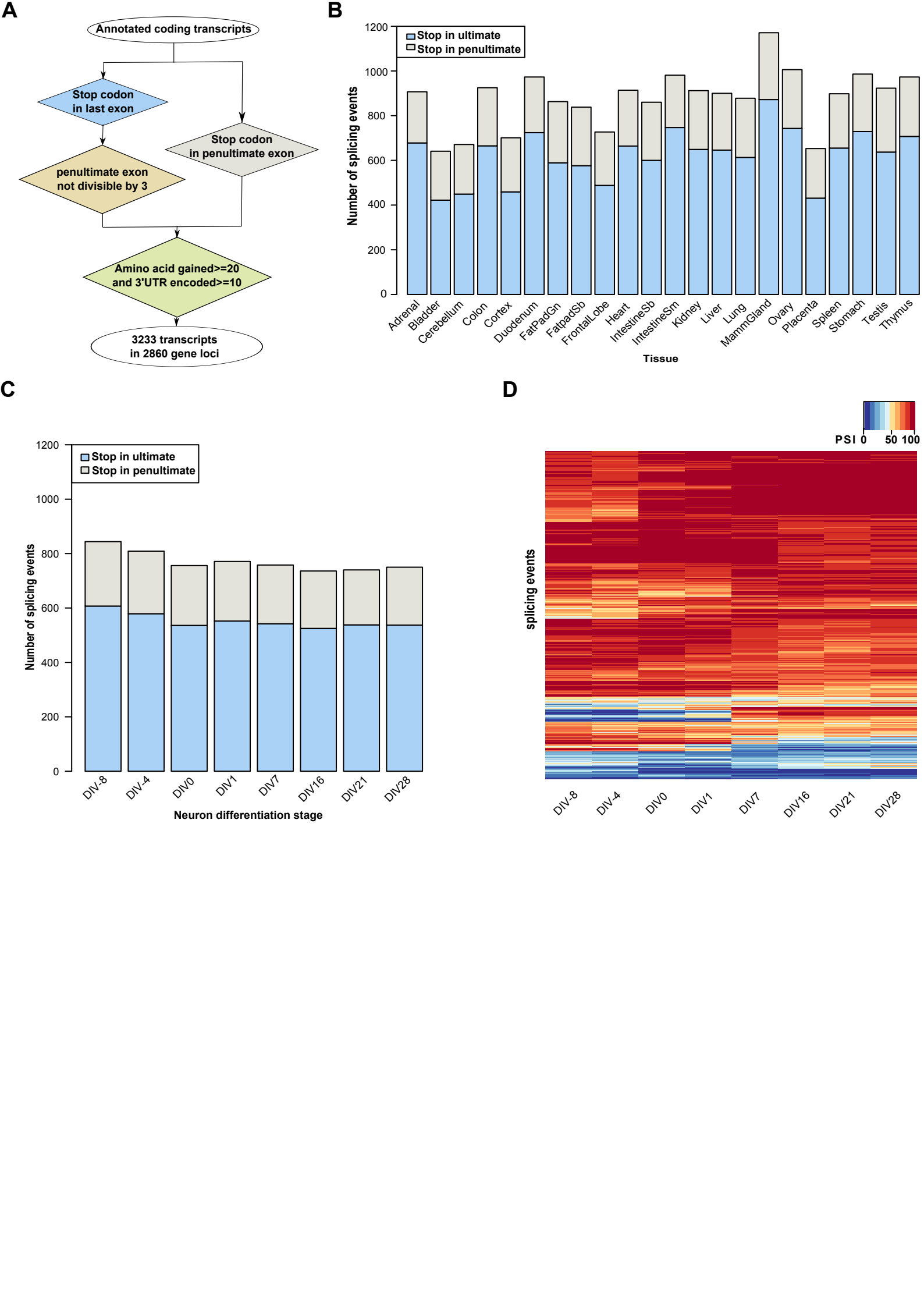
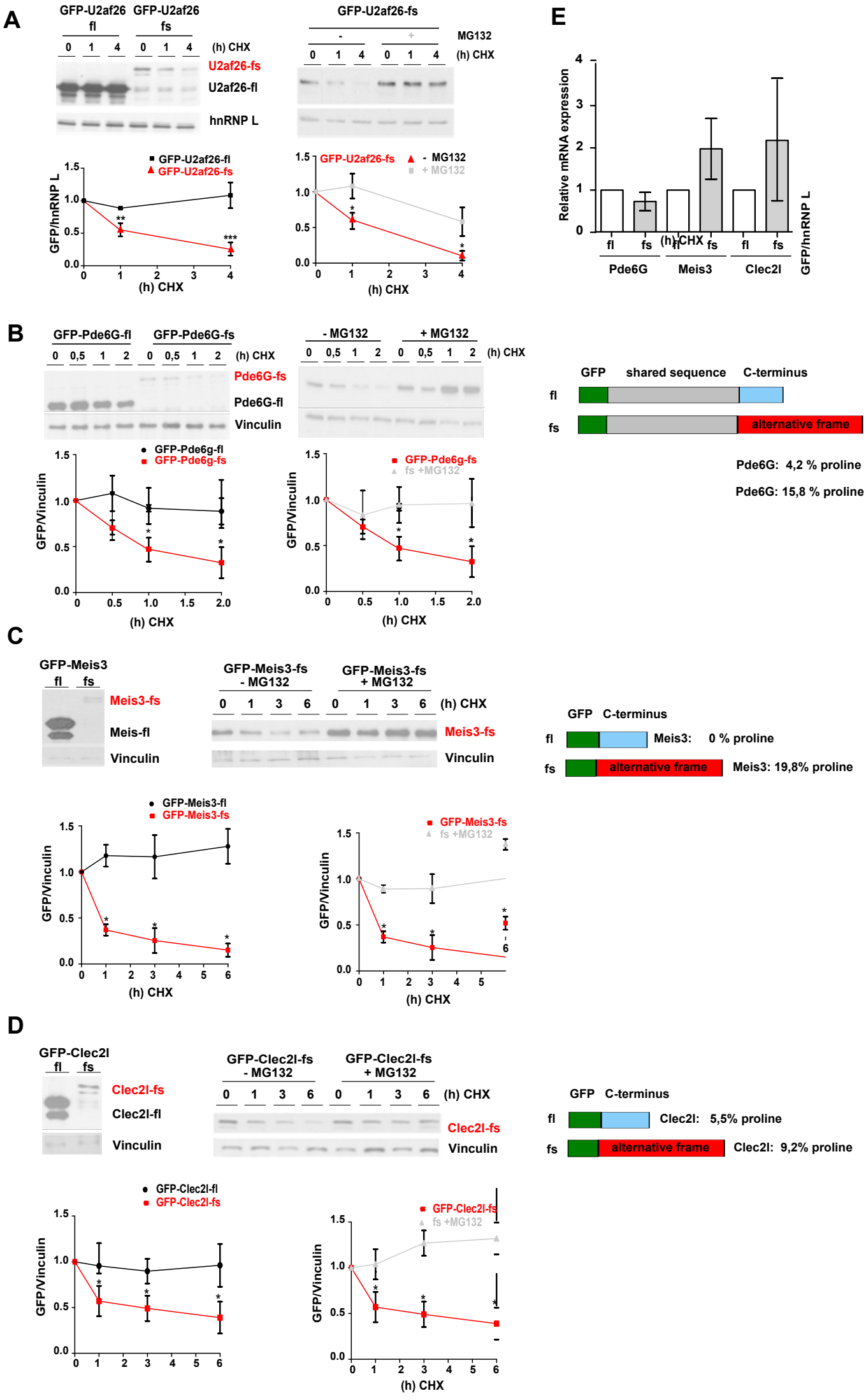
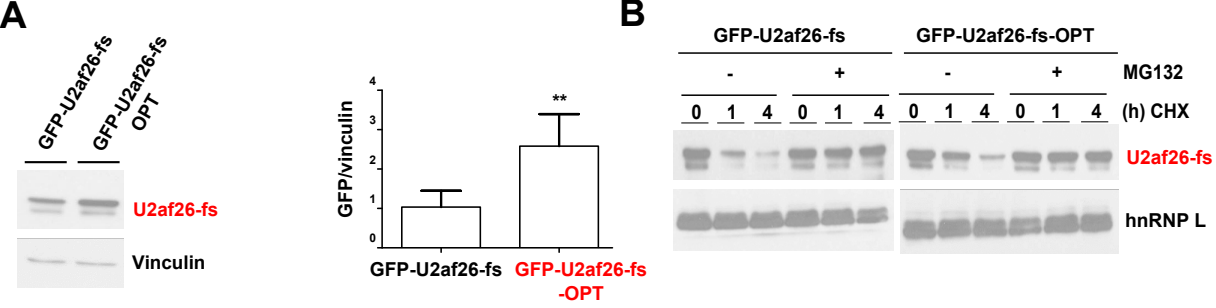

