Supplementary Information

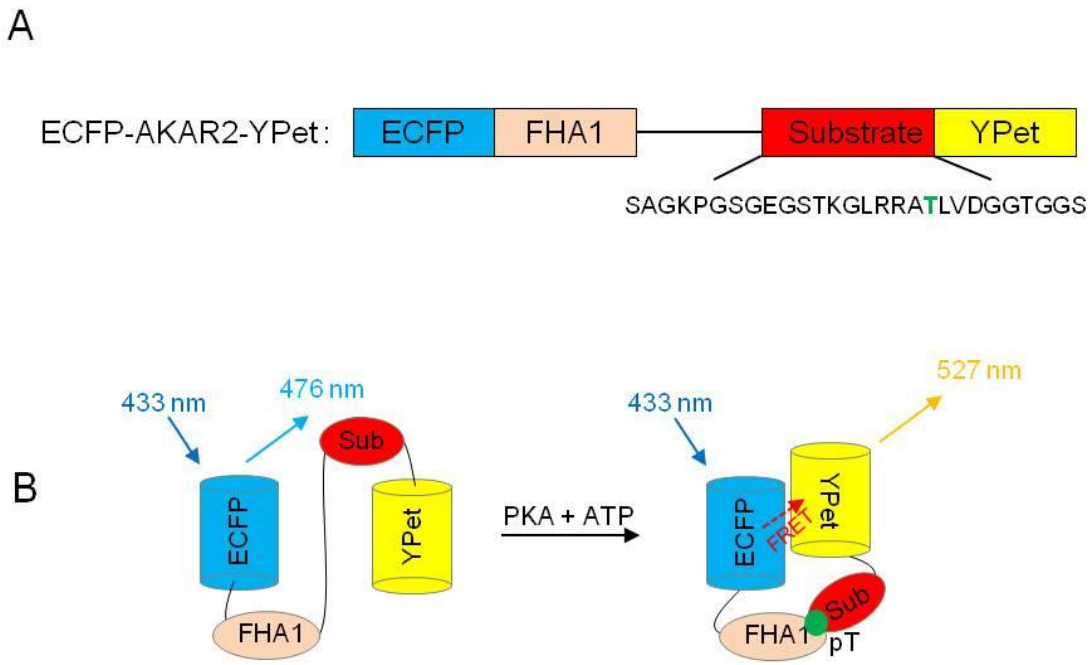

Figure S1. (A) The design of the modified AKAR2 reporter. Citrine in the original reporter is replaced by YPet. (B) The mechanism of the modified AKAR2 reporter. Upon PKA activation, the phosphorylation of the substrate allows its intramolecular binding to FHA1 domain, causing an increase of FRET efficiency.

