

Figure S1. UPF3b-depleted cell clones exhibit an increase in cell doubling time. The B31, B83, and Luc cell clones and the HeLa cell line were plated at 1×10^4 cells per well in triplicate in 12-well plates and cultured at 37°C for 4 days. Cells were stained with trypan blue, and viable cells were counted every 24 h. The values shown are the means of three independent experiments. The B31, B83, and Luc cell clones are described in Figure 1A and B. Error bars indicate standard deviation. * $p \leq 0.05$; ** $p \leq 0.01$.

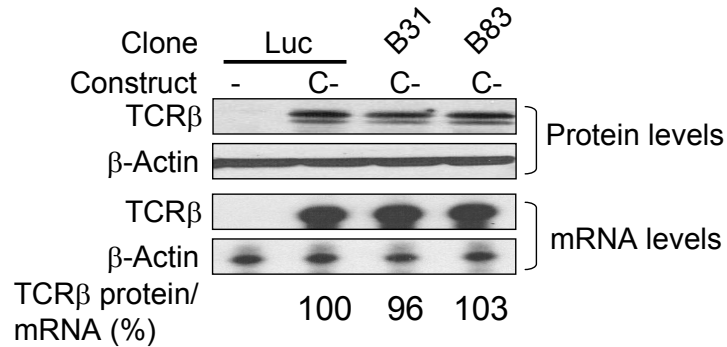


Figure S2. TCR β translation is unperturbed by depletion of UPF3b. UPF3b-depleted cell clones B31 and B83 were transfected with a plasmid encoding wild-type TCR β (construct C- in Figure 2A). Western blot and RNase protection analysis were performed 48 h after transfection on the cell lysates and the total cellular RNA from the transfected cells, respectively. The Luc clone and untransfected cells served as negative controls. Protein and mRNA levels were normalized to that of β -actin as the loading control. The numbers below the blot are the TCR β protein-to-mRNA ratio, a measure of translation efficiency. The results shown are representative of two independent experiments.

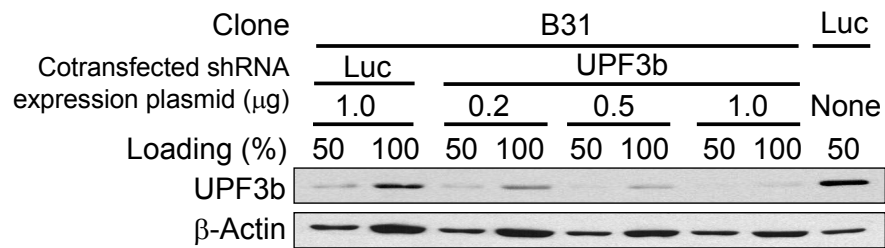


Figure S3. Depletion of UPF3b by ~90% was achieved using a “super-RNAi” procedure. Western blot analysis (performed as in Figure 1B) of the B31 cell clone transiently transfected with various amounts (μg) of the UPF3b shRNA expression plasmid. The Luc shRNA expression plasmid and Luc cell clone served as negative controls (described in Figure 1A and B).

