# Supporting Information

## Quintana et al. 10.1073/pnas.1009201107

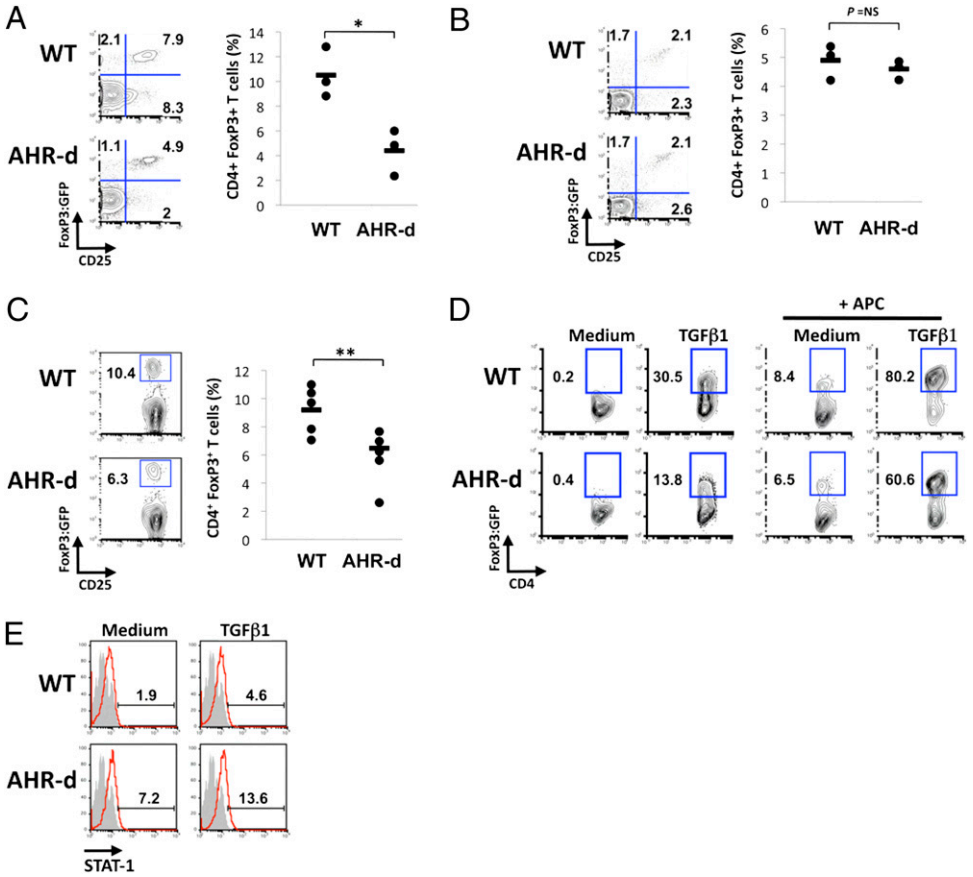


**Fig. S1.** AHR activation by endogenous ligands promotes the differentiation of FoxP3$^+$ iT$_{reg}$. (*A* and *B*) Frequency of CD4$^+$ Foxp3:GFP$^+$ T$_{reg}$ in the thymus (*A*) and the MLN (*B*) of naive AHR-d Foxp3$^{gpf}$ and Foxp3$^{gfp}$ knock-in mice. *$P < 0.05$ compared with WT mice. (*C*) CD4$^+$ Foxp3:GFP$^-$ T cells from naive AHR-d Foxp3$^{gpf}$ and Foxp3$^{gfp}$ mice were transferred into RAG-1-deficient hosts, the recipients were immunized with MOG$_{35–55}$ in IFA, and the frequency of CD4$^+$ FoxP3:GFP$^+$ T$_{reg}$ was analyzed in the spleen 3 wk after immunization. **$P < 0.01$ compared with recipients of WT cells. (*D*) Naive CD4$^+$ Foxp3:GFP$^-$ T cells from AHR-d Foxp3$^{gpf}$ and Foxp3$^{gfp}$ knock-in mice were stimulated for 5 d with plate-bound antibodies to CD3 and CD28, or with soluble antibodies to CD3 and irradiated APCs, in the presence of IL-2 and TGF-β1, and the frequency of CD4$^+$ Foxp3:GFP$^+$ T cells was quantified by FACS. (*E*) Naive CD4$^+$ Foxp3:GFP$^-$ T cells from naive AHR-d or WT mice were activated for 2 d with plate-bound antibodies to CD3 and CD28, with or without IL-2, TGF-β1, and IL-2 + TGF-β1, and the phosphorylation of Stat1 was analyzed by FACS. Representative data of one of at least three experiments that produced similar results.

