

Supplementary information

Novel EGFP reporter cell and mouse models for sensitive imaging and quantification of exon skipping

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Figure S1. Schematic diagram of the exon-skip therapy for *mdx*-type mutation by ASO

Figure S2. Chemical structure of ASOs used in this study

Figure S3. Result from modified ASOs transfection to WT primary myotubes

Figure S4. Determining the copy number of EGFP-transgenic mice by qPCR

Figure S5. Successful exon 23 skipping evaluated by RT-PCR in PMO-treated tibialis anterior (TA) muscles of EGFP-mdx23 transgenic mice.

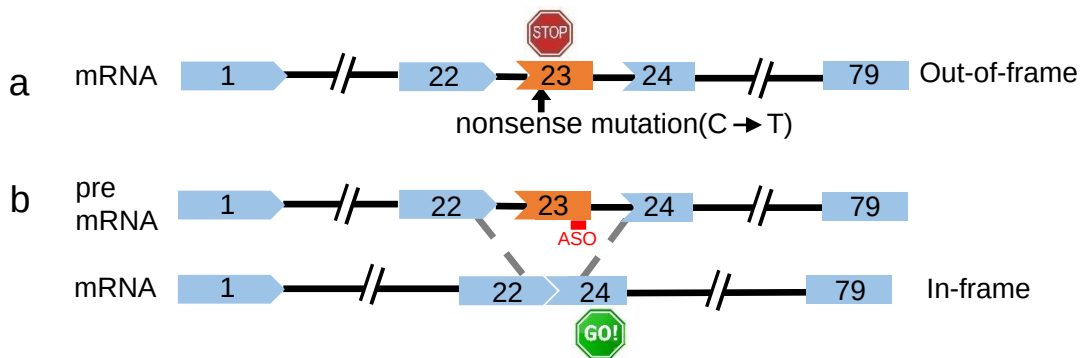


Figure S1. Schematic diagram of the exon-skip therapy for mdx-type mutation by ASO.

(a) Nonsense mutation of exon 23 (C > T) caused frameshift (out-of-frame) and produced a truncated dystrophin protein. (b) ASO targeted skipping exon 23 changes the out-of-frame transcript into in-frame one, allowing the synthesis of short but functional dystrophin. Antisense oligonucleotides: ASO.

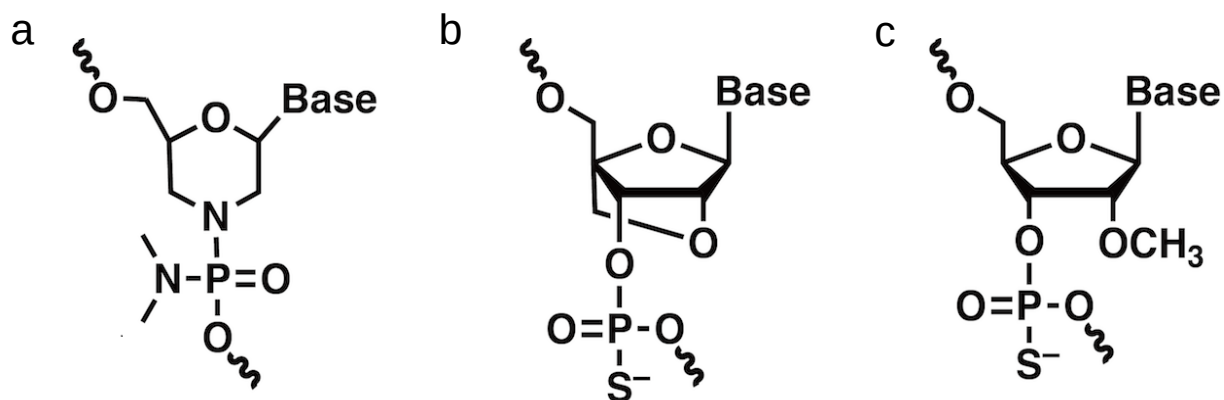


Figure S2. Chemical structure of antisense oligonucleotides using in this study.

- (a) The backbone structure of phosphorodiamidate morpholino oligomer (PMO)
- (b) The backbone structure of locked nucleic acid (LNA)
- (c) The backbone structure of 2'-O-methyl

