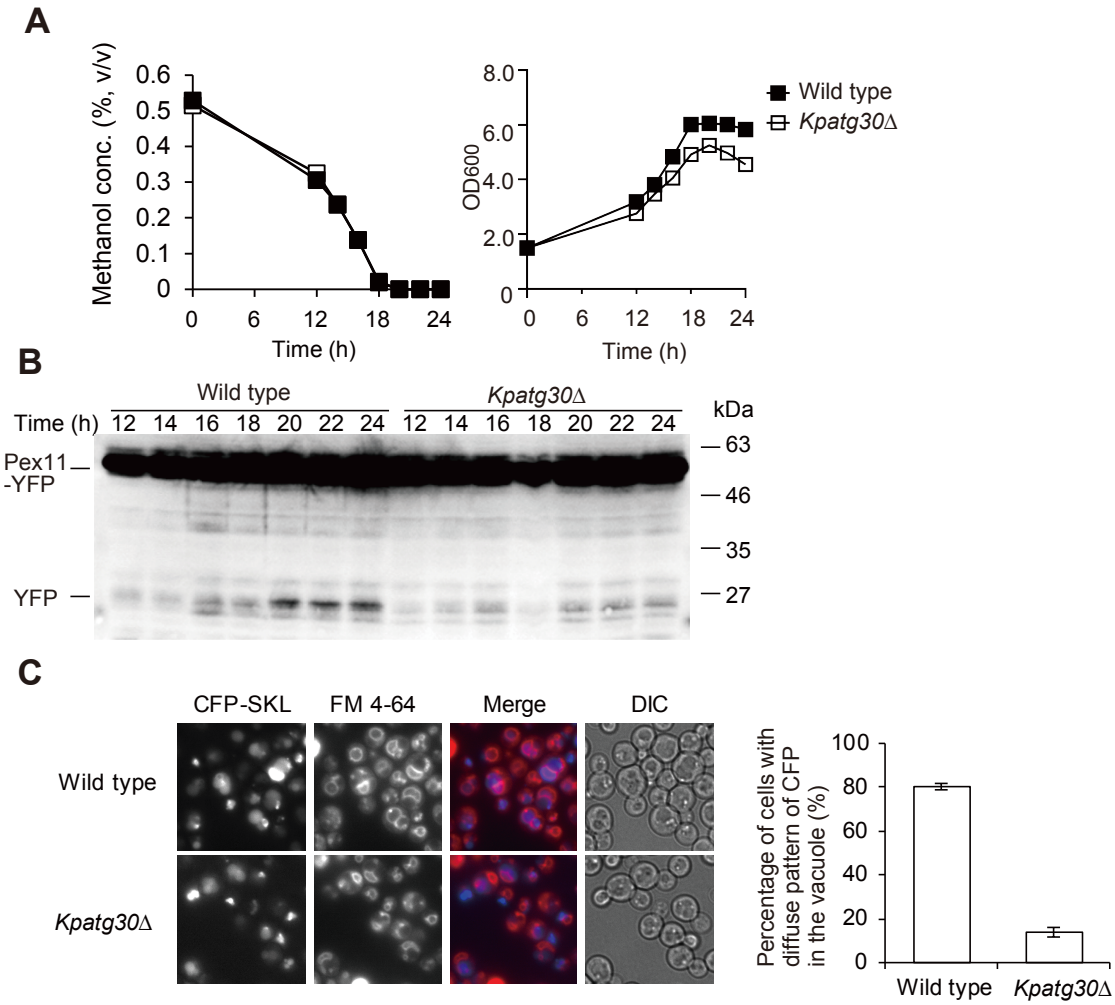

Fig. S1. Atg30-dependent pexophagy during methanol-consumption in batch-cultured growth. (A) Kinetics of methanol consumption (left) and growth of the wild-type and *Kpatg30*Δ strains (right) in 2 chambers of the *Beppu* flask. (B) Immunoblot analysis of KpPex11-YFP expressed in the wild-type and *Kpatg30*Δ strains. (C) Fluorescence microscopy of the wild-type and *Kpatg30*Δ cells with CFP-labeled peroxisomes (CFP-SKL) and FM4-64 -stained vacuolar membranes cultured on methanol-medium for 20 h in a *Beppu* flask. Brightfield images are shown as DIC. The graph shows the results of morphometric assays counting the percentage of the cells with CFP-SKL signal diffused in vacuole.

