Supporting Information

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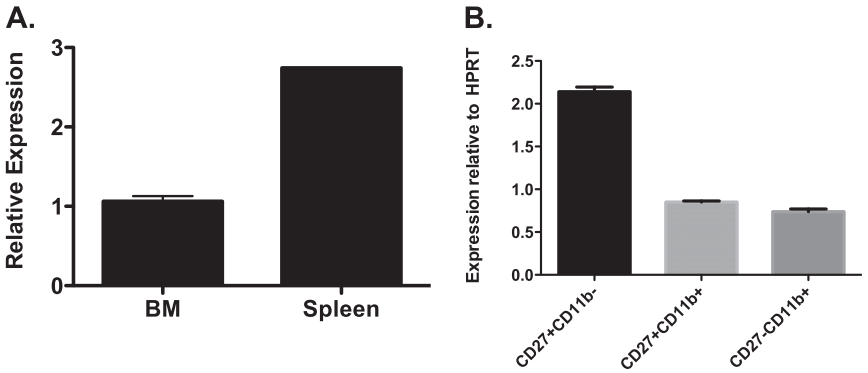

Fig. S1. Aryl hydrocarbon receptor (*AhR*) expression in mouse natural killer (NK) cells. (*A*) NK ($NKp46^+CD3^-$) cells were sorted by FACS from WT mice bone marrow (BM) and spleen to high purity, and *AhR* expression was assessed by Taqman quantitative RT-PCR (qRT-PCR). (*B*) CD27/CD11b subsets of NK ($NKp46^+$ $CD3^-$) cells were sorted by FACS from WT spleens to high purity, and *AhR* expression was assessed by Taqman qRT-PCR. Results in this figure were reproduced at least once.

