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METHODS

Cell culture and reagents. Human umbilical vein endothelial cells (ECs) were isolated from umbilical cord vein, characterized and grown as previously described[46]. In all experiments, ECs were used between passages two and five. To overcome the intrinsic biological variability of ECs, all data are shown as the mean of three independent experiments performed with a mix of ECs from three different umbilical cords.

293T (ATCC CRL-11268), B16F10 (ATCC CRL-6475) and HeLa (ATCC CCL-2) cell lines were obtained from the American Type Culture Collection and maintained as frozen stock. All experiments were performed on cell lines that had been passaged for <6 months after thaw.

Rat monoclonal function-blocking antibody against integrin α_6 (GoH3, MAB13501), aspecific rat IgGA2 antibody, VEGF-A165 and FGF-2 were obtained from R&D Systems. Function-blocking antibody against α_1 (FB12; MAB1973Z), α_2 (BHA2.1; MAB1998Z), α_4 (P1H4; MAB16983Z), α_5 (P1D6; MAB1956Z) and αv (LM609; MAB1976Z) were obtained from Millipore. Each treatment was carried out with a final concentration of 20 μg ml^{-1} of azide-free antibody. Phorbol-12-myristate-13-acetate (PMA) was provided by Calbiochem. Laminin (Sigma) was isolated from Engelbreth-Holm–Swarm murine sarcoma basement membrane. Nocodazole, primaquine, cycloheximide and porcine gelatin were obtained from Sigma-Aldrich.

***In vitro* podosome analysis.** Subconfluent ECs were cultured with M199 10% FCS for 24 h and kept with or without 30 ng ml^{-1} VEGF-A for 24 h. ECs were then trypsinized and allowed to adhere for 2 h in M199 20% FCS on glass coverslips, previously coated with 1% porcine gelatin for 1 h. Cell were starved with serum-free M199 for 1 h and then stimulated for 5–60 min with 10% FCS M199 plus 80 ng ml^{-1} of PMA. To visualize podosomes, cells were paraformaldehyde (PFA)-fixed—4% PFA in PBS—and stained with anti-cortactin antibody (4F11, 05-180, Millipore) and phalloidin. We imaged ECs using a confocal laser-scanning microscope (TCS SP5 AOBS; Leica) equipped with a ×63/1.30 HCX Plan-Apochromat oil-immersion objective. Podosome-rosette-positive cells were identified by co-localization of cortactin and F-actin in a ring-like structure. Individual-podosome-positive cells were characterized by co-localization of cortactin and F-actin in a dot-like distribution. All manual quantifications were performed in a double-blind manner.

Plasmid preparation. Lentivectors carrying short hairpin RNA (shRNA) sequences against human integrin α_6 or a scramble sequence (used as control) were purchased from the RNAi Consortium library (Sigma-Aldrich). For lentivectors carrying GFP-tagged integrin α_6 (α_6–GFP) and LifeAct–RubyFP (LifeAct–RFP), we used the In-Fusion 2.0 CF Dry-Down PCR Cloning Kit (Clonetech). Integrin α_6, cloned into the pWPXL lentiviral vector (Trono lab, http://tronolab.epfl.ch), was tagged with GFP, inserted in the C terminus. LifeAct–RubyFP (ref. 14) was provided by R. Wedlich-Söldner, Max Planck Institute of Biochemistry, Martinsried, Germany, and was inserted in the pLKO.1 lentiviral vector in the place of the puromycin resistance sequence.

Lentiviral preparation, purification and concentration. Lentiviruses were produced by calcium phosphate transfection of vector plasmids—pLKO.1 shRNAs (scramble control, TRCN0000057774 and TRCN0000057775; Sigma-Aldrich), pWPXL-ITGA6, α_6–GFP and FP-tagged LifeAct—together with packaging (pCMVdR8.74) and envelope (pMD2.G-VSVG) plasmids in 293T cells. Supernatant was collected 48 and 72 h post-transfection, filtered with 0.45 μm filters and concentrated (19,000*g* for 2 h at 20 °C). The multiplicity of infection (MOI) was determined by infecting HeLa cells, plus 8 μg ml^{-1} of Polybrene, followed by puromycin selection 24 h after the infection, and quantification of resistant cells or by flow cytometric quantification of GFP-positive cells.

