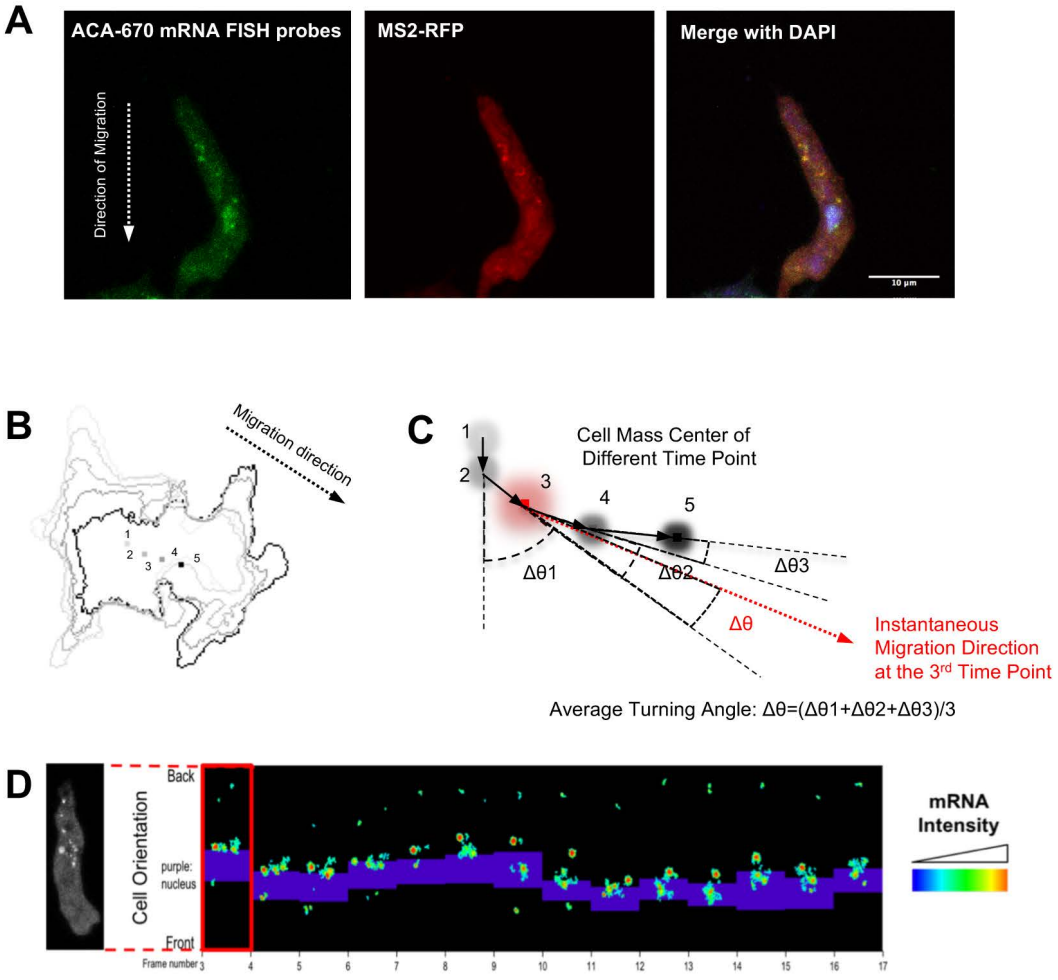


Fig. S1 Live cell ACA mRNA imaging system validation and quantification method
(A) Representative maximum intensity projections of confocal fluorescent images of individual polarized ACA-YFP-24xMS2/MS2-RFP/*aca*$^{-}$ cell depicting ACA mRNA (Green: ACA mRNA FISH probes conjugated with Quasar 670 Dye (Biosearch Technologies); Red: MS2-RFP) and DAPI (Blue).
(B) Representative cell mass center movement in 5 consecutive frames of one movie indicated by numbers. The cell outline in each frame is shown in gradient gray color.
(C) Cell instantaneous migration direction determination.
(D) Kymograph showing the relative ACA mRNA localization in each frame of one movie.