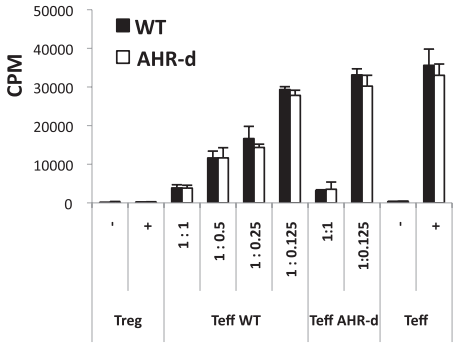


**Fig. S2.** Suppressive activity in WT and the d allele of *ahr* (aryl hydrocarbon receptor) (AHR-d) FoxP3$^+$ T$_{reg}$. CD4$^+$ Foxp3:GFP$^-$ T$_{reg}$ were isolated from WT or AHR-d mice and their suppressive activity was analyzed in coculture experiments using naive Foxp3:GFP$^-$ T cells activated with antibodies to CD3 and irradiated antigen presenting cells (APCs) as responder T cells. Representative data of one of two experiments that produced similar results.

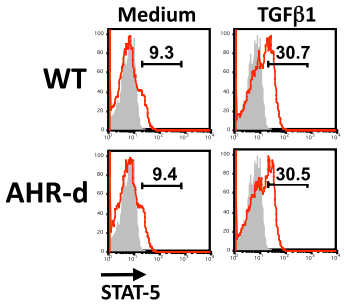


**Fig. S3.** AHR activation does not limit Stat 5 signaling. Naive CD4$^+$ Foxp3:GFP$^-$ T cells from naive AHR-d or WT mice were activated for 2 d with plate-bound antibodies to CD3 and CD28, with or without IL-2, TGF-β1, and IL-2 + TGFβ1, and the phosphorylation of Stat5 was analyzed by FACS. Representative data of three experiments that produced similar results.

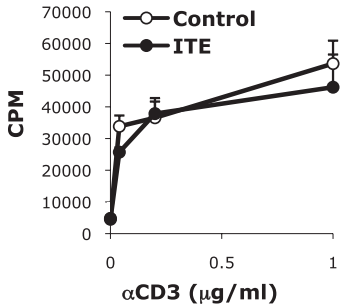


**Fig. S4.** AHR activation by the nontoxic endogenous ligand 2-(1′H-indole-3′-carbonyl)-thiazole-4-carboxylic acid methyl ester (ITE) does not result in general immunosupression. Proliferative response to anti-CD3 of lymph node cells from ITE- or control-treated animals 10 d after immunization with $MOG_{35–55}$ in complete Fruend's adjuvant (CFA). Cell proliferation is indicated as cpm + SD in triplicate wells.

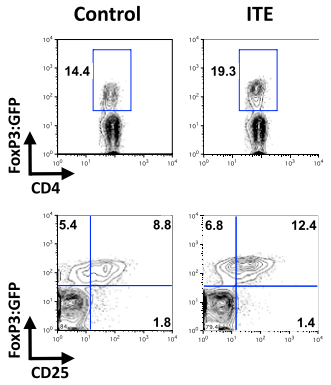


**Fig. S5.** Frequency of FoxP3$^+$ $T_{reg}$ in SJL treated mice. Frequency of CD4$^+$Foxp3:GFP$^+$ $T_{reg}$ in splenocytes from ITE or control treated SJL mice, 30 d after experimental autoimmune encephalomyelitis (EAE) induction. Representative data of one of two experiments that produced similar results.

