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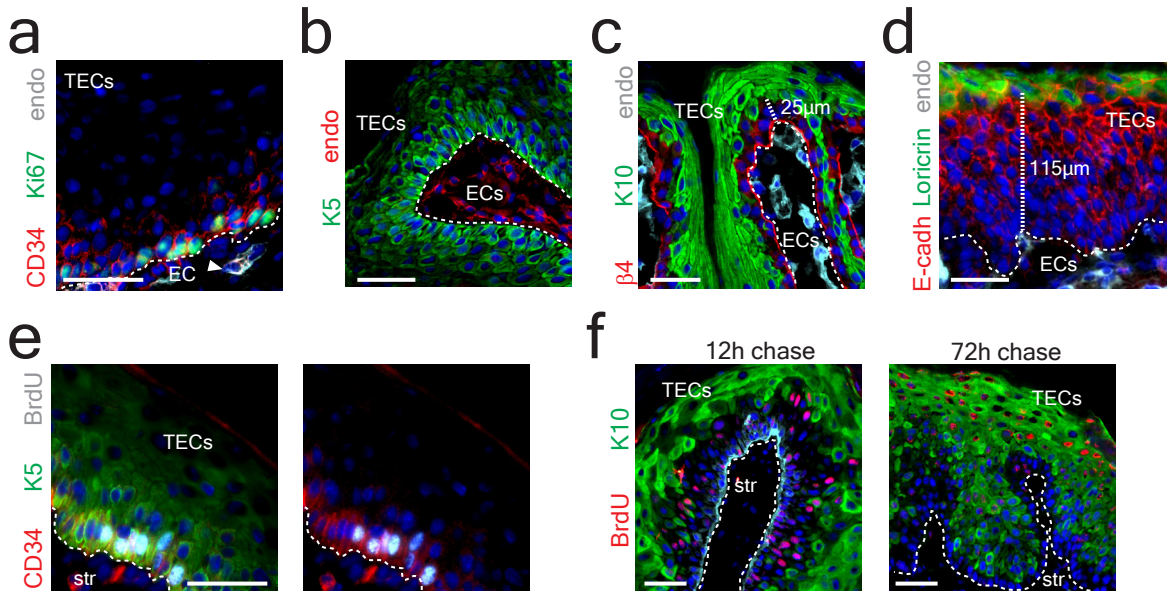
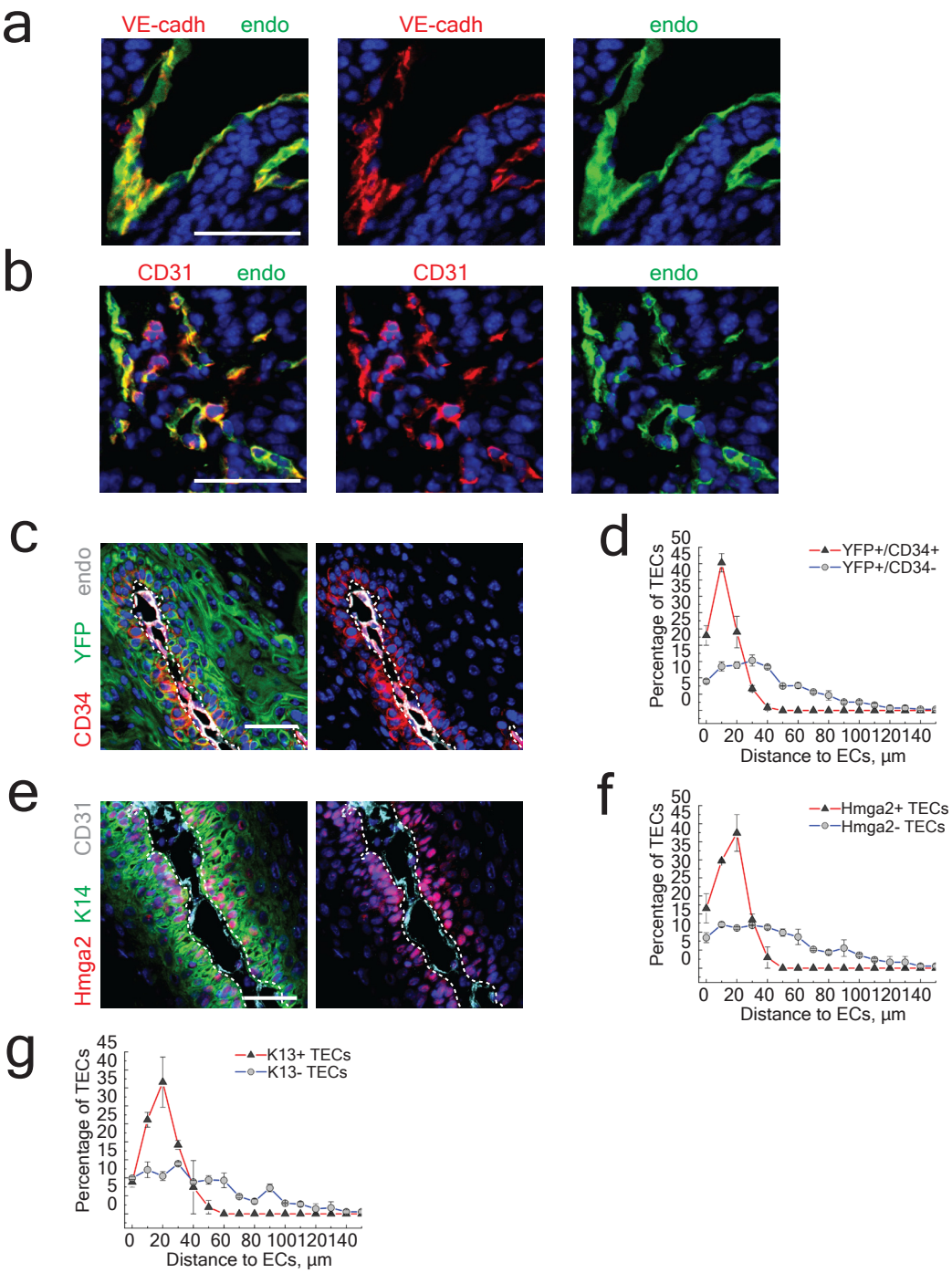
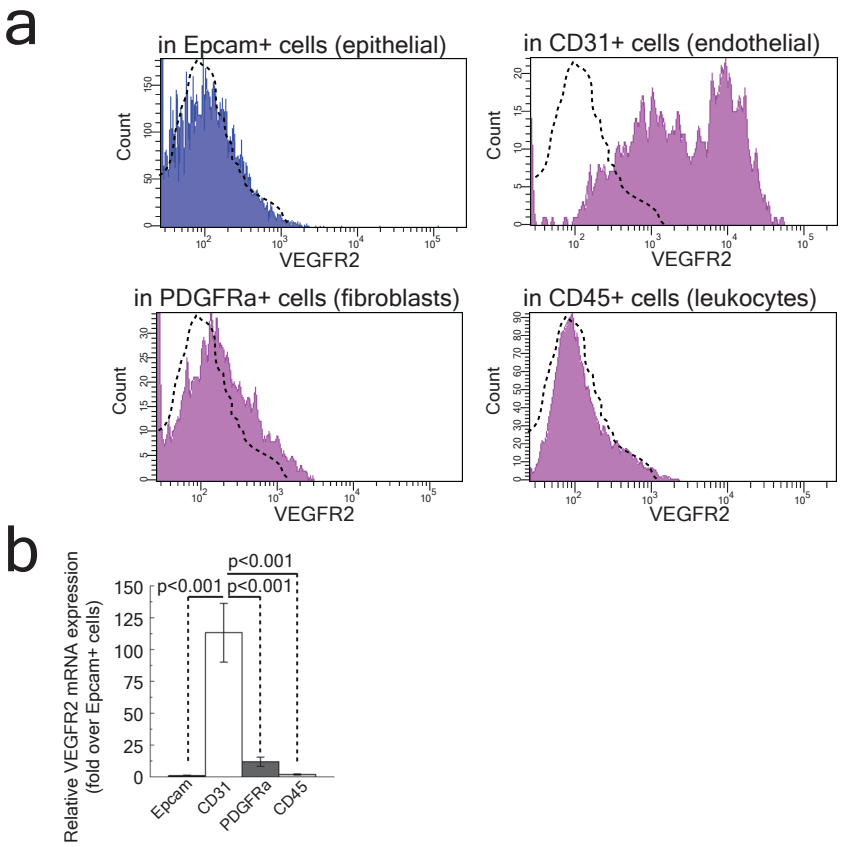


