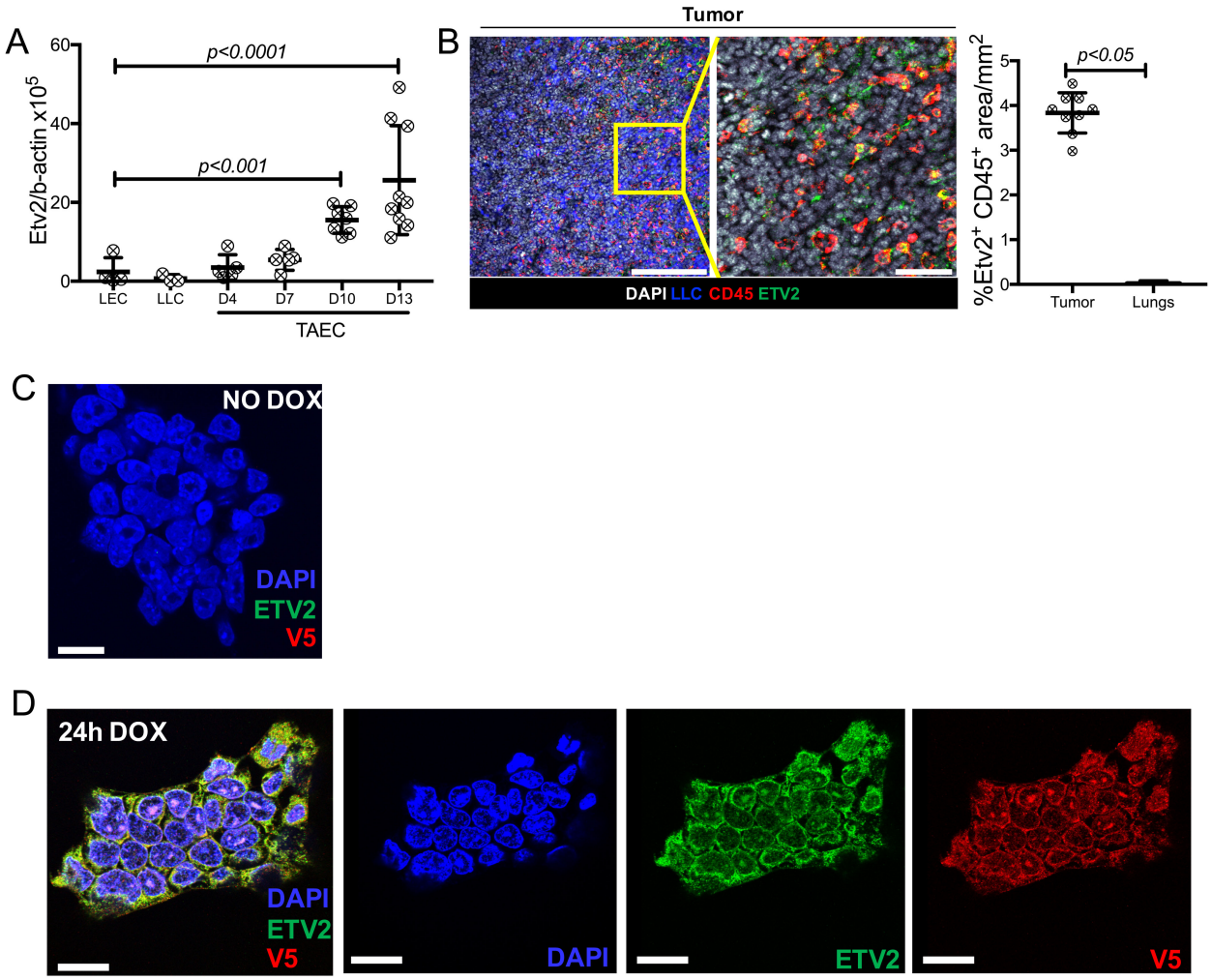
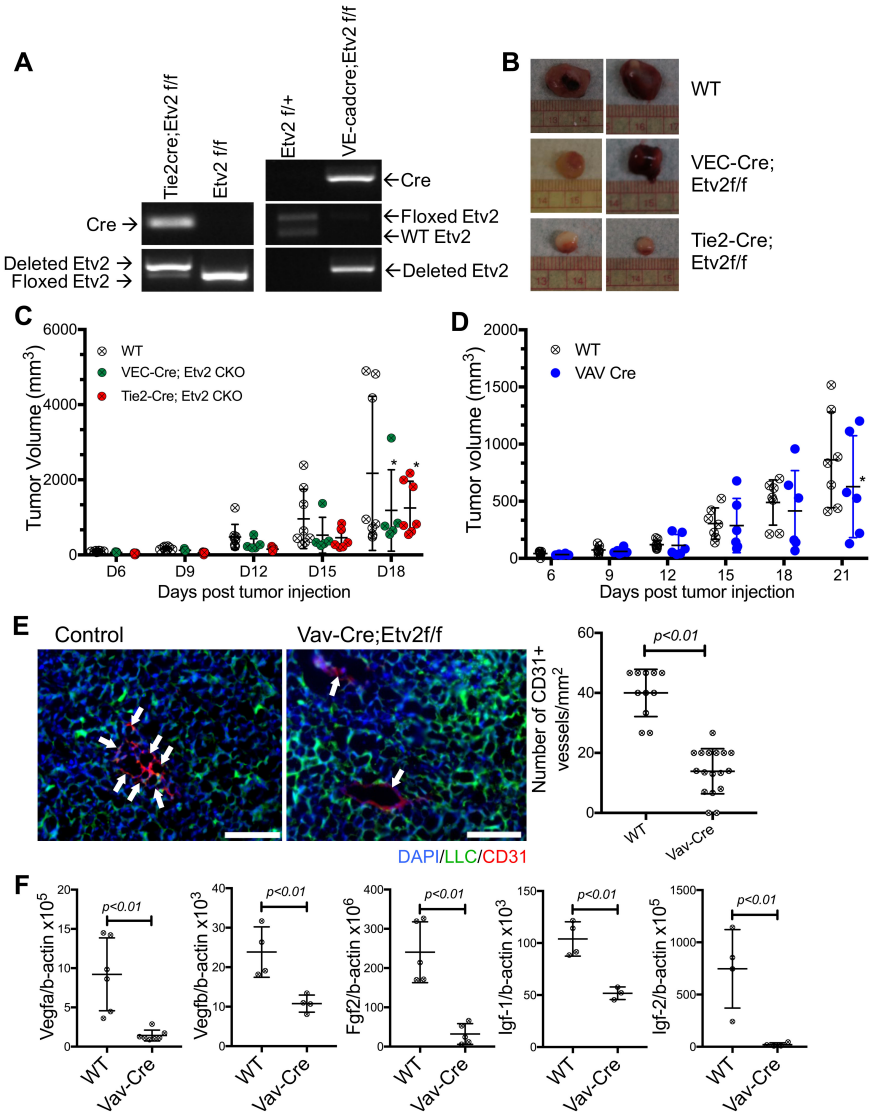
Supplemental figure 1. *Etv2* expression is upregulated in tumor associated endothelial and hematopoietic cells



