Supplementary Figure Legends

Figure S1. NMUP is intron dependent. (**A**) Schematic diagram indicating how the intron-deleted construct was generated from construct A. The nonsense mutation (A^{N2} and ΔA^{N2}) and missense mutation (A^{M2} or ΔA^{M2}) were introduced into exon 6 at codon. (**B-D**) Quantification of RNase protection analysis performed on total cellular RNA (10 μg) harvested from HeLa cells transiently transfected with the constructs shown. Probes 1, and 3 (panel **A**) were used to detect mature/IVS-A+ pre-mRNA and IVS-B+ pre-mRNA, respectively. Values were quantified from 3 or more independent experiments by the approach described in Figure 1. Error bars indicate standard error. (**E**) Splicing rate as measured by pre-mRNA-to-mRNA ratio, determined as in Figure 5E, using the values in panels **B**-**D**.

Figure S2. The NMUP substrate contains TCR introns IVS-A and -B, but not IVS-C or -D. (**A**) Schematic diagram showing construct A and the position of the nonsense and frameshift mutations in variant constructs A^{N1}, A^{I1+}, and A^{N2} (see also Figure 1A). The position of the intron probes used for the Northern blot analysis in panel B is also indicated. (**B**) Northern blot analysis of total cellular RNA (10 μg) isolated from HeLa cells stably transfected with constructs A, A^{N1}, A^{I1+}, and A^{N2}. The schematics indicate the introns present in the pre-mRNAs in each band, based on band migration and their hybridization with the different probes. The bands corresponding to spliced IVS-A, -B, -C, and -D migrated at a position consistent with their expected sizes (~0.5, ~0.8, ~0.25, and ~0.1 nt, respectively). The transcripts denoted by the symbols Δ, §, and * are explained in Figure 3. The results shown are representative of two independent experiments.

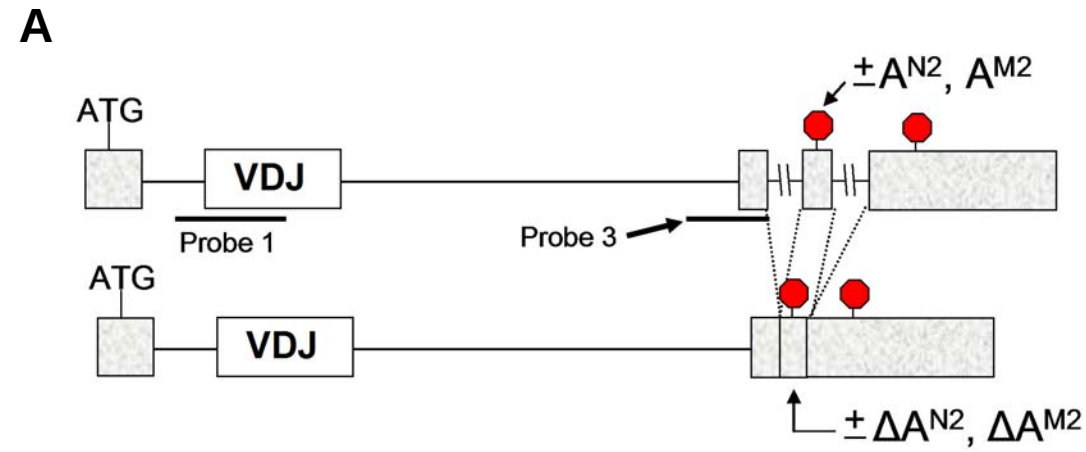
.

