

**Experimental Procedures for Supplementary material****siRNA transfection**

SMART pool siRNA was obtained from Dharmacon for the human homologs of *Atg6/Beclin1* (NM\_003766), *mTOR* (NM\_004958), *p70S6 kinase polypeptide 1* (NM\_003161), and *p70S6 kinase polypeptide 2* (NM\_003952). The siRNA sequence for *hVps34* (ACU CAA CAC UGG CUA AUU A within the sense strand of NM\_002647) was provided by J. Backer (Albert Einstein College of Medicine, New York, USA). Negative control siRNA was the siControl non-targeting pool (Dharmacon) and transfections were performed using Oligofectamine reagent as described in main text.

**Confirmation of Knockdown by RT-PCR and immunoblotting**

Knockdown of mTOR or p70S6 kinase was confirmed by immunoblotting cell samples with anti-mTOR (Cell signaling technologies #2972) or anti-pan-p70S6 kinase (Cell signaling technologies #9202) antibodies. Anti-beclin monoclonal antibody was from BD Transduction Labs. The polyclonal antibody for hVps34 was provided by A. Wandinger-Ness (Univ. New Mexico, USA).

**Legends to Supplementary Figures****Fig S1. Representative examples of raw immunoblot data from screen that include the candidate *ULK1*.**

Each siRNA in the library was analysed with duplicate cell samples that were resolved on 2 separate SDS-10% polyacrylamide gels. GFP-LC3 was detected after transfer to PVDF membranes using anti-GFP antibody and a secondary antibody coupled to an infra-red chromophore. A) Scan of the immunoblot showing 14 representative siRNA library members and the internal controls that were included on every gel. Quantification of band intensity was accomplished using a standardized-size box method that included a background correction step. Below the blot is the quantification of GFP-LC3 conversion =  $(\text{GFP-LC3-II}) / (\text{GFP-LC3-I} + \text{GFP-LC3-II})$ . B) Data for the same 14 library members in the replicate analysis showing general correlation of GFP-LC3 conversion data. siRNA “3E” represents the candidate *ULK1* that was analysed further.

**Fig S2. Normalisation procedure of primary screen data.**

The human kinome library of 753 genes was divided into ten 96-well format plates. Each plate (containing 75-80 genes) was analysed on two 48 well format SDS-PAGE gels (1st and 2nd halves). Since samples were analysed in two replicates (A and B), the total data of each plate can be divided into 4 groups, each corresponding to a discrete SDS-PAGE gel. Data from each immunoblot group have been color coordinated (as indicated in the legend) and positioned along the X axis of the plot. The raw experimental GFP-LC3 ratio values for cells which were transfected with the siRNA library members and then starved are plotted on the Y axis as shown in (c). Each immunoblot also included as internal controls samples from cells transfected

with siRNA towards *Atg7* or negative-control scrambled siRNA (SCR), which were then amino acid starved. These internal control data points are shown in (a) and (b) at the same position on the X axis as its corresponding set of experimental data.

In the raw data, there is variability between the groups that can be attributed most likely to immunoblotting. To correct for this, each ratio value within a group (both experimental and control) was adjusted by a constant value so that the median experimental value was equivalent across all groups (as shown in (d), (e) and (f)).

**Fig S3. Knockdown of the hVps34 class III PI3 kinase does not robustly modulate GFP-LC3 conversion.**

Upper panel: 293/GFP-LC3 cells were transfected with siRNA targeting *Atg7*, *Beclin*, or *hVps34* for 72 hours. Cells were then starved in EBSS/leupeptin for 2 hrs as indicated and lysed for analysis by SDS-PAGE. Conversion of GFP-LC3 was only modestly inhibited by knockdown of beclin or hVps34, and knockdown of beclin and hVps34 in combination did not lead to a cooperative effect. In contrast, inclusion of Wortmannin (100nM) into the starvation medium on mock transfected cells completely blocked GFP-LC3 conversion.

Lower panels: The same cells samples were immunoblotted with anti-Beclin, anti-hVps34, and anti-actin antibodies to monitor the efficiency of knockdown.

