SUPPLEMENTAL INFORMATION

Supplemental Data: Figures S1-S7

Flow Cytometry Methods for Figure S6C-H:

Splenocytes were suspended in PBS and stained for live/dead discrimination with Ghost Dye violet 510 (TONBO biosciences). Splenocytes were blocked in staining media (SM: 2% FCS, 5 mM EDTA, 0.05% sodium azide/PBS) with the FcR blocking antibody 2.4G2. Reagents used for staining were purchased from eBioscience: CD11c (HL3)-PE/Cy7; from Biolegend: CD4 (RM4-5)-A700, CD44 (IM7)-APC-Cy7, CD62L (MEL-14)-PECy7, I-A/I-E (M5/114.15.2)-APCCy7, TCRβ (H57-597)-PE; from BD-Horizon: CD19 (1D3)-BUV395, PD-1 (J43)-BV605; or from BD Pharmingen: Bcl-6 (K112-91)-A647. Surface stained cells were fixed with 1% PFA, washed and resuspended in SM. For intracellular staining of Bcl-6 the Foxp3 / Transcription Factor Staining Buffer Set was used (eBiosicence). Briefly, cells were fixed in Fixation/Permeabilization buffer, washed in permeabilization buffer and blocked with 5% rat serum prior to staining. Cytometry was performed on a Fortessa (BD) and data analyzed with FlowJo software.

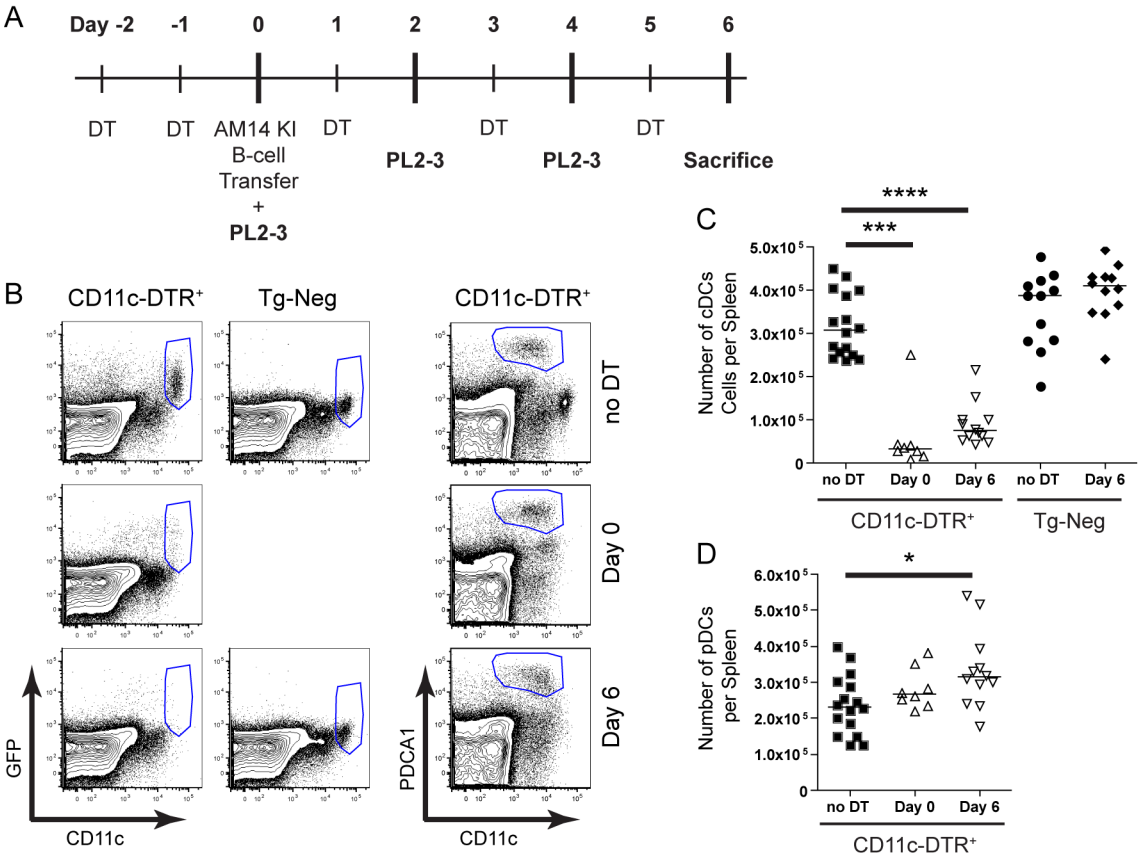

Figure S1, Related to Figure 1. Dendritic Cell Depletion and AM14 Response in CD11c-DTR.BALB/c BM Chimeras

