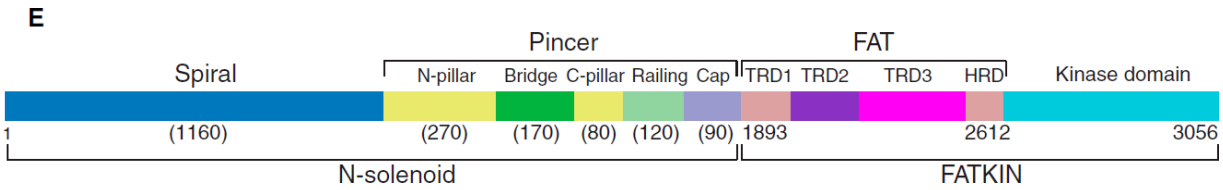
PVS1 Null variant (nonsense, frameshift, canonical +/-1 or 2 splice sites, initiation codon, single or multi-exon deletion) in a gene where loss of function (LOF) is a known mechanism of disease
Caveats:

1. Use caution interpreting LOF variants at the extreme 3' end of a gene
2. Use caution with splice variants that are predicted to lead to exon skipping but leave the remainder of the protein intact

Notes

1. The default RefSeq transcript for nucleotide (c.) annotation is **NM_000051.3/ENST00000278616.8.** All exons from this transcript can be considered constitutive exons without major alternate splice isoforms that could potentially rescue presumed LoF events (ENIGMA unpublished data).
   - Of note, ATM is occasionally annotated with multiple non-coding first exons so exon numbering must be carefully reviewed for variant interpretation using literature sources of data.
2. **The FAT/PI3K/FATC (collectively the FATKIN)** domains are considered *critical* for ATM protein function (PMID 28508083, 31740029, 31320732). PVS1 alterations that are predicted to escape NMD, but that adversely affect these domains can be granted PVS1 (as opposed to PVS1_Strong as the recommended base-line (PMID 30192042).
3. **The HEAT repeat domain** is considered *important* for protein function based on the appearance of many A-T affected individuals harboring a variant resulting in an in-frame, single exon loss in this domain (PMID 10980530, 19535770, 30819809, 15054841, 22927201, 19691550, 10330348, 17124347, 8845835, 16266405, 9463314, 24090759, 22213089). PVS1-eligible alterations that are predicted to escape NMD, but that adversely affect the HEAT repeat domain can be granted PVS1_Strong. They are limited to strong due to a lack of known missense pathogenic alterations in this domain.
4. The most 3'/C-Terminal residue considered to be pathogenic is p.R3047 (PMIDs: 8755918, 19691550, 18560558, 10980530, 26628246)
5. Below is the domain structure as annotated in PMID 28508083 and used in this body of work to delineate PVS1 boundaries

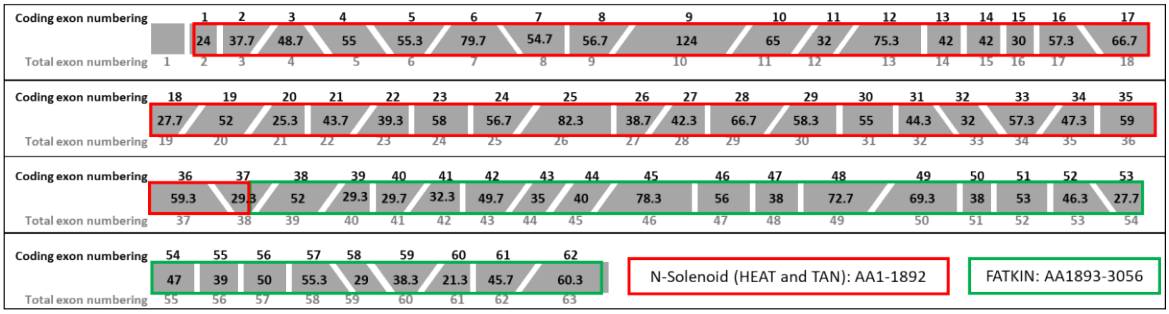


***ATM* Exon Map:** Use for Single- and Multi-Cassette Exon Losses and functional domain determination

- Number above exon in black text represents the coding exon number
- Number below exon in gray text represents total exon numbering
- The first exon is a non-coding 5'UTR-only exon
- Number within the exon represents the exon length (amino acid)

- Overhang on top: a two-nt overhang
- Overhang on bottom: a one-nt overhang
- Parallel lines represent in-frame changes (e.g. total EX6_7del is in-frame [5' of exon 6 and 3' of exon 7 are parallel]; however EX6_8del is out-of-frame [5' of exon 6 and 3' of exon 8 are not parallel)

ATM NM_000051.3/ENST00000278616.8



NOTE: Many diagrams for ATM show the FAT, PI3-K and FATC domains as separated by spacers, however these are not empirically derived and there is evidence of missense pathogenic alterations in the 'spacer' regions. This VCEP considers them a contiguous domain (PMID 28508083).