**Fig S4. Modulation of GFP-LC3 conversion by *p70S6 kinase* knockdown.**

Top panels: 293/GFP-LC3 cells were transfected, as indicated, with siRNA pools targeting *Atg7*, *mTOR*, *p70S6 kinase1* (also called *RPS6KB1*), *p70S6 kinase2* (also

called *RPS6KB2*), or both *B1+B2*, for 72 hrs. Cells were then starved in EBSS/leupeptin for 2 hrs as indicated, lysed, and analysed by SDS-PAGE. (\*) P<0.06 significant difference as compared to mock transfected starved cells. Bottom panels: Knockdown of *mTOR* or *p70S6 kinase* was confirmed by immunoblotting.

**Fig S5. Sequences of individual siRNA duplexes for the candidates that pass confirmatory criteria.**

Given are the 19 base sequences (D1-D4 as described in Fig 2 of main research paper) corresponding to the sense strand of the candidate genes.

**Fig S6. Highly expressed MYC-ULK1 localizes to large and irregular structures.**

a) 293/GFP-LC3 cells were transfected with MYC-ULK1 using FUGENE 6 reagent and then starved in EBSS/leupeptin for 2hrs before fixation. Starvation results in formation of many GFP-LC3-positive autophagosomes (left panel). This transfection protocol results in a range of MYC-ULK1 expression levels (right panel) (I - cell with intermediate expression; L - low expression; U - untransfected cell).

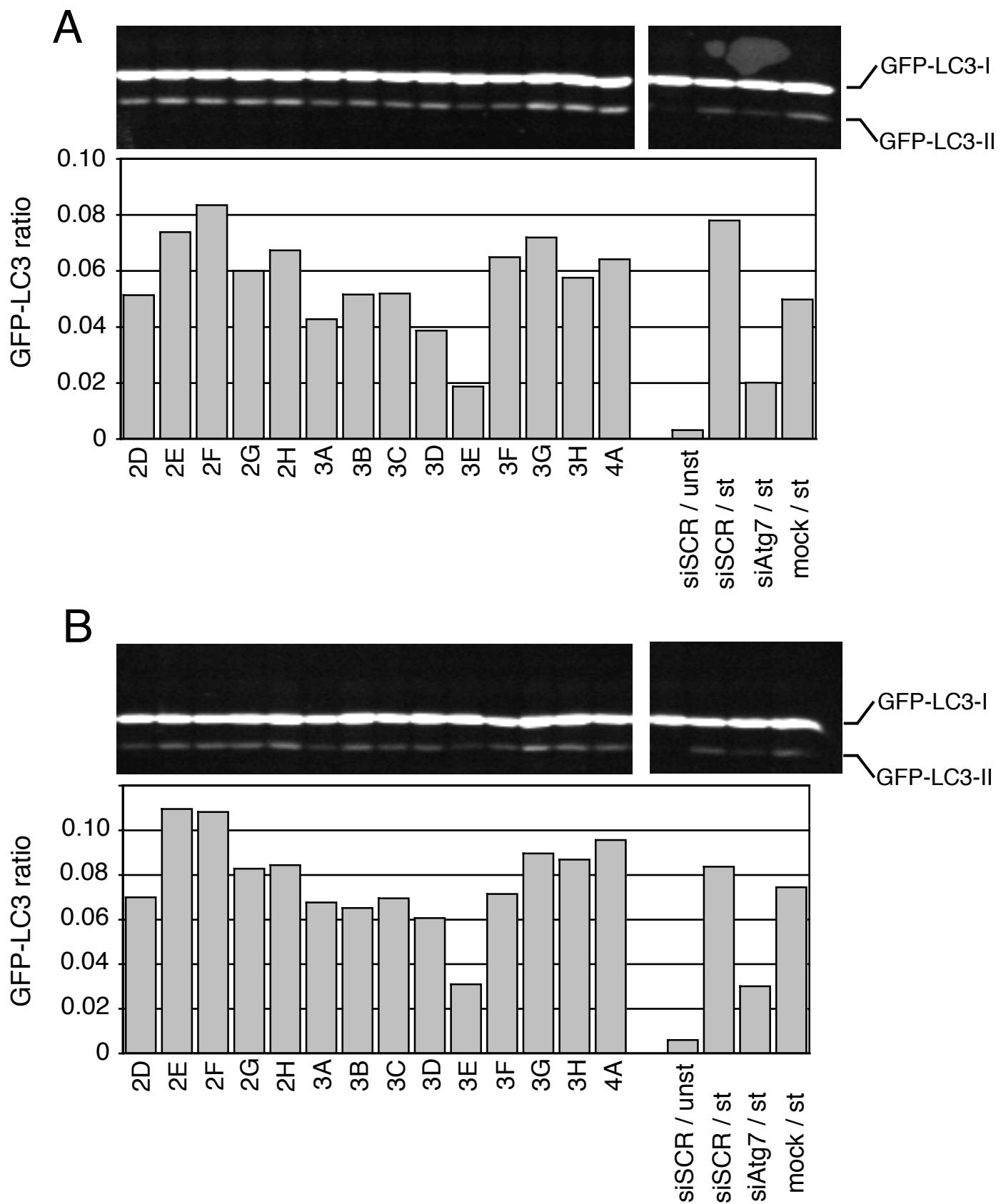
b) MYC-ULK1 expressed at intermediate levels in starved cells frequently localizes to irregularly-shaped cytoplasmic structures that co-localize with GFP-LC3 (arrows).

