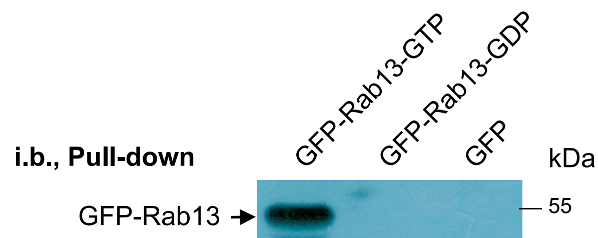
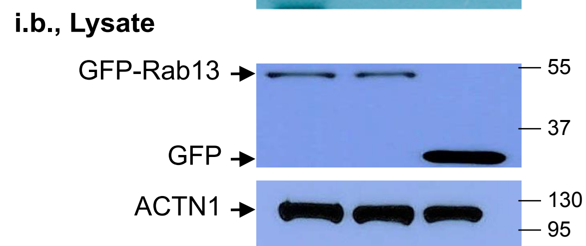
Supplemental Materials

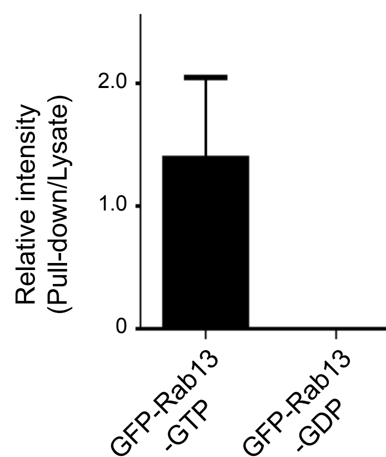
Molecular Biology of the Cell

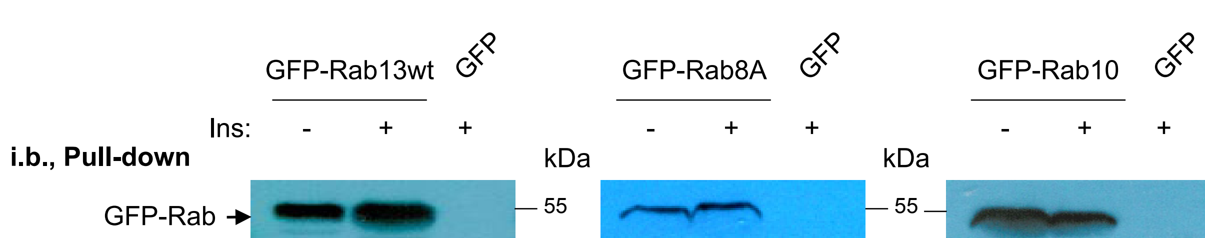
Sun et al.

