SUPPLEMENTARY DATA

SUPPLEMENTARY FIGURES AND LEGENDS

Figure S1 – Stromal ESDN protects from experimental melanoma metastasis formation and extravasation in mice.

(A) ESDN protein levels in B16 and B16-F10 cell lines in culture as measured via WB analysis. Protein expression of B16-F10 cells was calculated relative to B16 parental cells, normalized on a loading control and expressed as percentage (%). **(B)** Analyses of experimental lung metastasis formation derived from B16 cells injected in the tail vein of syngeneic WT and ESDN$^{-/-}$ mice evaluated 2 weeks postinjection as mean ± SEM of either lung surface metastasis number or percentage (%) of lung area occupied by metastases (24 fields over 6 sections per animal). Representative pictures of whole lungs (a, b; scale bar = 2 mm) or H&E-stained lung sections (c, d; bar = 500 µm) are presented; arrows indicate metastasis formations. **(C)** CMRA-labeled (red) B16 cells were injected into the tail vein of syngeneic WT and ESDN$^{-/-}$ mice and extravasation was evaluated 2 (a, c, d) or 48 (b, e, f) h later. (a, b) Representative fields of murine lung sections stained for CD31 (green) to highlight blood vessels and counterstained with DAPI (blue). Arrows indicate melanoma red cells within (a) or outside (b) the vessels; scale bar: 10 µm. (c-f) Representative pictures of whole lungs containing red B16 cells; scale bar: 1 mm. Top graph represents the lodging of injected cells at 2h as mean of fluorescence intensity ± SEM, bottom graph refers to the number of extravasated cells in the whole lungs at 48 h as mean ± SEM. **(A-C)** Three independent experiments were performed and a representative one is shown. SEM = Standard Error of the Mean; CMRA = CellTracker™ Orange; CD31 = cluster of differentiation 31; H&E = Haematoxylin & Eosin; WT = Wild Type; WB = Western Blot; *n* = number of

animals; DAPI = 4', 6-diamidino-2-dhenylindole; n = number. * =p<0.05; ** = p<0.01; ***= p<0.001 considered to be statistically significant.

Figure S2 – ESDN silencing in melanoma cells does not affect dissemination in mice.

(A) Evaluation of ESDN protein expression in B16 cells stably transduced with ESDN-targeting shRNA (pLKO.1-shESDN) or empty control (pLKO.1-empty) lentiviral vectors via WB analysis. Silencing of pLKO.1-shESDN transduced B16 cells was calculated relative to empty controls and expressed as percentage (%), following normalization on a loading control. **(B)** Analyses of experimental lung metastasis formation derived from B16 cells, stably transduced with pLKO.1-empty (a, c) or pLKO.1-shESDN (b, d) lentiviral vectors injected in the tail vein of syngeneic WT (a, b) and ESDN$^{-/-}$ (c, d) mice, 2 weeks postinjection, expressed as mean ± SEM of number of lung surface metastasis. Representative pictures of whole lungs (a-d; scale bar = 2 mm) are depicted; arrows indicate metastasis formations. (A, B) Three independent experiments were performed and a representative one is shown; SEM = Standard Error of the Mean; WT = Wild Type; WB = Western Blot; n = number of animals. * =p<0.05; ** = p<0.01; ***= p<0.001 considered to be statistically significant. NS indicates a nonstatistically significant p-value.

Figure S3 – Depletion of ESDN in the microenvironment does not favour tumor angiogenesis or vessel permeability in tumors or lungs.

(A) Analysis of blood vessels in B16-F10-derived subcutaneous tumors in WT and ESDN$^{-/-}$ mice, 10 days post-injection. Representative pictures of CD31 (green) stainings and DAPI (blue) counterstaing are depicted (a-d); scale bar = 100 µm. Graph refers to the number of CD31-positive vessels per mm^2 of tumor area presented as mean ± SEM (24 fields over 6

