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(54) Title: GPCR HETEROMER INHIBITORS AND USES THEREOF

(57) Abstract: This invention relates to inhibitors of CXC receptor 4 (CXCR4)-G protein-coupled receptor (GPCR) heteromers (CXCR4-GPCR heteromers) associated with cancers, where CXCR4 forms a functional heteromer with other G protein-coupled receptors (GPCRx). More specifically, this invention relates to GPCRx that form heteromers with CXCR4, which upon co-stimulation with CXCR4 agonists and GPCRx agonists leads to enhanced signaling downstream of CXCR4. This invention also provides for the use of inhibitors of the interacting GPCR partner of the CXCR4-GPCRx heteromer or CXCR4-GPCRx heteromer-specific inhibitors including inhibitors of the formation of the CXCR4-GPCRx heteromer and CXCR4-GPCRx heteromer-specific antibodies, and in the diagnosis and/or therapy for cancer.

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Description

Title of Invention: GPCR HETEROMER INHIBITORS AND USES THEREOF

Technical Field

[1] This application claims the benefit of priority from U.S. Provisional Application No. 62/607,876, filed December 19, 2017, and further claims the benefit of priority from U.S. Provisional Application No. 62/679,598, filed June 1, 2018, and further claims the benefit of priority from U.S. Provisional Application No. 62/732,946, filed September 18, 2018. Each of the foregoing related applications, in their entirety, are incorporated herein by reference.

[2] In addition, each of the references identified herein, in their entirety, are incorporated herein by reference.

Background Art

[3] The invention disclosed herein relates generally to inhibitors of novel, functional GPCR heteromers, and more specifically to inhibitors of CXC receptor 4 (CXCR4)-G protein-coupled receptor (GPCR) heteromers that display enhanced signaling downstream of CXCR4 as a result of functional heteromer formation and that are associated with cancers and other diseases.

[4] G protein-coupled receptors (GPCRs) are seven-transmembrane domain cell surface receptors that are coupled to G proteins. GPCRs mediate diverse sensory and physiological responses by perceiving stimuli including light, odorants, hormones, neurotransmitters, chemokines, small lipid molecules, and nucleotides. There are approximately 800 GPCR genes in human genome, and more than half of them are predicted to encode sensory receptors such as olfactory, visual, and taste receptors (Bjarnadottir, et al., 2006). The remaining 350 GPCRs have physiologically important roles in embryonic development, behavior, mood, cognition, regulation of blood pressure, heart rate, and digestive processes, regulation of immune system and inflammation, maintenance of homeostasis, and growth and metastasis of cancers (Filmore 2004, Overington, et al., 2006). GPCRs are associated with many diseases and are the targets of approximately 40% of all prescription drugs (Filmore 2004).

[5] CXC receptor 4 (CXCR4) is a member of the chemokine receptor family GPCR. CXCR4 is expressed on most of the hematopoietic cell types, bone marrow stem cells, endothelial progenitor cells, vascular endothelial cells, neurons and neuronal stem cells, microglia and astrocytes (Klein and Rubin 2004, Griffith, et al., 2014). CXCR4 responds to its ligand C-X-C Motif Chemokine ligand 12 (CXCL12), also known as Stromal cell-derived factor 1 (SDF-1), and has essential roles in the embryonic de-

velopment of the hematopoietic, cardiovascular, and nervous systems (Griffith, et al., 2014). CXCR4 was discovered as a co-receptor for human immunodeficiency virus (HIV), and has important roles in the homing of hematopoietic stem cells (HSCs) to the bone marrow, inflammation, immune surveillance of tissues, and tissue re-generation in adult (Chatterjee, et al. 2014). Mutations in the C-terminus of CXCR4 cause persistent CXCR4 activation, leading to a congenital immune deficiency called WHIM syndrome (Warts, Hypogammaglobulinemia, Infections, and Myelokathexis) characterized by neutropenia and B cell lymphopenia (Hernandez, et al., 2003; Kawai, 2009). CXCR4 also has essential roles in T and B lymphocyte development within lymphoid organs and the thymus during development and in adult (Allen, et al., 2004; Ara, et al., 2003).

[6] CXCR4 is implicated in various immune and autoimmune diseases, such as HIV infection, ischaemia, wound healing, rheumatoid arthritis, systemic lupus ery-thematosus (SLE), interstitial pneumonias, vascular disease, multiple sclerosis, pulmonary fibrosis, and allergic airway disease (Chu et al., 2017; Debnath, et al., 2013; Domanska, et al., 2013). The involvement of CXCR4 in rheumatoid arthritis was demonstrated by the increased accumulation of CXCR4-positive T-cells in arthritic joints, and a reduction in collagen-induced arthritis in CXCR4-deficient mice (Buckley et al., 2000; Chung et al., 2010). Moreover, CXCR4 antagonist, AMD3100, alleviated collagen-induced arthritis significantly in a mouse model (De Klerck et al., 2005). CXCR4 also regulates pulmonary fibrosis by recruiting circulatory fibroblasts and bone marrow-derived progenitor cells during lung injury, and AMD3100 demonstrated a preventive effect in bleomycin-induced mouse pulmonary fibrosis (Song et al., 2010). CXCR4/CXCL12 axis is also implicated in the pathogenesis of SLE. In mouse models of lupus and patients with SLE, inflammatory cells such as monocytes, neu-trophils, and B-cells showed increased expression of CXCR4 and migrated toward skin and lung that overexpress CXCL12 predominantly (Chong and Mohan, 2009; Wang et al., 2009; Wang et al., 2010). CXCR4 antagonist, CTCE-9908, prolonged survival and greatly improved disease conditions and nephritis in a mouse model of lupus (Wang et al., 2009). Furthermore, CXCR4 is also involved in brain and cardiac diseases including brain injury, stroke, myocardial infarction, atherosclerosis and injury-induced vascular restenosis (Cheng et al., 2017; Domanska, et al., 2013; Doring, et al., 2014).

[7] The involvement of CXCR4 in cancer was first noticed when B cells from patients with chronic lymphocytic leukemia (B-CCL) express high levels of functional CXCR4 on the surface, showing enhanced calcium mobilization and actin polymerization upon CXCL12 exposure, and migration towards bone marrow stromal cells that secret CXCL12 (Burger, et al., 1999).

[8] CXCR4 is subsequently characterized to be responsible for breast cancer metastasis to organs that express higher levels of CXCL12 such as lymph nodes, bone marrow, lung, and liver (Muller, et al., 2001). After initial discovery, increasing evidence indicates that CXCR4 is associated with a variety of different cancers and has multiple potential roles in malignancy. CXCR4 is overexpressed in more than 23 human cancers, including breast cancer, lung cancer, brain cancer, kidney cancer (or renal cell carcinoma), pancreatic cancer, ovarian cancer, prostate cancer, melanoma, leukemia, multiple myeloma, gastrointestinal cancers, and soft tissue sarcomas, and regarded as a poor prognosis marker (Domanska, et al., 2013; Chatterjee, et al., 2014; Furusato et al., 2010). CXCR4 is the only chemokine receptor that is expressed by the majority of cancer types (Liang et al., 2015), and stimulates tumor cell growth, survival, and invasiveness in response to CXCL12 secreted by the cancer cells and the surrounding cancer-associated cells (Burger and Kipps, 2006; Chatterjee et al., 2014; Domanska et al., 2013).

[9] Systematic meta-analyses using databases including PubMed, EMBASE, and Cochrane library indicate significant association between CXCR4 over-expression and poorer progression-free survival and overall survival in various cancers including hematological malignancy, breast cancer, colorectal cancer, esophageal cancer, head and neck cancer, renal cancer, lung cancer, gynecologic cancer, liver cancer, prostate cancer, and gallbladder cancer (Du et al., 2015; Hu et al., 2015; Li et al., 2017; Wang et al., 2016; Zhao et al., 2015).

[10] CXCR4/CXCL12 axis plays a central role in tumor growth, invasion, angiogenesis, vasculogenesis, metastasis, drug resistance, and cancer cell-tumor microenvironment interaction (D'Alterio et al., 2012; Domanska et al., 2013; Guo et al., 2016)

[11] The critical role of CXCR4 in cancer cell proliferation and tumor growth was demonstrated in various experimental models *in vitro* and *in vivo* such as orthotopic, subcutaneous human xenograft, and transgenic mouse models using CXCR4 antagonists (Domanska et al., 2013). The Daoy medulloblastoma cells and U87 glioblastoma cells showed CXCR4 expression and exhibited dose-dependent increase in proliferation to a gradient of CXCL12 *in vitro*, and systemic administration of AMD3100 inhibited the growth of intracranial U87 and Daoy cell xenografts (Rubin et al., 2003).

[12] Involvement of CXCR4 in the metastasis of cancer cells towards CXCL12 expressing organs was also demonstrated in pancreatic, thyroid, melanoma, prostate, and colon cancer xenograft models (Bartolome et al., 2009; De Falco et al., 2007; Taichman et al., 2002; Wang et al., 2008; Zeelenberg et al., 2003).

[13] The main mechanism of action described for the small molecules or peptides antagonists of CXCR4 is centered on their ability to mobilize malignant cells from the

BM, thereby sensitizing them to chemotherapy. These agents have shown limitations regarding short half-lives, making their adequate management over long periods of time difficult (Hendrix et al., 2000). In contrast, therapeutic monoclonal antibodies have the advantage of having more prolonged half-lives, and are suitable for less frequent dosing. Additionally, human IgG antibodies have the ability to induce cell death upon binding to their target protein on cancer cells, via interaction with Fc-receptor on effector cells, including antibody-dependent cell mediated cytotoxicity/ phagocytosis (ADCC/ADCP) (Jiang et al., 2011). Such cytotoxic mechanism of action are not inherent to small molecules or peptides, and have been demonstrated to play a key role in the clinical activity of several therapeutic antibodies (Wang et al., 2015).

[14] Targeting CXCR4 using a neutralizing anti-CXCR4 antibody or CXCR4 specific antagonists inhibited primary tumor growth as well as metastasis to secondary organs in breast cancer, colon cancer, hepatocellular carcinoma, osteosarcoma, and melanoma (De Falco et al., 2007; Hassan et al., 2011; Huang et al., 2009; Kim et al., 2008; Muller et al., 2001; Schimanski et al., 2006; Smith et al., 2004; Zeelenberg et al., 2003). In a transgenic breast cancer mouse model, inhibition of CXCR4 with CTCE-9908 reduced not only the growth of primary tumor but also the expression of vascular endothelial growth factor (VEGF) and AKT phosphorylation (Hassan et al., 2011).

[15] Cancer stem cells (CSCs) are a population of cancer cells with properties such as infinite self-renewal, potential to differentiate into multiple cancer lineages, ability to adopt a quiescent state, and intrinsic high resistance to chemo- and radio-therapy. CSCs are considered as the major cause of cancer relapse and recurrence after standard anti-proliferative therapy. Therefore, targeting cancer stem cells are expected to provide more effective therapeutic interventions to eradicate the cancer and prevent relapse (Batlle and Clevers, 2017; Reya et al., 2001; Wurth, 2016). Interestingly, CSCs also express CXCR4, and CXCR4 directs the trafficking and metastasis of these cells to the CXCL12-rich microenvironments such as bone marrow and subventricular zone in the brain that favor cancer stem cell maintenance, survival and growth. CXCR4 antagonists have been shown to mobilize CSCs from these protective microenvironments, and sensitize them to conventional chemo- and radiotherapy, and anti-angiogenic therapy (Burger and Kipps, 2006; Burger and Peled, 2009; Furusato et al., 2010; Redondo-Munoz et al., 2006; Walenkamp et al., 2017; Wurth, 2016).

[16] Increasing evidence showed that tumor mass contains various cell types such as stromal fibroblasts, immune cells, endothelial cells, connective tissue, and extracellular matrix in addition to cancer cells that constitute tumor microenvironment (TME) or cancer cell niches. CXCR4/CXCL12 axis plays pivotal roles in tumor cell-microenvironment interaction that support tumor structure, growth, angiogenesis, and evasion of immune surveillance in various cancers of both the hematopoietic and non-

hematopoietic system (Burger and Kipps, 2006; Burger and Peled, 2009; Walenkamp et al., 2017). CXCL12 can promote tumor angiogenesis by recruiting endothelial cells to the TME directly, or indirectly by attracting CXCR4-positive inflammatory cells to the tumor mass, and making them to secret proangiogenic factors (Owen and Mohamadzadeh, 2013; Walenkamp et al., 2017).

[17] Growing evidence indicates that CXCR4/CXCL12 axis contributes to the lack of tumor responsiveness to angiogenesis inhibitors. Vascular endothelial growth factor (VEGF) was considered as the major pro-angiogenic factor in cancer, and was targeted for anti-angiogenic therapy in patients with rectal carcinoma using an anti-VEGF antibody bevacizumab (Genentech). Surprisingly, bevacizumab increased the expression of CXCL12 and CXCR4 in cancer cells, and increased plasma levels of CXCL12 in these patients were associated with rapid disease progression and metastasis (Owen and Mohamadzadeh, 2013; Xu et al., 2009). Therefore, the efficacy of a combination therapy using bevacizumab and plerixafor was evaluated for recurrent glioma (ClinicalTrials.gov identifier: NCT01339039). However, the study was terminated due to a low accrual rate (Walenkamp et al., 2017).

[18] Inhibition of CXCR4/CXCL12 axis has been demonstrated to disrupt the tumor microenvironment (TME) and expose the tumor cells to immune attack by decreasing the infiltration of myeloid-derived suppressor cells, by increasing the ratio of CD8+ cytotoxic T cells to Treg cells, or eliminating tumor re-vascularization (Burger et al., 2011; Domanska et al., 2013; Walenkamp et al., 2017). Administration of CXCR4 antagonists, AMD3100 or T22, acted synergistically with immune checkpoint inhibitors such as cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) antibodies, programmed cell death protein 1 (PD-1), and programmed cell death ligand 1 (PD-L1) antibodies and greatly increased their antitumor activity in a model of pancreatic ductal adenocarcinoma, advanced hepatocellular carcinoma (HCC), and CXCR4-transduced B16 melanoma (Chen et al., 2015; Feig et al., 2013; Lee et al., 2006; Scala, 2015; Walenkamp et al., 2017). These results indicate that targeting CXCR4/CXCL12 axis offers benefit to the conventional immune checkpoint inhibitors.

[19] Various drugs targeting CXCR4 have been developed (Peled, et al., 2012; Debnath, et al., 2013; Walenkamp, et al., 2017). CXCR4 inhibitors can be divided into 5 categories:

[20] (1) non-peptide small molecule antagonists, such as AMD3100 (plerixafor, Mozobil™, Genzyme (MA, USA); (Cashen et al., 2007; Donzella et al., 1998), AMD070 (AMD11070; Crawford and Alan Kaller, 2008; Stone et al., 2007), AMD3465 (Genzyme Corp., Biochem Pharmacol. 2009 Oct 15;78(8):993-1000; Bodart et al., 2009; Ling et al., 2013), GSK812397 (Jenkinson et al., 2010; Planesas et al., 2015), KRH-3955 (Murakami et al., 2009; Nakasone et al., 2013), KRH-1636

(Ichiyama et al., 2003), D-[Lys3] GHRP-6 (Patel et al., 2012), TG-0054 (Burixafor, TaiGen Biotechnology Co., Ltd.; de Nigris et al., 2012; Hsu et al., 2015), WZ811 (Zhan et al., 2007), MSX-122 (Metastatix, Inc.; Liang et al., 2012), and 508MCl (Compound 26; Zhu et al., 2010);

[21] (2) small-peptide antagonists, such as anti-HIV peptides T22 (Masuda et al., 1992), T134 and T140 (Tamamura et al., 1998), BKT140 (a/k/a BL-8040; TF14016; 4F-Benzoyl-TN14003, Biokine Therapeutics Ltd. (Rehovot, Israel); Fahham et al., 2012; Otani et al., 2012), ALX40-4C (Canadian company Allelix Biopharmaceuticals (ON, Canada); Doranz et al., 2001), GST-NT21MP (Yang et al., 2014), FC131 (Tanaka et al., 2009; Tanaka et al., 2008), FC122 (Inokuchi et al., 2011), POL6326 (de Nigris et al., 2012), and LY2510924 (Lilly) (Peng et al., 2015);

[22] (3) antibodies to CXCR4, such as ulocuplumab (MDX1338/BMS-936564; Kuhne et al., 2013), PF-06747143 (Pfizer; Liu et al., 2017), 12G5 (Endres et al., 1996), i-bodies (AD-114, AD-114-6H, AD-114-Im7-FH, and AD-114-PA600-6H; AdAlta; Griffiths et al., 2016; Griffiths et al., 2018), nanobodies (238D2 and 238D4; Jahnichen et al., 2010; Proc. Natl Acad. Sci. 2010, USA 107(47), 20565-20570), and ALX-0651 (Ablynx, biparatopic nanobody, ClinicalTrials.gov Identifier: NCT01374503);

[23] (4) ligand (CXCL12) analogs with suppressive activity, such as CTCE-9908 (Chemokine Therapeutics (BC, Canada); Wong et al., 2014); and

[24] (5) radiolabeled CXCR4 ligands, such as [99mTc]O2-AMD3100 (Hartimath et al., 2013), [68Ga]pentixafor (Demmer et al., 2011; Gourni et al., 2011), [177Lu]pentixather, and [90Y]pentixather (Herrmann et al., 2016).

[25] AMD3100 (JM 3100, plerixafor, marketed as Mozobil) is a CXCR4-specific small molecule antagonist that inhibits CXCL12-mediated calcium mobilization and chemotaxis in various cell types (Hatse, Princen et al. 2002), and prevents tumor growth in mouse xenograft models (Rubin, Kung et al. 2003, Cho, Yoon et al. 2013, Liao, Fu et al. 2015). AMD3100 was originally developed for HIV therapy (as an HIV entry blocker that specifically antagonizes CXCR4, one of the HIV entry co-receptors), but was approved by the U.S. Food and Drug Administration only for stem cell mobilization in patients with lymphoma and multiple myeloma in 2008 (Keating 2011). During the initial clinical trial of AMD3100 for HIV infection, this compound was noted to cause hematopoietic stem cell (HSC, $CD34^+$) mobilization from bone marrow to peripheral blood (Broxmeyer et al., 2005; De Clercq, 2003; Liles et al., 2003).

[26] Development of AMD3100 for HIV therapy was suspended due to significant side effects including thrombocytopenia, premature ventricular contractions, and leukocytosis following long-term inhibition of CXCR4/CXCL12 axis (Hendrix, et al., 2004; Peled, et al., 2012). Although investigating the possibility of the use of AMD3100 as an anticancer drug is underway, lack of oral availability and some severe

side effects accompanying with long-term use need to be overcome (Peled, et al., 2012). Instead, subcutaneous AMD3100 was approved in combination with G-CSF for autologous stem cell mobilization and transplantation for patients in the US with non-Hodgkin lymphoma (NHL) or multiple mylenoma for up to 4 consecutive days, and for patients in Europe with multiple mylenoma or lymphoma for 2-4 consecutive days and for up to 7 consecutive days, respectively (DiPersio et al., 2009a; DiPersio et al., 2009b; Keating, 2011). So far, AMD3100 is the only CXCR4 antagonist approved by the FDA.

[27] AMD3100 has been shown to inhibit CXCL12-mediated calcium mobilization and chemotaxis in various cancer cell types expressing CXCR4 (Hatse et al., 2002), and has demonstrated significant anti-tumor activity with decreased metastasis and increased overall survival in various mouse xenograft models (Burger et al., 2011; Chatterjee et al., 2014; Cho et al., 2013; Debnath et al., 2013; Domanska et al., 2013; Liao et al., 2015; Rubin et al., 2003; Walenkamp et al., 2017).

[28] However, AMD3100 exhibits partial agonism for CXCR4 *in vitro* and increases the proliferation of meloma cells (Kim et al., 2010; Zhang et al., 2002). AMD3100 also acts as a positive allosteric modulator of CXCR7, an alternative chemokine receptor for CXCL12, and increases CXCL12 binding to CXCR7 (Kalatskaya et al., 2009). Like CXCR4, CXCR7 is also highly expressed by many types of cancers and is associated with tumor metastasis (Decaillot et al., 2011; Zabel et al., 2011). Due to the complex properties of AMD3100, the exact role of AMD3100 on cancer needs to be investigated carefully.

[29] AMD3100 has been clinically evaluated as anticancer drug in combination with conventional cytotoxic drugs such as mitoxantrone, etoposide, cytrabine, daunorubicin, azacitidine, lenalidomide, decitabine, clofarabine, fludarabine, and/or idarubicin; receptor tyrosine kinase inhibitors AC220 and sorafenib; HSP90 inhibitor (Ganetespib); G-CSF; proteasome inhibitor (bortezomib); or monoclonal antibody (rituximab) in hematological malignancies including acute myeloid leukemia (AML), MM, Myelodysplastic Syndromes (MDS), CLL, and small lymphocytic lymphoma (SLL) (ClinicalTrials.gov Identifier: NCT00512252, NCT00694590, NCT00903968, NCT00990054, NCT01065129, NCT01373229, NCT01352650, NCT01236144, NCT01301963, NCT01160354, NCT01027923, NCT00943943, and NCT01435343). AMD3100 was examined for interrupting lymphoid tumor microenvironment communication (NCT01610999) and for chemosensitization in combination with other anticancer drugs in patients with relapsed or refractory AML, ALL, and MDS (ClinicalTrials.gov identifier: NCT00906945 and NCT01319864).

[30] AMD3100 has been or is also being evaluated in solid tumors including high grade glioma, Ewing's sarcoma, neuroblastoma, pancreatic, ovarian, and colorectal cancers

alone or in combination with angiogenesis inhibitor bevacizumab (ClinicalTrials.gov identifier: NCT01339039, NCT01977677, NCT01288573, NCT02179970, and NCT03277209).

[31] AMD3100 has been tested or is currently being evaluated for the mobilization of the hematopoietic stem/progenitor/precursor cells in patients with various hematological malignancies and diseases including AML, MDS, neutropenia, beta-thalassemia, sickle cell disease, WHIMS, Fanconi anemia, Wiskott-Aldrich Syndrome, systemic mastocytosis ((Domanska et al., 2013), NCT01058993, NCT01206075, NCT03226691, NCT00967785, NCT02678533, NCT03019809, and NCT00001756). It has been or currently being evaluated for recruiting CD34+ cells, endothelial progenitor cells (EPC), and/or CD117+ progenitor cells in patients with diabetes, wounds, cirtical limb ischemia, chronic obstructive pulmonary disease (COPD), cystic fibrosis, pulmonary fibrosis (ClinicalTrials.gov identifier: NCT02056210, NCT02790957, NCT01916577, NCT03182426).

[32] Although investigating the possibility of the use of AMD3100 as an anticancer drug is underway, lack of oral availability and some severe side effects accompanying with long-term use need to be overcome (Peled et al., 2012).

[33] The orally available CXCR4 antagonist, AMD070 (also referred to herein as AMD-070, AMD11070, AMD-11070, or X4P-001; X4 Pharmaceuticals) has demonstrate antitumor activity in various tumor models (Morimoto et al., 2016; O'Boyle et al., 2013; Parameswaran et al., 2011) and has been studied in a phase I/II clinical trial in HIV infected subjects (ClinicalTrials.gov identifier: NCT00089466, (Debnath et al., 2013). AMD070 is now in Phase II/III clinical trial in patients with WHIM syndrome, advanced melanoma, and renal cell carcinoma alone or together with pembrolizumab, nivolumab, or axitinib (ClinicalTrials.gov identifier: NCT03005327, NCT02823405, NCT02923531, and NCT02667886).

[34] The efficacy of small peptide antagonists of CXCR4, T140 and its analogs TN14003, TC14012, and BKT140 (also referred to herein as BL-8040, TF14016, 4F-Benzoyl-TN14003, BioLineRx, Ltd.) for blocking CXCR4 has been demonstrated in numerous preclinical studies including stem cell mobilization, small-cell lung cancer (SCLC), breast cancer, melanoma, AML, chronic myeloid leukemia, MM, pancreatic cancer, and rheumatoid arthritis (Burger et al., 2011). Currently, BKT140 is under clinical development for hematopoietic stem and progenitor cell mobilization in various hematopoietic malignancies (ClinicalTrials.gov identifier: NCT02502968, NCT03154827, NCT02763384, NCT02639559, and NCT02462252). In addition, BKT140 is in Phase II clinical trials for interfering tumor microenvironment interaction in subjects with AML in combination with cytarabine (ClinicalTrials.gov identifier: NCT02502968). It is also investigated in various cancers such as AML,

gastric cancer, non-small-cell lung cancer (NSCLC), and metastatic pancreatic cancers together with immune checkpoint inhibitors such as Pembrolizumab (Keytruda, Merck) and Atezolizumab (Tecentriq, Genentech/Roche) (ClinicalTrials.gov Identifier: NCT03154827, NCT03281369, NCT03337698, NCT02907099, NCT03193190, and NCT02826486).

[35] Similarly, CTCE-9908 (Chemokine Therapeutics Corp., Canada), a peptide inhibitor of CXCR4, reduced metastases in mouse models of osteosarcoma and melanoma (Kim et al., 2008). After phase I study in patients with advanced solid cancers, CTCE-9908 was designated as an orphan drug for the treatment of osteosarcoma by the FDA in 2005. There is no further development after phase I/II clinical trial has been completed in 2008 (Debnath, et al., 2013).

[36] MSX-122 (Altiris Therapeutics), a small molecule CXCR4 antagonist, has been abandoned after clinical trial in patients with solid tumors (ClinicalTrials.gov identifier: NCT00591682). Two major setbacks have been reported in 2017 in the field of developing CXCR4 antagonists as anti-cancer drugs. Bristol-Myers Squibb (BMS) discontinued a phase I/II study of ulocuplumab (BMS-936564 / MDX1338, (Kuhne et al., 2013), a fully human anti-CXCR4 antibody, in patients with solid tumors for lack of efficacy (https://seekingalphacom/article/4057548-bristol-failure-makes-small-dent-cxcr4-blocking-approach?page=2). Also, Eli Lilly decided to abandon a few anti-cancer programs including LY2510924, which is a CXCR4 peptide antagonist (http://wwwfiercebiotechcom/biotech/lilly-puts-two-thirds-mid-phase-cancer-pipeline-up-for-sale-major-shake-up-r-d-priorities) after a series of clinical trials in solid tumors (ClinicalTrials.gov identifier: NCT02737072, NCT01391130, and NCT01439568). These events indicate that development of CXCR4 antagonists as anti-cancer drugs, especially for solid tumors, is an ongoing challenge.

[37] Other small molecule antagonists that are currently under clinical investigation, include:

[38] USL311 (Upsher-Smith) is under phase I and phase II in patients with solid tumors and glioblastoma multiforme (GBM), respectively (ClinicalTrials.gov identifier: NCT02765165); GMI-1359 (Glycomimetics) is in phase I trial (ClinicalTrials.gov identifier: NCT02931214).

[39] PF-06747143 (Pfizer), a humanized anti-CXCR4 antibody, is in phase I clinical trial in patients with AML either alone or combined with chemotherapy (ClinicalTrials.gov identifier: NCT02954653, (Liu et al., 2017)).

[40] AD-114 (i-body, AdAlta; (Griffiths et al., 2018) is a humanized shark anti-CXCR4 antibody and received orphan drug designation from united states FDA for a drug candidate used for treatment of Idiopathic Pulmonary Fibrosis in 2017

(https://www.reuters.com/article/idUSFWN1F70Y0). AD-114 study is mainly focused on fibrotic conditions, including wet age-related macular degeneration and non-alcoholic fatty liver disease (https://lungdiseasenews.com/2017/08/23/adalta-present-research-on-investigative-therapy-ad-114-at-ipf-summit/).

[41] POL6326 (balixafortide, Polyphor), a peptide CXCR4 antagonist, has been under clinical evaluation in subjects with breast cancer (ClinicalTrials.gov identifier: NCT01837095), hematologic malignancies (ClinicalTrials.gov identifier: NCT01413568), and stem cell mobilization and healing in patients with acute myocardial infarction (ClinicalTrials.gov identifier: NCT01905475).

[42] Radiolabeled CXCR4 ligands such as [99mTc]O2-AMD3100 and [68Ga]Pentixafor has been used for preclinical or clinical SPECT or PET imaging of CXCR4 expression (Demmer et al., 2011; Gourni et al., 2011; Hartimath et al., 2013; Walenkamp et al., 2017). PET imaging with [68Ga]Pentixafor has demonstrated increased expression of CXCR4 not only in hematologic and solid tumors such as leukemia, lymphoma, MM, adrenocortical carcinoma, and SCLC, but also in other pathological conditions such as splenosis, stroke, atherosclerosis, and myocardial infartion (Walenkamp et al., 2017).

[43] A peptide CXCR4 ligand labeled with α- or β-emitters ([177Lu]pentixather and [90Y]pentixather) has been tested in more than 30 therapies as CXCR4-directed endoradiotherapy together with standard chemotherapy in patients with hematologic malignancies (Walenkamp et al., 2017).

[44] Although various drugs antagonizing CXCR4 or antibody targeting CXCR4 have been developed and are under investigation, be it alone, in combination with conventional anticancer therapies, or with an immune checkpoint inhibitor, success has been limited so far (Peled, et al., 2012, Debnath, et al., 2013, Walenkamp, et al., 2017). Due to the pivotal roles of CXCR4 in B and T lymphocyte development and immune surveillance, long-term or persistent inhibition of CXCR4 with CXCR4 antagonist would potentially evoke immune system and hematopoietic dysfunctions and expose cancer patients to risk of immune suppression (Burger, 2009). Hematopoietic stem cells (HSCs) are normally protected in bone marrow niches. If CXCR4 antagonists are administered with cytotoxic drugs or radiotherapy, HSCs mobilized into periphery would be exposed to the effects of cytotoxic treatment which could exacerbate cytopenias. Cardiac complications observed in patients treated with AMD3100 also raised general concern in prolonged use of CXCR4 antagonists as anticancer drugs.

[45] To avoid potential side effects associated with conventional CXCR4 antagonists and to develop more efficient anti-cancer drugs targeting CXCR4, new paradigm for designing CXCR4 inhibitor is urgently required.

[46] Recently, GPCR heteromers offer new possibilities for developing more specific and

disease-restricted therapeutics. Traditionally, GPCRs were considered to be monomeric since monomeric GPCRs can activate G proteins by inducing conformational changes of the heptahelical domain upon ligand binding (Pin et al., 2008; Okada et al., 2001). However, increasing evidence demonstrates that GPCRs may form oligomers, either homo- or hetero-oligomers, and GPCR heteromers can exhibit specific properties that clearly distinguish them from existing well-defined monomers, such as signaling pathways, ligand binding affinities, internalization, and recycling (De Falco et al., 2007; Ferre et al., 2010; Gomes et al., 2016). Therefore, GPCR oligomerization may provide a way to increase the diversity of GPCR entities with a limited number of genes (Park and Palczewski, 2005). Thus, identification of novel GPCR heteromers would offer new possibilities for understanding the roles of GPCR heteromers in specific tissues and in specific diseases, as well as providing new opportunities for developing more efficient therapeutics with less side effects. (Milligan 2008; Rozenfeld and Devi 2010; Gomes et al., 2016; Farran 2017).

[47] CXCR4 also forms heteromers with different GPCRs. So far CXCR4 is known to interact with GPCRs in the chemokine receptor family (CCR2 (Rodriguez-Frade et al., 2004; Sohy et al., 2007; Sohy et al., 2009; Armando et al., 2014), CCR5 (Agrawal et al., 2004; Rodriguez-Frade et al., 2004; Sohy et al., 2007, Sohy et al., 2009; Martinez-Munoz et al., 2014), CXCR3 (Watts et al., 2013), and CXCR7 (Sierro et al., 2007; Levoye et al., 2009; Decaillot et al., 2011)), chemerin chemokine-like receptor 1 (CMKLR1) (de Poorter et al., 2013), δ-opioid receptor (OPRD) (Pello et al. 2008; Burbassi et al., 2010), and adrenergic receptor family (ADRA1A (Tripathi et al., 2015), ADRA1B (Tripathi et al., 2015), and ADRB2 (LaRocca et al. 2010; Nakai et al., 2014)).

[48] The existence of CXCR4-CCR2 and CXCR4-CCR5 heteromers was first noticed by studying HIV infection. Rodriguez-Frade et al. showed that CCR2-01, a CCR2-specific monoclonal antibody, did not compete with CCL2 for binding to CCR2 or trigger CCR2 signaling, but blocked replication of monotropic (R5) and T-tropic (X4) HIV strains by inducing oligomerization of CCR2 with CCR5 or CXCR4 (Rodriguez-Frade et al., 2004). Agrawal et al. also showed that CCR5D32 specifically inhibited CCR5 and CXCR4 cell surface expression by heteromerization with CCR5 and CXCR4, thereby inhibiting HIV coreceptor activity of CCR5 and CXCR4 in CD4+ cells (Agrawal et al., 2004). Co-expression of CCR5 was shown to prevent HIV-1 gp120 binding to the cell surface and to reduce X4 HIV-1 infectivity by inducing CCR5-CD4-CXCR4 oligomerization and conformational changes in CD4 and CXCR4 (Martinez-Munoz et al., 2014).

[49] Sohy et al. reported the existence of negative binding cooperativity between the subunits of CXCR4-CCR2 and CXCR4-CCR5 heteromers, i.e. the ligands of one

receptor competed for the binding of a specific tracer of the other, in recombinant cell lines and primary leukocytes (Sohy et al., 2007; Sohy et al., 2009). They also demonstrated that TAK-779, the CCR2 and CCR5 antagonist, prevented calcium mobilization and chemotaxis initiated by the CXCR4 agonist CXCL12 in cells co-expressing CCR2 and CXCR4 or CCR5 and CXCR4 (Sohy et al., 2007; Sohy et al., 2009). Armando et al. showed that CXCR4 and CCR2 can form homo- and hetero-oligomers, and co-activation of CCR2 and CXCR4 with the human monocyte chemotactic protein 1 (MCP-1) and CXCL12 caused a synergistic increase in calcium mobilization (Armando et al., 2014).

[50] Watts et al. identified CXCR4-CXCR3 heteromers in HEK293T cells and showed negative binding cooperativity for endogenous agonists and small CXCR3 agonist VUF10661, but not for CXCR3 antagonists VUF10085 nor CXCR4 antagonist AMD3100 (Watts et al., 2013).

[51] CXCR4 also interacts with ACKR3, also known as CXCR7, a chemokine family GPCR that binds the CXCL12 and CXCL11, but is unable to couple with G proteins upon agonist binding. Sierro et al. identified CXCR4-ACKR3 heteromers as functional heteromers that displayed enhanced CXCL12-induced calcium signaling and altered ERK1/2 signaling in HEK293 cells (Sierro et al., 2007). Lovoye et al., reported an opposite result showing that CXCR4-ACKR3 heteromers exhibited reduced Gαi and calcium responses upon exposure to CXCL12 in CHO-K1 cells (Levoye et al., 2009). Decaillot et al. noticed that co-expression of CXCR4 and ACKR3 constitutively recruited β-arrestin to CXCR4/ACKR3 heteromers and potentiated CXCL12-mediated β-arrestin-dependent downstream signaling pathways, including ERK1/2, p38 MAPK, and enhanced cell migration upon exposure to CXCL12 (Decaillot et al., 2011).

[52] CXCR4 also interacts with the chemerin chemokine-like receptor 1 (CMKLR1, also known as ChemR23) (de Poorter et al., 2013). Although CXCR4-CMKLR1 heteromers display negative agonist binding cooperativity similar to CXCR4-CCR2 and CXCR4-CCR5 heteromers reported by Sohy et al. (Sohy et al., 2007; Sohy et al., 2009), AMD3100 did not cross inhibit chemerin binding or inhibit calcium mobilization induced by CXCL12 (de Poorter et al., 2013).

[53] CXCR4 and the δ-opioid receptor (DOR) are widely distributed in brain tissues and immune cells. Pello et al. reported that CXCR4 and DOR can form heteromers, and simultaneous stimulation with both agonists suppressed the activation of Gαi signaling, and prevented cell migration toward CXCL12, although individual agonist elicit robust Gαi signaling (Pello et al., 2008). Burbassi et al. also noticed increased CXCR4-DOR heteromers and reduced coupling of CXCR4 to G-proteins in brain tissue and cultured glia from μ-opioid receptor (MOR)-deficient mice (Burbassi et al., 2010). CXCR4 function was rescued with a DOR antagonist, indicating that DOR is involved in the

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suppression of CXCR4 in glia by forming CXCR4-DOR heteromers (Burbassi et al., 2010).

[54] CXCR4 is also known to interact with α- and β-adrenergic receptors (a-AR and β-AR). LaRocca et al. reported that stimulation of CXCR4 with CXCL12 negatively regulates β-AR-induced cAMP accumulation and PKA-dependent phosphorylation of phospholamban in adult rat ventricular myocytes using non-selective β-AR agonist, isoproterenol (LaRocca et al., 2010). They showed the co-expression of CXCR4 and ADRB2 (β2-AR) on the cardiac myocytes, and physical association of CXCR4 with ADRB2 using co-immunoprecipitation and bioluminescence resonance energy transfer, suggesting the CXCR4-ADRB2 heteromer as a novel cardiac modulator.

[55] Nakai et al. studied the function of ADRB2 on lymphocytes. Stimulation of lymphocytes with ADRB2-selective agonists suppressed egress of lymphocytes from lymph nodes and produced lymphopenia in mice (Nakai et al., 2014). ADRB2 physically interacted with CCR7 and CXCR4, and activation of ADRB2 enhanced retention-promoting signals through CCR7-ADRB2 and CXCR4-ADRB2 heteromers, and subsequently reduced lymphocyte egress from lymph nodes.

[56] Tripathi et al. revealed the presence of CXCR4-ADRA1A (α1A-AR) and CXCR4-ADRA1B (α1B-AR) heteromers on the surface of vascular smooth muscle cells (VSMC) (Tripathi et al., 2015). A peptide derived from the second transmembrane helix of CXCR4 disrupted the interaction between ADRA1A/B and CXCR4, inhibited calcium mobilization and contraction of VSMC upon α1-AR stimulation. Activation of CXCR4 with CXCL12 increased the potency of α1-AR agonists on blood pressure response in rats, suggesting that CXCR4-ADRA1A/B heteromers could be a novel pharmacological target for blood pressure regulation.

[57] CXCR4 was reported to interact with CNR2 (Cannabinoid Receptor 2, aka CB2) (Coke et al., 2016; Scarlett et al., 2018). Simultaneous activation of CXCR4 and CB2 with both agonists resulted in reduction of ERK1/2 activation, calcium mobilization and cellular chemotaxis. These results show that cannabinoid system can negatively modulate CXCR4 function and tumor progression.

[58] As described above, GPCRs forming heteromers with CXCR4 have been studied within a limited number of GPCR families, such as chemokine, adrenergic, and opioid receptor families. Considering the major role and increased expression of CXCR4 in a variety of pathological conditions, there is a great potential for the existence of different CXCR4-GPCRx heteromers that confer unique features to a specific disease. However, due to the large number of GPCR genes (about 800) and difficulties in es-tablishing high-throughput proximity-based screening techniques and functional assays, the identification of new CXCR4-GPCRx heteromers has been a major challenge.

[58a] Any discussion of documents, acts, materials, devices, articles or the like which has been included in the present specification is not to be taken as an admission that any

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or all of these matters form part of the prior art base or were common general knowledge in the field relevant to the present disclosure as it existed before the priority date of each of the appended claims.

Disclosure of Invention

[59] Thus, there exists in the art a need for identifying functional GPCR heteromers, such as CXCR4-GPCRx, and developing their inhibitors for use as GPCR heteromer-targeting cancer therapeutics, preferably with higher efficacy and lower side effects.

[59a] In a first aspect, there is provided method for treating a cancer in a subject having a CXCR4-GPCRx heteromer, said method including the step of administering to the subject a therapeutically effective amount of a CXCR4 inhibitor and a GPCRx inhibitor, wherein:

(i) said CXCR4-GPCRx heteromer has enhanced signaling downstream of CXCR4; and

(ii) said CXCR4 inhibitor and said GPCRx inhibitor suppress the enhanced signaling downstream of CXCR4 from said CXCR4-GPCRx heteromer in the subject;

wherein said CXCR4 inhibitor is selected from the group consisting of: AMD3100 (plerixafor), ulocuplumab (MDX1338/BMS-936564), BKT140 (BL-8040; TF14016; 4F-Benzoyl- TN14003), AD-114, AD-114-6H, AD-114-Im7-FH, AD-114-PA600-6H, ALX-0651, ALX40-4C, AMD070 (AMD11070, X4P-001), AMD3465, ATI 2341, CTCE-9908, CX549, D-[Lys3] GHRP-6, FC122, FC131, GMI-1359, GSK812397, GST-NT21MP, isothiourea-1a, isothiourea-1t (IT1t), KRH-1636, KRH-3955, LY2510924, LY2624587, MSX-122, N-[11C]Methyl-AMD3465, PF- 06747143, POL6326, SDF-1 1-9[P2G] dimer, SDF1 P2G, T134, T140, T22, TC 14012, TG-0054 (Burixafor), USL311, viral macrophage inflammatory protein-II (vMIP-II), WZ811, 12G5, 238D2, 238D4, [64Cu]-AMD3100, [64Cu]-AMD3465, [68Ga]pentixafor, [90Y]pentixather, [99mTc]O2- AMD3100, [177Lu]pentixather, and 508MCl (Compound 26);

wherein said GPCRx inhibitor is an inhibitor selected from the group consisting of:

an ADRB2 inhibitor selected from the group consisting of Alprenolol, atenolol, betaxolol, bupranolol, butoxamine, carazolol, carvedilol, CGP 12177, cicloprolol, ICI 118551, ICYP, labetalol, levobetaxolol, levobunolol, LK 204-545, metoprolol, nadolol, NIHP, NIP, propafenone, propranolol, sotalol, SR59230A, and timolol,

an HRH1 inhibitor selected from the group consisting of (-)-chlorpheniramine, (+)-chlorpheniramine, (-)-*trans*-H2-PAT, (+)-*cis*-H2-PAT, (+)-*trans*-H2-PAT, (±)-*cis*-H2-PAT, (±)-*trans*- H2-PAT, (*R*)-cetirizine, (*S*)-cetirizine, 9-OH-risperidone, A-317920, A-349821, ABT-239, alimemazine, amitriptyline, aripiprazole, arpromidine, asenapine, astemizole, AZD3778, azelastine, BU-E 47, cetirizine, chlorpheniramine,

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chlorpromazine, ciproxifan, clemastine, clobenpropit, clozapine, conessine, cyclizine, cyproheptadine, desloratadine, diphenhydramine, dosulepin, doxepin, epinastine, fexofenadine, fluphenazine, fluspirilene, haloperidol, hydroxyzine, impromidine, INCB-38579, JNJ-39758979, ketotifen, loratadine, loxapine, MK- 0249, molindone, olanzapine, perphenazine, pimozide, pipamperone, pitolisant, promethazine, pyrilamine, quetiapine, risperidone, sertindole, terfenadine, thioridazine, thiothixene, trifluoperazine, tripelennamine, triprolidine, ziprasidone, and zotepine,

a TACR3 inhibitor selected from the group consisting of [Trp7, β-Ala8] neurokinin A-(4- 10), AZD2624, FK 224, GR138676, GSK 172981, GSK 256471, N',2-diphenylquinoline-4-carbohydrazide 8m, N′,2-diphenylquinoline-4-carbohydrazide, osanetant, PD 154740, PD 161182, PD157672, saredutant, SB 218795, SB 222200, SB 235375, SCH 206272, SSR 146977, and talnetant,

an ADCYAP1R1 inhibitor selected from the group consisting of M65, Max.d.4, MK-0893, N-stearyl-[Nle17] neurotensin-(6-11)/VIP-(7-28), PACAP-(6-38), and PG 97-269,

an ADORA2B inhibitor selected from the group consisting of 3-isobutyl-8-pyrrolidinoxanthine, alloxazine, AS16, AS70, AS74, AS94, AS95, AS96, AS99, AS100, AS101, ATL802, BW-A1433, caffeine, CGS 15943, CPX, CSC, CVT-6883, DAX, DEPX, derenofylline, DPCPX, FK-453, I-ABOPX, istradefylline, KF26777, LAS38096, LUF5981, MRE 2029F20, MRE 3008F20, MRS1191, MRS1220, MRS1523, MRS1706, MRS1754, MSX-2, OSIP339391, pentoxifylline, preladenant, PSB-10, PSB-11, PSB36, PSB603, PSB-0788, PSB1115, rolofylline, SCH 58261, SCH442416, ST-1535, theophylline, tonapofylline, vipadenant, xanthine amine congener, XCC, and ZM-241385,

an ADORA3 inhibitor selected from the group consisting of ATL802, BW-A1433, caffeine, CGS 15943, CSC, CVT-6883, derenofylline, dexniguldipine, DPCPX, FK-453, flavanone, flavone, galangin, I-ABOPX, istradefylline, KF26777, LAS38096, LUF5981, MRE 2029F20, MRE 3008F20, MRE 3010F20, MRS1041, MRS1042, MRS1067, MRS1088, MRS1093, MRS1097, MRS1177, MRS1186, MRS1191, MRS1191, MRS1220, MRS1476, MRS1486, MRS1505, MRS1523, MRS1754, MRS928, MSX-2, nicardipine, preladenant, PSB-10, PSB-11, PSB36, PSB603, PSB1115, rolofylline, sakuranetin, SCH 58261, SCH442416, ST-1535, theophylline, tonapofylline, vipadenant, visnagin, VUF5574, VUF8504, VUF8507, xanthine amine congener, and ZM-241385,

a C5AR1 inhibitor selected from the group consisting of A8Δ71-73, AcPhe-Orn-Pro-D-Cha-Trp-Arg, avacopan, C089, CHIPS, DF2593A, JPE1375, L-156,602, NDT9520492, *N*-methyl-Phe-Lys-Pro-D-Cha-Trp-D-Arg-CO_2H, PMX205, PMX53, RPR121154, and W54011,

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a CALCR inhibitor selected from the group consisting of α-CGRP-(8-37) (human), AC187, CT-(8-32) (salmon), and olcegepant,

a CHRM1 inhibitor selected from the group consisting of 3-Quinuclidinyl benzilate (QNB), 4-DAMP, aclidinium, AE9C90CB, AFDX384, amitriptyline, AQ-RA 741, atropine, benzatropine, biperiden, darifenacin, dicyclomine, dosulepin, ethopropazine, glycopyrrolate, guanylpirenzepine, hexahydrodifenidol, hexahydrosiladifenidol, hexocyclium, himbacine, ipratropium, lithocholylcholine, methoctramine, ML381, muscarinic toxin 1, muscarinic toxin 2, muscarinic toxin 3, N-methyl scopolamine, otenzepad, oxybutynin, *p*-F-HHSiD, pirenzepine, propantheline, (R,R)-quinuclidinyl-4-fluoromethyl-benzilate, scopolamine, silahexocyclium, solifenacin, telenzepine, tiotropium, tolterodine, trihexyphenidyl, tripitramine, UH-AH 37, umeclidinium, and VU0255035,

an EDNRB inhibitor selected from the group consisting of A192621, ambrisentan, atrasentan, bosentan (RO 470203, Tracleer), BQ788, IRL 2500, K-8794, macitentan, RES7011, Ro 46-8443, SB209670, SB217242 (enrasentan), TAK 044, and tezosentan (RO610612),

an MLNR inhibitor selected from the group consisting of GM-109, MA-2029, and OHM- 11526, and

an NTSR1 inhibitor selected from the group consisting of Meclinertant, SR48527, SR48692, and SR142948A, and

wherein the GPCRx of the subject having a CXCR4-GPCRx heteromer is selected from the group consisting of ADRB2, HRH1, TACR3, ADCYAP1R1, ADORA2B, ADORA3, C5AR1, CALCR, CHRM1, EDNRB, MLNR, and NTSR1, and wherein the GPCRx which the GPCRx inhibitor targets is the same as that of the subject having a CXCR4-GPCRx heteromer.

[59b] In a second aspect, the present provides relates to the use of a GPCRx inhibitor in the manufacture of a medicament for treating a cancer in a subject having a CXCR4-GPCRx heteromer, wherein the GPCRx inhibitor is formulated to be administered in combination with a CXCR4 inhibitor, wherein:

(i) said CXCR4-GPCRx heteromer has enhanced signaling downstream of CXCR4; and

(ii) said CXCR4 inhibitor and said GPCRx inhibitor suppress the enhanced signaling downstream of CXCR4 from said CXCR4-GPCRx heteromer in the subject;

wherein said CXCR4 inhibitor is selected from the group consisting of: AMD3100 (plerixafor), ulocuplumab (MDX1338/BMS-936564), BKT140 (BL-8040; TF14016; 4F-Benzoyl- TN14003), AD-114, AD-114-6H, AD-114-Im7-FH, AD-114-PA600-6H, ALX-0651, ALX40-4C, AMD070 (AMD11070, X4P-001), AMD3465, ATI 2341,

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CTCE-9908, CX549, D-[Lys3] GHRP-6, FC122, FC131, GMI-1359, GSK812397, GST-NT21MP, isothiourea-1a, isothiourea-1t (IT1t), KRH-1636, KRH-3955, LY2510924, LY2624587, MSX-122, N-[11C]Methyl-AMD3465, PF- 06747143, POL6326, SDF-1 1-9[P2G] dimer, SDF1 P2G, T134, T140, T22, TC 14012, TG-0054 (Burixafor), USL311, viral macrophage inflammatory protein-II (vMIP-II), WZ811, 12G5, 238D2, 238D4, [64Cu]-AMD3100, [64Cu]-AMD3465, [68Ga]pentixafor, [90Y]pentixather, [99mTc]O2- AMD3100, [177Lu]pentixather, and 508MCl (Compound 26);

wherein said GPCRx inhibitor is an inhibitor selected from the group consisting of:

an ADRB2 inhibitor selected from the group consisting of Alprenolol, atenolol, betaxolol, bupranolol, butoxamine, carazolol, carvedilol, CGP 12177, cicloprolol, ICI 118551, ICYP, labetalol, levobetaxolol, levobunolol, LK 204-545, metoprolol, nadolol, NIHP, NIP, propafenone, propranolol, sotalol, SR59230A, and timolol,

an HRH1 inhibitor selected from the group consisting of (-)-chlorpheniramine, (+)-chlorpheniramine, (-)-trans-H2-PAT, (+)-cis-H2-PAT, (+)-trans-H2-PAT, (±)-cis-H2-PAT, (±)-trans- H2-PAT, (R)-cetirizine, (S)-cetirizine, 9-OH-risperidone, A-317920, A-349821, ABT-239, alimemazine, amitriptyline, aripiprazole, arpromidine, asenapine, astemizole, AZD3778, azelastine, BU-E 47, cetirizine, chlorpheniramine, chlorpromazine, ciproxifan, clemastine, clobenpropit, clozapine, conessine, cyclizine, cyproheptadine, desloratadine, diphenhydramine, dosulepin, doxepin, epinastine, fexofenadine, fluphenazine, fluspirilene, haloperidol, hydroxyzine, impromidine, INCB-38579, JNJ-39758979, ketotifen, loratadine, loxapine, MK- 0249, molindone, olanzapine, perphenazine, pimozide, pipamperone, pitolisant, promethazine, pyrilamine, quetiapine, risperidone, sertindole, terfenadine, thioridazine, thiothixene, trifluoperazine, tripelennamine, triprolidine, ziprasidone, and zotepine,

a TACR3 inhibitor selected from the group consisting of [Trp7, β-Ala8] neurokinin A-(4- 10), AZD2624, FK 224, GR138676, GSK 172981, GSK 256471, N',2-diphenylquinoline-4-carbohydrazide 8m, N′,2-diphenylquinoline-4-carbohydrazide, osanetant, PD 154740, PD 161182, PD157672, saredutant, SB 218795, SB 222200, SB 235375, SCH 206272, SSR 146977, and talnetant,

an ADCYAP1R1 inhibitor selected from the group consisting of M65, Max.d.4, MK-0893, N-stearyl-[Nle17] neurotensin-(6-11)/VIP-(7-28), PACAP-(6-38), and PG 97-269,

an ADORA2B inhibitor selected from the group consisting of 3-isobutyl-8-pyrrolidinoxanthine, alloxazine, AS16, AS70, AS74, AS94, AS95, AS96, AS99, AS100, AS101, ATL802, BW-A1433, caffeine, CGS 15943, CPX, CSC, CVT-6883, DAX, DEPX, derenofylline, DPCPX, FK-453, I-ABOPX, istradefylline, KF26777, LAS38096, LUF5981, MRE 2029F20, MRE 3008F20, MRS1191, MRS1220,

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MRS1523, MRS1706, MRS1754, MSX-2, OSIP339391, pentoxifylline, preladenant, PSB-10, PSB-11, PSB36, PSB603, PSB-0788, PSB1115, rolofylline, SCH 58261, SCH442416, ST-1535, theophylline, tonapofylline, vipadenant, xanthine amine congener, XCC, and ZM-241385,

an ADORA3 inhibitor selected from the group consisting of ATL802, BW-A1433, caffeine, CGS 15943, CSC, CVT-6883, derenofylline, dexniguldipine, DPCPX, FK-453, flavanone, flavone, galangin, I-ABOPX, istradefylline, KF26777, LAS38096, LUF5981, MRE 2029F20, MRE 3008F20, MRE 3010F20, MRS1041, MRS1042, MRS1067, MRS1088, MRS1093, MRS1097, MRS1177, MRS1186, MRS1191, MRS1191, MRS1220, MRS1476, MRS1486, MRS1505, MRS1523, MRS1754, MRS928, MSX-2, nicardipine, preladenant, PSB-10, PSB-11, PSB36, PSB603, PSB1115, rolofylline, sakuranetin, SCH 58261, SCH442416, ST-1535, theophylline, tonapofylline, vipadenant, visnagin, VUF5574, VUF8504, VUF8507, xanthine amine congener, and ZM-241385,

a C5AR1 inhibitor selected from the group consisting of A8Δ71-73, AcPhe-Orn-Pro-D-Cha-Trp-Arg, avacopan, C089, CHIPS, DF2593A, JPE1375, L-156,602, NDT9520492, N-methyl-Phe-Lys-Pro-D-Cha-Trp-D-Arg-CO2H, PMX205, PMX53, RPR121154, and W54011,

a CALCR inhibitor selected from the group consisting of α-CGRP-(8-37) (human), AC187, CT-(8-32) (salmon), and olcegepant,

a CHRM1 inhibitor selected from the group consisting of 3-Quinuclidinyl benzilate (QNB), 4-DAMP, aclidinium, AE9C90CB, AFDX384, amitriptyline, AQ-RA 741, atropine, benzatropine, biperiden, darifenacin, dicyclomine, dosulepin, ethopropazine, glycopyrrolate, guanylpirenzepine, hexahydrodifenidol, hexahydrosiladifenidol, hexocyclium, himbacine, ipratropium, lithocholylcholine, methoctramine, ML381, muscarinic toxin 1, muscarinic toxin 2, muscarinic toxin 3, N-methyl scopolamine, otenzepad, oxybutynin, p-F-HHSiD, pirenzepine, propantheline, (R,R)-quinuclidinyl-4-fluoromethyl-benzilate, scopolamine, silahexocyclium, solifenacin, telenzepine, tiotropium, tolterodine, trihexyphenidyl, tripitramine, UH-AH 37, umeclidinium, and VU0255035,

an EDNRB inhibitor selected from the group consisting of A192621, ambrisentan, atrasentan, bosentan (RO 470203, Tracleer), BQ788, IRL 2500, K-8794, macitentan, RES7011, Ro 46-8443, SB209670, SB217242 (enrasentan), TAK 044, and tezosentan (RO610612),

an MLNR inhibitor selected from the group consisting of GM-109, MA-2029, and OHM- 11526, and

an NTSR1 inhibitor selected from the group consisting of Meclinertant, SR48527, SR48692, and SR142948A, and

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wherein the GPCRx of the subject having a CXCR4-GPCRx heteromer is selected from the group consisting of ADRB2, HRH1, TACR3, ADCYAP1R1, ADORA2B, ADORA3, C5AR1, CALCR, CHRM1, EDNRB, MLNR, and NTSR1, and wherein the GPCRx which the GPCRx inhibitor targets is the same as that of the subject having a CXCR4-GPCRx heteromer.

[59c] In a third aspect, the present disclosure provides for the use of a CXCR4 inhibitor in the manufacture of a medicament for treating a cancer in a subject having a CXCR4-GPCRx heteromer, wherein the CXCR4 inhibitor is formulated to be administered in combination with a GPCRx inhibitor, wherein:

(i) said CXCR4-GPCRx heteromer has enhanced signaling downstream of CXCR4; and

(ii) said CXCR4 inhibitor and said GPCRx inhibitor suppress the enhanced signaling downstream of CXCR4 from said CXCR4-GPCRx heteromer in the subject;

wherein said CXCR4 inhibitor is selected from the group consisting of: AMD3100 (plerixafor), ulocuplumab (MDX1338/BMS-936564), BKT140 (BL-8040; TF14016; 4F-Benzoyl- TN14003), AD-114, AD-114-6H, AD-114-Im7-FH, AD-114-PA600-6H, ALX-0651, ALX40-4C, AMD070 (AMD11070, X4P-001), AMD3465, ATI 2341, CTCE-9908, CX549, D-[Lys3] GHRP-6, FC122, FC131, GMI-1359, GSK812397, GST-NT21MP, isothiourea-1a, isothiourea-1t (IT1t), KRH-1636, KRH-3955, LY2510924, LY2624587, MSX-122, N-[11C]Methyl-AMD3465, PF- 06747143, POL6326, SDF-1 1-9[P2G] dimer, SDF1 P2G, T134, T140, T22, TC 14012, TG-0054 (Burixafor), USL311, viral macrophage inflammatory protein-II (vMIP-II), WZ811, 12G5, 238D2, 238D4, [64Cu]-AMD3100, [64Cu]-AMD3465, [68Ga]pentixafor, [90Y]pentixather, [99mTc]O2- AMD3100, [177Lu]pentixather, and 508MCl (Compound 26);

wherein said GPCRx inhibitor is an inhibitor selected from the group consisting of:

an ADRB2 inhibitor selected from the group consisting of Alprenolol, atenolol, betaxolol, bupranolol, butoxamine, carazolol, carvedilol, CGP 12177, cicloprolol, ICI 118551, ICYP, labetalol, levobetaxolol, levobunolol, LK 204-545, metoprolol, nadolol, NIHP, NIP, propafenone, propranolol, sotalol, SR59230A, and timolol,

an HRH1 inhibitor selected from the group consisting of (-)-chlorpheniramine, (+)-chlorpheniramine, (-)-*trans*-H2-PAT, (+)-*cis*-H2-PAT, (+)-*trans*-H2-PAT, (±)-*cis*-H2-PAT, (±)-*trans*- H2-PAT, (*R*)-cetirizine, (*S*)-cetirizine, 9-OH-risperidone, A-317920, A-349821, ABT-239, alimemazine, amitriptyline, aripiprazole, arpromidine, asenapine, astemizole, AZD3778, azelastine, BU-E 47, cetirizine, chlorpheniramine, chlorpromazine, ciproxifan, clemastine, clobenpropit, clozapine, conessine, cyclizine, cyproheptadine, desloratadine, diphenhydramine, dosulepin, doxepin, epinastine, fexofenadine, fluphenazine, fluspirilene, haloperidol, hydroxyzine, impromidine,

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INCB-38579, JNJ-39758979, ketotifen, loratadine, loxapine, MK- 0249, molindone, olanzapine, perphenazine, pimozide, pipamperone, pitolisant, promethazine, pyrilamine, quetiapine, risperidone, sertindole, terfenadine, thioridazine, thiothixene, trifluoperazine, tripelennamine, triprolidine, ziprasidone, and zotepine,

a TACR3 inhibitor selected from the group consisting of [Trp7, β-Ala8] neurokinin A-(4- 10), AZD2624, FK 224, GR138676, GSK 172981, GSK 256471, N',2-diphenylquinoline-4-carbohydrazide 8m, N′,2-diphenylquinoline-4-carbohydrazide, osanetant, PD 154740, PD 161182, PD157672, saredutant, SB 218795, SB 222200, SB 235375, SCH 206272, SSR 146977, and talnetant,

an ADCYAP1R1 inhibitor selected from the group consisting of M65, Max.d.4, MK-0893, N-stearyl-[Nle17] neurotensin-(6-11)/VIP-(7-28), PACAP-(6-38), and PG 97-269,

an ADORA2B inhibitor selected from the group consisting of 3-isobutyl-8-pyrrolidinoxanthine, alloxazine, AS16, AS70, AS74, AS94, AS95, AS96, AS99, AS100, AS101, ATL802, BW-A1433, caffeine, CGS 15943, CPX, CSC, CVT-6883, DAX, DEPX, derenofylline, DPCPX, FK-453, I-ABOPX, istradefylline, KF26777, LAS38096, LUF5981, MRE 2029F20, MRE 3008F20, MRS1191, MRS1220, MRS1523, MRS1706, MRS1754, MSX-2, OSIP339391, pentoxifylline, preladenant, PSB-10, PSB-11, PSB36, PSB603, PSB-0788, PSB1115, rolofylline, SCH 58261, SCH442416, ST-1535, theophylline, tonapofylline, vipadenant, xanthine amine congener, XCC, and ZM-241385,

an ADORA3 inhibitor selected from the group consisting of ATL802, BW-A1433, caffeine, CGS 15943, CSC, CVT-6883, derenofylline, dexniguldipine, DPCPX, FK-453, flavanone, flavone, galangin, I-ABOPX, istradefylline, KF26777, LAS38096, LUF5981, MRE 2029F20, MRE 3008F20, MRE 3010F20, MRS1041, MRS1042, MRS1067, MRS1088, MRS1093, MRS1097, MRS1177, MRS1186, MRS1191, MRS1191, MRS1220, MRS1476, MRS1486, MRS1505, MRS1523, MRS1754, MRS928, MSX-2, nicardipine, preladenant, PSB-10, PSB-11, PSB36, PSB603, PSB1115, rolofylline, sakuranetin, SCH 58261, SCH442416, ST-1535, theophylline, tonapofylline, vipadenant, visnagin, VUF5574, VUF8504, VUF8507, xanthine amine congener, and ZM-241385,

a C5AR1 inhibitor selected from the group consisting of A8Δ71-73, AcPhe-Orn-Pro-D-Cha-Trp-Arg, avacopan, C089, CHIPS, DF2593A, JPE1375, L-156,602, NDT9520492, *N*-methyl-Phe-Lys-Pro-D-Cha-Trp-D-Arg-CO_2H, PMX205, PMX53, RPR121154, and W54011,

a CALCR inhibitor selected from the group consisting of α-CGRP-(8-37) (human), AC187, CT-(8-32) (salmon), and olcegepant,

a CHRM1 inhibitor selected from the group consisting of 3-Quinuclidinyl benzilate

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(QNB), 4-DAMP, aclidinium, AE9C90CB, AFDX384, amitriptyline, AQ-RA 741, atropine, benzatropine, biperiden, darifenacin, dicyclomine, dosulepin, ethopropazine, glycopyrrolate, guanylpirenzepine, hexahydrodifenidol, hexahydrosiladifenidol, hexocyclium, himbacine, ipratropium, lithocholylcholine, methoctramine, ML381, muscarinic toxin 1, muscarinic toxin 2, muscarinic toxin 3, N-methyl scopolamine, otenzepad, oxybutynin, *p*-F-HHSiD, pirenzepine, propantheline, (R,R)-quinuclidinyl-4-fluoromethyl-benzilate, scopolamine, silahexocyclium, solifenacin, telenzepine, tiotropium, tolterodine, trihexyphenidyl, tripitramine, UH-AH 37, umeclidinium, and VU0255035,

an EDNRB inhibitor selected from the group consisting of A192621, ambrisentan, atrasentan, bosentan (RO 470203, Tracleer), BQ788, IRL 2500, K-8794, macitentan, RES7011, Ro 46-8443, SB209670, SB217242 (enrasentan), TAK 044, and tezosentan (RO610612),

an MLNR inhibitor selected from the group consisting of GM-109, MA-2029, and OHM- 11526, and

an NTSR1 inhibitor selected from the group consisting of Meclinertant, SR48527, SR48692, and SR142948A, and

wherein the GPCRx of the subject having a CXCR4-GPCRx heteromer is selected from the group consisting of ADRB2, HRH1, TACR3, ADCYAP1R1, ADORA2B, ADORA3, C5AR1, CALCR, CHRM1, EDNRB, MLNR, and NTSR1, and wherein the GPCRx which the GPCRx inhibitor targets is the same as that of the subject having a CXCR4-GPCRx heteromer.

[59d] In a fourth aspect, the present disclosure provides a kit comprising a CXCR4 inhibitor and a GPCRx inhibitor, when used in a method for treating a cancer in a subject having a CXCR4-GPCRx heteromer, wherein:

(i) said CXCR4-GPCRx heteromer has enhanced signaling downstream of CXCR4; and

(ii) said CXCR4 inhibitor and said GPCRx inhibitor suppress the enhanced downstream signaling from said CXCR4-GPCRx heteromer in the subject,

wherein the GPCRx of the subject having a CXCR4-GPCRx heteromer is selected from the group consisting of ADRB2, HRH1, TACR3, ADCYAP1R1, ADORA2B, ADORA3, C5AR1, CALCR, CHRM1, EDNRB, MLNR, and NTSR1, and wherein the GPCRx which the GPCRx inhibitor targets is the same as that of the subject having a CXCR4-GPCRx heteromer, and

wherein said CXCR4 inhibitor and said GPCRx inhibitor are as defined in the first, second or third aspects above.

[59e] Throughout this specification, the word “comprise’ or variations such as “comprises”

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or "comprising" will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

[60] By the use of adenovirus high-throughput system (AdHTS) that allows rapid and efficient generation of multiple recombinant adenoviruses simultaneously (Choi, et al., 2012), and the adenovirus-based bimolecular fluorescence complementation (BiFC) assay (patent; Song, 2014), inventors screened large number of GPCRs for their association with CXCR4 in U-2 OS cells.

[61] In one aspect, provided herein is a method for treatment, amelioration, prevention, or diagnosis, of a cancer in a subject having CXCR4-GPCRx heteromer, the method comprising: administering to the subject a therapeutically effective amount of an inhibitor of a CXCR4-GPCRx heteromer, wherein: GPCRx heteromerizes with CXCR4 in the subject, the heteromerization of GPCRx with CXCR4 is accompanied by enhancement of signaling downstream of CXCR4; and the enhancement of signaling downstream of CXCR4 is suppressed by the inhibitor of the CXCR4-GPCRx heteromer.

[62] In another aspect, provided herein, is a method for treating cancer in a patient having a cell containing a CXCR4-GPCRx heteromer, the method comprising: administering to the patient an inhibitor or a combination of inhibitors selected from the group consisting of: an inhibitor of CXCR4, an inhibitor of GPCRx, and an inhibitor of the CXCR4-GPCRx heteromer; wherein:

[63] i) the CXCR4-GPCRx heteromer has enhanced downstream signaling; and

[64] ii) the administered inhibitor or combination of inhibitors suppresses the enhanced downstream signaling from said CXCR4-GPCRx heteromer in the cancer patient.

[65] In another aspect, provided herein, is a method of suppressing enhanced downstream signaling from a CXCR4-GPCRx heteromer in a cell of a patient suffering from cancer, the method comprising: administering to the patient an inhibitor or a combination of inhibitors selected from the group consisting of: an inhibitor of CXCR4, an inhibitor of GPCRx, and an inhibitor of the CXCR4-GPCRx heteromer; wherein:

[66] i) the CXCR4-GPCRx heteromer has enhanced downstream signaling; and

[67] ii) the administered inhibitor or combination of inhibitors suppresses the enhanced downstream signaling from said CXCR4-GPCRx heteromer in the cancer patient.

[68] In another aspect, provided herein, is a pharmaceutical kit for use in treating cancer

in a patient having a cell containing a CXCR4-GPCRx heteromer, the pharmaceutical kit comprising: an inhibitor or a combination of inhibitors selected from the group consisting of: an inhibitor of CXCR4, an inhibitor of GPCRx, and an inhibitor of the CXCR4-GPCRx heteromer; wherein the CXCR4-GPCRx heteromer has enhanced downstream signaling.

[69] In another aspect, provided herein, is a pharmaceutical composition for use in treating cancer in a patient having a cell containing a CXCR4-GPCRx heteromer, the pharmaceutical composition comprising:

[70] i) an inhibitor or a combination of inhibitors selected from the group consisting of: an inhibitor of CXCR4, an inhibitor of GPCRx, and an inhibitor of the CXCR4-GPCRx heteromer; and

[71] ii) a pharmaceutically acceptable carrier;

[72] wherein the CXCR4-GPCRx heteromer has enhanced downstream signaling.

[73] In another aspect, provided herein, is a method for treating cancer in a patient having a cancer cell containing a CXCR4-GPCRx heteromer, wherein the CXCR4-GPCRx heteromer has enhanced downstream signaling, the method comprising:

[74] 1) determining whether the patient has the cancer cell containing the CXCR4-GPCRx heteromer having enhanced downstream signaling by: obtaining or having obtained a biological sample from the patient; and performing or having performed an assay on the biological sample to determine if:

[75] i) the patient's cancer cell containing said CXCR4-GPCRx heteromer; or

[76] ii) a CXCR4-GPCRx heteromer-selective reagent: alters heteromer-specific properties or function of said CXCR4-GPCRx heteromer in a patient derived cell(s); alters heteromer-specific properties of a patient derived cell(s) containing the CXCR4-GPCRx heteromer; or decreases cell proliferation of a patient derived cell(s) containing the CXCR4-GPCRx heteromer; and

[77] 2) if the patient has a cancer cell containing said CXCR4-GPCRx heteromer, then internally administering an inhibitor or a combination of inhibitors selected from the group consisting of: an inhibitor of CXCR4, an inhibitor of GPCRx, and an inhibitor of the CXCR4-GPCRx heteromer to the cancer patient.

[78] In another aspect, provided herein, is a method for treating cancer in a patient having a cancer cell containing a CXCR4-GPCRx heteromer, the method comprising:

[79] 1) determining whether the patient's cancer cell contains the CXCR4-GPCRx heteromer by: obtaining or having obtained a biological sample from the patient and performing or having performed an assay on the biological sample to determine if said CXCR4-GPCRx heteromer is present in the patient's cancer cell; wherein:

[80] a) the GPCRx of the CXCR4-GPCRx heteromer is selected from the group consisting of: ADCYAP1R1, ADORA2B, ADORA3, ADRB2, C5AR1, CALCR,

CHRM1, EDNRB, HRH1, MLNR, NTSR1, and TACR3; and

[81] b) the assay performed on the biological sample is or comprises one or more of the following: a co-internalization assay, a colocalization assay, in situ hybridization, immunohistochemistry, immunoelectron microscopy, a proximity-based assay, a co-immunoprecipitation assay, or a fluorescent animal assay; and

[82] 2) if the patient's cancer cell contains said CXCR4-GPCRx heteromer, then internally administering an inhibitor or a combination of inhibitors selected from the group consisting of: an inhibitor of CXCR4, an inhibitor of GPCRx, and an inhibitor of the CXCR4-GPCRx heteromer to the patient.

[83] In another aspect, provided herein, is a method for treating cancer in a patient having a cancer cell containing a CXCR4-GPCRx heteromer having enhanced downstream signaling, the method comprising:

[84] 1) determining whether the patient has the cancer cell containing the CXCR4-GPCRx heteromer having enhanced downstream signaling by: obtaining or having obtained a biological sample from the patient; and performing or having performed an assay on the biological sample to determine if:

[85] i) the patient's cancer cell contains said CXCR4-GPCRx heteromer; or

[86] ii) a CXCR4-GPCRx heteromer-selective reagent: alters heteromer-specific properties or function of said CXCR4-GPCRx heteromer in a patient derived cell(s); alters heteromer-specific properties of a patient derived cell(s) containing said CXCR4-GPCRx heteromer; or decreases cell proliferation of a patient derived cell(s) containing said CXCR4-GPCRx heteromer; and

[87] 2) if the patient has a cancer cell containing said CXCR4-GPCRx heteromer, then internally administering to the cancer patient a combination of inhibitors selected from the group consisting of: an inhibitor of CXCR4, an inhibitor of GPCRx, and an inhibitor of the CXCR4-GPCRx heteromer; and

[88] 3) if the patient does not have a cancer cell containing said CXCR4-GPCRx heteromer, then internally administering to the cancer patient either the CXCR4 inhibitor or the GPCRx inhibitor as a single inhibitor.

[89] In another aspect, provided herein, is a method for treating cancer in a patient having a cancer cell containing a CXCR4-GPCRx heteromer, the method comprising:

[90] 1) determining whether the patient's cancer cell contains the CXCR4-GPCRx heteromer by: obtaining or having obtained a biological sample from the patient and performing or having performed an assay on the biological sample to determine if said CXCR4-GPCRx heteromer is present in the patient's cancer cell; wherein:

[91] a) the GPCRx of the CXCR4-GPCRx heteromer is selected from the group consisting of: ADCYAP1R1, ADORA2B, ADORA3, ADRB2, C5AR1, CALCR, CHRM1, EDNRB, HRH1, MLNR, NTSR1, and TACR3; and

[92] b) the assay performed on the biological sample is or comprises one or more of the following: a co-internalization assay, a colocalization assay, in situ hybridization, immunohistochemistry, immunoelectron microscopy, a proximity-based assay, a co-immunoprecipitation assay, enzyme-linked immunosorbent assay (ELISA), flow cytometry, RNAseq, qRT-PCR, microarray, or a fluorescent animal assay; and

[93] 2) if the patient's cancer cell contains said CXCR4-GPCRx heteromer, then internally administering to the cancer patient a combination of inhibitors selected from the group consisting of: an inhibitor of CXCR4, an inhibitor of GPCRx, and an inhibitor of the CXCR4-GPCRx heteromer; and

[94] 3) if the patient's cancer cell does not contain said CXCR4-GPCRx heteromer, then internally administering to the cancer patient either the CXCR4 inhibitor or the GPCRx inhibitor as a single inhibitor.

[95] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the heteromerization of CXCR4 with GPCRx is evaluated by a proximity-based assay.

[96] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the proximity-based assay is selected from the group consisting of bimolecular fluorescence complementation (BiFC), proximity ligation assay (PLA), fluorescence resonance energy transfer (FRET), bioluminescence resonance energy transfer (BRET), cysteine crosslinking, co-immunoprecipitation, and. a combination of TR-FRET and SNAP-tag (Comps-Agrar et al., 2011).

[97] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the heteromerization of CXCR4 with GPCRx is evaluated by a co-internalization assay.

[98] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the enhancement of cell signaling downstream of CXCR4 is evaluated by an intracellular Ca2+ assay.

[99] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, GPCRx is selected from the group consisting of ADCYAP1R1, ADORA2B, ADORA3, ADRB2, APLNR, C5AR1, CALCR, CCR5, CHRM1, GALR1, EDNRB, HRH1, MLNR, NTSR1, PTGER2, PTGER3, SSTR2, and TACR3.

[100] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, GPCRx is selected from the group consisting of ADCYAP1R1, ADORA2B, ADORA3, ADRB2, C5AR1, CALCR, CHRM1, EDNRB, HRH1, MLNR, NTSR1, PTGER2, and TACR3.

[101] In certain embodiments of the method of treating, method of suppressing, pharma-

ceutical composition, or pharmaceutical kit, disclosed herein, GPCRx is selected from the group consisting of ADCYAP1R1, ADORA2B, ADORA3, ADRB2, C5AR1, CALCR, CHRM1, EDNRB, HRH1, MLNR, NTSR1, and TACR3.

[102] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, GPCRx is selected from the group consisting of ADCYAP1R1, ADORA2B, ADORA3, ADRB2, C5AR1, CHRM1, EDNRB, HRH1, MLNR, NTSR1, and TACR3.

[103] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, GPCRx is selected from the group consisting of ADCYAP1R1, ADORA2B, ADORA3, ADRB2, CHRM1, EDNRB, HRH1, MLNR, NTSR1, and TACR3.

[104] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, GPCRx is selected from the group consisting of ADCYAP1R1, ADORA2B, ADORA3, ADRB2, CHRM1, EDNRB, HRH1, NTSR1, and TACR3.

[105] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, GPCRx is selected from the group consisting of ADCYAP1R1, ADORA2B, ADORA3, ADRB2, CHRM1, HRH1, NTSR1, and TACR3.

[106] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, GPCRx is selected from the group consisting of ADORA2B, ADORA3, ADRB2, CHRM1, EDNRB, HRH1, NTSR1, and TACR3.

[107] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, GPCRx is selected from the group consisting of ADRB2, CHRM1, EDNRB, HRH1, NTSR1, and TACR3.

[108] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, GPCRx is selected from the group consisting of ADRB2, CHRM1, EDNRB, HRH1, and TACR3.

[109] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, GPCRx is selected from the group consisting of ADRB2, CHRM1, HRH1, and TACR3.

[110] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, GPCRx is selected from the group consisting of ADRB2, EDNRB, HRH1, and TACR3.

[111] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, GPCRx is selected from the group consisting of ADRB2, EDNRB, and HRH1.

[112] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, GPCRx is selected from the group consisting of ADRB2, CHRM1, and HRH1.

[113] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, GPCRx is selected from the group consisting of ADRB2, HRH1, and TACR3.

[114] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, GPCRx is selected from the group consisting of ADRB2 and HRH1.

[115] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the inhibitor of the CXCR4-GPCRx heteromer is, or comprises, an inhibitor of the CXCR4.

[116] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the inhibitor of CXCR4 is selected from the group consisting of: AD-114, AD-114-6H, AD-114-Im7-FH, AD-114-PA600-6H, ALX-0651, ALX40-4C, AMD070 (AMD11070, X4P-001), AMD3100 (plerixafor), AMD3465, ATI 2341, BKT140 (BL-8040; TF14016; 4F-Benzoyl-TN14003), CTCE-9908, CX549,,D-[Lys3] GHRP-6, FC122, FC131, GMI-1359, GSK812397, GST-NT21MP, isothiourea-1a, isothiourea-1t (IT1t), KRH-1636, KRH-3955, LY2510924, LY2624587, MSX-122, N-[^{11}C]Methyl-AMD3465, PF-06747143, POL6326, SDF-1 1-9[P2G] dimer, SDF1 P2G, T134, T140, T22, TC 14012, TG-0054 (Burixafor), USL311, ulocuplumab (MDX1338/BMS-936564), viral macrophage inflammatory protein-II (vMIP-II), WZ811, 12G5, 238D2, 238D4, [^{64}Cu]-AMD3100, [^{64}Cu]-AMD3465, [^{68}Ga]pentixafor, [^{90}Y]pentixather, [^{99m}Tc]O_2 -AMD3100, [^{177}Lu]pentixather, and 508MCl (Compound 26).

[117] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the inhibitor of the CXCR4-GPCRx heteromer is, or comprises, an antagonist of the CXCR4.

[118] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the antagonist of CXCR4 is selected from the group consisting of ALX40-4C, AMD070 (AMD11070, X4P-001), AMD3100 (plerixafor), AMD3465, ATI 2341, BKT140 (BL-8040; TF14016; 4F-Benzoyl-TN14003), CTCE-9908, CX549,,D-[Lys3] GHRP-6, FC122, FC131, GMI-1359, GSK812397, GST-NT21MP, isothiourea-1a, isothiourea-1t (IT1t), KRH-1636, KRH-3955, LY2510924, MSX-122, N-[11C]Methyl-AMD3465, POL6326, SDF-1 1-9[P2G] dimer, SDF1 P2G, T134, T140, T22, TC 14012, TG-0054 (Burixafor), USL311, viral macrophage inflammatory protein-II (vMIP-II), WZ811, [64Cu]-AMD3100, [64Cu]-AMD3465, [68Ga]pentixafor, [90Y]pentixather,

[99mTc]O2-AMD3100, [177Lu]pentixather, and 508MCl (Compound 26).

[119] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the inhibitor of the CXCR4-GPCRx heteromer is, or comprises, an antibody of the CXCR4.

[120] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the antibody of CXCR4 is selected from the group consisting of AD-114, AD-114-6H, AD-114-Im7-FH, AD-114-PA600-6H, ALX-0651, LY2624587, PF-06747143, ulocuplumab (MDX1338/BMS-936564), 12G5, 238D2, and 238D4.

[121] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the inhibitor of the CXCR4-GPCRx heteromer is, or further comprises, an inhibitor of the GPCRx.

[122] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the inhibitor of the GPCRx is concurrently or sequentially administered with the inhibitor of the CXCR4.

[123] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the inhibitor of the CXCR4-GPCRx heteromer is, or comprises, an antagonist, an inverse agonist, an allosteric modulator, an antibody or binding portion thereof, a ligand, or any combinations, of GPCRx.

[124] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the inhibitor of GPCRx is an inhibitor of a molecule selected from the group consisting of ADCYAP1R1, ADORA2B, ADORA3, ADRB2, C5AR1, CALCR, CHRM1, EDNRB, HRH1, MLNR, NTSR1, PTGER2, and TACR3.

[125] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the inhibitor of GPCRx is an inhibitor of a molecule selected from the group consisting of ADCYAP1R1, ADORA2B, ADORA3, ADRB2, C5AR1, CALCR, CHRM1, EDNRB, HRH1, MLNR, NTSR1, and TACR3.

[126] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the inhibitor of GPCRx is an inhibitor of a molecule selected from the group consisting of ADCYAP1R1, ADORA2B, ADORA3, ADRB2, C5AR1, CHRM1, EDNRB, HRH1, MLNR, NTSR1, and TACR3.

[127] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the inhibitor of GPCRx is an inhibitor of a molecule selected from the group consisting of ADCYAP1R1,

ADORA2B, ADORA3, ADRB2, CHRM1, EDNRB, HRH1, MLNR, NTSR1, and TACR3.

[128] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the inhibitor of GPCRx is an inhibitor of a molecule selected from the group consisting of ADCYAP1R1, ADORA2B, ADORA3, ADRB2, CHRM1, EDNRB, HRH1, NTSR1, and TACR3.

[129] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the inhibitor of GPCRx is an inhibitor of a molecule selected from the group consisting of ADCYAP1R1, ADORA2B, ADORA3, ADRB2, CHRM1, HRH1, NTSR1, and TACR3.

[130] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the inhibitor of GPCRx is an inhibitor of a molecule selected from the group consisting of ADORA2B, ADORA3, ADRB2, CHRM1, EDNRB, HRH1, NTSR1, and TACR3.

[131] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the inhibitor of GPCRx is an inhibitor of a molecule selected from the group consisting of ADRB2, CHRM1, EDNRB, HRH1, NTSR1, and TACR3.

[132] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the inhibitor of GPCRx is an inhibitor of a molecule selected from the group consisting of ADRB2, CHRM1, EDNRB, HRH1, and TACR3.

[133] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the inhibitor of GPCRx is an inhibitor of a molecule selected from the group consisting of ADRB2, CHRM1, HRH1, and TACR3.

[134] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the inhibitor of GPCRx is an inhibitor of a molecule selected from the group consisting of ADRB2, EDNRB, HRH1, and TACR3.

[135] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the inhibitor of GPCRx is an inhibitor of a molecule selected from the group consisting of ADRB2, EDNRB, and HRH1.

[136] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the inhibitor of GPCRx is an inhibitor of a molecule selected from the group consisting of ADRB2, CHRM1, and HRH1.

[137] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the inhibitor of GPCRx is an inhibitor of a molecule selected from the group consisting of ADRB2, HRH1, and TACR3.

[138] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the inhibitor of GPCRx is an inhibitor of a molecule selected from the group consisting of ADRB2 and HRH1.

[139] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the inhibitor of GPCRx is an inhibitor of ADCYAP1R1 selected from the group consisting of: M65, Max.d.4, MK-0893, N-stearyl-[Nle^{17}] neurotensin-(6-11)/VIP-(7-28), PACAP-(6-38), and PG 97-269.

[140] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the inhibitor of GPCRx is an inhibitor of ADORA2B selected from the group consisting of: 3-isobutyl-8-pyrrolidinoxanthine, alloxazine, AS16, AS70, AS74, AS94, AS95, AS96, AS99, AS100, AS101, ATL802, BW-A1433, caffeine, CGS 15943, CPX, CSC, CVT-6883, DAX, DEPX, derenofylline, DPCPX, FK-453, I-ABOPX, istradefylline, KF26777, LAS38096, LUF5981, MRE 2029F20, MRE 3008F20, MRS1191, MRS1220, MRS1523, MRS1706, MRS1754, MSX-2, OSIP339391, pentoxifylline, preladenant, PSB-10, PSB-11, PSB36, PSB603, PSB-0788, PSB1115, rolofylline, SCH 58261, SCH442416, ST-1535, theophylline, tonapofylline, vipadenant, xanthine amine congener, XCC, and ZM-241385.

[141] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the inhibitor of GPCRx is an inhibitor of ADORA3 selected from the group consisting of: ATL802, BW-A1433, caffeine, CGS 15943, CSC, CVT-6883, derenofylline, dexniguldipine, DPCPX, FK-453, flavanone, flavone, galangin, I-ABOPX, istradefylline, KF26777, LAS38096, LUF5981, MRE 2029F20, MRE 3008F20, MRE 3010F20, MRS1041, MRS1042, MRS1067, MRS1088, MRS1093, MRS1097, MRS1177, MRS1186, MRS1191, MRS1191, MRS1220, MRS1476, MRS1486, MRS1505, MRS1523, MRS1754, MRS928, MSX-2, nicardipine, preladenant, PSB-10, PSB-11, PSB36, PSB603, PSB1115, rolofylline, sakuranetin, SCH 58261, SCH442416, ST-1535, theophylline, tonapofylline, vipadenant, visnagin, VUF5574, VUF8504, VUF8507, xanthine amine congener, and ZM-241385.

[142] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the inhibitor of GPCRx is an inhibitor of ADRB2 selected from the group consisting of: Alprenolol, atenolol,

betaxolol, bupranolol, butoxamine, carazolol, carvedilol, CGP 12177, cicloprolol, ICI 118551, ICYP, labetalol, levobetaxolol, levobunolol, LK 204-545, metoprolol, nadolol, NIHP, NIP, propafenone, propranolol, sotalol, SR59230A, and timolol.

[143] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the inhibitor of ADRB2 is Carvedilol.

[144] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the inhibitor of GPCRx is an inhibitor of C5AR1 inhibitor is selected from the group consisting of: $A8^{\Delta 71-73}$, AcPhe-Orn-Pro-D-Cha-Trp-Arg, avacopan, C089, CHIPS, DF2593A, JPE1375, L-156,602, NDT9520492, *N*-methyl-Phe-Lys-Pro-D-Cha-Trp-D-Arg-CO_2H, PMX205, PMX53, RPR121154, and W54011.

[145] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the inhibitor of GPCRx is an inhibitor of CALCR selected from the group consisting of α-CGRP-(8-37) (human), AC187, CT-(8-32) (salmon), and olcegepant.

[146] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the inhibitor of CALCR is AC187.