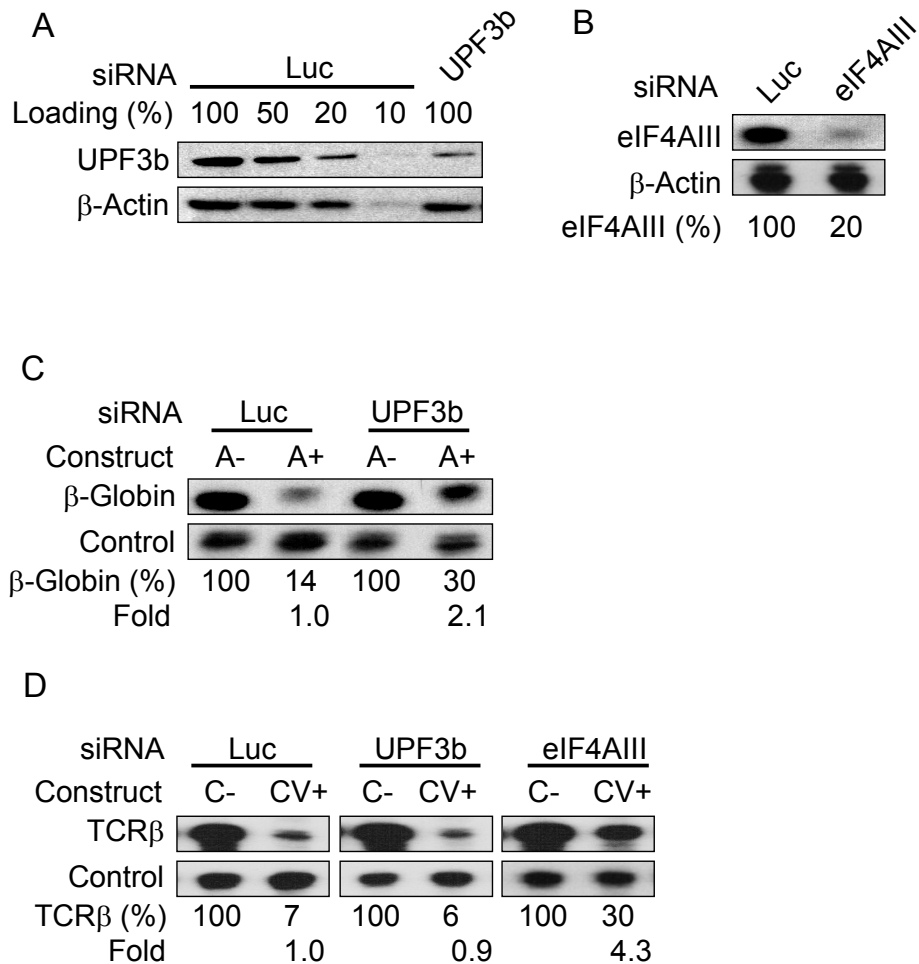


Figure S4. TCRβ NMD requires eIF4AIII. **(A)** Western blot analysis (performed as in Figure 1B) of HeLa cells depleted of UPF3b by siRNA. Firefly luciferase siRNA was used as a negative control. **(B)** RNase protection analysis (performed as in Figure 1A using probe h) of eIF4AIII mRNA levels in total cellular RNA isolated from HeLa cells depleted of eIF4AIII by siRNA. Firefly luciferase siRNA was used as a negative control. **(C)** RNase protection analysis (performed as in Figure 1C) of total cellular RNA isolated from HeLa cells transfected with siRNA against luciferase or UPF3b. Six hours later, the cells were transfected with constructs A- and A+ (Figure 1C). **(D)** RNase protection analysis (performed as in Figure 2C) of total cellular RNA isolated from HeLa cells transfected with siRNA against luciferase, UPF3b, or eIF4AIII. Six hours later, the cells were transfected with constructs C- and CV+ (Figure 2A). The results in panels C and D are representative of two independent experiments.

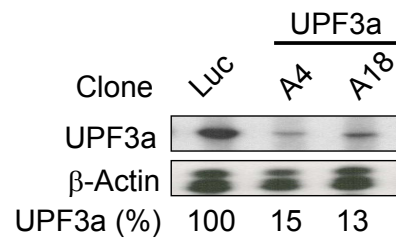


Figure S5. Establishment of HeLa cell clones stably depleted of UPF3a. RNase protection analysis (using probe g) of total cellular RNA from HeLa cell clones A4 and A18, which stably express the UPF3a shRNA plasmid. The Luc cell clone served as the negative control. Quantification was done as in Figure 1A.

