

Unsuppressed lipolysis in adipocytes is linked with enhanced gluconeogenesis and altered bile acid physiology in *Insr*^{P1195L/+} mice fed high-fat-diet

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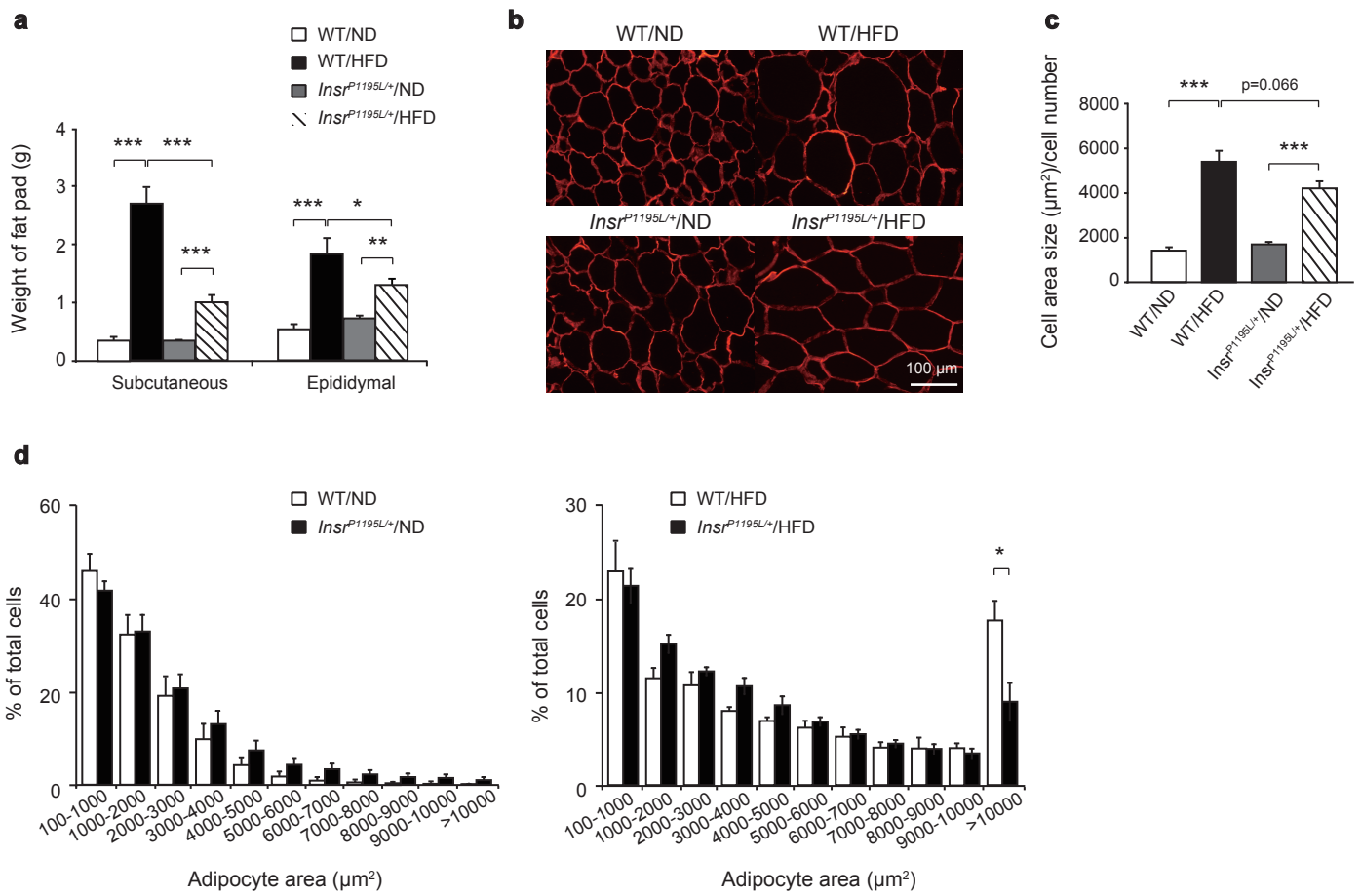