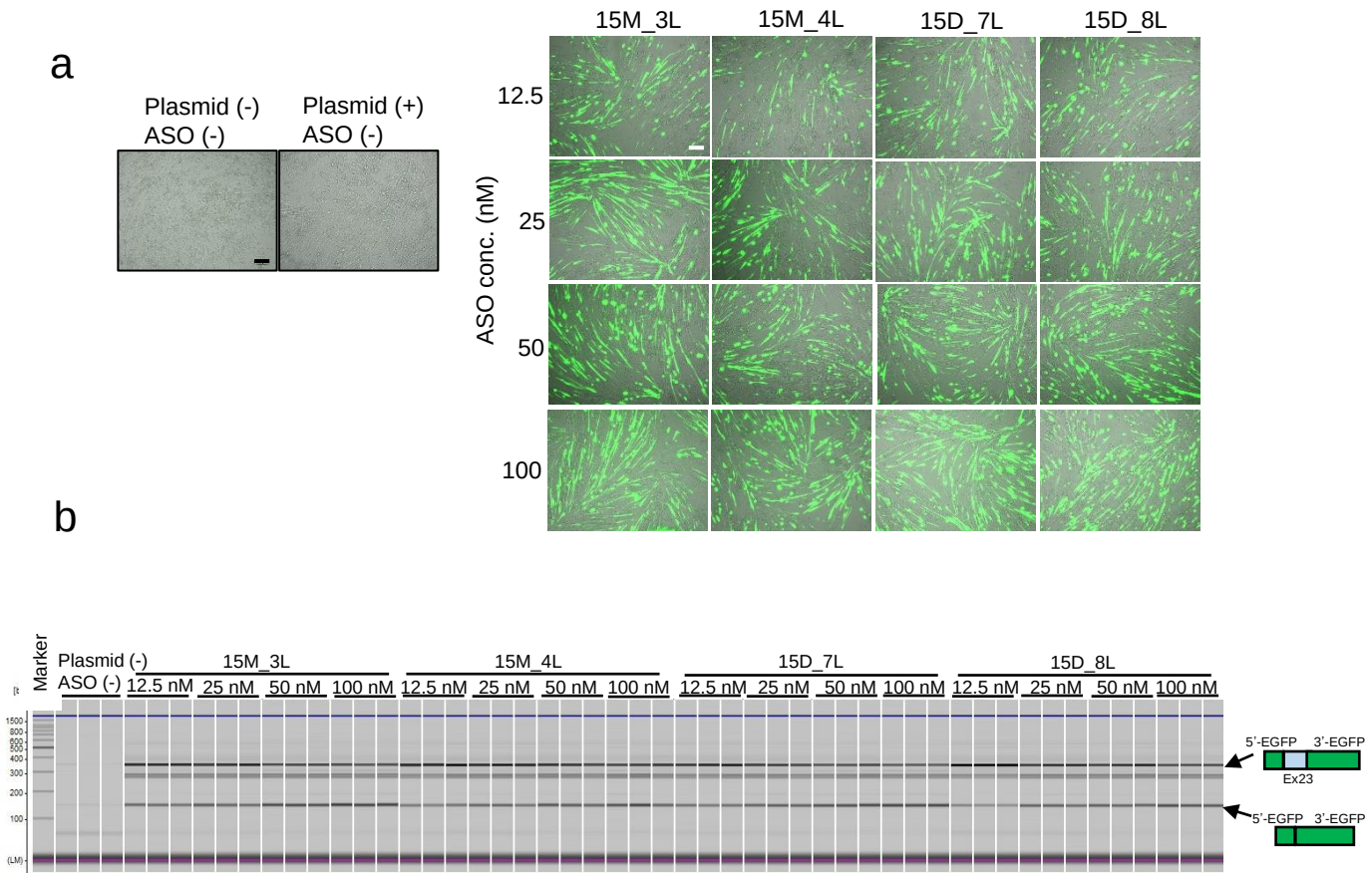


Figure S3. Transfection of LNA/2'-OMe mixmers in WT primary myotubes.

(a) Cells treated with various concentration of mixmers. Scale bar 100 μ m.

(b) The result from RT-PCR for the detection of exon 23 skipping are outlined. The PCR conditions were as follows: 32 cycles of 10 s at 98 $^{\circ}$ C, 30 s at 64 $^{\circ}$ C, and 60 s at 72 $^{\circ}$ C. Antisense oligonucleotides: ASO.

