Supplementary Information

Structure of the human ATG12~ATG5 conjugate required for LC3 lipidation in autophagy

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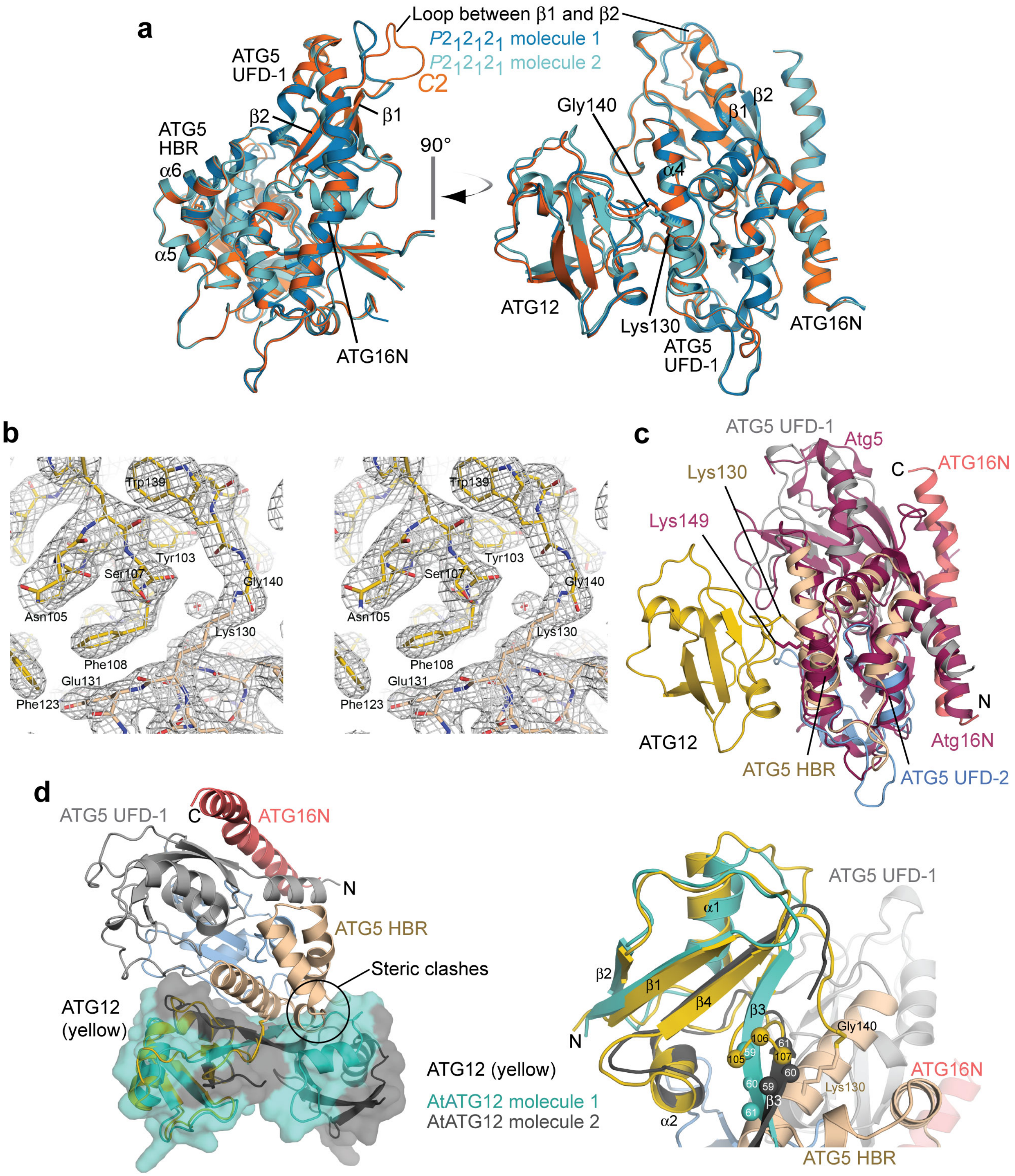
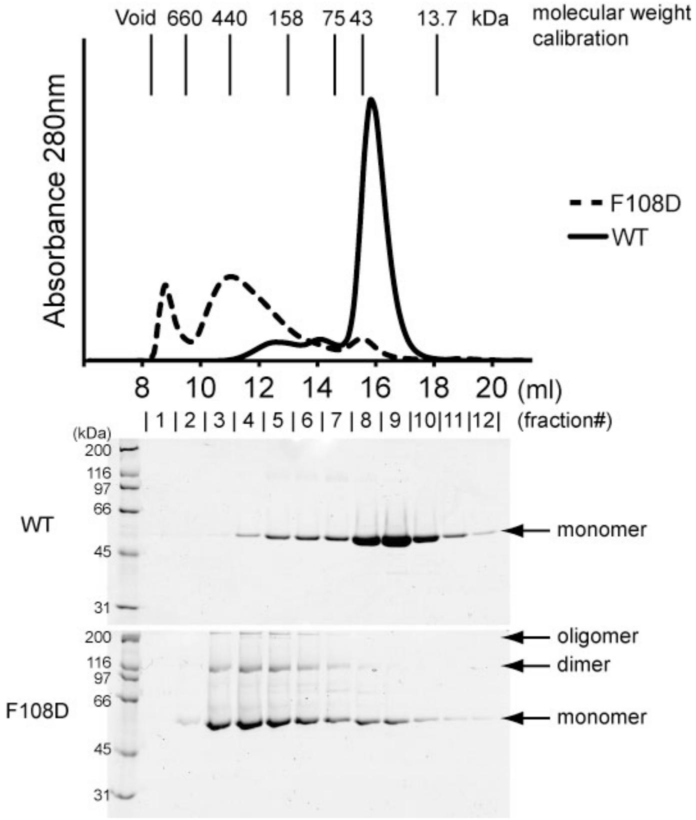
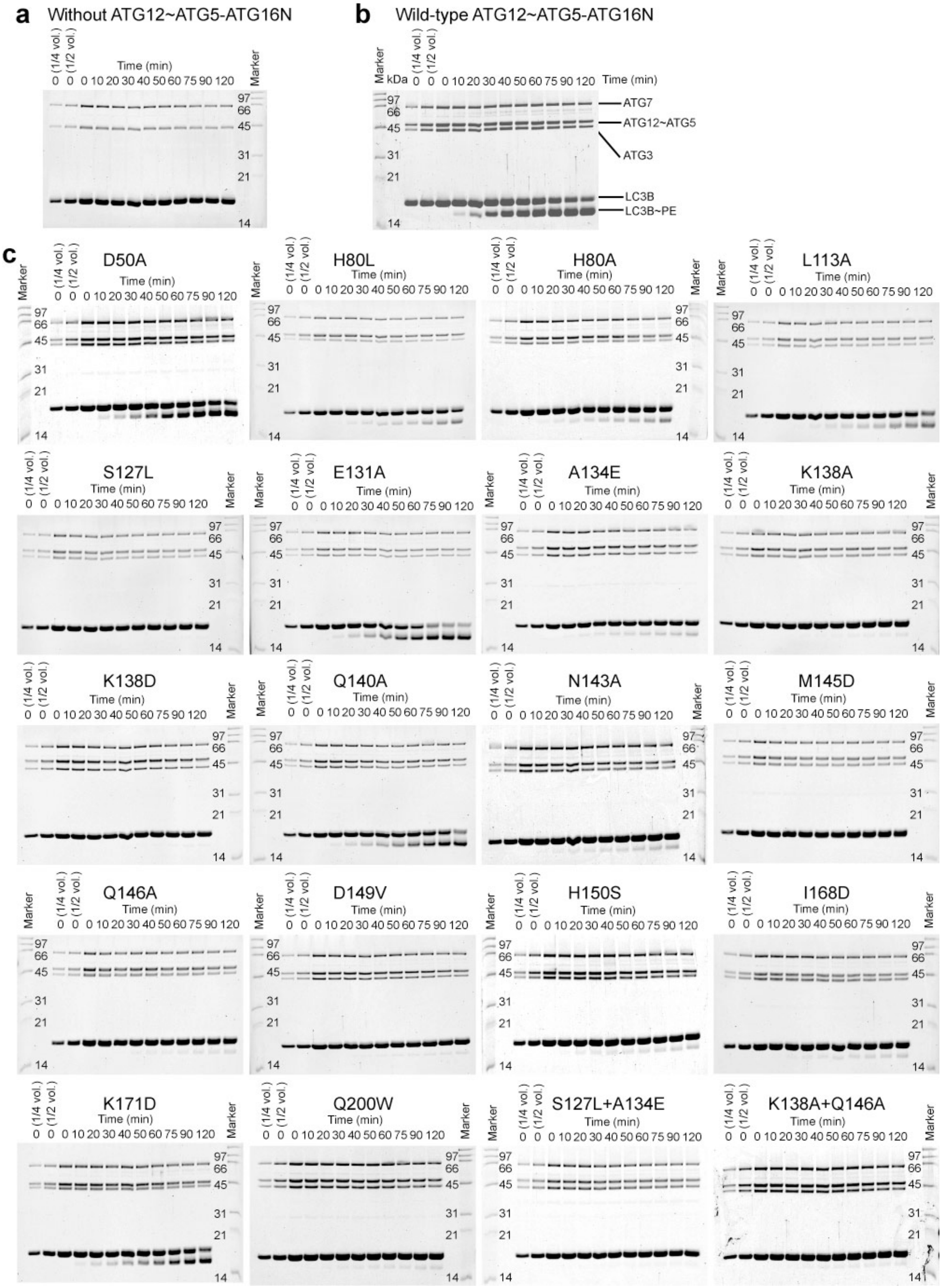
Supplementary Figures 1–5
Supplementary Note

