Supporting Information

Le Saout *et al.* 10.1073/pnas.0807743105

SI Methods

In Vitro Activation of TCR Transgenic T Cells. Purified Clone 4 $CD8^+$ were seeded in 24 well plates, 2×10^6 per well, in RPMI medium 1640 with GlutaMAX-I media (Invitrogen) containing 10% FCS, 5×10^{-5} M β-mercaptoethanol and 1% penicillin streptomycin (Invitrogen) (complete media). For priming, LPS blasts, prepared by incubation of BALB/c spleen cells in complete media containing 7 μg/mL dextran sulfate (Sigma) and 25 μg/mL LPS (Sigma) for 3 days, were used as APC. Cells were irradiated (3,000 rads) and pulsed for 1 h at 37 °C with 5 μg/mL of K^d HA (518–526) peptide (GL Biochem) in complete media. After washing, 2×10^6 cells per well were added to the T cell cultures. Cultures were maintained at 37 °C for 4 days and viable cells were then isolated by Ficoll-Paque separation.

Flow Cytometry. Seven, 10, or 13 days after adoptive transfer, the pancreas, spleen, pancreatic lymph nodes (pLN), and a mixture of inguinal, axillary, cervical, mandibular, popliteal, and mesenteric LN were excised and processed separately to obtain single cell suspensions by mechanic disruption on Nitex filters in PBS containing 5% FCS at 4 °C. After counting, all pLN cells and an equivalent number of cells from other LN and spleen were stained with the indicated Abs. Pancreatic LNs from 3 mice receiving the same treatment were generally pooled together for staining. For the pancreas, suspensions were subject to Histopaque separation and all cells obtained from a single pancreas were stained.

All mAbs and secondary reagents were purchased from BD PharMingen except anti-CD44-APC-Alexa Fluor 750 mAb (eBioscience). Donor Clone 4 and HNT T cells were detected and enumerated by virtue of their Thy1.1 expression. Lymphocytes were incubated with anti-CD8α-PerCP and anti-Thy1.1-PE mAbs in PBS containing 2% FCS and 0.02% sodium azide for 30 min at 4 °C. After washing, cells were analyzed with a FACSCanto II apparatus by using Diva software (BDB); 2×10^6 events were collected for each analysis.

For Clone 4 $CD8^+$ T cells, the intensity of CFSE fluorescence was analyzed in the $CD8^+$ $Thy1.1^+$ subpopulation of lymphocytes. For HNT cells, this was performed by gating on $CD8^-$ $Thy1.1^+$ or $CD4^+$ $Thy1.1^+$ T cells depending on the experiment. To assess the phenotype of proliferating T cells, they were also stained with anti-CD25-PE-Cy7, anti-CD62L-APC and anti-CD44-APC-Alexa Fluor 750 mAbs.

To assess production of IFN-γ in response to antigen by Clone 4 $CD8^+$ T cells, LN cells or splenocytes were incubated in complete media with 1 μg/mL of the K^d HA peptide and 10 μg/mL of brefeldin A (Sigma) for 5 h at 37 °C. Cells that did not receive peptide were used as a negative control. After washing, cells were stained to detect cell surface CD8 and Thy1.1 as described above. Cells were then permeabilized and stained to detect intracellular IFN-γ with an anti-IFN-γ-APC mAb using the Cytofix/Cytoperm kit (BD PharMingen) according to the manufacturer's instructions. To assess cytokine production by HNT $CD4^+$ T cells, lymphocytes from different lymphoid organs were incubated with equal numbers of A^d HA peptide-pulsed BALB/c spleen cells in complete media in the presence of 10 μg/mL of brefeldin A for 5 h at 37 °C. Cells were then washed, stained to detect CD4 and Thy1.1, and fixed and permeabilized by using the Cytofix/Cytoperm kit (BD PharMingen). Intracellular cytokine staining was performed using either an anti-IFN-γ-APC mAb or an anti-IL-2-APC mAb.