CD11c-DTR.BALB/c or Tg-negative littermate controls were used to make the BM chimeras experimental mice shown in Figure 1.

(A) Time line for injections of BM chimeras. cDCs were depleted with DT on days -1, -2, 1, 3 and 5. 9-10 x 10^6 AM14.BALB/c B-cells were transferred into the mice on day 0, and activated with PL2-3 on days 0, 2 and 4. Spleens were harvested on day 6. A test group of BM chimeras that received DT on days -2 and -1 were euthanized on day 0 to assay for splenic cDC depletion prior to B-cell transfer.

(B) Representative flow cytometry gating of live splenocytes for $CD11c^{Hi}$ cDCs (left), and $CD11c^{Intermediate}$, $PDCA1^+$ pDCs (right). cDCs in CD11c-DTR Tg^+ BM chimeras also express green fluorescent protein (GFP). cDCs in Tg-negative (Tg-Neg) BM chimeras are GFP negative.

(C) Number of cDCs ($CD11c^{Hi}$) per spleen

(D) Number of pDCs ($CD11c^{Intermediate}$, $PDCA1^+$) per spleen

Data were combined from 2 experiments. Each dot represents one mouse. Lines are median values.

*p < 0.05; ***p < 0.001; ****p < 0.0001.

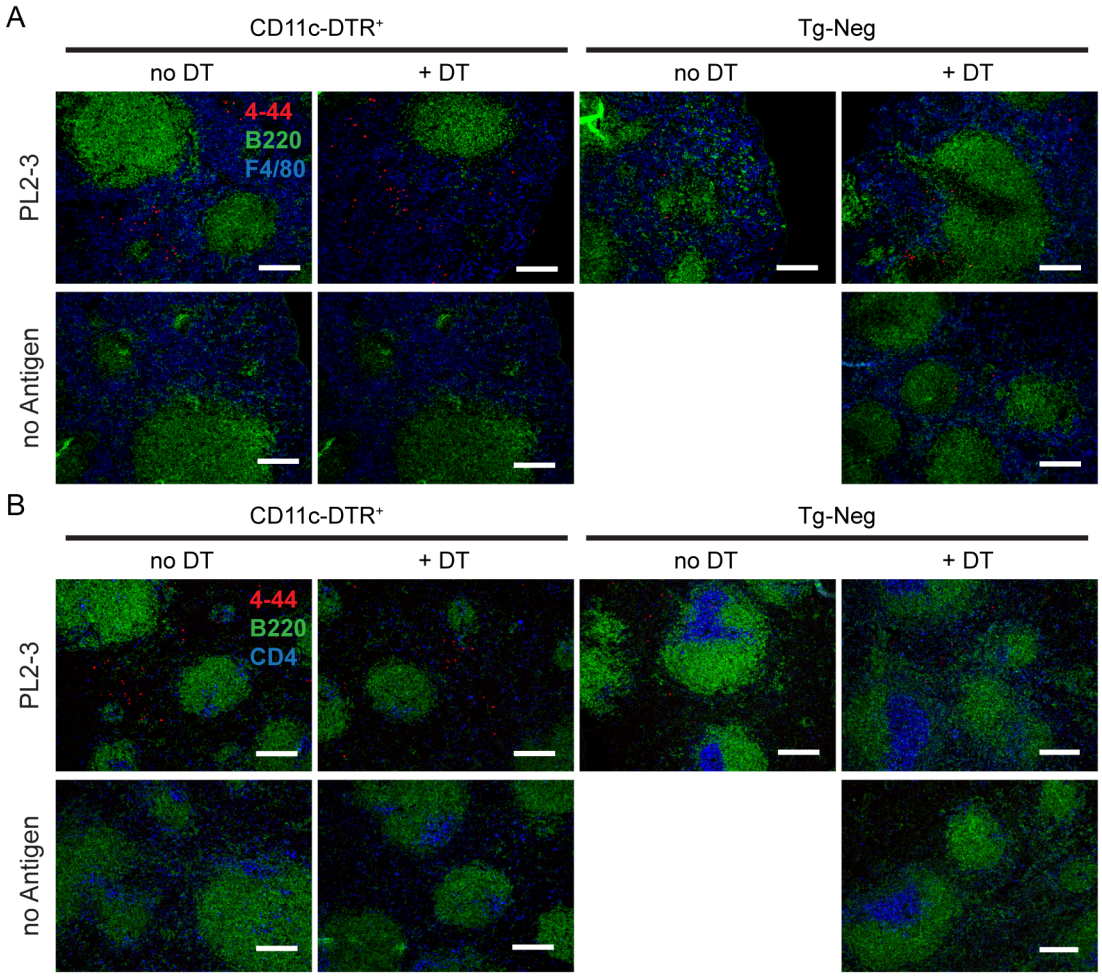

Figure S2, Related to Figure 1. Localization of AM14 Response in CD11c-DTR.BALB/c BM Chimeras

(A & B) Immunofluorescence histology was performed on spleens from experimental BM chimeras on day 6. Images were taken at a magnification of 100x.

(A) Red, AM14 B-cells (4-44$^+$); green, B-cell follicles (B220$^+$); blue, red pulp (F4/80$^+$).

(B) Red, AM14 B-cells (4-44$^+$); green, B-cell follicles (B220$^+$); blue, T-cell zones (CD4$^+$).

White scale bars shown on all images are 200 μm.

