β-arrestin1 and distinct CXCR4 structures are required for SDF-1 to down-regulate CXCR4 cell-surface levels in neuroblastoma
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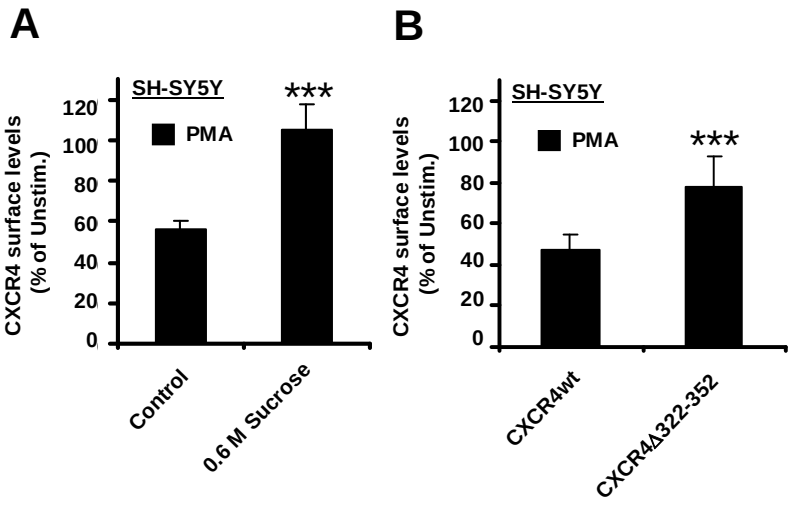
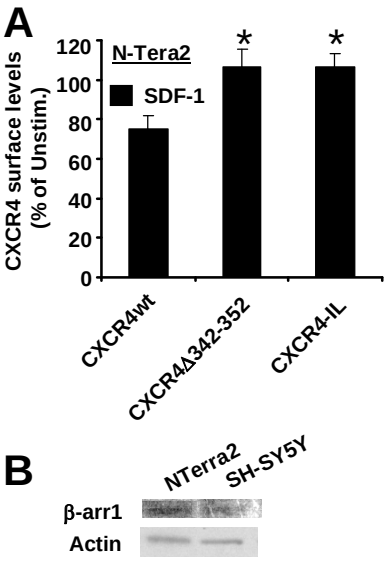
Molecular Pharmacology

Supplemental 1



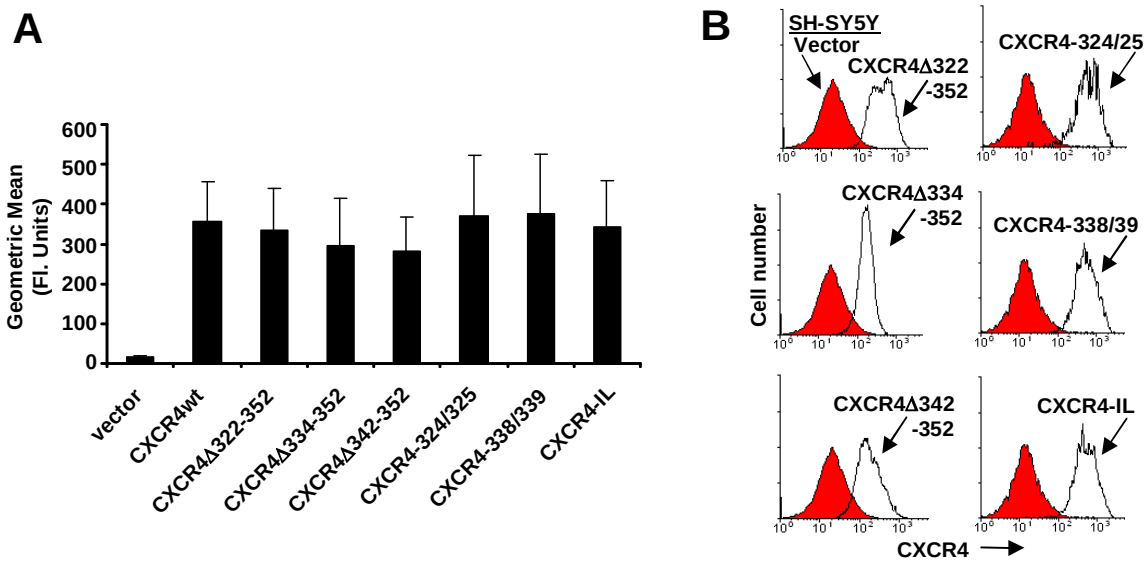
Supplemental Figure 1: **PMA mediated CXCR4 internalization is inhibited in the presence of sucrose and requires the CXCR4 intracellular tail**. SH-SY5Y neuroblastoma cells were treated with either nothing ("Unstim.") or PMA at 37 °C for 60 min. Cell-surface CXCR4 levels were assayed on individual cells via flow cytometry after staining of intact cells with APC-conjugated CXCR4 mAb. A) Surface levels of CXCR4 in PMA treated cells as a percentage of unstimulated ("Unstim.") in control and 0.6 M sucrose conditions. B) Surface levels of CXCR4 in PMA treated cells as a percentage of unstimulated ("Unstim.") in CXCR4wt GFP and CXCR4D322-352 GFP plasmid transfected overexpressions. Each point denotes the mean CXCR4 cell-surface level of PMA - treated as compared to unstimulated cells for three independent experiments $\pm$ S.E.M.; ***, significantly different from results using unstimulated cells, $p < 0.001$.