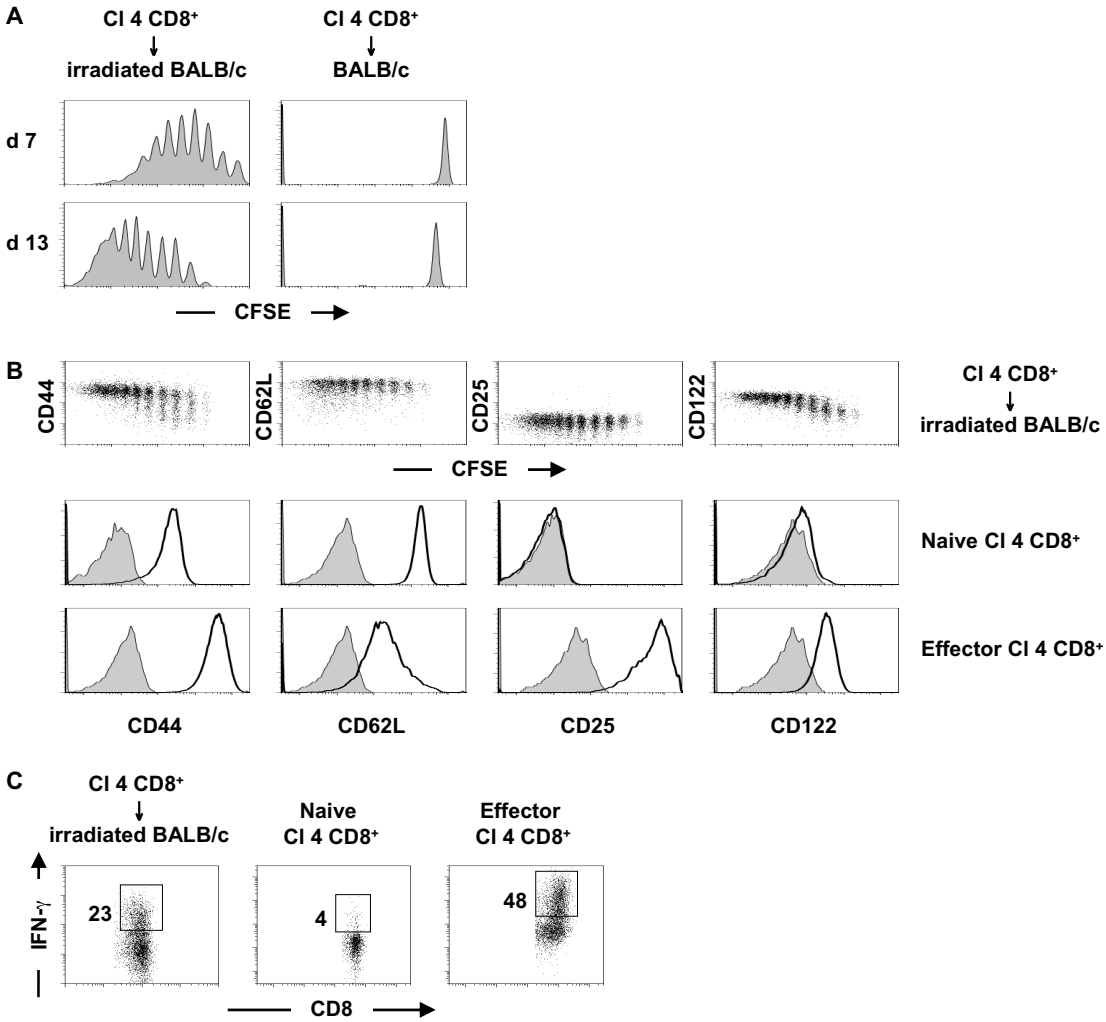

Fig. S1. Naive Clone 4 CD8$^+$ T cells proliferate and differentiate into memory-like T cells under lymphopenic conditions. (*A*) Irradiated and nonirradiated BALB/c mice were injected with 5×10^6 CFSE-labeled naive Clone 4 Thy1.1$^+$ CD8$^+$ T cells (Cl 4 CD8$^+$). Mice were killed at days 7 or 13 after transfer and splenocytes were analyzed by FACS. Histograms represent CFSE labeling on gated CD8$^+$ Thy1.1$^+$ lymphocytes. (*B*) CD8$^+$ Thy1.1$^+$ splenocytes from irradiated BALB/c mice were assessed for expression of the CD44, CD62L, CD25, and CD122 markers as a function of CFSE fluorescence at day 13. The phenotype of the naive Clone 4 CD8$^+$ cells before transfer as compared with in vitro activated (see *SI Methods*) Clone 4 CD8$^+$ T cell effectors are shown. Activation markers are presented as open histograms while isotype controls are shaded. (*C*) On day 13 after transfer, splenocytes from animals described in *B* were incubated with K^d HA peptide for 5 h at 37 °C and analyzed by FACS to detect IFN-γ production. Background in nonstimulated controls was <1%. Controls included naive and effector Clone 4 cells as indicated. The percentages of Clone 4 CD8$^+$ T cells producing IFN-γ are indicated. Data from one representative experiment of 3 are presented.

