

Supplemental Figure legends

Supplemental Figure 1: **Phagosome isolated at 5'0 are surrounded by an intact membrane** (A) (Upper panel) Electron microscopy (EM) picture of phagosomes purified at 5'0 showed that latex beads were enclosed in individual intact phagosomes even at very early time points. Little membranous contamination was seen, and no rough ER membranes were visible. Scale bar, 0.5 μm . (Lower panel) Magnified views of the membrane bilayer observed around intact phagosomes (5'0). Scale bar, 0.25 μm . Box width, 0.1 μm (B) Early 5'0 isolated phagosomes stained with the lipid dye FM4-64 (red). The phase contrast image of the beads was colored in green. Each phagosome was surrounded by an intact membrane.

Supplemental Figure 2: **Phagosomal protein part of the KEGG pathway “Protein processing in the endoplasmic reticulum”**. The KEGG pathway map 04141 available on <http://www.genome.jp/kegg/> has been modified as follows. Proteins labeled in green have homologs in *D. discoideum*. Proteins boxed in blue were identified in the phagosome proteome.

Supplemental Figure 3: **Representative 2D-DIGE gel images of phagosomes isolated according to the standard pulse/chase protocol** (A) A wild type to *myoK*-null comparison (B) A wild type to *abp1*-null comparison.

Supplemental Figure 4 : **Overall imbalance in the relative abundance of chaperone proteins in *abp1*-null phagosomes** Ratio profiles of the chaperones (A-B) on phagosomes from *myoK*-null cells (A) or *abp1*-null cells (B). ddj1 (DDB_G0280037), DnaJC7 (DDB_G0288639), HspE (DDB_G0273249). These proteins belong to the cytosolic heat shock protein family (1). HspE belongs to the Hsp70 family while DnaJC7 and ddj1 belong to the Hsp40 family. HspE and HspB are the two closest homologs of Hsc70 in *Dictyostelium* (2). In mammalian cells, Hsc70 together with auxilin are necessary for endocytosis and disassembly of the clathrin triskelion. Hsc70 function is regulated by the DnaJ-like protein Rme-8 which homolog in *Dictyostelium* is DnaJC13 (1, 2). Both HspE and HspB are found on phagosomes while DnaJC13 is not (*see* Supplemental List 1). Therefore, the Hsp40 proteins are suggested candidate for the

regulation of HspE and HspB possibly involved in the uncoating of flat clathrin lattices on *Dictyostelium* phagosomes.

Supplemental Fig. 5: **The acquisition of some small GTPases precedes the acquisition of the vacuolar H⁺-ATPase complex and accompanied by post-translational modifications.** (A-B) The ratio profiles of spots representing distinct post-translational forms of Rab11A and Rab11C did not overlap. A higher molecular weight isoform of Rab11C was transiently abundant on the phagosome and also appeared earlier than its low molecular weight counterpart. *myoK* null/wild type ratio profiles in log₂ scale representing both isoforms and an average total profile for the small GTPases Rab11A (A) and Rab11C (B), respectively. (C) The GTPase RabC and the Ca²⁺-regulated protein calmodulin (calA) have ratio profiles closer to the high MW isoform of Rab11C than to other Rab11 isoforms. (D) Gel images of Rab11A and Rab11C isoforms.

Supplemental Tables

Supplemental Table 1 **Analysis of *myoK*-null cells by 2D-DIGE**

Identified protein	K/WT or WT/K ratio	Spot number	p value	Standard deviation	
				wild type	<i>myoK</i> null
Lmc B	2.9	1724	0.0001	0.08	0.08
DDB_G0280445	1.4	1820	0.027	0.17	0.14

A complete list of proteins found differentially expressed in *myoK*-null cells after 2D-DIGE analyses of cell lysates. Three different cell lysates were analyzed in duplicates, with dye inversion, for a total of six gels. Two spots were found differentially expressed and represent less than 1% of the total 334 spots matched across at least four different gels. The Scaffold files containing MS identification details are named according to the corresponding 2D-DIGE spot number.

