

Supplementary Figures:

An anti-TNF- α antibody mimetic to treat ocular inflammation

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Figure S1

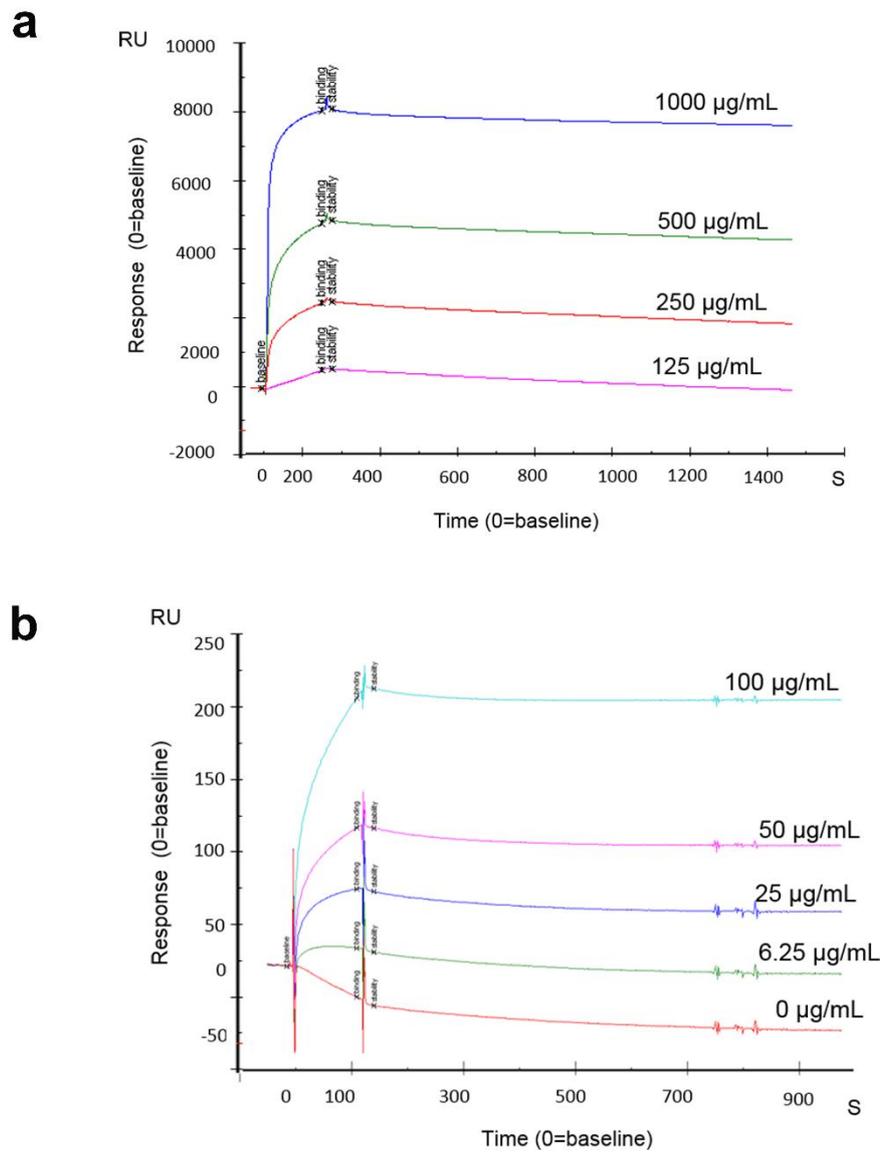
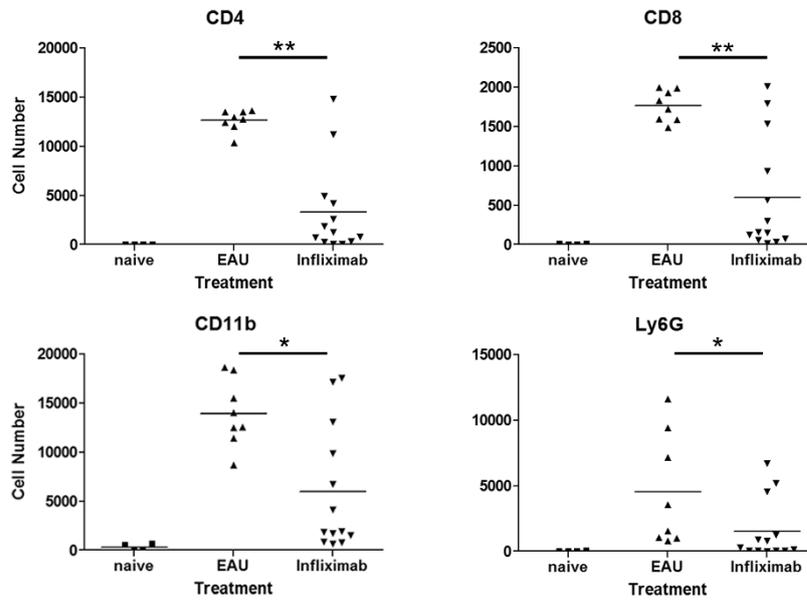


Figure S1: SPR binding assessment of infliximab and $\text{FpF}_{\text{infliximab}}$ to recombinant human $\text{TNF-}\alpha$

Graphs detailing the Surface Plasmon Resonance (SPR) binding sensograms, confirming that infliximab (**A**) and $\text{FpF}_{\text{infliximab}}$ (**B**) can both bind to human $\text{TNF-}\alpha$ in a concentration-dependent manner. The NTA chip was functionalized with Ni solution first and then his-tag $\text{TNF-}\alpha$ ($5\mu\text{g/mL}$) solution prior to loading an anti- $\text{TNF-}\alpha$ molecules (infliximab and $\text{FpF}_{\text{infliximab}}$).