Supplemental 2



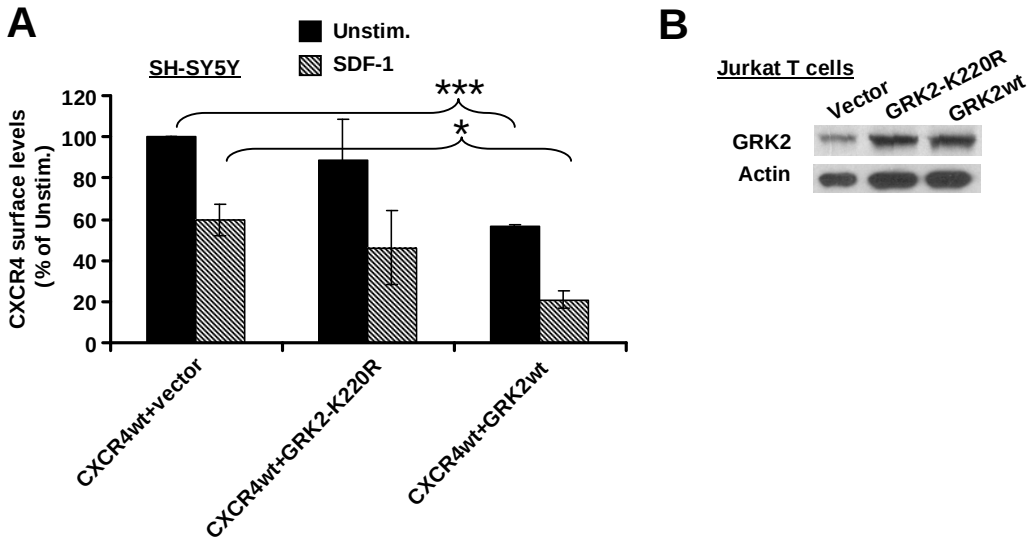
Supplemental Figure 2: **N-Tera2 cells contain β-arrestin1 and SDF-1 dependant CXCR4 internalization is inhibited by mutations to the CXCR4 tail region.** N-tera2 cells were treated with either nothing ("Unstim.") or SDF-1 at 37 °C for 60 min. Cell-surface CXCR4 levels were assayed on individual cells via flow cytometry after staining of intact cells with APC-conjugated CXCR4 mAb. A) Surface levels of CXCR4 in SDF-1 treated cells as a percentage of unstimulated ("Unstim.") in CXCR4wt-YFP, CXCR4Δ342-352, and CXCR4-IL plasmid transfected overexpressions. Each point denotes the mean CXCR4 cell-surface level of PMA - treated as compared to unstimulated cells for three independent experiments ± S.E.M.; *, significantly different from results using unstimulated cells, $p < 0.05$. B) Western blot showing the expression of β-arrestin1 in N-Tera2 and SH-SY5Y cells compared to Actin control.

Supplemental 3



Supplemental Figure 3: **CXCR4 surface levels in CXCR4wt and mutant plasmid overexpression vectors.** Shown as the geometric mean of flourescent units. A) Surface levels of CXCR4 in Vector transfected cells compared to CXCR4 plasmid overexpression mutants represented in mean geometric units; *n*>3. B) Representative FACS plots comparing vector transfected cells to individual mutant overexpressions as in A.

Supplemental 4



Supplemental Figure 4: **GRK2 requires K220-mediated kinase activity to participate in regulating CXCR4 cell-surface levels and internalization.** A) SH-SY5Y cells were transiently-transfected with either CXCR4wt alone or CXCR4wt plus GRK2 or point-mutated, kinase-inactive GRK2 (GRK2-K220R) expression plasmids, as indicated. The GRK2 expression plasmid, but not GRK2-K220R, significantly reduced CXCR4 cell-surface levels, and also enhanced SDF-1 - dependent CXCR4 internalization. Bars denote the mean CXCR4 cell-surface levels compared to unstimulated without GRK2 addition for 3 independent experiments $\pm$ S.E.M.; *** , $p < 0.001$; *, $p < 0.05$. B) Positive control western blot of whole cell lysates of Jurkat T cells showing that transfection with GRK2 or GRK2-K220R expression plasmids induce similar levels of GRK2 overexpression in comparison to actin control.