

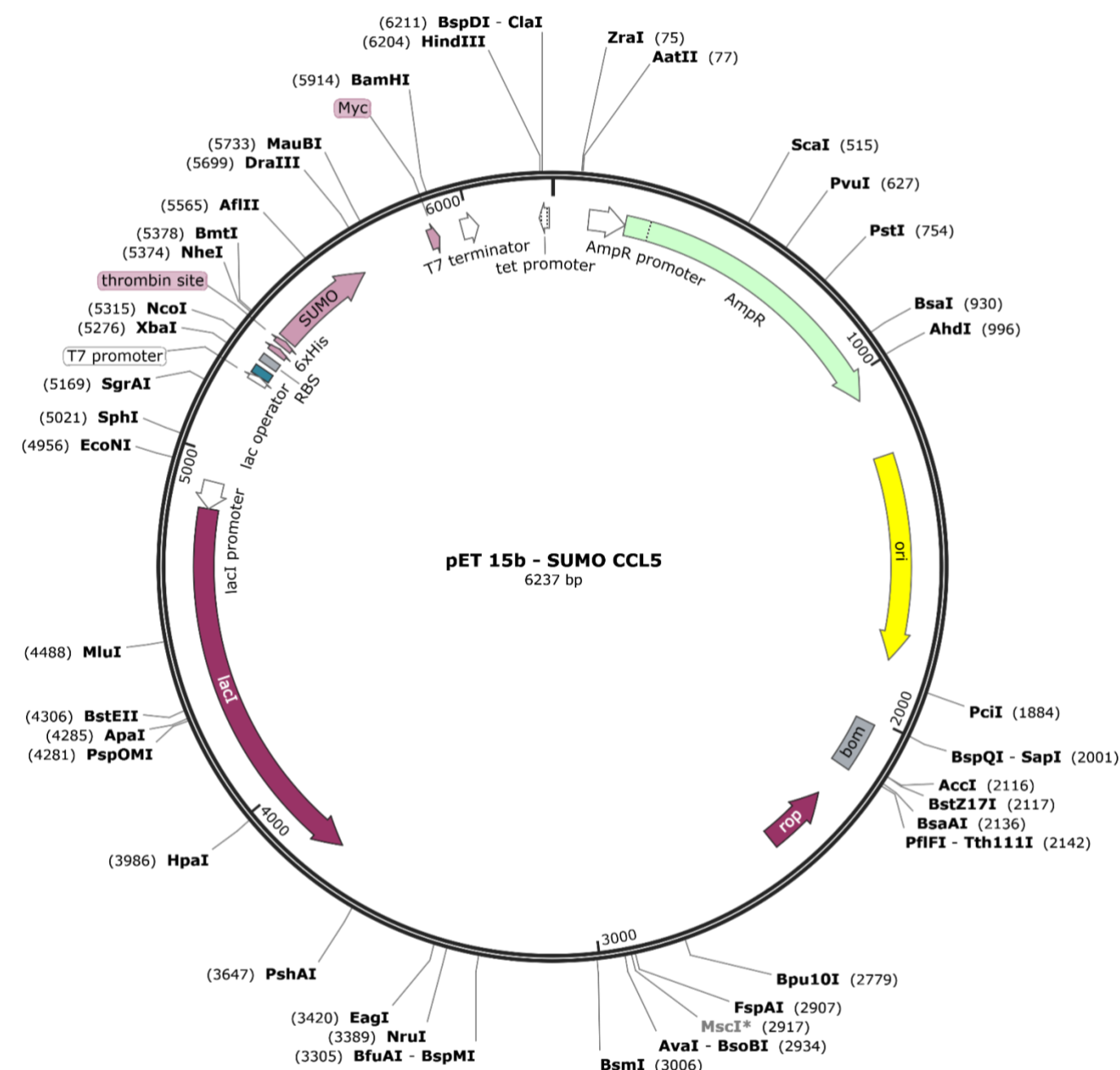
Supplementary Information

New insight into a high yielding and efficient method for the production of recombinant human CCL5

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Supporting Information: Construct design, mass spectrometry analysis of purified IH-CCL5, IH-CCL5 stimulated CHO-CCR5 calcium flux (plate reader method) and full-length blot for CCR5 detection with band shift after IH-CCL5 stimulation.



