Supplementary Data

Nonsense mutation-dependent reinitiation of translation in mammalian cells

Sarit Cohen[1], Lior Kramarski[2], Shahar Levi[1], Noa Deshe*[1], Oshrit Ben-David[1], and Eyal Arbely †[1,2]

[1]Department of Chemistry and National Institute for Biotechnology in the Negev, Ben Gurion University of the Negev, Beer-Sheva, 8410501, Israel

[2]Department of Life Sciences, Ben Gurion University of the Negev, Beer-Sheva, 8410501, Israel

*Current address: Department of Genetics, Silberman Institute of Life Sciences, Edmond J. Safra Campus, The Hebrew University, Jerusalem, Israel

†Corresponding Author: arbely@bgu.ac.il

Supplementary Tables

Primer number	name	Sequence
Pr 1	p53 EF1α_FW	GACTAGCGGCCGCATGCATCATCACCATCACCATGA GGAGCCGCAGTCAGATC
Pr 2	HA tag EF1α_Rev	CACTAGGTACCttaAGCGTAATCTGGAACATCGTATGGGTAC
Pr 3	p53 CMV_FW	GACTAGGATCCATGCATCATCACCATCACCATGAGGAG CCGCAGTCAGATC
Pr 4	HA tag CMV_Rev	CACTAGAATTCttaAGCGTAATCTGGAACATCGTATGGGTAC
Pr 5	Q38TAG_FW	GTCCCCCTTGCCGTCCtagGCAATGGATGATTTG
Pr 6	Q38TAG_Rev	ATCATCCATTGCctaGGACGGCAAGGGGGGACAG
Pr 7	Q52TAG_FW	CGGACGATATTGAAtagTGGTTCACTGAAGACCCAG
Pr 8	Q52TAG_Rev	GTCTTCAGTGAACCActaTTCAATATCGTCCGGGGAC
Pr 9	K120TAG_FW	GGGACAGCCTAGTCTGTGACTTGC
Pr 10	K120TAG_Rev	GCAAGTCACAGACTAGGCTGTCCC
Pr 11	K164TAG_FW	CATGGCCATCTACtagCAGTCACAGCACATGAC
Pr 12	K164TAG_Rev	ATGTGCTGTGACTGctaGTAGATGGCCATGG
Pr 13	Q52TGA_FW	GGACGATATTGAAtgaTGGTTCACTG
Pr 14	Q52TGA_Rev	GGTCTTCAGTGAACCAtcaTTCAATATC
Pr 15	Q52TAA_FW	GGACGATATTGAAtAaTGGTTCACTG
Pr 16	Q52TAA_Rev	GGTCTTCAGTGAACCAtTaTTCAATATC
Pr 17	Q38TGA_FW	CCCCCTTGCCGTCCtGAGCAATG
Pr 18	Q38TGA_Rev	CAAATCATCCATTGCTCaGGACGGCAAG
Pr 19	Q38TAA_FW	CCCCCTTGCCGTCCtAAGCAATGGATG
Pr 20	Q38TAA_Rev	CAAATCATCCATTGCTTaGGACGGCAAG
Pr 21	R273H_FW	AGCTTTGAGGTGCATGTTTGTGC
Pr 22	R273H_Rev	GACAGGCACAAACATGCACCTC
Pr 23	M133L_FW	CTGCCCTCAACAAGctaTTTTGCCAACTGGCCAAG
Pr 24	M133L_Rev	TGGCCAGTTGGCAAAAtagCTTGTTGAGGGCAG
Pr 25	M40L_FW	GTCCCAAGCAtTGGATGATTTGATGCTG
Pr 26	M40L_Rev	CATCAAATCATCCAaTGCTTGGGACGGCAAG
Pr 27	M40L M44L_FW	GTCCCAAGCAtTGGATGATTTGtTGCTGTC
Pr 28	M40L M44L_Rev	GTCCGGGGACAGCAaCAAATCATCCAaTGCTTG
Pr 29	Q38TAG frameshift_FW	CCTTGCCGTCCtagGGCAATGGATGATTTGATGCTG
Pr 30	Q38TAG frameshift_Rev	CATCAAATCATCCATTGCCctaGGACGGCAAGGGGGGAC

