# Supplemental Materials

***Molecular Biology of the Cell***

Levin et al.

SUPPLEMENTARY FIGURES

Figure S1. **2xP4M expression does not affect Golgi morphology. A)** Representative confocal micrograph of a RAW264.7 cell co-expressing the PtdIns4P-biosensor GFP-2xP4M and the Golgi-marker mCh-sialyltransferase. Main panel shows mCh-sialyltransferase fluorescence; the cell outline is drawn in yellow. Bottom left inset shows GFP-2xP4M fluorescence. **B)** Representative confocal micrograph of a RAW cell expressing only the Golgi-marker mCh-sialyltransferase. Top right insets show magnifications of the corresponding dotted white boxes. Scale bars = 5 μm.

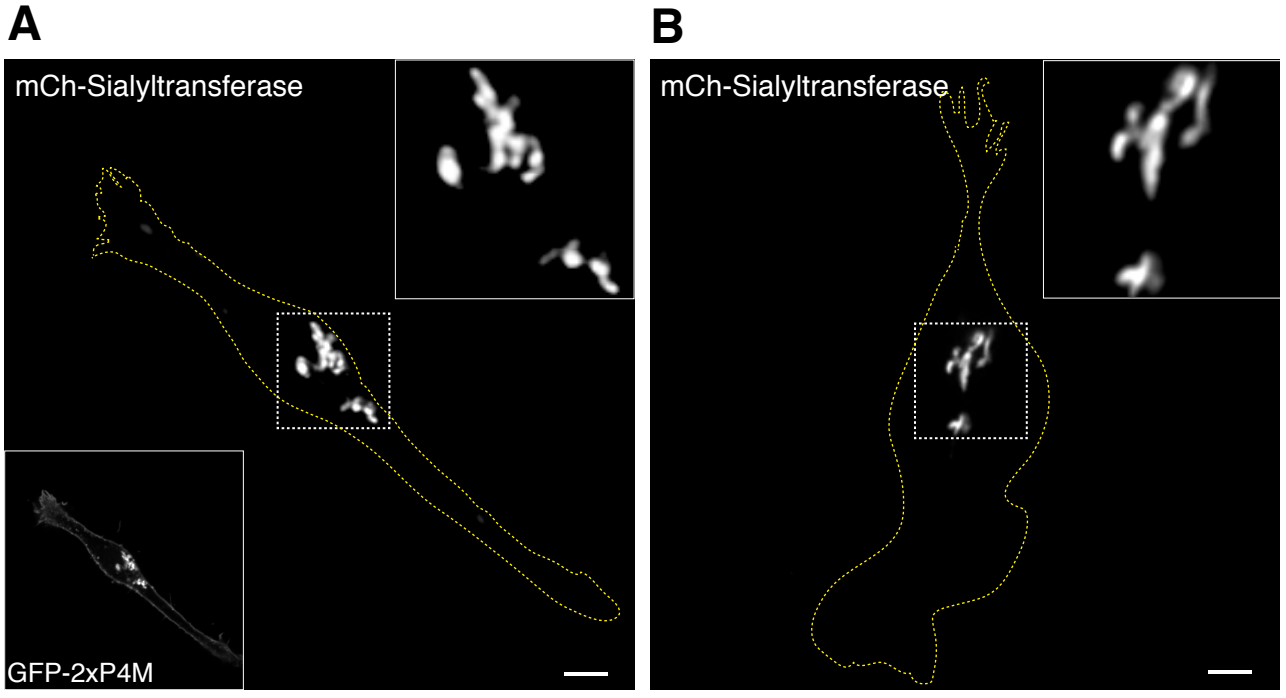
Figure S2. **Phosphoinositide metabolism during the early phagocytic stages. A)** Time-lapse gallery of confocal micrographs of RAW264.7 cells transiently co-expressing mCh-2xP4M and GFP-PH-Gab2 [a PtdIns(3,4,5)$P_3$-specific biosensor] during phagocytosis of IgG-SRBCs. The Roman numerals refer to the stages defined on Figure 3. Images are representative of at least 10 similar determinations. **B)** Schematic diagram illustrating possible enzymatic reactions whereby phagosomes can be depleted of PtdIns4P. **C)** Confocal micrographs of RAW264.7 cells co-expressing GFP-PH-TAPP1 [a PtdIns(3,4)$P_2$ biosensor] and mCh-2xP4M during the phases of initial increase (left) and disappearance of PtdIns4P (right); PtdIns(3,4)$P_2$ was not detected in phagosomes during PtdIns4P disappearance. **D)** Confocal micrographs acquired during phagocytosis of 1.6 μm TMR-IgG-coated latex beads by LY294002-treated (50 μM) RAW264.7 cells expressing GFP-2xP4M. Representative images of the phases of initial PtdIns4P increase (left) and its subsequent disappearance (right) are shown. LY294002 did not prevent PtdIns4P disappearance. Insets show magnifications of dotted white boxes. **E)** RAW264.7 cells were challenged with 3.87 μm IgG-coated latex beads. After 15 min, cells were washed and fixed, permeabilized and immunostained using anti-PI4KA antibodies. Confocal sections are shown. A forming phagosome is indicated with a yellow arrow while a maturing phagosome is indicated with a white arrow. Images are representative of at least 10 similar determinations. Scale bars = 5 μm.