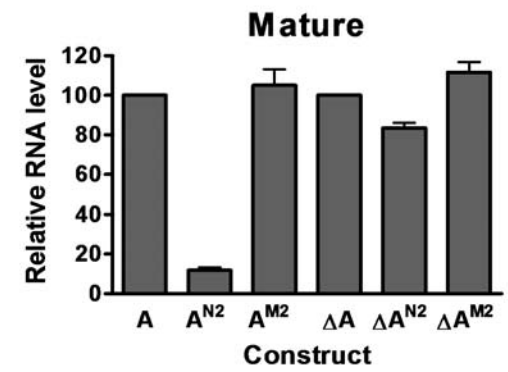
Figure S3. TCR/TPI pre-mRNA has a long half-life. (**A**) mRNA half-life analysis of TCR/TPI pre-mRNA expressed from construct A (Figure 1B) transiently transfected into HeLa cells. The cells were cultured for 2 days after transfection and then incubated with the transcriptional inhibitor actinomycin D (8 μg/ml) for the times indicated. RNase protection analysis was performed on 10 μg total cellular RNA using probe 1 for IVS-A+ pre-mRNA, probe 3 for IVS-B+ pre-mRNA, and probe 4 for IVS-D+ pre-mRNA. Values were quantified as described in Figure 1; similar results were obtained in two independent transfection experiments. (**B**) mRNA half-life analysis of TCR/TPI pre-mRNA expressed from the *c-fos* promoter-driven construct shown (construct A^{fos}) transiently transfected into NIH-3T3 cells. The cells were cultured for 1 day after transfection in serum-free conditions and then incubated with serum (the times indicated are 45 minutes post-serum addition, as serum-induced *c-fos* transcription ceases ~45 minutes after the addition of serum). RNase protection analysis was performed on 5 μg total cellular RNA using probe 1 for IVS-A+ pre-mRNA and probe 3 for IVS-B+ pre-mRNA. Values were quantified as described in Figure 1; similar results were obtained in two independent transfection experiments. (**C**) Real-time PCR analysis of endogenous β-actin and *GAPDH* pre-mRNA half-lives determined using total cellular RNA from HeLa cells incubated with actinomycin D (8 μg/ml) for the times indicated. Real-time PCR analysis was performed as previously described (63).

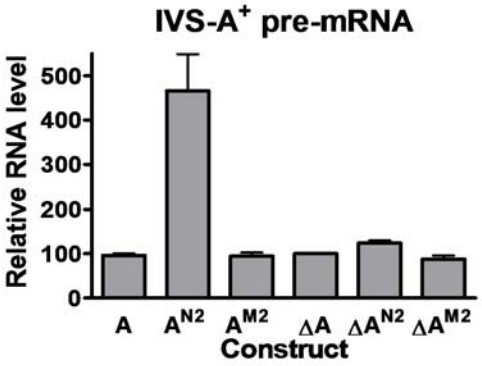
Figure S4. NMUP is not relieved by suppressor tRNAs. (**A**) Schematic indicating the location of the nonsense mutations in constructs A^{N1} and A^{N2}. (**B-D**) RNase protection analysis of total cellular RNA obtained from HeLa cells transiently co-transfected with the constructs shown in panel A and the nonsense codon-specific suppressor tRNAs indicated. Probes 1 and 3 were used to determine the levels of pre-mRNA harboring IVS-A+ and IVS-B+, respectively**.** Mature mRNA levels were determined using probe 1. Quantification was done as described in Figure 7: a β-globin expression construct was cotransfected into the cells to provide an internal control

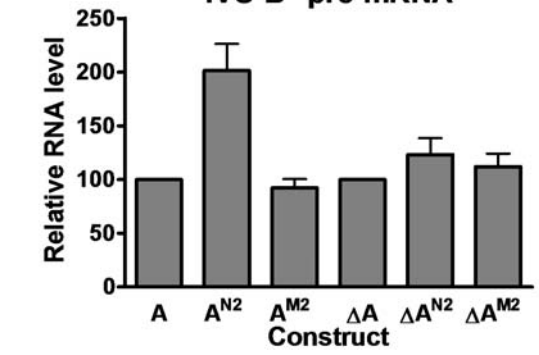
for transfection efficiency (β-globin mRNA levels were assayed by RNase protection analysis in the same gel that assayed TCR/TPI mRNA levels). Similar results were obtained in two independent experiments.