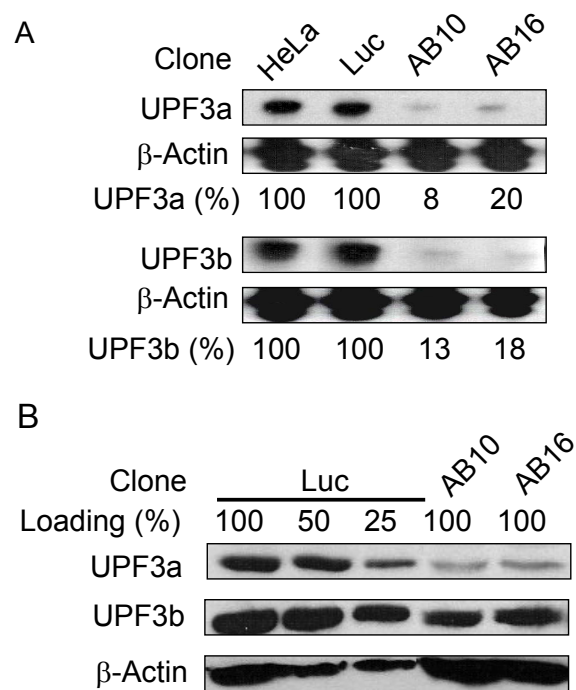


Figure S6. Establishment of HeLa cell clones stably depleted of UPF3a and UPF3b. **(A)** RNase protection analysis (using probes g and f for UPF3a and UPF3b, respectively) of total cellular RNA from HeLa cell clones AB10 and AB16, which both stably express both UPF3a and UPF3b shRNA plasmids. Untransfected HeLa cells and the Luc cell clone served as negative controls. Quantification was done as in Figure 1A. **(B)** Western blot analysis (performed as in Figure 1B) of the cells in panel A.

Supplementary Table I Transcripts downregulated by UPF3b

Listed are the 87 transcripts upregulated by 3 fold or more in UPF3b-depleted HeLa cells (p<0.01)