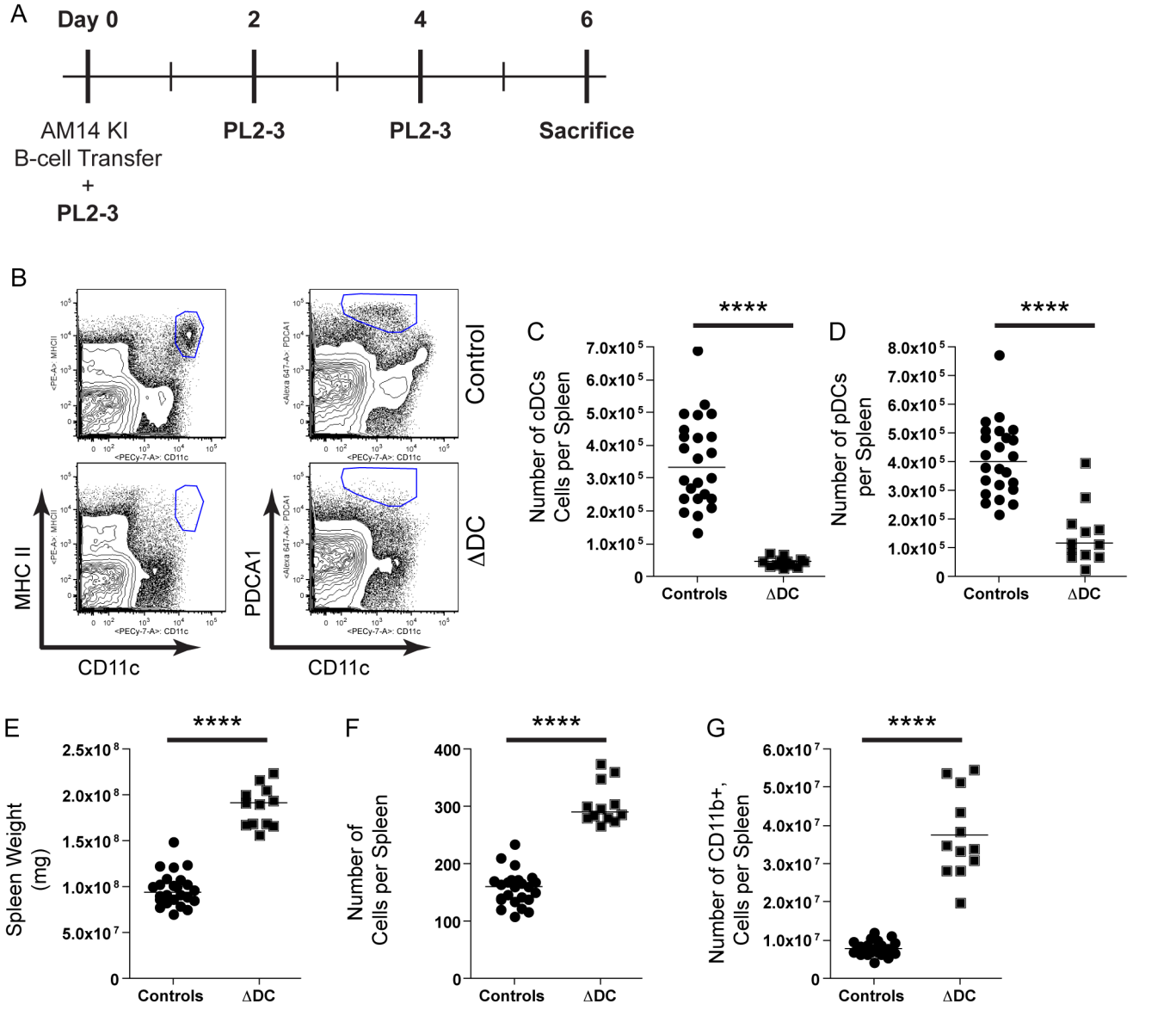

Figure S3, Related to Figure 2. Dendritic Cell Depletion and Macrophage Expansion in Mice that are Constitutively Deficient for Dendritic Cells

(A) Time line for injections of transgenic recipient mice, as performed in Figures 2-5. Recipient mice were either single Tg positive (CD11c-cre or Rosa26-flox-stop-DTA) or double Tg positive mice lacking cDCs (ΔDC mice). AM14.BALB/c B-cells were transferred into the mice on day 0, and activated with PL2-3 on days 0, 2 and 4. Spleens were harvested on day 6.

(B-G) Data from mice shown in Figure 3.

(B) Representative flow cytometry gating of live splenocytes for $CD11c^{Hi}$, $MHC\ II^{+}$ cDCs (left), and $CD11c^{Intermediate}$, $PDCA1^{+}$ pDCs (right).

(C) Number of cDCs ($CD11c^{Hi}$, $MHC\ II^{+}$) per spleen

(D) Number of pDCs ($CD11c^{Intermediate}$, $PDCA1^{+}$) per spleen

(E) Increase in spleen weight of mice lacking DCs.

(F) Increase in the numbers of cells per spleen in mice lacking DCs.

(G) Increase in $CD11b^{+}$ cells in mice lacking DCs

Each dot represents one mouse. Lines are median values. ****$p < 0.0001$.

