

Supplementary Data

Title: Systematic analysis of *CNGA3* splice variants identifies different mechanisms of aberrant splicing

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Supplementary Table S1: Primers for wildtype minigene constructs

Primer name	Sequence (5'→3')	Amplicon size (bp)		Flanking intronic region (upstream/downstream bp)
<i>CNGA3</i> -exon 2- <i>NotI</i>	TATATATAGCGGCCGCGCCCTTCATTTATTTATTCATTCACT	700		287/275
<i>CNGA3</i> -exon 2- <i>BamHI</i>	TATATATAGGATCCGCTGCATTTGAGTCTTCATTTTCT			
<i>CNGA3</i> -exon 3- <i>NotI</i>	TATATATAGCGGCCGCGCCAGGCCAGCATGTACT	693		269/310
<i>CNGA3</i> -exon 3- <i>BamHI</i>	TATATATAGGATCCTGGCTGCTCTTACCACCTTC			
<i>CNGA3</i> -exon 4- <i>NotI</i>	TATATATAGCGGCCGCCCTGCACCTTGGCAGTCATAA	850		379/291
<i>CNGA3</i> -exon 4- <i>BamHI</i>	TATATATAGGATCCGATTTCCAGGGGACCCTAAC			
<i>CNGA3</i> -exon 5- <i>NotI</i>	TATATATATAGCGGCCGCGTAACTAATCACAAAGCATTTTGG	487		183/250
<i>CNGA3</i> -exon 5- <i>BamHI</i>	TATATATATAGGATCCCTTTATGGATGAGAAAAATGAGAG			
<i>CNGA3</i> -exon 4-5- <i>NotI</i>	TATATATAGCGGCCGCCCTGCACCTTGGCAGTCATAA	3834		379/188
<i>CNGA3</i> -exon 4-5- <i>BamHI</i>	TATATATAGGATCCATCTCATCCCTCCCCGACTT			
<i>CNGA3</i> -exon 6- <i>NotI</i>	TATATATAGCGGCCGCAAGGGTGTGGATGTTGTCGT	687		291/279
<i>CNGA3</i> -exon 6- <i>BamHI</i>	TATATATAGGATCCCTGCATCCTCCTCGTACA			
<i>CNGA3</i> -exon 7- <i>NotI</i>	TATATATAGCGGCCGCCCTGGAAAACGGTACCTCCT	686		295/284
<i>CNGA3</i> -exon 7- <i>BamHI</i>	TATATATAGGATCCCAAGAACAAGGCCCAGAGAG			
Hybrid construct for exon 8				
<i>CNGA3</i> -exon 8- <i>NotI</i>	TATATATAGCGGCCGCGCCCTCTGTCACTTTTTGA	284		378/284
Overlap extension reverse	cctggggcacactcacCTTTAAGTAAGCCAGGTCCGGT			
Overlap extension forward	ACCGACCTGGCTTACTTAAAGgtgagtgtgccccagg	500	784	
<i>CNGA3</i> -exon 7- <i>BamHI</i>	TATATATAGGATCCCAAGAACAAGGCCCAGAGAG			

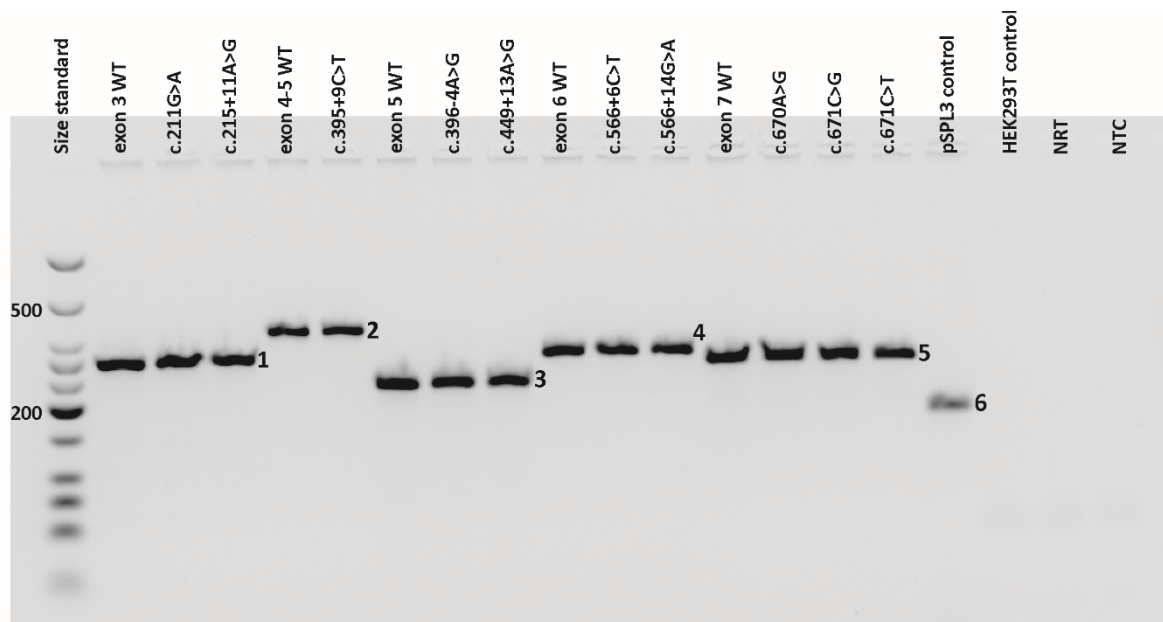
Binding sites for restriction enzymes are indicated in italics. 5'-extension tails to the restriction sites are indicated in bold.

