

Supplemental information for:
**Adipocyte PI3K links adipostasis with basal insulin secretion through
an adipoincretin effect**

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Seven supplemental figures.

Fig. S1

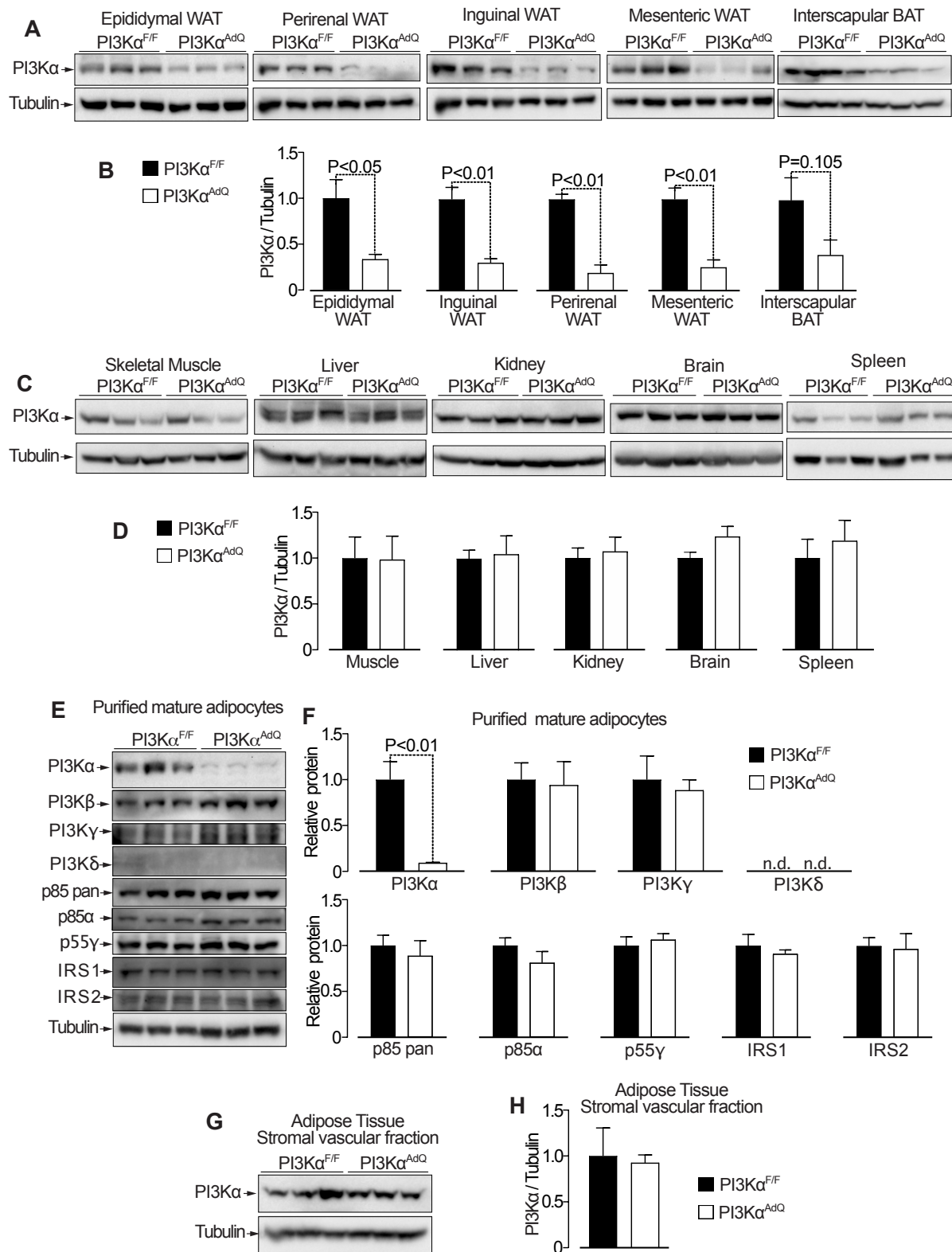


Figure S1. Efficient and specific PI3K α deletion in PI3K α^{AdQ} mice, related to Figure 1.

