SUPPLEMENTAL DATA

Supplementary Fig. 1. CpG elicits activation of IRF-3 and IRF-5 in a TLR9-dependent manner. (A) HEK cells expressing YFP-tagged moTLR9 were transiently cotransfected with plasmids encoding a luciferase reporter gene containing the Gal4 upstream activation sequence ($UAS_{(GAL)}$) and expression vectors for either Gal4-IRF-3, Gal4-IRF-5 or Gal4-DBD. 24 hours after transfection, cells were stimulated with 10 µg/ml CpG for the indicated times before determination of luciferase activity. (B) HEK cells expressing YFP-tagged moTLR9 were treated with 10 µg/ml CpG for the indicated times before immunoblotting with antibodies recognizing IRF3 phosphorylated at Ser^{396} or total IRF3. (C) HEK cells expressing YFP-tagged moTLR9 or non-transfected HEK cells were transiently cotransfected with plasmids encoding a luciferase reporter gene containing the Gal4 upstream activation sequence ($UAS_{(GAL)}$) and expression vectors for either Gal4-IRF-3 or Gal4-IRF-5. 24 hours after transfection, cells were stimulated with 10 µg/ml CpG for 16 hours before determination of luciferase activity. (D) HEK cells expressing $TLR3^{Flag}$ were transfected with the ($UAS_{(GAL)}$)–luciferase reporter and Gal4-IRF-3 prior to preincubation with 25 µM PP2 or PP3 and stimulation with 50 µg/ml pIC. Luciferase activity was determined after 16 hours treatment.

Supplementary Fig. 2. Inhibition of c-Src does not affect CpG- or pIC-stimulated NF-κB activation. HEK cells expressing $moTLR9^{YFP}$ (A) or $TLR3^{Flag}$ (B) were transfected with a NF-κB luciferase reporter construct containing four κB-elements. 24 hours after transfection, cells were preincubated with PP2 or 25 µM PP3 and stimulated with 10 µg/ml CpG (A) or 50 µg/ml pIC (B). Luciferase activity was determined after 16 hours treatment.

Supplementary Fig. 3. Effect of the Src inhibitor PP2 on TLR-induced expression of IRF-4, SOCS1 and SOCS3. RAW264.7 cells were pretreated with 25 uM PP2 prior to treatment with TLR ligands for 16 hours and assessment of mRNA levels of IRF-4 (A), SOCS1 and SOCS3 (B).

Table SI. Predicted transcription factor binding sites. Bioinformatics analysis were performed on aligned genomic data from human, mouse, and rat. Prediction of binding sites was done by combining information from several sources, including online datasets from CisRED (2), PReMod (3;4), ORegAnno (5) and UCSC TFBS Conserved (6) as well as predictions done locally using MotifScanner (7). None of the online datasets included predictions for all gene regions analyzed here. Although the TFBS Conserved set and the MotifScanner predictions turned out to be very useful, the other datasets often gave complementary information. The final predictions of putative binding sites in individual promoters are summarized in Table I, showing the source of each prediction and highlighting predictions that are found both in the online datasets as well as in our MotifScanner based predictions.

Supplementary Methods.

qRT-PCR analysis - Primers specific for IRF-5, IL-6, IP-10, IRF-3, ATF3, SOCS1 and SOCS3 were as following. For mouse: IRF-5; 5'-AATACCCCACCACCTTTTGA-3' (sense) and 5' TTGAGATCCGGGTTTGAGAT-3' (antisense), IL-6; 5'-ATGAAGTTCCTCTCTGCAAGAGACT-3' (sense) and 5'-CACTAGGTTTGCCGAGTAGATCTC-3' (antisense), IP-10; 5'-TCATCCTGCTGGGTCTGAGTGG-3' (sense) and 5'-CGCTTTCATTAAATTCTTGATGGTC-3' (antisense), IRF-3;

