

Figure S1: Related to Figure 1. The Rag GTPase inhibits TORC1 activity in WDR59-KO and MIOS-KO HeLa cells

(A) Immunoblots show that knockdowns of RAGA and RAGC increase pS6K level in WDR59-KO HeLa cells. Error bars represent standard deviation.

(B) Immunoblots show that knockdowns of RAGA and RAGC increase pS6K level in MIOS-KO HeLa cells. Error bars represent standard deviation.

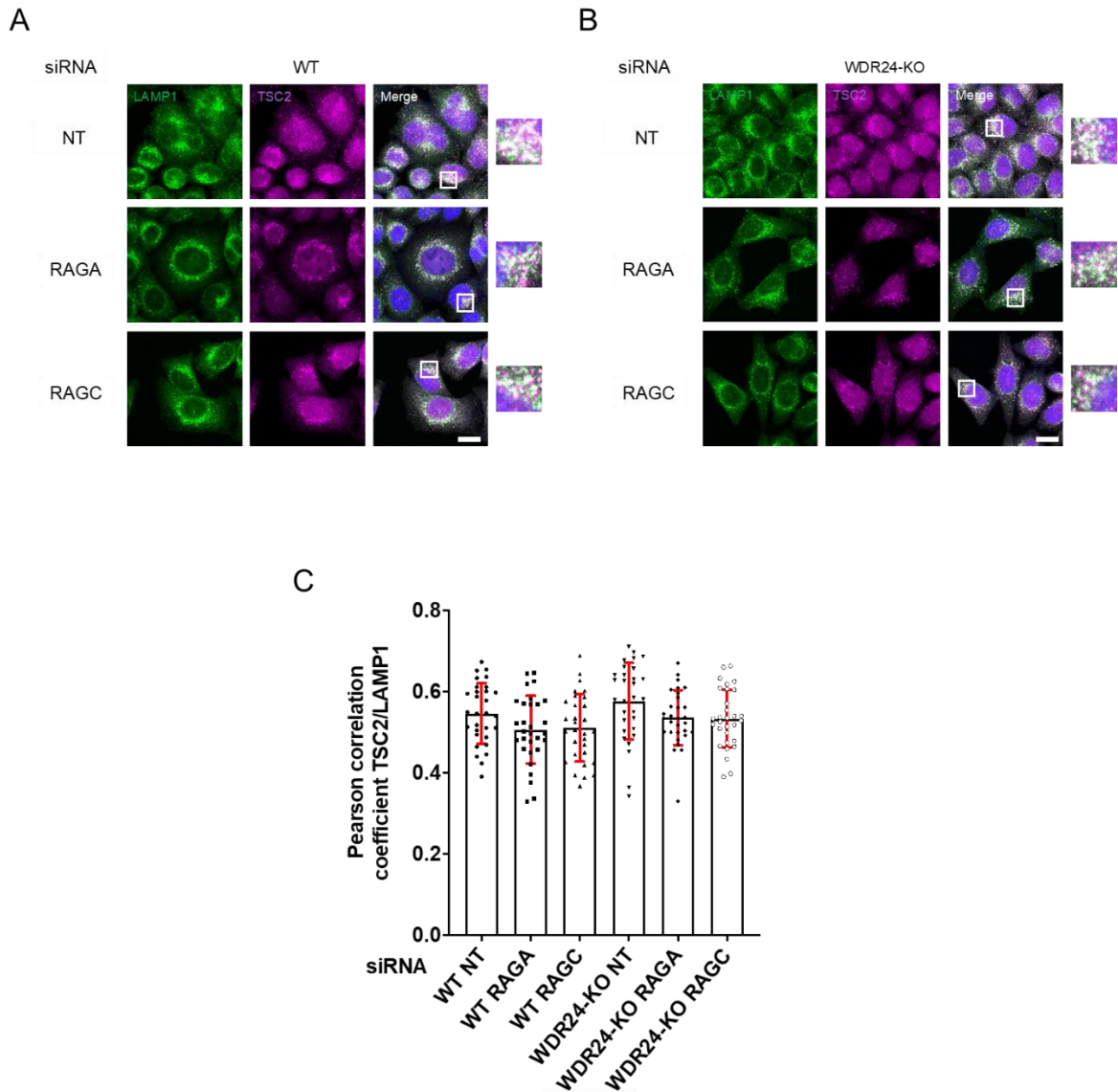


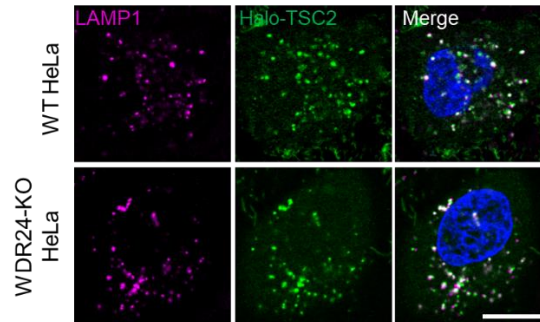
Figure S2: Related to Figure 2. The Rag GTPases are not required for TSC lysosomal localization in WT and WDR24-KO HeLa cells

(A) TSC2 and LAMP1 Immunofluorescence staining WT HeLa cells under FED (+AA +FBS) condition, with the treatments of control (NT), RAGA, RAGC siRNAs. Scale bar: 10 μ m.

