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# Supplemental Information

# A Genome-Wide siRNA Screen Reveals Multiple mTORC1 Independent Signaling Pathways Regulating Autophagy under Normal Nutritional Conditions

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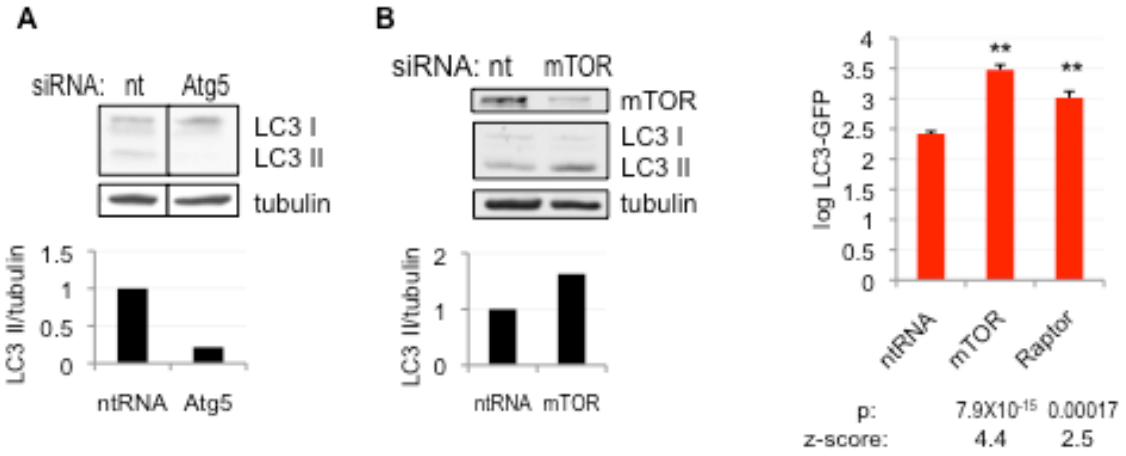


**Figure S1 (related to Figure 1). Confirmation of changes in autophagy levels following transfection of control siRNAs**

(**A-B**) H4 cells transfected with siRNA against Atg5 (**A**), mTOR (**B**) or non-targeting siRNA for 72 ours and treatment with 10μM E64d. Levels of autophagy were assessed by western blot with antibodies against LC3. Quantification of LC3 II to tubulin ratio is presented below.

(**C**) Quantification of LC3-GFP following transfection of H4 cells with non-targeting control siRNA or siRNA against mTOR or Raptor. ** p-values are based on two-tailed student t-test with equal variance. All error bars are s.e.m.

