

Supplementary Figures and Tables

Figure S1. Sequence analysis of ATG4 cysteine peptidases

A. Amino acid alignment of *L. major* ATG4 aligned with those of *T. brucei*, *S. cerevisiae*, *H. sapiens* and *A. thaliana*. Identical and conserved amino acids are shaded in black and grey, respectively. Gaps are indicated by hyphens and insertions removed from the alignment are indicated in parenthesis indicating the number of residues removed. Similarly, some N-terminal residues are not shown. The catalytic (β 1-7) and auxiliary (β 8-13) domains (based on Sugawara *et al.*,¹), the sequence linking the two domains, and the inhibitory loop are indicated by single, double and triple lines, respectively. Residues required for substrate recognition and hydrolysis according to Sugawara *et al.*¹ are marked: catalytic triads (*); oxyanion hole (Δ), substrate recognition (o, Π). Key: Lmj, *L. major*; Tb, *T. brucei*; Sc, *S. cerevisiae*; Hs, *H. sapiens*; At, *A. thaliana*. GenBank accession numbers and GeneDB systematic names are: LmjATG4.1, LmjF32.3890; LmjATG4.2, LmjF30.0270; TbATG4.1, Tb11.01.7970, TbATG4.2, Tb927.6.7690; ScATG4, P53867; HsATG4.1, autophagin-1, CAD43219; HsATG4.2, autophagin-2, CAD43218; HsATG4.3, autophagin-3, Q96DT6; HsATG4, autophagin-4, NP116274; AtATG4a, At2g44140.1; AtATG4b, At3g59950.1.

Figure S2. Analysis of ATG8 proteins.

A. Amino acid alignment of *L. major* ATG8 (i) and ATG12 (ii) proteins aligned with those of *S. cerevisiae*, *H. sapiens* and *A. thaliana*. Identical and conserved amino acids are shaded in black and grey, respectively. Gaps are indicated by hyphens. The alpha helices (α) and beta folds (β) of the ubiquitin-like fold conserved in *H. sapiens*

(GABARAP, ²; GATE-16, ³; MAP-LC3, ¹) and *A. thaliana* ⁴ATG8s are marked. The scissile glycine (*) hydrolysed by ATG4 and residues (#) that interact with the E1-like enzyme ATG7 ¹ are marked. Residues that recognise and interact with the active site of the peptidase is marked with ψ . ⁵ Residues used as the basis for the peptide substrates synthesised are underlined. Key: Lmj, *L. major*; Tb, *T. brucei*; Sc, *S. cerevisiae*; Hs, *H. sapiens*; At, *A. thaliana*. GenBank accession numbers are: Sc, P38182; Hs-GATE-16, 1EO_6_A; Hs-GABARAP, CAG47031; Hs-MAP-LC3A, Q62625; At-ATG8A, NP_567642; At-ATG8E, NP_850431; At-ATG8H, NP_566518. GeneDB systematic names for the *L. major* genes are: LmjF19.1630 (ATG8); LmjF22.1300 (ATG12); LmjF19.0820 (ATG8B.1); LmjF19.0840 (ATG8A.1); LmjF19.0842 (ATG8B.2); LmjF19.0844 (ATG8A.2); LmjF19.0846 (ATG8B.3); LmjF19.0848 (ATG8A.3); LmjF19.0850 (ATG8B.4); LmjF19.0860 (ATG8B.5); LmjF19.0870 (ATG8B.6); LmjF19.0880 (ATG8B.7); LmjF19.0900 (ATG8B.8); LmjF19.0910 (ATG8B.9); Lmj09.0150 (ATG8C.1); Lmj09.0152 (ATG8C.2); Lmj09.0154 (ATG8C.3); Lmj09.0156 (ATG8C.4); Lmj09.0158 (ATG8C.5); Lmj09.0160 (ATG8C.6); Lmj09.0162 (ATG8C.7); Lmj09.0164 (ATG8C.8); Lmj09.0166 (ATG8C.9); Lmj09.0170 (ATG8C.10); Lmj09.0172 (ATG8C.11); Lmj09.0174 (ATG8C.12); Lmj09.0180 (ATG8C.13).

B. Phylogenetic analysis of ATG8 paralogues. Conserved residues pruned of insertions and gaps were used for the analysis. Reliability values after bootstrapping are shown at the nodes. Key: GenBank accession numbers and GeneDB systematic names for *L. major* are as detailed in A. ScATG12, P38316; AtATG12, Q8S924; HsATG12, AAH12266; HIATG12, BAF75797.

