Supplementary Material for

A Xenograft Model for Venous Malformation

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Fig. S1. Morphology of cultured patient-derived EC at low and high passage.

Fig. S2. *TIE2* and *PIK3CA* sequencing of 7 VMK-EC clonal populations.

Fig. S3. Histology of patient Venous Malformation.

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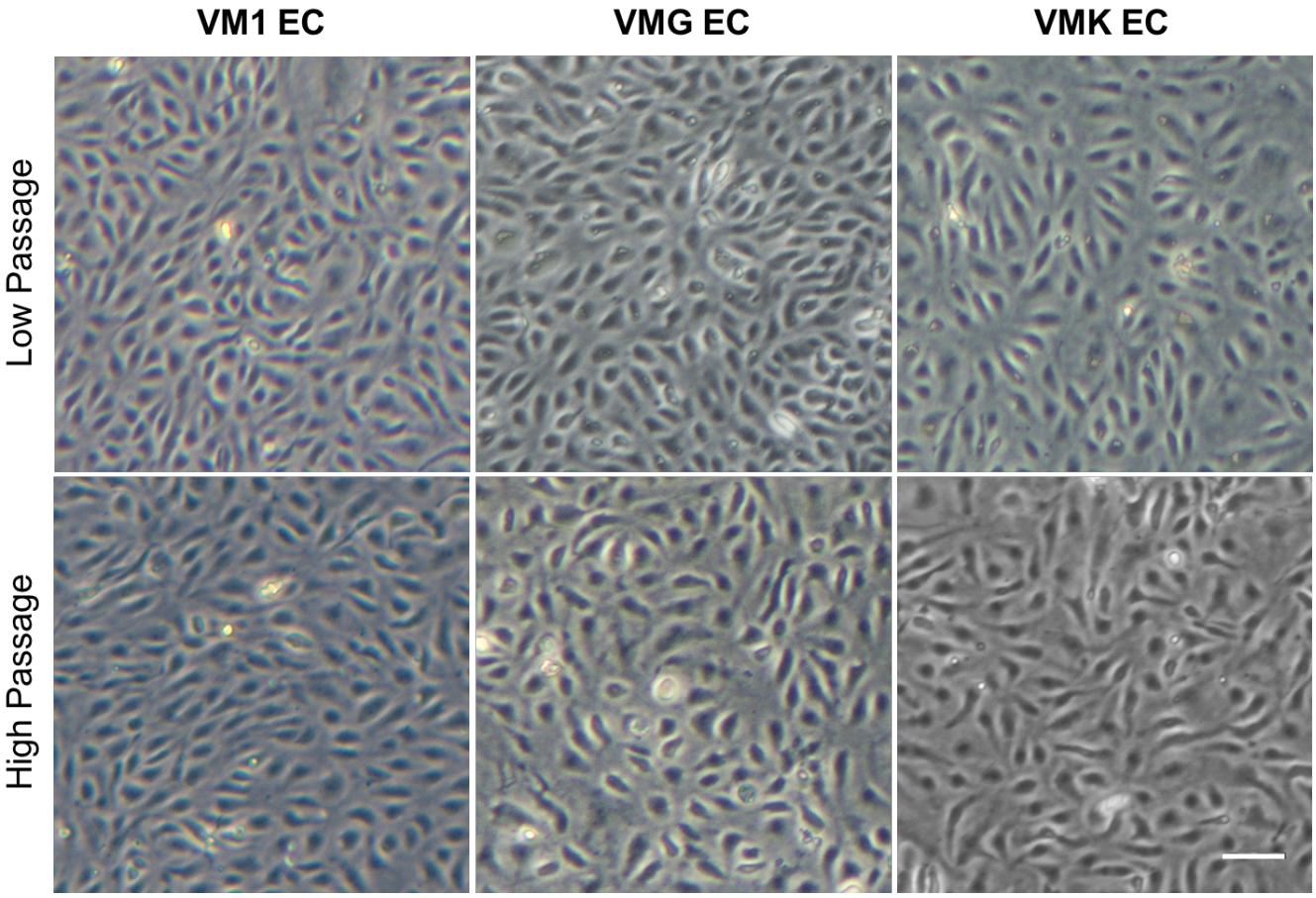
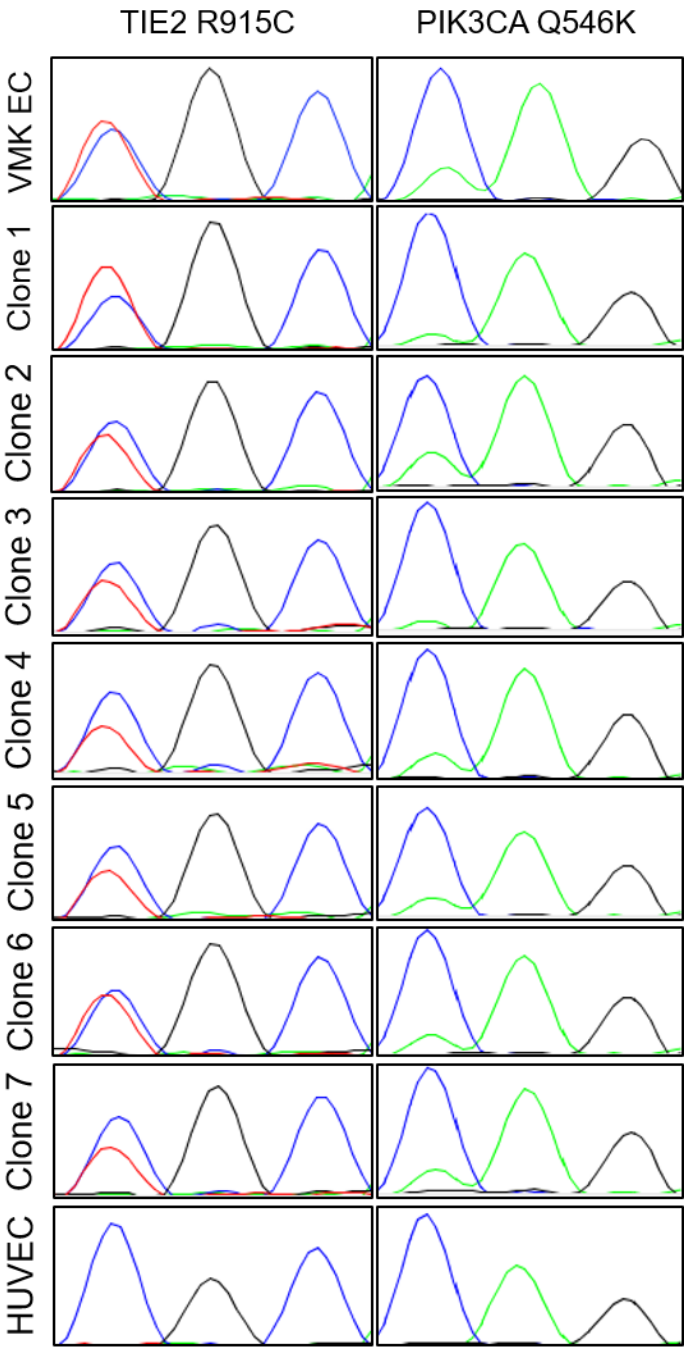
Fig. S5. IB4 staining of VM xenograft lesion sections.

