

Supplementary materials

Figure Legends

Figure S1 *SRGAP2* expression is correlated with tumors malignant behavior

(A) *SRGAP2* expression levels among tumor tissues and normal tissues from the Cancer Genome Atlas database. (B) Kaplan-Meier plots of overall survival of patients with breast cancer and triple-negative breast cancer.

Figure S2 SrGAP2 protein expression did not affect MF polymerization

(A) Mean ratios of F-actin/G-actin in TNBC cells transfected with pCMV-N or pCMV-srGAP2. No statistical difference between the two groups. (B) Actin tension probes were expressed in TNBC cells of control group, srGAP2 knockdown group and srGAP2 overexpression group. No statistical difference in actin tension among the three groups. (C) Immunoblot of MDA-MB-231 cells knocked down for FMNL1 and FMNL3. (D) Immunoblot of MDA-MB-231 cells knocked down for talin 1. (E) TNBC cells expressed GFP-srGAP2 WT, GFP-srGAP2 S206A and GFP-srGAP2 S206E.

Figure S3 Conformation of srGAP2 tension probes and their effective analysis

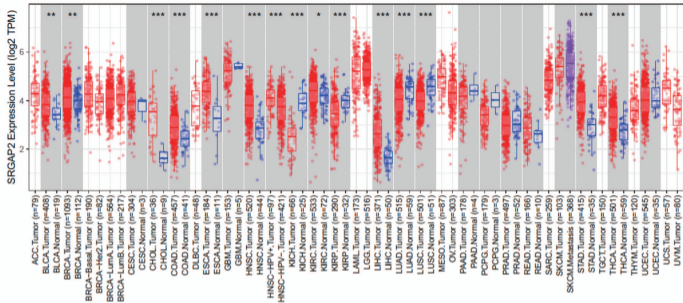
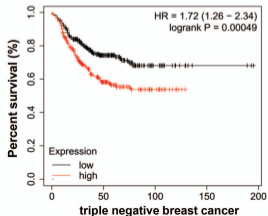
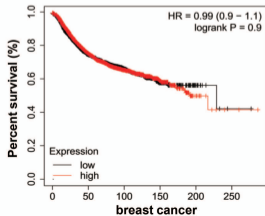
(A) The probe model consists of srGAP2-M-cpstFRET. The cpVenus is parallel to the cpCerulean when an external force across cpstFRET generates a certain angle, reducing FRET efficiency. (B) Insertion position of the probes in srGAP2 structure. (C) Testing the reliability of the srGAP2-M-cpstFRET probe using the acceptor bleaching FRET and fluorescence recovery after photobleaching tests. (D) and (E) Representative image of MDA-MB-231 cells expressing srGAP2-M-cpstFRET that recovered over time after laser bleaching. red box: bleached area. Normalized average fluorescence recovery of srGAP2-M-cpstFRET versus time (n = 8). Scale bar, 20 μ m.

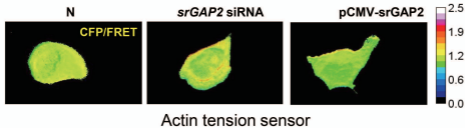
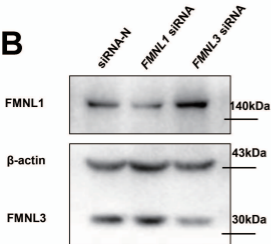
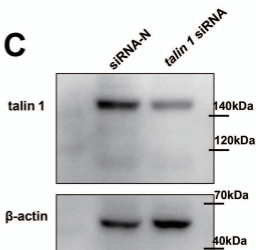
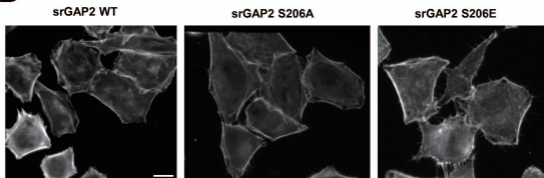
Figure S4 (A) Bioinformatics predicted serine phosphorylation sites in the F-Bar domain of srGAP2. Data from <https://services.healthtech.dtu.dk>

