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Supporting Material

Predictive Spatiotemporal Manipulation of Signaling Perturbations Using Optogenetics

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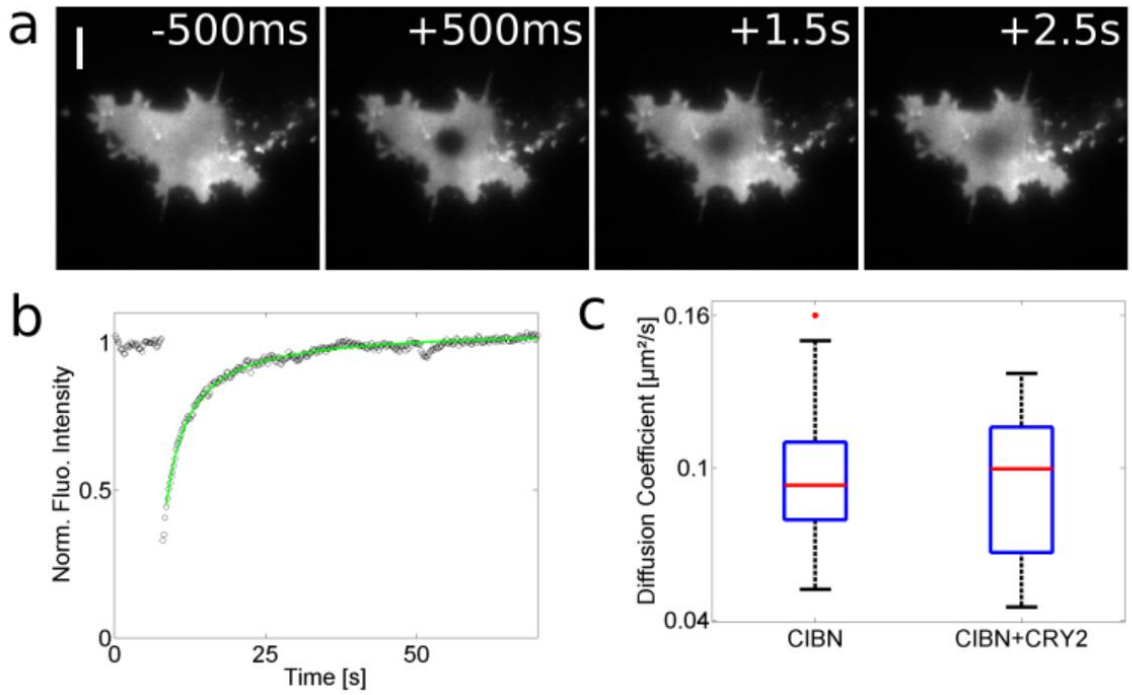
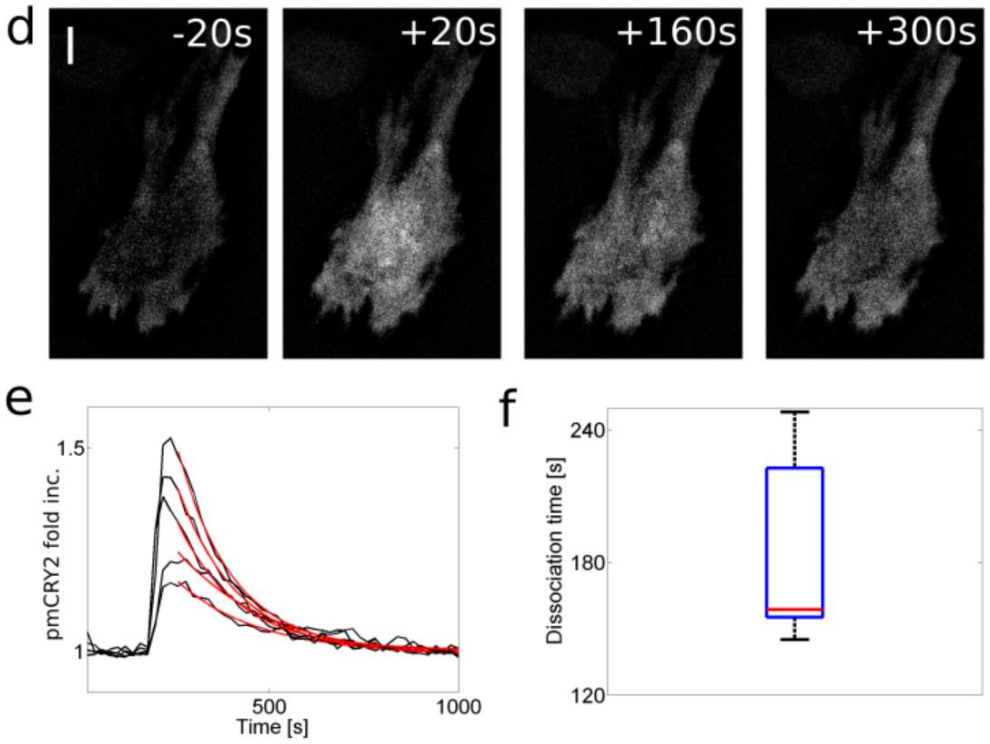
SUPPPLEMENTARY FIGURES



