Supporting Information

Development of FRET biosensor to characterize CSK subcellular regulations

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The list of supporting materials:

1. Supporting experimental procedures

2. Supporting figures S1-S5

3. Supporting movie

Supporting Experimental Procedures

Cell culture

HeLa cells were cultured in high-glucose DMEM (Gibco) supplemented with 10% fetal bovine serum (FBS), 2 mM L-Glutamine, 100 I.U./ml penicillin and 100 μg/ml streptomycin, and maintained at 37°C in 5% CO2 incubator.

Purification of the biosensor and molecular size with coomassie brilliant blue staining

The recombinant biosensor proteins with an N-terminal 6xHis tag in pRSETb vector were expressed in E. Coli (BL21) by culturing with 0.4 mM IPTG (Isopropyl β-D-1-thiogalactopyranoside, Sigma) at 25°C for 16-24 hours, and purified by nickel chelation chromatography[1].

The purified protein is subjected to 10% Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) to separate proteins based on their sizes. Following electrophoresis, the gel is washed with double-distilled water using agitation. Subsequently, the gel is immersed in a Coomassie Brilliant Blue staining solution and agitated for 30 minutes to facilitate binding of the dye with proteins. The gel is then incubated in the destaining soultion (containing 50% ethonal and 10% acetic acid) through multiple lasting washes to remove unbound dye. The picture of the protein bands in the gel is recorded by digital camera.

Supporting Figures

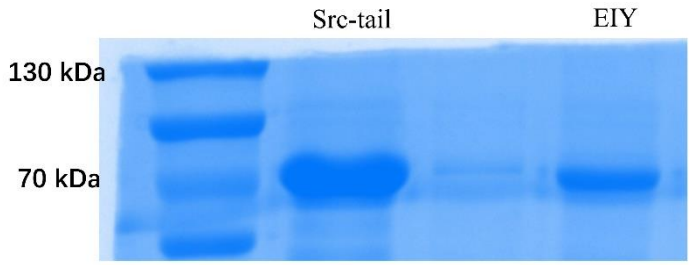

Figure S1. Molecular size of the purified biosensor proteins. The biosensor proteins were resolved on SDS-PAGE gel by electrophoresis and stained with Coomassie bright blue. The picture shows the successfully purified CSK biosensor proteins with Src-tail and EIY substrates, which displays a molecular size around 70 kDa.

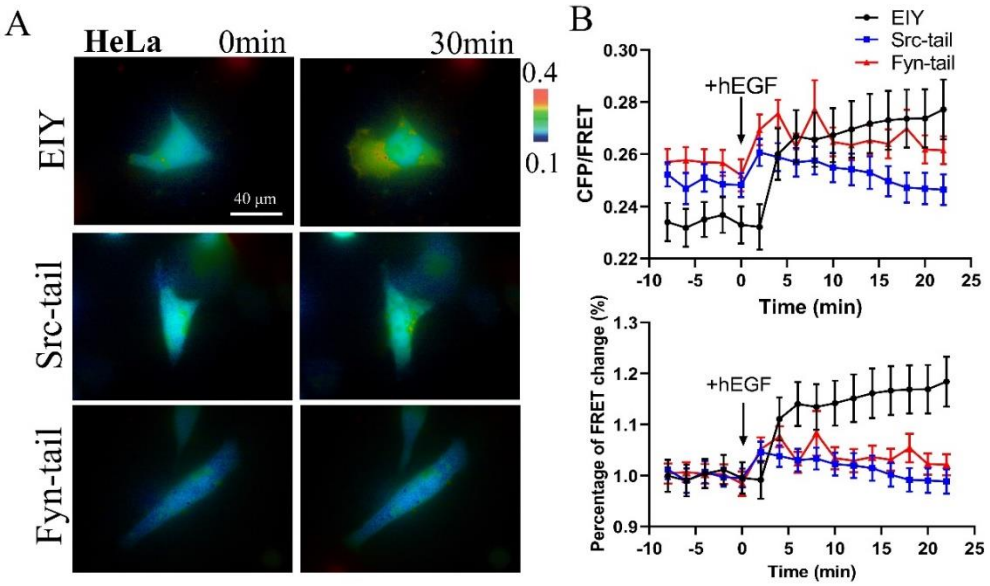

Figure S2. Characterizations of CSK biosensor with different substrate peptides in HeLa and MEF cells. (A) The ratiometric FRET images of HeLa cells expressing CSK biosensor with EIY, Src-tail or Fyn-tail substrate before and after EGF stimulation. **(B)** The quantified time courses of FRET ratio and normalized FRET changes from the cells in (A).

