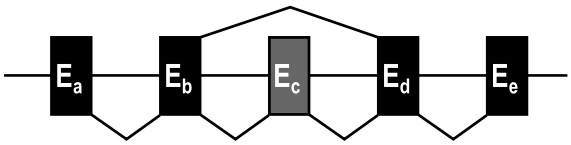
Supplemental Materials

Supplemental Methods

Calculation of the frequency of splicing variants

To account for the fact that in many cases the RNAseq reads are not distributed uniformly along the *PPP1R12A* cDNA, calculations were made relative to the regions where splicing events take place. Consider the following example where exon E_c can be alternatively spliced.



For each region, first the average number of reads spanning constitutive exon junctions was calculated using the following formula:

$$N = [E_aE_b + (E_bE_c + E_bE_d) + (E_cE_d + E_bE_d) + E_dE_e] / 4$$

where E_aE_b is the number of reads spanning exons E_a and E_b, and so on. Variations of the above formula were used for alternative splicing donor or acceptor sites and in regions involving splicing of more than one exon. The frequency (expressed as percentage) of skipping or splicing in of E_c is calculated as:

$$\%\text{Splicing out} = 100 * E_bE_d / N$$

$$\%\text{Splicing in} = 100 * [(E_bE_c + E_cE_d) / 2] / N$$

To calculate the frequency (expressed as percentage) of each variant we assumed only one exon is alternatively spliced in every variant, as there is no evidence of *PPP1R12A* variants resulting from simultaneous splicing of two or more exons other than E13+E14. The frequency of LZ^- variants was taken as the frequency of splicing in of E24 (LZ^-a) and E24b (LZ^-b). The frequency of all other (LZ^+) variants was calculated relative to (100 - LZ^-a - LZ^-b). For example, the frequency of the ΔE14 variant was calculated as:

$$\%\Delta E14 = \%\text{Splicing out E14} * (100\text{-}LZ^-a\text{-}LZ^-b) / 100$$

Finally, the frequency of the FL variant was calculated as 100 minus the sum of frequencies of all other variants.

Supplemental Tables

Table S1. Oligonucleotide pairs used for control PCR reactions. Primer pairs are given as forward (top)-reverse (bottom). In the amplicon size column (g) indicates that the primers were tested using genomic DNA as template; (p) indicates that the primers were tested using a plasmid containing the *PPP1R12A* variant encoding the FL MYPT1 variant as a template. The position of the primer pairs and their respective amplicons are shown in Fig. S5A. Primers in blue characters were used exclusively for testing the other member of the pair and not further used in RT-PCR reactions. E19 and E26' were used in combination to test each other and subsequently in separate RT-PCR reactions. Annealing temperature and number of cycles for the PCR reactions are indicated. Where the PCR buffer departed from the standard this is also indicated.

Primer pair	Sequence (5'-3')	Amplicon size (bp)	PCR conditions
E1^{-}–E1	ATCTGCCCTGTAGAGCCTTG TTCACCTGGTCTTCTGGCGC	632 (g)	55.4°C, 42x, GC-enriched buffer + DMSO
E1–E1	GGATACTGGAAGTCTCGAGCG TTCACCTGGTCTTCTGGCGC	265 (g)	55.4°C, 42x, GC-enriched buffer + DMSO
E1^{+}–I1	AGGGGCTAAGAGAAACACTGA CACAAACAGACAATGCACACA	158 (g)	58°C, 42x
E1'–E2	ATGAAATGGCGGACGCG GGTATCCAGCCTTCATTATCAGG	326 (p)	60°C, 35x
E4–E8	CGGCATGCAAAATCTGGAGG TCCTTCTTCTTCTTCATCAACC	465 (p)	52°C, 35x
E4'–E8	TACAGCACTTCACGTTGC TCCTTCTTCTTCTTCATCAACC	445 (p)	61°C, 35x
E8–E10	AGACGTTGATTATTGAACCAGAG GGGACTTGAAGCTGAACGTG	461 (p)	58°C, 35x
I9–E9^{+}	TGTTTGGTGGGGAAATCAGTAAA CCTTACTGGGCACAAGAACAATG	523 (g)	58°C, 42x
E12–E16	AGGAGAAAATGGGAAGATGATC CTCATCATACGTTCTGGAGTAC	705 (p)	56°C, 35x
E21–E26	AGAAAACCTTACAGCAGCAGG TCAAGGCCCCATTTTCATCC	316 (p)	56°C, 35x
E19–E26'	CAGACACAGAAGAGGGATCCA ATAACTCTGATCAAGGCCCC	463 (p)	56°C, 35x

Table S2. Oligonucleotide pairs used for RT-PCR reactions. Primer pairs are given as forward (top)-reverse (bottom). The position of the primer pairs and expected amplicons for each variant are shown in Figs. 5, 6, S7 and S8. In primers spanning exon boundaries the side of the upstream exon is underlined. Annealing temperature and number of cycles for the PCR reactions are indicated.

Primer pair	**Sequence (5'-3')**	**Variants (amplicon sizes in bp)**	**PCR conditions**
E1^{-}–E2	ATCTGCCCTGTAGAGCCTTG TTCACTTGGTCTTCTGGCGC	E1^{-} in (447) E1^{-} out (0)	55°C, 35x
E1–E2	GGATACTGGAAGTCTCGAGCG TTCACTTGGTCTTCTGGCGC	E1 in (481) E1 out (0)	56°C, 35x
E1^{+}–E2	AGGGGCTAAGAGAAACACTGA GGTATCCAGCCTTCATTATCAGG	E1^{+} in (159) E1^{+} out (0)	55°C, 35x
E4–E8	CGGCATGCAAAATCTGGAGG TCCTTCTTCTTCTTCATCAACC	E6 in (465) E6 out (389)	52°C, 35x
E8–E10	AGACGTTGATTATTGAACCAGAG GGGACTTGAAGCTGAACGTG	E9^{+} in (578) E9^{+} out (461)	58°C, 35x
E8–E9^{+}	AGACGTTGATTATTGAACCAGAG CCTTACTGGGCACAAGAACAATG	E9^{+} in (381) E9^{+} out (0)	58°C, 42x
E12–E16	AGGAGAAAATGGGAAGATGATC CTCATCATACGTTCTGGAGTAC	E13 in–E14 in (705) E13b in–E14 in (669) E13 out–E14 in (537) E13 in–E14 out (528) E13 out–E14 out (360)	54°C, 35x
E21–E26	AGAAAACCTTACAGCAGCAGG TCAAGGCCCCATTTTCATCC	E22 in–E24 in (347) E22 in–E24b in (329) E22 in–E24 out (316) E22 out–E24 out (211)	58°C, 35x
E4'–E5.7	TACAGCACTTCACGTTGC CCGTTTTTCACTATGGAGCACT	E6 in (0) E6 out (211)	61°C, 42x
E11–E12.13b	TGCACCTACAATACCAAGACGA GCTTGGAACACTAGAACTTTTATG	E13 in (0) E13b in (181)	53°C, 42x
E19–E21.23	CAGACACAGAAGAGGGATCCA CAGCAAATCTTTCTTGTCTCTTT	E22 in (0) E22 out (210)	55°C, 42x
E23.24–E26'	GAAAAAAGGGTGACCGGCAAG ATAACTCTGATCAAGGCCCC	E24 in (159) E24b in (141)	56°C, 42x

Table S3. *PPP1R12A* transcripts and protein variants as annotated in Ensemble and GenBank. All transcripts are supported by EST and/or mRNA sequences and for the protein coding variants also by RNAseq data, with the exception of transcript PPP1R12A-223, which is only supported by RNAseq data. The variant considered canonical in Uniprot uses all coding exons except $E9^+$, E24 and exons only found in nonsense-mediated decay transcripts ($E2^+$, $E20^+$ and $E22^+$). Transcript PPP1R12A-209 is annotated as protein coding but is more likely a nonsense-mediated decay transcript. Most Ensemble transcripts are incomplete and have been extended as shown in Fig. 2 to calculate the predicted protein isoforms.

