SUPPLEMETAL DATA

Base editing repairs an SGCA mutation in human primary muscle stem cells

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T+49 (0)30 450540523 (Gene editing strategies)

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T+49 (0)30 450540501 (Translational design, muscle stem cells)

SUPPLEMETAL FIGURE LEGENDS

Supplemental Figure 1. Genotyping of SGCA c.748-2A>G mutation in control 3. *SGCA* sequence analysis of control 3 shows a heterozygous mutation in the splice acceptor of exon 7 (c.748-2A>G).

Supplemental Figure 2. *SGCA* c.175G>A is an exonic splicing mutation. (A) Sequence analysis of the exon 2-3 junction in the full-length (412 bp) RT-PCR band from the c.157G>A carrier from Fig. 2A shows no detectable inclusion of the mutant exon 2. (B) RT-PCR analysis of *SGCA* mRNA in human muscle tissue from controls and the heterozygous c.175G>A carrier. The primer binding sites on the *SGCA* coding sequence and the expected band sizes are displayed above. Control 3 is a heterozygous carrier of the *SGCA* c.748-2A>G mutation. Splicing of *SGCA* exons 4-9 is unaffected in the c.157G>A carrier. (C) RT-PCR analysis of *SGCA* mRNA in human muscle tissue from controls and the heterozygous c.175G>A carrier was performed using a ³²P-labeled forward primer and the products were separated by denaturing PAGE. Splice isoforms were quantified using Phosphoimager analysis. The primer binding sites and expected band sizes are displayed above. fl: splice isoform containing exons 1-5; Δ2: splice isoform missing exon 2; Δ2/3: splice isoform missing exons 2/3.

Supplemental Figure 3. Minigene splicing assays in HEK293T cells. (A) Scheme of minigene constructs as in Fig. 2E. (B) Splicing pattern of minigene constructs in HEK293T cells analyzed by low-cycle RT-PCR with a ³²P-labeled forward primer, products were separated by denaturing PAGE. Quantification is shown in Fig. 2G. (C) Splicing pattern of minigene constructs in HEK293T cells analyzed by RT-PCR and agarose gel electrophoresis. The splice isoforms identified were confirmed by Sanger sequencing and are shown on the right. *HPRT* was used as a housekeeping control for RT-PCR analysis. (D) Quantification of the splice isoforms from (C). Quantified values are presented as mean \pm SD (n=3). *: intron-retention isoforms. 2*: truncated exon 2 (40 nt). 3*: truncated exon 3 (-70 nt). E: exon. I: intron.

Supplemental Figure 4. Generation and characterization of patient iPSC from patient primary myoblasts. (A) Immunostaining of iPSC colonies with pluripotency markers. Scale bars: 50 μ m. (B) Histopathological analysis of iPSC-derived teratomas containing tissues derived from the three germ layers. Scale bars: 50 μ m. (C) Virtual karyotype analysis of patient iPSC. Regions of gain (duplications) are shown in green, regions of loss (deletions) are shown in red and regions of uniparental disomy (loss of heterozygosity) are shown in grey. Reportable are copy number changes (gains and losses) greater than 0.4Mb compared to the human reference genome and regions of loss of heterozygosity above 3 Mb. (D) Histogram plots of flow cytometric analysis of the pluripotency markers SSEA4, Tra1-60, Nanog, Oct3/4, and the differentiation marker SSEA1 in patient iPSC.

Supplemental Figure 5. Gating strategies for FACS-sorting of Venus-positive iPSC and MuSC. Representative FACS plots of primary patient MuSC (A) and iPSC (B) transfected with ABE7.10_4.1/gRNA#1. Gates were defined to select for the viable cell population (P1) and for doublet exclusion (P2, P3). Venus-positive events (P4) were

separated according to their green fluorescence intensity using stringent gates to ensure a high purity sorting.

Supplemental Figure 6. Adenine base editing in iPSC with a homozygous SGCA c.157G>A mutation. (**A**) Experimental design. A plasmid encoding ABE7.10_4.1 or ABE7.10_3.1, a Venus reporter and a U6-driven gRNA expression cassette was transfected into patient iPSC where the c.157G>A mutation had been converted to homozygosis. Venus-positive cells were selected via FACS-sorting and the bulk sorted population was analyzed. (**B**) EditR analysis of nucleotide rates at each protospacer position in patient iPSC transfected with ABE7.10_4.1 and _3.1 in combination with gRNA#1. iPSC transfected with ABE7.10_4.1 without gRNA are shown as control. (**C**) c.157G/A nucleotide rates in EditR analysis from (B). The quantified values are presented as mean ± SD (n=2).

Supplemental Figure 7. Heatmap of A>G conversion rates at the target locus in patient and carrier MuSC. Crispresso2 analysis of amplicon sequencing data showing the percentage of A>G conversion at each position of the quantification window at the target sequence in Venus-positive patient (A) and carrier (B) MuSC. The target c.157A at protospacer position 6 is indicated in red and the bystander adenine at protospacer position 10 is indicated in orange. A range of concentrations for the ABE7.10_4.1/gRNA#1 vector is shown. Venus-positive cells transfected with the ABE7.10_4.1 vector without gRNA are shown as control.

Supplemental Figure 8. Allele frequencies at the target locus in patient and carrier **MuSC.** Crispresso2 analysis of allele frequencies in the quantification window at the target sequence in Venus-positive patient (**A**) and carrier (**B**) MuSC. Position c.157 of the *SGCA* CDS is indicated with a red arrow and the bystander adenine at protospacer position 10 is indicated with an orange arrow. The total number and percentage of reads for each allele type is shown on the right. A range of concentrations for the ABE7.10_4.1/gRNA#1 vector is shown. Venus-positive cells transfected with the ABE7.10_4.1 vector without gRNA are shown as control.

