Supplemental material

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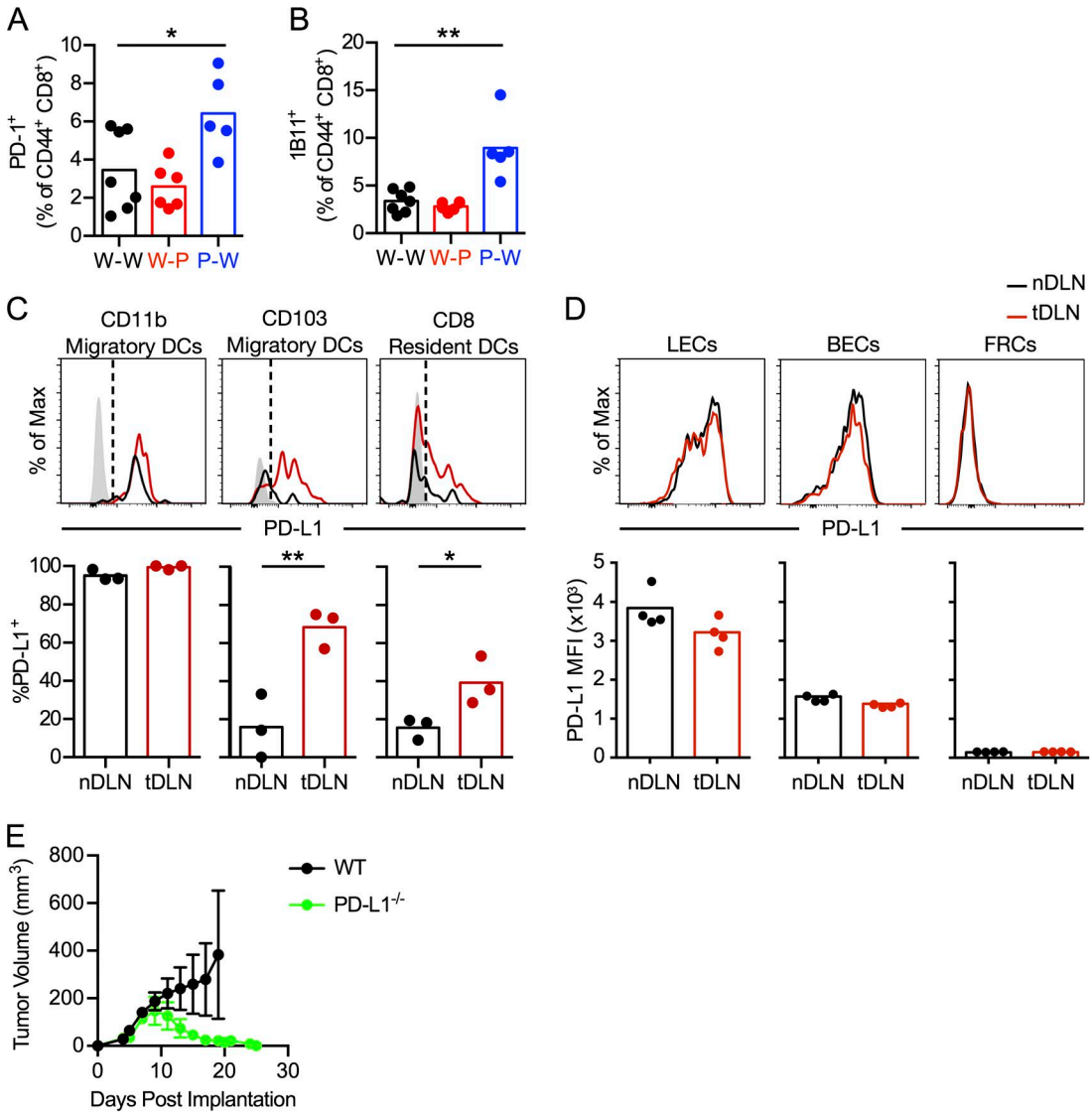

