Supplementary information

Documentation and Localization of Force-mediated Filamin A Domain Perturbations in Moving Cells

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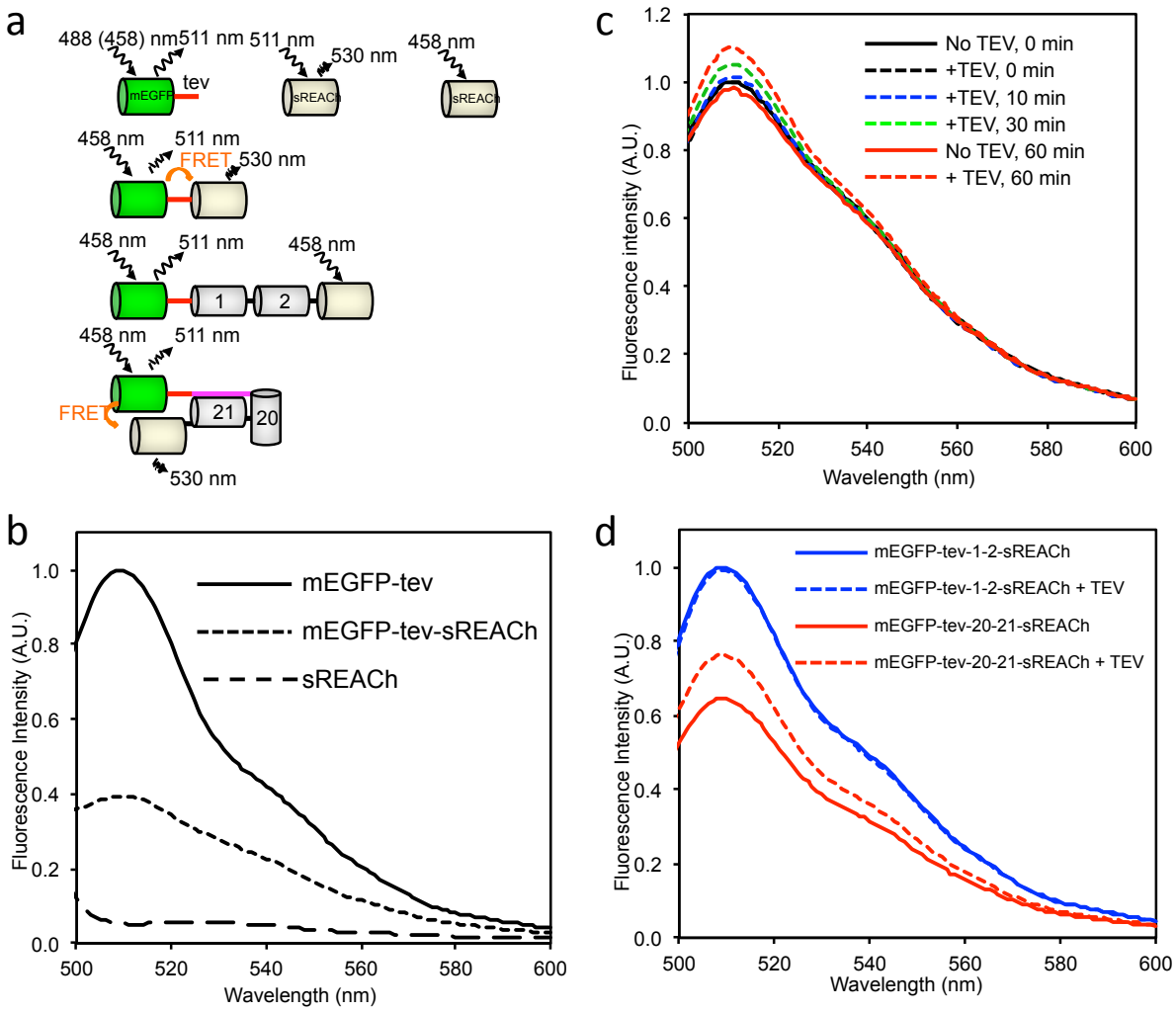
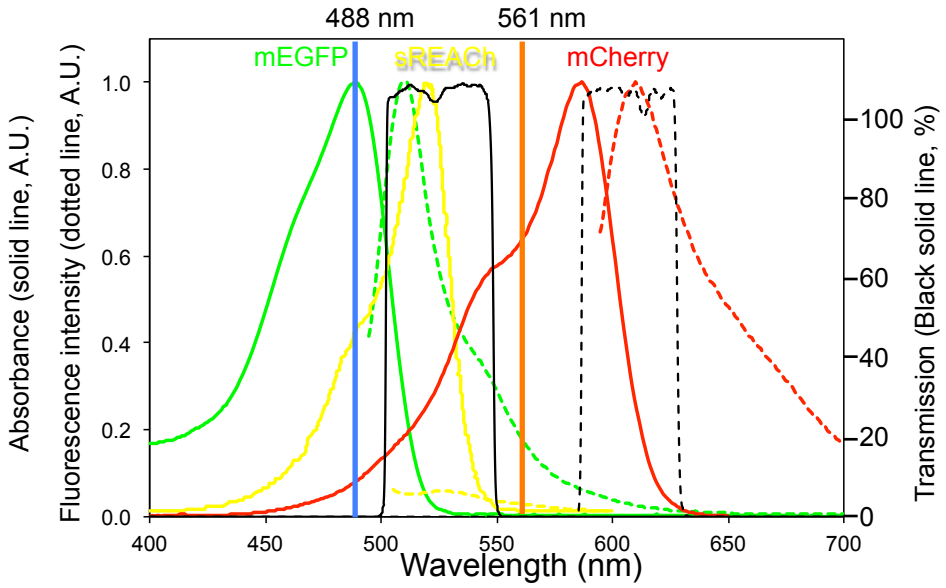
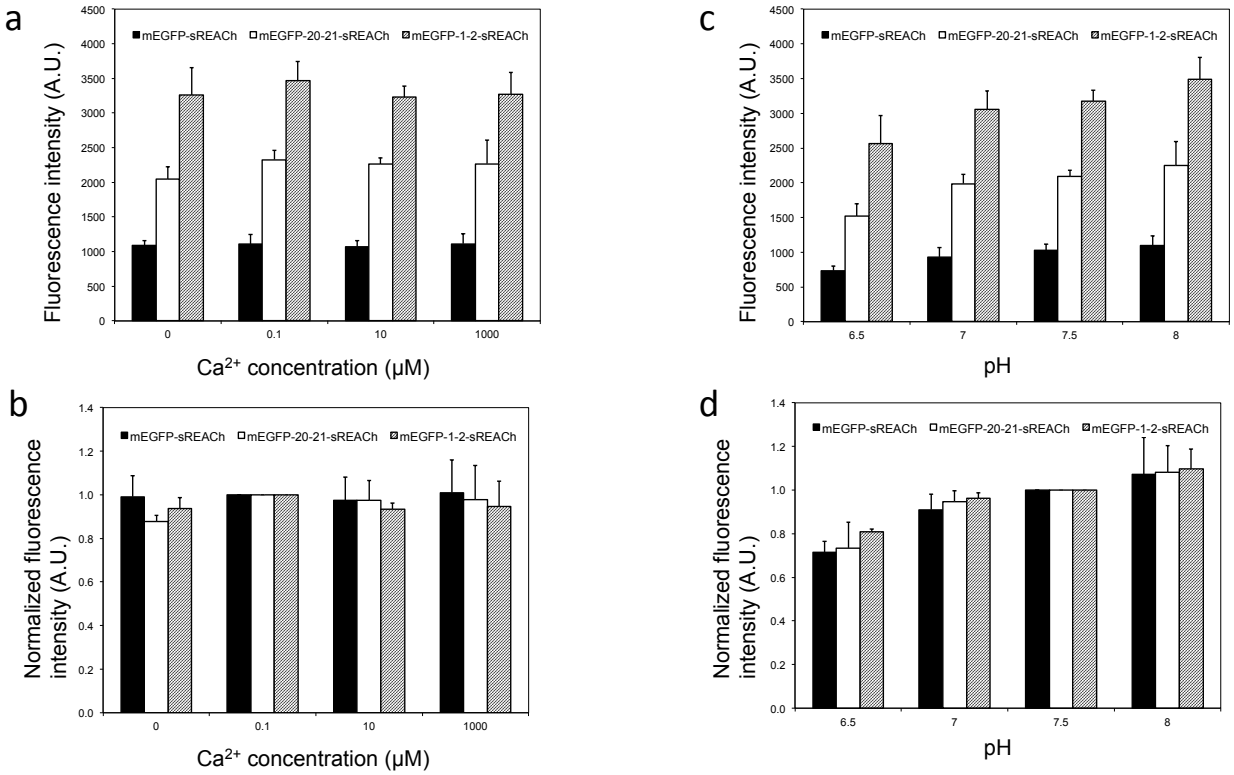


