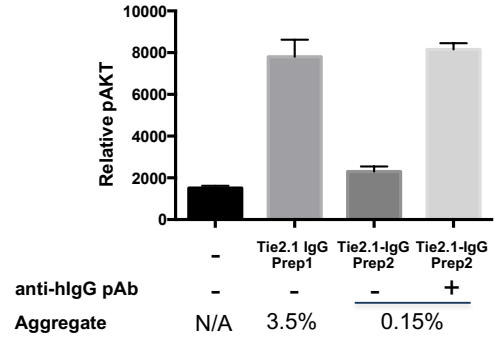
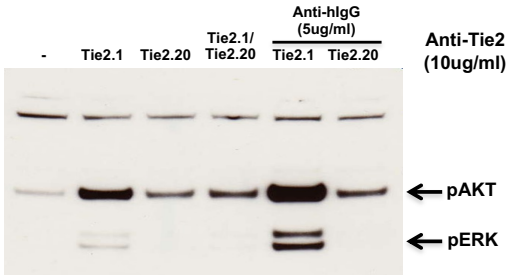
A.



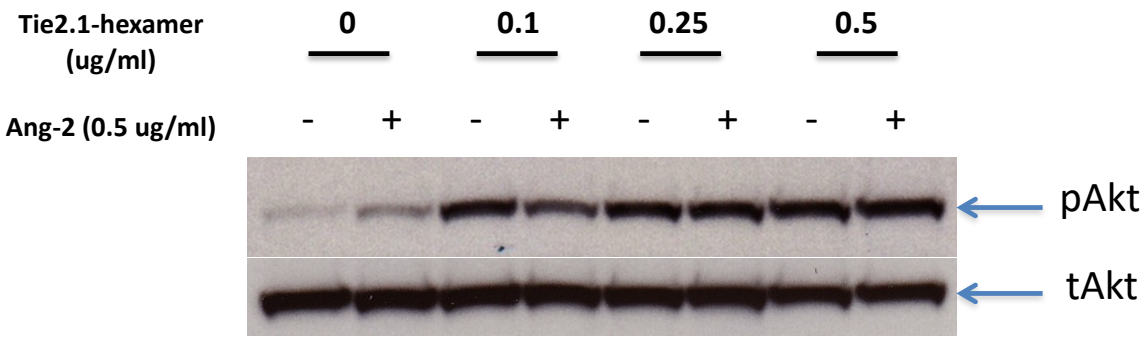
B.



