

**Table S1. Yeast strains used in this study.**

<b>Name</b>	<b>Genotype</b>	<b>Reference</b>
JMY322	WLY176 <i>ATG2-PA ATG7-PA ATG29-PA</i>	This study
SEY6210	MAT $\alpha$ <i>leu2-3,112 ura3-52 his3-<math>\Delta</math>200 trp1-<math>\Delta</math>901 suc2-<math>\Delta</math>9 lys2-801 GAL</i>	<sup>1</sup>
TVY1	SEY6210 <i>pep4<math>\Delta</math>::LEU2</i>	<sup>2</sup>
WLY176	SEY6210 <i>pho13<math>\Delta</math> pho8::pho8<math>\Delta</math>60</i>	<sup>3</sup>
XLY306	BY4742 <i>PGII-GFP::HIS3</i>	This study
XLY307	BY4742 <i>PGII-GFP::HIS3 atg1<math>\Delta</math>::URA3</i>	This study
XLY316	SEY6210 <i>atg1<math>\Delta</math>::HIS3 pRS406-ATG1::URA3</i>	<sup>4</sup>
XLY318	SEY6210 <i>atg1<math>\Delta</math>::HIS3 pRS406-ATG1(mutant)::URA3</i>	<sup>4</sup>
XLY324	SEY6210 <i>ATG1-ADHI 3' UTR::TRP1</i>	This study
XLY349	SEY6210 <i>atg1<math>\Delta</math>::HIS3 pRS406-ATG1-ATG7 3' UTR::URA3</i>	This study
XLY439	SEY6210 <i>KANMX6::ZEO1p-PSP2-PA::TRP1</i>	This study
XLY440	XLY306 <i>psp2<math>\Delta</math>::KANMX6</i>	This study
XLY441	XLY349 <i>psp2<math>\Delta</math>::KANMX6</i>	This study
XLY442	SEY6210 <i>atg1<math>\Delta</math>::HIS3 pRS406-ATG7p-ATG1::URA3</i>	This study
XLY443	XLY442 <i>psp2<math>\Delta</math>::KANMX6</i>	This study
XLY444	XLY324 <i>psp2<math>\Delta</math>::KANMX6</i>	This study
YKF527	WLY176 <i>atg9<math>\Delta</math>::LEU2</i>	This study
YZY050	SEY6210 <i>psp2<math>\Delta</math>::KANMX6</i>	This study
YZY051	SEY6210 <i>PSP2-PA::KANMX6</i>	This study
YZY059	SEY6210 <i>ATG14-PA::TRP1</i>	This study
YZY060	YZY050 <i>ATG14-PA::TRP1</i>	This study
YZY063	WLY176 <i>psp2<math>\Delta</math>::HIS3</i>	This study
YZY089	SEY6210 <i>PSP2<sup>S340A</sup>-PA::KANMX6</i>	This study
YZY090	SEY6210 <i>PSP2<sup>S340D</sup>-PA::KANMX6</i>	This study
YZY092	TVY1 <i>psp2<math>\Delta</math>::KANMX6</i>	This study
YZY114	SEY6210 <i>PSP2<math>\Delta</math>C56-PA::HIS3</i>	This study
YZY115	SEY6210 <i>PSP2<math>\Delta</math>C154-PA::HIS3</i>	This study
YZY116	SEY6210 <i>PSP2<math>\Delta</math>C175-PA::HIS3</i>	This study
YZY128	JMY322 <i>psp2<math>\Delta</math>::KANMX6</i>	This study
YZY131	YZY051 <i>CDC33-GFP::HIS3</i>	This study
YZY132	SEY6210 <i>CDC33-GFP::HIS3</i>	This study
YZY133	SEY6210 <i>PSP2-MYC::KANMX6</i>	This study
YZY139	YZY132 <i>PSP2(<math>\Delta</math>175)-PA::KANMX6</i>	This study
YZY142	SEY6210 <i>TIF4632-GFP::TRP1</i>	This study
YZY143	YZY051 <i>TIF4632-GFP::TRP1</i>	This study
YZY144	SEY6210 <i>PSP2<sup>S522A</sup>-PA::KANMX6</i>	This study
YZY145	SEY6210 <i>PSP2<sup>S522D</sup>-PA::KANMX6</i>	This study
YZY146	SEY6210 <i>PSP2<sup>SS236,238AA</sup>-PA::KANMX6</i>	This study
YZY147	SEY6210 <i>PSP2<sup>SS236,238DD</sup>-PA::KANMX6</i>	This study
YZY149	YZY133 <i>hmt1<math>\Delta</math>::HIS3</i>	This study

