Modified Anthrax Toxin Engineered to Selectively Target MMP-Expressing Activated Endothelial Cells as a Novel Therapeutic Approach for the Treatment of Corneal Neovascular Disease

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Authors 1 and 4 have no financial interest in the subject matter of this poster

Authors 2 and 3 have patents: 5,591,631 (P), 5,677,274 (P), 6,485,925 (P), 6,893,835 (P), 6,911,203 (P), 7,056,693 (P), 7,183,071 (P), 7,468,352 (P), 7,947,289 (P)

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Purpose:

•Corneal neovascularization (NV) is often insensitive to VEGF inhibition, prompting the exploration of other genes that contribute to this process. In this regard, the extensive interplay among endothelial cells (ECs), secreted factors, and the extracellular matrix (ECM) is an emerging target for anti-angiogenic therapies. In particular, ECM proteolysis has been implicated as one of the first and most sustained activities involved in pathological angiogenesis. Proteolysis by matrix metalloproteinases (MMPs) has previously been reported to play an important role in corneal NV. Our goal is to identify MMPs upregulated by activated ECs to develop targeted therapies for the treatment of corneal NV.

Methods:

•Immortalized human umbilical vein ECs (HUVECs) were exposed to recombinant human (rh)VEGF protein in culture, and expression of MMPs was assessed by quantitative PCR, and confirmed by zymography. These results were then corroborated *in vivo* using the rat corneal pocket (rCP) assay by immunohistochemistry (IHC). Expression of MMPs was confirmed in human tissue from patients with vascularized corneal grafts (IHC). A modified bacterial anthrax toxin (mBAT) engineered to be active only in the presence of specific MMPs was then assessed *in vitro* (Cell Titer Glo) and *in vivo* to evaluate its potential as a therapeutic approach for corneal NV.

Figure 1. VEGF promotes MMP-2 expression *in vivo.*

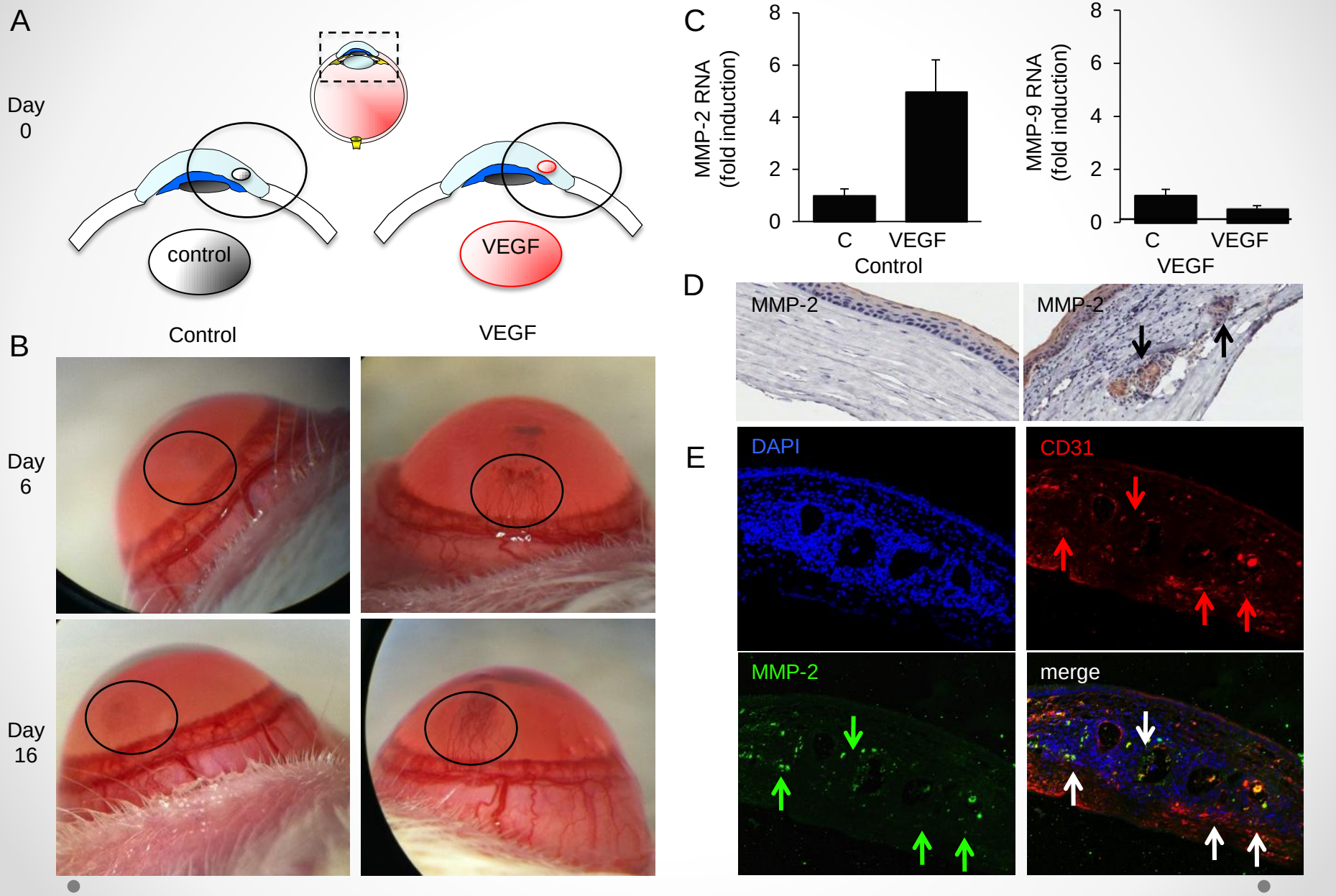


Figure 2. VEGF promotes MMP-2 expression by endothelial cells (HUVECs) *in vitro.*