sections per animal). **(B)** Evaluation of vessel permeability (a-f) in B16-F10-derived subcutaneous tumors (10 days postinjection) 10 minutes after tail vein injection of Texas Red-conjugated Dextran 40-kD in WT (a-c) and ESDN$^{-/-}$ (d-f) mice. Representative pictures showing vessel leakage (red) and vessels (green) are presented; scale bar = 100 µm. Arrows on merged images indicate sites of leakage (yellow, c, f). Graph refers to tumor blood vessel permeability as percentage (%) of green/red vessels compared to total green vessels expressed as mean ± SEM (24 fields over 6 sections per animal). **(C)** Evaluation of *in vivo* permeability (a-h) 10 minutes after tail vein injection of Evans blue solution in WT (a-d) and ESDN$^{-/-}$ (e-h) tumor-bearing mice (2 weeks postinjection of B16 cells). Representative pictures for the indicated tumors/organs are shown; scale bar = 2 mm (a-g); 1 mm (d, h). Graph refers to the extravasated Evans Blue dye (absorbance/mg of tissue at 630 nm) as mean ± SEM. **(D)** Evaluation of vessel permeability (a-d) in lungs of WT (a, c) and ESDN$^{-/-}$ (b, d) mice, injected in the tail vein 48 hours before with CMRA-labeled B16 cells (a, b), and subsequently (10 minutes before the analysis) with Evans Blue solution (c, d). Representative pictures of whole lungs (a-d) are presented; scale bar = 2 mm. The graph refers to the extravasated Evans blue dye evaluated as in (C). **(A-E)** Two or three independent experiments were performed and a representative one is shown. WT = Wild Type; NS = Nonstatistically Significant; *n* = number of animals; SEM = Standard Error of the Mean; CMRA = CellTracker™ Orange; CD31 = cluster of differentiation 31; DAPI = 4',6-diamidino-2-dhenylindole. * =p<0.05; ** = p<0.01; ***= p<0.001 considered to be statistically significant. NS indicates a nonstatistically significant *p*-value.

Figure S4 – Lung endothelial cell purification and characterization

(A) Schematic representation of the isolation protocol used for Murine Lung Endothelial Cells (MLECs). Cells were isolated from the lungs of 4 weeks old WT and ESDN$^{-/-}$ mice (a tissue

pool was generated by mixing three lungs for each genotype) by two rounds of immunoselection with the indicated monoclonal antibody-conjugated magnetic beads. The obtained cell suspension was cultured or lysed for RNA or protein extraction. **(B)** Representative phase-contrast images of WT (a) and ESDN$^{-/-}$ (b) MLECs; scale bar = 100 µm. **(C)** FAP mRNA levels were assessed in MEFs, WT and ESDN$^{-/-}$ MLECs via qRT-PCR. Results are shown as fold changes (mean ± SEM) relative to MEFs, normalized on β-actin mRNA levels. **(D)** Analysis of whole lungs in WT (a-c) and ESDN$^{-/-}$ (d-f) mice previously injected with CMRA-labeled B16-F10 (red) cells 6 (a, d), 24 (b, e) or 48 (c, f) h postinjection. Representative pictures are depicted; scale bar: 1 mm. **(E, F)** Analysis of FAP or TYR mRNA expression in MLECs isolated from WT and ESDN$^{-/-}$ mice, noninjected (basal) or 6, 24 and 48 hours after tail vein injection of CMRA-labeled B16-F10 cells via qRT-PCR. Data are shown as fold changes (mean ± SEM) relative to MEFs, normalized on β-actin. (B-F) Two independent experiments were performed in triplicate and a representative one is shown. SEM = Standard Error of the Mean; MEFs = Mouse Embryonic Fibroblasts; CMRA = CellTracker™ Orange; FAP = Fibroblast Associated Protein; TYR = Tyrosinase; WT = Wild Type. * =p<0.05; ** = p<0.01; ***= p<0.001 considered to be statistically significant.