[147] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the inhibitor of GPCRx is an inhibitor of CHRM1 selected from the group consisting of 3-Quinuclidinyl benzilate (QNB), 4-DAMP, aclidinium, AE9C90CB, AFDX384, amitriptyline, AQ-RA 741, atropine, benzatropine, biperiden, darifenacin, dicyclomine, dosulepin, ethopropazine, glycopyrrolate, guanylpirenzepine, hexahydrodifenidol, hexahydrosiladifenidol, hexocyclium, himbacine, ipratropium, lithocholylcholine, methoctramine, ML381, muscarinic toxin 1, muscarinic toxin 2, muscarinic toxin 3, N-methyl scopolamine, otenzepad, oxybutynin, *p*-F-HHSiD, pirenzepine, propantheline, (R,R)-quinuclidinyl-4-fluoromethyl-benzilate, scopolamine, silahexocyclium, solifenacin, telenzepine, tiotropium, tolterodine, trihexyphenidyl, tripitramine, UH-AH 37, umeclidinium, and VU0255035.

[148] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the inhibitor of CHRM1 is Oxybutynin, umeclidinium, and VU0255035.

[149] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the inhibitor of GPCRx is an inhibitor of EDNRB selected from the group consisting of A192621, ambrisentan, atrasentan, bosentan (RO 470203, Tracleer);, BQ788, IRL 2500, K-8794, macitentan,

RES7011, Ro 46-8443, SB209670, SB217242 (enrasentan), TAK 044, and tezosentan (RO610612).

[150] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the inhibitor of EDNRB is bosentan.

[151] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the inhibitor of GPCRx is an inhibitor of HRH1 selected from the group consisting of (-)-chlorpheniramine, (+)-chlorpheniramine, (-)-*trans*-H_2-PAT, (+)-*cis*-H_2-PAT, (+)-*trans*-H_2-PAT, (±)-*cis*-H_2-PAT, (±)-*trans*-H_2-PAT, (*R*)-cetirizine, (*S*)-cetirizine, 9-OH-risperidone, A-317920, A-349821, ABT-239, alimemazine, amitriptyline, aripiprazole, arpromidine, asenapine, astemizole, AZD3778, azelastine, BU-E 47, cetirizine, chlorpheniramine, chlorpromazine, ciproxifan, clemastine, clobenpropit, clozapine, conessine, cyclizine, cyproheptadine, desloratadine, diphenhydramine, dosulepin, doxepin, epinastine, fexofenadine, fluphenazine, fluspirilene, haloperidol, hydroxyzine, impromidine, INCB-38579, JNJ-39758979, ketotifen, loratadine, loxapine, MK-0249, molindone, olanzapine, perphenazine, pimozide, pipamperone, pitolisant, promethazine, pyrilamine, quetiapine, risperidone, sertindole, terfenadine, thioridazine, thiothixene, trifluoperazine, tripelennamine, triprolidine, ziprasidone, and zotepine.

[152] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the inhibitor of HRH1 is cetirizine, pyrilamine, hydroxyzine, or loratadine.

[153] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the inhibitor of GPCRx is an inhibitor of MLNR selected from the group consisting of GM-109, MA-2029, and OHM-11526.

[154] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the inhibitor of MLNR is MA-2029.

[155] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the inhibitor of GPCRx is an inhibitor of NTSR1 selected from the group consisting of Meclinertant, SR48527, SR48692, and SR142948A.

[156] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the inhibitor of NTSR1 is Merclinertant.

[157] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the inhibitor of GPCRx

is an inhibitor of TACR3 selected from the group consisting of [Trp^7, β-Ala^8] neurokinin A-(4-10), AZD2624, FK 224, GR138676, GSK 172981, GSK 256471, N',2-diphenylquinoline-4-carbohydrazide 8m, N′,2-diphenylquinoline-4-carbohydrazide, osanetant, PD 154740, PD 161182, PD157672, saredutant, SB 218795, SB 222200, SB 235375, SCH 206272, SSR 146977, and talnetant.

[158] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the inhibitor of TACR3 is SSR146977.

[159] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the inhibitor of the CXCR4-GPCRx heteromer is a protein-protein interaction (PPI) inhibitor.

[160] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the cancer is selected from the group consisting of breast cancer, lung cancer, brain cancer, kidney cancer, pancreatic cancer, ovarian cancer, prostate cancer, melanoma, multiple myeloma, gastrointestinal cancers, renal cell carcinoma, soft tissue sarcomas, hepatocellular carcinoma, stomach cancer, colorectal cancer, esophageal cancer, and leukemia.

[161] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the inhibitor of CXCR4-GPCRx heteromer is administered to the subject in a pharmaceutical composition.

[162] Further disclosed herein is a method for assessing response, or potential response, of a subject having CXCR4-GPCRx heteromer, to treatment, amelioration, or prevention of a cancer, the method comprising: obtaining a sample from the subject; detecting heteromerization of CXCR4 and GPCRx in the sample; and based at least in part on detection of the heteromerization of CXCR4 and GPCRx, assessing the subject's response, or potential response, to the treatment, amelioration, or prevention of a cancer.

[163] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the heteromerization of CXCR4 and GPCRx is accompanied by enhancement of signaling downstream of CXCR4.

[164] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the enhancement of cell signaling downstream of CXCR4 is evaluated by an intracellular Ca2+ assay.

[165] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the heteromerization of

CXCR4 with GPCRx is evaluated by a proximity-based assay.

[166] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the proximity-based assay is selected from the group consisting of bimolecular fluorescence complementation (BiFC), proximity ligation assay (PLA), fluorescence resonance energy transfer (FRET), bioluminescence resonance energy transfer (BRET), cysteine crosslinking, and co-immunoprecipitation.

[167] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the heteromerization of CXCR4 with GPCRx is evaluated by a co-internalization assay.

[168] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the CXCR4-GPCRx heteromer has, causes, or produces, the enhanced downstream signaling.

[169] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the enhanced downstream signaling results from the CXCR4-GPCRx heteromer, such as results from agonism of the CXCR4-GPCRx heteromer, results from CXCR4 agonism of the CXCR4-GPCRx heteromer, or results from GPCRx agonism of the CXCR4-GPCRx heteromer.

[170] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the enhanced downstream signaling is downstream of the CXCR4, the respective GPCRx, or the CXCR4-GPCRx heteromer.

[171] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the inhibitor or combination of inhibitors suppresses the enhanced downstream signaling from said CXCR4-GPCRx heteromer in the cancer patient, such as suppresses the enhanced downstream signaling from said CXCR4-GPCRx heteromer in the patient's cancer cells.

[172] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the enhanced downstream signaling from the CXCR4-GPCRx heteromer is determined by an intracellular Ca2+ assay.

[173] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the enhanced downstream signaling is an enhanced amount of calcium mobilization.

[174] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the enhanced amount of

calcium mobilization is a synergistic amount of calcium mobilization.

[175] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the CXCR4-GPCRx heteromer has two or more of the following characteristics:

[176] 1) the CXCR4-GPCRx heteromer components in a cell colocalize and physically interact, either directly or via intermediate proteins acting as conduits for allosterism, as determined via one or more of the following: a co-internalization assay, a colocalization assay, in situ hybridization, immunohistochemistry, immunoelectron microscopy, a proximity-based assay, a co-immunoprecipitation assay, or a fluorescent animal assay;

[177] 2) an enhanced amount of calcium mobilization, such that: a) either CXCR4 or the respective GPCRx in an individual protomer context in a cell, upon co-stimulation with CXCL12 and a respective selective GPCRx agonist, results in a calcium mobilization amount that is equal to or less than the sum of calcium mobilization amounts resulting from single agonist stimulation with either the CXCL12 or the respective selective GPCRx agonist; and b) the CXCR4-GPCRx heteromer exhibits an enhanced calcium mobilization upon co-stimulation with the CXCL12 and the respective selective GPCRx agonist relative to the sum of calcium mobilization amounts resulting from single agonist stimulation with either the CXCL12 or the respective selective GPCRx agonist; as determined via a calcium mobilization assay; or

[178] 3) a CXCR4-GPCRx heteromer-selective reagent: i) alters heteromer-specific properties of the CXCR4-GPCRx heteromer in a patient derived cell, ii) alters heteromer-specific function of the CXCR4-GPCRx heteromer in a patient derived cell; iii) alters heteromer-specific properties of a patient derived cell containing the CXCR4-GPCRx heteromer; or iv) decreases cell proliferation of a patient derived cell(s) containing the CXCR4-GPCRx heteromer.

[179] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the CXCR4-GPCRx heteromer components in the cell colocalize and physically interact, either directly or via intermediate proteins acting as conduits for allosterism, as determined via one or more of the following: a co-internalization assay, a colocalization assay, in situ hybridization, immunohistochemistry, immunoelectron microscopy, a proximity-based assay, a co-immunoprecipitation assay, or a fluorescent animal assay.

[180] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the proximity-based assay is, or comprises, resonance energy transfer (RET), bioluminescence RET (BRET), fluorescence RET (FRET), time-resolved fluorescence RET (TR-FRET), antibody-aided FRET, ligand-aided FRET, bimolecular fluorescence complementation

(BiFC), or a proximity ligation assay (PLA).

[181] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the CXCR4-GPCRx heteromer exhibits the enhanced amount of calcium mobilization, such that:

[182] a) either the CXCR4 or the respective GPCRx in an individual protomer context in the cell upon co-stimulation with CXCL12 and a respective selective GPCRx agonist results in a calcium mobilization amount that is equal to or less than the sum of calcium mobilization amounts resulting from single agonist stimulation with either the CXCL12 or the respective selective GPCRx agonist; and

[183] b) the CXCR4-GPCRx heteromer exhibits an enhanced calcium mobilization upon co-stimulation with the CXCL12 and the respective selective GPCRx agonist relative to the sum of calcium mobilization amounts resulting from single agonist stimulation with either the CXCL12 or the respective selective GPCRx agonist;

[184] as determined via a calcium mobilization assay.

[185] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the CXCR4-GPCRx heteromer-selective reagent: i) alters heteromer-specific properties of the CXCR4-GPCRx heteromer in the patient derived cell; ii) alters heteromer-specific function of the CXCR4-GPCRx heteromer in the patient derived cell; iii) alters heteromer-specific properties of the patient derived cell containing the CXCR4-GPCRx heteromer; or iv) decreases cell proliferation of the patient derived cell containing the CXCR4-GPCRx heteromer.

[186] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the CXCR4-GPCRx heteromer-selective reagent is a CXCR4 inhibitor, a GPCRx inhibitor, or a CXCR4-GPCRx heteromer inhibitor.

[187] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the CXCR4-GPCRx heteromer-selective reagent is a CXCR4 antagonist, a GPCRx antagonist, or a CXCR4-GPCRx heteromer antagonist.

[188] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the method administers an inhibitor selected from the group consisting of: the CXCR4 inhibitor, the GPCRx inhibitor, or the CXCR4-GPCRx heteromer inhibitor.

[189] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the inhibitor is administered as a pharmaceutical composition.

[190] In certain embodiments of the method of treating, method of suppressing, pharma-

ceutical composition, or pharmaceutical kit, disclosed herein, the administered inhibitor is the CXCR4 inhibitor.

[191] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the administered inhibitor is the GPCRx inhibitor.

[192] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the administered inhibitor is the inhibitor of the CXCR4-GPCRx heteromer.

[193] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the method administers the combination of inhibitors selected from the group consisting of: the CXCR4 inhibitor, the GPCRx inhibitor, and the inhibitor of the CXCR4-GPCRx heteromer.

[194] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the combination of inhibitors are administered sequentially, concurrently, or simultaneously.

[195] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the CXCR4 inhibitor is an antagonist of CXCR4, an inverse agonist of CXCR4, a partial antagonist of CXCR4, an allosteric modulator of CXCR4, an antibody of CXCR4, an antibody fragment of CXCR4, a ligand of CXCR4, or an antibody-drug conjugate of CXCR4.

[196] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the GPCRx inhibitor is an antagonist of GPCRx, an inverse agonist of GPCRx, a partial antagonist of GPCRx, an allosteric modulator of GPCRx, an antibody of GPCRx, an antibody fragment of GPCRx, a ligand of GPCRx, or an antibody-drug conjugate of GPCRx.

[197] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the inhibitor of the CXCR4-GPCRx heteromer is an antagonist of the CXCR4-GPCRx heteromer, an inverse agonist of the CXCR4-GPCRx heteromer, a partial antagonist of the CXCR4-GPCRx heteromer, an allosteric modulator of the CXCR4-GPCRx heteromer, an antibody of the CXCR4-GPCRx heteromer, an antibody fragment of the CXCR4-GPCRx heteromer, a ligand of the CXCR4-GPCRx heteromer, a protein-protein interaction (PPI) inhibitor of the CXCR4-GPCRx heteromer, or an antibody-drug conjugate of the CXCR4-GPCRx heteromer.

[198] In certain embodiments of the method of treating or method of suppressing, disclosed herein, the method further comprises detecting the CXCR4-GPCRx heteromer in the cancer patient.

[199] In certain embodiments of the method of treating or method of suppressing, disclosed

herein, the method further comprises identifying the CXCR4-GPCRx heteromer in the cancer patient.

[200] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the method further comprises:

[201] i) obtaining or having obtained a biological sample from the cancer patient;

[202] ii) conducting or having conducted a diagnostic assay to determine presence, identity, or presence and identity, of a CXCR4-GPCRx heteromer in the obtained biological sample from the cancer patient; and

[203] iii) selecting the inhibitor or combination of inhibitors to suppress enhanced downstream signaling from the CXCR4-GPCRx heteromer.

[204] In certain embodiments of the method of treating or method of suppressing, disclosed herein, the patient's biological sample is a biological fluid sample or a biological tissue sample.

[205] In certain embodiments of the method of treating or method of suppressing, disclosed herein, a liquid biopsy is performed on the biological fluid sample or the biological tissue sample.

[206] In certain embodiments, the biological fluid sample includes circulating tumor cells (CTCs), tumor-derived cell-free DNA (cfDNA), circulating small RNAs, and extracellular vesicles including exosomes, from bodily fluids as disclosed, for example, in Campos CDM et al., "Molecular Profiling of Liquid Biopsy Samples for Precision Medicine," Cancer J. 2018 Mar/Apr;24(2):93-103, which is incorporated hereby in its entirety.

[207] In certain embodiments of the method of treating or method of suppressing, disclosed herein, the biological fluid sample is a blood sample, a plasma sample, a saliva sample, a cerebral fluid sample, an eye fluid sample, or a urine sample.

[208] In certain embodiments of the method of treating or method of suppressing, disclosed herein, the biological tissue sample is an organ tissue sample, a bone tissue sample, or a tumor tissue sample.

[209] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the cancer cell contains the CXCR4-GPCRx heteromer.

[210] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, a normal, non-cancerous cell, does not contain the CXCR4-GPCRx heteromer.

[211] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the progression of the cancer in the patient having said cancer cell containing the CXCR4-GPCRx heteromer

is decreased in the range of 5-100% more upon administration of the combination of inhibitors, relative to administering the CXCR4 inhibitor or GPCRx inhibitor as the single inhibitor to said patient.

[212] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the efficacy of a CXCR4 inhibitor is increased in the range of 5-2000% when administered in combination with the GPCRx inhibitor to the patient having said cancer cell containing the CXCR4-GPCRx heteromer, relative to efficacy of the CXCR4 inhibitor when administered as a single inhibitor.

[213] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the efficacy of a GPCRx inhibitor is increased in the range of 5-2000% when administered in combination with the CXCR4 inhibitor to the patient having said cancer cell containing the CXCR4-GPCRx heteromer, relative to efficacy of the GPCRx inhibitor when administered as a single inhibitor.

[214] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the progression of the cancer in the patient having said cancer cell containing the CXCR4-GPCRx heteromer is decreased in the range of 5-100% more upon administration of the combination of inhibitors, relative to administering the CXCR4 inhibitor or GPCRx inhibitor as the single inhibitor to said patient.

[215] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the efficacy of a CXCR4 inhibitor is increased in the range of 5-2000% when administered in combination with the GPCRx inhibitor to the patient having said cancer cell containing the CXCR4-GPCRx heteromer, relative to efficacy of the CXCR4 inhibitor when administered as a single inhibitor.

[216] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the efficacy of a GPCRx inhibitor is increased in the range of 5-2000% when administered in combination with the CXCR4 inhibitor to the patient having said cancer cell containing the CXCR4-GPCRx heteromer, relative to efficacy of the GPCRx inhibitor when administered as a single inhibitor.

[217] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the method administers a combination of inhibitors selected from the group consisting of: an inhibitor of CXCR4, an inhibitor of GPCRx, and an inhibitor of the CXCR4-GPCRx heteromer.

[218] In certain embodiments of the method of treating, method of suppressing, pharma-

ceutical composition, or pharmaceutical kit, disclosed herein, the method administers a combination of a CXCR4 inhibitor and a GPCRx inhibitor.

[219] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the method administers a CXCR4-GPCRx heteromer inhibitor.

[220] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the administering of the combination of inhibitors suppresses the enhanced downstream signaling from said CXCR4-GPCRx heteromer in the cancer patient in the range of between 5-2000 fold, relative to single inhibitor administration.

[221] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the administering of the combination of inhibitors suppresses the enhanced downstream signaling from said CXCR4-GPCRx heteromer in the cancer patient in the range of between 5-1500 fold, relative to single inhibitor administration.

[222] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the administering of the combination of inhibitors suppresses the enhanced downstream signaling from said CXCR4-GPCRx heteromer in the cancer patient in the range of between 500-1500 fold, relative to single inhibitor administration.

[223] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the administering of the inhibitor or combination of inhibitors suppresses the enhanced downstream signaling from said CXCR4-GPCRx heteromer in the cancer patient in the range of between 5-2000 fold, relative to suppression of downstream signaling from either a CXCR4 protomer or a GPCRx protomer in their respective individual protomer context.

[224] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the administering of the inhibitor or combination of inhibitors suppresses the enhanced downstream signaling from said CXCR4-GPCRx heteromer in the cancer patient in the range of between 5-1500 fold, relative to suppression of downstream signaling from either a CXCR4 protomer or a GPCRx protomer in their respective individual protomer context.

[225] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the administering of the inhibitor or combination of inhibitors suppresses the enhanced downstream signaling from said CXCR4-GPCRx heteromer in the cancer patient in the range of between 500-1500 fold, relative to suppression of downstream signaling from either a CXCR4 protomer or a GPCRx protomer in their respective individual protomer context.

[226] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the cancer cell of the patient contains the CXCR4-GPCRx heteromer in a greater concentration than a normal, non-cancerous cell from said patient,

[227] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the presence of the CXCR4-GPCRx heteromer in the cancer cell of the patient identifies a sub-population of CXCR4-mediated cancer patients.

[228] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the presence of the CXCR4-GPCRx heteromer in the cancer cell of the patient is a biomarker of a sub-population of CXCR4-mediated cancer patients.

[229] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the biomarker of the sub-population of CXCR4-mediated cancer patients allows for precision medicine, patient stratification, or patient classification.

[230] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the biomarker of the sub-population of CXCR4-mediated cancer patients allows for GPCR-based precision cancer therapeutics.

[231] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the inhibitor is an antibody.

[232] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the CXCR4-GPCRx heteromer-selective reagent is an antibody.

[233] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the antibody is a bi-specific antibody of the CXCR4-GPCRx heteromer.

[234] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the antibody is a heteromer-specific antibody of the CXCR4-GPCRx heteromer.

[235] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the inhibitor is a bi-specific ligand(s) of the CXCR4-GPCRx heteromer.

[236] In certain embodiments of the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, disclosed herein, the antibody is an antibody-drug conjugate (ADC), as disclosed in, for example, Beck a et al., "Strategies

and challenges for the next generation of antibody-drug conjugates", Nature Reviews Drug Discovery, 16:315-337, (2017) and Lambert, et al., "Antibody-Drug Conjugates for Cancer Treatment", Annual Review of Medicine, 69:191-207 (2018), each of which are incorporated hereby in its entirety.

[237]

Brief Description of Drawings

[238] **FIGURE 1**: Shows schematic drawing of bimolecular fluorescence complementation (BiFC) assay. GPCR A is fused with N-terminal fragment of yellow fluorescent protein (YFP) Venus (VN) and GPCR B is fused with C-terminal fragment of Venus (VC). When GPCR A and B form heteromer, the complementary VN and VC are close enough to form functional Venus.

[239] **FIGURES 2a-2u**: Shows identification of CXCR4-interacting GPCRs using BiFC assay. Representative images that showed negative BiFC signal; CXCR4-VN and HA-VC (FIG. 2a), and CXCR4-VN and GCGR-VC (FIG. 2c). Representative images of CXCR4 and GPCRx that showed positive BiFC signal; CXCR4-VN and CXCR4-VC (FIGS. 2b), CXCR4-VN and ADCYAP1R1-VC (FIG. 2d), CXCR4-VC and ADORA2B-VN (FIG. 2e), CXCR4-VN and ADORA3-VC (FIG. 2f), CXCR4-VN and ADRB2-VC (FIG. 2g), CXCR4-VN and APLNR-VC (FIG. 2h), CXCR4-VN and C5AR1-VC (FIG. 2i), CXCR4-VN and CALCR-VC (FIG. 2j), CXCR4-VN and CCR5-VC (FIG. 2k), CXCR4-VN and CHRM1-VC (FIG. 2l), CXCR4-VN and GALR1-VC (FIG. 2m), CXCR4-VN and EDNRB-VC (FIG. 2n), CXCR4-VN and HRH1-VC (FIG. 2o), CXCR4-VN and MLNR-VC (FIG. 2p), CXCR4-VN and NTSR1-VC (FIG. 2q), CXCR4-VN and PTGER2-VC (FIG. 2r), CXCR4-VC and PTGER3-VN (FIG. 2s), CXCR4-VN and SSTR2-VC (FIG. 2t), and CXCR4-VN and TACR3-VC (FIG. 2u).

[240] **FIGURES 3a-3b**: Shows principle of GPCR co-internalization study. Cells co-expressing CXCR4-GFP and GPCRx are treated with GPCRx specific agonist. (FIG. 3a) CXCR4 and GPCRx does not physically interact with each other. GPCRx agonist induces internalization of GPCRx, but not CXCR4-GFP. (FIG. 3b) CXCR4 and GPCRx physically interact and forms heteromer. GPCRx agonist induces internalization of GPCRx, and CXCR4-GFP is co-internalized with GPCRx.

[241] **FIGURES 4a-4q**: Shows co-internalization of CXCR4-EGFP upon stimulation of GPCRx with its corresponding agonist (control: CXCR4-GFP (FIG. 4a)). U-2 OS cells were co-transduced with adenoviruses encoding CXCR-EGFP and GPCRx-VC, and the following GPCRx partners were examined to form heteromer with CXCR4-EGFP and to induce co-internalization of the CXCR4-EGFP: GPCRx represents ADCYAP1R1 (FIG. 4b), ADORA2B (FIG. 4c), ADORA3 (FIG. 4d), ADRB2 (FIG.

4e), APLNR (FIG. 4f), C5AR1 (FIG. 4g), CCR5 (FIG. 4h), CHRM1 (FIG. 4i), GALR1 (FIG. 4j), EDNRB (FIG. 4k), HRH1 (FIG. 4l), MLNR (FIG. 4m), NTSR1 (FIG. 4n), PTGER3 (FIG. 4o), SSTR2 (FIG. 4p), and TACR3 (FIG. 4q).

[242] **FIGURES 5a-5d**: Shows enhancement of the calcium response in cells co-expressing CXCR4 and ADRB2 upon co-stimulation with their respective selective agonists. MDA-MB-231 human breast cancer cells were transduced with adenoviruses encoding CXCR4 and HA-VC (FIG. 5a), ADRB2 and HA-VC (FIG. 5b), or CXCR4 and ADRB2 (FIG. 5c). Adenoviruses encoding HA-VC were used to adjust the total amount of adenoviruses transduced. Cells were allowed to express GPCR for 2 days, incubated with Cal-520 AM for 2 hr, and were treated with 15 nM of CXCL12, 100 nM of salmeterol (ADRB2-selective agonist), or CXCL12 and salmeterol together. Calcium mobilization was measured using FlexStation 3 Multi-Mode Microplate Reader. The results were normalized for base-line activity. Data represent three independent experiments (mean ± SEM). (FIG. 5d) Calcium mobilization was quantified by calculating the area-under-the-curve (AUC) of each graph in A-C. Data were normalized to CXCL12-stimulated calcium response in cells expressing CXCR4 alone. Sum represents the calculated additive effect of the responses obtained after stimulation of CXCL12 and salmeterol individually in cells co-expressing CXCR4 and ADRB2, to allow the visualization of the potentiation. ***$P < 0.001$; Student's t test.

[243] **FIGURES 6a-6l**: Shows enhancement of the calcium response in cells co-expressing CXCR4 and GPCRx upon co-stimulation with their respective selective agonists as shown in FIGS 5a-c. MDA-MB-231 cells were transduced with adenoviruses encoding CXCR4 and HA-VC, GPCRx and HA-VC, or CXCR4 and GPCRx. GPCRx represents ADCYAP1R1 (FIG. 6a), ADORA2B (FIG. 6b), ADORA3 (FIG. 6c), C5AR1 (FIG. 6d), CALCR (FIG. 6e), CHRM1 (FIG. 6f), EDNRB (FIG. 6g), HRH1 (FIG. 6h), MLNR (FIG. 6i), NTSR1 (FIG. 6j), PTGER2 (FIG. 6k), and TACR3 (FIG. 6l). Cells were incubated with Cal-520 AM, and were treated with CXCL12, GPCRx agonist, or CXCL12 and GPCRx agonist together. Calcium responses were quantified as described in FIG. 5. Data represent three independent experiments (mean ± SEM). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$; Student's t test.

[244] **FIGURES 7a-7e**: Shows examples of CXCR4-GPCRx heteromer that failed to show enhanced calcium signaling in cells co-expressing CXCR4 and GPCRx in the presence of agonists to both GPCRs. MDA-MB-231 cells were transduced with adenoviruses encoding CXCR4 and HA-VC, GPCRx and HA-VC, or CXCR4 and GPCRx. GPCRx represents APLNR (FIG. 7a), CCR5 (FIG. 7b), GALR1 (FIG. 7c), PTGER3 (FIG. 7d), and SSTR2 (FIG. 7e). Cells were incubated with Cal-520 AM, and were treated with CXCL12, GPCRx agonist, or CXCL12 and GPCRx agonist together. Calcium mobilization was quantified as described in FIG. 5. Data represent three independent ex-

periments (mean ± SEM). Student's t test. ns: not significant.

[245] **FIGURES 8a-8l**: Shows co-treatment of both antagonists efficiently suppressed the enhanced calcium response when cells expressing CXCR4-GPCRx heteromer were stimulated with CXCL12 and GPCRx agonists simultaneously. MDA-MB-231 cells were co-transduced with adenoviruses encoding CXCR4 and GPCRx. GPCRx represents ADRB2 (FIG. 8a), CHRM1 (FIGS. 8b-8d), EDNRB (FIG. 8e), HRH1(endo) (FIG. 8f and FIG. 8g), HRH1 (FIG. 8h and FIG. 8i), MLNR (FIG. 8j), NTSR1 (FIG. 8k), and TACR3 (FIG. 8l). In F &G, cells were transduced with adenoviruses encoding CXCR4, and endogenous HRH1 responses were examined with 50 nM of histamine. Cells were incubated with Cal-520 AM for 2 hr, GPCR antagonists or vehicle for 30 min, and stimulated with indicated amounts of CXCL12, GPCRx agonist, or CXCL12 and GPCRx agonist. Calcium mobilization was quantified as described in FIG. 5d. Data represent three independent experiments (mean ± SEM). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$; Student's t test.

[246] **FIGURE 9**: Shows principle of internalization inhibition study. Cells co-expressing CXCR4-GFP and GPCRx are treated with CXCL12 (SDF-1) and /or GPCRx specific antagonist. (scenario A) CXCR4 and GPCRx forms heteromer. CXCR4 agonist, CXCL12 (SDF-1) induced internalization of CXCR4-GFP alone or CXCR4-GFP with GPCRx. (scenario B) GPCRx antagonist does not induce internalization of CXCR4-GFP. (scenario C) Internalization of CXCL12 (SDF-1) stimulated CXCR4-GFP is inhibited by GPCRx specific antagonist.

[247] **FIGURES 10a-10c**: Shows inhibition of internalization by GPCRx antagonist for heteromers. (FIG. 10a) CXCR4-ADRB2; (FIG. 10b) CXCR4-CHRM1; and (FIG. 10c) CXCR4-HRH1. (FIG. 10a) CXCR4-GFP expressing U-2 OS cells were transduced with adenovirus encoding ADRB2. Treatment by CXCL12 (SDF-1), a CXCR4 agonist, induced CXCR4-ADRB2 internalization. But Carvedilol, an ADRB2 antagonist, did not induce internalization. Co-treatment with CXCL12 and Carvedilol partially inhibited CXCL12 induced CXCR4-ADRB2 internalization. (FIG. 10b) CXCR4-GFP expressing U-2 OS cells were transduced with adenovirus encoding CHRM1. Treatment by CXCL12 induced CXCR4-CHRM1 internalization. But Oxybutynin or Umeclidinium, CHRM1 antagonists, did not induce internalization. Co-treatment with CXCL12 and Oxybutynin or Umeclidinium inhibited CXCL12 induced CXCR4-CHRM1 internalization. (FIG. 10c) CXCR4-GFP expressing U-2 OS cells were transduced with adenovirus encoding HRH1. Treatment by CXCL12 induced CXCR4-HRH1 internalization. But Prometazine, Hydroxyzine, or Loratadine, HRH1 antagonists, did not induce internalization. Co-treatment with CXCL12 and Prometazine, Hydroxyzine, or Loratadine inhibited CXCL12 induced CXCR4-HRH1 internalization.

[248] **FIGURES 11a-11c**: Shows the effect of GPCRx antagonist on the survival of patient derived cells (PDCs) from cancer patients. (FIG. 11a) Effect of ADRB2 antagonist (Carvedilol) on the survival of PDC. Carvedilol induced significantly decreased survival of cells. (IC50=11.69 μM) (FIG. 11b) Effect of CHRM1 antagonists (Oxybutynin, Umeclidinium) on the survival of PDC. Oxybutynin and Umeclidinium each showed significantly decreased survival of cells at IC50=3.04 μM and 4.03 μM respectively. (FIG. 11c) Effect of HRH1 antagonists (Promethazine, Hydroxyzine, and Loratadine) on the survival of PDC. Promethazine, Hydroxyzine, and Loratadine each showed decreased survival of PDC at IC50=18.39 μM, 12.79 μM and 5.29 μM, respectively.

[249] **FIGURES 12a-12c**: Shows CXCR4-ADRB2 heterodimer detection by PLA and qRT-PCR in U-2 OS cells over-expressing CXCR4 and ADRB2. CXCR4-GFP expressing U-2 OS cells were transduced with adenovirus encoding ADRB2 at different MOIs for 2 days. Then CXCR4-ADRB2 co-expressing U-2 OS cells were performed PLA. (FIG. 12a) Image of CXCR4-ADRB2 heteromer detection by PLA. (FIG. 12b) Increase of the PLA signal proportionate to the expression level of ADRB2 in a dose dependent manner. (FIG. 12c) Data from qRT-PCR showing endogenous ADRB2 expression level of U-2 OS cells.

[250] **FIGURES 13a-13b**: Shows CXCR4-ADRB2 heteromer detection in PDC by PLA. GBM originated PDCs (sample IDs : 986T, 948T, 783T, 777T, 352T1, 352T2, 578T, 559T, 464T, 448T, 096T) were plated at chamber slide and CXCR4-ADRB2 heteromer were detected by PLA with CXCR4 and ADRB2 specific antibodies. (FIG. 13a) Image of CXCR4-ADRB2 heteromer detection. Nuclei were visualized with DAPI staining and CXCR4-ADRB2 heteromers were shown as small dots. (FIG. 13b) Ratio of CXCR4-ADRB2 heteromer in PDC.

[251] **FIGURES 14a-14b**: Shows CXCR4-CHRM1 heteromer detection in PDC by PLA. GBM originated PDCs (sample IDs : 986T, 948T, 783T, 777T, 352T1, 352T2, 578T, 559T, 464T, 448T, 096T) were plated at chamber slide and CXCR4-CHRM1 heteromer were detected by PLA with CXCR4 and CHRM1 specific antibodies. (FIG. 14a) Image of CXCR4-CHRM1 heteromer detection. Nuclei were visualized with DAPI staining and CXCR4-ADRB2 heteromers were shown as small dots. (FIG. 14b) Ratio of CXCR4-CHRM1 heteromer in PDC.

[252] **FIGURES 15a-15b**: Shows results from CXCR4-GPCRx heteromer detection in PDX. GBM originated PDXs (sample IDs; 777T, 783T, 948T, 559T) were detected CXCR4-ADRB2 heteromer by PLA with CXCR4 and ADRB2 specific antibodies. (FIG. 15a) Image of CXCR4-ADRB2 heteromer detection. Nuclei were visualized with DAPI staining and CXCR4-ADRB2 heteromers were shown as small dots. (FIG. 15b) Ratio of CXCR4-ADRB2 heteromer in PDX.

[253] **FIGURES 16a-16b**: Shows the enhancement of the calcium response in cells co-expressing CXCR4 and GPCRx upon co-stimulation with their respective agonists. MDA-MB-231 cells were transduced with adenoviruses encoding CXCR4 and ADRB2 (FIG. 16a) or CXCR4 and HRH1 (FIG. 16b). Cells were cultured for 3 days, stained with Cal-520 AM, and were treated with either CXCL12 (30 nM) alone, increasing doses of histamine or salmeterol alone, or increasing doses of histamine or salmeterol in combination with 30 nM of CXCL12. Calcium mobilization was measured using FlexStation 3. Sum represents the calculated additive value of the responses evoked by 30 nM of CXCL12 alone (open square) and GPCRx ligand alone at indicated doses (filled circle). Sum graph was depicted as a broken line with inverted triangles. Statistically significant differences between the sum (inverted triangle) and co-treatmemt (filled square) at each point were determined by Student's *t* test. *P < 0.05; **P < 0.01; ***P < 0.001; Data represent mean ± SD (n = 3).

[254] **FIGURES 17a-17c**: Shows no enhancement of the calcium response in cells co-expressing CXCR4 and GPCRx upon co-stimulation with their respective agonists. MDA-MB-231 cells were transduced with adenoviruses encoding CXCR4 and APLNR (FIG. 17a), CXCR4 and PTGER3 (FIG. 17b), or CXCR4 and SSTR2 (FIG. 17c). Cells were cultured for 3 days, stained with Cal 6 dye, and were treated with either CXCL12 alone (20 nM), increasing doses of Apelin-13, PGE2, or octreotide alone, or increasing doses of Apelin-13, PGE2 or octreotide in combination with 20 nM of CXCL12. Calcium mobilization was measured using FlexStation 3. Sum represents the calculated additive value of the responses evoked by 20 nM of CXCL12 alone (open square) and GPCRx ligand alone at indicated doses (filled circle). Sum graph was depicted as a broken line with inverted triangles. Statistically significant differences between the sum (inverted triangle) and co-treatment (filled square) at each point were determined by Student's t test. No statistical difference was observed at any point. Data represent mean ± SD (n = 3).

[255] **FIGURES 18a-18b**: Shows the enhancement of the calcium response in wild-type MDA-MB-231 cells upon co-stimulation with CXCL12 and histamine. (FIG. 18a) MDA-MB-231 cells were stimulated with CXCL12 (100 nM), histamine (10 nM), or CXCL12 and histamine together, and Ca2+ responses elicited by endogenous CXCR4 and HRH1 were measured. (FIG. 18b) Calcium mobilization was quantified by calculating the area-under-the-curve (AUC) of each graph. Sum represents the calculated additive value of the responses evoked by each agonist. The data represent three independent experiments done in triplicates (mean ± SEM). Statistically significant difference between the sum and co-treatmemt was determined by Student's t test. *P < 0.05.

[256] **FIGURES 19a-19b**: Shows enhanced migration of MDA-MB-231 cells in response

to a co-stimulation with CXCL12 (30 nM) and histamine (50 nM). MDA-MB-231 cells were transduced with 1 MOI of lentivirus encoding CXCR4, and chemotactic migration of the cells toward CXCL12, histamine, CXCL12 and histamine together in the presence or absence of pyrilamine (1 μM), a HRH1-selective inverse agonist, was evaluated. Chemotaxis was quantified by counting the migrated cells on the lower surface of the membrane of 10 fields per chamber. (FIG. 19a) A representative picture of each group. (FIG. 19b) Although histamine itself did not induce migration of the MDA-MB-231 cells, it significantly increased the migration of the cells upon co-stimulation with CXCL12. The enhanced migration of MDA-MB-231 cells was due to endogenous HRH1 since the addition of pyrilamine (1 μM), a HRH1-selective inverse agonist, completely abolished the enhanced response. The data represent mean ± SEM (n = 3 or 5). Statistically significant differences were determined by Student's *t* test. *P < 0.05.

[257] **FIGURE 20**: Shows efficient suppression of the enhanced calcium response by co-treatment of anti-CXCR4 antibody and ADRB2 antagonist when cells expressing CXCR4-ADRB2 heteromer were simultaneously stimulated with CXCL12 and ADRB2 agonist. MDA-MB-231 cells were co-transduced with adenoviruses encoding CXCR4 and ADRB2. Cells were treated with ADRB2 antagonist (Carvedilol), anti-CXCR4 antibody (12G5), or vehicle of indicated concentration and incubated with Cal 6 for 2 hours. Cells were subsequently stimulated with indicated amounts of CXCL12, ADRB2 agonist (Salmeterol), or CXCL12 and ADRB2 agonist.

[258] **FIGURES 21a-21b**: Shows detection of CXCR4-GPCR heteromer by PLA in U-2 OS cells over-expressing CXCR4 and GPCRx. U-2 OS cells expressing CXCR4-GFP were transduced with adenovirus encoding CHRM1 or HRH1 at different MOIs for 2 days. Subsequently, PLA was performed on U-2 OS cells co-expressing CXCR4-GPCRx using CXCR4- and GPCRx- specific antibodies. The PLA signal increased in proportion to the expression level of CHRM1 (FIG. 21a) and HRH1(FIG. 21b) in a dose-dependent manner.

[259] **FIGURES 22a-22b**: (FIG. 22a) Shows images of three mice that were transplanted with a parental cell A549, A549-CXCR4 overexpressing CXCR4 stably and A549-CXCR4-ADRB2 stably overexpressing CXCR4-ADRB2 heteromer, respectiviely, at 28 days after transplantation; (FIG. 22b) Shows graph comparing tumor growth between transplanted cells from FIG. 22a; tumor growth was monitored every third or fourth day by measuring the length (L) and width (W) of the tumor and calculating tumor volume in the basis of the following formula: Volume = 0.5 LW^2. The results are presented as the mean ± standard deviation of 3 individuals.

[260] **FIGURES 23a-23g**: Shows calcium response in MDAMB231 cells coexpressing CXCR4 and GPCRx upon co-stimulation with CXCL12 and endogenous ligand for

GPCRx. MDAMB231 cells were transduced with adenoviruses encoding CXCR4 and HA-VC, GPCRx and HA-VC, or CXCR4 and GPCRx where GPCRx represents ADCYAP1R1 (PAC1) (FIG. 23a), ADORA2B (FIG. 23b), ADORA3 (FIG. 23c), CHRM1 (FIG. 23d), EDNRB (FIG. 23e), MLNR (FIG. 23f), and TACR3 (FIG. 23g).

[261] **FIGURES 24a-24b**: Shows calcium response in MDAMB231 cells co-expressing CXCR4 and ADCYAP1R1 upon co-stimulation with their selective endogenous ligands. MDA-MB-231 cells were transduced with adenoviruses encoding CXCR4 and ADCYAP1R1. (FIG. 24a) Cells were treated with PACAP38 (1 nM, ADCYAP1R1 selective endogenous ligand) alone, increasing doses of CXCL12 alone, or increasing doses of CXCL12 in combination with 1 nM of PACAP38. (FIG. 24b) Cells were treated with CXCL12 alone, increasing doses of PACAP38 alone, or increasing doses of PACAP38 in combination with 15 nM of CXCL12.

[262] **FIGURES 25a-25b**: Shows calcium response in MDA-MB-231 cells co-expressing CXCR4 and TACR3 upon co-stimulation with their selective endogenous ligands. MDA-MB-231 cells were transduced with adenoviruses encoding CXCR4 and TACR3. (FIG. 25a) Cells were treated with Neurokinin B (0.4 nM, TACR3 selective endogenous ligand) alone, increasing doses of CXCL12 alone, or increasing doses of CXCL12 in combination with 0.4 nM of Neurokinin B. (FIG. 25b) Cells were treated with CXCL12 alone (30 nM), increasing doses of Neurokinin B alone, or increasing doses of Neurokinin B in combination with 30 nM of CXCL12.

[263] **FIGURES 26a-26b**: Shows validation of CRISPR/Cas9-mediated CXCR4 and HRH1 gene editing in MDA-MB-231 cells. (FIG. 26a) $MDA^{CXCR4+, HRH-}$ cells were generated by disrupting HRH1 gene in MDA-MB-231 cells stably expressing CXCR4 (MDA^{CXCR4+}), by transducing lentiviruses encoding Cas9 and guide RNA targeting HRH1. The absence of functional HRH1 was confirmed by measuring calcium responses upon exposure to histamine. (FIG. 26b) CXCR4 gene was edited in MDA-MB-231 cells using CRISPR/Cas-9 system and expression of CXCR4 was detected using immunoblotting.

[264] **FIGURES 27a-27b**: Shows enhanced calcium response in MDA-MB-231 cells upon co-treatment with CXCL12 and histamine is abrogated in the absence of HRH1. (FIG. 27a) MDAMB231 cells stably overexpressing CXCR4 (MDA^{CXCR4+}) or MDA^{CXCR4+} cells depleted with HRH1 using CRISPR/Cas9 system ($MDA^{CXCR4+, HRH1-}$) were treated with CXCL12 alone (50 nM), increasing concentrations of histamine, or histamine and CXCL12 together. (FIG. 27b) MDA^{CXCR4+} cells or $MDA^{CXCR4+, HRH1-}$ cells were treated with histamine alone (15 nM), increasing concentrations of CXCL12, or CXCL12 and histamine together.

[265] **FIGURES 28a-28b**: Shows enhanced calcium response in MDA-MB-231 cells upon co-treatment with CXCL12 and histamine is abrogated in the absence of CXCR4.

(FIG. 28a) MDA-MB-231 cells (MDA^{WT}) or MDA^{WT} cells depleted with CXCR4 using CRISPR/Cas9 system (MDA^{CXCR4-}) were treated with histamine alone (15 nM), increasing concentrations of CXCL12, or histamine and CXCL12 together. (FIG. 28b) MDA-MB-231 cells or MDA^{CXCR4-} cells were treated with CXCL12 alone (100 nM), increasing concentrations of histamine alone, or CXCL12 and histamine together, and Ca2+ responses were measured.

[266] **FIGURES 29a-29b**: Shows anti-tumor effect of CXCR4 inhibitor, ADRB2 inhibitor, or CXCR4 and ADRB2 inhibitor in mice transplanted with A549-CXCR4-ADRB2 cell line stably overexpressing CXCR4-ADRB2 heteromer. Figure 29a is a graph comparing tumor growth rates for the *in vivo* antitumor effect of CXCR4 inhibitor LY2510924, ADRB2 inhibitor Carvedilol, or combination of LY2510924 and Carvedilol. Figure 29b is a graph comparing tumor growth rates for the antitumor effect of CXCR4 inhibitor AMD070, ADRB2 inhibitor Carvedilol, or combination of AMD070 and Carvedilol. Tumor growth was monitored every third or fourth day by measuring the length (L) and width (W) of the tumor and calculating tumor volume in the basis of the following formula: Volume = 0.5 LW^2. The results are presented as the mean ± standard deviation of 10 individuals.

[267] Unless indicated otherwise, the following includes abbreviations for terms disclosed herein: acute myeloid leukemia (AML), Adenosine A3 Receptor (ADORA3), Adenosine Receptor A2b (ADORA2B), adenovirus high-throughput system (AdHTS), Adenylate Cyclase Activating Polypeptide 1 (Pituitary) Receptor Type I (ADCYAP1R1), Adrenoceptor Alpha 1A (ADRA1A), Adrenoceptor Beta 2 (ADRB2), Apelin Receptor (APLNR), Atypical chemokine receptor 3 (ACKR3), bimolecular fluorescence complementation (BiFC), Bioluminescence Resonance Energy Transfer (BRET), bovine serum albumin (BSA), Calcitonin Receptor (CALCR), Cancer stem cells (CSCs), C-C chemokine receptor type 2 (CCR2), chemerin chemokine-like receptor 1 (CMKLR1), Cholinergic Receptor Muscarinic 1 (CHRM1), chronic lymphocytic leukemia (CLL), chronic myeloid leukemia (CML), chronic obstructive pulmonary disease (COPD), Complement C5a Receptor 1 (C5AR1), C-terminal fragment of Venus (VC), C-X-C Motif Chemokine ligand 12 (CXCL12), CXC receptor 4 (CXCR4), cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4), δ-opioid receptor (OPRD), Endothelin Receptor Type B (EDNRB), enzyme-linked immunosorbent assay (ELISA), formalin-fixed paraffin-embedded (FFPE), fluorescence resonance energy transfer (FRET), G protein-coupled receptor (GPCR), Galanin Receptor 1 (GALR1), glioblastoma multiforme (GBM), Glucagon receptor (GCGR), GPCR heteromer identification technology (GPCR-HIT), Granulocyte-colony stimulating factor (G-CSF), hematopoietic stem cells (HSCs), hepatocellular carcinoma (HCC), Histamine Receptor H1 (HRH1), human immunodeficiency virus

(HIV), International Union of Basic and Clinical Pharmacology Committee on Receptor Nomenclature and Drug Classification (NC-IUPHAR), μ-opioid receptor (MOR), Motilin Receptor (MLNR), Multiple myeloma (MM), multiplicity of infection (MOI), Myelodysplastic Syndromes (MDS), Neurotensin Receptor 1 (NTSR1), non-Hodgkin lymphoma (NHL), non-small-cell lung cancer (NSCLC), N-terminal fragments of Venus (VN), patient derived cell (PDC), Patient-Derived Xenograft (PDX), positron emission tomography (PET), Computed Tomography (CT), programmed cell death ligand 1 (PD-L1), programmed cell death protein 1(PD-1), Prostaglandin E Receptor 2 (PTGER2), Prostaglandin E Receptor 3 (PTGER3), proximity ligation assay (PLA), reverse transcription-quantitative polymerase chain reaction (RT-qPCR), Single-photon emission computed tomography (SPECT), small lymphocytic lymphoma (SLL), small-cell lung cancer (SCLC), Somatostatin Receptor 2 (SSTR2), Stromal cell-derived factor 1 (SDF-1), systemic lupus erythematosus (SLE), Tachykinin Receptor 3 (TACR3), Threshold cycles (Ct), time-resolved FRET (TR-FRET), tumor microenvironment (TME), Vascular endothelial growth factor (VEGF), vascular smooth muscle cells (VSMC), WHIM syndrome (Warts, Hypogammaglobulinemia, Infections, and Myelokathexis), green fluorescence protein (GFP), and yellow fluorescence protein (YFP).

Mode for the Invention

[268] CXCR4 plays important roles in tumor formation and progression, but the development of CXCR4 antagonists as anticancer drugs has not been successful possibly due to side effects and lack of efficacy within acceptable dose ranges. Recently, various CXCR4-GPCR heteromers with distinct physiological and pharmacological properties were reported, but their roles in cancer biology or possibilities for developing anti-cancer therapeutics targeting CXCR4-GPCR heteromers have not been clearly understood.

[269] Traditionally GPCRs were believed to function as monomers that interact with hetero-trimeric G proteins upon ligand binding, and drugs were developed based on monomeric or homomeric GPCRs (Milligan 2008). This view has changed drastically by the discoveries that GPCRs can form heteromers, and heteromerization is obligatory for some GPCRs. GPCR heteromerization is known to alter GPCR maturation and cell surface delivery, ligand binding affinity, signaling intensity and pathways, as well as receptor desensitization and recycling (Terrillon and Bouvier 2004; Ferre et al., 2010; Rozenfeld and Devi 2010; Gomes et al., 2016; Farran 2017). Different GPCR heteromers display distinct functional and pharmacological properties, and GPCR heteromerization can vary depending on cell types, tissues, and diseases or pathological conditions (Terrillon and Bouvier 2004; Ferre et al., 2010;Rozenfeld and Devi 2010;

Gomes et al., 2016; Farran 2017). Now GPCR heteromerization is regarded as a general phenomenon, and deciphering GPCR heteromerization opens new avenues for understanding receptor function, physiology, roles in diseases and pathological conditions. Accordingly, identification of GPCR heteromers and their functional properties offers new opportunity for developing new pharmaceuticals or finding new use of old drugs with fewer side effects, higher efficacy, and increased tissue selectivity (Ferre et al., 2010; Rozenfeld and Devi 2010; Farran 2017).

[270] The identification of bona fide GPCR heteromer requires intensive and critical evaluation. To distinguish GPCR heteromers from simple association of GPCRs, researchers in this field and the International Union of Basic and Clinical Pharmacology Committee on Receptor Nomenclature and Drug Classification (NC-IUPHAR) have declared GPCR heteromer as "macromolecular complex composed of at least two (functional) receptor units [protomers] with biochemical properties that are demonstrably different from those of its individual components" and these heteromers exist in native tissue (Ferre et al., 2009; Gomes et al., 2016; Pin et al., 2007). They proposed three criteria to demonstrate GPCR heteromers: (1) Heteromers should exhibit appropriate co-localization and interaction to enable allosterism using co-immunoprecipitation, in situ hybridization, or proximity-based techniques including proximity ligation assays in cells/tissues that express both receptors and not in cells/tissues that lack one of the receptors; (2) Heteromers should exhibit distinct properties such as changes in signaling, ligand binding, and/or trafficking, only in cells/tissues expressing both receptors but not in cells/tissues that lack one of the receptors; and (3) Heteromer-selective reagents should alter heteromer-specific properties. Heteromer-selective reagents include heteromer-selective antibodies, membrane-permeable peptides, and bivalent/bifunctional ligands (Gomes et al., 2016; Pin et al., 2007). Although many GPCR heteromers have been identified *in vitro* using recombinant receptors expressed in heterologous cells, only a few have demonstrated novel properties, and very few have shown evidence for GPCR heteromerization in native tissue due to technical problems (Gomes et al., 2016). NC-IUPHAR announced that one should provide evidence that satisfies at least two of the above three criteria for approval of new GPCR heteromers (Pin et al., 2007).

[271] As disclosed herein, to establish whether criterion 1 of 3 is satisfied (relating to whether heteromer components colocalize and physically interact, either directly or via intermediate proteins acting as conduits for allosterism) in determining the presence/existence of a CXCR4-GPCRx heteromer, one or more of the following methods may be utilized to including, but not limited to: co-internalization assays; co-localization assays (determining co-localization of the receptor portomers within a cellular compartment) such as *in situ* hybridization, immunohistochemistry, or immunoelectron mi-

croscopy; proximity-based assays, such as proximity-based biophysical techniques, including resonance energy transfer (RET), bioluminescence RET (BRET), fluorescence RET (FRET), time-resolved fluorescence RET (TR-FRET), antibody-aided FRET, ligand-aided FRET, bimolecular fluorescence complementation (BiFC), and proximity ligation assays (PLA); co-immunoprecipitation assays; or fluorescent animals. For example, BiFC, co-internalization assay, or PLA, were utilized to evaluate whether a CXCR4-GPCRx heteromer satisfied criterion 1 of 3.

[272] As disclosed herein, to establish whether criterion 2 of 3 is satisfied (relating to whether a heteromer exhibits properties distinct from those of the individual protomers), such as a CXCR4-GPCRx heteromer that results in an enhanced downstream signaling, for example an enhanced calcium mobilization (such as determined by a calcium mobilization assay), a two-tiered approach was utilized on those CXCR4-GPCRx heteromers that satisfied criterion 1 of 3 discussed above: (1) determine the presence/absence of an enhanced downstream signaling, for example an enhanced calcium mobilization (synergism) in the individual protomer context - comparing calcium mobilization in cells co-expressing HA-VC and one of the protomers (either CXCR4 or GPCRx) upon (a) co-stimulation with CXCL12 and the respective selective agonist, relative to (b) stimulation with either CXCL12 alone or the respective selective agonist alone; and (2) determine the presence/absence of an enhanced downstream signaling, for example an enhanced calcium mobilization (synergism) in the CXCR4-GPCRx heteromer context - comparing calcium mobilization in cells co-expressing CXCR4 and GPCRx upon (a) co-stimulation with CXCL12 and the respective selective agonist, relative to (b) the sum of stimulation with either CXCL12 alone or the respective selective agonist alone. As disclosed herein, to satisfy criterion 2 of 3 and be considered a CXCR4-GPCRx heteromer that results in an enhanced downstream signaling, for example an enhanced calcium mobilization, there must be (1) an absence of an enhanced downstream signaling, for example an enhanced calcium mobilization in either protomer context (i.e., the CXCR4 and HA-VC context or the GPCRx and HA-VC context), and (2) a presence of an enhanced downstream signaling, for example an enhanced calcium mobilization in the CXCR4-GPCRx heteromer context. In both the protomer context and the CXCR4-GPCRx heteromer context, the concentration of CXCL12 utilized to stimulate the cells (either as a single agent or in combination with the respective selective GPCRx agonist) and the concentration of the selective GPCRx agonist (either an endogenous agonist or a known selective agonist for the respective GPCRx) utilized to stimulate the cells (either as a single agent or in combination with CXCL12) are independently at a concentration of 100x the EC50 concentration or lower. For example, the concentration of CXCL12 utilized to stimulate the cells (either as a single agent or

in combination with the respective selective GPCRx agonist) was at a concentration of 15 nM (which is approximately the EC50 concentration against CXCR4).

[273] As disclosed herein, to establish whether criterion 3 of 3 is satisfied (relating to whether heteromer-selective reagents should alter heteromer-specific properties) in determining the presence/existence of a CXCR4-GPCRx heteromer, patient derived cells, having satisfied criterion 1 of 3 and 2 of 3, are effected in the presence of an antagonist (a CXCR4 antagonist, a GPCRx antagonist, or a CXCR4-GPCRx heteromer antagonist), such as effecting cell proliferation of the patient derived cells containing a CXCR4-GPCRx heteromer.

[274] In some embodiments, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, as disclosed herein, include or rely upon establishing that an association of CXCR4 and a GPCRx in a cell satisfies at least two of the following criteria or characteristics to be considered a CXCR4-GPCRx heteromer, comprising: 1) the CXCR4-GPCRx heteromer components in a cell colocalize and physically interact, either directly or via intermediate proteins acting as conduits for allosterism, as determined via one or more of the following: a co-internalization assay, a colocalization assay, in situ hybridization, immunohistochemistry, immunoelectron microscopy, a proximity-based assay, a co-immunoprecipitation assay, or a fluorescent animal assay; 2) an enhanced amount of calcium mobilization, such that: a) either CXCR4 or the respective GPCRx in an individual protomer context in a cell, upon co-stimulation with CXCL12 and a respective selective GPCRx agonist, results in a calcium mobilization amount that is equal to or less than the sum of calcium mobilization amounts resulting from single agonist stimulation with either the CXCL12 or the respective selective GPCRx agonist; and b) the CXCR4-GPCRx heteromer exhibits an enhanced calcium mobilization upon co-stimulation with the CXCL12 and the respective selective GPCRx agonist relative to the sum of calcium mobilization amounts resulting from single agonist stimulation with either the CXCL12 or the respective selective GPCRx agonist; as determined via a calcium mobilization assay; or 3) a CXCR4-GPCRx heteromer-selective reagent: i) alters heteromer-specific properties of the CXCR4-GPCRx heteromer in a patient derived cell; ii) alters heteromer-specific function of the CXCR4-GPCRx heteromer in a patient derived cell; iii) alters heteromer-specific properties of a patient derived cell containing the CXCR4-GPCRx heteromer; or iv) decreases cell proliferation of a patient derived cell(s) containing the CXCR4-GPCRx heteromer. In some embodiments, the CXCR4-GPCRx heteromer-selective reagent alters heteromer-specific properties of the CXCR4-GPCRx heteromer in a patient derived cell, as determined by at least one of the following methods: PLA, radioligand binding assays, [35S]GTP-γS Binding assays, Calcuim assay, cAMP assay, GTPase assay, PKA activation, ERK1/2

and/or Akt/PKB Phosphorylation assays, Src and STAT3 phosphorylation assays, CRE-reporter assay, NFAT-RE-reporter assay, SRE-reporter assay, SRF-RE reporter assay, NF-kB-RE reporter assay, Secreted alkaline phosphatase Assay, Inositol 1-Phosphate Production assay, Adenylyl Cyclase Activity assay, analysis of target gene expression by RT-PCR, RT-qPCR, RNAseq, next generation sequencing (NGS), or microarray. In some embodiments, the CXCR4-GPCRx heteromer-selective reagent alters heteromer-specific function of the CXCR4-GPCRx heteromer in a patient derived cell, as determined by at least one of the following methods: PLA, radioligand binding assays, [35S]GTP-γS Binding assays, Calcuim assay, cAMP assay, GTPase assay, PKA activation, ERK1/2 and/or Akt/PKB Phosphorylation assays, Src and STAT3 phosphorylation assays, CRE-reporter assay, NFAT-RE-reporter assay, SRE-reporter assay, SRF-RE reporter assay, NF-kB-RE reporter assay, Secreted alkaline phosphatase Assay, Inositol 1-Phosphate Production assay, Adenylyl Cyclase Activity assay, analysis of target gene expression by RT-PCR, RT-qPCR, RNAseq, or microarray. In some embodiments, the CXCR4-GPCRx heteromer-selective reagent alters heteromer-specific properties of a patient derived cell containing the CXCR4-GPCRx heteromer, as determined by at least one of the following methods: assays on proliferation, migration, invasion, and drug resistance (survival) of cancer cells, modulation of immune cell function, angiogenesis, vasculogenesis, metastasis, drug resistance, tissue microarray (TMA), and cancer cell-tumor microenvironment (TME) interaction. For example, in some embodiments, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, as disclosed herein, include or rely upon establishing that an association of CXCR4 and a GPCRx in a cell satisfies at least two of the following criteria or characteristics to be considered a CXCR4-GPCRx heteromer, comprising: 1) the CXCR4-GPCRx heteromer components in a cell colocalize and physically interact, either directly or via intermediate proteins acting as conduits for allosterism, as determined via one or more of the following: a co-internalization assay, bimolecular fluorescence complementation (BiFC), or a proximity ligation assay (PLA); 2) an enhanced amount of calcium mobilization, such that: a) either CXCR4 or the respective GPCRx in an individual protomer context in a cell, upon co-stimulation with CXCL12 and a respective selective GPCRx agonist, results in a calcium mobilization amount that is equal to or less than the sum of calcium mobilization amounts resulting from single agonist stimulation with either the CXCL12 or the respective selective GPCRx agonist; and b) the CXCR4-GPCRx heteromer exhibits an enhanced calcium mobilization upon co-stimulation with the CXCL12 and the respective selective GPCRx agonist relative to the sum of calcium mobilization amounts resulting from single agonist stimulation with either the CXCL12 or the respective selective GPCRx agonist; as determined via

a calcium mobilization assay; or 3) a CXCR4-GPCRx heteromer-selective reagent: i) alters heteromer-specific properties of the CXCR4-GPCRx heteromer in a patient derived cell; ii) alters heteromer-specific function of the CXCR4-GPCRx heteromer in a patient derived cell; iii) alters heteromer-specific properties of a patient derived cell containing the CXCR4-GPCRx heteromer; or iv) decreases cell proliferation of a patient derived cell(s) containing the CXCR4-GPCRx heteromer. In some embodiments, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, as disclosed herein, include or rely upon establishing that an association of CXCR4 and a GPCRx in a cell satisfies criteria 1 and 2 to be considered a CXCR4-GPCRx heteromer. In some embodiments, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, as disclosed herein, include or rely upon establishing that an association of CXCR4 and a GPCRx in a cell satisfies criteria 1 and 3 to be considered a CXCR4-GPCRx heteromer. In some embodiments, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, as disclosed herein, include or rely upon establishing that an association of CXCR4 and a GPCRx in a cell satisfies criteria 2 and 3 to be considered a CXCR4-GPCRx heteromer. In some embodiments, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, as disclosed herein, include or rely upon establishing that an association of CXCR4 and a GPCRx in a cell satisfies criteria 1, 2, and 3, to be considered a CXCR4-GPCRx heteromer.

[275] As disclosed herein, the CXCR4-GPCRx heteromer satisfying at least two of the three criteria may have, cause, or produce an enhanced downstream signaling. The enhanced downstram signaling may be the result from the CXCR4-GPCRx heteromer, such as from agonism of the CXCR4-GPCRx heteromer, from CXCR4 agonism of the CXCR4-GPCRx heteromer, from GPCRx agonism of the CXCR4-GPCRx heteromer, and/or from CXCR4 agonism and GPCRx agonism of the CXCR4-GPCRx heteromer. In some embodiments, the enhanced downstram signaling may be downstream of the CXCR4, the respective GPCRx, or the CXCR4-GPCRx heteromer. In some embodiments, the enhanced downstram signaling may be from the CXCR4-GPCRx heteromer, relative to downstream signaling from a CXCR4 protomer or a respective GPCRx protomer in their respective individual protomer context. In some embodiments, the enhanced downstram signaling may be from the CXCR4-GPCRx heteromer, relative to downstream signaling from a CXCR4 protomer in an individual protomer context. In some embodiments, the enhanced downstram signaling may be from the CXCR4-GPCRx heteromer, relative to downstream signaling from a respective GPCRx protomer in an individual protomer context. In some embodiments, the enhanced downstram signaling may be from the CXCR4-GPCRx heteromer,

relative to downstream signaling from a CXCR4 protomer and a respective GPCRx protomer in their respective individual protomer context. The enhanced downstream signaling from said CXCR4-GPCRx heteromer, may in some embodiments, be suppressed in the cancer patient, such as suppressed in the patient's cancer cells. In some embodiments, the enhanced downstream signaling from said CXCR4-GPCRx heteromer, may be an enhanced amount of calcium mobilization (or synergistic amount of calcium mobilization), which may be determined by an intracellular Ca2+ assay, such as a calcium mobilization assay.

[276] As disclosed herein, the CXCR4-GPCRx heteromer satisfying at least two of the three criteria may have, cause, or produce an enhanced downstream signaling, wherein the enhanced downstream signaling is an enhanced amount of calcium mobilization. The enhanced amount of calcium mobilization from the CXCR4-GPCRx heteromer may be a calcium mobilization amount that, upon co-stimulation with the CXCL12 and the respective selective GPCRx agonist, is at least 10% greater, than the sum of calcium mobilization amounts resulting from single agonist stimulation of said cells with either the CXCL12 or the respective selective GPCRx agonist, as determined via a calcium mobilization assay. In some embodiments, the enhanced amount of calcium mobilization from the CXCR4-GPCRx heteromer may be a synergistic amount of calcium mobilization that, upon co-stimulation with the CXCL12 and the respective selective GPCRx agonist, is at least 10% greater, than the sum of calcium mobilization amounts resulting from single agonist stimulation of said cells with either the CXCL12 or the respective selective GPCRx agonist, as determined via a calcium mobilization assay. For example, the enhanced amount of calcium mobilization (or synergistic amount of calcium mobilization) from the CXCR4-GPCRx heteromer may be a calcium mobilization amount that, upon co-stimulation with the CXCL12 and the respective selective GPCRx agonist, is at least 20% greater, at least 30% greater, at least 40% greater, at least 50% greater, at least 75% greater, at least 90% greater, at least 100% greater, at least 150% greater, or at least 200% greater, than the sum of calcium mobilization amounts resulting from single agonist stimulation of said cells with either the CXCL12 or the respective selective GPCRx agonist, as determined via a calcium mobilization assay. In some embodiments, the enhanced amount of calcium mobilization (or synergistic amount of calcium mobilization) from the CXCR4-GPCRx heteromer may be a calcium mobilization amount that, upon co-stimulation with the CXCL12 and the respective selective GPCRx agonist, may be between 10-100% greater than the sum of calcium mobilization amounts resulting from single agonist stimulation of said cells with either the CXCL12 or the respective selective GPCRx agonist, as determined via a calcium mobilization assay, for example, may be a calcium mobilization amount that, upon co-stimulation with the CXCL12 and the re-

spective selective GPCRx agonist, may be between 25-100% greater, 50-100% greater, 75-100% greater , or 100-200% greater, than the sum of calcium mobilization amounts resulting from single agonist stimulation of said cells with either the CXCL12 or the respective selective GPCRx agonist, as determined via a calcium mobilization assay.

[277] In some embodiments, according to the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, as disclosed herein, the CXCR4-GPCRx heteromer satisfies at least two of the three criteria, thereby having, causing, or producing, an enhanced downstream signaling, wherein the enhanced downstream signaling is an enhanced amount of calcium mobilization, such that: a) either the CXCR4 or the respective GPCRx in an individual protomer context in the cell upon co-stimulation with CXCL12 and a respective selective GPCRx agonist results in a calcium mobilization amount that is equal to or less than the sum of calcium mobilization amounts resulting from single agonist stimulation with either the CXCL12 or the respective selective GPCRx agonist; and b) the CXCR4-GPCRx heteromer exhibits an enhanced calcium mobilization upon co-stimulation with the CXCL12 and the respective selective GPCRx agonist relative to the sum of calcium mobilization amounts resulting from single agonist stimulation with either the CXCL12 or the respective selective GPCRx agonist; as determined via a calcium mobilization assay. For example, in some embodiments, the enhanced downstream signaling from the CXCR4-GPCRx heteromer is an enhanced amount of calcium mobilization, such that: i) the calcium mobilization from the protomer CXCR4 or GPCRx, in the individual protomer context in the cell, is non-synergistic, as determined via calcium mobilization assay; and ii) the calcium mobilization from the CXCR4-GPCRx heteromer in the cell is synergistic, as determined via a calcium mobilization assay. In some embodiments, the individual protomer context may be: a) the individual protomer CXCR4 in the cell, in the absence of the respective individual protomer GPCRx; or b) the respective individual protomer GPCRx in the cell, in the absence of the individual protomer CXCR4; upon co-stimulation with CXCL12 and a respective selective GPCRx agonist results in a calcium mobilization amount that is equal to or less than the sum of calcium mobilization amounts resulting from single agonist stimulation with either the CXCL12 or the respective selective GPCRx agonist, as determined via a calcium mobilization assay. In some embodiments, the individual protomer context may be, independently: a) the individual protomer CXCR4 in the cell, in the absence of the respective individual protomer GPCRx; and b) the respective individual protomer GPCRx in the cell, in the absence of the individual protomer CXCR4; upon co-stimulation with CXCL12 and a respective selective GPCRx agonist results in a calcium mobilization amount that is equal to or less than the sum of calcium mobilization amounts resulting from single agonist stimulation with either the

CXCL12 or the respective selective GPCRx agonist, as determined via a calcium mobilization assay. For example, in some embodiments, the CXCR4-GPCRx heteromer, upon co-stimulation with the CXCL12 and the respective selective GPCRx agonist, may result in a calcium mobilization amount that is greater than the sum of calcium mobilization amounts resulting from single agonist stimulation of said cells with either the CXCL12 or the respective selective GPCRx agonist, as determined via a calcium mobilization assay.

[278] The term "CXCR4" as used herein refers to C-X-C Motif Chemokine Receptor 4, also identified by unique database identifiers (IDs) and alternate names as shown in Table 1 (Chatterjee et al., 2014; Debnath et al., 2013; Domanska et al., 2013; Guo et al., 2016; Peled et al., 2012; Roccaro et al., 2014; Walenkamp et al., 2017).

[279] The terms "GPCRx" as used herein refers to GPCRs that were used in this study to investigate if these GPCRs interact with CXCR4 and show properties distinct from those of individual protomers, including ADCYAP1R1 (ADCYAP Receptor Type I), ADORA2B (Adenosine A2b Receptor), ADORA3 (Adenosine A3 Receptor), ADRB2 (Adrenoceptor Beta 2), APLNR (Apelin Receptor), C5AR1 (Complement C5a Receptor 1), CALCR (Calcitonin Receptor), CCR5 (Chemokine (C-C Motif) Receptor 5), CHRM1 (Cholinergic Receptor Muscarinic 1), GALR1 (Galanin Receptor 1), EDNRB (Endothelin Receptor Type B), HRH1 (Histamine Receptor H1), MLNR (Motilin Receptor), NTSR1 (Neurotensin Receptor 1), PTGER2 (Prostaglandin E Receptor 2), PTGER3 (Prostaglandin E Receptor 3), SSTR2 (Somatostatin Receptor 2), and TACR3 (Tachykinin Receptor 3), also identified by unique database identifiers (IDs) and alternate names as shown in Table 1 and Table 2.

[280] Table 1 shown below provides the nomenclature of GPCRx that form heteromers with CXCR4 and synergistically enhance Ca2+ response upon co-stimulation with both agonists, and Table 2 shown below provides the nomenclature of GPCRx that form heteromers with CXCR4, but do not synergistically enhance Ca2+ response upon co-stimulation with both agonists.

[281] [Table 1]

Gene name	Full name	Other names	IDs
CXCR4	C-X-C Motif Chemokine Receptor 4	Leukocyte-Derived Seven Transmembrane Domain Receptor; Lipopolysaccharide-Associated Protein 3; Stromal Cell-Derived Factor 1 Receptor; Chemokine (C-X-C Motif) Receptor 4; LPS-Associated Protein 3 Seven Transmembrane Helix Receptor; C-X-C Chemokine Receptor Type 4; Neuropeptide Y Receptor Y3; Neuropeptide Y3 Receptor; Chemokine Receptor; Seven-Transmembrane-Segment Receptor, Spleen; Chemokine (C-X-C Motif), Receptor 4 (Fusin); SDF-1 Receptor; CD184 Antigen; Fusin; LAP-3; LESTR; NPYRL; FB22; HM89; LCR1; D2S201E; HSY3RR; NPYY3R;CXC-R4; CXCR-4; CD184; NPY3R; WHIMS; LAP3; NPYR; WHIM	GCID: GC02M136114HGNC: 2561Entrez Gene: 7852Ensembl: ENSG00000121966OMIM: 162643UniProtKB: P61073
ADCYAP1R1	ADCYAP Receptor Type I	Adenylate Cyclase Activating Polypeptide 1 (Pituitary) Receptor Type I; Pituitary Adenylate Cyclase-Activating Polypeptide Type 1	GCID: GC07P031058HGNC: 242Entrez Gene: 117Ensembl: ENSG00000078549OMIM: 102981UniProtKB:

		Receptor; PACAP Type I Receptor; PACAP Receptor 1; PACAP-R1; Pituitary Adenylate Cyclase Activating Polypeptide 1 Receptor Type I Hiphop; Pituitary Adenylate Cyclase-Activating Polypeptide Type I Receptor; PACAP-R-1; PACAPRI;PACAPR; PAC1R; PAC1	P41586
ADORA2B	Adenosine A2b Receptor	Adenosine Receptor A2b; ADORA2	GCID: GC17P015927HGNC: 264Entrez Gene: 136Ensembl: ENSG00000170425OMIM: 600446UniProtKB: P29275
ADORA3	Adenosine A3 Receptor	Adenosine Receptor A3; A3AR	GCID: GC01M111499HGNC: 268Entrez Gene: 140Ensembl: ENSG00000282608OMIM: 600445UniProtKB: P0DMS8
ADRB2	Adrenoceptor Beta 2	Adrenergic, Beta-2-, Receptor, Surface; Beta-2 Adrenoreceptor; Beta-2 Adrenoceptor; ADRB2R; B2AR; Adrenoceptor Beta 2, Surface; Adrenoceptor Beta 2 Surface; Beta-2 Adrenergic Receptor; Catecholamine Receptor;BETA2AR; ADRBR; BAR	GCID: GC05P148825HGNC: 286Entrez Gene: 154Ensembl: ENSG00000169252OMIM: 109690UniProtKB: P07550

C5AR1	Complement C5a Receptor 1	Complement Component 5a Receptor 1; Complement Component 5 Receptor 1 (C5a Ligand);C5a Ana-phylatoxin Chemotactic Receptor 1; C5a Ana-phylatoxin Chemotactic Receptor;Complement Component 5 Receptor 1; C5a Anaphylatoxin Receptor; C5a-R;C5R1; C5AR; CD88 Antigen; C5a Ligand; CD88; C5a	GCID: GC19P047290HGNC: 1338Entrez Gene: 728Ensembl: ENSG00000197405OM IM: 113995UniProtKB: P21730
CALCR	Calcitonin Receptor	CT-R; CTR1; CRT; CTR	GCID: GC07M093424HGNC: 1440Entrez Gene: 799Ensembl: ENSG00000004948OM IM: 114131UniProtKB: P30988
CHRM1	Cholinergic Receptor Muscarinic 1	Acetylcholine Receptor, Muscarinic 1;Muscarinic Acetylcholine Receptor M1; HM1; M1R; M1	GCID: GC11M062927HGNC: 1950Entrez Gene: 1128Ensembl: ENSG00000168539OM IM: 118510UniProtKB: P11229
EDNRB	Endothelin Receptor Type B	Endothelin Receptor Non-Selective Type; ET-BR; ET-B;ETRB;Endothelin Receptor Subtype B1; ABCDS; HSCR2;ETB1; ETBR; WS4A; HSCR; ETB	GCID: GC13M077895HGNC: 3180Entrez Gene: 1910Ensembl: ENSG00000136160OM IM: 131244UniProtKB: P24530
HRH1	Histamine Receptor H1	HH1R; H1R; Histamine Receptor, Subclass H1; Histamine H1 Receptor;	GCID: GC03P011113HGNC: 5182Entrez Gene:

		HisH1; H1-R	3269Ensembl: ENSG00000196639OM IM: 600167UniProtKB: P35367
MLNR	Motilin Receptor	G Protein-Coupled Receptor 38; GPR38; MTLR1; G-Protein Coupled Receptor 38; MTLR	GCID: GC13P049220HGNC: 4495Entrez Gene: 2862Ensembl: ENSG00000102539OM IM: 602885UniProtKB: O43193
NTSR1	Neurotensin Receptor 1	High-Affinity Levo-cabastine-Insensitive Neu-rotensin Receptor;Neurotensin Receptor 1 (High Affinity); NT-R-1; NTR1; NTRH; Neurotensin Receptor Type 1;NTRR; NTR	GCID: GC20P062708HGNC: 8039Entrez Gene: 4923Ensembl: ENSG00000101188OM IM: 162651UniProtKB: P30989
TACR3	Tachykinin Receptor 3	Neurokinin Beta Receptor; Neurokinin B Receptor; NK-3 Receptor; NK-3R; NK3RNKR; Neuromedin-K Receptor;TAC3RL; TAC3 R; HH11	GCID: GC04M103586HGNC: 11528Entrez Gene: 6870Ensembl: ENSG00000169836OM IM: 162332UniProtKB: P29371

[282] *GCID: Genecards identification

[283] HGNC: HUGO Gene Nomenclature Committee

[284]

[285] [Table 2]

Gene name	Full name	Other names	IDs
APLNR	Apelin Receptor	G-Protein Coupled Receptor HG11;Angiotensin II Receptor-Like 1;Angiotensin Receptor-Like 1; APJ (Apelin) Receptor; G Protein-Coupled Receptor APJ;G-Protein Coupled Receptor APJ;HG11 Orphan Receptor;APJ Receptor; AGTRL1; APJ; APJR;HG11	GCID: GC11M057233HG NC: 339Entrez Gene: 187Ensembl: ENSG00000134817 OMIM: 600052UniProtKB: P35414
CCR5	Chemokine (C-C Motif) Receptor 5	C-C Chemokine Receptor Type 5;Chemokine Receptor CCR5; Chemokine (C-C Motif) Receptor 5 (Gene/Pseudogene);C-C Motif Chemokine Receptor 5 A159A; Chemokine Recptor CCR5 Delta32; CD195 Antigen; HIV-1 Fusion Coreceptor; CC-CKR-5;ChemR13; CMKBR5; CCR-5;C-C CKR-5; CCCKR5; IDDM22;CD195; CKR-5; CKR5	GCID: GC03P046382HGN C: 1606Entrez Gene: 1234Ensembl: ENSG00000160791 OMIM: 601373UniProtKB: P51681
GALR1	Galanin Receptor 1	GALNR1; GAL1-R; GALR-1; GALNR; Galanin Receptor Type 1	GCID: GC18P077250HGN C: 4132Entrez Gene: 2587Ensembl: ENSG00000166573 OMIM: 600377UniProtKB: P47211
PTGER2	Prostaglandin E Receptor 2	Prostaglandin E Receptor 2 (Subtype EP2), 53kDa;	GCID: GC14P052314HGN

		Prostaglandin E Receptor 2 (Subtype EP2), 53kD; PGE2 Receptor EP2 Subtype; PGE Receptor EP2 Subtype; Prostanoid EP2 Receptor; Prostaglandin E2 Receptor EP2 Subtype; EP2	C: 9594Entrez Gene: 5732Ensembl: ENSG00000125384 OMIM: 176804UniProtKB: P43116
PTGER3	Prostaglandin E Receptor 3	Prostaglandin E Receptor 3 (Subtype EP3); PGE2 Receptor EP3 Subtype; Prostanoid EP3 Receptor;PGE2-R; Prostaglandin E Receotor EP3 Subtype 3 Isoform; Prostaglandin E2 Receptor EP3 Subtype; Prostaglandin Receptor (PGE-2); PGE Receptor, EP3 Subtype; PGE Receptor EP3 Subtype; EP3-III; EP3-II; EP3-IV;EP3-VI; EP3-I; EP3e; EP3	GCID: GC01M070852HG NC: 9595Entrez Gene: 5733Ensembl: ENSG00000050628 OMIM: 176806UniProtKB: P43115
SSTR2	Somatostatin Receptor 2	SRIF-1; SS2R; Somatostatin Receptor Type 2; SS-2-R; SS2-R	GCID: GC17P073165HGN C: 11331Entrez Gene: 6752Ensembl: ENSG00000180616 OMIM: 182452UniProtKB: P30874

[286] *GCID: Genecards identification

[287] HGNC: HUGO Gene Nomenclature Committee

[288]

[289] Further information regarding the GPCRs evaluated herein as heteromers with CXCR4 are detailed below:

[290] (1) ADCYAP1R1 -- Pituitary adenylate cyclase-activating polypeptide type I receptor also known as PAC1, is a protein that in humans is encoded by the

ADCYAP1R1 gene. This receptor binds pituitary adenylate cyclase activating peptide. ADCYAP1R1 is a membrane-associated protein and shares significant homology with members of the G-protein coupled class B glucagon/secretin receptor family. This receptor mediates diverse biological actions of adenylate cyclase activating polypeptide 1 and is positively coupled to adenylate cyclase (Vaudry et al., 2000). This is a receptor for PACAP-27 and PACAP-38. The activity of this receptor is mediated by G proteins which activate adenylyl cyclase. ADCYAP1R1 may regulate the release of adrenocorticotropin, luteinizing hormone, growth hormone, prolactin, epinephrine, and catecholamine. ADCYAP1R1 may play a role in spermatogenesis and sperm motility. ADCYAP1R1 causes smooth muscle relaxation and secretion in the gastrointestinal tract (Ogi et al., 1993).

[291] ADCYAP1R1 is expressed in the adrenal medulla, pancreatic acini, uterus, myenteric plexus and brain (Reubi, 2000; Reubi et al., 2000). It is also expressed in the trigeminal, otic and superior cervical ganglia (prejunctional) and cerebral arteries (postjunctional) (Knutsson and Edvinsson, 2002). Diseases associated with ADCYAP1R1 include post-traumatic stress disorder (Lowe et al., 2015) and anxiety disorder. Among its related pathways are G alpha(s) signalling events and RET signaling (Cooper et al., 2013).

[292] (2) ADORA2B -- The adenosine A2B receptor, also known as ADORA2B, is a G-protein coupled adenosine receptor, and also denotes the human adenosine A2b receptor gene which encodes it. Adenosine functions as a signaling molecule through the activation of four distinct adenosine receptors - ADORA1, ADORA2A, ADORA2B, and ADORA3, also called as A1, A2A, A2B, and A3, respectively. This integral membrane protein stimulates adenylate cyclase activity in the presence of adenosine. This protein also interacts with netrin-1, which is involved in axon elongation. These receptors are widely expressed and have been implicated in several biological functions, both physiological and pathological. Both ADORA2A and ADORA2B receptors are important in cancer immunology. The tumor microenvironment is hypoxic, which promotes the expression of CD39 and CD73 by immune cells. CD39 and CD73 are ectonucleotidases that convert ATP to adenosine, elevating concentrations of adenosine locally. Adenosine is a crucial mediator of altered immune function in cancer as it binds ADORA2A and ADORA2B receptors on lymphocytes silencing the antitumor immune response (Chen et al., 2013).

[293] ADORA2B has a role in regulating CXCR4 expression *in vivo* and in protecting against vascular lesion formation (Yang et al., 2008) and promotes progression of human oral cancer (Kasama et al., 2015). Identification of a pharmacologically tractable Fra-1/ADORA2B axis promoting breast cancer metastasis (Desmet et al., 2013). ADORA2B receptors display high expression levels in the cecum, colon and

bladder, with lower levels in the lung, blood vessels and eye (Fagerberg et al., 2014).

[294] (3) ADORA3 -- The adenosine A3 receptor, also known as ADORA3, is an adenosine receptor, but also denotes the human gene encoding it. ADORA3 receptors are G-protein coupled receptors that couple to Gi/Gq and are involved in a variety of intracellular signaling pathways and physiological functions. It mediates a sustained cardioprotective function during cardiac ischemia, it is involved in the inhibition of neutrophil degranulation in neutrophil-mediated tissue injury, it has been implicated in both neuroprotective and neurodegenerative effects, and it may also mediate both cell proliferation and cell death (Gao et al., 2008; Miwatashi et al., 2008).

[295] Cordycepin induces apoptosis in human bladder cancer cells via activation of ADORA3 receptors (Cao et al., 2017). Jafari et al. showed that ADORA3 receptor agonist inhibited survival of breast cancer stem cells via GLI-1 and ERK1/2 pathway (Jafari et al., 2017). ADORA3 is broadly expressed in adrenal (RPKM 4.4), small intestine (RPKM 2.9) and 20 other tissues containing brain, bladder, lymph node and colon (Fagerberg et al., 2014).

[296] (4) ADRB2 -- The beta-2 adrenergic receptor (β2 adrenoreceptor), also known as ADRB2, is a cell membrane-spanning beta-adrenergic receptor that interacts with epinephrine, a hormone and neurotransmitter (ligand synonym, adrenaline) whose signaling, via a downstream L-type calcium channel interaction, mediates physiologic responses such as smooth muscle relaxation and bronchodilation (Gregorio et al., 2017). ADRB2 functions in muscular system such as smooth muscle relaxation, motor nerve terminals, glycogenolysis and in circulatory system such as heart muscle contraction, cardiac output increase. In the normal eye, beta-2 stimulation by salbutamol increases intraocular pressure via net. In digestive system, the ADRB2 induces glycogenolysis and gluconeogenesis in liver and insulin secretion from pancreas (Fitzpatrick, 2004).

[297] ADRB2 signaling in the cardiac myocyte is modulated by interactions with CXCR4 (LaRocca et al., 2010). Norepinephrine attenuates CXCR4 expression and the corresponding invasion of MDA-MB-231 breast cancer cells via ADRB2 (Wang et al., 2015a). ADRB2 is expressed in several cancers such as pancreatic, prostate (Braadland et al., 2014; Xu et al., 2017), renal and breast cancer (Choy et al., 2016).

[298] (5) C5AR1 -- The C5a receptor also known as complement component 5a receptor 1 (C5AR1) or CD88 (Cluster of Differentiation 88) is a G protein-coupled receptor for C5a. It functions as a complement receptor. C5AR1 modulates inflammatory responses, obesity, development and cancers. (Gerard and Gerard, 1994).

[299] The C5AR1 is expressed on granulocytes, monocytes, dendritic cells, hepatoma-derived cell line HepG2, astrocytes, microglia (Klos et al., 2013). C5AR1 is related in several diseases such as inflammatory bowel disease, rheumatoid arthritis, psoriasis,

experimental allergic encephalomyelitis, multiple sclerosis, meningitis, and brain trauma (Lee et al., 2008).

[300] (6) CALCR -- The calcitonin receptor (CT) is a G protein-coupled receptor that binds the peptide hormone calcitonin and is involved in maintenance of calcium homeostasis, particularly with respect to bone formation and metabolism (Dacquin et al., 2004; Davey et al., 2008). CT works by activating the G-proteins Gs and Gq often found on osteoclasts, on cells in the kidney, and on cells in a number of regions of the brain. It may also affect the ovaries in women and the testes in men. The activity of this receptor is mediated by G proteins which activate adenylyl cyclase. The calcitonin receptor is thought to couple to the heterotrimeric guanosine triphosphate-binding protein that is sensitive to cholera toxin. The physiological effects of CT such as inhibition of osteoclast mediated bone resorption or increased Ca2+ excretion by the kidney are mediated by high affinity CTs (Albrandt et al., 1995).

[301] Loss of function mutation in CT identifies highly aggressive glioblastoma with poor outcome (Pal et al., 2018). Expression of CTRs in mammary gland, cartilage, nose and ear has been reported. The developmental regulation of CALCR gene expression in a variety of the above tissues suggests a role for CTRs in the morphogenesis of these tissues (Pondel, 2000).

[302] (7) CHRM1 -- The muscarinic cholinergic receptors belong to a larger family of G protein-coupled receptors. The functional diversity of these receptors is defined by the binding of acetylcholine and includes cellular responses such as adenylate cyclase inhibition, phosphoinositide degeneration, and potassium channel mediation. Muscarinic receptors influence many effects of acetylcholine in the central and peripheral nervous system (Rang, 2003). The muscarinic cholinergic receptor 1 is involved in mediation of vagally-induced bronchoconstriction and in the acid secretion of the gastrointestinal tract. It is predominantly found bound to G proteins of class Gq that use upregulation of phospholipase C and, therefore, inositol trisphosphate and intracellular calcium as a signaling pathway (Qin et al., 2011).

[303] Muscarinic receptors are widely distributed throughout the body and control distinct functions according to location and subtype (CHRM1 - CHRM5). They are predominantly expressed in the parasympathetic nervous system where they exert both inhibitory and excitatory effects. This receptor is found mediating slow excitatory postsynaptic potential at the ganglion in the postganglionic nerve, is common in exocrine glands and in the CNS (Johnson, 2002). Diseases associated with CHRM1 include asthma and heart block, congenital and Pelizaeus-Merzbacher disease. Activators of CHRM1 muscarinic acetylcholine receptors (mAChRs) may provide novel treatments for schizophrenia and Alzheimer's disease (Marlo et al., 2009).

[304] (8) EDNRB -- Endothelin receptor type B (ETB) receptor was cloned shortly after

the ETA receptor (EDBRA). Similarly to the EDNRA, it belongs to class A of G protein-coupled heptahelical receptors with an external amino terminus and internal carboxy terminus and binding sites intrinsic to the heptahelical portions of the receptor. Similar to the EDNRA, the endogenous agonist ET-1 has a high affinity for the EDNRB. The pharmacology of the EDNRB receptor is somewhat richer than that of the EDNRA in that multiple agonists of the EDNRB are recognized, including sarafotoxin 6c (S6c) and IRL1620. Selective antagonists of the EDNRB include BQ788, A192621, RES7011, and IRL2500. The EDNRB has been localized to cardio-vascular tissues, the pulmonary system, neurons, bone, pancreas, and kidney (Watts, 2010).

