Terrin et al., http://www.jcb.org/cgi/content/full/jcb.201201059/DC1

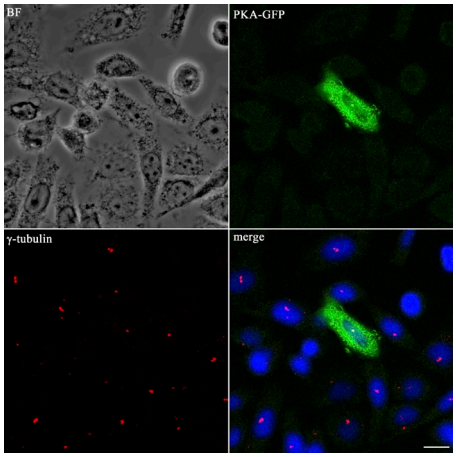

Figure S1. **PKA-GFP localizes at the centrosome, and its overexpression does not affect the centrosome morphology.** (A) CHO cells transiently expressing the PKA-GFP sensor immunostained with the centrosomal marker γ-tubulin. The brightfield image (BF) is also shown. The bottom right image shows the overlay between the PKA-GFP signal, γ-tubulin, and DAPI. The γ-tubulin staining clearly show that overexpression of PKA-GFP does not affect centrosome morphology. Bar, 10 µm.

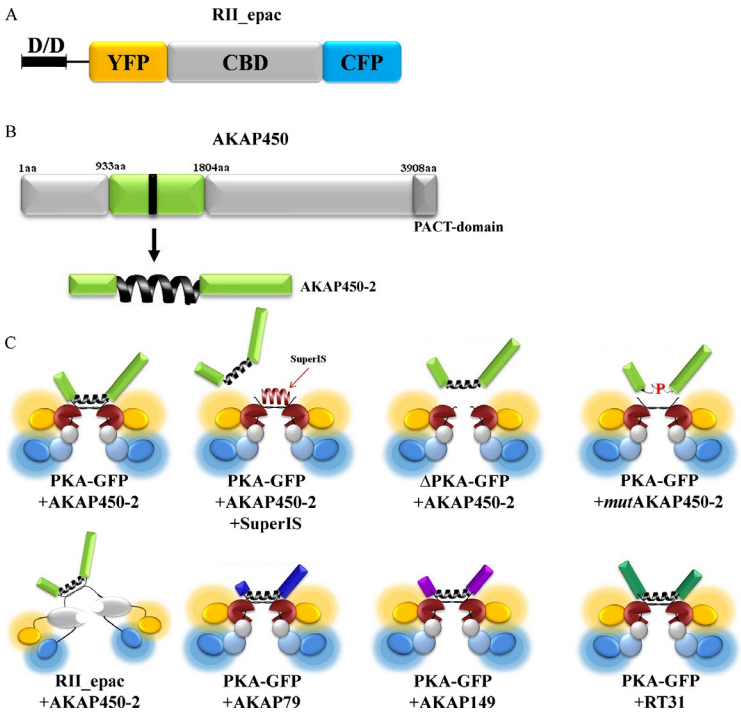

Figure S2. **Schematic representation of the RII_epac sensor, AKAP450, and AKAP450-2 fragment.** (A) Schematic representation of cAMP sensor RII_epac. Similarly to PKA-GFP, this sensor shows high FRET in the presence of low cAMP and vice versa. CBD, cAMP-binding domain. (B) Schematic representation of AKAP450 (amino acid 1–3,908) and AKAP450-2 fragment (amino acid 933–1,804; highlighted in green) encompassing the amphipathic helix (shown in black). The PACT domain is also indicated. (C) Schematic representation of the interaction between the FRET-based sensors and the AKAP constructs used in this study. The red P in the *mut*AKAP450-2 indicates the proline introduced in place of serine, resulting in disruption of the amphipathic helix in AKAP responsible for PKA binding.