Figure S1. **Hematopoietic but not nonhematopoietic PD-L1 mediates peripheral expansion of CD8⁺ T cells following tumor implantation. (A and B)** PD-1 (A) and 1B11 (B) expression by CD8⁺ T cells in B16F10.OVA tDLNs of PD-L1$^{-/-}$ bone marrow chimeric mice. **(C and D)** Representative histograms (top) and quantification (bottom) of PD-L1 expression by migratory and resident DCs (C), CD11c⁺MHCII^{hi}CD11b⁺, CD11c⁺MHCII^{hi}CD11b⁻CD103⁺, CD11c⁺MHCII^{int}CD8α⁺ and nonhematopoietic stromal cells (D), CD45⁻CD31⁺gp38⁺ LECs, CD45⁻CD31⁺gp38⁺ BECS, and CD45⁻CD31⁻gp38⁺ FRCs in tDLN and nondraining LNs (nDLN). Each point represents one mouse, and bars indicate the mean. **(E)** YUMMER 1.7 tumor growth in WT (black) and PD-L1$^{-/-}$ (green) mice. Shaded histogram represents isotype staining control. One-way ANOVA corrected for multiple comparisons (A and B). Student's *t* test (C–E); *, P < 0.05; **, P < 0.01.

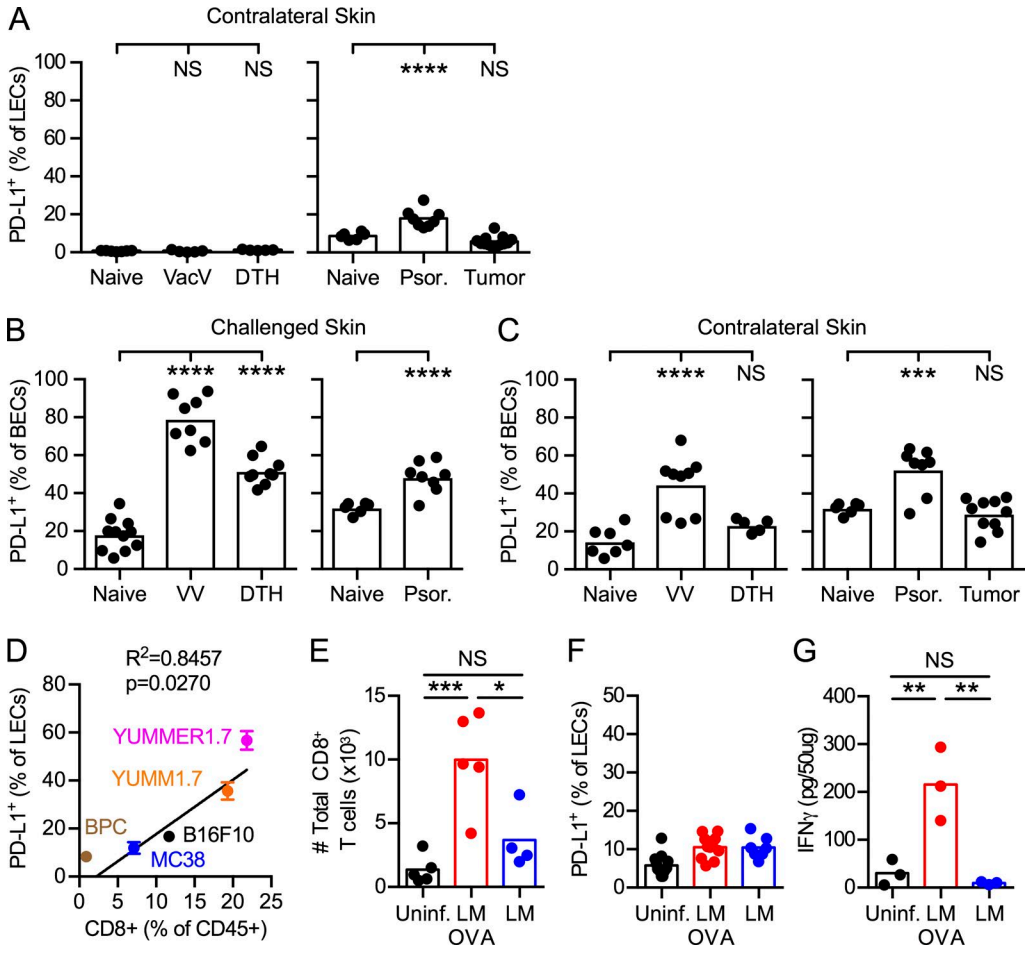

Figure S2. **LEC PD-L1 expression in contralateral skin. (A)** PD-L1 expression by LECs at sites distal to cutaneous challenge with VacV (VV), DTH, imiquimod-induced psoriasis (Psor.), or B16F10 melanoma, compared with skin of naive mouse. **(B and C)** PD-L1 expression by inflamed (B) or contralateral (C) skin of mice challenged with VacV, DTH, imiquimod-induced psoriasis, or B16F10 melanoma. **(D)** Correlation between $CD8^+$ T cell infiltration and tumor-associated LEC PD-L1 expression across various tumor models indicated on graph. **(E)** Total $CD8^+$ T cells in B16F10.OVA tumors of mice vaccinated with LM, LM-OVA, or no vaccination. **(F)** PD-L1 expression by cutaneous LECs in skin contralateral to B16F10.OVA tumors of mice vaccinated with LM, LM-OVA, or no vaccination. **(G)** IFNγ, measured by ELISA, in tumor lysates from B16F10.OVA tumors of mice vaccinated with LM, LM-OVA, or no vaccination. Each dot represents one mouse; bars represent mean. One-way ANOVA corrected for multiple comparisons (A–G). Pearson correlation (D). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$.