Fig. S6. Staining of control tissues.

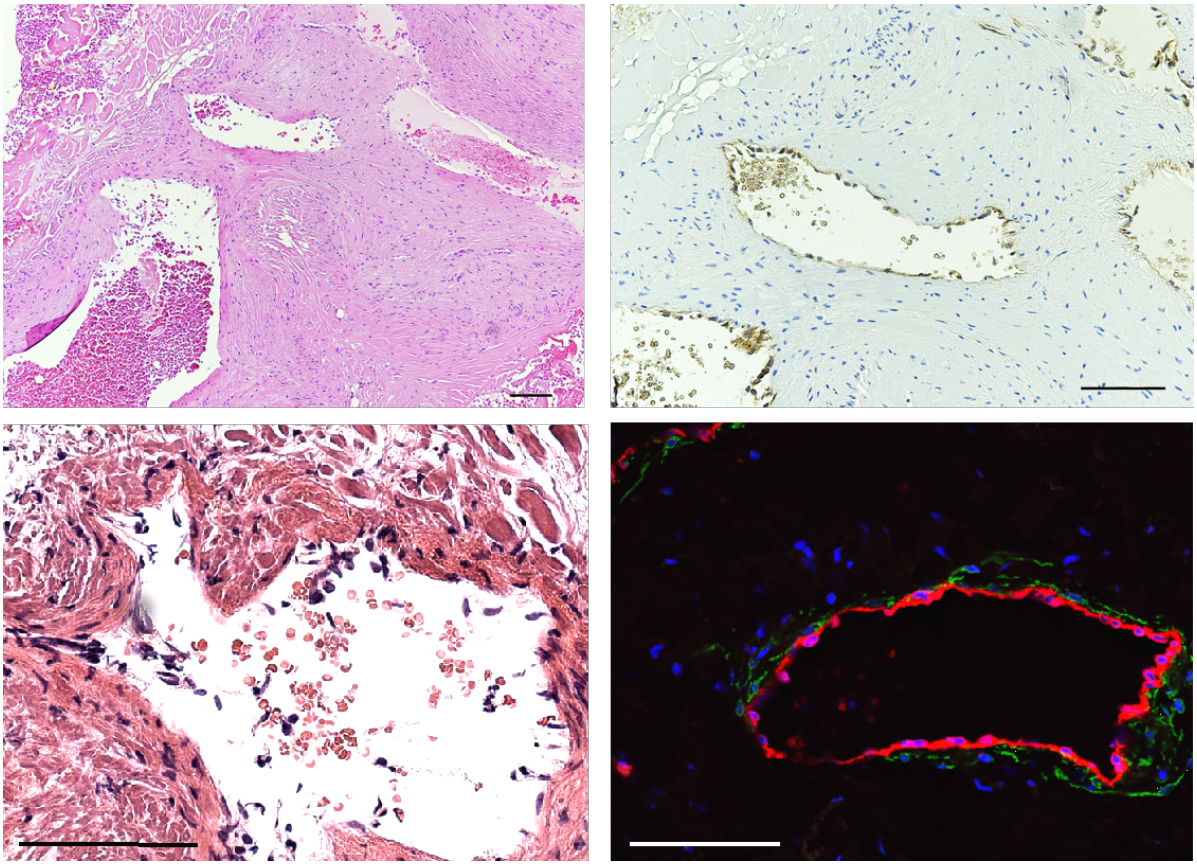
Table S1. List of Primers for DNA Sanger sequencing.



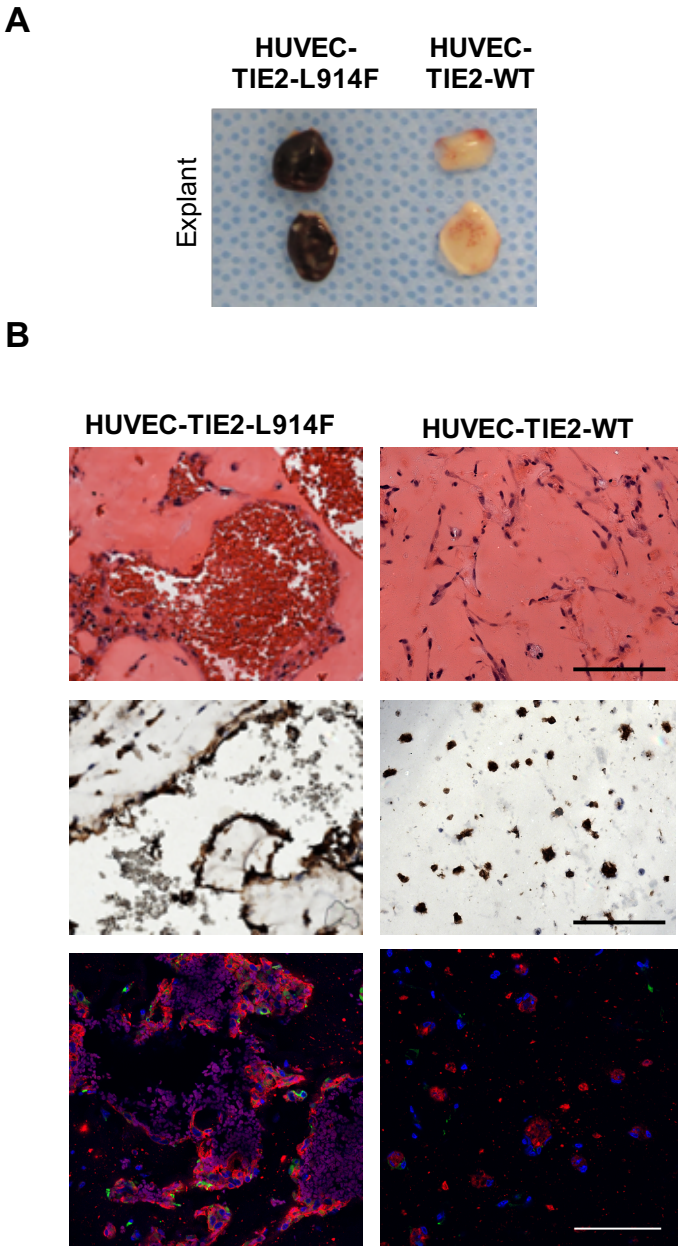
Supplemental Fig.S1. Morphology of cultured patient-derived EC at low and high passage.
Morphological analysis of Venous Malformation endothelial cells (VM-EC) from different patients (VM1, VMG, VMK) at low passage (p3) and high passage (p7).