A
Annotated coding transcripts
Stop codon in last exon
Stop codon in penultimate exon
penultimate exon not divisible by 3
Amino acid gained>=20 and 3'UTR encoded>=10
3233 transcripts in 2860 gene loci
B
Stop in ultimate
Stop in penultimate
Number of splicing events
0
200
400
600
800
1000
1200
Adrenal
Bladder
Cerebellum
Colon
Cortex
Duodenum
FatPadGn
FatpadSb
FrontalLobe
Heart
IntestineSb
IntestineSm
Kidney
Liver
Lung
MammGland
Ovary
Placenta
Spleen
Stomach
Testis
Thymus
Tissue
C
Stop in ultimate
Stop in penultimate
Number of splicing events
0
200
400
600
800
1000
1200
DIV-8
DIV-4
DIV0
DIV1
DIV7
DIV16
DIV21
DIV28
Neuron differentiation stage
D
PSI 0
50
100
splicing events
DIV-8
DIV-4
DIV0
DIV1
DIV7
DIV16
DIV21
DIV28


A
GFP-U2af26 fl
GFP-U2af26 fs
0 1 4 0 1 4 (h) CHX
U2af26-fs
U2af26-fl
hnRNP L
GFP-U2af26-fl
GFP-U2af26-fs
GFP/hnRNP L
(h) CHX
GFP-U2af26-fs
- MG132
+ MG132
0 1 4 0 1 4 (h) CHX
GFP-U2af26-fs - MG132
GFP-U2af26-fs + MG132
E
Relative mRNA expression
GFP/hnRNP L
fl fs fl fs fl fs (h) CHX
Pde6G
Meis3
Clec2l
B
GFP-Pde6G-fl
GFP-Pde6G-fs
0 0,5 1 2 0 0,5 1 2 (h) CHX
- MG132
+ MG132
Pde6G-fs
Pde6G-fl
Vinculin
GFP-Pde6g-fl
GFP-Pde6g-fs
GFP/Vinculin
(h) CHX
GFP-Pde6g-fs
fs +MG132
GFP shared sequence C-terminus
fl
fs
alternative frame
Pde6G: 4,2 % proline
Pde6G: 15,8 % proline
C
GFP-Meis3
fl fs
Meis3-fs
Meis-fl
Vinculin
GFP-Meis3-fs - MG132
GFP-Meis3-fs + MG132
0 1 3 6 0 1 3 6 (h) CHX
Meis3-fs
Vinculin
GFP-Meis3-fl
GFP-Meis3-fs
GFP/Vinculin
(h) CHX
GFP-Meis3-fs
fs +MG132
GFP C-terminus
fl
Meis3: 0 % proline
fs
alternative frame
Meis3: 19,8% proline
D
GFP-Clec2l
fl fs
Clec2l-fs
Clec2l-fl
Vinculin
GFP-Clec2l-fs - MG132
GFP-Clec2l-fs + MG132
0 1 3 6 0 1 3 6 (h) CHX
Clec2l-fs
Vinculin
GFP-Clec2l-fl
GFP-Clec2l-fs
GFP/Vinculin
(h) CHX
GFP-Clec2l-fs
fs +MG132
GFP C-terminus
fl
Clec2l: 5,5% proline
fs
alternative frame
Clec2l: 9,2% proline


A
GFP-U2af26-fs
GFP-U2af26-fs OPT
U2af26-fs
Vinculin
GFP/vinculin
0
1
2
3
4
**
GFP-U2af26-fs
GFP-U2af26-fs -OPT
B
GFP-U2af26-fs
GFP-U2af26-fs-OPT
-
+
-
+
MG132
0 1 4 0 1 4 0 1 4 0 1 4
(h) CHX
U2af26-fs
hnRNP L