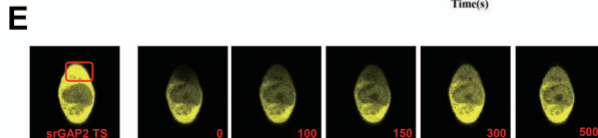
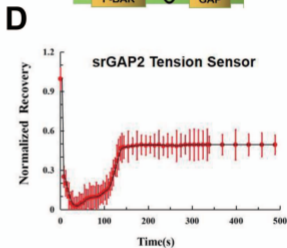
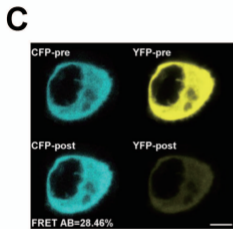
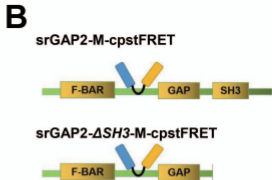
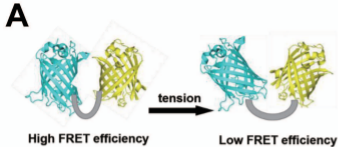
Figure S5 Representative images of TNBC cell expressing srGAP2-M-cpstFRET probes

(A) Representative images of TNBC cell expressing srGAP2-M-cpstFRET, srGAP2 S206A-M-cpstFRET and srGAP2 S206E-M-cpstFRET. The calibration bar: 0.3 to 0.9. (B) Representative images of TNBC cell expressing srGAP2-M-cpstFRET transfected with N siRNA or talin1 siRNA. PI3K inhibitor (LY-294002, 30 μ M) and Cytochalasin D (2 μ M) was pre-incubated 30 mins before the experiment.

Figure S6 (A) and (B) Endogenous PKC α interacted with SDC4 in the fresh human TNBC tissues and para tissues.

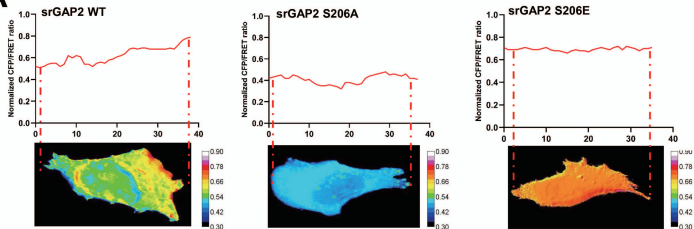
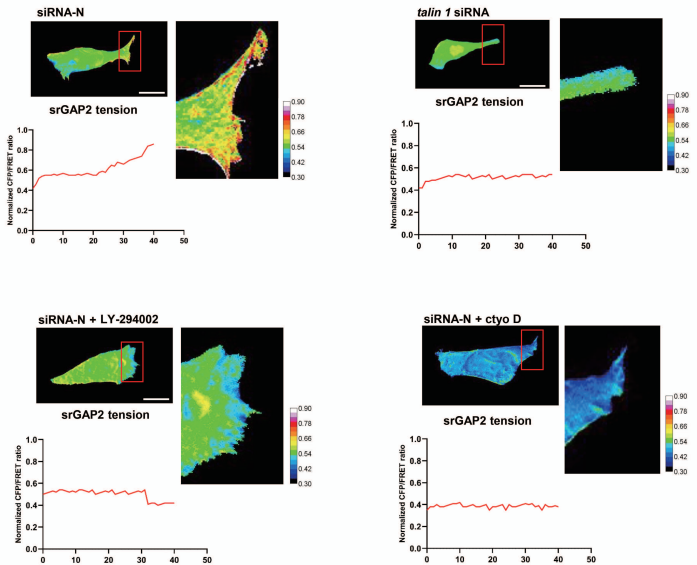
A**B**

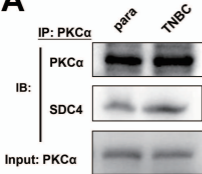
A**B****C****D**



A

SITE	CONTEXT	SCORE
64 S	EMDYSRNLE	0.507
80 S	AKTRSTKDQ	0.996
94 S	QNVLSPVNC	0.547
110 S	VKRESRDHT	0.991
117 S	HTTLSDIYL	0.593
132 S	FVQVSEDSG	0.941
135 S	VSEDSGRLF	0.715
142 S	LFKKSKEVG	0.874
162 S	NELYSVMKT	0.453
174 S	YNADSISAQ	0.827
176 S	ADSISAQSK	0.443
179 S	ISAQSKLKE	0.601
195 S	EMDYSRNLE	0.507
206 S	AKTRSTKDQ	0.996
209 S	QNVLSPVNC	0.547
224 S	VKRESRDHT	0.991
263 S	HTTLSDIYL	0.593
273 S	FVQVSEDSG	0.941
287 S	VSEDSGRLF	0.715
297 S	LFKKSKEVG	0.874
305 S	NELYSVMKT	0.453

A**B**

A**B**