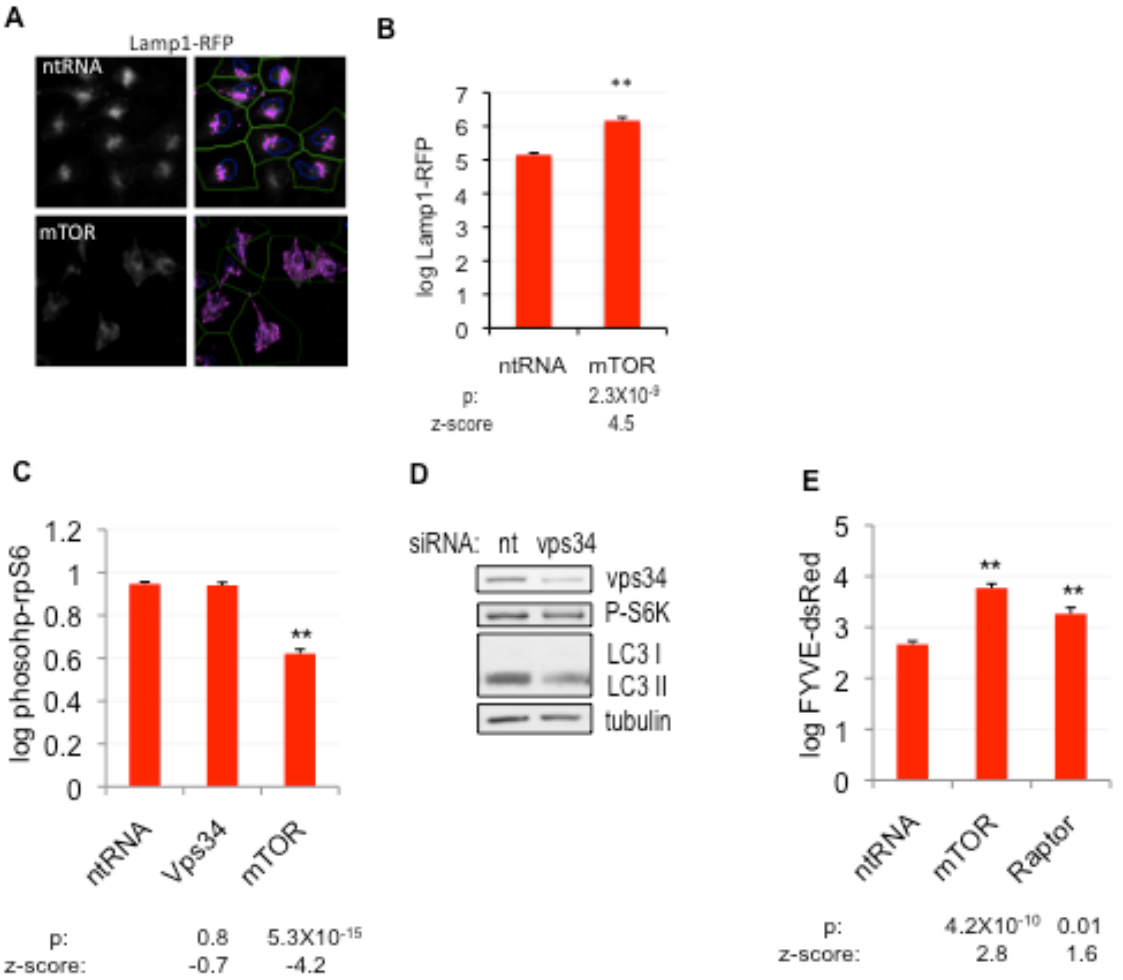


**Figure S2 (related to Figure 2). Controls for high-throughput characterization screens and assays**

**A-B**, Quantification of Lamp1-RFP to assess lysosomal function.

**A**, H4 cells stably expressing Lamp1-RFP were transfected with either non-targeting siRNA (ntRNA) or siRNA against mTOR. After 72 hours cells were fixed, counterstained with Hoechst and imaged on a high-throughput fluorescent microscope at 10X magnification, followed by quantification of Lamp-1-RFP levels. The right panels demonstrate results of image segmentation used for quantification: blue – nuclei, green – cell segmentation, pink – Lamp1-RFP.

**B**, Quantification of data from panel (**A**).

**C-D**, Type III PI3 kinase functions downstream of mTORC1

**C**, Quantification of in-cell-western assay for the function of mTORC1 following transfection of H4 cells with either non-targeting siRNA (ntRNA) or siRNA against Vps34 or mTOR. After 72 hours cells were fixed and stained with antibodies against phospho-rpS6.

**D**, Levels of autophagy and and activity of mTORC1 were assessed in H4 cells 48 hours after siRNA mediated knock-down of vps34, with antibodies against LC3 and phospho-S6 kinase, respectively. 10μg/mL E64d was added for 6 hours before cell harvest.

