Phosphodiesterase 4B negatively regulates endotoxin-activated interleukin-1 receptor antagonist responses in macrophages

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SUPPLEMENTARY FIGURE LEGENDS

sFigure 1. Effect of rolipram on LPS-induced icIL-1Ra mRNA expression in mouse macrophages. Raw 264.7 cells (A) and mouse peritoneal macrophages (B) were pretreated for 20 min with 10 μM rolipram prior to LPS (10 ng/ml) stimulation for 3 h. The icIL-1Ra mRNA levels in the cells were determined by real-time PCR and expressed as fold induction to the untreated cells. Data are the mean ± SEM (*n* = 4 in A; *n* = 5 in B).

sFigure 2. Rolipram does not alter cell viability in LPS-stimulated macrophages. Raw 264.7 cells (A) and mouse peritoneal macrophages (B) were pretreated for 20 min with 10 μM rolipram before LPS (10 ng/ml) stimulation for 8 h. Cell viability was determined by the MTT assay as described in the Methods. Data are the mean ± SEM (*n* = 4 in A; *n* = 5-6 in B).

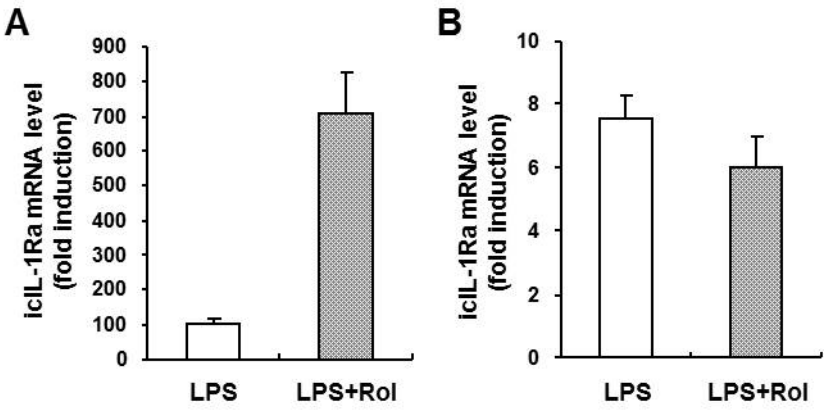
sFigure 3. PDE4 isoform expression and regulation of PDE4B ablation on IL-1Ra secretion in LPS-stimulated bone marrow-derived macrophages. (A-C) Bone marrow-derived macrophages (BMDM) were incubated with or without LPS (100 ng/ml) for 3 h. The levels of PDE4A (A), PDE4B (B), and PDE4D (C) mRNA were determined by real-time PCR and expressed as fold change to the untreated cells. (D) BMDM prepared from $PDE4B^{+/+}$ and $PDE4B^{-/-}$ mice were pretreated for 20 min with rolipram (10 μM) followed by LPS (100 ng/ml) stimulation for 8 h. IL-1Ra

accumulation in the medium supernatant was measured by ELISA. Data are the mean ± SEM (n = 5 in A-C; n = 4-6 in D).

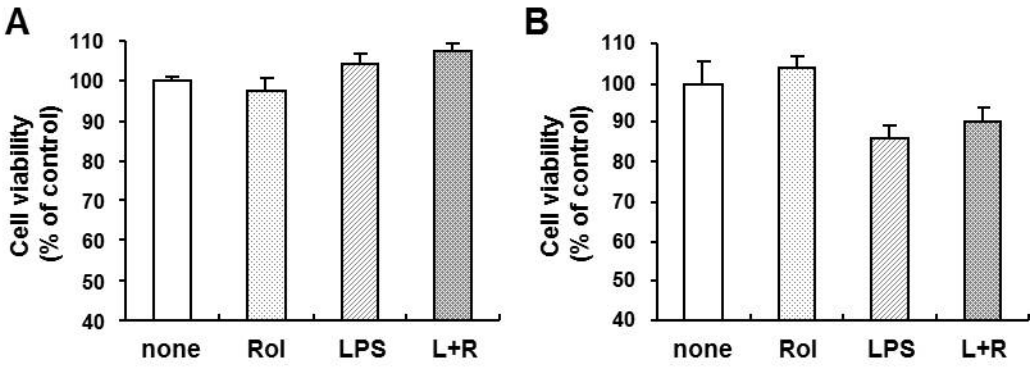
sFigure 4. Uncropped gel images of Western blots seen in Figure 7A. Two images with different brightness adjustments (25% and 50%) are shown.

sFigure 5. Uncropped gel images of Western blots seen in Figure 7C. Two images with different brightness adjustments (25% and 50%) are shown.

