Supplemental Figures

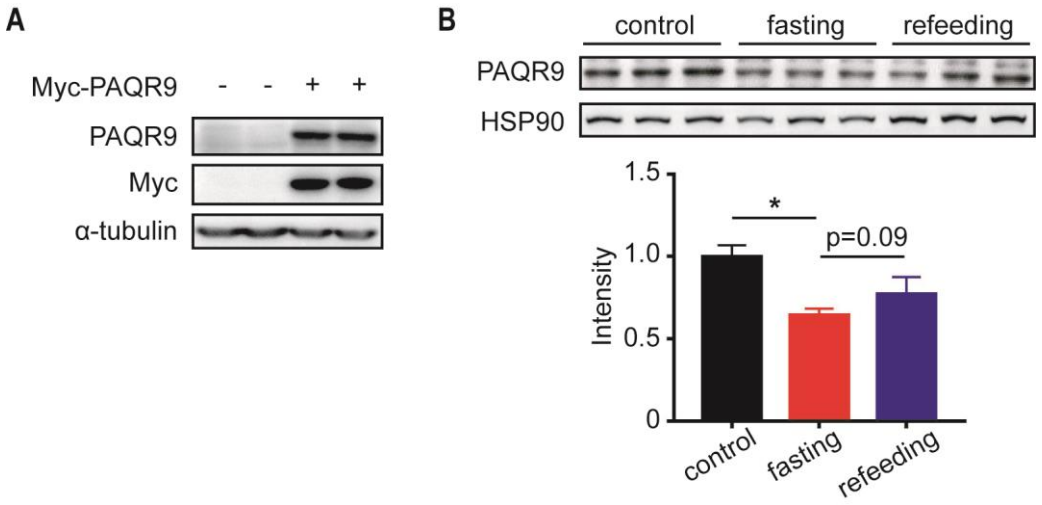

Fig. S1. Analysis of PAQR9 protein

A. Confirmation of the antibody using Western blotting to detect Myc-tagged PAQR9 overexpressed in HEK293T cells.

B. Western blotting to detect PAQR9 protein with the mouse liver samples as in Fig. 1B. Quantitation of the data is shown in the below panel. The data are shown as mean ± S.E.M., * for $P < 0.05$.

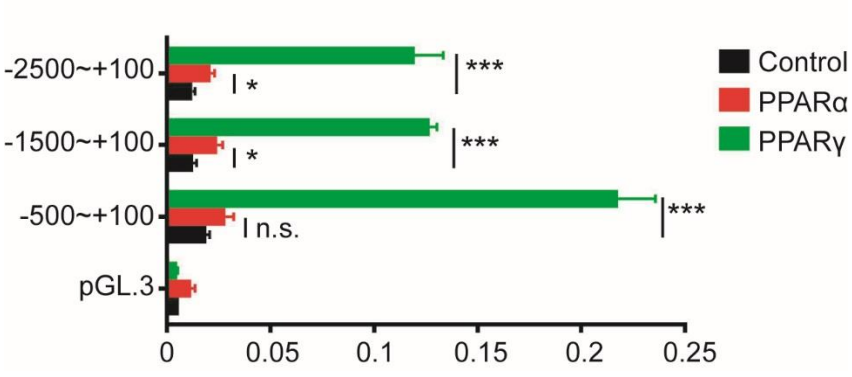

Fig. S2. The effect of PPARα and PPARγ overexpression on luciferase activity of *Paqr9* promoter

Different lengths of putative *Paqr9* promoter were transiently expressed in the Hep3B cells together with PPARγ-expressing or PPARα-expressing plasmids as indicated. Relative luciferase activity was calculated by dividing the firefly luciferase activity by renilla activity. The data are shown as mean ± S.E.M., ** for $P < 0.01$, *** for $P < 0.001$ and n.s. for non-significant as compared to the control group.

