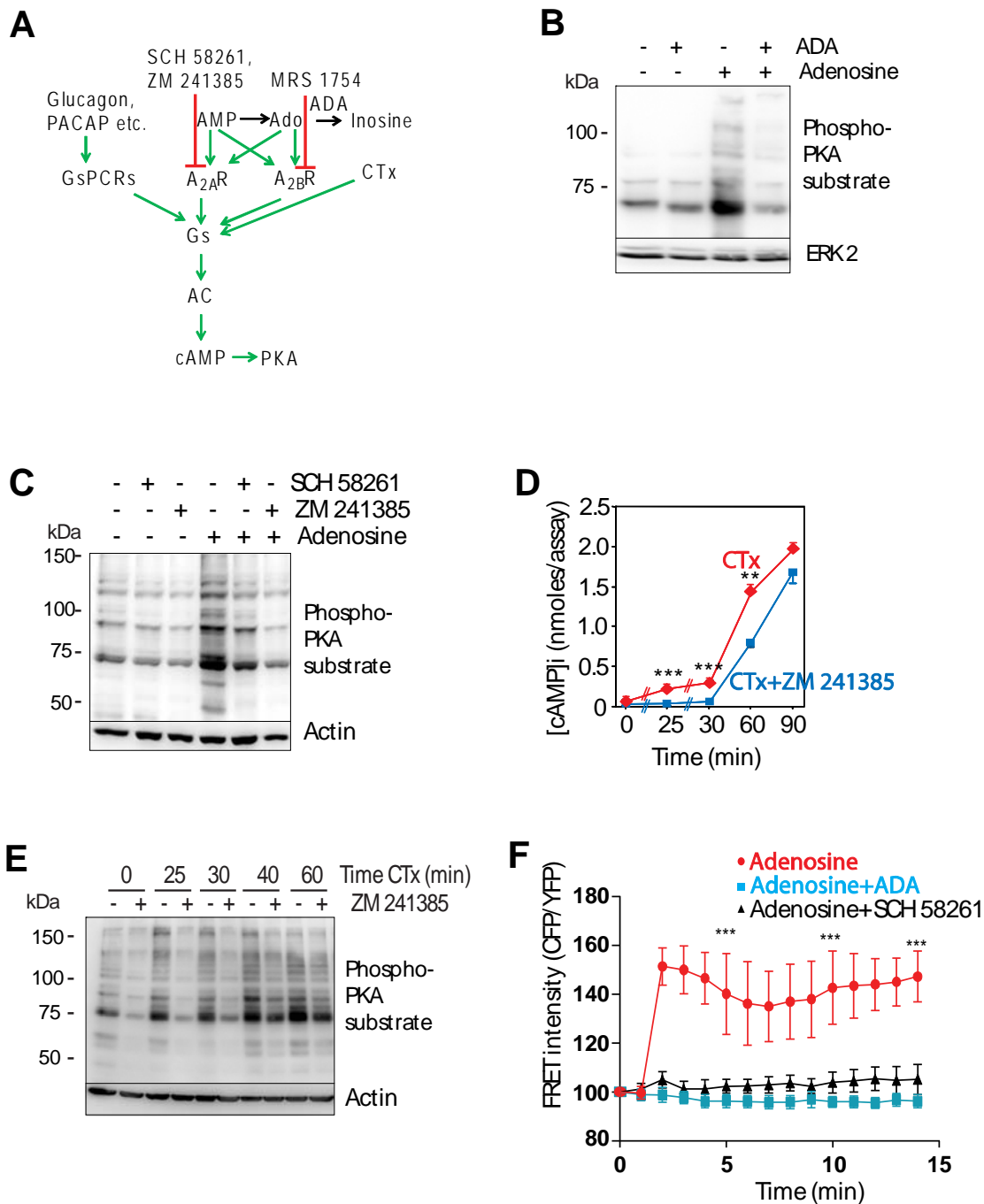


Supplementary Information

Activation of adenylyl cyclase causes stimulation of adenosine receptors

