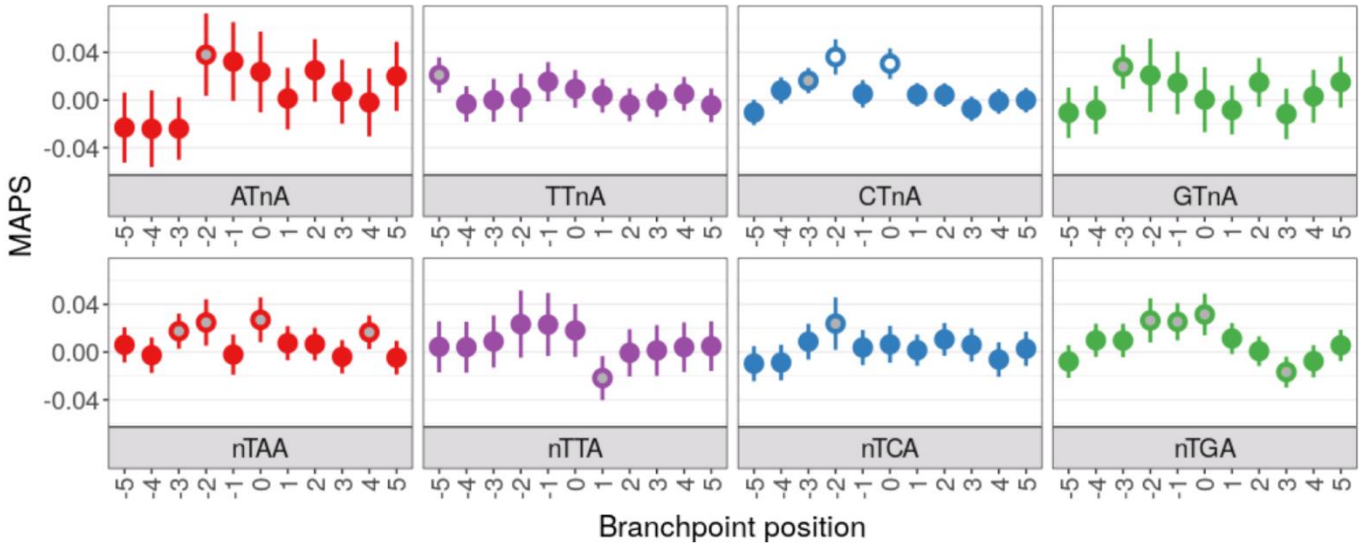
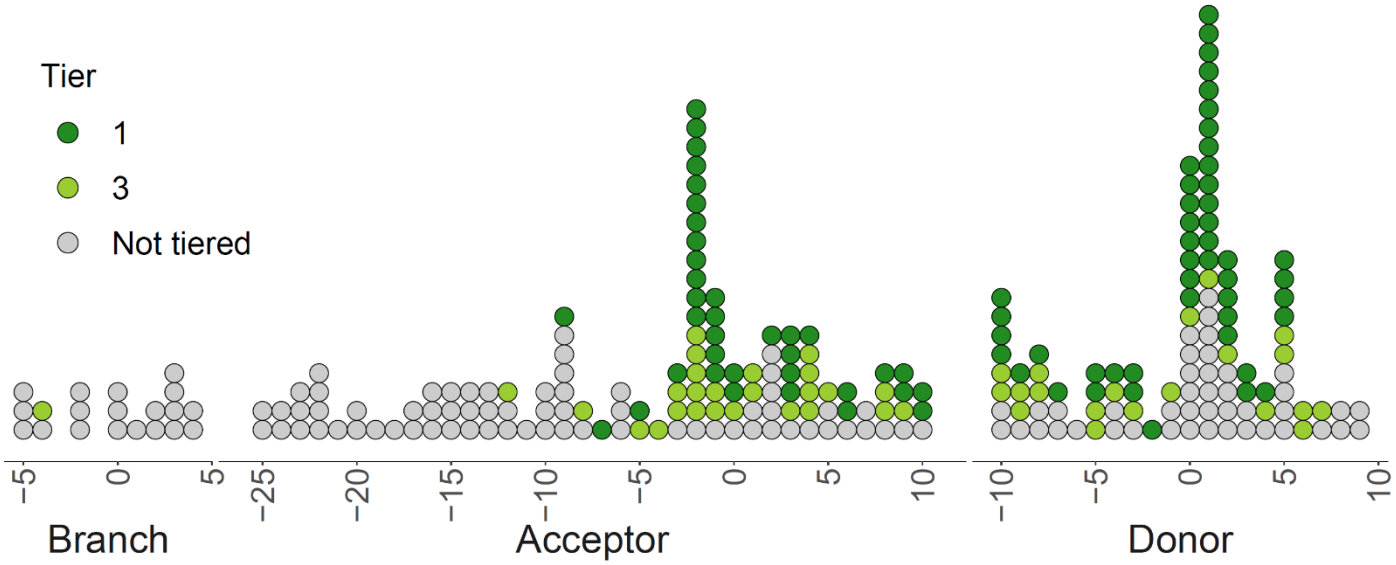
Fig S1



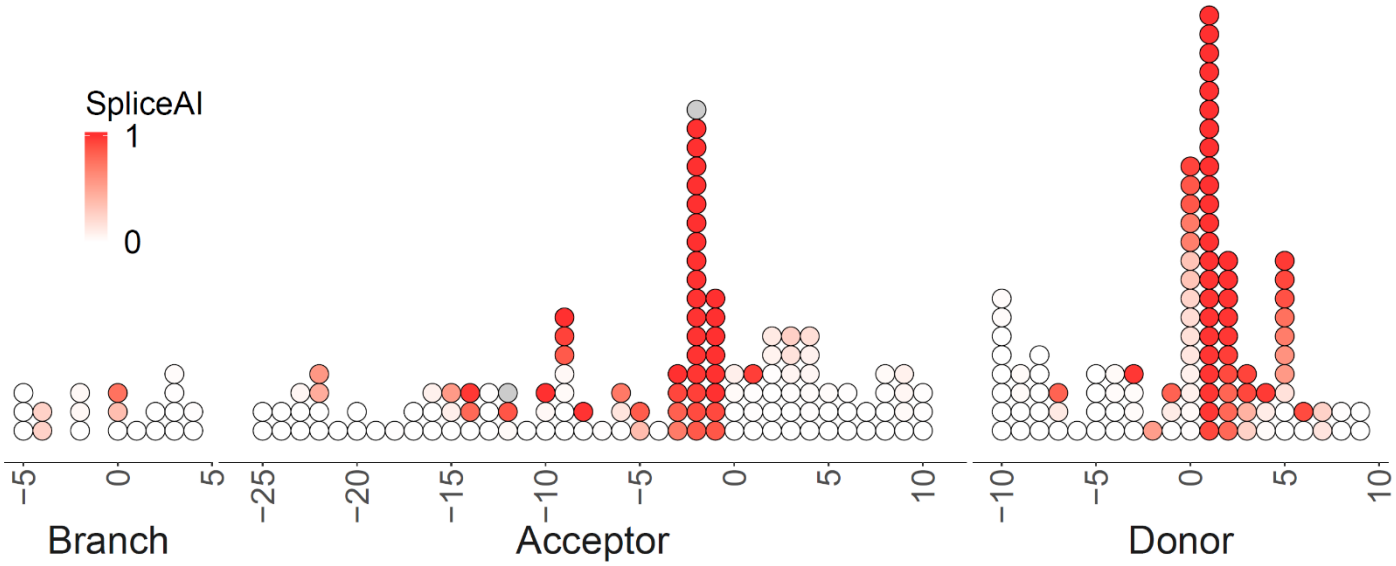
Constraint at predicted splicing branchpoints, stratified by reference allele at BP-3 and BP-1 positions. For LaBranchoR-predicted branchpoints with reference A and T nucleotides at the BP0 and BP-2 positions (nTnA motif, n = 165,564), we calculated MAPS after stratifying by the reference allele at BP-3 and BP-1 positions. Sites which are significantly constrained after Bonferroni correction (88 tests, alpha = 0.05, $p < 5.8 \times 10^{-4}$) are shown with white points. Sites with nominally significant constraint ($p < 0.05$) are shown with grey points. We are generally underpowered to detect motif-specific constraints at these loci.

