# Appendix

## Reverse Fountain Flow of PI(3,4)P2 Polarizes Cells

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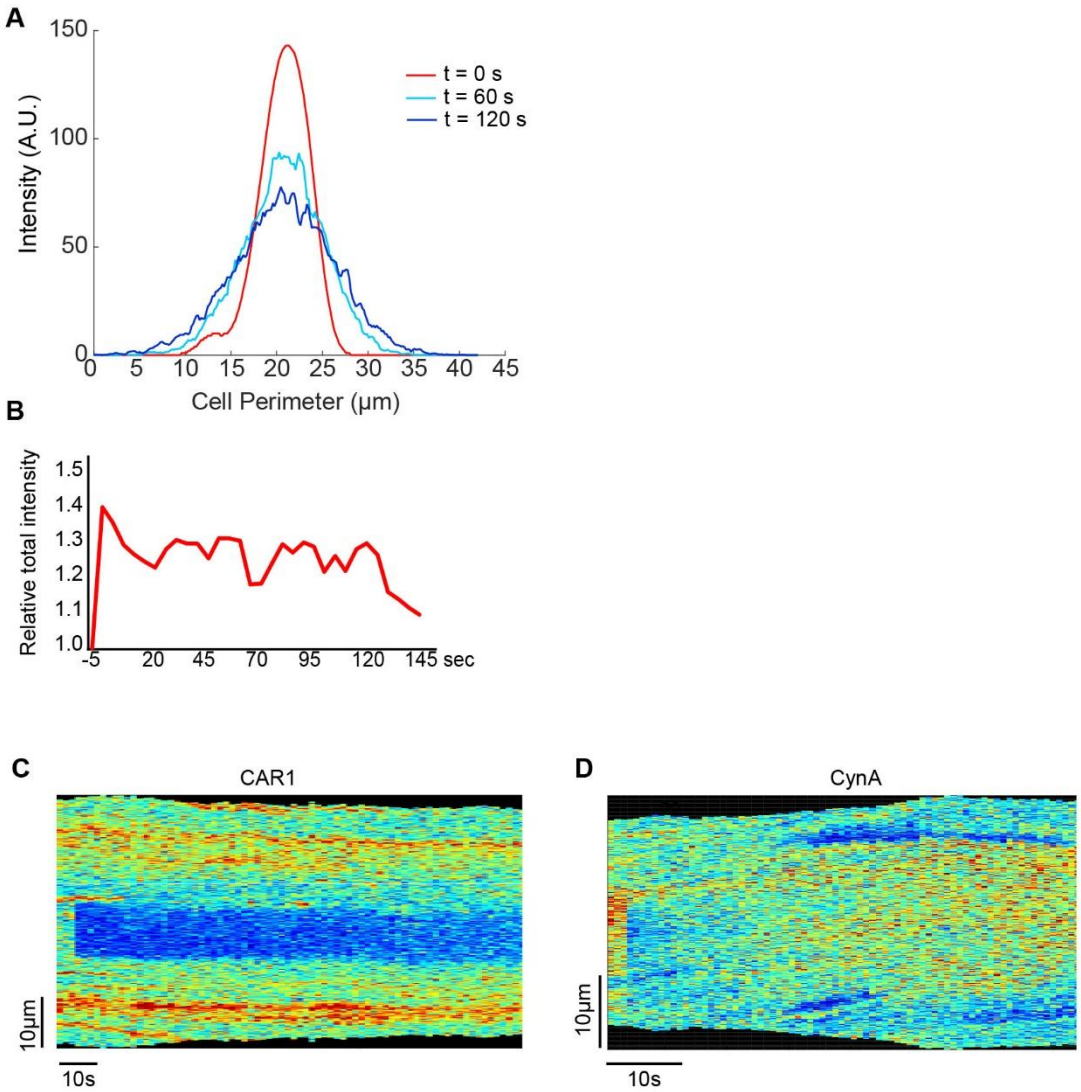
## Appendix Tables