(A) PI3K α^{AdQ} and PI3K $\alpha^{\text{F/F}}$ mice from Figure 1 were sacrificed, and PI3K α protein abundance in their adipose tissue pads was measured by immunoblotting. (B) Quantification of the immunoblots in A. (C) PI3K α protein abundance in muscle, liver, kidney, brain, and spleen was measured by immunoblotting. (D) Quantification of the immunoblots in C. (E) The abundances of class-1 PI3K catalytic and adapter subunits and of the insulin receptor substrates IRS1 and IRS2 were quantified by immunoblot in mature adipocytes purified from epididymal fat pads. (F) Quantification of the immunoblots in E. (G) Immunoblot analysis of PI3K α protein abundance in the stromal vascular fractions of the epididymal fat pads in E. (H) Quantification of the immunoblots in G.

n=3 mice per group for A-D and G-H, n=6 for E-F (n=3 for IRS1 and IRS2).

Data are mean $\pm$ s.e.m. Statistical analysis was performed using the unpaired t-test or Mann-Whitney for E (n=6).

Fig. S2

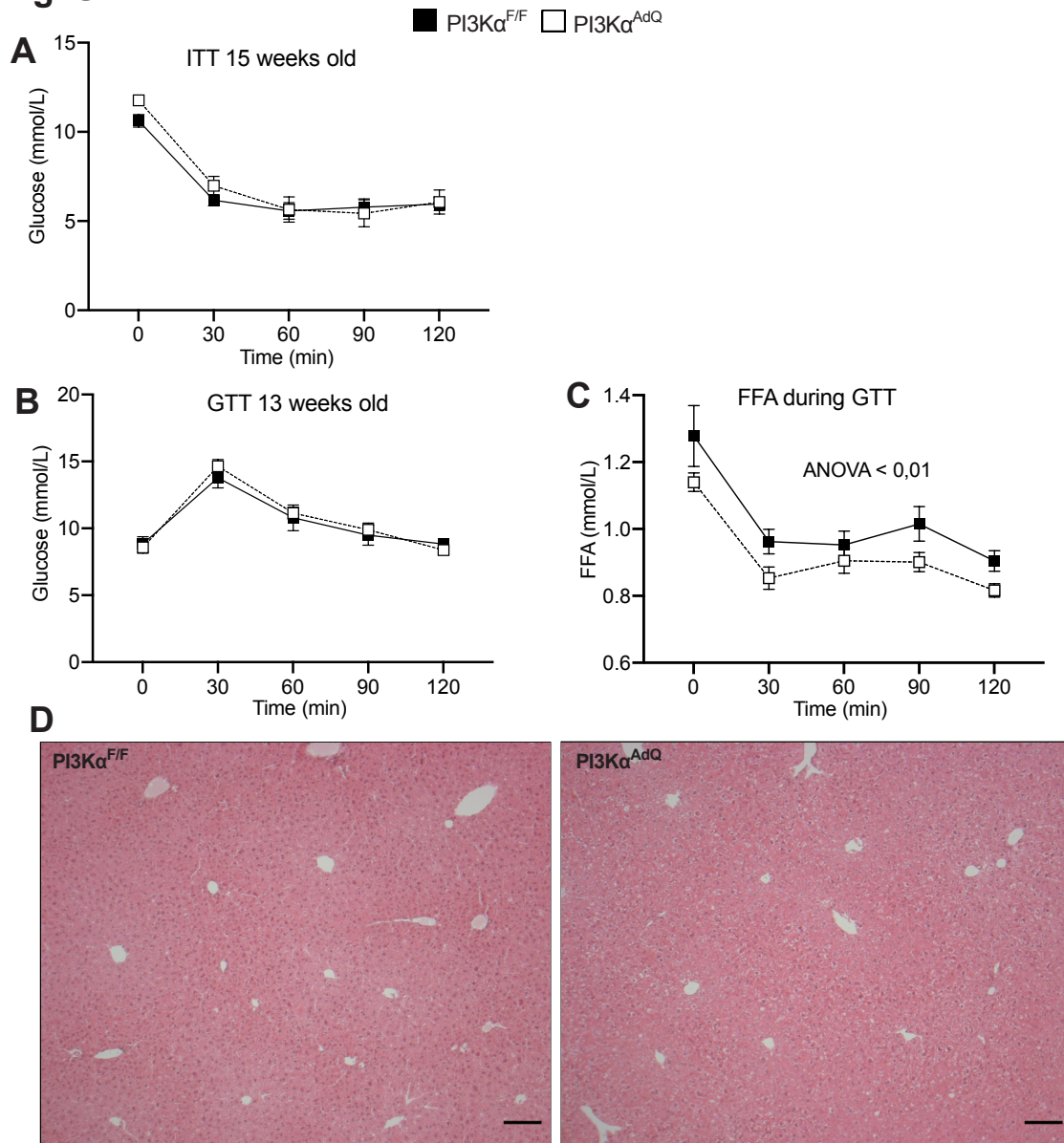


Figure S2. $PI3K\alpha^{AdQ}$ mice display normal glucose and lipid control, related to Figure 1.

(A) Insulin tolerance test (ITT): $PI3K\alpha^{AdQ}$ and $PI3K\alpha^{F/F}$ mice were fasted for 4 hours, and blood glucose was measured at the indicated time points after i.p. injection of 1 IU/kg of insulin.

(B) Glucose tolerance test (GTT): $PI3K\alpha^{AdQ}$ and $PI3K\alpha^{F/F}$ mice were fasted for 4 hours, and blood glucose was measured at the indicated time points after i.p. injection of 1 g/kg of glucose.

(C) Quantification of serum-free fatty acids during the GTT. (D) Representative image of liver histology. Scale bar 100 μ m. n=6 mice per group, data are mean $\pm$ s.e.m. Statistical analysis was performed using repeated measures two-way ANOVA.

