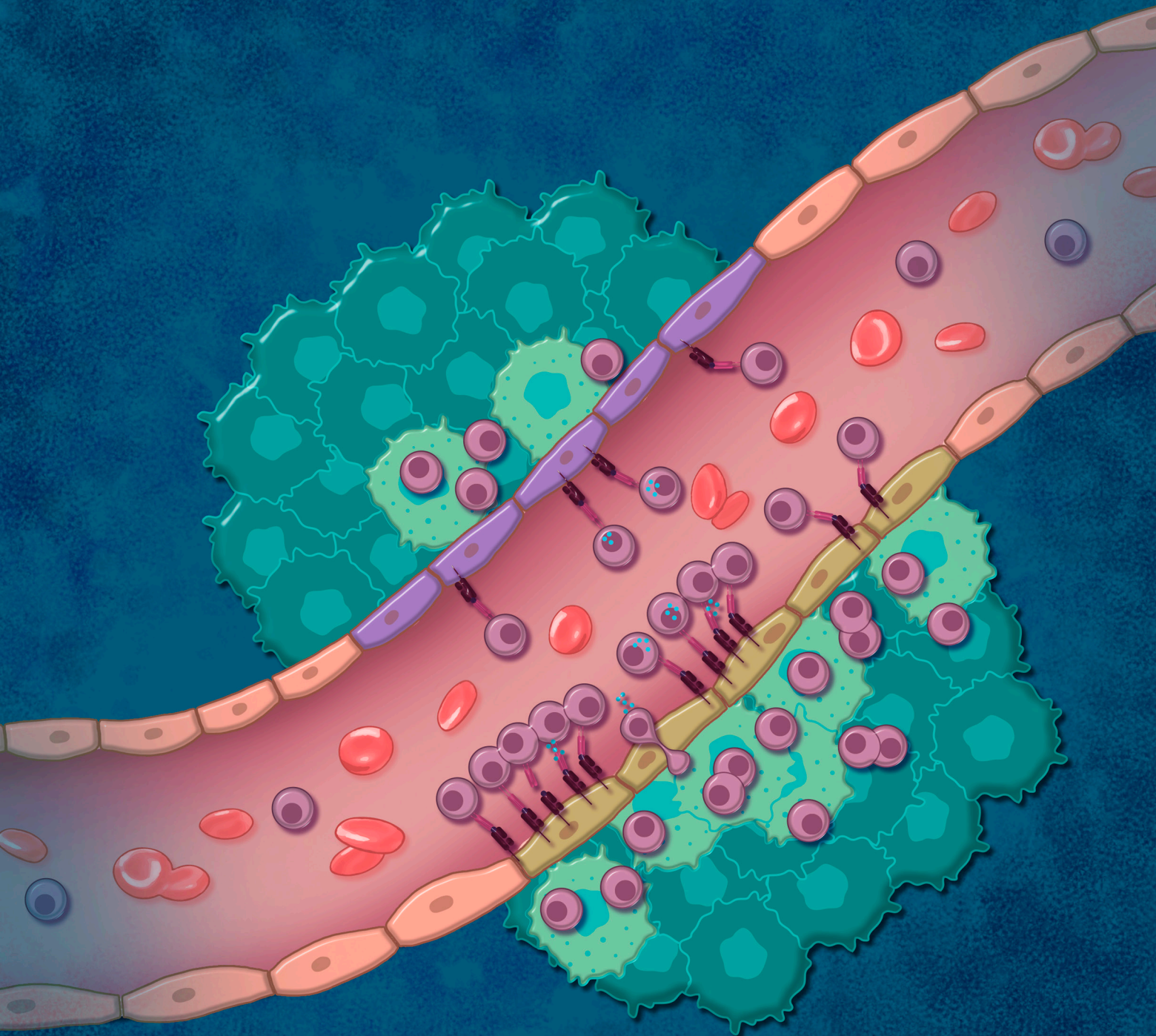


LPL-Positive Endothelial Cells Control T-cell Homing in Liver Metastasis



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ABSTRACT

Combination therapies involving vascular targeting drugs have shown promise in overcoming resistance to immunotherapy. However, the prerequisite for vascular modulation to evoke an effective antitumor T-cell response remains elusive. Tracing the transcriptional response of liver metastasis-associated peritumoral and tumor endothelial cells (TEC) to T-cell intervention, we discovered an immunomodulatory TEC subpopulation that highly expressed lipoprotein lipase (LPL). LPL⁺ TECs facilitated intratumoral homing of activated antitumor CD8⁺ T cells driving liver metastatic regression. Mechanistically, LPL enhanced MHC-I-dependent cross-presentation of tumor antigens on TECs for T-cell trafficking. Consequently, LPL⁺ TECs were recognized and targeted by T cells, further aiding antitumor response. Corresponding analyses of human liver metastasis samples identified a significant correlation between the presence of intratumoral LPL⁺ blood vessels and the accumulation of T cells. Altogether, the study identifies a decisive role of TECs in orchestrating an effective T-cell response by overcoming tumor's intrinsic insufficient antigen presentation.

SIGNIFICANCE: The study identifies LPL⁺ TECs as orchestrators of activated CD8⁺ T-cell homing into immunologically cold tumors with low baseline MHC-I expression. Enhancing tumor antigen exposure by MHC-I cross-presentation in TECs presents a promising approach to compensate the intrinsic inability of tumor cells and boost antitumor immunotherapy.

INTRODUCTION

Being potentially curative, immunotherapy, such as immune checkpoint blockade (ICB), has emerged as the most promising treatment modality for patients with cancer (1). Yet, only a limited number of patients benefit from its curative potential. Evoking an efficient antitumor response to immunotherapy relies not only on the tumor cell-intrinsic immunogenicity but also on the responsiveness of the tumor stroma, including endothelial cells (EC; refs. 2–4). Indeed, preclinical studies have shown that vascular remodeling, e.g., by targeting the VEGF-VEGFR pathway, can potentiate immunotherapy (5–11). Consistent with preclinical studies, the addition of bevacizumab, a VEGF-targeting antibody, substantially improved the outcome of ICB in patients with advanced liver cancer in the IMBrave150 trial (11). A subsequent report

analyzing the 12-month survival of patients receiving combination therapy (atezolizumab + bevacizumab) suggested a significant benefit in overall survival over the standard of care (sorafenib; ref. 12). Notably, only a subset of patients (~30%) showed an objective response to combinational therapy, with only 8% of patients displaying a complete response. Although the IMBrave150 trial demonstrated the impact of vascular modulation on improving the outcome of ICB therapy, it also highlighted the necessity to go beyond the VEGF-VEGFR pathway and better understand the underlying vascular mechanisms that can potentially extend the curative benefit of immunotherapy to a larger fraction of patients.

To systematically investigate the dependency of immunotherapy on vascular ECs, we here undertook spatiotemporal analyses of the liver metastatic vasculature and traced early

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and late vascular responses to T-cell intervention in peritumoral and intratumoral ECs. Elucidating the heterogeneous vascular responsiveness to T-cell intervention, we discovered a unique lipoprotein lipase (LPL)-expressing subpopulation of tumor ECs (TEC) that facilitates the infiltration of antitumor T cells into liver metastatic tumors. Further mechanistic analyses revealed that LPL⁺ TECs could enhance MHC-I-dependent cross-presentation of tumor cell antigens on vascular ECs, thereby promoting T-cell infiltration. Functional experiments with EC-specific genetic gain of function or loss of function of LPL substantiated the decisive role of vascular LPL in T-cell homing into MHC-I-low liver metastatic tumors. Lastly, retrospective analyses of human liver metastatic tumors highlighted a regulatory role of LPL⁺ TECs in driving T-cell infiltration. Beyond yielding fundamental insight into TEC–T cell interactions, the present study identifies and validates LPL as a potential vascular immunomodulatory signal that warrants further translational exploitation.

RESULTS

Mapping the Temporal Immune Response of Liver Metastatic TECs to T-cell Intervention

To investigate the contribution of ECs to T-cell infiltration into tumors, we challenged liver metastases of OVA-B16F10, a model with low-intrinsic MHC-I presentation ability, with adoptive cell transfer (ACT) of activated ovalbumin (OVA)-targeted CD8⁺ effector OT-I T cells and evaluated the EC responsiveness to antitumor T-cell intervention. In this study, PD-L1 and indoleamine 2,3-dioxygenase 1 (IDO) were used as markers of immune responsiveness as they have previously been described to be upregulated in response to antitumor CD8⁺ T cells (13). Although PD-L1 upregulation is widely regulated by multiple cytokines within the tumor microenvironment (14), IDO upregulation is dependent on IFN- γ secreted by effector T cells after peptide-loaded MHC-I (pMHC-I)–T-cell receptor (TCR) interaction at tumor sites and indicates a close interaction between antigen-presenting cells and activated T cells (15–17). Both tumor cells and ECs upregulated PD-L1 after ACT of OT-I T cells [Supplementary Fig. S1A (left and middle)]. In contrast, IDO upregulation was primarily detected in peritumoral areas or intratumoral vascular/perivascular niches [Supplementary Fig. S1A (right)]. Interestingly, a strong correlation between IDO⁺ blood vessels and OT-I T-cell infiltration was observed in the OVA-B16F10 model (Supplementary Fig. S1B), potentially indicating a prominent role of ECs in regulating the antitumor T-cell response.

To comprehensively characterize how ECs respond to and affect homing of T cells and the tumor response to T-cell intervention, we performed imaging-based spatiotemporal tracing of infiltrating OT-I T cells into OVA-B16F10 liver metastases at sequential time points after ACT (Fig. 1A). Infiltrating T cells accumulated in peritumoral and intratumoral areas with gradually increasing numbers over 4 days after ACT. Concurrently, the liver metastatic burden was comparable between liver samples collected 2 days after ACT and control PBS-treated mice, whereas the metastatic burden was reduced in samples collected 4 days after ACT (Supplementary Fig. S2A).

Next, we used single-cell RNA sequencing (scRNA-seq) to systematically investigate the transcriptional response of ECs to incoming antitumor T cells. To capture peritumoral and intratumoral ECs, liver metastatic nodules were macrodissected from the tumor-bearing livers. ECs were subsequently isolated from biological replicates at different stages of antitumor T-cell infiltration and multiplexed into scRNA-seq libraries for transcriptomic profiling. Relying on the infiltration status of injected OT-I T cells and the regression of liver metastatic tumors, we selected three experimental conditions—Ctrl (PBS-injected, termed as Ctrl-ECs), early (2 days after ACT, an early stage at which OT-I T cells just started infiltrating into the tumors, hereafter termed as Early-ECs), and late (4 days after ACT, a late stage at which OT-I T cells massively resided inside the tumors, hereafter termed as Late-ECs) for scRNA-seq library generation (Fig. 1B).

Following quality control, data integration, and demultiplexing of biological replicates, 9,699 ECs were obtained from three libraries. In line with the current EC transcriptomic literature (18–21), ECs were stratified into eight biologically distinct subpopulations, consisting of two subsets of tumor ECs and six subsets of peritumoral liver ECs (Fig. 1C; Supplementary Fig. S2B and S2C). The number of Late-ECs was strongly reduced, and clusters of Late-ECs were overlapping with Ctrl-ECs. In contrast, Early-ECs formed separate clusters, indicating a distinct early-stage vascular response to ACT.

Comparing the fraction of each EC subpopulation across Ctrl-, Early-, and Late-EC libraries, we observed a decrease in tumor EC I and a recovery of the cycling EC subset at the late stage after ACT (Fig. 1D; Supplementary Fig. S3A), suggesting the potential role of tumor EC I in facilitating adoptive T cell–driven tumor regression as observed at the Late-EC time point after ACT. Examining the top variable genes across EC libraries, we observed a strong upregulation of genes related to immune response in Early-ECs, but not in Late-ECs, compared with Ctrl-ECs (Fig. 1E). Accordingly, the tumor EC I subset showed the highest expression of guanylate-binding proteins (*Gbp*), IFN- γ –IFNGR downstream genes, whereas the cycling EC cluster had the lowest expression of *Gbps* (Fig. 1F), potentially hinting to a positive impact of tumor ECs and a negative impact of angiogenic ECs on vascular immune response at the early stage of T-cell intervention.

Divergent Response of Tumor EC Subsets to Antitumor T Cells

To delineate the role of tumor ECs in orchestrating T-cell infiltration and T cell–mediated tumor regression after ACT, we zoomed in on the metastatic vascular niche and focused on the identified TECs. TEC I and II subsets were segregated from all analyzed ECs and reclustered into four TEC populations, with TEC1, TEC2, and TEC3 from Tumor EC I and TEC4 from Tumor EC II (Fig. 2A; Supplementary Fig. S4A). Marker annotation based on the current literature suggested that TEC1 consisted of capillary EC signature, TEC2 closely mimicked the signature of arterial ECs, TEC3 reflected marker genes of sinusoidal ECs, and TEC4 resembled postcapillary venous ECs (Supplementary Fig. S4B).