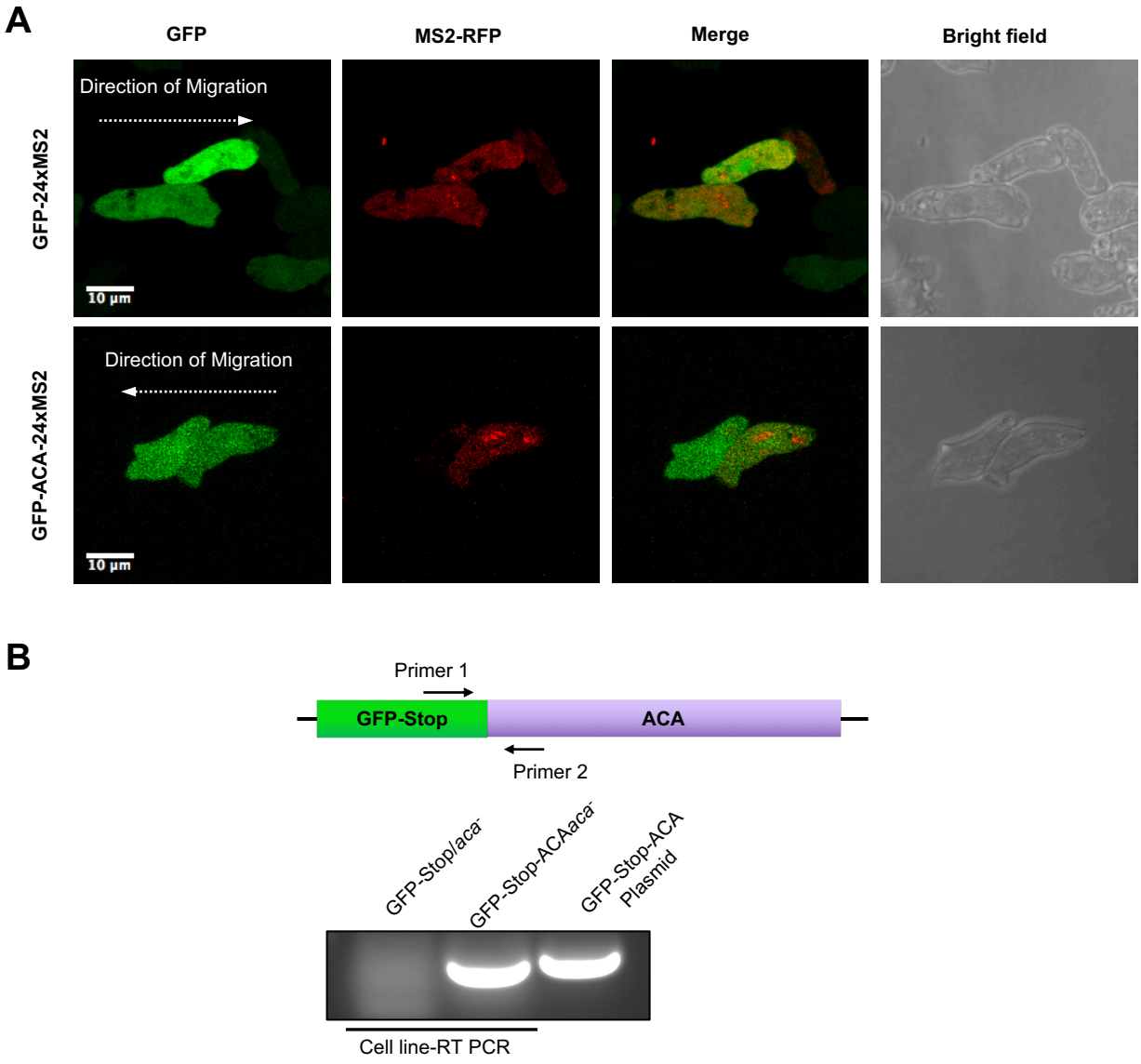


Fig. S2 Expression of chimeric fusion constructs for GFP-24xMS2 and GFP-ACA-24xMS2

(A) Representative maximum intensity projections of GFP-24xMS2/MS2-RFP/*aca*$^-$ and GFP-ACA-24xMS2/MS2-RFP/*aca*$^-$ cells as indicated.

(B) Top panel: Sheme representing primer locations against GFP-ACA construct. Bottom panel: RT-PCR results of mRNA extracted from cell lines and DNA plasmid as indicated using the primer pair in the top panel.

