## SUPPLEMENTAL MATERIAL

**Moriyama et al., http://www.jem.org/cgi/content/full/jem.20131666/DC1**

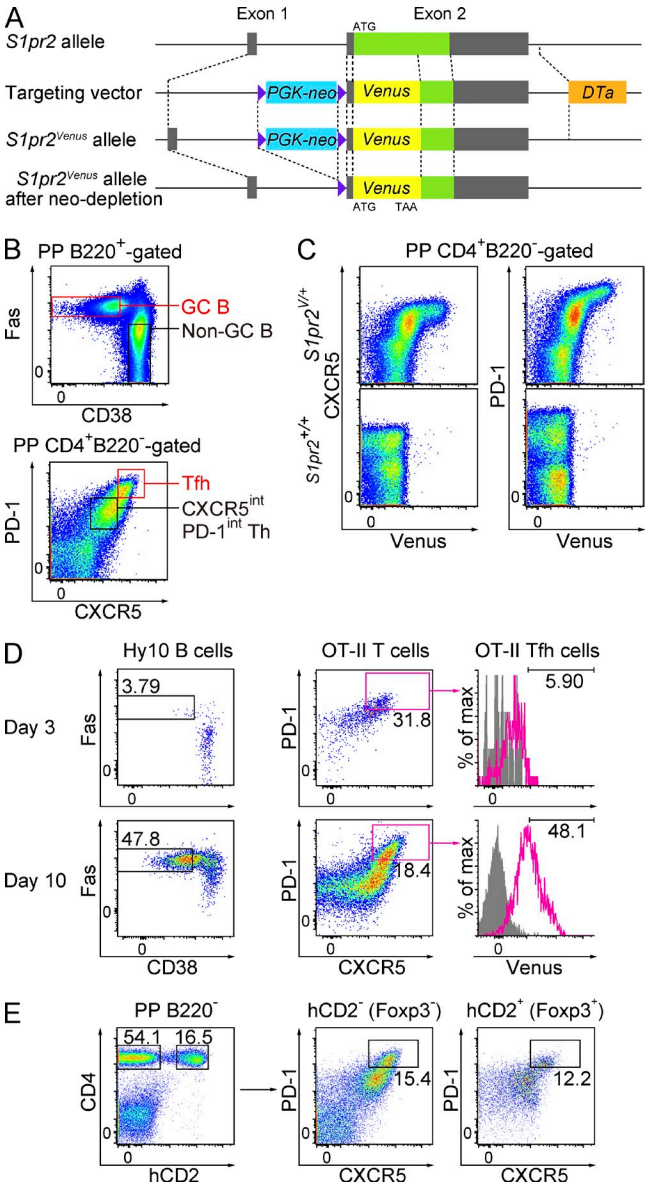



**Figure S1. Generation of the *S1pr2*$^{Venus}$ mice, and representative flow cytometry plots and gating strategy for the data shown in Fig. 1 (B–D).** (A) Schematic representation of the targeting vector and alleles. The first 498 bp of *S1pr2* protein coding region was replaced with the *Venus* gene. (B and C) Representative flow cytometry plots of B cells and CD4+ T cells shown in Fig. 1 B. (D) Representative flow cytometry plots of GC formation and Venus expression in Tfh cells at different time points. See the Fig. 1 C legend for details. (E) Representative flow cytometry plots of Tfr cells. See Fig. 1 D legend for details.

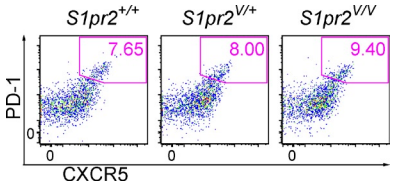



**Figure S2. Representative flow cytometry plots and gating strategy for the data shown in Fig. 2 C.** Shown are representative flow cytometry plots of OT-II $CD4^+$ T cells of the indicated genotypes and gating strategy for $CXCR5^{hi}PD1^{hi}$ cells. See the Fig. 2 C legend for details.