Supplementary figure 1: Papillomas are characterized by a hierarchy of proliferation and differentiation

a, Immunostaining for Ki67 (green), CD34 (red) and endoglin (white) in papilloma shows that CD34+ TECs expressing Ki67 are clustered along the basal lamina, near the endothelial cells (arrow). **b,** Immunostaining for K5 (green) and endoglin (red) in papilloma shows that cells along the basal lamina are positive for K5, a marker of basal epidermal cell and endoglin is expressed exclusively in ECs. **c,** Immunostaining for K10 (green), β4 (red) and endoglin (white) in papilloma shows that the cells in suprabasal layers express K10 and are far from ECs. **d,** Immunostaining for E-cadherin (red), Loricirin (green) and endoglin (white) shows that terminally differentiated TECs are far from ECs. **e,** Immunostaining for K5 (green), CD34 (red) and BrdU (white) in papilloma 4 hours after BrdU administration shows that basal CD34+ TECs incorporate BrdU. **f,** Immunostaining for K10 (green) and BrdU (red) in papilloma 12 and 72 hours after BrdU administration shows that over time, cells that had incorporated BrdU in the basal layer, migrate suprabasally and undergo terminal differentiation. Abbreviations: "TECs" means "tumour epithelial cells", "str" means "tumour stroma" "EC" means "endothelial cells"; on each immunostaining picture, Hoechst nuclear staining is presented in blue; scale bars represent 50µm.

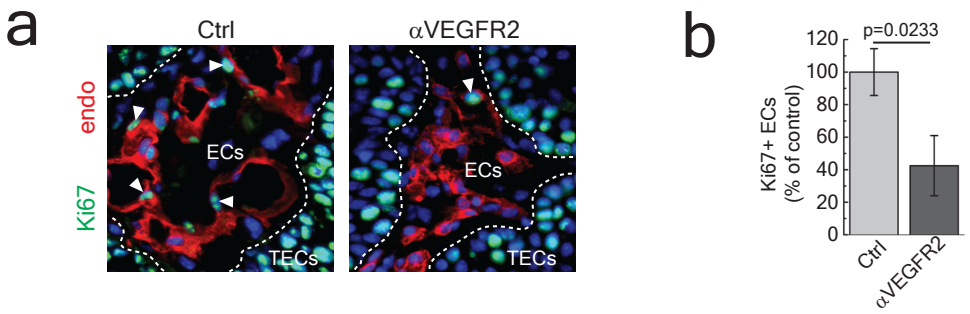


Supplementary figure 2: CSCs are localized in a perivascular microenvironment
a, Immunostaining for endoglin (green) and the endothelial cell marker VE-cadherin (red) in a representative papilloma shows the coexpression of endoglin and VE-cadherin in ECs. **b,** Immunostaining for endoglin (green) and the endothelial cell marker CD31 / PECAM-1 (red) in a representative papilloma shows the coexpression of endoglin and CD31 in ECs. **c,** Immunostaing for CD34 (red), YFP (green) and endoglin (white) in K14Cre:RosaYFP papilloma shows the proximity between CD34+/YFP+ TECs and ECs as compared to YFP+/CD34- TECs. **d,** Relative distance of CD34+/YFP+ and CD34-/YFP+ TECs to ECs. **e,** Immunostainings for Hmga2 or in red with K14 (green) and CD31 (white) show the proximity of CSCs to ECs. Relative distance of Hmga2+ and Hmga2- TECs (**f**) or K13+ and K13- TECs to ECs (**g**). Error bars mean s.e.m.

