

Supplemental Methods

Immunoblotting: MPMECs were isolated from *Rptor^{fl/fl}* mice and transduced with Cre adenovirus for 16-48 hrs. Ad-CMV-Null was used as a control. To assess VEGF-B activation of mTORC1, cells were serum starved in EBM-2 medium supplemented with 0.2% FBS overnight. A time course was performed on cells treated with 300 ng/mL of recombinant mouse VEGF-B₁₈₆ (R&D Systems #767-VE-010) for 0, 5, 15, or 30 minutes prior to harvest. Pre-cleared lysates were electrophoresed on a SDS-polyacrylamide gel and transferred to nitrocellulose membranes, as previously described (1). The following primary antibodies were used at 1:1000 dilution: Raptor (Cell Signaling Technology (CST) #2280), Rictor (Millipore #05-1471), phospho-S6K1 (T389, CST #9234), S6K1 (CST #9202), phospho-S6RP (S235/236, CST #2211), S6RP (CST #2217), phospho-4EBP1 (T37/46, CST #9459), phospho-4EBP1 (T70, CST #9455), 4EBP1 (CST #9644), phospho-AKT (T308, CST #4056), phospho-AKT (S473, CST #4060), AKT (CST #2920), CLSTN1 (Abcam #ab134130), and β -tubulin (Sigma #T4026). Blots were incubated with secondary antibodies IRDye 680LT goat anti-mouse (1:20,000; LI-COR #925-68020) or IRDye 800 CW goat anti-rabbit (1:10,000; LI-COR #925-32211) and imaged using LI-COR Odyssey.

Permeability assay: 1×10^5 *Rptor^{fl/fl}* MPMECs were plated onto the inner surface of a gelatin-coated transwell insert with a 0.4 μ m pore size. Cells were transduced with Ad-CMV-Null or Ad-CMV-iCre for 16 hours and stimulated with VEGF-B₁₈₆ (300 ng/mL) or VEGF-A (300 ng/mL) for 30 hr in EBM-2 supplemented with 1% BSA free of fatty acids (FFA-BSA). 70 kDa Texas Red-Dextran (1 mg/mL, Thermo #D1830) was placed in the

lower chamber. Ten microliter aliquots of medium were removed from the upper chamber at 0, 10, 30, and 60 min. A transwell without endothelial cells was used to determine maximum permeability intensity. A BioTek Synergy HT plate reader (595 excitation, 615 emission) was used to measure fluorescence intensity of Texas Red-Dextran that passed into the upper chamber. Permeability was calculated as a percent of the empty transwell control.

BODIPY uptake assay: *Rptor*^{fl/fl} MPMECs were transduced with Ad-CMV-Null or Ad-CMV-iCre for 24 hrs. Cells were stimulated with recombinant mouse VEGF-A (300 ng/mL, R&D Systems #493-MV-005), recombinant mouse VEGF-B₁₈₆ (300 ng/mL), mouse VEGFR1/Flt-1 antibody (1 µg/mL, R&D Systems, #AF471), or a combination of anti-VEGFR1 and VEGF-B for 30 hrs. For the combination, cells were pre-incubated with anti-VEGFR1 for 2 hr prior to addition of VEGF-B. Cells were then incubated with 10 µM of BODIPY FL C16 or BODIPY 500/510 C1,C12 (BODIPY-C12, Thermo #D3823) for 3 min. For imaging, cells were fixed in 4% PFA for 10 min and stained with DAPI (Invitrogen #R37606) as directed. Cells were imaged using an Olympus inverted microscope. For flow cytometry, cells were trypsinized and fixed in 4% PFA for 10 min. Flow cytometry was immediately performed as described above.

Immunohistochemistry: Formalin-fixed paraffin-embedded (FFPE) tumor-bearing lungs from WT or *Rptor*^{ECKO} inoculated with E0771 tumor cells were evaluated for phospho-H3 by fluorescent immunohistochemistry, while NDUFS1 was detected by immunohistochemistry, as previously described (1, 2). Antigen retrieval was achieved

using Retreivagen A (BD Biosciences #550524), according to manufacturer's directions. After blocking endogenous peroxidases and blocking with 3% goat serum, tissues were incubated with phospho-H3 (1:100, CST #9701) or NDUFS1 (1:200, Proteintech #12444-1-AP) overnight at 4°C. Phospho-H3 was detected by incubating with an Alexa Fluor 488 secondary antibody (1:500, Invitrogen #A11034), and tissues were mounted in anti-fade with DAPI (Invitrogen #P36941). To detect NDUFS1, sections were incubated with a biotinylated anti-rabbit IgG (1:500; Vector Laboratories, #BA-1000) for 1 hr at room temperature before incubating with streptavidin peroxidase (Vector Laboratories, #SA-5704). Protein was detected using ImmPACT diaminobenzidine (DAB) (Vector Laboratories #SK-4103), prepared according to the manufacturer's instructions. Nuclei were stained with hematoxylin (Vector Laboratories #H-3401), and tissues were mounted using Cytoseal XYL (Epredia #8312-4). At least 2-4 fields of view were obtained using an Olympus inverted microscope. Phospho-H3 positive nuclei and total nuclei were counted using ImageJ software. Total DAB intensity was determined using the deconvolution plug-in within ImageJ software. Percentages and intensities were averaged from all fields of view and normalized to littermate controls.

For H&E images, sections from formalin-fixed, paraffin-embedded lungs were stained with hematoxylin and eosin, as previously described (1). Images were obtained using an Olympus upright inverted microscope.

CLSTN1 immunohistochemistry images: CLSTN1 immunohistochemistry (Gene #ENSG00000171603; Antibody #HPA077705) images in breast lobular carcinoma (3)

(Patient ID #2900), lung squamous cell carcinoma (4) (Patient ID #4488), and melanoma (5) (Patient ID #4229) were obtained from The Human Protein Atlas (6) (www.proteinatlas.org).

Cytotoxicity assay: Cell-mediated cytotoxicity was determined as previously described, with some changes (1). Briefly, E0771-OVA or E0771 parental cells (1×10^4 cells per well) were plated in triplicate. OT-I splenocytes were isolated from 8-week-old OT-I female mice and activated with OVA257-264 (SIINFEKL) peptide ($1 \mu\text{g/mL}$) (Invivogen #vac-sin) for 48 hrs. CD8⁺ T cells were isolated using mouse CD8a microbeads (Miltenyi Biotec #130-045-201) as previously described (1). E0771-OVA or E0771 parental cells (1×10^4 cells per well) were co-cultured with activated CD8⁺ OT-I T cells (2×10^3 cells per well resulting in a 5:1 tumor cell to T cell ratio) in 0 or $50 \mu\text{M}$ palmitate-BSA for 48 hrs. Cytotoxicity was measured using the CyQUANT lactate dehydrogenase (LDH) cytotoxicity assay (Invitrogen #C20300), according to manufacturer's instructions and as previously indicated (1). Cytotoxicity was calculated as a percent of maximum LDH release upon cell lysis, accounting for spontaneous cell death under the respective culture conditions.

Sphere invasion assay: Invasion of E0771 cells was performed as previously described (7, 8), with some changes. Briefly, E0771 cells (2.5×10^4 cells/mL) were cultured as $20 \mu\text{L}$ hanging drops for 72 hrs in DMEM supplemented with 10% FBS. Spheres were then embedded in $200 \mu\text{L}$ of a mix of growth factor reduced Matrigel (50%, Corning #354230) and type I rat tail collagen (0.5 mg/mL , Gibco #A1048301)

supplemented with 0 or 50 μ M palmitate-BSA. Spheroids were incubated at 37°C for 30 min to allow Matrigel to solidify, followed by submersion in 1 mL of warm cell culture media supplemented with 0 or 50 μ M palmitate-BSA. Bright field images were taken at 48 hr using an inverted microscope. Invasive area was determined from 8-10 fields of view using ImageJ software, corrected for original sphere area.

Quantitative real-time PCR: RNA was collected from *Rptor*^{fl/fl} or *Tsc2*^{fl/fl} MPMECs transduced with Ad-CMV-Null or Ad-CMV-iCre, as described above. Following the manufacturer's directions, cDNA was generated using the iScript cDNA synthesis kit (Bio-Rad #1708891). Using the primers defined in Supplemental Table 4, PCR amplification was performed in triplicate using the StepOnePlus (Applied Biosystems) as previously described (1). The $\Delta\Delta C_t$ quantitation method was used.

