Human TNF-Luc reporter mouse: A new model for measuring inflammatory responses. Faisal Minshawi, Mike White, Werner Muller, Neil Humphreys, Dean Jackson, Barry J. Campbell, Antony Adamson, and Stamatia Papoutsopoulou.

Supplementary Information

Supplementary Table S1: **Blocking of TNFR1 or TNFR2 does not affect the human *TNF* promoter-induced luciferase activity in lipopolysaccharide stimulated hTNF.LucBAC cells.** Differentiated bone marrow-derived macrophages (BMDMs) or isolated splenocytes were pre-treated for 4h with either 50µg/mL isotype control antibody or neutralizing anti-TNFR1 or anti-TNFR2 antibodies prior to stimulation with 10ng/mL LPS (N=3-5 mice, with triplicate samples). Luciferase activity was monitored for 16h and expressed as mean area under the curve (AUC) ± standard error of mean (s.e.m.).

	BMDMs	**Splenocytes**
Unstimulated cells	41320 ±1656	24604 ± 334
LPS stimulated cells	1169169 ± 51884	107095 ± 21735
LPS + Isotype control antibody	870843 ± 71848	115323 ± 27866
LPS + anti-murine TNFR1 antibody	1027788 ± 55856	103326 ± 21070
LPS + anti-humanTNFR1 antibody	1080763 ± 56131	122319 ± 24259
LPS + anti-murine TNFR2 antibody	1017231 ± 22554	102298 ± 4825

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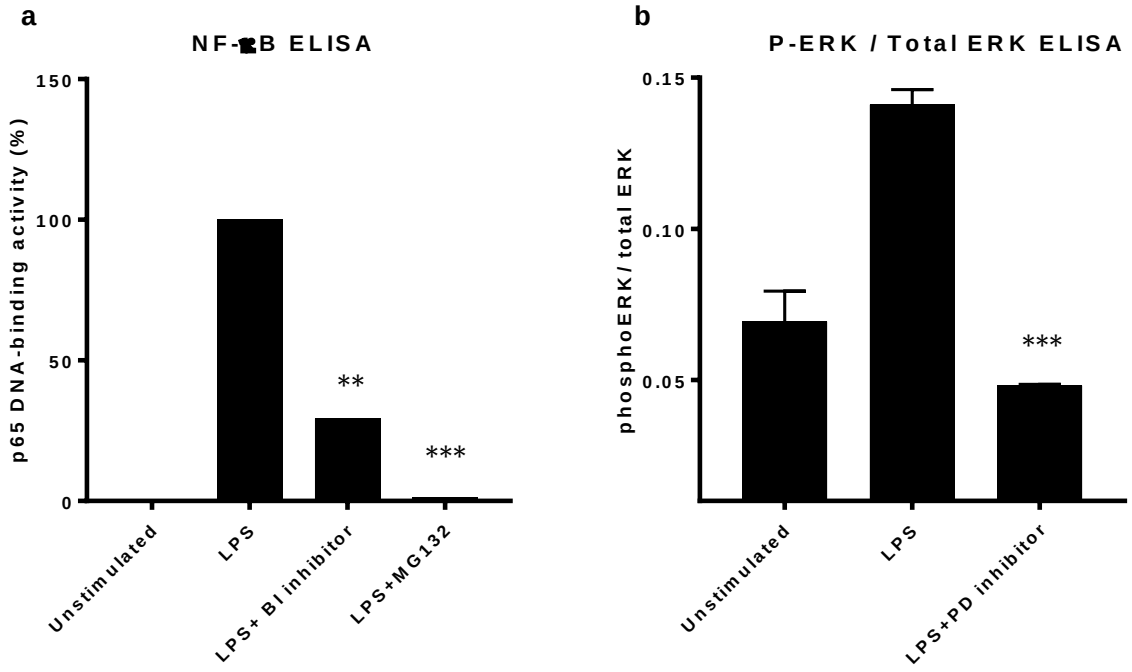
Supplementary Figure S1: **Effect of NF-κB and MEK/ERK pathway inhibitors on lipopolysaccharide-induced hTNF.LucBAC BMDMs.**

(a) Cells were left untreated or pre-treated for 30min with the IKK-2 inhibitor BI605906 or the proteasome inhibitor MG132 (both at 10μM), followed by stimulation with 10ng/mL LPS for 30min. At the end of the stimulation, cells were lysed with RIPA buffer and the cleared lysates were used for ELISA. p65 DNA binding activity was blocked by both inhibitors (**p<0.01 and ***p<0.001, ANOVA; N=6 mice). **(b)** Cells were pre-treated in the absence or presence of the MEK inhibitor PD0325901 (for 30min, at 100nM) and then stimulated with 10ng/mL LPS for 15min. phospho-ERK/total ERK was measured in lysates by ELISA. Phosphorylation of ERK was completely blocked by PD0325901 (***p<0.001, ANOVA; N=6).



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