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Supplemental information

mRNA-mediated delivery of gene editing tools to human primary muscle stem cells

Christian Stadelmann, Silvia Di Francescantonio, Andreas Marg, Stefanie Müthel, Simone Spuler, and Helena Escobar

SUPPLEMENTAL TABLES

Table S1. MuSC populations/lines used for each experiment

		Figure										
Donor ID	**MuSC line**	**1**	**2**	**3**	**4**	**5**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**
A	A1	A,B,C,E										
	A2	B,C,D,E	E,F,G		B		C,D,E,F				B	
	A3	E						B,C				
	A4			D	F							
	A5								C			
B	B	B,C,E										
C	C	B,C,E	E,F,G	C,D	B,D,F		C,D,E,F				B	
D	D	B,C	E,F,G		B		C,D,E,F				B	
E	E1							C				
	E2	E		D								
F	F					B,C						
G	G		E,F		A,B,C,E		C,F				A,B,C	
H	H		C,D,E,F		B,E		C,F				B	
I	I		E,F		B,E		B,C,F				B	
J	J								A			
K	K	E										
L	L	E										
M	M	E										

Table S2. Synthetic sgRNA sequences

Name	**Sequence 5' > 3'** (spacer)	**Target**
Sp_sgRNA_NCAM1 ex3wt#1_human	AACGCCAACAUCGACGACGCGUU UUAGAGCUAGAAAUAGCAAGUUAA AAUAAGGCUAGUCCGUUAUCAAC UUGAAAAAGUGGCACCGAGUCGG UGCUUUU	*NCAM1* exon 3 (SpCas9)
Sp_sgRNA_NCAM1 ex7ABE_3	CCCUACCAAAGACUUUGAGGGUU UUAGAGCUAGAAAUAGCAAGUUAA AAUAAGGCUAGUCCGUUAUCAAC UUGAAAAAGUGGCACCGAGUCGG UGCUUUU	*NCAM1* exon 7 splice donor (ABE)
Sp_sgRNA_SGCAe x2mut#1	AUGUCAGUGAGCGGCCUGACGUU UUAGAGCUAGAAAUAGCAAGUUAA AAUAAGGCUAGUCCGUUAUCAAC UUGAAAAAGUGGCACCGAGUCGG UGCUUUU	*SGCA* c.157G>A (ABE)

Table S3. Synthetic oligonucleotides used for PCR, sequencing and cloning

Oligo name	Sequence 5' > 3'	Purpose
HE_255	TCTTTGTGCACACCTTGGAC	*SGCA* c.157G>A ABE, amplicon & Sanger-sequencing
HE_256	GAGGACTCAGATACCAAATTAGAGG	
CS_149	CTGAGGAGTCTTTCCCATTG	*NCAM1* exon 7 splice donor site ABE, amplicon sequencing
CS_152	ACTAGGGCTTGGACTAGGTG	
CS_94	TGT GGA CGT TCA ACT TGG TG	*NCAM1* exon 7 splice donor site ABE, Sanger-sequencing
CS_95	AGG AGC TAG TTC ATC TCT GG	
SDF_29	CATTCCAGCAGCCATACTCAC	*NCAM1* exon 3 SpCas9, Sanger-sequencing
SDF_30	CGTAATAGCCCTCTGGGAAC	
CS_15	TTTTCCTACAGATCCTTAATTAAGCCG CCACCATGAGCGAGGTGGAATTCAG CCACGAGTACTGGATGCGGCACGCC CTGACACTGGCCAAAAGAGCTTGGGA CGAGAGGGAAGTGCCTGTGGGAGCT GTGCTGGTGCACAACAACAGAGTGAT CGGCGAAGGCTGGAACAGACCCATC GGCAGACACGATCCTACAGCTCACG CCGAGATCATGGCCCTGAGACAAGG CGGACTGGTCATGCAGAACTACCGG CTGATCGACGCCACACTGTACGTGAC CCTGGAACCTTGCGTGATGTGTGCCG GCGCTATGATCCACAGCAGAATCGGC AGAGTGGTGTTCGGCGCCAGAGATG CCAAAACAGGCGCTGCCGGAAGCCT GATGGATGTGCTGCATCACCCCGGC ATGAACCACAGAGTGGAAATCACCGA GGGCATCCTGGCCGATGAATGTGCC GCTCTGCTGAGCGACTTCTTCCGGAT GCGGCGGCAAGAGATCAAGGCCCAG AAGAAGGCCCAGTCCAGCACAGATA GCGGCGGATCTAGCGGAGGCAGCTC TGGATCTGAGACACCTGGCACAAGC GAGAGCGCCACACCTGAAAGTTCTG GCGGTTCTTCTGGCGGCAGCAGCGA GGTCGAGTTCTCTCACGAATATTGGA TGAGACACGCTCTCACCCTGGCTAAG AGAGCCAGGGACGAAAGAGAGGTGC	Gibson cloning of HE_ABE7.10co_4.1