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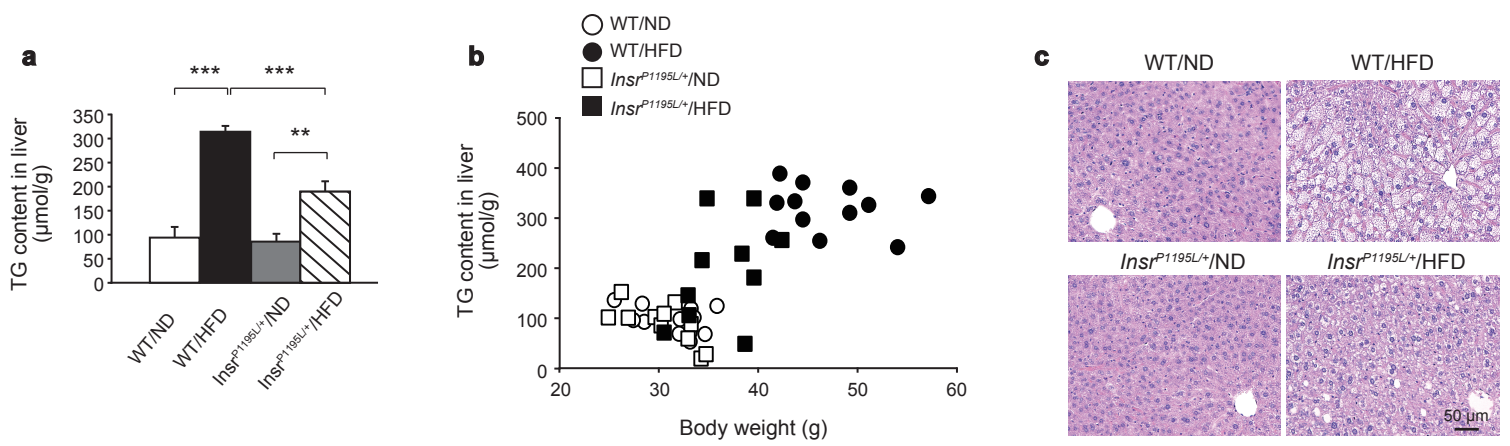
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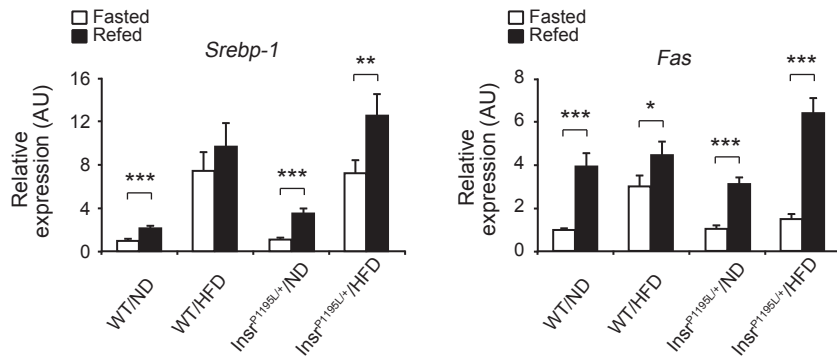
Supplementary Figure S1

