly	Cys	Glu	Leu	Arg	Val		
385															400	
Lys	Phe	Ser	Arg	Ser	Ala	Asp	Ala	Pro	Ala	Tyr	Gln	Gly	Gln	Asn		
405															415	
Gln	Leu	Tyr	Asn	Glu	Leu	Asn	Leu	Gly	Arg	Arg	Glu	Glu	Tyr	Asp	Val	

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420	425	430	
Leu Asp Lys Arg Arg Gly Arg Asp Pro Glu Met Gly Gly Lys Pro Arg			
435	440	445	
Arg Lys Asn Pro Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys			
450	455	460	
Met Ala Glu Ala Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg Arg Arg			
465	470	475	480
Gly Lys Gly His Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys			
485	490	495	
Asp Thr Tyr Asp Ala Leu His Met Gln Ala Leu Pro Pro Arg			
500	505	510	

<210> SEQ_ID NO 17
<211> LENGTH: 343
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Polypeptide

<400> SEQUENCE: 17

Met Trp Phe Leu Thr Thr Leu Leu Leu Trp Val Pro Val Asp Gly Gln			
1	5	10	15
Val Asp Thr Thr Lys Ala Val Ile Thr Leu Gln Pro Pro Trp Val Ser			
20	25	30	
Val Phe Gln Glu Glu Thr Val Thr Leu His Cys Glu Val Leu His Leu			
35	40	45	
Pro Gly Ser Ser Ser Thr Gln Trp Phe Leu Asn Gly Thr Ala Thr Gln			
50	55	60	
Thr Ser Thr Pro Ser Tyr Arg Ile Thr Ser Ala Ser Val Asn Asp Ser			
65	70	75	80
Gly Glu Tyr Arg Cys Gln Arg Gly Leu Ser Gly Arg Ser Asp Pro Ile			
85	90	95	
Gln Leu Glu Ile His Arg Gly Trp Leu Leu Leu Gln Val Ser Ser Arg			
100	105	110	
Val Phe Thr Glu Gly Glu Pro Leu Ala Leu Arg Cys His Ala Trp Lys			
115	120	125	
Asp Lys Leu Val Tyr Asn Val Leu Tyr Tyr Arg Asn Gly Lys Ala Phe			
130	135	140	
Lys Phe Phe His Trp Asn Ser Asn Leu Thr Ile Leu Lys Thr Asn Ile			
145	150	155	160
Ser His Asn Gly Thr Tyr His Cys Ser Gly Met Gly Lys His Arg Tyr			
165	170	175	
Thr Ser Ala Gly Ile Ser Val Thr Val Lys Glu Leu Phe Pro Ala Pro			
180	185	190	
Val Leu Asn Ala Ser Val Thr Ser Pro Leu Leu Glu Gly Asn Leu Val			
195	200	205	
Thr Leu Ser Cys Glu Thr Lys Leu Leu Leu Gln Arg Pro Gly Leu Gln			
210	215	220	
Leu Tyr Phe Ser Phe Tyr Met Gly Ser Lys Thr Leu Arg Gly Arg Asn			
225	230	235	240
Thr Ser Ser Glu Tyr Gln Ile Leu Thr Ala Arg Arg Glu Asp Ser Gly			
245	250	255	
Leu Tyr Trp Cys Glu Ala Ala Thr Glu Asp Gly Asn Val Leu Lys Arg			

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260	265	270	
Ser Pro Glu Leu Glu Leu Gln Val Leu Gly Ala Pro Arg Glu Lys Tyr			
275	280	285	
Trp Leu Gln Phe Phe Ile Pro Leu Leu Val Val Ile Leu Phe Ala Val			
290	295	300	
Asp Thr Gly Leu Phe Ile Ser Thr Gln Gln Val Thr Phe Leu Leu			
305	310	315	320
Lys Ile Lys Arg Thr Arg Lys Gly Phe Arg Leu Leu Asn Pro His Pro			
325	330	335	
Lys Pro Asn Pro Lys Asn Asn			
340			
 <210> SEQ_ID NO 18			
<211> LENGTH: 335			
<212> TYPE: PRT			
<213> ORGANISM: Artificial			
<220> FEATURE:			
<223> OTHER INFORMATION: Polypeptide			
 <400> SEQUENCE: 18			
Met Trp Phe Leu Thr Thr Leu Leu Leu Trp Val Pro Val Asp Gly Gln			
1	5	10	15
Val Asp Thr Thr Lys Ala Val Ile Thr Leu Gln Pro Pro Trp Val Ser			
20	25	30	
Val Phe Gln Glu Glu Thr Val Thr Leu His Cys Glu Val Leu His Leu			
35	40	45	
Pro Gly Ser Ser Ser Thr Gln Trp Phe Leu Asn Gly Thr Ala Thr Gln			
50	55	60	
Thr Ser Thr Pro Ser Tyr Arg Ile Thr Ser Ala Ser Val Asn Asp Ser			
65	70	75	80
Gly Glu Tyr Arg Cys Gln Arg Gly Leu Ser Gly Arg Ser Asp Pro Ile			
85	90	95	
Gln Leu Glu Ile His Arg Gly Trp Leu Leu Leu Gln Val Ser Ser Arg			
100	105	110	
Val Phe Thr Glu Gly Glu Pro Leu Ala Leu Arg Cys His Ala Trp Lys			
115	120	125	
Asp Lys Leu Val Tyr Asn Val Leu Tyr Tyr Arg Asn Gly Lys Ala Phe			
130	135	140	
Lys Phe Phe His Trp Asn Ser Asn Leu Thr Ile Leu Lys Thr Asn Ile			
145	150	155	160
Ser His Asn Gly Thr Tyr His Cys Ser Gly Met Gly Lys His Arg Tyr			
165	170	175	
Thr Ser Ala Gly Ile Ser Val Thr Val Lys Glu Leu Phe Pro Ala Pro			
180	185	190	
Val Leu Asn Ala Ser Val Thr Ser Pro Leu Leu Glu Gly Asn Leu Val			
195	200	205	
Thr Leu Ser Cys Glu Thr Lys Leu Leu Leu Gln Arg Pro Gly Leu Gln			
210	215	220	
Leu Tyr Phe Ser Phe Tyr Met Gly Ser Lys Thr Leu Arg Gly Arg Asn			
225	230	235	240
Thr Ser Ser Glu Tyr Gln Ile Leu Thr Ala Arg Arg Glu Asp Ser Gly			
245	250	255	
Leu Tyr Trp Cys Glu Ala Ala Thr Glu Asp Gly Asn Val Leu Lys Arg			