Figure S5 – Vascular gene expression analysis in ESDN$^{-/-}$ endothelial cells.
(A) Analysis of ESDN, VE-cadherin, VEGFR2, VEGFA, ICAM1, VCAM1, VAP1, E-selectin, N-cadherin and β-catenin mRNA expression in WT and ESDN$^{-/-}$ MLECs by qRT-PCR. Data are represented as fold changes (mean ± SEM) relative to WT MLECs, normalized on β-actin mRNA levels. **(B)** E-selectin and ESDN protein expression in WT and ESDN$^{-/-}$ MLECs in basal conditions evaluated by WB analysis. Protein expression has been normalized to the loading control and modulations of ESDN$^{-/-}$ cells are expressed as

percentages (%) relative to WT samples. **(C)** ESDN mRNA levels were assessed in MLECs isolated from WT and ESDN$^{-/-}$ mice, noninjected (basal) or 6, 24 and 48 hours after tail vein injections of CMRA-labeled B16-F10 cells via qRT-PCR. Results are presented as fold changes (mean ± SEM) relative to basal WT MLECs, normalized on β-actin mRNA levels. **(A-C)** Two or three independent experiments were performed and a representative one is shown. MLECs = Mouse Lung Endothelial Cells; SEM = Standard Error of the Mean; HUVECs = Human Umbilical Vein Endothelial Cells; WB = Western Blot; VEGFR2 = Vascular Endothelial Growth Factor Receptor-2; VEGFA = Vascular Endothelial Growth Factor; ICAM1 = Intercellular Adhesion Molecule 1; VCAM1 = Vascular Cell Adhesion Molecule 1; VAP1 = Vascular Adhesion Protein 1; WT = Wild Type. * =p<0.05; ** = p<0.01; ***= p<0.001 considered to be statistically significant.

Figure S6 – Stable depletion of ESDN in HUVECs favours melanoma cell adhesion, E-selectin and P-STAT3 expression upregulation.

(**A**) Evaluation of *in vitro* adhesion of B16-F10-GFP cells (a-d) on a confluent monolayer of shCtrl- and shESDN-transduced HUVECs, 15 minutes after seeding. Graph refers to the number (mean ± SEM) of tumor adherent cells per field (mm^2). Representative images of confluent shCtrl- or shESDN-transduced HUVECs (a, b) and adherent green B16-F10-GFP (c, d) cells are depicted; scale bar = 30 µm. (**B**) The cells used in (**A**) have been employed here to evaluate protein expression for ESDN, E-selectin, STAT3 and P-STAT3 (Y705) by WB analysis. Proteins have been normalized to a loading control and modulations of shESDN-transduced cells are expressed as percentages (%) relative to their shCtrl samples. **(A-B)** SEM = Standard Error of the Mean; WB = Western Blot. * =p<0.05; ** = p<0.01; ***= p<0.001 considered to be statistically significant.

1.Supplementary materials and methods

1.1 Reagents and antibodies

Murine RT-PCR primers: N-cadherin: 5'-TCCCTGAGATACAGCGTCACT-3' and 5'-ATAATGAAGATGCCCGTTGG-3', E-selectin: 5'-ACAGCAGGGCAACATGAAAT-3' and 5'-CAACTGGACCCATTTTGGAA-3', β-catenin: 5'-TGCAGATCTTGGACTGGACA-3' and 5'-AAGAACGGTAGCTGGGATCA-3', ICAM1: 5'-TGGTAGACAGCATTTACCCTCA-3' and 5'-GGCCACCATCCTGTTCTG-3', VCAM1: 5'-TCTTACCTGTGCGCTGTGAC-3' and 5'-GACCTCCACCTGGGTTCTCT-3', VAP1: 5'-ACCCACAGCGCTCACTTC-3' and 5'-GGGGACAAAAGCCATATCCT-3', β-actin: 5'-GGAGGGGGTTGAGGTGTT-3' and 5'-GTGTGCACTTTTATTGGTCTCAA-3', VEGF: 5'-AAAAACGAAAGCGCAAGAAA-3' and 5'-TTTCTCCGCTCTGAACAAGG-3', FAP: 5'-CGTGTATCGAAAACTGGGTGT-3' and 5'-AAACCCATTTCTATGAATTTTCTGAC-3', TYR: 5'-CACCATGCTTTTGTGGACAG-3' and 5'-GGCTTCTGGGTAAACTTCCAA-3', VE-cadherin: 5'-GTTCAAGTTTGCCCTGAAGAA-3' and 5'-GTGATGTTGGCGGTGTTGT-3', VEGFR2: 5'-TGGGCAGTCAAGTCCGAATC-3' and 5'-GTTGGTGAGGATGACCGTGT-3' (all from Sigma-Aldrich), Mm_DCBLD2/ESDN qRT-PCR QuantiTect Primer Assay QT00137207 and Mm_Actb_1_SG QuantiTect Primer Assay QT00095242 (all from Qiagen).

