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Supplemental Information

Gene Therapy via *Trans*-Splicing for *LMNA*-Related Congenital Muscular Dystrophy

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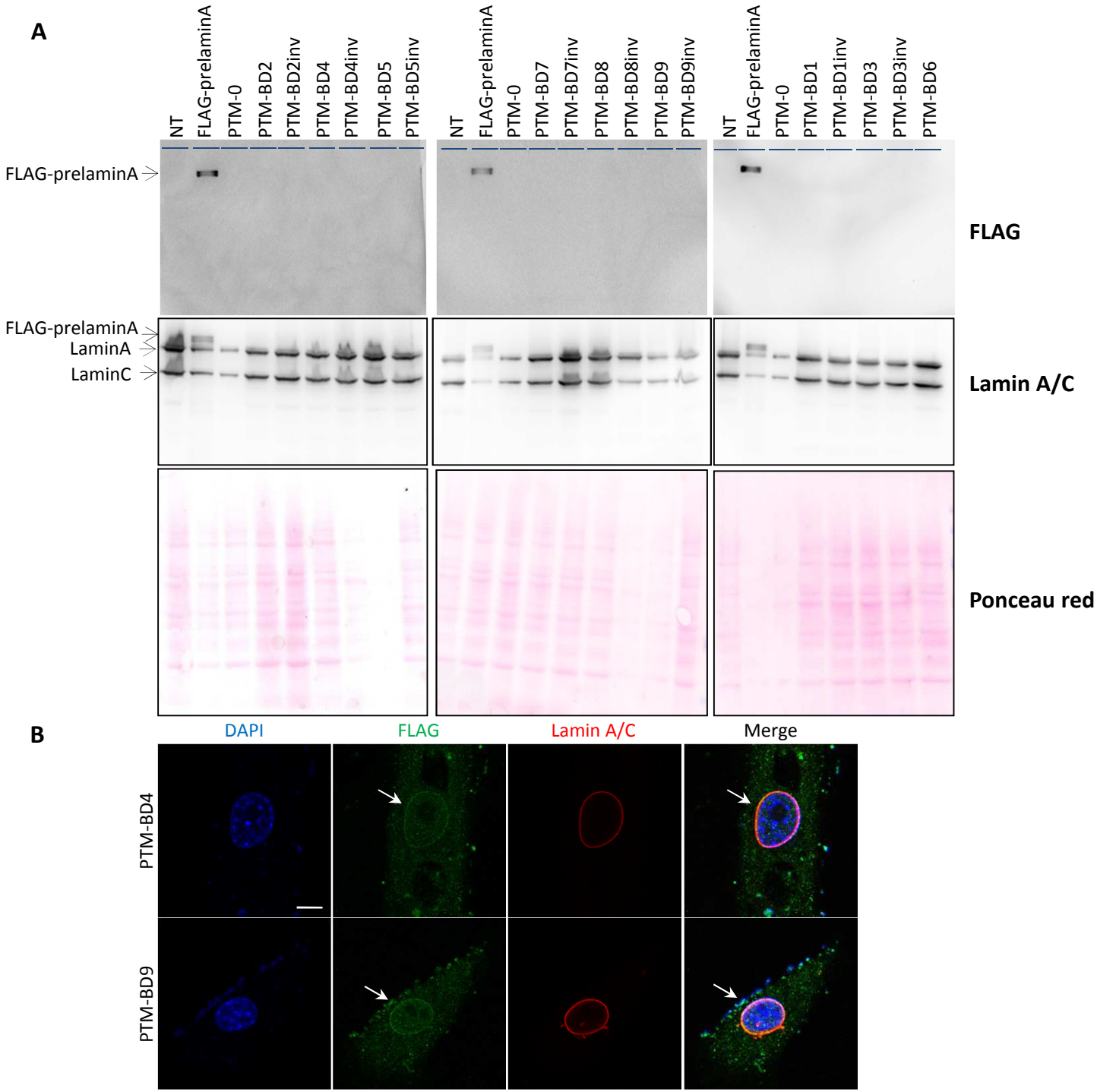
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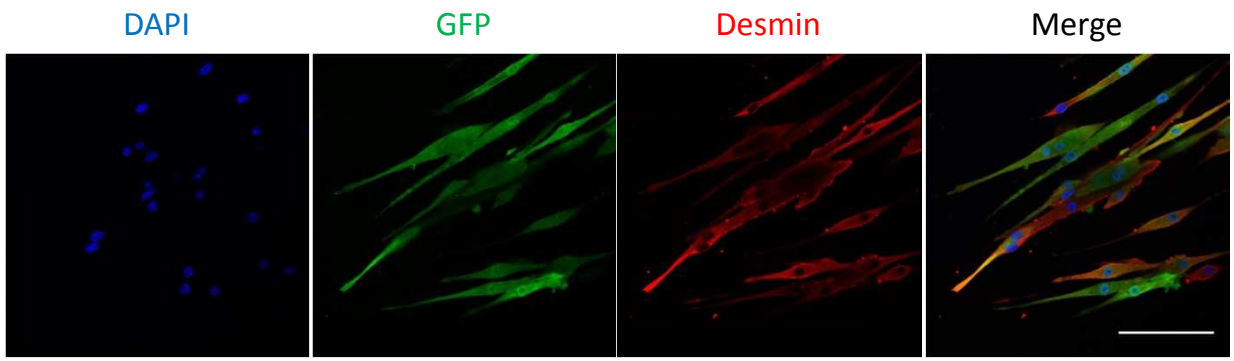
