# Science Advances

# Supplementary Materials for

## Activation of the human chemokine receptor CX3CR1 regulated by cholesterol

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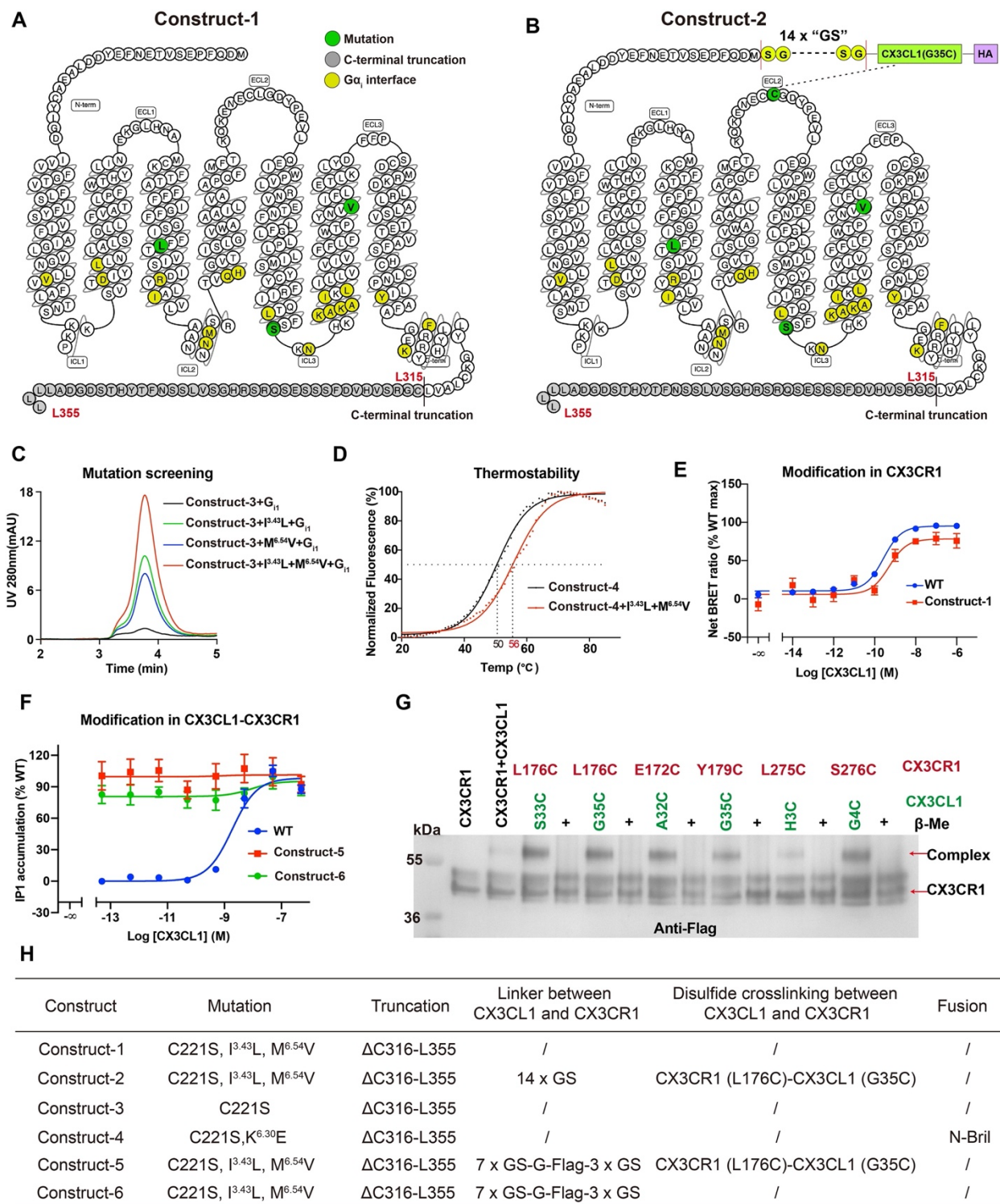**This PDF file includes:**

Figs. S1 to S6
Tables S1 to S5

**Fig. S1. Optimization and characterization of CX3CR1 constructs.** (**A** and **B**) Construct information of CX3CR1 used for CX3CR1-$G_{i1}$ (A) and CX3CL1-CX3CR1-$G_{i1}$ (B) structure determination. The mutated, truncated and involved in G protein interface residues are shown as