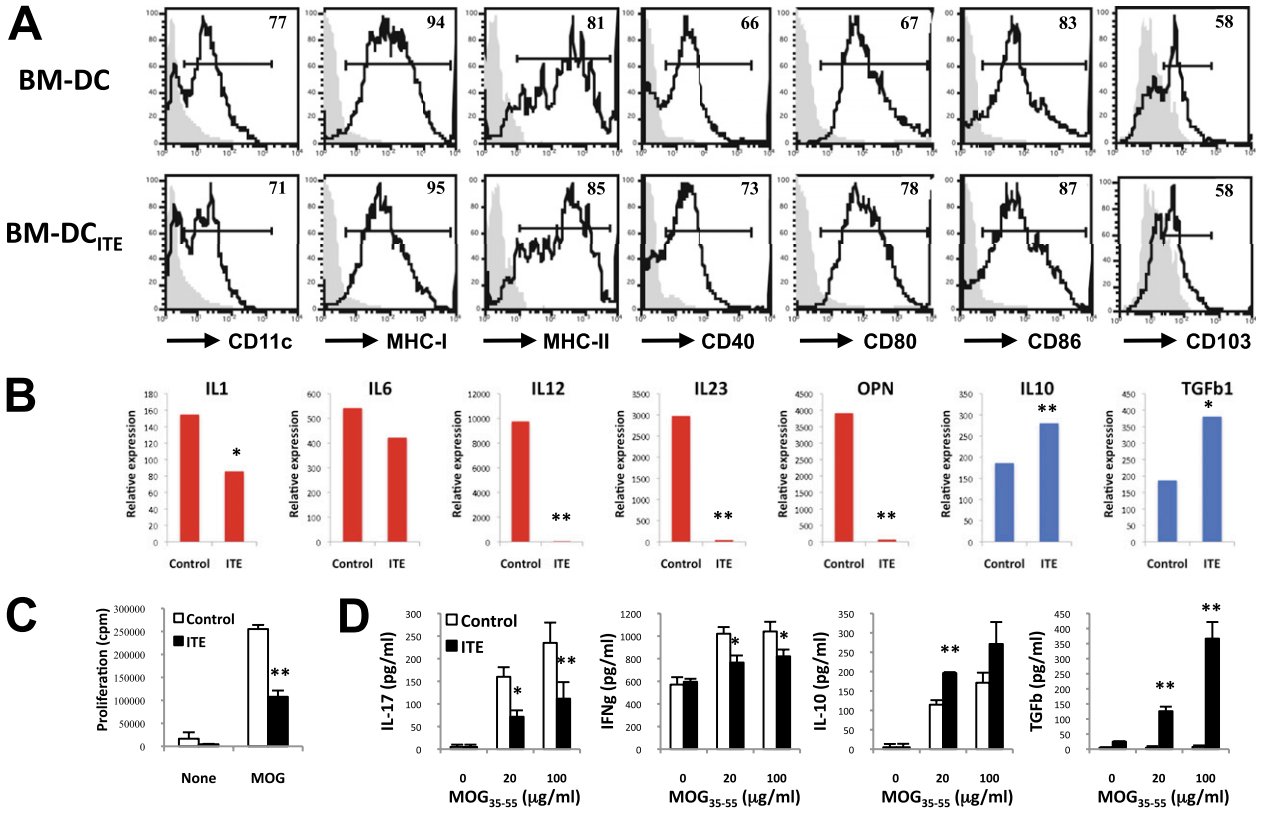


**Fig. S6.** Bone marrow-dendritic cell (BM-DC) differentiated in the presence of ITE are tolerogenic DCs. (*A*) FACS analysis of BM-DC treated with ITE (BM-DC$_{ITE}$) or vehicle (BM-DC) as control. Numbers in plots indicate the percent of positive cells; the staining obtained with isotype-matched control antibodies is shown in gray. (*B*) Quantitative PCR analysis of cytokine expression by splenic DC isolated from ITE- or control-treated mice; results are presented relative to GAPDH mRNA. $*P < 0.01$ and $**P < 0.001$ compared with BM-DC differentiated from WT mice. (*C* and *D*) Naive 2D2$^+$ CD4$^+$ FoxP3:GFP$^-$ T cells were stimulated with BM-DC$_{ITE}$ or BM-DC and MOG$_{35–55}$, and proliferation (*C*) and cytokine secretion (*D*) was analyzed. $*P < 0.01$ and $**P < 0.001$ when compared with T cells incubated with BM-DC differentiated from WT mice. Representative data of one of three experiments that produced similar results.

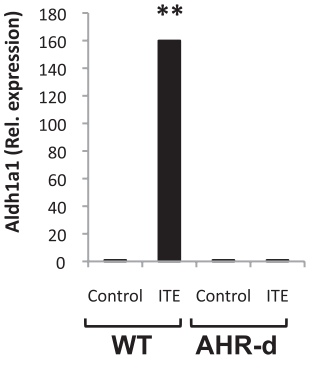


**Fig. S7.** Increased *aldh1a2* expression by BM-DC$_{ITE}$. Quantitative PCR analysis of *aldh1a2* expression by splenic DCs isolated from ITE- or control-treated WT or AHR-d mice; results are presented relative to GAPDH mRNA. **, $P < 0.001$ when compared with BM-DC taken from control-treated WT mice or BM-DC$_{ITE}$ from AHR-d mice. Representative data of one of two experiments that produced similar results.

**Table S1. Measurement of liver toxicity in ITE-treated mice**

| Test | Units | Control | ITE | Reference range |
|---|---|---|---|---|
| ALT (GPT) | U/L | 16 ± 2 | 19 ± 4 | 0–54 |
| AST (GOT) | U/L | 79 ± 29 | 94 ± 17 | 9–74 |
| Alkaline phosphatase | U/L | 75 ± 8 | 55 ± 11 | 36–300 |
| Total bilirubin | mg/dL | 0 ± 0 | 0 ± 0 | 0.1–1.2 |
| Direct bilirubin | mg/dL | 0 ± 0 | 0 ± 0 | 0.0–0.8 |
| Total protein | g/dL | 6 ± 0 | 5 ± 0 | 4.4–8.0 |
| Albumin | g/dL | 2 ± 0 | 3 ± 0 | 2.9–5.4 |
| Globulin | g/dL | 3 ± 0 | 3 ± 0 | 2.0–4.0 |

ALT, L-alanine: 2-oxoglutarate aminotransferase; AST, aspartate aminotransferase; GOT, glutamic oxalacetic transaminase; GPT, glutamyl pyruvic transaminase.