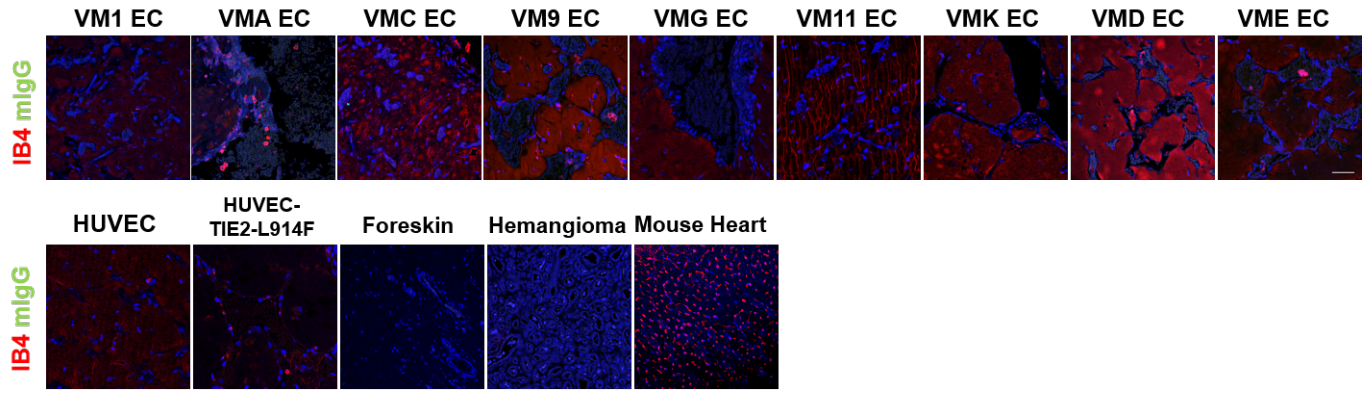
Supplemental Fig. S2. *TIE2* and *PIK3CA* sequencing of 7 VMK EC clonal populations.
DNA Sanger sequencing of VMK EC clone derived from 1 single cell. Presence of a double peak at *TIE2* c.2743C>T (p.R915C) (left) and c.1636C>A (p.Q546K) (right) in VMK EC population and in 7/7clonal populations. HUVEC did not show peaks for these variants.