Ensembl (GenBank) transcript	**Type**	**Splicing**	**Protein isoform (predicted length) (GenBank isoform)**
PPP1R12A-201 (NM_001143885.2, variant 2)	Protein coding	Canonical, start at $E1^-$	FL (1030) (isoform a)
PPP1R12A-202 (NM_001244990.2, variant 4)	Protein coding	E22 out, start at $E1^-$	ΔE22 (995) (isoform c)
PPP1R12A-203 (NM_002480.3, variant 1)	Protein coding	Canonical, start at E1	FL (1030) (isoform a)
PPP1R12A-204 (NM_001143886.2, variant 3)	Protein coding	Start at $E1^+$	ΔN (943) (isoform b)
PPP1R12A-205	Nonsense-mediated decay	$E22^+$ in	
PPP1R12A-206	Protein coding	E13+E14 out	ΔE13+14 (915)
PPP1R12A-207	Retained intron		
PPP1R12A-208	Protein coding	E14 out	ΔE14 (971)
PPP1R12A-209	Protein coding (Nonsense-mediated decay)	$E2^+$ in	
PPP1R12A-210	Retained intron		
PPP1R12A-211	Retained intron		
PPP1R12A-212	Retained intron		
PPP1R12A-213 (NM_001244992.1, variant 5)	Protein coding	E13 out	ΔE13 (974) (isoform d)
PPP1R12A-214	Protein coding	E24 in (31 bp variant)	LZ^-a (1005)
PPP1R12A-215	Retained intron		
PPP1R12A-216	Protein coding	E6 out	ΔE6 (1005)
PPP1R12A-217	Nonsense-mediated decay	$E20^+$ in	
PPP1R12A-218	Retained intron		
PPP1R12A-219	Retained intron		
PPP1R12A-220	Retained intron		
PPP1R12A-221	Protein coding	Alternative splicing acceptor site in E13	E13b (1018)
PPP1R12A-222	Protein coding	E24 in (13 bp variant)	LZ^-b (999)
PPP1R12A-223	Protein coding	$E9^+$ in	E9+ (1062)

Table S4. Collection of EST clones supporting terminator region T1 approximately 2100 bp downstream of the stop codon. Accession numbers are hyperlinked to the respective GenBank submission. Sequences at the end of each clone were taken directly from the GenBank submission, note that most are in antisense direction. Cleavage sites are highlighted in turquoise unless reliable identification was not possible. *Clones without a clear polyA stretch but with annotation that an oligo dT was used in the cDNA synthesis. §Clones without a clear polyA stretch and no annotation about an oligo dT being used in the cDNA synthesis.

Accession number	Tissue or cell type	Sequence at end of clone
BU620191.1	Chondrosarcoma cell line	tttttttttt tttttttaa tagttttatt catttattt gtatatgtca
CA312676.1	Lung epithelial cells	tttttttttt tttttttca cacatttaaa atagttttat tcatttatt
CN278743.1	Embryonic stem cell line	aaatgtattt tgacatatac caataaaatg aataaaacta aaa
BU677557.1	Lung epithelial cells	tttttttttt tttttttag ttttattcat tttatttgta tatgtcaaaa tacattttta
CA775922.1	Pancreatic islet	ttttttagg tttattcatt ttatttgtat atgtcaaaat acatttttat
BQ477501.1	Insulinoma	tttttttttt tttttagttt tattcatttt atttgtatat gtcaaaatat
CA310990.1	Lung epithelial cells	tttttttttt tttttttag ttttattcat tttatttgta tatgtcaaaa tacattttta
BM993875.1	Subchondral bone	tttttttttt tttttttat agntttattc attttatttg tatatgtcaa
CN479410.1	Subchondral bone	tttttttttt tttttttat agntttattc attttatttg tatatgtcaa
BM968622.1	Lung epithelial cells	tttttttttt tttttttca cacatttaaa atagttttat tcatttatt
AW001851.1	Thymus	tttttttttt tcacatttaa aatagtttta ttcattttat ttgtatatgt
CA440121.1	Chondrosarcoma	tttttttttt tttttttaa aatagtttta ttcattttat ttgtatatgt
AI828759.1	Endometrial adenocarcinoma	tttttttttt aaaatagttt tattcatttt atttgtatat gtcaaaatac
BF197980.1*	Skin	aaaatagttt tattcatttt atttgtatat gtcaaaatac atttttattt
AI342381.1	Uterus	tttttttttt atagttttat tcattttatt tgtatatgtc aaaatacatt
CD678763.1	Eye trabecular meshwork	acatatacaa ataaaatgaa taaaactaaa aaaaaaaaaa gggcgg
CD677602.1	Eye trabecular meshwork	tgacatatac aaataaaatg aataaaacta aaaaaaaaaa aaaaaaaaag
BU608452.1	Lung epithelial cells	gacatataca aataaaatga ataaaactaa aaaaaaaaaa aaaaaaaaaa
BU070239.1	Insulinoma	tttttttttt tttttagttt tattcatttt atttgtatat gtcaaaatac atttttattt
BQ272332.1	Insulinoma	tttttttttt tttttagttt tattcatttt atttgtatat gtcaaaatac atttttattt
BM970383.1	Lung epithelial cells	tttttttttt tttttttag ttttattcat tttatttgta tatgtcaaaa tacattttta
BM509287.1	Insulinoma	tttttttttt tttttagttt tattcatttt atttgtatat gtcaaaatac
BI492551.1	Fetal cochlea	tttttttttt tttttagttt tattcatttt atttgtatat gtcaaaatac
AW675588.1	Cervix	ttttttaatt tttttagttt tattcatttt atttgtatat gtcaaaatac
AW516268.1*	Endometrial adenocarcinoma	tagttttatt cattttattt gtatatgtca aaatacattt ttatttccaa

