SUPPLEMENTAL MATERIAL

GeurtsvanKessel et al., http://www.jem.org/cgi/content/full/jem.20090410/DC1

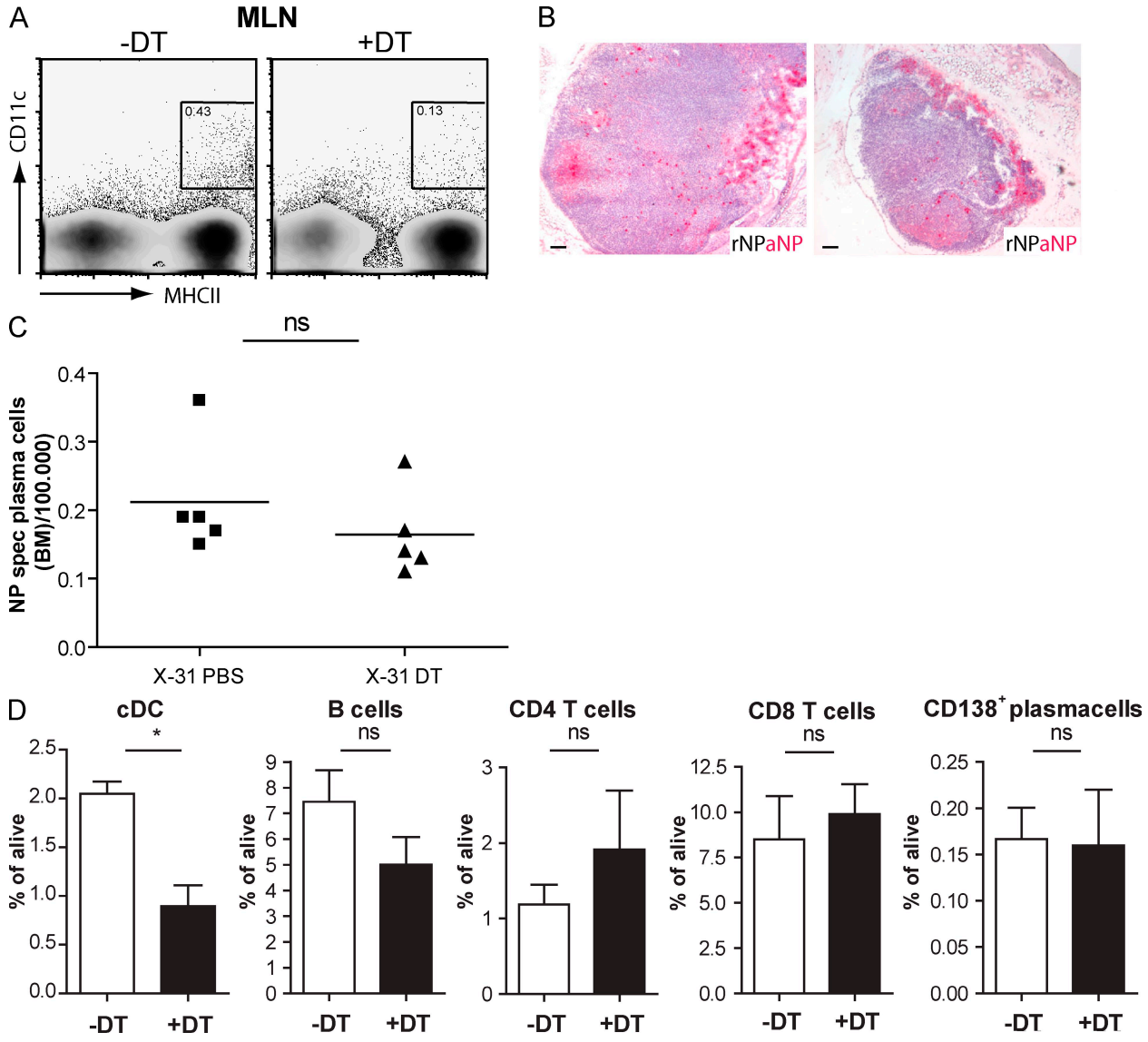

Figure S1. Depletion of cell populations 1 d after DT treatment in CD11c-DTR mice. i.t. treatment of CD11-DTR Tg mice with DT depletes CD11c$^+$ cells from lung and MLN. (A) FACS plots demonstrate depletion of CD11c$^+$MHCII$^+$ DCs in MLN 1 d after DT treatment at day 17. (B) Histology demonstrates intact NP-specific plasma cell differentiation in MLN, 1 wk after DT treatment (24 dpi). Bars, 100 µm. (C) NP-specific plasma cells in MLN measured by flow cytometry after treatment with DT or PBS. Horizontal bars indicate the mean value. (D) Depletion of cDCs, B cells, CD4 T cells, CD8 T cells, and CD138$^+$ plasma cells in lung tissue was analyzed by flow cytometry 1 d after treatment. All data are representative of at least two separate experiments. Bars represent mean values of at least five mice per group with error bars indicating SEM. *. $P < 0.05$; ns, not significant.

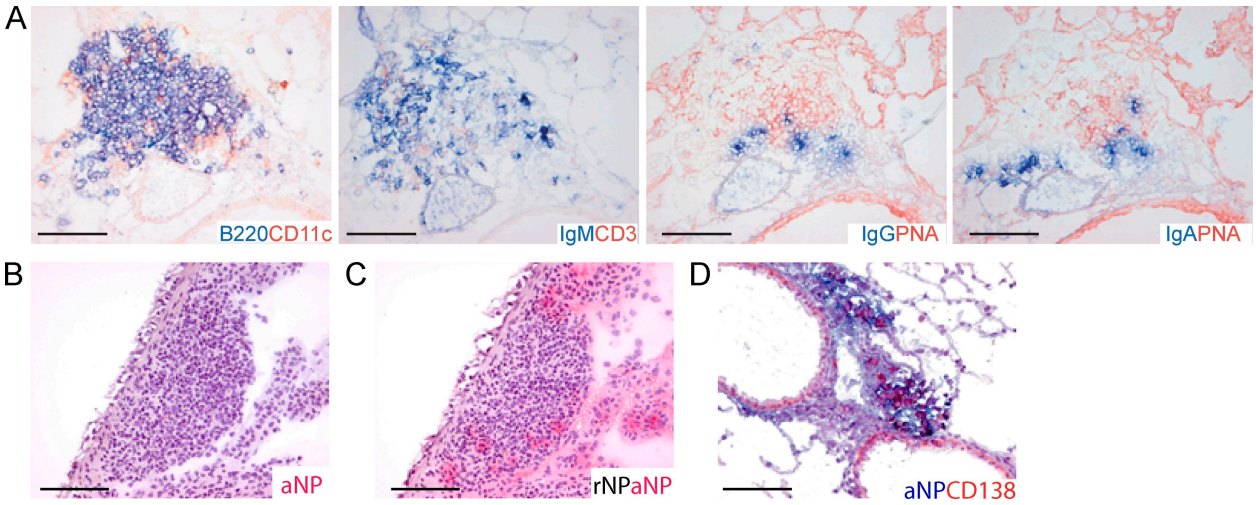

Figure S2. Detection of plasma cells in iBALT. (A) Local class switching in the lung is demonstrated on consecutive slides of lung tissue at 24 dpi which show IgM, IgG, and IgA in proximity of PNA-positive GCs. (B) Use of an antibody to NP detected infected cells of the lungs shortly after infection (not depicted; GeurtsvanKessel, C.H., M.A. Willart, L.S. van Rijt, F. Muskens, M. Kool, C. Baas, K. Thielemans, C. Bennett, B.E. Clausen, H.C. Hoogsteden, et al. 2008. *J. Exp. Med.* 205:1621–1634). At 24 dpi, staining with this antibody no longer detected NP-positive cells within iBALT, demonstrating that virus had been completely cleared from the lung and that NP antigen was not retained for prolonged periods in the FDC network. (C) Recombinant NP protein was used to reveal NP-specific B cells. Binding was demonstrated using NP-specific antibody. (D) Double staining in which slides were coated with recombinant NP, stained for NP positivity, and then double stained with CD138 (plasma cell marker). Bars, 100 µm. Photographs are representative of three separate experiments with at least five animals per group.