Transfection and lentiviral infection of ECs. ECs were transiently transfected with pTagRFP–vinculin (FP372, Evrogen) by Lipofectamine Plus (Invitrogen) according to the manufacturer's instructions.

For stable transduction, ECs were infected for 12–24 h at 1–6 MOI plus 8 μg ml^{-1} of Polybrene and mARs for 48 h with 1 million infecting virions.

Time-lapse total internal reflection fluorescence microscopy. ECs, transfected or transduced with FP-fusion-protein constructs, were incubated at 37 °C in a 5% CO_2 humidified atmosphere on gelatin-coated glass-bottom dishes (Willco, Intracel). Time-lapse experiments were performed using a Leica DMI6000 B MC TIRF system with a ×63/1.40 NA oil-immersion objective.

Blocking antibody and synthetic drug treatment in endothelial podosome rosette formation. After 24 h of VEGF treatment, subconfluent EC were trypsinized and seeded for 2 h in M199 20% FCS on glass coverslips, previously coated with 1% porcine gelatin for 1 h. Cells were starved with serum-free M199 for 30 min and then stimulated for 30 min with 10% FCS M199 plus 80 ng ml^{-1} of PMA.

Treatments with specific anti-integrin blocking antibodies were performed during a 2-hour-adhesion process in M199 20% FCS. Each treatment was carried out with a final concentration of 20 μg ml^{-1} of azide-free antibody.

Treatments with 1.25 μg ml^{-1} (4 μM) of nocodazole, 50 μg ml^{-1} (110 μM) of primaquine or 20 μg ml^{-1} of cycloheximide (CHX) were performed during a 30-min PMA stimulation. In the case of nocodazole washout experiments, ECs were treated with nocodazole for 30 min during serum-free M199 starving, and then rinsed twice in PBS and treated with PMA for 30 min. CHX pretreatment for 2 h showed results comparable to CHX treatment during PMA stimulation.

Matrix degradation assay. FITC-conjugated gelatin from pig skin was prepared according to the manufacturer's instructions (Invitrogen). ECs were plated on glass coverslips coated with FITC–gelatin (1 mg ml^{-1}), and were then fixed and stained with phalloidin for 20 min. The regions in which 488–gelatin was degraded were analysed using ImageJ (NIH), by quantifying the percentage of fluorescence reduction in the region underling ECs in comparison with zones where adhered ECs are not present.

Cytofluorimetric analysis. ECs were trypsinized and then incubated with PBS 1% BSA plus 5 μg ml^{-1} of mouse anti-integrin α_6 monoclonal antibody (4F10; sc-53356; Santa Cruz Biotechnology), active MT1-MMP monoclonal antibody (3G4.2; MAB1767; Millipore) or mouse IgG for 30 min at 4 °C. After three washes with PBS 1% BSA, cells were incubated with 2.5 μg ml^{-1} of Alexa 488-conjugated anti-mouse antibody (Invitrogen) for 30 min. After final rinses with PBS, samples were analysed with a CyAn ADP flow cytometer (Dako Cytomation) and data were analysed with Summit 4.3 software (Dako).

Integrin α_6–GFP expression analysis in ECs. We seeded integrin α_6–GFP-infected ECs on gelatin-coated dishes, with or without laminin (final concentration 20 μg ml^{-1}) for 3 h. After 30 min with M199 10% FCS with or without PMA treatment (80 ng ml^{-1}), cells were trypsinized and samples were acquired with a CyAn ADP flow cytometer (Dako Cytomation) and data were analysed with Summit 4.3 software (Dako).

