SUPPLEMENTAL MATERIALS

Supplemental Figure Legends

Figure S1. Body weight and food intake during 14 weeks of low fat diet and high fat diet. 6-week old wild type C57BL/6 and *Irak1* k/o mice were fed with low fat diet (panels A - B) or high fat diet (panels C - D) for 14 weeks. Body weight (A and C, n = 8 for each group) and food intake (B and D) were measured three times per week. Caloric intake (Kcal/mouse/d, B and D) was calculated based on caloric contents for low fat diet (3.845 kcal/g) and high fat diet (5.24 kcal/g). Data are presented as mean ± SEM. p-values indicated in the Figure refer to comparisons between WT vs. k/o mice fed on the same diet. When assessing the effect of diet, WT mice ate 22% more calories and gained 24% more weight on high fat vs. low fat diet. Similarly, k/o mice ate 16% more calories and gained 26% more weight on high fat vs. low fat diet.

Figure S2. Insulin tolerance tests (ITT): After 13 weeks of feeding with either low fat diet (panels A and B, n = 8) or high fat diet (panels C and D, n = 8), wild type and *Irak1* k/o mice were fasted for 5 h. Insulin (0.75 IU/kg) was injected intraperitoneally and blood glucose was measured at 0, 15, 30, 60 and 120 min after injection. Area above the curve (AAC) was calculated based on the trapezoidal rule (panels B and D). **A**. ANOVA for repeated measures, p = 0.67; **B**. AAC: 6915 ± 797 vs. 6621 ± 802 [(mg/dl) x min], p = 0.39; **C**. ANOVA for repeated measures, p = 0.95; **D**. AAC: 4885 ± 266 vs. 4441 ± 230 [(mg/dl) x min], p = 0.11. Data are expressed as mean ± SEM.

Figure S3. *In vivo* insulin-stimulated [^{18}F]fluorodeoxyglucose (^{18}F-FDG) distribution in mice fed with normal chow diet. After mice were fasted overnight, ^{18}F-FDG 11.1 MBq (300 µCi) and insulin (1.125U/kg) in 200 µL of saline were injected via tail vein under anesthesia. Dynamic imaging was acquired for 30 min by Siemens Inveon Small Animal PET-CT Imaging System. The data were obtained by drawing Regions of Interest (ROI) over target organs of muscle, abdominal subcutaneous white adipose tissue, liver, and brain (98,99). The ^{18}F-FDG bio-distribution in the target organs/tissues were analyzed at three time points: 10, 20 and 30 min. Coronal views are shown of ^{18}F-FDG imaging in the hind limb, liver, and abdominal subcutaneous white fat (indicated by arrows) in *Irak1* k/o mice (right column) and in wild-type mice (left column).

Figure S4. Immunoblotting analysis of IRAK-1 in muscle, liver and abdominal white adipose tissue (A-B) and GLUT4 in muscle (C). A. 20 week old wild type mice fed with chow diet were fasted for 5 h and skeletal muscle from hind limb, abdominal fat, and liver were immediately flash frozen and homogenized. These samples were centrifuged and supernatants of tissue lysates were analyzed (20 µg protein) by immunoblotting with anti-IRAK-1 antibody. B and C. 20 week old wild type mice and k/o mice fed with chow diet were fasted for 5 h and skeletal muscle from hind limb, abdominal fat, and liver were immediately flash frozen and homogenized. These samples were centrifuged and supernatants of tissue lysates were analyzed (20 µg protein) by immunoblotting with anti-IRAK-1 antibody (B) or anti-GLUT4 antibody (C).

SUPPLEMENTAL FIGURES

Figure S1.