Pr 31	Q52TAG frameshift_FW	GAAtagTGGTTCgACTGAAGACCCAGGTC
Pr 32	Q52TAG frameshift_Rev	GACCTGGGTCTTCAGTcGAACCActaTTCAATATC
Pr 33	K120TAG frameshift_FW	CAGCCtagTCTGTGgACTTGCACGTACTC
Pr 34	K120TAG frameshift_Rev	GTACGTGCAAGTcCACAGActaGGCTGTC
Pr 35	M66L _FW	GAAGCTCCCAGActaCCAGAGGCTGCTC
Pr 36	M66L _Rev	GCAGCCTCTGGtagTCTGGGAGCTTCATC
Pr 37	Enolase_FW	GACTAGCGGCCGCATGTCTATTCTCAAGATCCATG CCAGGGAGATC
Pr 38	Enolase_Rev	GAACATCGTATGGGTACATCTTGGCCAAGGGGTTTCTG
Pr 39	Enolase K80TAG_FW	ctgccctggttagctAGAAACTGAACGTCACAG
Pr 40	Enolase K80TAG_Rev	GTGACGTTCAGTTTCTagctaaccagggcagG
Pr 41	Enolase G156TAG_FW	CAATGTCATCAATGGCtagTCTCATGCTGGCAACAAG
Pr 42	Enolase G156TAG_Rev	GTTGCCAGCATGAGActaGCCATTGATGACATTGAAC
Pr 43	PFK WT _FW	ataagaatGCGGCCGCATGGACGCGGACG
Pr 44	PFK WT _Rev	GAACATCGTATGGGTACATGACACTCCAGGGCTGCACATG
Pr 45	PFK K25TAG _FW	CCGGGGCCGGCtagGCCATCGGCGTGCTGAC
Pr 46	PFK K25TAG _Rev	CACGCCGATGGCctaGCCGGCCCCGGAGAGGTG
Pr 47	p53 M1 bac_FW	GACTAcatATGGAGGAGCCGCAGTCAGATC
Pr 48	p53 M40 bac_FW	GACTAcatATGGATGATTTGATGCTGTCCCCGGAC
Pr 49	p53 M66 bac_FW	GACTAcatATGCCAGAGGCTGCTCCCCCCGTG
Pr 50	p53 M133 bac_FW	GACTAcatATGTTTTGCCAACTGGCCAAGACCTGC
Pr 51	p53 M160 bac_FW	GACTAcatATGGCCATCTACAAGCAGTCACAGCAC
Pr 52	p53 M169 bac_FW	GACTAcatATGACGGAGGTTGTGAGGCGCTGCC
Pr 53	p53 bac_Rev	CACTACTCGAGttaAGCGTAATCTGGAACATCGTATGGGTAC

Table S1. Sequences of primers used in cloning steps.

Primer number	name	Sequence
Pr 1	GAPDH_FW	agccacatcgctcagacac
Pr 2	GAPDH_Rev	aatacgaccaaatccgttgact
Pr 3	p53 N terminus (p-5'$_1$)_FW	gtcccaagcaatggatgatt
Pr 4	p53 N terminus (p-5'$_1$)_Rev	tctggacctgggtcttcagt
Pr 5	p53 N terminus (p-5'$_2$)_FW	aacaacgttctgtccccttg
Pr 6	p53 N terminus (p-5'$_2$)_Rev	tctggacctgggtcttcagt
Pr 7	p53 C terminus_FW	aggccttggaactcaaggat
Pr 8	p53 C terminus_Rev	ccctttttggacttcaggtg
Pr 9	p53 HA _FW	cactccagccacctgaag
Pr 10	p53 HA _Rev	ctggaacatcgtatgggtac
Pr 11	SC35 1.7_FW	gtgggcgtgtattggagcaga
Pr 12	SC35 1.7_Rev	ctgctacacaactgcgcc
Pr 13	CARS_FW	tgtgaatgttctccctccaga
Pr 14	CARS_Rev	ggcacatactcactaacccgg
Pr 15	SMG1_FW	cagagtccacgctgacatacaga
Pr 16	SMG1_Rev	cccaacgacttccgaccata

Table S2. Sequences of primers used in qPCR analyses.

