Supplementary material:

Functional characterization of a novel tick CC chemokine binding protein identifies a transportable CCL8 binding domain

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Contents:

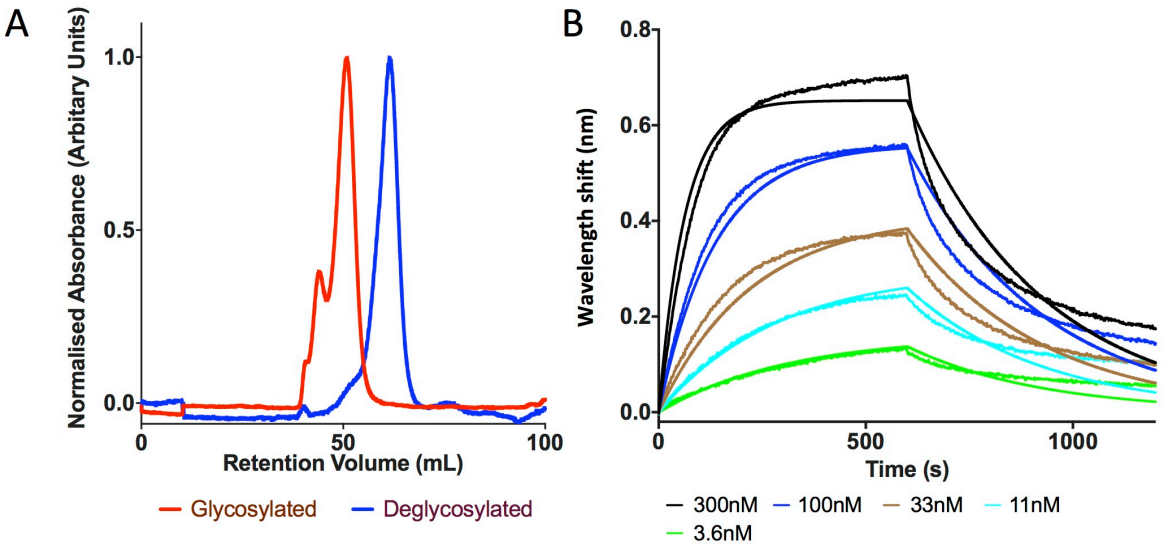

Fig. S1 – Characterisation of partially deglycosylated N-terminally tagged P672. **A**. Size exclusion chromatography traces of glycosylated P672 (red) and partially deglycosylated P672 (blue) using PNGaseF and EndoF1. **B.** Biolayer interferometry sensorgram of P672 (partially deglycosylated) binding to CCL8. Plots display wavelength shift (Y-axis, nm) versus time (X-axis, seconds). Solid lines indicate collected data, dashed lines indicate fitted data. K_d (M) = 7.25E-08, target residence time = 3.8 minutes.

