



Supplemental Material to:

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Identification of Atg3 as an intrinsically disordered polypeptide yields insights into the molecular dynamics of autophagy-related proteins in yeast

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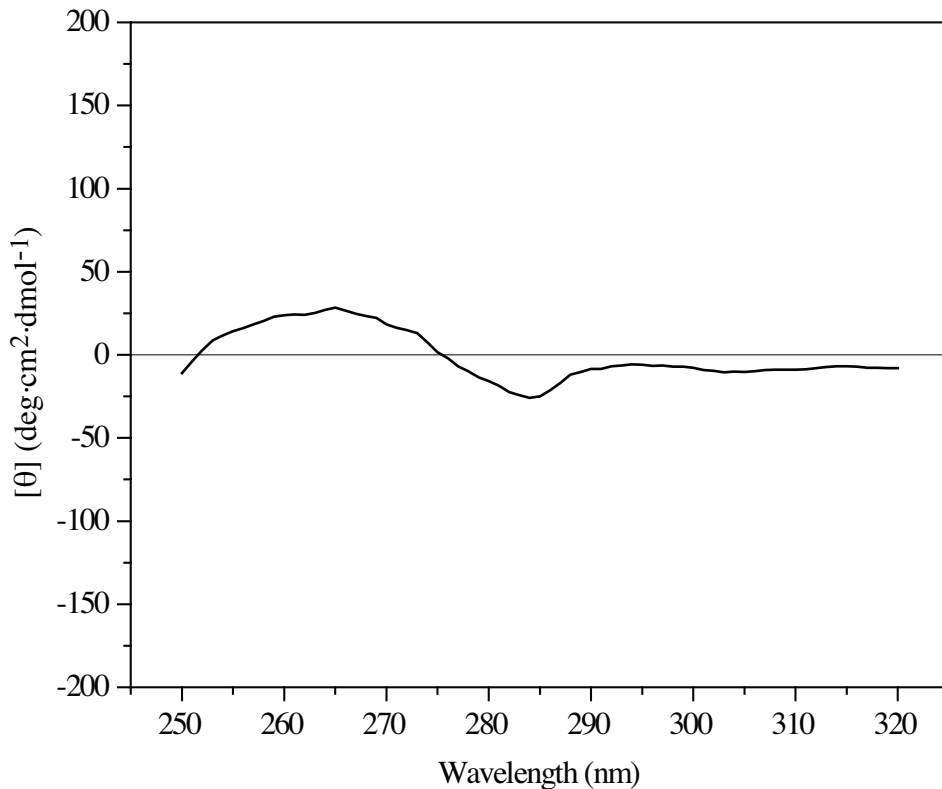


Figure S1. Near-UV CD spectrum (protein solution minus buffer) of Atg3 at 25 °C in 50 mM potassium buffer, pH 8.0. The spectrum is the average of 10 scans. Experimental conditions were as follows: time constant, 1 s; bandwidth, 1 nm; path length, 10 mm; sample volume, 1 ml.

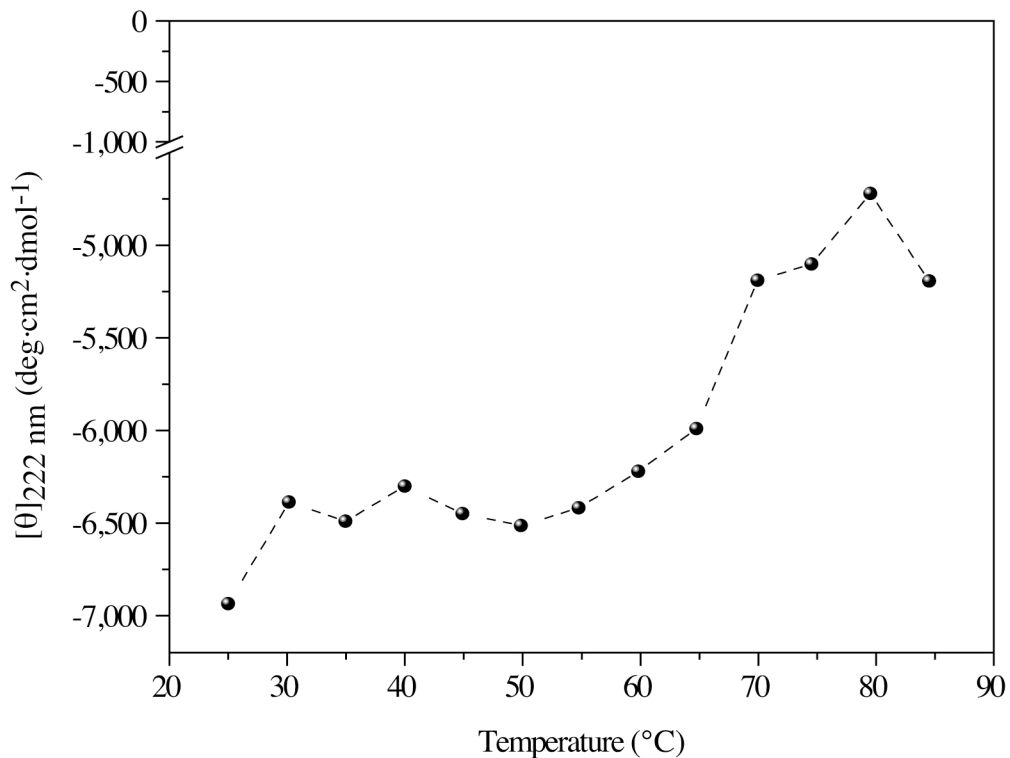


Figure S2. Melting curve (protein solution minus buffer) of Atg3. An increase in mean residue ellipticity at 222 nm ($[\theta]_{222 \text{ nm}}$) monitors the loss of the α -helical secondary structure element during heating. Experimental conditions were as follows: time constant, 1s; bandwidth, 1 nm; path length, 1 mm; sample volume, 300 μ l; temperature step, 5°C; temperature dead band, 0.5°C; temperature equilibration time, 0.1 min.



Figure S3. Amino acid sequence alignments between wild type and the Δ FR deletion mutant of Atg3 from yeast (A), wild-type and the recombinant FR fragment of Atg3 from yeast (B), and wild-type Atg3/ATG3 proteins from yeast and human (C). The alignment was created in the BioEdit Sequence Alignment Editor.¹