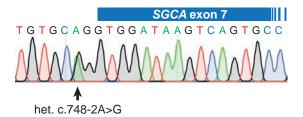
Supplemental Figure 9. Heatmap of A>G conversion rates at the predicted offtarget loci in patient MuSC. Crispresso2 analysis of amplicon sequencing data showing the percentage of A>G conversion at each position of the quantification window at the four predicted exonic off-target sites (A to D) in Venus-positive patient MuSC. Adenines located in the ABE activity window are indicated in orange and mismatched nucleotides between the protospacer sequence and gRNA#1 are indicated with blue stars above. A range of concentrations for the ABE7.10_4.1/gRNA#1 vector is shown. Venus-positive cells transfected with the ABE7.10_4.1 vector without gRNA are shown as control.

Supplemental Figure 10. Allele frequencies at the predicted off-target loci in patient MuSC. Crispresso2 analysis of allele frequencies in the quantification window at the four predicted exonic off-target sites (A to D) in Venus-positive patient MuSC. Mismatched nucleotides between the protospacer sequence and gRNA#1 are indicated with blue stars above. The total number and percentage of reads for each allele type is shown on the right. A range of concentrations for the ABE7.10_4.1/gRNA#1 vector is shown. Venus-positive cells transfected with the ABE7.10_4.1 vector without gRNA are shown as control.

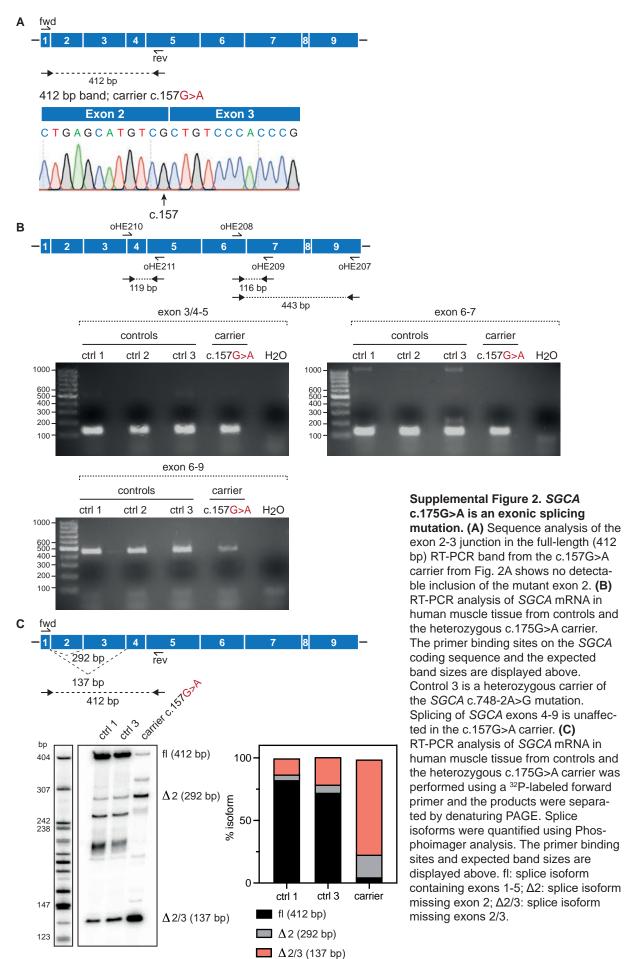
Supplemental Figure 11. Donor-derived myofibers express α -sarcoglycan. Grafted muscles were immunostained with antibodies against human Spectrin and α sarcoglycan. Scale bars: 50 µm.

SUPPLEMENTAL FIGURES

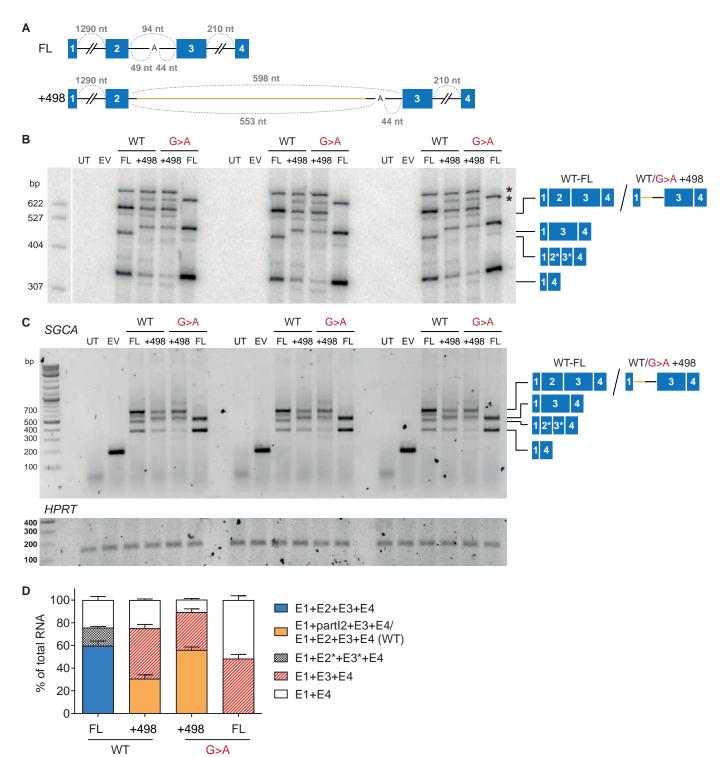
Control 3 (carrier SGCA c.742-2A>G)