Bar = 10um.

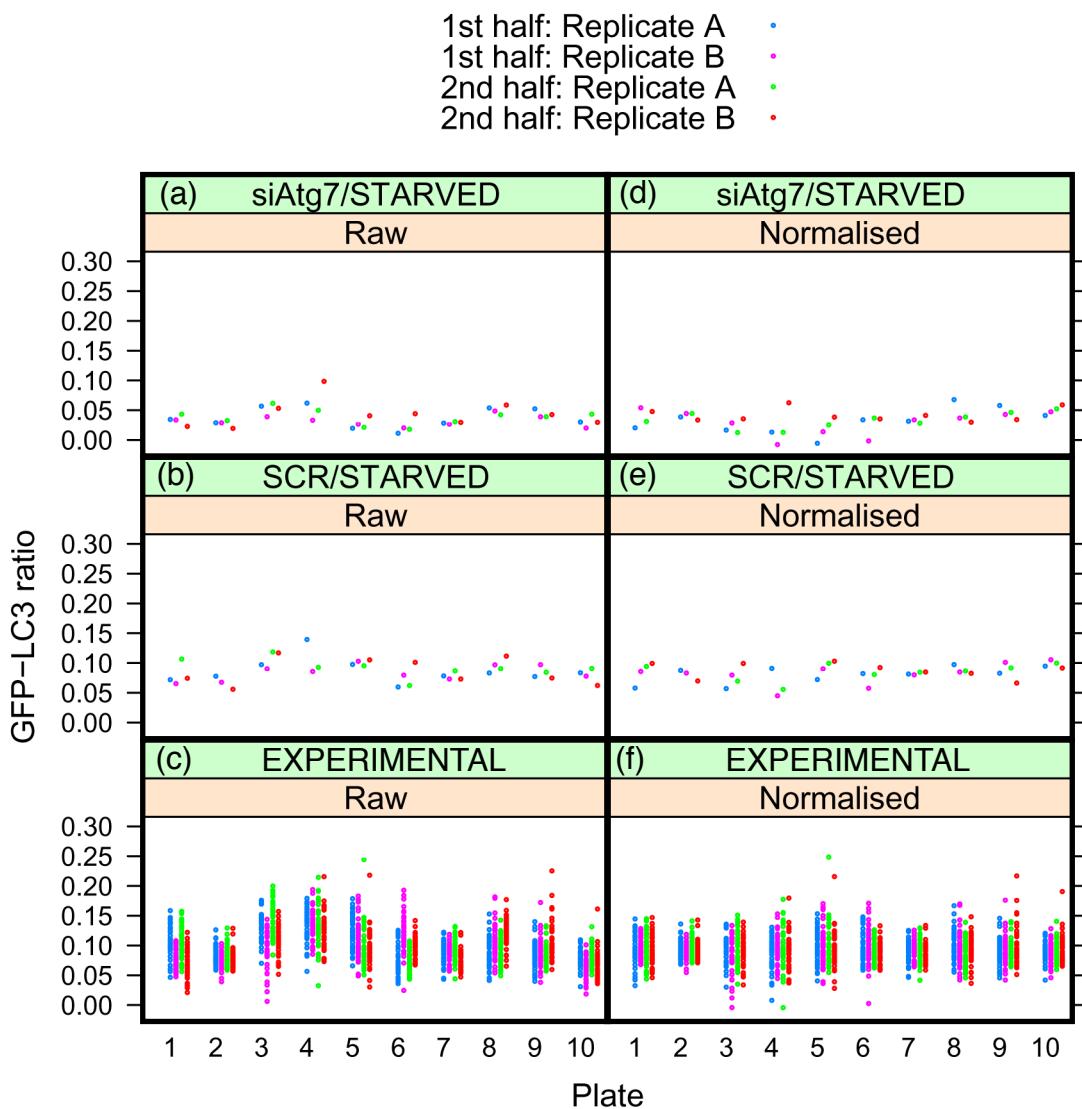
**Table S1. Lower stringency list of candidate genes showing significance of P<0.08.**