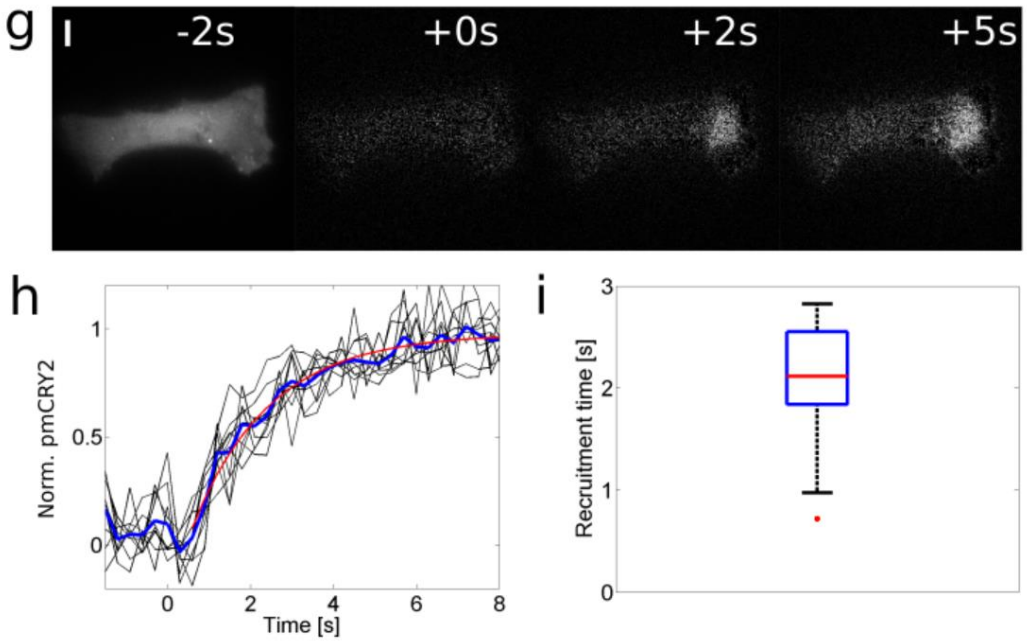
Supplementary Figure 1 (a-c): Determination of lateral diffusion coefficients

(a) TIRF images of CIBN-GFP during FRAP experiments with a bleaching area of 3 µm diameter. From left to right, t=500ms before and t= 500ms, t=1.5s, t=2.5s after photobleaching. Scale bar= 10µm. **(b)** Representative mean TIRF fluorescence intensity in the FRAP area over time (1 image every 0.2s) normalized by the mean of the 10 pre-bleached images and corrected for photobleaching (black curve). In green, fit of the FRAP data according to a diffusion-limited recovery of fluorescence. **(c)** Boxplot of lateral diffusion coefficients of CIBN-GFP-CAAX (N = 26) and CIBN-GFP-CAAX/CRY2-mCherry (N = 14) where the central mark is the median, the edges of the box are the 25th and 75th percentiles and the whiskers extend to the most extreme data points. We obtain a mean value of D = 0.1 µm²/s +/- 0.03 µm²/s and we cannot observe any significant difference between the diffusion of the membrane anchor alone and of the complex.