References

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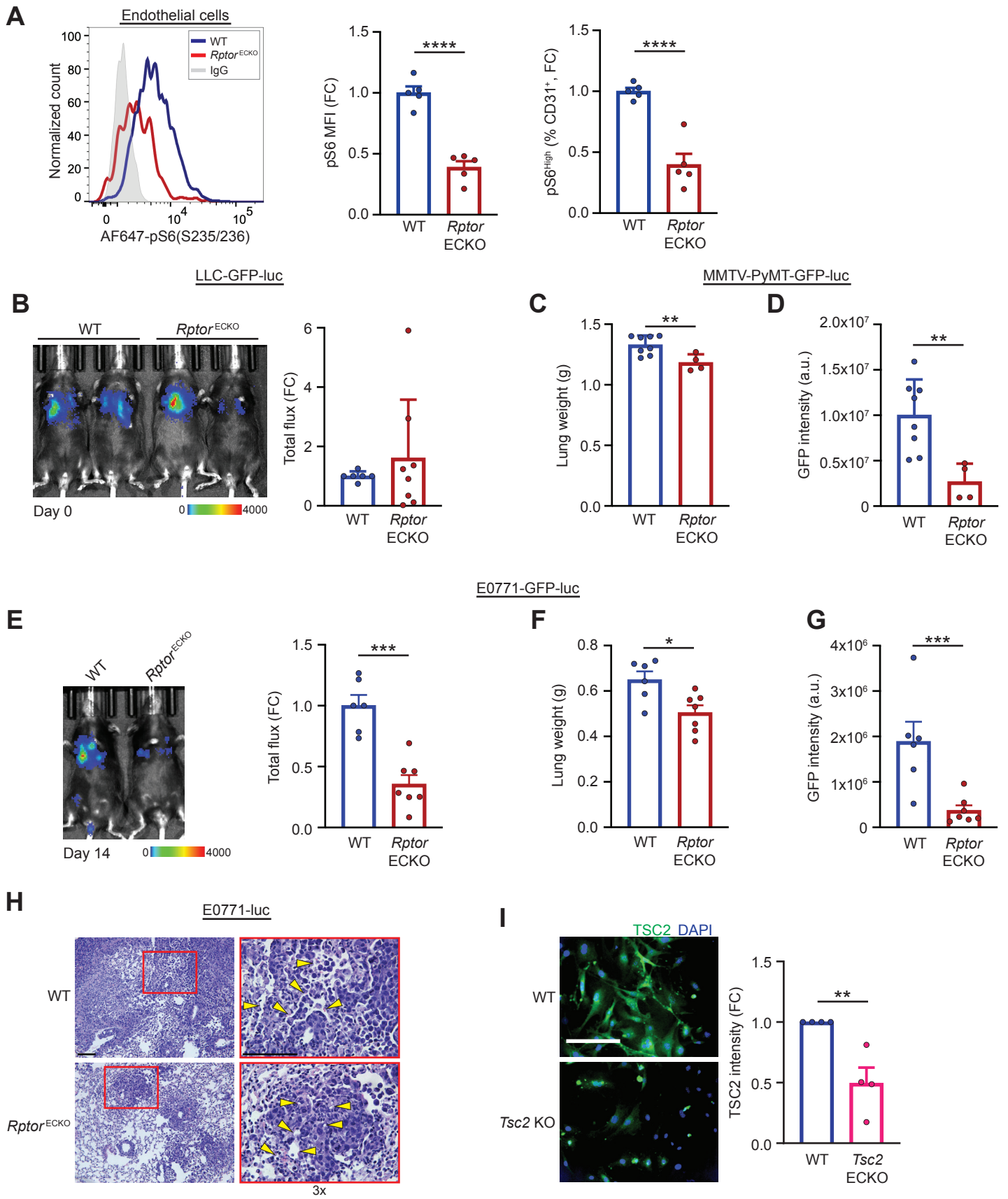
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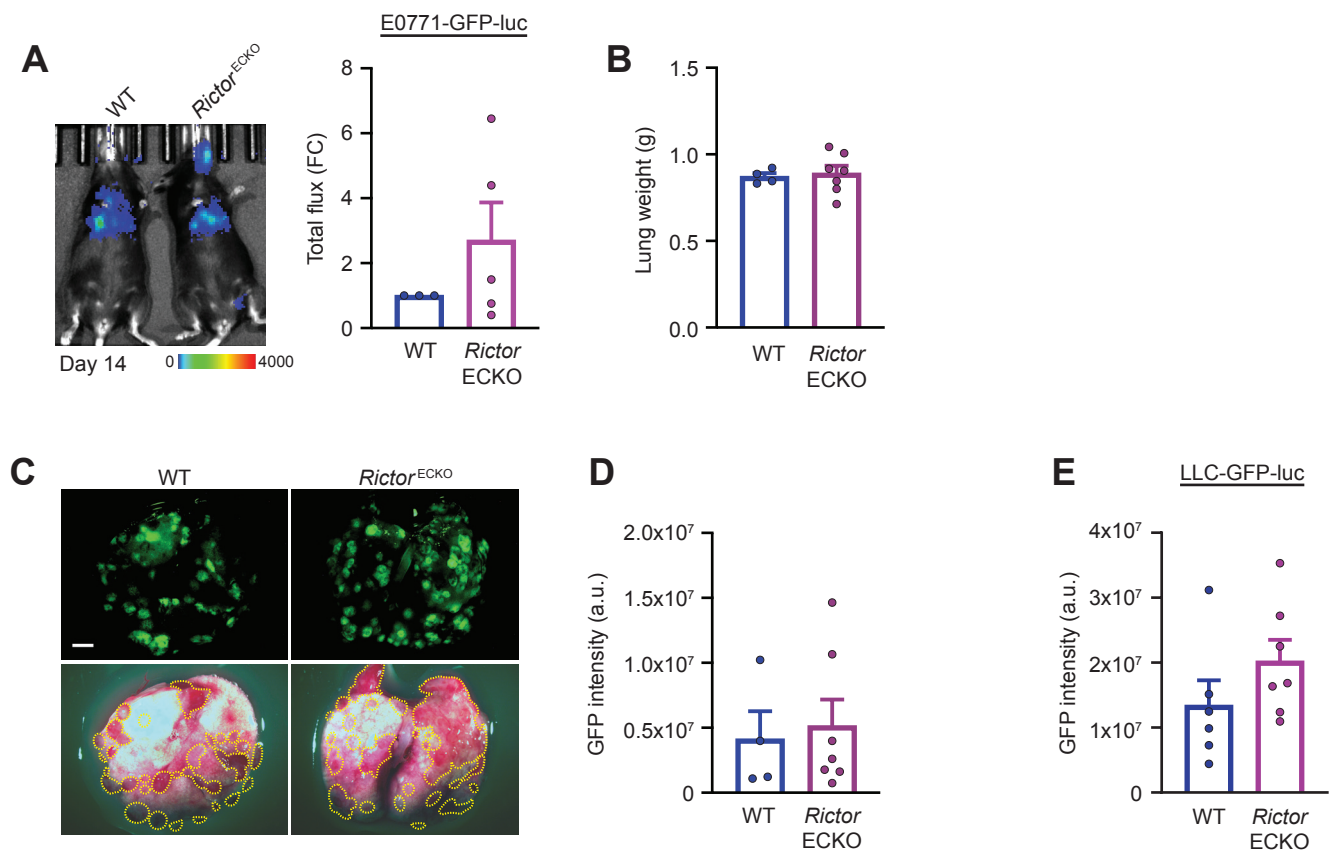
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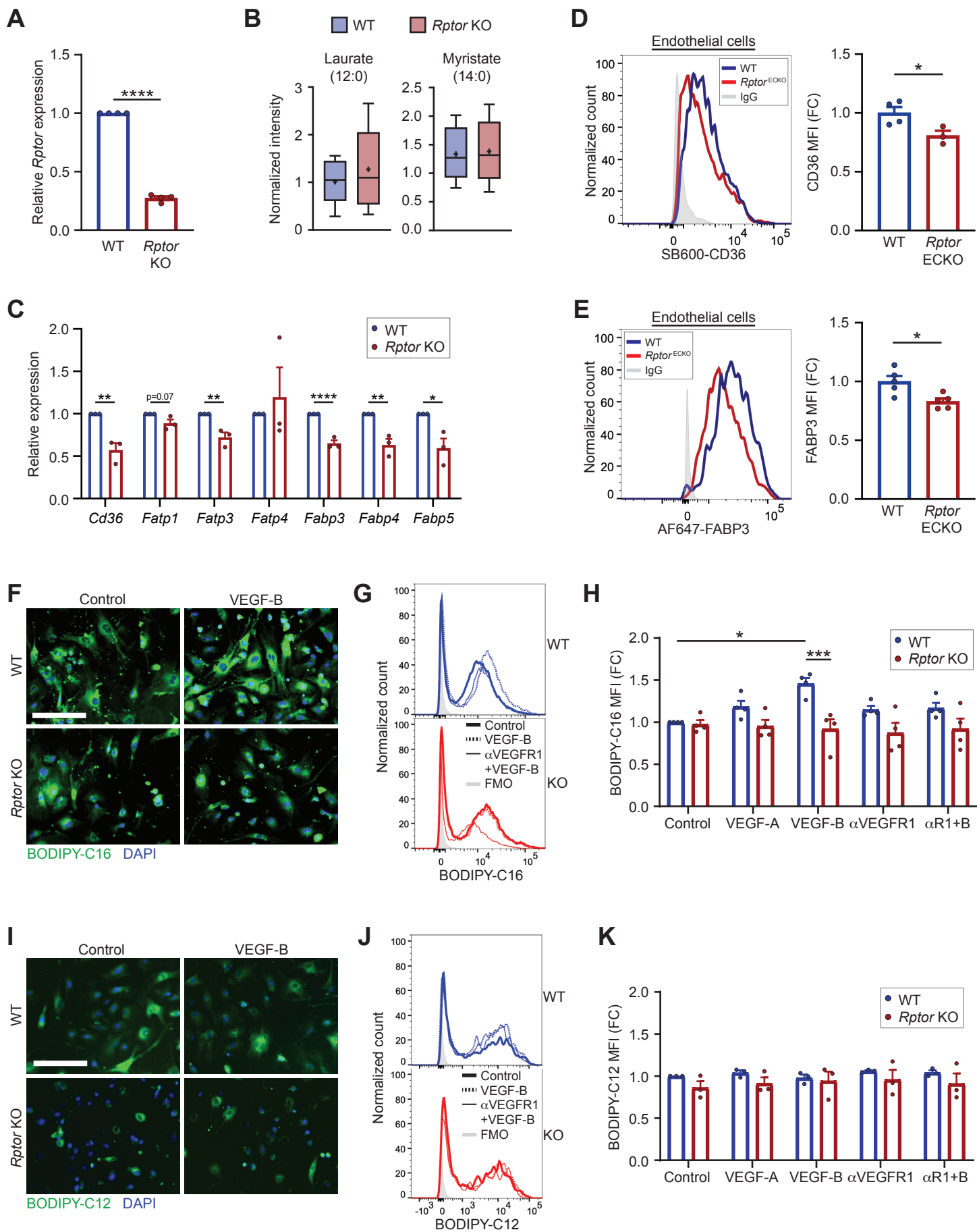
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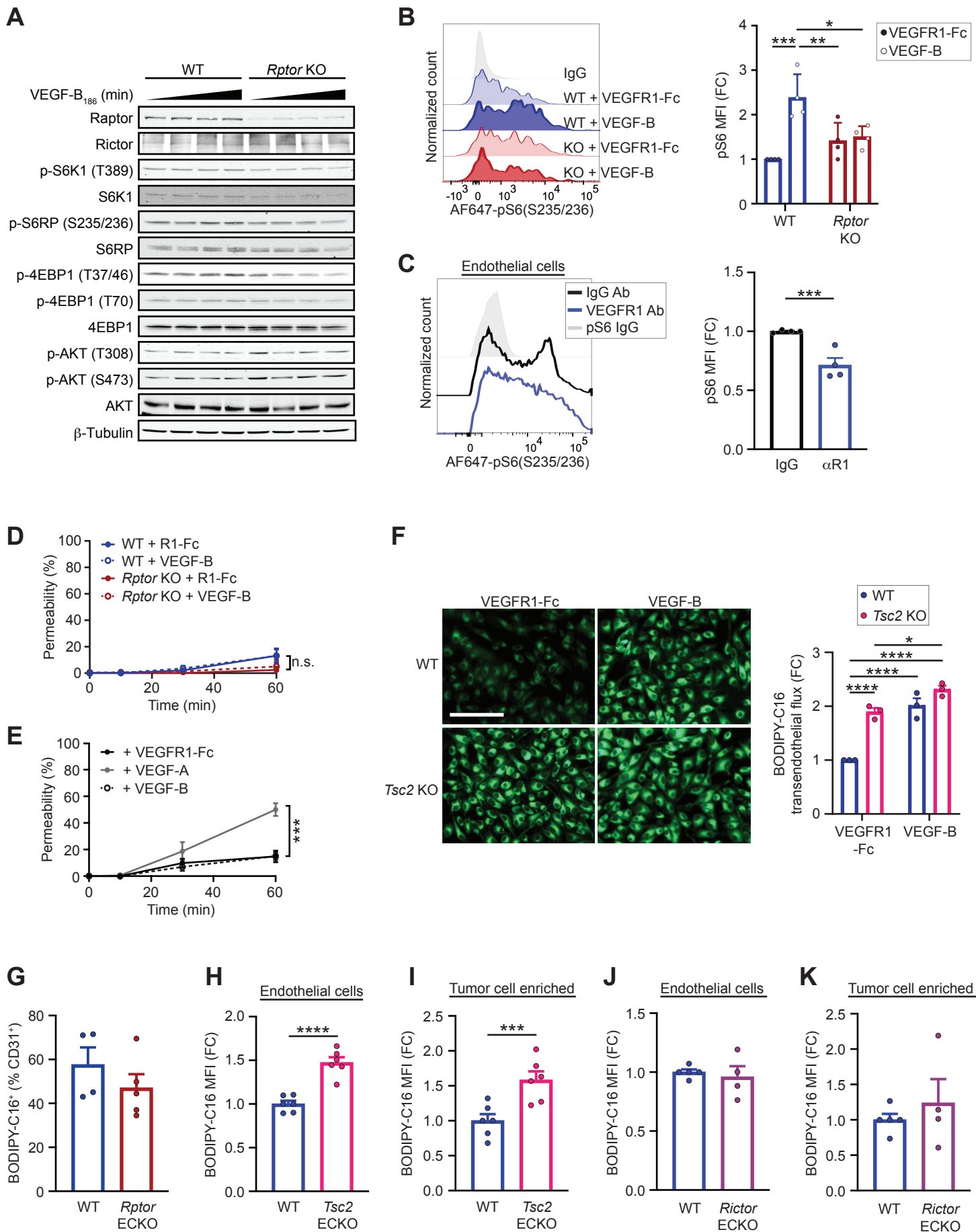
Supplemental Figure 1. Loss of Raptor/mTORC1 in endothelium reduces metastatic outgrowth of cancer cells in the lung. **(A)** WT (n=5) or *Rptor*^{ECKO} (n=7) female mice were treated with tamoxifen for 5 days and lungs were harvested 1 week following last treatment. Phospho-S6 (pS6) (S235/236) median fluorescence intensity (MFI) and percentage of pS6^{High} CD31⁺ endothelial cells was determined by flow cytometry, normalized to WT littermate controls. Unpaired t-test: pS6 MFI, $p=3.23 \times 10^{-5}$; pS6^{High}, $p=2.04 \times 10^{-4}$. **(B)** Representative bioluminescence images from Day 0 of LLC-GFP-luc inoculated WT or *Rptor*^{ECKO} male mice. Scale bar shows counts. Total radiance flux was normalized to WT controls. WT (n=6), *Rptor*^{ECKO} (n=8). Unpaired t-test, $p=0.472$. **(C-D)** WT (n=8) or *Rptor*^{ECKO} (n=4) female mice were inoculated with MMTV-PyMT-GFP-luc cells, followed by tamoxifen treatment starting on day 4. Tumors were harvested on day 14. **(C)** Lung weights were recorded in grams (g) at harvest and **(D)** GFP intensity was calculated as arbitrary units (a.u.). Unpaired t-test, $p=9.75 \times 10^{-3}$ for lung weights and $p=6.30 \times 10^{-3}$ for GFP intensity. **(E-G)** WT (n=6) or *Rptor*^{ECKO} (n=7) female mice were inoculated with E0771-GFP-luc cells as described in (B). **(E)** Representative bioluminescence images are shown from day 14. Scale bar shows counts. Total radiance flux was normalized to WT controls. Unpaired t-test, $p=1.65 \times 10^{-3}$. Tumors were harvested on day 18. **(F)** Lung weights were recorded in grams (g) at harvest and **(G)** GFP intensity was calculated. Unpaired t-test, $p=0.0131$ for lung weights and $p=3.97 \times 10^{-3}$ for GFP intensity. **(H)** WT (n=9) or *Rptor*^{ECKO} (n=8) female mice were inoculated with E0771-luc cells as described in (E-G). Representative lung H&E images are shown, with 3x magnified region indicated by the red box. Blood vessels are denoted by yellow arrows. Scale bars are 100 μ m. **(I)** Primary microvascular endothelial cells were isolated from *Tsc2*^{fl/fl} mice were transduced with control (WT) or cre-expressing (*Tsc2* KO) adenovirus. Immunofluorescence was used to confirm *Tsc2* deletion (n=4 per group). Representative images of TSC2 (green) are shown. Nuclei are stained with DAPI (blue). Scale bar is 100 μ m. Fluorescence intensity was normalized to WT control. Unpaired t-test, $p=8.11 \times 10^{-3}$. * $p<0.05$, ** $p<0.01$, *** $p<0.005$, **** $p<0.001$. Related to Figure 1.



