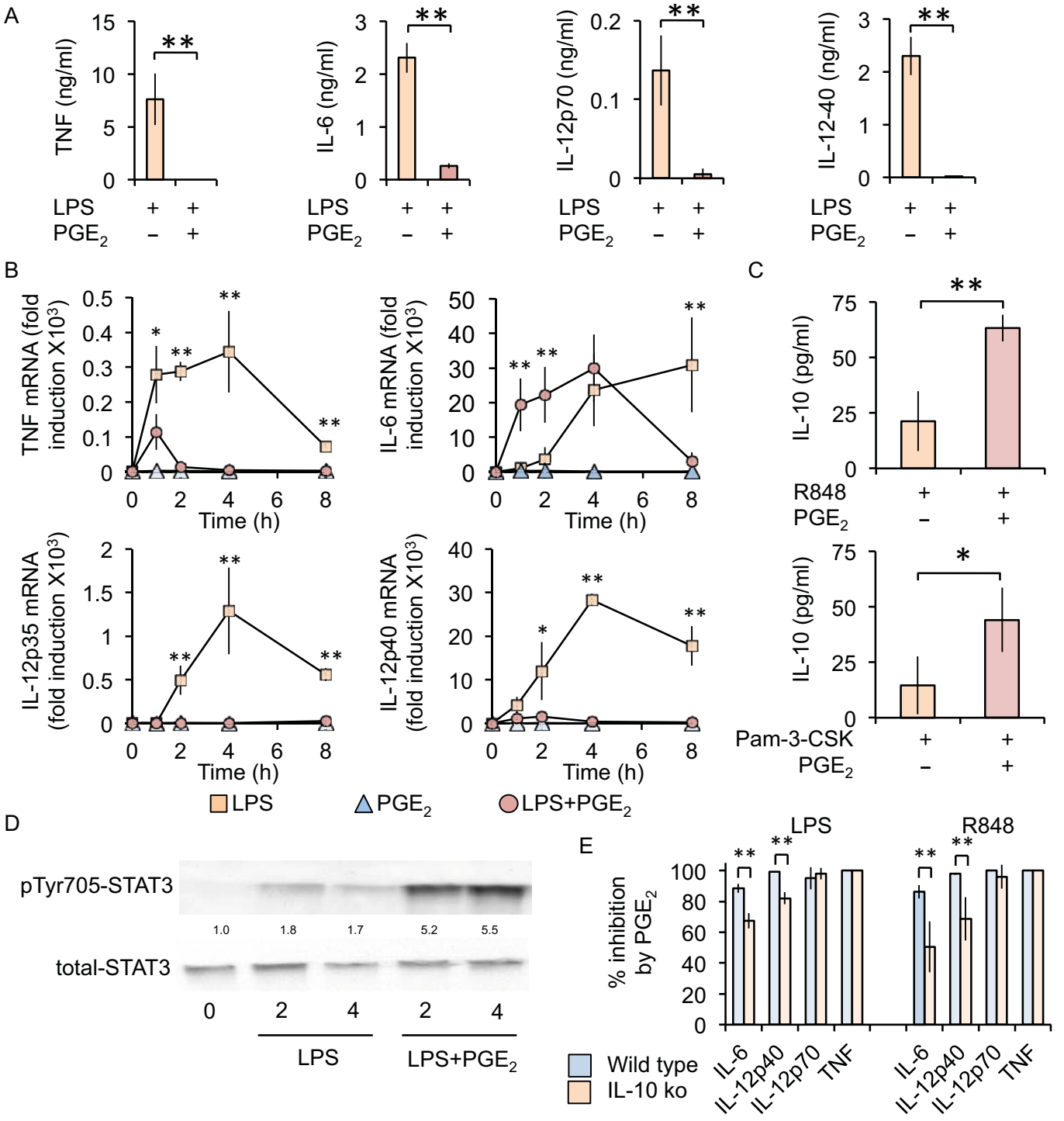
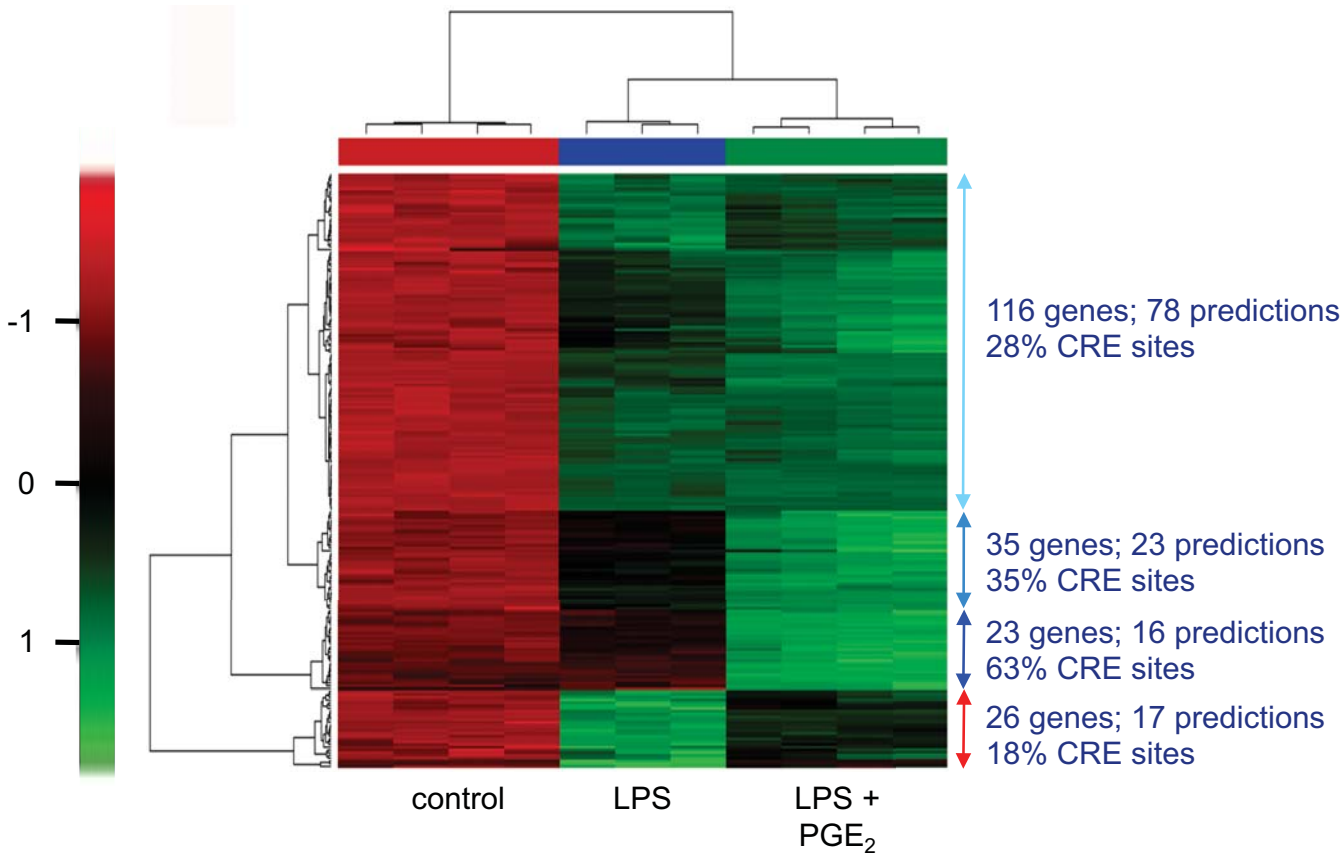