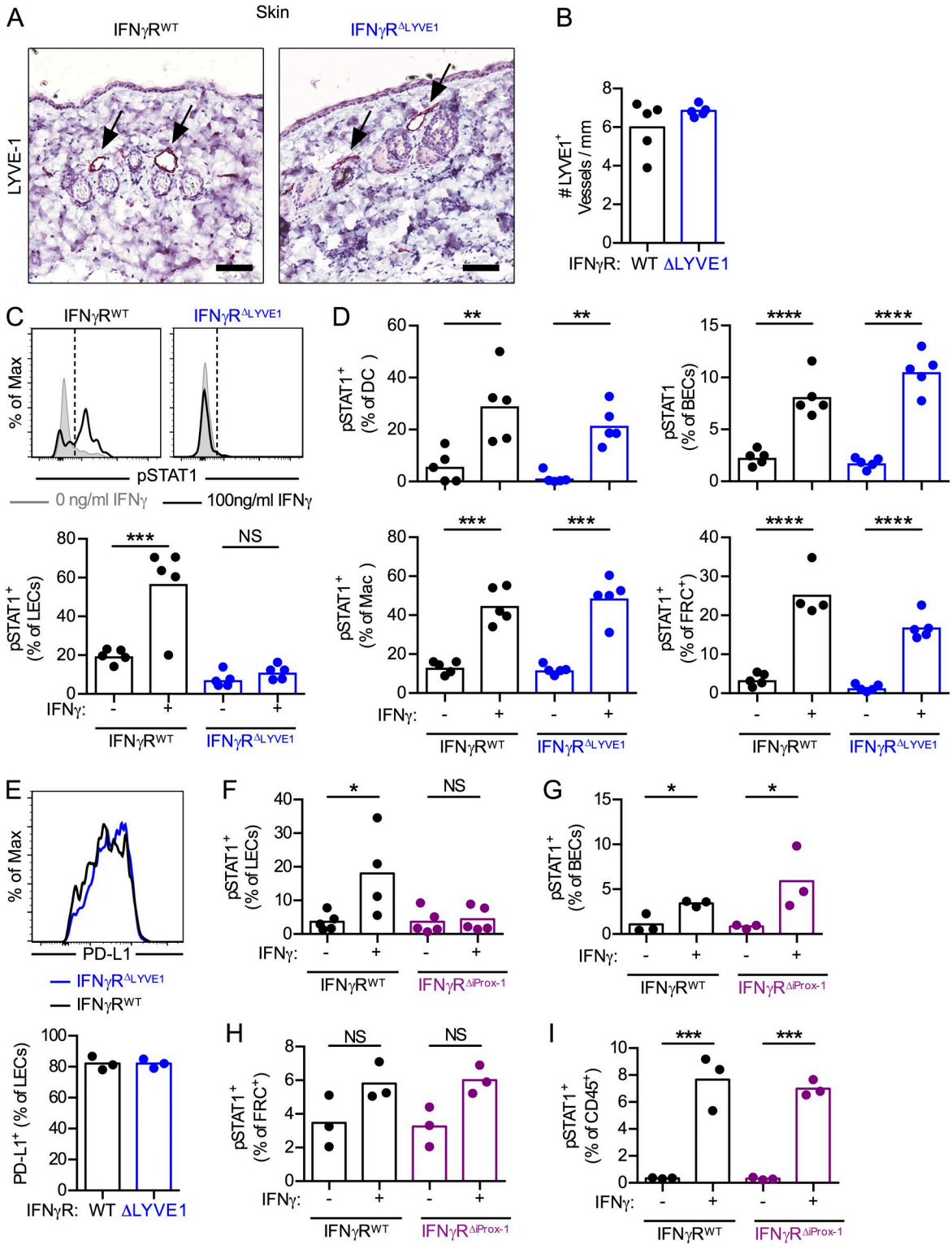

Figure S3. **LEC-specific loss of IFNγR. (A and B)** Representative images (A) and quantification (B) of LVD in naive skin of IFNγR$^{\Delta LYVE1}$ mice or littermate controls. **(C)** Representative histograms (top) and quantification (bottom) of STAT1 phosphorylation (pSTAT1) following IFNγ stimulation of ex vivo LECs (CD45$^-$CD31$^+$gp38$^+$) harvested from IFNγR$^{\Delta LYVE1}$ mice or littermate controls. **(D)** Quantification of STAT1 phosphorylation (pSTAT1) following IFNγ stimulation of ex vivo DCs (CD11c$^+$MHCII$^+$), macrophages (Mac; CD11c$^-$CD11b$^+$F4/80$^+$), BECs (CD45$^-$CD31$^+$gp38$^-$), and FRCs (CD45$^-$CD31$^-$gp38$^+$) harvested from IFNγR$^{\Delta LYVE1}$ mice or littermate controls. **(E)** PD-L1 expression by LECs in naive LNs of IFNγR$^{\Delta LYVE1}$ mice or littermate controls. **(F–I)** Quantification of pSTAT1 following ex vivo IFNγ stimulation of LEC (F), BEC (G), FRC (H), and CD45$^+$ cells (I) harvested from IFNγR$^{\Delta iProx\text{-}1}$ mice or littermate controls. Each point represents one mouse, bars indicate mean. Shaded histogram represents isotype staining control. One-way ANOVA corrected for multiple comparisons. *, P < 0.05; **, P < 0.01; ***, P < 0.001; ****, P < 0.0001.

