Supplementary Methods

Primary bone marrow stromal cells (BMSC) were obtained from 10-12 weeks old mice, following previously described procedures [31,38]. Briefly, the bone marrow from an entire bone (femur) was flushed with PBS, resuspended in culture media, filtered through 70-μ filter, and plated in a 15-mm plate. Cells were incubated in growth medium for 2 weeks until 70-80% confluence, then plated in 10mm plates and exposed to insulin 20 μg/mL for different times.

Supplementary Figure Legends

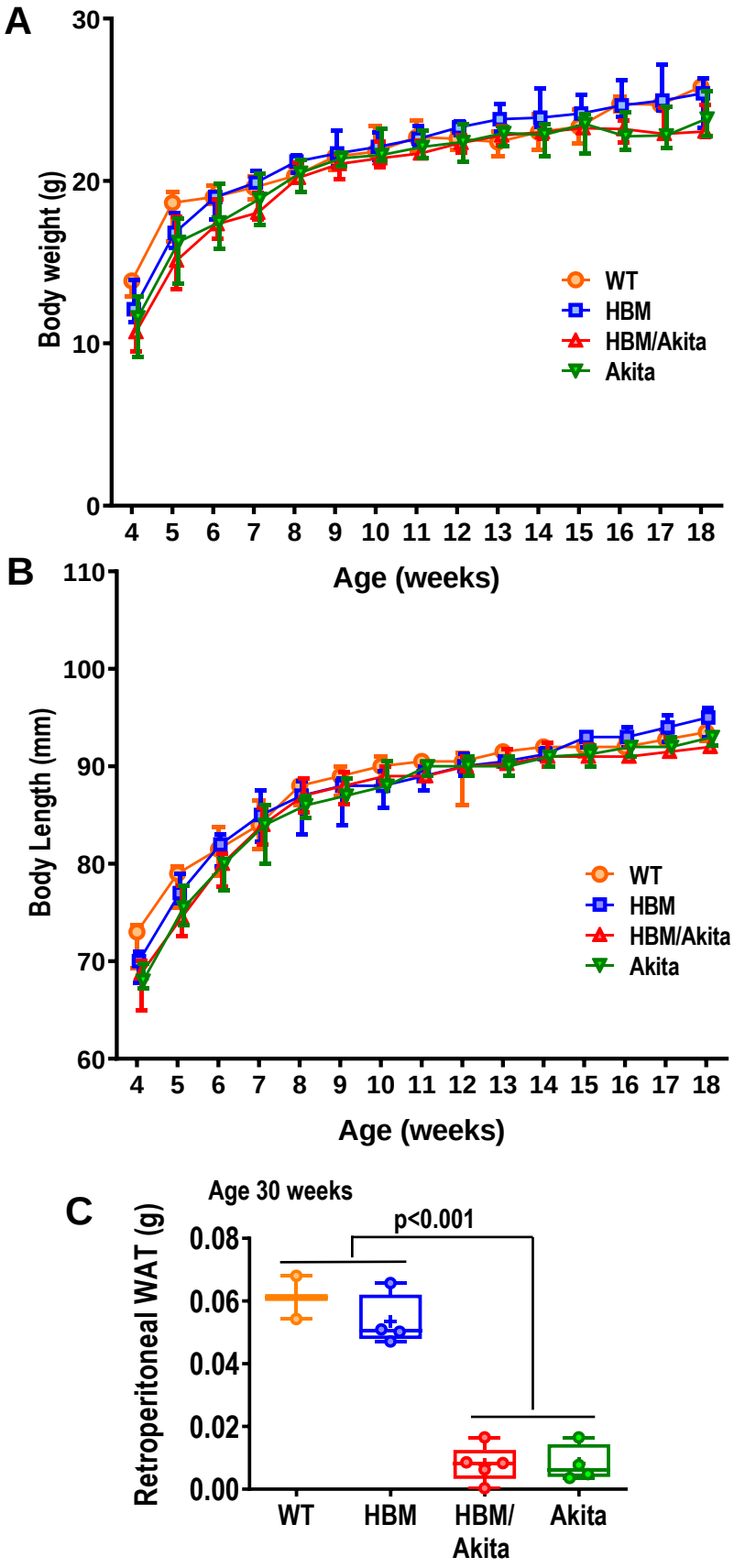
Supplemental Figure 1. Post-natal Growth and WAT Mass. (A) Body weight, and **(B)** length at different ages in the different mutants. N=11-16 at 4 weeks and 4-6 at 18 weeks. P>0.05 by 2-way ANOVA for genotype effect in both measures. **(C)** Retroperitoneal white adipose tissue (WAT) mass determined post-mortem at 30 weeks of age. Brackets represent adjusted p-values (Tukey's multiple comparison test) after one-way ANOVA, comparing all groups on one side with all groups on the other side.