**Appendix Table S1**: Parameter List

| Parameter | | Distribution | Nom. Value | Std Dev. | Unit |
|---|---|---|---|---|---|
| Arrival time of a vesicle | $t_{a,veg}$ | Exponential | 10 | 10 | s |
| | $t_{a,dev}$ | | 40 | 40 | s |
| Docking time | $t_{dock}$ | Normal | 10 | 2 | s |
| Radius of the vesicle | $r$ | Normal | 0.2 | 0.2 | μm |
| Molecules in vesicle per unit length | $n$ | Normal | 1000 | 50 | mol. |
| Cell perimeter | $L$ | | 40 | | μm |
| Biasing index | $n_v$ | | 3 | | N/A |
| Conversion rate | $k_{-u}$ | Normal | 0.4 | 0.04 | $s^{-1}$ |
| Basal degradation rate | $k_{1,veg}$ | Normal | 0.02 | 0.002 | $s^{-1}$ |
| | $k_{1,dev}$ | | 0.004 | 0.0004 | $s^{-1}$ |
| Maximal degradation rate | $k_{2,veg}$ | Normal | 0.004 | 0.0004 | $mol.^2s^{-1}$ |
| | $k_{2,dev}$ | | 0.0005 | 0.00005 | $mol.^2s^{-1}$ |
| Michaelis constant | $k_3$ | Normal | 0.45 | 0.04 | $mol.^2$ |
| Diffusion constant of V | $D_V$ | | $18x10^{-4}$ | | $\mu m^2s^{-1}$ |

## Appendix Figures



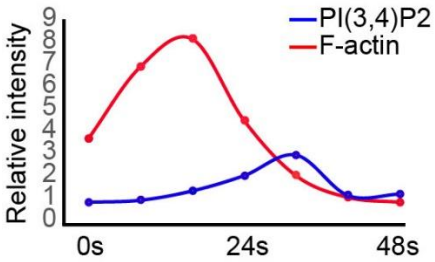

**Appendix Figure S1. Small PI(3,4)P2 vesicles supply PI(3,4)P2 to the back of migrating *Dictyostelium* cells**

(A) The simulated profiles for Fig. 1D for the computation of the diffusion coefficient.

(B) Relative intensity of converted red fluorescence at the side of the cell in Fig. 1F, decreases over time.

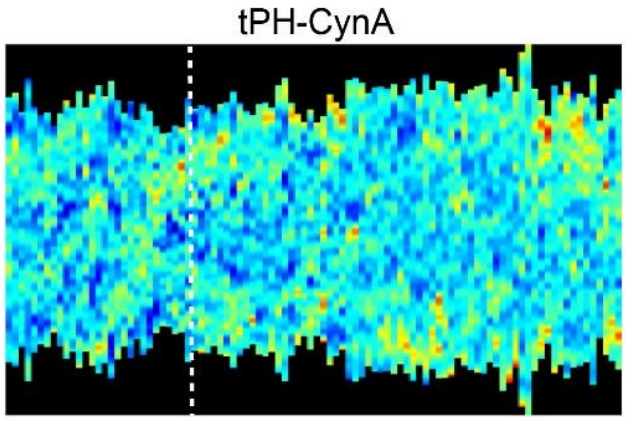
(C) Kymographs of converted red fluorescence in representative individual CAR1 expressing cell from Fig. 1H.

(D) Kymographs of converted red fluorescence in representative individual tPH-CynA expressing cell from Fig. 1H.