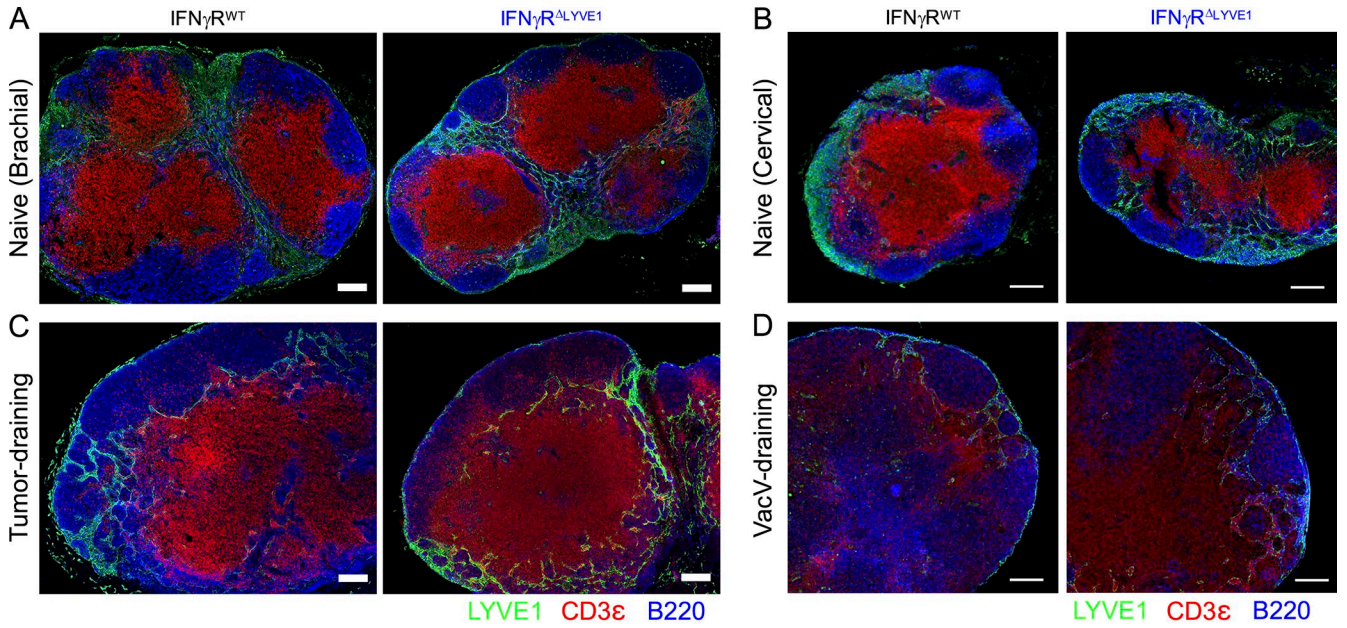

Figure S4. **Loss of IFNγ signaling on LECs does not affect LN lymphangiogenesis. (A and B)** Representative images of brachial (A) and cervical (B) LNs of naive IFNγR$^{\Delta LYVE1}$ mice or littermate controls. **(C)** Representative images of tDLN of IFNγR$^{\Delta LYVE1}$ mice or littermate controls (brachial). **(D)** Representative images of VacV-infected DLNs from IFNγR$^{\Delta LYVE1}$ mice or littermate controls (cervical). Lymphatic vessels (green, LYVE1), B cells (blue, B220), and T cells (red, CD3ε). Scale bars, 100 µm.

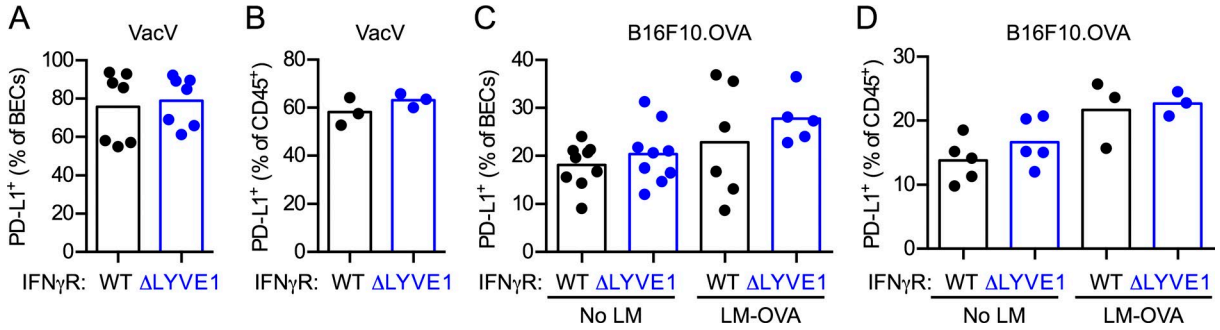

Figure S5. **PD-L1 expression in challenged skin of IFNγR$^{\Delta LYVE1}$ mice. (A and B)** PD-L1 expression by BECs (CD45$^-$CD31$^+$gp38$^-$; A) and CD45$^+$ cells (B) in skin of IFNγR$^{\Delta LYVE1}$ mice or littermate controls, 7 d after VacV infection. **(C and D)** PD-L1 expression by tumor-associated BECs (C) and CD45$^+$ cells (D) in B16F10.OVA tumors of IFNγR$^{\Delta LYVE1}$ mice