Supporting Information

Cho et al. 10.1073/pnas.1105118108

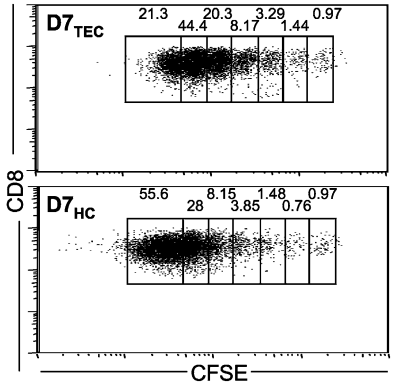

Fig. S1. D7 CD8$^+$ T cells selected on HCs demonstrate higher proliferative capacity on in vitro stimulation than those selected on TECs. Splenocytes from $D7_{TEC}$s and $D7_{HC}$s were labeled with 10 μM CFSE and incubated for 65 h with LemA pulsed stimulator cells. Proliferation of $D7_{TEC}$s or $D7_{HC}$s was measured by CFSE dilution. Events shown are gated on live $D7_{TEC}$s or $D7_{HC}$s. CFSE, carboxyfluorescein succinimidyl ester.

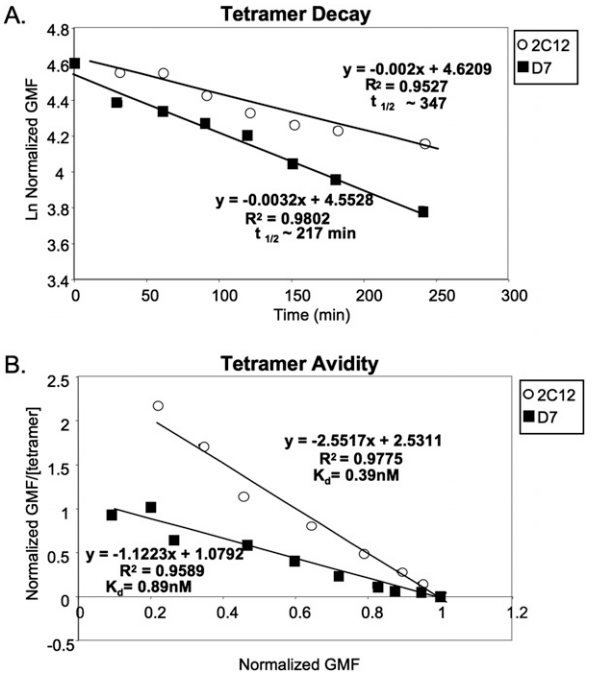

Fig. S2. Comparison of TCR affinity between D7 and iNKT cells. (*A*) D7 Tg mouse splenocytes and N38-2C12 iNKT cell hybridomas were incubated with 10 μM allophycocyanin-conjugated M3/LemA and CD1d/α-GalCer tetramers, respectively, for 45 min at 4 °C and then incubated at 37 °C with anti–TCR-β and excess anti-M3 or anti-CD1d mAb, respectively. At 20-min intervals, an aliquot of cells was washed, fixed with 4% paraformaldehyde, and analyzed via flow cytometry, gating on T cells. The natural log of percentage geometrical mean fluorescence of tetramer at each time point (compared with 0 min) was plotted against time. The half-life of each tetramer was derived from the slope as $t_{1/2}$ = ln2/slope. α-GalCer, α galactosylceramide; GMF, geometrical mean fluorescence, Ln, natural log. (*B*) D7 and iNKT cells were stained using 0.1–50 nM of the respective tetramers. Cells were stained for 45 min at 4 °C, washed, and analyzed with a FACSCanto II, gating on T cells. Equilibrium binding data were plotted as a Scratchard plot of GMF/concentration of tetramer used in staining against GMF. Tetramer avidity, K_D, is derived from the slope as K_D = 1/slope.

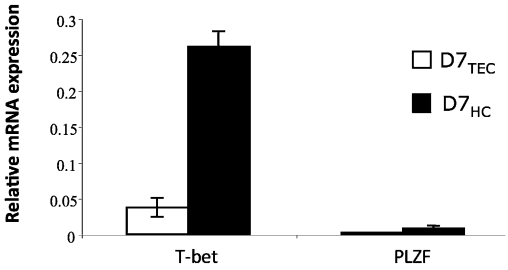

Fig. S3. D7 T cells selected on HCs express higher levels of *T-bet* than D7 T cells selected on TECs. Real-time RT-PCR of *T-bet* and *PLZF* mRNA of D7 T cells sorted from $D7_{TEC}$ and $D7_{HC}$ chimeric mice. The bar graph represents the mean ± SEM of triplicate determinants of *T-bet* and *PLZF* expression normalized to *GAPDH* expression levels. *T-bet* primers are: forward, 5′-CCAGCACCAGACAGAGATGA-3′; reverse, 5′-GCTTCCCAAATGAAACTTCC-3′. *PLZF* primers are: forward, 5′-CCCAGTTCTCAAAGGAGGATG-3′; reverse, 5′-TTCCCACACAGCAGACAGAAG-3′.

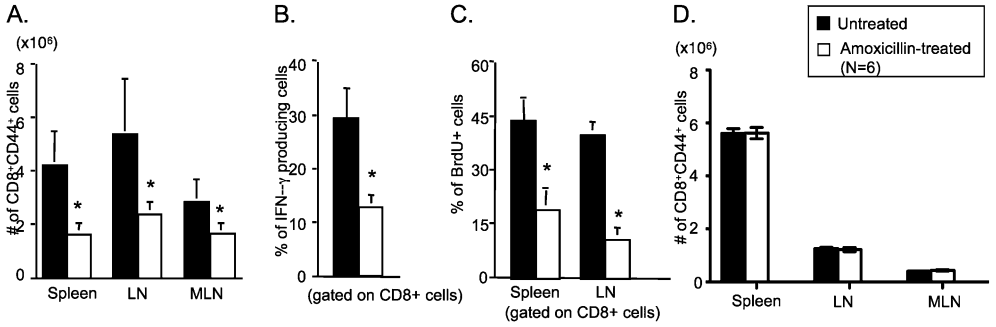

Fig. S4. Suppressed "innate-like" phenotype and effector functions in D7 mice fed with amoxicillin. Pregnant D7 Tg mice were treated with 3 mg/mL filter-sterilized amoxicillin solution at least 5 d before delivery. Treatment was continued until pups were 7 wk of age, when mice were killed and lymphoid tissues were harvested. (*A*) Number of activated ($CD44^{hi}$) $CD8^+$ T cells from amoxicillin-treated and untreated 7-wk-old mice were compared via flow cytometry. $*P < 0.05$. LN, lymph node; MLN, messenteric lymph node. (*B*) Pooled nucleated cells from the spleen and axillary and inguinal lymph nodes of treated and untreated 7-wk-old D7 mice were cultured with LemA for 8 h. Intracellular IFN-γ was analyzed by flow cytometry. $*P < 0.05$. (*C*) Relative proliferative capacity of $CD8^+$ T cells isolated from treated and untreated mice following treatment with LemA was analyzed via BrdU incorporation assay. $*P < 0.05$. (*D*) As a control, the number of $CD44^{hi}CD8^+$ T cells from treated and untreated histocompatibility-Y antigen (H-Y) Tg mice (expressing a TCR specific for an MHC Ia molecule) were compared by flow cytometry.