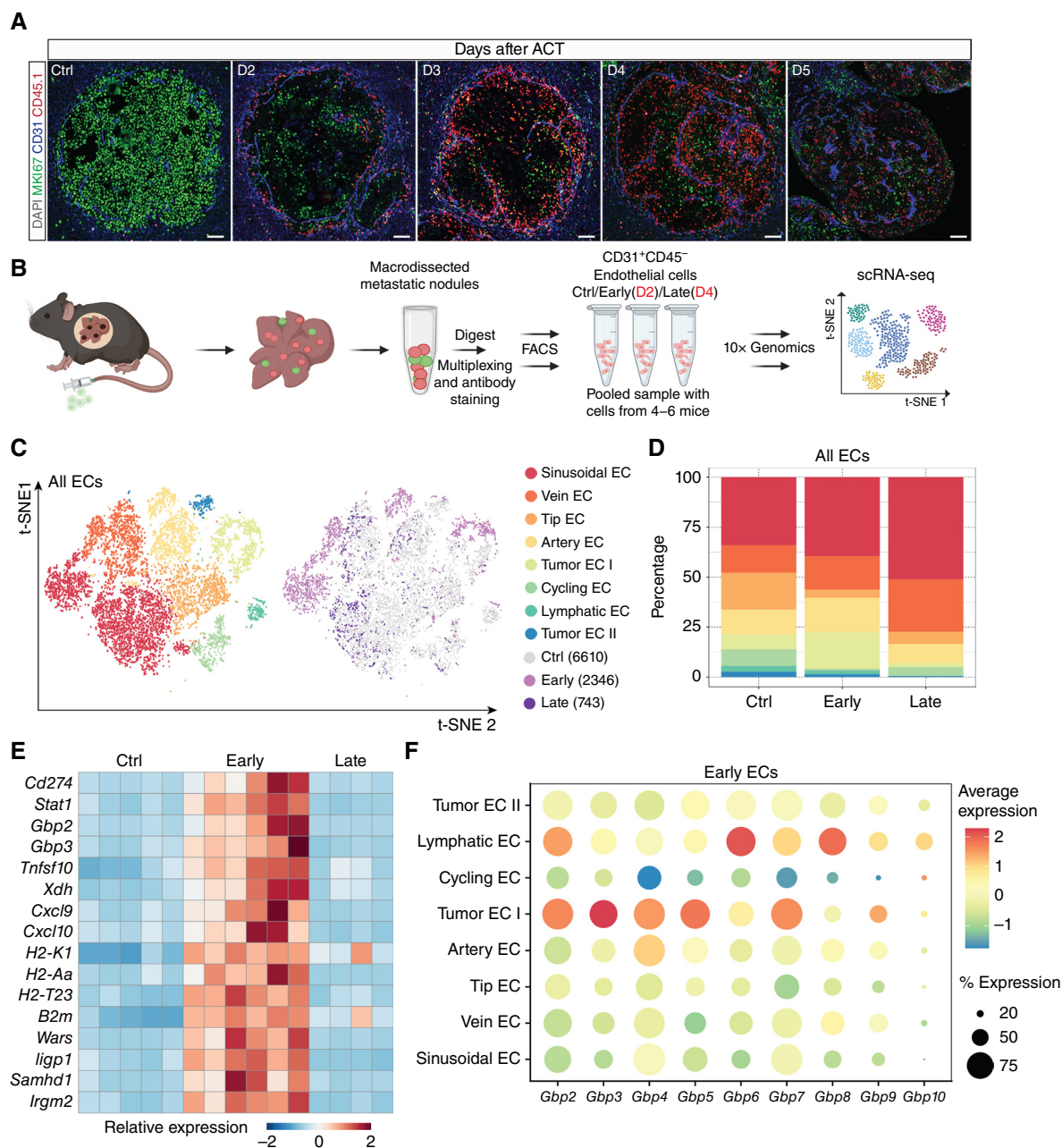


Figure 1. Temporal mapping of the immune response of liver metastatic tumor-associated ECs after ACT. **A**, Representative images of MKI67, CD31, CD45.1, and 4',6-diamidino-2-phenylindole (DAPI) stainings on OVA-B16F10 liver metastatic tumors collected at different time points (Ctrl, 2, 3, 4, and 5 days) after ACT of activated OT-I T cells. Scale bars, 100 μ m. **B**, Experimental strategy of the scRNA-seq experiments profiling tumor-associated ECs from macrodissected liver metastatic tumors. **C**, Left, t-distributed stochastic neighbor embedding (t-SNE) visualization of color-coded clusters of ECs retrieved from macrodissected liver metastatic tumors. Right, t-SNE visualization of color-coded ECs from Ctrl, Early, and Late libraries. ECs (6,610, 2,346, and 743) were collected from each library after quality control, respectively. **D**, Bar plot of the distribution of different EC clusters annotated in **C** across different single-cell libraries (Ctrl, Early, and Late time points). Changes in the cluster composition for each EC subset are shown in Supplementary Fig. S3. **E**, Heatmap showing gene expression of selected immune response genes among ECs from Ctrl, Early, and Late libraries. Each column represents a biological replicate. $N = 4$ –6 mice per library. **F**, Dot plot comparing gene expression of various *Gbp* genes across different EC clusters in Early library.

To characterize the TEC response to ACT, we next performed a comparative analysis with ingenuity pathway analysis (IPA) on differentially expressed genes (DEG) between Ctrl-TECs and Early-TECs. The top upregulated pathways in TECs at the early stage after ACT related to IFN- γ and

IFN- α/β signaling, along with an activation of pathways related to cell death, and mitochondrial dysfunction (Fig. 2B). Concurrently, stimulating ECs with IFN- γ *in vitro* showed a significant reduction in the active mitochondrial mass in ECs as observed with CMXRos staining (Supplementary Fig. S5A).

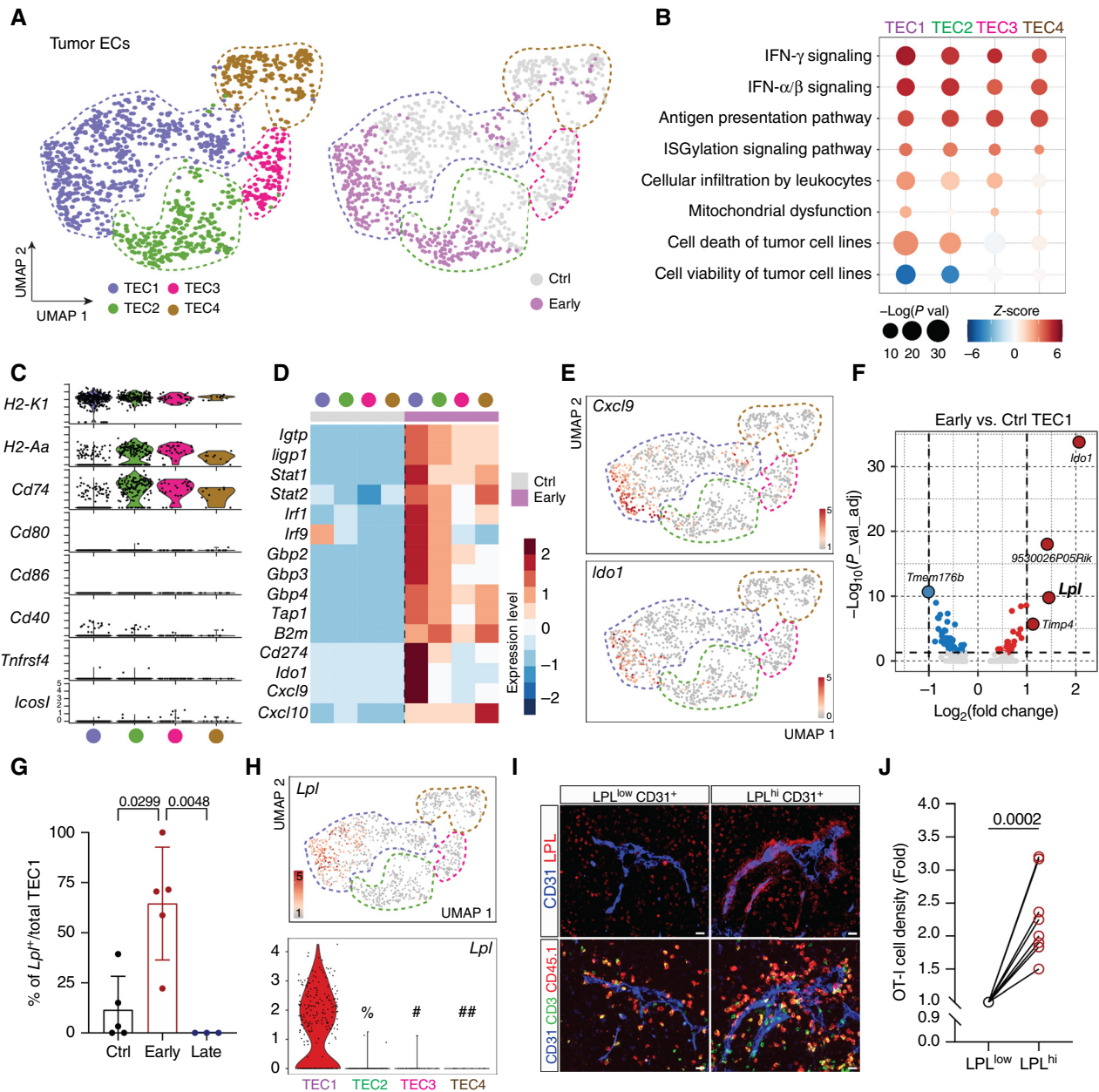


Figure 2. Identification of LPL⁺ TEC subset as a potential immunomodulator. **A**, UMAP visualization of color-coded clusters of TECs from Ctrl and Early libraries. **B**, Ingenuity pathway enrichment analysis showing top regulated pathways in Early-TECs compared with Ctrl-TECs. **C**, Violin plot showing the expression of antigen presentation and co-stimulation genes across different TEC subsets at the Early time point. **D**, Heatmap comparing gene expression of immune-activated genes across different TEC subpopulations. **E**, Feature plots showing *Cxcl9* and *Ido1* expression across different TEC clusters in Ctrl and Early libraries. **F**, Volcano plot of unique DEGs in Early-TEC1 versus Ctrl-TEC1 populations. **G**, Quantitation of *Lpl*⁺ TEC1 among biological replicates in Ctrl, Early, and Late libraries. N = 3–5 mice per library. One replicate from the Early library and one replicate from the Late library were removed due to lack of TEC1 capture in these samples. Data are presented as mean $\pm$ SD. One-way ANOVA test. **H**, Top, feature plot of *Lpl* expression across different TEC subsets. Bottom, violin plot of *Lpl* expression across different TEC subsets. Data are presented as mean $\pm$ SD. One-way ANOVA test. %: TEC1 versus TEC2: $P < 0.0002$; TEC2 versus TEC3 and TEC2 versus TEC4: $P > 0.9999$. #: TEC1 versus TEC3: $P < 0.0013$; TEC3 versus TEC4: $P > 0.9999$. ##: TEC1 versus TEC4: $P < 0.0003$. **I**, Serial sections of OVA-B16F10 liver metastatic tumors after ACT were stained for CD31 and LPL (top) and CD31, CD3, and CD45.1 (bottom). Scale bars, 20 μ m. **J**, Quantitation of perivascular OT-I T cells in the vicinity of LPL^{low} and LPL^{hi} blood vessels within the same liver tissue. N = 7 mice. Data are presented as matched pairs, normalized to LPL^{low} samples. Wilcoxon matched-pairs signed-rank test.

Intriguingly, although we observed no major difference in the expression of known vascular adhesion molecules (Supplementary Fig. S5B), we detected an enrichment of antigen presentation pathway in TECs (Fig. 2B and C). Here, TEC1

presented mainly MHC-I-related genes, whereas other TEC subsets presented both MHC-I- and MHC-II-related genes after ACT. Notably, neither TEC subset expressed costimulatory genes, indicating that TECs lack the ability to activate

naïve T cells as professional antigen-presenting cells, such as dendritic cells. These data suggest that antigen presentation by TECs potentially serves as a signal for T-cell recruitment at the tumor site, particularly for effector T cells.

Among the four TEC subpopulations, Early-TEC1 manifested the strongest response to ACT (Fig. 2D), with the highest expression of IFN- γ and IFN- α/β signaling genes, such as *Cd274*/*PD-L1* and *Cxcl9* [Fig. 2E (top); Supplementary Fig. S5C], which have been reported to be predictors of the outcome of immune checkpoint inhibitors (22). TEC1-derived CXCL9 potentially contributes to the homing of CXCR3⁺ activated OT-I T cells to the metastatic lesions (Supplementary Fig. S5D and S5E). Furthermore, abundant *Ido1* expression was enriched in Early-TEC1 [Fig. 2E (bottom)]. Immunofluorescence (IF)-stained images further confirmed this cellular heterogeneity among TECs, with only a fraction of intratumoral vascular ECs being IDO-positive at the early time point after ACT (Supplementary Fig. S5F). Moreover, we observed an increased accumulation of OT-I T cells in the vicinity of IDO⁺ intratumoral ECs at the early (2 days) time point after ACT (Supplementary Fig. S5G). These results further suggest that a direct interaction between TEC1 and antigen-specific effector T cells is critical for intratumoral recruitment of antitumor T cells.

Identification of *Lpl* as a T Cell-Induced Vascular Immunomodulator

To uncover the underlying molecular mechanism that resulted in the prominent response of TEC1 to ACT, we focused on genes that were specifically altered in the Early-TEC1 cluster by comparing DEGs from different TEC subsets between Early and Ctrl libraries (Fig. 2F; Supplementary Fig. S5H). One of the most antitumor T cell-induced genes in Early-TEC1 was *Lpl*, a gene encoding a metabolic enzyme that catalyzes the hydrolysis of triacylglycerol from circulating chylomicrons and very-low-density lipoproteins (23, 24). We confirmed that the fraction of *Lpl*⁺ TEC1 significantly increased at the early stage of ACT and decreased at the late stage, along with tumor regression (Fig. 2G). Moreover, upregulation of *Lpl* mRNA expression was only observed in the TEC1 population at the early stage after ACT (Fig. 2H). Correspondingly, IF staining confirmed the upregulation of LPL at the early stage and accumulated protein expression at the perivascular niche till the late stage of ACT (Supplementary Fig. S5I).

Previous studies have reported that deletion of LPL in myeloid cells leads to perigonadal white adipose fibrosis and that restoration of *Lpl* rescues the proinflammatory phenotype in *Abcg1*-deficient adipose tissue macrophages, suggesting an immunomodulatory role of LPL (25, 26). Yet, the role of EC-derived LPL in modulating the antitumor immune response remains unknown and has not been studied thus far. To investigate the potential contribution of EC-derived LPL, we stained serial sections of liver metastatic tumors and correlated the number of infiltrating OT-I T cells with LPL staining on blood vessels. Remarkably, a strong accumulation of perivascular OT-I T cells was observed in the vicinity of LPL^{hi} blood vessels compared with LPL^{low} blood vessels after ACT (Fig. 2I and J).

Taken together, these findings revealed the molecular response of metastatic tumor-associated ECs to an antitumor T-cell intervention and identified *Lpl* as a potential immune regulator of tumor ECs to mount an efficient antitumor T-cell response.

TEC-Derived LPL Facilitates T-cell Infiltration and Accumulation into Liver Metastases

To examine the role of EC-derived LPL in controlling T-cell infiltration and T cell-driven tumor regression, we established a transgenic mouse line with an EC-specific deletion of *Lpl* (Supplementary Fig. S6A and S6B). Ablation of *Lpl* expression in ECs neither affected metastatic growth in the liver (Fig. 3A and B) nor the intratumoral blood vessel area (Supplementary Fig. S6C and S6D). Next, we sought to investigate whether the deletion of *Lpl* affected the antitumor efficacy of adoptively transferred activated OT-I T cells. *Lpl*^{IECKO} mice showed a significantly higher liver metastatic burden as observed by macroscopic images of the livers and quantified by increased liver-to-body weight ratio (Fig. 3C and D). Microscopic analysis revealed that the number of tumor-infiltrated OT-I T cells was strongly decreased in liver metastatic tissues of *Lpl*^{IECKO} compared with *Lpl*^{WT} mice (Fig. 3E and F). These data highlight that the EC-specific loss of *Lpl* significantly dampened the homing of T cells and the efficacy of T cell-driven tumor regression.

To further assess the impact of EC-derived LPL on intratumoral T-cell infiltration and subsequent tumor regression, we next generated an EC-specific *Lpl* knock-in (*LSL-Lpl*^{IECKI}) mouse line (Supplementary Fig. S7A and S7B) and validated the overexpression of LPL in liver ECs (Supplementary Fig. S7C and S7D). Notably, the overexpression level of LPL was much higher in TECs than liver ECs (Supplementary Fig. S7C). Consistent with our findings in *Lpl*^{IECKO} mice, EC-LPL overexpression on its own did not affect the metastatic growth in the liver or the intratumoral blood vessel area (Fig. 3G and H; Supplementary Fig. S7E and S7F). In contrast, a reduced tumor burden was observed in livers with LPL overexpressing ECs after ACT of activated OT-I T cells (Fig. 3I and J). Concurrently, higher numbers of tumor-infiltrating OT-I T cells were detected in liver metastases in *LSL-Lpl*^{IECKI} mice (Fig. 3K and L).