A

(i)

$\alpha 1$ $\alpha 2$ $\beta 1$ $\beta 2$ $\alpha 4$ $\beta 4$
 ~~~~~#~~~~~  
 LmjF19.1630 (1) -----MSRVVCSYKHTLEARRDAEKV-ERRADRLVLCIKKENSVPVDDLK-----SKFLVPPDALTGGFLVSIIRRRITTEAEKALFFLVGD-  
 LmjF22.1300 (1) MHAPPQPPPPRHQYMHSEHRCCLLSKML-LRLGASTVPIVEPTE(58)-S-----KSTLKCILLKSKVAEVLTLRGLAIDSCQSTFLVSGEN-  
 Sc-ATG8 (1) -----MKSTKSEYFPEKKAESERI-ADREKNRIPVICHEKESDIPEIDK-----RKYLVPADLVGQFYVIRKRMLPPEKALFFLVND-  
 Hs-MAP-LC3A (1) -----MPSEKTKQRRSFQRNEIVR-LIEQHEPTKIPVILERYKGEK-----QLPVLDKTKFLVDPHVNMSELKILIRRRQLNANQAFFLVNG-  
 Hs-GABARAP (1) -----MKFVYKEEHFPEKRSSEGEKI-RKKVDRVPVIVEKPKARIGDLDK-----KKYLVPDILVGGQFYFLIRKRHRHAEADAFFLVNN-  
 Hs-GATE-16 (1) -----MKWMEKEDHSLHRCVHSAKI-RAKYEDRVPVIVEKVGSGQIVDIDK-----RKYLVPDITVAQFVWILIRKRQLPSEKALFFLVYDK-  
 At-ATG8A (1) -----MAKSSKINPLEARMSHSSRI-REKYDRIPVIVEKKGQSDVPDIDK-----KKYLVPADLVGQFYVVRKRKKGAEKALFFLVYKN-  
 At-ATG8E (1) -----MNKGIHEKMDDFEKKKASAGRI-REKYDRIPVIVEKKEKSEVPNIDK-----KKYLVPDILVGGQFYVIRKRKLSKAEKALFFLVYDN-  
 At-ATG8H (1) -----MKSEKEQYTLDELAESREI-LIKYETRIPVIAEKYCKTDLPAIEK-----KKFLVPRDMSVGGFYIISARHHSPEKALFFLVNN-  
 LmjF19.0840 (1) -----MSYQSLIEADARRAECERV-RRRFFQLPVVVESNN-----SSHVRELAVQRDAIVADLENEFQALGRTNKK-VLALAEF-  
 LmjF19.0844 (1) -----MSYQSLIEADARRAECERV-RRRFFQLPVVVESNN-----SSHVRELAVQRDAIVADLENEFQALGRTNKK-VLALAEF-  
 LmjF19.0848 (1) -----MSYQSLIEADARRAECERV-RRRFFQLPVVVESNN-----SSHVRELAVQRDAIVADLENEFQALGRTNKK-VLALAEF-  
 LmjF19.0820 (1) -----MSAYHSNFPVEARRAECARI-QAKYFGHAAVVEAEKA-----GSKVHELALPRDAIVAELEAAVRQALGTSARE-VTLAEF-  
 LmjF19.0842 (1) -----MSAYHSNFPVEARRAECARI-QAKYFGHAAVVEAEKA-----GSKVHELALPRDAIVAELEAAVRQALGTSARE-VTLAEF-  
 LmjF19.0846 (1) -----MSAYHSNFPVEARRAECARI-QAKYFGHAAVVEAEKA-----GSKVHELALPRDAIVAELEAAVRQALGTSARE-VTLAEF-  
 LmjF19.0950 (1) -----MSAYHSNFPVEARRAECARI-QAKYFGHAAVVEAEKA-----GSKVHELALPRDAIVAELEAAVRQALGTSARE-VTLAEF-  
 LmjF19.0860 (1) -----MSAYHSNFPVEARRAECARI-QAKYFGHAAVVEAEKA-----GSKVHELALPRDAIVAELEAAVRQALGTSARE-VTLAEF-  
 LmjF19.0870 (1) -----MSAYHSNFPVEARRAECARI-QAKYFGHAAVVEAEKA-----GSKVHELALPRDAIVAELEAAVRQALGTSARE-VTLAEF-  
 LmjF19.0880 (1) -----MSAYHSNFPVEARRAECARI-QAKYFGHAAVVEAEKA-----GSKVHELALPRDAIVAELEAAVRQALGTSARE-VTLAEF-  
 LmjF19.0900 (1) -----MSAYHSNFPVEARRAECARI-QAKYFGHAAVVEAEKA-----GSKVHELALPRDAIVAELEAAVRQALGTSARE-VTLAEF-  
 LmjF09.0150 (1) -----MSAVVLTPLEARVAKCASI-RAAN-AMPVVVEEQAR-----GKAYESALARETTVAQLVAAVRFGFRGDAKKEVALTVAG-  
 LmjF09.0152 (1) -----MSAVVLTPLEARVAKCASI-RAAN-AMPVVVEEQAR-----GKAYESALARETTVAQLVAAVRFGFRGDAKKEVALTVAG-  
 LmjF09.0154 (1) -----MSAVVLTPLEARVAKCASI-RAAN-AMPVVVEEQAR-----GKAYESALARETTVAQLVAAVRFGFRGDAKKEVALTVAG-  
 LmjF09.0156 (1) -----MSAVVLTPLEARVAKCASI-RAAN-AMPVVVEEQAR-----GKAYESALARETTVAQLVAAVRFGFRGDAKKEVALTVAG-  
 LmjF09.0158 (1) -----MSAVVLTPLEARVAKCASI-RAAN-AMPVVVEEQAR-----GKAYESALARETTVAQLVAAVRFGFRGDAKKEVALTVAG-  
 LmjF09.0160 (1) -----MSAVVLTPLEARVAKCASI-RAAN-AMPVVVEEQAR-----GKAYESALARETTVAQLVAAVRFGFRGDAKKEVALTVAG-  
 LmjF09.0162 (1) -----MSAVVLTPLEARVAKCASI-RAAN-AMPVVVEEQAR-----GKAYESALARETTVAQLVAAVRFGFRGDAKKEVALTVAG-  
