

Supplementary Material

Table S1. Primers used in the study.

Primer Name	Primer Sequence (5'-3')	Used for the experiment of
DMD exon 49-F	GTGCCCTTATGTACCAGGCA	PCR for the exon 49 of DMD (381 bp)
DMD exon 49-R	AAGACAGCTTTGCCTCTGCT	
DMD exon 50-F	AATTATGCCTGGAGAAAGGG	PCR for the exon 50 of DMD (588 bp)
DMD exon 50-R	CTTGTGCTCTTCTCTTCCTT	
DMD exon 51-F	ATTGGCTCTTTAGCTTGTGT	PCR for the exon 51 of DMD (437 bp)
DMD exon 51-R	TGTGTTTGCTGAGAGAGAAA	
LHA-F	CAAAGACATACAGTCATTCCG	PCR for the site-specific clones screening of LHA ~1.5 kb
LHA-R	GGCTCCAATAGTGGTCAGT	
neo-SHA-F	GGGCTTCGGAATCGTTT	PCR for the site-specific clones screening of SHA ~1.1 kb
neo-SHA-R	AGAATCAGGTAACCTACTGCTAAC	
RT-PCR-49-F	AGCAGTTCAAGCTAAACAACCGG	RT-PCR and Sanger sequencing in iPSCs stage (681 bp / 572 bp)
RT-PCR-54-R	CCTAAGACCTGCTCAGCTTCTTC	
RT-PCR-50-F	AAAAGACCTTGGGCAGCTTG	RT-PCR and Sanger sequencing in iCMs stage (390 bp)
RT-PCR-50-R	ACCACAGGTTGTGTCACCAG	

Table S2. Sequences of donor template fragment and primers for construction.

Fragment Name	Primer Name	Primer Sequence (5'-3')	Fragment Sequence (5'-3')
LHA 708 bp	DONOR- L-F	ATGCTTCAGCCTTCTC CAG	ATGCTTCAGCCTTCTCCAGACTGAAGAAAAGCAAAT AATAAACAAAATTAATCGTTCTTCAGAAGGCCTGTT TGACTCATTAATAAAATAATAAGCTTTGATACTGTGC TTTAAGTGTTTACCCTTTGGAAAAGAAAATAATTTTGA CAGTGATGTAGAAATAATTATTTGATATTTATTTCAA AACAAAATTTATATCCAATACTAAACACAGAATTTT GTAAAACAATAAGTGATAAAAGTAAATGAACATT AGGATTATTGAGATTATTGTAGCTAAAACTAGTGTT TATTCATATAAAATTATGTTAATAAATTGTATTGTCAT TATTGCATTTTACTTTTTTGAAGTAGTTAATGCCT GTGTTTCTATATGAGTATTATATAATTCAAGAAGAT