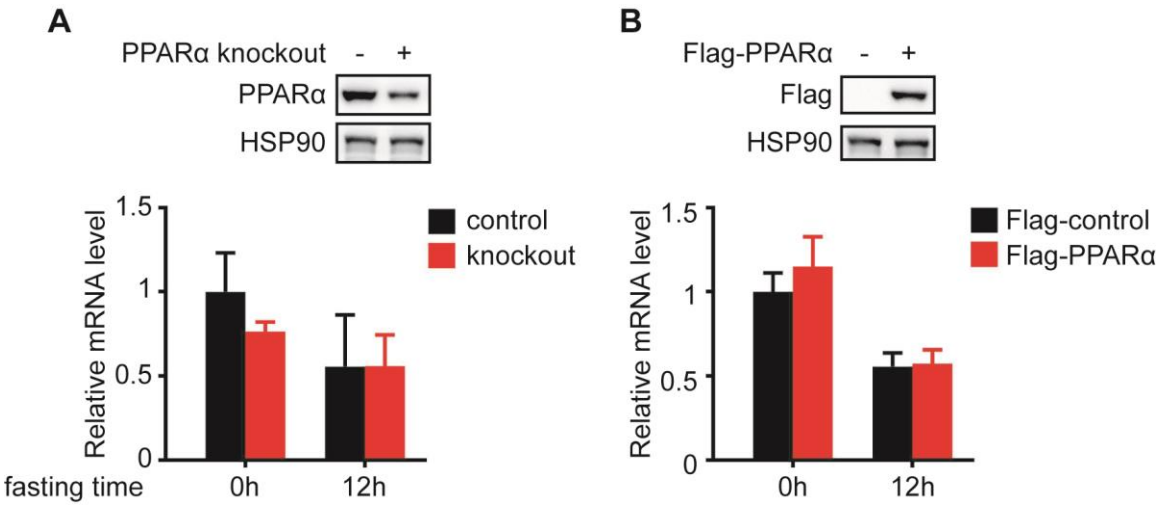

Fig. S3. PPARα could not affect PAQR9 expression in Hep3B cells

A. The mRNA levels of Paqr9 in PPARα-deleted Hep3B cells with low-glucose starvation for 0 and 12 hours. Western blotting of PPARα protein is shown in the upper panel.

B. The mRNA levels of Paqr9 in PPARα-overexpressing Hep3B cells with low-glucose starvation for 0 and 12 hours. Western blotting of PPARα protein is shown in the upper panel.

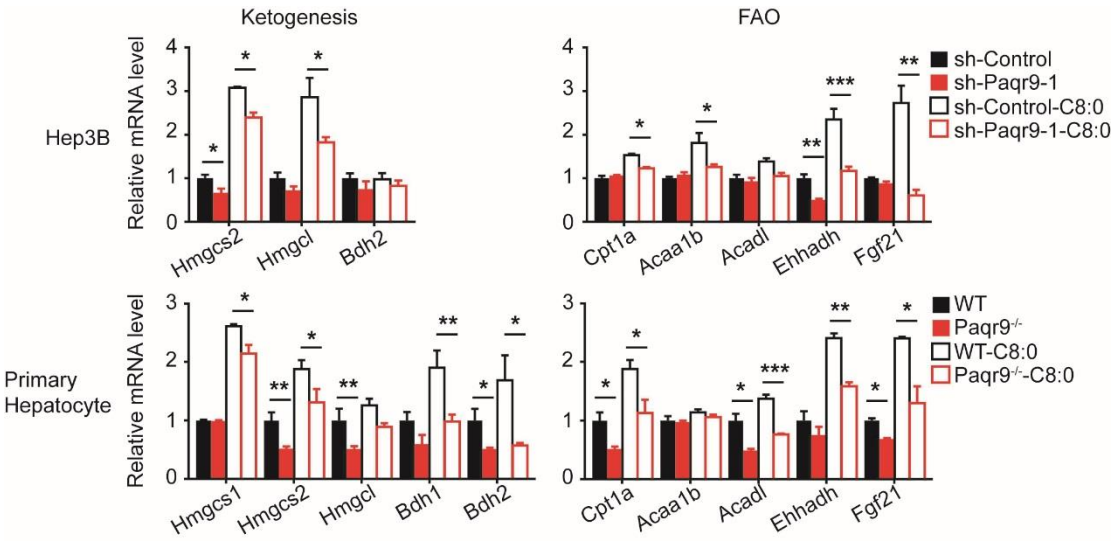

Fig. S4. Effect of *Paqr9* knockdown/knockout on expression of genes involved in ketogenesis and fatty acid oxidation in hepatocytes

The mRNA levels of ketogenesis and FAO genes in Hep3B cells and primary hepatocytes. For ketogenesis, the cells were treated with 500μM sodium caprylate (C8:0) for 24 h (n = 4 for each group). For FAO, the cells were treated with 1g/L glucose and 500μM sodium caprylate for 36 h (n = 4 for each group). The data are shown as mean ± S.E.M., * for $P < 0.05$, ** for $P < 0.01$ and *** for $P < 0.001$.