AW452563.1	B cells	ttttttttttt tttttttagt tttattcatt ttatttgtat atgtcaaaat acatttttat
AW021595.1	Fetal cochlea	acatatacaa ataaaatgaa taaaactaaa aaaaaaaaaa aaa
AI439179.1*	Lymphoma	tagttttatt cattttattt gtatatgtca aaatacattt ttatttccaa
AI453664.1*	Stomach adenocarcinoma	tagttttatt cattttattt gtatatgtca aaatacattt ttatttccaa
AA830346.1	B-cell	ttttttttttt tttagtttta ttcattttat ttgtatatgt caaaatacat
AI281137.1*	Colon adenocarcinoma	agttttattc attttatttg tatatgtcaa aatacatttt tatttccaaa
AI281011.1*	Colon adenocarcinoma	agttttattc attttatttg tatatgtcaa aatacatttt tatttccaaa
AA262664.1*	B-cell	agttttattc attttatttg tatatgtcaa aatacatttt tatttccaaa
AW968416.1§	Colon carcinoma	gttttattca ttttatttgt atatgtcaaa atacattttt atttccaaaa
AW967905.1§	Colon carcinoma	gttttattca ttttatttgt atatgtcaaa atacattttt atttccaaaa
AU310314.1§	Neuroblastomna	ttagttttat tcattttatt tgtatatgtc aaaatacatt tttatttcca
CK300902.1	Retina	ttttttttttt tttttttag ttttattcat tttatttgta tatgtcaaaa
BQ189797.1	Fetal eye	gacatatacа aataaaatga ataaaactaa aaaaaaaaaa aaaaaaggac
BE645430.1	Prostate	ttttagtttt attcatttta tttgtatatg tcaaaataca tttttatttc
AW316726.1*	Meningioma	tagttttatt cattttattt gtatatgtca aaatacattt ttatttccaa
AW264811.1*	Lung squamous cell carcinoma	tttaggttta ttcattttat ttgtatatgt caaaatacat ttttatttcc
AI678890.1	Stomach adenocarcinoma	ttttttttttt tttttagttt tattcatttt atttggatat gtcaaaatac
AI082593.1§	Pooled	tagttttatt cattttattt gtatatgtca aaatacattt ttatttccaa
BM511639.1§	Insulinoma	tttattcatt ttatttgtat atgtcaaaat acatttttat ttccaaaata
DB507086.2	Testis	taaaaatgta ttttgacata tacaaataaa acgaataaaa ctaaaaaaac g
CK300002.1	Retina	ttttttttttt agttttattc attttatttg aatatgtcaa aataaatttt
AI923578.1*	Endometrial adenocarcinoma	tagttttatt cattttattt gtatatgtca aaatacattt ttatttccaa
AI342464.1*	Uterus	tattcatttt atttgtatat gtcaaaatac atttttattt ccaaaatagt
AI926946.1*	Prostate	agttttattc attttatttg tatatgtcaa aatacatttt gtatttccaa
AI817061.1	Lung squamous adenocarcinoma	ttttagtttt attcatttta tttgtatatg tcaaaataca tttttatttc
BQ186919.1	Eye	aatttatttt gacatatacа aataaaatga ataaaactaa aaaaaaaaaa atataaggac
BE965957.2	Leiomyosarcoma	ttttttttttt tttttatag ttttattcat tttattggta tatgtcaaaa
AA282476.1*	B-cells	agttttattc attttatttg tatatgtcaa aatacatttt tatttccaaa
AI971777.1	Fibrothecoma	ttttttttttt tagttttatt cattttattt gtatatgtca aaatacattt
AI284479.1*	Esophagus squamous cell carcinoma	aaaatagttt tattcatttt atttgtatat gtcaaaatac atttttattt
H89328.1	Cochlea	Taaaaatgta ttttgacata tacaaataaa atggaataaa actaaaaaaa aa

AW149875.1*	Endometrial adenocarcinoma	tagttttatt cattttattt gtatatgtca aaatacattt ttatttccaa
H89329.1*	Cochlea	catttattt gtatatgtca aaatacattt ttatttccaa aatagtgggt
DB345733.1§	Thymus	cacatttaaa atagttttat tcattttatt tgtatatgtc aaaatacatt
AW131950.1*	Kidney tumour	ttagttttat tcattttatt tgtatatgtc aaaatacatt tttatttcca
AI868665.1§	Pooled	tattcatttt atttgtatat gtcaaaatac atttttattt ccaaaatagt
BI492771.1*	Fetal cochlea	ttatttgtat atgtcaaaat acatccttat ttccaaaata gtgggttttg
Z40397.1*	Brain	attcatttta tttgtatatg tcaaaataca tttttatttc caaaatagtg
AI472433.1*	Colon adenocarcinoma	aaaatagttt tattcatttt atttgtatat gtcaaaatac atttttattt
N057465.1*	Breast carcinoma	ataaaaatgt attttgacat atacaaataa aatgaataaa actatttaa
FN057465.1*	Breast carcinoma	ataaaaatgt attttgacat atacaaataa aatgaataaa actatttaa
BU960397.1	Pool	aaaatgtatt ttgacatata caaataaaat gaataaaact aaaaaaaaaa
BG169053.1	Hypernephroma	ataaaaatgt attttgacat atacaaataa aatgaataaa actaaaaaaa
AA903120.1	Leiomyosarcoma	ttttttttt tcacacattt aaaatagttt tattcatttt atttgtatat
BI966891.1	Pancreas	ttttttttt catggcaaaa tacattttta tttccaaaat agtgggtttt
DW461699.1	Liver	tacaaataaa atgaataaaa ctattttaaa tgcgcaaaaa aaaaaaaaaa

Table S5. Collection of EST clones supporting terminator region T2 approximately 2360 bp downstream of the stop codon. Accession numbers are hyperlinked to the respective GenBank submission. Sequences at the end of each clone were taken directly from the GenBank submission, note that most are in antisense direction. Cleavage sites are highlighted in turquoise unless reliable identification was not possible. *Clones without a clear polyA stretch but with annotation that an oligo dT was used in the cDNA synthesis. §Clones without a clear polyA stretch and no annotation about an oligo dT being used in the cDNA synthesis.

Accession number	Tissue or cell type	Sequence at end of clone
AI061415.1	Gessler Wilms tumour	tttttttttt tttttcaga ttctgcatac agacagcttt atgtaaaata
DB524753.2	Testis	atgtttatat attttacata aagctgtctg tatgcagaaa aaaaaag
BM968507.1	Lung epithelial cells	tttttttttt tttttctgc atacagacag ctttatgtaa aatatataaa
AW673535.1	Cervix	ttatatattt tacataaagc tgtctgtatg cagaaaaaaa aaaaaaag
AW273092.1	Colon carcinoma	ttttttctg catacagaca gctttatgta aaatatataa acatttgtcc
AA913505.1	Pooled	tttttttttt tttctgcata cagacagctt tatgtaaaat atataaacat
W32762.1	Parathyroid tumour	ntttttctgca tacagacagc tttatgtaaa atatataaac atttgtccaa
AA047292.1	Uterus	ntttttttttt tttctgcata cagacagctt tatgtaaaat atataaacat
DB356402.1 §	10-week embryo	ctgcatacag acagctttat gtaaaatata taaacatttg tccaaattgg
BE328265.1	Wilms tumours	tttgcataca gacagcttta tgtaaaatat ataaacattt gtccaaattg
AW731690.1 *	Cervix	tgcatacaga cagctttatg taaaatatat aaacatttgt ccaaattggt
AI004347.1	Fetus	tttttttttt ttttttttt ttctgcatac agacagcttt atgtaaaata
T89964.1 *	Lung	tgcatacaga cagctttatg taaaatatat aaacatttgt ccaaattggt
AA132555.1 *	Colon carcinoma cell line	gcatacagac agctttatgt aaaatatata aacatttgtc caaattggta
AI890988.1	Endometrial adenocarcinoma	tttttttttt ttttttttt ttctgcatac agacagcttt atgtaaaata
AI004376.1	Testis	ttttgcatac agacagcttt atgtaaaata tataaacatt tgtccaaatt
AA027817.1	Uterus	tttttttttt ttttnctgca nacagacagc tttatgtaaa atatataaac
DB373613.1 §	Placenta	ctgcatacag acagctttat gtnaaatata taaacatttg tccaaattgg
AW799002.1 §	Uterus	aaaacagctg atgtaaatat ataaacattg tccaatggta cacagatgca
DN914761.1 §	Breast cancer cell line	cggaaacaga cagctttatg taaaatatat aaacatttgt ccaaattggt

