

## Supplementary Data

### **A collagen IV-derived peptide disrupts $\alpha5\beta1$ integrin and potentiates Ang2-Tie2 signaling**

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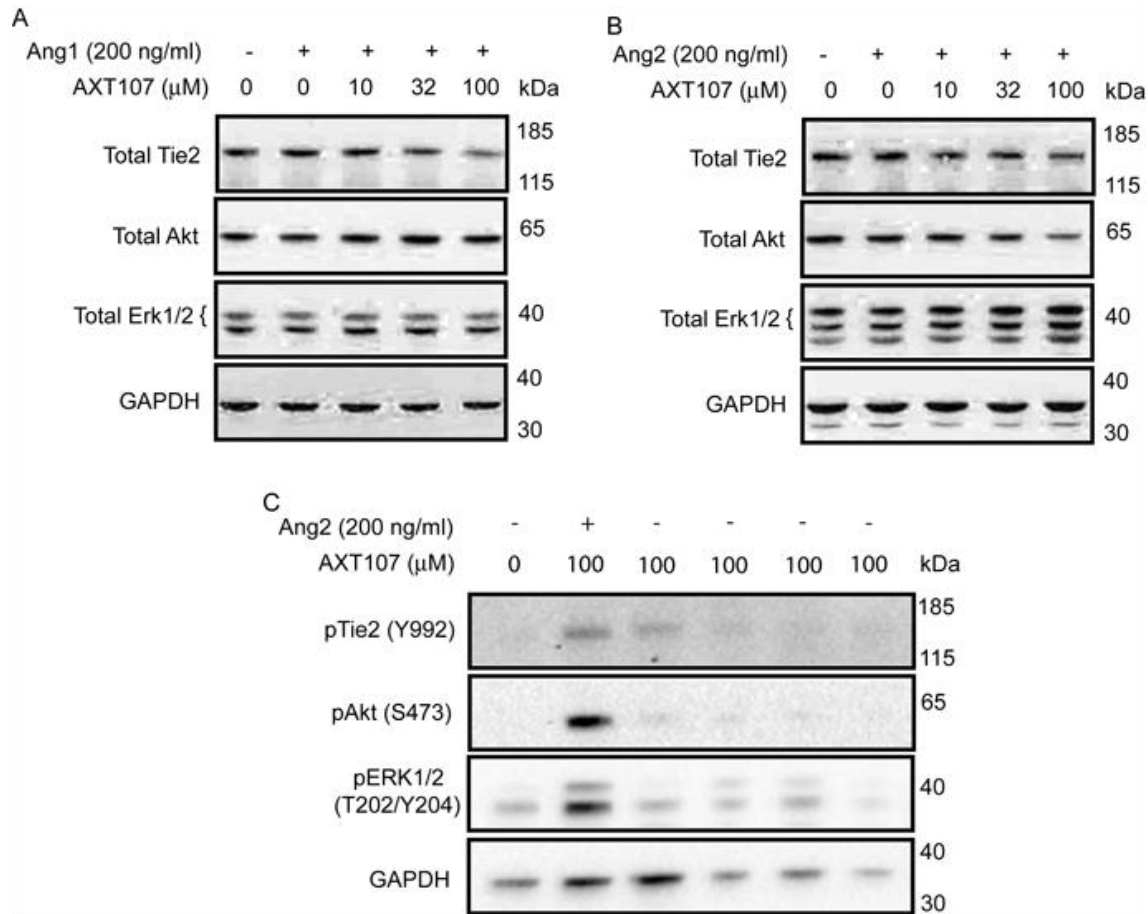