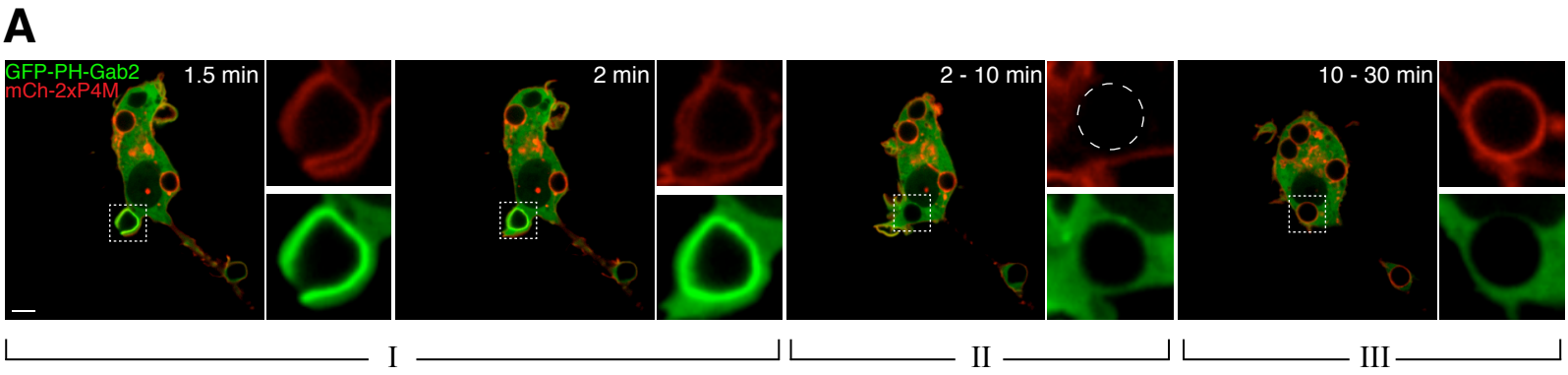
Figure S3. **PtdIns4P dynamics in COS-1-FcγRIIa cells. A)** Time-lapse gallery of confocal micrographs acquired during phagocytosis of TAMRA-labeled IgG-opsonized sheep red blood cells (IgG-SRBCs; shown in red) by COS-1-FcγRIIa cells transiently expressing GFP-2xP4M (green). The panels indicate the time elapsed from the moment the target was engaged. Insets show magnifications of the area delimited by dotted white boxes. **B)** Summary of the changes in PtdIns4P content of phagosomes following engagement of IgG-SRBCs. The intensity of GFP-2xP4M in phagosomes was quantified and normalized to that of the plasmalemma. The three phases defined in RAW264.7 cells were recapitulated in COS-1-FcγRIIa cells, with slower dynamics. Data are expressed relative to the maximum value attained during the initial transient increase, which was defined as time "0". Values are means ± SEM of 4 independent experiments. Scale bars = 5 μm.