(B) TSC2 and LAMP1 Immunofluorescence staining WDR24-KO HeLa cells under FED (+AA +FBS) condition, with the treatments of control (NT), RAGA, RAGC siRNAs. Scale bar: 10 μ m.

(C) Knocking-down RAGA or RAGC do not affect the Pearson correlation coefficient of TSC2/LAMP1 in WT and WDR24-KO HeLa cells. The correlation coefficient was calculated based on 30 cells per condition. Error bars represent standard deviation.

A



B

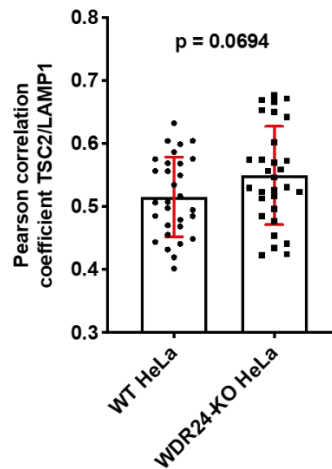
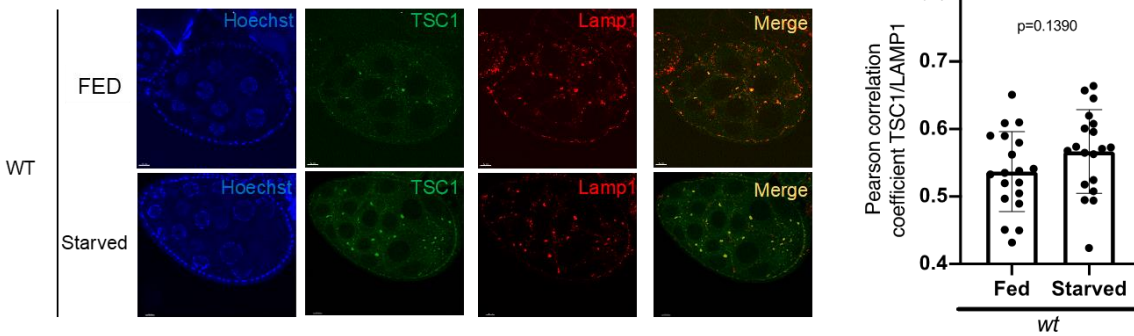


Figure S3: Related to Figure 2. The Halo-TSC2 is localized on lysosome in HeLa cells in nutrient replete condition.

(A) The Halo-TSC2 was localized with mPlum-LAMP1 in both WT and WDR24-KO HeLa cells. Scale bar: 10 μ m.

(B) WT and WDR24-KO HeLa cells have no significant difference in the correlation coefficient of Halo-TSC2 and mPlum-LAMP1. The Pearson correlation coefficient was calculated based on 30 cells per group. Error bars represent standard deviation. Unpaired Student's t-test was used to calculate p value.

A



B

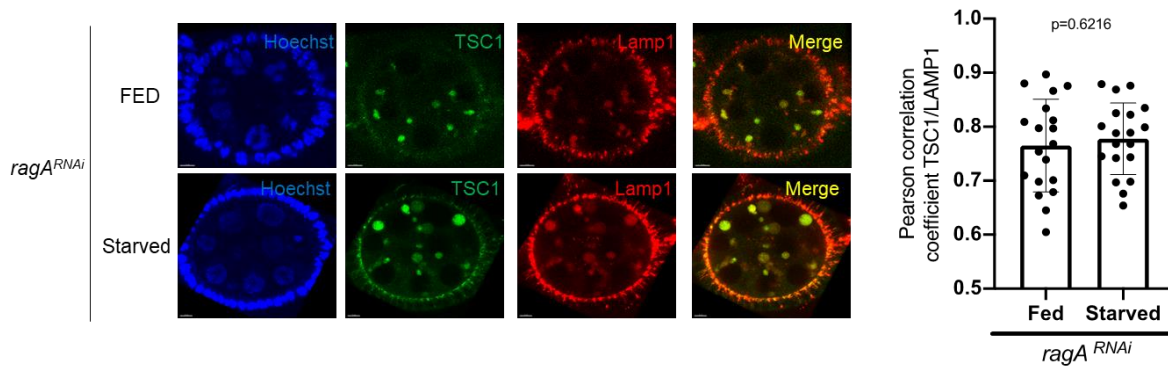


Figure S4: Related to Figure 2. The GFP tagged TSC1 is localized on lysosomes independent of RagA and nutrient conditions in *Drosophila* ovaries

(A) Immunofluorescence staining show TSC1-GFP co-localizes with lysosomal marker mCherry-Lamp1 in WT *Drosophila* ovaries in both fed and starvation conditions. Scale bar: 10 μ m. Error bars represent standard deviation. Unpaired Student's t-test was used to calculate p value.

