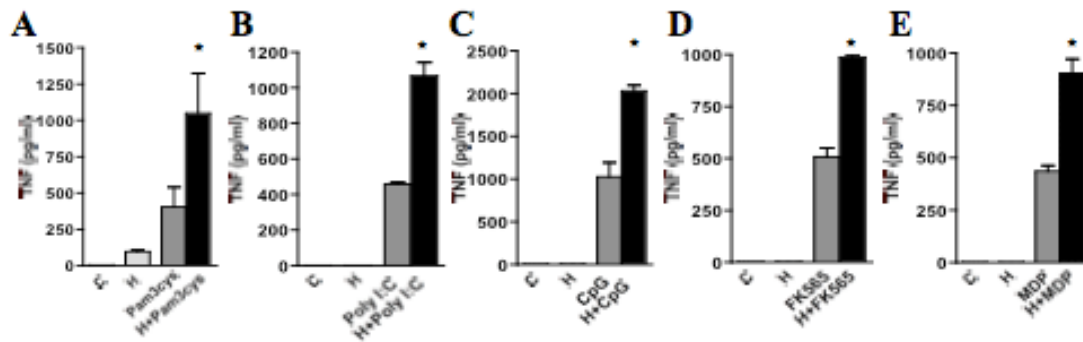
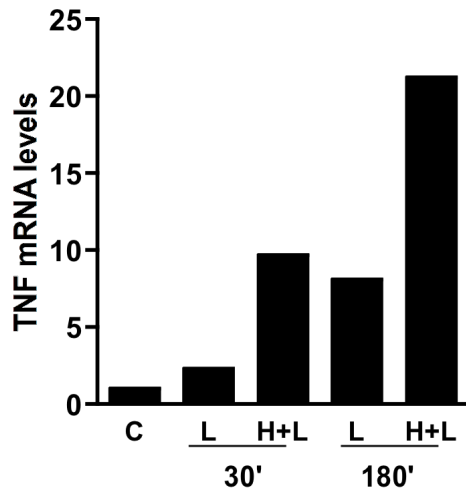


SUPPLEMENTARY FIGURE 1



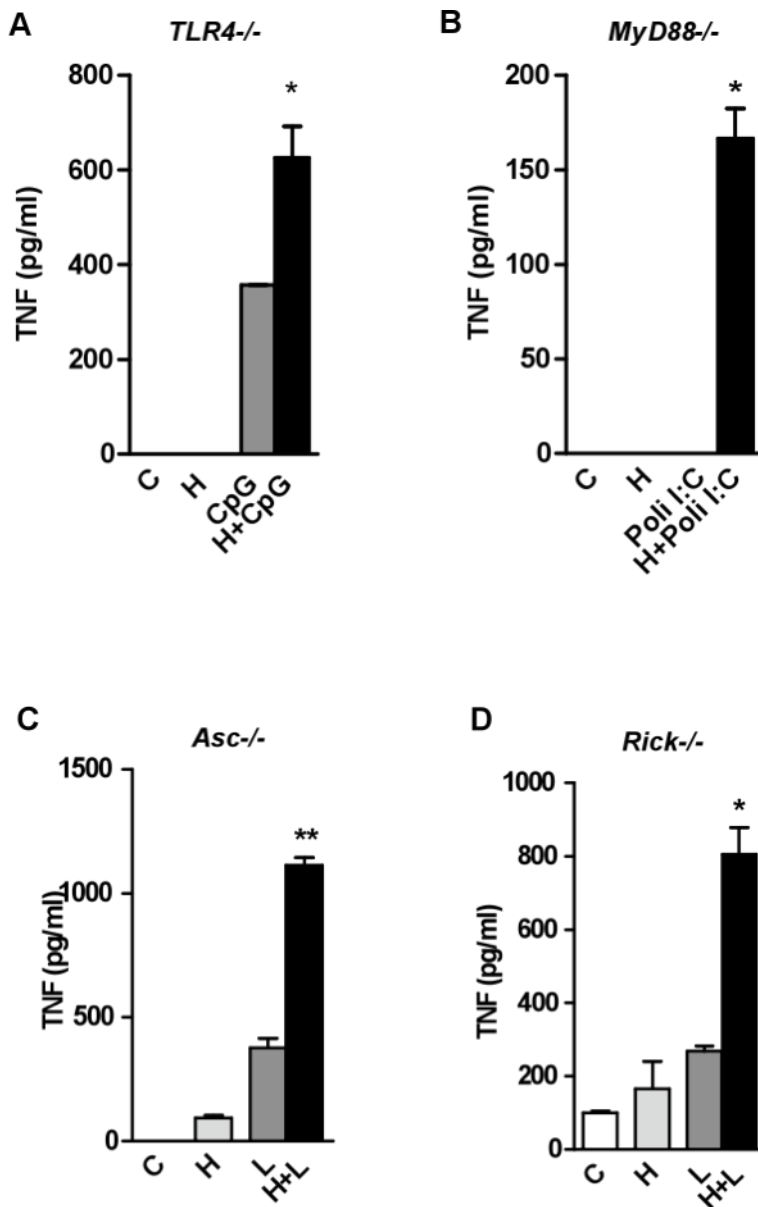
Heme increases the TNF secretion induced by different agonists of TLRs and NLRs. Elicited peritoneal macrophages from C57Bl/6 mice were stimulated with heme (100 μ M) one hour before the stimulation with Pam₃Cys (0.1ng/ml) (A), Poly (I:C) (10 μ g/ml) (B), CpG (10 μ g/ml) (C), FK 565 (10ng/ml) (D) or MDP (10 μ g/ml) (E). Supernatants were collected 4 hours after the stimuli with TLRs and NLRs ligands and TNF concentrations were evaluated by ELISA. Results represent mean \pm SEM and are representative of 3 different experiments. *P<0.05

