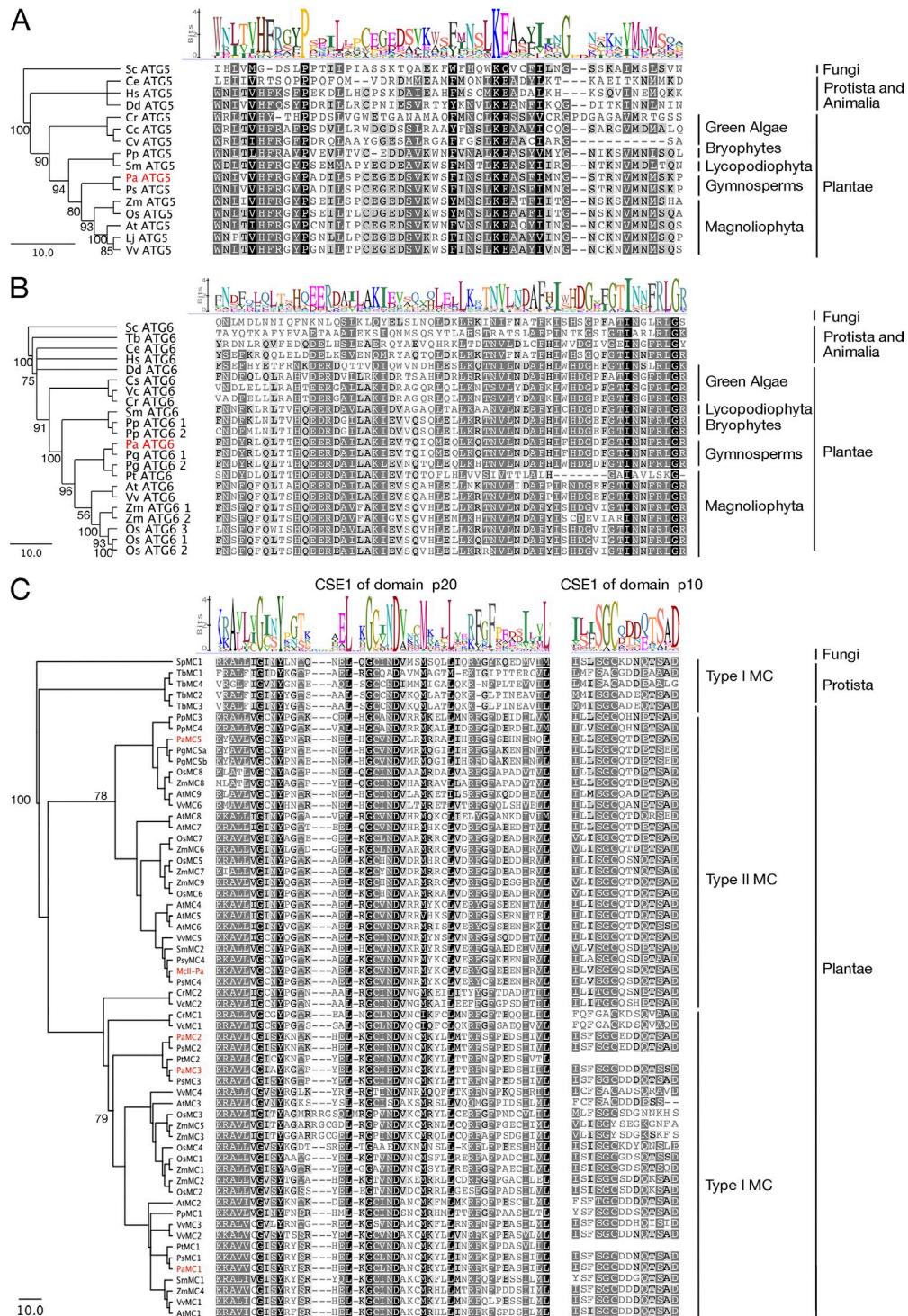
