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Optogenetic Rac1 engineered from membrane lipid-binding RGS-LOV for inducible lamellipodia formation

Erin E. Berlew, Ivan A. Kuznetsov, Keisuke Yamada, Lukasz J. Bugaj, and Brian Y. Chow *

Department of Bioengineering, University of Pennsylvania, 210 South 33rd Street, Philadelphia, PA 19104, United States; (*) Correspondence: bchow@seas.upenn.edu

SUPPLEMENTARY FIGURES

Domain annotations:

BcLOV4 (1-596)

- RGS domain: 124-234
- LOV domain: 256-356
- Amphipathic helix: 404-417
- DUF domain: 427-567

wild-type Rac1: 606-795

mCherry: 804-1039

Linkers: 597-604, 796-803

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MATDAIDHYY QNRDGPPSRS FSDEYDSYNK TAPLPPLSAC PERAQTPNSK RIPITPNSAK    60
STSESPRRIT FSRDAGSSTK GSTQTSPSNQ GSVSSRNGSN GNGLGLSSAG NFADFFSSEV   120
FYIVLHNPTT AHRFLKFCQS RACSENLEFL QKIDEYNRLI DEATRLLSTI YSTYVSSEAP   180
KQINISNSHA RRLSHDIKGA THNVLPGLED IFNGSQEQIE KLLASDLYPR FVKHQVTASA   240
TMALANDKER YPGLGDCFCL TDPRRADNPI VFASDGFVSV TGYSRSDIIP RNCRFLQGSF   300
TDRQATKRLR TSIENREETV ELLLNYRKNG DPFWNLLYVS PLLDGNGDVR FFLGGQINCS   360
