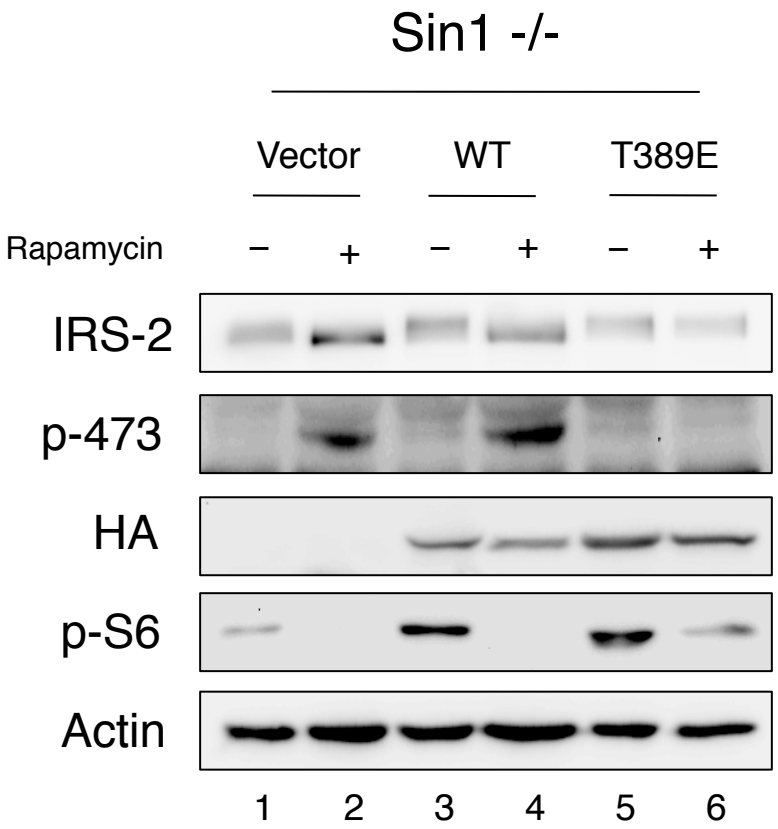
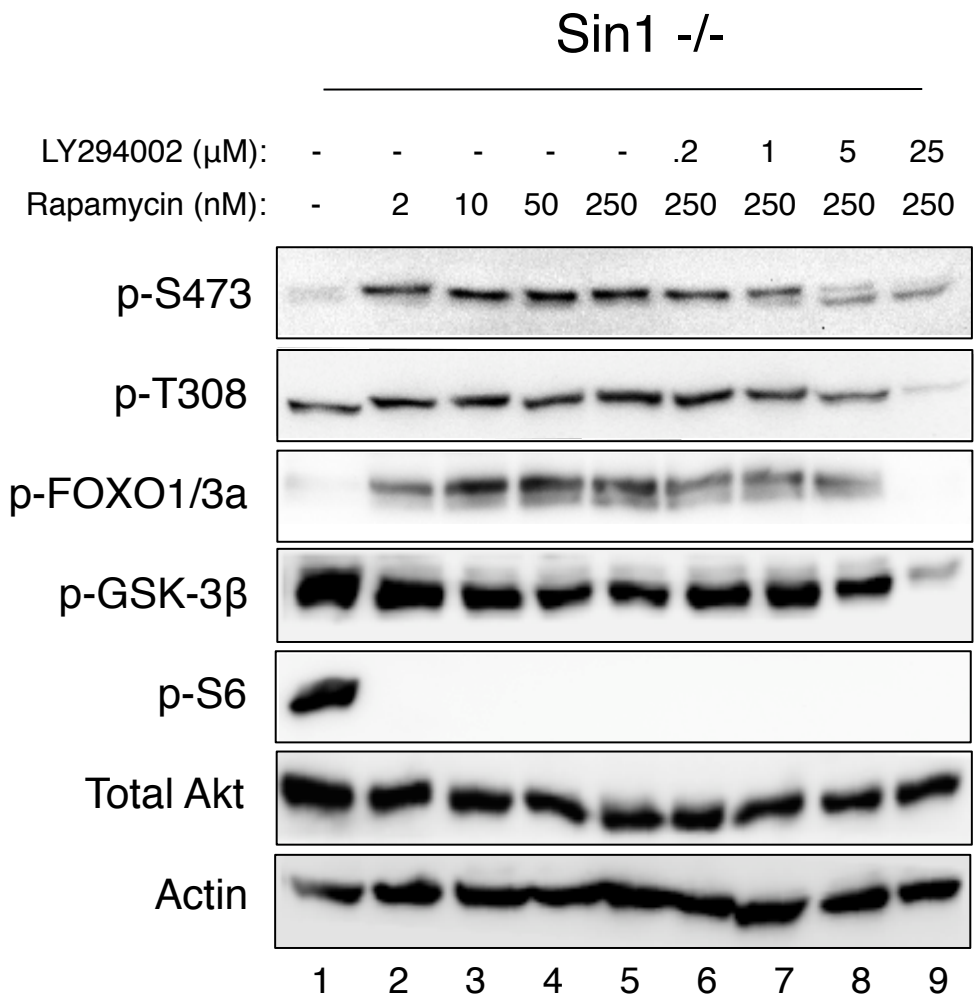
Supplemental Figure 1

