SUPPLEMENTAL INFORMATION

S1: Homotypic fusion between UBQLN-containing cytoplasmic structures.

Time-lapse microscopy was performed on cells transfected with GFP-UBQLN-2. Fusion events between UBQLN-positive structures were captured (frames). The sequence was acquired through IPLab on a Nikon TE300 microscope with a Nikon S Fluor 40x/0.90 objective. The incubation chamber was kept at 37ºC during the entire recording time. Frames were taken at 5-second intervals. Movie represents 15 minutes of "real time" (150x actual speed).

S2: Co-immunostaining of UBQLN-2 with endosomal markers.

HeLa cells expressing myc- or GFP- tagged UBQLN-2 were stained for EEA1 (marker for early endosomes), Rab7 or Lamp-1 (markers for late endosomes). Dual staining of UBQLN with each protein was analyzed by microscopy. Scale bar: 10 microns.

S3: Deletion mutagenesis of UBQLN-2 and its effect on colocalization with LC3.

Myc-tagged UBQLN-2 ΔUbL and UBQLN-2 ΔUBA deletion mutants were generated and their immunolocalization analyzed in cells co-expressing GFP-LC3.

S4: Recruitment of UBQLN around cytoplasmic aggregates.

Myc-tagged UBQLN (1or 2) was transfected in HeLa cells together with GFP-Htt72 (top and middle row) or GFP-250 (bottom row). Anti myc immunostaining shows UBQLN concentrated around aggregates formed by Huntingtin and GFP-250.

S5: Cell size and granularity of control and UBQLN-KD cells during starvation.

Control and UBQLN-KD cells were transferred to starvation medium, fixed, permeabilized with 70% cold ethanol, and stained with propidium iodide. Cells in G0-G1 were analyzed by flow cytometry for their size (reflected in the forward scatter, or FSC) and their granularity (measured by the side scatter, or SSC). Control and UBQLN-KD cells show comparable cell size (A). However, UBQLN-KD cells show a higher side scatter under starvation compared to controls (B), indicating their higher granularity. This is consistent with a lack of decrease in organelle content during autophagy, and with impaired autophagosomal maturation.

S6: Dual labeling of mitochondria with Mitotracker™ and pshooter™.

HeLa cells transfected with pshooter™ (a GFP cDNA cloned downstream of a mitochondrial promoter) were stained with Mitotracker™, and the localization of each mitochondrial probe analyzed by microscopy.

S7: Supplemental materials

1 - Antibodies and reagents

The following antibodies were used: myc (clone 9E10, Upstate Biotechnology), EEA1 (BD Biosciences), Lamp-1a (clone H4A3, ATCC), Atg7 (Santa Cruz Biotech), PLIC-1 (UBQLN-1) and Atg12 (Zymed), LC3 (clone 5F10, Axxora). UBQLN-2 antibody was previously described (Wu et *al*, 1999). Mitrotracker™ Red, pShooter™ (pEF/myc/mito/GFP) vector, Alexa- coupled secondary antibodies and Prolong mounting medium were from Invitrogen (Carlsbad, CA). Ubiquilin (PLIC) siRNAs were

previously described (N'Diaye E *et al*, 2008). Non-silencing siRNA and Atg5 siRNAs were from Dharmacon.

Lentiviral particles expressing shRNAs against Atg7 (target sequence GCCTGCTGAGGAGCTCTCC) were previously described (Fung *et al*, 2007). HeLa cells stably expressing a non-silencing shRNA or Atg7 shRNA were maintained under puromycin selection (2 μg/ml).

2 - DNA constructs

GFP-rab7 (Q67L) was from Ph. Stahl (Washington U.). GFP-250 was provided by E. Sztul (U. Alabama, Birmingham). GFP-Htt72 was from S. Finkbeiner (Gladstone Institute). LC3 cDNA (IMAGE 6185847) was inserted into the pEGFP-C3 vector (Clontech). Nucleotide sequencing was performed to verify the absence of errors. GFP- and myc-tagged UBQLN (PLIC) cDNAs were previously described (N'Diaye E *et al*, 2008; Wu *et al*, 1999). The GFP/mcherry/LC3 cDNA originally obtained from T. Johanssen (U. of Tromsø, Norway) (Pankiv et al., 2007) was transferred into the pBabe vector.