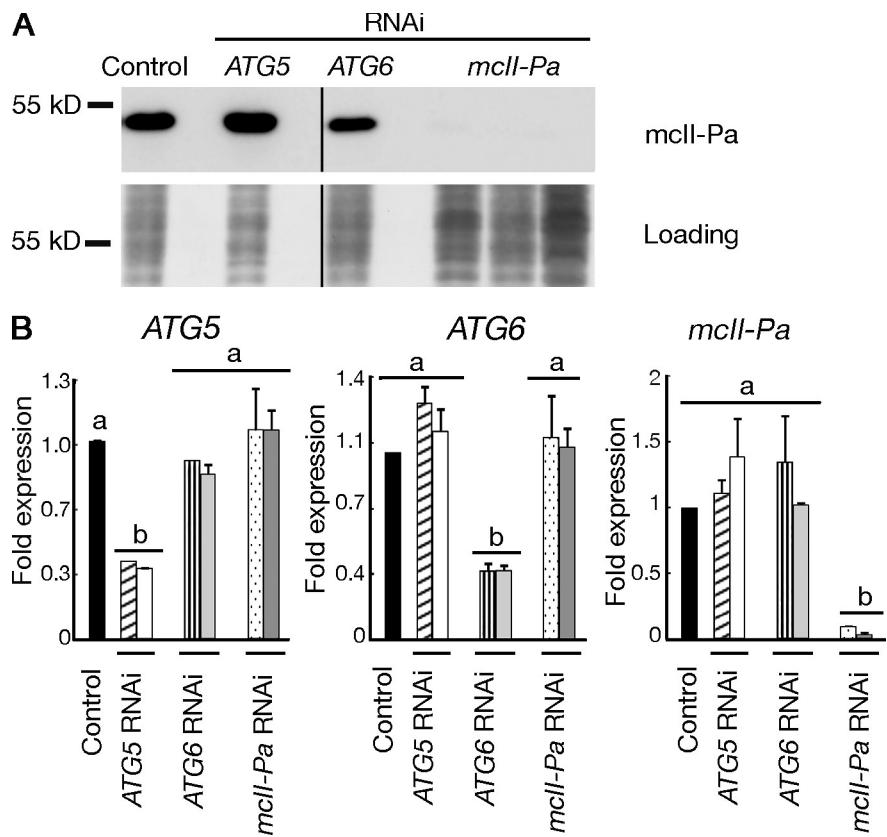