Treatment of 293/GFP-LC3 cells with siRNAs for these genes caused a decreased or increased response to amino acid starvation as measured by the GFP-LC3 conversion ratio. We also calculated the normalized (Norm) GFP-LC3 ratio that indicates fold-change relative to the average GFP-LC3 ratio observed over the entire library (set at 1.0). For each candidate siRNA, we list the genbank accession number, gene symbol and description. The subset of genes with P<0.06 chosen for further confirmatory experiments are indicated in bold.

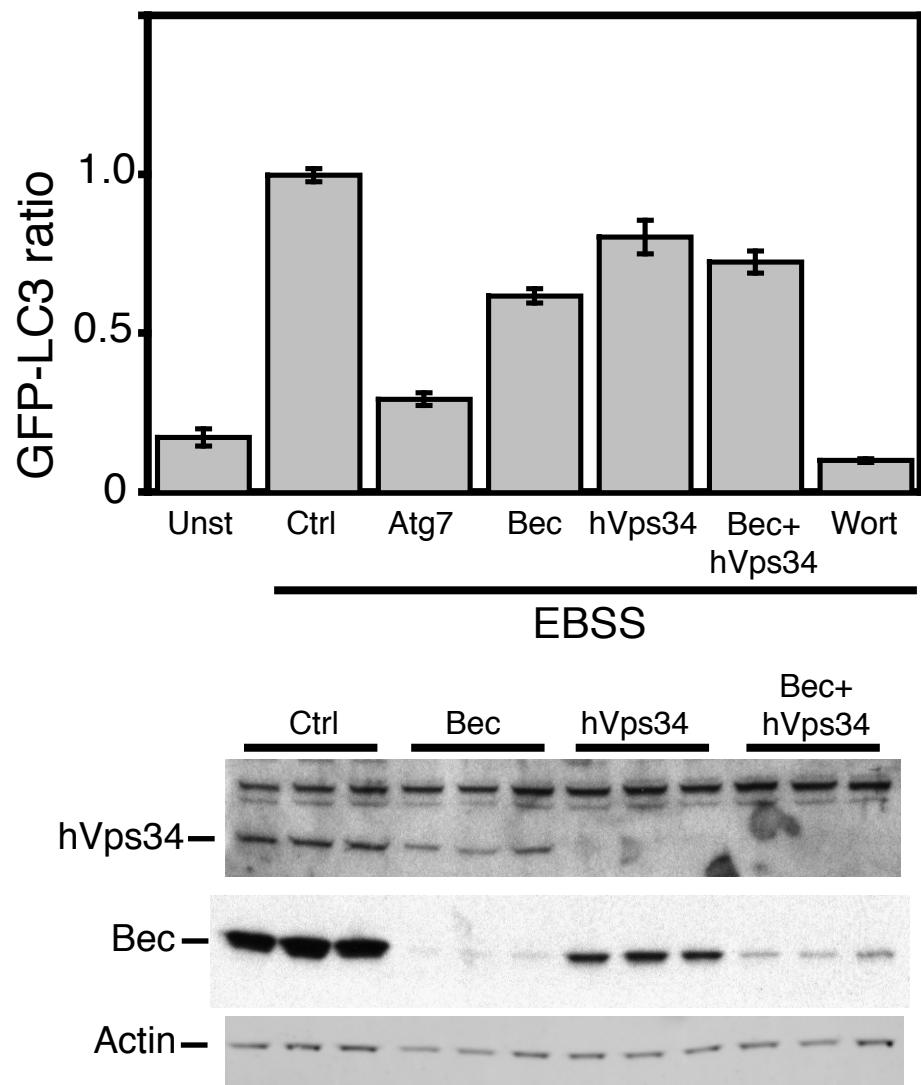
**Table S2. List of all 753 genes analysed in the human kinome as a Microsoft Excel document.**



