Supplemental Experimental Procedures

Mice lines

Hdac5$^{-/-}$ *mice* were described previously (Chang et al., 2004). *Hdac4* conditional mutant mice were generated by flanking exon 6 with loxP sites, which results in a frame shift mutation in the *Hdac4* allele (Potthoff et al., 2007). Transgenic mice harboring genomic DNA fragments ligated to the hsp68 basal promoter upstream of a LacZ reporter gene were generated as previously described (Kothary et al., 1989; Williams et al., 2009). Among various lines of transgenic mice, three out of five for *MuRF1*-WT-lacZ and three out of seven for a*trogin-1*-WT-lacZ expressed the lacZ transgene in myofibers. Among various lines of transgenic mice harboring mutant constructs, three out of nine for *MuRF1*-Emut-lacZ and two out of six for *atrogin-1*-Emut-lacZ expressed the lacZ transgene, as seen in a few denervated myofibers.

Mice harboring the *Myog*^{flox} allele mated to CAGGCre-ERTM transgenic mice, which ubiquitously express a conditional Cre-recombinase that is activated by intraperitoneal injection of tamoxifen (10 mg/40g body weight), have been previously described (Knapp et al., 2006). Tamoxifen injections were performed in two month-old mice. The deleted *Myog* allele was detected by quantitative PCR genotyping from genomic DNA one week after tamoxifen injection, using primer sequences described previously (Knapp et al., 2006). Denervation experiments were performed one month after tamoxifen injection and myogenin deletion was confirmed by quantitative real time PCR. Littermates injected with tamoxifen, but not expressing the Cre-recombinase,

were used as controls. All mouse studies were approved by the UT Southwestern Institutional Animal Care and Use Committee.

Plasmid Constructs

The following plasmids were used: pCMV-Snap25-GFP (provided by Tullio Pozzan, University of Padua, Padua, Italy); EMSV-myogenin; pcDNA-E12; pcDNA 3.1 and CMV-lacZ.

Immunoblot

Immunoblots were performed from GP muscles as previously described (Vega et al., 2004). Antibodies against HDAC4 (1:500 in TBST; Sigma) and α-tubulin (1:3,000 in 5% milk TBST; Sigma) were used. ECL Advance Western Blotting Detection Kit (Amersham Biosciences) was used for signal detection.

Electrophoretic Mobility Shift Assays

Oligonucleotides were synthesized (Integrated DNA Technology) corresponding to the E-box sites and mutated binding sites for generating probes used in the gel mobility shift assays. Unlabeled competitor was 200X of labeled probe. Myogenin and E12 were transcribed and translated together using a coupled *in vitro* transcription/translation system (Promega) and DNA binding assays were performed as described (Lu et al., 1999). Oligonucleotides were synthesized as follows (plus strand sequences are shown with the binding sites in bold and the mutations underlined):

MuRF1 E2: GGAATGCT**CAGCTG**GTCCCCTC, <u>TCTTGC</u>;

MuRF1 E3: CTGGGGCT**CATGTG**ACAGAGGT, TCGTGC;

atrogin-1 E-box: CCCGAGGC**CACGTG**GCTTTGTT, TCTTGC.

Taqman Primers

Hdac4: Mm01299542_g1

Hdac5: Mm00515917_m1

Myog: Mm00446194_m1

Myod1: Mm00440387_m1

MuRF1: Mm01185221_m1

Atrogin-1: Mm01207878_m1

GAPDH: Mm99999915_g1.