Human RT-PCR primers E-selectin: 5'-GGTTGGACAAGGCTGTGC-3' and 5'-ACCAGCCCAGGTTGAATG-3' (Eurofins), Hs_DCBLD2_1_SG QuantiTect Primer Assay QT00041790 and Hs_GAPDH_1_SG QuantiTect Primer Assay QT00079247 (all from Qiagen); 18S rRNA expression assay (TaqMan assay from Thermofisher).

Primary antibodies: anti-DCBLD2/ESDN pAb (Sigma-Aldrich), anti-E-selectin/CD62E pAb (R&D Systems), anti-murine CD62 (ab18981, Abcam), anti-hsp90 mAb F-8 (Santa Cruz Biotechnology), anti-α-tubulin mAb T5168 (Sigma-Aldrich); anti-GAPDH (MAB374, EMD Millipore Corp.), anti-phospho STAT3 Tyr705 (#9131, Cell Signaling), anti-STAT3 (#4904, and #9132 Cell Signaling), rat anti-mouse CD45 (550566, BD biosciences); rat anti-mouse F4/80 (MCA497, Serotec); rat anti-mouse CD31 (550274, BD biosciences). Secondary antibodies: HRP-conjugated goat anti-mouse IgG, goat anti-rabbit IgG and donkey anti-goat IgG (all from Santa Cruz Biotechnology); anti-rabbit Alexa Fluor 488 (Molecular Probes, Invitrogen Life Technologies). All antibodies were used at the producer's suggested concentration.

siRNAs: ON-TARGET plus Human DCBLD2 (131566) siRNA-SMARTpools (L016714-00-005), ON-TARGETplus Non-targeting Pool (D-001810-10-05) all from Dharmacon (GE Healthcare). STAT3 (siStat3) and control (siC) lipidoid siRNA-formulations were developed and provided by R. Bogorad and V. Koteliansky and used as described in [1].

Other reagents: 4',6-diamidino-2-dhenylindole (DAPI, Sigma-Aldrich); Texas Red-conjugated Dextran 40-kD (Molecular Probes, Invitrogen Life Technologies); Evans blue, Cimetidine, 5,6-Dichlorobenzimidazole 1-β-D-ribofuranoside, DRB, (all from Sigma-Aldrich).

1.2 Vectors, Generation of Stable Cell Lines, infections and transient transfection.

Stable ESDN downmodulated B16 and HUVECs were obtained following transduction of pLKO.1-shESDN lentiviral expression vectors (Thermo Scientific) Cat. No. RHS3979-97060656; pLKO.1 was used as empty control. GFP-transduced B16-F10 cells were obtained using pLenti-PGK-GFP-Puro (w509-5) (Addgene). Lentiviruses were produced according to Trono's lab protocol (http://tronolab.epfl.ch). Supernatants were harvested 48 hours post-

transfection, filtered with 0.45 μm filters, diluted and used to infect 3.5 x 10^5 cells in 6-well plates, in presence of 8 μg/mL Polybrene (Sigma-Aldrich). Infected cells underwent puromycin selection to obtain a pure population. For siRNA transient transfections, HUVECs were plated at 70% confluency in 6-well dish and transfected 24h later using Lipofectamine RNAiMAX reagent (Invitrogen Life Technologies) with 25 pmol/well of siRNA. To obtain transient silencing of STAT3, HUVECs were plated at 60% confluency in 6-well dish and treated 24h later using lipidoid siStat3- or siC-formulation in regular medium. For double silencing, HUVECs were plated at 60% confluency in 6-well dish and transfected 24h later for ESDN as describe above. After the overnight transfection overnight complete medium containing lipidoid siStat3- or siC-formulation was added.