Fig S2



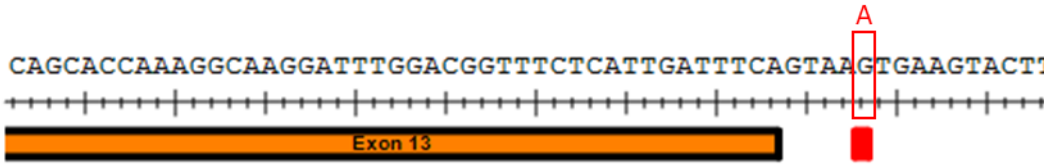
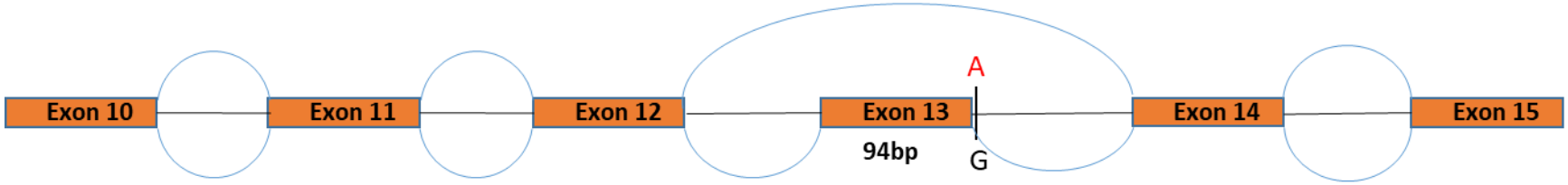
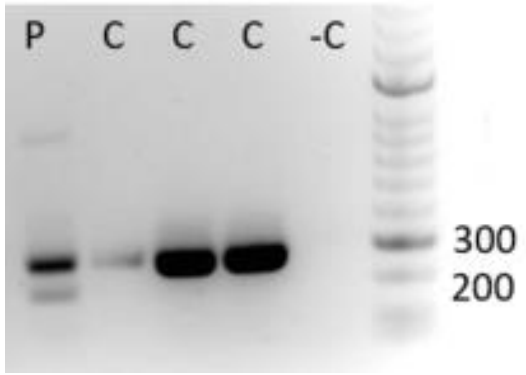
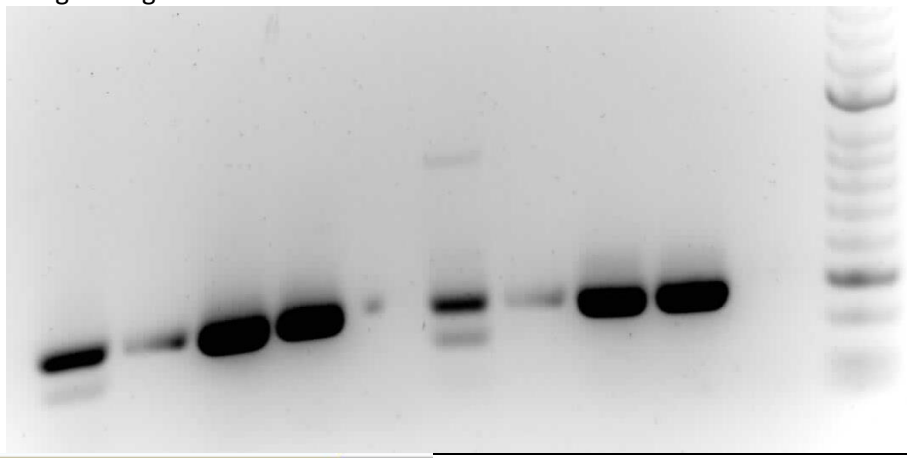
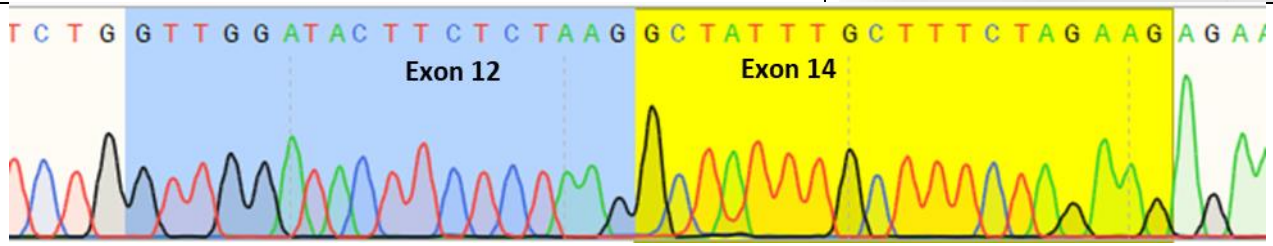
Tiering data for splicing DNVs in known monoallelic loss-of-function rare disease genes. Each point represents a DNV in a rare disease proband. Points are coloured by the "tier" of that variant in the GEL annotation pipeline (see Methods).