PVS1 can be applied as per the below decision tree.

PVS1_Variable(RNA) shall be used for observed splice defects, whether from canonical +/-1,2 positions or other spliceogenic regions (including mid-exonic missense/synonymous variants that cause splice defects) with baseline weight as per the below decision tree. Weight can be further modified based on the quality of the RNA study including consideration of concepts such as:

- Starting material (where patient material is preferable to in vitro minigene)
- Use of NMD inhibitors (where use of NMD inhibitors is critical in assays using cells vs. blood)
- Primer design (to make sure it's comprehensive to capture possible multicassette events)
- Method of quantification
  - where e.g. capillary electrophoresis is preferable to estimation by gel band density
  - where SNP analysis is most preferred (where analysis of exonic SNPs and their relative presence in aberrant and WT transcripts is informative)
- Quantification (where complete effects should have increased weight over incomplete effects)

Specific guidance on the use of RNA evidence in variant assessment is not a gene-specific consideration for *ATM* at this time, therefore discretion is left to assessors until further guidance is provided for this general concept from the Sequence Variant Interpretation group.

ATM PVS1/PVS1(RNA) Guide (Adapted from PMID 30192042)

1. PVS1 decision tree, based on ACMG/AMP rationale (Tayoun et al, 2018), introducing some code strength modifications (upgrades and downgrades, color coded as indicated), and a few instances not considered by Tayoun et al (**e.g. splice sites in non-coding exons,**. Color coded as indicated)

2. We have considered NM_000051 the clinically relevant reference transcript (63 exons, 62 coding exons, start codon located in total exon 2, coding a 3056aa protein)

3. We are not aware of any potential rescue transcripts (i.e. for the sake of simplicity, in the decision tree we will not refer to "exon is absent from biologically-relevant transcripts")

4. We define two clinically relevant domains: (i) an N-Solenoid (containing TAN and HEAT repeat domains) spaning residues 1-1892 (coded by total exons 2 to 38), and (ii) a C-terminal FATKIN domain spanning residues 1893-3056 (coded by total exons 38 to 63).

