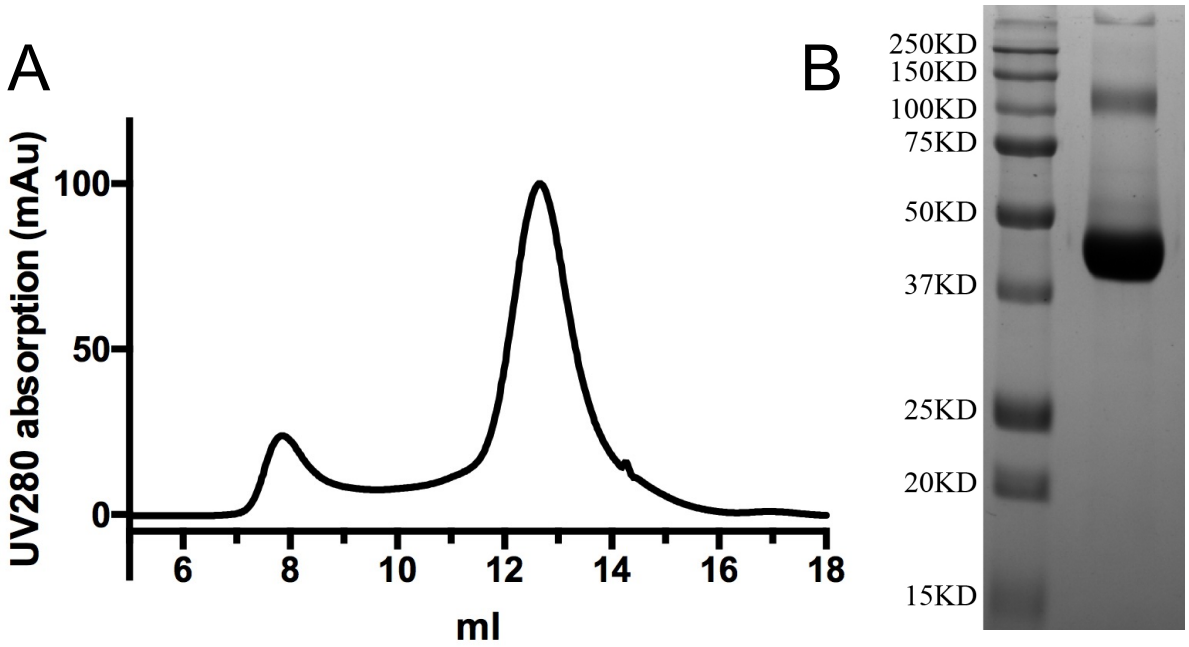

Figure S1. (A) Superdex 200 gel filtration profile of hNK$_1$R-PGS purified by Ni-IMAC and M1-FLAG immunoaffinity chromatography; (B) Coommassie stained PAGE of the isolated peak fraction from gel filtration.

Figure S2. Image of a lipidic cubic phase (LCP) sandwich plate setup containing representative microcrystals of the hNK$_1$R-PGS protein that were harvested to produce X-ray diffraction data.