To validate the findings from the OVA-B16F10 model in another tumor model, we performed Lewis lung carcinoma (LLC) tumor experiments in endothelial *Lpl* loss-of-function and gain-of-function mice. EC-specific deletion of LPL resulted in reduced OT-I T-cell infiltration in OVA-LLC liver metastases in *Lpl*^{IECKO} mice (Supplementary Fig. S8A and S8B). Conversely, overexpression of *Lpl* in ECs increased the infiltration of OT-I T cells into OVA-LLC liver metastases in *LSL-Lpl*^{IECKI} mice (Supplementary Fig. S8C and S8D). Taken together, these functional experiments reveal a critical role of EC-derived LPL in regulating T-cell infiltration and antitumor T-cell response in liver metastatic tumors.

EC-Derived LPL Does Not Alter the Tumor-Immune Microenvironment

Given that EC-derived LPL affected liver metastasis progression only after T-cell intervention, we next asked whether vascular EC-derived LPL alters the composition of immune

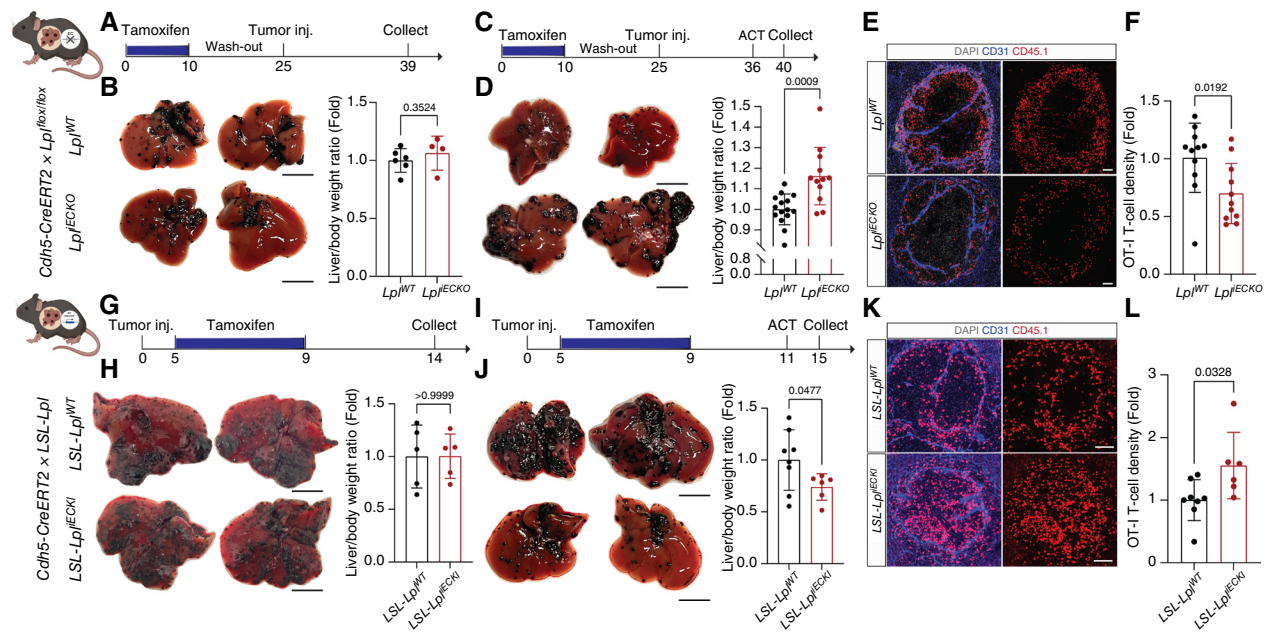


Figure 3. EC-derived LPL facilitates T-cell infiltration and drives liver metastatic regression. **A**, Experimental design. Tamoxifen was administered to induce the genetic deletion of *Lpl* in ECs. After a wash-out period, OVA-B16F10 cells were intrasplenically injected to initiate liver metastases. Livers were collected 14 days after tumor injection. **B**, Left, representative images of livers from *Lpl*^{WT} and *Lpl*^{IECKO} mice. Right, quantitation of the liver/body weight ratio. *N* = 4–6 mice per group. Data are presented as mean ± SD, normalized to *Lpl*^{WT} samples. Two-tailed Mann-Whitney U test. Scale bars, 1 cm. **C**, Experimental design. Tamoxifen was administered to induce genetic deletion of *Lpl* in ECs. After a wash-out period, OVA-B16F10 cells were intrasplenically injected to initiate liver metastases. ACT of OT-I T cells was intravenously performed into mice 11 days after tumor cell injection. Livers were collected 4 days after ACT. **D**, Left, representative images of livers from *Lpl*^{WT} and *Lpl*^{IECKO} mice. Right, quantitation of the liver/body weight ratio. *N* = 12–15 mice per group. Data are presented as mean ± SD, normalized to *Lpl*^{WT} samples. Two-tailed Mann-Whitney U test. Scale bars, 1 cm. **E** and **F**, Representative images (**E**) and quantitation (**F**) of intratumoral OT-I T cells in OVA-B16F10 liver metastatic tumors. *N* = 11 livers per group. Data are presented as mean ± SD, normalized to *Lpl*^{WT} samples. Two-tailed Mann-Whitney U test. Scale bars, 100 μm. For the T-cell analysis, 4 and 1 liver specimens were excluded in *Lpl*^{WT} and *Lpl*^{IECKO} groups, respectively, because of lack of detectable metastatic tumors in the tissue sections. **G**, Experimental design. OVA-B16F10 cells were intrasplenically injected to initiate liver metastases. Mice were treated with tamoxifen 5 days after tumor cell inoculation to overexpress *Lpl* in ECs. Livers were collected 14 days after tumor cell inoculation. **H**, Left, representative images of livers from *LSL-Lpl*^{WT} and *LSL-Lpl*^{IECKO} mice. Right, quantitation of the liver/body weight ratio. *N* = 5 mice per group. Data are presented as mean ± SD, normalized to *LSL-Lpl*^{WT} samples. Two-tailed Mann-Whitney U test. Scale bars, 1 cm. **I**, Experimental design. OVA-B16F10 cells were intrasplenically injected to initiate liver metastases. Mice were treated with tamoxifen 5 days after tumor cell injection to overexpress *Lpl* in ECs. ACT of OT-I T cells was intravenously performed into mice 11 days after tumor cell inoculation. Livers were collected 4 days after ACT. **J**, Left, representative images of livers from *LSL-Lpl*^{WT} and *LSL-Lpl*^{IECKO} mice. Right, quantitation of the liver/body weight ratio. *N* = 6–8 mice per group. Data are presented as mean ± SD, normalized to *LSL-Lpl*^{WT} samples. Two-tailed Mann-Whitney U test. Scale bars, 1 cm. **K** and **L**, Representative images (**K**) and quantitation (**L**) of intratumoral OT-I T cells in OVA-B16F10 liver metastatic tumors. *N* = 6–8 livers per group. Data are presented as mean ± SD, normalized to *LSL-Lpl*^{WT} samples. Two-tailed Mann-Whitney U test. Scale bars, 100 μm.

cells within the tumor microenvironment, which could influence subsequent T-cell homing and accumulation. To this end, we performed scRNA-seq analysis of FACS-sorted CD45⁺ immune cells from macrodissected liver metastatic tumors from *Lpl*^{IECKO}, *LSL-Lpl*^{IECKO}, and corresponding control mice, collected 2 days (Early) after ACT (Supplementary Fig. S9A). Neither LPL depletion nor gain of function in ECs led to major changes in the overall composition of myeloid or lymphoid cells at the early time point after ACT (Supplementary Fig. S9B–S9F).

To assess whether EC-derived LPL may alter the functional status of tumor-associated macrophages, we reclustered the identified monocyte/macrophage cluster into four subclusters, namely M1-like, M2-like, CD36⁺, and monocyte-like macrophages (Supplementary Fig. S10A and S10B). No overt changes in the composition of the four subclusters were observed with either LPL deletion or gain of LPL in ECs (Supplementary Fig. S10C and S10D). We next analyzed the polarization status of macrophages. Comparable expression of M1

(*Cxcl9* and *Nos2*) and M2 (*Arg1* and *Mrc1*) markers negated an obvious functional change in the polarization of macrophages upon endothelial LPL intervention (Supplementary Fig. S10E and S10F). These mRNA-level observations were further validated with FACS staining of an M2 marker (CD206/MRC1; Supplementary Fig. S10G–S10I). Likewise, we did not observe changes in the surface abundance of MHC-II on macrophages (Supplementary Fig. S10J–S10L).

Next, we zoomed in on T cells and reclustered them into four subsets as recently described (27), namely Tex1, Tpx1, Tpx2, and Teff (Supplementary Fig. S11A and S11B). No overt changes in the composition of the four subclusters were observed with either LPL deletion or gain of LPL in ECs (Supplementary Fig. S11C and S11D). Similarly, we observed comparable expression of an exhaustion marker (*Tox*) and proliferation marker (*Mki67*), negating an obvious functional change in T cells after endothelial LPL intervention (Supplementary Fig. S11E and S11F). These mRNA-level observations were further validated by FACS staining of TOX

(Supplementary Fig. S11G–S11I). Additionally, we analyzed T-cell memory markers CD44 and CD62L and observed no major changes in their surface abundance on tumor-associated T cells (Supplementary Fig. S11J–S11L) after endothelial LPL intervention.

Taken together, these data suggest that endothelial LPL does not directly affect the overall immune cell composition or alter their functional status but potentially contributes to the homing of activated T cells into liver metastatic tumors.

Endothelial LPL Enhances Surface MHC-I and Cross-Presentation of Tumor Antigens on TECs

To better understand the EC–T cell interaction, we first tested whether *in vitro*-cultured ECs upregulated *Lpl* when placed in direct contact with activated OT-I T cells. Consistent with the *in vivo* data, cultured ECs upregulated *Lpl* expression following coculture with OT-I T cells (Fig. 4A). The scRNA-seq analysis of Early TECs had suggested an activation of IFN- γ and IFN- α/β signaling (Fig. 2B). We therefore performed experiments aimed at unraveling the upstream stimulators that drive the expression of *Lpl* in ECs. To this end, ECs were treated with different cytokines that are known to be released along with the antitumor T-cell response. Intriguingly, stimulating ECs with IFN- α led to a robust transcriptional upregulation of *Lpl*, whereas neither IFN- γ nor TNF- α altered *Lpl* expression (Fig. 4B). Concurrent with the cellular experiments, mRNA velocity of *Lpl* in our scRNA-seq data showed a similar pattern with the mRNA velocity of *Irf9*, an IFN- α/β downstream gene, but not *Irf1*, an IFN- γ downstream gene (Supplementary Fig. S12A), supporting the finding that IFN- α/β potentially act as upstream regulators of *Lpl* expression in TECs.

To mechanistically dissect how LPL upregulation influences EC function, we performed transcriptomic analyses of cultured ECs overexpressing either *Lpl* or dsRed (Ctrl; Supplementary Fig. S12B and S12C). IPA analysis revealed an enrichment of genes related to the antigen processing and MHC-I-mediated antigen presentation in *Lpl*-overexpressing ECs (Fig. 4C and D). Relying on converging findings from cultured EC RNA-seq and the *in vivo* TEC scRNA-seq analyses (*Lpl*⁺ TEC1 subset preferentially expressed genes related to MHC-I complex), we focused on the effect of LPL on MHC-I antigen presentation by ECs.

Concurrent with transcriptional activation of gene related to MHC-I antigen presentation, an enhanced surface abundance of the H2-K^b/MHC-I complex was observed in *Lpl*-overexpressing compared with control ECs (Fig. 4E; Supplementary Fig. S12D). Consequently, *Lpl*-overexpressing ECs displayed significantly higher levels of OVA-derived SIINFEKL peptide loaded onto the H2-K^b/MHC-I complex (Fig. 4F; Supplementary Fig. S12E). To validate these *in vitro* findings in a relevant *in vivo* setting, we first analyzed the ability of TECs to present tumor cell-derived antigens. To circumvent the technical limitation of isolating pure intratumoral ECs from macrodissected liver metastases, we opted to analyze TECs isolated from subcutaneously grown OVA-B16F10 tumors. Consistent with previous reports (28), flow cytometric analysis of SIINFEKL peptide loaded onto the H2-K^b/MHC-I complex in

tumor ECs revealed the ability of tumor ECs to present tumor cell-derived antigen (Fig. 4G; Supplementary Fig. S12F). A significant increase in the tumor cell-derived antigen presentation by ECs after ACT was observed compared with PBS-treated mice (Fig. 4H), further validating the hypothesis that tumor ECs indeed present tumor cell-derived antigens to incoming T cells. Next, we assessed the effect of EC-derived LPL on MHC-I antigen presentation by tumor ECs. Here, tumor ECs isolated from OVA-B16F10 tumors grown in *LSL-Lpl*^{IECKI} mice displayed a higher surface abundance of total H2-K^b/MHC-I (Fig. 4I and J) and correspondingly presented higher levels of OVA-derived SIINFEKL peptide on the H2-K^b/MHC-I complex on their surface compared with tumor ECs isolated from *LSL-Lpl*^{WT} mice (Fig. 4K and L). Moreover, concurrent to our *in vitro* observations, tumor ECs isolated from *LSL-Lpl*^{IECKI} mice displayed higher expression of various genes involved in antigen processing and MHC-I-mediated presentation (Supplementary Fig. S12G).

Endothelial MHC-I-Mediated Antigen Presentation Facilitates T-cell Homing

Antigen presentation by the endothelial MHC-I complex has been reported by multiple studies including primary tumor ECs (28–33). However, its contribution to the homing of activated CD8⁺ T cells into tumor and subsequent antitumor T-cell response remain unclear. Building on the above mechanistic findings, we evaluated the contribution of TEC antigen presentation in defining the outcome of T-cell therapy for liver metastasis. *In vivo* administration of MHC-I-neutralizing (α -MHC-I) antibody has been reported to inhibit pMHC-I–TCR interaction (34). In line with these findings, we administered α -MHC-I antibody to *LSL-Lpl*^{IECKI} mice bearing liver metastases with the aim to block antigen presentation by TECs to incoming activated T cells (Fig. 5A). As expected, a reduced tumor burden was observed in livers of *LSL-Lpl*^{IECKI} mice following ACT compared with *LSL-Lpl*^{WT} controls (Fig. 5B and C). Confirming our hypothesis, this reduction in tumor burden with endothelial LPL gain of function in *LSL-Lpl*^{IECKI} mice was completely abolished upon administering α -MHC-I antibody. Concurrently, MHC-I blockade reduced the number of tumor-infiltrating OT-I T cells (Fig. 5D and E). Tracing the spatial binding of the administered α -MHC-I antibody across metastasis-bearing liver tissues, we observed preferential antibody binding to intratumoral ECs (Fig. 5F–H). As MHC-I is expressed on peritumoral liver ECs, we also observed α -MHC-I antibody bound to peritumoral liver ECs (Supplementary Fig. S13A–S13D).

