Supplementary data

Phosphorylation of Atg9 regulates movement to the phagophore assembly site and the rate of autophagosome formation

Yuchen Feng, Steven K. Backues, Misuzu Baba, Jin-mi Heo, J. Wade Harper and Daniel J. Klionsky

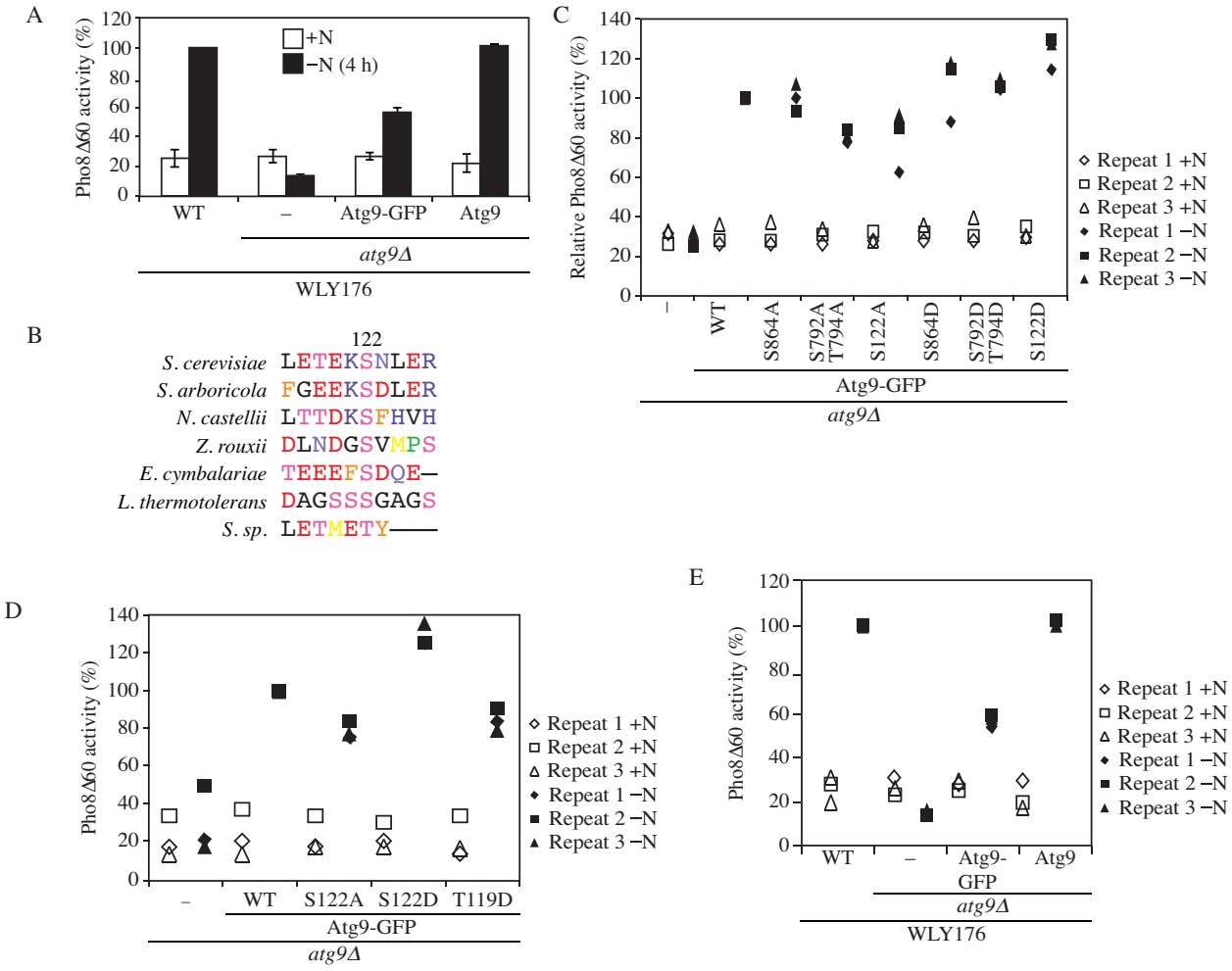

Figure S1. Phosphorylation of Atg9 S122 is important for nonselective autophagy. (**A**) Untransformed WLY176 (WT) or WLY176 *atg9*Δ cells transformed with an empty vector or a pRS406 plasmid expressing either untagged Atg9 or Atg9-GFP, and assayed for Pho8Δ60 activity after 4 h nitrogen starvation as described in *Materials and Methods.* Error bars correspond to the standard deviation and were obtained from 3 independent repeats. (**B**) Alignment of the N terminus of Atg9 throughout different species. (**C-E**) Results of (**C**) Figure 1B, (**D**) Figure 1C and (**E**) Figure S1 are displayed, showing 3 independent repeats as scatterplots.