(B) Immunofluorescence staining show TSC1-GFP co-localizes with mCherry-Lamp1 in *ragA*^{RNAi} *Drosophila* ovaries in both fed and starvation conditions. Scale bar: 10 μ m. Error bars represent standard deviation. Unpaired Student's t-test was used to calculate p value.

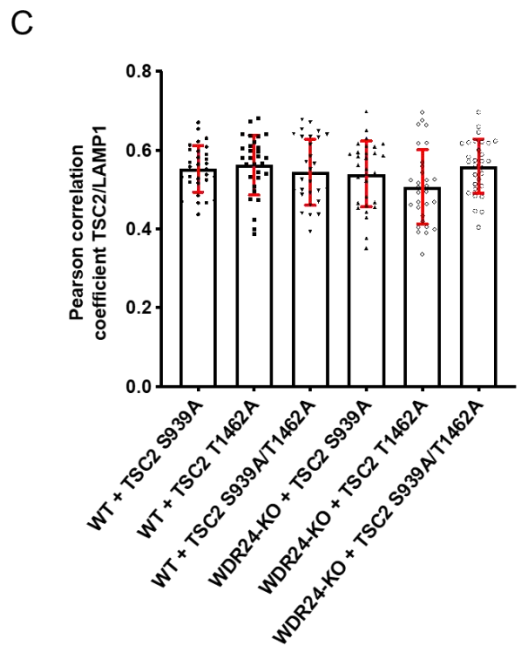
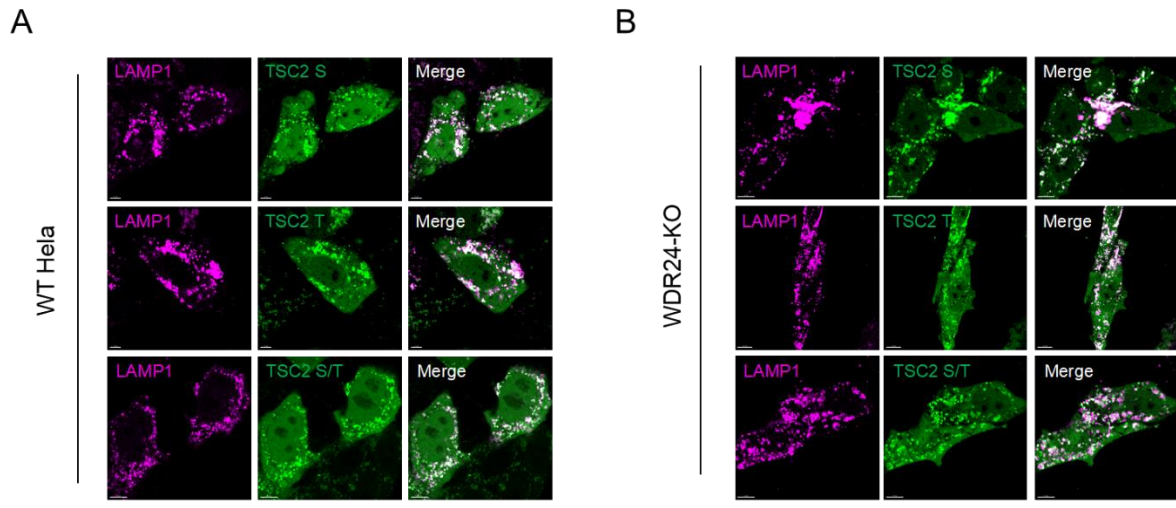


Figure S5: Related to Figure 7. The TSC2 phosphorylation-resistant point mutants localize to lysosomes in HeLa cells

(A) Halo-TSC2 T1462A, TSC2 S939A and TSC2 T1462A/S939A were colocalized with mPlum-LAMP1 in WT HeLa cells. Scale bar: 10 μ m.

(B) Halo-TSC2 triple mutant T1462A, S939A, T1462A/S939A protein colocalized with mPlum-LAMP1 in WDR24-KO HeLa cells. Scale bar: 10 μm .

(C) No significant differences were measured in the Halo-TSC2/mPlum-LAMP1 colocalization coefficient corresponding to the three TSC2 point mutants in WT and WDR24-KO HeLa cells. The Pearson correlation coefficient were calculated based on 30 cells per group. Error bars represent standard deviation.