YZY163	XLY316 <i>psp2Δ::KANMX6</i>	This study
YZY164	XLY318 <i>psp2Δ::KANMX6</i>	This study
YZY167	SEY6210 <i>PSP2(Δ419-427)-PA::TRP1</i>	This study
YZY168	SEY6210 <i>PSP2(Δ453-475)-PA::TRP1</i>	This study
YZY169	SEY6210 <i>PSP2(Δ551-564)-PA::TRP1</i>	This study
YZY170	SEY6210 <i>PSP2(Δ569-576)-PA::TRP1</i>	This study
YZY171	SEY6210 <i>PSP2(Δ440-449)-PA::TRP1</i>	This study
YZY172	SEY6210 <i>PSP2<sup>S236,238,340A</sup>-PA::TRP1</i>	This study
YZY173	SEY6210 <i>PSP2<sup>S236,238,340D</sup>-PA::TRP1</i>	This study
YZY177	YZY051 <i>hmt1Δ::HIS3</i>	This study
YZY184	XLY349 <i>PSP2-PA::KANMX6</i>	This study
YZY185	XLY442 <i>PSP2-PA::KANMX6</i>	This study
YZY195	SEY6210 <i>PSP2<sup>S236,238,340,522A</sup>::KANMX6</i>	This study
YZY196	SEY6210 <i>PSP2<sup>S236,238,340,522D</sup>-PA::KANMX6</i>	This study
YZY205	TVY1 <i>PSP2-PA::KANMX6</i>	This study
YZY211	SEY6210 <i>PGII-GFP::TRP1</i>	This study
YZY212	YZY211 <i>psp2Δ::KANMX6</i>	This study
YZY213	YZY212 <i>pRS406-PSP2-PA::URA3</i>	This study
YZY215	YZY050 <i>pRS406-PSP2<sup>R551,553A</sup>-PA::URA3</i>	This study
YZY216	YZY050 <i>pRS406-PSP2<sup>R557,559,563A</sup>-PA::URA3</i>	This study
YZY217	YZY212 <i>pRS406-PSP2<sup>R551,553,557,559,563A</sup>-PA::URA3</i>	This study
YZY218	YZY050 <i>pRS406-PSP2<sup>R440,443,447A</sup>-PA::URA3</i>	This study
YZY225	YZY205 <i>HIS3::ZEO1p-PSP2</i>	This study
YZY231	SEY6210 <i>ATG13-PA</i>	This study
YZY232	YZY231 <i>psp2Δ::KANMX6</i>	This study
YZY235	ZYY202 <i>psp2Δ::KANMX6</i>	This study
YZY236	ZYY202 <i>HIS3::ZEO1p-PSP2</i>	This study
YZY241	SEY6210 <i>ATG16-PA::TRP1</i>	This study
YZY242	YZY050 <i>ATG16-PA::TRP1</i>	This study
YZY243	SEY6210 <i>HMT1-PA::KANMX6</i>	This study
YZY248	YZY050 <i>pRS406-PSP2<sup>R551,553,557,559,563K</sup>-PA::URA3</i>	This study
YZY249	YZY050 <i>pRS406-PSP2<sup>R551,553,557,559,563F</sup>-PA::URA3</i>	This study
YZY252	YZY212 <i>pRS406-PSP2<sup>R551,553,557,559,563K</sup>-PA::URA3</i>	This study
YZY253	YZY212 <i>pRS406-PSP2<sup>R551,553,557,559,563F</sup>-PA::URA3</i>	This study
YZY271	SEY6210 <i>PSP2Δ175-MYC::KANMX6</i>	This study
YZY276	YZY132 <i>psp2Δ::KANMX6</i>	This study
YZY277	YZY276 <i>pRS406-PSP2-PA::URA3</i>	This study
YZY278	YZY276 <i>pRS406-PSP2(Δ2-51)-PA::URA3</i>	This study
YZY279	YZY276 <i>pRS406-PSP2(Δ51-100)-PA::URA3</i>	This study
YZY280	YZY276 <i>pRS406-PSP2(Δ224-252)-PA::URA3</i>	This study
YZY281	YZY276 <i>pRS406-PSP2(Δ274-322)-PA::URA3</i>	This study
YZY282	YZY276 <i>pRS406-PSP2(Δ323-418)-PA::URA3</i>	This study
YZY283	SEY6210 <i>PGK1-GFP::HIS3</i>	This study
YZY284	YZY050 <i>PGK1-GFP::HIS3</i>	This study
YZY285	SEY6210 <i>atg1Δ::KANMX6 PGK1-GFP::HIS3</i>	This study

YZY286	SEY6210 <i>FBA1-GFP::HIS3</i>	This study
YZY287	YZY050 <i>FBA1-GFP::HIS3</i>	This study
YZY288	SEY6210 <i>atg1Δ::KANMX6 FBA1-GFP::HIS3</i>	This study
ZYY202	JMY114 <i>pRS406-ATG13-PA::URA3</i>	<sup>4</sup>

### Supplemental References

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- 2 Wurmser, A. E. & Emr, S. D. Phosphoinositide signaling and turnover: PtdIns(3)P, a regulator of membrane traffic, is transported to the vacuole and degraded by a process that requires luminal vacuolar hydrolase activities. *EMBO J* **17**, 4930-4942, doi:10.1093/emboj/17.17.4930 (1998).
- 3 Mao, K. *et al.* Atg29 phosphorylation regulates coordination of the Atg17-Atg31-Atg29 complex with the Atg11 scaffold during autophagy initiation. *Proceedings of the National Academy of Sciences of the United States of America* **110**, E2875-2884, doi:10.1073/pnas.1300064110 (2013).
- 4 Liu, X. *et al.* Dhh1 promotes autophagy-related protein translation during nitrogen starvation. *PLoS Biol* **17**, e3000219, doi:10.1371/journal.pbio.3000219 (2019).