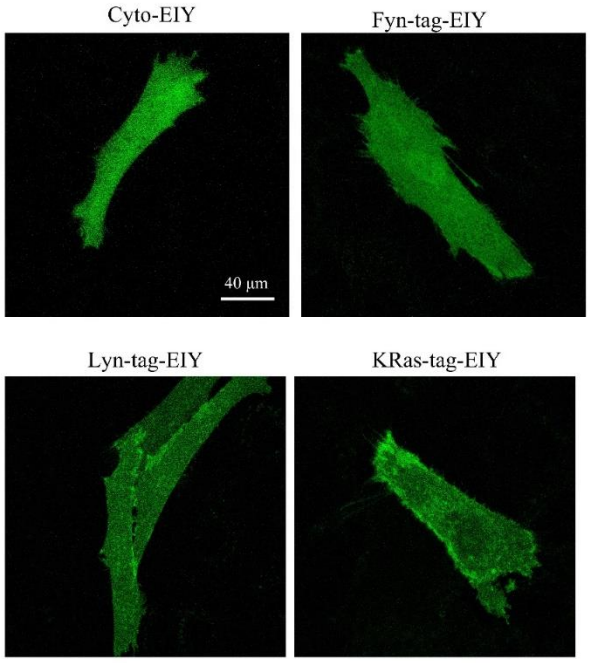

Figure S3. Confocal images of ASM cells show membrane localizations of CSK biosensor with or without membrane-targeting peptides. ASM cells were transfected with cytosolic or membrane microdomain-targeted CSK biosensors (Cyto, Fyn-tag, Lyn-tag, KRas-tag). After 24 h, cells were further seeded onto glass-bottom dishes overnight for confocal microscopic imaging.

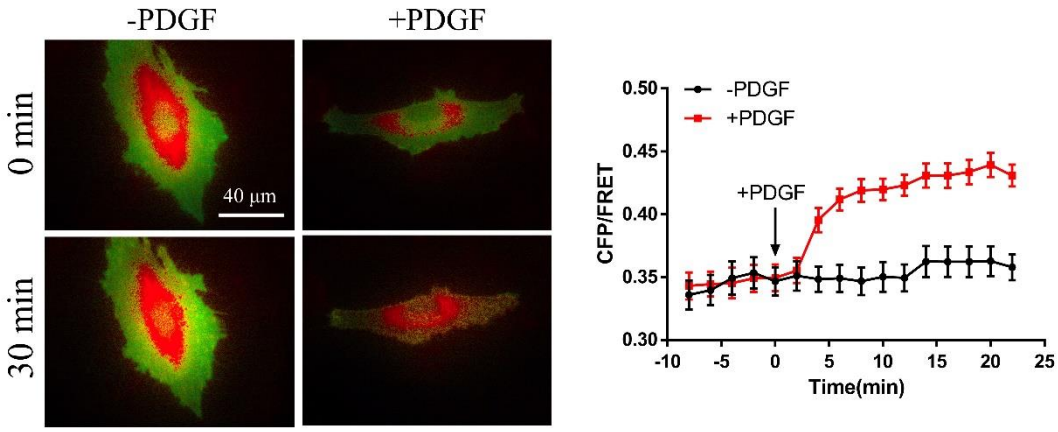

Figure S4. FRET change of CSK biosensor activated with PDGF. The KRas-tag-CSK FRET biosensor was transfected into ASM cells, followed by FRET imaging

without (control group) or with (experimental group) 10 μg/ml PDGF stimulation. The changes of FRET ratio were compared through the quantified time-courses with or without PDGF.

Supporting movie legend

Supporting Movie S1. PDGF-induced FRET responses of cytosolic and different membrane microdomain-localized CSK biosensors in ASM cells.

Reference:

1. Miyawaki, A.; Tsien, R. Y., Monitoring protein conformations and interactions by fluorescence resonance energy transfer between mutants of green fluorescent protein. *Methods in enzymology* **2000,** *327*, 472-500.