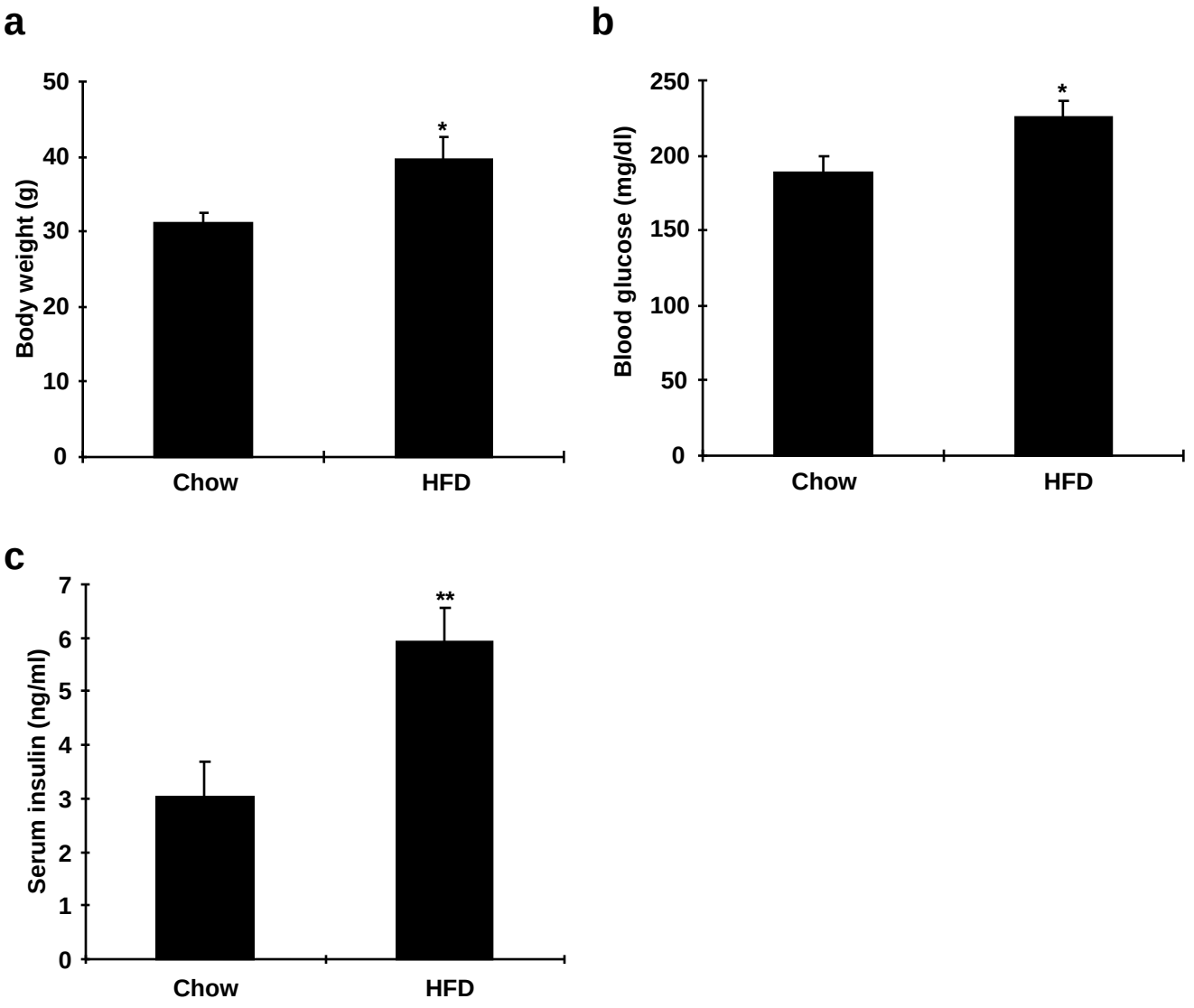
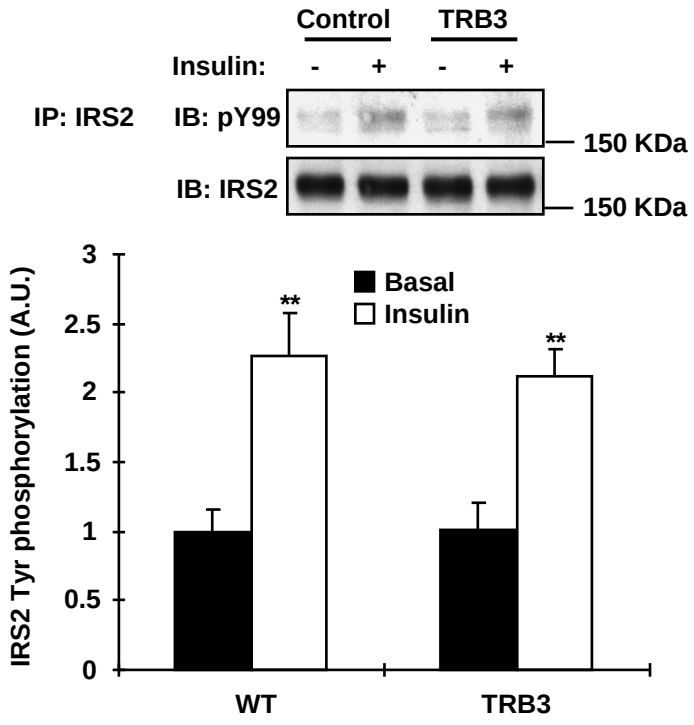
Koh et al. Supplementary Figure S1