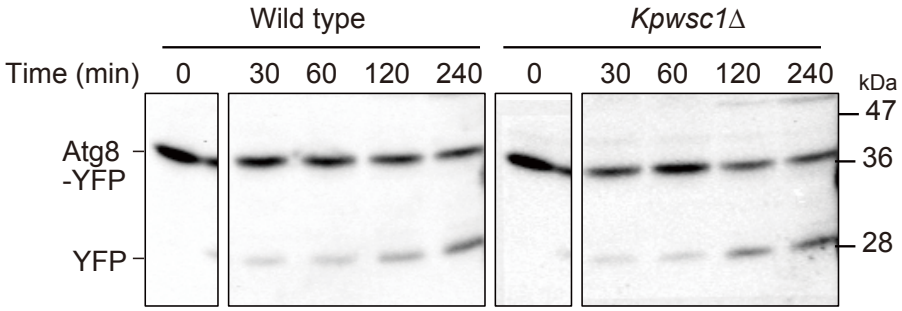

Fig. S2. Macropexophagy in *Kpwsc1*Δ strain. Immunoblot analysis of the lysates from the wild-type and *Kpwsc1*Δ strain expressing KpAtg8-YFP. The timepoints of sample acquisition after the shift from methanol medium to ethanol medium to induce macropexophagy are indicated. KpAtg8-YFP and cleavage YFP were detected using anti-GFP antibody.

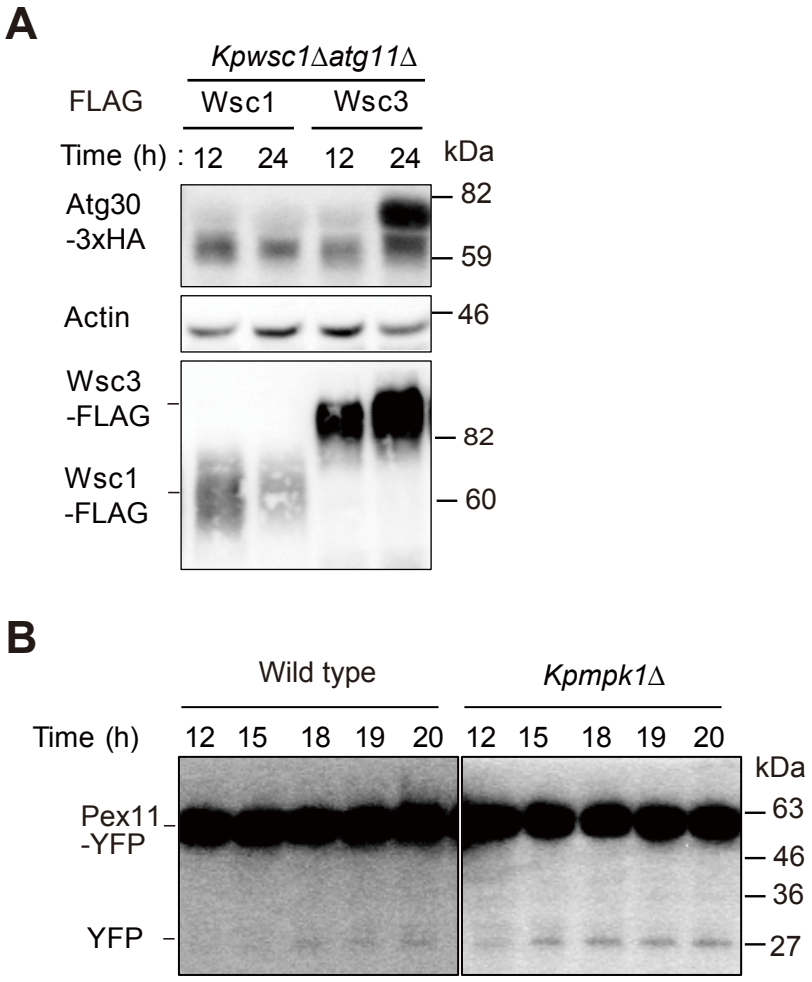

Fig. S3. Expression of KpWsc1-FLAG and KpWsc3-FLAG in *Kpwsc1*Δ*atg11*Δ strain, and pexophagy in *Kpmpk1*Δ strain. (A) Immunoblot analysis of the lysates from the *Kpwsc1*Δ*atg11*Δ strain expressing either KpWsc1-FLAG (KpWsc1) or KpWsc3-FLAG (KpWsc3). The timepoints of sample acquisition after the start of methanol culture are indicated. KpWsc1-FLAG and KpWsc3-FLAG bands were detected using anti-FLAG antibody reactive to FLAG. (B) Immunoblot analysis of Pex11-YFP expressed in the wild-type and *Kpmpk1*Δ strains cultured in a *Beppu* flask, as described in legend to Fig. 1.