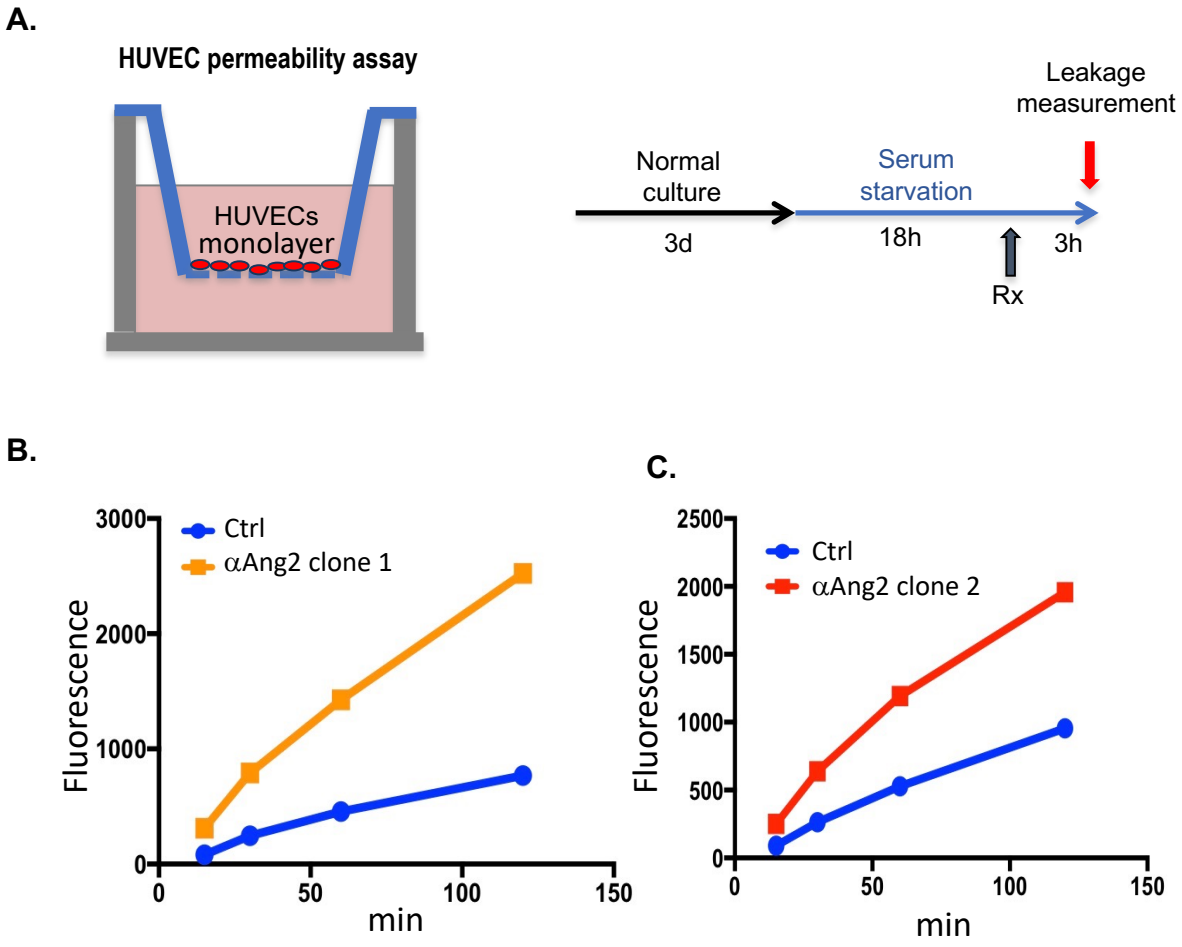
C.

	Binding to Tie-2								
	Human (nM) (SPR at 25C)	Rat (nM) (SPR at 25C)	Mouse (ELISA)	Cyno (ELISA)	Human (nM) (SPR at 37C)	Cyno (nM) (SPR at 37C)	Ang1 competitive	Ang2 Competitive	Agonist
Tie2.1	1200	1600	Yes	Yes	3000	3000	Yes	Yes	Yes
Tie2.20	7.6	20.7	Yes	Yes	N.D.	N.D.	Yes	Yes	No
Tie2.38	380	2200	Yes	Yes	N.D.	N.D.	No	No	No

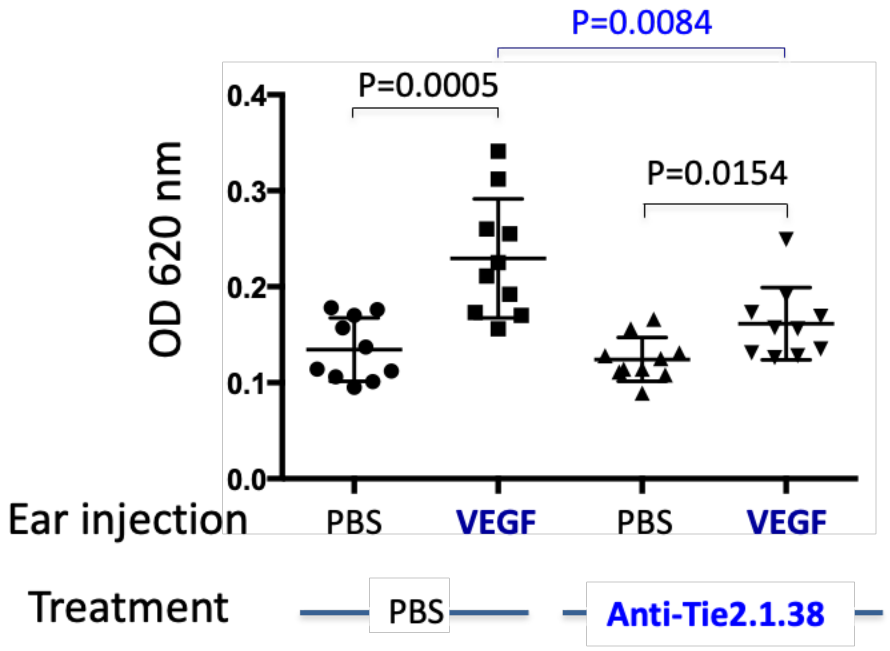
Supplementary Figure 1. Characterization of anti-Tie2 antibodies. Partially or highly purified anti-Tie2 (hIgG1) +/- polyclonal goat anti-hIgG1 was incubated for 15 min with RAECs. Cells were lysed and levels of pAKT were determined by HTRF (a). Anti-Tie2 antibodies were incubated with HUVEC cells for 15 minutes, lysed, and probed for the presence of pAKT and pERK by Western blot (b). Table describing cross-species binding, ligand competition, and agonistic activity of antibodies of interest (c).