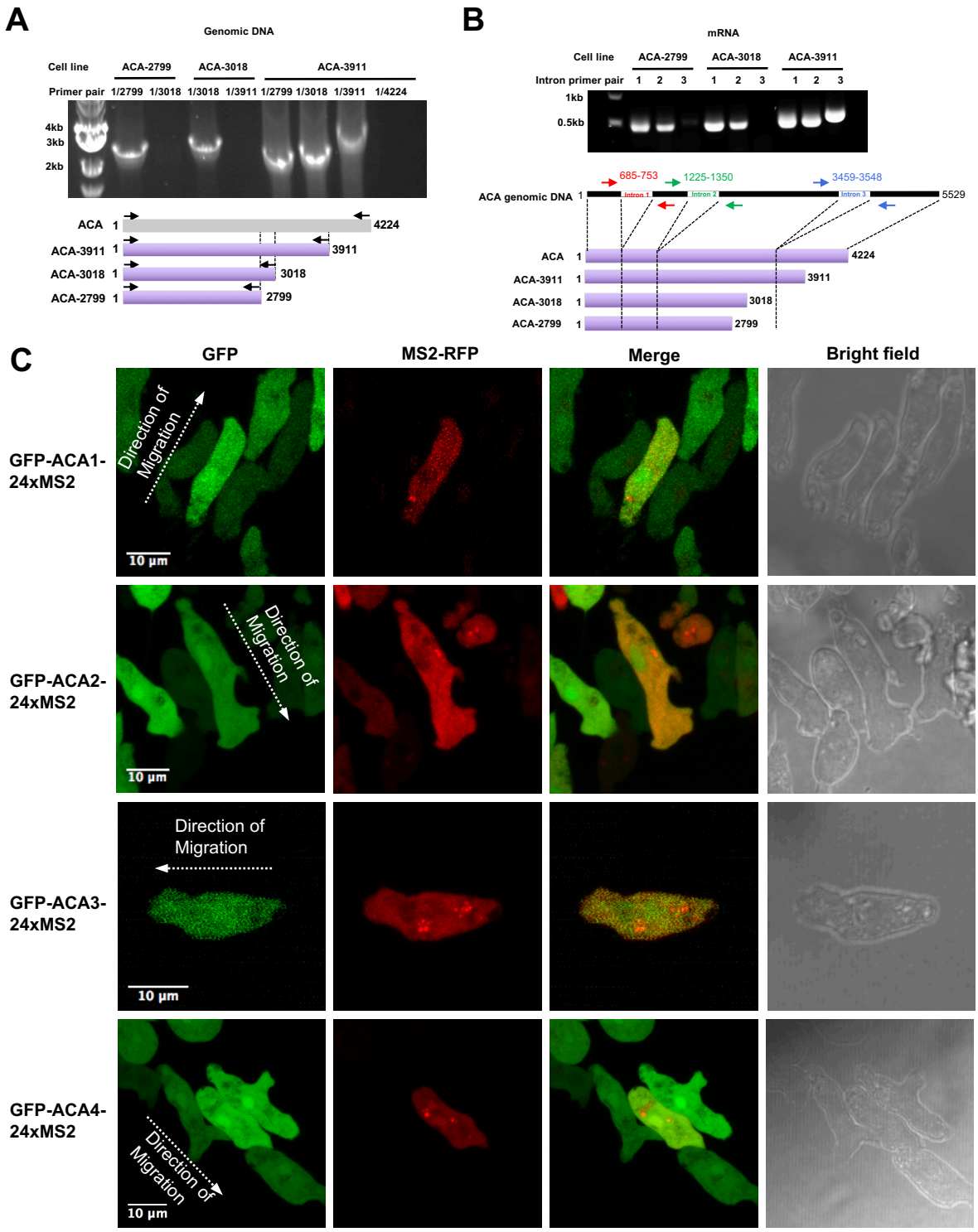


Fig. S3 Expression of chimeric fusion constructs for ACA deletion mutants
(A) PCR results of genomic DNAs from the cells indicated using the primer pairs depicted in the bottom panel.
(B) RT-PCR results of mRNA from the cells indicated using the primer pairs depicted in the bottom panel.
(C) Representative maximum intensity projections of GFP-ACA1~4/MS2-RFP/*aca*$^{-}$ cells as indicated.

