

Supplementary Figure 1. Generation of C2C12-knockout cell clones with CRISPR-Cas9

technique. (a) Electrophoresis of PCR product from C2C12^{Cas9} myoblasts transfected with or without gRNAs. (b) DNA sequence maps around the targeting locus of control and selected MyoD^{KO} cell clones. (c) Detection off-targets of *MyoD* sgRNA. (d) Immunostaining of MF20 (Red) and DAPI (Cyan) in control and MyoD^{KO} C2C12 myoblasts after 3 days induction of differentiation.

Supplementary Figure 2. Overexpression of miR-133 inhibits adipogenesis of MyoD^{KO}

C2C12 myoblasts. (a and b) qPCR analysis of miR-133a (a) and adipogenic marker genes (b).

N=3 different passages of myoblasts. Error bars represent SD, * indicates $p < 0.01$.

Supplementary Figure 3. Loss of MyoD facilitates adipogenic transdifferentiation of

myoblasts *in vivo*. (a) Immunostaining of BODIPY (green) and Dystrophin (red) in TA muscles

of mdx mice injected with control or MyoD^{KO} C2C12 cells. (b) Quantification of Dystrophin⁺

myofibers. (c) Triglyceride concentrations in TA muscles injected with control or MyoD^{KO}

C2C12 cells. N=3 independent biological samples. Four sections were analyzed for each sample.

Error bars represent SD, * indicates $p < 0.05$.

Supplementary Figure 4. MyoD transdifferentiates brown preadipocytes into myocytes. (a)

Immunostaining of BODIPY in MyoD^{OE} brown preadipocytes. (b) Immunostaining of MF20

(red) in control and MyoD^{OE} brown adipocytes. (c) Ratio of MF20 signal in elongated GFP⁺ cells.

N=3 different passages of myoblasts. Error bars represent SD, * indicates $p < 0.01$.

Supplementary Table 1 List of top 5 putative off-targets homologous to *MyoD*-sgRNA