Supplemental Table 2 **Analysis of *abp1*-null cells by 2D-DIGE**

Protein name	Description	Known or putative protein function	Spot number	A/WT or WT/A ratio	p value
Aco2, Tkt 1/2	Aconitase Transketolase	Citrate metabolism Pentose metabolism	536	1.7	0.039
Tkt 1/2	Transketolase	Pentose metabolism	537	1.7	0.013
CinB	Cycloheximide induced protein B	Lipase	1345	1.6	0.046
Qdpr DDB_ G0278293	Quinoid dihydropteridin reductase Regulator of microtubule dynamics protein 1	Recycling of the enzyme co-factor tetrahydrobiopterin	1759	1.6	0.018
PckA	Phosphoenolpyruvate carboxykinase	Gluconeogenesis	599	1.5	0.010
Dnpep SerS	Aspartyl aminopeptidase Serine t-RNA ligase	Protein degradation Protein synthesis	732	1.5	0.003
Vps5	Putative sorting nexin	Endosome-to-Golgi membrane trafficking	556	1.4	0.016
GrpE	Chaperone	Protein import to mitochondria	1817	1.6	0.023
Magoh	MAGO nashi protein homolog	mRNA nuclear export			

Ratios of proteins found differentially expressed in *abp1*-null cells after 2D-DIGE analyses of cell lysates are compared to ratios obtained by microarrays for the same DDB entry (Supplemental List 1). The complete list of proteins differential by 2D-DIGE is presented here. Three different cell lysates were analyzed by 2D-DIGE in duplicates with dye inversion for a total of six gels. Eight spots were found differentially expressed (1.0 % of the total 823 spots matched) and represent eleven different polypeptides. Positive *abp1*-null to wild type ratios (A/WT) are written in red while positive wild type to *abp1* null (WT/A) are written in black. Statistical significance is measured with the p value of the Student t test. The corresponding ratios found in microarrays are indicated following the same rules. Similar ratios found in both 2D-DIGE and microarrays are indicated in bold. For spots of mixed identities, the name of the protein for which microarray data were available is indicated in brackets. The Scaffold files containing MS identification details are named according to the corresponding 2D-DIGE spot number.

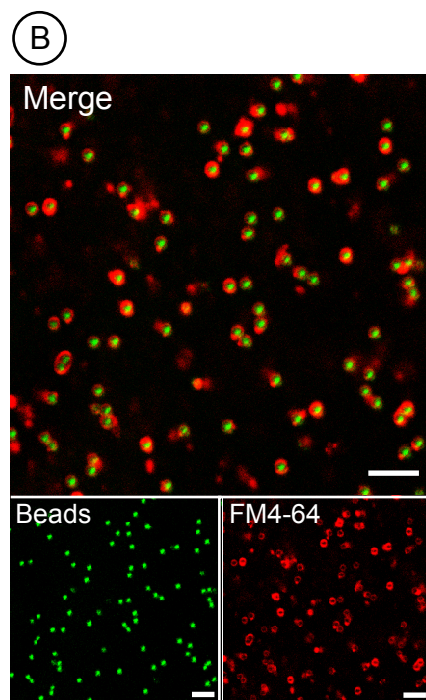
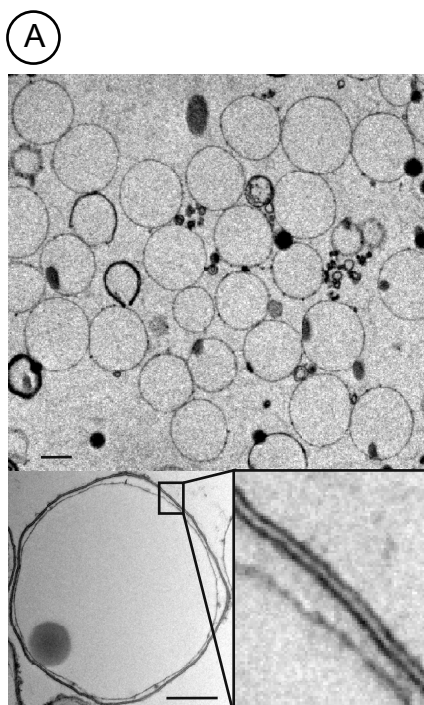
Supplemental Data