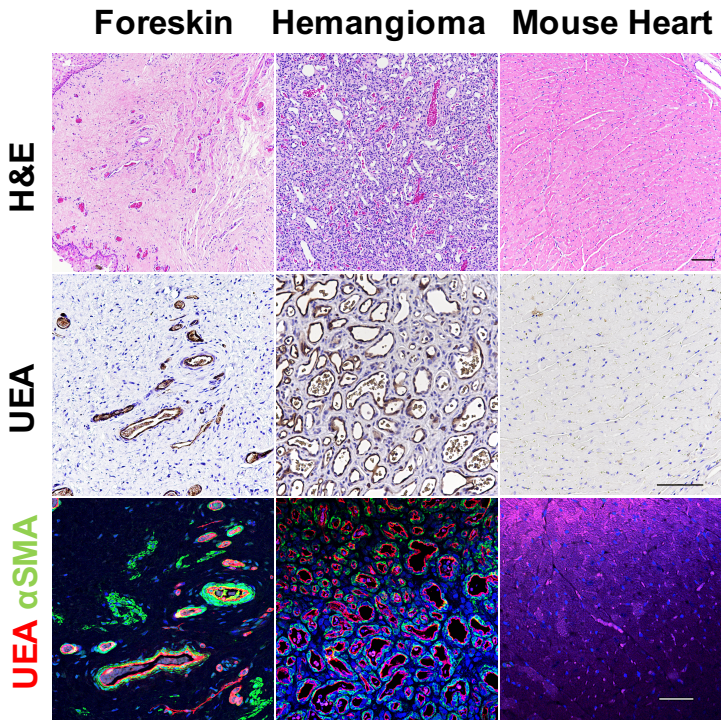
Supplemental Fig.S3. Histology of patient Venous Malformation.
Patient-derived Venous Malformation tissue subjected to hematoxylin and eosin staining (H&E) (left), immunohistochemical staining for Ulex europaeus Agglutinin I (UEA) (brown color) (top, right) and immunofluorescence staining for UEA (red) and smooth muscle alpha-actin (αSMA) (green) (bottom, right). Scale bar 100μm.



Supplemental Fig.S4. Lesion explants from injected HUVEC-TIE2-L914F and control HUVEC-TIE2-WT (wild type).
HUVEC-TIE2-L914F and HUVEC-TIE2-WT cells were injected subcutaneously on both backsides of immune-deficient mice. **A-** Lesion explant photo, **B-** staining of hematoxylin and eosin (H&E) (top), immunohistochemistry (IHC) of Ulex europaeus I (UEA) (middle), and immunofluorescence (IF) of UEA (red) and αSMA (green), nuclei (blue) (bottom); Scale bars: H&E**,** IHC and IF 100μm



Supplemental Fig.S5. IB4 staining of VM xenograft lesion sections.
VM-EC xenograft explants subjected to immunofluorescence staining for Griffonia simplicifolia Isolectin B4 (IB4) (red), DAPI for nuclei (blue). Controls are HUVEC, HUVEC-TIE2-L914F explants, human foreskin, patient-derived infantile hemangioma and mouse heart (positive control for IB4). Scale bar 50μm.



Supplemental Fig.S6. Staining of control tissues.
Human foreskin, patient-derived infantile hemangioma and mouse heart subjected to hematoxylin and eosin staining (H&E) (top), immunohistochemical (IHC) staining for Ulex europaeus Agglutinin I (UEA) (brown color) (center), and immunofluorescence (IF) staining for UEA (red) and smooth muscle alpha-actin (αSMA) (green) (bottom). Scale bar H&E, IHC 100μm; IF 50μm.

Supplemental Table S1. List of Primers for DNA Sanger sequencing.

Primer	Sequence
TIE2 Exon 17 mutations	F- 5' TGGTGTTGCTAGATGTGTTT R- 5' TTTTGGCTCAAGTAGTCCAT
PIK3CA Exon 21 mutations	F- 5' ACATTCGAAAGACCCTAGCC R- 5' ATGCTGTTCATGGATTGTGC
PIK3CA Exon 8 mutations [1]	F- 5' GGGGAAAAAGGAAAGAATGG R- 5' TGCTGAACCAGTCAAACTCC Seq- 5' TGAATTTTCCTTTTGGGGAAG
PIK3CA Exon 10 mutations	F- 5' GATTGGTTCTTTCCTGTCTCTG R- 5' CCACAAATATCAATTTACAACCATTG Seq- 5' TTGCTTTTTCTGTAAATCATCTGTG
CD31 (qRT-PCR)	F- 5' GACATGGCAACAAGGCTGTG R- 5' CGGGCTTGGAAAATAGTTCTGT
VWF (qRT-PCR)	F- 5' CCGATGCAGCCTTTTCGGA R- 5' TCCCCAAGATACACGGAGAGG
VE-Cadherin (qRT-PCR)	F- 5' GTTCACGCATCGGTTGTTCAA R- 5' CGCTTCCACCACGATCTCATA
18S (qRT-PCR)	F- 5' GTCTGTGATGCCCTTAGATG R- 5' AGCTTATGACCCGCACTTAC

BIBLIOGRAPHY

1. Samuels, Y., et al., *High frequency of mutations of the PIK3CA gene in human cancers.* Science, 2004. **304**(5670): p. 554.