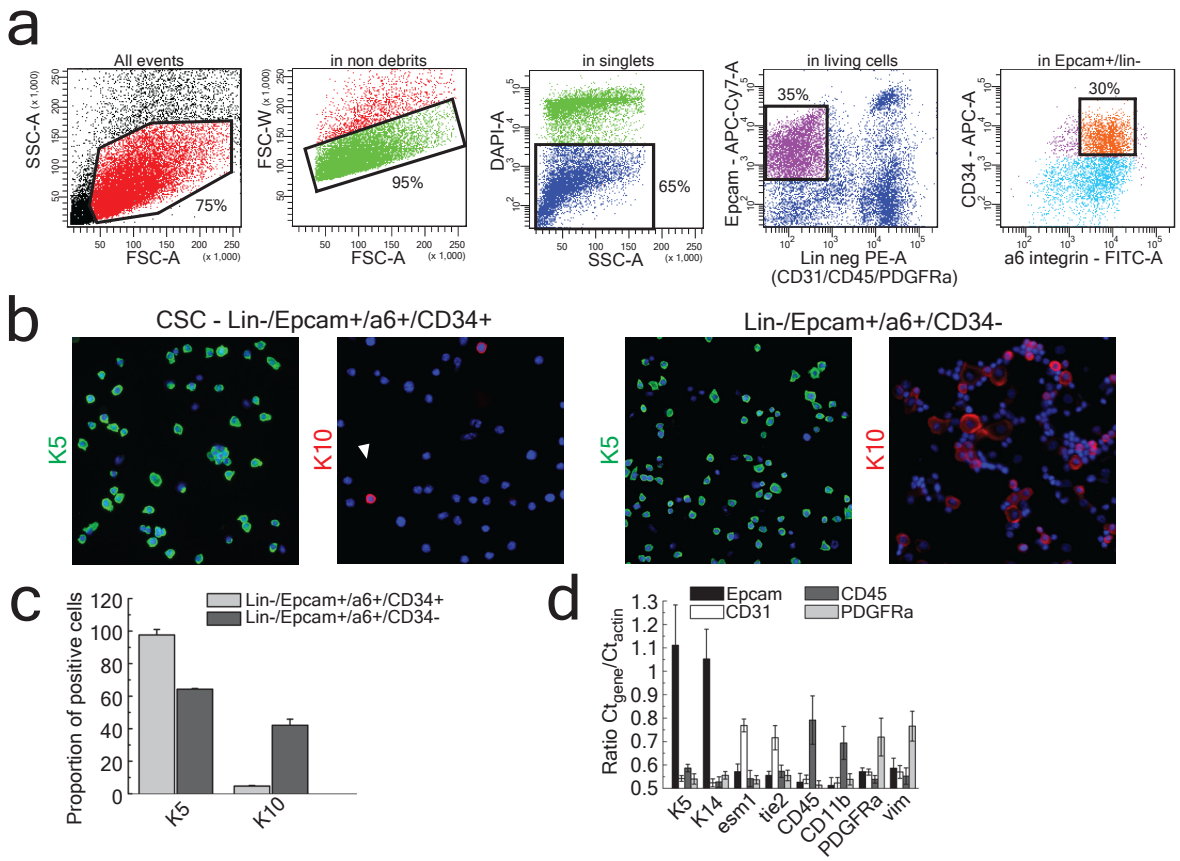


Supplementary figure 3: VEGFR2 marks specifically ECs

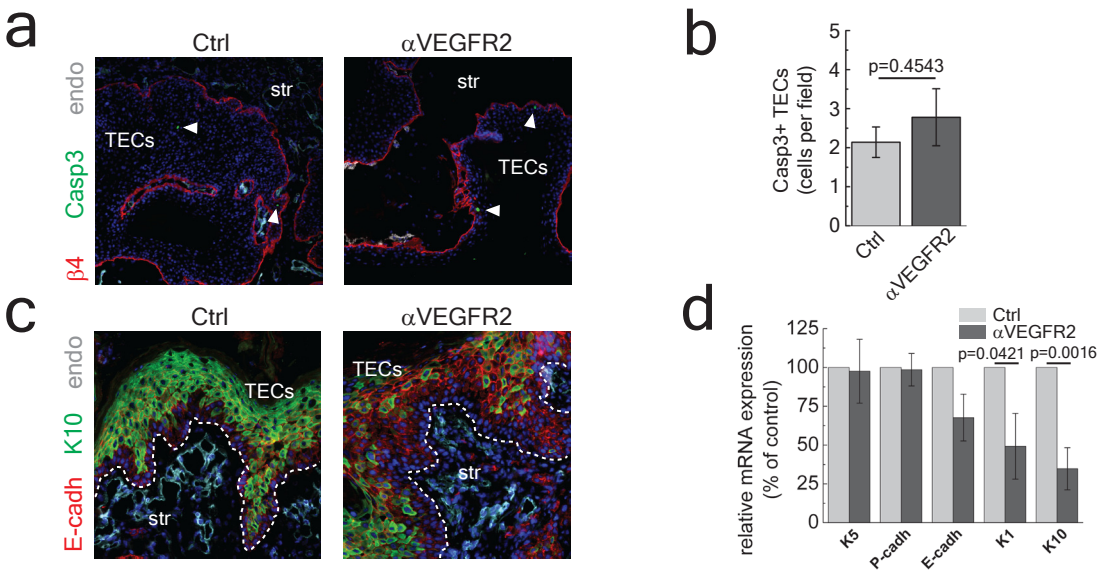
a, FACS analysis of VEGFR2 in different tumour cell populations. The different populations of tumour cells are stained with Epcam (TECs), CD31 (ECs), PDFGRa (mesenchyme) or CD45 (immune cells) in combination with anti-VEGFR2 (flk1). Dot line represents the control isotype (Rat IgG). This result shows that VEGFR2 expression is highly expressed in ECs and at low level in some mesenchymal cells. **b,** Relative VEGFR2 mRNA expression in Epcam+, CD31+, CD45+ and PDGFRa+ sorted cells. These data show that VEGFR2 mRNA is highly expressed in ECs. Data are normalized by the mRNA expression in Epcam+ cells. n=3 mRNA samples (ANOVA and tukey test were performed). Error bars mean s.e.m.



Supplementary figure 4: Blocking VEGFR2 inhibits EC proliferation
a, Immunostaining for endoglin (red) and Ki67 (green) in papillomas from mice treated for 2 weeks with a control IgG (Ctrl) or with the αVEGFR2 blocking antibody (αVEGFR2). **b,** Quantification of the percentage of Ki67+ ECs shows a decrease in EC proliferation 2 weeks after the beginning of αVEGFR2 treatment. n=12 from 3 different mice per condition, 1200 cells were counted per condition (t-student was performed). Abbreviations: "TECs" means "tumour epithelial cells", "str" means "tumour stroma"; on each immunostaining picture, Hoechst nuclear staining is presented in blue; scale bars represent 50μm. Error bars mean s.e.m.

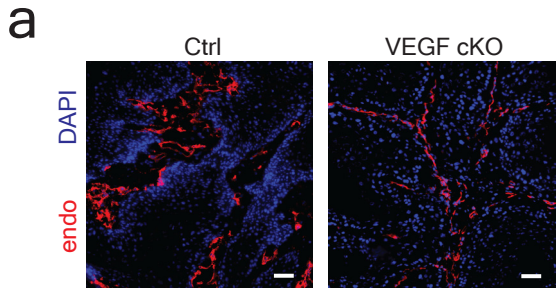


Supplementary figure 5: FACS purification of CD34+ TECs.
a, Gating strategy of CD34+ TECs: Debrits are discarded in P1, doublets are discarded in P2, living cells are gated in P3 by Hoechst dye exclusion, TECs are gated in P4 by a combination of a lineage negative (CD31, CD45 and CD140a in PE) and a positive marker Epcam (APC-Cy7). CSCs are then gated in P5 using CD34 (APC) and α6 integrin (FITC). **b,** Immunostaining for epithelial markers K5 (green) or K10 (red) of FACS isolated CD34+ TECs after cytospin. **c,** Quantification of the proportion of K5 and K10 positive cells in the CD34+ TECs and on the other epithelial cells isloated by FACS; n=600 cells counted per condition. **d,** Relative mRNA expression of markers of different cell lineages in the different FACS isolated cell populations. K5 and K14 are specific for epithelial lineage; esm1 and tie2 are specific for endothelial lineage, CD45 and CD11b are specific for immune lineage; and PDGFRa and Vimentin are specific of mesenchymal cells. Data represent the ratio bewteen the Ct of the target gene and the Ct of β-actin. These data show that transcripts for epithelial markers are enriched in the Epcam+ sorted cells, transcripts for endothelial markers are enriched in the CD31+ sorted cells, transcripts for immune markers are enriched in the CD45+ sorted cells, and transcripts for mesenchymal markers are enriched in the PDGFRa+ sorted cells. n=3. Hoechst nuclear staining is presented in blue; scale bars represent 50μm. Error bars mean s.e.m.