Supplemental Figure 2. Bone Microarchitecture in Vivo. (A) Trabecular thickness, **(B)** trabecular spacing**, (C)** cortical bone area**, (D)** cortical medullary area, **(E)** trabecular tissue mineral density, and **(D)** cortical tissue mineral density assessed by in vivo μCT in the 4 genotype groups at 6 and 20 weeks of age. Brackets represent adjusted p-values (Tukey's multiple comparison test) after two-way ANOVA. Brackets on multiple groups (C-F) indicate pairwise comparison of each genotype group at 6 and 20 weeks. Pairwise comparisons with p>0.10 are not indicated.

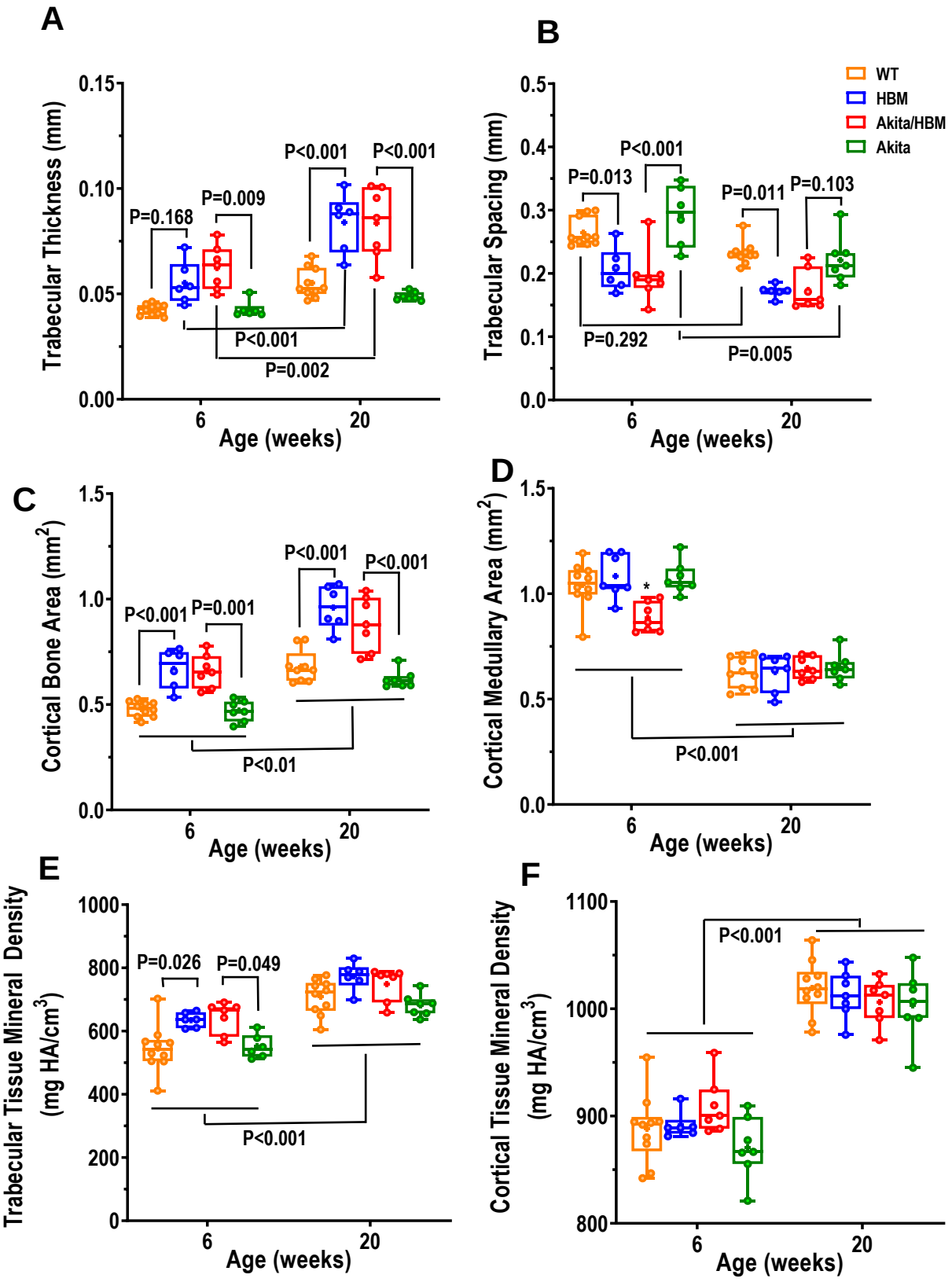
Supplemental Figure 3. Bone Microarchitecture Post-Mortem. (A) Volumetric trabecular bone volume/total volume (BV/TV), **(B)** trabecular bone mineral density, **(C)** cortical thickness, and **(D)** cortical bone area in the 4 genotype groups at 30 weeks of age measured by μCT. Brackets represent adjusted p-values (Tukey's multiple comparison test) after one-way ANOVA..