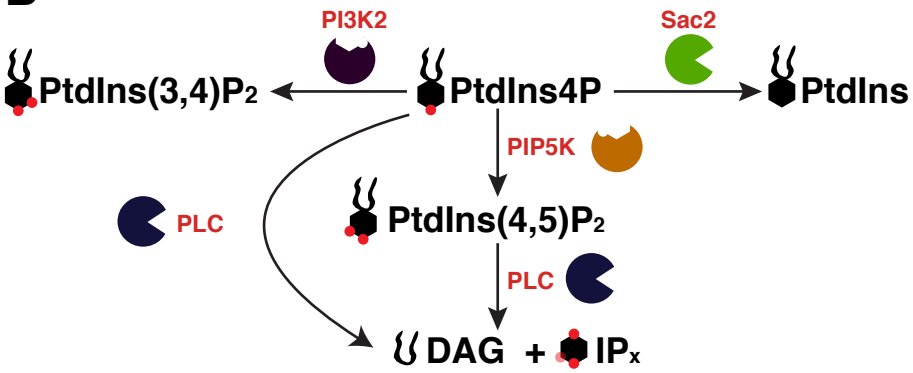
Figure S4. **Cresyl violet co-localizes with GFP-Rab7, but not with Rab5. A)** Representative confocal section of RAW264.7 cells transiently expressing GFP-Rab5 and treated with cresyl violet (1 μM for 2 min; shown in red). **B)** Single confocal section of

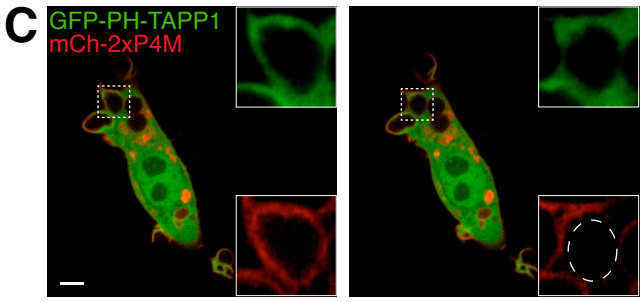
RAW264.7 cells transiently expressing GFP-Rab7 treated with cresyl violet as above. Insets show magnifications of dotted white boxes for each channel. Scale bars = 5 μm.

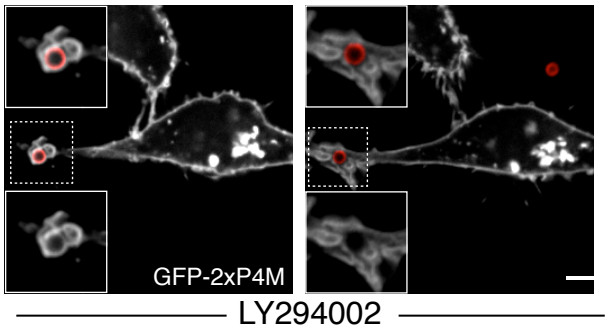
Figure S5. **Diagrammatic representation of the changes undergone by phosphoinositides during phagosome formation and maturation.** The changes in PtdIns4P (shades of blue) are compared with those undergone by PtdIns(4,5)$P_2$ (green), PtdIns(3,4,5)$P_3$ (red) and PtdIns3P (purple). Phagosome formation is divided into receptor engagement, pseudopod extension and vacuole sealing stages, while maturation is divided into early and late/lysosomal stages.