Supplementary Fig. 1. Spectrofluorimetric analysis of recombinant FLNA conformation sensor constructs.

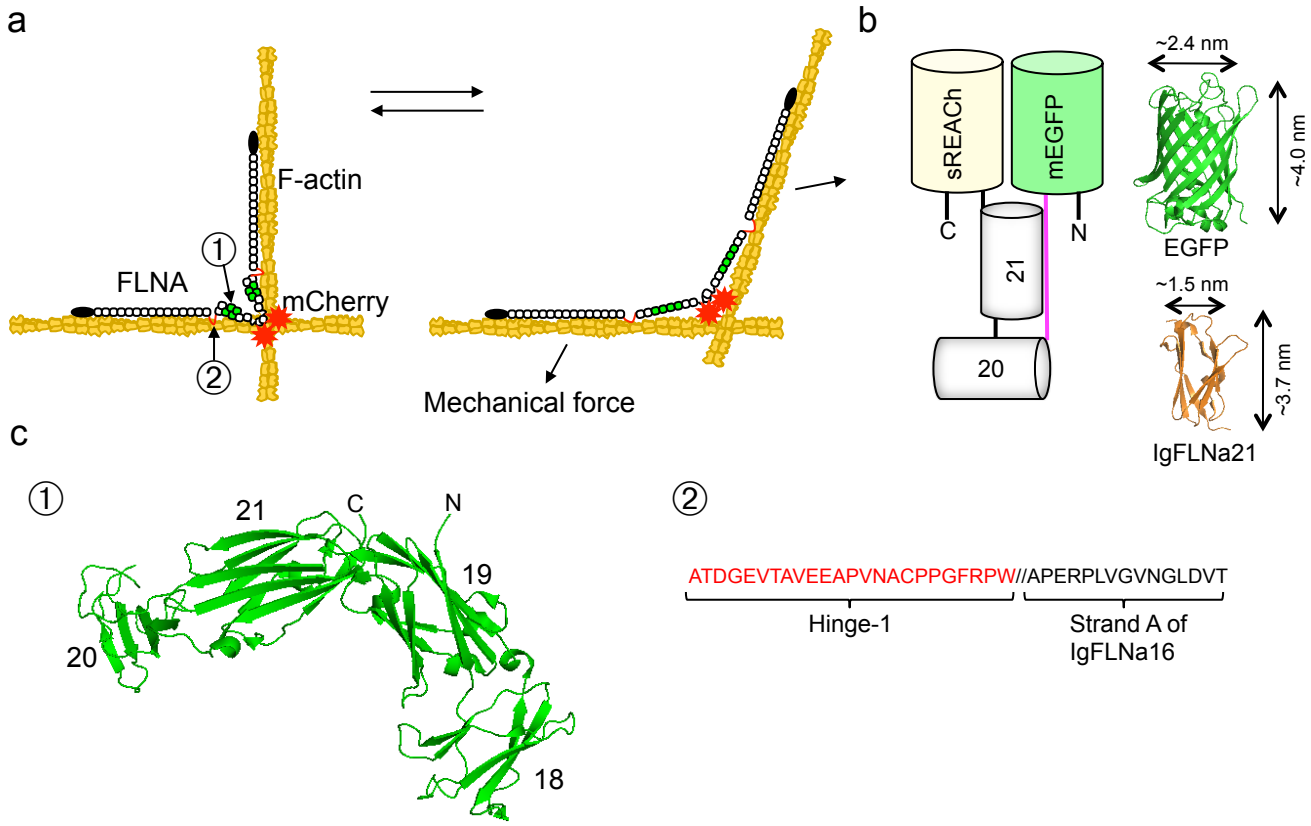
(**a**) Schematic representation of the PQ-FRET pair separated by different FLNA repeats. TEV protease-recognition sequence (ENLYFQ/G, "tev" in red) is inserted at the C-terminal of mEGFP. The strand A of repeat 20 is indicated in blue. (**b**) Emission spectra of recombinant PQ-FRET fluorophores (5 µM each). (**c**) Emission spectra of recombinant mEGFP-tev-sREACh construct before and after incubation with TEV protease. The constructs were incubated with or without 0.1 µg of TEV protease per µg of protein for the times indicated. (**d**) Emission spectra of the PQ-FRET pair separated by FLNA repeats before and after incubation with TEV protease. The constructs (5 µM each) were incubated with or without 0.1 µg of TEV protease per µg of protein for 30 min. (**b**-**d**) The emission spectra are shown for excitation at 457 nm and normalized. Fluorescence intensities are given in arbitrary units (A.U.).



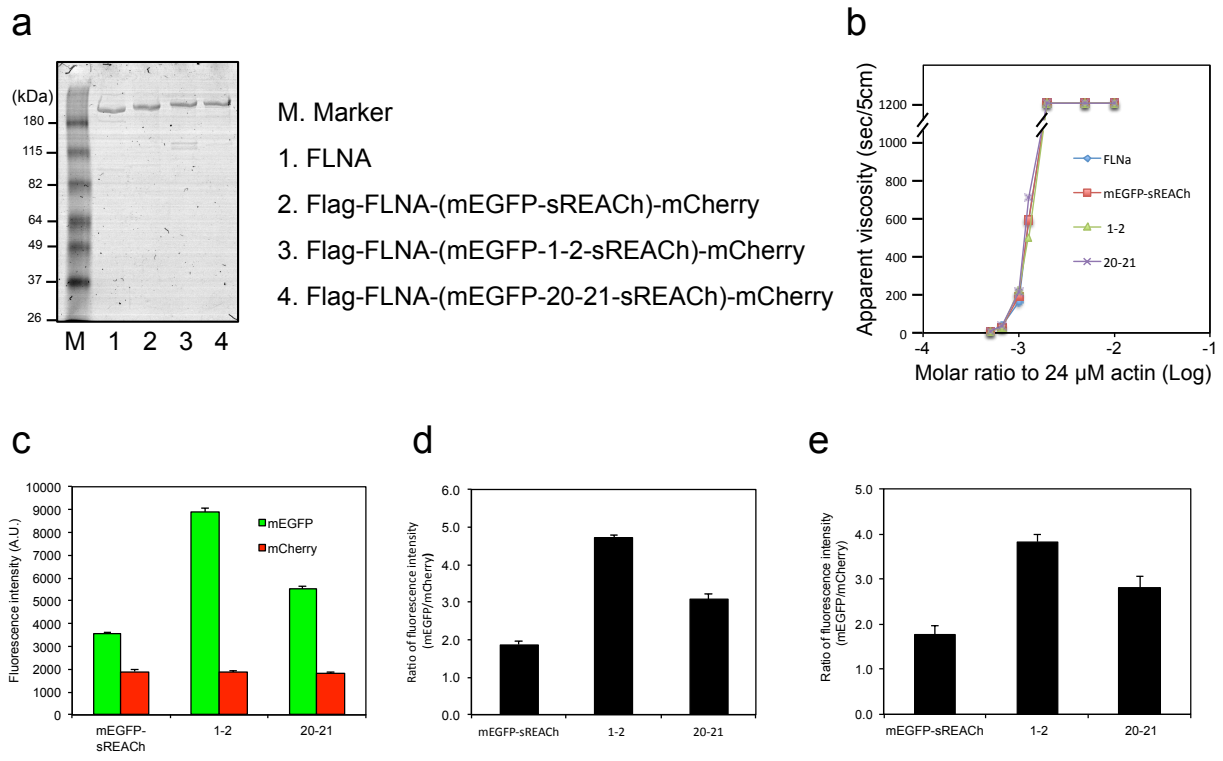
Supplementary Fig. 2. Absorbance (solid line) and fluorescence (dotted line) spectrum of mEGFP-tev (green), sREACh (yellow), and mCherry (red). The spectra are normalized. Transmission spectrum of 488/568 nm Yokogawa emission filters (FF02-525/40-25) and FF02-607/36-25, Semrock) used for spinning disk microscopy are plotted in black solid and dotted lines, respectively. mEGFP and mCherry were excited with a 488 and 561 nm laser, respectively.