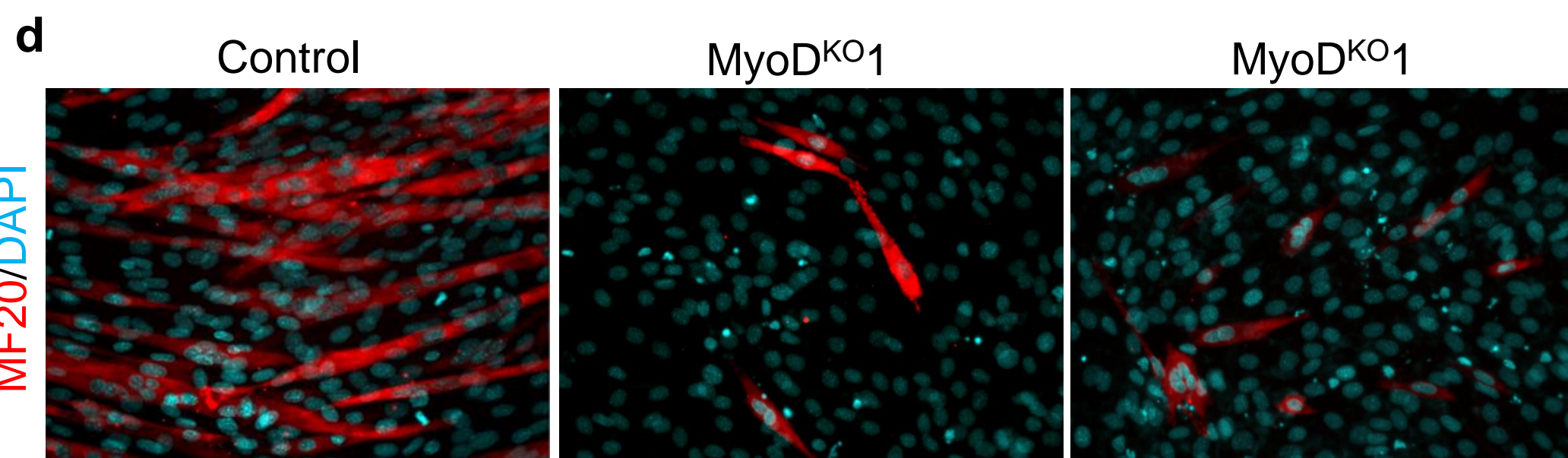
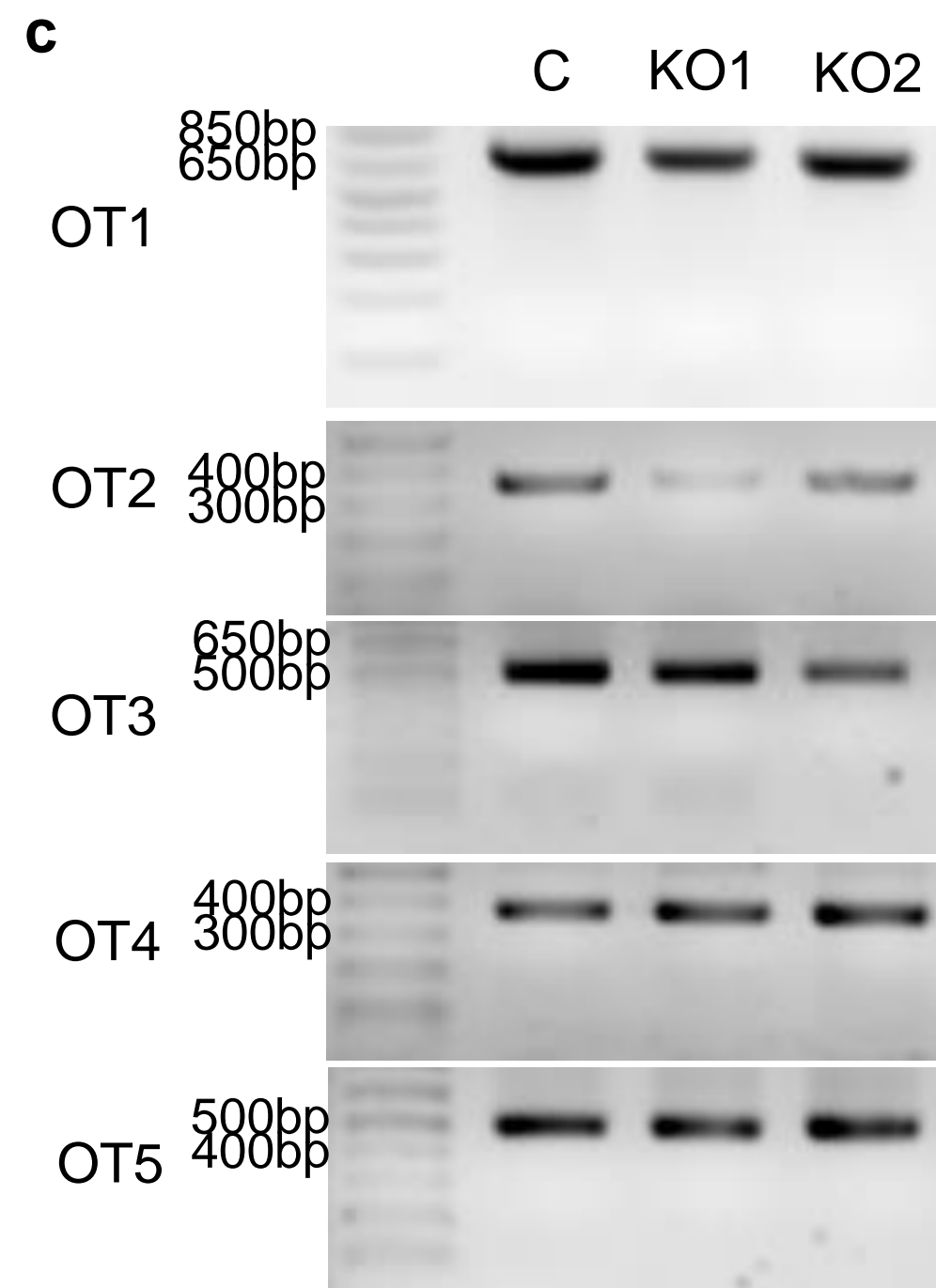
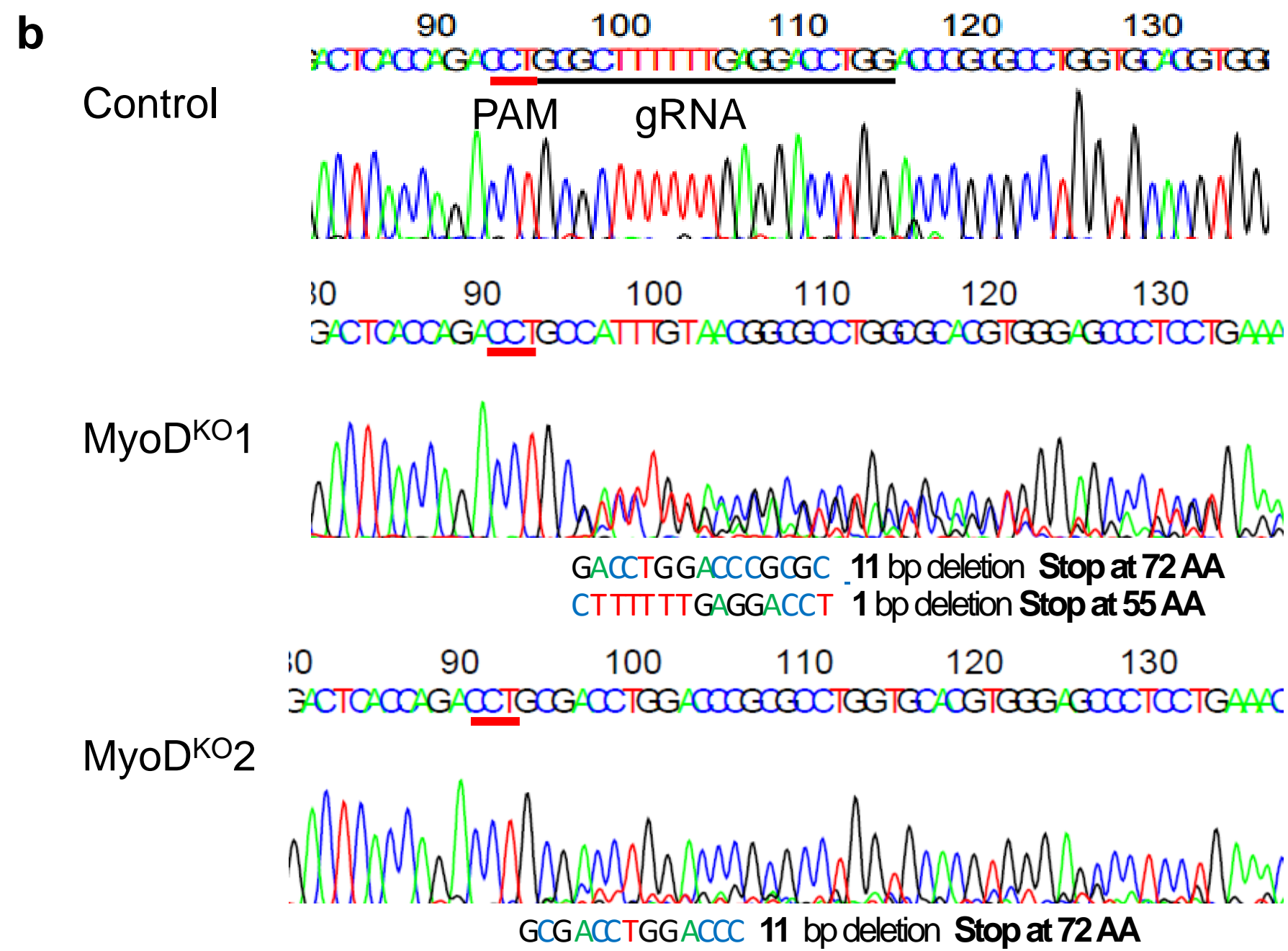
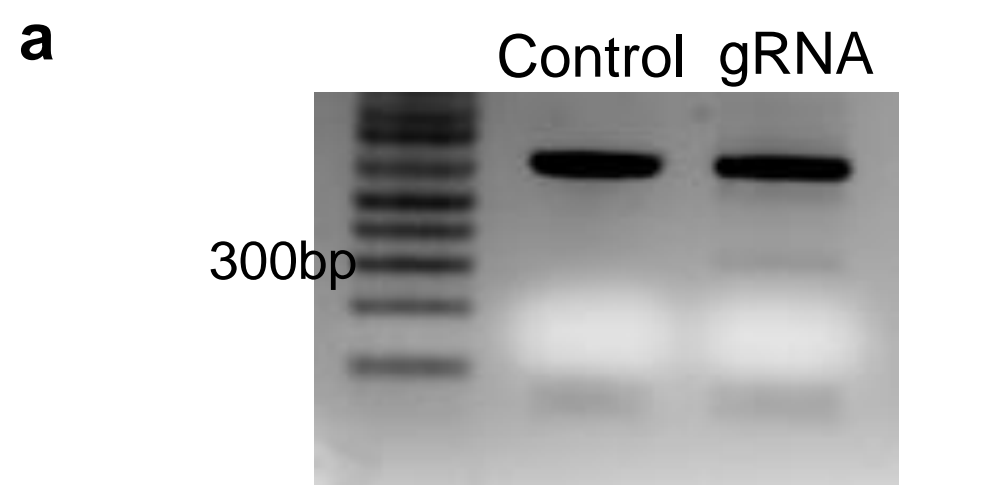
Position MyoD	20	19	18	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1	N	G	G	Locus Chro.:Start position
OT1	C	C	T	A	G	T	C	C	T	C	A	A	A	A	A	A	G	C	T	C	A	G	G	chr1:-106859905
OT2	C	T	A	A	G	T	C	A	T	C	A	A	A	A	A	A	G	C	A	C	T	G	G	chr13:+78086880
OT3	C	C	A	T	C	T	C	A	T	C	A	A	A	A	A	A	G	C	T	C	A	G	G	chr19:+50585297
OT4	A	C	A	C	G	T	G	C	T	C	A	A	A	A	A	A	G	C	A	C	G	A	G	chr1:-39340833
OT5	C	C	A	G	G	T	G	C	T	C	C	A	A	A	A	A	G	C	A	C	A	G	G	chr12:-113574844

