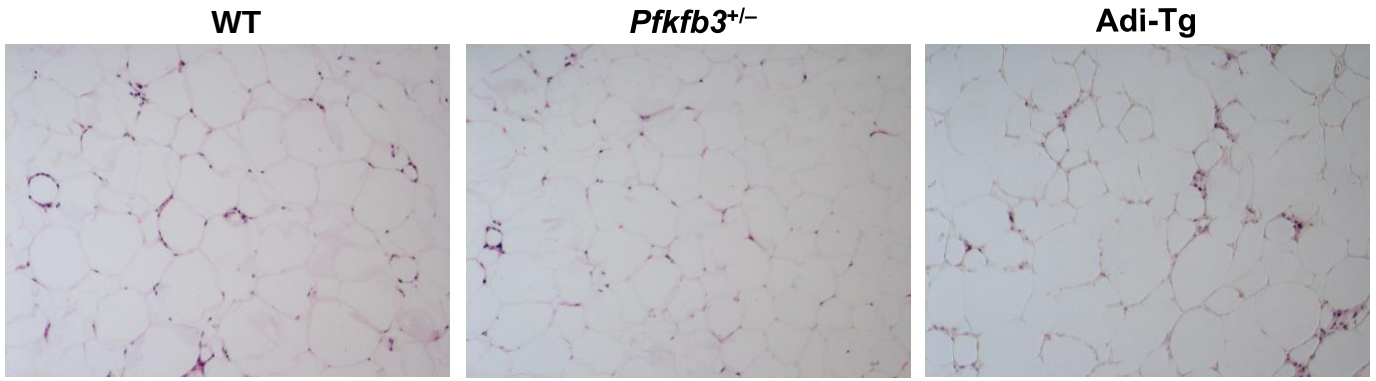
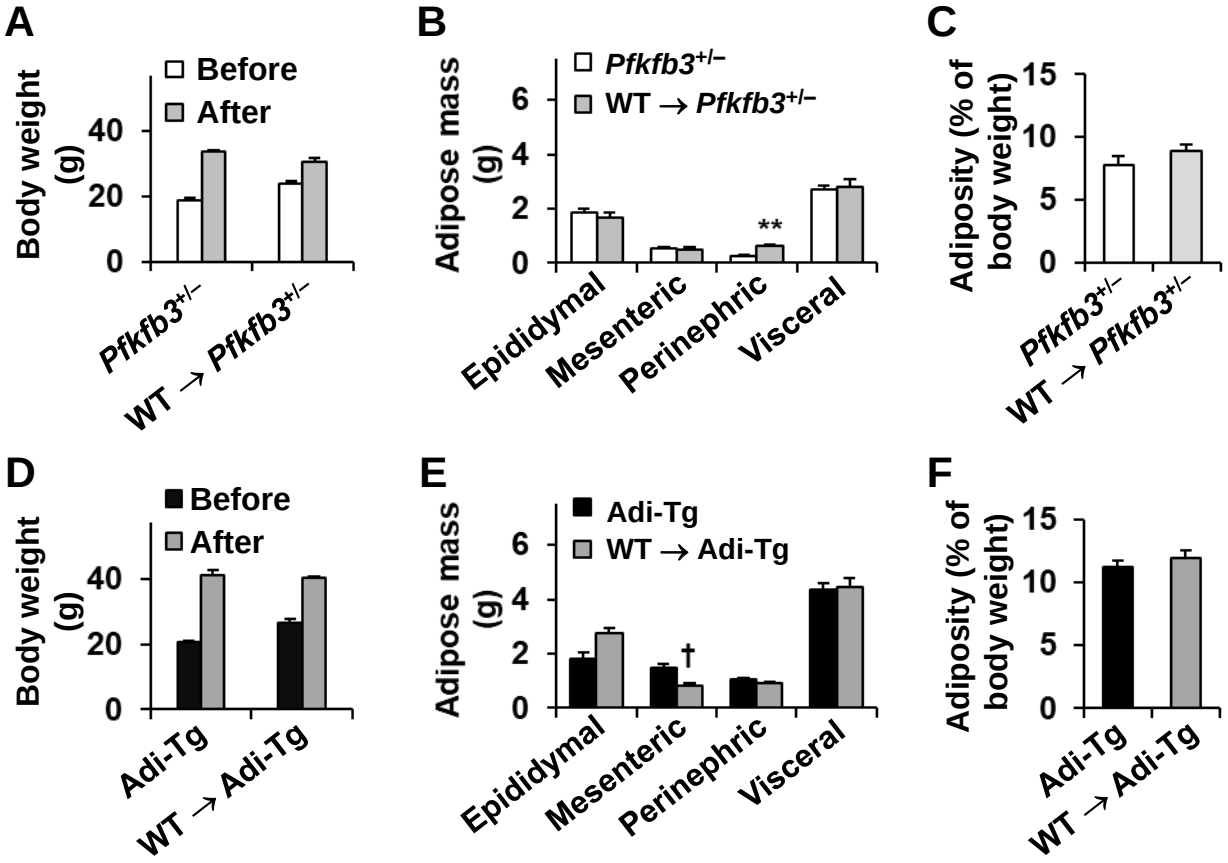