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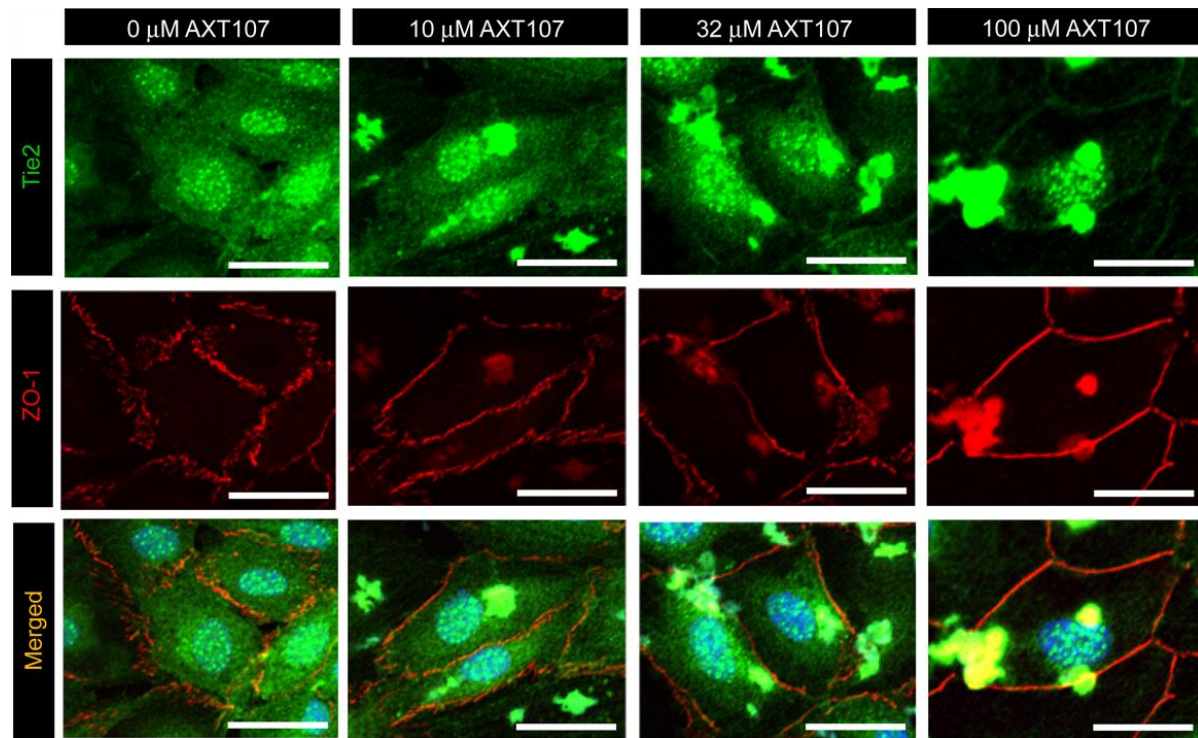
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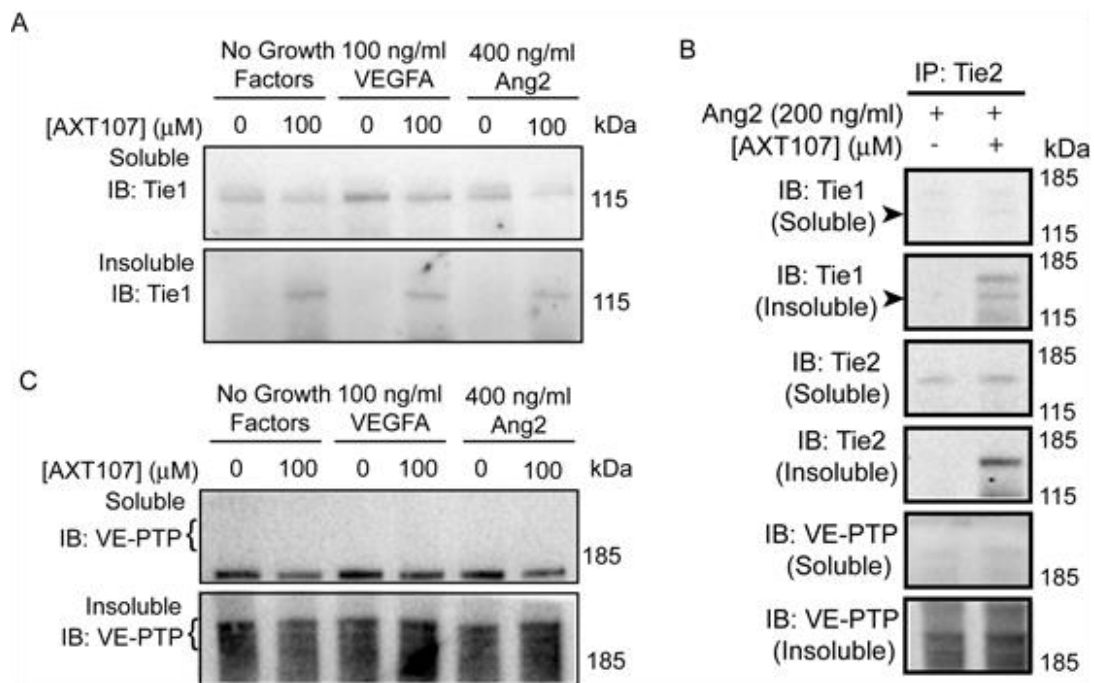


