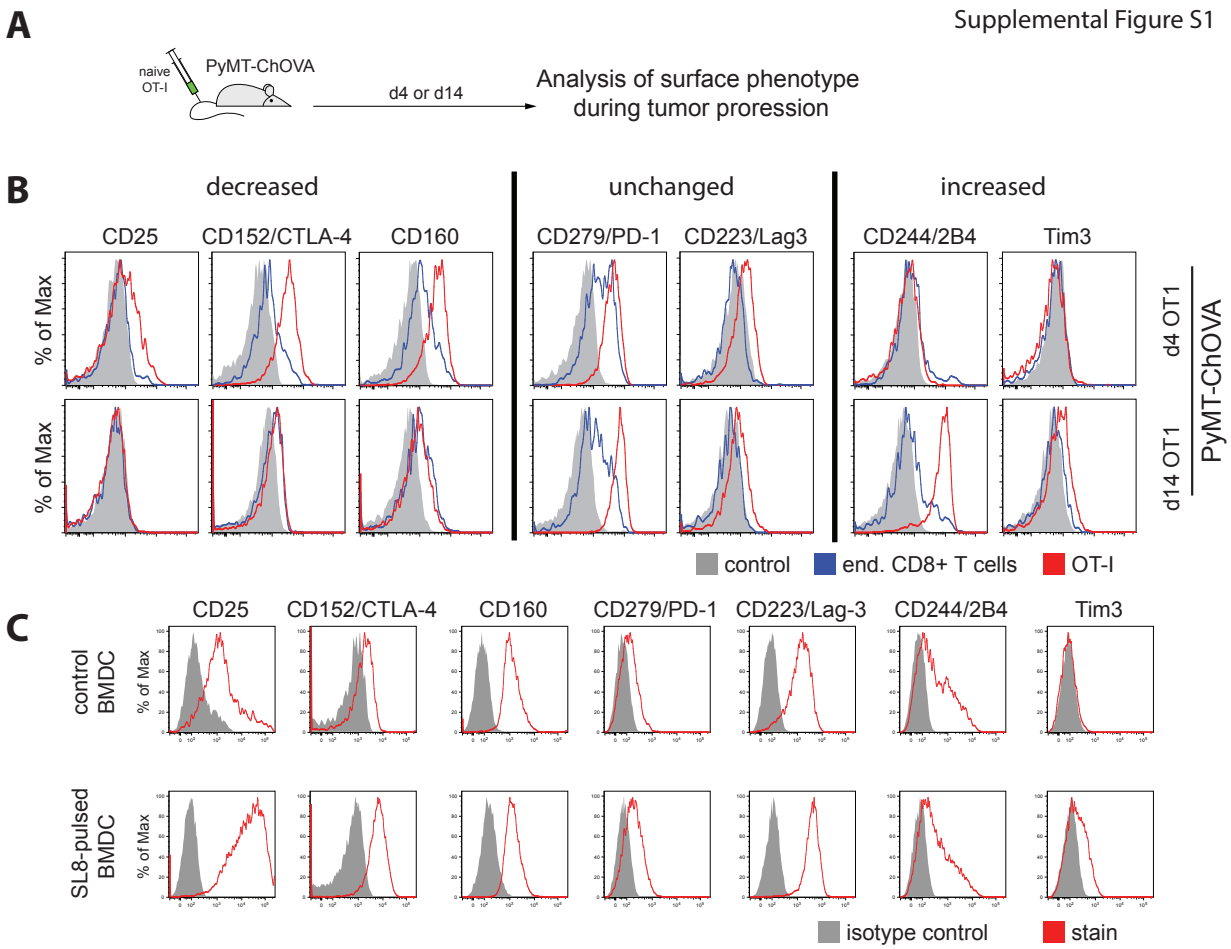
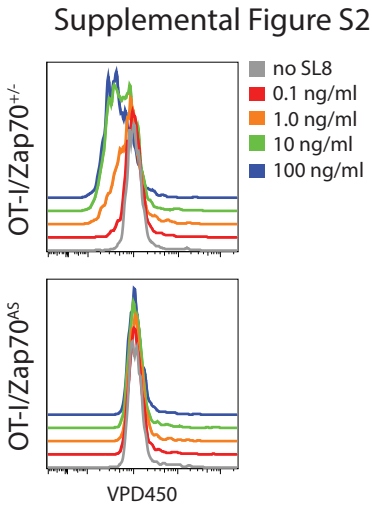