Supplemental Figure 4. Intraperitoneal Glucose Tolerance Test and Serum Osteocalcin. (A) Blood glucose before and after an intraperitoneal glucose load (1.5 mg/kg) at age 10-12 weeks (n=7-10). *p=0.0192 for WT vs. $Lrp5^{A214V/A214V}$; two-way ANOVA and Bonferroni post-hoc test. **(B)** Serum Gla13 (carboxylated) osteocalcin in ***-week-old mice. F=1.257, p=0.312 (ANOVA).

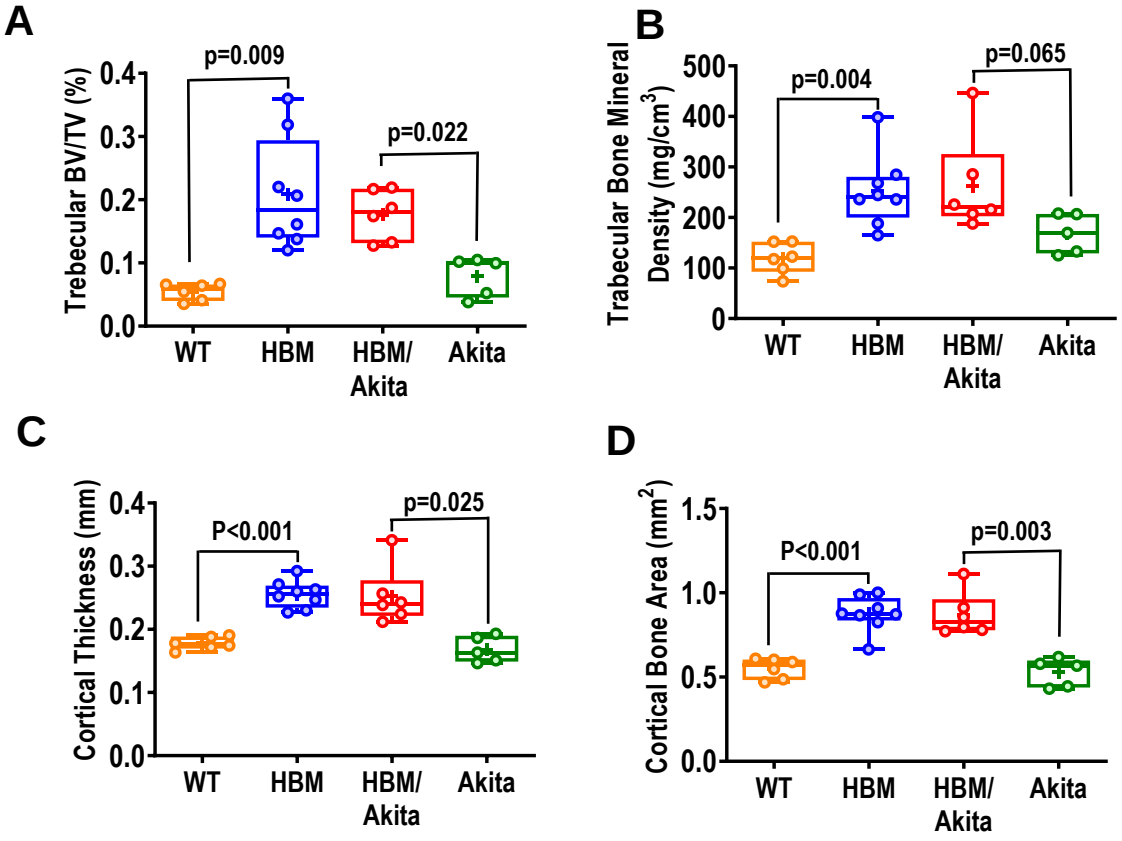
Supplemental Figure 5. Insulin Signaling. (A, B) Two replicates of immunoblots of whole cell lysates from external ear-derived adipocyte cultures before and after 5 or 30 minutes of exposure to 1 μg/ml of insulin. Quantitative band densitometry is shown in Fig. 8. **(C)** Immunoblot of bone marrow stromal cell lysates before and after 5 or 30 minutes of exposure to 20 μg/ml of insulin.