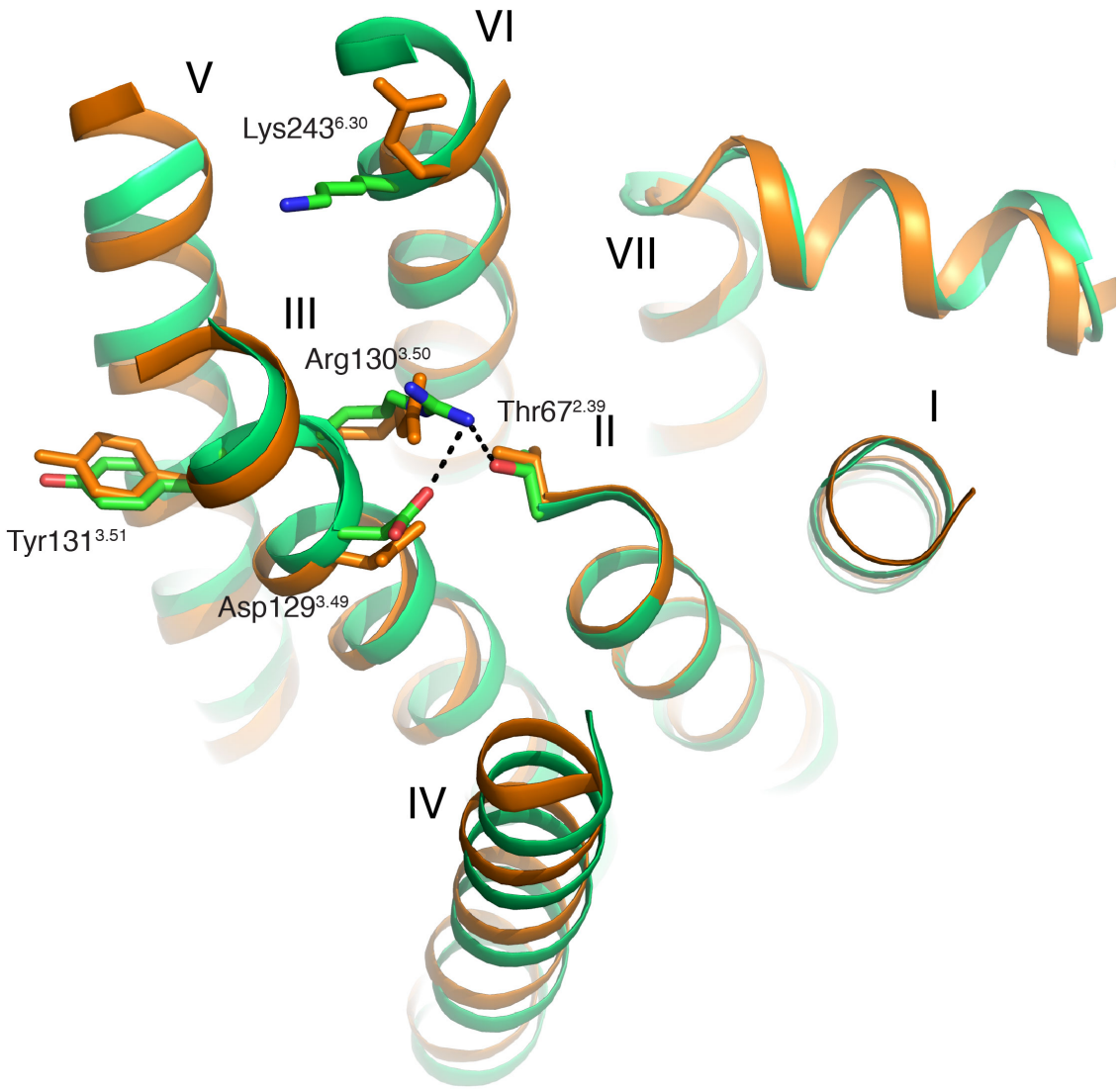

Figure S3. Superposition of inactive-state GPCRs at the intracellular side of the membrane. hNK_1R (green cartoon) and β_2 adrenergic receptor (orange cartoon) are viewed from the intracellular surface. Intracellular loops are removed for clarity. The hNK_1R DRY motif residues (TM3) and residues on TMs 2 and 6 are shown as green sticks. Dotted lines indicate electrostatic interactions and hydrogen bonds.