To examine the cell-specific impact of endothelial MHC-I presentation on T-cell homing in liver metastatic lesions, we next performed EC-specific deletion of *B2m*, a core component of the MHC-I complex, and EC-specific deletion of *Tap1*, a peptide transporter involved in antigen processing. EC-specific ablation of *B2m* resulted in significantly higher metastatic burden as reflected in a higher liver/body weight ratio in *B2m*^{IECKO} compared with *B2m*^{WT} controls (Fig. 5I and J; Supplementary Fig. S14A). Concurrently, there was reduced infiltration of OT-I T cells in liver metastatic tumors upon endothelial *B2m* deletion (Fig. 5K and L). FACS analyses revealed lower surface abundance of total H2-K^b/MHC-I

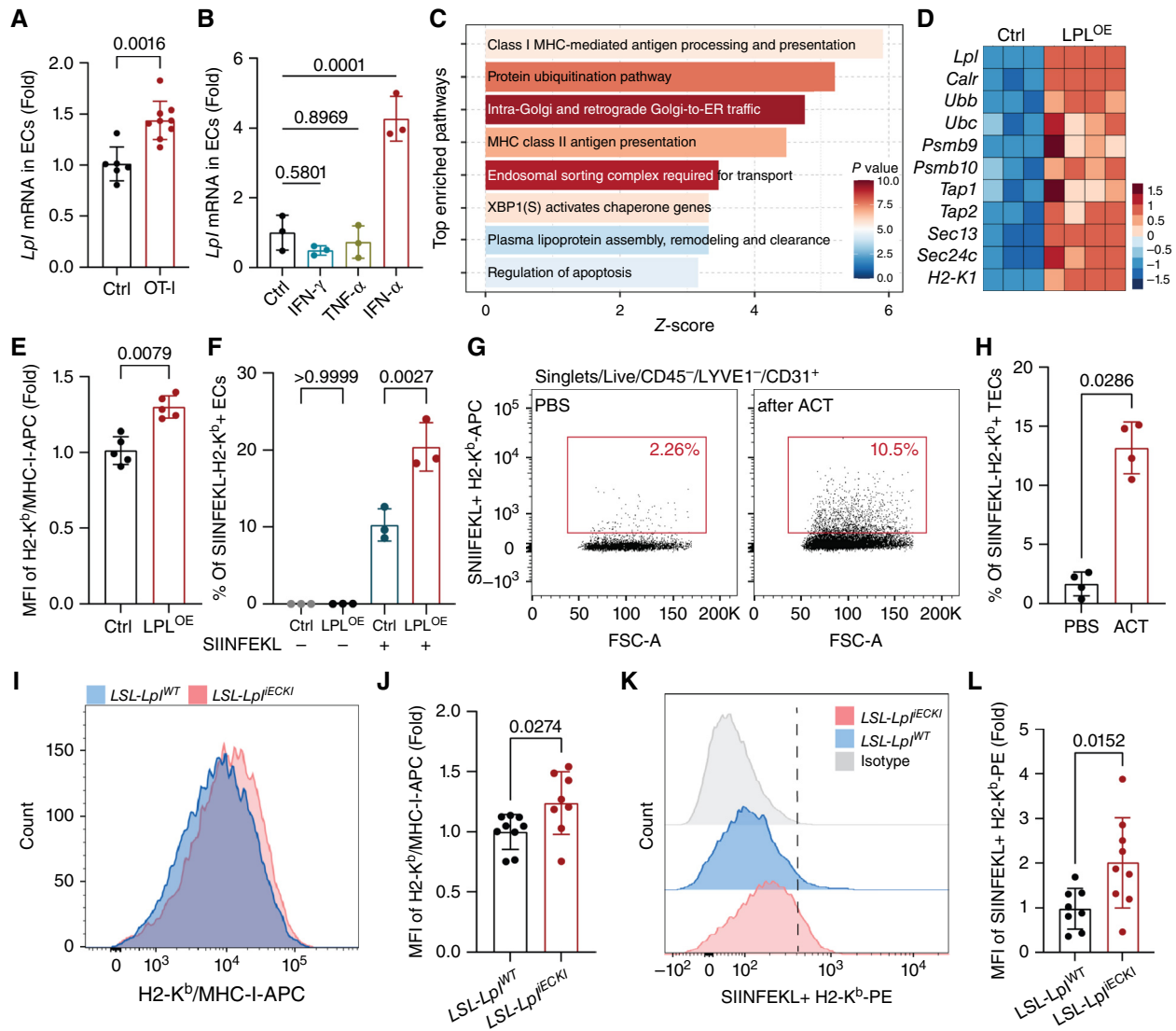


Figure 4. LPL enhances antigen presentation on TECs. **A**, qPCR quantitation of *Lpl* expression in ECs cocultured with either activated OT-I T cells or control PBS. *N* = 6–9 samples per group. Data are presented as mean $\pm$ SD, normalized to Ctrl samples. Two-tailed Mann-Whitney U test. **B**, qPCR quantitation of *Lpl* expression in ECs treated with PBS, 100 ng/mL IFN- γ , 100 ng/mL TNF- α , or 100 ng/mL IFN- α for 6 hours. *N* = 3 samples per group. Data are presented as mean $\pm$ SD, normalized to Ctrl samples. One-way ANOVA test. **C**, IPA analysis comparing enriched pathways between *Lpl*-overexpressing and control ECs. **D**, Heatmap comparing gene expression of antigen processing and MHC-I-mediated presentation between *Lpl*-overexpressing and control ECs. **E**, Cytometric analysis of H2-K^b/MHC-I surface presentation on *Lpl*-overexpressing and control ECs. *N* = 5 samples per group. Data are presented as mean $\pm$ SD, normalized to Ctrl samples. Two-tailed Mann-Whitney U test. **F**, Cytometric analysis of the frequency of SIINFEKL-H2-K^b surface presentation on *Lpl*-overexpressing and control ECs. *N* = 3 samples per group. Data are presented as mean $\pm$ SD. Two-way ANOVA test. **G** and **H**, Cytometric analysis (**G**) and quantitation (**H**) of OVA peptide SIINFEKL-loaded H2-K^b on TECs isolated from primary OVA-B16F10 tumors challenged with activated OT-I T cells or control PBS for 48 hours. *N* = 4 mice per group. Data are presented as mean $\pm$ SD. Two-tailed Mann-Whitney U test. **I** and **J**, Cytometric analysis (**I**) and quantitation (**J**) of H2-K^b/MHC-I presentation on the cell surface of TECs isolated from primary OVA-B16F10 tumors grown in *LSL-Lpl*^{WT} and *LSL-Lpl*^{IECKI} mice. *N* = 8–9 mice per group. Data are presented as mean $\pm$ SD, normalized to *LSL-Lpl*^{WT} samples. Two-tailed Mann-Whitney U test. **K** and **L**, Cytometric analysis (**K**) and quantitation (**L**) of OVA peptide SIINFEKL-loaded H2-K^b/MHC-I presentation on the cell surface of TECs isolated from primary OVA-B16F10 tumors grown in *LSL-Lpl*^{WT} and *LSL-Lpl*^{IECKI} mice. *N* = 8–9 mice per group. Data are presented as mean $\pm$ SD, normalized to *LSL-Lpl*^{WT} samples. Two-tailed Mann-Whitney U test. MFI, mean fluorescence intensity.

and correspondingly lower abundance of OVA-derived SIINFEKL-loaded H2-K^b/MHC-I complex on the surface of ECs isolated from *B2m*^{IECKO} mice (Fig. 5M; Supplementary Fig. S14B–S14D). Consistent with findings in *B2m*^{IECKO} mice, EC-specific deletion of *Tap1* resulted in blunted infiltration of T cells in liver metastatic tumors (Supplementary Fig. S14E–S14G). As anticipated, *Tap1* deletion did not affect the

surface levels of total H2-K^b/MHC-I but reduced the abundance of SIINFEKL-loaded H2-K^b/MHC-I on EC surface (Supplementary Fig. S14H–S14K). These data establish a crucial role of endothelial antigen presentation for T-cell homing.

Overall, these results uncovered a novel vascular mechanism by which LPL-expressing tumor ECs, in an MHC-I-dependent manner, cross-present tumor cell antigen to facilitate homing

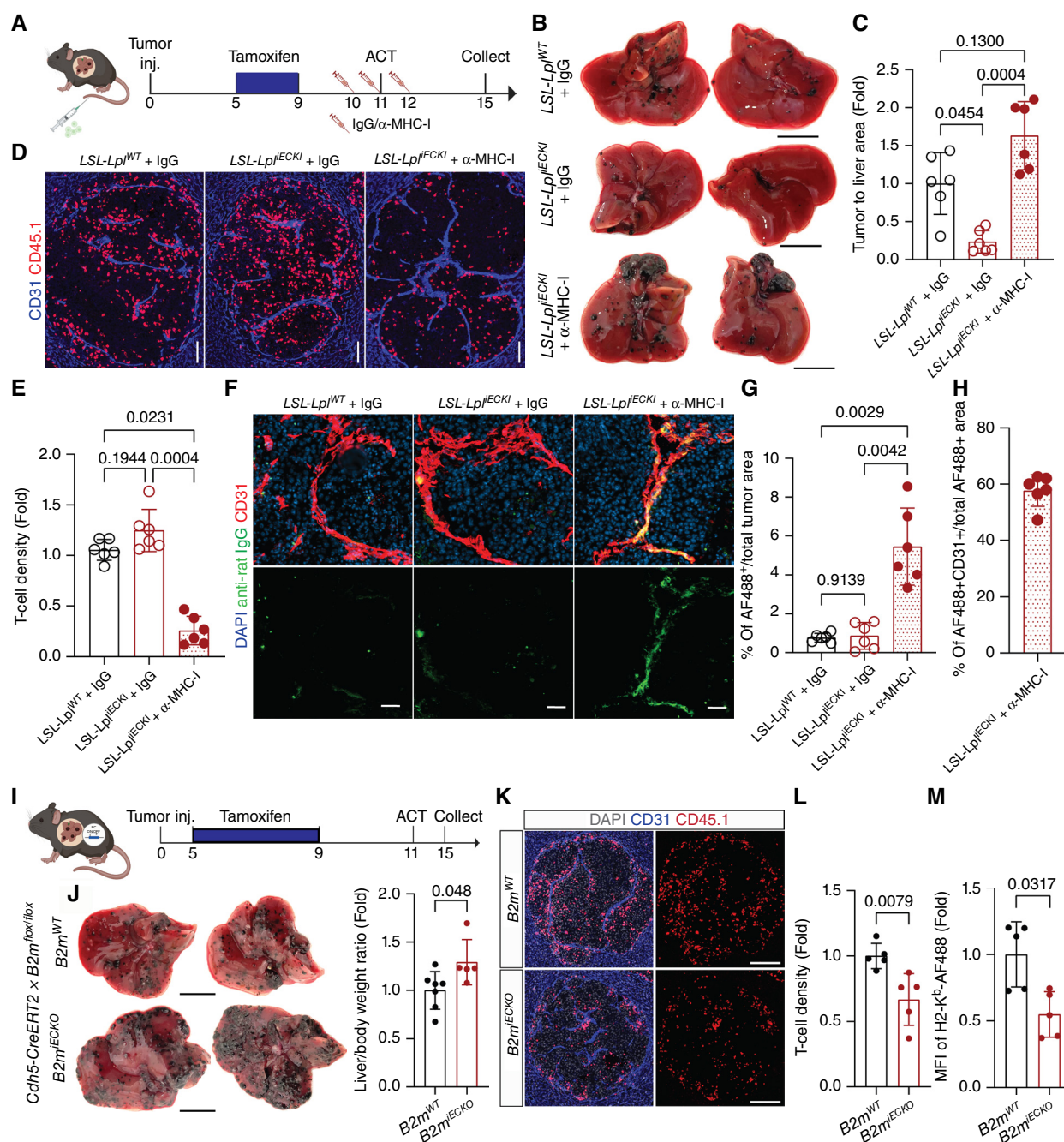


Figure 5. Endothelial antigen presentation facilitates intratumoral homing of activated T cells. **A**, Experimental strategy of the *in vivo* MHC-I blocking experiment. OVA-B16F10 cells were intrasplenically injected to initiate liver metastases. Five days after tumor cell injection, mice were treated with tamoxifen to induce *Lpl* overexpression in ECs. IgG and α-MHC-I antibodies were administered continuously 3 days as indicated. ACT of OT-I T cells was intravenously performed into mice 11 days after tumor cell injection. Livers were collected 4 days after ACT. **B** and **C**, Representative images of livers (**B**) and corresponding quantitation of tumor to liver area ratio (**C**) from LSL-Lp^l^{WT} + IgG, LSL-Lp^l^{IECKI} + IgG and LSL-Lp^l^{IECKI} + α-MHC-I mice. Scale bars, 1 cm. *N* = 6 mice per group. Data are presented as mean ± SD, normalized to LSL-Lp^l^{WT} + IgG samples. One-way ANOVA test. **D** and **E**, Representative images (**D**) and quantitation (**E**) of intratumoral OT-I T cells in OVA-B16F10 liver metastatic tumors. Scale bars, 100 μm. *N* = 6 livers per group. Data are presented as mean ± SD, normalized to LSL-Lp^l^{WT} + IgG samples. One-way ANOVA test. **F** and **G**, Representative images (**F**) of CD31 and anti-rat IgG (staining MHC-I-neutralizing antibody) in intratumoral area and quantitation (**G**) of percentage of the α-MHC-I-bound (AF488+) area of the total tumor area in intratumoral regions of liver tissues. Scale bars, 50 μm. *N* = 6 livers per group. Data are presented as mean ± SD. One-way ANOVA test. **H**, Quantitation of the α-MHC-I-bound CD31+ vascular area (AF488+ CD31+) to the total AF488+ area in intratumoral regions of liver tissues. *N* = 6 livers per group. Data are presented as mean ± SD. The analysis was only performed in the LSL-Lp^l^{IECKI} + α-MHC-I group and subjected to no statistical analysis. **I**, Experimental design. OVA-B16F10 cells were intrasplenically injected to initiate liver metastases. Mice were treated with tamoxifen 5 days after tumor cell inoculation to delete *B2m* in ECs. ACT of OT-I T cells was intravenously performed into mice 11 days after tumor cell injection. Livers were collected 4 days after ACT. **J**, Left, representative images of livers from B2m^{WT} and B2m^{IECKO} mice. Right, quantitation of the liver/body weight ratio. *N* = 5–7 mice per group. Data are presented as mean ± SD, normalized to B2m^{WT} samples. Two-tailed Mann-Whitney U test. (continued on following page)

of activated T cells. These findings are particularly noteworthy as vascular ECs are the first cell type that incoming T cells encounter when infiltrating tumors.

Endothelial LPL Promotes T-cell Extravasation and Cytotoxicity

Next, we performed assays to further investigate EC–T cell interaction and how endothelial LPL affects T-cell activity. As EC–T cell interactions occur during T-cell homing and extravasation (35), we examined whether *Lpl* overexpression in ECs would affect the process of T-cell extravasation. Pursuing Transwell transendothelial migration assays (Fig. 6A), transmigration of activated OT-I T cells was assessed after transient pulsing of the EC monolayer with or without the OVA peptide. No difference in the number of transendothelial-migrated OT-I T cells was detected in experiments without preconditioning of ECs with OVA peptide (Fig. 6B). In contrast, significantly higher numbers of transendothelial migrated T cells to the lower chamber were recorded in Transwells with SIINFEKL-primed *Lpl*-overexpressing ECs compared with indexed Transwells with control ECs (Fig. 6C and D), indicating that tumor antigen loading onto ECs plays a critical role in facilitating the infiltration of activated tumor-targeted T cells.