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260	265	270	
Ser Pro Glu Leu Glu Leu Gln Val Leu Gly Phe Phe Pro Pro Gly Tyr			
275	280	285	
Gln Val Ser Phe Cys Leu Val Met Val Leu Leu Phe Ala Val Asp Thr			
290	295	300	
Gly Leu Tyr Phe Ser Val Lys Thr Asn Ile Arg Gly Ala Gly Arg Asp			
305	310	315	320
Trp Lys Asp His Lys Phe Lys Trp Arg Lys Asp Pro Gln Asp Lys			
325	330	335	
 <210> SEQ_ID NO 19			
<211> LENGTH: 561			
<212> TYPE: PRT			
<213> ORGANISM: Artificial			
<220> FEATURE:			
<223> OTHER INFORMATION: Polypeptide			
 <400> SEQUENCE: 19			
Met Trp Phe Leu Thr Thr Leu Leu Leu Trp Val Pro Val Asp Gly Gln			
1	5	10	15
Val Asp Thr Thr Lys Ala Val Ile Thr Leu Gln Pro Pro Trp Val Ser			
20	25	30	
Val Phe Gln Glu Glu Thr Val Thr Leu His Cys Glu Val Leu His Leu			
35	40	45	
Pro Gly Ser Ser Ser Thr Gln Trp Phe Leu Asn Gly Thr Ala Thr Gln			
50	55	60	
Thr Ser Thr Pro Ser Tyr Arg Ile Thr Ser Ala Ser Val Asn Asp Ser			
65	70	75	80
Gly Glu Tyr Arg Cys Gln Arg Gly Leu Ser Gly Arg Ser Asp Pro Ile			
85	90	95	
Gln Leu Glu Ile His Arg Gly Trp Leu Leu Leu Gln Val Ser Ser Arg			
100	105	110	
Val Phe Thr Glu Gly Glu Pro Leu Ala Leu Arg Cys His Ala Trp Lys			
115	120	125	
Asp Lys Leu Val Tyr Asn Val Leu Tyr Tyr Arg Asn Gly Lys Ala Phe			
130	135	140	
Lys Phe Phe His Trp Asn Ser Asn Leu Thr Ile Leu Lys Thr Asn Ile			
145	150	155	160
Ser His Asn Gly Thr Tyr His Cys Ser Gly Met Gly Lys His Arg Tyr			
165	170	175	
Thr Ser Ala Gly Ile Ser Val Thr Val Lys Glu Leu Phe Pro Ala Pro			
180	185	190	
Val Leu Asn Ala Ser Val Thr Ser Pro Leu Leu Glu Gly Asn Leu Val			
195	200	205	
Thr Leu Ser Cys Glu Thr Lys Leu Leu Leu Gln Arg Pro Gly Leu Gln			
210	215	220	
Leu Tyr Phe Ser Phe Tyr Met Gly Ser Lys Thr Leu Arg Gly Arg Asn			
225	230	235	240
Thr Ser Ser Glu Tyr Gln Ile Leu Thr Ala Arg Arg Glu Asp Ser Gly			
245	250	255	
Leu Tyr Trp Cys Glu Ala Ala Thr Glu Asp Gly Asn Val Leu Lys Arg			
260	265	270	
Ser Pro Glu Leu Glu Leu Gln Val Leu Gly Ser Asn Leu Phe Val Ala			

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275	280	285
Ser Trp Ile Ala Val Met Ile Ile Phe Arg Ile Gly Met Ala Val Ala		
290	295	300
Ile Phe Cys Cys Phe Phe Pro Ser Gly Gly Ser Gly Gly Ser		
305	310	315
Gly Trp Arg Arg Lys Arg Lys Glu Lys Gln Ser Glu Thr Ser Pro Lys		
325	330	335
Glu Phe Leu Thr Ile Tyr Glu Asp Val Lys Asp Leu Lys Thr Arg Arg		
340	345	350
Asn His Glu Gln Glu Gln Thr Phe Pro Gly Gly Ser Thr Ile Tyr		
355	360	365
Ser Met Ile Gln Ser Gln Ser Ser Ala Pro Thr Ser Gln Glu Pro Ala		
370	375	380
Tyr Thr Leu Tyr Ser Leu Ile Gln Pro Ser Arg Lys Ser Gly Ser Arg		
385	390	395
Lys Arg Asn His Ser Pro Ser Phe Asn Ser Thr Ile Tyr Glu Val Ile		
405	410	415
Gly Lys Ser Gln Pro Lys Ala Gln Asn Pro Ala Arg Leu Ser Arg Lys		
420	425	430
Glu Leu Glu Asn Phe Asp Val Tyr Ser Gly Gly Ser Gly Gly Ser		
435	440	445
Gly Arg Val Lys Phe Ser Arg Ser Ala Asp Ala Pro Ala Tyr Lys Gln		
450	455	460
Gly Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu Gly Arg Arg Glu Glu		
465	470	475
Tyr Asp Val Leu Asp Lys Arg Arg Gly Arg Asp Pro Glu Met Gly Gly		
485	490	495
Lys Pro Arg Arg Lys Asn Pro Gln Glu Gly Leu Tyr Asn Glu Leu Gln		
500	505	510
Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile Gly Met Lys Gly Glu		
515	520	525
Arg Arg Arg Gly Lys Gly His Asp Gly Leu Tyr Gln Gly Leu Ser Thr		
530	535	540
Ala Thr Lys Asp Thr Tyr Asp Ala Leu His Met Gln Ala Leu Pro Pro		
545	550	555
Arg		
<210> SEQ ID NO 20		
<211> LENGTH: 575		
<212> TYPE: PRT		
<213> ORGANISM: Artificial		
<220> FEATURE:		
<223> OTHER INFORMATION: Polypeptide		
<400> SEQUENCE: 20		
Met Trp Phe Leu Thr Thr Leu Leu Leu Trp Val Pro Val Asp Gly Gln		
1	5	10
15		
Val Asp Thr Thr Lys Ala Val Ile Thr Leu Gln Pro Pro Trp Val Ser		
20	25	30
30		
Val Phe Gln Glu Glu Thr Val Thr Leu His Cys Glu Val Leu His Leu		
35	40	45
45		
Pro Gly Ser Ser Ser Thr Gln Trp Phe Leu Asn Gly Thr Ala Thr Gln		
50	55	60
60		