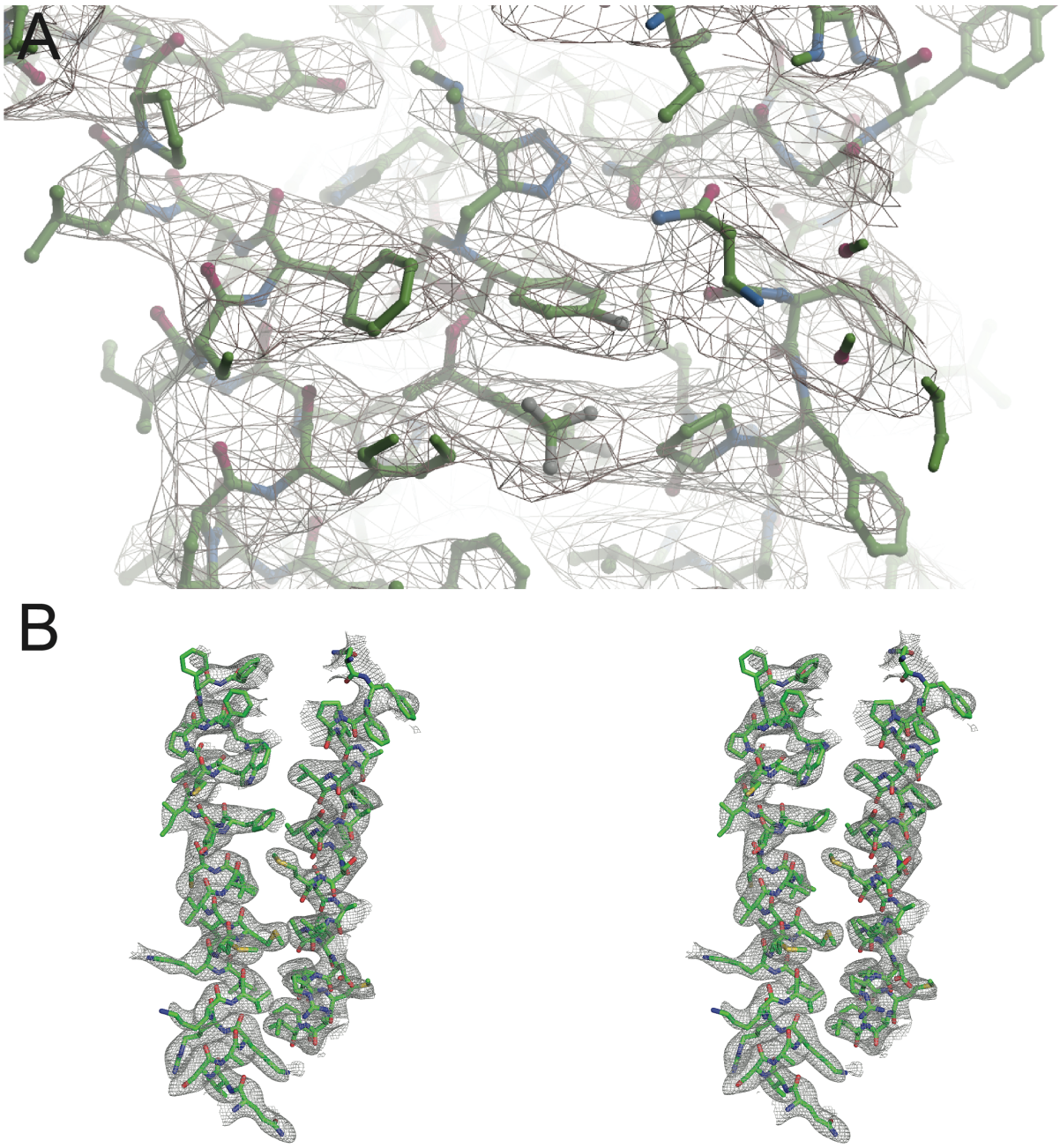

Figure S4. Electron density maps of the hNK_1R crystal structure. (A) 2Fo-Fc map for the orthosteric binding pocket region of hNK1R including the L760735 ligand at center. The contour level is 1.0 σ; (B) Stereo view of 2Fo-Fc electron density for TM helices 3 and 6. The contour level is 1.0 σ.