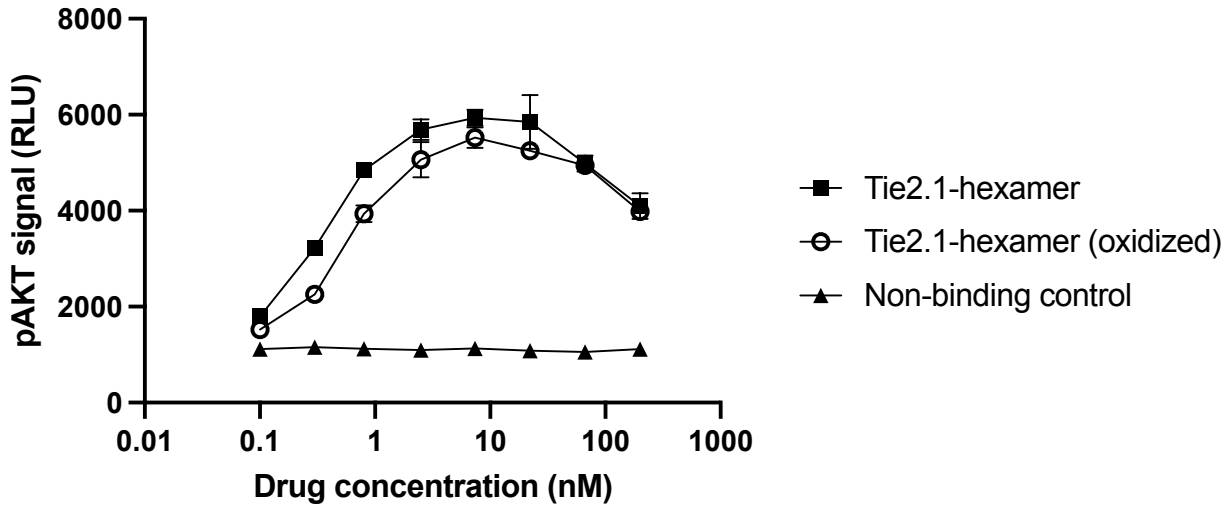
Supplementary Figure 2. Impact of Ang-2 on Tie2.1 hexamer activity. HUVECs were grown to confluency, transferred to serum-free basal medium for 4 h, and treated with the indicated agonists for 30 min. Phospho- (pAkt) and total Akt (tAkt) were assessed by Western blot.