 LmjF09.0164 (1) -----MSAVVLTPLEARVAKCASI-RAAN-AMPVVVEEQAR-----GKAYESALARETTVAQLVAAVRFGFRGDAKKEVALTVAG-  
 LmjF09.0166 (1) -----MSAVVLTPLEARVAKCASI-RAAN-AMPVVVEEQAR-----GKAYESALARETTVAQLVAAVRFGFRGDAKKEVALTVAG-  
 LmjF09.0170 (1) -----MSAVVLTPLEARVAKCASI-RAAN-AMPVVVEEQAR-----GKAYESALARETTVAQLVAAVRFGFRGDAKKEVALTVAG-  
 LmjF09.0172 (1) -----MSAVVLTPLEARVAKCASI-RAAN-AMPVVVEEQAR-----GKAYESALARETTVAQLVAAVRFGFRGDAKKEVALTVAG-  
 LmjF09.0174 (1) -----MSAVVLTPLEARVAKCASI-RAAN-AMPVVVEEQAR-----GKAYESALARETTVAQLVAAVRFGFRGDAKKEVALTVAG-  
 LmjF09.0180 (1) -----MSAVVLTPLEARVAKCASI-RAAN-AMPVVVEEQAR-----GKAYESALARETTVAQLVAAVRFGFRGDAKKEVALTVAG-

$\alpha 4$        $\beta 4$       \*  
 ~~~~~#~~~~~  
 LmjF19.1630 (87) -SVPANSTLMSDLFNRYKDEE-FLYVITYSGENTYCGQLH-----
 LmjF22.1300 (154) DVLVPGNSLLGDLYRERYNADP-FLYLGLLENTFCGDVRSAAEHAASHTVRRPVQR-----
 Sc-ATG8 (83) -TLEPTNALMSALYQEHKDEE-FLYVITYSGENTYCR-----
 Hs-MAP-LC3A (86) HSMVSVSTFISEVSESEDEDE-FLYVMVASQETFCALAVTYMALKATATGREPCL-----
 Hs-GABARAP (83) -VIEPTSATMGQLYQEHHEDE-FLYIAYSDSEVYEL-----
 Hs-GATE-16 (83) -TVPCSSLIMGQLYKEDKDEE-FLYVAYSSENTFCF-----
 At-ATG8A (84) -TLEPTNALMSALYQEHKDEE-FLYMTYSGENTYCSLTV-----
 At-ATG8E (85) -VLEPTGELMSSVYEDKDEE-FLYIITYSGENTYCSSI-----
 At-ATG8H (82) -TLEPTNALMSVYESYKDDDE-FLVMCYSSSEKTF-----
 LmjF19.0840 (76) -CSPAANTVMGDI FDACKQVDS-FLHVS CARESSVGRDLCCFENTGKYFADIENNPDLGLSL
 LmjF19.0844 (76) -CSPAANTVMGDI FDACKQVDS-FLHVS CARESSVGRDLCCFENTGKYFADIENNPDLGLSL
 LmjF19.0848 (76) -CSPAANTVMGDI FDACKQVDS-FLHVS CARESSVGRDLCCFENTGKYFADIENNPDLGLSL
 LmjF19.0820 (78) -SFAVVAIVGDIADACKRQDS-FLYYSVRTQAMGFASPCLSLY-----
 LmjF19.0842 (78) -SFAVVAIVGDIADACKRQDS-FLYYSVRTQAMGFASPCLSLY-----
 LmjF19.0846 (78) -SFAVVAIVGDIADACKRQDS-FLYYSVRTQAMGFASPCFSVA-----
 LmjF19.0850 (78) -SFAVVAIVGDIADACKRQDS-FLYYSVRTQAMGFAGLCLFASDGGI-----
 LmjF19.0860 (78) -SFAVVAIVGDIADACKRQDS-FLYYSVRTQAMGFAGLCLFASDGGI-----
 LmjF19.0870 (78) -SFAVVAIVGDIADACKRQDS-FLYYSVRTQAMGFASPCFSVA-----
 LmjF19.0880 (78) -SFAVVAIVGDIADACKRQDS-FLYYSVRTQAMGFAGLCLFASDGGI-----
 LmjF19.0900 (78) -SFAVVAIVGDIADACKRQDS-FLYYSVRTQAMGFASPCFSVA-----
 LmjF09.0150 (77) -CSVSPSATLGE LHDACRQADGDMLYVAYTAERCMAAVCTPCSSCWDGSSADDDVII-----
 LmjF09.0152 (77) -CSVSPSATLGE LHDACRQADGDMLYVAYTAERCMAAVCTPCSSCWN-----
 LmjF09.0154 (77) -CSVSPSATLGE LHDACRQADGDMLYVAYTAERCMAAVCTPCSSCWDGSSADDDVII-----
 LmjF09.0156 (77) -CSVSPSATLGE LHDACRQADGDMLYVAYTAERCMAAVCMSCSCLLN-----
 LmjF09.0158 (77) -CSVSPSATLGE LHDACRQADGDMLYVAYTAERCMAAVCTPCSSCWDGSSADDDVII-----
 LmjF09.0160 (77) -CSVSPSATLGE LHDACRQADGDMLYVAYTAERCMAAVCMSCSCLLN-----
 LmjF09.0162 (77) -CSVSPSATLGE LHDACRQADGDMLYVAYTAERCMAAVCTPCSSCWDGSSADDDVII-----
 LmjF09.0164 (77) -CSVSPSATLGE LHDACRQADGDMLYVAYTAERCMAAVCTPCSSCWN-----
 LmjF09.0166 (77) -CSVSPSATLGE LHDACRQADGDMLYVAYTAERCMAAVCTPCSSCWDGSSADDDVII-----
 LmjF09.0170 (77) -CSVSPSATLGE LHDACRQADGDMLYVAYTAERCMAAVCTPCSSCWDGSSADDDVII-----
 LmjF09.0172 (77) -CSVSPSATLGE LHDACRQADGDMLYVAYTAERCMAAVCMSCSCLLN-----
 LmjF09.0174 (77) -CSVSPSATLGE LHDACRQADGDMLYVAYTAERCMAAVCMPCDSCWDGSSADDDVII-----
 LmjF09.0180 (77) -CSVSPSATLGE LHDACRQADGDMLYVAYTAERCMAAVCTPCFLCA-----