5’GTGGCCTGGGTGAACAAGAG-3’ (sense) and 5’-CCATTGGTGTCCGGAGAGAGT-3’ (antisense); GADPH; 5-GGAAGGGCTCATGACCACA-3’ (sense) and 5’-CCGTTCAGCTCTGGGATGAC-3’ (antisense). ATF3; 5’-TTGTGCCAACAGAGGATGGA- 3’ (sense) 5’-gctgtgcccagggttcttc-3’ (antisense); SOCS3;5'-TCCCATGCCGCTCACAG-3' (sense) and 5'-ACAGGACCAGTTCCAGGTAATTG -3' (antisense); SOCS1; 5'-CCG TGG GTCGCGAGAAC -3' (sense) and 5'-AACTCAGGTAGTCACGGAGTACCG -3' (antisense).

Bioinformatics analysis - Databases for genome scale mappings of regulatory elements were accessed and visualized using the UCSC Genome Browser (8) as well as database-specific web services. This includes the Conserved Transcription Factor Binding Sites track (TFBS Conserved) (6) in the UCSC Genome Browser and data from CisRED (2), PReMod (3;4) and ORegAnno (5). TFBS Conserved is based on identification of binding sites conserved in an alignment of genomic data from human, mouse and rat, using Transfac (9) matrices. CisRED is based on motif discovery in regions around the transcription start site (from -1500 to +200) of known genes, using genome data from 41 vertebrate species. Discovered motifs are then been matched against known motifs from Transfac, Jaspar (10) and ORegAnno. PReMod is generated by a two-step process, where Transfac matrices first are matched against the human, mouse and rat genomes. Consensus predictions are then clustered to identify putative regulatory modules. ORegAnno consists of literature-curated data on regulatory regions, transcription factor binding sites and regulatory polymorphisms.

In addition to existing mappings the Transfac matrices were matched against regulatory regions for selected genes. Assumed regulatory regions for each gene (from -3000 to +500) from human and mouse orthologs were downloaded from the Ensembl (11) database with Toucan (7;12), and Transfac matrices were scanned against the sequences with MotifScanner (7) (prior 0.8, 3rd order background based on human-mouse conserved non-coding regions). The output was then filtered by searching for pairs of matrices with approximately equidistant hit positions in the regulatory regions of orthologous gene pairs from human and mouse. The final list of likely binding sites represents a combination of the available predictions.

