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Supplementary Materials for

REDD1 enhances protein phosphatase 2A–mediated dephosphorylation of Akt to repress mTORC1 signaling

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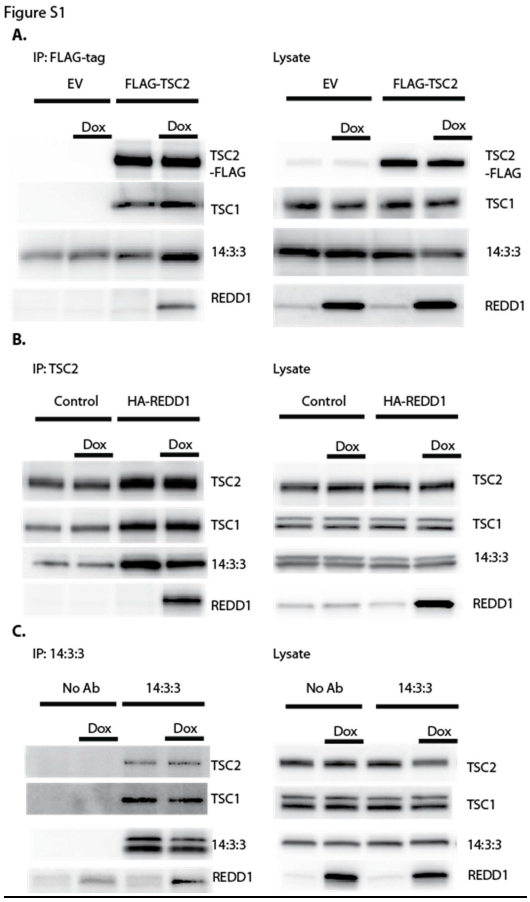
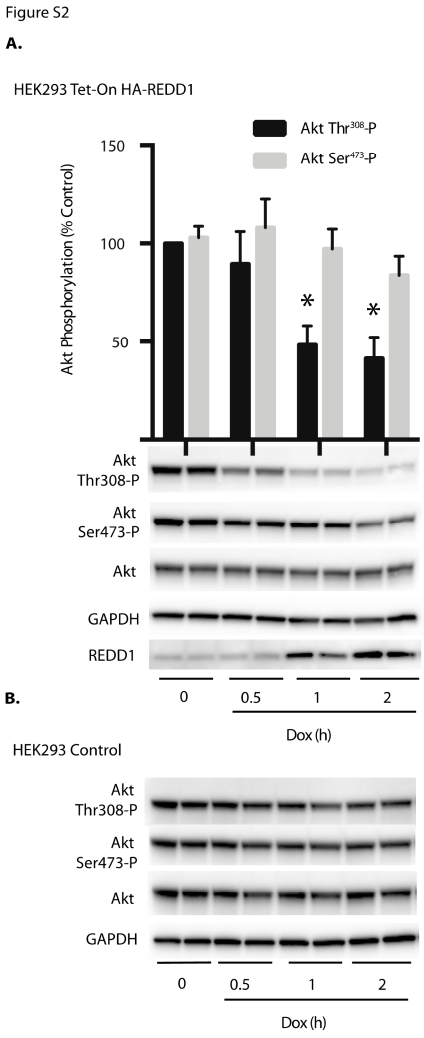
The PDF file includes:

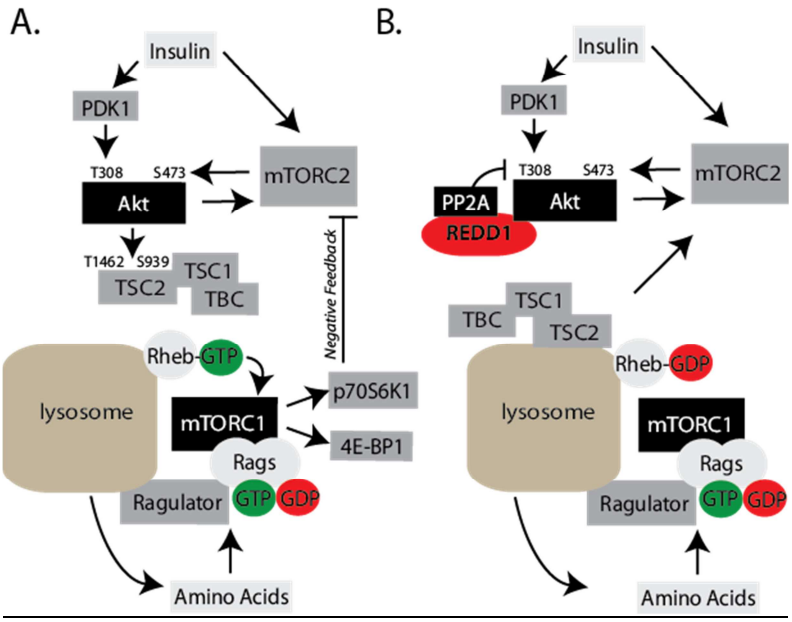


Supplemental Figure S1: Effect of REDD1 on the interaction of 14-3-3 protein with TSC2. (A) HEK293 Tet-On cells maintained in complete growth medium were transfected with either empty vector control plasmid (EV) or a plasmid expressing FLAG-tagged TSC2 (FLAG-TSC2). (B) Endogenous TSC2 was immunoprecipitated from HEK293 Tet-On (Control) or HEK293 Tet-On HA-REDD1 cells. (C) Endogenous 14:3:3 was immunoprecipitated from HEK293 Tet-On HA-REDD1 cells. Alternatively, a control immunoprecipitation lacking anti-14:3:3 antibody (No Ab) was used assess the background binding to beads. REDD1 expression was induced in cells using 1 μg/μl doxycycline (Dox) for 6 h as indicated. The abundance of TSC2, TSC1, 14:3:3, and REDD1 was evaluated in immunoprecipitates and supernatants of cell lysates by Western blot analysis. Blots shown in panels A-C are representative of results for two independent experiments.



Supplemental Figure S2: REDD1 promotes the dephosphorylation of Akt on Thr^{308}. HEK293 Tet-On HA-REDD1(A) and HEK293 Tet-On control cells (B) were serum deprived for 3 h and then treated with complete medium for 30 min to promote the phosphorylation of Akt. Cells were then treated with doxycycline (Dox) as indicated. Phosphorylation of Akt on Thr^{308} and Ser^{473} and the total abundance of Akt, HA-REDD1, and GAPDH were examined by Western blot analysis. Blots shown in A-B are representative of results from three independent experiments (N=3); within an experiment, two independent samples were analyzed. Results represent mean phosphorylation ± SEM for (A). *p<0.05 vs. time 0.

Figure S3



Supplemental Figure S3: Regulation of the phosphorylation of Akt at Thr^{308} and the TSC2-TBC complex by REDD1. (A) In the absence of REDD1, insulin stimulates phosphorylation of Akt on both Thr^{308} and Ser^{473} by PDK1 and mTORC2, respectively. Once active, Akt phosphorylates TSC2, and thus impairs the GAP activity of TSC2 leading to reduced Rheb GTPase activity and consequently increased Rheb GTP loading. On the other hand, amino acids signal to activate mTORC1 through the Rag/Ragulator complex, which promotes recruitment of the inactive kinase to the lysosomal surface where it contacts its essential activator Rheb-GTP. (B) An increase in REDD1 abundance facilitates recruitment of PP2A to Akt, leading to the dephosphorylation of Akt at Thr^{308}, and impaired TSC2 phosphorylation by Akt. Consequently, by impairing the GTP loading of Rheb, REDD1 acts as a dominant governor of mTORC1 signaling.