Supplementary Figures

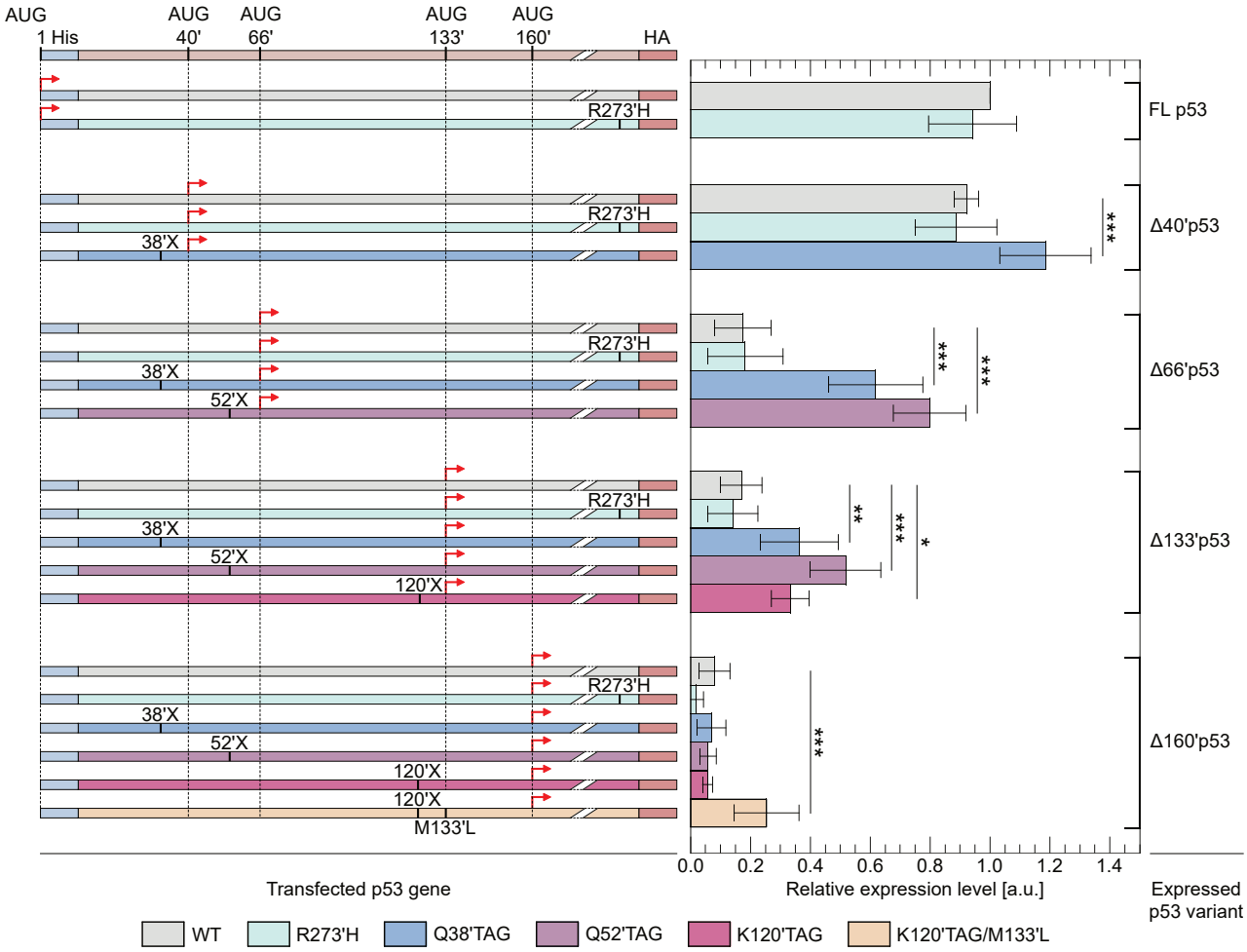

Figure S1. Alternative representation of data presented in Figure 1D. HEK293T cells were transfected with plasmids carrying the indicated gene, schematically presented on the left, and according to the color scheme described at the bottom. Red arrows mark possible translation initiation/reinitiation sites. Normalized expression levels displayed in the graph are grouped according to the expressed variant (full-length, Δ40', Δ66', Δ133', and Δ160' p53). For example, first two bars from the top, present the expression levels of full-length p53 in cells expressing wild-type p53 (top bar) or R273'H p53 (bottom bar).

