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Supplementary Materials for

Suppression of LPS-Induced TNF-α Production in Macrophages by cAMP Is Mediated by PKA-AKAP95-p105

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Published 16 June 2009, *Sci. Signal.* **2**, ra28 (2009)
DOI: 10.1126/scisignal.2000202

This PDF file includes:

Gene	Affymetrix Data	qRT-PCR Data
PKA-RIα	Present	Medium (Ct = 31)
PKA-RIβ	Marginal	Not detectable
PKA-RIIα	Present	Medium (Ct = 31)
PKA-RIIβ	Present	Low (Ct = 36)
PKA-Cα	Present	High (Ct = 26)
PKA-Cβ	Present	High (Ct = 25)
EPAC1	Marginal	Low (Ct = 34)
EPAC2	Marginal	Low (Ct = 36)

Fig. S1. Expression of *PKA* and *EPAC* mRNAs in RAW264.7 cells. Data from the analysis of the expression of *PKA* and *EPAC* mRNAs derived from Affymetrix gene chip arrays and qRT-PCR assays and a comparison of their abundance are presented. The Ct value denotes the cycle number at which mRNA became detectable in a 40-cycle PCR reaction with an input of 100 ng of total RNA.

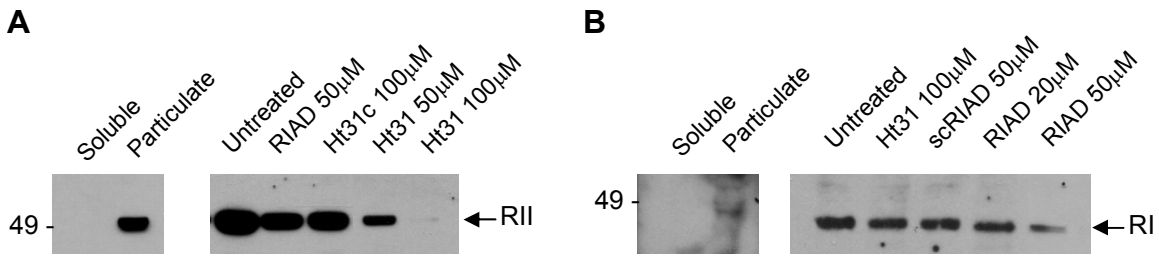

Fig. S2. Specificities of PKA-anchoring inhibitor peptides. (**A**) Subcellular fractionation of RAW264.7 cells and subsequent Western blotting analysis demonstrated that the PKA RIIβ subunit segregated with the particulate fraction (left panel). Preincubation of cells with PKA-anchoring inhibitor peptides resulted in a selective depletion of RIIβ from the particulate fraction with increasing concentrations of Ht31 peptide, and no effect with a control peptide (Ht31c) or an RI subunit–specific peptide (RIAD). (**B**) Subcellular fractionation of RAW264.7 cells and subsequent Western blotting analysis demonstrated that the PKA RIα subunit segregated with the particulate fraction (left panel). Preincubation of cells with PKA-anchoring inhibitor peptides resulted in a selective depletion of RIα from the particulate fraction with increasing concentrations of RIAD peptide, and no effect with a control peptide (scRIAD) or an RII subunit–specific peptide (Ht31).