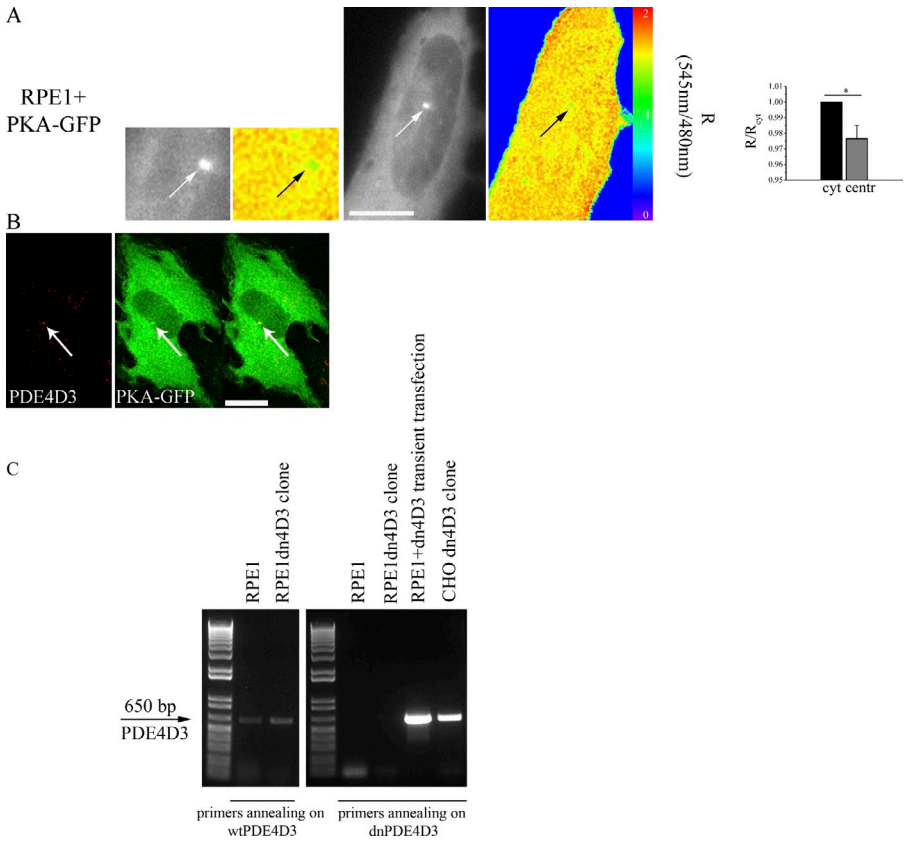

Figure S3. **Localization of the PKA-GFP sensor and PDE4D3 in the nontransformed cell line RPE1.** (A) Image in grayscale shows the subcellular localization of PKA-GFP in RPE1 cells. The FRET signal from the same cell is shown in pseudocolor. Images on the left show a higher magnification of the centrosomal region. Arrows point to the centrosome. Bars, 10 µm. The graph on the right shows the mean basal FRET signal measured in the bulk cytosol (cyt) and at the centrosome (centr) of RPE1 cells expressing the PKA-GFP sensor. FRET values are expressed relative to the FRET value measured in the cytosol. $n = 20$. Error bars represent SEM. A two-tailed paired *t* test was performed (*, $P < 0.05$). (B) Representative RPE1 cell expressing PKA-GFP (middle) and immunostained for PDE4D3 (left). The overlay between the PKA-GFP and the PDE4D3 signals is shown on the right. Arrows point to the centrosome. Bar, 10 µm. (C) RT-PCR analysis of PDE4D3 wild-type (wtPDE4D3) and dominant-negative (dnPDE3D3) variants in RPE1 cells, RPE1 cells selected for stable expression of dnPDE4D3mRFP, RPE1 cells transiently transfected with dnPDE4D3mRFP, and a CHO line stably expressing the dnPDE4D3mRFP. The 650-bp band expected from the wild-type isoform of the PDE4D3 is detectable in the entire set of samples analyzed (left), whereas only the RPE1 cells transiently expressing the dnPDE4D3mRFP and the CHO stably expressing the dnPDE4D3mRFP show amplification of the dominant-negative variant of PDE4D3. The aforementioned data indicate that while in cells transiently transfected with dnPDE4D3mRFP the transgene can be detected, RPE1 cells that have been selected for stable expression of dnPDE4D3mRFP have lost the transgene.

