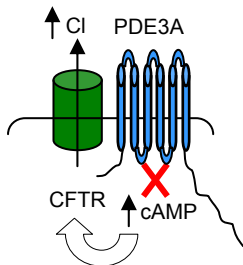
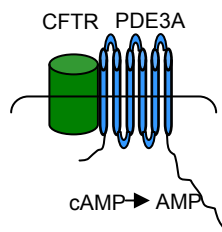


[i] PDE3A inhibition



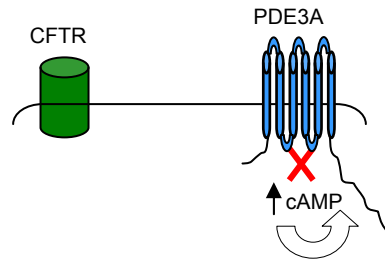
PDE3A inhibition increases compartmentalized cAMP at the plasma membrane. The increased cAMP activates CFTR channel function

[ii] PKA activation



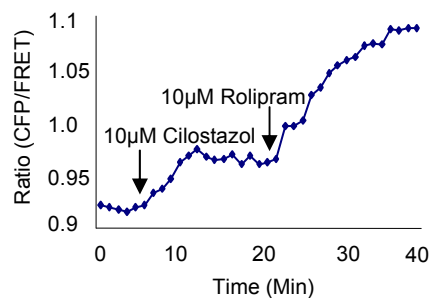
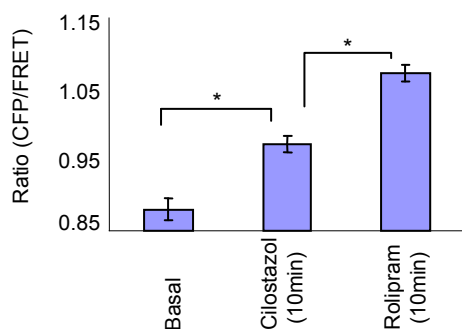
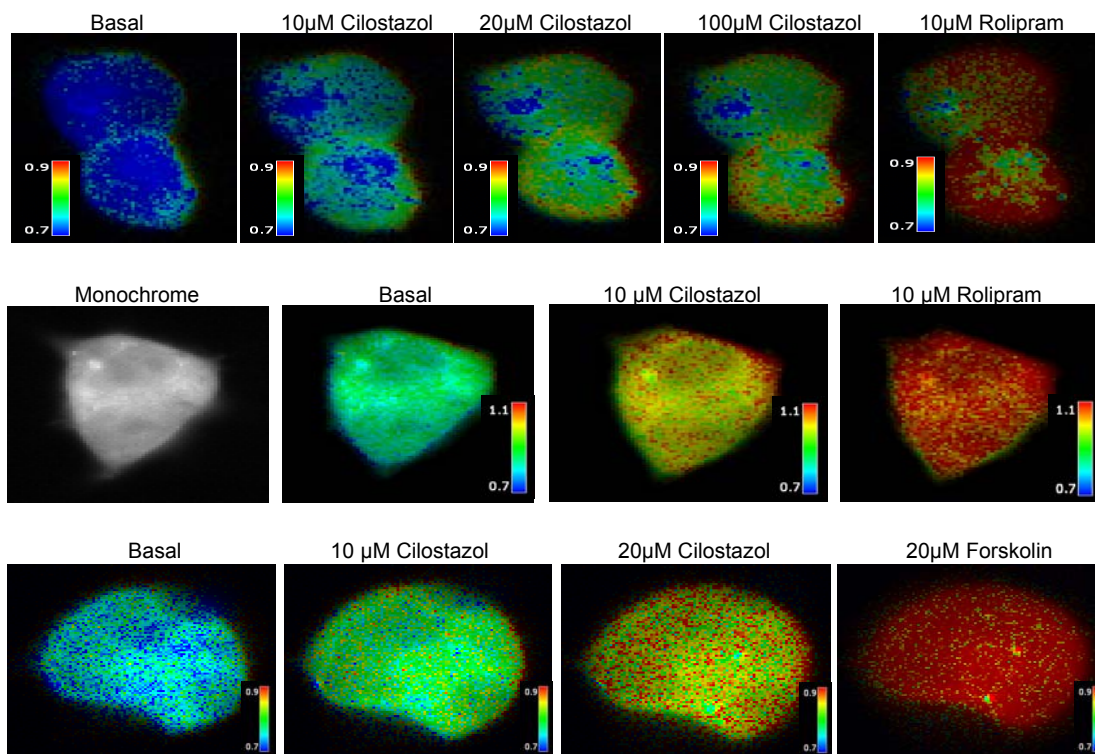
PKA activation clusters PDE3A and CFTR into microdomains and removes excess cAMP thus maintaining a high specificity

