Supplementary materials for

Mini-dCas13X-mediated RNA editing restores dystrophin expression in a humanized mouse model of Duchenne muscular dystrophy

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Figure S18. mxABE expression and dystrophin restoration level 6 weeks after systemic administration of AAV9-mxABE.

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Figure S21. Collagen staining shows no obvious fibrosis in hearts of treated or untreated DMD^{E30mut} mice 6 months after systemic administration of AAV-mxABE.

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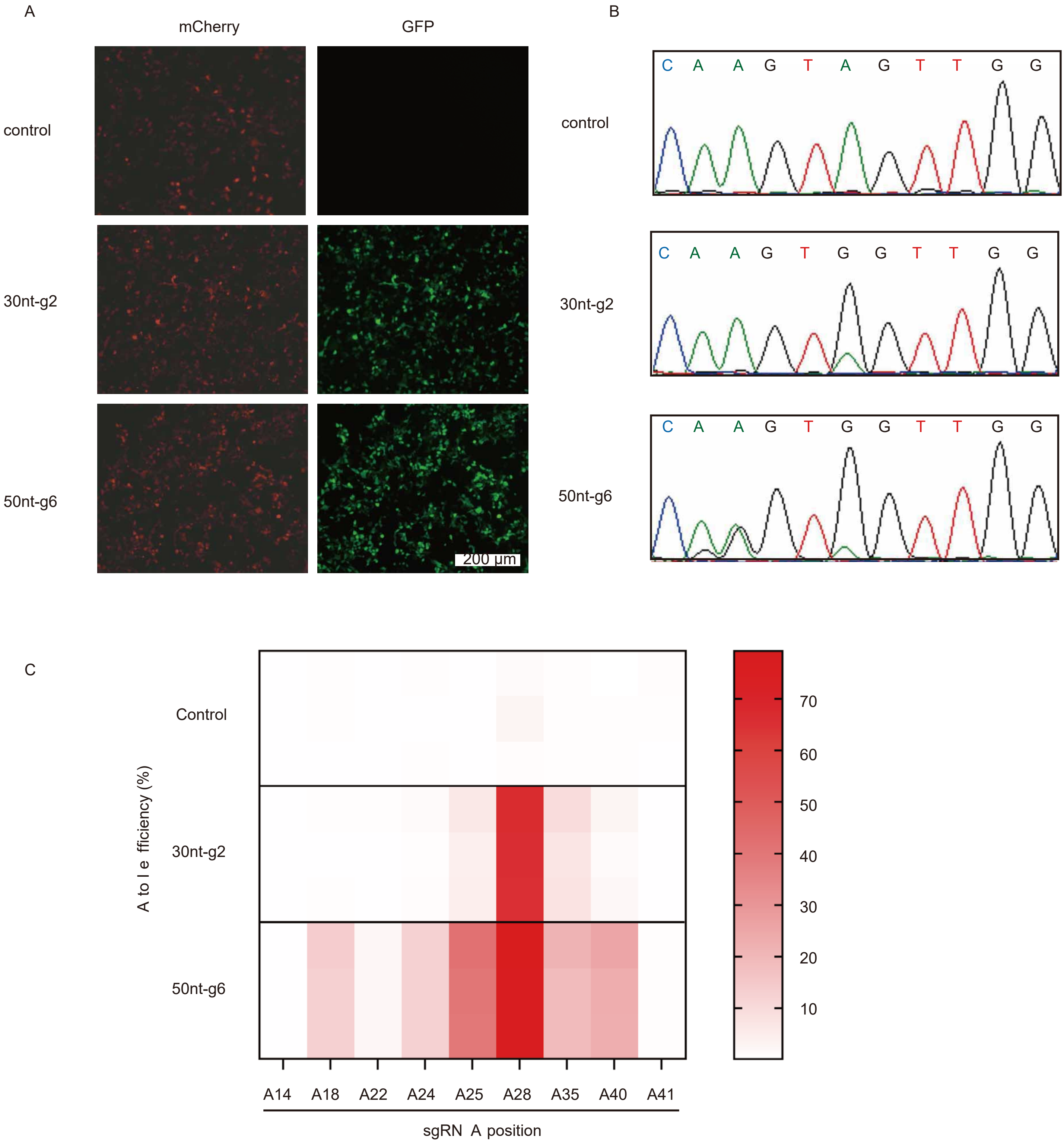
Figure S23. Systemic delivery of AAV-mxABE rescues dystrophin deficiency in adult DMD^{E30mut} mice.

Figure S24. Immunostaining of dystrophin in adult DMD^{E30mut} mice 6 weeks after systemic injection of AAV-mxABE.

Tables S1. Target sgRNA and primer sequences.

Supplemental Sequences

Figure S1

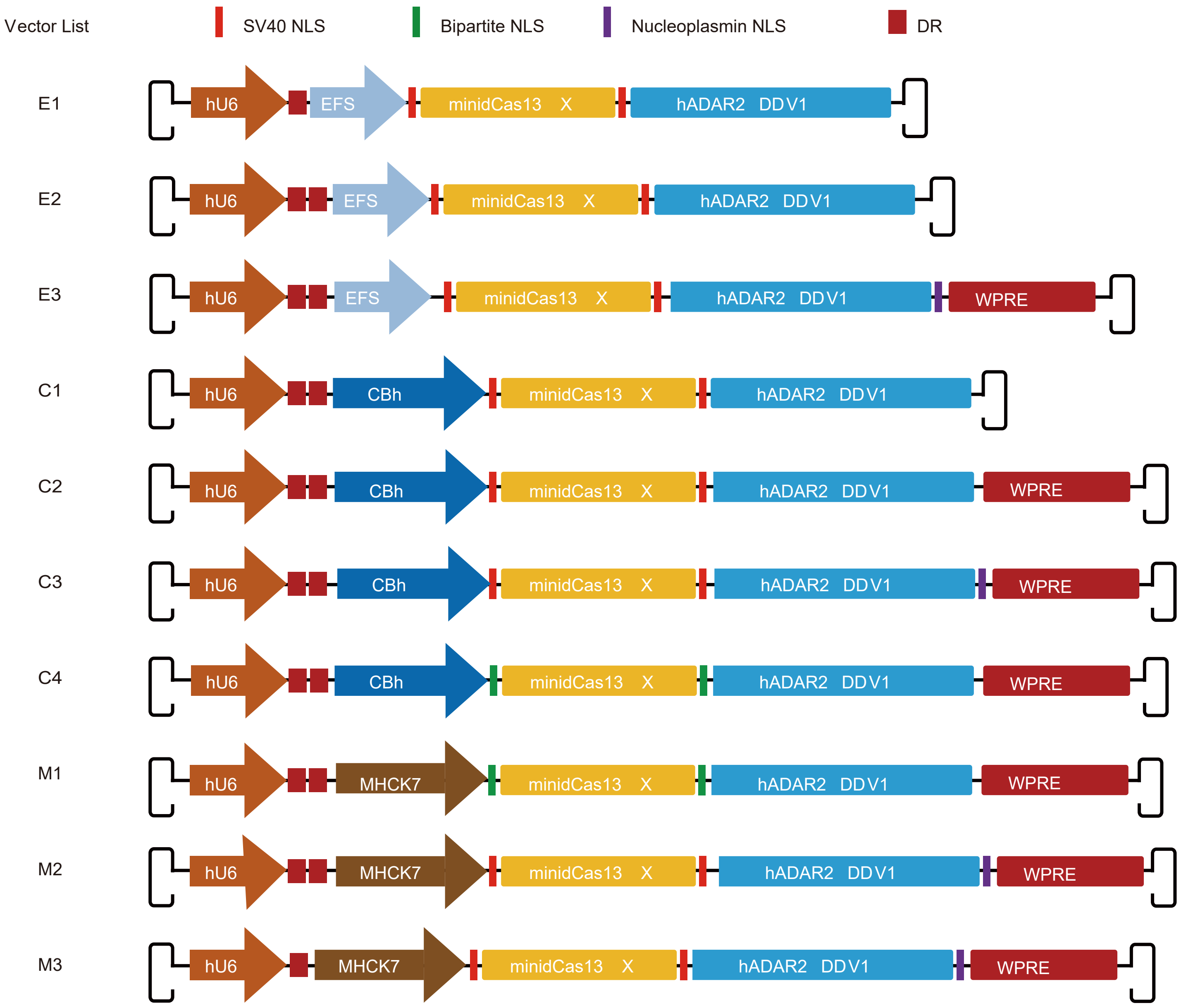

A
mCherry
GFP
control
30nt-g2
50nt-g6
200 μm
B
control
C A A G T A G T T G G
30nt-g2
C A A G T G G T T G G
50nt-g6
C A A G T G G T T G G
C
Control
30nt-g2
50nt-g6
A to I e fficiency (%)
70
60
50
40
30
20
10
A14
A18
A22
A24
A25
A28
A35
A40
A41
sgRN A position

SUPPLEMENTAL FIGURES

Figure S1. *In vitro* studies of c.4174C>T mutation correction using mxABE constructs.

A. Fluorescence microscopy images of HEK293T cells transfected with reporter alone, or reporter and mxABE constructs. Scale bar, 200 µm. B. Representative Sanger sequencing trace of reporter transcripts. C. Measurements of the representative gRNA (30 nt-g2 and 50 nt-g6) effect on bystander A to I editing efficiency (n=3).

Figure S2



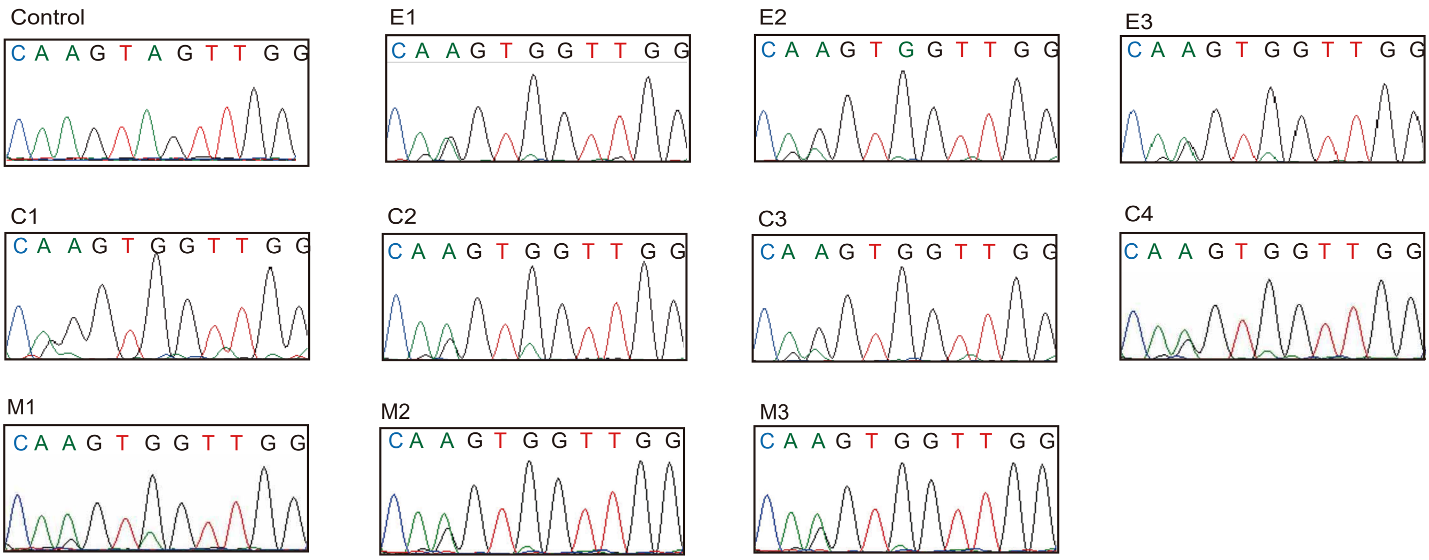
Vector List
SV40 NLS
Bipartite NLS
Nucleoplasmin NLS
DR
E1
hU6
EFS
minidCas13 X
hADAR2 DDV1
E2
hU6
EFS
minidCas13 X
hADAR2 DDV1
E3
hU6
EFS
minidCas13 X
hADAR2 DDV1
WPRE
C1
hU6
CBh
minidCas13 X
hADAR2 DDV1
C2
hU6
CBh
minidCas13 X
hADAR2 DDV1
WPRE
C3
hU6
CBh
minidCas13 X
hADAR2 DDV1
WPRE
C4
hU6
CBh
minidCas13 X
hADAR2 DDV1
WPRE
M1
hU6
MHCK7
minidCas13 X
hADAR2 DDV1
WPRE
M2
hU6
MHCK7
minidCas13 X
hADAR2 DDV1
WPRE
M3
hU6
MHCK7
minidCas13 X
hADAR2 DDV1
WPRE

Figure S2. Construction of 10 mxABE expression cassettes.

The mxABE expression cassettes contained different promoters (EFS, CBh, MHCK7), direct repeat (DR) numbers (single, dual DR), nuclear localization signals (NLS), and were constructed with or without a translational regulatory element (WPRE).

