Supplementary Table 1.

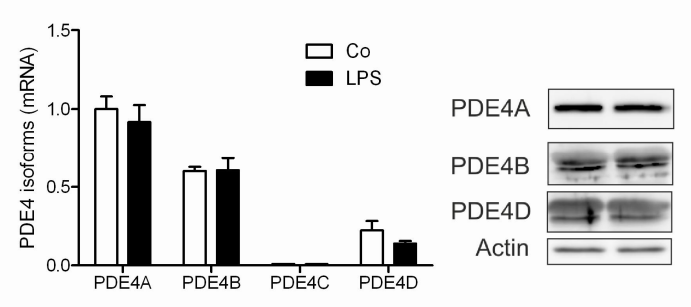
The effect of rolipram on cAMP levels in J774 macrophages		
treatment	*cAMP (pg mL^{-1})*	
Co	3.5 ± 2.3	
rolipram	367.0 ± 106.9	a
LPS	61.8 ± 22.2	b
rolipram + LPS	386.2 ± 51.2	c
IBMX (100 µM) + salbutamol (1 µM)	3900 ± 341.6	

J774 cells were preincubated with the vehicle (DMSO, v/v 0.1%), rolipram (2 µM) or IBMX (100 µM) for 1h and treated with LPS (10 ng mL^{-1}) or salbutamol (1 µM) for 1 min, and cAMP levels were measured by a method detecting acetylated cAMP using cAMP ELISA Kit (Cell Biolabs Inc., San Diego, CA, U.S.A.). Results are expressed as mean ± S.E.M., number of repeats = 4. One-way ANOVA with Bonferroni's post-test was performed. (a) $p < 0.01$ between Co and rolipram, (b) $p > 0.05$ (not significant) between Co and LPS, and (c) $p < 0.01$ between LPS and LPS+rolipram. The combination of IBMX and salbutamol was used as a positive control in the experiment.

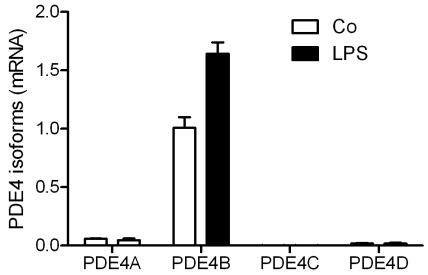
Supplementary Figure 1.

The expression of PDE4A, PDE4B, PDE4C and PDE4D in J774 macrophages and mouse primary peritoneal macrophages (PM) from WT and MKP-1(–/–) mice.

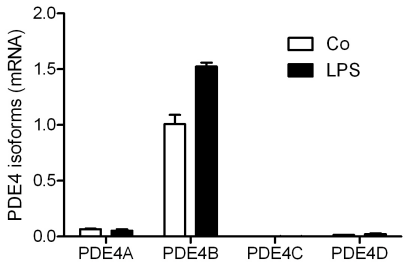
(**A**) In J774 cells, mRNA and protein levels of PDE4 isoforms were investigated in unstimulated cells (Co) or in cells stimulated with LPS (10 ng mL^{-1}) for 1h and mRNA and protein levels were measured by quantitative RT-PCR (number of repeats =4) and Western blot (number of repeats = 3), respectively. PDE4 mRNA levels were related to GAPDH mRNA. PDE4A in unstimulated cells was set as 1 and the other values were compared to the value.



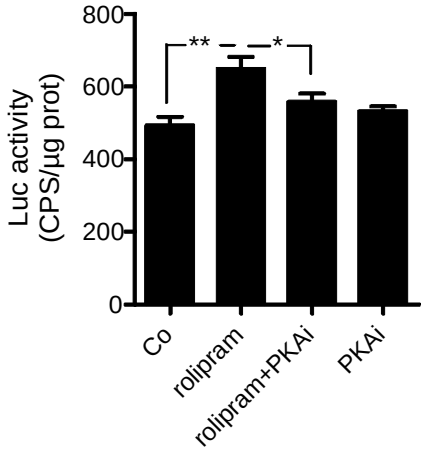
(**B**) mRNA levels of PDE4 isoforms in PM from WT mice. PM were stimulated with LPS (100 ng mL^{-1}) for 3h, and PDE4 isoform mRNA levels were measured with quantitative RT-PCR (number of repeats = 4). PDE4 mRNA levels were related to GAPDH mRNA. PDE4B in unstimulated cells was set as 1 and the other values were compared to the value.



(**C**) mRNA levels of PDE4 isoforms in PM from MKP-1(–/–) mice. PM were stimulated with LPS (100 ng mL^{-1}) for 3h, and PDE4 isoform mRNA levels were measured with quantitative RT-PCR (number of repeats = 4). PDE4 mRNA levels were related to GAPDH mRNA. PDE4B in unstimulated cells was set as 1 and the other values were compared to the value.



Supplementary Figure 2.



cAMP-responsive element-driven transcription was increased by rolipram and inhibited PKA inhibitor 6-22 amide (PKAi) in A549 human bronchial epithelial cells.

cAMP responsive element (CRE) reporter [pCRE(luc)neo], which also contained a neomycin resistance gene for mammalian selection, was kindly provided by Professor Hartmut Kleinert (Johannes Gutenberg University, Mainz, Germany). pCRE(luc)neo contained four CRE sites that bind CREB and thereby drive luciferase expression. A549 human bronchial epithelial cells (American Type Culture Collection, Manassas, VA) were cultured at 37°C in 5% CO_2 atmosphere in Ham's F-12K (Kaighn's modification) medium containing 5% heat-inactivated fetal bovine serum (all from Invitrogen, Paisley, UK). For the experiments, A549 cells ($1 \cdot 10^5$ cells/well) were seeded on a 24-well plate in 500 µL of fresh culture medium containing 5% fetal bovine serum (no antibiotics), and grown for 24h. Transfection complexes were prepared by mixing 1 µg of plasmid DNA and 2 µL Lipofectamine 2000 (Life Technologies Europe BV, Espoo Finland) in 100 µL of serum-free culture medium without antibiotics, and incubated in room temperature for 20 min. Transfection complexes were then added to the cells, and the cells

were further incubated for 24 h before the experiments. In the beginning of the experiment, vehicle (DMSO, v/v 0.1%), rolipram (2 μM) or PKAi (5 μM) were added to the cells in frexh medium and cells were incubated for 24h. Luciferase activity was then measured by luminometer using Luciferase Assay System (Promega, Madison, WI, USA). Luciferace activity was related to the total protein, and the results are expressed as mean ± S.E.M., number of repeats = 6. One-way ANOVA with Bonferroni's post-test was performed, and statistical significance is indicated as * $p < 0.05$ and ** $p < 0.01$.