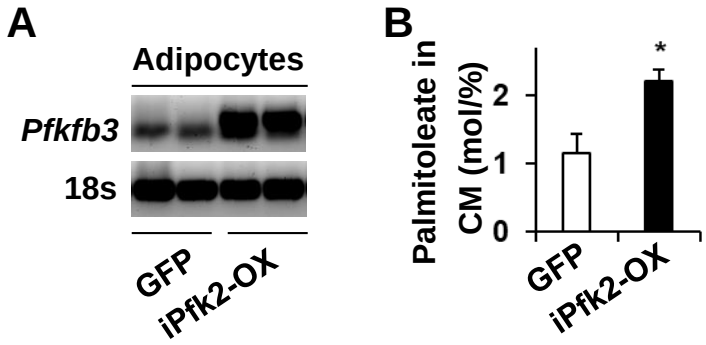
Supplemental Figure S1. Related to Figure 2
The iPfk2 in adipocytes determines adiposity

White adipose tissue (WAT) histology of WT, *Pfkfb3*$^{+/-}$, and Adi-Tg mice. At 5 - 6 weeks of age, the mice were fed a high-fat diet (HFD, 60% fat calories) for 12 weeks. WAT sections were stained with H&E. Similar data from the same sets of mice were published in J Biol Chem 2010,285:3713-3721 and J Biol Chem 2012,287:21492-21500.

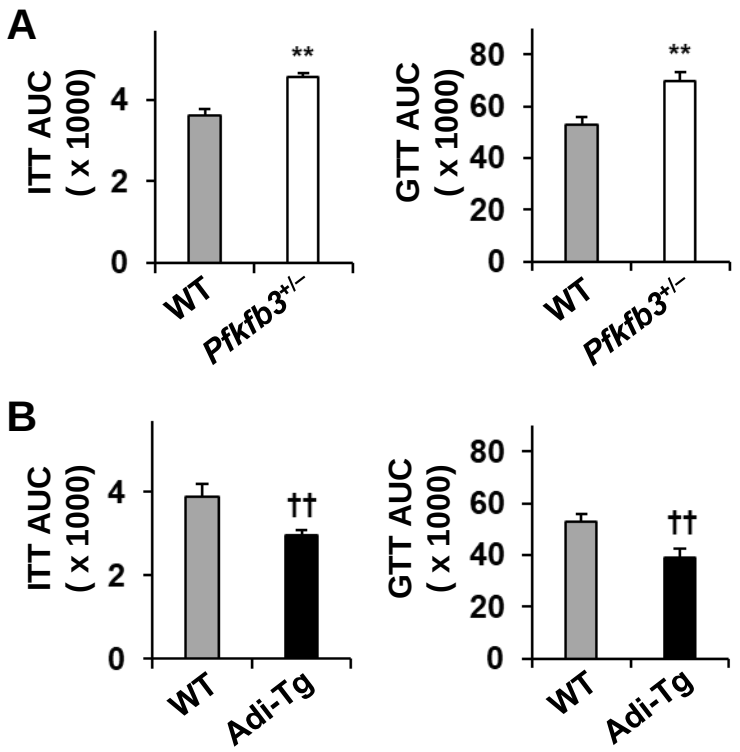


Supplemental Figure S2. Related to Figure 2
Adoptive transfer of wild-type hematopoietic cells does not alter adiposity

(A,**B**,**C)** Body weight (A), adipose tissue mass (B), and adiposity (C) of *Pfkfb3*-disrupted (*Pfkfb3*$^{+/-}$) mice and WT → *Pfkfb3*$^{+/-}$ mice. **(D**,**E**,**F)** Body weight (D), adipose tissue mass (E), and adiposity (F) of Adi-Tg mice and WT → Adi-Tg mice. For A - F, male *Pfkfb3*$^{+/-}$ and Adi-Tg mice, at 5 - 6 weeks of age, were lethally irradiated and transferred with bone marrow cells from wild-type (WT) C57BL/6J mice. After recovery for 4 weeks from bone marrow transplantation, chimeric mice were fed a high-fat diet (HFD, 60% fat calories) for 12 weeks. In separate studies, male *Pfkfb3*$^{+/-}$ and Adi-Tg mice, at 5 - 6 weeks of age, were fed an HFD for 12 weeks. For A and D, body weight was recorded before and after HFD feeding. For B and E, after harvest of mice, the mass of epididymal, mesenteric, and perinephric fats were weighed and estimated as visceral fat mass. For C and F, adiposity was calculated as the ratio of visceral fat mass to body weight. For A and B, data are means ± SEM. n = 7 - 10. **, $P < 0.01$ WT → *Pfkfb3*$^{+/-}$ vs. *Pfkfb3*$^{+/-}$ for the same type of fat mass; †, $P < 0.05$ WT → Adi-Tg vs. Adi-Tg for the same type of fat mass. Some of the original data for *Pfkfb3*$^{+/-}$ and Adi-Tg mice were published in J Biol Chem 2010,285:3713-3721 and J Biol Chem 2012,287:21492-21500.

