SUPPLEMENTAL INFORMATION

Supplemental Data:

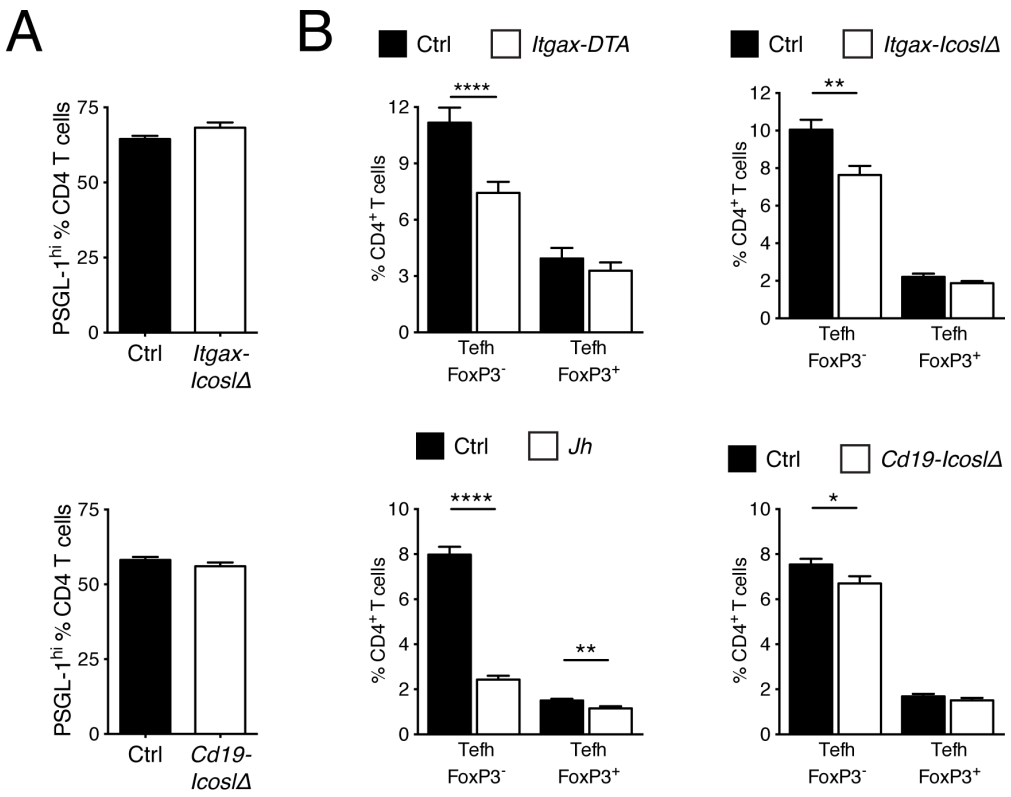

Figure S1, related to Figure 2. FoxP3⁺ Tefh cells are not reduced in lupus-prone mice deficient for *Icosl* in CD11c⁺ or B cells.

(A) Percentage of PSGL-1^{hi} cells among CD4^{+} T cells in spleens from *Itgax-Icosl*$^{\Delta}$ and *Cd19-Icosl*$^{\Delta}$ mice with their respective controls. n = 13 for *Itgax-Icosl*$^{\Delta}$ mice and n = 25 for controls; n = 20 for *Cd19-Icosl*$^{\Delta}$ mice and n = 29 for controls.

(B) Percentage of FoxP3^{-} and FoxP3^{+} Tefh cells among CD4^{+} T cells in spleens from *Itgax-Icosl*$^{\Delta}$, *Cd19-Icosl*$^{\Delta}$, *Itgax-DTA* and *Jh* MRL.*Fas*^{lpr} mice with their respective controls. n = 22 for *Itgax-Icosl*$^{\Delta}$ mice and n = 24 for controls; n = 21 for *Cd19-Icosl*$^{\Delta}$ mice and n = 23 for controls; n = 15 *Itgax-DTA* and n = 16 for controls; n = 19 for *Jh* MRL.-*Fas*^{lpr} mice and n = 21 for controls.