[305] EDNRB is a G protein-coupled receptor which activates a phosphatidylinositol-calcium second messenger system. Its ligand, endothelin, consists of a family of three potent vasoactive peptides: ET1, ET2, and ET3. In melanocytic cells the EDNRB gene is regulated by the microphthalmia-associated transcription factor. Mutations in either gene are links to Waardenburg syndrome (Sato-Jin et al., 2008). The multigenic disorder, Hirschsprung disease type 2, is due to mutation in EDNRB gene (Tanaka et al., 1998). Diseases associated with EDNRB include Waardenburg syndrome, Type 4A and Abcd syndrome. Among its related pathways are calcium signaling pathway and prostaglandin synthesis and regulation of Waardenburg Syndrome (Sato-Jin et al., 2008).

[306] (9) HRH1 -- The H1 receptor is a histamine receptor belonging to the family of rhodopsin-like G-protein-coupled receptors. This receptor is activated by the biogenic amine histamine. The HRH1 is linked to an intracellular G-protein (Gq) that activates phospholipase C and the inositol triphosphate (IP3) signaling pathway. Antihistamines, which act on this receptor, are used as anti-allergy drugs. The crystal structure of the receptor has been determined and used to discover new HRH1 ligands in structure-based virtual screening studies (de Graaf et al., 2011). A subunit of the G-protein subsequently dissociates and affects intracellular messaging including downstream signaling accomplished through various intermediaries such as cyclic AMP, cyclic GMP, calcium, and nuclear factor kappa B. It functions in immune-cell chemotaxis, pro-inflammatory cytokine production, expression of cell adhesion molecules, and other allergic and inflammatory conditions (Canonica and Blaiss, 2011).

[307] In peripheral tissues, the HRH1 mediates the contraction of smooth muscles, increase in capillary permeability due to contraction of terminal venules, and catecholamine release from adrenal medulla, as well as mediating neurotransmission in the central nervous system. It is also known to contribute to the pathophysiology of allergic diseases such as atopic dermatitis, asthma, anaphylaxis and allergic rhinitis (Xie and He, 2005). It is expressed in smooth muscles, on vascular endothelial cells, in the

heart, and in the central nervous system (de Graaf et al., 2011).

[308] (10) MLNR -- Motilin receptor is a G protein-coupled receptor that binds motilin. Motilin in turn is an intestinal peptide that stimulates contraction of gut smooth muscle. Also, the MLNR mediates progastrokinetic effects. MLNR is an important therapeutic target for the treatment of hypomotility disorders (Depoortere, 2001).

[309] It is found at its highest concentrations in the nerves of the antral wall of the stomach and is also found at significant levels throughout the smooth muscle of the upper gastrointestinal tract (Kitazawa et al., 1995). Diseases associated with MLNR include gastroparesis and diabetic autonomic neuropathy (Kitazawa et al., 1997).

[310] (11) NTSR1 -- Neurotensin receptor 1 belongs to the large superfamily of G-protein coupled receptors. Neurotensin is a 13-amino acid peptide originally isolated from hypothalamic and later from intestines of bovine. In the brain, neurotensin is exclusively found in nerve cells, fibers, and terminals, whereas the majority of peripheral neurotensin is found in the endocrine N-cells located in the intestinal. Central or peripheral injections of neurotensin produce completely different pharmacological effects indicating that the peptide does not cross the blood-brain barrier. NTSR1 mediates the multiple functions of neurotensin, such as hypotension, hyperglycemia, hypothermia, antinociception, and regulation of intestinal motility and secretion (Vincent, 1995).

[311] Signaling is effected via G proteins that activate a phosphatidylinositol-calcium second messenger system. Signaling leads to the activation of downstream MAP kinases and protects cells against apoptosis (Heakal et al., 2011). Swift et al. showed altered expression of neurotensin receptors is associated with the differentiation state of prostate cancer (Swift et al., 2010).

[312] (12) PTGER2 -- Prostaglandin E2 receptor 2, also known as EP2, is a prostaglandin receptor for prostaglandin E2 (PGE2) encoded by the human gene PTGER2. PTGER2 is classified as a relaxant type of prostanoid receptor based on its ability, upon activation, to relax certain types of smooth muscle. When initially bound to PGE2 or any other of its agonists, it mobilizes G proteins containing the Gαs-Gβγ complex. The Gαs- Gβγ complexes dissociate into their Gαs and Gβγ subunits which in turn regulate cell signaling pathways. In particular, Gαs stimulates adenyl cyclase to raise cellular levels of cAMP thereby activating PKA; PKA activates various types of signaling molecules such as the transcription factor CREB which lead to different types of functional responses depending on cell type (Markovic et al., 2017; Woodward et al., 2011). PTGER2 also activates the GSK-3 pathway which regulates cell migratory responses and innate immune responsesincluding pro-inflammatory cytokine and interleukin production and Beta-catenin pathway which regulates not only cell-cell adhesion but also activates the Wnt signaling pathway which, in turn, stimulates the transcription of genes responsible for regulating cell migration and proliferation

(Woodward et al., 2011).

[313] The PTGER2 receptor can act as a tumor promoter. PTGER2 gene knockout mice have less lung, breast, skin, and colon cancers following exposure to carcinogens. Knockout of this gene in mice with the adenomatous polyposis coli mutation also causes a decrease in the size and number of pre-cancerous intestinal polyps that the animals develop. These effects are commonly ascribed to the loss of PTGER2-mediated: vascular endothelial growth factor production and thereby of tumor vascularization; regulation of endothelial cell motility and survival; interference with transforming growth factor-β's anti-cell proliferation activity; and, more recently, regulation of host anti-tumor immune responses (O'Callaghan and Houston, 2015).

[314] PTGER2 is widely distributed in humans. Its protein is expressed in human small intestine, lung, media of arteries and arterioles of the kidney, thymus, uterus, brain cerebral cortex, brain striatum, brain hippocampus, corneal epithelium, corneal chorio-capillaries, Myometriuml cells, eosinophiles, sclera of the eye, articular cartilage, the corpus cavernosum of the penis, and airway smooth muscle cells; its mRNA is expressed in gingival fibroblasts, monocyte-derived dendritic cells, aorta, corpus cavernosum of the penis, articular cartilage, airway smooth muscle, and airway epithelial cells. In rats, the receptor protein and/or mRNA has been found in lung, spleen, intestine, skin, kidney, liver, long bones, and rather extensively throughout the brain and other parts of the central nervous system (Yagami et al., 2016).

[315] Pre-clinical studies indicate that PTGER2 may be a target for treating and/or preventing particular human disorders involving: allergic diseases such as asthma (particular aspirin and nonsteroidal inflammatory drug-induced asthma syndromes) and rhinitis (Machado-Carvalho et al., 2014); glaucoma (Doucette and Walter, 2017); various diseases of the nervous system (Yagami et al., 2016); fractures, osteoporosis, and other bone abnormalities (Li et al., 2007); pulmonary fibrosis; certain forms of malignant disease such as colon cancer including those that arise from Adenomatous polyposis coli mutations (O'Callaghan and Houston, 2015); and salt-sensitive forms of hypertension (Yang and Du, 2012); This receptor has also been suggested to be a target for contraception (Sugimoto et al., 2015).

[316] (13) TACR3 -- Tachykinin receptor 3, also known as TACR3, function as receptors for tachykinins. The tachykinins are a family of peptides that comprise substance P (SP), neurokinin A (NKA), neurokinin B (NKB) and the species divergent endokinins including endokinin B (EKB) in humans. These tachykinins are encoded on three different genes, preprotachykinin 1 (TAC1) encoding SP and NKA, TAC3 encoding NKB and TAC4 encoding EKB. Three tachykinin receptors have been identified, which interact with these tachykinins: TACR1, TACR2, and TACR3, also called as NK1, NK2 and NK3, respectively, whereby SP and EKB show the greatest potency for

TACR1, NKA for TACR2 and NKB for TACR3. Receptor affinities are specified by variations in the 5'-end of the sequence. Tachykinin receptor-3 (TACR3) is the mediator of biologic actions encoded by the C-terminal sequence of tachykinins, for which neurokinin B is a more potent agonist than neurokinin A or substance P (Page et al., 2003).

[317] It is reported that four human pedigrees with severe congenital gonadotropin deficiency and pubertal failure in which all affected individuals are homozygous for loss-of-function mutations in TAC3 (encoding Neurokinin B) or its receptor TACR3 (encoding NK3R) (Topaloglu et al., 2009). NKB, a member of the substance P-related tachykinin family, is known to be highly expressed in hypothalamic neurons that also express kisspeptin (Goodman et al., 2007), a recently identified regulator of gonadotropin-releasing hormone secretion (Gianetti and Seminara, 2008).

[318] (14) APLNR -- The apelin receptor (also known as the APJ receptor) is a G protein-coupled receptor which binds apelin and Apela/ELABELA/Toddler (Medhurst et al., 2003). Receptor for apelin receptor early endogenous ligand (APELA) and apelin (APLN) hormones coupled to G proteins that inhibit adenylate cyclase activity. APLNR plays a key role in early development such as gastrulation and heart morphogenesis by acting as a receptor for APELA hormone. APLNR plays also a role in various processes in adults such as regulation of blood vessel formation, blood pressure, heart contractility, and heart failure by acting as a receptor for APLN hormone. A decade of investigations has shown that the apelinergic system has a broad range of biological functions, playing an important role particularly in maintaining homeostasis of the cardiovascular system and fluid metabolism. The activation of APLNR causes a broad spectrum of biochemical changes including cAMP suppression, phosphorylation of protein kinase B (Akt), ERK1/25 and p70S6K, calcium mobilization7 and nitric oxide synthase (NOS). However, the specificity linking these biochemical activities to a certain biological function is yet to be determined (Wang et al., 2015c).

[319] Also, it functions in embryonic and tumor angiogenesis (Wu et al., 2017) and as a human immunodeficiency virus (HIV-1) coreceptor (Zhou et al., 2003). Both APLNR and apelin are expressed in many tissues including heart, lung, endothelium, kidney and brain (Wang et al., 2015c).

[320] (15) CCR5 -- C-C chemokine receptor type 5, also known as CCR5 or CD195, is a protein on the surface of white blood cells that is involved in the immune system as it acts as a receptor for chemokines. This is the process by which T cells are attracted to specific tissue and organ targets. Many forms of HIV, the virus that causes AIDS, initially use CCR5 to enter and infect host cells. Certain individuals carry a mutation known as CCR5-Δ32 in the CCR5 gene, protecting them against these strains of HIV.

Certain populations have inherited the Delta 32 mutation resulting in the genetic deletion of a portion of the CCR5 gene. Homozygous carriers of this mutation are resistant to M-tropic strains of HIV-1 infection (Hutter et al., 2009). CCR5's cognate ligands include CCL3, CCL4 (also known as MIP 1α and 1β, respectively), and CCL3L1. CCR5 furthermore interacts with CCL5 (Struyf et al., 2001).

[321] It is likely that CCR5 plays a role in inflammatory responses to infection, though its exact role in normal immune function is unclear. Regions of this protein are also crucial for chemokine ligand binding, functional response of the receptor, and HIV co-receptor activity (Barmania and Pepper, 2013). CCR5 is predominantly expressed on T cells, macrophages, dendritic cells, eosinophils, microglia and a subpopulation of ether breast or prostate cancer cells (Sicoli et al., 2014; Velasco-Velazquez et al., 2012).

[322] (16) GALR1 -- Galanin receptor 1 (GAL1) is a G-protein coupled receptor encoded by the GALR1 gene. The ubiquitous neuropeptide galanin controls numerous functions such as endocrine secretions, intestinal motility, and behavioral activities. These regulatory effects of galanin are mediated through the interaction with specific membrane receptors and involve the pertussis toxin-sensitive guanine nucleotide binding proteins Gi/Go as transducing elements (Habert-Ortoli et al., 1994).

[323] GALR1 has anti-proliferative effects in oral squamous cell carcinoma. GALR1 protein and mRNA expression and GAL secretion were detected at variable levels in immortalized human oral keratinocytes and human oropharyngeal squamous cell carcinoma cell lines. Upon competitive inhibition of GALR1, proliferation was up-regulated in immortalized and malignant keratinocytes (Henson et al., 2005)

[324] Stevenson et al. report the stem cell marker and regulator, galanin and GALR1 as key determinants of drug resistance and potential therapeutic targets for combating drug re-sistance. Mechanistically, they identify a novel role for the GALR1-galanin receptor-ligand axis as an important upstream regulator of expression of the anti-apoptotic protein FLIPL. Clinically, galanin mRNA was found to be overexpressed in colorectal tumours, and notably, high galanin mRNA expression correlated with poor disease-free survival in early stage disease (Stevenson et al., 2012). GALR1 is widely expressed in the brain and spinal cord, as well as in peripheral sites such as the small intestine and heart (Fagerberg et al., 2014)

[325] (17) PTGER3 -- Prostaglandin EP3 receptor (53kDa), also known as EP3, is a prostaglandin receptor for prostaglandin E2 (PGE2) encoded by the human gene PTGER3; it is one of four identified EP receptors, the others being PTGER1, PTGER2, and PTGER4, also called as EP1, EP2, and EP4, respectively, all of which bind with and mediate cellular responses to PGE2 and also, but generally with lesser affinity and responsiveness, certain other prostanoids. PTGER3 activation promotes duodenal secretion in mice; this function is mediated by PTGER3 activation in humans. EP

receptor functions can vary with species and most of the functional studies cited here have not translated their animal and tissue models to humans (Moreno, 2017).

[326] Studies of the direct effects of PTGER3 activation on cancer in animal and tissue models give contradictory results suggesting that this receptor does not play an important role in carcinogenesis. However, some studies suggest an indirect pro-carcinogenic function for the PTGER3: The growth and metastasis of implanted Lewis lung carcinoma cells, a mouse lung cancer cell line, is suppressed in PTGER3-deficient mice. This effect was associated with a reduction in the levels of Vascular endothelial growth factor and matrix metalloproteinase-9 expression in the tumor's stroma; expression of the pro-lymphangiogenic growth factor,VEGF-C and its receptor, VEGFR3; and a tumor-associated angiogenesis and lymphangiogenesis (O'Callaghan and Houston, 2015).

[327] PTGER3 is widely distributed in humans. Its protein and/or mRNA is expressed in kidney (i.e. glomeruli, Tamm-Horsfall protein negative late distal convoluted tubules, connecting segments, cortical and medullary collecting ducts, media and endothelial cells of arteries and arterioles); stomach (vascular smooth muscle and gastric fundus mucosal cells); thalamus (anterior, ventromedial, laterodorsal, paraventricular and central medial nuclei); intestinal mucosal epithelia at the apex of crypts; myometrium (stromal cells, endothelial cells, and, in pregnancy, placenta, chorion, and amnion); mouth gingival fibroblasts; and eye (corneal endothelium and keratocytes, trabecular cells, ciliary epithelium, and conjunctival and iridal stroma cells, and retinal M끼ller cells) (Norel, 2016).

[328] (18) SSTR2 -- Somatostatin receptor type 2 is a protein that in humans is encoded by the SSTR2 gene. Somatostatin acts at many sites to inhibit the release of many hormones and other secretory proteins. The biologic effects of somatostatin are probably mediated by a family of G protein-coupled receptors that are expressed in a tissue-specific manner. SSTR2 has been shown to interact with shank2 (Zitzer et al., 1999).

[329] SSTR2 encodes somatostatin receptor that can inhibit the cell proliferation of solid tumors. SSTR2 promoter hypermethylation is associated with the risk and progression of laryngeal squamous cell carcinoma in males (Shen et al., 2016). Most pituitary adenomas express SSTR2, but other somatostatin receptors are also found (Miller et al., 1995). Somatostatin analogs (i.e. Octreotide, Lanreotide) are used to stimulate this receptors, and thus to inhibit further tumor proliferation (Zatelli et al., 2007). SSTR2 is expressed in highest levels in cerebrum and kidney (Fagerberg et al., 2014).

[330] The term "inhibitor" as used herein refers to molecule that inhibits or suppresses the enhanced function of CXCR4-GPCRx heteromer. Non-limiting examples of the inhibitor of the invention that can be used for treatment, amelioration, or prevention of

a cancer or related symptoms include GPCRx antagonist, GPCRx inverse agonist, GPCRx positive and negative allosteric modulator, CXCR4-GPCRx heteromer-specific antibody or its antigen biding portions including single-domain antibody-like scaffolds, bivalent ligands which have a pharmacophore selective for CXCR4 joined by a spacer arm to a pharmacophore selective for GPCRx, bispecific antibody against CXCR4 and GPCRx, radiolabeled CXCR4 ligand linked with GPCRx ligand, and small molecule ligands that inhibit heteromer-selective signaling. Certain examples of inhibitors against GPCRx that form heteromers with CXCR4 and enhance Ca2+ response upon co-stimulation with both agonists are listed in Table 3.

[331] The term "antagonist" as used herein refers to a type of receptor ligand or drug that blocks or dampens a biological response by binding to and blocking a receptor, also called blockers. Antagonists have affinity but no efficacy for their cognate receptors, and their binding disrupts the interaction and inhibit the function of an agonist or inverse agonist at the cognate receptors. Certain examples of antagonists against GPCRx that form heteromers with CXCR4 and enhance Ca2+ response upon co-stimulation with both agonists are listed in Table 3.

[332] Table 3. Examples of inhibitors against GPCRx that form heteromers with CXCR4 and enhances Ca2+ response upon co-stimulation with both agonists.

[333] [Table 3]

Gene name	**Antagonists/Inverse agonists**	**Antibodies/**nanobodies/i-bodies/others
CXCR4	ALX40-4C, AMD070 (AMD11070, X4P-001), AMD3100 (plerixafor), AMD3465, ATI 2341, BKT140 (BL-8040; TF14016; 4F-Benzoyl-TN14003), CTCE-9908, CX549, D-[Lys3] GHRP-6, FC122, FC131, GMI-1359, GSK812397, GST-NT21MP, isothiourea-1a, isothiourea-1t (IT1t), KRH-1636, KRH-3955, LY2510924, MSX-122, N-[^{11}C]Methyl-AMD3465, POL6326, SDF-1 1-9[P2G] dimer, SDF1 P2G, T134, T140, T22, TC 14012, TG-0054 (Burixafor), USL311, viral macrophage inflammatory protein-II (vMIP-II), WZ811, [^{64}Cu]-AMD3100, [^{64}Cu]-AMD3465, [^{68}Ga]pentixafor, [^{90}Y]pentixather, [^{99m}Tc]O_2-AMD3100, [^{177}Lu]pentixather, and 508MCl (Compound 26).	AD-114, AD-114-6H, AD-114-Im7-FH, AD-114-PA600-6H, ALX-0651, LY2624587, PF-06747143, ulocuplumab (MDX1338/BMS-936564), 12G5, 238D2, and 238D4
ADCYAP1R1	M65, Max.d.4, MK-0893, N-stearyl-[Nle^{17}] neurotensin-(6-11)/VIP-(7-28), PACAP-(6-38), and PG 97-269	
ADORA2B	3-isobutyl-8-pyrrolidinoxanthine, alloxazine, AS16, AS70, AS74, AS94, AS95, AS96, AS99, AS100, AS101, ATL802, BW-A1433, caffeine, CGS 15943, CPX, CSC, CVT-6883, DAX, DEPX, derenofylline, DPCPX, FK-453, I-ABOPX, istradefylline, KF26777, LAS38096, LUF5981, MRE 2029F20, MRE 3008F20, MRS1191, MRS1220, MRS1523, MRS1706, MRS1754, MSX-2, OSIP339391, pentoxifylline, preladenant, PSB-10, PSB-11, PSB36, PSB603, PSB-0788, PSB1115, rolofylline, SCH 58261, SCH442416, ST-1535, theophylline, tonapofylline, vipadenant, xanthine amine congener, XCC, and ZM-241385.	
ADORA3	ATL802, BW-A1433, caffeine, CGS 15943, CSC, CVT-6883, derenofylline, dexniguldipine, DPCPX, FK-453, flavanone, flavone, galangin, I-ABOPX, istradefylline, KF26777, LAS38096, LUF5981, MRE 2029F20, MRE 3008F20, MRE 3010F20, MRS1041, MRS1042,	

	MRS1067, MRS1088, MRS1093, MRS1097, MRS1177, MRS1186, MRS1191, MRS1191, MRS1220, MRS1476, MRS1486, MRS1505, MRS1523, MRS1754, MRS928, MSX-2, nicardipine, preladenant, PSB-10, PSB-11, PSB36, PSB603, PSB1115, rolofylline, sakuranetin, SCH 58261, SCH442416, ST-1535, theophylline, tonapofylline, vipadenant, visnagin, VUF5574, VUF8504, VUF8507, xanthine amine congener, and ZM-241385	
ADRB2	Alprenolol, atenolol, betaxolol, bupranolol, butoxamine, carazolol, carvedilol, CGP 12177, cicloprolol, ICI 118551, ICYP, labetalol, levobetaxolol, levobunolol, LK 204-545, metoprolol, nadolol, NIHP, NIP, propafenone, propranolol, sotalol, SR59230A, and timolol.	
C5AR1	$A8^{\Delta 71-73}$, AcPhe-Orn-Pro-D-Cha-Trp-Arg, avacopan, C089, CHIPS, DF2593A, JPE1375, L-156,602, NDT9520492, *N*-methyl-Phe-Lys-Pro-D-Cha-Trp-D-Arg-CO_2H, PMX205, PMX53, RPR121154, and W54011.	
CALCR	α-CGRP-(8-37) (human), AC187, CT-(8-32) (salmon), and olcegepant.	
CHRM1	3-Quinuclidinyl benzilate (QNB), 4-DAMP, aclidinium, AE9C90CB, AFDX384, amitriptyline, AQ-RA 741, atropine, benzatropine, biperiden, darifenacin, di-cyclomine, dosulepin, ethopropazine, glycopyrrolate, guanylpirenzepine, hexahydrodifenidol, hexahydrosilad-ifenidol, hexocyclium, himbacine, ipratropium, litho-cholylcholine, methoctramine, ML381, muscarinic toxin 1, muscarinic toxin 2, muscarinic toxin 3, N-methyl scopolamine, otenzepad, oxybutynin, *p*-F-HHSiD, pirenzepine, propantheline, (R,R)-quinuclidinyl-4-fluoromethyl-benzilate, scopolamine, silahexocyclium, solifenacin, telenzepine, tiotropium, tolterodine, trihexyphenidyl, tripitramine, UH-AH 37, umeclidinium, and VU0255035.	
EDNRB	A192621, ambrisentan, atrasentan, bosentan (RO	

	470203, Tracleer);, BQ788, IRL 2500, K-8794, macitentan, RES7011, Ro 46-8443, SB209670, SB217242 (enrasentan), TAK 044, and tezosentan (RO610612).	
HRH1	(-)-chlorpheniramine, (+)-chlorpheniramine, (-)-*trans*-H_2-PAT, (+)-*cis*-H_2-PAT, (+)-*trans*-H_2-PAT, (±)-*cis*-H_2-PAT, (±)-*trans*-H_2-PAT, (*R*)-cetirizine, (*S*)-cetirizine, 9-OH-risperidone, A-317920, A-349821, ABT-239, alimemazine, amitriptyline, aripiprazole, arpromidine, asenapine, astemizole, AZD3778, azelastine, BU-E 47, cetirizine, chlorpheniramine, chlorpromazine, ciproxifan, clemastine, clobenpropit, clozapine, conessine, cyclizine, cyproheptadine, desloratadine, diphenhydramine, dosulepin, doxepin, epinastine, fexofenadine, fluphenazine, fluspirilene, haloperidol, hydroxyzine, impromidine, INCB-38579, JNJ-39758979, ketotifen, loratadine, loxapine, MK-0249, molindone, olanzapine, perphenazine, pimozide, pipamperone, pitolisant, promethazine, pyrilamine, quetiapine, risperidone, sertindole, terfenadine, thioridazine, thiothixene, trifluoperazine, tripelennamine, triprolidine, ziprasidone, and zotepine.	
MLNR	GM-109, MA-2029, and OHM-11526.	
NTSR1	Meclinertant, SR48527, SR48692, and SR142948A	
TACR3	[Trp^7, β-Ala^8] neurokinin A-(4-10), AZD2624, FK 224, GR138676, GSK 172981, GSK 256471, N',2-diphenylquinoline-4-carbohydrazide 8m, N′,2-diphenylquinoline-4-carbohydrazide, osanetant, PD 154740, PD 161182, PD157672, saredutant, SB 218795, SB 222200, SB 235375, SCH 206272, SSR 146977, and talnetant.	

[334] In some embodiments, the method for treating cancer in a patient having a cell containing a CXCR4-GPCRx heteromer, or the method of suppressing enhanced downstream signaling from a CXCR4-GPCRx heteromer in a cell of a patient suffereing from cancer, as disclosed herein, may comprise administering, or the pharmaceutical kit or pharmaceutical kit, comprise, administering to the patient an inhibitor

or a combination of inhibitors selected from the group consisting of: an inhibitor of CXCR4, an inhibitor of GPCRx, and an inhibitor of the CXCR4-GPCRx heteromer; wherein: i) the CXCR4-GPCRx heteromer has enhanced downstream signaling; and ii) the administered inhibitor or combination of inhibitors suppresses the enhanced downstream signaling from said CXCR4-GPCRx heteromer in the cancer patient. In some embodiments, the pharmaceutical kit or pharmaceutical composition for use in treating cancer in a patient having a cell containing a CXCR4-GPCRx heteromer, as disclosed herein, may comprise an inhibitor or a combination of inhibitors selected from the group consisting of: an inhibitor of CXCR4, an inhibitor of GPCRx, and an inhibitor of the CXCR4-GPCRx heteromer; wherein the CXCR4-GPCRx heteromer has enhanced downstream signaling. For example, in some embodiments, the CXCR4 inhibitor is an antagonist of CXCR4, an inverse agonist of CXCR4, a partial antagonist of CXCR4, an allosteric modulator of CXCR4, an antibody of CXCR4, an antibody fragment of CXCR4, a ligand of CXCR4, or an antibody-drug conjugate of CXCR4. For example, in some embodiments, the GPCRx inhibitor is an antagonist of GPCRx, an inverse agonist of GPCRx, a partial antagonist of GPCRx, an allosteric modulator of GPCRx, an antibody of GPCRx, an antibody fragment of GPCRx, a ligand of GPCRx, or an antibody-drug conjugate of GPCRx. For example, in some embodiments, the inhibitor of the CXCR4-GPCRx heteromer is an antagonist of the CXCR4-GPCRx heteromer, an inverse agonist of the CXCR4-GPCRx heteromer, a partial antagonist of the CXCR4-GPCRx heteromer, an allosteric modulator of the CXCR4-GPCRx heteromer, an antibody of the CXCR4-GPCRx heteromer, an antibody fragment of the CXCR4-GPCRx heteromer, a ligand of the CXCR4-GPCRx heteromer, a protein-protein interaction (PPI) inhibitor of the CXCR4-GPCRx heteromer, or an antibody-drug conjugate of the CXCR4-GPCRx heteromer.

[335] In some embodiments, the method for treating, method of suppressing, pharmaceutical kit, or pharmaceutical composition, as disclosed herein, includes a combination of inhibitors selected from the group consisting of: the CXCR4 inhibitor, the GPCRx inhibitor, and the inhibitor of the CXCR4-GPCRx heteromer, wherein the combination may be in a single pharmaceutical composition or a plurality of separate pharmaceutical compositions for each respective inhibitor. In some embodiments, the combination of inhibitors comprises the CXCR4 inhibitor and the GPCRx inhibitor. In some embodiments, the combination of inhibitors comprises the CXCR4 inhibitor and the inhibitor of the CXCR4-GPCRx heteromer. In some embodiments, the combination of inhibitors comprises the GPCRx inhibitor and the inhibitor of the CXCR4-GPCRx heteromer. In some embodiments, the combination of inhibitors are administered sequentially, concurrently, or simultaneously. In some embodiments, the combination of inhibitors are administered as a pharmaceutical composition further comprising a phar-

maceutically acceptable carrier. In some embodiments, the combination of inhibitors are administered as separate pharmaceutical compositions, wherein the separate pharmaceutical compositions independently further comprise a pharmaceutically acceptable carrier. In some embodiments, the method for treating, method of suppressing, pharmaceutical kit, or pharmaceutical composition, as disclosed herein, comprises a therapeutically effective amount or a sub-therapeutically effective amount of the CXCR4 inhibitor, such as administering a therapeutically effective amount of the CXCR4 inhibitor to the patient. In some embodiments, the method for treating, method of suppressing, pharmaceutical kit, or pharmaceutical composition, as disclosed herein, comprises a therapeutically effective amount or a sub-therapeutically effective amount of the GPCRx inhibitor, such as administering a therapeutically effective amount of the GPCRx inhibitor to the patient. In some embodiments, the method for treating, method of suppressing, pharmaceutical kit, or pharmaceutical composition, as disclosed herein, comprises a therapeutically effective amount or a sub-therapeutically effective amount of the inhibitor of the CXCR4-GPCRx heteromer, such as administering a therapeutically effective amount of the inhibitor of the CXCR4-GPCRx heteromer to the patient.

[336] In some embodiments, the method for treating cancer in a patient having a cancer cell containing a CXCR4-GPCRx heteromer, as disclosed herein, may comprise: 1) determining whether the patient has the cancer cell containing the CXCR4-GPCRx heteromer having enhanced downstream signaling by: obtaining or having obtained a biological sample from the patient; and performing or having performed an assay on the biological sample to determine if: i) the patient's cancer cell containing said CXCR4-GPCRx heteromer; or ii) a CXCR4-GPCRx heteromer-selective reagent: alters heteromer-specific properties or function of said CXCR4-GPCRx heteromer in a patient derived cell(s); alters heteromer-specific properties of a patient derived cell(s) containing the CXCR4-GPCRx heteromer; or decreases cell proliferation of a patient derived cell(s) containing the CXCR4-GPCRx heteromer; and 2) if the patient has a cancer cell containing said CXCR4-GPCRx heteromer, then internally administering an inhibitor or a combination of inhibitors selected from the group consisting of: an inhibitor of CXCR4, an inhibitor of GPCRx, and an inhibitor of the CXCR4-GPCRx heteromer to the cancer patient. In some embodiments, the method for treating cancer in a patient having a cancer cell containing a CXCR4-GPCRx heteromer, as disclosed herein, may comprise: 1) determining whether the patient's cancer cell contains the CXCR4-GPCRx heteromer by: obtaining or having obtained a biological sample from the patient and performing or having performed an assay on the biological sample to determine if said CXCR4-GPCRx heteromer is present in the patient's cancer cell; wherein: a) the GPCRx of the CXCR4-GPCRx heteromer is selected from the group

consisting of: ADCYAP1R1, ADORA2B, ADORA3, ADRB2, C5AR1, CALCR, CHRM1, EDNRB, HRH1, MLNR, NTSR1, and TACR3; and b) the assay performed on the biological sample is or comprises one or more of the following: a co-internalization assay, a colocalization assay, in situ hybridization, immunohistochemistry, immunoelectron microscopy, a proximity-based assay, a co-immunoprecipitation assay, or a fluorescent animal assay, such as via a co-internalization assay, bimolecular fluorescence complementation (BiFC), or a proximity ligation assay (PLA); and 2) if the patient's cancer cell contains said CXCR4-GPCRx heteromer, then internally administering an inhibitor or a combination of inhibitors selected from the group consisting of: an inhibitor of CXCR4, an inhibitor of GPCRx, and an inhibitor of the CXCR4-GPCRx heteromer to the patient. In some embodiments, the patient's biological sample is a biological fluid sample or is a biological tissue sample. In some embodiments, a liquid biopsy is performed on the biological fluid sample or performed on the biological tissue sample. In some embodiments, the biological fluid sample is a blood sample, a plasma sample, a saliva sample, a cerebral fluid sample, an eye fluid sample, or a urine sample. In certain embodiments, the biological fluid sample includes circulating tumor cells (CTCs), tumor-derived cell-free DNA (cfDNA), circulating small RNAs, and extracellular vesicles including exosomes, from bodily fluids as disclosed, for example, in Campos CDM et al., "Molecular Profiling of Liquid Biopsy Samples for Precision Medicine," Cancer J. 2018 Mar/Apr;24(2):93-103, which is incorporated hereby in its entirety. In some embodiments, the biological tissue sample is an organ tissue sample, a bone tissue sample, or a tumor tissue sample.

[337] The term "heteromer" as used herein refers to macromolecular complex composed of at least two GPCR units [protomerswith biochemical properties that are demonstrably different from those of its individual components. Heteromerization can be evaluated by in situ hybridization, immunohistochemistry, RNAseq, Reverse transcription-quantitative PCR (RT-qPCR, realtime PCR), microarray, proximity ligation assay (PLA), time-resolved FRET (TR-FRET), whole-body Single-photon emission computed tomography (SPECT) or Positron Emission Tomography/Computed Tomography (PET/CT).

[338] The phrase "effective amount" as used herein refers to an amount sufficient to effect beneficial or desired results. An effective amount can be administered in one or more administrations, applications or dosages. Such delivery is dependent on a number of variables including the time period for which the individual dosage unit is to be used, the bioavailability of the agent, the route of administration, etc.

[339] The phrase "therapeutically effective amount" as used herein refers to the amount of a therapeutic agent (e.g., an inhibitor, an antagonist, or any other therapeutic agent

provided herein) which is sufficient to reduce, ameliorate, and/or prevent the severity and/or duration of a cancer and/or a symptom related thereto. A therapeutically effective amount of a therapeutic agent can be an amount necessary for the reduction, amelioration, or prevention of the advancement or progression of a cancer, reduction, amelioration, or prevention of the recurrence, development or onset of a cancer, and/or to improve or enhance the prophylactic or therapeutic effect of another therapy (e.g., a therapy other than the administration of a inhibitor, an antagonist, or any other therapeutic agent provided herein).

[340] The phrase "therapeutic agent" refers to any agent that can be used in the treatment, amelioration, prevention, or management of a cancer and/or a symptom related thereto. In certain embodiments, a therapeutic agent refers to an inhibitor of CXCR4-GPCRx heteromer of the invention. A therapeutic agent can be an agent which is well known to be useful for, or has been or is currently being used for the treatment, amelioration, prevention, or management of a cancer and/or a symptom related thereto.

[341] The phrase "intracellular Ca2+ assay," "calcium mobilization assay," or their variants as used herein refer to cell-based assay to measure the calcium flux associated with GPCR activation or inhibition. The method utilizes a calcium sensitive fluorescent dye that is taken up into the cytoplasm of most cells. The dye binds the calcium released from intracellular store and its fluorescence increases. The change in the fluorescence intensity is directly correlated to the amount of intracellular calcium that is released into cytoplasm in response to ligand activation of the receptor of interest.

[342] The phrase "proximity-based assay" as used herein refers to biophysical and bio-chemical techniques that are able to monitor proximity and/or binding of two protein molecules *in vitro* (in cell lysates) and in live cells, including bioluminescence resonance energy transfer (BRET), fluorescence resonance energy transfer (FRET), bi-molecular fluorescence complementation (BiFC), Proximity ligation assay (PLA), cysteine crosslinking, and co-immunoprecipitation (Ferre et al., 2009; Gomes et al., 2016).

[343] Alternative methods for detecting heteromer formation include, but are not limited to: immunostaing (Bushlin et al., 2012; Decaillot et al., 2008); immunoelectron microscopy (Fernandez-Duenas et al., 2015); BRET (Pfleger and Eidne, 2006); Time-resolved FRET assays (Fernandez-Duenas et al., 2015); In Situ Hybridization (He et al., 2011); FRET (Lohse et al., 2012); β-arrestin recruitment assay using GPCR heteromer identification technology (GPCR-HIT, Dimerix Bioscience) (Mustafa and Pfleger, 2011) using BRET, FRET, BiFC, Bimolecular Luminescence Complementation, enzyme fragmentation assay, and Tango Tango GPCR assay system (Thermo Fisher Scientific) (Mustafa, 2010); PRESTO-Tango system (Kroeze et al., 2015); regulated secretion/aggregation technology (ARIAD Pharmaceuticals) (Hansen

et al., 2009); Receptor Selection and Amplification Technology (ACADIA Pharmaceuticals) (Hansen et al., 2009); DimerScreen (Cara Therapeutics) (Mustafa, 2010); Dimer/interacting protein translocation assay (Patobios) (Mustafa, 2010); Co-immunoprecipitation (Abd Alla et al., 2009); GPCR internalization assays using surface enzyme-linked immunosorbent assay (ELISA) (Decaillot et al., 2008) or Flow Cytometry (Law et al., 2005); Whole Cell Phosphorylation Assays (Pfeiffer et al., 2002); and Proximity-ligation assay (PLA) (Frederick et al., 2015).

[344] Alternative methods for detecting changes in pharmacological properties, signaling properties, and/or trafficking properties, in cells expressing both CXCR4 and GPCRx include, but are not limited to: Radioligand Binding Assays (Bushlin et al., 2012; Pfeiffer et al., 2002); Cell Surface Biotinylation and Immunoblotting (He et al., 2011); immunostaing (Bushlin et al., 2012; Decaillot et al., 2008); immunoelectron microscopy (Fernandez-Duenas et al., 2015); [35S]GTP S Binding assays (Bushlin et al., 2012); Calcuim imaging or assays using dyes such as Fura 2-acetomethoxy ester (Molecular Probes), Fluo-4 NW calcium dye (Thermo Fisher Scientific), or FLIPR5 dye (Molecular Devices); cAMP assays using radioimmunoassay kit (Amersham Biosciences); AlphaScreen (PerkinElmer Life Sciences); Parameter Cyclic AMP Assay (R&D Systems); femto cAMP kit (Cisbio); cAMP Direct Immunoassay Kit (Calbiochem) or GloSensor cAMP assay (Promega); GTPase assay (Pello et al., 2008); PKA activation (Stefan et al., 2007); ERK1/2 and/or Akt/PKB Phosphorylation Assays (Callen et al., 2012); Src and STAT3 phosphorylation assays (Rios et al., 2006); reporter assays such as cAMP response element (CRE); nuclear factor of activated T-cells response element (NFAT-RE); serum response element (SRE); serum response factor response element (SRF-RE); and NF-κB-response element luciferase reporter assays; Secreted alkaline phosphatase Assay (Decaillot et al., 2011); Measurement of Inositol 1-Phosphate Production Using TR-FRET or [3H]myo-Inositol (Mustafa et al., 2012); RT-qPCR for measuring downstream target gene expression (Mustafa et al., 2012); and Adenylyl Cyclase Activity (George et al., 2000); next generation sequencing (NGS); and any other assay that can detect a change in receptor function as a result of receptor heterodimerization.

[345] The phrase "protein-protein interaction inhibitor," "PPI inhibitor," or their variants as used herein refer to any molecules that can interfere with protein-protein interactions. Protein-protein interaction, unlike enzyme-substrate interaction involving well-defined binding pockets, is a transient interaction or association between proteins over relatively large areas and is often driven by electrostatic interactions, hydrophobic interactions, hydrogen bonds, and/or Van der Waals forces. PPI inhibitors may include, but not limited to, membrane-permeable peptides or lipid fused to a peptide sequence that disrupts the GPCR heteromeric interface, for example, transmembrane helix, intra-

cellular loop, or C-terminal tail of GPCRx. The PPI inhibitor of the CXCR4-GPCRx heteromer, for example, may be a membrane-permeable peptide or cell-penetrating peptide (CPP) conjugated with peptide that targets the CXCR4-GPCRx heteromeric interface(s), or may be a cell-penetrating lipidated peptide targeting the CXCR4-GPCRx heteromeric interface(s).

[346] For example, the membrane-permeable peptide or cell-penetrating peptide includes: HIV-1 TAT peptides, such as TAT_{48-60} and TAT_{49-57}; Penetratins, such as pAntp(43-58); Polyarginines (Rn such as R5 to R12); Diatos peptide vector 1047 (DPV1047, Vectocell®); MPG (HIV gp41 fused to the nuclear localization signal (NLS) of the SV40 large T antigen); Pep-1 (tryptophan-rich cluster fused to the NLS of SV40 large T antigen); pVEC peptide (vascular endothelial cadherin); p14 alternative reading frame (ARF) protein-based ARF(1-22); N-terminus of the unprocessed bovine prion protein BPrPr(1-28); Model amphipathic peptide (MAP); Transportans; Azurin-derived p28 peptide; amphipathic β-sheet peptides, such as VT5; proline-rich CPPs, such as Bac 7 (Bac1-24); hydrophobic CPPs, such as C105Y derived from α1-Antitrypsin; PFVYLI derived from synthetic C105Y; Pep-7 peptide (CHL8 peptide phage clone); and modified hydrophobic CPPs, such as stapled peptides and prenylated peptides (Guidotti et al., 2017; Kristensen et al., 2016). The membrane-permeable peptide or cell-penetrating peptide can further include, for example, TAT-derived cell-penetrating peptides, signal sequence-based (e.g., NLS) cell-penetrating peptides, hydrophobic membrane translocating sequence (MTS) peptides, and arginine-rich molecular transporters. The cell-penetrating lapidated peptide includes, for example, pepducins, such as ICL1/2/3, C-tail- short palmitoylated peptides (Covic et al., 2002; O'Callaghan et al., 2012).

[347] The peptide(s) that target the CXCR4-GPCRx heteromeric interface may be, for example, a transmembrane domain of CXCR4, transmembrane domain of GPCRx, intracellular loop of CXCR4, intracellular loop of GPCRx, C-terminal domain of CXCR4, or C-terminal domain of GPCRx., extracellular loop of CXCR4, extracellular loop of GPCRx, N-terminal region of CXCR4, or N-terminal region of GPCRx.

[348] In some embodiments, the invention provides a pharmaceutical composition (sometimes referred to herein as "pharmaceutical formulations") having an inhibitor of a CXCR4-GPCRx heteromer of the invention and a pharmaceutically acceptable carrier. A pharmaceutically acceptable carrier that can be used in the pharmaceutical compositions of the invention include any of the standard pharmaceutical carriers known in the art, such as physiologically acceptable carriers, excipients or stabilizers, for example, phosphate buffered saline solution, water and emulsions such as an oil and water emulsion, and various types of wetting agents. These pharmaceutical compositions can be prepared in liquid unit dose forms or any other dosing form that is

sufficient for delivery of the inhibitor of a CXCR4-GPCRx heteromer of the invention to the target area of the subject in need of treatment. For example, the pharmaceutical compositions can be prepared in any manner appropriate for the chosen mode of administration, e.g., intravascular, intramuscular, subcutaneous, intradermal, intrathecal, etc. Other optional components, e.g., pharmaceutical grade stabilizers, buffers, preservatives, excipients and the like can be readily selected by one of skill in the art. The preparation of a pharmaceutically composition, having due regard to pH, isotonicity, stability and the like, is within the level of skill in the art.

[349] Pharmaceutical formulations containing one or more inhibitors of a CXCR4-GPCRx heteromer of the invention provided herein can be prepared for storage by mixing the inhibitors having the desired degree of purity with optional physiologically acceptable carriers, excipients or stabilizers (Remington's Pharmaceutical Sciences (1990) Mack Publishing Co., Easton, PA), in the form of lyophilized formulations or aqueous solutions. Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol; low molecular weight polypeptides of less than about 10 residues; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counterions such as sodium; metal complexes, e.g., Zn-protein complexes; and/or non-ionic surfactants such as TWEEN™, PLURONICS™, or polyethylene glycol (PEG).

[350] Thus, in some embodiments, the invention provides a method for treatment, amelioration, or prevention of a disease in a subject in need thereof. The methods of the invention can include administering a therapeutically effective amount of a pharmaceutical composition provided herein to the subject. For example, the pharmaceutical composition can include one or more inhibitors of a CXCR4-GPCRx heteromer provided herein. Diseases that can be treated or prevented using the methods of the invention include cancer, tumor, metastasis, and/or angiogenesis. In particular, the methods of the invention are useful for treating cancers or related symptoms wherein the cells of cancer, tumor, and/or microenvironment expresses the CXCR4-GPCRx heteromer. Non-limiting examples of cancers or tumors that can be treated, ameliorated, or prevented using the methods of the invention include tumors of the gas-

trointestinal tract, for example, breast cancer, lung cancer, small cell carcinoma of the lung, hepatocellular carcinoma, brain cancer, kidney cancer, pancreatic cancer or pancreatic adenocarcinoma, ovarian cancer, prostate cancer, melanoma, lymphoma, leukemia, multiple myeloma, renal cell carcinoma, soft tissue sarcoma, gastrointestinal cancer, stomach cancer, colon cancer, colorectal cancer, colorectal adenocarcinoma, bladder adenocarcinoma, esophageal cancer, and adenocarcinoma of the stomach, esophagus, throat, and urogenital tract.

[351] In some embodiments, the invention provides a method for treating cancer in a patient having a cell containing a CXCR4-GPCRx heteromer, or provides a method of suppressing enhanced downstream signaling from a CXCR4-GPCRx heteromer in a cell of a patient suffereing from cancer, the method comprising: administering to the patient an inhibitor or a combination of inhibitors selected from the group consisting of: an inhibitor of CXCR4, an inhibitor of GPCRx, and an inhibitor of the CXCR4-GPCRx heteromer; wherein: i) the CXCR4-GPCRx heteromer has enhanced downstream signaling; and ii) the administered inhibitor or combination of inhibitors suppresses the enhanced downstream signaling from said CXCR4-GPCRx heteromer in the cancer patient.

[352] In some embodiments, the invention provides a pharmaceutical kit for use in treating cancer in a patient having a cell containing a CXCR4-GPCRx heteromer, the pharmaceutical kit comprising: an inhibitor or a combination of inhibitors selected from the group consisting of: an inhibitor of CXCR4, an inhibitor of GPCRx, and an inhibitor of the CXCR4-GPCRx heteromer; wherein the CXCR4-GPCRx heteromer has enhanced downstream signaling. In some embodiments, the invention provides a pharmaceutical composition for use in treating cancer in a patient having a cell containing a CXCR4-GPCRx heteromer, the pharmaceutical composition comprising: i) an inhibitor or a combination of inhibitors selected from the group consisting of: an inhibitor of CXCR4, an inhibitor of GPCRx, and an inhibitor of the CXCR4-GPCRx heteromer; and ii) a pharmaceutically acceptable carrier; wherein the CXCR4-GPCRx heteromer has enhanced downstream signaling.

[353] In some embodiments, according to the method of treating, method of suppressing, pharmaceutical kit, or pharmaceutical composition, provided herein, the progression of the cancer in the patient having said cancer cell containing the CXCR4-GPCRx heteromer is decreased in the range of 5-100% more upon administration of the combination of inhibitors, relative to administering the CXCR4 inhibitor or GPCRx inhibitor as the single inhibitor to said patient, such as decreased in the range of 5-100% more, 10-100% more, 20-100% more, 30-100% more, 40-100% more, 50-100% more, 60-100% more, 75-100% more, 5-75% more, 5-50% more, or 5-25% more, upon administration of the combination of inhibitors, relative to administering

the CXCR4 inhibitor or GPCRx inhibitor as the single inhibitor to said patient. In some embodiments, the GPCRx of the CXCR4-GPCRx heteromer is selected from the group consisting of: ADCYAP1R1, ADORA2B, ADORA3, ADRB2, C5AR1, CALCR, CHRM1, EDNRB, HRH1, MLNR, NTSR1, and TACR3; for example, selected from the group consisting of: ADCYAP1R1, ADORA2B, ADORA3, ADRB2, C5AR1, CHRM1, EDNRB, HRH1, MLNR, NTSR1, and TACR3; selected from the group consisting of: ADCYAP1R1, ADORA2B, ADORA3, ADRB2, CHRM1, EDNRB, HRH1, MLNR, NTSR1, and TACR3; selected from the group consisting of: ADCYAP1R1, ADORA2B, ADORA3, ADRB2, CHRM1, EDNRB, HRH1, NTSR1, and TACR3; selected from the group consisting of: ADCYAP1R1, ADORA2B, ADORA3, ADRB2, CHRM1, HRH1, NTSR1, and TACR3; selected from the group consisting of: ADORA2B, ADORA3, ADRB2, CHRM1, EDNRB, HRH1, NTSR1, and TACR3; selected from the group consisting of: ADRB2, CHRM1, EDNRB, HRH1, NTSR1, and TACR3; selected from the group consisting of: ADRB2, CHRM1, EDNRB, HRH1, and TACR3; selected from the group consisting of: ADRB2, CHRM1, HRH1, and TACR3; selected from the group consisting of: ADRB2, EDNRB, HRH1, and TACR3; selected from the group consisting of: ADRB2, EDNRB, and HRH1; selected from the group consisting of: ADRB2, CHRM1, and HRH1; or selected from the group consisting of: ADRB2, HRH1, and TACR3. In some embodiments, the GPCRx inhibitor is selected from the group consisting of: ADCYAP1R1 inhibitor, ADORA2B inhibitor, ADORA3 inhibitor, ADRB2 inhibitor, C5AR1 inhibitor, CALCR inhibitor, CHRM1 inhibitor, EDNRB inhibitor, HRH1 inhibitor, MLNR inhibitor, NTSR1 inhibitor, and TACR3 inhibitor; for example, selected from the group consisting of: ADCYAP1R1 inhibitor, ADORA2B inhibitor, ADORA3 inhibitor, ADRB2 inhibitor, C5AR1 inhibitor, CHRM1 inhibitor, EDNRB inhibitor, HRH1 inhibitor, MLNR inhibitor, NTSR1 inhibitor, and TACR3 inhibitor; selected from the group consisting of: ADCYAP1R1 inhibitor, ADORA2B inhibitor, ADORA3 inhibitor, ADRB2 inhibitor, CHRM1 inhibitor, EDNRB inhibitor, HRH1 inhibitor, MLNR inhibitor, NTSR1 inhibitor, and TACR3 inhibitor; selected from the group consisting of: ADCYAP1R1 inhibitor, ADORA2B inhibitor, ADORA3 inhibitor, ADRB2 inhibitor, CHRM1 inhibitor, EDNRB inhibitor, HRH1 inhibitor, NTSR1 inhibitor, and TACR3 inhibitor; selected from the group consisting of: ADCYAP1R1 inhibitor, ADORA2B inhibitor, ADORA3 inhibitor, ADRB2 inhibitor, CHRM1 inhibitor, HRH1 inhibitor, NTSR1 inhibitor, and TACR3 inhibitor; selected from the group consisting of: ADORA2B inhibitor, ADORA3 inhibitor, ADRB2 inhibitor, CHRM1 inhibitor, EDNRB inhibitor, HRH1 inhibitor, NTSR1 inhibitor, and TACR3 inhibitor; selected from the group consisting of: ADRB2 inhibitor, CHRM1 inhibitor, EDNRB inhibitor, HRH1 inhibitor, NTSR1 inhibitor, and TACR3 inhibitor;

selected from the group consisting of: ADRB2 inhibitor, CHRM1 inhibitor, EDNRB inhibitor, HRH1 inhibitor, and TACR3 inhibitor; selected from the group consisting of: ADRB2 inhibitor, CHRM1 inhibitor, HRH1 inhibitor, and TACR3 inhibitor; selected from the group consisting of: ADRB2 inhibitor, EDNRB inhibitor, HRH1 inhibitor, and TACR3 inhibitor; selected from the group consisting of: ADRB2 inhibitor, EDNRB inhibitor, and HRH1 inhibitor; selected from the group consisting of: ADRB2 inhibitor, CHRM1 inhibitor, and HRH1 inhibitor; or selected from the group consisting of: ADRB2 inhibitor, HRH1, inhibitor and TACR3 inhibitor.

[354] In some embodiments, according to the method of treating, method of suppressing, pharmaceutical kit, or pharmaceutical composition, provided herein, the efficacy of a CXCR4 inhibitor is increased in the range of 5-2000% when administered in combination with the GPCRx inhibitor to the patient having said cancer cell containing the CXCR4-GPCRx heteromer, relative to efficacy of the CXCR4 inhibitor when administered as a single inhibitor, such as increased in the range of 5-1750%, 5-1500%, 5-1250%, 5-1000%, 5-900%, 5-800%, 5-700%, 5-500%, 5-400%, 5-250%, 5-200%, 5-100%, 5-75%, 5-50%, 5-40%, 5-30%, 5-25%, 100-2000%, 200-2000%, 300-2000%, 500-2000%, 750-2000%, 1000-2000%, 1250-2000%, 1500-2000%, 5-1500%, 25-1500%, 50-1500%, 75-1500%, 100-1500%, 200-1500%, 300-1500%, 500-1500%, 750-1500%, 1000-1500%, or 1250-1500%, when administered in combination with the GPCRx inhibitor to the patient having said cancer cell containing the CXCR4-GPCRx heteromer, relative to efficacy of the CXCR4 inhibitor when administered as a single inhibitor. In some embodiments, the GPCRx of the CXCR4-GPCRx heteromer is selected from the group consisting of: ADCYAP1R1, ADORA2B, ADORA3, ADRB2, C5AR1, CALCR, CHRM1, EDNRB, HRH1, MLNR, NTSR1, and TACR3; for example, selected from the group consisting of: ADCYAP1R1, ADORA2B, ADORA3, ADRB2, C5AR1, CHRM1, EDNRB, HRH1, MLNR, NTSR1, and TACR3; selected from the group consisting of: ADCYAP1R1, ADORA2B, ADORA3, ADRB2, CHRM1, EDNRB, HRH1, MLNR, NTSR1, and TACR3; selected from the group consisting of: ADCYAP1R1, ADORA2B, ADORA3, ADRB2, CHRM1, EDNRB, HRH1, NTSR1, and TACR3; selected from the group consisting of: ADCYAP1R1, ADORA2B, ADORA3, ADRB2, CHRM1, HRH1, NTSR1, and TACR3; selected from the group consisting of: ADORA2B, ADORA3, ADRB2, CHRM1, EDNRB, HRH1, NTSR1, and TACR3; selected from the group consisting of: ADRB2, CHRM1, EDNRB, HRH1, NTSR1, and TACR3; selected from the group consisting of: ADRB2, CHRM1, EDNRB, HRH1, and TACR3; selected from the group consisting of: ADRB2, CHRM1, HRH1, and TACR3; selected from the group consisting of: ADRB2, EDNRB, HRH1, and TACR3; selected from the group consisting of: ADRB2, EDNRB, and HRH1; selected from the group consisting of: ADRB2,

CHRM1, and HRH1; or selected from the group consisting of: ADRB2, HRH1, and TACR3. In some embodiments, the GPCRx inhibitor is selected from the group consisting of: ADCYAP1R1 inhibitor, ADORA2B inhibitor, ADORA3 inhibitor, ADRB2 inhibitor, C5AR1 inhibitor, CALCR inhibitor, CHRM1 inhibitor, EDNRB inhibitor, HRH1 inhibitor, MLNR inhibitor, NTSR1 inhibitor, and TACR3 inhibitor; for example, selected from the group consisting of: ADCYAP1R1 inhibitor, ADORA2B inhibitor, ADORA3 inhibitor, ADRB2 inhibitor, C5AR1 inhibitor, CHRM1 inhibitor, EDNRB inhibitor, HRH1 inhibitor, MLNR inhibitor, NTSR1 inhibitor, and TACR3 inhibitor; selected from the group consisting of: ADCYAP1R1 inhibitor, ADORA2B inhibitor, ADORA3 inhibitor, ADRB2 inhibitor, CHRM1 inhibitor, EDNRB inhibitor, HRH1 inhibitor, MLNR inhibitor, NTSR1 inhibitor, and TACR3 inhibitor; selected from the group consisting of: ADCYAP1R1 inhibitor, ADORA2B inhibitor, ADORA3 inhibitor, ADRB2 inhibitor, CHRM1 inhibitor, EDNRB inhibitor, HRH1 inhibitor, NTSR1 inhibitor, and TACR3 inhibitor; selected from the group consisting of: ADCYAP1R1 inhibitor, ADORA2B inhibitor, ADORA3 inhibitor, ADRB2 inhibitor, CHRM1 inhibitor, HRH1 inhibitor, NTSR1 inhibitor, and TACR3 inhibitor; selected from the group consisting of: ADORA2B inhibitor, ADORA3 inhibitor, ADRB2 inhibitor, CHRM1 inhibitor, EDNRB inhibitor, HRH1 inhibitor, NTSR1 inhibitor, and TACR3 inhibitor; selected from the group consisting of: ADRB2 inhibitor, CHRM1 inhibitor, EDNRB inhibitor, HRH1 inhibitor, NTSR1 inhibitor, and TACR3 inhibitor; selected from the group consisting of: ADRB2 inhibitor, CHRM1 inhibitor, EDNRB inhibitor, HRH1 inhibitor, and TACR3 inhibitor; selected from the group consisting of: ADRB2 inhibitor, CHRM1 inhibitor, HRH1 inhibitor, and TACR3 inhibitor; selected from the group consisting of: ADRB2 inhibitor, EDNRB inhibitor, HRH1 inhibitor, and TACR3 inhibitor; selected from the group consisting of: ADRB2 inhibitor, EDNRB inhibitor, and HRH1 inhibitor; selected from the group consisting of: ADRB2 inhibitor, CHRM1 inhibitor, and HRH1 inhibitor; or selected from the group consisting of: ADRB2 inhibitor, HRH1, inhibitor and TACR3 inhibitor.

[355] In some embodiments, according to the method of treating, method of suppressing, pharmaceutical kit, or pharmaceutical composition, provided herein, the efficacy of a GPCRx inhibitor is increased in the range of 5-2000% when administered in combination with the CXCR4 inhibitor to the patient having said cancer cell containing the CXCR4-GPCRx heteromer, relative to efficacy of the GPCRx inhibitor when administered as a single inhibitor, such as increased in the range of 5-1750%, 5-1500%, 5-1250%, 5-1000%, 5-900%, 5-800%, 5-700%, 5-500%, 5-400%, 5-250%, 5-200%, 5-100%, 5-75%, 5-50%, 5-40%, 5-30%, 5-25%, 100-2000%, 200-2000%, 300-2000%, 500-2000%, 750-2000%, 1000-2000%, 1250-2000%, 1500-2000%, 5-1500%,

25-1500%, 50-1500%, 75-1500%, 100-1500%, 200-1500%, 300-1500%, 500-1500%, 750-1500%, 1000-1500%, or 1250-1500%, when administered in combination with the CXCR4 inhibitor to the patient having said cancer cell containing the CXCR4-GPCRx heteromer, relative to efficacy of the GPCRx inhibitor when administered as a single inhibitor. In some embodiments, the GPCRx of the CXCR4-GPCRx heteromer is selected from the group consisting of: ADCYAP1R1, ADORA2B, ADORA3, ADRB2, C5AR1, CALCR, CHRM1, EDNRB, HRH1, MLNR, NTSR1, and TACR3; for example, selected from the group consisting of: ADCYAP1R1, ADORA2B, ADORA3, ADRB2, C5AR1, CHRM1, EDNRB, HRH1, MLNR, NTSR1, and TACR3; selected from the group consisting of: ADCYAP1R1, ADORA2B, ADORA3, ADRB2, CHRM1, EDNRB, HRH1, MLNR, NTSR1, and TACR3; selected from the group consisting of: ADCYAP1R1, ADORA2B, ADORA3, ADRB2, CHRM1, EDNRB, HRH1, NTSR1, and TACR3; selected from the group consisting of: ADCYAP1R1, ADORA2B, ADORA3, ADRB2, CHRM1, HRH1, NTSR1, and TACR3; selected from the group consisting of: ADORA2B, ADORA3, ADRB2, CHRM1, EDNRB, HRH1, NTSR1, and TACR3; selected from the group consisting of: ADRB2, CHRM1, EDNRB, HRH1, NTSR1, and TACR3; selected from the group consisting of: ADRB2, CHRM1, EDNRB, HRH1, and TACR3; selected from the group consisting of: ADRB2, CHRM1, HRH1, and TACR3; selected from the group consisting of: ADRB2, EDNRB, HRH1, and TACR3; selected from the group consisting of: ADRB2, EDNRB, and HRH1; selected from the group consisting of: ADRB2, CHRM1, and HRH1; or selected from the group consisting of: ADRB2, HRH1, and TACR3. In some embodiments, the GPCRx inhibitor is selected from the group consisting of: ADCYAP1R1 inhibitor, ADORA2B inhibitor, ADORA3 inhibitor, ADRB2 inhibitor, C5AR1 inhibitor, CALCR inhibitor, CHRM1 inhibitor, EDNRB inhibitor, HRH1 inhibitor, MLNR inhibitor, NTSR1 inhibitor, and TACR3 inhibitor; for example, selected from the group consisting of: ADCYAP1R1 inhibitor, ADORA2B inhibitor, ADORA3 inhibitor, ADRB2 inhibitor, C5AR1 inhibitor, CHRM1 inhibitor, EDNRB inhibitor, HRH1 inhibitor, MLNR inhibitor, NTSR1 inhibitor, and TACR3 inhibitor; selected from the group consisting of: ADCYAP1R1 inhibitor, ADORA2B inhibitor, ADORA3 inhibitor, ADRB2 inhibitor, CHRM1 inhibitor, EDNRB inhibitor, HRH1 inhibitor, MLNR inhibitor, NTSR1 inhibitor, and TACR3 inhibitor; selected from the group consisting of: ADCYAP1R1 inhibitor, ADORA2B inhibitor, ADORA3 inhibitor, ADRB2 inhibitor, CHRM1 inhibitor, EDNRB inhibitor, HRH1 inhibitor, NTSR1 inhibitor, and TACR3 inhibitor; selected from the group consisting of: ADCYAP1R1 inhibitor, ADORA2B inhibitor, ADORA3 inhibitor, ADRB2 inhibitor, CHRM1 inhibitor, HRH1 inhibitor, NTSR1 inhibitor, and TACR3 inhibitor; selected from the group consisting of: ADORA2B inhibitor,

ADORA3 inhibitor, ADRB2 inhibitor, CHRM1 inhibitor, EDNRB inhibitor, HRH1 inhibitor, NTSR1 inhibitor, and TACR3 inhibitor; selected from the group consisting of: ADRB2 inhibitor, CHRM1 inhibitor, EDNRB inhibitor, HRH1 inhibitor, NTSR1 inhibitor, and TACR3 inhibitor; selected from the group consisting of: ADRB2 inhibitor, CHRM1 inhibitor, EDNRB inhibitor, HRH1 inhibitor, and TACR3 inhibitor; selected from the group consisting of: ADRB2 inhibitor, CHRM1 inhibitor, HRH1 inhibitor, and TACR3 inhibitor; selected from the group consisting of: ADRB2 inhibitor, EDNRB inhibitor, HRH1 inhibitor, and TACR3 inhibitor; selected from the group consisting of: ADRB2 inhibitor, EDNRB inhibitor, and HRH1 inhibitor; selected from the group consisting of: ADRB2 inhibitor, CHRM1 inhibitor, and HRH1 inhibitor; or selected from the group consisting of: ADRB2 inhibitor, HRH1, inhibitor and TACR3 inhibitor.

[356] In some embodiments, according to the method of treating, method of suppressing, pharmaceutical kit, or pharmaceutical composition, provided herein, the method administers a combination of inhibitors selected from the group consisting of: an inhibitor of CXCR4, an inhibitor of GPCRx, and an inhibitor of the CXCR4-GPCRx heteromer; or the pharmaceutical kit or pharmaceutical composition comprises a combination of inhibitors selected from the group consisting of: an inhibitor of CXCR4, an inhibitor of GPCRx, and an inhibitor of the CXCR4-GPCRx heteromer. In some embodiments, the combination of inhibitors is a combination of two inhibitors selected from the group consisting of: an inhibitor of CXCR4, an inhibitor of GPCRx, and an inhibitor of the CXCR4-GPCRx heteromer. In some embodiments, according to the method of treating, method of suppressing, pharmaceutical kit, or pharmaceutical composition, provided herein, the method administers a combination of a CXCR4 inhibitor and a GPCRx inhibitor; or the pharmaceutical kit or pharmaceutical composition comprises a combination of a CXCR4 inhibitor and a GPCRx inhibitor. In some embodiments, according to the method of treating, method of suppressing, pharmaceutical kit, or pharmaceutical composition, provided herein, the method administers a CXCR4-GPCRx heteromer inhibitor; or the pharmaceutical kit or pharmaceutical composition comprises a CXCR4-GPCRx heteromer inhibitor.

[357] In some embodiments, according to the method of treating or method of suppressing provided herein, or according to the use of the pharmaceutical kit or pharmaceutical composition provided herein, the administering of the combination of inhibitors suppresses the enhanced downstream signaling from said CXCR4-GPCRx heteromer in the cancer patient in the range of between 5-2000 fold, relative to single inhibitor administration, such as suppresses the enhanced downstream signaling from said CXCR4-GPCRx heteromer in the cancer patient in the range of between 5-1750 fold, 5-1500 fold, 5-1250 fold, 5-1000 fold, 5-900 fold, 5-800 fold, 5-700 fold, 5-500 fold,

5-400 fold, 5-250 fold, 5-200 fold, 5-100 fold, 5-75 fold, 5-50 fold, 5-40 fold, 5-30 fold, 5-25 fold, 100-2000 fold, 200-2000 fold, 300-2000 fold, 500-2000 fold, 750-2000 fold, 1000-2000 fold, 1250-2000 fold, 1500-2000 fold, 5-1500 fold, 25-1500 fold, 50-1500 fold, 75-1500 fold, 100-1500 fold, 200-1500 fold, 300-1500 fold, 500-1500 fold, 750-1500 fold, 1000-1500 fold, or 1250-1500 fold, relative to single inhibitor administration. In some embodiments, the GPCRx of the CXCR4-GPCRx heteromer is selected from the group consisting of: ADCYAP1R1, ADORA2B, ADORA3, ADRB2, C5AR1, CALCR, CHRM1, EDNRB, HRH1, MLNR, NTSR1, and TACR3; for example, selected from the group consisting of: ADCYAP1R1, ADORA2B, ADORA3, ADRB2, C5AR1, CHRM1, EDNRB, HRH1, MLNR, NTSR1, and TACR3; selected from the group consisting of: ADCYAP1R1, ADORA2B, ADORA3, ADRB2, CHRM1, EDNRB, HRH1, MLNR, NTSR1, and TACR3; selected from the group consisting of: ADCYAP1R1, ADORA2B, ADORA3, ADRB2, CHRM1, EDNRB, HRH1, NTSR1, and TACR3; selected from the group consisting of: ADCYAP1R1, ADORA2B, ADORA3, ADRB2, CHRM1, HRH1, NTSR1, and TACR3; selected from the group consisting of: ADORA2B, ADORA3, ADRB2, CHRM1, EDNRB, HRH1, NTSR1, and TACR3; selected from the group consisting of: ADRB2, CHRM1, EDNRB, HRH1, NTSR1, and TACR3; selected from the group consisting of: ADRB2, CHRM1, EDNRB, HRH1, and TACR3; selected from the group consisting of: ADRB2, CHRM1, HRH1, and TACR3; selected from the group consisting of: ADRB2, EDNRB, HRH1, and TACR3; selected from the group consisting of: ADRB2, EDNRB, and HRH1; selected from the group consisting of: ADRB2, CHRM1, and HRH1; or selected from the group consisting of: ADRB2, HRH1, and TACR3. In some embodiments, the GPCRx inhibitor is selected from the group consisting of: ADCYAP1R1 inhibitor, ADORA2B inhibitor, ADORA3 inhibitor, ADRB2 inhibitor, C5AR1 inhibitor, CALCR inhibitor, CHRM1 inhibitor, EDNRB inhibitor, HRH1 inhibitor, MLNR inhibitor, NTSR1 inhibitor, and TACR3 inhibitor; for example, selected from the group consisting of: ADCYAP1R1 inhibitor, ADORA2B inhibitor, ADORA3 inhibitor, ADRB2 inhibitor, C5AR1 inhibitor, CHRM1 inhibitor, EDNRB inhibitor, HRH1 inhibitor, MLNR inhibitor, NTSR1 inhibitor, and TACR3 inhibitor; selected from the group consisting of: ADCYAP1R1 inhibitor, ADORA2B inhibitor, ADORA3 inhibitor, ADRB2 inhibitor, CHRM1 inhibitor, EDNRB inhibitor, HRH1 inhibitor, MLNR inhibitor, NTSR1 inhibitor, and TACR3 inhibitor; selected from the group consisting of: ADCYAP1R1 inhibitor, ADORA2B inhibitor, ADORA3 inhibitor, ADRB2 inhibitor, CHRM1 inhibitor, EDNRB inhibitor, HRH1 inhibitor, NTSR1 inhibitor, and TACR3 inhibitor; selected from the group consisting of: ADCYAP1R1 inhibitor, ADORA2B inhibitor, ADORA3 inhibitor, ADRB2 inhibitor, CHRM1 inhibitor, HRH1 inhibitor, NTSR1 inhibitor, and

TACR3 inhibitor; selected from the group consisting of: ADORA2B inhibitor, ADORA3 inhibitor, ADRB2 inhibitor, CHRM1 inhibitor, EDNRB inhibitor, HRH1 inhibitor, NTSR1 inhibitor, and TACR3 inhibitor; selected from the group consisting of: ADRB2 inhibitor, CHRM1 inhibitor, EDNRB inhibitor, HRH1 inhibitor, NTSR1 inhibitor, and TACR3 inhibitor; selected from the group consisting of: ADRB2 inhibitor, CHRM1 inhibitor, EDNRB inhibitor, HRH1 inhibitor, and TACR3 inhibitor; selected from the group consisting of: ADRB2 inhibitor, CHRM1 inhibitor, HRH1 inhibitor, and TACR3 inhibitor; selected from the group consisting of: ADRB2 inhibitor, EDNRB inhibitor, HRH1 inhibitor, and TACR3 inhibitor; selected from the group consisting of: ADRB2 inhibitor, EDNRB inhibitor, and HRH1 inhibitor; selected from the group consisting of: ADRB2 inhibitor, CHRM1 inhibitor, and HRH1 inhibitor; or selected from the group consisting of: ADRB2 inhibitor, HRH1, inhibitor and TACR3 inhibitor.

[358] In some embodiments, according to the method of treating or method of suppressing provided herein, or according to the use of the pharmaceutical kit or pharmaceutical composition provided herein, the administering of the inhibitor or combination of inhibitors suppresses the enhanced downstream signaling from said CXCR4-GPCRx heteromer in the cancer patient in the range of between 5-2000 fold, relative to suppression of downstream signaling from either a CXCR4 protomer or a GPCRx protomer in their respective individual protomer context, such as suppresses the enhanced downstream signaling from said CXCR4-GPCRx heteromer in the cancer patient in the range of between 5-1750 fold, 5-1500 fold, 5-1250 fold, 5-1000 fold, 5-900 fold, 5-800 fold, 5-700 fold, 5-500 fold, 5-400 fold, 5-250 fold, 5-200 fold, 5-100 fold, 5-75 fold, 5-50 fold, 5-40 fold, 5-30 fold, 5-25 fold, 100-2000 fold, 200-2000 fold, 300-2000 fold, 500-2000 fold, 750-2000 fold, 1000-2000 fold, 1250-2000 fold, 1500-2000 fold, 5-1500 fold, 25-1500 fold, 50-1500 fold, 75-1500 fold, 100-1500 fold, 200-1500 fold, 300-1500 fold, 500-1500 fold, 750-1500 fold, 1000-1500 fold, or 1250-1500 fold, relative to suppression of downstream signaling from either a CXCR4 protomer or a GPCRx protomer in their respective individual protomer context. In some embodiments, the GPCRx of the CXCR4-GPCRx heteromer is selected from the group consisting of: ADCYAP1R1, ADORA2B, ADORA3, ADRB2, C5AR1, CALCR, CHRM1, EDNRB, HRH1, MLNR, NTSR1, and TACR3; for example, selected from the group consisting of: ADCYAP1R1, ADORA2B, ADORA3, ADRB2, C5AR1, CHRM1, EDNRB, HRH1, MLNR, NTSR1, and TACR3; selected from the group consisting of: ADCYAP1R1, ADORA2B, ADORA3, ADRB2, CHRM1, EDNRB, HRH1, MLNR, NTSR1, and TACR3; selected from the group consisting of: ADCYAP1R1, ADORA2B, ADORA3, ADRB2, CHRM1, EDNRB, HRH1, NTSR1, and TACR3; selected from the group consisting

of: ADCYAP1R1, ADORA2B, ADORA3, ADRB2, CHRM1, HRH1, NTSR1, and TACR3; selected from the group consisting of: ADORA2B, ADORA3, ADRB2, CHRM1, EDNRB, HRH1, NTSR1, and TACR3; selected from the group consisting of: ADRB2, CHRM1, EDNRB, HRH1, NTSR1, and TACR3; selected from the group consisting of: ADRB2, CHRM1, EDNRB, HRH1, and TACR3; selected from the group consisting of: ADRB2, CHRM1, HRH1, and TACR3; selected from the group consisting of: ADRB2, EDNRB, HRH1, and TACR3; selected from the group consisting of: ADRB2, EDNRB, and HRH1; selected from the group consisting of: ADRB2, CHRM1, and HRH1; or selected from the group consisting of: ADRB2, HRH1, and TACR3. In some embodiments, the GPCRx inhibitor is selected from the group consisting of: ADCYAP1R1 inhibitor, ADORA2B inhibitor, ADORA3 inhibitor, ADRB2 inhibitor, C5AR1 inhibitor, CALCR inhibitor, CHRM1 inhibitor, EDNRB inhibitor, HRH1 inhibitor, MLNR inhibitor, NTSR1 inhibitor, and TACR3 inhibitor; for example, selected from the group consisting of: ADCYAP1R1 inhibitor, ADORA2B inhibitor, ADORA3 inhibitor, ADRB2 inhibitor, C5AR1 inhibitor, CHRM1 inhibitor, EDNRB inhibitor, HRH1 inhibitor, MLNR inhibitor, NTSR1 inhibitor, and TACR3 inhibitor; selected from the group consisting of: ADCYAP1R1 inhibitor, ADORA2B inhibitor, ADORA3 inhibitor, ADRB2 inhibitor, CHRM1 inhibitor, EDNRB inhibitor, HRH1 inhibitor, MLNR inhibitor, NTSR1 inhibitor, and TACR3 inhibitor; selected from the group consisting of: ADCYAP1R1 inhibitor, ADORA2B inhibitor, ADORA3 inhibitor, ADRB2 inhibitor, CHRM1 inhibitor, EDNRB inhibitor, HRH1 inhibitor, NTSR1 inhibitor, and TACR3 inhibitor; selected from the group consisting of: ADCYAP1R1 inhibitor, ADORA2B inhibitor, ADORA3 inhibitor, ADRB2 inhibitor, CHRM1 inhibitor, HRH1 inhibitor, NTSR1 inhibitor, and TACR3 inhibitor; selected from the group consisting of: ADORA2B inhibitor, ADORA3 inhibitor, ADRB2 inhibitor, CHRM1 inhibitor, EDNRB inhibitor, HRH1 inhibitor, NTSR1 inhibitor, and TACR3 inhibitor; selected from the group consisting of: ADRB2 inhibitor, CHRM1 inhibitor, EDNRB inhibitor, HRH1 inhibitor, NTSR1 inhibitor, and TACR3 inhibitor; selected from the group consisting of: ADRB2 inhibitor, CHRM1 inhibitor, EDNRB inhibitor, HRH1 inhibitor, and TACR3 inhibitor; selected from the group consisting of: ADRB2 inhibitor, CHRM1 inhibitor, HRH1 inhibitor, and TACR3 inhibitor; selected from the group consisting of: ADRB2 inhibitor, EDNRB inhibitor, HRH1 inhibitor, and TACR3 inhibitor; selected from the group consisting of: ADRB2 inhibitor, EDNRB inhibitor, and HRH1 inhibitor; selected from the group consisting of: ADRB2 inhibitor, CHRM1 inhibitor, and HRH1 inhibitor; or selected from the group consisting of: ADRB2 inhibitor, HRH1, inhibitor and TACR3 inhibitor.

[359] Initial testing and evaluation of an inhibitor, or combination of inhibitors, regarding

whether effective, or therapeutically effective, in suppressing an enhanced downstream signaling from a CXCR4-GPCRx heteromer, according to the methods disclosed herein, and/or in determining an IC50 value according to the assays disclosed herein, may utilize a concentration of the inhibitor in the range of between 1-10 μM (or each of the inhibitors of the combination at concentrations in the range of between 1-10 μM), such as at a concentration of 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 μM. If suppression of the signal by the inhibitor is not appreciable enough for a determinable measurement, then a greater concentration of the inhibitor may be used to better evaluate a determinable measurement, such as an IC50 value. If suppression of the signal by the inhibitor is very strong, then a lower concentration of the inhibitor may be used to better evaluate a determinable measurement, such as an IC50 value.

[360] It is understood that modifications which do not substantially affect the activity of the various embodiments of this invention are also provided within the definition of the invention provided herein. Accordingly, the following examples are intended to illustrate but not limit the invention disclosed herein.

[361]

[362] **EXAMPLES**

[363] **Example 1. Evaluation of CXCR4-GPCRx heteromer formation by BiFC assay.**

[364] To identify novel CXCR4-GPCRx heteromers, we made recombinant adenoviruses encoding 143 GPCRs fused with N-terminal fragments of yellow fluorescent protein Venus (VN) and 147 GPCRs fused with C-terminal fragment of Venus (VC) as described in Song et al. (Song et al., 2014; SNU patent; Song, thesis). CXCR4-GPCR heteromers were identified using bimolecular fluorescence complementation (BiFC) assay (FIG. 1), in which two complementary VN and VC fragments of Venus reconstitute a fluorescent signal only when both fragments are close enough through interaction between two different proteins to which they are fused (Hu et al., 2002).

[365] U-2 OS cells were plated in 96-well plates, were co-transduced with 30 MOI each of adenoviruses encoding CXCR4-VN and GPCRx-VC or CXCR4-VC and GPCRx-VN, and were allowed to express GPCRs for 2 days. After staining the cells with Hoechst 33342, BiFC and nuclear images were obtained from three fields per well using IN Cell Analyzer 1000. Images of about 200 cells from each well were analyzed with Multi-target analysis software in IN Cell Developer ToolBox (GE Healthcare, Waukesha, WI). Cell boundary was marked based on Hoechst signal, and fluorescence intensity per cell was measured. Cells with fluorescence intensities higher than background level were considered as BiFC positive cells. Dead cells that showed extremely high intensities were excluded from the cell count. Positive cells were determined, and positive cell count ratio ("BiFC score") was calculated as (positive cells/ total cells) x 100 (see Table 4 below).

[366] When CXCR4-VN was co-expressed with HA-VC (FIG. 2a) or GCGR-VC, a GPCR encoding glucagon receptor (FIG. 2c), no yellow fluorescence protein (YFP) signal (BiFC signal) was observed. In contrast, when CXCR4-VN was co-expressed with CXCR4-VC (FIG. 2b), BiFC signal was observed in the plasma membrane and in the cytoplasm. Strong BiFC signal was observed in the plasma membrane and in the cytoplasm when CXCR4-VN was co-transduced with ADCYAP1R1-VC (FIG. 2d), ADORA3-VC (FIG. 2f), ADRB2-VC (FIG. 2g), APLNR-VC (FIG. 2h), C5AR1-VC (FIG. 2i), CALCR-VC (FIG. 2j), CCR5-VC (FIG. 2k), CHRM1-VC (FIG. 2l), GALR1-VC (FIG. 2m), EDNRB-VC (FIG. 2n), HRH1-VC (FIG. 2o), MLNR-VC (FIG. 2p), NTSR1-VC (FIG. 2q), PTGER2-VC (FIG. 2r), SSTR2-VC (FIG. 2t), and TACR3-VC (FIG. 2u). Robust BiFC signal was also observed when cells were co-transduced with CXCR4-VC and ADORA2B-VN (FIG. 2e) or PTGER3-VN (FIG. 2s). Cells that showed BiFC fluorescence signal higher than background level was counted as BiFC positive cells, and BiFC score was calculated.

[367] Protein-protein interaction can be affected by fusion tags such as fluorescence protein fragments in BiFC or renilla luciferase in BRET through interfering with the expression, folding, or localization of the partner protein. Partner protein can also impair the expression or folding of the fusion tags, and affect proximity-based assay result. Thus, the absence of signal between two proteins in specific combination does not necessarily imply that the proteins do not interact, but simply that the attached donor and acceptor molecules are in particular conformation that does not allow interaction to occur (Eidne et al., 2002; Kerppola, 2006).

[368] Therefore, CXCR4 and GPCRx that gave BiFC signal in either CXCR4-VN and GPCRx-VC or CXCR4-VC and GPCRx-VN combination were considered as interacting proteins. The BiFC score of CXCR4-VN and CXCR4-VC pair, a well-known homomer, was 9.9. Thus, CXCR4-GPCRx pairs that gave BiFC score equal or higher than 10 were selected as candidates for CXCR-GPCRx heteromer as shown in Table 4.

[369]

[370] Table 4. GPCRs that exhibited BiFC score equal or higher than 10 when co-expressed with CXCR4.

[371] [Table 4]

GPCRx		BiFC score CXCR4-VN & GPCRx-VC	GPCRx		BiFC score CXCR4-VN & GPCRx-VC
1	ADCYAP1R1	23	10	GALR1	60
2	ADORA2B	11*	11	EDNRB	41
3	ADORA3	43	12	HRH1	45
4	ADRB2	24	13	MLNR	10
5	APLNR	63	14	NTSR1	48
6	C5AR1	42	15	PTGER2	58
7	CALCR	53	16	PTGER3	13*
8	CCR5	44	17	SSTR2	30
9	CHRM1	24	18	TACR3	33

*CXCR4-VC and GPCRx-VN were used.

[372]

[373] Sixteen GPCRs, ADCYAP1R1, ADORA3, ADRB2, APLNR, C5AR1, CALCR, CCR5, CHRM1, GALR1, EDNRB, HRH1, MLNR, NTSR1, PTGER2, SSTR2, and TACR3, were identified as CXCR4-interacting GPCRs in CXCR4-VN and GPCRx-VC combination and two GPCRs, ADORA2B and PTGER3, gave BiFC score higher than 10 in CXCR4-VC and GPCRx-VN combination as shown in Table 4. Among those, ADRB2 and CCR5 have been reported to form heteromers with CXCR4 (LaRocca 2010, Nakai 2014, (Agrawal et al., 2004; LaRocca et al., 2010; Rodriguez-Frade et al., 2004; Sohy et al., 2007; Sohy et al., 2009; Martinez-Munoz et al., 2014)), and the remaining 16 GPCRs were found to be novel CXCR4-GPCRx heteromers that have not been reported to the best of our knowledge.

[374] Among GPCRs that have been known to interact with CXCR4, ADRA1A and CXCR3 were also included in our BiFC assay. These GPCRs were excluded from further investigation since they gave BiFC signal of less than 10 with CXCR4: ADRA1A (CXCR4-VN-ADRA1A-VC: BiFC score 7.85) and CXCR3 (BiFC score 1.16).

[375] Cannabinoid Receptor 2 (CB2, sometimes referred to as CNR2) was identified as a CXCR4-interacting GPCR in our BiFC assay (CNR2-VN-CXCR4-VC configuration: BiFC score 4.25; CNR2-VC-CXCR4-VN configuration: BiFC score 38.1), and co-internalization assay as described in FIGS. 3a-3b and 4a-4q. But CNR2 was not selected as the final candiadate because it failed to exhibit an enhanced downstream signaling in the calcium mobilization assay (see Example 3 below).

[376]

[377] **Example 2. Evaluation of CXCR4-GPCRx heteromer formation by co-internalization assay.**

[378] Some of the GPCR heteromers are known to exhibit, as a result of the heteromerization, altered trafficking properties such as maturation of the partner GPCR (GABA(B) receptor) (White, 1998), agonist-mediated internalization of the partner GPCR from the cell surface (DOR-GRPR, A2A-D2R) (Hillion et al., 2002; Liu et al., 2011; Torvinen et al., 2005), and changes in the localization of the partner GPCR from an intracellular compartment to the cell surface (DOR-CB1) (Rozenfeld et al., 2012).

[379] Co-internalization of pairs of co-expressed GPCRs in response to agonists selective for only one of the pair has been used to confirm GPCR heteromerization (Milligan, 2008). To examine if GPCRx modulates the trafficking of CXCR4 when co-expressed and forms CXCR4-GPCRx heteromer and also to confirm the physical interaction between CXCR4 and GPCRx identified using BiFC assay, cells were co-transduced with adenoviruses encoding CXCR4-GFP and GPCRx and GFP images were obtained before and 30 min after stimulation with GPCRx agonist. Loss of GFP expression on the cell surface or appearance of GFP granules inside the cells were considered as CXCR4-GFP co-internalization with GPCRx (FIGS. 3a-3b).

[380] In FIGS. 4a-4q, cells were stimulated with GPCRx agonists specific for CXCR4 (FIG. 4a), ADCYAP1R1 (FIG. 4b), ADORA2B (FIG. 4c), ADORA3 (FIG. 4d), ADRB2 (FIG. 4e), APLNR (FIG. 4f), C5AR1 (FIG. 4g), CCR5 (FIG. 4h), CHRM1 (FIG. 4i), GALR1 (FIG. 4j), EDNRB (FIG. 4k), HRH1 (FIG. 4l), MLNR (FIG. 4m), NTSR1 (FIG. 4n), PTGER3 (FIG. 4o), SSTR2 (FIG. 4p), and TACR3 (FIG. 4q) as follows: 10 nM of CXCL12 (FIG. 4a), 1 μM of vasoactive intestinal peptide (VIP) (FIG. 4b), 1 μM of BAY 60-6583 (FIG. 4c), 1 μM of CGS21680 (FIG. 4d), 100 nM of formoterol (FIG. 4e), 1 μM of apelin-13 (FIG. 4f), 100 nM of C5a (FIG. 4g), 100 nM of CCL2 (FIG. 4h), 1 μM of acetylcholine (FIG. 4i), 100 nM of galanin (FIG. 4j), 1 μM of endothelin 1 (FIG. 4k), 1 μM of histamine (FIG. 4l), 100 nM of motilin (FIG. 4m), 1 μM of neurotensin (FIG. 4n), 1 μM of PGE2 (FIG. 4o), 1 μM of SRIF-14 (FIG. 4p), and 1 μM of senktide (FIG. 4q), respectively. Images were obtained before and 30 min after agonist stimulation, and analyzed using IN Cell Analyzer 2000. The selection of the concentration of these GPCRx agonists was initially set at the EC50 concentration (or alternatively, the Ki or Kd concentration) for the respective specific GPCRx, and if the resulting signal was too intense, then the concertation was lowered below the EC50, and if the resulting signal was too weak, then the concertation was increased to no more than 10,000x the EC50 concentration.

[381] Stimulation of cells expressing CXCR4-GFP with CXCL12 resulted in re-location of GFP from the plasma membrane to distinct intracellular granules, showing internalization of surface CXCR4-GFP into the cytoplasm (FIG. 4a). Among 18 CXCR4-GPCRx heteromers identified by BiFC assay, 16 heteromers containing the following GPCRx induced internalization of CXCR4 as revealed by increased intra-

cellular GFP granules and reduced GFP signal in the plasma membrane in cells co-expressing CXCR4-GFP and GPCRx, confirming heteromer co-internalization: ADCYAP1R1, ADORA2B, ADORA3, ADRB2, APLNR, C5AR1, CCR5, CHRM1, GALR1, EDNRB, HRH1, MLNR, NTSR1, PTGER3, SSTR2, and TACR3 (FIG. 4b-q). CXCR4-GPCRx heteromers containing CALCR and PTGER2, on the other hand, did not show co-internalization of the heteromer upon GPCRx agonist treatment. These results demonstrate that certain GPCRx partners identified in the BiFC assay do form CXCR4-GPCRx heteromers in cells co-expressing both GPCRs, as evidenced by altered cellular trafficking properties of the heteromer.

