

PROTEIN KINASE R-DEPENDENT REGULATION OF INTERLEUKIN-10 IN RESPONSE TO DOUBLE-STRANDED RNA*

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Running head: PKR-dependent regulation of IL-10

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SUPPLEMENTAL FIGURE LEGENDS

FIGURE S1. Optimal concentrations of the chemical inhibitors. *A*, wt SM cells were treated with indicated doses of SB203580 (SB), PD98059 (PD), SP600125 (SP), or as a control the solvent DMSO (D) for 1 h, or left untreated (C) prior to the treatment with pIC (50 µg/ml) for 30 min. Cell lysates were used to measure phospho-MAPKAPK2, phospho-Erk1/2 and phospho-JNK levels by Western blot. *B*, wt SM were treated with indicated doses of BAY-11, or as a control the solvent DMSO (D) for 1 h, prior to the treatment with pIC (50 µg/ml) for 30 min, or left untreated (D). Total RNA was isolated and the levels of IL-10 mRNA quantitated by PCR.

FIGURE S2. Appearance of wt and pkr-ko SM cells. *A*, Phase-contrast microscopic images of wt and pkr-ko SM cells compared to the murine macrophage cell line *RAW264.7*. *B*, Western blot showing protein bands detected in lysates from the wt or pkr-ko SM cells using the anti-PKR polyclonal antibody D-20 (Santa Cruz Biotechnology).

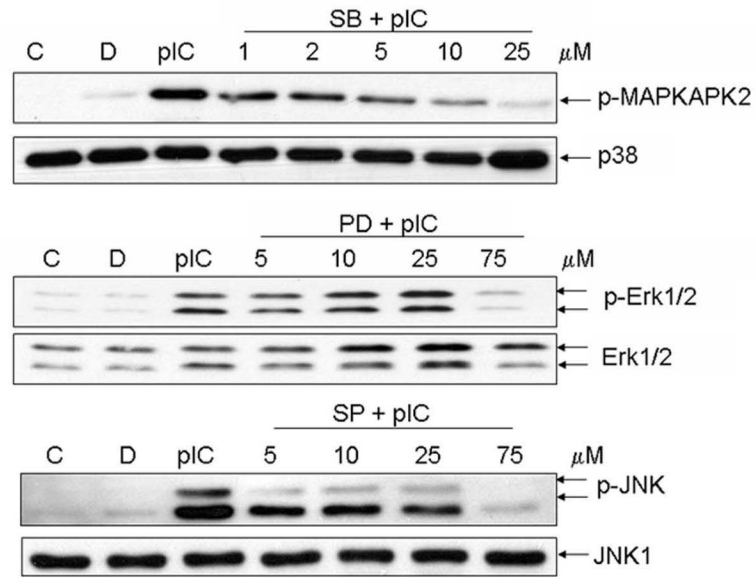
FIGURE S3. IL-10 induction is impaired in wt SM cells treated with an inhibitor or an RNAi targeting PKR. *A*, To further confirm the PKR dependence of the different induction of IL-10 demonstrated in cell lines wild-type or ablated for PKR, SM cells were left untreated (NT) or treated with the PKR inhibitor 2-aminopurine (2AP, 10 µM) for 1 h prior to stimulation with LPS (10 ng/ml) for 6 h. *B*, RNAi constructs targeting LacZ, as a nonspecific control, or PKR were transiently transfected into wt SM 36 h prior to stimulation with LPS (10 ng/ml) for 6 h. Total RNA was isolated and the levels of the IL-10 transcript were measured by real-time PCR. *C*, Phase-contrast and fluorescent microscope images of wt SM cells transiently transfected with constructs expressing the control, LacZ or PKR targeting RNAs. As the silencing RNA are inserted into the 3' UTR region of a GFP reporter gene, appropriate expression of the interfering RNA is confirmed by fluorescence in cells.

FIGURE S4. Sequence comparison between the murine and human IL-10 promoter sequences.