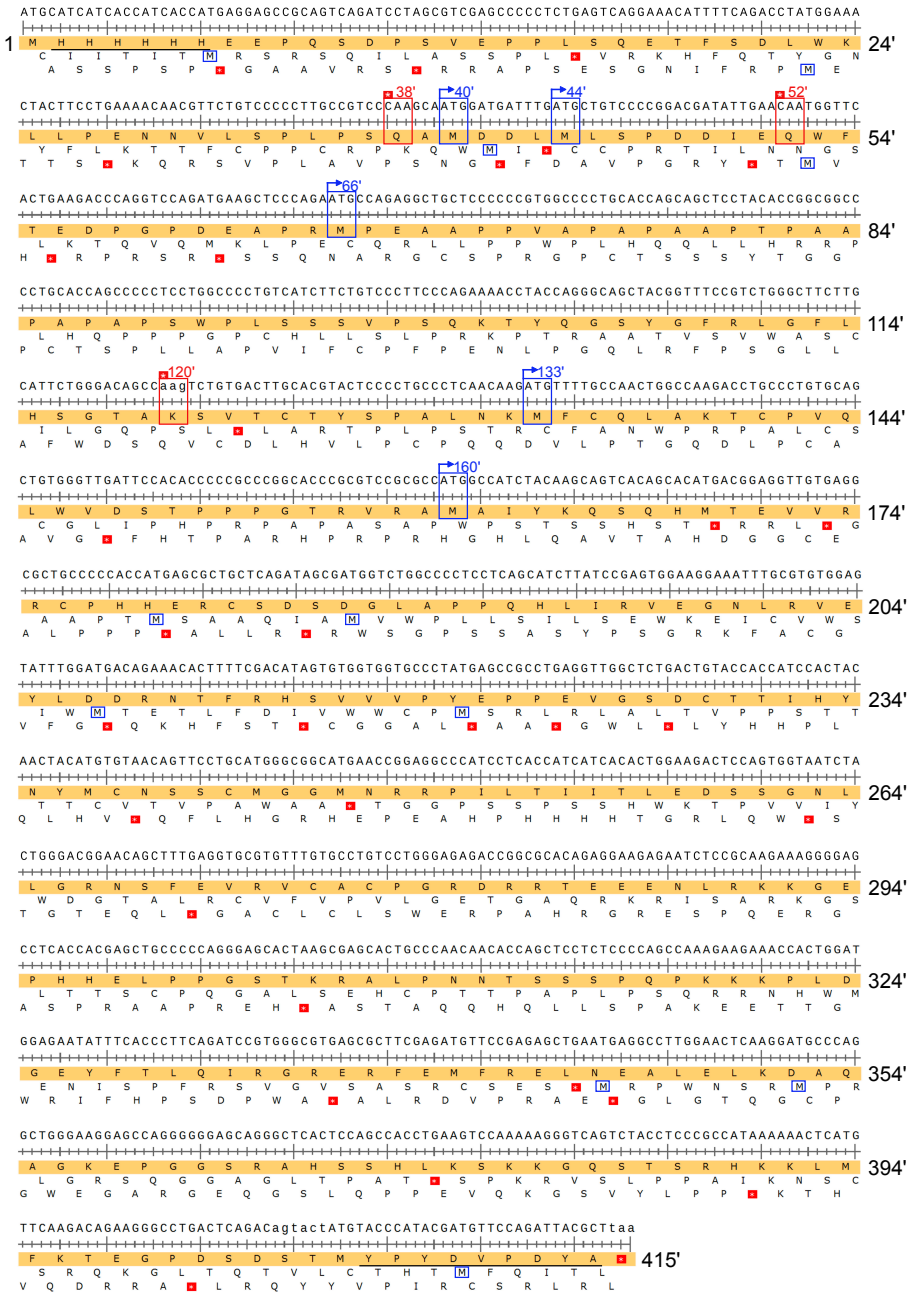

Figure S2. In-frame and out-of-frame ATG and stop codons. The cDNA sequence of p53 used in this study is displayed together with its translation in three frames (translation of in-frame codons is highlighted with orange background). Possible initiation sites and termination codons are marked in blue and red, respectively. Introduced stop codon mutations and suggested reinitiation sites, together with codon number-position, are marked with large rectangles. Translation of the two additional reading frames show that out-of-frame stop codons and initiation sites cannot account for the expression of Δ40', Δ66', Δ133', and Δ160' p53 seen in Figure 1C.

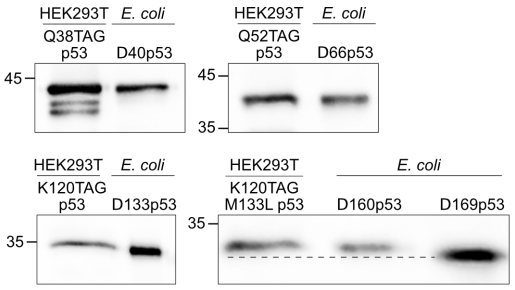

Figure S3. Size comparison between p53 variants expressed in cultured mammalian cells and truncated p53 recombinantly expressed in bacteria. The higher molecular weight band of Q38'TAG p53 mutant expressed in HEK293T cells was of the same size as bacterially expressed $p53_{(40–393)}$. Similarly, the higher molecular weight bands of Q52'TAG and K120'TAG p53 expressed in mammalian cells migrated together with bacterially expressed $p53_{(66–393)}$ and $p53_{(133–393)}$, respectively. The higher molecular weight band of K120'TAG M133'L p53 mutant expressed in mammalian cells was compared to $p53_{(160–393)}$ and $p53_{(169–393)}$ expressed in bacteria. Western blotting analysis suggests that the K120'TAG M133'L p53 mutant is expressed as Δ160'p53 and not as Δ169'p53.