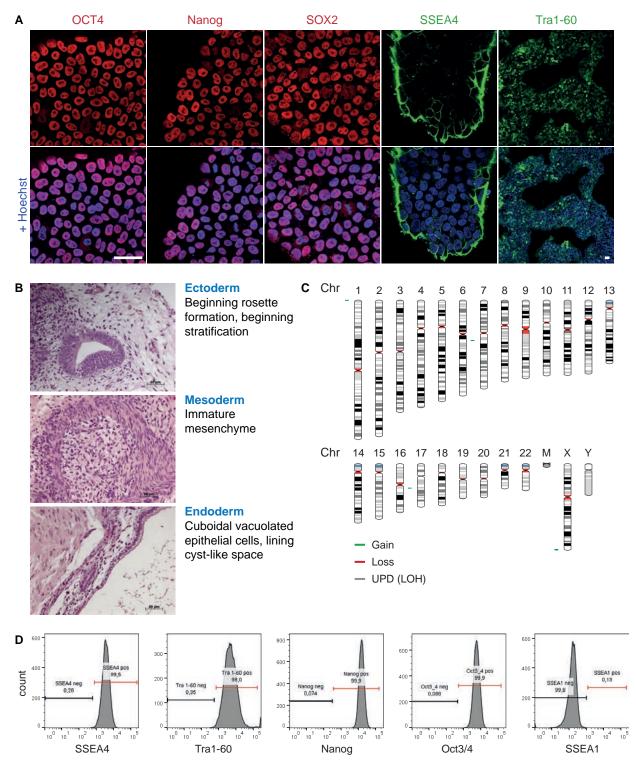
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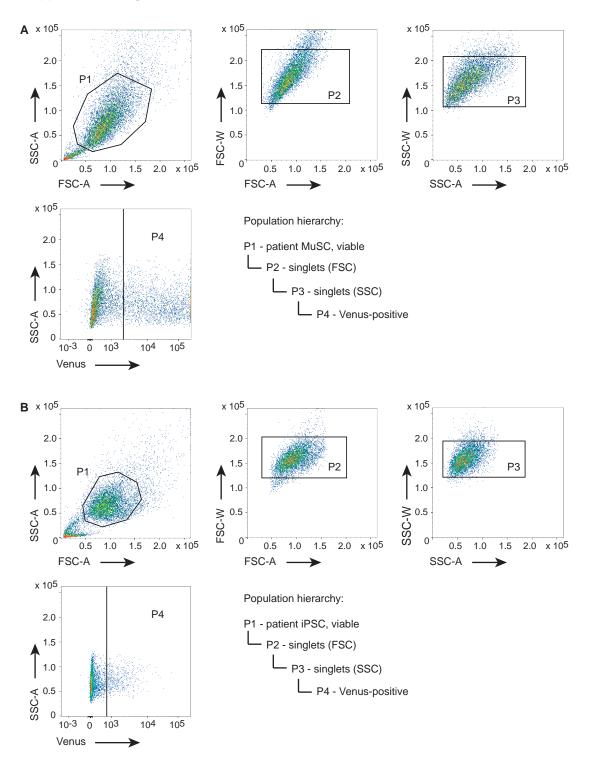




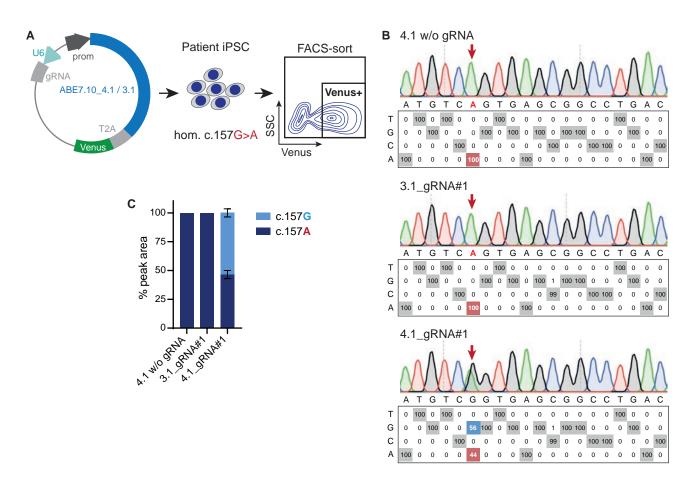
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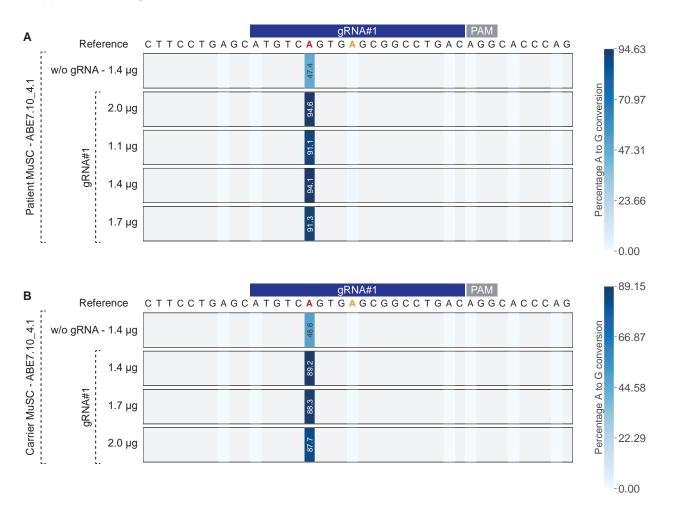