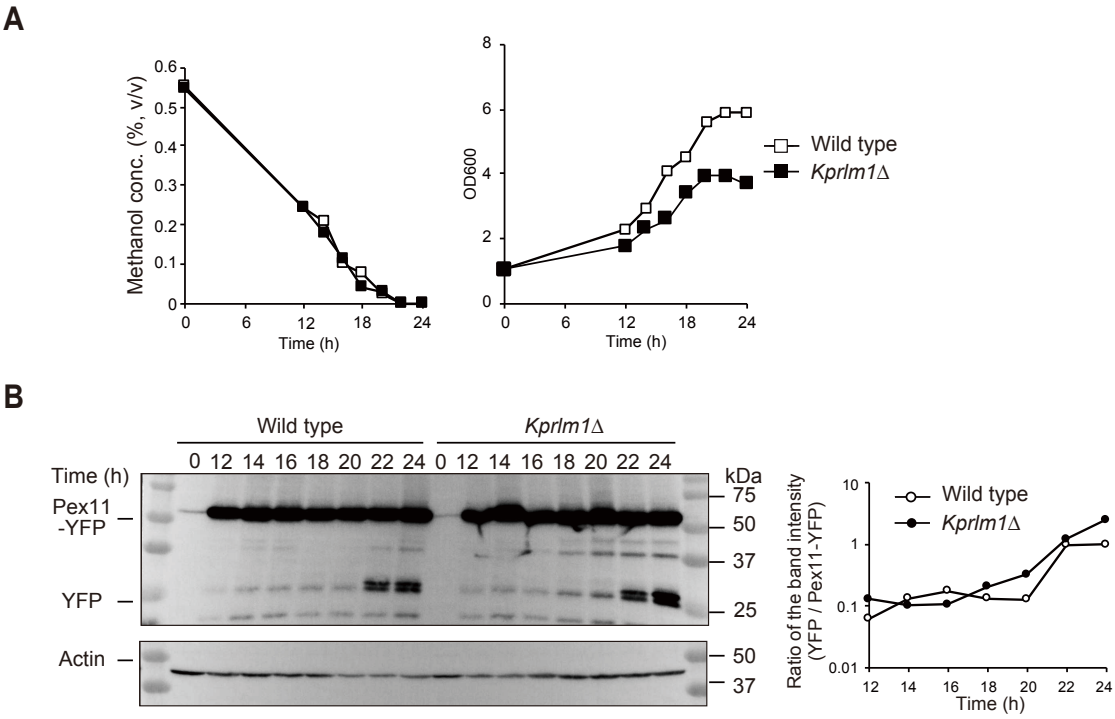

Fig. S4. Onset of pexophagy in the *rlm1*Δ strain during batch culture on methanol in a *Beppu* flask. (A) Kinetics of methanol consumption (left) and growth of the wild-type and *Kprlm1*Δ strains (right) in 2 chambers of the *Beppu* flask. (B) (Left panel) Immunoblot analysis of KpPex11-YFP expressed in the wild-type and *Kprlm1*Δ strains. (Right panel) Quantitative analysis of YFP-band intensity relative to KpPex11-YFP band intensity.

