

## Supplementary Data

Supplementary Material and Methods:

### *Southern Blot:*

Genomic DNA for southern blots was isolated by QIAamp DNA Blood mini kit (Qiagen). For Southern blots, 1.5 µg of DNA was digested and analyzed as previously described[1]. The single cross-over integrations were screened by AvrII/BclI digestion and probed using PfAtg7 ORF 3' end (1kb). Probe was synthesized by PCR using the AlkPhos direct Labeling system (GE Amersham) with the following primers and developed using CDPStar developing kit (Roche Diagnostics):

For: 5'-CTCGAGGGTGATAATGTGTTATGTGA-3'  
Rev: 5'-CCTAGGTTCCAATATTATAACATCATT -3'

### *Semi-quantitative PCR:*

*Amplification of mRNA* - RNA was purified using Trizol (Invitrogen) and purified using RNeasy kit (Qiagen). cDNA was synthesized using Superscript II reverse transcriptase (Invitrogen) by heating 10 µg total RNA with oligo-dT and dNTPs at 65° C, 5 min followed by addition of RT buffer and incubated at 42° C, 2 h. DNase-free RNase was added and incubated for 30 min, 37° C. The reaction was inactivated by heating at 65° C for 10 min.

*Semi-quantitative PCR*- Standard PCR conditions were used on a dilution series of the cDNA with annealing performed at 47° C. Primers were (forward and reverse, respectively):

PfATG7 (PF3D7\_1126100): 5'-CCTCCTTTAGGTATTACGGTAG -3' and 5'-CCTAGGTCATTCCAATATTATAACAT-3'.

Ribosomal protein (PF3D7\_1126200): 5'-GTCCTACAAGTAATAGATAATAATG -3' and 5'-CTCCTTTCTTTTTGGCAACAC -3'.

Predicted sizes of amplified products: PfATG7: 566bp for cDNA, 745bp for gDNA; ribosomal protein: 464bp for cDNA and 700bp for gDNA.

*Amplification of gDNA*: Genomic DNA was purified from parasites using QIAamp DNA blood mini kit (Qiagen) and used as a control.

*Quantification of Semi-quantitative PCR*: Pixel intensities of scanned images were determined using ImageJ software (NIH).

A

YeastATG8/1-118 1 MKSTFKSEYPFEKKAESERIDRFKNRIPVICEKAEKSDIPEIDKRRKYLVPADLT 56  
 PfATG8/1-124 1 MPS-LKDEVSFENRVAETHKIRSKYPNRIPVVCERANRSNLPIDKRRKFLVPMNML 55

YeastATG8/1-118 57 VGGFVYVIRKRIMLPP-----EKAIFIFVNDTLPPTAALMSAIYQEHKDKDG 103  
 PfATG8/1-124 56 VGEFKFILHQHINQSAYGSNMKLFERTIYLFVNNIVPKTGLLMQDLYEMYKDEDG 111

YeastATG8/1-118 104 FLYVTYSGENTFGR 117  
 PfATG8/1-124 112 YLYMEYSCSCLG- 124

B

PfATG4/1-1124 1 MSKEMKNFEDNQKKEIENSTIQEKKEENKTLDEIKNVVAINSNKINKKDNKYIKKKNSKKTKNFIIHKKNVDDLYSYQ 82  
 ScATG4/1-494 -----

PfATG4/1-1124 83 NKSKEYKILNYFNNFKYNSFKRYFKILVNVSLLNLYPLNLRNISNKVYIFGLCLNLKNDEMKIIFLILCKSKILFTYRSNFL 164  
 ScATG4/1-494 -----

PfATG4/1-1124 165 IRINDNNLYRSNNIMLVDDNVSQSNIFTYDNTTYNNSNITINNNNNNSNITINNNNNNNNSNITINNNNNNNNSNITINNNISNLCINNNNH 246  
 ScATG4/1-494 1-----MQRWLQLWKMDLVQK 15

