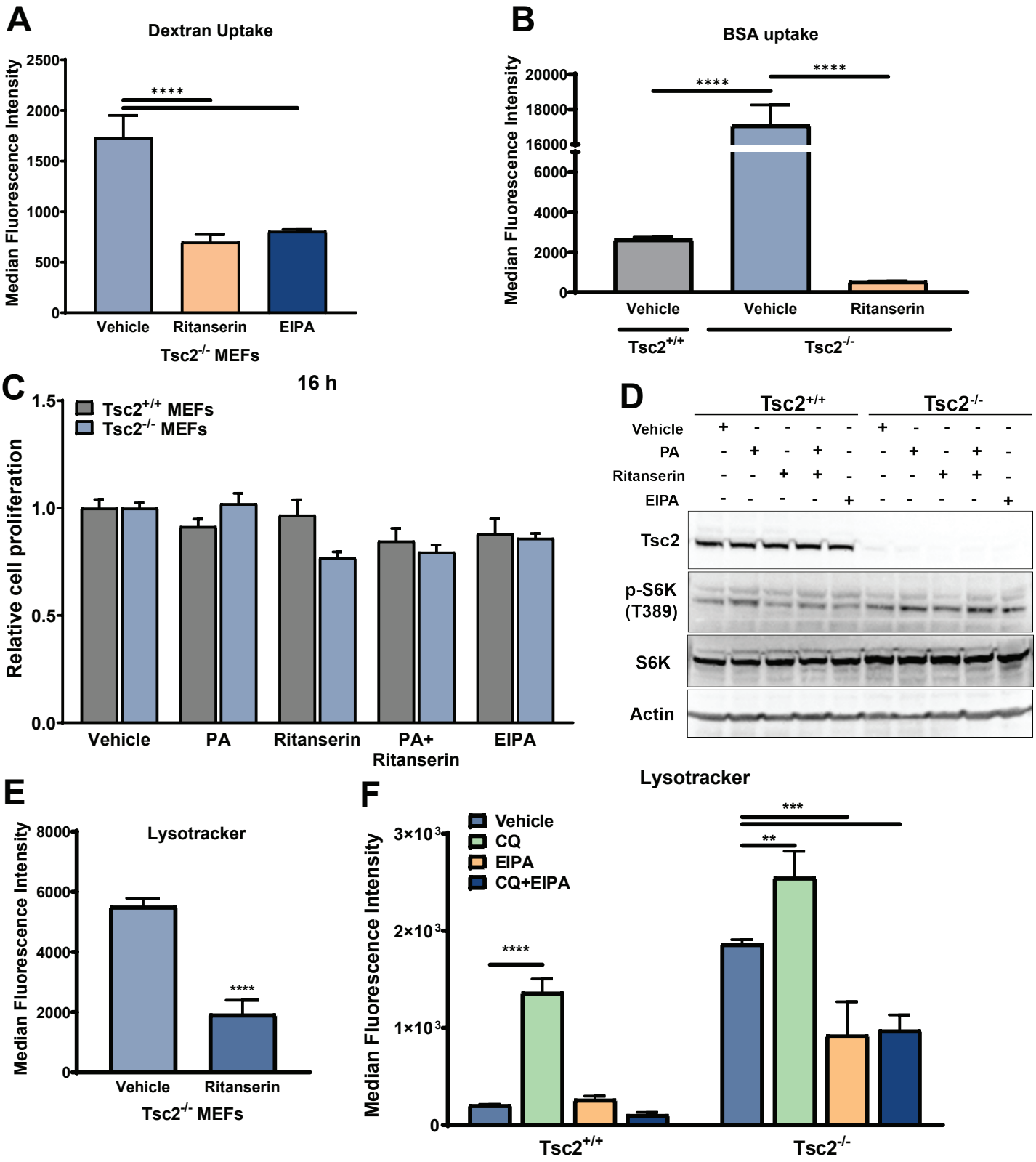
Supplementary Figure 3



Supplementary Figure 3. (A) Dextran uptake (0.5mg/ml, FITC-Dextran) was inhibited upon ritanserin treatment (10uM; 16 hours). As expected, EIPA (25uM;16 hours) inhibited macropinocytic dextran uptake. **(B)** Exogenous protein uptake (0.5mg/ml, BSA-TMR) was increased in $Tsc2^{-/-}$ MEFs compared to $Tsc2^{+/+}$ MEFs. Ritanserin (10uM; 4 hours) blocked macropinocytosis in Tsc2-/- MEFs. **(C)** Ritanserin treatment (10uM; 16 hours) has no impact on the viability of $Tsc2^{+/+}$ or $Tsc2^{-/-}$ MEFs. Data represented as mean +/- standard deviation of six biological replicates. Proliferation was quantified using crystal violet. Values are shown as fold change (FC) normalized to the day of seeding. Statistical significance was assessed using two-way and one-way ANOVA with Bonferroni correction. **(D)** Immunoblots show increased phosphorylation of the mTORC1 target phospho-S6 kinase upon PA treatment (100uM; 16 hours). **(E)** Lysotracker staining revealed that ritanserin (10uM; 16 hours) reduces lysosome numbers in $Tsc2^{-/-}$ MEFs. **(F)** Lysotracker staining shows that macropinocytosis inhibitor EIPA (25uM; 16 hours) inhibits lysosomal numbers in $Tsc2^{-/-}$ but not $Tsc2^{+/+}$ MEFs. CQ (5uM; 16 hours) increased lysotracker staining in both cell lines as expected.