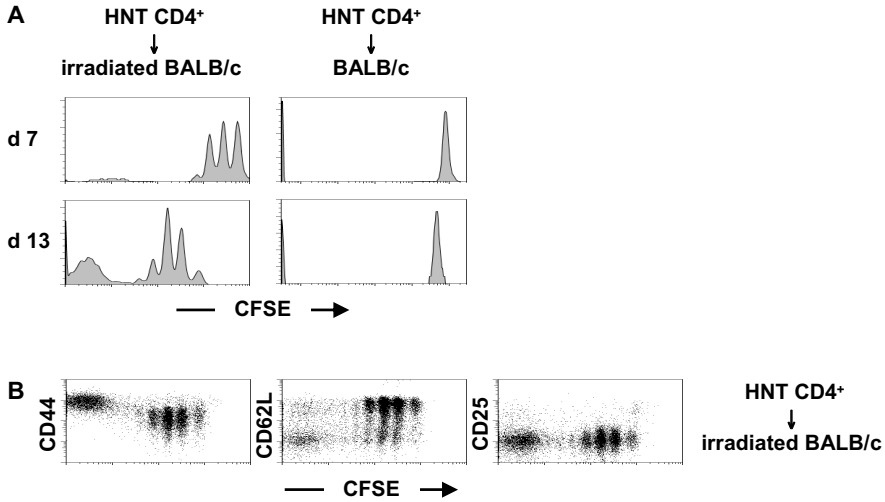

Fig. S2. Proliferation of HNT CD4$^+$ T cells in BALB/c mice. (*A*) Irradiated and nonirradiated BALB/c mice were injected with 5×10^6 purified CFSE-labeled HNT Thy1.1$^+$ CD4$^+$ T cells (HNT CD4$^+$) as described in *Materials and Methods*. Mice were killed at days 7 or 13 after transfer and splenocytes were analyzed by FACS. Histograms show CFSE labeling on gated CD4$^+$ Thy1.1$^+$ lymphocytes. (*B*) CD4$^+$ Thy1.1$^+$ splenocytes from irradiated BALB/c mice were assessed for expression of the CD44, CD62L, and CD25 markers as a function of CFSE fluorescence at day 13. Data from 1 representative experiment of 3 are presented. In preliminary experiments, naive HNT CD4$^+$ T cells were further purified by positive magnetic selection of CD62L^{hi} cells by using StemCell Tech. reagents (>98% purity), because their proliferation profiles and phenotype (data not shown) on injection into irradiated recipients were very similar to those obtained by using purified total CD4$^+$ T cells, those displayed in this figure, we used total CD4$^+$ T cells routinely throughout this study. Proliferating HNT cells contained a subpopulation of CD25$^+$ cells (3–4% of donor cells, as shown in *B*) that corresponds to Foxp3$^+$ Tregs (data not shown). They were most likely the progeny of the CD25$^+$ Foxp3$^+$ subpopulation present in the original inoculum.