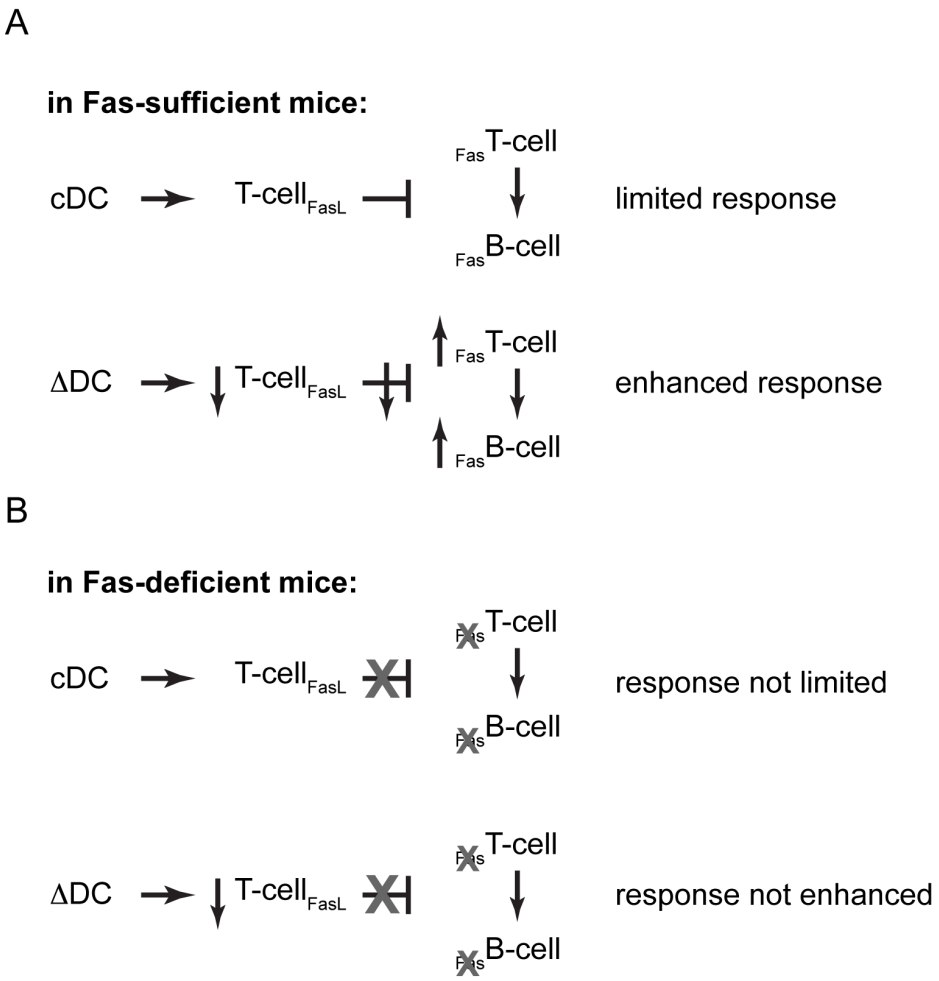

Figure S4, Related to Figures 1-3. Fas-Deficiency Could Obscure the Regulatory Effect of cDCs on the AM14 Response

A schematic diagram showing that mouse strain differences could explain how acute cDC depletion (Figure 1) and constitutive cDC depletion (Figure 2) resulted in different effects on AM14 B-cell activation. This reasoning served as rationale for testing the effect of constitutive cDC depletion on the AM14 response in Fas-intact mice (Figure 3).

(A) Mice used in the acute cDC depletion system were *Fas*$^{+/+}$. Normally, the AM14 response is limited by Fas-FasL interactions. Here, we show FasL expressed by a T-cell that is activated by a cDC. When cDCs are depleted (ΔDC), less FasL^{+} cells are activated, resulting in less inhibitory Fas-FasL interactions, and observation of an enhanced AM14 response. It would also be possible that FasL on the cDC itself could have a more direct effect (not shown).

(B) Mice used in the constitutive cDC depletion system were *Fas*$^{lpr/lpr}$. In this case, the AM14 response cannot be limited by Fas-FasL interactions. When cDCs are depleted from these mice, no enhancement of the response would be observed.