Surprisingly, ECs were found dead after coculture with T cells in Transwells prepulsed with the OVA peptide (Fig. 6E). Moreover, in line with higher SIINFEKL peptide presentation, *Lpl*-overexpressing ECs showed higher cell death than control ECs. Given the fact that IFN- γ -driven cell death is one of the mechanisms by which T cells eliminate target cells and the observed strong activation of ECs by T cell–derived IFN- γ , we performed additional cell death assays in which *Lpl* overexpression was found to enhance EC apoptosis after stimulation with recombinant IFN- γ (Fig. 6F and G). Concurrently, a positive cleaved caspase-3 (cCASP3) signal was also detected in CD31⁺ intratumoral blood vessels in liver metastatic tumors after ACT (Supplementary Fig. S15A).

We next performed multiplex staining for LPL, cCASP3, OT-I T cells, and ECs to explore the relationship between EC death and LPL expression. Here, we observed LPL⁺ cCASP3⁺ tumor vessels across both Early (2 days after ACT) and Late (4 days after ACT) liver metastatic tumors but rarely in Ctrl samples (Supplementary Fig. S15B). Focusing on the early EC–T cell interaction, we then assessed the multiplexed IF images. As shown in Fig. 6H, we observed an OT-I T cell localized in the middle of cCASP3⁺ LPL⁺ vascular area at the Early time point after ACT, suggesting a role of LPL⁺ tumor ECs in regulating T-cell homing. Further analysis of Ctrl and Early (2 days after ACT) liver metastatic tissues revealed that nearly 70% of all cCASP3⁺ tumor vessels after ACT were concurrently positive for LPL, suggesting that LPL⁺ tumor ECs were preferentially targeted by T cells (Fig. 6I). Moreover,

liver metastatic tumors with endothelial *Lpl* deletion displayed significantly reduced abundance of cCASP3⁺ intratumoral vessels (Fig. 6J and K). Likewise, EC-specific deletion of *B2m* resulted in a reduced fraction of cCASP3⁺ intratumoral vessels (Fig. 6L and M). These data together suggest that antigen-presenting LPL-expressing ECs were preferentially targeted by activated T cells. Moreover, the *in vitro* Transwell data combined with enhanced intratumoral accumulation of T cells with LPL gain of function in *LSL-Lpl^{flECK1}* mice support a crucial role of endothelial LPL in facilitating transendothelial migration of T cells in liver metastatic lesions while themselves being targeted by T cells.

Next, we evaluated the effect of EC-derived LPL on T-cell cytotoxicity. At baseline, i.e., without priming the EC monolayer with SIINFEKL peptide, we barely detected IFN- γ + OT-I T cells and observed no effect of EC-derived LPL on granzyme B expression (Fig. 6N and O). In contrast, assays with prior pulsing of ECs with SIINFEKL peptide, a significantly higher fraction of IFN- γ + OT-I T cells and higher levels of granzyme B were detected in T cells after coculture with LPL-overexpressing ECs compared with control ECs. To further substantiate the OVA-OT-I T cell findings, we used a clinically relevant premelanosomal protein gp100 (also termed Pmel17)-Pmel effector CD8⁺ T-cell system. Concurrently, a significantly higher fraction of IFN- γ + Pmel T cells and higher levels of granzyme B were detected in T cells after coculture with LPL-overexpressing ECs compared with control ECs after priming with gp100 peptide (Fig. 6P and Q).

Taken together, the *in vivo* and the *in vitro* experiments showed that LPL-expressing tumor ECs upregulated MHC-I surface abundance, leading to enhanced presentation of tumor cell–derived antigens (Fig. 6R). In turn, activated T cells engaged with antigen-presenting TECs, extravasated, and accumulated in perivascular LPL⁺ niches within liver metastatic tumors.

Clinical Relevance of LPL⁺ ECs for Immunotherapy

To ensure that the *in vivo* T-cell homing phenotype in LPL^{hi} perivascular tumor niches is not OVA (ectopic) antigen-restricted, we challenged mice bearing B16F10 melanoma metastasis with activated Pmel T cells (targeting endogenous gp100 antigen). Concurrent with the findings in the OVA-OT-I T-cell model, significantly higher numbers of activated Pmel T cells were found in LPL^{hi} compared with LPL^{low} perivascular niches (Fig. 7A and B). Consistently, we observed higher IDO staining on LPL^{hi} TECs. Additionally, we used the Hcmel12 melanoma tumor model which similarly harbors endogenous gp100 expression. In line with the B16F10 model, LPL⁺ blood vessels were detected at Pmel T cell-infiltrated regions in liver metastatic Hcmel12 tumors after ACT of activated CD8⁺ Pmel T cells (Supplementary Fig. S16A). Next, we examined whether endothelial LPL could

Figure 5. (Continued) Scale bars, 1 cm. **K** and **L**, Representative images (**K**) and quantitation (**L**) of intratumoral OT-I T cells in OVA-B16F10 liver metastatic tumors. *N* = 5 livers per group. Two samples were excluded *B2m^{WT}* group because of lack of detectable metastatic tumors in the tissue sections. Data are presented as mean $\pm$ SD, normalized to *B2m^{WT}* samples. Two-tailed Mann-Whitney U test. Scale bars, 200 μ m. **M**, Quantitation of H2-K^b/MHC-I presentation on the cell surface of ECs isolated from the corresponding metastatic livers. *N* = 5 mice per group. Data are presented as mean $\pm$ SD, normalized to *B2m^{WT}* samples. Two-tailed Mann-Whitney U test.

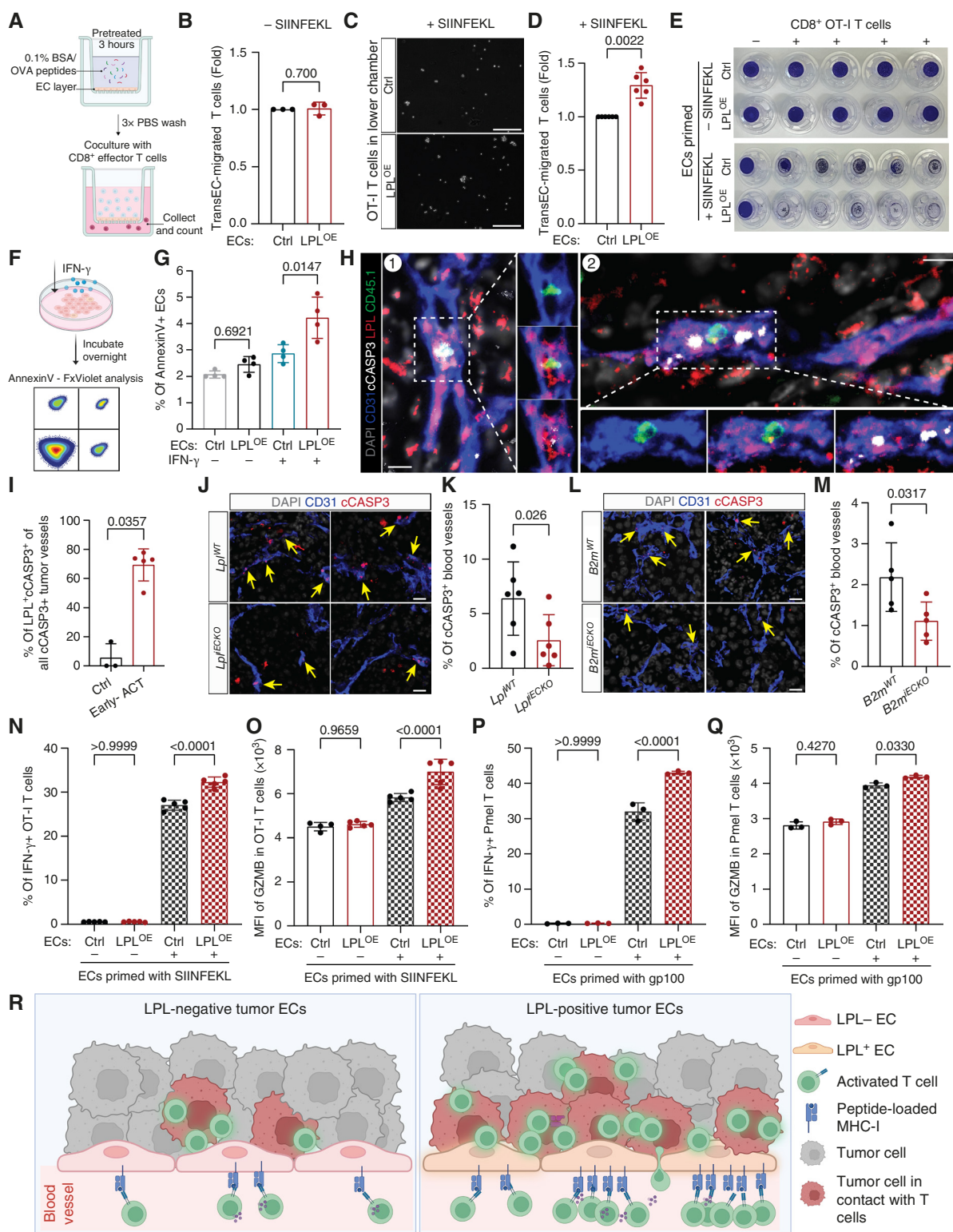


Figure 6. LPL promotes transendothelial migration and cytotoxicity of T cells. **A**, Experimental design of T-cell transendothelial migration assay. EC monolayers were prepulsed with or without OVA-SIINFEKL peptides prior to adding OT-I T cells. **B**, Quantitation of transendothelial migrated OT-I T cells. ECs were not prepulsed with OVA-SIINFEKL peptides. $N = 3$ independent experiments. Data are presented as mean $\pm$ SD, normalized to Ctrl samples. Two-tailed Mann-Whitney U test. **C** and **D**, Representative images (C) and corresponding quantitation (D) of transendothelial migrated OT-I T cells collected at the lower chamber and reseeded in a counting chip for imaging. Scale bars, 100 μ m. $N = 6$ independent experiments. Data are presented as mean $\pm$ SD, normalized to Ctrl samples. Two-tailed Mann-Whitney U test. **E**, Representative images of ECs stained with crystal violet on the upper membrane on Transwell inserts. Top, EC chambers without prepulsing with OVA-SIINFEKL peptides. (continued on following page)

enhance the homing of activated Pmel T cells (Supplementary Fig. S16B). Indeed, overexpression of *Lpl* in ECs increased the infiltration of Pmel T cells into B16F10 liver metastases in *LSL-Lpl^{IECKI}* compared with *LSL-Lpl^{WT}* mice (Fig. 7C and D).

Building on T-cell intervention experiments, we next asked whether combination therapy targeting VEGFR2 and PD-L1 would similarly affect the expression of *Lpl* in tumor ECs. Intriguingly, tumors treated with combination therapy (Supplementary Fig. S16C and S16D) displayed a higher abundance of LPL⁺ blood vessels 3 days after therapeutic intervention (Fig. 7E and F). Notably, higher *H2-k1* (gene coding for MHC-I) expression was also observed in TECs isolated from tumors treated with a combination of anti-VEGFR2 and anti-PD-L1 therapy compared with Ctrl-IgG-treated tumors (Fig. 7G).

Lastly, we examined the presence of LPL⁺ blood vessels in human liver metastatic tumors and analyzed whether T cells are located in the vicinity of LPL⁺ tumor vessels. Here, we analyzed the coexistence of LPL⁺CD31⁺ blood vessels and CD3⁺ T cells in surgically resected tumor sections from a cohort of patients with liver metastases ($n_{\text{total}} = 46$) derived from colorectal cancer ($n = 26$), melanoma ($n = 10$), and pancreatic cancer ($n = 10$). We detected LPL⁺CD31⁺ blood vessels in 8 of 26 colorectal cancer–derived, 1 of 10 pancreatic cancer–, and 3 of 10 melanoma–derived liver metastatic tumors. Interestingly, LPL⁺ CD31⁺ blood vessels were primarily located at the invasive front of the tumors and, to a lesser extent, in intratumoral regions of analyzed tumor sections (Supplementary Fig. S17A and S17B). Although we did not see any major differences in the vascular density in regions containing LPL^{hi} and LPL^{low} vessels (Supplementary Fig. S17C and S17D), a significant accumulation of CD3⁺ T cells was detected in the perivascular niche of LPL^{hi}CD31⁺ blood vessels compared with LPL^{low}CD31⁺ blood vessels in colorectal cancer–derived liver metastasis samples (Fig. 7H and I). The analysis of melanoma–derived and pancreatic cancer–derived liver metastases revealed a similar trend, but the limited number of cases precluded a statistical analysis (Fig. 7J and K).

Collectively, the data obtained in different tumor entities metastasizing to the liver provide a compelling proof of concept that LPL⁺ blood vessels are present in human liver metastases, potentially playing a crucial role in mounting the T cell–driven antitumor response.

DISCUSSION

Inefficient recruitment of antitumor T cells to the tumor microenvironment dampens the efficacy of immunotherapies in immunologically “cold” tumors (36). To overcome this critical limitation, vascular remodeling has been proposed to promote an antitumor immune response. Preclinical studies have so far focused on either normalizing tumor vessels to enhance perfusion, upregulate cytokines (e.g., CXCL10) or adhesive molecules (e.g., VCAM1), and differentiate tumor-associated vessels into high-endothelial venules to serve as gateways for leukocytes to emigrate into primary tumors (5, 8, 10). Although those studies offered a comprehensive understanding on how vascular targeted intervention contributes to improved recruitment for endogenous immune cells, including T cells and dendritic cells, it is still puzzling why only a fraction of patients benefit from immunotherapy with additional vascular-targeted intervention. For example, the IMpower130 clinical trial showed that the addition of bevacizumab to ICB only prolonged the overall survival in a subgroup of patients with lung cancer, who were additionally bearing liver metastasis (37).

Sufficient antigen presentation via MHC-I complex is a prerequisite for antitumor T-cell immunity, and previous reports have shown that MHC-I downregulation is observed during tumor progression (38, 39). Moreover, a retrospective molecular correlation analysis of patients from the GO30140a and IMbrave150 clinical trials suggested that tumor specimens from responders showed relatively higher expression of MHC class I on the surface of tumor cells than nonresponders, further indicating the importance of antigen presentation via MHC-I (40). With the above information and to explain why only a fraction of patients benefit from combination therapy, we hypothesized that early interactions between tumor ECs and antitumor T cells critically affect the efficacy of immunotherapy. Employing the OVA-B16F10 liver metastasis model with low baseline MHC-I surface expression on tumor cells, we uncovered a vital role of the vascular immune response in decisively controlling the T-cell infiltration after T-cell intervention. These findings suggest that vascular ECs potentially compensate for the intrinsic inability of MHC-I antigen presentation on tumor cells to evoke an efficient immune response.