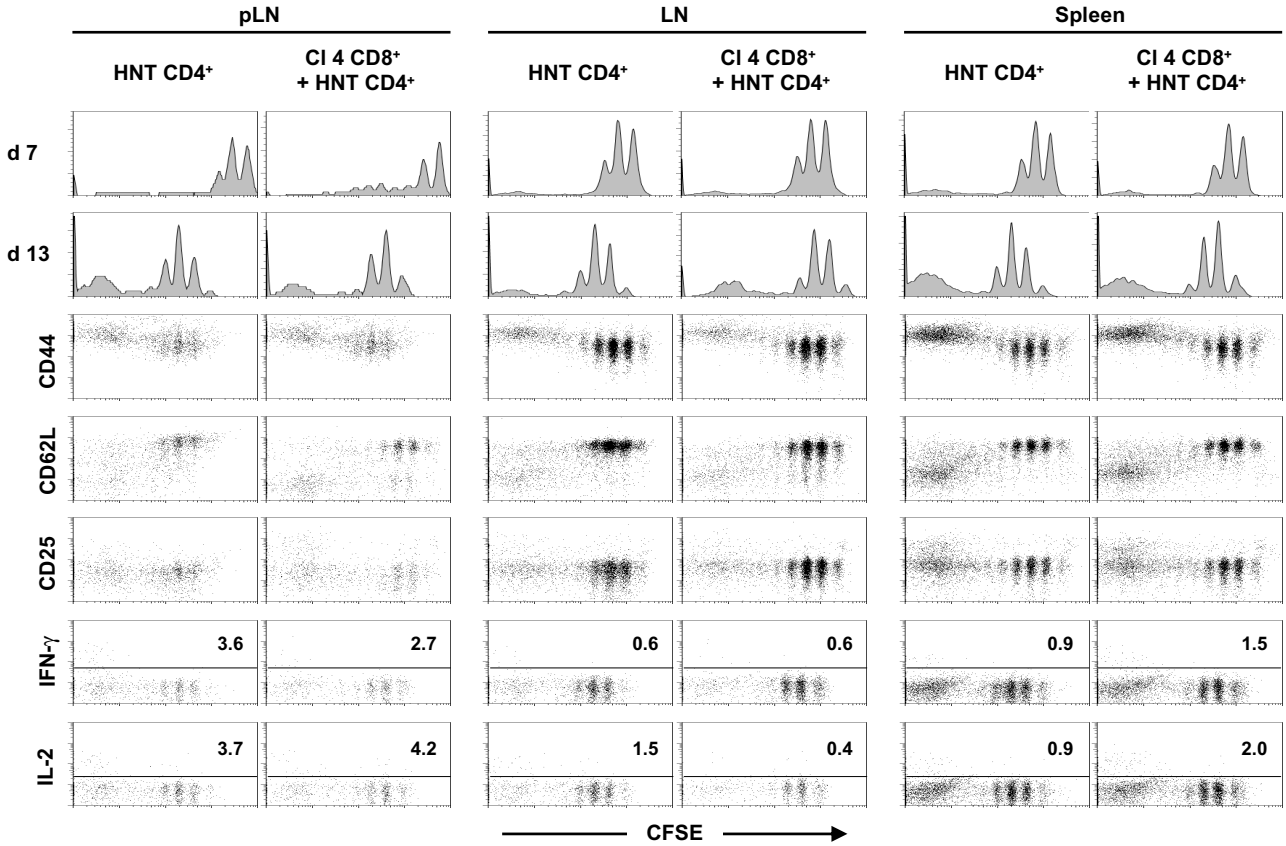

Fig. S3. Proliferation, phenotype, and responsiveness of HNT CD4$^+$ T cells in InsHA mice. Irradiated InsHA mice were injected with either 5×10^6 CFSE-labeled naïve HNT Thy1.1$^+$ CD4$^+$ T cells or with a combination of 5×10^6 Clone 4 CD8$^+$ T cells and 5×10^6 HNT CD4$^+$ T cells. Mice were killed on day 7 or 13 after transfer and CFSE profiles of gated CD4$^+$ Thy1.1$^+$ lymphocytes were assessed in pLN, LN, and spleen. Expression of CD44, CD62L, and CD25 was assessed as a function of CFSE fluorescence on day 13. On day 13 after transfer, to assess cytokine production by HNT CD4$^+$ T cells, peptide-stimulated lymphocytes from different lymphoid organs were analyzed by FACS. Plots represent CFSE fluorescence versus IFN-γ and IL-2 expression on gated donor CD4$^+$ Thy1.1$^+$ lymphocytes. The percentages of donor CD4$^+$ T cells expressing IFN-γ and IL-2 are indicated. Background levels in nonstimulated controls were $<$1%. Data from 1 representative experiment out of 4 are presented.

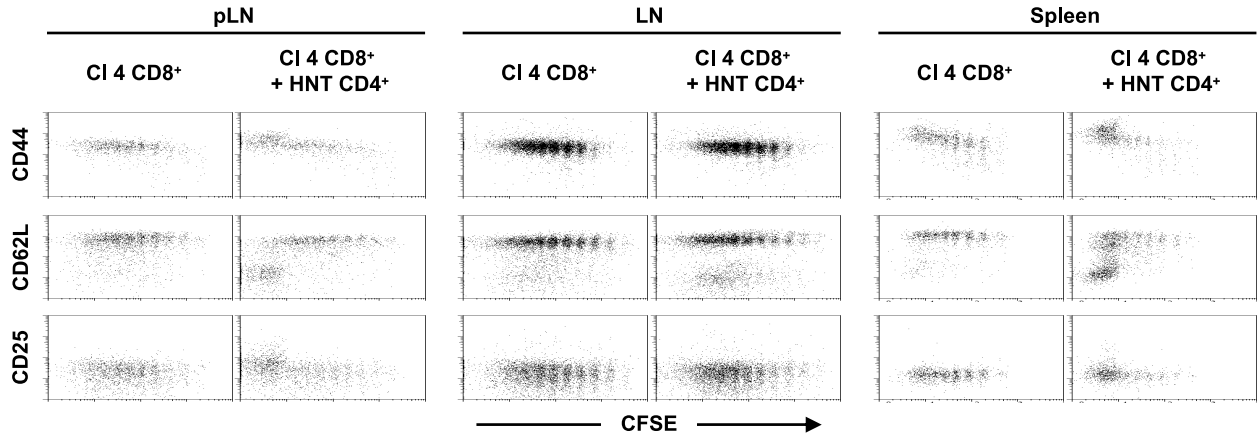

Fig. S4. Phenotype of Clone 4 $CD8^+$ T cells proliferating in irradiated InsHA mice. Thirteen days after transfer, expression of CD44, CD62L, and CD25 on $CD8^+$ donor T cells was assessed in pLN, LN, and spleen of mice from the groups described in Fig. 1. Plots represent CFSE fluorescence versus cell surface marker expression on gated $CD8^+$ $Thy1.1^+$ lymphocytes. Data of 1 representative experiment out of 3 are presented.