

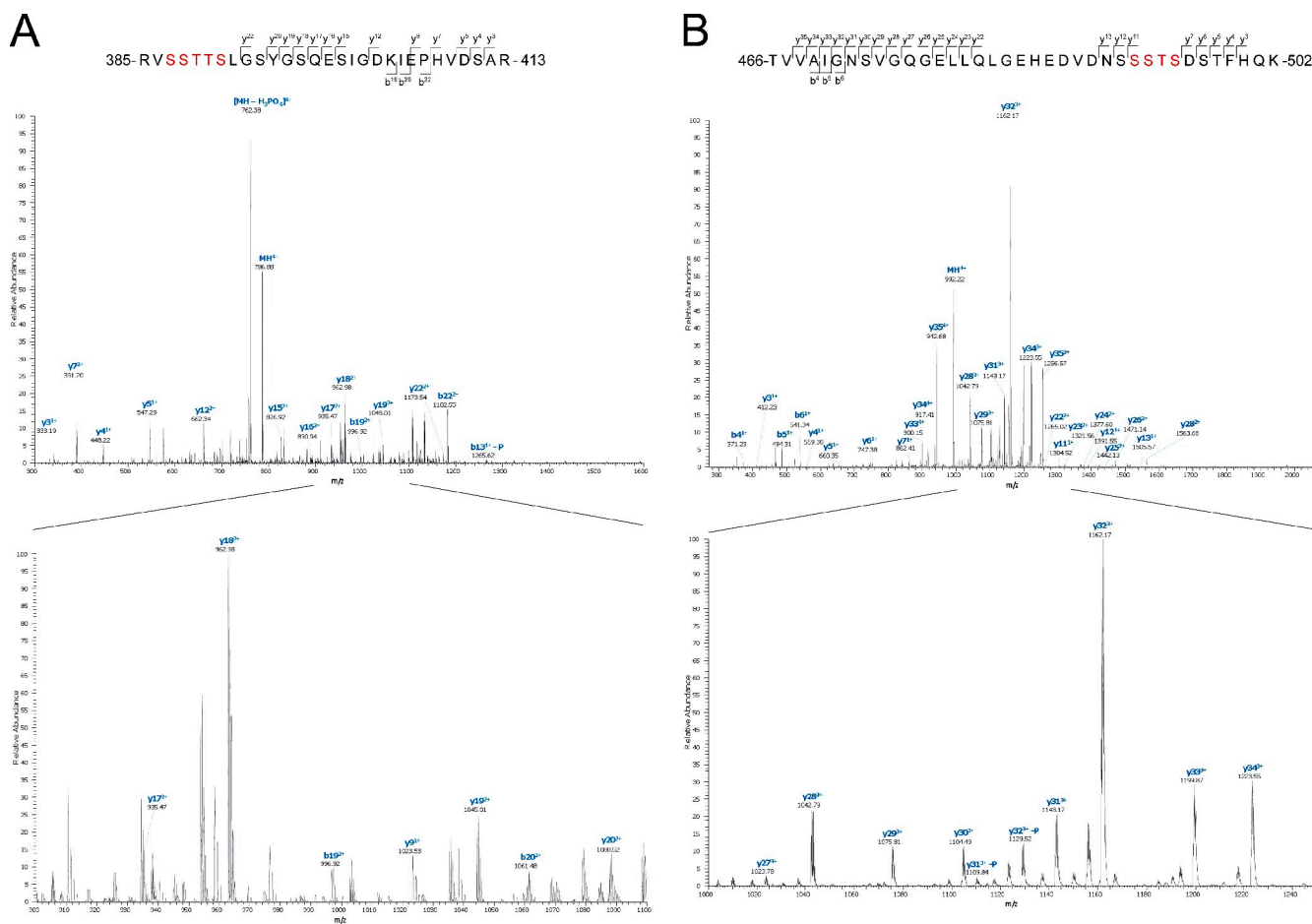
Tamura et al., <http://www.jcb.org/cgi/content/full/jcb.201302067/DC1>

Figure S1. Identification of phosphorylated regions by LC-MS/MS. To identify the phosphorylation sites on PpAtg18, the GST-fused protein was purified from *P. pastoris* and was analyzed by LC-MS/MS. The MS analysis covered 85% of the total PpAtg18 protein, and identified two monophosphorylated peptides, one corresponding to 385-RVSSSTTSLGSGYGSQESIGDKIEPHVDSAR-413 (A) and the other to 466-TVVAIGNSVGQGELLQLGHEHEDVDNNSSTSDSTFHQK-502 (B). Both peptides possess nine Ser/Thr residues. These two peptides were then subjected to targeted MS/MS analysis, which identified 387-SSTTS-391 and 492-SSTTS-495 as the phosphorylated regions in peptides A and B, respectively although the phosphorylated residue in these two regions could not be determined.