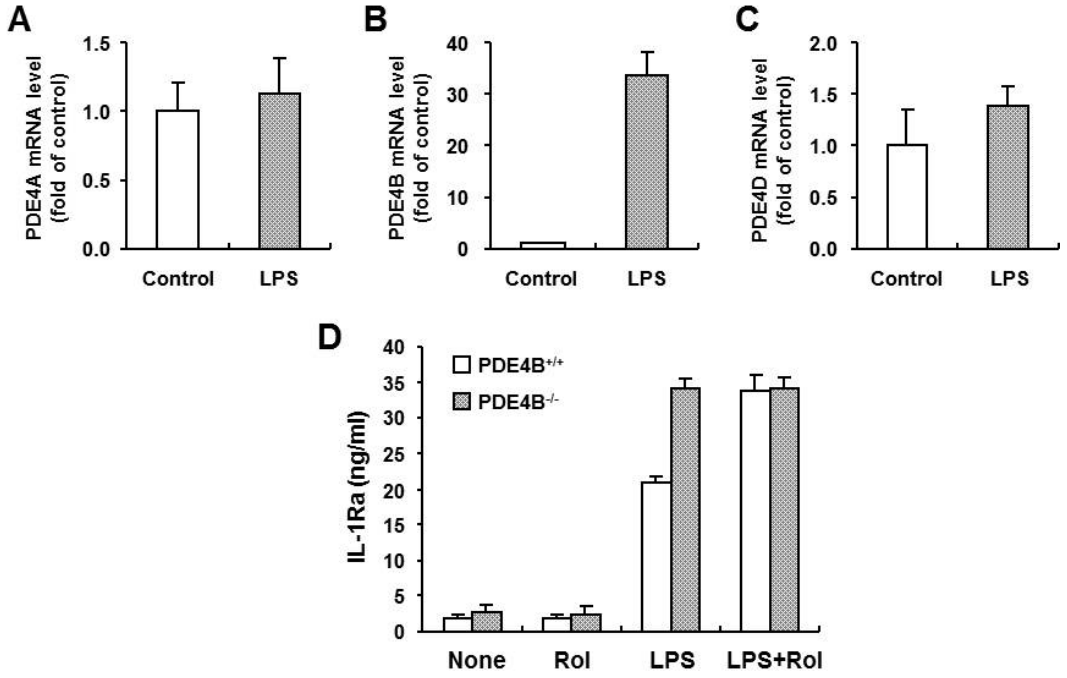
Supplementary Figure 1



Supplementary Figure 2

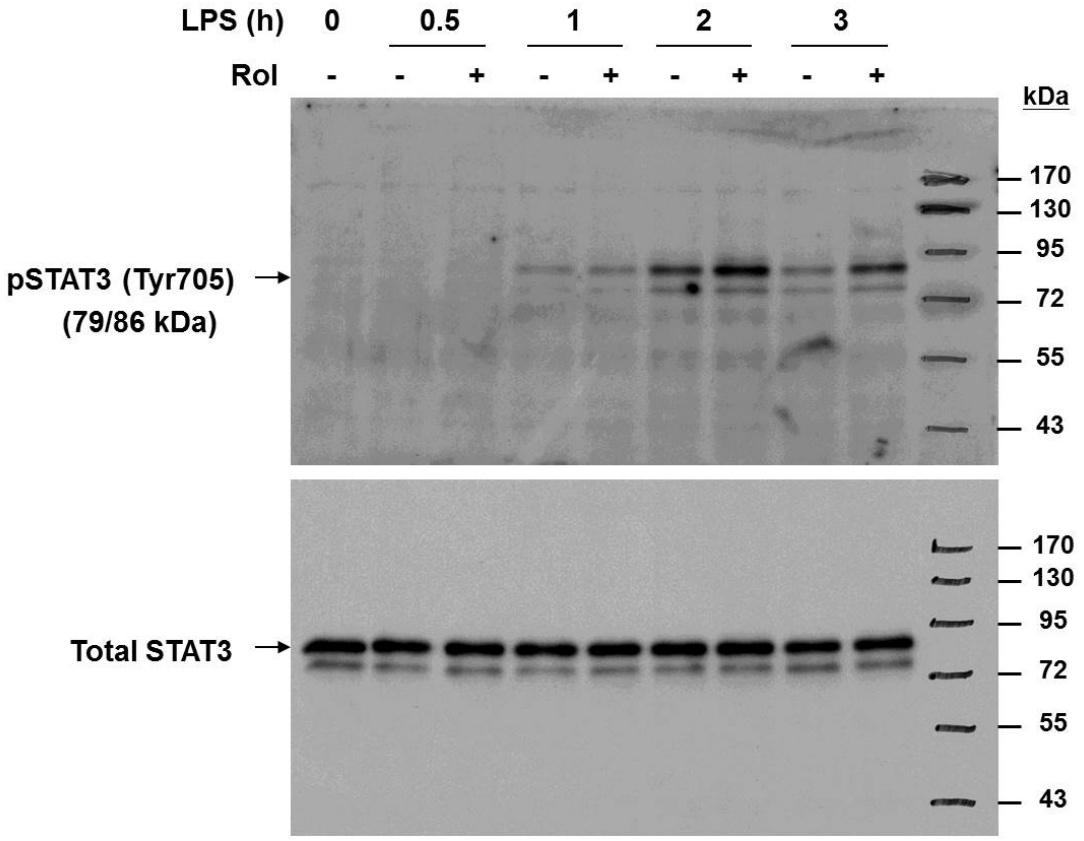


Supplementary Figure 3