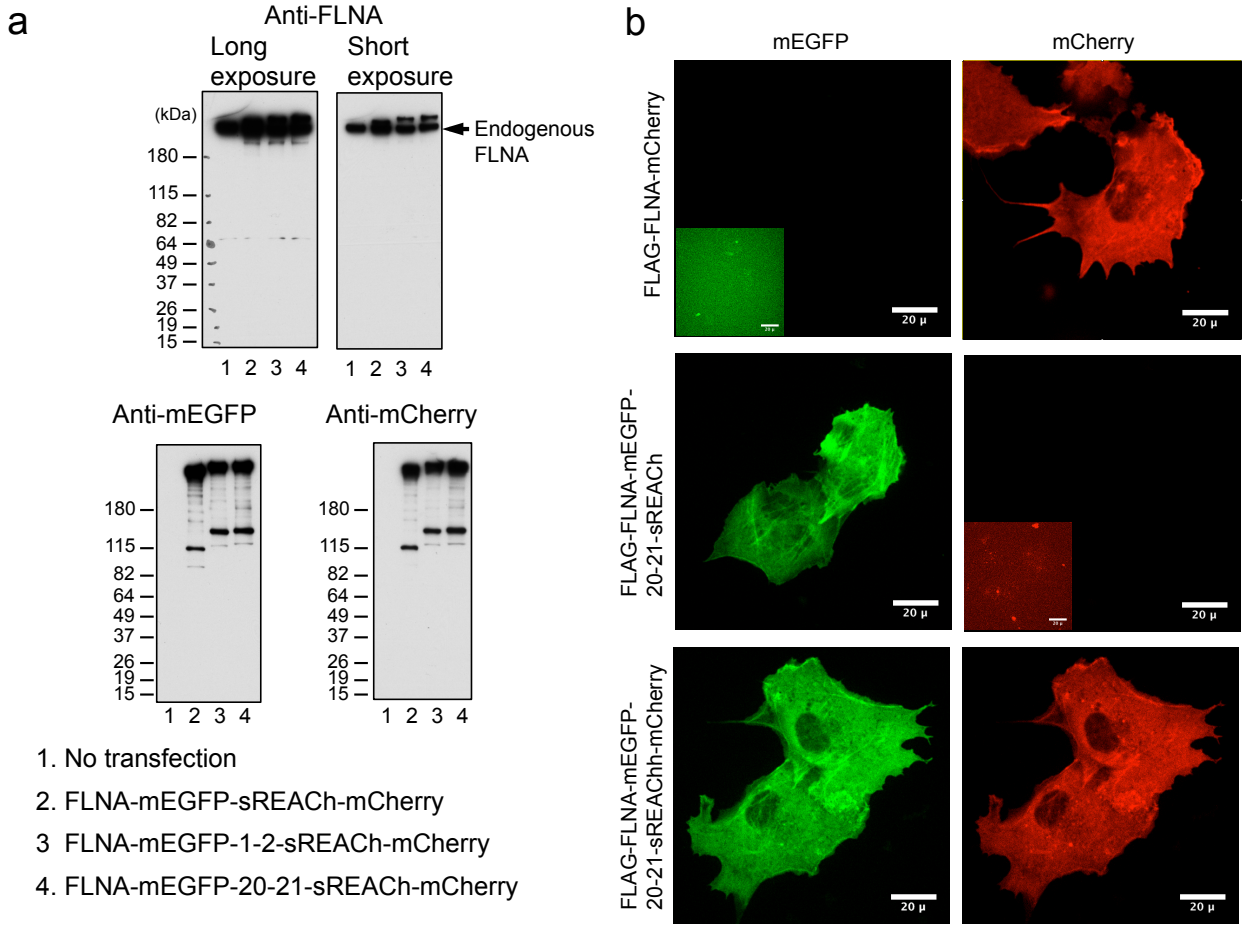
Supplementary Fig. 3. Effect of calcium concentration and pH on FRET.
(**a** and **b**) Effect of calcium concentration on FRET. The mEGFP emission of each construct (2 μM) at 511 nm upon excitation at 458 nm is plotted (**a**). Fluorescence intensities are normalized to the emissions of mEGFP in 0.1 μM of Ca^{2+} (**b**). Note that calcium concentrations in the physiological range have no significant effect on FRET. (**c** and **d**) Effect of pH on FRET. The mEGFP emission of of each construct (2 μM) at 511 nm upon excitation at 458 nm is plotted (**c**). Fluorescence intensities are normalized to the emissions of mEGFP at pH 7.5 (**d**). Note that fluorescence intensities increase as pH increases, independent of the insert between mEGFP and sREACh. Error bars represent SD (*n*=3).



