Supporting Information

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Supporting Materials and Methods

Flow Cytometry. For intracellular cytokine staining, cells were isolated and restimulated with PMA (10 ng/mL), ionomycin (1 μg/mL), and monensin (4 μM) for 4 h and stained with antibodies against CD4, IFN-γ, IL-4, IL-10, IL-17A, and TNF-α. Flow cytometry was performed on a FACSCalibur (Beckton Dickinson). To measure the ConA-induced T cell proliferative response in vivo, 2×10^7 CFSE-labeled splenocytes were injected into C57BL/6 mice along with ConA, and CFSE dilution in $CD4^+$ T cells in liver and spleen was measured 48 h after transfer by flow cytometry. For the analysis of antigen-specific proliferative responses in vivo, total spleen cells from OT-II TCR transgenic mice were labeled with CFSE and injected into Thy1.1 C57BL/6 mice. Those mice were further immunized with OVA protein emulsified with CFA (Sigma). Seven days after immunization, draining LN were isolated, and $Thy1.2^+$ $CD4^+$ cells were analyzed for CFSE dilution by flow cytometry.

ConA-Induced Hepatitis Model. Mice were i.v. injected with 10 μg/g of Con A (Sigma). Five to seven $RBP\text{-}J^{F/F}$-Cre or $RBP\text{-}J^{+/+}$-Cre mice were used in each experiment. In some experiments, recombinant IL-22 (Peprotech) was administered once at 2 μg per mouse along with ConA. After the indicated times, mice were killed and the livers were perfused. Isolated liver sections were frozen with O.C.T. compound (Tissue-Tek; Sakura) in liquid nitrogen for histological analysis by hematoxylin and eosin staining. To isolate liver-infiltrating lymphocytes, livers were collected after perfusion with PBS and smashed in serum-free RPMI medium 1640. After removing debris, cells were layered onto 4 mL of Lympholyte M (Cedarlane) and centrifuged at 1,100 × g for 25 min. After centrifugation, lymphocytes were collected and $CD4^+$ T cells were isolated by using biotin-conjugated anti-CD4 mAb (eBioscience) followed by streptavidin MACS beads (Miltenyi Biotech).

Real-Time PCR. Total RNA was extracted with TRIzol (Invitrogen). After reverse transcription using the Omniscript RT Kit (Qiagen), SYBR premix Ex Taq II (Takara Bio) was used for quantitative PCR. All data were normalized to HPRT (hypoxanthine-guanine phosphoribosyl transferase) and were presented as fold increase relative to the background value. The primers used in this study for real-time PCR are shown in Table S2.

DNA Microarray Analysis. Total RNA was isolated from cells by using an RNeasy Mini kit (Qiagen) and Affymetrix oligonucleotide GeneChips (Affymetrix) were probed, hybridized, stained, washed, and scanned according to the manufacturer's protocol.

Histology. Livers were fixed in 10% formalin, embedded in paraffin, and sectioned. Tissue sections were stained with hematoxylin and eosin.

Sep-Pak Solid Phase Extraction. Sep-Pak Plus C_{18} cartridges (Waters) were prepared by passing 10 mL of 100% methanol, 15 mL of acetone, and then washed with 15 mL of distilled water (DW). After preparation, 5 mL of supernatant were added to the column and the flow-through was collected. Finally, after washing with 15 ml of DW, the cartridge was eluted with 3 mL of acetone. The acetone was evaporated and dissolved in 300 μL of RPMI medium 1640.

Statistical Analysis. The distributed data from interval scales were analyzed with Student's *t* test; a *P* value <0.05 was considered statistically significant.