[iii] Cytoskeleton disruption

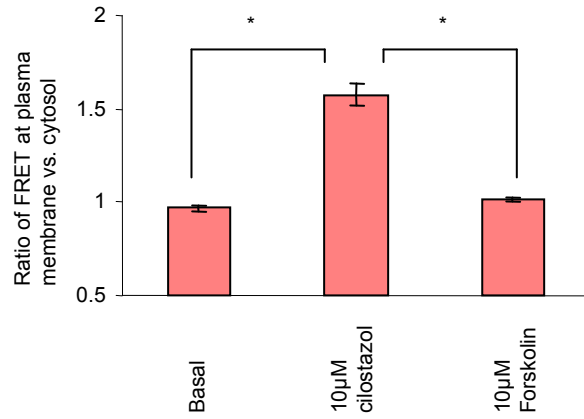


Cytoskeleton disruption scatters CFTR and PDE3A away from each other. Therefore, PDE3A inhibition no longer activates CFTR in compartmentalized fashion

Supplemental Figure S1. Pictorial representation of molecular mechanism of PDE3A coupling to CFTR chloride channel function.

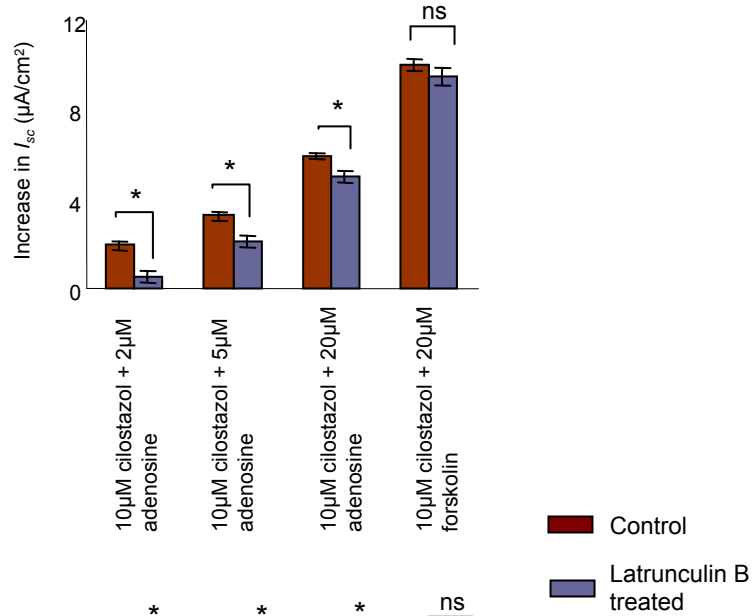


Supplemental Figure S2. Representative pseudocolor images of CFP/FRET emission ratio before (time = 0 min) and after addition of 10-20 μM PDE3 and PDE4 inhibitors cilostazol and rolipram (time = 10 min). Forskolin (20 μM) was added at the end of the experiment to monitor global cAMP response.

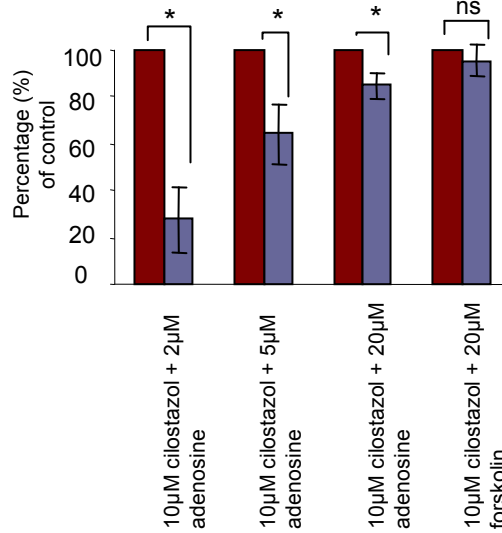


Supplemental Figure S3. Ratio of FRET at the plasma membrane vs. cytosol in Calu-3 cells (mean \pm SEM, $n=5$, $*p<0.001$) indicates compartmentalized distribution of cAMP upon PDE3 inhibition