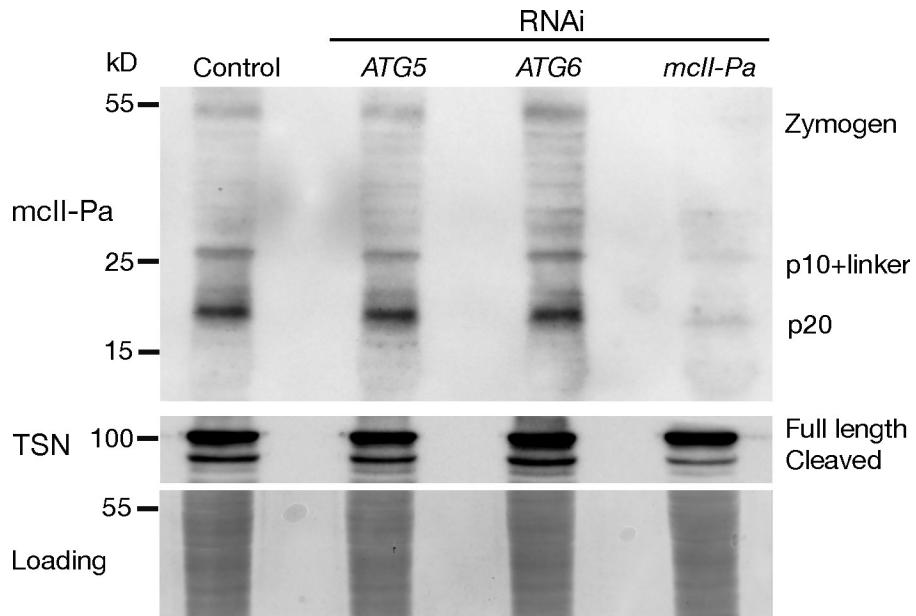
## Supplemental material

Minina et al., <http://www.jcb.org/cgi/content/full/jcb.201307082/DC1>

**Figure S1. Phylogenetic analysis of Atg5, Atg6, and metacaspase homologues in a range of species from Fungi, Animalia, Protista, and Plantae kingdoms.** (A) Phylogenetic tree of Atg5 homologues and a part of multiple alignment showing the region with conserved lysine residue required for conjugation with Atg12 (Mizushima et al., 1998). (B) Phylogenetic tree of Atg6 homologues and a part of multiple alignment showing a region of the Beclin-1 evolutionarily conserved domain (Kang et al., 2011). (C) Phylogenetic tree of metacaspase homologues and a part of multiple alignment showing first conserved sequence elements (CSE) for p20 and p10 domains. Unrooted trees were constructed based on multiple alignment of full-length protein sequences. Numbers indicate percentages of consensus support for each node. MC, metacaspase. P. abies protein homologues are highlighted red.



**Figure S2. Analysis of the efficiency of gene silencing in *P. abies* RNAi lines.** (A) Western blot analysis of protein extracts from control and RNAi lines using the anti-mcll-Pa antibody. Coomassie staining of the blotting membrane was used as the protein loading control. Black lines indicate that intervening lanes have been spliced out. (B) Real-time qPCR analysis of ATG5, ATG6, and *mcll-Pa* transcripts in control and RNAi lines. Data represent the means  $\pm$  SEM from three independent experiments. Different letters indicate statistically different mean values ( $P < 0.001$ ; Student's *t* test).



**Figure S3. Metacaspase activity is independent of autophagy.** Proteolytic activity of mcll-Pa in the control and RNAi lines was assessed by Western blot analyses of protein extracts from control and RNAi lines using anti-mcll-Pa and anti-TSN antibodies. The activity correlates with autoprocessing of mcll-Pa zymogen (formation of p10 + linker and p20 fragments) and cleavage of TSN (Bozhkov et al., 2005b; Sundström et al., 2009). Coomassie staining of the blotting membrane was used as the protein loading control.