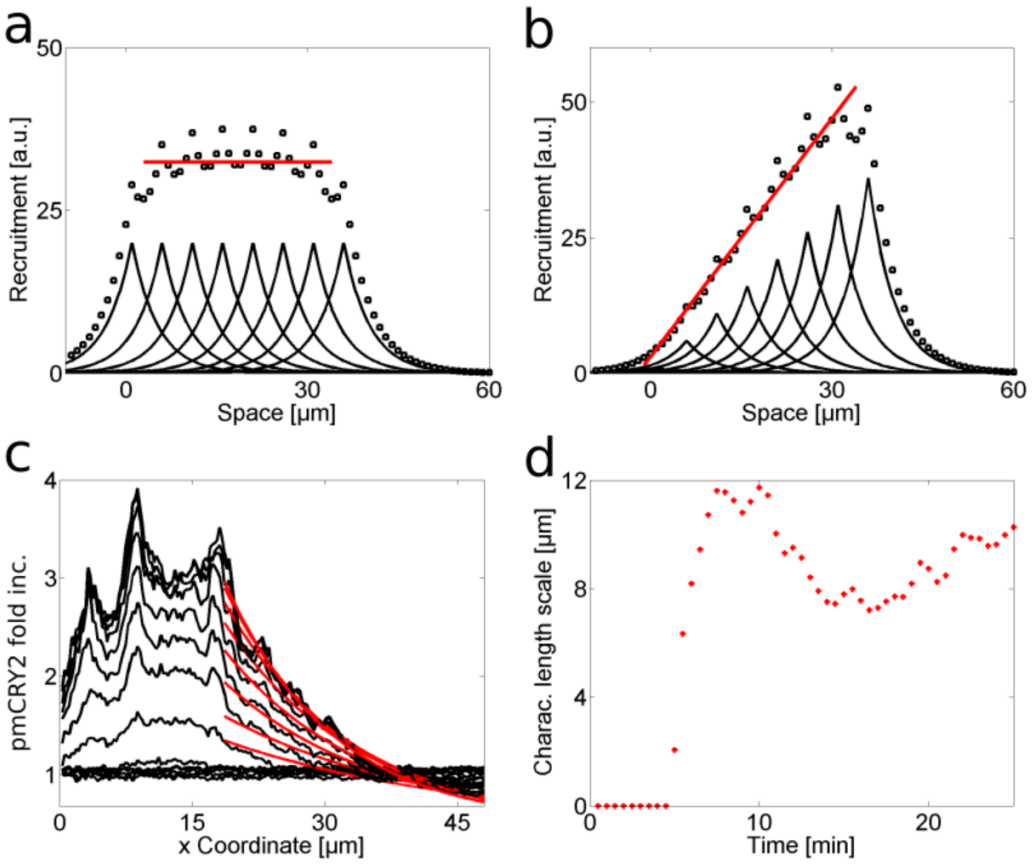
Supplementary Figure 1 (d-f): Determination of dissociation kinetics.

(d) TIRF images of pmCRY2 during a whole cell recruitment assay. From left to right, t=20s before, t= 20s, t=160s, t=300s after photoactivation. Scale bar= 10µm. **(e)** Total TIRF intensity of 5 cells over time minus the initial intensity (black curves). Activation is done between the 9^{th} and 10^{th} frame (t=200s, one frame every 20s). In red, fit of the curves with an exponentially decreasing function according to a simple dissociation model. **(f)** Boxplot of the characteristic time from the fits in (e). The dissociation time is 185s+/-40s.



Supplementary Figure 1 (g-i): Determination of dimerization kinetics.