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Thr Ser Thr Pro Ser Tyr Arg Ile Thr Ser Ala Ser Val Asn Asp Ser
 65 70 75 80
 Gly Glu Tyr Arg Cys Gln Arg Gly Leu Ser Gly Arg Ser Asp Pro Ile
 85 90 95
 Gln Leu Glu Ile His Arg Gly Trp Leu Leu Gln Val Ser Ser Arg
 100 105 110
 Val Phe Thr Glu Gly Glu Pro Leu Ala Leu Arg Cys His Ala Trp Lys
 115 120 125
 Asp Lys Leu Val Tyr Asn Val Leu Tyr Tyr Arg Asn Gly Lys Ala Phe
 130 135 140
 Lys Phe Phe His Trp Asn Ser Asn Leu Thr Ile Leu Lys Thr Asn Ile
 145 150 155 160
 Ser His Asn Gly Thr Tyr His Cys Ser Gly Met Gly Lys His Arg Tyr
 165 170 175
 Thr Ser Ala Gly Ile Ser Val Thr Val Lys Glu Leu Phe Pro Ala Pro
 180 185 190
 Val Leu Asn Ala Ser Val Thr Ser Pro Leu Leu Glu Gly Asn Leu Val
 195 200 205
 Thr Leu Ser Cys Glu Thr Lys Leu Leu Leu Gln Arg Pro Gly Leu Gln
 210 215 220
 Leu Tyr Phe Ser Phe Tyr Met Gly Ser Lys Thr Leu Arg Gly Arg Asn
 225 230 235 240
 Thr Ser Ser Glu Tyr Gln Ile Leu Thr Ala Arg Arg Glu Asp Ser Gly
 245 250 255
 Leu Tyr Trp Cys Glu Ala Ala Thr Glu Asp Gly Asn Val Leu Lys Arg
 260 265 270
 Ser Pro Glu Leu Glu Leu Gln Val Leu Gly Phe Phe Pro Pro Gly Tyr
 275 280 285
 Gln Val Ser Phe Cys Leu Val Met Val Leu Leu Phe Ala Val Asp Thr
 290 295 300
 Gly Leu Tyr Phe Ser Val Lys Thr Asn Ile Arg Ser Ser Thr Arg Asp
 305 310 315 320
 Trp Lys Asp His Lys Phe Lys Trp Arg Lys Asp Pro Gln Asp Lys Trp
 325 330 335
 Arg Arg Lys Arg Lys Glu Lys Gln Ser Glu Thr Ser Pro Lys Glu Phe
 340 345 350
 Leu Thr Ile Tyr Glu Asp Val Lys Asp Leu Lys Thr Arg Arg Asn His
 355 360 365
 Glu Gln Glu Gln Thr Phe Pro Gly Gly Ser Thr Ile Tyr Ser Met
 370 375 380
 Ile Gln Ser Gln Ser Ser Ala Pro Thr Ser Gln Glu Pro Ala Tyr Thr
 385 390 395 400
 Leu Tyr Ser Leu Ile Gln Pro Ser Arg Lys Ser Gly Ser Arg Lys Arg
 405 410 415
 Asn His Ser Pro Ser Phe Asn Ser Thr Ile Tyr Glu Val Ile Gly Lys
 420 425 430
 Ser Gln Pro Lys Ala Gln Asn Pro Ala Arg Leu Ser Arg Lys Glu Leu
 435 440 445
 Glu Asn Phe Asp Val Tyr Ser Gly Gly Ser Gly Gly Ser Gly Arg
 450 455 460

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Val	Lys	Phe	Ser	Arg	Ser	Ala	Asp	Ala	Pro	Ala	Tyr	Lys	Gln	Gly	Gln
465						470			475						480
Asn	Gln	Leu	Tyr	Asn	Glu	Leu	Asn	Leu	Gly	Arg	Arg	Glu	Glu	Tyr	Asp
	485					490				495					
Val	Leu	Asp	Lys	Arg	Arg	Gly	Arg	Asp	Pro	Glu	Met	Gly	Gly	Lys	Pro
	500					505				510					
Arg	Arg	Lys	Asn	Pro	Gln	Glu	Gly	Leu	Tyr	Asn	Glu	Leu	Gln	Lys	Asp
	515					520				525					
Lys	Met	Ala	Glu	Ala	Tyr	Ser	Glu	Ile	Gly	Met	Lys	Gly	Glu	Arg	Arg
	530					535			540						
Arg	Gly	Lys	Gly	His	Asp	Gly	Leu	Tyr	Gln	Gly	Leu	Ser	Thr	Ala	Thr
	545					550			555				560		
Lys	Asp	Asp	Thr	Tyr	Asp	Ala	Leu	His	Met	Gln	Ala	Leu	Pro	Pro	Arg
	565					570			575						

<210> SEQ ID NO 21
<211> LENGTH: 505
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Polypeptide

