SUPPLEMENTAL MATERIAL

Gotoh et al., http://www.jem.org/cgi/content/full/jem.20091776/DC1
Figure S1. The effect of CXCL16 deficiency, pertussis toxin, or PP2 on CpG-A uptake, actin polymerization, and cytokine production in pDCs. (A–D) Before assay, WT pDCs were pretreated for 1 h with 500 ng/ml pertussis toxin (PTx), 4 µM PP2, or vehicle (DMSO; Mock). (A and D) The uptake of CpG-A was compared between WT and Cxcl16−/− pDCs (A) or among WT pDCs treated with Mock, PTx, or PP2 (D). Cells were incubated with 3 µM CpG-A–Cy5 at 37°C (filled area) or 4°C (open area) for 1 h in the presence or absence of inhibitors. (B and E) CpG-A–induced actin polymerization was compared between WT and Cxcl16−/− pDCs (B) or among WT pDCs treated with Mock, PTx, or PP2 (E). After being stimulated with 3 µM CpG-A in the presence or absence of inhibitors, cells were fixed and stained with phalloidin. Bars, 10 µm. (C and F) Cytokine production was compared between WT and Cxcl16−/− pDCs (C) or among WT pDCs treated with Mock, pertussis toxin, or PP2 (F). Cells were stimulated with 3 µM CpG-A for 24 h in the presence or absence of inhibitors. Data indicate the levels of IFN-α and IL-12p40 in cell culture supernatants (mean ± SD of triplicate wells). All data are representative of two independent experiments.
Figure S2. DOCK2 deficiency does not affect CpG-A–induced actin polymerization and Rac activation in myeloid DCs. BM-derived WT and Dock2<sup>−/−</sup> myeloid DCs were left untreated or stimulated with 3 µM CpG-A for the indicated times. (A) Cells were then fixed and stained with phalloidin. Bar, 10 µm. (B) Activation of Rac was analyzed. Data are representative of two independent experiments.
Figure S3. **DOCK2 deficiency does not alter subcellular localization of TLR9 and CpG-B in pDCs.** BM-derived WT and Dock2^{−/−} pDCs were retrovirally transduced with TLR9–YFP. Cells were left untreated or stimulated with 1 μM CpG-B–Cy5 for the indicated times. After fixation with 4% paraformaldehyde, cells were stained for TfR (A) or LAMP-1 (B), and the area of TLR9–YFP or CpG-B–Cy5 merged with TfR (A) or LAMP-1 (B) was calculated in each cell. Data are pooled from two independent experiments and are expressed as the percentage of colocalization (mean ± SD) for 20 cells analyzed per group. Bars, 5 μm.
Figure S4. The serine phosphorylation of IKK-α at position 176/180 is critical for IRF-7–mediated type I IFN induction. 293T cells were transiently transfected with an Ifna4 promoter–driven reporter plasmid alone or together with a combination of the expression vector for MyD88, IRF-7, IKK-α (WT), IKK-α (Ser176/180Ala, SSAA), IKK-α (Lys44Ala, KA), or IKK-α (Ser176/180Glu, SSEE). After 24 h, cell lysates were prepared and promoter activity was measured by luciferase assay. Data are representative of three independent experiments.