[382]

[383] **Example 3. Evaluation by Ca2+ mobilization assay of enhanced CXCR4 downstream signaling upon CXCR4-GPCRx heteromer formation and inhibition of the enhanced signaling.**

[384] To further examine if these CXCR4-GPCRx heteromers exhibit properties distinct from those of the individual protomers (in cells lacking one of the receptors), we investigated calcium signaling in the presence of either or both agonists in cells expressing either GPCR or both GPCRs together. In this example, as noted in Example 2, the selection of the concentration of these GPCRx agonists was initially set at the EC50 concentration (or alternatively, the Ki or Kd concentration) for the respective specific GPCRx, and if the resulting Ca2+ signal was too intense, then the concertation was lowered below the EC50, and if the resulting Ca2+ signal was too weak, then the concertation was increased to no more than 100x the EC50 concentration.

[385] When MDA-MB-231 human breast cancer cells were transduced with adenoviruses encoding CXCR4, treatment of CXCL12 evoked intracellular calcium mobilization (FIG. 5a). Stimulation of the cells with salmeterol, an ADRB2-selective agonist, did not induce calcium response, demonstrating the calcium response evoked by CXCL12 was mediated by CXCR4. Co-treatment of the cells with CXCL12 and salmeterol induced similar calcium response compared to the one induced by CXCL12 alone. In cells overexpressing ADRB2 alone, CXCL12 did not induced calcium response while salmeterol induced calcium response, showing that salmeterol induces calcium response through ADRB2 (FIG. 5b). Co-treatment of both agonists induced similar calcium response to the one stimulated by salmeterol alone.

[386] In cells overexpressing both CXCR4 and ADRB2, stimulation with each agonist induced calcium responses similar to the ones shown in cells expressing CXCR4 or ADRB2 alone (FIGS. 5c vs 5a and 5b). In contrast, co-treatment of both agonists together significantly increased the calcium response compared to the ones evoked by individual agonists (FIGS. 5c & 5d). The enhanced calcium signaling was observed only in cells expressing both CXCR4 and ADRB2, but not in cells expressing either

CXCR4 or ADRB2 alone. These results clearly demonstrate that CXCR4-ADRB2 heteromer exhibit properties distinct from those of the individual GPCRs.

[387]

[388] To examine if the other GPCRs identified to interact with CXCR4 in BiFC and co-internalization assay also show distinct properties in calcium signaling, experiments shown in FIGS. 5a-5d were performed for other identified GPCRx partners. In cells co-expressing CXCR4 and either ADCYAP1R1 (FIG. 6a), ADORA2B (FIG. 6b), ADORA3 (FIG. 6c), C5AR1 (FIG. 6d), CALCR (FIG. 6e), CHRM1 (FIG. 6f), EDNRB (FIG. 6g), HRH1 (FIG. 6h), MLNR (FIG. 6i), NTSR1 (FIG. 6j), PTGER2 (FIG. 6k), or TACR3 (FIG. 6l), co-treatment of CXCL12 and respective GPCRx agonist significantly increased calcium responses compared with the sum of calcium responses that were induced by individual agonists as shown in FIGS. 5c and 5d, and as shown in FIGS. 6a-6l. The cells co-expressing the CXCR4 and the GPCRx (either ADCYAP1R1 (FIG. 6a), ADORA2B (FIG. 6b), ADORA3 (FIG. 6c), C5AR1 (FIG. 6d), CALCR (FIG. 6e), CHRM1 (FIG. 6f), EDNRB (FIG. 6g), HRH1 (FIG. 6h), MLNR (FIG. 6i), NTSR1 (FIG. 6j), PTGER2 (FIG. 6k), or TACR3 (FIG. 6l)) exhibited an enhanced calcium mobilization upon co-stimulation (or co-treatment) with the CXCL12 and the respective selective GPCRx agonist relative to the sum of calcium mobilization amounts resulting from single agonist stimulation with either the CXCL12 or the respective selective GPCRx agonist, as determined by the calcium mobilization assay. Notably, in cells co-expressing CXCR4 and CNR2, CXCL12-induced calcium response was significantly reduced, rather than enhanced, upon addition of CXCL12 alone or together with JWH-133, a selective CNR2 agonist, compared with the CXCL12-mediated calcium response observed in cells expressing CXCR4 alone (data not shown).

[389] Within the above set of co-expressed systems, it was also noted that an enhanced amount of calcium mobilization was observed in the individual protomer context in cells involving CXCR4 and PTGER2 (FIG. 6k) (an individual protomer context, *e.g.*, either CXCR4 expressed in the absence of the respective GPCRx, or the respective GPCRx expressed in the absence of the CXCR4), upon co-stimulation with CXCL12 and the respective selective GPCRx agonist. Specifically, an enhanced amount of calcium mobilization was observed for PTGER2 (FIG. 6k), in the individual protomer context, in both situations where CXCR4 was expressed in the absence of the respective GPCRx, and where the respective GPCRx (PTGER2) was expressed in the absence of the CXCR4, upon co-stimulation with CXCL12 and the respective selective GPCRx agonist (5 μM of PGE2), the calcium mobilization amount resulting from the individual protomer context systems were greater than the sum of calcium mobilization amounts resulting from single agonist stimulations with either the CXCL12 or the re-

spective selective GPCRx agonist (5 μM of PGE2).

[390] Accordingly, from these observations, those systems co-expressing CXCR4 and the GPCRx selected from the group consisting of ADCYAP1R1 (FIG. 6a), ADORA2B (FIG. 6b), ADORA3 (FIG. 6c), C5AR1 (FIG. 6d), CALCR (FIG. 6e), CHRM1 (FIG. 6f), EDNRB (FIG. 6g), HRH1 (FIG. 6h), MLNR (FIG. 6i), NTSR1 (FIG. 6j), and TACR3 (FIG. 6l), are considered to have: (i) exhibited an enhanced calcium mobilization upon co-stimulation (or co-treatment) with the CXCL12 and the respective selective GPCRx agonist relative to the sum of calcium mobilization amounts resulting from single agonist stimulation with either the CXCL12 or the respective selective GPCRx agonist, as determined by the calcium mobilization assay; (ii) exhibited a calcium mobilization amount equal to or less than the sum of calcium mobilization amounts resulting from single agonist stimulation with either the CXCL12 or the respective selective GPCRx agonist in an individual protomer context; and thus (iii) constituted CXCR4-GPCRx heteromers exhibiting properties distinct from those of the individual GPCR. The enhanced calcium responses were not observed in cells expressing individual GPCRs, thereby demonstrating that these CXCR4-GPCRx heteromers exhibit properties distinct from those of the individual GPCR.

[391] The enhanced calcium signaling induced by small amounts of individual agonists together clearly indicated that CXCR4-GPCRx heteromers would increase the sensitivity and dynamic range of these receptors at limiting ligand concentrations *in vivo*, and may be associated with poor prognosis. The enhanced calcium signaling by the CXCR4-GPCRx heteromers also indicates that the current diagnosis based on the level of CXCR4 expression alone needs to be changed to consider the expression levels of CXCR4 and GPCRx.

[392] In contrast, GPCRs such as APLNR (FIG. 7a), CCR5 (FIG. 7b), GALR1 (FIG. 7c), PTGER3 (FIG. 7d), and SSTR2 (FIG. 7e) did not show enhanced calcium responses when cells expressing both receptors were co-treated with both agonists, although these GPCRs were shown to interact with CXCR4 in BiFC (Table 5) and CXCR4-GFP co-internalization assay. These results clearly demonstrate that the synergistic increase in calcium response shown in FIGS. 5a-5d and 6a-6l is a unique feature of CXCR4-GPCRx heteromers, that is not shared by other CXCR4-GPCRx heteromers such as CXCR4-APLNR, CXCR4-CCR5, CXCR4-GALR1, CXCR4-PTGER3, and CXCR4-SSTR2.

[393] Table 5. GPCRs that exhibited BiFC score equal or higher than 10 when co-expressed with CXCR4 but did not show enhanced Ca2+ signaling upon co-treatment of both agonists.

[394] [Table 5]

CXCR4-GPCRx heteromers that do not show enhanced Ca^{2+} signaling upon co-treatment of both agonists		
GPCRx		BiFC score
		CXCR4-VN & GPCRx-VC
1	APLNR	63
2	CCR5	44
3	GALR1	60
4	PTGER3*	13
5	SSTR2	30

*CXCR4-VC and GPCRx-VN were used.

[395] It was further examined if enhanced calcium responses in cells co-expressing CXCR4 and GPCRx upon co-stimulation with CXCL12 and GPCRx ligands are inhibited by GPCRx antagonists. In cells co-expressing CXCR4 and either ADRB2 (FIG. 8a), CHRM1 (FIG. 8b & FIG. 8c), HRH1 (FIGS. 8f-8i), MLNR (FIG. 8j), or NTSR1 (FIG. 8k), treatment of 10 μM of ADRB2 antagonist carvedilol (FIG. 8a), 1 μM of CHRM1-selective antagonist VU0255035 (FIG. 8b), 10 μM of CHRM1 antagonist oxybutynin (FIG. 8c), 1 μM of HRH1-selective antagonist cetirizine (FIG. 8f), 1 μM of HRH1-selective antagonist pyrilamine (FIG. 8g), 10 μM of HRH1 antagonist hydroxyzine (FIG. 8h), 10 μM of HRH1-selective antagonist loratadine (FIG. 8i), 1 μM of MLNR-selective antagonist MA-2029 (FIG. 8j) and 1 μM of NTSR1-selective antagonist meclinertant (FIG. 8k), respectively, suppressed the enhanced calcium signaling significantly. And co-treatment of both antagonists together resulted in a more complete suppression (FIGS. 8a-8c, and 8f-8k). These results demonstrated that GPCRx antagonists could be used as an efficient therapeutics against CXCR4-GPCRx heteromers where GPCRx represents ADRB2, CHRM1, HRH1, MLNR, and NTSR1.

[396] In cells co-expressing CXCR4 and CHRM1, 10 μM of muscarinic acetylcholine receptor antagonist umeclidinium reduced the enhanced calcium signaling, although not statistically significant (FIG. 8d). In these cells, co-treatment of AMD3100 and umeclidinium almost completely inhibited calcium response induced by simultaneous addition of CXCL12 and bethanechol.

[397] In cells co-expressing CXCR4 and either EDNRB (FIG. 8e) or TACR3 (FIG. 8l), treatment of either 1 μM of AMD3100 alone (FIG. 8e and FIG. 8l), or 1 μM of endothelin receptor antagonist bosentan (FIG. 8e) or 1 μM of TACR3-selective antagonist SSR 146977 alone (FIG. 8l) failed to inhibit enhanced calcium response significantly. But when the cells were co-treated with AMD3100 and bosentan (FIG. 8e) or AMD3100 and SSR 146977 together (FIG. 8l), the enhanced calcium responses were significantly inhibited.

[398] In cells co-expressing CXCR4 and either CHRM1 (FIG. 8b), EDNRB (FIG. 8e), HRH1 (FIG. 8f and 8g), or MLNR (FIG. 8j), although 1 μM of AMD3100 alone failed to inhibit enhanced calcium signaling, co-treatment of 1 μM of both antagonists together significantly suppressed the enhanced calcium signaling.

[399] These results clearly demonstrate that co-treatment of small doses of antagonists targeting each protomer provides novel therapeutic tools to efficiently suppress CXCR4-GPCRx heteromer response while avoiding side effects associated with high doses of individual antagonists.

[400]

[401] **Example 4. Inhibition of internalization by GPCRx antagonists.**

[402] To further study if co-internalization of CXCR4 heterodimer was blocked by partner GPCRx antagonists, internalization inhibition assay was performed. As shown in FIGS. 4b-4q, CXCR4-GFP expressing U-2 OS cells were co-internalized by partner GPCRx specific agonist when CXCR4 and GPCRx were simultaneously transduced to cells (control: CXCR4-GFP (FIG. 4a)). If CXCR4 formed heterodimer with GPCRx and co-internalized by partner GPCRx, it can be blocked by GPCRx specific antagonist.

[403] U-2 OS cells stably expressing CXCR4-GFP were transduced with Adenoviruses encoding GPCRx (ADRB2, CHRM1, HRH1). After 2 days, images were obtained before and 20 minutes after stimulating cells with CXCR4-specific agonist, CXCL12 (SDF-1) (20 nM), and/ or GPCRx-specific antagonist (10 μM). Internalization of CXCR4-GFP was observed as GFP granules using IN Cell Analyzer 2500. Loss of GFP expression on the cell surface or appearance of GFP granules inside the cells were considered as CXCR4-GFP co-internalization. CXCR4 agonist, CXCL12 induced internalization of CXCR4-GFP with GPCRx (FIGS. 10a-c, first columns). GPCRx antagonists, treated as follows, had no effect on the internalization of the heteromer (FIGS. 10a-c, second columns): Carvedilol, an ADRB2 antagonist (FIG. 10a); Oxybutynin and Umeclidinium, CHRM1 antagonists (FIG. 10b); Prometazine, Hydroxyzine, and Loratadine, HRH1 antagonists (FIG. 10c). Internalization of CXCL12 stimulated CXCR4-GFP with GPCRx were inhibited by GPCRx specific antagonists (FIGS. 10a-c, third columns).

[404] These data demonstrate that CXCR4-GPCRx co-internalization was heteromer specific event and CXCR4 and GPCRx formed heterodimer. These data further demonstrate that, by inhibition of internalization of CXCR4 heteromer, abnormal downstream signal in CXCR4-GPCRx heteromer overexpressed cells, such as cancers, can be blocked for therapeutics purposes.

[405]

[406] **Example 5. Evaluation by cell proliferation assay of phenotypic effects of**

inhibitor of CXCR4-GPCRx heteromer signaling on tumor growth.

[407] Several CXCR4 antagonists have been developed, but none have been approved as anticancer drugs until now. To overcome the limitation of CXCR4 inhibitors and to develop CXCR4 heteromer-based therapeutic agent, we tested the effect of GPCRx antagonist on cell proliferation.

[408] We prepared single cell suspensions of resected and dissociated glioblastoma tissues from patients. (provided by Samsung Seoul hospital in Seoul, Korea) These cells were cultured under conditions optimal for propagation and non-differentiation of normal neuronal stem cell. Media were composed serum free Neurobasal media supplemented with basic FGF and EGF.

[409] The effect of GPCRx antagonist on the survival of patient derived cells (PDCs) were assessed using ATPlite (PerkinElmer, Cat. No. 6016739 reagent. ATPlite is an Adenosine Triphospate monitoring system based on firefly luciferase. This luminescence assay is the alternative to colorimetric, fluorometric and radioisotopic assays for the quantitative evaluation of proliferation and cytotoxicity of cultured mammalian cells. Cells were seeded in 384-well plate at 500 cells/well in 40 μl culture media. After overnight growth, the cells were cultured for 7 days in the presence of several dose of GPCRx antagonist or DMSO alone. After the 7-day incubation, 15 μl ATPlite was added into the each well and the plate were shaken for 5 minutes in an orbital shaker at 700 rpm. The luminescent signal was detected within 30 minutes at PerkinElmer TopCount detection instrument. The cell viability was calculated using the equation: Cell viability (%) = (OD of antagonist treatment/OD of DMSO only treatment) x 100%.

[410] As shown in FIGS. 11a-c, when Carvedilol, ADRB2 specific antagonist, was treated in PDC expressing CXCR4 and ADRB2, growth of cells was inhibited significantly. (IC50=11.69 μM, FIG. 11a). Oxybutynin and Umeclidinium, CHRM1 antagonists also inhibited on the survival of PDc. Oxybutynin or Umeclidinium each showed significantly decreased survival of cells at IC50=3.04 μM or 4.03 μM, respectively. (FIG. 11b). Promethazine, Hydroxyzine, and or Loratadine as a HRH1 antagonist each showed decreased survival of PDc at IC50=18.39 uM, 12.79 μM or 5.29 μM, respectively.(FIG. 11c).

[411] These results demonstrate that CXCR4 heteromer induced abnormal cell proliferation can be blocked by partner GPCRx specific antagonist in CXCR4-GPCRx heteromer expressing cells, indicating that inhibition of cancer cell growth using partner GPCRx antagonist in CXCR4-GPCRx heteromer bearing patients can overcome the limitations of CXCR4 inhibitors alone as cancer therapeutics.

[412]

[413] **Example 6. Evaluation of CXCR4-GPCRx heteromer formation in patient**

derived cells (PDC) using Proximity ligation assay (PLA).

[414] To investigate the existence of GPCR complexes in native tissues, various approaches such as atomic force microscopy (Fotiadis et al., 2006), co-immunoprecipitation (Gomes et al., 2004) and binding or functional assays (Wreggett and Wells, 1995) have been used. The most convenient methods to monitor interactions are based on resonance energy transfer performed with labeled proteins. The labeling can be performed by selective probes such as antibodies or fluorescent ligands (Roess et al., 2000; Patel et al., 2002).

[415] Bazin et al. employed a time-resolved fluorescence resonance energy transfer (TR-FRET)-based approach that offers a much higher signal-to-noise ratio (Bazin et al., 2002). FRET is based on the transfer of energy between two fluorophores, a donor and an acceptor, when in close proximity. Molecular interactions between biomolecules can be assessed by coupling each partner with a fluorescent label and by detecting the level of energy transfer. Introducing a time delay of approximately 50 to 150 μ seconds between the system excitation and fluorescence measurement allows the signal to be cleared of all non-specific short-lived emissions.

[416] Proximity ligation assay (PLA) is a technology that extends the capabilities of traditional immunoassays to include direct detection of proteins, protein interactions and modifications with high specificity and sensitivity (Gullberg et al., 2004). Two primary antibodies raised in different species recognize the target antigen on the proteins of interest. Secondary antibodies directed against the constant regions of the different primary antibodies, called PLA probes, bind to the primary antibodies. Each of the PLA probes has a unique short DNA strand attached to it, If the PLA probes are in close proximity (that is, if the two original proteins of interest are in close proximity, or part of a protein complex, as shown in the figures), the DNA strands can participate in rolling circle DNA synthesis when appropriate substrates and enzymes are added. The DNA synthesis reaction results in several-hundred fold amplification of the DNA circle. Next, fluorescent-labeled complementary oligonucleotide probes are added, and they bind to the amplified DNA. The resulting high concentration of fluorescence is easily visible as a distinct bright spot when viewed with a fluorescence microscope (Gustafsdottir et al., 2005).

[417] CXCR4 overexpressing cell line, U2OS-CXCR4, was infected with ADRB2 expressing adenovirus, Ad-ADRB2 at the dose of 0, 2.5, 10, 40 MOIs for 2 days. PLA was performed as described previously (Brueggemann et al., 2014; Tripathi et al., 2014). To perform PLA, infected cells were fixed with 4% paraformaldehyde (PFA) on sixteen-well tissue culture slides. Slides were blocked with blocking solution provided by Duolink and incubated with mouse anti-CXCR4 (1:200, Santacruz, Sc-53534), Rabbit anti-ADRB2(1:200, Thermoscientific, PA5-33333), Rabbit anti-CHRM1(1:200,

Ls bio, Ls-C313301) at 37°C for 1 h in a humidifying chamber. Slides were then washed and incubated (1 h at 37°C) with secondary anti-rabbit and anti-mouse antibodies conjugated with plus and minus Duolink II PLA probes. Slides were washed again and then incubated with ligation-ligase solution (30 min at 37°C) followed by incubation with amplification-polymerase solution (2 h at 37°C). Slides were then mounted with minimal volume of Duolink II mounting medium with 4′,6-diamidino-2phenylindole (DAPI) for 15-30 min, and PLA signals [Duolink In Situ Detection Reagents Green (λ excitation/emission 495/527 nm) or Red (λ excitation/ emission 575/623 nm) were identified as fluorescent spots under a IN Cell analyzer 2500.

[418] As shown in the FIGS. 12a-12b, the PLA signal increases in a dose dependent manner as the expression level of ADRB2. FIG. 12a: Images of PLA signal from U2OS cells expressing CXCR4-ADRB2 heteromer over a series of MOIs (multiplicity of infection). FIG. 12b: The red signal spots were counted and calculated by normalization against negative control. The PLA signal increased proportionate to the expression level of ADRB2 in a dose dependent manner. FIG. 12c: To investigate endogenous ADRB2 expression, qRT-PCR was performed with ADRB2 specific primers. As the results show, endogenous ADRB2 expression level of U2OS cell was quite high, indicating that the PLA signal is detected even in the section without virus infection (at 0 MOI for ADRB2).

[419] Traditionally Glioblastoma (GBM) is the most common and lethal primary brain tumor. Preclinical cancer biology has largely relied on the use of human cancer cell lines *in vitro* and the xenograft process of established these cell lines. However, the process of establishing conventional cell lines results in irreversible loss of important biological properties and, as a result, the xenograft tumor models do not maintain genomic and phenotypic characteristics present in the original tumor.

[420] Patient derived cell (PDC) derived directly from glioblastoma harbor extensive similarities to normal neural stem cells and recapitulate the genotype, gene expression patterns, and *in vivo* biology of human glioblastomas.

[421] To perform PLA with PDC samples, the patient derived cells were plated and fixed with 4 % PFA on sixteen-well tissue culture slides. Slides were blocked with blocking solution provided by Duolink and incubated with mouse anti-CXCR4 (1:200, Santa Cruz, Sc-53534), rabbit anti-ADRB2 (1:200, Thermo Scientific, PA5-33333), rabbit anti-CHRM1 (1:200, Lsbio, Ls-C313301) at 37 °C for 1 h in a humidifying chamber. Slides were then washed and incubated (1 h at 37 °C) with secondary anti-rabbit and anti-mouse antibodies conjugated with plus and minus Duolink II PLA probes. Slides were washed again and then incubated with ligation-ligase solution (30 min at 37 °C) followed by incubation with amplification-polymerase solution (2 h at 37 °C). Slides

were then mounted with minimal volume of Duolink II mounting medium with 4',6'-diamidino-2-phenylindole (DAPI) for 15-30 min, and PLA signals [Duolink In Situ Detection Reagents Green (λ excitation/emission 495/527 nm) or Red (λ excitation/emission 575/623 nm)were identified as fluorescent spots under the IN Cell analyzer 2500.

[422] As shown in FIGS. 13a-13b and FIGS.14a-14b, PLA ratio refers to the CXCR4 GPCRx heteromer, and the frequency of the heteromer formation varies depending on the patient. PLA ratio was calculated as: number of fluorescent spots in PDC sample / number of fluorescent spots in negative control. Negative control (NC) represents background fluorescence signal, indicated by the number of spots when only the secondary antibody conjugated with plus and minus Duolink II PLA probes is treated without primary antibody (mouse anti-CXCR4, rabbit anti-ADRB2, rabbit anti-CHRM1) treatment in PLA processing. These data demonstrate quantitative analysis of CXCR4-ADRB2 heterodimer in cancer patient samples.

[423] **Example 7. Evaluation of CXCR4-GPCRx heteromer formation** *in vivo* using PDX model.

[424] To perform PLA with PDX samples, the glioblastoma patient derived FFPE samples were used (provided by Samsung Seoul hospital in Seoul, Korea). After FFPE sample were de-paraffinized and performed heat induced antigen retrieval for 15 minutes at 100℃. Slides were blocked with blocking solution provided by Duolink and incubated with rabbit anti-CXCR4 (1:200, Thermoscientific, PA3305), mouse anti-ADRB2 (1:200, Santacruz, Sc-271322), at 37 ℃ for 1 h in a humidifying chamber. The other process was same as described above (PLA with PDC).

[425] In the FIG. 15a, nuclei were visualized with DAPI staining, and CXCR4-ADRB4 heteromers were stained with PLA as small dots. As shown in FIG. 15b, PLA ratio is different according to the patient and based on this result, indicating that it is possible to perform personalized medicine by the companion diagnostics.

[426]

[427] **Example 8. Evaluation by Ca2+ mobilization assay of enhanced CXCR4 downstream signaling upon CXCR4-GPCRx heteromer formation.**

[428] In cells overexpressing both CXCR4 and ADRB2, stimulation with salmeterol alone did not elicit calcium mobilization dose-dependently (FIG. 16a). But when the cells were co-stimulated with salmeterol in the presence of CXCL12, calcium signaling was greatly enhanced in a broad range of salmeterol concentrations, such as concentrations in the range of between 10 nM to 300 nM.

[429] Similarly, co-treatment with histamine and CXCL12 also significantly enhanced calcium responses in cells overexpressing both CXCR4 and HRH1 over a broad range of histamine concentrations (0.3 nM to 300 nM), even at histamine concentrations that

did not evoke any calcium responses when treated alone (0.3 nM and 1 nM) (FIG. 16b). Considering that the concentration of histamine in human plasma and glomeruli is below 10 nM and 2 μM, respectively (Sedor and Abboud, 1984), this result indicates that enhanced calcium signaling by CXCR4-HRH1 heteromer can occur at physiological levels of histamine concentration *in vivo*.

[430] In cells expressing CXCR4-APLNR (FIG. 17a), CXCR4-PTGER3 (FIG. 17b), or CXCR4-SSTR2 (FIG. 17c), stimulation with GPCRx agonist (Apelin-13, PGE2, or octreotide, respectively) alone increased calcium signal dose-dependently. However, unlike in cells expressing CXCR4-ADRB2 or CXCR4-HRH1, calcium signal was not enhanced any further upon co-stimulation with both agonists (CXCL12 and GPCRx agonist) in any dose ranges tested in cells expressing CXCR4-APLNR, CXCR4-PTGER3, or CXCR4-SSTR2. These results are consistent with the results shown in FIGS. 7a, 7d, 7e, repsectively, and show that the lack of signal enhancement in cells expressing CXCR4-APLNR or CXCR4-SSTR2 upon co-stimulation was not due to the use of a specific dose of GPCRx agonist, but due to the intrinsic nature of these heteromers. These results also clearly demonstrate that the enhanced calcium responses shown in FIG. 6a-6l and FIGS. 16a-16b are unique features of CXCR4-GPCRx heteromers such as CXCR4-ADRB2 or CXCR4-HRH1 described in FIGS. 5c-5d and FIG. 6h, respectively.

[431]

[432] **Example 9. Evaluation of enhanced Ca2+ mobilization upon co-stimulation of endogenous CXCR4-HRH1 heteromer in MDA-MB-231 cells.**

[433] Using RT-qPCR, the endogenous expression levels of CXCR4 and HRH1 in MDA-MB-231 cells were measured. The threshold cycles (Ct) of CXCR4 and HRH1 were 28.5 and 27.7, respectively, when 12.5 ng of total RNA was analyzed. Stimulation of MDA-MB-231 cells with either CXCL12 (100 nM), or histamine (10 nM) alone, elicit weak calcium responses (FIG. 18a). But when the cells were co-stimulated with CXCL12 and histamine together, greatly enhanced calcium response was observed similar to the enhancement observed in cells overexpressing CXCR4 and HRH1 together (FIGS. 18a and 18b, compared with FIG. 16b). The result clearly indicates that the enhanced calcium response upon co-stimulation with both agonists could occur in native cells expressing both CXCR4 and HRH1 together.

[434]

[435] **Example 10. Evaluation of enhanced migration of cancer cells by CXCR4-HRH1 heteromer upon co-stimulation with both ligands.**

[436] Since MDA-MB-231 cells express about twice more HRH1 mRNA compared with CXCR4 mRNA when measured by RT-qPCR, the MDA-MB-231 cells were transduced with small amount of lentivirus encoding CXCR4 (1 MOI) and chemotactic

migration of the cells toward CXCL12 and HRH1 was measured (FIGS. 19a and 19b). Histamine (50 nM) alone, sufficient to produce calcium signaling as shown in FIG. 27a (see below), did not induce cell migration. On the other hand, when treated together with CXCL12, histamine significantly enhanced the migration of MDA-MB-231 cells towards CXCL12. However, in the presence of pyrilamine, a HRH1-selective inverse agonist, co-stimulation of the cells with CXCL12 and histamine failed to enhance the cell migration elicited by CXCL12. The results clearly demonstrate that the enhancement of observed cancer cell migration was specifically induced by HRH1, and not by other histamine receptor subtypes.

[437]

[438] **Example 11. Evaluation by Ca2+ mobilization assay of enhanced CXCR4 downstream signaling upon CXCR4-GPCRx heteromer formation and inhibition of the enhanced signaling.**

[439] In cells overexpressing both CXCR4 and ADRB2, co-treatment of both agonists CXCL12, a CXCR4 agonist and salmeterol, an ADRB2-selective agonist significantly increased the calcium response compared to the ones evoked by individual agonists (FIG. 20). These results clearly demonstrate that CXCR4-ADRB2 heteromer exhibit properties distinct from those of the individual GPCRs.

[440] It was examined if enhanced calcium responses in cells co-expressing CXCR4 and ADRB2 upon co-stimulation with CXCL12 and ADRB2 ligands are inhibited by anti-CXCR4 antibody as a CXCR4 antagonist. In cells co-expressing CXCR4 and ADRB2, treatment of 2 μg of anti-CXCR4 antibody, 12G5, suppressed the enhanced calcium signaling as shown in FIG. 20. And co-treatment of both antagonists (Carvedilol, ADRB2 antagonist and 12G5, CXCR4 antagonist together) resulted in a more significant suppression. These results demonstrated that anti-CXCR4 antibody and ADRB2 antagonist could be used as an efficient therapeutics against CXCR4-ADRB2 heteromer.

[441] Utilizing the Calcium mobilization assay, MDA-MB-231 human breast cancer cells were seeded at 20,000 cells per well in a black clear bottom 96-well plate (Corning Costar, #3340) in 100 μL of RPMI 1640 supplemented with 10% FBS. The next day, cells were co-transduced with 10 MOI of CXCR4 and 30 MOI of GPCRx. After 2 days, cells were treated with ADRB2 antagonist, Carvedilol(Tocris), anti-CXCR4 antibody, 12G5 (Thermo Scientific, 35-8800) with indicated amounts and incubated with Cal 6 (FLIPR® Calcium 6 Assay Kit by Molecular Devices, Cat. R8191) for 2 hr. And then, cells were stimulated with indicated amounts of CXCL12, ADRB2 agonist, or CXCL12 and ADRB2 agonist. Calcium mobilization was measured using FlexStation 3 Multi-Mode Microplate Reader. The results were normalized for baseline activity. Calcium mobilization was quantified by calculating the area-

under-the-curve (AUC) of each graph. Data were normalized to CXCL12-stimulated calcium response in cells expressing CXCR4 alone. Data represent three independent experiments (mean ± SEM). *P < 0.05; Student's t test.

[442]

[443] **Example 12. Evaluation of CXCR4-GPCRx heteromer formation in CXCR4-GPCRx expressing cell using PLA.**

[444] A process for screening a CXCR4-GPCRx heteromer is essential for the treatment of CXCR4-GPCRx targeted anticancer drug. First, for the quantitative detection of CXCR4 and GPCRx heteromer, PLA was performed in CXCR4-GPCRx expressing cells. CXCR4 overexpressing cell line, the U2OS-CXCR4 was infected with GPCRx expressing adenovirus, Ad-GPCRx at the dose of 0, 2.5, 10, 40 MOIs for 2 days. And then, the transduced cells were fixed with 4% paraformaldehyde and performed PLA was performed. The number of PLA signals means quantitatively demonstrating the formation of CXCR4-GPCRx heteromerization.

[445] As shown in the FIGS. 21a-21b, the PLA signal increased proportionate to the expression level of CHRM1 (FIG. 21a) and HRH1 (FIG. 21b) in a dose dependent manner. These results demonstrate detection of different types of CXCR-GPCRx heteromer and quantitative analysis of CXCR4-GPCRx heteromer in cancer patient samples.

[446] Utilizing the PLA in cells, CXCR4 overexpression cell lines, the U2OS-CXCR4 was infected with GPCRx expressing adenovirus, Ad-GPCRx at the dose of 0, 2.5, 10, 40 MOIs for 2 days. PLA was performed as described previously (Brueggemann et al., 2014; Tripathi et al., 2014). To perform PLA, infected cells were fixed with 4% PFA on sixteen-well tissue culture slides. Slides were blocked with blocking solution provided by Duolink and incubated with mouse anti-CXCR4 (Santacruz, Sc-53534), rabbit anti-CHRM1 (LS Bio, LS-C313301), or rabbit anti-HRH1 (Thermoscientific, PA5-27817) at 37℃ for 1 h in a humidifying chamber. Slides were then washed and incubated (1 h at 37℃) with secondary anti-rabbit and anti-mouse antibodies conjugated with plus and minus Duolink II PLA probes. Slides were washed again and then incubated with ligation-ligase solution (30 min at 37℃) followed by incubation with amplification-polymerase solution (2 h at 37℃). Slides were then mounted with minimal volume of Duolink II mounting medium with 4′,6-diamidino-2phenylindole (DAPI) for 15-30 min, and PLA signals [Duolink In Situ Detection Reagents Green (λ excitation/emission 495/527 nm) or Red (λ excitation/emission 575/623 nm)were identified as fluorescent spots under a IN Cell analyzer 2500.

[447]

[448] **Example 13. Ca2+ Mobilization Inhibition of the Enhanced CXCR4 Downstream Signaling upon CXCR4-GPCRx heteromer Formation - Comparison**

of Single Inhibitor Treatment to Combination Inhibitor Treatment.

[449] The data shown above (see Example 3, FIG. 8a) demonstrated that an increased Ca2+ signal due to CXCR4-ADRB2 heteromer formation was significantly decreased when treated simultaneously with a CXCR4 inhibitor and an ADRB2 inhibitor. The degree of inhibition (measured as IC50 values for Ca^{2+} response) was compared for a series of CXCR4 inhibitors, evaluated as single treatment in cells expressing CXCR4 alone (individual protomer context), as single treatment in CXCR4-ADRB2 heteromer-expressing cells, and as co-treatment with an ADRB2 inhibitor (Carvedilol; 10 μM) in CXCR4-ADRB2 heteromer-expressing cells.

[450] MDA-MB-231 cells were transduced with adenoviruses encoding CXCR4 only, or CXCR4 and ADRB. The cells were cultured for 2 days and were treated CXCR4 inhibitor alone or co-treated with a CXCR4 inhibitor and an ADRB2 inhibitor (Carvedilol; 10 uM). The cells were then stained with Cal-6 for 2 hours and stimulated with CXCR4 agonsit (CXCL12; 20 nM) and ADRB2 agonist (Salmeterol; 1 μM). Calcium mobilization was measured using FlexStation3, the results of which are shown in Table 6, showing IC50 of $Ca2^{+}$ response in: (1) MDA-MB-231 cells expressing CXCR4 alone (individual protomer context), treated with CXCR4 inhibitor only (2^{nd} column); (2) MDA-MB-231 cells expressing CXCR4-ADRB2 heteromer, treated simultaneously with CXCR4 inhibitor and ADRB2 inhibitor Carvedilol (3^{rd} column); and (3) MDA-MB-231 cells expressing CXCR4-ADRB2 heteromer, treated only with CXCR4 inhibitor (4^{th} column).

[451]

[452] [Table 6]

CXCR4 inhibitor	CXCR4	CXCR4+ADRB2 (+ Carvedilol)	CXCR4+ADRB2 (- Carvedilol)
	IC_{50} [nM]	IC_{50} [nM]	IC_{50} [nM]
AMD3100	57.80±20.36	0.053±0.07	28.65±13.23
AMD070	43.37±0.57	1.40±2.2	24.69±8.88
12G5	80.19±48.5	2.784±0.86	13.95±10.04
Ulocuplumab	0.42±0.09	0.0008±0.0002	1.12±0.56
BKT140	434±4.38	0.81±0.41	130.40±49.78
TZ14011	23.93±3.58	0.02±0.02	17.78±7.84

[453]

[454] As shown in Table 6, the IC50 value of Ca^{2+} response was CXCR4 inhibitor dependent in the context of single treatment of MDA-MB-231 cells expressing CXCR4 alone, and in the single treatment context and the co-treatment context with an ADRB2 inhibitor of MDA-MB-231 cells expressing CXCR4-ADRB2 heteromer. Depending on the identity of the CXCR4 inhibitor, the IC50 value of Ca^{2+} response in

the CXCR4-ADRB2 heteromer context decreased by 1400 times or more when treated in combination with an ADRB2 inhibitor (3rd column), relative to single treatment with the CXCR4 inhibitor alone (4th column). For instance, the IC50 value of Ca^{2+} response in the CXCR4-ADRB2 heteromer context decreased about 540 fold (from 28.65 nM to 0.053 nM), about 1400 fold (from 1.12 nM to 0.0008 nM), or about 890 fold (from 17.78 nM to 0.02 nM), upon co-treatment of Carvedilol with AMD3100, Ulocuplumab, or TZ14011, relative to single treatement with AMD3100, Ulocuplumab, or TZ14011, respectively. These results suggest that co-treating with a CXCR4 inhibitor and ADRB2 inhibitor more effectively inhibits increased Ca^{2+} response than single treatment with a CXCR4 inhibitor alone in CXCR4-ADRB2 heteromer expressing cells.

[455] To determine the possibility that CXCR4-ADRB2 heteromer formation induces conformation change and/or changes the binding affinity for the CXCR4 inhibitor, the IC50 values of Ca^{2+} response were compared between single treatement with a CXCR4 inhibitor of cells expressing CXCR4 alone and cells expressing CXCR4-ADRB2 heteromer. The results showed that single treatement with a CXCR4 inhibitor altered the IC50 value only by about 0.4-5 fold in cells expressing CXCR4-ADRB2 heteromer (4th column), relative to cells expressing CXCR4 alone (2nd column). These results suggest that co-treatment with CXCR4 inhibitor and ADRB2 inhibitor can increase, to a dramatic extent for certain CXCR4 inhibitors, the therapeutic efficacy towards CXCR4-ADRB2 heteromer expressing patients and/or patient cells/tissues, compared with single treatment with CXCR4 inhibitor.

[456]

[457] **Example 14. Effect of CXCR4 -ADRB2 heteromer on Tumor Growth.**

[458] To investigate the effect of CXCR4-ADRB2 heteromer on tumor growth, cell lines stably overexpressing CXCR4 alone or CXCR4-ADRB2 heteromer were prepared in A549 lung cancer cell, and the same amount of cells ($1x10^7$ cell / head) were injected subcutaneously in nude mice to compare tumor growth rates. As shown in FIGS. 22a-22b, the tumor size at 28 days after transplantation was 351.4 ± 214.7 mm^3 for A549, 726.9 ± 259.6 mm^3 for A549-CXCR4, and 1012.2 ± 556.1 mm^3 for A549-CXCR4-ADRB2. The tumor growth rate of mice transplanted with the A549-CXCR4 overexpressing CXCR4 was faster than that of mice bearing only parental A549, and the fastest tumor growth was observed in mice transplanted with cells overexpressing the CXCR4-ADRB2 heteromer. These results suggest that the formation of CXCR4-ADRB2 heteromer synergistically increases the Ca^{2+} response and thus promotes tumor growth.

[459] Images of three mice which were transplanted with either parental cell A549, A549-CXCR4 stably overexpressing CXCR4, or A549-CXCR4-ADRB2 stably over-

expressing CXCR4-ADRB2 heteromer, at 28 days after transplantation, are shown in FIG. 22a. These images show that the tumor size of the mouse bearing the cell expressing CXCR4-ADRB2 heteromer is the largest (accelerated the most) among them. The tumor growth rates of these three different mice over time are graphically illustrated in FIG. 22b. Tumor growth was monitored every third or fourth day by measuring the length (L) and width (W) of the tumor and calculating tumor volume on the basis of the following formula: Volume = 0.5 LW^2. The mice bearing CXCR4-expressing cell show relatively fast tumor growth as compared to the mice bearing parental cell A549, and the tumor growth is the fastest in mice transplanted with cells overexpressing the CXCR4-ADRB2 heteromer.

[460]

[461] **Example 15. Evaluation by Ca2+ Mobilization Assay of Enhanced CXCR4 Downstream Signaling Upon Co-stimulation of CXCR4-GPCRx heteromer with Endogenous Ligands.**

[462] The enhancement of the calcium response in MDA-MB-231 cells co-expressing CXCR4 and GPCRx upon co-stimulation with CXCL12 and endogenous ligand for GPCRx, are shown in FIGS. 23a-23g. MDA-MB-231 cells were transduced with adenoviruses encoding CXCR4 and HA-VC, GPCRx and HA-VC, or CXCR4 and GPCRx where GPCRx represents ADCYAP1R1 (FIG. 23a), ADORA2B (FIG. 23b), ADORA3 (FIG. 23c), CHRM1 (FIG. 23d), EDNRB (FIG. 23e), MLNR (FIG. 23f), and TACR3 (FIG. 23g). Cells were treated with CXCL12 alone, GPCRx endogenous ligand alone, or CXCL12 and GPCRx ligand together. Calcium mobilization was calculated as described in FIG. 5d. Statistically significant difference between the sum (square with dots) and co-treatment (filled square) was determined by Student's *t* test. *P < 0.05; **P < 0.01; ***P < 0.001; Mean ± SEM (n = 3).

[463] In FIG. 6a, enhanced Ca^{2+} signaling was observed in cells expressing CXCR4 and ADCYAP1R1 together, but not in cells expressing individual GPCR alone, upon co-treatment with CXCL12 and VIP, a non-selective endogenous ADCYAP1R1 ligand. When the cells were co-treated with CXCL12 and PACAP-38, an ADCYAP1R1-selective endogenous ligand, enhanced Ca^{2+} signaling was also observed (FIG. 23a). The result demonstrates that enhanced signaling of CXCR4-ADCYAP1R1 heteromer upon co-stimulation with CXCL12 is not only limited to VIP, but also selective ADCYAP1R1 natural ligand. It further suggests this enhancement could happen *in vivo* in cells expressing CXCR4 and ADCYAP1R1 together by their natural ligands.

[464] Enhanced Ca^{2+} signaling was observed in cells expressing CXCR4 and ADORA2B (FIG. 6b) or CXCR4 and ADORA3 together (FIG. 6c), but not in cells expressing individual GPCR alone, upon co-treatment with CXCL12 and BAY 60-6583

(ADORA2B-selective agonist), or CXCL12 and Cl-IB-MECA (ADORA3-selective agonist), respectively. When these cells were co-treated with CXCL12 and adenosine, endogenous ligand for all adenosine receptors, enhanced Ca^{2+} signaling was also observed (FIGS. 23b and 23c). The result demonstrates that natural endogenous ligand, not only synthetic agonists, enhances downstream signaling of CXCR4-ADORA2B and CXCR4-ADORA3 heteromers upon co-stimulation with CXCL12. It further suggests that synergistic enhancement of downstream signaling could happen *in vivo* in cells expressing CXCR4 and ADORA2B or CXCR4 and ADORA3 together by their natural ligands.

[465] In FIG. 6f, enhanced Ca^{2+} signaling was observed in cells expressing CXCR4 and CHRM1 together, but not in cells expressing individual GPCR alone, upon co-treatment with CXCL12 and bethanechol, a synthetic CHRM1 agonist. When the cells were co-treated with CXCL12 and acetylcholine, endogenous ligand for all acetylcholine receptors, enhanced Ca^{2+} signaling was also observed (FIG. 23d). The result demonstrates that natural endogenous ligand, not only synthetic agonist, enhances signaling of CXCR4-CHRM1 heteromer upon co-stimulation with CXCL12. It further suggests that synergistic enhancement of downstream signaling could happen *in vivo* in cells expressing CXCR4 and CHRM1 together by their natural ligands.

[466] In FIG. 6g, enhanced Ca^{2+} signaling was observed in cells expressing CXCR4 and EDNRB together, but not in cells expressing individual GPCR alone, upon co-treatment with CXCL12 and BQ3020, an EDNRB-selective agonist. When the cells were co-treated with CXCL12 and endothelin-1, endogenous ligand for endothelin receptors, enhanced Ca^{2+} signaling was also observed (FIG. 23e). The result demonstrates that natural endogenous ligand, not only synthetic agonist, enhances signaling of CXCR4-EDNRB heteromer upon co-stimulation with CXCL12. It further suggests that synergistic enhancement of downstream signaling could happen *in vivo* in cells expressing CXCR4 and EDNRB together by their natural ligands.

[467] In FIG. 6i, enhanced Ca^{2+} signaling was observed in cells expressing CXCR4 and MLNR together, but not in cells expressing individual GPCR alone, upon co-treatment with CXCL12 and roxithromycin, an antibiotic as well as full MLNR agonist. When the cells were co-treated with CXCL12 and motilin, a selective endogenous ligand for MLNR, enhanced Ca^{2+} signaling was also observed (FIG. 23f). The result demonstrates that natural endogenous ligand, not only synthetic agonist, enhances signaling of CXCR4-MLNR heteromer upon co-stimulation with CXCL12. It further suggests that synergistic enhancement of downstream signaling could happen *in vivo* in cells expressing CXCR4 and MLNR together by their natural ligands.

[468] In FIG. 6l, enhanced Ca^{2+} signaling was observed in cells expressing CXCR4 and TACR3 together, but not in cells expressing individual GPCR alone, upon co-

treatment with CXCL12 and senktide, a TACR3-selective agonist. When the cells were co-treated with CXCL12 and neurokinin B, selective endogenous ligand for TACR3, enhanced Ca^{2+} signaling was also observed (FIG. 23g). The result demonstrates that natural endogenous ligand, not only synthetic agonist, enhances signaling of CXCR4-TACR3 heteromer upon co-stimulation with CXCL12. It further suggests that synergistic enhancement of downstream signaling could happen *in vivo* in cells expressing CXCR4 and EDNRB together by their natural ligands.

[469]

[470] **Example 16. Evaluation by Ca2+ Mobilization Assay of Enhanced CXCR4 Downstream Signaling Upon Co-stimulation of CXCR4-GPCRx heteromer.**

[471] In MDA-MB-231 cells co-expressing CXCR4 and ADCYAP1R1, stimulation with selective endogenous ligands CXCL12 alone or PACAP38 alone, respectively, produced calcium signaling dose-dependently (FIGS. 24a-24b). MDA-MB-231 cells were transduced with adenoviruses encoding CXCR4 and ADCYAP1R1. As shown in FIG. 24a, addition of small amount of ADCYAP1R1 selective endogenous ligand (PACAP38; 1 nM) significantly enhanced calcium response in a broad range of CXCL12 concentrations compared with the sum values calculated by adding the responses obtained by PACAP38 alone and CXCL12 alone at indicated doses (FIG. 24a). Maximal Ca^{2+} response evoked by CXCL12 alone was taken as 100%. Sum represents the calculated additive value of the responses evoked by 1 nM of PACAP38 and CXCL12 alone at indicated doses. Similarly, as shown in FIG. 24b, addition of CXCR4 selective endogenous ligand (CXCL12; 15 nM) significantly enhanced downstream response in a broad range of PACAP38 concentrations compared with the sum values calculated by adding the responses obtained by CXCL12 alone and PACAP38 alone at indicated doses, even at concentrations that did not evoke any response when treated alone (0.03 ~ 0.3 nM) (FIG. 24b). Maximal Ca^{2+} response evoked by PACAP38 alone was taken as 100%. Sum represents the calculated additive value of the responses evoked by 15 nM of CXCL12 alone and PACAP38 alone at indicated doses. Statistically significant differences between the sum and co-treatment at each point were determined by Student's *t* test. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; Mean ± SD (n = 3). These results suggest an enhanced response in cells co-expressing CXCR4 and ADCYAP1R1 *in vivo* in the presence of small amounts of CXCL12 and ADCYAP1R1 ligands together.

[472] The enhancement of calcium response in a broad range of ligand concentrations in cells co-expressing CXCR4 and TACR3 is shown in FIGS. 25a-25b. MDA-MB-231 cells were transduced with adenoviruses encoding CXCR4 and TACR3. Stimulation of the cells with CXCL12 alone or Neurokinin B alone, respectively, produced calcium signaling dose-dependently (FIGS. 25a-25b). As shown in FIG. 25a, addition of small

amount of Neurokinin B (0.4 nM, TACR3-selective endogenous ligand) significantly enhanced calcium response in a broad range of CXCL12 concentrations compared with the sum values calculated by adding the responses obtained by Neurokinin B alone and CXCL12 alone at indicated doses. Maximal calcium response evoked by CXCL12 alone was taken as 100%. Sum represents the calculated additive value of the responses evoked by 0.4 nM of Neurokinin B alone and CXCL12 alone at indicated doses. Similarly, as shown in FIG. 25b, addition of CXCL12 significantly enhanced downstream response in a broad range of Neurokinin B concentrations compared with the sum values calculated by adding the responses obtained by CXCL12 alone and Neurokinin B alone at indicated doses. Maximal response evoked by Neurokinin B alone was taken as 100%. Sum represents the calculated additive value of the responses evoked by 30 nM of CXCL12 alone and Neurokinin B alone at indicated doses. Statistically significant differences between the sum and co-treatment at each point were determined by Student's *t* test. *P < 0.05; **P < 0.01; Mean ± SD (n = 3). These results suggest an enhanced response in cells co-expressing CXCR4 and TACR3 *in vivo* in the presence of small amounts of CXCL12 and Neurokinin B together.

[473]

[474] **Example 17. Confirmation of a Loss of Heteromer-Specific Property Upon Deletion of One of the Protomers.**

[475] In FIG. 8f & 8g, enhanced calcium signaling was observed in MDA-MB-231 cells overexpressing CXCR4 alone upon co-treatment with CXCL12 and histamine. Using RT-qPCR, MDA-MB-231 cells were previsouly shown to express HRH1 as well as low level of CXCR4 mRNAs, with about twice more HRH1 mRNA being expressed compared with CXCR4 mRNA (Example 9). To confirm if the enhanced calcium signaling shown in FIG. 8f & 8g is due to the presence of endogenous HRH1 expression, and not to the presence of other histamine receptors, HRH1 gene in MDA $^{CXCR4+}$ cells was deleted using CRISPR/Cas9 system. MDA-MB-231 cells stably expressing CXCR4 (MDA^{CXCR4+}) were transduced with lentiviruses encoding Cas9 and guide RNA targeting HRH1. The presence of functional HRH1 was detected by measuring calcium responses upon exposure to histamine. While MDA^{CXCR4+} cells showed dose-dependent increase in calcium signaling upon exposure to histamine, MDA$^{CXCR4+, HRH1-}$ cells did not show any calcium response even at 1 μM histamine (FIG. 26a). The result implies almost complete absence of functional HRH1 in MDA $^{CXCR4+, HRH1-}$ cells.

[476] When MDA-MB-231 cells stably overexpressing CXCR4 (MDA^{CXCR4+} cells) were treated with histamine, dose-dependent increase in calcium signaling was observed (FIG. 27a). When the cells were treated with histamine in the presence of CXCL12 (50 nM), significantly enhanced calcium response was obtained in a broad range of

histamine concentrations, resulting in increased potency and efficacy as evidenced by the changes in EC_{50} and E_{max} values. However, in MDA^{CXCR4+} cells depleted with HRH1 using CRISPR/Cas9 system ($MDA^{CXCR4+, HRH1-}$ cells), no histamine-mediated response was obtained in the absence or presence of CXCL12 (50 nM), reconfirming the absence of functional HRH1 in these cells. Maximal calcium response evoked by histamine alone in MDA^{CXCR4+} cells was taken as 100%. Sum represents the calculated additive value of the responses evoked by 50 nM of CXCL12 alone and histamine alone at indicated doses.

[477] In MDA^{CXCR4+} cells, stimulation with CXCL12 produced calcium response dose-dependently (FIG. 27b). Addition of non-signaling concentration of histamine (15 nM) significantly enhanced calcium response in a broad range of CXCL12 concentrations, resulting in greatly increased potency and efficacy as evidenced by the changes in EC_{50} and E_{max} values. CXCL12-mediated calcium response in $MDA^{CXCR4+, HRH1-}$ cells was similar to the response observed in MDA^{CXCR4+} cells. However, addition of histamine failed to increase CXCL12-mediated calcium response in $MDA^{CXCR4+, HRH1-}$ cells, demonstrating the loss of heteromer-specific property upon deletion of HRH1. Maximal response evoked by CXCL12 alone in MDA^{CXCR4+} cells was taken as 100%. Sum represents the calculated additive value of the responses evoked by 15 nM of histamine alone and CXCL12 alone at indicated doses. Statistically significant differences between the sum and co-treatment at each point were determined by Student's *t* test. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; Mean ± SD (n = 3). EC_{50} and E_{max} values were calculated using GraphPad Prism software.

[478] In FIGS. 18a-18b, significant enhancement in calcium signaling was also observed in wildtype MDA-MB-231 cells in the presence of CXCL12 and histamine together although individual ligand alone only produced faint signals. To confirm if the enhanced response is due to the endogenous expression of CXCR4, CXCR4 gene was targeted using CRISPR/Cas9 system, and expression of CXCR4 was detected using immunoblotting. Expression of CXCR4 was significantly decreased in MDA-MB-231 cells treated with guide RNA targeting CXCR4 compared to the expression in cells treated with control non-targeting guide RNA (FIG. 26b).

[479] Enhanced calcium response in MDA-MB-231 cells upon co-treatment with CXCL12 and histamine is abrogated in the absence of CXCR4. As shown in FIG. 28a, in MDA-MB-231 cells (MDA-MB-231 cells (MDA^{WT}), but not in MDA^{WT} cells depleted with CXCR4 using CRISPR/Cas9 system (MDA^{CXCR4-})), enhanced signaling in the presence of small amount of histamine (15 nM) was evident in a broad range of CXCL12 concentrations although CXCL12-mediated dose-dependent increase in calcium response was not observed due to the low level expression of CXCR. Maximal calcium response evoked by CXCL12 alone in MDA^{WT} cells was taken as 100%. Sum represents the

calculated additive value of the responses evoked by 15 nM of histamine alone and CXCL12 alone at indicated doses. Deletion of CXCR4 completely abrogate the enhanced signaling in the presence of histamine, demonstrating loss of heteromer-specific property upon deletion of CXCR4.

[480] Similarly, as shown in FIG. 28b, addition of CXCL12 enhanced histamine-mediated response in a broad range of histamine concentrations in MDA-MB-231 cells compared with the sum values calculated by adding the responses obtained by CXCL12 alone and histamine alone at indicated doses. The enhanced signaling shown in MDA-MB-231 cells was not observed in MDA^{CXCR4-} cells, although MDA^{CXCR4-} cells retained histamine responses. Maximal response evoked by histamine alone in MDA^{WT} cells was taken as 100%. Sum represents the calculated additive value of the responses evoked by 100 nM of CXCL12 alone and histamine alone at indicated doses. Statistically significant differences between the sum and co-treatment at each point were determined by Student's *t* test. **P < 0.01; ***P < 0.001; Mean ± SD (n = 3). EC_{50} and E_{max} values were calculated using GraphPad Prism software. Taken together, these results demonstrate that CXCR4-HRH1 heteromer is responsible for the enhanced signaling upon co-treatment with CXCL12 and histamine in wildtype MDA-MB-231 cells.

[481]

[482] **Example 18. Ca2+ Mobilization Inhibition of the Enhanced CXCR4 Downstream Signaling upon CXCR4-GPCRx heteromer Formation - Comparison of Single Inhibitor Treatment to Combination Inhibitor Treatment.**

[483] Overview: As illustrated below (see Tables 7-14), co-treatment by various combinations of a CXCR4 inhibitor and a GPCRx inhibitor in a series of CXCR4-GPCRx heteromer-expressing cells results in a significantly reduced calcium response compared to single treatment by a CXCR4 inhibitor alone. The GPCRx protomers of the series of CXCR4-GPCRx heteromers evaluated in Tables 7-14 are: ADRB2, HRH1, ADCYAP1R1, C5AR1, CALCR, EDNRB, MLNR, and TACR3, respectively. MDA-MB-231 cells were transduced with adenoviruses encoding CXCR4 only, or CXCR4 and the specified GPCRx. The cells were cultured for 2 days and were treated CXCR4 antagonist alone or co-treatment of CXCR4 antagonist and the specified GPCRx antagonist. And then, cells were stained with Cal-6 for 2 hours and stimulated with CXCL12 (20 nM) and the specified GPCRx agonist. Calcium mobilization was measured using FlexStation3. A value in the column labeled "None" is the IC50 of Ca2+ response in the specified CXCR4-GPCRx heteromer-expressing MDA-MB-231 treated with CXCR4 inhibitor only (in the absence of a specified GPCRx antagonist). A value in the remaining columns is the IC50 of Ca2+ response in the specified CXCR4-GPCRx heteromer-expressing MDA-MB-231 from the simultaneous

treatment with CXCR4 antagonist and the specified GPCRx antagonist.

[484]

[485] The efficiency of CXCR4 inhibitors in suppressing the enhanced signaling of CXCR4-ADRB2 heteromer was measured in the absence or presence of the representative ADRB2 inhibitors Carazolol or Propranolol (**Table 7**). MDA-MB-231 cells were co-transduced with adenoviruses encoding CXCR4 and ADRB2, and Ca2+ signaling was measured upon co-stimulating the cells with CXCL12 (20 nM) and Salmeterol (1 μM). Inhibitors were treated 30 min before agonist stimulation. Ca2+ mobilization was measured as described in **Fig. 5**, and IC_{50} values were calculated using GraphPad Prism software.

[486]

[487] [Table 7]

CXCR4 inhibitors	ADRB2 inhibitors		
	None	Carazolol (10 μM)	Propranolol (10 μM)
	IC50 [nM]	IC50 [nM]	IC50 [nM]
AMD3100	22.10±8.53	2.69±3.41	6.23±4.90
Ulocuplumab	0.226±0.144	0.009±0.004	0.041±0.024
BKT140	123.86±76.91	26.66±19.32	66.14±53.77

[488]

[489] CXCR4 inhibitors AMD-3100, Ulocuplumab and BKT140 suppressed the CXCR4-ADRB2 signaling more efficiently (greater potency) in the presence of 10 μM of Carazolol or Propranolol as shown with the reduced IC_{50} values compared to the IC_{50} values of CXCR4 inhibitors in the absence of ADRB2 inhibitors. Depending on the identity of the CXCR4 inhibitor, the IC50 value of Ca response in the CXCR4-ADRB2 heteromer context decreased by up to about 25 times or more when treated in combination with an ADRB2 inhibitor (Carazolol or Propranolol), relative to single treatment with the CXCR4 inhibitor alone ("None" column). These results suggest that co-treating with a CXCR4 inhibitor and ADRB2 inhibitor more effectively inhibits increased Ca response than single treatment with a CXCR4 inhibitor alone in CXCR4-ADRB2 heteromer expressing cells.

[490] The efficiency of CXCR4 inhibitors in suppressing the enhanced signaling of CXCR4-HRH1 heteromer was measured in the absence or presence of the representative HRH1 inhibitors Hydroxyzine, Prometazine, or Cyproheptadine (**Table 8**). MDA-MB-231 cells were co-transduced with adenoviruses encoding CXCR4 and HRH1, and Ca2+ signaling was measured upon co-stimulating the cells with CXCL12 (20 nM) and Histamine (1 nM). Inhibitors were treated 30 min before agonist stimulation. Ca2+ mobilization was measured as described in **Fig. 5**, and IC_{50} values were calculated using GraphPad Prism software.

[491]

[492] [Table 8]

CXCR4 inhibitors	HRH1 inhibitors			
	None	Hydroxyzine (10 μM)	Promethazine (10 μM)	Cyproheptadine (10 μM)
	IC50 [nM]	IC50 [nM]	IC50 [nM]	IC50 [nM]
AMD3100	39.07±14.5	1.58±1.14	2.59±2.06	0.75±0.49
Ulocuplumab	0.5±0.44	0.003±0.003	0.000098±0.0001	0.003±0.001
BKT140	232.3±91.95	53.75±21.24	1.66±1.13	9.71±7.17

[493]

[494] CXCR4 inhibitors AMD-3100, Ulocuplumab and BKT140 suppressed the CXCR4-HRH1 signaling more efficiently (greater potency) in the presence of 10 μM of Hydroxyzine, Prometazine, or Cyproheptadine as shown with the reduced IC_{50} values compared to the IC_{50} values of CXCR4 inhibitors in the absence of HRH1 inhibitors. Depending on the identity of the CXCR4 inhibitor, the IC50 value of Ca response in the CXCR4-HRH1 heteromer context decreased by up to about 5,100 times or more when treated in combination with an HRH1 inhibitor (Hydroxyzine, Promethazine, or Cyproheptadine), relative to single treatment with the CXCR4 inhibitor alone ("None" column). These results suggest that co-treating with a CXCR4 inhibitor and HRH1 inhibitor more effectively inhibits increased Ca response than single treatment with a CXCR4 inhibitor alone in CXCR4-HRH1 heteromer expressing cells.

[495] The efficiency of CXCR4 inhibitors in suppressing the enhanced signaling of CXCR4-ADCYAP1R1 heteromer was measured in the absence or presence of the representative ADCYAP1R1 inhibitors M65 or PACAP-(6-38) (**Table 9**). MDA-MB-231 cells were co-transduced with adenoviruses encoding CXCR4 and ADCYAP1R1, and Ca2+ signaling was measured upon co-stimulating the cells with CXCL12 (20 nM) and PACAP-38 (1 nM). Inhibitors were treated 30 min before agonist stimulation. Ca2+ mobilization was measured as described in **Fig. 5**, and IC_{50} values were calculated using GraphPad Prism software.

[496]

[497] [Table 9]

CXCR4 inhibitors	ADCYAP1R1 inhibitors		
	None	M65 (1 μM)	PACAP-(6-38) (1 μM)
	IC50 [nM]	IC50 [nM]	IC50 [nM]
AMD3100	546.8±337.8	20.21±12.83	2.42±0.09
BKT140	213.08±142.72	7.46±1.93	19.56±3.1

[498]

[499] CXCR4 inhibitors AMD-3100 and BKT140 suppressed the CXCR4-ADCYAP1R1

signaling more efficiently (greater potency) in the presence of 1 μM of M65 or 1 μM of PACAP-(6-38) as shown with the reduced IC_{50} values compared to the IC_{50} values of CXCR4 inhibitors in the absence of ADCYAP1R1 inhibitors. Depending on the identity of the CXCR4 inhibitor, the IC50 value of Ca response in the CXCR4-ADCYAP1R1 heteromer context decreased by up to about 225 times or more when treated in combination with an ADCYAP1R1 inhibitor (M65 or PACAP-(6-38)), relative to single treatment with the CXCR4 inhibitor alone ("None" column). These results suggest that co-treating with a CXCR4 inhibitor and ADCYAP1R1 inhibitor more effectively inhibits increased Ca response than single treatment with a CXCR4 inhibitor alone in CXCR4-ADCYAP1R1 heteromer expressing cells.

[500] The efficiency of CXCR4 inhibitors in suppressing the enhanced signaling of CXCR4-C5AR1 heteromer was measured in the absence or presence of the representative C5AR1 inhibitor W54011 (**Table 10**). MDA-MB-231 cells were co-transduced with adenoviruses encoding CXCR4 and C5AR1, and Ca2+ signaling was measured upon co-stimulating the cells with CXCL12 (20 nM) and C5a (0.03 nM). Inhibitors were treated 30 min before agonist stimulation. Ca2+ mobilization was measured as described in **Fig. 5**, and IC_{50} values were calculated using GraphPad Prism software.

[501]

[502] [Table 10]

CXCR4 inhibitors	C5AR1 inhibitors	
	None	W54011 (10 μM)
	IC50 [nM]	IC50 [nM]
AMD3100	769.34±240.77	434.00±82.94
Ulocuplumab	3.80±0.22	1.06±0.25
BKT140	118.01±56.52	9.72±6.87

[503]

[504] CXCR4 inhibitors AMD-3100, BKT140, and Ulocuplumab suppressed the CXCR4-C5AR1 signaling more efficiently (greater potency) in the presence of 10 μM of W54011, as shown with the reduced IC_{50} values compared to the IC_{50} values of CXCR4 inhibitors in the absence of C5AR1 inhibitors. Depending on the identity of the CXCR4 inhibitor, the IC50 value of Ca response in the CXCR4-C5AR1 heteromer context decreased by up to about 12 times or more when treated in combination with an C5AR1 inhibitor (W54011), relative to single treatment with the CXCR4 inhibitor alone ("None" column). These results suggest that co-treating with a CXCR4 inhibitor and C5AR1 inhibitor more effectively inhibits increased Ca response than single treatment with a CXCR4 inhibitor alone in CXCR4-C5AR1 heteromer expressing

cells.

[505] The efficiency of CXCR4 inhibitors in suppressing the enhanced signaling of CXCR4-CALCR heteromer was measured in the absence or presence of the representative CALCR inhibitor CT-(8-32) (**Table 11**). MDA-MB-231 cells were co-transduced with adenoviruses encoding CXCR4 and CALCR, and Ca2+ signaling was measured upon co-stimulating the cells with CXCL12 (20 nM) and calcitonin (100 nM). Inhibitors were treated 30 min before agonist stimulation. Ca2+ mobilization was measured as described in **Fig. 5**, and IC_{50} values were calculated using GraphPad Prism software.

[506]

[507] [Table 11]

CXCR4 inhibitor	CALCR inhibitors	
	None	CT-(8-32) (salmon) (10 μM)
	IC50 [nM]	IC50 [nM]
AMD3100	2677.67±953.23	12.54±14.7
BKT140	1041.6±756.9	15.62±3.71

[508]

[509] CXCR4 inhibitors AMD-3100 and BKT140 suppressed the CXCR4-CALCR signaling more efficiently (greater potency) in the presence of 10 μM of CT-(8-32) as shown with the reduced IC_{50} values compared to the IC_{50} values of CXCR4 inhibitors in the absence of CALCR inhibitors. Depending on the identity of the CXCR4 inhibitor, the IC50 value of Ca response in the CXCR4-CALCR heteromer context decreased by up to about 210 times or more when treated in combination with an CALCR inhibitor (CT-(8-32)), relative to single treatment with the CXCR4 inhibitor alone ("None" column). These results suggest that co-treating with a CXCR4 inhibitor and CALCR inhibitor more effectively inhibits increased Ca response than single treatment with a CXCR4 inhibitor alone in CXCR4-CALCR heteromer expressing cells.

[510] The efficiency of CXCR4 inhibitors in suppressing the enhanced signaling of CXCR4-EDNRB heteromer was measured in the absence or presence of the representative EDNRB inhibitors ambrisentan or bosentan (**Table 12**). MDA-MB-231 cells were co-transduced with adenoviruses encoding CXCR4 and EDNRB, and Ca2+ signaling was measured upon co-stimulating the cells with CXCL12 (20 nM) and BQ3020 (0.5 nM). Inhibitors were treated 30 min before agonist stimulation. Ca2+ mobilization was measured as described in **Fig. 5**, and IC_{50} values were calculated using GraphPad Prism software.

[511] [Table 12]

CXCR4 inhibitor	EDNRB inhibitors		
	None	Ambrisentan (10 μM)	Bosentan (10 μM)
	IC50 [nM]	IC50 [nM]	IC50 [nM]
AMD3100	926.10±346.23	287.13±88.75	169.12±147.30
Ulocuplumab	14.11±2.31	2.24±0.18	4.68±2.81
BKT140	2680.5±3927.44	529.37±122.26	8.50±7.37

[512]

[513] CXCR4 inhibitors AMD-3100, BKT140, and Ulocuplumab suppressed the CXCR4-EDNRB signaling more efficiently (greater potency) in the presence of 10 μM of ambrisentan or 10 μM of bosentan, as shown with the reduced IC_{50} values compared to the IC_{50} values of CXCR4 inhibitors in the absence of EDNRB inhibitors. Depending on the identity of the CXCR4 inhibitor, the IC50 value of Ca response in the CXCR4-EDNRB heteromer context decreased by up to about 315 times or more when treated in combination with an EDNRB inhibitor (ambrisentan or bosentan), relative to single treatment with the CXCR4 inhibitor alone ("None" column). These results suggest that co-treating with a CXCR4 inhibitor and EDNRB inhibitor more effectively inhibits increased Ca response than single treatment with a CXCR4 inhibitor alone in CXCR4-EDNRB heteromer expressing cells.

[514] The efficiency of CXCR4 inhibitors in suppressing the enhanced signaling of CXCR4-MLNR heteromer was measured in the absence or presence of the representative MLNR inhibitor MA-2029 (**Table 13**). MDA-MB-231 cells were co-transduced with adenoviruses encoding CXCR4 and MLNR, and Ca2+ signaling was measured upon co-stimulating the cells with CXCL12 (20 nM) and motilin (0.2 nM). Inhibitors were treated 30 min before agonist stimulation. Ca2+ mobilization was measured as described in **Fig. 5**, and IC_{50} values were calculated using GraphPad Prism software.

[515] [Table 13]

CXCR4 inhibitor	MLNR inhibitors	
	None	MA-2029 (10uM)
	IC50 [nM]	IC50 [nM]
AMD3100	946.1±171.4	199.23±64.34
Ulocuplumab	25.51±16.48	2.25±1.30
BKT140	643.6±162.9	93.39±155.20

[516]

[517] CXCR4 inhibitors AMD-3100, BKT140, and Ulocuplumab suppressed the CXCR4-MLNR signaling more efficiently (greater potency) in the presence of 10 μM

of MA-2029 as shown with the reduced IC_{50} values compared to the IC_{50} values of CXCR4 inhibitors in the absence of MLNR inhibitor. Depending on the identity of the CXCR4 inhibitor, the IC50 value of Ca response in the CXCR4-MLNR heteromer context decreased by up to about 11 times or more when treated in combination with an MLNR inhibitor (MA-2029), relative to single treatment with the CXCR4 inhibitor alone ("None" column). These results suggest that co-treating with a CXCR4 inhibitor and MLNR inhibitor more effectively inhibits increased Ca response than single treatment with a CXCR4 inhibitor alone in CXCR4-MLNR heteromer expressing cells.

[518] The efficiency of CXCR4 inhibitors in suppressing the enhanced signaling of CXCR4-TACR3 heteromer was measured in the absence or presence of the representative TACR3 inhibitors SB 222200, osanetant, or talnetant (**Table 14**). MDA-MB-231 cells were co-transduced with adenoviruses encoding CXCR4 and TACR3, and Ca2+ signaling was measured upon co-stimulating the cells with CXCL12 (20 nM) and neurokinin B (0.3 nM). Inhibitors were treated 30 min before agonist stimulation. Ca2+ mobilization was measured as described in **Fig. 5**, and IC_{50} values were calculated using GraphPad Prism software.

[519] [Table 14]

CXCR4 inhibitor	TACR3 inhibitors			
	None	SB-222200 (10 μM)	Osanetant (10 μM)	Talnetant (10 μM)
	IC50 [nM]	IC50 [nM]	IC50 [nM]	IC50 [nM]
AMD3100	2680.33±210.49	343.13±199.54	567.53±322.61	179.63±52.11
Ulocuplumab	50.65±23.57	3.07±1.18	4.61±4.36	3.30±0.89
BKT140	1497.67±419.98	217.90±92.86	194.33±91.07	230.30±33.86

[520]

[521] CXCR4 inhibitors AMD-3100, BKT140, and ulocuplumab suppressed the CXCR4-TACR3 signaling more efficiently (greater potency) in the presence of 10 μM of SB-222200, 10 μM of osanetant, or 10 μM of talnetant as shown with the reduced IC_{50} values compared to the IC_{50} values of CXCR4 inhibitors in the absence of TACR3 inhibitors. Depending on the identity of the CXCR4 inhibitor, the IC50 value of Ca response in the CXCR4-TACR3 heteromer context decreased by up to about 16 times or more when treated in combination with an TACR3 inhibitor (SB-222200, osanetant, or talnetant), relative to single treatment with the CXCR4 inhibitor alone ("None" column). These results suggest that co-treating with a CXCR4 inhibitor and TACR3 inhibitor more effectively inhibits increased Ca response than single treatment with a CXCR4 inhibitor alone in CXCR4-TACR3 heteromer expressing cells.

[522]

[523] **Example 19. Anti-tumor effect of CXCR4-ADRB2 heteromer inhibitors on Tumor Growth.**

[524] In Figure 22, we observed that the CXCR4 and ADRB2 overexpressing cells bearing mice showed a dramatic increase in tumor proliferation compared to the A549 parent cell bearing mice. This suggests that CXCR4-ADRB2 heteromer promotes tumor proliferation.

[525] To investigate the anti-tumor effect of CXCR4-ADRB2 heteromer inhibitors on tumor growth, the A549-CXCR4-ADRB2, stably overexpressing CXCR4- ADRB2 heteromer ($1x10^7$ cell / head) was injected subcutaneously in nude mice. When tumor sizes were reached an average of 50-100mm^3, CXCR4 inhibitor, ADRB2 inhibitor alone or combination of CXCR4 and ADRB2 inhibitors were treated.

[526] Figure 29a is a graph comparing tumor growth rates for the *in vivo* antitumor effect of CXCR4 inhibitor LY2510924 (3 mg/kg), ADRB2 inhibitor Carvedilol (30 mg/kg), or combination of LY2510924 (3 mg/kg) and Carvedilol (30 mg/kg). Figure 29b is a graph comparing tumor growth rates for the antitumor effect of CXCR4 inhibitor AMD070 (10 mg/kg), ADRB2 inhibitor Carvedilol (30 mg/kg), or combination of AMD070 (10 mg/kg) and Carvedilol (30 mg/kg). The tumor growth was monitored every third or fourth day by measuring the length (L) and width (W) of the tumor and calculating tumor volume based on the following formula: Volume = 0.5 LW^2.

[527] As shown in **FIG. 29a**, CXCR4-ADRB2 overexpressing mice were more inhibited tumor growth by the CXCR4 inhibitor, LY2510924 or the ADRB2 inhibitor, Carvedilol than the control mice. In addition, the combination of LY2510924 and Carvedilol demonstrated that the tumor growth inhibitory effect was superior to the single administration group. More specifically CXCR4-ADRB2 overexpressing A549 bearing mice treated with vehicle as a control reached an average tumor volume of 554.2±152.7mm^3 at day 21 post-treatment, as compared to LY2510924, Carvedilol or LY2510924 and Carvedilol, which reached an average tumor volume of 488.9±135.2 mm^3, and 432.0±206.4 mm^3, 356.3±125.4 mm^3 respectively, in the same time period.

[528] A similar pattern was observed in the group treated with AMD070, another inhibitor of CXCR4.

[529] **FIG. 29b** shows CXCR4-ADRB2 heteromer-overexpressing cell lines were transplanted at nude mouse and when tumor size reached 50-100 mm^3, CXCR4 inhibitor, AMD070 or ADRB2 inhibitor, Carvedilol was administered orally alone or in combination for 23 days. As shown in **FIG. 29b**, the size of the tumor on the 23 days after administration of the drug was 618.5 ± 190.9 mm^3 in the control group, 543.2 ± 260.4 mm^3 or 510.4 ± 139.9 mm^3 in the group treated with AMD070 or Carvedilol, respectively. The tumor size of drug treated group is shown to be smaller than that of control group. In addition, in the combination of AMD070 and Carvedilol, the size of the tumor was 418.0 ± 238.4 mm^3, which indicates that the antitumor effect is superior to that of the single administration group.

[530] These results suggest that co-treatment of CXCR4 and ADRB2 inhibitors may provide a better therapeutic effect for CXCR4 and ADRB2 heteromer expressing patients.

[531]

[532] All publications and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

[533] While preferred embodiments have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. It is intended that the following claims define the scope of the invention and that methods and structures within the scope of these claims and their equivalents be covered thereby.

[534]

[535] ***Exemplary Embodiments***

[536] In an embodiment, a method for treating cancer in a subject having a cell containing a CXCR4-GPCRx heteromer, the method comprising: administering to the subject a therapeutically effective amount of an inhibitor or a combination of inhibitors selected from the group consisting of: an inhibitor of CXCR4, an inhibitor of GPCRx, and an inhibitor of the CXCR4-GPCRx heteromer.

[537] In an embodiment, a method for treating cancer in a subject having CXCR4-GPCRx heteromer, the method comprising: administering to the subject a therapeutically effective amount of an inhibitor or a combination of inhibitors selected from the group consisting of: an inhibitor of CXCR4, an inhibitor of GPCRx, and an inhibitor of the CXCR4-GPCRx heteromer; wherein the cell containing the CXCR4-GPCRx heteromer has enhanced downstream signaling.

[538] In an embodiment, a method for treatment, amelioration, or prevention of a cancer in a subject having CXCR4-GPCRx heteromer, the method comprising: administering to the subject a therapeutically effective amount of an inhibitor of a CXCR4-GPCRx heteromer, wherein: GPCRx heteromerizes with CXCR4 in the subject, the heteromerization of GPCRx with CXCR4 is accompanied by enhancement of signaling downstream of CXCR4; and the enhancement of signaling downstream of CXCR4 is suppressed by the inhibitor of the CXCR4-GPCRx heteromer.

[539] In an embodiment, a method for assessing response, or potential response, of a subject having CXCR4-GPCRx heteromer to treatment, amelioration, or prevention of a cancer, the method comprising: obtaining a sample from the subject; detecting heteromerization of CXCR4 and GPCRx in the sample; and based at least in part on detection of the heteromerization of CXCR4 and GPCRx, assessing the subject's response, or potential response, to the treatment, amelioration, or prevention of a

cancer.

[540] In an embodiment, a method for treating cancer in a patient having a cell containing a CXCR4-GPCRx heteromer, the method comprising: administering to the patient an inhibitor or a combination of inhibitors selected from the group consisting of: an inhibitor of CXCR4, an inhibitor of GPCRx, and an inhibitor of the CXCR4-GPCRx heteromer; wherein:

[541] i) the CXCR4-GPCRx heteromer has enhanced downstream signaling; and

[542] ii) the administered inhibitor or combination of inhibitors suppresses the enhanced downstream signaling from said CXCR4-GPCRx heteromer in the cancer patient.

[543] In an embodiment, a method of suppressing enhanced downstream signaling from a CXCR4-GPCRx heteromer in a cell of a patient suffereing from cancer, the method comprising: administering to the patient an inhibitor or a combination of inhibitors selected from the group consisting of: an inhibitor of CXCR4, an inhibitor of GPCRx, and an inhibitor of the CXCR4-GPCRx heteromer; wherein:

[544] i) the CXCR4-GPCRx heteromer has enhanced downstream signaling; and

[545] ii) the administered inhibitor or combination of inhibitors suppresses the enhanced downstream signaling from said CXCR4-GPCRx heteromer in the cancer patient.

[546] In an embodiment, a pharmaceutical kit for use in treating cancer in a patient having a cell containing a CXCR4-GPCRx heteromer, the pharmaceutical kit comprising: an inhibitor or a combination of inhibitors selected from the group consisting of: an inhibitor of CXCR4, an inhibitor of GPCRx, and an inhibitor of the CXCR4-GPCRx heteromer; wherein the CXCR4-GPCRx heteromer has enhanced downstream signaling.

[547] In an embodiment, a pharmaceutical composition for use in treating cancer in a patient having a cell containing a CXCR4-GPCRx heteromer, the pharmaceutical composition comprising:

[548] i) an inhibitor or a combination of inhibitors selected from the group consisting of: an inhibitor of CXCR4, an inhibitor of GPCRx, and an inhibitor of the CXCR4-GPCRx heteromer; and

[549] ii) a pharmaceutically acceptable carrier;

[550] wherein the CXCR4-GPCRx heteromer has enhanced downstream signaling.

[551] In an embodiment, a method for treating cancer in a patient having a cancer cell containing a CXCR4-GPCRx heteromer, wherein the CXCR4-GPCRx heteromer has enhanced downstream signaling, the method comprising:

[552] 1) determining whether the patient has the cancer cell containing the CXCR4-GPCRx heteromer having enhanced downstream signaling by: obtaining or having obtained a biological sample from the patient; and performing or having performed an assay on the biological sample to determine if:

[553] i) the patient's cancer cell contains said CXCR4-GPCRx heteromer; or

[554] ii) a CXCR4-GPCRx heteromer-selective reagent: alters heteromer-specific properties or function of said CXCR4-GPCRx heteromer in a patient derived cell(s); alters heteromer-specific properties of a patient derived cell(s) containing the CXCR4-GPCRx heteromer; or decreases cell proliferation of a patient derived cell(s) containing the CXCR4-GPCRx heteromer; and

[555] 2) if the patient has a cancer cell containing said CXCR4-GPCRx heteromer, then internally administering an inhibitor or a combination of inhibitors selected from the group consisting of: an inhibitor of CXCR4, an inhibitor of GPCRx, and an inhibitor of the CXCR4-GPCRx heteromer to the cancer patient.

[556] In an embodiment, a method for treating cancer in a patient having a cancer cell containing a CXCR4-GPCRx heteromer, the method comprising:

[557] 1) determining whether the patient's cancer cell contains the CXCR4-GPCRx heteromer by: obtaining or having obtained a biological sample from the patient and performing or having performed an assay on the biological sample to determine if said CXCR4-GPCRx heteromer is present in the patient's cancer cell; wherein:

[558] a) the GPCRx of the CXCR4-GPCRx heteromer is selected from the group consisting of: ADCYAP1R1, ADORA2B, ADORA3, ADRB2, C5AR1, CALCR, CHRM1, EDNRB, HRH1, MLNR, NTSR1, and TACR3; and

[559] b) the assay performed on the biological sample is or comprises one or more of the following: a co-internalization assay, a colocalization assay, in situ hybridization, immunohistochemistry, immunoelectron microscopy, a proximity-based assay, a co-immunoprecipitation assay, or a fluorescent animal assay; and

[560] 2) if the patient's cancer cell contains said CXCR4-GPCRx heteromer, then internally administering an inhibitor or a combination of inhibitors selected from the group consisting of: an inhibitor of CXCR4, an inhibitor of GPCRx, and an inhibitor of the CXCR4-GPCRx heteromer to the patient.

[561] In an embodiment, a method for treating cancer in a patient having a cancer cell containing a CXCR4-GPCRx heteromer having enhanced downstream signaling, the method comprising:

[562] 1) determining whether the patient has the cancer cell containing the CXCR4-GPCRx heteromer having enhanced downstream signaling by: obtaining or having obtained a biological sample from the patient; and performing or having performed an assay on the biological sample to determine if:

[563] i) the patient's cancer cell contains said CXCR4-GPCRx heteromer; or

[564] ii) a CXCR4-GPCRx heteromer-selective reagent: alters heteromer-specific properties or function of said CXCR4-GPCRx heteromer in a patient derived cell(s); alters heteromer-specific properties of a patient derived cell(s) containing said

CXCR4-GPCRx heteromer; or decreases cell proliferation of a patient derived cell(s) containing said CXCR4-GPCRx heteromer; and

[565] 2) if the patient has a cancer cell containing said CXCR4-GPCRx heteromer, then internally administering to the cancer patient a combination of inhibitors selected from the group consisting of: an inhibitor of CXCR4, an inhibitor of GPCRx, and an inhibitor of the CXCR4-GPCRx heteromer; and

[566] 3) if the patient does not have a cancer cell containing said CXCR4-GPCRx heteromer, then internally administering to the cancer patient either the CXCR4 inhibitor or the GPCRx inhibitor as a single inhibitor.

[567] In an embodiment, a method for treating cancer in a patient having a cancer cell containing a CXCR4-GPCRx heteromer having enhanced downstream signaling, the method comprising:

[568] 1) determining whether the patient has the cancer cell containing the CXCR4-GPCRx heteromer having enhanced downstream signaling by: obtaining or having obtained a biological sample from the patient; and performing or having performed an assay on the biological sample to determine if:

[569] i) the patient's cancer cell contains said CXCR4-GPCRx heteromer; or

[570] ii) a CXCR4-GPCRx heteromer-selective reagent: alters heteromer-specific properties or function of said CXCR4-GPCRx heteromer in a patient derived cell(s); alters heteromer-specific properties of a patient derived cell(s) containing said CXCR4-GPCRx heteromer; or decreases cell proliferation of a patient derived cell(s) containing said CXCR4-GPCRx heteromer; and

[571] 2) if the patient has a cancer cell containing said CXCR4-GPCRx heteromer, then internally administering to the cancer patient a combination of inhibitors selected from the group consisting of: an inhibitor of CXCR4, an inhibitor of GPCRx, and an inhibitor of the CXCR4-GPCRx heteromer; and

[572] 3) if the patient does not have a cancer cell containing said CXCR4-GPCRx heteromer, then internally administering to the cancer patient either the CXCR4 inhibitor or the GPCRx inhibitor as a single inhibitor;

[573] wherein:

[574] a) progression of the cancer in the patient having said cancer cell containing the CXCR4-GPCRx heteromer is decreased in the range of 5-100% more upon administration of the combination of inhibitors, relative to administering the CXCR4 inhibitor or GPCRx inhibitor as the single inhibitor to said patient;

[575] b) efficacy of a CXCR4 inhibitor is increased in the range of 5-2000% when administered in combination with the GPCRx inhibitor to the patient having said cancer cell containing the CXCR4-GPCRx heteromer, relative to efficacy of the CXCR4 inhibitor when administered as a single inhibitor; and/or

[576] c) efficacy of a GPCRx inhibitor is increased in the range of 5-2000% when administered in combination with the CXCR4 inhibitor to the patient having said cancer cell containing the CXCR4-GPCRx heteromer, relative to efficacy of the GPCRx inhibitor when administered as a single inhibitor.

[577] In an embodiment, a method for treating cancer in a patient having a cancer cell containing a CXCR4-GPCRx heteromer, the method comprising:

[578] 1) determining whether the patient's cancer cell contains the CXCR4-GPCRx heteromer by: obtaining or having obtained a biological sample from the patient and performing or having performed an assay on the biological sample to determine if said CXCR4-GPCRx heteromer is present in the patient's cancer cell; wherein:

[579] a) the GPCRx of the CXCR4-GPCRx heteromer is selected from the group consisting of: ADCYAP1R1, ADORA2B, ADORA3, ADRB2, C5AR1, CALCR, CHRM1, EDNRB, HRH1, MLNR, NTSR1, and TACR3; and

[580] b) the assay performed on the biological sample is or comprises one or more of the following: a co-internalization assay, a colocalization assay, in situ hybridization, immunohistochemistry, immunoelectron microscopy, a proximity-based assay, a co-immunoprecipitation assay, enzyme-linked immunosorbent assay (ELISA), flow cytometry, RNAseq, qRT-PCR, microarray, or a fluorescent animal assay; and

[581] 2) if the patient's cancer cell contains said CXCR4-GPCRx heteromer, then internally administering to the cancer patient a combination of inhibitors selected from the group consisting of: an inhibitor of CXCR4, an inhibitor of GPCRx, and an inhibitor of the CXCR4-GPCRx heteromer; and

[582] 3) if the patient's cancer cell does not contain said CXCR4-GPCRx heteromer, then internally administering to the cancer patient either the CXCR4 inhibitor or the GPCRx inhibitor as a single inhibitor.