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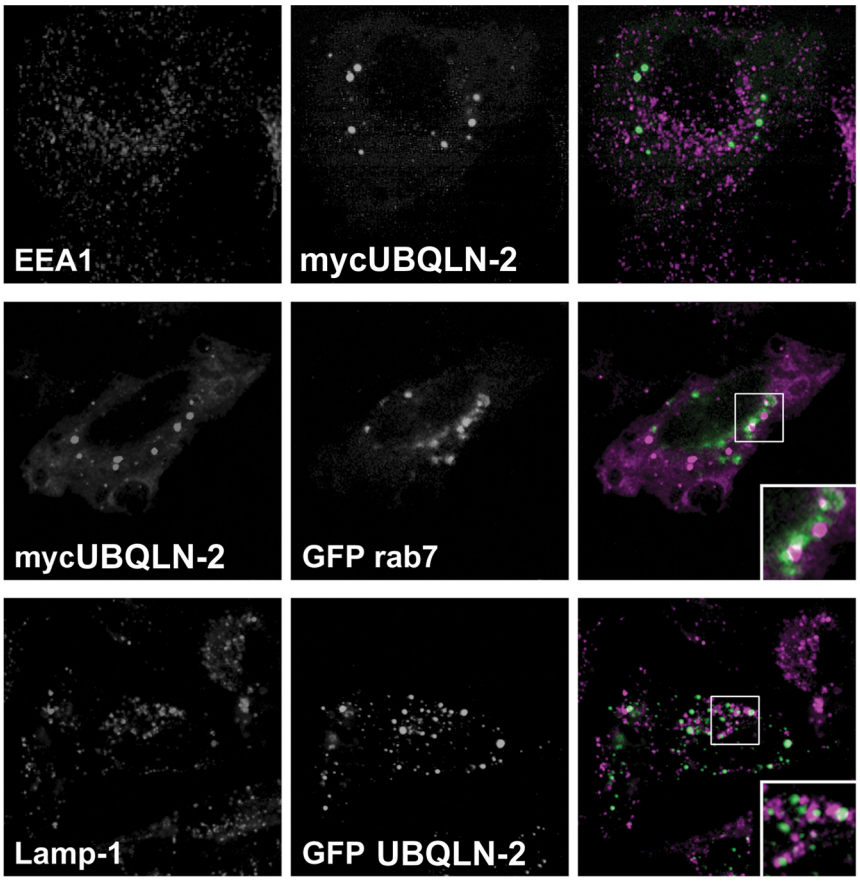
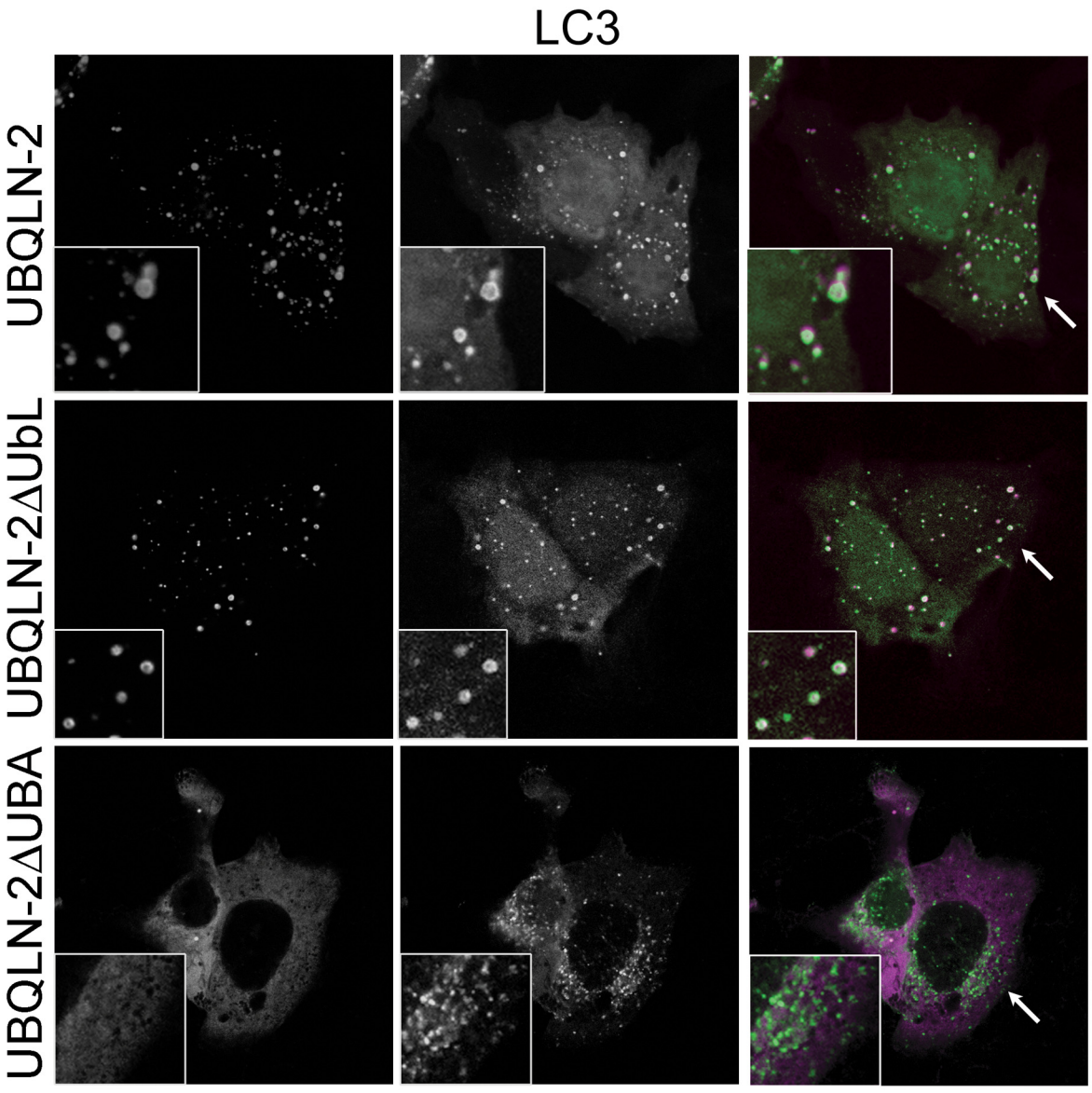


