Supplementary Table 1. Primers for amplification of the anti-VEGF-B V_L and V_H.

Primer name	Sequence 5`→3`
2H10VHback	GGCGGCGGCGGCTCCGGTGGTGGTGGATCCCAGGTGCAGCTGCAGCAGCC
2H10VHfor	GGAATTCGGCCCCCGAGGCCCTGGACACGGTCACGCT
2H10VLback	GCCATGGCGGACTACAAAGAGATCCAGATGACCCAGACC
2H10VLfor	GGAGCCGCCGCCGCCAGAACCACCACCACCAGAACCACCACCACCCCTCTTGATTTCCAGCTTTGT

Supplementary Table 2. Assessment of blink reflex in animals treated with anti-VEGF-B scFv.

Group	Time-point (weeks post-treatment)	Blink reflex assessed/confirmed (number of rats tested)
Untreated	1	8/8
	2	12/12
	12	4/4
Control scFv	1	8/8
	2	18/18
	12	6/6
2H10 scFv	1	8/8
	2	16/16
	12	6/6
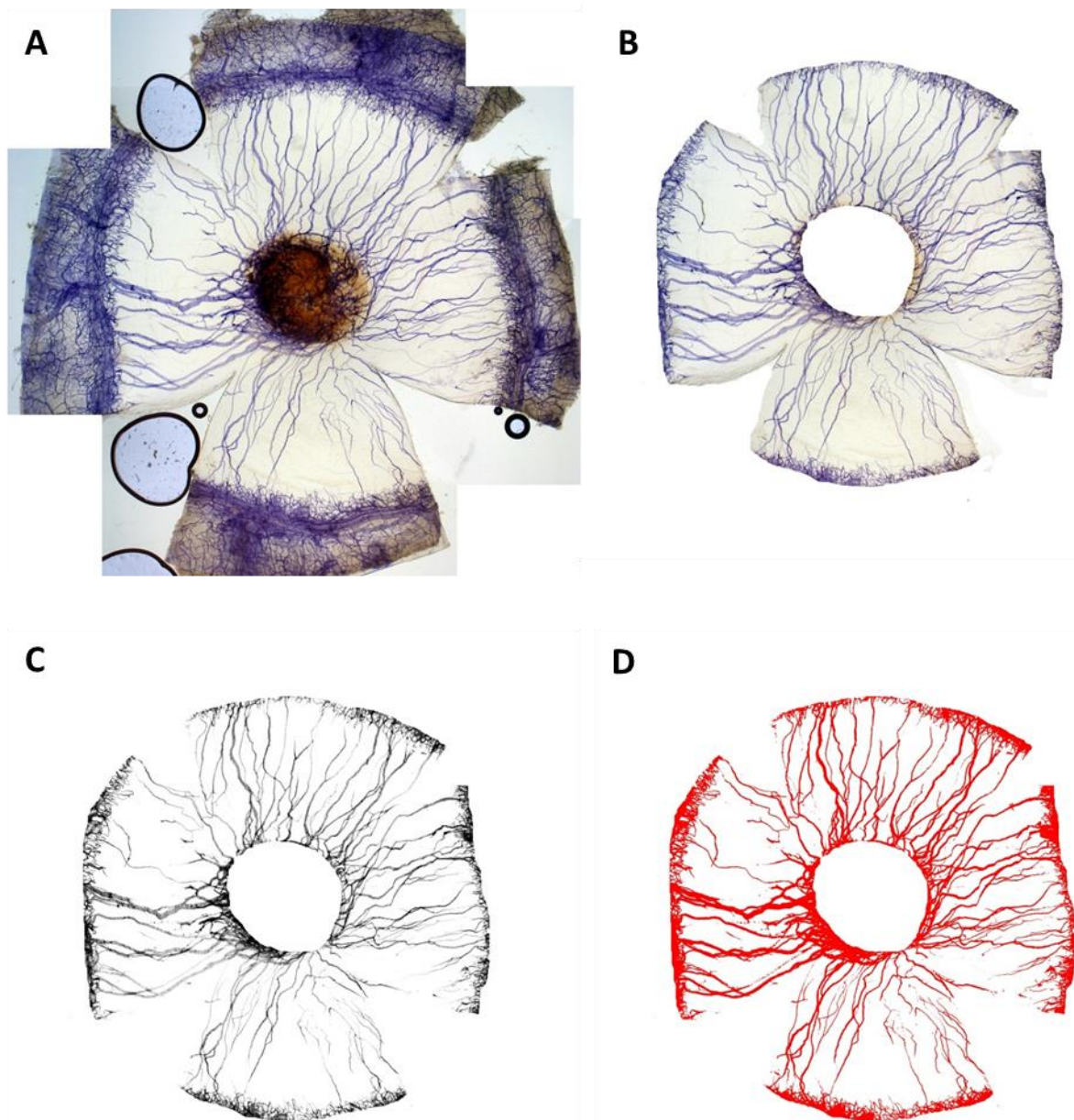
Supplementary Figure 1. Assessment of corneal vessel area using NIH ImageJ software (National Institutes of Health, USA). Images of corneal flat-mounts were processed in Photoshop CS2 (Adobe, San Jose, USA) using the auto level command. **(A)** Montages were prepared using the photomerge function. Montaged images were then coded so that the quantification of corneal vessel area was performed in a masked fashion. **(B)** Images were processed in ImageJ and the area of the central corneal burn was selected using the freehand selection tool. The