ATTGGATGAATTTTTTTTAAGTTTAATGTGTTTCACAT CTCTGTTTCTTTTCTCTGCACCAAAAGCTACATTTTGT TGCCCTTATGTACCAGGCAGAAATTGATCTGCAATA CATGTGGAGTCTCCAAGGGTATATTTAAATTTAGTA ATTTTATTGCTAACTGTGAAGTTAATCTGCACTATAT GGGTTCTTTTCCCCAGGAACTGAAATAGCAGTTCA AGCTAAACAACCGGATGTGGAAGAGATTTTGTCTA AAGGGCAGCATTTGTACAAGGAAAAACCAGCCACT CAGCCAGTGAAAGcaattg (Add MfeI restriction site)
	DONOR- L-R	caattgCTTCACTGGCTG AGTGGCT	
E50-SD 166 bp	DONOR- E50-F	cagccactcagccagtgaagAG GAAGTTAGAAGATCT GAGCT	AGGAAGTTAGAAGATCTGAGCTCTGAGTGGAAGGC GGTAAACCGTTTACTTCAAGAGCTGAGGGCAAAGC AGCCTGACCTAGCTCCTGGACTGACCACTATTGGAG
	DONOR- E50-R	ATTACgctagcgctagcACT TTTGAACAAATAGCT AGAGCCAAAG	CCTGTAAGTATACTGGATCCCATTCTCTTTGGCTCTA GCTATTTGTTCAAAGTgctagc (Add nheI restriction site)
SHA 652 bp	DONOR- S-F	AGTgctagcgctagcGTAA TGAAGCAACCTCT	(Add nheI restriction site) gctagcGTAATGAAGCAACCTCTAGCAATATCCATTAC CTCATAATGGGTTATGCTTCCCCTGTTGTACATTTGC CATTGACGTGGACTATTTATAATCAGTGAAATAACT TGTAAGGAAATACTGGCCATACTGTAATAGCAGAG GCAAAGCTGTCTTTTGTATCAGCATATCCTATTTATA TATTGTGATCTTAAGGCTATTAACGAGTCATTGCTTT AAAGGACTCATTCTGTCTGGTGTGCTGCCATCAA TACAAAAGTAGTCCCACCTTCAAGGTAGATTAAATT CTTTGGGGCTTTATTGCTTTGCTTGCCAGCCTTGATG CTTTTCATATTGTTTGGTTTAATTCAAATCAAGCTAC TGCATCATAGTGTCTGTCTCCAACAGCTGTAAAGAA TCACAATATGGTCCGTGACCTTTCTTTCTTTGTTGCT CTTTCTTATAAAAGAGTAAATCGGCATTGAGGCTT CAGTATTTTATTGGGTCAAGAATTTTATAGTGAAATGT CATGTAATTGCTTATTTCTTTGGCAAAAAGCTGGCCT GGGATAAACCTTTCTACTTTTTTTCTTCTCCATATCC ATGTTGAAGTCTAGGTCACATTTATTCACAGGCTATT ACATAAGGAGGGAGATGGAAAGCCAGG
	DONOR- S-R	gccgcgaattcactagtgatCCT GGCTTTCCATCTCC	

