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Comparison of T7E1 and Surveyor mismatch cleavage assays to detect mutations triggered by engineered nucleases

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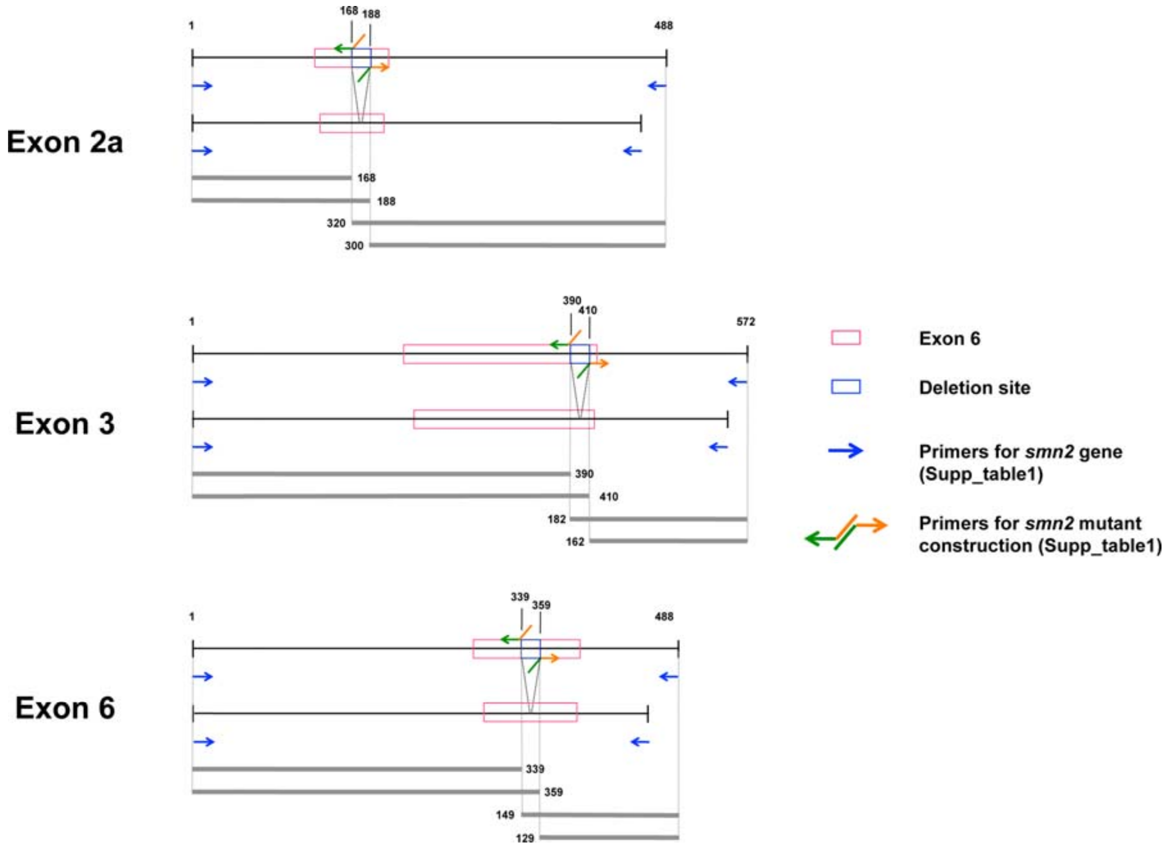

Figure S1 Structure of the PCR products corresponding to *smn* exon 2a, exon 3 and exon 6. Both the wild-type and constructed mutant forms are depicted.