Sybr Green Primers

Dach2 for: 5'-ACTGAAAGTGGCTTTGGATAA-3';

Dach2 rev: 5'-TTCAGACGCTTTTGCATTGTA-3'

ChIP Primers

Oligonucleotide primers for amplification of E-boxes on the *MuRF1* promoter are:

For: 5'-CGGCAGGGCAACAGCGATTT-3'

Rev: 5'-GTCTTGGTCTGAGGCCCCTC-3'

Oligonucleotide primers for amplification of the E-box on the *atrogin-1* promoter are:

For: 5'-CGCTGCCCCGTCTCTTTGTT-3'

Rev: 5'-GCTTCAAGTTTCCACGGACGC-3'

Oligonucleotide primers for amplification of *GAPDH* are:

For: 5’-ATC CAC GAC GGA CAC ATT GG-3’

Rev: 5’-TGGTGC TGC CAA GGCTGT GG-3’

Luciferase assay

The *MuRF1* reporter plasmid was constructed by ligating 600 bp of murine genomic region upstream of the 5’UTR of the *MuRF1* gene into the pGL3-Basic reporter (Promega). The *MuRF1* genomic fragment was generated by PCR using the following primers:

For: 5’-GGTACCGCAGCAAGCCCCTTTACCAC-3’

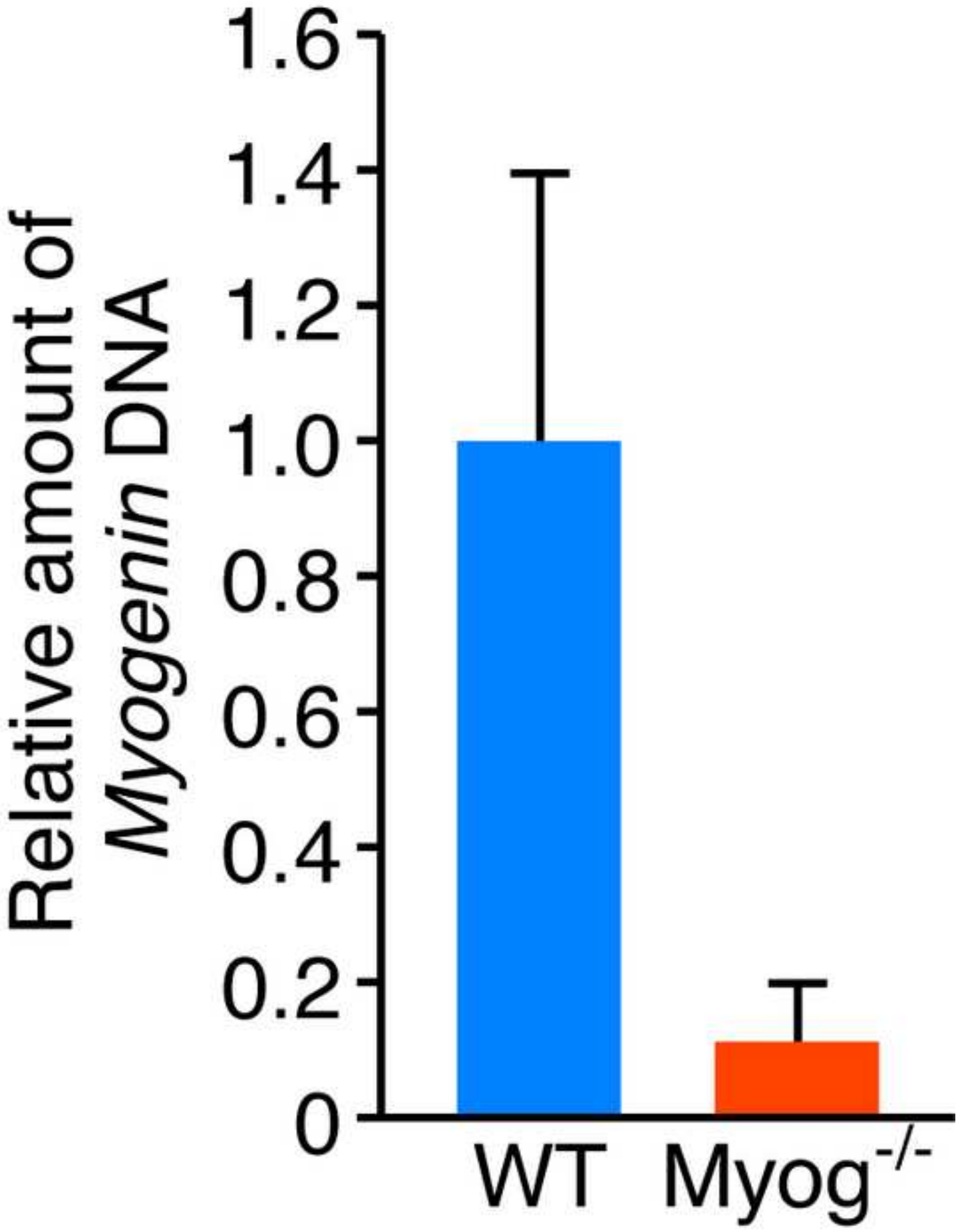
Rev: 5’-CTCGAGGCTGCACCTCTGTCACATGA-3’

