SUPPLEMENTARY MATERIALS

Supplementary Figure 1. Effects of *Usp2* knockout (KO) on insulin sensitivity of 3T3-L1 adipocytes

Usp2 KO adipocytes were generated using the CRISPR/Cas9 system [1]. The sequence of the *Usp2*-selective guide RNA is 5′- CTGGCTGGTCTTCGAAACCT-3′ (PAM sequence underlined). The guide RNA was ligated onto pSPCas9(BB)-2A-GFP plasmids (Addgene, Cambridge, MA, USA), and transfected into 3T3-L1 cells. After two days, GFP^+ cells were sorted using a FACS AriaII (BD Bioscience, San Jose, CA, USA), and subsequently cloned in 96-well plates. Genomic DNA from the cloned cells was extracted using the GeneElute Mammalian Genomic DNA Miniprep Kit (Sigma Aldrich), and sequenced by Hokkaido System Science (Sapporo, Japan). Both alleles at the *Usp2* locus in three resultant clones had missense mutations at the nucleophile amino acid, which are essential for ubiquitin isopeptidase activity [2].

Three independent *Usp2* KO clones and their parental 3T3-L1 cells were differentiated using the Adipoinducer cocktail (Takara Bio, Kusatsu, Japan) for one week. The cells were then incubated in a serum-free Dulbecco's modified Eagle medium (DMEM) for 24 h, and treated with human recombinant insulin (100 nM, Wako, Osaka, Japan) for 10 min. Western blot analysis of phosphorylated Akt (pAkt) , total Akt

and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was performed as described in the Materials and Methods Section.

Supplementary Figure 2. Effects of macrophage USP2A on metabolic states in lean mice

Transgenic (Tg) mice forcibly expressing *Usp2a* and their control littermate C57BL/6 mice (Ctrl) were fed a normal chow diet (NCD; MFG, Oriental Yeast, Tokyo, Japan) from the age of five weeks to three (A-C) and six (D-G) months. (A, D, E) Wet tissue weight of mesenteric and epididymal adipose tissues (A, E) and body weight gain and food intake (D). (B, F) Blood triglyceride, nonesterified fatty acids (NEFAs), glucose, and insulin were measured using Test Wako kits (Wako, Osaka, Japan). (C, G) an insulin tolerance test was conducted as previously described [3]. Values presented are the mean ± SD of four (A, B, E, F) or five (C, D, G) mice. Neither index yielded differences between the *Usp2a* Tg mice and their control littermates.

Supplementary Figure 3. Hematoxylin-eosin staining of adipose tissue from *Usp2a* transgenic mice

Usp2a transgenic (Tg) and control littermate mice were fed a 60% kcal HFD for six

months. Epididymal adipose tissues were fixed with 10% formaldehyde neutral buffer solution (Nacalai, Kyoto, Japan) at 4 ºC, dehydrated, and embedded in paraffin using a standard procedure. The 5-μm thick sections were stained with hematoxylin-eosin solution (Wako, Osaka,, Japan). Scale bar represents 200 μm.

Supplementary Figure 4. Effects of conditioned media from *Usp2* knockout adipocytes on Akt phosphorylation in myocytes

Usp2 knockout (KO) and control 3T3-L1 adipocytes were differentiated as shown in Supplementary Figure 1, and were then cultured in Dulbecco's modified Eagle medium (DMEM) for two additional days. The harvested media were diluted to half its original concentration with DMEM and then added to the differentiated C2C12 cells. After culturing for three days, the C2C12 cells were stimulated with insulin (200 nM) for 10 min. Subsequently, the cells were subjected to Western blot analyses of phosphorylated Akt (pAkt) , total Akt and glyceraldehyde 3-phosphate dehydrogenase (GAPDH)