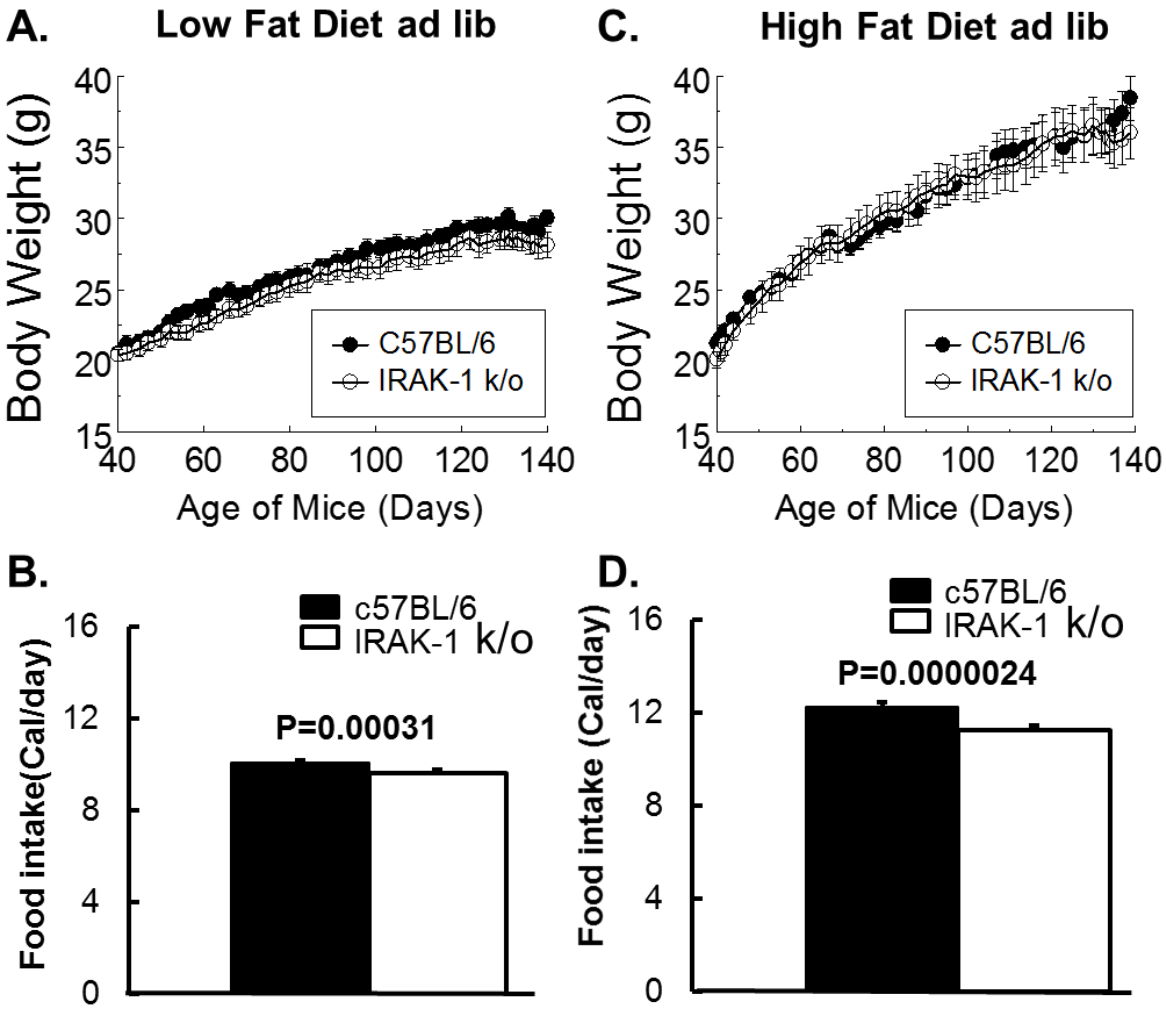


Figure S2.