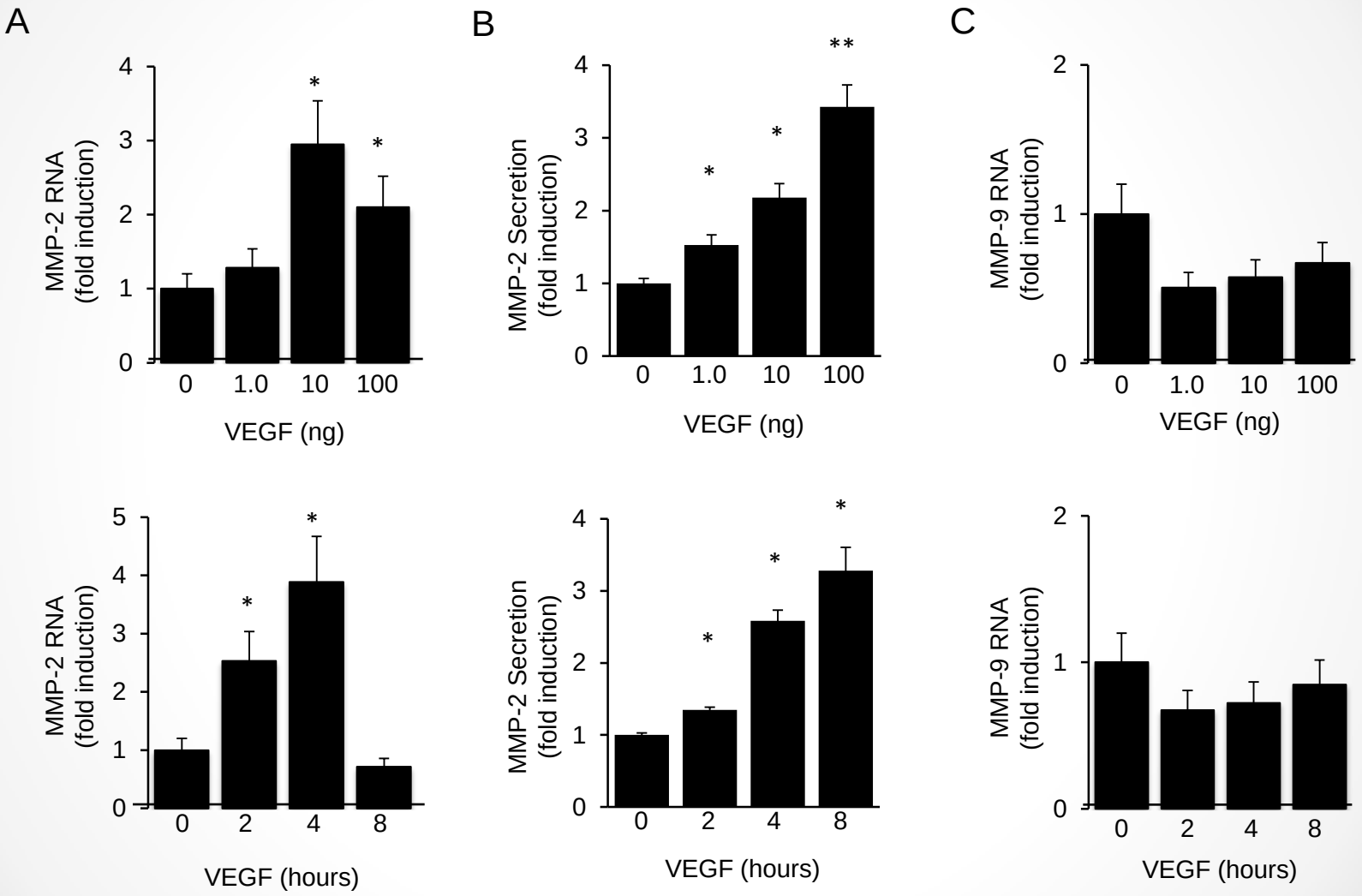


Figure 3. VEGF promotes expression of the MMP-2 activator, MT1-MMP, but not the MMP-2 inhibitor, TIMP-2, by HUVECs, resulting in increased MMP-2 enzymatic activity *in vitro.*

