

SUPPLEMENTARY DATA

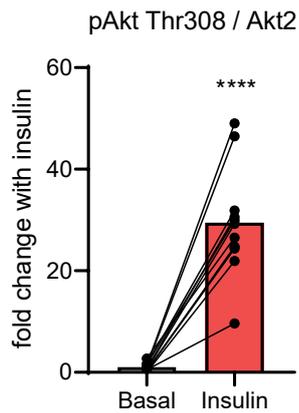
Supplementary Table 1 - List of mouse TBC1D4 interactors

Gene name	Significance based on	Gene name	Significance based on	Gene name	Significance based on
1700003F12Rik	t-test	Eif3e	Exclusive	Mybph	t-test
Acadl	t-test	Eif4a2	t-test	Myl4	Exclusive
Acads	Exclusive	Eno1	t-test	Ndrp2	Exclusive + t-test
Acadvl	t-test	Eno3	t-test	Nono	Exclusive
Aco2	t-test	Esd	Exclusive	Park7	t-test
Adsl	Exclusive + t-test	Etfb	t-test	Pebp1	Exclusive + t-test
Adssl1	Exclusive + t-test	Etfdh	Exclusive	Pgam2	t-test
Ak1	t-test	Fabp3	Exclusive + t-test	Pgk1	t-test
Akr1b1	Exclusive + t-test	Fabp4	Exclusive	Pgm1	t-test
Akr1b10	Exclusive	Fh	Exclusive	Pkm	t-test
Alb	t-test	Fkbp1a	Exclusive	Ppia	Exclusive + t-test
Aldoa	t-test	G3bp1	Exclusive	Prps1l3	Exclusive
Anxa1	Exclusive	Gbas	Exclusive + t-test	Psma6	t-test
Anxa6	Exclusive	Gdi2	Exclusive	Psmc4	Exclusive
Apoa1	Exclusive	Gm17669	Exclusive + t-test	Pvalb	t-test
Atp5d	t-test	Gm20390	Exclusive + t-test	Rab1b	Exclusive
Bsg	Exclusive + t-test	Gm20425	Exclusive	Retnlg	t-test
Ca3	t-test	Got2	Exclusive + t-test	Rps24	Exclusive + t-test
Cand2	Exclusive + t-test	Gpd1	t-test	Sfpq	Exclusive
Cfl1	Exclusive	Gpd2	Exclusive	Sod2	Exclusive
Cfl2	Exclusive + t-test	Gpx4	Exclusive	Sucla2	t-test
Ckm	t-test	Gstm2	Exclusive + t-test	Sucg1	t-test
Cox4i1	t-test	Gstp1	Exclusive + t-test	Taf15	Exclusive
Cox5a	Exclusive + t-test	Hadh	Exclusive + t-test	Tbc1d1	Exclusive
Cox5b	t-test	Hba-a1	t-test	Tpm3	Exclusive + t-test
Cox6b1	t-test	Hbbt1	t-test	Tpt1	Exclusive
Cox6c	t-test	Hmgb1	Exclusive	Trim72	Exclusive + t-test
Cox7a1	t-test	Hspb7	Exclusive + t-test	Ugp2	Exclusive
Cs	t-test	Hspe1	Exclusive	Uqcrb	Exclusive + t-test
Cycs	Exclusive + t-test	Idh2	Exclusive + t-test	Uqcrc1	t-test
D10Jhu81e	Exclusive + t-test	Idh3g	Exclusive	Vcp	t-test
Dbi	Exclusive + t-test	MacroD1	Exclusive + t-test	Vdac1	t-test
Dusp3	Exclusive	Mb	t-test	Vdac3	t-test
Echs1	Exclusive	Mdh1	t-test	Ywhag	t-test
Eef1a1	Exclusive	Mdh2	t-test	Ywhaz	Exclusive
Eef2	t-test	Msn	Exclusive		
Eif2s3x	Exclusive	Msrp3	Exclusive		

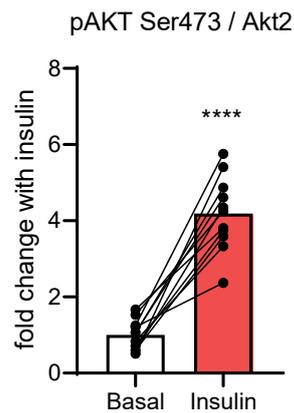
List of 109 significant TBC1D4 interactors in mouse skeletal muscle. Significance was based on exclusive identification and/or on Students' t-test (>1.5 fold change WT-D4KO and FDR <0.05).

**Supplementary Figure S1 – Intact insulin- and exercise-mediated phosphorylation of TBC1D1 in complex with TBC1D4.** Western blot on protein lysate from basal and insulin-stimulated human skeletal muscle of p-Akt Thr308 (A) and p-Akt Ser473 (B). Western blot on protein lysate from rested and acutely exercised human skeletal muscle of p-AMPK Thr172 (C). \*\*\*\* $P < 0.0001$ , \*\*\* $P > 0.001$  and \*\* $P > 0.01$ . Bars are means and lines indicate individual responses (n=11 in A-B, n=8 in C).

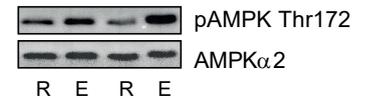
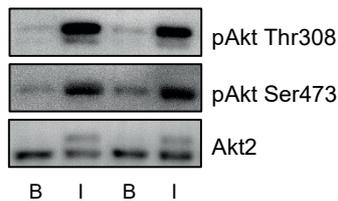
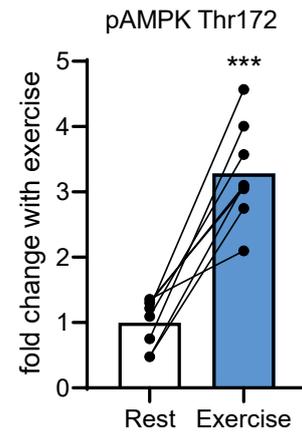
A



B



C





**Supplementary Figure S3 - Insulin-stimulated phosphorylation of the TBC1D1-TBC1D4 complex after exercise.** Western blot on protein lysate from rested, prior exercised human skeletal muscle at basal and in response to insulin stimulation followed by western blot of p-TBC1D4 Ser318 (A), p-TBC1D4 Thr642 (B) and p-TBC1D4 Ser704 (C). Representative western blot is shown in (D). Data were analyzed by repeated measures ANOVA. ANOVA significance is displayed with text on each graph. Where interaction was found between prior exercise and insulin Tukey's multiple comparisons test was used as post hoc test. \*\*\* $P < 0.0001$  and \*\* $P > 0.01$  indicate effect of insulin where † $P > 0.05$  indicates effect of prior exercise. Bars are mean. Values for each individual is marked with a unique asterisk and connected with lines within each leg ( $n=7$ ).