NEO
1949 bp

neo-F

CTAGCTATTTGTTCAA
AGTgctagcATAACTTCG
TATAGCATA

neo-R

GCTAGAGGTTGCTTCA
TTACgctagcTAACTTCG
TATAATGTATG

ATAACTTCGTATAGCATACATTATACGAAGTTATAC
ATGTCACAAAAGGAACTCACCTAACTGTAAAGT
AATTGTGTGTTTTGAGACTATAAATATCCCTTGGAG
AAAAGCCTTGTTAACGCGCGGTGACCCTCGAGTACT
AGGATCCATTAGGGAATTCGTCGACCTCGAAATTCT
ACCGGGTAGGGGAGGCGCTTTTCCCAAGGCAGTCTG
GAGCATGCGCTTTAGCAGCCCCGCTGGGCACTTGGC
GCTACACAAGTGGCCTCTGGCCTCGCACACATTCCA
CATCCACCGGTAGGCGCCAACCGGCTCCGTTCTTTG
GTGGCCCCCTTCGCGCCACCTTCTACTCCTCCCCTAGT
CAGGAAGTTCCCCCCCCGCCCGCAGCTCGCGTCGTG
CAGGACGTGACAAATGGAAGTAGCACGTCTCACTA
GTCTCGTGCAGATGGACAGCACCGCTGAGCAATGG
AAGCGGGTAGGCCTTTGGGGCAGCGGCCAATAGCA
GCTTTGCTCCTTCGCTTTCTGGGCTCAGAGGCTGGGA
AGGGGTGGGTCCGGGGGCGGGCTCAGGGGCGGGCT
CAGGGGCGGGGCGGGCGCCCGAAGGTCCTCCGGAG
GCCCCGCATTCTGCACGCTTCAAAGCGCACGTCTG
CCGCGCTGTTCTCCTCTTCCTCATCTCCGGGCCTTTC
GACCTGCATCCtctagaGCCACCATGATTGAACAAGAT
GGATTGCACGCAGGTTCTCCGGCCGCTTGGGTGGAG
AGGCTATTCGGCTATGACTGGGCACAACAGACAAT
CGGCTGCTCTGATGCCGCCGTGTTCCGGCTGTCAGC
GCAGGGGCGCCCCGGTTCTTTTTGTCAAGACCGACCT
GTCCGGTGCCCTGAATGAACTGCAGGACGAGGCAG
CGCGGCTATCGTGGCTGGCCACGACGGGCGTTCTT
GCGCAGCTGTGCTCGACGTTGTCACTGAAGCGGGAA
GGGACTGGCTGCTATTGGGCGAAGTGCCGGGGCAG
GATCTCCTGTCATCTCACCTTGCTCCTGCCGAGAAA
GTATCCATCATGGCTGATGCAATGCCGGCGGCTGCAT
ACGCTTGATCCGGCTACCTGCCCATTTCGACCACCAA
GCGAAACATCGCATCGAGCGAGCACGTACTCGGAT
GGAAGCCGGTCTTGTCGATCAGGATGATCTGGACGA
AGAGCATCAGGGGCTCGCGCCAGCCGAACTGTTCTG
CCAGGCTCAAGGCGCGCATGCCCGACGGCGAGGAT
CTCGTCGTGACCCATGGCGATGCCTGCTTGCCGAAT
GCTAGAGGTTGCTTCAATCATGGTGAAAAATGGCCGCTTTTCTGGATTTCATC
TTACgctagcTAACTTCGACTGTGGCCGGCTGGGTGTGGCGGACCGCTATCAG
TATAATGTATG GACATAGCGTTGGCTACCCGTGATATTGCTGAAGAG
CTTGGCGGCGAATGGGCTGACCGCTTCCTCGTGCTTT
ACGGTATCGCCGCTCCCGATTTCGAGCGCATCGCCT
TCTATCGCCTTCTTGACGAGTTCTTCTGAGCGGGACT
CTGGGGTTCGAAATGACCGACCAAGCGACGCCCAA
CCTGCCATCACGAGATTTCGATTCCACCGCCGCCTT
CTATGAAAGGTTGGGCTTCGGAATCGTTTTCCGGGA
CGCCGGCTGGATGATCCTCCAGCGCGGGGATCTCAT
GCTGGAGTTCTTCGCCCCACCCCAACTTGTTTATTGCA
GCTTATAATGGTTACAAATAAAGCAATAGCATCACA
AATTTACAAATAAAGCATTTTTTTCACTGCATTCTA
GTTGTGGTTTGTCCAAACTCATCAATGTATCTTATCA
TGTCTGTATACCGTCGACCTCTAGCTAGAGCTTGGC

GTAATCATGGTCATAGCTGTTTCCTGTGTGAAATTGT
TATCCGCTCACAATTCCACACAACATACGAGCCGG
AAGCAATAACTTCGTATAGCATACATTATACGAAGT
TAgctagc (Add nheI restriction site)

Table S3. 15 potential off-target sites predicted by CAS-OFFinder and primers for identification.

