Supplemental Materials

Molecular Biology of the Cell

Vitriol et al.

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Supplementary Figure 1. Fluorescence Recovery After Photobleaching (FRAP) analysis of cofilin-KR constructs.

Supplementary Figure 2. CALI of cofilin-mRFP is less effective than CALI of cofilin-KR.

Supplementary Figure 3. Automated image analysis of peripheral F-actin.

Supplementary Figure 4. CALI of cofilin$_{S3A}$ increases EGFP-actin localization to the lamellipodia.

Supplementary Figure 5. CALI of cofilin-KR, cofilin$_{S3E}$-KR, or KR by itself does not increase F-actin in the lamellipodia.

Supplementary Figure 6. Semi-automated image analysis of peripheral F-actin using kymographs.

Supplementary Figure 7. Kymograph analysis of F-actin before and after CALI of cofilin-KR constructs.

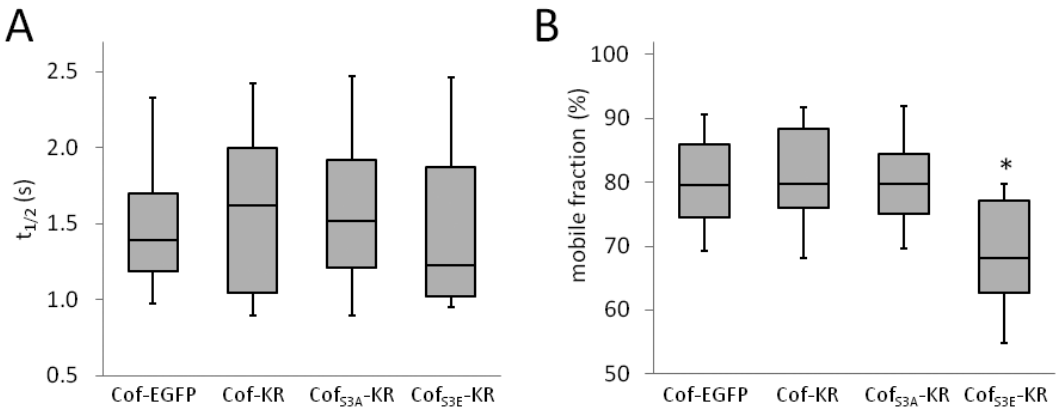
Supplementary Figure 8. Pre-CALI F-actin profiles of cells expressing different cofilin-KR constructs.

Supplementary Movie 1. Image sequence showing Lifeact-EGFP before and after CALI of cofilin$_{S3A}$-KR.

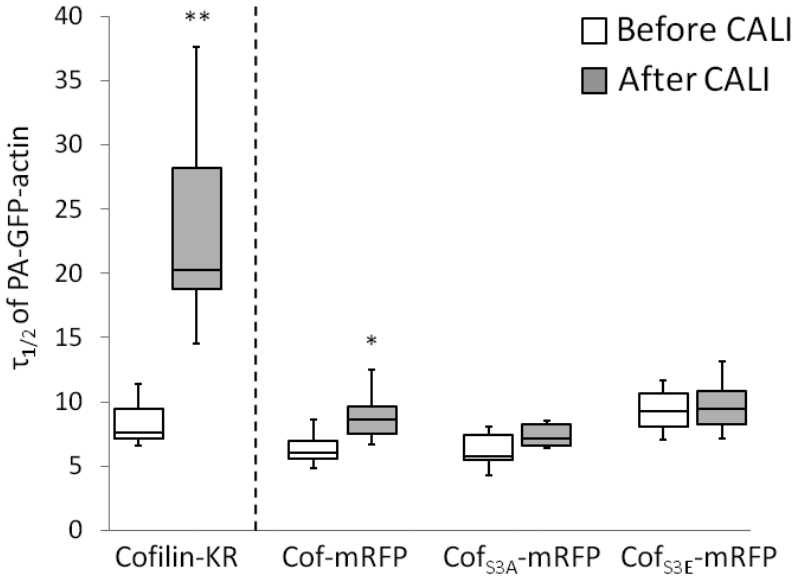
Supplementary Movie 2. Image sequence showing PA-GFP-actin before and after CALI of cofilin-KR. The images are psuedocolored to emphasize changes in fluorescent intensity.

Supplementary Movie 3. 3D-SIM image sequence showing Lifeact-EGFP before and after CALI of cofilin-KR in cofilin-KD cells.