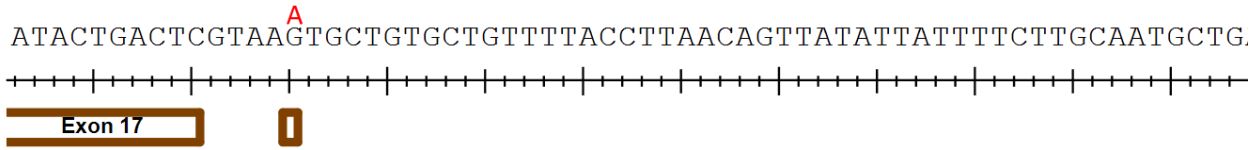
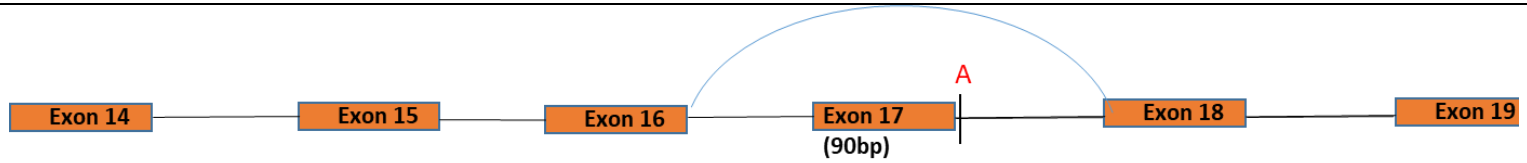
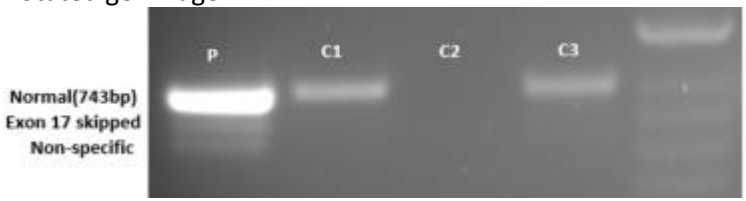
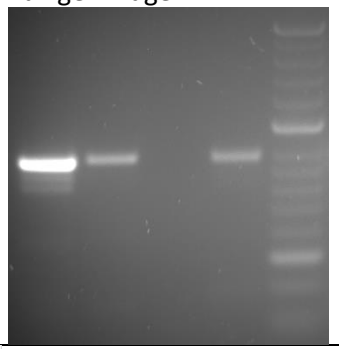
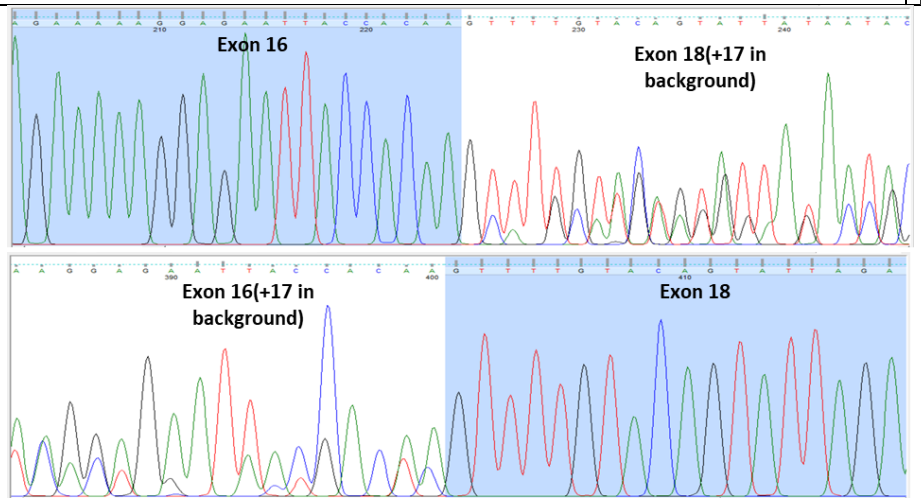
Fig S3

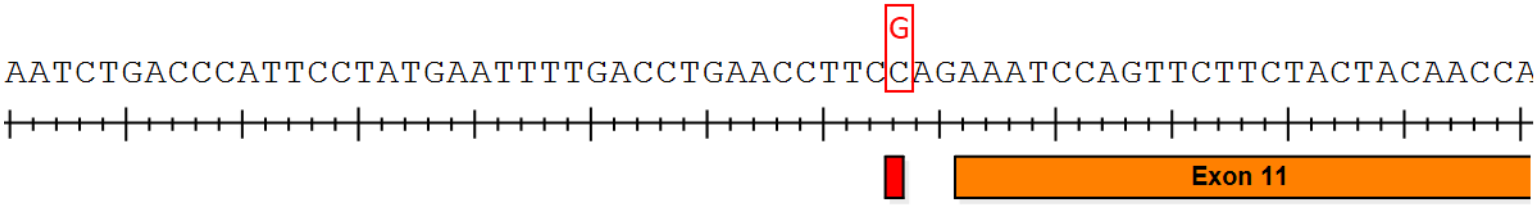
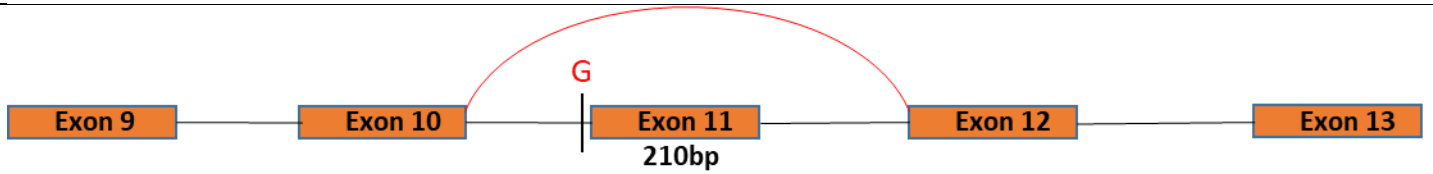
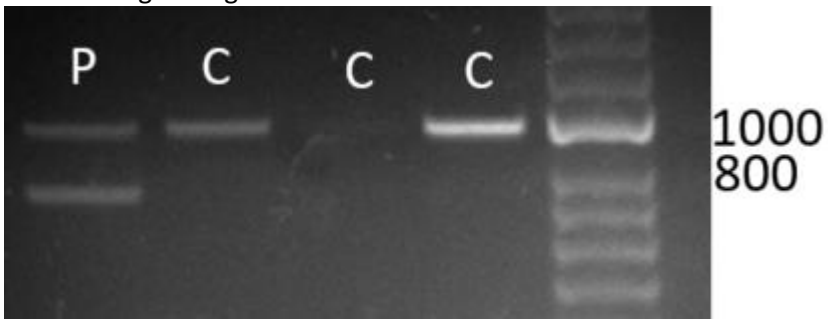
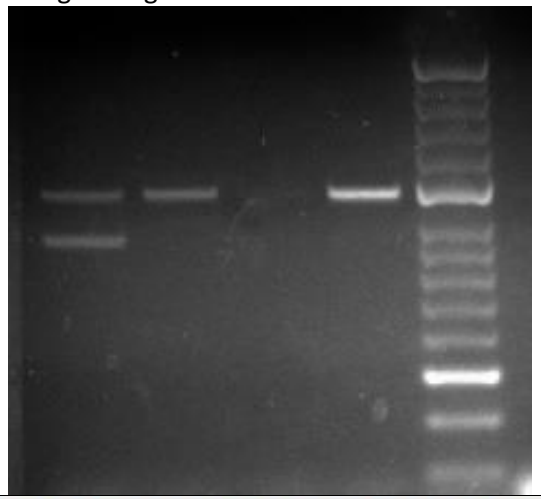
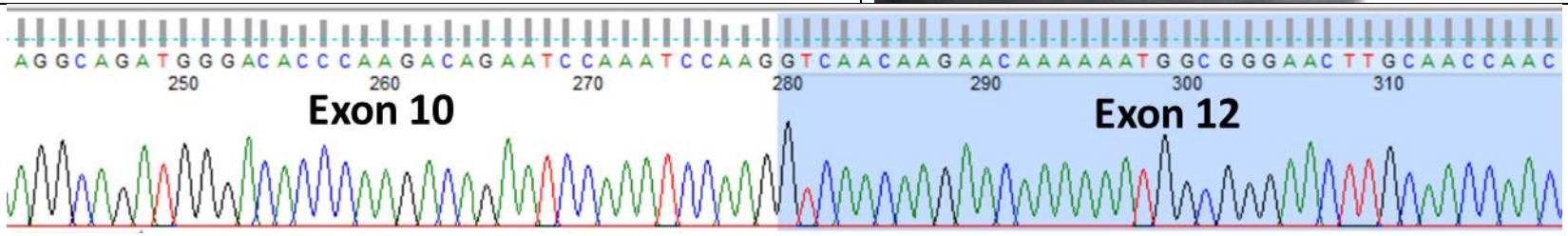


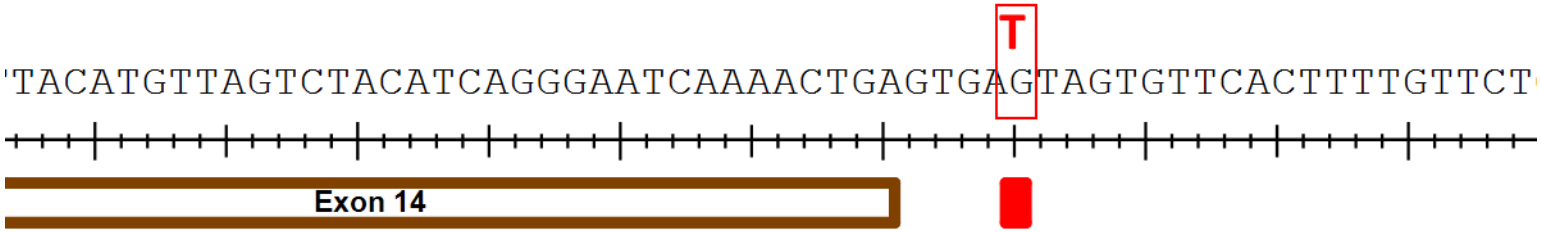
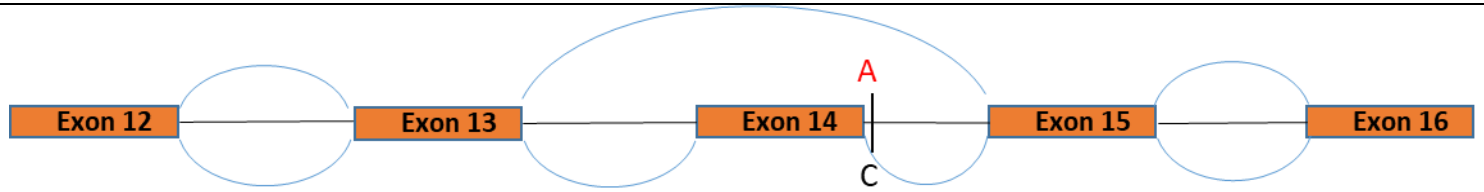
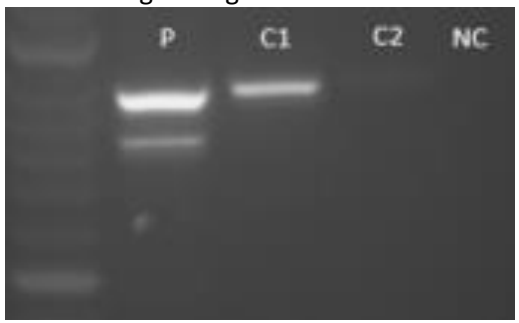
