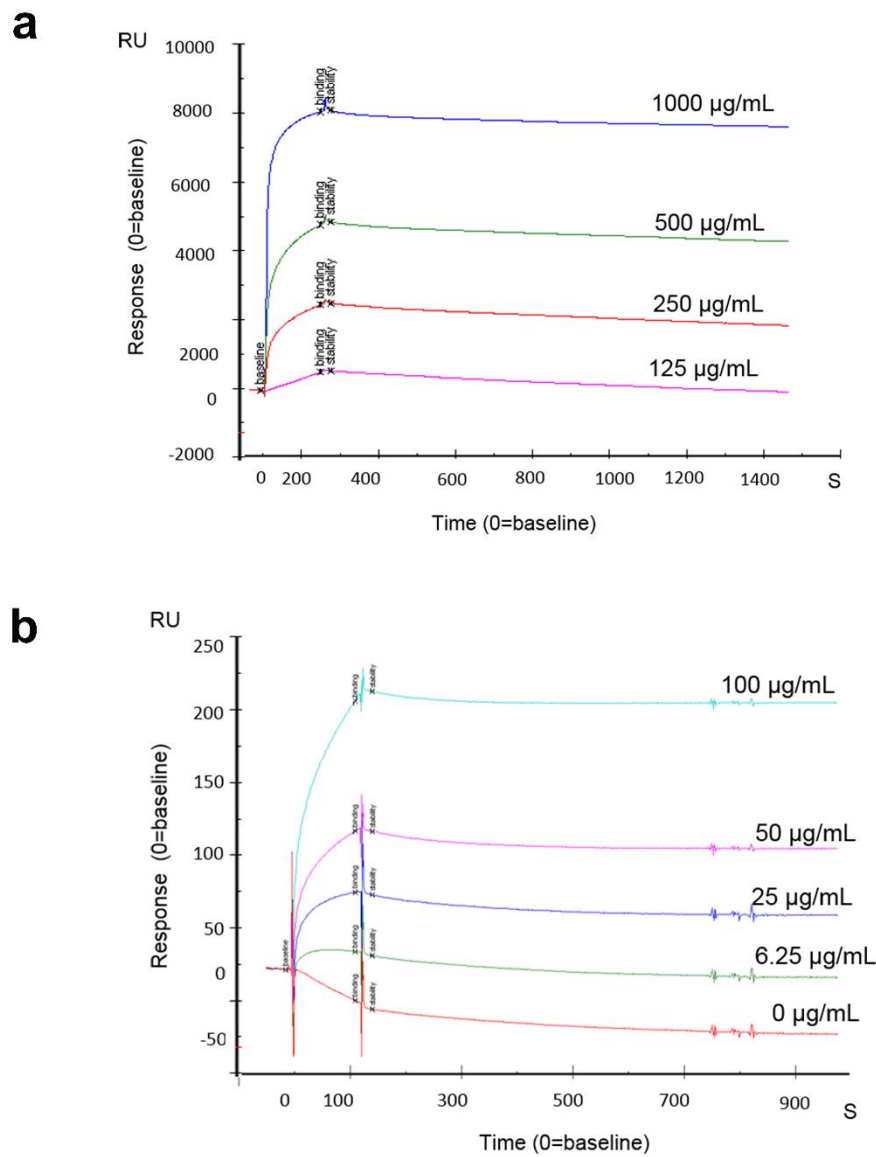


## Supplementary Figures:

### **An anti-TNF- $\alpha$ antibody mimetic to treat ocular inflammation**

Hanieh Khalilj<sup>2,3†</sup>, Richard W Lee<sup>1,3</sup>, Peng T Khaw<sup>3</sup>, Steve Brocchini<sup>2,3\*</sup>, Andrew D Dick<sup>1,3\*</sup>  
and David A Copland<sup>1,3†</sup>