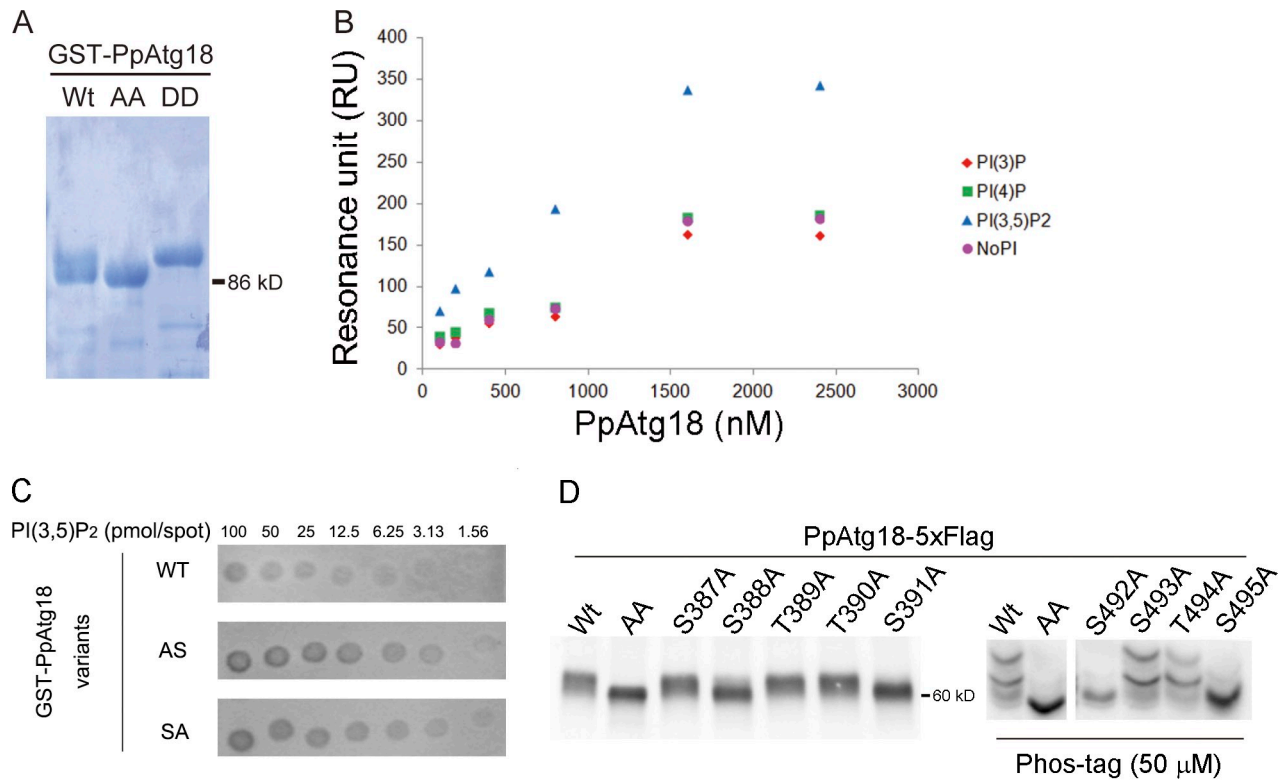


Figure S2. **Purification of PpAtg18 proteins from *P. pastoris*.** (A) SDS-PAGE of purified GST-PpAtg18 variants. The gel was stained with CBB. These proteins were purified as well as GST-PpAtg18Wt. An equal mole of each sample was applied to the gel. (B) Surface plasmon analysis. The interaction between PpAtg18 and liposomes was assessed by a Biacore system set L1 sensor chip (GE Healthcare). For surface plasmon, PpAtg18 was eluted from a GS 4B column with PreScission protease (GE Healthcare). (C) PI(3,5)P₂-binding activity of PpAtg18 SA and PpAtg18 AS proteins. The designated PpAtg18 variants fused to GST were incubated at a concentration of 1 μg/ml with nitrocellulose membrane onto which PI(3,5)P₂ was spotted with the same concentration gradient as PIP Array. (D) Identification of amino acid residues critical for phosphorylation of PpAtg18. Experiments were performed as described in Fig. 3 (A and B).