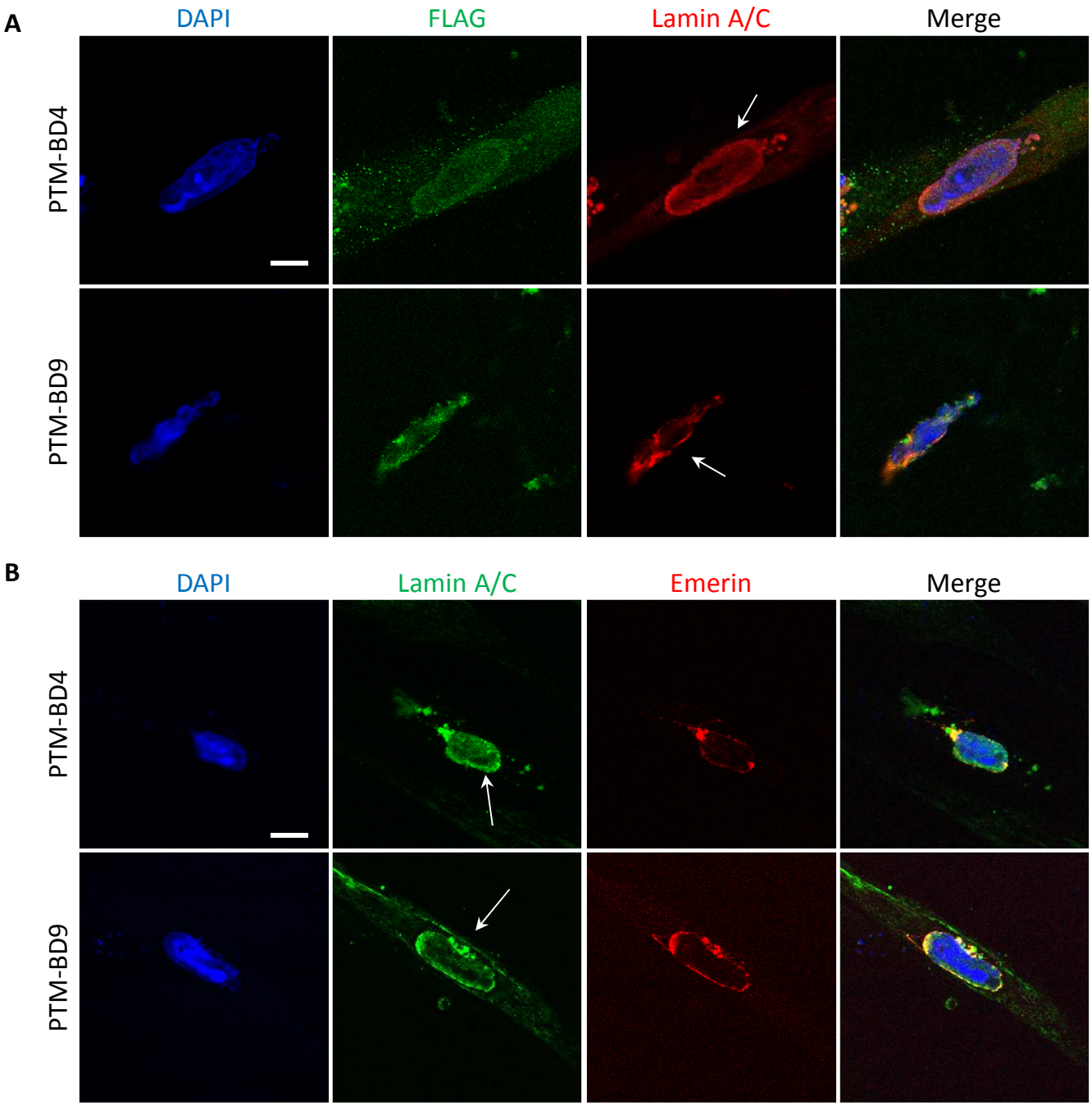
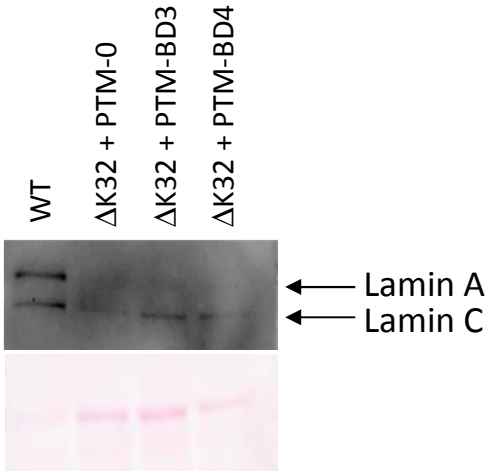
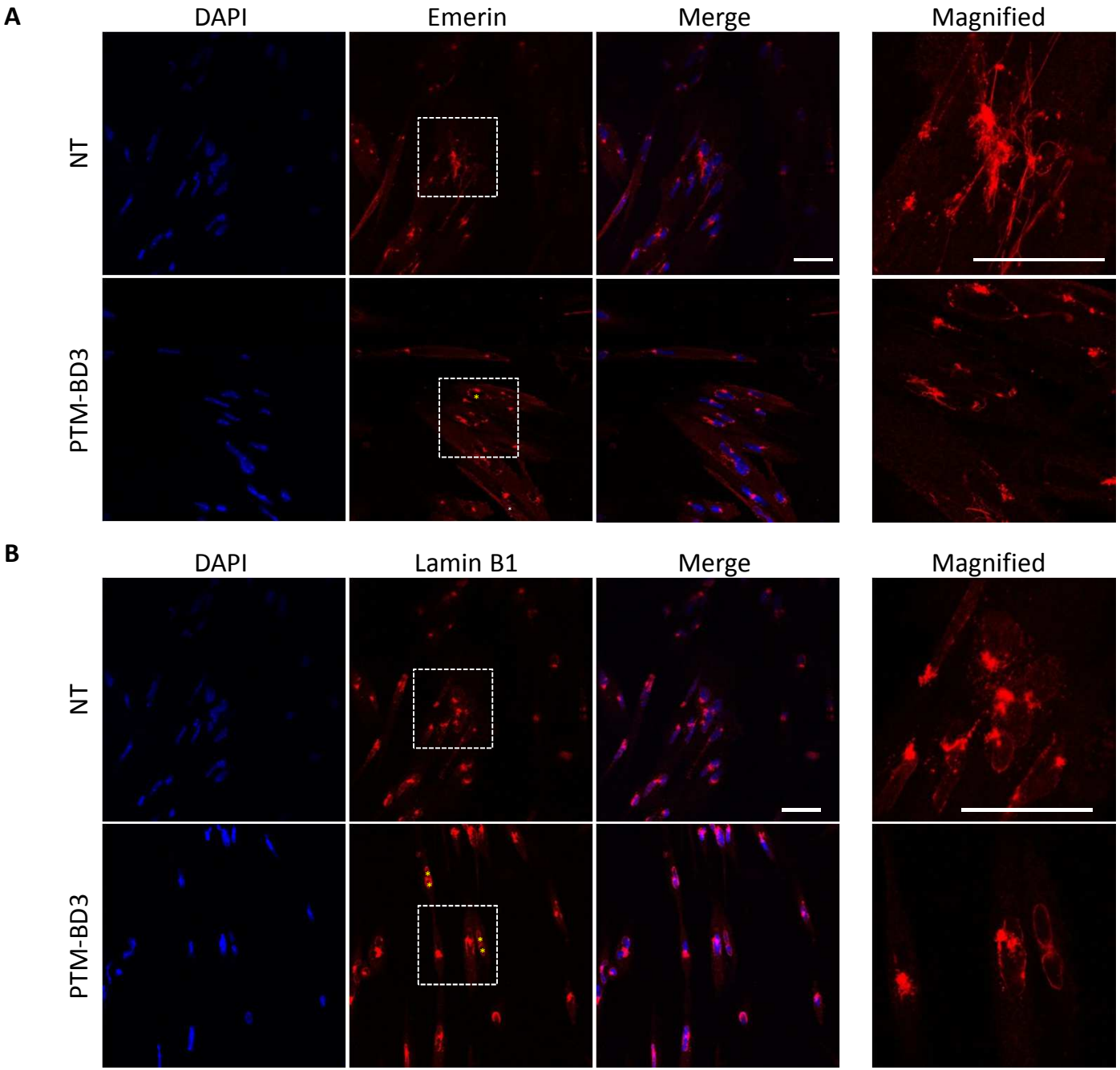
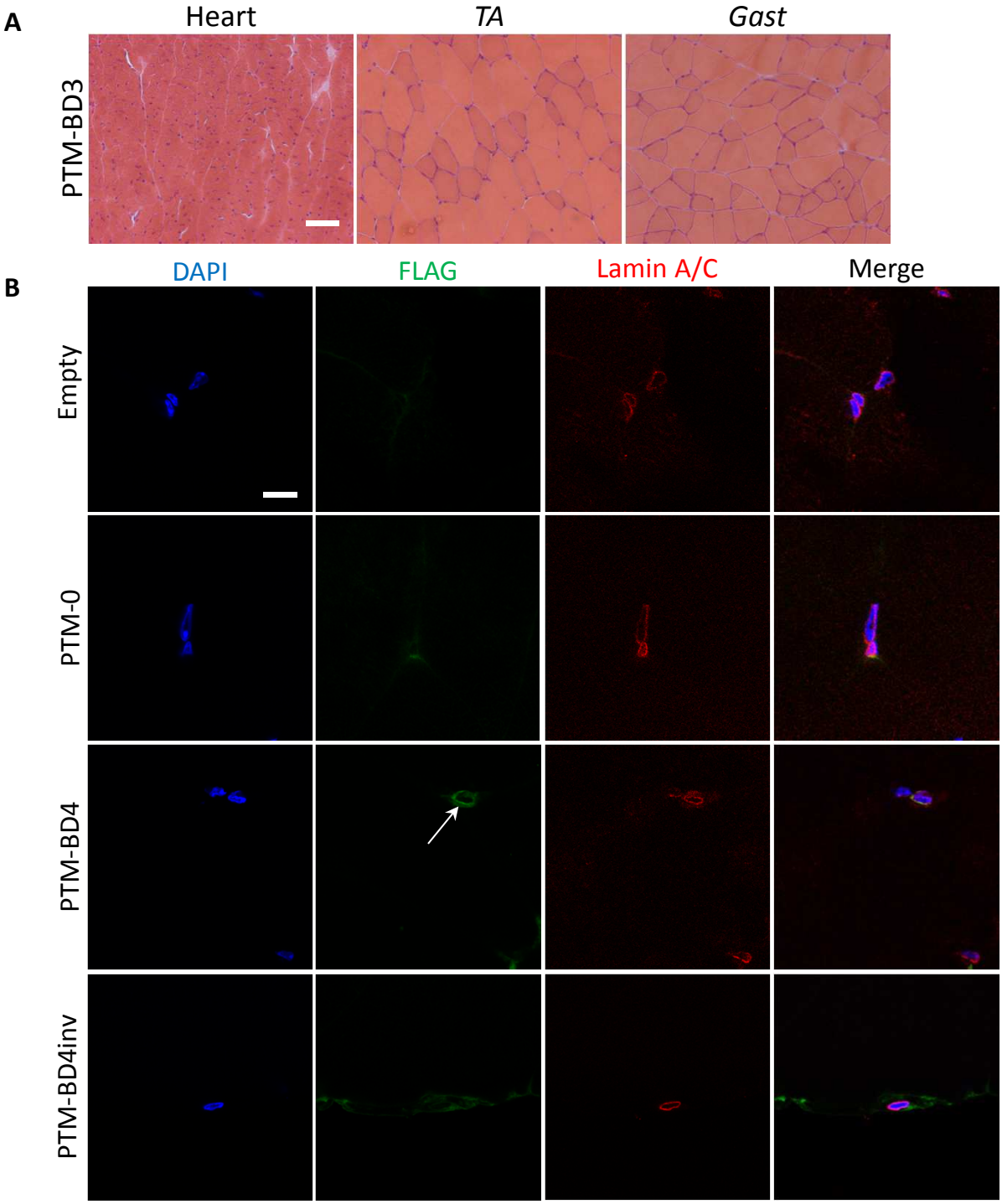
NT
FLAG-prelaminA
PTM-0
PTM-BD2
PTM-BD2inv
PTM-BD4
PTM-BD4inv
PTM-BD5
PTM-BD5inv
NT
FLAG-prelaminA
PTM-0
PTM-BD7
PTM-BD7inv
PTM-BD8
PTM-BD8inv
PTM-BD9
PTM-BD9inv
NT
FLAG-prelaminA
PTM-0
PTM-BD1
PTM-BD1inv
PTM-BD3
PTM-BD3inv
PTM-BD6
FLAG-prelaminA
FLAG
FLAG-prelaminA
LaminA
LaminC
Lamin A/C
Ponceau red
B
DAPI
FLAG
Lamin A/C
Merge
PTM-BD4
PTM-BD9