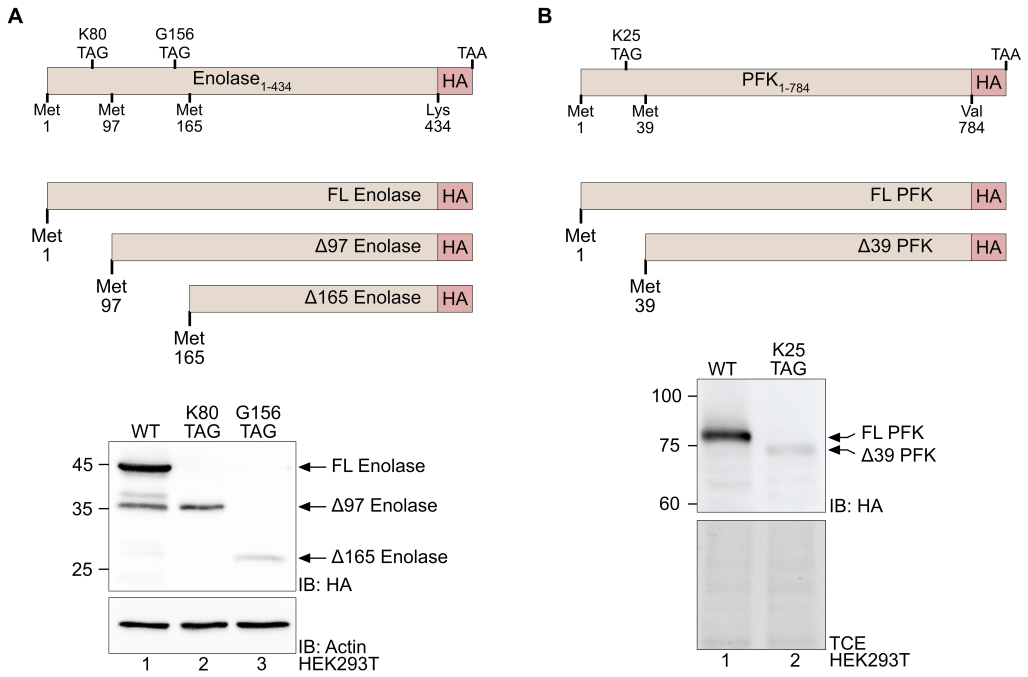

Figure S4. In-frame nonsense mutation-dependent expression of N-truncated proteins. (**A**) N-truncated variants of α-enolase. **Top:** Position of AUG codons (Met) that can serve as initiation or reinitiation sites. To test the effect of a PTC on reinitiation, TAG mutations were introduced at codons corresponding to K80 and G158. **Middle:** Full-length and possible N-truncated variants of α-enolase, expressed by initiation or reinitiation from the indicated AUG codons. **Bottom:** Western blot of total cell extracts from HEK293T cells expressing WT or TAG mutants of α-enolase. The codon for Met97 is part of a known internal ribosome entry site. Therefore, Δ97 enolase may be expressed by initiation from the AUG codon at position 97 (Met97). However, Δ165 enolase is expressed by premature termination codon-dependent reinitiation from the AUG codon at position 165. (**B**) N-truncated variants of human platelet-specific isoform of phosphofructokinase (PFK). In-frame nonsense mutation at position Lys25 promoted reinitiation of translation from downstream AUG codon at position 39.

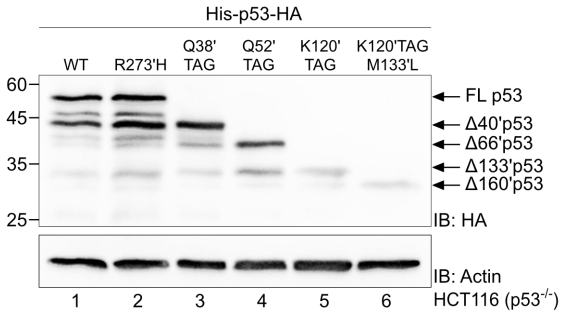

Figure S5. Expression of N-truncated variants of p53 in HCT116 ($p53^{-/-}$). Cells were transfected with plasmids carrying the indicated p53 variants and expression of N-truncated p53 was analysed by Western blot using antibodies against the C-terminal HA-tag.