PfATG4/1-1124 247 TTSNTTNSDNSLPILCQEV EHDQDDYLIVISK KKKKKK LKKKKY Y YQR I INFTD P E H F L K K Y F D D K E C F Y I N F K K 328  
 ScATG4/1-494 16 V H G V F E G S S E P A A L M N H D I V L G ----- E V Y P E D E E S G A E Q C E Q D C R Y R G ----- E A M S D G F L S S L F G ----- 76

PfATG4/1-1124 329 SSKGHNDTY YQKQHSQEIKENYSLQNNK K K S K E E I Y G D T C T Y T T S E N K T I Y L E N S K K C N S V D N K E I K N K N I S K D M H Y 410  
 ScATG4/1-494 77 ----- R E I S K Y T K E F L L D V Q S R V N F T Y R T R ----- F V P I A R A P D G P S P L S L N L L V R I N P I S I E D Y I A N ----- 135

PfATG4/1-1124 411 YREQKKIKKNKSENNTCDDDL ECFNNDNRNDNEEMK K K K K K I K I N N N N N N I E S E A T I K N I N I K T K K N S L K I L H S R S K 492  
 ScATG4/1-494 136 ----- P D C F N ----- 140

PfATG4/1-1124 493 KHSVLIK K K K N K N N I K N N N I N I K E Y L K Y V N Q N I T K S Q I Y E K D K K Y I C M S D N C W G C M I R V I Q M V L A N I L I H F N I S N R Y V Y F H 574  
 ScATG4/1-494 141 ----- T D I G W G C M I R T G S L L G N A L Q I L H L G R D E R V N G 173

PfATG4/1-1124 575 N--VNDYILYKNYINKLTTCTNKENKIQIEEKTMNKDKESIYLNKKNKDTKEMVKKHEKNNQCESNECNLNKNASQNNKE 654  
 ScATG4/1-494 174 NESLERESKFNWVNDTPEAFPSLHNFVSAGTELSDRPGEWFGPAATARSIQSLIYGFPECGIDDCIVSVSSGDIYENEV 254

PfATG4/1-1124 655 SDTLMIHPOQNTINGNNNNINSNNNNNNNSNNNNNNNNNNNSSSSTFFNSNTYLVPDSFKQEYKSSIKTDFLQSDK 736  
 ScATG4/1-494 255 EKVFAENPNRILFLLGVKGINAVNESYRESICGILSQTQSVGIAGGRSSS-----LYFFGYQNEFLHFD 323

PfATG4/1-1124 737 QITLNDTNINESGSDIYINDVNEITNKYNSDDLHHCENYNNFLNNKNNNDSNSEIMHINSLIYSITLSEFRDLEAKY 818  
 ScATG4/1-494 324 HIRQPAAVEDSFVES-----CHTSKFKLQLSEMDP-----SMLIGLKEKEDWQOWLU 372

PfATG4/1-1124 819 SIQNIYEMIKYKIDDDNQIEHFVHDLGPTSSAIIISNLINKKKYRFVKKKMKNNFRNTNIHMDKNIYEKEQKELTNYT 900  
 ScATG4/1-494 373 EVAESAIIINVLAKRMDDFDVSCSMDDVESVS-----NSMKKDA 411

PfATG4/1-1124 901 NNNKQTYKLLNQKENITISKSDDNINKDKKKYKLLFLKCKKRYTFFSVAFETVIYNNKVLKFFQIKQDIFIIIWICLK 982  
 ScATG4/1-494 412 SNNENLGVLEGDYVDIGAFPHHTNTEDVDEYDCQDILHCCKKQ-----LVVM 459

PfATG4/1-1124 983 LGIDSLNYSKYKSLSCFLKGFQGISGNTNTSAHYFYSANDNGLFYLDPHIKCQNAFIDFNENISSQFFMHKVKFLPWE 1064  
 ScATG4/1-494 460 GNTHIVNANLTDYEVGVVVEKTVGHSPIDEKC----- 494

