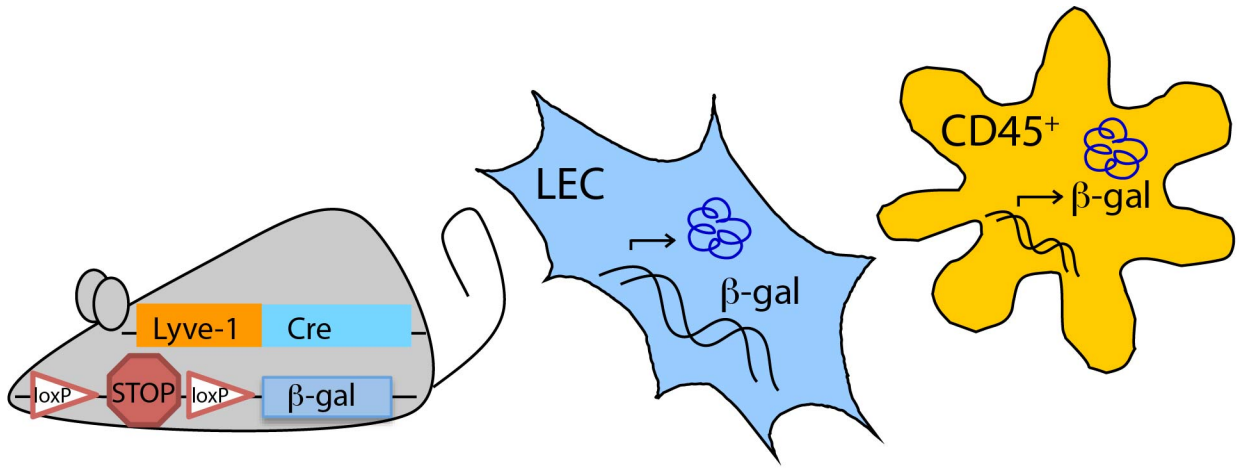
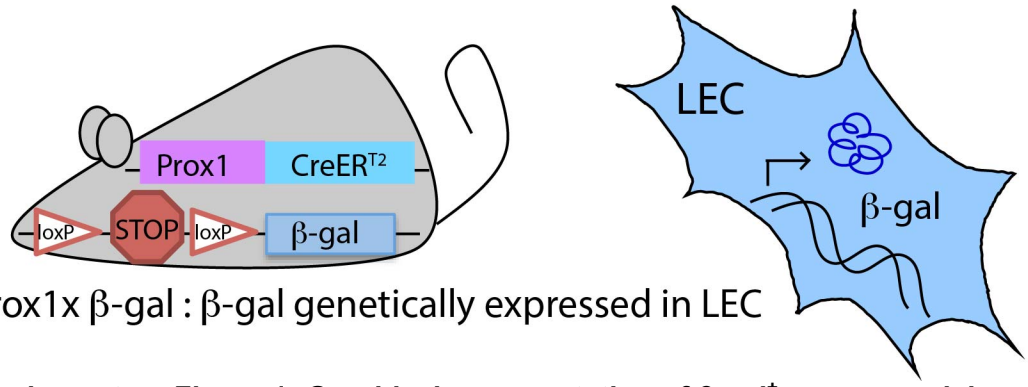