[583] In an embodiment, a method for treating cancer in a patient having a cancer cell containing a CXCR4-GPCRx heteromer, the method comprising:

[584] 1) determining whether the patient's cancer cell contains the CXCR4-GPCRx heteromer by: obtaining or having obtained a biological sample from the patient and performing or having performed an assay on the biological sample to determine if said CXCR4-GPCRx heteromer is present in the patient's cancer cell; wherein:

[585] a) the GPCRx of the CXCR4-GPCRx heteromer is selected from the group consisting of: ADCYAP1R1, ADORA2B, ADORA3, ADRB2, C5AR1, CALCR, CHRM1, EDNRB, HRH1, MLNR, NTSR1, and TACR3; and

[586] b) the assay performed on the biological sample is or comprises one or more of the following: a co-internalization assay, a colocalization assay, in situ hybridization, immunohistochemistry, immunoelectron microscopy, a proximity-based assay, a co-immunoprecipitation assay, enzyme-linked immunosorbent assay (ELISA), flow

cytometry, RNAseq, qRT-PCR, microarray, or a fluorescent animal assay; and

[587] 2) if the patient's cancer cell contains said CXCR4-GPCRx heteromer, then internally administering to the cancer patient a combination of inhibitors selected from the group consisting of: an inhibitor of CXCR4, an inhibitor of GPCRx, and an inhibitor of the CXCR4-GPCRx heteromer; and

[588] 3) if the patient's cancer cell does not contain said CXCR4-GPCRx heteromer, then internally administering to the cancer patient either the CXCR4 inhibitor or the GPCRx inhibitor as a single inhibitor;

[589] wherein:

[590] a) progression of the cancer in the patient having said cancer cell containing the CXCR4-GPCRx heteromer is decreased in the range of 5-100% more upon administration of the combination of inhibitors, relative to administering the CXCR4 inhibitor or GPCRx inhibitor as the single inhibitor to said patient;

[591] b) efficacy of a CXCR4 inhibitor is increased in the range of 5-2000% when administered in combination with the GPCRx inhibitor to the patient having said cancer cell containing the CXCR4-GPCRx heteromer, relative to efficacy of the CXCR4 inhibitor when administered as a single inhibitor; and/or

[592] c) efficacy of a GPCRx inhibitor is increased in the range of 5-2000% when administered in combination with the CXCR4 inhibitor to the patient having said cancer cell containing the CXCR4-GPCRx heteromer, relative to efficacy of the GPCRx inhibitor when administered as a single inhibitor.

[593] In certain embodiments, one or more than one (including for instance all) of the following further embodiments may comprise each of the other embodiments or parts thereof.

[594] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the CXCR4-GPCRx heteromer has, causes, or produces, the enhanced downstream signaling.

[595] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the enhanced downstream signaling results from the CXCR4-GPCRx heteromer.

[596] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the enhanced downstream signaling results from agonism of the CXCR4-GPCRx heteromer.

[597] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and

any one or more of the further embodiments herein, wherein the enhanced downstream signaling results from CXCR4 agonism of the CXCR4-GPCRx heteromer.

[598] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the enhanced downstream signaling results from GPCRx agonism of the CXCR4-GPCRx heteromer.

[599] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the enhanced downstream signaling results from CXCR4 agonism and GPCRx agonism of the CXCR4-GPCRx heteromer.

[600] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the enhanced downstream signaling is downstream of the CXCR4, the respective GPCRx, or the CXCR4-GPCRx heteromer.

[601] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the enhanced downstream signaling is downstream of the CXCR4.

[602] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the enhanced downstream signaling is downstream of the respective GPCRx.

[603] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the enhanced downstream signaling is downstream of the CXCR4-GPCRx heteromer.

[604] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the enhanced downstream signaling from the CXCR4-GPCRx heteromer is relative to downstream signaling from a CXCR4 protomer or a respective GPCRx protomer in their respective individual protomer context.

[605] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the enhanced downstream signaling from the CXCR4-GPCRx heteromer is relative to downstream signaling from

a CXCR4 protomer in an individual protomer context.

[606] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the enhanced downstream signaling from the CXCR4-GPCRx heteromer is relative to downstream signaling from a respective GPCRx protomer in an individual protomer context.

[607] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the enhanced downstream signaling from the CXCR4-GPCRx heteromer is relative to downstream signaling from a CXCR4 protomer and a respective GPCRx protomer in their respective individual protomer context.

[608] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the inhibitor or combination of inhibitors suppresses the enhanced downstream signaling from said CXCR4-GPCRx heteromer in the cancer patient.

[609] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the administered inhibitor or combination of inhibitors suppresses the enhanced downstream signaling from said CXCR4-GPCRx heteromer in the patient's cancer cells.

[610] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the method further comprises determining or diagnosing the presence of a CXCR4-GPCRx heteromer.

[611] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the method further comprises determining or diagnosing the presence of a CXCR4-GPCRx heteromer in a cancer patient.

[612] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the method further comprises determining or diagnosing the presence of a CXCR4-GPCRx heteromer in a cancer cell or cancer tissue.

[613] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and

any one or more of the further embodiments herein, wherein the method further comprises determining or diagnosing the presence of a CXCR4-GPCRx heteromer in a cancer cell or cancer tissue obtained from a cancer patient.

[614] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the method further comprises determining or diagnosing the presence of a CXCR4-GPCRx heteromer; and wherein the determining or diagnosing comprises:

[615] 1) obtaining or having obtained a biological sample (e.g., cells or tissue, such as cells or tissue from a cancer patient); and

[616] 2) performing or having performed an assay on the biological sample, wherin the assay is or comprises one or more of the following: a co-internalization assay, a colocalization assay, in situ hybridization, immunohistochemistry, immunoelectron microscopy, a proximity-based assay, a co-immunoprecipitation assay, or a fluorescent animal assay.

[617] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the enhanced downstream signaling from the CXCR4-GPCRx heteromer is determined by an intracellular Ca2+ assay.

[618] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the enhanced downstream signaling is an enhanced amount of calcium mobilization.

[619] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the enhanced amount of calcium mobilization from the CXCR4-GPCRx heteromer is a calcium mobilization amount that, upon co-stimulation with the CXCL12 and the respective selective GPCRx agonist, is at least 10% greater, at least 20% greater, at least 30% greater, at least 40% greater, at least 50% greater, at least 75% greater, or at least 90% greater, than the sum of calcium mobilization amounts resulting from single agonist stimulation of said cells with either the CXCL12 or the respective selective GPCRx agonist, as determined via a calcium mobilization assay.

[620] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the enhanced amount of calcium mobilization from the CXCR4-GPCRx heteromer is a calcium mobilization

amount that, upon co-stimulation with the CXCL12 and the respective selective GPCRx agonist, is between 10-100% greater than the sum of calcium mobilization amounts resulting from single agonist stimulation of said cells with either the CXCL12 or the respective selective GPCRx agonist, as determined via a calcium mobilization assay.

[621] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the enhanced amount of calcium mobilization from the CXCR4-GPCRx heteromer is a calcium mobilization amount that, upon co-stimulation with the CXCL12 and the respective selective GPCRx agonist, is between 25-100% greater than the sum of calcium mobilization amounts resulting from single agonist stimulation of said cells with either the CXCL12 or the respective selective GPCRx agonist, as determined via a calcium mobilization assay.

[622] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the enhanced amount of calcium mobilization is determined by an intracellular Ca2+ assay.

[623] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the intracellular Ca2+ assay is a calcium mobilization assay.

[624] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the enhanced amount of calcium mobilization is a synergistic amount of calcium mobilization.

[625] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the synergistic amount of calcium mobilization from the cells containing the CXCR4-GPCRx heteromer is a calcium mobilization amount that, upon co-stimulation with the CXCL12 and the respective selective GPCRx agonist, is at least 10% greater, at least 20% greater, at least 30% greater, at least 40% greater, at least 50% greater, at least 75% greater, or at least 90% greater, than the sum of calcium mobilization amounts resulting from single agonist stimulation of said cells with either the CXCL12 or the respective selective GPCRx agonist, as determined via a calcium mobilization assay.

[626] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and

any one or more of the further embodiments herein, wherein the CXCR4-GPCRx heteromer has two or more of the following characteristics:

[627] 1) the CXCR4-GPCRx heteromer components in a cell colocalize and physically interact, either directly or via intermediate proteins acting as conduits for allosterism, as determined via one or more of the following: a co-internalization assay, a colocalization assay, in situ hybridization, immunohistochemistry, immunoelectron microscopy, a proximity-based assay, a co-immunoprecipitation assay, or a fluorescent animal assay;

[628] 2) an enhanced amount of calcium mobilization, such that:

[629] a) either CXCR4 or the respective GPCRx in an individual protomer context in a cell, upon co-stimulation with CXCL12 and a respective selective GPCRx agonist, results in a calcium mobilization amount that is equal to or less than the sum of calcium mobilization amounts resulting from single agonist stimulation with either the CXCL12 or the respective selective GPCRx agonist; and

[630] b) the CXCR4-GPCRx heteromer exhibits an enhanced calcium mobilization upon co-stimulation with the CXCL12 and the respective selective GPCRx agonist relative to the sum of calcium mobilization amounts resulting from single agonist stimulation with either the CXCL12 or the respective selective GPCRx agonist;

[631] as determined via a calcium mobilization assay; or

[632] 3) a CXCR4-GPCRx heteromer-selective reagent:

[633] i) alters heteromer-specific properties of the CXCR4-GPCRx heteromer in a patient derived cell;

[634] ii) alters heteromer-specific function of the CXCR4-GPCRx heteromer in a patient derived cell;

[635] iii) alters heteromer-specific properties of a patient derived cell containing the CXCR4-GPCRx heteromer; or

[636] iv) decreases cell proliferation of a patient derived cell(s) containing the CXCR4-GPCRx heteromer.

[637] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the CXCR4-GPCRx heteromer-selective reagent alters heteromer-specific properties of the CXCR4-GPCRx heteromer in a patient derived cell, as determined by at least one of the following methods: PLA, radioligand binding assays, [35S]GTP-rS Binding assays, Calcuim assay, cAMP assay, GTPase assay, PKA activation, ERK1/2 and/or Akt/PKB Phosphorylation assays, Src and STAT3 phosphorylation assays, CRE-reporter assay, NFAT-RE-reporter assay, SRE-reporter assay, SRF-RE reporter assay, NF-kB-RE reporter assay, Secreted alkaline phosphatase Assay, Inositol 1-Phosphate Production

assay, Adenylyl Cyclase Activity assay, analysis of target gene expression by RT-PCR, RT-qPCR, RNAseq, or microarray.

[638] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the CXCR4-GPCRx heteromer-selective reagent alters heteromer-specific function of the CXCR4-GPCRx heteromer in a patient derived cell, as determined by at least one of the following methods: PLA, radioligand binding assays, [35S]GTP-rS Binding assays, Calcuim assay, cAMP assay, GTPase assay, PKA activation, ERK1/2 and/or Akt/PKB Phosphorylation assays, Src and STAT3 phosphorylation assays, CRE-reporter assay, NFAT-RE-reporter assay, SRE-reporter assay, SRF-RE reporter assay, NF-kB-RE reporter assay, Secreted alkaline phosphatase Assay, Inositol 1-Phosphate Production assay, Adenylyl Cyclase Activity assay, analysis of target gene expression by RT-PCR, RT-qPCR, RNAseq, next generation sequencing (NGS), or microarray.

[639] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the CXCR4-GPCRx heteromer-selective reagent alters heteromer-specific properties of a patient derived cell containing the CXCR4-GPCRx heteromer, as determined by at least one of the following methods: assays on proliferation, migration, invasion, and drug resistance (survival) of cancer cells, modulation of immune cell function, angiogenesis, vasculogenesis, metastasis, drug resistance, tissue microarray (TMA), and cancer cell-tumor microenvironment (TME) interaction.

[640] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the CXCR4-GPCRx heteromer components comprise individual protomers CXCR4 and GPCRx.

[641] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the cell containing either the CXCR4 or the respective GPCRx in an individual protomer context comprises, independently:

[642] i) the individual protomer CXCR4 in the absence of the respective individual protomer GPCRx; or

[643] ii) the respective individual protomer GPCRx in the absence of the individual protomer CXCR4;

[644] respectively.

[645] In a further embodiment, the method of treating, method of suppressing, pharma-

ceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the cell containing the CXCR4 in an individual protomer context comprises said individual protomer CXCR4 in the absence of the respective individual protomer GPCRx.

[646] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the cell containing the respective GPCRx in an individual protomer context comprises said respective individual protomer GPCRx in the absence of the individual protomer CXCR4.

[647] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the CXCR4-GPCRx heteromer components in the cell colocalize and physically interact, either directly or via intermediate proteins acting as conduits for allosterism, as determined via one or more of the following: a co-internalization assay, a colocalization assay, in situ hybridization, immunohistochemistry, immunoelectron microscopy, a proximity-based assay, a co-immunoprecipitation assay, or a fluorescent animal assay.

[648] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the proximity-based assay is, or comprises, resonance energy transfer (RET), bioluminescence RET (BRET), fluorescence RET (FRET), time-resolved fluorescence RET (TR-FRET), antibody-aided FRET, ligand-aided FRET, bimolecular fluorescence complementation (BiFC), or a proximity ligation assay (PLA).

[649] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the CXCR4-GPCRx heteromer components in the cell colocalize and physically interact, either directly or via intermediate proteins acting as conduits for allosterism, as determined via one or more of the following: a co-internalization assay, bimolecular fluorescence complementation (BiFC), or a proximity ligation assay (PLA).

[650] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the patient's cancer cell contains the CXCR4-GPCRx heteromer.

[651] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the CXCR4-GPCRx

heteromer exhibits the enhanced amount of calcium mobilization, such that:

[652] a) either the CXCR4 or the respective GPCRx in an individual protomer context in the cell upon co-stimulation with CXCL12 and a respective selective GPCRx agonist results in a calcium mobilization amount that is equal to or less than the sum of calcium mobilization amounts resulting from single agonist stimulation with either the CXCL12 or the respective selective GPCRx agonist; and

[653] b) the CXCR4-GPCRx heteromer exhibits an enhanced calcium mobilization upon co-stimulation with the CXCL12 and the respective selective GPCRx agonist relative to the sum of calcium mobilization amounts resulting from single agonist stimulation with either the CXCL12 or the respective selective GPCRx agonist;

[654] as determined via a calcium mobilization assay.

[655] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein:

[656] i) the calcium mobilization from the protomer CXCR4 or GPCRx, in the individual protomer context in the cell, is non-synergistic, as determined via calcium mobilization assay; and

[657] ii) the calcium mobilization from the CXCR4-GPCRx heteromer in the cell is synergistic, as determined via a calcium mobilization assay.

[658] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein in the individual protomer context:

[659] a) the individual protomer CXCR4 in the cell, in the absence of the respective individual protomer GPCRx; or

[660] b) the respective individual protomer GPCRx in the cell, in the absence of the individual protomer CXCR4;

[661] upon co-stimulation with CXCL12 and a respective selective GPCRx agonist results in a calcium mobilization amount that is equal to or less than the sum of calcium mobilization amounts resulting from single agonist stimulation with either the CXCL12 or the respective selective GPCRx agonist, as determined via a calcium mobilization assay.

[662] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein in the individual protomer context, independently:

[663] a) the individual protomer CXCR4 in the cell, in the absence of the respective individual protomer GPCRx; and

[664] b) the respective individual protomer GPCRx in the cell, in the absence of the individual protomer CXCR4;

[665] upon co-stimulation with CXCL12 and a respective selective GPCRx agonist results in a calcium mobilization amount that is equal to or less than the sum of calcium mobilization amounts resulting from single agonist stimulation with either the CXCL12 or the respective selective GPCRx agonist, as determined via a calcium mobilization assay.

[666] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the CXCR4-GPCRx heteromer upon co-stimulation with the CXCL12 and the respective selective GPCRx agonist results in a calcium mobilization amount that is greater than the sum of calcium mobilization amounts resulting from single agonist stimulation of said cells with either the CXCL12 or the respective selective GPCRx agonist, as determined via a calcium mobilization assay.

[667] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the calcium mobilization amount resulting from the co-stimulation of the CXCR4-GPCRx heteromer is an enhanced amount of calcium mobilization, relative to the sum of calcium mobilizations resulting from single agonist stimulation of said CXCR4-GPCRx heteromer, as determined via a calcium mobilization assay.

[668] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the enhanced amount of calcium mobilization is at least 10% greater, at least 20% greater, at least 30% greater, at least 40% greater, at least 50% greater, at least 75% greater, or at least 90% greater, than the sum of calcium mobilizations resulting from single agonist stimulation with either the CXCL12 or the respective selective GPCRx agonist, as determined via a calcium mobilization assay.

[669] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the enhanced amount of calcium mobilization is a synergistic amount of calcium mobilization.

[670] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the method administers a CXCR4-GPCRx heteromer-selective reagent.

[671] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the CXCR4-GPCRx heteromer-selective reagent:

[672] i) alters heteromer-specific properties of the CXCR4-GPCRx heteromer in the patient derived cell;

[673] ii) alters heteromer-specific function of the CXCR4-GPCRx heteromer in the patient derived cell;

[674] iii) alters heteromer-specific properties of the patient derived cell containing the CXCR4-GPCRx heteromer; or

[675] iv) decreases cell proliferation of the patient derived cell containing the CXCR4-GPCRx heteromer.

[676] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the CXCR4-GPCRx heteromer-selective reagent alters heteromer-specific properties of the CXCR4-GPCRx heteromer in the patient derived cell.

[677] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the CXCR4-GPCRx heteromer-selective reagent alters heteromer-specific function of the CXCR4-GPCRx heteromer in the patient derived cell.

[678] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the CXCR4-GPCRx heteromer-selective reagent alters heteromer-specific properties of the patient derived cell containing the CXCR4-GPCRx heteromer.

[679] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the CXCR4-GPCRx heteromer-selective reagent decreases cell proliferation of the patient derived cell containing the CXCR4-GPCRx heteromer.

[680] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the CXCR4-GPCRx heteromer-selective reagent is a CXCR4 inhibitor, a GPCRx inhibitor, or a CXCR4-GPCRx heteromer inhibitor.

[681] In a further embodiment, the method of treating, method of suppressing, pharma-

ceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the CXCR4-GPCRx heteromer-selective reagent is a CXCR4 antagonist, a GPCRx antagonist, or a CXCR4-GPCRx heteromer antagonist.

[682] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the CXCR4-GPCRx heteromer-selective reagent is administered as a pharmaceutical composition.

[683] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the method administers an inhibitor selected from the group consisting of: the CXCR4 inhibitor, the GPCRx inhibitor, or the CXCR4-GPCRx heteromer inhibitor.

[684] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the inhibitor is administered as a pharmaceutical composition.

[685] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the administered inhibitor is the CXCR4 inhibitor.

[686] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the administered inhibitor is the GPCRx inhibitor.

[687] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the administered inhibitor is the inhibitor of the CXCR4-GPCRx heteromer.

[688] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the method administers the combination of inhibitors selected from the group consisting of: the CXCR4 inhibitor, the GPCRx inhibitor, and the inhibitor of the CXCR4-GPCRx heteromer.

[689] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the combination of inhibitors is administered as a pharmaceutical composition.

[690] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the combination of inhibitors comprises the CXCR4 inhibitor and the GPCRx inhibitor.

[691] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the combination of inhibitors comprises the CXCR4 inhibitor and the inhibitor of the CXCR4-GPCRx heteromer.

[692] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the combination of inhibitors comprises the GPCRx inhibitor and the inhibitor of the CXCR4-GPCRx heteromer.

[693] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the combination of inhibitors are administered sequentially, concurrently, or simultaneously.

[694] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the combination of inhibitors are administered as a pharmaceutical composition further comprising a pharmaceutically acceptable carrier.

[695] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the combination of inhibitors are administered as separate pharmaceutical compositions, wherein the separate pharmaceutical compositions independently further comprise a pharmaceutically acceptable carrier.

[696] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the CXCR4 inhibitor is an antagonist of CXCR4, an inverse agonist of CXCR4, a partial antagonist of CXCR4, an allosteric modulator of CXCR4, an antibody of CXCR4, an antibody fragment of CXCR4, or a ligand of CXCR4.

[697] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the GPCRx inhibitor is an

antagonist of GPCRx, an inverse agonist of GPCRx, a partial antagonist of GPCRx, an allosteric modulator of GPCRx, an antibody of GPCRx, an antibody fragment of GPCRx, or a ligand of GPCRx.

[698] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the inhibitor of the CXCR4-GPCRx heteromer is an antagonist of the CXCR4-GPCRx heteromer, an inverse agonist of the CXCR4-GPCRx heteromer, a partial antagonist of the CXCR4-GPCRx heteromer, an allosteric modulator of the CXCR4-GPCRx heteromer, an antibody of the CXCR4-GPCRx heteromer, an antibody fragment of the CXCR4-GPCRx heteromer, a ligand of the CXCR4-GPCRx heteromer, or a protein-protein interaction (PPI) inhibitor of the CXCR4-GPCRx heteromer.

[699] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the CXCR4 inhibitor, the GPCRx inhibitor, or the inhibitor of the CXCR4-GPCRx heteromer, is an antibody-drug conjugate.

[700] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein a therapeutically effective amount of the inhibitor of the CXCR4-GPCRx heteromer is administered to the patient.

[701] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein a sub-therapeutically effective amount of the inhibitor of the CXCR4-GPCRx heteromer is administered to the patient.

[702] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein a therapeutically effective amount of the CXCR4 inhibitor is administered to the patient.

[703] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein a sub-therapeutically effective amount of the CXCR4 inhibitor is administered to the patient.

[704] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the CXCR4 inhibitor is

selected from the group consisting of:

[705] AD-114, AD-114-6H, AD-114-Im7-FH, AD-114-PA600-6H, ALX-0651, ALX40-4C, AMD070 (AMD11070, X4P-001), AMD3100 (plerixafor), AMD3465, ATI 2341, BKT140 (BL-8040; TF14016; 4F-Benzoyl-TN14003), CTCE-9908, CX549,,D-[Lys3] GHRP-6, FC122, FC131, GMI-1359, GSK812397, GST-NT21MP, isothiourea-1a, isothiourea-1t (IT1t), KRH-1636, KRH-3955, LY2510924, LY2624587, MSX-122, N-[^{11}C]Methyl-AMD3465, PF-06747143, POL6326, SDF-1 1-9[P2Gdimer, SDF1 P2G, T134, T140, T22, TC 14012, TG-0054 (Burixafor), USL311, ulocuplumab (MDX1338/BMS-936564), viral macrophage inflammatory protein-II (vMIP-II), WZ811, 12G5, 238D2, 238D4, [^{64}Cu]-AMD3100, [^{64}Cu]-AMD3465, [^{68}Ga]pentixafor, [^{90}Y]pentixather, [^{99m}Tc]O_2-AMD3100, [^{177}Lu]pentixather, and 508MCl (Compound 26).

[706] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein a therapeutically effective amount of the GPCRx inhibitor is administered to the patient.

[707] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein a sub-therapeutically effective amount of the GPCRx inhibitor is administered to the patient.

[708] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the GPCRx is selected from the group consisting of:

[709] ADCYAP1R1, ADORA2B, ADORA3, ADRB2, C5AR1, CALCR, CHRM1, EDNRB, HRH1, MLNR, NTSR1, and TACR3.

[710] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the GPCRx is ADRB2 or HRH1.

[711] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the GPCRx is ADCYAP1R1.

[712] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the ADCYAP1R1 inhibitor is selected from the group consisting of:

[713] M65, Max.d.4, MK-0893, N-stearyl-[Nle^{17}]neurotensin-(6-11)/VIP-(7-28), PACAP-(6-38), and PG 97-269.

[714] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the GPCRx is ADORA2B.

[715] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the ADORA2B inhibitor is selected from the group consisting of:

[716] 3-isobutyl-8-pyrrolidinoxanthine, alloxazine, AS16, AS70, AS74, AS94, AS95, AS96, AS99, AS100, AS101, ATL802, BW-A1433, caffeine, CGS 15943, CPX, CSC, CVT-6883, DAX, DEPX, derenofylline, DPCPX, FK-453, I-ABOPX, istradefylline, KF26777, LAS38096, LUF5981, MRE 2029F20, MRE 3008F20, MRS1191, MRS1220, MRS1523, MRS1706, MRS1754, MSX-2, OSIP339391, pentoxifylline, preladenant, PSB-10, PSB-11, PSB36, PSB603, PSB-0788, PSB1115, rolofylline, SCH 58261, SCH442416, ST-1535, theophylline, tonapofylline, vipadenant, xanthine amine congener, XCC, and ZM-241385.

[717] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the GPCRx is ADORA3.

[718] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the ADORA3 inhibitor is selected from the group consisting of:

[719] ATL802, BW-A1433, caffeine, CGS 15943, CSC, CVT-6883, derenofylline, dexniguldipine, DPCPX, FK-453, flavanone, flavone, galangin, I-ABOPX, istradefylline, KF26777, LAS38096, LUF5981, MRE 2029F20, MRE 3008F20, MRE 3010F20, MRS1041, MRS1042, MRS1067, MRS1088, MRS1093, MRS1097, MRS1177, MRS1186, MRS1191, MRS1191, MRS1220, MRS1476, MRS1486, MRS1505, MRS1523, MRS1754, MRS928, MSX-2, nicardipine, preladenant, PSB-10, PSB-11, PSB36, PSB603, PSB1115, rolofylline, sakuranetin, SCH 58261, SCH442416, ST-1535, theophylline, tonapofylline, vipadenant, visnagin, VUF5574, VUF8504, VUF8507, xanthine amine congener, and ZM-241385.

[720] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the GPCRx is ADRB2.

[721] In a further embodiment, the method of treating, method of suppressing, pharma-

ceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the ADRB2 inhibitor is selected from the group consisting of:

[722] Alprenolol, atenolol, betaxolol, bupranolol, butoxamine, carazolol, carvedilol, CGP 12177, cicloprolol, ICI 118551, ICYP, labetalol, levobetaxolol, levobunolol, LK 204-545, metoprolol, nadolol, NIHP, NIP, propafenone, propranolol, sotalol, SR59230A, and timolol.

[723] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the GPCRx is C5AR1.

[724] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the C5AR1 inhibitor is selected from the group consisting of:

[725] $A8^{\Delta 71\text{-}73}$, AcPhe-Orn-Pro-D-Cha-Trp-Arg, avacopan, C089, CHIPS, DF2593A, JPE1375, L-156,602, NDT9520492, *N*-methyl-Phe-Lys-Pro-D-Cha-Trp-D-Arg-CO_2H, PMX205, PMX53, RPR121154, and W54011.

[726] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the GPCRx is CALCR.

[727] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the CALCR inhibitor is selected from the group consisting of:

[728] α-CGRP-(8-37) (human), AC187, CT-(8-32) (salmon), and olcegepant.

[729] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the GPCRx is CHRM1.

[730] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the CHRM1 inhibitor is selected from the group consisting of:

[731] 3-Quinuclidinyl benzilate (QNB), 4-DAMP, aclidinium, AE9C90CB, AFDX384, amitriptyline, AQ-RA 741, atropine, benzatropine, biperiden, darifenacin, dicyclomine, dosulepin, ethopropazine, glycopyrrolate, guanylpirenzepine, hexahydrodifenidol, hexahydrosiladifenidol, hexocyclium, himbacine, ipratropium, lithocholylcholine, methoctramine, ML381, muscarinic toxin 1, muscarinic toxin 2, muscarinic toxin 3, N-methyl scopolamine, otenzepad, oxybutynin, *p*-F-HHSiD, pirenzepine, propantheline,

(R,R)-quinuclidinyl-4-fluoromethyl-benzilate, scopolamine, silahexocyclium, solifenacin, telenzepine, tiotropium, tolterodine, trihexyphenidyl, tripitramine, UH-AH 37, umeclidinium, and VU0255035.

[732] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the GPCRx is EDNRB.

[733] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the EDNRB inhibitor is selected from the group consisting of:

[734] A192621, ambrisentan, atrasentan, bosentan (RO 470203, Tracleer);, BQ788, IRL 2500, K-8794, macitentan, RES7011, Ro 46-8443, SB209670, SB217242 (enrasentan), TAK 044, and tezosentan (RO610612).

[735] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the GPCRx is HRH1.

[736] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the HRH1 inhibitor is selected from the group consisting of:

[737] (-)-chlorpheniramine, (+)-chlorpheniramine, (-)-*trans*-H_2-PAT, (+)-*cis*-H_2-PAT, (+)-*trans*-H_2-PAT, (±)-*cis*-H_2-PAT, (±)-*trans*-H_2-PAT, (*R*)-cetirizine, (*S*)-cetirizine, 9-OH-risperidone, A-317920, A-349821, ABT-239, alimemazine, amitriptyline, aripiprazole, arpromidine, asenapine, astemizole, AZD3778, azelastine, BU-E 47, cetirizine, chlorpheniramine, chlorpromazine, ciproxifan, clemastine, clobenpropit, clozapine, conessine, cyclizine, cyproheptadine, desloratadine, diphenhydramine, dosulepin, doxepin, epinastine, fexofenadine, fluphenazine, fluspirilene, haloperidol, hydroxyzine, impromidine, INCB-38579, JNJ-39758979, ketotifen, loratadine, loxapine, MK-0249, molindone, olanzapine, perphenazine, pimozide, pipamperone, pitolisant, promethazine, pyrilamine, quetiapine, risperidone, sertindole, terfenadine, thioridazine, thiothixene, trifluoperazine, tripelennamine, triprolidine, ziprasidone, and zotepine.

[738] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the GPCRx is MLNR.

[739] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the MLNR inhibitor is

selected from the group consisting of:

[740] GM-109, MA-2029, and OHM-11526.

[741] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the GPCRx is NTSR1.

[742] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the NTSR1 inhibitor is selected from the group consisting of:

[743] Meclinertant, SR48527, SR48692, and SR142948A.

[744] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the GPCRx is TACR3.

[745] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the TACR3 inhibitor is selected from the group consisting of:

[746] [Trp^7, β-Ala^8]neurokinin A-(4-10), AZD2624, FK 224, GR138676, GSK 172981, GSK 256471, N',2-diphenylquinoline-4-carbohydrazide 8m, N′,2-diphenylquinoline-4-carbohydrazide, osanetant, PD 154740, PD 161182, PD157672, saredutant, SB 218795, SB 222200, SB 235375, SCH 206272, SSR 146977, and talnetant.

[747] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the respective selective GPCRx agonist is natural ligand of the respective GPCRx., respectively.

[748] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the method further comprises detecting the CXCR4-GPCRx heteromer in the cancer patient.

[749] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the method further comprises identifying the CXCR4-GPCRx heteromer in the cancer patient.

[750] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the method further comprises:

[751] i) obtaining or having obtained a biological sample from the cancer patient;

[752] ii) conducting or having conducted a diagnostic assay to determine presence, identity, or presence and identity, of a CXCR4-GPCRx heteromer in the obtained biological sample from the cancer patient; and

[753] iii) selecting the inhibitor or combination of inhibitors to suppress enhanced downstream signaling from the CXCR4-GPCRx heteromer.

[754] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the cancer is selected from the group consisting of: breast cancer, lung cancer, brain cancer, kidney cancer, pancreatic cancer, ovarian cancer, prostate cancer, melanoma, multiple myeloma, gastrointestinal cancers, renal cell carcinoma, soft tissue sarcomas, hepatocellular carcinoma, stomach cancer, colorectal cancer, esophageal cancer, and leukemia.

[755] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the patient's biological sample is a biological fluid sample.

[756] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein a liquid biopsy is performed on the biological fluid sample. In some embodiments, the biological fluid sample includes circulating tumor cells (CTCs), tumor-derived cell-free DNA (cfDNA), circulating small RNAs, and extracellular vesicles including exosomes, from bodily fluids as disclosed, for example, in Campos CDM et al., "Molecular Profiling of Liquid Biopsy Samples for Precision Medicine," Cancer J. 2018 Mar/Apr;24(2):93-103, which is incorporated hereby in its entirety.

[757] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the biological fluid sample is a blood sample, a plasma sample, a saliva sample, a cerebral fluid sample, an eye fluid sample, or a urine sample.

[758] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the patient's biological sample is a biological tissue sample.

[759] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein a liquid biopsy is

performed on the biological tissue sample.

[760] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the biological tissue sample is an organ tissue sample, a bone tissue sample, or a tumor tissue sample.

[761] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the cancer cell contains the CXCR4-GPCRx heteromer.

[762] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein a normal, non-cancerous cell, does not contain the CXCR4-GPCRx heteromer.

[763] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the cancer cell of the patient contains the CXCR4-GPCRx heteromer in a greater concentration than a normal, non-cancerous cell from said patient, for example, 10% greater concentration than a normal, non-cancerous cell from said patient, such as 25% greater, 50% greater, 100% greater, 200% greater, or 300% greater, than a normal, non-cancerous cell from said patient.

[764] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the CXCR4-GPCRx heteromer contained in the cancer cell of the patient is in a greater concentration than a normal, non-cancerous cell from said patient, for example, 10% greater concentration than a normal, non-cancerous cell from said patient, such as 25% greater, 50% greater, 100% greater, 200% greater, or 300% greater, than a normal, non-cancerous cell from said patient; and wherein the GPCRx of the CXCR4-GPCRx heteromer contained in the cancer cell of the patient is selected from the group consisting of: ADCYAP1R1, ADORA2B, ADORA3, ADRB2, C5AR1, CALCR, CHRM1, EDNRB, HRH1, MLNR, NTSR1, and TACR3.

[765] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the presence of the CXCR4-GPCRx heteromer in the cancer cell of the patient identifies a sub-population of CXCR4-mediated cancer patients.

[766] In a further embodiment, the method of treating, method of suppressing, pharma-

ceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the presence of the CXCR4-GPCRx heteromer in the cancer cell of the patient is a biomarker of a sub-population of CXCR4-mediated cancer patients.

[767] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the biomarker of the sub-population of CXCR4-mediated cancer patients allows for precision medicine, patient stratification, or patient classification.

[768] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the biomarker of the sub-population of CXCR4-mediated cancer patients allows for GPCR-based precision cancer therapeutics.

[769] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the presence of the CXCR4-GPCRx heteromer in the cancer cell of the patient identifies a sub-population of CXCR4-mediated cancer patients; and wherein the GPCRx of the CXCR4-GPCRx heteromer present in the cancer cell of the patient is selected from the group consisting of: ADCYAP1R1, ADORA2B, ADORA3, ADRB2, C5AR1, CALCR, CHRM1, EDNRB, HRH1, MLNR, NTSR1, and TACR3.

[770] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the presence of the CXCR4-GPCRx heteromer in the cancer cell of the patient is a biomarker of a sub-population of CXCR4-mediated cancer patients; and wherein the GPCRx of the CXCR4-GPCRx heteromer present in the cancer cell of the patient is selected from the group consisting of: ADCYAP1R1, ADORA2B, ADORA3, ADRB2, C5AR1, CALCR, CHRM1, EDNRB, HRH1, MLNR, NTSR1, and TACR3.

[771] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the inhibitor is an antibody.

[772] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the CXCR4-GPCRx heteromer-selective reagent is an antibody.

[773] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the antibody is a bi-specific antibody of the CXCR4-GPCRx heteromer.

[774] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the antibody is a heteromer-specific antibody of the CXCR4-GPCRx heteromer.

[775] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the inhibitor is a bi-specific ligand(s) of the CXCR4-GPCRx heteromer.

[776] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the antibody is an antibody-drug conjugate (ADC), as disclosed in, for example, Beck a et al., "Strategies and challenges for the next generation of antibody-drug conjugates", Nature Reviews Drug Discovery, 16:315-337, (2017) and Lambert, et al., "Antibody-Drug Conjugates for Cancer Treatment", Annual Review of Medicine, 69:191-207 (2018), each of which are incorporated hereby in its entirety.

[777] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the method comprises: administering to the patient an inhibitor or a combination of inhibitors selected from the group consisting of: an inhibitor of CXCR4, an inhibitor of GPCRx, and an inhibitor of the CXCR4-GPCRx heteromer; wherein: i) the CXCR4-GPCRx heteromer has enhanced downstream signaling; and ii) the administered inhibitor or combination of inhibitors suppresses the enhanced downstream signaling from said CXCR4-GPCRx heteromer in the cancer patient.

[778] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the pharmaceutical kit or pharmaceutical composition, such as for use in treating cancer in a patient having a cell containing a CXCR4-GPCRx heteromer, comprises: an inhibitor or a combination of inhibitors selected from the group consisting of: an inhibitor of CXCR4, an inhibitor of GPCRx, and an inhibitor of the CXCR4-GPCRx heteromer; wherein the CXCR4-GPCRx heteromer has enhanced downstream signaling.

[779] In a further embodiment, the method of treating, method of suppressing, pharma-

ceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein progression of the cancer in the patient having said cancer cell containing the CXCR4-GPCRx heteromer is decreased in the range of 5-100% more, 10-100% more, 20-100% more, 30-100% more, 40-100% more, 50-100% more, 60-100% more, 75-100% more, 5-75% more, 5-50% more, or 5-25% more, upon administration of the combination of inhibitors, relative to administering the CXCR4 inhibitor or GPCRx inhibitor as the single inhibitor to said patient.

[780] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the efficacy of a CXCR4 inhibitor is increased in the range of 5-2000%, 5-1750%, 5-1500%, 5-1250%, 5-1000%, 5-900%, 5-800%, 5-700%, 5-500%, 5-400%, 5-250%, 5-200%, 5-100%, 5-75%, 5-50%, 5-40%, 5-30%, 5-25%, 100-2000%, 200-2000%, 300-2000%, 500-2000%, 750-2000%, 1000-2000%, 1250-2000%, 1500-2000%, 5-1500%, 25-1500%, 50-1500%, 75-1500%, 100-1500%, 200-1500%, 300-1500%, 500-1500%, 750-1500%, 1000-1500%, or 1250-1500%, when administered in combination with the GPCRx inhibitor to the patient having said cancer cell containing the CXCR4-GPCRx heteromer, relative to efficacy of the CXCR4 inhibitor when administered as a single inhibitor.

[781] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the efficacy of a GPCRx inhibitor is increased in the range of 5-2000%, 5-1750%, 5-1500%, 5-1250%, 5-1000%, 5-900%, 5-800%, 5-700%, 5-500%, 5-400%, 5-250%, 5-200%, 5-100%, 5-75%, 5-50%, 5-40%, 5-30%, 5-25%, 100-2000%, 200-2000%, 300-2000%, 500-2000%, 750-2000%, 1000-2000%, 1250-2000%, 1500-2000%, 5-1500%, 25-1500%, 50-1500%, 75-1500%, 100-1500%, 200-1500%, 300-1500%, 500-1500%, 750-1500%, 1000-1500%, or 1250-1500%, when administered in combination with the CXCR4 inhibitor to the patient having said cancer cell containing the CXCR4-GPCRx heteromer, relative to efficacy of the GPCRx inhibitor when administered as a single inhibitor.

[782] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the method administers a combination of inhibitors selected from the group consisting of: an inhibitor of CXCR4, an inhibitor of GPCRx, and an inhibitor of the CXCR4-GPCRx heteromer; or the pharmaceutical kit or pharmaceutical composition comprises a combination of in-

hibitors selected from the group consisting of: an inhibitor of CXCR4, an inhibitor of GPCRx, and an inhibitor of the CXCR4-GPCRx heteromer.

[783] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the combination of inhibitors is a combination of two inhibitors selected from the group consisting of: an inhibitor of CXCR4, an inhibitor of GPCRx, and an inhibitor of the CXCR4-GPCRx heteromer.

[784] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the method administers a combination of a CXCR4 inhibitor and a GPCRx inhibitor; or the pharmaceutical kit or pharmaceutical composition comprises a combination of a CXCR4 inhibitor and a GPCRx inhibitor.

[785] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the method administers a CXCR4-GPCRx heteromer inhibitor; or the pharmaceutical kit or pharmaceutical composition comprises a CXCR4-GPCRx heteromer inhibitor.

[786] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the administering of the combination of inhibitors suppresses the enhanced downstream signaling from said CXCR4-GPCRx heteromer in the cancer patient in the range of between 5-2000 fold, 5-1750 fold, 5-1500 fold, 5-1250 fold, 5-1000 fold, 5-900 fold, 5-800 fold, 5-700 fold, 5-500 fold, 5-400 fold, 5-250 fold, 5-200 fold, 5-100 fold, 5-75 fold, 5-50 fold, 5-40 fold, 5-30 fold, 5-25 fold, 100-2000 fold, 200-2000 fold, 300-2000 fold, 500-2000 fold, 750-2000 fold, 1000-2000 fold, 1250-2000 fold, 1500-2000 fold, 5-1500 fold, 25-1500 fold, 50-1500 fold, 75-1500 fold, 100-1500 fold, 200-1500 fold, 300-1500 fold, 500-1500 fold, 750-1500 fold, 1000-1500 fold, or 1250-1500 fold, relative to single inhibitor administration.

[787] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the administering of the inhibitor or combination of inhibitors suppresses the enhanced downstream signaling from said CXCR4-GPCRx heteromer in the cancer patient in the range of between 5-2000 fold, 5-1750 fold, 5-1500 fold, 5-1250 fold, 5-1000 fold, 5-900 fold, 5-800 fold, 5-700 fold, 5-500 fold, 5-400 fold, 5-250 fold, 5-200 fold, 5-100 fold, 5-75 fold,

5-50 fold, 5-40 fold, 5-30 fold, 5-25 fold, 100-2000 fold, 200-2000 fold, 300-2000 fold, 500-2000 fold, 750-2000 fold, 1000-2000 fold, 1250-2000 fold, 1500-2000 fold, 5-1500 fold, 25-1500 fold, 50-1500 fold, 75-1500 fold, 100-1500 fold, 200-1500 fold, 300-1500 fold, 500-1500 fold, 750-1500 fold, 1000-1500 fold, or 1250-1500 fold, relative to suppression of downstream signaling from either a CXCR4 protomer or a GPCRx protomer in their respective individual protomer context.

[788] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the GPCRx of the CXCR4-GPCRx heteromer is selected from the group consisting of: ADCYAP1R1, ADORA2B, ADORA3, ADRB2, C5AR1, CALCR, CHRM1, EDNRB, HRH1, MLNR, NTSR1, and TACR3; for example, selected from the group consisting of: ADCYAP1R1, ADORA2B, ADORA3, ADRB2, C5AR1, CHRM1, EDNRB, HRH1, MLNR, NTSR1, and TACR3; selected from the group consisting of: ADCYAP1R1, ADORA2B, ADORA3, ADRB2, CHRM1, EDNRB, HRH1, MLNR, NTSR1, and TACR3; selected from the group consisting of: ADCYAP1R1, ADORA2B, ADORA3, ADRB2, CHRM1, EDNRB, HRH1, NTSR1, and TACR3; selected from the group consisting of: ADCYAP1R1, ADORA2B, ADORA3, ADRB2, CHRM1, HRH1, NTSR1, and TACR3; selected from the group consisting of: ADORA2B, ADORA3, ADRB2, CHRM1, EDNRB, HRH1, NTSR1, and TACR3; selected from the group consisting of: ADRB2, CHRM1, EDNRB, HRH1, NTSR1, and TACR3; selected from the group consisting of: ADRB2, CHRM1, EDNRB, HRH1, and TACR3; selected from the group consisting of: ADRB2, CHRM1, HRH1, and TACR3; selected from the group consisting of: ADRB2, EDNRB, HRH1, and TACR3; selected from the group consisting of: ADRB2, EDNRB, and HRH1; selected from the group consisting of: ADRB2, CHRM1, and HRH1; or selected from the group consisting of: ADRB2, HRH1, and TACR3.

[789] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the GPCRx inhibitor is selected from the group consisting of: ADCYAP1R1 inhibitor, ADORA2B inhibitor, ADORA3 inhibitor, ADRB2 inhibitor, C5AR1 inhibitor, CALCR inhibitor, CHRM1 inhibitor, EDNRB inhibitor, HRH1 inhibitor, MLNR inhibitor, NTSR1 inhibitor, and TACR3 inhibitor; for example, selected from the group consisting of: ADCYAP1R1 inhibitor, ADORA2B inhibitor, ADORA3 inhibitor, ADRB2 inhibitor, C5AR1 inhibitor, CHRM1 inhibitor, EDNRB inhibitor, HRH1 inhibitor, MLNR inhibitor, NTSR1 inhibitor, and TACR3 inhibitor; selected from the group consisting of: ADCYAP1R1 inhibitor, ADORA2B inhibitor, ADORA3 inhibitor, ADRB2 inhibitor,

CHRM1 inhibitor, EDNRB inhibitor, HRH1 inhibitor, MLNR inhibitor, NTSR1 inhibitor, and TACR3 inhibitor; selected from the group consisting of: ADCYAP1R1 inhibitor, ADORA2B inhibitor, ADORA3 inhibitor, ADRB2 inhibitor, CHRM1 inhibitor, EDNRB inhibitor, HRH1 inhibitor, NTSR1 inhibitor, and TACR3 inhibitor; selected from the group consisting of: ADCYAP1R1 inhibitor, ADORA2B inhibitor, ADORA3 inhibitor, ADRB2 inhibitor, CHRM1 inhibitor, HRH1 inhibitor, NTSR1 inhibitor, and TACR3 inhibitor; selected from the group consisting of: ADORA2B inhibitor, ADORA3 inhibitor, ADRB2 inhibitor, CHRM1 inhibitor, EDNRB inhibitor, HRH1 inhibitor, NTSR1 inhibitor, and TACR3 inhibitor; selected from the group consisting of: ADRB2 inhibitor, CHRM1 inhibitor, EDNRB inhibitor, HRH1 inhibitor, NTSR1 inhibitor, and TACR3 inhibitor; selected from the group consisting of: ADRB2 inhibitor, CHRM1 inhibitor, EDNRB inhibitor, HRH1 inhibitor, and TACR3 inhibitor; selected from the group consisting of: ADRB2 inhibitor, CHRM1 inhibitor, HRH1 inhibitor, and TACR3 inhibitor; selected from the group consisting of: ADRB2 inhibitor, EDNRB inhibitor, HRH1 inhibitor, and TACR3 inhibitor; selected from the group consisting of: ADRB2 inhibitor, EDNRB inhibitor, and HRH1 inhibitor; selected from the group consisting of: ADRB2 inhibitor, CHRM1 inhibitor, and HRH1 inhibitor; or selected from the group consisting of: ADRB2 inhibitor, HRH1, inhibitor and TACR3 inhibitor.

[790] In a further embodiment, the method of treating, method of suppressing, pharmaceutical composition, or pharmaceutical kit, of any one of the above embodiments and any one or more of the further embodiments herein, wherein the initial concentration of the inhibitor is in the range of between 1-10 uM (or each of the inhibitors of the combination at concentrations in the range of between 1-10 uM), such as at a concentration of 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 uM, in testing and/or evaluating said inhibitor, or combination of inhibitors, regarding whether effective, or therapeutically effective, in suppressing an enhanced downstream signaling from a CXCR4-GPCRx heteromer.

[791]

[792] **Materials and Methods**

[793] **Reagents**

[794] BAY 60-6583, CGS 21680, C5a, acetylcholine, motilin, neurotensin, senktide, salmeterol, Cl-IB-MECA, salmon calcitonin, BQ-3020, octreotide, VU0255035, cetirizine, pyrilamine, MA-2029, meclinertant, and SSR 146977 were purchased from Tocris Bioscience (Ellisville, MO, USA). Galanin, endothelin-1, histamine, Prostaglandin E2 (PGE2), SRIF-14 (somatostatin), and bethanechol were purchased from Sigma-Aldrich (St Louis, Mo., USA). CXCL12, vasoactive intestinal peptide (VIP), and CCL2 were purchased from R&D systems (Minneapolis, Minnesota, USA). Formoterol, carvedilol, oxybutynin, bosentan, hydroxyzine, and loratadine were from

Prestwick Chemical (Illkirch, France). Apelin-13, roxithromycin, AMD3100, umeclidinium were obtained from Cayman Chemical Company (Ann Arbor, MI, USA), Selleckchem (Huston, TX, USA), Medchem express (Princeton, NJ, USA), and Santa Cruz Biotechnology (Santa Cruz, CA, USA), respectively.

[795]

[796] **Construction of adenoviral vectors containing GPCR cDNAs.**

[797] Vectors containing BiFC fragments, pCS2+VNm10 and pBiFC-VC155, were obtained from Chang-Deng Hu (Hu et al., 2002) and James Smith (Saka et al., 2007), respectively. The VNm10 is a Venus VN154 variant containing L46F and L64F mutations. To construct pAdBiFC-VN and pAdBiFC-VC vectors, DNAs containing VNm10 and VC155 were amplified by PCR and cloned into pShuttle-CMV. pAdBiFC-VN and pAdBiFC-VC vectors were obtained through homologous recombination between pAdEasy-1 and either pShuttle-CMV-VNm10 or pShuttle-CMV-VC155 using AdEasy vector system according to the manufacturer's instructions (Qbiogene, Carlsbad, CA). Human GPCR cDNA clones were obtained from the Missouri S&T cDNA Resource Center (Rolla, MO, USA). GPCR cDNAs were amplified by PCR and cloned into pDONR201 vector. Adenoviruses encoding GPCR, GPCR-VN, GPCR-VC, and GPCR-EGFP were obtained through *in vitro* LR recombination between entry clones containing GPCR and either pAdHTS, pAdBiFC-VN, pAdBiFC-VC, or pAdHTS-GFPC vectors, and subsequent transfection into 293A cells using AdHTS system as described previously (Choi et al., 2012; Song et al., 2014).

[798]

[799] **Cell culture**

[800] U-2 OS cells and MDA-MB-231 cells were purchased from the American Type Culture Collection (Manassas, VA, USA), and 293A cells were from Invitrogen (Carlsbad, CA, USA). U-2 OS cells, MDA-MB-231 cells, and 293A cells were grown in McCoy's 5A medium, RPMI 1640, and Modified Eagle's medium, respectively, in the presence of 10% fetal bovine serum (FBS), 100 units/ml of penicillin, and 100 μg/ml of streptomycin. Cells were cultured with 5% CO2 at 37 °C.

[801]

[802] **BiFC assay**

[803] U-2 OS cells were seeded at a density of 3000 cells per well in a black 96-well clear-bottom plate in 100 μl of McCoy's 5A medium supplemented with 10% FBS. On the following day, the cells were transduced with 30 MOI each of adenoviruses encoding GPCR-VN and GPCR-VC. Three days post-transduction, cells were fixed with 2 % formaldehyde and nuclei were stained with Hoechst 33342 (Invitrogen, Carlsbad, CA). Images were acquired using IN cell Analyzer 1000 and analyzed with IN Cell Developer ToolBox (GE Healthcare, Waukesha, WI). BiFC and nuclear images were

visualized using a ×20 objective and 360-nm (Hoechst) and 480-nm excitation filters, and monitored through 460- and 535-nm emission filters, respectively, with a 61002 trichroic mirror.

[804]

[805] **Co-internalization assay**

[806] U-2 OS cells were seeded at a density of 3000 cells per well in a black 96-well clear-bottom plate in 100 μl of McCoy's 5A medium supplemented with 10% FBS. On the following day, the cells were co-transduced with adenoviruses encoding CXCR4-EGFP (10 MOI) and GPCR-VC or GPCR-VN (30 MOI). Two days post-transduction, cells were stimulated with GPCRx agonists for 30 min and fixed with 2% formaldehyde. Images were obtained using IN cell analyzer 2000 using an excitation wavelength of 480 nm and an emission wavelength of 535 nm.

[807]

[808] **Calcium mobilization assay**

[809] MDA-MB-231 human breast cancer cells were seeded at 20,000 cells per well in a black clear bottom 96-well plate (Corning Costar, #3340) in 100 μl of RPMI 1640 supplemented with 10% FBS. The next day, cells were co-transduced with 10 MOI of CXCR4 and 30 MOI of HA-VC, 10 MOI of HA-VC and 30 MOI of GPCRx, or 10 MOI of CXCR4 and 30 MOI of GPCRx. Adenoviruses encoding HA-VC were used to adjust the total amount of adenoviruses transduced. After 2 days, cells were washed twice with assay buffer (Hank's balanced salt solution without phenol red, supplemented with 0.1% BSA and 20 mM HEPES, pH7.4), and stained with 5 μg/ml of Cal-520 AM (AAT Bioquest, Sunnyvale, CA, USA) diluted in assay buffer for 2 hr. Cells were washed with assay buffer three times, and incubated at 37 ˚C for another 30 min. Plate was loaded on a FlexStation 3 Multi-Mode Microplate Reader (Molecular Devices, Sunnyvale, CA, USA) and GPCRx agonists were added. Intracellular Ca2+ mobilization was measured at 37 ˚C using an excitation wavelength of 490 nm and an emission wavelength of 525 nm. Antagonists or vehicles were added 30 min before agonist treatment.

[810]

[811] **Internalization inhibition assay**

[812] CXCR4-GFP expressed U-2 OS cells were seeded at a density of 5000 cells per well in a black 96-well clear-bottom plate in 100 μl of McCoy's 5A medium supplemented with 10% FBS. On the following day, the cells were transduced with adenoviruses encoding GPCRx (30 MOI). Two days post-transduction, cells were stimulated with SDF-1 and/or GPCRx agonists for 20 min and fixed with 4% paraformaldehyde. Images were obtained using IN cell analyzer 2500 using an excitation wavelength of 480 nm and an emission wavelength of 535 nm.

[813]

[814] **Proliferation assay**

[815] PDCs were seeded in 384-well plate at 500 ea /well in 40 ul culture media. After overnight growth, the cells were cultured for 7 days in the presence of several dose of GPCRx antagonist or DMSO alone. After the 7-day incubation, 15 ul ATPlite (PerkinElmer, Cat. No. 6016739) was added into the each well and the plates were shaken for 5 minutes in an orbital shaker at 700 rpm. The luminescent signal was detected within 30 minutes at PerkinElmer TopCount detection instrument. The cell viability was calculated using the equation: Cell viability (%) = (OD of antagonist treatment/OD of DMSO only treatment) x 100%.

[816]

[817] **Proximity ligation assay in cell**

[818] CXCR4 overexpression cell lines, the U2OS-CXCR4 was infected with ADRB2 expressing adenovirus, Ad-ADRB2 at the dose of 0, 2.5, 10, 40 MOIs for 2 days. Proximity ligation assays (PLA) were performed as described previously (Brueggemann et al., 2014; Tripathi et al., 2014). To perform PLA, infected cells were fixed with 4% paraformaldehyde (PFA) on sixteen-well tissue culture slides. Slides were blocked with blocking solution provided by Duolink and incubated with mouse anti-CXCR4 (1:200, Santacruz, Sc-53534), Rabbit anti-ADRB2 (1:200, Thermoscientific, PA5-33333) at 37°C for 1 h in a humidifying chamber. Slides were then washed and incubated (1 h at 37°C) with secondary anti-rabbit and anti-mouse antibodies conjugated with plus and minus Duolink II PLA probes. Slides were washed again and then incubated with ligation-ligase solution (30 min at 37°C) followed by incubation with amplification-polymerase solution (2 h at 37°C). Slides were then mounted with minimal volume of Duolink II mounting medium with 4′,6-diamidino-2phenylindole (DAPI) for 15-30 min, and PLA signals [Duolink In Situ Detection Reagents Green (λ excitation/emission 495/527 nm) or Red (λ excitation/ emission 575/623 nm)were identified as fluorescent spots under a IN Cell analyzer 2500.

[819]

[820] **Proximity ligation assay in PDC**

[821] PDCs were seeded in 96-chamber slide at 100000 ea /well in 100 ul culture media. To perform PLA, PDCs were fixed with 4% paraformaldehyde (PFA) and were blocked with blocking solution provided by Duolink and incubated with mouse anti-CXCR4 (1:200, Santacruz, Sc-53534), Rabbit anti-ADRB2 (1:200, Thermoscientific, PA5-33333), Rabbit anti-CHRM1 (1:200, Ls bio, Ls-C313301) at 37°C for 1 h in a humidifying chamber. Slides were then washed and incubated (1 h at 37°C) with secondary anti-rabbit and anti-mouse antibodies conjugated with plus and minus

Duolink II PLA probes. Slides were washed again and then incubated with ligation-ligase solution (30 min at 37°C) followed by incubation with amplification-polymerase solution (2 h at 37°C). Slides were then mounted with minimal volume of Duolink II mounting medium with 4′,6-diamidino-2phenylindole (DAPI) for 15-30 min, and PLA signals [Duolink In Situ Detection Reagents Green (λ excitation/emission 495/527 nm) or Red (λ excitation/emission 575/623 nm)were identified as fluorescent spots under a IN Cell analyzer 2500.

[822]

[823] **Proximity ligation assay in PDX**

[824] To perform PLA with PDX samples, the glioblastoma patient derived FFPE samples were used (provided by Samsung Seoul hospital in Seoul, Korea). After FFPE sample were de-paraffinized and performed heat induced antigen retrieval for 15 minutes at 100°C. Slides were blocked with blocking solution provided by Duolink and incubated with rabbit anti-CXCR4 (1:200, Thermoscientific, PA3305), mouse anti-ADRB2 (1:200, Santacruz, Sc-271322), at 37 °C for 1 h in a humidifying chamber. The other process was same as described above (PLA with PDC).

[825]

[826] **Chemotactic Migration Assay**

[827] Transwell plates (8 μm pore size polycarbonate membrane, 6.5-mm diameter) were coated with 50 μg/mL collagen for 2 h at 37 °C (Corning Inc.). MDA-MB-231 cells were serum-starved 24 h and then plated in serum-free medium containing 0.5% BSA in the top chamber (20,000 cells/100 μL). Antagonists or inverse agonists were added to the cells 30 min before plating the cells in the transwell plates. Attractants were added in the bottom chamber. Antagonists or inverse agonists were also included in the bottom chamber. After 3 h at 37 °C, cells on the top transwell membrane were removed using a cotton swab, fixed, and stained with 0.1% Crystal violet. Chemotaxis was quantified by counting the migrated cells on the lower surface of the membrane of 10 fields per chamber at 10× objective.

[828]

[829] **Reverse Transcription-quantitative Polymerase Chain Reaction (RT-qPCR)**

[830] Total RNA was extracted using TRIzol (Invitrogen) and cDNA was synthesized from 1 μg of total RNA after treatment with DNase I (Sigma). RT-qPCR was conducted using SensiFAST SYBR kit (Bioline). Primer sequences are as follows:

[831] ADRB2-F: 5'-CTCTTCCATCGTGTCCTTCTAC-3' (SEQ ID NO:1),

[832] ADRB2-R: 5'-AATCTTCTGGAGCTGCCTTT-3' (SEQ ID NO:2);

[833] HRH1-F: 5'- CCTCTGCTGGATCCCTTATTTC-3' (SEQ ID NO:3),

[834] HRH1-R: 5'-GGTTCAGTGTGGAGTTGATGTA-3' (SEQ ID NO:4);

[835] CXCR4-F: 5'- CCACCATCTACTCCATCATCTTC-3' (SEQ ID NO:5),

[836] CXCR4-R: 5'-ACTTGTCCGTCATGCTTCTC-3' (SEQ ID NO:6);

[837] β-actin-F: 5'-GGAAATCGTGCGTGACATTAAG-3' (SEQ ID NO:7),

[838] β-actin-R: 5'-AGCTCGTAGCTCTTCTCCA-3' (SEQ ID NO:8); GAPDH-F:

[839] 5'-ATGACATCAAGAAGGTGGTGAA-3' (SEQ ID NO:9), GAPDH-R:

[840] 5'-GCTGTTGAAGTCAGAGGAGAC-3' (SEQ ID NO:10).

[841] Threshold cycles (Ct) were calculated by QuantStudio 3 Real-Time PCR system (Thermo Fisher Scientific).

[842]

[843] **Lentivirus production**

[844] pLenti CMV Hygro DEST (w117-1) was a gift from Eric Campeau & Paul Kaufman (Addgene plasmid # 17454) (Campeau et al., 2009). CXCR4 cDNA was inserted into the lentiviral vector using LR recombination. Lentiviruses encoding CXCR4 were produced using ViraPower Lentiviral Expression Systems (Invirtogen).

[845]

[846] **Construction of Stable Cell Lines Expressing CXCR4 alone or CXCR4 and ADRB2 heteromer**

[847] To establish stable CXCR4 expressing cell lines, lentiviral stock (containing the packaged pLenti6-CXCR4 expression construct which inserted with CXCR4 gene in pLenti-CMV Hygro DEST (Addgene, #17454)), were produced by co-transfecting the ViraPower Lentiviral Packaging Mix (Invitrogen, K497500) and pLenti6-CXCR4 expression construct into 293FT producer cell line. Transduction of this lentiviral stock into A549 cell line was performed and followed by selection with hygromycin (100 μg/mL) and blasticitin (5 μg/mL). To establish stable CXCR4-ADRB2 heteromer expressing cell lines, lentiviral stock (containing the packaged pLenti6/V5-ADRB2 expression construct which inserted with ADRB2 gene in pLenti6/V5-DEST Gateway™ Vector (Invitrogen, V49610)), were produced by co-transfecting the ViraPower Packaging Mix and pLenti6/V5-ADRB2 expression construct into 293FT producer cell line. Transduction of this lentiviral stock into A549-CXCR4 cell line was performed and followed by selection with hygromycin (100 μg/mL) and blasticitin (5 μg/mL). Then clones resistant to antibiotics were selected and performed RT-qPCR and immunofluorescence to confirm the expression of the inserted gene, CXCR4 and ADRB2.

[848]

[849] **Mouse Xenograft Model**

[850] Five week-old, female Balb/c-nu/nu mice were obtained from Envigo (France) and maintained in specific pathogen-free animal facility. All protocols for animal use and euthanasia were approved by the Qubest Bio (South Korea) Animal Experimental Ethics Committee based on the Animal Protection Act. The $1x10^7$ cells of A549,

A549-CXCR4 or A549-CXCR4-ADRB2 were suspended in 100 μL of phosphate-buffered saline (PBS) and were implanted by subcutaneously in axillary region between the clavicular and chest wall on the right side of the mouse. The tumor growth was monitored every third or fourth day by measuring the length (*L*) and width (*W*) of the tumor with an electronic caliper and calculating tumor volume in the basis of the following formula: Volume = 0.5 LW^2.

[851]

[852] **Generation of CXCR4 or HRH1 Knockout Cells Using CRISPR/Cas9 System**

[853] CRISPR guide RNAs targeting CXCR4 and HRH1, and non-targeting guide RNA cloned in lentiCRISPR v2 vector were purchased from GenScript (Piscataway, NJ) (Sanjana et al., 2014). The guide RNA sequences are as follows:

[854] TGTTGGCTGCCTTACTACAT (CXCR4 gRNA #1; (SEQ ID NO:11)),

[855] CGATCAAGTCCGCCACCGAG (HRH gRNA #3; (SEQ ID NO:12)), and

[856] ACGGAGGCTAAGCGTCGCAA (non-targeting gRNA; (SEQ ID NO:13)).

Lentiviruses were produced using ViraPower Lentiviral Expression Systems (Invirtogen). MDA-MB-231 cells and MDA-MB-231 cells overexpressing CXCR4 (MDA^{CXCR4+}) were transduced with lentiviruses encoding non-targeting gRNA, CXCR4 gRNA, or HRH1 gRNAs. Cells were selected with puromycin (3 μM) for 2 weeks and loss of CXCR4 and HRH1 was estimated with immunoblotting and calcium responses, respectively. For detecting CXCR4, anti-CXCR4 rabbit monoclonal antibody (Abcam, #ab124824) was used.

[857]

[858] **Sourceof GPCRx inhibitors**

[859] Ulocuplumab was purchased from Creative Biolabs (Shirley, NY, USA). BKT140 was purchased from Chem Scene (Monmouth Junction, NJ, USA). Carazolol was purchased from Santacruz Biotech (Dallas, TX, USA). Osanetant was purchased from Axon Medchem, (Groningen, Netherland). M65 and CT-(8-32) (salmon) were from Bachem (San Diego, CA, USA). PACAP-(6-38), W54011, PMX205, PMX53, AC187, SB 222200, and Talnetant were from Tocris Bioscience (Ellisville, MO, USA). Pro-pranolol, Prometazine, Cyproheptadine, Hydroxyzine, Ambrisentan and macitentan were from Prestwick Chemical (Illkirch, France), and Selleckchem (Huston, TX, USA), respectively.

[860]

[861] ***In vivo*** studies

[862] For the antitumor efficacy test using CXCR4 and ADRB2 inhibitor, A549-CXCR4-ADRB2 cell line overexpressing CXCR4 and ADRB2 in A549, lung cancer cell line was subcutaneously administered ($1x10^7$ cell in 100 μL PBS / head) to BALB/c-nu. When Tumor size reached 50-100 mm^3, groups of ten mice were admin-

istrated with vehicle (1% dimethylcellulose in PBS), CXCR4 inhibitor (3 mg/kg of LY2510924 formulated in PBS, 10 mg/kg of AMD070 formulated in DMSO), ADRB2 inhibitor (30 mg/kg of Carvedilol formulated in 1% dimethylcellulose in PBS) or combination of CXCR4 and ADRB2 inhibitor, AMD070 and Carvedilol, or LY2510924 and Carvedilol once a day for 21-23 days. LY2510924 was subcutaneously injected and AMD070 or Carvedilol was administrated orally. The tumor growth was monitored every third or fourth day by measuring the length(L) and width(W) of the tumor: Volume = 0.5 LW^2.

[863]

[864] **References**

[865] Each of the following references, in their entirety, are incorporated herein by reference:

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WHAT IS CLAIMED IS:

1. A method for treating a cancer in a subject having a CXCR4-GPCRx heteromer, said method including the step of administering to the subject a therapeutically effective amount of a CXCR4 inhibitor and a GPCRx inhibitor, wherein:

(i) said CXCR4-GPCRx heteromer has enhanced signaling downstream of CXCR4; and

(ii) said CXCR4 inhibitor and said GPCRx inhibitor suppress the enhanced signaling downstream of CXCR4 from said CXCR4-GPCRx heteromer in the subject;

wherein said CXCR4 inhibitor is selected from the group consisting of: AMD3100 (plerixafor), ulocuplumab (MDX1338/BMS-936564), BKT140 (BL-8040; TF14016; 4F-Benzoyl-TN14003), AD-114, AD-114-6H, AD-114-Im7-FH, AD-114-PA600-6H, ALX-0651, ALX40-4C, AMD070 (AMD11070, X4P-001), AMD3465, ATI 2341, CTCE-9908, CX549, D-[Lys3] GHRP-6, FC122, FC131, GMI-1359, GSK812397, GST-NT21MP, isothiourea-1a, isothiourea-1t (IT1t), KRH-1636, KRH-3955, LY2510924, LY2624587, MSX-122, N-[^{11}C]Methyl-AMD3465, PF- 06747143, POL6326, SDF-1 1-9[P2G] dimer, SDF1 P2G, T134, T140, T22, TC 14012, TG-0054 (Burixafor), USL311, viral macrophage inflammatory protein-II (vMIP-II), WZ811, 12G5, 238D2, 238D4, [^{64}Cu]-AMD3100, [^{64}Cu]-AMD3465, [^{68}Ga]pentixafor, [^{90}Y]pentixather, [^{99m}Tc]O2- AMD3100, [^{177}Lu]pentixather, and 508MCl (Compound 26);

wherein said GPCRx inhibitor is an inhibitor selected from the group consisting of:

an ADRB2 inhibitor selected from the group consisting of Alprenolol, atenolol, betaxolol, bupranolol, butoxamine, carazolol, carvedilol, CGP 12177, cicloprolol, ICI 118551, ICYP, labetalol, levobetaxolol, levobunolol, LK 204-545, metoprolol, nadolol, NIHP, NIP, propafenone, propranolol, sotalol, SR59230A, and timolol,

an HRH1 inhibitor selected from the group consisting of (-)-chlorpheniramine, (+)-chlorpheniramine, (-)-*trans*-H2-PAT, (+)-*cis*-H2-PAT, (+)-*trans*-H2-PAT, (±)-*cis*-H2-PAT, (±)-*trans*- H2-PAT, (*R*)-cetirizine, (*S*)-cetirizine, 9-OH-risperidone, A-317920, A-349821, ABT-239, alimemazine, amitriptyline, aripiprazole, arpromidine, asenapine, astemizole, AZD3778, azelastine, BU-E 47, cetirizine, chlorpheniramine, chlorpromazine, ciproxifan, clemastine, clobenpropit, clozapine, conessine, cyclizine, cyproheptadine, desloratadine, diphenhydramine, dosulepin, doxepin, epinastine, fexofenadine, fluphenazine, fluspirilene, haloperidol,

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hydroxyzine, impromidine, INCB-38579, JNJ-39758979, ketotifen, loratadine, loxapine, MK-0249, molindone, olanzapine, perphenazine, pimozide, pipamperone, pitolisant, promethazine, pyrilamine, quetiapine, risperidone, sertindole, terfenadine, thioridazine, thiothixene, trifluoperazine, tripelennamine, triprolidine, ziprasidone, and zotepine,

a TACR3 inhibitor selected from the group consisting of [Trp^7, β-Ala^8] neurokinin A-(4- 10), AZD2624, FK 224, GR138676, GSK 172981, GSK 256471, N',2-diphenylquinoline-4-carbohydrazide 8m, N′,2-diphenylquinoline-4-carbohydrazide, osanetant, PD 154740, PD 161182, PD157672, saredutant, SB 218795, SB 222200, SB 235375, SCH 206272, SSR 146977, and talnetant,

an ADCYAP1R1 inhibitor selected from the group consisting of M65, Max.d.4, MK-0893, N-stearyl-[Nle^{17}] neurotensin-(6-11)/VIP-(7-28), PACAP-(6-38), and PG 97-269,

an ADORA2B inhibitor selected from the group consisting of 3-isobutyl-8-pyrrolidinoxanthine, alloxazine, AS16, AS70, AS74, AS94, AS95, AS96, AS99, AS100, AS101, ATL802, BW-A1433, caffeine, CGS 15943, CPX, CSC, CVT-6883, DAX, DEPX, derenofylline, DPCPX, FK-453, I-ABOPX, istradefylline, KF26777, LAS38096, LUF5981, MRE 2029F20, MRE 3008F20, MRS1191, MRS1220, MRS1523, MRS1706, MRS1754, MSX-2, OSIP339391, pentoxifylline, preladenant, PSB-10, PSB-11, PSB36, PSB603, PSB-0788, PSB1115, rolofylline, SCH 58261, SCH442416, ST-1535, theophylline, tonapofylline, vipadenant, xanthine amine congener, XCC, and ZM-241385,

an ADORA3 inhibitor selected from the group consisting of ATL802, BW-A1433, caffeine, CGS 15943, CSC, CVT-6883, derenofylline, dexniguldipine, DPCPX, FK-453, flavanone, flavone, galangin, I-ABOPX, istradefylline, KF26777, LAS38096, LUF5981, MRE 2029F20, MRE 3008F20, MRE 3010F20, MRS1041, MRS1042, MRS1067, MRS1088, MRS1093, MRS1097, MRS1177, MRS1186, MRS1191, MRS1191, MRS1220, MRS1476, MRS1486, MRS1505, MRS1523, MRS1754, MRS928, MSX-2, nicardipine, preladenant, PSB-10, PSB-11, PSB36, PSB603, PSB1115, rolofylline, sakuranetin, SCH 58261, SCH442416, ST-1535, theophylline, tonapofylline, vipadenant, visnagin, VUF5574, VUF8504, VUF8507, xanthine amine congener, and ZM-241385,

a C5AR1 inhibitor selected from the group consisting of $A8^{\Delta 71\text{-}73}$, AcPhe-Orn-Pro-D-Cha-Trp-Arg, avacopan, C089, CHIPS, DF2593A, JPE1375, L-156,602, NDT9520492, *N*-methyl-Phe-Lys-Pro-D-Cha-Trp-D-Arg-CO_2H, PMX205, PMX53, RPR121154, and W54011,

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a CALCR inhibitor selected from the group consisting of α-CGRP-(8-37) (human), AC187, CT-(8-32) (salmon), and olcegepant,

a CHRM1 inhibitor selected from the group consisting of 3-Quinuclidinyl benzilate (QNB), 4-DAMP, aclidinium, AE9C90CB, AFDX384, amitriptyline, AQ-RA 741, atropine, benzatropine, biperiden, darifenacin, dicyclomine, dosulepin, ethopropazine, glycopyrrolate, guanylpirenzepine, hexahydrodifenidol, hexahydrosiladifenidol, hexocyclium, himbacine, ipratropium, lithocholylcholine, methoctramine, ML381, muscarinic toxin 1, muscarinic toxin 2, muscarinic toxin 3, N-methyl scopolamine, otenzepad, oxybutynin, *p*-F-HHSiD, pirenzepine, propantheline, (R,R)-quinuclidinyl-4-fluoromethyl-benzilate, scopolamine, silahexocyclium, solifenacin, telenzepine, tiotropium, tolterodine, trihexyphenidyl, tripitramine, UH-AH 37, umeclidinium, and VU0255035,

an EDNRB inhibitor selected from the group consisting of A192621, ambrisentan, atrasentan, bosentan (RO 470203, Tracleer), BQ788, IRL 2500, K-8794, macitentan, RES7011, Ro 46-8443, SB209670, SB217242 (enrasentan), TAK 044, and tezosentan (RO610612),

an MLNR inhibitor selected from the group consisting of GM-109, MA-2029, and OHM- 11526, and

an NTSR1 inhibitor selected from the group consisting of Meclinertant, SR48527, SR48692, and SR142948A, and

wherein the GPCRx of the subject having a CXCR4-GPCRx heteromer is selected from the group consisting of ADRB2, HRH1, TACR3, ADCYAP1R1, ADORA2B, ADORA3, C5AR1, CALCR, CHRM1, EDNRB, MLNR, and NTSR1, and wherein the GPCRx which the GPCRx inhibitor targets is the same as that of the subject having a CXCR4-GPCRx heteromer.

2. Use of a GPCRx inhibitor in the manufacture of a medicament for treating a cancer in a subject having a CXCR4-GPCRx heteromer, wherein the GPCRx inhibitor is formulated to be administered in combination with a CXCR4 inhibitor, wherein:

(i) said CXCR4-GPCRx heteromer has enhanced signaling downstream of CXCR4; and

(ii) said CXCR4 inhibitor and said GPCRx inhibitor suppress the enhanced signaling downstream of CXCR4 from said CXCR4-GPCRx heteromer in the subject;

wherein said CXCR4 inhibitor is selected from the group consisting of: AMD3100 (plerixafor), ulocuplumab (MDX1338/BMS-936564), BKT140 (BL-8040; TF14016; 4F-Benzoyl-

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TN14003), AD-114, AD-114-6H, AD-114-Im7-FH, AD-114-PA600-6H, ALX-0651, ALX40-4C, AMD070 (AMD11070, X4P-001), AMD3465, ATI 2341, CTCE-9908, CX549, D-[Lys3] GHRP-6, FC122, FC131, GMI-1359, GSK812397, GST-NT21MP, isothiourea-1a, isothiourea-1t (IT1t), KRH-1636, KRH-3955, LY2510924, LY2624587, MSX-122, N-[^{11}C]Methyl-AMD3465, PF- 06747143, POL6326, SDF-1 1-9[P2G] dimer, SDF1 P2G, T134, T140, T22, TC 14012, TG-0054 (Burixafor), USL311, viral macrophage inflammatory protein-II (vMIP-II), WZ811, 12G5, 238D2, 238D4, [^{64}Cu]-AMD3100, [^{64}Cu]-AMD3465, [^{68}Ga]pentixafor, [^{90}Y]pentixather, [^{99m}Tc]O2- AMD3100, [^{177}Lu]pentixather, and 508MCl (Compound 26); wherein said GPCRx inhibitor is an inhibitor selected from the group consisting of:

an ADRB2 inhibitor selected from the group consisting of Alprenolol, atenolol, betaxolol, bupranolol, butoxamine, carazolol, carvedilol, CGP 12177, cicloprolol, ICI 118551, ICYP, labetalol, levobetaxolol, levobunolol, LK 204-545, metoprolol, nadolol, NIHP, NIP, propafenone, propranolol, sotalol, SR59230A, and timolol,

an HRH1 inhibitor selected from the group consisting of (-)-chlorpheniramine, (+)-chlorpheniramine, (-)-*trans*-H2-PAT, (+)-*cis*-H2-PAT, (+)-*trans*-H2-PAT, (±)-*cis*-H2-PAT, (±)-*trans*- H2-PAT, (*R*)-cetirizine, (*S*)-cetirizine, 9-OH-risperidone, A-317920, A-349821, ABT-239, alimemazine, amitriptyline, aripiprazole, arpromidine, asenapine, astemizole, AZD3778, azelastine, BU-E 47, cetirizine, chlorpheniramine, chlorpromazine, ciproxifan, clemastine, clobenpropit, clozapine, conessine, cyclizine, cyproheptadine, desloratadine, diphenhydramine, dosulepin, doxepin, epinastine, fexofenadine, fluphenazine, fluspirilene, haloperidol, hydroxyzine, impromidine, INCB-38579, JNJ-39758979, ketotifen, loratadine, loxapine, MK-0249, molindone, olanzapine, perphenazine, pimozide, pipamperone, pitolisant, promethazine, pyrilamine, quetiapine, risperidone, sertindole, terfenadine, thioridazine, thiothixene, trifluoperazine, tripelennamine, triprolidine, ziprasidone, and zotepine,

a TACR3 inhibitor selected from the group consisting of [Trp^7, β-Ala^8] neurokinin A-(4- 10), AZD2624, FK 224, GR138676, GSK 172981, GSK 256471, N',2-diphenylquinoline-4-carbohydrazide 8m, N′,2-diphenylquinoline-4-carbohydrazide, osanetant, PD 154740, PD 161182, PD157672, saredutant, SB 218795, SB 222200, SB 235375, SCH 206272, SSR 146977, and talnetant,

an ADCYAP1R1 inhibitor selected from the group consisting of M65, Max.d.4, MK-0893, N-stearyl-[Nle^{17}] neurotensin-(6-11)/VIP-(7-28), PACAP-(6-38), and PG 97-269,

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an ADORA2B inhibitor selected from the group consisting of 3-isobutyl-8-pyrrolidinoxanthine, alloxazine, AS16, AS70, AS74, AS94, AS95, AS96, AS99, AS100, AS101, ATL802, BW-A1433, caffeine, CGS 15943, CPX, CSC, CVT-6883, DAX, DEPX, derenofylline, DPCPX, FK-453, I-ABOPX, istradefylline, KF26777, LAS38096, LUF5981, MRE 2029F20, MRE 3008F20, MRS1191, MRS1220, MRS1523, MRS1706, MRS1754, MSX-2, OSIP339391, pentoxifylline, preladenant, PSB-10, PSB-11, PSB36, PSB603, PSB-0788, PSB1115, rolofylline, SCH 58261, SCH442416, ST-1535, theophylline, tonapofylline, vipadenant, xanthine amine congener, XCC, and ZM-241385,

an ADORA3 inhibitor selected from the group consisting of ATL802, BW-A1433, caffeine, CGS 15943, CSC, CVT-6883, derenofylline, dexniguldipine, DPCPX, FK-453, flavanone, flavone, galangin, I-ABOPX, istradefylline, KF26777, LAS38096, LUF5981, MRE 2029F20, MRE 3008F20, MRE 3010F20, MRS1041, MRS1042, MRS1067, MRS1088, MRS1093, MRS1097, MRS1177, MRS1186, MRS1191, MRS1191, MRS1220, MRS1476, MRS1486, MRS1505, MRS1523, MRS1754, MRS928, MSX-2, nicardipine, preladenant, PSB-10, PSB-11, PSB36, PSB603, PSB1115, rolofylline, sakuranetin, SCH 58261, SCH442416, ST-1535, theophylline, tonapofylline, vipadenant, visnagin, VUF5574, VUF8504, VUF8507, xanthine amine congener, and ZM-241385,

a C5AR1 inhibitor selected from the group consisting of $A8^{\Delta 71\text{-}73}$, AcPhe-Orn-Pro-D-Cha-Trp-Arg, avacopan, C089, CHIPS, DF2593A, JPE1375, L-156,602, NDT9520492, *N*-methyl-Phe-Lys-Pro-D-Cha-Trp-D-Arg-CO_2H, PMX205, PMX53, RPR121154, and W54011,

a CALCR inhibitor selected from the group consisting of α-CGRP-(8-37) (human), AC187, CT-(8-32) (salmon), and olcegepant,

a CHRM1 inhibitor selected from the group consisting of 3-Quinuclidinyl benzilate (QNB), 4-DAMP, aclidinium, AE9C90CB, AFDX384, amitriptyline, AQ-RA 741, atropine, benzatropine, biperiden, darifenacin, dicyclomine, dosulepin, ethopropazine, glycopyrrolate, guanylpirenzepine, hexahydrodifenidol, hexahydrosiladifenidol, hexocyclium, himbacine, ipratropium, lithocholylcholine, methoctramine, ML381, muscarinic toxin 1, muscarinic toxin 2, muscarinic toxin 3, N-methyl scopolamine, otenzepad, oxybutynin, *p*-F-HHSiD, pirenzepine, propantheline, (R,R)-quinuclidinyl-4-fluoromethyl-benzilate, scopolamine, silahexocyclium, solifenacin, telenzepine, tiotropium, tolterodine, trihexyphenidyl, tripitramine, UH-AH 37, umeclidinium, and VU0255035,

an EDNRB inhibitor selected from the group consisting of A192621, ambrisentan,

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atrasentan, bosentan (RO 470203, Tracleer), BQ788, IRL 2500, K-8794, macitentan, RES7011, Ro 46-8443, SB209670, SB217242 (enrasentan), TAK 044, and tezosentan (RO610612),

an MLNR inhibitor selected from the group consisting of GM-109, MA-2029, and OHM- 11526, and

an NTSR1 inhibitor selected from the group consisting of Meclinertant, SR48527, SR48692, and SR142948A, and

wherein the GPCRx of the subject having a CXCR4-GPCRx heteromer is selected from the group consisting of ADRB2, HRH1, TACR3, ADCYAP1R1, ADORA2B, ADORA3, C5AR1, CALCR, CHRM1, EDNRB, MLNR, and NTSR1, and wherein the GPCRx which the GPCRx inhibitor targets is the same as that of the subject having a CXCR4-GPCRx heteromer.

3. Use of a CXCR4 inhibitor in the manufacture of a medicament for treating a cancer in a subject having a CXCR4-GPCRx heteromer, wherein the CXCR4 inhibitor is formulated to be administered in combination with a GPCRx inhibitor, wherein:

(i) said CXCR4-GPCRx heteromer has enhanced signaling downstream of CXCR4; and

(ii) said CXCR4 inhibitor and said GPCRx inhibitor suppress the enhanced signaling downstream of CXCR4 from said CXCR4-GPCRx heteromer in the subject;

wherein said CXCR4 inhibitor is selected from the group consisting of: AMD3100 (plerixafor), ulocuplumab (MDX1338/BMS-936564), BKT140 (BL-8040; TF14016; 4F-Benzoyl-TN14003), AD-114, AD-114-6H, AD-114-Im7-FH, AD-114-PA600-6H, ALX-0651, ALX40-4C, AMD070 (AMD11070, X4P-001), AMD3465, ATI 2341, CTCE-9908, CX549, D-[Lys3] GHRP-6, FC122, FC131, GMI-1359, GSK812397, GST-NT21MP, isothiourea-1a, isothiourea-1t (IT1t), KRH-1636, KRH-3955, LY2510924, LY2624587, MSX-122, N-[^{11}C]Methyl-AMD3465, PF- 06747143, POL6326, SDF-1 1-9[P2G] dimer, SDF1 P2G, T134, T140, T22, TC 14012, TG-0054 (Burixafor), USL311, viral macrophage inflammatory protein-II (vMIP-II), WZ811, 12G5, 238D2, 238D4, [^{64}Cu]-AMD3100, [^{64}Cu]-AMD3465, [^{68}Ga]pentixafor, [^{90}Y]pentixather, [^{99m}Tc]O2- AMD3100, [^{177}Lu]pentixather, and 508MCl (Compound 26);

wherein said GPCRx inhibitor is an inhibitor selected from the group consisting of:

an ADRB2 inhibitor selected from the group consisting of Alprenolol, atenolol, betaxolol, bupranolol, butoxamine, carazolol, carvedilol, CGP 12177, cicloprolol, ICI 118551,

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ICYP, labetalol, levobetaxolol, levobunolol, LK 204-545, metoprolol, nadolol, NIHP, NIP, propafenone, propranolol, sotalol, SR59230A, and timolol,

an HRH1 inhibitor selected from the group consisting of (-)-chlorpheniramine, (+)-chlorpheniramine, (-)-*trans*-H2-PAT, (+)-*cis*-H2-PAT, (+)-*trans*-H2-PAT, (±)-*cis*-H2-PAT, (±)-*trans*- H2-PAT, (*R*)-cetirizine, (*S*)-cetirizine, 9-OH-risperidone, A-317920, A-349821, ABT-239, alimemazine, amitriptyline, aripiprazole, arpromidine, asenapine, astemizole, AZD3778, azelastine, BU-E 47, cetirizine, chlorpheniramine, chlorpromazine, ciproxifan, clemastine, clobenpropit, clozapine, conessine, cyclizine, cyproheptadine, desloratadine, diphenhydramine, dosulepin, doxepin, epinastine, fexofenadine, fluphenazine, fluspirilene, haloperidol, hydroxyzine, impromidine, INCB-38579, JNJ-39758979, ketotifen, loratadine, loxapine, MK-0249, molindone, olanzapine, perphenazine, pimozide, pipamperone, pitolisant, promethazine, pyrilamine, quetiapine, risperidone, sertindole, terfenadine, thioridazine, thiothixene, trifluoperazine, tripelennamine, triprolidine, ziprasidone, and zotepine,

a TACR3 inhibitor selected from the group consisting of [Trp^7, β-Ala^8] neurokinin A-(4- 10), AZD2624, FK 224, GR138676, GSK 172981, GSK 256471, N',2-diphenylquinoline-4-carbohydrazide 8m, N′,2-diphenylquinoline-4-carbohydrazide, osanetant, PD 154740, PD 161182, PD157672, saredutant, SB 218795, SB 222200, SB 235375, SCH 206272, SSR 146977, and talnetant,

an ADCYAP1R1 inhibitor selected from the group consisting of M65, Max.d.4, MK-0893, N-stearyl-[Nle^{17}] neurotensin-(6-11)/VIP-(7-28), PACAP-(6-38), and PG 97-269,

an ADORA2B inhibitor selected from the group consisting of 3-isobutyl-8-pyrrolidinoxanthine, alloxazine, AS16, AS70, AS74, AS94, AS95, AS96, AS99, AS100, AS101, ATL802, BW-A1433, caffeine, CGS 15943, CPX, CSC, CVT-6883, DAX, DEPX, derenofylline, DPCPX, FK-453, I-ABOPX, istradefylline, KF26777, LAS38096, LUF5981, MRE 2029F20, MRE 3008F20, MRS1191, MRS1220, MRS1523, MRS1706, MRS1754, MSX-2, OSIP339391, pentoxifylline, preladenant, PSB-10, PSB-11, PSB36, PSB603, PSB-0788, PSB1115, rolofylline, SCH 58261, SCH442416, ST-1535, theophylline, tonapofylline, vipadenant, xanthine amine congener, XCC, and ZM-241385,

an ADORA3 inhibitor selected from the group consisting of ATL802, BW-A1433, caffeine, CGS 15943, CSC, CVT-6883, derenofylline, dexniguldipine, DPCPX, FK-453, flavanone, flavone, galangin, I-ABOPX, istradefylline, KF26777, LAS38096, LUF5981, MRE 2029F20, MRE 3008F20, MRE 3010F20, MRS1041, MRS1042, MRS1067, MRS1088,

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MRS1093, MRS1097, MRS1177, MRS1186, MRS1191, MRS1191, MRS1220, MRS1476, MRS1486, MRS1505, MRS1523, MRS1754, MRS928, MSX-2, nicardipine, preladenant, PSB-10, PSB-11, PSB36, PSB603, PSB1115, rolofylline, sakuranetin, SCH 58261, SCH442416, ST-1535, theophylline, tonapofylline, vipadenant, visnagin, VUF5574, VUF8504, VUF8507, xanthine amine congener, and ZM-241385,

a C5AR1 inhibitor selected from the group consisting of $A8^{\Delta 71\text{-}73}$, AcPhe-Orn-Pro-D-Cha-Trp-Arg, avacopan, C089, CHIPS, DF2593A, JPE1375, L-156,602, NDT9520492, *N*-methyl-Phe-Lys-Pro-D-Cha-Trp-D-Arg-CO_2H, PMX205, PMX53, RPR121154, and W54011,

a CALCR inhibitor selected from the group consisting of α-CGRP-(8-37) (human), AC187, CT-(8-32) (salmon), and olcegepant,

a CHRM1 inhibitor selected from the group consisting of 3-Quinuclidinyl benzilate (QNB), 4-DAMP, aclidinium, AE9C90CB, AFDX384, amitriptyline, AQ-RA 741, atropine, benzatropine, biperiden, darifenacin, dicyclomine, dosulepin, ethopropazine, glycopyrrolate, guanylpirenzepine, hexahydrodifenidol, hexahydrosiladifenidol, hexocyclium, himbacine, ipratropium, lithocholylcholine, methoctramine, ML381, muscarinic toxin 1, muscarinic toxin 2, muscarinic toxin 3, N-methyl scopolamine, otenzepad, oxybutynin, *p*-F-HHSiD, pirenzepine, propantheline, (R,R)-quinuclidinyl-4-fluoromethyl-benzilate, scopolamine, silahexocyclium, solifenacin, telenzepine, tiotropium, tolterodine, trihexyphenidyl, tripitramine, UH-AH 37, umeclidinium, and VU0255035,

an EDNRB inhibitor selected from the group consisting of A192621, ambrisentan, atrasentan, bosentan (RO 470203, Tracleer), BQ788, IRL 2500, K-8794, macitentan, RES7011, Ro 46-8443, SB209670, SB217242 (enrasentan), TAK 044, and tezosentan (RO610612),

an MLNR inhibitor selected from the group consisting of GM-109, MA-2029, and OHM- 11526, and

an NTSR1 inhibitor selected from the group consisting of Meclinertant, SR48527, SR48692, and SR142948A, and

wherein the GPCRx of the subject having a CXCR4-GPCRx heteromer is selected from the group consisting of ADRB2, HRH1, TACR3, ADCYAP1R1, ADORA2B, ADORA3, C5AR1, CALCR, CHRM1, EDNRB, MLNR, and NTSR1, and wherein the GPCRx which the GPCRx inhibitor targets is the same as that of the subject having a CXCR4-GPCRx heteromer.