green, dark gray and dark yellow solid circles, respectively. (**C**) Analytical size-exclusion chromatography (aSEC) result of purified CX3CR1-$G_{i1}$ complex with different CX3CR1 mutants. (**D**) Thermostability assay of CX3CR1 mutants. (**E**) CX3CL1-induced G protein activation of the WT and modified CX3CR1. At least three independent experiments (n) were performed in triplicate, data are shown as mean ± SEM. See table S3 for detailed statistical evaluation. (**F**) CX3CL1-induced IP accumulation of the WT CX3CR1 and the fused form of CX3CL1-CX3CR1 with or without disulfide crosslinking mutations [CX3CL1 (G35C)-CX3CR1 (L176C)]. At least three independent experiments (n) were performed in triplicate, data are shown as mean ± SEM. See table S2 for detailed statistical evaluation. (**G**) The result of anti-Flag western blot in disulfide bond crosslinking screening of CX3CL1 and CX3CR1. The western blot was performed with mouse anti-Flag antibody and polyclonal goat anti-mouse IgG antibody to specifically identify Flag-tagged CX3CR1. The red arrows indicate the bands of CX3CL1-CX3CR1 complex and CX3CR1. (**H**) Modifications of constructs used in structure determination and optimization. Construct 1, the CX3CR1 construct used to determine the CX3CR1-$G_{i1}$ structure; construct 2, the CX3CR1 construct used to determine the CX3CL1-CX3CR1-$G_{i1}$ structure; construct 3 and construct 4 are the CX3CR1 constructs used in construct optimization; construct 5 and construct 6 are the fused form of CX3CL1-CX3CR1 with and without disulfide crosslinking mutations respectively, and are used to validate the effect of "14 × Gly-Ser" linker and disulfide bond (CX3CL1 (G35C)-CX3CR1 (L176C)).
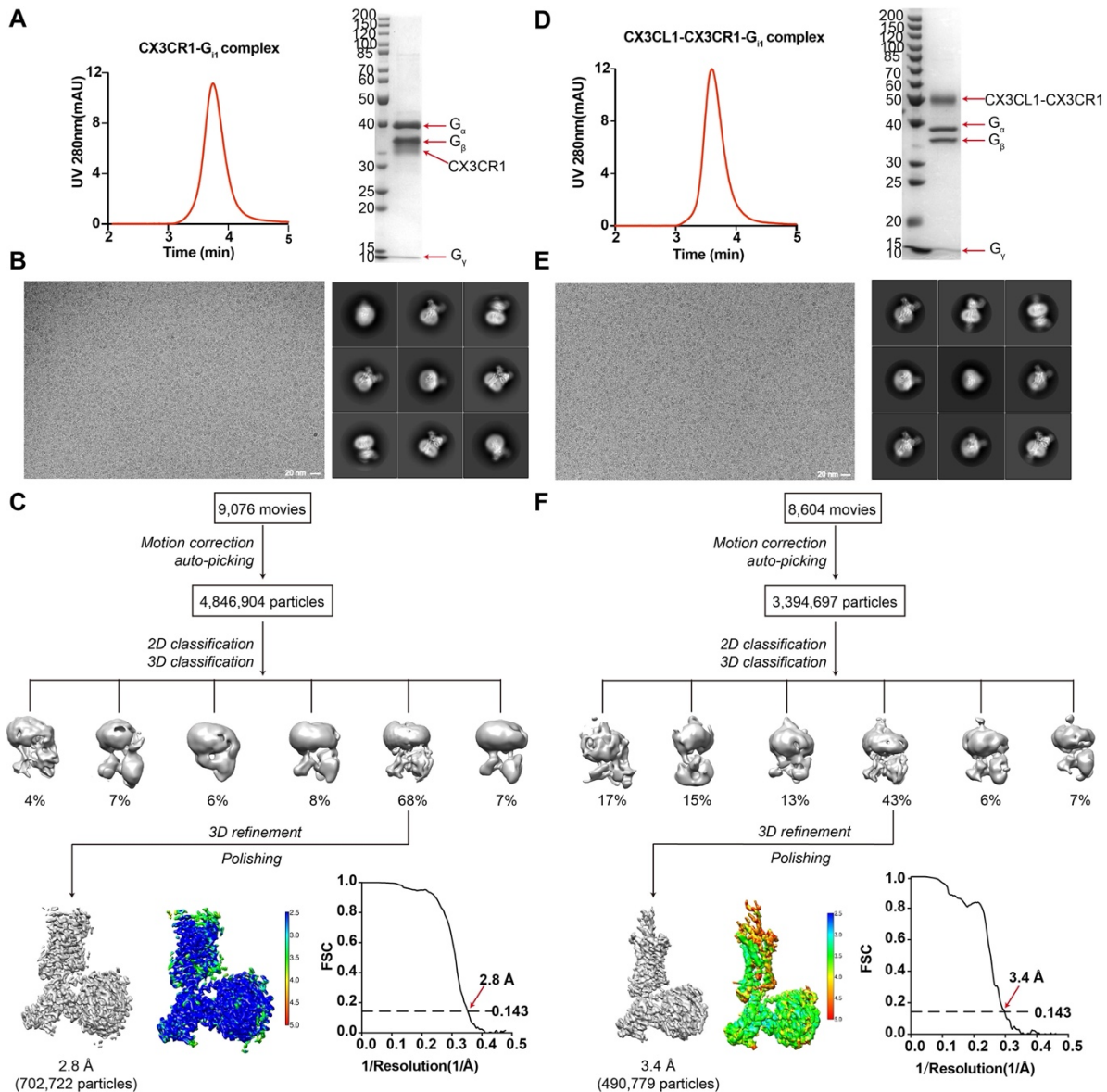
**Fig. S2. Sample preparation and cryo-EM data processing of G$_{i1}$-bound CX3CR1 complexes.**

**(A-C)** Results of CX3CR1-G$_{i1}$ complex. **(A)** aSEC and SDS-PAGE results of CX3CR1-G$_{i1}$ complex. **(B)** Representative cryo-EM image and 2D average of CX3CR1-G$_{i1}$ complex. **(C)** Workflow of cryo-EM data processing with cryo-EM map colored according to local resolution (Å). Gold-standard FSC curve showing an overall resolution at 2.8 Å (red arrow) for CX3CR1-G$_{i1}$. **(D-F)** Results of CX3CL1-CX3CR1-G$_{i1}$ complex. **(D)** aSEC and SDS-PAGE results of CX3CL1-CX3CR1-G$_{i1}$ complex. **(E)** Representative cryo-EM image and 2D average of CX3CL1-

CX3CR1-G$_{i1}$ complex. **(F)** Workflow of cryo-EM data processing with cryo-EM map colored according to local resolution (Å). Gold-standard FSC curve showing an overall resolution at 3.4 Å (red arrow) for CX3CL1-CX3CR1-G$_{i1}$.