SUMO-CCL5 protein sequence

MGSSHHHHHHGSGLVPRGSASMSDSEVNQEAKPEVKPEVKPETHINLKVSDGSSEIFFKIKKTTPLRRLMEAFKR
QKGEMDSLRLFLYDGIRIQADQTPEDLDMEDNDIIEAHREQIGGSPYSSDTPCCFAYIARPLPRAHIKEYFYTSGKCS
NPAVVFVTRKNRQVCANPEKKWVREYINSLEMS*

Figure Supplementary 1. SUMO-CCL5 plasmid map

SUMO-CCL5 gene was purchased codon optimised from GenScript Biotech, subcloned into pET15b vector with flanked NcoI and BamHI restriction sites at the N and T termini. Plasmid map generated using SnapGene.

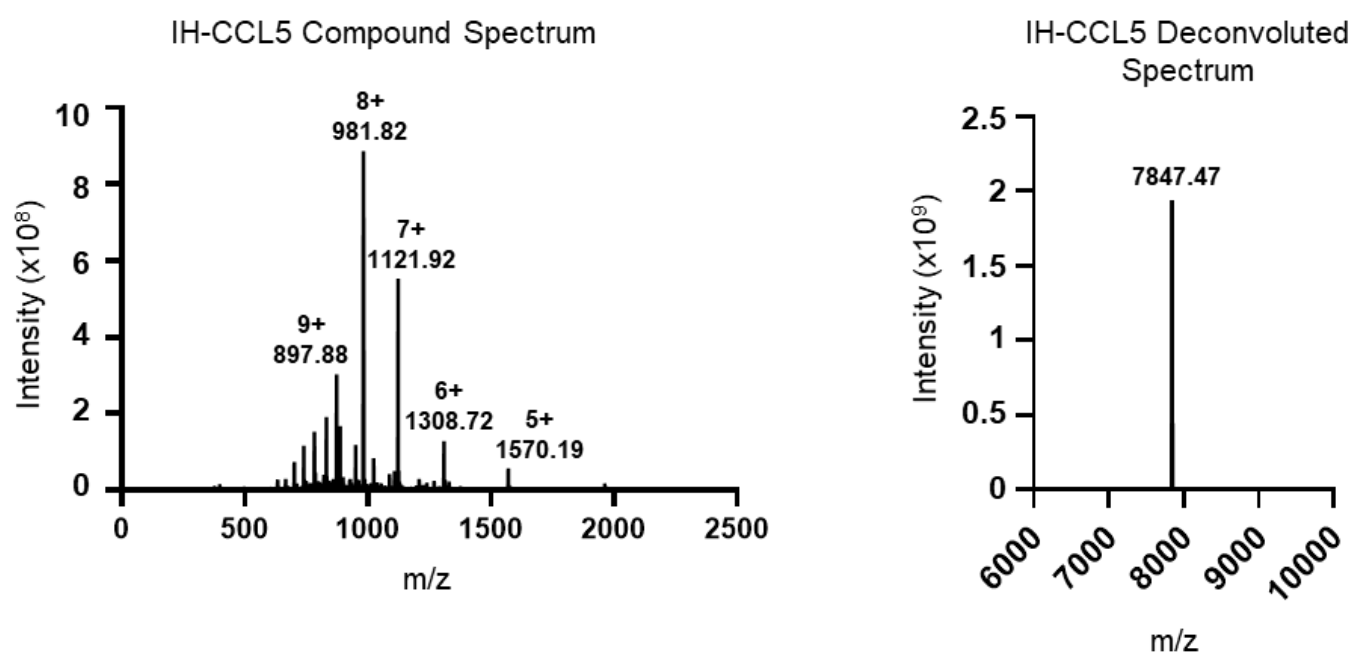


Figure Supplementary 2. Positive ESI mass spectrum of purified CCL5

Protein samples were prepared in 1 : 1 water : acetonitrile + 1% formic acid (v/v/v) and run on positive ESI mode. Charge ladder for IH-CCL5 showing a mass of 7847.47 Da (required mass 7851.01 Da) indicating the formation of two disulphide bonds.