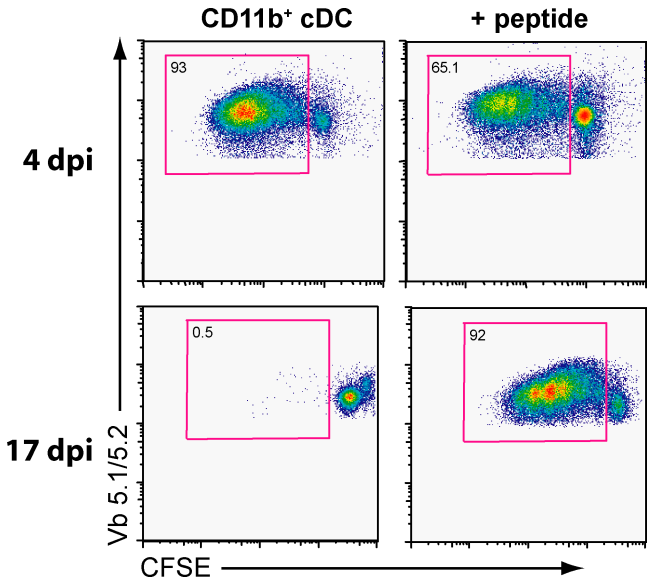

Figure S3. Antigen presentation by CD11c⁺CD11b⁺ DCs in the lung after infection. We infected mice with a recombinant influenza X-31 strain in which the MHCII $OVA_{323-339}$ epitope was inserted in the HA gene of the virus. At 4 and 17 dpi, CD11b⁺ DCs (defined as B220⁻CD11c⁺CD11b⁺MHCII⁺120G8$^{lo\ w}$) were purified from lung tissue and cocultured with CFSE-labeled OVA-specific CD4 T cells (OT-II) taken from OT-II transgenic animals. 4 d later, CFSE dilution in T cells was measured by flow cytometry and expressed as the percentage of cells that had divided at least once. At 4 dpi, lung CD11b⁺ DCs were capable of efficient OVA antigen presentation to CD4 T cells ex vivo. At 17 dpi, however, sorted CD11b⁺ DCs could no longer induce CD4 T cell division, whereas addition of preprocessed OVA peptide showed that they still possessed antigen-presenting capacity for naive T cells. In the negative control experiment, in which the coculture was set up with OVA-specific CD8 T cells (OT-1) at both time points, DCs did not induce any cell division (not depicted). These data indicate that the function of DCs within iBALT at 17 dpi is not the presentation of viral antigen to surrounding CD4 T cells. At 4 dpi (left), cDCs (B220⁻CD11c⁺MHCII⁺CD11b⁺120G8⁻) efficiently presented the OVA antigen to OT-II T cells, but at 17 dpi (right), both subsets had lost this capacity. However, when peptide was added DCs still showed to have APC function at 17 dpi. Boxes line the cells in division and the percentage of cells in division is indicated in the top left corner. The experiments were performed at least two times and at each time point lungs of 10 animals were pooled .

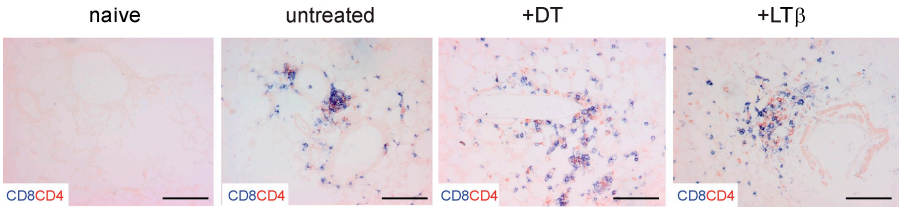

Figure S4. T cell clusters in lung tissue after depletion of DCs or treatment with LTβR–Fc. Mice were infected with influenza virus. At 17 dpi, either DCs were depleted from the lung using a CD11c-DTR mouse model or LTβR signaling was blocked using LTβR-Fc. 1 wk after treatment, snap-frozen lung sections were stained for CD4 and CD8 T cells. Bars, 100 µm. Photographs are representative of two separate experiments with at least five animals per group.