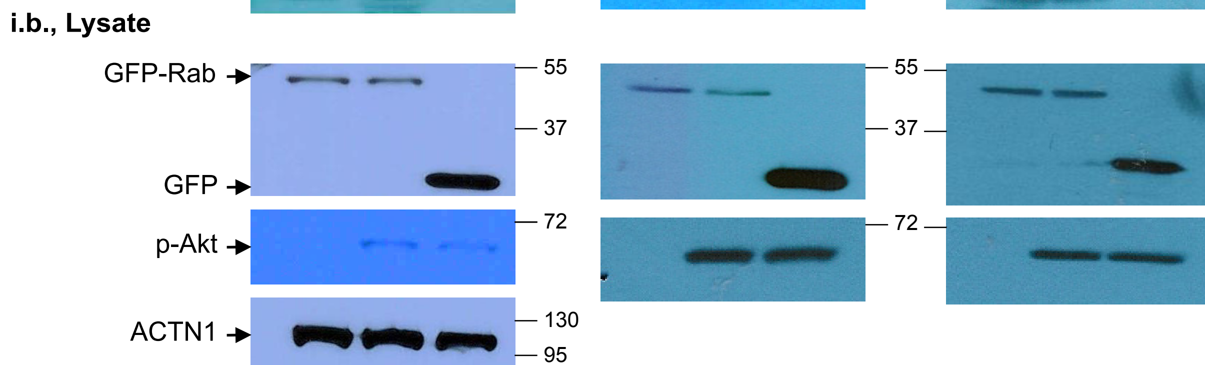
Suppl. 1

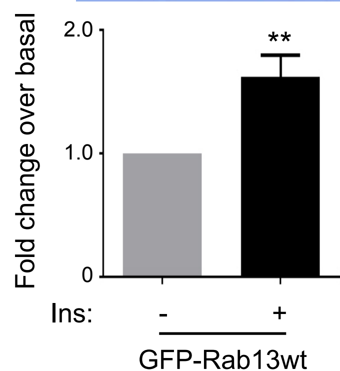


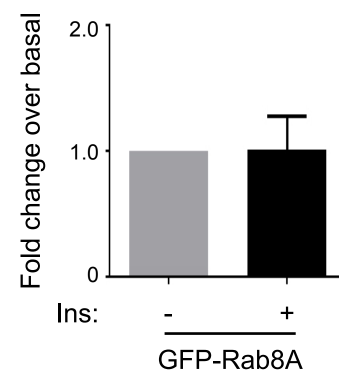


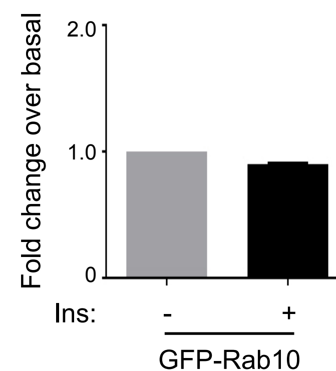






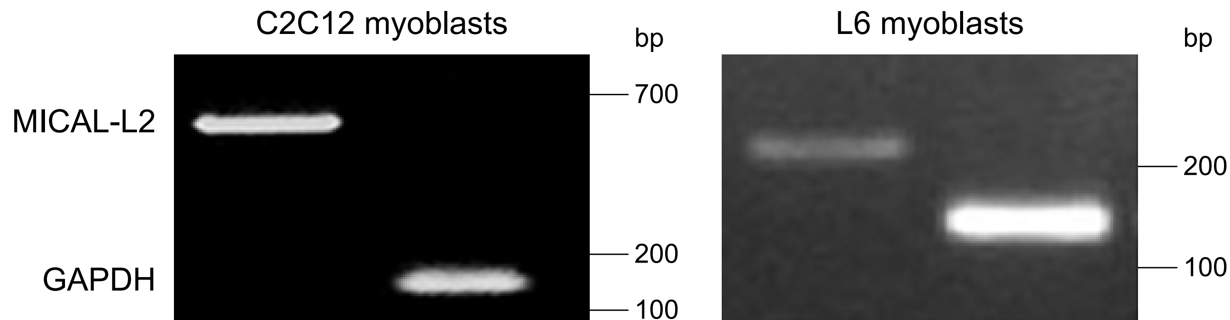


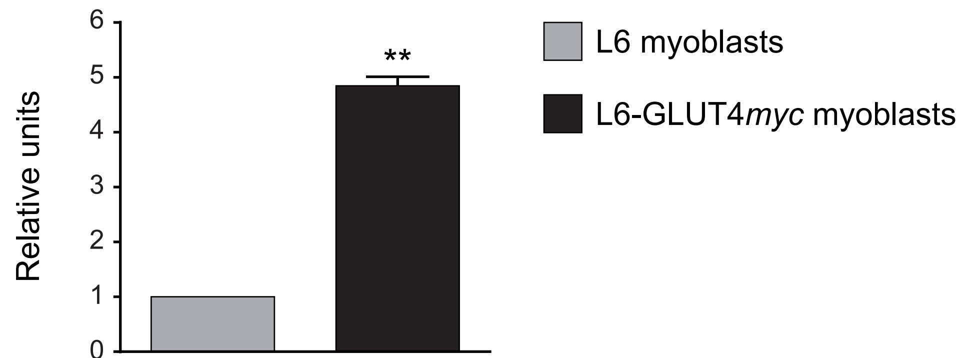




Supplemental Figure 1. MICAL-L2-CT binds Rab13 in an insulin-dependent fashion. (A) Lysates from CHO-IR cells expressing GTP-locked Rab13Q67L (GFP-Rab13-GTP), GDP-locked Rab13T22N (GFP-Rab13-GDP) or GFP were incubated with glutathione Sepharose (GSHS) beads loaded with MICAL-L2-CT. Proteins bound to beads were pulled down and subjected to SDS-PAGE and immunoblotting with anti-GFP along with lysate aliquots. Graph: Pixel intensity of pulled-down bands relative to lysates (n=2). **(B)** CHO-IR cells expressing GFP-Rab13, GFP-Rab8A, GFP-Rab10 or GFP were serum-starved and stimulated with/without