

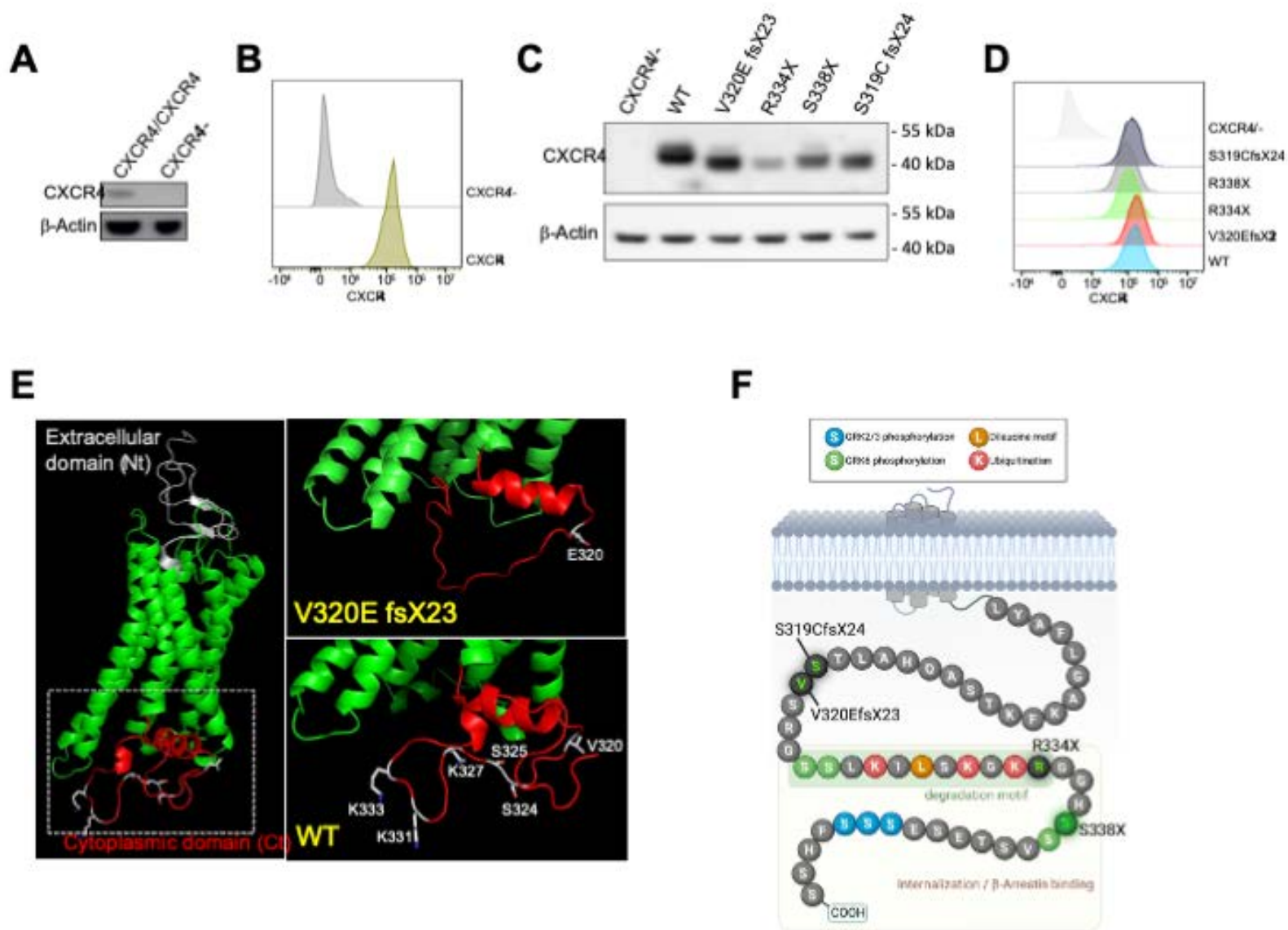
Supplemental Table 1. Mutagenesis primers

Primer	Sequence
V320fx23 FWD	GCACGCACTCACCTCTG*AGCAGAGGGTCCAG
V320fx23 REV	CTGGACCCTCTGCT*CAGAGGTGAGTGCGTGC
R334X FWD	CTCTCCAAAGGAAAGTGAGGTGGACATTCAT
R334X REV	ATGAATGTCCACCTCACTTTCTTTGGAGAG
S338X FWD	AGCGAGGTGGACATTGATCTGTTTCCACTGA
S338X REV	TCAGTGGAACAGATCAATGTCCACCTCGCT
S319CfsX24 FWD	CACGCACTCACCT*GTGAGCAGAGGGT
S319CfsX24 REV	ACCCTCTGCTCAC*AGGTGAGTGCGTG

Bold: change of base
 * : deletion site

Legend:

Supplemental Figure 1. Structure and signaling of CXCR4 mutants. (A) CXCR4 expression in HEK-293T cells WT and CXCR4^{-/-} after CRISPR analyzed by WB. **(B)** Representative flow cytometry histograms showing CXCR4 expression in NALM6 cells WT and CXCR4^{-/-} after CRISPR. **(C)** CXCR4 expression in HEK-293T CXCR4^{-/-} and transduced with WT or CXCR4 variants by WB. **(D)** Representative flow cytometry histograms of NALM6 CXCR4^{-/-} cells transduced with WT or CXCR4 mutants. **(E)** Three-dimensional structure of CXCR4. Left panel: Nt domain (gray) is in the extracellular space. The Ct domain (Red) is in the cytoplasmic compartment. Lower-right panel: WT CXCR4 Ct domain shows S324 and S325, the phosphorylation sites after ligand binding. It is also indicated the position of V320. Upper-right panel: molecular structure predicted for the Ct domain of CXCR4^{V320fsX23}. **(F)** The schematic structure of C-terminus CXCR4 that reports sites of mutation (noted and highlighted in green); ubiquitination motif is indicated in red and dileucine motif in orange; degradation motif and internalization/b-arrestin binding domains are shown.



Supplemental figure 1