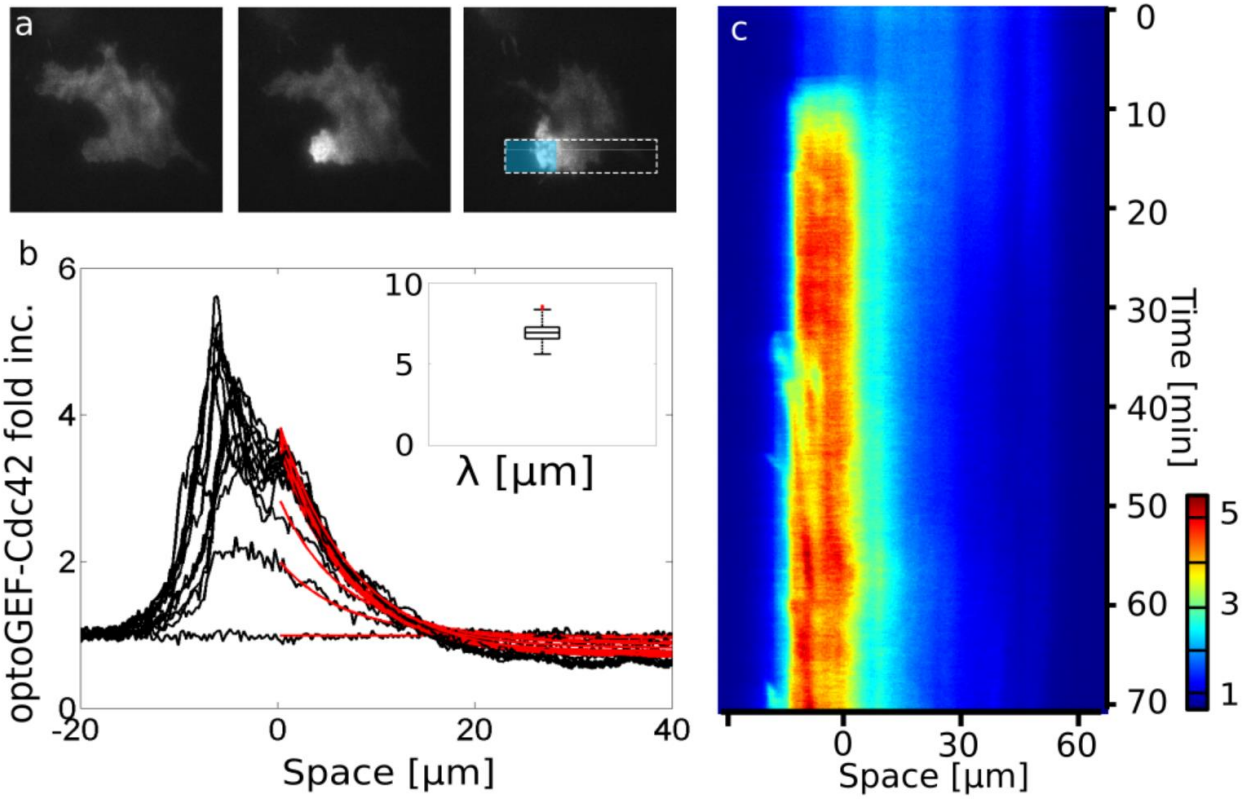
(g) TIRF images of pmCRY2 during a local recruitment assay. From left to right, t=2s before, t= 0s, t=2s, t=5s after photoactivation. Scale bar= 10µm. **(h)** Total TIRF intensity in the activated region over time for N=10 cells (black), average recruitment curve (blue) and exponential fit (red). **(i)** Boxplot of the characteristic time from the fits in (h). The characteristic dimerization time is 2.2s+/-0.4s.



Supplementary Figure 2: Theoretical and experimental distribution of pmCRY2 in response to constant and linear patterns of light.

(a,b) Theoretical pmCRY2 distributions for a constant and a linearly increasing pattern of light. The theoretical pmCRY2 total intensities sampled in space are represented with black dots. They are the sum of the individual responses for every activating points defining the region of activation (black curve) for D = 0.1µm²/s and $\tau = 180$s. **(a)** Response at steady state for a constant pattern of illumination in a restricted segment of space consisting of 8 point-like activations between 0 and 35µm. The linear fit (red line) shows that the response is almost flat between 5 and 30µm. **(b)** Response at steady state for a linear pattern of illumination consisting of 7 point-like activations between 5 and 35µm. The linear fit (red line) shows the linearity of the response between 2 and 30µm

(c,d) Experimental pmCRY2 distribution for a uniform activation in a rectangular region. (c) TIRF signal (black) along the cell long axis observed for a rectangular illumination at different time before and after an activation routine (one activation pulse in a rectangle ROI every 30s). Each black curve is separated by 30s (5 curves before activation and 8 curves after). In red, fit with exponentially decreasing functions of the profiles next to the activation region. **(d)** Evolution in time of the characteristic length of the exponential fits for experiment (c).