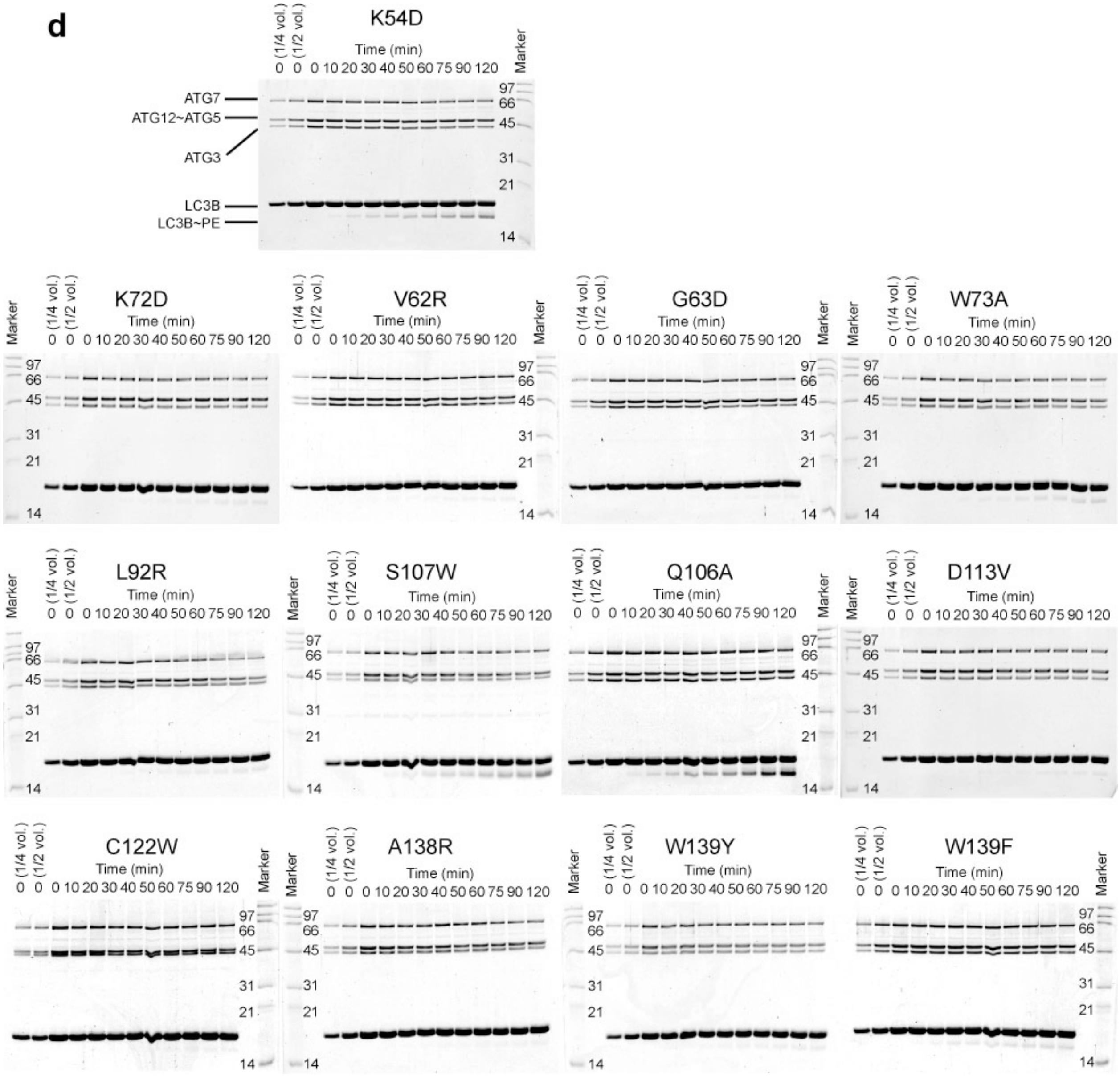


Supplementary Figure 1 Structural comparisons of the ATG12~ATG5–ATG16N complex with related structures. (**a**) Overlay of the crystal structures of the ATG12~ATG5–ATG16N complex. The molecules 1 and 2 in $P2_12_12_1$ structure are shown in blue and light blue, respectively, and the structure from the $C2$ data is in orange. The pair-wise r.m.s.d.s of all Cα atoms between two of the three structures are 0.86 Å, 1.07 Å, and 1.18 Å between $P2_12_12_1$ molecules 1 and 2, $P2_12_12_1$ molecule 1 and $C2$ structure, and $P2_12_12_1$ molecule 2 and $C2$ structure, respectively. The location of the loop between β1 and β2 of UFD-1 in ATG5, which is the only appreciable difference between the $P2_12_12_1$ and $C2$ structures, is indicated. (**b**) Stereo view of the simulated annealing omit map (contoured at 1.5σ) calculated using a model lacking ATG12 Gly140 and ATG5 Lys130 is shown with the final complete model. (**c**) Overlay of the yeast Atg5–Atg16N complex (PDB ID: 2DYO) on the ATG12~ATG5–ATG16N structure. Atg5–Atg16N is shown in magenta and ATG12~ATG5–ATG16N is in the same colors as in **Figure 2**. The average pair-wise r.m.s.d. of the Cα atoms between the Atg5–Atg16N complex and the ATG5–ATG16N part of the complex is 3.45 Å. (**d**) Overlay of AtATG12b domain-swapped dimer (PDB ID: 1WZ3) on the ATG12~ATG5–ATG16N structure. An overview with surface representation for AtATG12b is shown in the left panel. Steric clashes between the HBR of ATG5 and molecule 1 (turquoise) of AtATG12b dimer are indicated. An enlarged view is shown in the right panel. Each of the two molecules of AtATG12b dimer is shown in turquoise or dark gray. The "turn-loop-turn-α2-β4-tail" segments following β3 strands are swapped in the AtATG12b dimer. The extended β3 strands from both molecules form a β sheet by interacting each other. The three residues in the first turn of the "turn-loop-turn-α2-β4-tail" segment of ATG12 (Asn105-Gln106-Ser107) as well as the corresponding ones in the extended β3 of AtATG12b (Asn59-Ser60-Ala61) are indicated with Cα spheres. The average pair-wise r.m.s.d. of all Cα atoms between ATG12 and the "monomeric" AtATG12b is 1.8 Å.

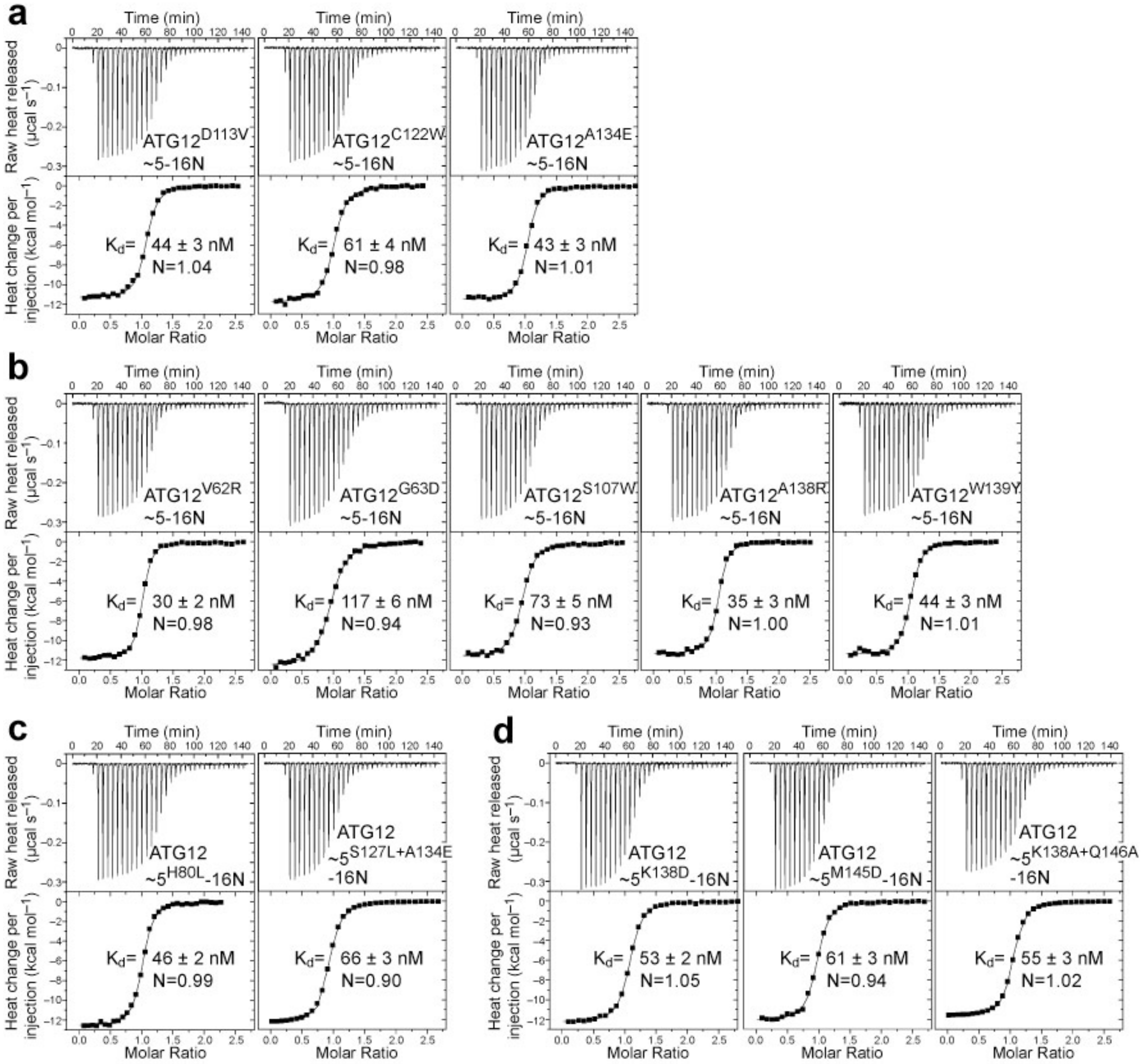


Supplementary Figure 2 The F108 mutation in ATG12 causes aggregation of the recombinant ATG12 protein. The size exclusion column profiles of recombinant maltose binding protein-fused wild-type ATG12 or ATG12 F108D protein are shown. One hundred µL of 10 µM protein was injected into a Superdex 200 gel filtration column equilibrated with 10 mM Hepes, pH 7.0, 300 mM NaCl, and 1 mM DTT (top). SDS-PAGE gels for the fractions are shown in the bottom panels. The data indicate that high-molecular-weight species of ATG12 including SDS-resistant dimers and oligomers are generated by the F108D mutation. The wild-type ATG12 also exhibits an aggregation tendency.


a
Without ATG12~ATG5-ATG16N
(1/4 vol.)
(1/2 vol.)
Time (min)
0 0 0 10 20 30 40 50 60 75 90 120
Marker
97
66
45
31
21
14
b
Wild-type ATG12~ATG5-ATG16N
Marker
kDa
(1/4 vol.)
(1/2 vol.)
0 0 0 10 20 30 40 50 60 75 90 120
Time (min)
ATG7
ATG12~ATG5
ATG3
LC3B
LC3B~PE
c
D50A
H80L
H80A
L113A
S127L
E131A
A134E
K138A
K138D
Q140A
N143A
M145D
Q146A
D149V
H150S
I168D
K171D
Q200W
S127L+A134E
K138A+Q146A



Supplementary Figure 3 SDS-PAGE gels of *in vitro* LC3 lipidation assays carried out with mutant ATG12~ATG5–ATG16N complexes. (**a–d**) Kinetic LC3B~phosphatidylethanolamine formation assays were performed in the absence of the ATG12~ATG5–ATG16N complex (**a**), or in the presence of the wild-type complex (**b**) or complexes containing mutations in ATG5 (**c**) or ATG12 (**d**). The same assay condition as described in **Figure 1a** was used in these experiments. The bands corresponding LC3B~phosphatidylethanolamine conjugate were quantified as described in Supplemental Experimental Procedures and are reported in **Figures 4b**,**4c**.



Supplementary Figure 4 ITC data for the ATG12~ATG5–ATG16N mutant complexes showing no major effects of the mutations on the high-affinity binding to ATG3. **(a–d)** ATG3 was injected into cells filled with solutions containing mutant ATG12~ATG5–ATG16N complexes that have mutations in ATG12 at the interface to ATG5 (**a**) or on the exposed surface (**b**), or in ATG5 at the interface to ATG12 (**c**) or on the exposed surface (**d**).

ConSurf color-coded MSA of ATG12 family members

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Q9US24|ATG12_SCHPO|44-132   NRRVNLRFKAIG-RTPLLRKTVFSINAS-QRFEKVTRFLKKELGLPMN--
A5E1F1|ATG12_LODEL|122-210  PEKVTIRFQPIG-STTAIHPKVFKISSV-QSILTVNRFLSQKLKNNE---
A8E622|A8E622_XENLA|44-132  KRKIDVLLKAVG-DTPIMKTKKWTIEKT-RSVQGLMDFIKKYLKMDA---
Q6CUD5|ATG12_KLULA|104-189  QVKVKIRLQPIG-AIPQIQPRVCQISAH-QQFLALTRFLCKRLKRKH---
Q8S924|AT12A_ARATH|11-96    VRKVVVHLRATG-GAPILKQSKFKIPGT-DKFAKVIDFLRRQLHSD----
Q1E8C2|ATG12_COCIM|84-182   AGKVTVRFHPLP-SAPILRNRVFKISAS-QKFETVVRFLRKKLDCSE---
A7RKL8|A7RKL8_NEMVE|42-130  KDKVEVLLKAAG-DAPIMKRKKWAVEGS-KTVAYLIEFIRKYIRCEP---
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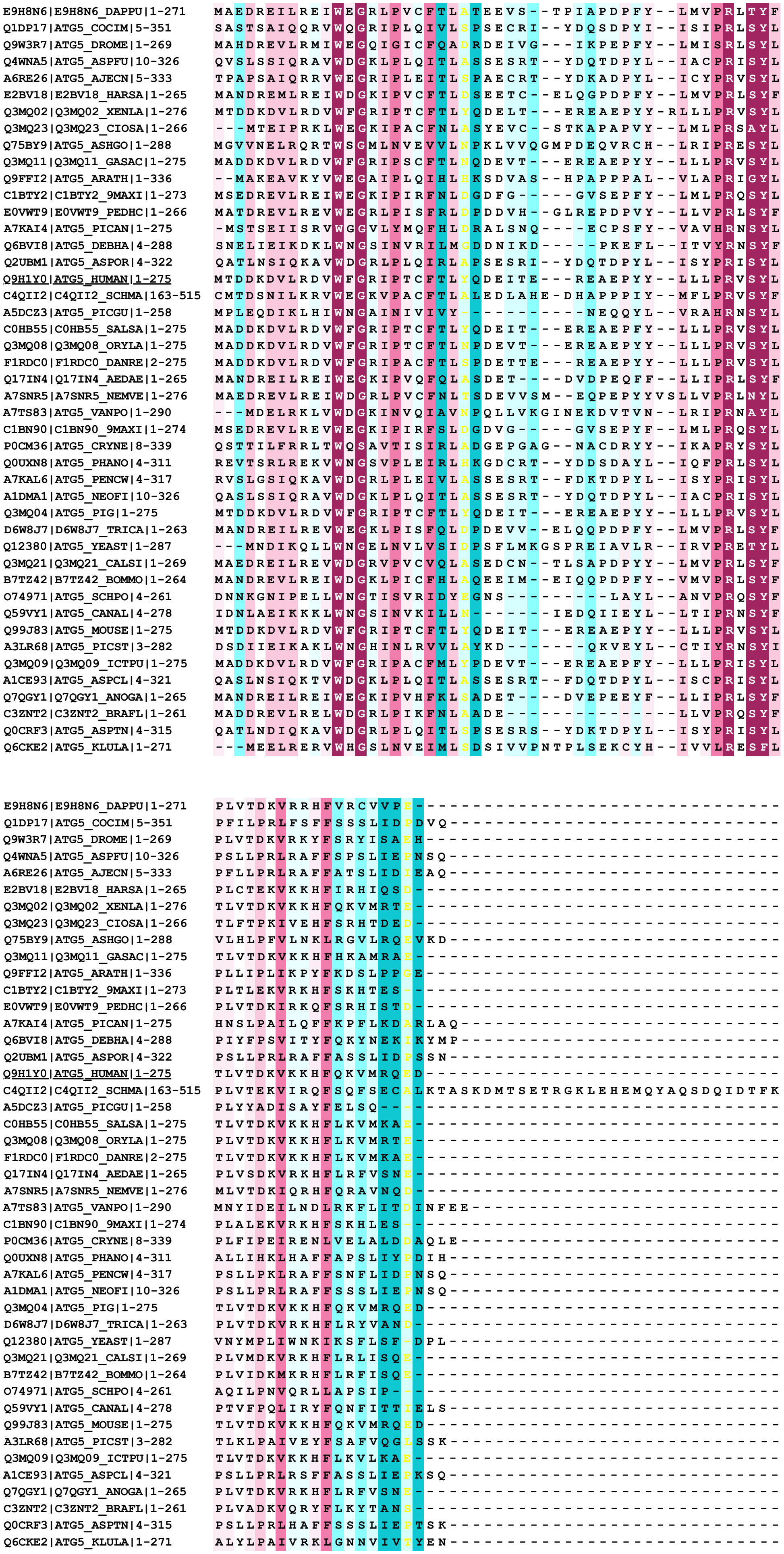
X - Insufficient data - the calculation for this site was performed on less than 10% of the sequences.