	CAGTTGGCGCTGTCCTGGTGTTGAAC AATCGCGTCATCGGAGAAGGATGGAA TCGCGCCATTGGCCTGCACGATCCAA CCGCACATGCCGAAATTATGGCTCTG CGGCAAGGCGGCCTCGTGATGCAAA ATTACAGACTGATCGATGCTACCCTC TACGTCACCTTCGAGCCCTGTGTCAT GTGTGCTGGGGCAATGATTCACTCCC GGATTGGCCGCGTGGTGTTTGGAGT GCGGAATGCCAAGACTGGCGCCGCT GGATCTCTGATGGACGTCCTGCACTA TCCTGGGATGAACCACCGGGTCGAG ATCACAGAGGGAATTCTGGCTGACGA GTGCGCTGCCCTGCTGTGCTACTTCT TTAGAATGCCCAGACAGGTGTTCAAC GCCCAGAAAAAAGCTCAGAGCAGCA CCGATTCCGGCGGAAGCAGCGGAGG ATCTTCTGGAAGCGAAACCCCAGGCA CCAGCGAGTCTGCCACACCAGAATCA TCTGGCGGTAGCTCTGGCGGATCTG ATAAAAAGTATTCTATTGGTTTAGCCA TCGGCACTAATTCCGTTGGATGGGCT GTCATAACCGATGAATACAAAGTACC TTCAAAGAAATTTAAGGTGTTGGGGA ACACAGACCGTCATTCGATTAAAAAG AATCTTATCGGTGCCCTCCTATTCGAT AGTGGCGAAACGGCAGAGGCGACTC GCCTGAAACGAACCGCTCGGAGAAG GTATACACGTCGCAAGAACCGAATAT GTTACTTACAAGAGATCTTCAGCAAC GAGATGGCCAA	
CS_58	CCCTACCAAAGACTTTGAGGgtttt	sgRNA cloning into HE_ABE7.10co_4.1
CS_59	CCTCAAAGTCTTTGGTAGGGggtgt	
CS_184	AGAAGTCCTCCAGGTGATGG	RT-PCR *NCAM1 exon 7* splice donor site
CS_186	ATCGCTGTGAGGGCAGAATC	

Table S4. Primary antibodies

Antibody	Manufacturer (catalogue number)	Working dilution
PAX7	Santa Cruz (sc-81648)	1:200
KI-67	Thermo Fisher Scientific (MA5-14520)	1:300
MYF5	Santa Cruz (sc-302)	1:2000
MYOD 5.8A	Santa Cruz (sc 32758)	1:50
Desmin	Dako (M0760)	1:100
Desmin	Abcam (ab15200)	1:2000
NCAM1 (CD56)	Miltenyi Biotec (130-090-955)	1:200
Skeletal Myosin (fast)	Sigma-Aldrich (M4276)	1:500

