

Supplementary Materials for
**Spatial Control of Epac2 Activity by cAMP and Ca²⁺-Mediated
Activation of Ras in Pancreatic β Cells**

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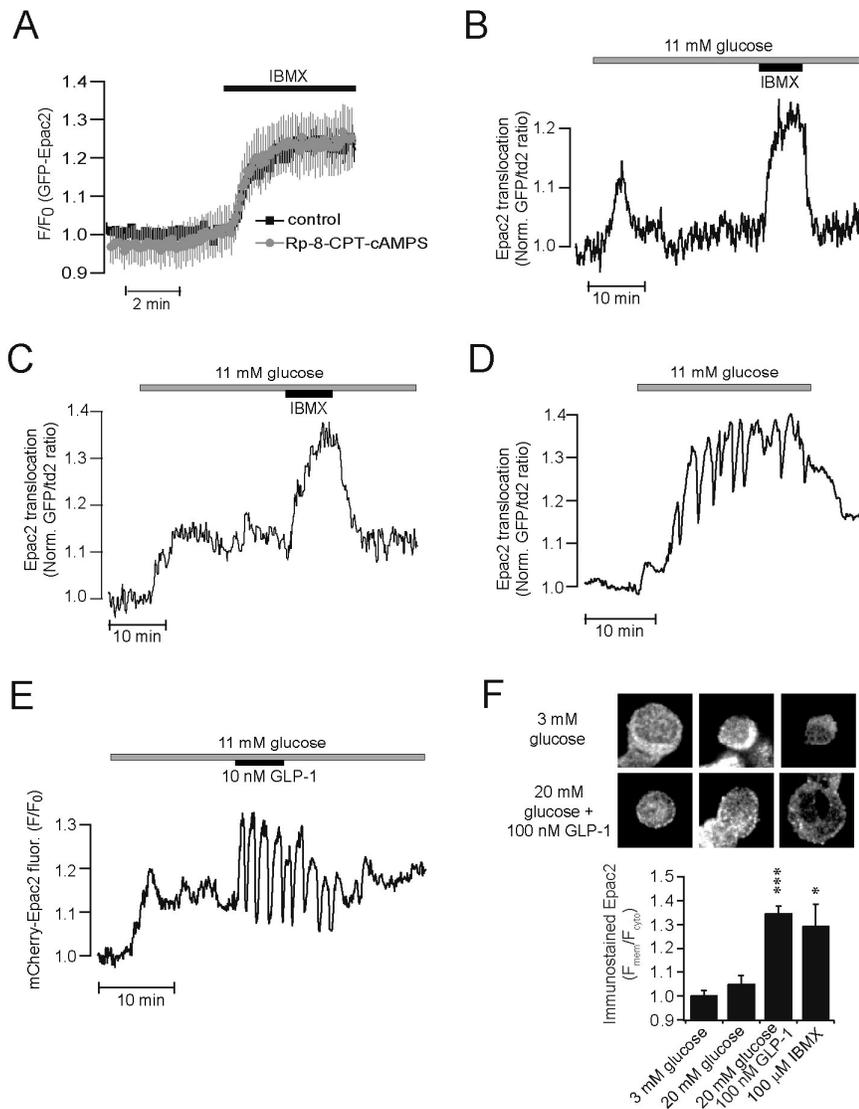


Fig. S1: Glucose- and cAMP-induced Epac2 translocation.

(A) TIRF microscopy recordings of IBMX-induced translocation of GFP-Epac2 to the plasma membrane in MIN6 β -cells exposed or not to the protein kinase A inhibitor Rp-8-CPT-cAMPS. The traces represent mean values \pm S.E. for 10 cells in each group. (B-D) TIRF recordings of GFP-Epac2 plasma membrane translocation (GFP/td2 ratio) in MIN6 β -cells during glucose stimulation. Examples of transient (B; representative of 10% of the cells), stable (C; representative of 23% of the cells) and fast oscillatory (D; representative of 8% of the cells) translocation responses. The glucose-induced GFP-Epac2 translocation is amplified by IBMX (B, C). $n=60$ cells. (E) TIRF microscopy recording of GFP-Epac2 membrane fluorescence from a MIN6 β -cell during stimulation with a combination of glucose and GLP-1. Representative of 8 cells. (F) Confocal images of MIN6 β -cells fixed and immunostained for endogenous Epac2 under unstimulated conditions (3 mM glucose; $n=46$ cells) and after stimulation with a combination of 20 mM glucose and 100 nM GLP-1 ($n=35$ cells). The bar graph shows quantifications of the membrane-to-cytoplasmic intensity ratio of Epac2