Figure 6. (Continued) Bottom, EC chambers with prepulsing with OVA-SIINFEKL peptides. **F**, Experimental design for the Annexin-V apoptosis analysis. **G**, FxCycle violet/Annexin-V cytometric analysis of ECs treated with PBS or 100 ng/mL IFN- γ overnight. $N = 4$ samples per group. Data are presented as mean $\pm$ SD. Two-way ANOVA test. **H**, Representative stained images of CD45.1 (OT-I T cells), CD31, cCASP3, and LPL in OVA-B16F10 liver metastatic tumors collected at the Early time point after ACT. Scale bars, 10 μ m. **I**, Quantitation of percentage of LPL⁺ and cCASP3⁺ double-positive blood vessels among total cCASP3⁺ blood vessels in OVA-B16F10 liver metastatic tumors collected at Ctrl and Early time points after ACT. $N = 3$ –5 livers per group. Data are presented as mean $\pm$ SD. Two-tailed Mann-Whitney U test. **J** and **K**, Representative images (**J**) and quantitation (**K**) of percentage of cCASP3⁺ blood vessels in OVA-B16F10 liver metastatic tumors in *Lpl^{WT}* and *Lpl^{IECKO}* mice, collected 3 days after ACT. Yellow arrowheads mark cCASP3⁺ vessels. $N = 6$ livers per group. Data are presented as mean $\pm$ SD, normalized to *Lpl^{WT}* samples. Two-tailed Mann-Whitney U test. Scale bars, 20 μ m. **L** and **M**, Representative images (**L**) and quantitation (**M**) of percentage of cCASP3⁺ blood vessels in OVA-B16F10 liver metastatic tumors in *B2m^{WT}* and *B2m^{IECKO}* mice, collected 4 days after ACT. Yellow arrowheads mark cCASP3⁺ vessels. $N = 5$ livers per group. Data are presented as mean $\pm$ SD, normalized to *B2m^{WT}* samples. Two-tailed Mann-Whitney U test. Scale bars, 20 μ m. **N**, Quantitation of IFN- γ -positive effector OT-I T cells after cocultured with *Lpl*-overexpressing or control ECs, with or without priming with SIINFEKL peptide. $N = 5$ samples per group. Data are presented as mean $\pm$ SD. Two-way ANOVA test. **O**, Quantitation of granzyme B protein level in effector OT-I T cells after cocultured with *Lpl*-overexpressing or control ECs, with or without priming with SIINFEKL peptide. $N = 4$ –5 samples per group. Data are presented as mean $\pm$ SD. Two-way ANOVA test. **P**, Quantitation of IFN- γ -positive effector Pmel T cells after cocultured with *Lpl*-overexpressing or control ECs, with or without priming with gp100 peptide. $N = 3$ samples per group. Data are presented as mean $\pm$ SD. Two-way ANOVA test. **Q**, Quantitation of granzyme B protein level in effector Pmel T cells after cocultured with *Lpl*-overexpressing or control ECs, with or without priming with gp100 peptide. $N = 3$ samples per group. Data are presented as mean $\pm$ SD. Two-way ANOVA test. **R**, Graphical depiction of EC-derived LPL, in an MHC-I-dependent manner, facilitating homing and accumulation of activated T cells in liver metastatic tumors. **[R]**, Created in BioRender. Pari, A. (2026) <https://BioRender.com/wwwi5hc>.

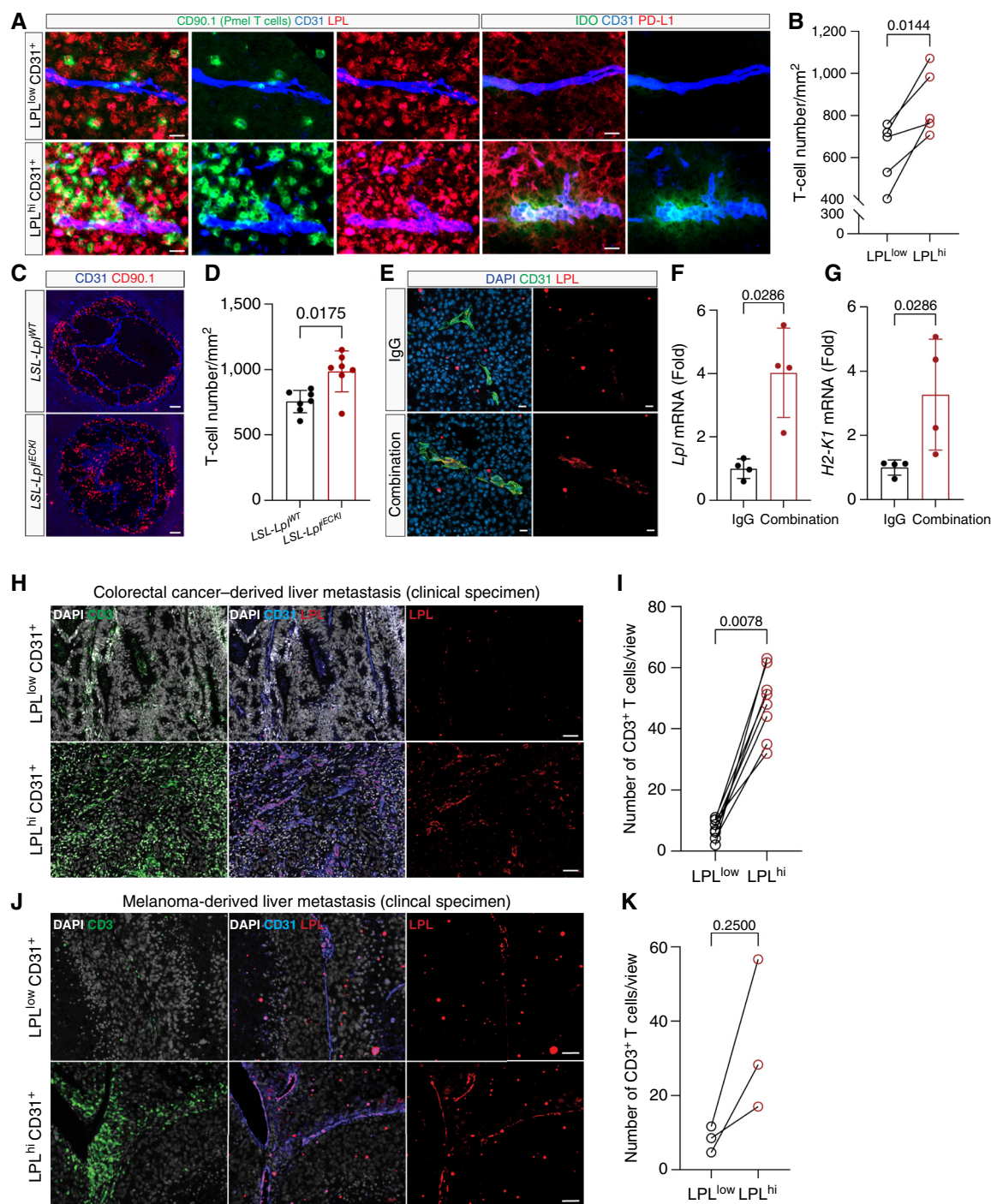


Figure 7. Relevance of LPL⁺ ECs in preclinical and clinical settings. **A** and **B**, Representative images (**A**) and quantification (**B**) of Pmel T-cell density in the vicinity of LPL^{low} and LPL^{hi} tumor vessels in B16F10 liver metastatic tumors after ACT of activated Pmel T cells. Scale bars, 20 μ m. $N = 5$ mice per group. Data are presented as matched pairs. Paired t test. **C** and **D**, Representative images (**C**) and quantification (**D**) of intratumoral Pmel T-cell density in B16F10 liver metastatic tumors. $N = 7$ livers per group. Data are presented as mean $\pm$ SD, normalized to LSL-Lpl^{WT} samples. Two-tailed Mann-Whitney U test. Scale bars, 100 μ m. **E**, Representative images of LPL staining in OVA-B16F10 tumors, treated with either combination therapy or control IgG. Scale bars, 20 μ m. **F** and **G**, qPCR-based quantification of *Lpl* (**F**) and *H2-K1* (**G**) expression in TECs isolated from OVA-B16F10 tumors treated with either combination therapy or control IgG. $N = 4$ mice per group. Data are presented as mean $\pm$ SD, normalized to IgG samples. Two-tailed Mann-Whitney U test. **H**, Representative stained images of CD3, CD31, LPL, and DAPI on human colorectal cancer-derived liver metastatic tumors. Scale bars, 50 μ m. **I**, Quantitation of CD3⁺ T cells in the perivascular niches of either LPL^{low} or LPL^{hi} intratumoral blood vessels in human colorectal cancer-derived liver metastatic tumors. $N = 8$ human tumors. Data are presented as matched pairs. Paired t test. **J**, Representative stained images of CD3, CD31, LPL, and DAPI on human melanoma-derived liver metastatic tumors. Scale bars, 50 μ m. **K**, Quantitation of CD3⁺ T cells in the perivascular niches of either LPL^{low} or LPL^{hi} intratumoral blood vessels in human melanoma-derived liver metastatic tumors. $N = 3$ human tumors. Data are presented as matched pairs. Paired t test.

Spatiotemporal tracing of EC-T cell interaction after ACT at single-cell resolution, we identified an early response program of tumor ECs during the initial infiltration of T cells into liver metastatic tumors. Along with expressing migratory factors and adhesive molecules, such as *Cxcl9* and *Vcam1*, a subset of TECs (TEC1) showed the strongest response to T cells and displayed the potential ability of MHC-I antigen presentation. However, TEC1 showed low or lack of MHC-II-related gene expression, distinguishing TEC1 endothelium from previously reported MHC-II-dependent antigen-presenting TEC subsets in scRNA-seq atlases of brain (41), breast (42), and lung (20) cancers. Moreover, considering the enrichment of capillary genes, the TEC1 subset is molecularly and functionally distinct from high endothelial venules in brain (9) and breast (7) cancers, ACKR1⁺ postcapillary venules in rectal cancers (resembling TEC4 subset in our data; ref. 43), and tumor lymphatic ECs (29, 44), all of which possess varying degrees of immunomodulatory functions.

Comparative analyses uncovered *Lpl* as a key DEG associated with TEC1. Subsequent *in vivo* and *in vitro* experiments, using genetic loss or gain of *Lpl* in ECs, revealed the functional impact of vascular LPL on T-cell accumulation in liver metastatic tumors and subsequent antitumor response. Mechanistically, LPL upregulation in TECs significantly enhanced the abundance of surface MHC-I on ECs and thereby increased the cross-presentation of tumor cell antigens to administered antitumor T cells in an antigen-loaded MHC-I-dependent manner, along with enhancing IFN- γ and granzyme B expression in T cells. Enhanced immune responsiveness of LPL⁺ TECs observed in our study aligns with a previous study suggesting that LPL could enhance STAT1 signaling in ECs after IFN- γ stimulation (45). Along with guiding antitumor T cells to infiltrate into the liver metastatic tumors, tumor antigen-presenting ECs were also targeted by activated T cells, altogether contributing to T cell-driven tumor regression. We further validated these observations with Pmel T cells which target endogenously expressed gp100 antigen on melanoma tumor cells. It is noteworthy that although EC-derived LPL boosted the antitumor efficacy of T cells, LPL on its own did not affect the growth of liver metastases (in the absence of T-cell intervention), revealing a previously unknown function of LPL in eliciting a vascular immunomodulatory response. LPL overexpression in ECs manifested in transcriptional upregulation of molecules involved in antigen processing and MHC-I-mediated antigen presentation; however, future studies will need to investigate the exact molecular connection between LPL and the observed upregulation of antigen presentation ability of ECs. In particular, it will be intriguing to investigate whether LPL-induced metabolomic alterations could influence the cell state of capillary ECs, thereby driving the observed immunomodulatory functions of TECs.

Our findings are in line with previous reports describing the antigen-presenting capacity of ECs, especially liver sinusoidal ECs, via the MHC-I complex (28–33, 46). Although many of the previous studies compared ECs with professional antigen-presenting cells, such as dendritic cells, and investigated their role in activating naïve T cells, our study suggests that TECs are only able to present antigen via MHC-I without coactivation. In line with the location of TECs, we conclude that TECs function as target cells that exposed tumor

antigens via MHC-I to circulating activated T cells and play a critical role in regulating extravasation and infiltration of activated CD8⁺ T cells in the metastatic lesions. Similar antigen presentation features were recently reported for the brain endothelium, in which inflamed ECs capture T cells in experimental models of cerebral malaria (46) and neuroinflammation (30), albeit in very different pathologic contexts and different organ vasculatures. Functionally, it is noteworthy that although MHC-I-mediated interaction between ECs and T cells leads to the disruption of the blood-brain barrier in the brain and worsens neuroinflammatory diseases, TEC1-T cell interaction is critical in the tumor context and results in an antitumor response, restricting metastatic growth. Indeed, EC-specific targeting of MHC-I, via depletion of *B2m*, suppressed T-cell homing, emphasizing the crucial importance of endothelial antigen presentation for antitumor response.

Altogether, the data of this study support the concept that enhancing vascular LPL expression can on-demand recruit antitumor T cells to the perivascular niches via enhancing MHC-I antigen presentation on ECs in tumor models in which tumor cell express little to low surface MHC-I. These findings may offer an explanation why only a fraction of patients benefits from combination therapy. In conclusion, expanding the scope of responsive and instructive functions of the vasculature in regulating tumor growth, progression, and metastasis (47, 48), the present study conceptualizes and presents proof of concept of a novel strategy to enhance tumor antigen MHC-I presentation on TECs via upregulation of LPL in immunologically cold tumors.

METHODS

Patient Samples

Archived paraffin-embedded tissue samples were provided by the tissue bank of the National Center for Tumor Diseases (NCT) Heidelberg (liver metastatic tumors derived from colorectal cancers, pancreatic cancers, and melanomas) and from the Institute of Pathology, Technical University Munich (liver metastatic tumors derived from colorectal cancers). Analyses in the current study were performed in accordance with the regulations of the tissue bank and approved by the ethics committees of Heidelberg University (S-206/2005) and the Technical University of Munich (2022-140_1-S-KH).

Animals

Mice were housed on a 12-hour light/dark cycle with free access to food and drinking water in specific pathogen-free animal facilities. All animal experiments were approved by the governmental (G-25/20 and G-124/21 from Regierungspräsidium Karlsruhe) and Institutional (DKFZ370 and DKFZ434) Animal Care and Use Committees. All experiments were performed in accordance with the respective institutional guidelines for the care and use of laboratory animals. Eight- to 18-week-old female C57BL/6N mice and transgenic mice of both sexes were used in this study unless otherwise indicated. C57BL/6N mice were purchased from either Janvier-Labs or Charles River Laboratories. *Lpl*^{fl/fl} (RRID: IMSR_JAX:006503), *B2m*^{fl/fl} (RRID: IMSR_JAX:034858), and CD90.1 Pmel (cat. #005023, RRID: IMSR_JAX:005023) mouse lines were purchased from The Jackson Laboratory. The *Tap1*^{fl/fl} (RRID: IMSR_EUMMCR:20868) mouse cell line was originally generated within the European Conditional Mouse Mutagenesis Program and was shared by M. Platten (German Cancer Research Center). The CD45.1 OT-I mouse cell line was kindly provided by Prof. Rienk Offringa (German Cancer Research Center).