SUPPLEMENTAL FIGURES AND LEGENDS

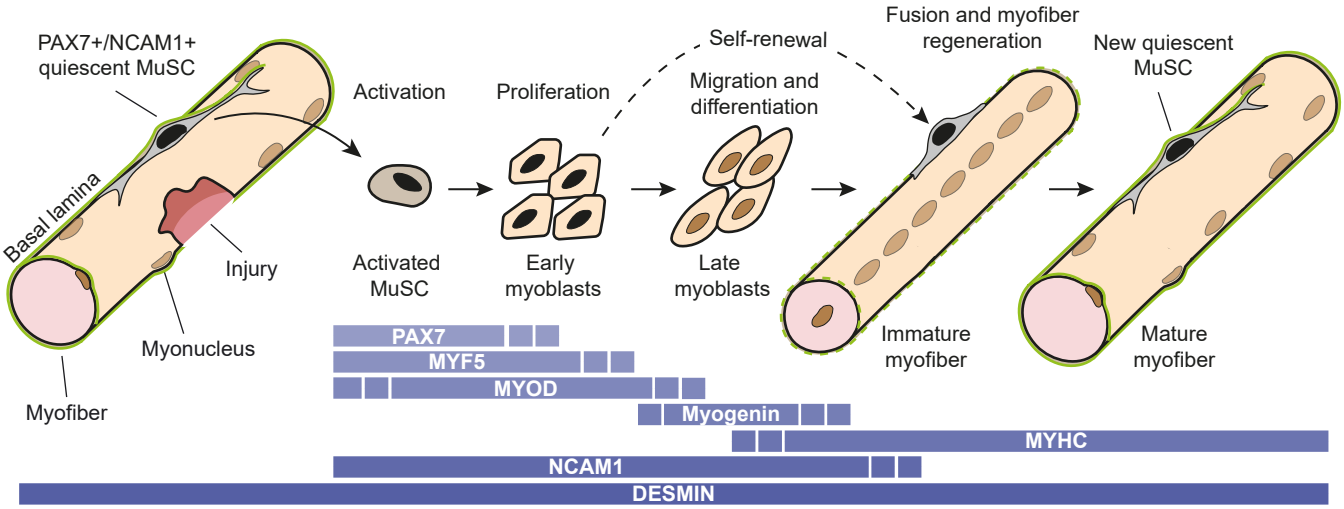

Figure S1. The role of MuSC in muscle regeneration. Adult skeletal muscle owns a population of quiescent MuSC responsible for muscle regeneration. Upon muscle damage or during homeostasis, MuSC activate and divide, giving rise to myogenic progenitor cells (early myoblasts). These cells can either re-enter quiescence to maintain the MuSC pool, or commit to differentiation, exit the cell cycle (late myoblasts) and fuse into post-mitotic multinucleated cells called myotubes or early myofibers. This process is regulated, amongst others, by the tightly timed expression of key myogenic regulatory transcription factors (i.e. PAX7, MYF5, MYOD, Myogenin) that control the downstream expression of muscle-related genes (i.e. the intermediate filament protein Desmin or the sarcomeric protein Myosin heavy chain, MYHC). Finally, myotubes mature into myofibers with peripherally located nuclei and a highly organized contractile apparatus, namely the functional units of skeletal muscle.

