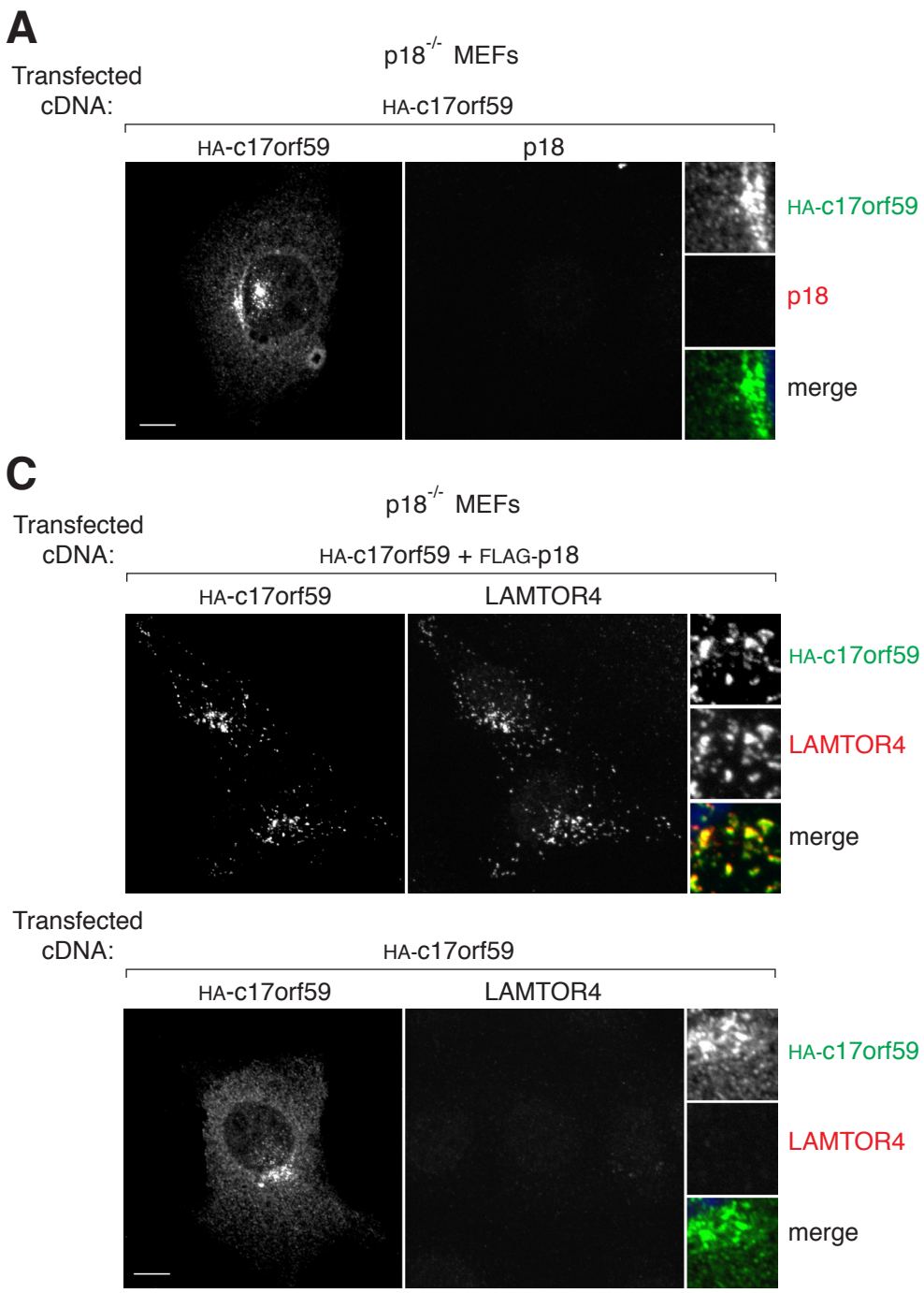
Cell Reports

Supplemental Information

Disruption of the Rag-Ragulator Complex by c17orf59 Inhibits mTORC1

Lawrence D. Schweitzer, William C. Comb, Liron Bar-Peled, and David M. Sabatini

Supplemental Figure 1 related to Figure 2: C17orf59 localizes to the lysosome with Ragulator