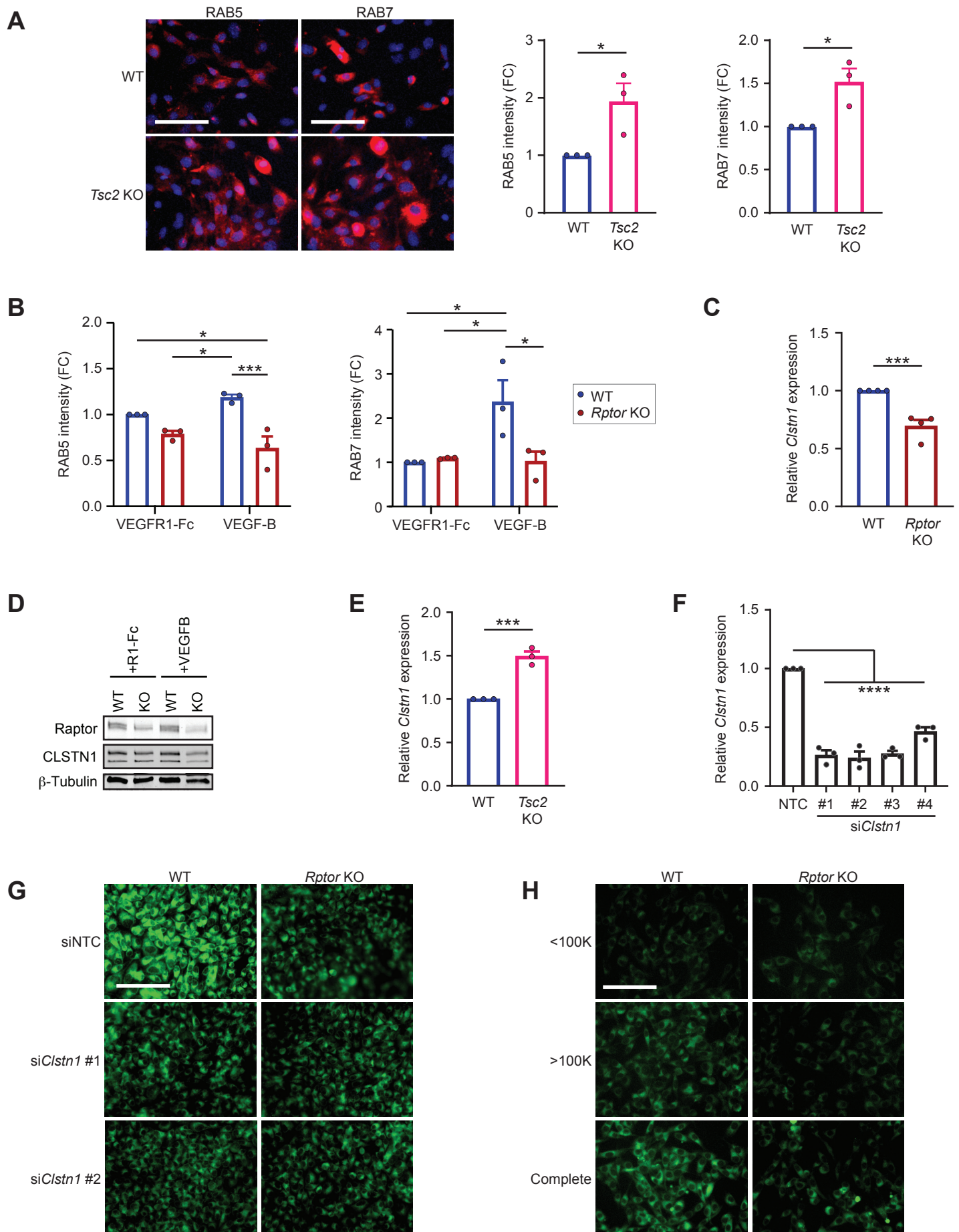
Supplemental Figure 2. Loss of Rictor/mTORC2 in endothelium does not significantly alter metastatic outgrowth of cancer cells in the lung. (A-D) WT (n=3) or *Rictor*^{ECKO} (n=5) female mice were inoculated with E0771-GFP-luc cells and treated with tamoxifen as described in Figure 1. (A) Representative bioluminescence images from day 14 are shown. Scale bar shows counts. Total radiance flux was normalized to WT controls. Unpaired t-test, p=0.318. (B) Tumors were harvested on day 18, and lung weights were recorded in grams (g). WT (n=4), *Rictor*^{ECKO} (n=7). Unpaired t-test, p=.767. (C) Representative GFP (top) and gross (bottom) lung images are shown. Scale bar is 5 mm. Visible tumor area is outlined by yellow line. (D) GFP intensity was calculated as arbitrary units (a.u.). WT (n=4), *Rictor*^{ECKO} (n=7). Unpaired t-test, p=0.753. (E) WT (n=6) or *Rictor*^{ECKO} (n=7) male mice were inoculated with LLC-GFP-luc cells as described above. Tumors were harvested on day 18. Lung weights were recorded. Unpaired t-test, p=0.204. Related to Figure 1.