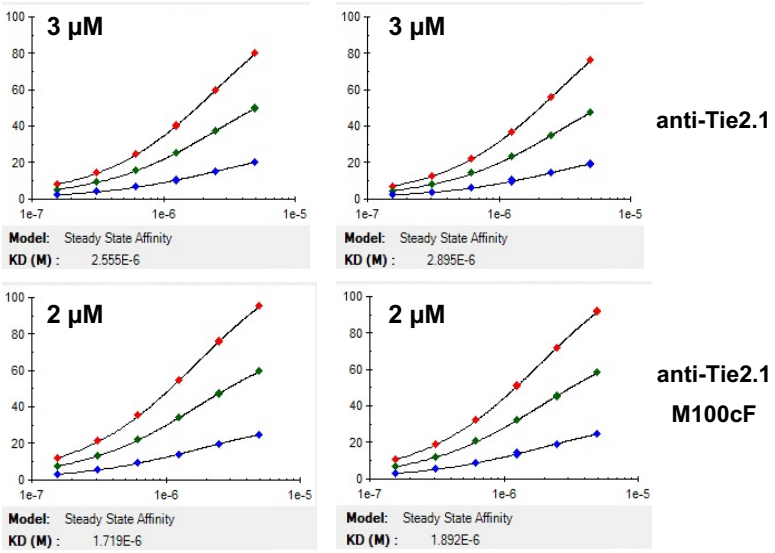
Supplementary Figure 3. Impact of anti-Ang2 on HUVEC permeability. Schematic of experimental design (a). Transit of FITC-labeled dextran through the HUVEC monolayer is monitored by fluorescence (n=3, error bars (standard deviation) are smaller than the indicated symbols). Two different anti-Ang-2 antibodies both reported to block interactions with Tie2 were added at 10 ug/ml and compared to a PBS control (b-c).



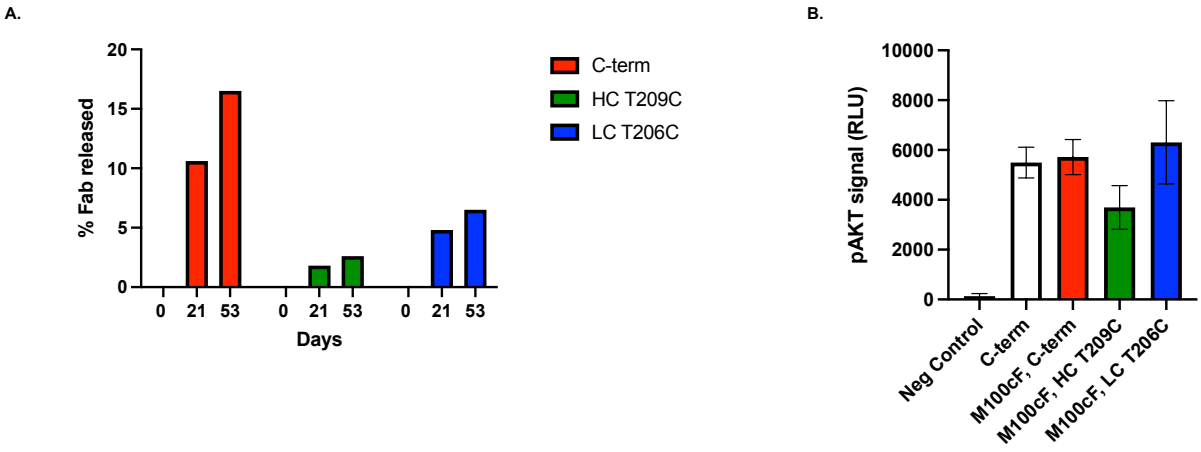
Supplementary Figure 4. *Tie2 agonists reduce permeability of murine vasculature*. Balb/C mice were administered Tie2.1.38 (10 mg/kg) or control intravenously. Three hours following administration of drug, Evans Blue dye was administered intravenously followed by a VEGF or PBS intradermally into the ears. Thirty minutes following intradermal injection the mice were sacrificed, dye was extracted from the ears, and quantified spectrophotometrically. Data shown are mean +/- standard deviation.