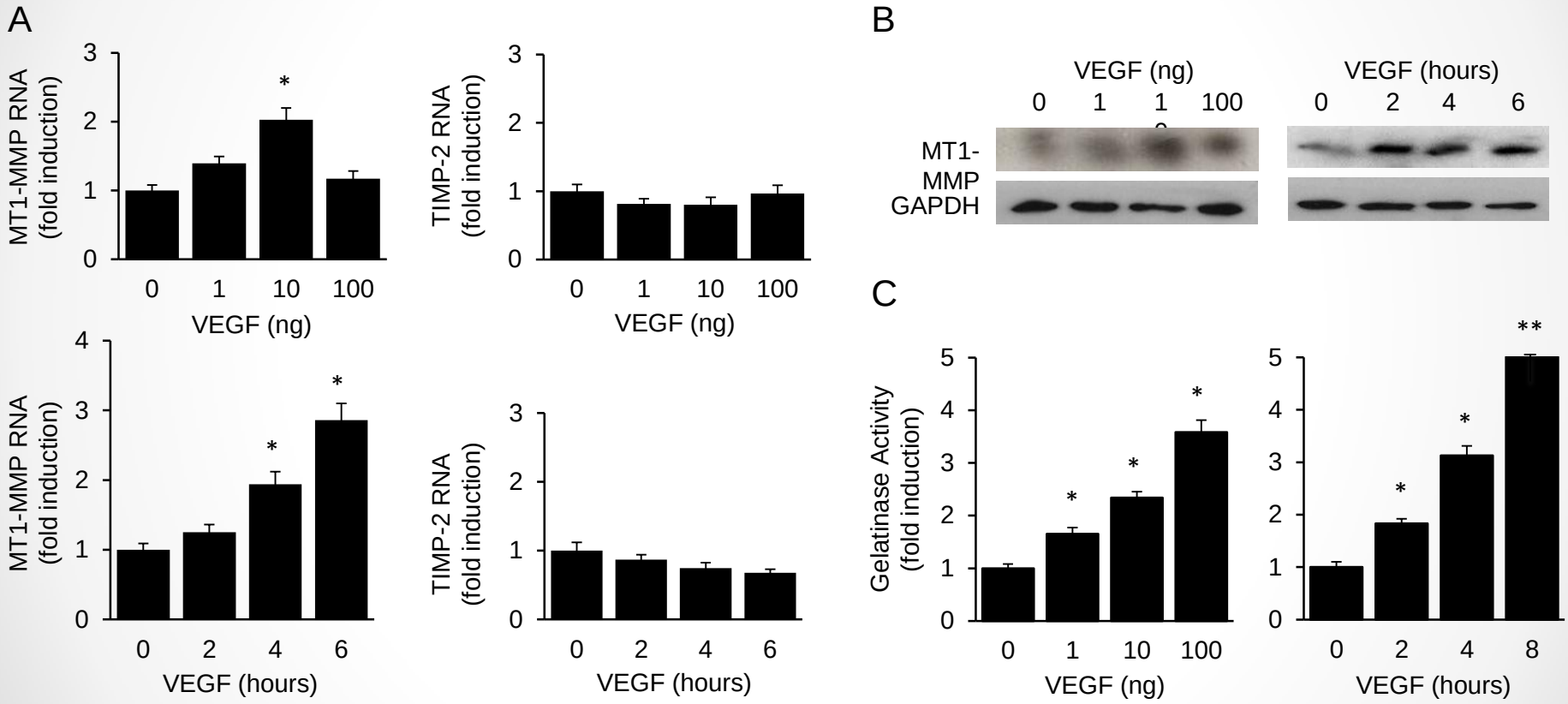
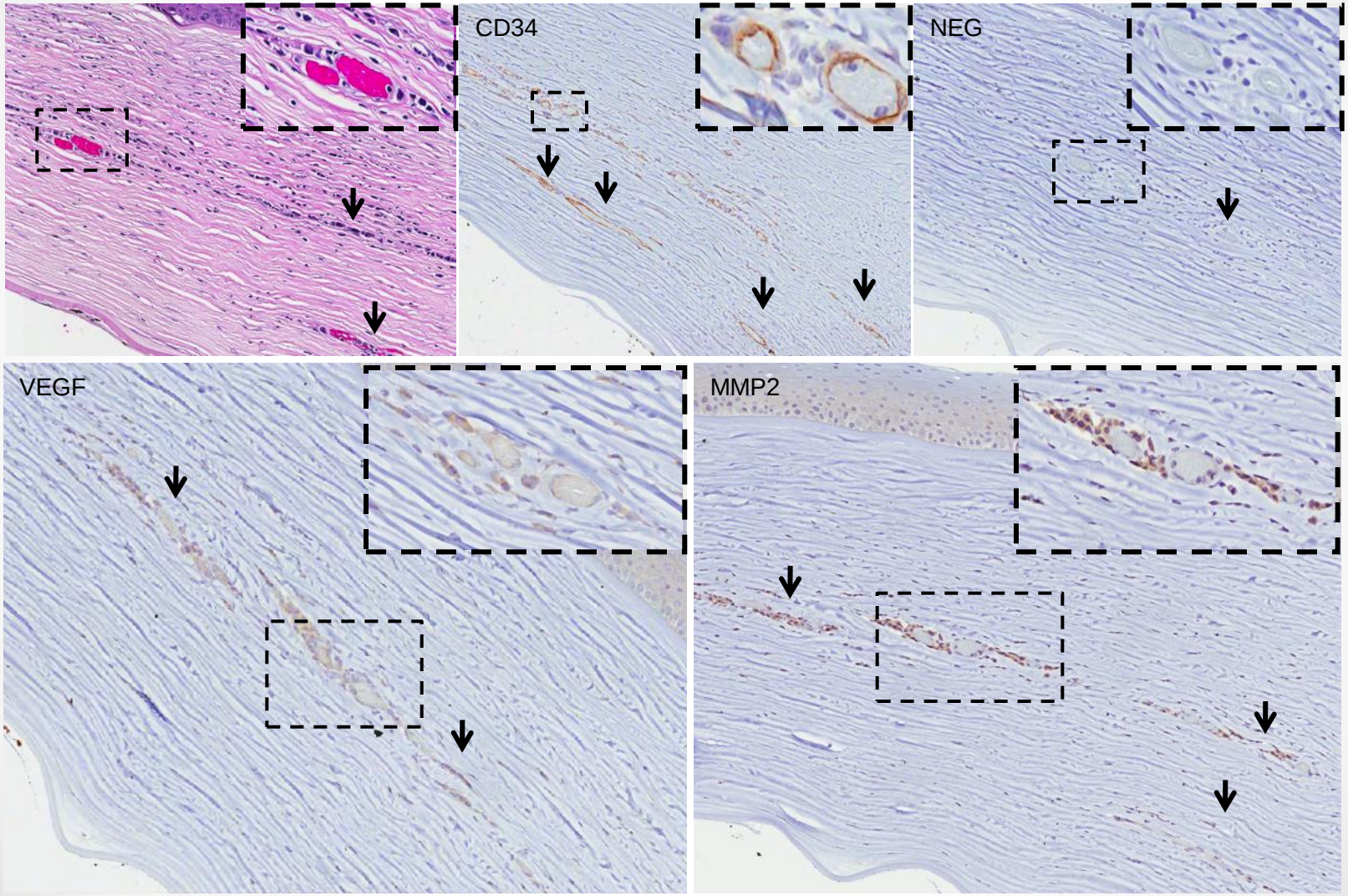