1. The total list of *Dictyostelium discoideum* phagosomal protein (Supplemental List 1, SuppData1_Dictyostelium_phagosomal_proteins_Dieckmann.xls) is used to build Fig. 1B. This list encompasses all proteins identified by us in isolated phagosomes at all different time-points in maturation in *Dictyostelium* in this study and others (3, 4). Proteins are listed according to the gene ID, the accession numbers and gene name (columns A, B & C) assigned to each protein sequence in Dictybase (www.dictybase.org). The gene product section is a compilation of the gene product and gene description fields on Dictybase (www.dictybase.org) as well as personal bibliographic searches. Proteins have been identified after MS sequencing from 2D spots [2D this study, (4)] or from 1D gel bands [1D this study, Boulais et al. (3)]. The protein classification is based on (4) and further refined to highlight novel functions associated with the phagosome. The classification key is presented in column O and the class assigned to each protein is presented in column B. Our comparison with the macropinosome proteome is based on the comparison with data published by Journet et al. (5), column K.
2. Raw data and complete analysis of the 2D-DIGE analysis of *Dictyostelium* phagosomes in the *myoK* null and *abp1* null mutant backgrounds (SuppData2_Dictyostelium_phagosome_2DDIGE_Dieckmann.xls). This table lists all spot ratios, including raw data, used to build up the ratio profiles. It also contains all ratio profiles for all the spot available on the gel with their associated protein identifications when available. The “graph” sheet present all ratio profiles classified by function or profile similarity. The legends for this table are present on row 3 and 4.
3. List of protein identifications with the number of unique peptides and sequence coverage (SuppData3_Lists_of_protein_identifications)
4. List of all peptide sequence assigned (SuppData4_Lists_of_peptide_sequences_assigned)

Supplemental References

1. Qiu, X. B., Shao, Y. M., Miao, S., and Wang, L. (2006) The diversity of the DnaJ/Hsp40 family, the crucial partners for Hsp70 chaperones. *Cell Mol Life Sci* 63, 2560-2570.
2. Fey, P., Gaudet, P., Curk, T., Zupan, B., Just, E. M., Basu, S., Merchant, S. N., Bushmanova, Y. A., Shaulsky, G., Kibbe, W. A., and Chisholm, R. L. (2009) dictyBase--a Dictyostelium bioinformatics resource update. *Nucleic Acids Res* 37, D515-519.
3. Boulais, J., Trost, M., Landry, C. R., Dieckmann, R., Levy, E. D., Soldati, T., Michnick, S. W., Thibault, P., and Desjardins, M. (2010) Molecular characterization of the evolution of phagosomes. *Mol Syst Biol* 6, 423.
4. Gotthardt, D., Blancheteau, V., Bosserhoff, A., Ruppert, T., Delorenzi, M., and Soldati, T. (2006) Proteomics fingerprinting of phagosome maturation and evidence for the role of a Galpha during uptake. *Mol Cell Proteomics* 5, 2228-2243.
5. Journet, A., Klein, G., Brugiere, S., Vandenbrouck, Y., Chapel, A., Kieffer, S., Bruley, C., Masselon, C., and Aubry, L. (2012) Investigating the macropinocytic proteome of Dictyostelium amoebae by high-resolution mass spectrometry. *Proteomics* 12, 241-245.

supplemental Figure 1
Dieckmann et al.