Supplementary Figure S1







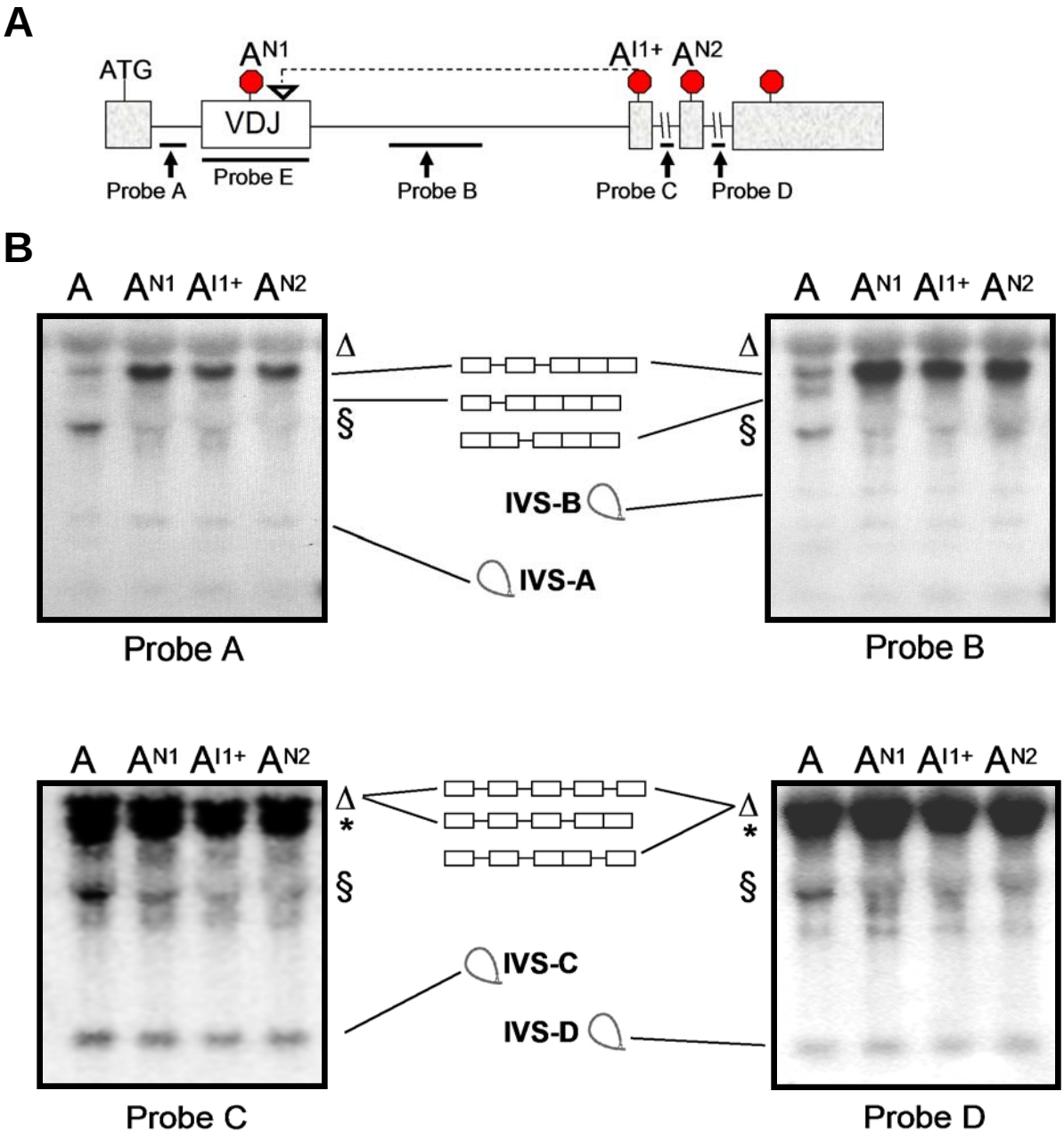


E

IVS-A+ pre-mRNA/mRNA ratio

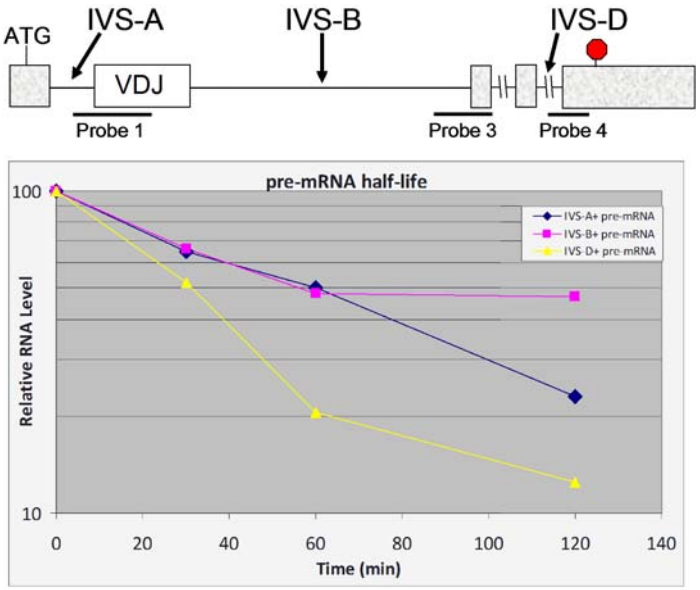
A	A^{M2}	ΔA	ΔA^{M2}
1.0	0.9	1.0	0.8

Supplementary Figure S2

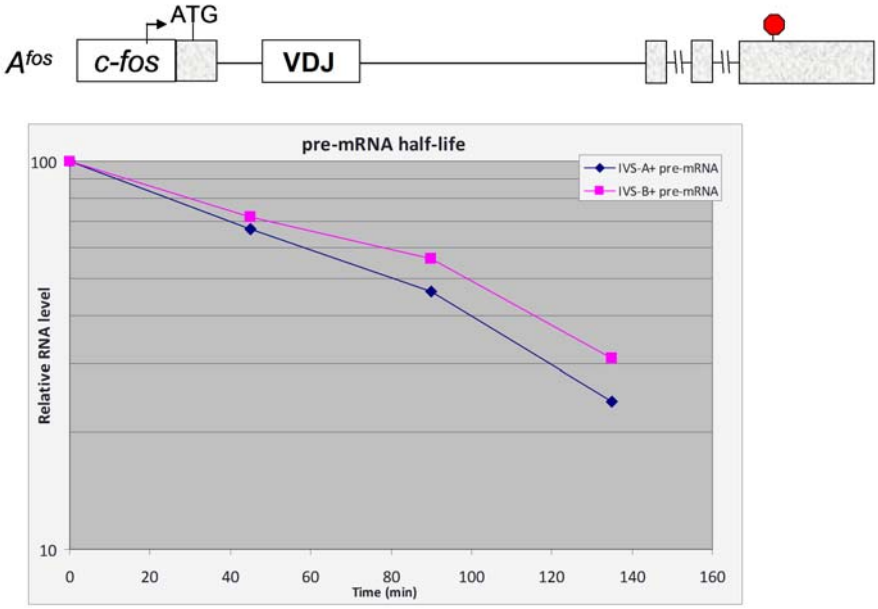


Supplementary Figure S3

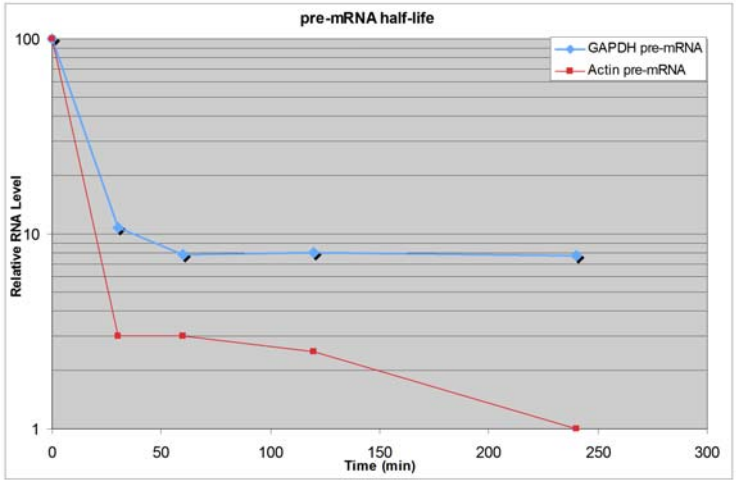
A



B



C



Supplementary Figure S4

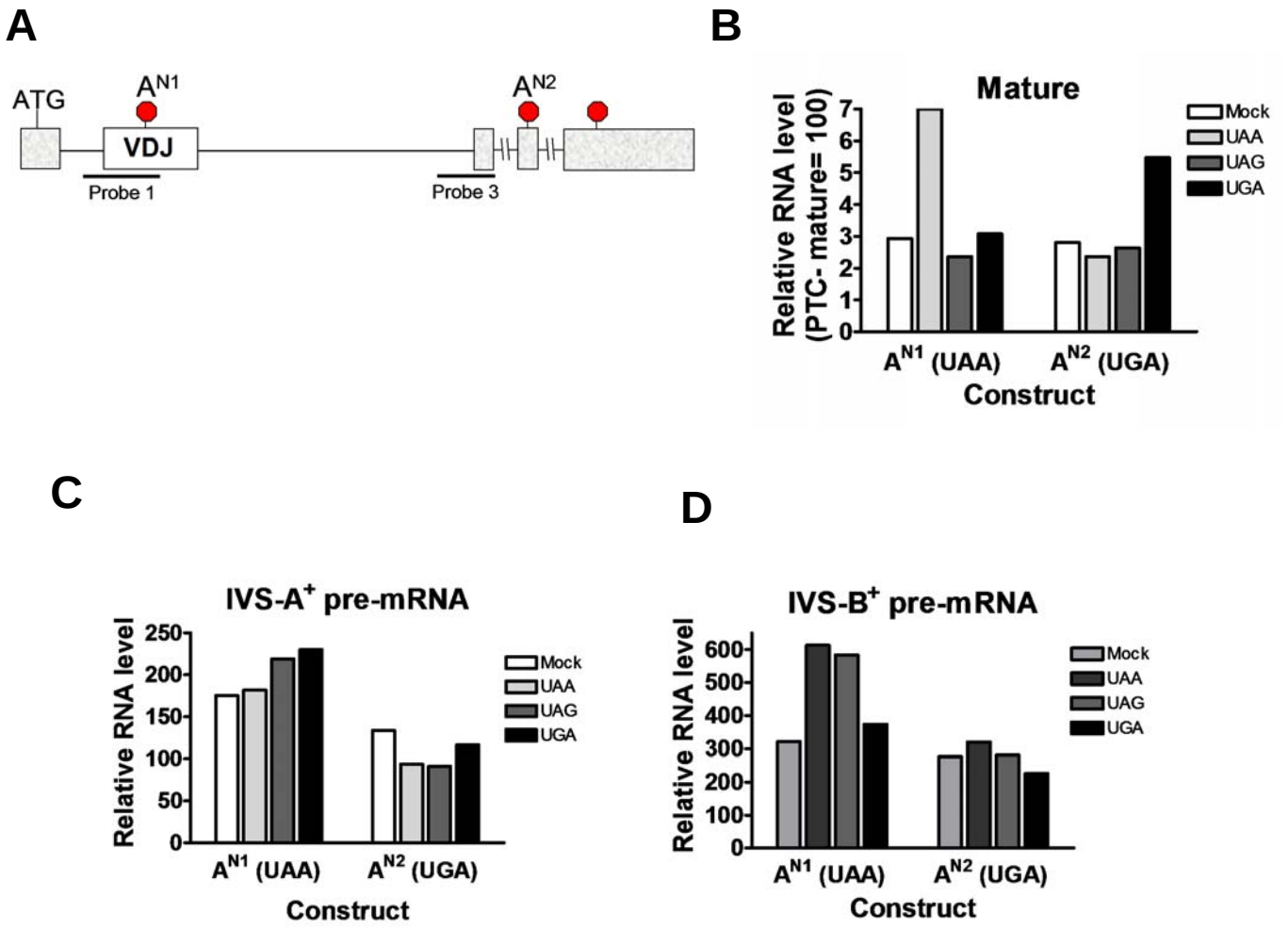


Table S1. Constructs

Construct	Name	Mutation	Primer (5'-3')
A	β-652	none	Gudikote *et al.* 2002 (8)
A^{N1}	β-653	Codon 91, nonsense	Gudikote *et al.* 2002 (8)
A^{M1}	β-1149	Codon 91, missense	MDA 1924: CTGATCCATTACTCATACGTCGCTGACAGCACGGAG
			MDA 1925: CTCCGTGCTGTCAGCGACGTATGAGTAATGGATCAG
