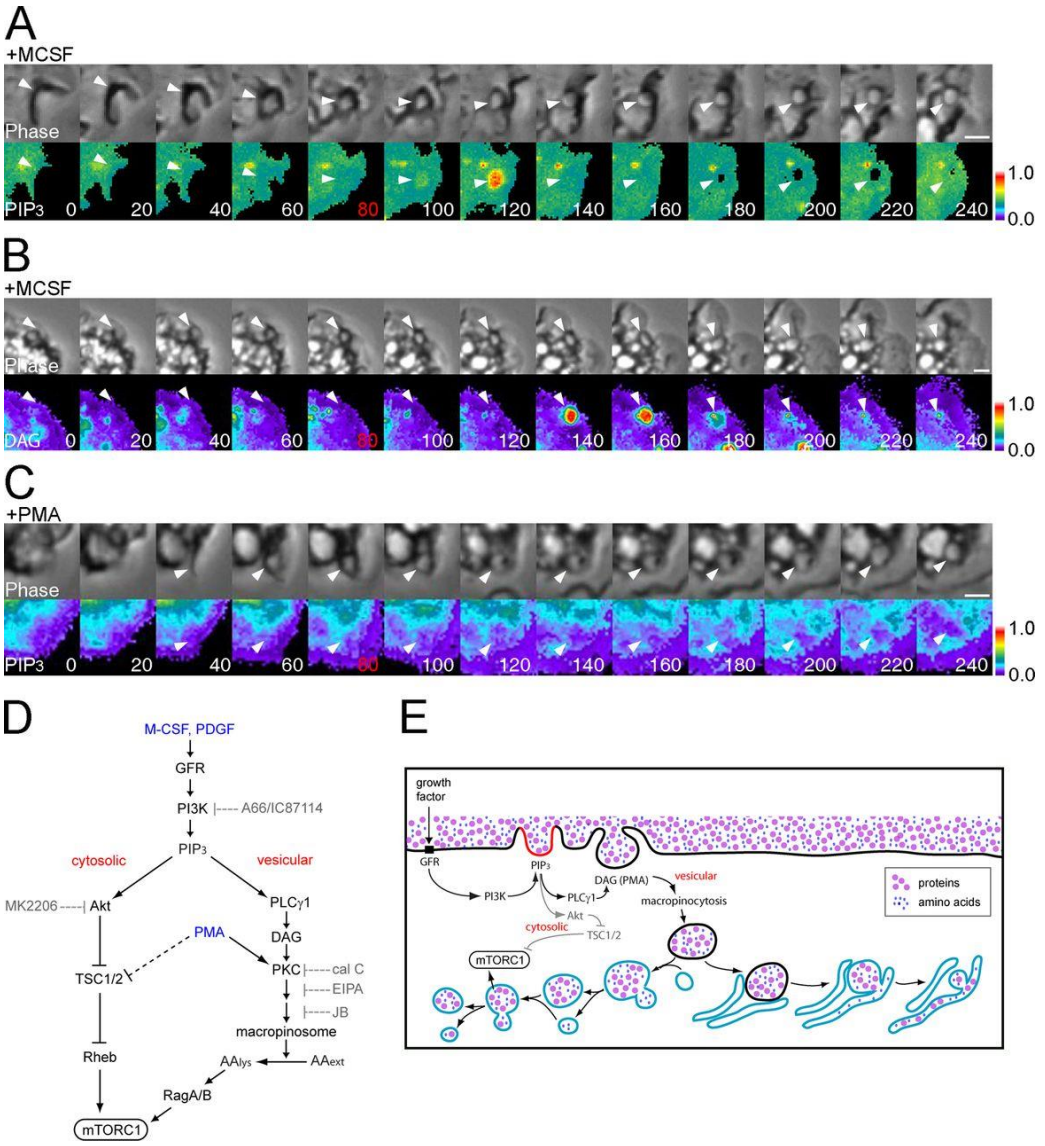
Signaling for mTORC1 activation is localized to macropinocytic cups.



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Signaling for mTORC1 activation is localized to macropinocytic cups. (A) BMMs expressing CFP and YFP-BtkPH, a probe for PIP_3, were imaged during M-CSF–stimulated macropinocytosis. The image series are aligned such that circular ruffle closure occurs at *t* = 80 s (top row). Pseudocolor ratio images (YFP-BtkPH/CFP) show strong YFP-BtkPH recruitment to the macropinocytic cup at *t* = 120 s (bottom row). (B) Ratiometric imaging of the DAG probe C1δ-YFP in BMMs, as described in (A). *t* = 80 s marks the end of ruffle closure. Maximal C1δ-YFP recruitment occurs at *t* = 140 and 160 s (C) Ratiometric imaging of the PIP_3 probe YFP-BtkPH during PMA-stimulated macropinocytosis. YFP-BtkPH was not recruited to the macropinocytic cup. Bars, 5 μm. (D) Two pathways of growth factor receptor (GFR) signaling to mTORC1. GFR signaling activates PI3K, which activates mTORC1 by a cytosolic pathway, involving Akt, TSC2, and Rheb, and a vesicular pathway, involving PKC-dependent, macropinosome-mediated delivery of leucine to endolysosomes. PMA activates both pathways independent of PI3K. Stimuli are indicated in blue type; inhibitors are indicated in gray type. (E) The macropinosome as a discrete unit of GFR signaling. PI3K-generated PIP_3 accumulates in macropinocytic cups (red line), activating Akt (cytosolic pathway) and PLCγ. PLCγ generates DAG in the cup, leading to PKC-dependent macropinocytosis (vesicular pathway). Extracellular solutes internalized by macropinocytosis are delivered rapidly into LAMP-1 (blue lines)–enriched endolysosomes by piranhalysis or after tubular endolysosomes wrap around macropinosomes. Small solutes exchange more rapidly between macropinosomes and endolysosomes than large solutes, providing a rapid mechanism for activation of mTORC1 by amino acids inside macropinosomes and endolysosomes.