<400> SEQUENCE: 21

Met	Trp	Phe	Leu	Thr	Thr	Leu	Leu	Trp	Val	Pro	Val	Asp	Gly	Gln	
1						5			10				15		
Val	Asp	Thr	Thr	Lys	Ala	Val	Ile	Thr	Leu	Gln	Pro	Pro	Trp	Val	Ser
						20			25				30		
Val	Phe	Gln	Glu	Glu	Thr	Val	Thr	Leu	His	Cys	Glu	Val	Leu	His	Leu
						35			40				45		
Pro	Gly	Ser	Ser	Ser	Thr	Gln	Trp	Phe	Leu	Asn	Gly	Thr	Ala	Thr	Gln
						50			55				60		
Thr	Ser	Thr	Pro	Ser	Tyr	Arg	Ile	Thr	Ser	Ala	Ser	Val	Asn	Asp	Ser
						65			70				80		
Gly	Glu	Tyr	Arg	Cys	Gln	Arg	Gly	Leu	Ser	Gly	Arg	Ser	Asp	Pro	Ile
						85			90				95		
Gln	Leu	Glu	Ile	His	Arg	Gly	Trp	Leu	Leu	Leu	Gln	Val	Ser	Ser	Arg
						100			105				110		
Val	Phe	Thr	Glu	Glu	Pro	Leu	Ala	Leu	Arg	Cys	His	Ala	Trp	Lys	
						115			120				125		
Asp	Lys	Leu	Val	Tyr	Asn	Val	Leu	Tyr	Tyr	Arg	Asn	Gly	Lys	Ala	Phe
						130			135				140		
Lys	Phe	Phe	His	Trp	Asn	Ser	Asn	Leu	Thr	Ile	Leu	Lys	Thr	Asn	Ile
						145			150				160		
Ser	His	Asn	Gly	Thr	Tyr	His	Cys	Ser	Gly	Met	Gly	Lys	His	Arg	Tyr
						165			170				175		
Thr	Ser	Ala	Gly	Ile	Ser	Val	Thr	Val	Lys	Glu	Leu	Phe	Pro	Ala	Pro
						180			185				190		
Val	Leu	Asn	Ala	Ser	Val	Thr	Ser	Pro	Leu	Leu	Glu	Gly	Asn	Leu	Val
						195			200				205		
Thr	Leu	Ser	Cys	Glu	Thr	Lys	Leu	Leu	Leu	Gln	Arg	Pro	Gly	Leu	Gln
						210			215				220		
Leu	Tyr	Phe	Ser	Phe	Tyr	Met	Gly	Ser	Lys	Thr	Leu	Arg	Gly	Arg	Asn
						225			230				235		240

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Thr Ser Ser Glu Tyr Gln Ile Leu Thr Ala Arg Arg Glu Asp Ser Gly
 245 250 255
 Leu Tyr Trp Cys Glu Ala Ala Thr Glu Asp Gly Asn Val Leu Lys Arg
 260 265 270
 Ser Pro Glu Leu Glu Leu Gln Val Leu Gly Phe Phe Pro Pro Gly Tyr
 275 280 285
 Gln Val Ser Phe Cys Leu Val Met Val Leu Leu Phe Ala Val Asp Thr
 290 295 300
 Gly Leu Tyr Phe Ser Val Lys Thr Asn Ile Arg Ser Ser Thr Arg Asp
 305 310 315 320
 Trp Lys Asp His Lys Phe Lys Trp Arg Lys Asp Pro Gln Asp Lys Trp
 325 330 335
 Arg Arg Lys Arg Lys Glu Lys Gln Ser Glu Thr Ser Pro Lys Glu Phe
 340 345 350
 Leu Thr Ile Tyr Glu Asp Val Lys Asp Leu Lys Thr Arg Arg Asn His
 355 360 365
 Glu Gln Glu Gln Thr Phe Pro Gly Gly Ser Thr Ile Tyr Ser Met
 370 375 380
 Ile Gln Ser Gln Ser Ser Ala Pro Thr Ser Gln Glu Pro Ala Tyr Thr
 385 390 395 400
 Leu Tyr Ser Leu Ile Gln Pro Ser Arg Lys Ser Gly Ser Arg Lys Arg
 405 410 415
 Asn His Ser Pro Ser Phe Asn Ser Thr Ile Tyr Glu Val Ile Gly Lys
 420 425 430
 Ser Gln Pro Lys Ala Gln Asn Pro Ala Arg Leu Ser Arg Lys Glu Leu
 435 440 445
 Glu Asn Phe Asp Val Tyr Ser Gly Gly Ser Gly Gly Ser Gly Arg
 450 455 460
 Leu Lys Ile Gln Val Arg Lys Ala Ala Ile Thr Ser Tyr Glu Lys Ser
 465 470 475 480
 Asp Gly Val Tyr Thr Gly Leu Ser Thr Arg Asn Gln Glu Thr Tyr Glu
 485 490 495
 Thr Leu Lys His Glu Lys Pro Pro Gln
 500 505

<210> SEQ ID NO 22
<211> LENGTH: 485
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Polypeptide

<400> SEQUENCE: 22

Met	Trp	Phe	Leu	Thr	Thr	Leu	Leu	Leu	Trp	Val	Pro	Val	Asp	Gly	Gln
1					5					10					15

Val	Asp	Thr	Thr	Lys	Ala	Val	Ile	Thr	Leu	Gln	Pro	Pro	Trp	Val	Ser
20								25					30		

Val Phe Gln Glu Glu Thr Val Thr Leu His Cys Glu Val Leu His Leu
35 40 45

Pro Gly Ser Ser Ser Thr Gln Trp Phe Leu Asn Gly Thr Ala Thr Gln
50 55 60

Thr	Ser	Thr	Pro	Ser	Tyr	Arg	Ile	Thr	Ser	Ala	Ser	Val	Asn	Asp	Ser
65					70					75					80

