Fig S1 – Yeast interolog network centered on autophagy proteins

(A) First-order network generated using interaction data of yeast proteins (light-beige circles). Blue circles represent a subset of the yeast proteins that could be mapped to human orthologs. Orange squares denote autophagy proteins conserved between human and yeast. (B) Extension of the yeast network showing both first- and second-order interactions between yeast proteins mapped onto human orthologs (blue circles). Orthologs associated with the cytoskeleton (in the 'FNBP1L' sub-network cluster) or components of proteosomes are represented by golden-yellow or light-blue circles respectively. Autophagy proteins are denoted by orange squares.

Fig S2 – FNBP1L interacts with ATG3 directly and siRNA-mediated knockdown of FNBP1L has no effect upon autophagy induced by classical stimuli

(A) To confirm the FNBP1L/ATG3 interaction full-length FNBP1L-Myc and human ATG3-flag, or appropriate control vectors were co-expressed in HEK293T cells and subjected to flag pull-down, followed by anti-Myc blotting. In this reciprocal i.p. FNBP1L was immunoprecipitated by ATG3, but not the control vector. (B) To demonstrate that ATG3 interacted directly with FNBPL1 and not via either ATG12 or ATG5, we attempted to co-i.p. full-length FNBP1L-myc with flag-tagged ATG3, 5 12 and 16L1. Only ATG3 was able to co-i.p. FNBP1L, establishing that the FNBP1L/ATG3 interaction is not mediated via any of the other ATG proteins tested. (C) Following 48 hours of siRNA knockdown, HeLa cells stably expressing LC3-GFP were subjected to 2 hours of serum starvation in the presence of 50 mM ammonium chloride (to block lysosomal vesicle fusion). Control-incubated cells (control panels are repeated from

Figure 3C for comparison), showed little LC3-positive vesicle accumulation (green in merge). However, ammonium chloride-treated cells accumulated LC3-positive vesicles and aggregates regardless of siRNA treatment. Actin cytoskeletal morphology appeared normal in all cases (actin is shown in red in merged panels). Images are projections of confocal z-stacks and the scale bar represents 10 μm.

Fig S3 - Anti-*Salmonella* autophagosomes in cdc42-deficient HeLa cells.

(A) Cdc42-targeted siRNA yields robust knockdown in HeLa cells.

HeLa cells were transfected with control or cdc42-targeted siRNA duplexes, 48 hours later cells were harvested and examined by quantitative RT-PCR. Mean cdc42 mRNA abundance, normalised to GAPDH control reactions, are shown (+/- s.e.m), p-values were calculated using a two-tailed Student's T-test, comparing to siControl values, none reached significance. Data was calculated from two separate wells for each condition, with PCR reactions performed in triplicate. A single experiment, representative of two separate experiments, is shown. (B) Cdc42 is not essential for anti-*Salmonella* autophagy in HeLa cells. Control or cdc42-deficient HeLa LC3-GFP cells were infected with *S.* Typhimurium 48 hours following transfection. After one hour of infection, cells were fixed and mounted and the percentage of internalized bacteria enclosed within autophagosomes calculated. No significant differences between conditions were observed. At least 50 cells per condition were used to count total internalized SL1344 and LC3-GFP+ SL1344. Data is shown as means, +/- s.e.m, significance was assessed using a two-tailed Student's T-test. Representative of two independent experiments. (C) Anti-*Salmonella* autophagosomes in cdc42-deficient HeLa cells. High-magnification

crops of infected control- and cdc42-directed siRNA transfected HeLa LC3-GFP cells are shown. LC3 (green in merge) and *S.* Typhimurium (red in merge) are shown. All images were taken one hour post-infection, following fixation as described. Images are maximum projections from high-resolution z-stacks, scale bars represent 10 μm.

Supplementary Table 1 – Annotation of potential ancillary factors of autophagy (AFA)

This table summarizes cellular localization, function and pathway information about potential ancillary factors of autophagy (AFA) identified from yeast-human interolog mapping and protein network extensions from two-hybrid screen data. The various information was extracted and integrated bioinformatically from a number of knowledge databases including KEGG (1), Panther (2), Gene Ontology (3), NCBI (4) and HGNC (5).

References:

1. Kanehisa, M., M. Araki, S. Goto, M. Hattori, M. Hirakawa, M. Itoh, T. Katayama, S. Kawashima, S. Okuda, T. Tokimatsu, and Y. Yamanishi. 2008. KEGG for linking genomes to life and the environment. *Nucleic Acids Res* 36:D480-484.
2. Mi, H., N. Guo, A. Kejariwal, and P. D. Thomas. 2007. PANTHER version 6: protein sequence and function evolution data with expanded representation of biological pathways. *Nucleic Acids Res* 35:D247-252.
3. Gene Ontology Consortium. 2008. The Gene Ontology project in 2008. *Nucleic Acids Res* 36:D440-444.
4. Wheeler, D. L., T. Barrett, D. A. Benson, S. H. Bryant, K. Canese, V. Chetvernin, D. M. Church, M. Dicuccio, R. Edgar, S. Federhen, M. Feolo, L. Y. Geer, W. Helmberg, Y. Kapustin, O. Khovayko, D. Landsman, D. J. Lipman, T. L. Madden, D. R. Maglott, V. Miller, J. Ostell, K. D. Pruitt, G. D. Schuler, M. Shumway, E. Sequeira, S. T. Sherry, K. Sirotkin, A. Souvorov, G. Starchenko, R. L. Tatusov, T. A. Tatusova, L. Wagner, and E. Yaschenko. 2008. Database resources of the National Center for Biotechnology Information. *Nucleic Acids Res* 36:D13-21.

5. Bruford, E. A., M. J. Lush, M. W. Wright, T. P. Sneddon, S. Povey, and E. Birney. 2008. The HGNC Database in 2008: a resource for the human genome. *Nucleic Acids Res* 36:D445-448.