

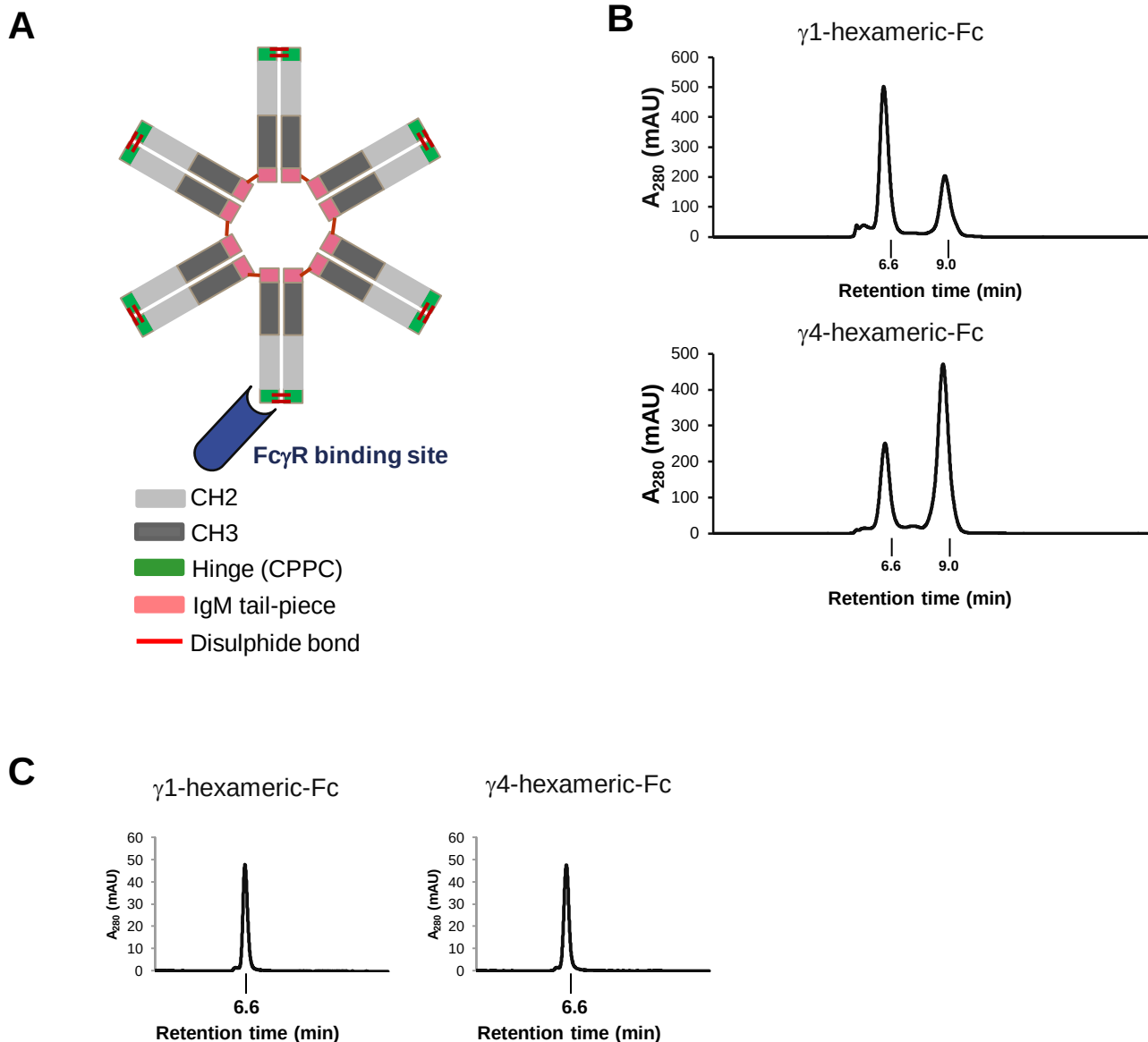
Multivalent Fc γ -receptor engagement by a hexameric Fc-fusion protein triggers Fc γ -receptor internalisation and modulation of Fc γ -receptor functions