PfATG4/1-1124 1065 YLNSLSLIFVQVSKDDYFNLIQNLKLI DSSLFEIYHEEPQYVYKNELNLYDTDDSGLVVL 1124  
 ScATG4/1-494 -----

C

*PfATG7/1-1316* 1 MKKK FEEFNKPSYILKHNNEFKIDISYFTQLHEHKINIKLQSNVNLCSSTYVNKIKLGFKY 64  
*ScATG7/1-630* 1 ----MSSERVLSYAPAFKS---FLDTSFFQELSRKLDVLLKLDSTCQPLTVNLDLHNI PKSADQ 57

*PfATG7/1-1316* 65 KLLNRYLIEFAHPFIHVRTIEINKKSFLKYENFDNEDEKNNMEPNDCTKTIEERNHINNINDG 128  
*ScATG7/1-630* 58 VPL-----F-----FLTNRSFKHNNKRTNEVP----- 79

*PfATG7/1-1316* 129 NKKVQKIWIYIMNNYRNNYLGVLLENFNTLEEF LKCNKDDHINYLEP LKCYINNEKNDICKDMNL 192  
*ScATG7/1-630* 80 -----LQGSIFNFVLDLDFKLNLDK--QLFLHQRALCEWEDG----- 113

*PfATG7/1-1316* 193 YIHDNIYDDTFWEYKENC LTVLEKINKYVILSFFDLKRYICYYSIANPIIKPKDNYKLIKNST 256  
*ScATG7/1-630* 114 -----IKDINKCVS FVILS FADLKKYRFYWLGVPCFQRP----- 148

*PfATG7/1-1316* 257 RYFFYIDSKYVYINTENRHINIIDIFYLSYKIDDYFNKYKMF LNTNIFLL LKFDNIP LHTMNNQ 320  
*ScATG7/1-630* 149 -----SSTVLHVRPEPS LKGLFSKCG 169

*PfATG7/1-1316* 321 DYYDEYINKLYTNIECEEDQKSKKEFYQINSFYKLF EYLK LNDISQNSYHPMGNKSFNNHYNNN 384  
*ScATG7/1-630* 170 KWFVNVNWKWVCILOADDEIVN-----YD 193

*PfATG7/1-1316* 385 SSMHLKNDYDMVILPINALSELKEDTKNSKDKILRYIKKDFFDLYICFIDINYIFNSLSWDFRNL 448  
*ScATG7/1-630* 194 KCIIRKTKVLAIRDSTMENVP SALTKNFLSVLQYDVPDLIDFKLLIIRQ----- 243

*PfATG7/1-1316* 449 LYCLTLKYKLYDFQIDVLAFRDISLLRQQYVGT FKSQEGFIWSYPKVVMKRGSINNPRNYNDED 512  
*ScATG7/1-630* ----- 512

*PfATG7/1-1316* 513 KNNDDNNYDDKNNDDNNYDDSHNNYDDKNNDDNNYDDSHNNYEDSHNNYDDSDLHKDIDMD 576  
*ScATG7/1-630* ----- 576

*PfATG7/1-1316* 577 KDKNNSFHYNPINNCLSHQDVSFCSVTKMCKVNYNSIKDCKNDWRDDL N EYSHDMNPIHEDI E 640  
*ScATG7/1-630* 244 -----NEGSFALNATFASID 258

*PfATG7/1-1316* 641 HSSSQYENNMVSNNTYKKDNRNIKHNHNIYHNHLVKYILNSSLFQVTVPKVHF IYDNGSNYV 704  
*ScATG7/1-630* 259 PQSSSNPDMKVS----- 271

*PfATG7/1-1316* 705 DINLNGKKDDSL NKQDIHILEKKKEGDTCCIINSYLKSF SDEKKNDCIDVSSNLGFSINIRKEDN 768  
*ScATG7/1-630* ----- 768

*PfATG7/1-1316* 769 HFTTRVKYKDEEMDV LHI SEGDENENNMNATNNNINNNIKNYKTFCCDNKVYDILCGWKYYE 832  
*ScATG7/1-630* 272 -----GWERNV 277