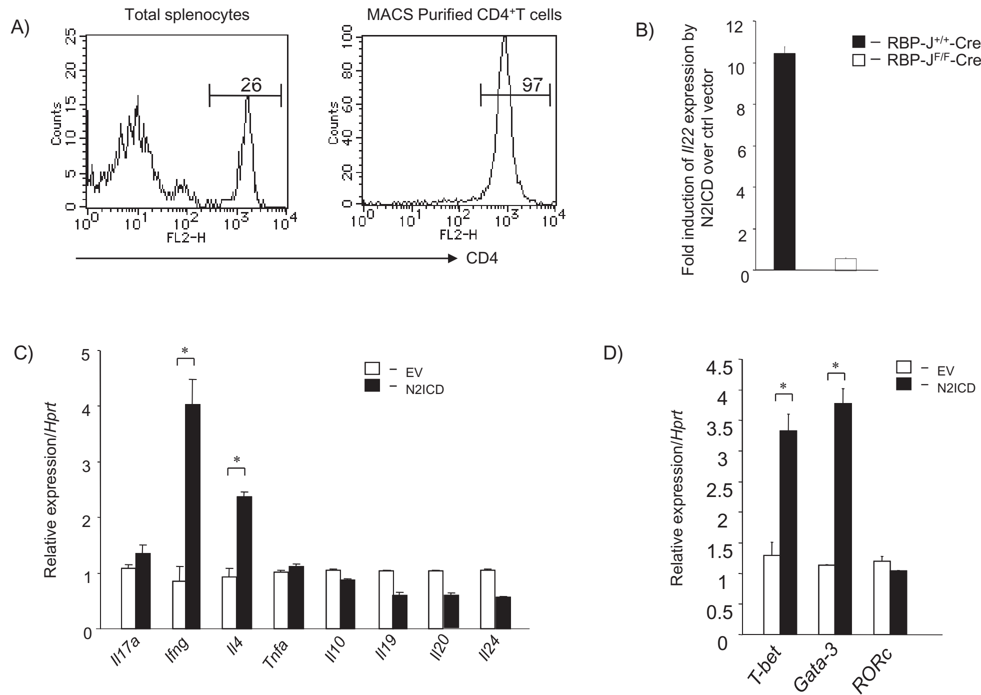

Fig. S1. Expression profile of cytokines and transcription factors in purified CD4⁺T cells. (*A*) Total splenocytes were stimulated with anti-CD3 mAb for 48 h and then sorted out CD4⁺T cells with MACS beads. Percentages of CD4⁺T cells before and after MACS purification were checked by staining with anti-CD4 mAb. (*B*) Naive CD4⁺ T (CD4⁺ CD62L⁺) cells from RBP-J$^{+/+}$ crossed with CD4-Cre and RBP-J$^{F/F}$ crossed with CD4-Cre mice were stimulated with plate-coated anti-CD3 mAb for 24 h and transduced with a retrovirus carrying N2ICD or a control virus. The expression of *Il22* was analyzed 48 h after infection by real-time PCR. Data are shown as the fold increase in *Il22* expression by N2ICD over empty vector. (*C* and *D*) Total spleen cells from C57BL/6 mice were stimulated with anti-CD3 mAb for 24 h and transduced with a retrovirus carrying N2ICD or a control virus. The expression of cytokines (*C*) and *T-bet*, *Gata-3*, and *Rorc* (*D*) in MACS-enriched CD4⁺ T cells was analyzed 48 h after infection by real-time PCR. *, $P < 0.05$, indicates a statistically significant difference. Data are representative of at least four independent experiments.