immunostaining in MIN6 β -cells stimulated under various conditions. n=36 cells for 3 mM glucose, 10 for 20 mM glucose, 35 for 20 mM + GLP-1 and 10 for IBMX. *, P<0.05; ***, P<0.001 for difference from 3 mM glucose (Student's t-test).

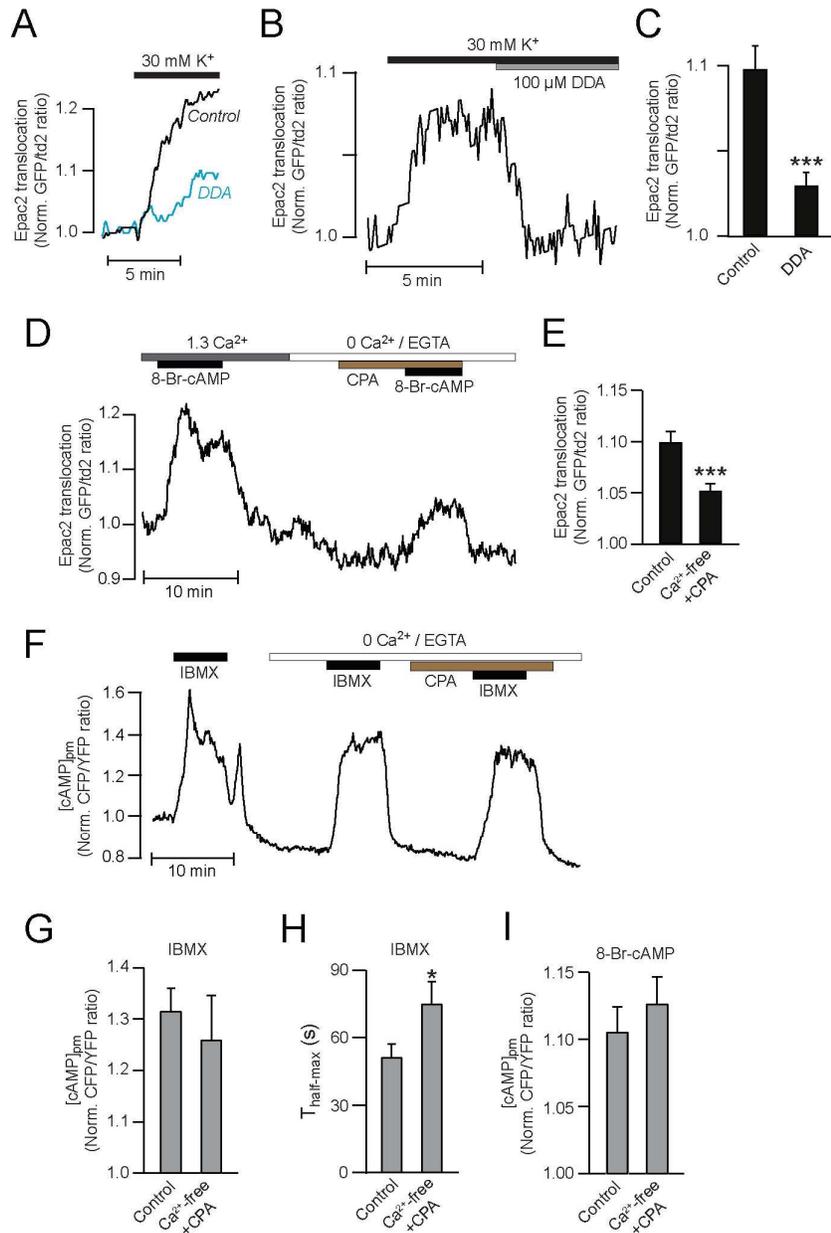


Fig. S2: Permissive effect of Ca^{2+} on cAMP-induced translocation of Epac2.