Figure 4. MMP-2 is highly expressed in by vascular ECs in patients with failed corneal grafts with corneal neovascularization. MMP-2 may therefore be a valid therapeutic target for the treatment of corneal neovascularization.



Summary: Hypoxic inflammatory cells release VEGF (1) which acts on neighboring vascular Ecs, leading to an increase in the expression of MMP-2 and MT1-MMP (2), and resulting in an increase in MMP-2 enzymatic activity (3), and corneal NV (4).

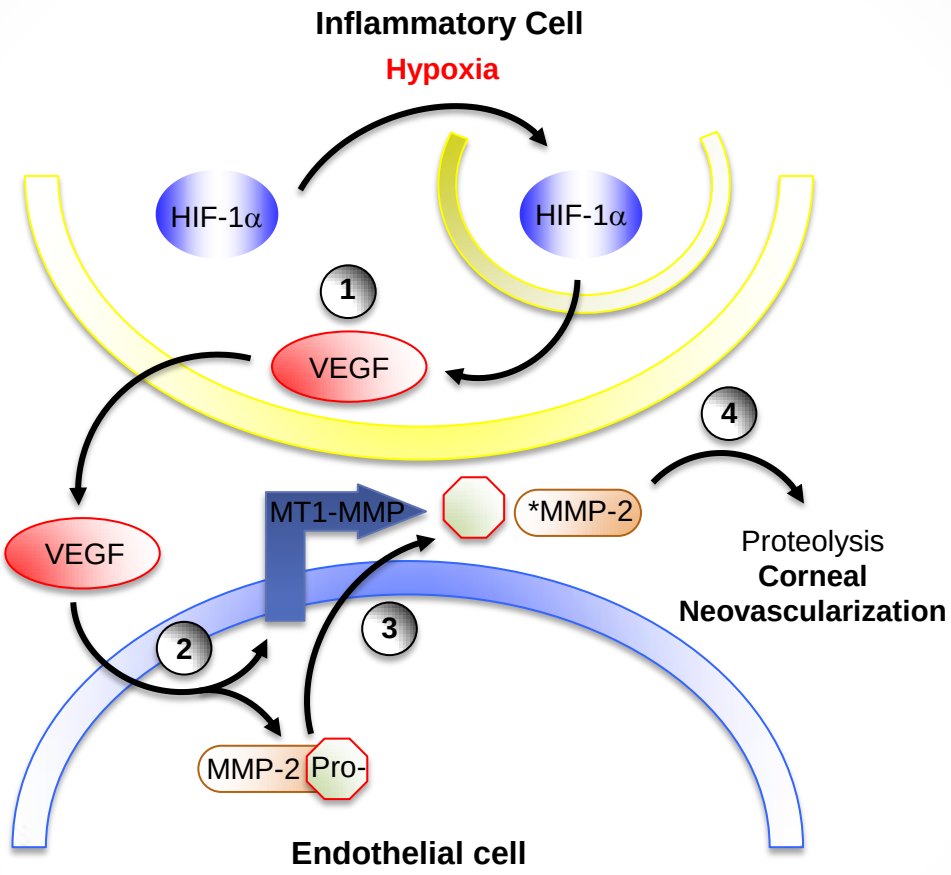


Figure 5. Genetically modified anthrax toxin (mBAT) engineered to be active in the presence of active MMP-2.