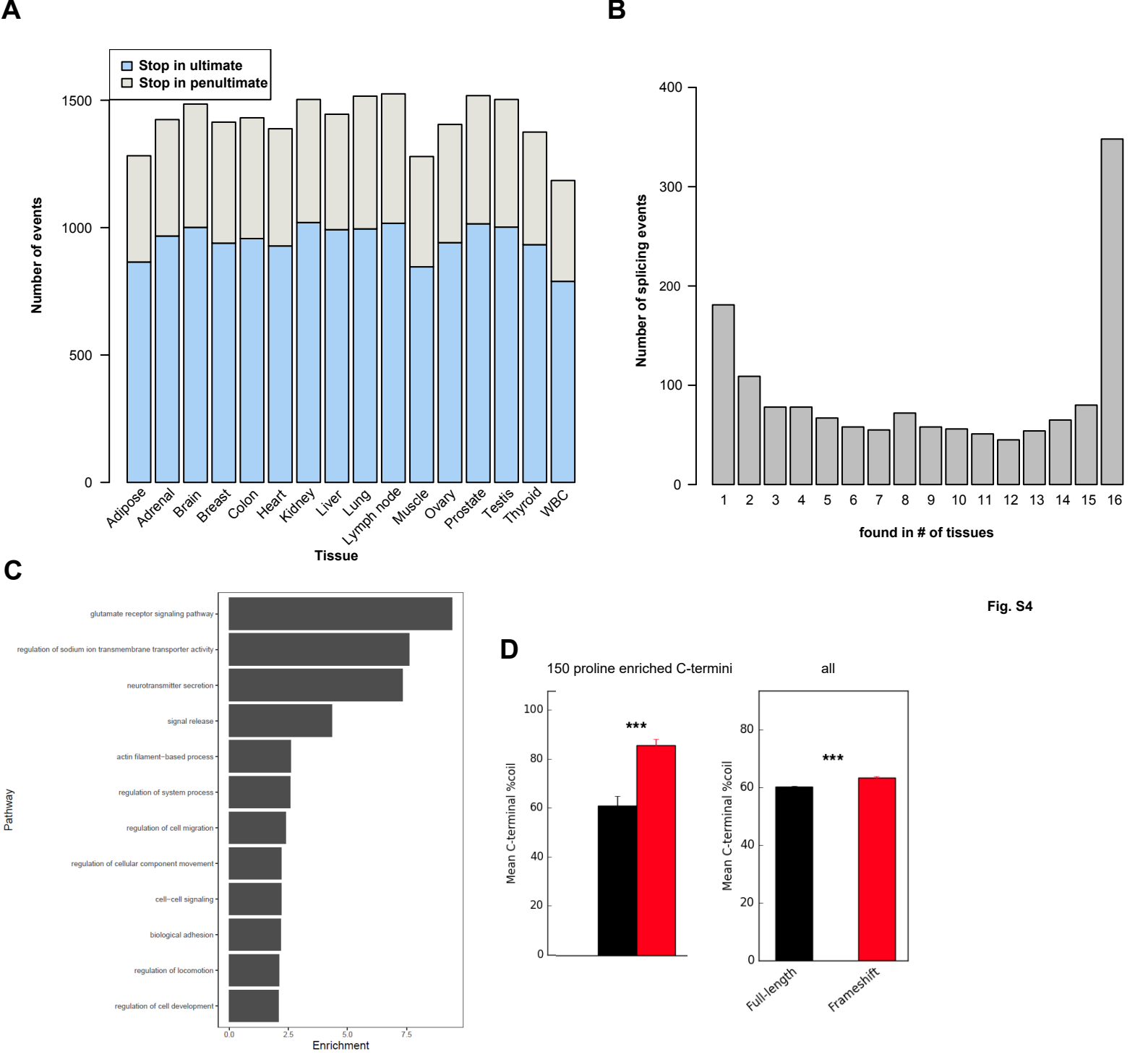
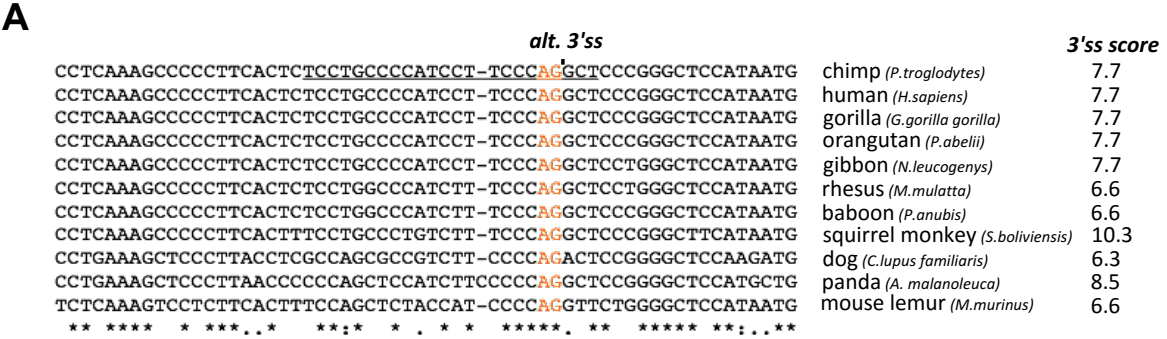