4. The method of claim 1 or the use of claim 2 or claim 3, wherein said GPCRx inhibitor

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is:

(i) an ADRB2 inhibitor selected from the group consisting of: Alprenolol, atenolol, betaxolol, bupranolol, butoxamine, carazolol, carvedilol, CGP 12177, cicloprolol, ICI 118551, ICYP, labetalol, levobetaxolol, levobunolol, LK 204-545, metoprolol, nadolol, NIHP, NIP, propafenone, propranolol, sotalol, SR59230A, and timolol; or

(ii) an HRH1 inhibitor selected from the group consisting of: (-)-chlorpheniramine, (+)-chlorpheniramine, (-)-trans-H 2-PAT, (+)-cis-H 2-PAT, (+)-trans-H 2-PAT, (±)-cis-H 2-PAT, (±)- trans-H 2-PAT, (R)-cetirizine, (S)-cetirizine, 9-OH-risperidone, A-317920, A-349821, ABT-239, alimemazine, amitriptyline, aripiprazole, arpromidine, asenapine, astemizole, AZD3778, azelastine, BU-E 47, cetirizine, chlorpheniramine, chlorpromazine, ciproxifan, clemastine, clobenpropit, clozapine, conessine, cyclizine, cyproheptadine, desloratadine, diphenhydramine, dosulepin, doxepin, epinastine, fexofenadine, fluphenazine, fluspirilene, haloperidol, hydroxyzine, impromidine, INCB-38579, JNJ-39758979, ketotifen, loratadine, loxapine, MK- 0249, molindone, olanzapine, perphenazine, pimozide, pipamperone, pitolisant, promethazine, pyrilamine, quetiapine, risperidone, sertindole, terfenadine, thioridazine, thiothixene,trifluoperazine, tripelennamine, triprolidine, ziprasidone, and zotepine; or

(iii) a TACR3 inhibitor selected from the group consisting of: [Trp ^{7}, β-Ala ^{8}] neurokinin A-(4-10), AZD2624, FK 224, GR138676, GSK 172981, GSK 256471, N',2-diphenylquinoline-4-carbohydrazide 8m, N′,2-diphenylquinoline-4-carbohydrazide, osanetant, PD 154740, PD 161182, PD 157672, saredutant, SB 218795, SB 222200, SB 235375, SCH 206272, SSR 146977, and talnetant; or

(iv) an ADCYAP1R1 inhibitor selected from the group consisting of: M65, Max.d.4, MK-0893, N-stearyl-[Nle ^{17}] neurotensin-(6-11)/VIP-(7-28), PACAP-(6-38), and PG 97-269; or

(v) an ADORA2B inhibitor selected from the group consisting of: 3-isobutyl-8-pyrrolidinoxanthine, alloxazine, AS16, AS70, AS74, AS94, AS95, AS96, AS99, AS100, AS101, ATL802, BW-A1433, caffeine, CGS 15943, CPX, CSC, CVT-6883, DAX, DEPX, derenofylline, DPCPX, FK-453, I-ABOPX, istradefylline, KF26777, LAS38096, LUF5981, MRE 2029F20, MRE 3008F20, MRS1191, MRS1220, MRS1523, MRS1706, MRS1754, MSX-2, OSIP339391, pentoxifylline, preladenant, PSB-10, PSB-11, PSB36, PSB603, PSB-0788, PSB1115, rolofylline, SCH 58261, SCH442416, ST-1535, theophylline, tonapofylline,

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vipadenant, xanthine amine congener, XCC, and ZM-241385; or

(vi) an ADORA3 inhibitor selected from the group consisting of: ATL802, BW-A1433, caffeine, CGS 15943, CSC, CVT-6883, derenofylline, dexniguldipine, DPCPX, FK-453, flavanone, flavone, galangin, I-ABOPX, istradefylline, KF26777, LAS38096, LUF5981, MRE 2029F20, MRE 3008F20, MRE 3010F20, MRS1041, MRS1042, MRS1067, MRS1088, MRS1093, MRS1097, MRS1177, MRS1186, MRS1191, MRS1191, MRS1220, MRS1476, MRS1486, MRS1505, MRS1523, MRS1754, MRS928, MSX-2, nicardipine, preladenant, PSB-10, PSB-11, PSB36, PSB603, PSB1115, rolofylline, sakuranetin, SCH 58261, SCH442416, ST-1535, theophylline, tonapofylline, vipadenant, visnagin, VUF5574, VUF8504, VUF8507, xanthine amine congener, and ZM-241385; or

(vii) a CALCR inhibitor selected from the group consisting of α-CGRP-(8-37) (human), AC187, CT-(8-32) (salmon), and olcegepant; or

(viii) a CHRM1 inhibitor selected from the group consisting of: 3-Quinuclidinyl benzilate (QNB), 4-DAMP, aclidinium, AE9C90CB, AFDX384, amitriptyline, AQ-RA 741, atropine, benzatropine, biperiden, darifenacin, dicyclomine, dosulepin, ethopropazine, glycopyrrolate, guanylpirenzepine, hexahydrodifenidol, hexahydrosiladifenidol, hexocyclium, himbacine, ipratropium, lithocholylcholine, methoctramine, ML381, muscarinic toxin 1, muscarinic toxin 2, muscarinic toxin 3, N-methyl scopolamine, otenzepad, oxybutynin, p-F-HHSiD, pirenzepine, propantheline, (R,R)-quinuclidinyl-4-fluoromethyl-benzilate, scopolamine, silahexocyclium, solifenacin, telenzepine, tiotropium, tolterodine, trihexyphenidyl, tripitramine, UH-AH 37, umeclidinium, and VU0255035; or

(ix) an EDNRB inhibitor selected from the group consisting of: A192621, ambrisentan, atrasentan, bosentan (RO 470203, Tracleer), BQ788, IRL 2500, K-8794, macitentan, RES7011, Ro 46-8443, SB209670, SB217242 (enrasentan), TAK 044, and tezosentan (RO610612); or

(x) an MLNR inhibitor selected from the group consisting of GM-109, MA-2029, and OHM-11526; or

(xi) an NTSR1 inhibitor selected from the group consisting of Meclinertant, SR48527, SR48692, and SR142948A.

5. The method or use of claim 4(i), wherein said ADRB2 inhibitor is carazolol, carvedilol, or propranolol.

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6. The method or use of claim 4(i), wherein said ADRB2 inhibitor is propranolol.

7. The method or use of any one of claims 1 to 6, wherein said CXCR4- GPCRx heteromer components in a cancer cell colocalize and physically interact, either directly or *via* intermediate proteins acting as conduits for allosterism, as determined *via* one or more of the following: a co-internalization assay, a colocalization assay, *in situ* hybridization, immunohistochemistry, immunoelectron microscopy, a proximity-based assay, a co-immunoprecipitation assay, enzyme-linked immunosorbent assay (ELISA), flow cytometry, RNAseq, qRT-PCR, microarray, or a fluorescent animal assay.

8. The method or use of any one of claims 1 to 7, wherein said CXCR4- GPCRx heteromer has enhanced amount of calcium mobilization, such that:

(i) either the CXCR4 or the respective GPCRx in an individual protomer context in a cancer cell, upon co-stimulation with CXCL12 and a respective selective GPCRx agonist, results in a calcium mobilization amount that is equal to or less than the sum of calcium mobilization amounts resulting from single agonist stimulation with either said CXCL12 or said respective selective GPCRx agonist; and

(ii) said CXCR4-GPCRx heteromer exhibits an enhanced calcium mobilization upon co-stimulation with said CXCL12 and said respective selective GPCRx agonist relative to the sum of calcium mobilization amounts resulting from single agonist stimulation with either said CXCL12 or said respective selective GPCRx agonist;

as determined via a calcium mobilization assay.

9. The method or use of claim 8, wherein said enhanced amount of calcium mobilization is at least 10% greater, at least 20% greater, at least 30% greater, at least 40% greater, at least 50% greater, at least 75% greater, or at least 90% greater, than the sum of calcium mobilization amounts resulting from single agonist stimulation with either the CXCL12 or the respective selective GPCRx agonist, as determined via a calcium mobilization assay.

10. The method or use of claim 9, wherein said calcium mobilization assay is an intracellular Ca^{2+} assay.

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11. The method or use of any one of claims 1 to 10, wherein:

(i) progression of said cancer is decreased in the range of 5 to 100% more, relative to administering said CXCR4 inhibitor or said GPCRx inhibitor as a single inhibitor;

(ii) efficacy of said CXCR4 inhibitor is increased in the range of 5 to 2000%, relative to efficacy of said CXCR4 inhibitor when administered as a single inhibitor;

(iii) efficacy of said GPCRx inhibitor is increased in the range of 5 to 2000%, relative to efficacy of said GPCRx inhibitor when administered as a single inhibitor; and/or

(iv) said enhanced downstream signaling from said CXCR4-GPCRx heteromer is suppressed in the range of between 5 to 2000 fold, relative to administration of said CXCR4 inhibitor or said GPCRx inhibitor as a single inhibitor.

12. The method of any one of claims 1 and 4 to 11, further comprising the step of determining the presence of said CXCR4-GPCRx heteromer in a biological sample obtained from said subject using one or more of the following: a co-internalization assay, a colocalization assay, *in situ* hybridization, immunohistochemistry, immunoelectron microscopy, a proximity-based assay, a co-immunoprecipitation assay, enzyme- linked immunosorbent assay (ELISA), flow cytometry, RNAseq, qRT-PCR, microarray, or a fluorescent animal assay; or the use of any one of claims 2 to 11, wherein the presence of said CXCR4-GPCRx heteromer in a biological sample obtained from said subject has been determined using one or more of the following: a co-internalization assay, a colocalization assay, *in situ* hybridization, immunohistochemistry, immunoelectron microscopy, a proximity-based assay, a co-immunoprecipitation assay, enzyme- linked immunosorbent assay (ELISA), flow cytometry, RNAseq, qRT-PCR, microarray, or a fluorescent animal assay.

13. The method or use of claim 12, wherein said biological sample is a biological fluid sample or a biological tissue sample.

14. The method or use of claim 13, wherein said biological sample is a blood sample, a plasma sample, a saliva sample, a cerebral fluid sample, an eye fluid sample, a urine sample, an organ tissue sample, a bone tissue sample, or a tumor tissue sample.

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15. The method or use of any one of claims 1 to 14, wherein said cancer is selected from the group consisting of: tumors of the gastrointestinal tract, breast cancer, lung cancer, small cell carcinoma of the lung, hepatocellular carcinoma, brain cancer, kidney cancer, pancreatic cancer or pancreatic adenocarcinoma, ovarian cancer, prostate cancer, melanoma, lymphoma, leukemia, multiple myeloma, renal cell carcinoma, soft tissue sarcoma, gastrointestinal cancer, stomach cancer, colon cancer, colorectal cancer, colorectal adenocarcinoma, bladder adenocarcinoma, esophageal cancer, and adenocarcinoma of the stomach, esophagus, throat, and urogenital tract.

16. The method of any one of claims 1 and 4 to 15, wherein said CXCR4 inhibitor and said GPCRx inhibitor are administered sequentially, concurrently, or simultaneously; or the use of any one of claims 2 to 15, wherein said CXCR4 inhibitor and said GPCRx inhibitor are formulated to be administered sequentially, concurrently, or simultaneously.

17. The method of any one of claims 1 and 4 to 15, wherein said CXCR4 inhibitor and said GPCRx inhibitor are administered as: (a) separate formulations; or (b) one formulation; or the use of any one of claims 2 to 15, wherein said CXCR4 inhibitor and said GPCRx inhibitor are formulated to be administered as; (a) separate formulations; or (b) one formulation.

18. The use of any one of claims 2 to 15, wherein the medicament is formulated to comprise both said CXCR4 inhibitor and said GPCRx inhibitor.

19. A kit comprising a CXCR4 inhibitor and a GPCRx inhibitor, when used in a method for treating a cancer in a subject having a CXCR4-GPCRx heteromer, wherein:

(i) said CXCR4-GPCRx heteromer has enhanced signaling downstream of CXCR4; and

(ii) said CXCR4 inhibitor and said GPCRx inhibitor suppress the enhanced downstream signaling from said CXCR4-GPCRx heteromer in the subject,

wherein the GPCRx of the subject having a CXCR4-GPCRx heteromer is selected from the group consisting of ADRB2, HRH1, TACR3, ADCYAP1R1, ADORA2B, ADORA3, C5AR1, CALCR, CHRM1, EDNRB, MLNR, and NTSR1, and wherein the GPCRx which the GPCRx inhibitor targets is the same as that of the subject having a CXCR4-GPCRx heteromer,

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and

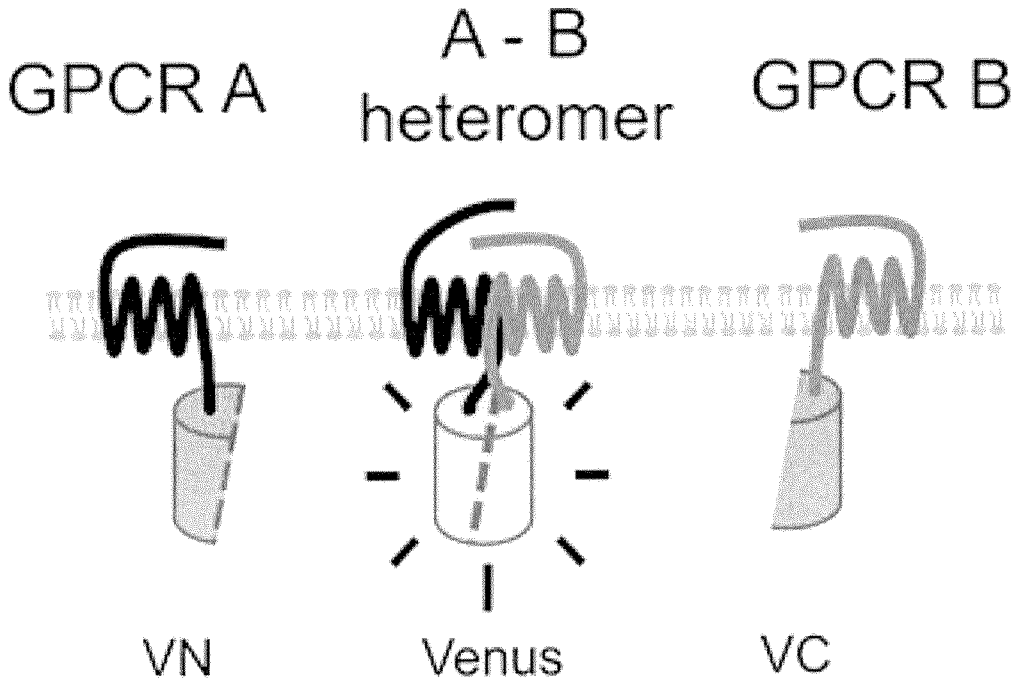
wherein said CXCR4 inhibitor and said GPCRx inhibitor are as defined in any one of claims 1 to 6.

20. The kit of claim 19, wherein the kit is when used such that said CXCR4 inhibitor and said GPCRx inhibitor are administered sequentially, concurrently, or simultaneously.

21. The kit of claim 19, wherein the kit is when used such that said CXCR4 inhibitor and said GPCRx inhibitor are administered as: (a) separate formulations; or
(b) one formulation.

22. The kit of any one of claims 19 to 21, when used in the method of any one of claims 1 and 4 to 17.

[FIG. 1]



[FIG. 2a]

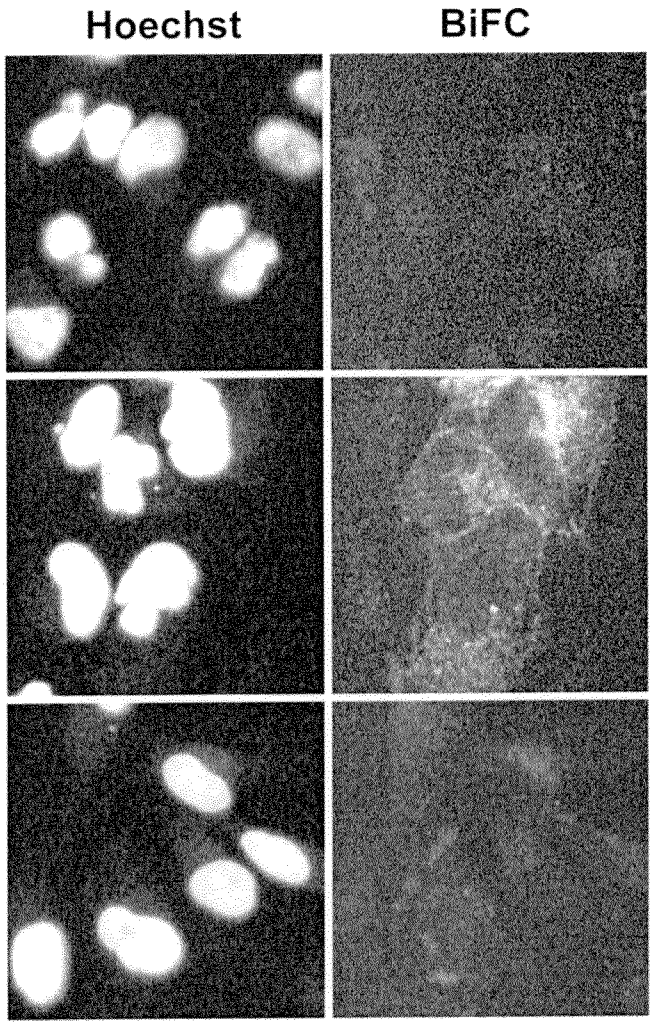
CXCR4-VN
& HA-VC

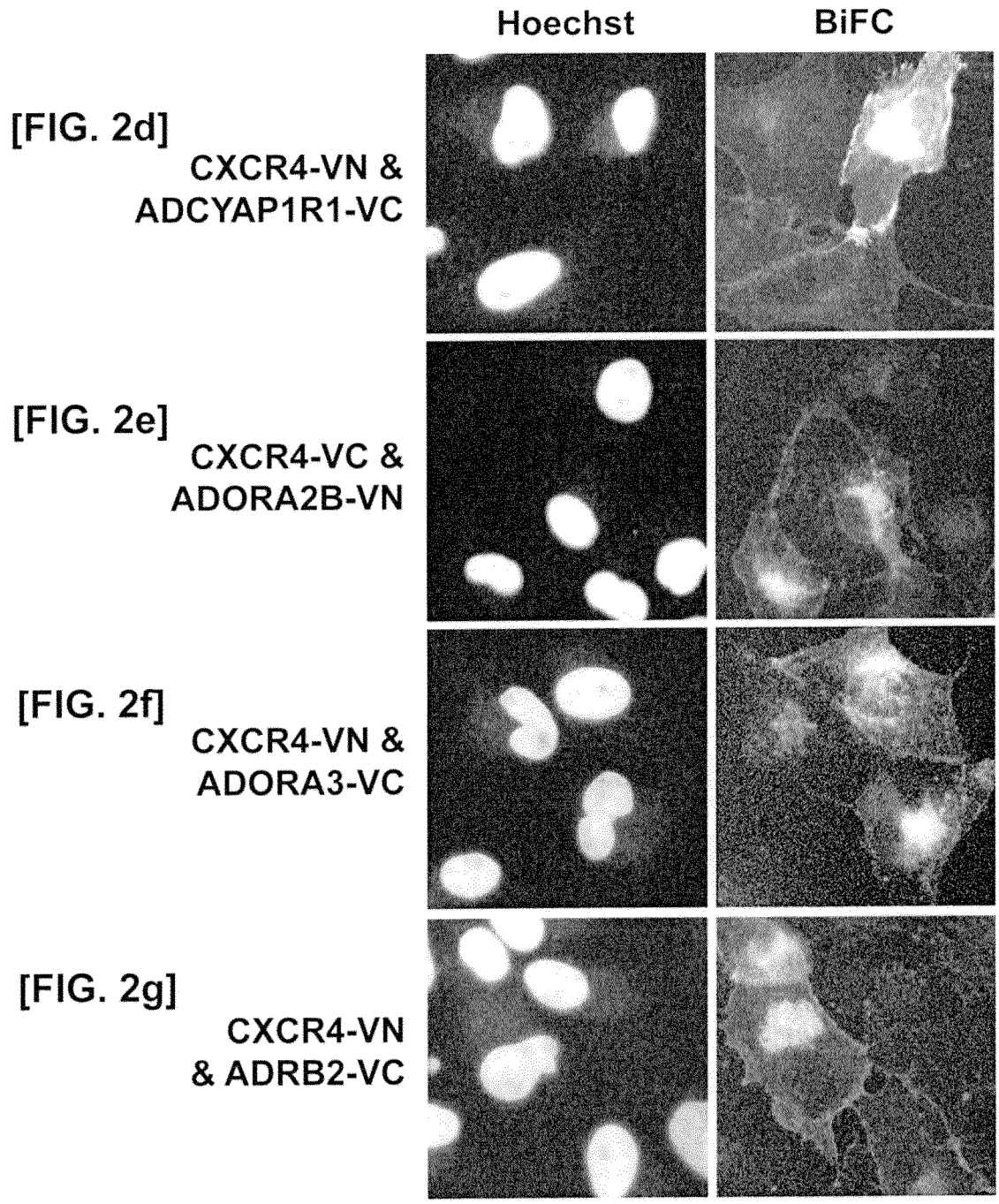
[FIG. 2b]

CXCR4-VN
& CXCR4-VC

[FIG. 2c]

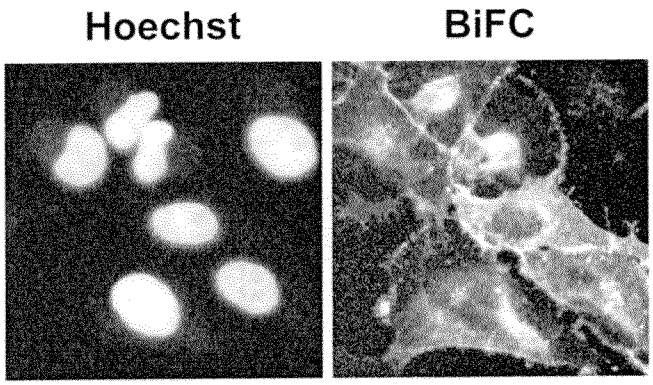
CXCR4-VN
& GCGR-VC




Hoechst
BiFC
[FIG. 2d]
CXCR4-VN &
ADCYAP1R1-VC
[FIG. 2e]
CXCR4-VC &
ADORA2B-VN
[FIG. 2f]
CXCR4-VN &
ADORA3-VC
[FIG. 2g]
CXCR4-VN
& ADRB2-VC

[FIG. 2h]

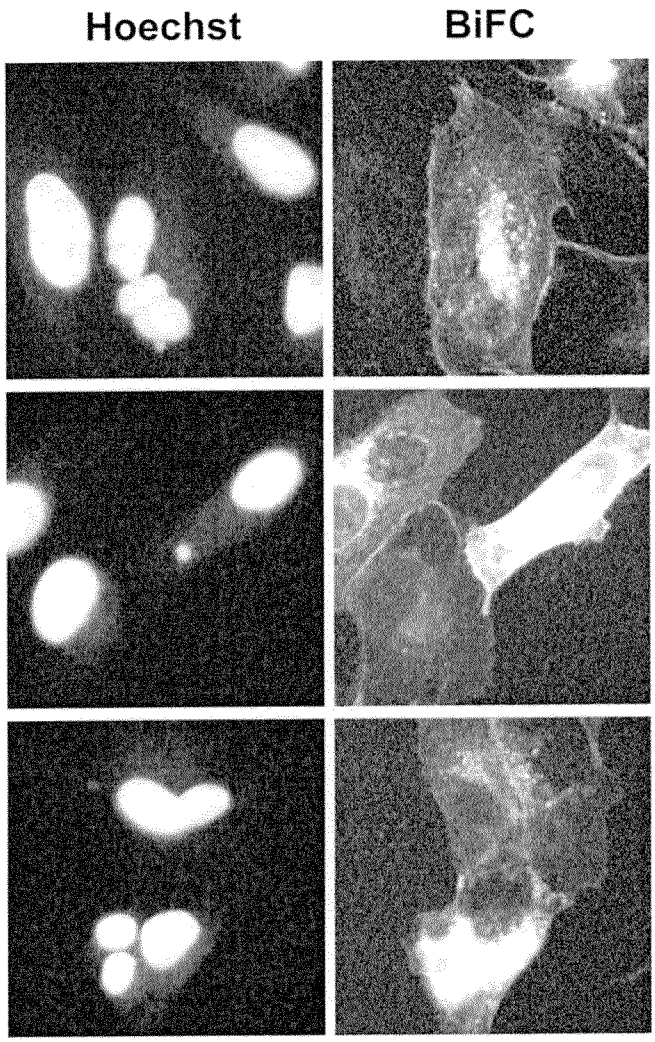
CXCR4-VN
& APLNR-VC



[FIG. 2i]
CXCR4-VN
& C5AR1-VC

[FIG. 2j]
CXCR4-VN
& CALCR-VC

[FIG. 2k]
CXCR4-VN
& CCR5-VC

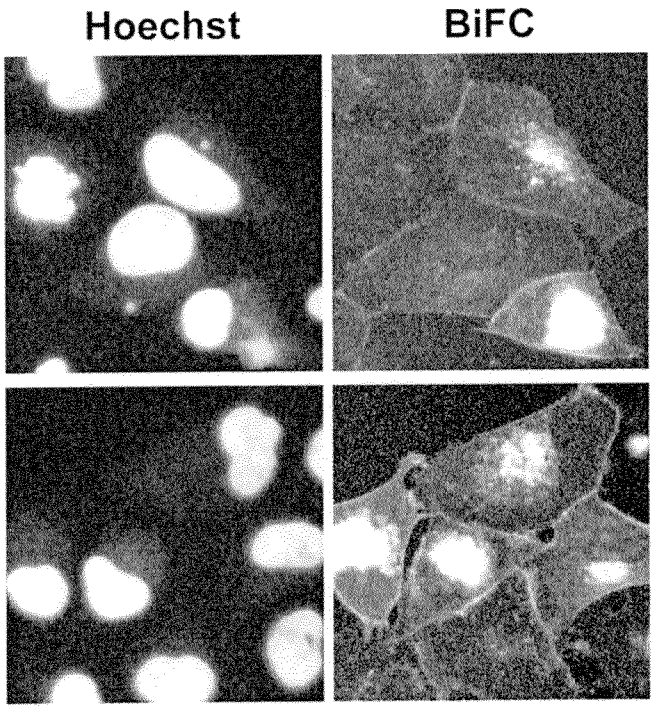


[FIG. 2l]

CXCR4-VN
& CHRM1-VC

[FIG. 2m]

CXCR4-VN
& GALR1-VC

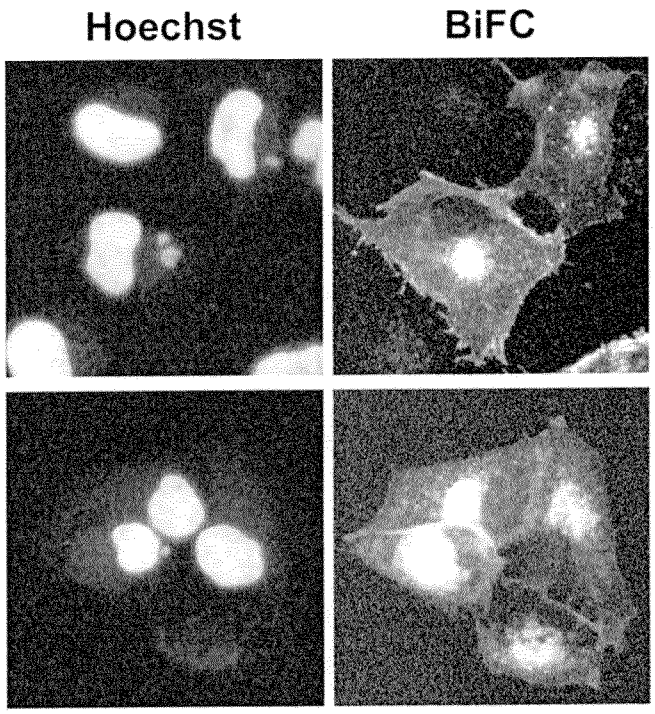


[FIG. 2n]

CXCR4-VN
& EDNRB-VC

[FIG. 2o]

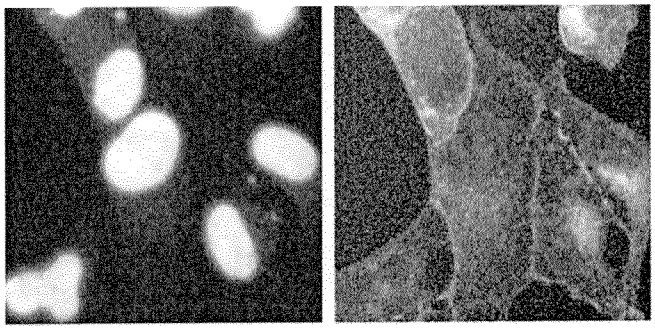
CXCR4-VN
& HRH1-VC



Hoechst BiFC

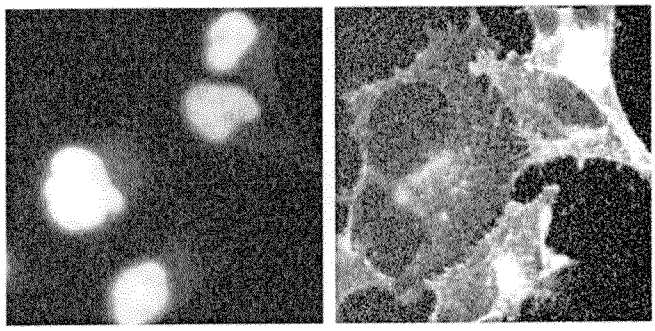
[FIG. 2p]

CXCR4-VN
& MLNR-VC



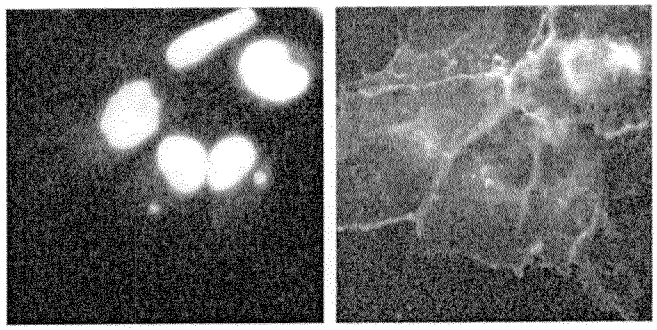
[FIG. 2q]

CXCR4-VN
& NTSR1-VC



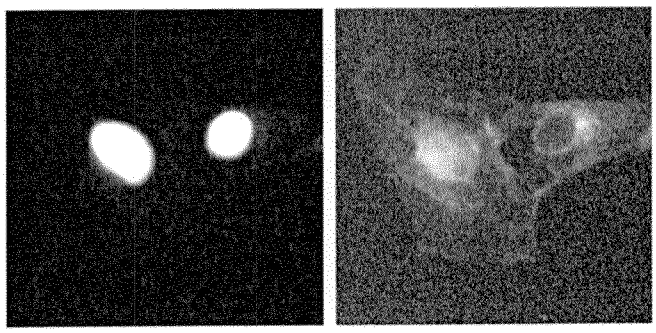
[FIG. 2r]

CXCR4-VN
& PTGER2-VC



[FIG. 2s]

CXCR4-VC
& PTGER3-VN

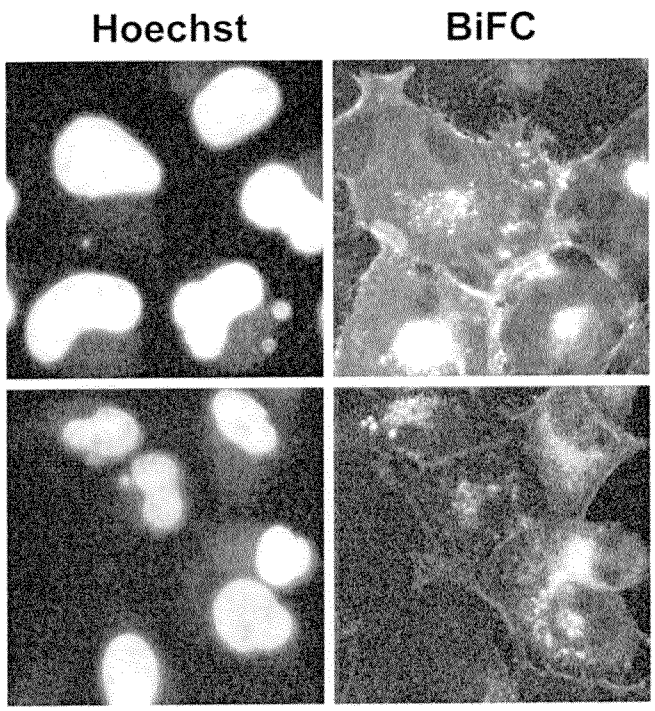


[FIG. 2t]

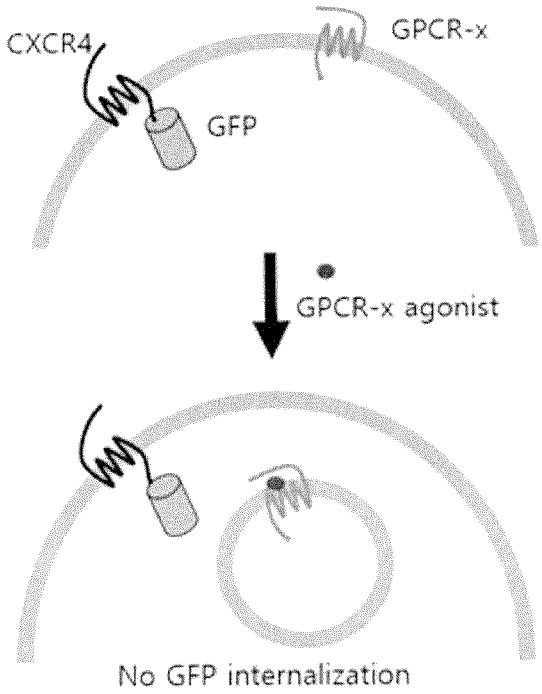
CXCR4-VN
& SSTR2-VC

[FIG. 2u]

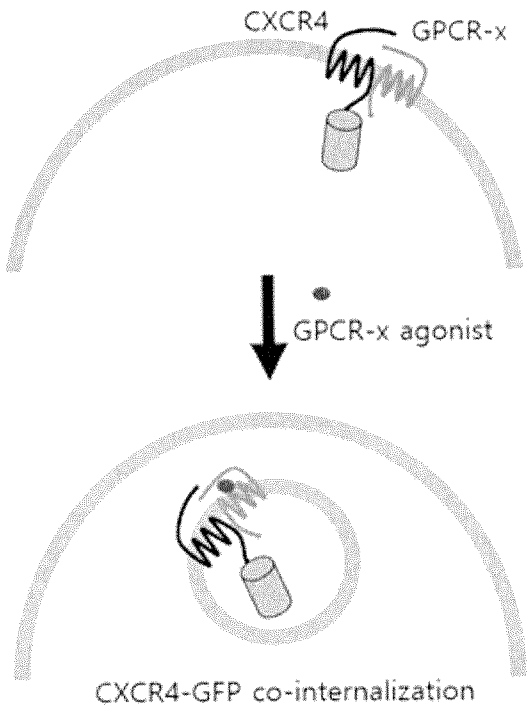
CXCR4-VN
& TACR3-VC



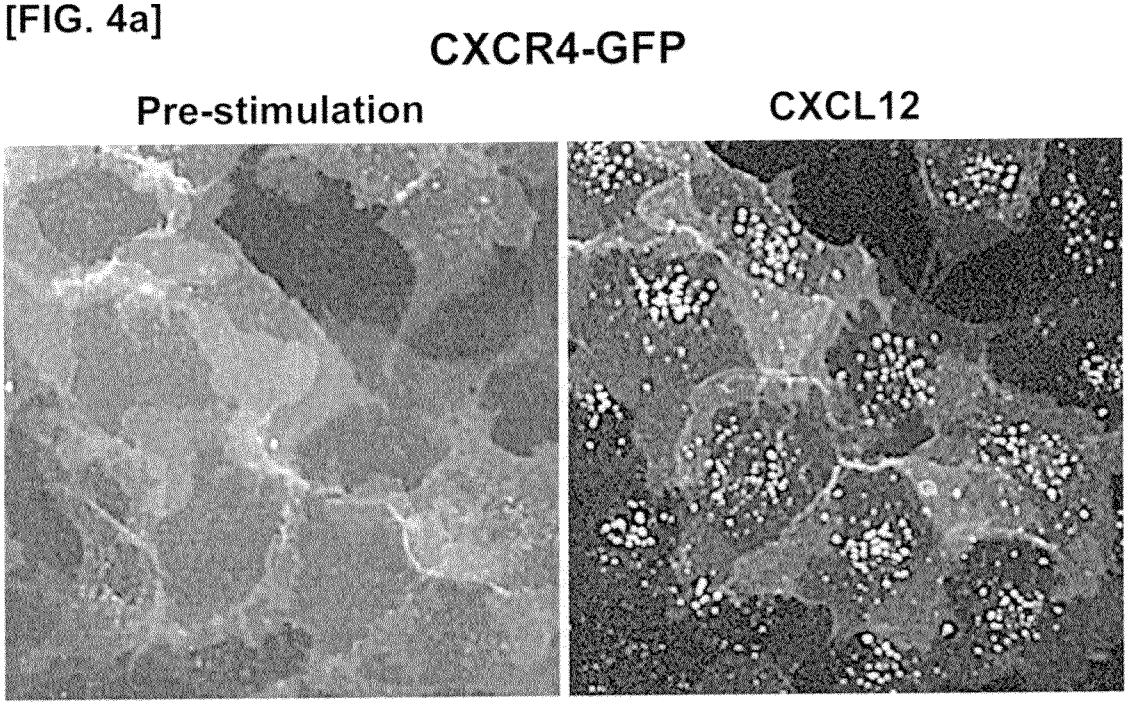
[FIG. 3a]



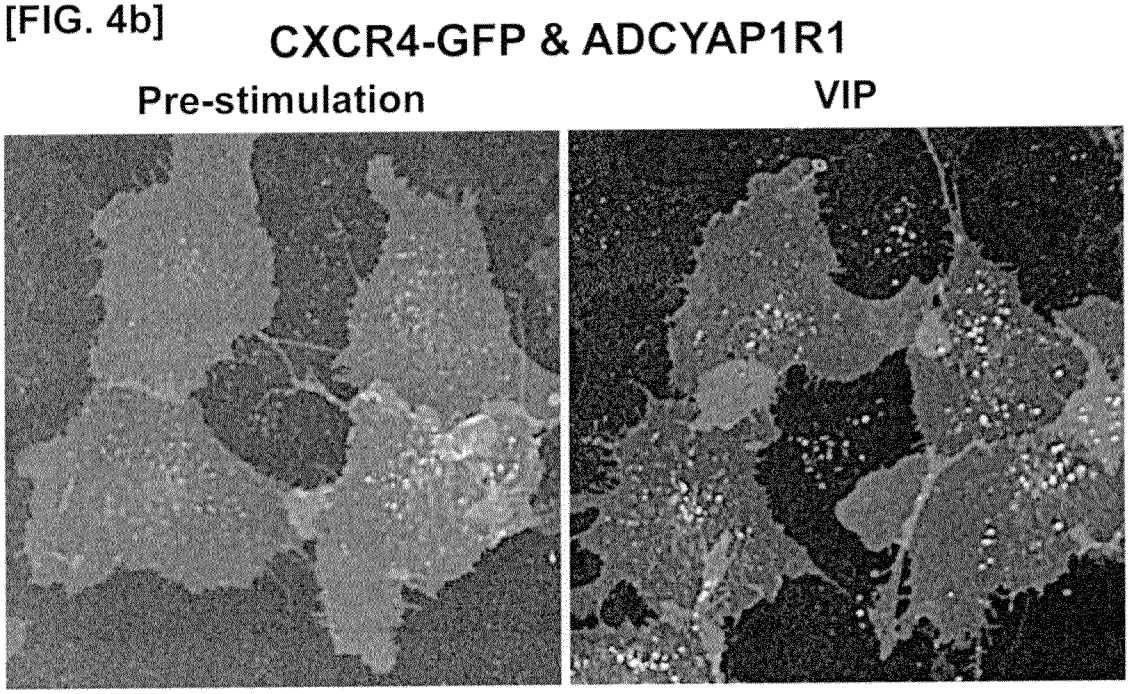
[FIG. 3b]



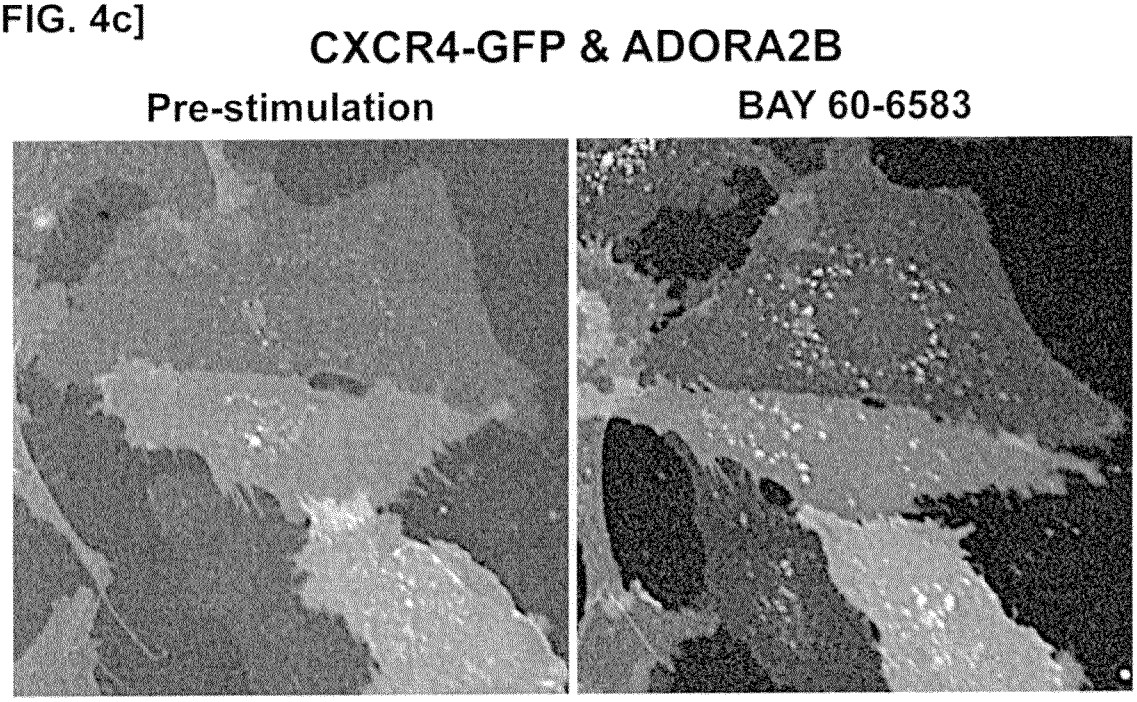
[FIG. 4a]


CXCR4-GFP
Pre-stimulation
CXCL12

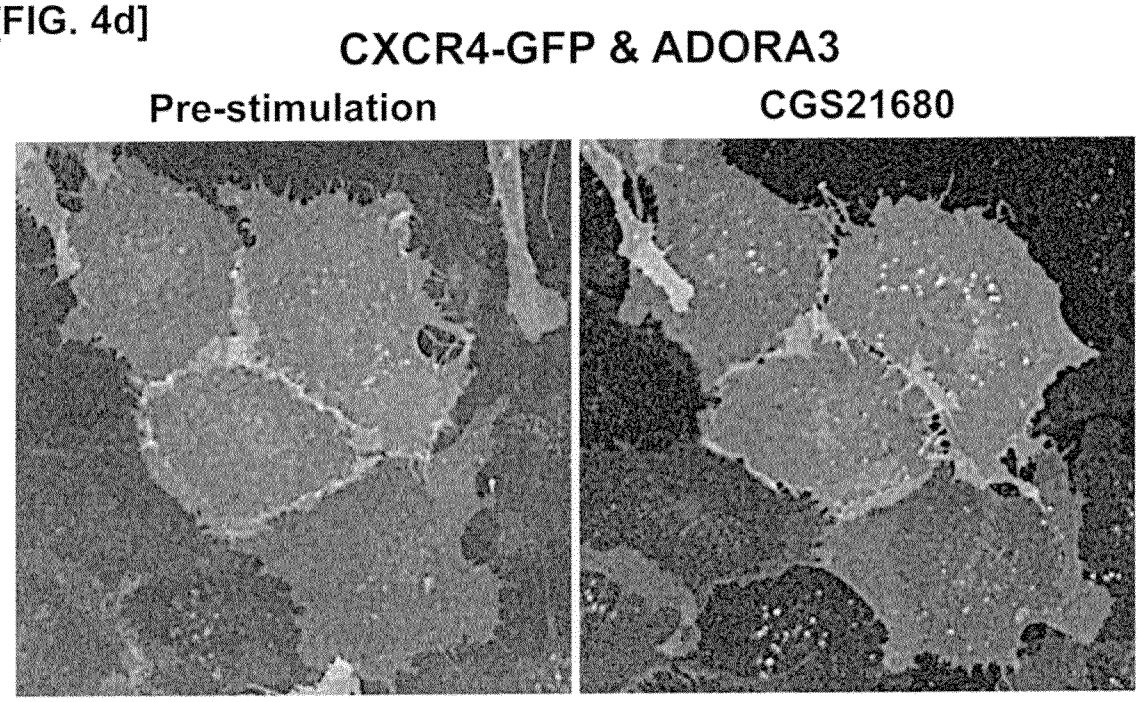
[FIG. 4b]


CXCR4-GFP & ADCYAP1R1
Pre-stimulation
VIP

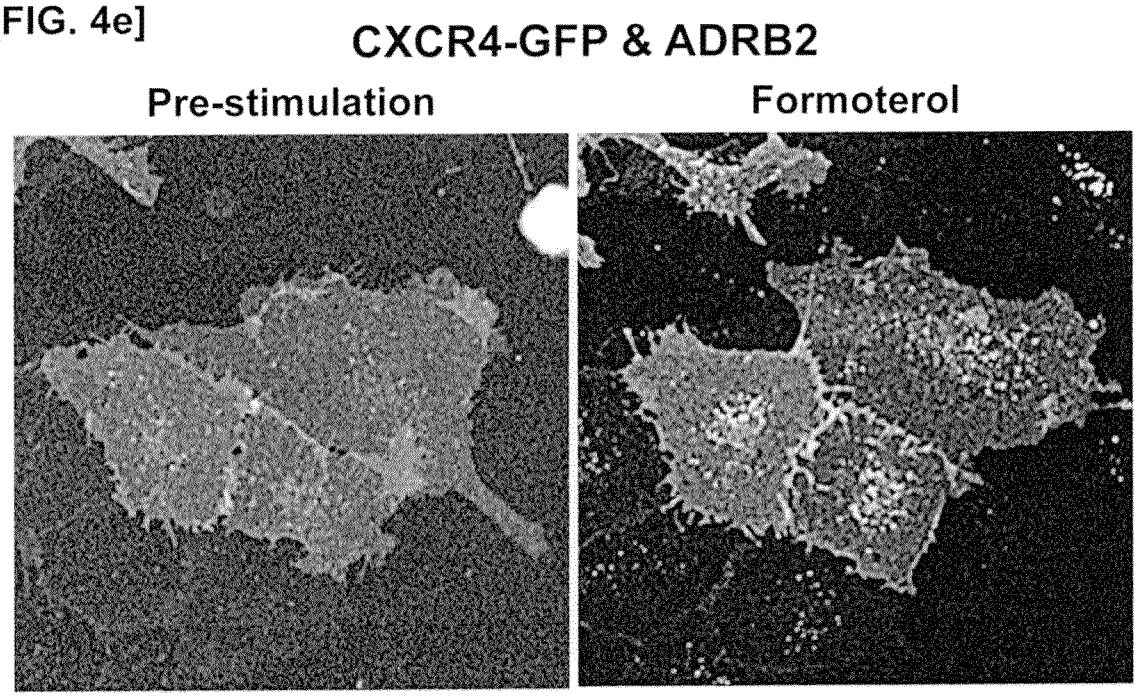
[FIG. 4c]


CXCR4-GFP & ADORA2B
Pre-stimulation
BAY 60-6583

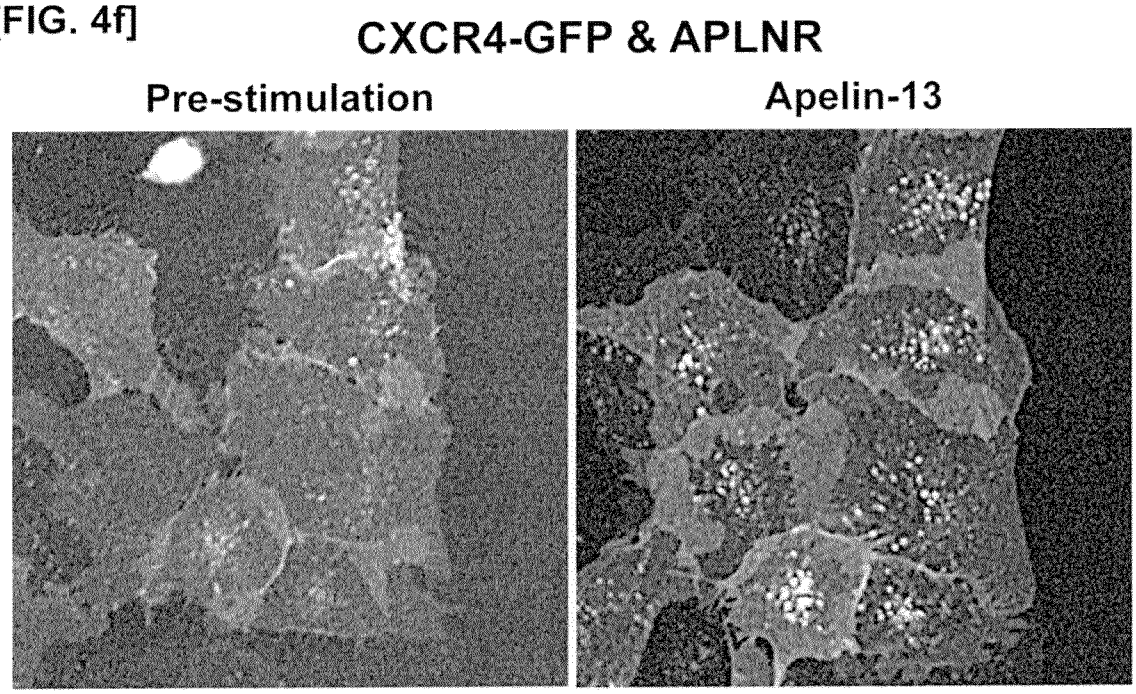
[FIG. 4d]


CXCR4-GFP & ADORA3
Pre-stimulation
CGS21680

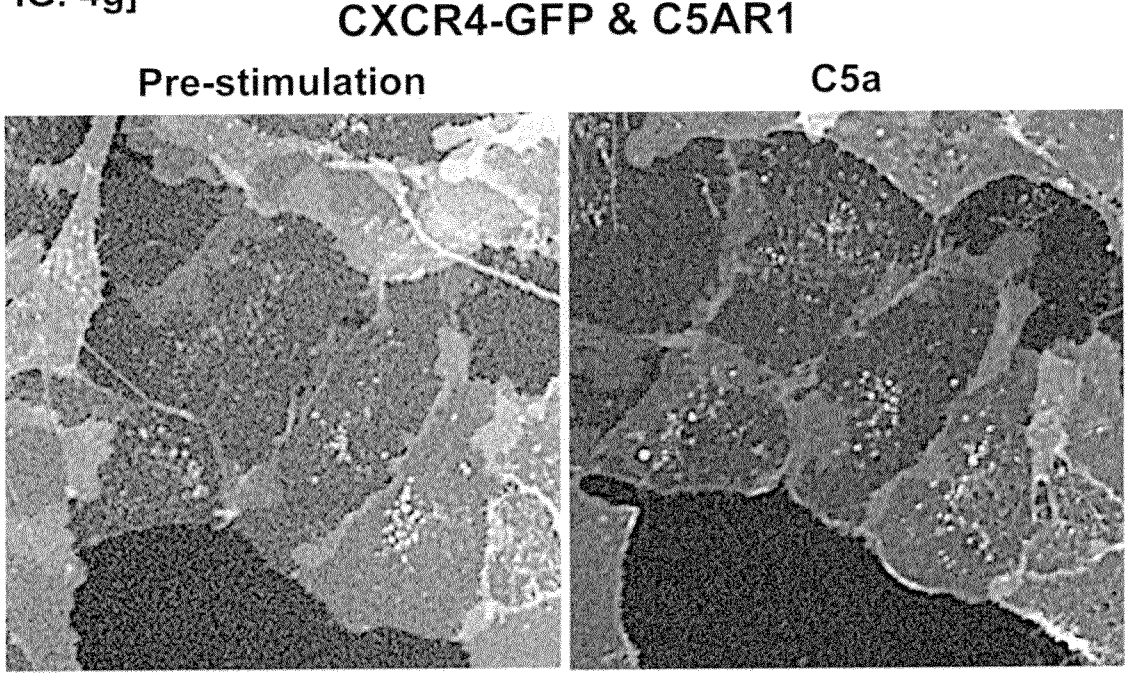
[FIG. 4e]



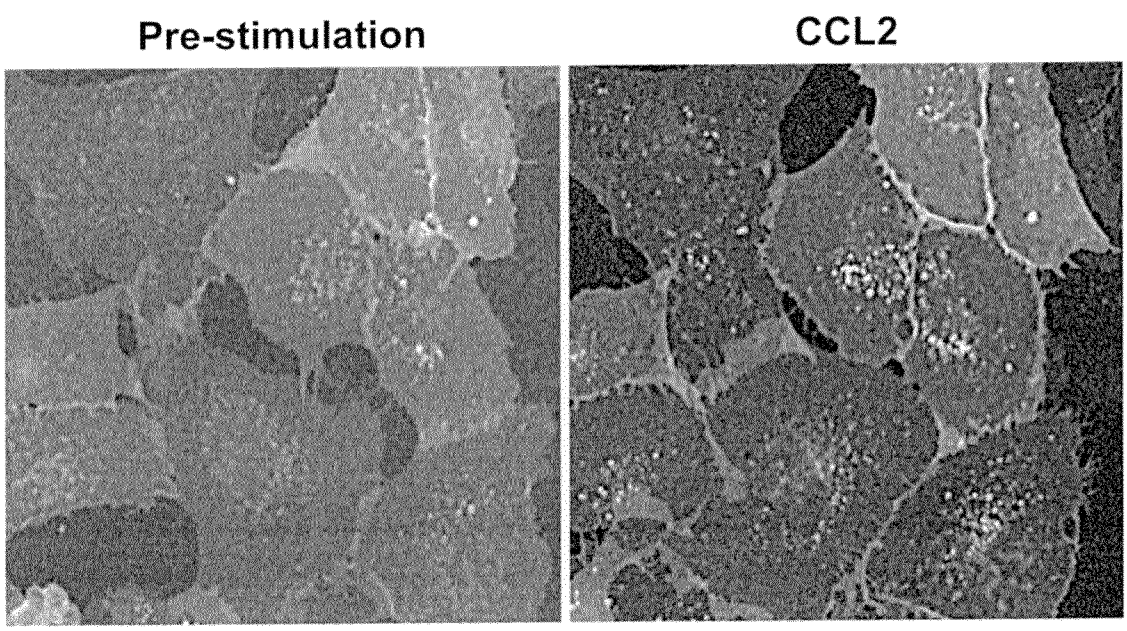
[FIG. 4f]



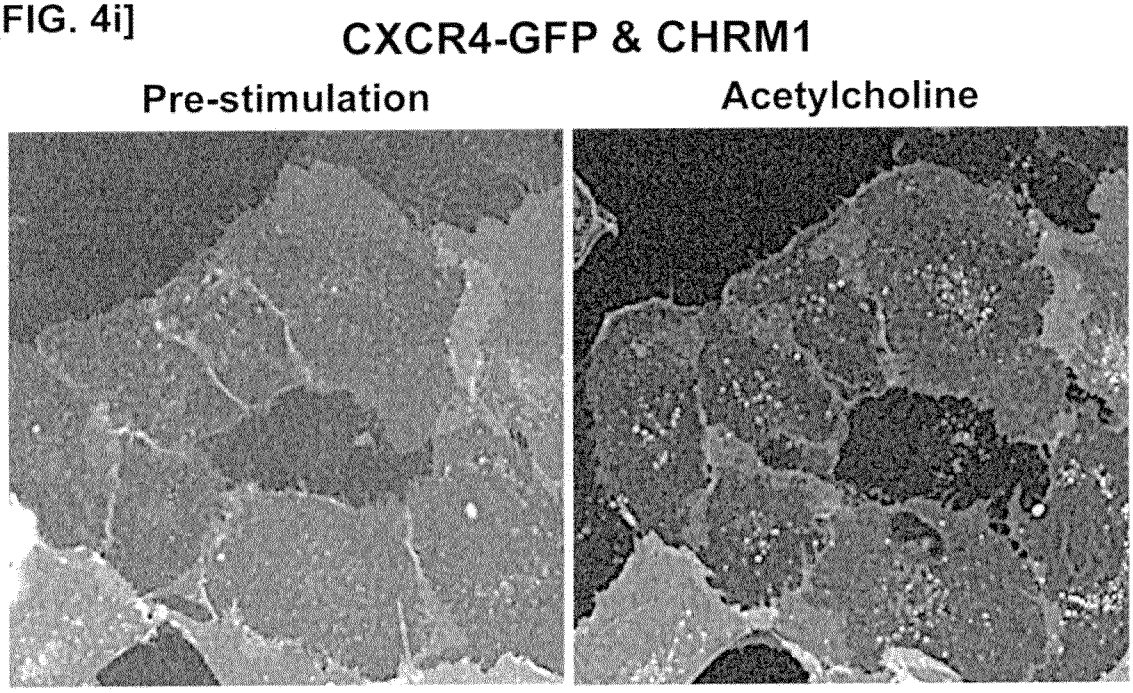
[FIG. 4g]


CXCR4-GFP & C5AR1
Pre-stimulation
C5a

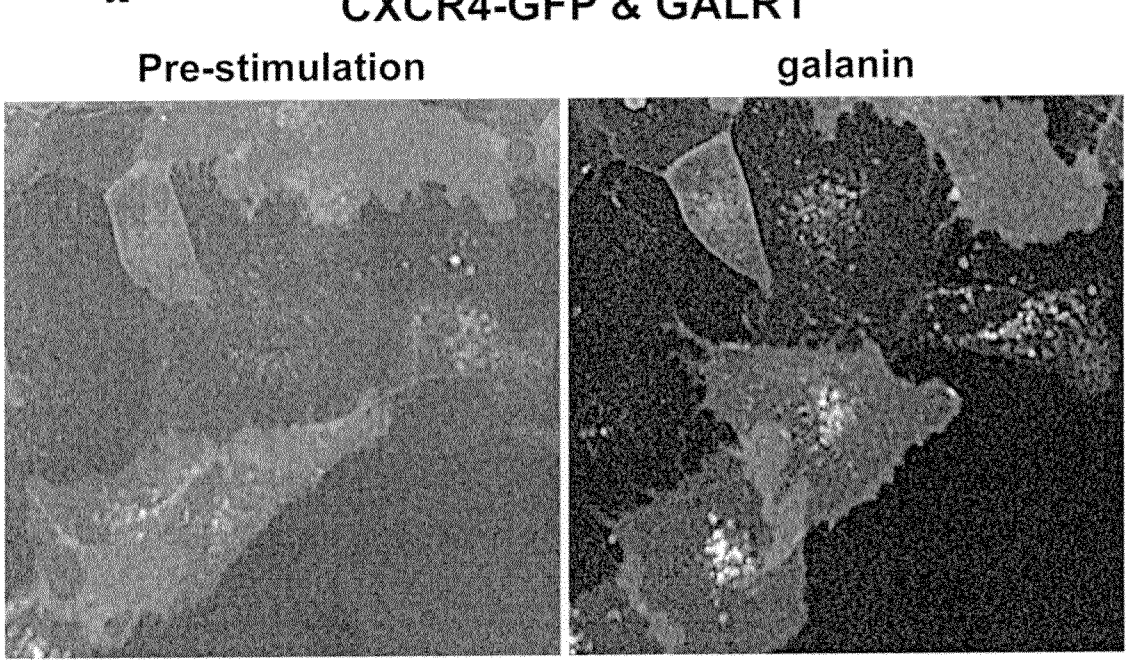
[FIG. 4h]


CXCR4-GFP & CCR5
Pre-stimulation
CCL2

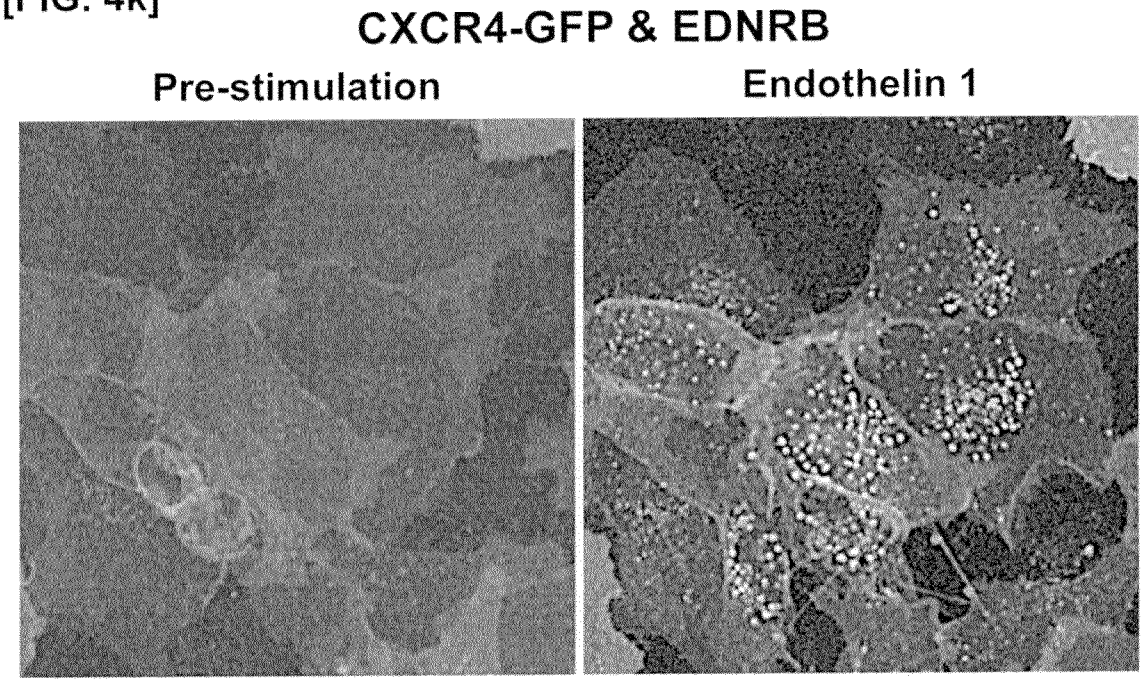
[FIG. 4i]


CXCR4-GFP & CHRM1
Pre-stimulation
Acetylcholine

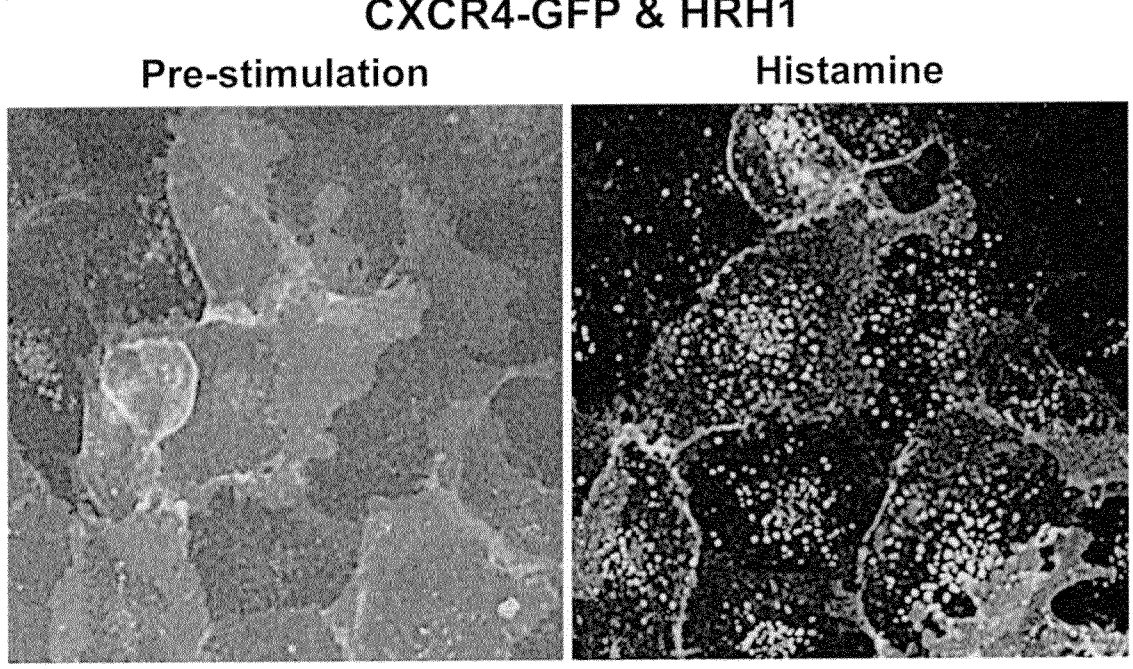
[FIG. 4j]


CXCR4-GFP & GALR1
Pre-stimulation
galanin

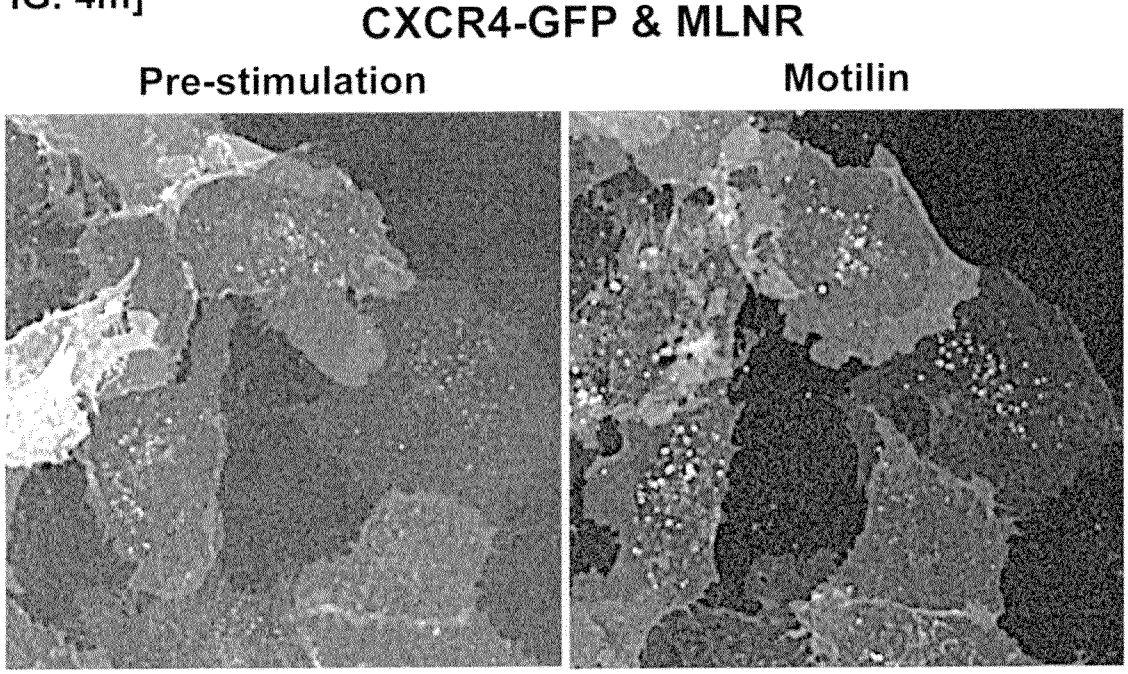
[FIG. 4k]


CXCR4-GFP & EDNRB
Pre-stimulation
Endothelin 1

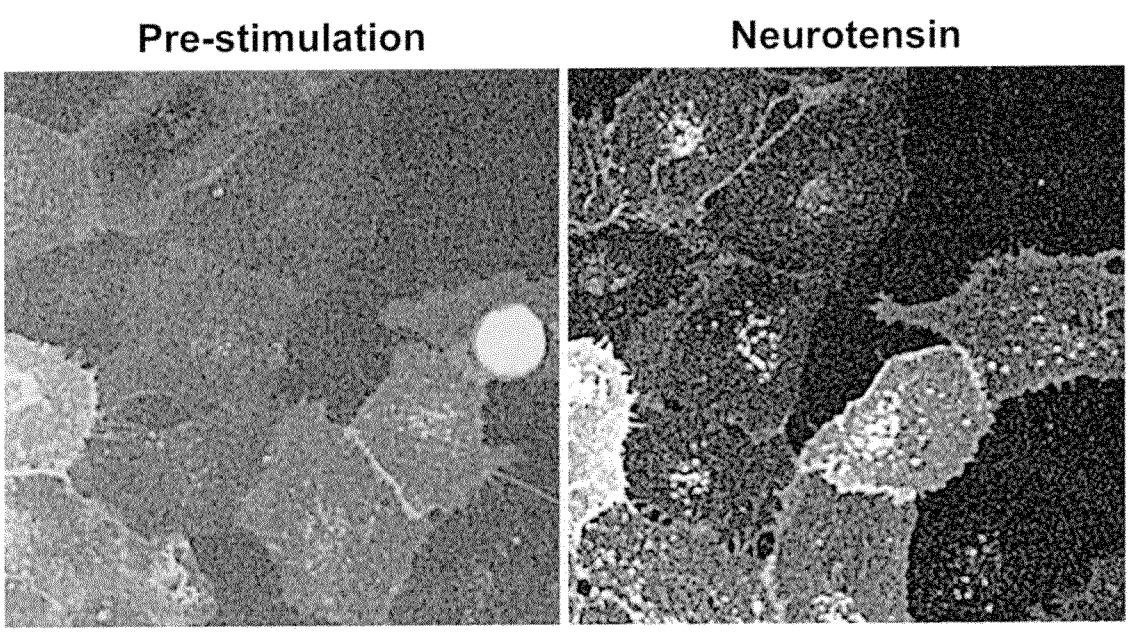
[FIG. 4l]


CXCR4-GFP & HRH1
Pre-stimulation
Histamine

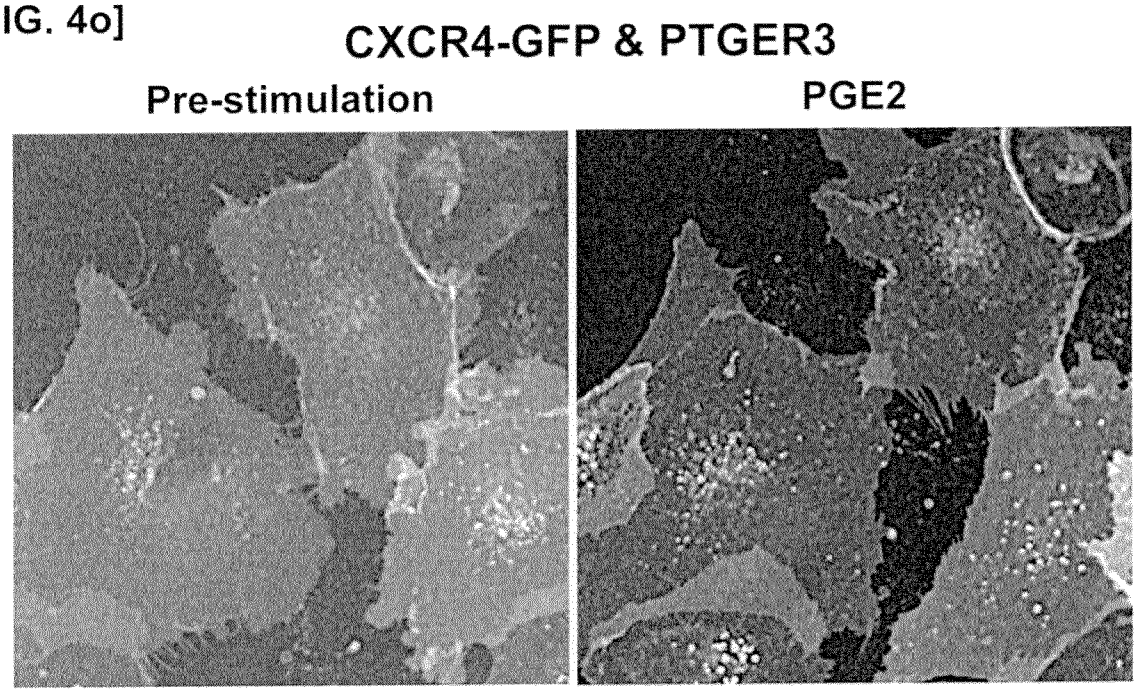
[FIG. 4m]


CXCR4-GFP & MLNR
Pre-stimulation
Motilin

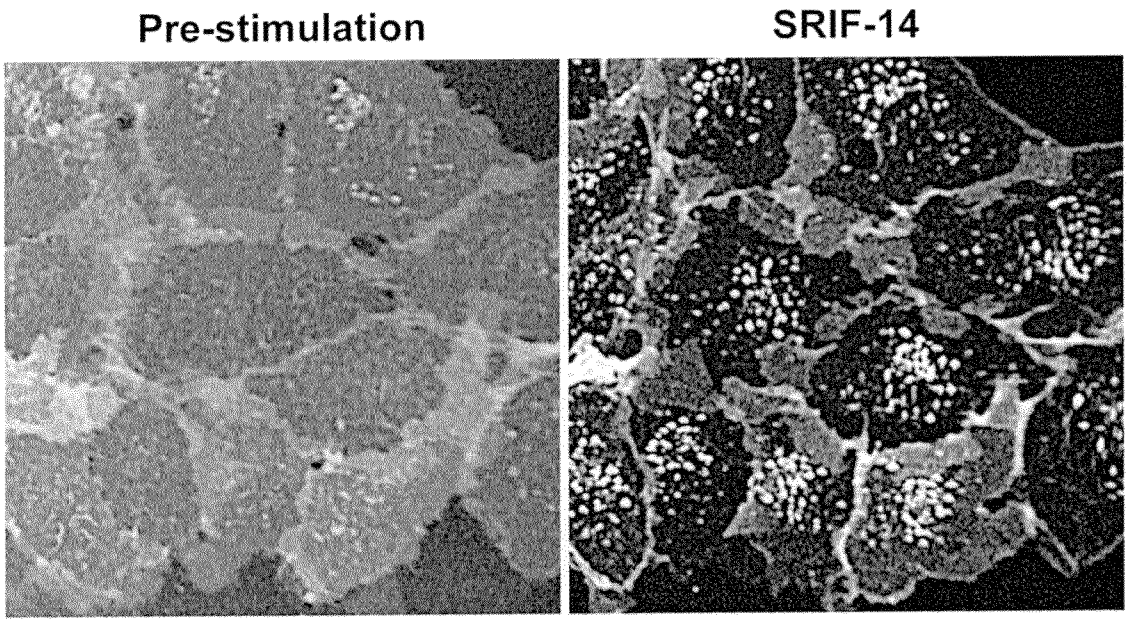
[FIG. 4n]


CXCR4-GFP & NTSR1
Pre-stimulation
Neurotensin

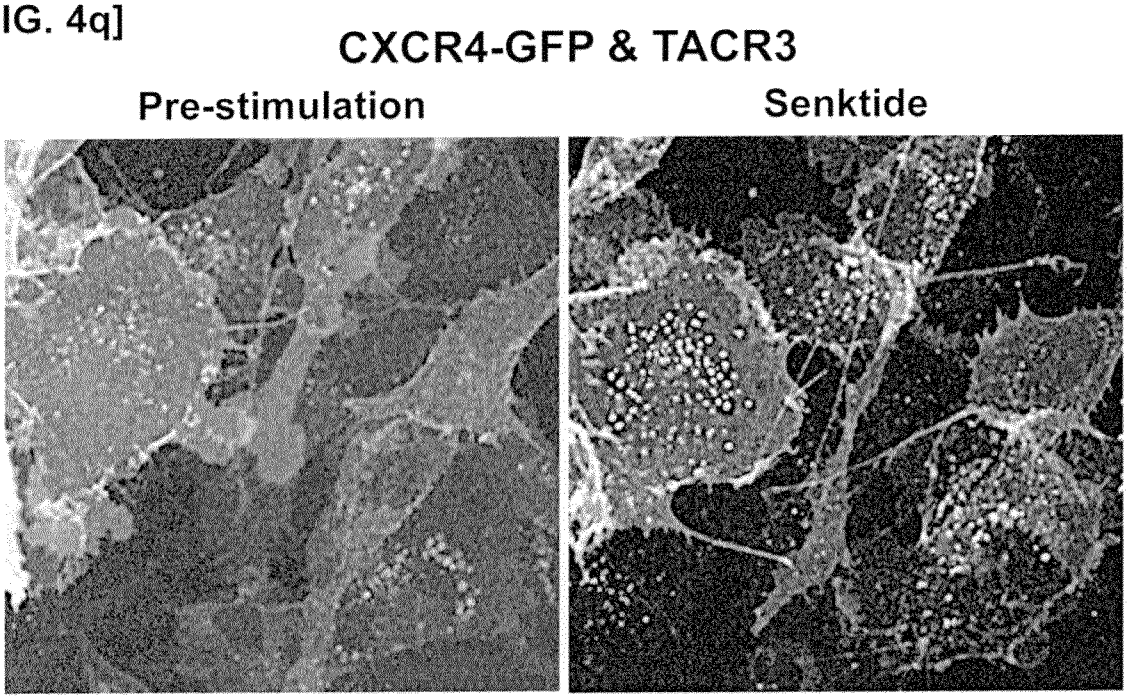
[FIG. 4o]


CXCR4-GFP & PTGER3
Pre-stimulation
PGE2

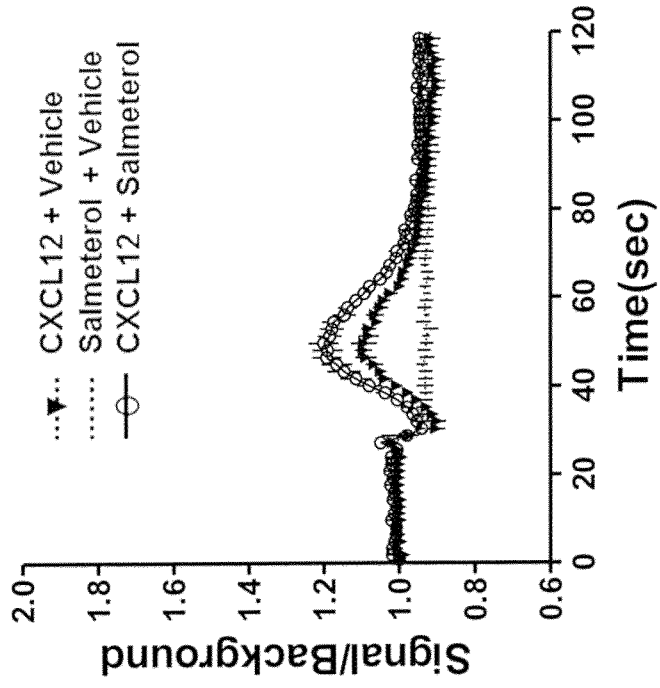
[FIG. 4p]


CXCR4-GFP & SSTR2
Pre-stimulation
SRIF-14

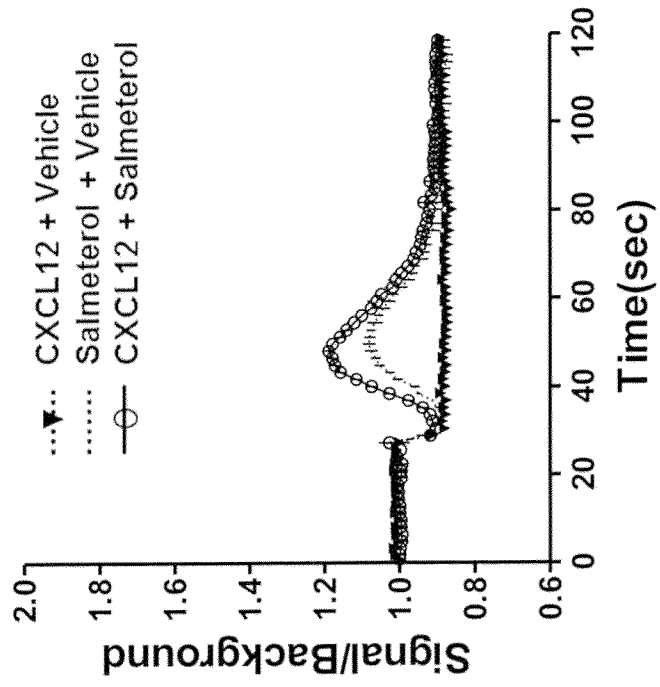
[FIG. 4q]



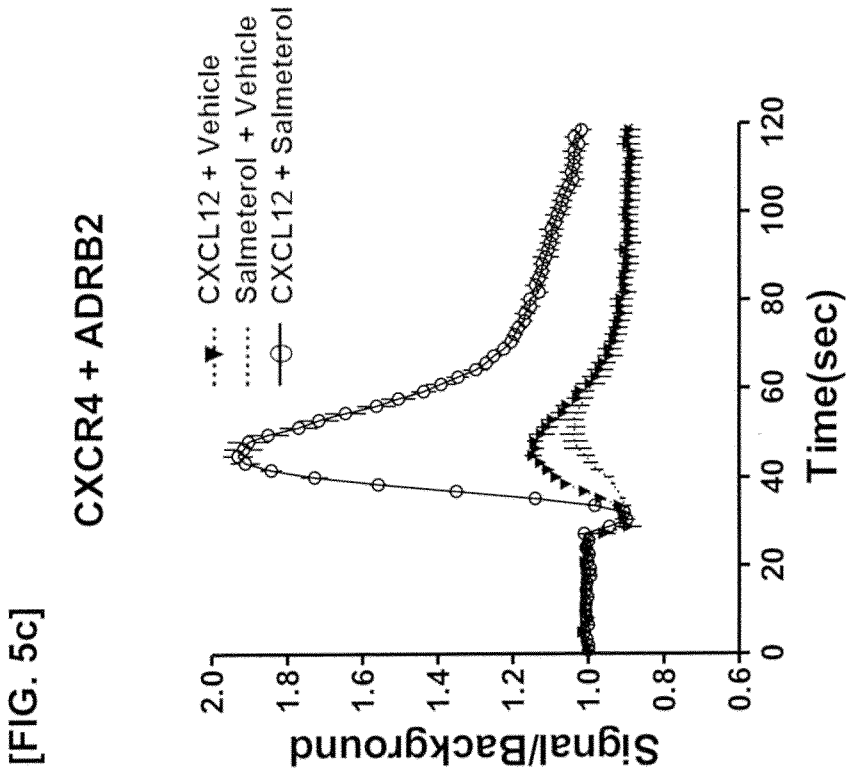
[FIG. 5a]


CXCR4
CXCL12 + Vehicle
Salmeterol + Vehicle
CXCL12 + Salmeterol
Signal/Background
Time(sec)
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0
20
40
60
80
100
120

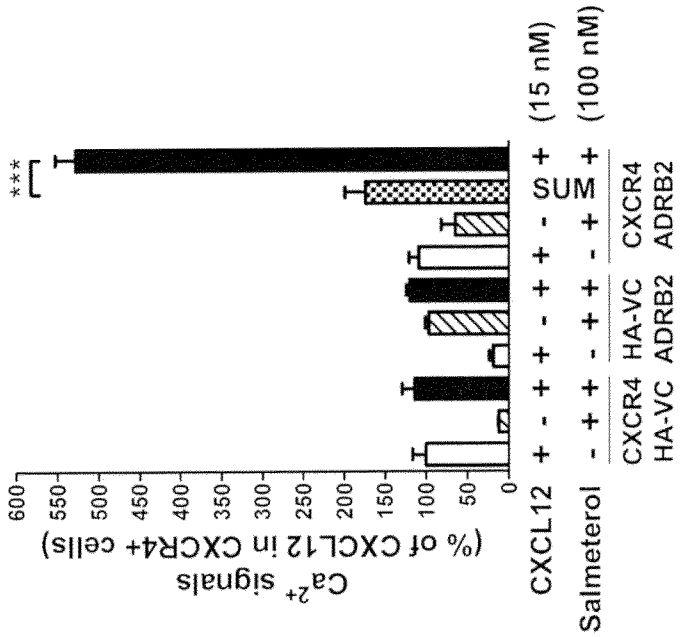
[FIG. 5b]