Table S1. Yeast strains used in this study

Strain	Genotype	Reference
PPY12	*Kparg4 Kphis4* (parental strain)	Sakai *et al.* (1998)
GS115	*Kphis4*	Cregg *et al*. (1985)
OH2002	GS115, *Kphis4*::(P_{KpACT1}*KpATG30-3xHA*)	This study
IS20000	PPY12, P_{PEX11}*KpPex11-YFP::KpARG4*	This study
IS20009	IS20000, *Kphis4::KpHIS4* $P_{KpPEX11}$*KpPEX11-YFP::KpARG4*	This study
IS20010	IS20009, *Kpatg30*::*Zeo*^{r}	This study
IS20011	IS20009, *Kpwsc1*::*Zeo*^{r}	This study
IS20012	IS20009*, Pprlm1::Zeor*	This study
IS10020	GS115, P_{KpAOX1}*CFP-SKL::KpHIS4*	This study
IS17020	IS10020, *Kpatg30::BSD*	This study
IS20110	PPY12, *Kpatg11:: BSD*, $P_{KpATG30}$*KpATG30-3xHA:: KpHIS4*	This study
IS20111	IS20110, *Kparg4::KpARG4*	This study
IS24110	IS20110, *Kpwsc1::Zeo*^{r}	This study
IS24111	IS24110, *Kparg4::KpARG4*	This study
IS24112	IS24110, P_{KpACT1}*KpWSC1-5xFLAG::KpARG4*	This study
IS24113	IS24110, P_{KpACT1}*KpWSC1(310-316Δ)-5xFLAG::KpARG4*	This study
IS24116	IS24110, P_{KpACT1}*KpWSC1(Y53A)-5xFLAG::KpARG4*	This study
IS24117	IS24110, P_{KpACT1}*KpWSC1(Y53F)-5xFLAG::KpARG4*	This study
IS22110	IS20110, *Kpmpk1::Zeo*^{r}	This study
IS22111	IS22110, *Kparg4::KpARG4*	This study
IS22112	IS22110, P_{KpMPK1}*KpMPK1-YFP::KpARG4*	This study
IS22113	IS22110, P_{KpMPK1}*KpMPK1(TAYF)-YFP::KpARG4*	This study
IS23110	IS20110, *Kprlm1::Zeo*^{r}	This study
IS23111	IS23110, *Kparg4::KpARG4*	This study
IS23116	IS20110, *Kpswi4::Zeo*^{r}	This study
IS23117	IS23116, *Kparg4::KpARG4*	This study
IS23118	IS20110, *Kpmsg5::Zeo*^{r}	This study
IS23119	IS23118, *Kparg4::KpARG4*	This study
IS23120	IS20110, *Kpptp2a::Zeo*^{r}	This study
IS23121	IS23120, *Kparg4::KpARG4*	This study
IS23122	IS23120, *Kpmsg5::KpARG4*	This study
IS24118	IS24110, P_{KpACT1}*KpWSC3-5xFLAG::KpARG4*	This study
SA1017	PPY12*, arg4::pSAP115(PATG8YFP-PpATG8, ARG4)*	Mukaiyama et al. (2004)
IS24119	SA1017, *his4*::*HIS4*	This study
IS24120	SA1017*, Ppwsc1::Zeor*	This study
IS24121	IS24120*, his4::HIS4*	This study

Table S2. Plasmids used in this study

Designation	Discription	Reference
pIB1	*KpHIS4*	Sears *et al*. (1998)
SK+Zeo^r	Zeo^r	Yano *et al*. (2009)
pOH100	Δ*Kpwsc1::Zeo^r*	Ohsawa *et al.* (2017)
pOH103	Δ*Kprlm1::Zeo^r*	This study
pOH104	*ΔKpatg30::Bsd^r*	This study
pOH105	Δ*Kpatg30::Zeo^r*	This study
pOH106	Δ*Kpswi4::Zeo^r*	This study
pOH107	Δ*Kpmsg5*Δ*::Zeo^r*	This study
pOH108	Δ*Kpptp2a*Δ*::Zeo^r*	This study
pOH109	Δ*Kpmsg5*Δ*::ScARG4*	This study
pIS100	*ΔKpatg11::Bsd^r*	This study
pIS101	*ΔKpmpk1::Zeo^r*	This study
pSY302	$P_{KpPEX11}$*KpPEX11-YFP KpARG4*	Laboratory collection
pSY8200	pIB1 *KpHIS4::ScARG4*	Ohsawa *et al.* (2017)
pSY006	pIB1 3xHA *KpHIS4*	Ohsawa *et al.* (2017)
pRN001	$P_{KpATG30}$*KpATG30-3xHA KpHIS4*	This study
pYA006	P_{KpAOX1}*CFP-SKL KpHIS4*	Ano *et al.* (2005)
pOH202	P_{KpACT1}*KpWSC1-3xHA KpHIS4*	Ohsawa *et al.* (2017)
pOH203	P_{KpACT1}*KpWSC3-3xHA KpHIS4*	Ohsawa *et al.* (2017)
pOH205	P_{KpACT1}*KpWSC1(310-316*Δ*)-3xHA KpHIS4*	Ohsawa *et al.* (2017)
pOH208	P_{KpACT1}*KpWSC1(Y53A)-3xHA KpHIS4*	Ohsawa *et al.* (2017)
pOH209	P_{KpACT1}*KpWSC1(Y53F)-3xHA KpHIS4*	Ohsawa *et al.* (2017)
pNT206	pIB1 5xFLAG *KpARG4*	Tamura *et al.* (2013)
pOH213	P_{KpACT1}*KpWSC1-5xFLAG KpARG4*	This study
pOH214	P_{KpACT1}*KpWSC1(310-316*Δ*)-5xFLAG KpARG4*	This study
pOH215	P_{KpACT1}*KpWSC1(Y53A)-5xFLAG KpARG4*	This study
pOH216	P_{KpACT1}*KpWSC1(Y53F)-5xFLAG KpARG4*	This study
pOH217	P_{KpACT1}*KpWSC3-5xFLAG KpARG4*	This study
pNT204	pIB1 *KpARG4*	Tamura *et al.* (2010)
pNT205	YFP-pIB1 *KpARG4*	Tamura *et al.* (2010)
pIS001	P_{KpMPK1}*KpMPK1-YFP KpARG4*	This study
pIS002	P_{KpMPK1}*KpMPK1(TAYF)-YFP KpARG4*	This study