Supplemental reference

1. Hall TA. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp.* 1999; 41:95-8.

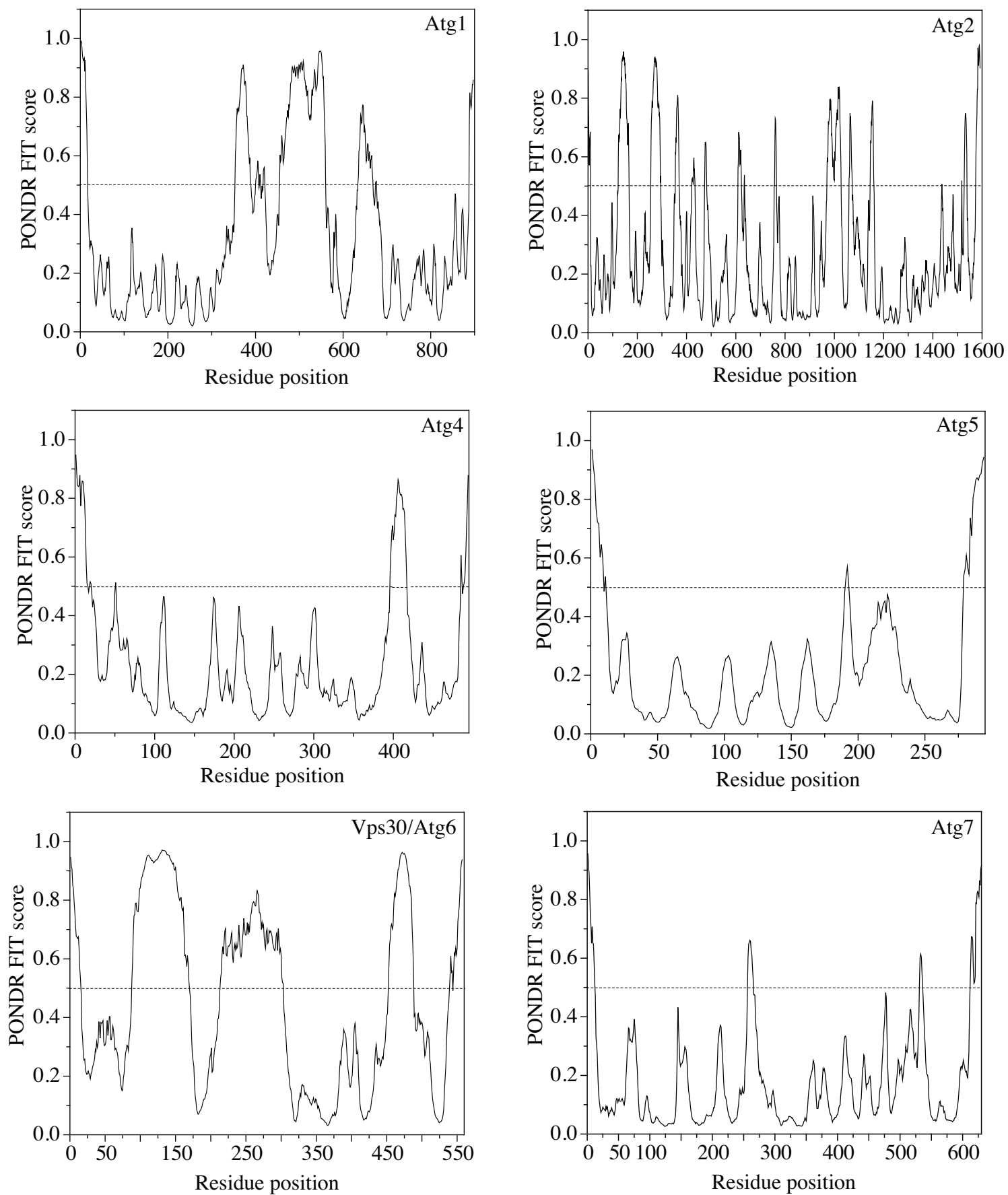


Figure S4. PONDR-FIT analysis of yeast Atg1, Atg2, Atg4, Atg5, Vps30/Atg6, and Atg7.

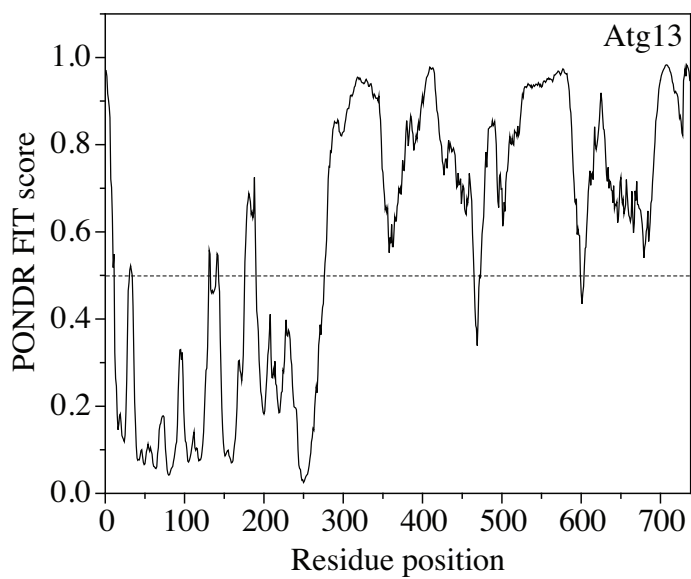
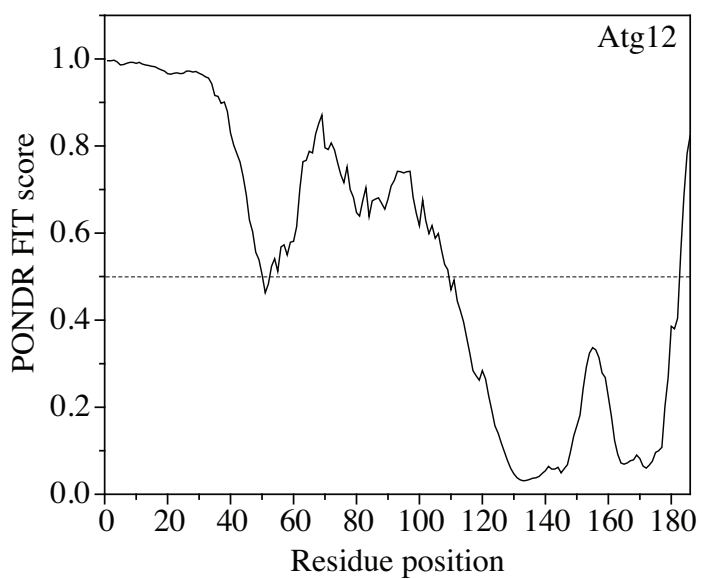
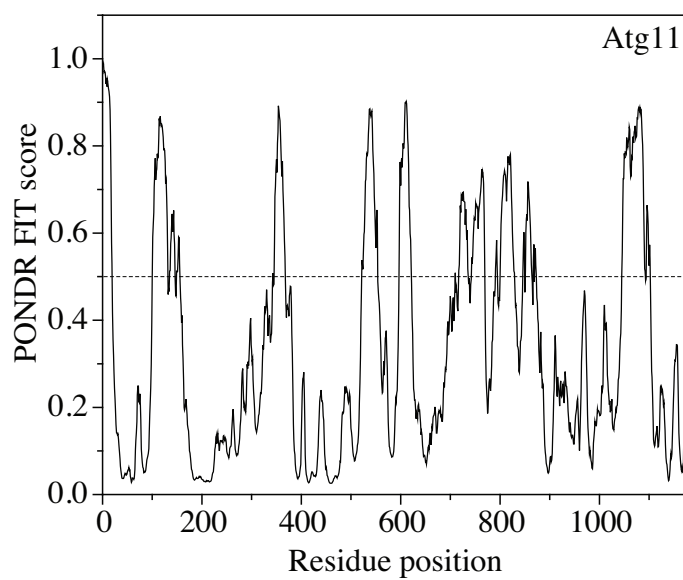
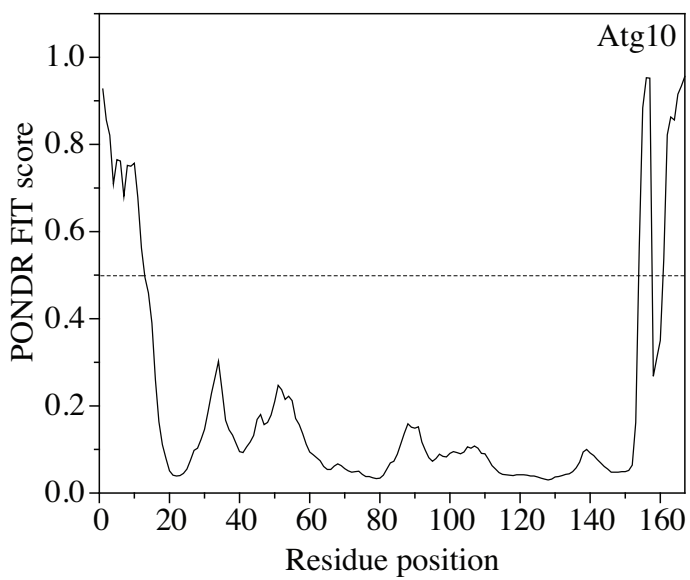
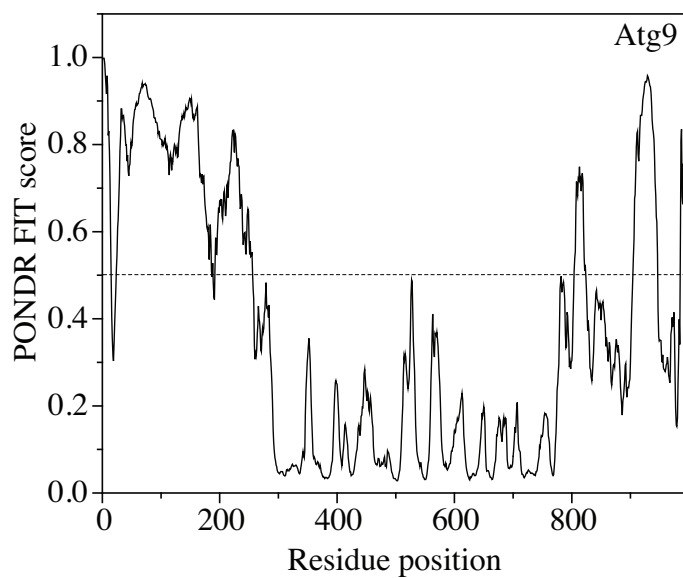
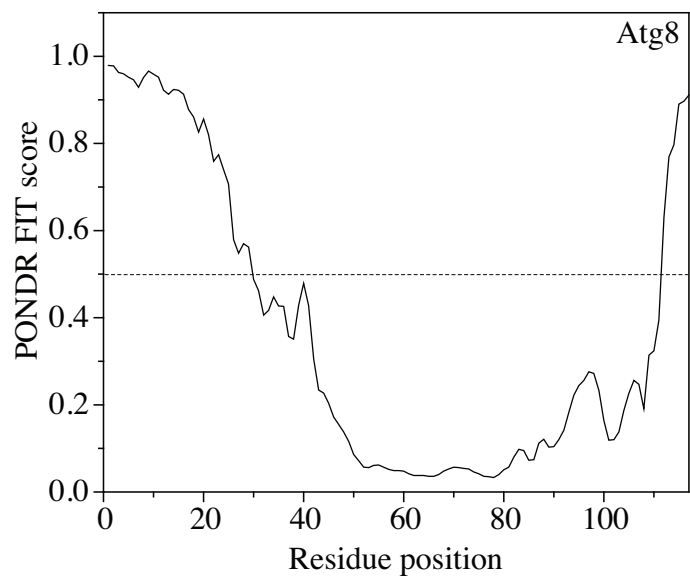