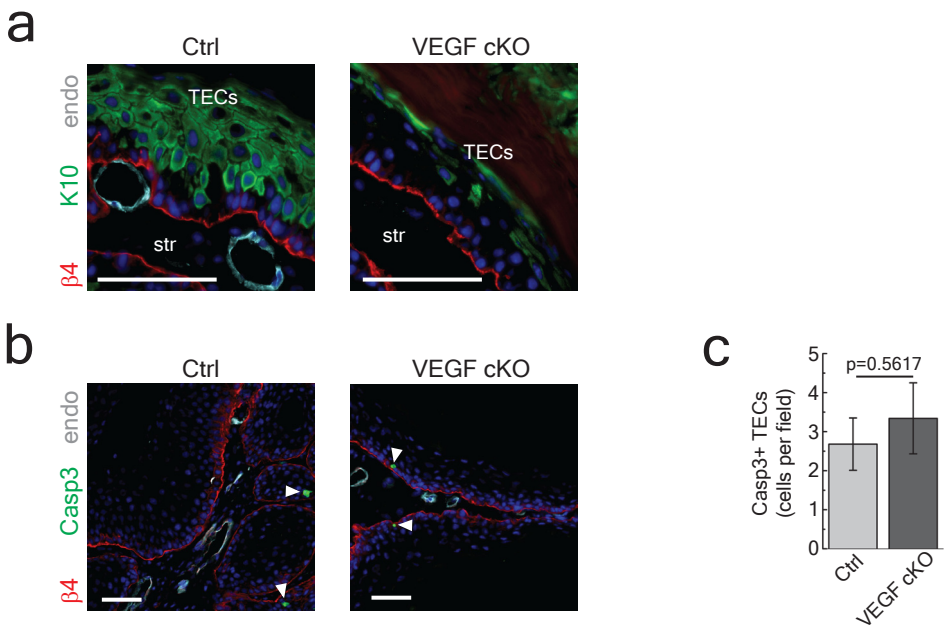


Supplementary figure 6: Blocking VEGFR2 inhibits tumour epithelial cell differentiation without inducing apoptosis

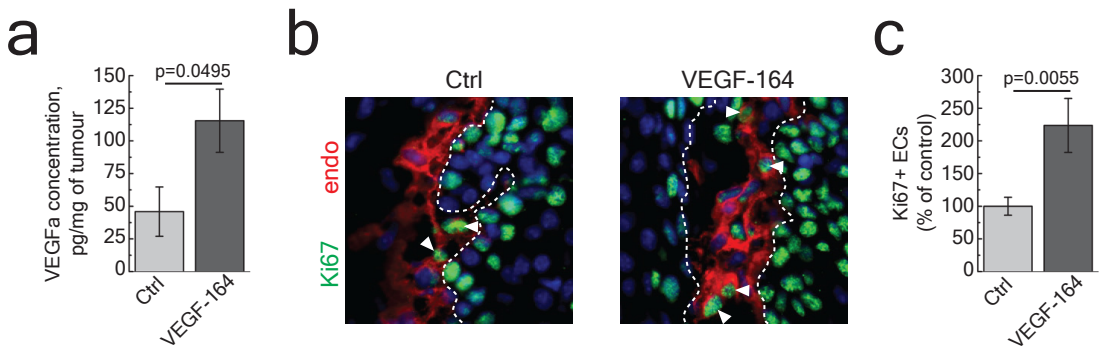
a, Immunostaining for β4 integrin (red), active Caspase 3 (green) and endoglin (white) in a representative papilloma treated either with IgG1 (Ctrl) or with the αVEGFR2 for 1 week. **b,** Quantification of the average number of active caspase 3 positive TECs per field (10x) in papilloma treated either with the control IgG1 or with the αVEGFR2 shows no significant increase of apoptosis after αVEGFR2 administration for 1 week. n=9 tumours from 3 different mice per condition, 90 fields were counted per condition (t-student was performed). **c,** Immunostaining for E-cadherin (red), K10 (green) and endoglin (white) of papillomas treated with a control IgG (Ctrl) or with the αVEGFR2 shows a decrease of TEC differentiation after αVEGFR2 administration for 2 weeks. **d,** qRT-PCR for the basal epithelial markers K5 and P-cadherin, epithelial cell marker E-cadherin and differentiated epithelial cell markers K1 and K10 performed on tumours one week after treatment with antibody, shows a decrease of TEC differentiation. n=6 tumours from 3 different animals per conditions. Data were normalized by the mRNA expression of each marker in IgG treated animals (ctrl) (t-student was performed) .Abbreviations: "TECs" means "Tumour epithelial cells", "str" means "tumour stroma"; on each immunostaining picture, Hoechst nuclear staining is presented in blue; scale bars represent 50μm. Error bars mean s.e.m.



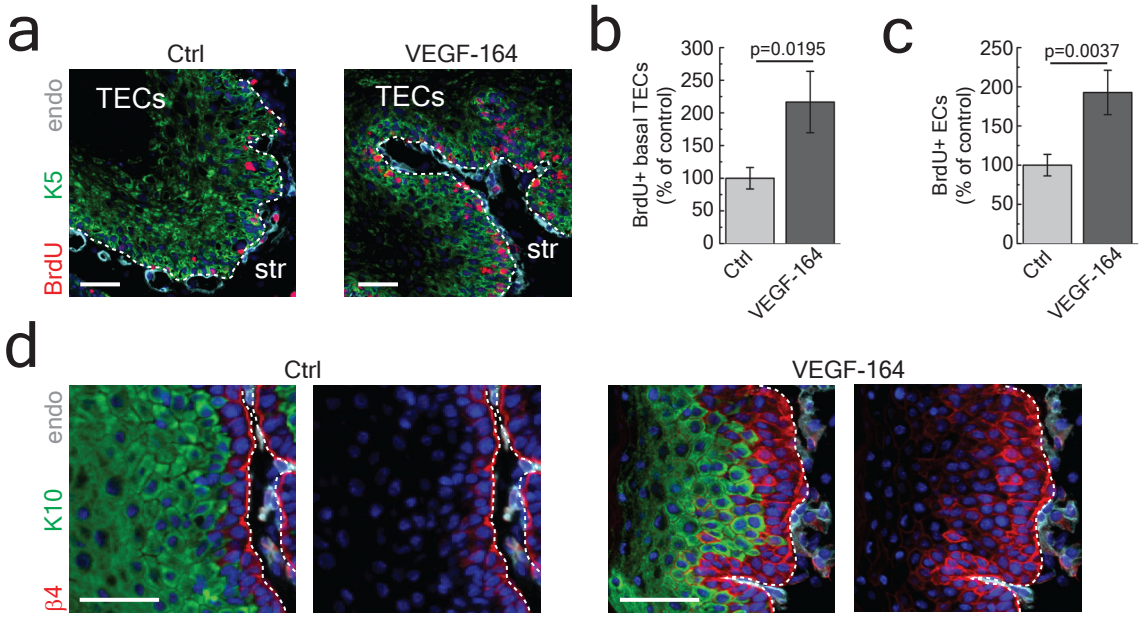
Supplementary figure 7: Deletion of VEGF-A in tumour epithelial cells decreases MVD.
a. Immunostaining for endoglin (red) in representative papillomas from control and VEGFcKO mice shows a decrease in the MVD 1 week after TAM administration. Hoechst nuclear staining is presented in blue; scale bars represent 50μm.