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Gly Glu Tyr Arg Cys Gln Arg Gly Leu Ser Gly Arg Ser Asp Pro Ile
 85 90 95
 Gln Leu Glu Ile His Arg Gly Trp Leu Leu Leu Gln Val Ser Ser Arg
 100 105 110
 Val Phe Thr Glu Gly Glu Pro Leu Ala Leu Arg Cys His Ala Trp Lys
 115 120 125
 Asp Lys Leu Val Tyr Asn Val Leu Tyr Tyr Arg Asn Gly Lys Ala Phe
 130 135 140
 Lys Phe Phe His Trp Asn Ser Asn Leu Thr Ile Leu Lys Thr Asn Ile
 145 150 155 160
 Ser His Asn Gly Thr Tyr His Cys Ser Gly Met Gly Lys His Arg Tyr
 165 170 175
 Thr Ser Ala Gly Ile Ser Val Thr Val Lys Glu Leu Phe Pro Ala Pro
 180 185 190
 Val Leu Asn Ala Ser Val Thr Ser Pro Leu Leu Glu Gly Asn Leu Val
 195 200 205
 Thr Leu Ser Cys Glu Thr Lys Leu Leu Gln Arg Pro Gly Leu Gln
 210 215 220
 Leu Tyr Phe Ser Phe Tyr Met Gly Ser Lys Thr Leu Arg Gly Arg Asn
 225 230 235 240
 Thr Ser Ser Glu Tyr Gln Ile Leu Thr Ala Arg Arg Glu Asp Ser Gly
 245 250 255
 Leu Tyr Trp Cys Glu Ala Ala Thr Glu Asp Gly Asn Val Leu Lys Arg
 260 265 270
 Ser Pro Glu Leu Glu Leu Gln Val Leu Gly Phe Phe Pro Pro Gly Tyr
 275 280 285
 Gln Val Ser Phe Cys Leu Val Met Val Leu Leu Phe Ala Val Asp Thr
 290 295 300
 Gly Leu Tyr Phe Ser Val Lys Thr Asn Ile Arg Ser Ser Thr Arg Asp
 305 310 315 320
 Trp Lys Asp His Lys Phe Lys Trp Arg Lys Asp Pro Gln Asp Lys Trp
 325 330 335
 Arg Arg Lys Arg Lys Glu Lys Gln Ser Glu Thr Ser Pro Lys Glu Phe
 340 345 350
 Leu Thr Ile Tyr Glu Asp Val Lys Asp Leu Lys Thr Arg Arg Asn His
 355 360 365
 Glu Gln Glu Gln Thr Phe Pro Gly Gly Ser Thr Ile Tyr Ser Met
 370 375 380
 Ile Gln Ser Gln Ser Ser Ala Pro Thr Ser Gln Glu Pro Ala Tyr Thr
 385 390 395 400
 Leu Tyr Ser Leu Ile Gln Pro Ser Arg Lys Ser Gly Ser Arg Lys Arg
 405 410 415
 Asn His Ser Pro Ser Phe Asn Ser Thr Ile Tyr Glu Val Ile Gly Lys
 420 425 430
 Ser Gln Pro Lys Ala Gln Asn Pro Ala Arg Leu Ser Arg Lys Glu Leu
 435 440 445
 Glu Asn Phe Asp Val Tyr Ser Gly Gly Ser Gly Gly Ser Gly Ala
 450 455 460
 Arg Pro Arg Arg Ser Pro Ala Gln Glu Asp Gly Lys Val Tyr Ile Asn
 465 470 475 480
 Met Pro Gly Arg Gly

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485

<210> SEQ_ID NO 23
<211> LENGTH: 512
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Polypeptide