Supplemental Figures

Figure S1. Exon composition of *PPP1R12A* and location of PCR primers used in this study. Nucleotide numbering is as per Ensemble transcript PPP1R12A-202. Exons are in upper case except for the part of E1 that is spliced out when transcription starts at E1^{-}. Introns are in lower case. Coding sequences are highlighted in turquoise except when E24 is spliced in, in which case the sequence after the alternative stop codon in E25 and E26 is non-coding. Non-coding sequences are highlighted in yellow. Exons of non-sense mediated decay transcripts are highlighted in grey. Translation start and stop codons are highlighted in green and red, respectively. Forward PCR primers are in green characters, reverse PCR primers in red characters except E26'r, that overlaps with E26r and is underlined. Primers spanning exon boundaries are not indicated. Note duplication of E13, E24, E25 and E26 to accommodate alternative splicing variants.

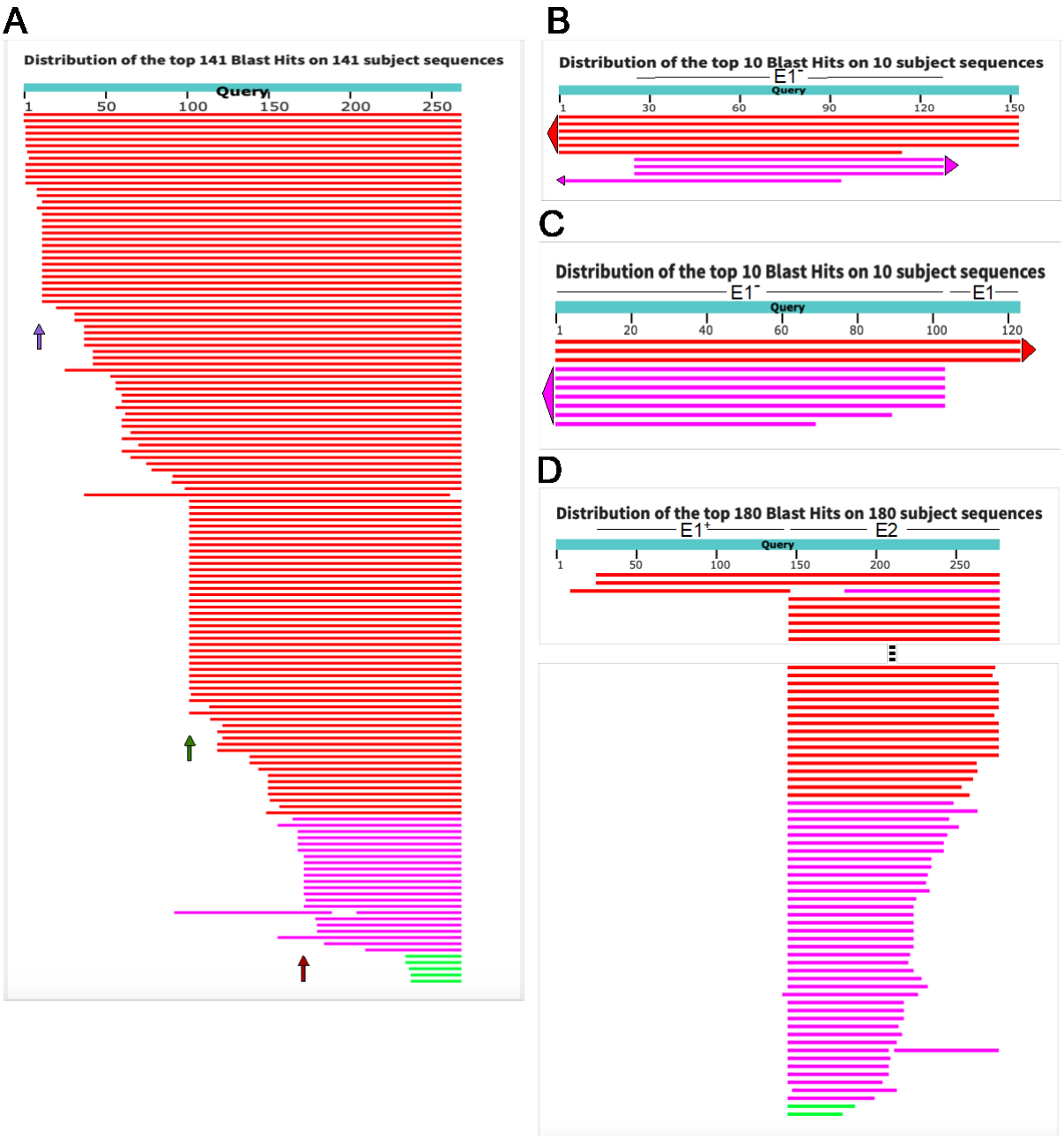


Figure S2. Results of BLASTn searches for ESTs in the transcription start regions of *PPP1R12A*. Horizontal bars represent the extent of the alignment of the database sequences to the query sequence and are color-coded by score: red, pink and green from highest to lowest. (A) EST support for a transcription start in E1. The human EST database was interrogated with the sequences corresponding to the UTR of E1 as per transcript PPP1PR12-208. Arrows indicate predicted transcription start sites and are colored like in Fig. 1C. (B and C) EST support for a transcription start variant at E1^{-}. In (B) the human EST database was interrogated with the sequence corresponding to E1^{-} flanked by 25 bases of genomic/intron sequence. The arrowheads indicate the directionality of the clones. Three hits read towards the right and encompass only the E1^{-} sequence. The rest read in the opposite direction and correspond to the overlapping antisense gene *PPP1R12A-AS1*. In (C) the human EST database was interrogated with the sequences corresponding to E1^{-} and 20 bases of E1 as per transcript PPP1R12A-201. The hits are the same as in panel B: the three top ones correspond to EST clones that contain E1^{-} and splice E1^{-} into E1, the rest correspond to *PPP1R12A-AS1*. (D) Support for a transcription start variant at E1^{+}. The human EST database was interrogated with the sequences corresponding to E1^{+}–E2 flanked by 25 bases of genomic/intron sequence before E1^{+}. The output has been truncated (dashed line). The two top hits correspond to EST clones that include E1^{+}. One EST that covers only E1^{+} is flanked by intron sequences and may correspond to amplification of contaminating genomic DNA.