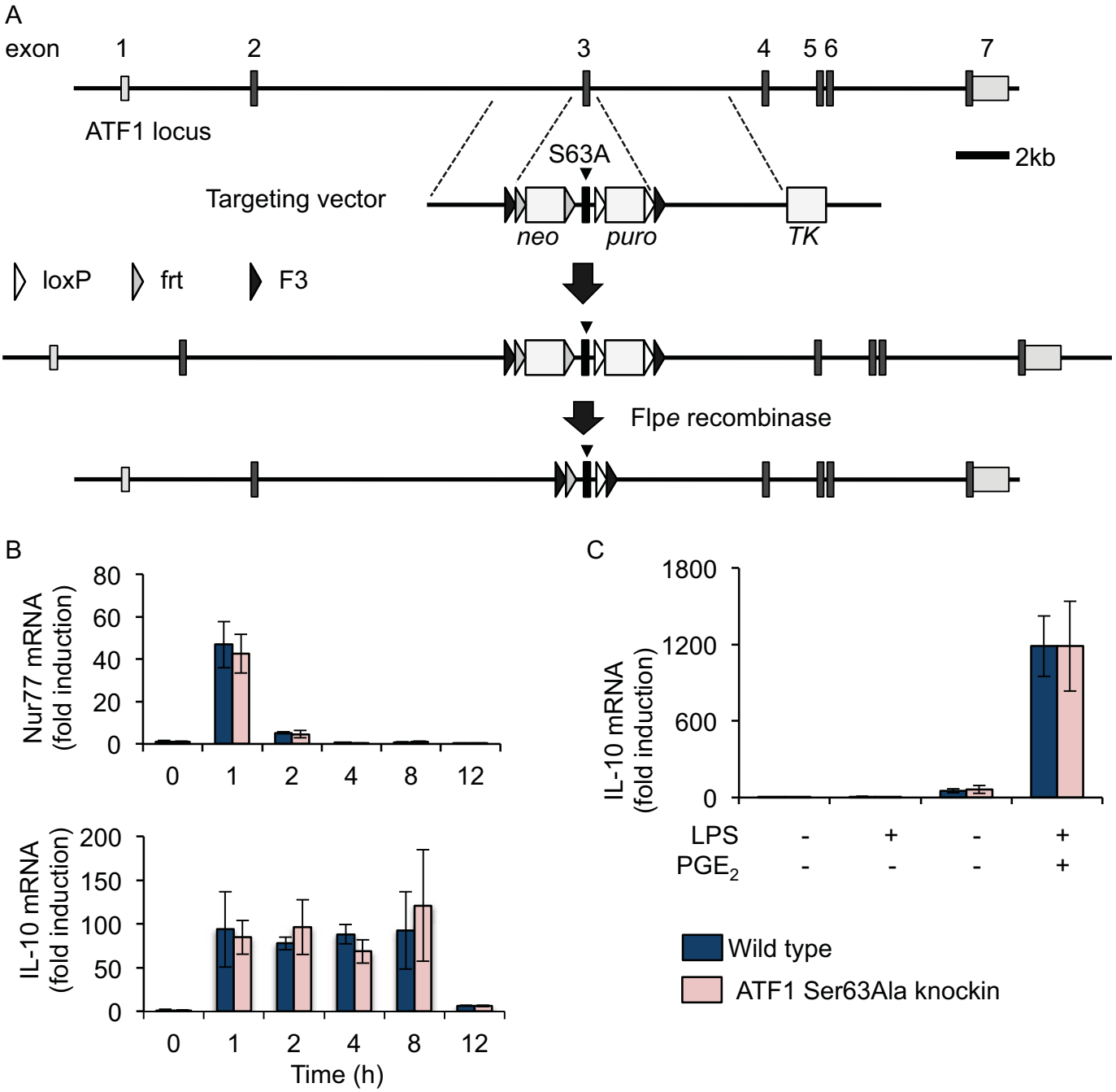
Supp. Fig. 1 PGE_2 represses pro-inflammatory cytokine production but induces IL-10.