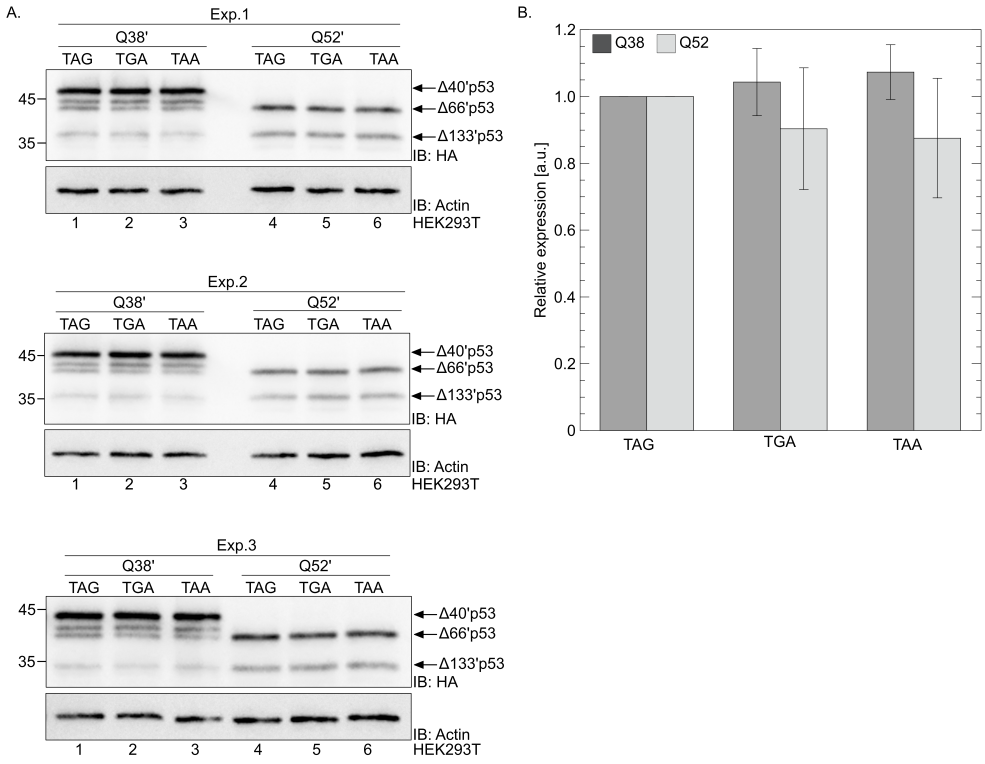

Figure S6. (**A**) Expression of N-truncated p53 as a function of premature stop codon type. Indicated stop codon mutations (TAG, TGA, or TAA) were introduced at codons for positions Q38' or Q52', and expression of N-truncated p53 was evaluated by Western blotting using antibodies against the C-terminal HA-epitope. The experiment was performed in triplicate (Exp. 1, 2, or 3). (**B**) Quantification of expression levels of Δ40' and Δ66' measured in triplicate as described in panel A. Displayed values are mean ±SD.

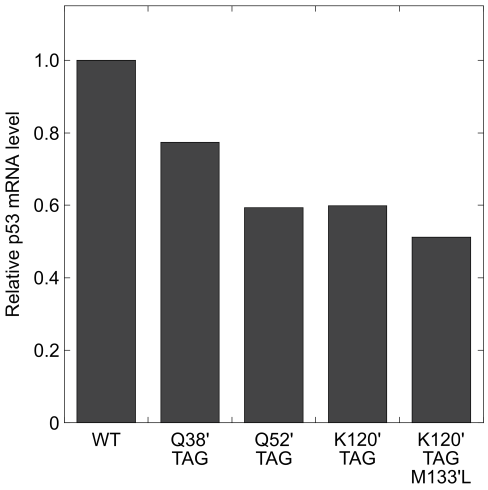

Figure S7. Relative mRNA levels of p53 mRNA transcripts with an in frame nonsense mutation. mRNA levels in HCT116 ($p53^{-/-}$) cells expressing indicated constructs were measured by RT-qPCR. Values are displayed relative to mRNA transcript without stop codon (WT).

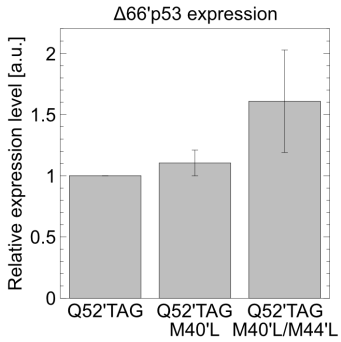

Figure S8. Quantification of the Western blot analysis displayed in Figure 3 panel A, lanes 4–6. Data are presented relative to expression levels of Q52'TAG (n=3 ± SD).