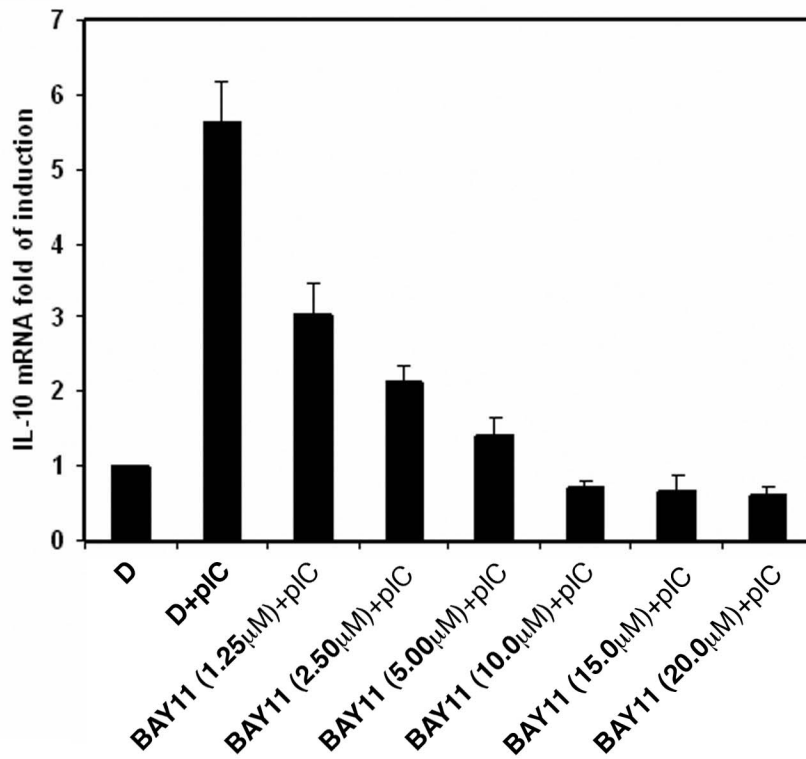
Genomic sequence alignment produced by Ensembl (www.ensembl.org) for *Homo sapiens* IL-10 (ENSG00000136634) and *Mus musculus* IL-10 (ENSMUSG00000016529). Nucleotides mutated in the distal (-861/-851) and proximal (-59/-39) NF-κB binding sites are underlined.