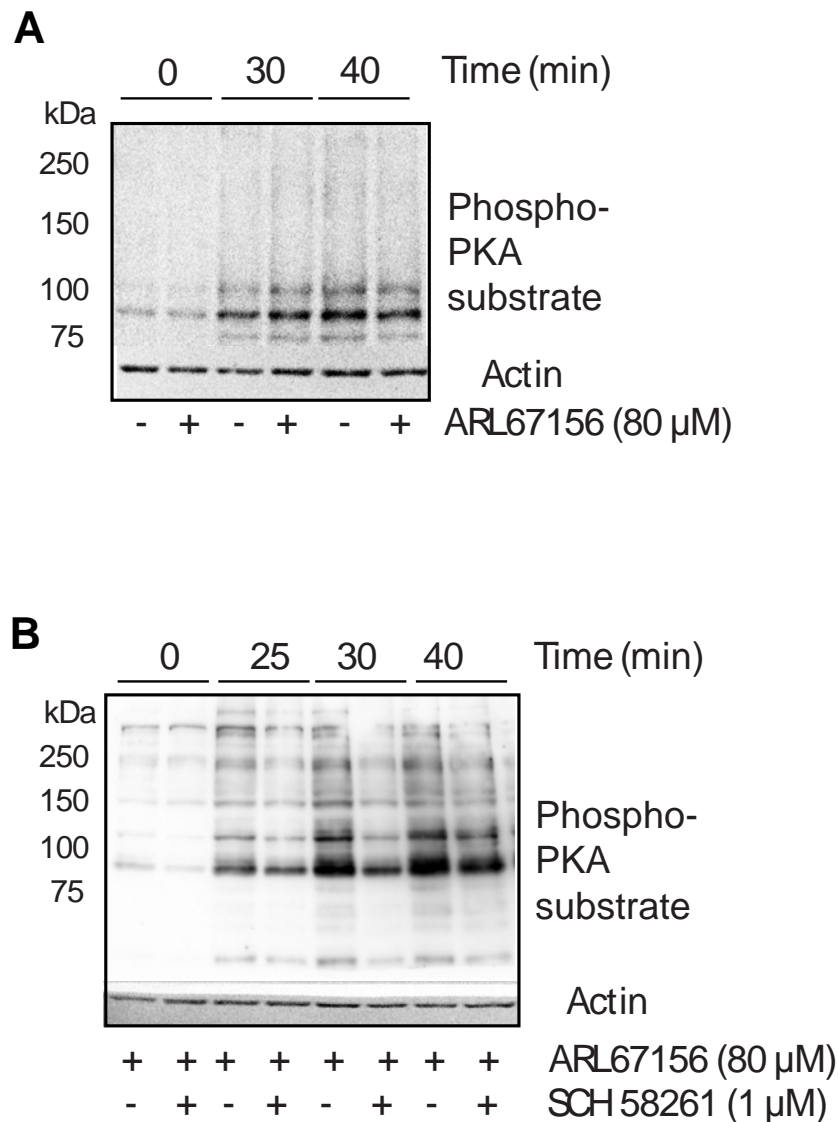
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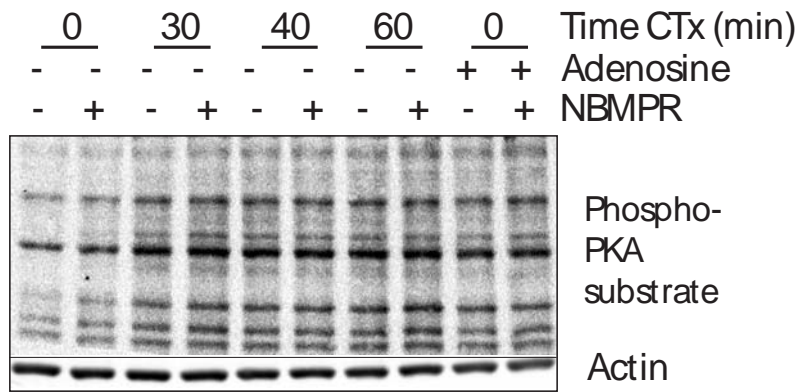


