

Supplementary Materials for
**A Physical Interaction Between the Adaptor Proteins DOK3 and
DAP12 Is Required to Inhibit Lipopolysaccharide Signaling in
Macrophages**

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Fig. S1. The abundance of pDAP12 is not increased in response to LPS.

Fig. S2. Stimulation of peritoneal macrophages with LPS in vivo fails to induce a stable complex containing DOK3 and pDAP12.

Fig. S3. DAP12 and DOK3 mediate inhibition of LPS-dependent cytokine production.

Sup Figure1

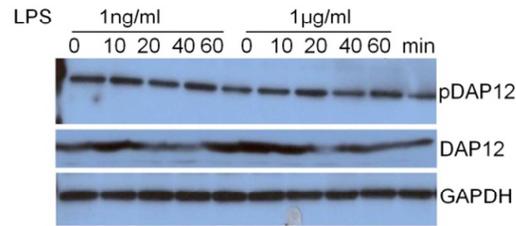


Fig S1. The abundance of pDAP12 is not increased in response to LPS. RAW 264.7 cells were stimulated with LPS (1 ng/ml or 1 µg/ml) for the indicated times. Whole-cell lysates were analyzed by Western blotting with antibodies specific for pDAP12 and GAPDH, as a loading control. Data are representative of three independent experiments.

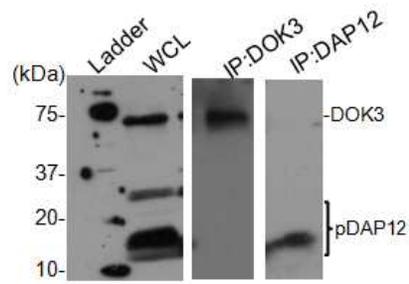


Fig S2. Stimulation of peritoneal macrophages with LPS in vivo fails to induce a stable complex containing DOK3 and pDAP12. Peritoneal macrophages were isolated from WT mice 40 min after they received an intraperitoneal injection of LPS (10 mg/kg). Cells were lysed and subjected to immunoprecipitation (IP) with antibodies specific for DOK3 or DAP12, and then were analyzed by Western blotting with antibodies against DOK3 and pDAP12. Whole-cell lysate (WCL) from each sample indicates the presence of DOK3 (~50 to 60 kD band) and pDAP12 (~15 kD). Data are representative of two independent experiments.

Sup Fig 3

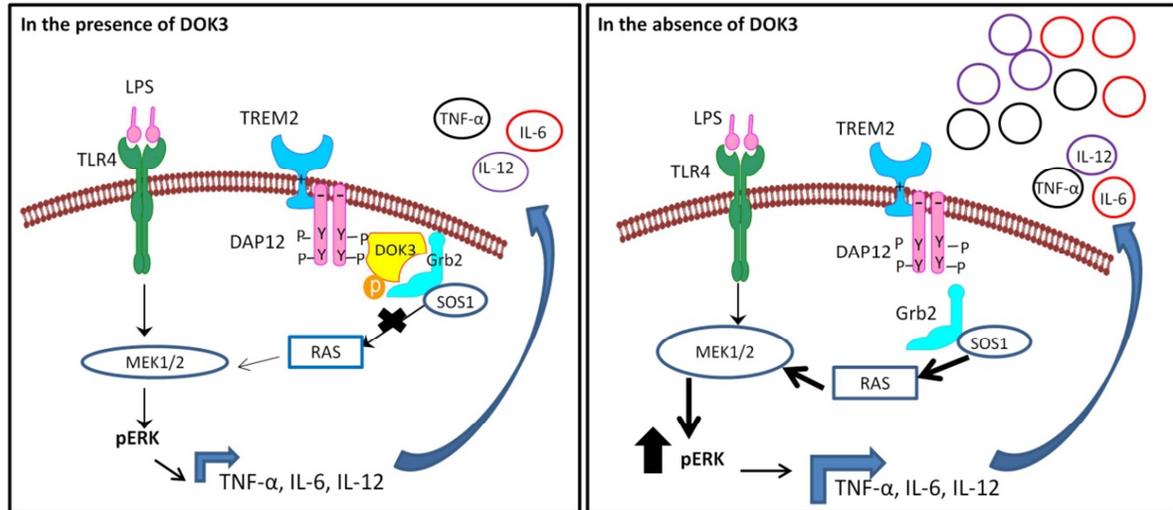


Fig S3. DAP12 and DOK3 mediate inhibition of LPS-dependent cytokine production. Left: LPS stimulates the phosphorylation of DOK3 and its recruitment to the plasma membrane in a DAP12-dependent manner. In a Src-dependent manner, the PTB of DOK3 associates with the phosphorylated ITAM of DAP12. DOK3 associates with Grb2 and Sos1, preventing the activation of Ras and inhibiting the activation of ERK and the production of the proinflammatory cytokines TNF- α , IL-6, and IL-12. Right: In the absence of DOK3, Grb2 and Sos1 are available to activate the Ras-ERK pathway to enhance proinflammatory cytokine production downstream of LPS.