RipTag2, endothelial ITGA6 KO and *Lama4*$^{-/-}$ mice. Generation of RipTag2 mice as a model of pancreatic islet cell carcinogenesis has been previously reported[47]. RipTag2 mice were maintained in the C57Bl/6J background (Jackson Laboratory). From 12 weeks of age, all RipTag2 mice received 50% sugar food (Harlan Teklad) and 5% sugar water to relieve hypoglycaemia induced by the insulin-secreting tumours. LifeAct–EGFP mice were generated previously[33], and provided by R. Wedlich-Söldner (Max-Planck Institute of Biochemistry, Martinsried, Germany) and L. M. Machesky (Beatson Institute for Cancer Research, Glasgow, UK). Mice were housed under the approval and the institutional guidelines governing the care of laboratory mice of the University of Torino Committee on Animal Research and in compliance with the international laws and policies.

Generation of α_6 floxed mice (α_6*fl/fl-Tie2Cre*+) has been reported previously[19]. For mouse breeding, both combinations were used: α_6*fl/fl-Tie2Cre*+ (KO) male with α_6*fl/fl-Tie2Cre*− (WT) female or α_6*fl/fl-Tie2Cre*− (WT) male with α_6*fl/fl-Tie2Cre*+ (KO) female. Integrin α_6 KO female mice were fertile and we did not observe any evident differences during pregnancy or in newborn number or size, as described in ref. 19. All protocols were approved by the Regional Ethics Committee on Animal Experimentation (P2.CBV.031.07, CEEA34.CB.041.11 and CEEA34.CB.011.11) and all experiments complied with Directive 2010/63/EU of the European Parliament.

Lama4$^{-/-}$ mice were generated previously[28,29].

Melanoma tumour subcutaneous injection. One million B16F10 melanoma cells were suspended in 100 μl of PBS and injected subcutaneously into the right flank of 8-week-old α_6*fl/flTie2Cre*+ and α_6*fl/flTie2Cre*− male mice. Twelve days later the mice were anaesthetized with a single intraperitoneal injection of ketamine (80 mg kg^{-1}) and xylazine (16 mg kg^{-1}), then killed by cervical dislocation. Tumours were collected and frozen in isopentane solution cooled in liquid nitrogen before being stored at −80 °C until immunohistological analysis.

Unilateral hindlimb ischaemia. The hindlimb ischaemia experiment was performed as previously described[19,48]. Male mice aged 7–8 weeks were anaesthetized with a single intraperitoneal injection of ketamine (80 mg kg^{-1}) and xylazine (16 mg kg^{-1}). The femoral artery and vein were separated from the femoral nerve, ligated, and excised from proximal to the superficial epigastric artery to proximal to the bifurcation of the saphena and popliteal arteries. On day 14 after ischaemia induction, ischaemic and non-ischaemic gastrocnemius muscles were collected and

frozen slowly in isopentane solution cooled in liquid nitrogen, before being stored at −80 °C.

Human lung tumour data. Endothelial podosome rosette levels, microvessel density (MVD) and VEGF area fraction were measured in the lung tumour samples of a cohort of patients collected between 1992 and 1993 before surgical resections with the approval of the Massachusetts General Hospital Institutional Review Board and previously analysed in ref. 49. The Massachusetts General Hospital Institutional Review Board determined that our investigation did not meet the definition of 'human subjects research'. We did not obtain data through an intervention or interaction with individual subjects or identifiable private information about living individuals. The patients had a variety of pre- and post-surgical treatments and tumour stages. We analysed 11 biopsies, 3 vessels per tumour for rosette quantification and 20 different regions of interest (ROIs) per biopsy for MVD and VEGF.