Figure S3. Results of BLASTn searches for ESTs in support of transcripts with non-canonical exons. Horizontal bars represent the extent of the alignment of the database sequences to the query sequence and are color-coded by score: red, pink and green from highest to lowest. Separate aligned regions on the same database sequence are connected by a thin grey line. (A) EST support for a splicing variant with 144 bp exon $E2^+$ (transcript PPP1R12A-209). The EST database was interrogated with the sequences corresponding to E2–$E2^+$–E3. The search was limited to 100 hits and only the top 42 hits are shown. A single EST (BP279682.1, asterisk) supports this transcript. The annotation in Ensembl identifies a start codon in E3 that matches the sequence of MYPT1 and interprets the sequences upstream as UTR. It appears more likely that the inclusion of the extra $E2^+$ would cause a shift to the canonical reading frame, introducing a premature stop codon, therefore this variant would be a nonsense mediated decay mRNA. (B) EST support for a splicing variant with 83 bp exon $E20^+$ (PPP1R12A-217). This exon causes a frame shift and a premature stop. The EST database was

interrogated with the sequences corresponding to E19–E20–E20^{+}–E22. Here E19 was included due to the small size of E20, insufficient for significant alignments. This search revealed two clones that spliced in E20^{+} (asterisks). One clone that only covers E20^{+} (arrowhead) contains intron sequences upstream and downstream and may correspond to an intron retain clone. (C) EST support for a splicing variant with 25 bp exon E22^{+} (PPP1R12A-205). This exon causes a frame shift and a premature stop. The human EST database was interrogated with the sequences corresponding to E22–E22^{+}–E23. This search revealed a single sequence, BG180627.1, containing E22^{+} (top hit with an asterisk). The second hit appears to correspond to a clone (BF980059.1) with anomalous splicing at the E22^{+}–E23 boundary. One clone that appears to include E22^{+} (at the bottom, asterisk) corresponds to a misaligned sequence and two more that only cover E22^{+} (arrowheads) contain intron sequences upstream and downstream and may correspond to intron retain transcripts.

Figure S4. Results of BLASTn searches for ESTs in support of alternatively spliced exons. Separate aligned regions on the same database sequence are connected by a thin grey line. Vertical tick marks indicate intervening sequences not present in the query sequence. (A) Splicing of E6. The EST database was interrogated with the sequences corresponding to E5–E7. The boundary between E5 and E7 is placed at bp 145. Two ESTs were found in which E6 was spliced out (asterisks). (B) Splicing of 117 bp exon $E9^+$ (transcript PPP1R12A-223). The EST database was interrogated with the sequences corresponding to E9–$E9^+$–E10. This search and a further search with the $E9^+$ sequence alone failed to reveal any ESTs including $E9^+$. The sequence marked with an arrow showed anomalous splicing of E10. (C) Splicing of E13. The EST database was interrogated with the

sequences corresponding to E12–E14. The boundary between E12 and E14 is placed at bp 105. Twelve ESTs were found in which E13 was spliced out (asterisks). (D) An alternative splicing acceptor site in E13. The EST database was interrogated with the sequences corresponding to E12 plus E13b, a 32 bp truncated E13. The boundary between E12 and E13b is placed at bp 105. Four ESTs were found in support of this variant (asterisks). (E) Splicing of E14. The EST database was interrogated with the sequences corresponding to E13–E15. The boundary between E13 and E15 is placed at bp 168. One EST was found in which E14 was spliced out (asterisk). (F) Splicing of E13+E14. The EST database was interrogated with the sequences corresponding to E2–E15. The boundary between E12 and E15 is placed at bp 105. Four ESTs was found in which E13 and E14 were spliced out together (asterisks). (G) Splicing of E22. The EST database was interrogated with the sequences corresponding to E21–E23–E25. The boundary between E21 and E23 is placed at bp 136. Note the absence of EST clones splicing out E22. The clone marked with a black arrow head is anomalous, it contains E21 in sense fused to a fragment composed of E23+E24+E25 plus part of the coding region of E26 in antisense. The clones marked with red arrow heads splice in the 31 bp variant of E24 (tick mark at bp 184). (H) Splicing of E24. The EST database was interrogated with the sequences corresponding to E23–E24–E25 using the 31 bp variant of E24 (nucleotides 49–79 in the graph). Inspection of the six top hit alignments revealed three that include the 31 bp variant and three that include the 13 bp variant (the 18 bp difference is too short to be displayed in the graph). Five ESTs matching almost only E24 at the bottom of the graph (in green) probably correspond to intron-retaining transcripts (PPP1R12A-210 or 220).