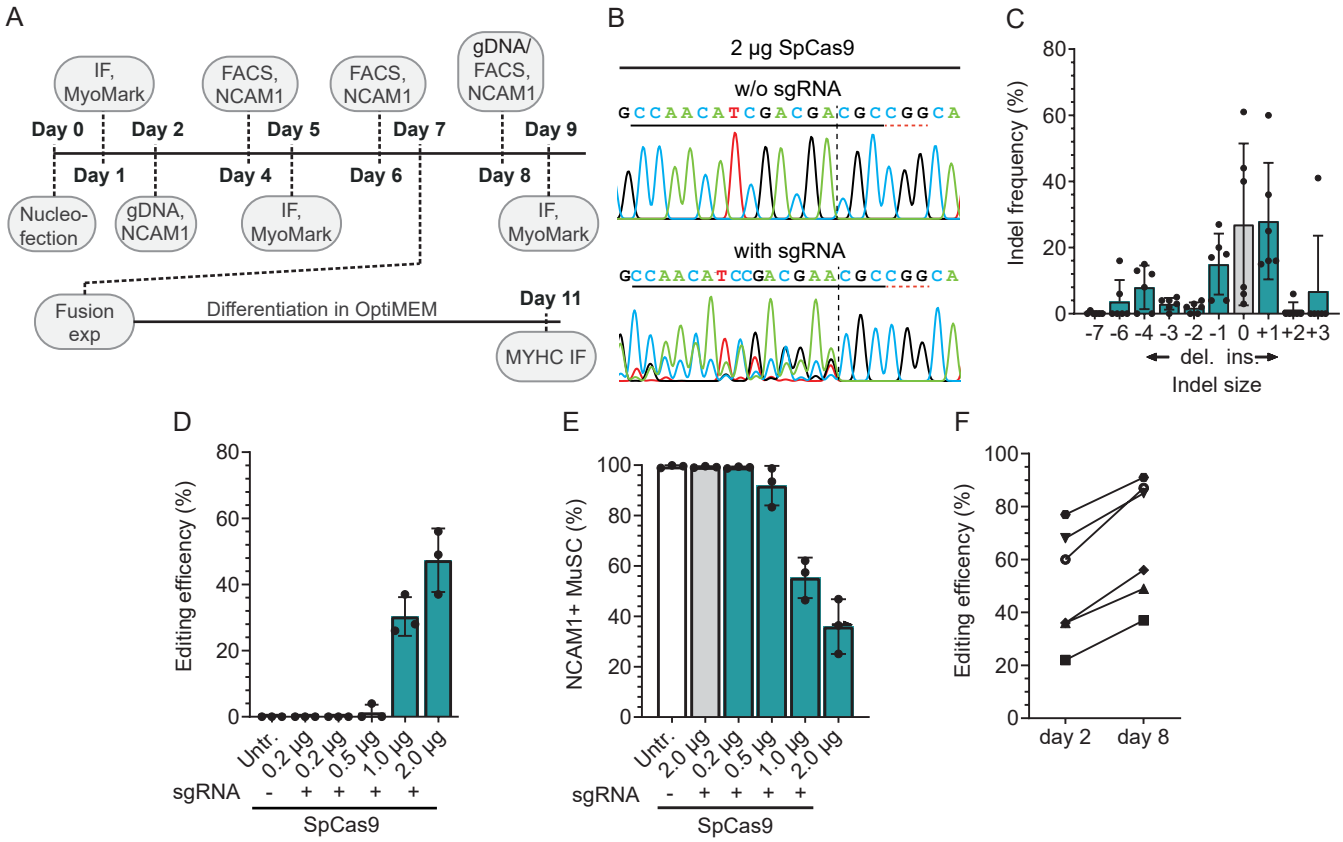

Figure S2. SpCas9 mRNA transfection of primary MuSC induces a knock-out of the *NCAM1* gene. A) Schematics of experimental plan. **B)** Representative Sanger-sequencing chromatograms of the region surrounding the sgRNA sequence obtained from MuSC from donor #I. The protospacer and PAM sequences are underlined. The discontinuous vertical line indicates the expected DSB site, 3 bp distal to the PAM. **C)** Indel plot from ICE analysis showing the predicted indel frequencies at day 8 post transfection (*n* = 6; mean ± SD). **D)** Editing efficiency with increasing amounts of SpCas9 mRNA (and sgRNA), day 8 post transfection (*n* = 3; mean ± SD). **E)** Percentage of NCAM1 positive cells with increasing amounts of SpCas9 mRNA (and sgRNA) (*n* = 3, mean ± SD). **F)** Editing efficiency variations between day 2 and day 8 post transfection for each individual MuSC population (*n* = 6).