Fold change	Gene symbol	Gene title	Putative NMD-inducing features	Biological function	Probe set ID
23.0	EDNRA	endothelin receptor type A (204464 s at)	AS	G-protein coupled receptor	204463 s at
17.8	POLR2B	Polymerase (RNA) II (DNA directed) polypeptide B	unknown	transcription	232544 at
15.7	FAM31B	family with sequence similarity 31, member B	uORF	---	1552835 at
14.2	NAP1L3	nucleosome assembly protein 1-like 3	unknown (no intron)	nucleosome assembly	204749 at
13.7	HBE1	hemoglobin, epsilon 1	uORF	oxygen transport	205919 at
13.5	---	Homo sapiens, clone IMAGE:4818734, mRNA	unknown	---	1562389 at
13.0	CGA	glycoprotein hormones, alpha polypeptide	unknown	cell-cell signaling	204637 at
12.8	PASD1	PAS domain containing 1	uORF	signal transduction	240687 at
12.6	---	M41 mRNA, complete sequence; alternatively spliced	unknown	---	1562821 s at
12.3	NEGR1	neuronal growth regulator 1	unknown	cell adhesion	229461 x at
11.3	C14orf159	chromosome 14 open reading frame 159	uORF, AS	---	218298 s at
10.9	FBN2	fibrillin 2 (congenital contractural arachnodactyly)	unknown	morphogenesis	203184 at
10.9	IGSF10	immunoglobulin superfamily, member 10	unknown	protein amino acid phosphorylation	230670 at
10.6	GRIK2	GRIK2=Glur6 kainate receptor	uORF	---	213845 at
9.2	SHANK3	SH3 and multiple ankyrin repeat domains 3	unknown	---	227923 at
9.0	HMGGA2	high mobility group AT-hook 2	uORF	chromatin architecture, transcription	208025 s at
7.7	EMR1	egf-like module containing, mucin-like, hormone receptor-like 1	unknown	cell adhesion	207111 at
7.6	MGC10646	hypothetical protein MGC10646	unknown	---	210603 at
7.5	PCDH10	protocadherin 10	uORF	cell adhesion, cell adhesion	228635 at
7.2	FSTL1	Follistatin-like 1	unknown	---	208782 at
7.1	EDNRA	endothelin receptor type A (204463 s at)	AS	G-protein coupled receptor	204464 s at
6.8	FSTL1	Follistatin-like 1 (208782 at)	unknown	---	240891 at
6.6	BLVRA	Biliverdin reductase A	unknown	electron transport	229109 s at
6.5	TIMP3	tissue inhibitor of metalloproteinase 3	uORF	tyrosine kinase signaling	201150 s at
6.2	PDE1A	phosphodiesterase 1A, calmodulin-dependent	uORF	signal transduction	208396 s at
5.8	GPR87	G protein-coupled receptor 87	uORF	signal transduction	219936 s at
5.6	---	Similar to CG9996-PA	unknown	---	222288 at
5.4	EDNRA	endothelin receptor type A (204463 s at)	AS	G-protein coupled receptor	216235 s at
5.3	---	Homo sapiens, clone IMAGE:4398657, mRNA	uORF	---	1568795 at
5.2	---	Transcribed locus	unknown	---	230356 at
5.2	---	Transcribed locus	unknown	---	244636 at
5.1	DERP12	dermal papilla derived protein 12	unknown	---	1555623 at
4.9	MAN1A1	Mannosidase, alpha, class 1A, member 1	uORF	carbohydrate metabolism	221760 at
4.9	RGMB	RGM domain family, member B	uORF	---	242450 at
4.8	BMPER	BMP binding endothelial regulator	unknown	---	241986 at
4.6	PAPSS2	3'-phosphoadenosine 5'-phosphosulfate synthase 2 (203058 s at)	unknown	nucleic acid metabolism	203060 s at
4.6	CGA	Glycoprotein hormones, alpha polypeptide	unknown	cell-cell signaling	233615 at
4.4	C6orf155	chromosome 6 open reading frame 155	intron >50	---	220324 at
4.4	IGFBP7	insulin-like growth factor binding protein 7 (201163 s at)	unknown	cell proliferation	201162 at
4.3	IGFBP7	insulin-like growth factor binding protein 7 (201162 at)	unknown	cell proliferation	201163 s at
4.2	PAPSS2	3'-phosphoadenosine 5'-phosphosulfate synthase 2 (203060 s at)	unknown	nucleic acid metabolism	203058 s at