Supplemental Figure 1 related to Figure 2

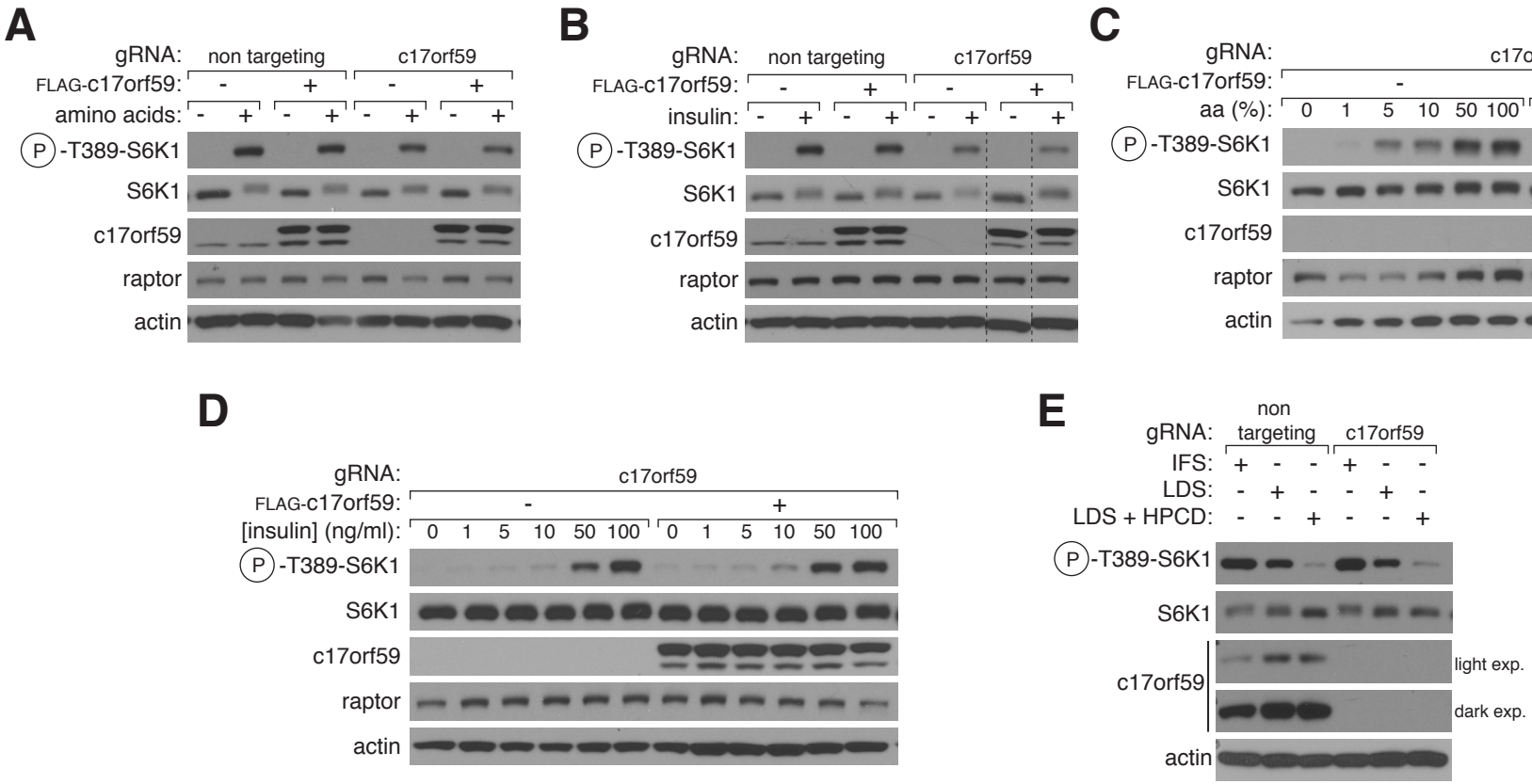
c17orf59 localizes to lysosomes with Ragulator

A) Recombinant c17orf59 localization in p18-null MEFs and validation of the anti-p18 antibody. p18-null MEFs were transfected with the HA-c17orf59 cDNA. Cells were immunostained with antibodies against the HA epitope tag (psuedocolored green) and against p18 (pseudocolored red) and the HA epitope tag (red), and imaged using confocal microscopy. Insets represent selected fields that have been magnified as well as the overlay of the fields. Scale bar represents 10 micrometers.