A^{I1+}	β-725	VDJ exon frameshift +10 at Codon 100	Gudikote *et al.* 2002 (8)
A^{N2}	β-726	Codon 194 exon 6 nonsense	MDA 2908: CACGAGAAGCTCTGAGGATGGCTG
			MDA 2909: CAGCCATCCTCAGAGCTTCTCGTG
A^{M2}	β-1076	Codon 194 exon 6 missense	MDA 1834: GAAGTACACGAGAAGCTCGGAGGATGGCTGAAGTCCAAC
			MDA 1835: GTTGGACTTCAGCCATCCTCCGAGCTTCTCGTGTACTTC
A^{N3}	β-1075	Codon 112 VDJ nonsense	MDA 1830: CAAGGCCTCCAGACCAAGCDAAGAGAATTTCTCTCTCATTCTG
			MDA 1831: CAGAATGAGAGAGAAATTCTCTTHGCTTGGTCTGGAGGCCTTG
A^{M3}	β-1077	Codon 112 VDJ missense	MDA 1926: GGCCTCCAGACCAAGCGAAGAGAATTTCTCTCTCATTCTG
			MDA 1927: CAGAATGAGAGAGAAATTCTCTTCGCTTGGTCTGGAGGCC
A^{N4}	β-1163	Codon 146 VDJ nonsense	MDA 2449: GGTGCAGAAACGCTGTAATTTGGCTCAGGAACCAGACTG
