SUPPLEMENTARY INFORMATION

Ansarizadeh, Nguyen *et al*. Microfluidic vessel-on-chip platform for investigation of cellular defects in venous malformations and responses to various shear stress and flow conditions.

Cell lysis and western blot analysis

To assess the TIE2 and Akt total protein and phosphorylation levels in control, *TIE2*^{L914F} expressing HUVECs and in iECs western blot analysis was performed on cell lysates.

Cells were washed with ice-cold 1x PBS twice and incubated in 500 µL ice-cold cell lysis buffer: 9.1 mM Na_2HPO_4, 1.7 mM NaH_2PO_4, pH 7.2; 1% NP-40, 0.25% sodium deoxycholate, 150 mM NaCl, 0.1% SDS, 1 mM EDTA; protease and phosphatase inhibitors (1:100, P8340 and P5726, Sigma-Aldrich) for 5 min at +4°C on a racker. To homogenize lysates, the suspension was drawn up and down with a syringe and 20G needle several times, the supernatant of lysate was collected after 15 min by centrifugation at +4°C, aliquoted and stored at -80°C.

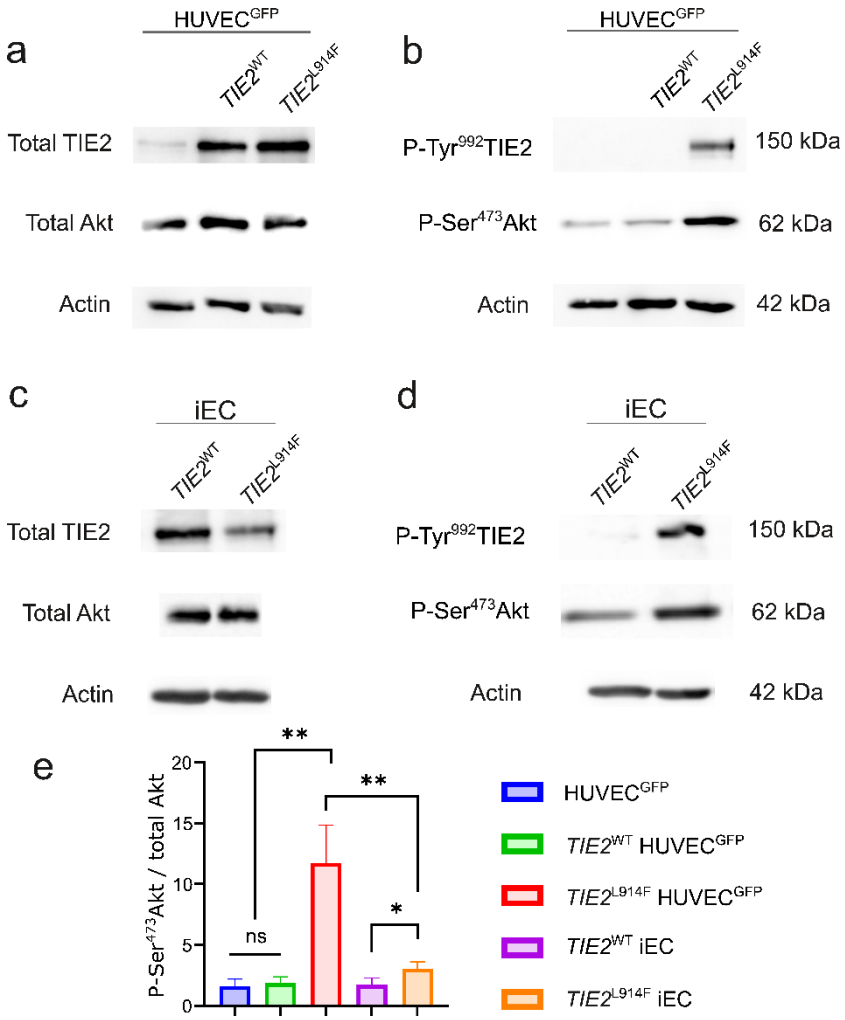
Each lysate sample (30 µL) was mixed with 10 µL of 4x loading buffer (0.25 M Tris HCL pH 6.8, 8% SDS, 40% glycerol, 135 mM bromophenol blue) with 1% β-mercaptoethanol and denatured at 95°C for 5 min. The proteins were separated in polyacrylamide gel (4% stacking and 7.5% resolving) in a 1x running buffer (5x running buffer; 0.25 M Tris, 1.92 M glycin, 35 mM SDS) and 0.2% SDS, run for 10 minutes at 100 V followed by 45 minutes at 200 V. Protein transfer to a nitrocellulose membrane was carried out in a transfer buffer (20% ethanol, 25 mM Tris, 920 mM glycine) at 100 V for 1 h and 30 min at +4°C. The transfer setup included a sandwich of sponges, Whatman papers, the gel, and the nitrocellulose membrane, all soaked in ice-cold transfer buffer. The membrane was blocked with 5% milk in PBST for 1 h, followed by washing and incubation with primary antibodies (Supplementary Table 1) in 3% BSA PBST overnight at +4°C. The next day, after washing, secondary antibodies were applied at RT for 1 h. Proteins were visualized using enhanced chemiluminescence and detected with Azure-600 Biosystems. The signal intensities were quantified using Gels tool in FIJI software, normalized to β-actin or total protein levels.