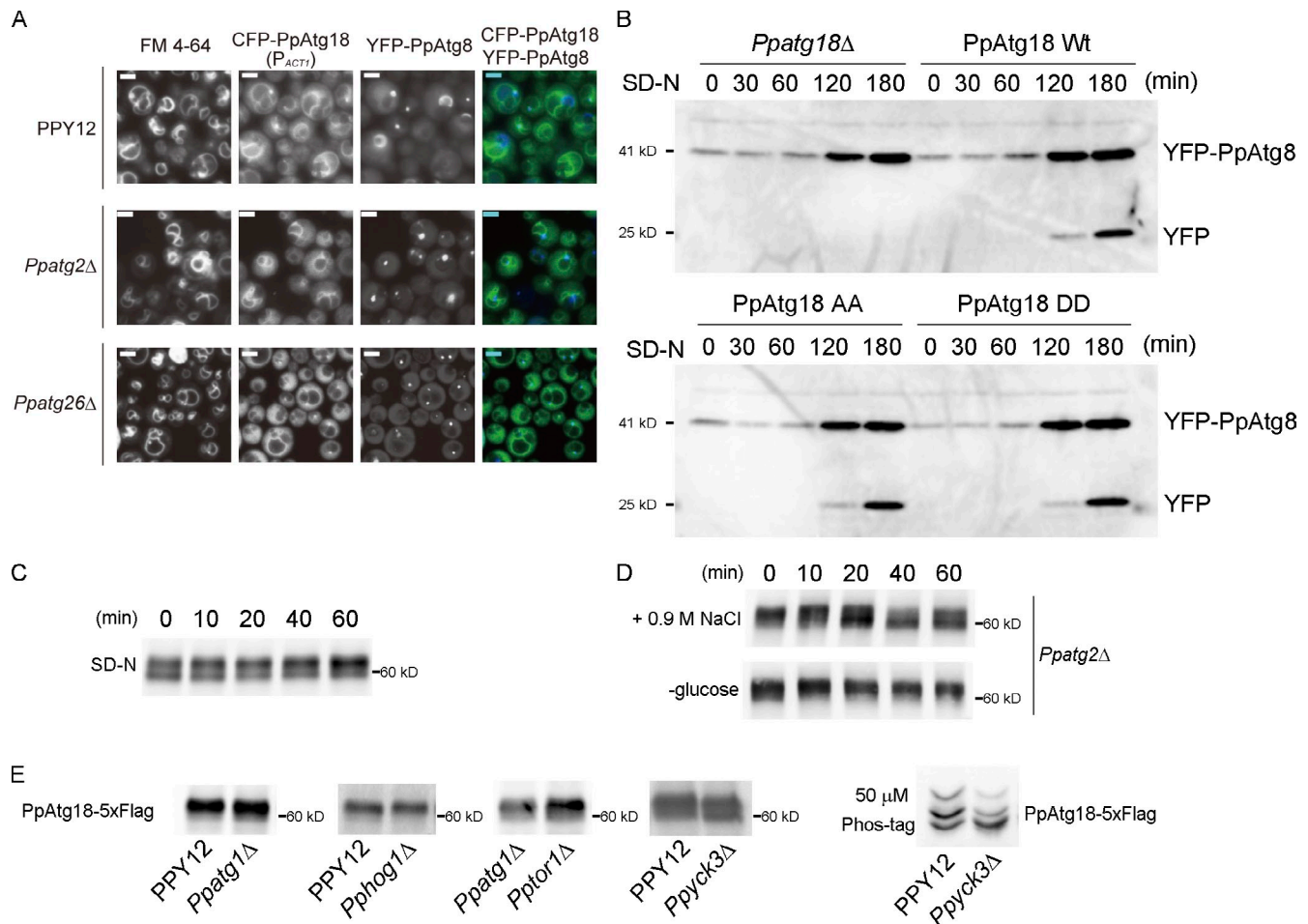


Figure S3. **Role of PpAtg18 in autophagic pathways.** (A) Colocalization of PpAtg18 and PpAtg8. In this experiment, PpAtg18-tagged CFP at the N terminus was expressed under the *PpACT1* promoter. Each strain was transferred from methanol medium to glucose medium for 30–60 min. Vacuoles were stained with FM 4-64. Bars, 2 μ m. (B) YFP-PpAtg8 procession assay during nitrogen starvation in PpAtg18 AA and DD mutant strains. This procession experiment was referred from a previous report (Yamashita et al., 2009). Cells grown in SD were shifted to SD-N medium and collected at indicated time point. These blots were incubated with anti-GFP monoclonal antibody (Molecular Probes). (C) Western blotting of PpAtg18-5xFlag under nitrogen starvation. Cells grown on SD were shifted to SD-N. (D) Western blotting of PpAtg18-5xFlag in *Ppatg2Δ* cells. Cells grown on SD medium were shifted to SD-glucose or SD + 0.9 M NaCl similar to Fig. 4 A. (E) Western blotting of PpAtg18-5xFlag with a Phos-tag in *Ppyck3Δ*. Cell lysates were obtained from SD culture of the designated strains harboring PpAtg18-5xFLAG, and were subjected to immunoblot analysis as described in Fig. 3 A.

Table S1. Yeast strains used in this study

Designation	Description	Genotype	Reference
PPY12	Wild type	<i>arg4 his4</i>	Sakai et al., 1998
SMD1163	<i>pep4Δ prbΔ</i>	<i>arg4::Δpep4 prb his4</i>	Tuttle and Dunn, 1995
NTP0101	<i>atg1Δ</i>	<i>Δatg1::Zeo^r arg4 his4</i>	Tamura et al., 2010
PAZ6	<i>atg2Δ</i>	<i>Δatg2::Zeo^r arg4 his4</i>	Mukaiyama et al., 2002
PZO101	<i>atg26Δ</i>	<i>Δatg26::Zeo^r arg4 his4</i>	Oku et al., 2003
NTP0201	YFP-ATG8 <i>atg2Δ</i>	PAZ6 <i>arg4::pSAP115 his4</i>	This study