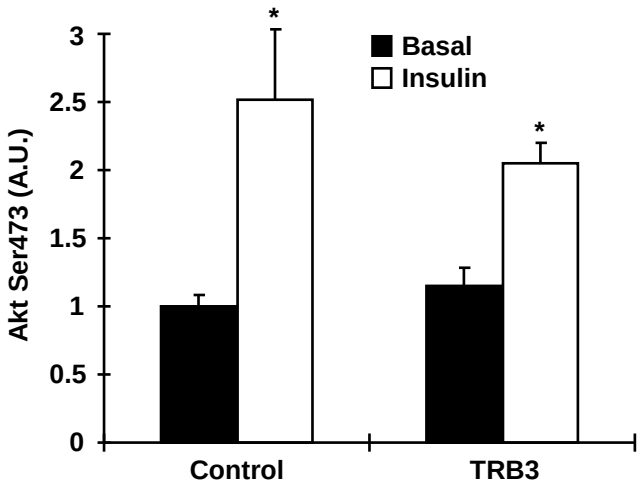
Supplementary Figure S1. Characterization of high fat fed mice. (**A-C**) C57BL/6 male mice were fed a chow (Chow) or a high fat (HFD) diet (60% fat) for 6 weeks beginning at 8 weeks of age. Body weights (A), blood glucose concentrations (B), and serum insulin concentrations (C) were measured in mice at 14 weeks old. Data are the means ± S.E.M., n=6/group. * indicates $p<0.05$ and ** indicates $p<0.01$ vs. chow diet group, using Student's t-test.

Koh et al. Supplementary Figure S2

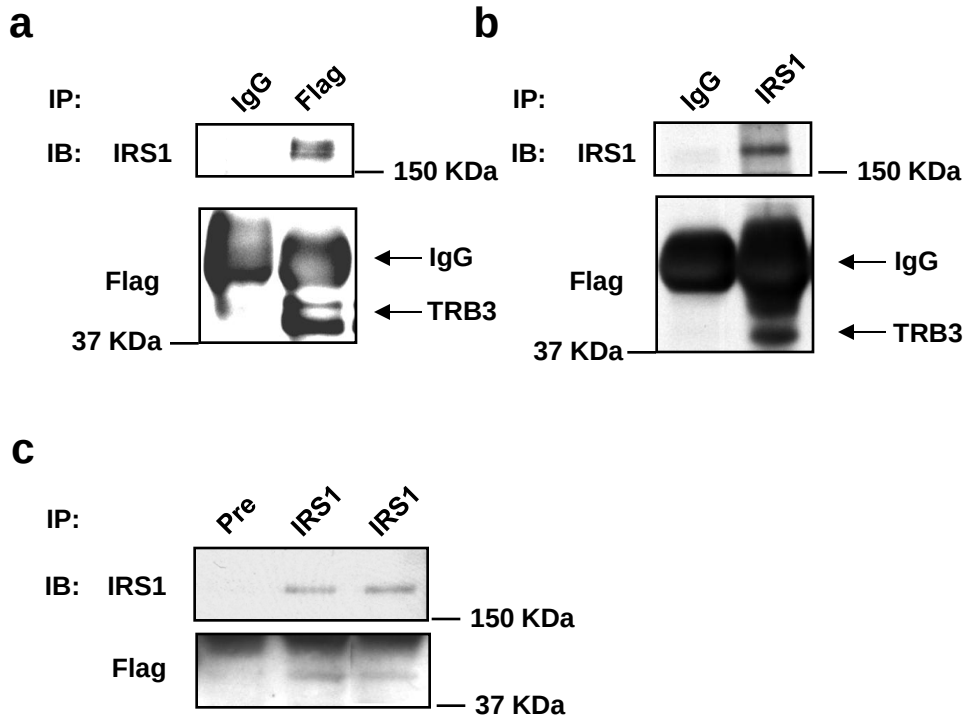


Supplementary Figure S2. The effect of TRB3 overexpression on IRS2 tyrosine phosphorylation. C2C12 lysates (500 μg) from Fig 2 a and b were immunoprecipitated with IRS2 antibody and immunoblotted with pY99 and IRS2 antibodies (n=3). Data are the means ± S.E.M. ** indicates $p<0.01$ vs. basal in the same group, using two way ANOVA with Bonferroni correction.