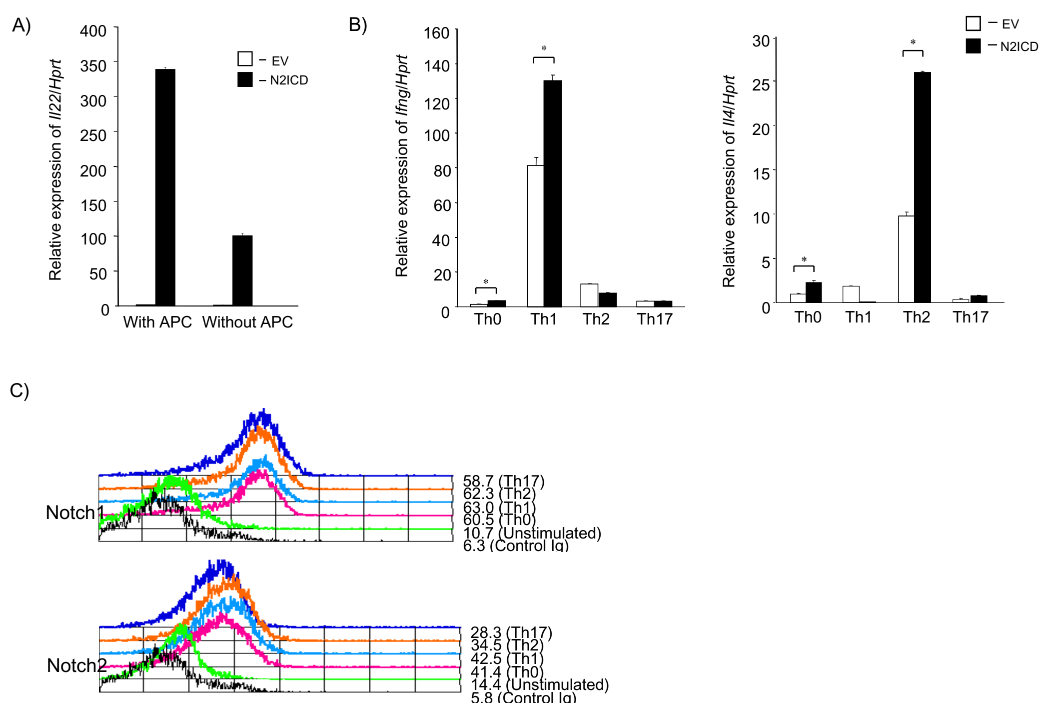

Fig. S2. Expression of cytokines by CD4$^+$T cells after Notch induction. (*A*) Total spleen cells from C57BL/6 mice were stimulated with anti-CD3 mAb for 24 h and transduced with a retrovirus carrying N2ICD or a control virus. The expression of *Il22* in MACS-enriched CD4$^+$ T cells was analyzed 48 h after infection by real-time PCR. MACS-purified CD4$^+$ T cells were infected with the same procedure as that for total splenocytes except that cells were stimulated with plate-bound anti-CD3 mAb instead of soluble anti-CD3. The expression of *Il22* in CD4$^+$ T cells was analyzed 48 h after infection by real-time PCR. (*B*) Total spleen cells were stimulated with anti-CD3 mAb in Th0, Th1, Th2, and Th17 conditions for 24 h and then transduced with N2ICD or control virus. After 48 h of further culture under the same conditions, *Ifng* and *Il4* expression in MACS-enriched CD4$^+$ T cells was measured by real-time PCR. (*C*) Total spleen cells were isolated from C57BL/6 mice and stimulated with anti-CD3 mAb under Th0 (neutral), Th1, Th2, and Th17 culture conditions. Forty-eight hours after stimulation, the expression of Notch1 and Notch2 receptors was checked by flow cytometry. Data are representative of at least four independent experiments.

Fig. S3. DL1 on DC can induce IL-22 and IFN-γ production. (*A*) ELISA for the detection of IFN-γ secretion from naive CD4$^+$T cells (CD4$^+$CD62L$^+$) isolated from OT-II TCR transgenic mice stimulated with OVA peptide-pulsed Cont-DC (open) or DL1-DC (filled) for 3 days. (*B*) Naive CD4$^+$T cells (CD4$^+$CD62L$^+$) isolated from OT-II TCR transgenic mice were stimulated under Th0, Th1, Th2, and Th17 conditions with or without OVA peptide-pulsed Cont-DC (open) or DL1-DC (filled). IL-22 secretion was measured in the culture supernatant by ELISA after 3 days. (*C*) Naive CD4$^+$ T cells isolated from RBP-J$^{+/+}$-Cre OT-II (filled) or RBP-J$^{F/F}$-Cre OT-II (open) mice were stimulated with OVA peptide-pulsed BMDCs for 3 days. IFN-γ concentrations in the supernatants were evaluated by ELISA. (*D*) Purified and CFSE-labeled naive CD4$^+$ T cells from RBP-J$^{+/+}$-Cre OT-II or RBP-J$^{F/F}$-Cre OT-II were stimulated with peptide-pulsed BMDCs for 2 days (*Upper*) or 3 days (*Lower*). The intensity of CFSE in CD4$^+$ T cells was analyzed by flow cytometry. (*E*) Naive CD4$^+$ T cells isolated from wild-type OT-II TCR transgenic mice were stimulated with OVA peptide-pulsed BMDCs in presence or absence or γ-secretase inhibitor (20 μM) for 3 days. IL-22 concentrations in the supernatants were evaluated by ELISA. *, $P < 0.05$, indicates a statistically significant difference. Data are representative of at least four independent experiments.

