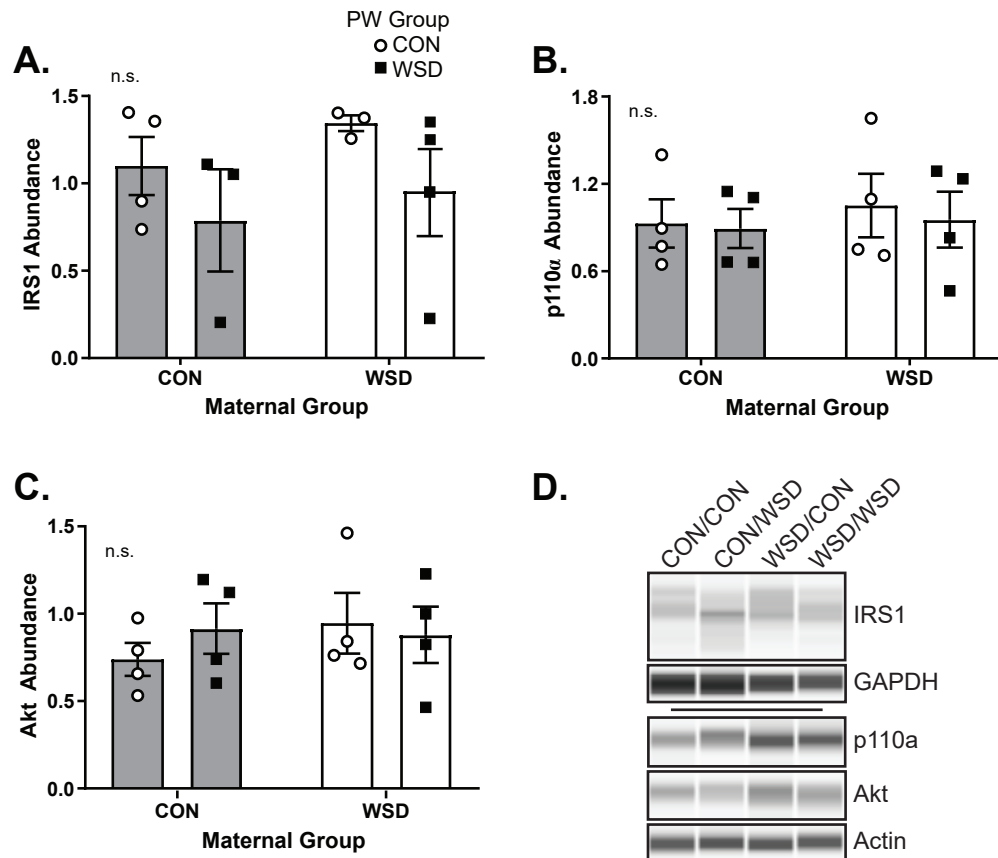
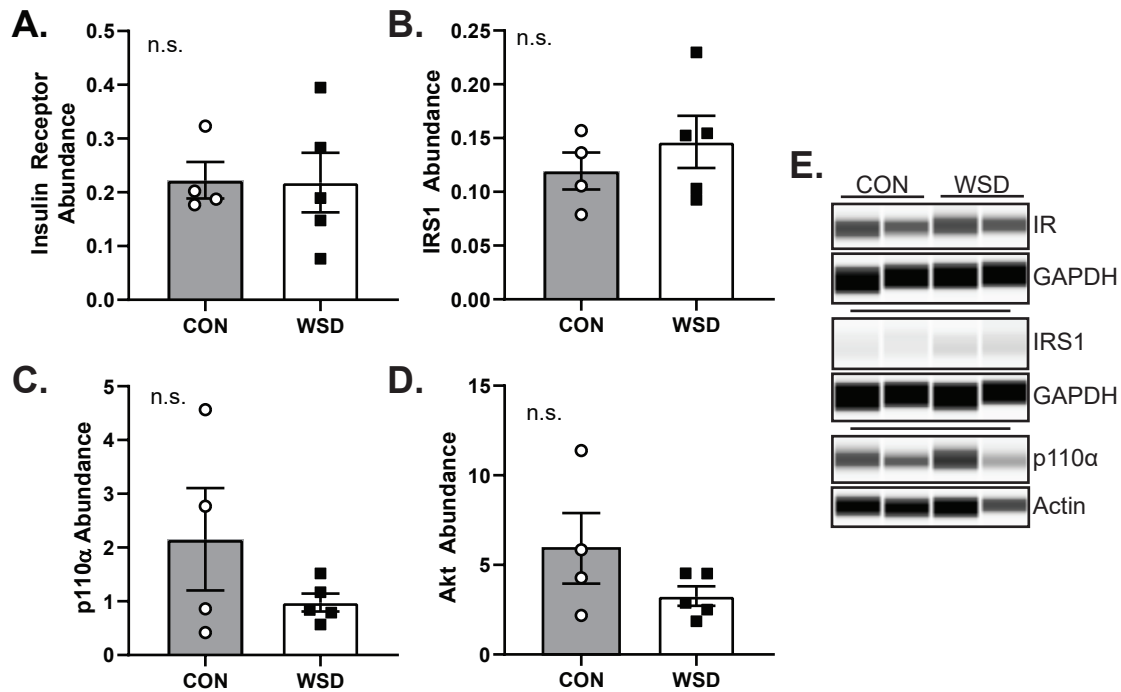