or littermate controls receiving LM-OVA vaccination on day 4 after implantation or not. Each point represents one mouse, bars indicate mean. Student's *t* test (A and B) or one-way ANOVA corrected for multiple comparisons (C and D).

Table S1. **Baseline characteristics of primary melanoma cohort at inclusion**

Baseline characteristics in cohort at inclusion	
Number of patients	**17**
Age (yr)	
Median (range)	55 (27–98)
Primary site	
Trunk	8 (47.1%)
Upper limb	6 (35.3%)
Lower limb	2 (11.8%)
Other and face	1 (5.9%)
Tumor size (mm)	
Median (range)	1.7 (1.0–6.9)
TNM staging	
1A	1 (5.9%)
1B	8 (47.1%)
2A	3 (17.6%)
2B	3 (17.6%)
2C	2 (11.8%)
Lymphovascular invasion	
Not present	7 (41.2%)
Unknown/indeterminate	10 (58.8%)
Metastasis	
No regional LN	17 (100%)
Distal metastasis	0 (0%)
Ulceration	
Ulcerated	6 (35.3%)
Non-ulcerated	11 (64.7%)
Mitoses	
Present	7 (41.2%)
Absent	3 (17.6%)
N/A	7 (41.2%)
Clark level	
I	0 (0%)
II	0 (0%)
III	2 (11.8%)
IV	2 (11.8%)
Unknown	13 (76.5%)

TNM, classification of malignant tumors.