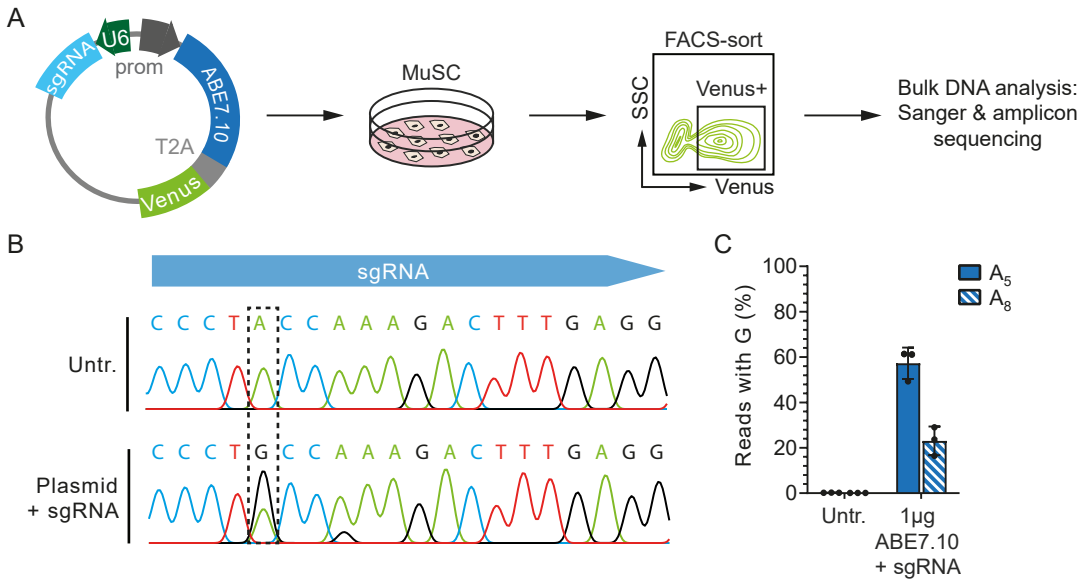

Figure S3. Plasmid-based adenine base editing at the *NCAM1* locus targeting a splice donor site in human MuSC. A) Schematic overview of experimental workflow. MuSC were transfected with a plasmid encoding ABE7.10 and a Venus fluorescence reporter, FACS-sorted and processed for DNA analysis via Sanger and amplicon sequencing. **B)** Sanger-sequencing chromatograms of edited samples (bottom) compared to the non-edited sequence (top). **C)** Percentage of NGS reads with A>G conversion at the target site (*n* = 3, mean ± SD).