*PfATG7/1-1316* 833 DKKKEKRSISITINLDFINKDTIQRI SLELNKLIKWKILKDLKFEHKKLKLILIGLTLGC 896  
*ScATG7/1-630* 278 QGKLAPR---VVDLSSLLDPLKADQSVDLNLKLMKWRILPDLNLDIKNTKVLLL GAGTLGC 337

*PfATG7/1-1316* 897 MVARNCVSWG IQHYTFV DNRV SFSNISRQYLYTLEDAEKYGNIG EYKCVAAKKNL KICPDLN 960  
*ScATG7/1-630* 338 YVSRALIAWGVK IITFVDNGTVSYSNPVRQALYNFEDCGKPI-----KAELAAASLKRIFPLMD 395

*PfATG7/1-1316* 961 ITAKVMDIPMPGHLNYLNE NLEDITINELDNLI NNHDVVFLITDSKESRYFPCLMIAEKQYNSLK 1024  
*ScATG7/1-630* 396 ATGVKLSIPMIGHKLVNEEAQH KDFDRLRALIKEHDIIFLLVDSRESRWLP SLLSN IENKT---- 456

*PfATG7/1-1316* 1025 ELQESVNHNHNNNNNNSSSSSSG SNKFRKGDVNLCEEENMITHEYIENIKCTKIMDKSLNNIL 1088  
*ScATG7/1-630* ----- 1088

*PfATG7/1-1316* 1089 LYEQNNNIYKSLNNIHMYDRYQEIFYNNILTSVKRLCKMPPLGITVAISFDSFVVLRHSGWLYFK 1152  
*ScATG7/1-630* 457 -----VINAALGFDSYLVMRHGNRDEQ 478

*PfATG7/1-1316* 1153 G----ACYFCNDMHCP SDSLRYRTLDEKCTVTRCGISNIS SSIAT ELLLAL TQHLYFFAPHID 1212  
*ScATG7/1-630* 479 SSKQLGCYFCHDVVAPTDSLTDRLDQMCVTRPGVAMMASLAVELMTSLLDT----- 532

*PfATG7/1-1316* 1213 RDQYIYNYDNDMNQKNSDISNIFTSCLGATPHIMNFNLANFTIKKIFCEPFEKCMCCSERVIL 1276  
*ScATG7/1-630* 533 -----KYSGETTVLGDIPHQIRGFLHNF SIKLETPAYEHCPACSPKVI E 578

*PfATG7/1-1316* 1277 KYQEDKMDFIRNVI RDS SILERITNMDQLKVEEN---DVIILE----- 1316  
*ScATG7/1-630* 579 AFTDLGWEFVKKALEHPLYLEEISGLSVIKQEVERLGNDFVEWEDDESDEIA 630

D

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PfATG3/1-313      1 MSDQ I NVKHK I GDT CRKLYSYFKTVNNTSTFIQNGTLTPSEFVDSG 46
ScATG3/1-310     1 -----M I R S T L S S W R E Y L T P I T H K S T F L T T G Q I T P E E F V Q A G 37

PfATG3/1-313     47 DFLVYKFKTWEWQEADKDR-VVPYLPENKQFLITKNVPCQQR IKDL 91
ScATG3/1-310     38 DY LCHMFPTWKWNEESSDISYRDFLPKNKQFLIIRKVPCKDKRAEQC 83

PfATG3/1-313     92 NNIVHDLKIVDNDWLLPSYEEDNNPDIYEYLPNSEYTIINDKNIYN 137
ScATG3/1-310     84 -----VEVEGPDVIMKGF AEDGDEDVLE YIGSETEHVQS----- 118

PfATG3/1-313    138 YEEEEEDDNCDEAIDINNFYME NNLIKEHDPASINSTSCYSKNMLH 183
ScATG3/1-310    119 ---PAGGTKDSSIDDIDELIQDMEIKEED-ENDDTEEFNAKGLA 160

