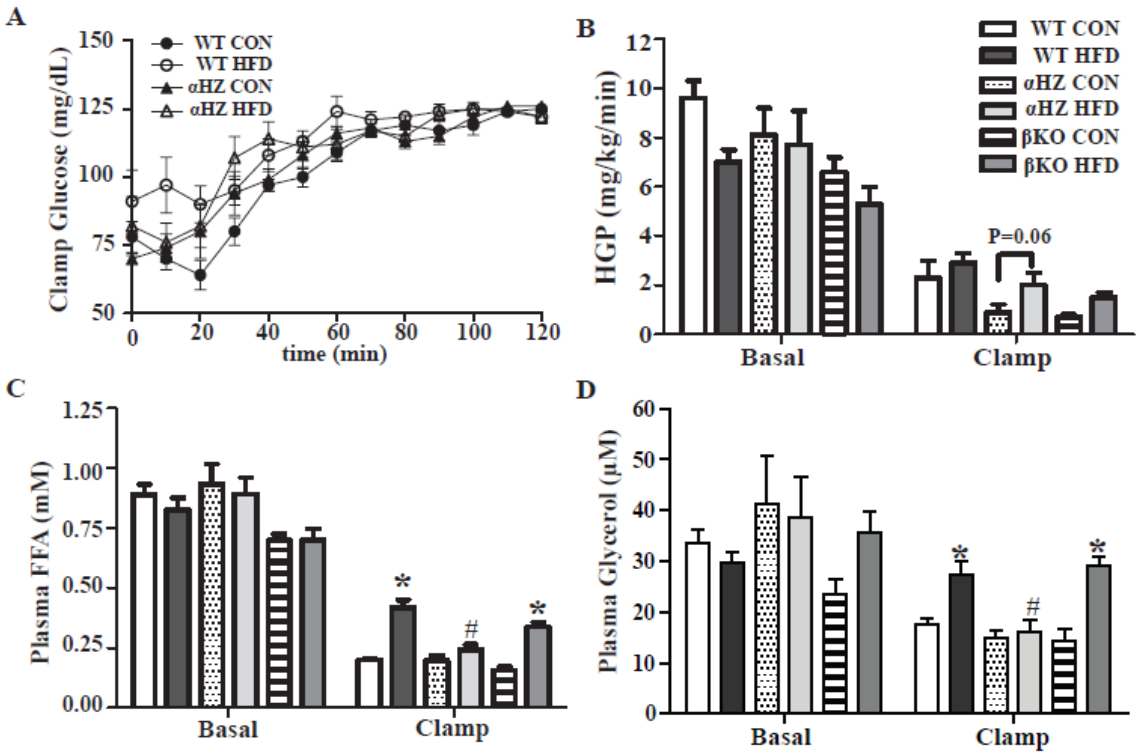
SUPPLEMENTARY DATA

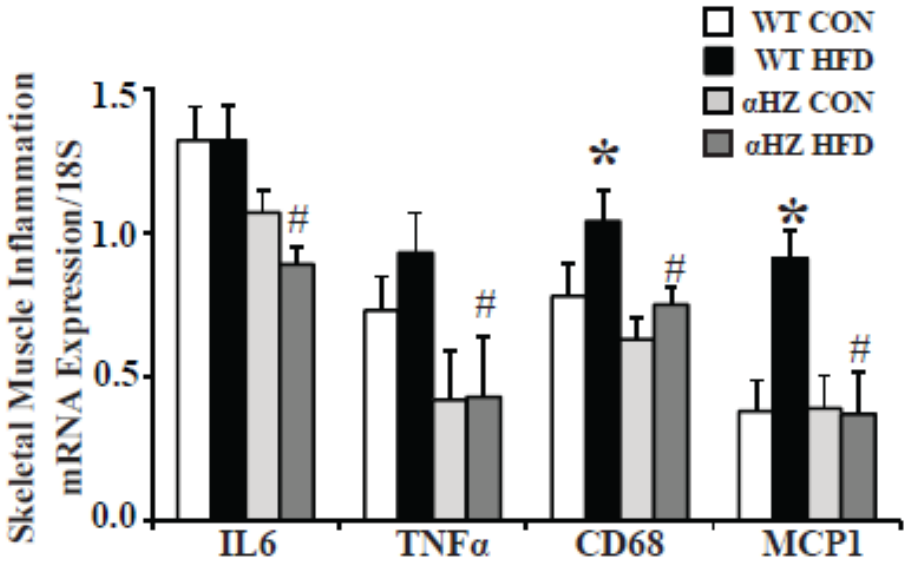
XBP-1 Splicing. eAT RNA was isolated and reverse transcribed as above. Semi-quantitative PCR for XBP1 (94°C for 3min 1 cycle, followed by 30cycles 94°C for 30s, 58°C for 30s, 72°C 1min, then 72°C 3min) was run on an Eppendorf Mastercycler Gradient instrument. PCR products were then subjected to *apal1* restriction enzyme digestion at 37°C overnight as previously described (30). Samples (20 µl) were run on 2.5% agarose gel. The presented data show quantitation of spliced XBP-1, which does not contain the restriction enzyme site.

Supplementary Figure 1. Insulin sensitivity measurements during hyperinsulinemic-euglycemic clamp. (A) Blood glucose was measured by tail sampling over the time course of the clamp to determine when glucose infusion rate during the insulin clamp had reached steady state levels. Blood was taken (100ul) from the tail at the end of the basal tracer infusion period (time 0) and at the end of the clamp (time 120) for measurement (B) hepatic glucose production (HGP), (C) free fatty acids and (D) glycerol in WT, αHZ and βKO mice after 16 weeks on a Control (CON) or high fat diet (HFD). n=6-10/group. Data were analyzed by 2-way ANOVA for main effects of diet and genotype with a Tukey posthoc test, *P<0.05 within same genotype; #P<0.05 within same diet group.



