Supplemental Methods :

Erk 1/2 phosphorylation

U373 cells were lysed in culture plate using Laemmli reducing buffer (75mM Tris-HCl pH 6.8, 3% SDS, 15% glycerol, 0.15mM DTT with bromophenol blue). Protein samples were resolved on a 10% SDS-polyacrylamide gel electrophoresis (PAGE) then electrotransferred onto polyvinylidene fluoride (PVDF) membranes (Roche Diagnostics) for immunoblotting. Following transfer, membranes were blocked with 5% nonfat milk in PBS-T (1X PBS + 0,1% Tween20) for 1h at room temperature then probed with primary antibody (anti-Phospho-p44/42 (Thr202/Tyr204) Erk1/2 (Cell Signaling Technology) diluted in 5% milk PBS-T overnight at 4ºC. The blots were washed three times in PBS-T, and then incubated for 1h at room temperature with horseradish peroxidise-conjugated (HRP) secondary antibody (HRP-conjugated sheep anti-rabbit IgG, Millipore) diluted in 5% milk PBS-T. Labels were detected by chemiluminescence using the western lightning™ plus ECL system (PerkinElmer). Membranes were then stripped using stripping buffer (62,5mM Tris-HCl, pH 6.8, 100mM Beta-mercaptoethanol, 10% SDS), at 50°C in a rotating oven for 30 minutes, and reblotted using antibody against total erk 1/2 antibody (Cell Signaling Technology) as a primary antibody as a loading control.