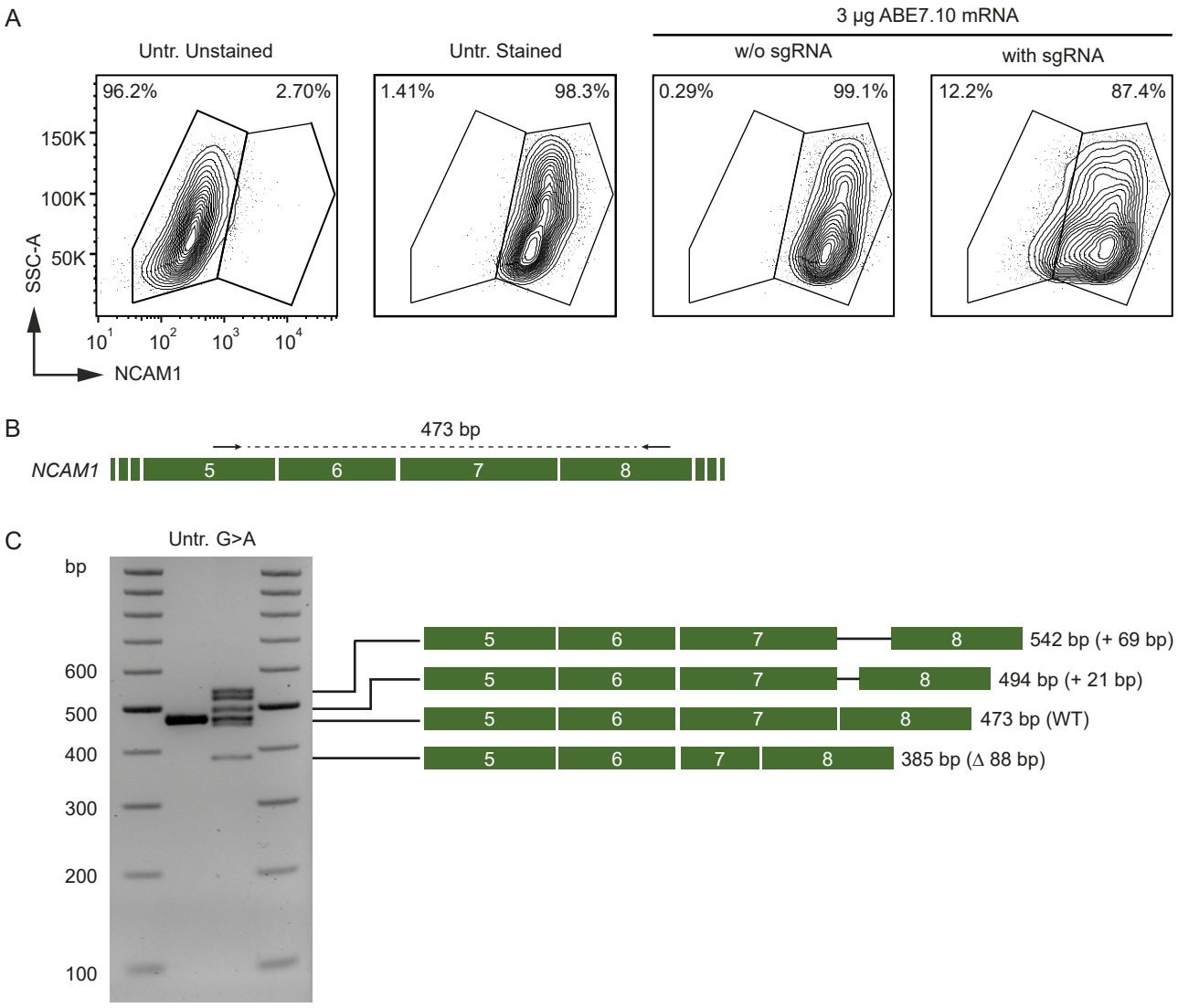

Figure S4. Analysis of NCAM1 protein and mRNA in MuSC after editing the *NCAM1* exon 7 splice donor site. A) Representative NCAM1 staining of ABE7.10-edited MuSC and controls. Flow cytometry performed at day 9 after transfection. **B)** Primer binding sites on the *NCAM1* coding sequence with the expected PCR-band size for unedited MuSC. Exons are represented as boxes. **C)** RT-PCR analysis of *NCAM1* mRNA for ABE7.10 mRNA-edited MuSC (G>A) and unedited MuSC. The splice isoforms whose identity was confirmed by Sanger sequencing are indicated on the right. Exons are represented as boxes. Interjacent intronic sequences are represented as lines. Untr.: Untransfected. WT: wild-type.

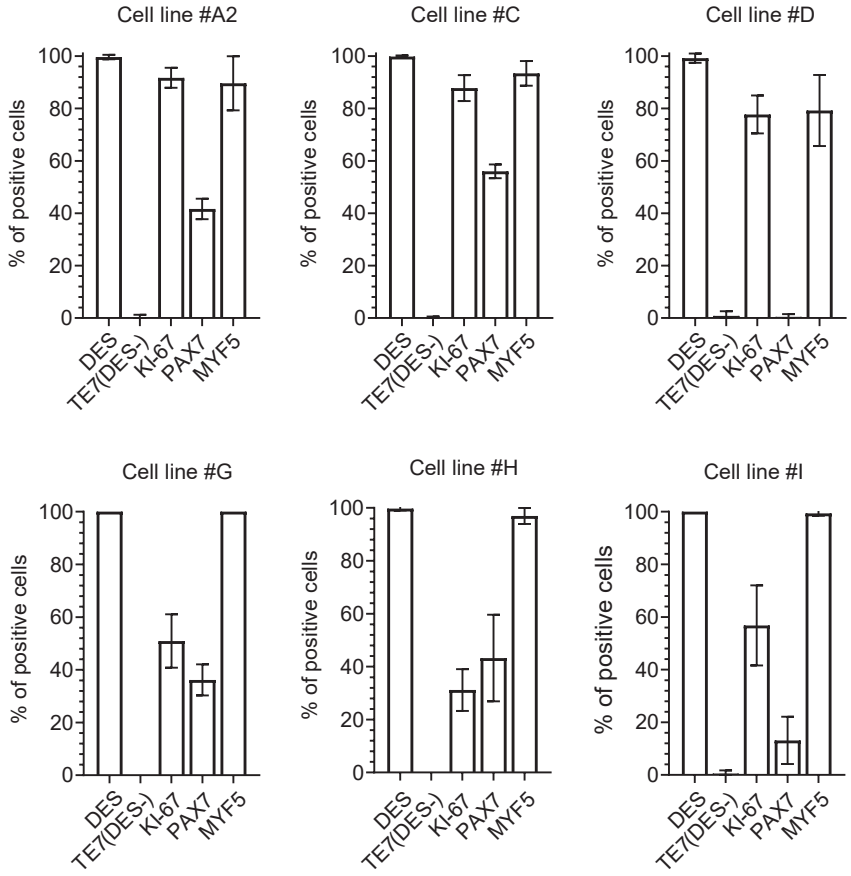

Figure S5. Characterization of primary human MuSC used for SpCas9 mRNA-based editing. Quantification of myogenic and proliferation markers in cells from all donors used for the SpCas9 mRNA-experiment before nucleofection (*n* = 6; mean ± SD of ≥ 5 images).