The *atrogin-1* reporter plasmid was constructed by linking 712 bp of murine genomic region upstream of the 5’UTR of the *atrogin-1* gene into the pGL3-Basic reporter. The *atrogin-1* genomic fragment was generated by PCR using the following primers:

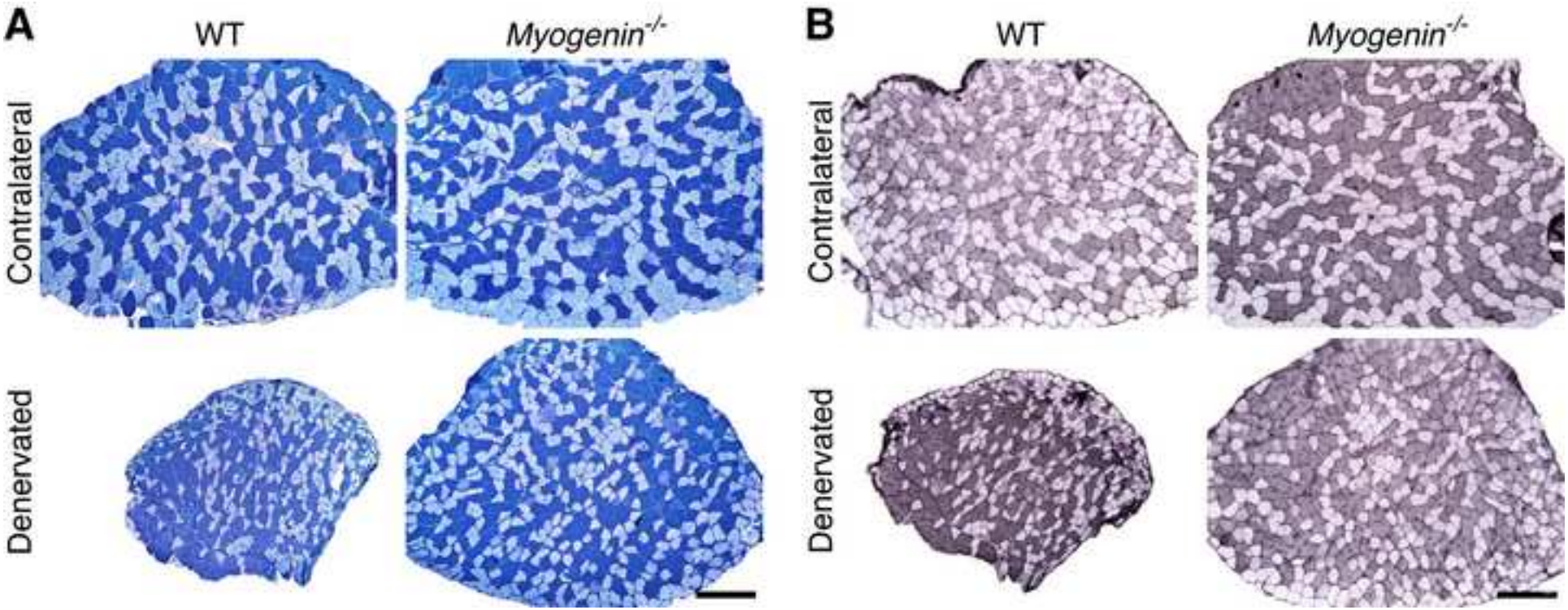
For: 5’-GGTACCCAGAGCGCGGACGCGACCGA-3’