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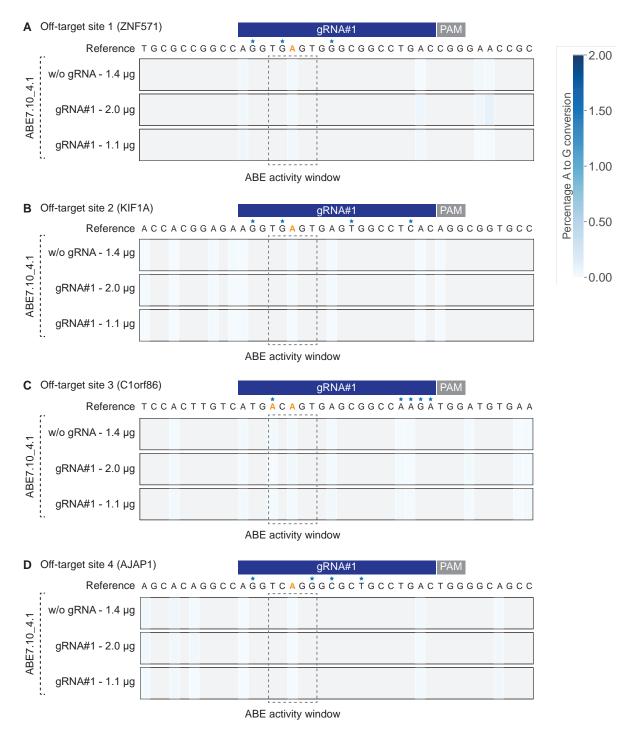
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														ç	gR	NA	#1							PAI	M						i	bol	 d	Sul	 ostitu	ution	s ¦
Re	eference	СТ СТ																																Ins	ertio etio	ns	
Α	c.157A c.157G c.157A c.157A c.157G	C T C T	T C T C T C	C C C	T (T (T (G A G A	G G G	C /	A T A T	G G	T T	C G	G	T T	G G	A C		G G	G G	T	GG	A		A G A G	G G	C .	A C	C C	C C	A G A T	46 0.4	.58% 19%) (1 (10	026 7 re	4 rea ads)	ids)	
0_4.1	c.157 G c.157 A indel c.157 G A ₁₀ >G indel	C T C T C T	T C T C T C T C T C	00000		G A A A A A A A	GGGG		TTT	6666		AAGGG	0000	T O T O T O		AG	0.00	G G G G		T • T T	G · G G		A	GGG	G (G (G (0000	0000		G	7.7 0.7 0.6		(15 (14 (12 (11	18 r 0 re 1 re 3 re	eads ads) ads) ads)		
Patient MuSC - ABE7.10_	c.157 G c.157 A A ₁₀ >G c.157 G	СТСТ	T C T C T C	CCC		G A G A G A	G	CI	A T A T	G	T	C A C G	G	T T	G	A C G C	C C	G G	G G	T	G	A		A G A G	G G	C	A C A C	C	C C	A G A G	5.7	73% 10%	(13 51	95 r 0 re	eads ads)	s) ´	
Patient	c.157 G c.157 A c.157 G A ₁₀ >G indel	СТСТ	T C T C T C	0000		G A G A G A G A	GGG	CACA	T	GGG	T (T (GGGG	GGG	T T T	G	A G A G	CCC	GGG	GGG	TTT	GGG	ACAC	AAAA	G	GGG			000	C C C	A G A T A G	8.2 0.6	21% 55% 50%	(21 (17 (13	98 r 3 re 3 re	eads ads) ads)	a í	
	c.157 G c.157 A c.157 G A ₁₀ >G	СТСТ	T C T C T C	CCC	T (T (T (G A G A G A	G	CI	A T A T	G	T	C A C G	G	T T	G	AC	C C	G G	G G	T	G	A		A G A G	G G	C	A C A C	C	C C	AGAT	5.2 0.7	29% 76%	(11 16	17 r 0 re	eads ads))	
В	c.157 A c.157 G c.157 G c.157 A	СТСТ	T C T C T C	CCC		G A G A G A	GGG	CI	A T A T	GG	T	C G	G	T T	G	AC	G C	G G	GG		GG	A		A G A G	G	C	A C A C	C	C C	AGAT	47 0.4	.80% 13%) (1 (11	276 4 re	0 rea ads)	ads) ads)	
ABE7.10_4.1	c.157 G c.157 A c.157 G A ₁₀ >G *c.157 G	gRN c T c T c T c T c T		0000	T (T (T (GAGAGA	GGG	000	A T A T	GGG	T	G	GGG	T T T	G		C C C	GGG	G	T	GGG	ACAC	ACA	G	GGG	0	AC	C C C	CCC	A G A T A G	10 0.6 0.2	.40% 57% 26%) (3 (19 (75	023 5 re 7 rea	reac ads) ds)	ids) Is)	
Carrier MuSC - ABE7.10	c.157G c.157A c.157G A ₁₀ >G *c.157G	C T C T C T		0000	T O T O T O	G A G A G A G A	GGG	000		GGG	T T		GGG	T T T	G			G G G	GGG	T	GGG	A		A G A G	GGG	C	ACAC	C C C	CCC	A G A T A G	11 0.6 0.4	.41% 57% 14%	(3 (21 (14	591 0 re 0 re	read ads) ads)	ls) ́	
	c.157 G c.157 A c.157 G A ₁₀ >G	СТ	T C T C T C	C C C	T (T (T (G A G A G A	G	C	A T A T	GG	T T	C A C G	G	T T	G G	A C		G G	G G	C T C T	G	A		A G A G	GG	C .	A C	C C	C C	A G A T	11 0.6	.77% 66%	(2 (12	213 5 re	read ads)		