ADRB2
CXCL12 + Vehicle
Salmeterol + Vehicle
CXCL12 + Salmeterol
Signal/Background
Time(sec)
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0
20
40
60
80
100
120

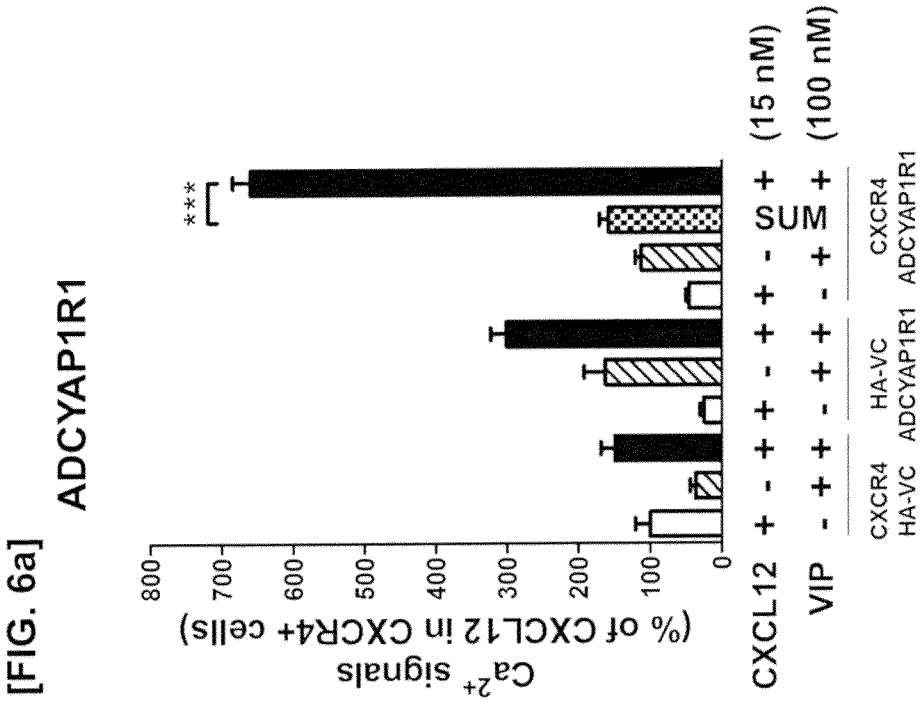
[FIG. 5c]


CXCR4 + ADRB2
CXCL12 + Vehicle
Salmeterol + Vehicle
CXCL12 + Salmeterol
Signal/Background
Time(sec)
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0
20
40
60
80
100
120

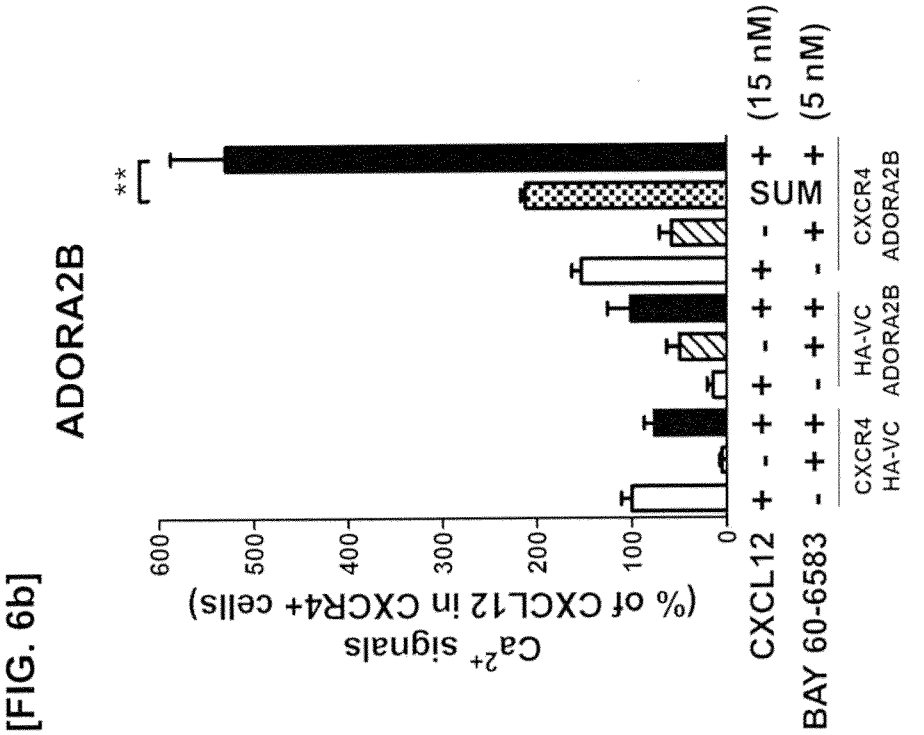
[FIG. 5d]


Quantification
Ca2+ signals
(% of CXCL12 in CXCR4+ cells)
600
550
500
450
400
350
300
250
200
150
100
50
0
***
CXCL12 + - + + - + + - + (15 nM)
Salmeterol - + + - + + - + + (100 nM)
CXCR4 HA-VC CXCR4
HA-VC ADRB2 ADRB2
SUM

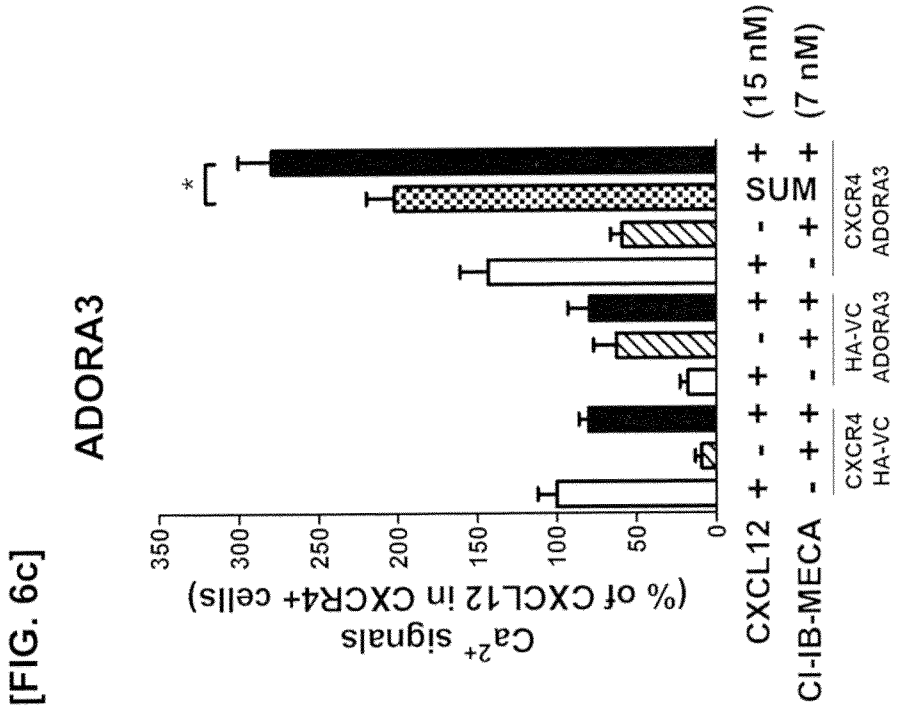
[FIG. 6a]


ADCYAP1R1
Ca2+ signals
(% of CXCL12 in CXCR4+ cells)
800
700
600
500
400
300
200
100
0
***
CXCL12 + - + + - + + - SUM + (15 nM)
VIP - + + - + + - + (100 nM)
CXCR4 HA-VC CXCR4
HA-VC ADCYAP1R1 ADCYAP1R1

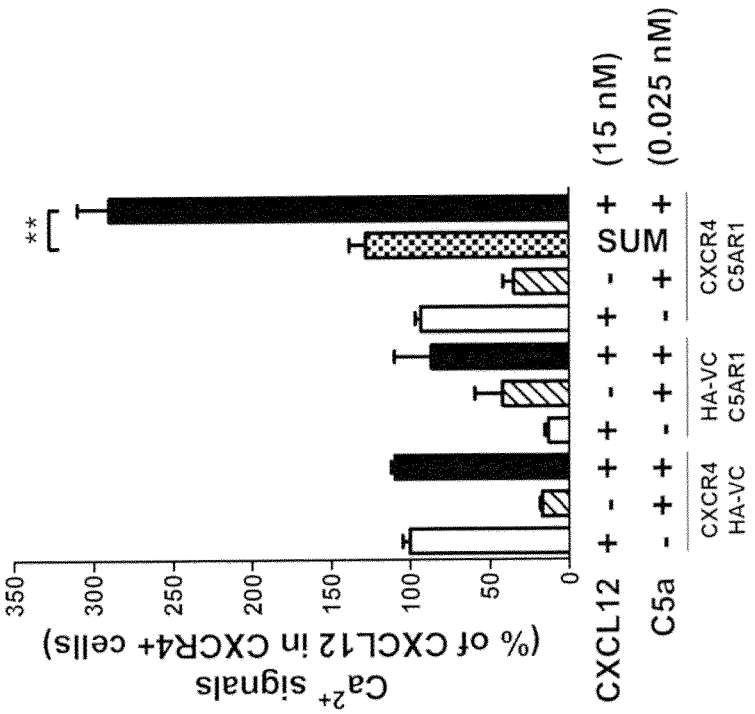
[FIG. 6b]


ADORA2B
Ca2+ signals
(% of CXCL12 in CXCR4+ cells)
600
500
400
300
200
100
0
**
CXCL12 + - + + - + + - SUM + (15 nM)
BAY 60-6583 - + + - + + - + (5 nM)
CXCR4 HA-VC CXCR4
HA-VC ADORA2B ADORA2B

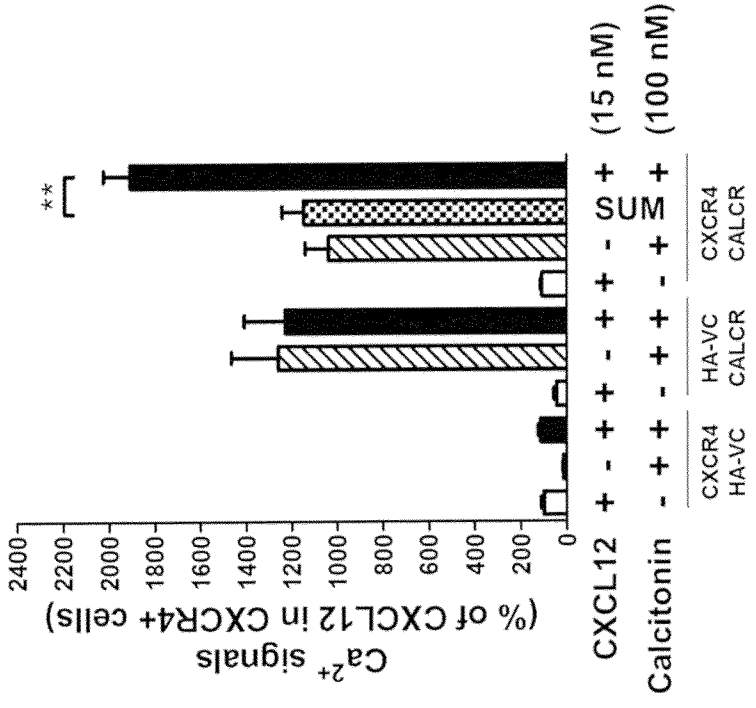
[FIG. 6c]


ADORA3
Ca2+ signals
(% of CXCL12 in CXCR4+ cells)
350
300
250
200
150
100
50
0
*
CXCL12 + - + + - + + - SUM +
CI-IB-MECA - + + - + + - + +
(15 nM)
(7 nM)
CXCR4 HA-VC CXCR4
HA-VC ADORA3 ADORA3

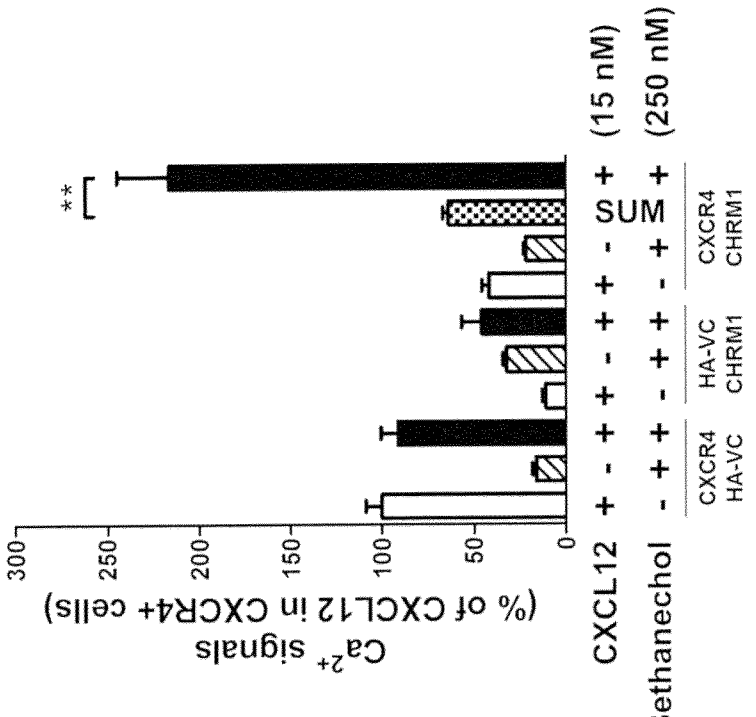
[FIG. 6d]


C5AR1
Ca2+ signals
(% of CXCL12 in CXCR4+ cells)
350
300
250
200
150
100
50
0
**
CXCL12 + - + + - + + - SUM +
C5a - + + - + + - + +
(15 nM)
(0.025 nM)
CXCR4 HA-VC CXCR4
HA-VC C5AR1 C5AR1

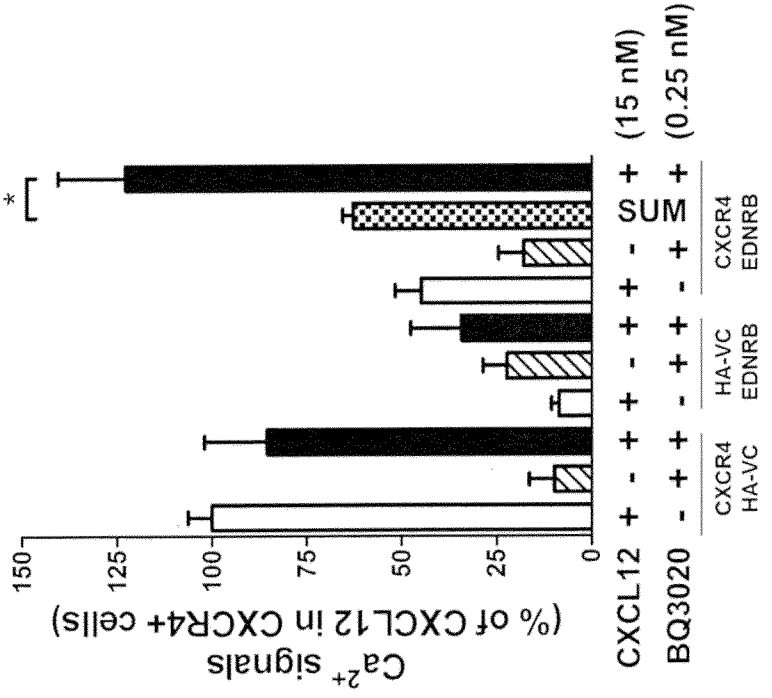
[FIG. 6e]


CALCR
Ca2+ signals
(% of CXCL12 in CXCR4+ cells)
2400
2200
2000
1800
1600
1400
1200
1000
800
600
400
200
0
**
CXCL12 + - + + + - + + - SUM +
Calcitonin - + + - + + - + + (15 nM) (100 nM)
CXCR4 - + + + + + - + +
HA-VC + + - + + - + + -
HA-VC CALCR CXCR4 CALCR

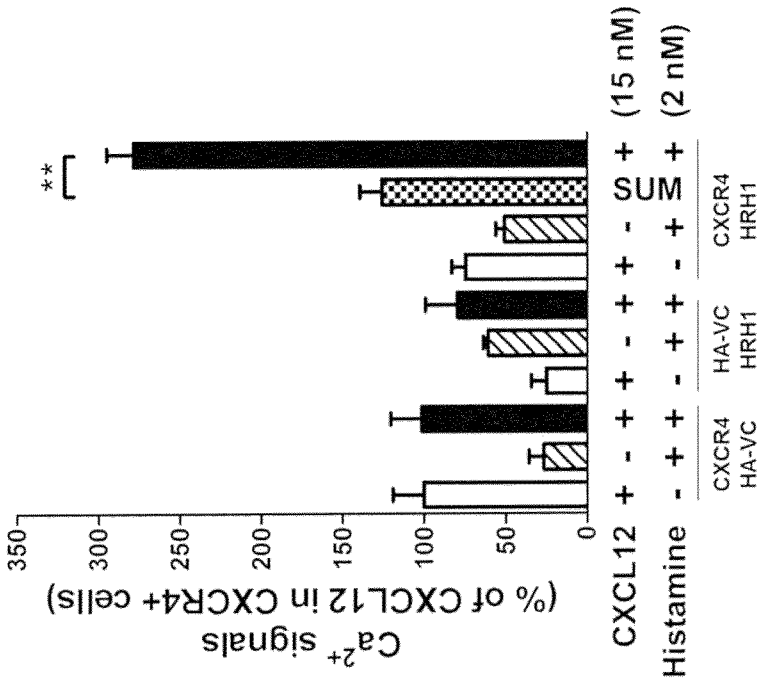
[FIG. 6f]


CHRM1
Ca2+ signals
(% of CXCL12 in CXCR4+ cells)
300
250
200
150
100
50
0
**
CXCL12 + - + + + - + + - SUM +
Bethanechol - + + - + + - + + (15 nM) (250 nM)
CXCR4 - + + + + + - + +
HA-VC + + - + + - + + -
HA-VC CHRM1 CXCR4 CHRM1

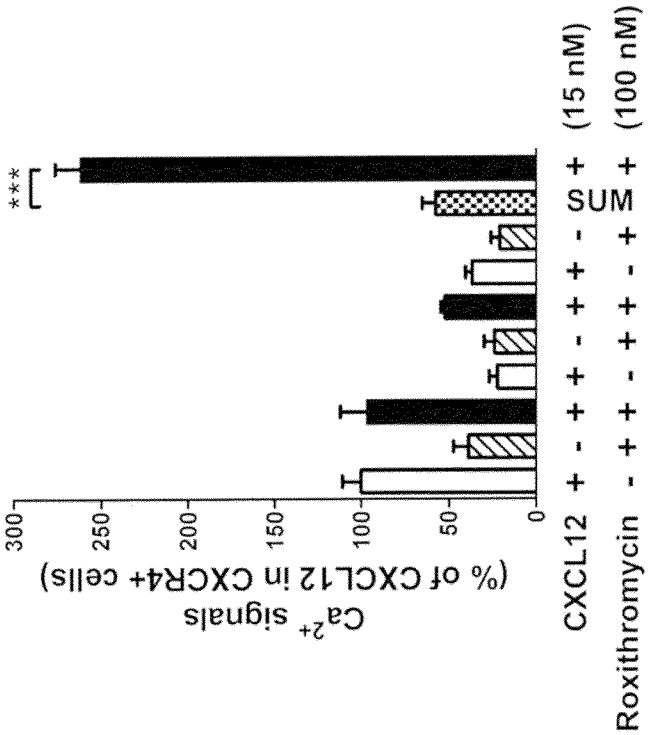
[FIG. 6g]


EDNRB
Ca2+ signals
(% of CXCL12 in CXCR4+ cells)
150
125
100
75
50
25
0
*
CXCL12 + - + + - + + - SUM +
BQ3020 - + + - + + - + (0.25 nM)
CXCR4 HA-VC CXCR4
HA-VC EDNRB EDNRB
(15 nM)

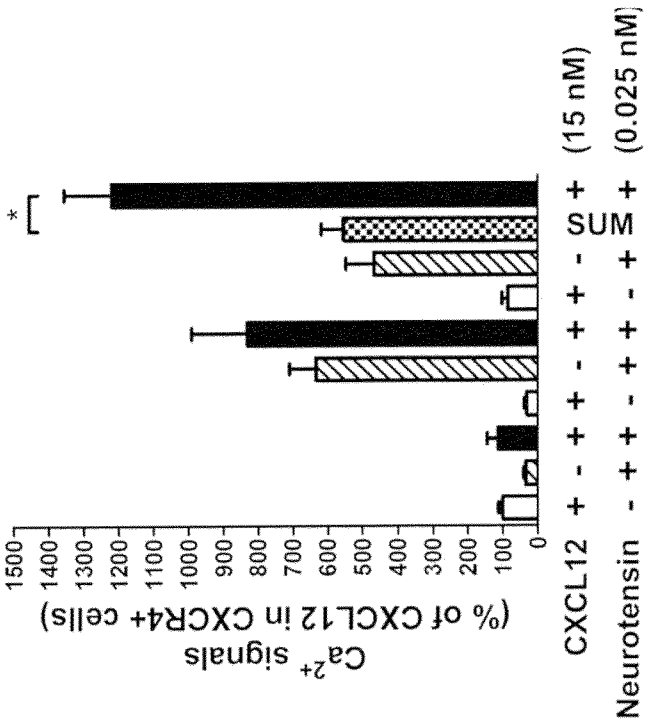
[FIG. 6h]


HRH1
Ca2+ signals
(% of CXCL12 in CXCR4+ cells)
350
300
250
200
150
100
50
0
**
CXCL12 + - + + - + + - SUM +
Histamine - + + - + + - + (2 nM)
CXCR4 HA-VC CXCR4
HA-VC HRH1 HRH1
(15 nM)

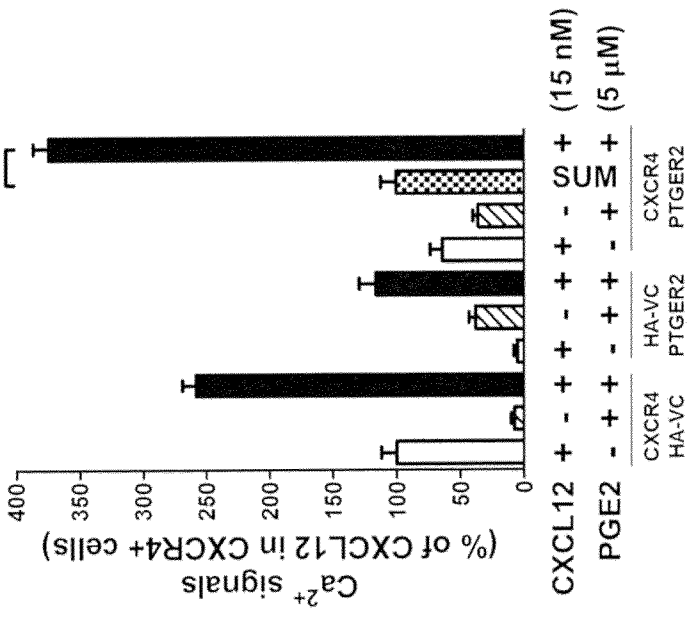
[FIG. 6i]


MLNR
Ca^{2+} signals
(% of CXCL12 in CXCR4+ cells)
300
250
200
150
100
50
0
***
CXCL12 + - + + - + + - SUM +
Roxithromycin - + + - + + - + +
(15 nM)
(100 nM)
CXCR4 HA-VC
HA-VC MLNR
CXCR4 MLNR

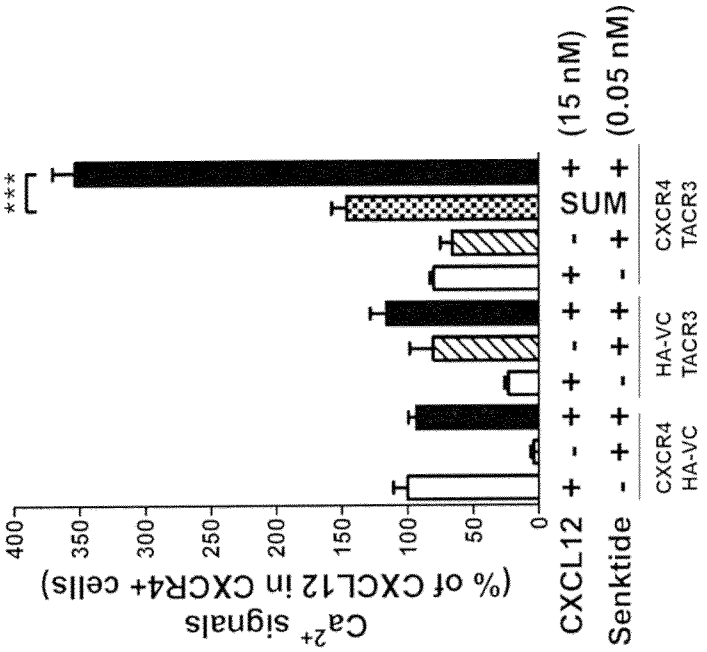
[FIG. 6j]


NTSR1
Ca^{2+} signals
(% of CXCL12 in CXCR4+ cells)
1500
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
*
CXCL12 + - + + - + + - SUM +
Neurotensin - + + - + + - + +
(15 nM)
(0.025 nM)
CXCR4 HA-VC
HA-VC NTSR1
CXCR4 NTSR1

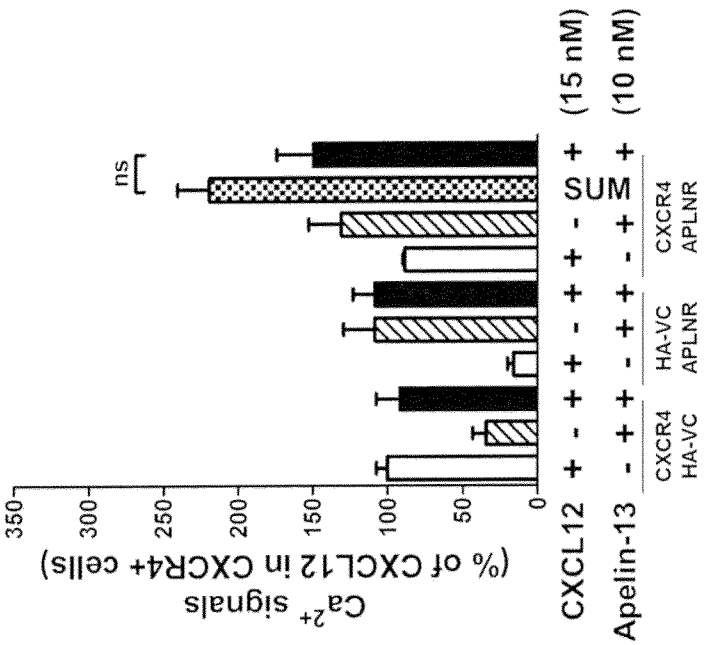
[FIG. 6k]


PTGER2
Ca2+ signals
(% of CXCL12 in CXCR4+ cells)
400
350
300
250
200
150
100
50
0
***
CXCL12 + - + + - + + - SUM + (15 nM)
PGE2 - + + - + + - + + (5 μM)
CXCR4 HA-VC CXCR4 PTGER2

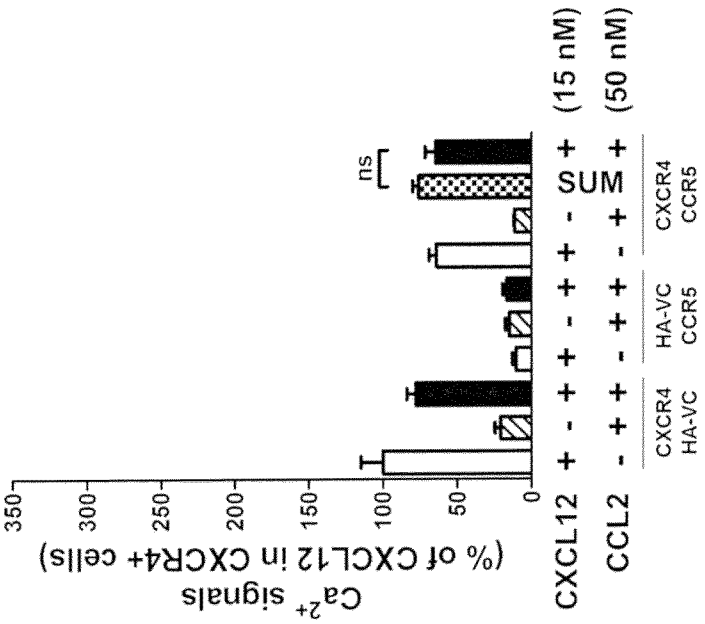
[FIG. 6l]


TACR3
Ca2+ signals
(% of CXCL12 in CXCR4+ cells)
400
350
300
250
200
150
100
50
0
***
CXCL12 + - + + - + + - SUM + (15 nM)
Senktide - + + - + + - + + (0.05 nM)
CXCR4 HA-VC CXCR4 TACR3

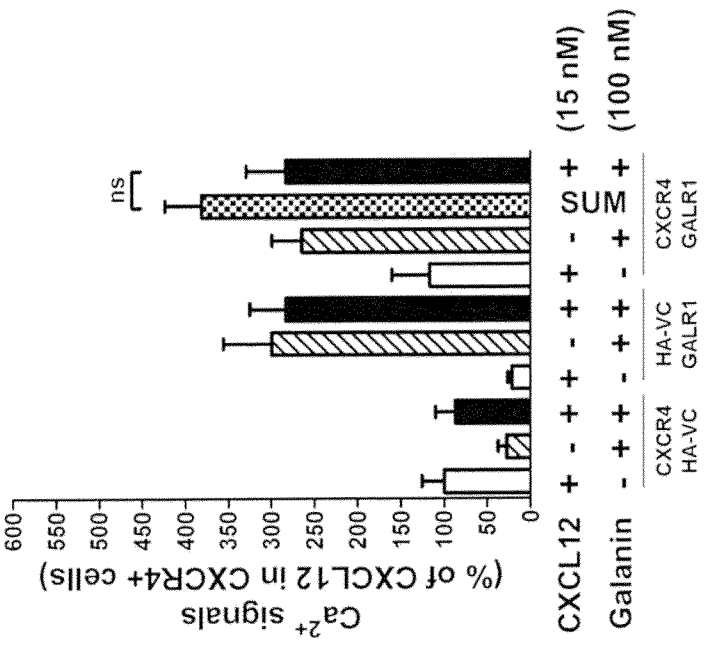
[FIG. 7a]


APLNR
Ca2+ signals
(% of CXCL12 in CXCR4+ cells)
350
300
250
200
150
100
50
0
ns
CXCL12 + - + + - + + - + (15 nM)
Apelin-13 - + + - + + - + + (10 nM)
CXCR4 HA-VC
HA-VC APLNR
CXCR4 APLNR
SUM

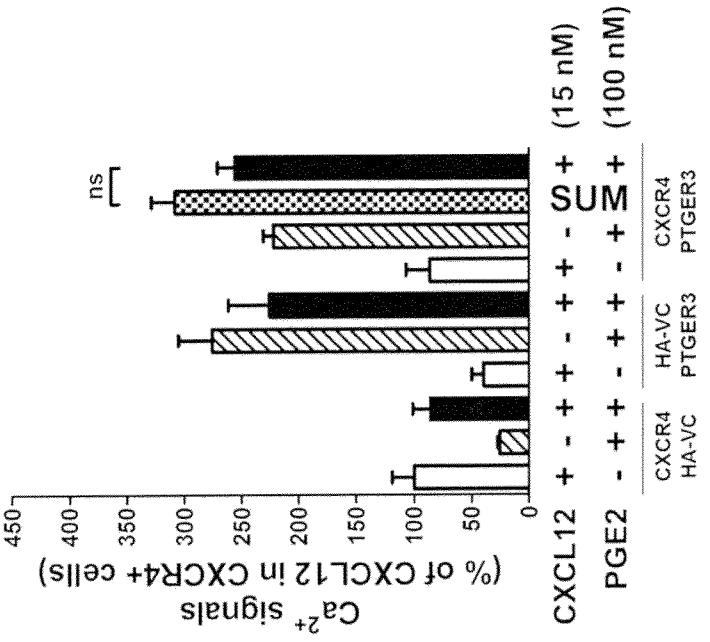
[FIG. 7b]


CCR5
Ca2+ signals
(% of CXCL12 in CXCR4+ cells)
350
300
250
200
150
100
50
0
ns
CXCL12 + - + + - + + - + (15 nM)
CCL2 - + + - + + - + + (50 nM)
CXCR4 HA-VC
HA-VC CCR5
CXCR4 CCR5
SUM

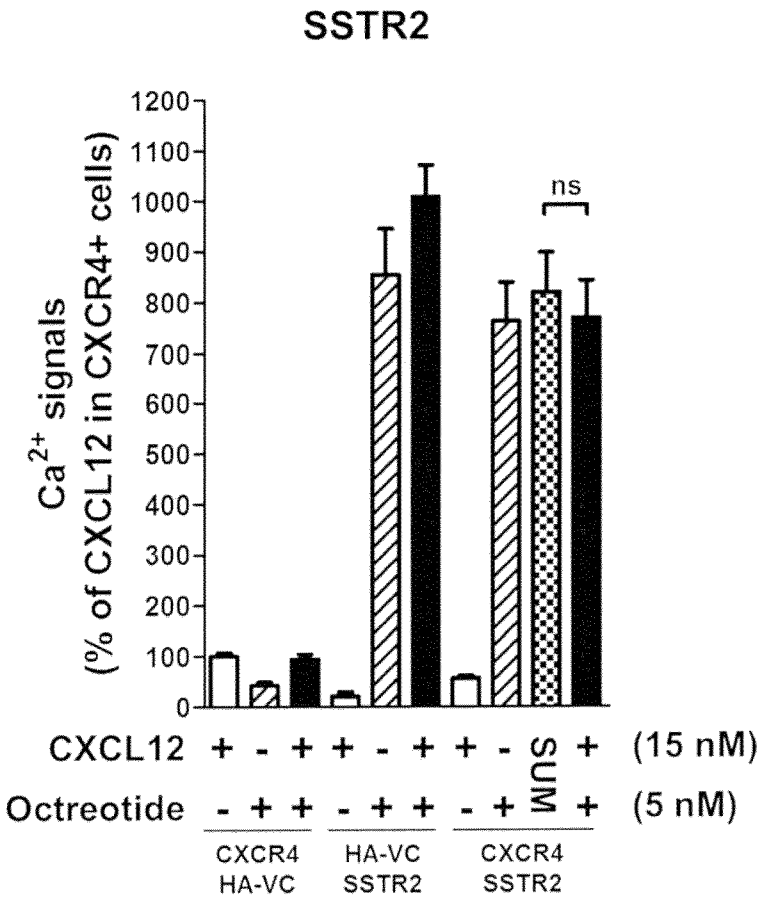
[FIG. 7c]


GALR1
Ca2+ signals
(% of CXCL12 in CXCR4+ cells)
600
550
500
450
400
350
300
250
200
150
100
50
0
ns
CXCL12 + - + + - + + - + (15 nM)
Galanin - + + - + + - + + (100 nM)
SUM
CXCR4 HA-VC CXCR4
HA-VC GALR1 GALR1

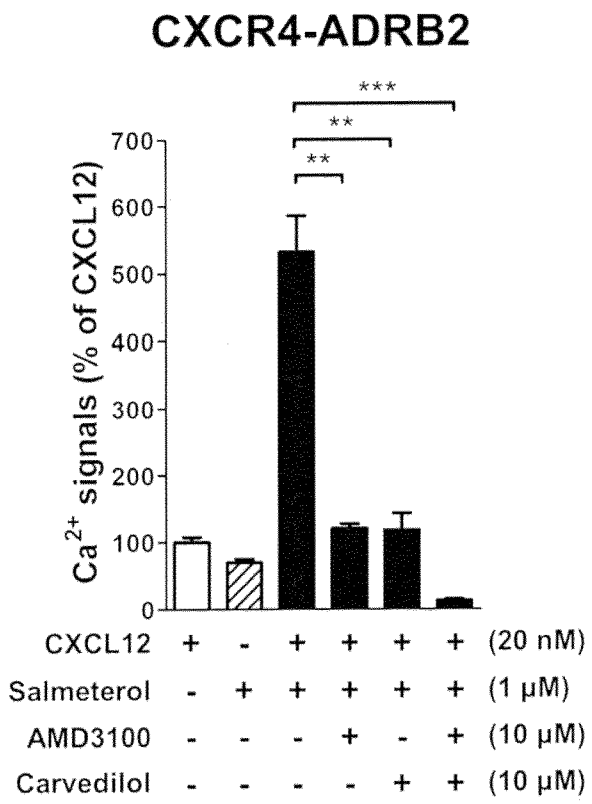
[FIG. 7d]


PTGER3
Ca2+ signals
(% of CXCL12 in CXCR4+ cells)
450
400
350
300
250
200
150
100
50
0
ns
CXCL12 + - + + - + + - + (15 nM)
PGE2 - + + - + + - + + (100 nM)
SUM
CXCR4 HA-VC CXCR4
HA-VC PTGER3 PTGER3

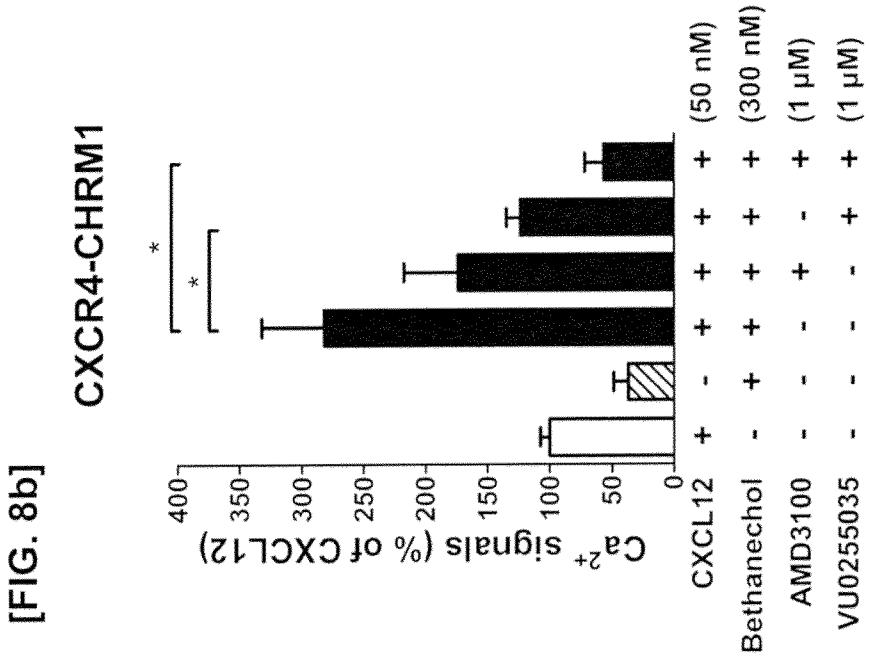
[FIG. 7e]


SSTR2
Ca2+ signals
(% of CXCL12 in CXCR4+ cells)
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
ns
CXCL12 + - + + - + + - + + (15 nM)
Octreotide - + + - + + - + + (5 nM)
SUM
CXCR4
HA-VC
HA-VC
SSTR2
CXCR4
SSTR2

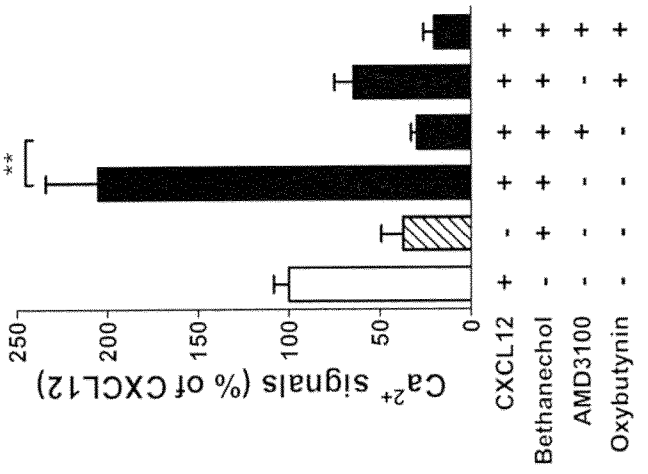
[Fig. 8a]


CXCR4-ADRB2
***
**
**
700
600
500
400
300
200
100
0
Ca2+ signals (% of CXCL12)
CXCL12 + - + + + + (20 nM)
Salmeterol - + + + + + (1 μM)
AMD3100 - - - + - + (10 μM)
Carvedilol - - - - + + (10 μM)

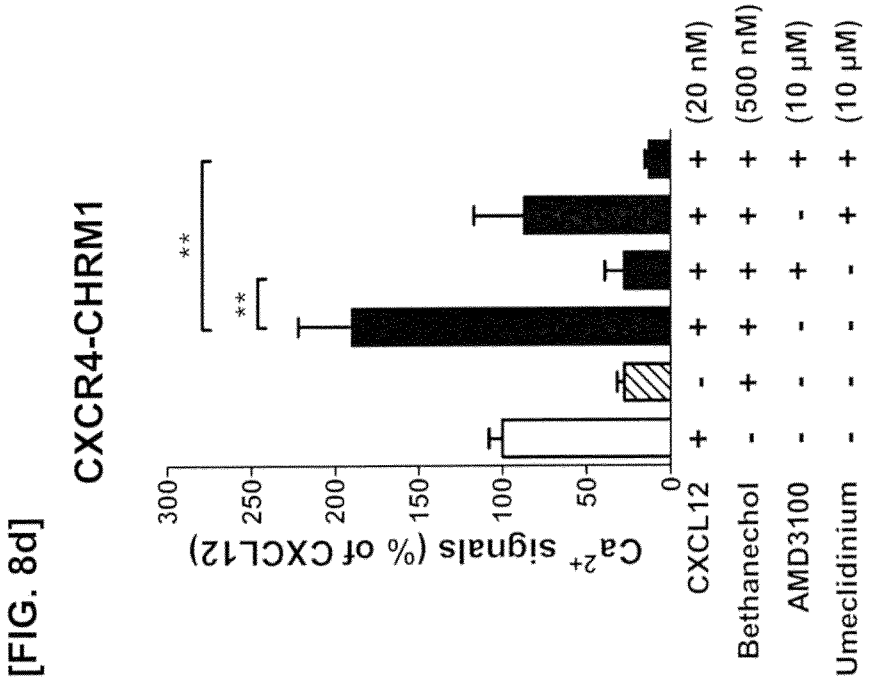
[FIG. 8b]


CXCR4-CHRM1
Ca2+ signals (% of CXCL12)
0
50
100
150
200
250
300
350
400
*
*
CXCL12 + - + + + + (50 nM)
Bethanechol - + + + + + (300 nM)
AMD3100 - - - + - + (1 μM)
VU0255035 - - - - + + (1 μM)

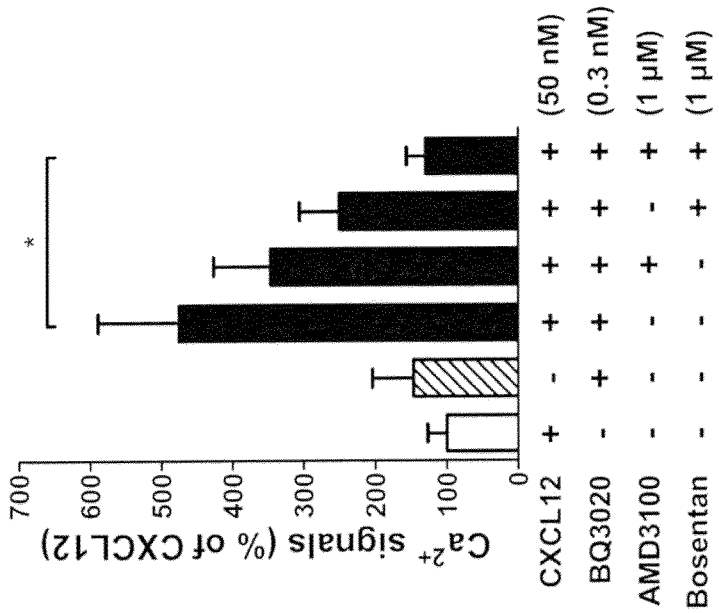
[FIG. 8c]


CXCR4-CHRM1
Ca2+ signals (% of CXCL12)
0
50
100
150
200
250
**
**
**
CXCL12 + - + + + + (20 nM)
Bethanechol - + + + + + (500 nM)
AMD3100 - - - + - + (10 μM)
Oxybutynin - - - - + + (10 μM)

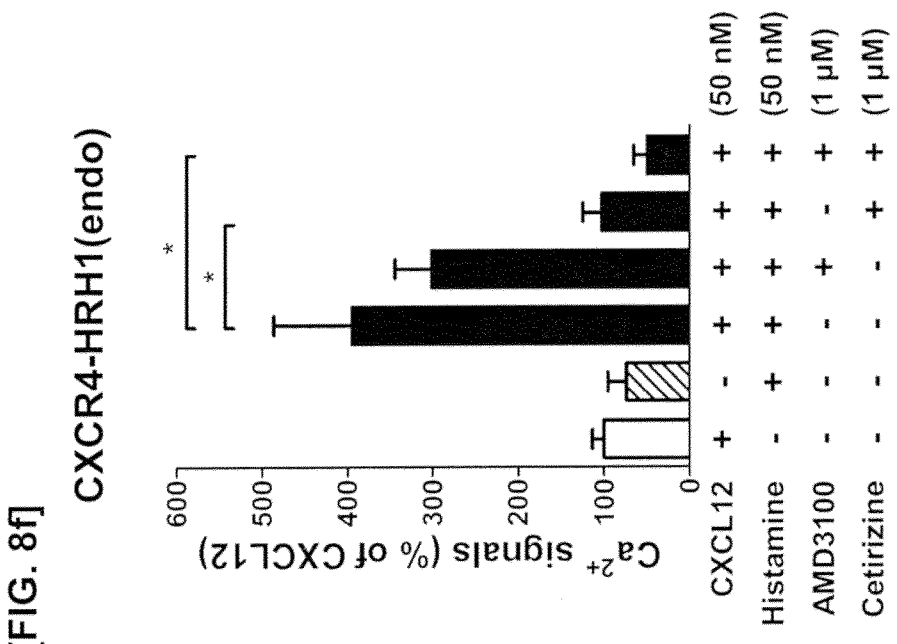
[FIG. 8d]


CXCR4-CHRM1
Ca2+ signals (% of CXCL12)
300
250
200
150
100
50
0
**
**
CXCL12 + - + + + + (20 nM)
Bethanechol - + + + + + (500 nM)
AMD3100 - - + - + + (10 μM)
Umeclidinium - - - + + + (10 μM)

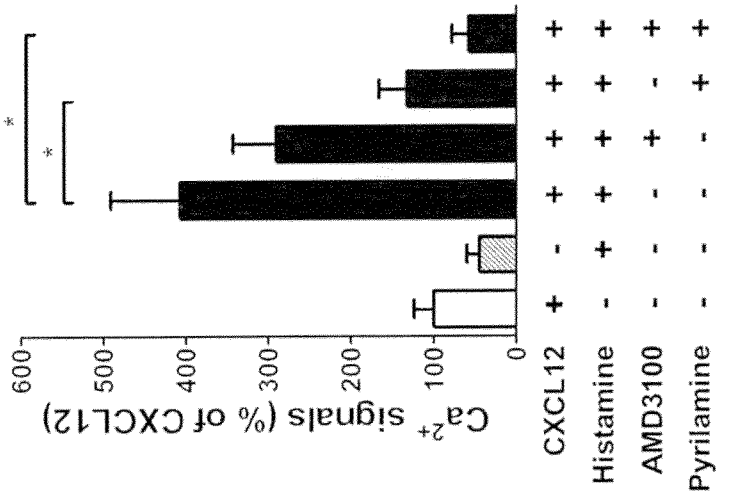
[FIG. 8e]


CXCR4-EDNRB
Ca2+ signals (% of CXCL12)
700
600
500
400
300
200
100
0
*
CXCL12 + - + + + + (50 nM)
BQ3020 - + + + + + (0.3 nM)
AMD3100 - - + - + + (1 μM)
Bosentan - - - + + + (1 μM)

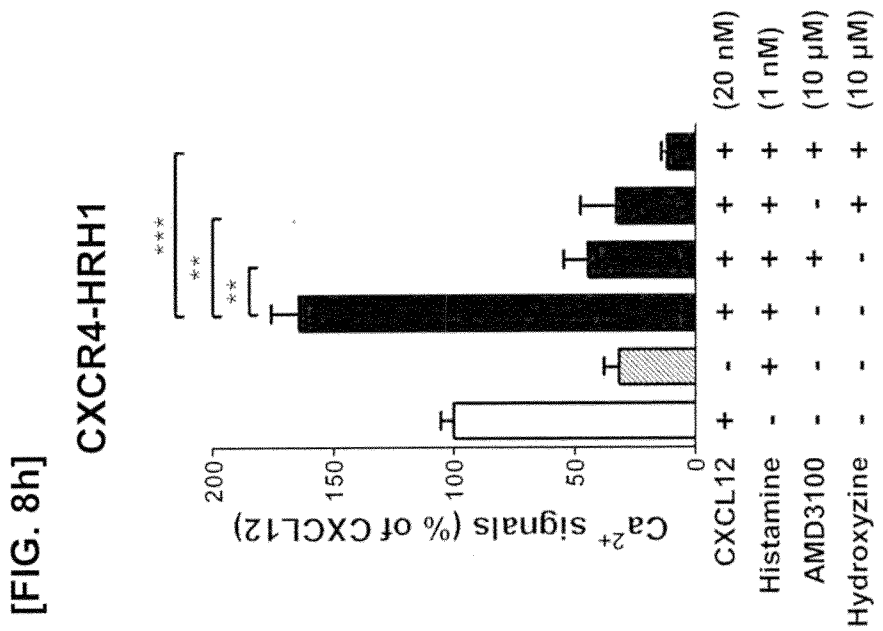
[FIG. 8f]


CXCR4-HRH1(endo)
Ca^{2+} signals (% of CXCL12)
0
100
200
300
400
500
600
*
*
CXCL12 + - + + + + (50 nM)
Histamine - + + + + + (50 nM)
AMD3100 - - + - + + (1 μM)
Cetirizine - - - + + + (1 μM)

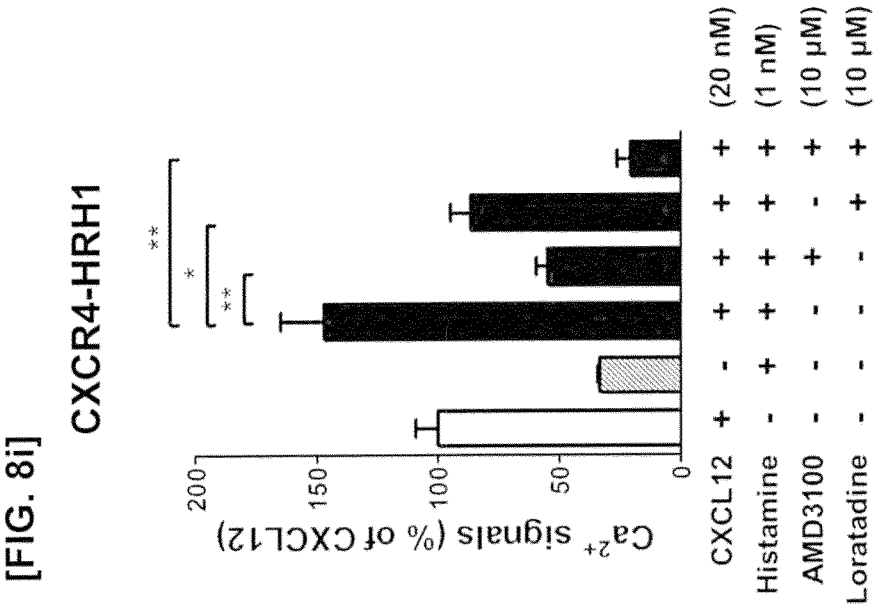
[FIG. 8g]


CXCR4-HRH1(endo)
Ca^{2+} signals (% of CXCL12)
0
100
200
300
400
500
600
*
*
CXCL12 + - + + + + (50 nM)
Histamine - + + + + + (50 nM)
AMD3100 - - + - + + (1 μM)
Pyrilamine - - - + + + (1 μM)

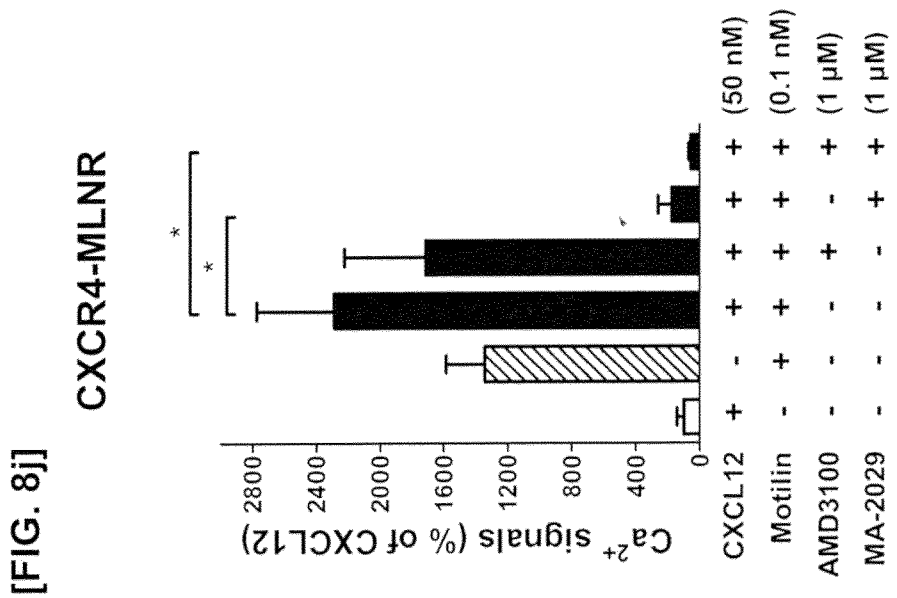
[FIG. 8h]


CXCR4-HRH1
Ca2+ signals (% of CXCL12)
0
50
100
150
200
***
**
**
CXCL12 + - + + + + (20 nM)
Histamine - + + + + + (1 nM)
AMD3100 - - + - + + (10 μM)
Hydroxyzine - - - + + + (10 μM)

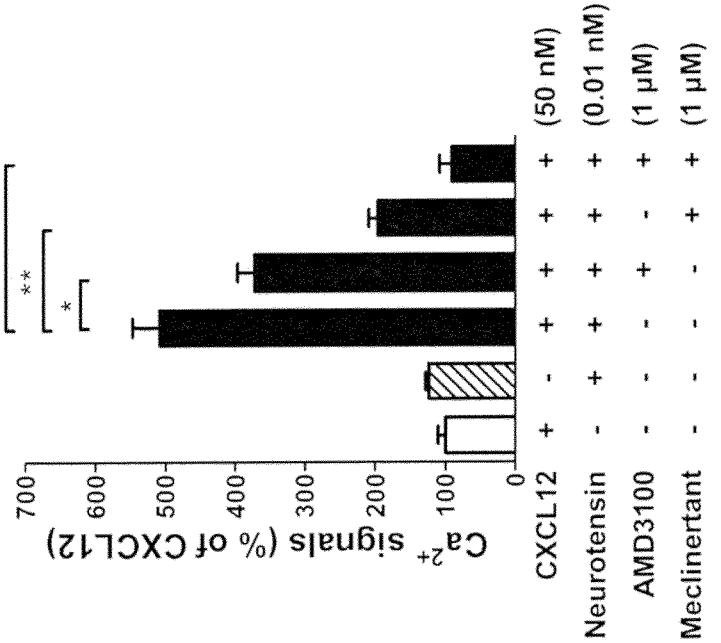
[FIG. 8i]


CXCR4-HRH1
Ca2+ signals (% of CXCL12)
0
50
100
150
200
**
*
**
CXCL12 + - + + + + (20 nM)
Histamine - + + + + + (1 nM)
AMD3100 - - + - + + (10 μM)
Loratadine - - - + + + (10 μM)

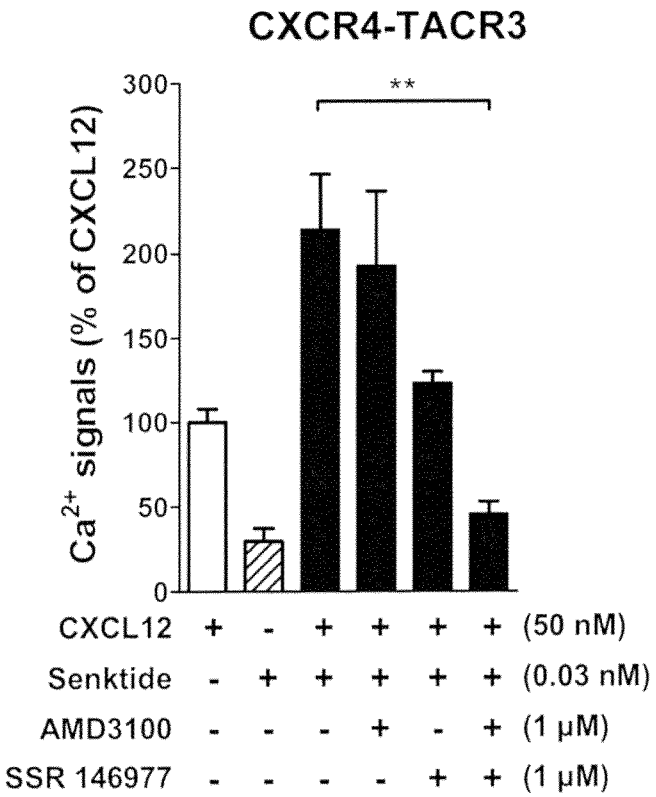
[FIG. 8j]


CXCR4-MLNR
Ca^{2+} signals (% of CXCL12)
0
400
800
1200
1600
2000
2400
2800
*
*
CXCL12 + - + + + + (50 nM)
Motilin - + + + + + (0.1 nM)
AMD3100 - - - + - + (1 μM)
MA-2029 - - - - + + (1 μM)

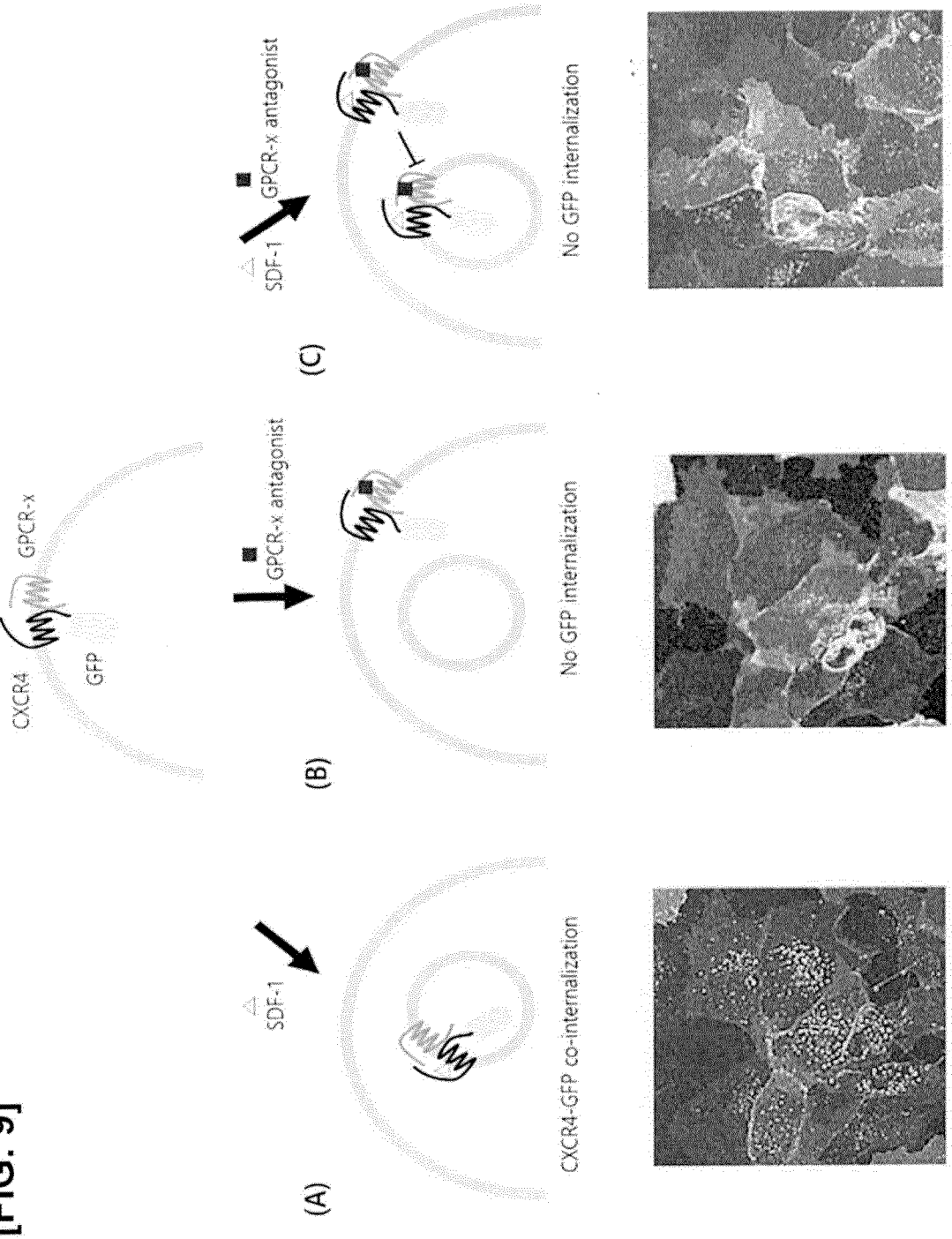
[FIG. 8k]


CXCR4-NTSR1
Ca^{2+} signals (% of CXCL12)
0
100
200
300
400
500
600
700
*
**
***
CXCL12 + - + + + + (50 nM)
Neurotensin - + + + + + (0.01 nM)
AMD3100 - - - + - + (1 μM)
Meclinertant - - - - + + (1 μM)

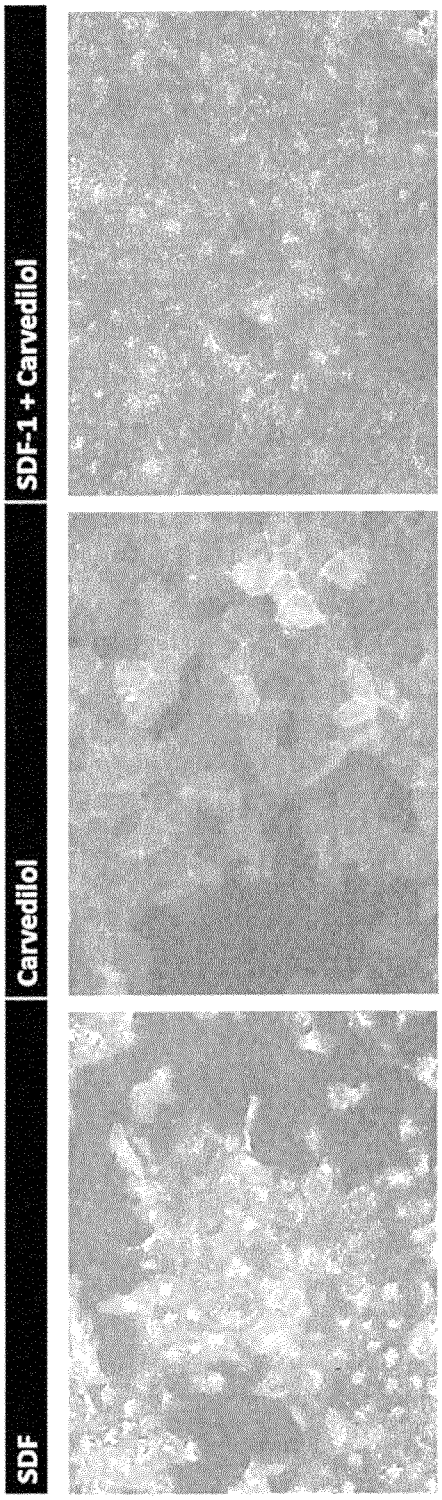
[Fig. 8l]


CXCR4-TACR3
**
300
250
200
150
100
50
0
Ca2+ signals (% of CXCL12)
CXCL12 + - + + + + (50 nM)
Senktide - + + + + + (0.03 nM)
AMD3100 - - - + - + (1 μM)
SSR 146977 - - - - + + (1 μM)

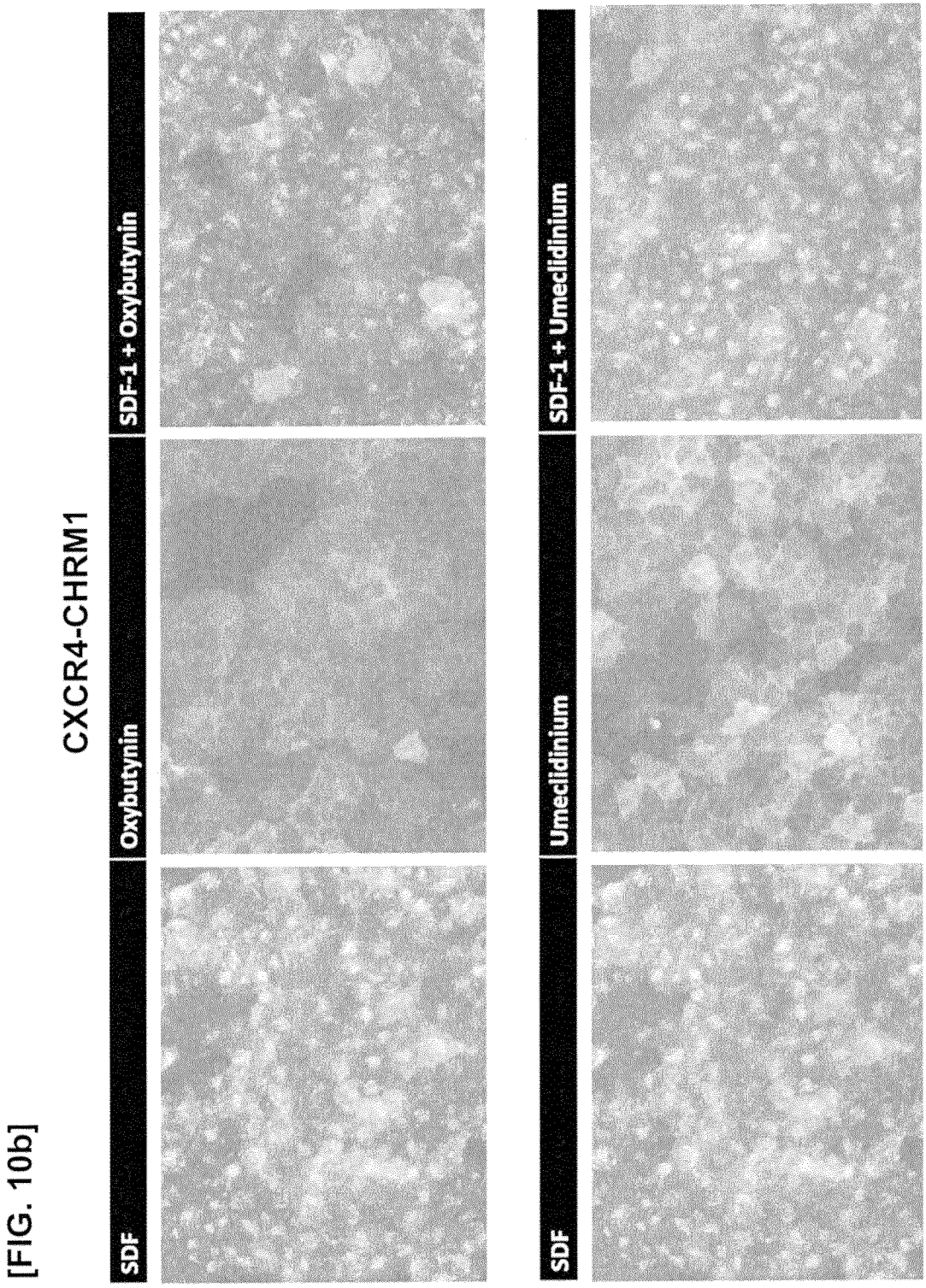
[FIG. 9]


CXCR4
GPCR-x
GFP
(A)
SDF-1
CXCR4-GFP co-internalization
(B)
GPCR-x antagonist
No GFP internalization
(C)
SDF-1
GPCR-x antagonist
No GFP internalization

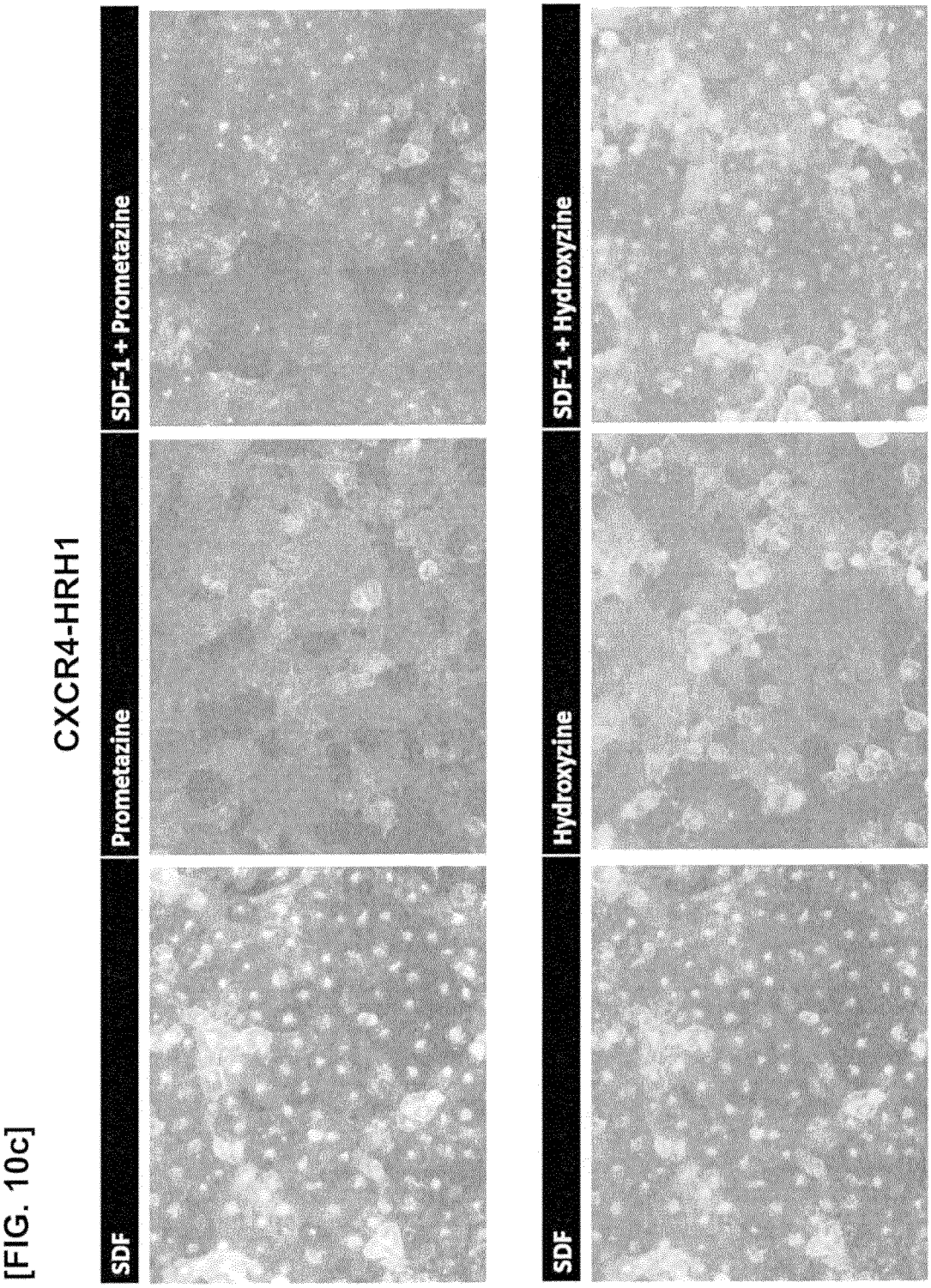
[FIG. 10a]



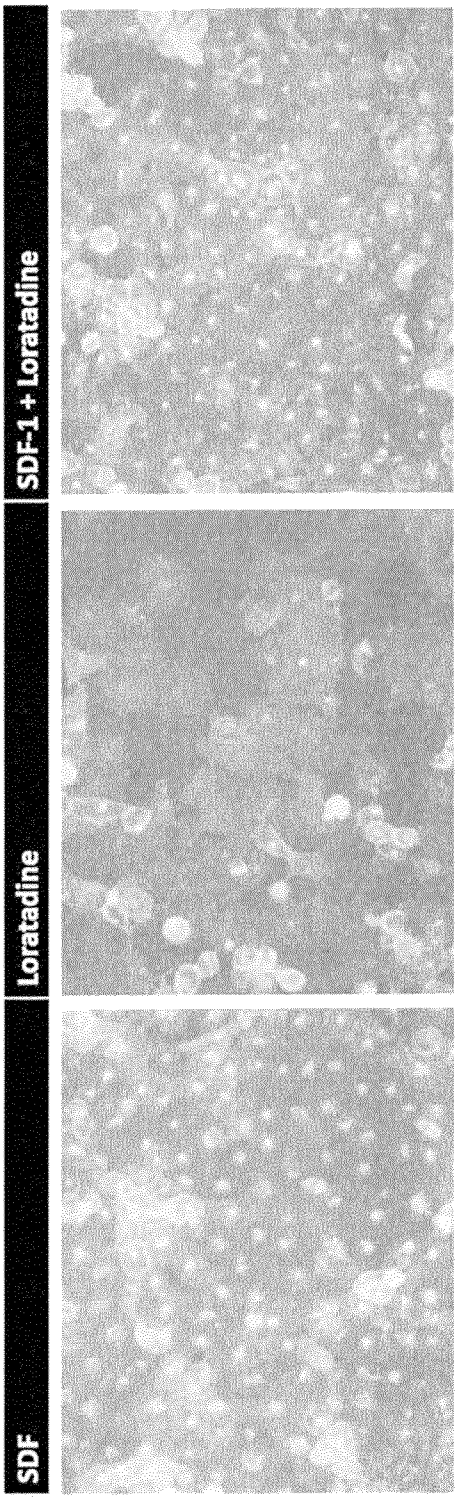
[FIG. 10b]


CXCR4-CHRM1
SDF
Oxybutynin
SDF-1 + Oxybutynin
SDF
Umeclidinium
SDF-1 + Umeclidinium

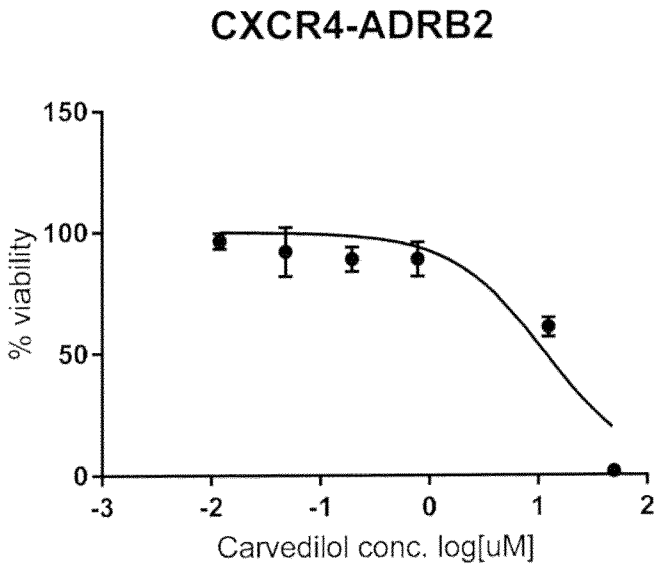
[FIG. 10c]


CXCR4-HRH1
SDF
Prometazine
SDF-1 + Prometazine
SDF
Hydroxyzine
SDF-1 + Hydroxyzine

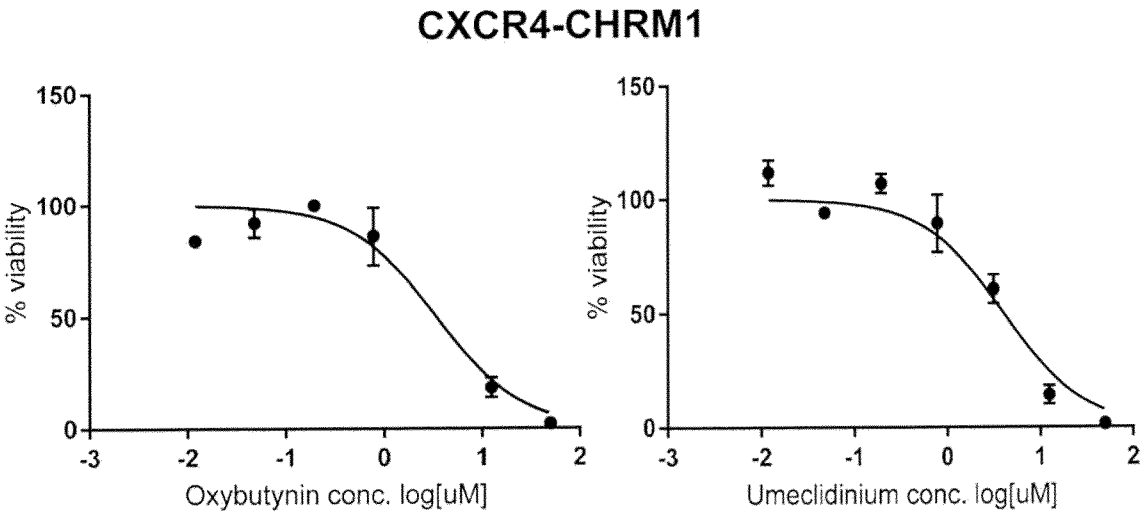
[FIG. 10c (continued)]



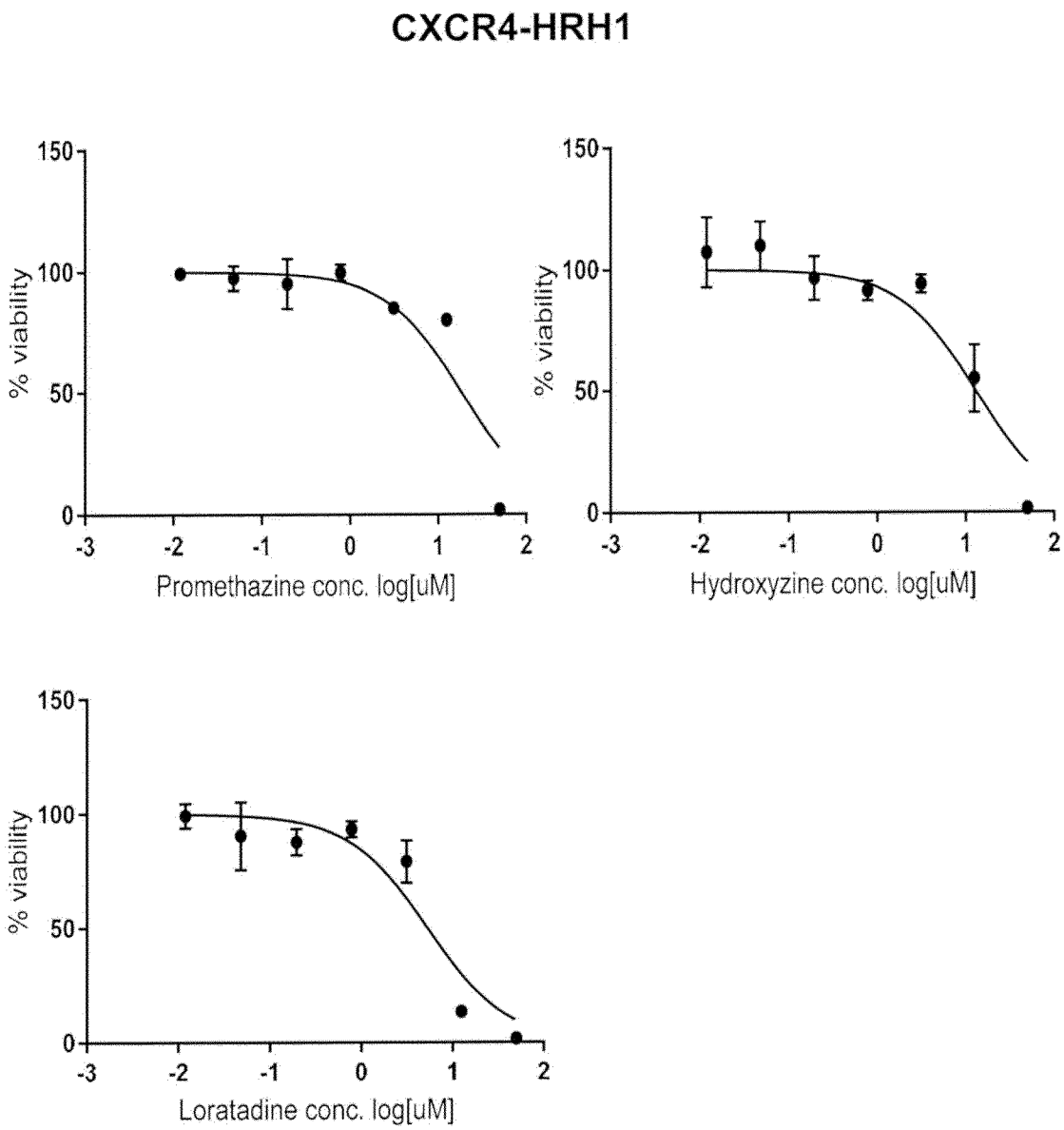
[FIG. 11a]


CXCR4-ADRB2
150
100
50
0
% viability
-3
-2
-1
0
1
2
Carvedilol conc. log[uM]

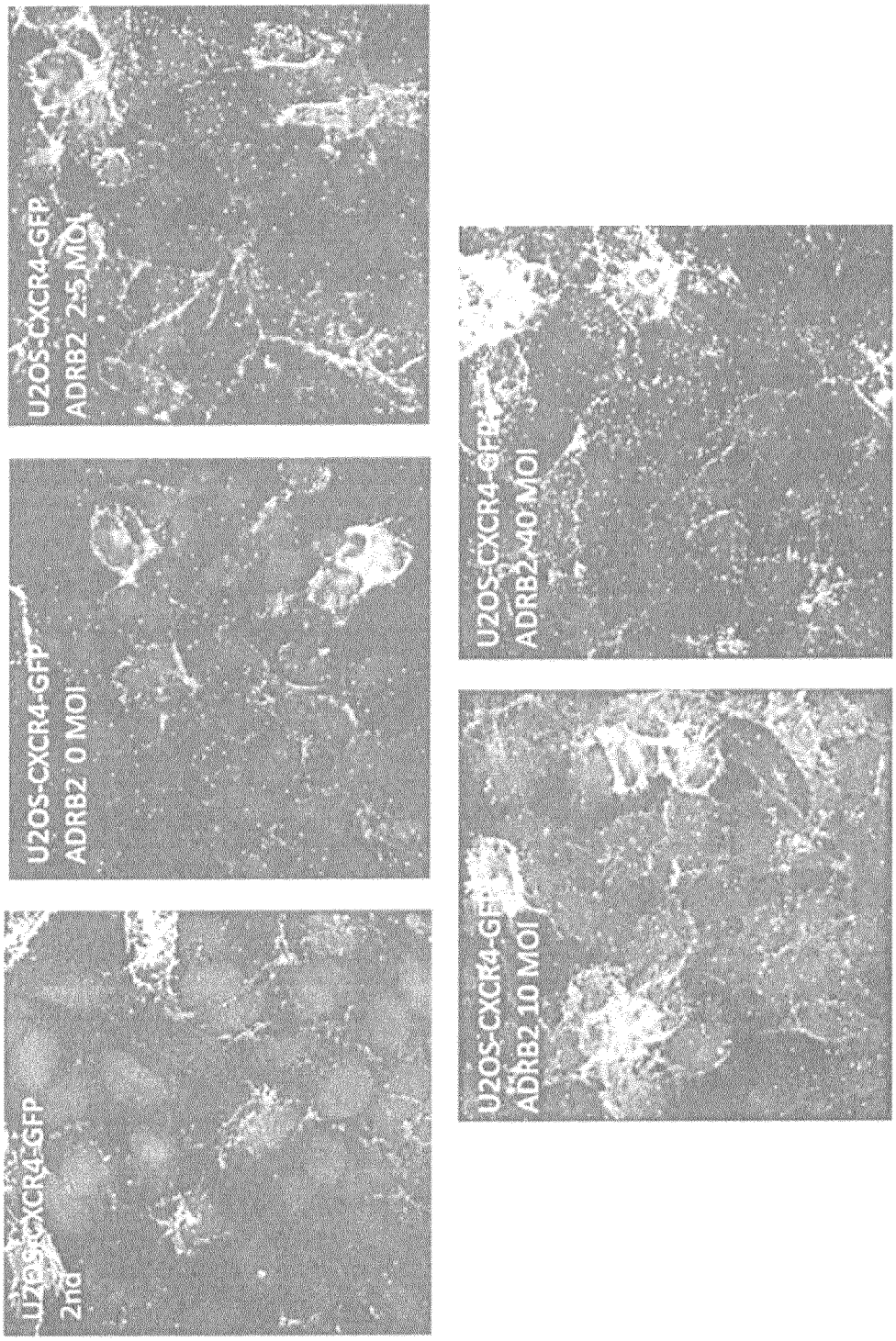
[FIG. 11b]


CXCR4-CHRM1
150
100
50
0
% viability
-3
-2
-1
0
1
2
Oxybutynin conc. log[uM]
150
100
50
0
% viability
-3
-2
-1
0
1
2
Umeclidinium conc. log[uM]

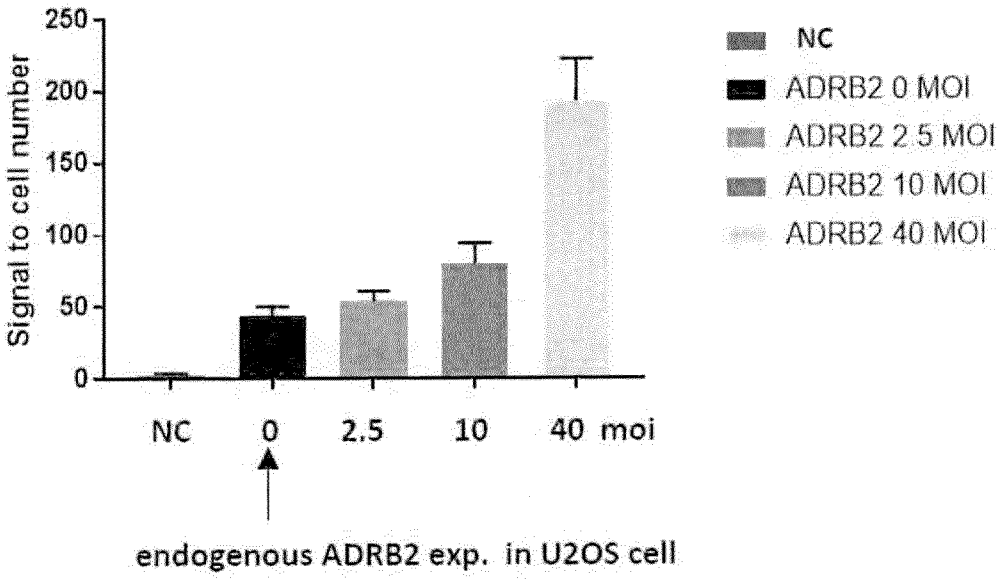
[FIG. 11c]