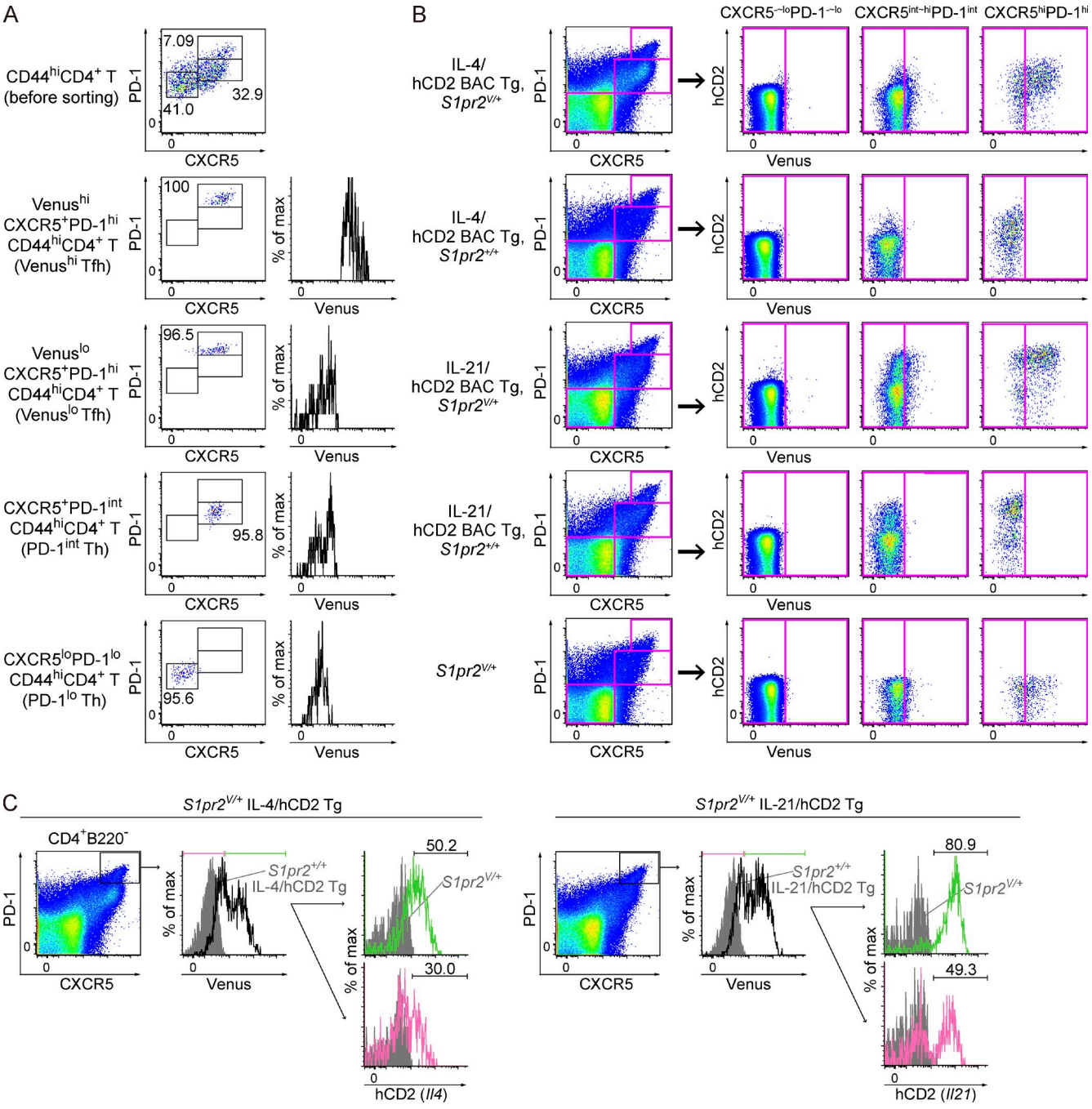



**Figure S3. Gating strategy and representative flow cytometry plots for the data shown in Fig. 4.** (A) Gating strategy for cell sorting. Microarray analysis data of sorted cells were shown in Fig. 4 A. (B and C) Representative flow cytometry plots of CD4+ T cells from immunized mice of the indicated genotypes. Refer to the Fig. 4 B legend for details.