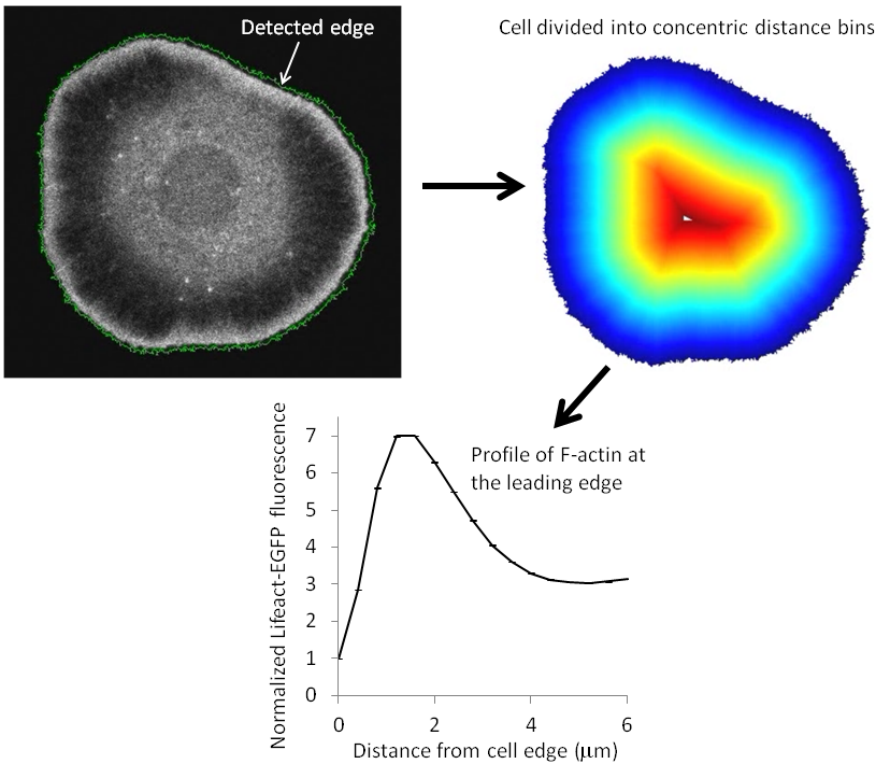
Supplementary Figures – Vitriol et al.



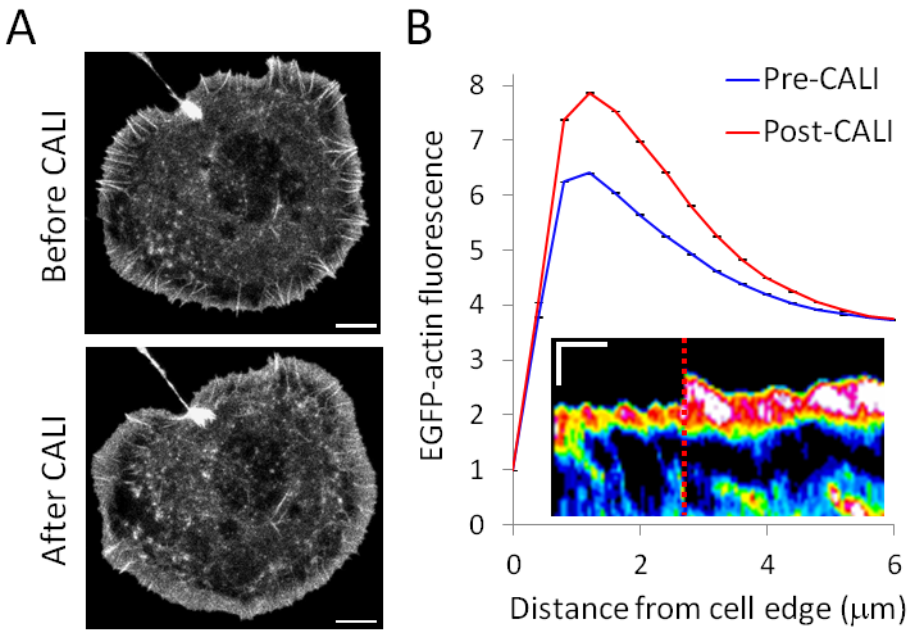
Supplementary Figure 1. Fluorescence Recovery After Photobleaching (FRAP) analysis of cofilin-KR constructs. (**A**) Box and whisker plots showing $t_{1/2}$ values and (**B**) mobile fractions for cofilin constructs used in this study. There was a significant decrease in the $cofilin_{S3E}$-KR mobile fraction ($p < 0.01$, student's t-test), otherwise all values were identical. Plots denote 95th (top whisker), 75th (top edge of box), 25th (bottom edge of box), and 5th (bottom whisker) percentiles and the median (bold line in box). The number of cells used for this analysis is as follows: cof-EGFP (20), cof-KR (20), cof_{S3A}-KR (19), and cof_{S3E}-KR (16).