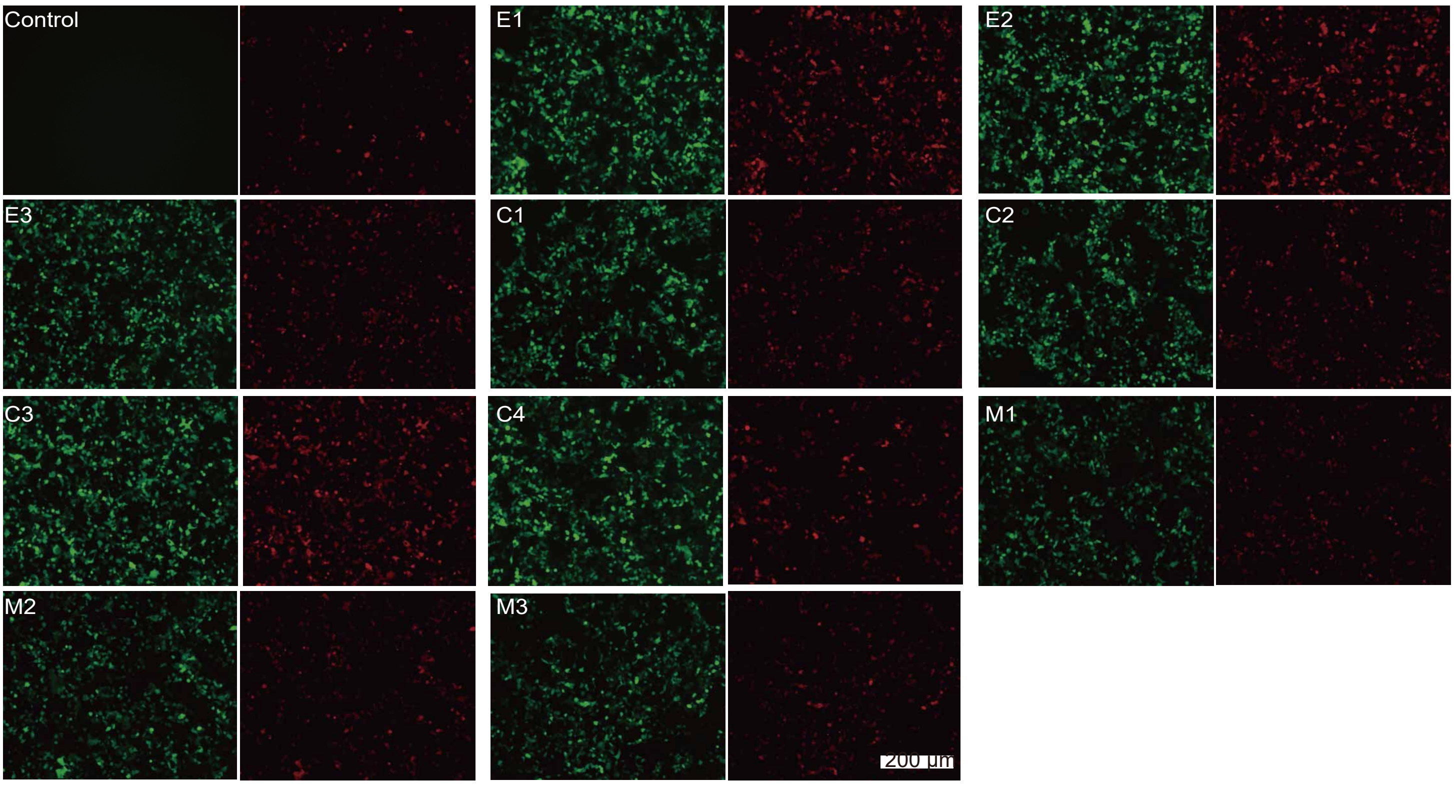
Figure S3

A



Control
C A A G T A G T T G G
E1
C A A G T G G T T G G
E2
C A A G T G G T T G G
E3
C A A G T G G T T G G
C1
C A A G T G G T T G G
C2
C A A G T G G T T G G
C3
C A A G T G G T T G G
C4
C A A G T G G T T G G
M1
C A A G T G G T T G G
M2
C A A G T G G T T G G
M3
C A A G T G G T T G G

B



Control
E1
E2
E3
C1
C2
C3
C4
M1
M2
M3
200 μm

Figure S3. Representative Sanger sequencing trace of reporter transcripts.

A. Sanger sequencing results showing representative A to I conversion on reporter transcripts by different mxABE editors. B. Fluorescence microscopy images of HEK293T cells transfected with reporter alone or with reporter and mxABE construct. Scale bar, 200 µm. Figure S3B and S1A showed similar results but from different experiments investigating the effect of gRNA length and construct expression elements on mutation correction efficiency respectively.

Figure S4

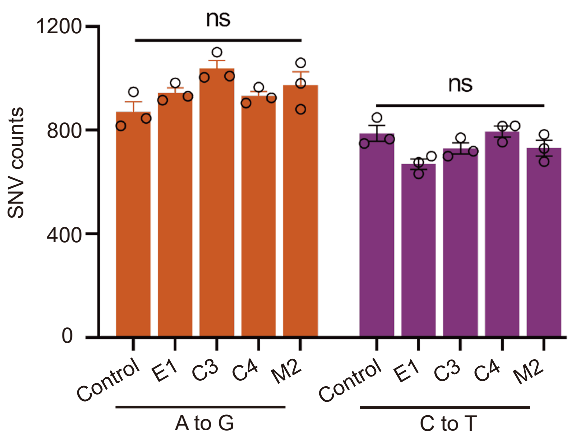
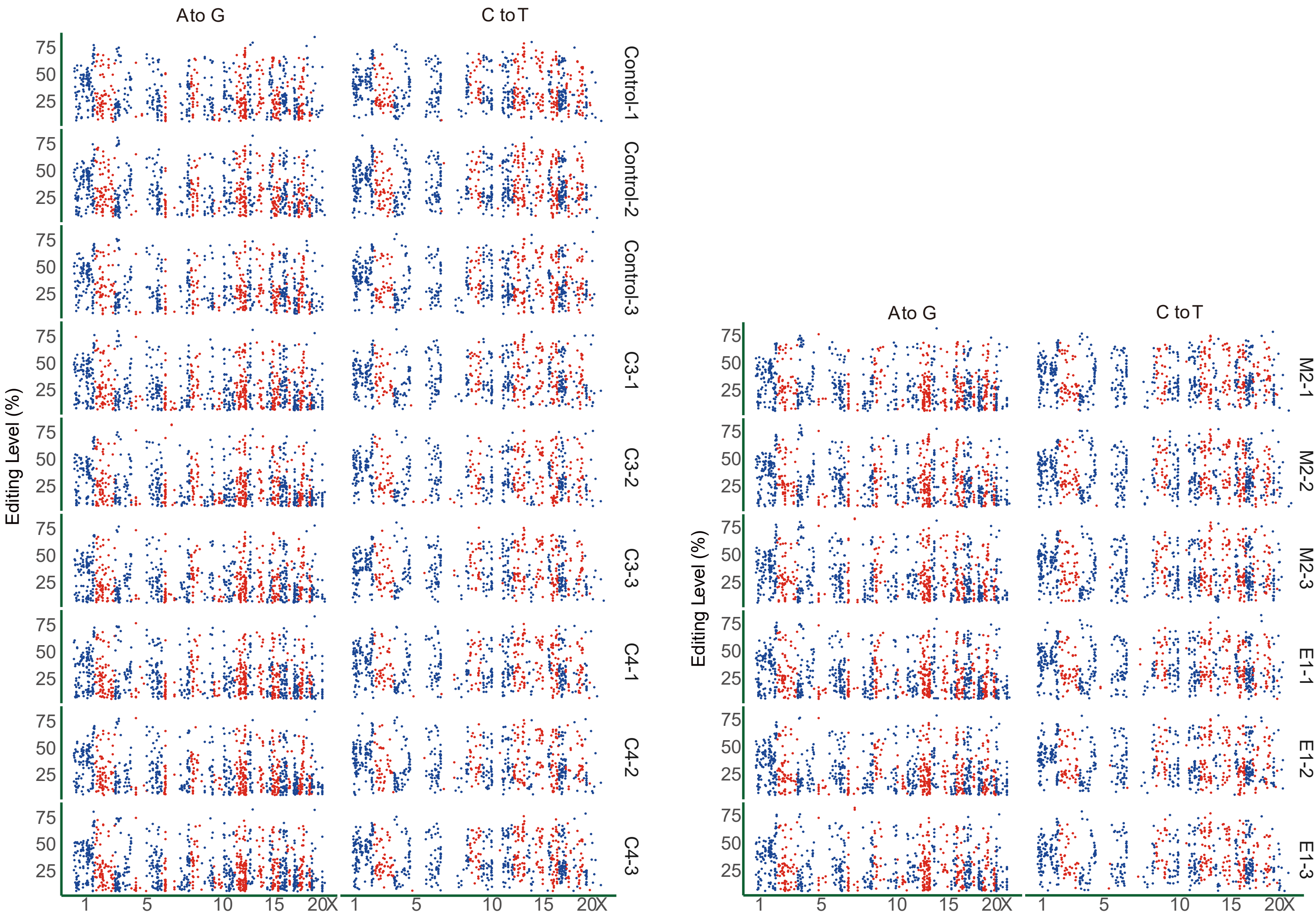

ns
ns
1200
800
400
0
SNV counts
Control
E1
C3
C4
M2
Control
E1
C3
C4
M2
A to G
C to T

Figure S4. Evaluation of SNV counts *in vitro* using transcriptome-wide off-target analysis.

The transcriptome-wide off-target effect of different mxABE constructs targeting the DMD c.4174C>T mutation were performed *in vitro*. The E1 mxABE construct was driven by an EFS promoter; C3 and C4 were driven by an CBh promoter; and M2 was driven by an MHCK7 promoter. Non-targeting mxABE construct was analyzed as a control. Values are shown as mean ± SEM (n=3). NS, not statistically significant in multiple comparison test using ANOVA ($P < 0.01$).

Figure S5



A to G
C to T
Editing Level (%)
75
50
25
1
5
10
15
20X
Control-1
Control-2
Control-3
C3-1
C3-2
C3-3
C4-1
C4-2
C4-3
M2-1
M2-2
M2-3
E1-1
E1-2
E1-3

Figure S5. Manhattan plots of transcriptome-wide off-target RNA editing analysis for different mxABE constructs.

Non-targeting mxABE construct (control) and E1, C3, C4, and M2 constructs were transfected into HEK293T cells. mCherry-positive cells were sorted and endogenous transcripts were analyzed by deep sequencing. The x and y axes are proportionally enlarged with each Manhattan plot to make the axis legend clear (n=3).

Figure S6

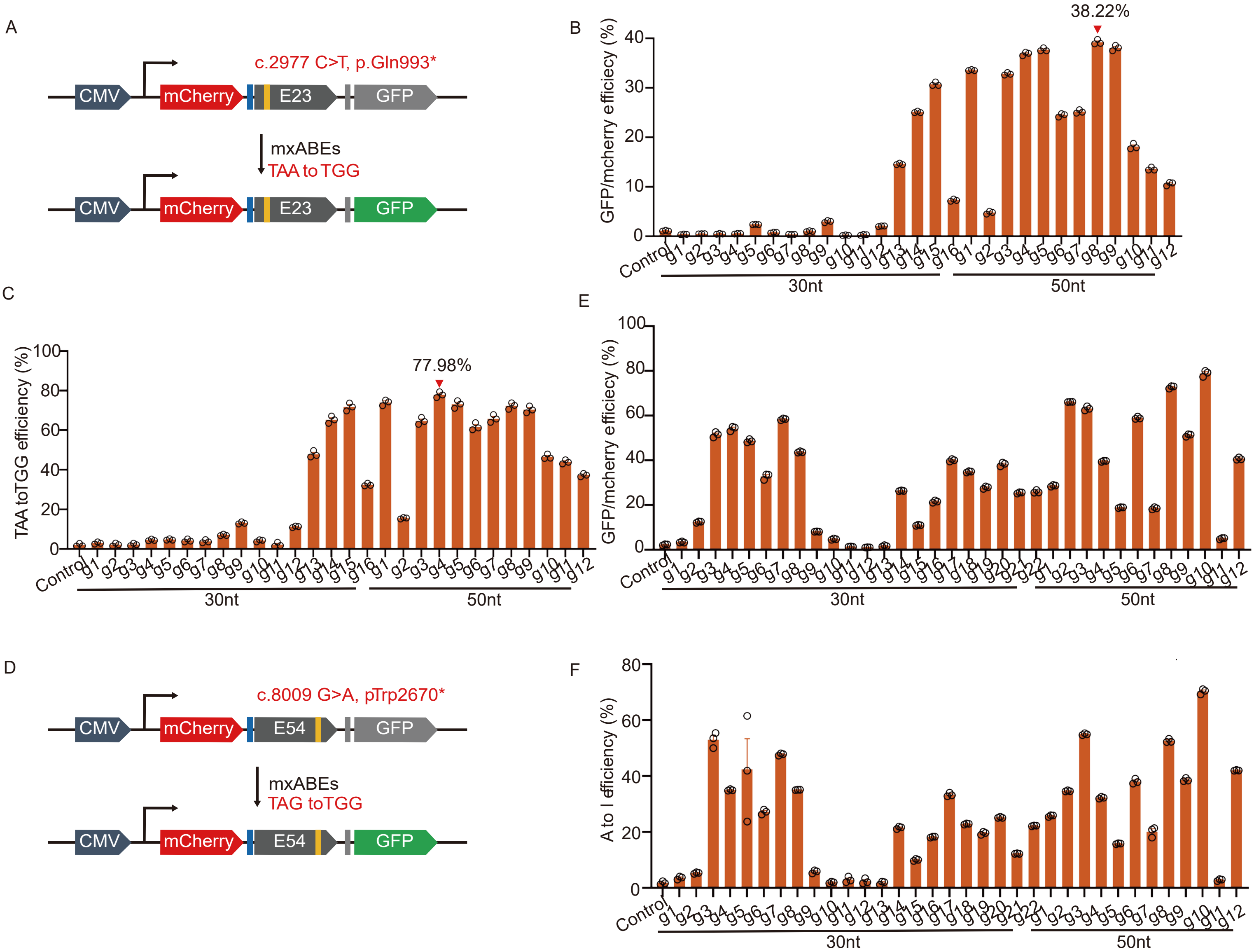

A
c.2977 C>T, p.Gln993*
CMV
mCherry
E23
GFP
mxABEs
TAA to TGG
B
38.22%
GFP/mcherry efficiecy (%)
30nt
50nt
C
77.98%
TAA toTGG efficiency (%)
D
c.8009 G>A, pTrp2670*
E54
TAG toTGG
E
F
A to I efficiency (%)

Figure S6. mxABE-mediated A to I correction of other mutations in the *DMD* gene.

Schematic diagram of reporter cassettes containing an mCherry sequence fused with 2A peptide, mutant human *DMD* exon 23 (A) or exon 54 (D) and ATG-removed GFP. Correction of the stop codon within the target sequence would allow EGFP expression. Flow cytometry analysis of GFP expression in HEK293T cells transfected with 28 gRNAs targeting exon 23 (B) and exon 54 (E). Deep sequencing of the reporter RNA after GFP rescue experiment in exon 23 (C) and exon 54 (F). Data are represented as mean ± SEM (n=3).