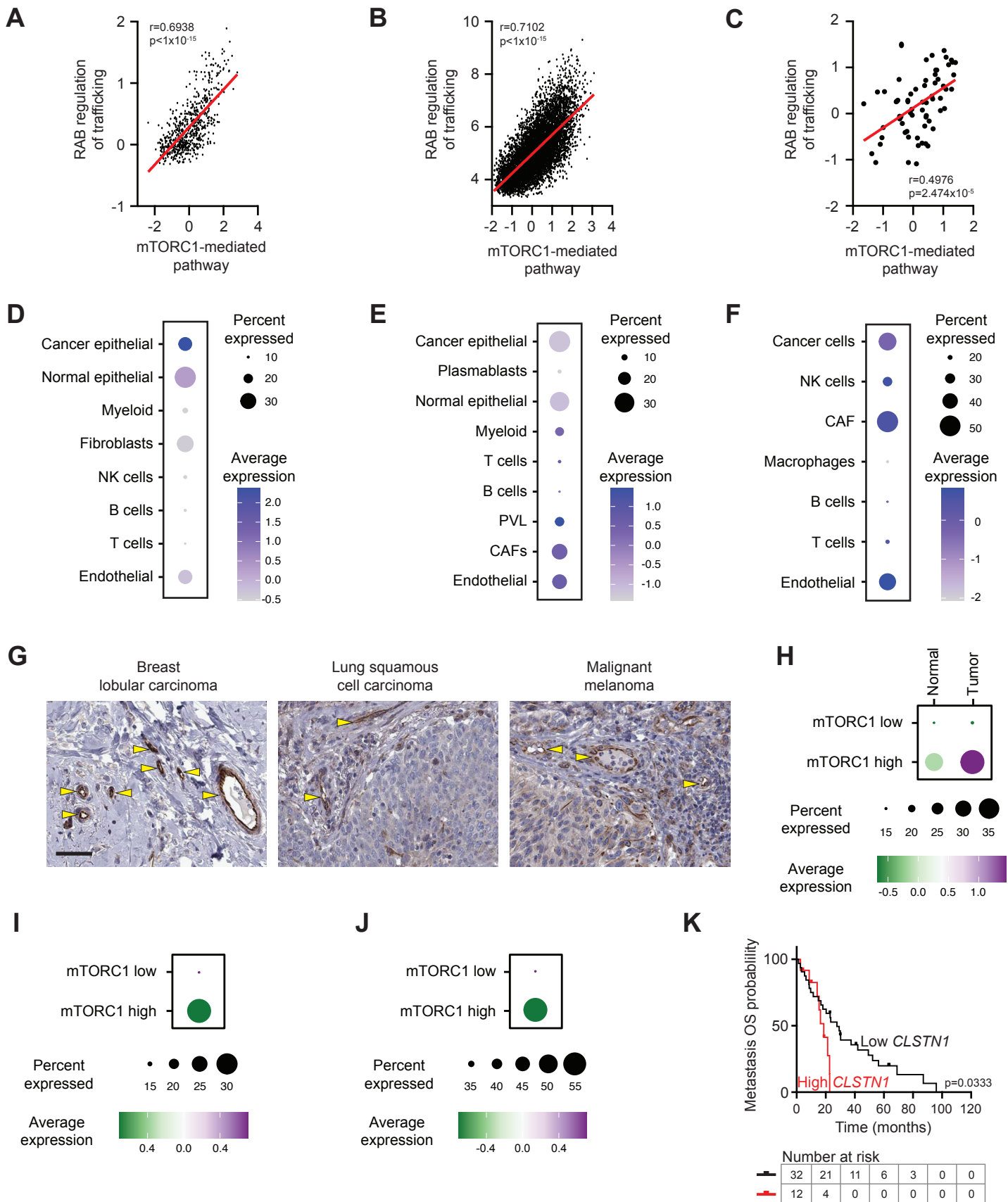
Supplemental Figure 3. Targeting Raptor/mTORC1 reduces fatty acid long-chain fatty acid content in endothelial cells. (A-B) Metabolomics was performed on primary microvascular endothelial cells isolated from *Rptor*^{fl/fl} mice transduced with control (WT) or cre recombinase (KO) adenoviruses, as described in Figure 2. (A) Rptor deletion was confirmed by reduced *Rptor* RNA expression by qRT-PCR (n=4 per group). Unpaired t-test, $p=6.19 \times 10^{-9}$. (B) Normalized intensities ($\log_2+1$) of representative medium chain fatty acid (MCFA) metabolites (n=6 per group). The median along with the 25th and 75th percentile hinges are indicated within the box. The whiskers indicate minimum and maximum values within each group. The mean is shown as a plus sign (“+”). Welch’s t-test; 12:0, $p=0.533$; 14:0, $p=0.879$. (C) Relative expression of fatty acid transporters and fatty acid binding proteins was determined by qRT-PCR (n=4 per group). Unpaired t-test, *Cd36*, $p=0.00619$; *Slc27a1/Fatp1*, $p=0.0685$; *Slc27a3/Fatp3*, $p=0.00619$; *Slc27a4/Fatp4*, $p=0.610$; *Fabp3*, $p=0.000741$; *Fabp4*, $p=0.00672$; *Fabp5*, $p=0.0236$. (D-E) WT or *Rptor*^{ECKO} female mice were treated with tamoxifen for 5 days, and lungs were harvested 1 week following last treatment. Median fluorescence intensity (MFI) of (D) CD36 and (E) FABP3 was determined by flow cytometry in CD31⁺ endothelial cells, normalized to WT littermate controls. CD36: WT (n=4), *Rptor*^{ECKO} (n=3); FABP3: WT (n=5), *Rptor*^{ECKO} (n=5). Unpaired t-test; CD36, $p=0.0392$; FABP3, $p=0.0140$. (F-K) Primary microvascular endothelial cells were isolated from *Rptor*^{fl/fl} mice, and deletion was carried out by transduction with control (WT) or cre-expressing (KO) adenovirus for 48 hours. Cells were then treated with VEGF-B (300 ng/mL), VEGF-A (300 ng/mL), anti-VEGFR1 (1 μ g/mL), or anti-VEGFR1+VEGF-B (α R1+B) for 30 hours in free fatty acid BSA (FFA-BSA) supplemented media. Control cells were untreated. Cells were immediately incubated with (F-H) BODIPY FL C16 (BODIPY-C16, 20 μ g/mL) or (I-K) BODIPY-C12 (20 μ g/mL) for 3 min. (F) Representative images of BODIPY-C16 (green) are shown. Nuclei were stained with DAPI (blue). Scale bar is 100 μ m. (G-H) BODIPY-C16 fluorescence was analyzed by flow cytometry (n=4 per group). (G) Representative histogram plots of Control, VEGF-B, and anti-VEGFR1+VEGF-B groups are shown. (H) BODIPY-C16 median fluorescence intensity (MFI) was normalized to WT Control. Two-way ANOVA ($p=1.56 \times 10^{-5}$) with Tukey’s post hoc. (I) Representative images of BODIPY-C12 (green) are shown. Nuclei were stained with DAPI (blue). Scale bar is 100 μ m. (J-K) BODIPY-C12 fluorescence was analyzed by flow cytometry (n=3 per group). (J) Representative histogram plots of Control, VEGF-B, and anti-VEGFR1+VEGF-B are shown. (K) BODIPY-C12 MFI was normalized to WT Control. Two-way ANOVA ($p=0.0339$) with Tukey’s post hoc, which revealed no significant comparisons. * $p<0.05$, ** $p<0.01$, *** $p<0.005$, **** $p<0.001$. Related to Figure 2 and Supplemental Table 1.



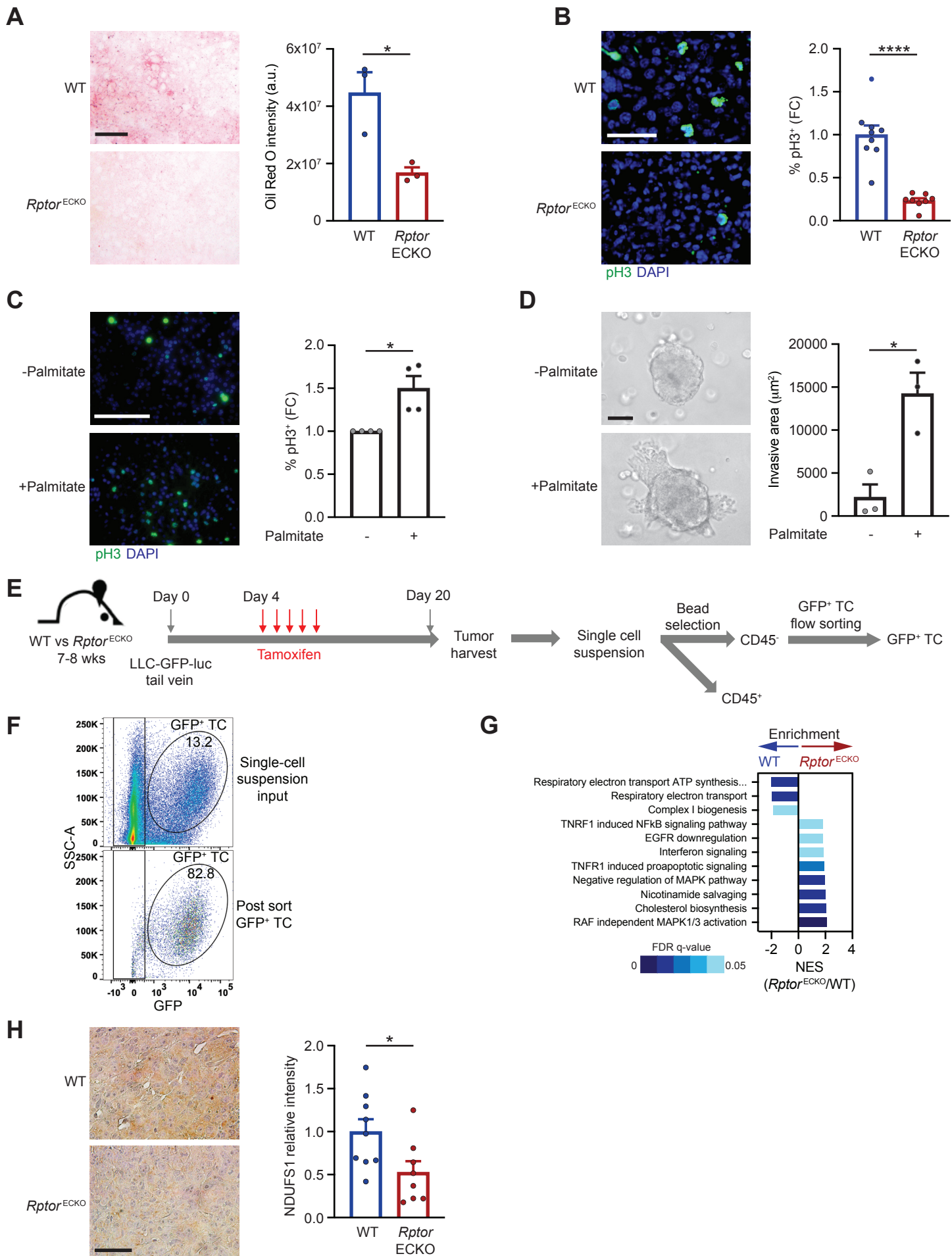
Supplemental Figure 4. Endothelial Raptor/mTORC1 promotes transendothelial transport of fatty acids in response to VEGF-B. (A-B) Primary microvascular endothelial cells were isolated from *Rptor*^{fl/fl} mice, and deletion was carried out by transduction with control (WT) or cre-expressing (KO) adenovirus for 48 hrs. **(A)** Cells were serum starved overnight and stimulated with VEGF-B (300 ng/mL) for 0, 5, 15, or 30 min. Lysates were harvested for signaling analysis by immunoblotting. Blot is representative of two independent experiments. **(B)** Cells were serum starved as described in (A) and treated with VEGFR1-Fc (1 μ g/mL) or VEGF-B (300 ng/mL) for 30 hr. Cells were analyzed for pS6 (S235/236) by flow cytometry (n=4 per group). Two-way ANOVA (p=0.00316) with Tukey's post hoc. **(C)** Mice bearing E0771-GFP-luc lung metastases were treated with anti-VEGFR1 antibody or IgG control (n=4 per group). CD31⁺ endothelial cells from harvested tumors were analyzed for pS6 as in (B). Unpaired t-test, p=0.003259. **(D-E)** Endothelial permeability assay was performed by detecting diffusion of Texas Red (TR)-Dextran across a transwell coated with a confluent top layer of WT or *Rptor* KO primary microvascular endothelial cells. Cells were treated with VEGFR1-Fc, VEGF-B, or VEGF-A for 30 hrs immediately before performing the assay. Media was removed from the upper chamber at 0, 10, 30, and 60 min and fluorescence was analyzed on a plate reader (n=3-4 per group). Two-way ANOVA (D: p=0.2809; E: p=2.949x10⁻⁶) with Tukey's post hoc. **(F)** Transendothelial transport assay of WT or *Tsc2* KO primary vascular endothelial cells (n=3 per group), as described in Figure 3. Representative images of BODIPY-C16 (green) in LLC tumor cells. Scale bar is 100 μ m. BODIPY-C16 intensity was normalized to WT + VEGFR1-Fc control. Two-way ANOVA (p=8.04x10⁻³) with Tukey's post hoc. **(G)** WT (n=4) or *Rptor*^{ECKO} (n=5) male mice were inoculated with LLC cells and BODIPY-C16 fluorescence was determined by flow cytometry as described in Figure 3E-F. Percentage (%) of BODIPY⁺ CD31⁺ endothelial cells are shown. Unpaired t-test, p=0.3194. **(H-I)** WT (n=6) or *Tsc2*^{ECKO} (n=6) female mice were inoculated with E0771-luc cells and injected with BODIPY FL C16 (BODIPY-C16), as described in Figure 3. BODIPY-C16 median fluorescence intensity (MFI) was determined by flow cytometry in **(H)** CD45⁻CD31⁺ endothelial cells and **(I)** CD45⁻CD31⁺FSC^{hi} tumor-cell enriched populations and normalized to WT controls. Representative histograms are shown. Unpaired t-test; CD31⁺, p=6.26x10⁻⁵; tumor-cell enriched, p=3.90x10⁻³. **(J-K)** WT (n=5) or *Rictor*^{ECKO} (n=4) male mice were inoculated with LLC cells and injected with BODIPY FL C16 (BODIPY-C16), as described above. BODIPY-C16 MFI was determined by flow cytometry in **(J)** CD45⁻CD31⁺ endothelial cells and **(K)** CD45⁻CD31⁺FSC^{hi} tumor-cell enriched populations and normalized to WT controls. Representative histograms are shown. Unpaired t-test; CD31⁺, p=0.577; tumor-cell enriched, p=0.376. *p<0.05, **p<0.01, ***p<0.005, ****p<0.001. Related to Figure 3.