References

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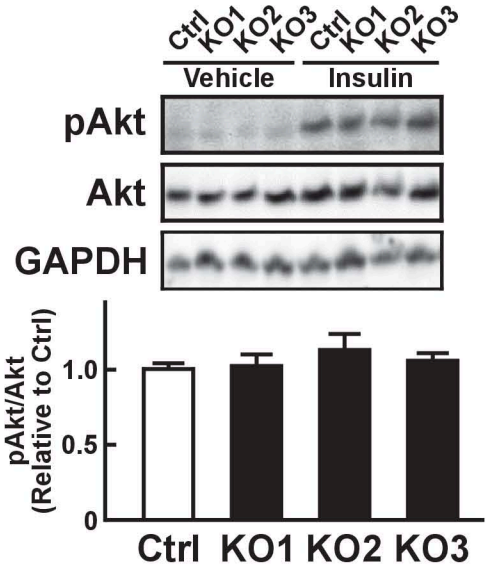
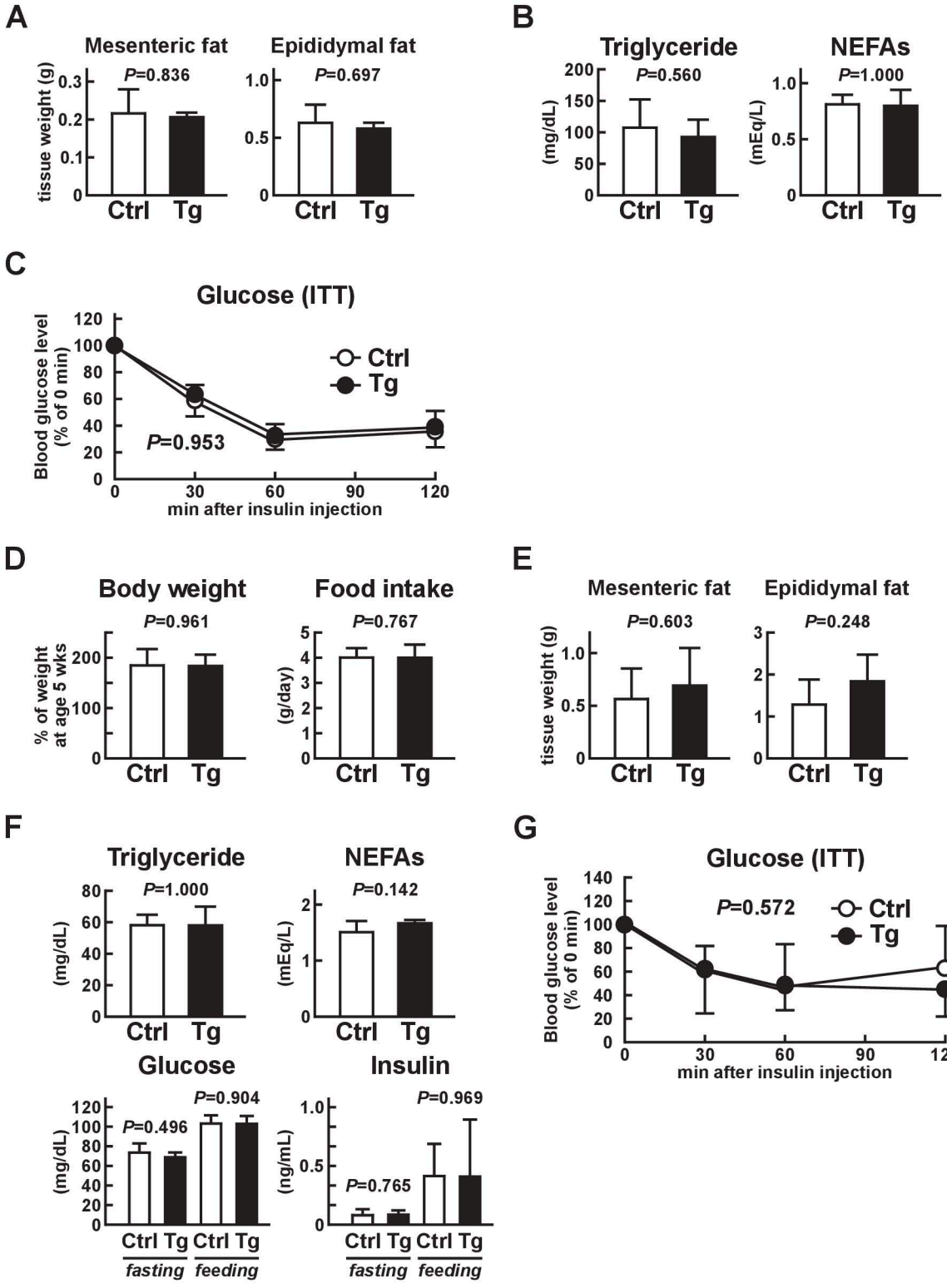
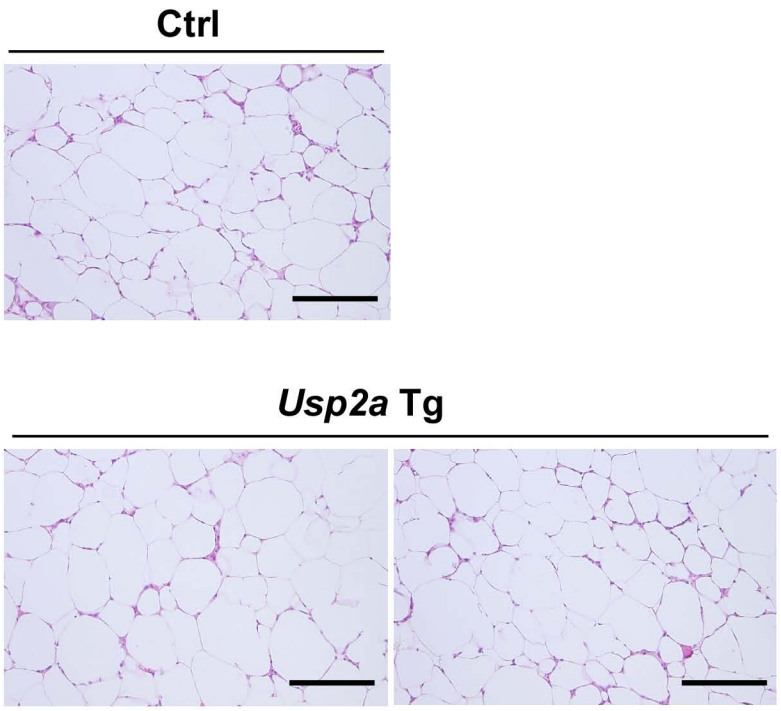


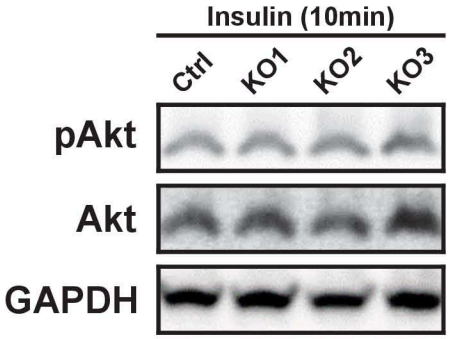
Saito et al. Supplementary Figure1



Saito et al. Supplementary Figure 2



Saito et al. Supplementary Figure 3



Saito et al. Supplementary Figure 4