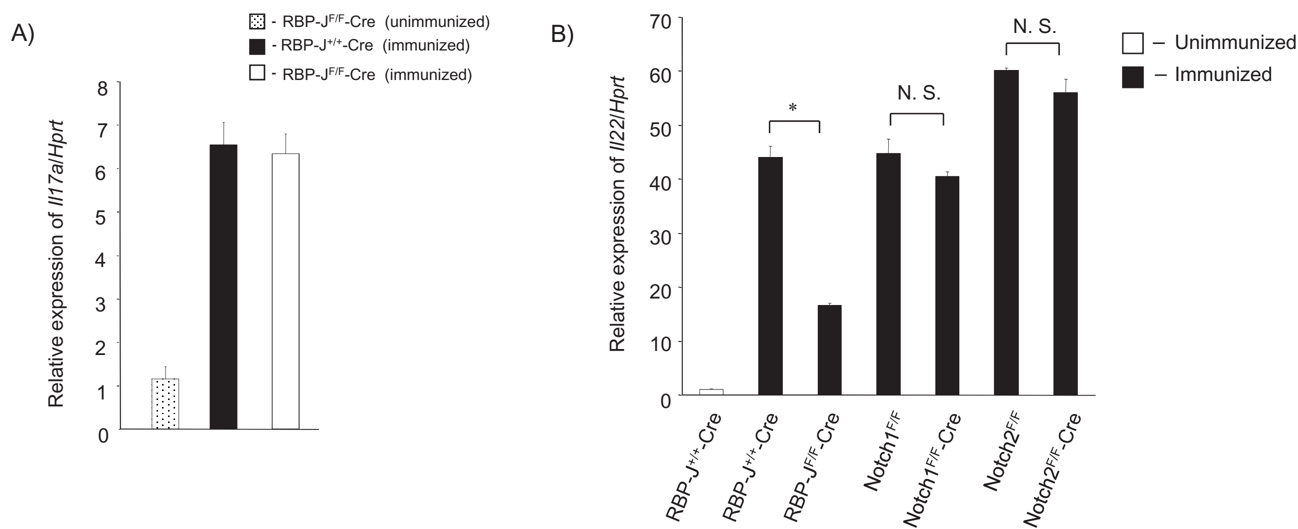

Fig. S4. Cytokine expression after OVA immunization. (*A*) CD4$^+$ T cells were isolated from RBP-J$^{+/+}$-Cre (filled) and RBP-J$^{F/F}$-Cre (open) mice 7 days after immunization with OVA emulsified in CFA. As a control, CD4$^+$ T cells from unimmunized RBP-J$^{F/F}$-Cre mice (dot) were isolated. *Il17a* expression in CD4$^+$ T cells was evaluated by real-time PCR. (*B*) CD4$^+$ T cells were isolated from RBP-J$^{+/+}$-Cre, RBP-J$^{F/F}$-Cre, N1$^{F/F}$, N1$^{F/F}$-Cre, N2$^{F/F}$, and N2$^{F/F}$-Cre mice 7 days after immunization with OVA emulsified in CFA. As a control, CD4$^+$ T cells from unimmunized RBP-J$^{+/+}$-Cre mice (dot) were isolated. *Il22* expression in CD4$^+$ T cells was evaluated by real-time PCR. *, $P < 0.05$, indicates a statistically significant difference. Data are representative of at least four independent experiments. N.S., not significant.