Cell viability assay

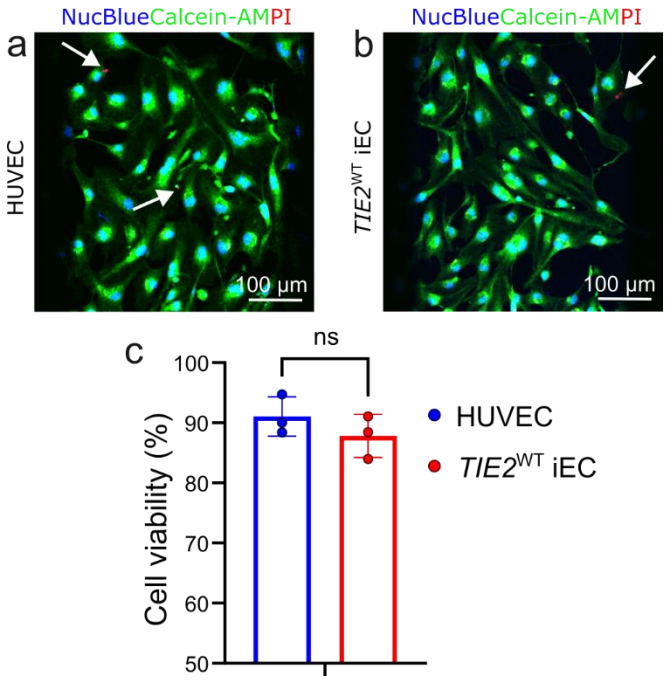
The medium was removed from the reservoirs of the channels and calcein AM (at the final concentration 4 µM, Thermo Fisher, 65-0853-81), propidium iodide (PI, 4 µg mL^{-1}, Sigma-Aldrich, P4170) and NucBlue™ Live (2 drops mL^{-1}, Thermo Fisher, R37605) were added in ECGM2 medium to the channels and incubated for 20 min. Then the chips were imaged by Leica SP8 FALCON laser scanning confocal microscope using HC PL APO 20×/0.75 CS2 (air) lens. Living cells were identified by calcein AM, the number of dead cells by PI positive nuclei, and the number of all cells based on NucBlue™ staining. Nuclei were counted using particle analysis in FIJI, a distribution of ImageJ.

Supplementary Table 1. Primary and secondary antibodies in western blot.

Primary antibody (Manufacturer, catalog number)	**Dilution** In 1% BSA-PBST	**Secondary antibody** (Manufacturer, catalog number)	**Dilution** In 1% BSA-PBST
Total-TIE2 (Clone Ab33, mouse monoclonal, Merck Millipore, 05-584)	1:1000	HRP-conjugated goat anti-mouse IgG (Affinipure, 115-035-003)	1:10 000
Phospho-TIE2 (Y992, rabbit polyclonal, Cell Signaling Technology, 4221S)	1:1000	HRP-conjugated goat anti-rabbit IgG (Affinipure, 111-035-003)	1:10 000
Total-Akt (rabbit polyclonal, Cell Signaling Technology, 9272S)	1:1000	HRP-conjugated goat anti-rabbit IgG (Affinipure, 111-035-003)	1:10 000
Phospho-Akt (Ser 473, rabbit polyclonal, Cell Signaling Technology, 4060B)	1:1000	HRP-conjugated goat anti-rabbit IgG (Affinipure, 111-035-003)	1:10 000
β-Actin (mouse monoclonal, Sigma-Aldrich, A5441)	1:1000	HRP-conjugated goat anti-mouse IgG (Affinipure, 115-035-003)	1:10 000



Supplementary Fig. 1. Western blot analysis of TIE2 and Akt phosphorylation states in HUVECs and iECs. HUVECs were transduced with bicistronic TIE2-IRES-GFP or only GFP-expressing retroviruses as the control, iECs were generated using CRISPR Cas9 technology. TIE2 and Akt protein expression and activation (phosphorylation states) were investigated using western blotting from cell lysates of HUVEC^{GFP}, *TIE2*^{WT} HUVEC^{GFP} and *TIE2*^{L914F} HUVEC^{GFP} (a, b) and from *TIE2*^{WT} iECs and *TIE2*^{L914F} iECs (c, d). The signal intensity quantification of phosphorylated Akt (e). Note that both retroviral expression of *TIE2*^{L914F} in HUVECs and *TIE2* gene locus targeted L914F gain-of-function mutation in iECs increased TIE2 and Akt phosphorylation (downstream from mutated TIE2). The level of activation of Akt, quantified as P-Ser^{473}Akt / total Akt ratio, is significantly higher in *TIE2*^{L914F} HUVEC^{GFP} than in *TIE2*^{L914F} iECs. *P<0.05, **P<0.01; ns, statistically not significant in one-way ANOVA. Mean ± SD. n, three independent experiments.



Supplementary Fig. 2. Cell viability of HUVECs and *TIE2*^{WT} iECs in microfluidic chip after five days of culture under bidirectional flow. (a-b) Calcein AM stains the living cells (green), PI (red) penetrates cell membranes of dead/dying cells and stains DNA (pyknotic or fragmented nuclei pointed by white arrows). All nuclei were stain with NucBlue™ Live (blue). (c) The quantification of the percentage of living cells. ns, statistically not significant in t-test, mean ± SD. n, three independent experiments.