

Figure S1

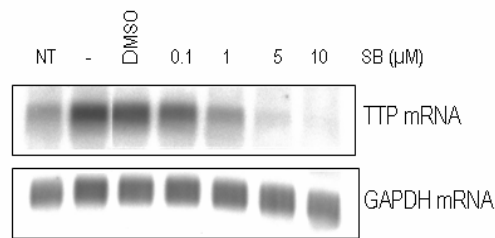
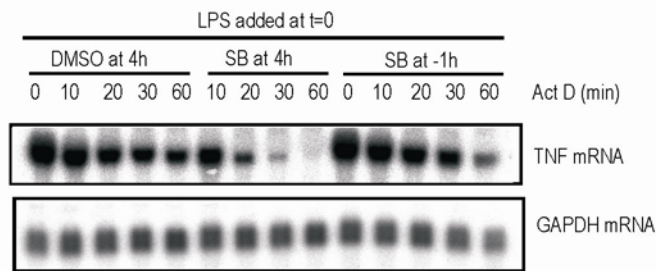
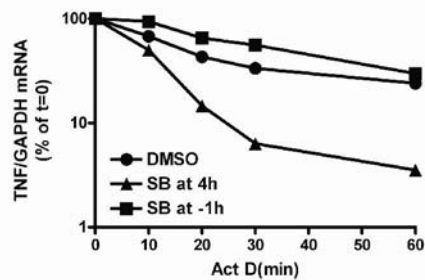
The effect of adding SB 202190 before or after LPS treatment on MK2 activity and concentration dependence of inhibition of p38 MAPK by SB 202190 in BMDM

A) BMDM were left untreated or were pretreated with 0.1% DMSO or SB 202190 (final concentration 1 μ M) for 1h and then LPS was added for a further 4h. After 4h LPS treatment, cells were left untreated or 0.1% DMSO or SB 202190 (final concentration 1 μ M) was added for a further 1h. Cells were harvested at 5h post-LPS and MK2 was immunoprecipitated from lysates and assayed for its activity using recombinant HSP27 protein according to Dean et al. (1999) J Biol Chem, 274, 264-269.

B) Graph shows mean MK2 activity from three independent experiments (\pm SEM) as percentage of the maximum activity.

C) BMDM were left untreated or were pretreated with 0.1% DMSO (vehicle) or SB 202190 at the concentrations indicated for 1h and then treated with LPS for 4h. MK2 was assayed as in (A) as a measure of inhibition of the upstream kinase p38 MAPK.

D) Graph shows mean MK2 activity \pm SEM for three separate experiments as in (C).

A**B****C****Figure S2**

Prior p38 MAPK activity is required for the induction by LPS of TTP mRNA and the decay of TNF mRNA upon SB 202190 addition.

A) BMDM were left untreated (NT) or incubated with 0.1% DMSO or SB 202190 at the concentrations indicated for 1h and then treated with LPS for 4h. Cells were then harvested and RNA was extracted and subjected to northern blotting for TTP and GAPDH mRNA.

B) BMDM were either pretreated with SB 202190 (final concentration 5 μM) for 1h or left untreated then LPS was added for a further 4 h. After 4 h LPS treatment, actinomycin D was added either alone or together with either SB 202190 (final concentration 5 μM) or vehicle (0.1% DMSO). RNA was isolated at the times shown and subjected to northern blotting for TNF. The blot was stripped and re-probed for GAPDH mRNA.

C) Plot of TNF normalised to GAPDH mRNA expressed as a percentage of t=0 actinomycin D treatment. Similar data were obtained in two independent experiments.