Supplementary Figure 1: Fetal Skeletal Muscle Insulin Signaling. Total abundance of components of the insulin signaling pathway measured by simple western in homogenates of fetal soleus muscle. (A) Insulin receptor abundance as a ratio to the control protein Actin. (B) p85 α abundance as a ratio to the control protein GAPDH. (C) Akt abundance relative to the control protein Actin. (D) GSK3 β abundance relative to the control protein Vinculin. (E) GLUT1 abundance relative to the control protein GAPDH. (F) Example simple western probing of each of the above proteins. Data is expressed as mean \pm SEM, with individual data points shown, and analyzed by unpaired T test (n=6-10 per group).



Supplementary Figure 2: Juvenile Skeletal Muscle Ex Vivo Insulin Response. Total abundance of proteins in the insulin signaling cascade were measured in basal skeletal muscle homogenates measured by simple western. (A) IRS1 abundance is expressed relative to GAPDH. (B) p110 α abundance is expressed relative to Actin. (C) Akt abundance is expressed relative to Actin. (D) Example simple western probing of IRS1, p110 α , Akt, GAPDH, and Actin. Data is expressed as the mean \pm SEM, with individual data points shown, and analyzed by two-way ANOVA with Tukey multiple comparisons test (n=3-4 per group).



Supplementary Figure 3: Juvenile Skeletal Muscle In Vivo Insulin Response. Abundance of total proteins in the insulin signaling cascade were measured by simple western in homogenates of gastrocnemius biopsies from fasted seven-month-old animals. Insulin receptor (A), IRS1 (B), p110α (C), and Akt (D) abundance was expressed relative to either GAPDH or Actin. Data is expressed as the mean \pm SEM, with individual data points shown, and analyzed by unpaired T test (n=4-5 per group).

Supplementary Table 1

Target	Manufacturer	Catalog Number	Dilution	Host Species	Immunogen Species	Supplier Reported Reactivity
IRS1	Cell Signaling Technology	3407	1:25	Rabbit	Human	Human, Mouse, Rat, Monkey
p110 α	Cell Signaling Technology	4249	1:50	Rabbit	Human	Human, Mouse, Rat, Bovine
IRS1 (pY896)	Abcam	ab46800	1:25	Rabbit	Human	Human
Akt (pT308)	Cell Signaling Technology	4056	1:50	Rabbit	Mouse	Human, Mouse, Rat, Monkey
Akt (pS473)	Cell Signaling Technology	4060	1:50	Rabbit	Human	Human, Mouse, Rabbit, Hamster, Monkey, Drosophila, Zebrafish, Bovine
AS160 (pT642)	Cell Signaling Technology	4288	1:25	Rabbit	Human	Human
GSK3 β (pS9)	Cell Signaling Technology	9323	1:100	Rabbit	Human	Human, Mouse, Rabbit, Monkey
Insulin Receptor	Cell Signaling Technology	3025	1:20	Rabbit	Human	Human, Mouse Rat
p85 α	Cell Signaling Technology	4257	1:25	Rabbit	Human	Human, Mouse, Rabbit
Akt	Cell Signaling Technology	4691	1:1000	Rabbit	Mouse	Human, Mouse, Rabbit, Monkey, Drosophila
GSK3 β	Cell Signaling Technology	9315	1:2000	Rabbit	Human	Human, Mouse, Rabbit, Monkey
GLUT1	Cell Signaling Technology	12939	1:25	Rabbit	Human	Human, Mouse, Rat