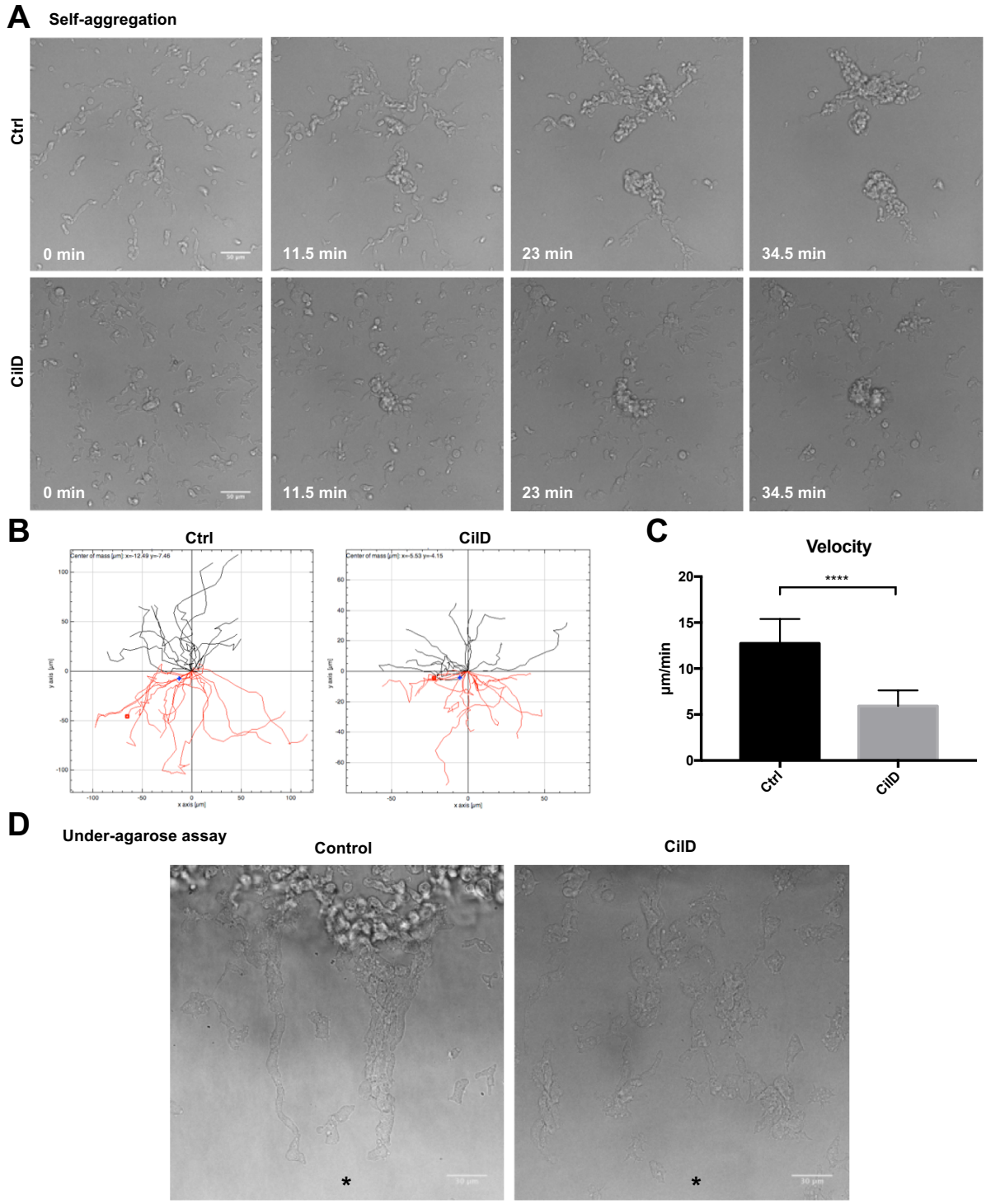


Fig. S4 CilD abolishes streaming and decreases the cell migration speed
(A) Sequential bright field images of control and 40 µM CilD-treated ACA-YFP/*aca*$^{-}$ cells under self-aggregation.
(B) Plots showing the tracks of cell migration in (A) (n=25).
(C) Velocity of the cell migration in (A) (n=25).
(D) Bright field image of control and 40 µM CilD treated ACA-YFP/*aca*$^{-}$ cells chemotaxing towards a gradient of cAMP.