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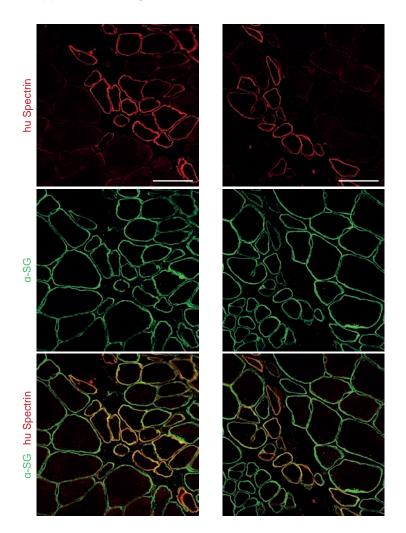


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Α	Off-target site 1 (ZNI	-571)	g	RNA#1	PAM	bold Substitutions
	Reference				G A C C G G G G A A C C G C C A C C G G G A A C C G C	 Insertions Deletions
4.1	w/o gRNA - 1.4 µg	TGCGCCGGCC	AGGTGAGTO	GGCGGCCTC	CACCGGGAACCGC	98.35% (10978 reads)
ABE7.10_4.1	gRNA#1 - 2.0 µg	TGCGCCGGCC	AGGTGAGTO	GGCGGCCT	CACCGGGAACCGC	98.09% (11725 reads)
ABE	gRNA#1 - 1.1 μg	TGCGCCGGCC	AGGTGAGT	GGCGGCCT	C A C C G G G A A C C G C	98.67% (9171 reads)
		4.4.)				
В	Off-target site 2 (KIF	,		RNA#1		
	Reference				CACAGGCGGTGCC CACAGGCGGTGCC	
4.1	w/o gRNA - 1.4 μg	ACCACGGAGA	AGGTGAGT	AGTGGCCT	C A C A G G C G G T G C C	98.38% (216560 reads)
ABE7.10	gRNA#1 - 2.0 µg	A C C A C G G A G A	AGGTGAGTO	AGTGGCCT	CACAGGCGGTGCC	98.38% (260276 reads)
ABE	gRNA#1 - 1.1 μg	ACCACGGAGA	AGGTGAGTO	AGTGGCCT	CACAGGCGGTGCC	98.22% (215057 reads)
C	Off-target site 3 (C1c	,	*	RNA#1	PAM	
C		TCCACTTGTC	ATGÂCAGTG	ассссса́	PAM A G A T G G A T G T G A A A G C T G G A T G T G A A	
_ [Reference	T C C A C T T G T C T C C A C T T G T C	A T G Å C A G T G A T <mark>G</mark> A C A G T G	A G C G G C C Å Å	Ă Ğ Ă T G G A T G T G A A A <mark>G C</mark> T <mark>G G</mark> A T <mark>G T G</mark> A A	98.99% (45674 reads)
_ [Reference w/o gRNA - 1.4 µg	T C C A C T T G T C T C C A C T T G T C T C C A C T T G T C	A T G A C A G T G A T G A C A G T G A T G A C A G T G	A G C G G C C Å Å	À Ġ Ă T G G A T G T G A A A <mark>G C T G G A T G T G A A</mark> A <mark>G C T G G A T G T G A A</mark>	
.10_4.1	Reference w/o gRNA - 1.4 μg gRNA#1 - 2.0 μg	T C C A C T T G T C T C C A C T T G T C T C C A C T T G T C T C C A C T T G T C	A T G Å C A G T G A T G A C A G T G A T G A C A G T G A T G A C A G T G	A G C G G C C A A A G C G G C C A A A G C G G C C A A A G C G G C C A A	À Ĝ À T G G A T G T G A A A G C T G G A T G T G A A A G C T G G A T G T G A A A G C T G G A T G T G A A	99.20% (24535 reads)
_ [Reference w/o gRNA - 1.4 μg gRNA#1 - 2.0 μg	T C C A C T T G T C T C C A C T T G T C T C C A C T T G T C T C C A C T T G T C	A T G Å C A G T G A T G A C A G T G A T G A C A G T G A T G A C A G T G	A G C G G C C A A A G C G G C C A A A G C G G C C A A A G C G G C C A A	À Ġ Ă T G G A T G T G A A A <mark>G C T G G A T G T G A A</mark> A <mark>G C T G G A T G T G A A</mark>	99.20% (24535 reads)
ABE7.10_4.1	Reference w/o gRNA - 1.4 μg gRNA#1 - 2.0 μg gRNA#1 - 1.1 μg	T C C A C T T G T C T C C A C T T G T C T C C A C T T G T C T C C A C T T G T C T C C A C T T G T C T C C A C T T G T C	A T G Å C A G T G A T G A C A G T G	A G C G G C C A A A G C G G C C A A	À Ĝ Ă T G G A T G T G A A A G C T G G A T G T G A A A G C T G G A T G T G A A A G C T G G A T G T G A A A G C T G G A T G T G A A	99.20% (24535 reads)
ABE7.10_4.1	Reference w/o gRNA - 1.4 μg gRNA#1 - 2.0 μg	T C C A C T T G T C T C C A C T T G T C T C C A C T T G T C T C C A C T T G T C T C C A C T T G T C T C C A C T T G T C	A T G Å C A G T G A T G A C A G T G	A G C G G C C A A A G C G G C C A A RNA#1	A G A T G G A T G T G A A G C T G G A T G T G T G A A A G C T G G A T G T G T G A A A G C T G G A T G T G A A A G C T G G A T G T G A A A G C T G G A T G T G A A	99.20% (24535 reads)