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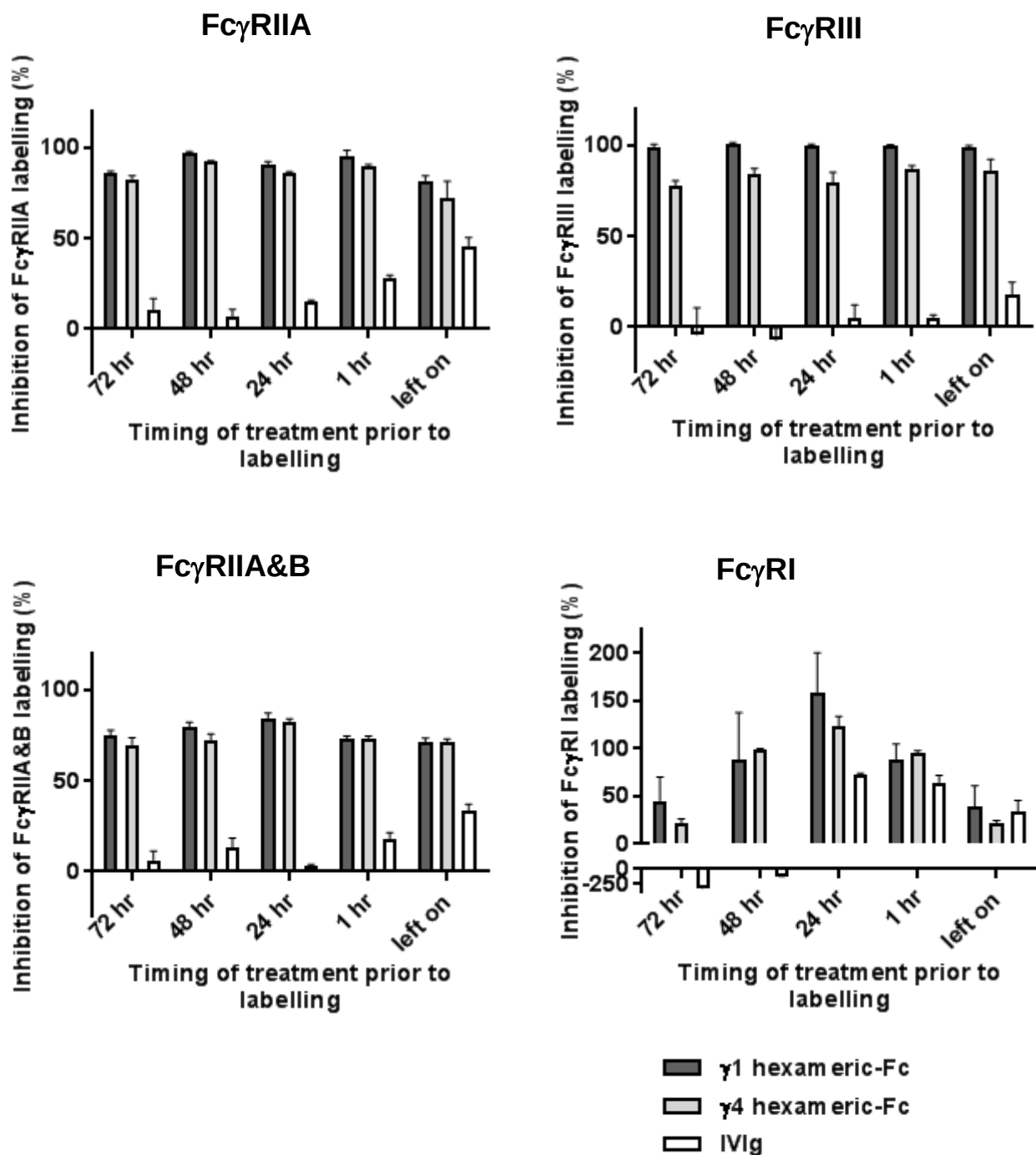
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