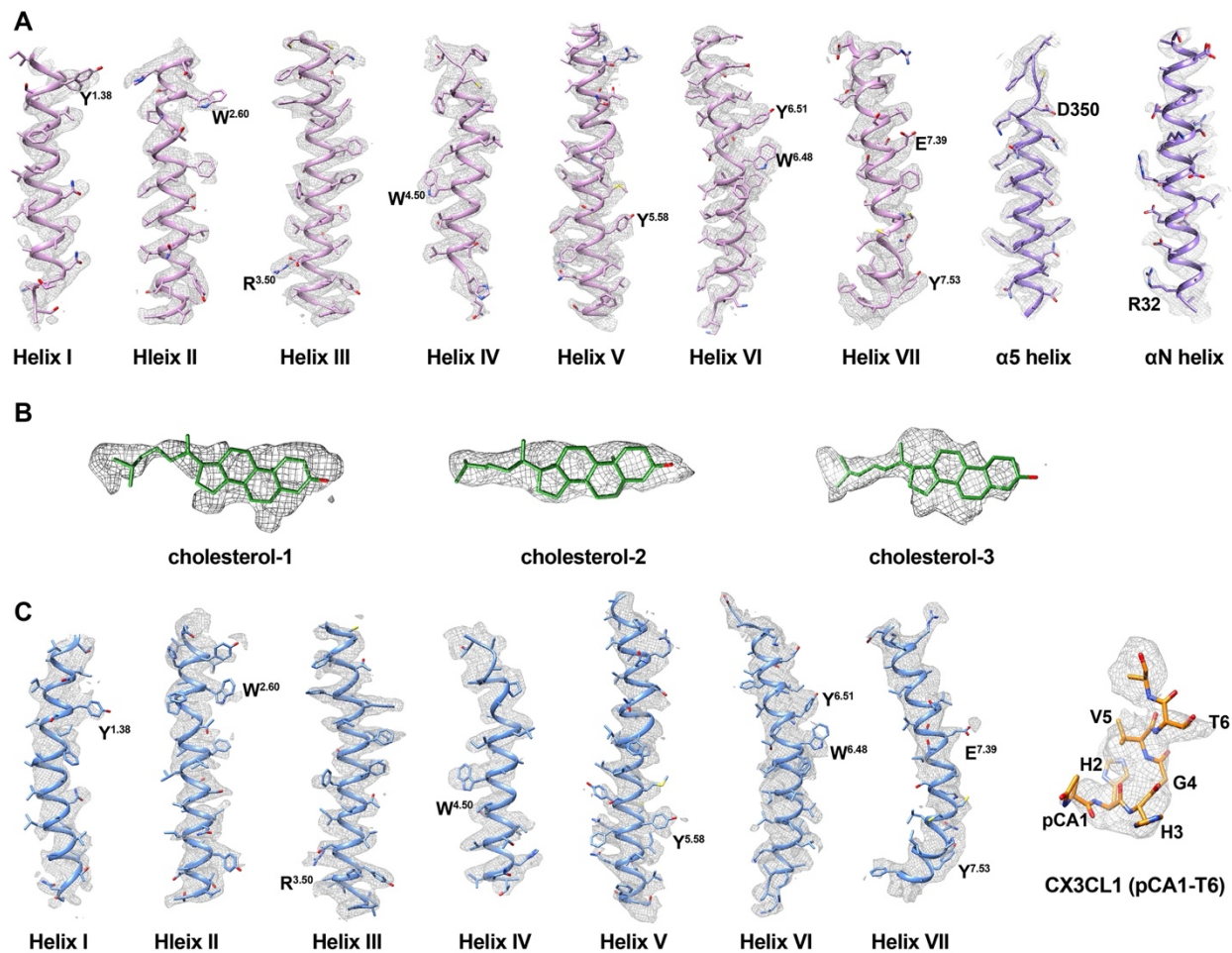**Fig. S3. Electron density maps of the CX3CR1 structures. (A)** Cryo-EM density map and model of all transmembrane helices of CX3CR1, α5- and αN-helixes of Gα$_{i1}$ in CX3CR1-G$_{i1}$ complex. **(B)** Cryo-EM density map and model of cholesterol molecules. **(C)** Cryo-EM density map and model of the CX3CL1-CX3CR1-G$_{i1}$ complex structure are displayed for all transmembrane helices, and residues (pE1-T6) of CX3CL1.
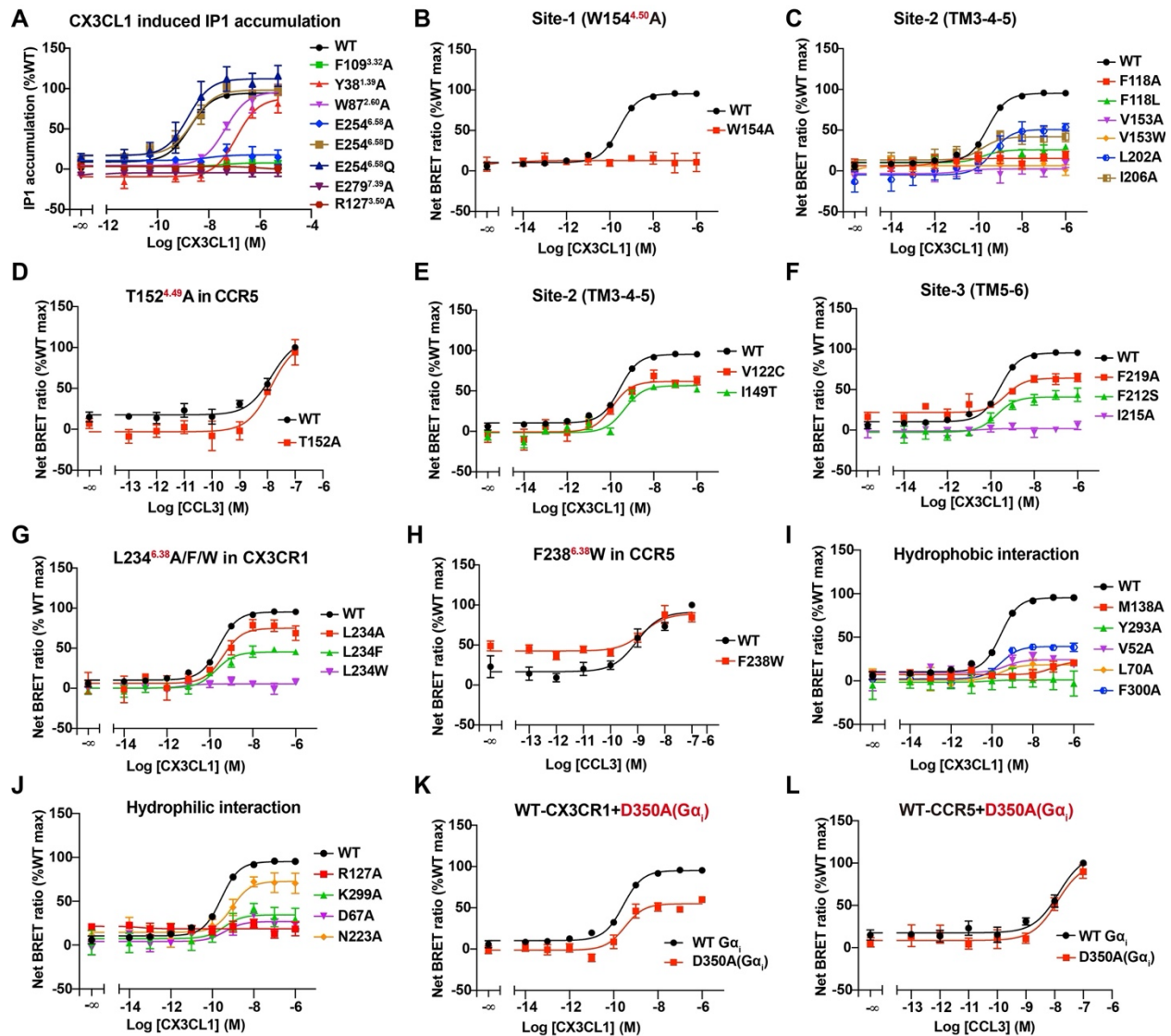
**Fig. S4. Signaling assays of CX3CR1 and CCR5. (A)** CX3CL1-induced IP accumulation of WT and mutant CX3CR1. **(B)** CX3CL1-induced G protein activation tested by TRUPATH sensor, reflecting the effects of W154$^{4.50}$A. **(C and E)** CX3CL1-induced G protein activation of WT and mutant CX3CR1 in site-2 tested by TRUPATH sensor, reflecting the effects of receptor mutants in site-2. **(D)** CCL3-induced G protein activation of WT CCR5 and T152$^{4.49}$A. **(F-G)** CX3CL1-induced G protein activation of CX3CR1 tested by TRUPATH sensor, reflecting the effects of receptor mutants in site-3. **(H)** CCL3-induced G protein activation of WT CCR5 and F238$^{6.38}$W. **(I-J)** CX3CR1-induced G protein activation of WT and mutant CX3CR1. **(K)** CX3CL1-induced