Supplementary Table S2: Primers used for site-directed mutagenesis

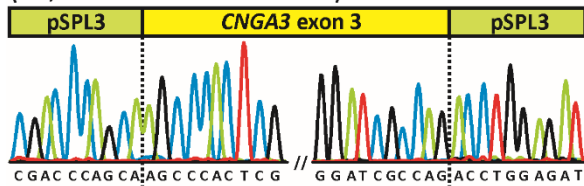
Primer name	Sequence (5'→3')
CNGA3_IVMc.101+1G>A_F	GAAAATGGCCTCAGCAGataagatgggctaagatgg
CNGA3_IVMc.101+1G>A_R	ccatcttagcccatcttat CTGCTGAGGCCATTTTC
CNGA3_IVMc.211G>A_F	GGCCAGGGGATC ACCAG gtaactgacc
CNGA3_IVMc.211G>A_R	ggtcagttac CTGGTGATCCCCTGGCC
CNGA3_IVMc.215+11A>G_F	CAGgtaactgaccggcctcagtcctac
CNGA3_IVMc.215+11A>G_R	gtagggactgagg ccggtcagttacCTG
CNGA3_IVMc.395+1G>T_F	GCAGACAGAGGGAGAAG ttaaggaacggaaaag
CNGA3_IVMc.395+1G>T_R	cttttcggttccttaa CTTCTCCCTCTGTCTGC
CNGA3_IVMc.395+9C>T_F	GGGAGAAGgtaaggaatggaaaagaagaaggggc
CNGA3_IVMc.395+9C>T_R	gccccttctcttttccattccttac CTTCTCCC
CNGA3_IVMc.396-11C>G_F	gatgttctcttagcttcccagCGCC
CNGA3_IVMc.396-11C>G_R	GGCGctgcggaagctagagagaacatc
CNGA3_IVMc.396-2_398dup_F	cctccccag CGCagCGCCTGGC
CNGA3_IVMc.396-2_398dup_R	GCCAGGCgct GCgctg cggaagg
CNGA3_IVMc.396-4G>A_F	ctctacctcccacagCGCCTGGCC
CNGA3_IVMc.396-4G>A_R	GGCCAGGCGct gtggaaggtagag
CNGA3_IVMc.449+13A>G_F	gtaagtaccacgcaccacagcagagcc
CNGA3_IVMc.449+13A>G_R	ggctctgctgggtg ctg gggtacttac
CNGA3_IVMc.450-15T>G_F	gcgctgttgtgtaggtgtgggttccagG
CNGA3_IVMc.450-15T>G_R	Cctggaaacccacacctacacaaacagcgc
CNGA3_IVMc.450-1G>A_F	gtatgtgtgggtttccaaGAAGAAGACGAAAAAGAAGG
CNGA3_IVMc.450-1G>A_R	CCTCTTTTTTCGTCTTCTT Cttg aaaccacacatac
CNGA3_IVMc.566+14G>A_F	gtaagcgacagggatggaaggtgcagcg
CNGA3_IVMc.566+14G>A_R	cgctgcaccttccatccctgtcgttac
CNGA3_IVMc.566+6C>T_F	CTTATTTGCAGgtaagtacaggggtggaaggtg
CNGA3_IVMc.566+6C>T_R	cacctccaccctgtcacttac CTGCAAATAAG
CNGA3_IVMc.566G>A_F	GTATCTGCTTATTTGCA Ag taagcgacaggggtgg
CNGA3_IVMc.566G>A_R	ccaccctgtcgttac TTGCAAATAAGCAGATAC
CNGA3_IVMc.567-11G>A	ccatctcccacatagcttctttagGGCC
CNGA3_IVMc.567-11G>A	GGCCctaaagaagctatgtgggagatgg
CNGA3_IVMc.670A>G_F	GTACGAGCTCGGGCAGgtgagtgtgc
CNGA3_IVMc.670A>G_R	gcacactcac CTGCCGAGCTCGTAC
CNGA3_IVMc.671C>G_F	GTACGAGCTCGGAGAGgtgagtgtgcc
CNGA3_IVMc.671C>G_R	ggcactcac CTCTCCGAGCTCGTAC
CNGA3_IVMc.671C>T_F	GTACGAGCTCGGATAGgtgagtgtgcc
CNGA3_IVMc.671C>T_R	gggcactcac CTATCCGAGCTCGTAC
CNGA3_IVMc.673+5G>T_F	CGGACAGgtgattgtccccagggc
CNGA3_IVMc.673+5G>T_R	ggcctggggcacaatcac CTGTCCG
CNGA3_IVMc.674-2A>C_F	cctcatcttcttcttcgGTTTTCTCGAGCAAG
CNGA3_IVMc.674-2A>C_R	CTTGCTCGAGAAAACcgaagaagaagatggagg