Koh et al. Supplementary Figure S3

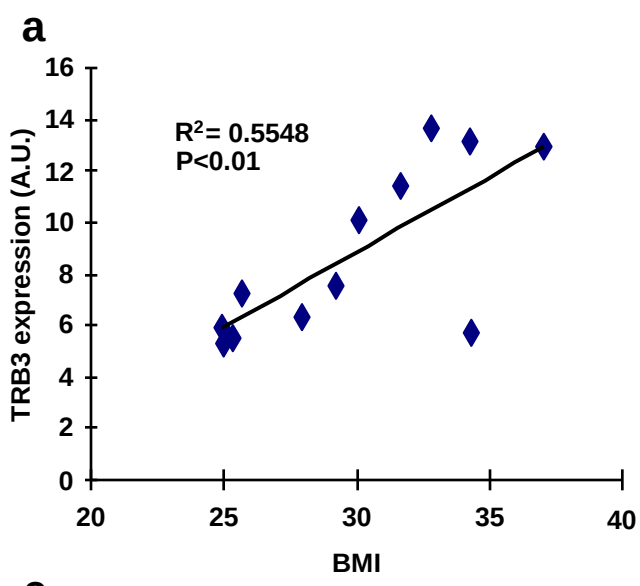


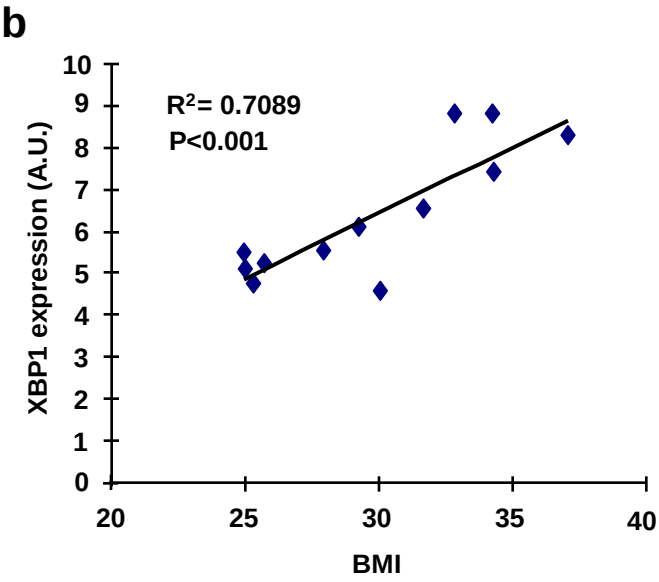
Supplementary Figure S3. The effect of TRB3 overexpression on Akt Ser473 phosphorylation. TA muscle lysates from Fig 2 c and d were used to determine phosphorylation of Akt S473 (n=6). Data are the means ± S.E.M. * indicates $p<0.05$ vs. basal in the same group, using two way ANOVA with Bonferroni correction.

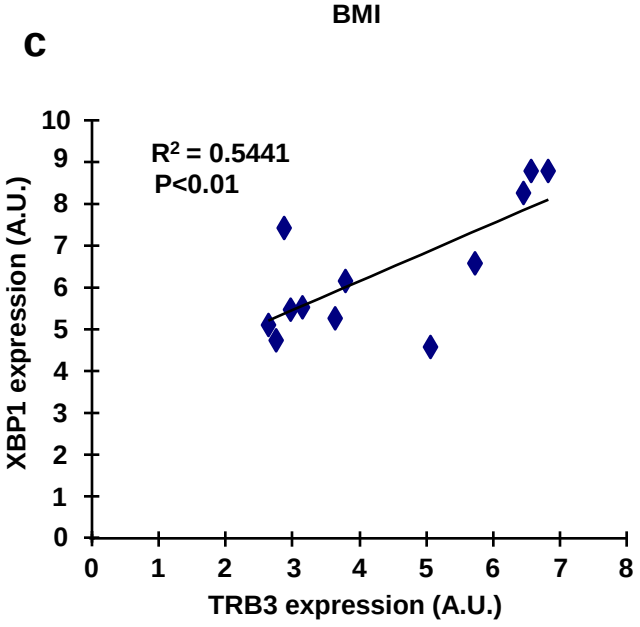


Supplementary Figure S4. Association of TRB3 with IRS1. (**A and B**) 293T cells were transfected with Flag-TRB3 and lysates were immunoprecipitated with IgG, Flag or IRS1 antibodies. Precipitates were subjected to gel electrophoresis followed by immunoblotting with IRS1, TRB3, or Flag antibodies. (**C**) Lysates from tibialis anterior muscles over-expressing Flag-TRB3 by electroporation were immunoprecipitated with IgG (pre) or IRS1 and immunoblotted with Flag to detect Flag-TRB3.