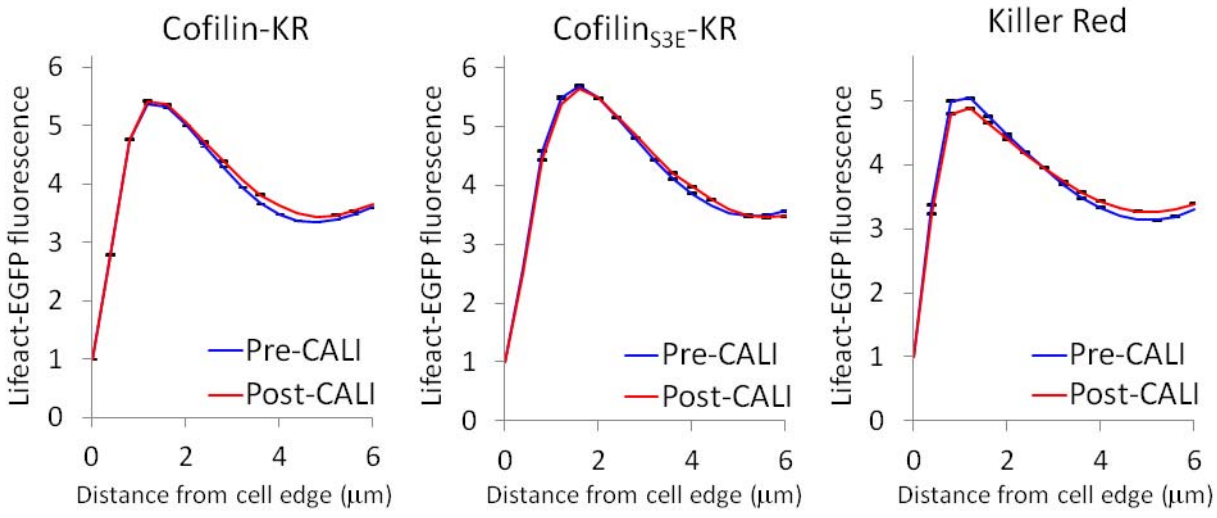
Supplementary Figure 2. CALI of cofilin-mRFP is less effective than CALI of cofilin-KR. PA-GFP-actin was photoactivated in a 10µm circular region of the cytoplasm before and after CALI. The box and whisker plot shows $\tau_{1/2}$ of PA-GFP-actin in cells expressing cofilin-KR and and cofilin-mRFP constructs before and after CALI. Though there is a significant decrease in actin mobility after CALI of cofilin-mRFP, the effect is reduced from a 271% change (8.0 ± 1.3 to 21.7 ± 7.0 sec average time to reach $\tau_{1/2}$ for cofilin-KR) to a 33% change (6.0 ± 0.9 to 8.2 ± 1.3 sec average time to reach $\tau_{1/2}$ for cofilin-mRFP). There was also no effect after CALI of $cofilin_{S3A}$-mRFP or $cofilin_{S3E}$-mRFP. Plots denote 95th (top whisker), 75th (top edge of box), 25th (bottom edge of box), and 10th (bottom whisker) percentiles and the median (bold line in box). Double asterisk indicates $p < 0.0001$, single asterisk indicates $p < 0.05$ (student's t-test). The number of cells used for this analysis is as follows: cof-KR (9), cof-mRFP (11), cof_{S3A}-mRFP (10), and cof_{S3E}-mRFP (11).