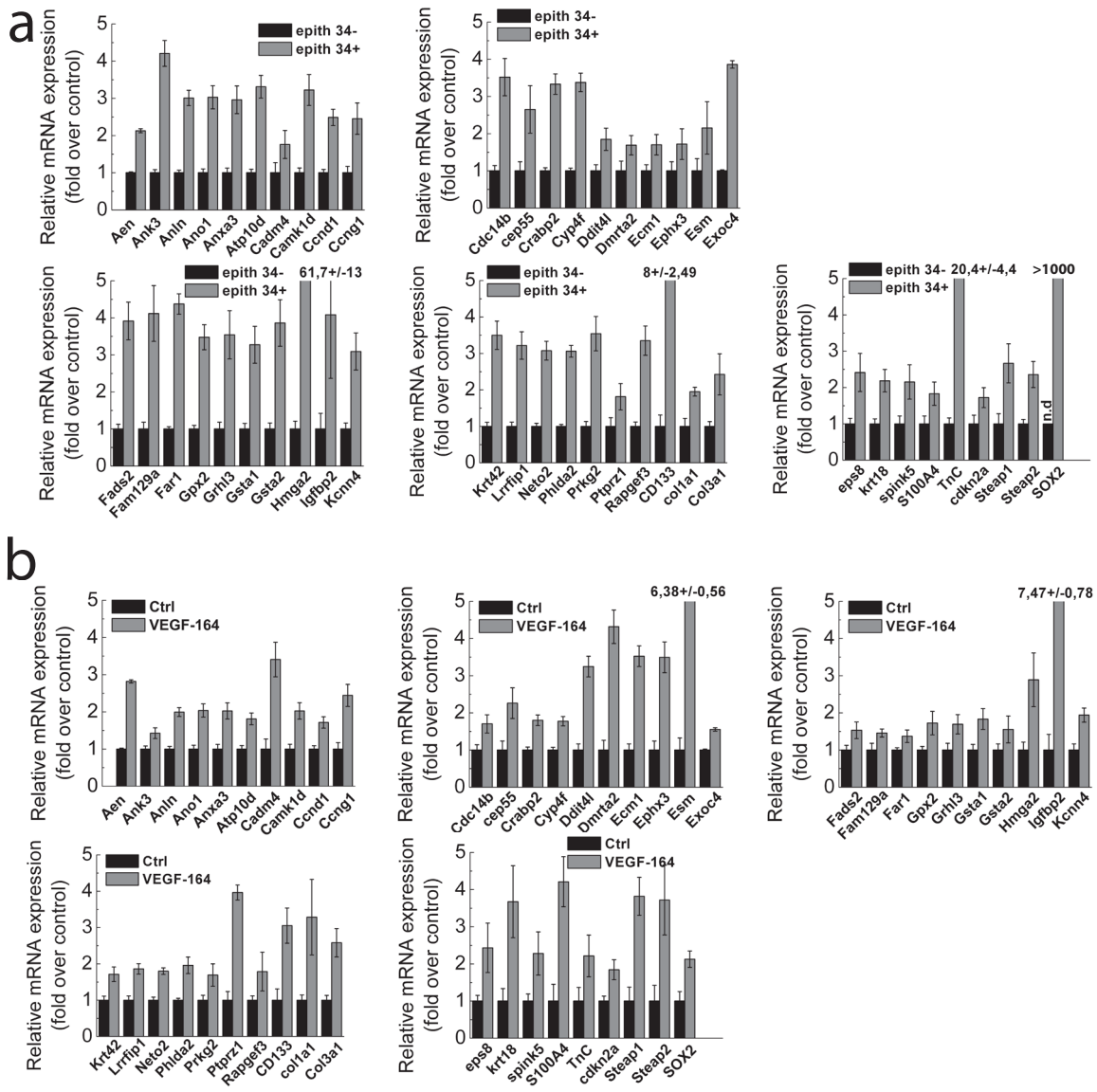
Supplementary figure 8: Deletion of VEGF-A in tumour epithelial cells decreases the number of differentiated layers without inducing apoptosis.
a. Immunostaining for β4 integrin (red), K10 (green) and endoglin (white) in representative papillomas from mice of each genotype. **b.** Immunostaining for β4 integrin (red), endoglin (white) and active caspase 3 (green). **c.** Quantification of the average number of active caspase 3+ TECs per field (10x) in papillomas from mice of each genotype. n=30 fields from 3 different animals per condition (t-student was performed). Abbreviations: "TECs" means "tumour epithelial cells" and "str" means "tumour stroma"; on each immunostaining picture, Hoechst nuclear staining is presented in blue; scale bars represent 50μm. Error bars mean s.e.m.



Supplementary figure 9: Increasing VEGF-A expression in TECs increases ECs proliferation.
a. Measurement of VEGF-A protein expression in papilloma from control and VEGF-164 mice by ELISA. **b.** Immunostaining for endoglin (red) and Ki67 (green) in papillomas from control or VEGF-164 mice (VEGF-164 overexpression) treated for 2 weeks with TAM (10mg/week); n=6 (t-student). **c.** Quantification of the percentage of Ki67+ endothelial cells (ECs) shows that the proportion of ECs in cell cycle is increased following VEGF-A overexpression by TECs. n=240 from 3 different mice per condition (t-student was performed). On each immunostaining picture, Hoechst nuclear staining is presented in blue; scale bars represent 50µm. Error bars mean s.e.m.