Intronic nucleotides are indicated in lowercase letters, exonic nucleotides in capital letters. The introduced nucleotides are indicated in bold. F, forward; R, reverse.

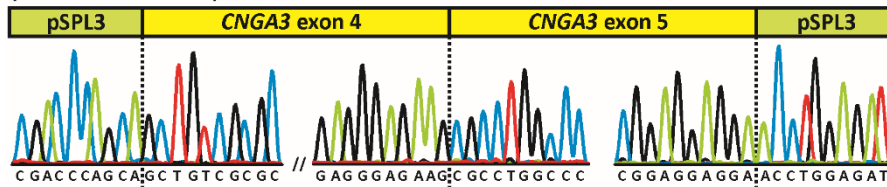
Supplementary Figure S1



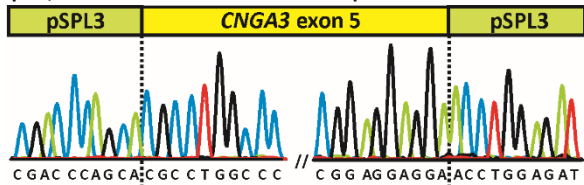
Product 1: Transcript from exon 3 minigenes
(WT, c.211G>A and c.215+11A>G)



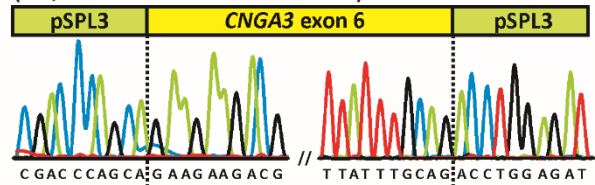
Product 2: Transcript from exon 4-5 minigenes
(WT and c.395+9C>T)



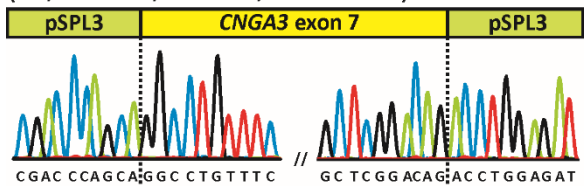
Product 3: Transcript from exon 5 minigenes
(WT, c.396-4G>A and c.449+13A>G)



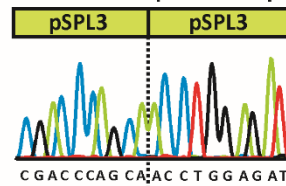
Product 4: Transcript from exon 6 minigenes
(WT, c.566+6C>T and c.566+14G>A)



Product 5: Transcript from exon 7 minigenes
(WT, c.670A>G, c.671C>G, and c.671C>T)



Product 6: Transcript from empty pSPL3



Supplementary Figure S2

Figure 2A transcript 1: Correct splicing (exon 2 wild-type minigene)

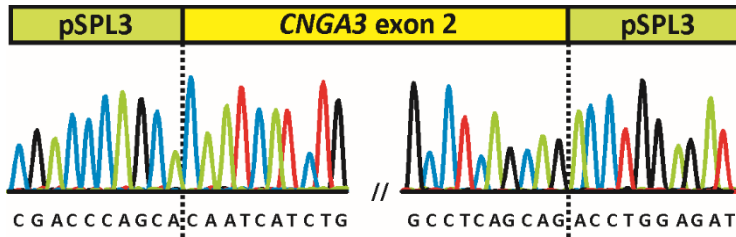


Figure 2A transcript 2: Deletion of the last 15 nucleotides of exon 2 (c.101+1G>A minigene)