<400> SEQUENCE: 23

Met Trp Phe Leu Thr Thr Leu Leu Leu Trp Val Pro Val Asp Gly Gln
1 5 10 15

Val Asp Thr Thr Lys Ala Val Ile Thr Leu Gln Pro Pro Trp Val Ser
20 25 30

Val Phe Gln Glu Glu Thr Val Thr Leu His Cys Glu Val Leu His Leu
35 40 45

Pro Gly Ser Ser Ser Thr Gln Trp Phe Leu Asn Gly Thr Ala Thr Gln
50 55 60

Thr Ser Thr Pro Ser Tyr Arg Ile Thr Ser Ala Ser Val Asn Asp Ser
65 70 75 80

Gly Glu Tyr Arg Cys Gln Arg Gly Leu Ser Gly Arg Ser Asp Pro Ile
85 90 95

Gln Leu Glu Ile His Arg Gly Trp Leu Leu Leu Gln Val Ser Ser Arg
100 105 110

Val Phe Thr Glu Gly Glu Pro Leu Ala Leu Arg Cys His Ala Trp Lys
115 120 125

Asp Lys Leu Val Tyr Asn Val Leu Tyr Tyr Arg Asn Gly Lys Ala Phe
130 135 140

Lys Phe Phe His Trp Asn Ser Asn Leu Thr Ile Leu Lys Thr Asn Ile
145 150 155 160

Ser His Asn Gly Thr Tyr His Cys Ser Gly Met Gly Lys His Arg Tyr
165 170 175

Thr Ser Ala Gly Ile Ser Val Thr Val Lys Glu Leu Phe Pro Ala Pro
180 185 190

Val Leu Asn Ala Ser Val Thr Ser Pro Leu Leu Glu Gly Asn Leu Val
195 200 205

Thr Leu Ser Cys Glu Thr Lys Leu Leu Leu Gln Arg Pro Gly Leu Gln
210 215 220

Leu Tyr Phe Ser Phe Tyr Met Gly Ser Lys Thr Leu Arg Gly Arg Asn
225 230 235 240

Thr Ser Ser Glu Tyr Gln Ile Leu Thr Ala Arg Arg Glu Asp Ser Gly
245 250 255

Leu Tyr Trp Cys Glu Ala Ala Thr Glu Asp Gly Asn Val Leu Lys Arg
260 265 270

Ser Pro Glu Leu Glu Leu Gln Val Leu Gly Phe Phe Pro Pro Gly Tyr
275 280 285

Gln Val Ser Phe Cys Leu Val Met Val Leu Leu Phe Ala Val Asp Thr
290 295 300

Gly Leu Tyr Phe Ser Val Lys Thr Asn Ile Arg Ser Ser Thr Arg Asp
305 310 315 320

Trp Lys Asp His Lys Phe Lys Trp Arg Lys Asp Pro Gln Asp Lys Trp
325 330 335

Arg Arg Lys Arg Lys Glu Lys Gln Ser Glu Thr Ser Pro Lys Glu Phe

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340	345	350	
Leu Thr Ile Tyr Glu Asp Val Lys Asp Leu Lys Thr Arg Arg Asn His			
355	360	365	
Glu Gln Glu Gln Thr Phe Pro Gly Gly Ser Thr Ile Tyr Ser Met			
370	375	380	
Ile Gln Ser Gln Ser Ser Ala Pro Thr Ser Gln Glu Pro Ala Tyr Thr			
385	390	395	400
Leu Tyr Ser Leu Ile Gln Pro Ser Arg Lys Ser Gly Ser Arg Lys Arg			
405	410	415	
Asn His Ser Pro Ser Phe Asn Ser Thr Ile Tyr Glu Val Ile Gly Lys			
420	425	430	
Ser Gln Pro Lys Ala Gln Asn Pro Ala Arg Leu Ser Arg Lys Glu Leu			
435	440	445	
Glu Asn Phe Asp Val Tyr Ser Gly Gly Ser Gly Gly Ser Gly Gly			
450	455	460	
Arg Leu Val Pro Arg Gly Arg Gly Ala Ala Glu Ala Ala Thr Arg Lys			
465	470	475	480
Gln Arg Ile Thr Glu Thr Glu Ser Pro Tyr Gln Glu Leu Gln Gly Gln			
485	490	495	
Arg Ser Asp Val Tyr Ser Asp Leu Asn Thr Gln Arg Pro Tyr Tyr Lys			
500	505	510	

<210> SEQ ID NO 24
<211> LENGTH: 615
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Polypeptide

<400> SEQUENCE: 24

Met Trp Phe Leu Thr Thr Leu Leu Leu Trp Val Pro Val Asp Gly Gln			
1	5	10	15
Val Asp Thr Thr Lys Ala Val Ile Thr Leu Gln Pro Pro Trp Val Ser			
20	25	30	
Val Phe Gln Glu Glu Thr Val Thr Leu His Cys Glu Val Leu His Leu			
35	40	45	
Pro Gly Ser Ser Ser Thr Gln Trp Phe Leu Asn Gly Thr Ala Thr Gln			
50	55	60	
Thr Ser Thr Pro Ser Tyr Arg Ile Thr Ser Ala Ser Val Asn Asp Ser			
65	70	75	80
Gly Glu Tyr Arg Cys Gln Arg Gly Leu Ser Gly Arg Ser Asp Pro Ile			
85	90	95	
Gln Leu Glu Ile His Arg Gly Trp Leu Leu Leu Gln Val Ser Ser Arg			
100	105	110	
Val Phe Thr Glu Gly Glu Pro Leu Ala Leu Arg Cys His Ala Trp Lys			
115	120	125	
Asp Lys Leu Val Tyr Asn Val Leu Tyr Tyr Arg Asn Gly Lys Ala Phe			
130	135	140	
Lys Phe Phe His Trp Asn Ser Asn Leu Thr Ile Leu Lys Thr Asn Ile			
145	150	155	160
Ser His Asn Gly Thr Tyr His Cys Ser Gly Met Gly Lys His Arg Tyr			
165	170	175	
Thr Ser Ala Gly Ile Ser Val Thr Val Lys Glu Leu Phe Pro Ala Pro			

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180	185	190
Val Leu Asn Ala Ser Val Thr Ser Pro Leu Leu Glu Gly Asn Leu Val		
195	200	205
Thr Leu Ser Cys Glu Thr Lys Leu Leu Leu Gln Arg Pro Gly Leu Gln		
210	215	220
Leu Tyr Phe Ser Phe Tyr Met Gly Ser Lys Thr Leu Arg Gly Arg Asn		
225	230	235
240		
Thr Ser Ser Glu Tyr Gln Ile Leu Thr Ala Arg Arg Glu Asp Ser Gly		
245	250	255
Leu Tyr Trp Cys Glu Ala Ala Thr Glu Asp Gly Asn Val Leu Lys Arg		
260	265	270
Ser Pro Glu Leu Glu Leu Gln Val Leu Gly Leu Gln Leu Pro Thr Pro		
275	280	285
Val Trp Phe His Val Leu Phe Tyr Leu Ala Val Gly Ile Met Phe Leu		
290	295	300
Val Asn Thr Val Leu Trp Val Thr Ile Arg Lys Glu Leu Lys Arg Lys		
305	310	315
320		
Lys Lys Trp Asp Leu Glu Ile Ser Leu Asp Ser Gly His Glu Lys Lys		
325	330	335
Val Ile Ser Ser Leu Gln Glu Asp Arg His Leu Glu Glu Leu Lys		
340	345	350
Cys Gln Glu Gln Lys Glu Glu Gln Leu Gln Glu Gly Val His Arg Lys		
355	360	365
Glu Pro Gln Gly Ala Thr Gly Trp Arg Arg Lys Arg Lys Glu Lys Gln		
370	375	380
Ser Glu Thr Ser Pro Lys Glu Phe Leu Thr Ile Tyr Glu Asp Val Lys		
385	390	395
400		
Asp Leu Lys Thr Arg Arg Asn His Glu Gln Glu Gln Thr Phe Pro Gly		
405	410	415
Gly Gly Ser Thr Ile Tyr Ser Met Ile Gln Ser Gln Ser Ser Ala Pro		
420	425	430
Thr Ser Gln Glu Pro Ala Tyr Thr Leu Tyr Ser Leu Ile Gln Pro Ser		
435	440	445
Arg Lys Ser Gly Ser Arg Lys Arg Asn His Ser Pro Ser Phe Asn Ser		
450	455	460
Thr Ile Tyr Glu Val Ile Gly Lys Ser Gln Pro Lys Ala Gln Asn Pro		
465	470	475
480		
Ala Arg Leu Ser Arg Lys Glu Leu Glu Asn Phe Asp Val Tyr Ser Gly		
485	490	495
Gly Ser Gly Gly Ser Gly Arg Val Lys Phe Ser Arg Ser Ala Asp		
500	505	510
Ala Pro Ala Tyr Lys Gln Gly Gln Asn Gln Leu Tyr Asn Glu Leu Asn		
515	520	525
Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg Arg Gly Arg		
530	535	540
Asp Pro Glu Met Gly Gly Lys Pro Arg Arg Lys Asn Pro Gln Glu Gly		
545	550	555
560		
Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu		
565	570	575
Ile Gly Met Lys Gly Glu Arg Arg Gly Lys Gly His Asp Gly Leu		
580	585	590

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Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp Ala Leu His
595 600 605

