Figure S1, related to Fig. 1.

Evolution of Atg13 and Atg101 in the eukarya. Phylogenetic trees for Atg13 (A) and Atg101 (B). Species lacking Atg101 are colored in red. Phylogenetic trees were generated using the iTOL (http://itol.embl.de/) and Phylogeny.fr (http://www.phylogeny.fr) servers.

Figure S2, related to Fig. 2.

Structure based sequence alignments of Atg13 were carried out by ClustalW2 and PROMALS3D. Hm:*Homo sapiens*; Bt: *Bos taurus*; Mm: *Mus musculus*; Xt: *Xenopus tropicalis*; Da: *Danio rerio*; Dm: *Drosophila melanogaster*; Sp: *Schizosaccharomyces pombe*; Sc:*Saccharomyces cerevisiae*; Lt: *Lachancea thermotolerans*. Green numbers in parentheses indicates the number of unshown residues inserted relative to the human sequences. Basic residues of the interface are labeled in blue while the hydrophobic residues and polar uncharged residues are labeled as orange. “Δ” indicates the region of Atg13 that was deleted in αA-αB connector mutation. “*” indicates the residues mutated in this paper. “H” on top of the sequences indicates the residues contribute to hydrogen bonds discussed in the text. “+” shows residues that contribute to the positively charged groove. The binding sites of benzamidine are colored in magenta. The αA-αB connector of Atg13 is colored light orange.

Figure S3, related to Fig. 2.

Structure based sequence alignments of Atg101 were carried out by ClustalW2 and PROMALS3D. “W” indicates the residues that sequester the WF finger. The WF finger residues are colored red. Other labels are as described for Fig. S1.

