Supplemental Material for

The third coiled coil domain of Atg11 is required for shaping mitophagy initiation sites

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*Equal Contribution

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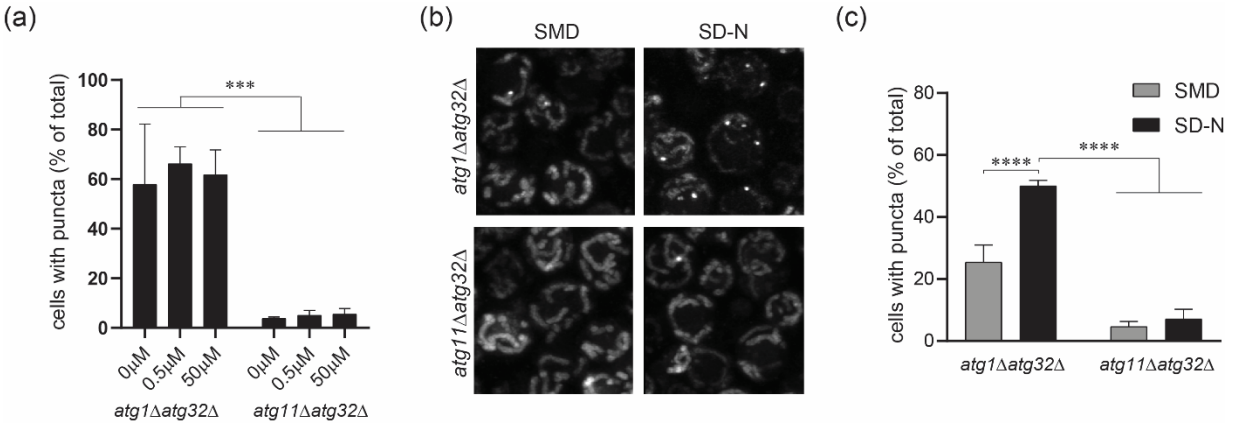

Figure S1. The formation of Atg32 puncta is independent of Atg32 concentration and fluorophore. (a) Quantification of puncta prevalence observed in *atg1Δatg32Δ* or *atg11Δatg32Δ* cells expressing GFP-Atg32 driven by the CUP1 promoter, induced with the indicated copper concentrations and incubated in SD-N for 1 hr. (b) Representative images showing mCherry-Atg32 driven by the CUP1 promoter with 0.5 μM copper in *atg1Δatg32Δ* or *atg11Δatg32Δ* cells. Cells were grown in SMD, then transferred to SD-N for 1 hr. (c) Quantification of the percent of cells containing puncta from b.

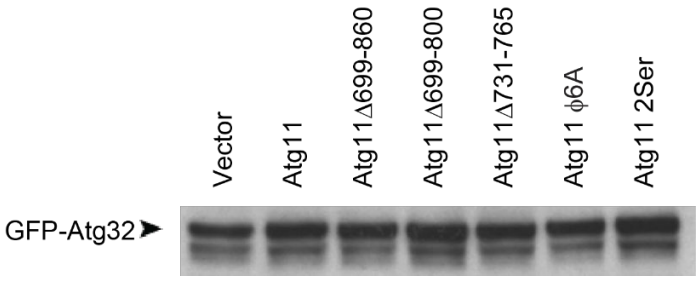

Figure S2. Expression of GFP-Atg32 when co-expressed with different Atg11 variants. GFP-Atg32 was co-expressed with Atg11 variants in *atg11Δatg32Δ* cells grown in SMD. Samples were blotted against GFP.

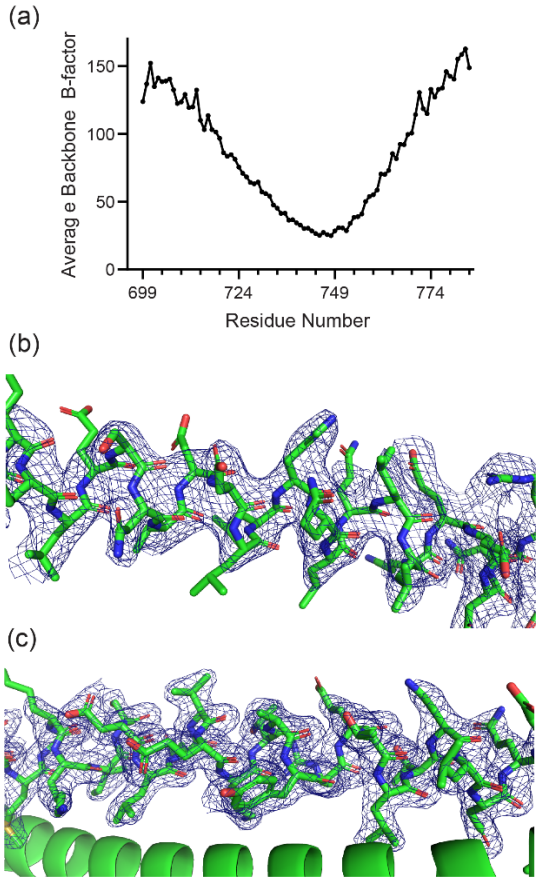

Figure S3. B-factors and electron density for Atg11$_{699\text{-}800}$. (a) Average B-factor values for backbone atoms determined by BAVERAGE in CCP4 and plotted per residue. (b) 2Fo-Fc map contoured at 1.0 σ for residues 704-726. (c) 2Fo-Fc map contoured at 2.0 σ for residues 734-757.

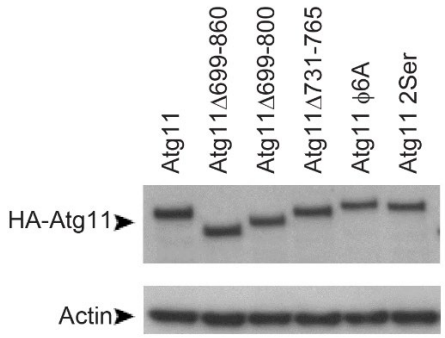

Figure S4. Expression of different Atg11 variants. HA-Atg11 variants were expressed in *atg11Δatg32Δ* cells grown in SMD. Samples were blotted against HA.