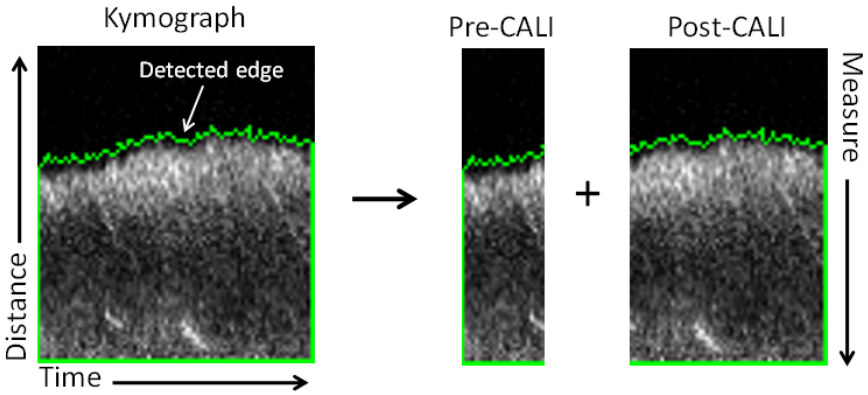
Supplementary Figure 3. Automated image analysis of peripheral F-actin. From a movie of Lifeact-EGFP, the edge is automatically detected (left). The movie is separated into Pre- and Post-CALI frames. Concentric lines of variable width ("distance bins") are drawn, beginning at the edge of the cell and then inward to the center (right). The average fluorescence intensity of each distance bin is obtained, and the result is an intensity profile of EGFP-Lifeact (bottom). Intensity profiles are normalized to the value of the first distance bin. Pre- and Post-CALI intensity profiles are averaged together for all movies.



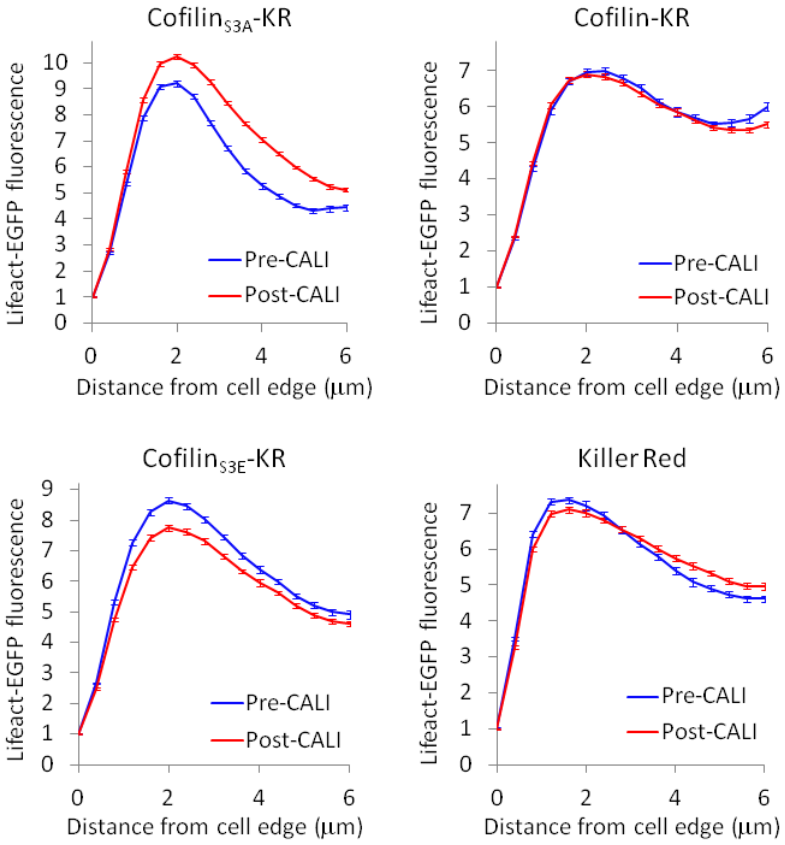
Supplementary Figure 4. CALI of cofilin$_{S3A}$ increases EGFP-actin localization to the lamellipodia. (**A**) Representative images of EGFP-actin before and after CALI of cofilin$_{S3A}$-KR in a CAD cell. Scale bars are 10 μm. (**B**) Average leading edge profile of EGFFP-actin before and after CALI of cofilin$_{S3A}$-KR (n = 5 cells). After CALI, there is a significant increase in EGFP-actin fluorescence. Error bars represent 95% confidence intervals. The graph inset shows a representative kymograph of EGFP-actin from a cofilin$_{S3A}$-KR CALI experiment. The kymograph is psuedocolored to emphasize changes in fluorescent intensity. Time of CALI is indicated with a dotted red line. Kymograph scale bar is 3μm (vertical) and 3min (horizontal).