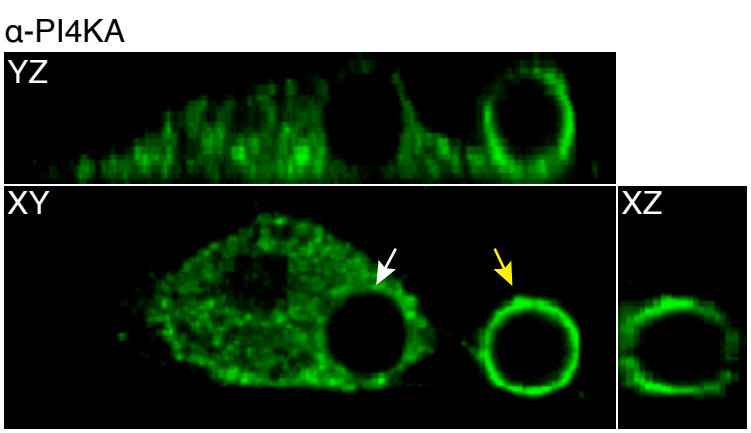

A
mCh-Sialyltransferase
GFP-2xP4M
B
mCh-Sialyltransferase

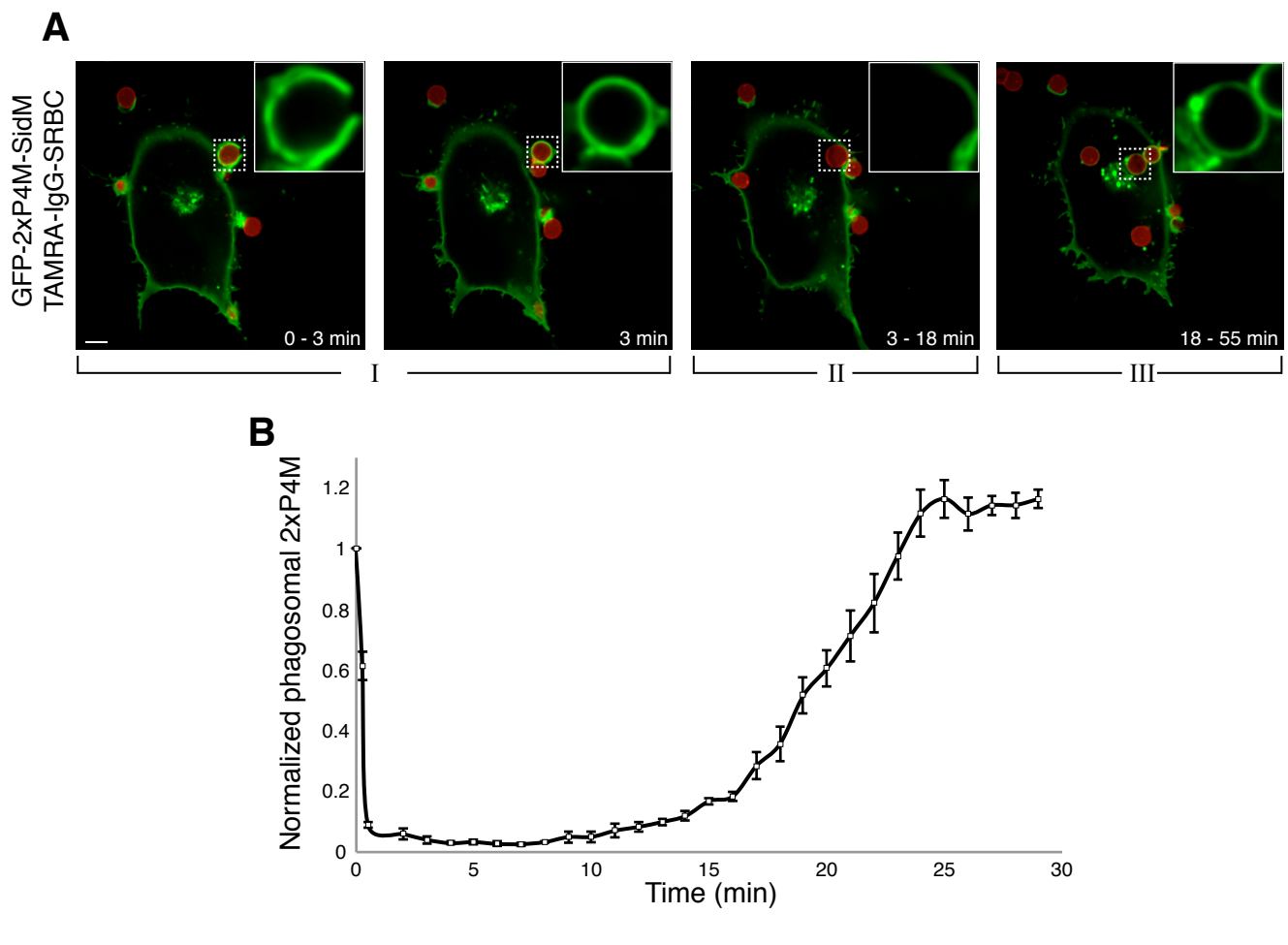

A
GFP-PH-Gab2
mCh-2xP4M
1.5 min
2 min
2 - 10 min
10 - 30 min
I
II
III

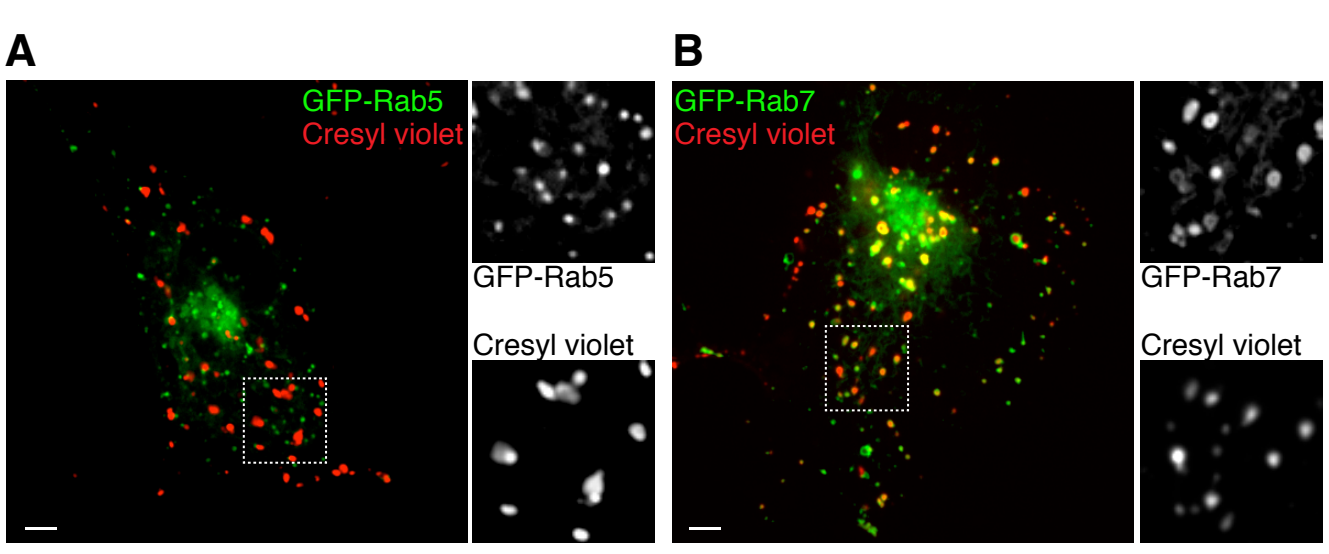

B
PI3K2
Sac2
PtdIns(3,4)P2
PtdIns4P
