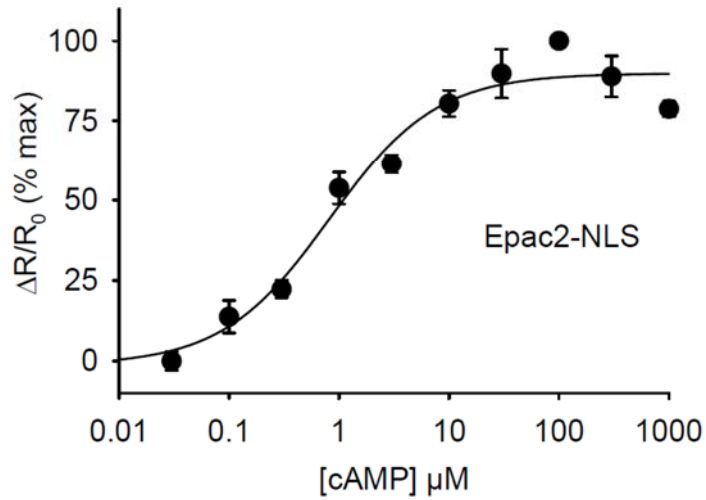


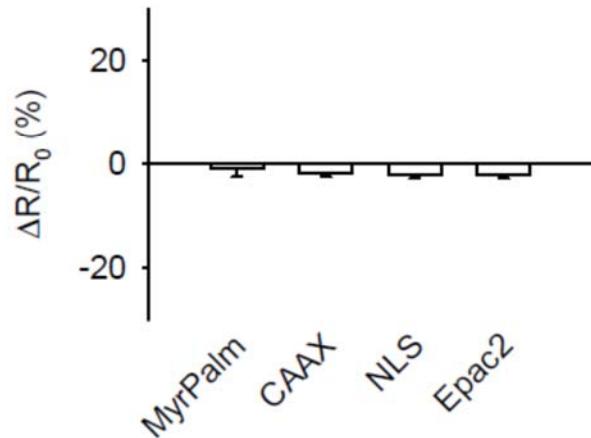
Supplemental Figure S1



Supplemental Figure S1. *In vitro* calibration of Epac2-NLS biosensor.

Concentration-response curve for cAMP activation of Epac2-NLS (n = 5). EC_{50} and Hill slope were $1.04 \pm 0.13 \mu\text{M}$ and 0.84 ± 0.087 , respectively. Experiments were conducted as described previously (Agarwal et al., 2014).

Supplemental Figure S2



Supplemental Figure S2. Exposure to the adenylyl cyclase inhibitor MDL-12,330A (MDL). FRET response ($\Delta R/R_0$) observed following 10 min exposure to 100 μ M MDL. Because MDL alone had no effect, subsequent exposure to IBMX plus forskolin was used at the end of each experiment as a positive control. There was no statistical difference ($p > 0.7$, One Way ANOVA) in the FRET responses detected by MyrPalm ($-1.0 \pm 1.6\%$, $n = 5$), CAAX ($-1.9 \pm 0.73\%$, $n = 5$), NLS ($-2.2 \pm 0.62\%$, $n = 7$), and Epac2 ($-2.2 \pm 0.78\%$, $n = 6$).

Supplemental Table 1

cAMP affinity of FRET-based biosensors			
	EC₅₀ (μM)	Hill coefficient	ref
Epac2-camps	0.31	0.84	Agarwal et al. 2014
Epac2-MyrPalm	0.43	0.82	
Epac2-CAAX	0.16	1.1	
Epac2-NLS	1.04	0.84	figure S1