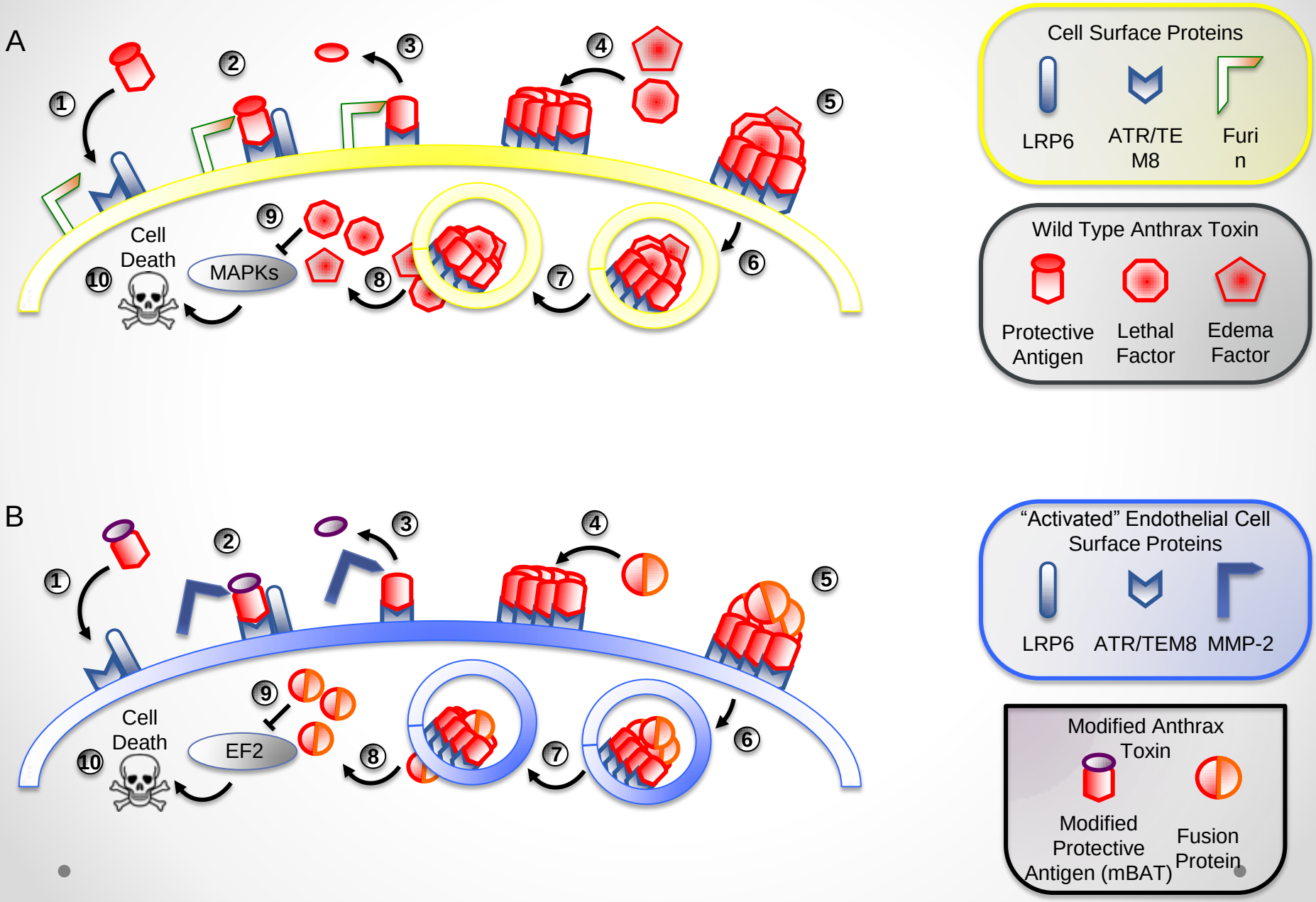


Figure 6. mBAT potently inhibits cell proliferation of active but not quiescent ECs *in vitro.*