Supplemental Figure 1

A

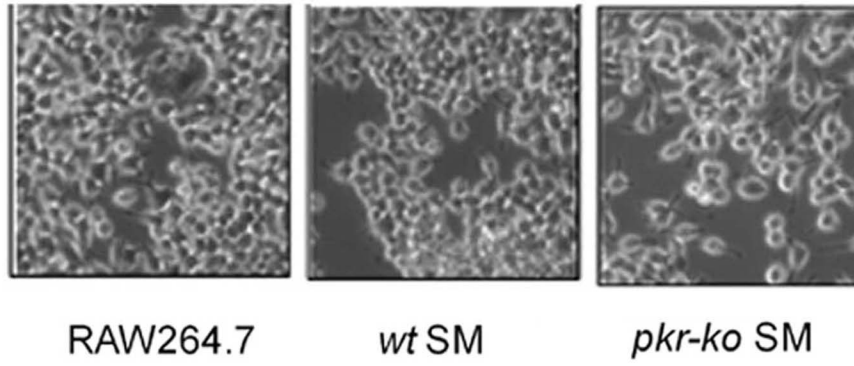


B

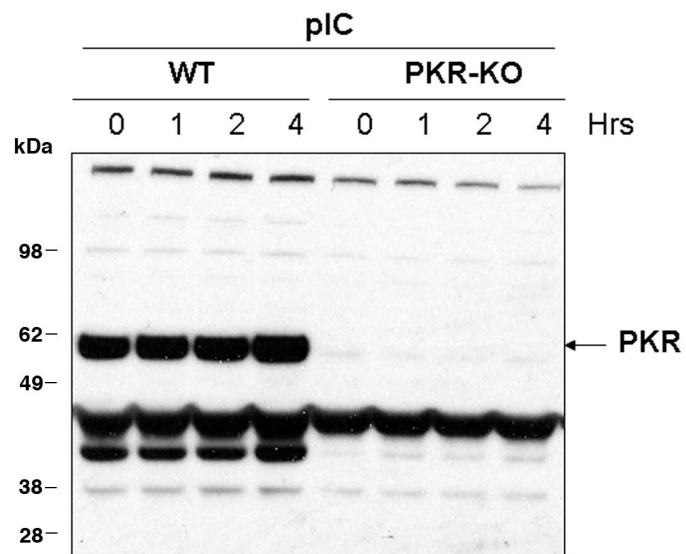


Supplemental Figure 2

A

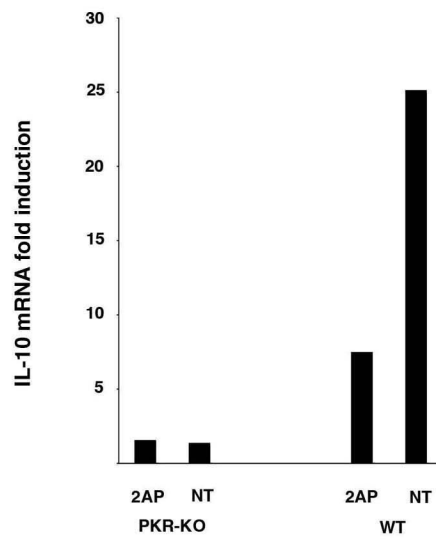


B

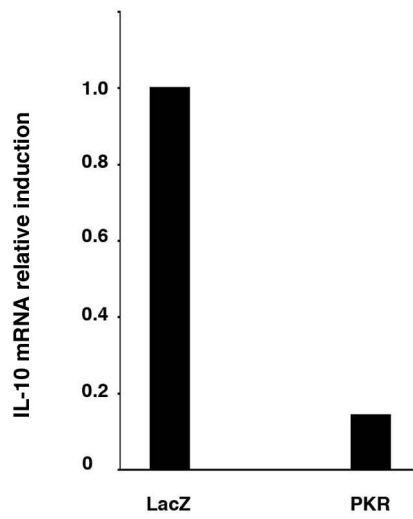


Supplemental Figure 3

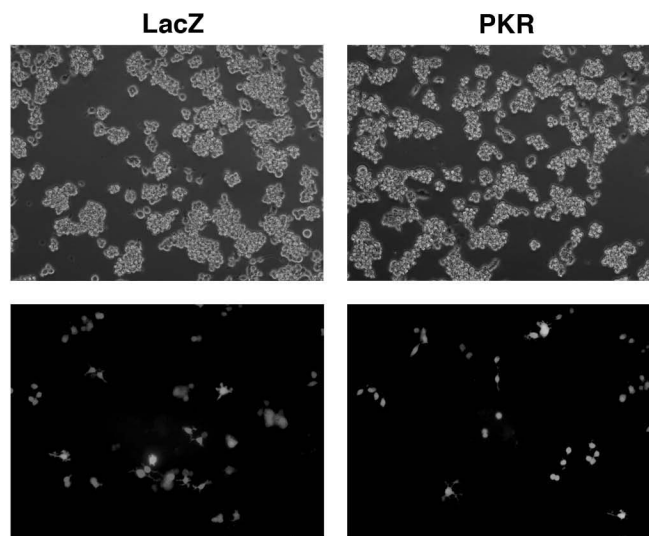
A



B



C



Supplemental Figure 4

Homo_sapiens CCTCCATCTGGGTCCATGGCTACTTAAGCTCAATGCTCCCTGGCAGGCAGGAGACAGGTGCTATTGCCCTGTTGGGACAGATGAAAAACAGACACAGGGAGGATGAGTGATTTGCCCTG
Mus_musculus CCTCTACATGGGTCTACTTTTATTTAAGC-AAACATTCCCTGGT---CAACAGGACGTGTAGCATTGCCCCCTTGGGTACACAGAAAACAGGTACCAGGAGGACAAGTAGTTTGCCCG

Homo_sapiens ACTATAGAGTGGCAGGGCCAAGGCAGAGCCCAGGCCCTCTGCACCTAGGTCACTGTTCCCTCCAGTTACAGTCTAAACTGGAATGGCAGGCAAAGCCCTGTGGAAGGGGAAGGTGAAGG
Mus_musculus GGTACAGAATGAAAGGCAATAGG-----GGACT-----CTAGGCGAATGTTCTTCCCA-----CCCAAAGTGGGTAGTAGGAGAAGTCCCTACTGAA-GGGAAGGTCCAGA

Homo_sapiens CTCAATCAAAGGATCCCCAGAGACTTTCAGATATCTGAAGAAGTCTGTACTGCCCCGGTCTTCCCCAGGTAGAGCAACACTCCTCGCCGCAACCCAAGTGGCTCCCCTTACCT
Mus_musculus CATAATCAAAGGACTACCAGAGATCTCCAGGTATCTGTAGAAGTACTAACATCTCC-----ATCCTTCAACAGCTACAG.....

