

FIGURE 1A

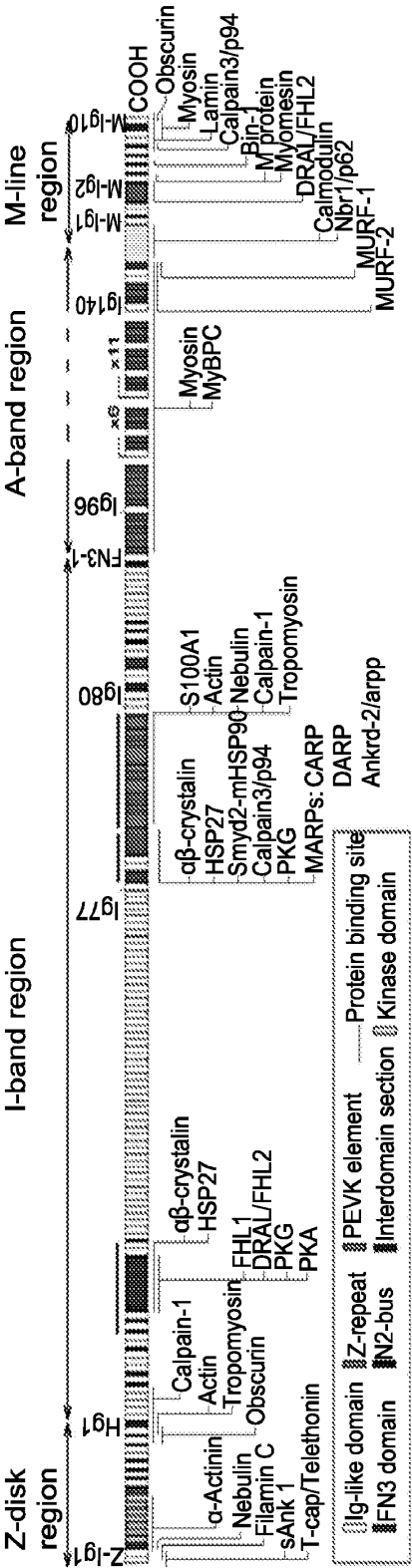


FIGURE 1B

Repair Strategy 1: Repair titin mutation c.32854G>C using donor template

Human specific SaCas9		Spacer Sequence	PAM	Specificity Score	OLIGOS ORDERED	
Position	Strand					
Guide 1: chr2 +178,645,987 *	-1	<u>GGAGGAGGTGGGGGTCTTGGT</u> (SEQ ID NO: 1)	TTGAGT (SEQ ID NO: 2)	55	CACCGGAGGAGGTGGGGGTCTTGGT (SEQ ID NO: 3)	
					AAACACCAAGACCCCACTCTCTCC (SEQ ID NO: 4)	
	reverse					
Guide 2: chr2 +178,645,975	-1	<u>GGTGGAGCAGGTGGAGGAGGT</u> (SEQ ID NO: 5)	GGGGGT (SEQ ID NO: 6)	25	CACCGGTGGAGCAGGTGGAGGAGGT (SEQ ID NO: 7)	
					AAACACCTCTCTCCACTGCTCCACC (SEQ ID NO: 8)	
	reverse					
Guide 3: chr2 +178,645,924	-1	<u>TGGCATTTTACCTTTAAGT</u> (SEQ ID NO: 9)	TGGAAT (SEQ ID NO: 10)	14	CACCTGGCATTTTACCTTTAAGT (SEQ ID NO: 11)	
					ACCGTAAAAAATGGAAATTCACAAA (SEQ ID NO: 12)	
	reverse					
Guide 4: chr2 +178,646,032 *	-1	<u>TTTCAGGTTCAGGTTCTTGA</u> (SEQ ID NO: 13)	AAGAGT (SEQ ID NO: 14)	36	CACCTTTTCAGGTTTCAGGTTCTTGA (SEQ ID NO: 15)	
					AAAAGTCCAAGTCCAAGAACTCAAA (SEQ ID NO: 16)	
	reverse					

* Best performing guide sequences; Underlined letter of the spacer sequence corresponds to the location of double-stranded break made in the target sequence

Oligos ordered corresponds to primer sequences used for cloning the spacer sequence into the Cas9 plasmid (px601 Addgene plasmid #61591)

Figure 2



Human donor template sequence (for repair of c.32854G>C mutation) with 450 nt homology arms on either side of repair region:

ctttacatataccaaatacaacaacatgtatatacatatacagacacagggtaaacacattttacactcggagtaagaatccaagggtacattgaaacatgacacaaagagatcacagttga
cacggaacagacctaagaaacaaacaataaacaacacacggaacacacataaaagagtagtagaagaacattttcccaactataaaaaatacaaaaatacaagacacaaaatacaaacctatgaccataatgcatgga
attttcatttgaacatgaagtatatatatatatatatatatatatatttaaagataattttatgatgtatagggtacgattgtatatgtatgtatgtggagactttatgagaaagtcttTT
GGACTTGGGACTTTTCCAATAACTCTTCGGTTTTGAGTTTGGTCTCGGGGGTGGAGGAGGTGGAGGATTCTCTTACACTTCTCTTTTATAAGGTTGA
ATTTCatttttttacgg'tgaagggttcaaaaataatcggaacgaagtttgaagatagggtcgtagactttactacgaacgttctttacatact'gaaatcttcacacat'gaaagtctgtactaaacatgacactctaaacgt
atctgcagagagaagacgacgggtaccaggacccaacgaacagtagcggtacggacgacgacgttttcttcagttcttcatgtaactatcgtagggccctatagacttacgttccgtgacagggttaattgaaagaa
aacacctcaccctcttctcaaaaagactaaagtctcggaactaaacgatatgtatagtaaacgctagaatacgaattccttcacattctttaggaacggttctataaaaaattataac
SEQ ID NO: 18

(lower case denotes intronic sequences; upper case denotes exonic sequence; underline bold letter denotes exact repair of mutation)

Figure 4

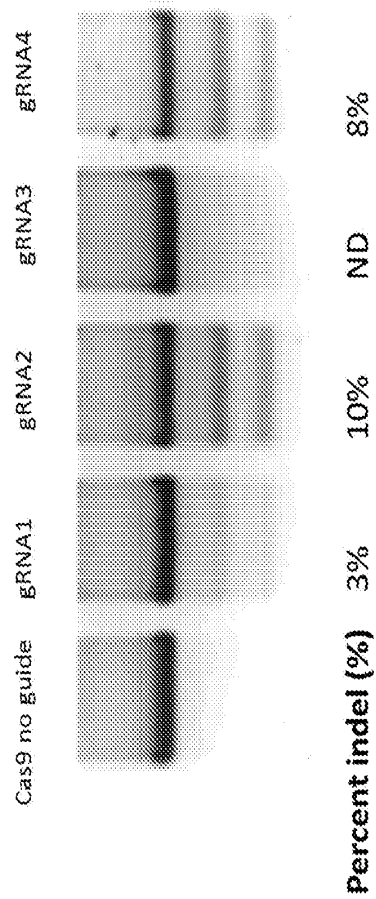


Figure 5

Repair Strategy 2: Remove exon containing mutation c.32854G>C using CRISPR/Cas9 to restore reading frame

Human specific SaCas9						
Position	Location	Spacer Sequence	PAM	Specificity Score	OLIGOS ORDERED	
Chr 2: 178,646,056	Intron 218	AGTATATGTATATGTTAGCAT (SEQ ID NO: 19)	GTGGAT (SEQ ID NO: 20)	79	CACCAGTATATGTATATGTTAGCAT (SEQ ID NO 21)	
	-1				TCATATACATATACAATCGTACAAA (SEQ ID NO: 22)	
	reverse					
chr2 +178,645,863	Intron 219	TGCAAGCATCATTTCAGATGG (SEQ ID NO: 23)	CTGGAT (SEQ ID NO: 24)	61	CACCTGCAAGCATCATTTCAGATGG (SEQ ID NO: 25)	
	-1				ACGTTCTAGTAAAGTCTACCCAAA (SEQ ID NO: 26)	
	reverse					

Underlined letter of the spacer sequence corresponds to the location of double-stranded break made in the target sequence

Oligos ordered corresponds to primer sequences used for cloning the spacer sequence into the Cas9 plasmid (px601 Addgene plasmid #61591)

Figure 6

Deletion of exon 219

Position	Strand	Spacer Sequence	SEQ ID NO	PAM	SEQ ID NO	Specificity Score
Chr 2:178646456	-1	AGAAGATTAAAGTCCACT <u>GGA</u>	27	TTGAAT	42	44.6132083
Chr 2:178646451	-1	GATAAAGAAAGATTAAAGT <u>CCA</u>	28	CTGGAT	43	39.3753798
Chr 2:178646346	-1	ATGAGGCTCACATTTACA <u>ACA</u>	29	AAGAAT	44	43.2076588
Chr 2:178645704	1	TGATAGCGTCATCCGGGA <u>IAT</u>	30	CTGAAT	45	49.4369454
Chr 2:178646321	-1	GTACAAGTTACATGGAA <u>ACT</u>	31	AAGAAT	46	43.4406842
Chr 2:178646213	1	TTTTATGTTCTGTGTTT <u>ATG</u>	32	TTGGAT	47	42.9694116
Chr 2:178645669	1	TGTCCAATTAACTTTCTT <u>ITG</u>	33	TGGAGT	48	36.6155577
Chr 2:178645656	1	TTCTTTTGTGGAGTGGGG <u>GGA</u>	34	AAGAGT	49	28.0044261
Chr 2:178645712	1	AAGTACATTGATAGCGT <u>CATC</u>	35	CGGGAT	50	48.8316012
Chr 2:178645776	1	TCTTCTGCTGCCCCATGGT <u>TC</u>	36	CTGGGT	51	42.8850669
Chr 2:178645598	1	ATCATTGCGATCTTATG <u>CTTA</u>	37	AGGAAT	52	46.915383
Chr 2:178646253	1	GTATTTCTCATCATCTTT <u>GTGA</u>	38	AAGGGT	53	36.4064834
Chr 2:178645688	-1	CAGTGCTGCATTCAGAT <u>ATC</u>	39	CCGGAT	54	44.6971424
Chr 2:178646313	1	CTCATGTCAACTGTGGCT <u>IGT</u>	40	CTGGAT	55	43.7754718
Chr2:178,646,422	-1	AACCATATACATTTCAAG <u>AGA</u>	41	AAGAAT	56	37.7929693