PAM is in grey. Bases different from sgRNA are in red.

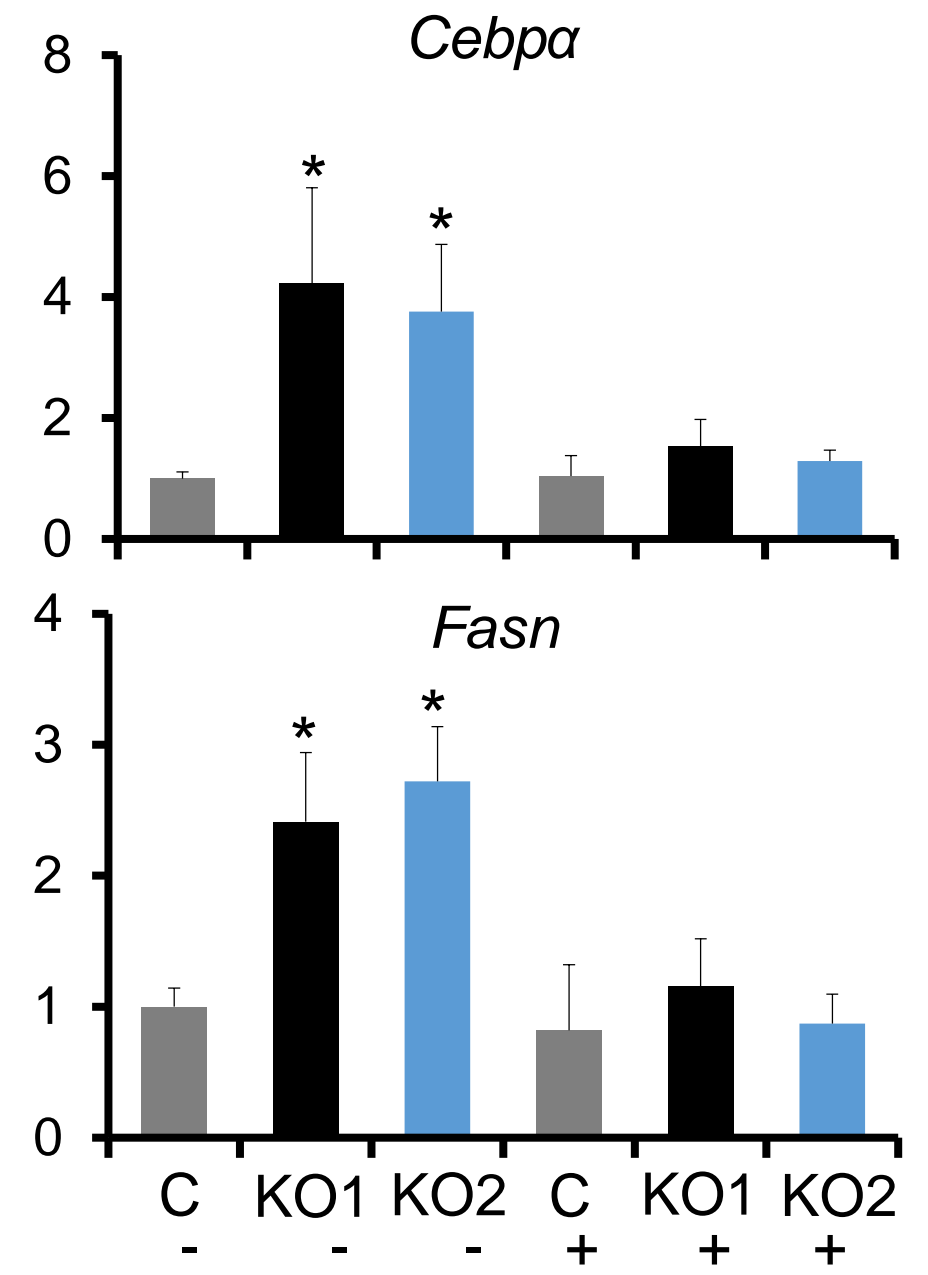