Met Gln Ala Leu Pro Pro Arg
610 615

<210> SEQ ID NO 25

<211> LENGTH: 248

<212> TYPE: PRT

<213> ORGANISM: Canis lupus familiaris

<400> SEQUENCE: 25

Met Trp Gln Leu Val Ser Ser Thr Ala Leu Leu Leu Val Ser Ala
1 5 10 15

Gly Thr Gln Ala Asp Val Pro Lys Ala Val Val Val Leu Glu Pro Lys
20 25 30

Trp Asn Arg Val Leu Thr Met Asp Ser Val Thr Leu Lys Cys Gln Gly
35 40 45

Asp His Leu Leu Arg Asp Asn Tyr Thr Trp Leu His Asn Gly Arg Pro
50 55 60

Ile Ser Asn Gln Ile Ser Thr Tyr Ile Ile Lys Asn Ala Ser Ile Lys
65 70 75 80

Asn Ser Gly Glu Tyr Arg Cys Gln Thr Asp Gln Ser Lys Leu Ser Asp
85 90 95

Pro Val Gln Leu Glu Val His Thr Gly Trp Leu Leu Leu Gln Val Pro
100 105 110

Arg Leu Val Phe Gln Glu Gly Glu Leu Ile Gln Leu Lys Cys His Ser
115 120 125

Trp Lys Asn Thr Pro Val Arg Asn Val Gln Tyr Phe Gln Asn Gly Arg
130 135 140

Gly Lys Lys Phe Phe Tyr Asn Asn Ser Glu Tyr His Ile Pro Ala Ala
145 150 155 160

Thr Ser Glu His Asn Gly Ser Tyr Phe Cys Arg Gly Ile Ile Gly Lys
165 170 175

Lys Asn Glu Ser Ser Glu Ala Val Asn Ile Ile Ile Gln Gly Ser Ser
180 185 190

Leu Pro Ser Thr Ser Leu Leu Leu Ser His Trp Pro Gln Ile Pro Phe
195 200 205

Ser Leu Val Met Ala Leu Leu Phe Ala Val Asp Thr Gly Leu Tyr Phe
210 215 220

Ala Val Gln Arg Asp Leu Arg Ser Ser Met Gly Asn Leu Lys Asn Ser
225 230 235 240

Lys Val Ile Trp Ser Gln Gly Ser
245

<210> SEQ ID NO 26

<211> LENGTH: 372

<212> TYPE: PRT

<213> ORGANISM: Canis lupus familiaris

<400> SEQUENCE: 26

Met Trp Leu Leu Thr Val Leu Leu Trp Val Pro Ala Gly Ala Gln
1 5 10 15

Thr Asp Pro Val Lys Ala Val Ile Thr Leu Gln Pro Pro Trp Val Ser
20 25 30

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Val	Phe	Gln	Glu	Glu	Ser	Val	Thr	Leu	Trp	Cys	Glu	Gly	Pro	His	Leu
35						40					45				
Pro	Gly	Asp	Ser	Ser	Thr	Gln	Trp	Phe	Leu	Asn	Gly	Thr	Ala	Thr	Gln
50						55				60					
Thr	Leu	Thr	Pro	Arg	Tyr	Arg	Ile	Ala	Ala	Ala	Ser	Val	Asn	Asp	Asn
65						70				75			80		
Gly	Glu	Tyr	Arg	Cys	Gln	Thr	Gly	Leu	Ser	Val	Leu	Ser	Asp	Pro	Ile
	85					90				95					
Gln	Leu	Gly	Ile	His	Arg	Asp	Trp	Leu	Ile	Leu	Gln	Val	Ser	Gly	Arg
	100					105				110					
Val	Phe	Thr	Glu	Gly	Glu	Pro	Leu	Thr	Leu	Arg	Cys	His	Gly	Trp	Asn
115						120				125					
Asn	Lys	Leu	Val	Tyr	Asn	Val	Leu	Phe	Tyr	Gln	Asn	Gly	Thr	Val	Leu
130						135				140					
Lys	Phe	Ser	Pro	Gln	Asn	Ser	Glu	Phe	Thr	Ile	Leu	Lys	Thr	Thr	Leu
145						150				155			160		
His	His	Asn	Gly	Ile	Tyr	His	Cys	Ser	Ala	Met	Gly	Lys	His	Arg	Tyr
	165					170				175					
Glu	Ser	Ala	Gly	Val	Ser	Ile	Thr	Ile	Lys	Glu	Leu	Phe	Pro	Ala	Pro
	180					185				190					
Val	Leu	Lys	Ala	Ser	Leu	Ser	Ser	Pro	Ile	Leu	Glu	Gly	His	Val	Val
	195					200				205					
Asn	Leu	Ser	Cys	Glu	Thr	Lys	Leu	Leu	Gln	Arg	Pro	Gly	Leu	Gln	
	210					215				220					
Leu	Tyr	Phe	Ser	Phe	Tyr	Met	Gly	Ser	Lys	Thr	Leu	Leu	Ser	Arg	Asn
225						230				235			240		
Thr	Ser	Ser	Glu	Tyr	Gln	Ile	Leu	Thr	Ala	Lys	Lys	Glu	Asp	Ser	Gly
	245					250				255					
Leu	Tyr	Trp	Cys	Glu	Ala	Thr	Thr	Glu	Asp	Gly	Asn	Val	Val	Lys	Arg
	260					265				270					
Ser	Pro	Glu	Leu	Glu	Leu	Gln	Val	Val	Gly	Pro	Gln	Thr	Leu	Thr	Pro
	275					280				285					
Val	Trp	Phe	His	Val	Leu	Phe	Tyr	Val	Ala	Met	Gly	Met	Ile	Phe	Leu
	290					295				300					
Val	Asp	Thr	Ile	Phe	Cys	Met	Ile	Ile	His	Lys	Glu	Leu	Gln	Arg	Lys
	305					310				315			320		
Lys	Lys	Trp	Asn	Leu	Glu	Ile	Ser	Leu	Tyr	Ser	Gly	Leu	Glu	Lys	Arg
	325					330				335					
Val	Asp	Ser	Tyr	Leu	Gln	Lys	Glu	Arg	Asp	Leu	Glu	Glu	Pro	Lys	Tyr
	340					345				350					
Gln	Glu	Leu	Glu	Gln	Leu	Gln	Glu	Lys	Thr	Pro	Gln	Lys	Pro	Pro	Glu
	355					360				365					
Gly	Glu	Gln	Gln												
	370														