selected area was then removed. The freehand selection tool was used to select the corneal area including some sclera. The selection was copied to a new image file. The paintbrush tool was used to remove all areas beyond the limbal arcades. **(C)** The image was converted to a RGB stack and the green channel, which had the maximum contrast, was copied to a new file. The brightness and contrast of the file were optimized to show vessels. **(D)** The color threshold function was applied and the level was adjusted to select the vessels with minimal background. The "analyse particles" function was used to measure the area covered by vessels. The freehand selection and measure tools were used to measure the total corneal area. The following formula was used to calculate the percentage of corneal area covered by vessels.

$$Percent\ area\ of\ vessels = \left(\frac{Measured\ vessel\ area}{Total\ corneal\ area - cauterised\ area}\right) * 100$$

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2H10 ScFv    GGCCCAGCCGGCCATGGCGGACTACAAAGAGATCCAGATGACCCAGACCACCTCCAGCCT    60
2H10 VL      --------------------------GAGATCCAGATGACCCAGACCACCTCCAGCCT    32
2H10 VH      ------------------------------------------------------------    0

2H10 scFv    GAGCGCCAGCCTGGGCGACAGAGTGACCATCAGCTGCCGGGCCAGCCAGGACATCAGCAA   120
2H10 VL      GAGCGCCAGCCTGGGCGACAGAGTGACCATCAGCTGCCGGGCCAGCCAGGACATCAGCAA    92
2H10 VH      ------------------------------------------------------------    0

2H10 scFv    CTTTCTGAACTGGTATCAGCAGAAACCCGACGGCACCGTGAAGCTGCTGATCTACTACAC   180
2H10 VL      CTTTCTGAACTGGTATCAGCAGAAACCCGACGGCACCGTGAAGCTGCTGATCTACTACAC   152
2H10 VH      ------------------------------------------------------------    0

2H10 ScFv    CAGCACCCTGCACAGCGGCGTGCCCAGCCGGTTTAGCGGCAGCGGCTCCGGCACCGACTA   240
2H10 VL      CAGCACCCTGCACAGCGGCGTGCCCAGCCGGTTTAGCGGCAGCGGCTCCGGCACCGACTA   212
2H10 VH      ------------------------------------------------------------    0

2H10 ScFv    CAGCCTGACCATCTCCAACCTGGAACAGGAAGATATTGCCACCTACTTTTGCCAGCAGGG   300
2H10 VL      CAGCCTGACCATCTCCAACCTGGAACAGGAAGATATTGCCACCTACTTTTGCCAGCAGGG   272
2H10 VH      ------------------------------------------------------------    0