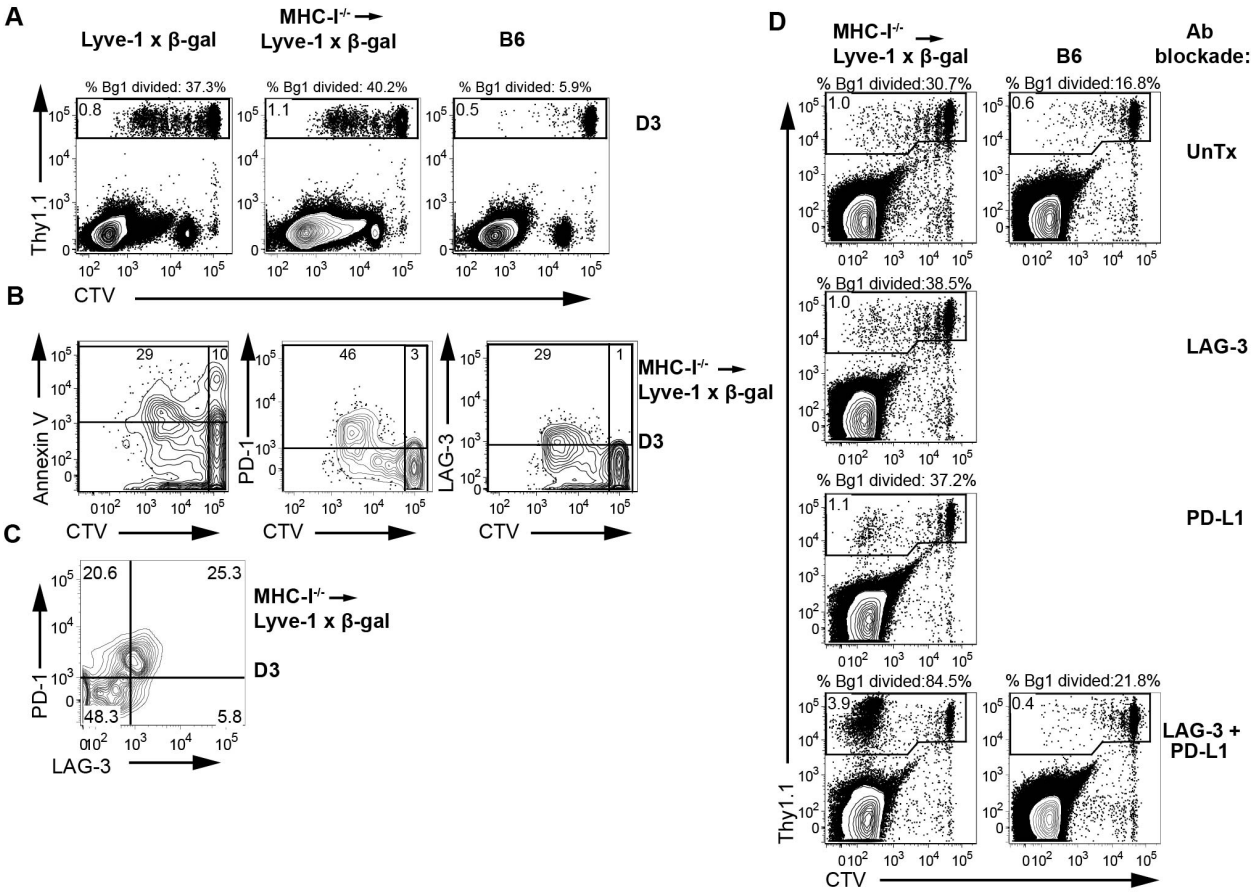