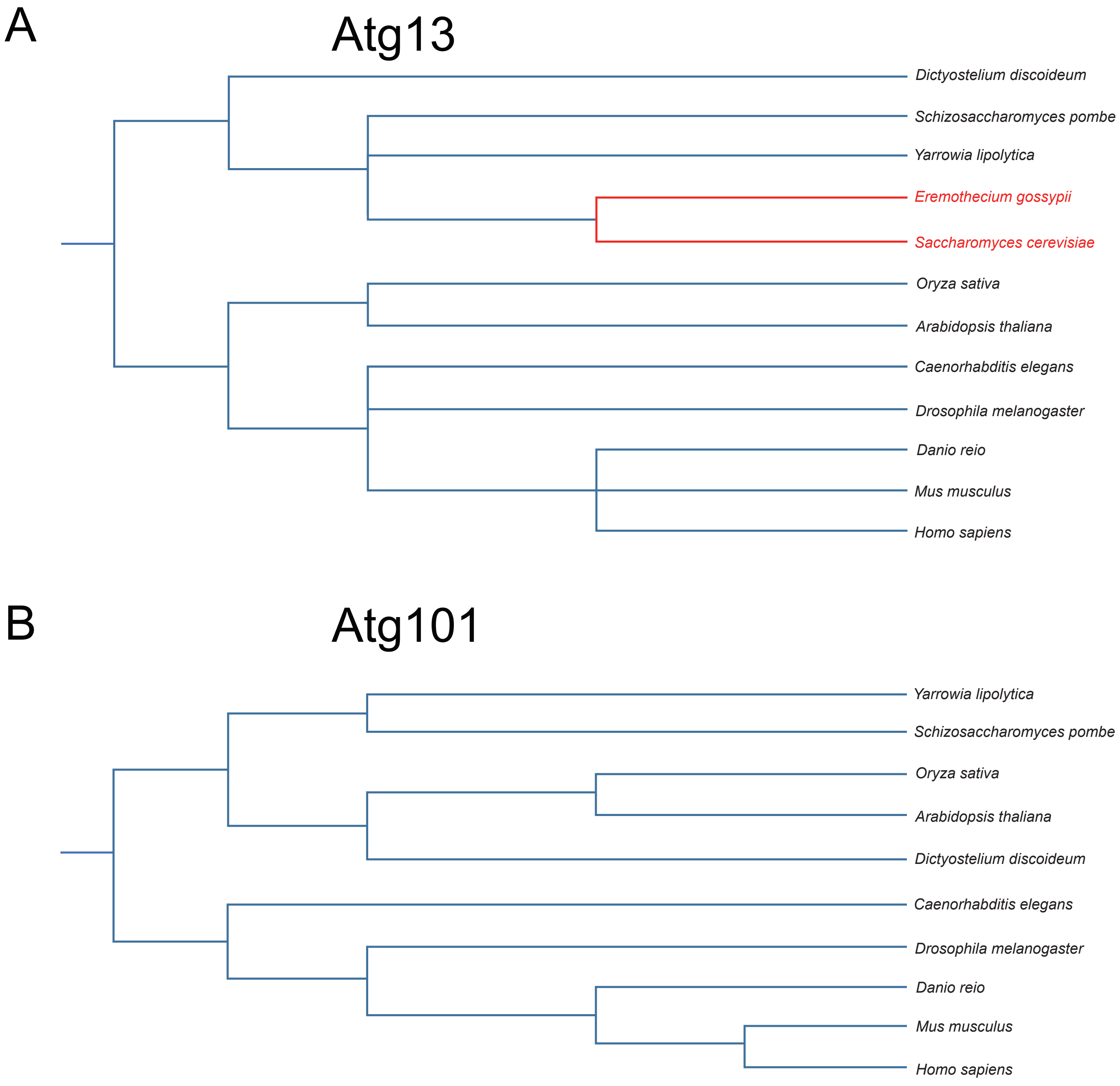
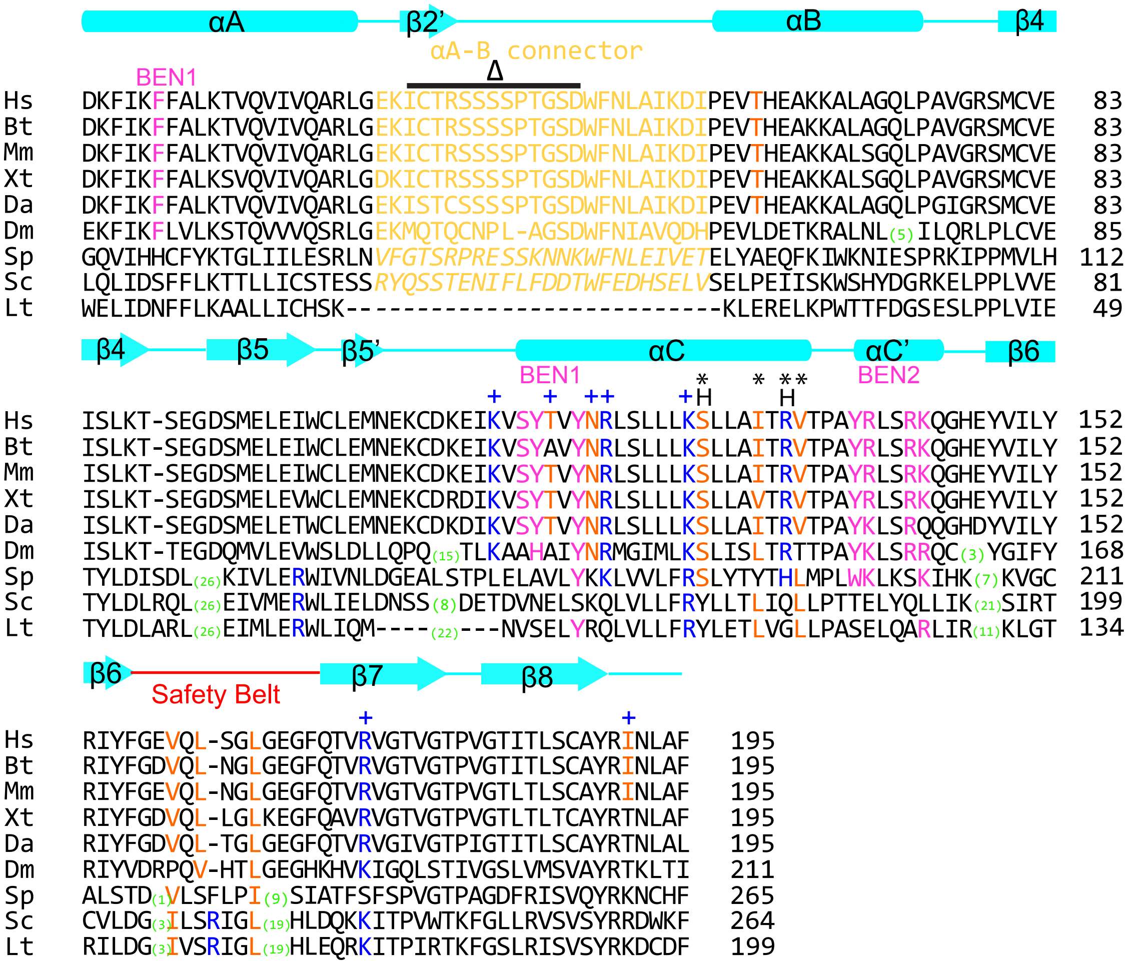