4.1	FST	follistatin	unknown	regulation of FSH secretion	226847 at
4.0	FGF13	fibroblast growth factor 13	uORF	cell-cell signaling, neurogenesis	205110 s at
4.0	NRG1	neuregulin 1	uORF, AS	embryonic development	206343 s at
4.0	MYOCD	myocardin	uORF	transcription	237206 at
3.9	IGSF10	immunoglobulin superfamily, member 10 (230670 at)	unknown	protein phosphorylation	155679 s at
3.9	TUBA3	tubulin, alpha 3	unknown	microtubule-based movement	209118 s at
3.9	STXBP6	syntaxin binding protein 6 (amisyn)	uORF	vesicle-mediated transport	220994 s at
3.9	SANG	GNAS1 antisense	unknown	---	232881 at
3.8	---	CDNA clone IMAGE:4815026, partial cds	unknown	---	232478 at
3.7	LOC151878	Hypothetical protein LOC151878	unknown	---	222372 at
3.6	SMARCE1	SWI/SNF related, matrix associated regulator of chromatin e1	uORF	nucleosome disassembly	229511 at
3.6	LOC375010/LOC401131	hypothetical LOC375010/hypothetical LOC401131	intron >50	---	237737 at
3.5	TIMP3	tissue inhibitor of metalloproteinase 3	uORF	tyrosine kinase signaling	201147 s at
3.5	KIAA0527	KIAA0527 protein	unknown	cell adhesion	214954 at
3.5	TMEM20	transmembrane protein 20	unknown	---	239265 at
3.5	TSPAN2	tetraspanin 2	unknown	cell motility, adhesion, proliferation	227236 at
3.5	LOC389129	similar to CG9996-PA	unknown	---	235371 at
3.5	---	Transcribed locus	unknown	---	241535 at
3.4	GDA	guanine deaminase	AS	nucleic acid metabolism	1569555 at
3.4	KLF7	Kruppel-like factor 7	uORF	transcription	204334 at
3.4	ABCA8	ATP-binding cassette, sub-family A (ABC1), member 8	uORF	---	204719 at
3.4	SH3GL2	SH3-domain GRB2-like 2	unknown	signal transduction	205751 at
3.4	CYP24A1	cytochrome P450, family 24, subfamily A1	uORF	electron transport	206504 at
3.4	---	Homo sapiens, clone IMAGE:4401608, mRNA	unknown	---	233142 at
3.4	SLC1A4	solute carrier family 1, member 4	unknown	neutral amino acid transport	212810 s at
3.3	LPIN2	lipin 2	uORF	---	202460 s at
3.3	DNAJC12	DnaJ (Hsp40) homolog, subfamily C, member 12	uORF	protein folding	223722 at
3.2	MYL9	myosin, light polypeptide 9, regulatory	unknown	muscle development	201058 s at
3.2	DISP1	dispatched homolog 1 (Drosophila)	unknown	---	228184 at
3.1	ADRB2	adrenergic, beta-2-, receptor, surface	unknown (no intron)	Protein tyrosine kinase	206170 at
3.1	GADD45B	growth arrest and DNA-damage-inducible, beta	intron >50	apoptosis	209304 x at
3.1	SFRS11	splicing factor, arginine/serine-rich 11	unknown	RNA splicing	213742 at
3.1	FBN2	fibrillin 2 (congenital contractural arachnodactyly)	unknown	morphogenesis	215717 s at
3.1	---	Putative ORF3 gene, 5'	unknown	---	227030 at
3.1	---	Transcribed locus	unknown	---	228214 at
3.1	---	Full length insert cDNA Y137C01	unknown	---	236657 at
3.1	APG7L	APG7 autophagy 7-like	unknown	ubiquitin cycle, membrane fusion	1569827 at
3.0	GBP1	guanylate binding protein 1, interferon-inducible	unknown	---	202270 at
3.0	NCF2	neutrophil cytosolic factor 2	uORF	superoxide metabolism	209949 at
3.0	GARNL1	GTPase activating Rap/RanGAP domain-like 1	intron >50	---	237216 at
3.0	IGF1R	Insulin-like growth factor 1 receptor	unknown	cell cycle, anti-apoptosis	237377 at
3.0	BDNF	Brain-derived neurotrophic factor	uORF	neurogenesis	244503 at
3.0	MATN3	matrilin 3	unknown	extracellular matrix	206091 at
3.0	BEX2	brain expressed X-linked 2	unknown	---	224367 at
3.0	---	Hypothetical LOC401093	uORF	---	232298 at
3.0	---	Transcribed locus	unknown	---	239069 s at