TTIHSRTDVL RILSLNDEDT GSFIEGSNKT PSVRSKESNN ASNYGKTSFF KSFKKYKANT   420
VEIRDEAGME GELIDRIGKL QFKTQVEEFY TAYSKYLVLS YQPQSQNLIV KYFSPGIVDM   480
LNLNMPNGLV APIVDKDIFK VLTEHSPSSV PRTFKNVVRE GIKAGRAVSV ETGLLTGIEE   540
IKKSQNIFSG TTANRERGWK KAEEKYVCHF TPCKDEMGRV GWVVLTVAPK LERFEFGGGS   600
GGGSMQAIKC VVVGDGAVGK TCLLISYTTN AFPGEYIPTV FDNYSANVMV DGKPVNLGLW   660
DTAGQEDYDR LRPLSYPQTD VFLICFSLVS PASFENVRAK WYPEVRHHCP NTPIILVGTK   720
LDLRDDKDTI EKLKEKKLTP ITYPQGLAMA KEIGAVKYLE CSALTQRGLK TVFDEAIRAV   780
LCPPPVKKRK RKCLLGGGSG GGSMVSKGEE DNMAIIKEFM RFKVHMEGSV NGHEFEIEGE   840
GEGRPYEGTQ TAKLKVTKGG PLPFAWDILS PQFMYGSKAY VKHPADIPDY LKLSFPEGFK   900
WERVMNFEDG GVVTVTQDSS LQDGEFIYKV KLRGTNFPSD GPVMQKKTMG WEASSERMYP   960
EDGALKGEIK QRLKLKDGGH YDAEVKTTYK AKKPVQLPGA YNVNIKLDIT SHNEDYTIVE  1020
QYERAEGRHS TGGMDELYK                                              1039
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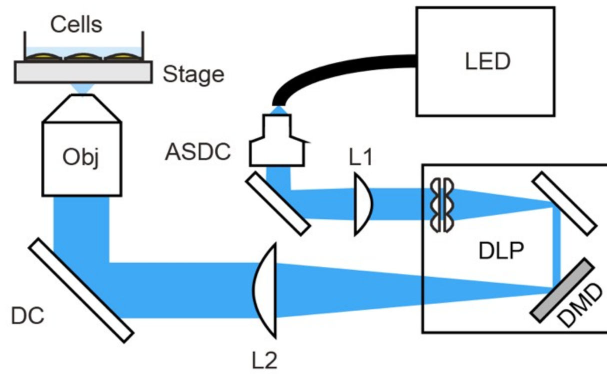
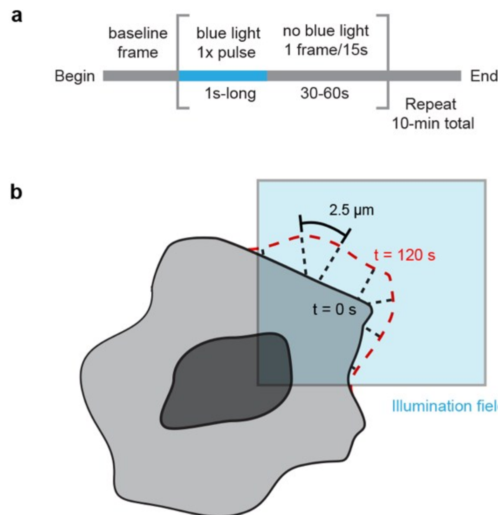
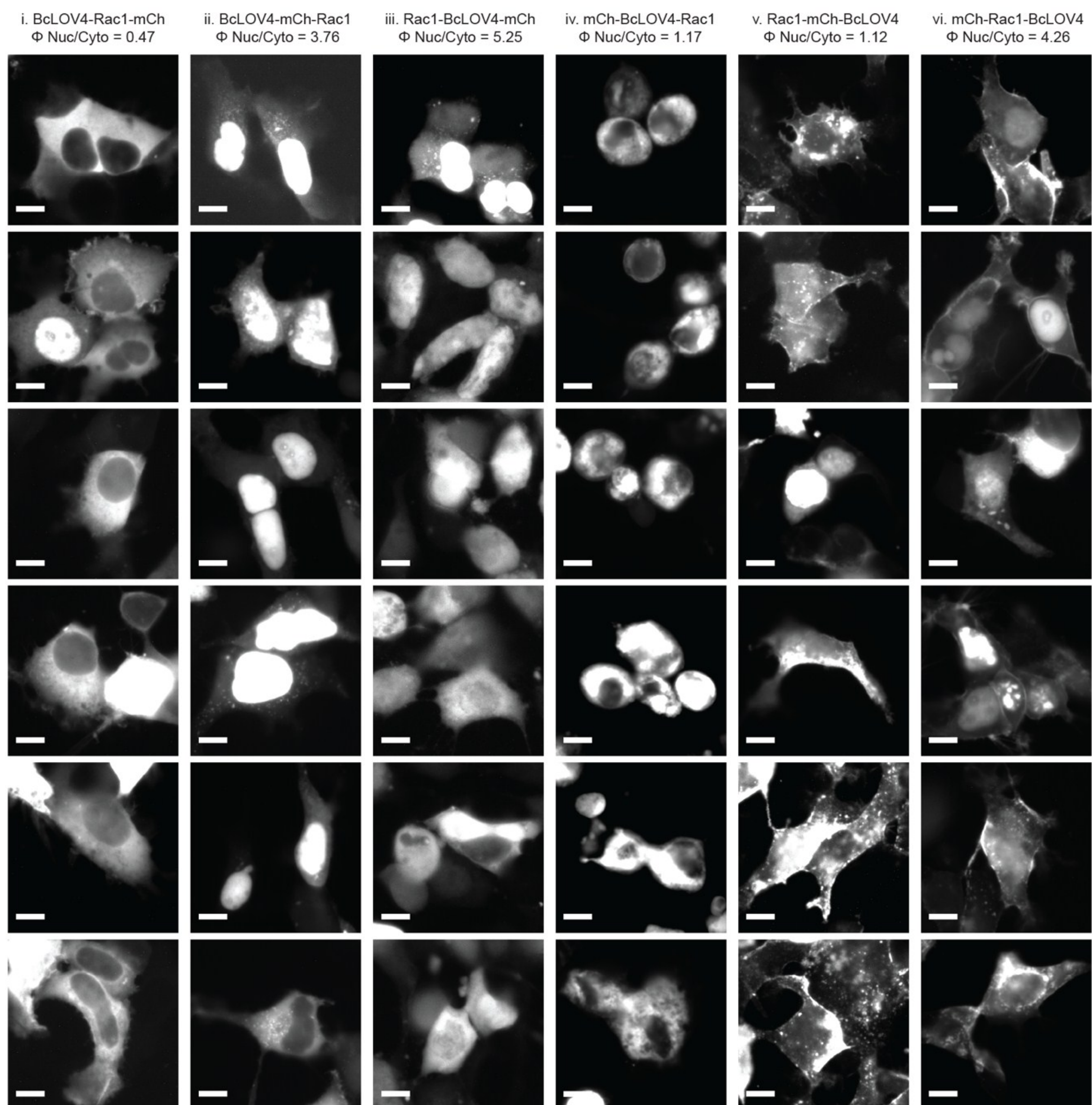
Supplementary Figure 1. Opto-Rac1 protein sequence.