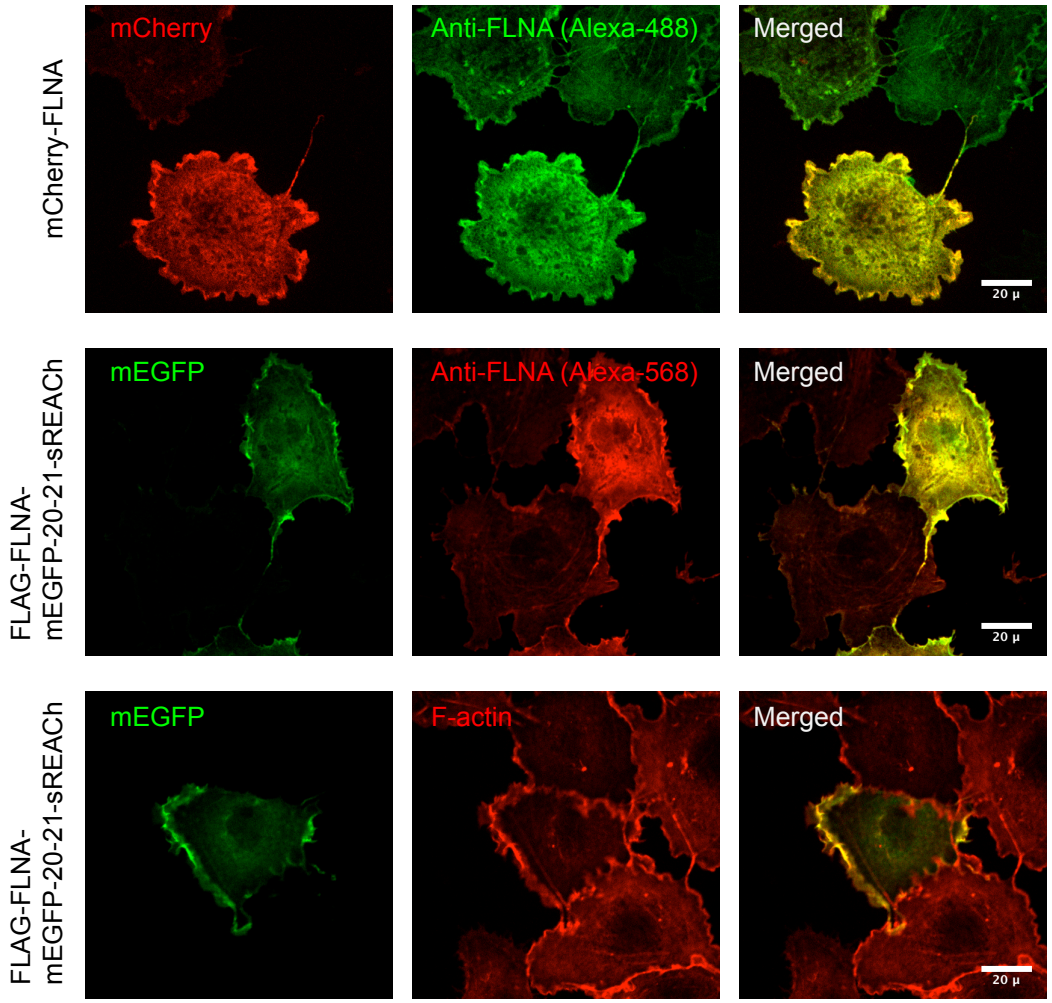
Supplementary Fig. 4. Molecular design of FLNA-CS.
(**a**) Cartoons show conformation sensor insertion points. (**b**) An illustration of the photo-quenching tension sensor. Strand A of repeat 20 is indicated in magenta. Structures of EGFP (PDB, 2YoG) and IgFLNa21 (PDB, 2BRQ) were displayed on PyMol (http://www.pymol.org/). (**c**) ①: Structure of FLNA repeats 19-21 adopted from *J. Am. Chem. Soc.*, DOI: 10.1021/ja2114882, 2012. ②: Primary amino acid sequence of hinge-1 and strand A of repeat 16. Location of tension sensor insertion point is indicated with double slash.