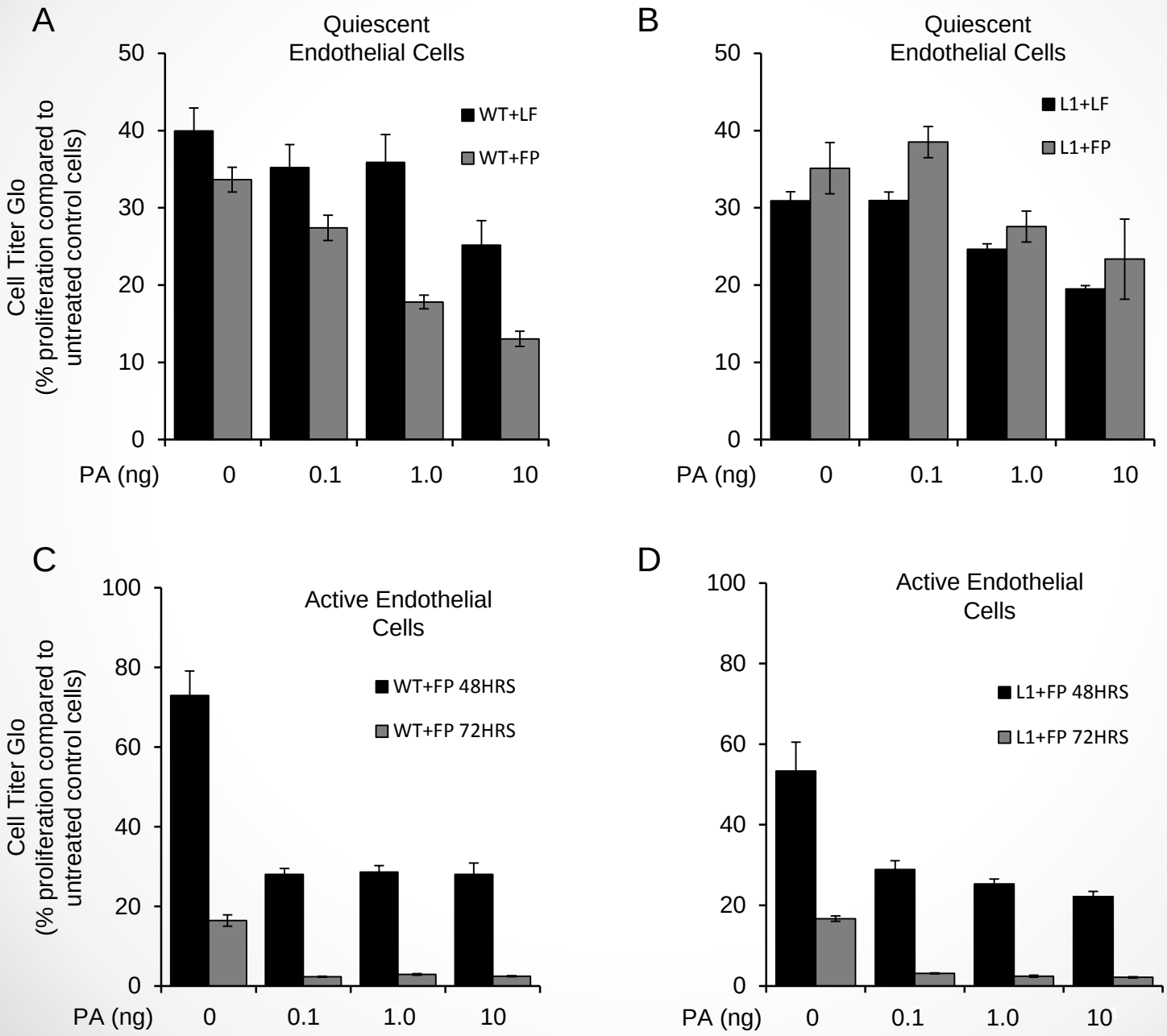


Figure 7. mBAT potently causes minimal corneal toxicity *in vivo.*