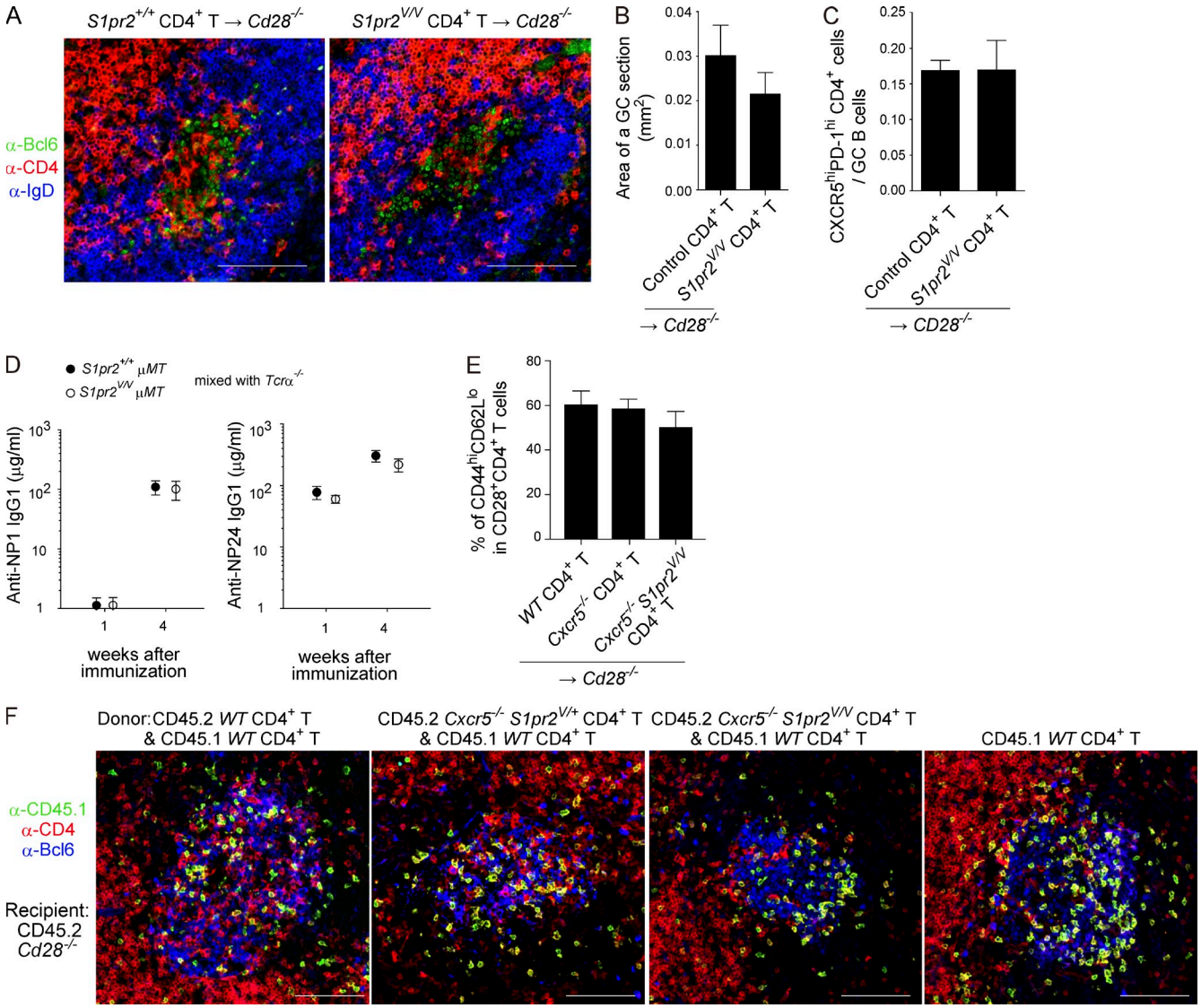
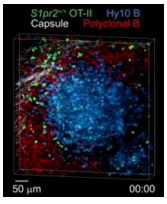
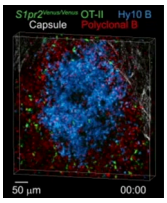