**Figure S1**

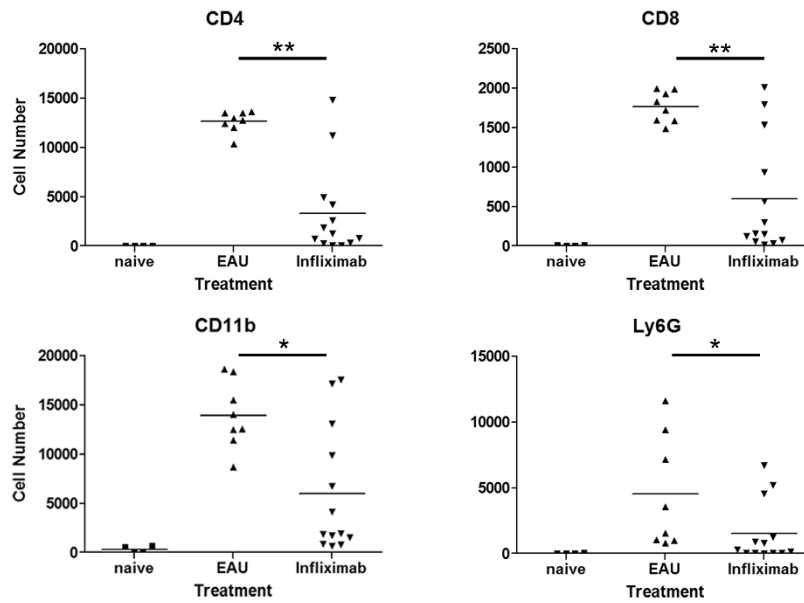


**Figure S1: SPR binding assessment of infliximab and  $\text{FpF}_{\text{infliximab}}$  to recombinant human  $\text{TNF-}\alpha$**

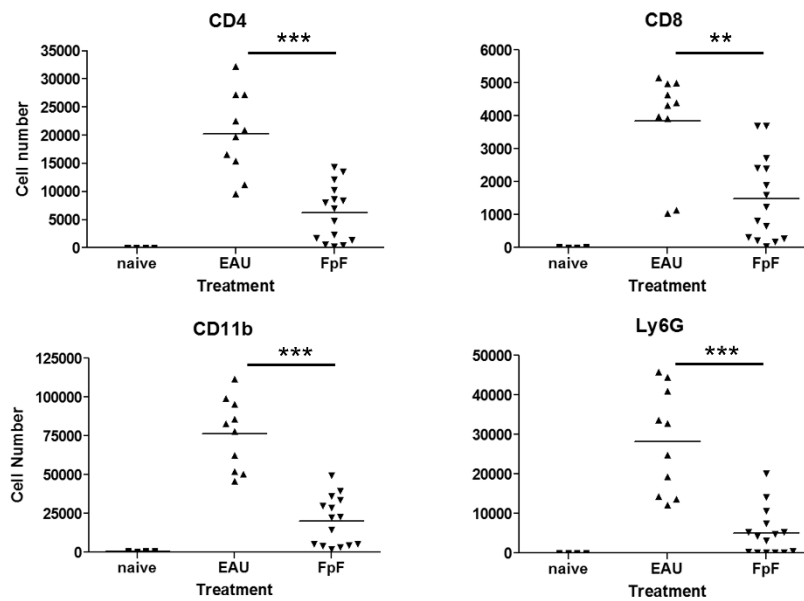
Graphs detailing the Surface Plasmon Resonance (SPR) binding sensograms, confirming that infliximab (**A**) and  $\text{FpF}_{\text{infliximab}}$  (**B**) can both bind to human  $\text{TNF-}\alpha$  in a concentration-dependent manner. The NTA chip was functionalized with Ni solution first and then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



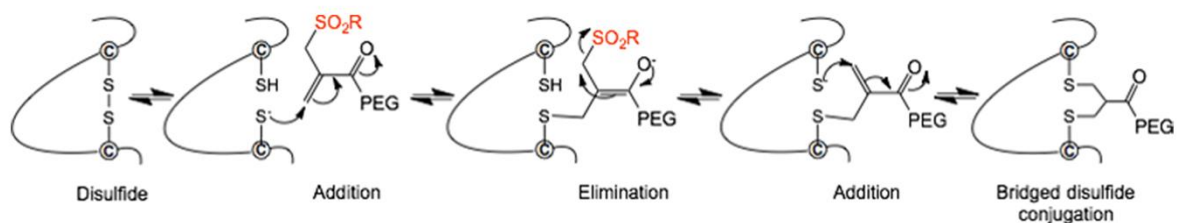
b



**Figure S2: Analysis of retinal infiltrate following infliximab and FpF<sub>infiximab</sub> treatment**

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

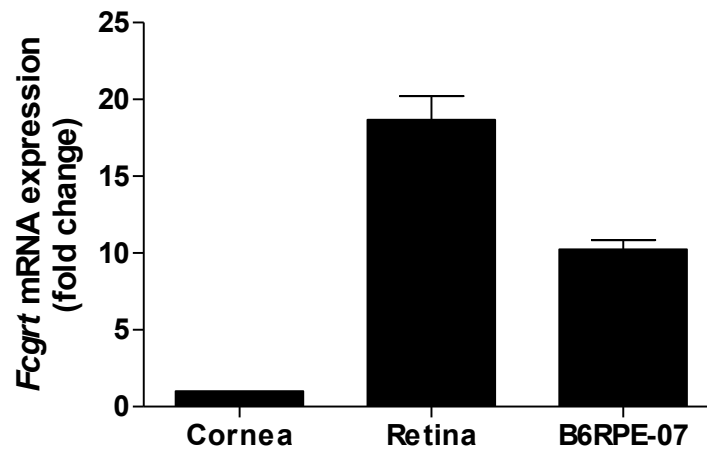
**Figure S3**



**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.