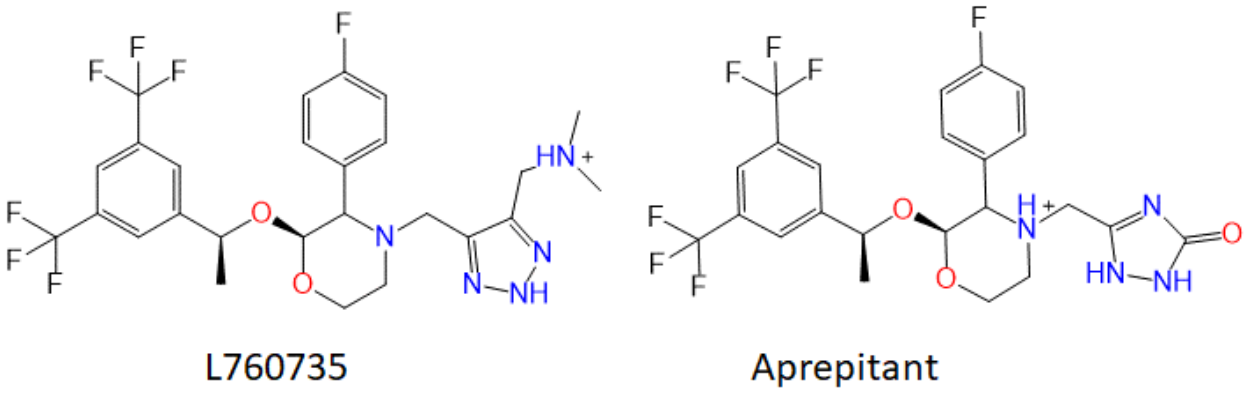

Figure S5. Chemical structures of the hNK$_1$R antagonists L760735 and aprepitant, drawn as conjugate acids. The predicted pK_a's using the program Epik are 7.4 for L760735 and 5.2 for aprepitant.