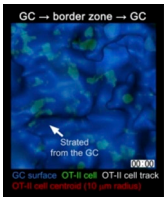

**Figure S4. Representative histological images and supporting data for Fig. 5.** (A) Representative histological images of the data shown in Fig. 5 B. Bars, 100 µm. (B) Area quantification of GCs analyzed in Fig. 5 B. Data are presented as mean ± SEM. (C) Comparison of Tfh cell numbers normalized by GC B cell numbers between the indicated genotypes. See the Fig. 5 A legend for experimental settings. Data are presented as mean ± SEM. (D) Enumeration of anti-NP1 IgG1 and anti-NP24 IgG1 concentrations. Refer to the Fig. 5 C legend for details. Data are presented as mean ± SEM. (E) Comparison of CD4+ T cell activation between the indicated genotypes. See Fig. 5 D legend for experimental settings. Data are presented as mean ± SEM. (F) Representative histological images of the data shown in Fig. 5 E. Bars, 100 µm.




**Video 1. Dynamics of *S1pr2*$^{+/+}$ OT-II T cells in the GC and FM.** Time-lapse sequence of 84 µm z-projection images 7 d after immunization. GFP+ *S1pr2*$^{+/+}$ OT-II T cells were visualized in follicles containing GCs formed by CFP+ Hy10 B cells. CMTMR-labeled polyclonal B cells were also visualized for demarcation of the follicular regions. Second harmonic generation signals show collagen fibers in the LN capsule. Time is shown in minutes:seconds. Data are representative of two independent experiments with 8 recipient mice in total.




**Video 2. Dynamics of *S1pr2$^{V/V}$* OT-II T cells in the GC and FM.** Time-lapse sequence of 96 µm z-projection images 7 d after immunization. GFP$^+$ *S1pr2$^{V/V}$* OT-II T cells were visualized in follicles containing GCs formed by CFP$^+$ Hy10 B cells. CMTMR-labeled polyclonal B cells were also visualized for demarcation of the follicular regions. Second harmonic generation signals show collagen fibers in the LN capsule. Time is shown in minutes:seconds. Note that compared with *S1pr2$^{+/+}$* OT-II T cells (Video 1), *S1pr2$^{V/V}$* cells were less abundantly found in the GC. Data are representative of two independent experiments with 5 recipient mice in total.




**Video 3. Tracking of OT-II T cells that entered the GC–FM interface zone.** Time-lapse sequence of 78 µm or 84 µm z-projection images 7 d after cell transfer and immunization. Representative tracks of GFP$^+$ OT-II T cells approaching GC surfaces are shown. Red spheres with the 10-µm radius are placed around centroids of GFP$^+$ cells to determine whether the centroids exist in the 20-µm-thick interface zone between the GC and FM.

**Table S1.** Entrez gene ID and probe set ID of genes shown in Fig. 4 A

| Gene symbol | Entrez gene ID | Probe set ID |
|---|---|---|
| Bcl6 | 12053 | 1450381_a_at |
| Pdcd1 | 18566 | 1449835_at |
| Cxcr5 | 12145 | 1422003_at |
| Foxp3 | 20371 | 1420765_a_at |
| Gata3 | 14462 | 1448886_at |
| Tbx21 | 57765 | 1449361_at |
| Rorc | 19885 | 1425792_a_at |
| Prdm1 | 12142 | 1420425_at |
| Ccr6 | 12458 | 1450357_a_at |
| Ccr7 | 12775 | 1423466_at |
| Gpr18 | 110168 | 1439141_at |
| Gpr183 | 321019 | 1457691_at |
| S1pr1 | 13609 | 1423571_at |
| S1pr2 | 14739 | 1428176_at |
| Sell | 20343 | 1419481_at |
| Sh2d1a | 20400 | 1449393_at |