Supplementary Material

Front. Cell. Infect. Microbiol., 25 June 2019 | https://doi.org/10.3389/fcimb.2019.00210

Residue Mutations in Murine Herpesvirus 68 Immunomodulatory Protein M3 Reveal Specific Modulation of Chemokine Binding

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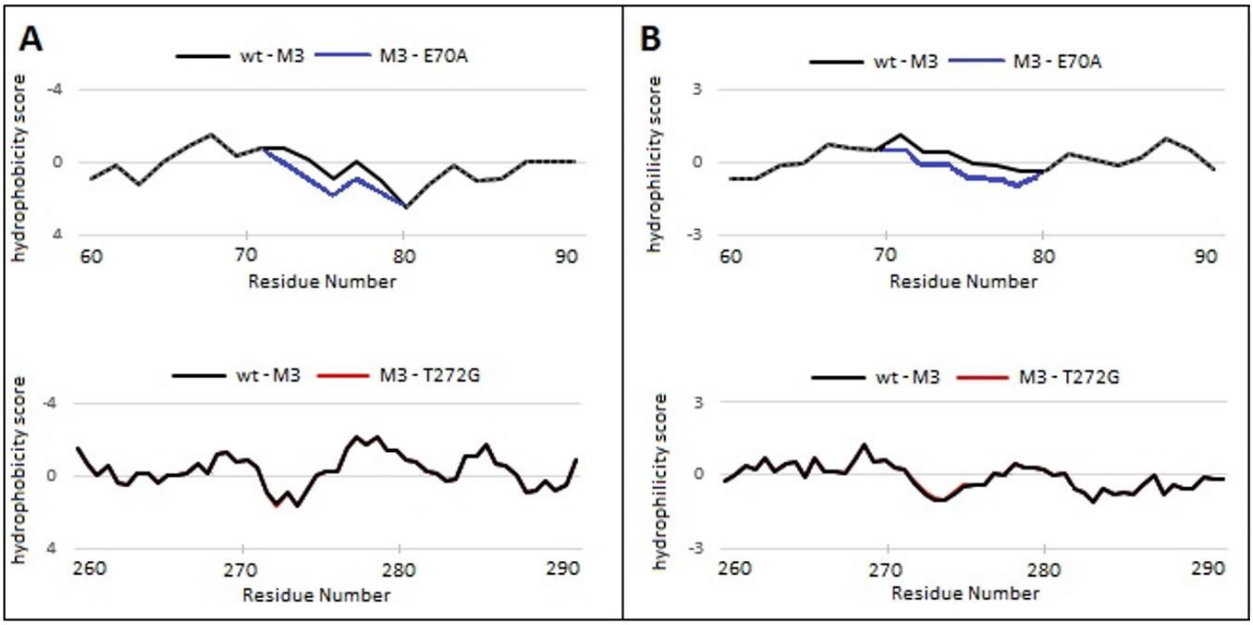

Figure S1. Hydrophobicity/hydrophilicity profile in the vicinity of the mutation sites. The mutation of M3 residues E70 to A70 and T272 to G272 resulted in changes to the predicted hydrophobicity (A) and hydrophilicity (B) of the protein in the area surrounding the mutations. A reduction in hydrophilicity was associated with the E70 mutation (indicated by blue).

