Supplementary Information

Manipulation of Endogenous Kinase Activity in Living Cells using Photoswitchable Inhibitory Peptides

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Supplemental Methods

Active Rac pulldown assay.

HEK293 cells were seeded at a density of 1.5 x 10^6 in 60 mm culture dishes. Cells were transfected with the appropriate PA-Rac constructs along with FLAG-tagged mCherry-p21 PBD constructs using Lipofectamine 2000. After 20 – 24 hours of expression, cells were lysed at 4°C in lysis buffer composed of 25 mM Tris pH 7.4, 50 mM NaCl, 1% Triton X-100, and supplemented with protease inhibitors. Lysates were cleared by centrifugation at 3000 x g for 10 minutes and the supernatant removed. Active Rac and FLAG-mCherry-p21 PBD was co-immunoprecipitated using EZview Red anti-FLAG M2 affinity gel (Sigma) in lysis buffer at 4°C in the presence or absence of light for 1.5 hours. Beads were collected by centrifugation at 0.1 x g for 1 minute. The complex was washed three times with lysis buffer, resuspended in sample buffer, resolved by SDS-PAGE, and subjected to immunoblot analysis.

Peptide Studies

Cos-7 cells were transiently transfected with LifeAct-mCherry 20 – 28 hours before imaging. The cells were imaged as described in the main text. Myosin Light Chain Kinase Inhibitor Peptide 18 (EMD Millipore) was dissolved in PBS and added directly to the imaging chamber after 15 minutes of basal acquisition. Cells were imaged for 30 minutes after peptide addition. Myristoylated PKI (14-22; Enzo Life Sciences) was dissolved in purified H_2O. PKI fragment (8-22) was fused to mCherry by polymerase chain reaction and cloned into pmCherry-C1 using AgeI and EcoRI sites. Primer sequences used are as follows: mCherry sense 5' – ACCGGTCGCCACCATGGTGAGCAAGGGCGAGGAGGATAAC – 3', mCherry-PKI

antisense, 5’ –
GAATTCCTAAATAGCATTACGACGACCAGTACGACCAGAAGCAATAAAATCAGCAT
AAGTCTTGTACAGCTCGTCCATGCCGCCGG – 3’.

Supplemental Figures

Figure S1. Incorporation of PKI into the Jα loop region perturbs the photoresponse of PA-Rac. (A) Crystal structure of PA-Rac*1* showing the PAS fold of LOV2 (blue), the Jα helix (yellow), and Rac (red). The site of incorporation of the PKI peptide is indicated by the arrowhead. (B) Schematic showing the biochemical assay used to determine Rac activation. Upon irradiation, the Jα helix unfolds, allowing Rac to bind to target effector domains. A FLAG-tagged CRIB domain from p-21 activated kinase is used to bind active Rac. (C) Western blot showing the results of the pulldown assay. Whereas little PA-Rac is pulled down in the dark, this response is robust upon irradiation of cell lysate (Left panels). In contrast, when the peptide is inserted, response is lost in the light, the dark, or when using mutants mimicking the dark-state (C450A) or the light-state (I539E).

Figure S2. PA-PKI inhibits PKA signaling comparably to existing reagents. (A) Western blot showing levels of phosphorylated PKA substrates in HEK293 cells after the indicated treatments. PKA activity was stimulated with forskolin for 1.5 hours. Cells expressing PA-PKI were irradiated for 15 minutes prior to forskolin addition. Transfections were performed 24 hours prior to the experiment. (B) Quantification of (A), n=3, *p<0.01, Student's t-test.

Figure S3. PA-MKI activation does not perturb membrane retraction. Box plots showing normalized distributions of retraction persistence (duration of retraction phase; red boxes) and maximum velocity per retraction event (green boxes) for cells expressing LOV2. The three plots represent normalized distributions of the >75th (left), < 25th (center), and total (right). Boxes within each plot represent the distributions before (left; gray fill), during (center; white fill), and after (right; gray fill) light stimulation. Data was derived from n = 6 cells expressing PA-PKI.