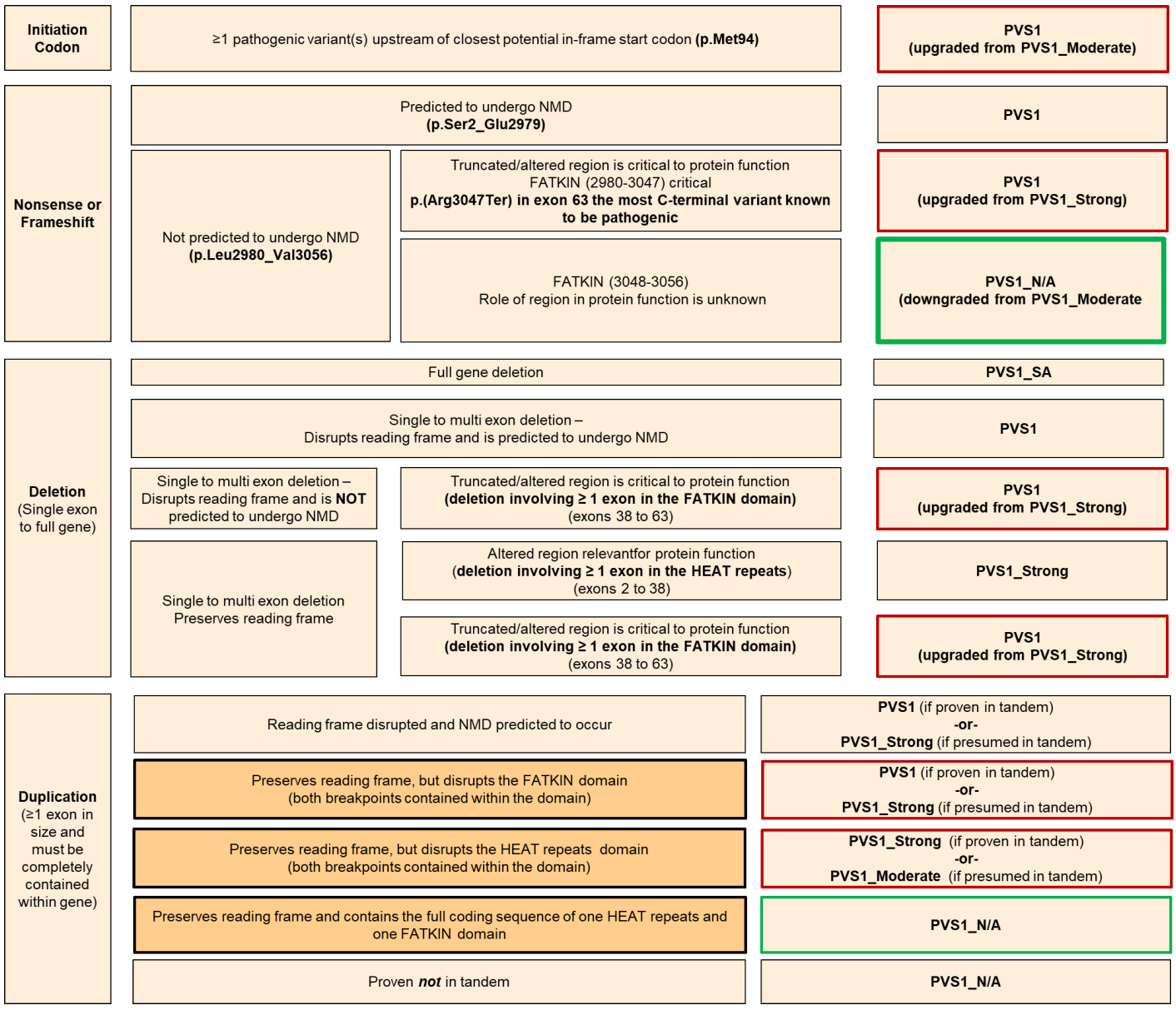
5. Based on clinical and structural data, we have considered in-frame alterations targeting HEAT repeats as **PVS1_Strong,** the only exception being any very small in-frame alterations with PROVEAN score sugesting pathogenic, that were considered **PVS1_Supporting**

