Supporting Information

Nguyen et al. 10.1073/pnas.1014465107

SI Materials and Methods

Splenic DC Preparation and Stimulation Conditions. Spleen cells were isolated from WT and Ahr$^{-/-}$ mice and counted (Fig. S2*A*). DC from mouse spleen were prepared with Collagenase D according to the protocol of the manufacturer (Miltenyi Biotec). CD11c^{+} splenic DC were selected by MACS. Splenic DC were seeded in 10 mL of complete RPMI medium 1640 (5×10^5 cells/mL) and stimulated for 24 h with or without LPS (1 μg/mL; Sigma) or CpG (1 μM; Gene Design Inc.).

Measurement of Kyn Concentrations. BMDC were cultured in complete RPMI medium 1640 plus L-Trp (100 μM). The cells were then stimulated with LPS or CpG for 18 h. Kyn levels in culture supernatants were quantified using a colorimetric method as previously described (1) with minor modifications. Briefly, 60 μL sample/standard was added to 30 μL of 30% TCA. Then, 75 μl of supernatant was added to 75 μL of freshly prepared Ehrlich Reagent (100 mg *p*-dimethylbenzaldehyde in 5 mL glacial acetic acid) in a microplate. The OD of the samples was measured at 490 nm using a microplate reader (Biorad). The Kyn concentration was determined using a Kyn standard curve as a reference. All chemicals were purchased from Sigma.

Western Blotting. BMDC were stimulated with LPS or CpG for 24 h. Cells were lysed in lysis buffer (1% Nonidet P-40, 20 mM Tris·HCl, pH 7.5, 150 mM NaCl, 10 mM Na_2VO_4, 0.5 mM DTT, and 1/100 protease inhibitor mixture). Whole-cell lysates were analyzed by Western blotting with anti-Ahr antibodies (BIOMOL International).

Cytokine ELISA. BMDC were stimulated with LPS or CpG. After 24 h, cytokine levels, including those of IL-6, TNF-α, IL-12p40, TGF-β1, and IL-10, were measured in culture supernatants using ELISAs (R&D Systems).

CFSE Dilution Assay. Naive T cells were incubated in 3 μM CFSE at 37 °C for 10 min following the instructions of the manufacturer (Invitrogen). CFSE-labeled naive T cells were cocultured with LPS- or CpG-stimulated BMDC. After 4 d, T-cell proliferation was assessed based on CFSE dilution using FACS analysis with a Cytomics FC500 system (Beckman Coulter).

1. Däubener W, et al. (1994) A new, simple, bioassay for human IFN-γ. *J Immunol Methods* 168:39–47.

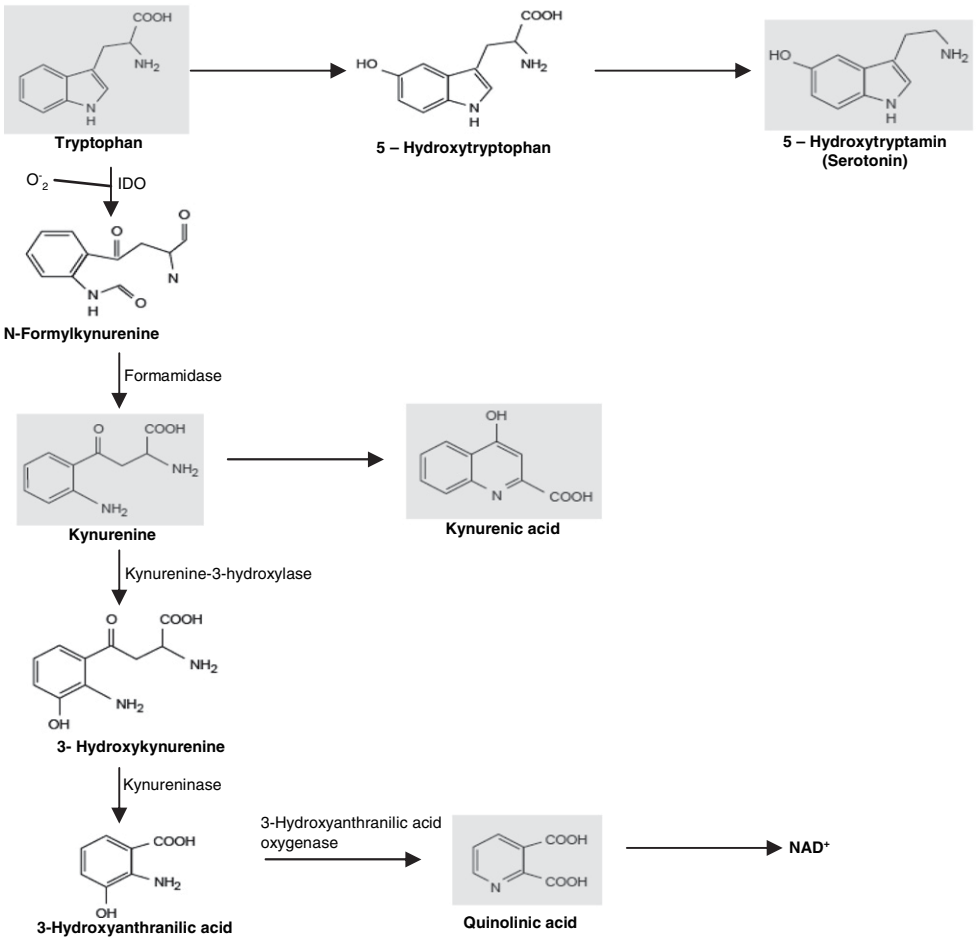

Fig. S1. Schematic of the Trp catabolism pathways. Trp is mainly converted into Kyn and other metabolites in the Kyn pathway via IDO and the other indicated enzymes.