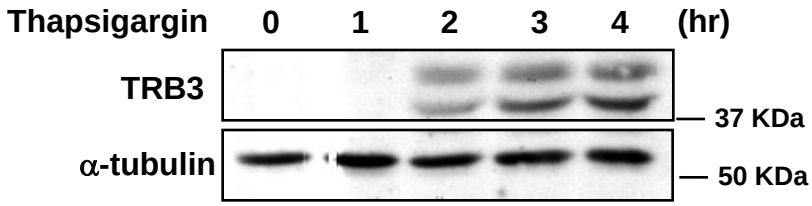
Koh et al. Supplementary Figure S5





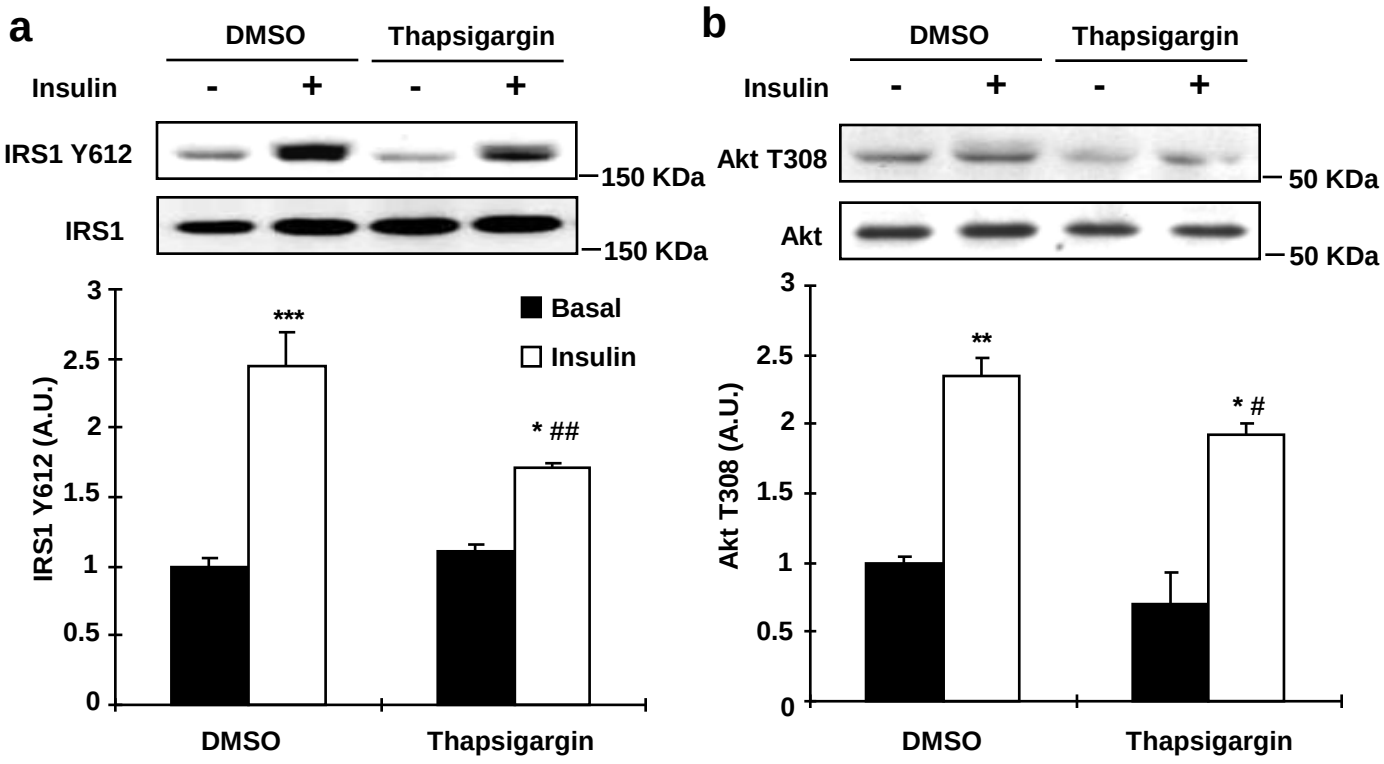


Supplementary Figure S5. Comparison of Body mass index (BMI) and TRB3 (A), XBP1 (B), and TRB3 and XBP1 (C) in human subjects. Vastus lateralis muscle biopsies were taken from 29 subjects under resting conditions (11 males and 18 females). Muscle lysates were used to determine the protein expression of TRB3 and XBP1. Protein expression was compared with BMI and TRB3 was compared with XBP1. R^2 represents pearson's correlation coefficient. The relations differ significantly between the comparisons.