Figure S5. PONDR-FIT analysis of yeast Atg8, Atg9, Atg10, Atg11, Atg12, and Atg13.

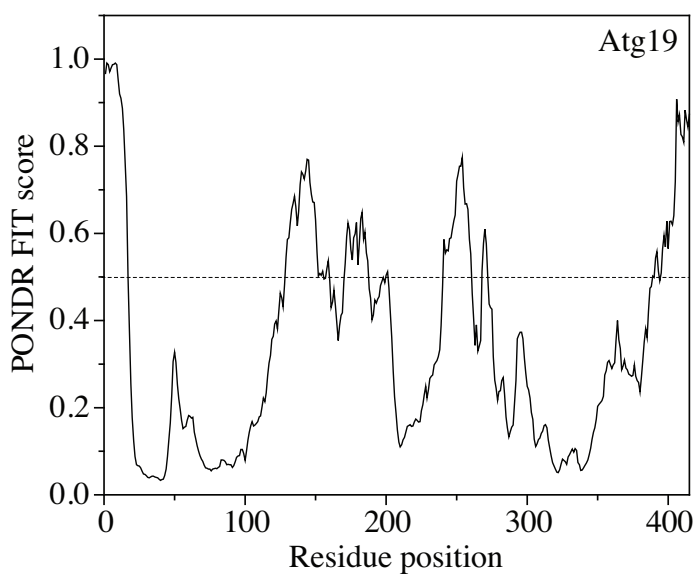
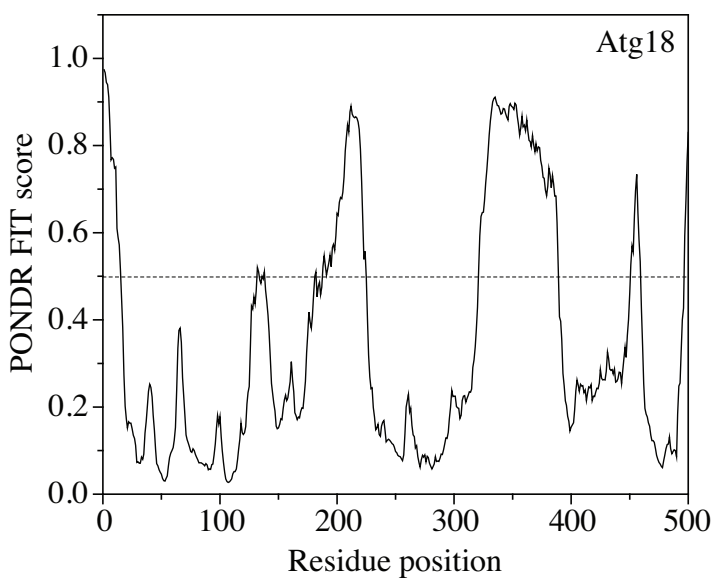
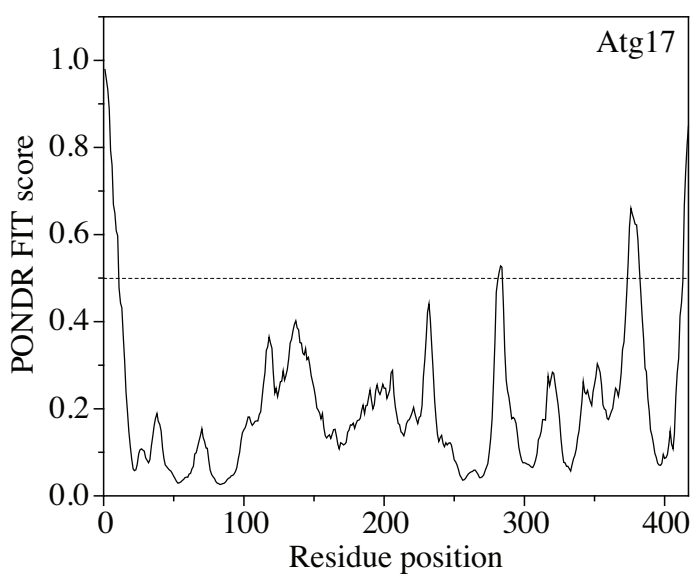
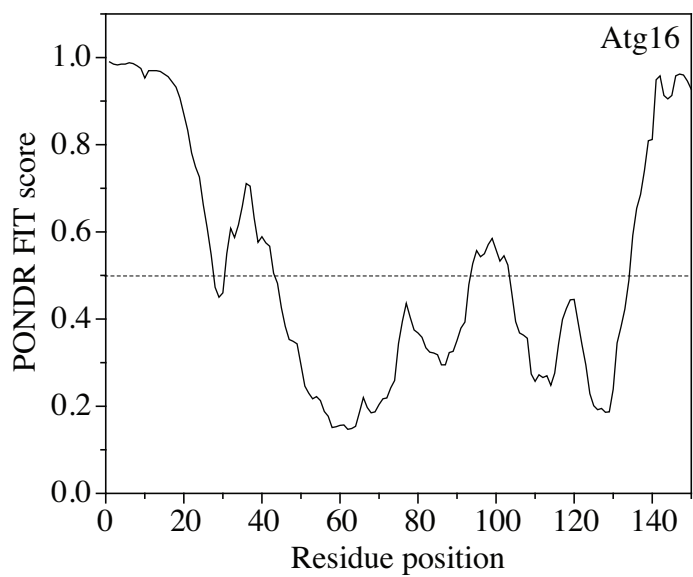
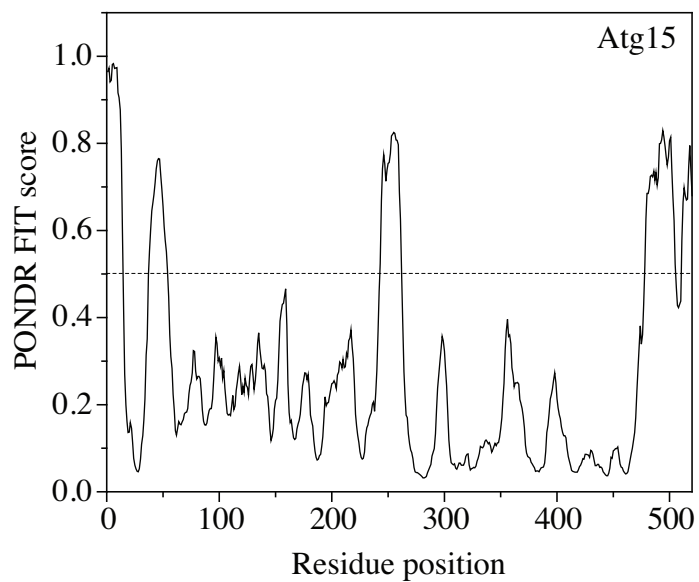
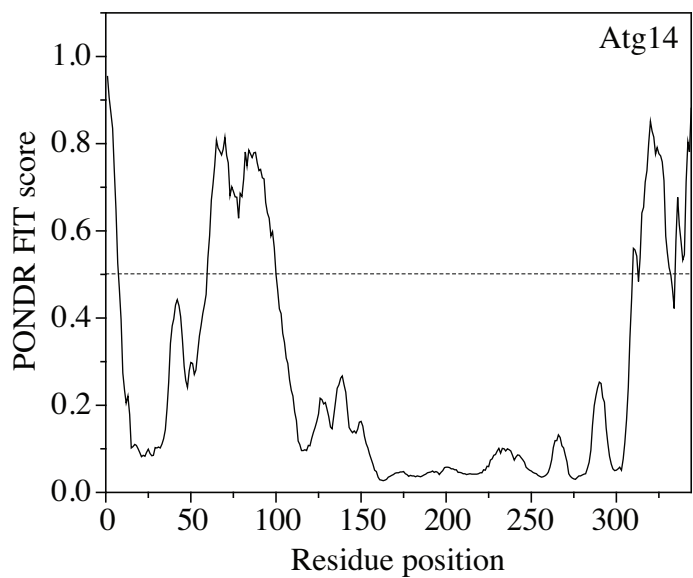