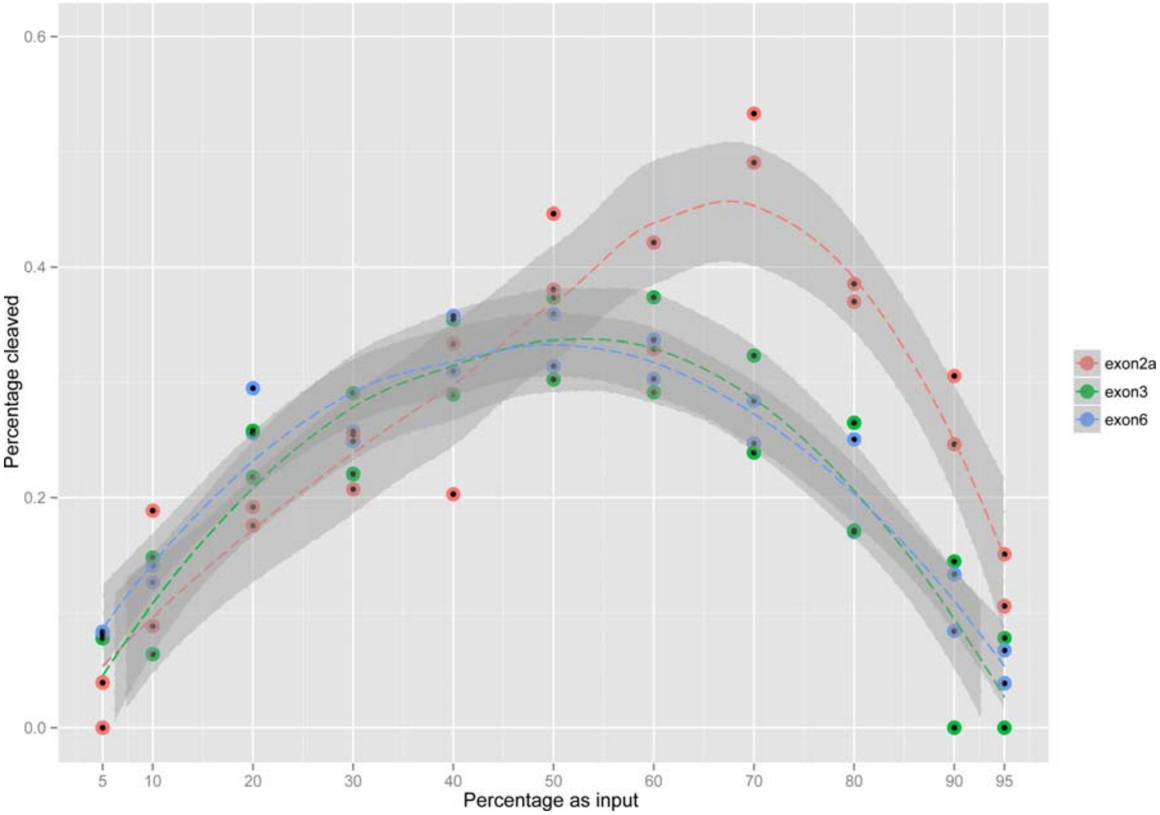

Figure S2 This graph shows the fraction of cleaved products from all products (y axis) in a mixture composed of various quantities of deletion mutants of exon 2a, 3 and 6 in a population of mutant and wild-type DNA molecules (x axis). Both T7E1 and Surveyor digestion products are shown. The grey intervals correspond to the variation and the dotted line corresponds to a loess regression model fitting.

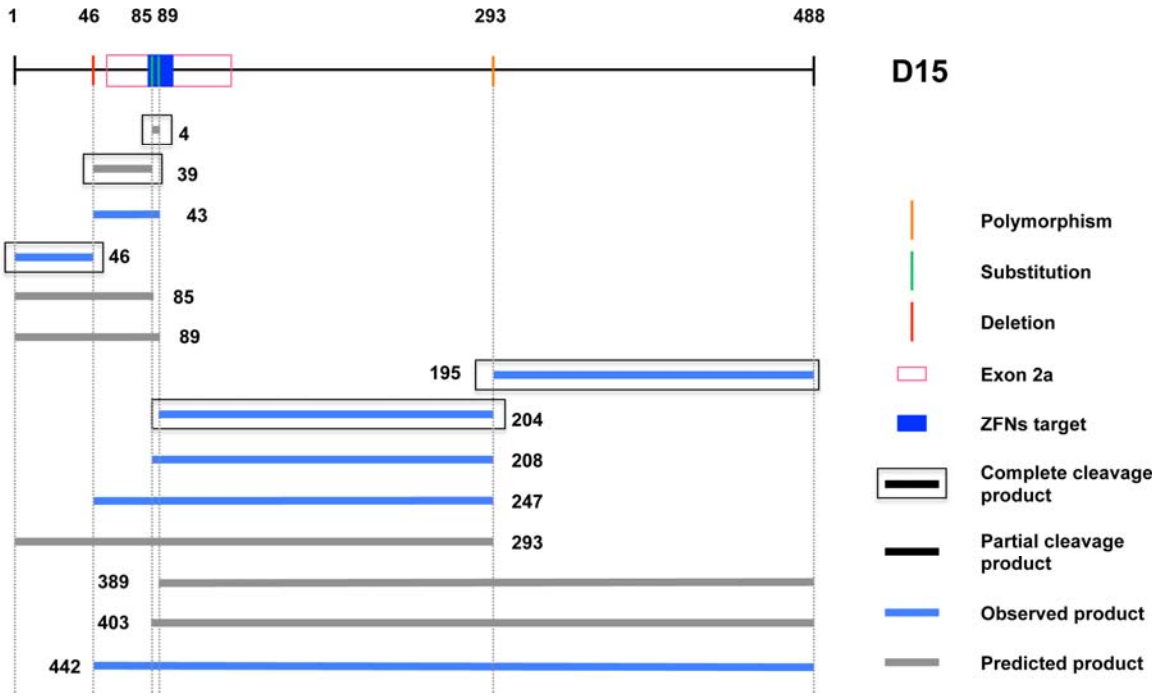

Figure S3 Predicted cleavage map of all mismatches between D15 and wild-type *smn* exon 2a alleles.