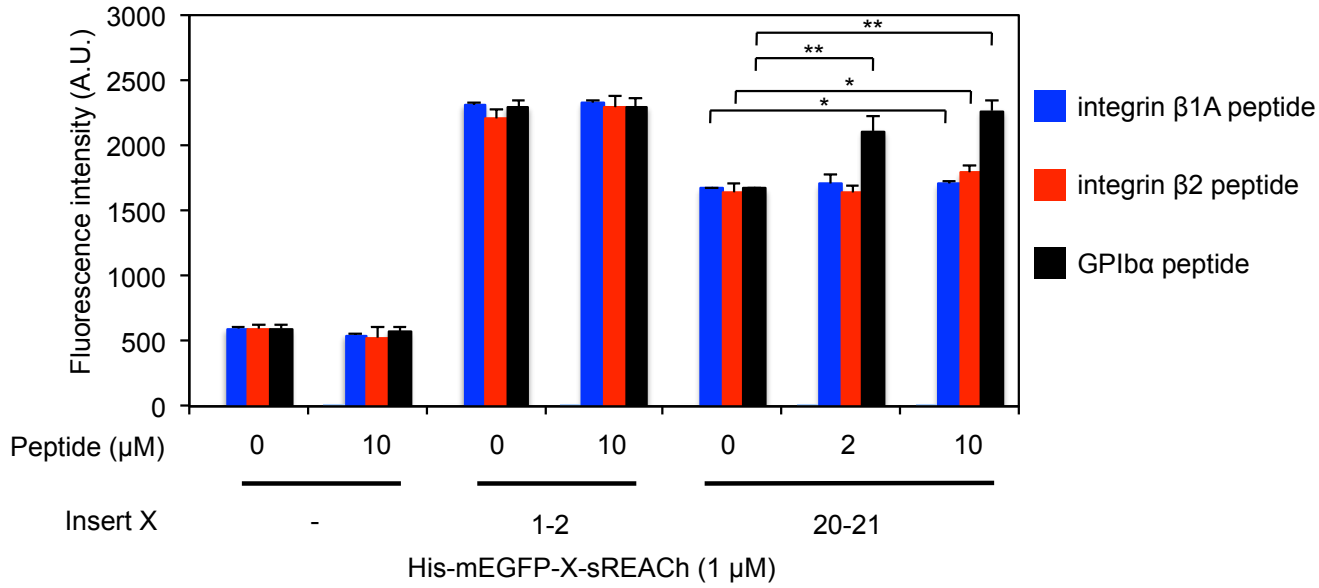
Supplementary Fig. 5. Characterization of recombinant full-length FLNA-TS.
(**a**) Coomassie blue stain of SDS-PAGE gel of purified proteins used in this study. (**b**) Gel point assay of the purified FLNA proteins. (**c**) The fluorescence intensity of purified protein constructs (1 μM) was measured in a fluorescence microplate reader with excitation/emission wavelengths of 458/511 (mEGFP) and 561/610 nms (mCherry). (**d**) Ratio of fluorescence intensity (mEGFP/mCherry) from (**c**) is plotted. (**e**) The fluorescence intensity of purified protein constructs (0.12 μM) was measured by spinning disk confocal microscopy. mEGFP and mCherry were excited with 488 and 568 nm lasers, respectively. Error bars represent SD (*n*=3).



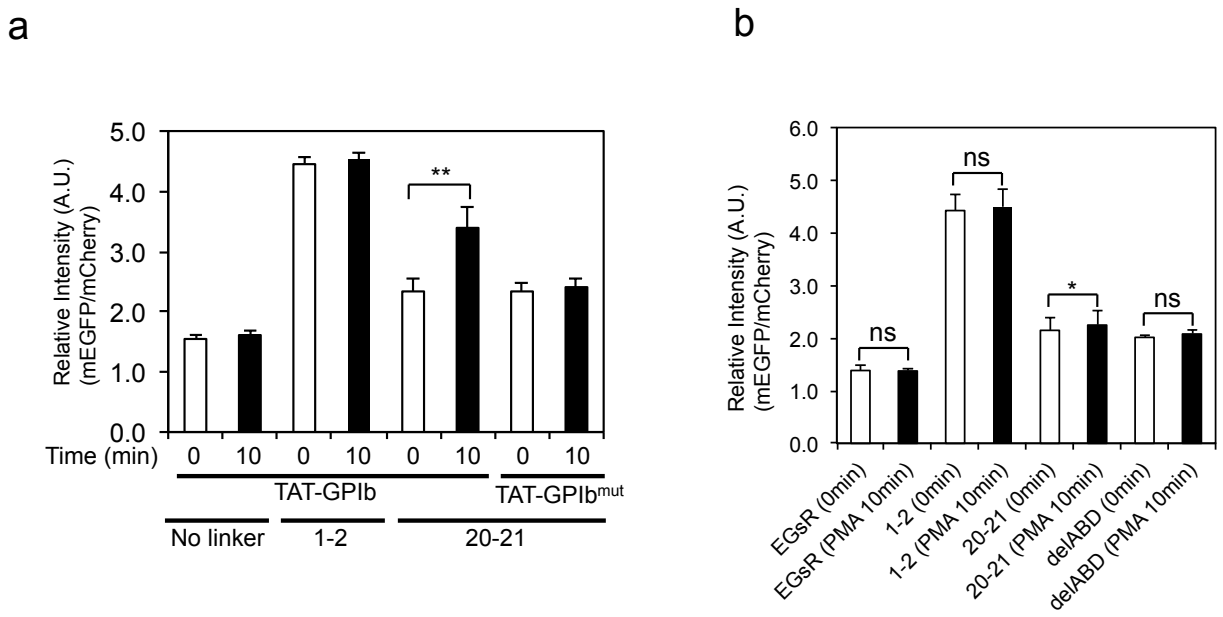
Supplementary Fig. 6. Expression of FLNA-CS in COS-7 cells.
(**a**) Western blot of lysates from COS-7 cells expressing mCherry-FLNA-CS. (**b**) Fluorescence images showing expression of mCherry-FLNA-CS. Insets: Brightness enhanced fluorescence images. Note that fluorescence bleed-through between mEGFP and mCherry is not detectable in the fluorescence microscope used in this study. Scale bar = 20µm