ConSurf color-coded MSA for ATG5 family members



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F1RDC0|F1RDC0_DANRE|2-275       MADDKDVLRDVWFGRIPACFTLSPDETTE---REAEPYY--LLLPRVSYL
Q17IN4|Q17IN4_AEDAE|1-265       MANDREILREIWEGKIPVQFQLASDET----DVDPEQFF--LLIPRLSYF
A7SNR5|A7SNR5_NEMVE|1-276       MAEDREVLREIWDGRLPVCFNLTSDEVVSM--EQPEPYYVSLLVPRLNYL
A7TS83|ATG5_VANPO|1-290         ---MDELRKLVWDGKINVQIAVNPQLLVKGINEKDVTVN--LRIPRNAYL
C1BN90|C1BN90_9MAXI|1-274       MSEDREVLREVWEGKIPIRFSLDGDVG-----GVSEPYF--LMLPRQSYL
P0CM36|ATG5_CRYNE|8-339         QSTTILFRRLTWQSAVTISIRLADGEPGAG--NACDRYY--IKAPRYSYL
Q0UXN8|ATG5_PHANO|4-311         REVTSRLREKVWNGSVPLEIRLHKGDCRT--YDDSDAYL--IQFPRLSYL
A7KAL6|ATG5_PENCW|4-317         RVSLGSIQKAVWDGRLPLEIVLASSESRT--FDKTDPYL--ISYPRISYL
A1DMA1|ATG5_NEOFI|10-326        QASLSSIQRAVWDGKLPLQITLASSESRT--YDQTDPYL--IACPRISYL
Q3MQ04|ATG5_PIG|1-275           MTDDKDVLRDVWFGRIPTCFTLYQDEIT---EREAEPYY--LLLPRVSYL
D6W8J7|D6W8J7_TRICA|1-263       MANDREILREVWEGKLPISFQLDPDEVV--ELQQPDPFY--LMVPRLSYF
Q12380|ATG5_YEAST|1-287         ---MNDIKQLLWNGELNVLVSIDPSFLMKGSPREIAVLR--IRVPRETYL
Q3MQ21|Q3MQ21_CALSI|1-269       MAEDREILREVWDGRVPVCVQLASEDCN--TLSAPDPYY--LMVPRLSYF
B7TZ42|B7TZ42_BOMMO|1-264       MANDREVLREIWDGKLPICFHLAQEEIM--EIQQPDPFY--VMVPRLSYF
O74971|ATG5_SCHPO|4-261         DNNKGNIPELLWNGTISVRIDYEGNS---------LAYL--ANVPRQSYF
Q59VY1|ATG5_CANAL|4-278         IDNLAEIKKKLWNGSINVKILLN-------IEDQIIEYL--LTIPRNSYF
Q99J83|ATG5_MOUSE|1-275         MTDDKDVLRDVWFGRIPTCFTLYQDEIT---EREAEPYY--LLLPRVSYL
A3LR68|ATG5_PICST|3-282         DSDIIEIKAKLWNGHINLRVVLAYKD-------QKVEYL--CTIYRNSYI
Q3MQ09|Q3MQ09_ICTPU|1-275       MADDKDVLRDVWFGRIPACFMLYPDEVT---EREAEPFY--LLLPRISYL
A1CE93|ATG5_ASPCL|4-321         QASLNSIQKTVWDGKLPLQITLASSESRT--FDQTDPYL--ISCPRISYL
Q7QGY1|Q7QGY1_ANOGA|1-265       MANDREILREIWEGKIPVHFKLSADET----DVEPEEYF--LLIPRLSYF
C3ZNT2|C3ZNT2_BRAFL|1-261       MADDREVLRELWDGRLPIKFNLAADE---------------LLVPRQSYF
Q0CRF3|ATG5_ASPTN|4-315         QATLNDIQKAVWDGRLPLQITLSPSESRS--YDKTDPYL--ISYPRISYL
Q6CKE2|ATG5_KLULA|1-271         ---MEELRERVWHGSLNVEIMLSDSIVVPNTPLSEKCYH--IVVLRESFL


E9H8N6|E9H8N6_DAPPU|1-271       PLVTDKVRRHFVRCVVPE--------------------------------
Q1DP17|ATG5_COCIM|5-351         PFILPRLFSFFSSSLIDPDVQ-----------------------------
Q9W3R7|ATG5_DROME|1-269         PLVTDKVRKYFSRYISAEH-------------------------------
Q4WNA5|ATG5_ASPFU|10-326        PSLLPRLRAFFSPSLIEPNSQ-----------------------------
A6RE26|ATG5_AJECN|5-333         PFLLPRLRAFFATSLIDIEAQ-----------------------------
E2BV18|E2BV18_HARSA|1-265       PLCTEKVKKHFIRHIQSD--------------------------------
Q3MQ02|Q3MQ02_XENLA|1-276       TLVTDKVKKHFQKVMRTE--------------------------------
Q3MQ23|Q3MQ23_CIOSA|1-266       TLFTPKIVEHFSRHTDED--------------------------------
Q75BY9|ATG5_ASHGO|1-288         VLHLPFVLNKLRGVLRQEVKD-----------------------------
Q3MQ11|Q3MQ11_GASAC|1-275       TLVTDKVKKHFHKAMRAE--------------------------------
Q9FFI2|ATG5_ARATH|1-336         PLLIPLIKPYFKDSLPPGE-------------------------------
C1BTY2|C1BTY2_9MAXI|1-273       PLTLEKVRKHFSKHTES---------------------------------
E0VWT9|E0VWT9_PEDHC|1-266       PLVTDKIRKQFSRHISTD--------------------------------
A7KAI4|ATG5_PICAN|1-275         HNSLPAILQFFKPFLKDARLAQ----------------------------
Q6BVI8|ATG5_DEBHA|4-288         PIYFPSVITYFQKYNEKIKYMP----------------------------
Q2UBM1|ATG5_ASPOR|4-322         PSLLPRLRAFFASSLIDPSSN-----------------------------
Q9H1Y0|ATG5_HUMAN|1-275         TLVTDKVKKHFQKVMRQED-------------------------------
C4QII2|C4QII2_SCHMA|163-515     PLVTEKVIRQFSQFSECALKTASKDMTSETRGKLEHEMQYAQSDQIDTFK
A5DCZ3|ATG5_PICGU|1-258         PLYYADISAYFELSQ-----------------------------------
C0HB55|C0HB55_SALSA|1-275       TLVTDKVKKHFLKVMKAE--------------------------------
Q3MQ08|Q3MQ08_ORYLA|1-275       PLVTDKVKKHFLKVMRTE--------------------------------
F1RDC0|F1RDC0_DANRE|2-275       TLVTDKVKKHFLKVMKAE--------------------------------
Q17IN4|Q17IN4_AEDAE|1-265       PLVSDKVRKHFLRFVSNE--------------------------------
A7SNR5|A7SNR5_NEMVE|1-276       MLVTDKIQRHFQRAVNQD--------------------------------
A7TS83|ATG5_VANPO|1-290         MNYIDEILNDLRKFLITDINFEE---------------------------
C1BN90|C1BN90_9MAXI|1-274       PLALEKVRKHFSKHLES---------------------------------
P0CM36|ATG5_CRYNE|8-339         PLFIPEIRENLVELALDDAQLE----------------------------
Q0UXN8|ATG5_PHANO|4-311         ALLIHKLHAFFAPSLIYPDIH-----------------------------
A7KAL6|ATG5_PENCW|4-317         PSLLPKLRAFFSNFLIDPNSQ-----------------------------
A1DMA1|ATG5_NEOFI|10-326        PSLLPRLRAFFSSSLIEPNSQ-----------------------------
Q3MQ04|ATG5_PIG|1-275           TLVTDKVKKHFQKVMRQED-------------------------------
D6W8J7|D6W8J7_TRICA|1-263       PLVTDKVRKHFLRYVAND--------------------------------
Q12380|ATG5_YEAST|1-287         VNYMPLIWNKIKSFLSF-DPL-----------------------------
Q3MQ21|Q3MQ21_CALSI|1-269       PLVMDKVRKHFLRLISQE--------------------------------
B7TZ42|B7TZ42_BOMMO|1-264       PLVIDKMKRHFLRFISQE--------------------------------
O74971|ATG5_SCHPO|4-261         AQILPNVQRLLAPSIP----------------------------------
Q59VY1|ATG5_CANAL|4-278         PTVFPQLIRYFQNFITTIELS-----------------------------
Q99J83|ATG5_MOUSE|1-275         TLVTDKVKKHFQKVMRQED-------------------------------
A3LR68|ATG5_PICST|3-282         TLKLPAIVEYFSAFVQGLSSK-----------------------------
Q3MQ09|Q3MQ09_ICTPU|1-275       TLVTDKVKKHFLKVLKAE--------------------------------
A1CE93|ATG5_ASPCL|4-321         PSLLPRLRSFFASSLIEPKSQ-----------------------------
Q7QGY1|Q7QGY1_ANOGA|1-265       PLVTDKVRKHFLRFVSNE--------------------------------
C3ZNT2|C3ZNT2_BRAFL|1-261       PLVADKVQRYFLKYTANS--------------------------------
Q0CRF3|ATG5_ASPTN|4-315         PSLLPRLHAFFSSSLIEPTSK-----------------------------
Q6CKE2|ATG5_KLULA|1-271         ALYLPAIVRKLGNNVIVTYEN-----------------------------
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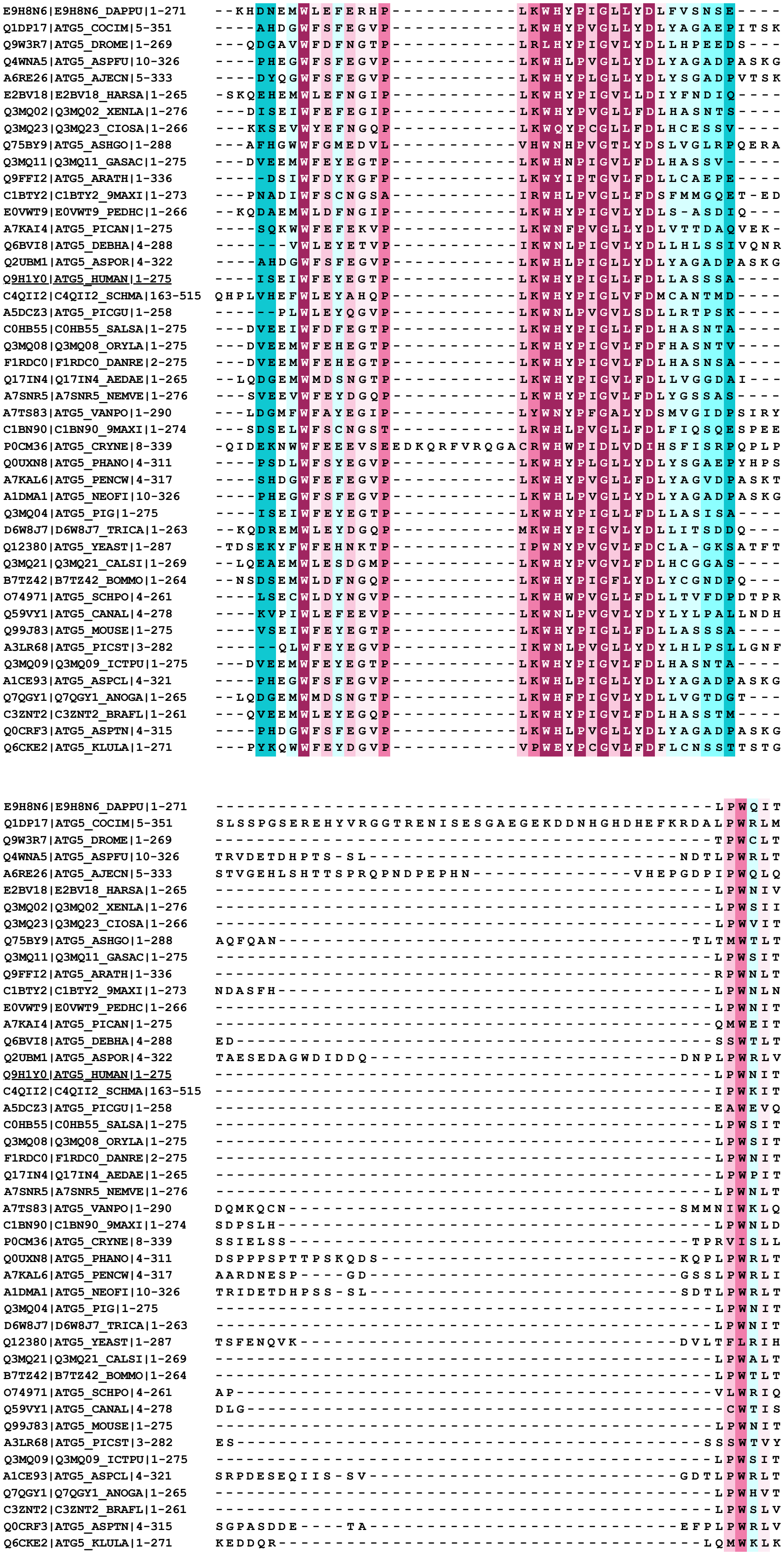

