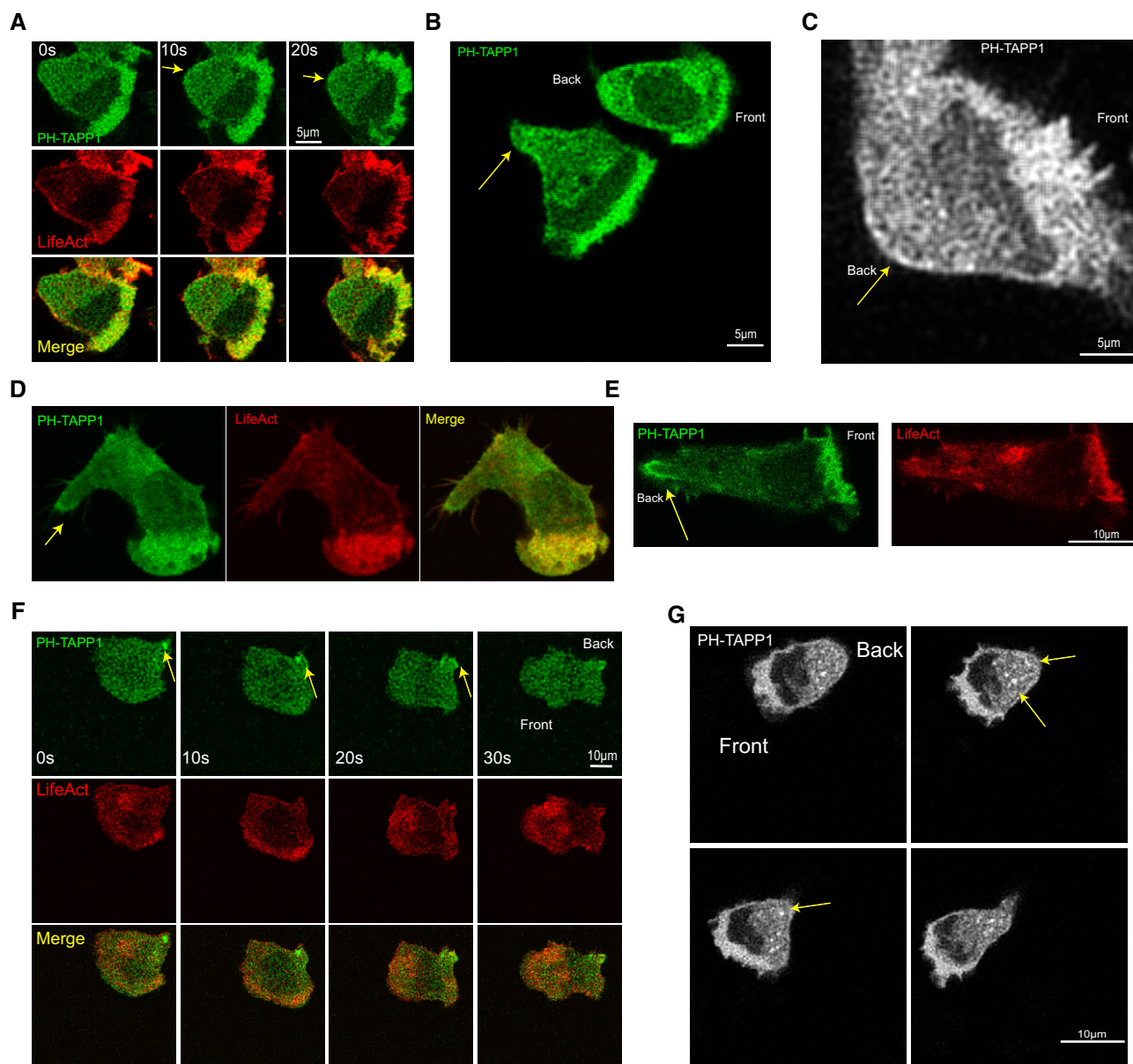


## Expanded View Figures



**Figure EV1. Dynamic localization of PI(3,4)P2 in neutrophils.**

- A Time-lapse confocal images of human leukemia neutrophil-like HL-60 cell line stably co-expressing PI(3,4)P2 biosensor, trimers of the isolated cPH (residues 169–329 in human TAPP1) (cPHx<sub>3</sub><sub>TAPP1</sub>-GFP), and RFP-LifeAct (labeling F-actin) showing front and back localization of PI(3,4)P2. Yellow arrow points to PI(3,4)P2 localization at the back of the membrane. Front and back of the cell are shown.
- B, C Confocal images of HL-60 cell line stably co-expressing PI(3,4)P2 biosensor cPHx<sub>3</sub><sub>TAPP1</sub>-GFP and RFP-LifeAct. Yellow arrow points to PI(3,4)P2 localization at the back of the membrane. Front and back of the cell are shown.
- D Confocal images showing front and back localization of PI(3,4)P2 in fixed HL-60 cells. Yellow arrow points to PI(3,4)P2 localization at the back of the membrane.
- E A maximum intensity projection of Z-stack of confocal images showing front and back localization of PI(3,4)P2 in fixed HL-60 cells. Yellow arrow points to PI(3,4)P2 localization at the back of the membrane.
- F Time-lapse confocal images of HL-60 cell co-expressing cPHx<sub>3</sub><sub>TAPP1</sub>-GFP and RFP-LifeAct showing PI(3,4)P2 vesicles at the back of cells. Yellow arrow points to PI(3,4)P2 vesicle localization at the back of the HL-60 cell.
- G Time-lapse confocal images of HL-60 cell expressing cPHx<sub>3</sub><sub>TAPP1</sub>-GFP showing PI(3,4)P2 vesicles at the back of cells. Yellow arrow points to some PI(3,4)P2 vesicles at the back of the HL-60 cell. Front and back of the cell are shown.