Supplementary Figure 3: distribution of optoGEF-Cdc42 for a local photoactivation (related to Figure 6 and Supplementary Movie 5).

(a) TIRF images of a fibroblast before, 5 min, and 25 min after photoactivation in a rectangular ROI (1 pulse every 20s, in the blue region represented in the third image). **(b)** TIRF signal (black) projected along a horizontal line (dashed white rectangle in (a)) at successive time points before and after the activation routine. Each black curve is separated by 2 min (1 curve before activation and 13 curves after). In red, fit with exponentially decreasing functions of the profiles next to the activation region. Inset: boxplot of the decay lengths measured for each time points at steady state (n=118), λ_exp= 7 ± 0.5 µm. **(c)** kymograph of the TIRF signal projected along a horizontal line (dashed white rectangle in (a)).

SUPPPLEMENTARY MOVIES

Movie S1:

Maintenance of a spatially restricted distribution of pmCRY2

TIRF mCherry images of a HeLa cell transfected with CRY2-mCherry and CIBN-GFP and locally activated with 2 times 6 pulses of blue light. The area of activation is localized in the red then in the green region. Scale bar is 10µm.

Movie related to Figure 1.

Movie S2:

pmCRY2 distribution following single pulses of localized light with increasing exposure times

TIRF mCherry images of a HeLa cell transfected with CRY2-mCherry and CIBN-GFP. The area of activation (circle of 12 pixels diameter) is represented each time activating light is shined. Scale bar is 10µm.

Movie related to Figure 2.

Movie S3:

Remote control of pmCRY2 exponential gradients

TIRF mCherry images of a HeLa cell transfected with CRY2-mCherry and CIBN-GFP and plated on round pattern of fibronectin. The area of activation is represented each time blue light is shined. The area of activation is first on the right side of the cell then on the left side of it and finally at both sides. Scale bar is 10µm.

Movie related to Figure 4.

Movie S4:

Maintained pmCRY2 exponential gradient

First panel: TIRF mCherry images of a HeLa cell transfected with CRY2-mCherry and CIBN-GFP and plated on round pattern of fibronectin. The area of activation is represented each time blue light is shined. Scale bar is 10µm. Second panel: kymograph corresponding to the quantification of the gradient along the horizontal axis. The actual time is represented by the moving line. Third panel: instantaneous pmCRY2 gradient (black curve) fitted by an exponentially decaying function (red curve).

Movie related to Figure 4.

Movie S5:

Local recruitment of OptoGEF-Cdc42 in a NIH 3T3 fibroblast

Fibroblast cells transfected with OptoGEF-Cdc42 and CIBN-GFP. The area of activation is represented each time blue light is shined. Left: DIC images. Right: TIRF mCherry images. Scale bar is 10µm.

Movie related to Figure 6.

Movie S6:

Separation of two cells in contact by local recruitment of OptoGEF-Cdc42 in a HeLa cell

DIC image of a HeLa cell transiently transfected with OptoGEF-Cdc42 and CIBN-GFP. The area of activation is represented each time blue light is shined. Scale bar is 10µm.

Movie S7:

Induction of spreading and migration in an initially blebbing HeLa cell by local recruitment of OptoGEF-Cdc42

DIC image of a HeLa cell transiently transfected with OptoGEF-Cdc42and CIBN-GFP. The area of activation is represented each time blue light is shined. Scale bar is 10µm.

Movie S8:

Local recruitment of OptoGEF-Cdc42 in a HeLa cell

HeLa cells transfected with OptoGEF-Cdc42, CIBN-GFP and Histone 2B-iRFP. The area of activation is represented each time blue light is shined. Left: DIC images. Right: TIRF mCherry images (red) and brightfield iRFP signal (blue).

Scale bar is 10µm. Movie related to Figure 7a-c.

Movie S9:

Local recruitment of OptoGEF-Cdc42 in four HeLa cell

HeLa cells transfected with OptoGEF-Cdc42-mCherry, CIBN-GFP and H2B-iRFP for two of them. The area of activation is represented each time blue light is shined. Top: DIC images. Bottom: TIRF mCherry images in red, and brightfield iRFP in blue. Scale bar is 10µm.

Movie related to Figure 7f, g.