(A-B) BMDMs were isolated from wild type mice and stimulated for the indicated times with either 100 ng/ml LPS, 10 μM PGE_2 or a combination of LPS and PGE_2. (A) TNF, IL-6, IL-12p70 and IL-12p40 levels secreted into the media were measured after 8h as described in the methods. (B) Total RNA was extracted at the indicated times and the levels of TNF, IL-6, IL-12p35 and IL-12p40 mRNA determined by qPCR. Results are expressed as fold change relative to the unstimulated control (C) BMDMs were stimulated with 1 μg/ml R848 (TLR7/8 agonist) or 1 μg/ml Pam-3CSK4 (TLR1/2 agonist) with or without 10 μM PGE_2 for 8 h and the levels of IL-10 secreted determined. (D) Wild type BMDMs were stimulated with the indicated combinations of LPS and PGE2 for the indicated times, and the levels of phospho and total STAT3 determined by immunoblotting. Levels of phospho STAT3 were quantified relative to total STAT3 protein and represented as a ratio of unstimulated control. (E) BMDMs were isolated from wild type of IL-10 knockout mice and cytokine secretion measured after 8h of stimulation with either LPS or a combination of either 100 ng/ml LPS or 1 μg/ml R848 either alone or in combination with 10 μM PGE_2. Results are expressed as the % inhibition of cytokine levels by PGE_2 for each mouse. In each panel, error bars represent the average and standard deviation of independent cultures from 4 mice. ** indicates a *p* value (students t-test) of less than 0.01 and * less than 0.05.

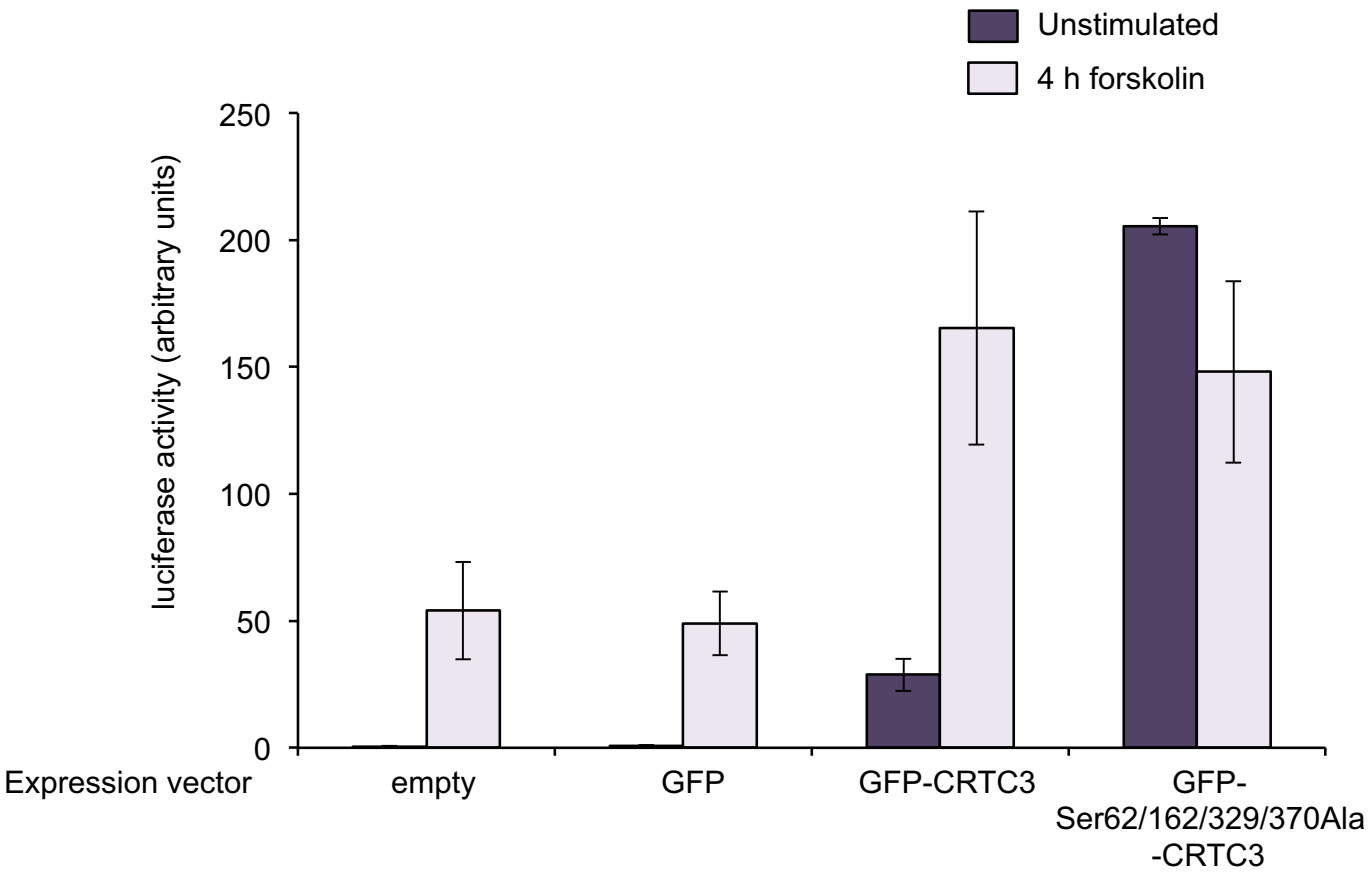


Supplementary figure 2. PGE_2 synergizes with LPS to promote CREB dependent transcription

(A) BMDMs were stimulated with 100 ng/ml LPS or a combination of LPS and 10 μM PGE_2 for 1h. Total RNA was isolated and subject to microarray analysis as described in the methods. Data was then filtered to identify genes that were upregulated at least 4 fold by LPS or LPS and PGE_2. Complete linkage agglomerative hierarchical clustering method with Pearson correlation as distance metric was used to cluster the genes into 4 groups based on the ability of PGE_2 to promote their transcription. The number of genes in each cluster for which predictions were available is indicated, as is the % of these genes that had potential CRE sites.