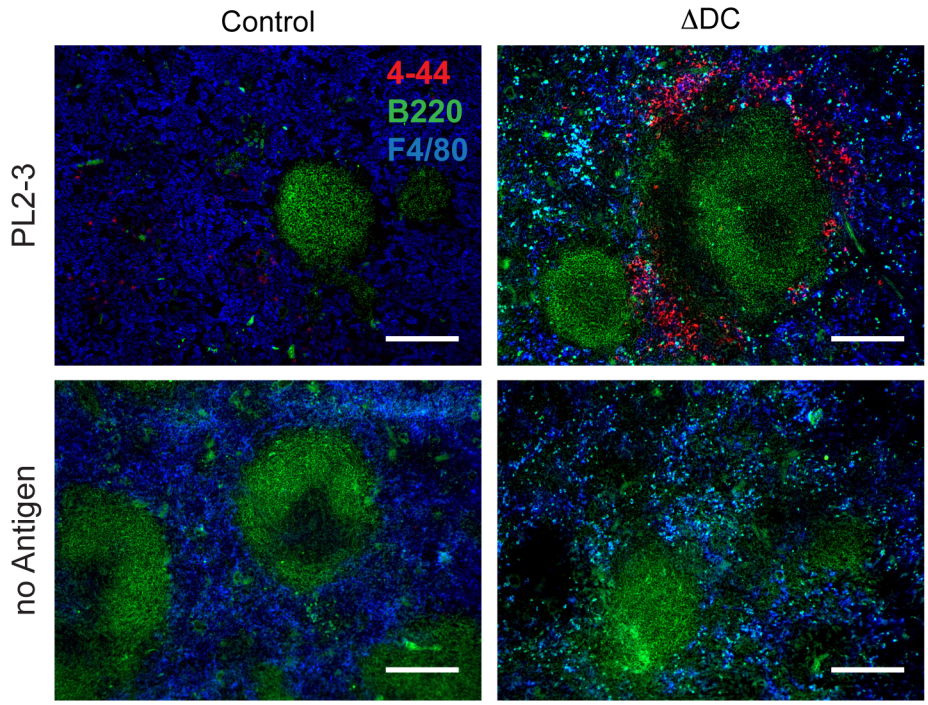

Figure S5, Related to Figure 3. Localization of AM14 B cells in Fas-Sufficient MRL.*Fas*$^{lpr/+}$ Mice that are Constitutively Deficient of cDCs is Extrafollicular
Immunofluorescence histology was performed on spleens from experimental mice shown in Figure 3. Images were taken at a magnification of 100x.
Red, AM14 B-cells (4-44^{+}); green, B-cell follicles (B220^{+}); blue, red pulp (F4/80^{+}).
White scale bars shown on all images are 200 μm.