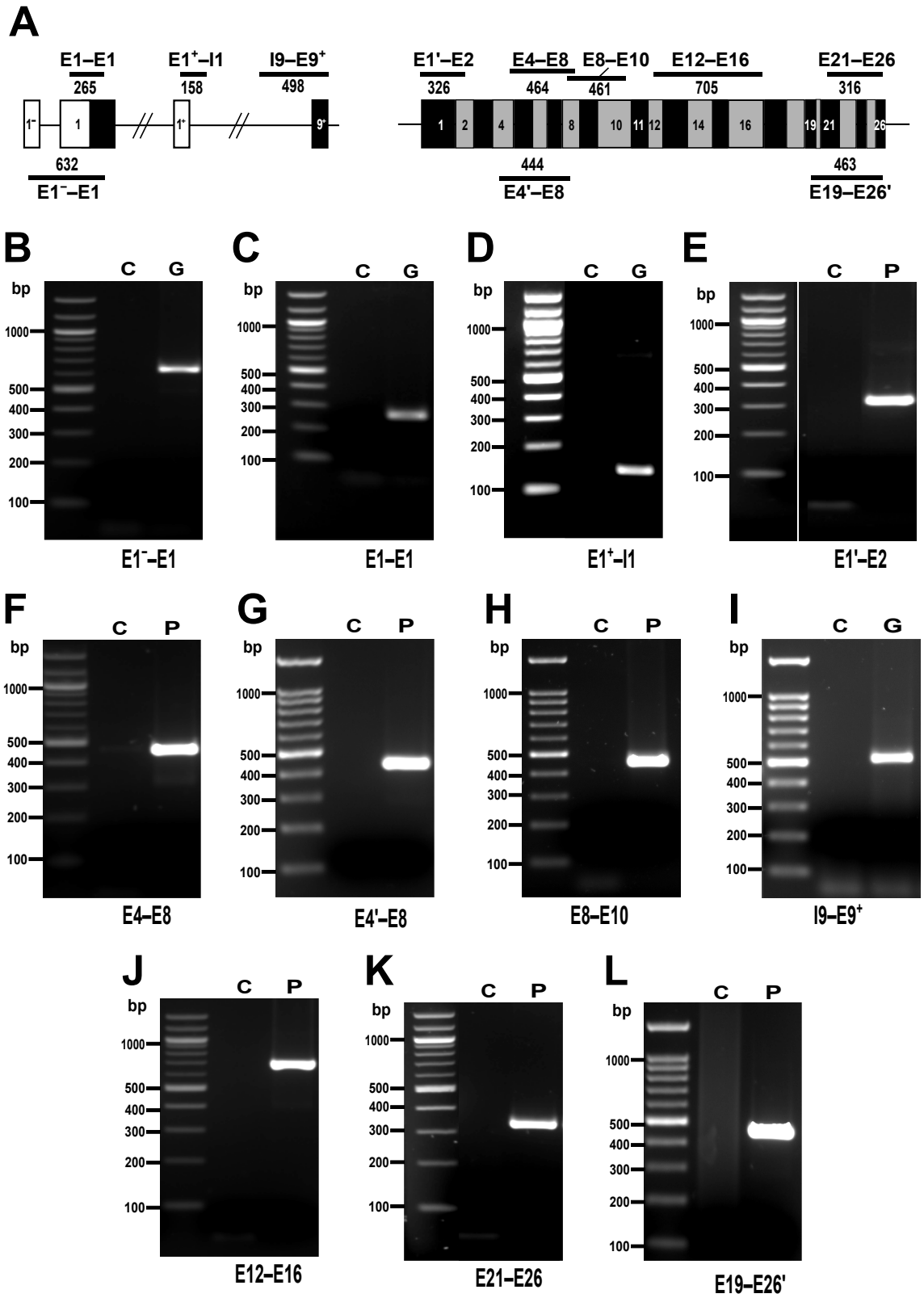


Figure S5. Test PCR reactions of *PPP1R12A* primers. (A) Position and expected size of PCR products. On the left, scheme of the genomic sequence around exons E1 and E9^{+}; white and black boxes represent untranslated and translated regions, respectively. On the right, coding region of the canonical *PPP1R12A* FL variant (lacking E24); exons are shown at scale alternating black and grey. Primer pairs in the forward-reverse sequence and size of expected PCR products are indicated above and under the DNA schemes. Primer sequences and PCR conditions are shown in Table S1. (B-L) Results of PCR reactions using the primer combinations of the top panel. G, reactions were run using genomic DNA; P, reactions were run using a plasmid carrying the coding sequence of the FL *PPP1R12A* variant as template; C, control reaction without template.

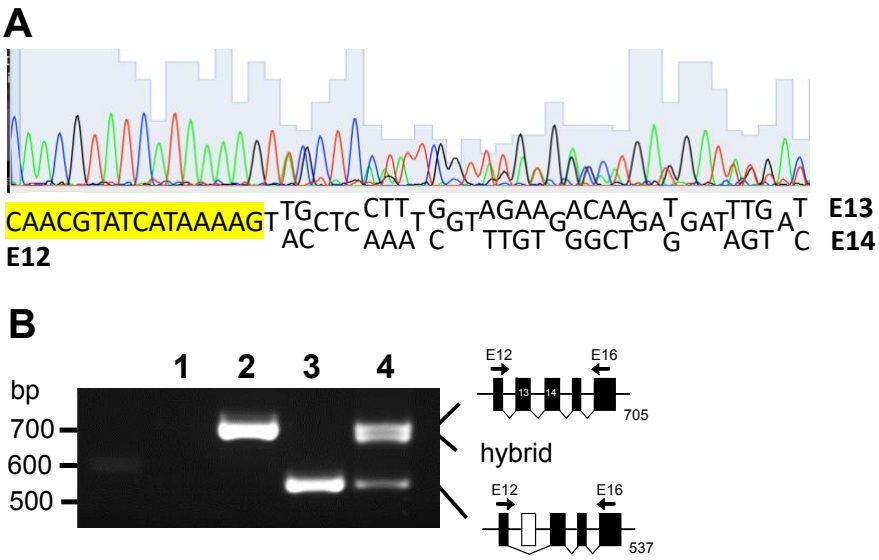


Figure S6. Hybridization of PCR products of reaction targeting E13 and E14 variants. (A) Fragment of the sequencing reaction chromatogram of a hybrid product (intermediate band) of the PCR reaction with primer pair E12-E16 (Fig. 5D). The hybrid band was purified from an agarose gel and sequenced with E12 forward primer. The 3' end of E12 is highlighted in yellow. It is followed by mixed sequences compatible with E13 and E14, confirming that the band is a hybrid of E13in and E13out DNA fragments. (B) Hybridization of E13in and E13out amplicons in a PCR reaction using plasmid DNA as template. Lane 1, negative control (no template); lane 2, plasmid containing full length MYPT1 cDNA as template; lane 3, plasmid containing an MYPT1 cDNA fragment lacking E13 as template; lane 4, mix of both plasmids as template.