	Number of mismatched bases; match values	location on the chromosome		primer(5'-3')
	0; 1	ontarget_mm0_exon_DMD_chrX_3183 6716	F R	GTGCCCTTATGTACCAGGCA AAGACAGCTTTGCCTCTGCT
1	4; 0.64	mm4_intron_GUCY2F_chrX_10937446 3	F R	GCAAGTAACAGCAACAGAGGAGTG CTGACTCACTGACACCTGGGG
2	2; 0.61	mm2_intergenic_RNU6-1078P RGAG 4_chrX_72073232	F R	GGGAAGATGCGATGTGAGATAG TAGGCGAAAGGTGAGAGTTGGT
3	4; 0.56	mm4_intergenic_MIR548AP RP11-553 E24.3_chr15_85947521	F R	GGGATTAGGAGGCATTAGAAGA CAAGGATAGAGAAAAGGGGAAC
4	4; 0.53	mm4_intron_GRK4_chr4_3030352	F R	GTATTTTGAGGTGGGTAAAGGTT GGGACTTCTGTTCTATTGCTGC
5	4; 0.51	mm4_intron_CPLX2_chr5_175803726	F R	AAAGAGAGGAGTGTGCCGAGG TTTATTTCCCAGCAGGTGAGTC
6	3; 0.50	mm3_intergenic_CNTNAP4 AC01052 8.1_chr16_76356648	F R	GAGACTGGGAAAGAGGCA AGCAGGGCTACTTCTCTCAA
7	4; 0.45	mm4_intron_CTB-113P19.3_chr5_1517 62937	F R	GTGAAACCTATTTCAGTTAAAGTCCT GCAGGTTTGTCTGGGTGTC
8	3; 0.43	mm3_intron_SYNDIG1_chr20_2458916 1	F R	TTTTGGAAGAAATGACCCCT TGTGCCCATACTTGCTACCT
9	4; 0.39	mm4_intron_AC004691.5_chr7_308004 12	F R	TTTTTGTGCCCTCTTAGTCAT TCCTGCCCCCTCAATAGC
10	4; 0.39	mm4_intron_TMEM163_chr2_1344977 42	F R	ACTCTGAATCCCAAGTGCCT TCAAGGTTGCCCCAAATAGT
11	4; 0.37	mm4_intron_MPHOSPH8_chr13_1966 3742	F R	GGCTTGTTCTGTGTTCTGG CTGGCTGTGCTCTTTTGCTT
12	4; 0.37	mm4_intergenic_RNU5D-2P C9orf171 _chr9_132423670	F R	TTACAGATGGGGAAGCCGA GGTCCTTGGAGAACAGAAAACA
13	3; 0.36	mm3_intergenic_CCDC185 CAPN8_c hr1_223482694	F R	AGAGACAGAAAGTTGAATGGTGG CAGGCAGTCTAATAGCAGTCCA
14	4; 0.36	mm4_intergenic_MED10 UBE2QL1_c hr5_6404252	F R	GCTGGGATGTGTTTCTGGTG ATCTGGAATGAACAGTCTTGGG
15	4; 0.35	mm4_intergenic_RP11-267C16.1 RP11 -25O3.1_chr18_51961725	F R	GTCCTGCTTTGGCATT CAT AGTAAGATAGGGGTGGGGG