PfATG3/1-313    184 DNLMKIRTYDVSITYDKYQTPRIWLF GY NENGDP LKSEEIFEDIL 229
ScATG3/1-310    161 KDMAQERYYDLYIAYSTSYRVPKMYIVGFNSNGSP LSP EQMFEDIS 206

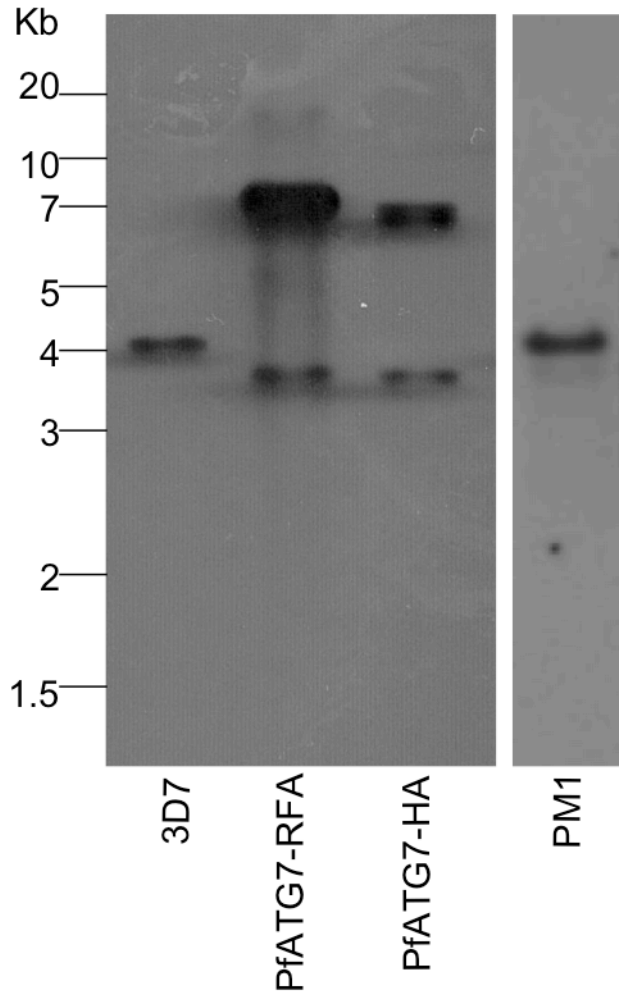
PfATG3/1-313    230 SDYSYKTVTYDPHPCTG--VMTASIHPC KHA----- 258
ScATG3/1-310    207 ADYRTKTATIEKLPFYKNSVLSVSIHPCKHANVMKILLDKVRVVRQ 252

PfATG3/1-313    259 -----EAILNVVNNWIS E E K E P -----RHDLYLLFLLK F I S G V I 292
ScATG3/1-310    253 RRRKELQEEQELDGVGDWEDLQDDIDDSL RVDQYLIVFLK F I T S V T 298

PfATG3/1-313    293 PTIEYDFTTDIEIPRDSNAGL 313
ScATG3/1-310    299 PSIQHDYTM EGW----- 310

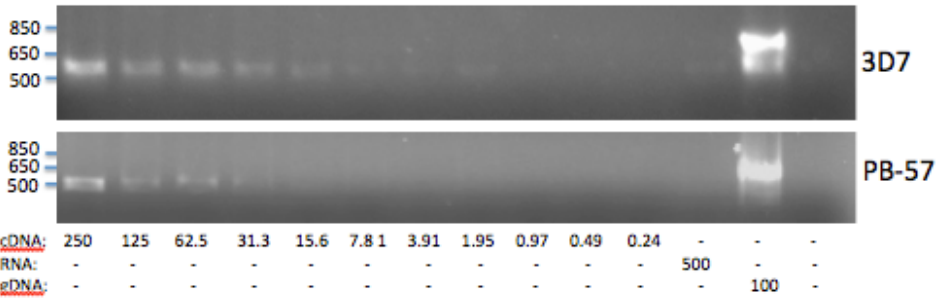
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**Figure S1. Alignments show similarity between yeast and *P. falciparum* Atg proteins.** Atg8 (A), Atg4 (B), Atg7 (C) and Atg3 (D) alignments between *P. falciparum* and *S. cerevisiae* performed through ClustalW. PfAtg protein sequences identified by a PlasmDB blastp using ScAtg protein sequences as described in M&M in the main manuscript.