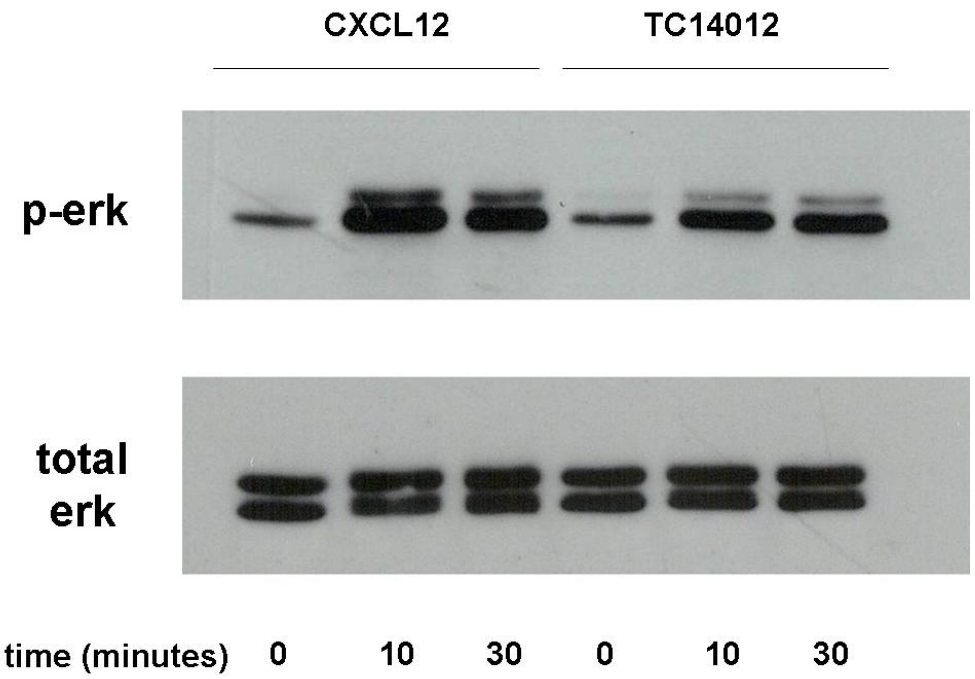
Quantitative RT-PCR

Total RNA was extracted from U373 cells using TRIzol reagent (Invitrogen, Life Technologies) according to manufacturer's instruction. RNA was quantified using a spectrophotometer. First strand cDNA was synthesized using 1 µg of total RNA and the Moloney Murine Leukemia Virus Reverse Transcriptase (M-MLV RT) ((Invitrogen, Life Technologies) with an oligo-dT_{18} primer. The expression of CXCR4 and CXCR7 were analyzed by quantitative PCR (qPCR) with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as an internal control. Gene expression level for endogenous control was determined using pre-validated Taqman Gene Expression Assays (Applied Biosystems). QPCR reactions in 384 well plate were performed using 1.5 µl of cDNA samples (5-25 ng), 5µl of the TaqMan Fast Universal PCR Master Mix (Applied Biosystems), 0.5 µl of the TaqMan Gene Expression Assay (20X) (Applied Biosystems, human GAPDH #Hs00266705) and 3 µl of water in a total volume of 10 µl. CXCR4 and CXCR7 gene expression levels were determined using assays designed with the Universal Probe Library from Roche (www.universalprobelibrary.com). QPCR reactions for 384-well plate format were performed using 1.5 µl of cDNA samples (5-25 ng), 5 µl of the TaqMan Fast Universal PCR Master Mix (Applied Biosystems), 2 µM of each primer and 1 µM of a UPL probe in a total volume of 10 µl. The following probes as well as pairs of forward and reverse primer sets were used: CXCR4 UPL probe # 18, 5'-ggtggtctatgttggcgtct-3' and 5'actgacgttggcaaagatga-3; CXCR7 UPL probe 37, 5'-cggagtactctgccttggag-3' and 5'-caagtaaacccgtcccagag-3'. The ABI PRISM® 7900HT Sequence Detection System (Applied Biosystems) was used to detect the amplification level and was programmed with an initial step at 95°C for 3 minutes, followed by 40 cycles at 95˚C for 5 sec and 60˚C for 30 sec. All reactions were run in triplicate. The relative abundance of CXCR4 and CXCR7 in U373 cell line was calculated relative to the expression of the internal control GAPDH. Relative expression = $2^{(-\Delta Ct)}$ x1000, where ΔC_t is the difference between the Ct values of CXCR4 or CXCR7 and GAPDH. Quantitative RT-PCR was performed at the IRIC (Institute de recherché en immunologie and cancerologie, Université de Montréal) genomics core facility.

Supplemental Figure 1:

TC14012 leads to erk 1/2 phosphorylation in U373 cells, which express endogenous CXCR7 but not CXCR4.

Supplemental Figure S1A:



Supplemental Figure S1B:

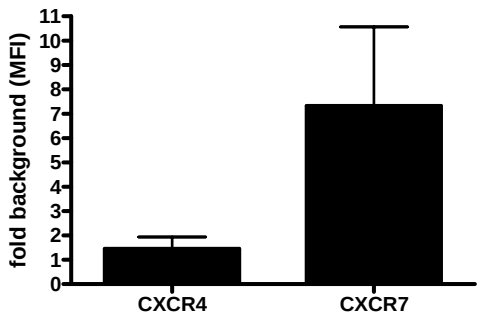
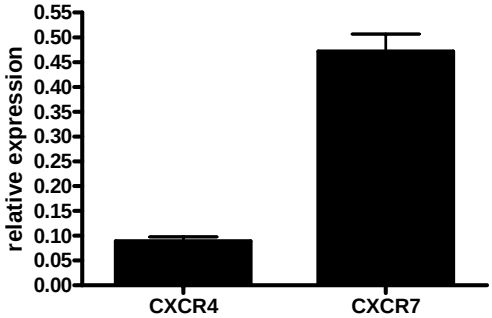


Fig Supplemental Figure S1C:



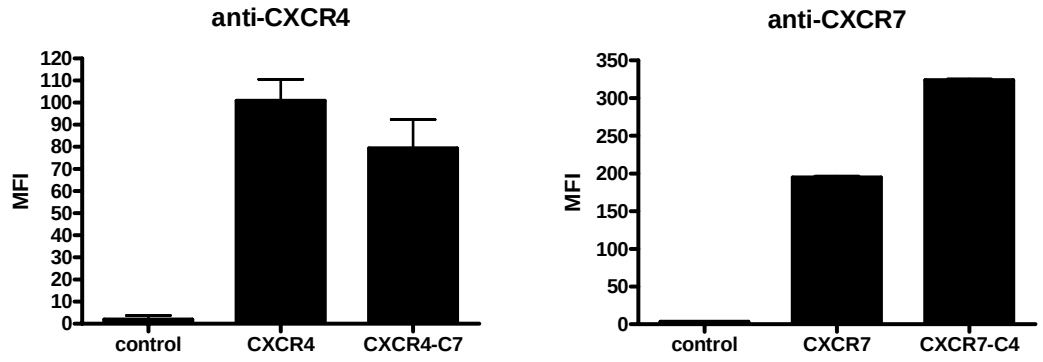
Supplemental figure S1: erk 1/2 phophorylation. Figure S3A: TC14012, like SDF-1, leads to erk phosphorylation in U373 cells, indicating the relevance of beta-arrestin recruitment to CXCR7 by TC14012 for productive signalling. One representative experiment out of three is shown. U373 glioblastoma cells were chosen because they express endogenous CXCR7, but not CXCR4. CXCR4 and CXCR7 protein expression are shown in Figure S1B, detected by flow cytometry using monoclonal antibodies 12G5 (anti-CXCR4) and 358426 (anti-CXCR7). Values are expressed as fold increase of mean fluorescence intensity (MFI) compared to isotype control. The results are coherent with CXCR4 and CXCR7 mRNA expression in U373 cells (Figure S1C), detected by quantitative RT-PCR). Expression levels of the two receptors were normalized to the expression of the internal control GAPDH. Data shown are means of duplicates of three (flow cytometry) or two (Q-PCR) independent experiments and error bars correspond to standard error of mean.