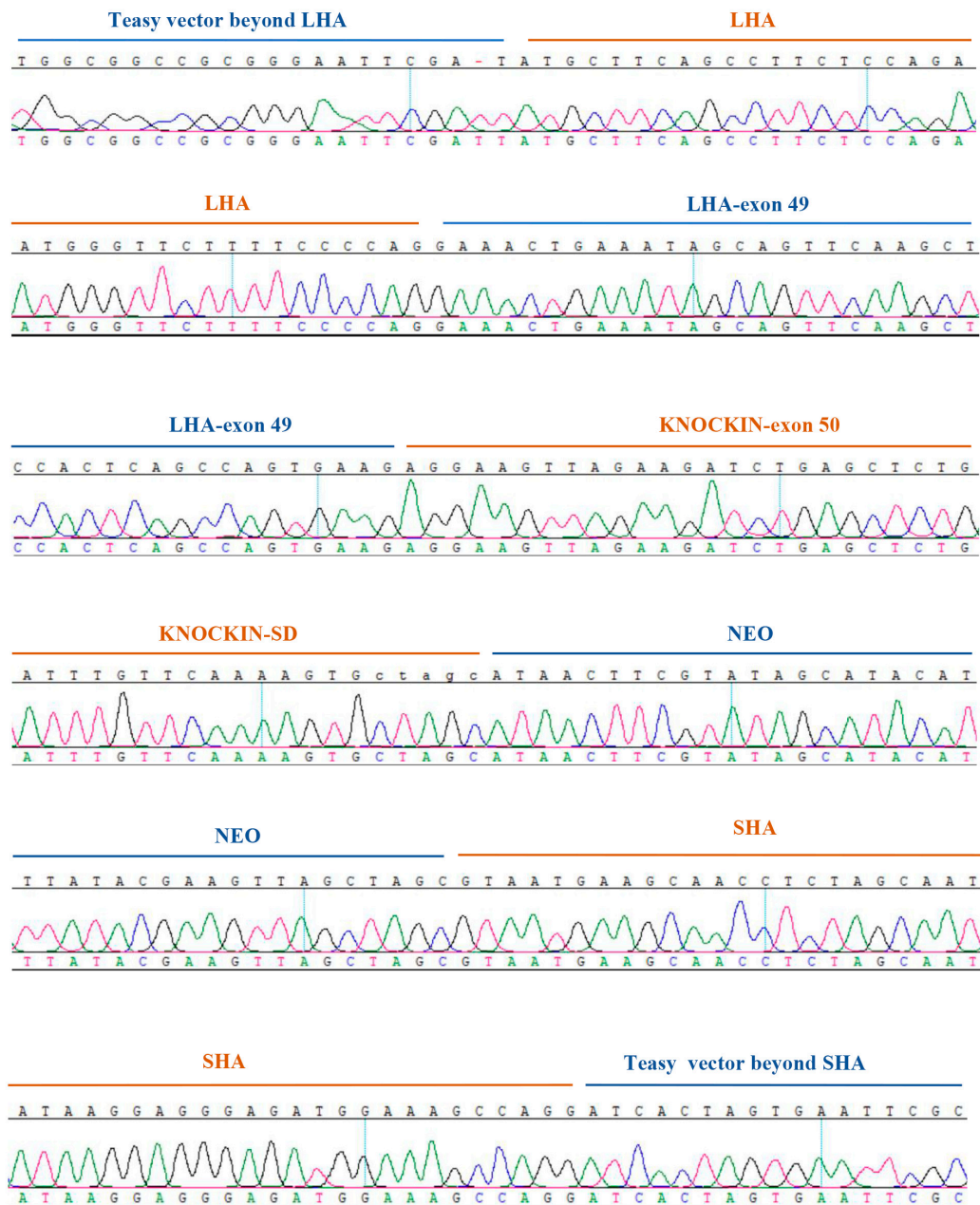


Figure S1. Sanger sequencing of the constructed donor vector.

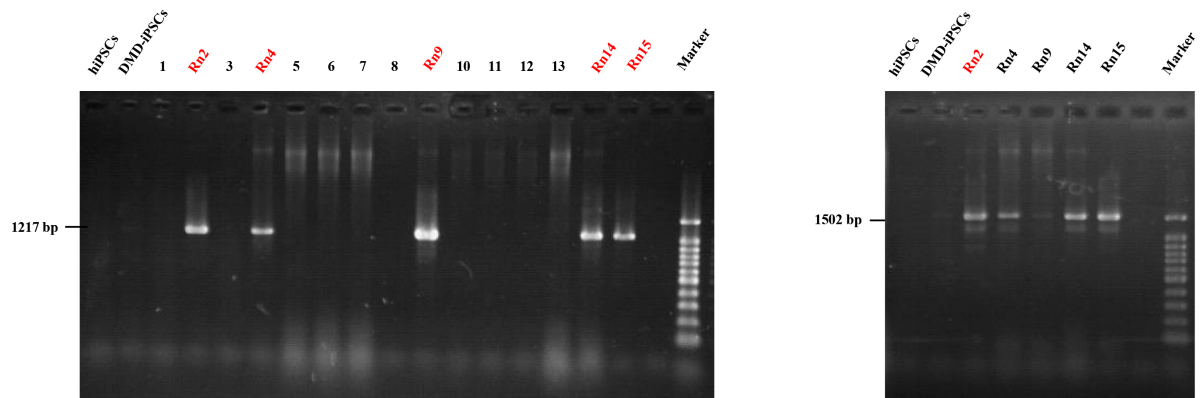


Figure S2. Identification of targeted clones by PCR products using primers across the homology arms, hiPSCs and DMD-iPSCs were used as controls. The results of agarose gel electrophoresis showed that 5 (2, 4, 9, 14, and 15) out of 15 clones existed the predicted band.