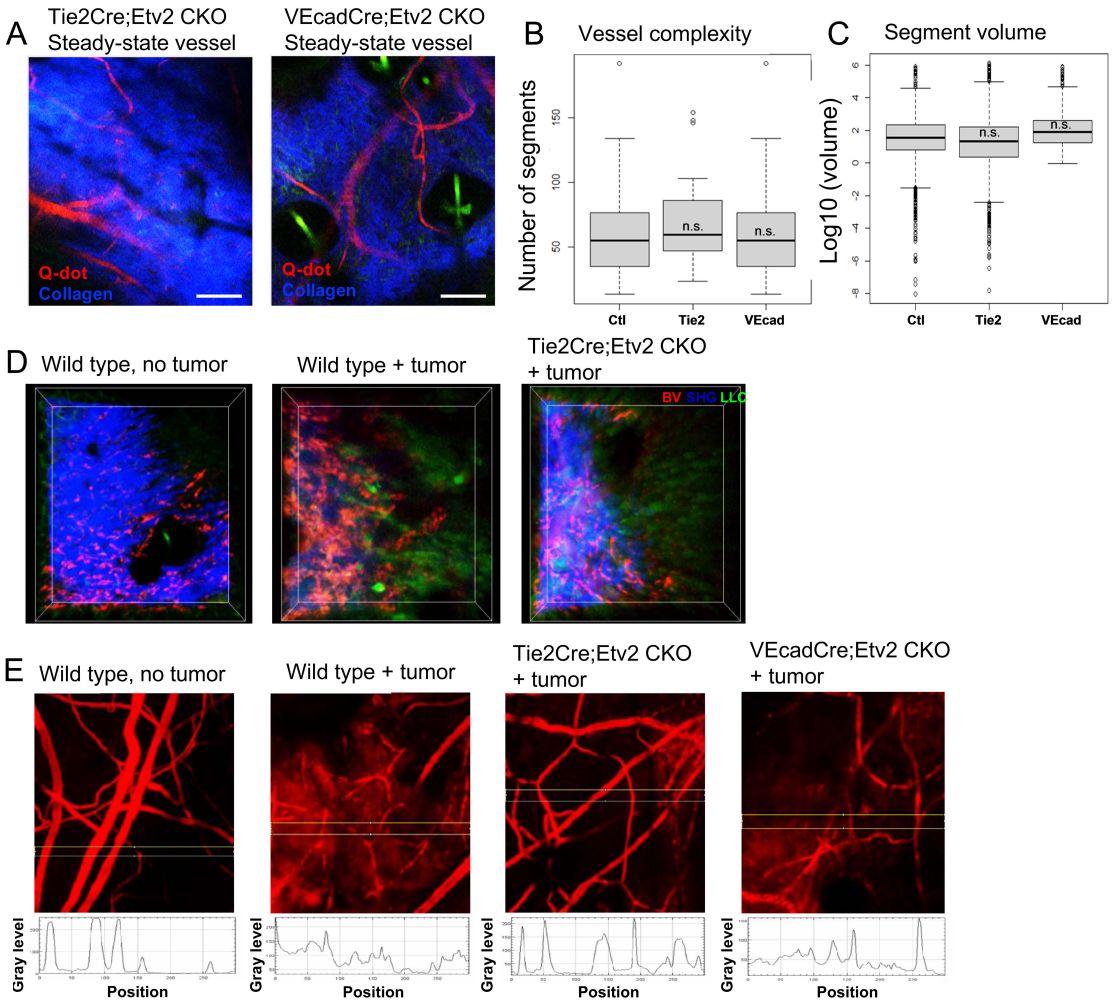
(**A**) qRT-PCR analysis on *Etv2* expression in tumor cells (LLC, n=4), lung endothelial cells (LEC, n=4), and tumor endothelial cells (TAEC, n=6 or more/time point). Tumors were collected on 4, 7, 10 and 13 days post tumor transplantation (ptt). (**B**) Representative images for ETV2 (green) and CD45 (red) immunofluorescence and quantification (n=9/group) of mouse tumor sections, processed after 20 days of tumor transplantation. LLC-GFP cells (blue) and nuclei counterstained with DAPI (grey) are shown. Scale bars: 150μm (70μm for zoomed-in sections). (**C,D**) Representative imgase for ETV2 (green) and V5 (red) immunofluorescence of the *iEtv2* ES cells (**C**) without or (**D**) with 24hour DOX treatment. Sacale bars: 20μm. Data are presented as mean with standard deviation for all measurements. Statistical significances were analyzed by either a two-tailed Student's *t*-test (**B**) or One-way ANOVA with Tukey's multiple-comparison test (**A**).