Figure S7

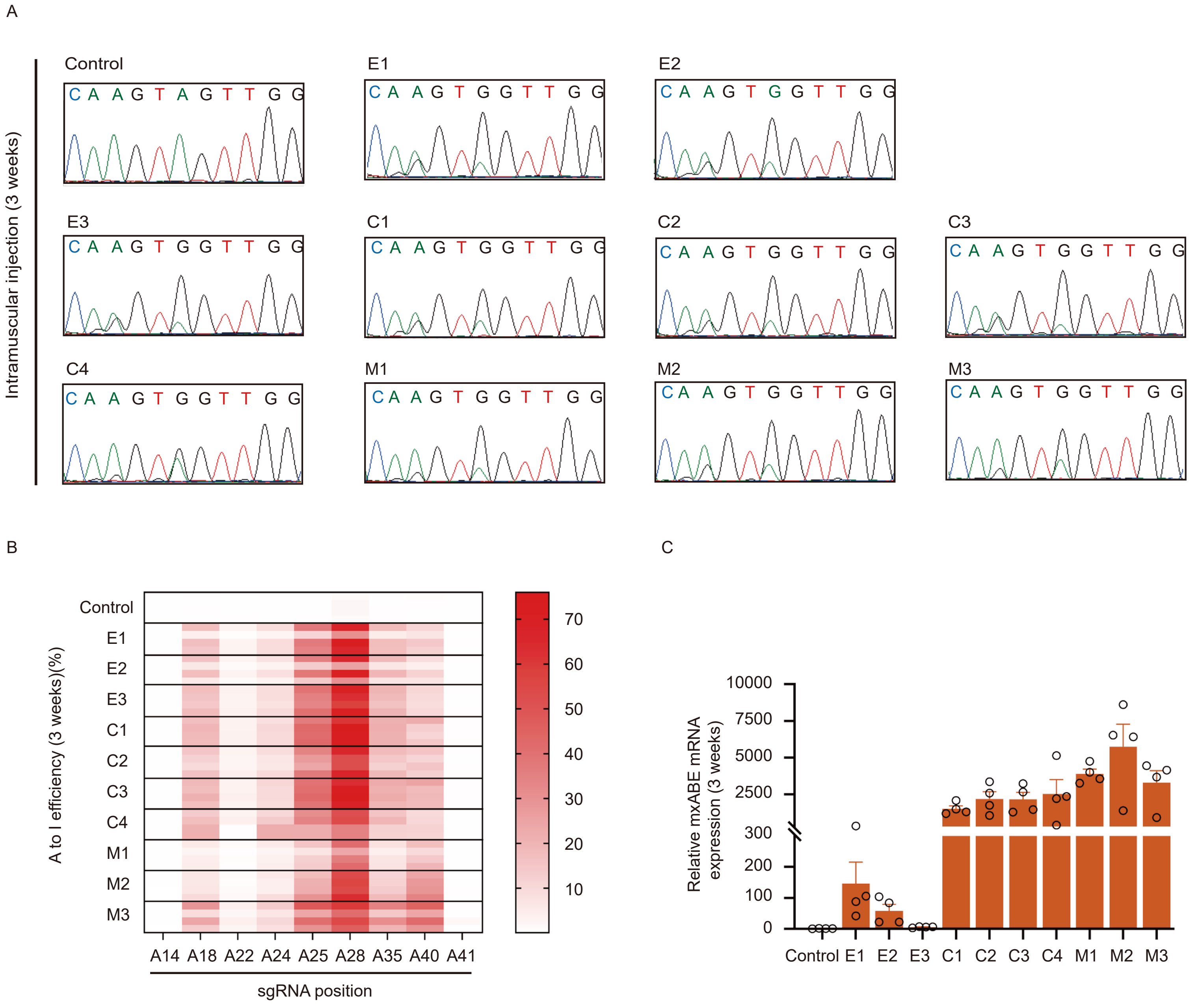
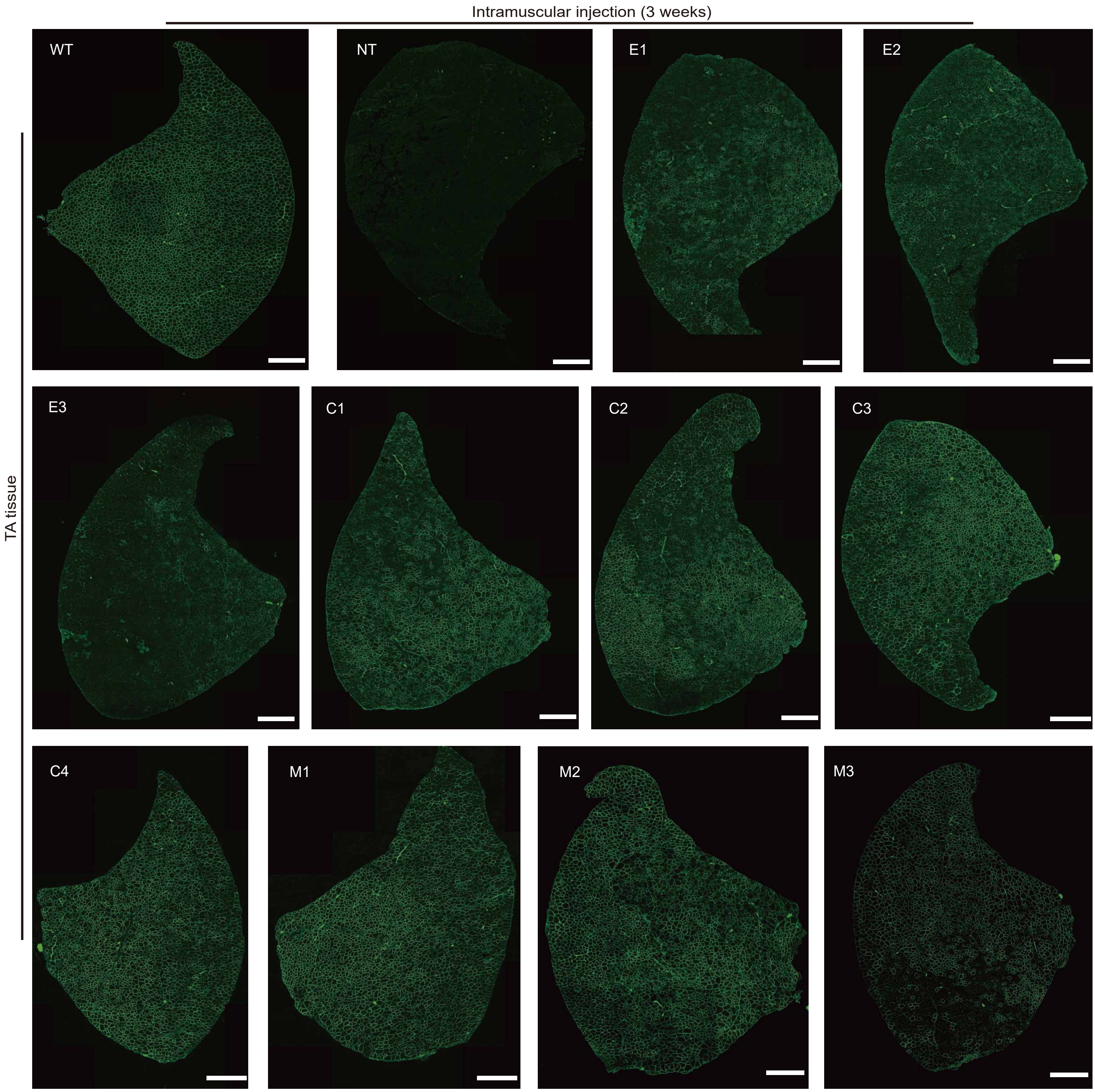

A
Intramuscular injection (3 weeks)
Control
E1
E2
E3
C1
C2
C3
C4
M1
M2
M3
B
A to I efficiency (3 weeks)(%)
sgRNA position
A14 A18 A22 A24 A25 A28 A35 A40 A41
70
60
50
40
30
20
10
C
Relative mxABE mRNA expression (3 weeks)
10000
7500
5000
2500
300
200
100
0
Control E1 E2 E3 C1 C2 C3 C4 M1 M2 M3

Figure S7. *In vivo DMD* gene editing by IM injection with mxABEs at 3 weeks.

A. Representative Sanger sequencing trace of *DMD* transcripts 3 weeks after IM injection with ten AAV9-mxABE particles. B. Heat map indicates the A>G edits in the vicinity of the target. C. qPCR analysis of mxABE expression in TA muscles 3 weeks after intramuscular injection. Values are shown as mean ± SEM (n=4).

Figure S8



Intramuscular injection (3 weeks)
TA tissue
WT
NT
E1
E2
E3
C1
C2
C3
C4
M1
M2
M3

Figure S8. Rescue of dystrophin expression following IM injection of mxABEs in DMD^{E30mut} mice.

Dystrophin immunohistochemistry of entire TA muscle. Control mice were injected with saline. Dystrophin (Abcam, ab15277) is shown in green. Scale bar, 500 µm.

Figure S9

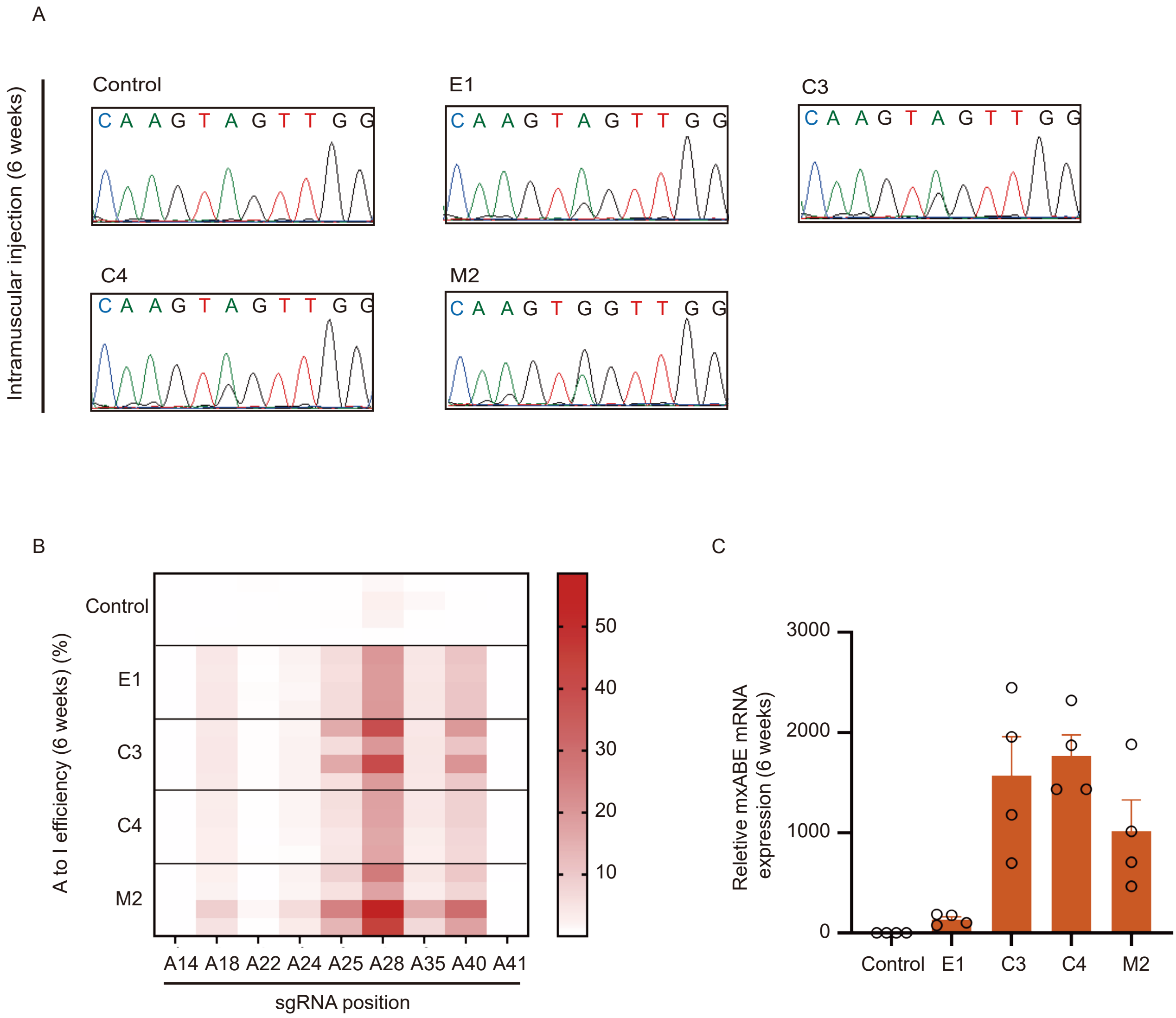

A
Intramuscular injection (6 weeks)
Control
C A A G T A G T T G G
E1
C A A G T A G T T G G
C3
C A A G T A G T T G G
C4
C A A G T A G T T G G
M2
C A A G T G G T T G G
B
A to I efficiency (6 weeks) (%)
Control
E1
C3
C4
M2
A14 A18 A22 A24 A25 A28 A35 A40 A41
sgRNA position
50
40
30
20
10
C
Reletive mxABE mRNA expression (6 weeks)
3000
2000
1000
0
Control
E1
C3
C4
M2

Figure S9. In vivo *DMD* gene editing by IM injection with mxABEs after 6 weeks.

A. Representative Sanger sequencing trace of *DMD* transcripts 6 weeks after IM injection with four AAV9-mxABE particles. B. Heat map indicates the A>G edits in the vicinity of the target. C. qPCR analysis of mxABE expression in TA muscles 6 weeks after intramuscular injection. Values are shown as mean ± SEM (n=4).