			MDA 2450: CAGTCTGGTTCCTGAGCCAAATTACAGCGTTTCTGCACC
A^{M4}	β-1164	Codon 146 VDJ missense	MDA 2447: GGTGCAGAAACGCTGTACTTTGGCTCAGGAACCAGACTG
			MDA 2448: CAGTCTGGTTCCTGAGCCAAAGTACAGCGTTTCTGCACC
A^{I2+}	β-1154	Codon 114 VDJ Frameshift +1	MDA 1725: CTCCAGAATGAGAGAGAAATTTCTCTTGGCTTGGTCTGGAG
			MDA 1726: CTCCAGAATGAGAGAGAAATTTCTCTTGGCTTGGCTTGGAG
A^{I3}	β-1080	Codon 114 VDJ Frameshift +3	MDA 1733: CTCCAGACCAAGCCAAGAGAAAAATTTCTCTCTCATTCTGGAG
			MDA 1734: CTCCAGAATGAGAGAGAAATTTTTCTCTTGGCTTGGTCTGGAG
A^{D1+}	β-1155	Codon 146 VDJ Frameshift -1	MDA 1735: GGTGCAGAAACGCTGTATTTGGCTCAGGAACCAGACTG MDA 1736: CAGTCTGGTTCCTGAGCCAAATACAGCGTTTCTGCAAA
A^{I2D1}	β-1156	Codon 114,146 VDJ Frameshift +1/-1	