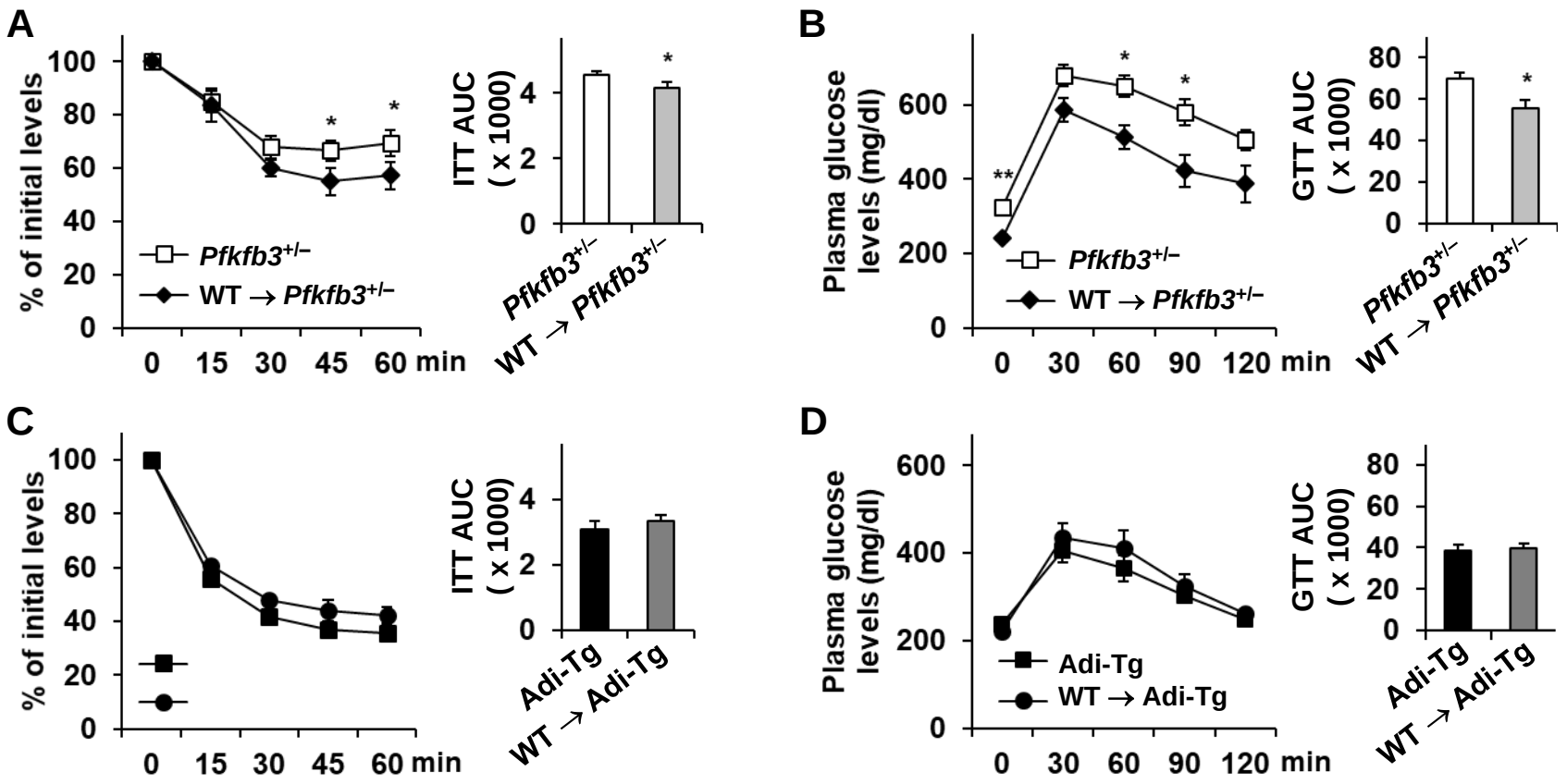


Supplemental Figure S3. Related to Figure 4
Palmitoleate is a *Pfkfb3*-driven adipocyte bioactive lipid
Palmitoleate production by adipocytes in response to *Pfkfb3* overexpression. Stable *Pfkfb3*-overexpressing 3T3-L1 (iPfk2-OX) were differentiated into adipocytes and examined for palmitoleate production. (**A**) Representative images of adipocyte *Pfkfb3* mRNAs. (**B**) Quantification of palmitoleate levels in adipocyte conditioned media (the original data were published in *J Biol Chem*. 2012 Jun 15;287(25):21492-500. doi: 10.1074/jbc.M112.370379).