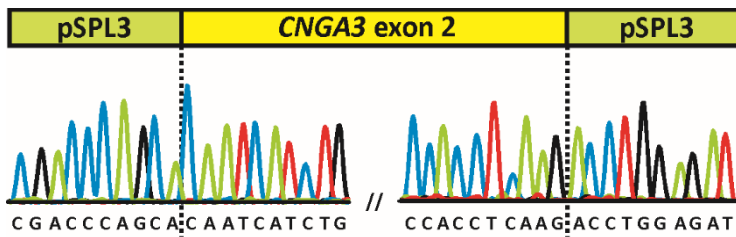


Figure 2B transcript 1: Correct splicing (exon 4-5 wild-type minigene)

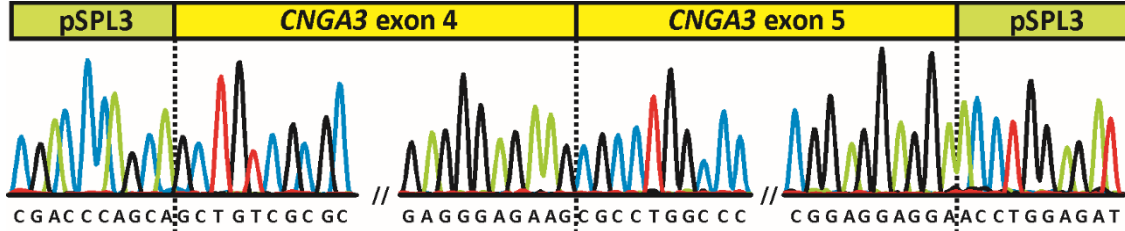


Figure 2B transcript 2: Retention of the first 488 nucleotides of intron 4 (c.395+1G>A minigene)

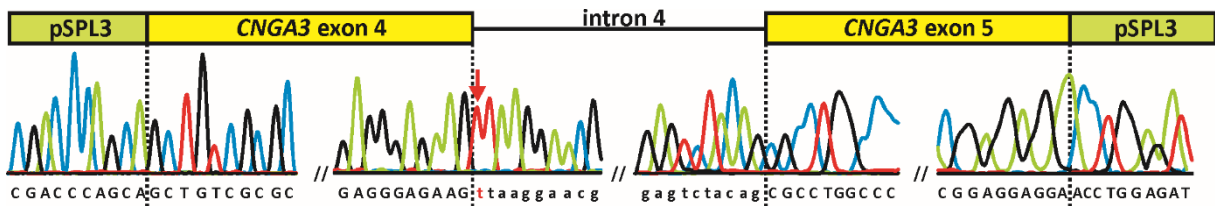


Figure 2B transcript 3: Deletion of the last 41 nucleotides of exon 4 (c.395+1G>A minigene)

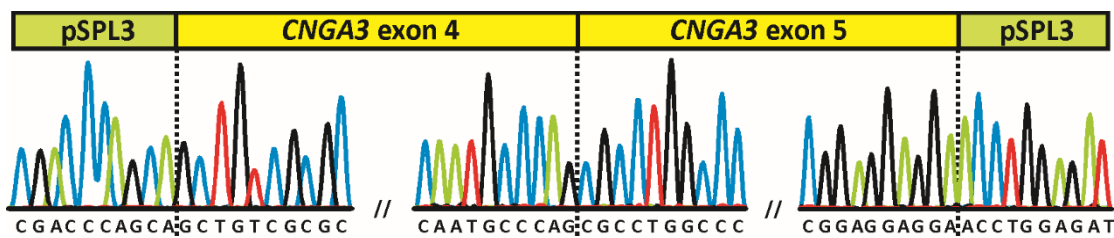


Figure 2B transcript 4: Deletion of the last 138 nucleotides of exon 4 (c.395+1G>A minigene)

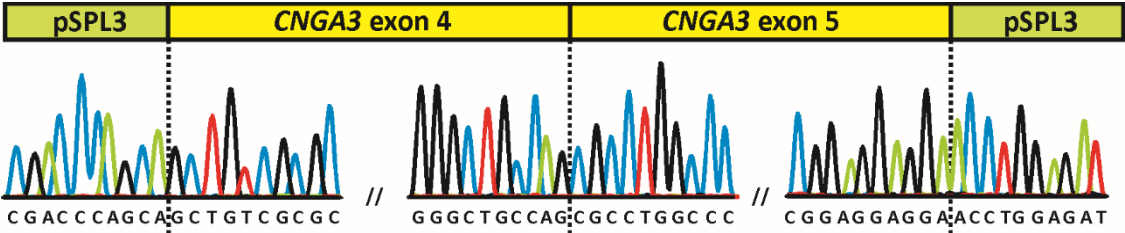


Figure 2B transcript 5: Deletion of the last 150 nucleotides of exon 4 (c.395+1G>A minigene)