Fig. S3

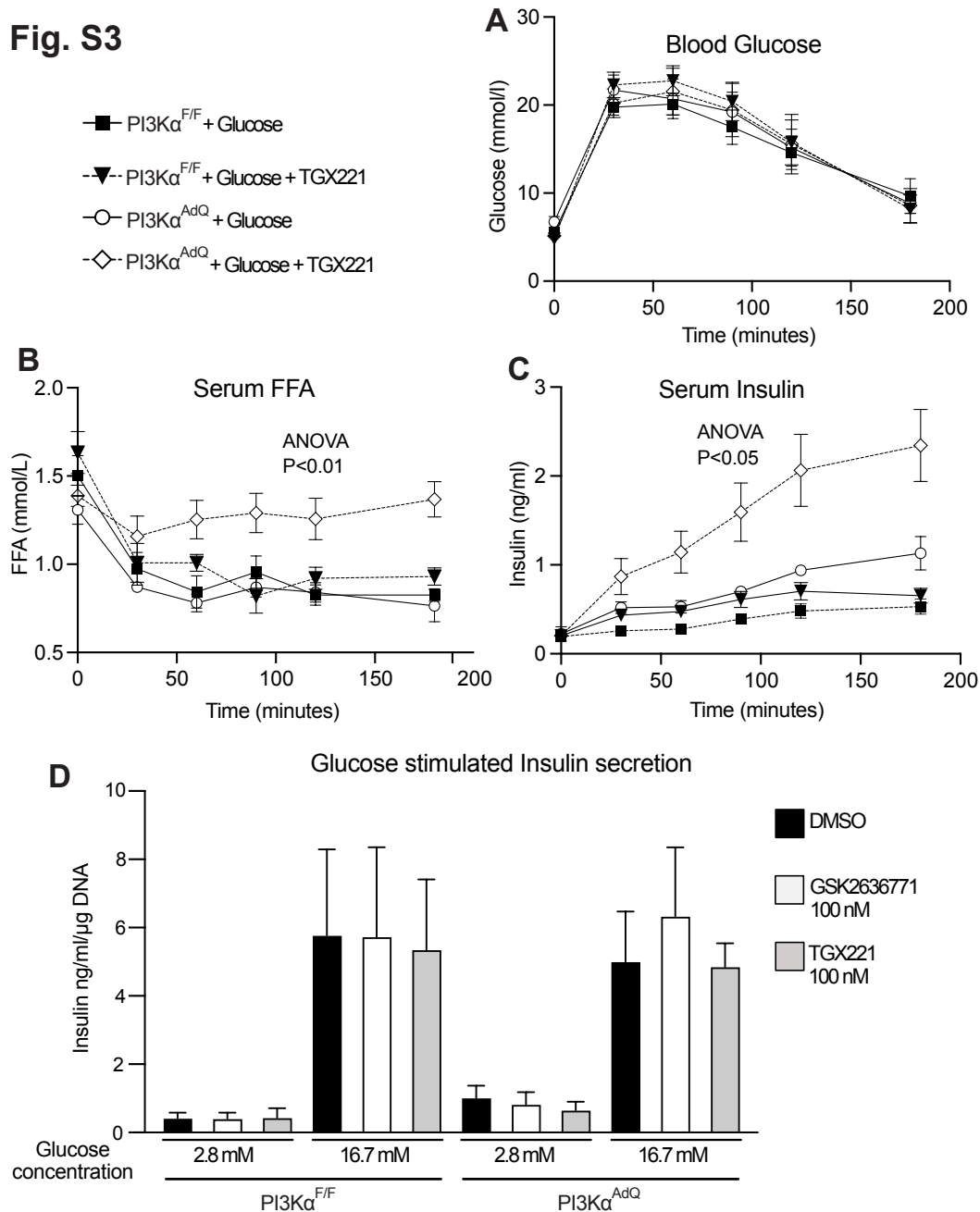


Figure S3. Adipocyte PI3K activity links adipostasis with insulin secretion through an adipoincretin effect, related to Figure 2.

(A) $PI3K^{\alpha AdQ}$ and $PI3K^{\alpha F/F}$ mice were fasted for 12 hours and injected i.p. with 2 g/Kg of glucose with either vehicle or 8 mg/Kg of the PI3K β -selective inhibitor TGX221. Blood glucose was measured at the indicated time points. (B) Serum FFA, and (C) serum insulin of the mice from

A. **(D)** Glucose stimulated insulin secretion of islets isolated from $PI3K\alpha^{AdQ}$ and $PI3K\alpha^{F/F}$ mice kept in the presence of either vehicle, the $PI3K\beta$ -selective inhibitors GSK2636771 or TGX221. $n=6$ mice per group in A-C and $n=3$ for D. Data are mean $\pm$ s.e.m. Statistical analysis was performed using repeated measures two-way ANOVA for A-C and unpaired t-test for D. For B, glucose significantly reduced FFA levels in all groups except for $PI3K\alpha^{AdQ}$ mice co-treated with TGX221. The P-value in B is for $PI3K\alpha^{AdQ}$ + Glucose + TGX221 vs $PI3K\alpha^{AdQ}$ + Glucose. C, the indicated P-value is the most conservative for the following comparisons for $PI3K\alpha^{AdQ}$ + Glucose + TGX221 vs. $PI3K\alpha^{AdQ}$ + Glucose, and $PI3K\alpha^{AdQ}$ + Glucose + TGX221 vs. $PI3K\alpha^{F/F}$ + Glucose + TGX221.