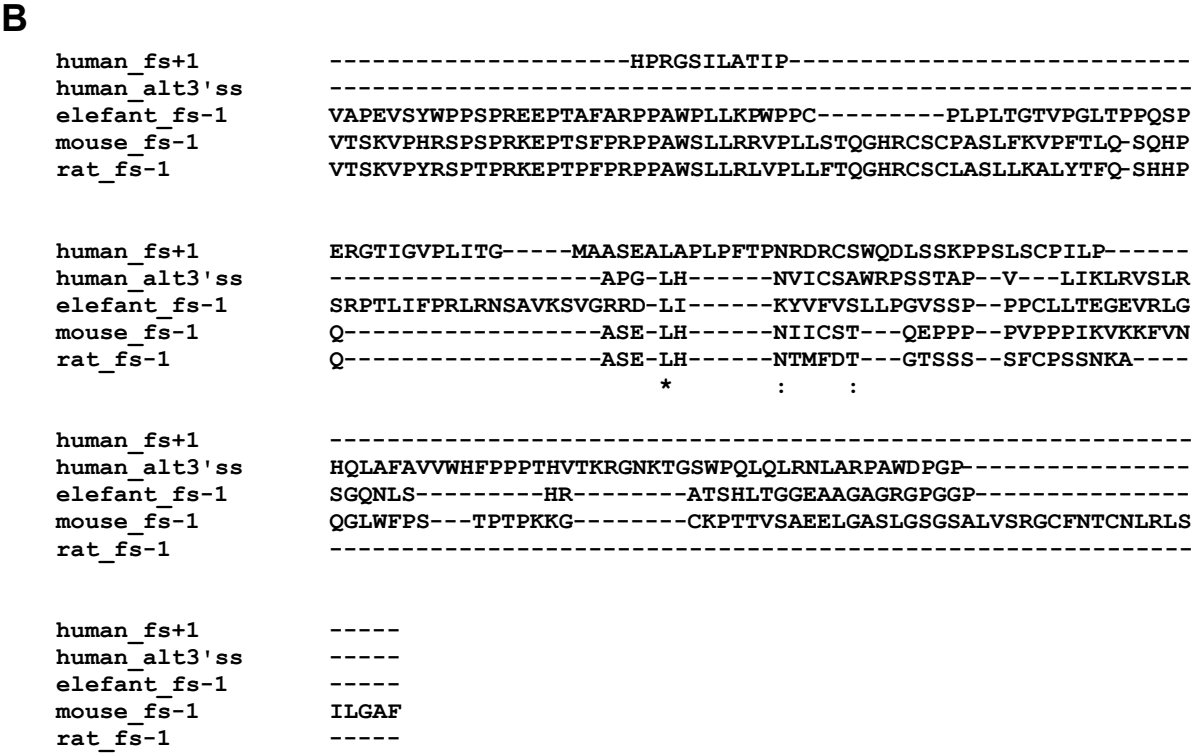