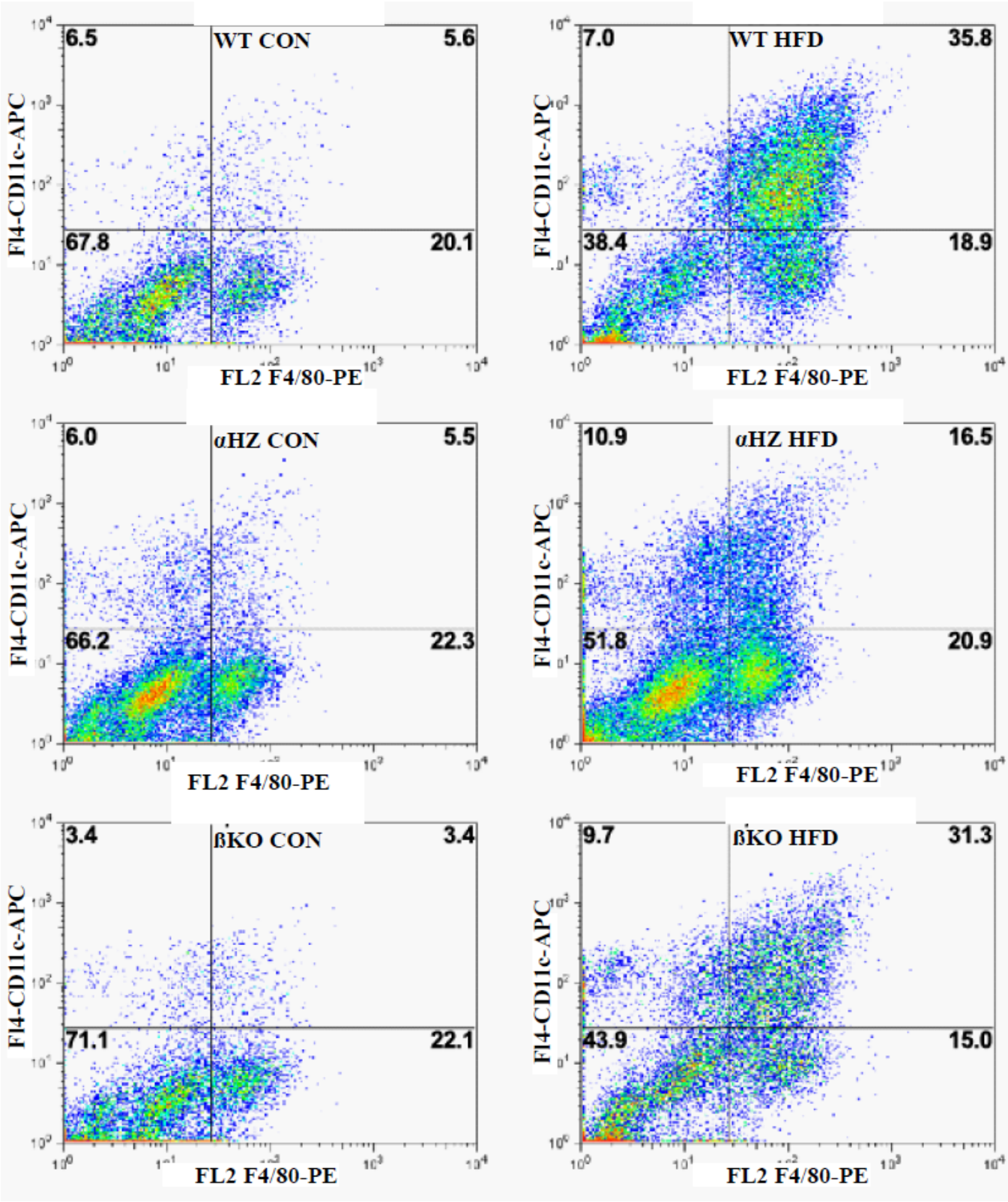
SUPPLEMENTARY DATA

Supplementary Figure 2. *Pik3r1+/-* knockdown prevent skeletal muscle inflammation. (A) Changes in gene expression for IL6, TNFα, CD68, and MCP were measured in skeletal muscle by qPCR in WT and αHZ mice fed either a CON or HFD for 16 weeks. All data are shown as the Mean + SEM. n=6-10/group. Data were analyzed by 2-way ANOVA for main effects of diet and genotype with a Tukey posthoc test, *P<0.05 within same genotype; #P<0.05 within same diet group.