2H10 scFv    CAAGACACTGCCCCCCACCTTTGGCGGCGGAACAAAGCTGGAAATCAAGAGG<u>GGTGGTGG</u>   360
2H10 VL      CAAGACACTGCCCCCCACCTTTGGCGGCGGAACAAAGCTGGAAATCAAGAGG--------   324
2H10 VH      ------------------------------------------------------------    0

2H10 scFv    <u>TGGTTCTGGTGGTGGTGGTTCTGGCGGCGGCGGCTCCGGTGGTGGTGGATCC</u>CAGGTGCA   420
2H10 VL      ------------------------------------------------------------    -
2H10 VH      -------------------------------------------------CAGGTGCA    8

2H10 scFv    GCTGCAGCAGCCCGGCACCGAGCTGGTGAAGCCTGGCGCCTCCGTGAAACTGTCCTGCAA   480
2H10 VL      ------------------------------------------------------------    -
2H10 VH      GCTGCAGCAGCCCGGCACCGAGCTGGTGAAGCCTGGCGCCTCCGTGAAACTGTCCTGCAA    68

2H10 scFv    GGCCTCCGGCTACACCTTCACCGGCTTTTGGATCCACTGGGTGAAACAGAGACCAGGACA   540
2H10 VL      ------------------------------------------------------------    -
2H10 VH      GGCCTCCGGCTACACCTTCACCGGCTTTTGGATCCACTGGGTGAAACAGAGACCAGGACA   128

2H10 scFv    GGGCCTGGAATGGATCGGCCACATCAACCCCGGCAACGGCGGCACCAACTACAACGAGAA   600
2H10 VL      ------------------------------------------------------------    -
2H10 VH      GGGCCTGGAATGGATCGGCCACATCAACCCCGGCAACGGCGGCACCAACTACAACGAGAA   188

2H10 scFv    GTTCAAGCGGATGGCCACCCTGACCGTGGACAAGAGCAGCAGCACCGCCTACATGCAGCT   660
2H10 VL      ------------------------------------------------------------    -
2H10 VH      GTTCAAGCGGATGGCCACCCTGACCGTGGACAAGAGCAGCAGCACCGCCTACATGCAGCT   248

2H10 scFv    GTCCAGCCTGACCAGCGAGGACAGCGCCGTGTACTACTGCGCCCGCAGCTACAGCAACTA   720
2H10 VL      ------------------------------------------------------------    -
2H10 VH      GTCCAGCCTGACCAGCGAGGACAGCGCCGTGTACTACTGCGCCCGCAGCTACAGCAACTA   308

2H10 scFv    CGTGCGGGCCATGGACTACTGGGGCCAGGGCACCAGCGTGACCGTGTCCAGGGCCTCGGG   780
2H10 VL      ------------------------------------------------------------    -
2H10 VH      CGTGCGGGCCATGGACTACTGGGGCCAGGGCACCAGCGTGACCGTGTCCAG[C]-------   360

2H10 scFv    GGCCGAT***CACCATCATCACCATCAT***           807
2H10 VL      -----------------------           -
2H10 VH      -----------------------           -
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Supplementary Figure 2. The sequence of the anti-VEGF-B scFv was aligned to the known sequence of the V_L and V_H regions of the 2H10 hybridoma. The linker region is underlined and the histidine tag is represented by bold and italicized text.