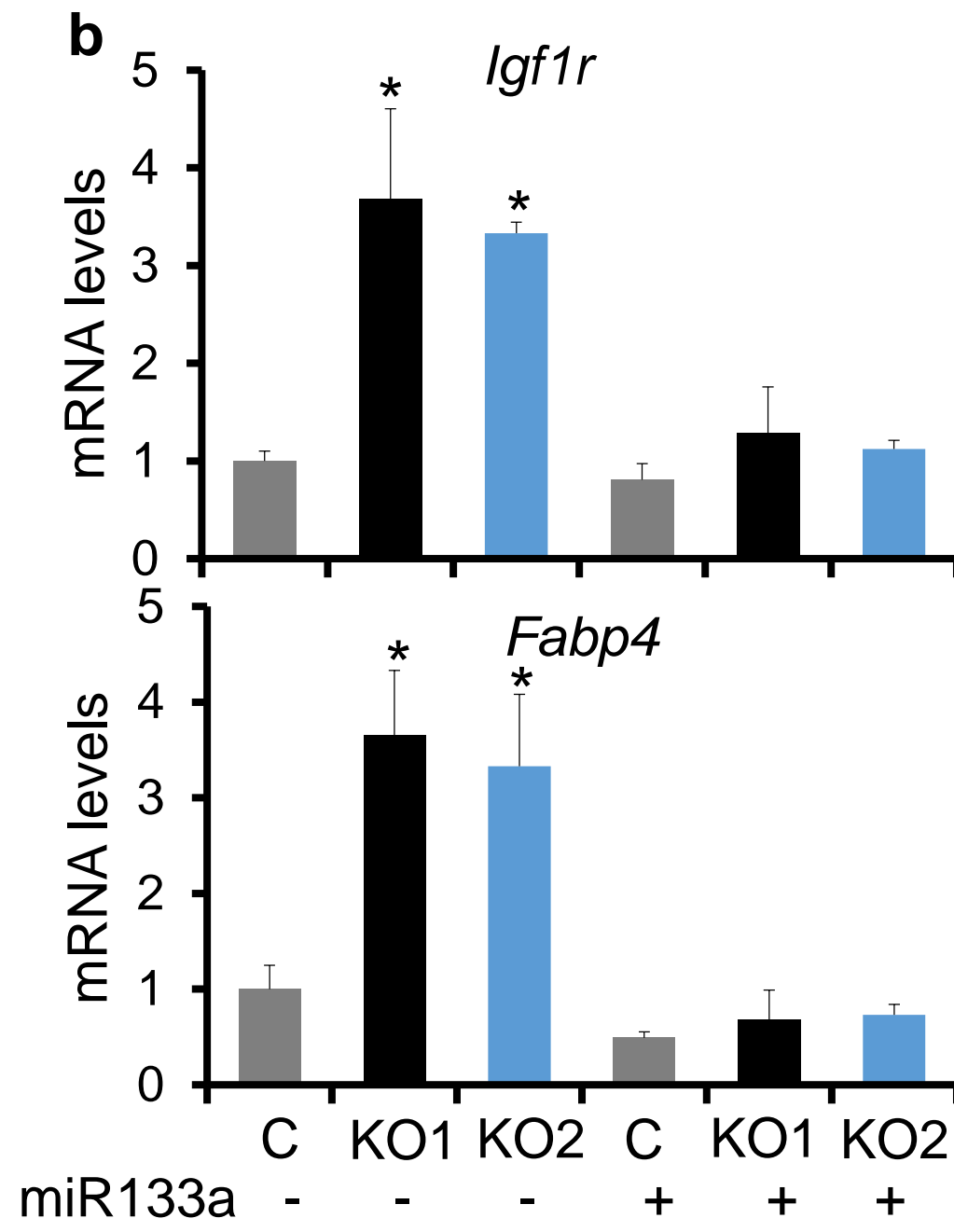
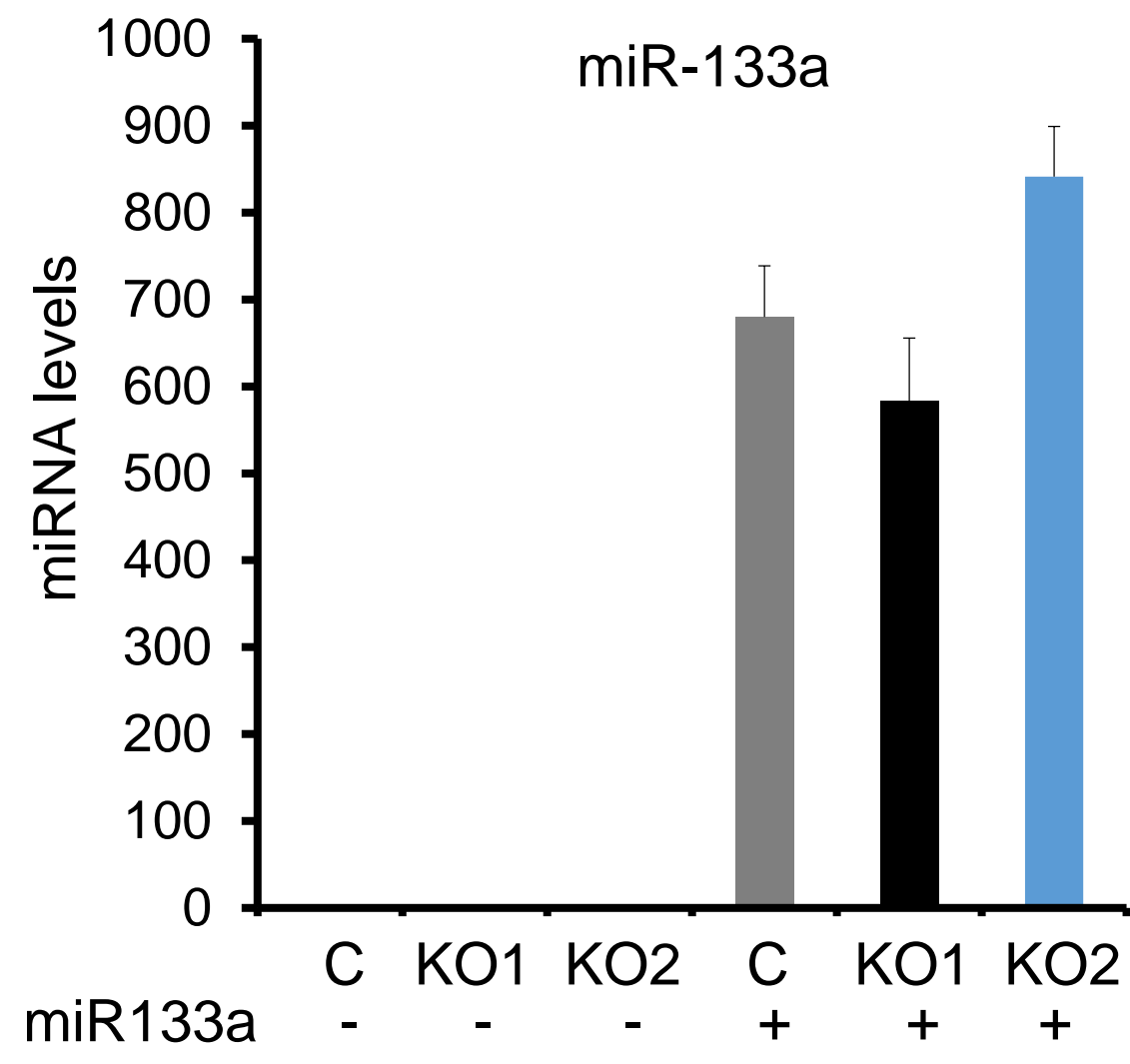
Supplementary Table 2 Primer sequences (5'-3')

