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online data

SUPPLEMENTARY ONLINE DATA

Contraction regulates site-specific phosphorylation of TBC1D1 in skeletal muscle

Kanokwan VICHAIWONG*[1,2], Suneet PUROHIT*[1], Ding AN*, Taro TOYODA*[3], Niels JESSEN*[4], Michael F. HIRSHMAN* and Laurie J. GOODYEAR*†[5]

*Research Division, Joslin Diabetes Center, One Joslin Place, Boston, MA 02215, U.S.A., and †Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, 75 Francis Street, Boston, MA 02115, U.S.A.

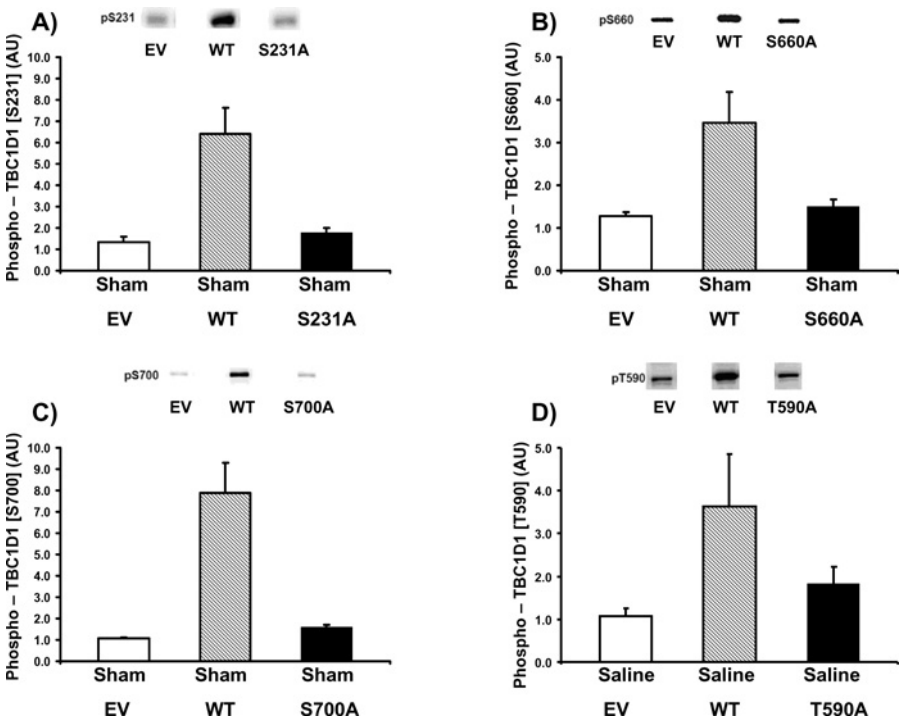

Figure S1 Specificity of anti-phospho-TBC1D1 Ser^{231}, Thr^{590}, Ser^{660} and Ser^{700} antibodies

Point mutations of the TBC1D1 phosphorylation sites Ser^{231} (S231A), Thr^{590} (T590A), Ser^{660} (S660A) and Ser^{700} (S700A) to alanine were generated separately. Empty vector (EV) control, TBC1D1 WT and the four TBC1D1 single alanine mutants were injected into tibialis anterior muscles followed by *in vivo* electroporation. Muscles were collected 1 week later. Immunoblotting was with anti-phospho-TBC1D1 (**A**) Ser^{231}, (**B**) Ser^{660}, (**C**) Ser^{700} and (**D**) Thr^{590} antibodies. Data are means ± S.E.M.; $n = 3$–6/group. Au, arbitrary units.

[1] These authors contributed equally to this work.
[2] Present address: Graduate Program in Exercise Science, Department of Physiology, Faculty of Science, Mahidol University, Bangkok, Thailand.
[3] Present address: Department of Cell Growth and Differentiation, Center for iPS Cell Research and Application, Kyoto University, 53 Kawahara-cho, Shogoin Yoshida, Sakyo-ku, Kyoto 606-8507, Japan.
[4] Present address: Department of Clinical Pharmacology, Aarhus University Hospital, Aarhus, Denmark.
[5] To whom correspondence should be addressed (email laurie.goodyear@joslin.harvard.edu).