Figure S6. POND-RFIT analysis of yeast Atg14, Atg15, Atg16, Atg17, Atg18, and Atg19.

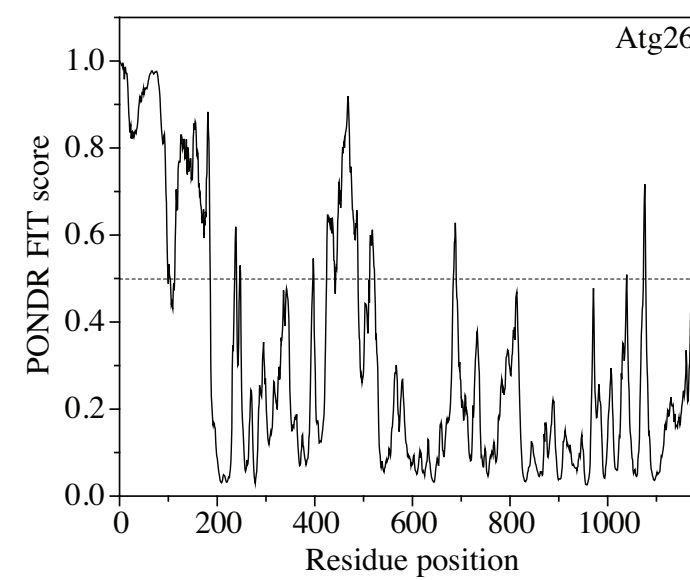
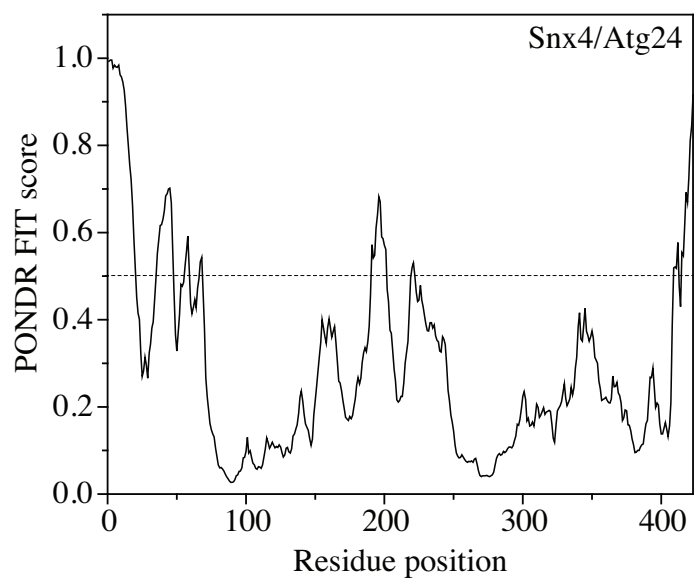
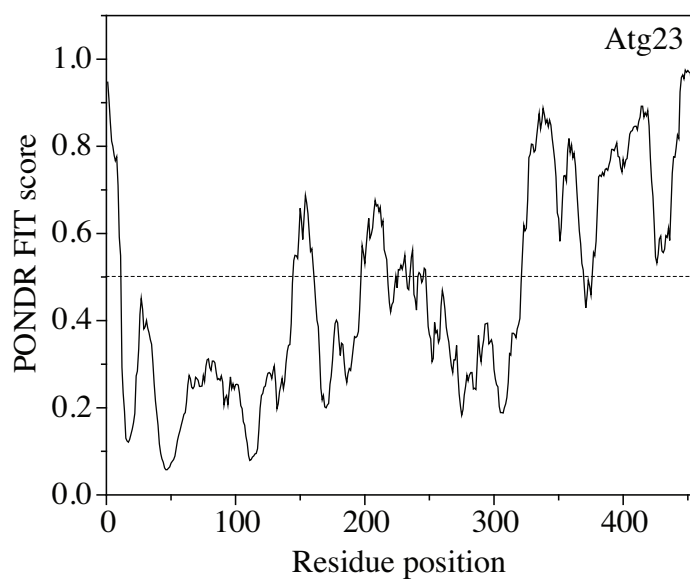
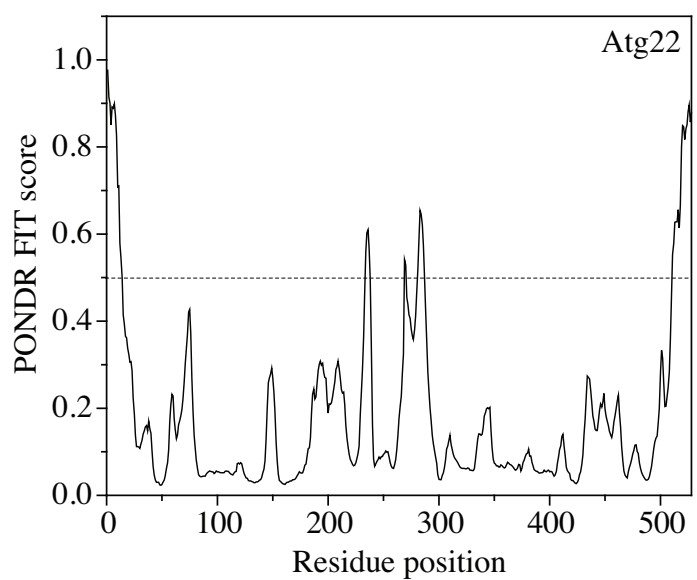
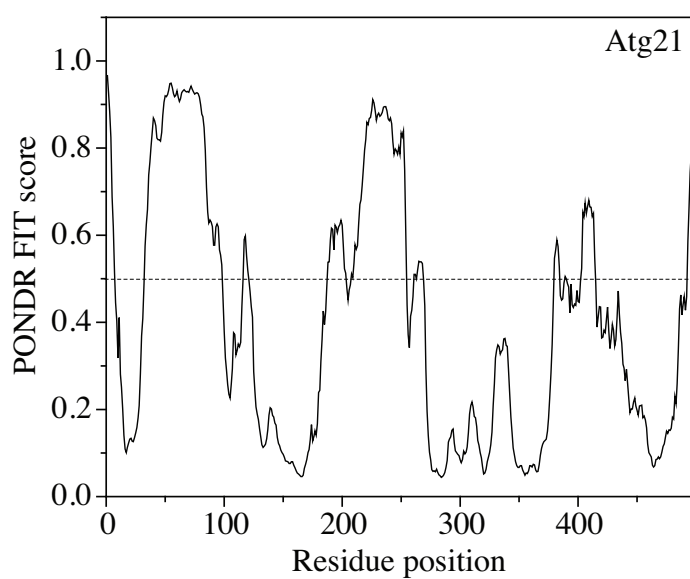
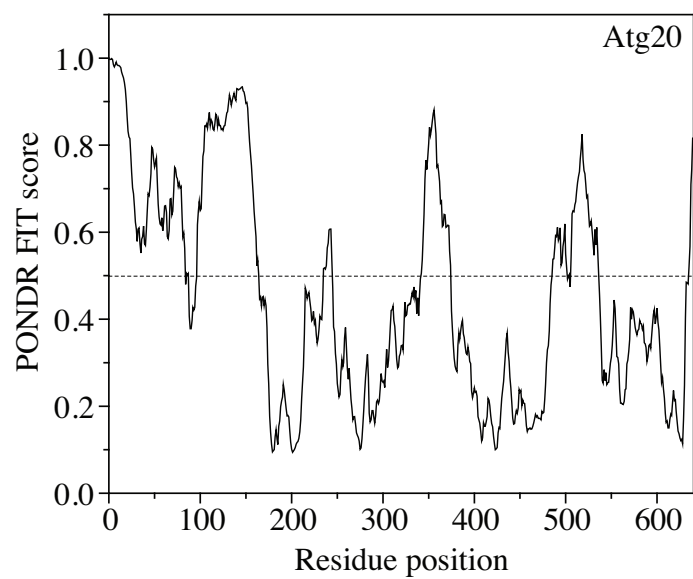