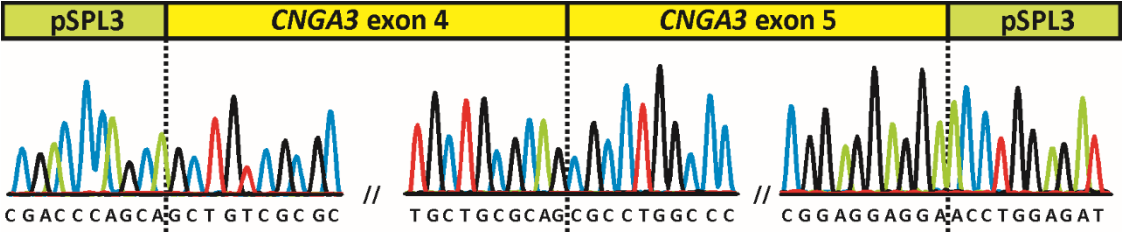


Figure 2B transcript 6: Skipping of exon 4 (c.395+1G>A minigene)

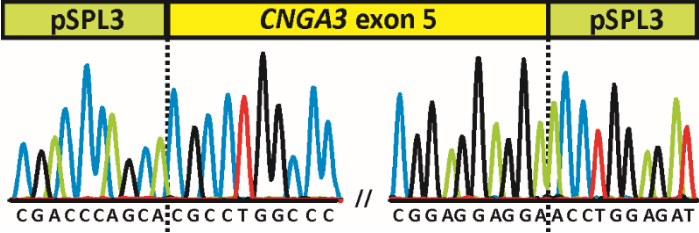


Figure 2B transcript 7: Skipping of exon 4 and exon 5 (c.395+1G>A minigene)

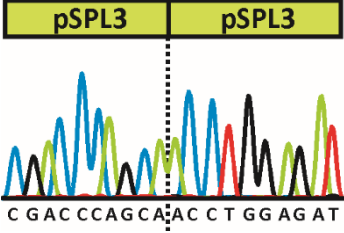


Figure 2C transcript 1: Correct splicing (exon 5 wild-type minigene)

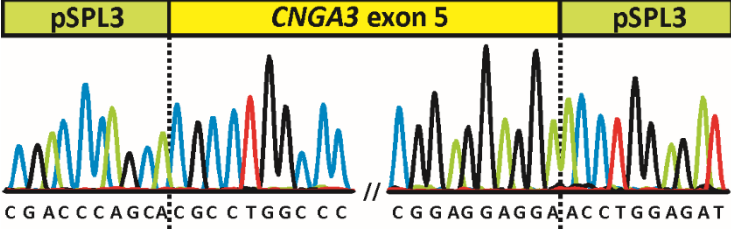


Figure 2C transcript 2: Retention of the last 10 nucleotides of intron 4 (c.396-11C>G minigene)

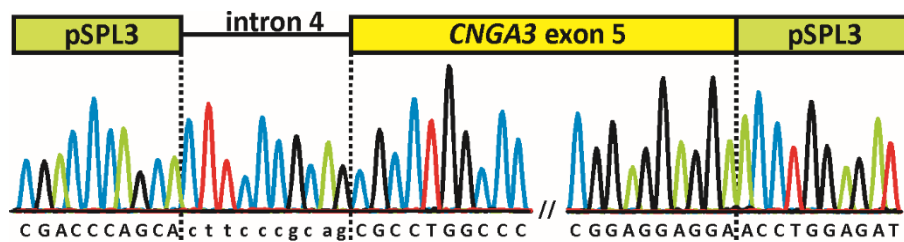


Figure 2C transcript 3: Skipping of exon 5 (c.396-11C>G minigene)

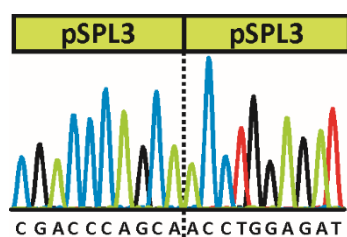


Figure 2C transcript 4: Retention of the last 4 nucleotides of intron 4 (c.396-2_398dup minigene)

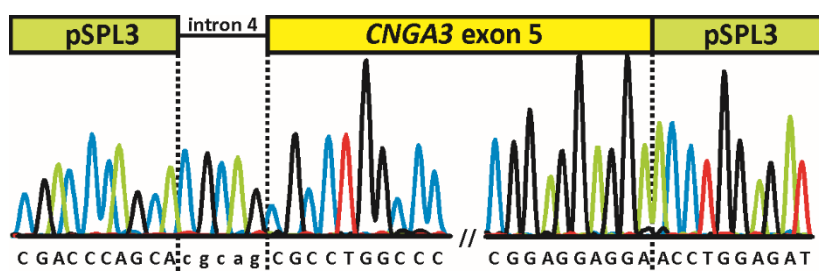


Figure 3A transcript 1: Correct splicing (exon 6 wild-type and c.450-15T>G minigenes)