Fig. S4

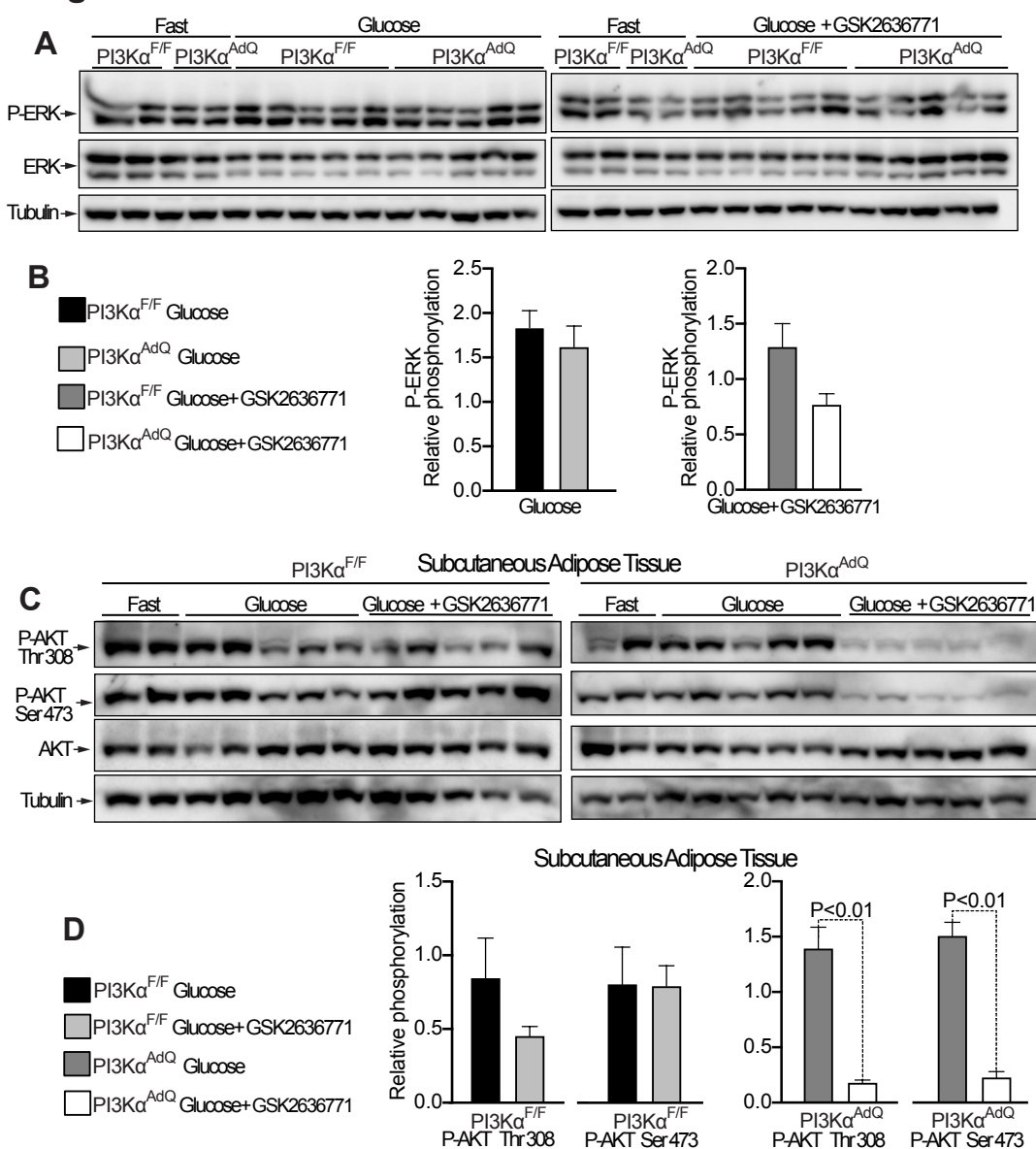


Figure S4. Effects of adipose tissue-selective inhibition on ERK and AKT phosphorylation in fat tissues, related to Figure 2.

(A) Immunoblot analysis of ERK phosphorylation in the epididymal adipose tissues from $PI3K\alpha^{AdQ}$ and $PI3K\alpha^{F/F}$ mice described in Figure 2. (B) Quantification of the immunoblots in A. (C) Immunoblot analysis of AKT phosphorylation in the subcutaneous inguinal adipose tissues from $PI3K\alpha^{AdQ}$ and $PI3K\alpha^{F/F}$ mice described in Figure 2. (D) Quantification of the immunoblots in C. $n=2-5$ mice per group, Data are mean $\pm$ s.e.m. Statistical analysis was performed using the Mann–Whitney test.