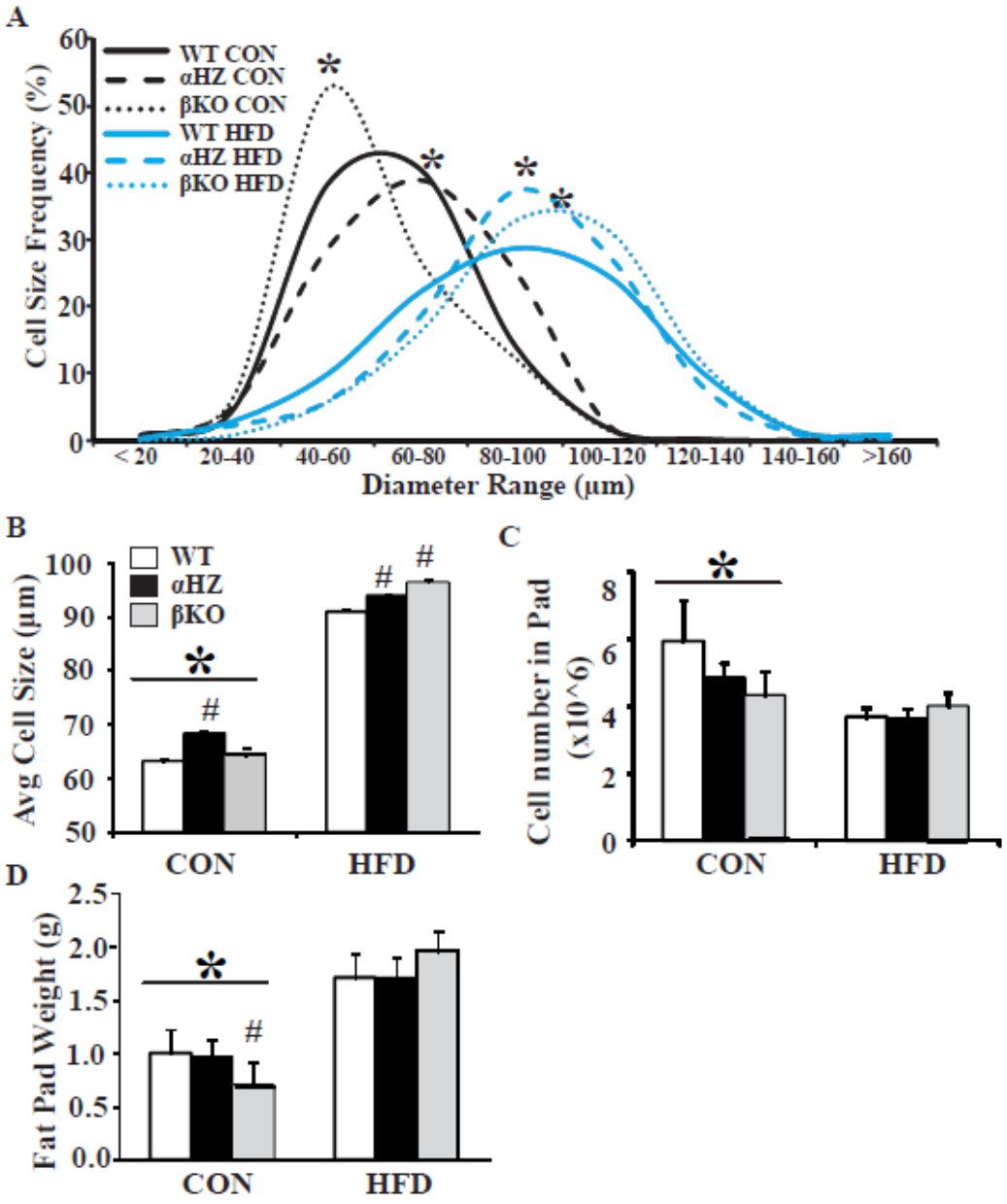
Supplementary Figure 3. Flow cytometry scatter plots for AT macrophages. Cells were isolated from the stromal vascular fraction of epididymal fat pads and incubated with antibodies for F4/80 and CD11c. Antibody positive cells were quantitated by flow cytometry and mean percent cells for each quadrant are shown.