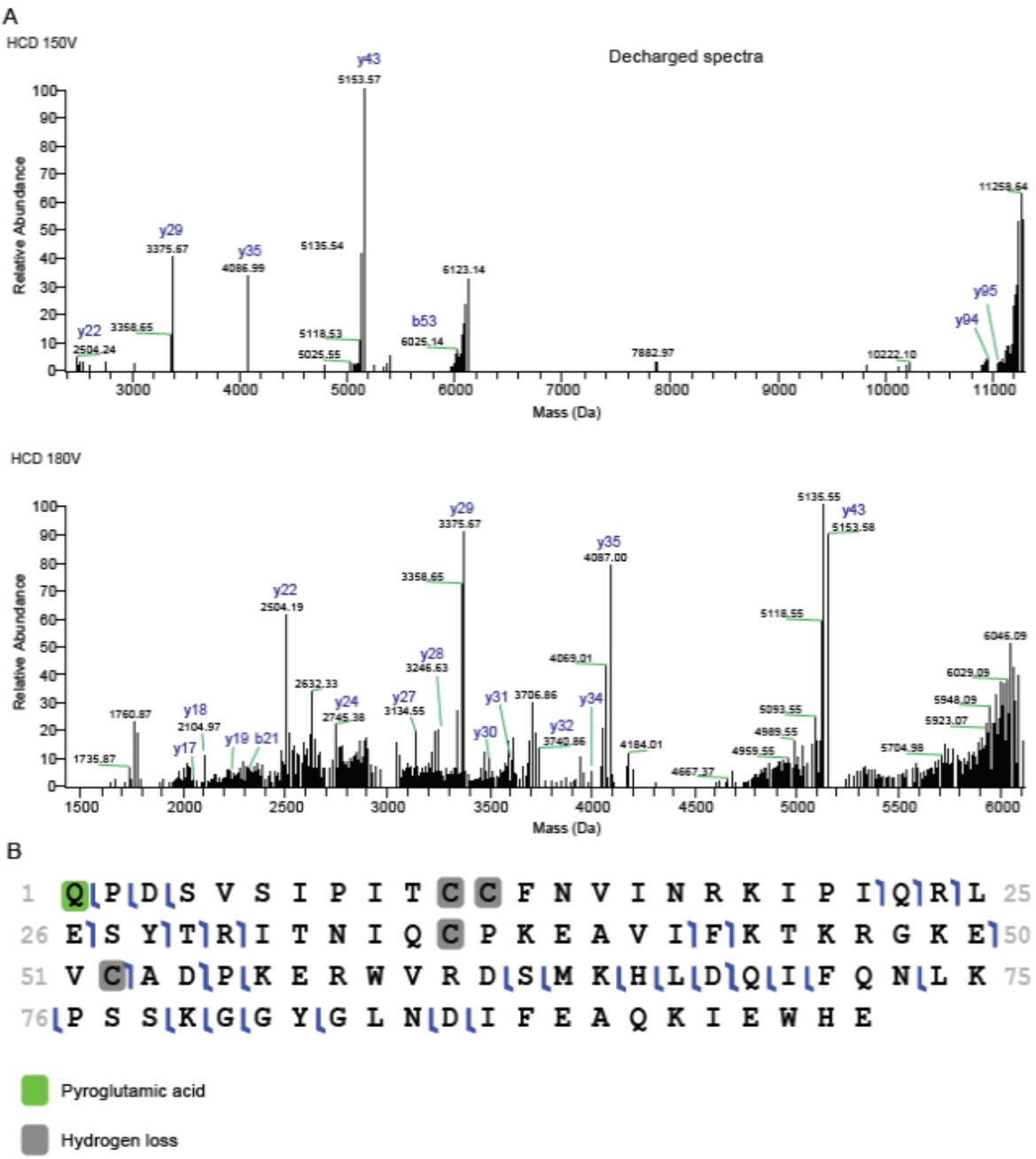

Fig. S2 - CCL8 top-down MS/MS HCD fragmentation spectra and annotated sequence. CCL8 monomer was characterized by selecting precursor ions (isolation window of 5 m/z) and fragmenting them using higher collision induced dissociation (HCD). **A.** Decharged monoisotopic spectra obtained for *m/z* 2821 (z=5) when fragmented with 150 and 180 V. **B.** Annotated sequence fragmentation pattern showing a 33% residue cleavage. The protein sequence included the presence of 2 disulfide bonds and the formation of pyroglutamic acid at the N-terminal glutamine during ionization (monoisotopic mass of 11276.76 Da).

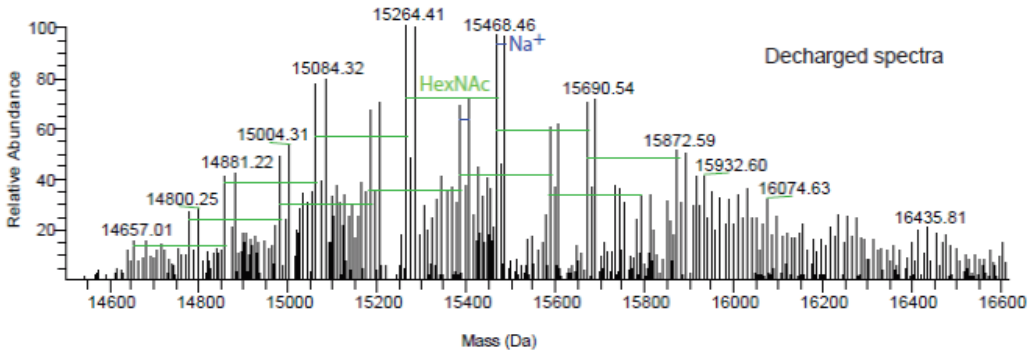

Fig. S3 - Native MS decharged monoisotopic spectra of partially deglycosylated P672. The decharged spectra shows monoisotopic masses of several P672-RHIPU modified forms. Mass differences suggesting the presence of sodium adducts and 5 to 6 HexNAc mass additions (+203 Da) were observed. The presence of partial glycosylations was supported by shotgun proteomics analyses.