Figure S7. PONDR-FIT analysis of yeast Atg20, Atg21, Atg22, Atg23, Snx4/Atg24, and Atg26.

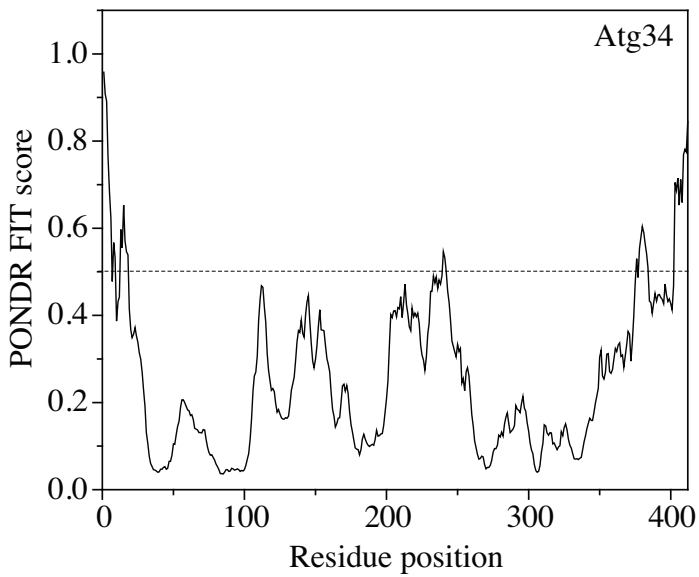
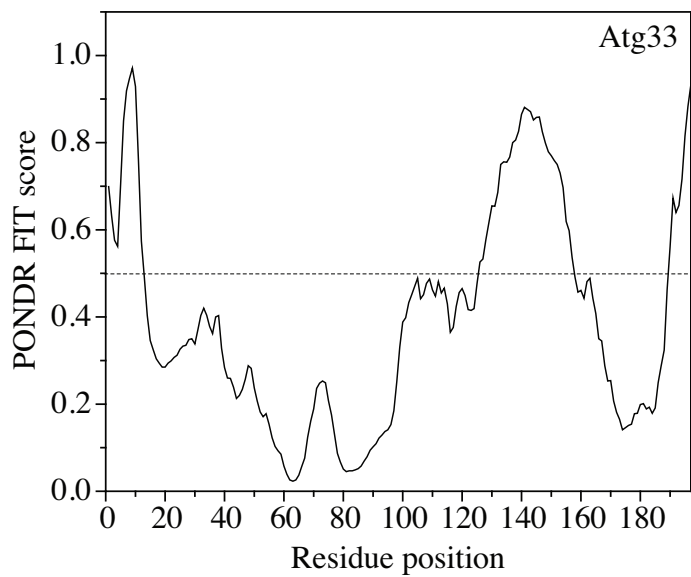
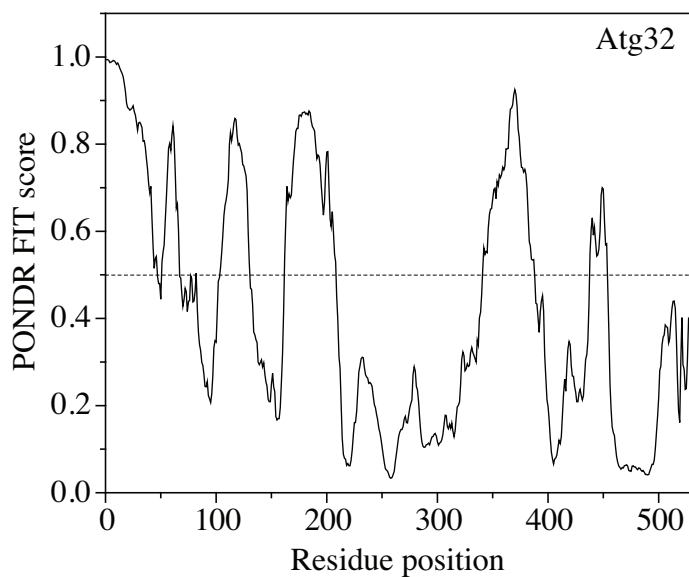
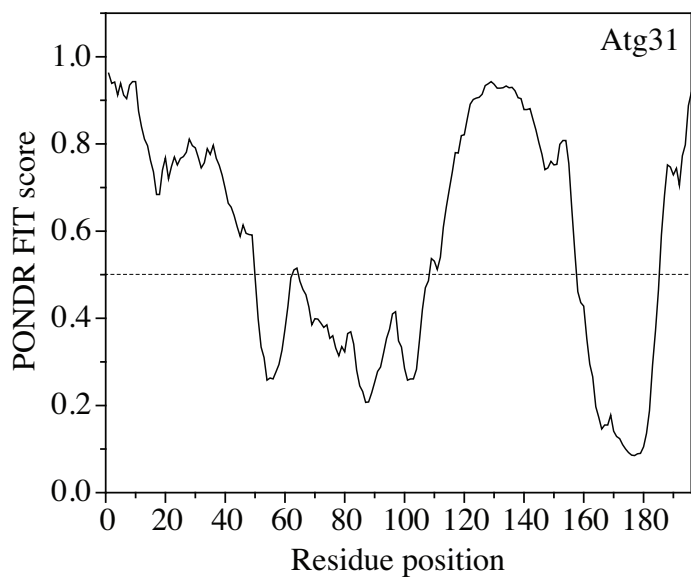
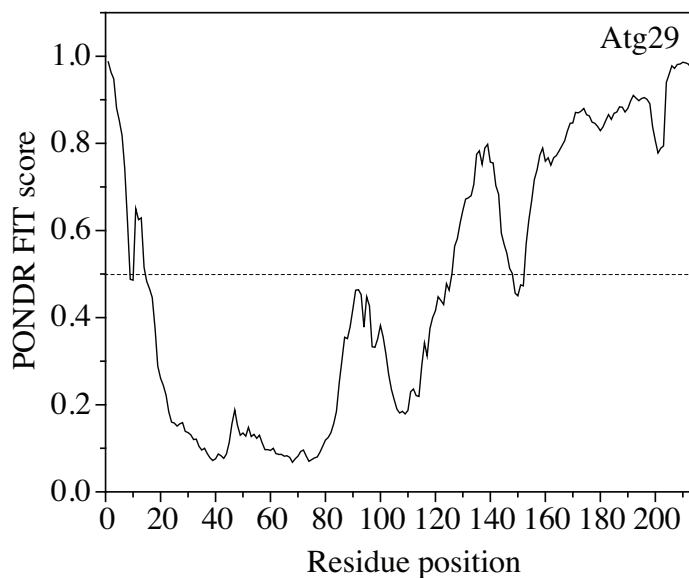
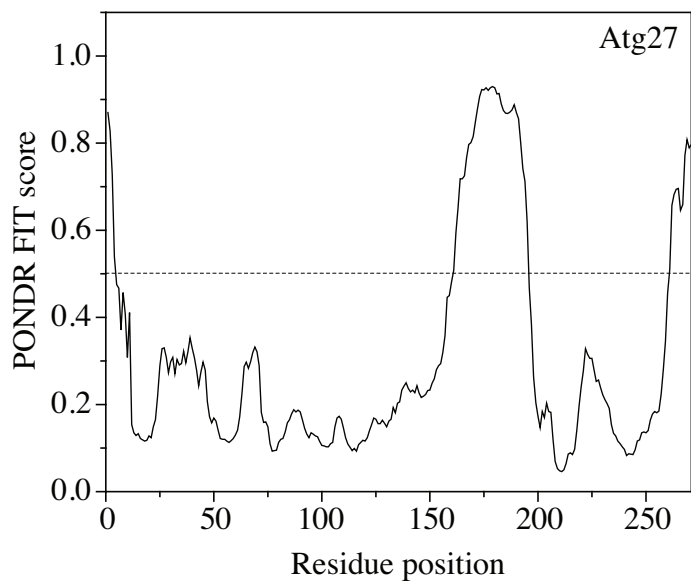