Supplementary figure 3. ATF1 phosphorylation is not required for IL-10 transcription.
The targeting strategy used to generate a Ser63 to Ala knockin mutation in the endogenous ATF1 gene is shown (A). Mice were generated using targeting of C57/Bl6n embryonic stem cells using standard techniques by TaconicArtemis. Details of vector sequence and targeting are available on request. Routine genotyping was carried out by PCR across the 5' loxP site using the primers *CTGACCTGCAGATGATGTAGACG* and *TGCTAGGGATGGAGCTCTGG*. This gives rise to a 374bp targeted band and 255bp wild type band. ATF1 Ser63Ala knockin mice were viable and fertile with no apparent adverse effects when maintained under specific pathogen free conditions.
BMDMs were isolated from wild type or ATF1 Ser63Ala knockin mice and stimulated with 100 ng/ml LPS for the indicated times. Total RNA was then extracted and IL-10 mRNA and nur77 levels determined by qPCR (B) Alternatively cells were stimulated for 1h with either 100ng/ml LPS or 10 μM PGE2 as indicated and IL-10 mRNA levels determined (C). Error bars represent the standard deviation of independent cultures from 4 mice per genotype.



Supplementary Figure 4. Mutation of SIK phosphorylation sites in CRTC3 promotes CREB dependent transcription in Hek293 cells.

Four phosphorylation sites for SIKs in CRTC3 have been identified, Ser62, Ser162, Ser239 and Ser370. Hek293 cells were transfected with a CREB dependent luciferase reporter in combination with either an empty expression vector or expression vectors for GFP, wild type GFP tagged CRTC3 or Ser62Ala/Ser162Ala/Ser329Ala/Ser370Ala GFP tagged CRTC3. Cells were either left unstimulated or stimulated with 20 μM forskolin to induce PKA activation for 4h. Error bars represent the Standard deviation of 4 stimulations.