The sequence of the 2H10 scFv was identical to the expected sequence of the V_L and V_H except for one mismatched base shown in bold text within a box. This change caused a substitution of a serine in place of an arginine in the framework region. However, the change did not abolish the binding to the scFv to VEGF-B.
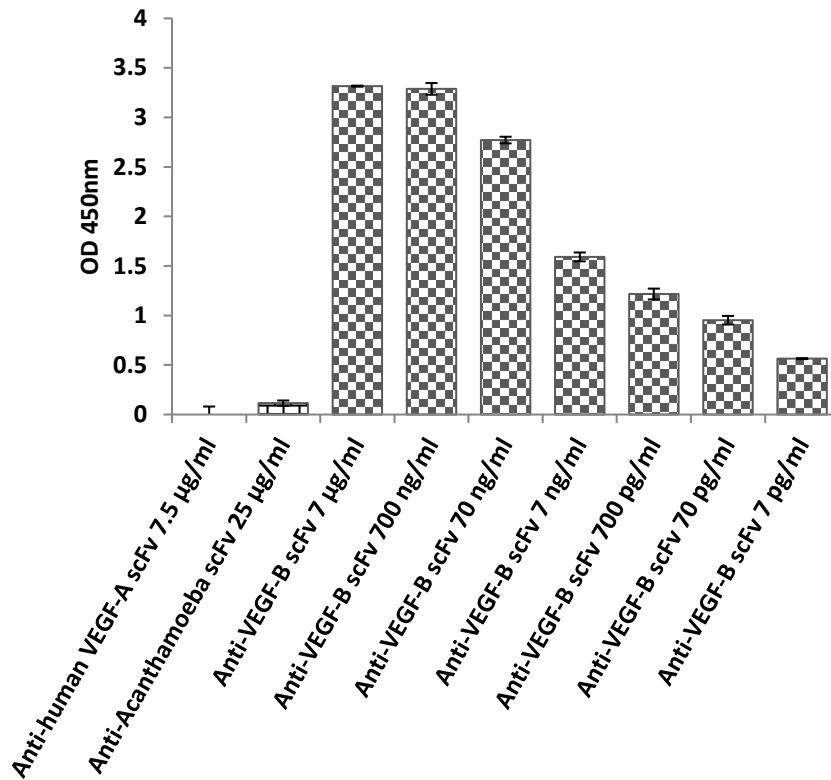
Supplementary Figure 3. Titration of the binding of the anti-VEGF-B scFv to human VEGF-B. Anti-VEGF-B and control scFvs (anti-VEGF-A and anti-*Acanthamoeba*) were expressed in *E. coli*. Recombinant scFv was purified from bacterial lysates by IMAC, and pooled fractions were dialyzed against PBS. Purified anti-VEGF-B scFv was diluted from 7 µg/ml to 7 pg/ml and binding to human VEGF-B was assayed by a direct