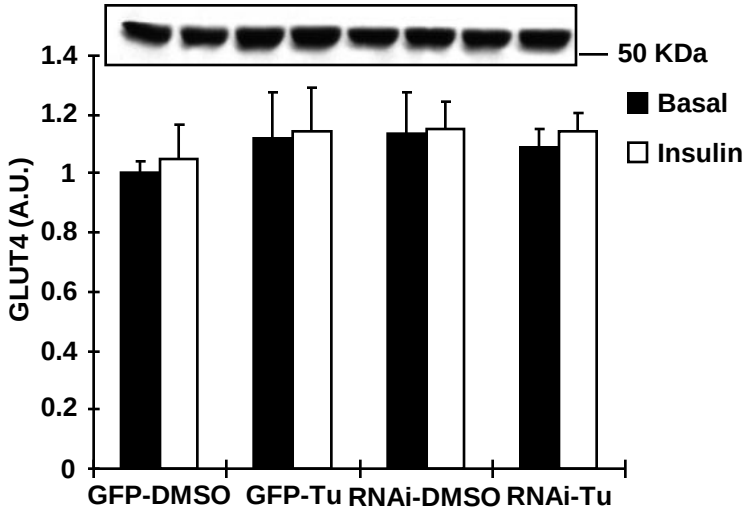


Supplementary Figure S6. The effects of thapsigargin on TRB3 expression. C2C12 myotubes were incubated with thapsigargin (1.3 μg/ml) and cells were harvested at indicated times. Lysates w ere subjected to gel electrophoresis for immunobloted with TRB3 antibodies. α-tubulin was used for the loading control.