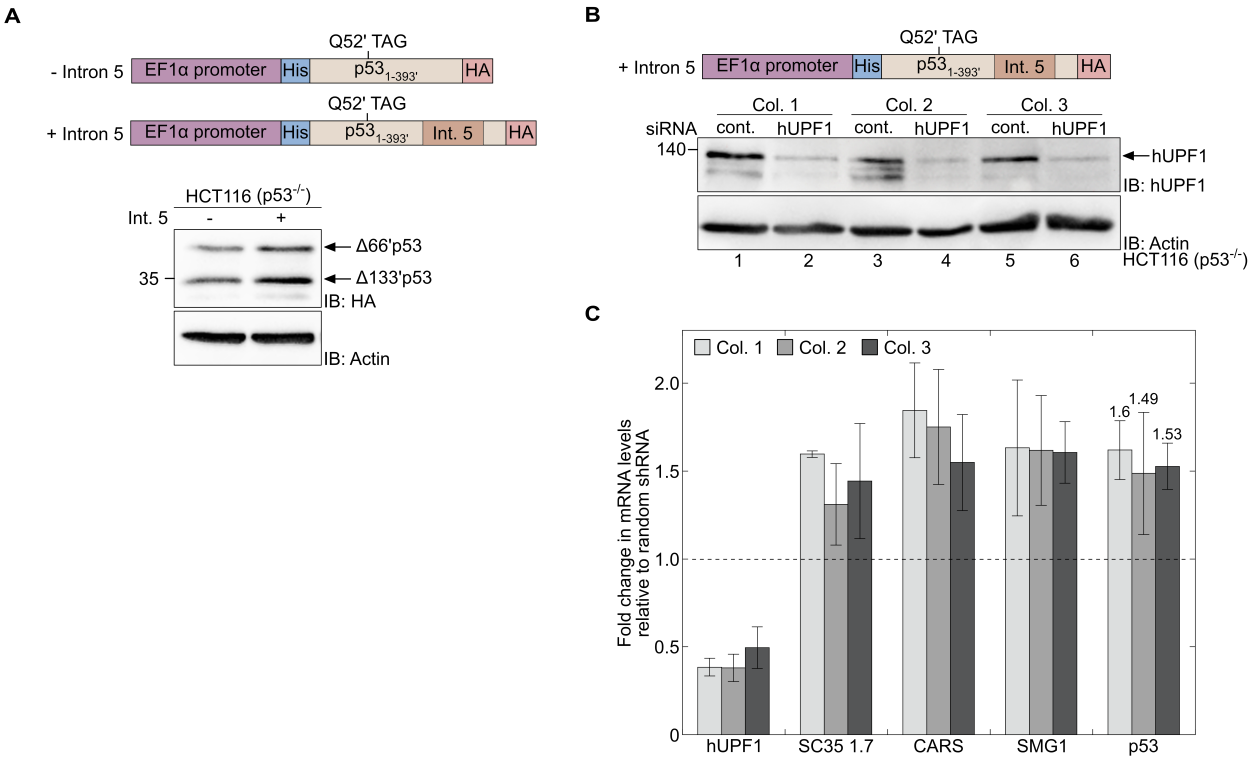

Figure S9. (**A**) The effect of intron 5 and NMD on the expression of Δ66'p53. Expression of N-truncated p53 variants in HCT116 ($p53^{-/-}$) cells transfected with p53 Q52'TAG cDNA (-intron 5) or Q52'TAG minigene (+intron 5), evaluated by Western blot analysis using antibodies against the C-terminal HA tag. (**B**) Knockdown of hUPF1. Three monoclonal HCT116 ($p53^{-/-}$) cell lines were stably transfected with p53 Q52'TAG minigene, and treated with siRNA directed against hUPF1 or control siRNA (cont.). Protein expression levels were evaluated by immunoblotting using a specific antibody against hUPF1. (**C**) Levels of SC35 1.7, CARS, SMG1 and p53 mRNA transcripts in cells stably expressing Q52'TAG minigene, as described in panel B. Data represent the fold change in mRNA levels, following treatment with hUPF1-specific siRNA, relative to cells treated with control siRNA. Values are the mean± SD of three independent experiments, each measured in triplicates.