N'Diaye et al, Suppl. S3



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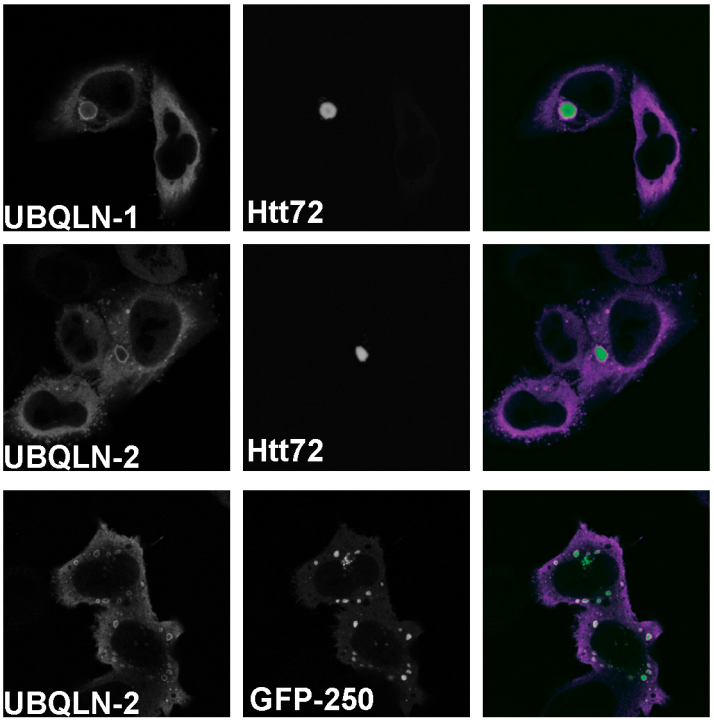

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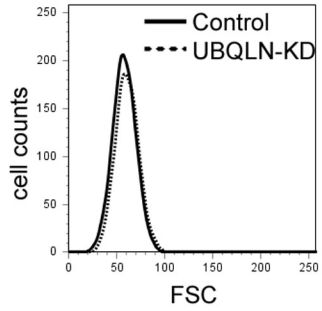
A



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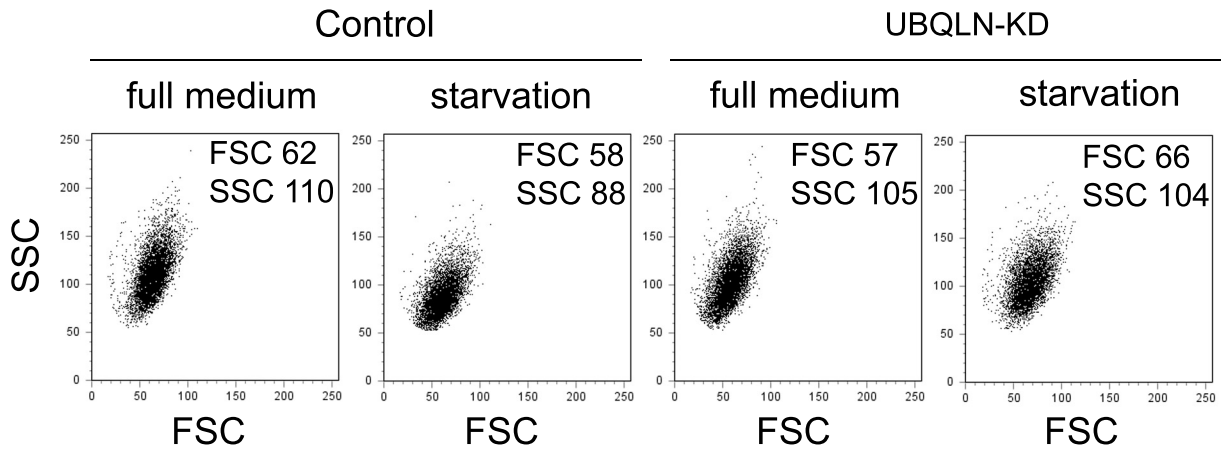

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