SUPPLEMENTAL MATERIALS

Figure S1. The FKBP-C domain in FKBP38 mediates its interaction with Rheb. Myc tagged deletion mutants of FKBP38 were expressed together with Flag tagged Rheb in HEK293 cells. Cell lysates were precipitated with Protein A bead conjugated anti-myc antibody and the precipitates were analyzed by western blotting. **A.** Co-purified myc-FKBP38 mutants (upper panel) and Flag-Rheb (lower panel). **C.** Input lysates representing 5% of the amount used for precipitation.

Figure S2. Effect of FKBP38 deletion mutants on mTORC1 activity. Myc tagged deletion mutants of FKBP38 were transfected together with HA-S6K into HEK293 cells. Transfected cells were incubated for 24 hr. Cells were starved for amino acids for 1hr followed by re-addition of amino acids. Cells were harvested after incubation for another 30 min. **A.** The levels of HA-S6K (lower panels) and phosphorylation at T389 (upper panels) were determined by western blotting and quantified by densitometry. The ratio of phosphorylation versus protein level of each sample was compared to that of the control (lane 1) and the relative values were shown. Data are the mean ± S.D. of values from three independent experiments. **B.** The expression levels of the FKBP38 mutant proteins in the lysates.

Figure S3. Localization of the FKBP38 mutant proteins. HEK293 cells expressing different myc tagged FKBP38 mutants were stained with mitotracker (red), followed by fixation and staining with anti-myc antibody in conjunction with Alexa Fluor labeled secondary antibody (green), and imaged with confocal microscopy.

Figure S4. Effect of FKBP38 on Rheb mitochondria association. HEK293 cells expressing GFP tagged Rheb (Green) together with wild type FKBP38 (upper row) or FKBP38 lacking its transmembrane domain (lower row) were stained with mitotracker (red). Cells were imaged with confocal microscopy.

Figure S5. Mutations in the effector domain of Rheb affect its signaling activity. A. Stimulation of S6K(T389) phosphorylation by the alanine substitution mutants under serum deprivation condition. HEK293 cells were transfected with Rheb mutants together with HA-S6K. After 24 hr, cells were serum deprived (0.2% serum) for 16 hr. Cells were collected and lysed. The levels of HA-S6K (2^{nd} panel), phosphorylation at T389 of HA-S6K (top panel), phosphorylation at T37/46 of 4E-BP1 (3^{rd} panel), the endogenous 4E-BP1 (4^{th} panel) and expressed Rheb (bottom panel) were determined by western blotting. **B.** The ability of the Rheb mutants to antagonize the inhibitory effect of FKBP38. Rheb mutants were transfected together with FKBP38 into HEK293 cells. Upon incubation for 24 hr, cells were serum-deprived for 16 hr followed by repletion of serum (20%). Cells were harvested after incubation for another 30 min. The levels of HA-S6K (2^{nd} panel), phosphorylation at T389 of HA-S6K (top panel), phosphorylation at T37/46 of 4E-BP1 (3^{rd} panel), the endogenous 4E-BP1 (4^{th} panel) and expressed Rheb (bottom panel) were determined by western blotting.