NTP2601	YFP-ATG8 <i>atg26Δ</i>	PZO101 <i>arg4::pSAP115 his4</i>	This study
NTP1801	<i>atg18Δ</i>	<i>Δatg18::Zeo^r arg4 his4</i>	This study
NTP1802	<i>fab1Δ</i>	<i>Δfab1::Zeo^r arg4 his4</i>	This study
NTP1803	<i>vac14Δ</i>	<i>Δvac14::Zeo^r arg4 his4</i>	This study
NTP1804	<i>yck3Δ</i>	<i>Δyck3::Zeo^r arg4 his4</i>	This study
NTP1805	<i>hog1Δ</i>	<i>Δhog1::Zeo^r arg4 his4</i>	This study
YAP0004	YFP-ATG8 CFP-SKL PPY12	PPY12 <i>arg4::pSAP115 his4::pYA006</i>	Ano et al., 2004
NTP1806	YFP-ATG8 CFP-SKL <i>atg18Δ</i>	YAP004 <i>Δatg18::Zeo^r</i>	This study
NTP1807	YFP-ATG8 CFP-SKL <i>atg2Δ</i>	PAZ6 <i>arg4::pSAP115 his4::pYA006</i>	This study
NTP1808	YFP-ATG8 CFP-SKL <i>fab1Δ</i>	YAP004 <i>Δfab1::Zeo^r</i>	This study
NTP1809	PGAPDH-GST-ATG18 SMD1163	SMD1163 <i>Zeo^r::pNT1815 his4</i>	This study
NTP1810	PGAPDH-GST-ATG18AA SMD1163	SMD1163 <i>his4::pNT1813</i>	This study
NTP1811	PGAPDH-GST-ATG18DD SMD1163	SMD1163 <i>his4::pNT1814</i>	This study
NTP1812	PATG18-ATG18-5xFLAG Wt <i>atg18Δ</i>	NTP1801 <i>arg4::pNT1801 his4</i>	This study
NTP1813	PATG18-ATG18-5xFLAG AA <i>atg18Δ</i>	NTP1801 <i>arg4::pNT1802 his4</i>	This study
NTP1814	PATG18-ATG18-5xFLAG DD <i>atg18Δ</i>	NTP1801 <i>arg4::pNT1803 his4</i>	This study
NTP1815	PATG18-ATG18-5xFLAG AS <i>atg18Δ</i>	NTP1801 <i>arg4::pNT1804 his4</i>	This study
NTP1816	PATG18-ATG18-5xFLAG SA <i>atg18Δ</i>	NTP1801 <i>arg4::pNT1805 his4</i>	This study
NTP1817	PATG18-ATG18-5xFLAG DS <i>atg18Δ</i>	NTP1801 <i>arg4::pNT1806 his4</i>	This study
NTP1818	PATG18-ATG18-5xFLAG SD <i>atg18Δ</i>	NTP1801 <i>arg4::pNT1807 his4</i>	This study
NTP1819	YFP-ATG8 <i>atg18Δ</i>	NTP1801 <i>his4::pNT1812 arg4</i>	This study
NTP1820	YFP-ATG8 ATG18-5xFLAG Wt <i>atg18Δ</i>	NTP1819 <i>arg4::pNT1801</i>	This study