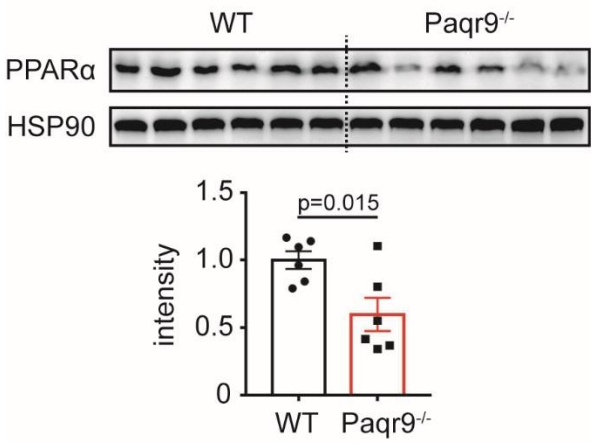

Fig. S5. *Paqr9* deletion reduces PPARα protein level in the mouse liver

Western blotting to detect PPARα protein in the liver of the mice. Quantitation of the data is shown in the lower panel.

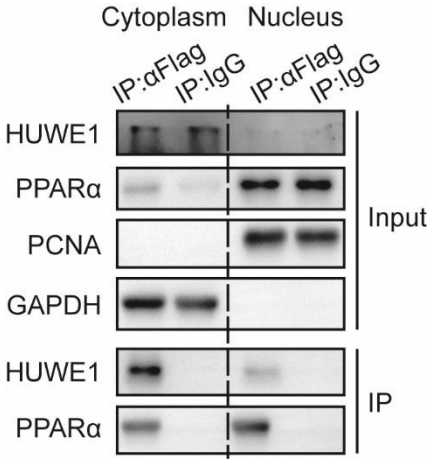

Fig. S6. The interaction of PPARα with HUWE1 in both cytoplasm and nucleus

HepB2 cells were transiently transfected with Flag-tagged Pparα, separated into cytoplasm and nucleus fractions, and then used in immunoprecipitation and immunoblotting with the antibodies as indicated.

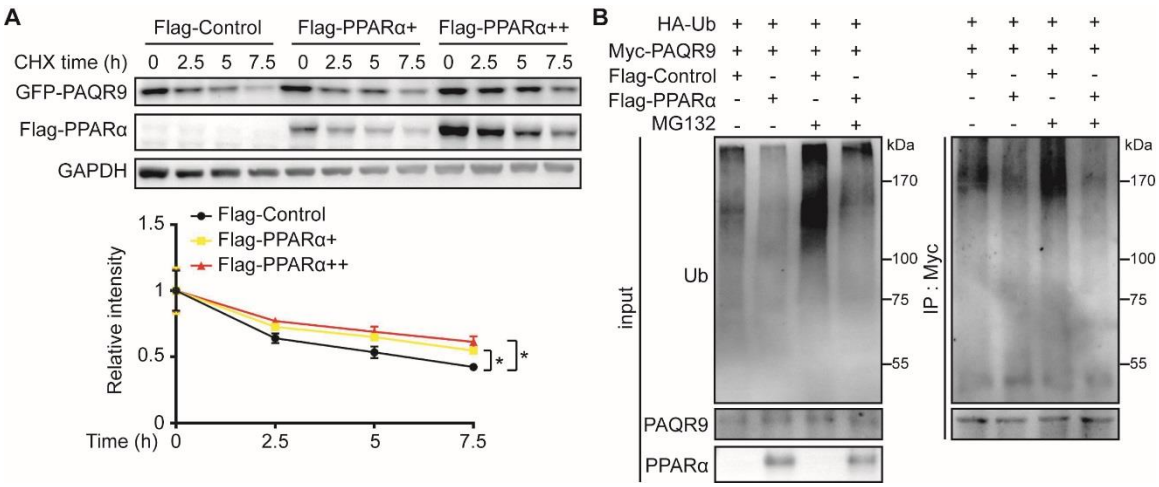

Fig. S7. The stability and ubiquitination of PAQR9 protein is affected by PPARα

A. The protein stability of PAQR9 is affected by PPARα. HEK293T cells were transiently transfected with GFP-fused PAQR9 and different amount of Flag-tagged PPARα, and then treated with 100μg/mL CHX for various time, followed by IB with the antibodies as indicated. Quantitation of the immunoblotting results from three independent experiments is shown in the lower panel. The data are shown as mean ± S.E.M., * for $P < 0.05$.