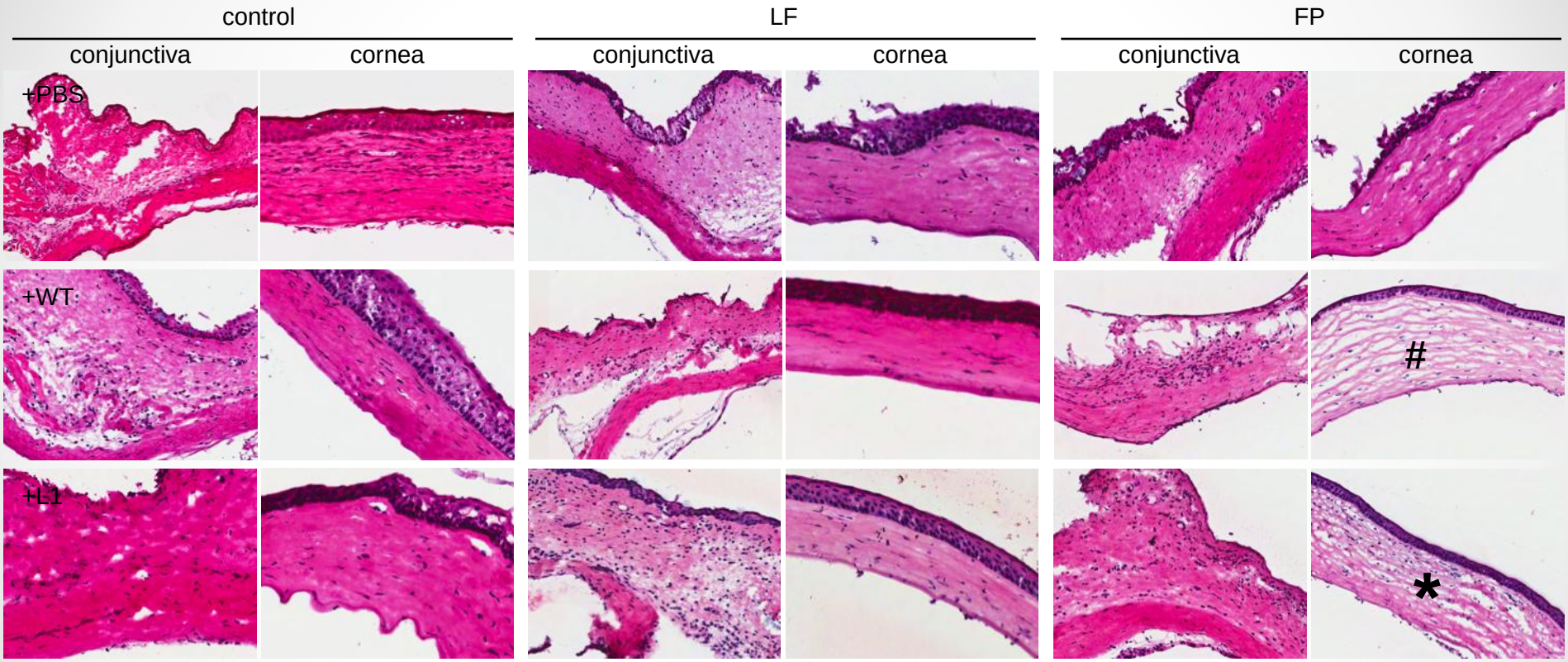
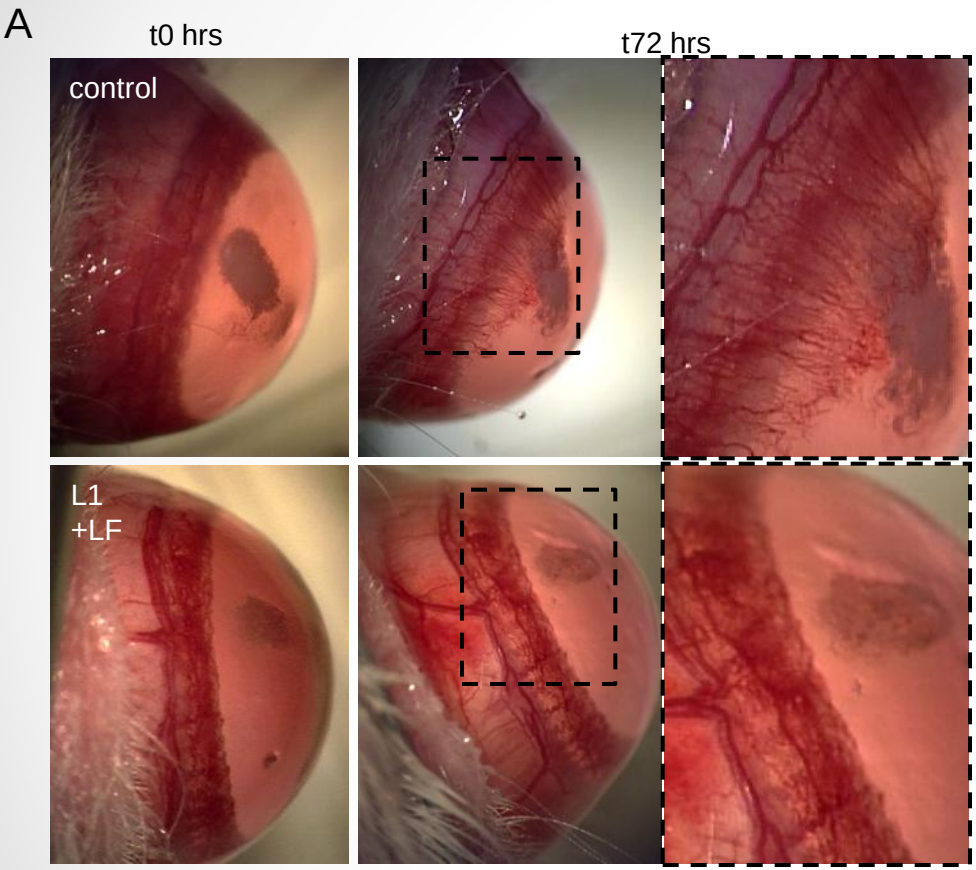


