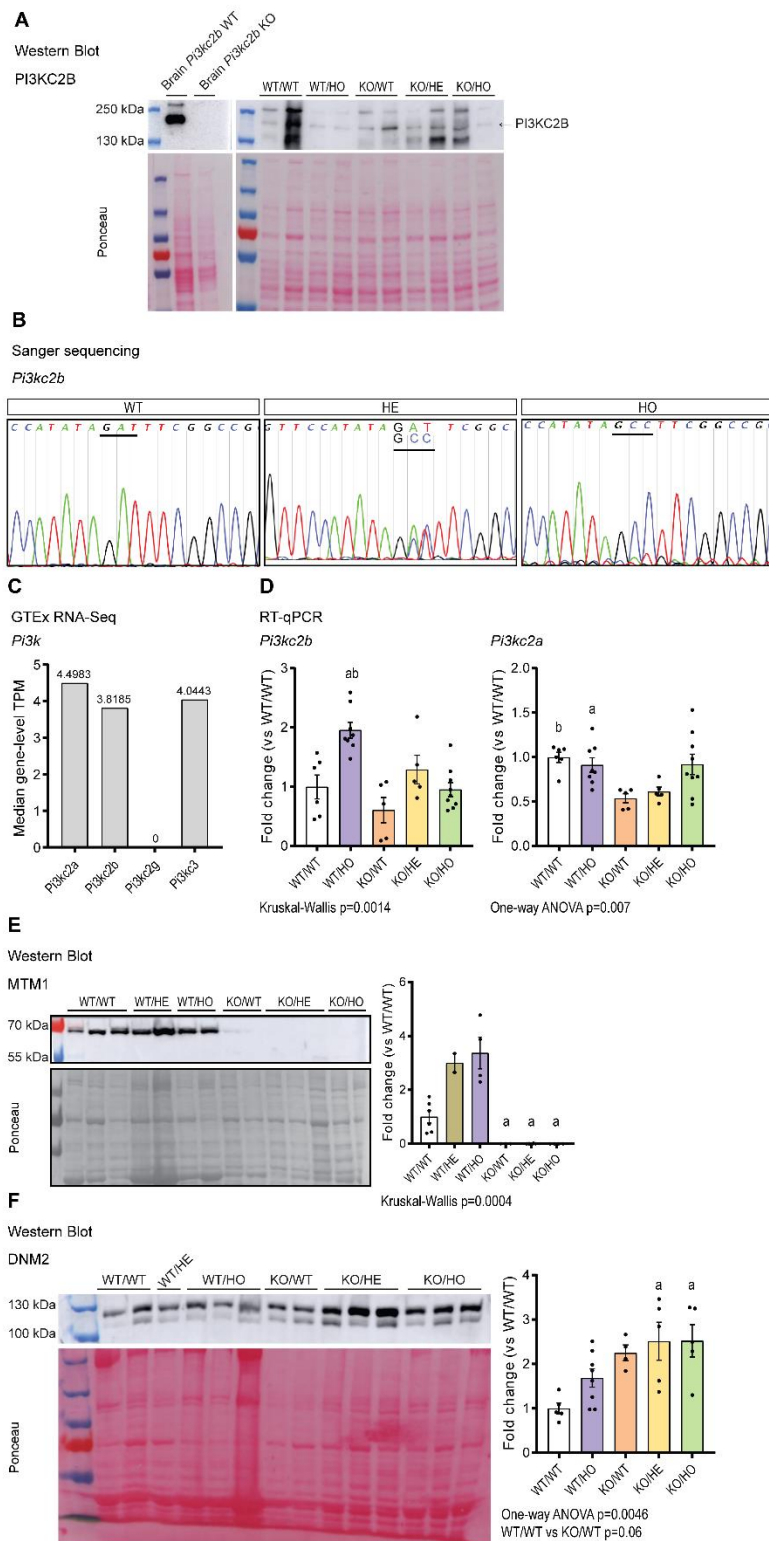