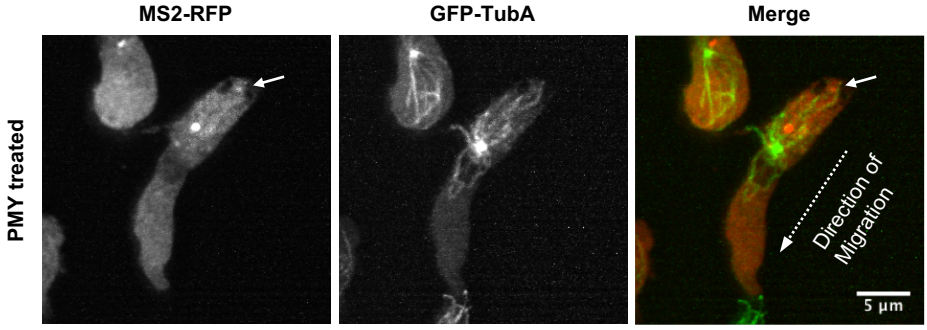


Fig. S5 ACA mRNA remains at the cell back under PMY treatment
Representative maximum intensity projection image of ACA-24xMS2/MS2-RFP/aca- cells migrate on glass coverslip taken by lattice light-sheet microscope. Left: MS2-RFP; center: GFP-TubA; right: merge.

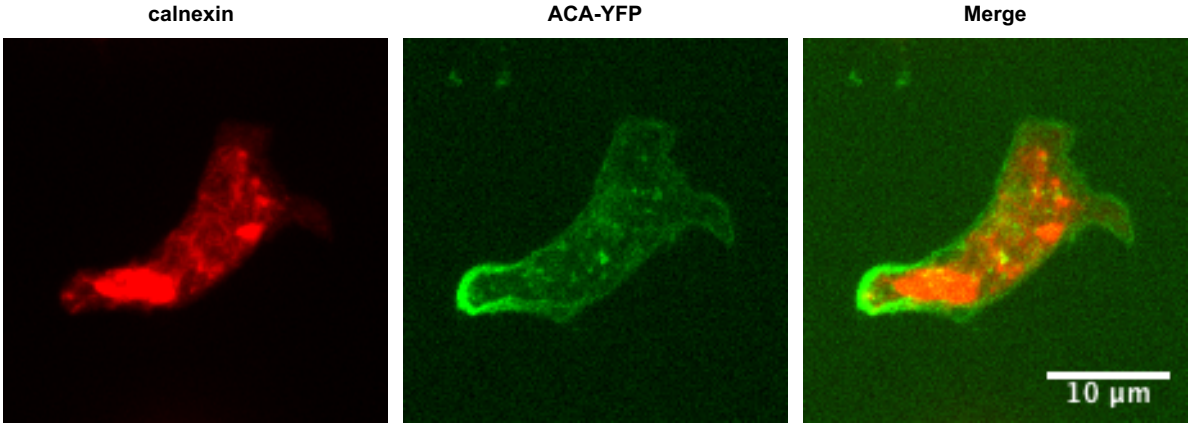
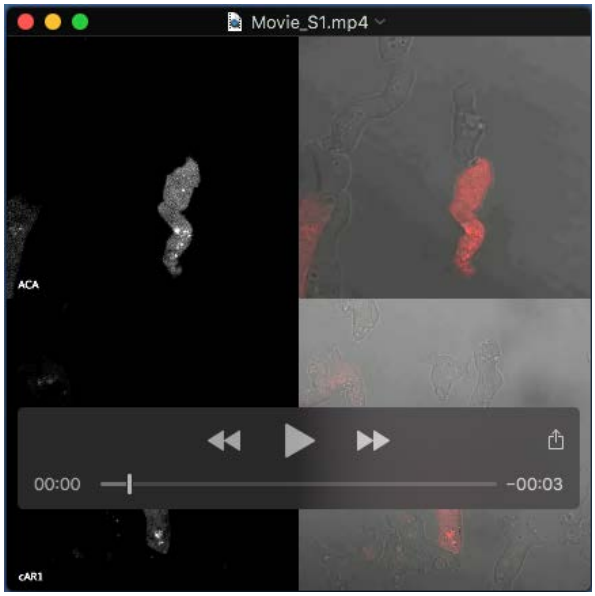