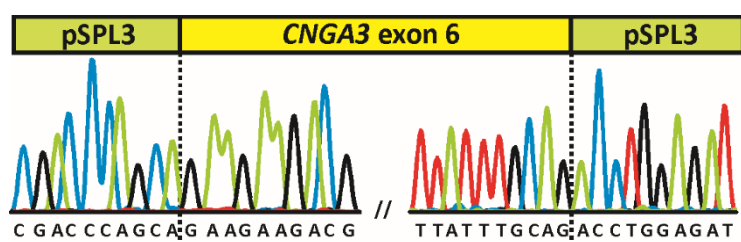


Figure 3A transcript 1: Correct splicing (c.566G>A minigene)

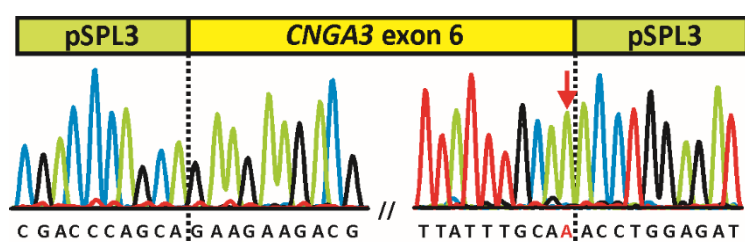


Figure 3A transcript 2: Retention of the last 14 nucleotides of intron 5 (c.450-15T>G minigene)

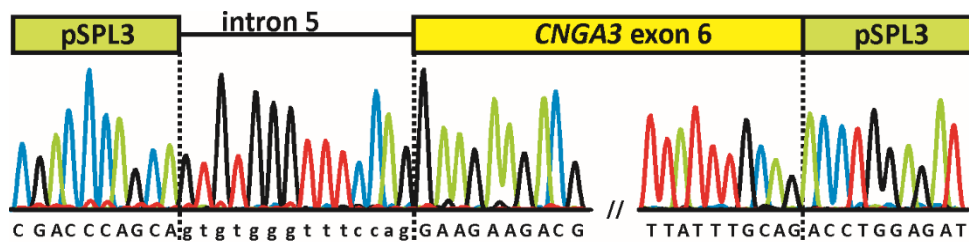


Figure 3A transcript 3: Retention of the last 49 nucleotides of intron 5 (c.450-15T>G minigene)

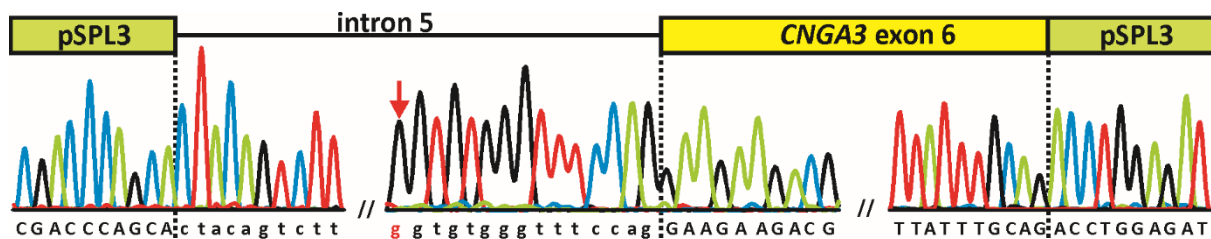


Figure 3A transcript 3: Retention of the last 49 nucleotides of intron 5 (c.450-1G>A minigene)

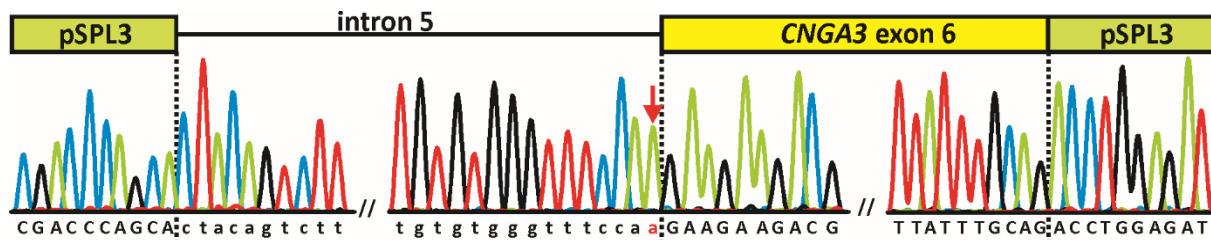


Figure 3A transcript 4: Skipping of exon 6 (c.450-15T>G and c.450-1G>A minigenes)