ABE7.10_4.1	Reference w/o gRNA - 1.4 μg gRNA#1 - 2.0 μg gRNA#1 - 1.1 μg Dff-target site 4 (AJA	T C C A C T T G T C T C C A C T T G T C T C C A C T T G T C T C C A C T T G T C T C C A C T T G T C T C C A C T T G T C T C C A C T T G T C	A T G Å C A G T G A T G A C A G T G G T C A G Ğ G	A G C G G C C Å Å A G C G G C C A Å A G C G G C C A Å A G C G G C C A Å A G C G G C C A Å A G C G G C C A Å A G C G G C C A Å A G C G G C C A Å A G C G G C C A Å A G C G G C C A Å A G C G G C C A Å A G C G G C C A Å A G C G G C C A Å A G C G G C C A Å A G C G G C C A Å A G C G G C C A Å A G C G G C C A Å A G C G G C C A Å	À Ĝ Ă T G G A T G T G A A A G C T G G A T G T G A A A G C T G G A T G T G A A A G C T G G A T G T G A A A G C T G G A T G T G A A	99.20% (24535 reads) 99.13% (23385 reads)
ABE7.10_4.1	Reference w/o gRNA - 1.4 μg gRNA#1 - 2.0 μg gRNA#1 - 1.1 μg Dff-target site 4 (AJA Reference	T C C A C T T G T C T C C A C T T G T C T C C A C T T G T C T C C A C T T G T C T C C A C T T G T C T C C A C T T G T C A C A C A C A G G C C A G C A C A G G C C	A T G Å C A G T G A T G A C A G T G G T C A G Ĝ G A G G T C A G Ĝ G	A G C G G C C A A A G C G G C C A A B A G C G G C C G G C C A A	A G A T G G A T G T G A A G C T G G A T G T G T G A A A G C T G G A T G T G T G A A A G C T G G A T G T G T G A A A G C T G G A T G T G A A PAM B A C T G G G G C A G C C	99.20% (24535 reads) 99.13% (23385 reads)
.10_4.1 D ABE7.10_4.1	Reference w/o gRNA - 1.4 μg gRNA#1 - 2.0 μg gRNA#1 - 1.1 μg Off-target site 4 (AJA Reference	T C C A C T T G T C T C C A C T T G T C T C C A C T T G T C T C C A C T T G T C T C C A C T T G T C T C C A C T T G T C T C C A C T T G T C A C A C A C A G G C C A G C A C A G G C C	A T G Å C A G T G A T G A C A G T G G T C A G G G A G G T C A G G G A G G T C A G G G	A G C G G C C A A A G C G G C C A A RNA#1 C G C T G C C T G C G C T G C C T G C G C T G C C T G	A G A T G G A T G T G A A G C T G G A T G T G T G A A A G C T G G A T G T G T G A A A G C T G G A T G T G T G A A A G C T G G A T G T G T G A A A G C T G G G C A G C C G A C T G G G G C A G C C	99.20% (24535 reads) 99.13% (23385 reads) 99.13% (25565 reads)
4.1 D ABE7.10_4.1	Reference w/o gRNA - 1.4 μg gRNA#1 - 2.0 μg gRNA#1 - 1.1 μg Off-target site 4 (AJA Reference w/o gRNA - 1.4 μg gRNA#1 - 2.0 μg	T C C A C T T G T C T C C A C T T G T C T C C A C T T G T C T C C A C T T G T C T C C A C T T G T C T C C A C T T G T C T C C A C T T G T C A C C A C A C A G G C C A G C A C A G G C C A G C A C A G G C C	A T G Å C A G T G A T G A C A G T G G T C A G G G A G G T C A G G G A G G T C A G G G A G G T C A G G G	A G C G G C C Å Å A G C G G C C Å Å A G C G G C C A Å A G C G G C C A Å A G C G G C C A Å A G C G G C C A Å A G C G G C C A Å A G C G G C C A Å A G C G G C C A Å A G C G G C C A Å A G C G G C C A Å A G C G G C C A Å A G C G G C C A Å C G C T G C C T G C C T G C G C T G C C T G C G C T G C C T G C G C T G C C T G	A G A T G G A T G T G A A G C T G G A T G T G T G A A A G C T G G A T G T G T G A A A G C T G G A T G T G T G A A A G C T G G A T G T G A A A G C T G G G C A G C C G A C T G G G G C A G C C G A C T G G G G C A G C C	99.20% (24535 reads) 99.13% (23385 reads) 99.13% (25565 reads) 99.29% (23765 reads)