Supplemental figure 2. *Etv2* expression is required for tumor growth.



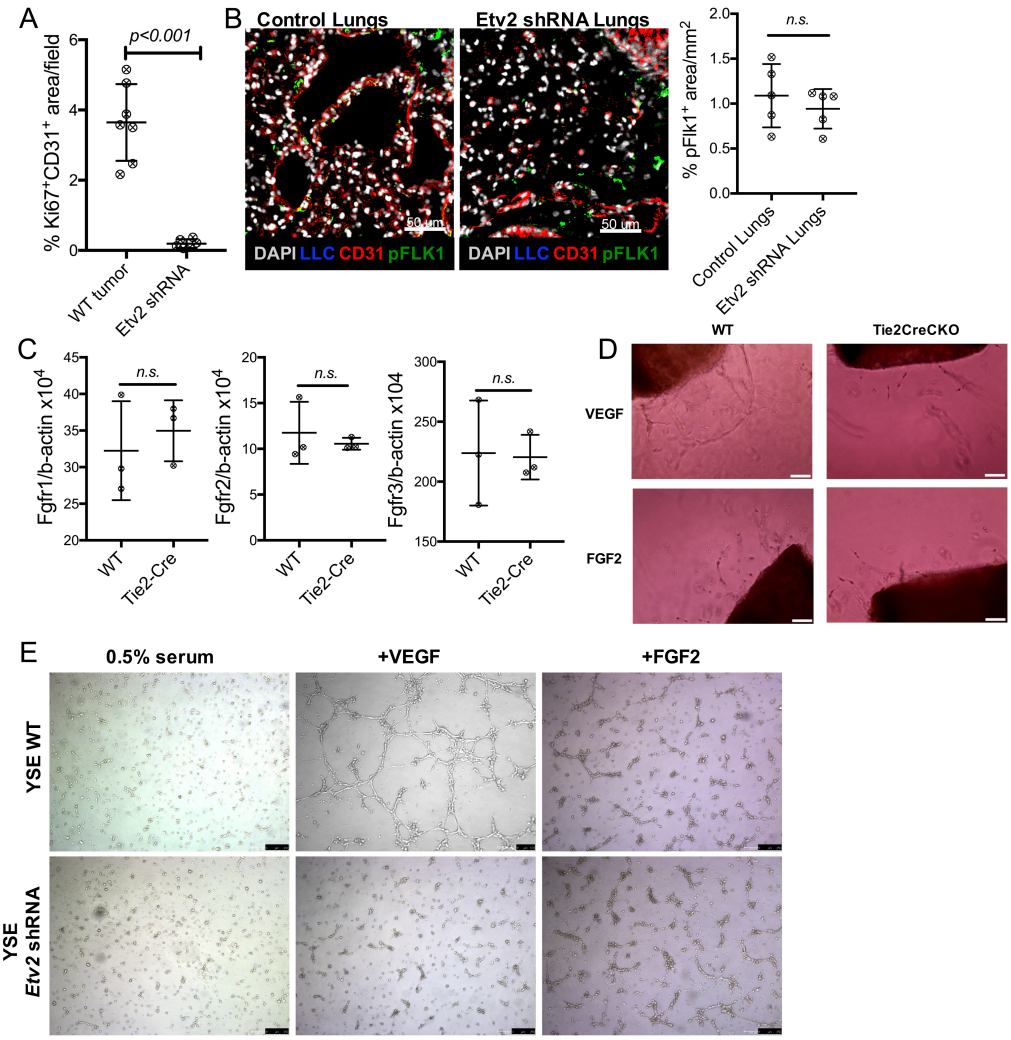
(**A**) Genomic DNA PCR analysis with the lung CD31^{+}CD45^{-} endothelial cells of control, *VEC-Cre;Etv2,* and and Tie2-Cre; *Etv2* CKO mice. (**B**) Representative images of tumors harvested from littermate control (WT), VEC-Cre;*Etv2,* and and Tie2-Cre;*Etv2* CKO mice, on day 21ptt. (**C**) Tumor growth of littermate control, *VEC-Cre;Etv2 and Tie2-Cre;Etv2 CKO* mice using B16 melanoma cells. n=8(control), 7(*Tie2-Cre),* and 5(*VEC-Cre)*; **P<0.05* (**D**) Tumor growth of littermate control, *Vav-Cre;Etv2 CKO* mice using LLC-GFP cells. n=7(control) and 6(*Vav-Cre)*; **P<0.05* (**E**) Representative images for CD31 (red) immunofluorescence and quantification of CD31^{+} vessel density of the tumor sections, processed after 21 days of transplantation (n=10/group). LLC-GFP cells (green) and nuclei counterstained with DAPI (blue) are shown. Scale bars: 50μm. (**F**) qRT-PCR analysis of *Vegfa, Vegfb, Fgfr2, Igf1, and Igf2* expression in CD45+CD31- hematopoietic cells of littermate control (WT) and *Vav-Cre;Etv2* CKO mice, on day 16ptt (n=4/group). Data are presented as mean with standard deviation for all measurements. Statistical significances were analyzed by either a two-tailed Student's *t*-test (**E,F**) or Two-way repeated-measures ANOVA with Sidak's multiple-comparison test (C,D).