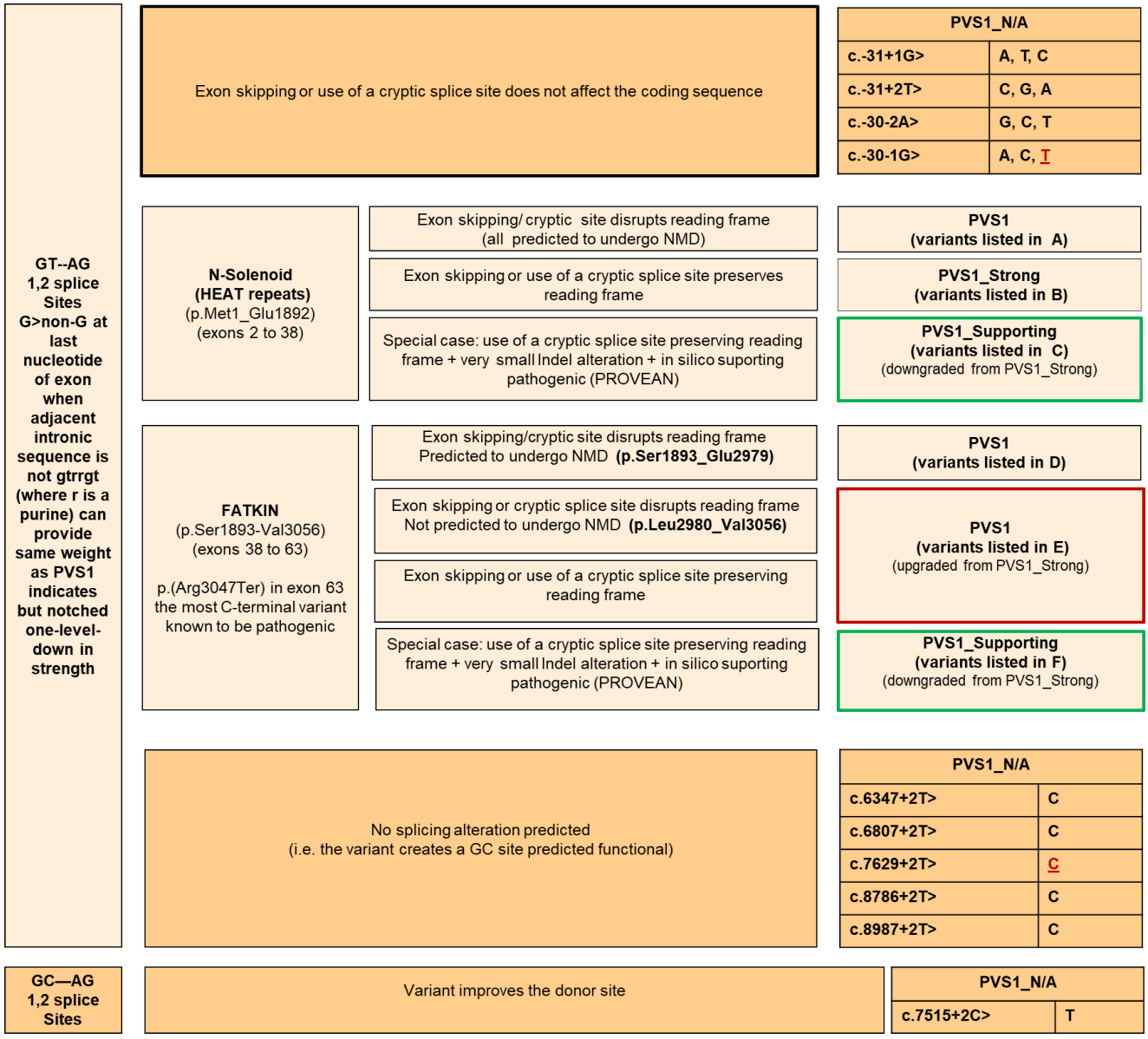
6. Based on clinical and structural data, we have considered in-frame alterations targeting FATKIN as **PVS1**, the only exception being very small in-frame alterations with PROVEAN score sugesting pathogenic, that were considered **PVS1_Supporting**

7. As far as we know, p.Arg3047Ter is the last PTC variant known to be pathogenic

8. The existence of experimental data (literature and/or personal communication from HBOP VCEP members) supporting the PVS1 weight are denoted by **red-underline** in the PVS1 decision tree.

Related publication(s):


Initiation Codon
≥1 pathogenic variant(s) upstream of closest potential in-frame start codon (p.Met94)
PVS1 (upgraded from PVS1_Moderate)
Nonsense or Frameshift
Predicted to undergo NMD (p.Ser2_Glu2979)
PVS1
Not predicted to undergo NMD (p.Leu2980_Val3056)
Truncated/altered region is critical to protein function FATKIN (2980-3047) critical p.(Arg3047Ter) in exon 63 the most C-terminal variant known to be pathogenic
PVS1 (upgraded from PVS1_Strong)
FATKIN (3048-3056) Role of region in protein function is unknown
PVS1_N/A (downgraded from PVS1_Moderate
Deletion (Single exon to full gene)
Full gene deletion
PVS1_SA
Single to multi exon deletion – Disrupts reading frame and is predicted to undergo NMD
PVS1
Single to multi exon deletion – Disrupts reading frame and is NOT predicted to undergo NMD
Truncated/altered region is critical to protein function (deletion involving ≥ 1 exon in the FATKIN domain) (exons 38 to 63)
PVS1 (upgraded from PVS1_Strong)
Single to multi exon deletion Preserves reading frame
Altered region relevantfor protein function (deletion involving ≥ 1 exon in the HEAT repeats) (exons 2 to 38)
PVS1_Strong
Truncated/altered region is critical to protein function (deletion involving ≥ 1 exon in the FATKIN domain) (exons 38 to 63)
PVS1 (upgraded from PVS1_Strong)
Duplication (≥1 exon in size and must be completely contained within gene)
Reading frame disrupted and NMD predicted to occur
PVS1 (if proven in tandem) -or- PVS1_Strong (if presumed in tandem)
Preserves reading frame, but disrupts the FATKIN domain (both breakpoints contained within the domain)
PVS1 (if proven in tandem) -or- PVS1_Strong (if presumed in tandem)
Preserves reading frame, but disrupts the HEAT repeats domain (both breakpoints contained within the domain)
PVS1_Strong (if proven in tandem) -or- PVS1_Moderate (if presumed in tandem)
Preserves reading frame and contains the full coding sequence of one HEAT repeats and one FATKIN domain
PVS1_N/A
Proven *not* in tandem
PVS1_N/A