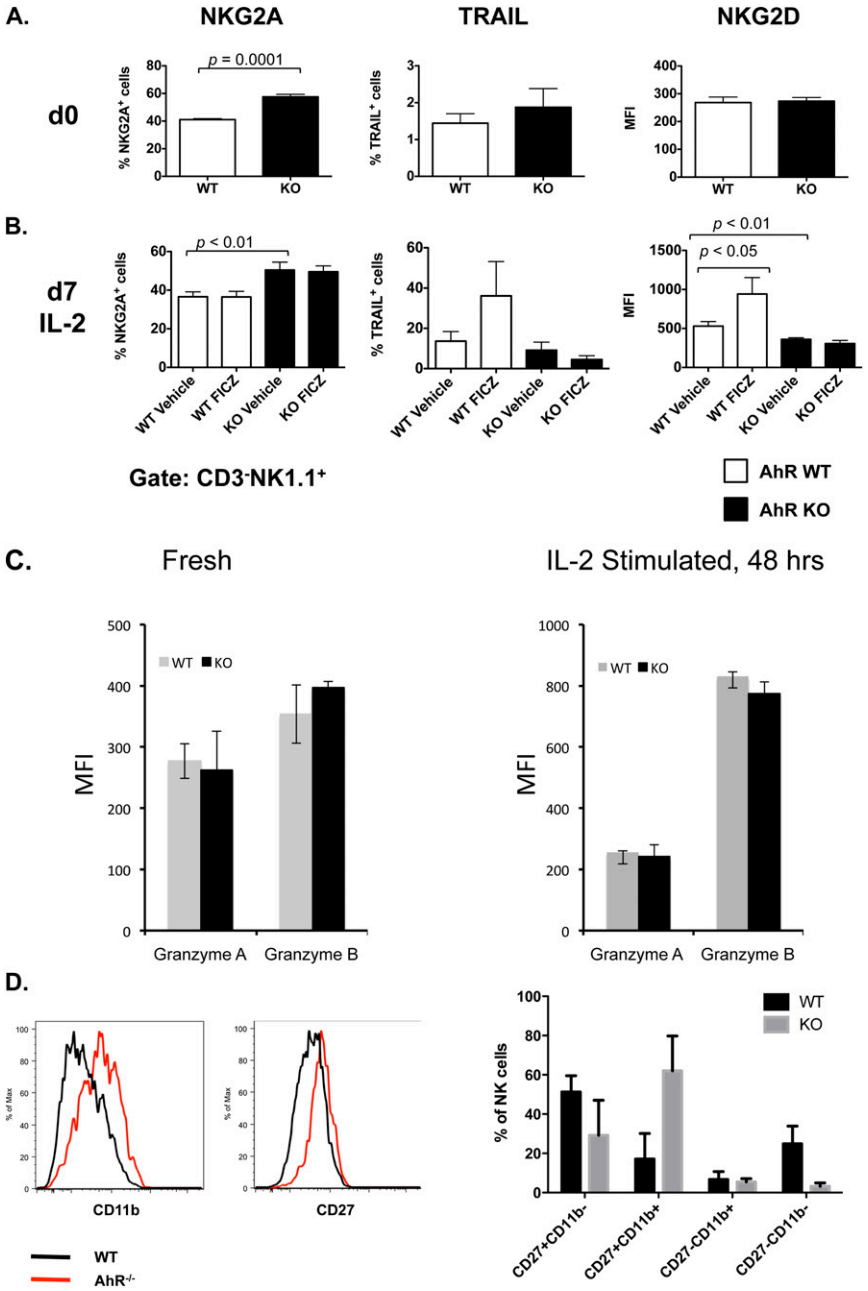

Fig. S2. Profiling of NK cells from AhR$^{-/-}$ mice. (*A* and *B*) Splenic NK cells from AhR$^{-/-}$ and AhR$^{+/+}$ mice were enriched by magnetic bead negative selection and cultured in IL-2 (1,000 U/mL) for 7 d, either in the presence of 6-formylindolo[3,2-*b*]carbazole (FICZ) (200 nM) or vehicle control. The NK cells (NK1.1^{+}CD3^{-}) were analyzed for NKG2A, TRAIL, and NKG2D by FACS on day 0 and day 7. (*C*) Intracellular staining of Granzyme A and B and FACS analysis was performed on splenocytes from 12- to 16 wk-old AhR$^{+/+}$ and AhR$^{-/-}$ mice immediately after harvesting (fresh) and after 48 h in culture with IL-2 (1,000 U/mL). Gate, CD3^{-}NK1.1^{+}. (*D*) NK (NK1.1^{+}CD3^{-}) cells were isolated by negative isolation from the spleens of AhR$^{-/-}$ and AhR$^{+/+}$ mice and cultured in 1,000 U/mL IL-2. Cells were analyzed for CD27 and CD11b expression by FACS on day 11 (n = 3 mice per condition). Results in this figure were reproduced at least once.