Immunostaining. ECs plated on coated glass coverslips, whole-mount mouse aortic explants, whole-mount mARs or 30–50 µm cryosections of mice tumours were equilibrated in PBS, PAF-fixed and permeabilized with PBS 0.3% Triton X-100. For biopsy, samples from human patients, 10-µm-formalin-fixed paraffin-embedded (FFPE) sections were de-waxed and permeabilized with PBS 0.3% Triton-X100. Primary antibodies—mouse anti-cortactin (1:100, 4F11; 05-180; Millipore), rabbit anti-paxillin (1:100, Y113; 04-581; Millipore), goat anti-MT1-MMP (1:100, 3G4.2; MAB1767; Millipore), rabbit anti-MT1-MMP (1:150, AB53712, Abcam), mouse anti-dynamin (1:100, E-11; sc-74532; Santa Cruz Biotechnology), rabbit anti-phospho-cortactin (1:100, Tyr 421; AB3852; Millipore), mouse anti-phospho-FAK (1:80, Tyr 397; ABT135; Chemicon), mouse anti-vinculin (1:1,000, hVIN-1; V3191; Sigma-Aldrich), mouse anti-α_1 integrin (1:100, FB12; MAB1973; Millipore), mouse anti-$\alpha_2\beta_1$ integrin (1:100, BHA2.1; MAB1998; Millipore), mouse anti-α_3 integrin (1:100, P1B5; MAB1952; Millipore), mouse anti-α_4 integrin (1:100, P4C2; MAB1955; Millipore), mouse anti-α_5 integrin (1:50, P1D6; MAB1956; Millipore), mouse anti-$\alpha v\beta_3$ integrin (1:100, LM609; MAB1976; Millipore), rat anti-α_6 integrin (1:50, GoH3; MAB13501; R&D System), mouse anti-β_4 integrin (1:100, 450-11, a gift from R. Falcioni, National Cancer Institute 'Regina Elena', Rome, Italy), and rabbit anti-laminin (1:80, AB2034, Millipore)—were diluted in PBS 5% donkey serum and incubated overnight at 4 °C in a humidified chamber. Coverslips or specimens were washed with PBS and incubated for 30 min at room temperature with secondary antibodies and counterstained with Alexa488-conjugated phalloidin (Invitrogen) and 4′,6-diamidino-2-phenylindole (DAPI). Coverslips or specimens were analysed using a confocal laser-scanning microscope (TCS SP5 AOBS; Leica) equipped with a ×63/1.30 HCX Plan-Apochromat oil-immersion objective. Confocal stack images were digitally post-processed with blind deconvolution algorithms.

Endothelial podosome density in tumours. We stained tumour slices with anti-laminin (1:80, AB2034, Millipore) and anti-cortactin (4F11, 05-180, Millipore) antibody and phalloidin and performed confocal imaging with a laser-scanning microscope (TCS SP5 AOBS; Leica) equipped with a ×63/1.30 HCX Plan-Apochromat oil-immersion objective. After three-dimensional (3D) blind deconvolution algorithms, tumour vessel volumes were recognized by using laminin staining using Imaris 6.3 software (Bitplane, AG). To count endothelial podosome rosettes, several discriminating criteria were followed: endothelial podosome rosettes were identified as ring-like structures within vessels with co-staining for F-actin and cortactin, close to a region depleted in laminin staining, and with a diameter ranging between 2 and 6 µm. All manual quantifications were performed in a double-blind manner.

***In situ* zymography.** Tissue sections (RipTag2 or human lung tumours) or whole-mount mARs were fixed in acetone and immunostained as indicated. Substrate for *in situ* zymography was prepared by diluting DQ gelatin (0.1% in deionized H_2O, Invitrogen) 1:50 in a reaction buffer containing PBS 5 mM $CaCl_2$. Tissue sections or whole-mount mARs were incubated in a dark humidity chamber at 37 °C in gelatin solution for 2 h. Samples were then carefully rinsed with PBS and PFA-fixed for 10 min in the dark. To verify the contribution of metalloproteases, control slides were pre-incubated with 20 mM EDTA or protease inhibitor mix (leupeptin, aproptin and pepstatin) for 1 h. Specimens and whole-mount mARs were analysed using a confocal laser-scanning microscope (TCS SP5 AOBS; Leica) equipped with a ×40/0.95 or ×63/1.30 HCX Plan-Apochromat oil-immersion objective. Confocal stack images were digitally post-processed with deconvolution algorithms.

Immunohistochemistry on human tissues. FFPE sections (5 µm thick) were immunostained following the manufacturer's recommendations and standard protocols with antibodies against the following antigens: mouse anti-human CD31 (JC70A; 102870; Dako) and mouse anti-human VEGF (Ab-7; MS-1467-P0; Thermo Scientific). Sections were visualized by the avidin–biotin complex immunoperoxidase method, observed with a bright-field microscope and photographed. MVD and VEGF-positive area were estimated in 20 ROIs of the tumour on CD31- or VEGF-stained sections using a customized analysis with ImageJ (NIH).