Supplemental Figure S4. Related to Figure 5
***Pfkfb3*/iPfk2 regulates systemic inulin sensitivity**

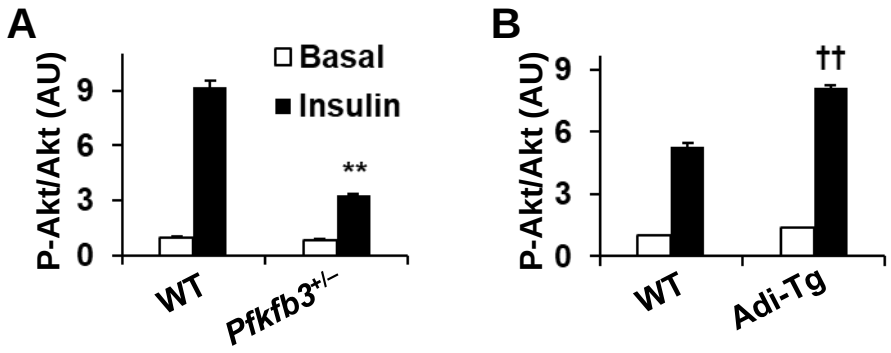
(A,**B)** Area under curve (AUC) of insulin sensitivity and glucose tolerance tests in global *Pfkfb3*-disrupted (*Pfkfb3*$^{+/-}$) mice (A) and aP2-*Pfkfb3* transgenic mice (Adi-Tg) mice (B). For A and B, male WT, *Pfkfb3*$^{+/-}$, and Adi-Tg mice, at 5 - 6 weeks of age, were fed a high-fat diet (60% fat calories) for 12 weeks. After the feeding period, the mice were fasted for 4 hr and subjected to an intraperitoneal injection of insulin (1 U/kg body weight) or glucose (2 g/kg body weight). For A and B, data are means ± SEM. n = 10. *, $P < 0.05$ and **, $P < 0.01$ *Pfkfb3*$^{+/-}$ vs. WT; ††, $P < 0.01$ Adi-Tg vs. WT. The original data for glucose and insulin tolerance tests were published in J Biol Chem 2010,285:3713-3721 and J Biol Chem 2012,287:21492-21500.



Supplemental Figure S5. Related to Figure 5

Hematopoietic *Pfkfb3* regulates systemic insulin sensitivity and glucose tolerance

Mice were as described in Figure S2. (**A**,**B**) Insulin (A) and glucose (B) tolerance tests for *Pfkfb3*-disrupted (*Pfkfb3*$^{+/-}$) mice and WT → *Pfkfb3*$^{+/-}$ mice. **(C**,**D)** Insulin (C) and glucose (D) tolerance tests for Adi-Tg mice and WT → Adi-Tg mice. For A - D, after the feeding period, mice were fasted for 4 hr and received an intraperitoneal injection of insulin (1 U/kg body weight) (A and C) or glucose (2 g/kg body weight) (B and D). For A - D, data are means ± SEM. n = 7 - 10. *, $P < 0.05$ WT → *Pfkfb3*$^{+/-}$ vs. *Pfkfb3*$^{+/-}$ in A and B (bar graphs) for the same point (line graphs).



Supplemental Figure S6. Related to Figure 6
***Pfkfb3*/iPfk2 regulates WAT insulin signaling**

(**A**,**B**) Ratios of P-Akt/Akt in WAT from *Pfkfb3*$^{+/-}$ mice (A) and Adi-Tg mice (B) were quantified based on WAT insulin signaling (published in J Biol Chem 2010,285:3713-3721 and J Biol Chem 2012,287:21492-21500). At 5 - 6 weeks of age, male WT, *Pfkfb3*$^{+/-}$, and Adi-Tg mice were fed a high-fat diet (60% fat calories) for 12 weeks. After the feeding period, some mice were fasted for 4 hr and given a bolus injection of insulin (1 U/kg body weight) into the portal vein for 5 min prior to harvest. WAT lysates were analyzed for the phosphorylation states of Akt and total amount of AKT. Data are means ± SEM. n = 4 (without insulin) or 6 (with insulin). **, $P < 0.01$ *Pfkfb3*$^{+/-}$ vs. WT; ††, $P < 0.01$ Adi-Tg vs. WT.