Figure S10

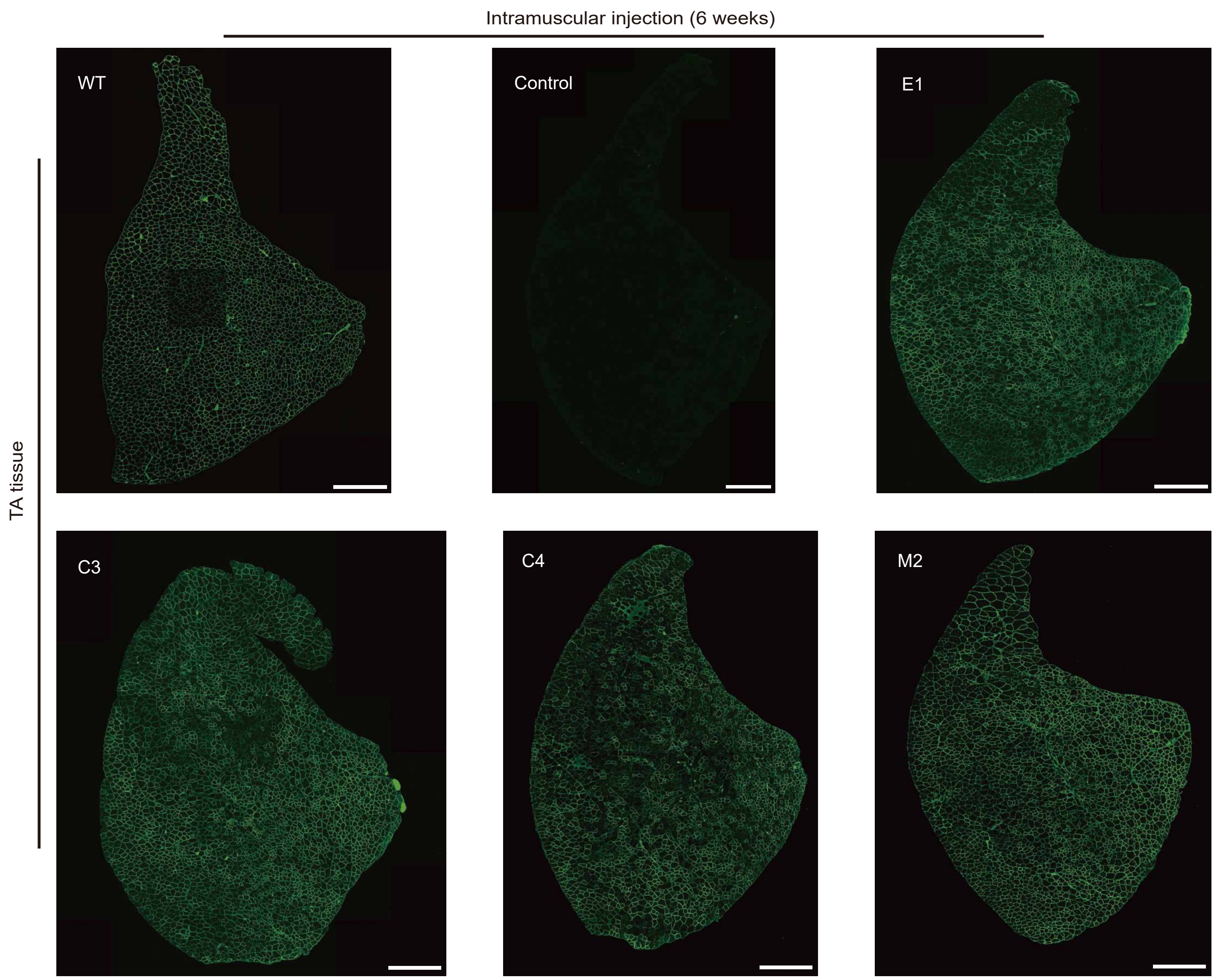
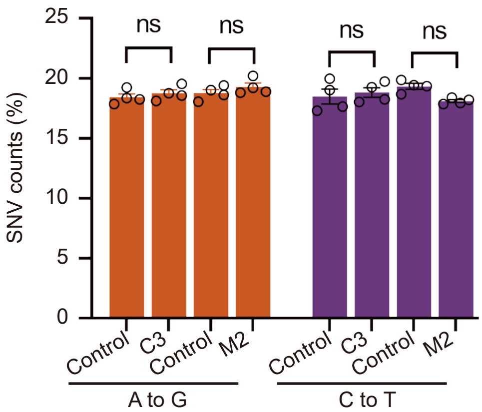

Intramuscular injection (6 weeks)
TA tissue
WT
Control
E1
C3
C4
M2

Figure S10. Intramuscular AAV9 delivery of gene editing components rescues dystrophin expression.

Immunohistochemistry of dystrophin in entire TA muscle 6 weeks after intramuscular AAV9 delivery. Control mice were injected with saline. Dystrophin (Abcam, ab15277) is showed in green. Scale bar, 500 µm.

Figure S11

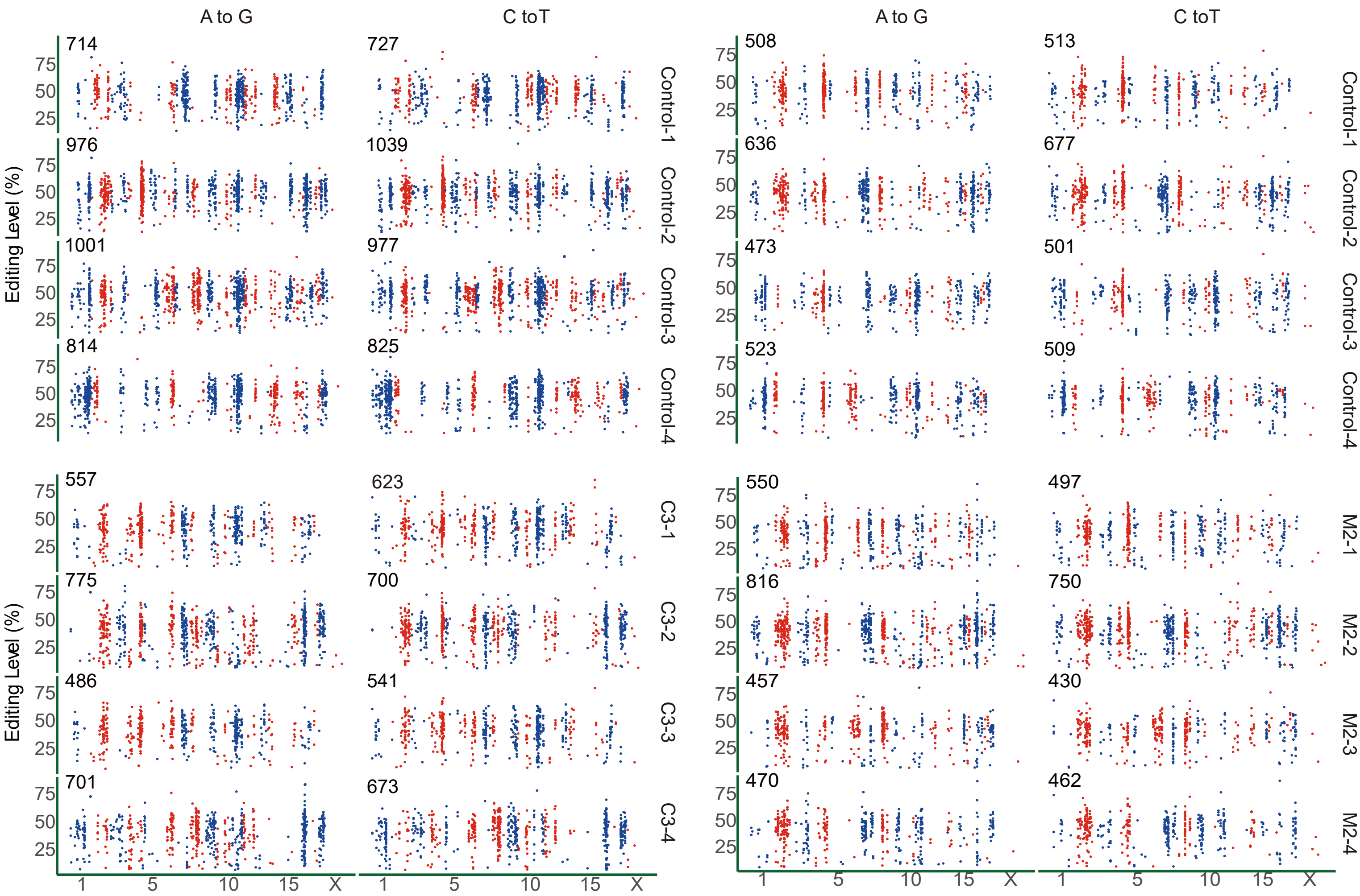


25
20
15
10
5
0
SNV counts (%)
ns
ns
ns
ns
Control
C3
Control
M2
Control
C3
Control
M2
A to G
C to T

Figure S11. Calculation of SNV counts by transcriptome-wide off-target analysis *in vivo*.

The transcriptome-wide off-target effect of two AAV9-mxABEs (C3 and M2) were analyzed *in vivo*. Control mice were injected with saline. Values are shown as mean ± SEM (n=4). NS, not statistically significant using unpaired two-tailed Student’s t tests (P<0.01).

Figure S12

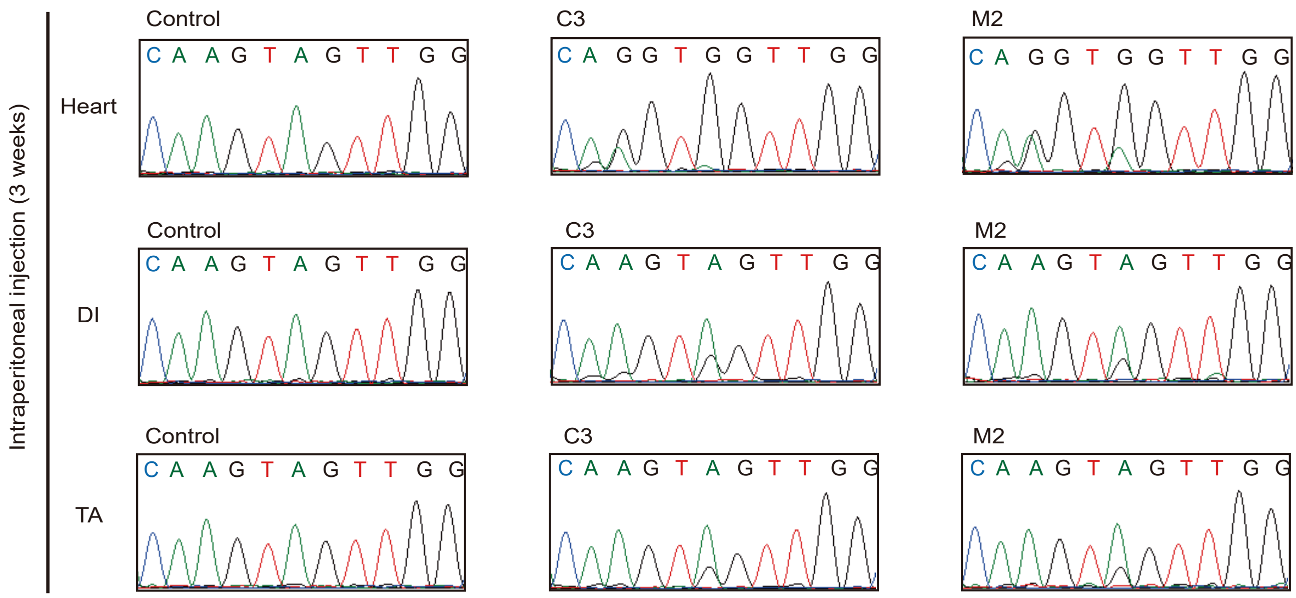


A to G
C toT
A to G
C toT
Editing Level (%)
Editing Level (%)
714
727
508
513
976
1039
636
677
1001
977
473
501
814
825
523
509
557
623
550
497
775
700
816
750
486
541
457
430
701
673
470
462
Control-1
Control-2
Control-3
Control-4
C3-1
C3-2
C3-3
C3-4
M2-1
M2-2
M2-3
M2-4
75
50
25
1
5
10
15
X

Figure S12. Manhattan plots of transcriptome-wide off-target RNA editing analysis *in vivo*.
Control mice were injected with saline. The x and y axes are proportionally enlarged with each Manhattan plot to make the axis legend clear. n=4.

Figure S13

A



B

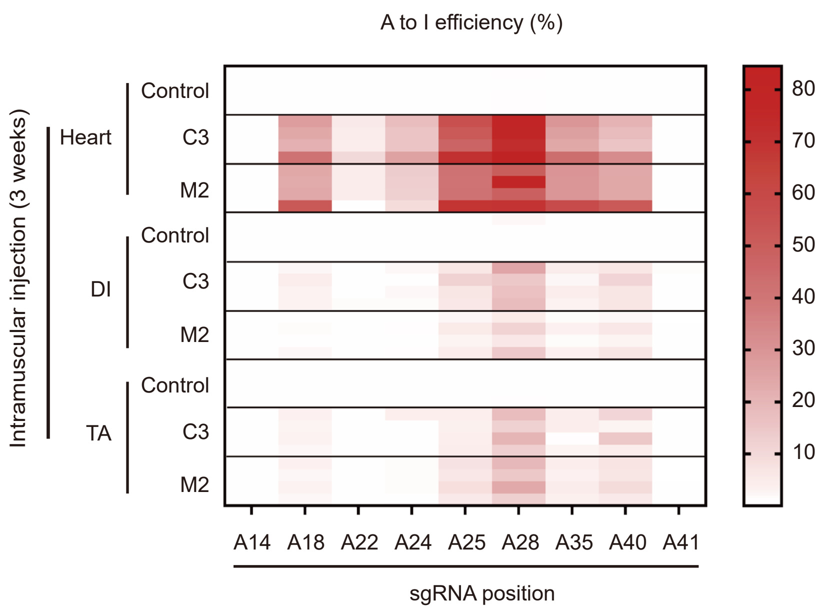


Figure S13. Gene editing 3 weeks after systemic delivery of mxABEs in DMD^{E30mut} mice.

A. Representative Sanger sequencing trace of *DMD* transcripts in TA, DI, and cardiac muscle 3 weeks after intraperitoneal (IP) injection with C3 and M2 particles. B. Heat map indicates the A>G edits in the vicinity of the target in indicated tissues. n=4.

