Supplementary Material for:

Evaluation and Extension of the Two-site, Two-step Model for Binding and Activation of Chemokine Receptor CCR1

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Supplementary Table

Table S1. Affinity for binding of CC chemokines to Fl-R2D determined using direct fluorescence anisotropy binding assay.

Supplementary Figures

Figure S1. Binding of CC chemokines to Fl-R2D.

Figure S2. Correlations between chemokine binding affinities of CCR1 (sulfo)peptides.

Figure S3. Binding of CC chemokines to non-sulfated CCR1.

Table S1. Affinity for binding of CC chemokines to Fl-R2D determined using direct fluorescence anisotropy binding assay. Binding constants are reported as pK_d values (-$\log_{10}$ of the K_d; in M) ± S.E. The corresponding K_d values (in nM) are in parentheses. CCL7 was used as a reference for statistical analysis. Data represent mean ± SEM from at least three independent experiments performed in duplicate.

Chemokine	**pK_d**
CCL2	6.73 ± 0.04 (186)***
CCL5	6.89 ± 0.06 (129)***
CCL7	7.67 ± 0.04 (21.3)
CCL8	7.09 ± 0.03 (82.3)***
CCL15	5.90 ± 0.05 (1245)***
CCL26	7.61 ± 0.05 (24.5)

Figure S1. Binding of CC chemokines to Fl-R2D. Binding was measured using a direct fluorescence anisotropy assay. The concentration of Fl-R2D was 10 nM. Data points represent mean ± SEM of at least three independent experiments performed in duplicate.

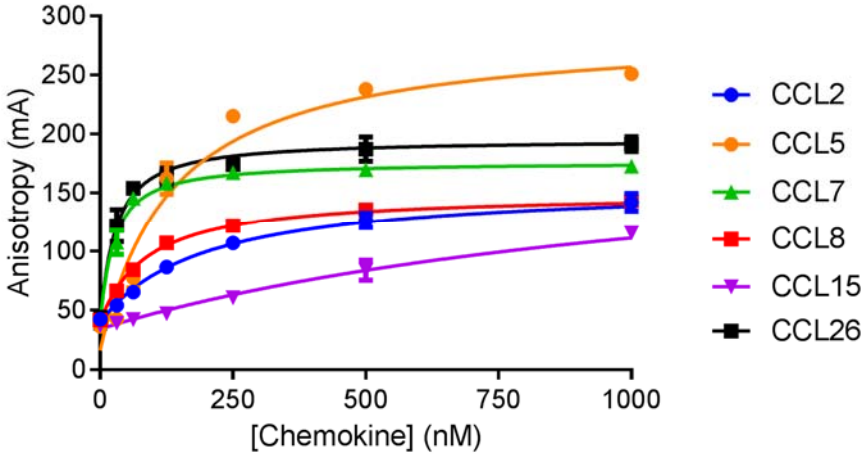

Figure S2. Correlations between chemokine binding affinities of CCR1 (sulfo)peptides. Each panel shows the pK_d values for binding of two CCR1 (sulfo)peptides to each of several chemokines: **(A)** R1A versus R1B, **(B)** R1A versus R1C, **(C)** R1A versus R1D, **(D)** R1B versus R1C, **(E)** R1B versus R1D and **(F)** R1C versus R1D. The linear correlation coefficient (r^2) is given in the bottom right corner of each graph. Data points represent mean ± SEM of at least three independent experiments.