Figure S8. PONDR-FIT analysis of yeast Atg27, Atg29, Atg31, Atg32, Atg33, and Atg34.

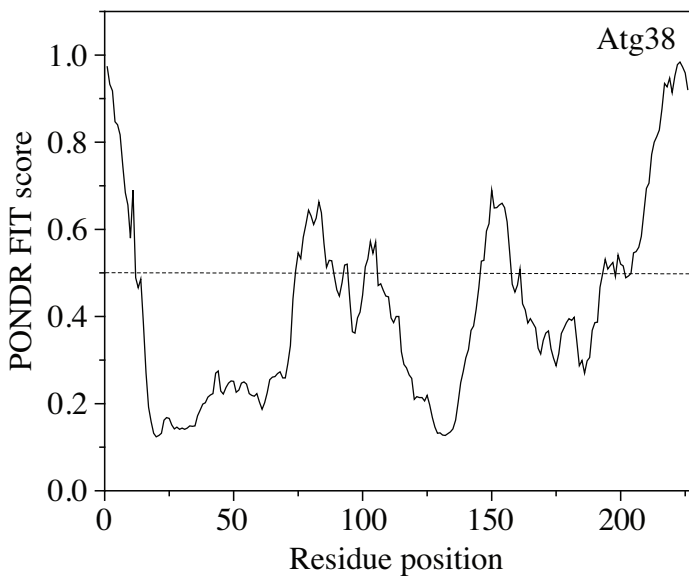
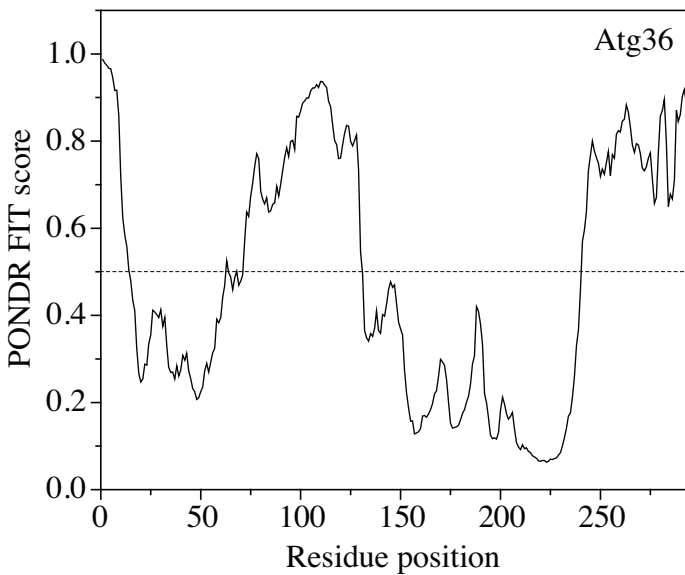


Figure S9. PONDR-FIT analysis of yeast Atg36 and Atg38.

10 20 30 40 50 60 70 80 90 100 (93)

Baker's yeast -----MIRSTLSSWRREYLTPTIHKSTELTTCQITPEEFVQAGDYLCHEMFTWKNNEESSDTSYRDELKPKQELIIRKVECDKRAEQCVVEGPD-VIM
Diploid fungus -----MSLRKSLSSLRREYLTPIHNHNSNVTTCFISPEEFVKACDYLYVYKFTWQGNDCPKNLQKSFLEPDKQYLVLRHRVPSYQRASNYLTGEDDKGANP
Roundworm of pigs MDALVKTMKSAALNVGEMLTPILRESKQKETVLTPEEFVIAADHLLVHHCPTWSWAKAADASRTKTYLDDKQFLIIRNVPCYRRCI--EMDYDPTQEKV
Wheat -----MGQVKQKVELYEYKGTVERVTPRTVSALEKQVLSVPEFTLADNLVSKCPTWSWAGDPS-KRKEPFLPANKQFLVIRNVECLRRAVAVEEYDAAAGAEV
Arabidopsis thaliana -----MVLSQLKHEAFKGTVERITGPRTISAKREKQVLSVSEFVLAADNLVSKCPTWSWESGDAS-KRKEPFLPANKQFLIIRNVPCLRRAASVAEDYEAAGGEV
Corn -----MQVQKQVVEYFYKGTVERVTPRTVSALEKQVLSVPEFTLADNLVHHCPTWSWAGDPS-KRKEPFLPANKQFLVIRNVECLRRAISVEEYDAAAGAEV
Fruit fly -----MQSVLNTVKGKALNVAEYLTPLVKESKQKRETGVITPEEFVAAADHLLVHHCPTWSWAGDDET-KTKPEYLPDKQFLVIRNVECLRRAVAVEEYDAAAGAEV
Zebrafish -----MQNVINSVKGKALGVAEYLTPLVKESKQKRETGVITPEEFVAAADHLLVHHCPTWQWASGEEA-KVKAYLPNDKQFLIIRNVPCYKRCK--QMEYSDELEAI
Channel catfish -----MQDVFNKVKGTALGVAEYLTPLVKESKQKRETGVITPEEFVAAADHLLVHHCPTWQWASGEEA-KVKAYLPNDKQFLIIRNVPCYKRCK--QMEYSDELEAI
Frog -----MQSVINTVKGKALEVAEYLTPLVKESKQKRETGVITPEEFVAAADHLLVHHCPTWQWASGEEA-KVKAYLPNDKQFLIIRNVPCYKRCK--QMEYSDELEAI
King cobra -----MQNVINTVKGKALEVAEYLTPLVKESKQKRETGVITPEEFVAAADHLLVHHCPTWQWATGEEL-KVKAYLPNDKQFLVIRNVECLRRAISVEEYDAAAGAEV
Red Junglefowl -----MQNVINTVKGKALEVAEYLTPLVKESKQKRETGVITPEEFVAAADHLLVHHCPTWQWASGEEA-KVKAYLPNDKQFLVIRNVECLRRAISVEEYDAAAGAEV
Mouse -----MQNVINTVKGKALEVAEYLTPLVKESKQKRETGVITPEEFVAAADHLLVHHCPTWQWATGEEL-KVKAYLPNDKQFLVIRNVECLRRAISVEEYDAAAGAEV
Rat -----MQNVINTVKGKALEVAEYLTPLVKESKQKRETGVITPEEFVAAADHLLVHHCPTWQWATGEEL-KVKAYLPNDKQFLVIRNVECLRRAISVEEYDAAAGAEV
Rhesus macaque -----MQNVINTVKGKALEVAEYLTPLVKESKQKRETGVITPEEFVAAADHLLVHHCPTWQWATGEEL-KVKAYLPNDKQFLVIRNVECLRRAISVEEYDAAAGAEV
Chimpanzee -----MQNVINTVKGKALEVAEYLTPLVKESKQKRETGVITPEEFVAAADHLLVHHCPTWQWATGEEL-KVKAYLPNDKQFLVIRNVECLRRAISVEEYDAAAGAEV
Human -----MQNVINTVKGKALEVAEYLTPLVKESKQKRETGVITPEEFVAAADHLLVHHCPTWQWATGEEL-KVKAYLPNDKQFLVIRNVECLRRAISVEEYDAAAGAEV