Rev: 5’-CTCGAGTGCACCCCTACCCGTCCCCA-3’

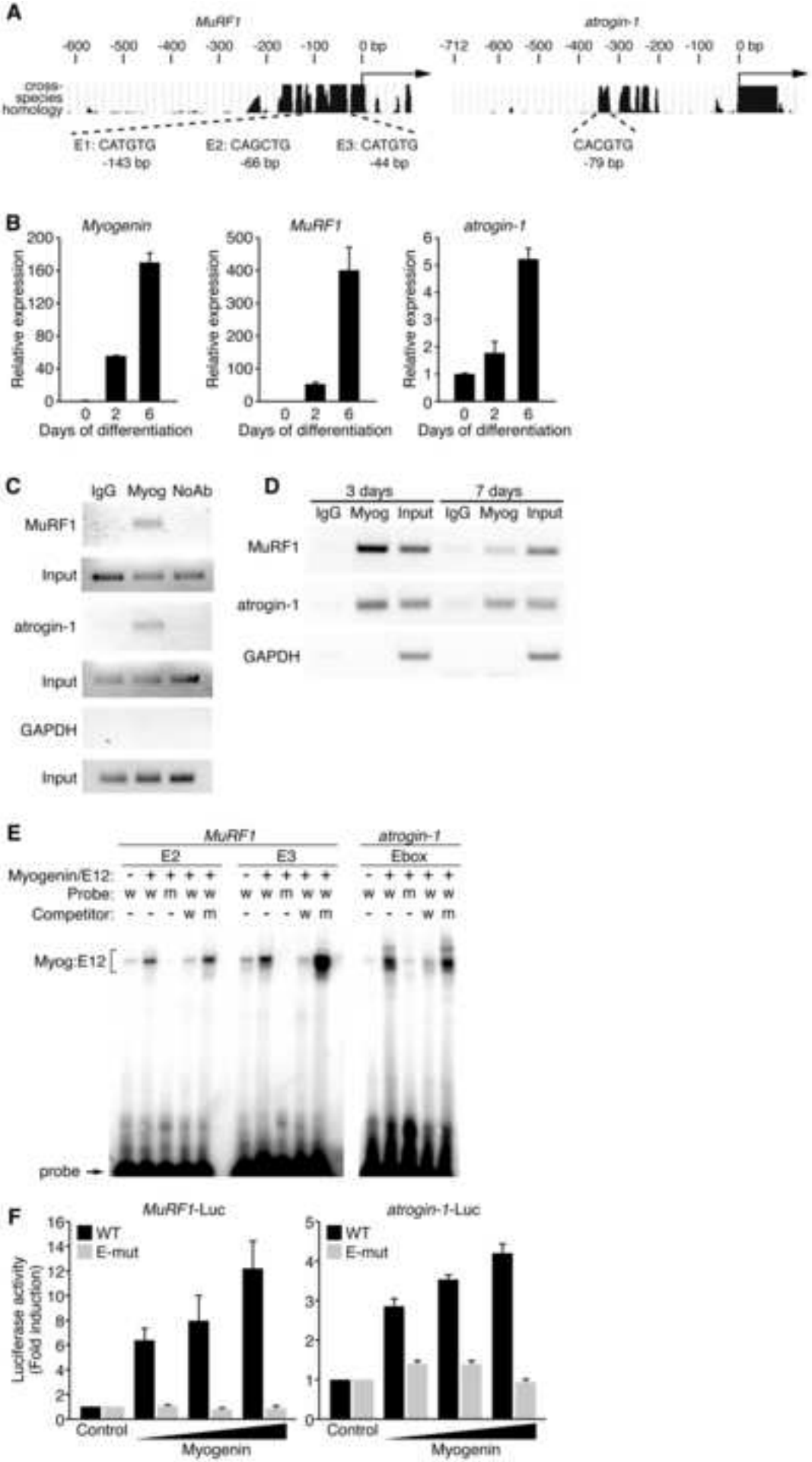
Supplemental Figure 1



Supplemental Figure 2