Supplementary Figure S1. $A_{2A}R$ antagonists inhibit adenosine-, CTx- and PACAP-induced increase in $[cAMP]_i$ or PKA substrate motif phosphorylation. (A) Model of the action of $A_{2A}R$ agonists and antagonists and of adenosine deaminase (ADA). Green: stimulatory effect; red: inhibitory effects. (B-E) PC12 cells were pre-incubated with ADA (1 U/ml) (B) ($n=3$), ZM 241385 (10 μ M) or SCH 58261 (100 nM) (C) ($n=3$), followed by stimulation with adenosine (10 μ M) and immunoblotting with antibodies recognizing phosphorylated PKA substrate motif or β -actin, respectively. (C and D) The cells were stimulated with CTx in the absence and presence of ZM 241385 (1 μ M) for the indicated time, followed by determination of the cellular cAMP (D) or immunoblotting with anti-PKA substrate motif phosphorylation and anti- β -actin (E) ($n=5$). (F) FRET trace obtained

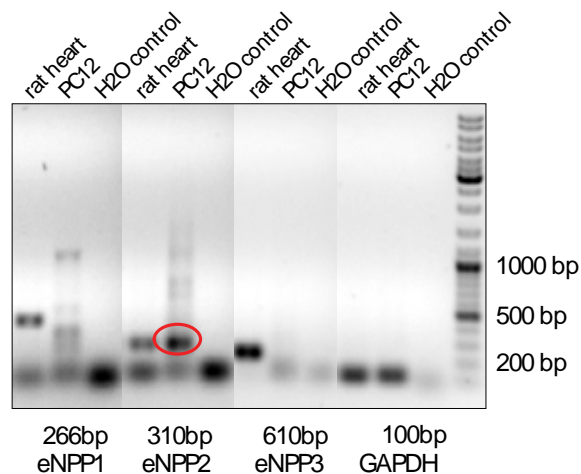
from PC12 cells expressing T Epac^{vv} (representing means \pm SD), stimulated with adenosine with and without ADA or SCH 58261. Following recording of a baseline with (n=3) or without (n=3) ADA (1 U/ml) or SCH 58261 (1 μ M), the cells were incubated with adenosine (1 μ M). (D) Numbers are means \pm SD. Asterisks indicate significant differences between the curves at those time points. **P < 0.01, ***P < 0.001.



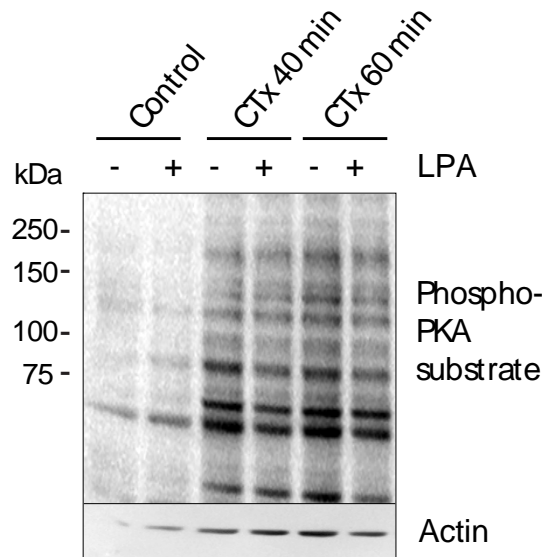
Supplementary Figure S2. The CD39 inhibitor ARL67156 does not prevent the inhibitory effect of $A_{2A}R$ inhibition on CTx-induced PKA substrate motif phosphorylation and did not affect CTx-induced PKA substrate motif phosphorylation itself. PC12 cells were pre-incubated with ARL67156 (80 μ M) or vehicle (A), or with ARL67156 with and without SCH 58261 (1 μ M) (B) for 10 min followed by stimulation with CTx for the indicated time. Cell lysates were subjected to immunoblotting with antibodies recognizing phosphorylated PKA substrate motif or β -actin, respectively (n=3 each).