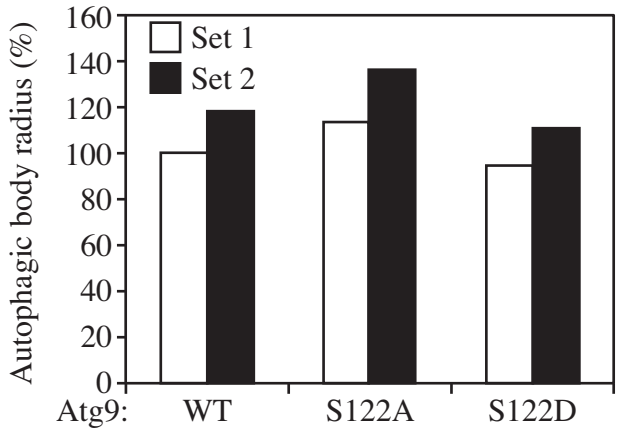

Figure S2. Phosphorylation of Atg9 S122 does not affect the size of autophagosomes. Estimated average radius of autophagic bodies in WT and mutant Atg9 after 4 h of nitrogen starvation. The estimation was based on the radius of autophagic body cross sections observed by TEM in 2 independent experiments done by 2 different labs of more than 100 cells each for each strain.

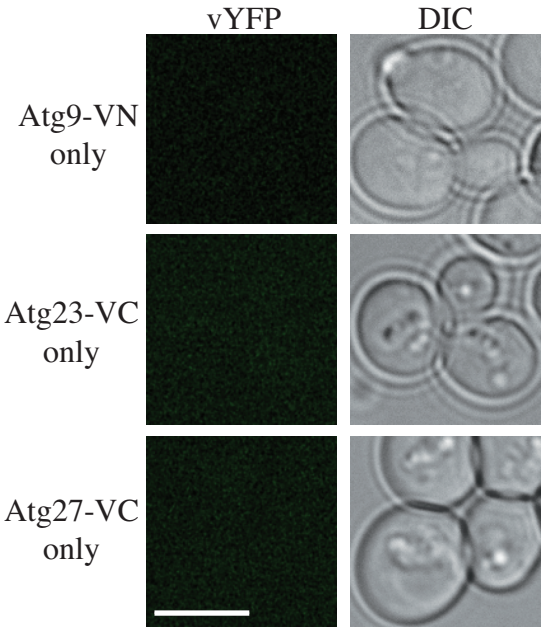

Figure S3. Phosphorylation of S122 is important for the interaction of Atg9 with Atg23 and Atg27. Representative florescence microscopy images of cells expressing only WT Atg9-VN, Atg23-VC or Atg27-VC. Cells were cultured, collected and imaged as in Figure 5. Scale bars: 5 μm.