GT--AG 1,2 splice Sites G>non-G at last nucleotide of exon when adjacent intronic sequence is not gtrrgt (where r is a purine) can provide same weight as PVS1 indicates but notched one-level-down in strength
Exon skipping or use of a cryptic splice site does not affect the coding sequence
PVS1_N/A
c.-31+1G> A, T, C
c.-31+2T> C, G, A
c.-30-2A> G, C, T
c.-30-1G> A, C, T
N-Solenoid (HEAT repeats) (p.Met1_Glu1892) (exons 2 to 38)
Exon skipping/ cryptic site disrupts reading frame (all predicted to undergo NMD)
PVS1 (variants listed in A)
Exon skipping or use of a cryptic splice site preserves reading frame
PVS1_Strong (variants listed in B)
Special case: use of a cryptic splice site preserving reading frame + very small Indel alteration + in silico suporting pathogenic (PROVEAN)
PVS1_Supporting (variants listed in C) (downgraded from PVS1_Strong)
FATKIN (p.Ser1893-Val3056) (exons 38 to 63)
p.(Arg3047Ter) in exon 63 the most C-terminal variant known to be pathogenic
Exon skipping/cryptic site disrupts reading frame Predicted to undergo NMD (p.Ser1893_Glu2979)
PVS1 (variants listed in D)
Exon skipping or cryptic splice site disrupts reading frame Not predicted to undergo NMD (p.Leu2980_Val3056)
PVS1 (variants listed in E) (upgraded from PVS1_Strong)
Exon skipping or use of a cryptic splice site preserving reading frame
Special case: use of a cryptic splice site preserving reading frame + very small Indel alteration + in silico suporting pathogenic (PROVEAN)
PVS1_Supporting (variants listed in F) (downgraded from PVS1_Strong)
No splicing alteration predicted (i.e. the variant creates a GC site predicted functional)
PVS1_N/A
c.6347+2T> C
c.6807+2T> C
c.7629+2T> C
c.8786+2T> C
c.8987+2T> C
GC—AG 1,2 splice Sites
Variant improves the donor site
PVS1_N/A
c.7515+2C> T

N-terminal HEAT repeats
(exon2 to exon 38)

C-terminal FATKIN
(exon 38 to exon 63)
p.(Arg3047Ter) in exon 63 the most C-terminal variant known to be pathogenic

Exon skipping or use of a cryptic splice site disrupts reading frame
(all predicted to undergo NMD)

PVS1 (list A)	
c.72+1G>	A, C, T
c.72+2T>	A, C, G
c.73-2A>	C, G, T
c.73-1G>	A, C, T
c.185+1G>	A, C, T
c.185+2T>	A, C, G
c.186-2A>	C, G, T
c.186-1G>	A, C, T
c.331+1G>	A, C, T
c.331+2T>	A, C, G
c.497-2A>	C, G, T
c.497-1G>	A, C, T
c.662+1G>	A, C, T
c.662+2T>	A, C, G
c.663-2A>	C, G, T
c.663-1G>	A, C, T
c.901+1G>	A, C, T
c.901+2T>	A, C, G
c.902-2A>	C, G, T
c.902-1G>	A, C, T
c.1065+1G>	A, C, T
c.1065+2T>	A, C, G
c.1066-2A>	C, G, T
c.1066-1G>	A, C, T
c.1235+1G>	A, C, T
c.1235+2T>	A, C, G
c.1236-2A>	C, G, T
c.1236-1G>	A, C, T
c.1803-2A>	C,G,T
c.1803-1G>	A,C,T
c.1899-2A>	C, G, T
c.1899-1G>	A, C, T
c.2124+1G>	A,C,T
c.2124+2T>	A,C,G
c.2125-2A>	C, G, T
c.2125-1G>	A, C, T
c.2251-2A>	C, G, T
c.2251-1G>	A, C, T