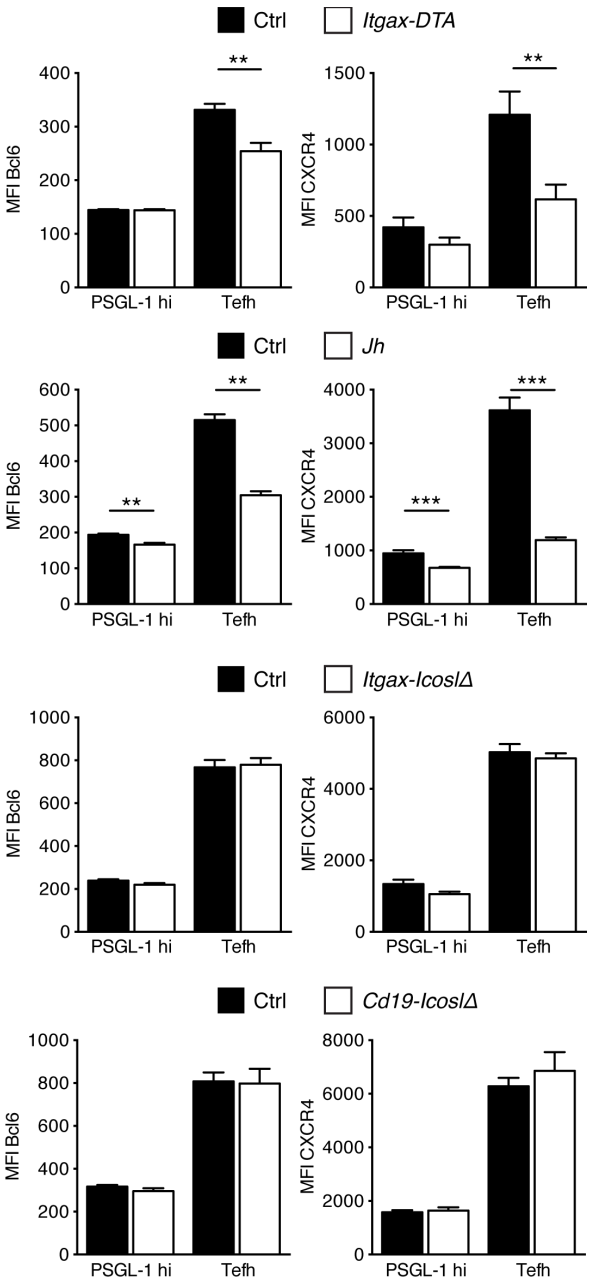

Figure S2, related to Figure 3. Bcl6 and CXCR4 expression by Tefh cells is unimpaired in MRL.*Fas*^{lpr} mice lacking ICOSL on CD11c^{+} or B cells.

Mean fluorescence intensity (MFI) for Bcl6 (left column) and CXCR4 (right column) of Tefh cells in spleens from *Itgax-DTA, Jh* MRL.*Fas*^{lpr}, *Itgax-Icosl*$^{\Delta}$ and *Cd19-Icosl*$^{\Delta}$ mice with their respective controls. n = 6 for *Itgax-Icosl*$^{\Delta}$ mice and n = 6 for controls; n = 10 for *Cd19-Icosl*$^{\Delta}$ mice and n = 23 for controls; n = 7 *Itgax-DTA* and n = 6 for controls; n = 4-6 for *Jh* MRL.*Fas*^{lpr} mice and n = 8 for controls. Data are represented as mean ± SEM.