A
Stop in ultimate
Stop in penultimate
Number of events
1500
1000
500
0
Adipose
Adrenal
Brain
Breast
Colon
Heart
Kidney
Liver
Lung
Lymph node
Muscle
Ovary
Prostate
Testis
Thyroid
WBC
Tissue
B
Number of splicing events
400
300
200
100
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
found in # of tissues
C
glutamate receptor signaling pathway
regulation of sodium ion transmembrane transporter activity
neurotransmitter secretion
signal release
actin filament−based process
regulation of system process
regulation of cell migration
regulation of cellular component movement
cell−cell signaling
biological adhesion
regulation of locomotion
regulation of cell development
Pathway
0.0
2.5
5.0
7.5
Enrichment
D
150 proline enriched C-termini
all
Mean C-terminal %coil
***
***
Full-length
Frameshift

Fig. S4

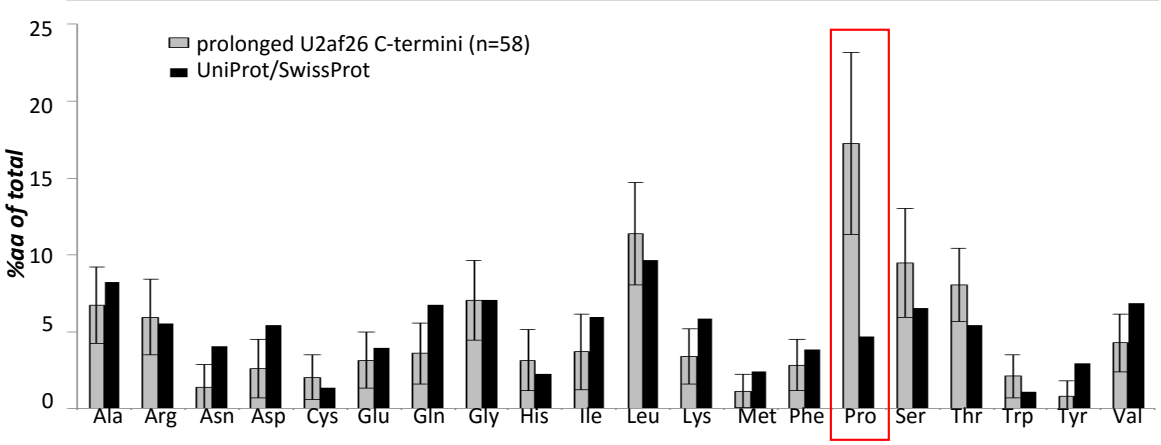
A


alt. 3'ss
3'ss score
CCTCAAAGCCCCCTTCACTCTCCTGCCCCATCCT-TCCCAGGCTCCCGGGCTCCATAATG chimp (P.troglodytes) 7.7
CCTCAAAGCCCCCTTCACTCTCCTGCCCCATCCT-TCCCAGGCTCCCGGGCTCCATAATG human (H.sapiens) 7.7
CCTCAAAGCCCCCTTCACTCTCCTGCCCCATCCT-TCCCAGGCTCCCGGGCTCCATAATG gorilla (G.gorilla gorilla) 7.7
CCTCAAAGCCCCCTTCACTCTCCTGCCCCATCCT-TCCCAGGCTCCCGGGCTCCATAATG orangutan (P.abelii) 7.7
CCTCAAAGCCCCCTTCACTCTCCTGCCCCATCCT-TCCCAGGCTCCTGGGCTCCATAATG gibbon (N.leucogenys) 7.7
CCTCAAAGCCCCCTTCACTCTCCTGGCCCATCTT-TCCCAGGCTCCTGGGCTCCATAATG rhesus (M.mulatta) 6.6
CCTCAAAGCCCCCTTCACTCTCCTGGCCCATCTT-TCCCAGGCTCCCGGGCTCCATAATG baboon (P.anubis) 6.6
CCTCAAAGCCCCCTTCACTTTCCTGCCCTGTCTT-TCCCAGGCTCCCGGGCTTCATAATG squirrel monkey (S.boliviensis) 10.3
CCTGAAAGCTCCCTTACCTCGCCAGCGCCGTCTT-CCCCAGACTCCGGGGCTCCAAGATG dog (C.lupus familiaris) 6.3
CCTGAAAGCTCCCTTAACCCCCCAGCTCCATCTTCCCCCAGGCTCCGGGGCTCCATGCTG panda (A. malanoleuca) 8.5
TCTCAAAGTCCTCTTCACTTTCCAGCTCTACCAT-CCCCAGGTTCTGGGGCTCCATAATG mouse lemur (M.murinus) 6.6