Table S1. List of proteins used for phylogenetic analysis

Organism	Protein	Accession no.	Type of metacaspase
<i>A. thaliana</i>	At ATG5	NP_197231	NA
<i>A. thaliana</i>	At ATG6	NP_974475	NA
<i>A. thaliana</i>	AtMC1	AT1G02170	Type I
<i>A. thaliana</i>	AtMC2	AT4G25110	Type I
<i>A. thaliana</i>	AtMC3	AT5G64240	Type I
<i>A. thaliana</i>	AtMC4	AT1G79340	Type II
<i>A. thaliana</i>	AtMC5	AT1G79330	Type II
<i>A. thaliana</i>	AtMC6	AT1G79320	Type II
<i>A. thaliana</i>	AtMC7	AT1G79310	Type II
<i>A. thaliana</i>	AtMC8	AT1G16420	Type II
<i>A. thaliana</i>	AtMC9	AT5G04200	Type II
<i>Coccomyxa subellipsoidea</i>	Cc ATG5	EIE21973	NA
<i>C. subellipsoidea</i>	Cc ATG6	EIE26749	NA
<i>Caenorhabditis elegans</i>	Ce ATG5	NP_490885	NA
<i>C. elegans</i>	Ce ATG6	CCD62215	NA
<i>Chlamydomonas reinhardtii</i>	Cr ATG5	XM_001692610	NA
<i>C. reinhardtii</i>	Cr ATG6	XP_001689505	NA
<i>C. reinhardtii</i>	CrMC1	XP_001696956	Type I
<i>C. reinhardtii</i>	CrMC2	XP_001691826	Type II
<i>Chlorella variabilis</i>	Cv ATG5	EFN59373	NA
<i>D. discoideum</i>	Dd ATG5	AAO39075	NA
<i>D. discoideum</i>	Dd ATG6	XP_636942	NA
<i>Homo sapiens</i>	Hs ATG5	NP_004840	NA
<i>H. sapiens</i>	Hs ATG6	AAH10276	NA
<i>Lotus japonicus</i>	Lj ATG5	AFK43433	NA
<i>O. sativa</i>	Os ATG5	NP_001045690	NA
<i>O. sativa</i>	Os ATG6 1	NP_001043880	NA
<i>O. sativa</i>	Os ATG6 2	NP_001173355	NA
<i>O. sativa</i>	Os ATG6 3	NP_001050756	NA
<i>O. sativa</i>	OsMC1	NP_001050270	Type I
<i>O. sativa</i>	OsMC2	NP_001050268	Type I
<i>O. sativa</i>	OsMC3	AAR06359	Type I
<i>O. sativa</i>	OsMC4	NP_001050269	Type I
<i>O. sativa</i>	OsMC5	NP_001044530	Type II
<i>O. sativa</i>	OsMC6	NP_001055939	Type II
<i>O. sativa</i>	OsMC7	NP_001055938	Type II
<i>O. sativa</i>	OsMC8	NP_001065680	Type II
<i>P. abies</i>	Pa ATG5	CCG89180	NA
<i>P. abies</i>	Pa ATG6	HG326275	NA
<i>P. abies</i>	PaMC1	HG421141	Type I
<i>P. abies</i>	PaMC2	comp91450_c0_seq2 <sup>a</sup>	Type I
<i>P. abies</i>	PaMC3	HG421142	Type I
<i>P. abies</i>	MclI-Pa (PaMC4)	CAD59226	Type II
<i>P. abies</i>	PaMC5	comp95097_c0_seq1,2,3 <sup>a</sup>	Type II
<i>P. glauca</i>	Pg ATG6 1	BT117701	NA
<i>P. glauca</i>	Pg ATG6 2	BT116151	NA
<i>P. glauca</i>	PgMC5a	BT108991	Type II
<i>P. glauca</i>	PgMC5	BT116587	Type II
<i>Physcomitrella patens</i>	Pp ATG5	XP_001777836	NA
<i>P. patens</i>	Pp ATG6 1	XP_001761841	NA
<i>P. patens</i>	Pp ATG6 2	XP_001779419	NA
<i>P. patens</i>	PpMC1	XP_001764027	Type I
<i>P. patens</i>	PpMC2	Phpat.024G040800.1.p <sup>b</sup>	Type I
<i>P. patens</i>	PpMC3	XP_001773634	Type II
<i>P. patens</i>	PpMC4	XP_001760554	Type II
<i>P. patens</i>	PpMC5	PPhp.at001G075700.1.p <sup>b</sup>	Type II
<i>P. patens</i>	PpMC6	Phpat.021G059400.1.p <sup>b</sup>	Type II
<i>P. sitchensis</i>	Ps ATG5	ABR17459	NA

Table S1. List of proteins used for phylogenetic analysis (Continued)