Intravenous injection of antibody and detection. Rapid accessibility of antibody was analysed as previously described[26,50]. 25 µg of anti-α_6 (GoH3, R&D) or nonspecific IgG, diluted to 125 µl final volume with 0.9% NaCl, were injected through the tail vein. Antibodies were allowed to circulate for 10 min and the tissues were fixed by vascular perfusion. The chest was opened rapidly, and the vasculature perfused for 3 min with PFA fixative from a cannula inserted into the aorta through an incision in the left ventricle. The right atrium was incised to provide an exit for the fixative. After the perfusion, tissues were removed, stored and obtained as OCT-embedded sections. The localization of antibodies was detected by incubating sections with Alexa-647 goat anti-rat antibody, phalloidin and anti-cortactin antibody. Confocal stack images were post-processed with blind deconvolution algorithms with Autodeblur (Media Cybernetics) and ImageJ (NIH).

Therapeutic antibody treatment. The therapeutic antibody treatment was performed in RipTag2 mice as previously described[26]. In brief, the dosage regimen used was 0.125 mg of anti-α_6 integrin antibody (GoH3, R&D) per mouse through tail vein injection. Antibody treatment started when mice reached the age of 9 weeks and continued for 15 days. Control animals were treated with purified rat IgG2A (R&D) at a dose of 0.125 mg per mouse every 2 days for 2 weeks. Cohorts of 6 mice were treated for each arm of the trial study.

Vascular branching index in tumours. The vessel branching index (also 'vessel branching incidence') is the number of manually counted branching points/vascular volume and is independent of the vascularization rate. We stained tumour slices with anti-laminin (1:80, AB2034, Millipore) or anti-CD31 (JC70A; 102870; Dako) antibody and analysed them using a confocal laser-scanning microscope (TCS SP5 AOBS; Leica) equipped with a ×20/1.40 HCX Plan-Apochromat oil-immersion objective. After maximum projection of 30-µm-slices, we manually counted branching points and divided them by a given vessel volume as described above. All manual quantifications were performed in a double-blind manner.

***Ex vivo* podosome stimulation in aortic explants.** Aortas were explanted as previously described[26]. After isolation from fibro-adipose tissue, aortae were cut along their long axis and then sectioned in 1 mm^2 squares and then incubated for 24 h in serum-free medium with antibiotics. Aortic segments were incubated for 48 h in M199 10% FCS plus antibiotics with or without 30 ng ml^{-1} of VEGF-A. In the case of knockdown experiments media were supplied with Polybrene and lentiviral supernatants.

To visualize podosome-positive cells, we PFA-fixed aortic explants and stained them with anti-cortactin (4F11, 05-180, Millipore) antibody and phalloidin and imaged them using a confocal laser-scanning microscope (TCS SP5 AOBS; Leica) equipped with a ×40/0.95 HCX Plan-Apochromat oil-immersion objective. After 3D blind deconvolution algorithms (ImageJ plugins), podosome-positive cells in the endothelial layer were recognized by identifying regions of co-localization of cortactin and F-actin in ring-like structures in the endothelial layer. The endothelial layer was easily identified as ECs are not as highly stained by phalloidin as smooth muscle cells (SMCs); the endothelial layer and the SMC layer are divided by a highly autofluorescing layer of elastin; moreover, the circularity of nuclei allowed to distinguish ECs from SMCs because endothelial nuclei are circular and not elongated[11]. These criteria were previously described[11] and are summarized in Supplementary Fig. 1d. All manual quantifications were performed in a double-blind manner.

Mouse aortic ring angiogenesis assay. The mouse aortic ring (mAR) assay was performed as previously described[26,31] with the following with modifications. After explant, mARs were incubated for 2 days in serum-free medium. Aortic explants were then kept in place on glass-bottom dishes (Willco, Intracel) with a drop of 20 µl of type-I collagen gel (from rat tail, Roche) and covered with Endothelial Basal Medium (EBM, Clonetics) 5% FCS with VEGF-A (20 ng/ml, R&D) and FGF-2 (10 ng ml^{-1}, R&D).