Supplemental figure 3. 2PM assessment of steady-state vessel morphology of *Etv2* conditional knockout mice.



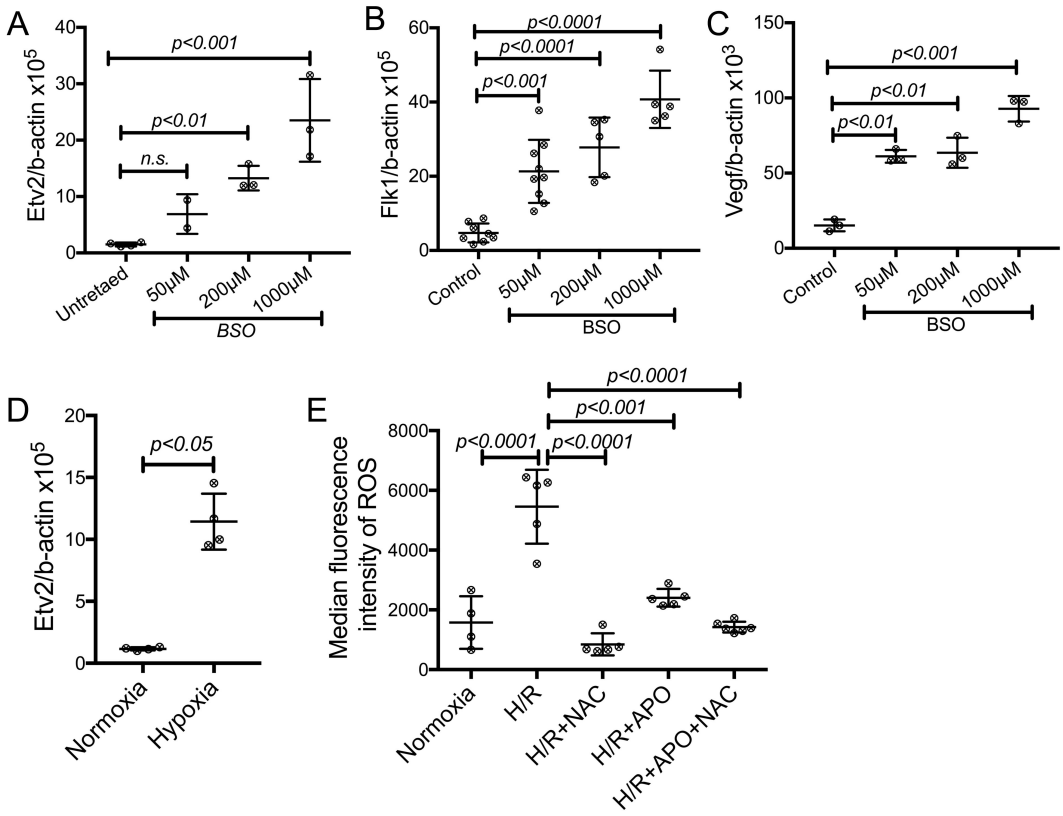
2PM was performed non-invasively on anesthetized mice to assess vessel morphology. (**A**) Images of steady-state vessels in (**left**) *Tie2-Cre*;*Etv2*$^{f/f}$ CKO and (**right**) *VEC-Cre*;*Etv2*$^{f/f}$ CKO mice showing steady-state capillaries (red) and collagen fibers (second harmonic generation signal, blue); Scale bar, 10μm. (**B-C**), Steady-state vessels in littermate control wild-type (WT), *Tie2-Cre*;*Etv2*$^{f/f}$ CKO (Tie2) and *VEC-Cre*;*Etv2*$^{f/f}$ CKO mice (VEcad) were assessed for (**B**) vessel complexity and (**C**) volume (n=6/group; n.s.= not significant). (**D**) Images of blood vessels focusing on the tumor margin in littermate control wild-type and *Tie2-Cre*;*Etv2*$^{f/f}$ CKO mice. BV=Blood vessels, SHG=second harmonic generation, LLC=tumor cells. (**E**) Representative images of vessel integrity analyzed using kymographs. Intensity profiles were measured for the boxed regions in the images. Data are presented as mean with standard deviation for all measurements. Statistical significances were analyzed by One-way ANOVA with Bonferroni's multiple-comparison test (**B,C**).

Supplemental figure 4. *Etv2* CKO mice or lentiviral *Etv2* shRNA treated YSE cells show defects in VEGF, but not FGF, induced angiogenic responses.