SUPPLEMENTARY DATA



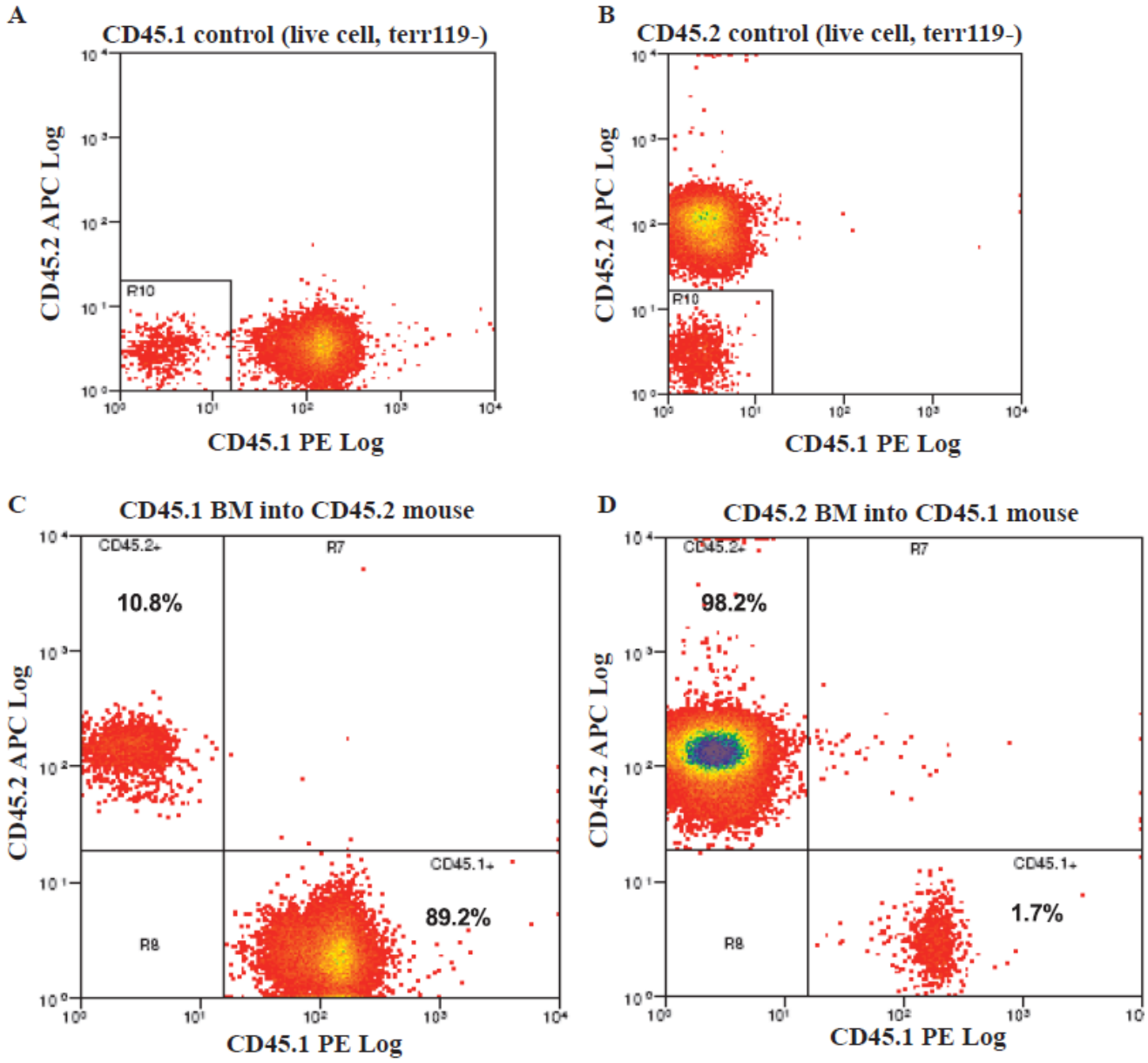
SUPPLEMENTARY DATA

Supplementary Figure 4. Adipocytes were isolated from the epididymal adipose tissue (eAT) of WT, αHZ and βKO mice after 16 weeks on a Control (CON) or high fat diet (HFD). Cell diameter was measured by microscopy and data expressed as (A) cell size frequency over a range of diameters, (B) average cell size per eAT, and (C) calculated cell number. (D) Average epididymal fat pad weight for each group. Data were analyzed by 2-way ANOVA for main effects of diet and genotype with a Tukey posthoc test, *P<0.05 within same genotype; #P<0.05 within same diet group vs. control. n=8/group.