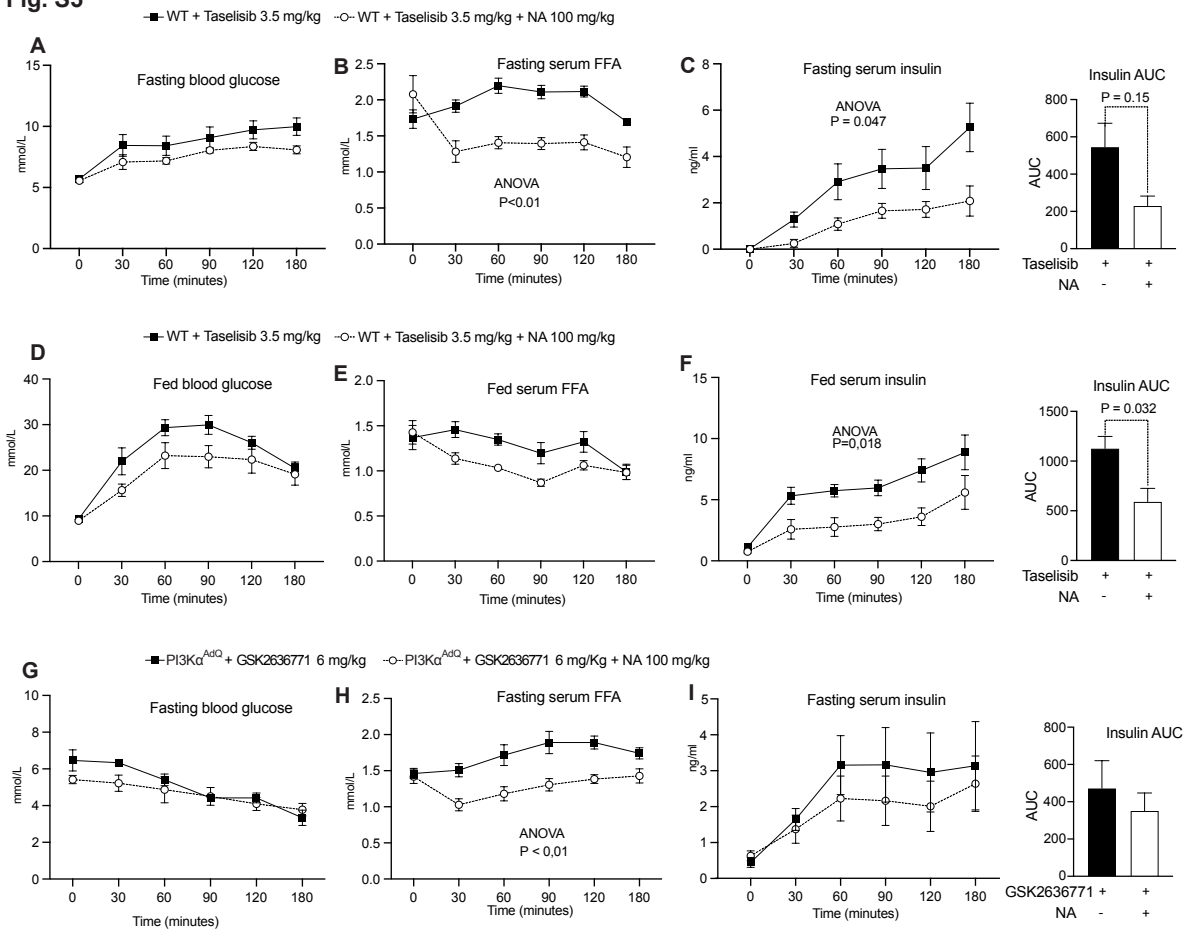
Fig. S5

Figure S5. The hyperinsulinemia induced by systemic PI3K inhibition and the adiponectin effect are partially dependent on lipolysis, related to Figure 4.

Wild-type C57BL/6 mice were fasted for 12 hours and injected with pan-PI3K inhibitor Taselisib in the presence or absence of nicotinic acid (NA). (A) Blood glucose, (B) serum FFA, (C) serum insulin of the mice above. The same C57BL/6 mice above were kept in fed conditions and injected with pan-PI3K inhibitor Taselisib in the presence or absence of nicotinic acid (NA). (D) Blood glucose, (E) serum FFA, (F) serum insulin of the mice above.

$PI3K^{\text{AdQ}}$ mice were fasted for 12 hours and injected with GSK2636771, and one group of $PI3K^{\text{AdQ}}$ received GSK2636771 together with nicotinic acid (NA). (G) Blood glucose, (H) serum FFA, (I) serum insulin of the mice above.

n=5 mice for A-F and for G-H: n=5 GSK2636771 and n=6 for GSK2636771 with NA.

Data are mean $\pm$ s.e.m. Statistical analysis was performed using repeated measures two-way ANOVA and Mann-Whitney for insulin AUC.