PVS1 (list A)	
c.2467-2A>	G
c.2467-1G>	A
c.2638+1G>	A, C, T
c.2638+2T>	A, C, G
c.2639-2A>	C, G, T
c.2639-1G>	A, C, T
c.2838+1G>	A, C, T
c.2838+2T>	A, C, G
c.2921+1G>	A, C, T
c.2921+2T>	A, C, G
c.2922-2A>	C, G, T
c.2922-1G>	A, C, T
c.3077+1G>	A, C, T
c.3077+2T>	A, C, G
c.3078-2A>	C, G, T
c.3078-1G>	A, C, T
c.3153+1G>	A, C, T
c.3153+2T>	A, C, G
c.3154-2A>	C, G, T
c.3154-1G>	A, C, T
c.3284+1G>	A, C, T
c.3284+2T>	A, C, G
c.3285-2A>	C, G, T
c.3285-1G>	A, C, T
c.3402+1G>	A, C, T
c.3402+2T>	A, C, G
c.3403-2A>	C, G, T
c.3403-1G>	A, C, T
c.3577-2A>	C, G, T
c.3577-1G>	A, C, T
c.3746+1G>	A, C, T
c.3746+2T>	A, C, G
c.3747-2A>	C, G, T
c.3747-1G>	A, C, T
c.3994-2A>	C, G, T
c.3994-1G>	A, C, T
c.4109+1G>	A, C, T
c.4109+2T>	A, C, G

PVS1 (list A)	
c.4110-2A>	C, G, T
c.4110-1G>	A, C, T
c.4236+1G>	A, C, T
c.4236+2T>	A, C, G
c.4237-1G>	A
c.4436+1G>	A, C, T
c.4436+2T>	A, C, G
c.4437-1G>	A
c.4611+1G>	A, C, T
c.4611+2T>	A, C, G
c.4777-2A>	C, G, T
c.4777-1G>	A, C, T
c.4909+1G>	A, C, T
c.4909+2T>	A, C, G
c.5006-2A>	C, G, T
c.5006-1G>	A, C, T
c.5177+1G>	A, C, T
c.5177+2T>	A, C, G
c.5178-2A>	C, G, T
c.5178-1G>	A, C, T
c.5319+1G>	A, C, T
c.5319+2T>	A, C, G
c.5320-2A>	C, G, T
c.5320-1G>	A, C, T
c.5496+2T>	A, C, G
c.5497-2A>	C, G, T
c.5497-1G>	A, C, T
c.5674+1G>	A, C, T
c.5674+2T>	A, C, G
c.5675-2A>	G
c.5675-1G>	A, C, T
c.5762+1G>	A, C, T
c.5762+2T>	A, C, G

PVS1 (list D)	
c.5674+2T>	A, C, G
c.5675-2A>	G
c.5675-1G>	A, C, T
c.5762+1G>	A, C, T
c.5763-2A>	C, G, T
c.5763-1G>	A, C, T
c.6006+1G>	A, C, T
c.6006+2T>	A, C, G
c.6007-2A>	C, G, T
c.6007-1G>	A, C, T
c.6095+1G>	A, C, T
c.6095+2T>	A, C, G
c.6096-2A>	C, G, T
c.6096-1G>	A, C, T
c.6198+1G>	A, C, T
c.6198+2T>	A, C, G
c.6199-1G>	A
c.6347+1G>	A, C, T
c.6347+2T>	A, G
c.6453-2A>	C, G, T
c.6453-1G>	A, C, T
c.6573-2A>	C, G, T
c.6573-1G>	A, C, T
c.6807+1G>	A, C, T
c.6807+2T>	A, G
c.7090-2A>	C, G, T
c.7090-1G>	A, C, T
c.7307+1G>	A, C, T
c.7307+2T>	A, C, G
c.7308-2A>	C, G, T
c.7308-1G>	A, C, T
c.7515+1G>	A, C, T
c.7515+2C>	A, G
c.7516-2A>	C, G, T
c.7516-1G>	A, C, T
c.7789-2A>	C, G, T
c.7789-1G>	A, C, T
c.7927+1G>	A, C, T
c.7927+2T>	A, C, G

PVS1 (list D)	
c.8010+1G>	A, C, T
c.8010+2T>	A, C, G
c.8011-2A>	C, G, T
c.8011-1G>	A, C, T
c.8152-2A>	G
c.8152-1G>	A
c.8419-2A>	G
c.8419-1G>	A
c.8584+1G>	A, C, T
c.8584+2T>	A, C, G
c.8672-2A>	C, G, T
c.8672-1G>	A, C, T
c.8786+1G>	A, C, T
c.8786+2T>	A, G
c.8787-2A>	C, G, T
c.8787-1G>	A, C, T
c.8850+1G>	A, C, T
c.8850+2T>	A, C, G
c.8851-1G>	A