Supplemental Figure 5. mTORC1 increases RAB endosomes and regulates CLSTN1 expression to support transendothelial transport of fatty acids in endothelial cells. (A) Immunofluorescence of RAB5 (left) or RAB7 (right) was performed on WT or *Tsc2* KO primary microvascular endothelial cells cultured in complete endothelial cell media (n=3 per group). Representative immunofluorescence images of RAB5 and RAB7 are shown (both red). Nuclei are stained with DAPI (blue). Scale bars are 100 μ m. RAB5 or RAB7 intensities were normalized to WT controls. Unpaired t-test; RAB5, p=0.0371; RAB7, p=0.0255. (B) WT or *Rptor* KO primary microvascular endothelial cells cultured in basal media supplemented with FFA-BSA were stimulated with VEGFR1-Fc (1 μ g/mL) or VEGF-B (300 ng/mL) for 30 hours (n=3 per group). Immunofluorescence and intensities were performed as in (A). Two-way ANOVA (RAB5, p=0.0372; RAB7, p=0.0283) with Tukey's post hoc. (C-D) WT or *Rptor* KO primary microvascular endothelial cells were assessed for *Clstn1* expression by (C) qRT-PCR (n=4 per group) and (D) immunoblotting (image representative of 3 independent experiments). Unpaired t-test: *Clstn1* expression, p=1.27x10⁻³. (E) *Clstn1* expression was analyzed in WT or *Tsc2* KO primary microvascular endothelial cells by qRT-PCR (n=3 per group). Unpaired t-test, p=9.96x10⁻⁴. (F) Knockdown of *Clstn1* using siRNA was confirmed by reduced *Clstn1* expression in *Rptor*^{fl/fl} primary microvascular endothelial cells treated with control adenovirus (n=3 per group). One-way ANOVA (p=1.84x10⁻⁷) with Dunnett's post hoc. (G) Representative images of LLC tumor cells from transendothelial transport of BODIPY-C16 (green) in non-targeting control (NTC) or *Clstn1* knockdown endothelial cells from Figure 4. Scale bar is 100 μ m. (H) Representative images of LLC tumor cells cultured in small (<100 kDa) or large (>100 kDa) fractionated endothelial cell conditioned media from Figure 4. Images from complete conditioned media are also shown. Scale bar is 100 μ m. *p<0.05, ***p<0.005, ****p<0.001. Related to Figure 4.

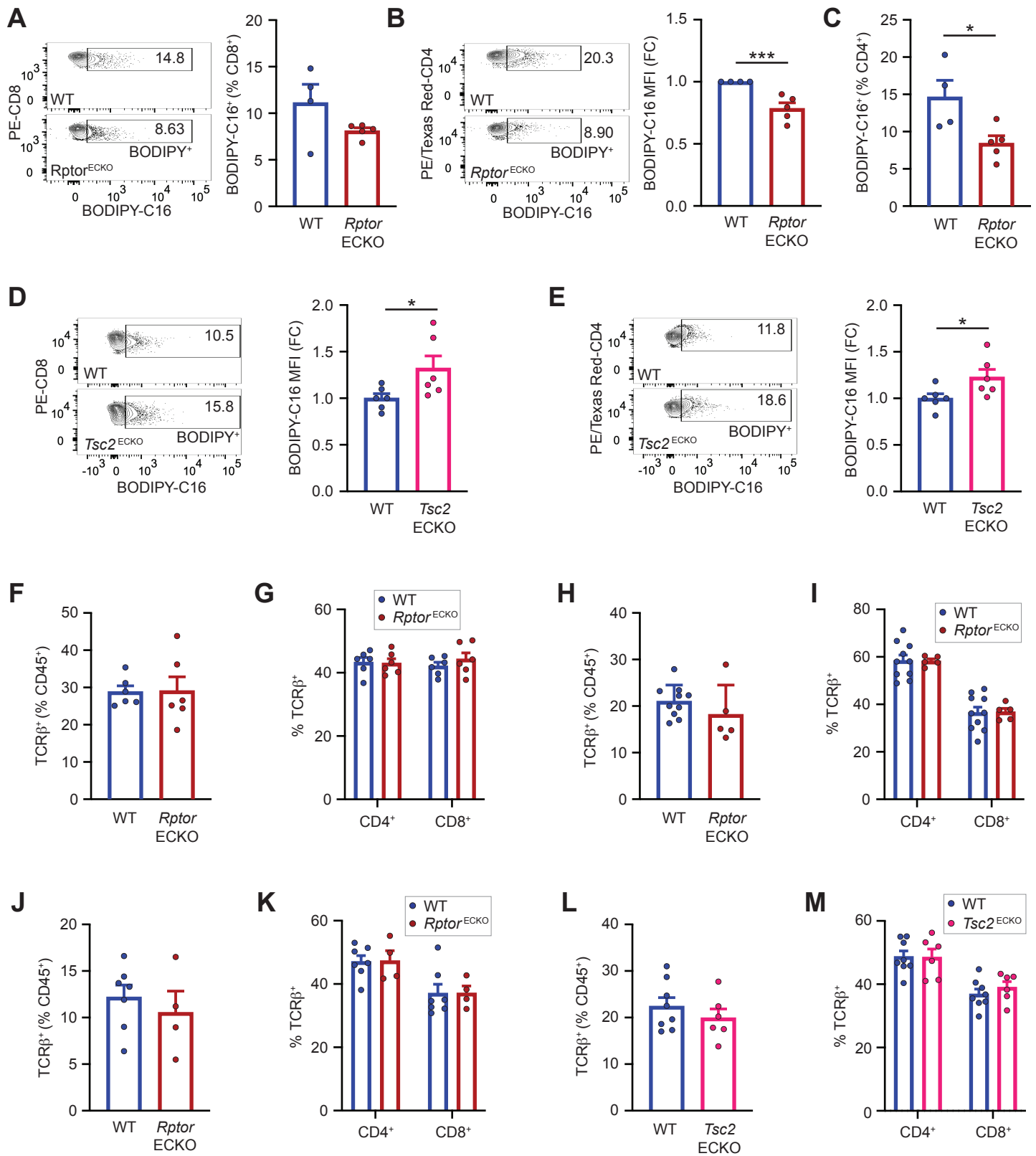


Supplemental Figure 6. mTORC1 signaling correlates with RAB trafficking and *CLSTN1* expression in human tumor-associated endothelial cells. (A-C) mTORC1 signaling positively correlates with RAB regulation of trafficking gene set in endothelial cells from the (A) lung adenocarcinoma (Bischoff, et al.), (B) breast cancer (GSE176078), and (C) melanoma (GSE72056) single-cell RNA-seq datasets. The x and y axis represents z-scores of ssGSEA enrichment of indicated gene sets. (D-E) Dot plots of *CLSTN1* expression in cell populations from (D) lung adenocarcinoma, (E) breast cancer, and (F) melanoma datasets above. (G) Immunohistochemistry of *CLSTN1* from patient samples showing strong staining in vascular endothelial regions, denoted by yellow arrows. Images were acquired from the Human Protein Atlas. (H) Dot plot of *CLSTN1* expression in normal or tumor-associated endothelial cells, stratified based on mTORC1 signaling ssGSEA enrichment scores, in the lung adenocarcinoma dataset used above. (I-J) Dot plot of *CLSTN1* expression in tumor-associated endothelial cells in (I) breast cancer and (J) melanoma datasets described above. mTORC1 signaling stratification was performed as described in (H). (K) Overall survival (OS) of metastatic breast cancer patients stratified by low (quartiles 1-3, black) or high (quartile 4, red) *CLSTN1:PECAM1* ratio. Data collected from the AURORA dataset (GSE209998). Hazard ratio is 2.114 (95% CI: 0.8157 - 5.481). Related to Figure 4 and Supplemental Table 6.