Supplemental Figure S2A:

```
   CXCR7  NPVLYSFINRNYRYELMKA--FIFKYSAKTGLTKLIDASRVS-ETEYSALEQSTK
362
          NP+LY+F+   ++     A   + + S+   L+K       S  TE  +    +
   CXCR4  NPILYAFLGAKFKTSAQHALTSVSRGSSLKILSKGKRGGHSSVSTESESSSFHSS
352
```

Supplemental Figure 2A: Alignment of the C-termini of CXCR7 and CXCR4. The reciprocally swapped residues in the chimera are highlighted.

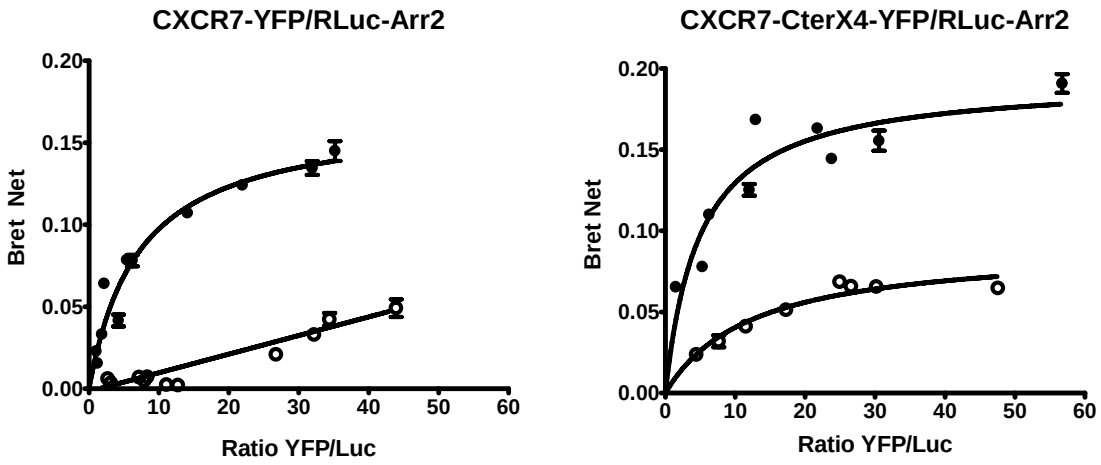
Supplemental Figure S2B:



Supplemental Figure 2B: Surface expression of chimeric receptors. HEK293 cells transiently expressing the receptors were stained with anti-CXCR4 or anti-CXCR7 monoclonal antibodies coupled to the fluorochrome allophycocyanin (APC) and receptor surface expression measured by flow cytometry (FACSCalibur, BD biosciences). Results from two independent experiments are expressed as mean fluorescence intensity (MFI).

Supplemental Figure 3:

Recruitment of β-arrestin by CXCL12 to CXCR4 and CXCR7 chimeras



Supplemental Figure 3: BRET [acceptor]/[donor] titrations. BRET [acceptor]/[donor] titrations between receptor-YFP and β-arrestin 2-RLuc fusions in the absence (open symbols) and presence of 100 nM CXCL12 (closed symbols). One representative out of four independent experiments, performed in triplicate, is shown. In the presence of the chemokine, the BRET values follow hyperbolic saturation curves, indicating specific interaction between the receptor and arrestin. Chimera CXCR7-Cter4 yielded a hyperbolic saturation curve also in the absence of the chemokine, whereas CXCR7 yielded straight lines in the absence of CXCL12, indicating the absence of specific interaction with arrestin. This effect was seen in all four experiments, with hyperbolic being the preferred fitting model over a straight line during simultaneous curve fitting in four out of four experiments with CXCR7-Cter4, suggesting constitutive recruitment of arrestin by this chimera. The $BRET_{50}$ of the curve yielded by the chimera in the absence of chemokine was consistently significantly lower than that of the curve in the presence of CXCL12 ($p<0.001$ in 3 out of 4 experiments, and $p<0.01$ in 1 out of 4), indicating that constitutive arrestin recruitment is further increased by the chemokine.