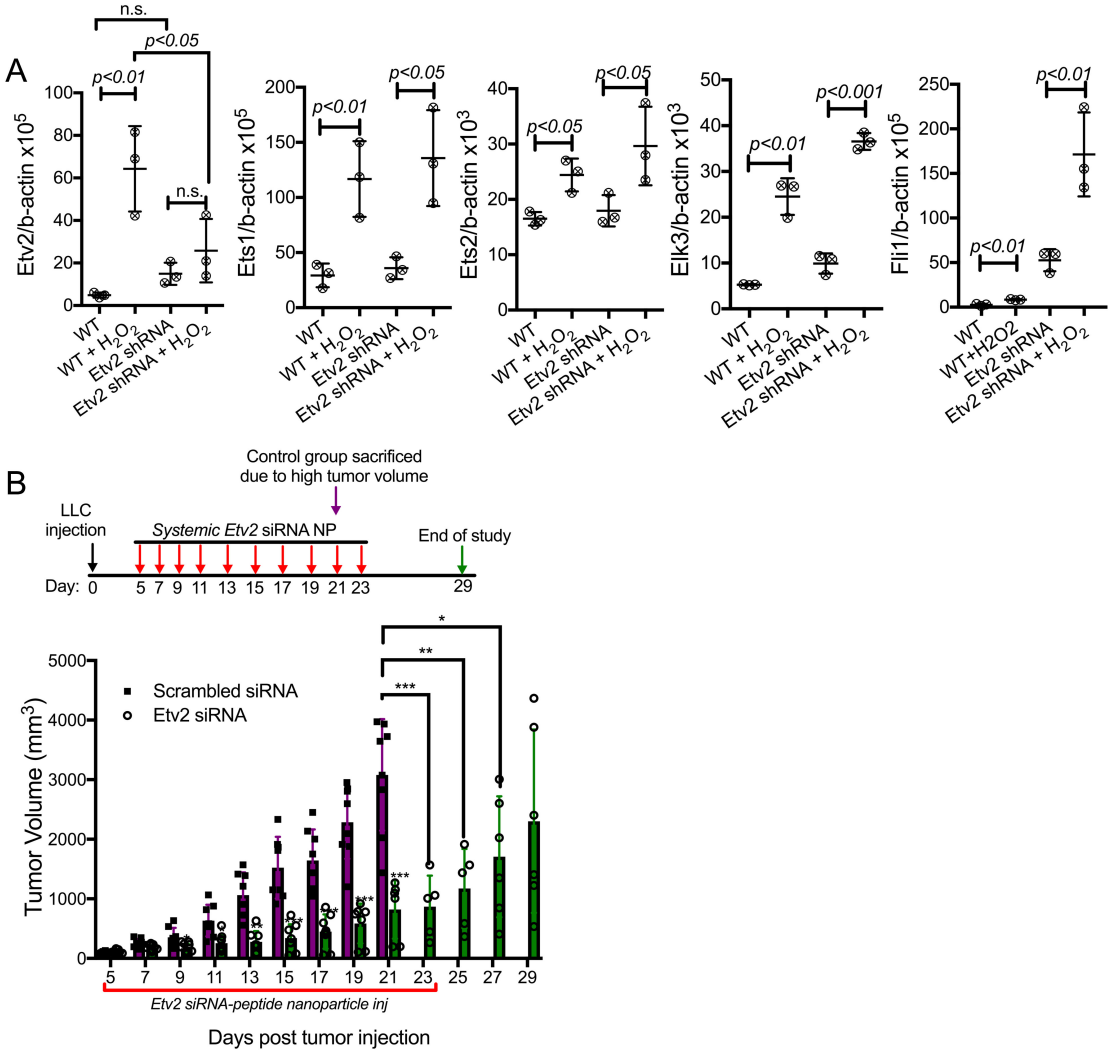
(**A**) Quantification of Ki67^{+} vessels in tumor sections from control and *Etv2* shRNA treated mice, harvested on day 15ptt (n=5/group). Data are presented as percentage of CD31^{+} vessels. (**B**) Representative images and quantification of pFLK1+ vessels in lungs sections harvested from control and *Etv2* shRNA treated mice on day 15 after tumor transplantation (n=5/group). Scale bars: 50μm. (**C**) qRT-PCR analysis of *Fgfr1, Fgfr2, and Fgfr3* expression in CD31^{+}CD45^{-} ECs obtained from lungs of wild-type littermate control and Tie2-Cre;*Etv2* CKO mice, 15ptt (n=3/group). (**D**) Representative bright field images of the aorta of the littermate control and *Tie2-Cre; Etv2* CKO mice. VEGF; vascular endothelial growth factor. FGF2; fibroblast growth factor 2. Scale bars: 100μm (**E**) Representative images from tube formation assay with wild type yolk sac derived endothelial cells (YSE) and lentiviral *Etv2* shRNA infected YSE cells to probe VEGF and FGF responsiveness. Scale bars: 200μm. Data are presented as mean with standard deviation for all measurements. Statistical significances were analyzed by a two-tailed Student's *t*-test.

Supplemental figure 5. ROS trigger *Etv2, Flk1, and Vegf* expression.