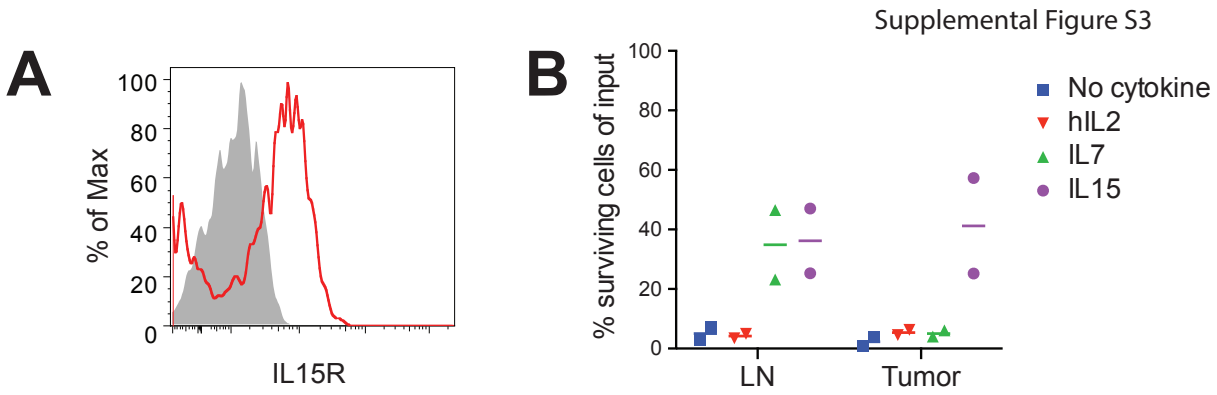
Supplementary Figure S1: Analysis of T cell surface marker expression in non-regressing spontaneous mouse tumors and upon stimulation in vitro.

(A) Schematic of analysis of interrogation of exhaustion marker analysis on high-affinity T cells in dthe PyMT-ChOVA tumor setting. **(B)** Representative examples of the expression of surface markers of endogenous $CD44^{hi}$ $CD8^{+}$ T cells (blue) and high-affinity OT-I T cells (red) as determined by flow cytometry in at least 3 experiments. Markers are grouped according to their change in expression in the PyMT-ChOVA model upon establishment of residence. Filled histograms: matched isotype controls. **(C)** Upregulation of surface markers on in vitro-activated OT-I T cells in response to TCR signaling. OT-I T cells were incubated for 24 hours with SL8-pulsed BMDC or unpulsed control BMDC and analyzed using flow cytometry. Data are representative of 2 experiments.



Supplementary Figure S2: Controlled Inhibition of T cell proliferation by ZAP70AS inhibitor.

In vitro-activated OT-I/ZAP70AS and OT-I/ZAP70$^{+/-}$ T cells were labeled with cell proliferation dye VPD450 and incubated with BDMCs pulsed with increasing concentrations of SL8 peptide in the presence of the HXJ42 inhibitor. Dye dilution as a result of T cell proliferation was assessed by flow cytometry 48 hours later.



Supplementary Figure S3: Intact IL15 signaling in tumor-resident OT-I T cells.

(A) FACS analysis of of IL15R on OT-I T cells isolated from B78-CherryOVA tumors, 14 days after transfer.