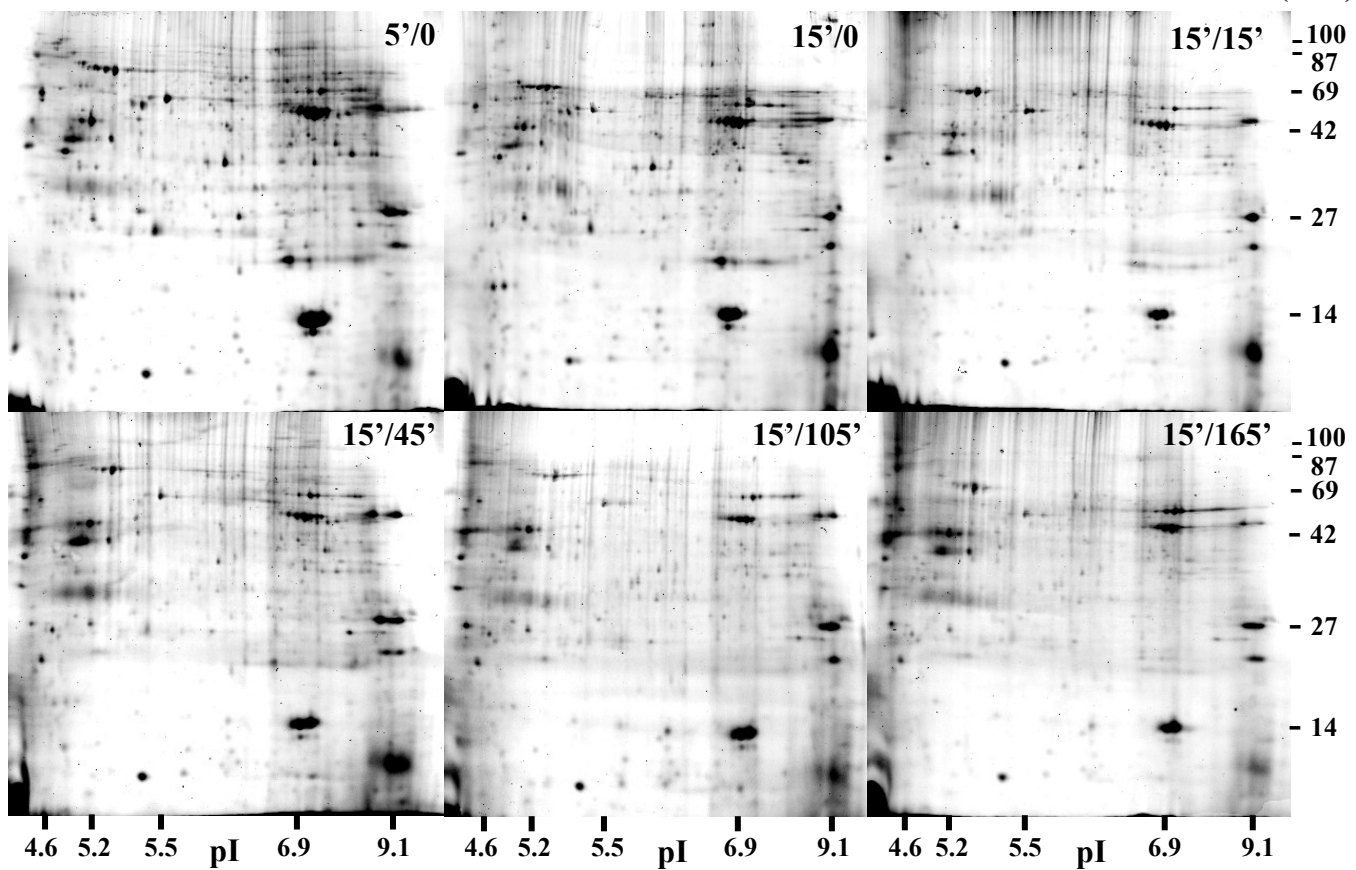


Supplementary Figure 3