PtdIns
PIP5K
PLC
PtdIns(4,5)P2
PLC
DAG + IPx

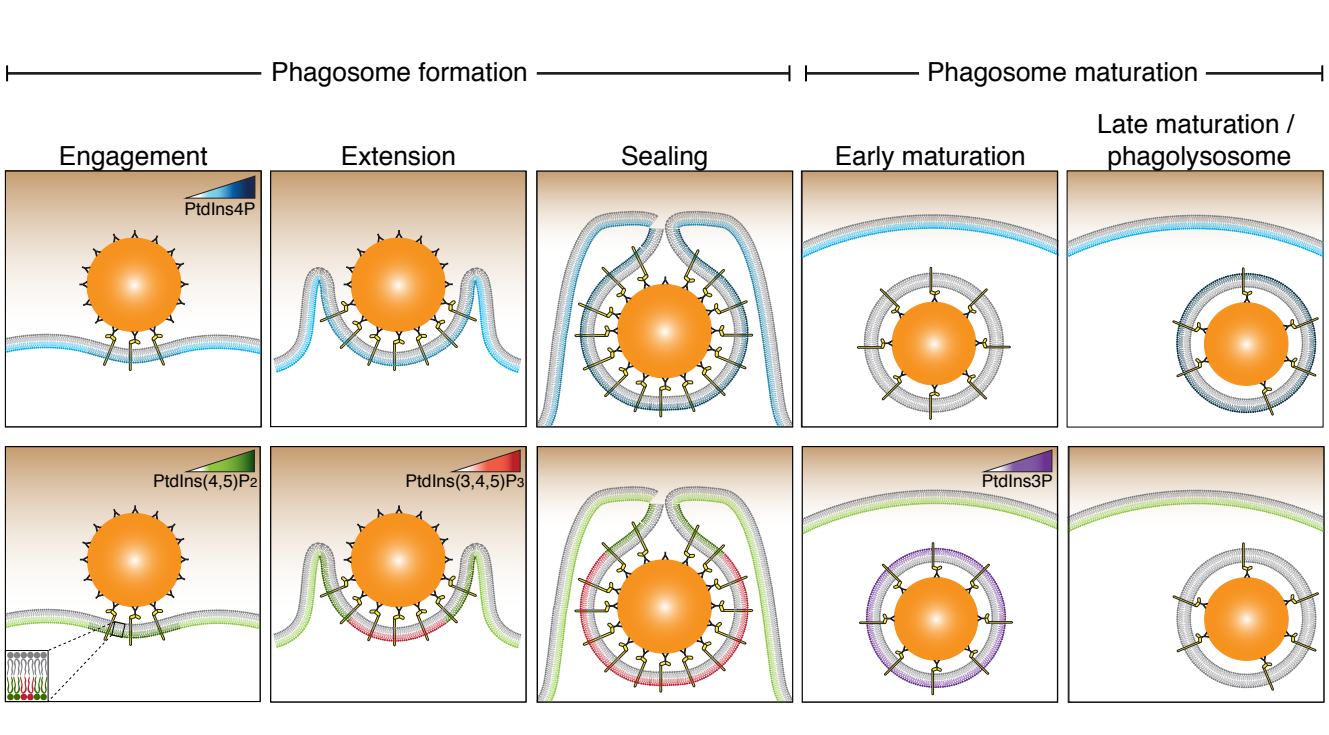

C
GFP-PH-TAPP1
mCh-2xP4M


D
GFP-2xP4M
LY294002


E
α-PI4KA
YZ
XY
XZ


A
GFP-2xP4M-SidM
TAMRA-IgG-SRBC
0 - 3 min
3 min
3 - 18 min
18 - 55 min
I
II
III
B
Normalized phagosomal 2xP4M
1.2
1
0.8
0.6
0.4
0.2
0
0
5
10
15
20
25
30
Time (min)


A
GFP-Rab5
Cresyl violet
GFP-Rab5
Cresyl violet
B
GFP-Rab7
Cresyl violet
GFP-Rab7
Cresyl violet


Phagosome formation
Phagosome maturation
Engagement
Extension
Sealing
Early maturation
Late maturation / phagolysosome
PtdIns4P
PtdIns(4,5)P2
PtdIns(3,4,5)P3
PtdIns3P