**E**, Effects of mTOR or Raptor knock-down on the levels of PtdIns3P as assessed by accumulation of vesicular FYVE-dsRed. All error bars are s.e.m. ** p-values are based on two-tailed student t-test with equal variance. n≥6

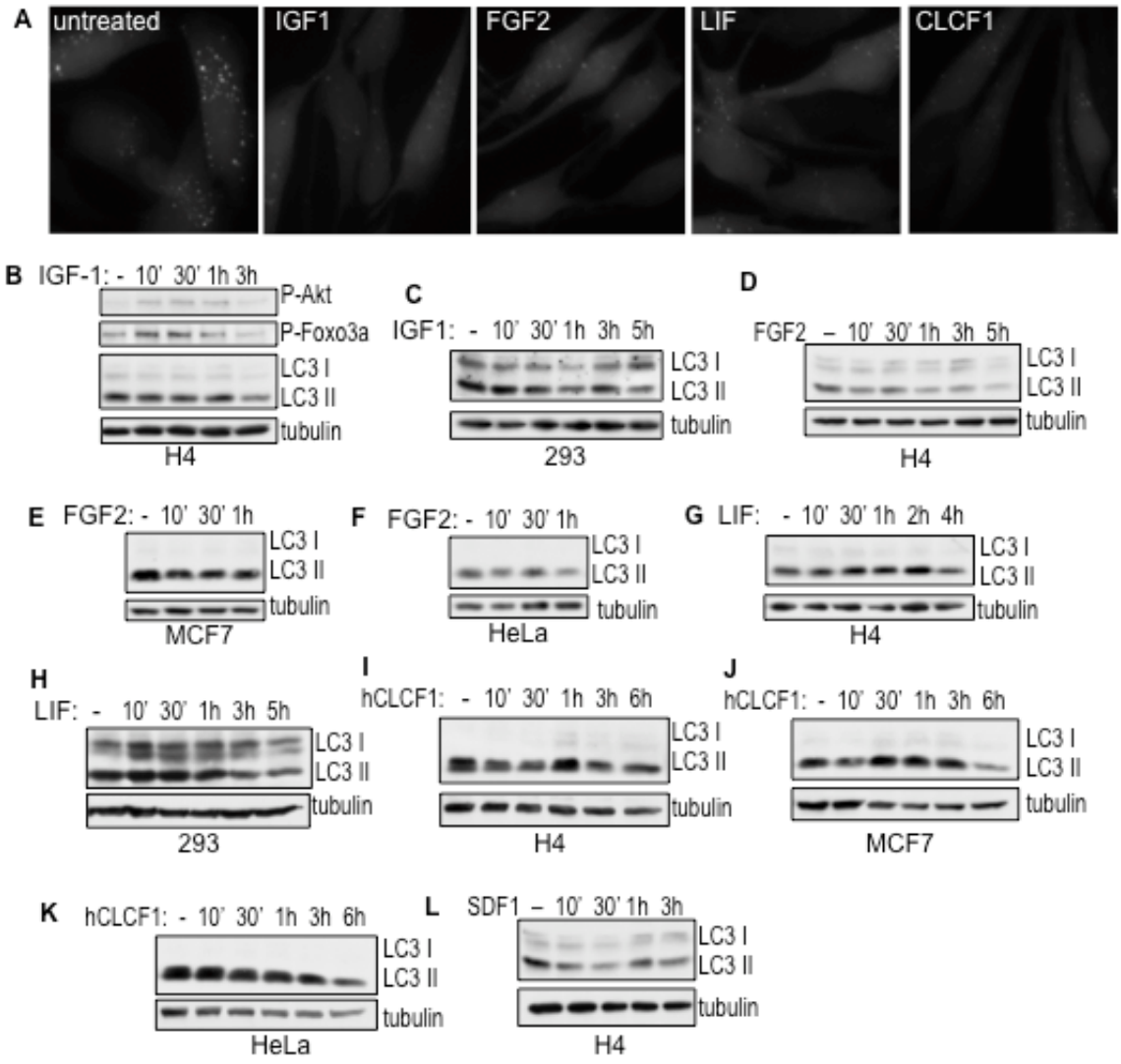


**Figure S3 (related to Figure 4). Cytokines identified in the screen negatively regulate autophagy**

**A,** H4 LC3-GFP cells were grown on cover slips in serum-free medium, followed by addition of 100ng/mL IGF1, 50ng/mL FGF2, 50ng/mL LIF or 50ng/mL CLCF1. After 24 hours cells were fixed, counterstained with Hoechst, mounted and imaged on a fluorescent microscope at 40X magnification.