1.3 In vivo permeability assays

Vessel leakage was analyzed following tail vein injection of 0.25 mg/mice (in 100 μL of PBS) Texas Red-conjugated Dextran 40-kD in 8-10 weeks old female and male WT and ESDN$^{-/-}$ mice, previously injected (10 days before) in the right flank with $5x10^5$ B16-F10 cells. 10 minutes later, mice were perfused with PBS and 2% PFA. Subcutaneous tumors were then harvested and frozen in OCT. Quantification of tumor blood vessel permeability was evaluated as percentage of CD31-Texas Red-Dextran-positive vessels compared to total CD31-positive vessels per tumor area section, following blood vessels immunofluorescence staining with an anti-CD3 as described above. Alternatively, 100 μl of a 3% Evans blue (Sigma-Aldrich) solution (in PBS) were injected into the tail vein of 8-10 weeks old female and male WT and ESDN$^{-/-}$ mice, previously injected in the right flank with $5x10^5$ B16 cells (2 weeks before) or intravenously with $1x10^6$ CMRA-labeled B16 cells (48 hours before). After 10 minutes mice were perfused, subcutaneous tumors and organs were collected in formamide (Sigma-Aldrich) and digested for 24 hours at 70°C. Concentration of the

extravasated dye was determined spectrophotometrically at 630 nm and expressed as absorbance/mg of tissue.

1.4 Murine Lung Endothelial Cells isolation protocol

Murine Lung Endothelial Cells (MLECs) were isolated from the lungs of 4 weeks old female and male WT and ESDN$^{-/-}$ mice. A tissue pool was generated by mixing three lungs for each genotype. Collected lungs were chopped into small pieces and dispersed in a single-cell suspension by incubation (37°C, 45 minutes) in 0.1% Collagenase type I, 0.2% Dispase, DNAase (1:100) digestion solution in RPMI 1640 Medium containing 10 mM Glutamax and 4.5 g/mL glucose (RPMI-1640 Glutamax™, GIBCO Invitrogen Life Technologies), supplemented with 10% heat-inactivated FCS (Seromed) and 100 μg/mL Penicillin-Streptomycin (all from GIBCO Invitrogen Life Technologies). Then, MLECs were derived by two rounds of immunoselection with the monoclonal antibodies indicated below previously conjugated with magnetic beads (Dynabeads sheep anti-rat, Life Technologies) as described in the datasheet. Rat anti-mouse CD45 and rat anti-mouse F4/80-conjugated magnetic beads were used for negative immunoselection and rat anti-mouse CD31-conjugated magnetic beads was used for positive immunoselection. The obtained MLECs suspension was lysed for total RNA extraction or cultured. The purity of MLEC isolations was assessed by qRT-PCR analysis to evaluate the expression of various endothelial-specific or fibroblast-specific genes.

1.5 RNA isolation and qRT-PCR for mRNA detection

Total RNA was isolated from cells or tumors using TRIzol® Reagent (Invitrogen Life Technologies). For mRNA detection, 250 ng of DNAse-treated RNA (RQ1, Promega) was retrotranscribed with High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems).

qRT-PCRs for mRNA detection were performed with the SYBR® Green PCR Master Mix or with Platinum™ Quantitative PCR SuperMix-UDG w/ROX (Applied Biosystems) on cDNAs according to the manufacturer's instructions. qRT-PCRs were carried out using gene-specific primers, using a 7900HT Fast Real Time PCR System (Applied Biosystems). Quantitative normalization was performed on the expression of β-actin or GAPDH mRNA or 18S rRNA. The relative expression levels between samples were calculated using the comparative delta CT (threshold cycle number) method ($2-\Delta\Delta CT$) with a control sample or the median expression of the analyzed genes as reference point [2]. For inhibition of transcription, 42 hours following transfection, ESDN-silenced (siESDN) or control (siCtrl) HUVECs were treated with 75 µm DRB (Sigma-Aldrich) for six 6 hours and RNA extracted and analyzed as described above.