Time-lapse analysis of mAR model. LifeAct–EGFP, endothelial α_6 null and *Lama4*$^{-/-}$ mARs were embedded in type-I collagen gel, stimulated as described previously and kept at 37 °C in a 5% CO_2 humidified atmosphere for 24–72 h on glass-bottom dishes.

LifeAct–EGFP, endothelial α_6 null or *Lama4*$^{-/-}$ mARs were imaged with a ×20/0.75 dry objective (Leica Microsystems) with an inverted photomicroscope (DM IRB HC; Leica Microsystems) in phase-contrast or epifluorescence.

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LifeAct–EGFP mARs were imaged with a ×20/0.50 dry objective (Leica Microsystems) with a multiphoton microscope Leica TCSII SP5. *Z* stacks were acquired at 512 × 512 resolution, scan speed of 400 Hz, and 1 µm *z*-step size.

To investigate the relation between endothelial podosome rosettes and lateral sprouting, we analysed 8 lateral protrusion events from 3 different mARs. We measured the time of persistence and diameter of ring-like structures. Data obtained in LifeAct–GFP mARs were corroborated by the quantification of rosettes in fixed confocal sections of mARs identified by co-staining of cortactin (4F11, 05-180, Millipore) and F-actin. Rates of rosettes per unit length and time were calculated by knowing the duration of rosettes and assuming a uniform density over every branch of the mAR. Lateral sprouting in mARs was instead quantified in live bright-field movies, where we identified protrusions emerging from pre-existing branches and either retracting or developing into full secondary branches.

Integrin α_6 membrane localization in mARs. After 10 days of culture, live mARs were treated with the rat anti-antibody GoH3 (0.2 µg ml^{-1} MAB13501; R&D System) for 1 h and then PFA-fixed. The localization of the anti-a6 antibody (GoH3) was detected by whole-mount incubation with Alexa-647-conjugated goat anti-rat antibody, anti-cortactin (4F11, 05-180, Millipore) antibody, phalloidin and DAPI. We quantified the anti-α_6 antibody in cells with or without endothelial rosettes by using phalloidin and DAPI staining to distinguish the cell edges and phalloidin and cortactin for podosome rosettes (shown in Supplementary Fig. 7e). Confocal stacks of images were quantified using ImageJ (NIH).

Image analysis. Immunostained cryosections of mouse tumours or whole-mounted mARs were imaged using a confocal laser-scanning microscope (TCS SP5 AOBS; Leica) equipped with a ×40/0.95 (*z*-step = 0.4 µm) or ×63/1.30 HCX Plan-Apochromat oil-immersion objective (*z*-step = 0.3 µm). To increase the signal to noise ratio, confocal images were obtained by using a high line average and a low scan speed.

To reduce optical distortions, images were filtered with blind deconvolution algorithms by means of Autodeblur (Media Cybernetics). We applied 5 iterations for light microscopy, 10 for confocal stacks, and 3 for multiphoton 4D analysis. Podosome rosettes were identified in deconvoluted stacks and checked, *a posteriori*, in each raw stack.

For ease of visualization, we reconstructed the 3D geometry of confocal stacks by isosurface rendering using Imaris 6.3 (Bitplane, AG). Briefly, each channel was binarized with a threshold level chosen automatically by the software.

Fluorescence image quantification. Fluorescence intensity quantification was performed using Leica Confocal Software (Leica), ImageJ (NIH) or Imaris 6.3 (Bitplane, AG). Image acquisition was performed maintaining the same laser power, gain and offset settings. In the case of *in vitro* experiments, we analysed 10 different cells for each experimental point, in three independent experiments. Integrin α_6 localization in podosome rosettes was detected as mean fluorescence in podosome ROIs. Podosome ROIs were manually selected by using co-localization of cortactin and phalloidin staining.