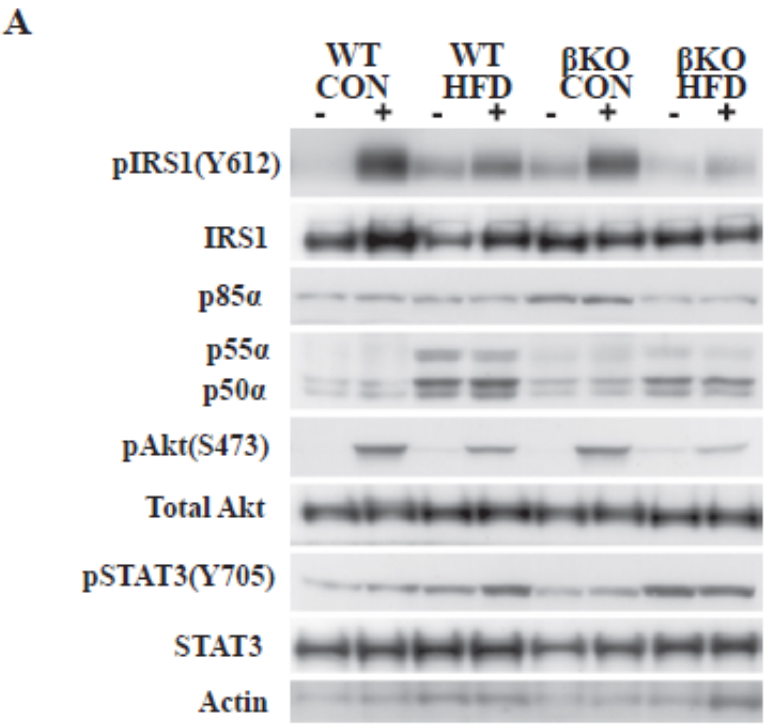
SUPPLEMENTARY DATA

Supplementary Figure 5. Bone Marrow Engraftment. Bone marrow engraftment was assayed in irradiated mice after 6weeks of recovery by flow cytometry for reciprocal CD45.1 or CD45.2 cell markers in peripheral blood immune cells. (A) Control gating for a CD45.1 non-transplant mouse. (B) Control gating for a CD45.2 non-transplant mouse. (C) Representative scatter plot of engraftment from αHZ (CD45. 2) recipient mouse receiving WT bone marrow (CD45.1). (D) Representative scatter plot of engraftment from a WT (CD45.1) recipient mouse for αHZ bone marrow (CD45.2). . Mice were included in study analysis only if engraftment was > 80%.