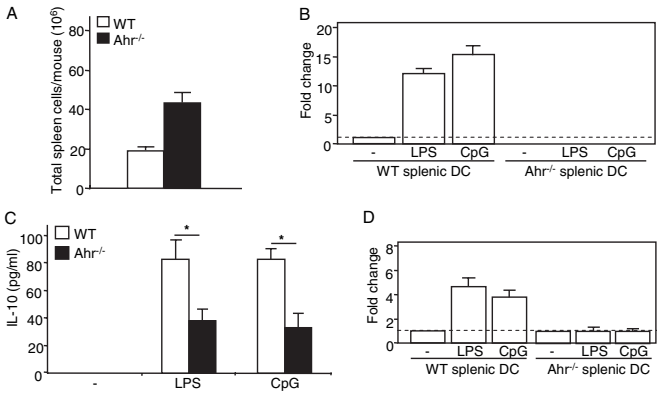

Fig. S2. LPS or CpG stimulated Ahr and IDO expression in splenic DC. (*A*) Total spleen cells per mouse. (*B*) Ahr expression in splenic DC stimulated with LPS or CpG. (*C*) IL-10 production was inhibited in Ahr$^{-/-}$ splenic DC stimulated with LPS or CpG. (*D*) IDO expression in splenic DC stimulated with LPS or CpG.

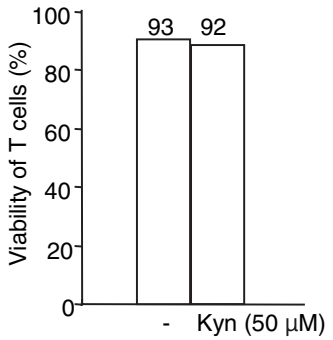

Fig. S3. Synthetic L-Kyn at a concentration of 50 μM did not reduce the viability of T cells. In the BMDC–T cell-coculture system, synthetic L-Kyn (50 μM) was added to the indicated samples. Viability of the cells was determined after 4 d using trypan blue staining and an automated cell counter.

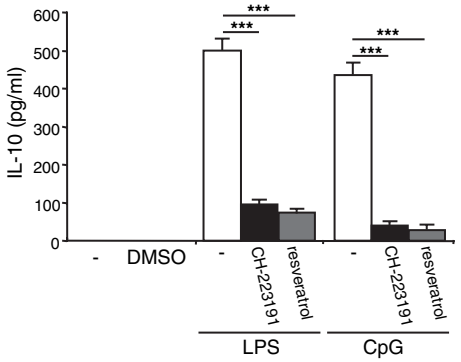

Fig. S4. Ahr antagonists CH-223191 and resveratrol blocked IL-10 production in LPS- or CpG-stimulated WT BMDC. WT BMDC were stimulated with LPS or CpG with or without CH-223191 or resveratrol, and culture supernatants were harvested after 24 h. IL-10 levels were measured using ELISA. Data show mean ± SD from three independent experiments. ***$P < 0.005$.

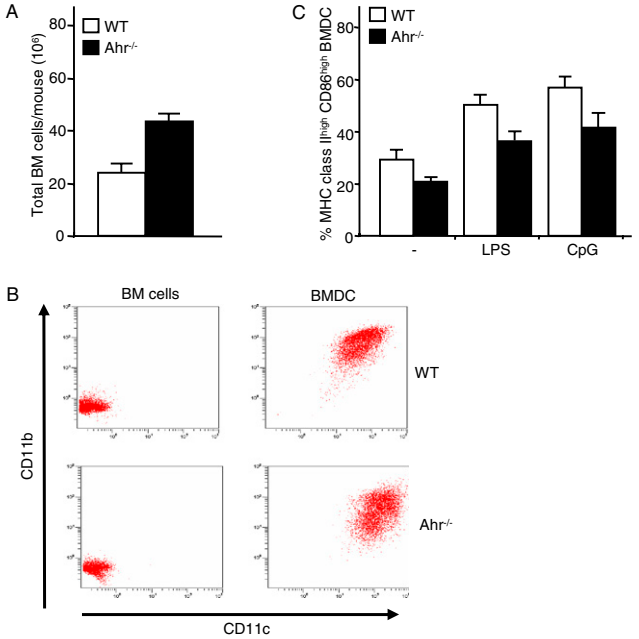

Fig. S5. Phenotypes of BMDC unstimulated or stimulated with LPS or CpG. (*A*) Total bone marrow (BM) cells per mouse. (*B*) Generation of BMDC from BM cells after 9 d culture in the presence of GM-CSF. More than 80% of the BMDC at day 9 were $CD11b^+$ and $CD11c^+$. (*C*) Double staining for MHC class II and CD86 of the BMDC stimulated with LPS or CpG.