The *Cdh5-CreERT2* mouse cell line was kindly provided by Prof. Ralf Adams (Max Planck Institute [MPI] of Molecular Biomedicine). *LSL-Lpl* knock-in transgenic mice were generated by GemPharmatech. In brief, the *CAG-loxP-Stop-loxP-mLpl-Wpre-polyA* cassette was inserted between exons 1 and 2 of the endogenous *Rosa26* locus of C57BL/6NGpt mice by homologous recombination using the CRISPR/Cas9 technology. To generate inducible EC-specific gene deletion or overexpression mouse models, homozygous *Lpl^{fl/fl}* and *LSL-Lpl* mouse lines were bred with *Cdh5-CreERT2^{+/+}* to generate 50% *CreERT2^{+/+}-positive* (*Cdh5-CreERT2^{+/+} × Lpl^{fl/fl}* mice, termed *Lpl^{fl/fl}*, and *Cdh5-CreERT2^{+/+} × LSL-Lpl* mice, termed *LSL-Lpl^{fl/fl}*) and 50% *CreERT2^{+/+}-negative* (*Lpl^{fl/fl}* mice, termed *Lpl^{WT}*, *LSL-Lpl* mice, termed *LSL-Lpl^{WT}*) littermates. Tamoxifen (Sigma-Aldrich, cat. #T5648) was dissolved in corn oil (40 mg/mL) and was administered (0.1 mg/g) by intraperitoneal injection at the indicated time points.

Cells

Mouse tumor cell lines LLC (ATCC cat. #CRL-1642, RRID: CVCL_4358) and B16F10 (ATCC cat. #CRL-6475, RRID: CVCL_0159), HEK293T (ATCC cat. #CRL-3216, RRID: CVCL_0063), and the endothelial cell line MS1 (ATCC cat. #CRL-2279, RRID: CVCL_6502) were maintained according to ATCC standard culture instructions. Hcme12 cells were kindly gifted by Prof. Thomas Tüting (University of Magdeburg). All cell lines except Hcme12 were cultured in DMEM media supplemented with 10% fetal calf serum (FCS), 1% penicillin-streptomycin, and nonessential amino acids. Hcme12 cells were cultured in RPMI 1640 medium supplemented with 10% FCS, 1% penicillin-streptomycin, and nonessential amino acids. B16F10 and LLC cells were transduced to overexpress OVA. MS1 cells were transduced to overexpress either *Lpl* or dsRed (or empty) as control. All cells were cultured at 37°C with 5% CO₂ and routinely tested for *Mycoplasma* contamination by a polymerase chain reaction (PCR)-based test.

Tumor Models

For experimental liver metastasis models, an adapted version of a previously published protocol was used (49). OVA-LLC [3×10^5 for wild-type (WT) and *Lpl^{fl/fl}*; 3.5×10^5 for *LSL-Lpl* mice], OVA-B16F10 (1.3×10^5 for WT, *B2m^{fl/fl}*, *Lpl^{fl/fl}*, and *Tap1^{fl/fl}*; 1.5×10^5 for *LSL-Lpl* mice), B16F10 (1.25×10^5 for *LSL-Lpl* mice), or Hcme12 (7.5×10^5) cells were intrasplenically injected into the mice. Splenectomy was performed 10 minutes after tumor cell inoculation.

For the subcutaneous primary tumor model, OVA-B16F10 (1×10^6) cells were subcutaneously inoculated at the abdominal area as previously described (50). Tumor volumes were determined by caliper measurements ($3 \times$ per week; tumor volume = $\frac{1}{2} \times$ length $\times$ width $\times$ height).

All mice were routinely checked for ethical experimental endpoint criteria: (i) enlarged peritoneum (liver metastasis), (ii) weight loss of 20% (compared with the original mouse weight), (iii) lack of food or water intake, (iv) noticeable respiratory symptoms, (v) abnormal posture or crouching, (vi) apathy or immobility, and (vii) pale extremities.

ACT

A single-cell suspension was prepared from the spleens of CD45.1 OT-I or CD90.1 Pmel mice. Cells were subsequently activated overnight with 10 nmol/L of OVA257-264 (SIINFEKL, Sigma cat. #S7951) or 2 μ g/mL hgp100 (25-33) peptide (GenScript cat. #RP20344) in RPMI 1640 medium containing 10% FCS in the presence of 50 μ mol/L β -mercaptoethanol (Sigma-Aldrich cat. #M3701) and 10 ng/mL murine IL-2 (BioLegend cat. #575406). Cells were then cultured in the presence of 50 μ mol/L β -mercaptoethanol and 5 ng/mL IL-2 for an additional 3 to 4 days. CD8⁺ T cells were later isolated and

purified via untouched magnetically labeled antibodies (Miltenyi Biotec cat. #130-104-075). Activated CD8⁺ OT-I T cells (2×10^6) or Pmel T cells ($5-10 \times 10^6$) were intravenously injected into mice with liver metastases at the indicated time points. OT-1 cells were identified and analyzed by CD45.1 expression on T cells. For Pmel models, mice were vaccinated with 10 μ g hgp100 6 hours before the ACT.

In Vivo Antibody Blockade

For combination therapy experiments of anti-VEGFR2 and anti-PD-L1 blockade, subcutaneously tumor-bearing mice (volume ~ 150 to 200 mm^3) were randomly assigned to treatment or control groups. A combination of 40 mg/kg anti-VEGFR2 (clone DC101, Bio X Cell cat. #BE0060, RRID: AB_1107766) and 20 mg/kg anti-PD-L1 (clone 10F.9G2, Bio X Cell cat. #BE0101, RRID: AB_10949073) or control rat IgG2b (clone LTF2, Bio X Cell cat. #BE0090, RRID: AB_1107780) was intraperitoneally injected every day for 3 days. Tumors were collected 72 hours after the start of therapy.

For H-2/MHC-I blockade experiments, mice were treated with 500 μ g anti-mouse MHC-I antibody (clone M1/42.3.9.8, Bio X Cell cat. #BE0077, RRID: AB_1125537) or control IgG2a (clone 2A3, Bio X Cell cat. #BE0089, RRID: AB_1107769) via intraperitoneal injection at 1 day before, on the day of, and 1 day after ACT. MHC-I blocking efficacy was detected with Alexa Fluor (AF) 488 donkey anti-rat IgG antibody (Thermo Fisher Scientific cat. #A-21208, RRID: AB_2535794).

EC and Immune Cell Isolation

Tissues were dissociated into single-cell suspension with Liberase digestion enzyme mix (Roche cat. #05401127001) and DNase I (Roche cat. #11284932001) as previously reported (50). In brief, chopped tissues were digested with Liberase for 20 to 30 minutes depending on the tissue size with resuspension every 10 minutes. For liver sinusoidal ECs, cells were after ACK lysis enriched using CD146 microbeads (Miltenyi Biotec cat. #130-092-007) according to the manufacturer's instructions. Enriched ECs were stained with fluorophore-conjugated antibodies and sorted for the surface marker profile CD45-TER119-CD146⁺CD31⁺ (51). For macrodissected liver metastatic tumors, subcutaneous primary tumors, and heart tissues, following ACK lysis, the single-cell suspension was directly stained with FACS antibodies. ECs from metastatic tumors were isolated via FACS sorting for cells with a surface marker profile CD45-TER119-CD31⁺. Primary tumor and heart ECs were FACS-sorted with a panel of CD45-LYVE1-TER119-CD31⁺. Immune cells were sorted with the surface marker CD45⁺. OT-I T cells were sorted with a surface marker profile CD45⁺CD45.1⁺. Dead cells were excluded by FxCycle Violet (Invitrogen cat. #F10347) staining. The following FACS antibodies were used: FITC anti-mouse CD45 (30-F11; BD Biosciences cat. #553080, RRID: AB_394610), FITC anti-mouse CD45.1 (A20; BD Biosciences cat. #553775, RRID: AB_395043), allophycocyanin (APC) anti-mouse CD45.1 (A20; BD Biosciences cat. #558701, RRID: AB_1645214), PE anti-mouse CD31 (MEC 13.3; BD Biosciences cat. #553373, RRID: AB_394819), APC anti-mouse CD31 (MEC 13.3; BD Biosciences cat. #551262, RRID: AB_398497), FITC anti-mouse TER-119 (TER-119; BD Biosciences cat. #557915, RRID: AB_396936), Pacific Blue anti-mouse TER-119 (TER-119; BioLegend cat. #116232, RRID: AB_2251160), AF488 anti-mouse LYVE1 (ALY7; Thermo Fisher Scientific cat. #53-0443-82, RRID: AB_1633415), and PE/Cyanine7 anti-mouse CD146 (ME-9F1; BioLegend cat. #134714, RRID: AB_2563109). Stained cells were sorted using a BD Aria or Fusion cell sorting platform.

Flow Cytometry Analysis

For SIINFEKL-H2-K^b/MHC-I and total H2-K^b/MHC-I analyses, single-cell suspensions were stained with FITC anti-mouse CD45 (30-F11), Brilliant Violet (BV)711 anti-mouse CD31 (MEC 13.3;

BD Biosciences cat. #740680, RRID: AB_2740367), AF647 anti-mouse H2-K^b antibody (AF6-88.5; BioLegend cat. #116512, RRID: AB_492917), PE anti-mouse H2-K^b bound to SIINFEKL (25-D1.16; BioLegend cat. #141604, RRID: AB_10895905), APC anti-mouse H-2K^b bound to SIINFEKL (25-D1.16; BioLegend cat. #141606, RRID: AB_11219595), and AF488 anti-mouse H2-K^b antibody (AF6-88.5; BioLegend cat. #116510, RRID: AB_492915) for 20 minutes. Dead cells were excluded by FxCycle Violet staining. Cells were analyzed using BD Symphony SE5, Fusion I, or Aria II cytometers.

For Annexin V and FxCycle Violet analysis, MS1 ECs were treated overnight with 100 ng/mL IFN- γ or PBS as Ctrl. Cells were collected and stained with AF647-Annexin V (Invitrogen cat. #A23204) and FxCycle for 15 minutes. Samples were immediately analyzed by BD Symphony SE5 cytometers after washing and resuspension.

For 5-ethynyl-2'-deoxyuridine (EdU) analysis, CD8⁺ OT-I T cells and ECs were cocultured in medium that contained 10 μ mol/L EdU for 3 hours. Cells then were digested and collected. Cell pellets were subsequently stained with FITC anti-CD45.1 for 15 minutes to label OT-I T cells, followed by being fixed, permeabilized using a fixation/permeabilization kit (BD Pharmingen cat. #554722), and finally stained using the AF647 Click-iT EdU kit (Life Technologies cat. #C10340) according to the kit instruction. Cell cycle of OT-I T cells was analyzed using BD Canto flow cytometry.

For T-cell status, differentiation, cytotoxic, and exhaustion analyses, single-cell suspension were stained with FITC anti-mouse CD45.1 (A20), APC anti-mouse CD45.1 (A20), AF488 anti-rat CD90/mouse CD90.1 (Thy1.1; BioLegend cat. #202506, RRID: AB_492882), BV605 anti-mouse CD45.1 (A20; BioLegend cat. #110737, RRID: AB_11204076), FITC anti-mouse CD45 (30-F11), BUV805 anti-mouse CD45 (30-F11; Thermo Fisher Scientific cat. #368-0451-82, RRID: AB_2896112), Spark UV 387 anti-mouse CD3 (17A2; BioLegend cat. #100283, RRID: AB_3083252), PE-Cy7 anti-mouse CD3 (145-2C11; BioLegend cat. #100319, RRID: AB_10897937), APC anti-mouse CD8a (53-6.7; BioLegend cat. #100712, RRID: AB_312751), REAfinity APC anti-mouse CD183 (CXCR3, REA724; Miltenyi Biotec cat. #130-111-088, RRID: AB_2655748), FITC anti-mouse/human CD44 (IM7; BioLegend cat. #103006, RRID: AB_312957), PE/Cyanine7 anti-mouse CD62L (MEL-14; BioLegend cat. #104418, RRID: AB_313103), and Fixable Viability Dye eFluor 780 (eBioscience cat. #65-0865-14) for 15 minutes. Cell pellet was later washed for analysis or fixed and permeabilized using a fixation/permeabilization kit (BD Pharmingen cat. #554722) for 20 minutes. Fixed cells were subsequently stained with BV421 anti-mouse IFN- γ (XMG1.2; BioLegend cat. #505829, RRID: AB_10897937), PE-Cy7 anti-mouse Granzyme B (NGZB; Thermo Fisher Scientific cat. #25-8898-82, RRID: AB_10853339), and PE anti-mouse TOX (TXRX10; Thermo Fisher Scientific cat. #12-6502-80, RRID: AB_10853657) for 30 minutes. Dead cells in nonfixed samples were excluded by FxCycle Violet staining. After final wash and resuspension in PBS, cells were analyzed by BD SE5 flow cytometry.

For analysis of tumor-associated macrophages/innate immune cells, single-cell suspension from macrodissected liver metastases was stained with BUV805 anti-mouse CD45 (30-F11), BV605 anti-mouse CD11b (M1/70; BioLegend cat. #101237, RRID: AB_11126744), PE anti-mouse F4/80 (BM8; BioLegend cat. #123110, RRID: AB_893486), AF488 anti-mouse CD206 (MMR; Thermo Fisher Scientific cat. #53-2061-82, RRID: AB_2784749), and AF647 anti-mouse I-A/I-E (M5/114.15.2; BioLegend cat. #107618, RRID: AB_493525). Dead cells were excluded by FxCycle Violet staining. Cells were analyzed using BD Symphony SE5.

scRNA-seq Library Preparation and Data Analysis

Sample Multiplexing. To enable tracking of sample identity of ECs and immune cells in scRNA-seq collections, we integrated Smart-Hash labeling (provided by Single-cell Open Lab, DKFZ) into the EC

(WT) and immune cell (iECKO) and TotalSeq labeling into immune cell (iECKI) isolation protocols. For each library, tumor nodules were collected from up to six individual livers from adult mice and were enzymatically dissociated and processed in parallel. In brief, digested single-cell suspensions from each liver were incubated with a unique Smart-Hash or TotalSeq barcoding tag along with FACS antibodies in 0.3% BSA/PBS buffer for 30 minutes at 4°C. Cells were washed three times with 0.3% BSA/PBS buffer after incubation. Dead cells were excluded by FxCycle Violet staining. Stained cells were sorted using a BD Aria or Fusion cell sorting platform. Smart-Hash used in this study are given as follows: Hash1-GTCAACTCTTTAGCG, Hash2-TGATGGCCTATTGGG, Hash3-TTCCGCCTCTCTTTG, Hash4-AGTAAGTTCAGCGTA, Hash5-AAGTATCGTTTCGCA, Hash6-GGTTGCCAGATGTCA, and Hash7-TGTCTTCTCTGCCAG. TotalSeq antibodies used in this study are given as follows: TotalSeq-C0301 anti-mouse Hashtag 1 (BioLegend cat. #155861, RRID: AB_2800693), TotalSeq-C0302 anti-mouse Hashtag 2 (BioLegend cat. #155863, RRID: AB_2800694), TotalSeq-C0303 anti-mouse Hashtag 3 (BioLegend cat. #155865, RRID: AB_2800695), TotalSeq-C0304 anti-mouse Hashtag 4 (BioLegend cat. #155867, RRID: AB_2800696), TotalSeq-C0305 anti-mouse Hashtag 5 (BioLegend cat. #155869, RRID: AB_2800697), and TotalSeq-C0306 anti-mouse Hashtag 6 (BioLegend cat. #155871, RRID: AB_2819910).