Table S3. Primers used in this study

Designation	DNA Sequence
PpATG11-BSD-F	5'-GGATCTCAACAAACTGCGGTAGCCCACACACCATAGCTTC-3'
PpATG11-BSD-R	5'-CAGTCCATCGATCTCCGTTTTTGTAATAGAACAAGAAAAATGAAACTGA-3'
EcoRI-PpATG11-1-F	5'-CGGAATTCAACGCAACACAAGTCCTTCC-3'
PpATG11-1-R	5'-GAAGCTATGGTGTGTGGGCTACCGCAGTTTGTTGAGATCC-3'
PpATG11-2-F	5'-TCAGTTTCATTTTTCTTGTTCTATTACAAAAACGGAGATCGATGGACTG-3'
BamHI-PpATG11-2-R	5'-CGGGATCCGGAGACGACACCACATTGAA-3'
HindIII-PpATG30-1-F	5'-CCCAAGCTTTGCCATTTAGCTCCCTGATT-3'
PpATG30-1-R	5'-GAAGCTATGGTGTGTGGGCTATATTCTTGCTCGGCATCGT-3'
PpATG30-2-F	5'-CGAAGGCTTTAATTTGCAAGCTCCAATTCCCAGTCCACATCT-3'
PstI-PpATG30-2-R	5'-TGCACTGCAGTGCCAAGTCTGACTCCCTTT-3'
PpATG30-BSD-F	5'-ACGATGCCGAGCAAGAATATAGCCCACACACCATAGCTTC-3'
PpATG30-BSD-R	5'-AGATGTGGACTGGGAATTGGAGCTTGCAAATTAAAGCCTTCG-3'
NotI-PpMPK1-1-F	5'-CGATTATTTCTTCGGTGCCTGCGGCCGCCCTGAAGAGGGGAAAGAAGG-3'
KpnI-PpMPK1-1-R	5'-GGGGTACCCACCTTTTTGATGGCCACTT-3'
SacI-PpMPK1-2-F	5'-CCCGAGCTCCGGATTGGATCGGTATGGTA-3'
NotI-PpMPK1-2-R	5'-CCTTCTTTCCCCTCTTCAGGGCGGCCGCAGGCACCGAAGAAATAATCG-3'
NotI-PpRLM1-1-F	5'-ATTGCCAGAAAGCAACGTCTGCGGCCGCAACTCATCAGGCGTGCTTTT-3'
KpnI-PpRLM1-1-R	5'-GGGGTACCAAAGCCCAGCTTTCCTCTTC-3'
SacI-PpRLM1-2-F	5'-CCCGAGCTCCGAGATTTCCAAGCAGTGTG-3'
NotI-PpRLM1-2-R	5'-AAAAGCACGCCTGATGAGTTGCGGCCGCAGACGTTGCTTTCTGGCAAT-3'
NotI-PpSWI4-1-F	5'-GAGTGGACGTCAGCATTTCAGCGGCCGCGGAGCATCGAGTGTGTTGTG-3'
KpnI-PpSWI4-1-R	5'-GGGGTACCACCTCCTTGGATCCTCTGGT-3'
SacI-PpSWI4-2-F	5'-CCCGAGCTCCGCATGAAGCTGGTAAATGA-3'
NotI-PpSWI4-2-R	5'-CACAACACACTCGATGCTCCGCGGCCGCTGAAATGCTGACGTCCACTC-3'
PpMSG5-1-F	5'-GACCAAAGACGTGGGAAGAA-3'
PpMSG5-1-R	5'-TTTGAAGCTATGGTGTGTGGGCGGTTCTTCTTCGAAACCTG-3'
PpMSG5-2-F	5'-CGAAGGCTTTAATTTGCAAGCTATCAGCCTACCTGCATCACC-3'
PpMSG5-2-R	5'-TACGTTTGGCATCTGGAGTG-3'
PpMSG5-ZEO-F	5'-CAGGTTTCGAAGAAGAACCGCCCACACACCATAGCTTCAAA-3'
PpMSG5-ZEO-R	5'-GGTGATGCAGGTAGGCTGATAGCTTGCAAATTAAAGCCTTCG-3'
PpPTP2A-1-F	5'-GTTTGGGGGCTACAACTTGA-3'
PpPTP2A-1-R	5'-TTTGAAGCTATGGTGTGTGGGCGAGATTCCTCGTACGCATT-3'
PpPTP2A-2-F	5'-CGAAGGCTTTAATTTGCAAGCTCGAAGACTCAGGGTATCAATGG-3'
PpPTP2A-2-R	5'-TCTTCGCTGTTCGTCTACCC-3'