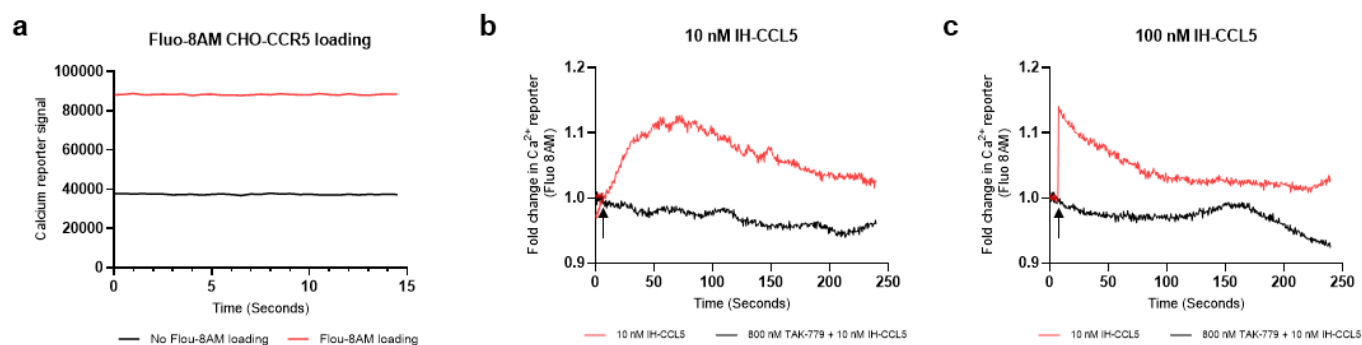


Figure Supplementary 3. Plate read based calcium assay using IH-CCL5

Cells were loaded with Fluo-8AM (calcium reporter dye) and stimulated with either 10 nM or 100 nM chemokine +/- TAK-779 (800 nM) and calcium release was measured using Clariostar Plus (BMG Labtech) using excitation 490 nm and emission 525 nm. Arrows on graph shows time at which IH-CCL5 was added.

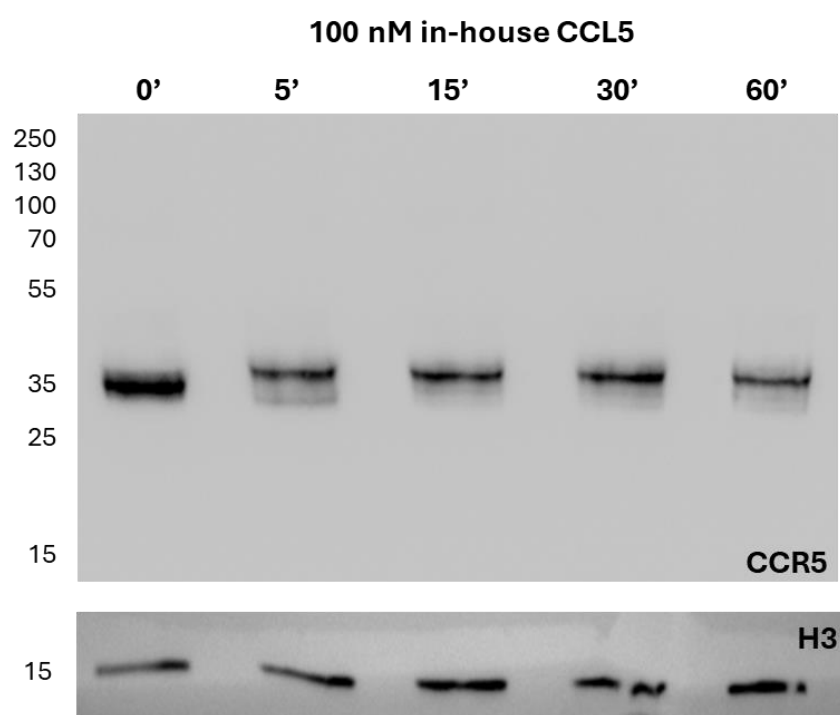


Figure Supplementary 4. Full-length blot for phosphorylation of CCR5 after IH-CCL5 stimulation

CHO-CCR5 immunoblot (full-length) after 100 nM IH-CCL5 stimulation for up to 60 minutes. Anti-CCR5 mAb MC5 (1 µg/mL) was used to detect CCR5 and histone-3 as a loading control.