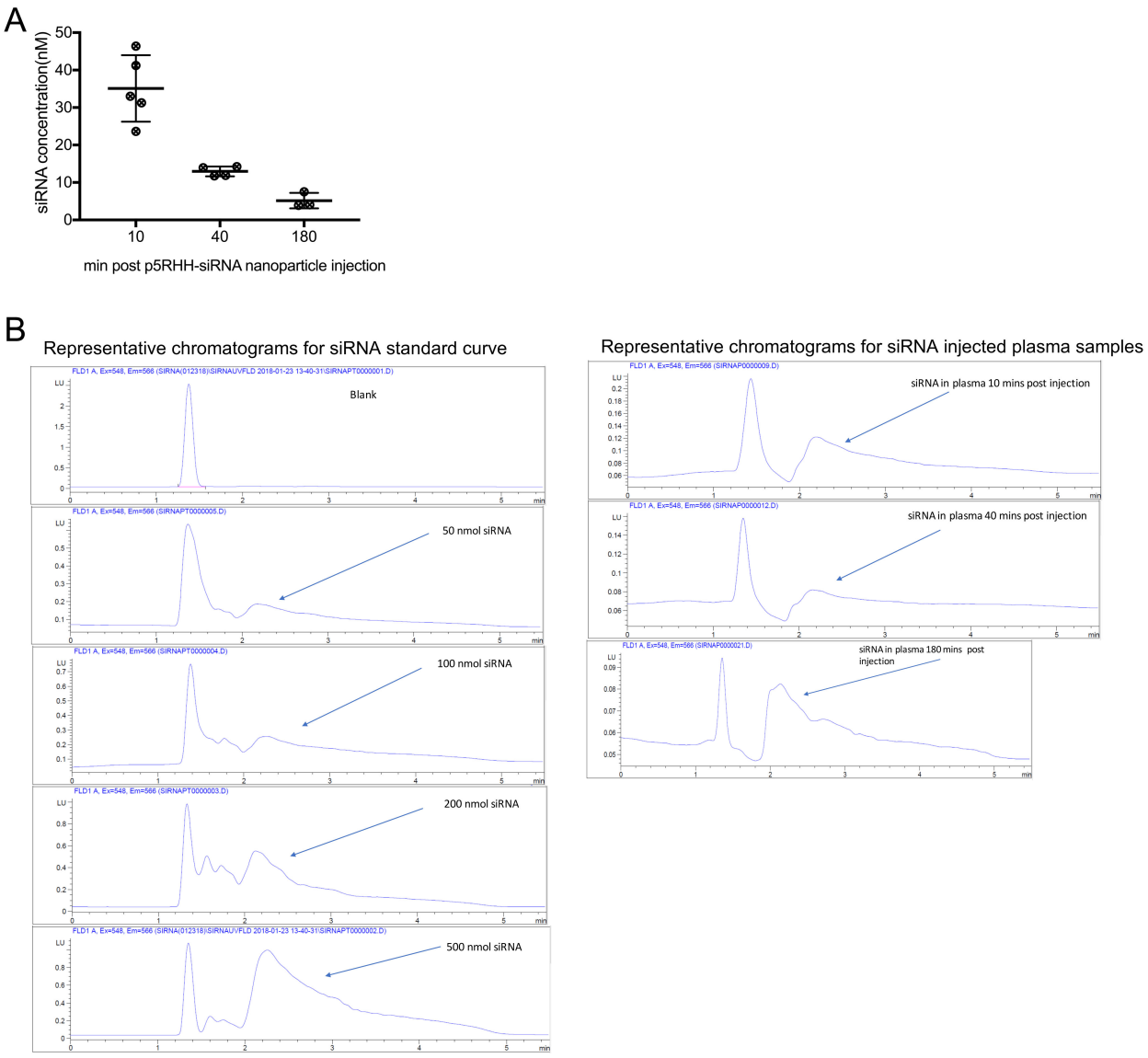
YSE cells were treated with BSO (L-buthionine-S,R-sulfoximine) for 24h and (**A**) *Etv2,* **(B)** *Flk1,* and (**C**) *Vegf* expression were analyzed using qRT-PCR (n=4 or more/group). (**D**) qRT-PCR analysis of *Etv2* expression in YSE cells following 24h hypoxia (<1% oxygen) (n=4/group). (**E**) ROS levels in H/R (24h hypoxia, followed by 24h nomoxia) treated YSE cells with/without ROS scavengers N-Acetyl cysteine (NAC; 5mM) and Apocynin (APO; 100mM) (n=4 or more/group). Data are presented as mean with standard deviation for all measurements. Statistical significances were analyzed by either a two-tailed Student's *t*-test (**D**) or One-way ANOVA with Dunnett's multiple-comparison test (**A,B,C,E**).

Supplemental figure 6. Lentiviral *Etv2* shRNA selectively inhibits *Etv2* expression, and extended treatment of *Etv2* siRNA-peptide nanoparticle keeps tumor growth restricted.



(**A**) qRT-PCR analysis of *Etv2, Fli1, Ets1, Ets2,* and *Elk3* expression in wild type YSE cells (WT) and lentiviral *Etv2* shRNA infected YSE cells (shRNA) with/without 10μM H_2O_2 treatment (n=3/group; n.s.=not significant). (**B**) Tumor growth in mice treated with scrambled and *Etv2* siRNA nanoparticle for an extended period. Line fitted to the bars showing the trend of the growth curve. Scrambled and *Etv2* siRNA nanoparticles were injected through the tail-vein of the mice on days 5, 7, 9, 11, 13, 15, 17, 19, 21, and 23ptt (n=8/group; **p<0.05,**p<0.01,***p<0.001)*. Data are presented as mean with standard deviation for all measurements. Statistical significances were analyzed by One-way ANOVA with Bonferroni's multiple-comparison test (**A**) or Two-way repeated-measures ANOVA with Sidak's multiple-comparison test (**B**).

Supplemental figure 7. Pharmacokinetic study of *Etv2* siRNA-p5RHH peptide nanoparticles.