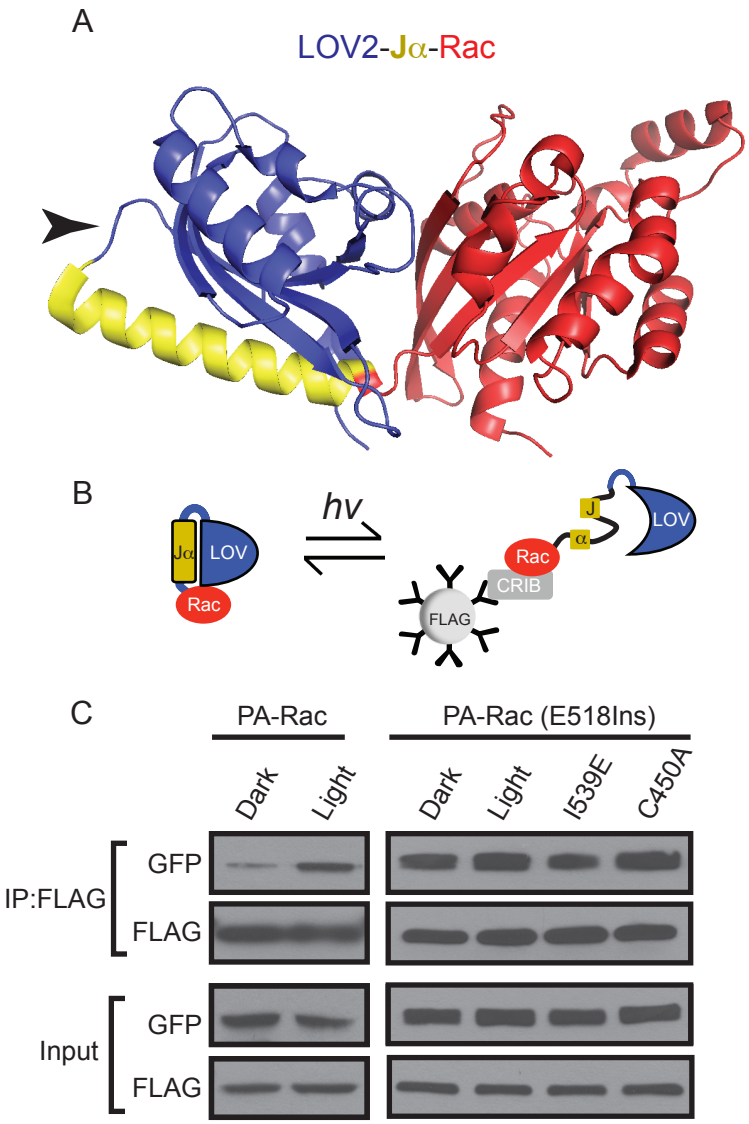
Figure S4. Cell permeable MKI peptide also diminishes cell protrusion velocities. Box plots showing normalized distributions of protrusion persistence (duration of protrusion phase; red boxes) and maximum velocity per protrusion event (green boxes) in cells expressing LifeAct-mCherry. The three plots represent normalized distributions of the >75th (left), < 25th (center), and total (right). Boxes within each plot represent the distributions before (left), first 15 minutes

after MKI treatment (center), and second 15 minutes after MKI treatment (right). (A – C) Treatment with 1 μM MKI. (D – F) Treatment with 10 μM MKI. (G – I) Treatment with 100 μM MKI. Data was derived from n = 6 cells expressing PA-PKI. n = 7, 1 μM; n = 8, 10 μM; n = 11, 100 μM, *$p < 0.001$, **$p < 0.03$, Anderson-Darling test.