A
Atg13
Dictyostelium discoideum
Schizosaccharomyces pombe
Yarrowia lipolytica
Eremothecium gossypii
Saccharomyces cerevisiae
Oryza sativa
Arabidopsis thaliana
Caenorhabditis elegans
Drosophila melanogaster
Danio reio
Mus musculus
Homo sapiens
B
Atg101
Yarrowia lipolytica
Schizosaccharomyces pombe
Oryza sativa
Arabidopsis thaliana
Dictyostelium discoideum
Caenorhabditis elegans
Drosophila melanogaster
Danio reio
Mus musculus
Homo sapiens

Figure S1



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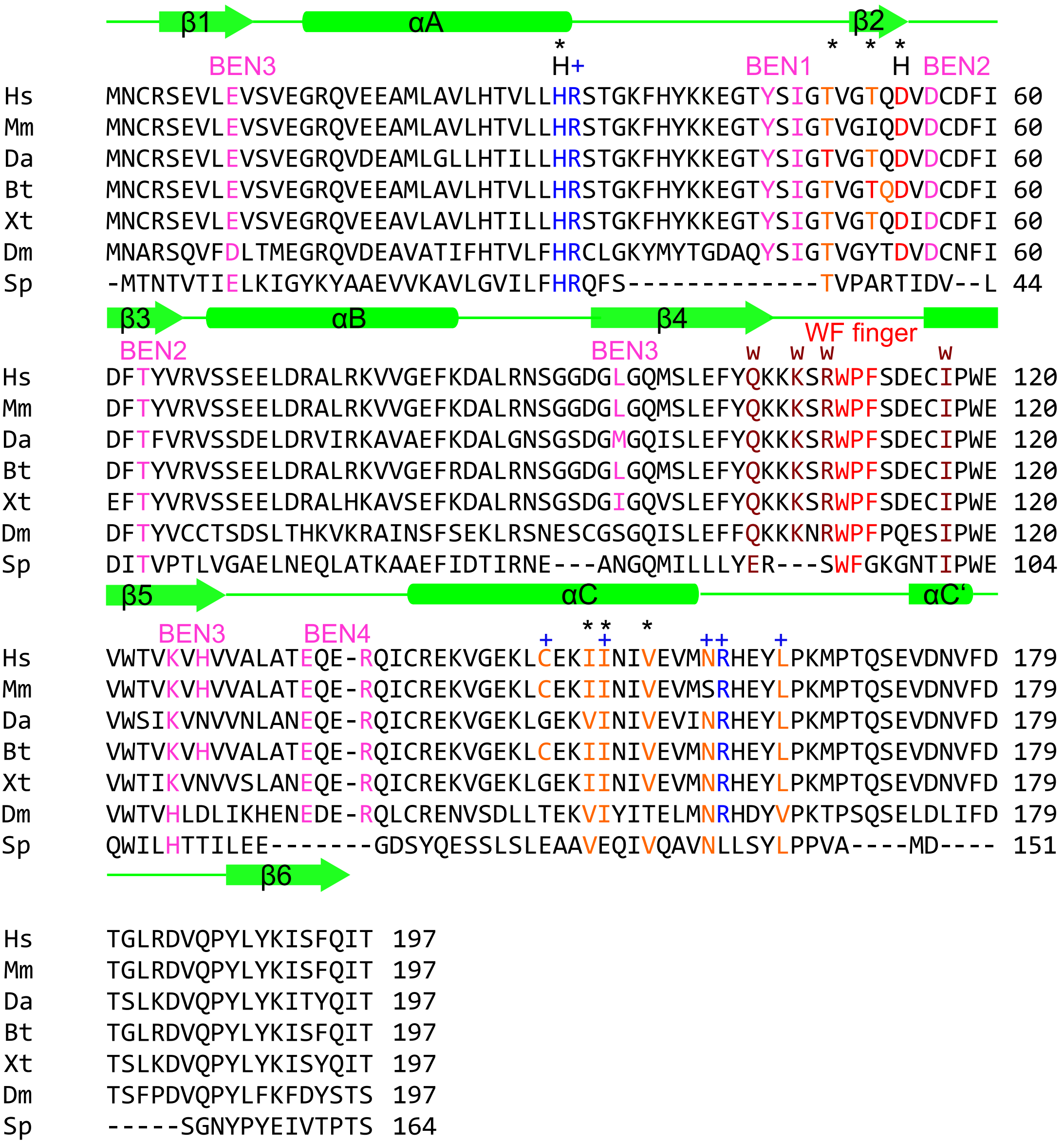
                                              BEN1
Hs    DKFIKFFALKTVQVIVQARLGEKICTRSSSSPTGSDWFNLAIKDIPEVTHEAKKALAGQLPAVGRSMCVE   83
Bt    DKFIKFFALKTVQVIVQARLGEKICTRSSSSPTGSDWFNLAIKDIPEVTHEAKKALAGQLPAVGRSMCVE   83
Mm    DKFIKFFALKTVQVIVQARLGEKICTRSSSSPTGSDWFNLAIKDIPEVTHEAKKALSGQLPAVGRSMCVE   83
Xt    DKFIKFFALKSVQVIVQARLGDKICTRSSSSPTGSDWFNLAIKDIPEVTHEAKKALSGQLPAVGRSMCVE   83
Da    DKFIKFFALKTVQVIVQARLGEKISTCSSSSPTGSDWFNLAIKDIPEVTHEAKKALAGQLPGIGRSMCVE   83
Dm    EKFIKFLVLKSTQVVVQSRLGEKMQTQCNPL-AGSDWFNIAVQDHPEVLDETKRALNL(5)ILQRLPLCVE   85
Sp    GQVIHHCFYKTGLIILESRLNVFGTSRPRESSKNNKWFNLEIVETELYAEQFKIWKNIESPRKIPPMVLH  112
Sc    LQLIDSFFLKTTLLICSTESSRYQSSTENIFLFDDTWFEDHSELVSELPEIISKWSHYDGRKELPPLVVE   81
Lt    WELIDNFFLKAALLICHSK--------------------------KLERELKPWTTFDGSESLPPLVIE   49