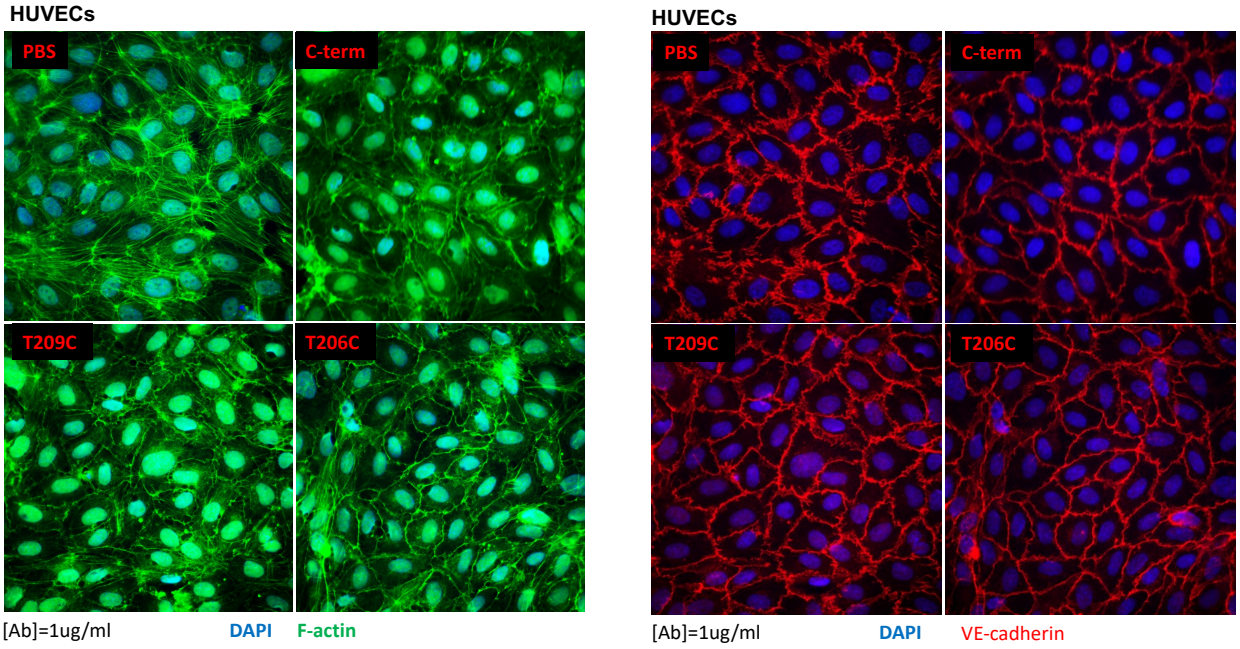
Supplementary Figure 5. Impact of M100c oxidation on Tie2.1-hexamer activity. Tie2.1-hexamer was treated with hydrogen peroxide resulting in an average of 65% oxidation of M100c. The untreated and oxidized hexamers were added to HUVECs at the indicated concentrations for 15 minutes, the cells were lysed and pAKT levels were assessed. Datapoints are the average of n=3 with error bars indicating the standard deviation. Error bars smaller than the datapoints are not plotted.



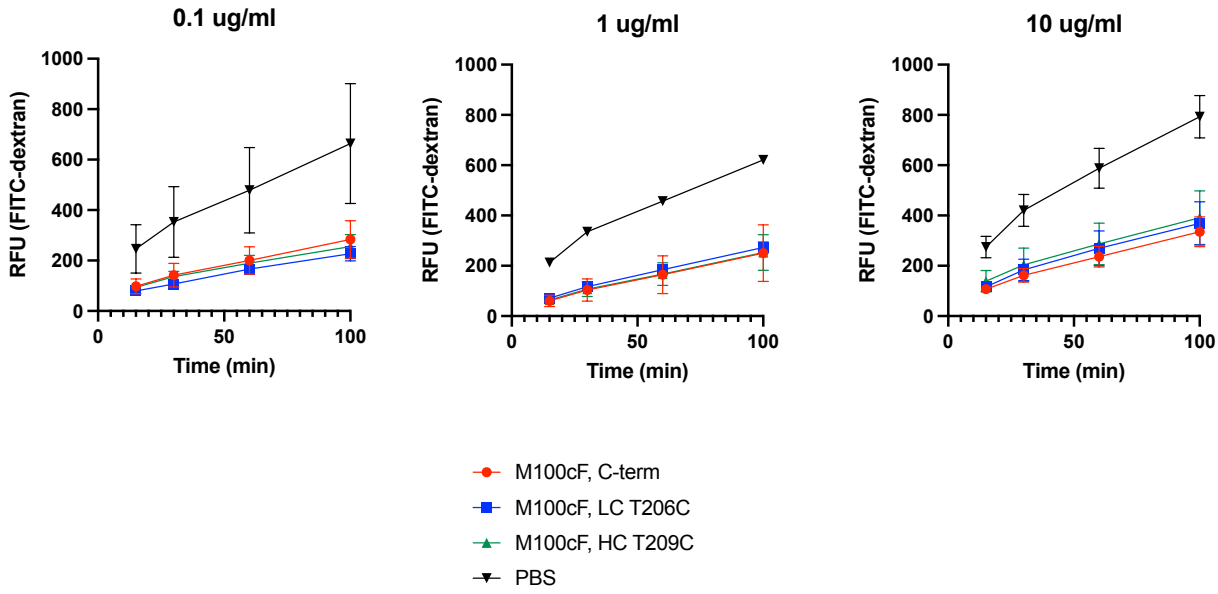
Supplementary Figure 6. SPR characterization of anti-Tie2.1 and anti-Tie2.1 M100cF binding to human (left) and cynomolgus monkey (right) Tie2. Due to the low affinity at 37 degrees C, steady state characterization was performed.