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Q1DP17|ATG5_COCIM|5-351      ----AHDGWFSFEGVP-----------LKWHYPVGLLYDLYAGAEPITSK
Q9W3R7|ATG5_DROME|1-269      ---QDGAVWFDFNGTP-----------LRLHYPIGVLYDLLHPEEDS---
Q4WNA5|ATG5_ASPFU|10-326     ----PHEGWFSFEGVP-----------LKWHLPVGLLYDLYAGADPASKG
A6RE26|ATG5_AJECN|5-333      ----DYQGWFSFEGVP-----------LKWHYPLGLLYDLYSGADPVTSK
E2BV18|E2BV18_HARSA|1-265    -SKQEHEMWLEFNGIP-----------LKWHYPIGVLLDIYFNDIQ----
Q3MQ02|Q3MQ02_XENLA|1-276    ---DISEIWFEFEGIP-----------LKWHYPVGLLFDLHASNTS----
Q3MQ23|Q3MQ23_CIOSA|1-266    ---KKSEVWYEFNGQP-----------LKWQYPCGLLFDLHCESSV----
Q75BY9|ATG5_ASHGO|1-288      ---AFHGWWFGMEDVL-----------VHWNHPVGTLYDSLVGLRPQERA
Q3MQ11|Q3MQ11_GASAC|1-275    ---DVEEMWFEYEGTP-----------LKWHNPIGVLFDLHASSV-----
Q9FFI2|ATG5_ARATH|1-336      -----DSIWFDYKGFP-----------LKWYIPTGVLFDLLCAEPE----
C1BTY2|C1BTY2_9MAXI|1-273    ---PNADIWFSCNGSA-----------IRWHLPVGLLFDSFMMGQET-ED
E0VWT9|E0VWT9_PEDHC|1-266    --KQDAEMWLDFNGIP-----------LKWHYPIGVLYDLS-ASDIQ---
A7KAI4|ATG5_PICAN|1-275      ----SQKWWFEFEKVP-----------LKWNFPVGLLYDLVTTDAQVEK-
Q6BVI8|ATG5_DEBHA|4-288      -------VWLEYETVP-----------IKWNLPIGVLYDLLHLSSIVQNR
Q2UBM1|ATG5_ASPOR|4-322      ----AHDGWFSFEGVP-----------LKWHLPIGLLYDLYAGADPASKG
Q9H1Y0|ATG5_HUMAN|1-275      ----ISEIWFEYEGTP-----------LKWHYPIGLLFDLLASSSA----
C4QII2|C4QII2_SCHMA|163-515  QHPLVHEFWLEYAHQP-----------LKWHYPIGLVFDMCANTMD----
A5DCZ3|ATG5_PICGU|1-258      ------PLWLEYQGVP-----------LKWNLPVGVLSDLLRTPSK----
C0HB55|C0HB55_SALSA|1-275    ---DVEEIWFDFEGTP-----------LKWHYPIGLLFDLHASNTA----
Q3MQ08|Q3MQ08_ORYLA|1-275    ---DVEEMWFEHEGTP-----------LKWHYPIGVLFDFHASNTV----
F1RDC0|F1RDC0_DANRE|2-275    ---DVEEMWFEHEGTP-----------LKWHYPIGVLFDLHASNSA----
Q17IN4|Q17IN4_AEDAE|1-265    --LQDGEMWMDSNGTP-----------LKWHYPIGVLFDLLVGGDAI---
A7SNR5|A7SNR5_NEMVE|1-276    ---SVEEVWFEYDGQP-----------LKWHYPIGVLFDLYGSSAS----
A7TS83|ATG5_VANPO|1-290      ---LDGMFWFAYEGIP-----------LYWNYPFGALYDSMVGIDPSIRY
C1BN90|C1BN90_9MAXI|1-274    ---SDSELWFSCNGST-----------LRWHLPVGLLFDLFIQSQESPEE
P0CM36|ATG5_CRYNE|8-339      -QIDEKNWWFEEEVSEEDKQRFVRQGACRWHWPIDLVDIHSFISRPQPLP
Q0UXN8|ATG5_PHANO|4-311      ----PSDLWFSYEGVP-----------LKWHYPLGLLYDLYSGAEPYHPS
A7KAL6|ATG5_PENCW|4-317      ----SHDGWFEFEGVP-----------LKWHYPVGLLFDLYAGVDPASKT
A1DMA1|ATG5_NEOFI|10-326     ----PHEGWFSFEGVP-----------LKWHLPVGLLYDLYAGADPASKG
Q3MQ04|ATG5_PIG|1-275        ----ISEIWFEYEGTP-----------LKWHYPIGLLFDLLASISA----
D6W8J7|D6W8J7_TRICA|1-263    --KQDREMWLEYDGQP-----------MKWHYPIGVLYDLLITSDDQ---
Q12380|ATG5_YEAST|1-287      -TDSEKYFWFEHNKTP-----------IPWNYPVGVLFDCLA-GKSATFT
Q3MQ21|Q3MQ21_CALSI|1-269    --LQEAEMWLESDGMP-----------LKWHYPVGVLFDLHCGGAS----
B7TZ42|B7TZ42_BOMMO|1-264    --NSDSEMWLDFNGQP-----------LKWHYPIGFLYDLYCGNDPQ---
O74971|ATG5_SCHPO|4-261      ----LSECWLDYNGVP-----------LKWHWPVGLLFDLLTVFDPDTPR
Q59VY1|ATG5_CANAL|4-278      ----KVPIWLEFEEVP-----------LKWNLPVGVLYDYLYLPALLNDH
Q99J83|ATG5_MOUSE|1-275      ----VSEIWFEYEGTP-----------LKWHYPIGLLFDLLASSSA----
A3LR68|ATG5_PICST|3-282      ------QLWFEYEGVP-----------IKWNLPVGLLYDYLHLPSLLGNF
Q3MQ09|Q3MQ09_ICTPU|1-275    ---DVEEMWFEYEGTP-----------LKWHYPIGVLFDLHASNTA----
A1CE93|ATG5_ASPCL|4-321      ----PHEGWFSFEGVP-----------LKWHLPIGLLYDLYAGADPASKG
Q7QGY1|Q7QGY1_ANOGA|1-265    --LQDGEMWMDSNGTP-----------LKWHFPIGVLYDLLVGTDGT---
C3ZNT2|C3ZNT2_BRAFL|1-261    ---QVEEMWLEYEGQP-----------LKWHYPIGVLFDLHASSTM----
Q0CRF3|ATG5_ASPTN|4-315      ----PHDGWFSFEGVP-----------LKWHLPVGLLYDLYAGADPASKG
Q6CKE2|ATG5_KLULA|1-271      ---PYKQWWFEYDGVP-----------VPWEYPCGVLFDFLCNSSTTSTG


E9H8N6|E9H8N6_DAPPU|1-271    --------------------------------------------LPWQIT
Q1DP17|ATG5_COCIM|5-351      SLSSPGSEREHYVRGGTRENISESGAEGEKDDNHGHDHEFKRDALPWRLM
Q9W3R7|ATG5_DROME|1-269      --------------------------------------------TPWCLT
Q4WNA5|ATG5_ASPFU|10-326     TRVDETDHPTS-SL---------------------------NDTLPWRLT
A6RE26|ATG5_AJECN|5-333      STVGEHLSHTTSPRQPNDPEPHN--------------VHEPGDPIPWQLQ
E2BV18|E2BV18_HARSA|1-265    --------------------------------------------LPWNIV
Q3MQ02|Q3MQ02_XENLA|1-276    --------------------------------------------LPWSII
Q3MQ23|Q3MQ23_CIOSA|1-266    --------------------------------------------LPWVIT
Q75BY9|ATG5_ASHGO|1-288      AQFQAN------------------------------------TLTMWTLT
Q3MQ11|Q3MQ11_GASAC|1-275    --------------------------------------------LPWSIT
Q9FFI2|ATG5_ARATH|1-336      --------------------------------------------RPWNLT
C1BTY2|C1BTY2_9MAXI|1-273    NDASFH--------------------------------------LPWNLN
E0VWT9|E0VWT9_PEDHC|1-266    --------------------------------------------LPWNIT
A7KAI4|ATG5_PICAN|1-275      --------------------------------------------QMWEIT
Q6BVI8|ATG5_DEBHA|4-288      ED------------------------------------------SSWTLT
Q2UBM1|ATG5_ASPOR|4-322      TAESEDAGWDIDDQ---------------------------DNPLPWRLV
Q9H1Y0|ATG5_HUMAN|1-275      --------------------------------------------LPWNIT
C4QII2|C4QII2_SCHMA|163-515  --------------------------------------------IPWKIT
A5DCZ3|ATG5_PICGU|1-258      --------------------------------------------EAWEVQ
C0HB55|C0HB55_SALSA|1-275    --------------------------------------------LPWSIT
Q3MQ08|Q3MQ08_ORYLA|1-275    --------------------------------------------LPWSIT
F1RDC0|F1RDC0_DANRE|2-275    --------------------------------------------LPWNIT
Q17IN4|Q17IN4_AEDAE|1-265    --------------------------------------------LPWPIT
A7SNR5|A7SNR5_NEMVE|1-276    --------------------------------------------LPWNLT
A7TS83|ATG5_VANPO|1-290      DQMKQCN----------------------------------SMMNIWKLQ
C1BN90|C1BN90_9MAXI|1-274    SDPSLH--------------------------------------LPWNLD
P0CM36|ATG5_CRYNE|8-339      SSIELSS-----------------------------------TPRVISLL
Q0UXN8|ATG5_PHANO|4-311      DSPPPSPTTPSKQDS--------------------------KQPLPWRLT
A7KAL6|ATG5_PENCW|4-317      AARDNESP----GD---------------------------GSSLPWRLI
A1DMA1|ATG5_NEOFI|10-326     TRIDETDHPSS-SL---------------------------SDTLPWRLT
Q3MQ04|ATG5_PIG|1-275        --------------------------------------------LPWNIT
D6W8J7|D6W8J7_TRICA|1-263    --------------------------------------------LPWNIT
Q12380|ATG5_YEAST|1-287      TSFENQVK---------------------------------DVLTFLRIH
Q3MQ21|Q3MQ21_CALSI|1-269    --------------------------------------------LPWALT
B7TZ42|B7TZ42_BOMMO|1-264    --------------------------------------------LPWTLT
O74971|ATG5_SCHPO|4-261      AP------------------------------------------VLWRIQ
Q59VY1|ATG5_CANAL|4-278      DLG------------------------------------------CWTIS
Q99J83|ATG5_MOUSE|1-275      --------------------------------------------LPWNIT
A3LR68|ATG5_PICST|3-282      ES-----------------------------------------SSSWTVY
Q3MQ09|Q3MQ09_ICTPU|1-275    --------------------------------------------LPWSIT
A1CE93|ATG5_ASPCL|4-321      SRPDESEQIIS-SV---------------------------GDTLPWRLT
Q7QGY1|Q7QGY1_ANOGA|1-265    --------------------------------------------LPWHVT
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Q0CRF3|ATG5_ASPTN|4-315      SGPASDDE----TA---------------------------EFPLPWRLV
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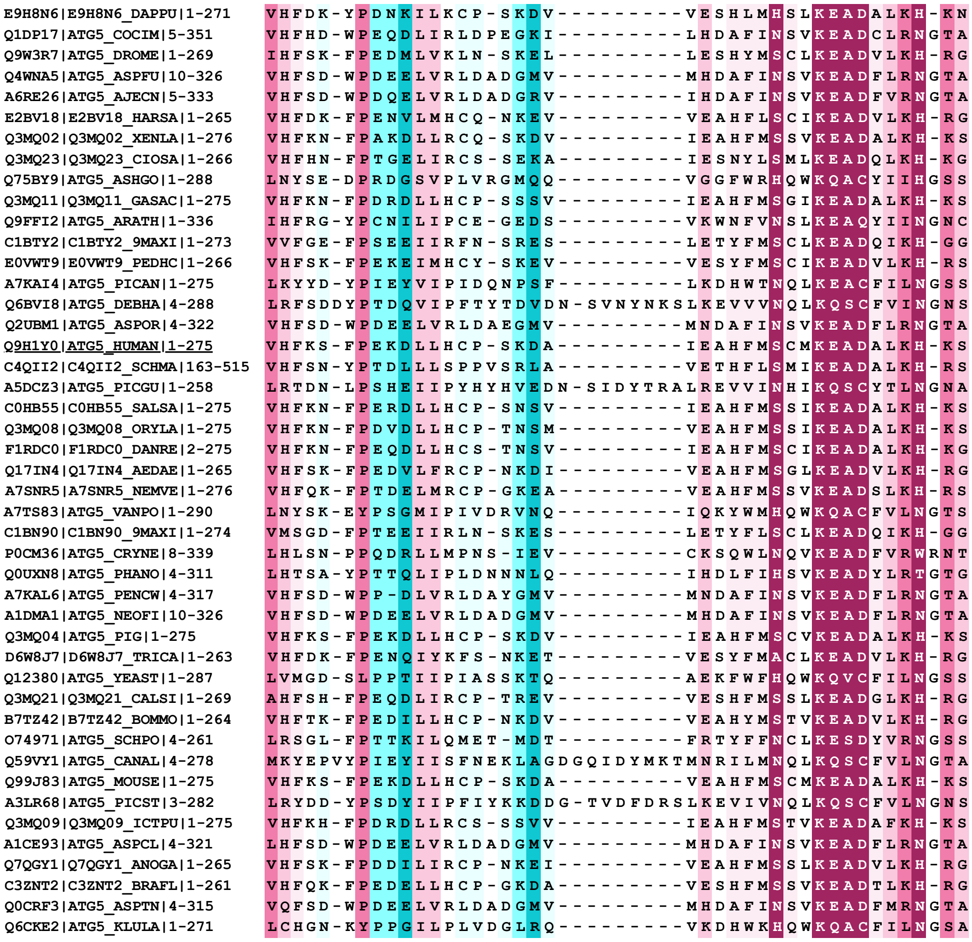