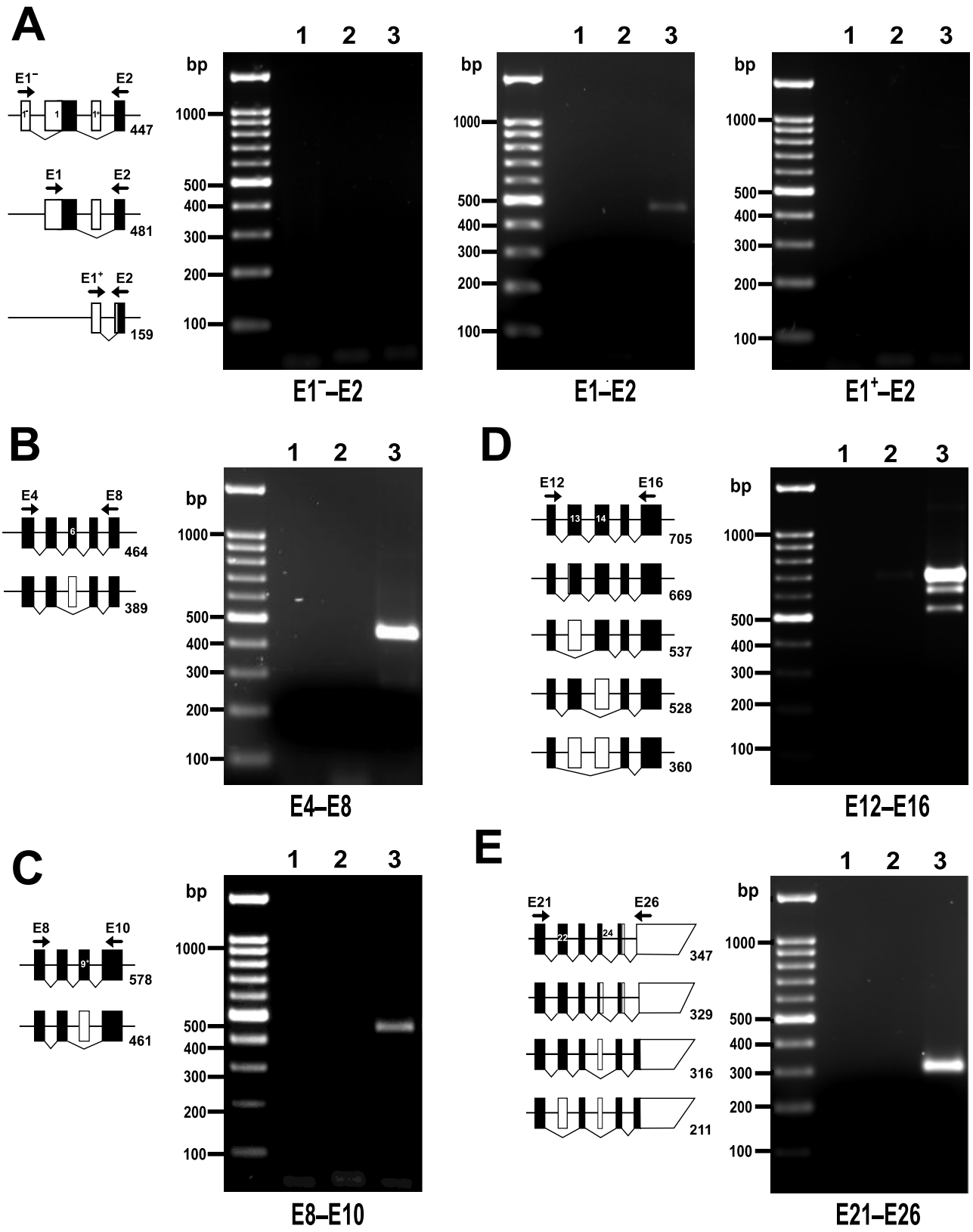


Figure S7. *PPP1R12A* transcripts in human saphenous vein smooth muscle cells as determined by RT-PCR. The diagrams accompanying each panel depict the expected size of PCR products corresponding to all possible alternatively spliced variants sorted by size. White and black boxes represent untranslated and translated exons, respectively. Exons are depicted at scale. Position of primer pairs is indicated schematically. (A) PCR reactions targeting alternative transcription start sites. A transcription start on E1 is the only site used by HSVSMCs. PCR reactions targeting (B) E6, (C) E9^{+}, (D) E13 and E14, (E) E22 and E24 splicing variants. PCR reactions were run using no template (lane 1), negative control for reverse transcriptase (lane 2) or cDNA (lane 3) as templates. Primer sequences and PCR conditions are shown in Table S2.

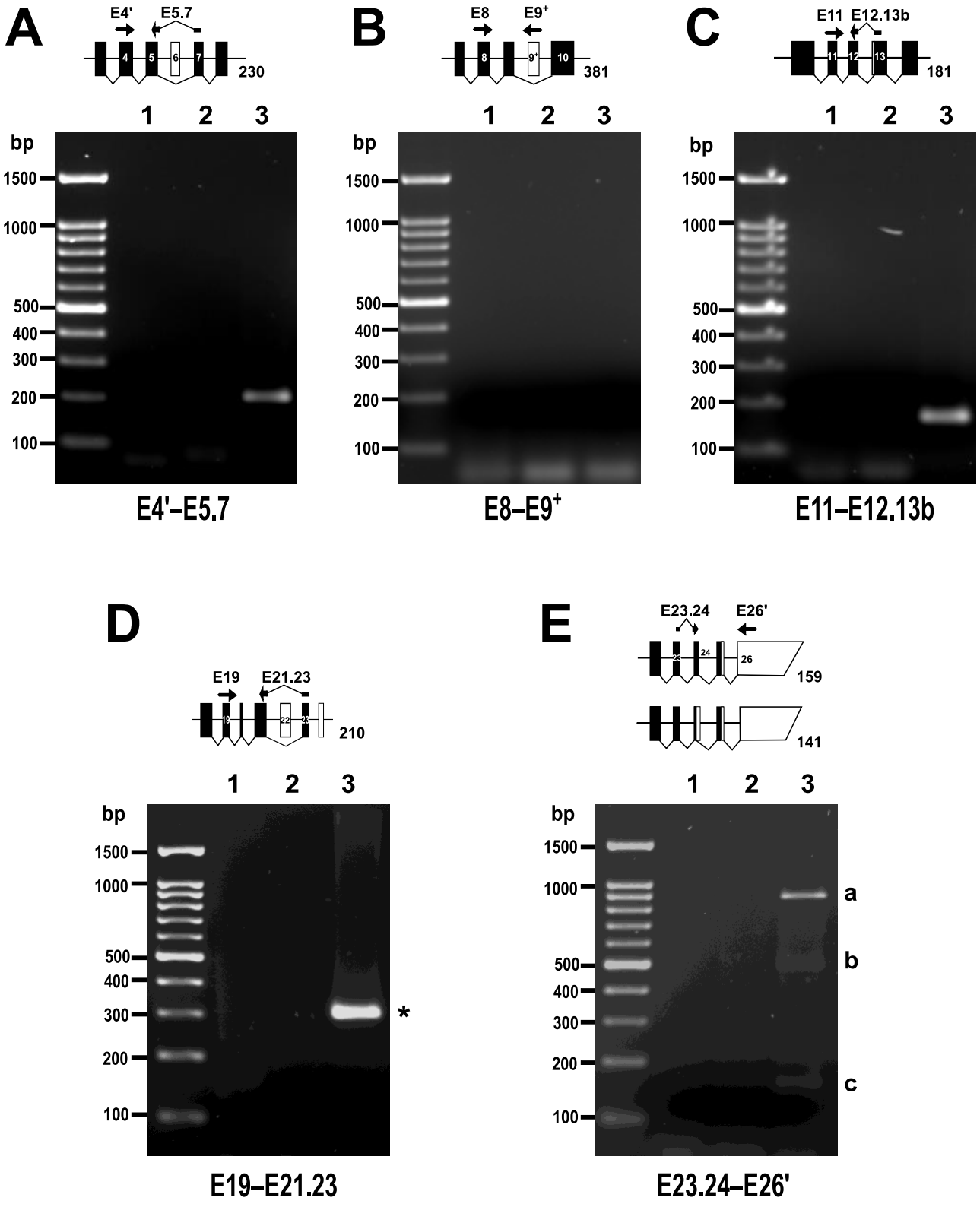


Figure S8. Rare *PPP1R12A* transcripts in human saphenous vein smooth muscle cells as determined by RT-PCR. The diagrams accompanying each panel depict the expected size of PCR products corresponding to the rare alternatively spliced variants. White and black boxes represent untranslated and translated exons, respectively. Exons are depicted at scale. Position of primer pairs is indicated schematically. PCR reactions targeting the (A) ΔE6, (B) $E9^{+}$, (C) E13b and (D) ΔE22 splicing variants. The asterisk indicates an unexpected PCR product. (E) PCR reaction targeting LZ^{-} splicing variants. Bands a and b are unexpected amplicons, band c corresponds to the 159 bp product. PCR reactions were run using no template (lane 1), negative control for reverse transcriptase (lane 2) or cDNA (lane 3) as templates. Primer sequences and PCR conditions are shown in Table S2.