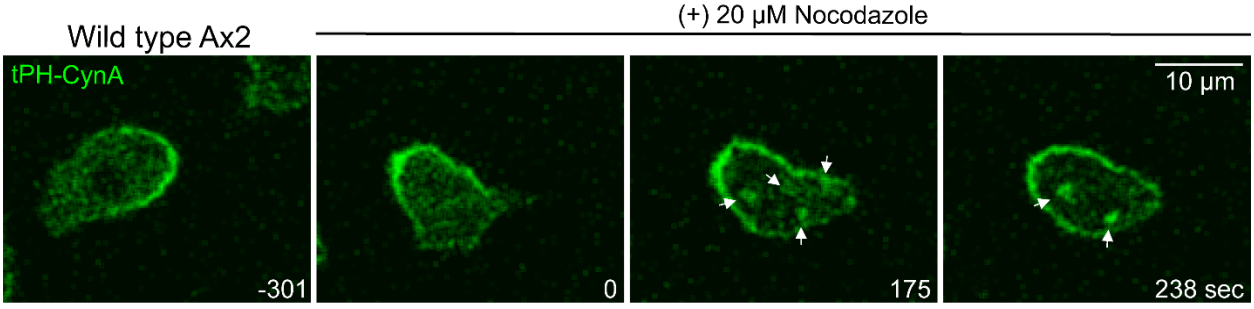

**Appendix Figure S2. PI(3,4)P2 trails F-actin on the leading edge macropinosomes**

Relative intensities of tPHCynA-KikGR and LimE-RFP on macropinosomes in the cell of Fig. 2A



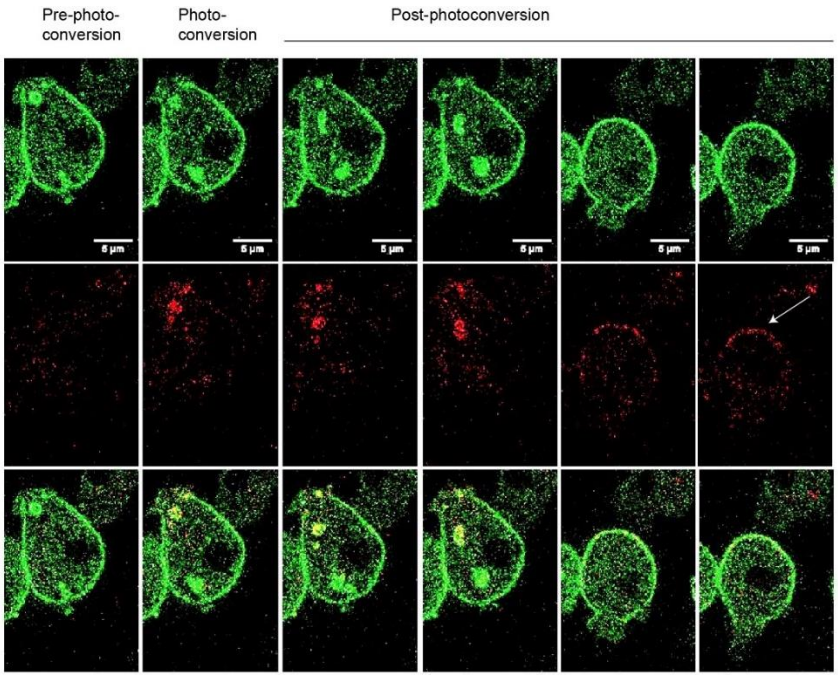

**Appendix Figure S3. PI(3,4)P2 appears in absence of PIP3**

(A) Kymograph of tPHCynA-KikGR intensity on the perimeter of one cell undergoing random migration in Fig. 3A.