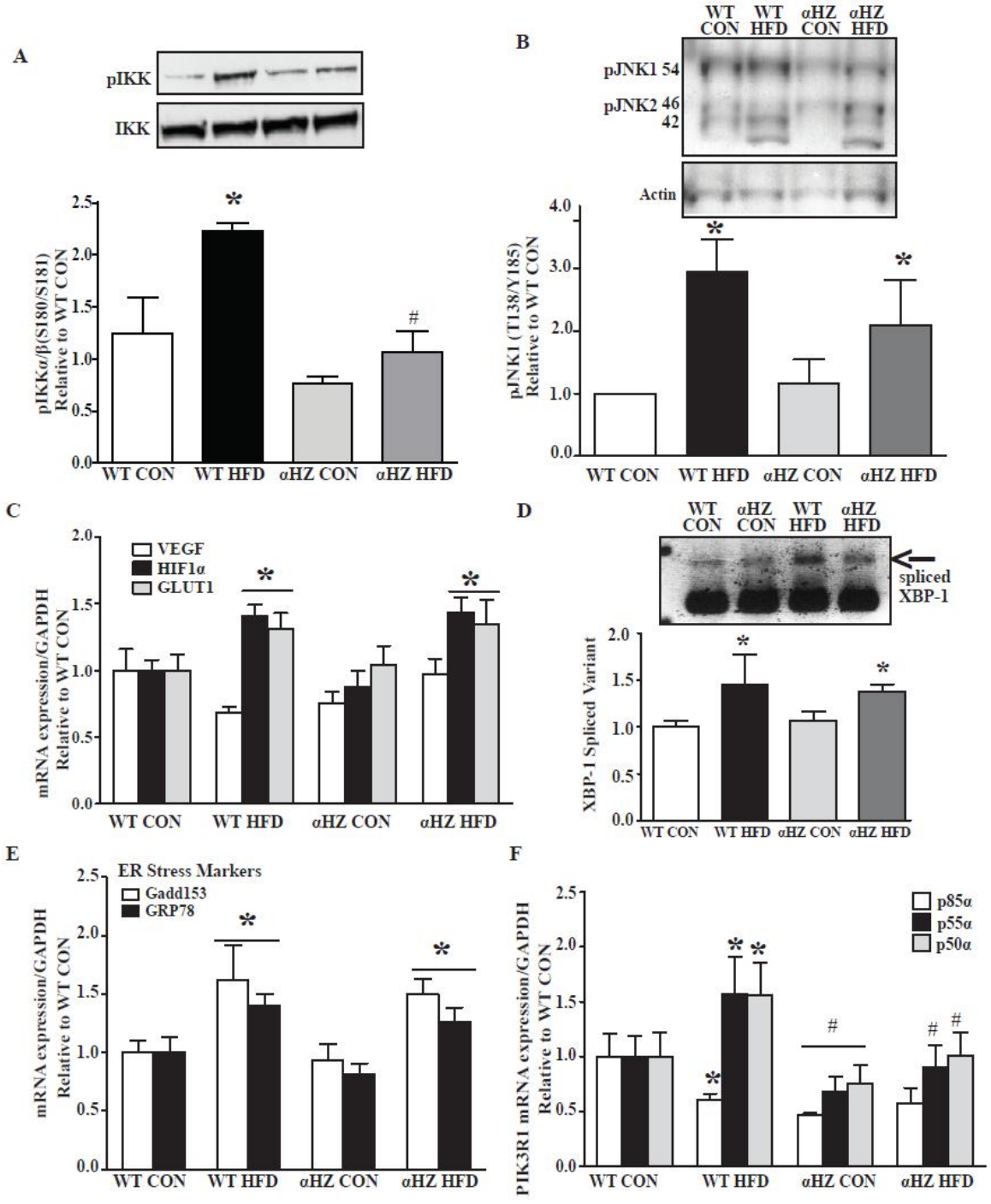
SUPPLEMENTARY DATA

Supplementary Figure 6. *Pik3r2* knockout does not prevent impairments in AT insulin signaling. (A) Insulin signaling proteins were measured in adipose tissue from WT and BKO mice before and after insulin stimulation. No differences were found between WT-HFD and BKO-HFD mice. Representative blots are shown.



SUPPLEMENTARY DATA

Supplementary Figure 7. Obesity activates markers of inflammation, hypoxia and ER stress in WT and αHZ. Activation of inflammatory signaling pathways (A) IKKα/β(S180/181) phosphorylation and (B) JNK(T183/Y185) was measured in adipose tissue by immunoblot. (A) A 2-fold increase in IKKα/β(S180/181) phosphorylation was found WT-HFD but not αHZ-HFD while pJNK was significantly elevated in both HFD groups. Representative blots are shown for pIKK, IKK, pJNK and actin. Changes in gene expression were measured in adipose tissue by qPCR for (C) hypoxia factors, HIF1α, GLUT1 and VEGF, (D) XBP1 splicing, (E) ER stress genes, GADD145 (also CHOP) and GRP78 (also Bip) and (F) *Pik3r1* subunits, p85α p55α, p50. All data are presented as the mean + SEM (n=5-6/group). Data were analyzed by 2-way ANOVA for main effects of diet and genotype with a Tukey posthoc test, *P<0.05 within same genotype; #P<0.05 within same diet group.



SUPPLEMENTARY DATA

Supplementary Figure 8. *Pik3r1+/-* knockdown protects insulin signaling in obese skeletal muscle. Insulin signaling proteins were measured in skeletal muscle before and after insulin stimulation from WT and αHZ mice fed either a CON or HFD for 16 weeks. Quantitation of signaling proteins from immunoblots for (A) IRS1(Y612) phosphorylation, (B) Akt(S473) phosphorylation, (C) p85α abundance, (D) p110 abundance and (F) PY-associated PI 3-kinase activity was assayed in basal and insulin-stimulated skeletal muscle. (E) Representative blots are shown. All data are shown as the mean + SEM. n=6-10/group. Data were analyzed by 2-way ANOVA for main effects of diet and genotype with a Tukey posthoc test, *P<0.05 within same genotype; #P<0.05 within same diet group.