Supplemental Figure 10. Allele frequencies at the predicted off-target loci in patient

MuSC. Crispresso2 analysis of allele frequencies in the quantification window at the four predicted exonic off-target sites (A to D) in Venus-positive patient MuSC. Mismatched nucleotides between the protospacer sequence and gRNA#1 are indicated with blue stars above. The total number and percentage of reads for each allele type is shown on the right. A range of concentrations for the ABE7.10_4.1/gRNA#1 vector is shown. Venus-positive cells transfected with the ABE7.10_4.1 vector without gRNA are shown as control.



Supplemental Figure 11. Donor-derived myofibers express α -sarcoglycan. Grafted muscles were immunostained with antibodies against human Spectrin and α -sarcoglycan. Scale bars: 50 µm.

SUPPLEMENTAL TABLES

Supplemental	Table 1:	Myogenic	marker	expression	in SGCA	c.157Grep	patient
MuSC							

Patient MuSC		g vector	% positive cells						
populations	۳ ۱	greetor	Desmin	Pax7	Myf5	MyoD	Ki67		
Unedited		-	89	60	58	54	37		
ABE7.10_4.1 (w/o sgRNA)		1.4 µg	97	13	46	71	37		
		1.1 µg	54	16	30	30	57		
	1.4 µg	cryopreserv.	99	27	40	66	25		
ABE7.10_4.1/gRNA#1	1. 4 µy	after recovery	99	34	45	64	39		
(SGCA c.175G rep)	1.7 µg	cryopreserv.	98	20	53	44	39		
	1.7 P9	after recovery	99	23	24	60	28		
		2.0 µg	100	3	34	53	23		

Supplemental Table 2: Synthetic oligonucleotides for genomic PCR, RT-PCR / RTqPCR, sequencing and cloning of ABE and minigene vectors

Oligo	Name	Sequence 5' > 3'	Purpose
oHE24	HE_SGCA i1 F	CGCTTCTCTCGGTCCCTTAG	Genotyping
oHE25	HE_SGCA e3 R	GTAGAGGAAGCCAGGGTGGT	SGCA
			c.157G>A
			mutation / ABE
			analysis
oHE26	HE_SGCA i6 F	TGGATTCAAACCAGGAGGTC	Genotyping
oHE27	HE_SGCA ei7 R	CTTCATGCCCACATTCACCT	SGCA c.748-
			2A>G mutation
oHE28	Bpll-site_oligo1	CACCGGGTCTTCGAGAAGACCTc	exchange of
		gtcatcta	BbsI to BpII site
oHE29	Bpll-site_oligo2	AAACtagatgacgAGGTCTTCTCGAA	in HE_p4.1
		GACCC	
oHE55	Sp_sgRNA_SGCAe	ATGTCAGTGAGCGGCCTGACgtttt	sgRNA#1
	x2mut#1_for		cloning into
oHE56	Sp_sgRNA_SGCAe	GTCAGGCCGCTCACTGACATggtgt	ABE7.10_4.1
	x2mut#1_rev		and _3.1
oHE206	SGCA-	ATGGCTGAGACACTCTTCTG	RT-PCR SGCA
	201_CCDS_ex1 F1		
oHE207	Sgca_CCDS_ex9	TCAGTGCTGGTCCAGGATAA	
	R1		
oHE208	SGCA_Sgca_ex6 F1	GCGTTGACTGGTGCAATGT	
oHE209	SGCA_Sgca_ex7	AGTGGGTGGGCAGAAGAAC	
	R1		
oHE210	SGCA_Sgca_ex3-4	GAGGTCACAGCCTACAATCG	
	F1		
oHE211	SGCA_Sgca_ex5	TGGCTGCGCACCAGGAACT	
	R1		
oHE238	SGCA-	TCTGGACTCCTCTCCTCGTG	
	201_CCDS_ex1 F2		
oHE243	ZNF571_Fwd1	GGTGCGGAGGAACACAACTA	ABE OFF-target
oHE246	KIF1A_Rev1	CACGCCGTCTTCAACATCATC	analysis;
oHE248	C1orf86_Rev1	CTCACCAACTGCCCAGATGTG	primers for

oHE249	AJAP1_Fwd1	CCCCGGTTGGTTTGAAAACG	amplicon
			sequencing
oHE255	SGCA_NGS_ex2	TCTTTGTGCACACCTTGGAC	ABE ON-target
	fwd5		analysis;
oHE256	SGCA_NGS_ex2	GAGGACTCAGATACCAAATTAGA	primers for
	rev1	GG	amplicon
			sequencing
oHE259	ZNF571_Rev2	AGTCCCACAGTTCGAGCAAG	ABE OFF-target
oHE260	KIF1A_Fwd2	GGCAGGGGACAGAGTAAGAGA	analysis;
oHE261	C1orf86_Fwd2	GCCTGTCCTCTCTGAGTTGC	primers for
oHE262	AJAP1_Rev2	TCAGGTGAGGCTTGTGAGG	amplicon
			sequencing
oHE263	SGCA_NGS_ex2	ACACTCTTTCCCTACACGACGCT	ABE ON- and
	fwd5_ILLUM-ADAPT	CTTCCGATCTTCTTTGTGCACACC	OFF-target;
		TTGGAC	primers for
oHE264	SGCA_NGS_ex2	GACTGGAGTTCAGACGTGTGCTC	amplicon
	rev1_ILLUM-ADAPT	TTCCGATCTGAGGACTCAGATAC	sequencing
		CAAATTAGAGG	containing the
oHE265	ZNF571_Fwd1_ILLU	ACACTCTTTCCCTACACGACGCT	Illumina
	M-ADAPT	CTTCCGATCTGGTGCGGAGGAAC	adaptares
		ACAACTA	(underlined) as
oHE266	ZNF571_Rev2_ILLU	GACTGGAGTTCAGACGTGTGCTC	5'- extension
	M-ADAPT	TTCCGATCTAGTCCCACAGTTCG	
		AGCAAG	
oHE267	KIF1A_Fwd2_ILLUM	ACACTCTTTCCCTACACGACGCT	
	-ADAPT	CTTCCGATCTGGCAGGGGGACAGA	
		GTAAGAGA	
oHE268	KIF1A_Rev1_ILLUM	GACTGGAGTTCAGACGTGTGCTC	
	-ADAPT	TTCCGATCTCACGCCGTCTTCAA	
		CATCATC	
oHE269	C1orf86_Fwd2_ILLU	ACACTCTTTCCCTACACGACGCT	
	M-ADAPT	CTTCCGATCTGCCTGTCCTCTCT	
		GAGTTGC	
oHE270	C1orf86_Rev1_ILLU	GACTGGAGTTCAGACGTGTGCTC	
	M-ADAPT	TTCCGATCTCTCACCAACTGCCC	
		AGATGTG	