**B-C**, IGF1 suppresses autophagy in H4 (**B**) and HEK293 (**C**) cells.

**D-F**, FGF2 treatment down-regulates autophagy in H4 (**D**), MCF7 (**E**) and HeLa (**F**) cells.

**G-H**, Suppression of autophagy by LIF in H4 (**G**) and HEK293 (**H**) cells.

**I-K**, CLCF1 down-regulates autophagy in H4 (**I**), MCF7 (**J**) and HeLa (**K**) cells.

**L**, Chemokine SDF1 suppresses autophagy in H4 cells. All cells were incubated in serum-free DMEM overnight, followed by addition of 10μg/mL E64d and treatment with 200ng/mL human IGF1, 50ng/mL FGF2, 50ng/mL LIF, 50ng/mL CLCF1 or 100ng/mL SDF1. Following lysis, levels of autophagy were assessed by western blot with antibodies against LC3.

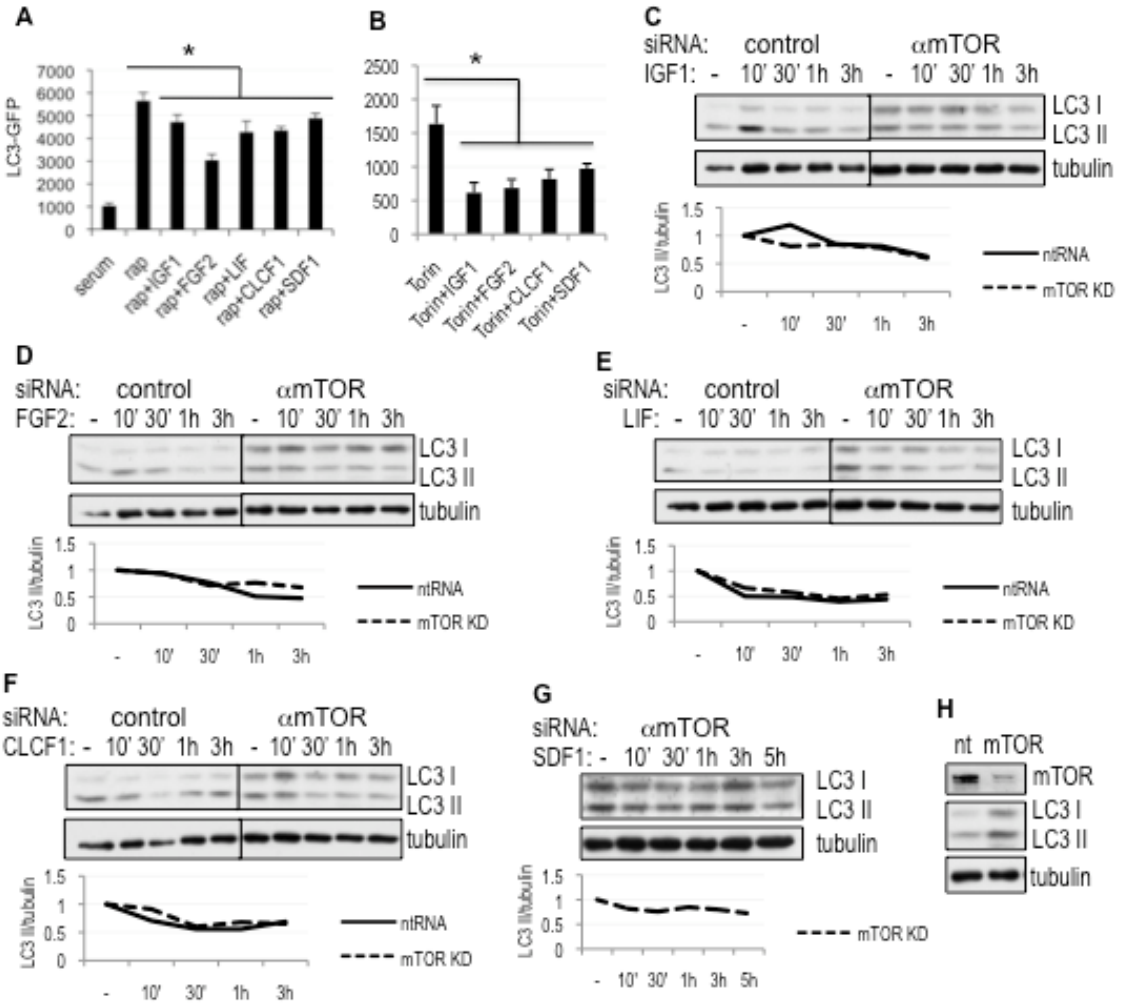


**Figure S4 (related to Figure 4). Cytokines suppress autophagy independently of mTOR**

**A-B**, Autophagy is suppressed by cytokines in the presence of rapamycin (**A**) or Torin 1 (**B**) in LC3-GFP H4 cells as assessed by LC3-GFP translocation to autophagosomes. Cells grown in serum-free media were treated with 50nM rapamycin or 50nM Torin 1 and cultured in the absence or presence of 100ng/mL human IGF1, 50ng/mL human FGF2, LIF, CLCF1 or SDF1 for 6 hours, followed by fixation and counterstaining with Hoechst. Cells were imaged at 10X of a high throughput automated microscope. All error bars are s.e.m. * $p<0.07$ based on two-tailed student t-test.

**C-G**, Down-regulation of autophagy by IGF1 (**C**), FGF2 (**D**), LIF E), CLCF1 (**F**) and SDF1 (**G**) in HEK293 transfected with siRNA against mTOR. 48h after transfection cells were incubated in serum-free DMEM overnight, followed by addition of 10μg/mL E64d and treatment with 200ng/mL human IGF1, 50ng/mL FGF2, 50ng/mL LIF, 50ng/mL CLCF1 or 100ng/mL SDF1. Following lysis, levels of autophagy were assessed by western blot with antibodies against LC3. Quantification of LC3 II to tubulin ratio is shown for each blot.