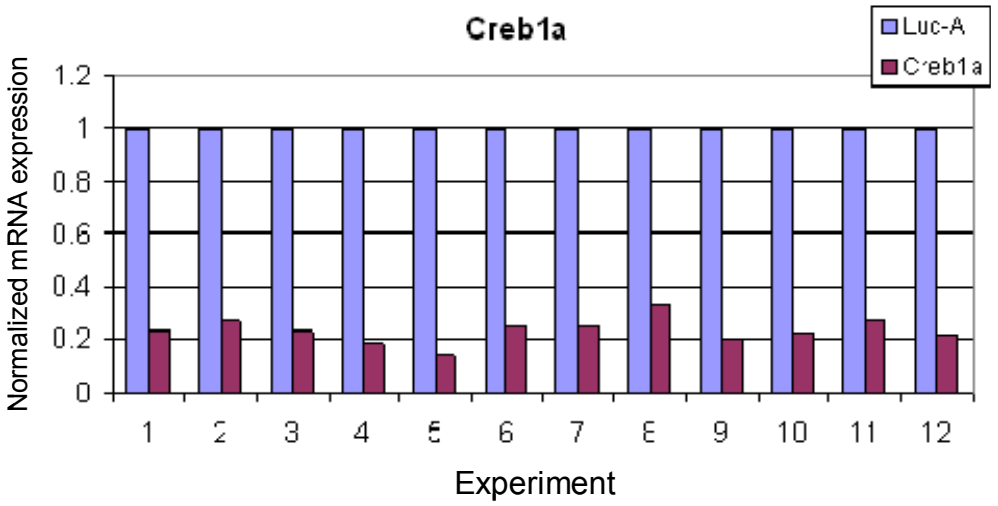

Fig. S3. RNAi-mediated knockdown of *Creb1a* mRNA in RAW 264.7 cells. RAW 264.7 cell lines were generated that stably expressed shRNAs against either Creb1a or luciferase (Luc-A). The abundance of *Creb1a* mRNA in the shCreb cell line is shown relative to that in the shLuc control cell line for 12 experiments carried out over a period of 4 weeks.