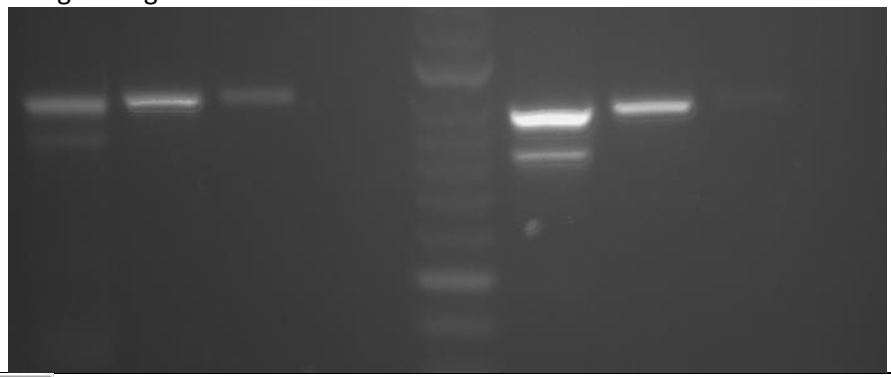
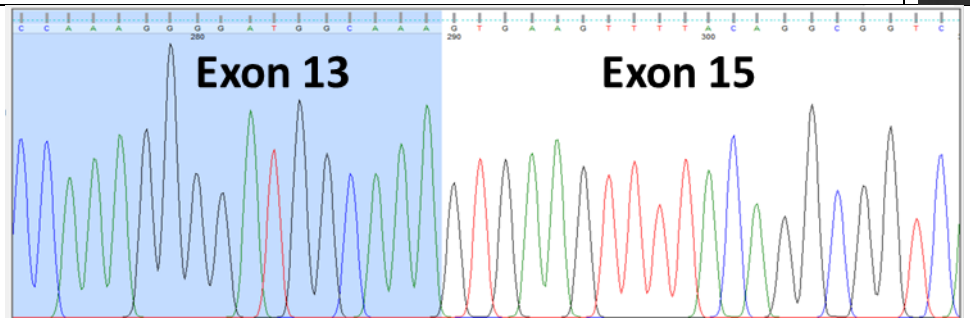
SpliceAI scores of splicing DNVs in known monoallelic loss-of-function rare disease genes. Each point represents a DNV in a rare disease proband. Points are coloured by SpliceAI score; grey points indicate that no SpliceAI annotation is available.

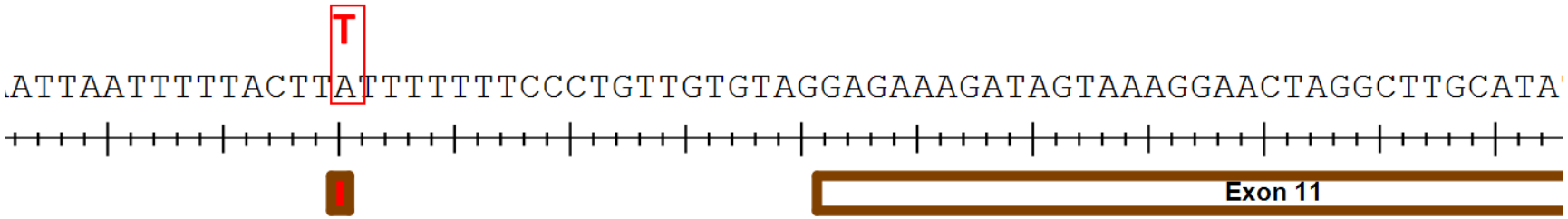
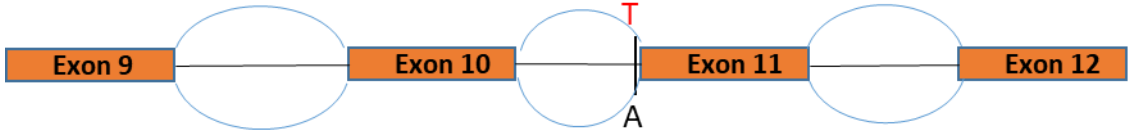
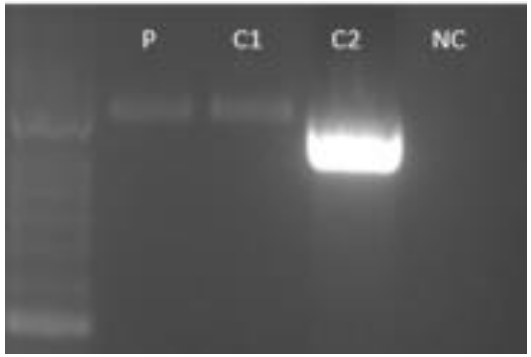
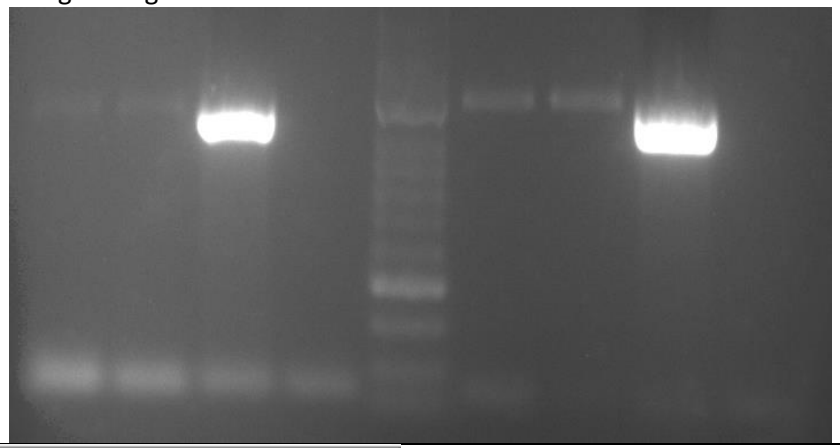
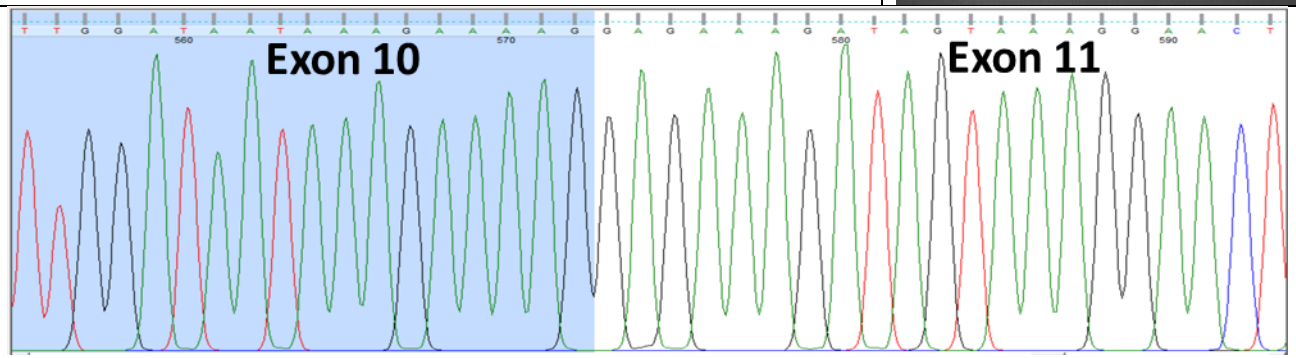