Figure S14

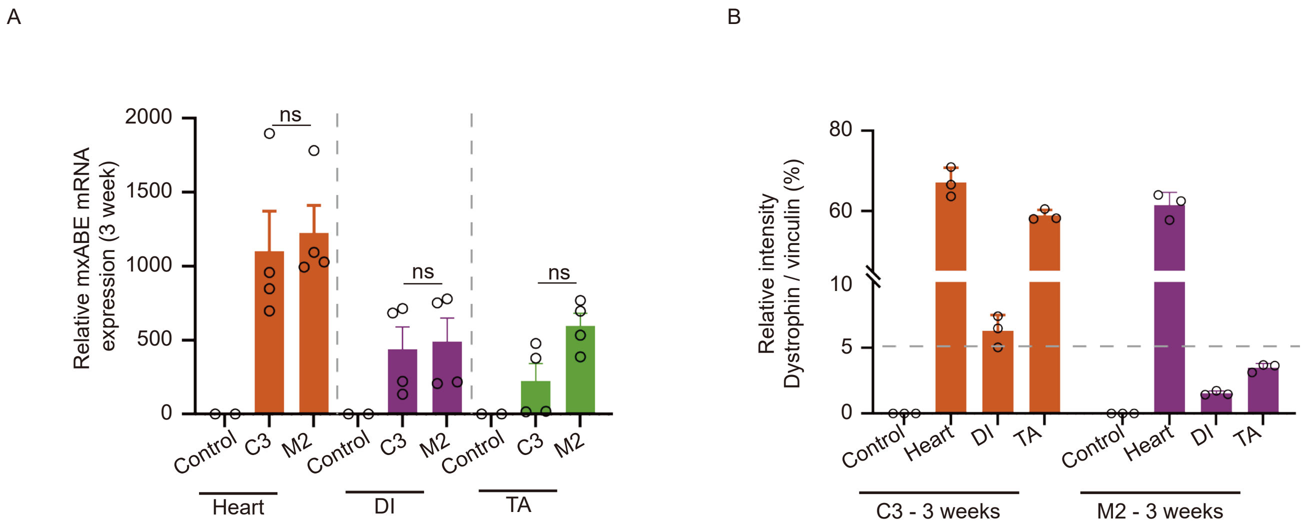

A
Relative mxABE mRNA expression (3 week)
2000
1500
1000
500
0
ns
ns
ns
Control
C3
M2
Control
C3
M2
Control
C3
M2
Heart
DI
TA
B
Relative intensity Dystrophin / vinculin (%)
80
60
10
5
0
Control
Heart
DI
TA
Control
Heart
DI
TA
C3 - 3 weeks
M2 - 3 weeks

Figure S14. AAV9-mxABE expression and dystrophin protein levels after systemic delivery in $\mathbf{DMD^{E30mut}}$ mice.

A. qPCR analysis of mxABE expression in TA, DI, and heart muscles 3 weeks after systemic delivery (n=4). B. Relative dystrophin (Sigma, D8168) intensity was calibrated against a vinculin (CST, 13901S) internal control before normalizing to the WT control. The results showed restoration of dystrophin in TA, DI, and heart muscles of DMD^{E30mut} mice 3 weeks after systemic delivery of AAV9-packaged C3 and M2 (n=3). Values are shown as mean ± SEM. NS, not statistically significant using unpaired two-tailed Student’s t tests (P<0.01).

Figure S15

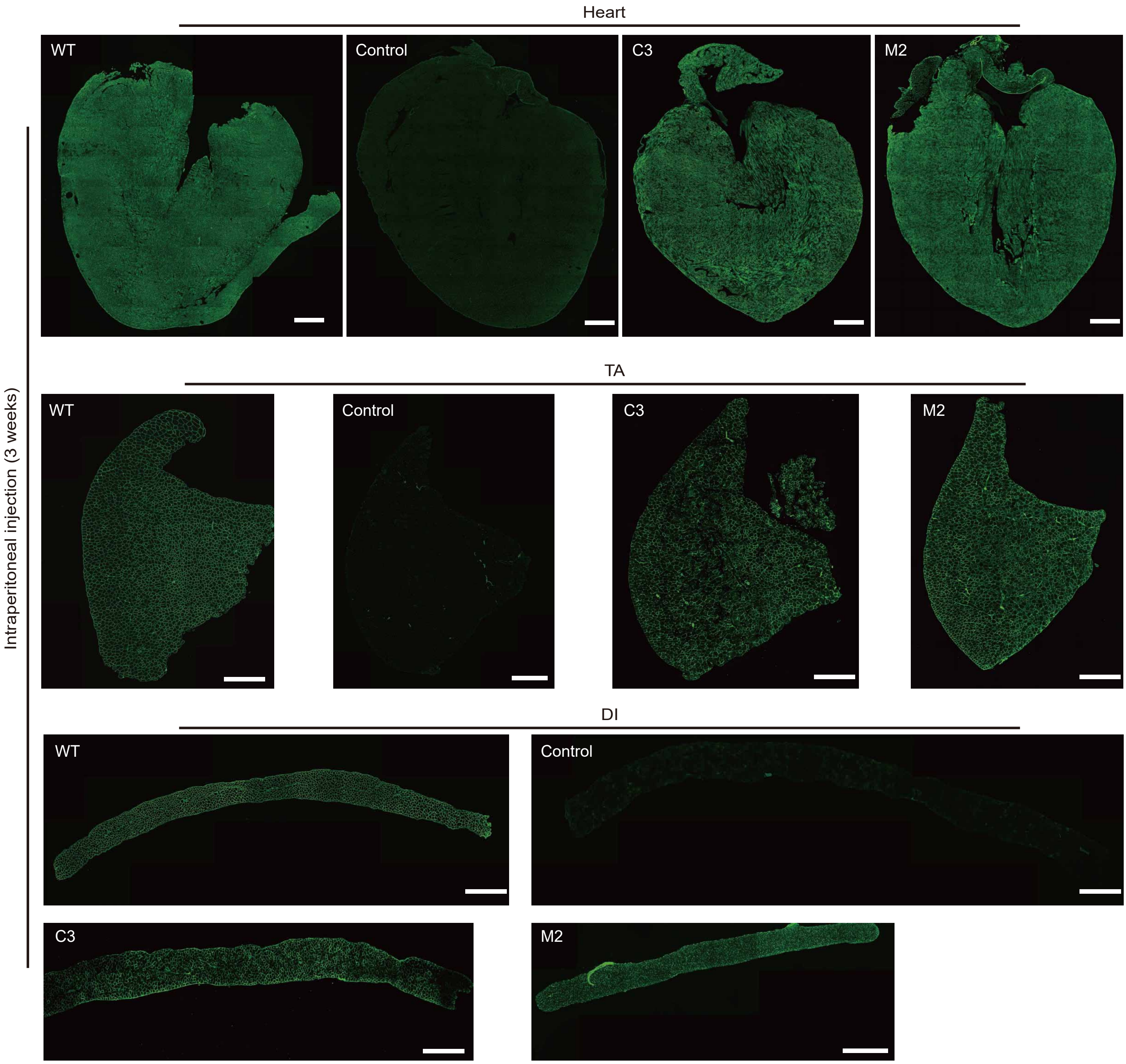
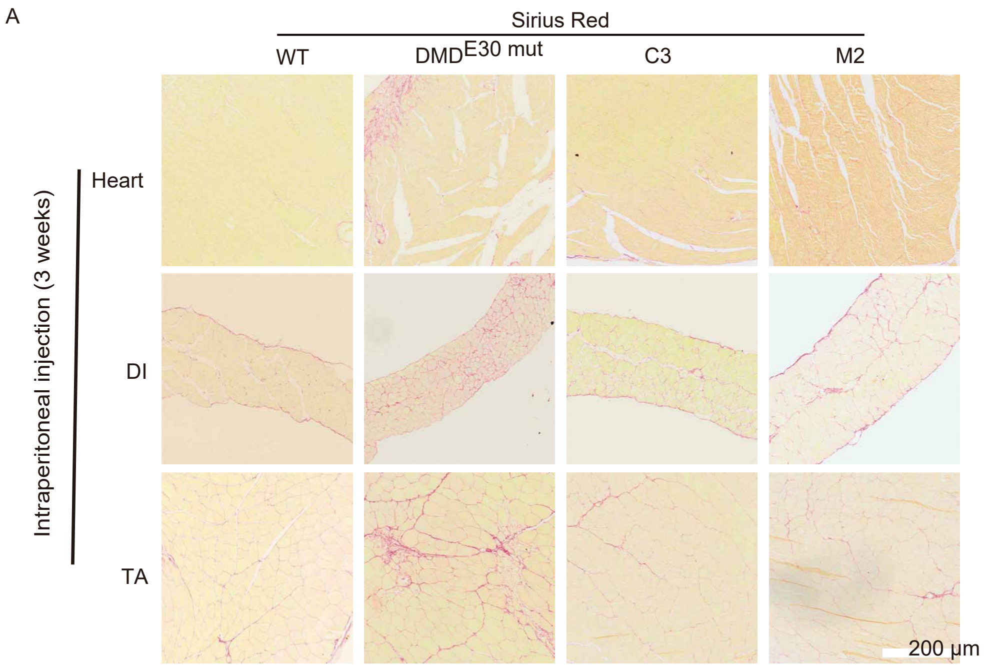

Heart
WT
Control
C3
M2
TA
WT
Control
C3
M2
DI
WT
Control
C3
M2
Intraperitoneal injection (3 weeks)

Figure S15. Histological analysis of dystrophin expression 3 weeks after systemic delivery of AAV9-mxABE in DMD^{E30mut} mice.

Whole-muscle scanning of TA, DI, and heart of DMD^{E30mut} mice 3 weeks after systemic delivery AAV9-C3 and M2 particles. Control mice were injected with saline. Dystrophin (Abcam, ab15277) is showed in green. Scale bar, 500 μm.

Figure S16


A
Sirius Red
WT
DMD^E30 mut
C3
M2
Intraperitoneal injection (3 weeks)
Heart
DI
TA
200 μm

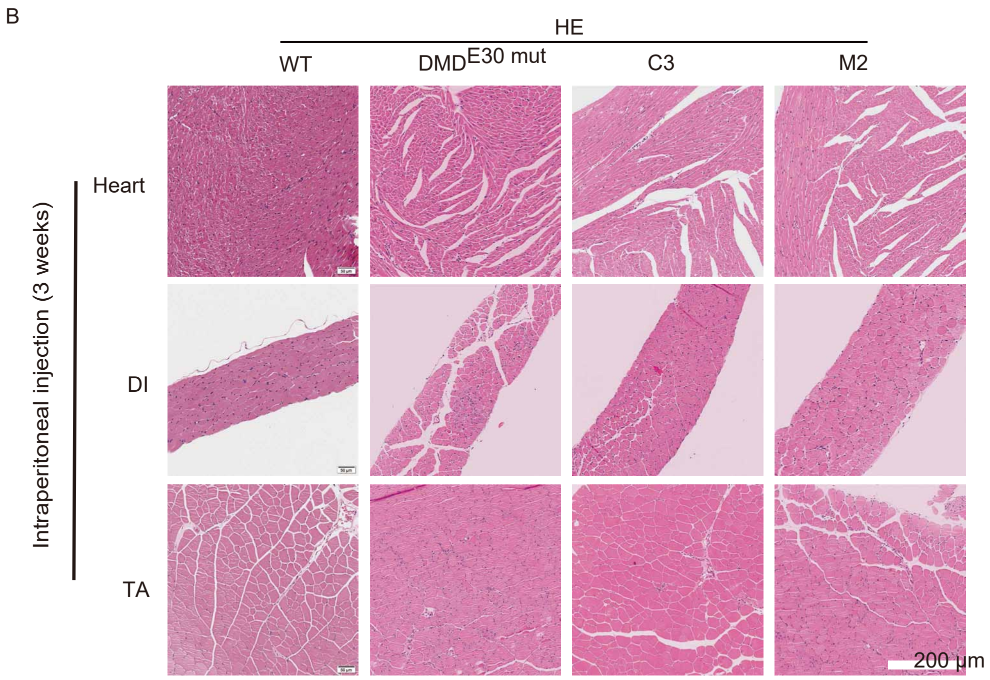

B
HE
WT
DMD^E30 mut
C3
M2
Intraperitoneal injection (3 weeks)
Heart
DI
TA
200 μm

Figure S16. Histological analysis 3 weeks after systemic delivery of AAV9-mxABE.

Sirius red staining (A) and HE staining (A) of TA, DI, and heart of WT, untreated DMD^{E30mut} mice and DMD^{E30mut} mice treated with AAV9-mxABE 3 weeks after systemic injection. n=4 for each group. Scale bars, 200 μm.

Figure S17

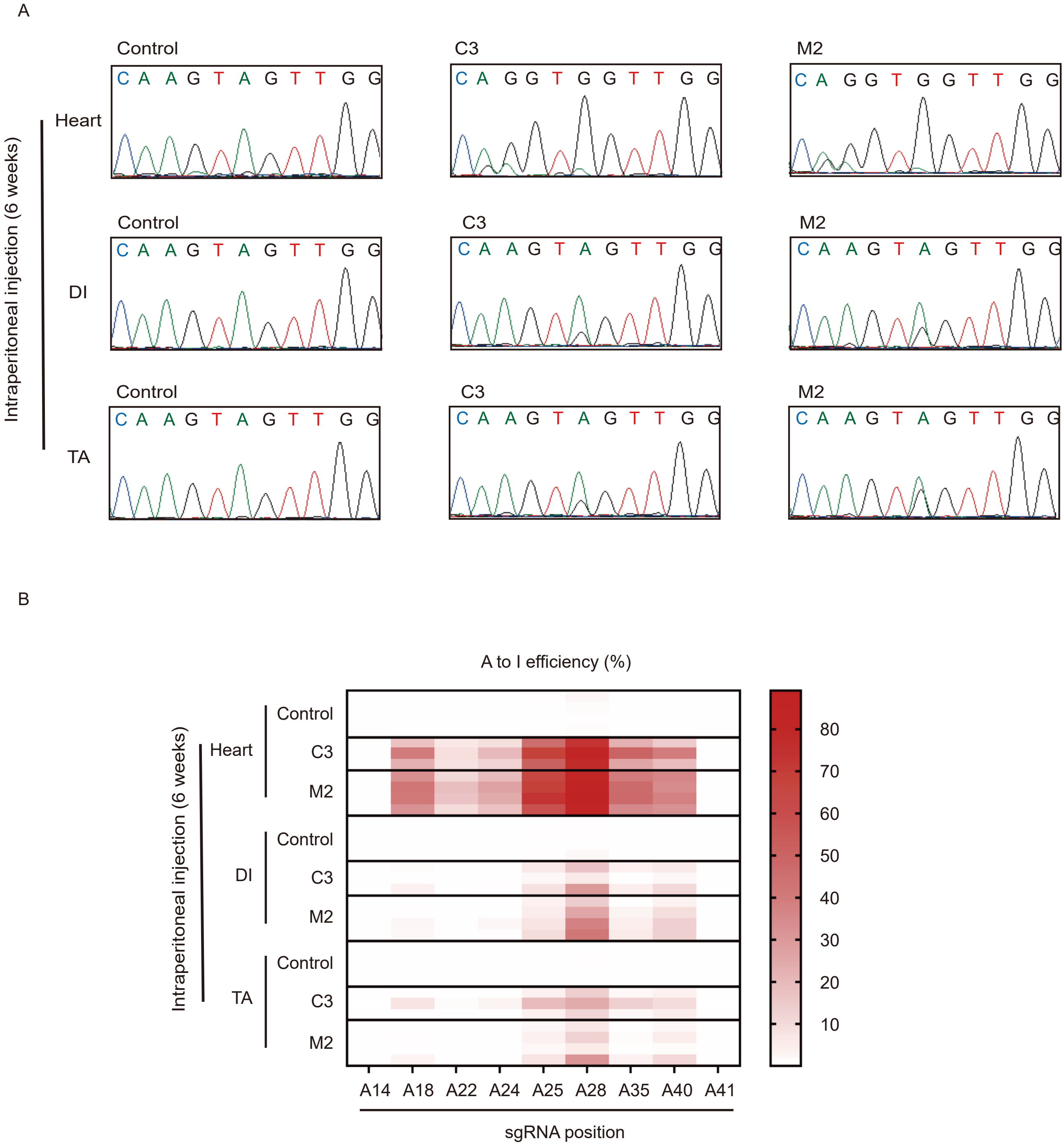

A
Intraperitoneal injection (6 weeks)
Heart
DI
TA
Control
C3
M2
C A A G T A G T T G G
C A G G T G G T T G G
B
A to I efficiency (%)
Control
C3
M2
80
70
60
50
40
30
20
10
A14 A18 A22 A24 A25 A28 A35 A40 A41
sgRNA position

Figure S17. Base editing efficiency achieved by systemic delivery of mxABE after 6 weeks.