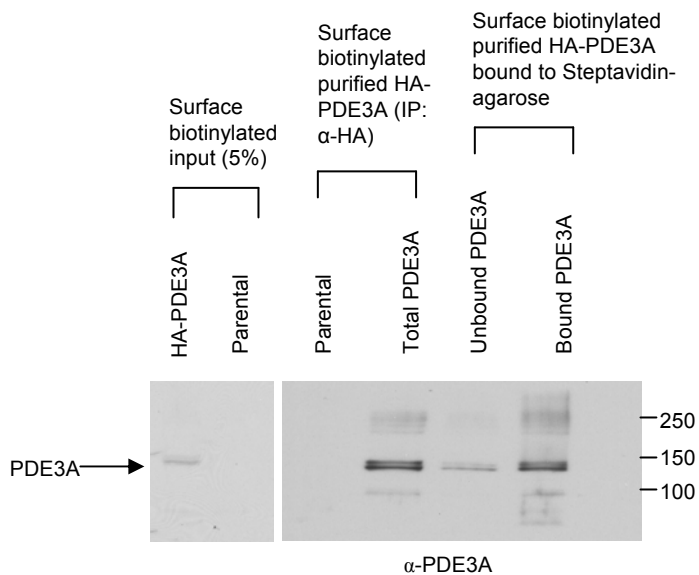
A



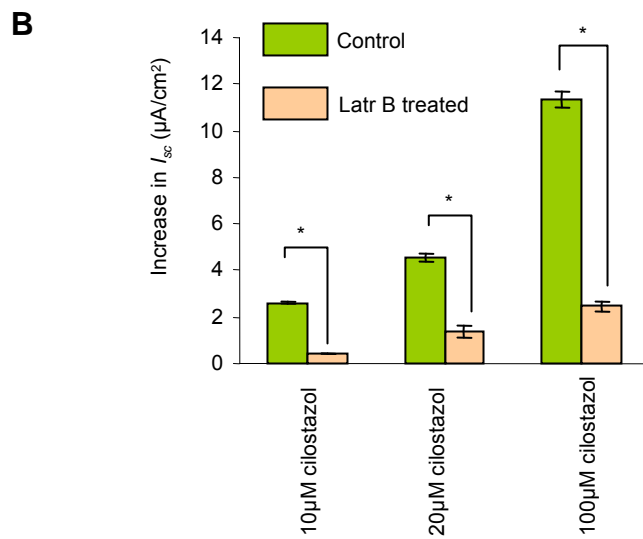
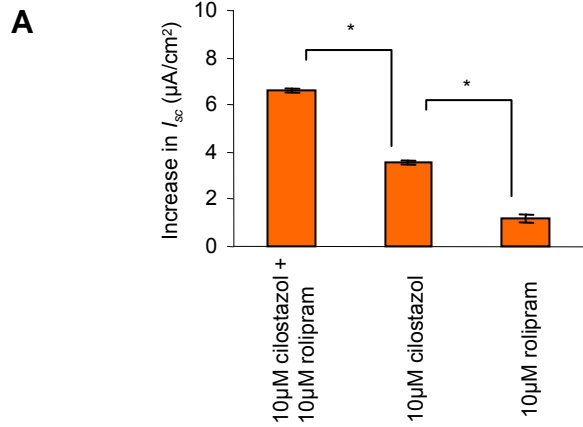
B



Supplemental Figure S4. (A) Adenosine mediated CFTR short-circuit currents (I_{sc}) in the presence of PDE3 inhibitor cilostazol are significantly inhibited by actin cytoskeleton disruptor latrunculin B (mean \pm SEM, $n=3$, $*p<0.001$). (B) The data in Figure S4A is converted to percentage (%) of control where control is taken as maximal 100%. The bar graph shows averaged maximal I_{sc} after latrunculin B treatment as a percentage of the control. The data shows mean \pm SEM, $n=3$, ns: not significant. $*p<0.001$ compared with control for each dose.



Supplemental Figure S5. Immunoblot for PDE3A expression on the plasma membrane of HEK293 cells expressing HA-PDE3A. Bound PDE3A clearly shows that about 90% of PDE3A is present at the plasma membrane.



Supplemental Figure S6. (A) Quantification of Ussing chamber experimental data in Figure 2 (mean \pm SEM, $n=3$, $*p<0.001$). (B) Quantification of Ussing chamber experimental data in Figure 7 (mean \pm SEM, $n=3$, $*p<0.001$).