(B) Survival of OT-I T cells in vitro, isolated from tumor-draining LN and tumors of two PyMT-ChOVA mice 14 days after T cell transfer. T cells were isolated from LN and tumors and incubated for 24 hours in the presence of 100 U/ml human IL2, 100 ng/ml recombinant mouse IL7 (Peprotech) or 100 ng/ml recombinant mouse IL15 (Peprotech). Data are representative of 2 experiments.

Supplementary Video Legends:

Supplementary Video 1: High-affinity T cells arrest upon arrival in the tumor microenvironment and differ in their behavior from tumor-resident endogenous T cells. Video of the data represented in Figure 1A-D. GFP-labeled OT-I $CD8^+$ T cells (green) were transferred into PyMT-ChOVA/CD2-RFP/CD11c-YFP (red) recipient mice 4 days before imaging. Blood vessels were labeled by injection of Evans Blue (magenta) immediately prior to surgery. Tumors were surgically exposed and imaged by timelapse 2-photon microscopy. Scale bar = 20 μm. Framerate = 10 minutes per second.

Supplementary Video 2: Arrest of T cells upon tumor arrival does not depend on TCR affinity. Video of the data represented in Figure 1E-OT-I. GFP-labeled OT-I $CD8^+$ T cells (green) and RFP-labeled OT-3 CD8+ T cells (red) were transferred into PyMT-ChOVA recipient mice 4 days before imaging. Blood vessels were labeled by injection of Evans Blue (magenta) immediately prior to surgery. Tumors were surgically exposed and imaged by timelapse 2-photon microscopy. Second harmonic signal of collagen (fibers) can also be seen in green. Scale bar = 20 μm. Framerate = 10 minutes per second.

Supplementary Video 3: Tumor-resident high-affinity T cells do not arrest and exhibit similar behavior as tumor-resident endogenous T cells. Video of the data represented in Figure 2A-D. GFP-labeled OT-I $CD8^+$ T cells (green) were transferred into PyMT-ChOVA/CD2-RFP (red) recipient mice 14 days before imaging. Blood vessels were labeled by injection of Evans Blue (magenta) immediately prior to surgery. Tumors were surgically exposed and imaged by timelapse 2-photon microscopy. Scale bar = 20 μm. Framerate = 10 minutes per second.

Supplementary Video 4: High-affinity T cells arrest upon arrival in the tumor microenvironment in the presence of tumor-resident high-affinity T cells. Video of the data represented in Figure 2E-OT-I. GFP-labeled OT-I $CD8^+$ T cells (green) were transferred into PyMT-ChOVA recipient mice 14 days before imaging, while RFP-labeled OT-1 CD8+ T cells (red) were transferred 4 days before imaging. Blood vessels were labeled by injection of Evans Blue (magenta) immediately prior to surgery. Tumors were surgically exposed and imaged by timelapse 2-photon microscopy. Second harmonic signal of collagen can be seen in green. Scale bar = 20 μm. Framerate = 10 minutes per second.

Supplementary Video 5: Division of an endogenous T cell in the tumor microenvironment. Video of the data represented in Figure 4E. GFP-labeled OT-I $CD8^+$ T cells (green) were transferred into PyMT-ChOVA/CD2-RFP/CD11c-YFP recipient mice 4 days before imaging. Blood vessels were labeled by

injection of Evans Blue (magenta) immediately prior to surgery. Tumors were surgically exposed and imaged by timelapse 2-photon microscopy. The dividing endogenous T cell (red) is in the center of the frame. CD11c-expressing antigen-presenting cells are labeled in yellow. Scale bar = 20 μm. Framerate = 10 minutes per second.

Supplementary Video 6: Division of a high-affinity T cell in the tumor microenvironment. Video of the data represented in Figure 4D. GFP-labeled OT-I $CD8^+$ T cells were transferred into PyMT-ChOVA/CD2-RFP (red) recipient mice 14 days before imaging. Tumors were surgically exposed and imaged by timelapse 2-photon microscopy. The dividing high-affinity T cell (green) is in the center of the frame. Scale bar = 10 μm. Framerate = 10 minutes per second.