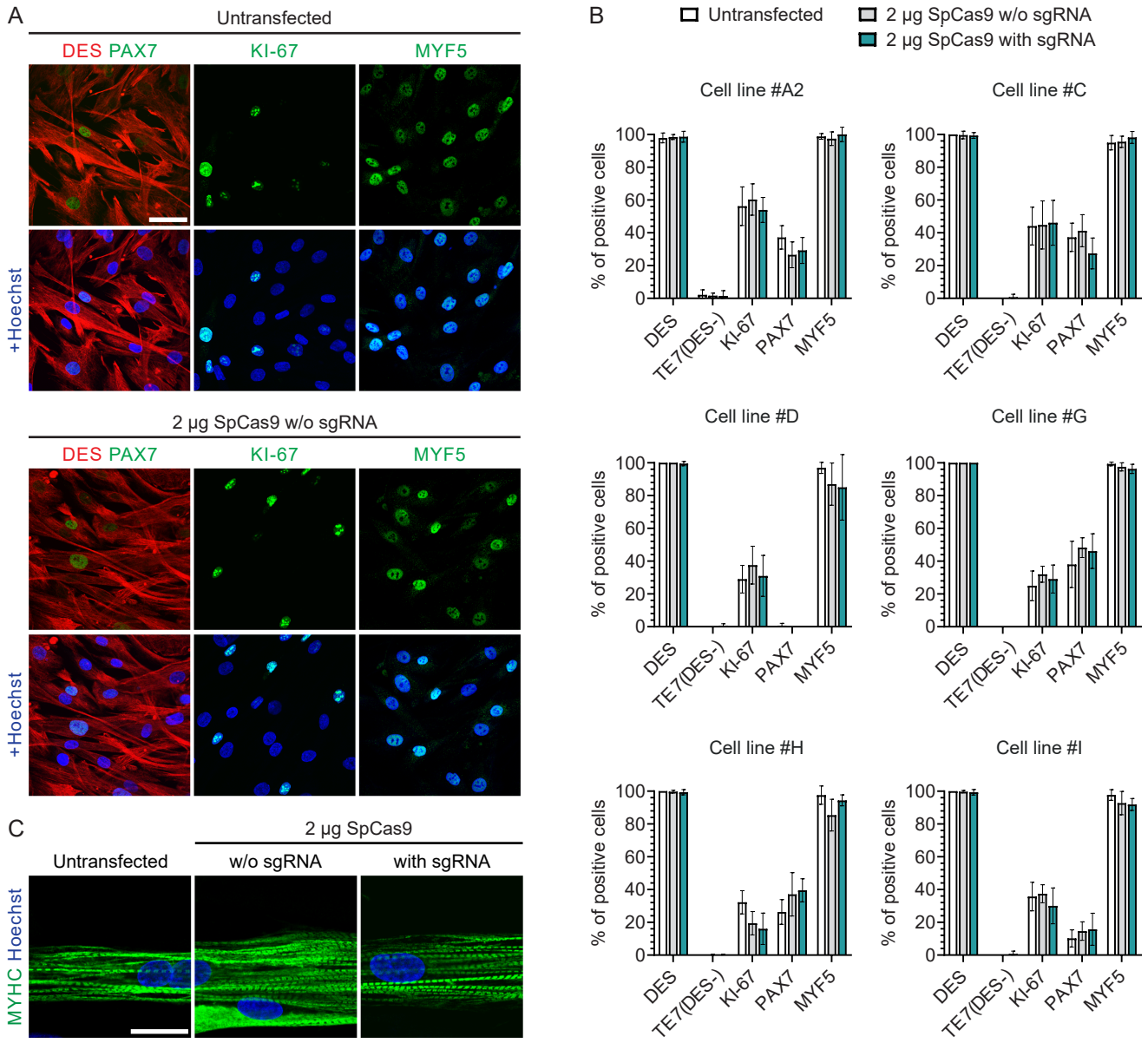

Figure S6. SpCas9 mRNA transfection and *NCAM1* editing do not influence myogenic marker expression and differentiation of human MuSC. A) Representative immunofluorescence staining for DES, PAX7, KI-67, and MYF5 of MuSC transfected with 2 µg SpCas9 mRNA w/o sgRNA, and untransfected control (day 5 post transfection). Scale bar: 50 µm. **B)** Quantification of myogenic and proliferation markers in SpCas9 mRNA-edited cells and controls (day 5 post transfection; $n = 6$; mean ± SD of ≥ 5 images; P value calculated with multiple unpaired t-tests; no significant changes). **C)** Zoom of representative confocal microscopy images of MuSC differentiated into myotubes after *NCAM1* knock-out using SpCas9 mRNA, and immunostained for MYHC. Scale bar: 50 µm. Nuclei were counterstained with Hoechst (blue).