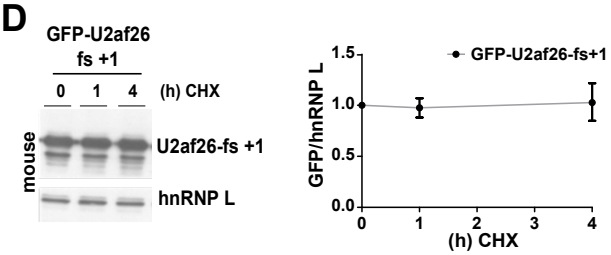
B

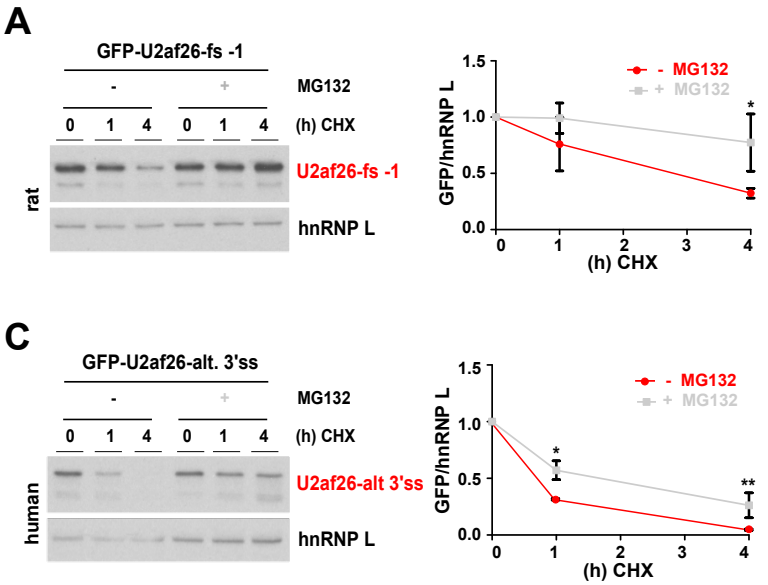

human_fs+1 ----------HPRGSILATIP----------
human_alt3'ss ----------
elefant_fs-1 VAPEVSYWPPSPREEPTAFARPPAWPLLKPWPPC---------PLPLTGTVPGLTPPQSP
mouse_fs-1 VTSKVPHRSPSPRKEPTSFPRPPAWSLLRRVPLLSTQGHRCSCPASLFKVPFTLQ-SQHP
rat_fs-1 VTSKVPYRSPTPRKEPTPFPRPPAWSLLRLVPLLFTQGHRCSCLASLLKALYTFQ-SHHP
human_fs+1 ERGTIGVPLITG-----MAASEALAPLPFTPNRDRCSWQDLSSKPPSLSCPILP------
human_alt3'ss ----------APG-LH------NVICSAWRPSSTAP--V---LIKLRVSLR
elefant_fs-1 SRPTLIFPRLRNSAVKSVGRRD-LI------KYVFVSLLPGVSSP--PPCLLTEGEVRLG
mouse_fs-1 Q----------ASE-LH------NIICST---QEPPP--PVPPPIKVKKFVN
rat_fs-1 Q----------ASE-LH------NTMFDT---GTSSS--SFCPSSNKA----
human_fs+1 ----------
human_alt3'ss HQLAFAVVWHFPPPTHVTKRGNKTGSWPQLQLRNLARPAWDPGP----------
elefant_fs-1 SGQNLS---------HR--------ATSHLTGGEAAGAGRGPGGP----------
mouse_fs-1 QGLWFPS---TPTPKKG--------CKPTTVSAEELGASLGSGSALVSRGCFNTCNLRLS
rat_fs-1 ----------
human_fs+1 -----
human_alt3'ss -----
elefant_fs-1 -----
mouse_fs-1 ILGAF
rat_fs-1 -----