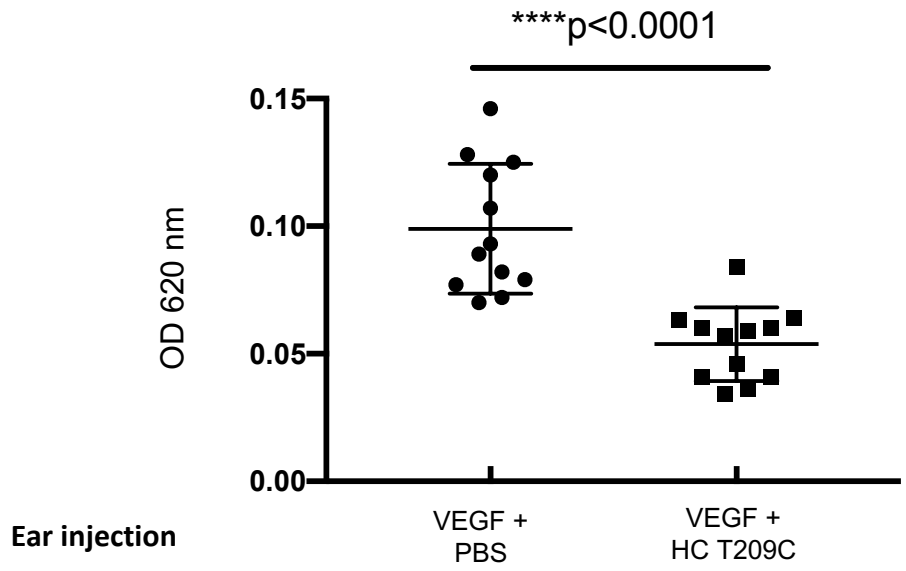
Supplementary Figure 7. Introduction of alternative conjugation sites prevents deconjugation of Tie2.1 Fabs over time, and maintains activity. Tie2.1-hexamers conjugated through the indicated cysteines were incubated in PBS at 37 degrees C for the up to 53 days. At the indicated times, samples were analyzed by analytical size exclusion chromatography to determine the fraction of total protein present as a low molecular weight species (Fab) versus high molecular weight species (at least a trimer) (a). The indicated Tie2.1-hexamers were incubated at 10 ug/ml with serum starved HUVEC cells for 24h, lysed, and analyzed for pAKT levels by HTRF.



Supplementary Figure 8. Tie2.1-hexamer variants stabilize cellular junctions. Immunofluorescence imaging of HUVEC cells treated with 1 ug/ml of Tie2.1-hexamer for 3h, and stained with DAPI (blue) and phalloidin (green) (*left*) or anti-VE-cadherin (red) analyzed by fluorescent microscopy (b).



Supplementary Figure 9. Tie2.1-hexamer variants suppress HUVEC permeability. Transit of FITC-labeled dextran through the HUVEC monolayer is monitored by fluorescence. Three Tie2.1-hexamers were added at indicated concentrations for 7 h and compared to a PBS control.



Supplementary Figure 10. Tie2.1 M100cF VH T209C-hexamer reduces vascular permeability in mice. Balb/C mice were administered Evans Blue dye intravenously followed by a combination of VEGF and a drug or negative control intradermally into the ears. Thirty minutes following intradermal injection the mice were sacrificed, dye was extracted from the ears, and quantified by spectrophotometrically. P values are calculated using unpaired t test with Welch's correction.