Homo_sapiens TCTACAAATCCAAGACAACACTACTAAGGCTTCTTTGGGAAGGGGAAGTAGGGATAGGTAAGAGGAAAGTAAGGGACC
Mus_musculus

Homo_sapiens TCCTATCCAGCCTCCATGGAATCCTGACTTCTTTTCTTGTATTTCACACTTCTTCCACCCATCTTTTAAACTTTAGACTCCAGCCACAGAAGCTTACAATAAAAGAACTCTAAGGC
Mus_musculus

Homo_sapiens CAATTTAATCCAAGGTTTCATTCTATGTGCTGGAGATGGTGTACAGTAGGGTGAGGAAACCAAATCTCAGTTGGCACTGGTGTACCCTTGTACAGGTGATGTAATATCTCTGTGCCTCA
Mus_musculus

Homo_sapiens GTTTGCTCACTATAAAATAGAGACGGTAGGGTCTGTTGAGCACTACCTGACTAGCATATAAGAAGCTTTCAGCAAGTGCAGACTACTCTTACCCACTTCCCCAAGCACAGTTGGGGT
Mus_musculusCATTGTAAACAG-----GGCCATGGTAAGGTCTACCCGAC-AGCACAGAGCAAGCCTCCAGAAGTCTGAGTTCCTTCTCCTAACTTCTCAT-----GCTGGGAT

Homo_sapiens GGGGGACAGCTGAAGAGGTGGAACATGTGCCTGAGAATCCTAATGAAATCGGGGTAAAGGAGCCTGGAACACATCCTGTGACCCCGCCTGTACTGTAGGAAGCCAGTCTCTGGAAGTA
Mus_musculus -----CTGAGCTTCTCGTGAAACCGGGGACAGGAGACCAGAATC-TCCTCTGACCCGCCCATATCCTCAAAGGATAGTCTT--GAATACG

Homo_sapiens AAATGGAAGGGCTGCTTGGGAACCTTGAGGATATTTAGCCCACCCCTCATTTTTACTTGGGGAACTAAGGCCACAGACCTAAGGTGACTGCCTAAGTTAGCAAGGAGAAGTCTTGGG
Mus_musculus TGATGGAAGAATTGAGAGTGAGGTCTGAAGAAAATCAGCCCTCTCG---GGGTTTCTTTGGGTAAGT-----GAGTGCTAAGGTGACTTCC-GAGTCAGCAAG---AAATATCGGA

Homo_sapiens TATTCATCCCAGGTTGGGGGACCCAATTATTCTCAATCCATTGTATTCTGGAATGGGCAATTTGTCCACGTCACTGTGACCTAGGAACACGGAATGAGAACCACAGCTGAGGGCC
Mus_musculus CGTTCAACCCAGGTTGGGAGGAAACAATTATTCTCAATCCTAATATGTTCTGGAATAGCCATTTATCCACGTCAATATGACCTGGGAGTGCGTGAATG-GAATCCACAGATGAGGGCC

Homo_sapiens TCTGCGCACAGAACAGCTGTCTCCCAGGAAATCAACTTTTTTAAATGAGAAGCTAAAAAATTATTCTAAGAGAGGTAGCCCATCCTAAAAATAGCTGTAATGCAGAAGTTCATGTTT
Mus_musculus TCTGTACATAGAACAGCTGTCTGCCCTCAGGAAATCAACTTTTAGTA-TTGAGAAGCTAAAAAATAAAAGAGAGGTAGCCATACTAAAAATAGCTGTAATGCAGAAGTTCAT-TCC

Homo_sapiens AACCAATCATTTTTGCTTACGATGCAAAAATGAAAATAAGTTTATTAGAGAGGTAGAGAAGGAGGAGCTTAAGCAGAAAAATCCTGTGCCGGGAAACCTTGATTGTGGCTTTTTTA
Mus_musculus GACCAGTTCTTTGCGCTTACAATGCAAAAAAAGAAATTAAGTCAATGTCATGTTTAGAGGAGGAGGAGCCTGAATAACAAAA--CCTTTGCCAGGAA-----

Homo_sapiens ATGAATGAAGAGGCCCTCCCTGAGCTTACAATATAAAAGGGGACAGAGAGGTGAAGGTCTACACATCAGGGGCTTGCTCTTGCAAAACCAAACCACAAGACAGACTTGCAAAAGAAGGC
Mus_musculus -----GGCCCACTGAGCCTTCAGTATAAAAGGGGACCAAGAACAGGAGGTCTACAT-TTAGAGACTTGCTCTTGCACTACCAAACCACAAGGACGCTTGCAAAAAGAGC