1.6 Protein preparation and Immunoblotting

Total protein extracts were obtained by lysing cells or xenograft samples using a boiling buffer (Laemmli buffer) containing 0.125 M Tris/HCl, pH 6.8 and 2.5% sodium dodecyl sulphate (SDS) (Sigma-Aldrich). Western blotting (WB) was performed separating 30-50 µg of proteins by SDS polyacrylamide gel electrophoresis (PAGE) and electroblotting onto nitrocellulose membranes (BioRad). Membranes were blocked in 5% non-fat milk PBS-Tween 0.1% buffer for 1 h at 37 °C, then incubated with appropriate primary and secondary antibodies in PBS-Tween 0.1% buffer, respectively, overnight at 4 °C or for 1 h at room temperature and developed using Chemidoc Touch Imaging System (Bio Rad). Quantitations were made by densitometry, using Image Lab software (Bio-Rad) and normalization was performed on loading control (hsp90, α-tubulin or GAPDH) levels.

1.7 Immunofluorescence and confocal microscopy

For tissue immunofluorescence (IF) staining, 10 µM-thick frozen B16 or B16-F10 subcutaneous tumor sections or 6 µM-thick frozen lung sections, embedded in OCT (Killik, BioOptica) were stained. Tumor sections were fixed in 4% paraformaldehyde (PFA) for 5 min, while lung sections were acetone-fixed for 10 minutes. Blocking buffer composed of 5% bovine serum albumin (Sigma-Aldrich) in PBS was used to eliminate nonspecific protein binding (sections were incubated at room temperature for 1h). IF staining was performed using an anti-CD31 primary antibody (1:100 dilution) at 4 °C overnight in 1% bovine serum albumin in PBS and then with an anti-rabbit Alexa Fluor 488 secondary antibody (1:800 dilution) at room temperature for 1 h. Nuclei were stained with DAPI. Specimens were examined and photographed using a Zeiss AxioObserver microscope with the ApoTome Module (Zeiss). For IF and confocal microscopy on HUVECs, cells were plated on 0.17 mm glass coverslips coated with 3 µg/ml Fibronectin (FN, R&D Systems) in PBS and allowed to adhere overnight. HUVECs were washed in PBS, fixed in 4% paraformaldehyde (PFA) for 15 min, permeabilized in 0.1% Triton X-100 for 2 min on ice and incubated with primary antibodies (STAT3, 1:50 or E-selectin 1:50) for 1 h at RT. Cells were incubated with the appropriate Alexa-Fluor-tagged secondary antibody (Life Technologies) for 45 min at RT in the dark. Subsequently, cells were incubated with the nuclei marker TO-PRO-3 (Life technologies) for 15 min at RT in the dark and mounted with Mowiol 4-88 mounting. Cells were analyzed by using a Leica TCS SP8 confocal laser-scanning microscope (Leica Microsystems). Fluorochromes and fluorescent proteins were excited at the optimal wave-length by means of 80 MHz pulsed white light laser (470-670 nm), allowing time gating of fluorescence life times. Fluorescence channels were scanned sequentially and emission was revealed by means of hybrid spectral detectors (HyD SP Leica Microsystems). Image acquisition was performed by adopting a laser power, gain and offset settings that allowed maintaining pixel intensities (grey scale) within the 0–255 range and hence avoided