SUPPLEMENTARY FIGURE 2



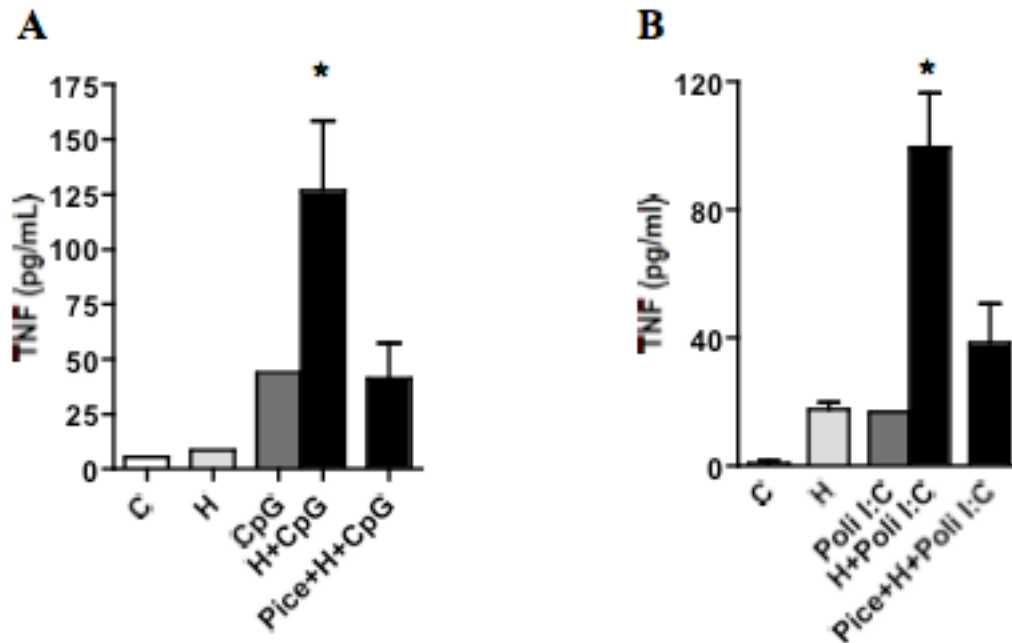
Heme increases the TNF transcription induced by LPS - Peritoneal macrophages from C57Bl/6 mice were stimulated with heme (100 μ M) one hour before the stimulus with LPS (0.1 ng/ml). After 30 or 180 minutes of LPS treatment, macrophages were lysed and RNA prepared with Trizol. Content of mRNA for TNF was determined by real-time RT-PCR. Results were normalized by HPRT expression and are presented as fold induction of mRNA expression relative to control samples.

SUPPLEMENTARY FIGURE 4



Heme increases the secretion of TNF induced by agonists of TLRs in the absence of TLR4 MyD88, ASC or RICK. (A) Elicited peritoneal macrophages were obtained from *Tlr4^{-/-}* mice and were stimulated with heme (100 μ M) one hour before the stimulation with CpG (10 μ g/ml). Supernatants were collected after 4 hours and TNF concentrations were evaluated by ELISA. Bone marrow-derived macrophages from *Myd88^{-/-}* (B), *Asc^{-/-}* (C) and *Rick^{-/-}* (D) mice were stimulated with heme (100 μ M) and one hour later were stimulated with Poly (I:C) (10 μ g/ml) (B) or LPS (C and D). The supernatants were collected 4 hours after Poli (I:C) and LPS stimulus and the TNF concentrations were determined by ELISA. The stimuli were performed in the present of 10%FCS. Results represent mean \pm SEM for TNF determination and are representatives of 2 different experiments. *P<0.005 and **P<0.001 compared to stimulus with TLRs ligands alone.

SUPPLEMENTARY FIGURE 4



Syk is involved in the synergistic effect of heme on cytokine secretion induced by TLR ligands. Peritoneal macrophages from C57Bl/6 were stimulated with heme (100 μ M) and /or CpG (10 μ g/ml) (A) or Poli (I:C) (10 μ g/ml) (B) 30 minutes after the treatment with 10 μ M of Piceatannol. TNF was quantified by ELISA in supernatants collected 4 hour after de stimuli with heme and LPS. The stimuli were performed in the presence of 10% FCS. Results represent mean \pm SEM for cytokines determination and are representative of 2 different experiments. *P<0.05