110 120 130 140 150 160 170 (125) 180 190 200

Baker's yeast -----KG-----FAEDGDEDDV-----LEYIGSETEHVQSTPAGG-----TKDSSIDDTELIQDMIEKBEEDND-----
Diploid fungus -----EE-----DDEEEEEDE-----EGWVKSKKIHKVIDDTHDS-----QINKGEEINDTDDFDENAEQEQHDQIGDH-----
Roundworm of pigs -----LITNAEMGVVEGFDGADDDGGWVDT-----HFFAPESALQKPV-----MDEPATTEPEVATG-----NASADDDGPAMDMSFIDSGLEQDDPN-----
Wheat -----VLDDD-----EDGEGLWAT-----HGLQASESKEEEDIPSMDT-LDIGKVEEIKSIPSYFGASEKPDDEEDIPDMOTYEDTG-----DHSTATPOP-----
Arabidopsis thaliana -----LVDD-----EDNDGLWAT-----HGKPKDKGKEEDNLPSMDA-LDINEKNTQSIPTFYFGE-----EDDDIPDMEEFDEADNVNENDPATLQS-----
Corn -----VLD-----EDGEGLWAT-----HGVAQSKTEEEDIPSMDT-LDIGRSDGKLSIPSYFSGG-KDEEEDIPDMOTYEDTG-----DN-LAAAEF-----
Fruit fly -----VE-----EESGGGGVETHQLNDGTTQLEDKICELTMEETKEEMHTPDSKSAAGGQAEDEDDDEAIDMDFEESGMLLELVDPAVATTRKP-----
Zebrafish -----IE-----EDDGGGGWVDT-----FHNSGGVTGTEAVREISLDN-KDNMNMVKTGACGNSG-DEEEDIPDMOTYEDTG-----DDEEEDIPDMOTYEDTG-----
Channel catfish -----IE-----EDDGGGGWVDT-----FHNSGGVTGTEAVREISLDN-KDNMNMVKTGACGNSG-DEEEDIPDMOTYEDTG-----DDEEEDIPDMOTYEDTG-----
Frog -----IE-----EDDGGGGWVDT-----FHNSGGVTGTEAVREISLDN-KDNMNMVKTGACGNSG-DEEEDIPDMOTYEDTG-----DDEEEDIPDMOTYEDTG-----
King cobra -----IE-----EDDGGGGWVDT-----FHNSGGVTGTEAVREISLDN-KDNMNMVKTGACGNSG-DEEEDIPDMOTYEDTG-----DDEEEDIPDMOTYEDTG-----
Red Junglefowl -----IE-----EDDGGGGWVDT-----FHNSGGVTGTEAVREISLDN-KDNMNMVKTGACGNSG-DEEEDIPDMOTYEDTG-----DDEEEDIPDMOTYEDTG-----
Mouse -----IE-----EDDGGGGWVDT-----FHNSGGVTGTEAVREISLDN-KDNMNMVKTGACGNSG-DEEEDIPDMOTYEDTG-----DDEEEDIPDMOTYEDTG-----
Rat -----IE-----EDDGGGGWVDT-----FHNSGGVTGTEAVREISLDN-KDNMNMVKTGACGNSG-DEEEDIPDMOTYEDTG-----DDEEEDIPDMOTYEDTG-----
Rhesus macaque -----IE-----EDDGGGGWVDT-----FHNSGGVTGTEAVREISLDN-KDNMNMVKTGACGNSG-DEEEDIPDMOTYEDTG-----DDEEEDIPDMOTYEDTG-----
Chimpanzee -----IE-----EDDGGGGWVDT-----FHNSGGVTGTEAVREISLDN-KDNMNMVKTGACGNSG-DEEEDIPDMOTYEDTG-----DDEEEDIPDMOTYEDTG-----
Human -----IE-----EDDGGGGWVDT-----FHNSGGVTGTEAVREISLDN-KDNMNMVKTGACGNSG-DEEEDIPDMOTYEDTG-----DDEEEDIPDMOTYEDTG-----

210 220 230 (170) 240 250 260 270 280 290 300 (240)