Insertion

(ii)

LmjF22.1300 (39) TVPVIIVEPTESHLRLSPPSPPLHGESKSSAGSAGGPGGYARHGQLSSSGRAGAGVTAASLASAFTSPSAKSTLKCILLKSKSVA
 Sc-ATG12 (73) SSGTYIQEETIKTNAQTSKQKS-----HKDEKNIQIKIQFPIGSIQQLKPSVCKTMSMSQSF
 Hs-ATG12 (62) IAAGGGLTLDVSPETTTPEPPSSA-----AVSPGTEEPAGDKKKIDILLKAVGDTPIKTKKVVETRIQ
 At-ATG12 (11) VRRVWHLRATG-----GAPILKOSKFKIPKIDKFA
 LmjF19.1630 (33) ILMICKEKENSVPVDDLK-----SKFLVPPDALTGG-
 Sc-ATG8 (29) IPVICKEKESDIPEIDK-----K-----RKYLVPADLVG-
 Hs-MAP-LC3A (31) IPVILIRYKGEK-----QLPVLDKTKFLVDPHVNMS-
 Hs-GATE-16 (29) VPIVIVEKVGSGQIVDIDK-----K-----RKYLVPDITVA-
 Hs-GABARAP (29) VPIVIVEKPKARIGDLDK-----K-----KKYLVPDILVGG-
 At-ATG8A (30) IPVIVEKKGQSDVPDIDK-----K-----KKYLVPADLVG-
 At-ATG8E (31) IPVIVEKKEKSEVPNIDK-----K-----KKYLVPDILVGG-
 At-ATG8H (28) IPVIAEKYCKTDLPAIE-----KFLVPRDMSVGG-
 LmjF19.0840 (28) LPPVVESNN-----SSHVRELAVQRDAIVADLENEFQALGRTNKK-VLALAEF-
 LmjF19.0820 (28) AAHVVEAEKA-----GSKVHELALPRDAIVAELEAAVRQALGTSARE-VTLAEF-
 LmjF09.0150 (26) VPVVVEEQAR-----GKAYESALARETTVAQLVAAVRFGFRGDAKKEVALTVAG-