B) Recombinant c17orf59 has a highly significant correlation in signal distribution and intensity with p18. Correlation of p18 and c17orf59 signal distribution and intensity was compared between p18-null MEFs expressing the cDNA for HA-c17orf59 with or without expression of the cDNA coding for FLAG-p18. Pearson's correlation between HA-c17orf59 and p18 from 12 images in FLAG-p18 expressing cells and 6 images in null cells was quantified and statistical significance was calculated using Student's two-tailed t-test.

C) Recombinant c17orf59 co-localizes with LAMTOR4 in a p18-dependent manner. p18-null MEFs were transfected with the HA-c17orf59 cDNA alone (bottom panels) or with the FLAG-p18 cDNA (top panels). Cells were processed and imaged as in (A). Insets represent selected fields that have been magnified as well as the overlay of the fields. Scale bar represents 10 micrometers.

Supplemental Figure 2 related to Figure 3:
Loss of c17orf59 does not alter mTORC1 signaling in response to amino acids or insulin



Supplemental Figure 2 related to Figure 3

Loss of c17orf59 does not alter mTORC1 signaling in response to amino acids or insulin

A) c17orf59-null cells do not have alterations in amino acid-sensitive mTORC1 activity. c17orf59-null HeLa cells were generated by CRISPR/Cas9 technology. Cells were starved of amino acids for one hour and re-stimulated with amino acids for 10 minutes. Lysates were analyzed by immunoblotting for the indicated proteins.