Library Preparation. Sorted viable cells were resuspended in PBS and loaded onto a 10x Genomics Chromium Chip for one well per sample pool. The chip was run on a 10x Genomics Chromium instrument to generate GEM bead emulsions according to the manufacturer's instructions. cDNA synthesis and amplification and library preparation steps were then performed to generate scRNA-seq libraries according to the Chromium Next GEM Single Cell 3' v3.1 or 5' v3 protocol (10x Genomics). For multiplexing libraries, feature barcoding primers were added during cDNA synthesis. Quality control for amplified cDNA and final sequencing libraries was performed using D5000 and D1000 TapeStation chemistry, respectively (Agilent Technologies). The pooled libraries were sequenced on the Illumina NovaSeq 6000 platform.

Data Processing and Cell-Type Annotation. Sequenced reads were processed and aligned to the mouse reference genome (mm10, 2020) using the 10x Genomics Cell Ranger multi (6.0.0 or 6.1.2 or 9.0.1) workflow. The Cell Ranger multi output data were subsequently processed by Cell Ranger aggr to merge the files and filter low-quality cells. Cell Ranger aggr output files imported and analyzed by Seurat (52). Filters of (i) nFeature_RNA < 200, (ii) nCount_RNA > 40,000, and (iii) percent_mito $\geq$ 10% were used to eliminate low-quality cells. The filtered data were then normalized and scaled based on the Seurat instruction and decomposed using PCA. Neighborhood graph computing was performed with FindNeighbors() with dimension of 1:15, and clusters were determined using FindClusters(). t-distributed stochastic neighbor embedding and Uniform Manifold Approximation and Projection (UMAP) were then computed. Contaminating non-ECs and doublet clusters were removed from further analyses. Multiple classic cell type-specific markers were used jointly to annotate each cluster. Cell subtypes were distinguished by evaluating the expression of well-known markers and top marker genes defining each cluster. To assess differential expression, the "FindAllMarkers" and "FindMarkers" functions in Seurat were used with the default Wilcoxon rank-sum test. The lists of DEGs were typically refined based on a corrected *P* value threshold ("p_val_adj") $\leq$ 0.05 with absolute log₂ (fold change) $\geq$ 0.25. Comparison of DEGs across different TEC clusters was performed using the Venn diagram webtool (<https://bioinformatics.psb.ugent.be/webtools/Venn/>).

RNA Velocity Analysis. Cell Ranger output-aligned bam files were used as the input for velocity (v0.17.17; ref. 53) to derive the counts of spliced and unspliced reads in loom format. Next, the

UMAP embedding from the previously produced integrated Seurat object was converted into a .h5ad file for analysis using the scVelo (v0.2.4; ref. 54) package. Data were filtered, normalized, and calculated as described in scVelo using default parameters. Velocities were then projected on previously calculated UMAP embedding using the `scv.pl.velocity()` function.

Bulk RNA-seq and Data Analysis

Total RNA was isolated from Ctrl- or *Lpl*-overexpressing MS1 ECs using an RNeasy Mini Kit (Qiagen, #74104) according to the manufacturer's instructions. Quality control was performed by Bioanalyzer (Agilent Technologies) measurements. The sequencing library was generated with TruSeq stranded total-RNA library prep kit according to the manufacturer's protocol. Sequencing reads (75-bp single-read) were generated on the NextSeq 550 (Illumina) platform. The sequenced reads were aligned to the mouse reference genome mm10 with STAR aligner (55). Only transcripts with reads per kilobase of transcript per million mapped reads ≥ 1 in at least one sample were considered for the downstream analysis. IPA was undertaken to study regulated molecular pathways and corresponding biological functions.

Generation of Stable OVA- or LPL-overexpressing Cells

Entry plasmid of the open reading frame (ORF) of mouse *Lpl* for gateway assay was provided by Core Facility Cellular Tools, DKFZ. Entry plasmid of *Ova* ORF was kindly gifted by M. Platten, DKFZ. The ORF was inserted into pLenti-CMV-Puro-DEST (Addgene, cat. #17452, RRID: Addgene_17452) via Gateway LR Clonase II Enzyme Mix (Invitrogen, #11791020). Lentiviruses were generated in HEK-293T cells with second-generation packaging plasmids. Tumor or EC cell lines were infected with concentrated lentivirus, and puromycin-resistant (Gibco, cat. #A1113803) cells were selected and maintained for experiments.

In Vitro Coculture Assay

Ctrl- or *Lpl*-overexpressing ECs were seeded overnight to form a monolayer. OT-I or Pmel T cells were activated and expanded for 3 days as previously described before coculture. EC monolayer was primed with 10 nmol/L of OVA₂₅₇₋₂₆₄ or 100 ng/mL hgp100 (25–33) peptide for 3 hours and then washed 3 times with PBS. Purified CD8⁺ OT-I or Pmel T cells were then added with 1:3 EC:T cell ratio and cocultured with ECs for 3 to 4 hours. Cells were subsequently collected for FACS analysis. For the T-cell cytotoxicity analysis, Brefeldin A (BioLegend cat. #420601) was added into the coculture medium.

Transendothelial Migration Assay

Transwell inserts (6.5 mm) with 3- μ m pore polycarbonate membrane in a 24-well plate (Corning cat. #CLS3415) were used in this experiment. ECs (1×10^5) were seeded in 0.1% gelatin-coated Transwell inserts. After 24 hours, the EC monolayer was treated with 10 ng/mL OVA-SIINFEKL peptide or 0.1% BSA as a control for 3 hours. After washing 3 times with PBS to remove the remaining peptide, 2×10^5 OT-I T cells in 250 μ L serum-free DMEM medium were added into the upper chamber. DMEM (500 μ L) containing 10% FCS was added to the lower chamber. After 4 hours (for migrated T-cell measurement) or 6 hours (for EC death measurement) of incubation, media in the lower chamber were collected, and live cells were resuspended in Trypan Blue solution after spinning down and counted with Hemocytometer or Countess automated cell counter (Invitrogen). Transwell chambers were washed 3 times with PBS, followed by fixation with methanol for 15 minutes at -20°C . Crystal violet solution was then used to stain the remaining ECs on the upper membrane of the Transwell.

IF Staining

For human liver metastatic tumor samples, epitopes were retrieved after rehydration of the sections using sodium citrate pH 6 buffer at 99.5°C for 30 minutes. The sections were blocked for 1 hour in 5% FCS at room temperature (RT) and incubated with primary antibodies against anti-human CD3 (Agilent Technologies cat. #A0452, RRID: AB_2335677), anti-human CD31 (JC70A; R&D Systems cat. #AF806, RRID: AB_355617), and anti-human LPL (LPL.A4; Abcam cat. #ab21356, RRID: AB_446221). The sections were subsequently incubated with secondary antibodies: AF488 donkey anti-rabbit IgG (Thermo Fisher Scientific cat. #A-21206, RRID: AB_2535792), AF647 donkey anti-mouse IgG (Thermo Fisher Scientific cat. #A-31571, RRID: AB_162542), and AF568 donkey anti-sheep IgG (Thermo Fisher Scientific cat. #A-21099, RRID: AB_10055702).

For mouse liver and primary tumor staining, isolated and collected livers were frozen in optimal cutting temperature compound (Sakura Bio cat. #4583). Cryosections (10 or 12 μ m) were prepared and air dried at -20°C for 1 hour. Unstained slides were kept in a -80°C freezer. Frozen sections were fixed in ice-cold methanol for 15 minutes at -20°C . The slides were blocked in 5% FCS for 30 minutes at room temperature (RT) and subsequently stained with primary antibodies anti-mouse CD31 (MEC13.3; BD Biosciences cat. #553370, RRID: AB_394816), anti-mouse CD31 (R&D Systems cat. #AF3628, RRID: AB_2161028), AF594 anti-mouse CD31 (MEC13.3; BioLegend cat. #102520, RRID: AB_2563319), anti-mouse MKI67 (Bethyl Laboratories cat. #IHC-00375, RRID: AB_1547959), anti-human/mouse LPL (R&D Systems cat. #AF7197, RRID: AB_10972480), anti-mouse IDO (D8W5E; Cell Signaling Technology cat. #51851, RRID: AB_2799402), anti-mouse PD-L1 (R&D Systems cat. #AF1019, RRID: AB_354540), anti-human/mouse CD3 (Dako), APC anti-mouse CD45.1 (A20), AF647 anti-mouse CD90.1 (OX-7; BioLegend cat. #202507, RRID: AB_492885), AF488 anti-mouse CD90.1 (OX-7), and anti-mouse cCASP3 (Asp175; Cell Signaling Technology cat. #9661, RRID: AB_2341188) at 4°C overnight, followed by staining with secondary antibodies AF488 donkey anti-rabbit IgG (Thermo Fisher Scientific cat. #A-21206, RRID: AB_2535792), DyLight 550 donkey anti-Rat IgG (Thermo Fisher Scientific cat. #SA5-10027, RRID: AB_2556607), AF568 donkey anti-goat IgG (Thermo Fisher Scientific cat. #A-11057, RRID: AB_2534104), AF647 donkey anti-goat IgG (Thermo Fisher Scientific cat. #A-21447, RRID: AB_2535864), AF647 donkey anti-rabbit IgG (Thermo Fisher Scientific cat. #A-31573, RRID: AB_2536183), and SRM Identifyn AF750 donkey anti-rabbit IgG (Thermo Fisher Scientific cat. #SA000082).

For *in vitro*-cultured cell staining, cover slides were inserted into 12-well or 24-well plates and coated with 0.2% gelatin for 30 minutes at 37°C in the incubator. Cells were seeded on the top and allowed to grow to 85% to 95% confluency. Thereafter, cells were fixed with 4% paraformaldehyde (Thermo Fisher Scientific cat. #28909) or ice-cold methanol for 15 minutes. After washing with PBS, cells were blocked with 5% FCS for 60 minutes at RT and subsequently stained with primary antibodies (listed above) at 4°C overnight, followed by the staining with secondary antibodies (listed above).

Phalloidin and DAPI (Merck-Sigma) were used for cellular actin staining and nuclear counterstaining, respectively. Slides were mounted with fluorescence mounting medium (Dako cat. #S2023) and coverslips. Images were obtained using the ZEISS LSM 710, ZEISS LSM 910, and ZEISS Axio Scan Z1 microscopes, and image analysis was performed using ZEISS ZEN blue edition v3.8 and Fiji software.

Image Processing and Analysis

All ZEISS (.czi) files were exported using ZEN 3.8 [ZEN Blue; ZEISS ZEN Microscopy Software (RRID: SCR_013672)] as single-channel tagged image file format (TIFF) files and used as the input for subsequent analysis in Fiji (ImageJ, RRID: SCR_002285).

For the quantification of T cells, blood vessel density, and cCASP3⁺ area, the tumor area of each individual liver metastatic nodule was first manually selected to generate a region of interest (ROI), and its area was measured. Subsequently, CD45.1⁺ cells, CD3⁺CD8⁺ cells, or CD31⁺ areas were identified following background adjustment, and corresponding ROIs were generated. The number of T cells and the CD31⁺ area within each previously defined tumor ROI were then quantified. cCASP3⁺ area within each previously defined tumor ROI and the following CD31⁺ ROI was identified and measured after background adjustment. T-cell density and CD31⁺ blood vessel density was calculated by normalizing these values to the respective tumor area. For each mouse, the reported value represents the mean of all calculated measurements.

For the analysis of the anti-MHC-I blocking antibody area, thresholds were set and kept for each slide by comparing unstained section with section stained using AF488-conjugated anti-rat IgG antibodies from the same sample on the same slide. Images were exported with the applied display settings and channel colors. The tumor or peritumoral liver area was first manually selected to generate an ROI, and its area was measured. Following background adjustment, the AF488-positive area was identified, and a corresponding ROI was generated for area measurement. The CD31⁺ area was then selected, and its ROI was created within the previously generated AF488⁺ ROI, thus marking CD31⁺AF488⁺ area. Area measurements were performed to quantify the CD31⁺ AF488⁺ area. Finally, the percentage of anti-MHC-I blocking area was calculated by dividing the CD31⁺ AF488⁺ area by the total AF488⁺ area. For each mouse, the reported value represents the mean of all calculated measurements. Given that we analyzed only one biological group (*LSL-Lpl^{IECK1}* + α -MHC-I), no statistical comparison was performed.

Gene Expression Analysis

Total mRNA was transcribed into cDNA using the QuantiTect Reverse Transcription kit (QIAGEN, #205313). Quantitative PCRs were performed with the TaqMan master mix (Applied Biosystems, #4444965). TaqMan primers (*Actb*-Mm00607939_S1, *Pecam1*-Mm01242584_m1, *Ptprc*-Mm01293577_m1, *H2-k1*-Mm01612247_mH, *Lpl*-Mm00434764_m1, *Tap1*-Mm00443188_m1, *B2m*-Mm00437762_m1, *Psmb9*-Mm00479004_m1, and *Psmb10*-Mm00479052_g1) were ordered from Thermo Fisher Scientific. Gene expression was calculated based on the $\Delta\Delta C_t$ relative quantitation method. mRNA abundances were normalized to *Actb* expression as indicated.

Statistics Analysis

Statistical analyses were performed with GraphPad Prism 10 software. Data values are expressed as mean $\pm$ SD, unless otherwise stated. A *P* value of less than 0.05 was considered statistically significant.

Data Availability

Datasets generated in this study are available at the Gene Expression Omnibus under accession numbers GSE289731 (bulk RNA-seq data) and GSE289961 (scRNA-seq data). No new codes were generated in this study.

Authors' Disclosures

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Authors' Contributions

X. Zhang: Conceptualization, formal analysis, investigation, methodology, writing—original draft, writing—review and editing. **M. Kamiyama:** Investigation, methodology. **M. Tulesin:** Resources. **L. Rinck:** Software, formal analysis. **J.-P. Mallm:** Investigation. **M. Kilian:** Investigation. **D.A. Agardy:** Resources. **M. Heikenwälder:** Investigation. **M. Platten:** Investigation. **C. Mogler:** Resources, investigation. **M. Singhal:** Conceptualization, resources, formal analysis, supervision, funding acquisition, investigation, methodology, writing—original draft, project administration, writing—review and editing. **H.G. Augustin:** Conceptualization, formal analysis, funding acquisition, methodology, writing—original draft, project administration, writing—review and editing.

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Note

Supplementary data for this article are available at Cancer Discovery Online (<http://cancerdiscovery.aacrjournals.org/>).

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