B. Poly-ubiquitination of PAQR9 is reduced by PPARα overexpression. HEK293T cells were transiently transfected with the plasmids as indicated, and then treated with MG132 (10 μM) for 6 h, followed by IP and IB with the antibodies as indicated.

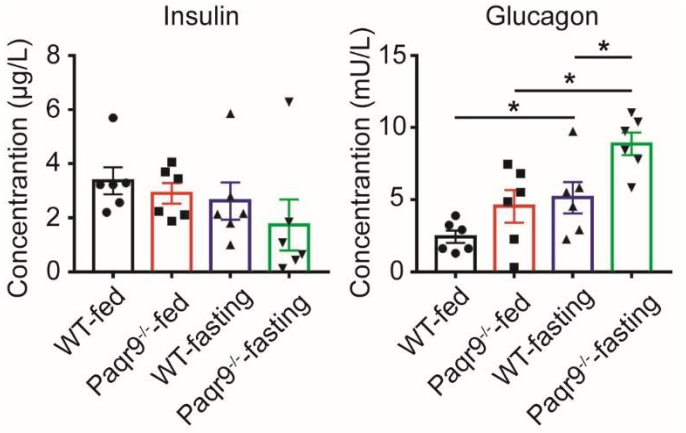

Fig. S8. The levels of insulin and glucagon in the serum of wild type and *Paqr9*-deleted mice

The mice were fasted 24 hours (n = 6 for each group). The data are shown as mean ± S.E.M., * for $P < 0.05$.

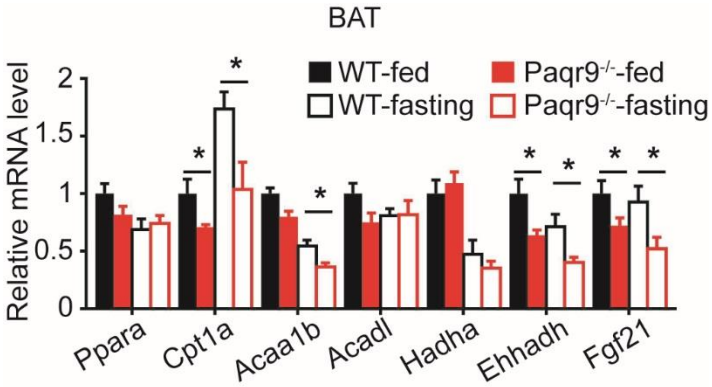

Fig. S9. Effect of *Paqr9* deletion on genes involved in fatty acid oxidation in brown adipose tissue

The mice were under fed condition or fasting condition for 24 h (n = 6 for each group). The mRNA levels of FAO genes in brown adipose tissue was analyzed by quantitative RT-PCR. The data are shown as mean ± S.E.M., * for $P < 0.05$.

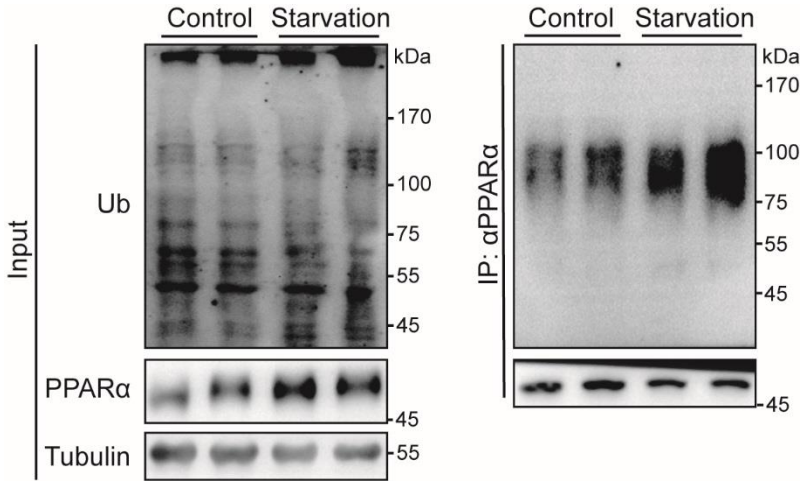

Fig. S10. Poly-ubiquitination of PPARα is elevated by starvation

Western blotting to detect PPARα in Hep3B cells cultured in full medium or in starvation medium (5% FBS with 1g/L glucose for 24 h), followed by immunoprecipitation and immunoblotting with the antibodies as indicated.