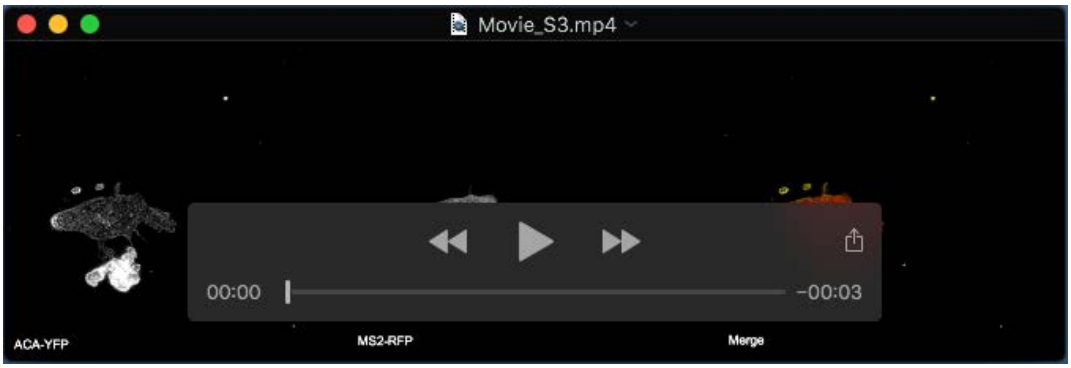
Fig. S6 ER distribution in ACA-YFP/*aca*$^{-}$ cell
Representative maximum intensity projection image of ACA-YFP/aca- cell stained with calnexin.

SUPPLEMENTAL MOVIES



Movie S1. Maximum intensity projection time-lapse images of ACA-YFP-24xMS2/MS2-RFP/*aca*$^{-}$ cells and cAR1-YFP-24xMS2/MS2-RFP/*car1/3*$^{-/-}$ cells - Related to Figure 1.
ACA-YFP-24xMS2/MS2-RFP/*aca*$^{-}$ (top) and cAR1-YFP-24xMS2/MS2-RFP/*car1/3*$^{-/-}$ (bottom) cells migrating towards a cAMP source under agarose. Left: MS2-RFP; Right: Merge with bright field. Images were taken every 15 s and presented at 3 frames/s (fps).

Movie S2. Maximum intensity projection time-lapse images of GFP-24xMS2/MS2-RFP/*aca*$^{-}$ and GFP-ACA-24xMS2/MS2-RFP/*aca*$^{-}$ cells - Related to Figure 2.
GFP-24xMS2/MS2-RFP/*aca*$^{-}$ (top) and GFP-ACA-24xMS2/MS2-RFP/*aca*$^{-}$ (bottom) cells migrating towards a cAMP source under agarose. Left: MS2-RFP; Right: Merge with bright field. Images were taken every 15 s and presented at 7 fps.

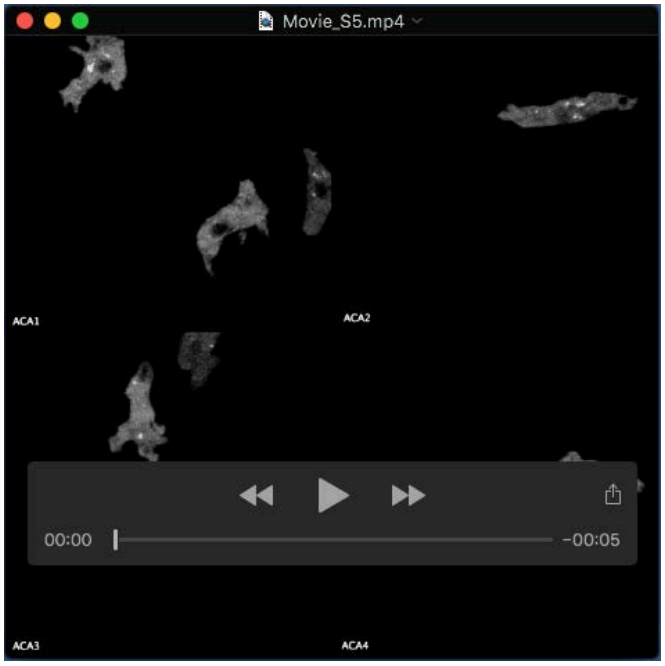


Movie S3. Maximum intensity projection time-lapse images of a ACA-YFP-24xMS2/MS2-RFP/*aca*$^{-}$ cell - Related to Figure 2.