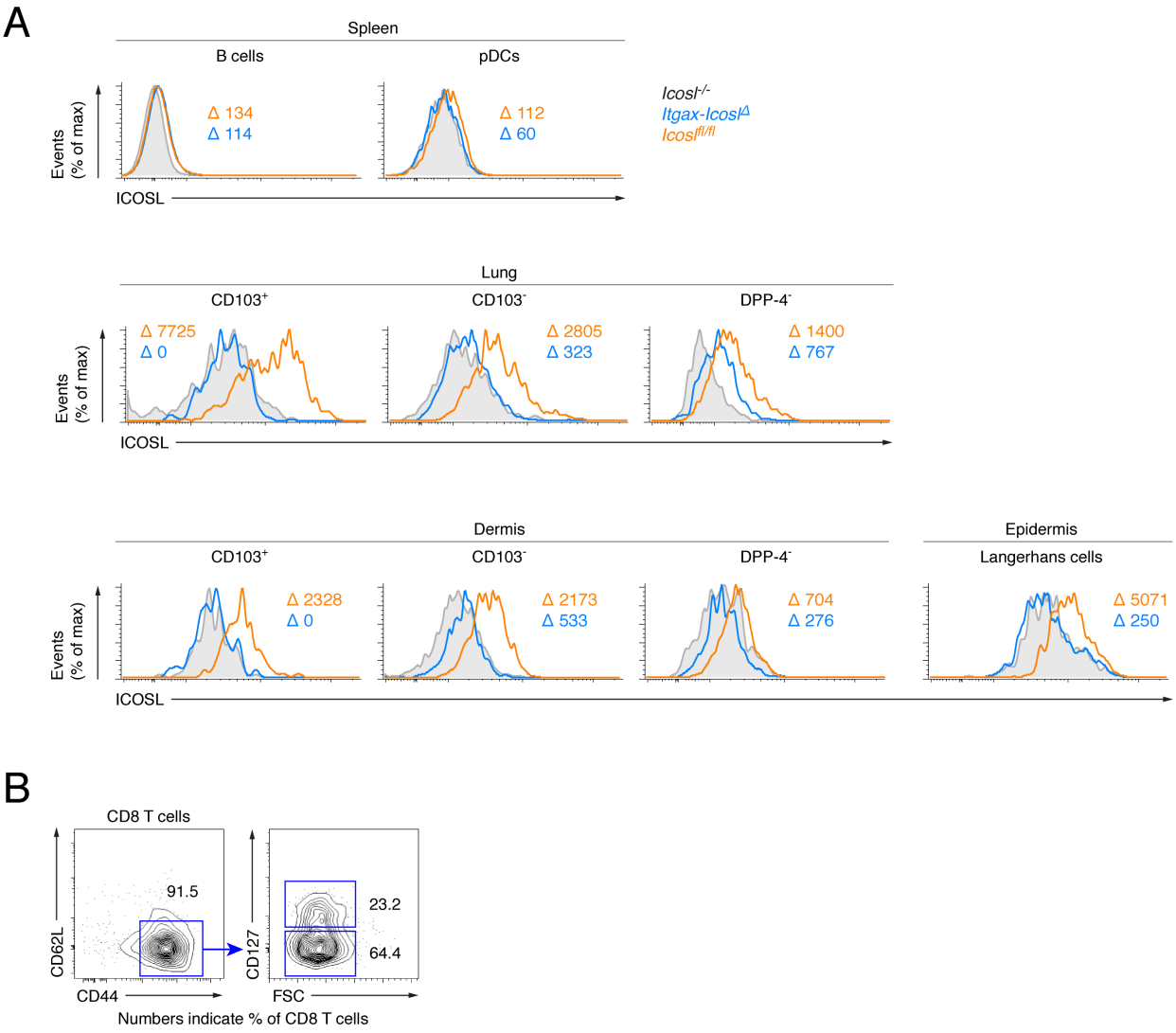

Figure S3, related to Figure 5. CD11c⁺ cells in inflamed peripheral organs express high levels of ICOSL.

(A) Histograms for ICOSL expression of B cells and pDCs in spleens, several CD11c⁺ MHCII⁺ subsets (CD103⁺DPP-4⁺, CD103⁻DPP-4⁺ and DPP-4⁻) in lungs and dermis, and Langerhans cells in epidermis from control MRL.*Fas*^{lpr} (*Icosl*$^{fl/fl}$, n = 4), *Itgax-Icosl*$^{\Delta}$ (n = 4) and *Icosl*$^{-/-}$ (n = 2) mice. Values indicate MFI minus background staining (MFI of *Icosl*$^{-/-}$).

(B) Flow cytometry plots showing CD44, CD62L and CD127 staining characteristics of gated CD8⁺ T cells in kidney cell suspensions of wild type MRL.*Fas*^{lpr} mice (n = 5). Values indicate percentage of CD8⁺ T cells (mean).