insulin. Complexes were pulled-down and analyzed as in (A). Phospho-Akt S473 (p-Akt) in lysates confirmed insulin effectiveness. Data are the mean ± SE of 3-5 independent experiments (**$p<0.01$).

Suppl. 2

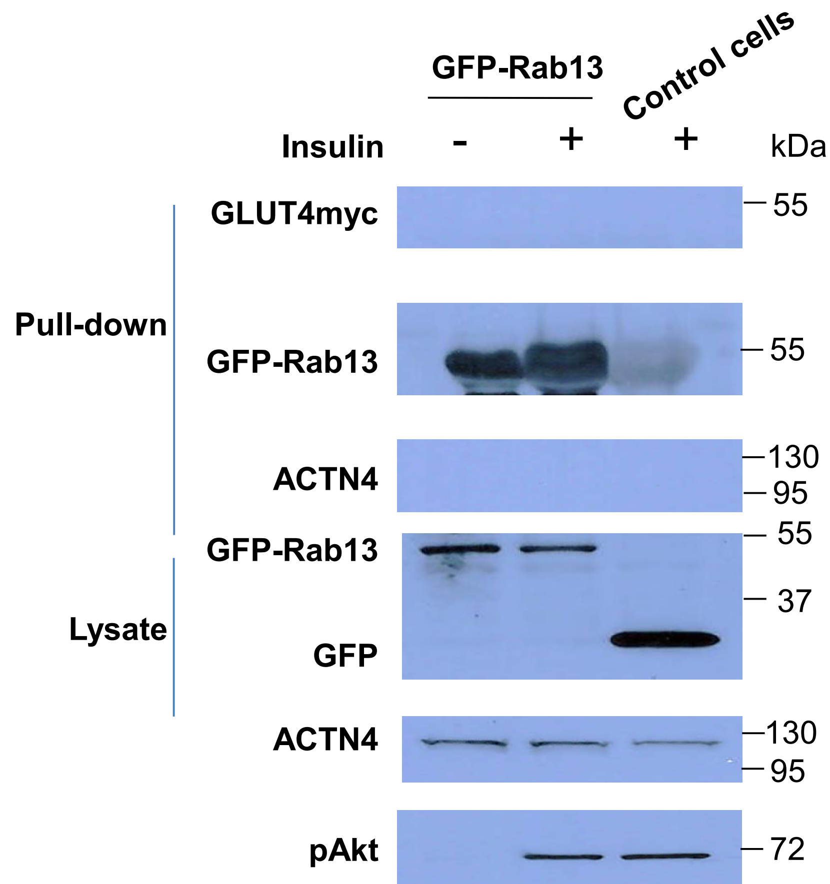




Supplemental Figure 2. A) MICAL-L2 expression in L6 and C2C12 muscle cells. RT-PCR was performed with cDNA from rat L6 muscle cells and mouse C2C12 muscle cells using pairs of primers of MICAL-L2 with specificity for rat or mouse orthologues, respectively. **B) MICAL-L2 expression in L6 and L6-GLUT4*myc* muscle cells.** qPCR was performed with cDNA from L6 and L6-GLUT4*myc* muscle cells **($p<0.01$). Gene expression was normalized to that of the housekeeping gene *Hprt*. All qPCR reactions were performed using the experimental conditions previously described (Chan et al., J Biol Chem. 2015 Jul 3;290(27):16979-88).