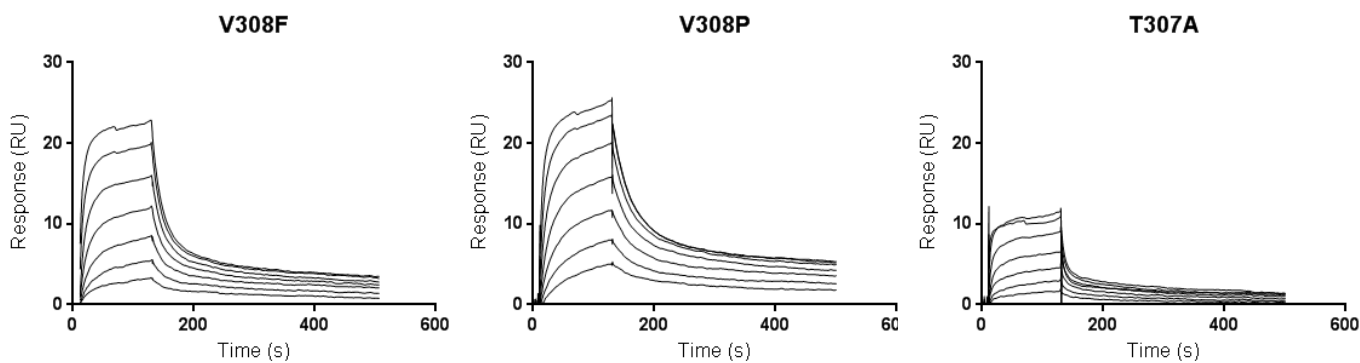
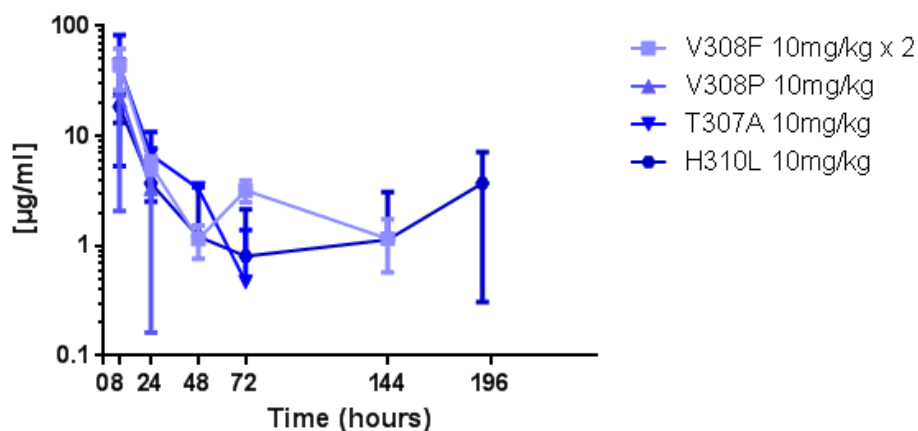
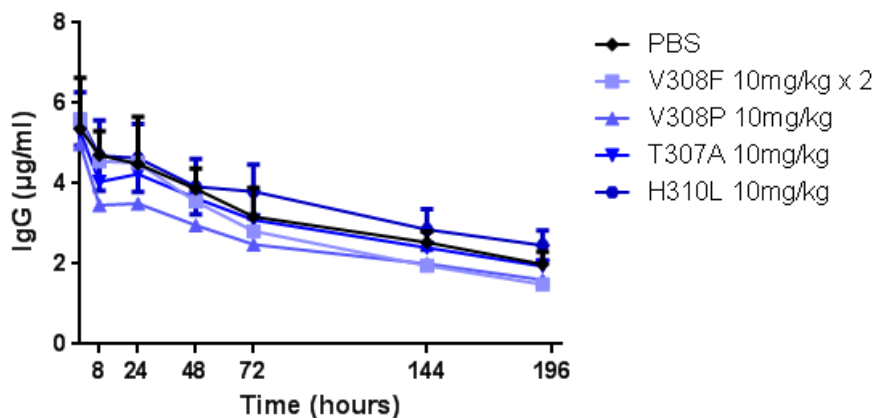
Supplementary Figure 1 – Generation of hexameric-Fc