<210> SEQ_ID NO 27
<211> LENGTH: 287
<212> TYPE: PRT
<213> ORGANISM: Canis lupus familiaris

<400> SEQUENCE: 27

Met Trp Leu Leu Thr Val Leu Leu Leu Trp Val Pro Ala Gly Ala Gln

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1	5	10	15
Thr Asp Trp Leu Ile Leu Gln Val Ser Gly Arg Val Phe Thr Glu Gly			
20	25	30	
Glu Pro Leu Thr Leu Arg Cys His Gly Trp Asn Asn Lys Leu Val Tyr			
35	40	45	
Asn Val Leu Phe Tyr Gln Asn Gly Thr Val Leu Lys Phe Ser Pro Gln			
50	55	60	
Asn Ser Glu Phe Thr Ile Leu Lys Thr Thr Leu His His Asn Gly Ile			
65	70	75	80
Tyr His Cys Ser Ala Met Gly Lys His Arg Tyr Glu Ser Ala Gly Val			
85	90	95	
Ser Ile Thr Ile Lys Glu Leu Phe Pro Ala Pro Val Leu Lys Ala Ser			
100	105	110	
Leu Ser Ser Pro Ile Leu Glu Gly His Val Val Asn Leu Ser Cys Glu			
115	120	125	
Thr Lys Leu Leu Gln Arg Pro Gly Leu Gln Leu Tyr Phe Ser Phe			
130	135	140	
Tyr Met Gly Ser Lys Thr Leu Leu Ser Arg Asn Thr Ser Ser Glu Tyr			
145	150	155	160
Gln Ile Leu Thr Ala Lys Lys Glu Asp Ser Gly Leu Tyr Trp Cys Glu			
165	170	175	
Ala Thr Thr Glu Asp Gly Asn Val Val Lys Arg Ser Pro Glu Leu Glu			
180	185	190	
Leu Gln Val Val Gly Pro Gln Thr Leu Thr Pro Val Trp Phe His Val			
195	200	205	
Leu Phe Tyr Val Ala Met Gly Met Ile Phe Leu Val Asp Thr Ile Phe			
210	215	220	
Cys Met Ile Ile His Lys Glu Leu Gln Arg Lys Lys Lys Trp Asn Leu			
225	230	235	240
Glu Ile Ser Leu Tyr Ser Gly Leu Glu Lys Arg Val Asp Ser Tyr Leu			
245	250	255	
Gln Lys Glu Arg Asp Leu Glu Glu Pro Lys Tyr Gln Glu Leu Glu Gln			
260	265	270	
Leu Gln Glu Lys Thr Pro Gln Lys Pro Pro Glu Gly Glu Gln Gln			
275	280	285	

<210> SEQ ID NO 28

<211> LENGTH: 34

<212> TYPE: DNA

<213> ORGANISM: Artificial

<220> FEATURE:

<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 28

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34

<210> SEQ ID NO 29

<211> LENGTH: 42

<212> TYPE: DNA

<213> ORGANISM: Artificial

<220> FEATURE:

<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 29

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42

-continued

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<210> SEQ ID NO 30
<211> LENGTH: 42
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 30
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<210> SEQ ID NO 31
<211> LENGTH: 33
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 31
ccggaattct catttgtctt gagggtcctt tct                  33

<210> SEQ ID NO 32
<211> LENGTH: 34
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 32
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<210> SEQ ID NO 33
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<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 33
ccggaattct catttgtctt gagggtcctt tct                  33

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What is claimed is:

1. A chimeric IgG Fc receptor comprising:
an extracellular domain comprising a sufficient portion of CD64 to bind to an IgG Fc region;
a transmembrane domain; and
an intracellular domain comprising a sufficient portion of an Fc receptor immunoreceptor tyrosine-based activation motif (ITAM) to initiate cell signaling when an IgG Fc region binds to the extracellular domain.
2. The chimeric IgG Fc receptor of claim 1, wherein the intracellular domain comprises at least a portion of the intracellular region of CD16A.
3. The chimeric IgG Fc receptor of claim 1, wherein the intracellular domain comprises at least a portion of the intracellular region of CD27, CD28, CD134 (OX40), CD137 (4-1BB), FcεR1, NKG2D, CD244 (2B4), FcRγ, DAP10, DAP12, or CD3ζ.
4. The chimeric IgG Fc receptor of any preceding claim, wherein the extracellular domain comprises the CD16A cleavage site.
5. The chimeric IgG Fc receptor of any preceding claim, wherein the intracellular domain comprises a signaling domain.
6. A polynucleotide encoding the chimeric receptor of any preceding claim.
7. A recombinant cell comprising the polynucleotide of claim 6.
8. A recombinant cell expressing the IgG Fc chimeric receptor of any one of claims 1-5.
9. The recombinant cell of claim 8, wherein the recombinant cell is a natural killer (NK) cell.
10. A recombinant natural killer (NK) cell comprising a polynucleotide that encodes CD64.
11. A recombinant cell comprising a natural killer (NK) cell genetically modified to express CD64.
12. A method of killing a tumor cell, the method comprising:
contacting the tumor cell with an antibody that specifically binds to the tumor cell; and

contacting the tumor cell with the recombinant cell of any one of claims **7-11** under conditions effective for the recombinant cell to kill the tumor cell.

13. A method of treating a subject having a tumor, the method comprising:

administering to the subject an antibody that specifically binds to cells of the tumor; and

administering to the subject a composition comprising the recombinant cell of any one of claims **7-11** under conditions effective for the recombinant cell to kill cells of the tumor.

14. A composition comprising:

the recombinant cell of any one of claims **7-11**; and
an antibody bound to the chimeric receptor.

15. A method of treating a subject having a tumor, the method comprising:

administering to the subject the composition of claim **14** wherein the antibody specifically binds to cells of the tumor.

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