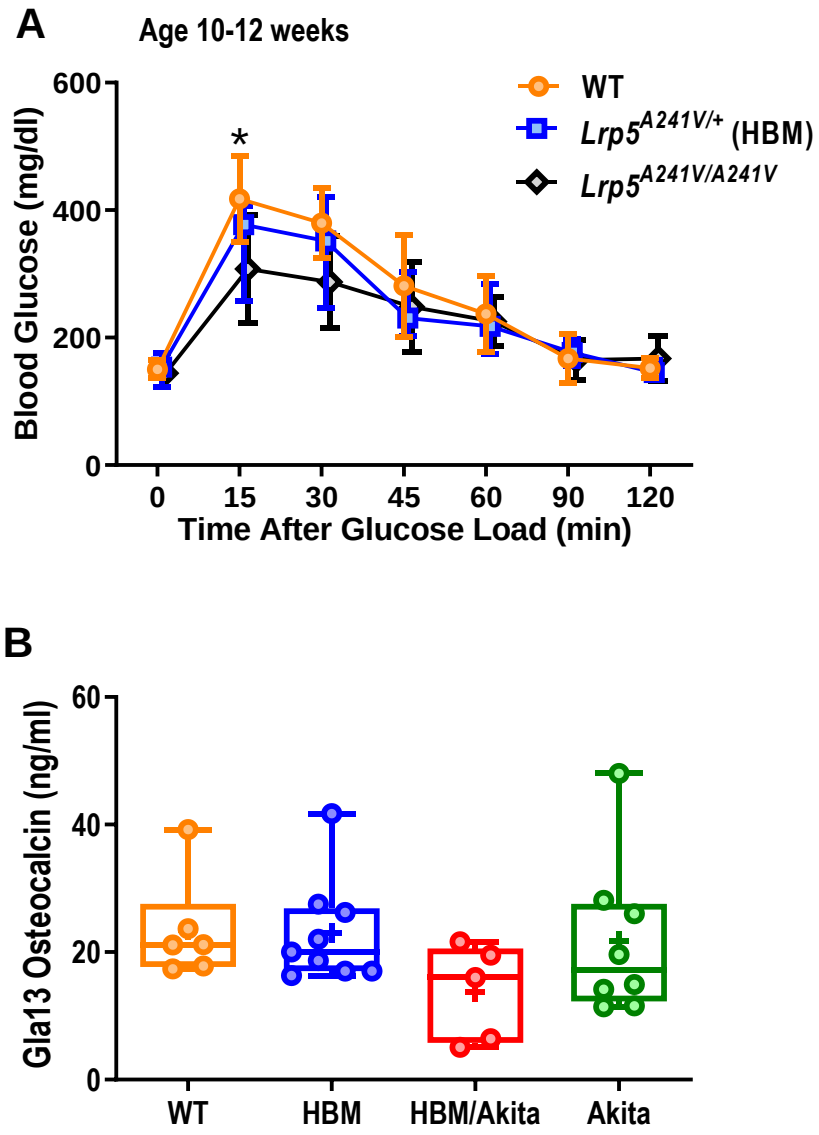
Supplemental Figure 1



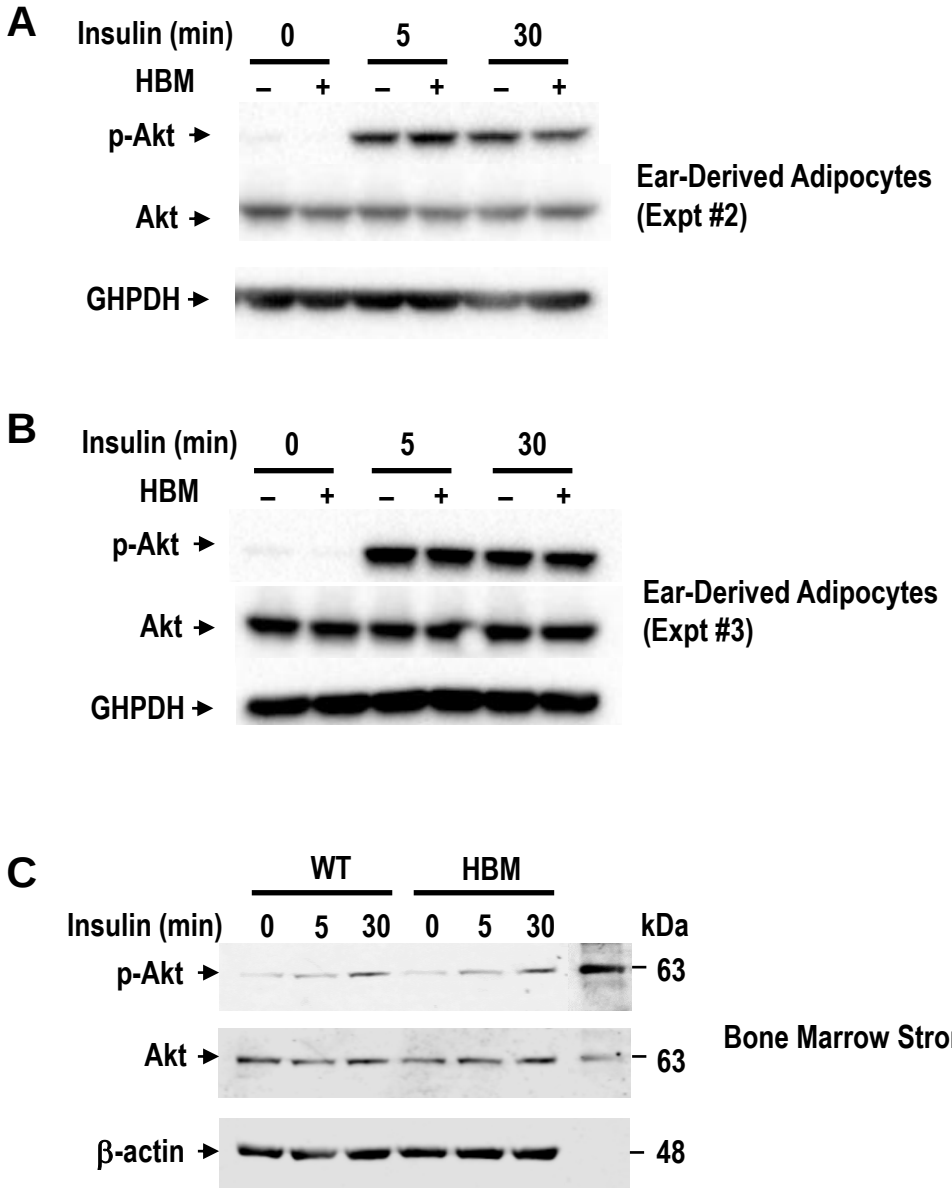
Supplemental Figure 2



Supplemental Figure 3



Supplemental Figure 4



Supplemental Figure 5