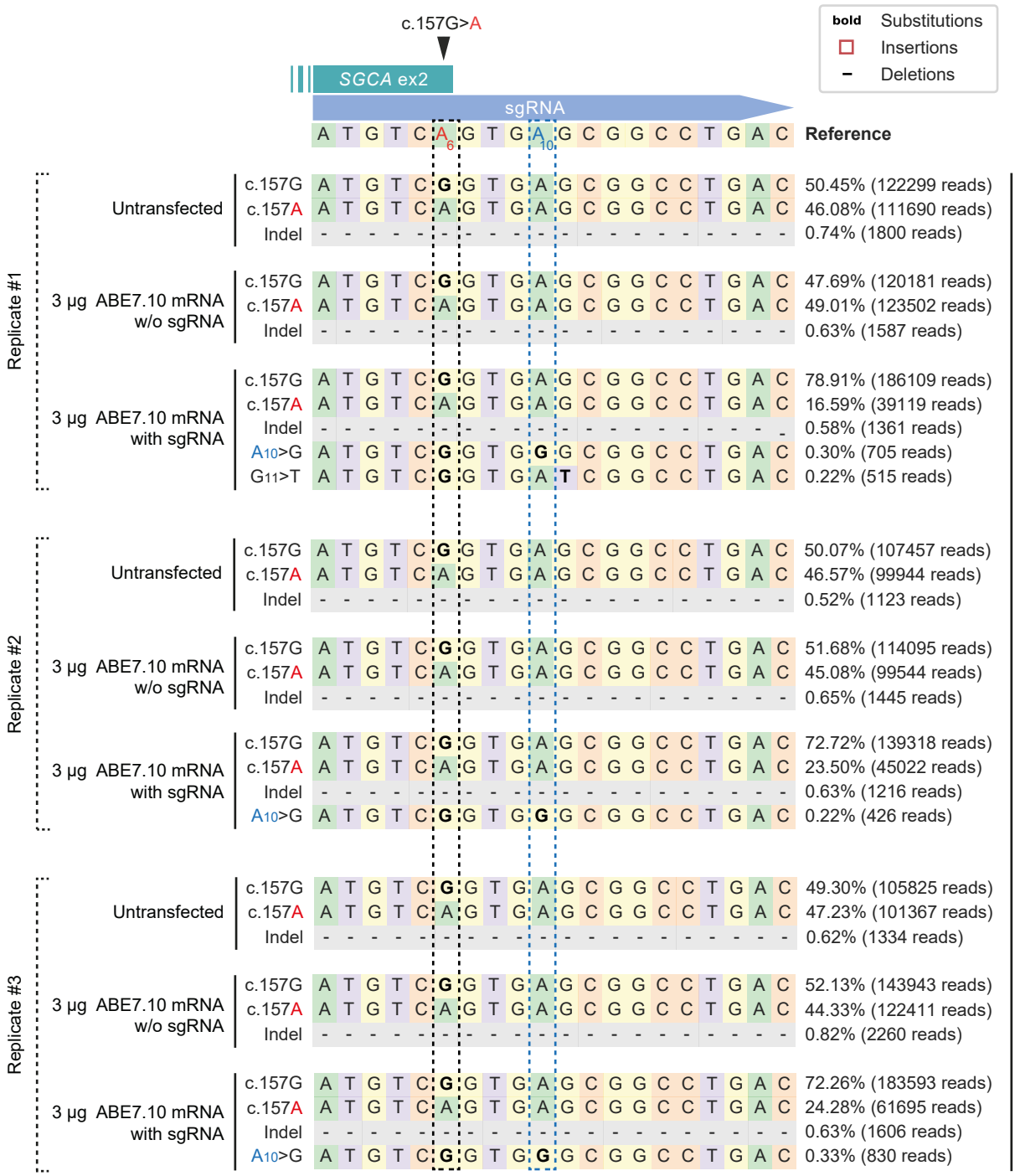

Figure S7. Allele frequencies at the target site in *SGCA* c.157G>A carrier MuSC. Analysis of amplicon sequencing data using CRISPResso2 shows allele frequencies within the sgRNA target site for untransfected and ABE7.10 mRNA +/- sgRNA transfected samples (n = 3 repetitions). The *SGCA* c.157 target site is highlighted with a black dotted rectangle and an arrow. The bystander edit at protospacer position 10 is indicated with a blue dotted rectangle. The percentages of reads assigned to each allele type are shown on the right alongside the total number of aligned reads (in brackets).