Fig. S1

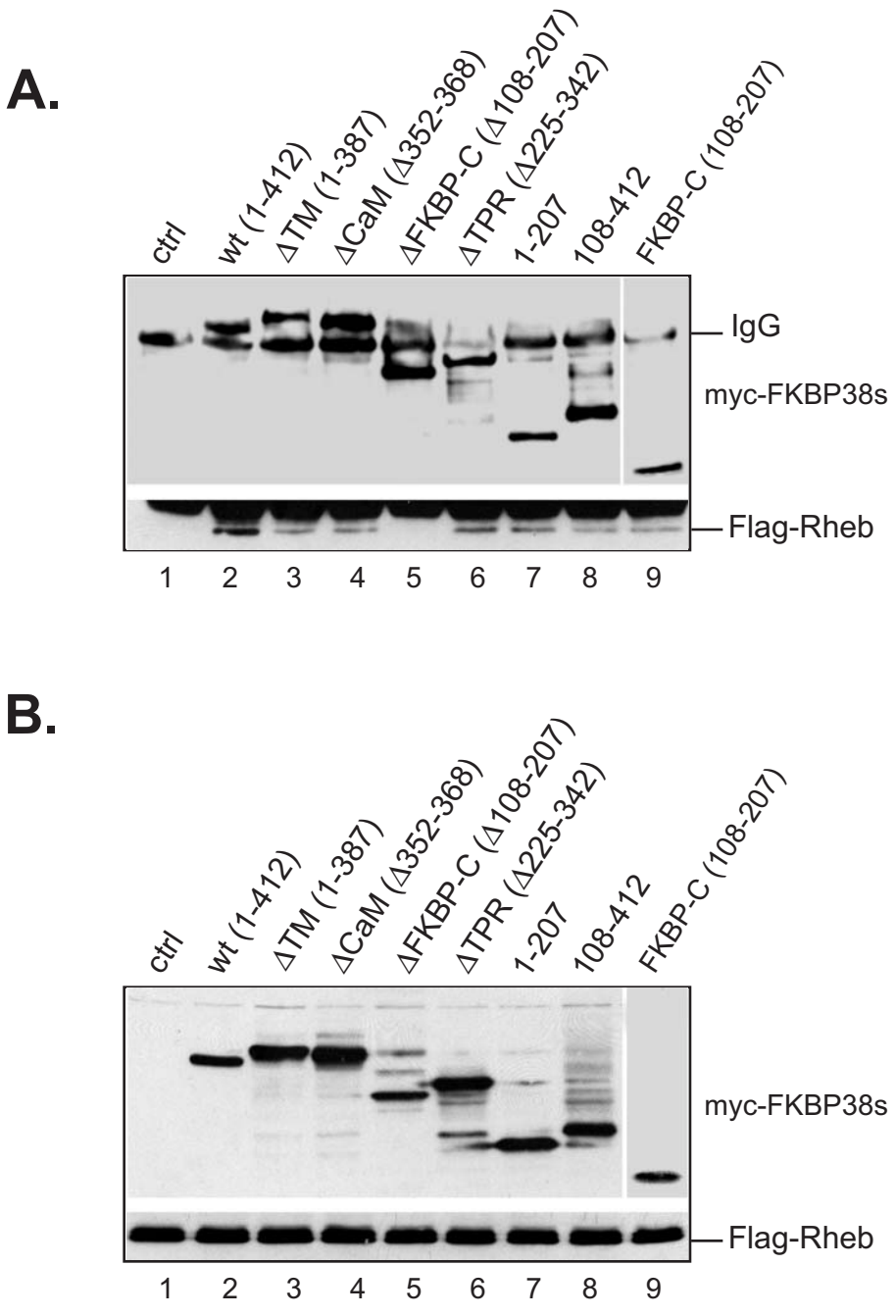
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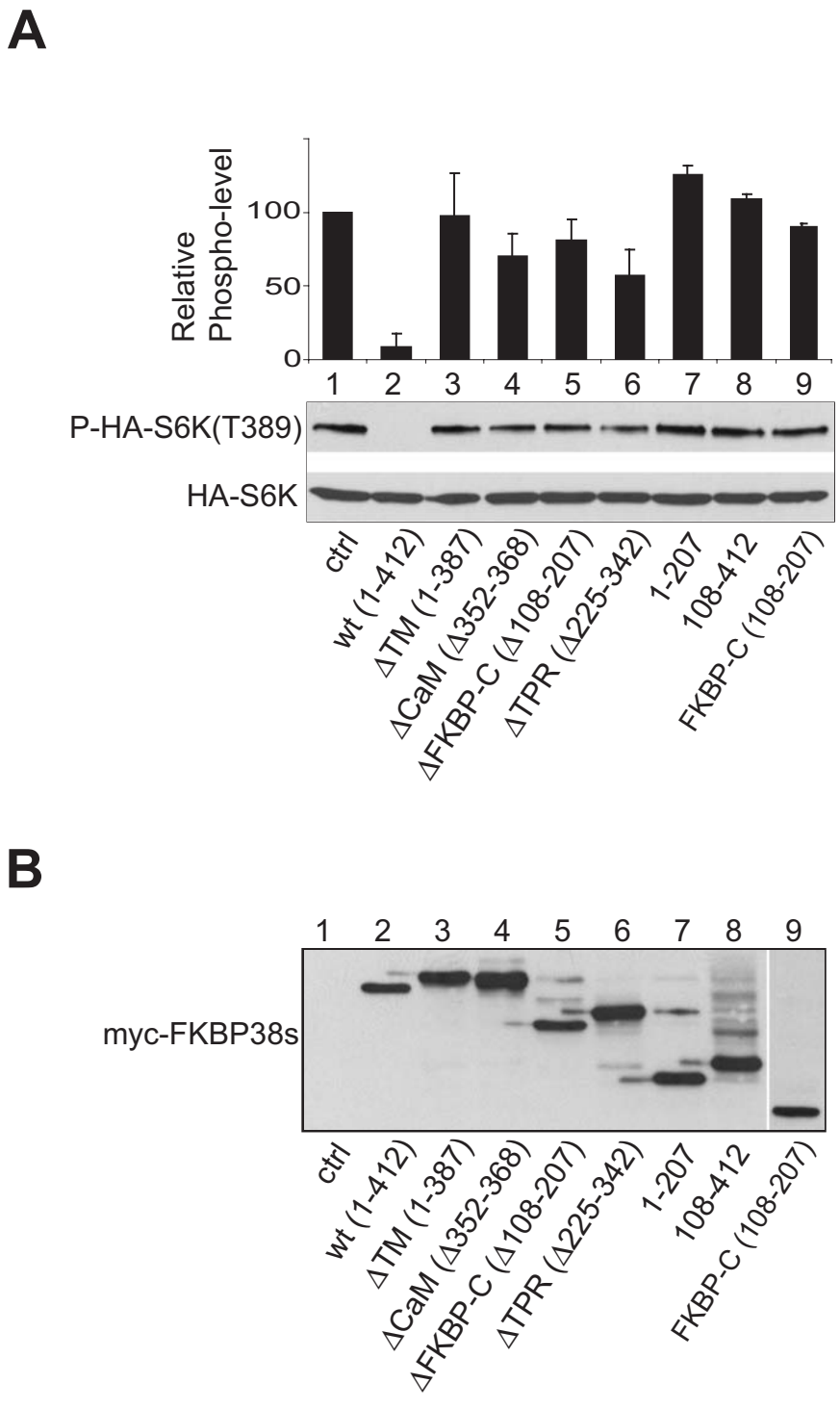
Fig. S2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B
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Fig. S3