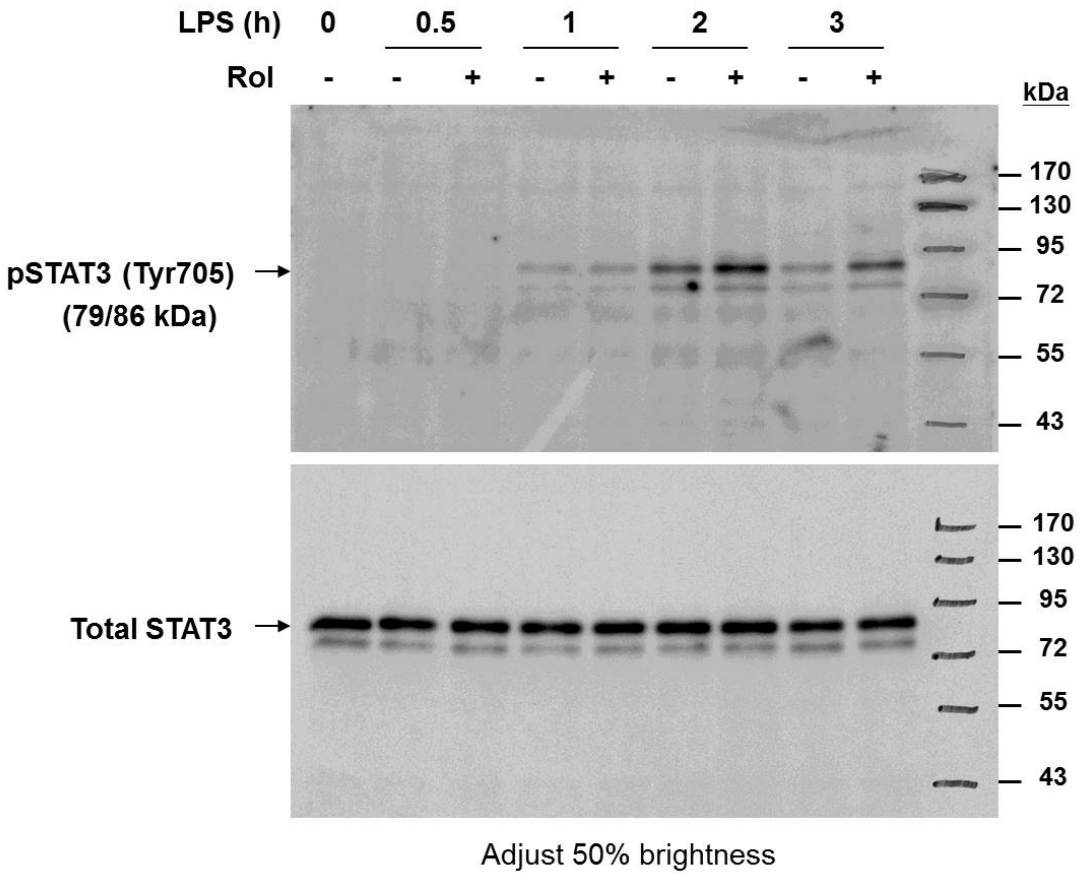
Supplementary Figure 4

25% brightness adjustment



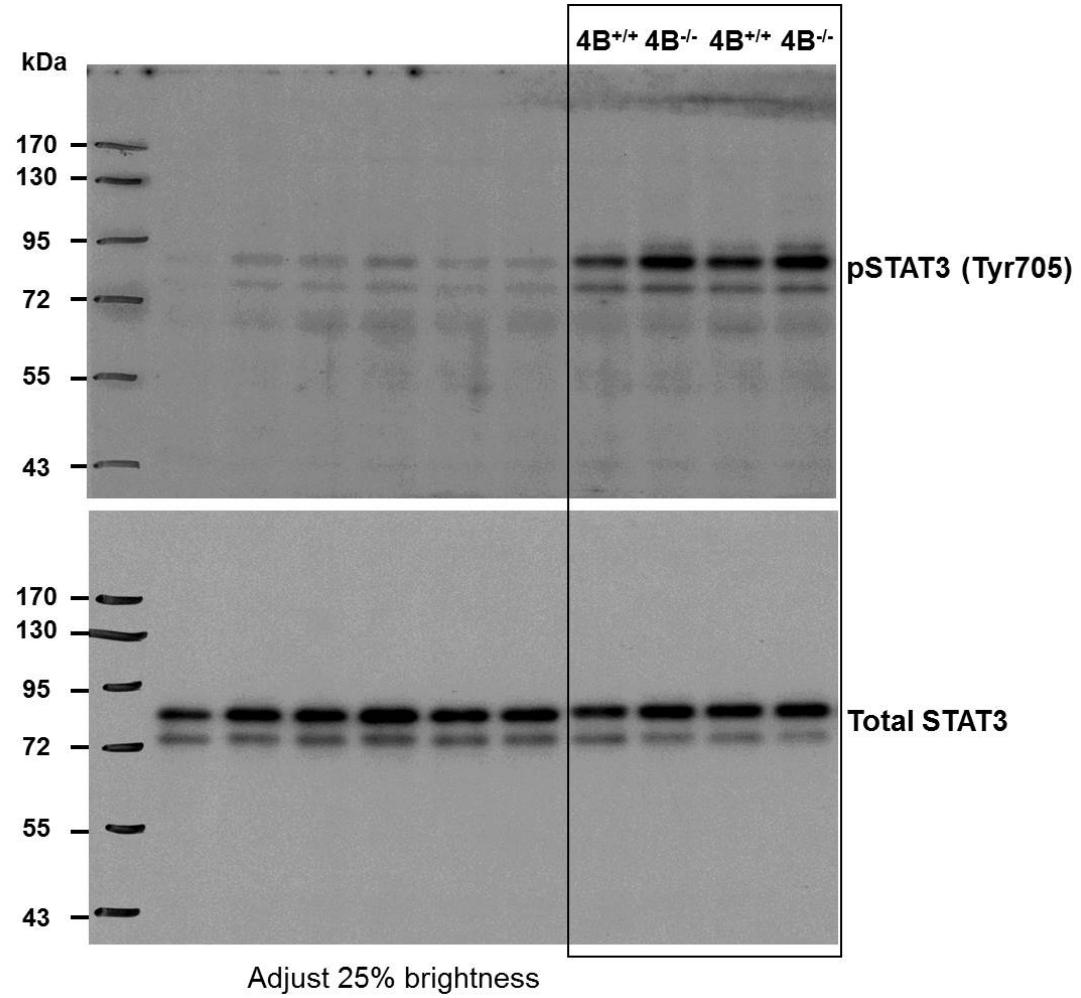
Supplementary Figure 4

50% brightness adjustment



Supplementary Figure 5

<u>25% brightness adjustment</u>



Supplementary Figure 5

<u>50% brightness adjustment</u>