ELISA. Binding above background levels was observed at each dilution tested. The control scFvs did not bind to human VEGF-B. The bars represent the mean $OD_{450\,nm}$ of three technical replicates and the error bars represent the standard deviation.
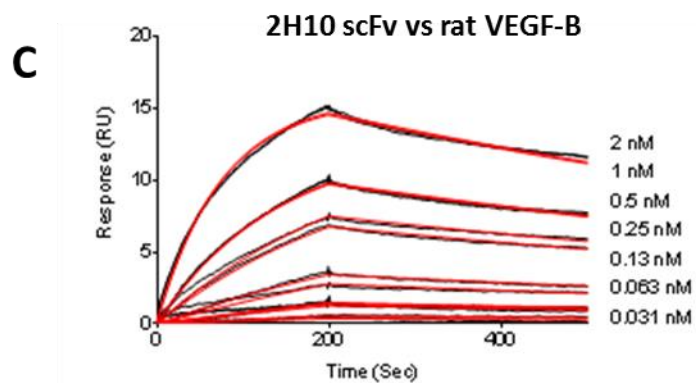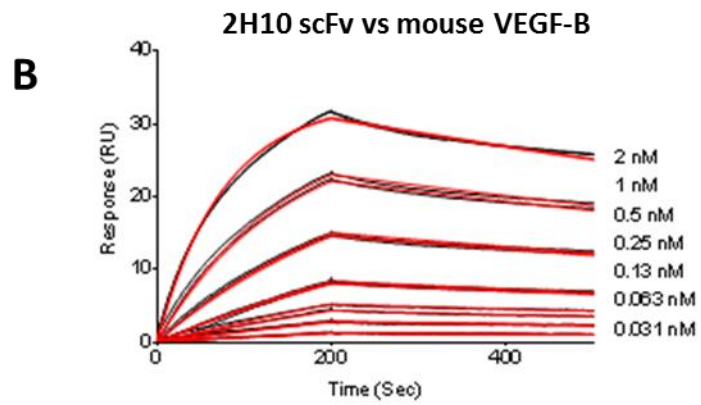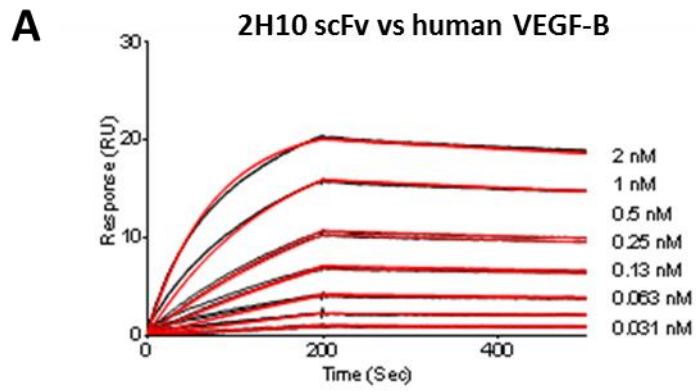
Supplementary Figure 4. Representative surface plasmon resonance sensorgrams of scFv 2H10 binding to **(A)** human, **(B)** mouse and **(C)** rat VEGF-B.
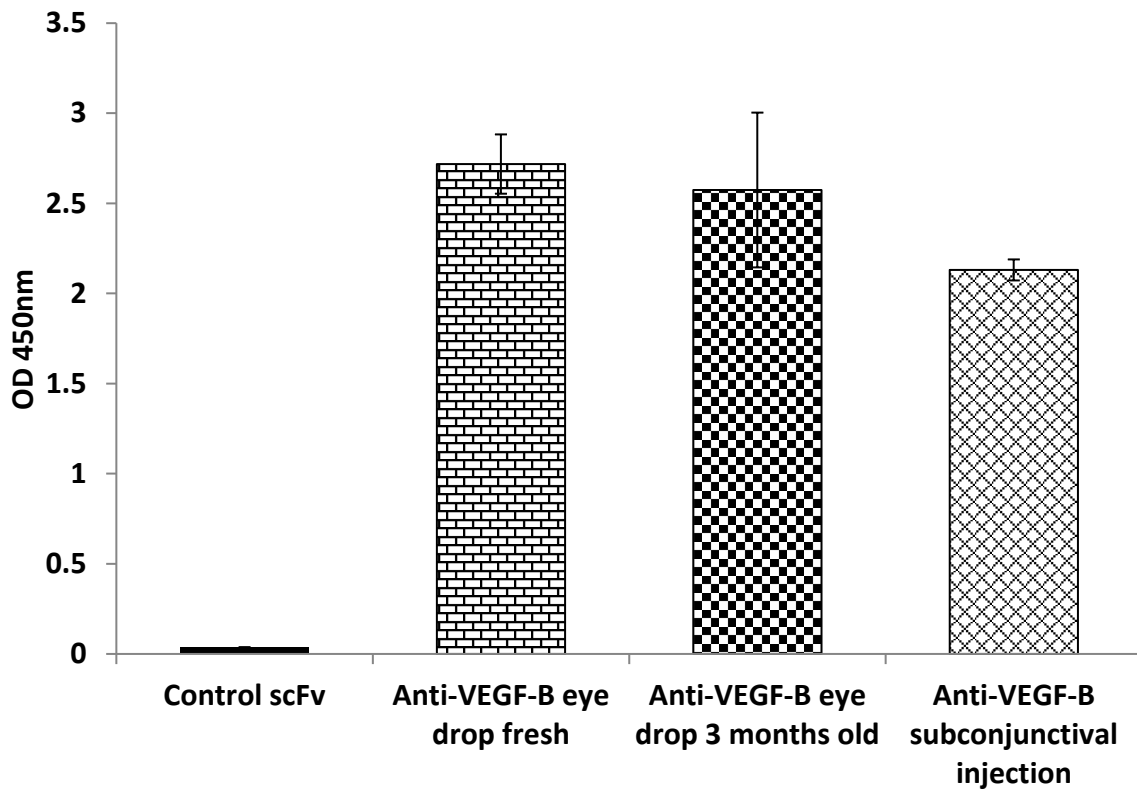
Supplementary Figure 5. Maintenance of anti-VEGF scFv activity after formulation into eye drops and for subconjunctival injection. Anti-VEGF-B scFv was formulated into eye drops, which contained 1.5% hypromellose, a