NTP1821	YFP-ATG8 ATG18-5xFLAG AA <i>atg18Δ</i>	NTP1819 <i>arg4::pNT1802</i>	This study
NTP1822	YFP-ATG8 ATG18-5xFLAG DD <i>atg18Δ</i>	NTP1819 <i>arg4::pNT1803</i>	This study
NTP1823	PATG18-ATG18-YFP Wt <i>atg18Δ</i>	NTP1801 <i>arg4::pNT1808 his4</i>	This study
NTP1824	PATG18-ATG18-YFP AA <i>atg18Δ</i>	NTP1801 <i>arg4::pNT1809 his4</i>	This study
NTP1825	PATG18-ATG18-YFP DD <i>atg18Δ</i>	NTP1801 <i>arg4::pNT1810 his4</i>	This study
NTP1826	PATG18-ATG18-YFP Wt <i>fab1Δ</i>	NTP1802 <i>arg4::pNT1808 his4</i>	This study
NTP1827	PATG18-ATG18-5xFLAG <i>atg1Δ</i>	NTP0101 <i>arg4::pNT1801 his4</i>	This study
NTP1828	PATG18-ATG18-5xFLAG <i>atg2Δ</i>	NTP0101 <i>arg4::pNT1801 his4</i>	This study
NTP1829	PATG18-ATG18-5xFLAG <i>yck3Δ</i>	NTP1804 <i>arg4::pNT1801 his4</i>	This study
NTP1830	PATG18-ATG18-5xFLAG <i>hog1Δ</i>	NTP1805 <i>arg4::pNT1801 his4</i>	This study
NTP1831	PACT1-CFP-ATG18 YFP-ATG8 PPY12	PPY12 <i>arg4::pSAP115 his4::pNT1811</i>	This study
NTP1832	PATG18-ATG18-5xFLAG S387A <i>atg18Δ</i>	NTP1801 <i>arg4::pNT1817 his4</i>	This study
NTP1833	PATG18-ATG18-5xFLAG S388A <i>atg18Δ</i>	NTP1801 <i>arg4::pNT1818 his4</i>	This study
NTP1834	PATG18-ATG18-5xFLAG T389A <i>atg18Δ</i>	NTP1801 <i>arg4::pNT1819 his4</i>	This study
NTP1835	PATG18-ATG18-5xFLAG T390A <i>atg18Δ</i>	NTP1801 <i>arg4::pNT1820 his4</i>	This study
NTP1836	PATG18-ATG18-5xFLAG S391A <i>atg18Δ</i>	NTP1801 <i>arg4::pNT1821 his4</i>	This study
NTP1837	PATG18-ATG18-5xFLAG S492A <i>atg18Δ</i>	NTP1801 <i>arg4::pNT1822 his4</i>	This study
NTP1839	PATG18-ATG18-5xFLAG S493A <i>atg18Δ</i>	NTP1801 <i>arg4::pNT1823 his4</i>	This study
NTP1840	PATG18-ATG18-5xFLAG T494A <i>atg18Δ</i>	NTP1801 <i>arg4::pNT1824 his4</i>	This study