Koh et al. Supplementary Figure S7

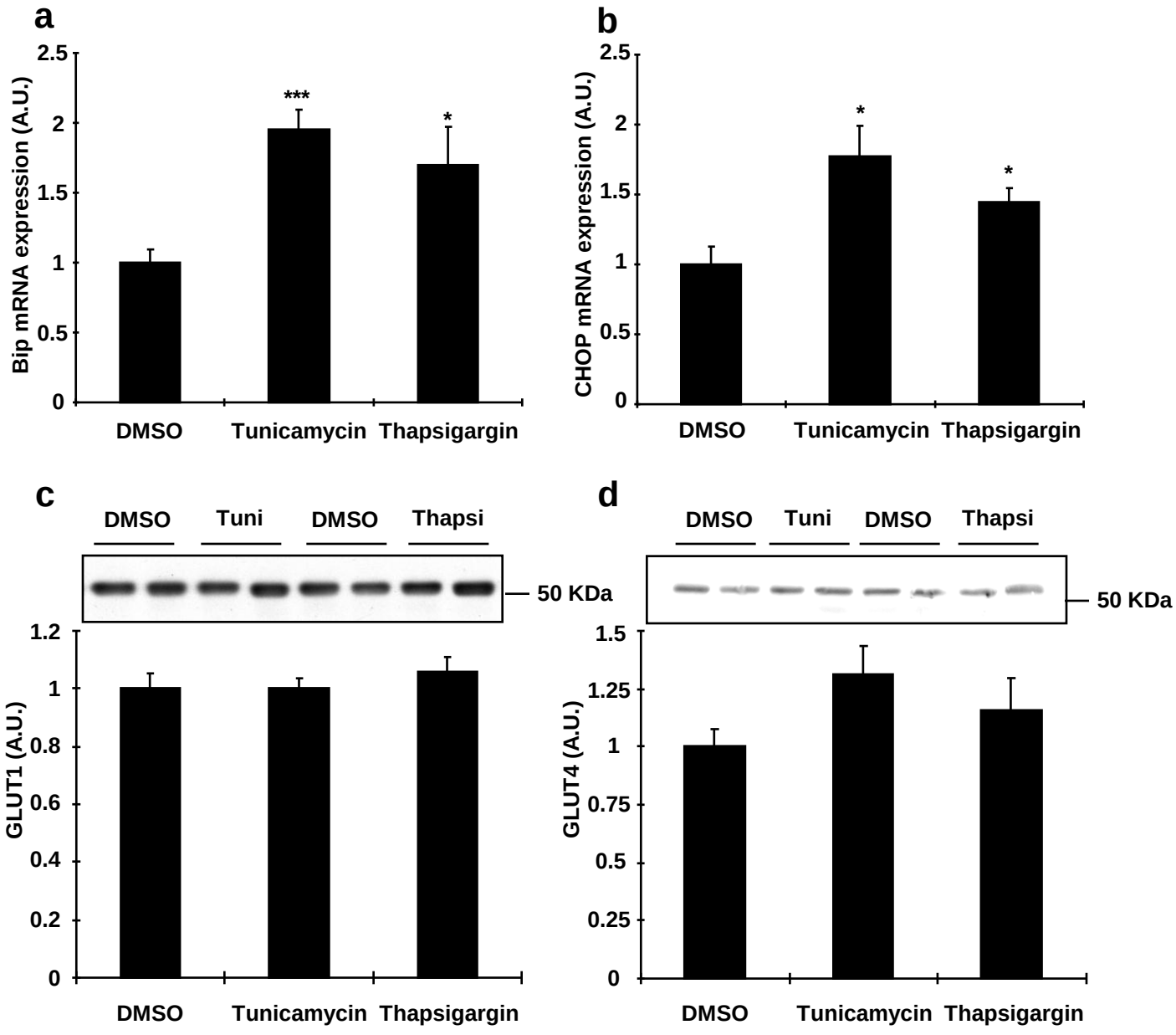


Supplementary Figure S7. Effects of thapsigargin on insulin signaling. (**A and B**) C2C12 myotubes were incubated with thapsigargin (1.3 μg/ml) for 4 hrs in the presence and absence of 100 nM of insulin for 10 min. Lysates were subjected to immunoblot analysis. Basal and insulin-stimulated IRS1 tyrosine phosphorylation at 612 (A) and Akt phosphorylation at theonine 308 (B) were determined. Data are the means ± S.E.M., n=3/group. * indicates $p<0.05$, ** indicates $p<0.01$, and *** indicates $p<0.001$ vs. basal in the same group. # indicates $p<0.05$ and ## indicates $p<0.01$ vs. corresponding Control, using two way ANOVA with Bonferroni correction.



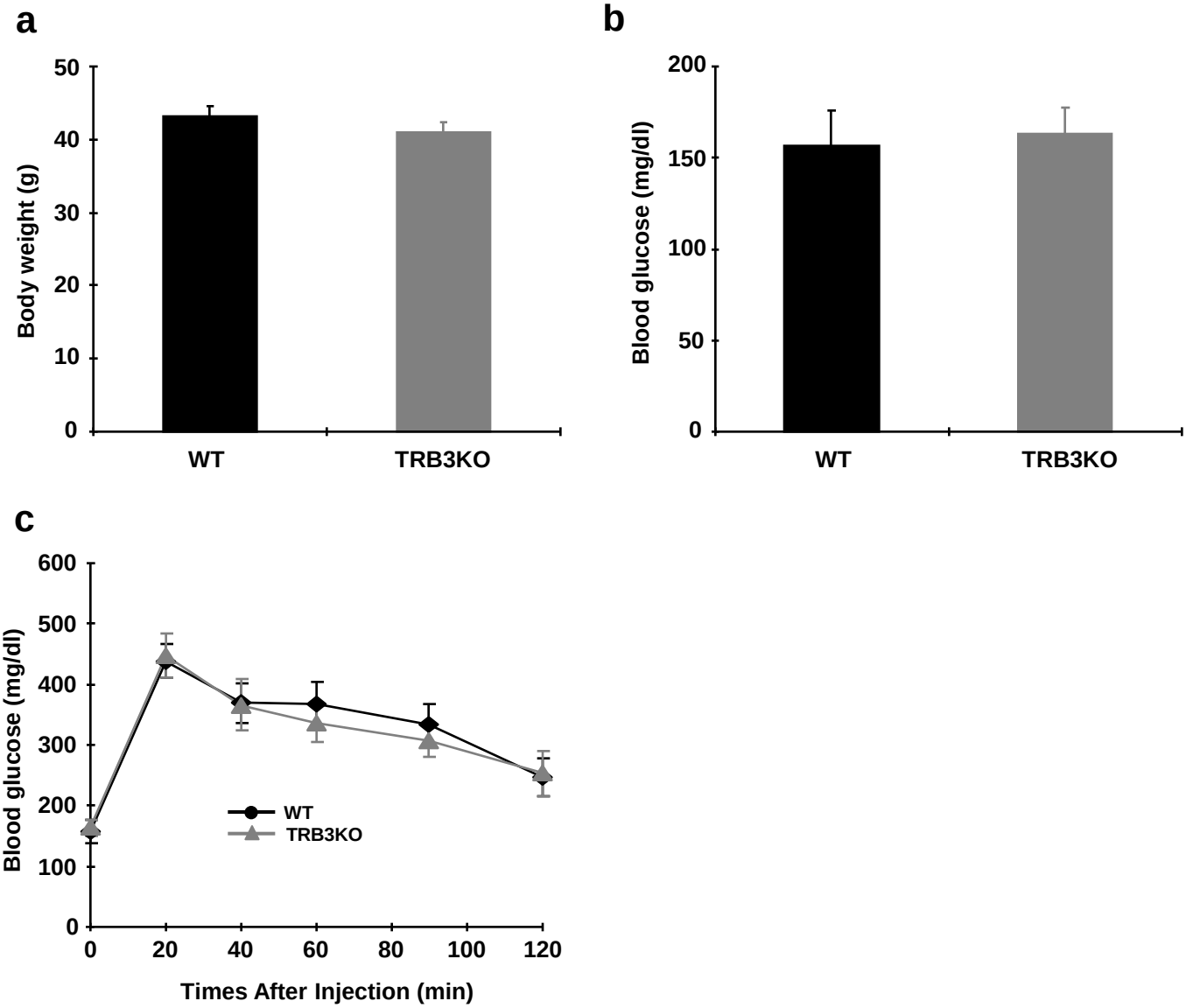
Supplementary Figure S8. Effects of ER stress and knockdown of TRB3 on GLUT4 expression. C2C12 myotubes were infected with adenovirus containing scramble (GFP) or RNAi for TRB3 (RNAi) and incubated with DMSO or tunicamycin (Tu) for 4 hrs. Myotubes were incubated in the presence and absence of 100 nM of insulin for 10 min. GLUT4 expression was determined by immunoblot analysis. Data are the means ± S.E.M., n=3/group.