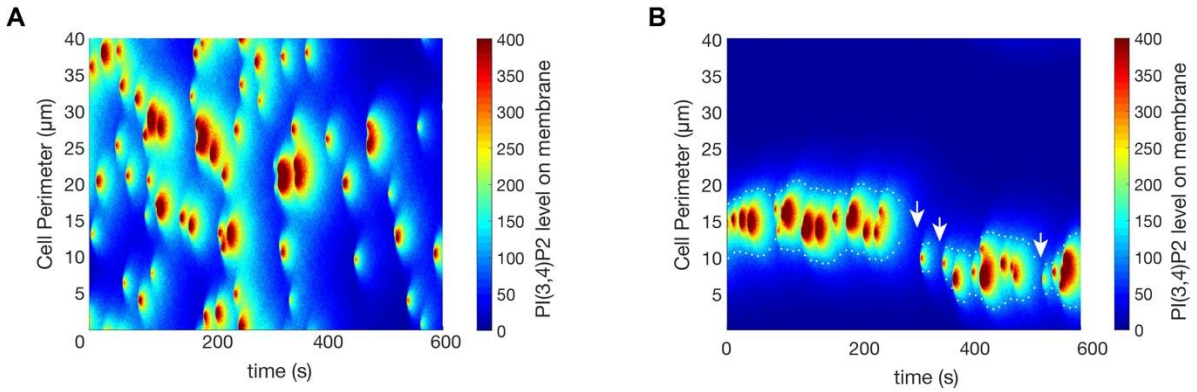

**Appendix Figure S4. Macropinocytic vesicle processing is necessary for PI(3,4)P2 enrichment at the back of migrating cells.**

Time-lapse confocal images showing localization of PI(3,4)P2 (tPH-CynA-KiKGR ; green) in wild type *Dictyostelium* Ax2 cells in absence or presence of 20 µM Nocodazole. Nocodazole was added at time 0 during the live cell imaging. Images were captured every 7 sec.




**Appendix Figure S5. A link between PI(3,4)P2 on front macropinosomes and the back to front gradient of PI(3,4)P2**

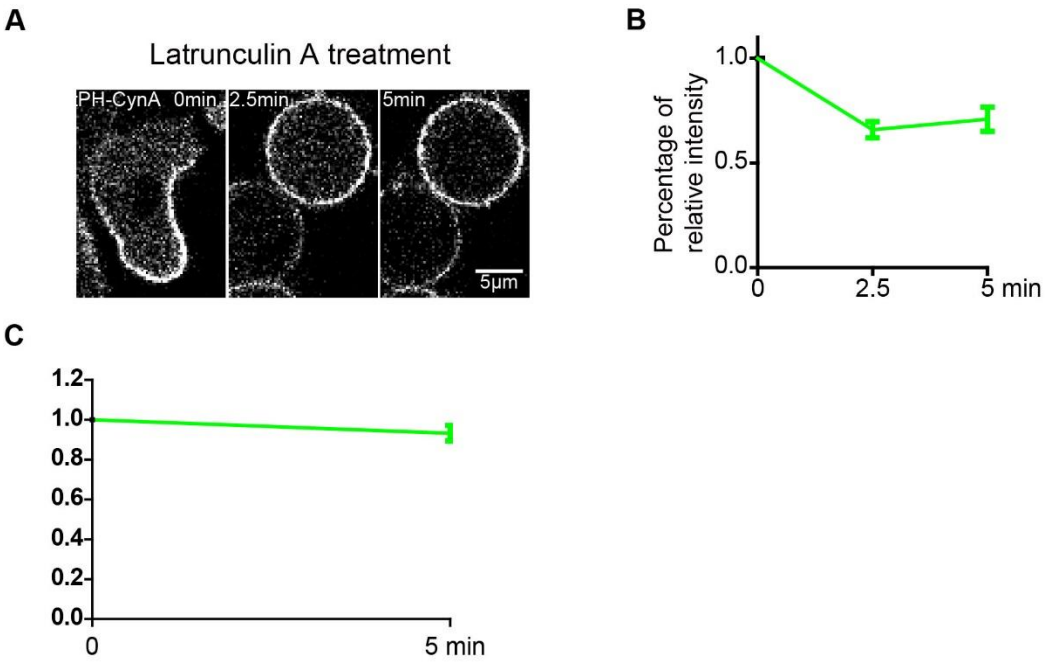
Time-lapse confocal images of $tPH_{CynA}$-KikGR (green) expressing AX3 cells. A macropinosome at the front of the cell was photoconverted at time 0 (Similar to Fig. 5B). The cell changed direction after photo-conversion.




**Appendix Figure S6**. **Simulated kymographs.**

(A) Kymograph with random insertion of the satellite vesicles with same set of parameters as used in the case of less polarized cell (see Fig 6A, see Appendix table S1).