viscosity enhancer, and 1% capric acid, a penetration enhancer. ScFv for subconjunctival injection was formulated with 20% Pluronic F127. Binding to human VEGF-B was assayed by direct ELISA. Freshly prepared eye drops maintained binding to human VEGF-B. The eye drops were then stored at 4°C for 3 months and binding retested. The eye drops maintained binding to human VEGF-B. ScFv in Pluronic F127 maintained binding to human VEGF-B. The bars represent mean the mean optical density at 450 nm, of three technical replicates, and the error bars represent the standard deviation.
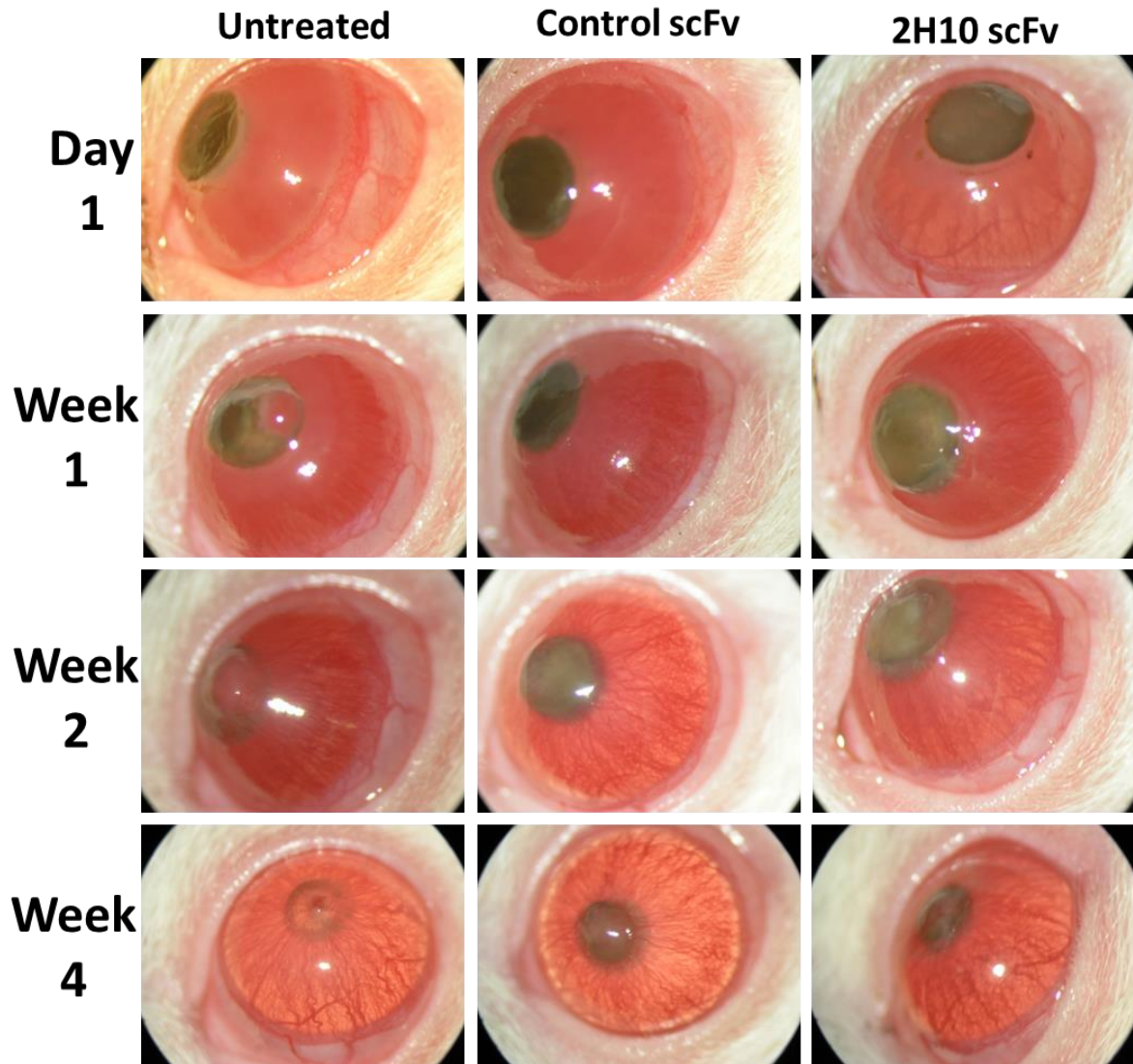
Supplementary Figure 6. Representative images of rat eyes post-cautery, captured at the operating microscope. Edema was apparent following cautery in all