G protein activation of WT CX3CR1 using the mutant Gα$_i$ (D350A). **(L)** CCL3-induced G protein activation of WT CCR5 using the mutant Gα$_i$ (D350A). At least three independent experiments (n) were performed in triplicate, data are shown as mean ± SEM. See table S2, S3 and S4 for detailed statistical evaluation.
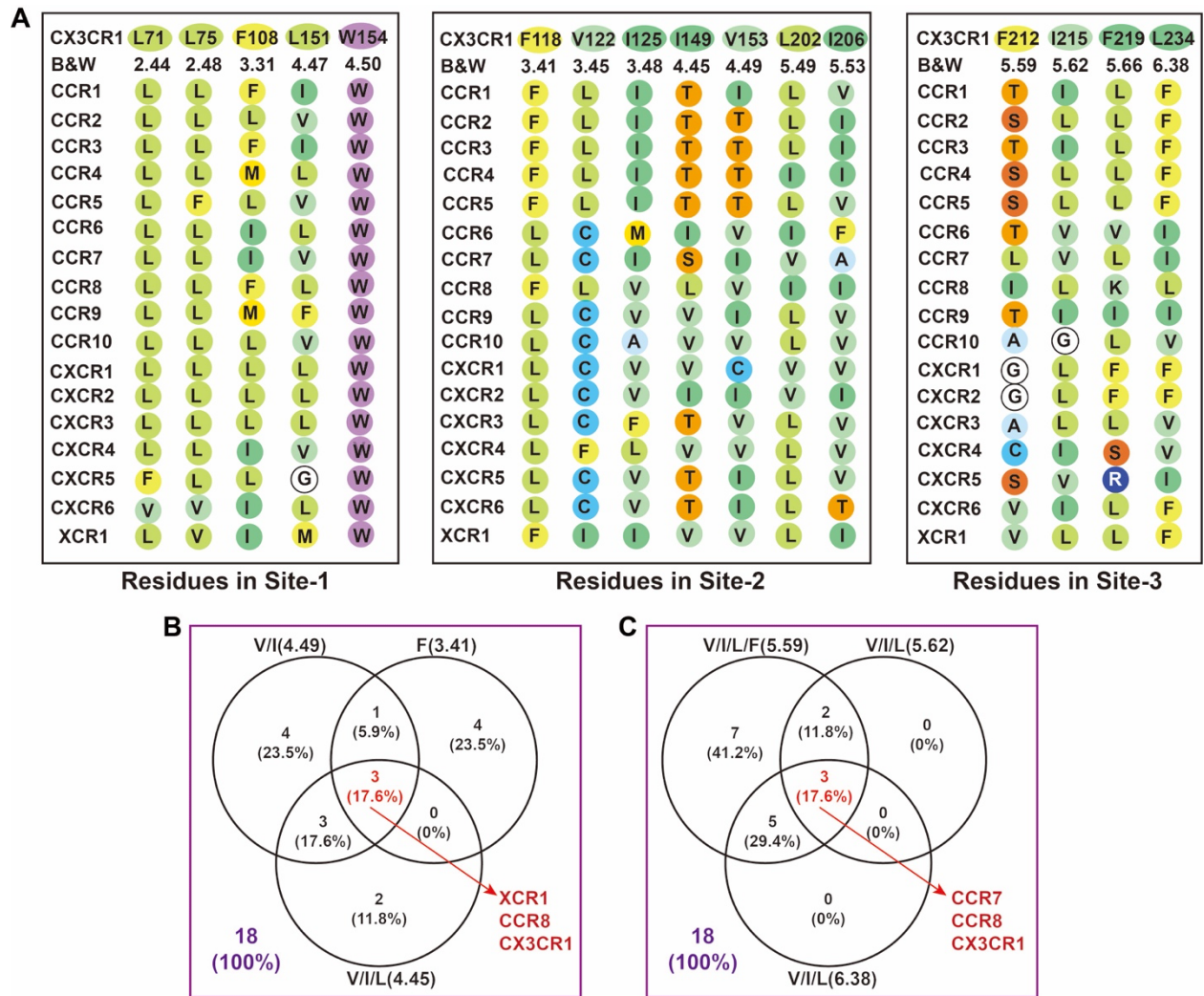
**Fig. S5. Sequence alignment of equivalent residues in CX3CR1 cholesterol binding pockets of human CKRs. (A)** Sequence alignment results of cholesterol binding pockets in human CKRs. Different residues are labeled with distinct colors. **(B-C)** Venn diagrams illustrating the abundance of key elements involved in binding at site-2 and site-3 among CKRs.
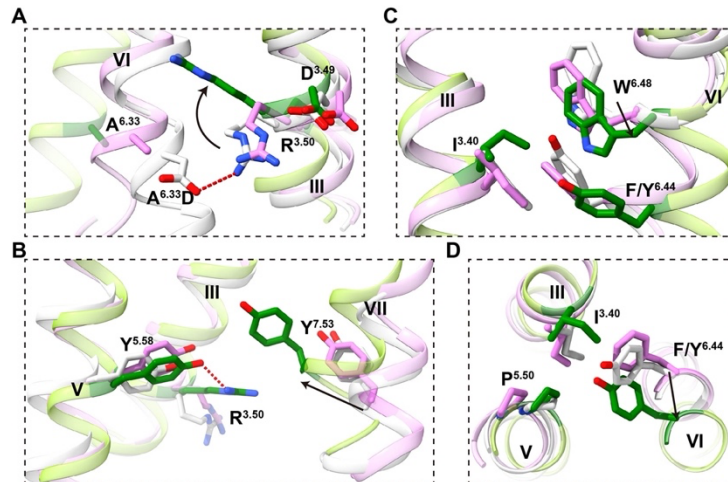
**Fig. S6. Structural comparison of conserved motifs in CX3CR1 and CCR5. (A-D)** Superposition of the inactive CCR5 (PDB ID: 4MBS), active CCR5 (PDB ID: 7F1Q) and active CX3CR1, which are shown in gray, green and pink with cartoon representations, respectively. Residues in each motif are shown as sticks. The red dashed line shows the salt bridge. The black arrows indicate conformational changes of conserved motifs between inactive and active CCR5. **(A)** Conformational changes of "DRY" motif. **(B)** Rearrangement of $Y^{7.53}$ in "NPxxY" motif. **(C)** Conformational changes of "toggle switch"-$W^{6.48}$. **(D)** Rearrangement of "PIF" motif.