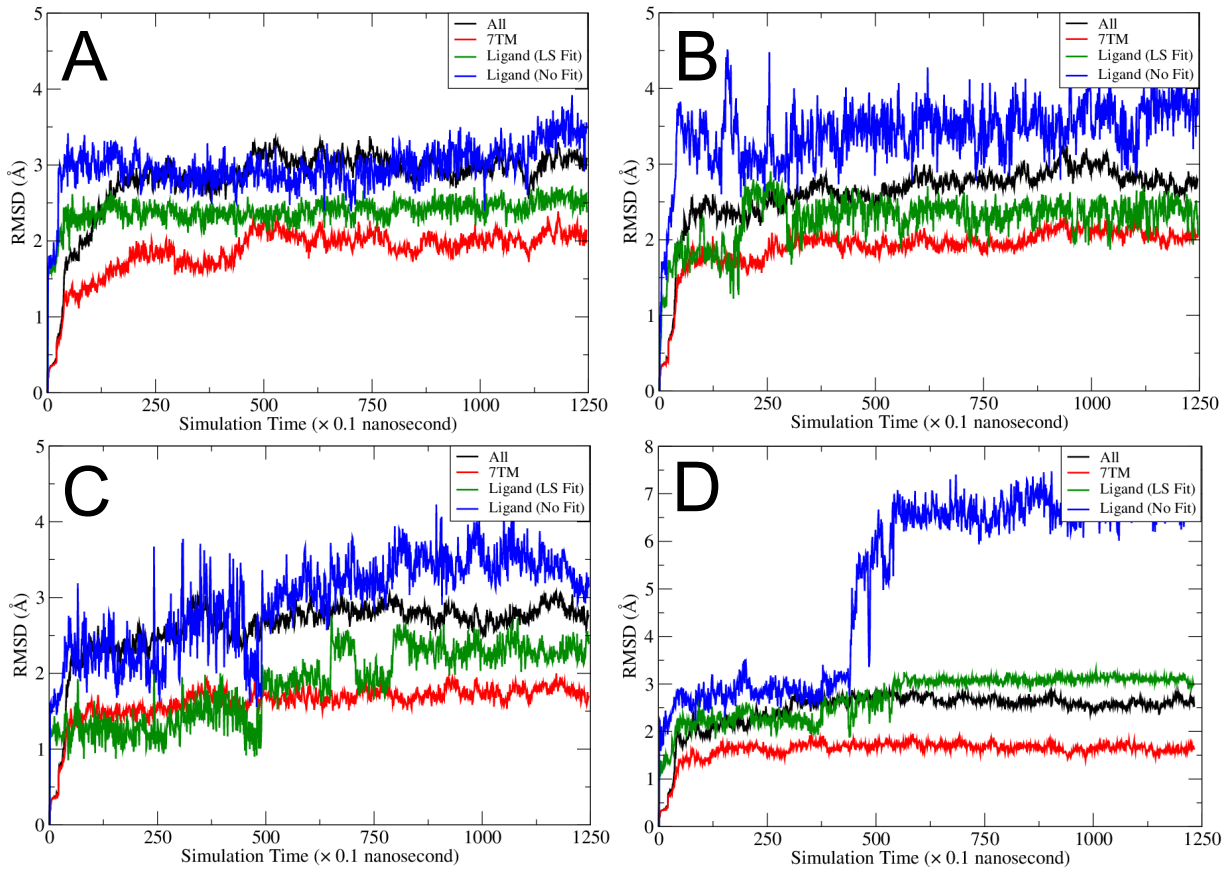

Figure S6. Root-mean-square deviation (RMSD) vs simulation time curves for four MD systems. (A) hNK$_1$R/L760735 (neutral form); (B) hNK$_1$R/L760735 (charged form); (C) hNK$_1$R/aprepitant (neutral form); and (D) hNK$_1$R/aprepitant (charged form). The Ligand (LS Fit) curve (green) indicates the extent of ligand conformational changes during the MD simulations, while the Ligand (No Fit) curve (blue) measures both conformational changes and translational/rotational movement of the ligand in the binding pocket.