(B) Kymograph with directed insertion of the satellite vesicles with increased mean arrival time (20 sec) and the same decay rates as of the less polarized cell (Fig 6A). The longer arrival time results discontinuities in the back profile (indicated by the white arrows) as explained in the results section of the manuscript.

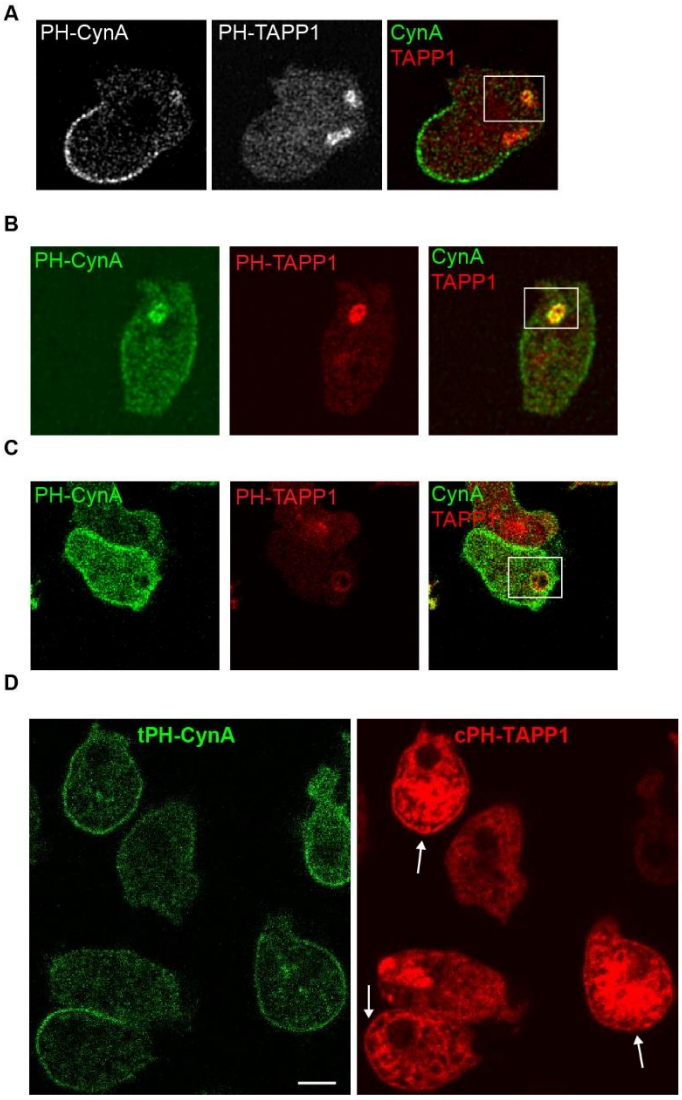



**Appendix Figure S7.**

(A) Confocal fluorescent images of tPH-CynA-KikGR in *Dictyostelium* AX3 cell before and after Latrunculin A treatment were shown. Latrunculin A was added during images at time 0.

(B) Temporal profile of normalized intensity of total fluorescence of tPH-CynA-KiKGR on the membrane of cells in (A) was shown (Mean ± SEM).

(C) Small membrane region of Latrunculin A treated cells was photoconverted at time 0. Temporal profile of normalized intensity of total converted red fluorescence was shown (Mean ± SEM). The signal decreased by less than 8 percent in the first 5 minutes following photoconversion.




**Appendix Figure S8**. **Comparison of PH-TAPP1 and tPH-CynA in *Dictyostelium***

(A-C) Three examples of confocal images showing localization of PI(3,4)P2 ( tPH-CynA-KiKGR; green) and cPH-TAPP1-RFP (red) in wild type *Dictyostelium* AX3 live cells.

(D) Confocal images showing localization of PI(3,4)P2 ( tPH-CynA-KiKGR ; green) and cPH-TAPP1 (red) in fixed wild type *Dictyostelium* AX3 cells.