# Supplementary information

**LPS-induced NF-kappa B activation as described in the literature.**

The pathway can be split into three modules: 1) The MyD88-dependent pathway, 2) The TRIF-dependent pathway, 3) IκB-NF-κB signalling.

The reactions that describe LPS activation of its receptor Toll Like Receptor 4 (TLR4) are common to both modules 1 and 2: LPS binds to LPS-binding protein (LBP)[1] and this complex binds to and activates CD14 on the cell membrane [2]. CD14 then helps transport the LPS-LBP complex to TLR4 where LPS activates the receptor [3–6].

Further downstream signalling occurs via two pathways: one dependent on Myeloid Differentiation Primary Response Gene 88 (MyD88) and the other dependent on Tir-Domain-Containing Adapter-Inducing Interferon-β (TRIF). The MyD88-dependent pathway is largely responsible for the expression of proinflammatory cytokines [7–12], and the main role of the TRIF-dependent pathway is to induce expression of co-stimulatory molecules and IFN-inducible genes [13–16]. NF-κB can be activated via either pathway, and *in vivo* the two pathways probably interact to maximise expression of inflammatory cytokines [17].

Module 1) The MyD88-dependent pathway

The MyD88-dependent pathway begins when TIRAP (toll-interleukin 1 receptor domain containing adaptor protein) is recruited to the cytoplasmic domain of TLR4 [18,19]. TIRAP recruits MyD88 [18–20], which binds IRAK4 (interleukin-1 receptor-associated kinase 4),[21,22] IRAK1 (interleukin-1 receptor-associated kinase 1)[23] and TRAF6 (TNF receptor associated factor 6) [24,25]. IRAK1 is phosphorylated by IRAK4 [21,26,27] and then the IRAK1/TRAF6 complex dissociates from the receptor[23,27]. TRAF6 then interacts with a complex consisting of the MAPK kinase kinase transforming growth factor-beta-activated kinase 1 (TAK1) and its binding proteins TAB1 and TAB2 [28]. The TRAF6/IRAK1/TAK1/TAB1/TAB2 complex is inactive until it translocates to the cytoplasm and forms a larger complex with the ubiquitin-conjugating enzymes Ubc13 and Uev1A. This ubiquitination activates TAK1 and marks IRAK1 for degradation by the proteasome [28,29]. TAK1 phosphorylates an IκB kinase (IKK) complex consisting of two catalytic subunits (IKKα and IKKβ) and a regulatory subunit (IKKγ) [30–33]. This phosphorylated IKK complex feeds into the IκB-NF-κB signalling module of the pathway.

Module 2) The TRIF-dependent pathway

Compared to the MyD88-dependent pathway, less is known about the TRIF-dependent module. Here we have modelled the pathway according to the suggestions put forward by Covert *et al*.[34]. They observed delayed NF-κB activation in MyD88-deficient mice embryo fibroblasts (MEFs) treated with LPS. They hypothesised the delay occurs because the TRIF-dependent pathway requires protein synthesis before NF-κB can be activated via a secondary signal in an autocrine manner. Microarray analysis of LPS stimulated MyD88-deficient MEFs showed significant upregulation of the tumour necrosis factor alpha (TNFα) transcript, which (along with the findings of additional validation experiments) led them to suggest that TNFα is the autocrine signal responsible for the late-phase activation of NF-κB in response to LPS.

The pathway they put forward begins when TRIF-related adapter molecule (TRAM) binds to active TLR4[35–37] and acts as a bridging adaptor connecting TLR4 to TRIF [36,38]. This complex activates two noncanonical IKKs, TANK-binding kinase 1(TBK1) and IKKε, [39–41] which phosphorylate Interferon Regulatory Factor 3 (IRF3) [39,40,42]. IRF3 then translocates to the nucleus and regulates transcription of TNFα. TNFα is secreted into the extracellular space where it binds to its membrane receptor TNFR1[43]. This leads to the recruitment of TRAF2, TRADD and RIP1. TRAF2 then recruits the IKK complex, which allows it to be phosphorylated by RIP1 [44–48].

Module 3) IκB-NF-κB signalling

In resting cells, NF-κB is sequestered in an inactive state in the cytoplasm by nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor (IκB). Active IKK can bind to and phosphorylate IκB, which causes it to degrade and therefore release NF-κB [41,49]. Free NF-κB then translocates to the nucleus where it regulates transcription of target genes, including the IκB isoform IκBα [50–52]. The production of IκBα results in a negative feedback loop that causes oscillations in NF-κB activity [53,54]. Two other IκB isoforms (IκBβ and IκBε) are also included in the model. They act in the same way as IκBα, but their production is not modelled as NF-κB-dependent and their activity is in antiphase with IκBα activity. This acts to dampen NF-κB oscillations.

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