Fig. S6

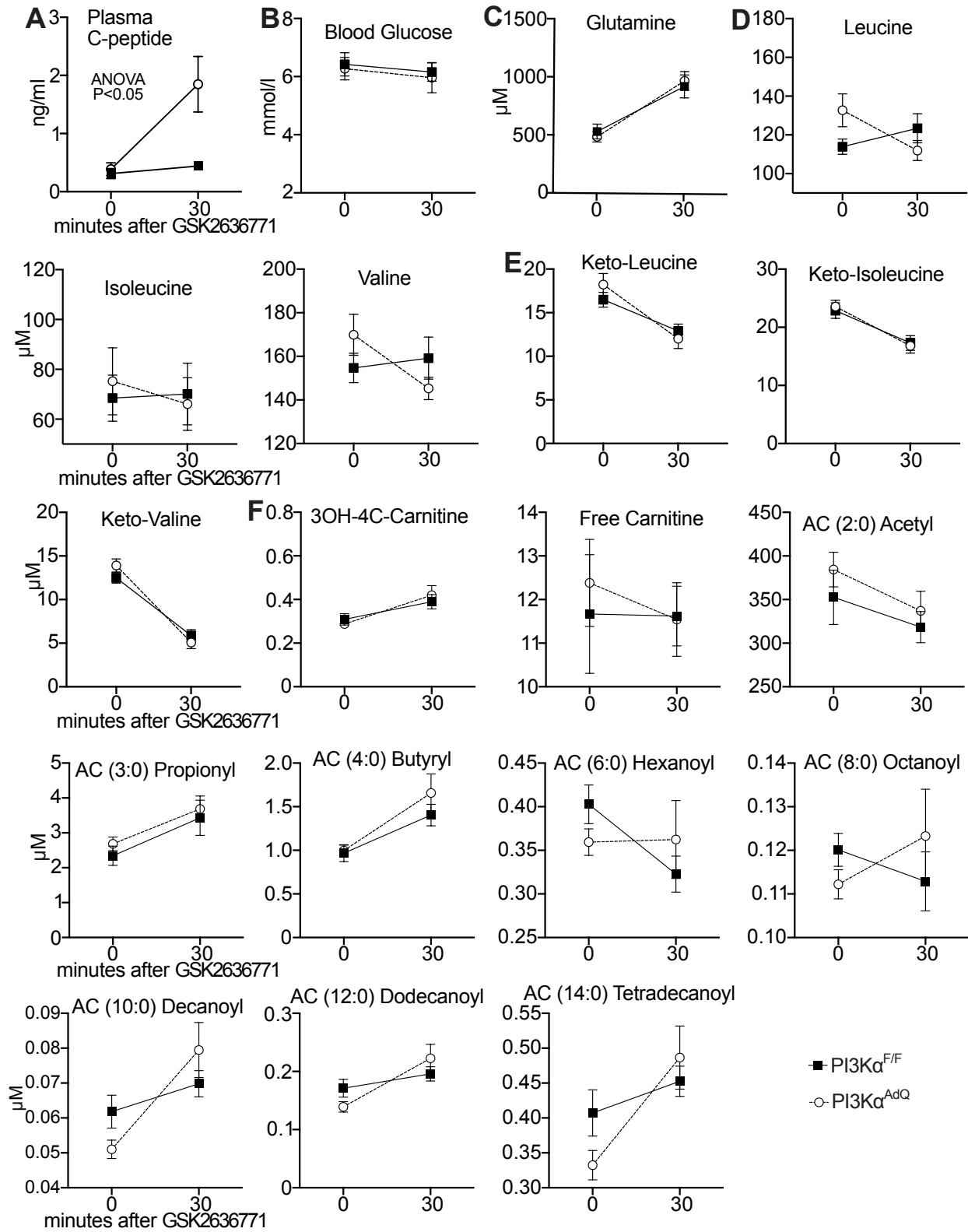


Figure S6. Targeted metabolomics, related to Figure 5.

PI3K α^{AdQ} and PI3K $\alpha^{F/F}$ mice fasted for 12 hours and were injected with GSK2636771, and plasma was collected at 0 minutes (before GSK2636771 injection) and at 30 minutes post-injection. Plasma levels of **(A)** C-peptide, **(B)** glucose, **(C)** glutamine, **(D)** branched-chain amino acids, **(E)** ketoacids, and **(F)** acylcarnitines were measured. n=10 mice. Data are mean $\pm$ s.e.m. Statistical analysis was performed using repeated measures two-way ANOVA.