Fat weight and adipocyte size. a. Fat weight of the mice at 25 weeks of age ($n = 3-8$). Epididyma fat weight in *Insr*^{P1195L/+}/HFD mice was significantly decreased compared with that of WT/HFD mice. b. Immunostaining of epididymal fat tissue with Caveolin-1 (1:1000, BD biosciences). c. Mean adipocyte size. d. Histogram of the cell area of WT/ND and *Insr*^{P1195L/+}/ND mice (left) and that of WT/HFD and *Insr*^{P1195L/+}/HFD mice (right). The adipocyte size was not different between of WT/ND and *Insr*^{P1195L/+}/ND mice (left). By contrast, the number of large (>10000 μm²) adipocytes of *Insr*^{P1195L/+}/HFD mice was significantly decreased compared with that of WT/HFD mice (right). Data are mean ± SEM. One-way ANOVA plus Bonferroni *post-hoc* analysis. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.



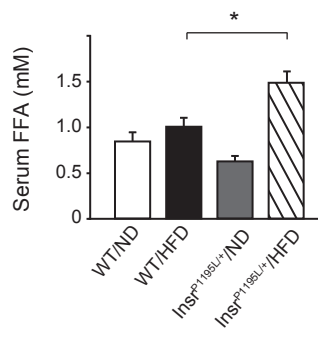
Supplementary Figure S2

Fat accumulation in the liver is reduced in *Insr*^{P1195L/+}/HFD mice. (a) TG content in *Insr*^{P1195L/+}/HFD liver was significantly less than that in WT/HFD liver ($n = 10-12$ per each group). (b) Relationship between body weight and TG content in liver. (c) HE staining of *Insr*^{P1195L/+}/HFD liver shows the amount of lipid droplet is decreased compared with that of WT/HFD liver. Data are mean \pm SEM. Two-way ANOVA plus Bonferroni *post-hoc* analysis. ** $P < 0.01$, *** $P < 0.001$.



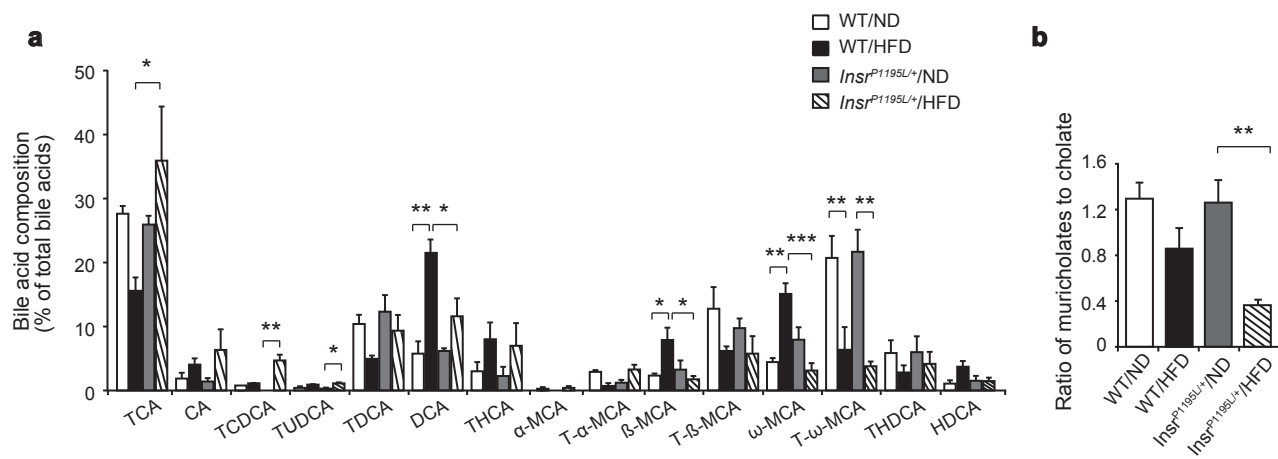
Supplementary Figure S3

mRNA expressions of genes regulated by insulin are not decreased in liver of *Insr*^{P1195L/+}/HFD mice. mRNA expressions of *Srebp-1* in *Insr*^{P1195L/+}/ND liver is not different from that in WT/ND liver, including its feeding-induced increase. In addition, mRNA expression of *Fas*, a target of *Srebp-1*, is not decreased in *Insr*^{P1195L/+}/HFD liver, indicating that induction of *Srebp-1* and *Fas* by refeeding or HFD remains 'sensitive' under insulin resistance in *Insr*^{P1195L/+} mice ($n = 10-12$ per each group). Data are mean \pm SEM. Significance was analyzed by two-tailed Student's t -test between fasted and refeed conditions. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.



Supplementary Figure S4

Serum FFA levels. Serum FFA levels were measured by using the kit from BioVision, Inc. (San Francisco, CA, USA) ($n = 8-13$ per each group). Data are mean \pm SEM. One-way ANOVA plus Bonferroni *post-hoc* analysis. * $P < 0.05$.