Underlined letter of the spacer sequence corresponds to the location of double-stranded break made in the target sequence

Figure 7

Repair Strategy 3: Cut out and repair Titin mutation c.37112 G>A

Position	Strand	Spacer Sequence	PAM	Specificity Score
Chr 2: 178622784	-1	GGAGCTGTAAGAGAATGT <u>C</u> AT (SEQ ID NO: 57)	CAGAAT (SEQ ID NO: 58)	41.758739
Chr 2: 178622773	-1	ATCCACATGAGGAGCTG <u>T</u> AA (SEQ ID NO: 59)	GAGAAT (SEQ ID NO: 60)	44.4639886
Chr 2: 178622779	1	ATTCTTACAGCTCCTC <u>T</u> ATG (SEQ ID NO: 61)	TGGAAT (SEQ ID NO: 62)	42.8443275

Underlined letter of the spacer sequence corresponds to the location of double-stranded break made in the target sequence

Donor Template:

ccatagtcgtcacgtgtgttctatcttgaattctgctaatttctcaagattgtgtaagtgcccttaataagaacaagtgttcttgccactgggtctgaagtttcttgacattatcttcaagtcgtggcagtgcaaa
tgaagatttttctccagctattatccaagaactgagatggctccctagtatatactgtacacacacatagcacactagtgaacatagatgccacataatttttaigtaaacctaaagagagatatctaaaaa
gcaaaagcatcatcacgtgaggcagctcaagtgatttcaaaagtcagttgtagcttattaagtggaataccctaaagagtcacttttcttaagtatactatgttaagtgcagagtaaatctccagaaaaacct
catttgaattctgatgacattctcttacaGCTCCTCATGTGGAAATCTTAAGACCCACTCACCGACCTTCAAGTTAGAGAAAAAGAAATGGCTCGATTGAGTGTGAACTTT
CCCCGAGAAAAATGCTAAGgtctgtgactgtataccgtgcatctgtactgtcaataaactttatatttctgttagccaaaaatggaaaaaaagttagtcacaacttctctggtcaccactttactttt
aaagataattgtagtataaaaaatgtattcttgactttaatgtctctcttttttagtatttaataattactatctgaaaaaacactggagactcaaatcaggagatttagattagtttgaaccaatgaaaagtgctgttttc
ttagatcagaaaagtcaaaatatacagcaattatattctttcaactaagtaacctctgtgtgctatttaactaccattggcaagtttcttccaagtc
SEQ ID NO: 63

* lowercase denotes intronic sequences; uppercase denotes exonic sequences; **Underlined bold** denotes the repair

Figure 8

Repair Strategy 4: Exon Skipping of Exon 326 – Repair of 2 bp insertion in exon 326 (c.4362insAT, p.Ser14450fsX4) which causes a frameshift with premature stop codon in the A band truncating more than half the A band and the entire M band (accounts for ~20% of known DCM causing TTN mutations)

Set 1

Position	Strand	Spacer Sequence	PAM	Specificity Score
Chr 2: 178576493	-1	TTAGATAAAATATTGGCA <u>CTC</u> (SEQ ID NO: 64)	TGGAAT (SEQ ID NO: 65)	43.073943
Chr 2: 178576463	-1	TATATATTCAGAGTTTGG <u>CTT</u> (SEQ ID NO: 66)	TTGGGT (SEQ ID NO: 67)	38.9577076
Chr 2: 178576474	1	AAATTACCCAAAAGCCAA <u>ACT</u> (SEQ ID NO: 68)	CTGAAT (SEQ ID NO: 69)	40.3960258
Chr 2: 178576450	-1	AAAGACACAAAAGTATAT <u>TAT</u> (SEQ ID NO: 70)	CAGAGT (SEQ ID NO: 71)	28.3422717

Set 2

Position	Strand	Sequence	PAM	Specificity Score
Chr 2: 178559283	-1	AGCAAAATTAACGTGGAT <u>ATG</u> (SEQ ID NO: 72)	TAGAAT (SEQ ID NO: 73)	36.7538162
Chr 2: 178559284	1	ACATATCCACGTTAATT <u>TIGC</u> (SEQ ID NO: 74)	TAGAAT (SEQ ID NO: 75)	45.3645981
Chr 2: 178559275	1	CGTTAATTGCTAGAAT <u>AGT</u> (SEQ ID NO: 76)	GTGAGT (SEQ ID NO: 77)	39.5941154

Underlined letter of the spacer sequence corresponds to the location of double-stranded break made in the target sequence

Figure 9