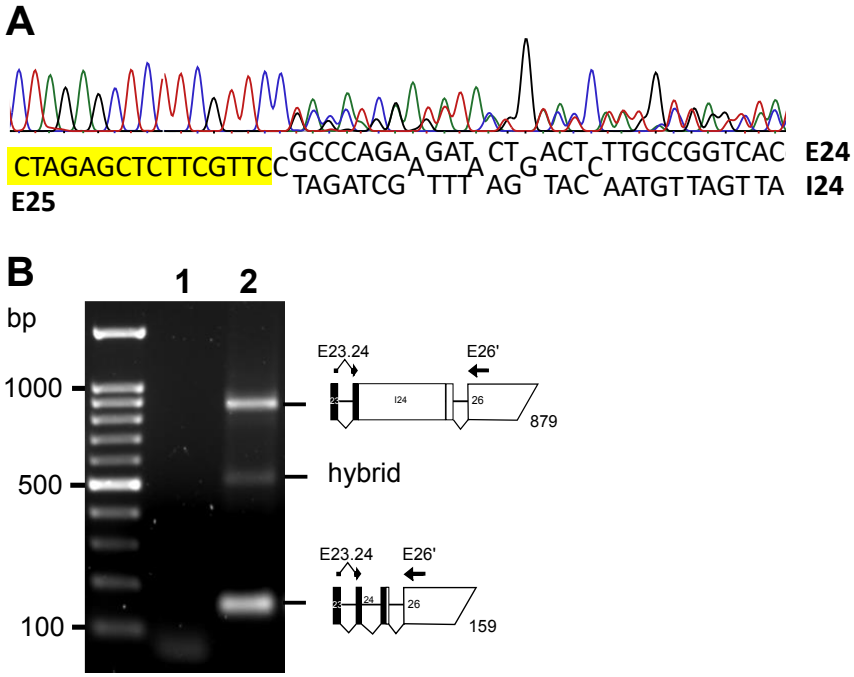


Figure S9. Hybridization of PCR products of the reaction targeting E24. (A) Fragment of the sequencing reaction chromatogram of a hybrid product (intermediate band) of the PCR reaction with primer pair E23.24–E26' (Fig. S8E). The hybrid band was purified from an agarose gel and sequenced with 26' reverse primer. The 5' end of E25 is highlighted in yellow. It is followed by mixed sequences compatible with E24 and I24, confirming that the band is a hybrid of E24in and retained intron DNA fragments. (B) Hybridization of E24in (159 bp) and a retained intron (879 bp) DNA in a PCR reaction using the hybrid band as template (lane 2). Lane 1 is a negative control (no template). This reaction yielded the 159 bp and the 879 bp along with a small amount of hybrid product.

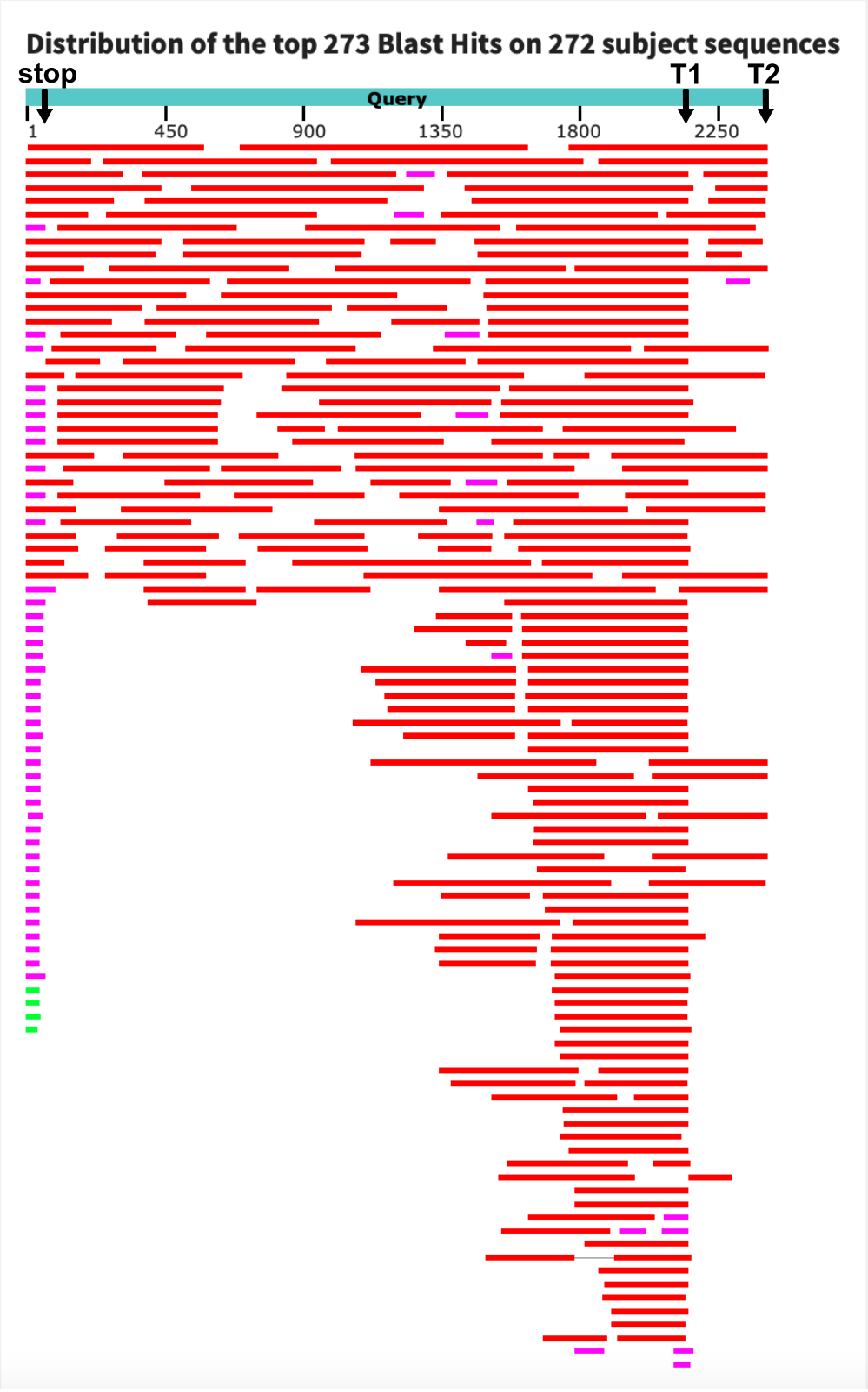


Figure S10. Results of BLASTn search for ESTs in the 3'-UTR region of *PPP1R12A*. The human EST database was interrogated with a BLASTn search using the sequences corresponding to the last 52 coding bp followed by the complete 3'-UTR. Horizontal bars represent the extent of the alignment of the database sequences to the query sequence and are color-coded by score: red, pink and green from highest to lowest. An accumulation of ESTs that terminate sharply approximately 2100 bp downstream of the stop codon suggests the presence of a terminator in that area (T1). A less common terminator (T2) is suggested 260 bp downstream of the first termination site.