Figure S2

B

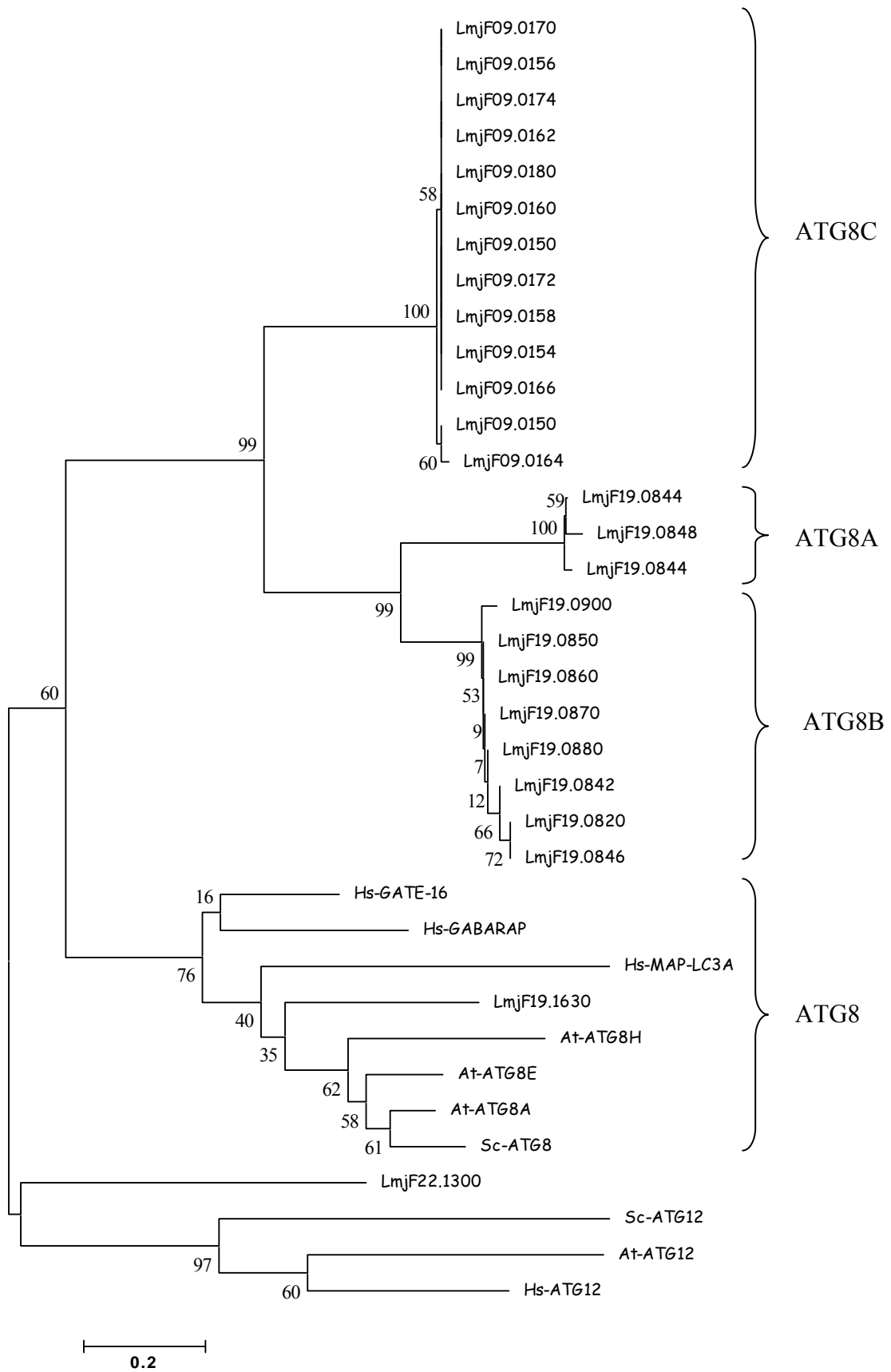


Figure S2

Table S1. Plasmids used in this study**(i) For transforming *E. coli***

| Plasmid | Characteristic | Primer combination |
|------------------------------------|---|---|
| pET21a ⁺ ATG4.1 | Plasmid containing a 1.1 kb ORF of LmjF32.3890 modified with the <i>NdeI/XhoI</i> restriction sites. | NT158 5'CAT ATG GGC ACG AAC GCC AAA GTG GCA GAG3'
NT5159 5'CTC GAG GCT CGG TGG AGA AGA GAT TGA ATT CGA GC3' |
| pET21a ⁺ ATG4.2 | Plasmid containing a 1.2 kb ORF of LmjF30.0270 modified with the <i>NdeI/XhoI</i> restriction sites. | NT160 5'CAT ATG CTC CGC TAC GTG CAA GAT T3'
NT161 5'CTC GAG ATC CAG ATA CTC CCA CGA A3' |
| pET28a ⁺ ATG8-HA | Plasmid containing a 0.39 kb ORF of LmjF19.1630 containing the HA tag and modified with the <i>NdeI/XhoI</i> restriction sites. | NT208 5'CAT ATG TCT TCC AGA GTA GCT GGG TGG TA3'
NT209 5' CTC GAG TCA AGC GTA GTG TGG GAC GTC GTA TGG GTA GTG CAG CCC CTG CCC GCC GTA3' |
| pET28a ⁺ ATG8A-HA | Plasmid containing a 0.42 kb ORF of LmjF19.0840 containing the HA tag and modified with the <i>NdeI/XhoI</i> restriction sites. | NT212 5'CAT ATG TCC ATG TAC CAG TCG CTG ATC CCT GCC G3'
NT213 5'CTC GAG TCA AGC GTA GTG TGG GAC GTG GTA TGG GTA CAA GGA GCC AAG CAG GTC CGG GT3' |
| pET28a ⁺ ATG8B-HA | Plasmid containing a 0.38 kb ORF of LmjF19.0850 containing the HA tag and modified with the <i>NdeI/XhoI</i> restriction sites. | NT210 5' CAT ATG TCC GCC TAC CAC AGC AGC AAC C3'
NT211 5' CTC GAG TCA AGC GTA GTG TGG GAC GTC GTA TGG GTA AGC GAC GGA GAA GCA CGG ACT C3' |
| pET28a ⁺ ATG8C-HA | Plasmid containing a 0.42 kb ORF of LmjF09.0150 containing the HA tag and modified with the <i>NdeI/XhoI</i> restriction sites. | NT390 5'ATC GTA TGG GTA AAT GAT CAC GTC ATC GTC CGC AGA GCT GC3'
NT391 5' TGG CAT ATG TCC GCC TAC GTG TTG TCG ACG CCG CTG GAG3' |
| pET28a ⁺ LmjF22.1300-HA | Plasmid containing a 0.63 kb ORF of LmjF22.1300 containing the HA tag and modified with the <i>NdeI/XhoI</i> restriction sites. | NT388 5' AAC ATC GTA TGG GTA CCG CTG CAC CGG TCG CCT CAC CGT ATG AG3'
NT389 5' TGG CAT ATG CAC GCG CCA CCA CAG CCG CCG CCG CCA CCT CG3' |