Organism	Protein	Accession no.	Type of metacaspase
<i>P. sitchensis</i>	PsMC1	ABR16279	Type I
<i>P. sitchensis</i>	PsMC2	ABK26561	Type I
<i>P. sitchensis</i>	PsMC3	ABK26795	Type I
<i>P. sitchensis</i>	PsMC4	ABK24525	Type II
<i>Pinus sylvestris</i>	PsyMC4	ACB11499	Type II
<i>Pinus taeda</i>	PtMC1	AFG50895	Type I
<i>P. taeda</i>	PtMC2	AFG62232	Type I
<i>Saccharomyces cerevisiae</i>	Sc ATG5	BAA11937	NA
<i>S. cerevisiae</i>	Sc ATG6	EDN61024	NA
<i>Selaginella moellendorffii</i>	Sm ATG5	XP_002993284	NA
<i>S. moellendorffii</i>	Sm ATG6	EFJ13904	NA
<i>S. moellendorffii</i>	SmMC1	XP_002987241	Type I
<i>S. moellendorffii</i>	SmMC2	XP_002970440	Type II
<i>Schizosaccharomyces pombe</i>	SpMC1	NP_588503	Type I
<i>Trypanosoma brucei</i>	Tb ATG6	XP_844999	NA
<i>T. brucei</i>	TbMC1	AJ437301	Type I
<i>T. brucei</i>	TbMC2	CAD24803	Type I
<i>T. brucei</i>	TbMC3	CAD24804	Type I
<i>T. brucei</i>	TbMC4	CAD24805	Type I
<i>T. brucei</i>	TbMC5	XP_827616	Type I
<i>Volvox carteri f. nagariensis</i>	Vc ATG6	EFJ46852	NA
<i>V. carteri f. nagariensis</i>	VcMC1	XP_002948071	Type I
<i>V. carteri f. nagariensis</i>	VcMC2	XP_002946738	Type II
<i>Vitis vinifera</i>	Vv ATG5	XP_002276604	NA
<i>V. vinifera</i>	Vv ATG6	XP_002277370	NA
<i>V. vinifera</i>	VvMC1	AGJ94048	Type I
<i>V. vinifera</i>	VvMC2	AGJ94049	Type I
<i>V. vinifera</i>	VvMC3	AGJ94050	Type I
<i>V. vinifera</i>	VvMC4	AGJ94051	Type I
<i>V. vinifera</i>	VvMC5	AGJ94052	Type II
<i>V. vinifera</i>	VvMC6	AGJ94053	Type II
<i>Zea mays</i>	Zm ATG5	NP_001105827	NA
<i>Z. mays</i>	Zm ATG6 1	NP_001105883	NA
<i>Z. mays</i>	Zm ATG6 2	GRMZM2G027857_T01 <sup>c</sup>	NA
<i>Z. mays</i>	ZmMC1	DAA45630	Type I
<i>Z. mays</i>	ZmMC2	AFW88009	Type I
<i>Z. mays</i>	ZmMC3	NP_001147740	Type I
<i>Z. mays</i>	ZmMC4	AFW87827	Type I
<i>Z. mays</i>	ZmMC5	DAA45632	Type I
<i>Z. mays</i>	ZmMC6	NP_001151968	Type II
<i>Z. mays</i>	ZmMC7	DAA57249	Type II
<i>Z. mays</i>	ZmMC8	AFW64729	Type II
<i>Z. mays</i>	ZmMC9	NP_001140337	Type II

Accession numbers were obtained from GenBank unless otherwise noted. NA, not applicable.

<sup>a</sup>*P. abies* genome project (ConGenIE).

<sup>b</sup>*P. patens* genome v3 (Phytozome).

<sup>c</sup>*Z. mays* genome (Phytozome).