oHE271	AJAP1 Fwd1 ILLU	ACACTCTTTCCCTACACGACGCT	
	M-ADAPT		
		GAAAACG	
oHE272	AJAP1 Rev2 ILLU	GACTGGAGTTCAGACGTGTGCTC	
	M-ADAPT		
		TGAGG	
oSS114	ABE Cas9 BgIII re	ttggccatctcgttgctgaagatctcttgtaagtaa	Gibson cloning
000114	V	catattcggttc	of ABE7.10 4.1
oSS121	ABE+kozak fwd		
033121	ADE+KUZAK_IWU	ttttcctacagatccttaattaagccgccaccatgt	
- AK10			
oAK19	SGCA_ex1 F1	GCTGAGACACTCTTCTGGAC	RT-qPCR
oAK21	SGCA_ex2 R1	CATGGTCCAAGGTGTGCAC	SGCA and
oAK23	SGCA_Sgca_ex7	CAGAAGAACGGGTCATGCTC	GAPDH
	R2		
oAK30	GAPDH_ex1-Fwd	GAAGGTGAAGGTCGGAGTC	
oAK31	GAPDH_ex3-Rev	GAAGATGGTGATGGGATTTC	
-	MYH2_V1_Fwd	GGAACGGGCTGACATTGCTG	RT-qPCR
-	MYH2_V1_Rev	GTCATTCCATGGCATCAGGACA	MYH2
-	HindIII_SGCA_F	AATTTaagcttCCCTGTCTCTGTCAC	Cloning of
		TCACC	minigine
-	EcoRI_SGCAPart1_	ATTAgaattcGCCTGTCAGGCCGCT	constructs
	all_R	CACCGACAT	
-	EcoRI_SGCAPart1	ATTAgaattcGCCTGTCAGGCCGCT	
	mut_all_R	CACTGACAT	
-	EcoRI_Hbbl2_all_F	ATTAgaattcAGTGTGGAAGTCTCA	
		GGATCG	
-	Hbbl2_498_R	GAATGGTGCAAAGAGGCATG	
-	SGCAPart2_498_F	catgcctctttgcaccattcACCCAGGCGG	
		GCGGGCTGGGGTGTA	
-	Xhol_SGCA_R	AATTTctcgagCAAGCCTCTCCTGT	
		CCACAG	
-	T7 seq F	TAATACGACTCACTATAGGG	PCR & seq.,
-	BGH R	TAGAAGGCACAGTCGAGG	minigene
			assays

Antibodies used for immunocytochemistry/flow-cytometry							
	Antibody	Dilution	Company & Cat #				
Pluripotency	Rabbit anti-OCT4	1:1000	Abcam #ab19857				
Markers	Rabbit anti-SOX2	1:300	Abcam #ab97959				
(Immunostaining)	Rabbit anti-NANOG	1:100	Abcam #ab21624				
	Mouse anti-TRA-1-60	1:500	Abcam #ab16288				
Pluripotency	Anti-OCT3/4 APC	1:50	Miltenyi Biotec #130-117-709				
Markers (Flow	Anti-NANOG PE	1:100	Cell Signaling #14955S				
Cytometry)	Anti-TRA-1-60 Vio488	1:600	Miltenyi Biotec #130-106-872				
	Anti-SSEA4 VioBlue	1:20	Miltenyi Biotec #130-098-366				
	Anti-CD15 Vio770	1:100	Miltenyi Biotec #130-113-486				
Primers	I	1					
	Target	Forward/F	Reverse primer (5'-3')				
Sendai-virus (RT-	SeV (total)	GGATCAC	CTAGGTGATATCGAGC /				
PCR)		ACCAGAG	CAAGAGTTTAAGAGATATGTAT				
		С					
	SeV-KOS	ATGCACO	CGCTACGAGTGAGCGC /				
		ACCTTGA	CAATCCTGATGTGG				
	SeV-KLF-4	TTCCTGC	CATGCCAGAGGAGCCC /				
		AATGTAT	CGAAGGTGCTCAA				
	SeV-c-Myc	TAACTGA	CTAGCAGGCTTGTCG /				
		TCCACAT	ACAGTCCTGGATGATGATG				
Housekeeping	Hu18SRNA	GTAACCO	CGTTGAACCCCATT /				
		CCATCCAATCGGTAGTAGCG					

Supplemental Table 3: Reagents for iPSC generation and characterization

Antibody	Clone	Company & Cat No.	Working dilution
α-sarcoglycan	EPR14773	Abcam, #ab189254	IF cells/tissue -
			1:500; WB – 1:2000
PAX7		Santa Cruz Biotechnology,	IF cells - 1: 200 /
		#sc-81648	tissue - 1: 100
Desmin		Abcam, #ab15200	IF cells - 1:2,000
Desmin		Dako #M0760	IF tissue – 1:50
Ki-67		ThermoFisher Scientifitc,	IF cells - 1:300
		#RM-9106-S0	
MYOD	5.8A	Santa Cruz Biotechnology,	IF cells - 1:50
		#sc-32758	
MYF-5	C20	Santa Cruz Biotechnology,	IF cells - 1:2,000
		#sc-302	
МуНС	MF20	Developmental Studies	WB – 1:1000
		Hybridoma Bank, #MF20	
Skeletal Myosin	MY-32	Sigma-Aldrich, #M4276	IF cells - 1:100
(FAST)			
Hu Lamin A+C	EPR4100	Abcam, #ab108595	IF tissue - 1:4,000
Hu Spectrin	RBC2/3D5	Leica Biosystems, #NCL-	IF tissue - 1:100
		SPEC1	
Vinculin	VIN-11-5	Sigma-Aldrich, #V4505	WB – 1:200

Supplemental Table 4: Antibodies