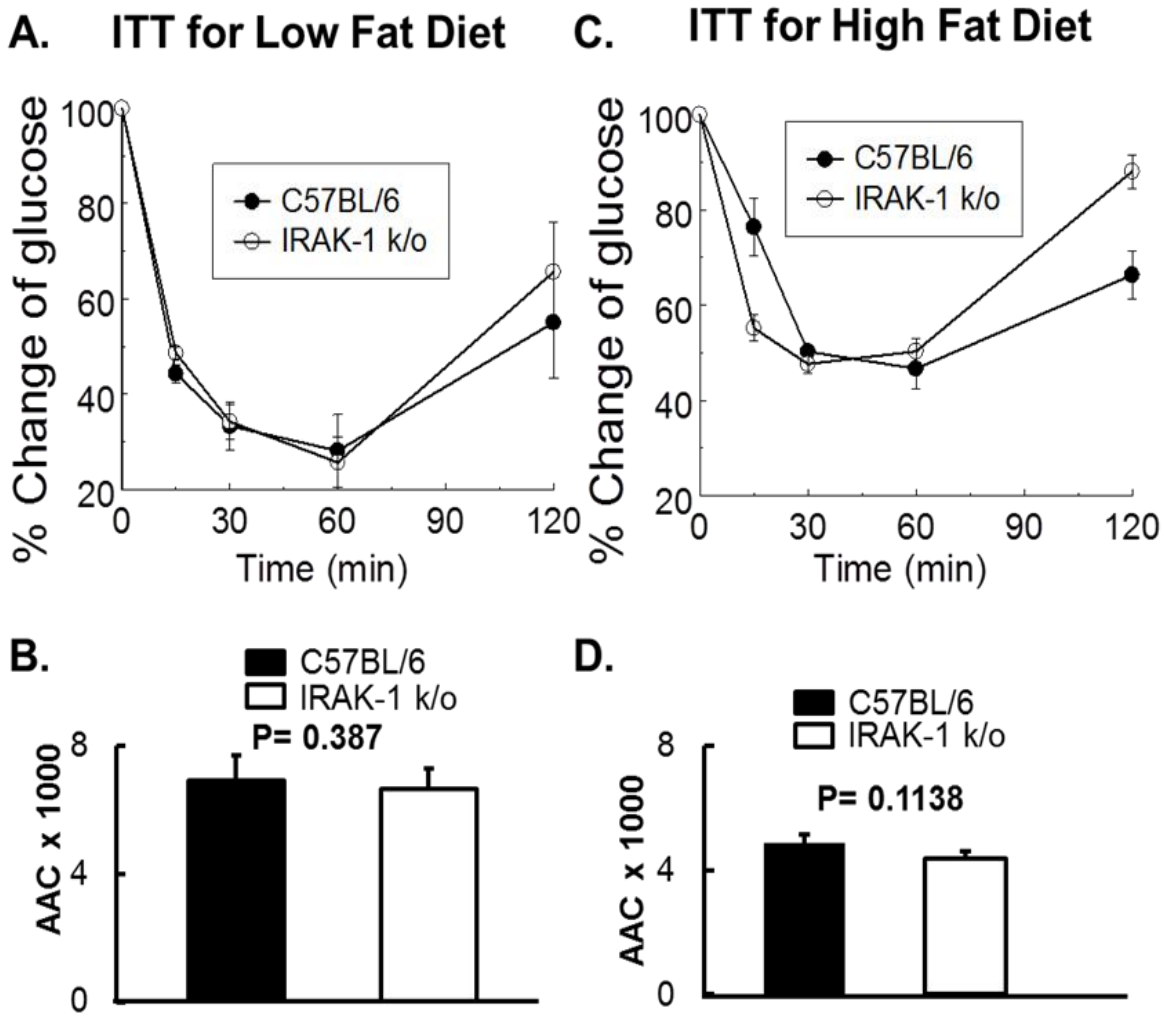

A.
ITT for Low Fat Diet
% Change of glucose
100
80
60
40
20
0
30
60
90
120
Time (min)
C57BL/6
IRAK-1 k/o
C.
ITT for High Fat Diet
% Change of glucose
100
80
60
40
20
0
30
60
90
120
Time (min)
C57BL/6
IRAK-1 k/o
B.
C57BL/6
IRAK-1 k/o
P= 0.387
AAC x 1000
8
4
0
D.
C57BL/6
IRAK-1 k/o
P= 0.1138
AAC x 1000
8
4
0

Figure S3.