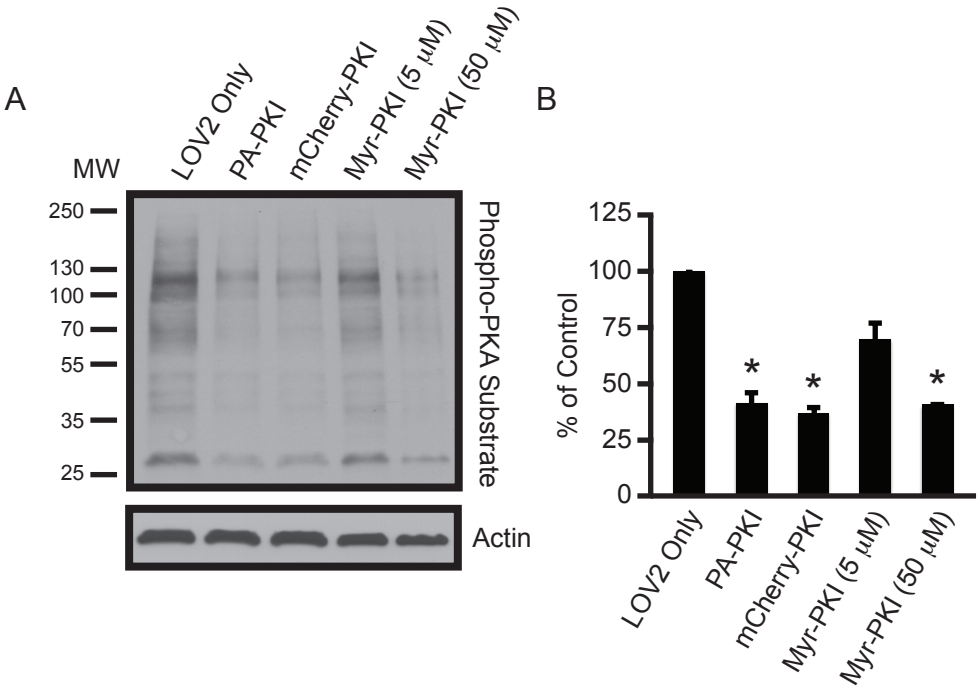
References

1. Wu, Y. I., Frey, D., Lungu, O. I., Jaehrig, A., Schlichting, I., Kuhlman, B., and Hahn, K. M. (2009) A genetically encoded photoactivatable Rac controls the motility of living cells, *Nature 461*, 104-108.

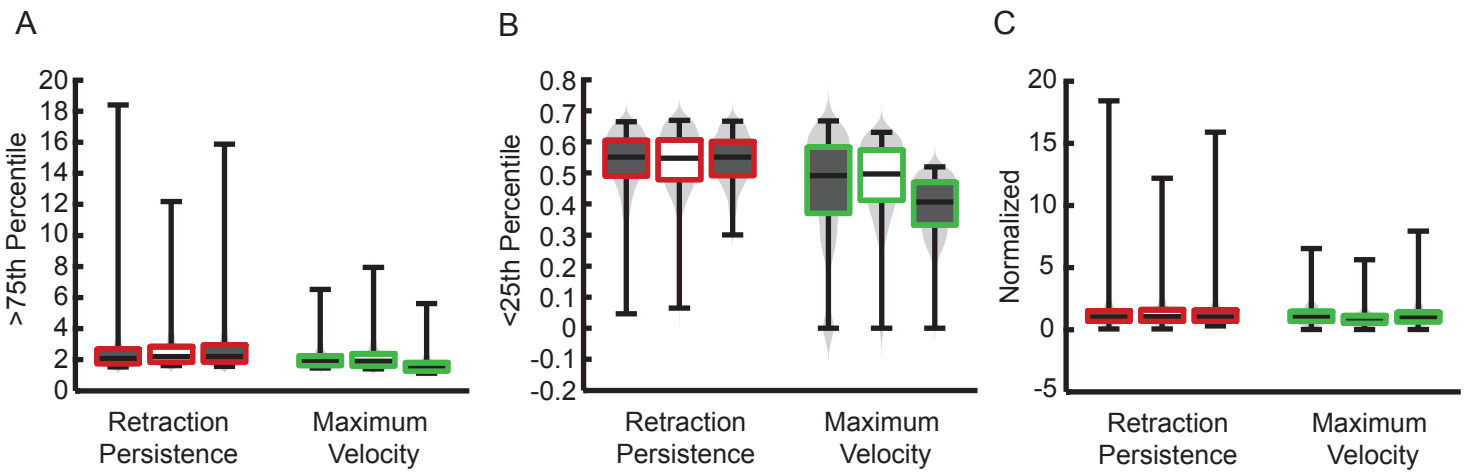
Yi et al., Supplemental Figure 1



Yi et al., Supplemental Figure 2



Yi et al., Supplemental Figure 3



Yi et al., Supplemental Figure 4