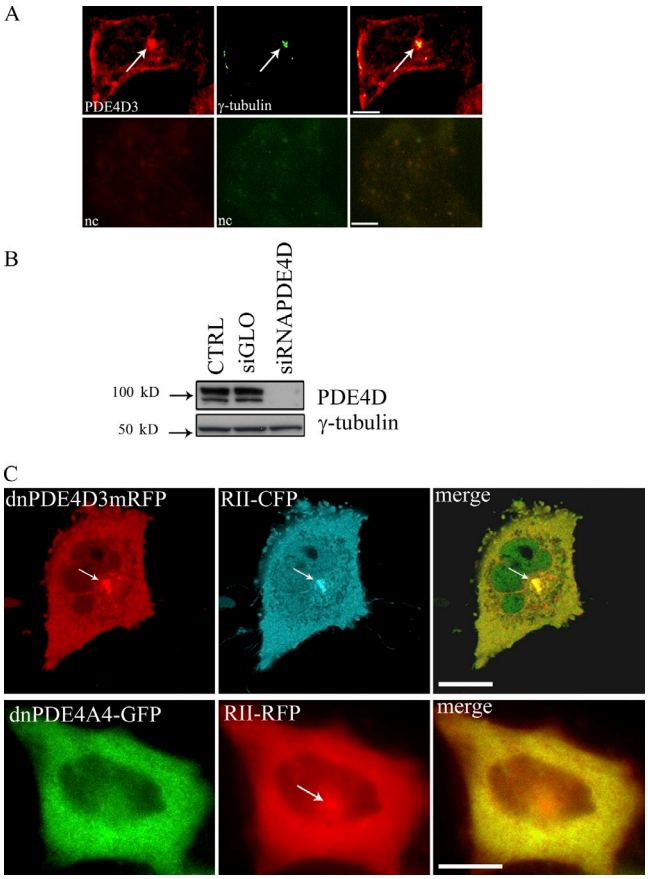

Figure S4. **Localization of endogenous PDE4D3 in CHO cells, the efficiency of its knockdown, and the localization of the overexpressed catalytically dead PDE4D3 isoform in CHO cells.** (A) Representative CHO cell coimmunostained with a PDE4D3-specific antibody and with the centrosome-specific antibody γ-tubulin. A negative control (nc) in which the primary antibody is omitted is shown in the bottom images. The overlay between the PDE4D3 (shown on the left) and the γ-tubulin signal is shown on the right. Arrows point to the centrosome. Bars, 10 µm. (B) Representative Western blot analysis of PDE4 expression in CHO cells treated as indicated. Lysates from control CHO cells (CTRL) and CHO cells overexpressing either the siRNA of PDE4D (siRNAPDE4D) or the control sequence siGLO (siGLO) were blotted and probed with specific antibody for PDE4D and γ-tubulin. Bands above and below 100 kD correspond respectively to PDE4D5/7 and PDE4D3/8/9 isoforms. γ-tubulin detection, indicated by the 50-kD band, was used as a control for the amount of protein loaded. (C, top row) Representative CHO cell stably expressing the RFP-tagged and catalytically inactive PDE4D3 (dnPDE4D3mRFP) and transiently transfected with CFP-tagged PKA type II regulatory subunit (RII-CFP). (bottom row) Representative CHO cell stably expressing the GFP-tagged and catalytically inactive PDE4A4 (dnPDE4A4-GFP) and transiently transfected with RFP-tagged PKA type II regulatory subunit (RII-RFP). Pictures on the right show the overlay between the two signals. Arrows point to the centrosome. Bars, 10 µm.

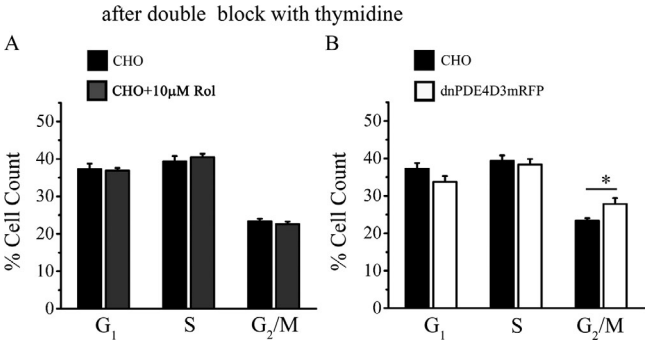

Figure S5. **Cell synchronization confirms an arrest of the cell cycle progression in G_2/M of cells expressing the dnPDE4D3.** (A) Quantification of flow cytometry scan analysis of control CHO cells and CHO cells treated with 10 µM rolipram (Rol). (B) Quantification of flow cytometry scan analysis for CHO and CHO stably expressing the catalytic inactive mutant of PDE4D3 (dnPDE4D3mRFP). Histograms indicate the mean percentages of cells in various phases of the cell cycle. Data are the mean of at least three independent experiments. Error bars represent SEM. A two-tailed unpaired *t* test was performed (*, $P < 0.05$).