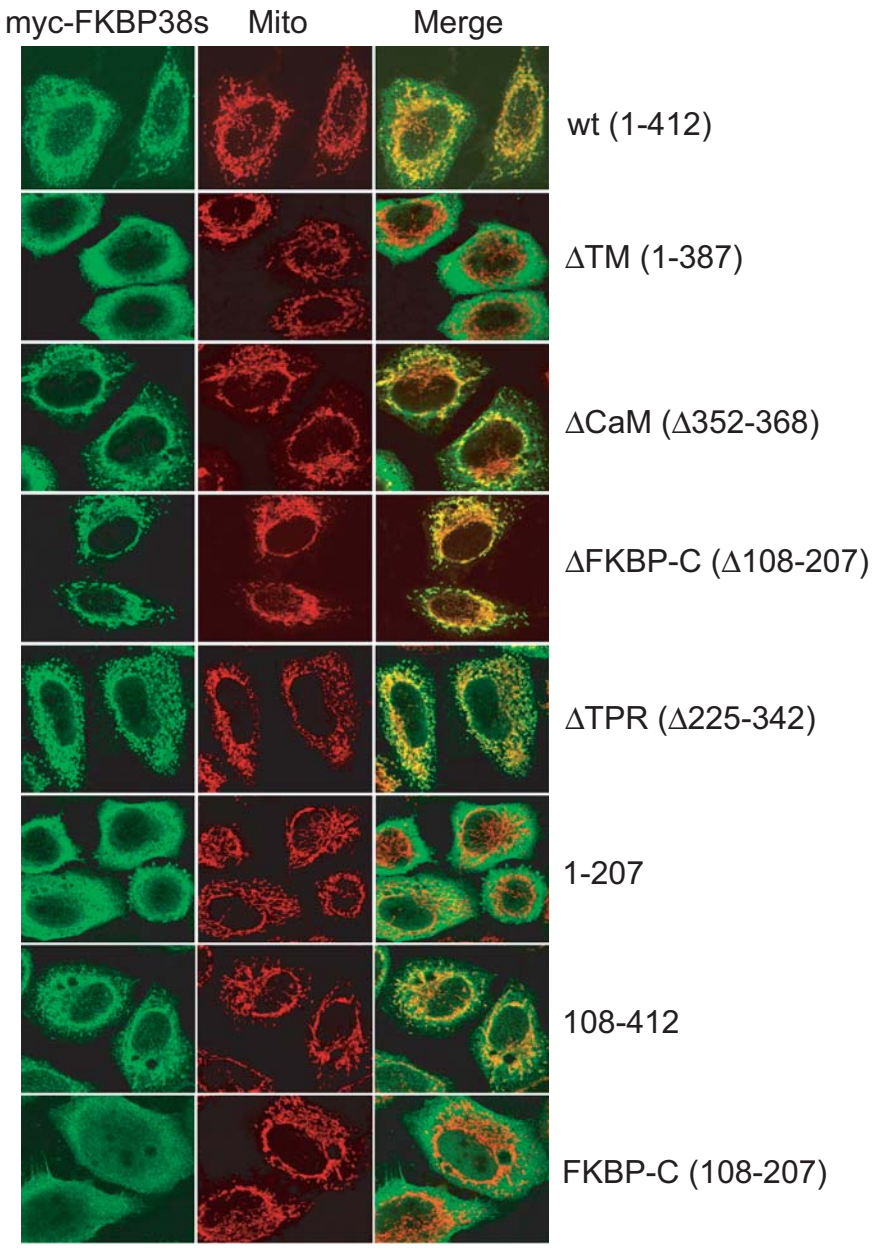
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Fig. S4