Supplemental Figure 3



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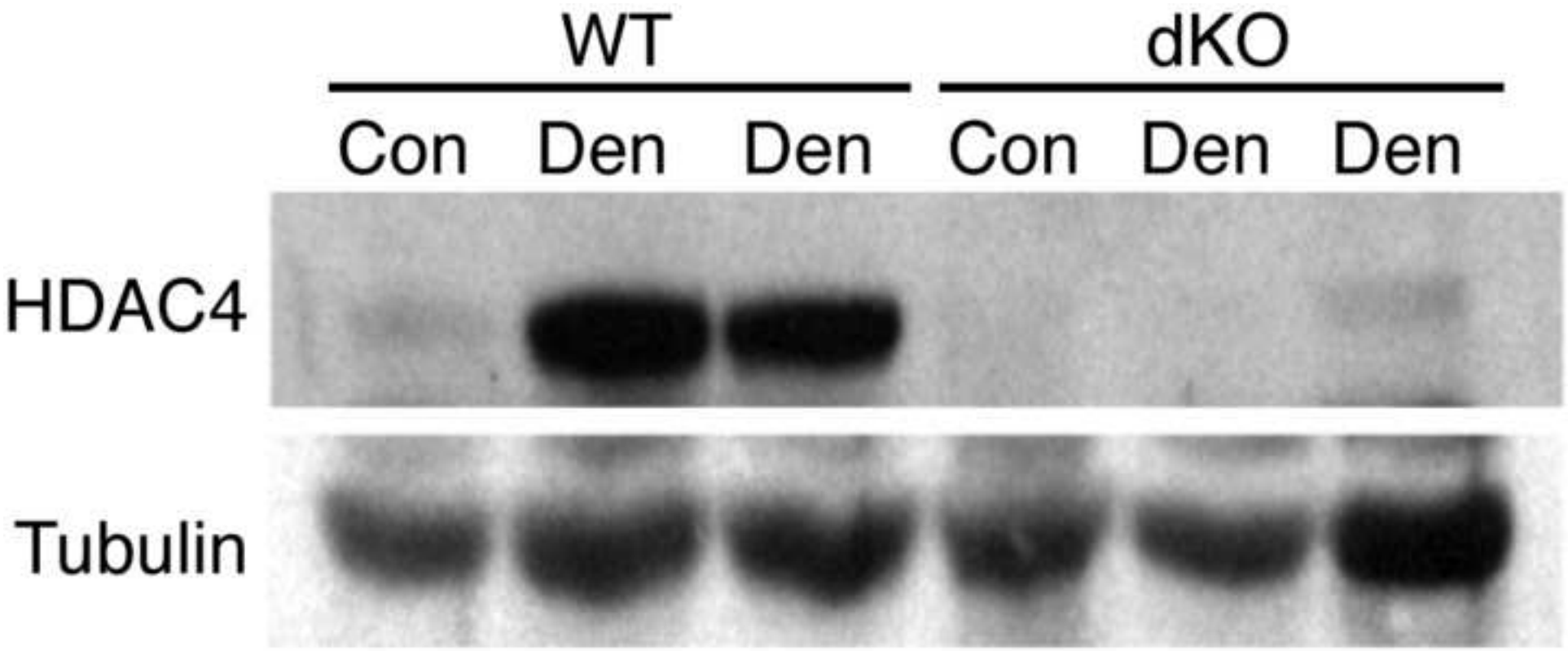