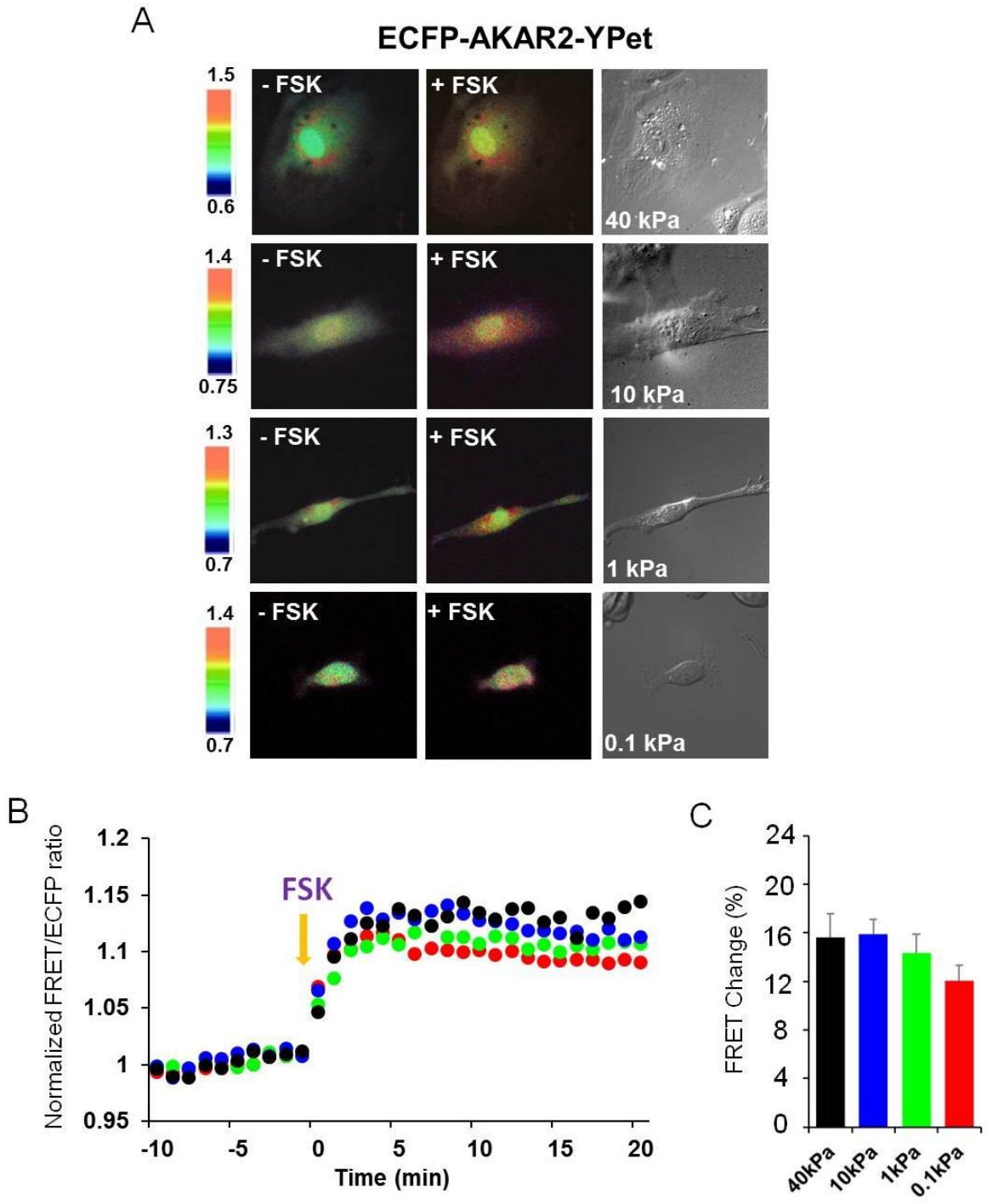

Figure S2. (A) PKA activation upon FSK treatment in HMSCs cultured on different substrate stiffness. Blue and red colors represent low and high FRET ratios, respectively. The cells expressing ECFP-AKAR2-YPet sensor show the change in FRET/ECFP emission ratio before and after 20 min treatment with FSK. (B, C) Time courses and bar graphs indicate the average of normalized FRET/ECFP emission ratio changes of the reporter from multiple cells in response to FSK (n=6).