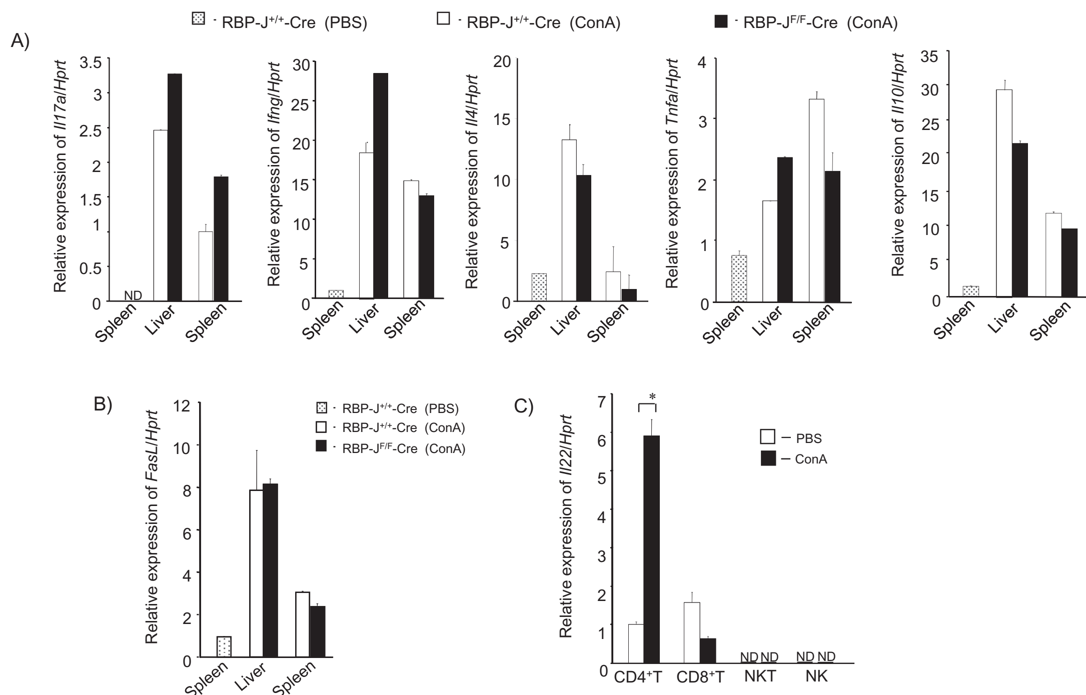

Fig. S5. Expression of different cytokines and Fas ligand (FasL) after ConA injection. (*A* and *B*) RBP-J$^{+/+}$-Cre or RBP-J$^{F/F}$-Cre mice received ConA (10 μg/g). CD4^{+} T cells were purified from livers and spleens 48 h after ConA injection by Lympholyte M and MACS beads, and expressions of different cytokines (*A*) and FasL (*B*) were measured by real-time PCR. (*C*) C57BL/6 mice received ConA (10 μg/g). Forty-eight hours after ConA injection CD4, CD8, NK (NK1.1 positive), and NKT (NK1.1 plus anti-CD3 positive) cells were sorted and checked *Il22* mRNA expression by real-time PCR. *, $P < 0.05$, indicates a statistically significant difference. Data are representative of at least four independent experiments. N.D., not detected.