(**A**) Systemic disposition kinetics of siRNA delivered by i.v. injection of p5RHH-siRNA nanoparticles to wild-type mice. Blood samples were collected at 10, 40, and 180 minutes after the nanoparticles injection (n=4 or more/time point). Data is presented as Mean with standard deviation. (**B**) Representative chromatograms of siRNA standard curve (left) and siRNA in plasma samples (right) collected from siRNA-nanoparticle inected subjects (n=4 or more)

Supplementary Table 1. Sequences of primers used in the study

Supplementary table 1: qRT-PCR primer sequences used in the study

Gene	Forward primer	Reverse primer
Etv2	CTGGGAGCGGAATTTGGTTTC	GTAAAGCGGGGTTCCAGTCC
Flk1	TTTGGCAAATACAACCCTTCAGA	GCAGAAGATACTGTCACCACC
Fgfr1	GCAGAGCATCAACTGGCTG	GGTCACGCAAGCGTAGAGG
Fgfr2	GCCTCTCGAACAGTATTCTCCT	ACAGGGTTCATAAGGCATGGG
Fgfr3	GCCTGCGTGCTAGTGTTCT	TACCATCCTTAGCCCAGACCG
Fli1	ATGGACGGGACTATTAAGGAGG	GAAGCAGTCATATCTGCCTTGG
Ets1	ACAGACTACTTTCGGATCAAGCA	ACGCTCTCAAAAGAGTCCTGG
Ets2	CCTGTCGCCAACAGTTTTCG	TGGAGTGTCTGATCTTCACTGA
Elk3	TCCTCACGCGGTAGAGATCAG	GTGGAGGTACTCGTTGCGG
Erg	ACCTCACCCCTCAGTCCAAA	TGGTCGGTCCCAGGATCTG
Vegfa	GCACATAGAGAGAATGAGCTTCC	CTCCGCTCTGAACAAGGCT
Vegfb	GCCAGACAGGGTTGCCATAC	GGAGTGGGATGGATGATGTCAG
Fgf2	GCGACCCACACGTCAAACTA	TCCCTTGATAGACACAACTCCTC
Igf1	CTGGACCAGAGACCCTTTGC	GGACGGGGACTTCTGAGTCTT
Igf2	GTGCTGCATCGCTGCTTAC	ACGTCCCTCTCGGACTTGG
B-actin	GGCTGTATTCCCCTCCATCG	CCAGTTGGTAACAATGCCATGT

Supplementary Table 2: Pathological review of the malignant tissues used in the study.

Supplementary File 1: SOP for Pathology Specimen Review by Tissue Procurement Core (TPC)-Siteman Cancer Center at Washington University School of Medicine in St. Louis

Supplementary Movie 1. Movie shows a montage of z-series animations from representative 3D images of WT (UL), WT-LLC (UR), Tie2-LLC (LL) and VEcad-LLC (LR). LLC (green), blood vessels (red) and collagen (blue).

Supplementary Movie 2. Movie shows a montage of representative 3D contour surfaces generated by Imaris (Bitplane) that were used to identify vessels in WT (UL), WT-LLC (UR), Tie2-LLC (LL) and VEcad-LLC (LR) mice. These surfaces were used to count the number of discrete vessel segments for complexity measurements and measure vessel segment volumes. Surface quality was optimized using background subtraction and thresholding and common settings used across all images.

DCR#: 2014-074
SOP-306.01

Effective Date: 06/25/2015
Supersedes: SOP-306

TITLE: TPC PATHOLOGY SPECIMEN REVIEW

1. PURPOSE

A histopathological review of an H&E stained section of a tissue specimen is done to characterize and confirm the pathological diagnosis of a tissue specimen. Secondary to this, a review can provide assurance that a specimen has been properly fixed, processed for embedding and adequately sectioned and H&E stained based on the quality of histological detail. A histopathological review of a tissue specimen is considered best practice for specimen evaluation and quality assurance prior to submitting a tissue specimen, or its associated derivatives (nucleic acids, proteins, unstained slides), to a variety of downstream assessments including, but not limited to, whole genome sequencing, whole exome sequencing, transcriptome expression (RNA Seq), microarray analysis, proteomics, histochemistry and immunohistochemistry. In addition to general pathology findings (i.e. malignant, non-malignant, metastatic) and over-all histological quality of the specimen (poor, fair, good, excellent), endpoints captured from a TPC pathology specimen review include % neoplastic cellularity, % necrosis, % lymphocyte cellularity and % total cellularity (neoplastic and non-neoplastic).

2. SCOPE

This SOP applies to all pathology specimen reviews on H&E stained frozen tissue slides and fixed tissue slides in the Tissue Procurement Core.

3. REFERENCES

Comprehensive Genomic Studies: Emerging Regulatory, Strategic, and Quality Assurance Challenges for Biorepositories. Am J Clin Pathol. 2012 July; 138(1): 31–41

4. SAFETY

Use universal precautions and don appropriate personal protective equipment when handling human specimens.

5. MATERIALS

5.1 H&E Stained frozen tissue slide or fixed tissue slide

5.2 Leica DM1000 Light Microscope (or similar)

6. DEFINITIONS

TPC	Tissue Procurement Core
LCM	Laser Capture Microscopy

7. RELATED DOCUMENTS

SOP-103	Biospecimen Identity Management
SOP-304	H&E Staining and Coverslipping
Form-926	TPC Pathology Specimen Review Form

8. PROCEDURE

8.1 Pathology Review

8.1.1 All TPC specimen reviews are completed by a board certified pathologist.

8.1.2 Unless directed by a client, it is the policy of the TPC to provide a histological characteristics review of all tissue specimens distributed from the TPC biorepository. In some instances clients will have an established research team which includes a board certified pathologist that will provide the review.

8.2 H&E Stained Slide Sources

8.2.1 All TPC pathology specimen reviews are performed on H&E stained fixed tissue slides or frozen tissue slides.

8.2.2 H&E slides can be generated by the TPC (See SOP-304 H&E Staining and Coverslipping) or provided to the TPC from an outside source (i.e. outside lab, Surgical Pathology).