Table S2. Primers used in this study

Primer	5' → 3' sequence
<b>Primers for cloning</b>	
hpATG5-P-sense	AAGGCCGGCCCTTCCCTCTTACTCCTGTC
hpATG5-M-antisense	TTTAATAAACCTTCCCTTACTCCTG
ATG5-Re	TCATCTCCAACATCAATGTGAAT
attB1-pAHC25-Fw	GGGGACAAGTTGACAAAAAAGCAGGCTGGCGAATTGGTACGTTAA
attB2-pAHC25-Rev	GGGGACCACTTGACAGAAAGCTGGTAAAGCTGGAGCTTACCTGCAGG
Beclin-Ascl-sense	AAAAGGCGGCCAGCACATGGATGTCAAACATC
Beclin-Ascl-anti	AAAAGGCGCGCTTAAGGTTATGAAGCATGTCTTG
attB2-hpATG6	GGGGACCACTTGACAGAAAGCTGGTCATAGCGAGTGCTCCAGAACAA
attB1-hpATG6	GGGGACAAGTTGACAAAAAAGCAGGCTAGCGAGTGCTCCAGAACAA
attB1-hpmcl-Pa	GGGGACAAGTTGACAAAAAAGCAGGCTATCGCATATAATGGAGCATTGCAA
attB2-hpmcl-Pa	GGGGACCACTTGACAGAAAGCTGGTATCGCATATAATGGAGCATTGCAA
s-hpmcl-Pa-EcoRI	TTGAATTGCGCTTCAGAGACCTTTATCTGAGA
as-hpmcl-Pa-EcoRI	TTGAATTGCGATTGCCCCGTGACATGAATCT
attB1-ATG8-Pa	GGGGACAAGTTGACAAAAAAGCAGGCTGATGGCAAGAAGTTCTCAAGCAA
attB2-ATG8-Pa	GGGGACCACTTGACAGAAAGCTGGTTAAGTAGATGTGACATCAAGGAA
attB1	GGGGACAAGTTGACAAAAAAGCAGGCTG
PaATG5-Fw	TCCCACCTCGCTTGATTACGG
picA-Fw	GACGACATCCGCGCTGCTCGA
picA-Re	ACTGGGGAGCTGGCGAAG
VirG-Fw	GACGATGCGTATGCGGCATCTATA
VirG-Re	CGAACACATCCACCGTCAGCGTCAAAGAA
attB1-GUS-Fw	GGGGACAAGTTGACAAAAAAGCAGGCTAIGTTACGTCTGTAGAA
attB2-GUS-Re	GGGGACCACTTGACAGAAAGCTGGTTCATGTTGCCTCCCTG
<b>Primers for qPCR</b>	
qPCR-Pa-Beclin-Fw	GTTCCCTCTCGTCCTCGAAC
qPCR-Pa-Beclin-Re	CACTGGAGGAGAGCCAGGG
qPCR-ATG5-Pa-F2	AGTATGTGGATGGCAGCA
qPCR-ATG5-Pa-R2	CCGATTCGTCAAATGAACCT
cdc2-ref-Fw	TCCGACGGGTGCAGAGAA
cdc2-ref-Re	GCTCCATTGAGCCTGATTCAA
Phosphogluc-ref-Fw	AATGCAGTTGAAGGCCATTCC
Phosphogluc-ref-Re	CCAGTGCGAAACTCTCTTC
qPCRmcl-Pa-Fw	GCTGGTGGGATGCAATTACCCA
qPCRmcl-Pa-Re	AAGCCGTAGCGCTTACGAGGCA

Re, reverse; Fw, forward.

## References

- Bozhkov, P.V., M.F. Suarez, L.H. Filonova, G. Daniel, A.A. Zamyatin Jr., S. Rodriguez-Nieto, B. Zhivotovsky, and A. Smertenko. 2005b. Cysteine protease mclI-Pa executes programmed cell death during plant embryogenesis. *Proc. Natl. Acad. Sci. USA*. 102:14463–14468. <http://dx.doi.org/10.1073/pnas.0506948102>
- Kang, R., H.J. Zeh, M.T. Lotze, and D. Tang. 2011. The Beclin 1 network regulates autophagy and apoptosis. *Cell Death Differ.* 18:571–580. <http://dx.doi.org/10.1038/cdd.2010.191>
- Mizushima, N., T. Noda, T. Yoshimori, Y. Tanaka, T. Ishii, M.D. George, D.J. Klionsky, M. Ohsumi, and Y. Ohsumi. 1998. A protein conjugation system essential for autophagy. *Nature*. 395:395–398. <http://dx.doi.org/10.1038/26506>
- Sundström, J.F., A. Vaculova, A.P. Smertenko, E.I. Savenkov, A. Golovko, E. Minina, B.S. Tiwari, S. Rodriguez-Nieto, A.A. Zamyatin Jr., T. Välineva, et al. 2009. Tudor staphylococcal nuclease is an evolutionarily conserved component of the programmed cell death degradome. *Nat. Cell Biol.* 11:1347–1354. <http://dx.doi.org/10.1038/ncb1979>