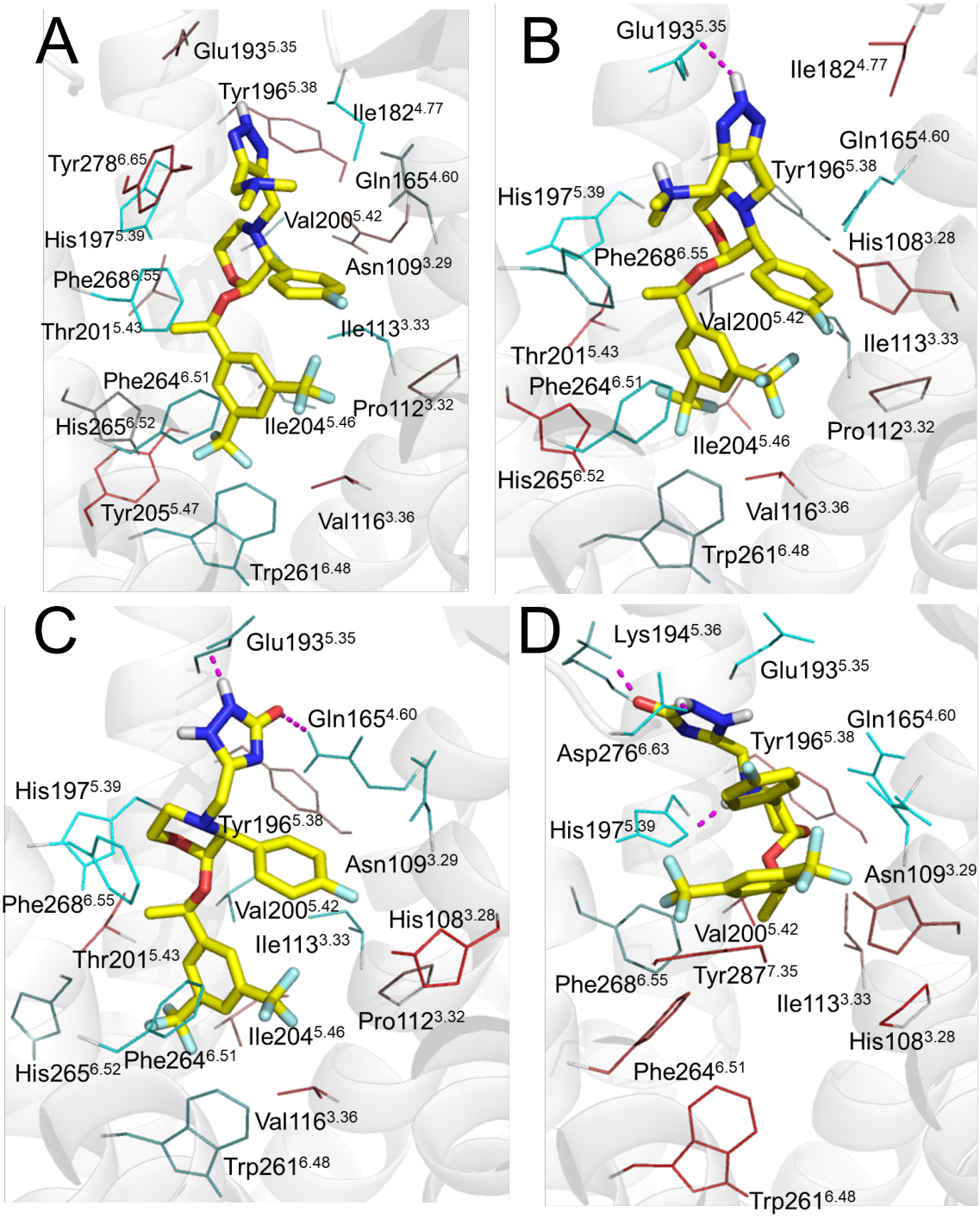

Figure S7. Detailed interactions between ligands and hNK$_1$R hotspot residues derived from MD simulations. Hotspot residues were identified by MM/GBSA decomposition analysis and colored by spectrum from cyan to red. The stronger the interaction with the ligand, the closer to cyan the residue. Only hotspot residues that have $\Delta G_{MM/GBSA}$ better than -0.5 kcal/mol are shown in wireframe. The conformations shown are representative MD snapshots that have the smallest mainchain RMSDs compared to the average MD structures. (A) hNK$_1$R/L7650735; (B) hNK$_1$R/L7650735 (charged form); (C) hNK$_1$R/aprepitant; (D) hNK$_1$R/aprepitant (charged form).