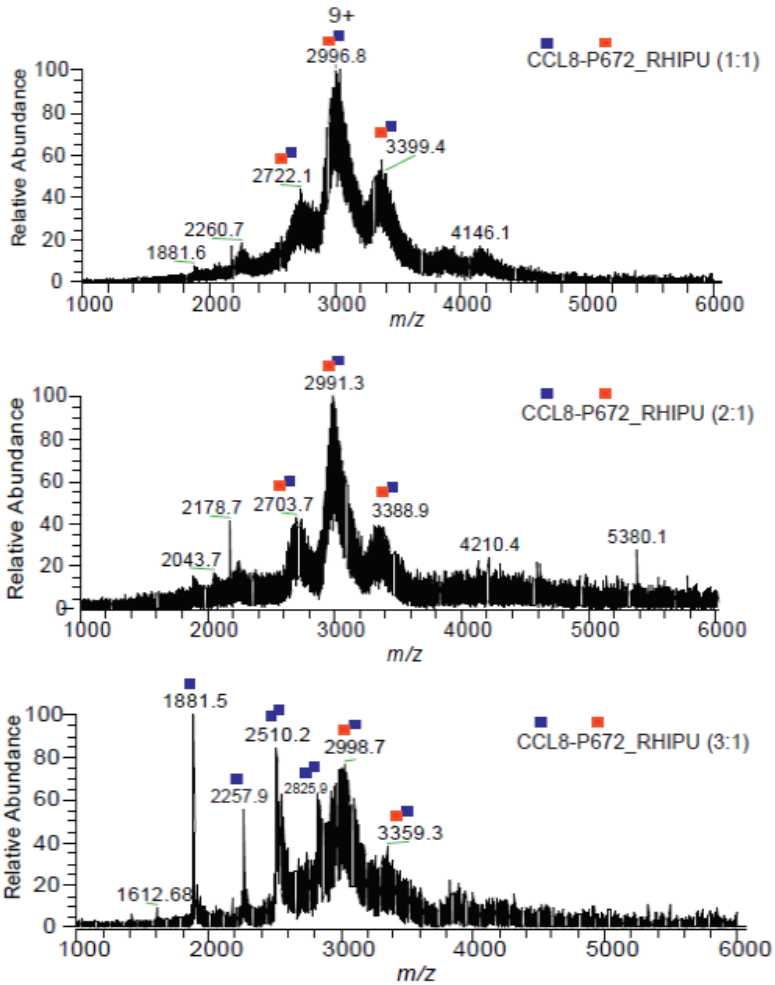

Fig. S4 - Native MS of CCL8:P672 complex with increasing amounts of CCL8

The P672:CCL8 complex was formed at ratios 1:1, 2:1 and 3:1 as indicated in top, middle and bottom panels. At 1:1 and 2:1 ratios the CCL8:P672 heterodimer is the prominent component observed and, as the ratio increase to 3:1, CCL8 monomer and homodimer are also observed.

Peaks are indicated as CCL8 (blue squares) or P672 (red squares). Monomers are indicated as a single square, and homo or heterodimers as double squares. Y-axis indicates relative abundance, X-axis indicates *m/z* (mass/charge) ratio.