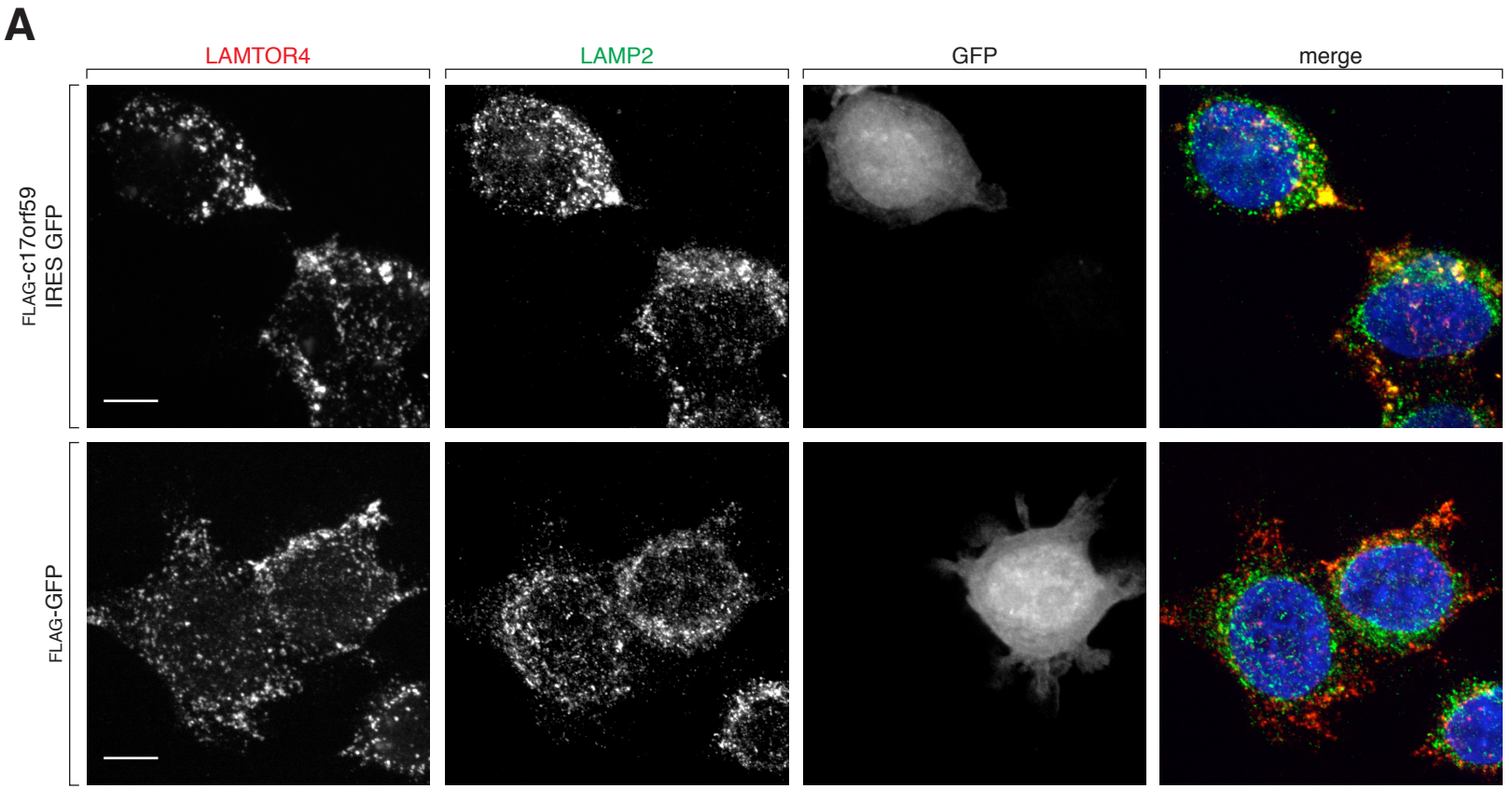
B) c17orf59-null cells do not have alterations in growth factor-senstive mTORC1 activity. c17orf59-null HeLa cells were starved of serum for three hours and re-stimulated with 100ng/ml insulin for 10 minutes. Lysates were analyzed as in (A).

C) c17orf59-null cells do not have altered mTORC1 activity in response to lower doses of amino acids. c17orf59-null HeLa cells were starved of amino acids for one hour and re-stimulated with the indicated dilution of all amino acids for 10 minutes. 100 percent amino acid stimulation represents the concentration in full RPMI and the concentration used in other experiments. Lysates were analyzed as in (A).

D) c17orf59-null cells do not have altered mTORC1 activity in response to lower doses of insulin. c17orf59-null HeLa cells were starved of serum for one hour and re-stimulated with the indicated concentration of insulin for 10 minutes. The highest dose (100ng/ml) represents that concentration of insulin used to stimulate cells in previous experiments. Lysates were analyzed as in (A).

E) c17orf59-null HeLa cells do not have alterations in mTORC1 signaling in response to cholesterol deprivation. c17orf59-nll HeLa were incubated in media containing 10% IFS (“IFS” lanes), 0.5% lipid-depleted serum overnight (“LDS” lanes), or 0.5% lipid-depleted serum overnight followed by 5 hours in 2% hydroxylpropyl-beta-cyclodextrin (“LDS + HPCD” lanes). Lysates were analyzed as in (A).

Supplemental Figure 3 related to Figure 4: c17orf59 disrupts the Rag-Ragulator interaction



Supplemental Figure 3 related to Figure 4

c17orf59 disrupts the Rag-Ragulator interaction

A) c17orf59 overexpression does not alter the localization of LAMTOR4. HEK-293T cells were transfected with 1μg of the cDNA encoding FLAG-c17orf59-IRES-GFP (top panels) or FLAG-GFP controls (bottom panels). Cells were starved of amino acids for one hour and re-stimulated with amino acids for 10 minutes, and immunostained with antibodies against LAMTOR4 (pseudocolored red) and the LAMP1 (green) and imaged for GFP (white) using confocal microscopy. GFP-positive cells (third column) represent transfected cells.