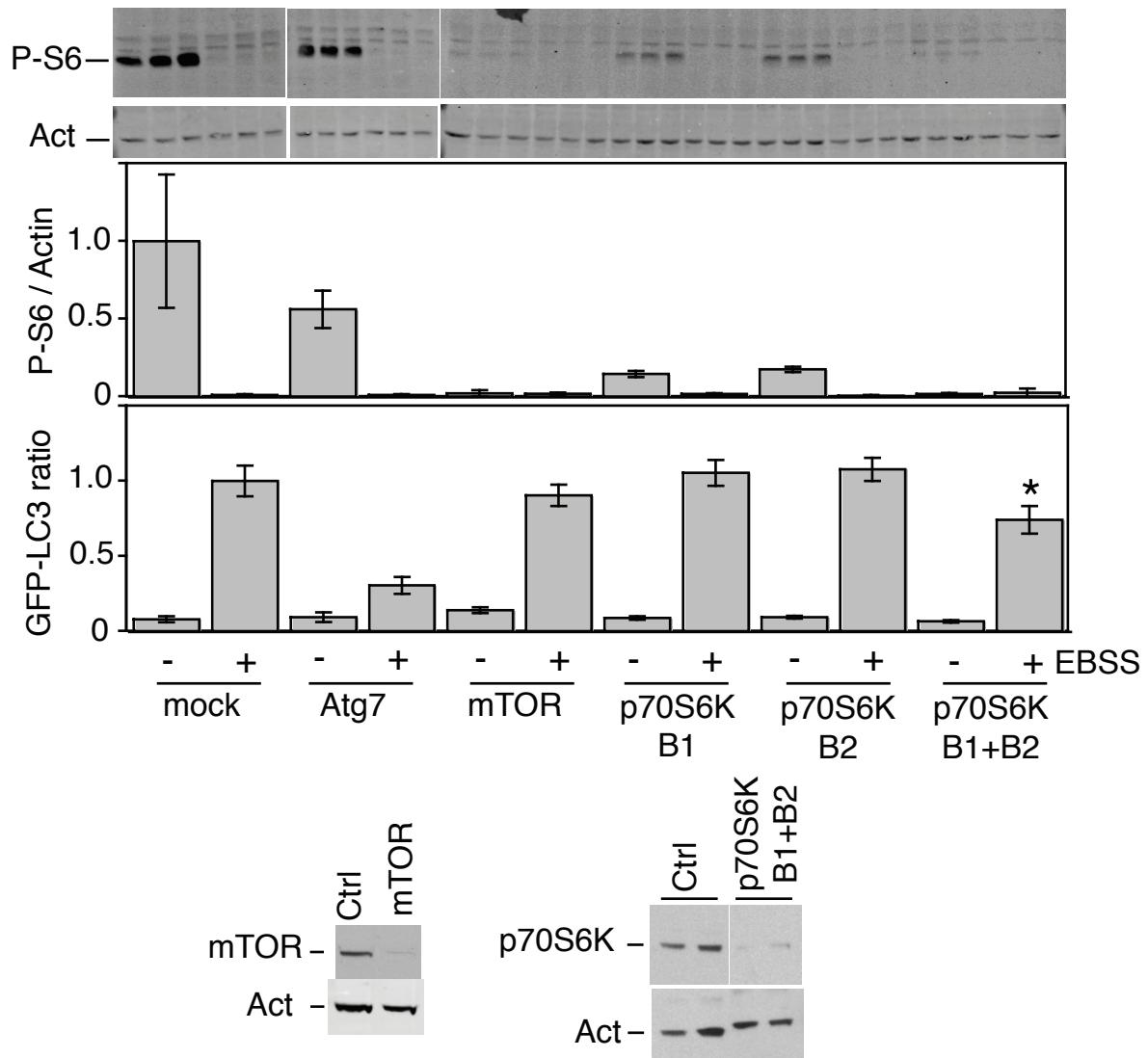
Chan et al, Figure S1



Chan et al, Figure S2



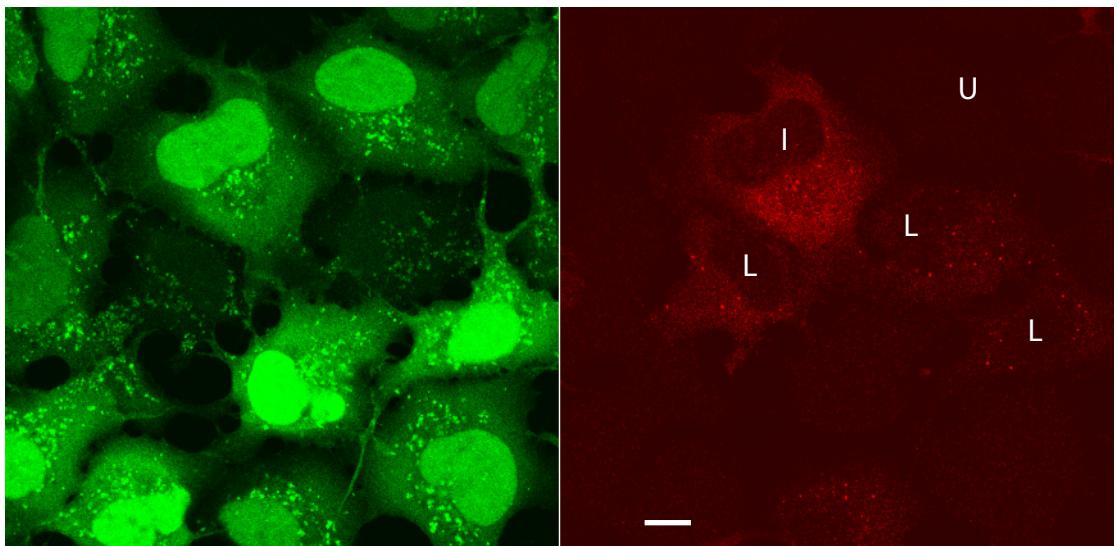