NTP1841	PATG18-ATG18-5xFLAG S495A <i>atg18Δ</i>	NTP1801 <i>arg4::pNT1825 his4</i>	This study
NTP1842	PGAPDH-GST-ATG18AS <i>atg18Δ</i>	NTP1801 <i>his4::pNT1826 arg4</i>	This study
NTP1843	PGAPDH-GST-ATG18SA <i>atg18Δ</i>	NTP1801 <i>his4::pNT1827 arg4</i>	This study
NTP0202	PACT1-CFP-ATG18 YFP-ATG8 <i>atg2Δ</i>	PAZ6 <i>arg4::pSAP115 his4::pNT1811</i>	This study
NTP2602	PACT1-CFP-ATG18 YFP-ATG8 <i>atg26Δ</i>	PZO101 <i>arg4::pSAP115 his4::pNT1811</i>	This study

Table S2. Oligonucleotides used in this study

Designation	DNA sequence
FLAGORF(+ATG)FwXhoI	5'-CCGCTCGAGATCCCCGGGTTAATTAATCATATGGACTAC-3'
FLAGORF(+TGA)RvPstI	5'-AACTGCAGTCATAAATCATAAGAAAATTCGCTTATTAGAAGTGGCG-3'
PpATG18promoterFwBamHI	5'-CGGGATCCCATCATTACGTGTAGGTCAGCACAC-3'
PpATG18ORF(-TAA)RvXhoI	5'-CCGCTCGAGCCCGTCATTGACATCAGTCAGCA-3'
PpATG18ORF(-ATG)FwBamHI	5'-CGGGATCCTCGCAACCTACAGATGAGGCT-3'
PpATG18 ST387-391AFw	5'-GTTAACGGTGCCTAGAAGGGTAGCCGCAGCCGCCGCTCTTGGCTCATATGGAAGTCAAG-3'
PpATG18 ST387-391ARv	5'-CTTGACTTCCATATGAGCCAAGAGCGGGCTGCGGCTACCCCTTAGGCACCGTTAAC-3'
PpATG18 ST387-391DFw	5'-GTTAACGGTGCCTAGAAGGGTAGACGAAGACGACGATCTTGGCTCATATGGAAGTCAAG-3'
PpATG18 ST387-391DRv	5'-CTTGACTTCCATATGAGCCAAGATCGTCTCGTCTTCCCTTCTAGGCACCGTTAAC-3'
PpATG18 ST491-495AFw	5'-GATGTCGACAATGCTGCTGCCGCTGCTGATTCCACCTTTCATCAAAAAGCTTCTTCATGT-3'
PpATG18 ST491-495ARv	5'-AAAGGTGGAATCAGCAGCGGCAGCAGCATTGTCGACATCTTCGTGCTCACCCAATTGAAG-3'
PpATG18 ST491-495DFw	5'-GATGTCGACAATGAGGATGACGAGGAGGATTCCACCTTTCATCAAAAAGCTTCTTCATGT-3'
PpATG18 ST491-495DRv	5'-AAAGGTGGAATCCTCCTCGTCATCCTCATTGTCGACATCTTCGTGCTCACCCAATTGAAG-3'
PpATG18 S387AFw	5'-AGGGTAGCCTCAACCACCTCTCTTGGC-3'
PpATG18 S387ARv	5'-GGTTGAGGCTACCCCTTAGGCACCGT-3'
PpATG18 S388AFw	5'-GTATCCGCTACCACCTCTCTTGGCTCA-3'
PpATG18 S388ARv	5'-GGTGGTAGCGGATACCCCTTAGGCAC-3'