**H**, Confirmation of mTOR knock down 72 hours following siRNA transfection.

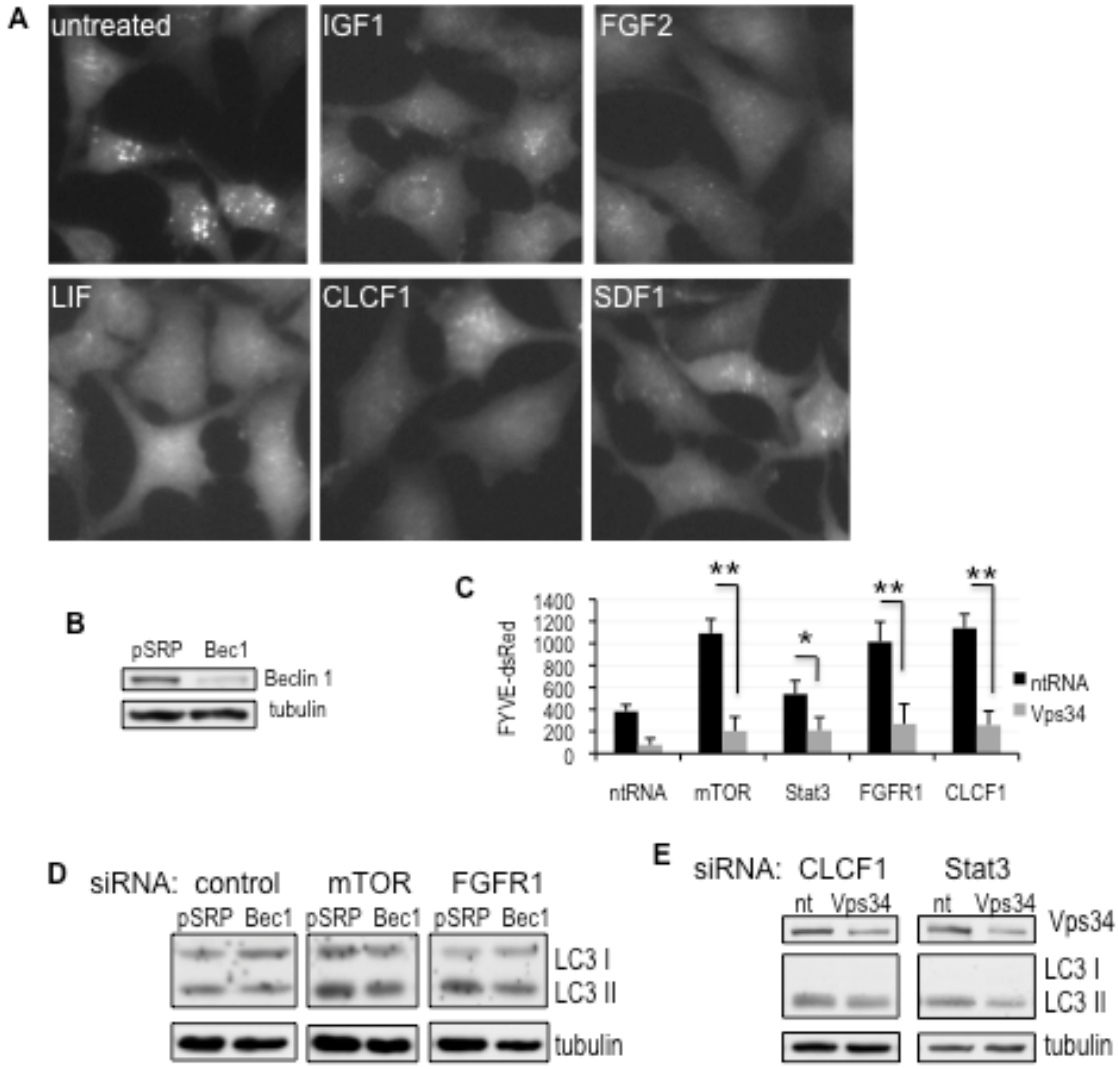


**Figure S5 (related to Figure 6). Function of the type III PI3 kinase in mediation of autophagy downstream of cytokine signaling**

**A**, H4 FYVE-dsRed cells were grown in serum-free medium, followed by addition of 100ng/mL IGF1, 50ng/mL FGF2, 50ng/mL LIF, 50ng/mL CLCF1 or 100ng/mL SDF1. After 24 hours cells were fixed, counterstained with Hoechst, mounted and imaged on a high-throughput microscope at 10X magnification.

**B**, Confirmation of beclin 1 knock-down in H4 LC3-GFP cells stably transfected with pSRP-Beclin 1 (Bec1) or empty vector control (pSRP).

**C**, Increase in PtdIns3P levels following knock down of mTOR, Stat3, FGFR1 or CLCF1 is attenuated in cells deficient for Vps34. H4 FYVE-dsRed cells were transfected with siRNA against Vps34 or non-targeting control siRNA. After 24 hours cells were re-transfected with indicated siRNAs for 48 hours, followed by fixation and imaging. n=10, *$p<0.05$, **$p<0.01$ All error bars are s.e.m.

**D-E**, Knock-down of the type III PI3 kinase components, Beclin 1 (**D**) or Vps34 (**E**) attenuated induction of autophagy following knock-down of mTOR, FGFR1, CLCF1 or Stat3 in H4 cells.