SUPPORTING INFORMATION

Modulation of CXCR4-mediated Gi1 activation by EGF receptor and GRK2

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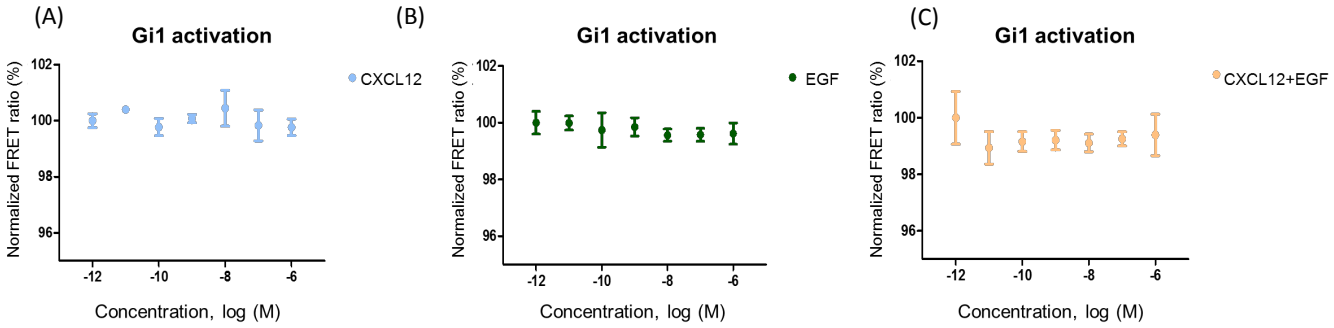

Figure S1. *CXCL12, EGF or combination of both ligands does not activate Gi1 in the absence of overexpressed receptors*. Normalized FRET ratio/dose response curves were obtained as detailed in Methods in HEK293 cells expressing the Gi sensor. Cells were challenged with 0.001nM to 1μM of EGF (A), CXCL12 (B) or the indicated combinations of CXCL12 and EGF (C). Data are mean ± SEM of quadruplicate determinations in FRET assays.

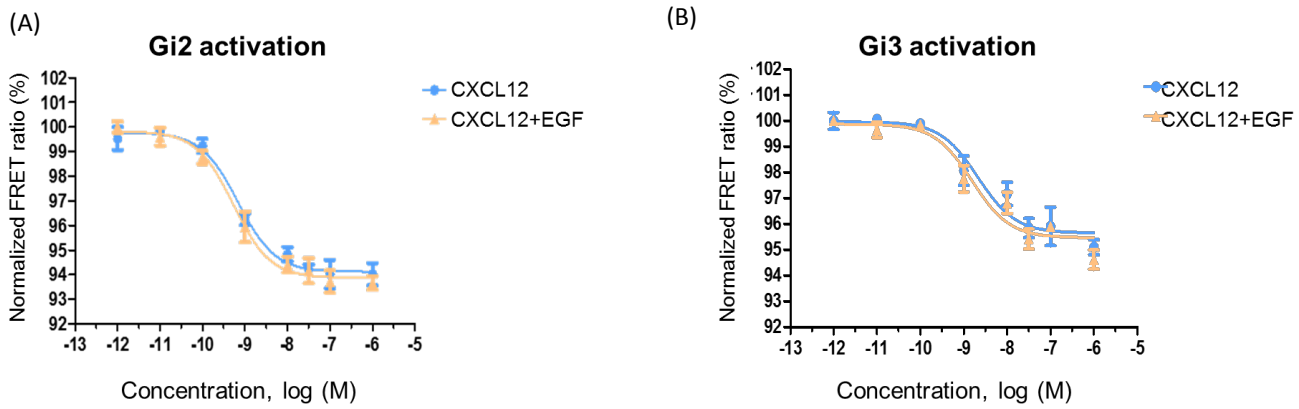

Figure S2. *EGF at endogenous levels of EGFR expression does not affect activation of Gi2 or Gi3 by CXCR4 in response to increasing concentrations of CXCL12*. Normalized FRET ratio/dose response curves were obtained as detailed in Methods in HEK293 cells expressing CXCR4 and either the Gi2 or Gi3 sensor and challenged with 0.001nM to 1μM of CXCL12 or both CXCL12 and EGF (0.001nM to 1μM, same concentration of each ligand). Data are mean ± SEM of quadruplicate determinations in paired FRET assays. In this particular experiment, EC50 values were 0.68 nM (CXCL12) and 0.53 nM (CXCL12+EGF condition) (panel A, Gi2), and 2.1 nM (CXCL12) and 12 nM (CXCL12+EGF condition) (panel B, Gi3).

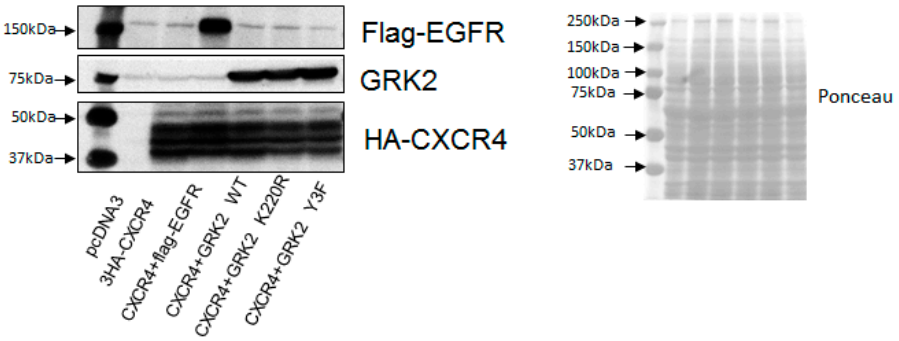

Figure S3. *Expression levels of 3HA-CXCR4, GRK2 constructs and Flag-EGFR in HEK293 cells*. Lysates (20 μg) from HEK293 cells expressing Gi sensors and either a control vector (pcDNA) or the indicated combinations of 3HA-tagged CXCR4, Flag-tagged EGFR or different GRK2 constructs were analyzed with the appropriate tag or protein antibodies as detailed in Methods. Ponceau staining was used as loading control. A representative blot is shown.