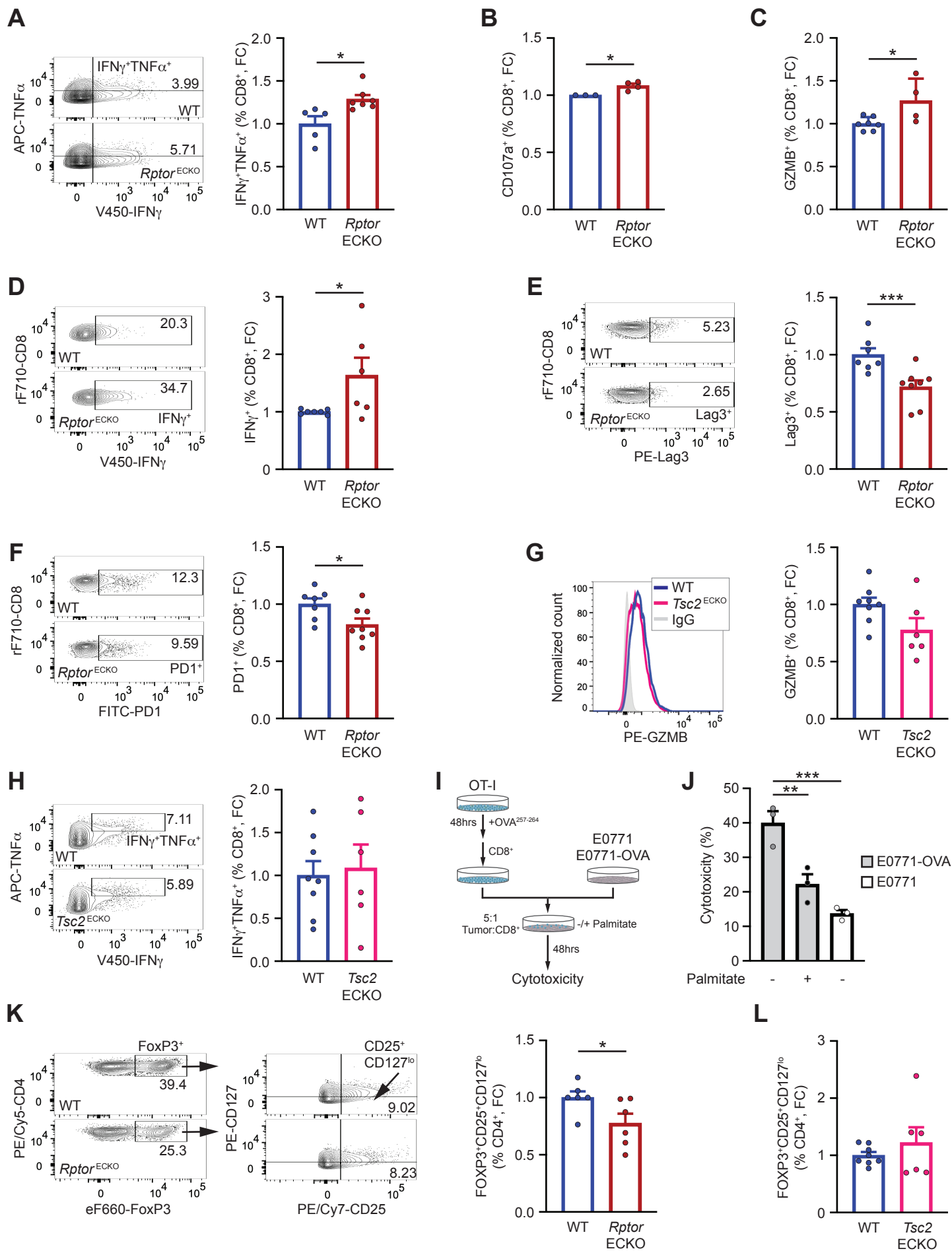


Supplemental Figure 7. Endothelial mTORC1 promotes tumor cell invasion and proliferation.

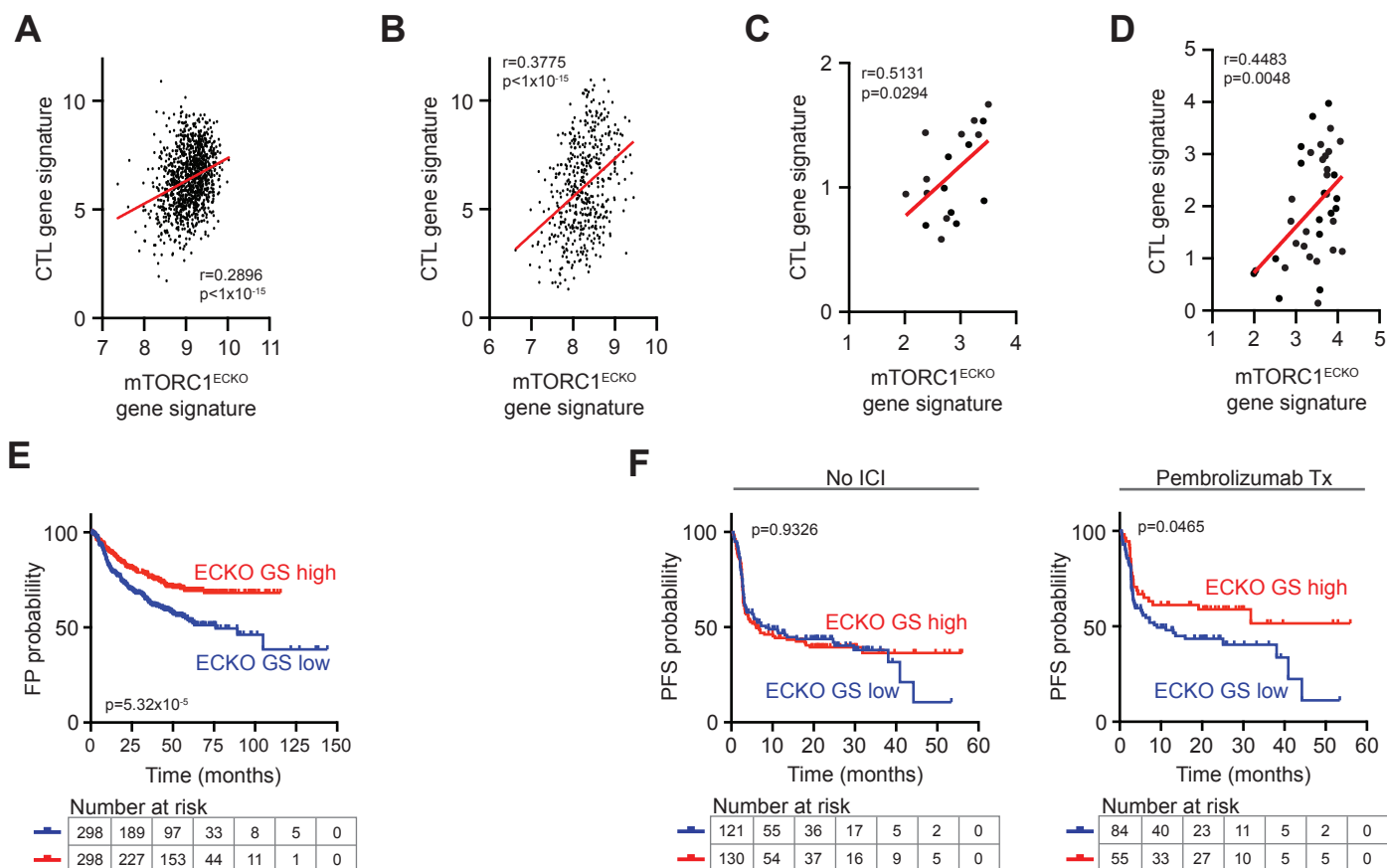
(A) WT or *Rptor*^{ECKO} male mice were inoculated with LLC-GFP-luc cells, as in Figure 1A. Harvested lung were cryopreserved, and sections were stained with Oil Red O (n=3 per group). Representative images are shown. Scale bar is 50µm. Oil Red O intensities are shown. Unpaired t-test, p=0.0204. **(B)** WT (n=9) or *Rptor*^{ECKO} (n=8) female mice were inoculated with E0771-luc cells as in Figure 1. Fluorescent immunohistochemistry for phospho-H3 (pH3, green) was performed, and percent positive nuclei was determined. Nuclei are stained with DAPI (blue). Representative images are shown. Scale bar is 50 µm. Unpaired t-test, p=8.91x10⁻⁶. **(C-D)** E0771 cells were cultured in the absence or presence of 50 µM of palmitate-BSA conjugate for 48 hrs. **(C)** Immunofluorescence of phospho-H3 (pH3, green) was performed (n=4 per group). Representative images are shown. Nuclei are stained with DAPI (blue). Scale bar is 100 µm. Percentages of positive cells are shown. Unpaired t-test, p=0.0128. **(D)** 3D sphere invasion assay was performed (n=3 per group). Mean invasive area (µm²) of spheroids is shown. Unpaired t-test, p=0.0138. **(E)** Schematic of GFP⁺ tumor cell sorting of WT and *Rptor*^{ECKO} lung metastatic tumors. **(F)** Flow cytometry confirmed enrichment of GFP⁺ tumor cells (TC) after sorting. Representative data are shown. **(G)** Sorted GFP⁺ tumor cells from WT or *Rptor*^{ECKO} lung metastatic tumors were analyzed by RNA-seq (n=4 per group) and differentially expressed genes were identified. GSEA was performed to identify pathway enrichment. Normalized enrichment score (NES) and false discovery rate (FDR) q-values are indicated. **(H)** Immunohistochemistry of NDUFS1 (brown) in WT (n=9) or *Rptor*^{ECKO} (n=8) metastatic tumors formed from E0771-luc cell inoculation as in Figure 1A. Nuclei were stained with hematoxylin (blue). Scale bar is 50 µm. Intensities were normalized to controls. Unpaired t-test, p=0.0290. *p<0.05, ****p<0.001. Related to Figure 5.