In the case of vBM fluorescence quantification in proximity of endothelial podosome rosettes, we analysed 5 different fields for each mouse. After 3D blind deconvolution algorithms, tumour vessel volumes were recognized by using isosurface of vBM staining. vBM was detected as laminin staining. Volumes of vBM were subdivided in 1,000-µm^3-volumes. The volumes of vBM were then classified in vBM volumes with or without endothelial rosettes. Mean fluorescence of laminin staining in vessel volumes was quantified by Imaris 6.3 (Bitplane, AG).

Statistical analysis. No statistical method was used to predetermine sample size, but the sample size was conceived to obtain a 95% confidence level and a confidence interval of 5%, which were verified *a posteriori* once the experiment was performed. For cells and aortic explants, we used the experiments in Fig. 1 to set the maximum (VEGF-A stimulation) and the minimum (unstimulated) for the following experiments. The animal numbers for anti-α_6 treatments and for integrin α_6 null mice experiments are based on similar experiments in the past[19,26]. The investigators were not blinded during the treatments, but they were blinded for all image analyses and manual quantifications. The experiments were not randomized.

Data are presented as means ± standard error (s.e.m.) of three independent experiments. For *in vitro* assays, each experiment was performed with a mix of ECs from three different umbilical cords. For digital quantification of fluorescence in specific ROIs, we measured 6 fields per experimental point in three independent experiments. For *in vivo* studies, cohorts of 6 mice were treated for each arm of the anti-α_6 antibody treatment study in RipTag2 mice, cohorts of 7 mice were analysed for each arm of the B16F10 tumours in α_6 null mice and cohorts of 3 mice for ischaemic experiments in α_6 null mice. For human sample analysis, we studied 11 biopsies, three different vessels per biopsy and the measurement of 20 ROIs per slide for MVD and VEGF.

Prism (GraphPad Software) was used for analysis. Statistical analyses were performed using unpaired *t*-tests or, when more than two groups were assessed, by ANOVA followed by Bonferroni-adjusted *post hoc t*-tests. *F*-tests were used to determine whether groups had equal variance; if equality was not established unpaired *t*-tests with Welch's correction were performed. A D'Agostino–Pearson test was used to assess normality. A Mann–Whitney test was used when normality was not achieved. A Pearson test was used for correlation analyses in human tissues because all three sets (rosette densities, MVD and VEGF areas) passed the normality test. Statistical significance was achieved when *P* was less than 0.05.

For representative images, we repeated the experiments multiple times: Fig. 1a (10 cells), Fig. 1d (5 cells), Fig. 1e (5 aortic explants), Fig. 2a (5 fields per mouse in 3 mice, 3 fields per biopsy for a total of 33 images), Fig. 2b (2 fields per mouse in 3 mice), Fig. 3f (6 aortic explants), Fig. 5a (5 different cells), Fig. 6a (3 mARs), Fig. 6b (3 mARs), Fig. 6c (4 sprouts), Fig. 6d (8 lateral sprouts), Fig. 7a (2 fields per mouse in 3 mice). Supplementary Fig. 1a (5 cells per podosomal marker), Supplementary Fig. 1b (3 cells), Supplementary Fig. 1e (5 aortic explants), Supplementary Fig. 2b (2 fields), Supplementary Fig. 2c (9 fields), Supplementary Fig. 2e (2 fields), Supplementary Fig. 2f (3 fields in 2 different biopsies), Supplementary Fig. 3a (5 fields per mouse in 3 mice, 3 fields per biopsy for a total of 33 images), Supplementary Fig. 3b (2 fields), Supplementary Fig. 3c (20 fields per slide for MVD and VEGF), Supplementary Fig. 4a (at least 3 cells per marker), Supplementary Fig. 5b (2 cells), Supplementary Fig. 5c (6 aortic explants), Supplementary Fig. 6e (3 cells), Supplementary Fig. 7a (2 mARs), Supplementary Fig. 7b (2 mARs), Supplementary Fig. 7c (3 mARs), Supplementary Fig. 7c (3 mARs), Supplementary Fig. 7e (12 fields), Supplementary Fig. 7f (2 mARs), Supplementary Fig. 7i (2 fields in 2 different mice), Supplementary Fig. 7l (3 fields in 2 different biopsies).

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