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Q1DP17|ATG5_COCIM|5-351       VHFHD-WPEQDLIRLDPEGKI---------LHDAFINSVKEADCLRNGTA
Q9W3R7|ATG5_DROME|1-269       IHFSK-FPEDMLVKLN-SKEL---------LESHYMSCLKEADVLKH-RG
Q4WNA5|ATG5_ASPFU|10-326      VHFSD-WPDEELVRLDADGMV---------MHDAFINSVKEADFLRNGTA
A6RE26|ATG5_AJECN|5-333       VHFSD-WPDQELVRLDADGRV---------IHDAFINSVKEADFVRNGTA
E2BV18|E2BV18_HARSA|1-265     VHFDK-FPENVLMHCQ-NKEV---------VEAHFLSCIKEADVLKH-RG
Q3MQ02|Q3MQ02_XENLA|1-276     VHFKN-FPAKDLLRCQ-SKDV---------IEAHFMSSVKEADALKH-KS
Q3MQ23|Q3MQ23_CIOSA|1-266     VHFHN-FPTGELIRCS-SEKA---------IESNYLSMLKEADQLKH-KG
Q75BY9|ATG5_ASHGO|1-288       LNYSE-DPRDGSVPLVRGMQQ---------VGGFWRHQWKQACYIIHGSS
Q3MQ11|Q3MQ11_GASAC|1-275     VHFKN-FPDRDLLHCP-SSSV---------IEAHFMSGIKEADALKH-KS
Q9FFI2|ATG5_ARATH|1-336       IHFRG-YPCNILIPCE-GEDS---------VKWNFVNSLKEAQYIINGNC
C1BTY2|C1BTY2_9MAXI|1-273     VVFGE-FPSEEIIRFN-SRES---------LETYFMSCLKEADQIKH-GG
E0VWT9|E0VWT9_PEDHC|1-266     VHFSK-FPEKEIMHCY-SKEV---------VESYFMSCIKEADVLKH-RS
A7KAI4|ATG5_PICAN|1-275       LKYYD-YPIEYVIPIDQNPSF---------LKDHWTNQLKEACFILNGSS
Q6BVI8|ATG5_DEBHA|4-288       LRFSDDYPTDQVIPFTYTDVDN-SVNYNKSLKEVVVNQLKQSCFVINGNS
Q2UBM1|ATG5_ASPOR|4-322       VHFSD-WPDEELVRLDAEGMV---------MNDAFINSVKEADFLRNGTA
Q9H1Y0|ATG5_HUMAN|1-275       VHFKS-FPEKDLLHCP-SKDA---------IEAHFMSCMKEADALKH-KS
C4QII2|C4QII2_SCHMA|163-515   VHFSN-YPTDLLLSPPVSRLA---------VETHFLSMIKEADALKH-RS
A5DCZ3|ATG5_PICGU|1-258       LRTDN-LPSHEIIPYHYHVEDN-SIDYTRALREVVINHIKQSCYTLNGNA
C0HB55|C0HB55_SALSA|1-275     VHFKN-FPERDLLHCP-SNSV---------IEAHFMSSIKEADALKH-KS
Q3MQ08|Q3MQ08_ORYLA|1-275     VHFKN-FPDVDLLHCP-TNSM---------VEAHFMSSIKEADALKH-KS
F1RDC0|F1RDC0_DANRE|2-275     VHFKN-FPEQDLLHCS-TNSV---------IEAHFMSCIKEADALKH-KG
Q17IN4|Q17IN4_AEDAE|1-265     VHFSK-FPEDVLFRCP-NKDI---------VEAHFMSGLKEADVLKH-RG
A7SNR5|A7SNR5_NEMVE|1-276     VHFQK-FPTDELMRCP-GKEA---------VEAHFMSSVKEADSLKH-RS
A7TS83|ATG5_VANPO|1-290       LNYSK-EYPSGMIPIVDRVNQ---------IQKYWMHQWKQACFVLNGTS
C1BN90|C1BN90_9MAXI|1-274     VMSGD-FPTEEIIRLN-SKES---------LETYFLSCLKEADQIKH-GG
P0CM36|ATG5_CRYNE|8-339       LHLSN-PPQDRLLMPNS-IEV---------CKSQWLNQVKEADFVRWRNT
Q0UXN8|ATG5_PHANO|4-311       LHTSA-YPTTQLIPLDNNNLQ---------IHDLFIHSVKEADYLRTGTG
A7KAL6|ATG5_PENCW|4-317       VHFSD-WPP-DLVRLDAYGMV---------MNDAFINSVKEADFLRNGTA
A1DMA1|ATG5_NEOFI|10-326      VHFSD-WPDEELVRLDADGMV---------MHDAFINSVKEADFLRNGTA
Q3MQ04|ATG5_PIG|1-275         VHFKS-FPEKDLLHCP-SKDV---------IEAHFMSCVKEADALKH-KS
D6W8J7|D6W8J7_TRICA|1-263     VHFDK-FPENQIYKFS-NKET---------VESYFMACLKEADVLKH-RG
Q12380|ATG5_YEAST|1-287       LVMGD-SLPPTIIPIASSKTQ---------AEKFWFHQWKQVCFILNGSS
Q3MQ21|Q3MQ21_CALSI|1-269     AHFSH-FPEQDLIRCP-TREV---------VESHFMSSLKEADGLKH-RG
B7TZ42|B7TZ42_BOMMO|1-264     VHFTK-FPEDILLHCP-NKDV---------VEAHYMSTVKEADVLKH-RG
O74971|ATG5_SCHPO|4-261       LRSGL-FPTTKILQMET-MDT---------FRTYFFNCLKESDYVRNGSS
Q59VY1|ATG5_CANAL|4-278       MKYEPVYPIEYIISFNEKLAGDGQIDYMKTMNRILMNQLKQSCFVLNGTA
Q99J83|ATG5_MOUSE|1-275       VHFKS-FPEKDLLHCP-SKDA---------VEAHFMSCMKEADALKH-KS
A3LR68|ATG5_PICST|3-282       LRYDD-YPSDYIIPFIYKKDDG-TVDFDRSLKEVIVNQLKQSCFVLNGNS
Q3MQ09|Q3MQ09_ICTPU|1-275     VHFKH-FPDRDLLRCS-SSVV---------IEAHFMSTVKEADAFKH-KS
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Q7QGY1|Q7QGY1_ANOGA|1-265     VHFSK-FPDDILIRCP-NKEI---------VEAHFMSSLKEADVLKH-RG
C3ZNT2|C3ZNT2_BRAFL|1-261     VHFQK-FPEDELLHCP-GKDA---------VESHFMSSVKEADTLKH-RG
Q0CRF3|ATG5_ASPTN|4-315       VQFSD-WPDEELVRLDADGMV---------MHDAFINSVKEADFMRNGTA
Q6CKE2|ATG5_KLULA|1-271       LCHGN-KYPPGILPLVDGLRQ---------VKDHWKHQWKQACFILNGSA
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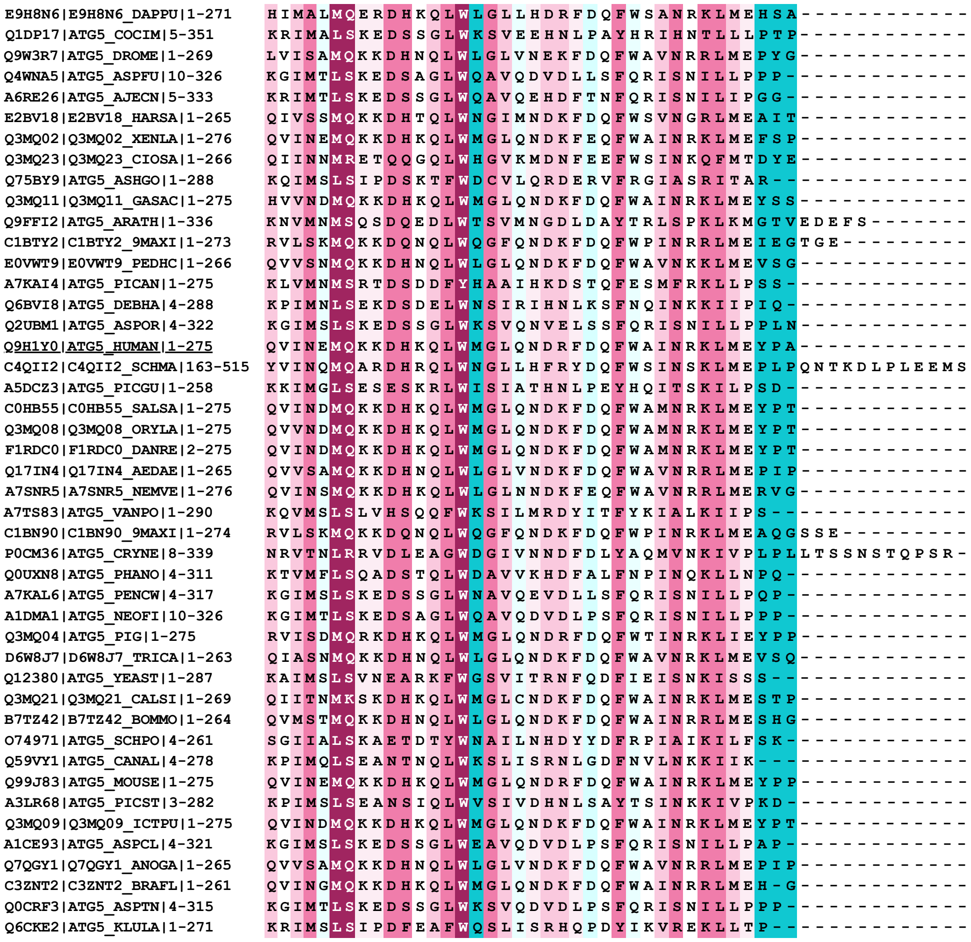