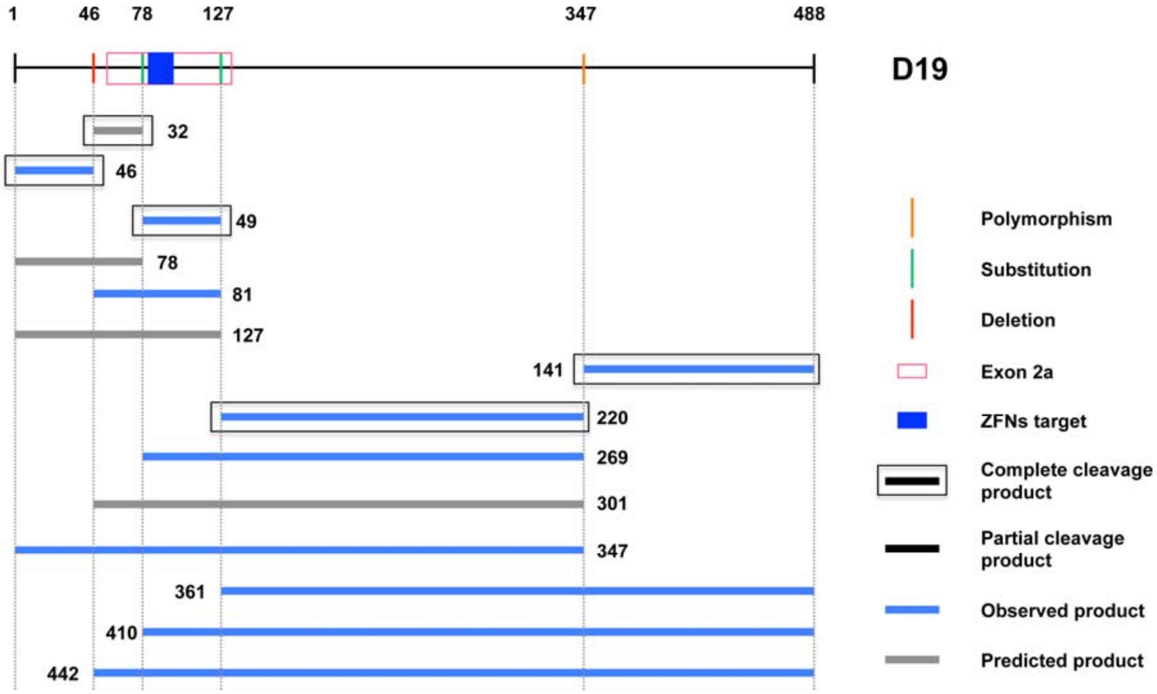

Figure S4 Predicted cleavage map of all mismatches between D19 and wild-type *smn* exon 2a alleles.

Table S1 Sequence of primers used in this work

smn2 gene	Primer sequences 5'-3'		
	Forward	Reverse	Ta (°C)
exon2a	**TCTCCAACTCCTTGTGTTTGTG**	**TATTCCAACACAGGGGCCAC**	58
exon3	**TGCCAAACGAATGAATGTTG**	**CCTTAGCACCTAGGCAGTGG**	50
exon6	**TTCACATTCAAGAAGTCGATAGAA**	**GAATTGATAGATGCAGATCAACC**	50
Constructions of mutants	Primer sequences 5'-3'		
	Forward	Reverse	Ta (°C)
20del_exon2a	**GATACAGCTCTCATT**TCTTCTTTTAAGGTAAGAAT	**TACCTTAAAAGAAGA**AATGAGAGCTGTATCGTCCC	58
20del_exon3	**TGGCTGACCTGCGCT**GAAAGTGACCAGAGGGAGCA	**CCTCTGGTCACTTTC**AGCGCAGGTCAGCCAAACTT	50
20del_exon6	**TCCTCCAATGAGCCC**AGGCATTGGGCAGTATGCTG	**TACTGCCCAATGCCT**GGGCTCATTGGAGGAGGTGG	50

Table S2 List of fragments obtained after T7E1 or Surveyor assays on D15 and D19 clones.

		Predicted size of matching fragment (bp)		
Sample	**Observed Size (bp)***	**Complete digestion**	**Partial digestion**	**Uncleaved**
D15 T7E1	23			
D15 T7E1	**47**	**46**	**43**	
D15 T7E1	**210**	**195, 204**	**208**	
D15 T7E1	**250**		**247**	
D15 T7E1	480			
D15 T7E1	*516*			488
D15 Surveyor	51	46		
D15 Surveyor	55			
D15 Surveyor	197	195		
D15 Surveyor	**212**	**204**	**208**	
D15 Surveyor	**251**		**247**	
D15 Surveyor	**426**		**442**	
D15 Surveyor	*516*			488
D19 T7E1	53	46, 49		
D19 T7E1	**91**		**81**	
D19 T7E1	223	220		
D19 T7E1	**257**		**269**	
D19 T7E1	**357**		**347**	
D19 T7E1	**373**		**361**	
D19 T7E1	**388**		**410**	
D19 T7E1	**471**		**442**	
D19 T7E1	*518*			488
D19 Surveyor	143	141		
D19 Surveyor	**253**		**269**	
D19 Surveyor	**373**		**361**	
D19 Surveyor	**438**		**442**	
D19 Surveyor	472			
D19 Surveyor	*513*			488

ND: Non determined

* The observed size is according to the Agilent fragment analysis Expert software report. Sizing accuracy coefficient of variation is +/- 10%, and increases with size.