**Table S1. Cryo-EM data collection and refinement statistics of CX3CR1-$G_{i1}$ and CX3CL1-CX3CR1-$G_{i1}$ complex structures.**

| | CX3CR1-$G_{i1}$ (PDB-7XBW) (EMDB-33107) | CX3CL1-CX3CR1-$G_{i1}$ (PDB-7XBX) (EMDB-33108) |
|---|---|---|
| **Data collection and processing** | | |
| Magnification | 81,000 | 81,000 |
| Voltage (kV) | 300 | 300 |
| Electron exposure (e-/$\text{Å}^2$) | 70 | 70 |
| Defocus range (μm) | -0.8 ~ -1.5 | -0.8 ~ -1.5 |
| Pixel size (Å) | 1.045 | 1.045 |
| Symmetry imposed | C1 | C1 |
| Initial particle images (no.) | 4,846,904 | 3,394,697 |
| Final particle images (no.) | 702,722 | 490,779 |
| Map resolution (Å) | 2.8 | 3.4 |
| FSC threshold | 0.143 | 0.143 |
| Map resolution range (Å) | 2.5-5.0 | 2.5-5.0 |
| **Refinement** | | |
| Initial model used (PDB code) | 6DDE,4XT1 | 6DDE, 4XT1 |
| Model resolution (Å) | 3.0 | 4.1 |
| Map sharpening B factor ($\text{Å}^2$) | -101 | -128 |
| Model composition | | |
| Receptor residues | 288 (2,260 atoms) | 287 (2,119 atoms) |
| Chemokine residues | / | 61 (402 atoms) |
| $G_{i1}$ residues | 598 (4,405 atoms) | 560 (4,087 atoms) |
| B-factors ($\text{Å}^2$) | | |
| Receptor | 70.41 | 151.50 |
| Chemokine | / | 115.15 |
| $G_{i1}$ | 43.99 | 75.71 |
| R.m.s. deviations | | |
| Bond lengths (Å) | 0.002 | 0.002 |
| Bond angles (°) | 0.494 | 0.430 |
| Validation | | |
| MolProbity score | 1.40 | 1.43 |
| Clash score | 6.14 | 6.35 |
| Poor rotamers (%) | 0.0 | 0.0 |
| Ramachandran plot | | |
| Favored (%) | 97.71 | 97.65 |
| Allowed (%) | 2.29 | 2.35 |
| Disallowed (%) | 0.0 | 0.0 |

**Table S2. CX3CL1-induced IP accumulation of WT and mutant CX3CR1 using a chimeric Gα protein Gα$_{qi5}$.**

| Mutants | EC$_{50}$ (nM) | Ratio[a] | pEC$_{50}$ ± SEM[b] | Span[b,c] (% of WT) | n[d] | Expression[e] (% of WT) Mean± SEM |
|---|---|---|---|---|---|---|
| WT | 1.82 | 1 | 8.74 ± 0.08 | 100 ± 3 | 14 | 100 |
| Y38$^{1.39}$A[f] | 119.84 | 66 | 6.92 ± 0.19* | 114 ± 10 | 5 | 104 ± 21 |
| W87$^{2.60}$A | 44.67 | 25 | 7.35 ± 0.13 | 107 ± 6 | 3 | 153 ± 9 |
| F109$^{3.32}$A | ND | ND | ND | ND | 4 | 106 ± 21 |
| E254$^{5.38}$A | ND | ND | ND | ND | 3 | 60 ± 17 |
| E254$^{5.38}$D | 2.32 | 1 | 8.64 ± 0.18 | 95 ± 7 | 5 | 164 ± 16 |
| E254$^{5.38}$Q | 1.56 | 0.8 | 8.81 ± 0.26 | 112 ± 11 | 3 | 75 ± 10 |
| E279$^{7.39}$A | ND | ND | ND | ND | 4 | 93 ± 16 |
| R127$^{3.50}$A | ND | ND | ND | ND | 4 | 96 ± 21 |
| Construct-5[g] | ND | ND | ND | ND | 6 | 304 ± 8*** |
| Construct-6[h] | 8.9 | 4.9 | 8.05 ± 1.18 | 15 ± 8*** | 5 | 169 ± 11 |

[a]The EC50 ratio, EC$_{50(mutant)}$/EC$_{50(WT)}$, refers to the shift between the WT CX3CR1 and mutant curves, characterizing the effect of the mutations on receptor signaling.

[b]Data are shown as mean ± SEM from at least three independent experiment each performed in technical triplicate. *$P<0.01$; **$P<0.001$; ***$P<0.0001$ by one-way ANOVA followed by Dunnett's post-test, compared with the response of the WT.

[c]The span is defined as the window between the maximal CX3CL1 response (E$_{max}$) and the vehicle (no ligand). "ND" (not determined) refers to data where a robust concentration response curve could not be established within the concentration range tested, such that an E$_{max}$ was not reached and therefore span could not be calculated.

[d]Sample size: the number of independent experiments performed in triplicate.

[e]Protein expression levels of CX3CR1 constructs at the cell surface were determined in parallel by flow cytometry with an anti-FLAG antibody and reported as per cent compared to the WT form three independent measurements performed in duplicate.

[f]All mutations were introduced in the WT CX3CR1.

[g]The construct used to validate the effect of "14 × Gly-Ser" linker and disulfide bond between CX3CL1 (G35C) and CX3CR1 (L176C).

[h]The construct used to validate the effect of "14 × Gly-Ser" linker between CX3CL1 and CX3CR1.