groups, but had reduced by week 1 and resolved by week 2. Blood vessels had sprouted from the limbal plexus by week 1 and had reached the site of cautery by week 2. Remodeling of blood vessels took place between weeks 2-4. Treatment with either the control or the 2H10 scFv (weeks 3-4) did not cause further edema. Original magnification 340X.
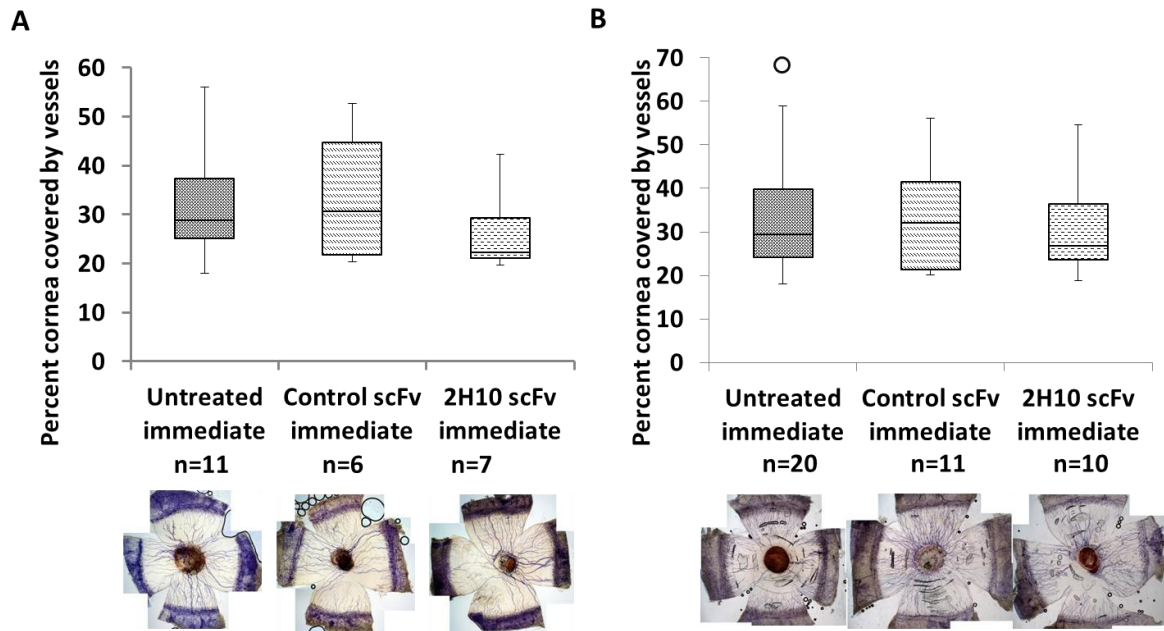
Supplementary Figure 7. Effect of (A) topical and (B) combined topical and subconjunctival injection of anti-VEGF-B scFv on growing corneal blood vessels. There was no significan difference in corneal blood vessel area in untreated, control scFv treated or anti-VEGF-B scFv treated groups (topical p=0.46, combined p=0.80). Representative corneal flat-mounts are displayed under each group.