A. Representative Sanger sequencing trace of *DMD* transcripts in TA, DI, and heart tissues 6 weeks after intraperitoneal (IP) injection with C3 and M2 particles. B. Heat map shows base editing rates in tissues indicated after IP injection with C3 (n=3) and M2 (n=4).

Figure S18

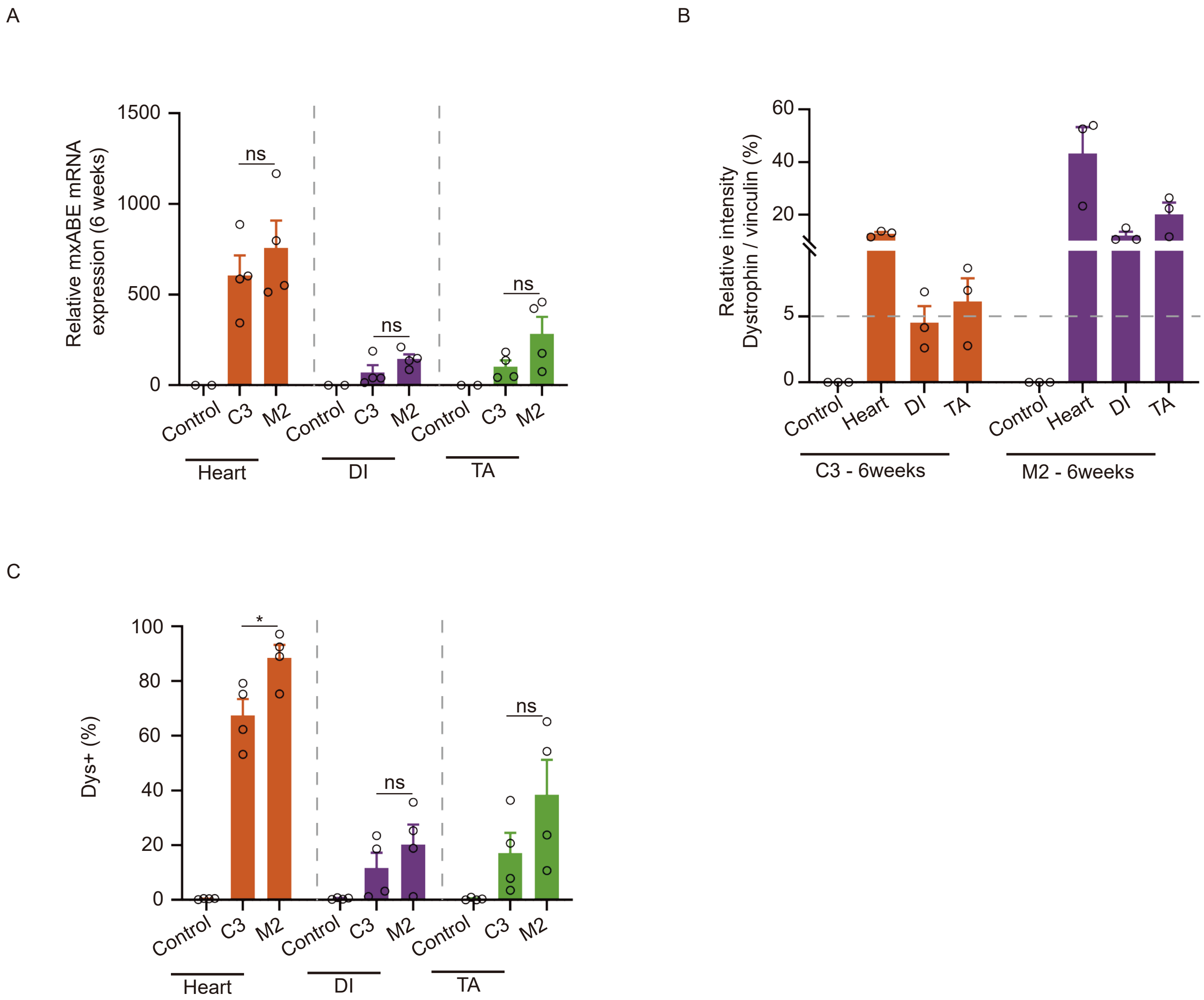

A
Relative mxABE mRNA expression (6 weeks)
1500
1000
500
0
ns
ns
ns
Control
C3
M2
Heart
DI
TA
B
Relative intensity Dystrophin / vinculin (%)
60
40
20
5
0
Control
Heart
DI
TA
C3 - 6weeks
M2 - 6weeks
C
Dys+ (%)
100
80
60
40
20
0
*
ns
ns
Control
C3
M2
Heart
DI
TA

Figure S18. mxABE expression and dystrophin restoration level after 6-week administration of AAV-mxABE systemically.

A. qPCR analysis of mxABE expression in TA, DI, and heart muscles 6 weeks after systemic delivery (n=4). B. Relative dystrophin (Sigma, D8168) intensity was calibrated against a vinculin (CST, 13901S) internal control before normalizing to the WT control. The results showed restoration of dystrophin in TA, DI, and cardiac muscles of DMD^{E30mut} mice 6 weeks after systemic delivery of C3 and M2 AAV-mxABE (n=3). C. Percentage of Dys+ tissue area in TA, DI, and heart from treated and untreated DMD^{E30mut} mice (n=4). Values are shown as mean ± SEM. NS, not statistically significant using unpaired two-tailed Student’s t test (P<0.01).

Figure S19

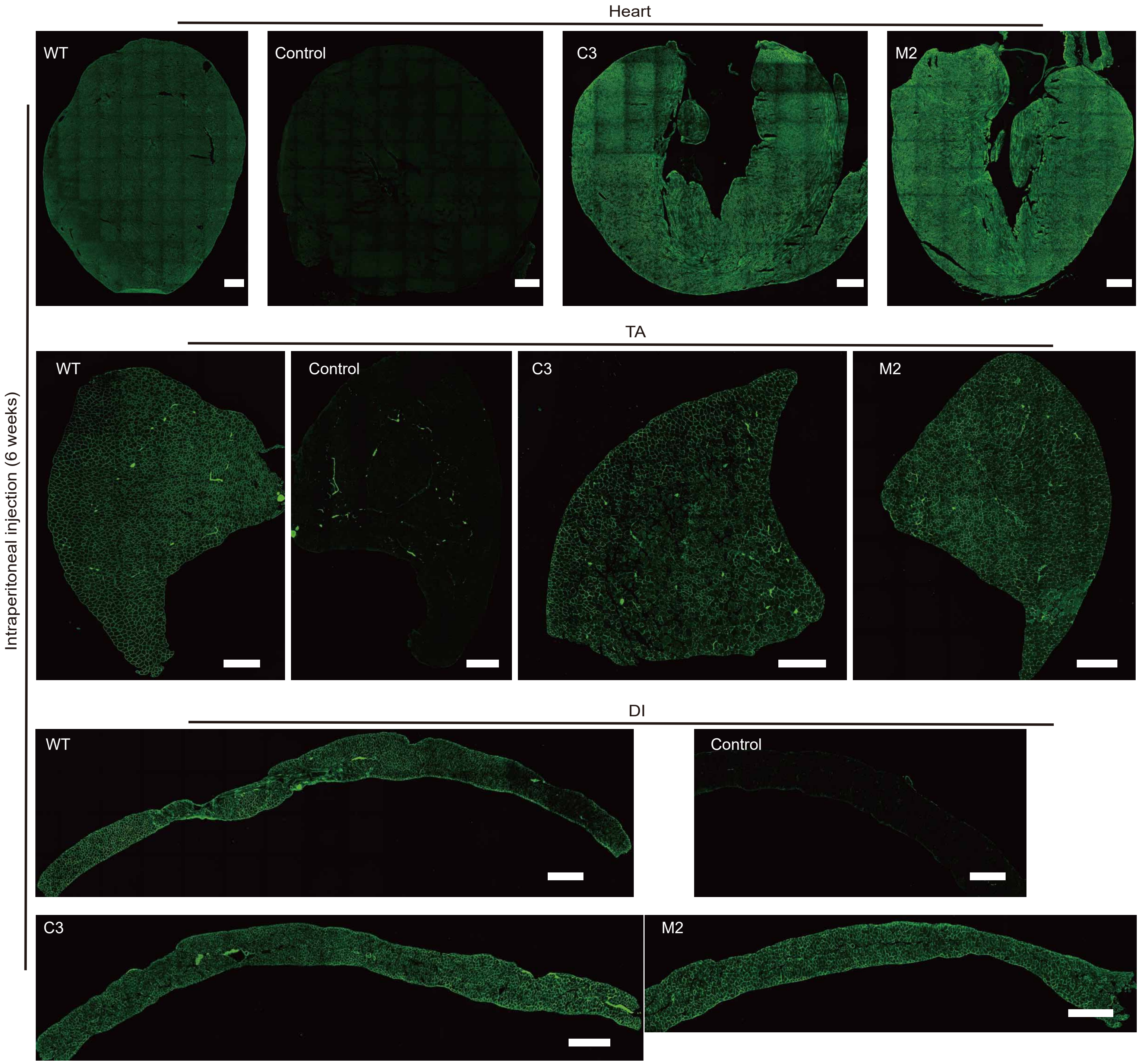

Heart
WT
Control
C3
M2
TA
WT
Control
C3
M2
DI
WT
Control
C3
M2
Intraperitoneal injection (6 weeks)

Figure S19. Immunostaining of dystrophin in heart, TA, and DI tissues 6 weeks after systemic administration of AAV-mxABE.

Whole-muscle scanning of TA, DI, and heart muscle of DMD^{E30mut} mice 6 weeks after systemic delivery of AAV9-C3 and M2 particles. Control mice were injected with saline. Dystrophin (Abcam, ab15277) is shown in green. Scale bar, 500 µm. Images shown in both Figure 6C and Figure S19 were obtained from the same tissue at 20x magnification. Figure 6C showed the local region staining image rather than the reconstituted whole-tissue scanning image in Figure S19.

Figure S20

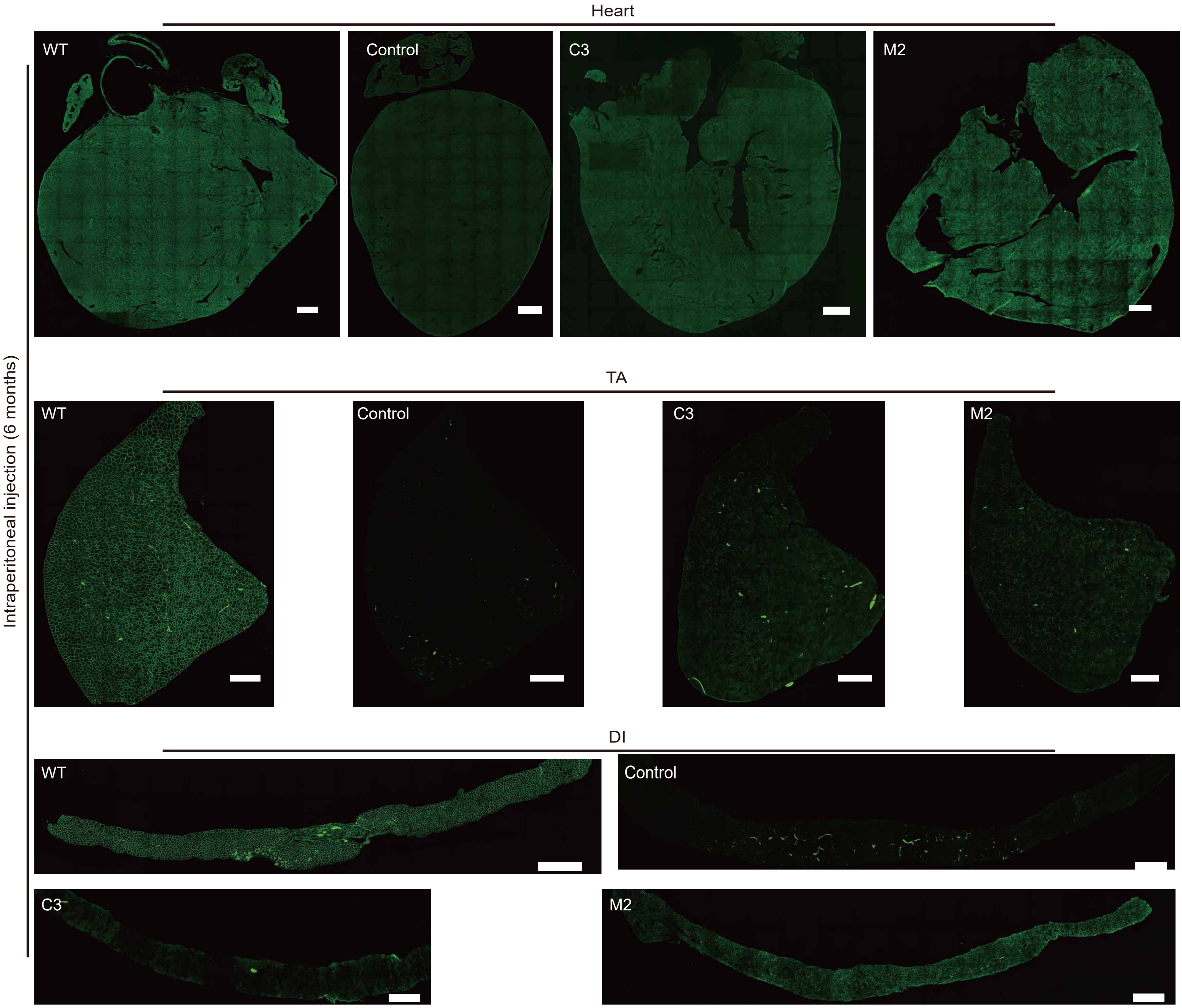

Heart
WT
Control
C3
M2
TA
WT
Control
C3
M2
DI
WT
Control
C3
M2
Intraperitoneal injection (6 months)

Figure S20. Immunostaining of dystrophin in heart, TA, and DI 6 months after systemic administration of AAV-mxABE.