DAPI
GFP
Desmin
Merge


A
DAPI
FLAG
Lamin A/C
Merge
PTM-BD4
PTM-BD9
B
DAPI
Lamin A/C
Emerin
Merge
PTM-BD4
PTM-BD9


WT
ΔK32 + PTM-0
ΔK32 + PTM-BD3
ΔK32 + PTM-BD4
Lamin A
Lamin C


A
DAPI
Emerin
Merge
Magnified
NT
PTM-BD3
B
DAPI
Lamin B1
Merge
Magnified
NT
PTM-BD3


A
Heart
TA
Gast
PTM-BD3
B
DAPI
FLAG
Lamin A/C
Merge
Empty
PTM-0
PTM-BD4
PTM-BD4inv
C
Heart
Liver
Gast/Sol
NT
WT
Lmna^ΔK32/ΔK32
WT
Lmna^ΔK32/ΔK32
WT
Lmna^ΔK32/ΔK32
Trans-spliced Lmna
PTM
Total Lmna

Supplemental information

Figure S1: Protein evaluation of *trans*-splicing *in vitro*.

(**A**) Representative western blot analysis for FLAG and lamin A/C expression relative to Ponceau red from C2C12 cells transfected or not (NT) with FLAG-prelaminA, various PTM-BD and PTM-BDinv. No trans-splicing event is detected by western blot. (**B**) Immunofluorescence analysis of C2C12 transfected with PTM-BD4 and PTM-BD9. Cells were double-stained with anti-FLAG (green) and anti-Lamin A/C (red) antibodies. Nuclei were stained with DAPI (blue). Arrows point on FLAG+ nuclei. (Scale bar: 10 µm).

Figure S2: Evaluation of the transduction rate in mouse primary myotubes.

Immunofluorescent confocal micrographs of WT mouse primary myotubes transduced with AAV2/9-CMV-GFP and stained for GFP (green) and Desmin (red). Nuclei were stained with DAPI (blue). (Scale bar: 100 µm).

Figure S3: Detection of repaired *Lmna* mRNA induced by 5' *trans*-splicing in mouse *Lmna*$^{\Delta K32/\Delta K32}$ primary myotubes.

Immunofluorescence analysis of *Lmna*$^{\Delta K32/\Delta K32}$ myotubes transduced with AAV2/9-PTM-BD4 and PTM-BD9. Cells were double-stained with anti-FLAG (green) and anti-lamin A/C (red) antibodies (**A**) or with anti-lamin A/C (green) and anti-emerin (red) antibodies (**B**). Nuclei were stained with DAPI (blue). Arrows show relocalization of lamin A/C to nuclear envelope. (Scale bar: 10 µm).

<u>Figure S4:</u> *In vitro* validation of *Lmna* 5' *trans*-splicing in primary mouse mutant myotubes.

Western blot analysis for lamin A/C expression relative to Ponceau red from primary mouse WT myotubes and mutant (ΔK32) myotubes transduced either with AAV2/9-PTM-0, -BD3 or -BD4. Transduction with AAV2/9-PTM-BD3 and -BD4 induces a small increase in lamin A/C protein level.

Figure S5: Improve in emerin and lamin B1 localization after *Lmna* 5' *trans*-splicing in vitro.

Immunofluorescence against emerin (**A**) or lamin B1 (**B**) in non-transduced (NT) or PTM-BD3-transduced primary mutant (ΔK32) myotubes. Nuclei are counterstained with DAPI. Stars highlighted nuclei with corrected emerin or lamin B1 localization. Pictures on the right are a magnification of areas delimited with white doted squares. (Scale bar: 50 µm).

<u>Figure S6:</u> Evaluation of *trans*-splicing efficiency *in vivo*.

(**A**) Hematoxylin eosin staining of striated muscles sections from WT mice 50 days post-systemic injection with AAV2/9 expressing PTM-BD3. (Scale bar: 50 µm). *TA*: *Tibialis anterior*; *Gast*: *Gastrocnemius*. (**B**) Immunofluorescence analysis of *Tibialis anterior* sections from WT mice 50 days post-systemic injection with AAV2/9-PTM-0, -PTM-BD4 and PTM-BD4inv. Cells were double-stained with anti-FLAG (green) and anti-Lamin A/C (red) antibodies. Nuclei were stained with DAPI (blue). (Scale bar: 10 µm). (**C**) PCR analysis was performed with primers specific for *trans*-spliced *Lmna* (F-FLAG and R-*Lmna* exon 7), PTM (F-FLAG and R-*Lmna* exon 1) or total *Lmna* (F-*Lmna* exon 1 and R-*Lmna* exon 1) on RNA extracts from 15 days-old mouse heart, *Gastrocnemius* and liver after AAV2/9-PTM-BD4.

<u>Supplemental Table 1</u>: Sequence of the different binding domains targeting intron 5 of *Lmna* pre-mRNA.