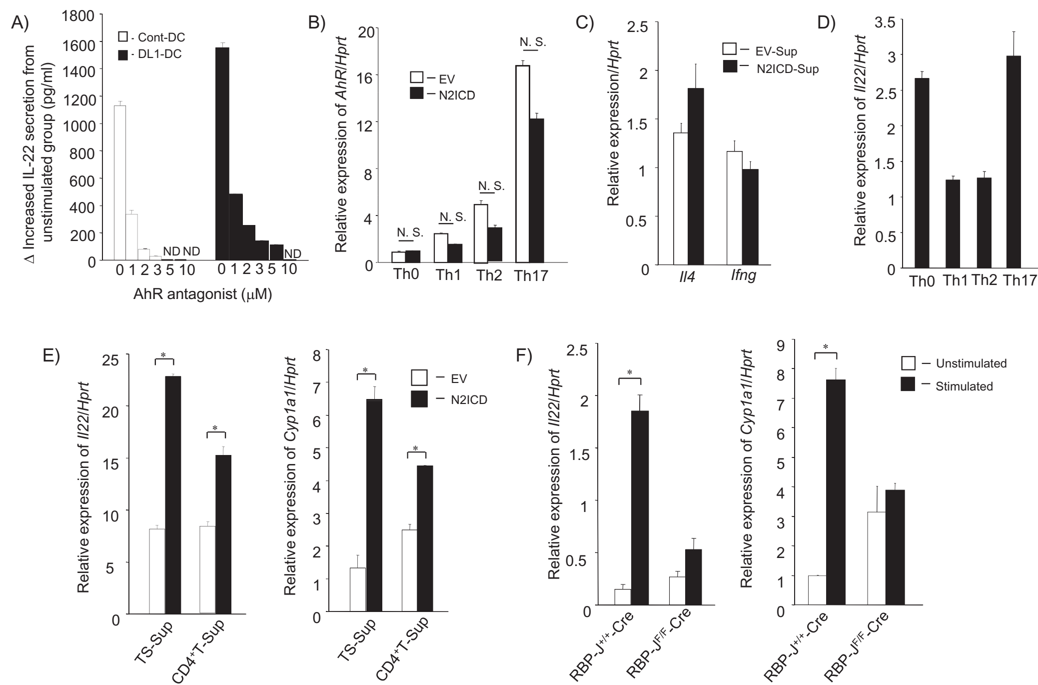

Fig. S6. Notch signaling regulates AhR stimulator production. (*A*) CD4$^+$ T cells from OT-II transgenic mice were stimulated with Cont-DC or DL1-DC in the presence of OVA peptide for 72 h with the indicated concentrations of an AhR antagonist. IL-22 concentration in the supernatants was measured by ELISA. (*B*) Total spleen cells were stimulated with anti-CD3 mAb under Th0, Th1, Th2, and Th17 culture conditions for 24 h and transduced with a retrovirus carrying N2ICD (filled) or a control vector (open). After infection, cells were restimulated under the same culture conditions with anti-CD3 mAb for 48 h and then *AhR* expression in MACS-enriched CD4$^+$T cells was checked by quantitative real-time PCR. (*C*) The supernatant from total spleen cells transduced with N2ICD or EV was collected 72 h after initial stimulation. CD4$^+$ T cells from C57BL/6 mice were stimulated with anti-CD3 mAb in the presence of 40% of such supernatant. The expression of *Il4* and *IFNg* in mature CD4$^+$ T cells was tested 48 h after initial stimulation. (*D*) Total spleen cells were stimulated with anti-CD3 mAb under Th0, Th1, Th2, and Th17 culture conditions for 24 h and transduced with a retrovirus carrying N2ICD. After infection, cells were restimulated under the same culture conditions with anti-CD3 mAb for 48 h and then cell culture supernatants were collected. Total spleen cells were restimulated with the supernatant for 48 h in the presence of anti-CD3Ab. Finally, *Il22* expression was checked by quantitative real-time PCR. (*E*) Supernatants from total spleen cells or CD4$^+$ T cells transduced with N2ICD or EV were collected 72 h after initial stimulation. Purified CD4$^+$ T cells from C57BL/6 mice were restimulated for 48 h with 40% of such supernatants in the presence of anti-CD3 mAb. The expression of *Il22* (*Left*) and *Cyp1a1* (*Right*) were tested by using real time PCR. (*F*) RBP-J$^{+/+}$-Cre or RBP-J$^{F/F}$-Cre mice received ConA (10 μg/g). The CD4$^+$ T cells in the liver were isolated and restimulated in presence anti-CD3 mAb or left unstimulated for 24 h. Supernatants were collected and used to stimulate splenic purified CD4$^+$ T cells of wild-type C57BL/6 mice in presence of anti-CD3 mAb. After 48 h of stimulation mRNA expressions of *Il22* and *Cyp1a1* were tested by real-time PCR. *, $P < 0.05$, indicates a statistically significant difference. Data are representative of at least four independent experiments. N.D., not detected; N.S., not significant.