A^{N5}	β-1161	Codon 164 E5 nonsense	MDA 2308: GACTGGAGCTAGGTCGTCCTG
			MDA 2309: CAGGACGACCTAGCTCCAGTC
A^{M5}	β-1162	Codon 164 E5 missense	MDA 2310: GACTGGAGCGAGGTCGTCCTG
			MDA 2311: CAGGACGACCTCGCTCCAGTC
ΔA	β-1177	Codon 194 IVS 5,6 PTC-	MDA 476: GGTTGGATCCCTCACATGGGTGGTTCA MDA 480: GACAATCGATGCTTGGGGCCTATGACT
ΔA^{N2}	β-1178	Codon 194 ΔIVS 5,6 nonsense	
ΔA^{M2}	β-1179	Codon 194 ΔIVS 5,6 missense	
A^{N6}	β-1184	Codon 191 E6 nonsense	MDA 2866: GGAAGTACACTAGAAGCTCCGAG MDA 2867: CTCGGAGCTTCTAGTGTACTTCC
A^{M6}	β-1185	Codon 191 E6 missense 1	MDA 2868: GGAAGTACACMAGAAGCTCCGAG MDA 2869: CTCGGAGCTTCTKGTGTACTTCC
$A^{M6'}$	β-1186	Codon 191 E6 missense 2	Same as A^{M6}
A^{N7}	β-1187	Codon 192 E6 nonsene	MDA 2870: GAAGTACACGAGTAGCTCCGAGG