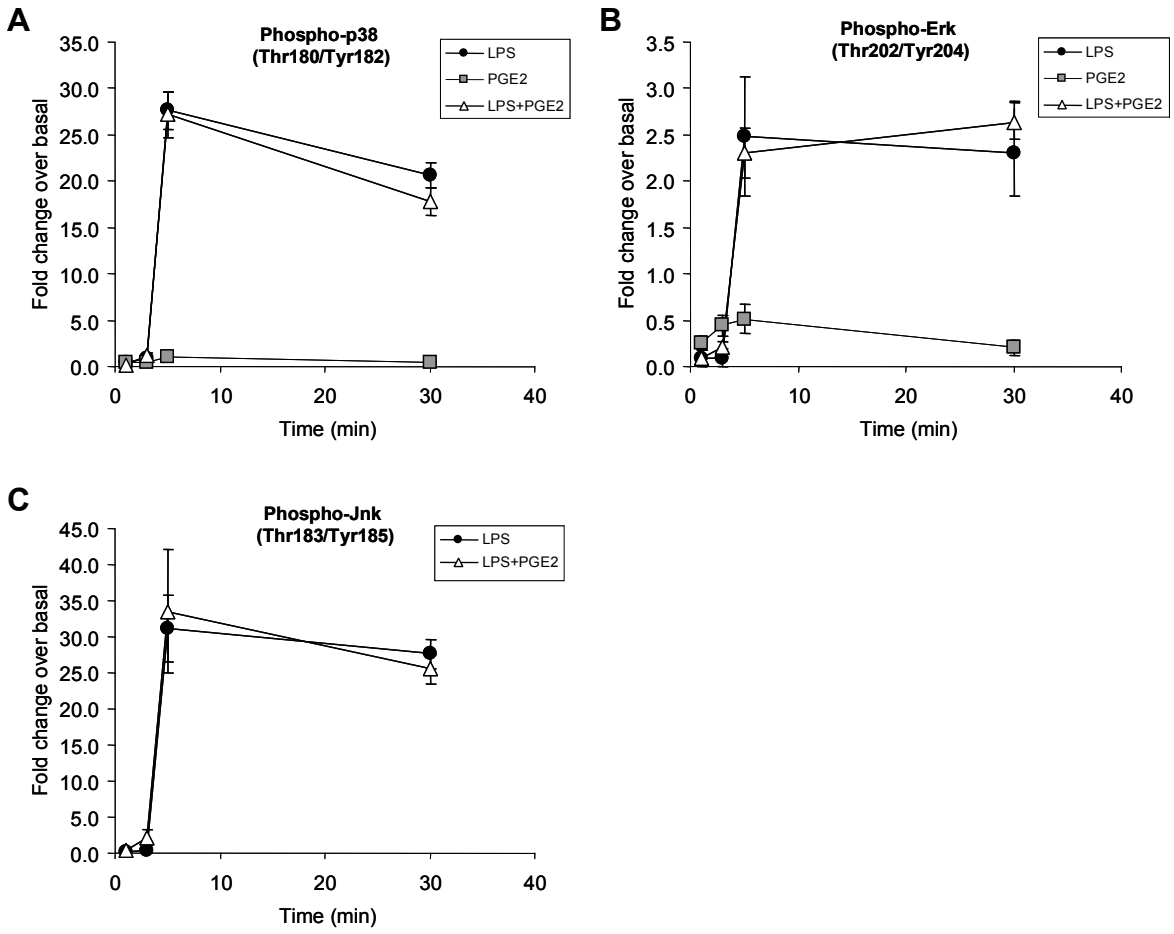

Fig. S4. The effect of PGE_2 on LPS-induced phosphorylation of MAPKs. The fold changes in the phosphorylation of (**A**) p38, (**B**) ERK, and (**C**) JNK in response to LPS (100 ng/ml LPS + 250 pM LBP), PGE_2 (10 μM), or both ligands for the indicated times are shown and relative to the abundance of each phosphoprotein in unstimulated cells.

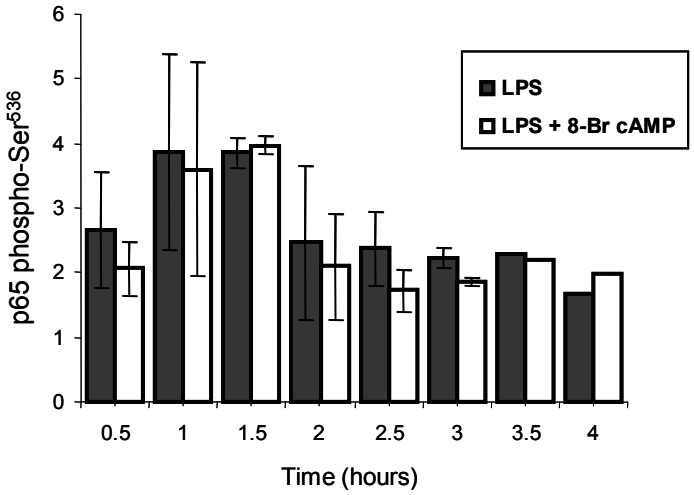

Fig. S5. The effect of cAMP on LPS-induced phosphorylation of p65 at Ser^{536}. Phosphorylation of p65 at Ser^{536} in RAW 264.7 cells in response to either LPS (100 ng/ml + 250pM LBP) or LPS and 8Br-cAMP (100 μM) for the indicated times is shown as the fold-increase in the abundance of phosphorylated p65 in treated cells relative to that in unstimulated cells.

A

Gene	*Affymetrix level*	*qRT-PCR level*	*Location*
D-AKAP550/LBA	High	High	Endosome
D-AKAP1	High	High	Mitochondria
AKAP-Lbc (Ht31)	High	High	Cytoskeletal
AKAP95	High	High	Nucleus/Cytoplasm
AKAP350	Medium	Medium	Centrosome
AKAP220	Unclear	Medium	Vesicular
D-AKAP2	Medium	Medium	Mitochondria
AKAP79/150	Unclear	Low	Plasma membrane
AKAP18	Unclear	Low	Plasma membrane
Gravin	Medium	Low	Plasma membrane

B

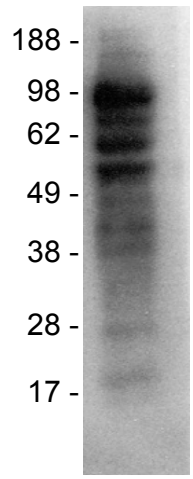

Fig. S6. Relative abundance of endogenous AKAPs in RAW 264.7 cells. (**A**) Transcript data for AKAP expression in RAW cells derived from Affymetrix gene chip arrays and quantitative PCR. (**B**) Detection of AKAP proteins expressed in RAW cells by overlay assay using RII alpha PKA regulatory subunit.