BD1	CCCTGGAAAGCCAGACAGGCATCAGATTCAGAACCATAGCATGTGGTCCTGGAGTCCCTCCCCCACCC CCTTCTCAGTGGGCTTCTGCAGTATCTGCTTAGACCAGAGCCAAGGTGGGAGGGGTAAACTGAGGGTC ACCTG
BD2	CATGTCCAGTCCAAGGGAAGCTCTATCTCCAAGTAGGGAATGCGGCTGGGTTTGTAACTCAGTGAGAA AGCACCTATCTACCAAGCAAGAGGCCTCTGGTTCAGTCTCCGGCAGGCACAGAAGAAACATGAAAAAC CCTTCATGCACA
BD3	TCAGTGAGAAAGCACCTATCTACCAAGCAAGAGGCCTCTGGTTCAGTCTCCGGCAGGCACAGAAGAAACATG AAAAACCCTTCATGCACAGTAAAATAGAGGCTCTGAGGATCAGGGCTAGCTAAAGGAGAGCCCTGGAAAGCC AGACAG
BD4	AGTTAAGGGTGACCTTGAATCTCTGATACTTCAACCTGAGATAAAAGGCATGCACCACCCAGTTCCCTGTGG TCCTGGGGATAGAAGCCAGGCCATCGTGTATAATAAGCAAGCACTGTACCAACTGAGCTACATGTCCAGTCC AAGGGA
BD5	CTGCCTCCCGAGTGCTGGGATTAAAGGCCTGTGCTATTACAACCTGGCTTCTTTTCTTTCTTTTTTGAGATA AGGTCTGGTAGTCTAGGCTGGTCTCAAACTTGCTGTATAGTTAAGGGTGACCTTGAATCTCTGATACTTCAA CCTGAG
BD6	TGTTTGTTTGTGGTATTTTGGTTTGGTTTGGTTTTTGGTTTTTCAAGACAGGGTTTCTCTGTATAGCCCTGG CTGTCCTGGAACTCACTTTGTAGACCAGGCTGGCCTCGAACTCAGAAATCCTCCTGCCTCTGCCTCCCGAGT GCTGGG
BD7	CACTGCAGTGTCATGACAGACCTGGGAGTCCCGGAGGACAAGAATGTTCATCATTTTCCACTTTTTTTTTTA AGGATTTTTATTTATGTGTTGTTTGTTTGTTTGTTTGTGGTATTTTGGTTTGGTTTGGTTTTTGGTTTTTCA AGACAG
BD8	TCTGTCTGAAACCTCTCATTCCGAGGCTAGACCCCCATCTTCTTTTGTCACAAATAGTGTAGCTATTGTGAC AAATAATATCGACACAAGTAACATTTCACACTGCAGTGTCATGACAGACCTGGGAGTCCCGGAGGACAAGAA TGTTCA
BD9	AAGGGCTGATGTCGATGAAGAGGGAGGGGCACAGGGGCAGGCAGAGGGACACTCAGGATCTGTAGGCTCTCT CTAGAGCCACCATGGCCTTATCCAGTGACCAGGAAGCCCTTCCTAATGTCTGTCTGAAACCTCTCATTCCGA GGCTAG

<u>Supplemental Table 2:</u> PCR primers used to amplify trans-spliced *Lmna* mRNA, total *Lmna* mRNA and PTM mRNA.

Primer	**Sequence**
F-FLAG	ATGGACTACAAGGACGACGA
F-*Lmna* ex1	GCCAGCTCTACCCCACTGT
R-*Lmna* ex1	CAGACTCAGTGATGCGAAGG
F-*Lmna* ex2	GGGGACTTGTTGGCTGCGCA
R-*Lmna* ex6-1	TCCAGGGCCAGCTTGATGTCCAG
R-*Lmna* ex9-2	CCATCTCTCGCTCTTTCTCA
R-*Lmna* ex7	CCGCACGAACTTTCCCTCTT