**Supplementary Figure 1. AXT107 does not influence total levels of Tie2 or downstream effectors or alters their phosphorylation in absence of angiopoietins.** A, B) Representative Western blots of MEC lysates treated with 200 ng/ml Ang1 (A) or 200 ng/ml Ang2 (B) and 0-100  $\mu$ M AXT107 showing the total protein levels of Tie2 and downstream effectors Akt and ERK1/2 with GAPDH as a loading control (repeated at least three times). C) Representative Western blots of MEC lysates treated with 200 ng/ml Ang2 or vehicle and 0 or 100  $\mu$ M AXT107 showing phosphorylation of Tie2 (Y992) and downstream effectors Akt (S473) and ERK1/2 (T202/Y204) with GAPDH as a loading control.

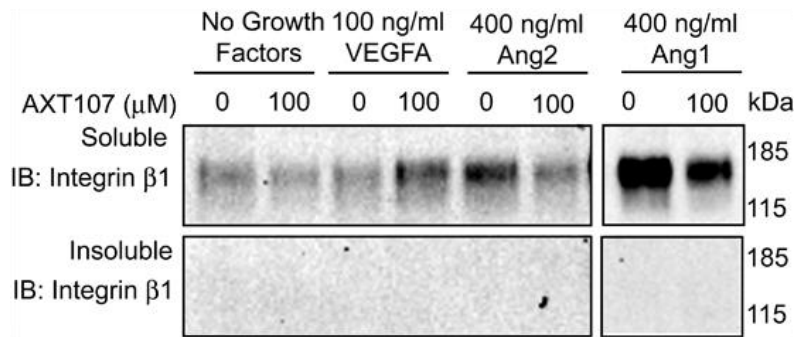


**Supplementary Figure 2. AXT107 alters Tie2 intracellular distribution.**

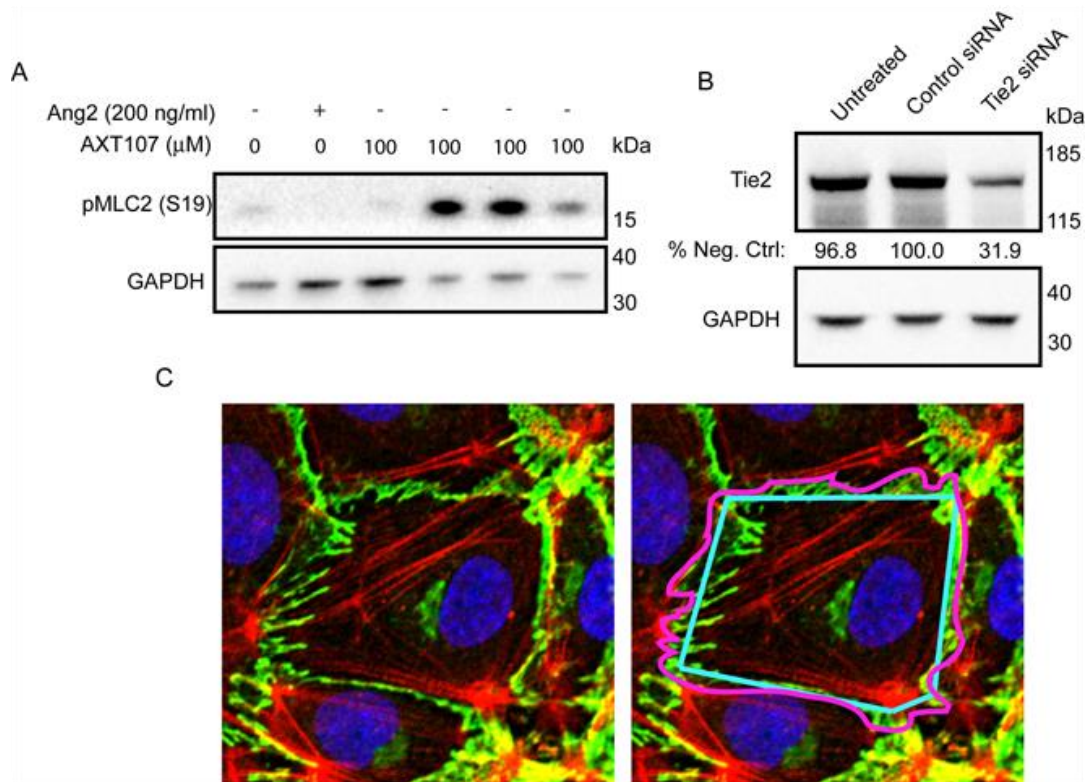
Immunofluorescence images of MECs monolayers treated with 200 ng/ml Ang2 for 15 min at varying concentrations of AXT107 and stained for total Tie2 (green), ZO-1 (red), and DAPI (blue) (n=2). Scale bars represent 25μm.