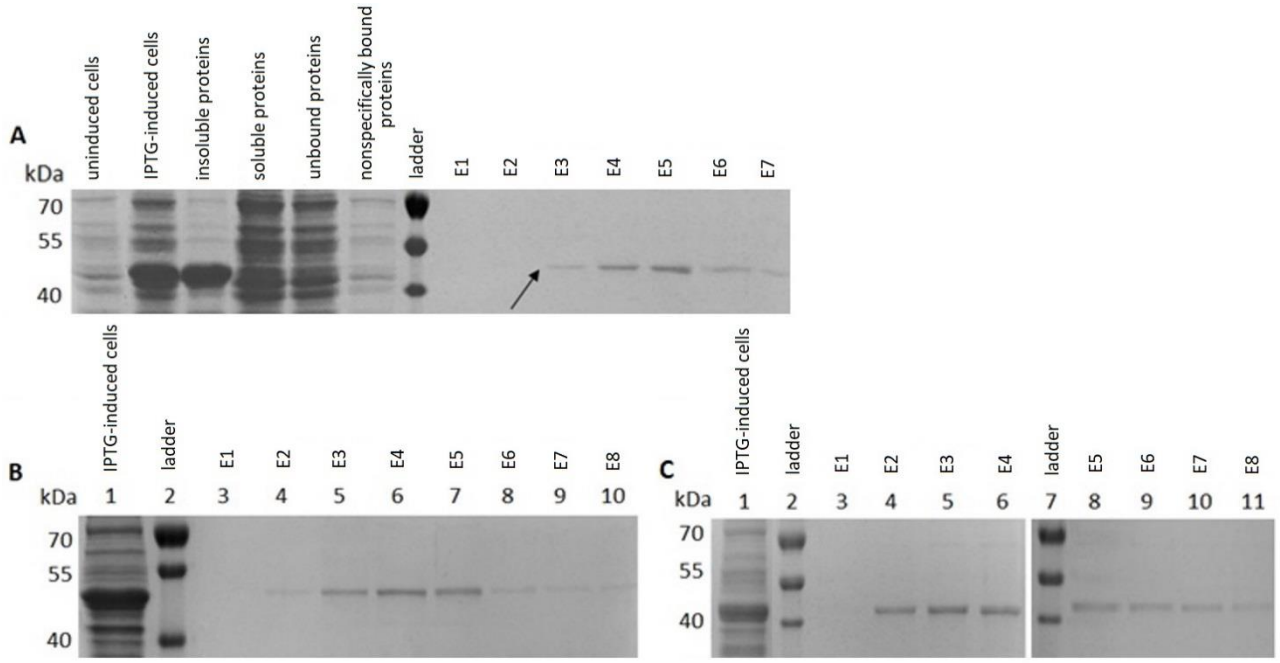

Figure S2. SDS-PAGE (12.5%) analysis of the expression in *E. coli* Rosetta-gami 2 (DE3) cells and purification by IMAC of wtM3 and its mutants. (A) wtM3; (B) M3-E70A; (C) M3-T272G. E indicates fractions eluted from the affinity column, the arrow indicates wtM3 size (44 kDa).

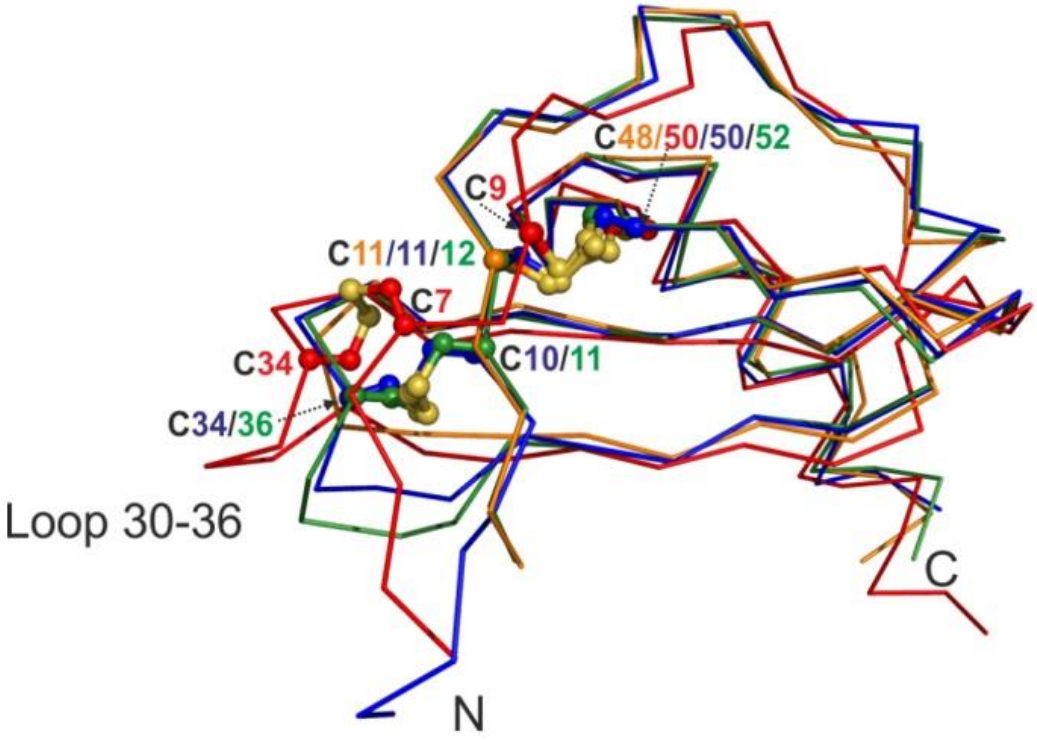

Figure S3. Superposition of Cα traces of chemokines CCL5 (blue), **CCL2** (green), **CXCL8** (red) **and XCL1** (orange). The greatest differences are seen at the N-terminus and in the loop containing residues 30–36 (CCL5 numbering). Details of the superposition are given in Table 1. Cysteines forming disulfide bonds are indicated as balls and sticks and numbered; Sγ atoms are coloured in yellow, Cα and Cβ are colored according to the chemokine.

Table S1. Forward and reverse primers used to prepare the M3 mutant constructs.

Mutation	Forward primer (5´-3´)	Reverse primer (3´-5´)
E70A	GAAACTGAAGTGACTG**C**GTGTGCTGGCATTCTG	CAGAATGCCAGCACAC**G**CAGTCACTTCAGTTTC
T272G	GATGTCTCTGCTC**GG**TCCATTTGGAGGCCC	GGGCCTCCAAATGGA**CC**GAGCAGAGACATC

Table S2. Predicted properties of each M3 protein.

Protein	wt-M3	M3-E70A	M3-T272G
Molecular Weight (Da)*	44 917.17	44 859.13	44 873.12
Theoretical pI*	4.93	4.97	4.93
Instability index*	54.33	53.86	54.33
Solubility index$	86%	93.9%	95.8%
Location of mutation in secondary structure	-	α-helix	loop

Note:

* Parameters were calculated by ProtParam algorithm (Gasteiger *et al.*, 2005).

$ Parameters were calculated according Diaz *et al.* (2009).

Gasteiger E, Hoogland C, Gattiker A, Duvaud S, Wilkins MR, Appel RD & Bairoch A (2005) Protein Identification and Analysis Tools on the ExPASy Server. In *John M. Walker (ed): The Proteomics Handbook, Humana Press,* pp. 571-607.

Diaz A, Tomba E, Lennarson R, Richard R, Bagahewicz M & Harrison RG (2009) Prediction of Protein Solubility in *Escherichia coli* Using Logistic Regression. *Biotechnol Bioeng* 105, 374-383.