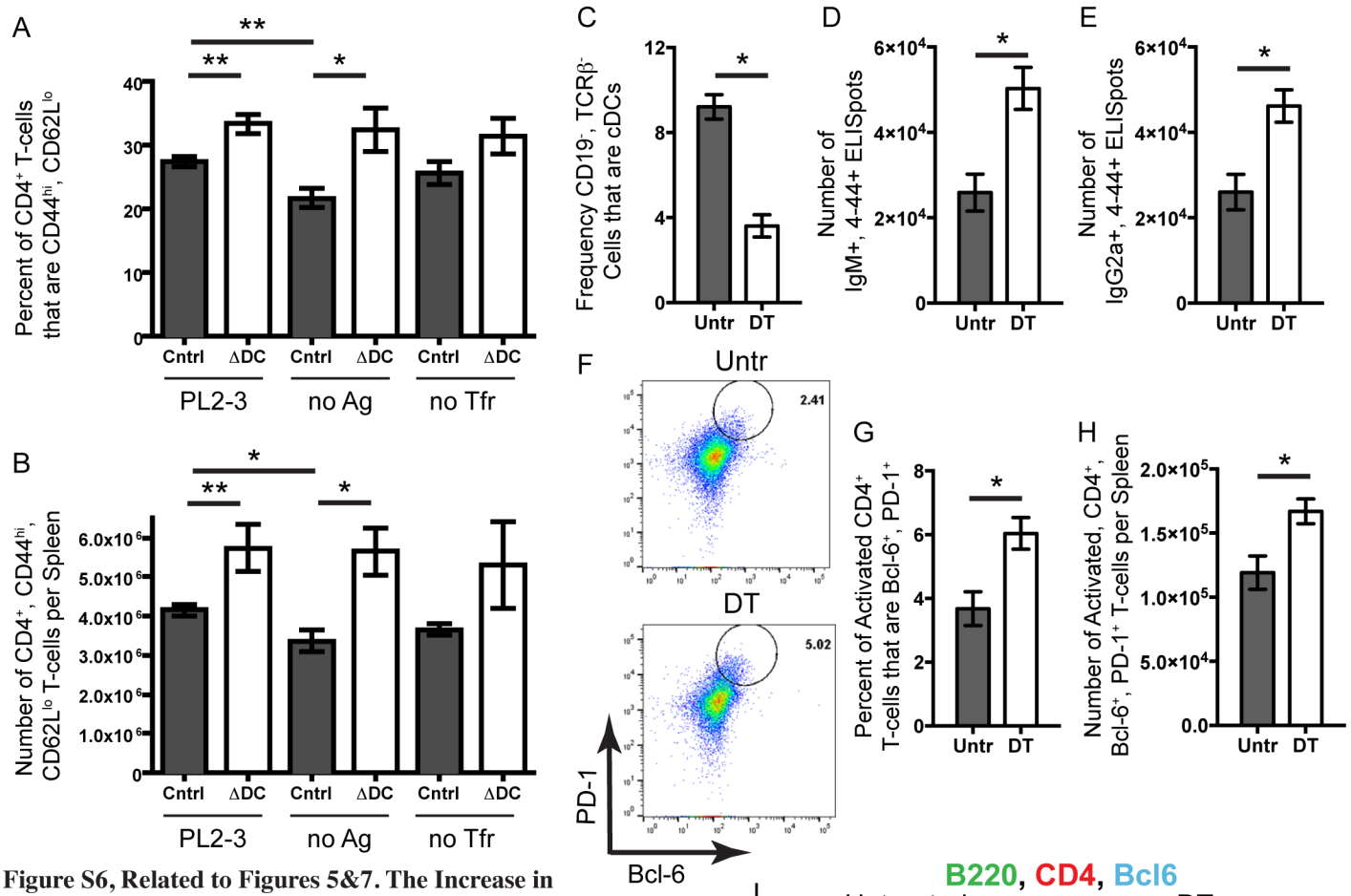

Figure S6, Related to Figures 5&7. The Increase in Activated CD4⁺ T cells in DC-Deficient Mice Does Not Depend on AM14 B-Cell Transfer or PL2-3 Antigen Administration, and DC-Depleted Mice Have Higher Numbers of CD4⁺ T cells with a T_{EFH} Phenotype.

(A&B) Splenic T-cells from Fas-sufficient MRL.*Fas*$^{lpr/+}$ experimental mice shown in Figures 3 were analyzed by flow cytometry as shown in Figure 5. Note that the first two columns of data in panels (A) and (B) are the same as the first two columns of data in shown in Figure 5C and D, respectively: mice that received AM14 B-cell transfer and antigen (PL2-3). The third and fourth columns of data are controls that received AM14 B-cell transfer but no antigen (no Ag). The fifth and sixth columns of data are controls that received no B-cell transfer or antigen (no Tfr). (A) Percent of CD4⁺, TCRβ⁺ cells that are activated (CD44^{hi}, CD62L^{lo}). (B) Number of CD4⁺, TCRβ⁺, CD44^{hi}, CD62L^{lo} cells per spleen. ΔDC, mice that lack cDCs; Cntrl, littermate controls. *$p < 0.05$; **$p < 0.01$ by Mann-Whitney U test.

(C-I) As described in Figure 7G-H, the AM14 B cell response was observed in CD11c-DTR.BALB/c BM chimeras. Data for untreated control versus DT-treated mice are shown, 3 animals per group.

(C) Frequency of CD19⁻TCRβ⁻ cells that were cDCs (CD11c⁺, MHCII⁺).

(D&E) ELISpot assays for the number of 4-44⁺, IgM⁺ or IgG2a⁺ AFCs per spleen. (F) Representative flow cytometry gating of live, activated (CD44^{hi}, CD62L^{lo}) CD4⁺, TCRβ⁺ cells to quantitate cells with a T_{EFH} phenotype (PD-1⁺ and Bcl-6⁺). (G) Percent of activated T cells that have a T_{EFH} phenotype. (H) Number of T_{EFH} cells per spleen. (I) Representative histology showing T_{EFH} cells in the spleen, taken at a magnification of 200x. Scale bars are 50 μm. Arrowheads indicate CD4⁺, Bcl-6⁺ cells. Untr, untreated; DT, diptheria toxin. *$p < 0.05$ by unpaired T-test. Bars represent mean and SEM.