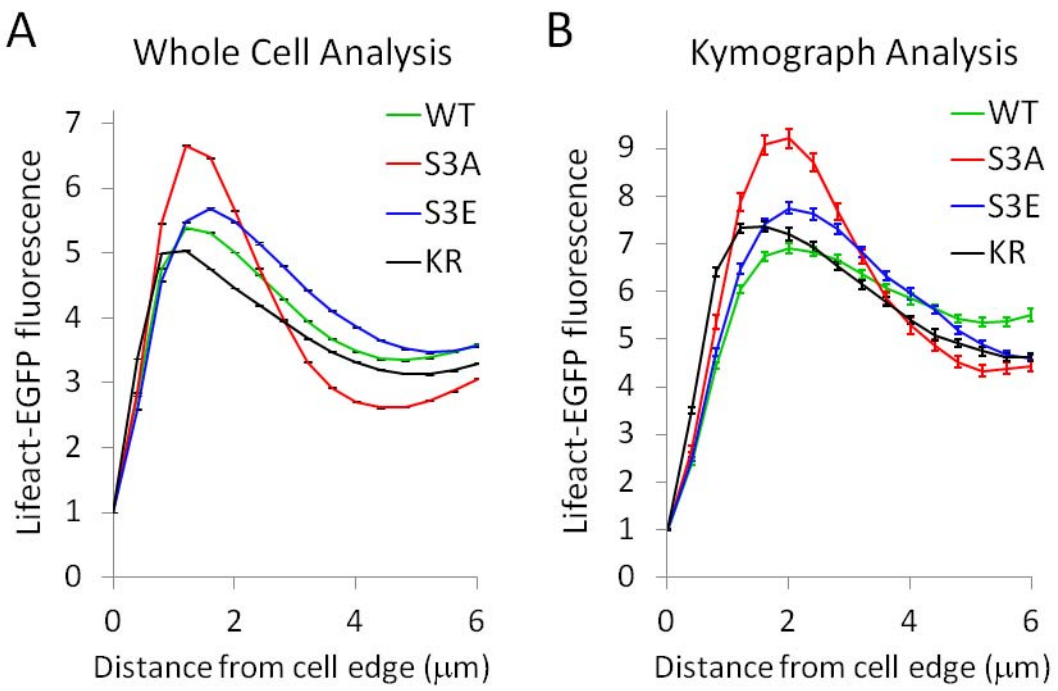
Supplementary Figure 5. CALI of cofilin-KR, cofilin$_{S3E}$-KR, or KR by itself does not increase F-actin in the lamellipodia. Cells were co-transfected with Killer Red constructs and Lifeact-EGFP to label F-actin and analyzed as described in Supplementary Figure 3. Average leading edge profile of Lifeact-EGFP before and after CALI of cofilin-KR (n = 5 cells), cofilin$_{S3E}$-KR (n = 6 cells), and Killer Red by itself (n = 7 cells). Error bars represent 95% confidence intervals.



Supplementary Figure 6. Semi-automated image analysis of peripheral F-actin using kymographs. A kymograph is extracted from a movie of Lifeact-EGFP from a CALI experiment. The edge is automatically detected. The kymograph is separated into Pre- and Post-CALI sections and individual intensity scans are generated starting from the edge going toward the cell body. Intensity values are obtained from manually set "distance bins." Intensity profiles are normalized to the value of the first distance bin. Pre- and Post-CALI intensity profiles are averaged together for all kymographs.



Supplementary Figure 7. Kymograph analysis of F-actin before and after CALI of cofilin-KR constructs. Cells were co-transfected with Killer Red constructs and Lifeact-EGFP to label F-actin and analyzed as described in Supplementary Figure 5. Average leading edge profile of Lifeact-EGFP before and after CALI of cofilin$_{S3A}$-KR (n = 11 cells, 60 kymographs), cofilin-KR (n = 10 cells, 62 kymographs), cofilin$_{S3E}$-KR (n = 11 cells, 74 kymographs), and Killer Red by itself (n = 7 cells, 52 kymographs). CALI of cofilin$_{S3A}$-KR shows a large and significant increase in F-actin, while CALI of cofilin$_{S3E}$-KR shows a small decrease in F-actin. There was no change in F-actin amount after CALI of cofilin-KR or Killer Red by itself. Error bars represent 95% confidence intervals.



Supplementary Figure 8. Pre-CALI F-actin profiles of cells expressing different cofilin-KR constructs. Cells were co-transfected with cofilin-KR constructs and Lifeact-EGFP to label F-actin and analyzed as described in Supplementary Figure 3 (Whole Cell Analysis) or Supplementary Figure 7 (Kymograph Analysis). Error bars represent 95% confidence intervals. The number of cells measured for Whole Cell Analysis were: WT (n = 5 cells), S3A (n = 6 cells), S3E (n = 6 cells), and KR (n = 7 cells). The number of cells measured for Kymograph Analysis were WT (n = 10 cells, 62 kymographs), S3A (n = 11 cells, 60 kymographs), S3E (n = 11 cells, 74 kymographs), and KR (n = 7 cells, 52 kymographs).