Whole-muscle scanning of TA, DI, and heart muscle of DMD^{E30mut} mice 6 months after systemic delivery of AAV9-C3 and M2 particles. Control mice were injected with saline. Dystrophin (Abcam, ab15277) is shown in green. Scale bar, 500 µm.

Figure S21

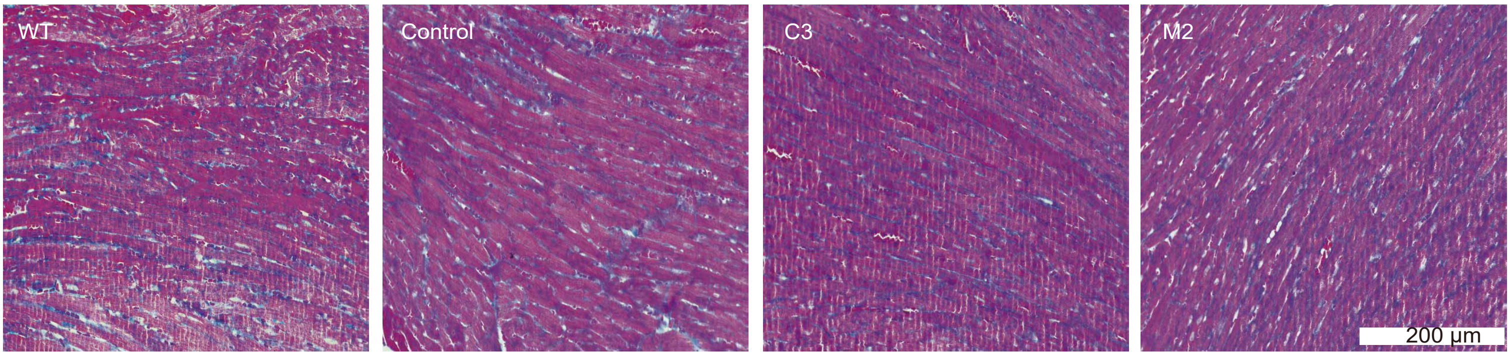
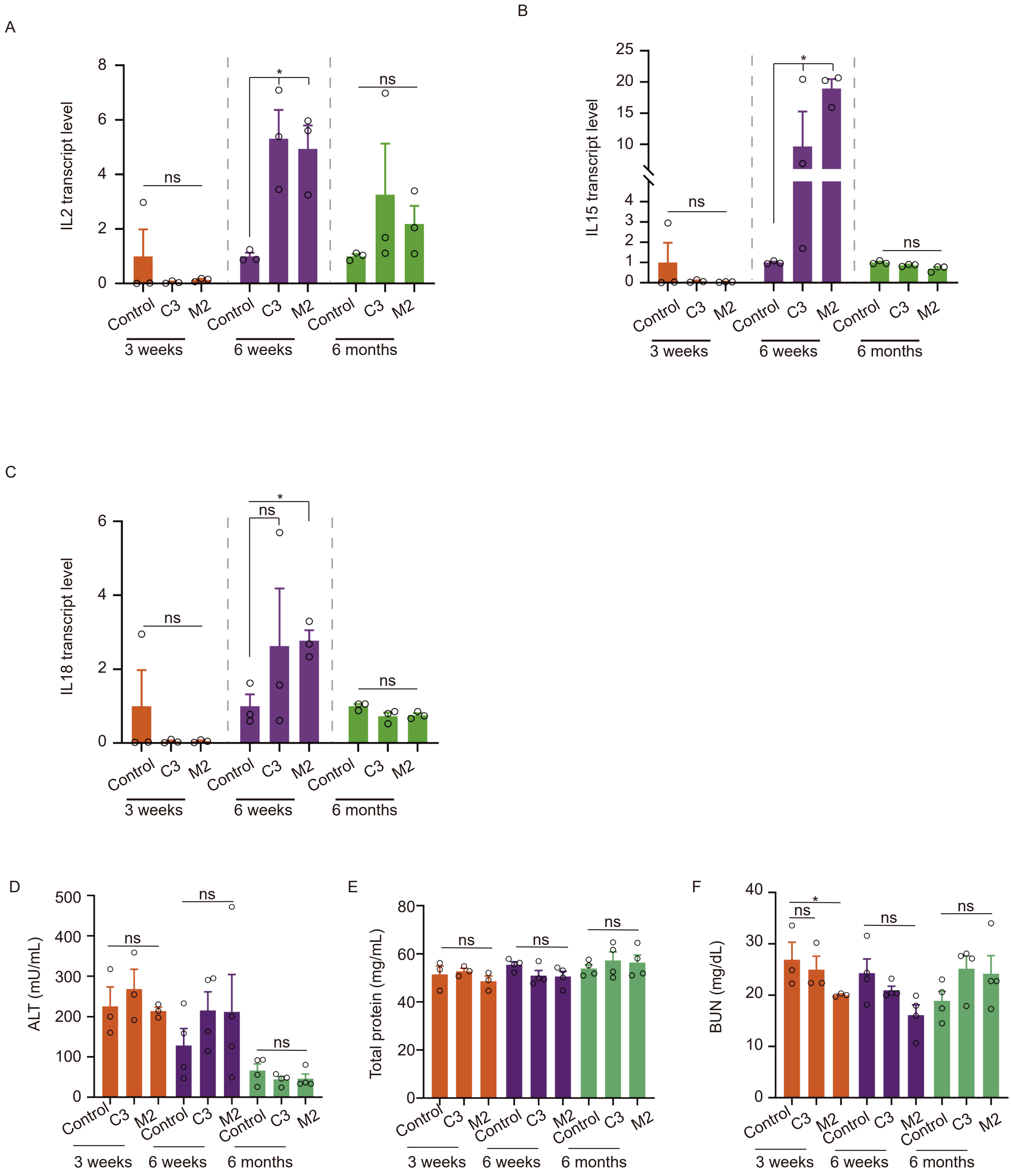


Figure S21. Collagen staining shows no obvious fibrosis in treated and untreated hearts 6 months after systemic administration of AAV-mxABE. Scale bar, 200 µm.

Figure S22

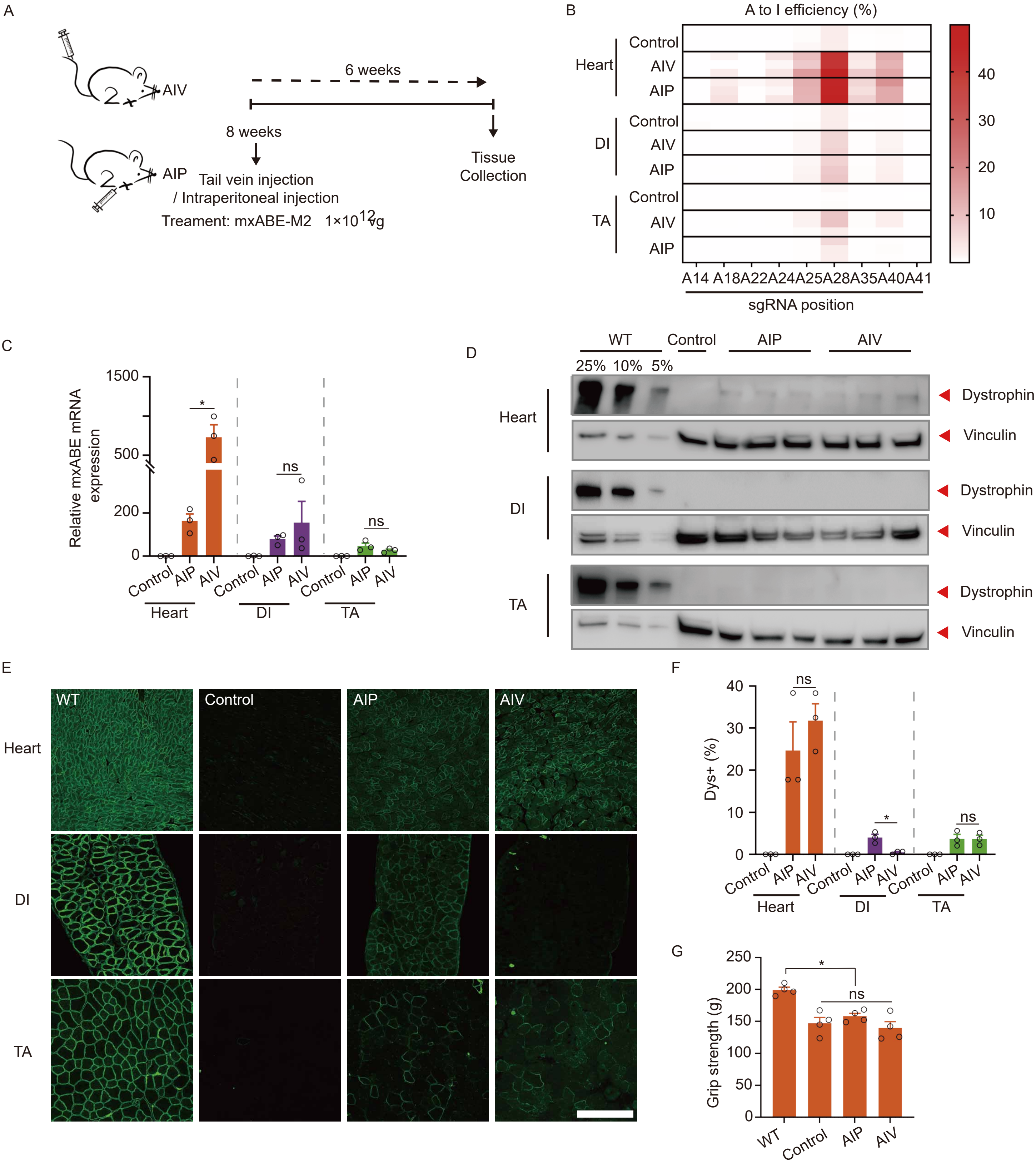


A
IL2 transcript level
8
6
4
2
0
ns
*
ns
Control
C3
M2
3 weeks
6 weeks
6 months
B
IL15 transcript level
25
20
15
10
4
3
2
1
0
ns
*
ns
C
IL18 transcript level
6
4
2
0
ns
*
ns
ns
D
ALT (mU/mL)
500
400
300
200
100
0
ns
ns
ns
E
Total protein (mg/mL)
80
60
40
20
0
ns
ns
ns
F
BUN (mg/dL)
40
30
20
10
0
ns
*
ns
ns

Figure S22. Host immune response to AAV-mxABE treatment indicates no overt toxicity after 6 months.

(A-C) IL2, IL15, and IL18 transcript levels at baseline for untreated mice, 3 weeks, 6 weeks, and 6 months for treated mice (n=3). (D-F) No obvious change in ALT, total protein and BUN level after AAV-mxABE treatment (n=3). Significance is indicated by asterisk ($P < 0.05$). NS represents not statistically significant using unpaired two-tailed Student's t test.

Figure S23

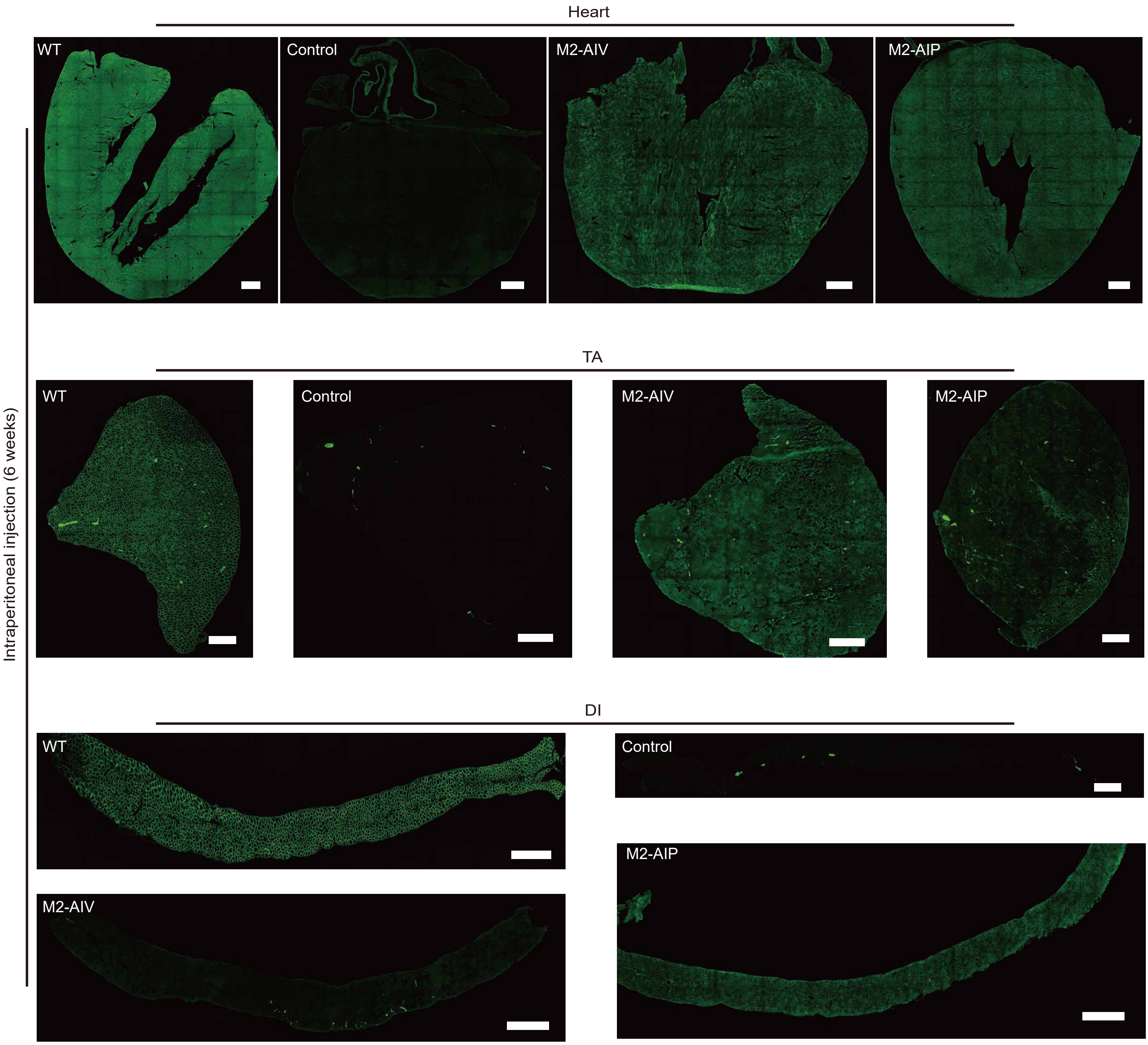


A
AIV
AIP
6 weeks
8 weeks
Tail vein injection
/ Intraperitoneal injection
Treament: mxABE-M2 1×10^12 vg
Tissue
Collection
B
A to I efficiency (%)
Heart
DI
TA
Control
AIV
AIP
A14 A18 A22 A24 A25 A28 A35 A40 A41
sgRNA position
40
30
20
10
C
Relative mxABE mRNA expression
1500
500
200
0
*
ns
ns
Control
AIP
AIV
Heart
DI
TA
D
WT
Control
AIP
AIV
25% 10% 5%
Heart
DI
TA
Dystrophin
Vinculin
E
WT
Control
AIP
AIV
Heart
DI
TA
F
Dys+ (%)
40
30
20
10
0
ns
*
ns
Control
AIP
AIV
Heart
DI
TA
G
Grip strength (g)
250
200
150
100
50
0
*
ns
WT
Control
AIP
AIV

Figure S23. Systemic delivery of AAV-mxABE rescued dystrophin deficiency in adult DMD^{E30mut} mice.