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Q1DP17|ATG5_COCIM|5-351       KRIMALSKEDSSGLWKSVEEHNLPAYHRIHNTLLLPTP------------
Q9W3R7|ATG5_DROME|1-269       LVISAMQKKDHNQLWLGLVNEKFDQFWAVNRRLMEPYG------------
Q4WNA5|ATG5_ASPFU|10-326      KGIMTLSKEDSAGLWQAVQDVDLLSFQRISNILLPPP-------------
A6RE26|ATG5_AJECN|5-333       KRIMTLSKEDSSGLWQAVQEHDFTNFQRISNILIPGG-------------
E2BV18|E2BV18_HARSA|1-265     QIVSSMQKKDHTQLWNGIMNDKFDQFWSVNGRLMEAIT------------
Q3MQ02|Q3MQ02_XENLA|1-276     QVINEMQKKDHKQLWMGLQNDKFEQFWAINRKLMEFSP------------
Q3MQ23|Q3MQ23_CIOSA|1-266     QIINNMRETQQGQLWHGVKMDNFEEFWSINKQFMTDYE------------
Q75BY9|ATG5_ASHGO|1-288       KQIMSLSIPDSKTFWDCVLQRDERVFRGIASRITAR--------------
Q3MQ11|Q3MQ11_GASAC|1-275     HVVNDMQKKDHKQLWMGLQNDKFDQFWAINRKLMEYSS------------
Q9FFI2|ATG5_ARATH|1-336       KNVMNMSQSDQEDLWTSVMNGDLDAYTRLSPKLKMGTVEDEFS-------
C1BTY2|C1BTY2_9MAXI|1-273     RVLSKMQKKDQNQLWQGFQNDKFDQFWPINRRLMEIEGTGE---------
E0VWT9|E0VWT9_PEDHC|1-266     QVVSNMQKKDHNQLWLGLQNDKFDQFWAVNKKLMEVSG------------
A7KAI4|ATG5_PICAN|1-275       KLVMNMSRTDSDDFYHAAIHKDSTQFESMFRKLLPSS-------------
Q6BVI8|ATG5_DEBHA|4-288       KPIMNLSEKDSDELWNSIRIHNLKSFNQINKKIIPIQ-------------
Q2UBM1|ATG5_ASPOR|4-322       KGIMSLSKEDSSGLWKSVQNVELSSFQRISNILLPPLN------------
Q9H1Y0|ATG5_HUMAN|1-275       QVINEMQKKDHKQLWMGLQNDRFDQFWAINRKLMEYPA------------
C4QII2|C4QII2_SCHMA|163-515   YVINQMQARDHRQLWNGLLHFRYDQFWSINSKLMEPLPQNTKDLPLEEMS
A5DCZ3|ATG5_PICGU|1-258       KKIMGLSESESKRLWISIATHNLPEYHQITSKILPSD-------------
C0HB55|C0HB55_SALSA|1-275     QVINDMQKKDHKQLWMGLQNDKFDQFWAMNRKLMEYPT------------
Q3MQ08|Q3MQ08_ORYLA|1-275     QVVNDMQKKDHKQLWMGLQNDKFDQFWAMNRKLMEYPT------------
F1RDC0|F1RDC0_DANRE|2-275     QVINDMQKKDHKQLWMGLQNDKFDQFWAMNRKLMEYPT------------
Q17IN4|Q17IN4_AEDAE|1-265     QVVSAMQKKDHNQLWLGLVNDKFDQFWAVNRRLMEPIP------------
A7SNR5|A7SNR5_NEMVE|1-276     QVINSMQKKDHKQLWLGLNNDKFEQFWAVNRRLMERVG------------
A7TS83|ATG5_VANPO|1-290       KQVMSLSLVHSQQFWKSILMRDYITFYKIALKIIPS--------------
C1BN90|C1BN90_9MAXI|1-274     RVLSKMQKKDQNQLWQGFQNDKFDQFWPINRKLMEAQGSSE---------
P0CM36|ATG5_CRYNE|8-339       NRVTNLRRVDLEAGWDGIVNNDFDLYAQMVNKIVPLPLLTSSNSTQPSR-
Q0UXN8|ATG5_PHANO|4-311       KTVMFLSQADSTQLWDAVVKHDFALFNPINQKLLNPQ-------------
A7KAL6|ATG5_PENCW|4-317       KGIMSLSKEDSSGLWNAVQEVDLLSFQRISNILLPQP-------------
A1DMA1|ATG5_NEOFI|10-326      KGIMTLSKEDSAGLWQAVQDVDLPSFQRISNILLPPP-------------
Q3MQ04|ATG5_PIG|1-275         RVISDMQRKDHKQLWMGLQNDRFDQFWTINRKLIEYPP------------
D6W8J7|D6W8J7_TRICA|1-263     QIASNMQKKDHNQLWLGLQNDKFDQFWAVNRKLMEVSQ------------
Q12380|ATG5_YEAST|1-287       KAIMSLSVNEARKFWGSVITRNFQDFIEISNKISSS--------------
Q3MQ21|Q3MQ21_CALSI|1-269     QIITNMKSKDHKQLWMGLCNDKFDQFWAINRKLMESTP------------
B7TZ42|B7TZ42_BOMMO|1-264     QVMSTMQKKDHNQLWLGLQNDKFDQFWAINRRLMESHG------------
O74971|ATG5_SCHPO|4-261       SGIIALSKAETDTYWNAILNHDYYDFRPIAIKILFSK-------------
Q59VY1|ATG5_CANAL|4-278       KPIMQLSEANTNQLWKSLISRNLGDFNVLNKKIIK---------------
Q99J83|ATG5_MOUSE|1-275       QVINEMQKKDHKQLWMGLQNDRFDQFWAINRKLMEYPP------------
A3LR68|ATG5_PICST|3-282       KPIMSLSEANSIQLWVSIVDHNLSAYTSINKKIVPKD-------------
Q3MQ09|Q3MQ09_ICTPU|1-275     QVINDMQKKDHKQLWMGLQNDKFDQFWAINRKLMEYPT------------
A1CE93|ATG5_ASPCL|4-321       KGIMSLSKEDSSGLWEAVQDVDLPSFQRISNILLPAP-------------
Q7QGY1|Q7QGY1_ANOGA|1-265     QVVSAMQKKDHNQLWLGLVNDKFDQFWAVNRRLMEPIP------------
C3ZNT2|C3ZNT2_BRAFL|1-261     QVINGMQKKDHKQLWMGLQNDKFDQFWAINRRLMEH-G------------
Q0CRF3|ATG5_ASPTN|4-315       KGIMTLSKEDSSGLWKSVQDVDLPSFQRISNILLPPP-------------
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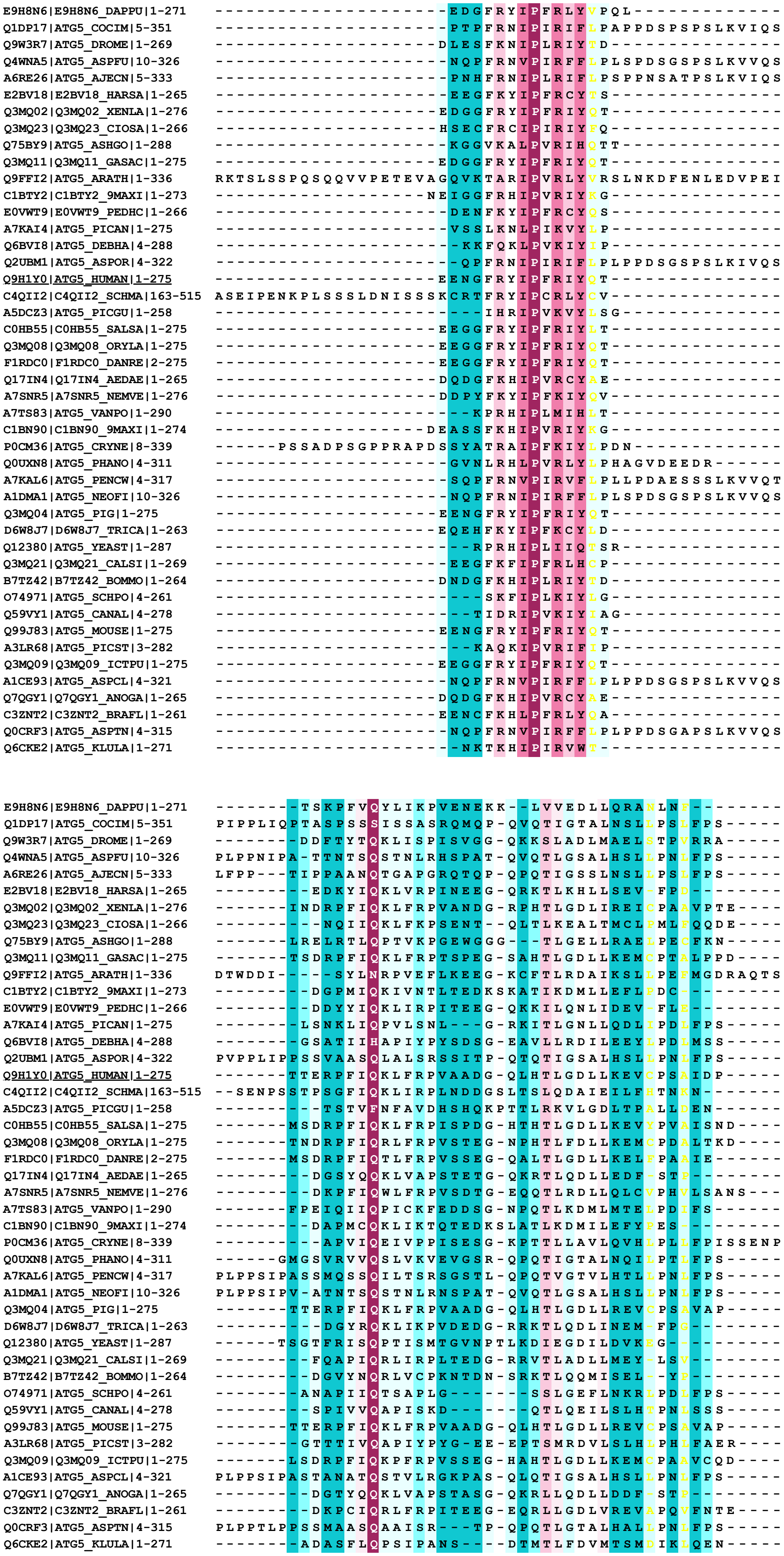