Baker's yeast -----DTEEFNAKGGGLAKD-MAQERYLDLITAVSTSVRVPKMYIVGFNSGSLSPQMGEDISADVTRKTAATIEKLPFYKNSVLVSIHPCCKHANVM
Diploid fungus -----ELDDDEFNDLIDINLKSNNKLRRLDLYITVSTSVRVPKLYLGGDFNSGILPLPQCMFEDINSDVTRKTAATIEKLPFYKNSVLVSIHPCCKHANVM
Roundworm of pigs -----RFIPTNRVMCGSQG-----DTIIHTRTYDLEITVYDKYQVPRVWLGGYDQAKKPLSVEKMNEDFSODHANKTITITHTPHLHN-TQMASIHPCRHAQVM
Wheat -----SYFVAEEPDD-----DNILHTRTYDLEITVYDKYQVPRVWLGGYDQAKKPLSVEKMNEDFSODHANKTITITHTPHLHN-TQMASIHPCRHAQVM
Arabidopsis thaliana -----TYLVAHEPDD-----DNILHTRTYDLEITVYDKYQVPRVWLGGYDQAKKPLSVEKMNEDFSODHANKTITITHTPHLHN-TQMASIHPCRHAQVM
Corn -----SYFVAEEPDD-----DNILHTRTYDLEITVYDKYQVPRVWLGGYDQAKKPLSVEKMNEDFSODHANKTITITHTPHLHN-TQMASIHPCRHAQVM
Fruit fly -----EPEAKASPVAAASGDAEASGDSVLHTRTYDLEITVYDKYQVPRVWLGGYDQAKKPLSVEKMNEDFSODHANKTITITHTPHLHN-TQMASIHPCRHAQVM
Zebrafish -----KMAADLSKTKAEAGG-EDAILQTRTYDLEITVYDKYQVPRVWLGGYDQAKKPLSVEKMNEDFSODHANKTITITHTPHLHN-TQMASIHPCRHAQVM
Channel catfish -----KMAADLSKTKAEAGG-EDAILQTRTYDLEITVYDKYQVPRVWLGGYDQAKKPLSVEKMNEDFSODHANKTITITHTPHLHN-TQMASIHPCRHAQVM
Frog -----KMAADLSKTKAEAGG-EDAILQTRTYDLEITVYDKYQVPRVWLGGYDQAKKPLSVEKMNEDFSODHANKTITITHTPHLHN-TQMASIHPCRHAQVM
King cobra -----KMAADLSKTKAEAGG-EDAILQTRTYDLEITVYDKYQVPRVWLGGYDQAKKPLSVEKMNEDFSODHANKTITITHTPHLHN-TQMASIHPCRHAQVM
Red Junglefowl -----KMAADLSKTKAEAGG-EDAILQTRTYDLEITVYDKYQVPRVWLGGYDQAKKPLSVEKMNEDFSODHANKTITITHTPHLHN-TQMASIHPCRHAQVM
Mouse -----KMAADLSKTKAEAGG-EDAILQTRTYDLEITVYDKYQVPRVWLGGYDQAKKPLSVEKMNEDFSODHANKTITITHTPHLHN-TQMASIHPCRHAQVM
Rat -----KMAADLSKTKAEAGG-EDAILQTRTYDLEITVYDKYQVPRVWLGGYDQAKKPLSVEKMNEDFSODHANKTITITHTPHLHN-TQMASIHPCRHAQVM
Rhesus macaque -----KMAADLSKTKAEAGG-EDAILQTRTYDLEITVYDKYQVPRVWLGGYDQAKKPLSVEKMNEDFSODHANKTITITHTPHLHN-TQMASIHPCRHAQVM
Chimpanzee -----KMAADLSKTKAEAGG-EDAILQTRTYDLEITVYDKYQVPRVWLGGYDQAKKPLSVEKMNEDFSODHANKTITITHTPHLHN-TQMASIHPCRHAQVM
Human -----KMAADLSKTKAEAGG-EDAILQTRTYDLEITVYDKYQVPRVWLGGYDQAKKPLSVEKMNEDFSODHANKTITITHTPHLHN-TQMASIHPCRHAQVM

310 320 330 340 350 360 370 380 (307)

Baker's yeast -----KILLLDKVRVVR---QRRRRELQEEQELDGVGD-----WEDLQDDIDDSLVRVQVLLIHLKHTITSVTPSFOHDYTMEGW-----
Diploid fungus -----KVLMLKHSKLNKKNLQOKDESLSDDLKSLSVNEKKTQDEHSQINNDREEEEEGRGDHMLIIFLKFITASDTPMEYEVYTMDDAL-----
Roundworm of pigs -----KRLLEQLAESG-----KELTVEHVLIIIFLKFVQAVIPPTIEYDYTRSIQL-----
Wheat -----KKIIDVIVSQG-----GAPEVDKLEIIFLKFEMASVIPPTIEYDYTMDFDLGSTST-----
Arabidopsis thaliana -----KKIIDVIMSRG-----VEPEVDKLEIIFLKFEMASVIPPTIEYDYTMDFDLGSSST-----
Corn -----KKIIDVPMSSG-----VEPEVDKLEIIFLKFEMASVIPPTIEYDYTMDFDLGSSST-----
Fruit fly -----KKIITQVVEEGG-----GQLGVHMLLIIIFLKFVQAVIPPTIEYDYTRHFTM-----
Zebrafish -----KKIITQVVEEGG-----GQLGVHMLLIIIFLKFVQAVIPPTIEYDYTRHFTM-----
Channel catfish -----KKIITQVVEEGG-----GQLGVHMLLIIIFLKFVQAVIPPTIEYDYTRHFTM-----
Frog -----KKIITQVVEEGG-----GQLGVHMLLIIIFLKFVQAVIPPTIEYDYTRHFTM-----
King cobra -----KKIITQVVEEGG-----GQLGVHMLLIIIFLKFVQAVIPPTIEYDYTRHFTM-----
Red Junglefowl -----KKIITQVVEEGG-----GQLGVHMLLIIIFLKFVQAVIPPTIEYDYTRHFTM-----
Mouse -----KKIITQVVEEGG-----GQLGVHMLLIIIFLKFVQAVIPPTIEYDYTRHFTM-----
Rat -----KKIITQVVEEGG-----GQLGVHMLLIIIFLKFVQAVIPPTIEYDYTRHFTM-----
Rhesus macaque -----KKIITQVVEEGG-----GQLGVHMLLIIIFLKFVQAVIPPTIEYDYTRHFTM-----
Chimpanzee -----KKIITQVVEEGG-----GQLGVHMLLIIIFLKFVQAVIPPTIEYDYTRHFTM-----
Human -----KKIITQVVEEGG-----GQLGVHMLLIIIFLKFVQAVIPPTIEYDYTRHFTM-----

Table S1. Distribution of the Atg proteins from yeast in DCH-DCDF plot based on PONDR-VSL2.

Quadrant in DCH-DCDF plot	Core autophagy machinery protein from <i>S. cerevisiae</i>
Q1	<i>Atg3</i>
Q2	Atg1, Atg2, Atg4, Atg5, Atg7, Atg10, Atg11, Atg14, Atg15, Atg17, Atg18, Atg22, Atg26, <i>Atg27, Atg33, Atg34</i>
Q3	Atg6, Atg8, Atg9, Atg12, Atg19, Atg20, <i>Atg21</i> , Atg23, Atg24, Atg32, Atg36, Atg38
Q4	Atg13, Atg16, Atg29, Atg31

Q1, structured based on CDF, unstructured based on CH; Q2, structured based on both CDF and CH; Q3, structured according to CH, unstructured based on CDF; Q4, unstructured based on both CH and CDF. The CDF function was calculated from the PONDR-VSL2 score. Proteins in italic exhibit different localizations arising from different PONDR methods.

Table S2. Distribution of the Atg proteins from yeast in the DCH-DCDF plot based on PONDR-VL3.

Quadrant in DCH-DCDF plot	Core autophagy machinery protein from <i>S. cerevisiae</i>
Q1	-
Q2	Atg1, Atg2, Atg4, Atg5, Atg7, Atg10, Atg11, Atg14, Atg15, Atg17, Atg18, <i>Atg21</i> , Atg22, Atg26, Atg27
Q3	Atg6, Atg8, Atg9, Atg12, Atg19, Atg20, Atg23, Atg24, Atg32, <i>Atg33, Atg34</i> , Atg36, Atg38
Q4	<i>Atg3</i> , Atg13, Atg16, Atg29, Atg31

Q1, structured based on CDF, unstructured based on CH; Q2, structured based on both CDF and CH; Q3, structured according to CH, unstructured based on CDF; Q4, unstructured based on both CH and CDF. The CDF function was calculated from the PONDR-VL3 score. Proteins in italic exhibit different localizations arising from different PONDR methods.

Table S3. IDPRs of Atg proteins from yeast as predicted by PONDR-FIT.

Protein	IDPRs of ≥ 30 consecutive disordered residues
Atg1	353-390; 455-560; 632-668
Atg2	122-167; 255-295; 970-1027
Atg3	110-165
Atg6	88-170; 213-303
Atg8	1-30
Atg9	24-256; 904-946
Atg11	100-156; 524-554; 717-769; 791-828; 1045-1102
Atg12	1-110
Atg13	276-465; 473-738
Atg14	60-100; 310-344
Atg16	1-44
Atg18	181-225; 321-389
Atg19	128-160
Atg20	1-87; 96-164; 341-374; 485-536
Atg21	32-98; 187-254
Atg23	321-453
Atg26	1-100; 111-186; 424-489
Atg27	160-196
Atg29	126-213
Atg31	1-50; 108-158
Atg32	1-68; 102-132; 162-208; 341-388
Atg33	125-158
Atg36	63-131; 240-293
Atg38	193-226

The residues with the PONDR-FIT score near to 0.5 or higher were considered disordered. The numbers indicate the residue position in the amino acid sequence of a particular protein.