Gene	Sequence
OT1	GGCTGAAAGGCTTGTACTCG GGAGCATTTCAGGCATCAAT
OT2	GGTGGAAAGGCTGTTGATGT GCCAGAGTGATCGGAATGAT
OT3	TCCCTGGCTATGCAAAATTC CATTCATGGAGCCTGTGTGT
OT4	ACATGTATGCCAGATGCAA GTGCTCCAACGAGTGAGACA
OT5	CCCAAGTTCATGTCCCTAA TGAGCAAGGTTTGCAGTTTG
<i>MyoD-f</i>	AAGCGGCCGCATGGAGCTTCTATCGCCGC
<i>MyoD-r</i>	TTTCTAGATCAAAGCACCTGATAAATC

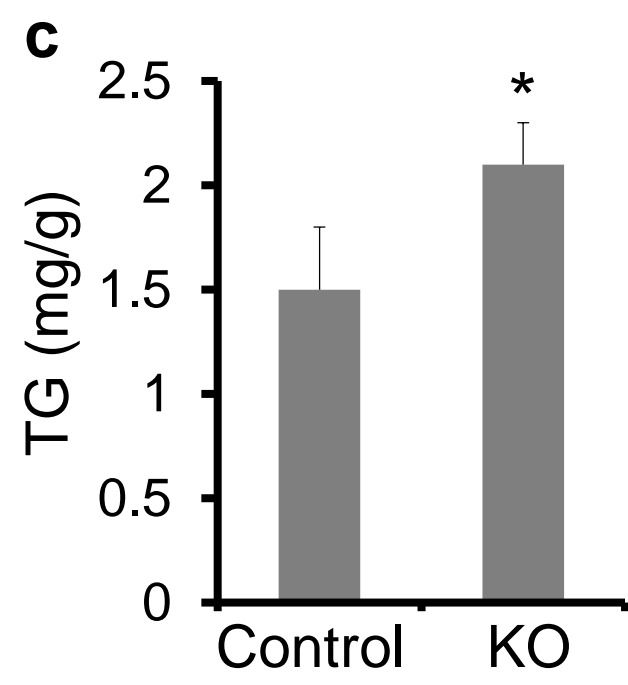
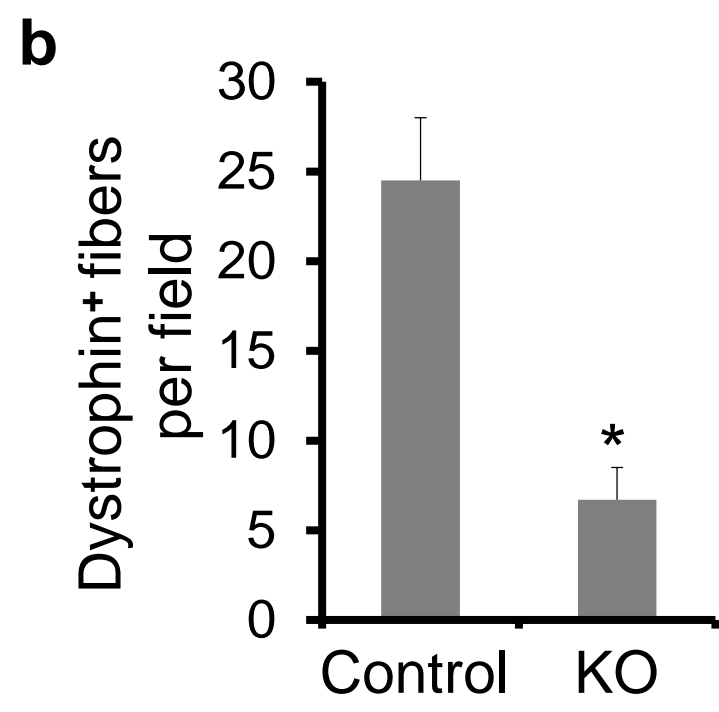
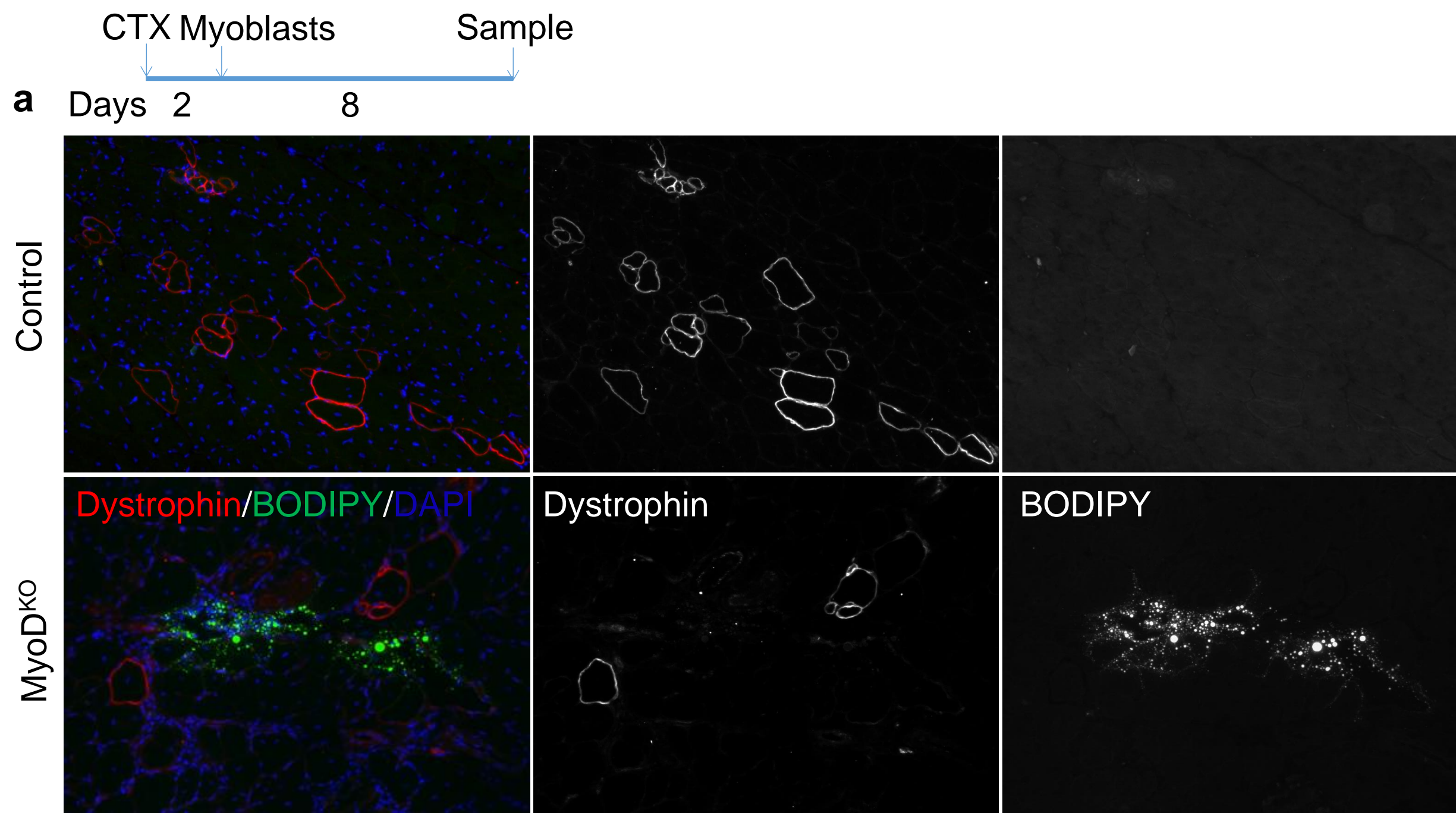
Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3



Supplementary Figure 4