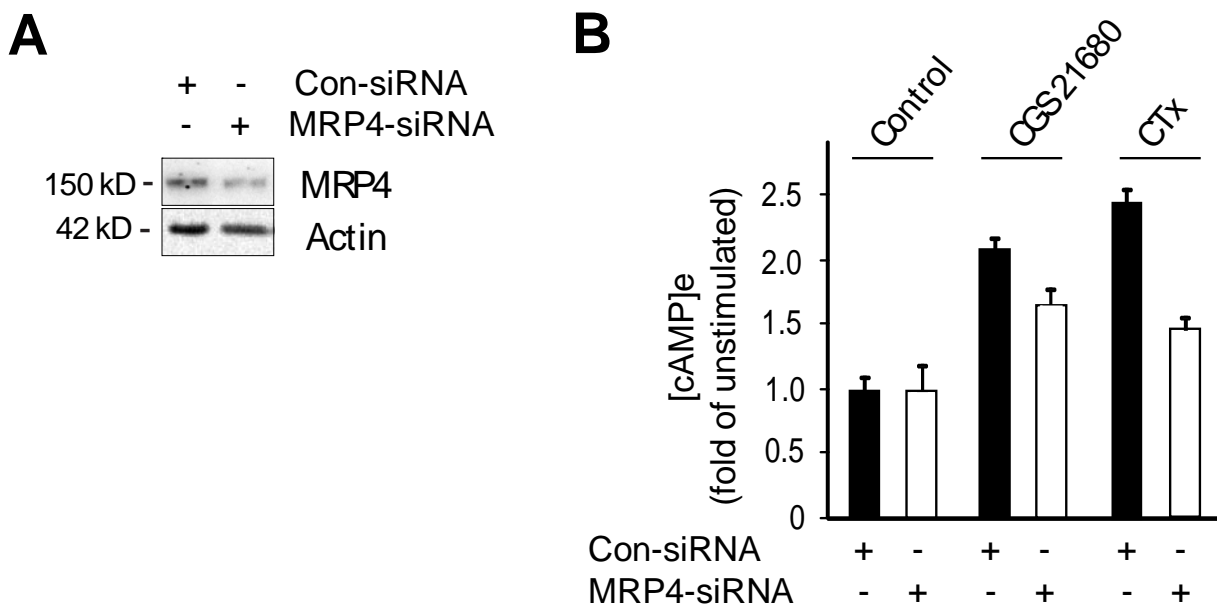
Supplementary Figure S3. Lack of effect of NBMPR on CTx- and adenosine-induced PKA substrate motif phosphorylation. PC12 cells were pre-incubated with NBMPR (500 nM) for 30 min followed by addition of CTx (200 U/ml) or adenosine (1 μ M) to the cells for the time indicated (CTx) or for 15 min (adenosine). Cell lysates were subjected to immunoblotting with antibodies recognizing phosphorylated PKA substrate motif or β -actin, respectively (n=3 each).



Supplementary Figure S4. Analysis of the PC12 cells for the expression of eNPP isoenzymes and GAPDH by RT-PCR. RNA was extracted from rat heart (serving as positive control) and PC12 cells. The expression of eNPP isoenzymes was performed by isoenzyme-specific RT-PCR.



Supplementary Figure S5. Effect of LPA on CTx-induced PKA substrate motif phosphorylation in PC12 cells. The cells were stimulated LPA (1 μ M) for the indicated time. Cellular lysates were analyzed by immunoblotting and antibodies recognizing phosphorylated PKA substrate motif or β -actin (n=3).



Supplementary Figure S6. Inhibition of MRP4 expression by siRNA reduces cAMP extrusion in response to CTx and PACAP. (A) Analysis of the cellular lysates of MRP4-siRNA- and control-siRNA-treated cells by anti-MRP4 and anti- β -actin immunoblotting (n=5). (B) Cells pre-treated with MRP4-siRNA or control siRNA were incubated with CGS 21680 (1 μ M) or CTX for one hour followed by determination of the cAMP concentration in the medium ([cAMP]_e). The numbers are fold of [cAMP]_e in the media of the unstimulated cells (n=5). Con-siRNA, control-siRNA.