(ii) For transfecting *L. major*.

| Plasmid | Characteristic | Primer combination |
|-------------------|---|--|
| pGLATG4.2-HYG5'3' | Plasmid containing the 5' and 3' flanks of <i>L. major</i> ATG4.2 gene (LmjF30.0270) for genetic manipulation. | Besteiro <i>et al.</i> , 2006 |
| pGLATG4.2-BSD5'3' | Plasmid containing the 0.39 kb ORF of LmjF19.1630 modified with the <i>BglIII/XhoI</i> restriction sites for cloning into the pNUS-nHGFP vector. | Besteiro <i>et al.</i> , 2006 |
| pN-[GFP-ATG8] | Plasmid containing the 0.42 kb ORF of LmjF19.0849 modified with the <i>BglIII/KpnI</i> restriction sites for cloning into the pNUS-nGFP vector. | NT250 5'AGA TCT ATG TCC ATG TAC CAG TCG CTG ATC CCT GCC GAC3'
NT251 5'GGT ACC CAA GGA GCC AAG CAG GTC CGG GTT ATT CTC3' |
| pN-[GFP-ATG8A] | Plasmid containing the 0.38 kb ORF of LmjF19.0850 modified with the <i>BglIII/KpnI</i> restriction sites for cloning into the pNUS-nHGFP vector. | NT252 5'AGA TCT ATG TCC GCC TAC CAC AGC AGC AAC CCT GTC GAG GCC C3'
NT253 5'GGT ACC AGC GAC GGA GAA GCA CGG ACT CGC AAA GGC3' |
| pN-[GFP-ATG8B] | Plasmid containing the 0.42 kb ORF of LmjF09.0150 modified with the <i>BglIII/XhoI</i> restriction sites for cloning into the pNUS-nHGFP vector. | NT256 5'AGA TCT ATG TCC GCC TAC GTG TTG TCG ACG CCG CTG3'
NT257 5'CTC GAG AAT GAT CAC GTC ATC GTC CGC AGA GCT GCC3' |
| pN-[GFP-ATG8C] | Plasmid containing the 0.63 kb ORF of LmjF22.1300 modified with the <i>BglIII/XhoI</i> restriction sites for cloning into the pNUS-nHRFP vector. | NT350 5'CTC GAG TCA CCG CTG CAC CGG TCG CC3'
NT351 5'AGA TCT ATG CAC GCG CCA CCA CAG CC3' |
| pN[RFP-ATG12] | Plasmid containing the 1.39 kb ORF of LmjF30.0270 modified with the <i>NdeI/XhoI</i> restriction sites for cloning into the pNUS-nHGFP vector and used to replace GFP in the plasmid. | Besteiro <i>et al.</i> , 2006 |

(iii) For transforming yeast strains.

| Plasmid | Characteristic | Primer combination |
|----------------|--|--|
| pCM185[ATG5] | Plasmid containing a 1.38 kb ORF of LmjF30.0980 and modified with the <i>BamHI/Not1</i> restriction sites. | NT364 5'GGA TCC ATG CTT CTC GCC ATT GTG CGG GAC CTC CTT3'
NT365 5'GCG GCC GCT CAC ACC TGA ACC GTG ACA AAA AT3' |
| pCM185[ATG10] | Plasmid containing a 0.78 kb ORF of LmjF31.3105 and modified with the <i>BamHI/Not1</i> restriction sites. | NT362 5'GGA TCC GAC TGC TCT GAG GCG GTC CTC GTG3'
NT363 5'GCG GCC GCT CAT GCA CGT GCG GTA TCG AA3' |
| pCM185[ATG8] | Plasmid containing a 0.39 kb ORF of LmjF19.1630 (LmjATG8) and modified with the <i>BamHI/Not1</i> restriction sites. | NT352 5'GGA TCC ATG TCT TCC AGA GTA GCT GGG TCG TAC AAG3'
NT353 5'GCG GCC GCC TAG TGC AGC CCC TGC CC3' |
| pCM185[ATG8g] | Plasmid containing a 0.39 kb ORF of LmjF19.1630 (LmjATG8) terminating at the scissile glycine and modified with the <i>BamHI/Not1</i> restriction sites. | NT352 5'GGA TCC ATG TCT TCC AGA GTA GCT GGG TCG TAC AAG3'
NT357 5'GCG GCC GCC TAG TGC AGC CCC TGC CC3' |
| pCM185[ATG8A] | Plasmid containing a 0.42 kb ORF of LmjF19.0840 (LmjATG8A.1) and modified with the <i>BamHI/Not1</i> restriction sites. | NT354 5'GGA TCC ATG TCC ATG TAC CAG TGC CTG ATC CCT GCC GAC3'
NT355 5'GCG GCC GCT TAC AAG GAG CCA AGC AGG TCC GGG TTA TTC TC3' |
| pCM185[ATG8B] | Plasmid containing a 0.38 kb ORF of LmjF19.0850 (LmjATG8B.4) and modified with the <i>BamHI/Not1</i> restriction sites. | NT356 5'GGAT CCA TGT CCG CCT ACC ACA GCA GCA ACC CTG TCG AGG CCC3'
NT367 5'GCG GCC GCC TAA GCG ACG GAG AAG CAC GGA CTC GCA AAG GC3' |
| pCM185[ATG8C] | Plasmid containing a 0.42 kb ORF of LmjF09.0150 (LmjATG8C.1) and modified with the <i>BamHI/Not1</i> restriction sites. | NT358 5'GGA TCC ATG TCC GCC TAC GTG TTG TCG ACG CCG CTG3'
NT359 5'GCG GCC GCT TAA ATG ATC ACG TCA TCG TCC GCA GAG CTG CC3' |
| pCM185[ATG12] | Plasmid containing a 0.63 kb ORF of LmjF22.1300 and modified with the <i>BamHI/Not1</i> restriction sites. | NT360 5'GGA TCC ATG CAC GCG CCA CCA CAG CC3'
NT361 5'GCG GCC GCT CAC CGC TGC ACC GGT CGC C3' |
| pCM185[ATG12g] | Plasmid containing a 0.63 kb ORF of LmjF22.1300 terminating at the scissile glycine and modified with the <i>BamHI/Not1</i> restriction sites. | NT360 5'GGA TCC ATG CAC GCG CCA CCA CAG CC3'
NT368 5' GCG GCC TCC GCC GAA GGT GTT TTC CAG CAA GTA3' |