(A) TIRF microscopy recordings of GFP-Epac2 translocation (GFP/td2 ratio) from an individual MIN6 β -cell depolarized with K^+ in the absence (black trace) or presence (blue trace) of the adenylyl cyclase inhibitor 2',5'-dideoxyadenosine (DDA). (B) TIRF microscopy recording showing the acute effect of DDA on GFP-Epac2 membrane translocation induced by depolarization with 30 mM K^+ . (C) Means \pm S.E. for the peak GFP-Epac2 translocation in response to K^+ depolarization in the absence (n=24 cells) or presence (n=37 cells) of 100 μ M DDA. *** P<0.001 for difference from control (Student's t-test). (D) TIRF microscopy recordings of $[cAMP]_{pm}$ from a MIN6 β -cell following exposure to 8-Br-cAMP under control

conditions and when increases in the cytosolic Ca^{2+} concentration was prevented by removal of extracellular Ca^{2+} , addition of EGTA and depletion of intracellular stores with cyclopiazonic acid (CPA). **(E)** Means \pm S.E. of the peak Epac2 translocation induced by 8-Br-cAMP under control or Ca^{2+} -deficient conditions. n=41 cells. **(F)** TIRF microscopy recording of $[\text{cAMP}]_{\text{pm}}$ from a MIN6 β -cell following an IBMX-induced increase in the cAMP concentration under control or Ca^{2+} -deficient conditions. **(G, H)** Means \pm S.E. of the peak $[\text{cAMP}]_{\text{pm}}$ response (G; n=15 cells) and time to half-maximal $[\text{cAMP}]_{\text{pm}}$ elevation (H; n=32 cells) following addition of IBMX in control or Ca^{2+} -deficient conditions. * $P < 0.05$ for difference from control (Student's t-test). **(I)** Means \pm S.E. of the peak $[\text{cAMP}]_{\text{pm}}$ elevation induced by 8-Br-cAMP (n=11 cells).

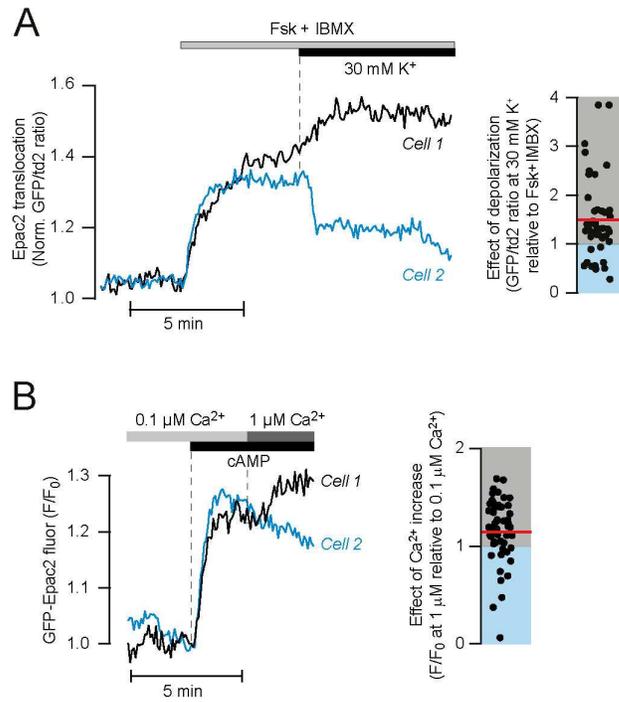


Fig. S3: Dual effects of Ca^{2+} on cAMP-induced translocation of Epac2.

(A) TIRF microscopy recordings from two MIN6 β -cells within the same view-field showing either a stimulatory or an inhibitory effect of membrane depolarization on cAMP-induced Epac2 membrane translocation. The plot to the right shows the effect of depolarization in each individual cell exposed to a combination of IBMX and forskolin. The horizontal bar indicates the average response of 45 cells. (B) TIRF microscopy recordings from two α -toxin-permeabilized MIN6 β -cells exposed to cAMP followed by a step increase in medium Ca^{2+} concentration. The plot to the right shows the response of individual cells to this increase in $[\text{Ca}^{2+}]$. The red horizontal bar indicates the average response of 53 cells.