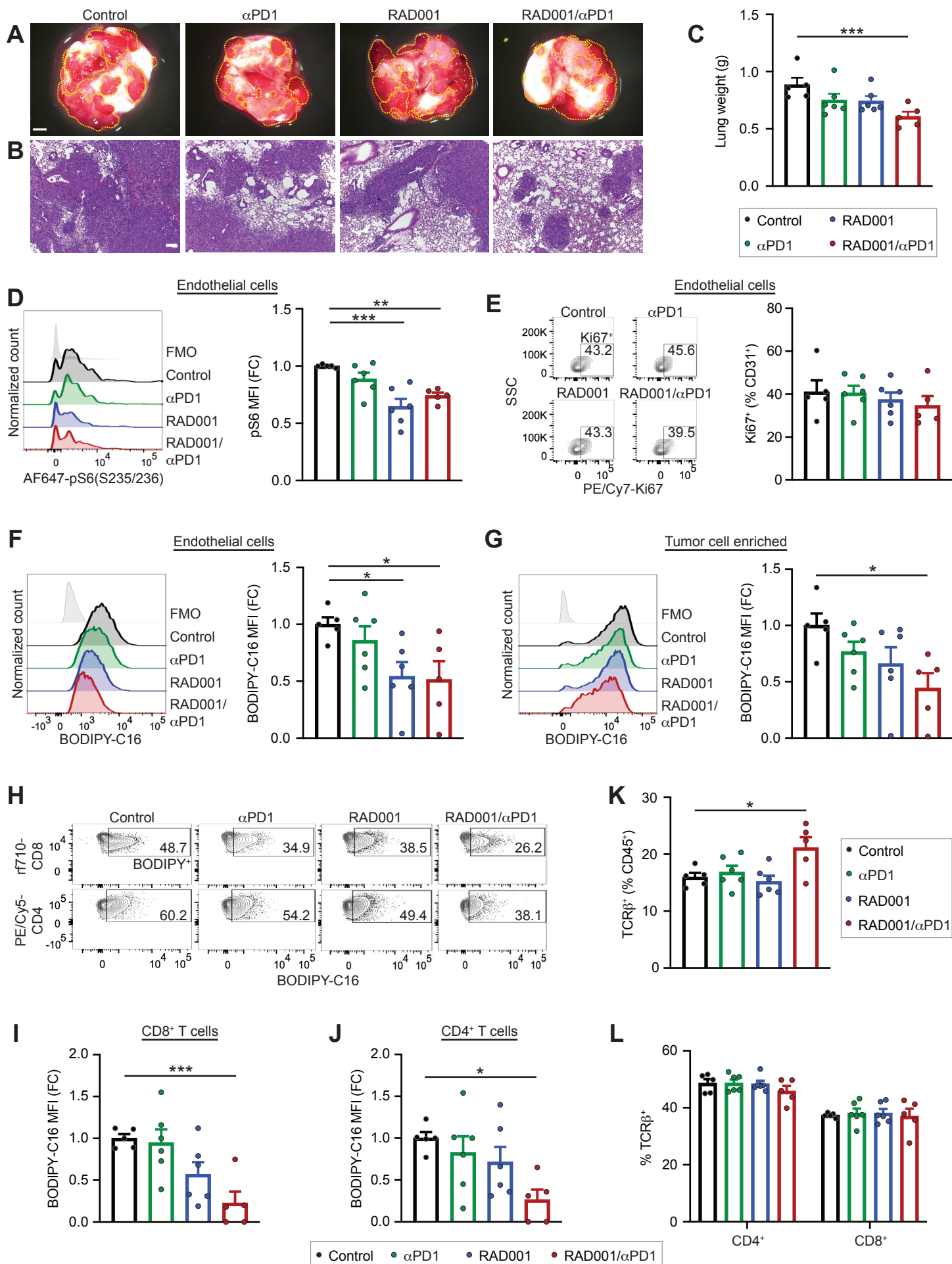
Supplemental Figure 8. Endothelial mTORC1 promotes BODIPY-C16 uptake in tumor-associated T cells. (A-C) WT (n=4) or *Rptor*^{ECKO} (n=5) male mice were inoculated with LLC tumors cells and injected with BODIPY-C16, as described in Figure 5. BODIPY-C16⁺ cells are shown as (A) a percent of CD8⁺ T cells, (B) median fluorescence intensity (MFI) in CD4⁺ T cells, or (C) percent of CD4⁺ T cells. Representative plots are shown. BODIPY-C16 MFI was normalized to WT controls, presented as fold change (FC). Unpaired t-test, BODIPY⁺ (% CD8⁺), p=0.132; CD4⁺ MFI, p=4.33x10⁻³; BODIPY⁺ (% CD4⁺), p=0.030. (D-E) WT (n=6) or *Tsc2*^{ECKO} (n=6) female mice were inoculated with E0771-luc tumor cells and injected with BODIPY-C16, as in (A-C). Representative plots of BODIPY-C16 in (D) CD8⁺ T cells or (E) CD4⁺ T cells are shown. BODIPY-C16 median fluorescence intensity (MFI) was calculated and normalized to WT controls, presented as fold change (FC). Unpaired t-test; CD8⁺ T cells, p=0.0464; CD4⁺ T cells, p=0.0422. (F-G) WT (n=6) or *Rptor*^{ECKO} (n=6) female mice were inoculated with E0771-GFP-luc tumor cells. Metastatic tumors were analyzed by flow cytometry. (F) T cells (TCRβ⁺) and (G) CD4⁺/CD8⁺ T cells are shown as percentage (%) of CD45⁺ or TCRβ⁺, respectively. Unpaired t-test: TCRβ⁺, p=0.946; CD4⁺, p=0.868; CD8⁺, p=0.369. (H-I) WT (n=10) or *Rptor*^{ECKO} (n=5) female mice were inoculated with MMTV-PyMT-GFP-luc tumor cells. Metastatic tumors were analyzed for (H) T cells (TCRβ⁺) and (I) CD4⁺/CD8⁺ T cells, as described in (F-G). Unpaired t-test: TCRβ⁺, p=0.275; CD4⁺, p=0.960; CD8⁺, p=0.932. (J-K) WT (n=7) or *Rptor*^{ECKO} (n=4) male mice were inoculated with LLC-GFP-luc tumor cells. Metastatic tumors were analyzed for (J) T cells (TCRβ⁺) and (K) CD4⁺/CD8⁺ T cells, as described above. Unpaired t-test: TCRβ⁺, p=0.515; CD4⁺, p=0.944; CD8⁺, p=0.997. (L-M) WT (n=8) or *Tsc2*^{ECKO} (n=6) female mice were inoculated with E0771-GFP-luc tumor cells. Metastatic tumors were analyzed for (L) T cells (TCRβ⁺) and (M) CD4⁺/CD8⁺ T cells, as described above. Unpaired t-test: TCRβ⁺, p=0.376; CD4⁺, p=0.958; CD8⁺, p=0.396. *p<0.05, ***p<0.005. Related to Figure 5.



Supplemental Figure 9. Endothelial Raptor/mTORC1 loss improves anti-tumor immune activity of CD8⁺ T cells. (A-B) WT or *Rptor*^{ECKO} female mice were inoculated with E0771-luc cells and lung metastatic tumors were analyzed by flow cytometry, as described in Figure 5. (A) TNF α ⁺IFN γ ⁺ and (B) CD107a⁺ CD8⁺ T cells were normalized to WT controls and shown as fold change (FC). TNF α ⁺IFN γ ⁺: WT (n=5), *Rptor*^{ECKO} (n=7); CD107a⁺: WT (n=3), *Rptor*^{ECKO} (n=4). Unpaired t-test; TNF α ⁺IFN γ ⁺, p=0.0128; CD107⁺, p=0.0144. (C) WT (n=7) or *Rptor*^{ECKO} (n=4) female mice were inoculated with MMTV-PyMT cells and lung metastatic tumors were analyzed by flow cytometry. CD8⁺GZMB⁺ cells were normalized, as described in (A). Unpaired t-test, p=0.0274. (D) WT (n=7) or *Rptor*^{ECKO} (n=6) male mice were inoculated with LLC tumor cells and lung metastatic tumors were analyzed by flow cytometry. CD8⁺IFN γ ⁺ cells were normalized as in (A). Unpaired t-test, p=0.0473. (E-F) Metastatic tumor samples from (A) were analyzed for (E) Lag3 or (F) PD1. WT (n=7), *Rptor*^{ECKO} (n=8). Unpaired t-test; Lag3⁺, p=0.00415; PD1⁺, p=0.0303. (G-H) WT (n=8) or *Tsc2*^{ECKO} (n=6) female mice were inoculated and analyzed for (G) GZMB or (H) IFN γ /TNF α , as described in (A). Unpaired t-test; GZMB⁺, p=0.0709; IFN γ ⁺TNF α ⁺, p=0.781. (I-J) The effects of palmitate on T cell cytotoxicity was determined. (I) Schematic of co-culture cytotoxicity assay. OT-I splenocytes were activated with OVA²⁵⁷⁻²⁶⁴ (SIINFEKL) peptide for 48 hrs and positively selected for CD8. CD8⁺ OT-I T cells were co-cultured with E0771-OVA cells in the absence or presence of 50 μ M palmitate for 48 hrs, in a 5:1 tumor cell to T cell ratio. (J) Percent cytotoxicity was determined by LDH release (n=3 per group). E0771 parental cells do not express ovalbumin and were used as an antigen-free background control. One-way ANOVA (p=0.00126) with Tukey's post hoc. (K) WT (n=6) or *Rptor*^{ECKO} (n=6) female mice were inoculated with E0771 tumor cells, and lung metastatic tumors were analyzed by flow cytometry, as described in Figure 5. CD4⁺FoxP3⁺ Tregs were assessed for the activation markers CD25⁺CD127^{lo}. Activated Tregs were normalized to WT controls and shown as fold change (FC). Unpaired t-test, p=0.0443. (L) WT (n=8) or *Tsc2*^{ECKO} (n=6) female mice were inoculated and analyzed as in (I). Unpaired t-test, p=0.375. *p<0.05, ***p<0.005. Related to Figure 5.



Supplemental Figure 10. Human tumors with a high mTORC1^{ECKO} gene signature correlate with improved anti-tumor T cell activity and improved progression-free survival. Differentially expressed genes in *Rptor*^{ECKO} tumor cells identified in Supplemental Figure 7G were used to generate an mTORC1^{ECKO} gene signature. **(A-D)** Correlations between the mTORC1^{ECKO} gene signature and a cytotoxic lymphocyte (CTL) gene signature in **(A)** TCGA LUNG (n=1129), **(B)** TCGA SKCM (n=474), **(C)** the Metastatic Breast Cancer Project (n=18), and **(D)** Metastatic Melanoma (n=38) datasets. **(E)** First progression (FP) survival of lung cancer patients, stratified by low (blue) or high (red) mTORC1^{ECKO} gene signature (ECKO GS). Number of at-risk patients in each group is shown. Hazard ratio (HR) is 0.5726 (95% CI: 0.4371 - 0.7502). **(F)** Progression-free survival (PFS) of melanoma patients treated with no immune checkpoint inhibitors (ICI, left) or pembrolizumab therapy (right). Patients were stratified by low (blue) or high (red) mTORC1^{ECKO} gene signature (ECKO GS). Number of at-risk patients in each group is shown. Hazard ratios (HR) for No ICI and Pembrolizumab therapy is 1.014 (95% CI: 0.7353 - 1.398) and 0.6101 (95% CI: 0.3839 - 0.9697), respectively. Related to Figure 5 and Supplemental Table 5.