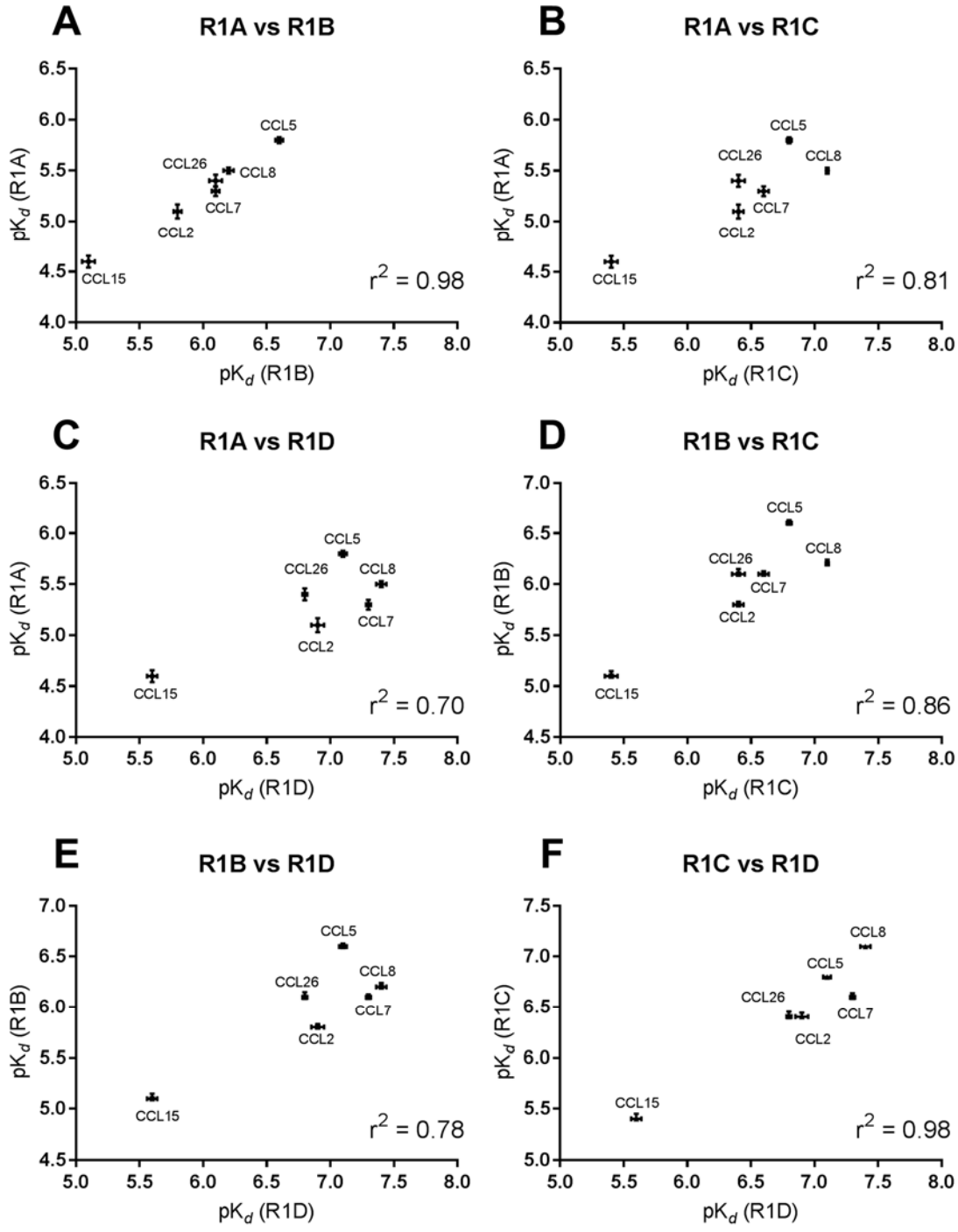

Figure S3. Chlorate treatment effectively blocks sulfation. Chemokine receptors bearing N-terminal cMyc and FLAG epitope tags were expressed in Flp-In T-Rex HEK 293 cells. Sulfation of the tyrosine residue in the FLAG epitope sequence (DYKDDDDK) prevents detection by the M2 anti-FLAG monoclonal antibody, whereas cMyc cannot be sulfated. Receptor expression was induced 24 hours prior to each experiment by addition of 10 μg/mL tet to cell media. To inhibit sulfation, cells were incubated in media containing 30 mM sodium chlorate for 48 hours prior to each experiment. ELISA was performed using tag-specific mouse primary antibody, either anti-cmyc (9E10, Sigma) or anti-FLAG (M2, CSIRO) and anti-mouse-IgG-horseradish peroxidase secondary antibody (Sigma-Aldrich). Shown are the levels of epitope tags detected for cMyc-FLAG CCR2 (top panel) and cMyc-FLAG CCR3 (bottom panel) for cells grown in the absence (gray bars) or presence (colored bars) or chlorate. Data bars represent means ± SEM of at three independent experiments performed in triplicate. *$p<0.05$, paired t-test. Cell growth in the presence of 30 mM chlorate did not affect the level of cMyc tag detected, indicating that the receptor expression levels were unchanged. In contrast, the FLAG tag was not detectable for receptors expressed in the absence of chlorate, consistent with the presence of tyrosine sulfation, but was robustly detected for the chlorate-treated cells, indicating effective inhibition of sulfation.