Chan et al, Figure S3



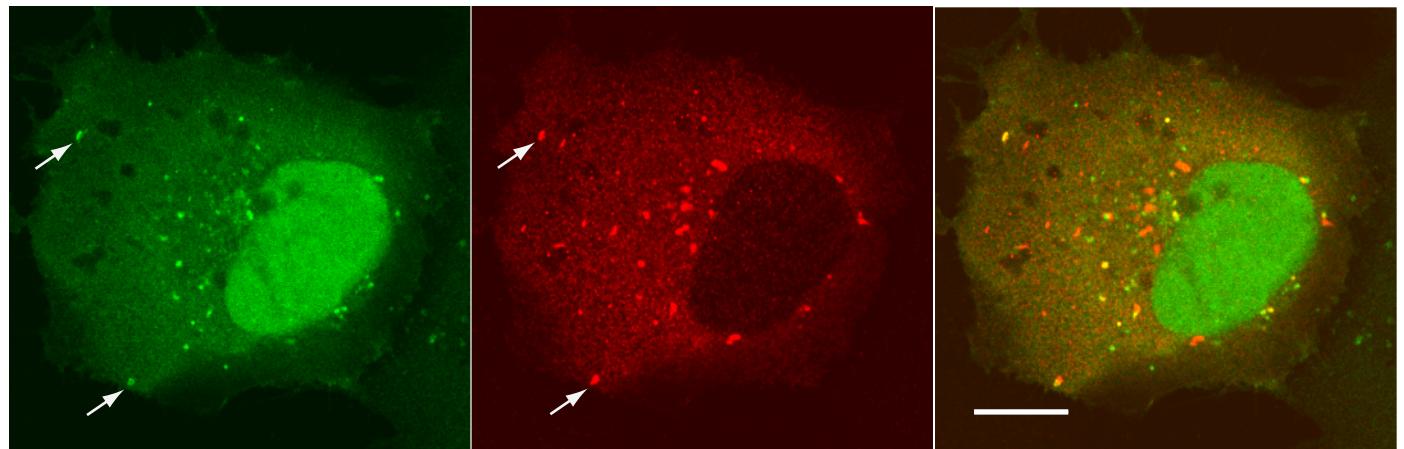
Chan et al, Figure S4



A



B



Chan et al, Figure S6