intron >50: termination codon is >50 nt upstream of an intron

AS: alternative splicing creates a termination codon >50 nt upstream of the last intron

uORF: upstream open reading frame

Supplementary Table II Transcripts downregulated by UPF1 but not UPF3b

Listed are the 43 transcripts upregulated by 4-fold or more in UPF1 depleted HeLa cells but not changed in UPF3b depleted HeLa cells (p<0.01).

Gene title	Gene symbol	Biological functions	Putative NMD features	Probe set ID	Fold change in UPF1 RNAi cells
plasminogen-like A1, B1, B2	PLGLA1, PLGLB1, PLGLB2	plasmin activity	A1: intron>50	205871_at	45.3
tripartite motif-containing 73, 74	TRIM73, TRIM74	protein binding, ion binding	uORFs	1554182_at	25.5
interleukin 8	IL8	angiogenesis, chemotaxis, signal transduction, cell motility, cell cycle	unknown	202859_x_at 211506_s_at	16 9.5
KIAA1199	KIAA1199	sensory perception of sound	unknown	212942_s_at	16
Phosphodiesterase 5A, cGMP-specific	PDE5A	signal transduction, cyclic nucleotide metabolism	unknown	1561761_x_at 1561760_s_at 1561759_at	13.9 8.1 6.1
ovary-specific acidic protein	OSAP	---	unknown	223734_at	11.7
stanniocalcin 2	STC2	signal transduction, response to nutrient	uORFs	203438_at	11.5
CDNA FLJ34873 fis, clone NT2NE2014950	---	---	unknown (no intron)	229296_at	11.1
chromosome 3 open reading frame 62	C3orf62	---	unknown	241817_at	11.1
serpin peptidase inhibitor, clade E member 2	SERPINE2	nervous system development, cell differentiation, proteolysis, cell migration	unknown	212190_at	10.9
R-spondin 3 homolog (Xenopus laevis)	RSPO3	electron transport, iron ion binding	uORF	228186_s_at	10.7
hypothetical protein MGC11332, MGC11332	MGC11332	tetracycline transport	unknown	224483_s_at	10.6
Hypothetical gene supported by BC040598	LOC400960	---	unknown	1568640_at	8.9
nischarin	NISCH	intracellular signaling cascade	unknown	227392_at	8.6
phosphatidylinositol glycan, class L	PIGL	GPI anchor biosynthesis	unknown	232262_at	8
chaperonin containing TCP1, subunit 6A-like	LOC441244, LOC641740, LOC643253	---	intron>50	227301_at	7.6
similar to PDZ domain containing 1	LOC653397	---	unknown	240634_x_at	7.3
angiopoietin-like 4	ANGPTL4	angiogenesis, response to hypoxia and starvation, cell differentiation, apoptosis, lipid metabolism	intron>50	223333_s_at 221009_s_at	6.2 5.2
similar to centaurin gamma-like family, member 1, similar to Ribosome biogenesis protein BMS1 homolog	LOC653468, LOC653471, LOC654000	regulation of GTPase activity	uORFs, AS	243937_x_at	6
neuroblastoma breakpoint family, member 9.8	NBPF9, NBPF8	---	uORFs	237917_at	6
CDNA FLJ34964 fis, clone NTONG2004095	---	---	unknown	1558750_a_at	5.9
hypothetical protein LOC441528, similar to Protein kinase PKX1, similar to hypothetical protein LOC441528	LOC441528, LOC644379, LOC645216	---	unknown	1558046_x_at	5.9
hypothetical protein LOC92497	LOC92497	DNA integration	intron>50	232611_at	5.9
two pore segment channel 2	TPCN2	cation transport	unknown	231978_at	5.7
Ets variant gene 5 (ets-related molecule)	ETV5	transcription	unknown	230102_at 203349_s_at	5.5 4.3
hypothetical protein FLJ39575	FLJ39575	---	uORFs	239203_at	5.5
serine protease inhibitor	SPINK5L3	---	uORFs	233340_at	5.4
KIAA1267 /// KIAA1267	KIAA1267	---	unknown	224490_s_at	5.2
chromosome 20 open reading frame 133	C20orf133	---	uORFs	1553564_at 222088_s_at	5 4.4
brain and reproductive organ-expressed (TNFRSF1A modulator)	BRE	apoptosis	uORF	1568768_s_at	4.9
U2 small nuclear RNA auxiliary factor 1-like 4	U2AF1L4	---	uORFs	226700_at	4.9
Ribosomal protein L37	RPL37	protein biosynthesis	unknown	224763_at	4.8
similar to Protein kinase PKX1	LOC389906	---	uORFs	1569629_x_at	4.5
v-jun sarcoma virus 17 oncogene homolog (avian)	JUN	transcription	unknown (no intron)	201466_s_at	4.4
secretogranin II (chromogranin C)	SCG2	protein secretion	uORF	204035_at	4.2
Transcribed locus	---	---	unknown	1558045_a_at	4.1
CDNA FLJ36588 fis, clone TRACH2013991	---	---	unknown	1557667_at	4.1
hypothetical LOC221442	LOC221442	---	intron>50	236832_at	4.1
N-acetyltransferase 9	NAT9	---	unknown	204382_at	4.1
pleckstrin homology-like domain, family A, member 1	PHLDA1	apoptosis	intron>25	217997_at	4.1
Erythrocyte membrane protein band 4.1 like 4A	EPB41L4A	---	unknown	228259_s_at	4
epiregulin	EREG	cell cycle, signaling, proliferation and differentiation, transcription, translation, DNA replication	unknown	205767_at	4
Fibronectin type III and SPRY domain containing 1-like	FSD1L	---	unknown	242586_at	4

intron>50: Termination codon is >50 nt upstream of an intron

AS: alternative splicing creates termination codon >50 nt upstream of an intron

uORF: upstream open reading frame