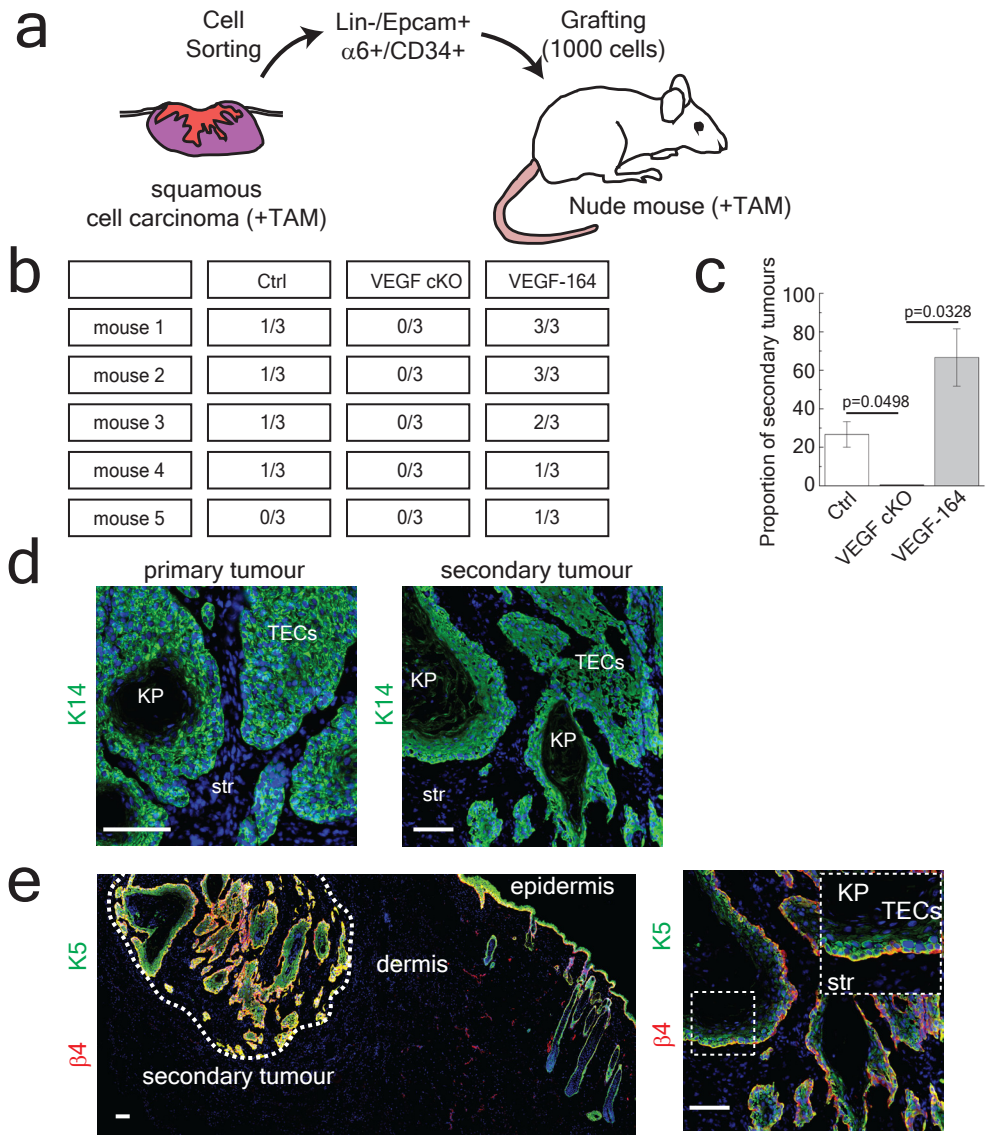


Supplementary figure 10: Increasing VEGF-A expression in TECs increases TECs and ECs proliferation and leads to an accumulation of undifferentiated TECs.
a. Immunostaining for K5 (green), BrdU (red) and endoglin (white) in papillomas from control mice and VEGF-164 mice treated for 2 weeks with TAM (10mg/week). **b.** TECs proliferation: quantification of the percentage of BrdU+ K5+ tumour epithelial cells. n=450 from 3 different mice per condition (t-student was performed). **c.** ECs proliferation: quantification of the percentage of BrdU+ endoglin+ ECs. n=210 cells counted from 3 different mice per condition (t-student was performed). **d.** Immunostaining for β4 integrin (red), the differentiation marker K10 (green) and endoglin (white) in a representative papilloma shows an accumulation of undifferentiated cells in papillomas overexpressing VEGF-164. Abbreviations: "TECs" means "tumour epithelial cells", "str" means "tumour stroma"; on each immunostaining picture, Hoechst nuclear staining is presented in blue; scale bars represent 50µm. Error bars represent s.e.m.



Supplementary figure 11: VEGF-A overexpression in TECs increases expression of genes from the "CD34+ signature".

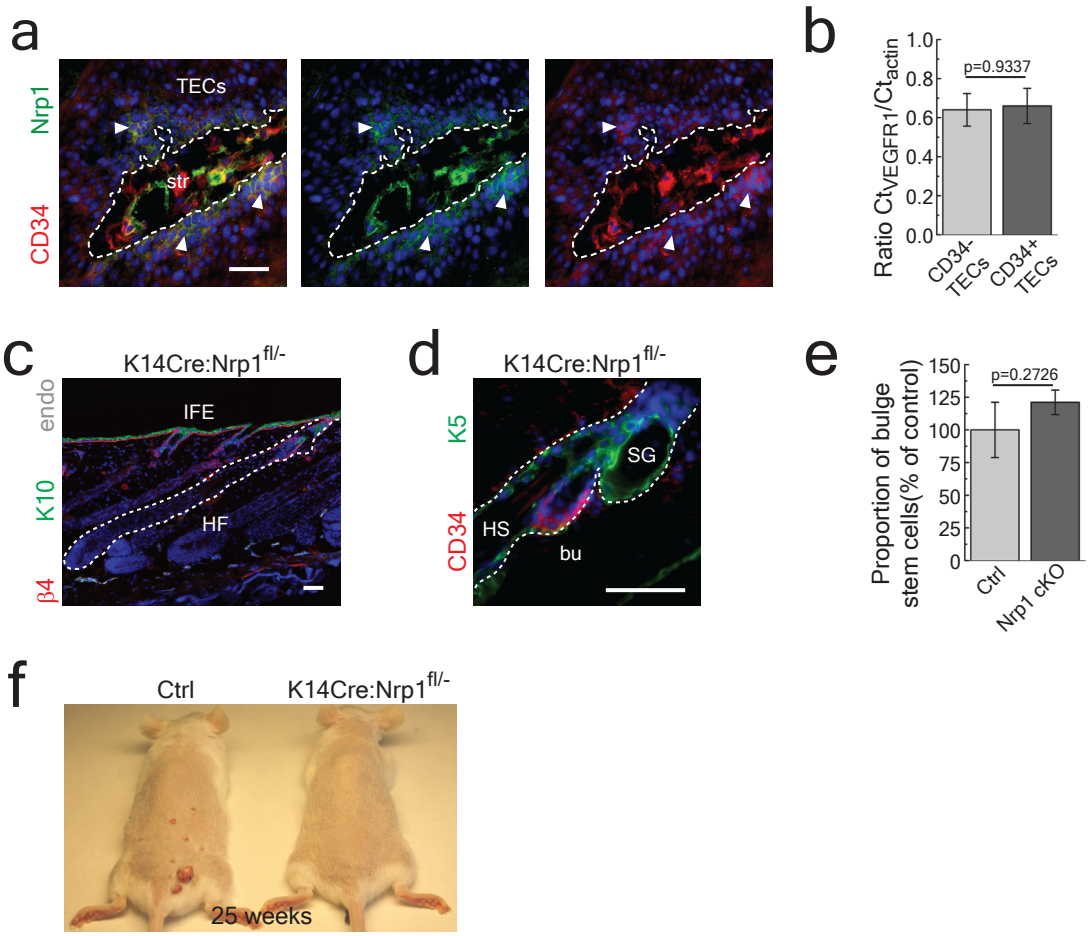
a, mRNA expression of 49 transcripts from the "CD34+ signature " in sorted epithelial CD34- and CD34+ cells. Data represent the mean of the fold of change. Data are normalized to the gene expression in epithelial CD34- cells. **b,** mRNA expression of the "CD34 signature" in CD34+ isolated TECs from control and K14CreER:RosaVEGF-164 mice 2 weeks following TAM administration. n=3 mice per genotype. Data represent the mean of the fold of change. Data are normalized to the gene expression in epithelial CD34+ TECs from control mice. These data show that 47 genes of the "CD34 signature" are modulated by VEGF expression in CD34+ TECs.



