



Supplemental figure 1 Accumulation of PLD2 surrounding macropinosomes at relatively later stages of macropinocytosis. COS7 cells were transiently transfected with expression vectors encoding GFP-PLD1 (A), GFP-PLD2 (B) or GFP-LN-PLD2 (C). Three days after transfection cells were serum-starved for 1 h and stimulated with 100 ng/ml EGF in the presence of tetramethylrhodamine-labeled dextran for 8 min. After washing extracellular dextran, cells were incubated for additional 0, 10 or 20 min at 37 °C, fixed and analyzed by confocal microscopy. Bars, 10 μ m. Note that PLD1 accumulated around macropinosomes at earlier stages (0 time), whereas PLD2 co-localized well with macropinosomal membranes at relatively later stages of macropinocytosis and that macropinocytosed dextran stayed longer in the cytoplasm in cells expressing PLD2 compared with the cell expressing PLD1, suggesting functional differences between the two isozymes. In the case of LN-PLD2, however, macropinocytosis was suppressed compared with cells expressing wild-type PLD2 and that LN-PLD2 was not co-localized well with macropinosomal membranes (see arrows), suggesting the necessity of catalytic activity of PLD2 for the phenomena seen in (B).