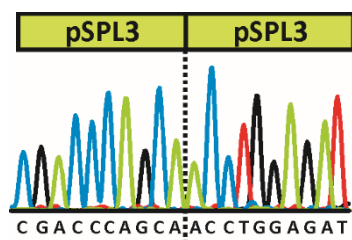


Figure 3A transcript 5: Retention of the last 154 nucleotides of intron 5 (c.450-1G>A minigene)

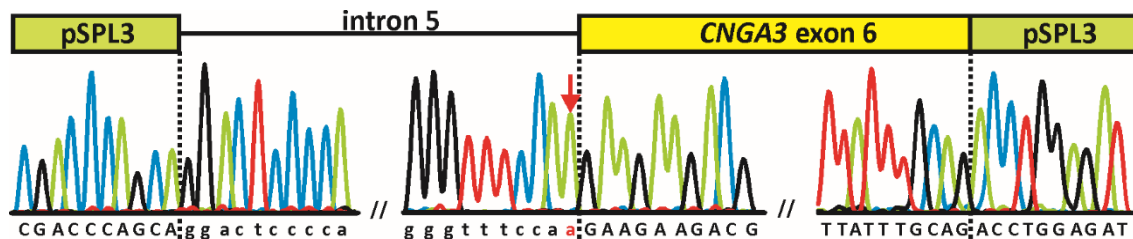


Figure 3A transcript 6: Retention of the last 70 nucleotides of intron 5 (c.450-1G>A minigene)

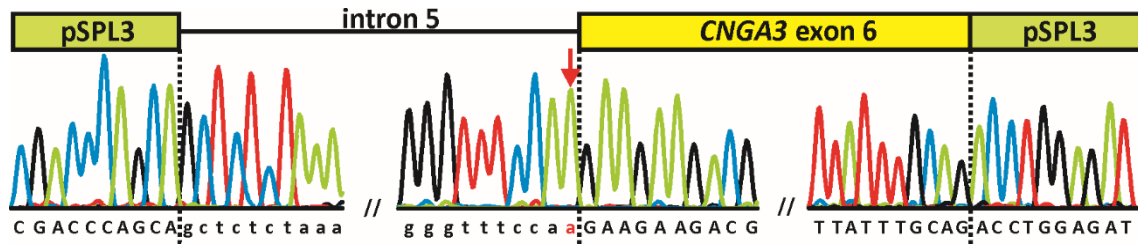


Figure 3A transcript 7: Deletion of the last 18 nucleotides of exon 6 (c.566G>A minigene)

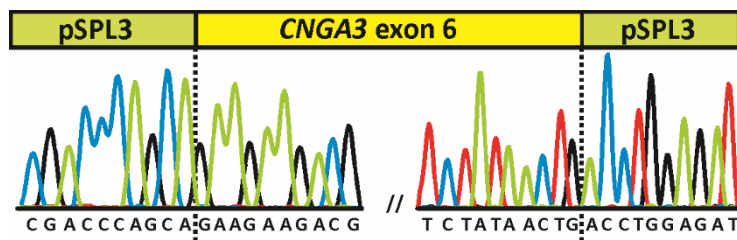


Figure 3B transcript 1: Correct splicing (exon 7 wild-type and c.673+5G>T minigenes)

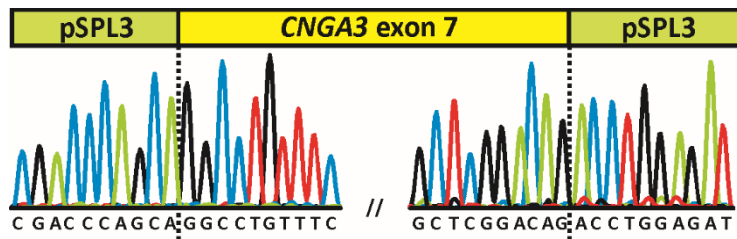


Figure 3B transcript 2: Retention of the last 9 nucleotides of intron 6 (c.567-11G>A minigene)

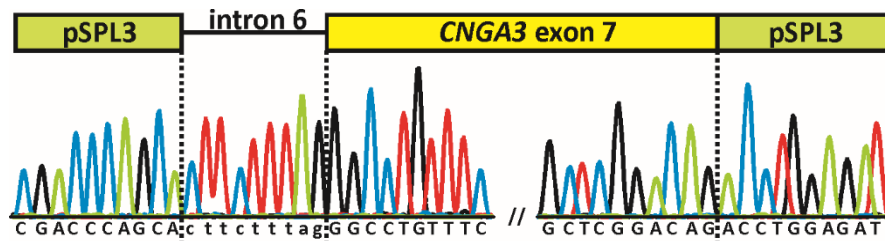


Figure 3B transcript 3: Skipping of exon 7 (c.673+5G>T minigene)