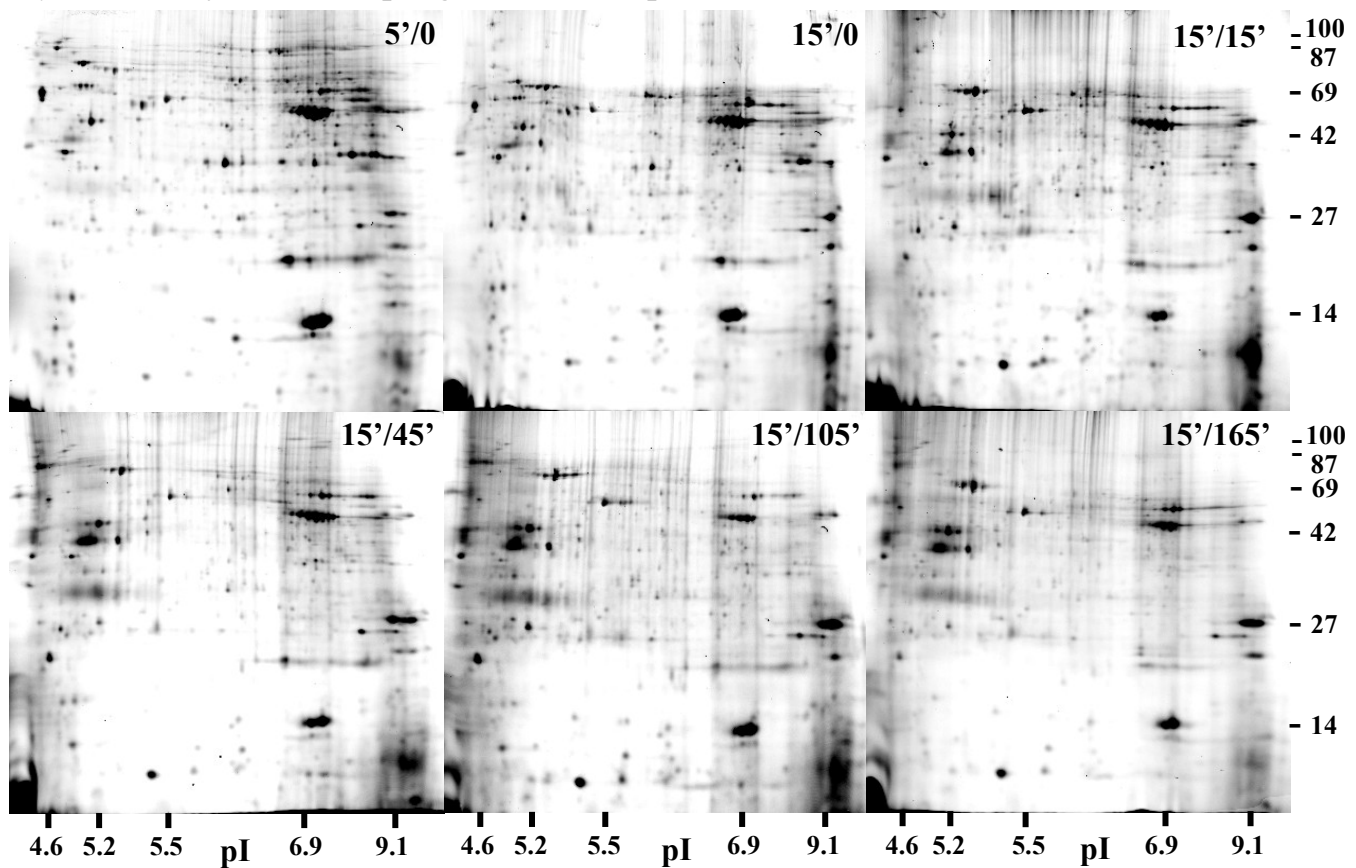
A

Wild type Cy3-labelled phagosomal time profile

MW
(kDa)