* pCM vectors were obtained from EUROSCARF

* described previously in Besteiro *et al.*, 2006

Table S2. Strains used in this study**(i) *L. major***

| Strain | Genotype and characteristics | Reference |
|-------------------------------|--|-------------------------------|
| <i>Δatg4.2</i> | <i>L. major</i> deficient in the cysteine peptidase ATG4.2 | Besteiro <i>et al.</i> , 2006 |
| <i>Δatg4.2</i> [pN-GFP-ATG8] | <i>L. major Δatg4.2</i> transfected with pN- GFP-ATG8 | Besteiro <i>et al.</i> , 2006 |
| <i>Δatg4.2</i> [pN-GFP-ATG8A] | <i>L. major Δatg4.2</i> transfected with pN-GFP-ATG8A | This study |
| <i>Δatg4.2</i> [pN-GFP-ATG8B] | <i>L. major Δatg4.2</i> transfected with pN-GFP-ATG8B | This study |
| <i>Δatg4.2</i> [pN-GFP-ATG8C] | <i>L. major Δatg4.2</i> transfected with pN-GFP-ATG8C | This study |
| WT[pN-GFP-ATG8] | <i>L. major</i> transfected with pN-GFP-ATG8 | Besteiro <i>et al.</i> , 2006 |
| WT[pN-GFP-ATG8A] | <i>L. major</i> transfected with pN-GFP-ATG8A | This study |
| WT[pN-GFP-ATG8B] | <i>L. major</i> transfected with pN-GFP-ATG8B | This study |
| WT[pN-GFP-ATG8C] | <i>L. major</i> transfected with pN-GFP-ATG8C | This study |
| WT[pN-ATG4.2/pN-GFP-ATG8] | <i>L. major</i> co-transfected with pN-GFP-ATG8 and pN-ATG4.2 | This study |
| WT[pN-GFP-ATG8/pN-RFP-ATG12] | <i>L. major</i> co-transfected with pN-GFP-ATG8 and pN-RFP-ATG12 | This study |

(ii) *E. coli*

| Strain | Genotype and characteristics | Reference |
|-------------------------------|---|------------------|
| BL21-ATG4.1-His | BL21(DE3) transformed with pET21a ⁺ ATG4.1 | This study |
| BL21-ATG4.2-His | BL21(DE3) transformed with pET21a ⁺ ATG4.2 | This study |
| BL21-His-ATG8-HA | BL21(DE3) transformed with pET28a ⁺ His-ATG8-HA | This study |
| BL21-His-ATG8A-HA | BL21(DE3) transformed with pET28a ⁺ ATG8A-HA | This study |
| BL21-His-ATG8B-HA | BL21(DE3) transformed with pET28a ⁺ ATG8B-HA | This study |
| BL21-His-ATG8C-HA | BL21(DE3) transformed with pET28a ⁺ ATG8C-HA | This study |
| BL21-His-ATG8-HA[ATG4.1-His] | BL21(DE3) co-transformed with pET28a ⁺ ATG8-HA and pET21a ⁺ ATG4.1 | This study |
| BL21-His-ATG8-HA[ATG4.2-His] | BL21(DE3) co-transformed with pET28a ⁺ ATG8-HA and pET21a ⁺ ATG4.2 | This study |
| BL21-His-ATG8A-HA[ATG4.1-His] | BL21(DE3) co-transformed with pET28a ⁺ ATG8A-HA and pET21a ⁺ ATG4.1 | This study |
| BL21-His-ATG8A-HA[ATG4.2-His] | BL21(DE3) co-transformed with pET28a ⁺ ATG8A-HA and pET21a ⁺ ATG4.2 | This study |
| BL21-His-ATG8B-HA[ATG4.1-His] | BL21(DE3) co-transformed with pET28a ⁺ ATG8B-HA and pET21a ⁺ ATG4.1 | This study |
| BL21-His-ATG8B-HA[ATG4.2-His] | BL21(DE3) co-transformed with pET28a ⁺ ATG8B-HA and pET21a ⁺ ATG4.2 | This study |
| BL21-His-ATG8C-HA[ATG4.1-His] | BL21(DE3) co-transformed with pET28a ⁺ ATG8C-HA and pET21a ⁺ ATG4.1 | This study |
| BL21-His-ATG8C-HA[ATG4.2-His] | BL21(DE3) co-transformed with pET28a ⁺ ATG8C-HA and pET21a ⁺ ATG4.2 | This study |