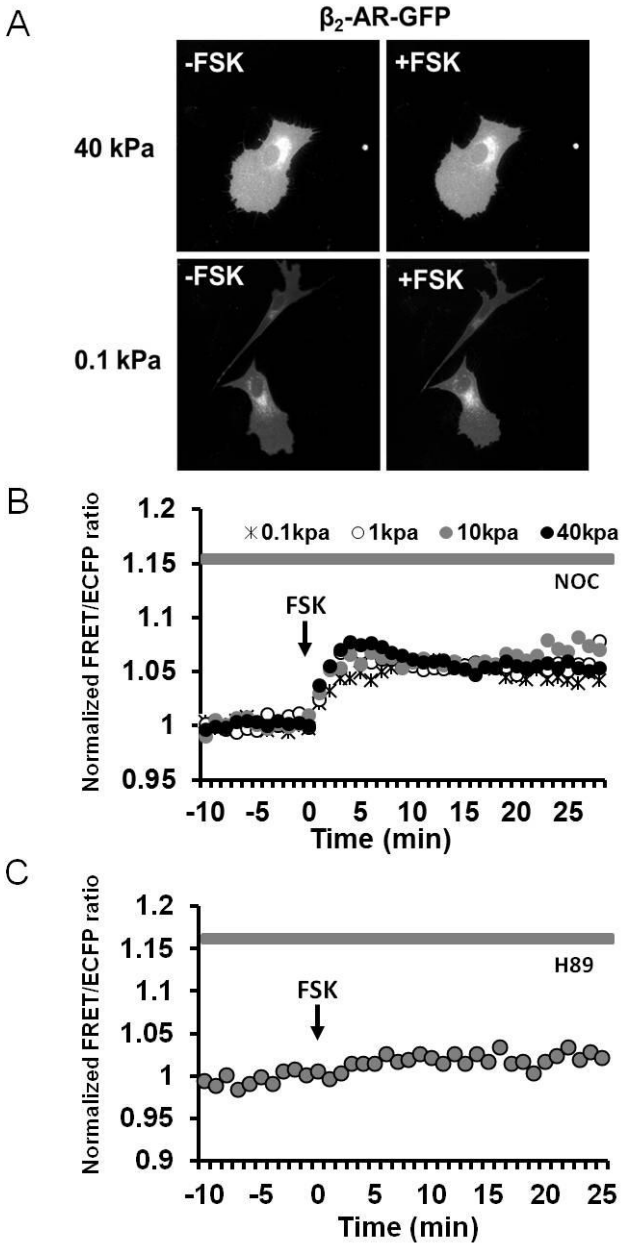

Figure S3. (A) Effect of FSK on β_2-AR internalization in response to substrate stiffness. FSK does not stimulate β_2-AR internalization, suggesting that PKA activation alone is not sufficient for the internalization of β_2-AR. Similar results were observed in three independent experiments. (B)Time courses of emission ratio in HMSCs pretreated with NOC (5 μM), a microtubule inhibitor, in response to FSK. NOC didn't affect FSK-induced PKA activation. Similar results were observed in three independent experiments. (C)Time courses of emission ratio in the cells pretreated with H89 (20 μM), a PKA inhibitor, in response to FSK. H89 inhibits FRET emission ratio in ECFP-AKAR2-YPet transfect cells. Similar results were observed in three independent experiments.

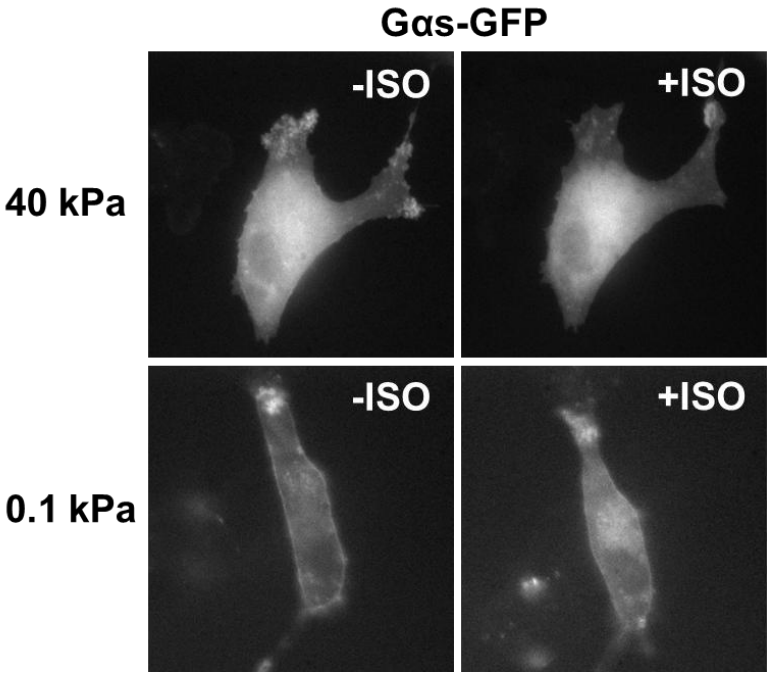

Figure S4. Substrate stiffness has no effect on Gαs recruitment or internalization in response to ISO in HMSCs. Similar results were observed in three independent experiments.