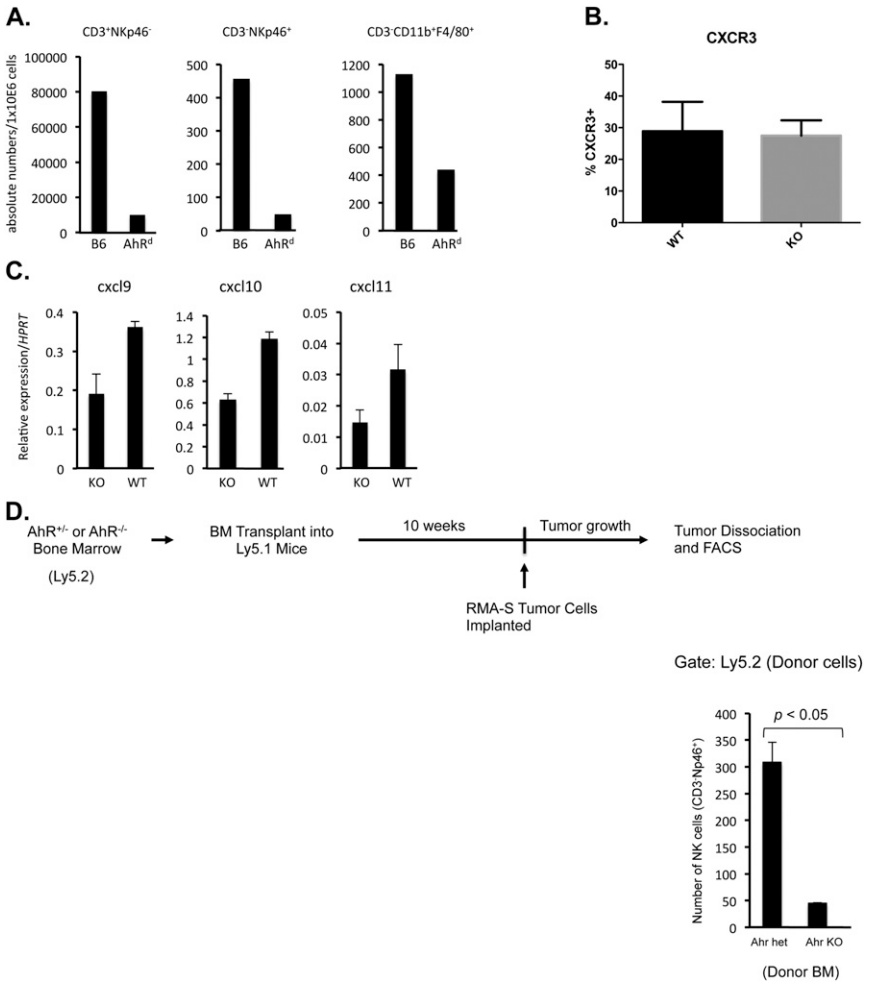

Fig. S3. Increased tumor growth in AhR-deficient mice is correlated with a decrease in tumor infiltrating lymphocytes. (*A*) RMA-S tumors established in WT and AhR^d mice were dissociated and stained with CD3, NKp46, CD11b, and F4/80 antibodies. The absolute numbers of each population were analyzed with flow cytometry. (*B*) PBMCs were isolated from the blood of non–tumor-bearing adult $AhR^{-/-}$ and $AhR^{+/+}$ mice, and unstimulated NK cells ($NK1.1^+CD3^-$) were analyzed for CXCR3 expression by FACS. $n = 4$ mice per group. (*C*) RMA-S tumors were established in $AhR^{-/-}$ and $AhR^{+/+}$ mice, and cDNA was synthesized from RNA preparations of the tumors. Chemokine expression was assessed by qRT-PCR, normalized to *Hprt* expression. (*D*) BM cells (10^7) from $AhR^{+/-}$ or $AhR^{-/-}$ mice (Ly5.2) were transplanted i.v. into irradiated (10 Gy) C57BL/6 (B6) Ly5.1-congenic mice. After 10 wk, the recipients were implanted s.c. over the flanks with RMA-S tumor cells (5×10^4 cells per mouse). After tumors formed, the tumors were harvested, dissociated, stained for Ly5.2, CD3, and NKp46, and analyzed by FACS. $n = 3$ per cohort. Results were reproduced at least once.

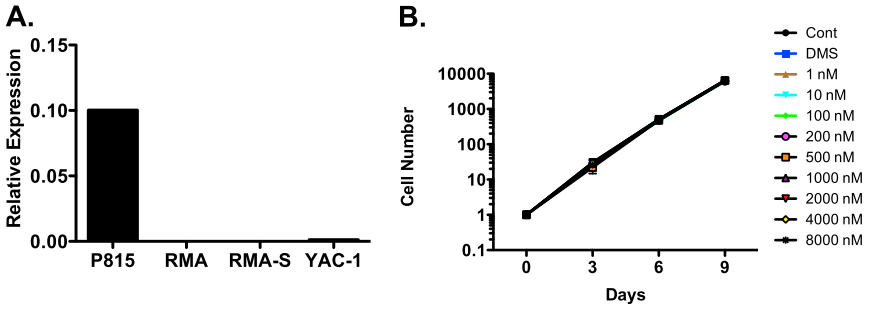

Fig. S4. RMA-S cells do not express AhR and are not affected by FICZ. (*A*) *AhR* expression was assessed by qRT-PCR in P815, RMA, RMA-S, and Yac-1 cells, normalized to *Hprt* expression. (*B*) RMA-S cells were cultured in various concentrations of FICZ, and cell proliferation was assessed by cell counting of the cultured cells.