An ACA-YFP-24xMS2/MS2-RFP/*aca*$^{-}$ cell migrating towards a cAMP source under agarose. Left: ACA-YFP; Center: MS2-RFP; Right: Merge. Images were taken every 15 s and presented at 3 fps.

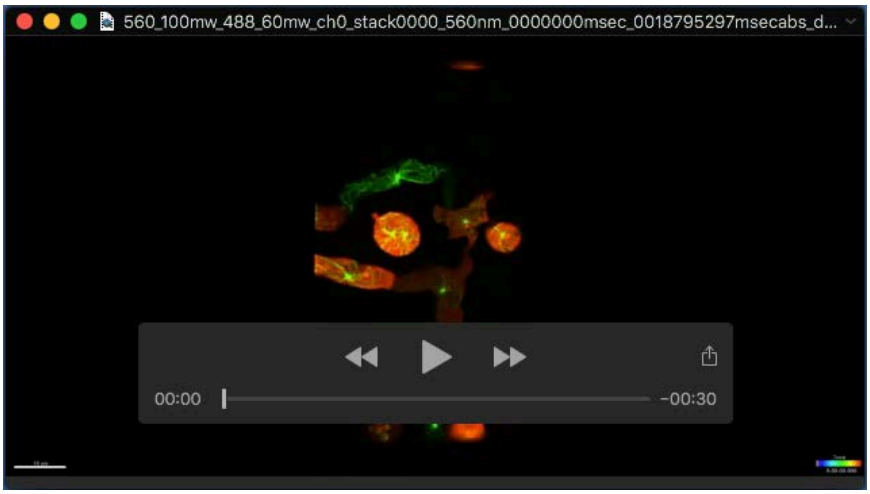
Movie S4. Maximum intensity projection time-lapse images of ACA-2799-24xMS2/MS2-RFP/*aca*$^{-}$, ACA-3018-24xMS2/MS2-RFP/*aca*$^{-}$, and ACA-3911-24xMS2/MS2-RFP/*aca*$^{-}$ cells - Related to Figure 3.

ACA-2799-24xMS2/MS2-RFP/*aca*$^{-}$ (left), ACA-3018-24xMS2/MS2-RFP/*aca*$^{-}$ (center) and ACA-3911-24xMS2/MS2-RFP/*aca*$^{-}$ (right) cells migrating towards a cAMP source under agarose. Images are showing MS2-RFP only. Images were taken every 15 s and presented at 6 fps.



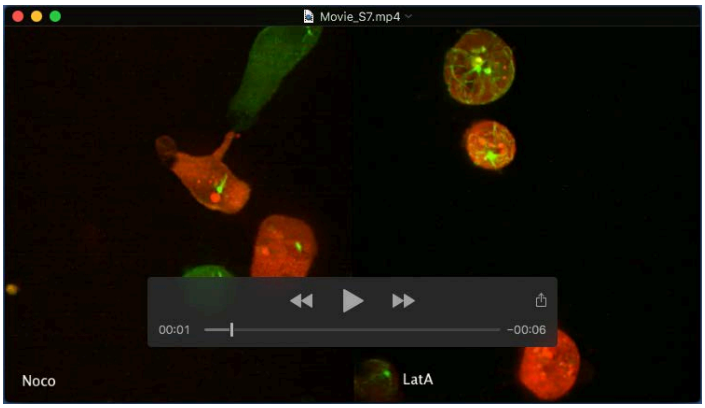
Movie S5. Maximum intensity projection time-lapse images of GFP-ACA1~4-24xMS2/MS2-RFP/*aca*⁻ cells - Related to Figure 3.

GFP-ACA1~4-24xMS2/MS2-RFP/*aca*⁻ (ACA1~4 as depicted) cells migrating towards a cAMP source under agarose. Images are showing MS2-RFP only. Images were taken every 15 s and presented at 6 fps.