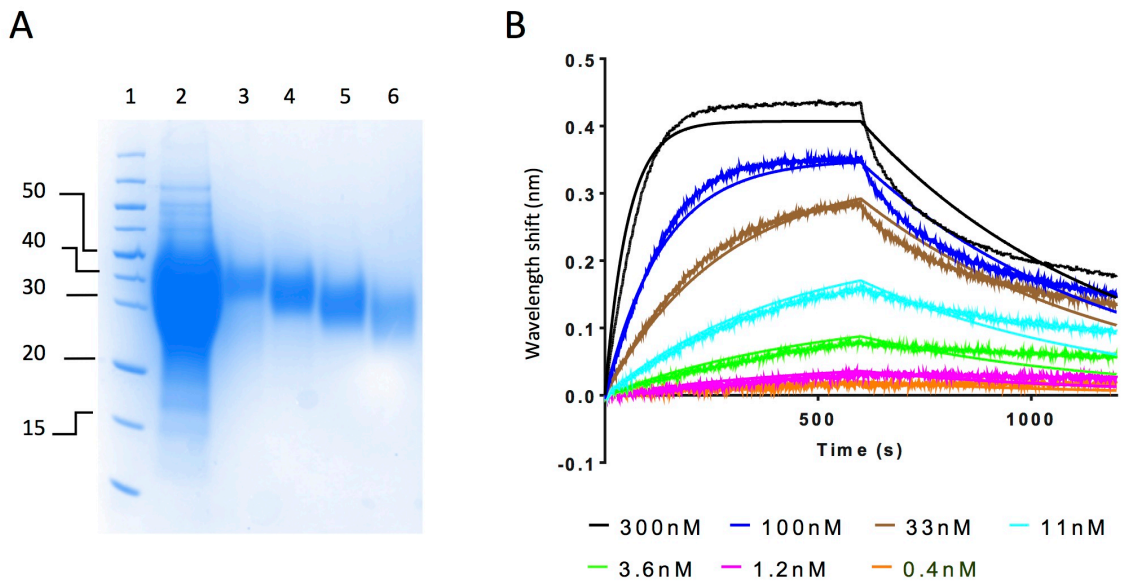

Fig. S5 - Expression and characterization of P672(17_104)

A. Expression and purification of C-terminally tagged P672(17_104). SDS-PAGE gel showing molecular weight marker (lane 1), Ni-NTA captured protein (lane 2) and size exclusion chromatography fractions (lanes 3-6) of C-terminally tagged P672(17-104) stained with colloidal Coomassie blue.

B. Biolayer interferometry sensorgram of P672(17_104) binding to CCL8. Plots display wavelength shift (Y-axis, nm) versus time (X-axis, seconds). Solid lines indicate collected data, dashed lines indicate fitted data. K_d (M) = 3.04E-08, target residence time = 6.7 minutes.

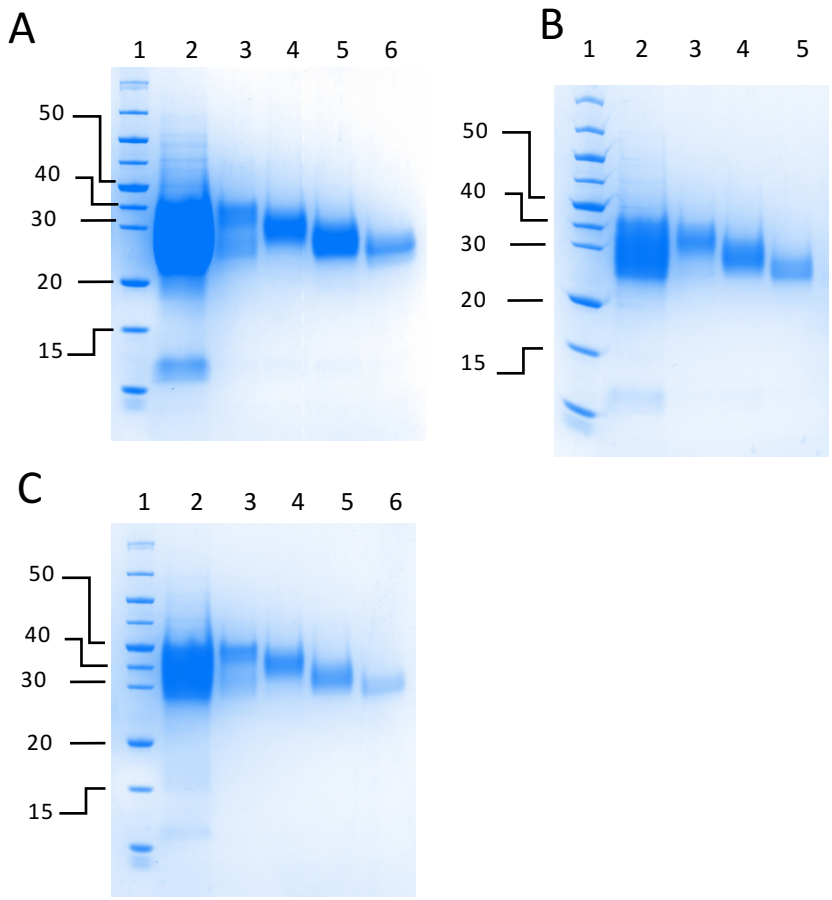

Fig. S6 - Expression and purification of hybrid evasin proteins. A. SDS-PAGE gel of molecular weight marker (lane 1), Ni-NTA captured protein (lane 2) and size exclusion chromatography fractions (lanes 3-6) of P672(1_44): EVA1(29_94). **B.** SDS-PAGE gel of molecular weight marker (lane 1), Ni-NTA captured protein (lane 2) and size exclusion chromatography fractions (lanes 3-5) of P672(1_66): EVA1(47_94). **C.** SDS-PAGE gel of molecular weight marker (lane 1), Ni-NTA captured protein (lane 2) and size exclusion chromatography fractions (lanes 3-6) of P672(1_92): EVA1(74_94).