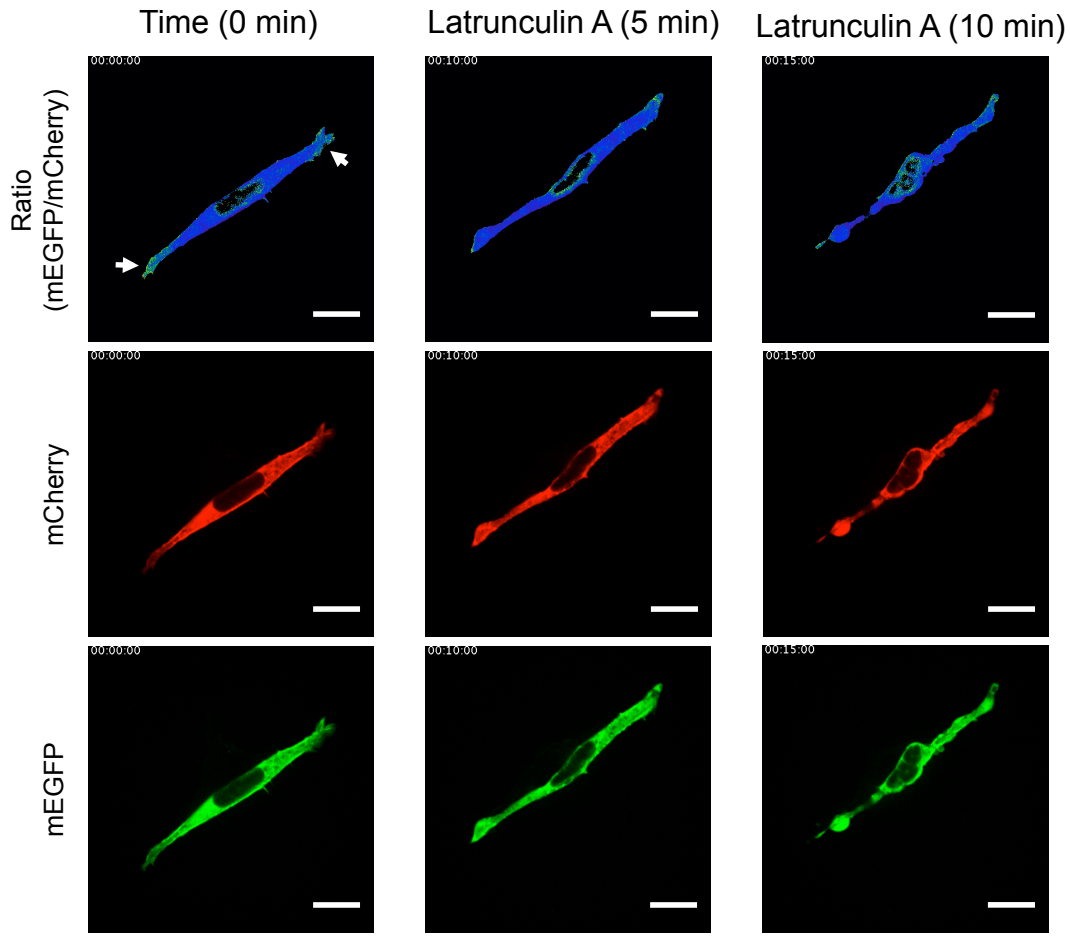
Supplementary Fig. 7. Comparison of the localization of expressed FLNA and FLNA-CS with total FLNA in COS-7 cells.
mCherry-FLNA and FLNA-CS were transfected into COS-7 cells. Total (endogenous and experessed) FLNA was stained with an anti-FLNA antibody. F-actin was stained with Alexa-568 phalloidin. Scale bar = 20μm.



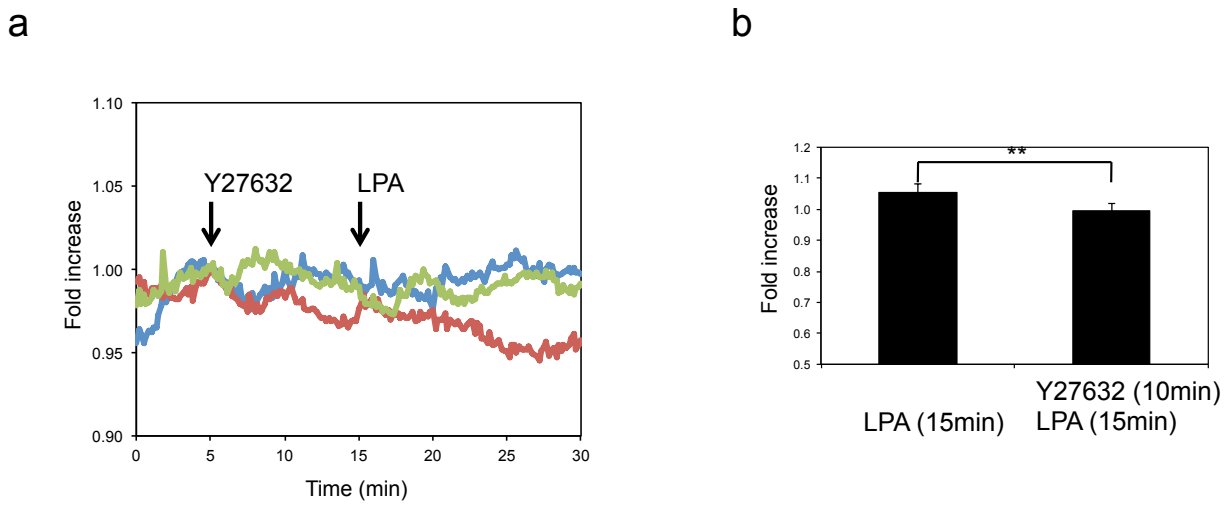
Supplementary Fig. 8. The FLNA PQ-FRET sensor is opened by the GPIbα peptide.
The fluorescence intensity of purified PQ-FRET constructs (1.0 μM) with increasing amounts of GPIbα (556LRGSLPTFRSSLFLWVRPNGRV577), integrin β1A (774KWDTGENPIYKSAVTTVVNPKYEGK798), integrin β2 (745SQWNNDNPLFKSATTTVMNPKFAES769) peptides (0 - 10 μM) was measured in a fluorescence microplate reader using excitation/emission wavelengths of 458/511 nms (mEGFP). Error bars represent SD (n=3). *P<0.05, **P<0.005 (t test).