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Q4WNA5|ATG5_ASPFU|10-326       ---------------------NQPFRNVPIRFFLPLSPDSGSPSLKVVQS
A6RE26|ATG5_AJECN|5-333        ---------------------PNHFRNIPLRIFLPSPPNSATPSLKVIQS
E2BV18|E2BV18_HARSA|1-265      ---------------------EEGFKYIPFRCYTS---------------
Q3MQ02|Q3MQ02_XENLA|1-276      --------------------EDGGFRYIPFRIYQT---------------
Q3MQ23|Q3MQ23_CIOSA|1-266      --------------------HSECFRCIPIRIYFQ---------------
Q75BY9|ATG5_ASHGO|1-288        ---------------------KGGVKALPVRIHQTT--------------
Q3MQ11|Q3MQ11_GASAC|1-275      --------------------EDGGFRYIPFRIYQT---------------
Q9FFI2|ATG5_ARATH|1-336        RKTSLSSPQSQQVVPETEVAGQVKTARIPVRLYVRSLNKDFENLEDVPEI
C1BTY2|C1BTY2_9MAXI|1-273      -------------------NEIGGFRHIPVRIYKG---------------
E0VWT9|E0VWT9_PEDHC|1-266      ---------------------DENFKYIPFRCYQS---------------
A7KAI4|ATG5_PICAN|1-275        ---------------------VSSLKNLPIKVYLP---------------
Q6BVI8|ATG5_DEBHA|4-288        ----------------------KKFQKLPVKIYIP---------------
Q2UBM1|ATG5_ASPOR|4-322        ----------------------QPFRNIPIRIFLPLPPDSGSPSLKIVQS
Q9H1Y0|ATG5_HUMAN|1-275        --------------------EENGFRYIPFRIYQT---------------
C4QII2|C4QII2_SCHMA|163-515    ASEIPENKPLSSSLDNISSSKCRTFRYIPCRLYCV---------------
A5DCZ3|ATG5_PICGU|1-258        ------------------------IHRIPVKVYLSG--------------
C0HB55|C0HB55_SALSA|1-275      --------------------EEGGFRYIPFRIYLT---------------
Q3MQ08|Q3MQ08_ORYLA|1-275      --------------------EEGGFRYIPFRIYQT---------------
F1RDC0|F1RDC0_DANRE|2-275      --------------------EEGGFRYIPFRIYQT---------------
Q17IN4|Q17IN4_AEDAE|1-265      --------------------DQDGFKHIPVRCYAE---------------
A7SNR5|A7SNR5_NEMVE|1-276      --------------------DDPYFKYIPFKIYQV---------------
A7TS83|ATG5_VANPO|1-290        -----------------------KPRHIPLMIHLT---------------
C1BN90|C1BN90_9MAXI|1-274      -------------------DEASSFKHIPVRIYKG---------------
P0CM36|ATG5_CRYNE|8-339        ------PSSADPSGPPRAPDSSYATRAIPFKIYLPDN-------------
Q0UXN8|ATG5_PHANO|4-311        ---------------------GVNLRHLPVRLYLPHAGVDEEDR------
A7KAL6|ATG5_PENCW|4-317        ---------------------SQPFRNVPIRVFLPLLPDAESSSLKVVQT
A1DMA1|ATG5_NEOFI|10-326       ---------------------NQPFRNIPIRFFLPLSPDSGSPSLKVVQS
Q3MQ04|ATG5_PIG|1-275          --------------------EENGFRYIPFRIYQT---------------
D6W8J7|D6W8J7_TRICA|1-263      --------------------EQEHFKYIPFKCYLD---------------
Q12380|ATG5_YEAST|1-287        -----------------------RPRHIPLIIQTSR--------------
Q3MQ21|Q3MQ21_CALSI|1-269      ---------------------EEGFKFIPFRLHCP---------------
B7TZ42|B7TZ42_BOMMO|1-264      --------------------DNDGFKHIPLRIYTD---------------
O74971|ATG5_SCHPO|4-261        ------------------------SKFIPLKIYLG---------------
Q59VY1|ATG5_CANAL|4-278        -----------------------TIDRIPVKIYIAG--------------
Q99J83|ATG5_MOUSE|1-275        --------------------EENGFRYIPFRIYQT---------------
A3LR68|ATG5_PICST|3-282        -----------------------KAQKIPVRIFIP---------------
Q3MQ09|Q3MQ09_ICTPU|1-275      --------------------EEGGFRYIPFRIYQT---------------
A1CE93|ATG5_ASPCL|4-321        ---------------------NQPFRNVPIRFFLPLPPDSGSPSLKVVQS
Q7QGY1|Q7QGY1_ANOGA|1-265      --------------------DQDGFKHIPVRCYAE---------------
C3ZNT2|C3ZNT2_BRAFL|1-261      --------------------EENCFKHLPFRLYQA---------------
Q0CRF3|ATG5_ASPTN|4-315        ---------------------NQPFRNVPIRFFLPLPPDSGAPSLKVVQS
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E9H8N6|E9H8N6_DAPPU|1-271      --------TSKPFVQYLIKPVENEKK--LVVEDLLQRANLNF--------
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Q9W3R7|ATG5_DROME|1-269        --------DDFTYTQKLISPISVGG-QKKSLADLMAELSTPVRRA-----
Q4WNA5|ATG5_ASPFU|10-326       PLPPNIPA-TTNTSQSTNLRHSPAT-QVQTLGSALHSLLPNLFPS-----
A6RE26|ATG5_AJECN|5-333        LFPP---TIPPAANQTGAPGRQTQP-QPQTIGSSLNSLLPSLFPS-----
E2BV18|E2BV18_HARSA|1-265      ---------EDKYIQKLVRPINEEG-QRKTLKHLLSEV-FPD--------
Q3MQ02|Q3MQ02_XENLA|1-276      -------INDRPFIQKLFRPVANDG-RPHTLGDLIREICPAAVPTE----
Q3MQ23|Q3MQ23_CIOSA|1-266      ----------NQIIQKLFKPSENT--QLTLKEALTMCLPMLFQQDE----
Q75BY9|ATG5_ASHGO|1-288        -------LRELRTLQPTVKPGEWGGG---TLGELLRAELPECFKN-----
Q3MQ11|Q3MQ11_GASAC|1-275      -------TSDRPFIQKLFRPTSPEG-SAHTLGDLLKEMCPTALPPD----
Q9FFI2|ATG5_ARATH|1-336        DTWDDI-----SYLNRPVEFLKEEG-KCFTLRDAIKSLLPEFMGDRAQTS
C1BTY2|C1BTY2_9MAXI|1-273      ---------DGPMIQKIVNTLTEDKSKATIKDMLLEFLPDC---------
E0VWT9|E0VWT9_PEDHC|1-266      ---------DDYYIQKLIRPITEEG-QKKILQNLIDEV-FLE--------
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Q6BVI8|ATG5_DEBHA|4-288        --------GSATIIHAPIYPYSDSG-EAVLLRDILEEYLPDLMSS-----
Q2UBM1|ATG5_ASPOR|4-322        PVPPLIPPSSVAASQLALSRSSITP-QTQTIGSALHSLLPNLFPS-----
Q9H1Y0|ATG5_HUMAN|1-275        -------TTERPFIQKLFRPVAADG-QLHTLGDLLKEVCPSAIDP-----
C4QII2|C4QII2_SCHMA|163-515    --SENPSSTPSGFIQKLIRPLNDDGSLTSLQDAIEILFHTNKN-------
A5DCZ3|ATG5_PICGU|1-258        ----------TSTVFNFAVDHSHQKPTTLRKVLGDLTPALLDEN------
C0HB55|C0HB55_SALSA|1-275      -------MSDRPFIQKLFRPISPDG-HTHTLGDLLKEVYPVAISND----
Q3MQ08|Q3MQ08_ORYLA|1-275      -------TNDRPFIQRLFRPVSTEG-NPHTLFDLLKEMCPDALTKD----
F1RDC0|F1RDC0_DANRE|2-275      -------MSDRPFIQTLFRPVSSEG-QALTLGDLLKELFPAAIE------
Q17IN4|Q17IN4_AEDAE|1-265      ---------DGSYQQKLVAPSTETG-QKRTLQDLLEDF-STP--------
A7SNR5|A7SNR5_NEMVE|1-276      ---------DKPFIQWLFRPVSDTG-EQQTLRDLLQLCVPHVLSANS---
A7TS83|ATG5_VANPO|1-290        -------FPEIQIIQPICKFEDDSG-NPQTLKDMLMTELPDIFS------
C1BN90|C1BN90_9MAXI|1-274      ---------DAPMCQKLIKTQTEDKSLATLKDMILEFYPES---------
P0CM36|ATG5_CRYNE|8-339        ----------APVIQEIVPPISESG-KPTTLLAVLQVHLPLLFPISSENP
Q0UXN8|ATG5_PHANO|4-311        ------GMGSVRVVQSLVKVEVGSR-QPQTIGTALNQILPTLFPS-----
A7KAL6|ATG5_PENCW|4-317        PLPPSIPASSMQSSQILTSRSGSTL-QPQTVGTVLHTLLPNLFPS-----
A1DMA1|ATG5_NEOFI|10-326       PLPPSIPV-ATNTSQSTNLRNSPAT-QVQTLGSALHSLLPNLFPS-----
Q3MQ04|ATG5_PIG|1-275          -------TTERPFIQKLFRPVAADG-QLHTLGDLLREVCPSAVAP-----
D6W8J7|D6W8J7_TRICA|1-263      ----------DGYRQKLIKPVDEDG-RRKTLQDLINEM-FPG--------
Q12380|ATG5_YEAST|1-287        ------TSGTFRISQPTISMTGVNPTLKDIEGDILDVKEG----------
Q3MQ21|Q3MQ21_CALSI|1-269      ---------FQAPIQRLIRPLTEDG-RRVTLADLLMEY-LSV--------
B7TZ42|B7TZ42_BOMMO|1-264      ---------DGVYNQRLVCPKNTDN-SRKTLQQMISEL--YP--------
O74971|ATG5_SCHPO|4-261        --------ANAPIIQTSAPLG-------SSLGEFLNKRLPDLFPS-----
Q59VY1|ATG5_CANAL|4-278        ---------SPIVVQAPISKD-------QTLQEILSLHTPNLSSS-----
Q99J83|ATG5_MOUSE|1-275        -------TTERPFIQKLFRPVAADG-QLHTLGDLLREVCPSAVAP-----
A3LR68|ATG5_PICST|3-282        --------GTTTIVQAPIYPYG-EE-EPTSMRDVLSLHLPHLFAER----
Q3MQ09|Q3MQ09_ICTPU|1-275      -------LSDRPFIQKPFRPVSSEG-HAHTLGDLLKEMCPAAVCQD----
A1CE93|ATG5_ASPCL|4-321        PLPPSIPASTANATQSTVLRGKPAS-QLQTIGSALHSLLPNLFPS-----
Q7QGY1|Q7QGY1_ANOGA|1-265      ---------DGTYQQKLVAPSTASG-QKRLLQDLLDDF-STP--------
C3ZNT2|C3ZNT2_BRAFL|1-261      ---------DKPCIQRLFRPITEEG-EQRLLGDLVREVAPQVFNTE----
Q0CRF3|ATG5_ASPTN|4-315        PLPPTLPPSSMAASQAAISR---TP-QPQTLGTALHALLPNLFPS-----
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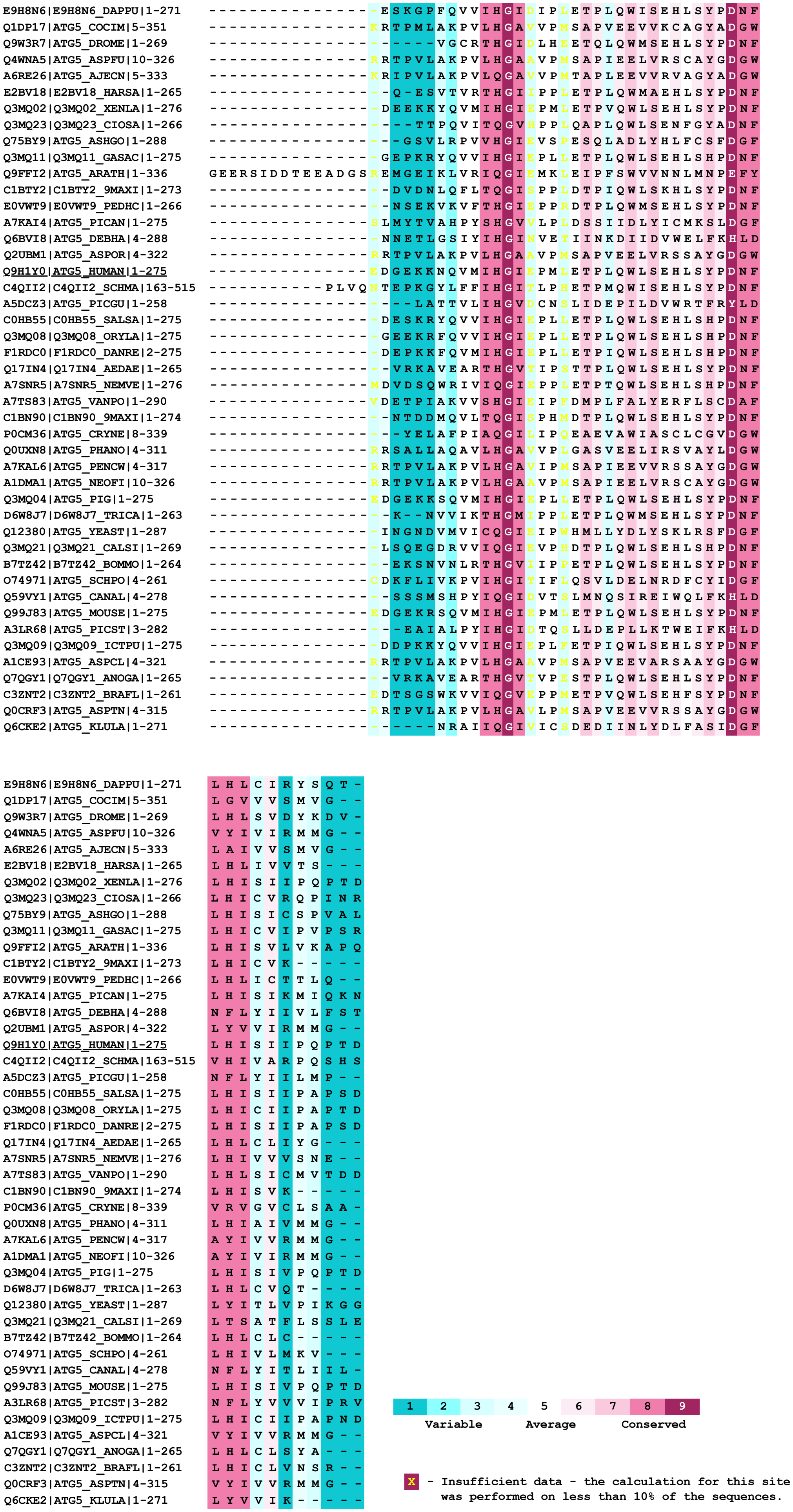