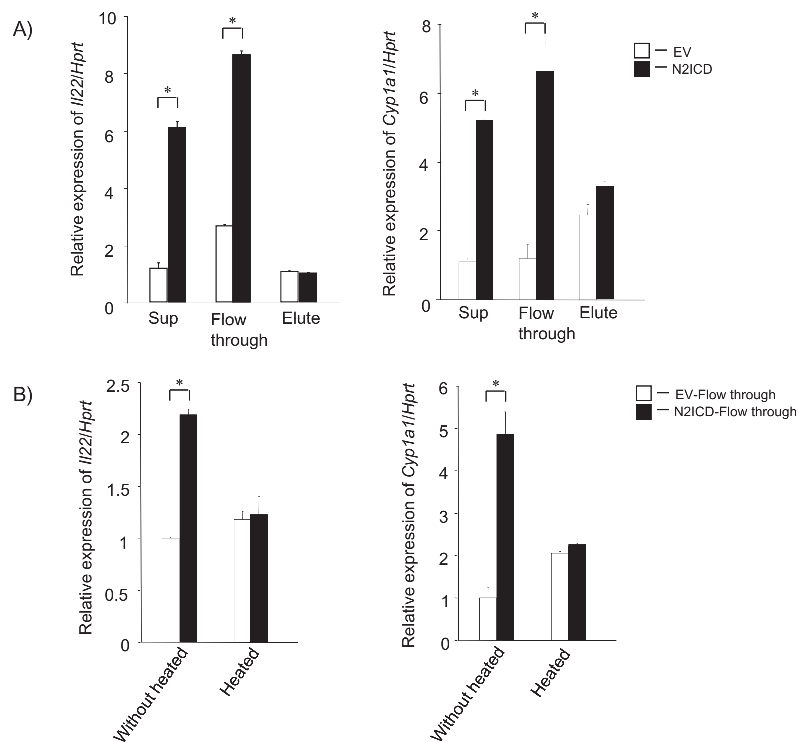

Fig. S7. Notch-mediated AhR stimulator for IL-22 production is not FICZ but heat labile. (*A*) The supernatants from total spleen cells transduced with N2ICD (filled) or EV (open) were collected 48 h after infection and stimulation. Then Sep-Pak C_{18} Plus cartridges were used to collect the flow-through from supernatants and finally eluates were collected in acetone. After evaporation of acetone from eluates, the pellets were dissolved in RPMI media. Total spleen cells from C57BL/6 mice were restimulated with the supernatants, flow-through, and eluates in the presence of anti-CD3 mAb. *Il22* and *Cyp1a1* expression was measured by quantitative real-time PCR after 48 h of stimulation. (*B*) The flow-throughs collected from the protocol described in Fig. S6*A* were heat inactivated, and total splenocytes were stimulated with the heat-inactivated flow-through in the presence of anti-CD3 mAb. *Il22* and *Cyp1a1* expression was measured by quantitative real-time PCR after 48 h. *, $P < 0.05$, indicates a statistically significant difference. Data are representative of at least four independent experiments.

Table S1. Fold increases of genes up-regulated after N2ICD transduction into DO11.10 T cell hybridoma

Developmental process		Metabolic process		Signal transduction	
Gene	Fold increase	Gene	Fold increase	Gene	Fold increase
Wisp2	61.1	Rhox6	77.6	Cd22	49.1
Evl	44.8	Fabp4	50.0	Evl	44.8
Chd7	26.4	Evl	44.8	Tgm2	33.3
Dtx1	26.3	Pou2af1	28.9	Rhov	27.9
Agtr1a	24.7	Arhgef10l	27.4	Arhgef10l	27.4
Sgk	19.6	Chd7	26.4	Arl4c	27.3
Sepp1	19.1	Brdt	19.0	Dtx1	26.3
Flnb	19.1	Pdgfrb	17.2	Agtr1a	24.7
CD3g	18.0	Parp14	16.8	Pdzk1	20.7
Rag1	17.2	Rhox9	16.6	CD3g	18.0
Prf1	16.9	Klf2	15.9	Pdgfrb	17.2
Hrb	16.0	Hes1	15.8	Unc13b	15.6
Hes1	15.8	Apol6	15.4	Arhgef3	15.0
Gzmb	14.9	Igh-6	13.9	Depdc6	14.5
Gzmd	13.9	Ifi204	11.3	Igh-6	13.9
Snrk	13.9	Lmo4	10.1	Rasal1	13.6
Casp4	13.8			Garnl4	12.1
Ifi204	11.3			Ahnak	10.5
Ifih1	11.2				
Fhl1	10.5				
Lmo4	10.1				

Table S2. Primers used in this study

Cytokine	Forward	Reverse
IL-22	5′-CATGCAGGAGGTGGTACCTT-3′	5′-AGCTTCTTCTCGCTCAGACG-3′
IL-17A	5′-GCTCCAGAAGGCCCTCAGA-3′	5′- CTTTCCCTCCGCATTGACA-3′
IFN-γ	5′- ACTGGCAAAAGGATGGTGA-3′	5′- TGAGCTCATTGAATGCTTG-3′
IL-4	5′- GAGCTGCAGAGACTCTTTCG-3′	5′- ACTCATTCATGGTGCAGCTTA-3′
TNF-α	5′- CATCTTCTCAAAATTCGAG-3′	5′-TGGGAGTAGACAAGGTACAACCC-3′
IL-10	5′-TGTGAAAATAAGAGCAAGGCAGTG-3′	5′- CATTCATGGCCTTGTAGACACC-3′
IL-19	5′- AGCCTGGATTGACAGGAATC-3′	5′- GATAATCAGACGAGGCGTTTC-3′
IL-20	5′- TTTGGGAGAACTAGGCATTCTT-3′	5′- TCTTGGACAGGAGTGTTCTCA-3′
IL-24	5′- GCTCTCCATGCCATTTCAA-3′	5′- TGGCCAAGGGTCTGAAGT-3′
CYP1A1	5′- GCAGCCTTCTTCATCTGCTT-3′	5′- TGGTTTTGTTTTATTTTTCACACA-3′
HPRT	5′-AGCCTAAGATGAGCGCAAGT-3′	5′-TTACTAGGCAGATGGCCACA-3′