PpPTP2A-ZEO-F	5'-AATGCGTACGAGGAATCTCGCCCACACACCATAGCTTCAAA-3'
PpPTP2A-ZEO-R	5'-CCATTGATACCCTGAGTCTTCGAGCTTGCAAATTAAAGCCTTCG-3'
(PpMSG5)-ScARG4-F	5'-CAGGTTTCGAAGAAGAACCGGATCTGCCAAGGCTCCATCA-3'
(PpMSG5)-ScARG4-R	5'-GGTGATGCAGGTAGGCTGATTATAAACTAAGACAACTGCTAAGTTGGTTAAC-3'
(ScArg4)-PpMSG5-1-R	5'-TGATGGAGCCTTGGCAGATCCGGTTCTTCTTCGAAACCTG-3'
(ScArg4)-PpMSG5-2-F	5'-GTTAACCAACTTAGCAGTTGTCTTAGTTTATAATCAGCCTACCTGCATCACC-3'
XhoI-PpATG30-subclo-F	5'-CCGCTCGAGGGCGATGAGAGGAAGCATTA-3'
SphI-PpATG30-subclo-R	5'-ACATGCATGCTAAAATCTCCTGTTTGAGCTTTGA-3'
KpnI-P_{ACTI}-F	5'-GGGGTACCTCGCTGGTAATCCCGGCT-3'
SpeI-PpWSC1-subclo-R	5'-GGACTAGTAGCATCATCAGGATTTGCTACC-3'
SpeI-PpWSC1(310-316Δ)-R	5'-GGACTAGTAGCATCCACCTTCCTGGAGTAATCTGCT-3'
BamHI-P_{ACTI}-F	5'-CGGGATCCTCGCTGGTAATCCCGGCT-3'
SpeI-PpWSC3-R	5'-GGACTAGTAACTTCATCATCTGTGGGGTT-3'
XmaI-MPK1-F	5'-TTCCCCCCGGGTCGAGAAAACGCAAACTCTG-3'
(YFP)-PpMPK1-R	5'-CATGCCTGCAGCTCGAGCTGTGTACCATACCGATCCAATC-3'
(PpMPK1)-YFP-F	5'-GATTGGATCGGTATGGTACACAGCTCGAGCTGCAGGCATG-3'
BamHI-YFP-R	5'-CGCGGATCCTTACTTGTACAGCTCGTCCATGC-3'
PpMPK1(T188AY190F)-F	5'-TTTCTTGCTGAATTTGTTGCTACCAGGTGGTAT-3'
PpMPK1(T188AY190F)-R	5'-AGCAACAAATTCAGCAAGAAAGCCAGCATTCTT-3'
RT-GAP1-F	5'-CCACCGGTGTTTTCACCACT-3'
RT-GAP1-R	5'-CACCGACAACGAACATTGGA-3'
RT-PpMSG5-F	5'-ACCGATCCCGGAATACCAAG-3'
RT-PpMSG5-R	5'-TCCAGTTCTGTGGCGGACTT-3'
RT-PpPTP2A-F	5'-TGGCTTCTCCTGGATGTGGT-3'
RT-PpPTP2A-R	5'-GGTCTTTGGCACTTGCTGCT-3'

Table S4: Supplementary Figure 1C cell count

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