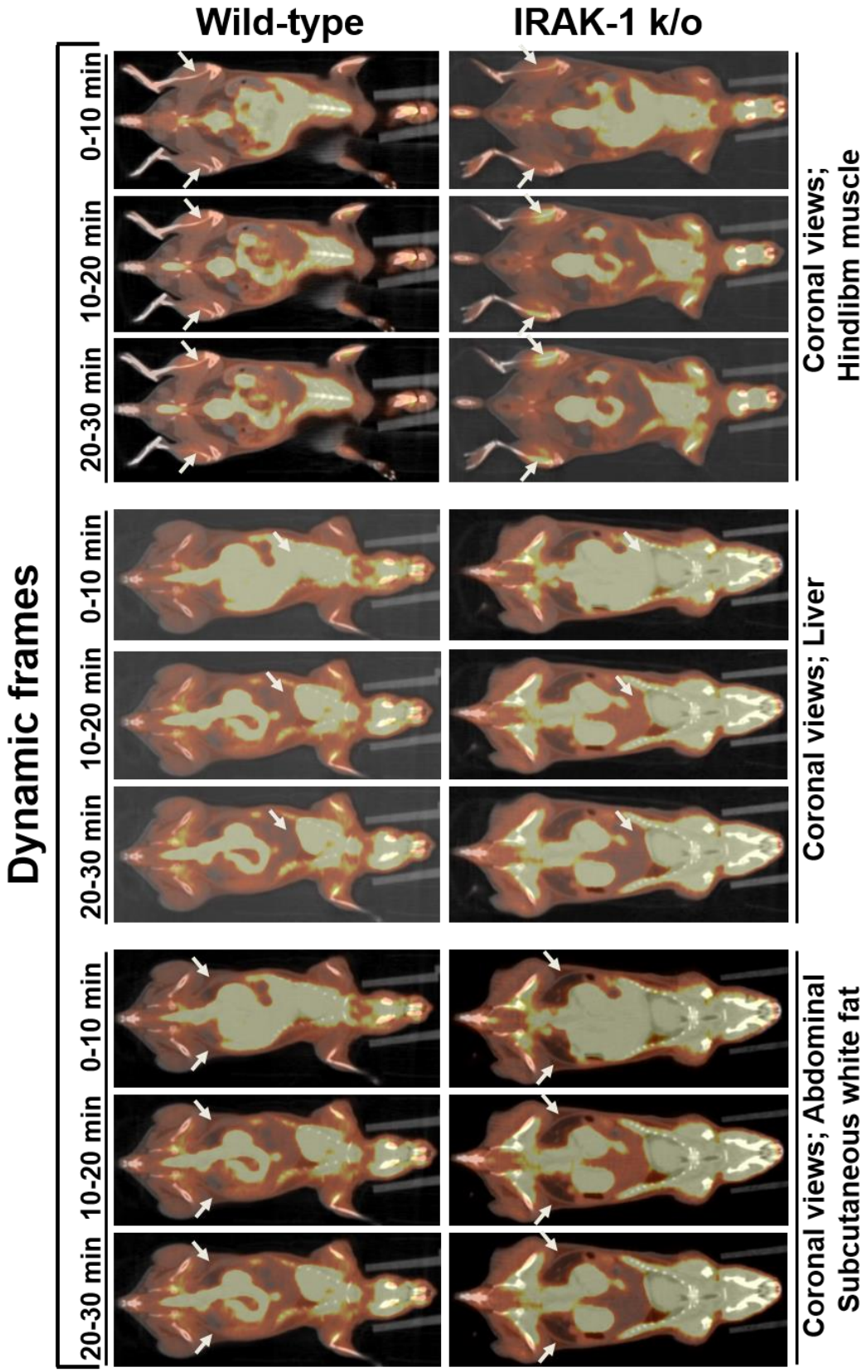
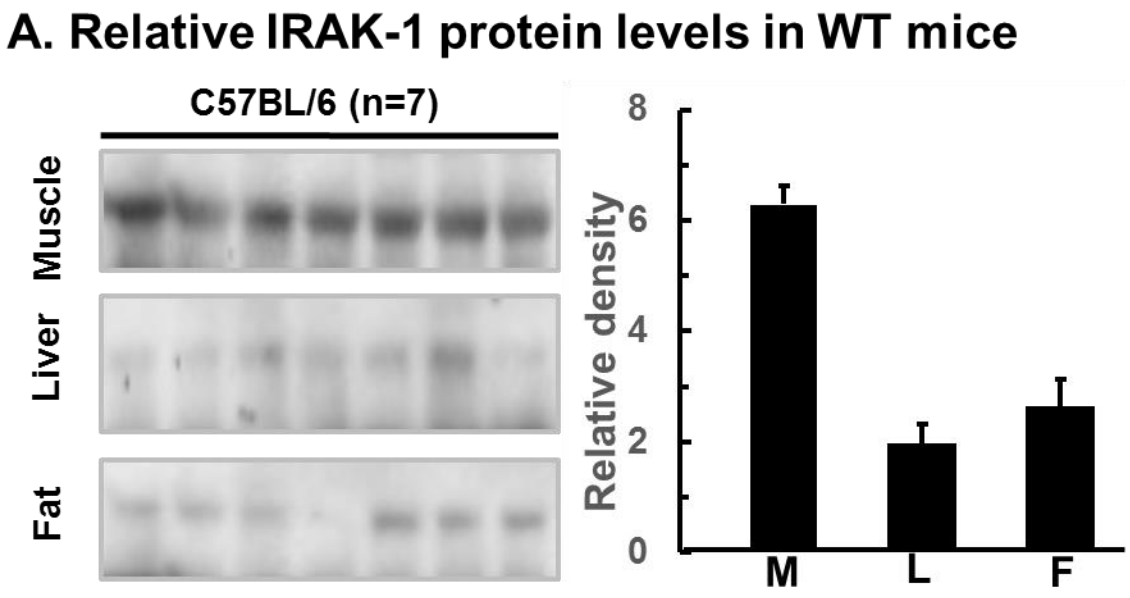
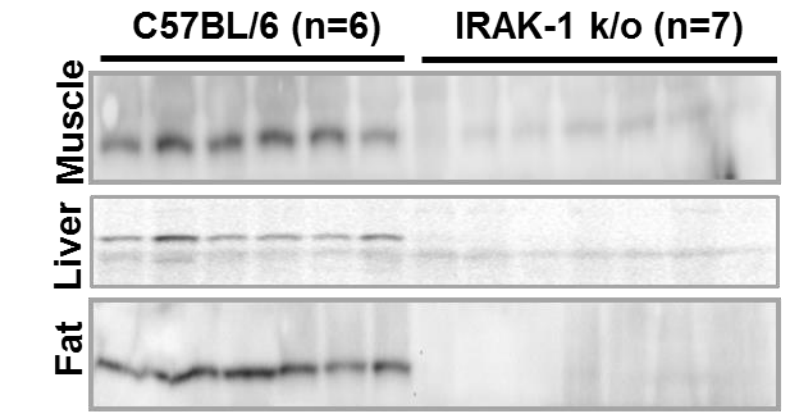
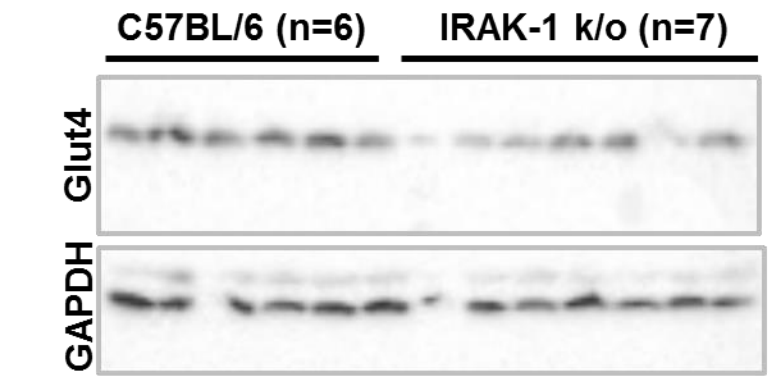

Wild-type
IRAK-1 k/o
Dynamic frames
0-10 min
10-20 min
20-30 min
Coronal views; Hindlibm muscle
Coronal views; Liver
Coronal views; Abdominal Subcutaneous white fat

Figure S4.


A. Relative IRAK-1 protein levels in WT mice
C57BL/6 (n=7)
Muscle
Liver
Fat
Relative density
8
6
4
2
0
M
L
F


B. IRAK-1 protein level in WT and k/o mice
C57BL/6 (n=6)
IRAK-1 k/o (n=7)
Muscle
Liver
Fat


C. Muscle Glut4 level
C57BL/6 (n=6)
IRAK-1 k/o (n=7)
Glut4
GAPDH