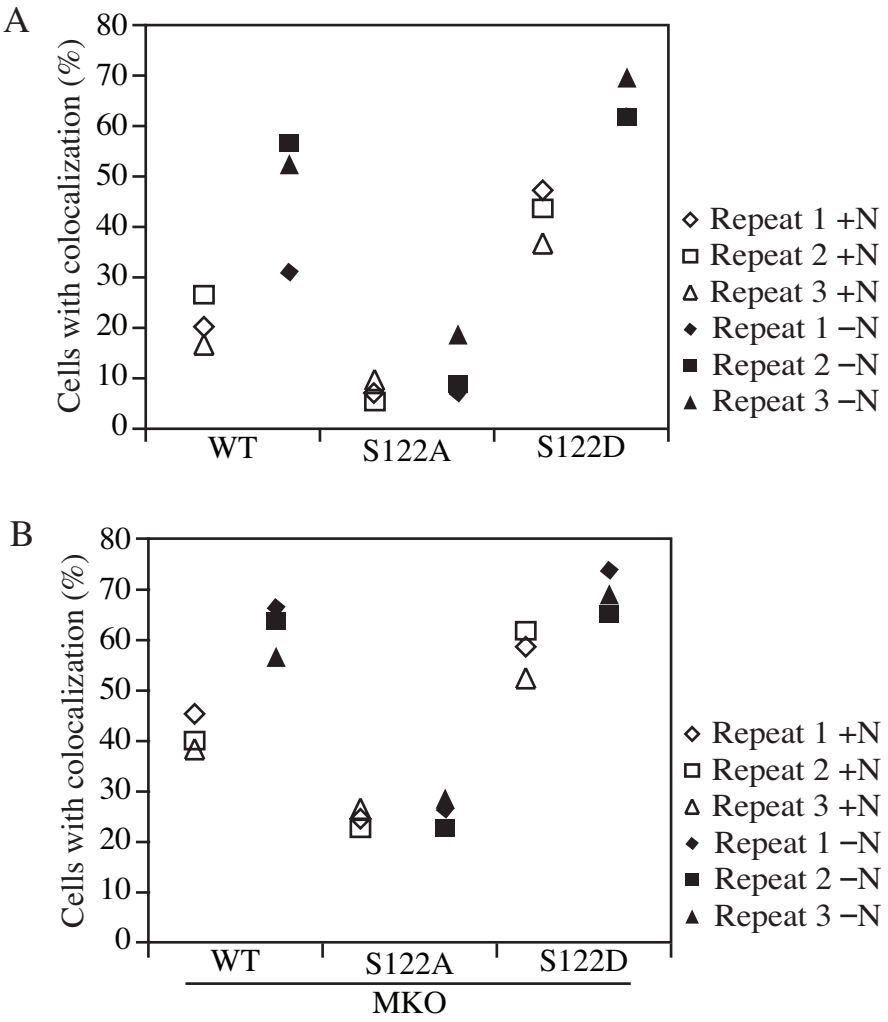

Figure S4. Phosphorylation of S122 is important for Atg9 anterograde trafficking. (**A-B**) Results of the quantification of colocalization between Atg9-GFP and RFP-Ape1 from (**A**) Figure 4B and (**B**) Figure 4D are displayed, showing 3 independent repeats as scatterplots.

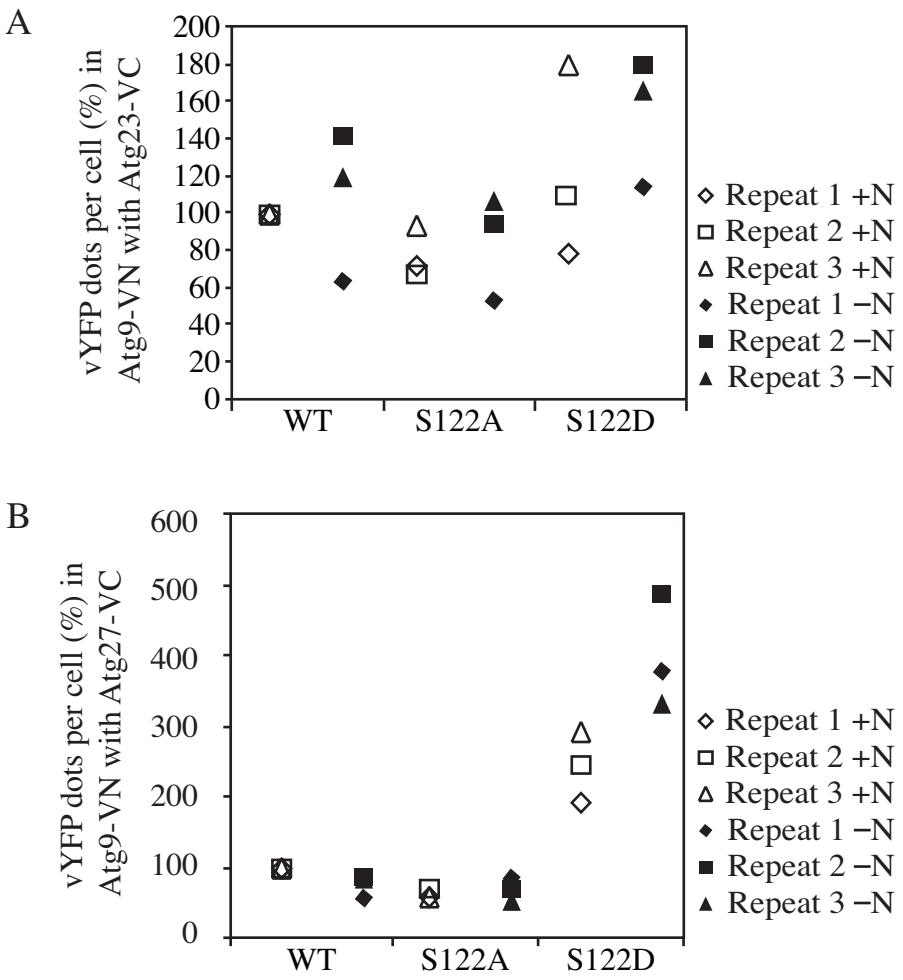

Figure S5. Phosphorylation of Atg9 S122 is important for the interaction of Atg9 with Atg23 and Atg27. Results of the quantification of colocalization between Atg9-GFP and RFP-Ape1 in (**A**) Figure 5B and (**B**) Figure 5D are displayed, showing 3 independent repeats as scatterplots.