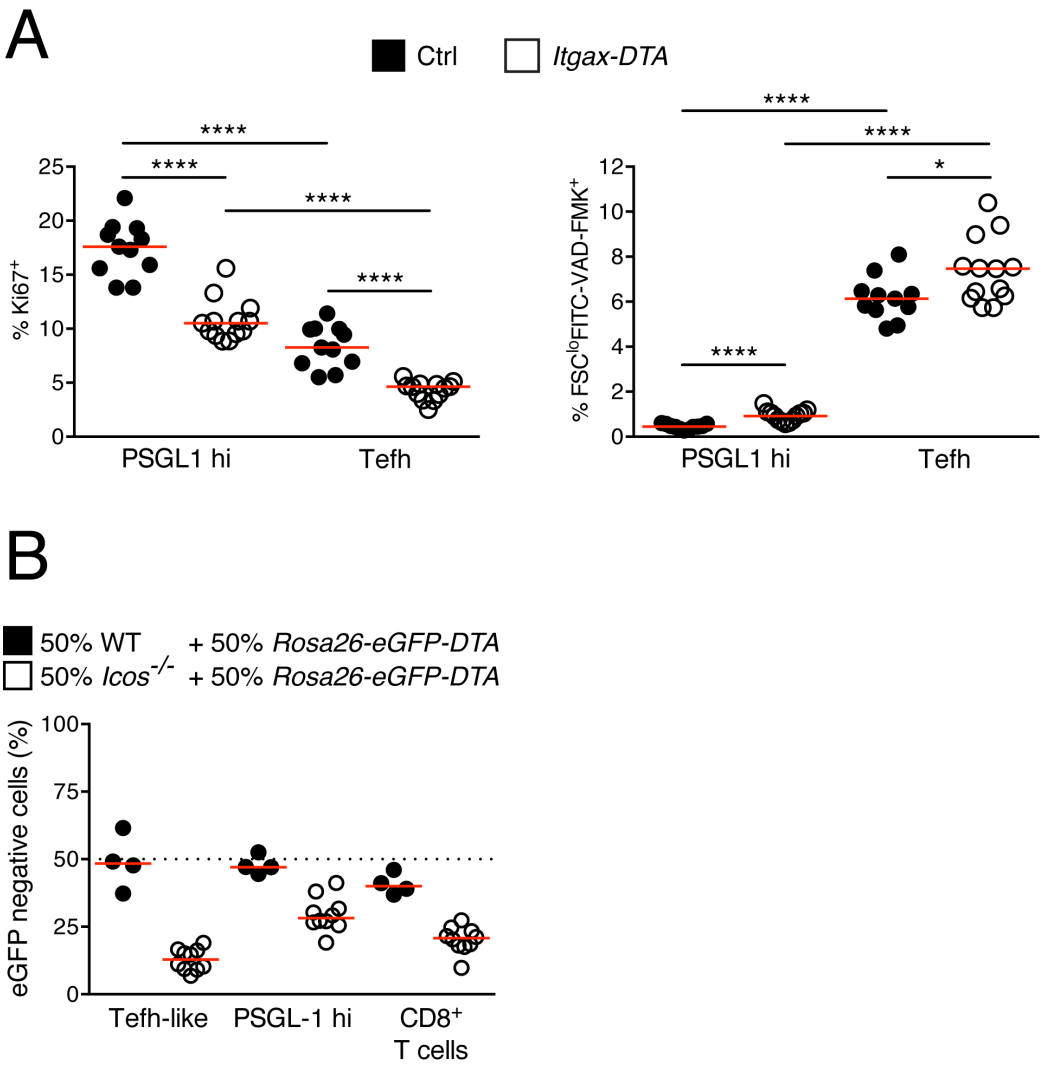

Figure S4, related to Figure 6. ICOS-deficiency impairs T cell accrual in kidneys in a cell-intrinsic manner.

(A) Percentage of cycling (Ki67+, left) and apoptotic (FSC^{lo}FITC-VAD-FMK+, right) PSGL1^{hi} CD4+ T cells and Tefh cells in spleens from control (black) and *Itgax-DTA* (white) mice.

(B) *Rosa-eGFP-DTA* MRL.*Fas*^{lpr} mice were lethally irradiated and reconstituted with a mixture of 50% wild type MRL.*Fas*^{lpr} and 50% *Rosa26-eGFP-DTA* MRL.*Fas*^{lpr} (black), or 50% *Icos*$^{-/-}$ MRL.*Fas*^{lpr} and 50% *Rosa26-eGFP-DTA* MRL.*Fas*^{lpr} (white) bone marrow cells. The scatter plot shows the percentage of eGFP negative cells among renal Tefh-like, PSGL-1^{hi} CD4+, and CD8+ T cells. Note that *Rosa26-eGFP-DTA* MRL.*Fas*^{lpr} mice display generalized expression of eGFP (DTA transcription is prevented by a transcriptional stop sequence).