A Schematic showing the design of hexameric-Fc. **B** Representative size exclusion chromatogram showing separation between hexamer and monomer post protein A purification. For γ1-hexameric-Fc, hexamer and monomer levels were observed around 70% and 30% respectively. For γ4-hexameric-Fc, typically 40% hexamer and 60% monomer was observed. **C** Representative size exclusion chromatograms of hexameric-Fc post gel filtration. All traces show pure hexamer fractions of at least 98%.



Supplementary Figure 2 – Prolonged disruption of FcγR surface labelling

Macrophages were incubated with hexameric-Fcs or IVIg at 75µg/ml for 1 hour. Cells were then washed and incubated for the indicated period. Cells were then labelled with fluorescently-conjugated antibodies against the indicated Fcγ-receptors. Data shows means from three donors ± SEM.

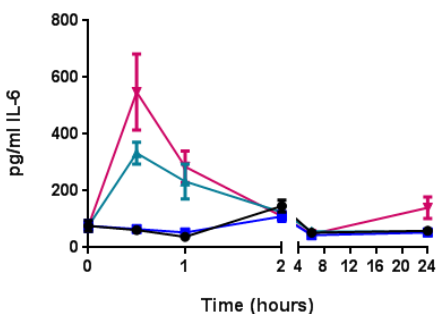
A**B****PK****C****IgG**

Supplementary Figure 3 – Effects of altering FcRn binding of hexameric-Fc

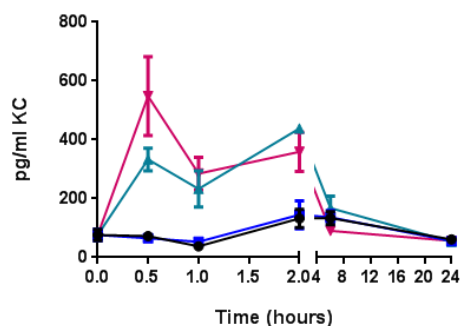
A Representative sensorgrams showing binding of hexameric-Fc variants binding to immobilised FcRn. Hexameric-Fc was titrated in a two-fold dilution series from 2.5 μ M to 39 nM. **B** PK measurements in mice dosed with the indicated hexameric-Fcs. **C** Measurement of human IgG levels in mice dosed with indicated hexameric-Fcs.

● No treatment
 ■ IVIg
 ▲ $\gamma 1$ hexameric-Fc
 ▼ $\gamma 4$ hexameric-Fc

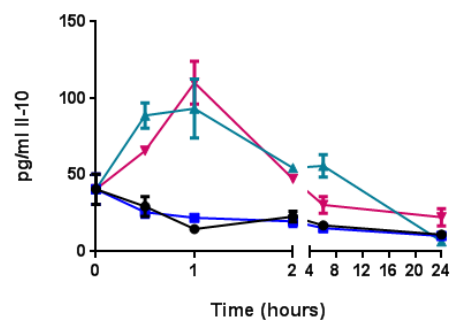
IL-6



KC



IL-10



Supplementary Figure 4 – Effects of hexameric-Fc on cytokine release in mice

To assess the effect of hexameric-Fc on cytokine release, 10mg/kg hexameric-Fc was administered to mice IV. At the timepoints indicated, whole blood samples were taken and cytokine release measured by Meso Scale Discovery electrochemiluminescence assay. n = 5 mice per group.