Supplemental Figure 11. Low-dose RAD001 combined with anti-PD1 reduces intratumoral BODIPY-C16 transport in lung metastatic tumors. Female wild type mice were inoculated with E0771-luc cells, as described in Figure 5. Control (n=5), α PD-1 (n=6), RAD001 (n=6), RAD001/ α PD-1 (n=5). **(A)** Representative lung images show reduced surface tumors in RAD001/ α PD1 animals. Scale bar is 5 mm. Visible tumor area is outlined by the yellow line. **(B)** Representative images H&E staining indicates reduced micro-metastatic tumor burden in RAD001/ α PD1 lungs. Scale bar is 100 μ m. **(C)** Lung weights were recorded at harvest in grams (g). One-way ANOVA ($p=0.0167$) with Dunnett's post hoc. **(D-E)** Within the CD45⁺CD31⁺ endothelial cell population, **(D)** phospho-S6 (S235/236) median fluorescence intensity (MFI) and **(E)** percent Ki67⁺ cells was determined by flow cytometry. pS6 MFI was normalized to Control. One-way ANOVA (pS6, $p=4.26 \times 10^{-4}$; Ki67, $p=0.688$) with Dunnett's post hoc where appropriate. **(F-G)** BODIPY-C16 median fluorescence intensity (MFI) was determined by flow cytometry in **(F)** CD45⁺CD31⁺ endothelial cells and **(G)** CD45⁺CD31⁺FSC^{hi} tumor-cell enriched populations and normalized to Control. Representative histograms are shown. One-way ANOVA (CD31⁺, $p=0.0372$; tumor-cell enriched, $p=0.0443$) with Dunnett's post hoc. **(H-J)** BODIPY-C16 MFI was determined in **(I)** CD8⁺ and **(J)** CD4⁺ T cells, as described in (D-E). Representative plots are shown in **(H)**. One-way ANOVA (CD8⁺, $p=0.00327$; CD4⁺, $p=0.0367$) with Dunnett's post hoc. **(K-L)** T cell populations were determined from treated lung metastatic tumors. **(K)** T cells (TCR β ⁺) and **(L)** CD4⁺/CD8⁺ T cells are shown as percentage (%) of CD45⁺ or TCR β ⁺, respectively. One-way ANOVA (TCR β ⁺, $p=0.0182$; CD4⁺, $p=0.459$; CD8⁺, $p=0.959$) with Dunnett's post hoc where appropriate. * $p<0.05$, ** $p<0.01$, *** $p<0.005$. Related to Figure 5.

Table S2. Genotyping primers used in study.

Primer Name		Primer Sequence
iCdh5-Cre	Forward	5'-TCCTGATGGTGCCTATCCTC-3'
	Reverse	5'-CGAACCTGGTCGAAATCAGT-3'
Rptor	Forward	5'-CTCAGTAGTGGTATGTGCTCAG-3'
	Reverse	5'-GGGTACAGTATGTCAGCACAG-3'
Rictor	Forward	5'-GAAGTTATTCAGATGGCCCAGC-3'
	Reverse	5'-ACTGAATATGTTTCATGGTTGTG-3'
Tsc2	Forward	5'-TGGCAGGACAGAGGGTCATCATGG-3'
	Reverse	5'-TTCAGAGTCACCTGGCAGGCTCG-3'

Table S3. Mouse flow cytometry antibodies used in study.

Antibody	Clone	Dilution	Company	Catalog #
APC/Cy7-CD45	30-F11	1:500	BD Biosciences	557659
PerCP/Cy5.5-TCR β	H57-597	1:500	Tonbo/Cytek	65-5961
PE/Cy5-CD4	GK1.5	1:500	Tonbo/Cytek	55-0041
PE/Dazzle 594-CD4	GK1.5	1:500	Biolegend	100455
Red Fluor 710-CD8	53-6.7	1:400	Tonbo/Cytek	80-0081
PE-CD8	53-6.7	1:400	BD Biosciences	553033
V450-CD107a	1D4B	1:200	BD Biosciences	560648
PE-Lag3	C9B7W	1:400	eBioscience	12-2231-81
FITC-PD1	29F.1A12	1:100	Biolegend	135213
PE/Cy7-CD25	PC61.5	1:500	Tonbo/Cytek	60-0251
PE-CD127	A7R34	1:500	Tonbo/Cytek	50-1271
BV421-CD31	390	1:200	Biolegend	102423
PE/Cy7-EpCAM	G8.8	1:500	Biolegend	118215
Super Bright 600-CD36	HM36	1:500	eBioscience	63-0362-82
PE/Cy7-Ki67	B56	1:100	BD Biosciences	561283
PE-GZMB	NGZB	1:20	eBioscience	12-8898-82
V450-IFN γ	XMG1.2	1:100	Tonbo/Cytek	75-7311
APC-TNF α	MP6-XT22	1:50	eBioscience	17-7321-82
eFluor 660-FoxP3	FJK-16s	1:250	eBioscience	50-5773-82
PE-IgG2 α , κ	eBR2a	1:20	eBioscience	12-4321-81
APC-IgG1, κ	eBRG1	1:50	eBioscience	17-4301-82
FABP3		1:100	Invitrogen	PA5-13461
pS6 (S235/236)		1:100	Cell Signaling Tech.	2211

Table S4. Quantitative RT-PCR primers used in study.

Primer Name		Primer Sequence	Source
Mm- <i>C1stn1</i>	Forward	5'-GATGCCGTGGTAGTGGATAAG-3'	
	Reverse	5'-CCTGGATGGTGAATGTGTAGTC-3'	
Mm- <i>Rptor</i>	Forward	5'-CCTCTGTCCATATACGACCT-3'	
	Reverse	5'-CTGTGCAGTGCAAACCTGT-3'	
Mm- <i>Cd36</i>	Forward	5'-GATGAGCATAGGACATACTTAGATGTG-3'	Hagberg CE, et al. <i>Nature</i> . 464:917-921 (2010).
	Reverse	5'-CACCACTCCAATCCCAAGTAAG-3'	
Mm- <i>Fatp1</i>	Forward	5'-TCAATGTACCAGGAATTACAGAAGG-3'	Hagberg CE, et al. <i>Nature</i> . 464:917-921 (2010).
	Reverse	5'-GAGTGAGAAGTCGCCTGCAC-3'	
Mm- <i>Fatp3</i>	Forward	5'-CCTCGGTTTCTCAGGCTCCA-3'	Hagberg CE, et al. <i>Nature</i> . 464:917-921 (2010).
	Reverse	5'-CTGTACCGGGCAGGTGTGA-3'	
Mm- <i>Fatp4</i>	Forward	5'-GCAAGTCCCATCAGCAACTG-3'	Hagberg CE, et al. <i>Nature</i> . 464:917-921 (2010).
	Reverse	5'-GGGGGAAATCACAGCTTCTC-3'	
Mm- <i>Fabp3</i>	Forward	5'-TTCAGCTGGGAATAGAGTTTCG-3'	Hagberg CE, et al. <i>Nature</i> . 464:917-921 (2010).
	Reverse	5'-CTGCACATGGATGAGTTTGC-3'	
Mm- <i>Fabp4</i>	Forward	5'-GATGGTGACAAGCTGGTGGT-3'	Hagberg CE, et al. <i>Nature</i> . 464:917-921 (2010).
	Reverse	5'-AATTTCCATCCAGGCCTCTT-3'	
Mm- <i>Fabp5</i>	Forward	5'-GGAAGATGGCGCCTGGTGG-3'	Hagberg CE, et al. <i>Nature</i> . 464:917-921 (2010).
	Reverse	5'-CCGAGTACAGGTGACATTGT-3'	
Mm- <i>Actb</i>	Forward	5'-AGAGGGAAATCGTGCGTGAC-3'	Edwards DN, et al. <i>J Clin Invest</i> . 131:e140100 (2021).
	Reverse	5'-CAATAGTGATGACCTGGCCGT-3'	

Table S5. Gene sets used in correlation and survival analysis of bulk RNA datasets.

Gene Sets	mTORC1 ^{ECKO} Gene Signature		CTL Gene Signature	
	DEGs from sorted tumor cells		Modified from Edwards et al. 2021.	
Gene Lists	Gene Name	Gene ID	Gene Name	Gene ID
	<i>LCN2</i>	ENSG00000148346	<i>CD8A</i>	ENSG00000153563
	<i>PADI1</i>	ENSG00000142623	<i>GZMA</i>	ENSG00000145649
	<i>LYPD5</i>	ENSG00000159871	<i>GZMB</i>	ENSG00000100453
	<i>ARRB1</i>	ENSG00000137486	<i>GZMM</i>	ENSG00000197540
	<i>AMPD3</i>	ENSG00000133805	<i>PRF1</i>	ENSG00000180644
	<i>FAT2</i>	ENSG00000086570	<i>IFNG</i>	ENSG00000111537
	<i>MXD1</i>	ENSG00000059728		
	<i>VEGFA</i>	ENSG00000112715		
	<i>CRYAB</i>	ENSG00000109846		
	<i>DUSP10</i>	ENSG00000143507		
	<i>C3AR1</i>	ENSG00000171860		
	<i>IER2</i>	ENSG00000160888		
	<i>PTGS2</i>	ENSG00000073756		
	<i>SCN8A</i>	ENSG00000196876		
	<i>SGK1</i>	ENSG00000118515		
	<i>ERRFI1</i>	ENSG00000116285		
	<i>GPR35</i>	ENSG00000178623		
	<i>BDNF</i>	ENSG00000176697		
	<i>RCAN1</i>	ENSG00000159200		
	<i>GPR39</i>	ENSG00000183840		
	<i>DUSP1</i>	ENSG00000120129		
	<i>SOCS3</i>	ENSG00000184557		
	<i>TSC22D1</i>	ENSG00000102804		
	<i>ST3GAL1</i>	ENSG00000008513		
	<i>FILIP1L</i>	ENSG00000168386		
	<i>BACH1</i>	ENSG00000156273		
	<i>DNAJB9</i>	ENSG00000128590		
	<i>ARRDC3</i>	ENSG00000113369		
	<i>RBPJ</i>	ENSG00000168214		
	<i>HSPA5</i>	ENSG00000044574		
	<i>BIN1</i>	ENSG00000136717		
	<i>ITGB2</i>	ENSG00000160255		
	<i>TEAD2</i>	ENSG00000074219		
	<i>CGREF1</i>	ENSG00000138028		
	<i>FGFR1</i>	ENSG00000077782		
	<i>RAP1GAP</i>	ENSG00000076864		
	<i>CD109</i>	ENSG00000156535		
	<i>MICAL1</i>	ENSG00000135596		
	<i>PAPLN</i>	ENSG00000100767		
	<i>ACSL6</i>	ENSG00000164398		
	<i>CD22</i>	ENSG00000012124		