**Table S3. CX3CL1-induced TRUPATH assay of WT and mutant CX3CR1.**

| | | | CX3CL1-induced TRUPATH assay of CX3CR1 | | | |
|---|---|---|---|---|---|---|
| Mutants | $EC_{50}$ (nM) | Ratio[a] | $pEC_{50} \pm$ SEM[b] | Span[b,c] (% of WT) | n[d] | Expression[e] (% of WT) Mean $\pm$ SEM |
| WT | 0.27 | 1 | $9.57 \pm 0.07$ | $100 \pm 2$ | 25 | 100 |
| Construct-1[f] | 0.17 | 0.6 | $9.76 \pm 0.23$ | $80 \pm 6$* | 5 | $123 \pm 17$ |
| F118[3.41]A[g] | ND | ND | ND | ND | 4 | $86 \pm 20$ |
| F118[3.41]L | 0.16 | 0.6 | $9.79 \pm 0.46$ | $19 \pm 3$*** | 4 | $115 \pm 12$ |
| V122[3.45]C | 0.12 | 0.4 | $9.91 \pm 0.24$ | $74 \pm 7$** | 4 | $142 \pm 24$ |
| I149[4.45]T | 0.45 | 2 | $9.35 \pm 0.19$ | $69 \pm 5$*** | 4 | $154 \pm 46$ |
| V153[4.49]A | ND | ND | ND | ND | 4 | $216 \pm 21$*** |
| V153[4.49]W | ND | ND | ND | ND | 4 | $73 \pm 3$ |
| L202[5.49]A | 0.45 | 2 | $9.35 \pm 0.35$ | $64 \pm 5$*** | 3 | $226 \pm 15$*** |
| I206[5.53]A | 0.10 | 0.4 | $9.98 \pm 0.26$ | $37 \pm 4$*** | 4 | $101 \pm 2$ |
| F212[5.59]S | 0.19 | 1 | $9.71 \pm 0.27$ | $51 \pm 5$*** | 3 | $130 \pm 28$ |
| I215[5.62]A | ND | ND | ND | ND | 3 | $64 \pm 11$ |
| F219[5.66]A | 0.50 | 2 | $9.30 \pm 0.23$ | $50 \pm 4$*** | 4 | $267 \pm 28$*** |
| L234[6.38]A | 0.27 | 1 | $9.37 \pm 0.26$ | $81 \pm 8$* | 4 | $185 \pm 21$*** |
| L234[6.38]F | 0.20 | 0.7 | $9.71 \pm 0.23$ | $53 \pm 5$*** | 3 | $144 \pm 21$ |
| L234[6.38]W | ND | ND | ND | ND | 5 | $56 \pm 8$ |
| W154[4.50]A | ND | ND | ND | ND | 3 | $84 \pm 13$ |
| M138[34.54]A | 83.30 | 309 | $7.08 \pm 0.57$*** | $18 \pm 6$*** | 3 | $462 \pm 28$*** |
| Y293[7.53]A | ND | ND | ND | ND | 4 | $121 \pm 10$ |
| V52[1.53]A | 0.48 | 2 | $9.31 \pm 0.58$ | $18 \pm 4$*** | 3 | $99 \pm 20$ |
| L70[2.43]A | 0.28 | 1 | $9.56 \pm 0.65$ | $21 \pm 4$*** | 3 | $80 \pm 14$ |
| F300[8.50]A | 0.28 | 1 | $9.55 \pm 0.19$ | $44 \pm 3$*** | 3 | $76 \pm 8$ |
| N223[ICL3]A | 0.93 | 3 | $9.03 \pm 0.24$ | $69 \pm 7$*** | 4 | $157 \pm 16$ |
| R127[3.50]A | ND | ND | ND | ND | 3 | $104 \pm 8$ |
| K299[8.49]A | 0.37 | 1 | $9.43 \pm 0.38$ | $33 \pm 4$*** | 3 | $127 \pm 16$ |
| D67[2.40]A | 0.40 | 1 | $9.39 \pm 0.49$ | $27 \pm 5$*** | 3 | $134 \pm 13$ |
| D350A ($G\alpha_i$)[h] | 0.32 | 1 | $9.50 \pm 0.20$ | $66 \pm 5$*** | 5 | $102 \pm 10$ |

[a]The EC50 ratio, $EC_{50(mutant)}/EC_{50(WT)}$, refers to the shift between the WT CX3CR1 and mutant curves, characterizing the effect of the mutations on receptor signaling.

[b]Data are shown as mean $\pm$ SEM from at least three independent experiment each performed in technical triplicate. *P<0.01; **P<0.001; ***P<0.0001 by one-way ANOVA followed by Dunnett's post-test, compared with the response of the WT.

[c]The span is defined as the window between the maximal CX3CL1 response ($E_{max}$) and the vehicle (no ligand). "ND" (not determined) refers to data where a robust concentration response curve could not be established within the concentration range tested, such that an $E_{max}$ was not reached and therefore span could not be calculated.

[d]Sample size: the number of independent experiments performed in triplicate.

[e]Protein expression levels of CX3CR1 constructs at the cell surface were determined in parallel by flow cytometry with an anti-FLAG antibody and reported as per cent compared to the WT form three independent measurements performed in duplicate.

[f]The construct used for complex formation with $G_{i1}$, containing the mutations I120[3.43]L, C221[ICL3]S and M250[6.54]V and 40 residues truncated at C terminus.

[g]All mutations were introduced in the WT CX3CR1.

[h]D350A was introduced into $G\alpha_{i1}$-Rluc8.

**Table S4. CCL3-induced TRUPATH assay of WT and mutant CCR5.**

| CCL3-induced TRUPATH assay of CCR5-1[a] | | | | | | |
|---|---|---|---|---|---|---|
| Mutants | $EC_{50}$ (nM) | Ratio[b] | $pEC_{50} \pm$ SEM[c] | Span[c,d] (% of WT) | n[e] | Expression[f] (% of WT) Mean $\pm$ SEM |
| WT | 0.93 | 1 | $9.03 \pm 0.21$ | $100 \pm 10$ | 4 | 100 |
| F238[6.38]W[g] | 1.63 | 2 | $8.79 \pm 0.29$ | $62 \pm 8$* | 4 | $65 \pm 1$ |

| CCL3-induced TRUPATH assay of CCR5-2[a] | | | | | | |
|---|---|---|---|---|---|---|
| Mutants | $EC_{50}$ (nM) | Ratio[b] | $pEC_{50} \pm$ SEM[h] | Span[h,d] (% of WT) | n[e] | Expression[f] (% of WT) Mean $\pm$ SEM |
| WT | 13.68 | 1 | $7.86 \pm 0.15$ | $100 \pm 10$ | 4 | 100 |
| T152[4.49]A[g] | 13.68 | 1 | $7.86 \pm 0.25$ | $119 \pm 17$ | 3 | $124 \pm 4$ |
| D350A ($G\alpha_i$)[i] | 12.35 | 1 | $7.91 \pm 0.22$ | $98 \pm 13$ | 4 | $122 \pm 10$ |

[a]Assays of CCR5-1 and CCR-2 were carried out in totally different batch of cells, ligand and assay buffer. All the results of mutant were compared to the corresponding WT.