Figure 8. mBAT potently inhibits corneal NV *in vivo.*



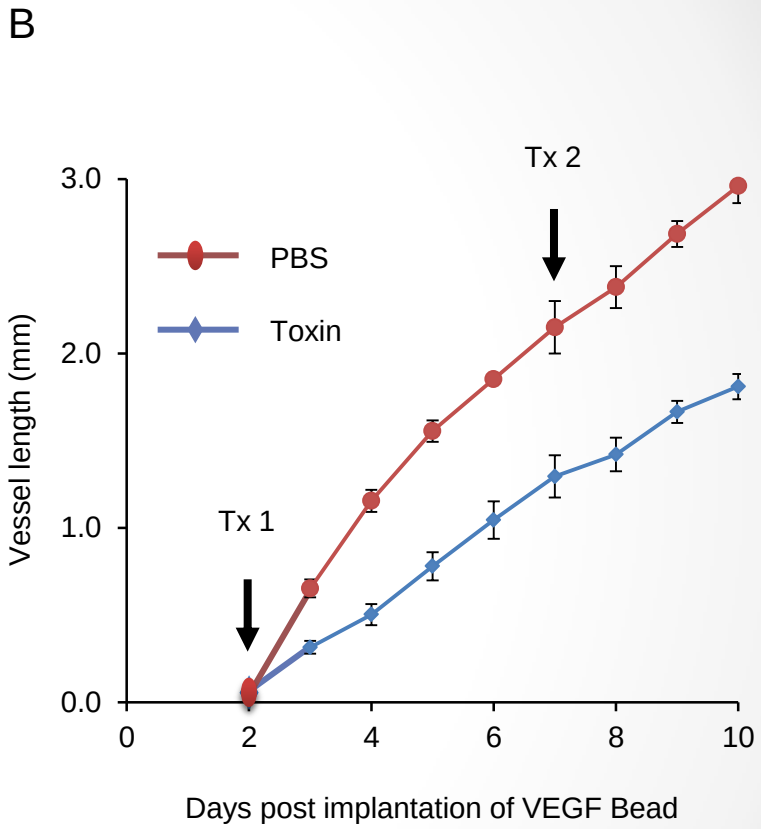
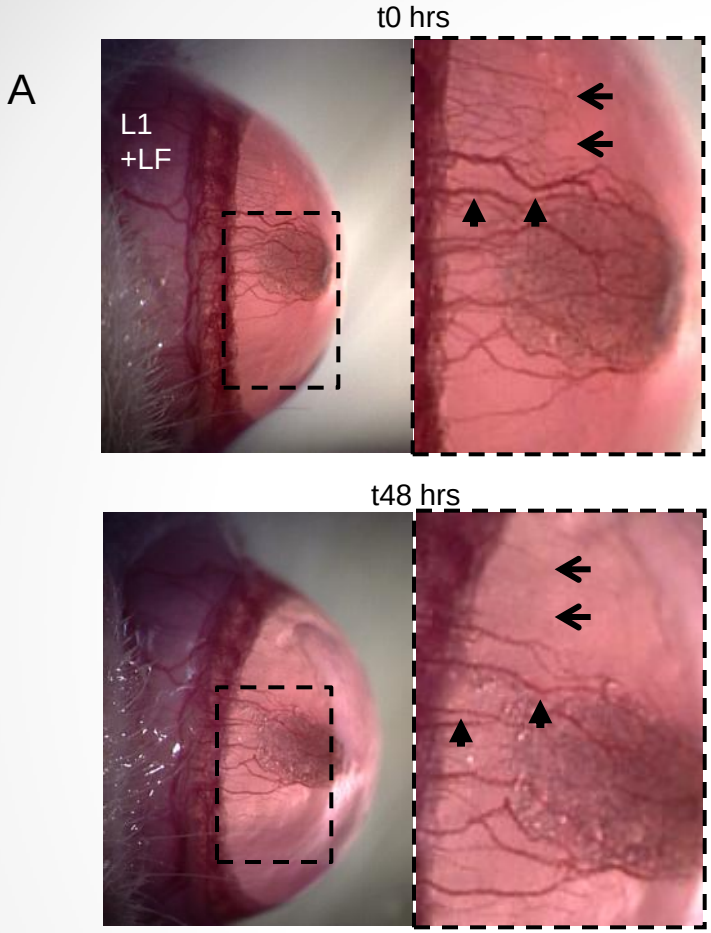
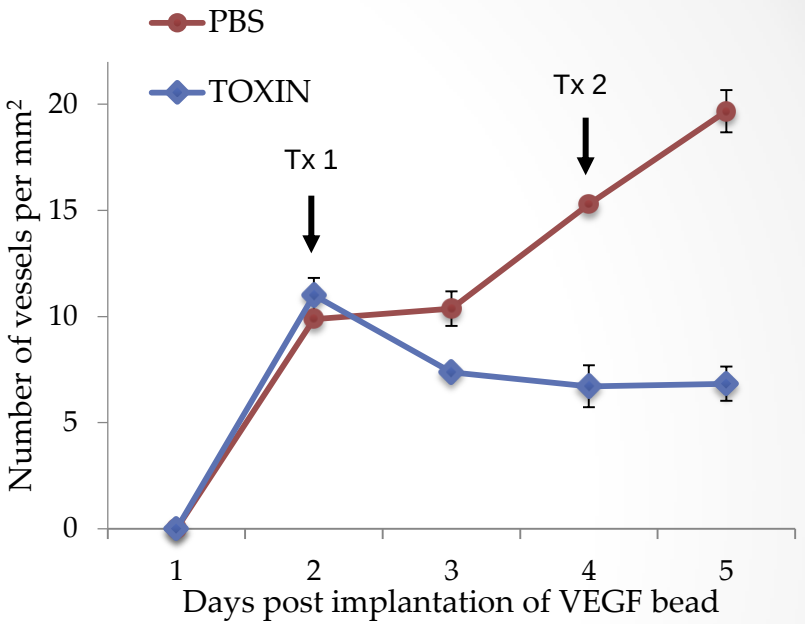


Figure 9. mBAT causes regression of corneal NV *in vivo*





Conclusion: We observed that treatment of HUVECs with rhVEGF results in an increase in the gelatinase, MMP-2 RNA levels (approximately 4 fold; $p < 0.05$) and enzymatic activity (approximately 6 fold; $p < 0.05$) in treated cells in a dose and time-dependent manner. We further observed an increase in the expression of MMP-2 in ECs in the rCP assay in response to VEGF and in human failed (vascularized) corneal grafts. Use of a mBAT specifically targeted and killed activated (dividing) ECs *in vitro* ($p < 0.05$) and effectively inhibited and treated corneal NV *in vivo* ($p < 0.05$).