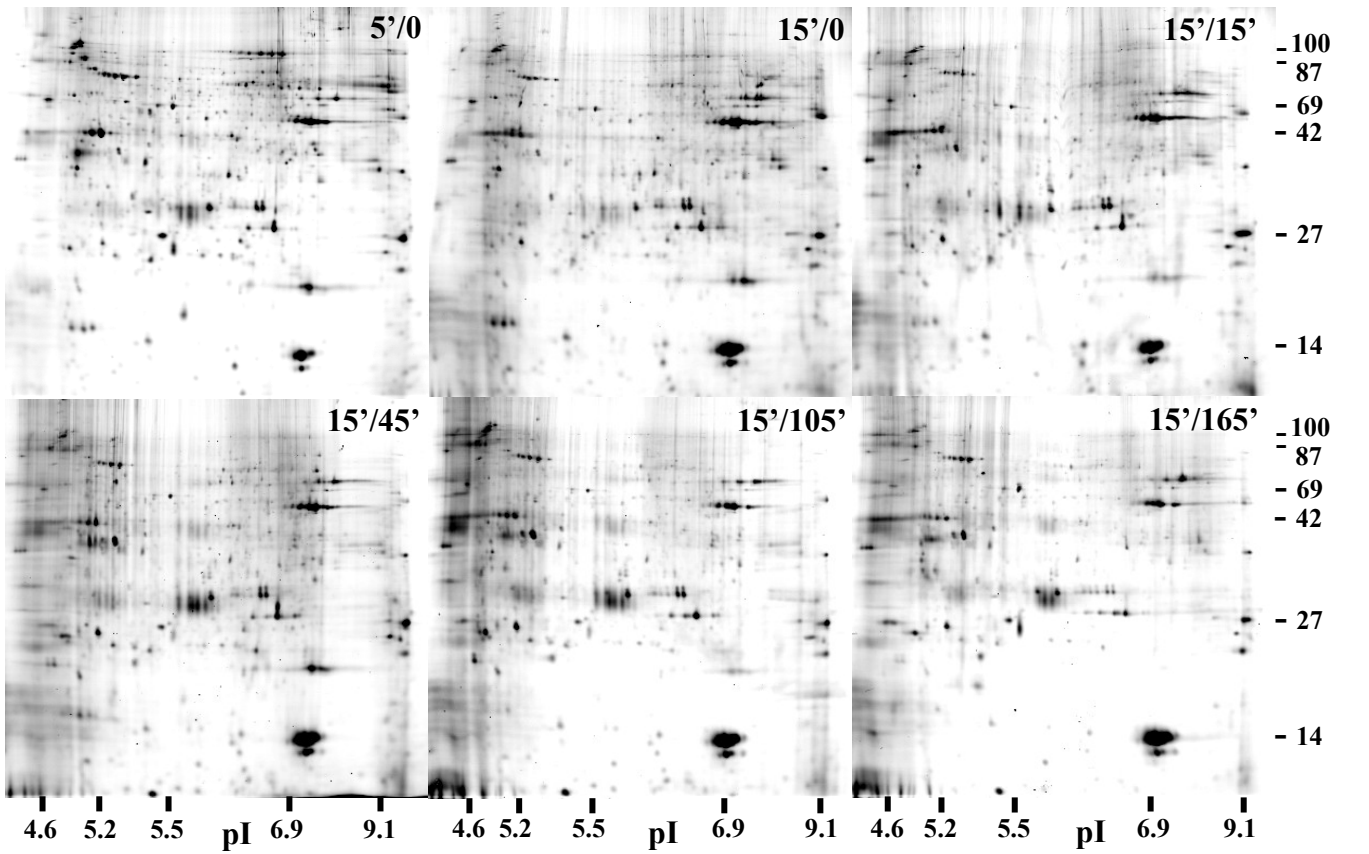
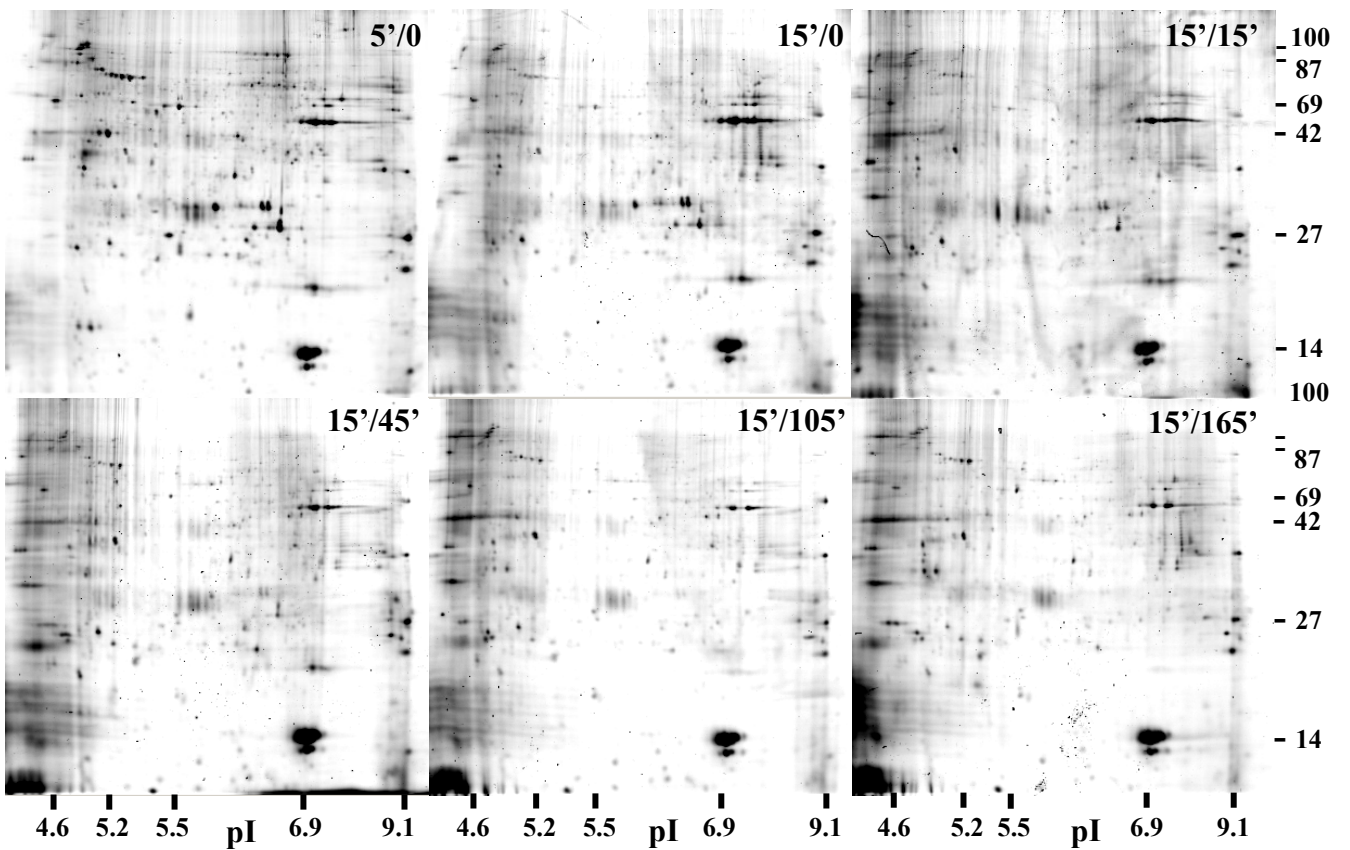