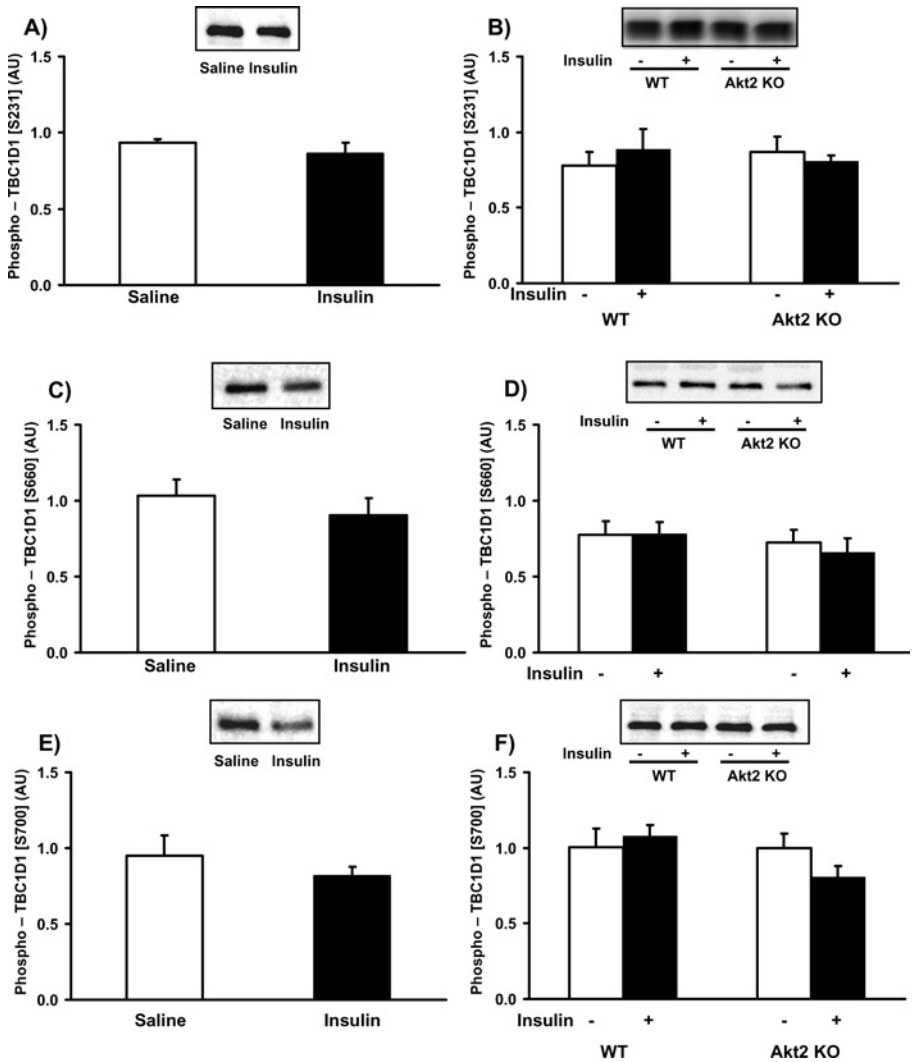

Figure S2 TBC1D1 Ser^{231}, Ser^{660} and Ser^{700} phosphorylation are not increased by insulin

(**A**) Maximal insulin injection did not affect TBC1D1 Ser^{231} phosphorylation in ICR mice. Mice were fasted and injected intraperitoneally with maximal insulin, and tibialis anterior muscles were dissected 10 min later. (**B**) Akt2 KO had normal levels of basal and insulin-stimulated TBC1D1 Ser^{231} phosphorylation. (**C**) Maximal insulin injection did not affect TBC1D1 Ser^{660} phosphorylation in ICR mice. (**D**) Akt2 KO had normal levels of basal and insulin-stimulated TBC1D1 Ser^{660} phosphorylation. (**E**) Maximal insulin injection did not affect TBC1D1 Ser^{700} phosphorylation in ICR mice. (**F**) Akt2 KO had normal levels of basal and insulin-stimulated TBC1D1 Ser^{700} phosphorylation. Data are means ± S.E.M.; $n = 5$–7/group. Au, arbitrary units.