Lyve-1x β-gal : β-gal genetically expressed in LEC and hematopoietically-derived CD45^{+} cells



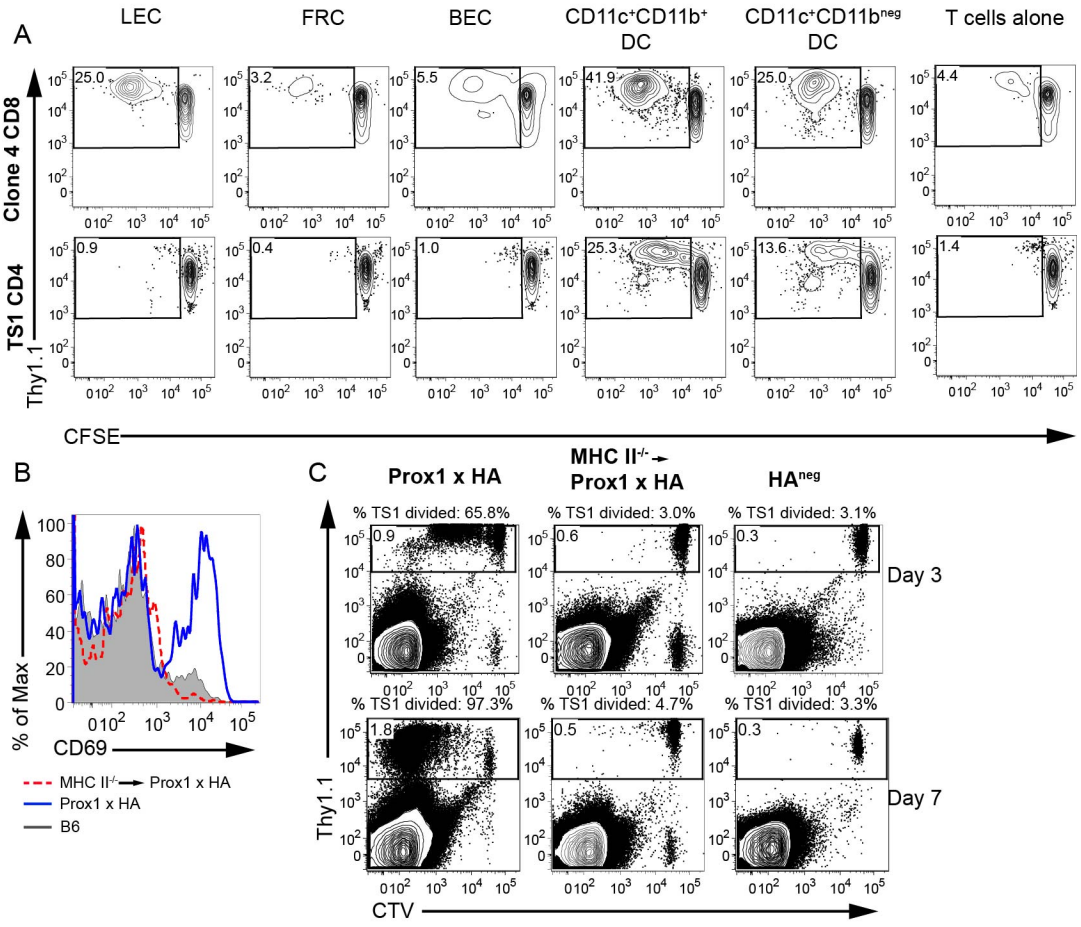
Prox1x β-gal : β-gal genetically expressed in LEC

Supplementary Figure 1: Graphical representation of β-gal^{+} mouse models used. Lyve-1-cre x Rosa26$^{stop\text{-}LacZ}$ (Lyve-1x β-gal) mice genetically express β-gal in LECs and some CD45^{+} cells, whereas Prox1-creER^{T2} x Rosa26$^{stop\text{-}LacZ}$ (Prox1x β-gal) mice genetically express β-gal in LECs but not CD45^{+} cells.



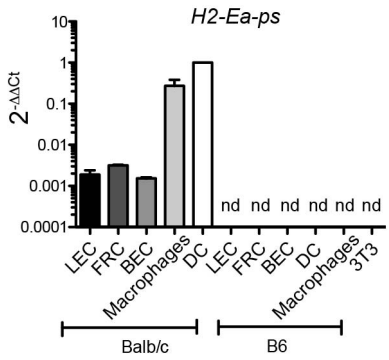
Supplementary Figure 2: β-gal specific CD8 T-cells delete via the PD-1/PD-L1 and LAG-3/MHC-II pathways after recognizing antigen expressed by LECs.

(A-C) CTV-labeled Thy1.1^{+} Bg1 cells were adoptively transferred along with CTV-labeled Thy1.1^{neg} cells as an injection control. Skin-draining LN were analyzed for Bg1 proliferation and the indicated markers 3 days later. (D) Representative data from Figure 3D is shown. Plots are gated on total CD8^{+} T-cells in (A, D), CD8^{+}Thy1.1^{+} Bg1 cells in (B), and on proliferating CD8^{+}Thy1.1^{+} Bg1 cells in (C). Numbers in (B) represent the percent of dividing (left number) or undivided (right number) Bg1 cells that express the indicated marker. Data representative of 2-3 experiments with 1-3 mice each (A-C) or 2 experiments with 1-2 mice each (D).