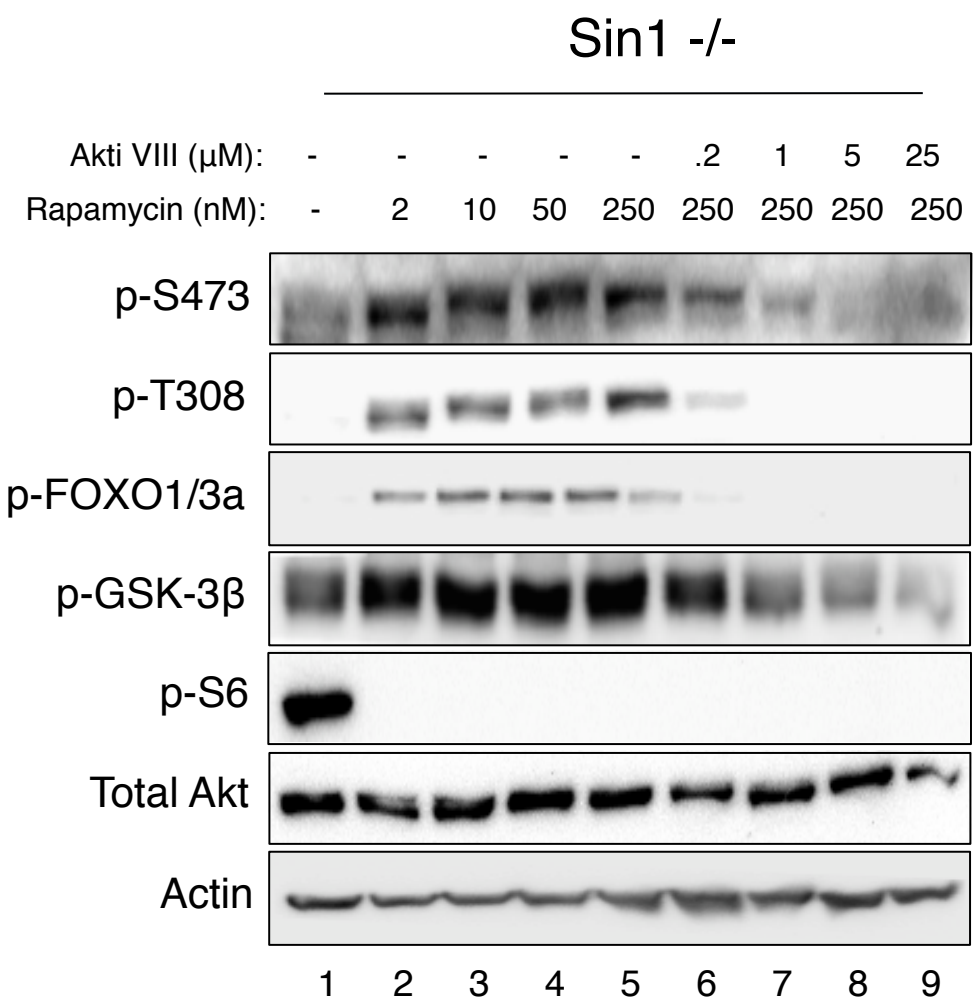


293t

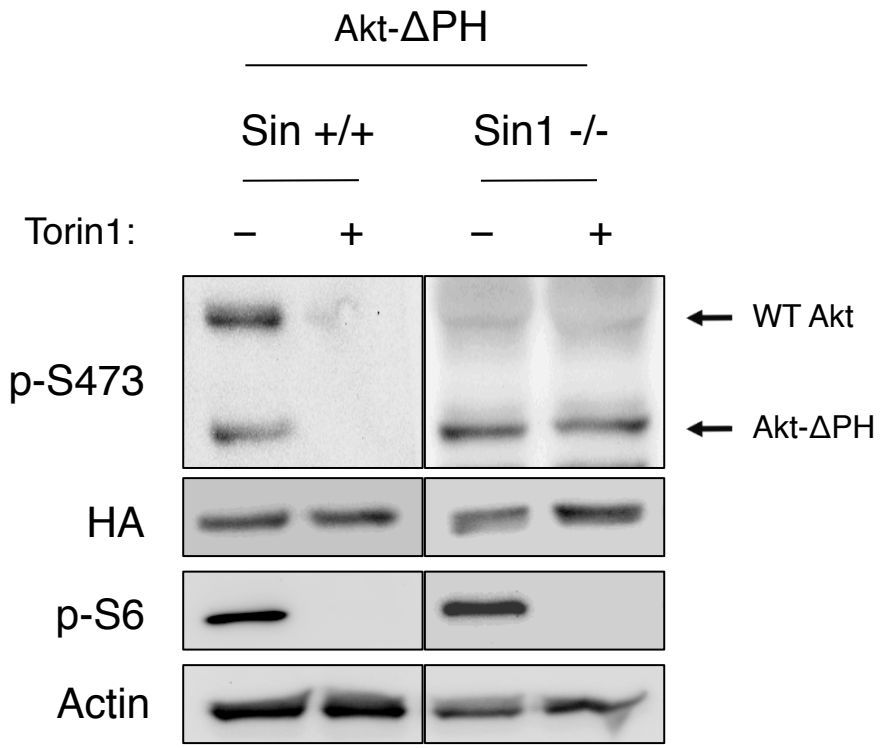
Supplemental Figure 2a



Supplemental Figure 2b



Supplemental Figure 3



Supplemental Figure 1. Inactivation of S6K is necessary to restore S473 phosphorylation in Sin1 -/- MEFs. Sin1 -/- MEFs were transfected with vector control, WT-p70S6K, or a rapamycin-insensitive construct, p70S6K (T389E) prior to treatment with rapamycin (100 nM) for 24 hours. Immunoblotting was used to monitor the relative level of total and phosphorylated proteins.

Supplemental Figure 2. Rescue of Akt (S473) phosphorylation in cells lacking mTORC2 is dependent upon PI3K and the conformation of Akt. (a) Sin1 -/- MEFs were treated with increasing concentrations of rapamycin alone or in combination with increasing doses of LY294002 for 24 hours. (b) Sin1 -/- MEFs were treated with increasing concentrations of rapamycin alone or in combination with increasing doses of Akti VIII for 24 hours. The phosphorylation state of Akt, GSK-3, FOXO1/3a, and S6 were monitored by immunoblotting.

Supplemental Figure 3. Regulation of Akt (S473) phosphorylation is specifically mediated by mTORC2. Sin1 +/+ MEFs or Sin1 -/- MEFs were transfected with Akt-ΔPH for approximately 30 hours prior to treatment with Torin1 (50 nm) for 1-hour and the phosphorylation state of Akt (S473) and S6 were monitored by immunoblotting.