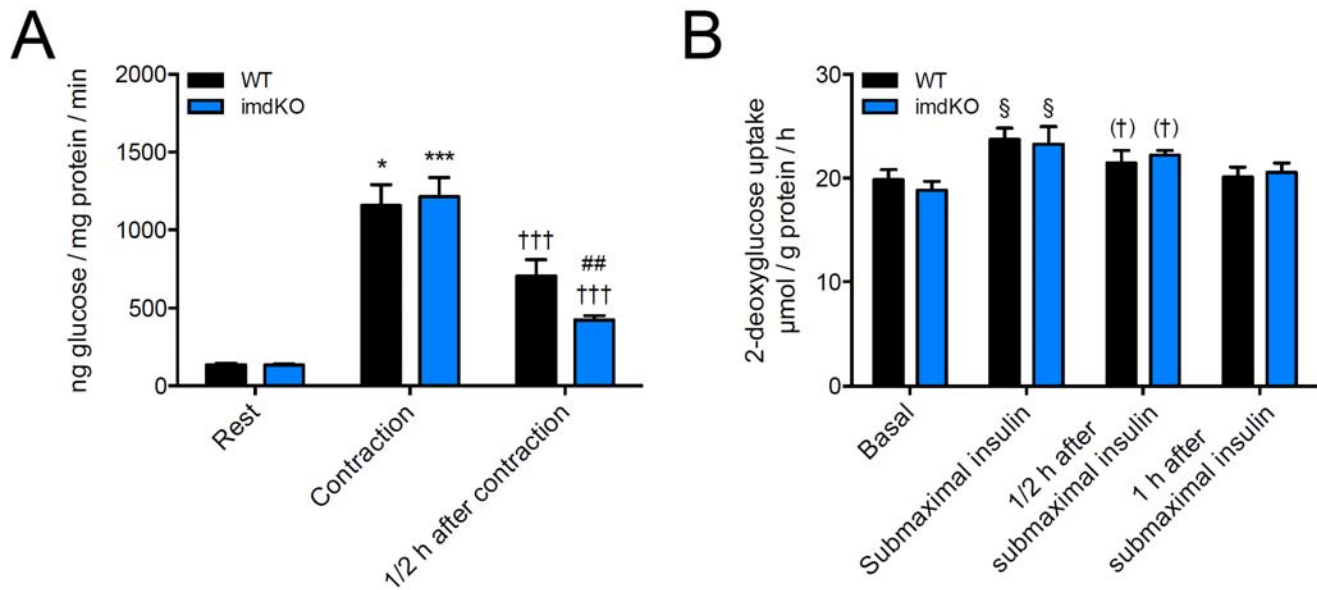


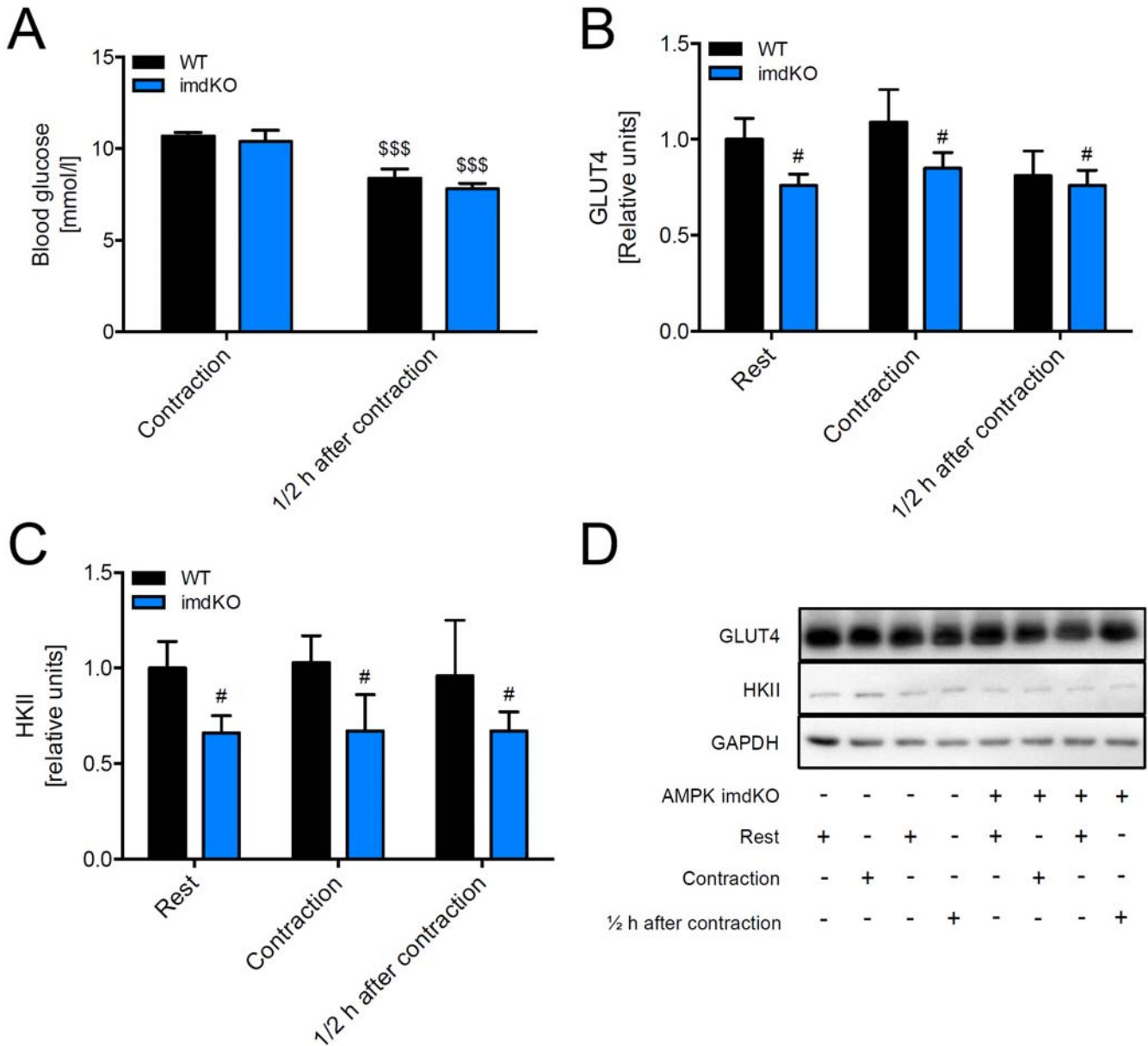
SUPPLEMENTARY DATA

**Supplementary Figure S1 - Glucose uptake after *in situ* contraction is decreased in EDL muscle from AMPK imdKO mice.** **A**, *In vivo* glucose uptake in EDL muscle from AMPK imdKO mice (blue bars) and WT littermates (black bars) at rest, during *in situ* contraction, and ½ hour after contraction (Rest: n = 9 and 20, Contraction: n = 4 and 10, ½ hour after contraction: n = 5 and 10 in WT and imdKO mice, respectively). **B**, *Ex vivo* glucose uptake in isolated EDL muscle from AMPK imdKO mice and WT littermates during submaximal insulin stimulation (100 µU/ml) as well as ½ hour and 1 hour after submaximal insulin stimulation (n = 6). Significantly different from WT is indicated as follows: ##p<0.01. Significantly different from Rest and ½ hour after exercise is indicated as follows: \*\*\*p<0.001 and \*p<0.05. Significantly different from Basal and 1 hour after insulin stimulation is indicated as follows: §p<0.05. Significantly different from Rest/Basal is indicated as follows: †††p<0.001 and (†)p=0.050. Statistical symbols presented in **b** indicate main effect of intervention. Data in **B** and **B** are means ± s.e.m. h, hour.