Koh et al. Supplementary Figure S9



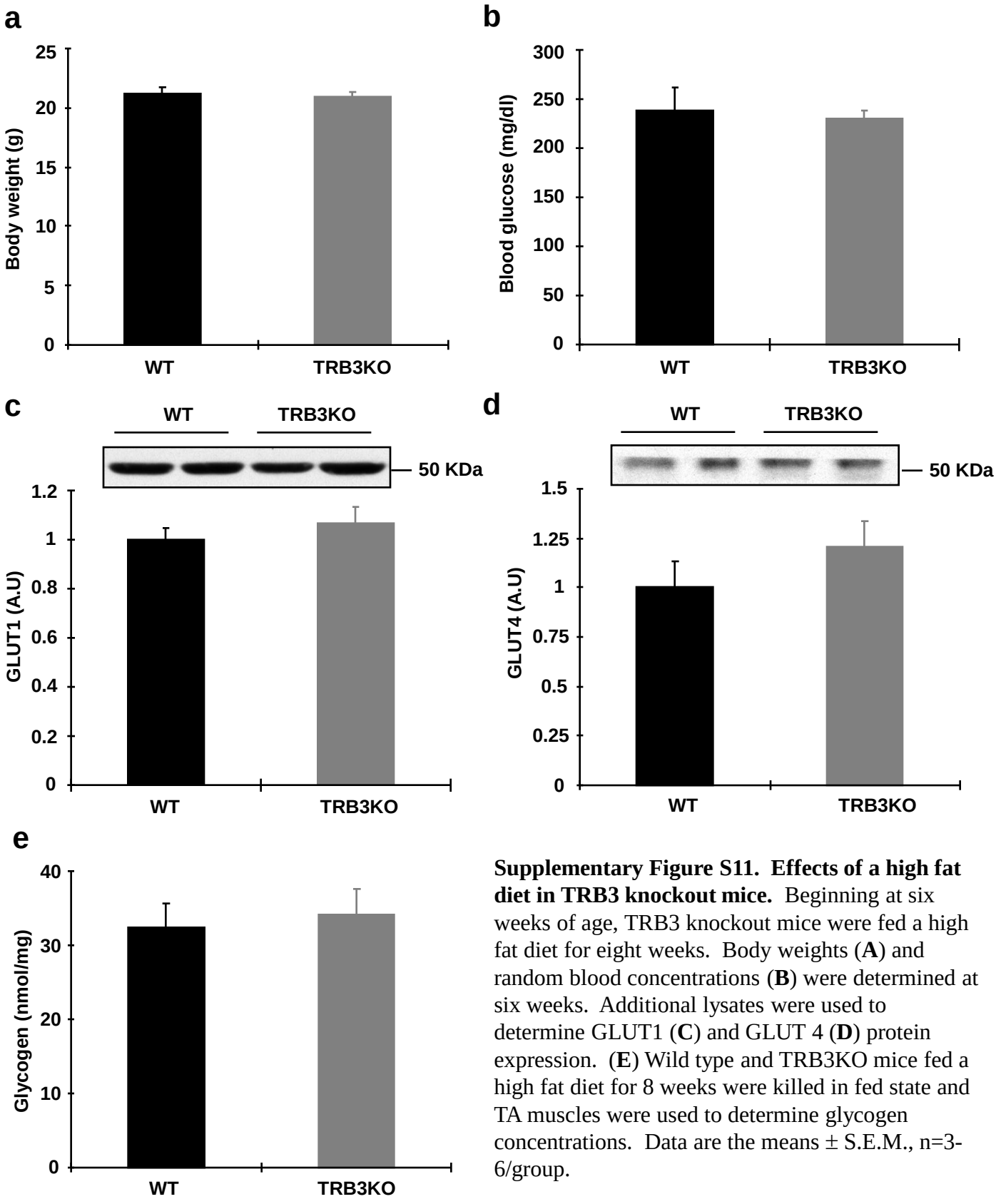
Supplementary Figure S9. Effects of ER stressors on the expression of ER stress markers and glucose transporter proteins in mouse skeletal muscle. (**A-D**) Extensor digitorum longus (EDL) muscles were dissected and incubated in the presence or absence of ER stressors, tunicamycin (1 μg/ml) and thapsigargin (1.3 μg/ml) for 4 hrs. Muscles were collected and used for mRNA measurement for Bip (A) and CHOP (B), or GLUT1 (C) and GLUT4 (D) protein expression by immunoblot analysis. Data are the means ± S.E.M., n=6/group. * indicates $p<0.05$ and *** indicates $p<0.001$ vs. DMSO control, using Student's t-test.