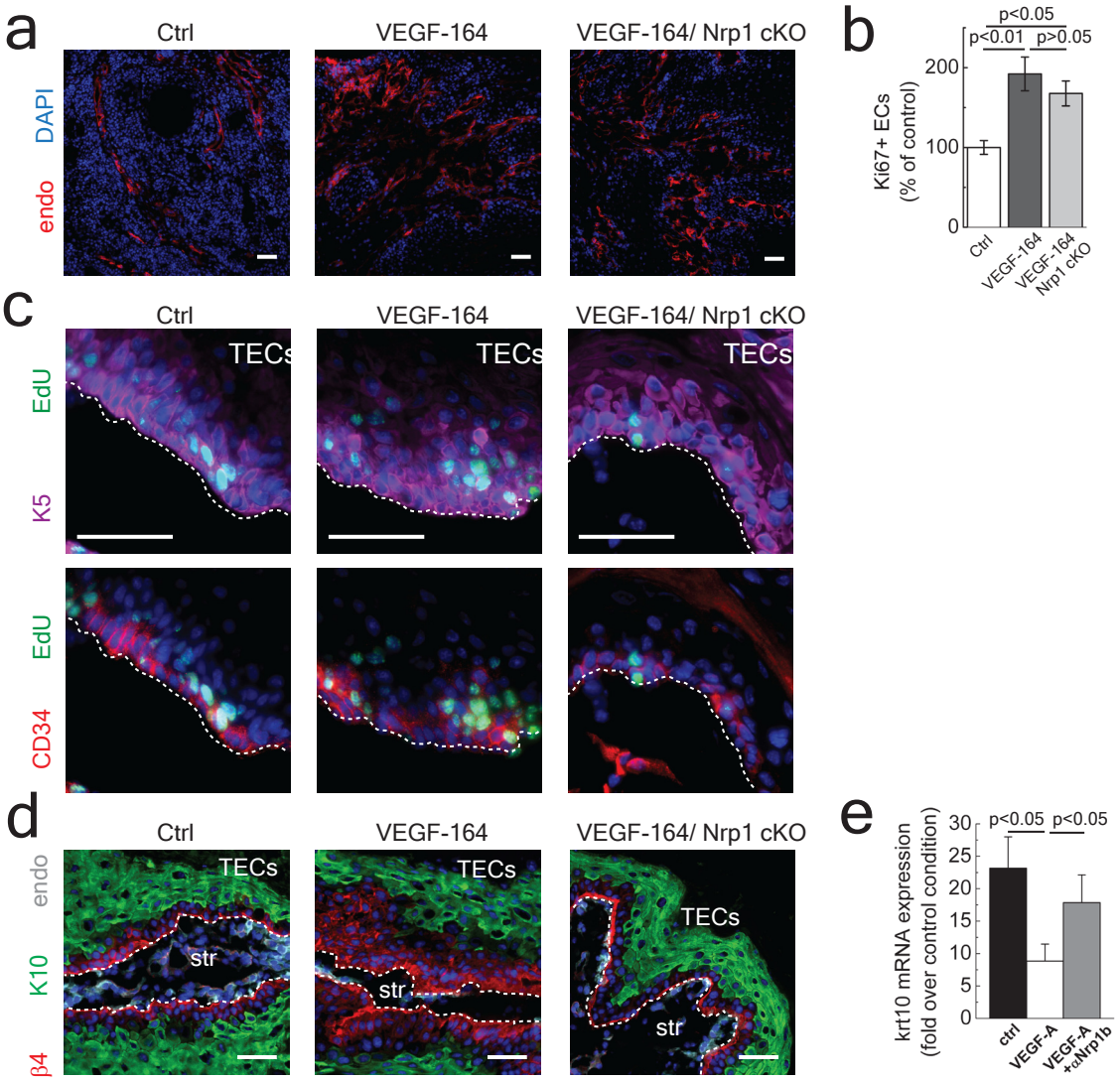
Supplementary figure 12: VEGF-A expression by TECs modulates their ability to form secondary tumours following transplantation into immunodefficient mice.

a, Scheme summarizing the strategy used to transplant 1000 FACS isolated CD34+ TECs from squamous cell carcinoma into immunodefficient mice. **b,** Table summarizing the frequency of secondary tumours formation following the transplantation of FASC isolated epithelial CD34+ cells (lin-/epcam+/a6+/CD34+) from control, VEGF cKO and VEGF-164 mice. 1000 cells were injected subcutaneously and tumours were detected by palpation. **c,** Incidence of secondary tumour formation by CD34+ TECs from control, VEGF cKO and K14CreER:Rosa-VEGF-164 mice 4 weeks following transplantation. n=15 from 5 different mice (Fisher's exact test was performed). These data show that VEGF cKO mice fail to form secondary tumours while VEGF-A overexpressing cells form more frequently secondary tumours. **d,** Immunostaining for K14 (green) in primary and secondary tumours formed by FACS isolated CD34+ TECs (lin-/epcam+/α6+/CD34+). These data show that CD34+ TECs give rise to secondary tumours with identical K14 expression when grafted subcutaneously into nude mice. Note the presence of keratin pearl, a typical feature of squamous tumours, are found both in the primary and the secondary tumours. **e,** Immunostaining for K5 (green) and β4 (red) in a secondary tumour formed by FACS isolated CD34+ TECs (lin-/epcam+/α6+/CD34+) at low and high magnification. These data show a secondary tumours that formed within the dermis. The cells along the basal lamina are positive for K5, a marker of basal tumour cells (inset on the right). Abbreviations: "TECs" means "tumour epithelial cells", "str" means "tumour stroma" and "KP" means keratin pearl. On each immunostaining picture, Hoechst nuclear staining is presented in blue; scale bars represent 100μm. Error bars mean s.e.m.