                                                  *     * **
                                      +    +  ++     +H       H
Hs    ISLKT-SEGDSMELEIWCLEMNEKCDKEIKVSYTVYNRLSLLLKSLLAITRVTPAYRLSRKQGHEYVILY  152
Bt    ISLKT-SEGDSMELEIWCLEMNEKCDKEIKVSYAVYNRLSLLLKSLLAITRVTPAYRLSRKQGHEYVILY  152
Mm    ISLKT-SEGDSMELEIWCLEMNEKCDKEIKVSYTVYNRLSLLLKSLLAITRVTPAYRLSRKQGHEYVILY  152
Xt    ISLKT-SEGDSMELEVWCLEMNEKCDRDIKVSYTVYNRLSLLLKSLLAVTRVTPAYRLSRKQGHEYVILY  152
Da    ISLKT-SEGDSMELETWCLEMNEKCDKDIKVSYTVYNRLSLLLKSLLAITRVTPAYKLSRQQGHDYVILY  152
Dm    ISLKT-TEGDQMVLEVWSLDLLQPQ(15)TLKAAHAIYNRMGIMLKSLISLTRTTPAYKLSRRQC(3)YGIFY  168
Sp    TYLDISDL(26)KIVLERWIVNLDGEALSTPLELAVLYKKLVVLFRSLYTYTHLMPLWKLKSKIHK(7)KVGC  211
Sc    TYLDLRQL(26)EIVMERWLIELDNSS(8)DETDVNELSKQLVLLFRYLLTLIQLLPTTELYQLLIK(21)SIRT  199
Lt    TYLDLARL(26)EIMLERWLIQM----(22)---NVSELYRQLVLLFRYLETLVGLLPASELQARLIR(11)KLGT  134