Koh et al. Supplementary Figure S10



Supplementary Figure S10. Body weight, fasting blood glucose concentrations, and glucose tolerance in TRB3 knockout mice fed a chow fat diet. (**A-C**) Twenty six week old male wild type and TRB3 knockout mice maintained on a chow fat diet were fasted for 14 hours and used to determine body weight (A), blood glucose concentrations (B), and glucose tolerance (C). Data are the means ± S.E.M., n=3-7/group.

Koh et al. Supplemental Figure 11



Supplementary Figure S11. Effects of a high fat diet in TRB3 knockout mice. Beginning at six weeks of age, TRB3 knockout mice were fed a high fat diet for eight weeks. Body weights (**A**) and random blood concentrations (**B**) were determined at six weeks. Additional lysates were used to determine GLUT1 (**C**) and GLUT 4 (**D**) protein expression. (**E**) Wild type and TRB3KO mice fed a high fat diet for 8 weeks were killed in fed state and TA muscles were used to determine glycogen concentrations. Data are the means ± S.E.M., n=3-6/group.

Supplementary Table 1. Metabolic parameters of TRB3 knockout mice under fasting conditions

	WT	TRB3KO
Glucose (mg/dl)	205.3±9.4	151.3±14.2*
Leptin (ng/ml)	7.68±0.64	4.84±1.13*
FFA (mEq/l)	0.64±0.08	0.55±0.04
Insulin (ng/ml)	3.89±0.96	1.09±0.29*
Adiponectin (ng/ml)	11836±1345	23562±2482**
Triglycerides (mg/ml)	19.0±1.4	20.7±6.8
Total Cholesterol (mg/dl)	154.9±4.6	103.6±12.4**

Each value represents the mean ± S.E.M. of 3-6 mice.

* $p<0.05$, ** $p<0.01$, and *** $p<0.001$ vs. wild type using Student's t-test

SupplementaryTable 2. Characteristics of human subjects

	Lean (n=11)	Obese (n=10)	T2DM (n=13)
Age (yr)	46.2±3.2	41.7±2.9	47.9±3.3
Body mass index (kg/m^2)	25.1±0.7	32.6±1.5*	32.8±1.1*
Fasting glucose (mg/dl)	91±3	88±3	179±20*#
Fasting insulin (μU/ml)	4.4±0.8	12.3±6.2	11.6±1.8*
M value (mg/kg·min)	11.1±0.6	9.0±0.8*	6.1±0.9*#

Each value represents the mean ± S.E.M.

* $p<0.05$ vs. lean and # $p<0.05$ vs. obese using one way ANOVA.

Supplementary Table 3. Primer sequences for Real-time PCR

Gene	Forward primer	Reverse primer
TBP	5′-ACCCTTCACCAATGACTCCTATG-3′	5′-TGACTGCAGCAAATCGCTTGG-3′
TRB3	5′-TCTCCTCCGCAAGGAACCT-3′	5′-TCTCAACCAGGGATGCAAGAG-3′
Bip	5′-CTGGACTGAATGTCATGAGGATCA-3′	5′-CTCTTATCCAGGCCATATGCAATAG-3′
CHOP	5′-CCACCACACCTGAAAGCAGAA-3′	5′-GGTGCCCCCAATTTCATCT-3′
PCK	5′-GGCGATGACATTGCCTGGATGA-3′	5′-TGTCTTCACTGAGGTGCCAGGA-3′
G6PC	5′-AGGTCGTGGCTGGAGTCTTGTC-3′	5′-GTAGCAGGTAGAATCCAAGCGC-3′
FBP1	5′-TGCTGAAGTCGTCCTACGCTAC-3′	5′-TTCCGATGGACACAAGGCAGTC-3′
GCK	5′-GCATCTCTGACTTCCTGGACAAG-3′	5′-CTTGGTCCAGTTGAGCAGGATG-3′
Mlxipl	5′-GAGTGCTTGAGCCTGGCTTACA-3′	5′-GCTCTCCAGATGGCGTTGTTCA-3′
SREBP1	5′-CGACTACATCCGCTTCTTGCAG-3′	5′-GCTCTCCAGATGGCGTTGTTCA-3′
FAS	5′-CACAGTGCTCAAAGGACATGCC-3′	5′-CACCAGGTGTAGTGCCTTCCTC-3′
ACACA	5′-GTTCTGTTGGACAACGCCTTCAC-3′	5′-GGAGTCACAGAAGCAGCCCATT-3′
SCD1	5′-GCAAGCTCTACACCTGCCTCTT-3′	5′-CGTGCCTTGTAAGTTCTGTGGC-3′