Supplementary Fig. 9. Quantification of the conformational changes in FLNA-CSs expressed in HEK-293 cells.
The ratio of fluorescence intensities of mEGFP versus mCherry was plotted before and after treatment with 100 μM of the cell-permeable peptides (**a**) or 100 nM PMA (**b**). Corresponding time lapse movies are in the supplemental material (movies S2 and S4, respectively). Error bars represent SD (n=3). *P<0.05, **P<0.005; ns, not significant by a two-tailed t test.

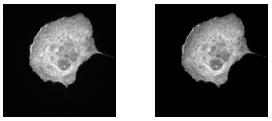
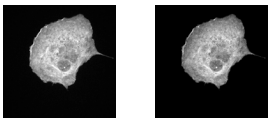
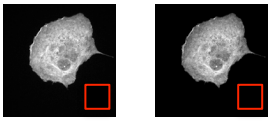
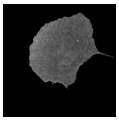
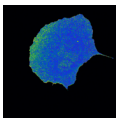


Supplementary Fig. 10. Effect of an actin depolymerization on the conformational changes of FLNA-CS(20-21) expressed in HEK-293 cells.
Ratio images of FLNA-CS(20-21) expressed in HEK-293 cells before and after treatment with 500 nM latrunculin A (Time, hh:mm:ss, Total 15 min:00 sec). Latrunculin A was added at the 5 min. Scale bar is 20 μm. Vorresponding movies are shown in supplemental as movie S7.



Supplementary Fig. 11. Effect of the Rho-kinase inhibitor, Y27632, on the conformational changes of FLNA-CS(20-21) probe expressed in MEF cells.
(**a**) Quantification of spatial conformational changes in the FLNA-CS(20-21) probe expressed in MEF cells before and after treatment with 10 μM Y27632 for 10min then stimulated with 1 μM LPA for an additional 15min. Intensities of the ratio images of the whole cells are quantified, normalized (relative to the intensity at 5 min), and plotted as a function of time. Plots from three independent experiments are shown. The corresponding time lapse movie is supplemental movie S9. (**b**). Changes of average intensities of more than 5 independent ratio images after LPA stimulation with or without Y27632 treatment were quantitated and plotted (**c**). Error bars represent SD (n=5~10). **P<0.005 by a two-tailed t test.

Supplementary Table 1. Ratio image generation procedure.

Processing step	ImageJ command	Outcome
Open mEGFP and mCherry channel images* (Optional: Crop the images)	(Image>Crop: Use ROI manager, "Command+T", to select the same regions)	mEGFP channel mCherry channel 
32-Bit conversion (if not converted)	Image>Type>32 bit	
Measure background values	Select a region next to a cell that has no fluorescence (use ROI manager). Analyze>Measure (mean grey value)	
(Optional: Smooth filter)	(Process>Smooth)	
Threshold: only mEGFP image	Image>Adjust>Threshold Select "Default", "B&W", and "Dark background". Press "Apply". Check "Set Background Pixels to NaN" and press "OK".	
Ratio: mEGFP/mCherry	Plugins>Ratio Plus** Image1: mEGFP Image2: mCherry Add background values measured above.	
LUT assignment	Plugins>NucMed>Lookup Tables**> Blue_Green_Red	
Range adjustment	Image>Adjust>Brightness/Contrast: set the range. Normalization: Open all ratio images and press "Set" Check "Propagate all other open images" and "OK.	
Prepare image for presentation	Image>Type>RGB color Save as…	

Modified from *Nature Protocols 6, 1835–1846 (2011)*

* Metamorph nd & ROI files importer (nd stack builder) plugin can be used to import nd file.

**Note that "Ratio Plus" and "Lookup Tables " (in "NucMed)" plugins are not included in the basic ImageJ package and should be downloaded from the ImageJ plugins website.

Supplementary Table 2. Amino acid sequences of synthetic peptides

Name	Amino acid sequences
GPIbα (556-577)	LRGSLPTFRSSLFLWVRPNGRV
Integrin β1A (774-798)	KWDTGENPIYKSAVTTVVNPKYEGK
Integrin β2 (745-769)	SQWNNDNPLFKSATTTVMNPKFAES
TAT- GPIbα (561-573)	RRKKRRQRRR-PTFRSSLFLWVRP
TAT- GPIbα^{mut} (561-573)	RRKKRRQRRR-PTFRSSLAAAVRP

Underlines indicate FLNA-binding sites.