(iii) Yeast stains obtained from EUROSCARF

| Name | Strain | Genotype |
|---------|----------------|--|
| YPL149w | <i>atg5</i> Δ | BY4741; Mat a; his3Δ0; leuΔ0; met15Δ0; ura3Δ0; YPL149w::KANmx4 |
| YBL078c | <i>atg8</i> Δ | BY4741; Mat a; his3Δ0; leuΔ0; met15Δ0; ura3Δ0; YBL078C::KANmx4 |
| YLL047c | <i>atg10</i> Δ | BY4741; Mat a; his3Δ0; leuΔ0; met15Δ0; ura3Δ0; YLL047c::KANmx4 |
| YBR217c | <i>atg12</i> Δ | BY4741; Mat a; his3Δ0; leuΔ0; met15Δ0; ura3Δ0; YBR217c::KANmx4 |
| Y00000 | Wild type | BY4741; Mat a; his3Δ1; leu2Δ0; met15Δ0 |

(iv) Yeast strains generated for this study

| Strain | Genotype and characteristics | Reference |
|--------------------------------|--|------------|
| <i>atg5</i> Δ [pCM185-ATG5] | <i>atg5</i> Δ transformed with pCM185-LmjATG5 [LmjF30.0980] | This study |
| <i>atg10</i> Δ [pCM-ATG10] | <i>atg10</i> Δ transformed with pCM185-LmjATG10 [LmjF31.3105] | This study |
| <i>atg8</i> Δ [pCM185-ATG8] | <i>atg8</i> Δ transformed with pCM185-ATG8 [LmjF19.1630] | This study |
| <i>atg8</i> Δ [pCM185-ATG8g] | <i>atg8</i> Δ transformed with pCM185-ATG8 [LmjF19.1630 terminating at the scissile glycine residue] | This study |
| <i>atg8</i> Δ [pCM185-ATG8A] | <i>atg8</i> Δ transformed with pCM185-ATG8 [LmjF19.0840] | This study |
| <i>atg8</i> Δ [pCM185-ATG8B] | <i>atg8</i> Δ transformed with pCM185-ATG8 [LmjF19.0850] | This study |
| <i>atg8</i> Δ [pCM185-ATG8C] | <i>atg8</i> Δ transformed with pCM185-ATG8 [LmjF09.0150] | This study |
| <i>atg8</i> Δ [pCM185-ATG12] | <i>atg8</i> Δ transformed with pCM185-ATG12 [LmjF22.1300] | This study |
| <i>atg8</i> Δ [pCM185-ATG12g] | <i>atg8</i> Δ transformed with pCM185-ATG12 [LmjF22.1300 terminating at the scissile glycine residue] | This study |
| <i>atg12</i> Δ [pCM185-ATG8] | <i>atg12</i> Δ transformed with pCM185-ATG8 [LmjF19.1630] | This study |
| <i>atg12</i> Δ [pCM185-ATG8g] | <i>atg12</i> Δ transformed with pCM185-ATG8 [LmjF19.1630 terminating at the scissile glycine residue] | This study |
| <i>atg12</i> Δ [pCM185-ATG8A] | <i>atg12</i> Δ transformed with pCM185-ATG8 [LmjF19.0840] | This study |
| <i>atg12</i> Δ [pCM185-ATG8B] | <i>atg12</i> Δ transformed with pCM185-ATG8 [LmjF19.0850] | This study |
| <i>atg12</i> Δ [pCM185-ATG8C] | <i>atg12</i> Δ transformed with pCM185-ATG8 [LmjF09.0150] | This study |
| <i>atg12</i> Δ [pCM185-ATG12] | <i>atg12</i> Δ transformed with pCM185-ATG12 [LmjF22.1300] | This study |
| <i>atg12</i> Δ [pCM185-ATG12g] | <i>atg12</i> Δ transformed with pCM185-ATG12 [LmjF22.1300 terminating at the scissile glycine residue] | This study |

Sequences alignments and phylogenetic analyses

Amino acid sequences from the ATG8 genes were aligned using the Clustal W algorithm with AlignX program included in the Vector NTI 10.1 package (<http://www.invitrogen.com>). The data set were converted to a multiple sequence file (MSF) format and the MEGA2 software ⁶ used to generate a phylogenetic tree using the Neighbor Joining method and Poisson-corrected amino acid distance. The reliability of clustering patterns in the tree was tested by bootstrapping (100 pseudoreplicates).

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