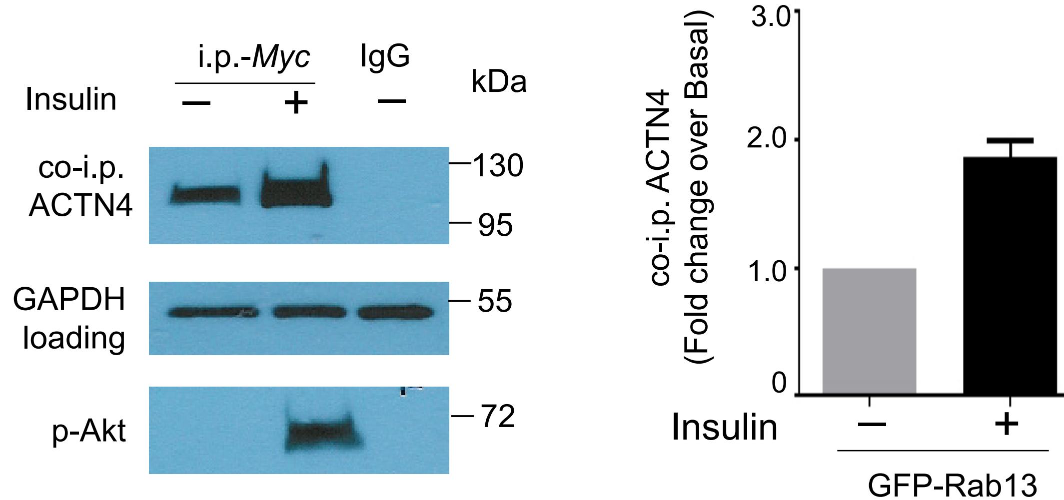
Suppl. 3

GST-MICAL-L2-CT Pull-Down



Supplemental Figure 3. GST-MICAL-L2-CT pulls down Rab13 but not ACTN4 or GLUT4*myc*. Cell lysates prepared from L6-GLUT4*myc* cells expressing GFP-Rab13 or GFP, treated with/without insulin, were incubated with GSHS beads loaded with GST-MICAL-L2-CT (containing the Rab but not the ACTN4 binding domain). Proteins in the Pull-down assays or aliquots of cell lysates were subjected to SDS-PAGE and immunoblotted with anti-GFP, anti-ACTN4 or anti-*myc* as indicated. Phospho-Akt illustrates insulin effectiveness. Shown are the results from one experiment representative of three. GFP-Rab13 association with GST-MICAL-L2-CT was detected, consistent with the findings in Suppl. 1, but ACTN4 and GLUT4*myc* were not present in the pulled down material.

Suppl. 4



Supplemental Figure 4. Insulin promotes ACTN4 co-immunoprecipitation with GLUT4 in L6 muscle cells. L6 GLUT4*myc*-IR myoblasts were treated with the cell-permeant cross-linker, DSP (1.5 mM for 30 min) prior to stimulation with or without insulin (100 nM, 15 min). Cells were lysed and were subjected to immunoprecipitation with mouse anti-*myc* antibody or IgG. Immunoprecipitates (IP) were immunoblotted with rabbit anti-ACTN4 antibody. Aliquots of lysates were immunoblotted with anti-GAPDH and anti-phospho-Akt S473 was detected to confirm equal inputs and insulin effectiveness. The bar graph shows the quantification of pixel intensity of immunoblotted protein bands (n=2).