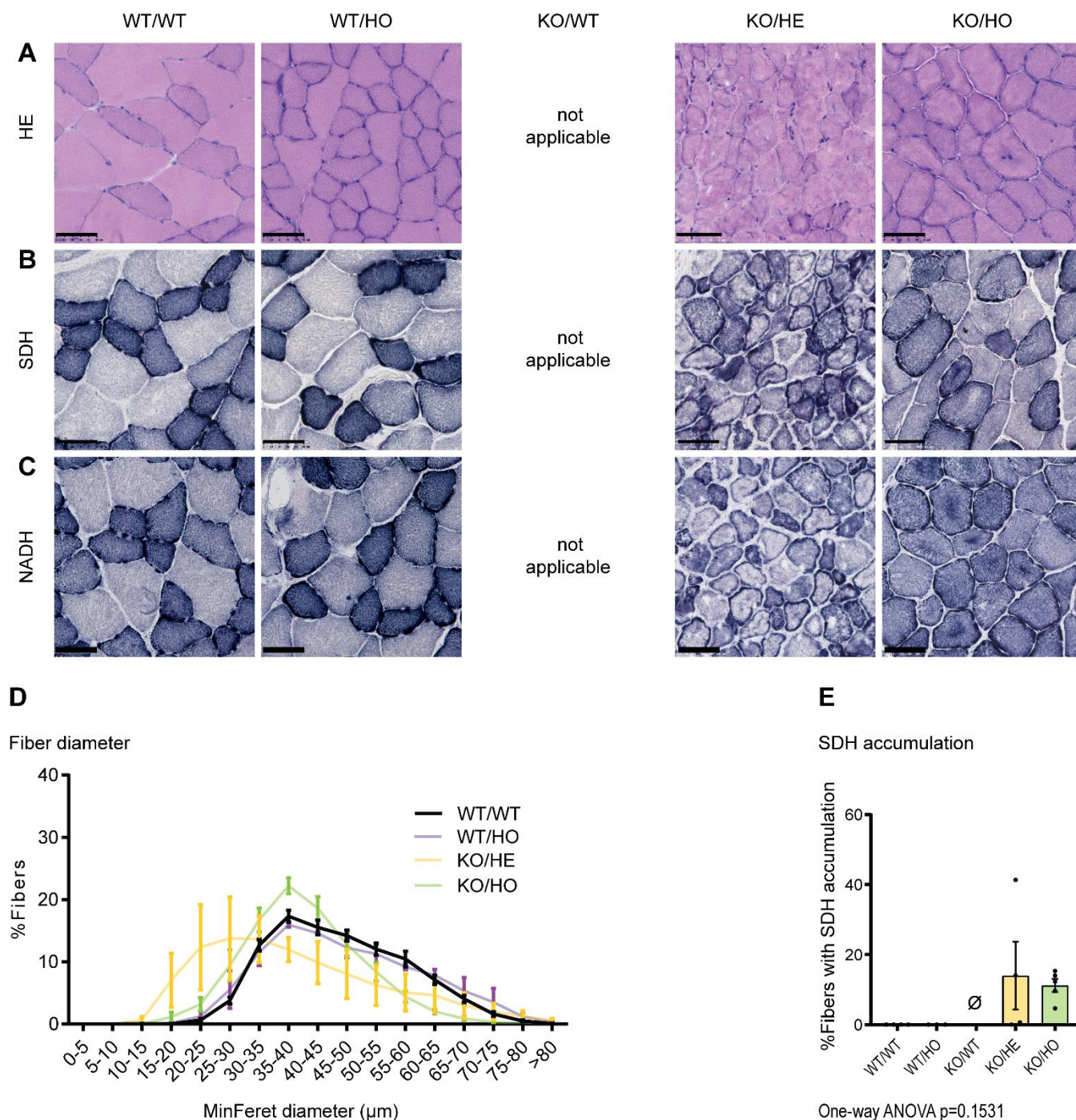