Supplementary figure 12

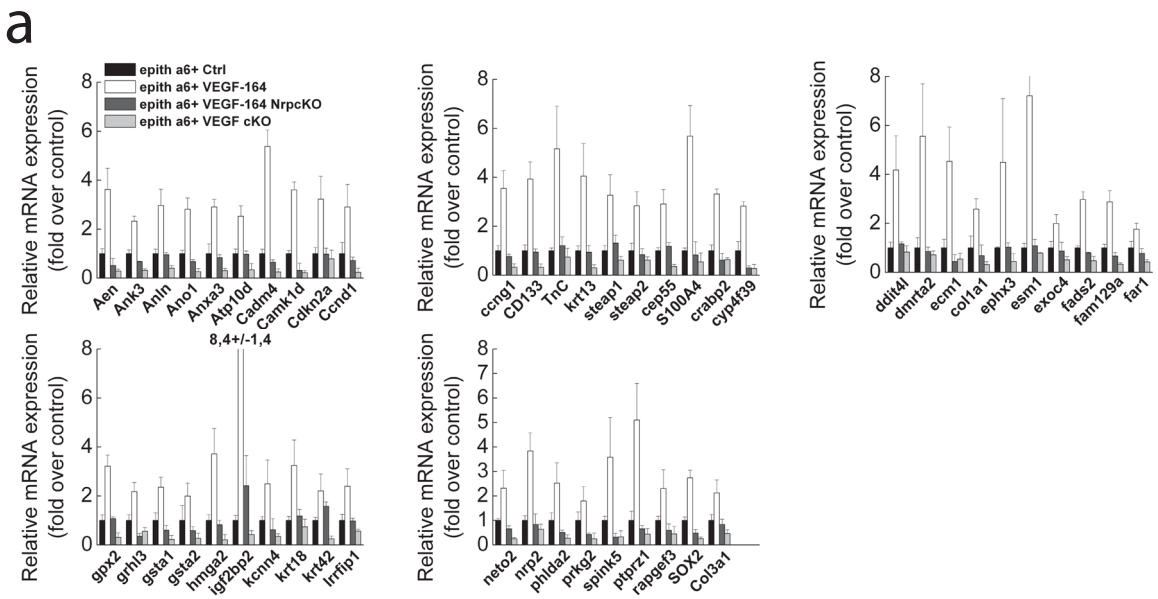


Supplementary figure 13: Deletion of Nrp1 in epidermal cells inhibits skin tumour initiation.
a, Immunostaining for CD34 (red) and Nrp1 (green) in papilloma shows the colocalisation of these two markers in CSCs. **b,** VEGFR1 mRNA expression in CD34+ and CD34- TECs (lin-/epcam+/α6 integrin+). Data represent the ratio between the Ct of VEGFR1 and the Ct of actin measured by real time PCR (t-student was performed). These data show that VEGFR1 is not preferentially expressed by CD34+ TECs. n=3 from 3 different mice. **c,** Immunostaining for β4 integrin (red), K10 (green) and endoglin (white) in skin from K14Cre+/-:Nrp1-/fl mice showing a normal morphology and differentiation of the interfollicular epidermis (IFE) with hair follicles (HF). **d,** Immunostaining for CD34 (red) and K5 shows a normal bulge stem cell compartment (bu) on normal hair follicles morphology. **e,** FACS quantification of the CD34+ bulge cell from control and K14Cre:Nrp-/fl mice shows no variation in the pool of hair follicle SC in the absence of Nrp1 expression (t-student was performed). **f,** Pictures of two representative mice 25 weeks after treatment with DMBA/TPA show an abscence of skin tumours on mice with a conditionnal deletion of Nrp1 in the epidermis. Abbreviations: “bu” means “bulge”, “SC” means “stem cells”, “HF” means “hair follicle”, “HS” means hair shaft and “SG” means “sebaceous gland”; on each immunostaining picture, Hoechst nuclear staining is presented in blue; scale bars present 50µm. Error bars mean s.e.m



Supplementary figure 14: Deletion of Nrp1 in TECs inhibits the VEGF promoting effect on TECs proliferation but not on MVD.

a. Immunostaining for endoglin (red) in papillomas from K14CreER-:Rosa26-VEGF-164+:Nrp1fl/- (Ctrl) K14CreER+:Rosa26-VEGF-164+:Nrp1fl/+ (mice overexpressing VEGF-164) and K14CreER+:Rosa26-VEGF-164+:Nrp1fl/- (mice overexpressing VEGF-164 and deficient for Nrp1 in TECs) 3 weeks after the beginning of TAM administration. These data show that the loss of Nrp1 in the TECs does not affect VEGF-A promotion on MVD. **b,** ECs proliferatioin measured by the proportion of Ki67+ ECs in control, VEGF-164 and Nrp1 cKO mice; n=6 tumours (ANOVA and tukey test were performed). These data show that VEGF-164 stimulates ECs proliferation in the absence of Nrp1 expression in TECs. **c.** Immunostaining for CD34 (red), EdU (green) and keratin5 (magenta) in papillomas from mice of each genotype 3 weeks after the beginning of TAM administration. These data show that VEGF-164 overexpression does not increase CD34+ TECs proliferation in the absence of Nrp1 expression. **d.** Immunostaining for b4 integrin (red), K10 (green) and endoglin (white) in a representative papilloma from mice of each genotype. These data show that VEGF-164 overexpression does not promote undifferentiated basal cell expansion in the absence of Nrp1 expression in TECs. **e,** Quantification of the relative krt10 mRNA expression 48hours after calcium treatment in epithelial CD34+ sorted cells cultured in vitro in a normal medium (ctrl), or supplemented with VEGF-A (50ng/mL) or a combination of VEGF-A and a Nrp1 blocking antibody (aNrp1b). These results show that VEGF-A treatment delays the expression of krt10 following calcium treatment in CD34+ TECs and this effect is blocked by the addition of anti-Nrp1 or VEGF blocking antibodies. n=3 (ANOVA and tukey test). Abbreviations: “TECs” means “tumour epithelial cells”, “str” means “tumour stroma”. On each immunostaining picture, Hoechst nuclear staining is presented in blue; scale bars present 50µm. Error bars mean s.e.m



Supplementary figure 15: Deletion of Nrp1 in TECs blocks the VEGF promoting effect on the expression of the "CD34+ signature" genes.

a, Expression of 49 transcripts from the "CD34+ signature" that are upregulated by VEGF in sorted basal TECs (Lin-/Epcam+/α6itg+) from control, VEGF-164, VEGF-164/Nrp1 cKO and VEGF cKO mice. 3 different mRNA samples from sorted Epcam+/α6itg+ cells isolated from a dozen of papillomas (3 mice per genotype). Data represent the mean of the fold of change. Data are normalized to the mRNA expression in sorted basal TECs (Lin-/Epcam+/α6itg+) from control mice. These data show that the genes of the "CD34+ signature" upregulated 2 weeks after VEGF overexpression are no longer upregulated in the absence of Nrp1 in TECs. The downregulation of these transcripts in VEGF cKO mice suggests that the autocrine VEGF expression is important for the expression of these "CD34+ signature" markers in TECs. Error bars mean s.e.m.