

## Supplemental Material

# **Skeletal Muscle Tissue Trib3 Links Obesity with Insulin Resistance by Autophagic Degradation of AKT2**

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**Supplementary Fig. S1.** Immunoblots showing elevation of Trib3 expression in C2C12 myotubes by cellular stress such as (a) thapsigargin and (b) tunicamycin treatments. (c) BSA-conjugated palmitic acid upregulates Trib3 expression in C2C12 myotubes.

**Supplementary Fig. S2.** (a) Schematic illustration of a Trib3TG construct. (b) Genotyping results of control litter mates and Trib3TG mice. (c) Trib3 mRNA expression level in gastrocnemius (Gas), soleus (Sol), and extensor digitorum longus (EDL) muscle tissues. (d) Trib3 protein in multiple tissues of Trib3TG mice was analyzed.

**Supplementary Fig. S3.** Metabolic analysis of (a) relative oxygen consumption, (b) carbon dioxide production, (c) energy expenditure, and (d) physical activity in control litter mates and Trib3TG mice using indirect calorimetry.

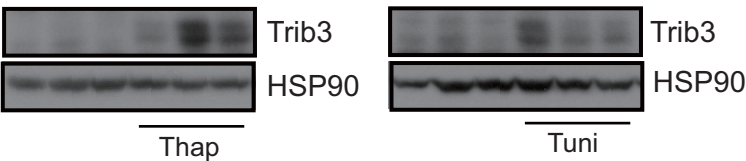
**Supplementary Fig. S4.** Analysis of (a) body weight and (b) weight of major peripheral metabolic organs of 1-year-old Trib3TG and wild-type litter mates that were fed a normal chow diet (n = 4 mice per group). MRI analysis of these mice showed relative (c) fat and (d) lean mass.

**Supplementary Fig. S5.** (a) Effect of hyperglycemia induced by STZ injection on AKT2 and Trib3 expression in quadriceps muscle tissue of control and Trib3TG mice. (b) Immunoblots showing the dosage effects of Trib3 expression on the endogenous protein level of AKT1 and AKT2 in HeLa cells. (c) Immunoblots were used to determine the effect of Trib3 and AKT1/2 co-expression on the protein level of AKT1/2 in C2C12 myoblasts.

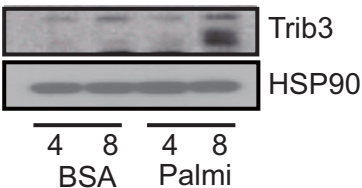
**Supplementary Fig. S6.** Development of ATG7 knockout in HEK293T cells using the CRISP/Cas9 system with ATG7-specific guide RNA. Deletion of the ATG7 gene was examined using an immunoblot, and an autophagy pathway blockade was also confirmed with p62 and LC3b expressions.