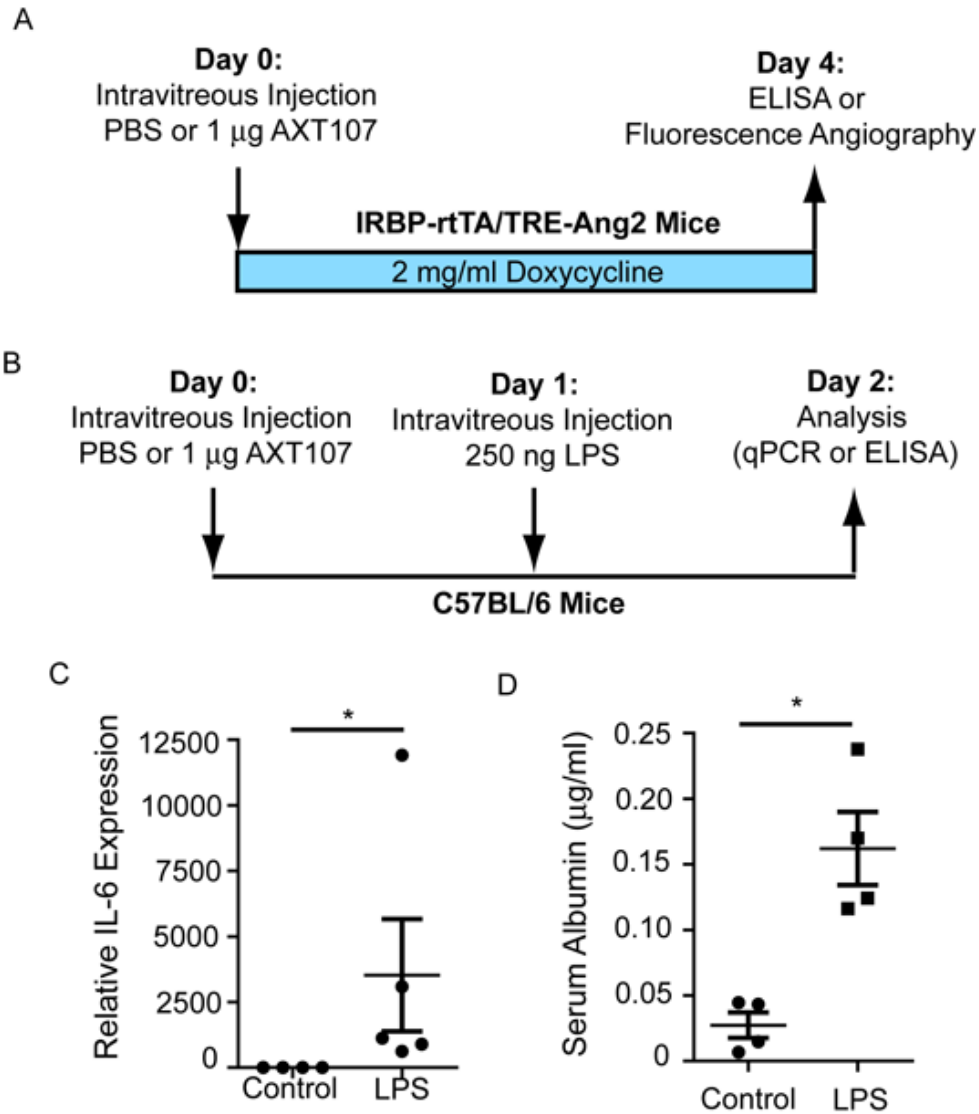
**Supplementary Figure 3. The effects of AXT107 on Tie1 and VE-PTP intracellular distribution.** A) MEC lysates treated with various growth factors and 100  $\mu$ M AXT107 or DMSO vehicle and fractionated into Triton X-100 soluble and insoluble pools. Samples were immunoblotted for Tie1. B) Lysates from Triton X-100 fractionated MECs treated with 200 ng/ml Ang2 that were immunoprecipitated with anti-Tie2 antibodies and A/G agarose beads and immunoblotted for Tie1, Tie2, and VE-PTP. C) As (A) except that membranes were stained for VE-PTP.



**Supplementary Figure 4. AXT107 disrupts interactions between  $\alpha_5$  and  $\beta_1$  integrin.** MEC lysates treated with various growth factors and 100  $\mu$ M AXT107 or DMSO vehicle and fractioned into Triton X-100 soluble and insoluble pools and blotted for  $\beta_1$  integrin (epitope corresponding to a site near Pro680, near C-terminus) (n=1).



**Supplementary Figure 5. AXT107-treatment strengthens endothelial cell junctions.** A). Representative western blot images of lysates from MECs treated with 200 ng/ml Ang2 or vehicle and 0 or 100  $\mu$ M AXT107 showing phosphorylation of MLC2 (S19) with GAPDH as a loading control. B) Representative western blot images from HUVECs that were untreated or treated with negative control siRNA or Tie2 siRNA and blotted for total Tie2 and GAPDH as a loading control. Values beneath Tie2 bands indicate band intensities by densitometry as percentages relative to the negative control siRNA band. C) Schematic showing representative traces of the total perimeter (magenta) compared to the minimal perimeter (cyan) for quantifying jaggedness of VE-cadherin arrangement.



**Supplementary Figure 6. AXT107 potentiates Tie2 phosphorylation *in vivo*.** A) Schematic depicting treatment and analysis of the transgenic IRBP-rtTA/TRE-Ang2 mouse model experiments. B) Schematic depicting treatment and analysis of LPS-induced uveitis mouse model. C) IL-6 expression from mouse eyes treated with intravitreal injection of 125 ng LPS or PBS for 24 hours. D) Serum albumin concentrations from vitreous taps of mouse eyes treated with intravitreal injected 250 ng LPS or PBS determined by ELISA.