Supplementary Figure S5

Serum bile acid composition. Measurement of BAs in serum revealed that BA composition was altered in *Insr^{P1195L/+}/HFD* mice (n=4 per each group). Data are mean ± SEM. One-way ANOVA plus Bonferroni *post-hoc* analysis. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Figure 2h

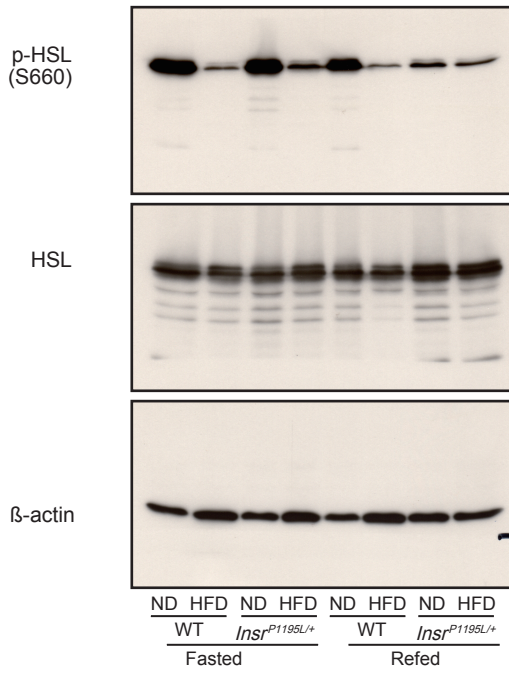


Figure 2i

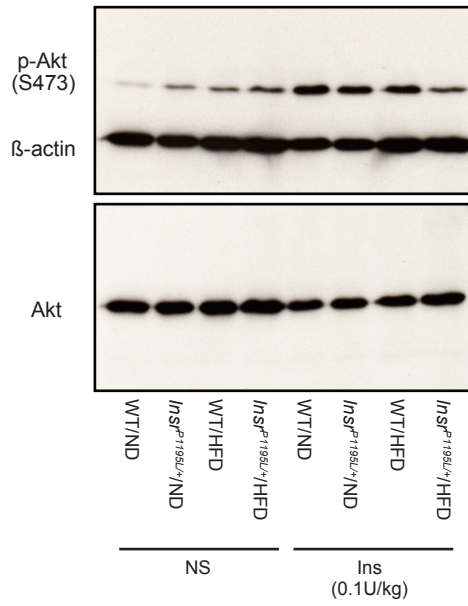


Figure 2j

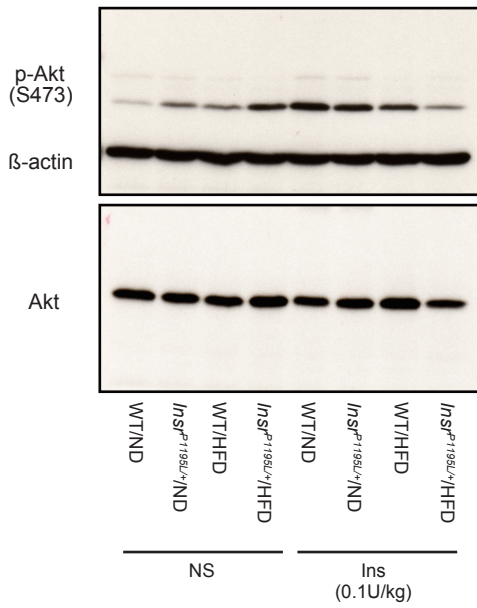
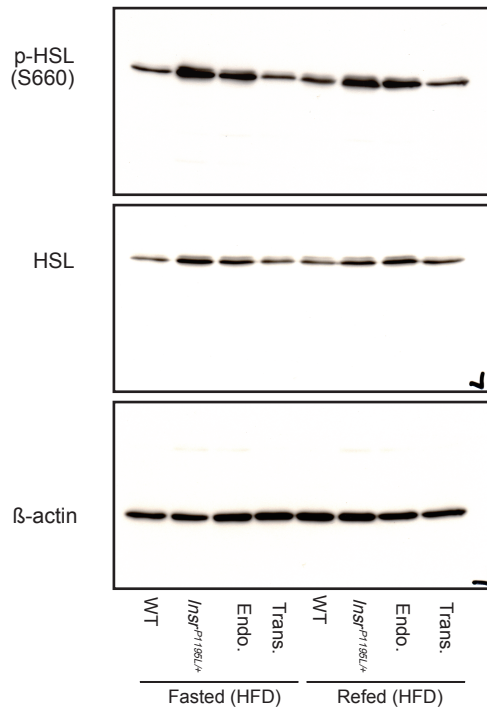


Figure 5d



Full-length blots of Figure 2h, 2i, 2j, 5d.

Supplementary Figure S6