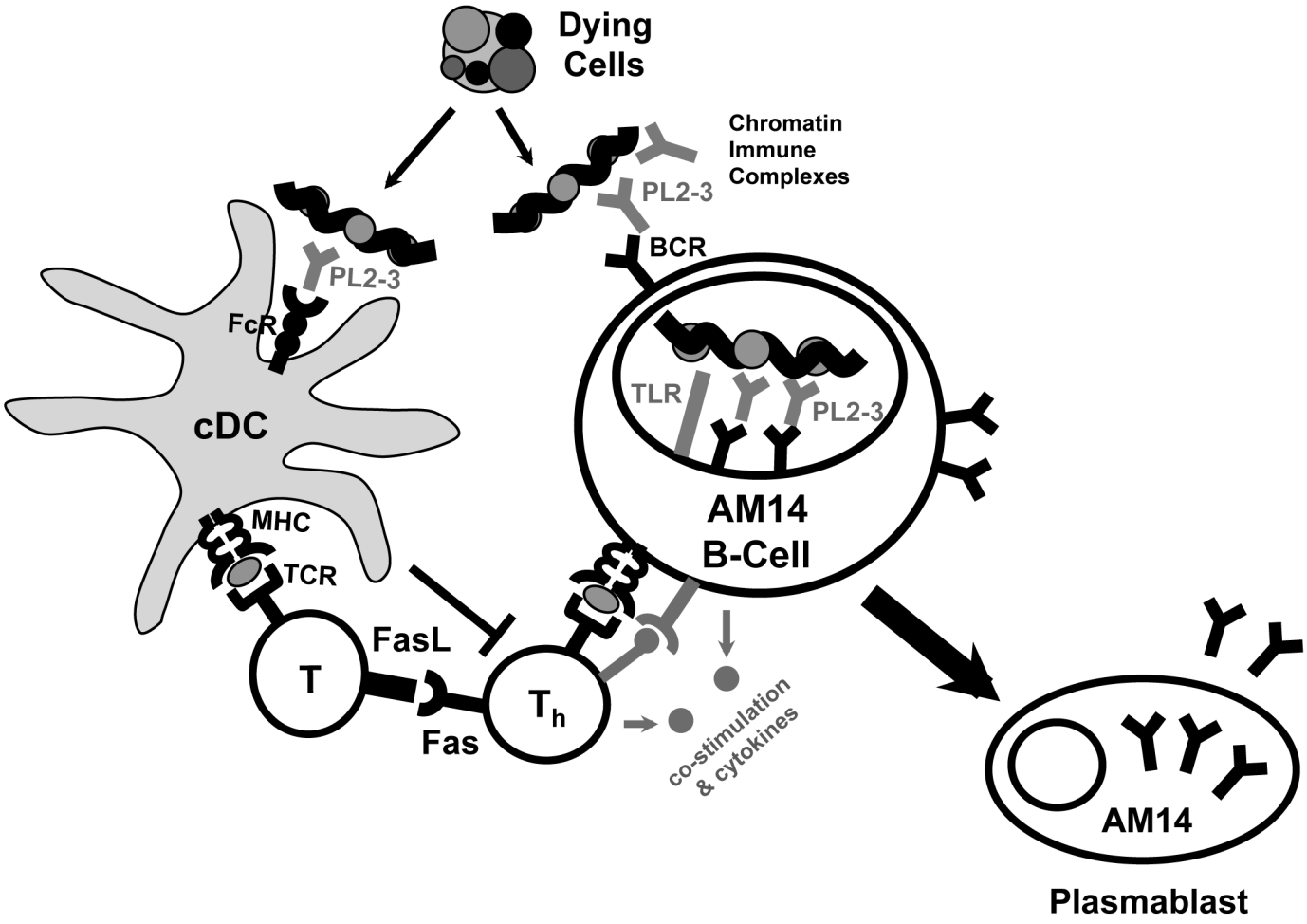

Figure S7, Related to Figures 1-7. Proposed Model for the Role of cDCs and Fas in Regulating the AM14 B-Cell Response

Antigens that promote dual-ligation of AM14 BCRs and TLRs activate the extrafollicular (EF) AM14 plasmablast response. In this case, the antigen consists of chromatin-containing ICs that include the anti-chromatin IgG2a^a antibody, PL2-3, which is itself a ligand for the AM14 BCR. cDCs regulate the EF response, by supporting a FasL-expressing T cell that kills Fas-expressing EF T helper (T_h) cells. T_h cells in turn help the AM14 B cell response. T-B interactions produce an auto-amplifying loop. In the absence of cDCs, or in the absence of Fas, there are less inhibitory Fas-FasL interactions, resulting in more activating T_h cells, and ultimately enhancement of the EF plasmablast response. BCR, B cell receptor; TCR, T cell receptor; TLR, Toll-like receptor; FcR, Fc eceptor; MHC, major histocompatibility complex