Each dot represents an individual mouse and horizontal lines represent the median.

Table S1, related to Figure 1. Deletion efficacy of *Icosl*

Mice	Cell Population	Tissue	n	Deletion efficacy (%)
Itgax-Icosl$^{\Delta}$	cDCs	Spleen	2	95.2
	pDCs	Spleen	2	61.8
	Red pulp macrophages	Spleen	2	5.9
	Neutrophils	Spleen	2	0
	B cells	Spleen	2	23.1
	CD11c+MHCII+	Kidney	2	91.2
Cd19-Icosl$^{\Delta}$	B cells	Spleen	3	95.8
	Ab-forming cells	Spleen	3	90.3
	cDCs	Spleen	3	0
	pDCs	Spleen	3	2.2
	Red pulp macrophages	Spleen	3	0
	Neutrophils	Spleen	3	0
	B cells	Kidney	3	89.9

Deletion efficacy of *Icosl* in various cell types isolated from spleens and kidneys was determined by measuring the amount of residual *Icosl*^{fl} by qRT-PCR. Cell populations were purified by FACS. Spleen: cDCs (CD11c^{hi}MHCII^{+}CD19^{-}), pDCs (Siglec-H^{+}BST2^{+} CD11c$^{int\text{-}hi}$), red pulp macrophages (F4/80^{+}CD11b^{+}), neutrophils (GR-1^{hi}CD11b^{+}), B cells (CD19^{+}CD22^{+}), Ab-forming cells (intracellular-κ^{hi}CD138^{hi}CD19^{int}CD44^{+}TCRβ^{-}). Kidney: CD11c^{+}MHCII^{+} (CD11c^{+}MHCII^{+}CD45^{+}CD19^{-}TCRβ^{-}), B cells (CD19^{+}MHCII^{+} CD45^{+}). To calculate deletion efficacy values for qRT-PCR the equation (1 — residual *Icosl*) x 100 was used. Residual *Icosl* values were calculated as $2^{-\Delta\Delta Ct}$. Negative deletion efficacy values were set to 0.

Supplemental Experimental Procedures:

Ab clones used for FACS

Anti-Bcl6 (K112-91), anti-BST2 (927), anti-CD4 (GK1.5), anti-CD8 (TIB 105), anti-CD11b (M1/70), anti-CD11c (N418), anti-CD19 (1D3), anti-CD22 (Cy34.1), anti-CD25 (PC61), anti-CD44 (1M7), anti-CD45 (30-F11), anti-CD45R (RA3-6B2), anti-CD62L (Mel-14), anti-CD103 (2E7), anti-CD127 (A7R34), anti-CXCR4 (2B11), anti-DPP-4 (H194-112), anti-F4/80 (BM8), anti-FoxP3 (FJK-16s), anti-I-A/I-E (M5/114), anti-IFN-γ (XMG1.2), anti-PSGL1 (2PH1), anti-Siglec-H (eBio440c), and anti-TCRβ (H57-597).

qRT-PCR

To quantify deletion efficacy of *Icosl*, genomic DNA was extracted from FACS purified cells and used as template for qRT-PCR. The amount of *Icosl* in each sample was normalized to the unaffected gene *Il10*. Primer sequences (5'—3') for *Icosl* were forward ACCTACACCTGCATGTCCAA and reverse TCTCTACGCAGCACAGAACA, and for *Il10* forward GCTCTTACTGACTGGCATGAG and reverse CGCAGCTCTAGGAGCATGTG. qRT-PCR was performed with the Agilent Brilliant II SYBR Green QPCR kit on a Stratagene Mx3000P instrument.

Mixed bone marrow chimeras

For mixed bone marrow chimeras 6-7 wk old *Rosa26-eGFP-DTA* MRL.*Fas*^{lpr} mice (Teichmann et al., 2010) were irradiated with 800 cGy and injected intravenously with 5 x 10^6 *Icos*$^{-/-}$ MRL.*Fas*^{lpr} (Odegard et al., 2008) together with 5 x 10^6 *Rosa26-eGFP-DTA* MRL.*Fas*^{lpr} mice bone marrow cells. Controls were generated in the same experiments using 5 x 10^6 wild type MRL.*Fas*^{lpr} instead of *Icos*$^{-/-}$ MRL.*Fas*^{lpr} bone marrow cells. Chimeras were analyzed 21 wks after transplantation.