Fig. S7

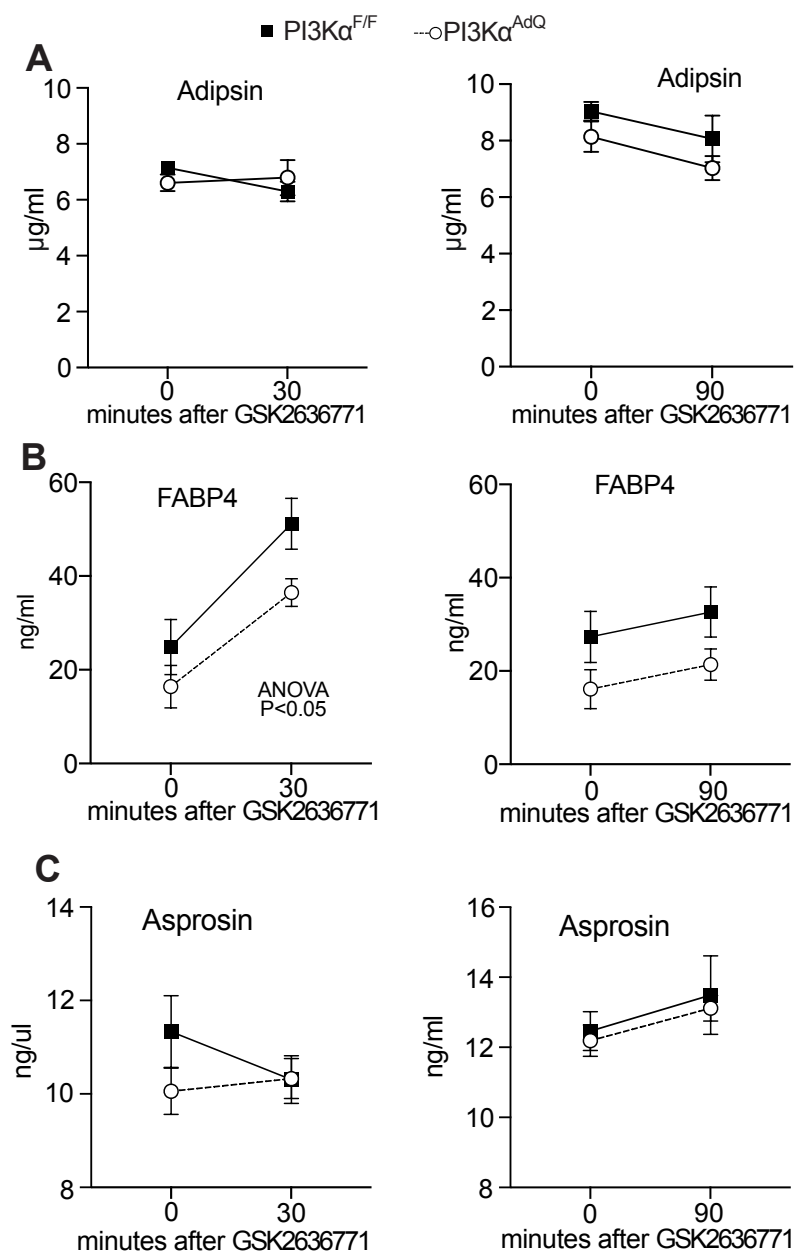


Figure S7. The adiponcretin effect is not associated with plasma abundances of Adipsin, FABP4, or Asprosin, related to Figure 5.

$PI3K\alpha^{AdQ}$ and $PI3K\alpha^{F/F}$ mice were fasted for 12 hours, and injected with GSK2636771, and plasma was collected at 0 minutes (before GSK2636771 injection) and at 30 minutes post-injection or, in another cohort, at 0' (before GSK2636771 injection) and at 90 minutes post-injection. Plasma levels of (A) Adipsin, (B) FABP4, and (C) Asprosin were measured by ELISA.

n=7 mice for A, n=9 mice for B and n=8 for C. Data are mean $\pm$ s.e.m. Statistical analysis was performed using repeated measures two-way ANOVA. P value in B refers to an effect of GSK2636771 independent from the genotype.