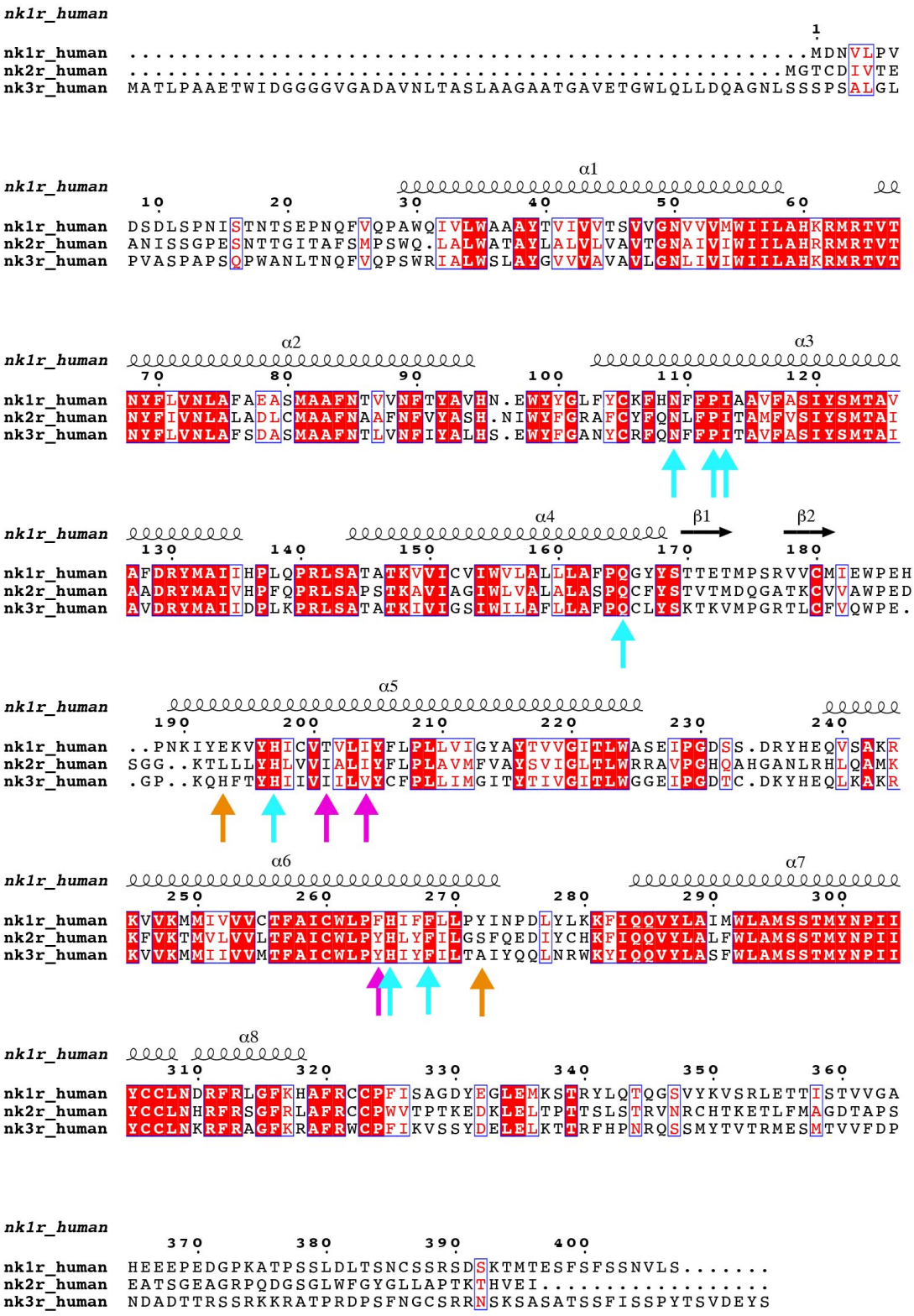

Figure S8. Multiple sequence alignment of the 3 human tachykinin receptors. Arrows identify residues making contacts to the ligand. Colors correspond to those in Fig. S9: cyan arrows show positions where all are identical, magenta arrows show positions where two subtypes are identical, and orange arrows show positions that are not conserved between any of the three receptors.