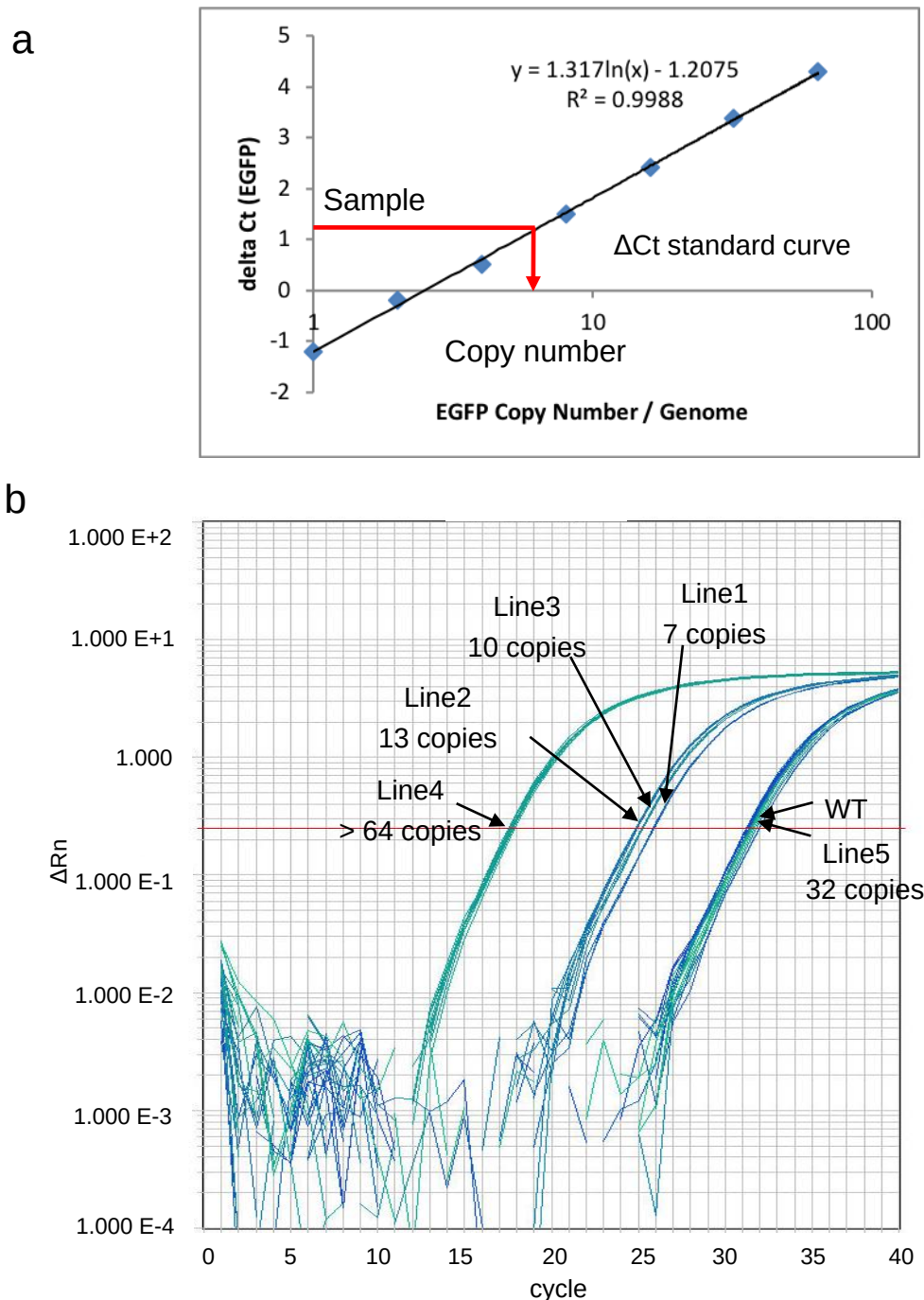


Figure S4. Determining the copy number of EGFP-mdx23 transgenic mice by qPCR

(a) A standard curve is drawn using EGFP-mdx23 / pCAGGS plasmid DNA.

(b) The copy number is calculated from the ΔC_t value of the sample obtained by qPCR.

Although Line 4 mice are estimated to carry more than 64 copies of the transgene, line 2 mice are mainly used for the study. This is because too many exogenous copies may cause instability of the integration sites as well as integrated gene cassettes over the crossing.

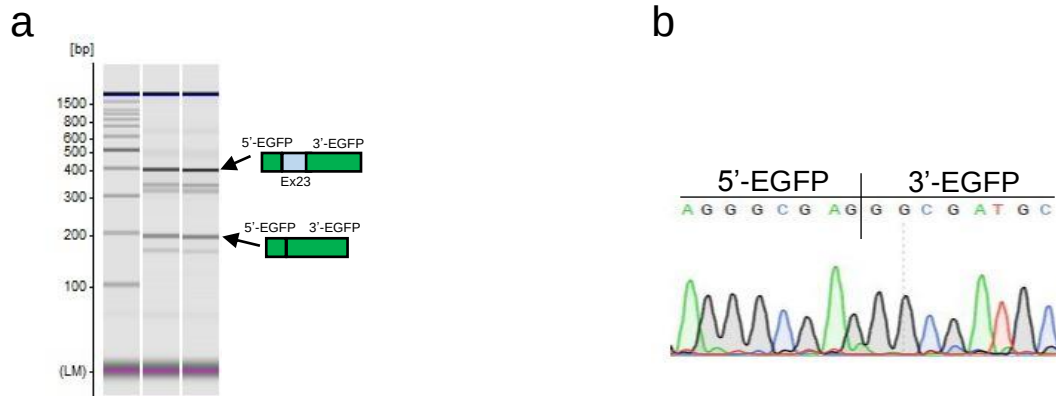


Figure S5. Successful exon 23 skipping evaluated by RT-PCR in PMO-treated tibialis anterior (TA) muscles of EGFP-mdx23 transgenic mice. (a) Result from RT-PCR for exon 23 skipping in TA muscles are summarised. (b) Confirmation of the sequence with exon 23 skipping. Note that 5' - and 3' - fragments of EGFP are correctly fused.