Related publication(s):

N-terminal HEAT repeats
(exons 2 to 38)

Exon skipping or use of a cryptic splice site preserves reading frame

PVS1_Strong (list B)	
c.332-2A>	C, G, T
c.332-1G>	A, C, T
c.496+1G>	A, C, T
c.496+2T>	A, C, G
c.1607+1G>	A, C, T
c.1607+2T>	A,C,G
c.1608-2A	C,G,T
c.1608-1G>	A,C,T
c.1802+1G>	A, C, T
c.1802+2T>	A, C, G
c.1898+1G>	A, C, T
c.1898+2T>	A, C, G
c.2250+1G>	A, C, T
c.2250+2T>	A, C, G
c.2376+1G>	A, C, T
c.2376+2T>	A, C, G
c.2377-2A>	C, G, T
c.2377-1G>	A, C, T
c.2466+1G>	A, C, T
c.2466+2T>	A, C, G
c.3576+1G>	A, C, T
c.3576+2T>	A, C, G
c.3993+1G>	A, C, T
c.3993+2T>	A, C, G
c.4612-2A>	C, G, T
c.4612-1G>	A, C, T
c.4776+1G>	A, C, T
c.4776+2T>	A, C, G

PVS1_Strong (list B)	
c.4910-2A>	C, G, T
c.4910-1G>	A, C, T
c.5005+1G>	A, C, T
c.5005+2T>	A, C, G
c.5496+1G>	A, C, T

Very small Indel predicted damaging by PROVEAN

PVS1_Supporting (list C)		
		PROVEAN score
c.2467-2A>	C,T	-8.91
c.2467-1G>	C,T	
c.2839-2A>	C,G,T	-17.71
c.2839-1G>	A,C,T	
c.4237-2A>	C, G, T	-19.00
c.4237-1G>	C, T	
c.4437-2A>	C, G, T	-20.08
c.4437-1G>	C, T	
c.5675-2A	C, T	-4.98

C-terminal FATKIN
(exon 38 to 63)

Exon skipping or use of a cryptic splice site preserves reading frame, or PTC not predcited to undergo NMD

PVS1 (list E)	
c.5918+1G>	A, C, T
c.5918+2T>	A, C, G
c.5919-2A>	C, G, T
c.5919-1G>	A, C, T
c.6348-2A>	C, G, T
c.6348-1G>	A, C, T
c.6452+1G>	A, C, T
c.6452+2T>	A, C, G
c.6572+1G>	A, C, T
c.6572+2T>	A, C, G
c.6808-2A>	C, G, T
c.6808-1G>	A, C, T
c.6975+1G>	A, C, T
c.6975+2T>	A, C, G
c.6976-2A>	C, G, T
c.6976-1G>	A, C, T
c.7089+1G>	A, C, T
c.7089+2T>	A, C, G
c.7629+1G>	A, C, T
c.7629+2T>	A, G
c.7630-2A>	C, G, T
c.7630-1G>	A, C, T
c.7788+1G>	A, C, T
c.7788+2T>	A, C, G
c.8151+1G>	A, C, T
c.8151+2T>	A, C, G

PVS1 (list E)	
c.8268+1G>	A, C, T
c.8268+2T>	A, C, G
c.8269-1G>	A
c.8418+1G>	A, C, T
c.8418+2T>	A, C, G
c.8585-2A>	C, G, T
c.8585-1G>	A, C, T
c.8671+1G>	A, C, T
c.8671+2T>	A, C, G
c.8851-2A>	C, G, T
c.8851-1G>	C, T
c.8987+1G>	A, C, T
c.8987+2T>	A, G
c.8988-2A>	C, G, T
c.8988-1G>	A, C, T

Very small Indel predicted damaging by PROVEAN

PVS1_Supporting (list F)		
		PROVEAN score
c.6199-2A>	C, G, T	-14.76
c.6199-1G>	C, T	
c.7928-2A>	C, G, T	-6.13
c.7928-1G>	A, C, T	
c.8152-2A>	C, T	-73.69
c.8152-1G>	C, T	
c.8269-2A>	C, G, T	-34.54
c.8269-1G>	C, T	
c.8419-2A>	C, T	-6.32
c.8419-1G>	C, T	

Related publication(s):