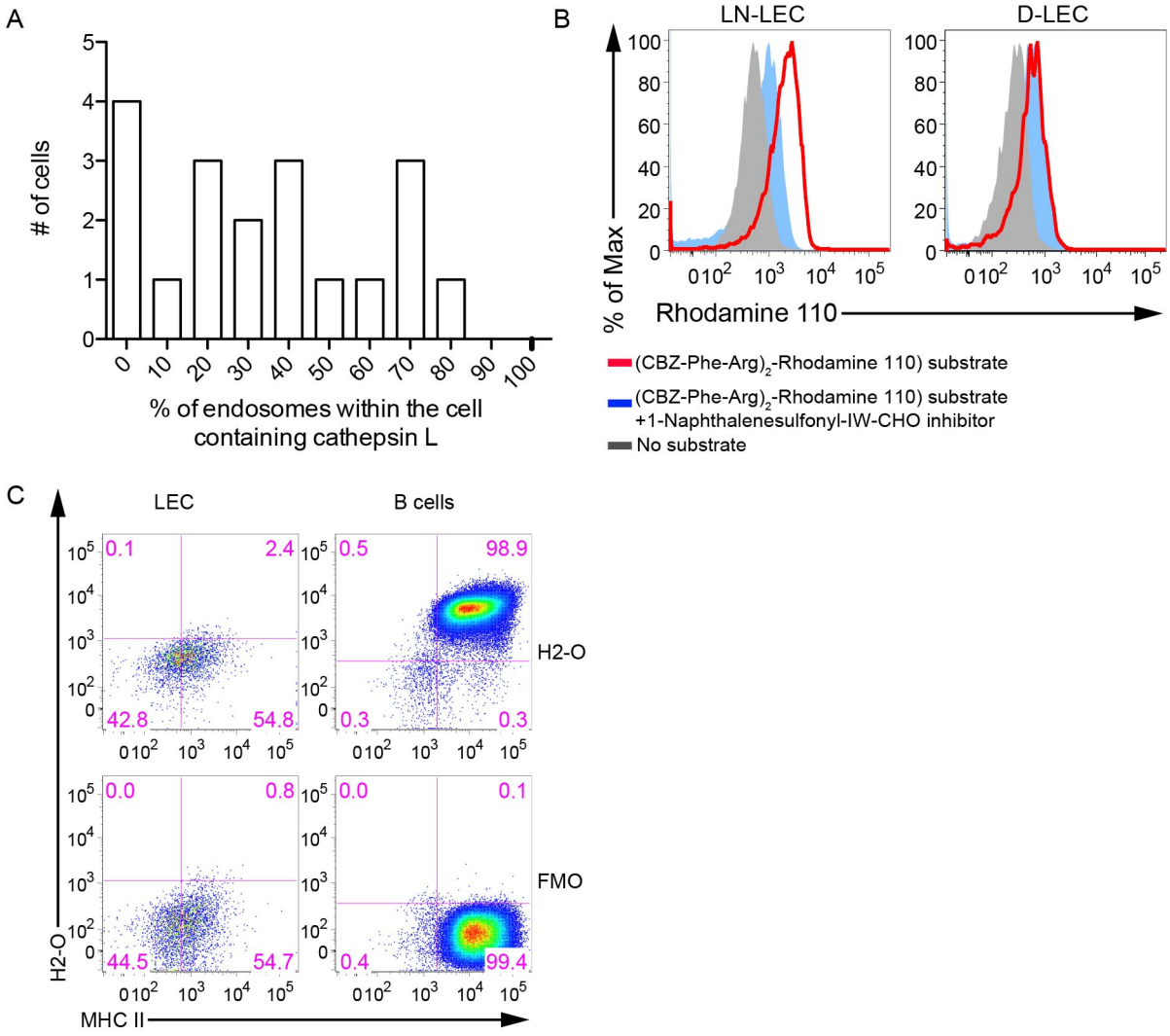


Supplementary Figure 3: LECs do not present HA on MHC-II molecules

(A) Representative data from co-culture in Figure 5A is shown. (B) CTV-labeled Thy1.1^{+} TS1 cells were transferred into the indicated recipients, and CD69 was analyzed 16 hours after transfer. Plot is gated on Thy1.1^{+} CD4^{+} cells. Data representative of 2 experiments with 1-2 mice each. (C) CTV-labeled Thy1.1^{+} TS1 cells were transferred into the indicated recipients, along with CTV-labeled Thy1.1^{neg} cells as an injection control. Proliferation was measured 3 or 7 days later. Plot is gated on CD4^{+} cells. Data representative of 1-2 mice from 1 experiment.



Supplementary Figure 4: BALB/c LECs express I-Eα, but B6 LECs do not. LECs, FRCs, BECs, macrophages, and DCs from Balb/c or B6 mice were sorted by flow cytometry as in Figure 1, and cultured 3T3 cells were harvested. mRNA was purified and qPCR was performed for *H2-Ea-ps.* Data from 2 independent experiments with LN pooled from 4-5 mice. Nd, not detected.



Supplementary Figure 5: LECs express cathepsin L but not H-2O

(A) The percent of endosomes containing cathepsin L in each LEC was calculated using ImageJ, and histograms were computed using Prism. A representative immunofluorescent image is shown in Figure 6B. (B) LN-LECs or D-LECs were preincubated with the cathepsin L inhibitor 1-naphthalenesulfonyl-IW-CHO or DMSO vehicle control for 20 minutes at 37°C, followed by a 20 minute incubation with the cathepsin L substrate $(CBZ\text{-}Phe\text{-}Arg)_2$-rhodamine 110 at 37°C, and rhodamine 110 fluorescence was measured by flow cytometry. (C) LECs and B cells were stained for MHC-II and H-2O intracellularly. (A) shows data from 17 cells from 2

separate experiments. Data in B is from 2 experiments with LN pooled from 1-2 mice, and (C) is representative of 2 independent experiments.

Mouse strain	Mouse background	Constitutive or induced cre	Antigen	Antigen genetically expressed in:	Notes
Lyve-1xβ-gal	B6	Constitutive	β-gal	LECs, $CD45^+$ cells	
MHC-$I^{-/-}$→Lyve-1xβ-gal bone marrow chimeras	B6	Constitutive	β-gal	LECs	No genetic antigen or MHC-I expression in $CD45^+$ cells
MHC-$II^{-/-}$→Lyve-1xβ-gal bone marrow chimeras	B6	Constitutive	β-gal	LECs	No genetic antigen or MHC-II expression in $CD45^+$ cells
Prox1xβ-gal	B6	Tamoxifen induced	β-gal	LECs	
MHC-$II^{-/-}$→Prox1xβ-gal bone marrow chimeras	B6	Tamoxifen induced	β-gal	LECs	Bone marrow-derived $CD45^+$ cells are MHC-$II^{-/-}$
Prox1xHA	(B6 x Balb/c) F1	Tamoxifen induced	HA	LECs	
MHC-$II^{-/-}$→Prox1xHA bone marrow chimeras	(B6 x Balb/c) F1	Tamoxifen induced	HA	LECs	Bone marrow-derived $CD45^+$ cells are MHC-$II^{-/-}$

Supplementary Table 1: Antigen-expressing mouse strains used

Mouse strain	Mouse background	Antigen recognized	CD4 or CD8	Restriction element
Bg1	B6	β-gal	CD8	$H2\text{-}K^b$
Bg2	B6		CD4	$I\text{-}A^b$
Clone 4	(B6 x Balb/c) F1	HA	CD8	$H\text{-}2K^d$
TS1	(B6 x Balb/c) F1		CD4	$I\text{-}E^d$

Supplementary Table 2: TCR-transgenic mouse strains used