saturation. To quantify the localization of STAT3 in in the nucleus, we analyzed the Mean Fluorescence Intensity (MFI) of anti-STAT3 staining using ImageJ Fiji Software. To quantify the amount of E-selectin on the apical membrane, we measure the MFI values of E-selectin staining on apical membrane using ImageJ Fiji Software and normalized it on the apical membrane length.
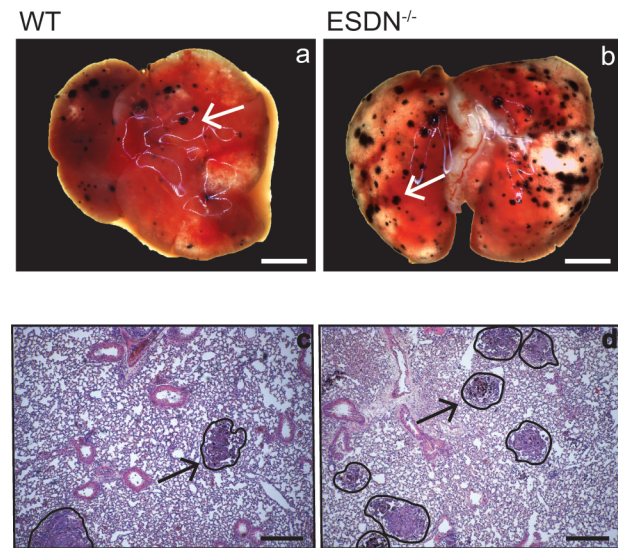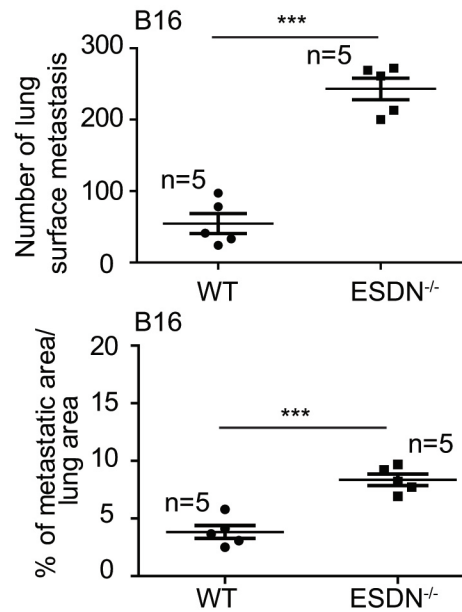
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Figure S1

A

B16

B

A

Number of vessels (CD31/mm²)

*

n=6   n=6

WT   ESDN⁻/⁻

B16-F10 tumor

WT   a   b

ESDN⁻/⁻   c   d

CD31/DAPI   CD31

B

% of Texas Red-Dextran extravasation/CD31

NS

n=6   n=6

WT   ESDN⁻/⁻

B16-F10 tumor

WT   a   b   c

ESDN⁻/⁻   d   e   f

CD31   Texas Red-Dextran   Merge

C

Leakage of Evans Blue (ABS/mg of tissue)

NS   n=4   NS   n=4   NS   n=4   n=4

Tumor   Lung   Liver   Brain

§   §

B16 tumor   Lung   Liver   Brain

WT   a   b   c   d

ESDN⁻/⁻   e   f   g   h

Evans blue

D

Leakage of Evans Blue (ABS/mg of tissue)

NS

n=5   n=5

WT   ESDN⁻/⁻

B16-CMRA lung

WT   a   2 h   c

ESDN⁻/⁻   b   2 h   d

Evans blue

A

Isolation of MLECs from WT or ESDN⁻/⁻ mice (three for each genotype)

Collected lungs are chopped into small pieces and dispersed in a single-cell suspension by incubation in collagenase/ dispase digestion solution

Negative (CD45 and F4/80) and positive (CD31) immunoselection with monoclonal antibody-conjugated magnetic beads

Total RNA extraction

MLECs in culture

Figure S4

B

MLECs

WT

ESDN⁻/⁻

C

Relative FAP mRNA level (Fold change)

MEFs   WT MLECs   ESDN⁻/⁻ MLECs

***   ***

D

B16-F10-CMRA

WT

6 h   24 h   48 h

ESDN⁻/⁻

6 h   24 h   48 h

E

Relative FAP mRNA level (Fold change)

MEFs   0   6 h   24 h   48 h

***   ***   ***   ***   ***   ***

☐ WT MLECs   ▨ ESDN⁻/⁻ MLECs

F

Relative TYR mRNA level (Fold change)

B16-F10   6 h   24 h   48 h

***   ***   ***   ***   ***

☐ WT MLECs   ▨ ESDN⁻/⁻ MLECs

A

B16-F10-GFP adhesion (number of cells/mm²)

HUVECs

***

shCtrl   shESDN

shCtrl   shESDN

HUVECs

a   b

B16-F10-GFP

c   d

B

HUVECs

shCtrl   shESDN

ESDN
−57%

E-selectin
+60%

P-Stat3 (Y705)
+50%

Stat3
+13%

Loading control