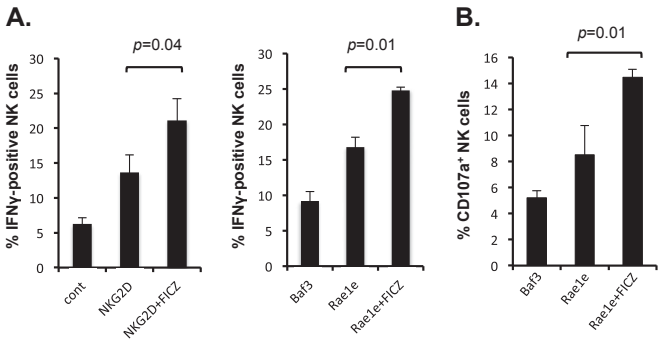

Fig. S5. FICZ enhances NKG2D-mediated NK cell activation. (*A*) Splenocytes from Rag1$^{-/-}$ mice, injected i.p. with either FICZ (3 μg per mouse, i.p.) or vehicle control, were cultured on anti-NKG2D antibody (A10; eBioscience)-coated plates or with Baf cells stably transfected with Rae1ε, for 8 h (ratio 1:1). IFN-γ production was assessed by intracellular cytokine staining and FACS analysis, similar to Fig. 4. Baf3, parental Baf3 cells; NKG2D, anti-NKG2D antibody and vehicle control i.p. treatment; NKG2D+FICZ, anti-NKG2D antibody and FICZ i.p. treatment; Rae1ε, Baf3-Rae1ε transfectant and vehicle control i.p. treatment; Rae1ε + FICZ, Baf3-Rae1ε transfectant and FICZ i.p. treatment. (*B*) Splenocytes from WT mice, treated i.p. with FICZ (3 μg per mouse, i.p.) or vehicle control, were incubated 1:1 with Baf3-Rae1ε transfectant with or without FICZ, as in *A*, and stained for CD107a surface expression. Gate is on NK1.1^{+}CD3^{-} cells.

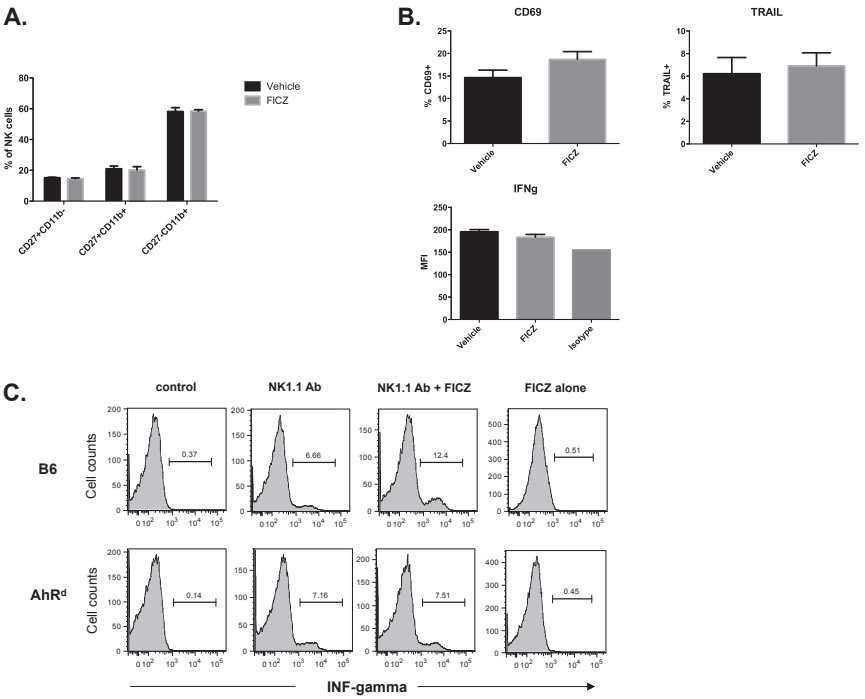

Fig. S6. No effect of FICZ alone on naïve NK cell maturation and activation markers and IFN-γ production. (*A*) Adult WT mice were injected with 3 μg of FICZ per mouse or vehicle control. Spleens were harvested at 48 h. NK cells (CD3-NK1.1+) were analyzed by FACS for surface expression of CD27 and CD11b ($n = 3$ mice per group). (*B*) Splenocytes from mice treated, as in *A*, were analyzed for CD69 and TRAIL expression ($n = 3$ mice per group), or were incubated with Monensin for 4 h, and then NK cells (CD3-NK1.1+) were assessed by FACS for intracellular staining of IFN-γ ($n = 3$ mice per group). (*C*) Splenocytes from B6 and AhR^{d} mice were stimulated ex vivo with plate-bound anti-NK1.1 antibody in the absence and presence of FICZ and stained for intracellular IFN-γ, and analyzed by FACS. Gate: DAPI^{-}CD3^{-}NKp46^{+}.