Figure S2

a



b

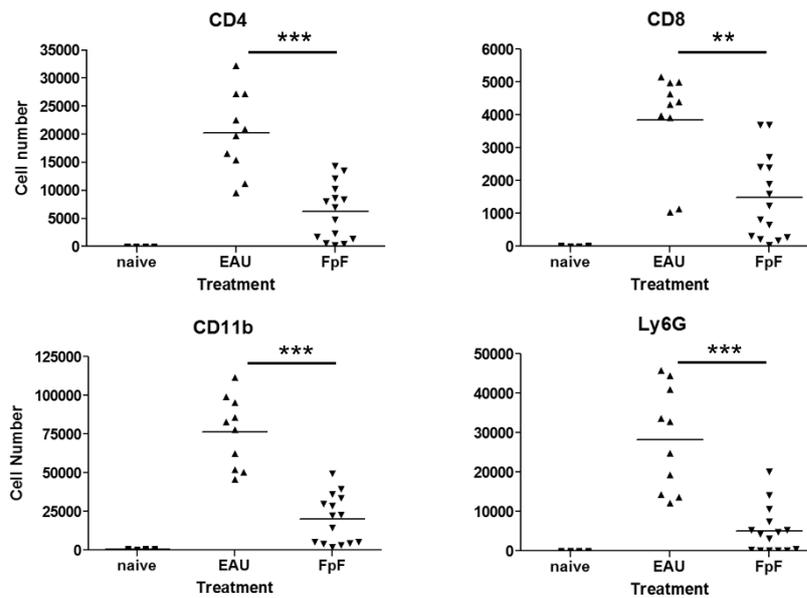


Figure S2: Analysis of retinal infiltrate following infliximab and FpF_{infiximab} treatment

Groups of immunized mice received intravitreal injection of 15 μ g infliximab or FpF_{infiximab} or vehicle control (EAU) on day 10. Eyes were enucleated on day 14, and retinal infiltrate characterized and quantified. Graphs detailing the specific CD45⁺ subsets of retinal infiltrate (CD4⁺, CD8⁺, CD11b⁺ and Ly6G⁺). *P<0.05, **P<0.005, ***P<0.0005; Data presented as means \pm SEM, and representative of two independent experiments.

Figure S3

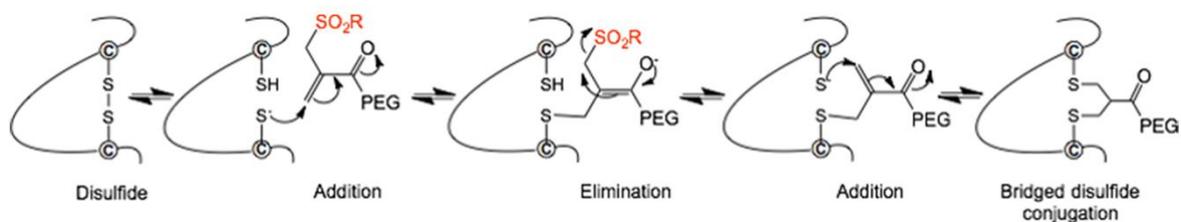


Figure S3: Bis-alkylation mechanism to generate three-carbon bridge site-specific conjugation between Fab and PEG scaffold.

The *mono*-sulfones **3** are latently crossed functionalized reagents capable of sequential and interactive addition-elimination reactions capable of bis-alkylation. In the case of disulfides, first the cysteine thiols are liberated by reduction (e.g. TCEP or DTT) and then conjugation involves (i) a first thiol addition to the mono-sulfone reagent **3**, (ii) sulphinic acid elimination to generate a second double bond, and (iii) a second thiol addition.

Figure S4

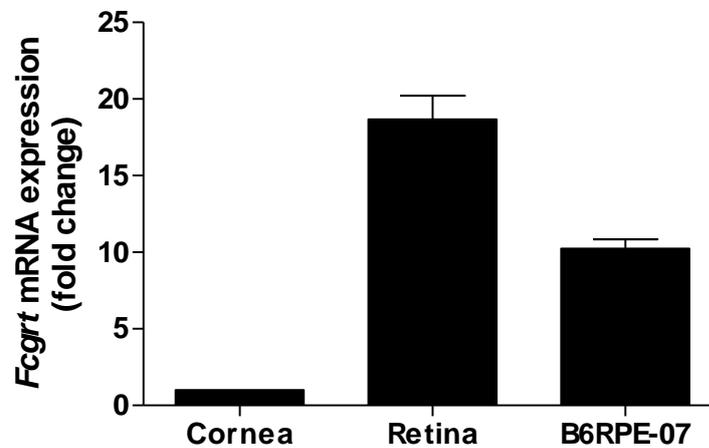


Figure S4: B6RPE-07 cell line expression *Fcgrt* mRNA

To confirm expression of the neonatal Fc receptor in the B6RPE-07 cell line, *Fcgrt* mRNA levels from 3 separate samples of cultured cells, as well as ex vivo retina and cornea tissues were determined by RT-qPCR. The ex-vivo mouse tissues were controls for *Fcgrt* expression, with retina (positive) and cornea (negative). Values were normalized to *Gapdh*, and the relative expression (fold change to negative control cornea) calculated.