Fig S4

Variant, gene	chr10:74989117 G>A, *KAT6B*
Sequence schematic	
Splicing schematic	
Gel electrophoresis	Annotated gel image  P=Proband, C=Control, -C=DNA free control Full gel image 
Sanger sequencing proband specific band	 Exon 13 skipped

Variant, gene	chr17:62596679 G>A, *TLK2*
Sequence schematic	
Splicing schematic	
Gel electrophoresis	Annotated gel image  P=Proband, C=Control Full gel image 
Sanger sequencing proband specific band	 Exon 17 skipped

Variant, gene	chr1:26767787 C>G, *ARID1A*
Sequence schematic	
Splicing schematic	
Gel electrophoresis	Annotated gel image  P=proband, C=Control Full gel image 
Sanger sequencing proband specific band	 Exon 11 skipped

Variant, gene	chr16:8905182 C>A, *USP7*
Sequence schematic	
Splicing schematic	
Gel electrophoresis	Annotated gel image  P=proband, C=Control, NC = no template control Full gel image 
Sanger sequencing proband specific band	 Exon 14 skipped

Variant, gene	chr12:79808598 T>A, *PPP1R12A*
Sequence schematic	
Splicing schematic	
Gel electrophoresis	Annotated gel image  P=proband, C=Control, NC = no template control Full gel image 
Sanger sequencing	 Normal splicing

Functional outcomes for participant samples which were characterised by RT-PCR. Each page illustrates the following: a schematic of variant position relative to the exon/intron junction, a schematic of the splicing consequence of each variant, gel electrophoresis of amplified RT-PCR products for participant and control samples (cropped annotated image for clarity, plus full gel image), Sanger sequencing trace for proband-specific bands.