PpATG18 T389AFw	5'-TCCTCAGCCACCTCTCTTGGCTCATAT-3'
PpATG18 T389ARv	5'-AGAGGTGGCTGAGGATACCCCTTAGG-3'
PpATG18 T390AFw	5'-TCAACCGCCTCTCTTGGCTCATATGGA-3'
PpATG18 T390ARv	5'-AAGAGAGGCGGTTGAGGATACCCCTTCT-3'
PpATG18 S391AFw	5'-ACCACCGCTCTTGGCTCATATGGAAGT-3'
PpATG18 S391ARv	5'-GCCAAGAGCGGTGGTTGAGGATACCCCT-3'
PpATG18 S492AFw	5'-AATTGGCTAGCACGTCGGATTCCACC-3'
PpATG18 S492ARv	5'-CGTGCTAGCCGAATTGTCGACATCTTC-3'
PpATG18 S493AFw	5'-TCGAGTGCCACGTCGGATTCCACCTT-3'
PpATG18 S493ARv	5'-CGACGTGGCACTCGAATTGTCGACATC-3'
PpATG18 T494AFw	5'-AGTAGCGCCTCGGATTCCACCTTTCAT-3'
PpATG18 T494ARv	5'-ATCCGAGGCGCTACTCGAATTGTCGAC-3'
PpATG18 S495AFw	5'-AGCACGGCCGATTCCACCTTTCATCA-3'
PpATG18 S495ARv	5'-GGAATCGCCGTGCTACTCGAATTGTC-3'
PpATG18FTTGFw	5'-GGTGTCAAATGTATCAGTTCACAACAGGCACCTACCCACAAAAGATC-3'
PpATG18FTTGRv	5'-GATCTTTGTGGGGTAAGTGCCTGTTGTGAACGATACAATTTGACACC-3'
pDVATG18UPFw	5'-CGGGATCCTTAACTGGGTTACGACATCATTACGT-3'
pDVATG18UPRv	5'-GGGGTACCTAGATGGTTATCTGAATGGTATTTGTAAGGA-3'
pDVATG18DWFw	5'-TCCCCGCGGAAAAAATAAACTAGCAGCCGGCGG-3'
pDVATG18DWRv	5'-CGGGATCCCTGAGAGACAGTTTTTTAATTGGCATG-3'
pDVFAB1UPFw	5'-GGAATCCGAATTGATGAAACAGCAAACATTGTCAC-3'
pDVFAB1UPRv	5'-CCGCTCGAGAGATGGATAAATACACAGGTGAAATGAATC-3'
pDVFAB1DWFw	5'-CGGGATCCACATTCTCTTTTTTTTTCAGAG-3'
pDVFAB1DWRv	5'-GGAATCCATCCATATTAGTAAATCATCGTTCAGATT-3'
pDVVAC14UPFw	5'-CGGGATCCTCATCCGACTGTGATGCCTTGAAC-3'
pDVVAC14UPRv	5'-GGAATCTGTGTTGGTGGGACGGAGGTGT-3'
pDVVAC14DWFw	5'-GGAATTCAGTATAGTGTATGCTGTCCGAAAC-3'
pDVVAC14DWRv	5'-CCGCTCGAGGAAGCTAGCCCAATTGGAACAGTCG-3'
pDVYCK3UPFw	5'-CGGGATCCTGAGACTGGAAGTGTGAGACTTCA-3'
pDVYCK3UPRv	5'-GGAATTCGGTTTATAGTCCCTGCAATATCGTAC-3'
pDVYCK3DWFw	5'-GGAATTCGTTTTTCCACTACGGAGTTAATTC-3'
pDVYCK3DWRv	5'-CCGCTCGAGTGCAAATATCCTTCACTGGGTGCCG-3'

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