A. Experimental design for mxABE administration and therapeutic analysis in adult DMD^{E30mut} mice. Both intravenous (AIV group) and intraperitoneal (AIP group) injection of AAV-mxABE were evaluated. B. Heatmap shows base editing rate in heart, DI, and TA muscles (n=3). C. mxABE expression level in heart, DI, and TA muscles. D. Dystrophin restoration level analyzed by western blot in heart, DI, and TA muscles of treated and untreated mice. E. Tissue section immunostaining for dystrophin in heart, DI, and TA muscles of treated and untreated mice. Scale bar, 200 µm. F. Quantification of Dys+ immunostaining in (E) (n=3). G. Forelimb grip strength was measured in WT, DMD^{E30mut} mice, and DMD^{E30mut} mice treated with AAV9-M2 (n=4). Significance is indicated by asterisk ($P < 0.05$). NS represents not statistically significant using unpaired two-tailed Student’s t test.

Figure S24



Heart
WT
Control
M2-AIV
M2-AIP
TA
WT
Control
M2-AIV
M2-AIP
DI
WT
Control
M2-AIV
M2-AIP
Intraperitoneal injection (6 weeks)

Figure S24. Immunostaining of dystrophin in adult DMD^{E30mut} mice 6 weeks after systemic injection of AAV-mxABE.
Scale bar, 500 µm.

Supplementary Table S1: Target sgRNA and primer sequence.

Experiment	Primer name	Primer sequence (5'-3')
sgRNA for generation DMD^{E30mut} model	DMD-T7sgRNA1	CTTCTTCTATTTAGGCTGTG
	DMD-T7sgRNA2	AGCTTATATCACTGACAAGG
Genotyping of DMD^{E30mut} mice	DMD^{E30mut}-2333F	ATTCATATAGGGCTTCAGTTCC
	DMD^{E30mut}-2333R	CATCTGTTTTAATAGTGTGCAT
RT-PCR primer flanking exon 30	RT-DMD-358F	AATCAGATTCGTCTATTGGCACA
	RT-DMD-358R	CCCTTTGGTTGGCATCCTT
RNA base editing sgRNA for exon 30	30nt-g1	GCTGCCAACCACTTGTCAATGAATGTGAGG
	30nt-g2	AGCTGCCAACCACTTGTCAATGAATGTGAG
	30nt-g3	TGCAATATAAGCTGCCAACCACTTGTCAAT
	30nt-g4	CTGCAATATAAGCTGCCAACCACTTGTCAA
	30nt-g5	AAGCTGCCAACCACTTGTCAATGAATGTGA
	30nt-g6	TAAGCTGCCAACCACTTGTCAATGAATGTG
	30nt-g7	ATAAGCTGCCAACCACTTGTCAATGAATGT
	30nt-g8	TATAAGCTGCCAACCACTTGTCAATGAATG
	30nt-g9	ATATAAGCTGCCAACCACTTGTCAATGAAT
	30nt-g10	AATATAAGCTGCCAACCACTTGTCAATGAA
	30nt-g11	CAATATAAGCTGCCAACCACTTGTCAATGA
	30nt-g12	GCAATATAAGCTGCCAACCACTTGTCAATG
	50nt-g1	TGCAATATAAGCTGCCAACCACTTGTCAATGAATGTGAGGGACTCCTGGA
	50nt-g2	CTGCAATATAAGCTGCCAACCACTTGTCAATGAATGTGAGGGACTCCTGG
	50nt-g3	CCACCTTGTCTGCAATATAAGCTGCCAACCACTTGTCAATGAATGTGAGG
	50nt-g4	TCCACCTTGTCTGCAATATAAGCTGCCAACCACTTGTCAATGAATGTGAG
	50nt-g5	TCTGCAATATAAGCTGCCAACCACTTGTCAATGAATGTGAGGGACTCCTG
	50nt-g6	GTCTGCAATATAAGCTGCCAACCACTTGTCAATGAATGTGAGGGACTCCT
	50nt-g7	TGTCTGCAATATAAGCTGCCAACCACTTGTCAATGAATGTGAGGGACTCC

	50nt-g8	TTGTCTGCAATATAAGCTGCCAACCACTTGTCAATGAATGTGAGGGACTC
	50nt-g9	CTTGTCTGCAATATAAGCTGCCAACCACTTGTCAATGAATGTGAGGGACT
	50nt-g10	CCTTGTCTGCAATATAAGCTGCCAACCACTTGTCAATGAATGTGAGGGAC
	50nt-g11	ACCTTGTCTGCAATATAAGCTGCCAACCACTTGTCAATGAATGTGAGGGA
	50nt-g12	CACCTTGTCTGCAATATAAGCTGCCAACCACTTGTCAATGAATGTGAGGG
RT-PCR primer for Reporter	RT-rep765F	CCACAACGAGGACTACACCA
	RT-rep765R	TCCTTGAAGTCGATGCCCTT
qPCR primer	GAPDH-119F	TCAACGACCCCTTCATTGACC
	GAPDH-119R	CTTCCCGTTGATGACAAGCTTC
	Cas13X-140F	GCCATGCAGAAATAATATCTCGG
	Cas13X-140R	CATTCTCCTTCAGCCTAAACCC
RNA base editing sgRNA for exon 23	50nt-g1	CTGAGATAGTATAGGCCACTccATTGCTCTTGCAGAGAACTTTGTAAAGC
	50nt-g2	GCTGAGATAGTATAGGCCACTCCATTGCTCTTGCAGAGAACTTTGTAAAG
	50nt-g3	TGCTGAGATAGTATAGGCCACTCCATTGCTCTTGCAGAGAACTTTGTAAA
	50nt-g4	GTGCTGAGATAGTATAGGCCACTCCATTGCTCTTGCAGAGAACTTTGTAA
	50nt-g5	GGTGCTGAGATAGTATAGGCCACTCCATTGCTCTTGCAGAGAACTTTGTA
	50nt-g6	TGGTGCTGAGATAGTATAGGCCACTCCATTGCTCTTGCAGAGAACTTTGT
	50nt-g7	GTGGTGCTGAGATAGTATAGGCCACTCCATTGCTCTTGCAGAGAACTTTG
	50nt-g8	CACAGTGGTGCTGAGATAGTATAGGCCACTCCATTGCTCTTGCAGAGAAC
	50nt-g9	TCACAGTGGTGCTGAGATAGTATAGGCCACTCCATTGCTCTTGCAGAGAA
	50nt-g10	AGTGGTGCTGAGATAGTATAGGCCACTCCATTGCTCTTGCAGAGAACTTT
	50nt-g11	CAGTGGTGCTGAGATAGTATAGGCCACTCCATTGCTCTTGCAGAGAACTT
	50nt-g12	ACAGTGGTGCTGAGATAGTATAGGCCACTCCATTGCTCTTGCAGAGAACT
	30nt-g1	TAGTATAGGCCACTCCATTGCTCTTGCAGA
	30nt-g2	ATAGTATAGGCCACTCCATTGCTCTTGCAG
	30nt-g3	GATAGTATAGGCCACTCCATTGCTCTTGCA
	30nt-g4	AGATAGTATAGGCCACTCCATTGCTCTTGC
	30nt-g5	GAGATAGTATAGGCCACTCCATTGCTCTTG
	30nt-g6	TGAGATAGTATAGGCCACTCCATTGCTCTT

	30nt-g7	CTGAGATAGTATAGGCCACTCCATTGCTCT
	30nt-g8	GCTGAGATAGTATAGGCCACTCCATTGCTC
	30nt-g9	TGCTGAGATAGTATAGGCCACTCCATTGCT
	30nt-g10	GTGCTGAGATAGTATAGGCCACTCCATTGC
	30nt-g11	GGTGCTGAGATAGTATAGGCCACTCCATTG
	30nt-g12	AGTATAGGCCACTCCATTGCTCTTGCAGAG
	30nt-g13	TAGGCCACTCCATTGCTCTTGCAGAGAACT
	30nt-g14	ATAGGCCACTCCATTGCTCTTGCAGAGAAC
	30nt-g15	TATAGGCCACTCCATTGCTCTTGCAGAGAA
	30nt-g16	GTATAGGCCACTCCATTGCTCTTGCAGAGA
RNA base editing sgRNA for exon 54	50nt-g1	TGTAATTCATACCTTTTATGAATGCTTCTCCAAGAGGCATTGATATTCTC
	50nt-g2	GTAATTCATACCTTTTATGAATGCTTCTCCAAGAGGCATTGATATTCTCT
	50nt-g3	CATACCTTTTATGAATGCTTCTCCAAGAGGCATTGATATTCTCTGTTATC
	50nt-g4	TCATACCTTTTATGAATGCTTCTCCAAGAGGCATTGATATTCTCTGTTAT
	50nt-g5	TTCATACCTTTTATGAATGCTTCTCCAAGAGGCATTGATATTCTCTGTTA
	50nt-g6	ATTCATACCTTTTATGAATGCTTCTCCAAGAGGCATTGATATTCTCTGTT
	50nt-g7	AATTCATACCTTTTATGAATGCTTCTCCAAGAGGCATTGATATTCTCTGT
	50nt-g8	TAATTCATACCTTTTATGAATGCTTCTCCAAGAGGCATTGATATTCTCTG
	50nt-g9	ATACCTTTTATGAATGCTTCTCCAAGAGGCATTGATATTCTCTGTTATCA
	50nt-g10	TACCTTTTATGAATGCTTCTCCAAGAGGCATTGATATTCTCTGTTATCAT
	50nt-g11	ACCTTTTATGAATGCTTCTCCAAGAGGCATTGATATTCTCTGTTATCATG
	50nt-g12	CCTTTTATGAATGCTTCTCCAAGAGGCATTGATATTCTCTGTTATCATGT
	30nt-g1	CATACCTTTTATGAATGCTTCTCCAAGAGG
	30nt-g2	TCATACCTTTTATGAATGCTTCTCCAAGAG
	30nt-g3	TTCATACCTTTTATGAATGCTTCTCCAAGA
	30nt-g4	ATACCTTTTATGAATGCTTCTCCAAGAGGC
	30nt-g5	TACCTTTTATGAATGCTTCTCCAAGAGGCA
	30nt-g6	ACCTTTTATGAATGCTTCTCCAAGAGGCAT
	30nt-g7	TCTCCAAGAGGCATTGATATTCTCTGTTAT

	30nt-g8	TTCTCCAAGAGGCATTGATATTCTCTGTTA
	30nt-g9	TATGAATGCTTCTCCAAGAGGCATTGATAT
	30nt-g10	TTATGAATGCTTCTCCAAGAGGCATTGATA
	30nt-g11	TTTATGAATGCTTCTCCAAGAGGCATTGAT
	30nt-g12	TTTTATGAATGCTTCTCCAAGAGGCATTGA
	30nt-g13	CTTTTATGAATGCTTCTCCAAGAGGCATTG
	30nt-g14	CCTTTTATGAATGCTTCTCCAAGAGGCATT
	30nt-g15	CTTCTCCAAGAGGCATTGATATTCTCTGTT
	30nt-g16	GCTTCTCCAAGAGGCATTGATATTCTCTGT
	30nt-g17	TGCTTCTCCAAGAGGCATTGATATTCTCTG
	30nt-g18	AATGCTTCTCCAAGAGGCATTGATATTCTC
	30nt-g19	GAATGCTTCTCCAAGAGGCATTGATATTCT
	30nt-g20	TGAATGCTTCTCCAAGAGGCATTGATATTC
	30nt-g21	ATGAATGCTTCTCCAAGAGGCATTGATATT
	30nt-g22	ATGCTTCTCCAAGAGGCATTGATATTCTCT

Supplemental sequences AAV constructs' sequences of C1 to C4, E1 to E4 and M1 to M3

C1 to C4 AAV constructs are CBh-driven mxABE. CBh promoter sequence is highlighted in red

E1 to E4 AAV constructs are EFS-driven mxABE. EFS promoter sequence is highlighted in green

M1 to M3 AAV constructs are MHCK7-driven mxABE. MHCK7 promoter sequence is highlighted in blue

>C1
CCTGCAGGCAGCTGCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCG
TCGGGCGACCTTTGGTCGCCCGGCCTCAGTGAGCGAGCGAGCGCGCA
GAGAGGGAGTGGCCAACTCCATCACTAGGGGTTCCTGCGGCCTCTAGAg
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>C2
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