### Supplementary Figure 1. Additional data on the characterization of *Pik3c2b* kinase-dead mice

**(A)** Western blotting of brain and TA muscle extracts probed with anti-PI3KC2 $\beta$  antibody ( $n=2$ ). **(B)** Chromatopherograms of wild-type, heterozygous or homozygous *Pik3c2b* kinase-dead mice. **(C)** GTEx RNA-seq data showing *Pik3* class II and III expression in skeletal muscle ( $n=803$ ). **(D)** RT-qPCR data assessing mRNA levels of *Pik3c2b* and *Pik3c2a* ( $5 \leq n \leq 9$ ). **(E)** Western blotting of TA muscle extracts probed with anti-MTM1 antibody, and quantification as a ratio to Ponceau red ( $2 \leq n \leq 6$ ). **(F)** Western blotting of TA muscle extracts probed with anti-DNM2 antibody, and quantification as a ratio to Ponceau red ( $4 \leq n \leq 8$ ). In the graphs, a:  $p < 0.05$  vs WT/WT; b:  $p < 0.05$  vs KO/WT.

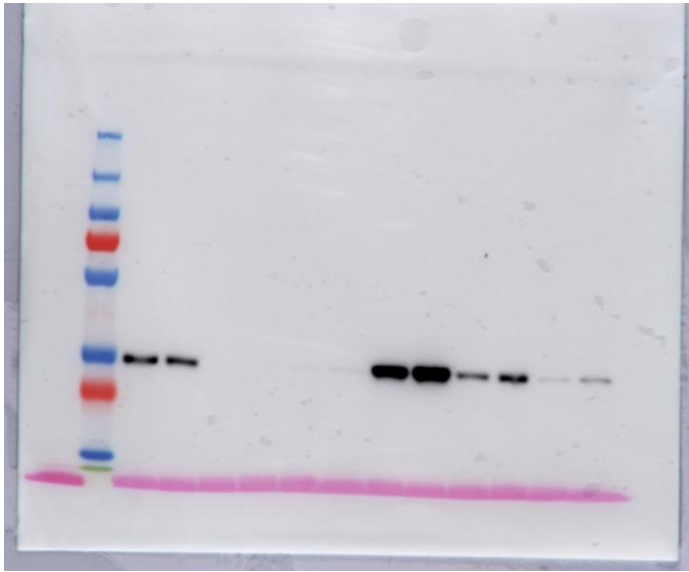


## Supplementary Figure 2. Histology at 16 weeks

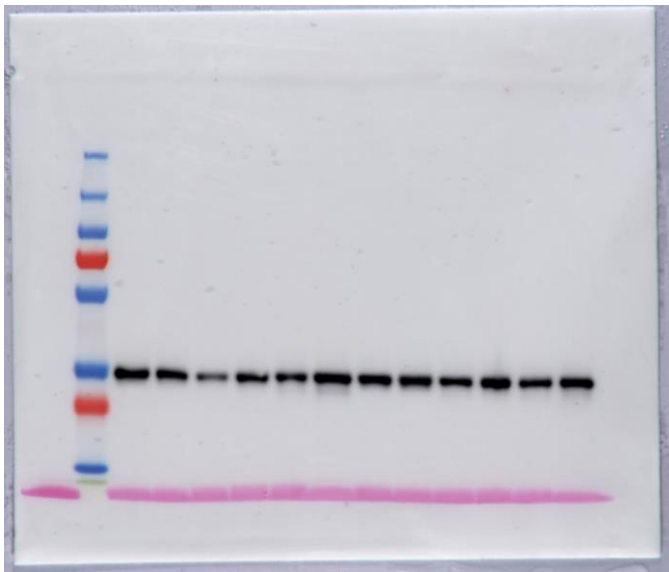
Muscle sections stained for **(A)** HE, **(B)** SDH and **(C)** NADH. Scale bar = 50 μm. **(D)** Quantifications of fiber diameter. **(E)** Percentage of fibers with SDH staining central accumulation. Note that all KO/WT mice died by 12 weeks of age, before these analyses, and thus histology imaging and quantification is not applicable. All mice ( $3 \leq n \leq 5$ ) analyzed at 16 weeks of age.

**Full unedited gels for Figure 5D**

Lane p-S6RP

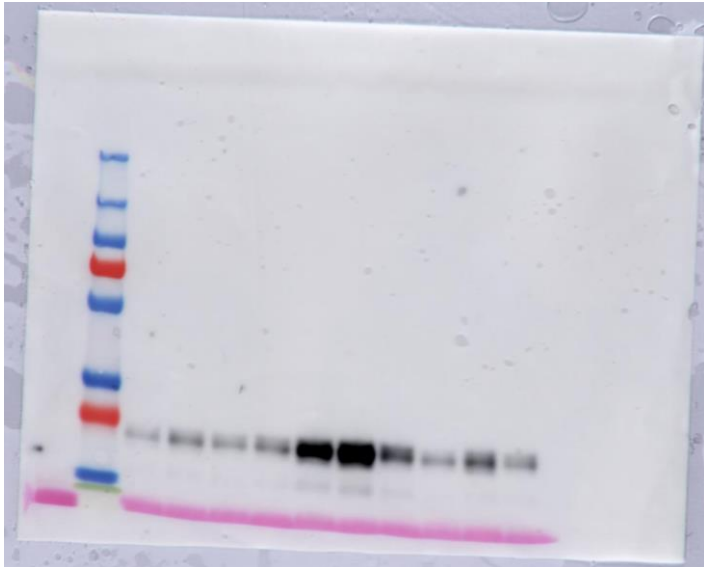


Lane S6RP



**Full unedited gels for Figure 5E**

Lane p-4EBP1



Lane 4EBP1