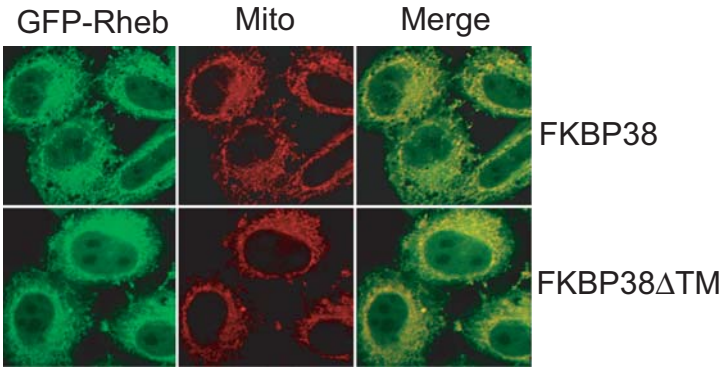
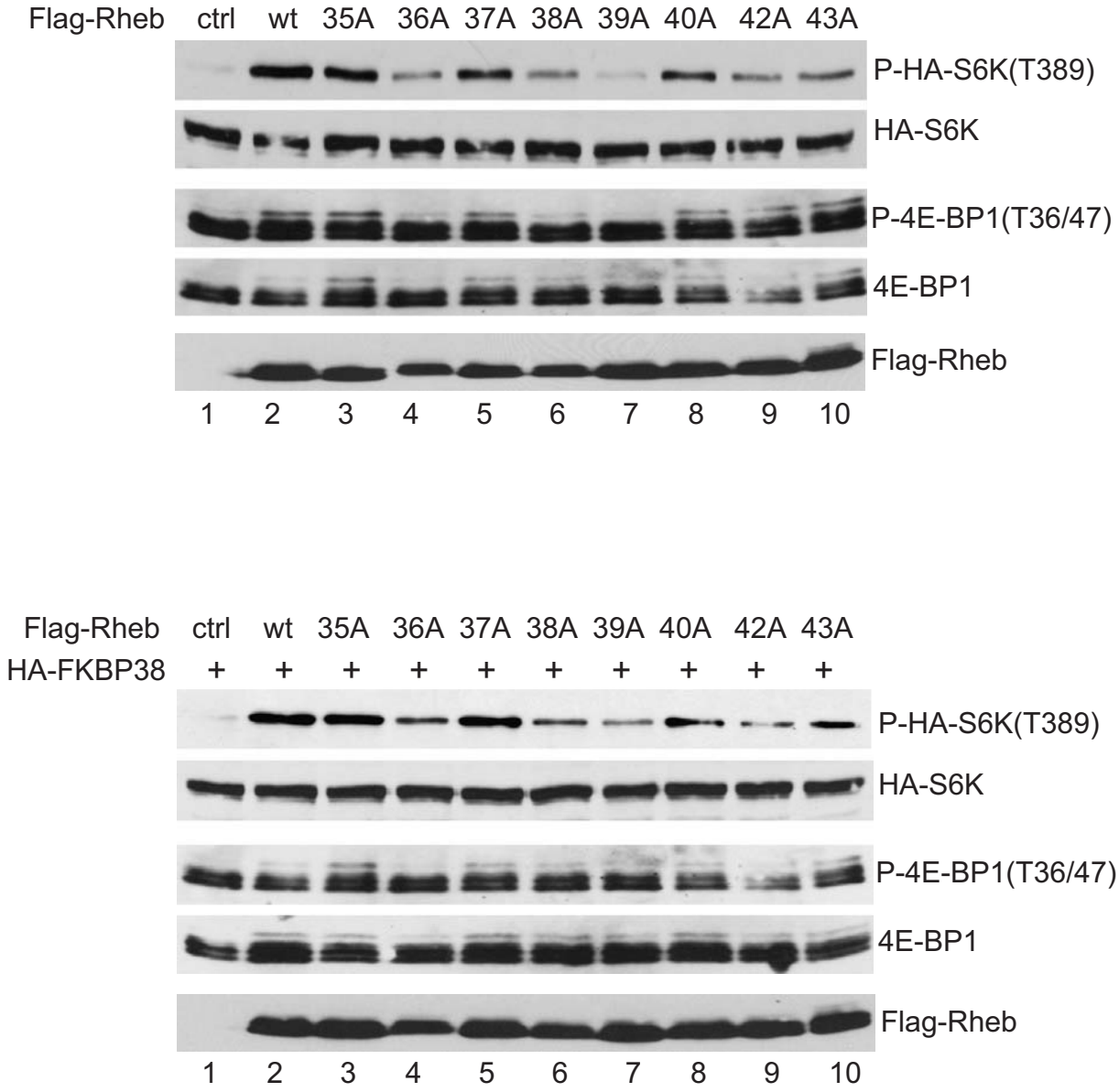


Fig. S5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4E-BP1
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