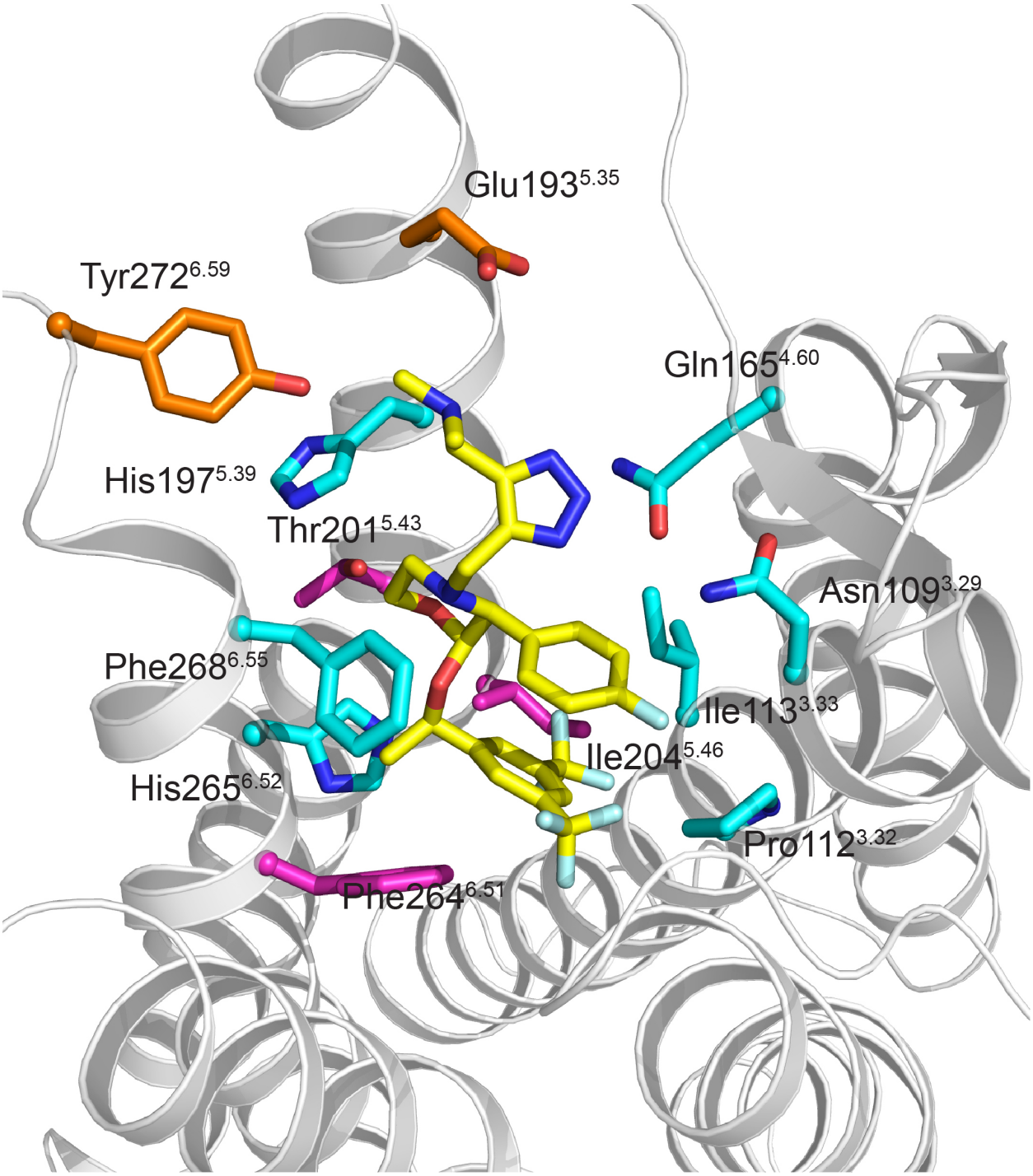

Figure S9. Subtype divergence at the antagonist binding pocket. Colors correspond to the arrows in Fig. S8: cyan sticks show residues that are identical between the three human receptor subtypes (hNK$_1$R, hNK$_2$R, hNK$_3$R), magenta sticks show residues where two of the subtypes are identical, and orange sticks show residues that are not conserved between any of the three receptors. L7650735 is in yellow.

	hNK_1R-PGS/L760735[¶]
Data collection	
Space group	C2
Cell dimensions	
a, *b*, *c* (Å)	124.0, 62.0, 142.9
β (°)	100.2
Resolution (Å)	34.44 (3.40) [†]
Limit[§] along a*	3.9
b*	3.7
c*	3.4
R_{sym} or R_{merge}	0.15 (N/A) [‡]
I/σI	6.98 (1.14)
Completeness (%)	94.1 (94.6)
Redundancy	4.0 (3.9)
$CC_{1/2}$ in the highest shell	0.41
Refinement	
Resolution (Å)	34.44 -3.40 (3.49-3.40)
No. reflections	11310
R_{work}/ R_{free}	0.24/0.30 (0.27/0.34)
No. atoms	
Protein	3844
Ligand	40
B-factors	
Protein	105.9
Fusion protein	18.9
Ligand	118.5
R.m.s deviations	
Bond lengths (Å)	0.011
Bond angles (°)	1.51
Ramachandran statistics (%)	
Favored regions	95.8
Allowed regions	4.2
Disallowed regions	0
Clash score	8.21 (97th percentile)
Molprobity score	1.74 (100th percentile)

[¶] Diffraction data from 36 crystals were merged together.
[†] Highest-resolution shell is shown in parenthesis.
[§] Limits along a*, b*, c* from anisotropic truncation in HKL3000.
[‡] R_{merge} higher than 1 is statistically meaningless.

Table S1. Crystallographic data collection and refinement statistics