SUPPLEMENTARY DATA

**Supplementary Figure S2 - AMPK imdKO mice exhibit a minor but significant decrease in total protein abundance of GLUT4 and HKII in skeletal muscle.** **A**, Blood glucose concentration (mmol/l) in AMPK imdKO mice (blue bars) and WT littermates (black bars) during and ½ hour after *in situ* contraction (Contraction: n = 4 and 10, ½ hour after contraction: n = 5 and 10 in WT and imdKO mice, respectively). **B,C**, GLUT4 and HKII protein abundance in TA muscle from AMPK imdKO mice and WT littermates at rest, during contraction as well as ½ hour after contraction (Rest: n = 9 and 20, Contraction: n = 4 and 10, ½ hour after contraction: n = 5 and 10 in WT and imdKO mice, respectively). **D**, Representative immunoblots. Significantly different from WT is indicated as follows: #p<0.05. Significantly different from Contraction is indicated as follows: \$\$\$p<0.001. Statistical symbols presented in **A-C** indicate main effect of intervention/genotype. Data in **A-C** are means ± s.e.m. h, hour.



## SUPPLEMENTARY DATA

**Supplementary Figure S3 - AMPK, TBC1D1, and TBC1D4 signaling in skeletal muscle from AMPK mdKO and imdKO mice.** **A,B**, AMPK $\alpha$ 2 $\beta$ 1-associated activity (**A**) and AMPK $\alpha$ 1-associated activity (**B**) in TA muscle from AMPK mdKO mice (red bars) and WT littermates (black bars) at rest, during *in situ* contraction as well as ½ hour after and 1 hour after contraction (Rest: n = 19-23, Contraction: n = 5-7, ½ hour after contraction: n = 8-10, 1 hour after contraction: n = 6). **C,D**, Phosphorylation of AMPK Thr172 (**C**) and ACC Ser212 (**D**) in TA muscle from AMPK imdKO mice (blue bars) and WT littermates (black bars) at rest, during *in situ* contraction as well as ½ hour after contraction (Rest: n = 9 and 20, Contraction: n = 4 and 10, ½ hour after contraction: n = 5 and 10 in WT and imdKO mice, respectively). **E,F**, Pearson correlations between glucose uptake and phosphorylation of TBC1D1 Ser231 in TA muscle from AMPK mdKO (**E**) and AMPK imdKO mice (**F**) ½ hour after and 1 hour after *in situ* contraction (n = 16 and 6, respectively). R and significance level are indicated in the respective panel. **G,H**, Phosphorylation of TBC1D4 Thr649 in TA muscle from AMPK mdKO mice (**G**), AMPK imdKO mice (**H**), and WT littermates at rest, during *in situ* contraction as well as ½ hour and 1 hour (only AMPK mdKO mice) after contraction (Rest: n = 9-23, Contraction: n = 4-10, ½ hour after contraction: n = 5-10, 1 hour after contraction: n = 6). **I**, GLUT4 protein abundance in TA muscle from TBC1D1 KO mice (green bars) and WT littermates (black bars) at rest, during *in situ* contraction as well as ½ hour after contraction (Rest: n = 12 and 11, Contraction: n = 6 and 6, ½ hour after contraction: n = 6 and 5 in WT and TBC1D1 KO mice, respectively). **J**, Representative immunoblots. Significantly different from WT is indicated as follows: ####p<0.001, ##p<0.01 and #p<0.05. Significantly different from Rest, ½ hour after and 1 hour after contraction is indicated as follows: \*\*\*p<0.001, \*\*p<0.01, and \*p<0.05. Significantly different from Rest and 1 hour after contraction is indicated as follows: §p<0.05. Significantly different from Rest is indicated as follows: †††p<0.001 and ††p<0.01. Statistical symbols presented in figure S3A, B, G, H, and I indicate main effect of intervention/genotype. Data in **A-D** and **G-I** are means  $\pm$  s.e.m. h, hour.

SUPPLEMENTARY DATA