[b]The $EC_{50}$ ratio, $EC_{50(mutant)}/EC_{50(WT)}$, refers to the shift between the WT CCR5 and mutant curves, characterizing the effect of the mutations on receptor signaling.

[c]Data are shown as mean $\pm$ SEM from at least three independent experiment each performed in technical triplicate. *P<0.05 by unpaired t-test, compared with the response of the WT.

[d]The span is defined as the window between the maximal CCL3 response ($E_{max}$) and the vehicle (no ligand). "ND" (not determined) refers to data where a robust concentration response curve could not be established within the concentration range tested, such that an $E_{max}$ was not reached and therefore span could not be calculated.

[e]Sample size: the number of independent experiments performed in triplicate.

[f]Protein expression levels of CCR5 constructs at the cell surface were determined in parallel by flow cytometry with an anti-FLAG antibody and reported as per cent compared to the WT form three independent measurements performed in duplicate.

[g]F238[6.38]W and T152[4.49]A were introduced in the WT CCR5.

[h]Data are shown as mean $\pm$ SEM from at least three independent experiment each performed in technical triplicate. *P<0.01; **P<0.001; ***P<0.0001 by one-way ANOVA followed by Dunnett's post-test, compared with the response of the WT.

[i]D350A was introduced into $G\alpha_{i1}$-Rluc8.

**Table S5. DNA and primer sequences of CX3CR1, CCR5 and DNGα$_i$.**

| CX3CR1-WT DNA sequence |
|---|
| ATGGATCAATTCCCAGAGAGCGTTACGGAGAACTTCGAGTACGACGACTTGGCAGAGGCTTGCTACATCGGTGACATTGTGGTCTTCGGTACCGTGTTCCTGAGCATCTTCTACTCTGTGATCTTCGCTATTGGTTTGGTCGGCAACCTGCTCGTGGTCTTCGCTCTGACAAACTCAAAGAAACCTAAGAGTGTTACGGACATCTACTTGCTGAACCTCGCCTTGTCAGATCTCTTGTTCGTCGCAACACTGCCCTTCTGGACGCACTACCTCATCAACGAGAAGGGCTTGCATAACGCGATGTGCAAATTCACCACTGCTTTCTTCTTCATTGGATTCTTCGGTTCGATCTTCTTCATTACTGTCATCTCCATTGACAGGTACTTGGCTATCGTTCTGGCTGCCAACAGCATGAACAACAGAACCGTGCAGCACGGCGTCACTATCTCTCTGGGAGTTTGGGCAGCGGCTATTCTCGTGGCCGCACCACAGTTCATGTTCACAAAGCAAAAAGAGAACGAATGTCTGGGCGATTACCCTGAGGTGCTCCAGGAAATCTGGCCCGTTTTGCGCAACGTGGAGACAAACTTCCTGGGATTCCTGCTCCCGTTGCTGATCATGTCGTACTGCTACTTCCGTATCATTCAAACGCTCTTCTCCTGTAAGAACCACAAGAAAGCGAAGGCTATCAAACTCATTCTCTTGGTTGTGATCGTGTTCTTCTTGTTCTGGACCCCATACAACGTCATGATTTTCCTGGAAACTCTGAAGCTCTACGACTTCTTCCCCGTCATGCGACATGCGCAAGGATTTGCGTCTGGCTCTCAGCGTCACAGAGACGGTTGCCTTCTCTCATTGCTGTTTGAACCCACTGATCTACGCCTTCGCAGGTGAAAAGTTCCGCCGTTACCTCTACCACTTGTACGGTAAATGCCTGGCCGTGCTCTGTGGCAGGAGTGTCCATGTTGACTTCTCCAGCTCTGAATCGCAAAGGTCCAGACACGGATCAGTCCTGTCAAGTAACTTCACCTACCATACTAGTGACGGTGACGCTCTGCTCTTGCTG |

| WT-F | GTGTTCGCCGGCGCGCCGATGGATCAATTCCCAGAGAGC |
|---|---|
| C-truncation-R | CAGGAATTCGAGCACGGCCAGGCATTTACCGTACAAGTG |
| Y38A-R | GAAGATCACAGACGCGAAGATGCTCAGGAACAC |
| W87A-R | GAGGTAGTGCGTCGCGAAGGGCAGTGTTGCGAC |
| F109A-R | GAATCCAATGAACGCGAAAGCAGTGGTGAATTT |
| E254A-R | GAGCTTCAGAGTCGCCAGGAAAATCATGACGTT |
| E254D-R | GAGCTTCAGAGTGTCCAGGAAAATCATGACGTT |
| E254Q-R | GTCGTAGAGCTTCAGAGTTTGCAGGAAAATCATGAC |
| E279A-R | GAAGGCAACCGTCGCTGTGACGCTGAGAGCCAG |
| R127A-R | GATAGCCAAGTACGCGTCAATGGAGATGACAGT |
| F118A-R | GGAGATGACAGTAATGAAAGCGATCGAACCGAAGAA |
| F118L-R | GATGACAGTAATGAACAGGATCGAACCGAAGAATCC |
| V122C-R | GTACCTGTCAATGGAGATAGCAGTAATGAAGAAGAT |
| I149T-R | CCAAACTCCCAGAGAGGTAGTGACGCCGTGCTGCAC |
| V153A-R | GAGAATAGCCGCTGCCCAAGCTCCCAGAGAGATAGT |
| V153W-R | GAGAATAGCCGCTGCCCACCATCCCAGAGAGATAGT |
| L202A-R | CGACATGATCAGCAACGGAGCCAGGAATCCCAGGAA |
| I206A-R | GAAGTAGCAGTACGACATAGCCAGCAACGGGAGCAG |
| F212A-R | GAGCGTTTGAATGATACGTGCGTAGCAGTACGACAT |
| I215A-R | ACAGGAGAAGAGCGTTTGAGCGATACGGAAGTAGCA |
| F219A-R | CTTGTGGTTCTTACAGGAAGCGAGCGTTTGAATGAT |
| L234A-R | GAACACGATCACAACCAAAGCAATGAGTTTGATAGC |
| L234F-R | GAACACGATCACAACCAAGAAAATGAGTTTGATAGC |
| L234W-R | GAACACGATCACAACCAACCAAATGAGTTTGATAGC |
| W154A-R | CACGAGAATAGCCGCTGCCGCAACTCCCAGAGAGATAGT |
| M138A-R | GGTTCTGTTGTTCGCGCTGTTGGCAGCCAGAAC |
| Y293A-R | TGCGAAGGCCGCGATCAGTGGGTTCAAACAGCA |
| V52A-R | AGCGAAGACCGCGAGCAGGTTGCCGACCAAACC |
| L70A-R | GAGGTTCAGCGCGTAGATGTCCGTAACACTCTT |
| F300A-R | GTGGTAGAGGTAACGGCGCGCGCCTTTTCACCTGCGAAGGC |
| N223A-R | CGCTTTCTTGTGCGCCTTACAGGAGAAGAGCGT |
| K299A-R | GTAGAGGTAACGGCGGAAAGCTTCACCTGCGAAGGC |
| D67A-R | CAGCAAGTAGATCGCCGTAACACTCTTAGGTTT |