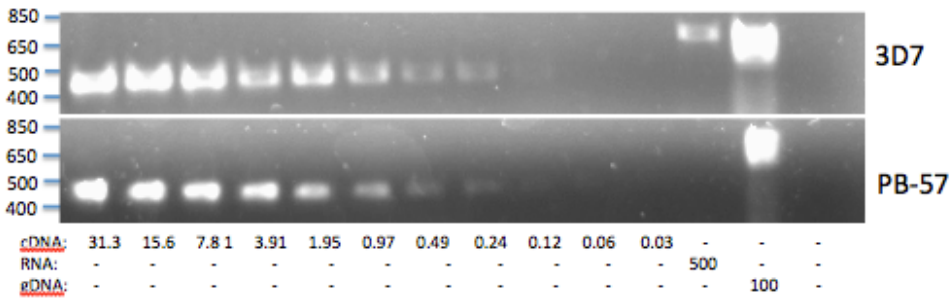


**Figure S2. Southern blot confirms integration into correct locus.** DNA was purified from transgenic parasites: PfATG7-RFA (clone A1) and PfATG7-HA (clone C1). Southern blot analyzed 1.5ug of DNA and single cross-over integrations were screened by AvrII/BclI digestion and probed using PfAtg7 ORF 3' end (1kb). The expected fragment for 3D7 and PM1 parasites is 4039 bp and the expected size fragments for successful integration of PfATG7-HA are 7653 bp and 3635 bp and for PfATG7-RFA parasites are 7528 bp and 3635 bp. Lack of wildtype locus in PfAtg7-HA and PfAtg-RFA clones indicates successful integration. High intensity band in the PfAtg7-RFA clone possibly represents concatamerization of the plasmid prior to integration into the genome.

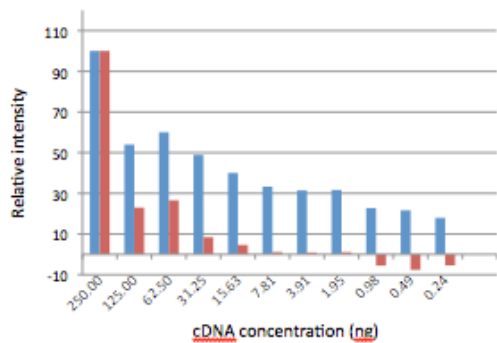
**A PfAtg7 (PF3D7\_1126100)**



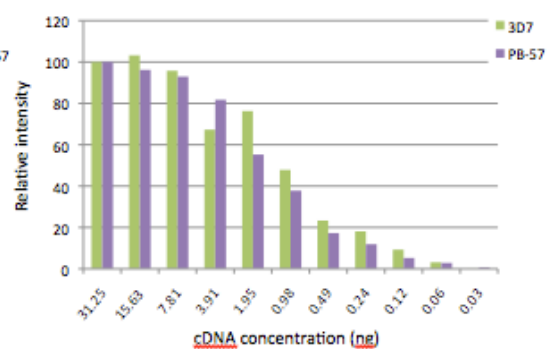
**B Ribosomal protein (PF3D7\_1126200)**



**C Relative intensity of PfAtg7 (PF3D7\_1126100)**



**D Relative intensity of ribosomal protein (PF3D7\_1126200)**



**Figure S3. Semi-quantitative PCR confirms lower expression levels of PfAtg7 in PB-57 parasites.** Standard PCR conditions were used to establish expression levels of PfAtg7 (A) and the ribosomal protein upstream of PfAtg7 (B) across a dilution series of cDNA (nanogram amounts) for 3D7 and PB-57 parasites, with quantification also shown (C,D). The ribosomal protein is used as a control as the transposable element was inserted between it and PfAtg7. Predicted sizes of amplified products: PfATG7: 566bp for cDNA, 745bp for gDNA; ribosomal protein: 464bp for cDNA and 700bp for gDNA.