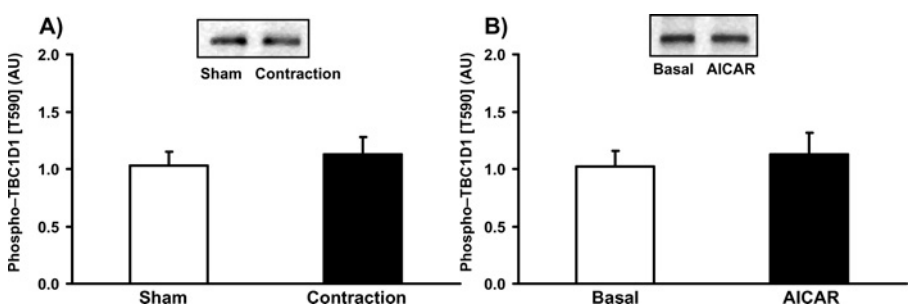

Figure S3 TBC1D1 Ser^{590} was not increased by contraction or AICAR

(**A**) Contraction *in situ* for 15 min did not increase TBC1D1 Ser^{590} phosphorylation in tibialis anterior muscles from ICR mice. (**B**) AICAR incubation did not increase TBC1D1 Ser^{590} phosphorylation in EDL muscles from ICR mice. Data are means ± S.E.M.; $n = 5$–7/group. Au, arbitrary units.

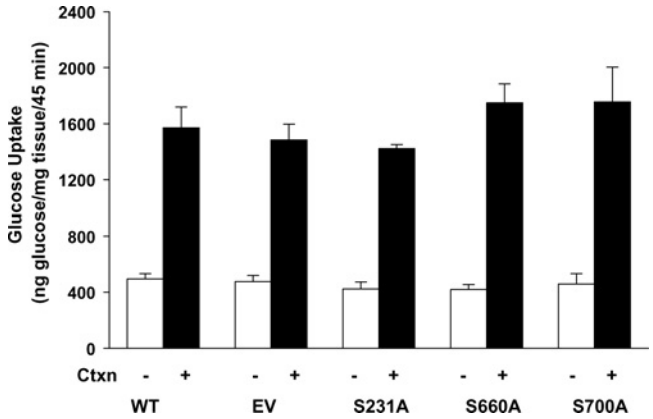

Figure S4 Single point mutations of TBC1D1 do not alter glucose uptake

A separate cohort of mice, including different control empty vector (EV) and WT TBC1D1-injected mice were used to characterize the effects of single mutations on glucose uptake. Individual point mutations of the TBC1D1 phosphorylation sites Ser^{231} (S231A), Thr^{590} (T590A), Ser^{660} (S660A) and Ser^{700} (S700A) to alanine were generated. EV control, TBC1D1 WT and the four TBC1D1 single alanine mutants were injected into tibialis anterior muscles followed by *in vivo* electroporation and 7 days later basal and contraction-stimulated glucose uptake were measured *in vivo*. Basal and contraction-stimulated glucose uptake were not affected by overexpression of WT TBC1D1 or single mutant TBC1D1. Data are means ± S.E.M.; $n = 4$–12/group.

Table S1 Characteristics of high-fat-fed animals

Mice were fed a high-fat diet (60% kcal of fat) for 9 weeks. Mice fed the high-fat diet had significantly higher body weights, fasting blood glucose concentrations and fasting insulin concentrations compared with chow-fed mice. Data are means ± S.E.M.; $n = 6$/group. $^*P < 0.05$ compared with the chow diet.

Characteristic	Chow ($n = 6$)	High fat ($n = 6$)
Body weight (g)	25.2 ± 1.2	33.4 ± 1.2*
Glucose levels (mg/dl)	142.0 ± 17.4	185.2 ± 8.5*
Insulin levels (ng/ml)	0.68 ± 0.12	2.42 ± 0.16*

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