| CCR5-WT DNA sequence |
|---|
| GATTATCAAGTGTCAAGTCCAATCTATGACATCAATTATTATACATCGGAGCCCTGCCAAAAAATCAATGTGAAGCAAATCGCAGCCCGCCTCCTGCCTCCGCTCTACTCACTGGTGTTCATCTTTGGTTTTGTGGGCAACATGCTGGTCATCCTCATCCTGATAAACTGCAAAAGGCTGAAGAGCATGACTGACATCTACCTGCTCAACCTGGCCATCTCTGACCTGTTTTTCCTTCTTACTGTCCCCTTCTGGGCTCACTATGCTGCCGCCCAGTGGGACTTTGGAAATACAATGTGTCAACTCTTGACAGGGCTCTATTTTATAGGCTTCTTCTCTGGAATCTTCTTCATCATCCTCCTGACAATCGATAGGTACCTGGCTGTCGTCCATGCTGTGTTTGCTTTAAAAGCCAGGACGGTCACCTTTGGGGTGGTGACAAGTGTGATCACTTGGGTGGTGGCTGTGTTTGCGTCTCTCCCAGGAATCATCTTTACCAGATCTCAAAAAGAAGGTCTTCATTACACCTGCAGCTCTCATTTTCCATACAGTCAGTATCAATTCTGGAAGAATTTCCAGACATTAAAGATAGTCATCTTGGGGCTGGTCCTGCCGCTGCTTGTCATGGTCATCTGCTACTCGGGAATCCTtAAgACTCTGCTTCGGTGTCGAAATGAGAAGAA |

| | |
|---|---|
| GAGGCACAGGGCTGTGAGGCTTATCTTCACCATCATGATTGTTTATTTTCTCTTCTGGGCTCCCTACAACATTGTCC TTCTCCTGAACACCTTCCAGGAATTCTTTGGCCTGAATAATTGCAGTAGCTCTAACAGGTTGGACCAAGCTATGCA GGTGACAGAGACTCTTGGGATGACGCACTGCTGCATCAACCCCATCATCTATGCCTTTGTCGGGGAGAAGTTCAG AAACTACCTCTTAGTCTTCTTCCAAAAGCACATTGCCAAACGCTTCTGCAAATGCTGTTCTATTTTCCAGCAAGAG GCTCCCGAGCGAGCAAGCTCAGTTTACACCCGATCCACTGGGGAGCAGGAAATATCTGTGGGCTTG | |
| WT-F | GACGATGATGACGATTATCAAGTGTCAAGTCCAATCTAT |
| T152A-R | CACAGCCACCACCCACGCGATCACACTTGTCACCAC |
| F238W-R | AACAATCATGATGGTCCAGATAAGCCTCACAGCCCT |
| DNGα<sub>i</sub>-DNA sequence (S47C G202T G203A E245A A326S) | |
| ATGGGCTGCACGCTGAGCGCCGAGGACAAGGCGGCGGTGGAGCGGAGTAAGATGATCGACCGCAACCTCCGTG AGGACGGCGAGAAGGCGGCGCGCGAGGTCAAGCTGCTGCTGCTCGGTGCTGGTGAATCTGGTAAATGTACAATT GTGAAGCAGATGAAAATTATCCATGAAGCTGGTTATTCAGAAGAGGAGTGTAAACAATACAAAGCAGTGGTCTAC AGTAACACCATCCAGTCAATTATTGCTATCATTAGGGCTATGGGGAGGTTGAAGATAGACTTTGGTGACTCAGCCC GGGCGGATGATGCACGCCAACTCTTTGTGCTAGCTGGAGCTGCTGAAGAAGGCTTTATGACTGCAGAACTTGCTG GAGTTATAAAGAGATTGTGGAAAGATAGTGGTGTACAAGCCTGTTTCAACAGATCCCGAGAGTACCAGCTTAATG ATTCTGCAGCATACTATTTGAATGACTTGGACAGAATAGCTCAACCAAATTACATCCCGACTCAACAAGATGTTCT CAGAACTAGAGTGAAAACTACAGGAATTGTTGAAACCCATTTTACTTTCAAAGATCTTCATTTTAAAATGTTTGAT GTGACCGCCCAGAGATCTGAGCGGAAGAAGTGGATTCATTGCTTCGAAGGAGTGACGGCGATCATCTTCTGTGTA GCACTGAGTGACTACGACCTGGTTCTAGCTGAAGATGAAGAAATGAACCGAATGCATGCCAGCATGAAATTGTTT GACAGCATATGTAACAACAAGTGGTTTACAGATACATCCATTATACTTTTTCTAAACAAGAAGGATCTCTTTGAAG AAAAAATCAAAAAGAGCCCTCTCACTATATGCTATCCAGAATATGCAGGATCAAACACATATGAAGAGGCAGCTG CATATATTCAATGTCAGTTTGAAGACCTCAATAAAAGAAAGGACACAAAGGAAATATACACCCACTTCACATGTTC CACAGATACTAAGAATGTGCAGTTTGTTTTTGATGCTGTAACAGATGTCATCATAAAAAATAATCTAAAAGATTGT GGTCTCTTT | |
| DNGα<sub>i</sub>-F | ATCGGGCGCGGATCCATGGGCTGCACGCTGAGCGCC |
| D350A-R | CTCGACAAGCTTTTAAAAGAGACCACAATCTTTTAG |