	<b>Atg protein</b>	<b><i>P. falciparum</i> orthologue</b>	<b>BLAST P(N)</b>	<b>function</b>
Autophagosome formation	Atg3	PF3D7_0905700.2 (prev PF10280C)	3.30E-40	E2 type ligase for Atg8 lipidation
	Atg4	PF3D7_1417300 (prev PF14_0171)	5.00E-09	cysteine protease for Atg8 processing and delipidation
	Atg7	PF3D7_1126100 (prev PF11_0271)	4.00E-51	E1 type ligase or activating enzyme of Atg8 and Atg12
	Atg8	PF3D7_1019900 (prev PF10_0193)	3.10E-24	ubiquitin-like modifier that regulates autophagosome size
	Atg5	nd		forms complex with Atg12
	Atg10	nd		E2-like ligase Atg12-modifier protein
	Atg12	PF3D7_1470000 (prev PF14_0667a)	3.50E-09	forms complex with Atg5
	Atg16	nd		stabilises the Atg5-Atg12 complex
Pre-autophagosomal structure	Atg9	nd		Protein of the pre-autophagosomal structure (PAS), recruits Atg8
	Atg23	PF3D7_1126700 (prev PF11_0277)	1.20E-07	recycles Atg9, cytoplasm to vacuole transport (cvt) protein
	Atg1	PF3D7_1450000 (prev PF14_0476)	2.40E-22	serine/threonine kinase, interacts with Atg13
	Atg11	PF3D7_0216700.2	4.00E-14	interacts with Atg1, Cvt
	Atg17	PF3D7_1120000 (prev PF11_0207)	1.80E-11	interacts with Atg1, 20 and 24
	Atg31	nd		interacts with Atg17
	Atg13	nd		phosphoprotein, dephosphorylated under starvation
	Atg2	nd		interacts with Atg9
	Atg29	nd		formation of PAS
	Atg19	nd		cargo receptor, Cvt
	Atg18	PF3D7_1012900 (prev PF10_0126)	5.20E-31	Needed for Atg2 localisation and Atg8 recycling
	Atg27	nd		PI3P-binding protein, Cvt
	Atg21	PF3D7_1012900 (prev PF10_0126)	6.10E-12	required to recruit Atg8 to the PAS, Cvt

	Atg20	PF3D7_0216700.2	1.40E-06	interacts with Atg24, 17, Cvt
	Atg24	PF3D7_0216700.2	5.80E-07	interacts with Atg1, 17, 20, Cvt
Upstream signalling	Bif-1	?		
	UVRAG	?		
	Ambra1	?		
	Bcl-2	?		
	Vps34	PF3D7_0515300 (prev PFE0765w)	9.2E-75	PI3kinase catalytic subunit, positive regulator of autophagy
	Vps15	PF3D7_0823000 (prev MAL8P1.42)	2.20E-14	Protein kinase required for Vps34
	Atg6	nd		Beclin 1, Interacts with Atg14, VPS34, VPS15
	TOR kinase	?		protein kinase, negative regulator of autophagy

**Table S1. Results of bioinformatic analysis of ATG genes in *P. falciparum*.** See M&M in main manuscript for search parameters.

1. Liu J, Gluzman IY, Drew ME, Goldberg DE (2005) The role of Plasmodium falciparum food vacuole plasmepsins. J Biol Chem 280: 1432–1437. doi:10.1074/jbc.M409740200.