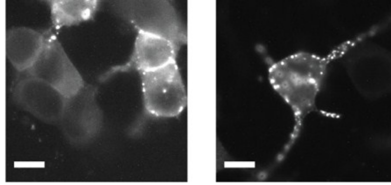
Supplementary Figure 2. **Structured illumination schematic**. Light from a liquid light guide-coupled λ = 455 nm LED was collimated into the (DMD) digital micromirror device-based DLP (digital light processor, Digital Light Innovations CEL5500) via an air-spaced doublet collimator (ASDC, Thorlabs F810APC-405) and a plano-convex lens (L1, f = 200 mm). After the DLP the projected illumination pattern was infinity-corrected using another plano-convex lens (L2, f = 150 mm). The light was then redirected to the objective (Obj, 63x / 1.40 NA, Leica #506187) using a shortpass dichroic (DC, λ < 900 nm, Thorlabs DMLP900R) mounted in a custom K type laser cube (Nuhsbaum). The LED was from Mightex (LCS-0455-3-22).



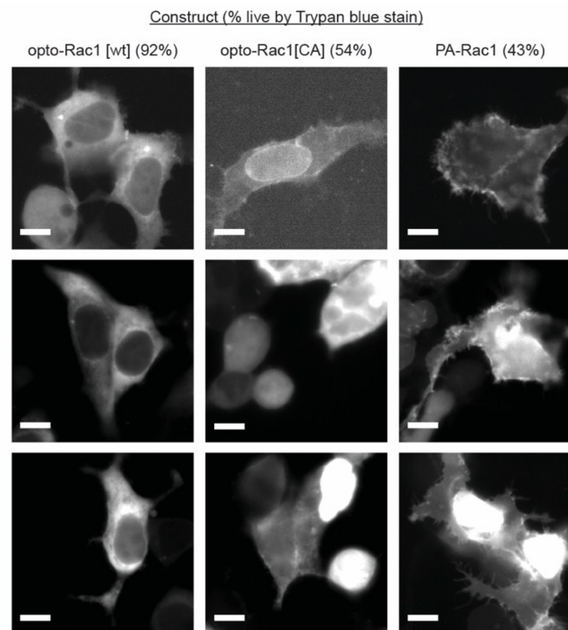
Supplementary Figure 3: Summary of optogenetic assays and analysis. (a) Stimulation epochs using a digital micromirror device to spatially pattern illumination fields. **(b)** Analysis methodology to determine distance moved by cell. The cell border was manually segmented at 0 seconds post-illumination (black solid border) and at 120 seconds post-illumination (red dashed line). The distance moved by the cell was defined as the mean length of line segments (black dashed line) that were normal to the cell border at 120 seconds, with one endpoint at the t = 0 border and the other endpoint at the t = 120 s border. Line segments were spaced at 2.5 µm intervals along the t = 120 s border.



Supplementary Figure 4. Representative distribution of expression profile in HEK cells of domain arrangements screened in engineering opto-Rac1. Fluorescence micrographs of transfected HEK cells in the dark-adapted state. (ϕ Nuc/Cyto) = ratio of observed average fluorescence intensity in the nucleus vs. cytoplasm (N = 25 – 35 each). The dominant preference for nuclear localization of genetic constructs ii. and iii., plus their lack of plasma membrane localization, indicates that their nuclear localization is not a consequence of cell cycle phase or sequestration by the plasma membrane. Scale = 10 μm.



Supplementary Figure 5. Fluorescence micrographs of mKoKappa-BcLOV4 expressed in HEK cells. The BcLOV4 fusion with mKoKappa as a signaling inert N-terminal protein is aggregated and membrane localized in the dark-adapted state, consistent with opto-Rac1 domain arrangements screened with C-terminal BcLOV4. Scale = 10 μm.



Supplementary Figure 6: Expression of constitutively active Rac1 constructs in HEK cells. Multiple representative cells were imaged in the dark-adapted state. Constitutively active [CA] Rac1 fused to BcLOV4 reduced cell health based on Trypan Blue live/dead cell count, and compromised morphology with evidence of spurious activity in the dark-adapted state. The top-center image, chosen to demonstrate permanent membrane localization in non-rounded cells, shows high background after auto-contrast because of dimmer raw fluorescence. For reference, photoactivatable Rac1 (PA-Rac1), a fusion of AsLOV2 and constitutively active Rac1, shows spurious sheet-like protrusions in the dark-adapted state under similar laboratory conditions. Scale bar = 10 μm.

[For Video File]

Supplementary Video 1. Focal induction of lamellipodia formation in opto-Rac1 transducing cells in response to spatially patterned illumination. Field of view = 60 x 60 μm. Box = Illumination field. λ = 455 nm @ 15 mW/cm^2, 1.6% duty ratio.