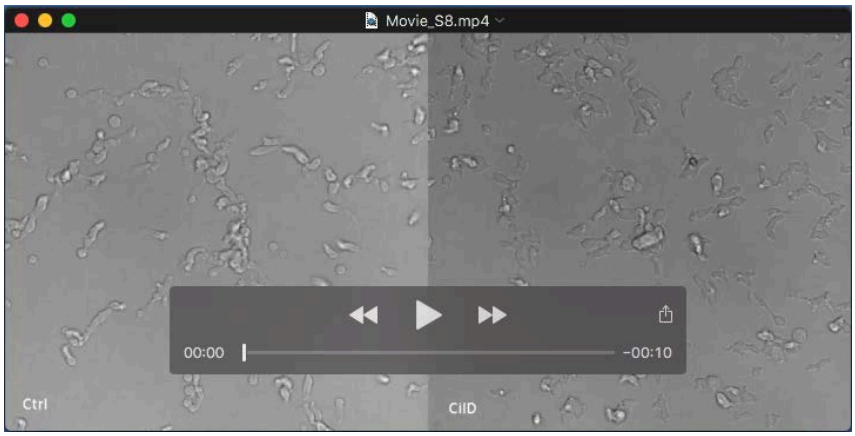
Movie S6. Three-dimensional lattice light-sheet microscopy time-lapse images of ACA-24xMS2/MS2-RFP/GFP-TubA/*aca*⁻ cells - Related to Figure 4.

ACA-24xMS2/MS2-RFP/GFP-TubA/*aca*⁻ cells streaming and self-aggregating on a glass coverslip. Red: MS2-RFP; Green: GFP-TubA. Images were presented at 10 fps.



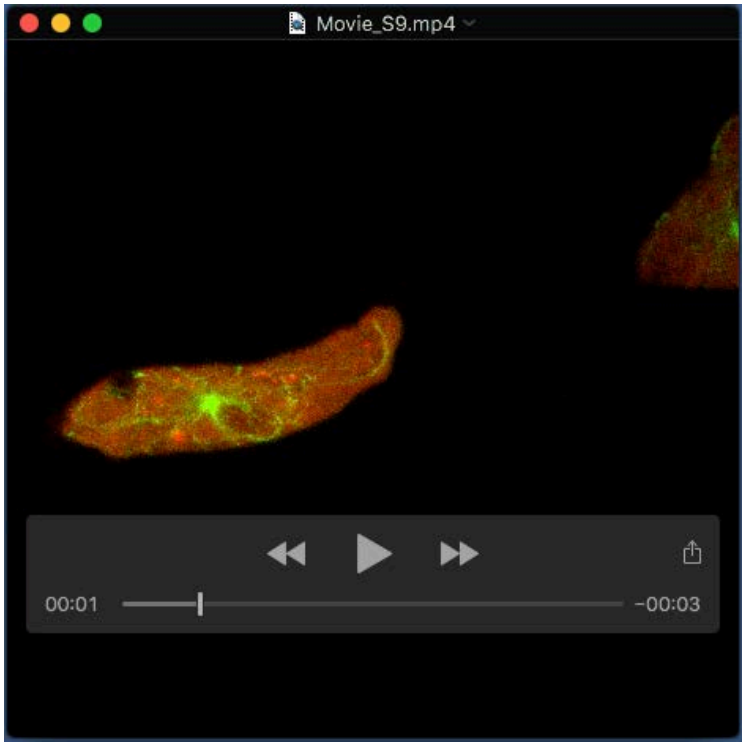
Movie S7. Maximum intensity projection time-lapse images acquired using lattice light-sheet microscopy of ACA-24xMS2/MS2-RFP/GFP-TubA/*aca*$^{-}$ cells treated with Noco (left) and LatA (Right)- Related to Figure 5.

ACA-24xMS2/MS2-RFP/GFP-TubA/*aca*$^{-}$ cells treated with Noco or LatA migrating on a glass coverslip. Red: MS2-RFP; Green: GFP-TubA. Images were presented at 7 fps.



Movie S8. Time-lapse images of ACA-YFP/*aca*$^{-}$ cells self-aggregating in the presence and absence of CilD - Related to Figure 5.

ACA-YFP/*aca*$^{-}$ cells were treated with CilD and allowed to self-aggregate on glass. Images were taken every 30 s and presented at 7 fps.



Movie S9. Maximum intensity projection time-lapse images of ACA-24xMS2/MS2-RFP/GFP-TubA/*aca*$^{-}$ cells treated with CilD - Related to Figure 5.

ACA-24xMS2/MS2-RFP/GFP-TubA/*aca*$^{-}$ cell migrating towards a cAMP source under agarose. Red: MS2-RFP; Green: GFP-TubA. Images were taken every 15 s and presented at 4 fps.