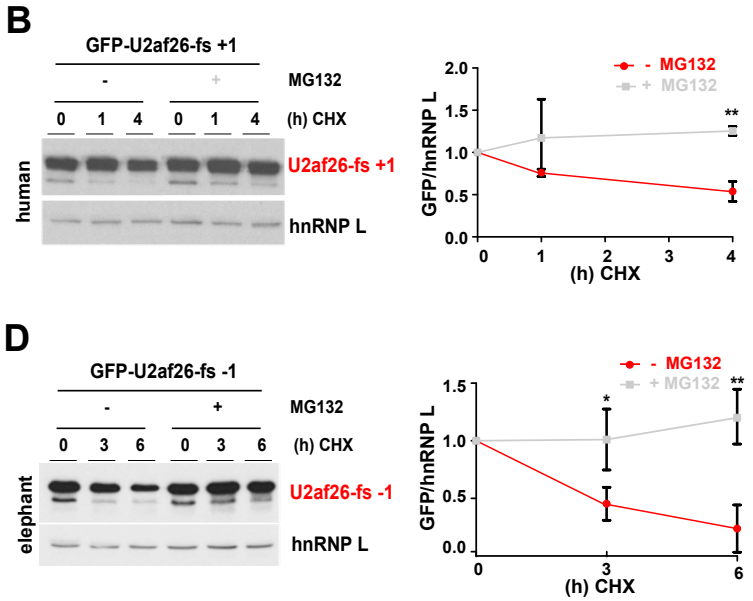
C


prolonged U2af26 C-termini (n=58)
UniProt/SwissProt
%aa of total
25
20
15
10
5
0
Ala Arg Asn Asp Cys Glu Gln Gly His Ile Leu Lys Met Phe Pro Ser Thr Trp Tyr Val

D

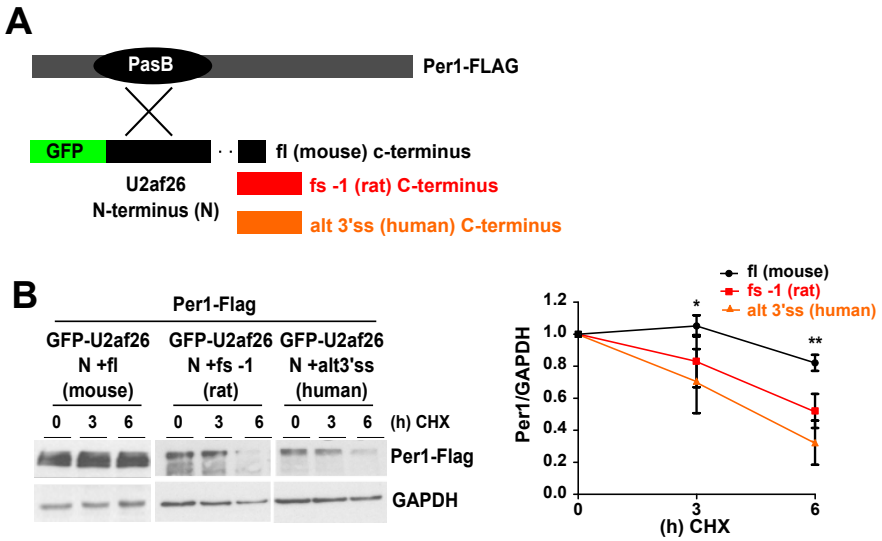

GFP-U2af26
fs +1
0 1 4 (h) CHX
mouse
U2af26-fs +1
hnRNP L
GFP-U2af26-fs+1
GFP/hnRNP L
1.5
1.0
0.5
0.0
0 1 2 3 4
(h) CHX


A
GFP-U2af26-fs -1
MG132
(h) CHX
rat
U2af26-fs -1
hnRNP L
GFP/hnRNP L
- MG132
+ MG132
(h) CHX


B
GFP-U2af26-fs +1
MG132
(h) CHX
human
U2af26-fs +1
hnRNP L
GFP/hnRNP L
- MG132
+ MG132
(h) CHX

C
GFP-U2af26-alt. 3'ss
MG132
(h) CHX
human
U2af26-alt 3'ss
hnRNP L
GFP/hnRNP L
- MG132
+ MG132
(h) CHX

D
GFP-U2af26-fs -1
MG132
(h) CHX
elephant
U2af26-fs -1
hnRNP L
GFP/hnRNP L
- MG132
+ MG132
(h) CHX


A
PasB
Per1-FLAG
GFP
fl (mouse) c-terminus
U2af26
N-terminus (N)
fs -1 (rat) C-terminus
alt 3'ss (human) C-terminus
B
Per1-Flag
GFP-U2af26 N +fl (mouse)
GFP-U2af26 N +fs -1 (rat)
GFP-U2af26 N +alt3'ss (human)
0 3 6 0 3 6 0 3 6 (h) CHX
Per1-Flag
GAPDH
fl (mouse)
fs -1 (rat)
alt 3'ss (human)
Per1/GAPDH
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
**
0
3
6
(h) CHX