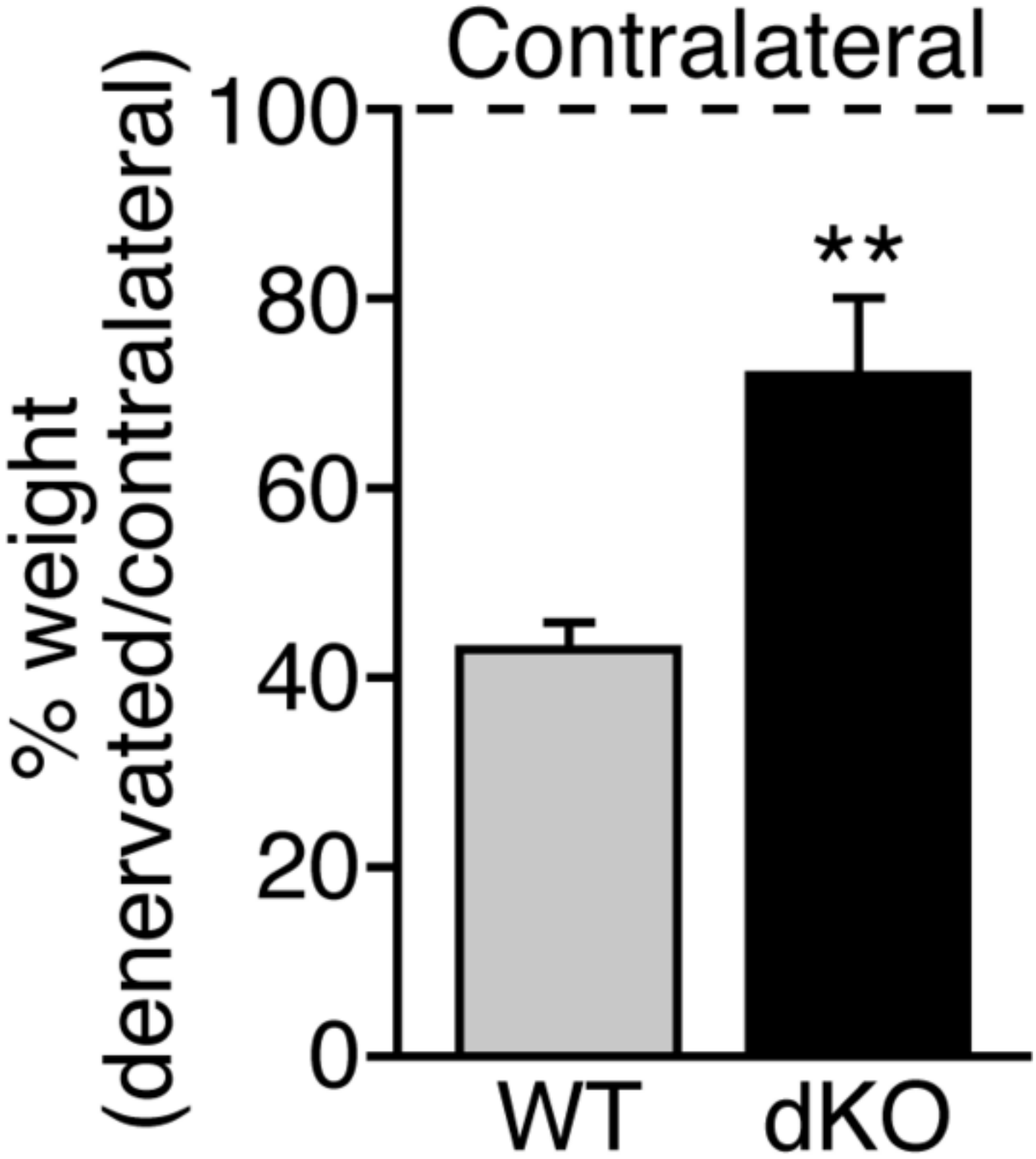


Supplemental Figure 5



Supplemental Figure 6

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