			MDA 2871: CCTCGGAGCTACTCGTGTACTTC
$\mathbf{A^{M7}}$	β-1188	Codon 192 E6 missense 1	MDA 2872: GAAGTACACGAGMAGCTCCGAGG MDA 2873: CCTCGGAGCTKCTCGTGTACTTC
$\mathbf{A^{M7'}}$	β-1189	Codon 192 E6 missense 2	Same as A^{M7}
$\mathbf{A^{N8}}$	β-1190	Codon 195 E6 nonsense	MDA 2874: GAAGCTCCGATGATGGCTGAAG MDA 2875: CTTCAGCCATCATCGGAGCTTC
$\mathbf{A^{M8}}$	β-1191	Codon 195 E6 missense 1	MDA 2876: GAAGCTCCGAMGATGGCTGAAG MDA 2877: CTTCAGCCATCKTCGGAGCTTC
$\mathbf{A^{M8'}}$	β-1192	Codon 195 E6 missense 2	Same as A^{M8}
$\mathbf{A^{D2+}}$	β-1193	Codon 190 E6 frameshift -1	MDA 2878: CCCAGGAAGTAACGAGAAGCTC MDA 2879: GAGCTTCTCGTTACTTCCTGGG

Table S2. RNase protection analysis probes

Probe	Target	Primers (5'-3') or Source
1	IVS-A and VDJ exon	Probe *b* in Gudikote *et al.* 2005 (10)
2	Exon 1 and IVS-A	MDA 1446: GAAGCAGAGTCTGGGGGAGC MDA 1447: CTCCTAACCCCGTCGCCT
3	IVS-B and exon 5	MDA 1448: GTACTTCGGTCCCGGCAC MDA 1449: GTACCAATGGCCACACAG
4	IVS-D and exon 7	MDA 2016: GGGCAGACTCATCCCATT MDA 2189: GTTTGGCATTGATGATGTCCAC

Table S3. Northern blot analysis probes

Probe	Target	Primers (5'-3') or Source
A	IVS-A	Generated by restriction enzyme digestion as detailed in the Materials & Methods
B	IVS-B	Generated by restriction enzyme digestion as detailed in the Materials & Methods
C	IVS-C	MDA 1629: GTAACCGGGCCCAGGAG MDA 1630: CTTCCTGGGCCTAGAACAAG
D	IVS-D	MDA 1631: GTGAGTGGCTTTGGTTCCCG MDA 1632: CTGGGAAGGGAGCAGAACAAG
E	VDJ exon	VDJ exon probe from Gudikote *et al.* 2002 (8)