                              +                        +
Hs    RIYFGEVQL-SGLGEGFQTVRVGTVGTPVGTITLSCAYRINLAF   195
Bt    RIYFGDVQL-NGLGEGFQTVRVGTVGTPVGTITLSCAYRINLAF   195
Mm    RIYFGEVQL-NGLGEGFQTVRVGTVGTPVGTLTLSCAYRINLAF   195
Xt    RIYFGDVQL-LGLKEGFQAVRVGTVGTPVGTLTLTCAYRTNLAF   195
Da    RIYFGDVQL-TGLGEGFQTVRVGIVGTPIGTITLSCAYRTNLAL   195
Dm    RIYVDRPQV-HTLGEGHKHVKIGQLSTIVGSLVMSVAYRTKLTI   211
Sp    ALSTD(1)VLSFLPI(9)SIATFSFSPVGTPAGDFRISVQYRKNCHF   265
Sc    CVLDG(3)ILSRIGL(19)HLDQKKITPVWTKFGLLRVSVSYRRDWKF   264
Lt    RILDG(3)IVSRIGL(19)HLEQRKITPIRTKFGSLRISVSYRKDCDF   199
```

Figure S2



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Hs     MNCRSEVLEVSVEGRQVEEAMLAVLHTVLLHRSTGKFHYKKEGTYSIGTVGTQDVDCDFI 60
Mm     MNCRSEVLEVSVEGRQVEEAMLAVLHTVLLHRSTGKFHYKKEGTYSIGTVGIQDVDCDFI 60
Da     MNCRSEVLEVSVEGRQVDEAMLGLLHTILLHRSTGKFHYKKEGTYSIGTVGTQDVDCDFI 60
Bt     MNCRSEVLEVSVEGRQVEEAMLAVLHTVLLHRSTGKFHYKKEGTYSIGTVGTQDVDCDFI 60
Xt     MNCRSEVLEVSVEGRQVEEAVLAVLHTILLHRSTGKFHYKKEGTYSIGTVGTQDIDCDFI 60
Dm     MNARSQVFDLTMEGRQVDEAVATIFHTVLFHRCLGKYMYTGDAQYSIGTVGYTDVDCNFI 60
Sp     -MTNTVTIELKIGYKYAAEVVKAVLGVILFHRQFS-------------TVPARTIDV--L 44

Hs     DFTYVRVSSEELDRALRKVVGEFKDALRNSGGDGLGQMSLEFYQKKKSRWPFSDECIPWE 120
Mm     DFTYVRVSSEELDRALRKVVGEFKDALRNSGGDGLGQMSLEFYQKKKSRWPFSDECIPWE 120
Da     DFTFVRVSSDELDRVIRKAVAEFKDALGNSGSDGMGQISLEFYQKKKSRWPFSDECIPWE 120
Bt     DFTYVRVSSEELDRALRKVVGEFRDALRNSGGDGLGQMSLEFYQKKKSRWPFSDECIPWE 120
Xt     EFTYVRVSSEELDRALHKAVSEFKDALRNSGSDGIGQVSLEFYQKKKSRWPFSDECIPWE 120
Dm     DFTYVCCTSDSLTHKVKRAINSFSEKLRSNESCGSGQISLEFFQKKKNRWPFPQESIPWE 120
Sp     DITVPTLVGAELNEQLATKAAEFIDTIRNE---ANGQMILLLYER---SWFGKGNTIPWE 104

Hs     VWTVKVHVVALATEQE-RQICREKVGEKLCEKIINIVEVMNRHEYLPKMPTQSEVDNVFD 179
Mm     VWTVKVHVVALATEQE-RQICREKVGEKLCEKIINIVEVMSRHEYLPKMPTQSEVDNVFD 179
Da     VWSIKVNVVNLANEQE-RQICREKVGEKLGEKVINIVEVINRHEYLPKMPTQSEVDNVFD 179
Bt     VWTVKVHVVALATEQE-RQICREKVGEKLCEKIINIVEVMNRHEYLPKMPTQSEVDNVFD 179
Xt     VWTIKVNVVSLANEQE-RQICREKVGEKLGEKIINIVEVMNRHEYLPKMPTQSEVDNVFD 179
Dm     VWTVHLDLIKHENEDE-RQLCRENVSDLLTEKVIYITELMNRHDYVPKTPSQSELDLIFD 179
Sp     QWILHTTILEE-------GDSYQESSLSLEAAVEQIVQAVNLLSYLPPVA----MD---- 151

Hs     TGLRDVQPYLYKISFQIT 197
Mm     TGLRDVQPYLYKISFQIT 197
Da     TSLKDVQPYLYKITYQIT 197
Bt     TGLRDVQPYLYKISFQIT 197
Xt     TSLKDVQPYLYKISYQIT 197
Dm     TSFPDVQPYLFKFDYSTS 197
Sp     -----SGNYPYEIVTPTS 164
```

Figure S3