myoK null Cy5-labelled phagosomal time profile



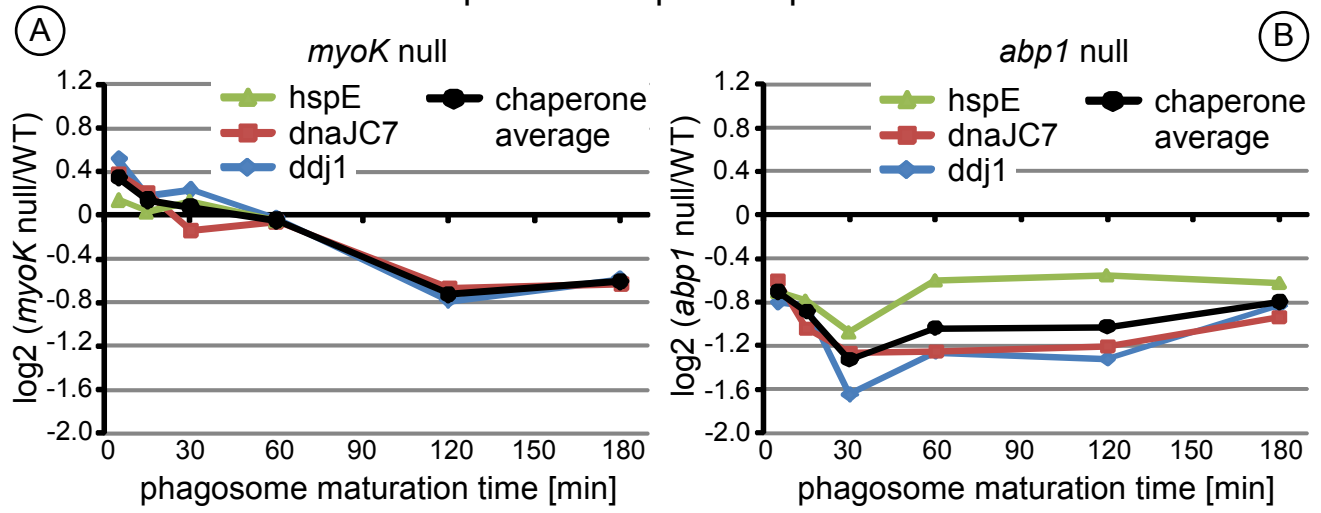
B

Wild type Cy5-labelled phagosomal time profile

*abp1* null Cy3-labelled phagosomal time profile

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Hsp40 and Hsp70 chaperones



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