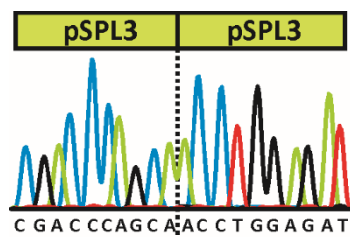


Figure 3C transcript 1: Correct splicing (exon 8 wild-type minigene)

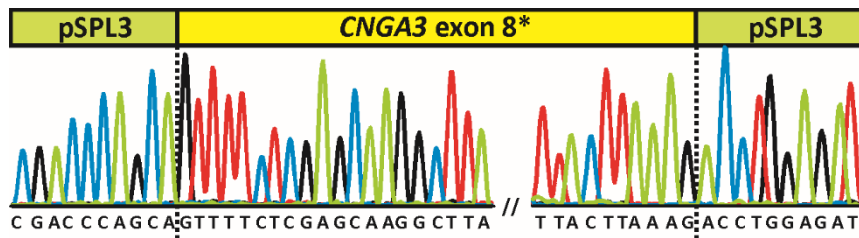


Figure 3C transcript 2: Deletion of the first 15 nucleotides of exon 8 (c.674-2A>C minigene)

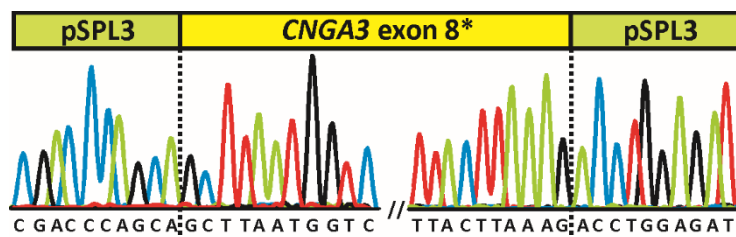
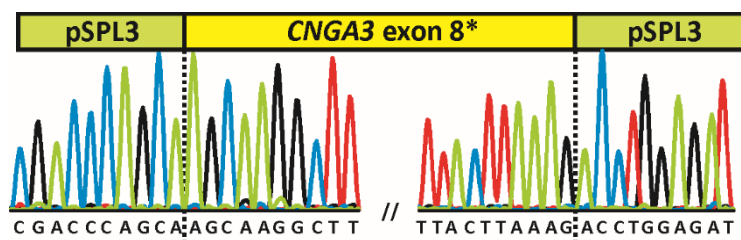


Figure 3C transcript 3: Deletion of the first 9 nucleotides of exon 8 (c.674-2A>C minigene)



*Note that the minigene construct for exon 8 does not comprise the full-length exon.

Figure legends

Supplementary Figure S1: Minigene assays of variants that showed no splice defect.

The agarose gel (uncropped image) shows the RT-PCR products obtained upon transfection of HEK293T cells with the wildtype (WT) minigene constructs for *CNGA3* exon 3 (lane 2), exon 4 (lane 5), exon 5 (lane 7), exon 6 (lane 10), and exon 7 (lane 13). Also shown are the RT-PCR products from the mutant minigene constructs harboring variants c.211G>A (lane 3), c.215+11A>G (lane 4), c.395+9C>T (lane 6), c.396-4G>A (lane 8), c.449+13A>G (lane 9), c.566+6C>T (lane 11), c.566+14G>A (lane 12), c.670A>G (lane 14), c.671C>G (lane 15), and c.671C>T (lane 16). A size standard (low molecular weight DNA ladder, NEB) is loaded in the leftmost lane. RT-PCRs from transfection with empty pSPL3 vector (lane 17) and untransfected HEK293T cells (lane 18) served as controls. NRT (lane 19), no reverse transcriptase control; NTC (lane 20), no template control. Sequence electropherograms below the agarose gel show correct splicing (i.e., splicing of the respective *CNGA3* exon between the pSPL3 resident exons) for all minigene constructs. Note that only one representative sequence electropherogram is shown for minigenes harboring the same exon.

Supplementary Figure S2: Splice products of *CNGA3* variants inducing missplicing. Sequence electropherograms showing the splice junctions of all subcloned transcripts presented in Figure 2 and Figure 3. The pSPL3 exons are depicted in green, the *CNGA3* exons in yellow and intronic regions as a

black line. Variants are indicated by a red arrow if present in the corresponding transcript. Exonic nucleotides are shown in upper case and intronic nucleotides in lower case.