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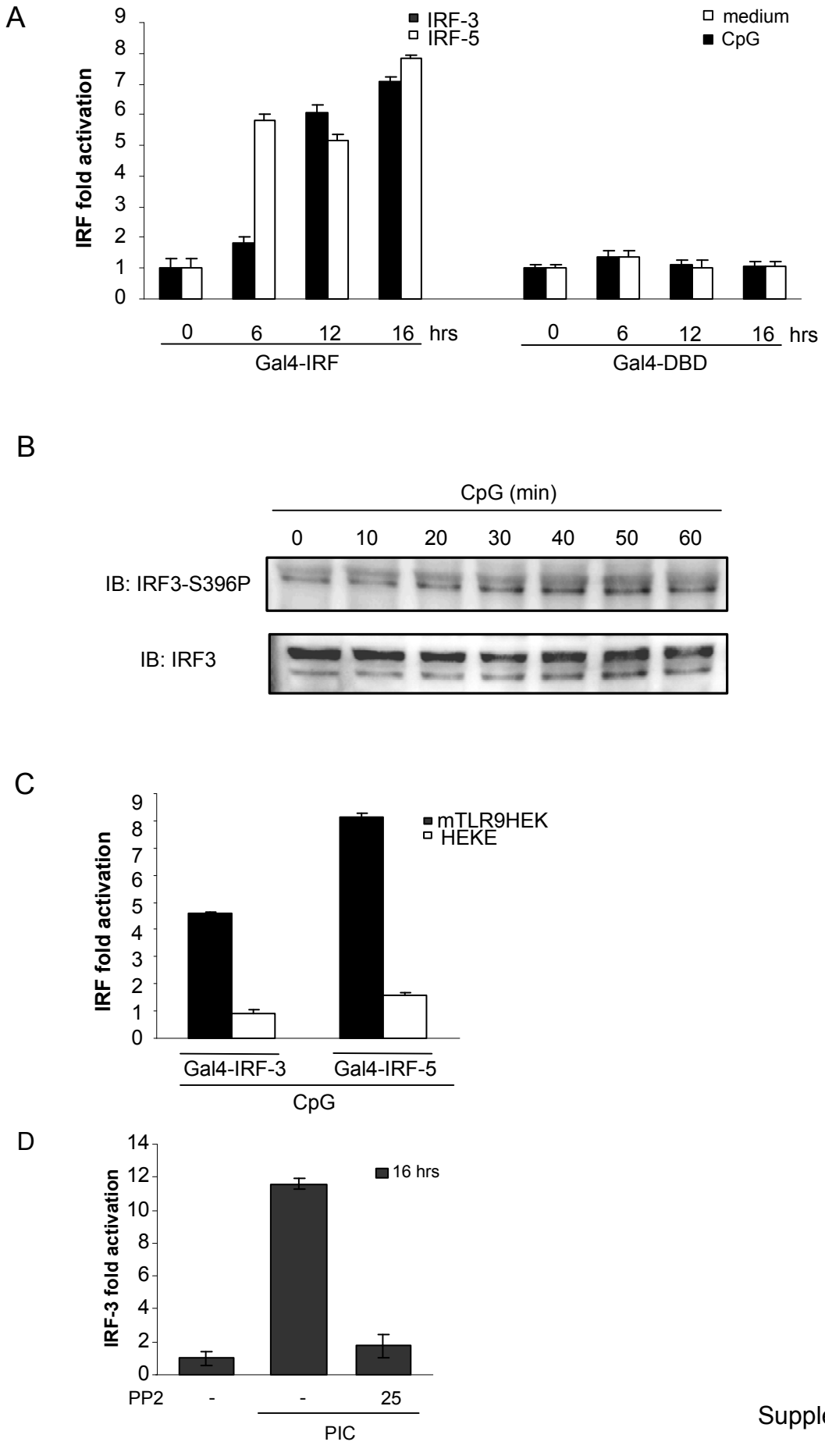
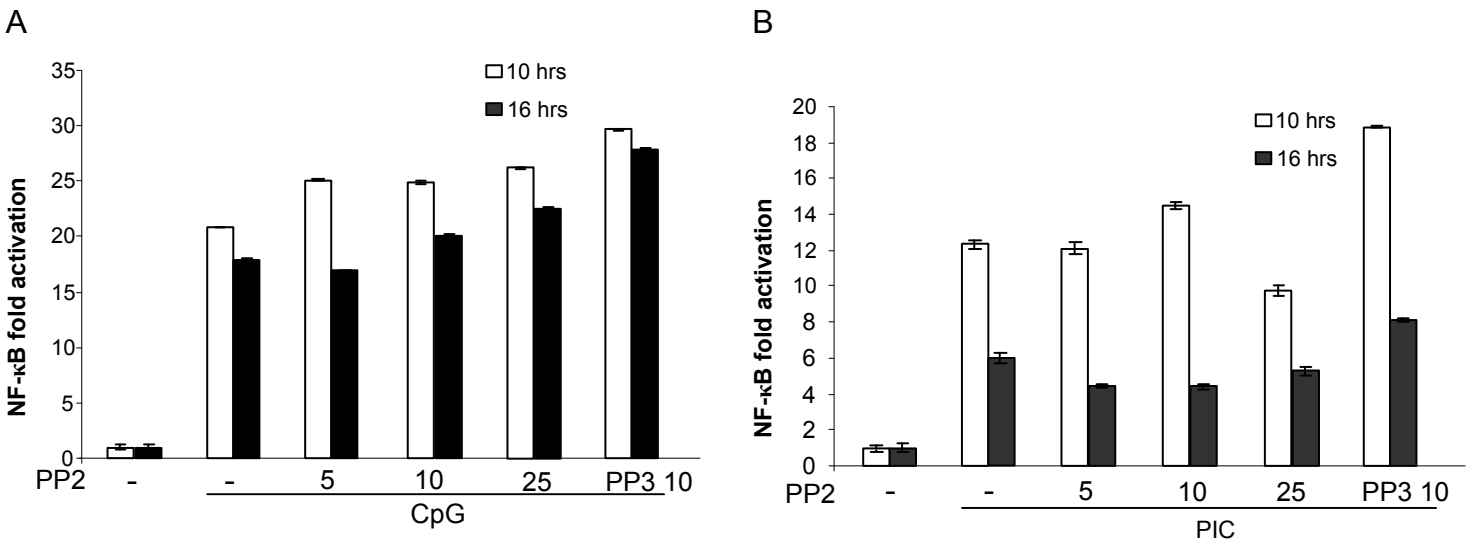
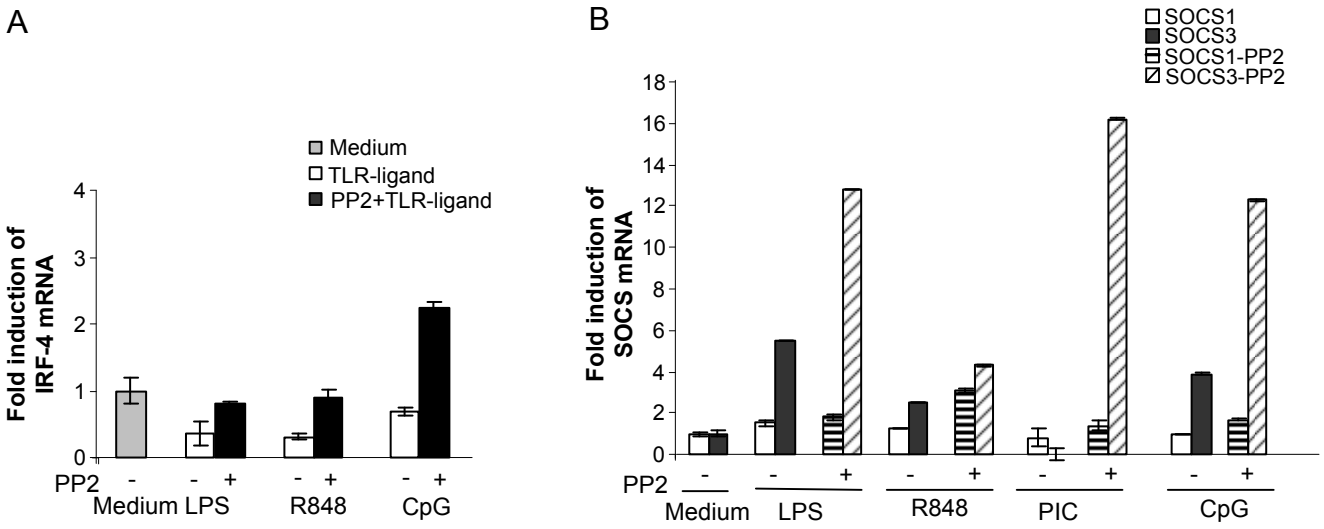


Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3

Table SI. Predicted transcription factor binding sites.

	ATF	CREB	**NF-κB**	AP1	ISRE	IRF	c-Rel	SP1
IL-6	ATF[3]	**CREB**[134]	**NF-κB**[134]	**AP1**[14]	ISRE[3]	IRF[123]	**CREL**[34]	**SP1**[24]
IP10			**NF-κB**[1234]		**ISRE**[134]	**IRF**[134]	**CREL**[34]	
IRF-3			NF-κB[4]					SP1[2]
IL-12 β		CREB[2]	**NF-κB**[24]			IRF[2]	CREL[4]	

The source of each prediction is indicated as 1 – TFBS Conserved, 2 – CisRED, 3 – PreMod and 4 – MotifScanner (local). Binding sites identified by any of the existing mappings (1-3) as well by our MotifScanner based mapping (4) are shown in **bold**.