# Supplementary Figure 1

a



b

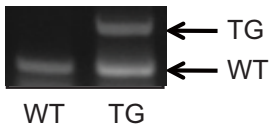


# Supplementary Figure 2

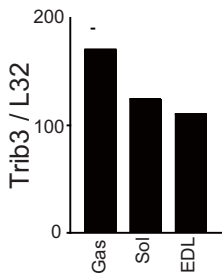
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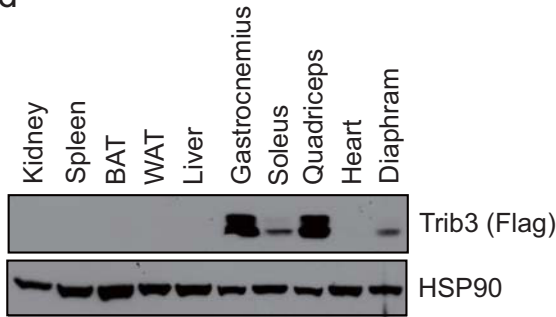
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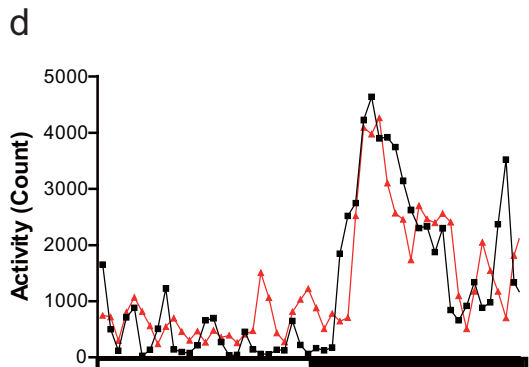
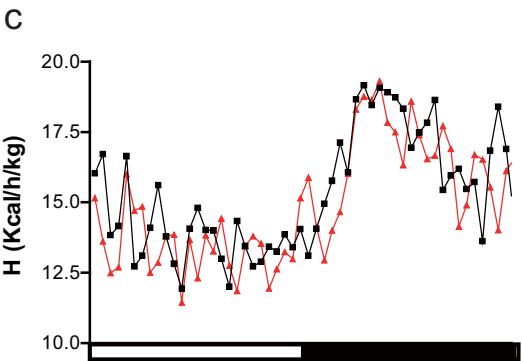
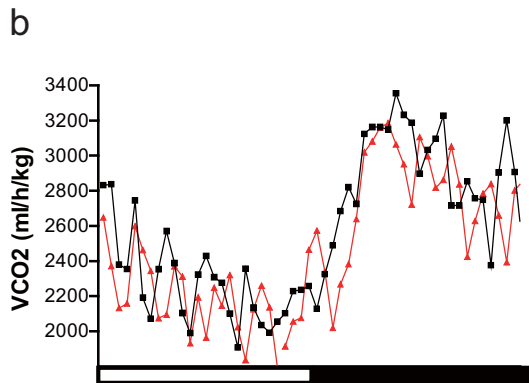
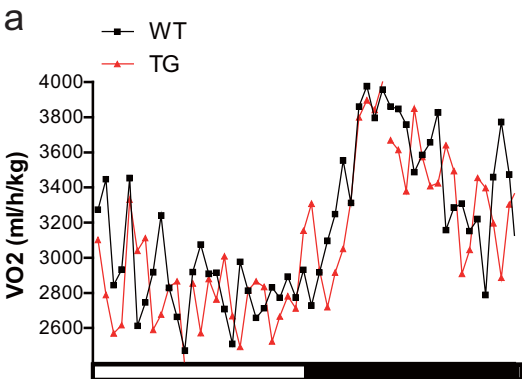
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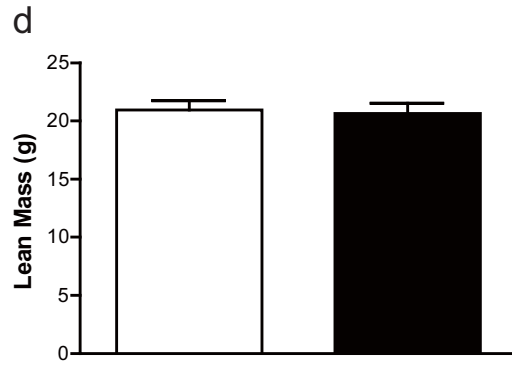
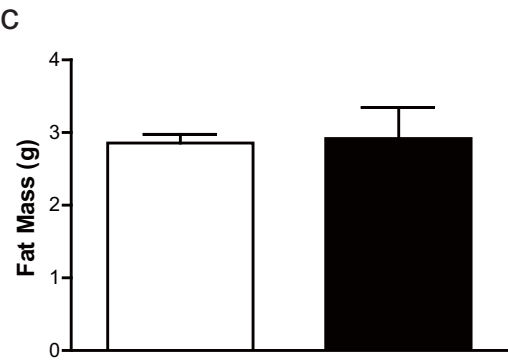
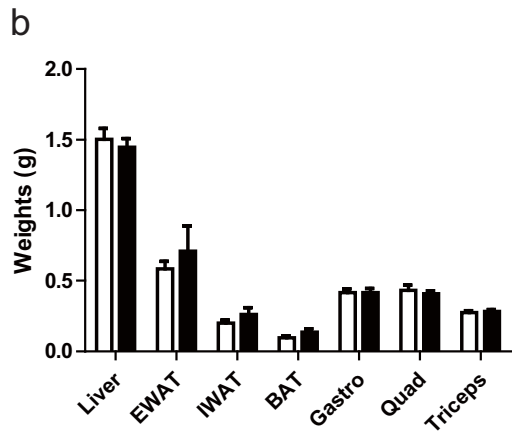
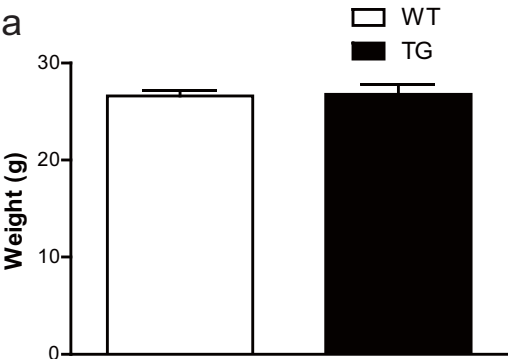
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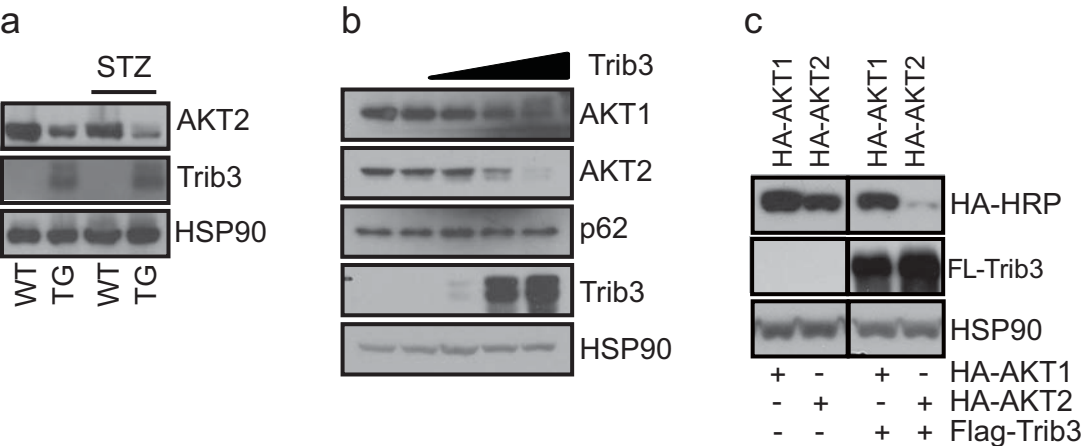
# Supplementary Figure 3



Supplementary Figure 4



# Supplementary Figure 5



# Supplementary Figure 6