8.2.3 All H&E stained slides generated within the TPC, or provided to the TPC from clients, are accessioned into the TPC through its inventory management system and labeled with a unique specimen accession number. See SOP-103 Biospecimen Identity Management for details regarding specimen receiving, accessioning and labeling.

8.3 TPC Pathology Review Form

8.3.1 A copy of the unique H&E slide label is affixed to the "Slide Label" column of the TPC Pathology Specimen Review Form. See Form-926 TPC Pathology Specimen Review Form.

8.3.1.1 If applicable, the Participant ID is entered into the "Participant ID" column of the TPC Pathology Specimen Review Form.

8.4 TPC Specimen Review Type

8.4.1 Standard Review:

8.4.1.1 Review intended to capture general pathology findings (i.e. malignant, non-malignant, metastatic) and over-all histological quality of the specimen (poor, fair, good, excellent). In addition to this, % of the specimen area occupied by necrosis, % of nuclei associated with neoplastic cells, % of nuclei associated with lymphocytes and % of the total specimen area occupied by nucleated cells (neoplastic and non-neoplastic) are captured.

8.4.2 Targeted Dissection Review:

8.4.2.1 In addition to noting and recording all of the parameters associated with a standard review, this review is additionally intended to identify specific regions within a tissue section for macrodissection and microdissection methods (i.e. LCM and paraffin block core punches) in an effort to enhance the percentage of neoplastic cellularity for a tumor or other desired component(s) of a tissue specimen based on the intended downstream scientific method (e.g. Whole Genome Sequencing).

8.4.2.2 To note that the review guided macrodissection and microdissection methods were able to enrich neoplastic cellularity and/or reduce necrosis, a statement of "Specimen is suitable for Macrodissection" will be entered into the "comments" field of the Specimen Review Event.

8.4.2.3 Region(s) on the slide are identified (outlined in marker) and overlaid on the originating block for core punching or used to isolate regions on additional slides cut from the block for LCM.

8.5 TPC Specimen Review Attributes

8.5.1 The pathologist will review each slide and record values on Form-926 TPC Pathology Specimen Review Form for the following:

8.5.1.1 Specimen Attributes

- **% Necrosis:** Percentage of the total tissue specimen area that is necrotic.
- **% Neoplastic Cellularity:** Percentage of total nuclei in the specimen that are associated with neoplastic cells.
- **% Lymphocytes:** Percentage of total nuclei in the specimen that are associated with lymphocytes.
- **% Total Cellularity:** Percentage of the total specimen area occupied by nucleated cells (neoplastic and non-neoplastic).

8.5.1.2 Pathological Status:

- **(M) Malignant:** ≥1 neoplastic cell in section.
- **(Mt) Metastatic:** ≥1 neoplastic cell in section and informed (or histologically evident) that specimen under review is suspected distant metastasis of primary malignancy.
- **(N) Non-malignant:** No neoplastic cells in section.
- **(NS) Not Specified:** Histological quality is Poor and insufficient for accurate diagnosis. See section 8.5.1.3 Histological Quality.

8.5.1.3 Histological Quality:

- **(NS) Not Specified:** No definable specimen on slide. Histological cannot be specified.
- **(P) Poor-No Definable Features:** Histology is so poor that it is difficult to identify cell or tissue type.

- **(F) Fair-Definable Tissue Architecture:** Able to discern tissue and cell type but histomorphology is poor.
- **(G) Good-Definable Cellular Detail:** Specimen is of good quality and cellular histomorphology is reasonably preserved.
- **(E) Excellent-Definable Nuclear Detail:** Specimen is of excellent quality with good cellular, nuclear and tissue histomorphology.

8.5.1.4 Comments: Values are variable, depends on client instructions and intent of request (e.g. Please define tumor type and grade), and may not be defined for every specimen evaluated.

8.6 Biospecimen Inventory Management System

8.6.1 Upon completing the TPC Specimen Review, data is uploaded into the biospecimen inventory management system.

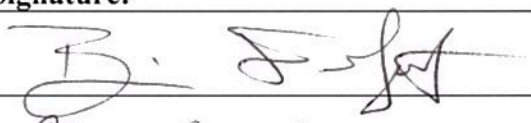
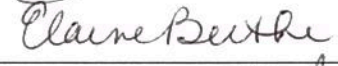
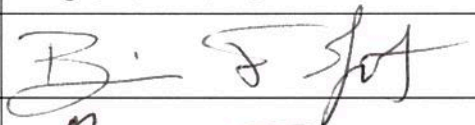
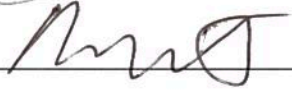
8.6.1.1 Specimen Review Event is completed in the biospecimen inventory management system to attach data to the accessioned parent specimen (i.e. fixed tissue block, frozen tissue block) that gave rise to the H&E slide reviewed.

8.6.1.2 The unique slide label for the specimen reviewed will be entered into the "Comments" section of the Specimen Review Event for the originating biospecimen block (i.e. Fixed Tissue Block, Frozen Tissue Block).

9. DOCUMENTATION HISTORY

Version #	Revision History
SOP-306	New SOP
SOP-306.01	Added section 8.1.2 and re-formatted section 8.5

10. APPROVALS

Title:	Signature:	Date:
Author		06/19/2015
Quality Assurance		06/19/2015
Laboratory Manager		06/19/2015
Laboratory Director		06/19/2015.