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Q9W3R7|ATG5_DROME|1-269       ---------------  ------VGCRTHGIDLHEETQLQWMSEHLSYPDNF
Q4WNA5|ATG5_ASPFU|10-326      ---------------  RRTPVLAKPVLHGAAVPMSAPIEELVRSCAYGDGW
A6RE26|ATG5_AJECN|5-333       ---------------  KRIPVLAKPVLQGAVVPMTAPLEEVVRVAGYADGW
E2BV18|E2BV18_HARSA|1-265     ---------------  --Q-ESVTVRTHGIIPPLETPLQWMAEHLSYPDNF
Q3MQ02|Q3MQ02_XENLA|1-276     ---------------  -DEEKKYQVMIHGIEPMLETPVQWLSEHLSYPDNF
Q3MQ23|Q3MQ23_CIOSA|1-266     ---------------  ----TTPQVITQGVHPPLQAPLQWLSENFGYADNF
Q75BY9|ATG5_ASHGO|1-288       ---------------  ---GSVLRPVVHGIEVSPESQLADLYHLFCSFDGF
Q3MQ11|Q3MQ11_GASAC|1-275     ---------------  -GEPKRYQVVIHGIEPLLETPLQWLSEHLSHPDNF
Q9FFI2|ATG5_ARATH|1-336       GEERSIDDTEEADGS  REMGEIKLVRIQGIEMKLEIPFSWVVNNLMNPEFY
C1BTY2|C1BTY2_9MAXI|1-273     ---------------  --DVDNLQFLTQGISPPLDTPIQWLSKHLSYPDNF
E0VWT9|E0VWT9_PEDHC|1-266     ---------------  --NSEKVKVFTHGIEPPRDTPLQWMSEHLSYPDNF
A7KAI4|ATG5_PICAN|1-275       ---------------  SLMYTVAHPYSHGVVLPLDSSIIDLYICMKSLDGF
Q6BVI8|ATG5_DEBHA|4-288       ---------------  -NNETLGSIYIHGINVETIINKDIIDVWELFKHLD
Q2UBM1|ATG5_ASPOR|4-322       ---------------  RRTPVLAKPVLHGAAVPMSAPVEELVRSSAYGDGW
Q9H1Y0|ATG5_HUMAN|1-275       ---------------  EDGEKKNQVMIHGIEPMLETPLQWLSEHLSYPDNF
C4QII2|C4QII2_SCHMA|163-515   -----------PLVQ  NTEPKGYLFFIHGITLPHETPMQWISEHLSYPDNF
A5DCZ3|ATG5_PICGU|1-258       ---------------  ----LATTVLIHGVDCNSLIDEPILDVWRTFRYLD
C0HB55|C0HB55_SALSA|1-275     ---------------  -DESKRYQVVIHGIEPLLETPLQWLSEHLSHPDNF
Q3MQ08|Q3MQ08_ORYLA|1-275     ---------------  -GEEKRFQVVIHGIEPLLETPLQWLSEHLSHPDNF
F1RDC0|F1RDC0_DANRE|2-275     ---------------  -DEPKKFQVMIHGIEPLLETPIQWLSEHLSHPDNF
Q17IN4|Q17IN4_AEDAE|1-265     ---------------  --VRKAVEARTHGVTIPSTTPLQWLSEHLSYPDNF
A7SNR5|A7SNR5_NEMVE|1-276     ---------------  MDVDSQWRIVIQGIEPPLETPTQWLSEHLSHPDNF
A7TS83|ATG5_VANPO|1-290       ---------------  VDETPIAKVVSHGIEIPFDMPLFALYERFLSCDAF
C1BN90|C1BN90_9MAXI|1-274     ---------------  --NTDDMQVLTQGISPHMDTPLQWLSEHLSYPDNF
P0CM36|ATG5_CRYNE|8-339       ---------------  ---YELAFPIAQGILIPQEAEVAWIASCLCGVDGW
Q0UXN8|ATG5_PHANO|4-311       ---------------  RRSALLAQAVLHGAVVPLGASVEELIRSVAYLDGW
A7KAL6|ATG5_PENCW|4-317       ---------------  RRTPVLAKPVLHGAVIPMSAPIEEVVRSSAYGDGW
A1DMA1|ATG5_NEOFI|10-326      ---------------  RRTPVLAKPVLHGAAVPMSAPIEELVRSCAYGDGW
Q3MQ04|ATG5_PIG|1-275         ---------------  EDGEKKSQVMIHGIEPLLETPLQWLSEHLSYPDNF
D6W8J7|D6W8J7_TRICA|1-263     ---------------  --K--NVVIKTHGMIPPLETPLQWMSEHLSYPDNF
Q12380|ATG5_YEAST|1-287       ---------------  -INGNDVMVICQGIEIPWHMLLYDLYSKLRSFDGF
Q3MQ21|Q3MQ21_CALSI|1-269     ---------------  -LSQEGDRVVIQGIEVPHDTPLQWLSEHLSHPDNF
B7TZ42|B7TZ42_BOMMO|1-264     ---------------  --EKSNVNLRTHGVIIPPETPLQWLSEHLSYPDNF
O74971|ATG5_SCHPO|4-261       ---------------  CDKFLIVKPVIHGITIFLQSVLDELNRDFCYIDGF
Q59VY1|ATG5_CANAL|4-278       ---------------  --SSSMSHPYIQGIDVTSLMNQSIREIWQLFKHLD
Q99J83|ATG5_MOUSE|1-275       ---------------  EDGEKRSQVMIHGIEPMLETPLQWLSEHLSYPDNF
A3LR68|ATG5_PICST|3-282       ---------------  ---EAIALPYIHGIDTQSLLDEPLLKTWEIFKHLD
Q3MQ09|Q3MQ09_ICTPU|1-275     ---------------  -DDPKKYQVVIHGIEPLFETPIQWLSEHLSYPDNF
A1CE93|ATG5_ASPCL|4-321       ---------------  RRTPVLAKPVLHGAAVPMSAPVEEVARSAAYGDGW
Q7QGY1|Q7QGY1_ANOGA|1-265     ---------------  --VRKAVEARTHGVTVPESTPLQWLSEHLSYPDNF
C3ZNT2|C3ZNT2_BRAFL|1-261     ---------------  EDTSGSWKVVIQGVEPPMETPVQWLSEHFSYPDNF
Q0CRF3|ATG5_ASPTN|4-315       ---------------  RRTPVLAKPVLHGAVLPMSAPVEEVVRSSAYGDGW
Q6CKE2|ATG5_KLULA|1-271       ---------------  ------NRAIIQGIVICSDEDIINLYDLFASIDGF

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Q9W3R7|ATG5_DROME|1-269       LHLSVDYKDV-
Q4WNA5|ATG5_ASPFU|10-326      VYIVIRMMG--
A6RE26|ATG5_AJECN|5-333       LAIVVSMVG--
E2BV18|E2BV18_HARSA|1-265     LHLIVVTS---
Q3MQ02|Q3MQ02_XENLA|1-276     LHISIIPQPTD
Q3MQ23|Q3MQ23_CIOSA|1-266     LHICVRQPINR
Q75BY9|ATG5_ASHGO|1-288       LHISICSPVAL
Q3MQ11|Q3MQ11_GASAC|1-275     LHICVIPVPSR
Q9FFI2|ATG5_ARATH|1-336       LHISVLVKAPQ
C1BTY2|C1BTY2_9MAXI|1-273     LHICVK-----
E0VWT9|E0VWT9_PEDHC|1-266     LHLICTTLQ--
A7KAI4|ATG5_PICAN|1-275       LHISIKMIQKN
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A5DCZ3|ATG5_PICGU|1-258       NFLYIILMP--
C0HB55|C0HB55_SALSA|1-275     LHISIIPAPSD
Q3MQ08|Q3MQ08_ORYLA|1-275     LHICIIPAPTD
F1RDC0|F1RDC0_DANRE|2-275     LHISIIPAPSD
Q17IN4|Q17IN4_AEDAE|1-265     LHLCLIYG---
A7SNR5|A7SNR5_NEMVE|1-276     LHIVVVSNE--
A7TS83|ATG5_VANPO|1-290       LHLSICMVTDD
C1BN90|C1BN90_9MAXI|1-274     LHISVK-----
P0CM36|ATG5_CRYNE|8-339       VRVGVCLSAA-
Q0UXN8|ATG5_PHANO|4-311       LHIAIVMMG--
A7KAL6|ATG5_PENCW|4-317       AYIVVRMMG--
A1DMA1|ATG5_NEOFI|10-326      AYIVIRMMG--
Q3MQ04|ATG5_PIG|1-275         LHISIVPQPTD
D6W8J7|D6W8J7_TRICA|1-263     LHLCVQT----
Q12380|ATG5_YEAST|1-287       LYITLVPIKGG
Q3MQ21|Q3MQ21_CALSI|1-269     LTSATFLSSLE
B7TZ42|B7TZ42_BOMMO|1-264     LHLCLC-----
O74971|ATG5_SCHPO|4-261       LHIVLMKV---
Q59VY1|ATG5_CANAL|4-278       NFLYITLIIL-
Q99J83|ATG5_MOUSE|1-275       LHISIVPQPTD
A3LR68|ATG5_PICST|3-282       NFLYVVVIPRV
Q3MQ09|Q3MQ09_ICTPU|1-275     LHICIIPAPND
A1CE93|ATG5_ASPCL|4-321       VYIVVRMMG--
Q7QGY1|Q7QGY1_ANOGA|1-265     LHLCLSYA---
C3ZNT2|C3ZNT2_BRAFL|1-261     LHICLVNSR--
Q0CRF3|ATG5_ASPTN|4-315       VYIVVRMMG--
Q6CKE2|ATG5_KLULA|1-271       LYVVIK-----
```

1	2	3	4	5	6	7	8	9
Variable				Average				Conserved

X - Insufficient data - the calculation for this site was performed on less than 10% of the sequences.

Supplementary Figure 5 Multiple sequence alignments of ATG12 and ATG5 family members used for ConSurf analyses in **Figure 3**. Each sequence is labeled with its UniProtKB, entry name for Uniprot, and residue range at the left, middle, and right, respectively, in the left column. Fifty and 45 sequences of ATG12 and ATG5, respectively, were used to generate the multiple sequence alignments.

Supplementary Note

Cloning, expression and purification of recombinant proteins.

ATG3: The full-length ATG3 coding DNA sequence was cloned into the BamHI-XhoI site of a glutathione S-transferase (GST)-fusion vector modified from pGEX4T3 (GE). Protein expression was induced by the addition of 0.2 mM IPTG to transformed BL21 (λDE3) cells growing in LB media at an OD_{600} of 0.8, after which the cells were grown at 18ºC for 12–16 hours and then harvested. The protein was purified by glutathione affinity and Source 15Q (GE) anion-exchange chromatography followed by TEV cleavage and passage over a SD200 column. ATG12: The $ATG12_{52–140}$ coding DNA sequence was subcloned into pTXB1 (NEB) using NdeI and BspQI and then was transferred to a modified pET-Duet-1 vector using NdeI and BamHI; the resulting vector expresses the 6×His-MBP-TEVs-$ATG12_{52–140}$-intein-chitin binding domain fusion. Protein expression was performed as described for ATG3. The fusion protein was purified by chitin affinity (NEB) and SD200 size-exclusion chromatography followed by dithiothreitol (DTT)-induced intein cleavage according to the manufacturer's instructions (NEB). The cleaved protein was further purified on a Source 15S column. The 6×His-MBP tag was removed by TEV protease, and the protein was further purified on Source 15S and Superdex 75 (GE) gel-filtration columns. Because ATG12 tended to aggregate over time, the purified protein was used within 2 days after the final gel-filtration step. The ATG5–ATG16N complex: The full-length ATG5 coding DNA sequence was cloned into a 6×His-MBP vector based on pET Duet-1. The fusion protein was expressed as described for ATG3. The 6×His-MBP-TEVs-ATG5 fusion protein was purified by nickel affinity and SD200 size-exclusion chromatography, treated with TEV protease followed by Source 15Q anion-exchange and butyl sepharose (GE) hydrophobic chromatography. The purified ATG5 was then complexed with 6×His-MBP-TEVs-ATG16N. Upon completion of TEV cleavage, the complex was further purified on Source 15Q and SD200 columns. The ATG12-ATG5 fusion: The $ATG12_{52–140}$ and full-length ATG5 coding DNA sequences were joined with a linker coding GlyGlySerGlyGlySer hexa-peptide by PCR and the resulting fusion sequence was cloned into a MBP-6×His vector modified from pET15b (Novagen). The fusion protein was expressed, purified, and complexed with ATG16N as described for ATG5. The BMOE-cross-linked ATG12~ATG5 conjugate mimic: The ATG12$_{52–140}^{C122N\ C134S\ G140C}$ and ATG5$^{C19Q\ C115L\ C128S\ C223L\ K130C}$ mutant proteins were purified as described above for the wild-type of each protein. The purified proteins were buffer-exchanged into 20 mM Hepes, pH 7.5, 300 mM NaCl, and 5 mM EDTA and then mixed at a concentration of ~250 µM of each protein. A 1.5-fold molar excess of BMOE (Pierce) was added to the protein solution, which was then incubated at room temperature for 2 hours. The reaction was quenched by the addition of 10 mM DTT. The reaction solution was mixed with the 6×His-MBP-TEVs-ATG16N protein, followed by purification as described for the wild-type ATG12~ATG5–ATG16N complex. ATG7: The full-length ATG7 coding DNA sequence was cloned into the NcoI-SalI site of a modified pFastBac1 vector (Invitrogen); the resulting vector expresses 6×His-TEVs-ATG7 in insect cells. The baculovirus was generated using Bac-to-Bac Baculovirus Expression System (Invitrogen) with this vector and protein expression was carried out in Sf9 (Spodoptera frugiperda) cells at 27ºC for 2.5 days. The expressed protein was purified by nickel affinity chromatography followed by passage over Source 15Q and SD200 columns. LC3B: The LC3B$_{1\text{-}120}$ (matured form) coding DNA sequence was cloned into the Nde-BamHI site of a GST-fusion vector modified from pGEX2T (GE). Protein expression was carried out as described for ATG3. LC3B was purified on glutathione-sepharose and Source 15S columns followed by TEV cleavage and SD200 size-exclusion chromatography.