CXCR4-HRH1
150
100
50
0
% viability
-3
-2
-1
0
1
2
Promethazine conc. log[uM]
Hydroxyzine conc. log[uM]
Loratadine conc. log[uM]

[FIG. 12a]


U2OS-CXCR4-GFP
2nd
U2OS-CXCR4-GFP
ADRB2 0 MOI
U2OS-CXCR4-GFP
ADRB2 2.5 MOI
U2OS-CXCR4-GFP
ADRB2 10 MOI
U2OS-CXCR4-GFP
ADRB2 40 MOI

[FIG. 12b]

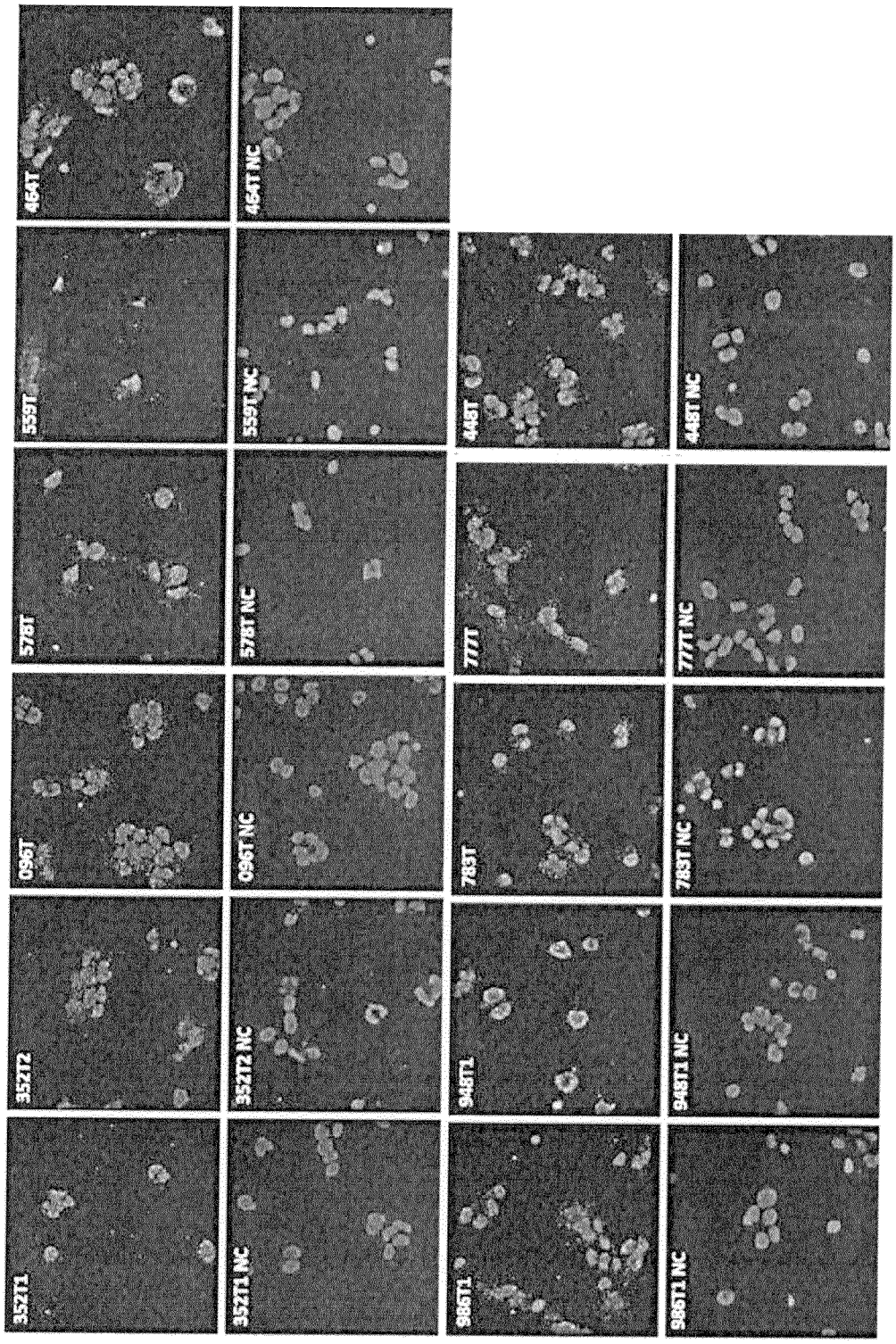


[FIG. 12c]

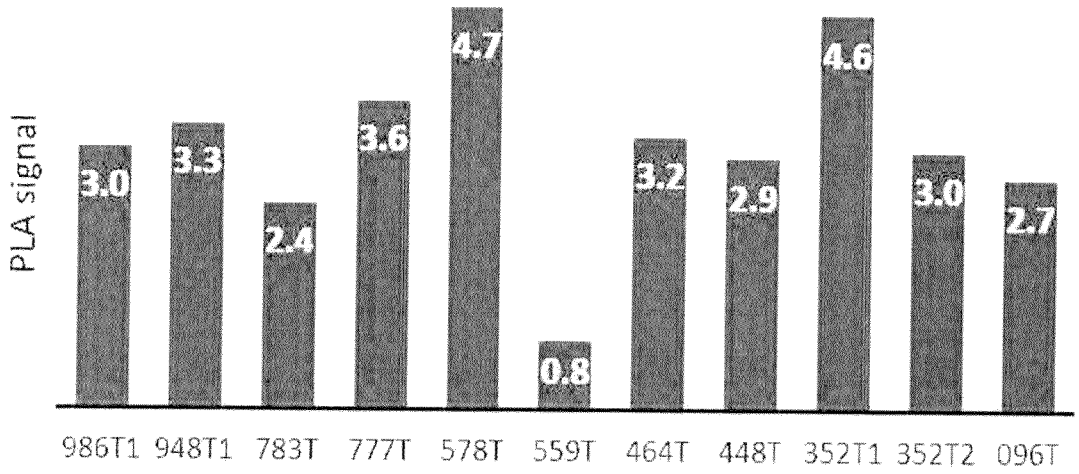
sample	actin	ADRB2
U2OS	*14.2	*22.3

*qPCR (Ct value)

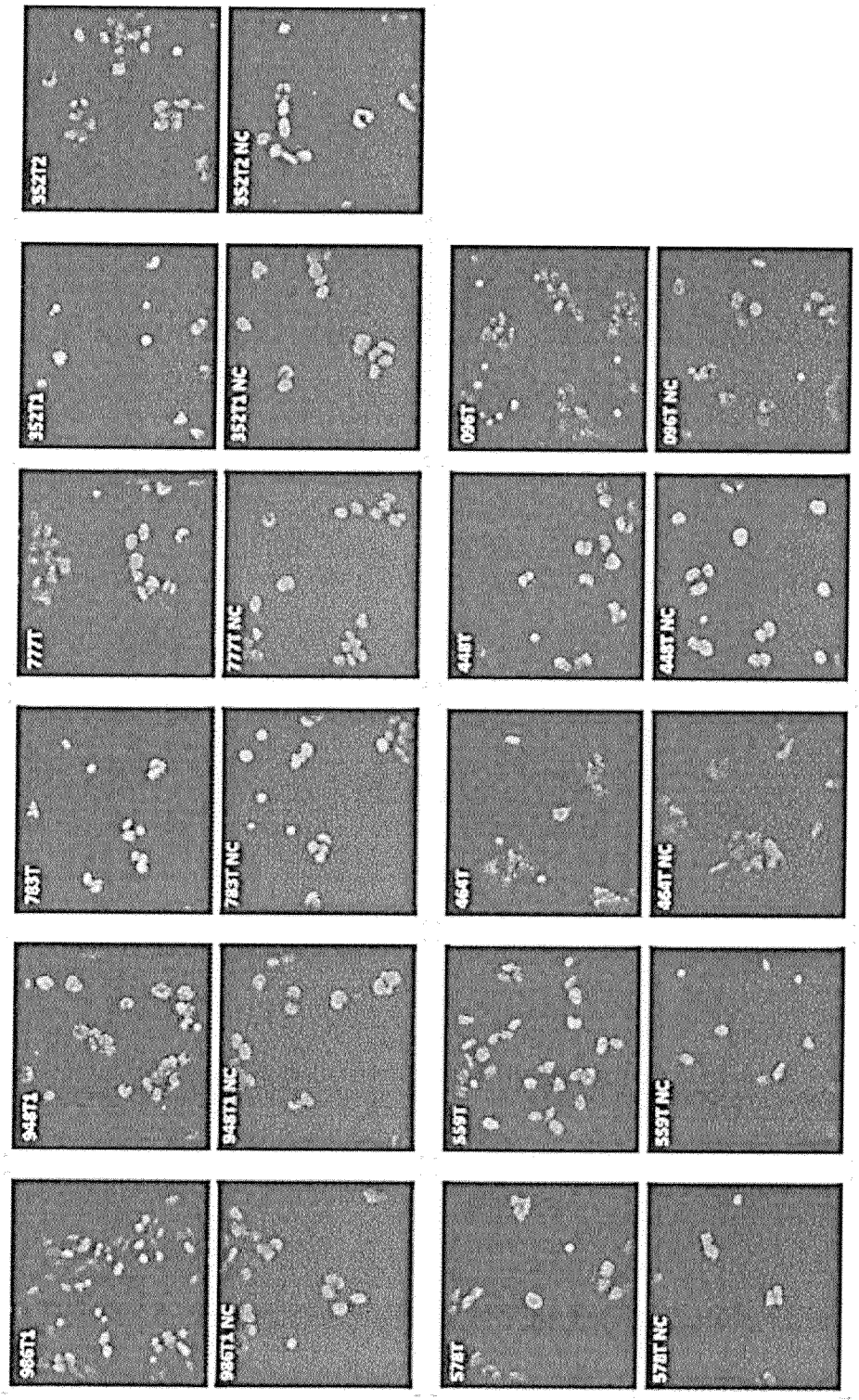
[FIG. 13a]


464T
464T NC
559T
559T NC
578T
578T NC
096T
096T NC
352T2
352T2 NC
352T1
352T1 NC
448T
448T NC
777T
777T NC
783T
783T NC
948T1
948T1 NC
986T1
986T1 NC

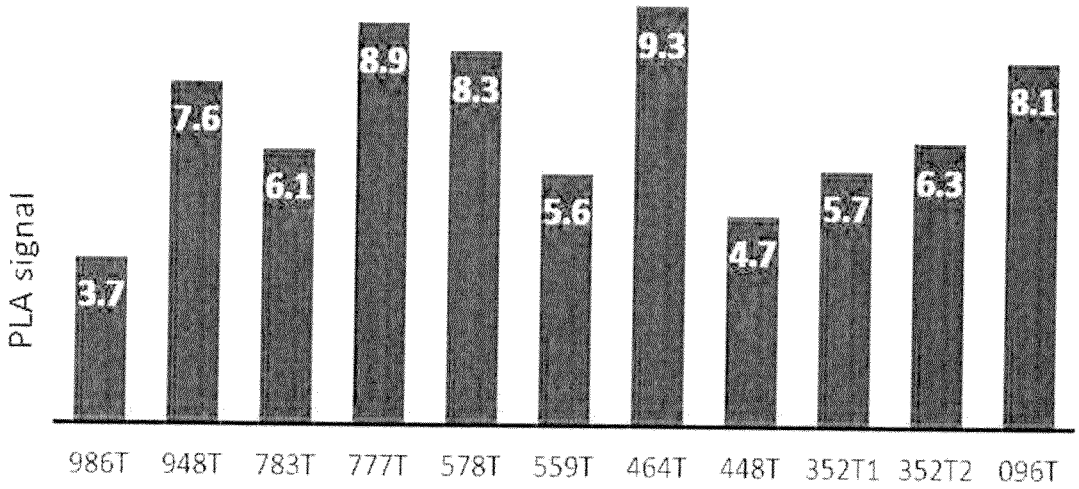
[FIG. 13b]


PLA signal
3.0
3.3
2.4
3.6
4.7
0.8
3.2
2.9
4.6
3.0
2.7
986T1
948T1
783T
777T
578T
559T
464T
448T
352T1
352T2
096T

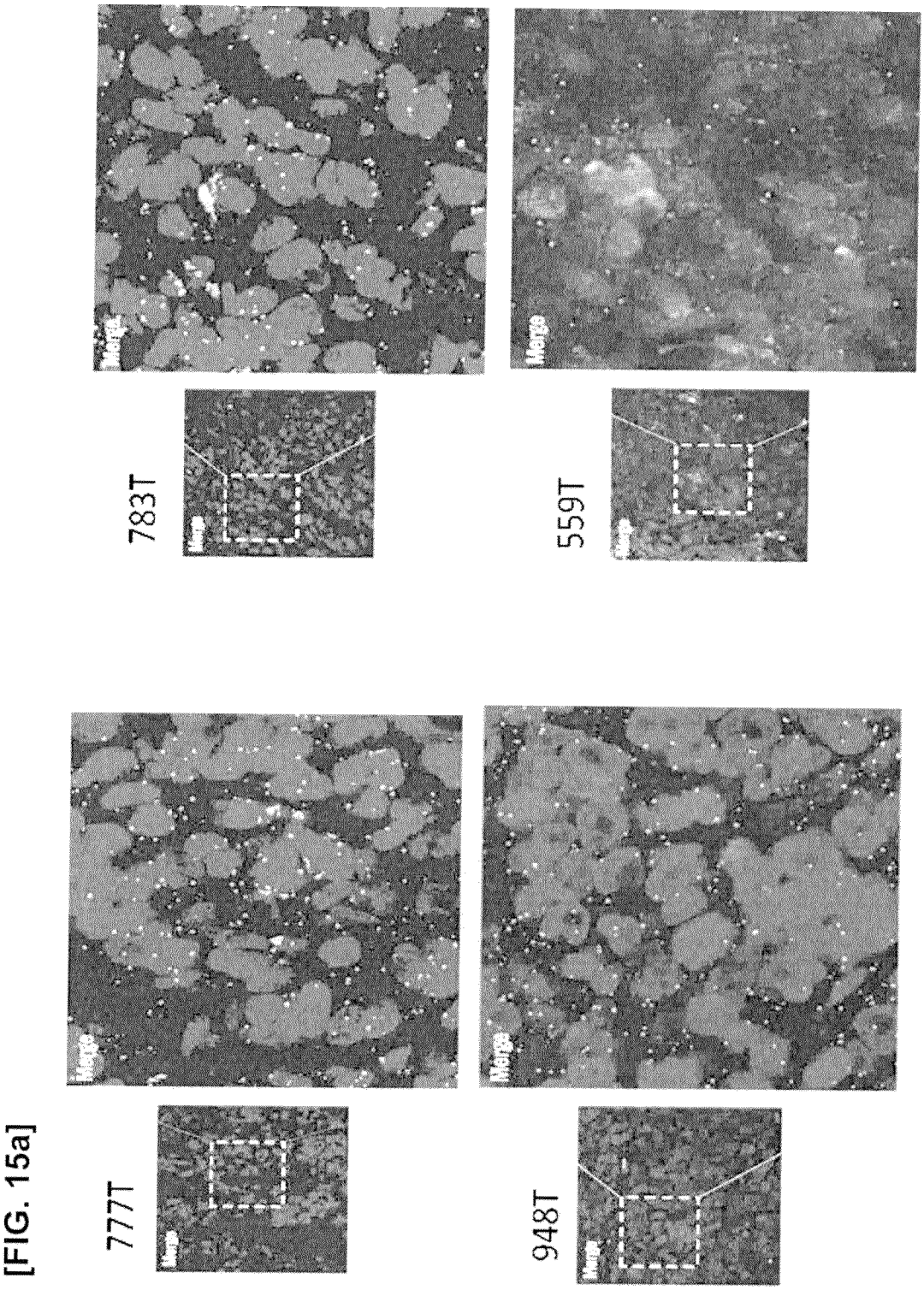
[FIG. 14a]


9857T
9857T NC
5781T
5781T NC
9487T
9487T NC
5591T
5591T NC
7851T
7851T NC
4641T
4641T NC
7777T
7777T NC
4485T
4485T NC
3521T
3521T NC
0965T
0965T NC
3527T
3527T NC

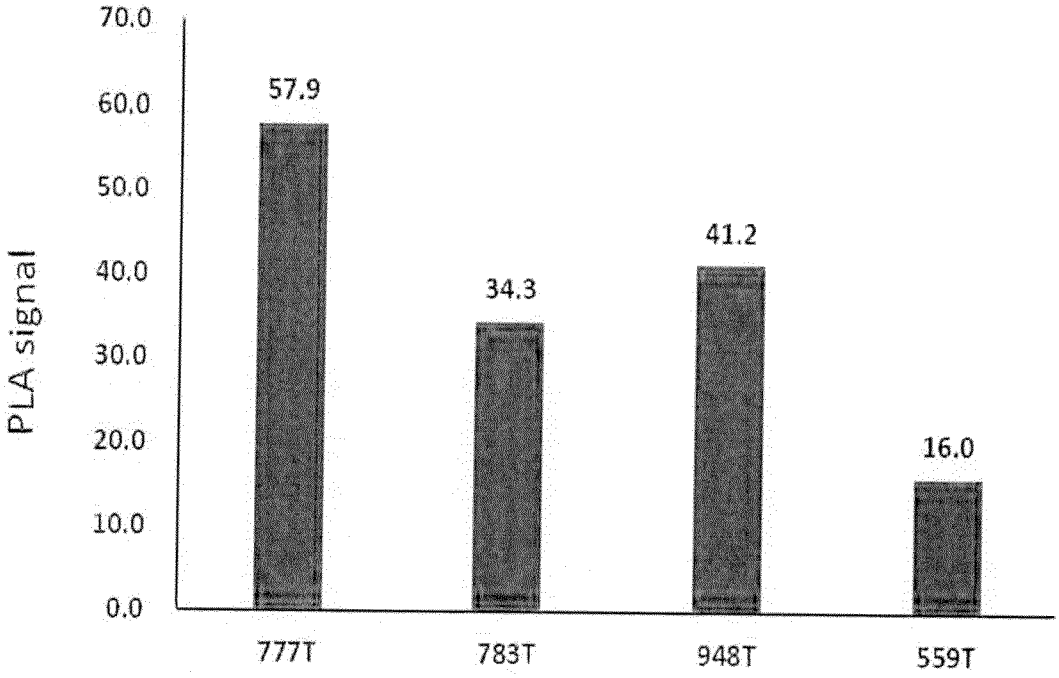
[FIG. 14b]


PLA signal
3.7
7.6
6.1
8.9
8.3
5.6
9.3
4.7
5.7
6.3
8.1
986T
948T
783T
777T
578T
559T
464T
448T
352T1
352T2
096T

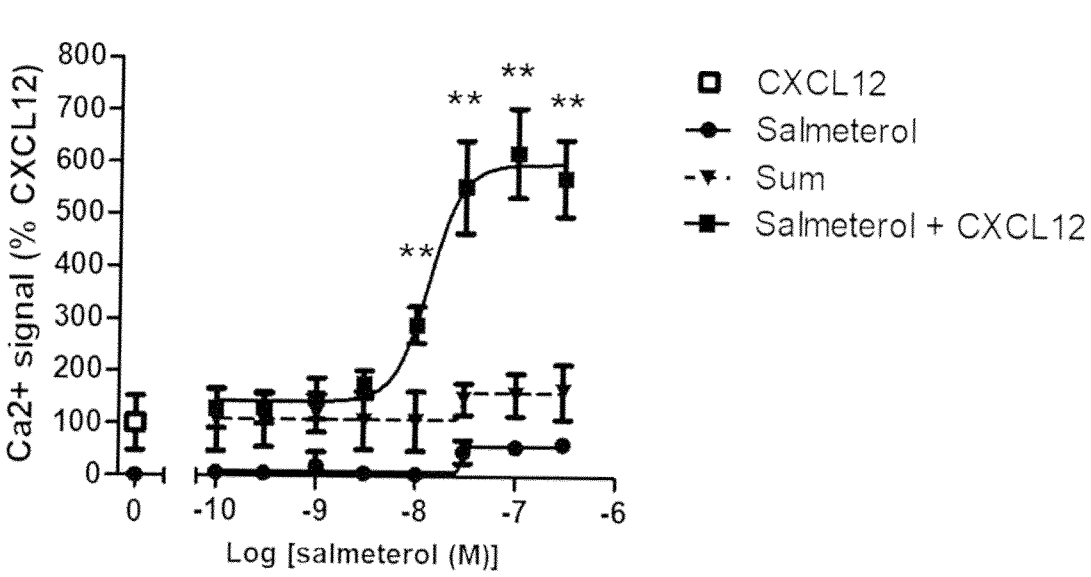
[FIG. 15a]



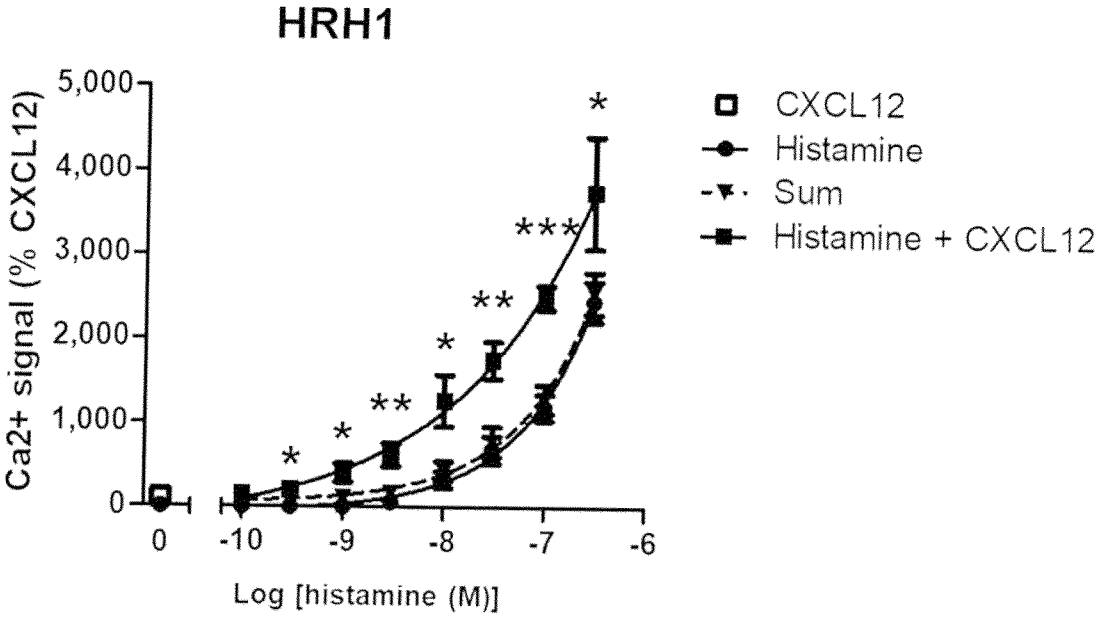
[FIG. 15b]


70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
PLA signal
57.9
34.3
41.2
16.0
777T
783T
948T
559T

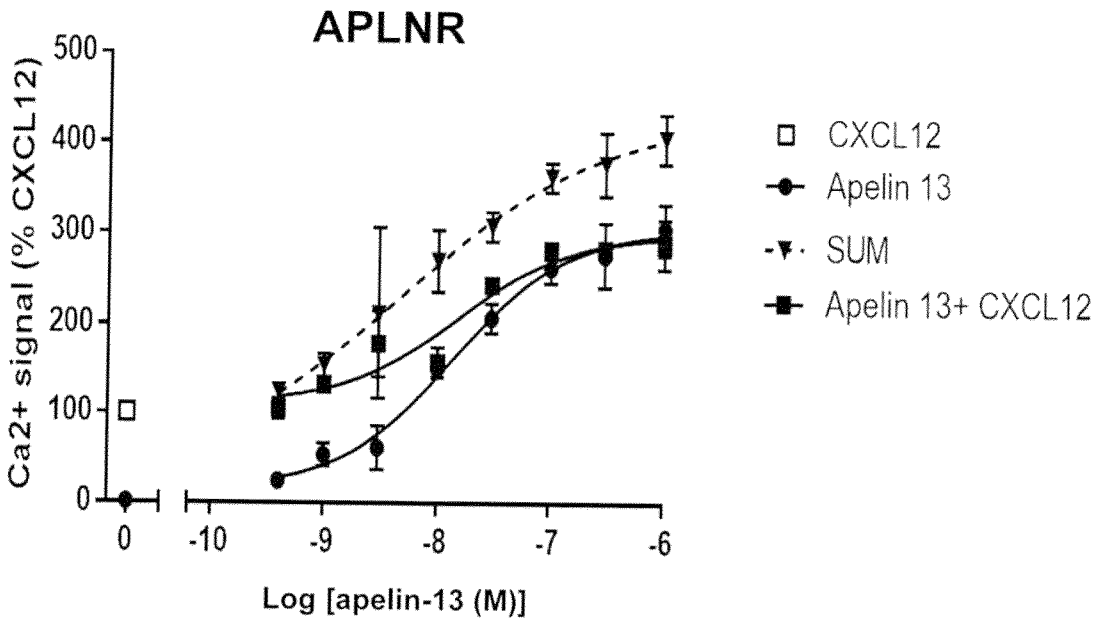
[FIG. 16a]


ADRB2
Ca2+ signal (% CXCL12)
800
700
600
500
400
300
200
100
0
0
-10
-9
-8
-7
-6
Log [salmeterol (M)]
**
**
**
**
CXCL12
Salmeterol
Sum
Salmeterol + CXCL12

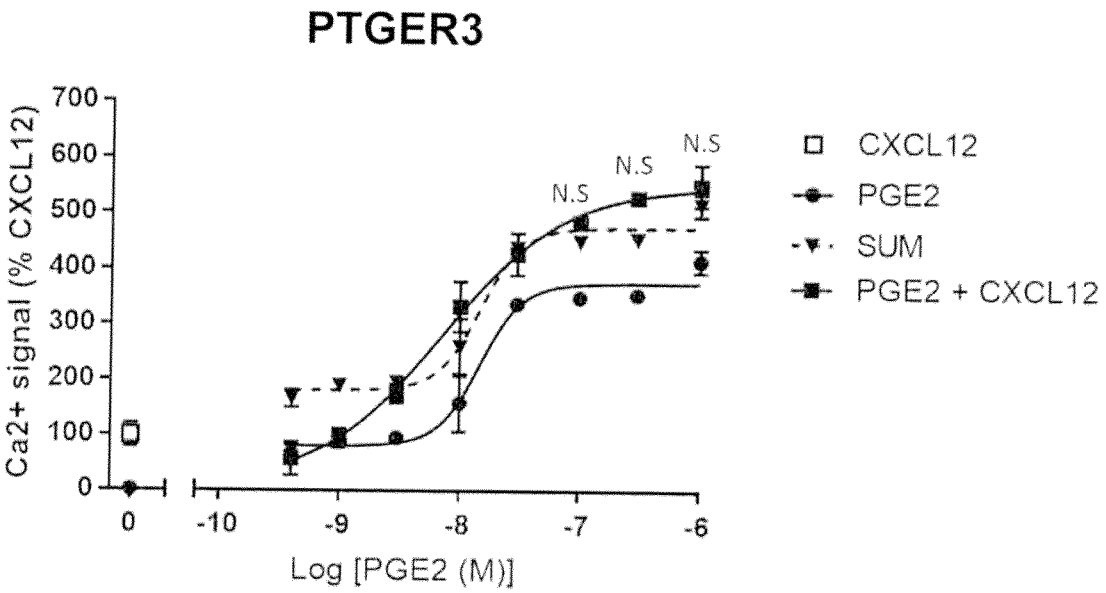
[FIG. 16b]


HRH1
Ca2+ signal (% CXCL12)
5,000
4,000
3,000
2,000
1,000
0
0
-10
-9
-8
-7
-6
Log [histamine (M)]
*
*
**
*
**
***
CXCL12
Histamine
Sum
Histamine + CXCL12

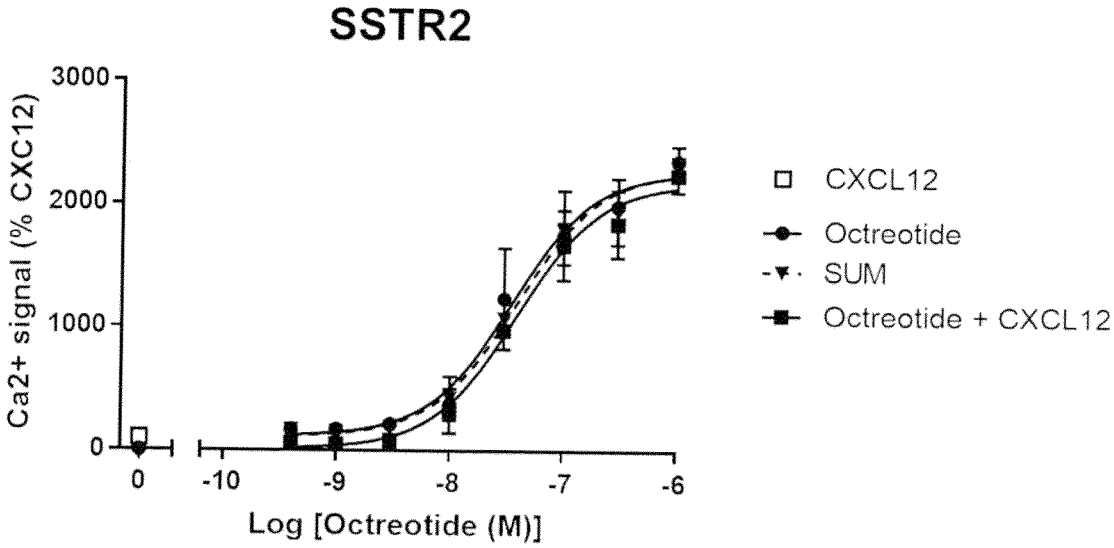
[FIG. 17a]



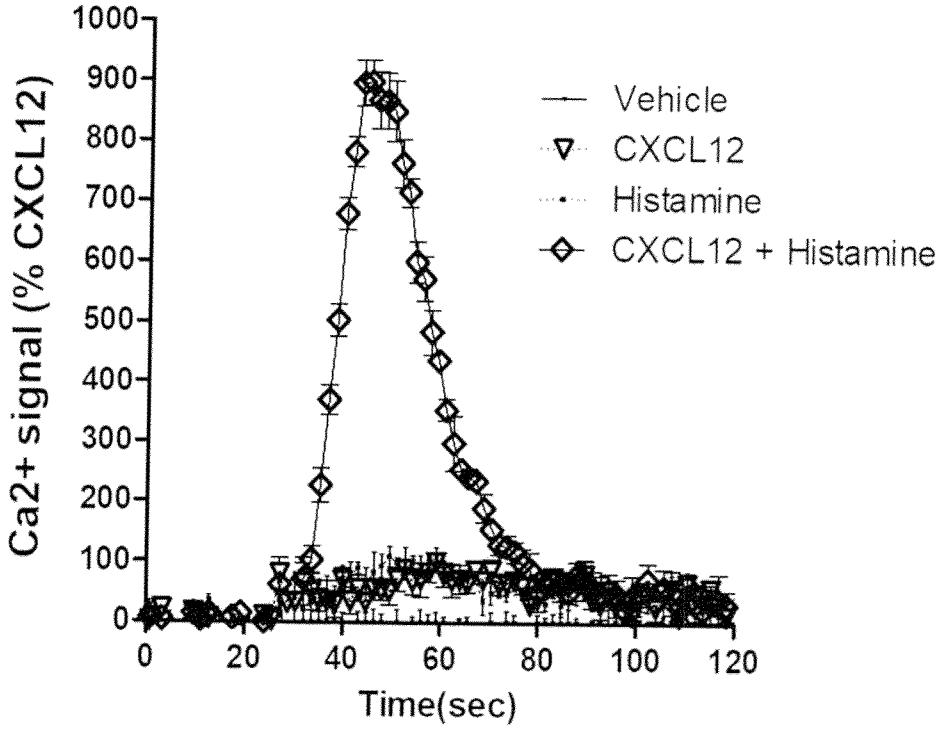
[FIG. 17b]



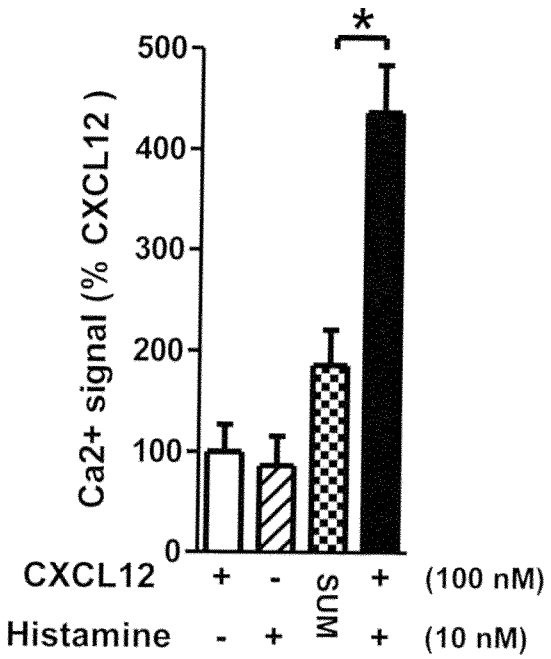
[FIG. 17c]


SSTR2
Ca2+ signal (% CXC12)
3000
2000
1000
0
0
-10
-9
-8
-7
-6
Log [Octreotide (M)]
CXCL12
Octreotide
SUM
Octreotide + CXCL12

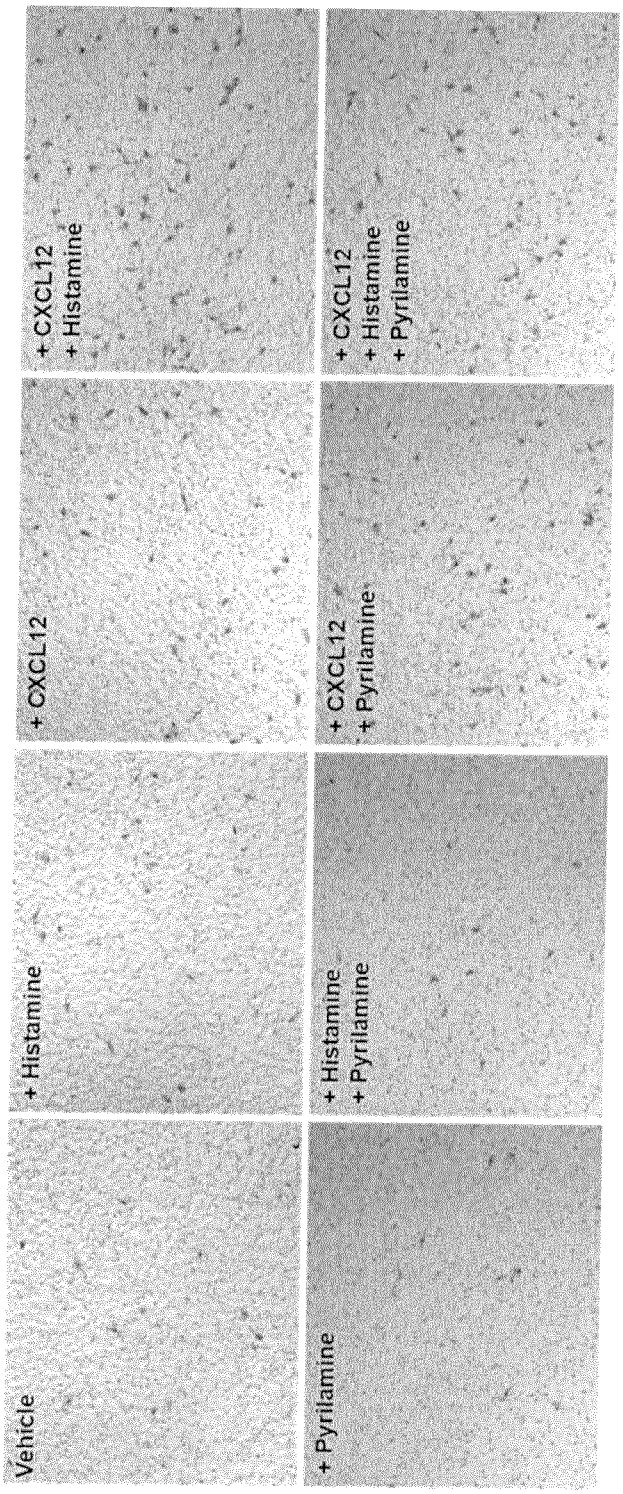
[FIG. 18a]


Ca2+ signal (% CXCL12)
1000
900
800
700
600
500
400
300
200
100
0
0
20
40
60
80
100
120
Time(sec)
Vehicle
CXCL12
Histamine
CXCL12 + Histamine

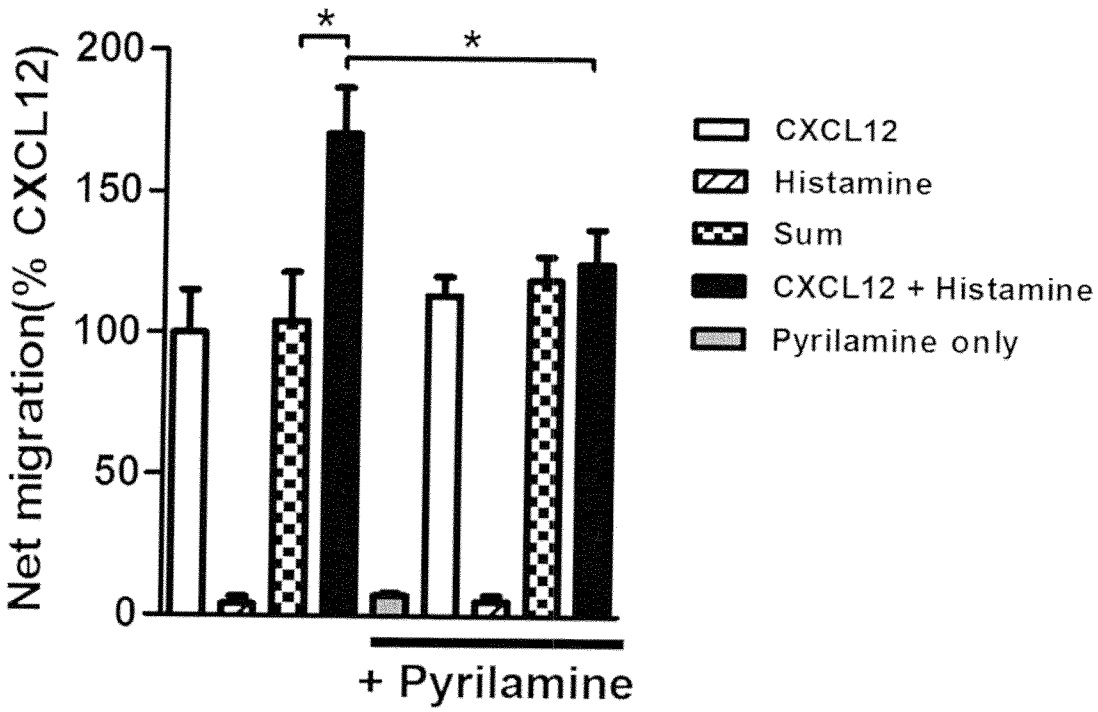
[FIG. 18b]


*
500
400
300
200
100
0
Ca2+ signal (% CXCL12)
CXCL12 + - SUM + (100 nM)
Histamine - + + (10 nM)

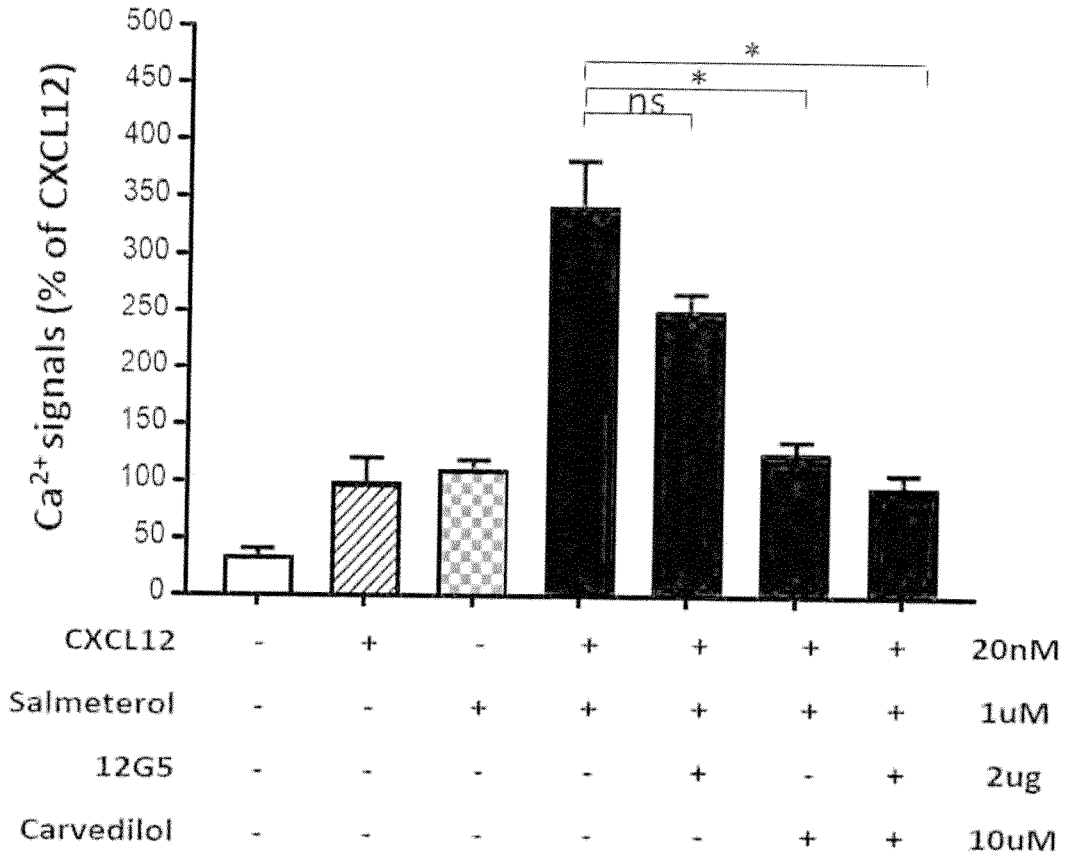
[FIG. 19a]


Vehicle
+ Histamine
+ CXCL12
+ CXCL12
+ Histamine
+ Pyrilamine
+ Histamine
+ Pyrilamine
+ CXCL12
+ Pyrilamine
+ CXCL12
+ Histamine
+ Pyrilamine

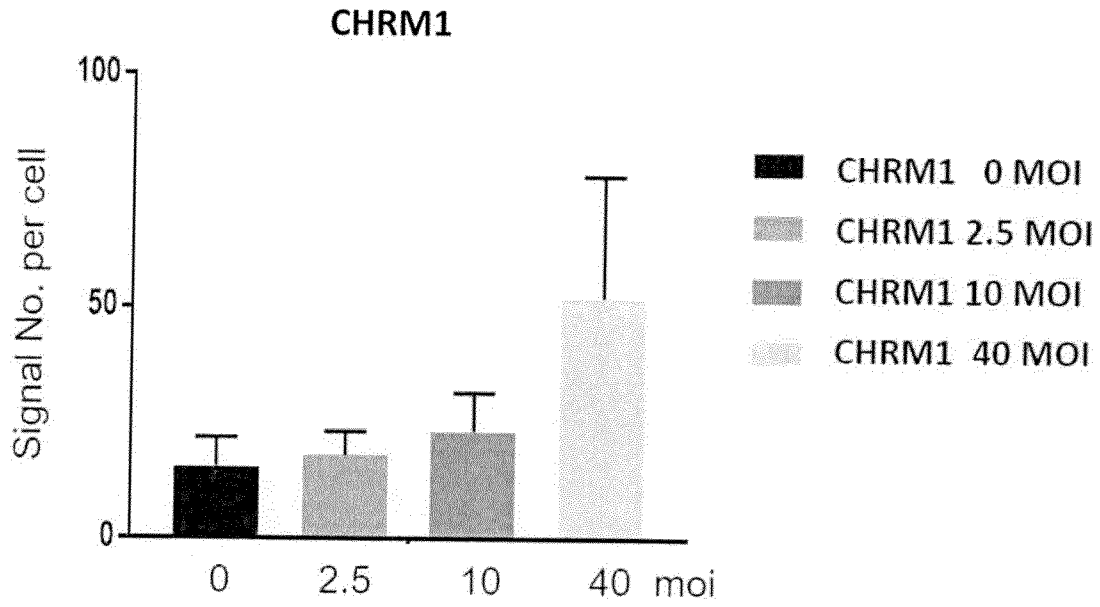
[FIG. 19b]


Net migration(% CXCL12)
200
150
100
50
0
*
*
+ Pyrilamine
CXCL12
Histamine
Sum
CXCL12 + Histamine
Pyrilamine only

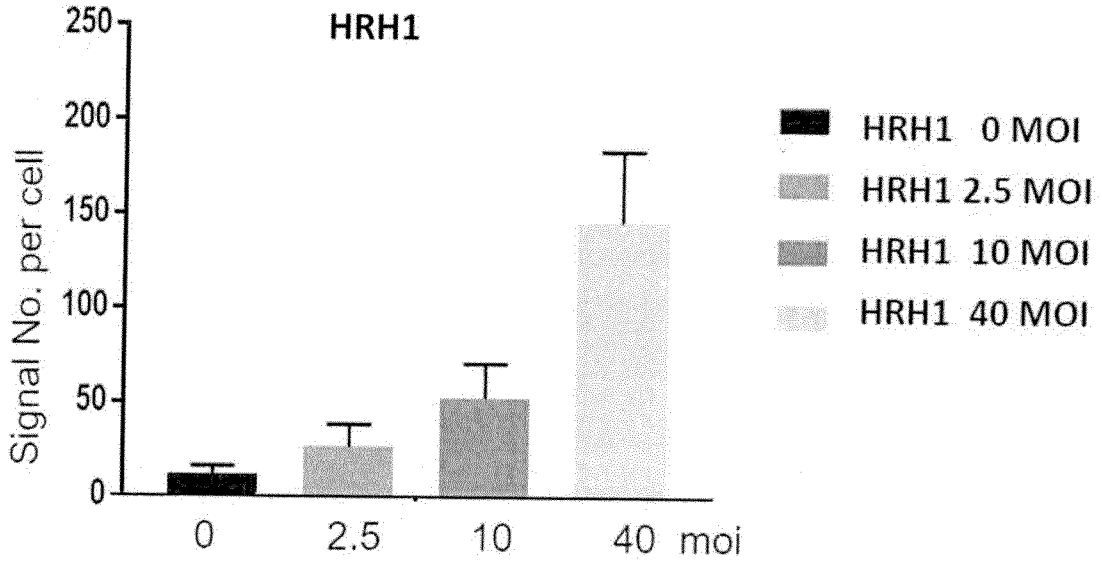
[FIG. 20]


Ca2+ signals (% of CXCL12)
500
450
400
350
300
250
200
150
100
50
0
*
*
ns
CXCL12 - + - + + + + 20nM
Salmeterol - - + + + + + 1uM
12G5 - - - - + - + 2ug
Carvedilol - - - - - + + 10uM

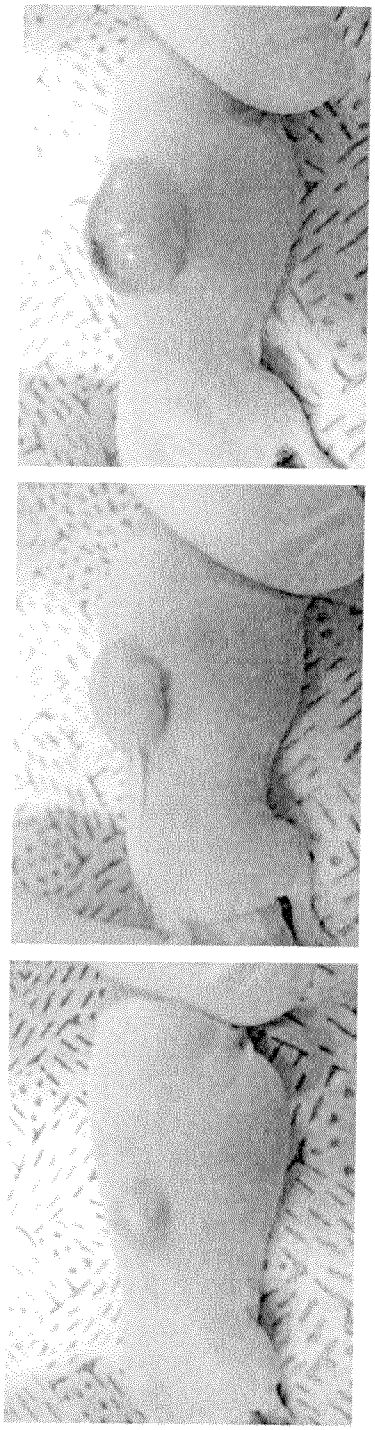
[FIG. 21a]



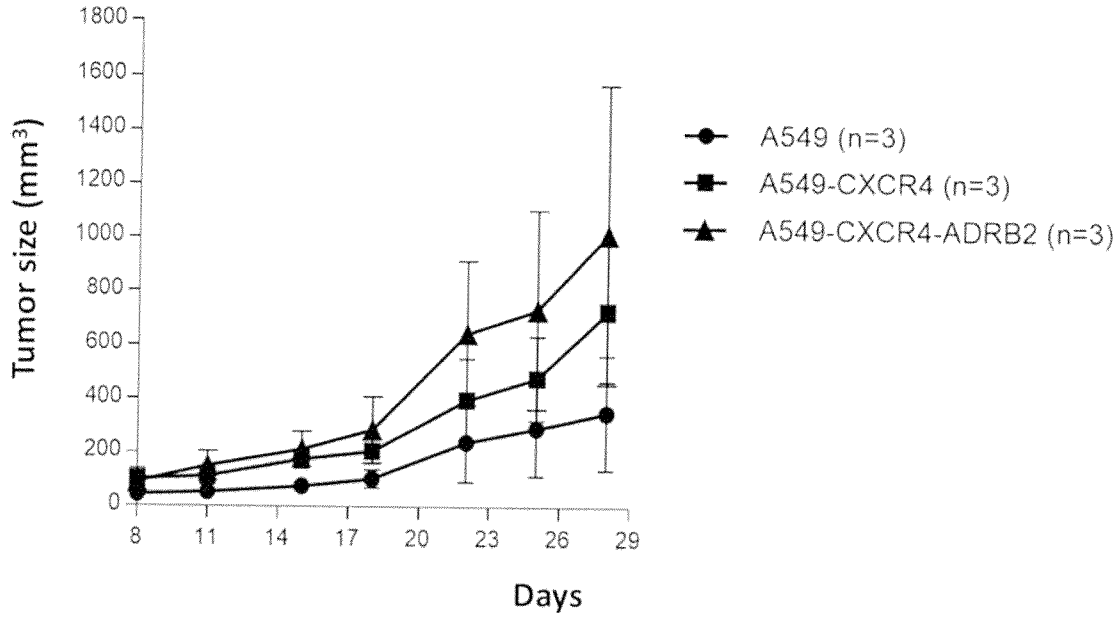
[FIG. 21b]



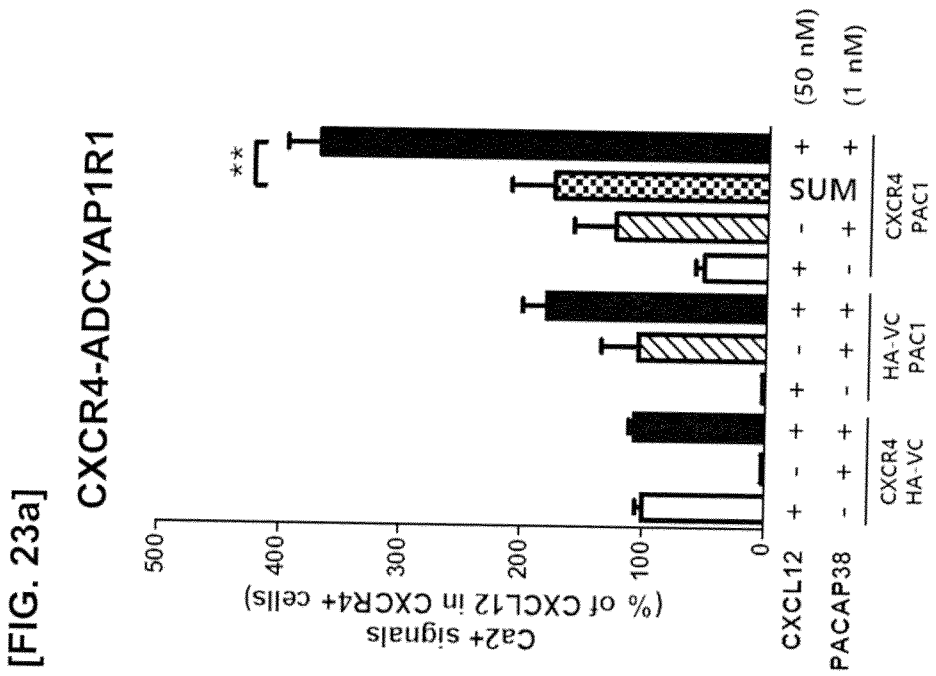
[FIG. 22a]



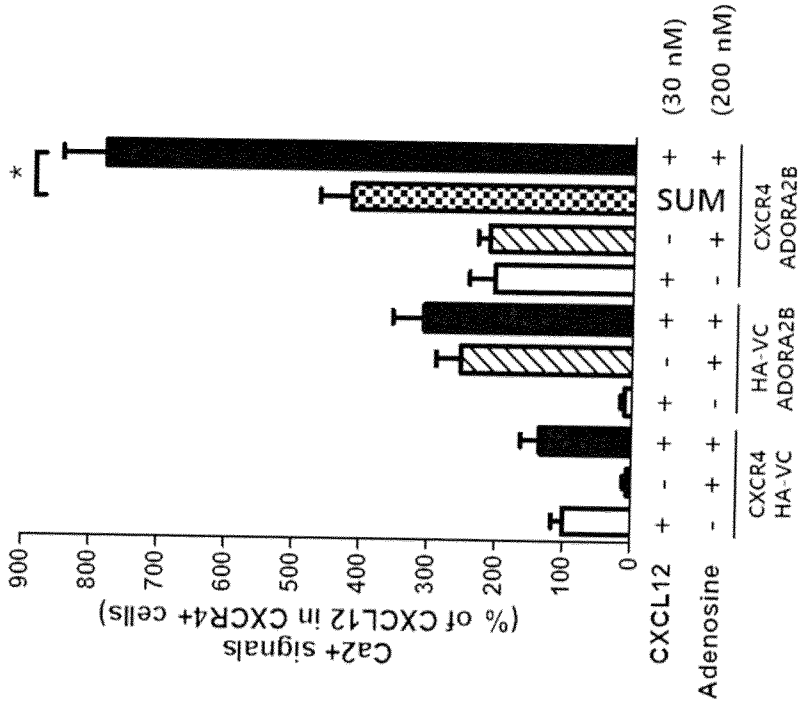
[FIG. 22b]


Tumor size (mm³)
1800
1600
1400
1200
1000
800
600
400
200
0
8
11
14
17
20
23
26
29
Days
A549 (n=3)
A549-CXCR4 (n=3)
A549-CXCR4-ADRB2 (n=3)

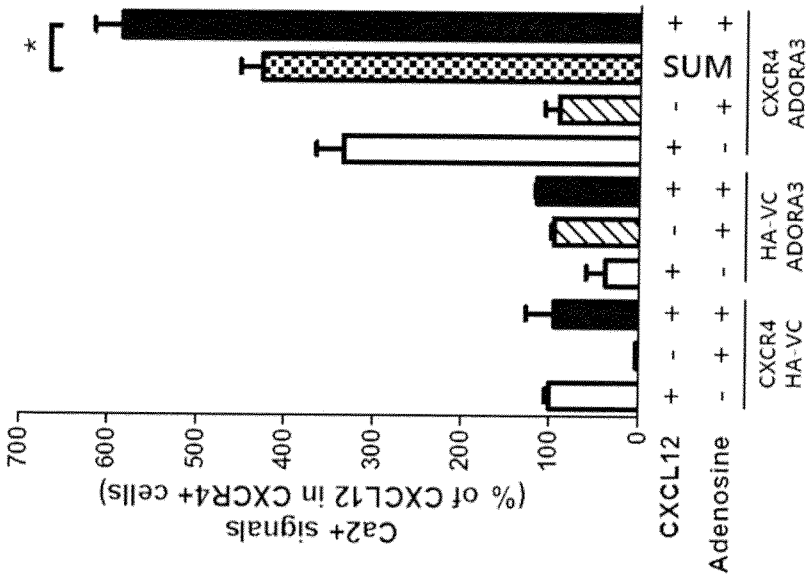
[FIG. 23a]



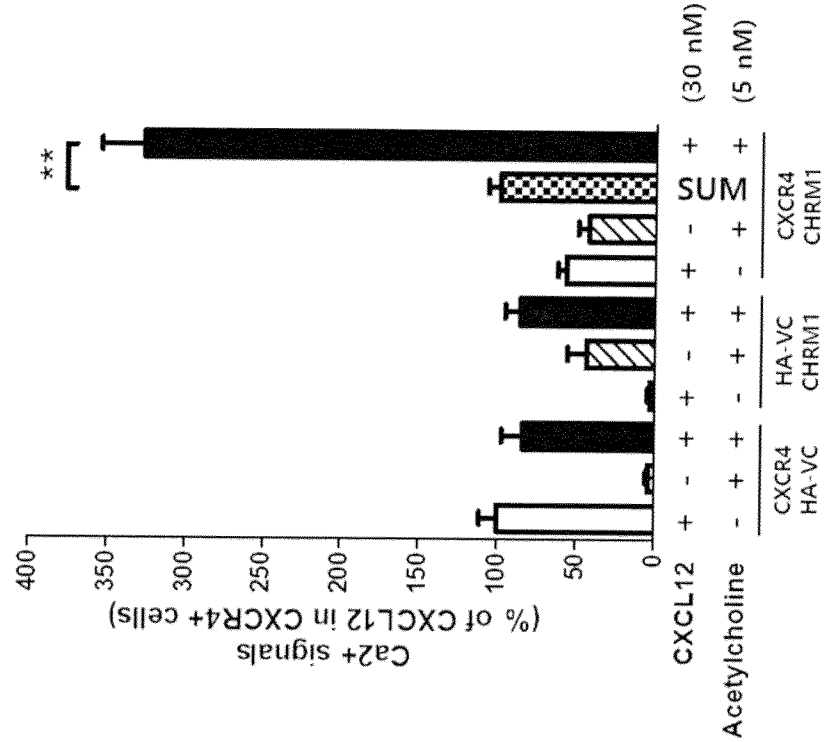
[FIG. 23b]



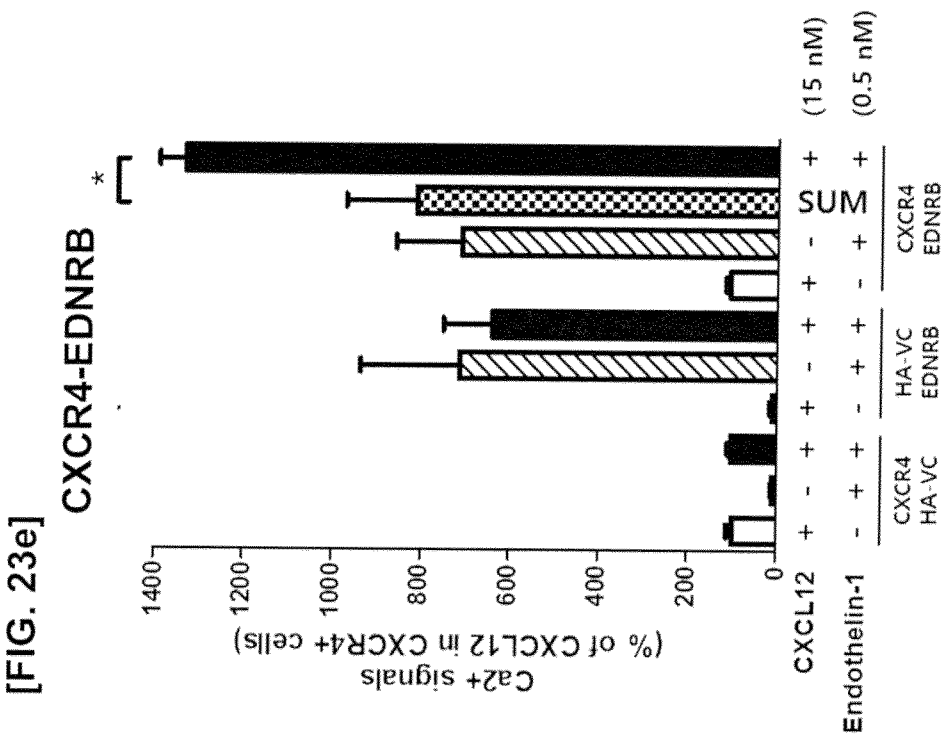
[FIG. 23c]



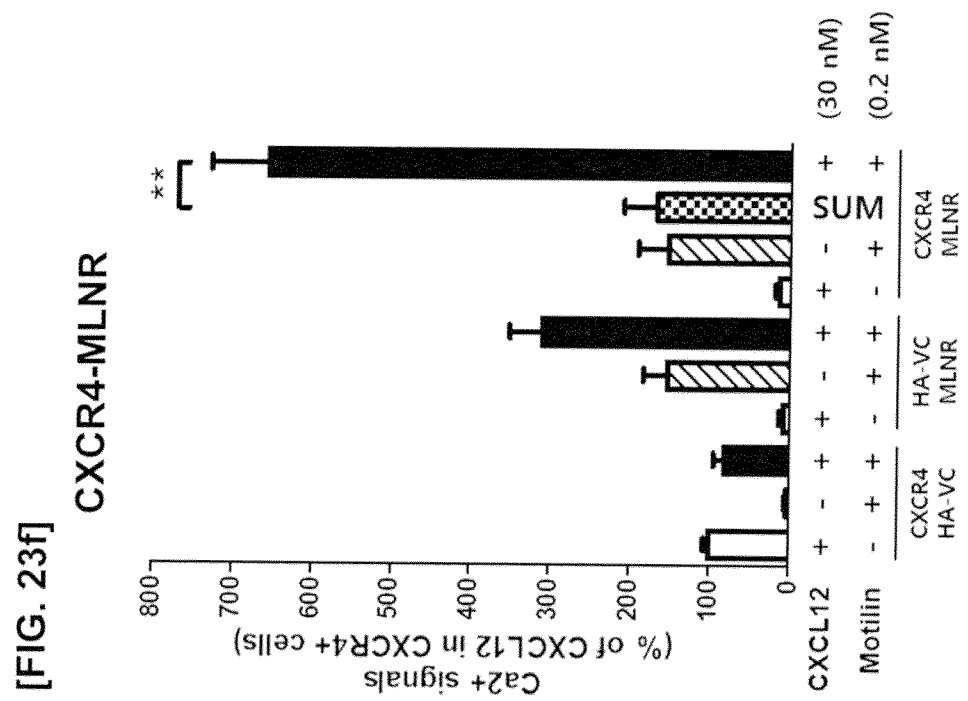
[FIG. 23d]



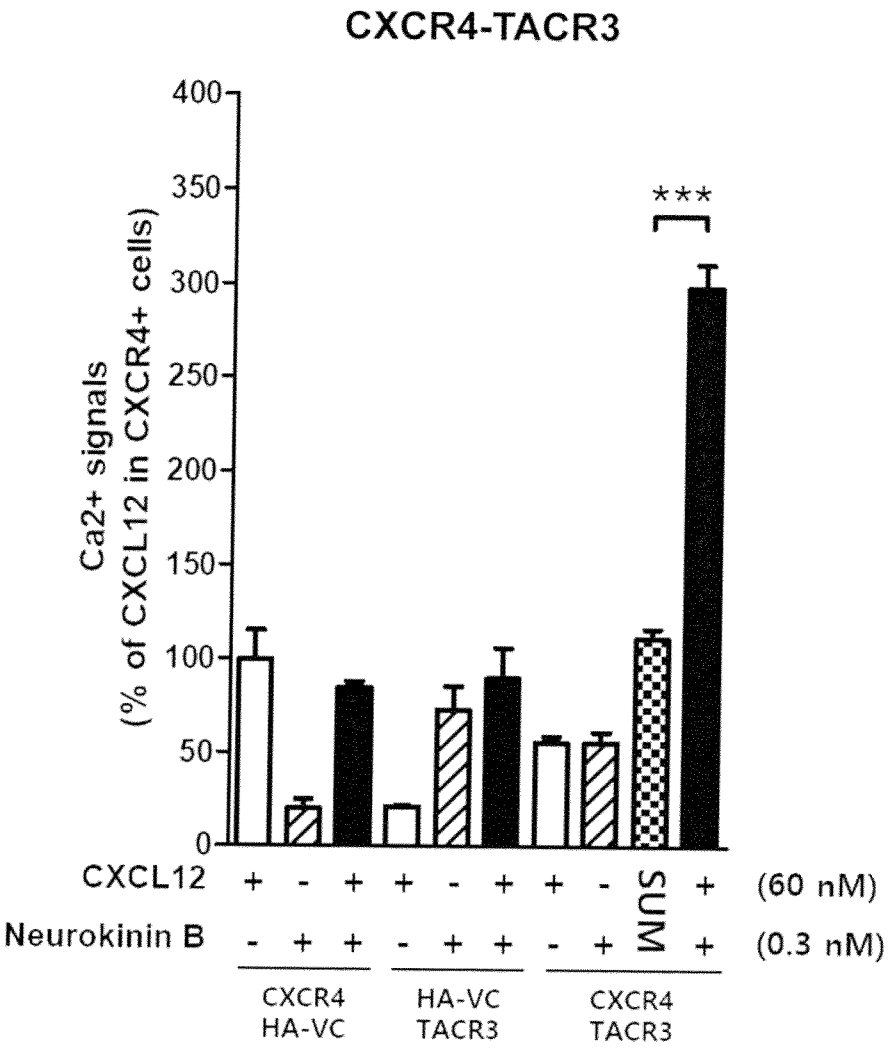
[FIG. 23e]



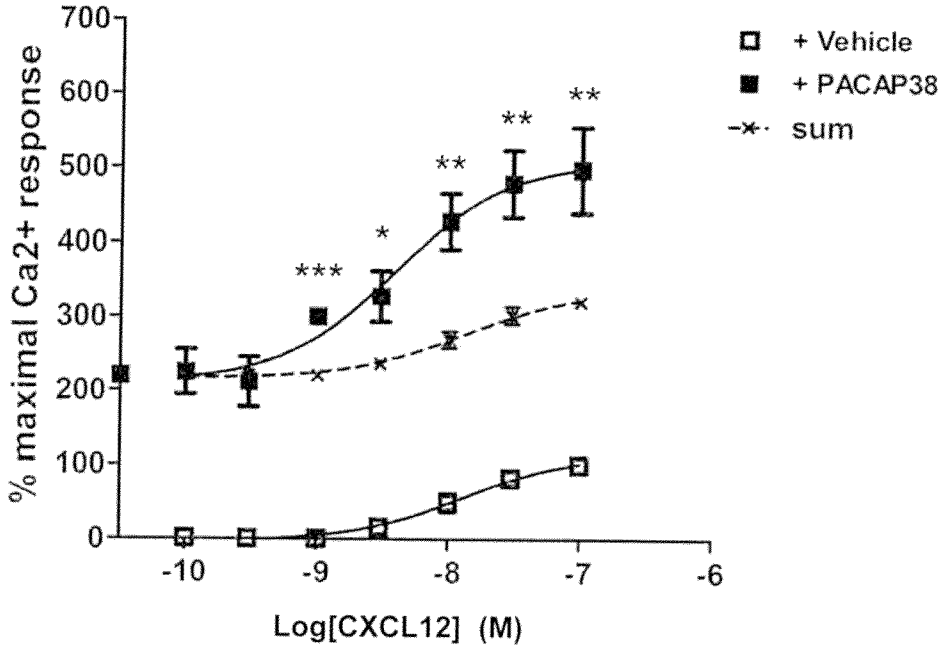
[FIG. 23f]



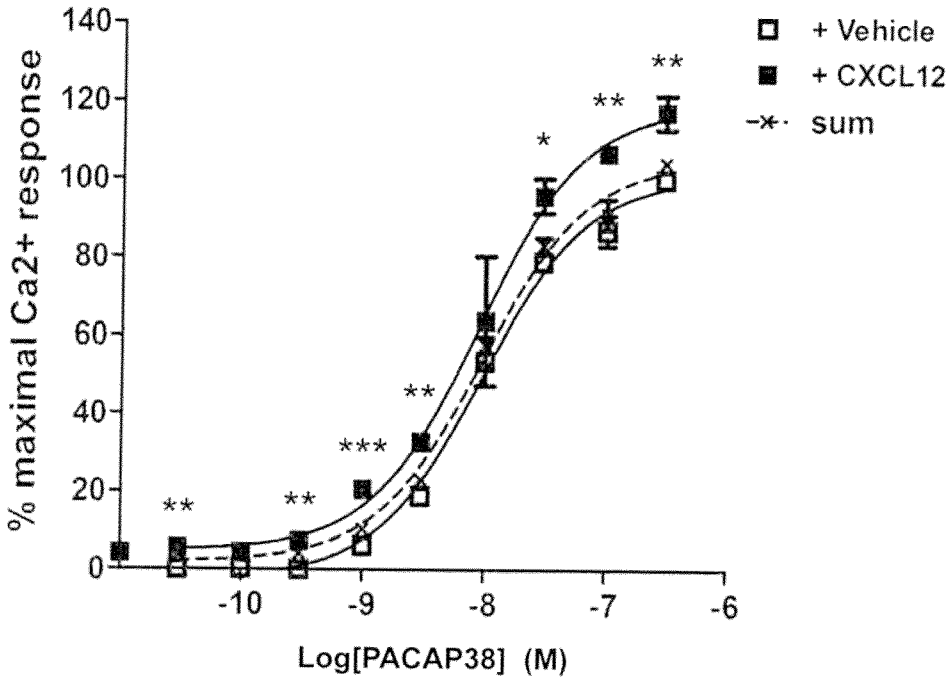
[FIG. 23g]


CXCR4-TACR3
Ca2+ signals
(% of CXCL12 in CXCR4+ cells)
400
350
300
250
200
150
100
50
0
***
CXCL12 + - + + - + + - SUM +
(60 nM)
Neurokinin B - + + - + + - + + +
(0.3 nM)
CXCR4
HA-VC
HA-VC
TACR3
CXCR4
TACR3

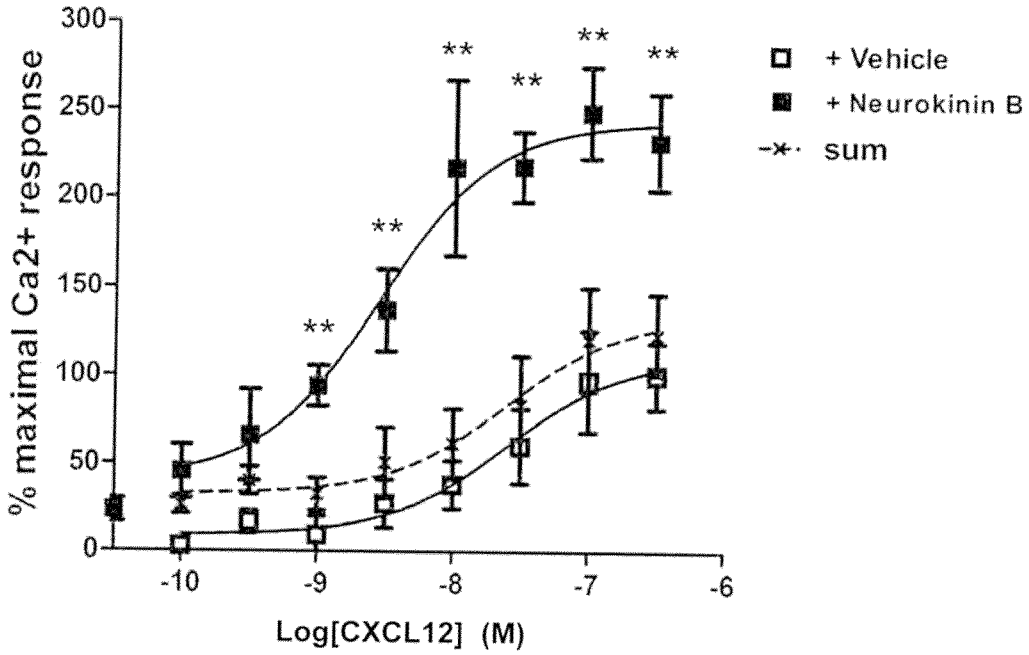
[FIG. 24a]


700
600
500
400
300
200
100
0
% maximal Ca2+ response
***
*
**
**
**
-10
-9
-8
-7
-6
Log[CXCL12] (M)
+ Vehicle
+ PACAP38
sum

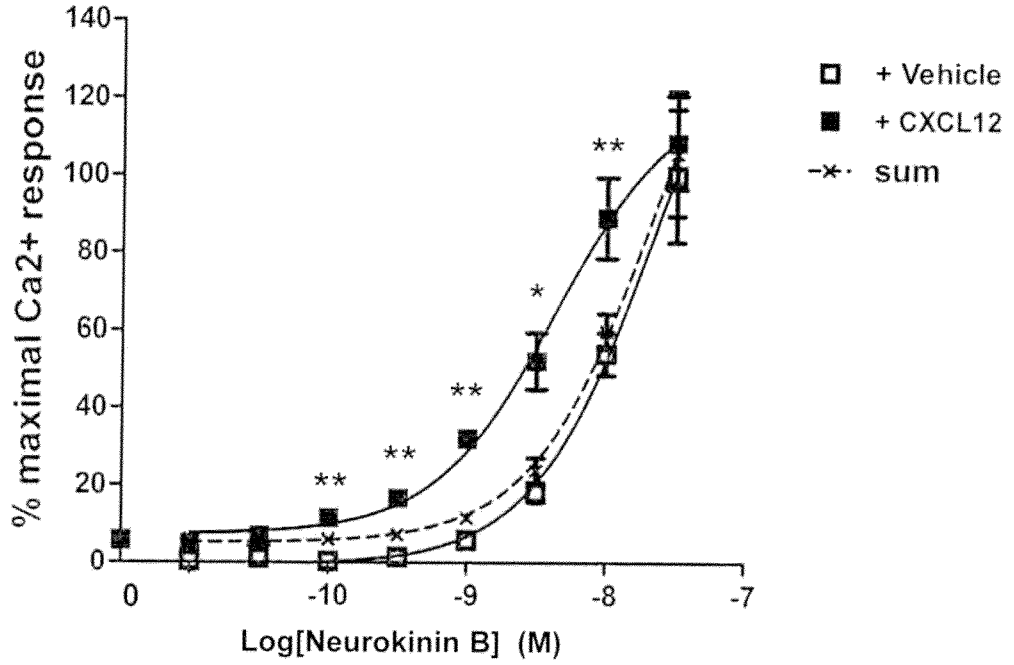
[FIG. 24b]


% maximal Ca2+ response
140
120
100
80
60
40
20
0
-10
-9
-8
-7
-6
Log[PACAP38] (M)
+ Vehicle
+ CXCL12
sum
**
**
***
**
*
**
**

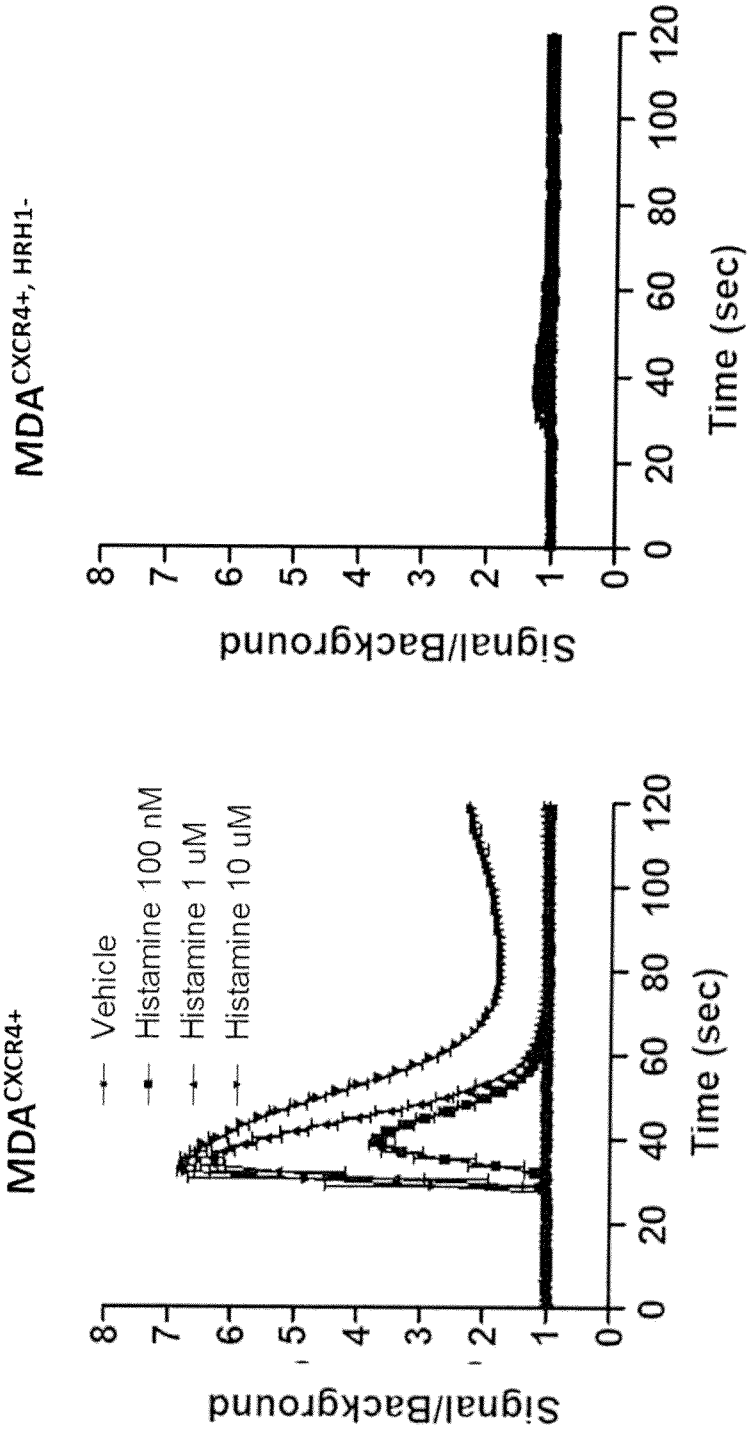
[FIG. 25a]


300
250
200
150
100
50
0
% maximal Ca2+ response
**
**
**
**
**
-10
-9
-8
-7
-6
Log[CXCL12] (M)
+ Vehicle
+ Neurokinin B
sum

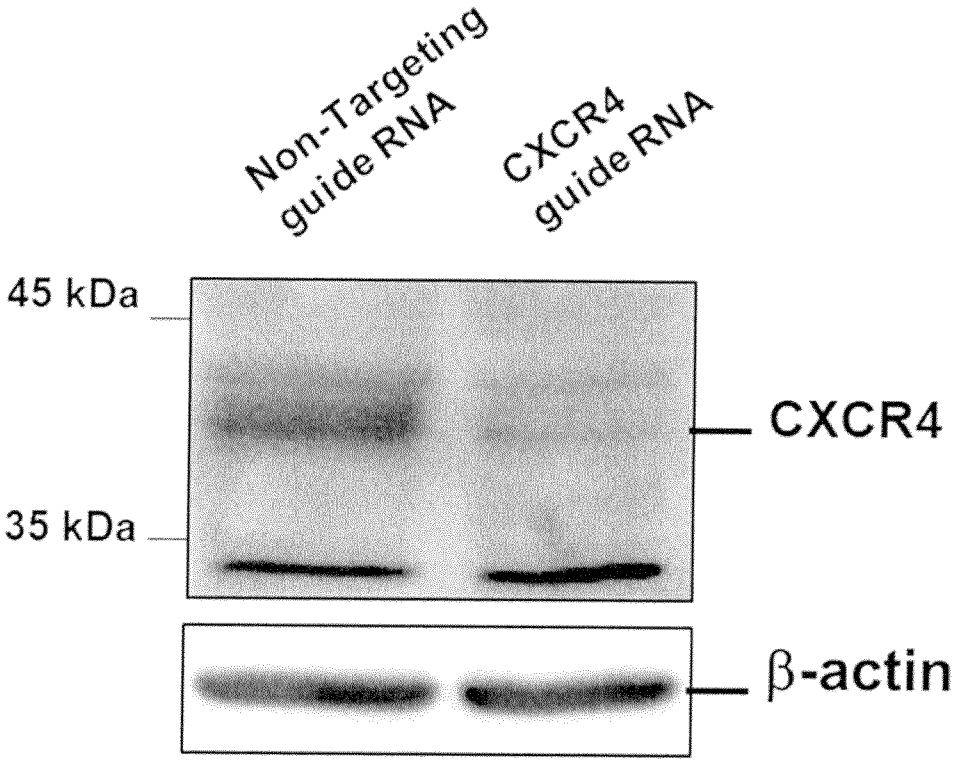
[FIG. 25b]


% maximal Ca2+ response
140
120
100
80
60
40
20
0
0
-10
-9
-8
-7
Log[Neurokinin B] (M)
**
**
**
*
**
+ Vehicle
+ CXCL12
sum

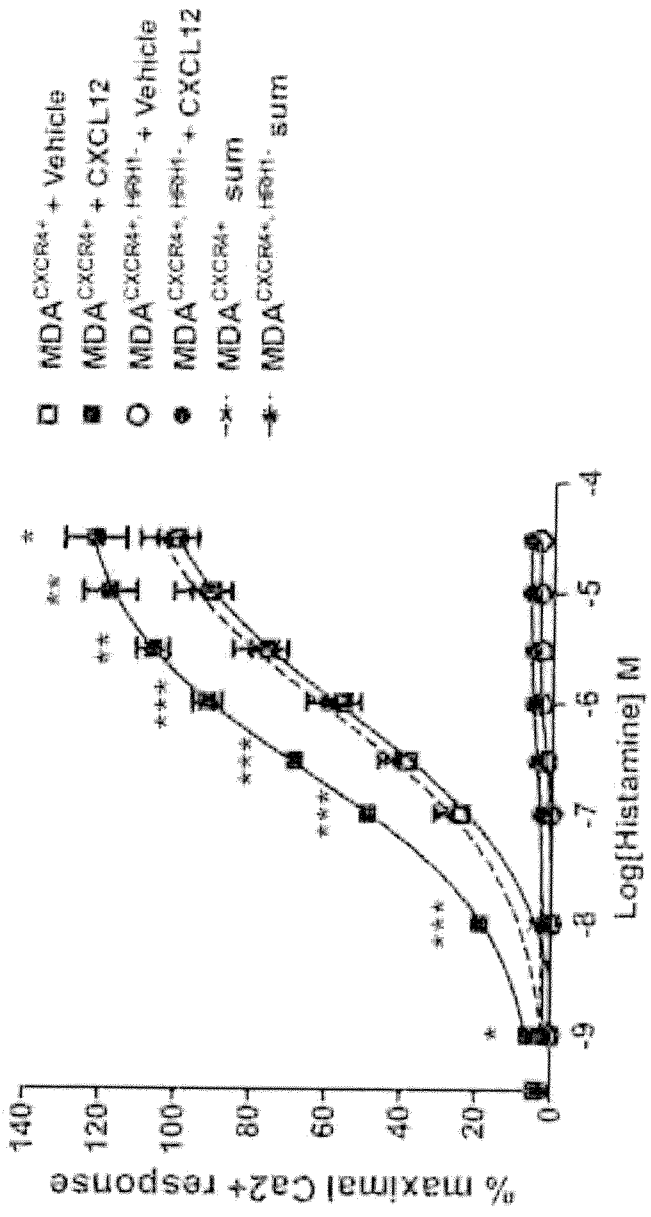
[FIG. 26a]


MDA CXCR4+
Vehicle
Histamine 100 nM
Histamine 1 uM
Histamine 10 uM
Signal/Background
Time (sec)
MDA CXCR4+, HRH1-
Signal/Background
Time (sec)

[FIG. 26b]

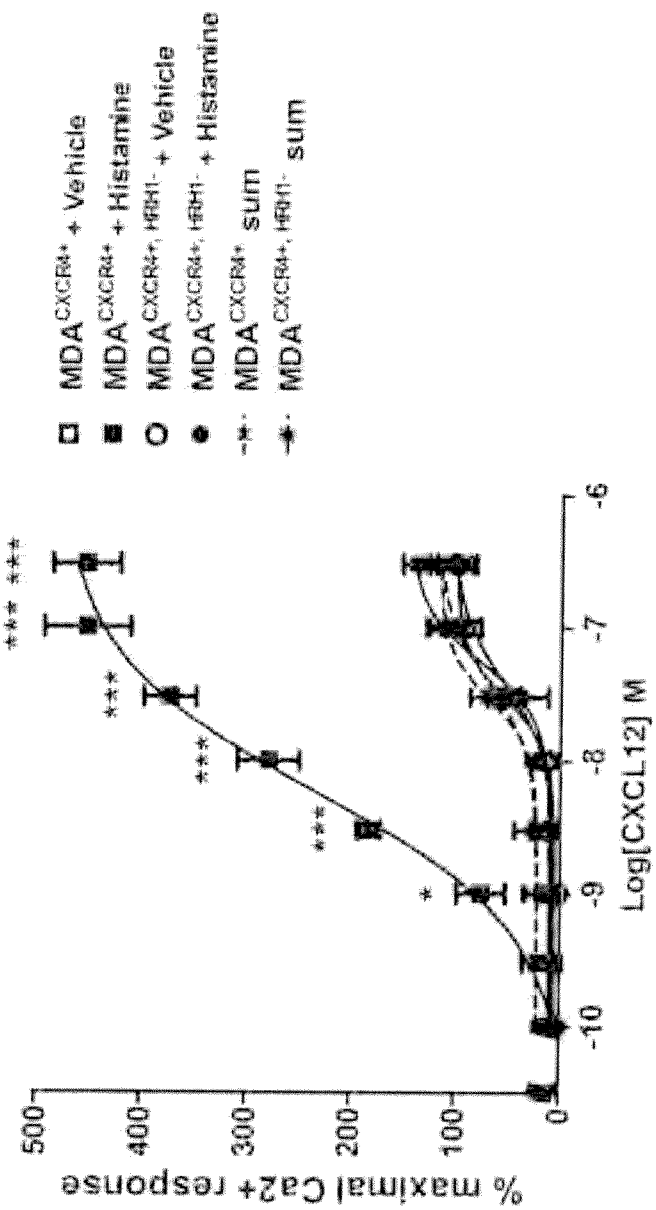

Non-Targeting guide RNA
CXCR4 guide RNA
45 kDa
35 kDa
CXCR4
β-actin

[FIG. 27a]



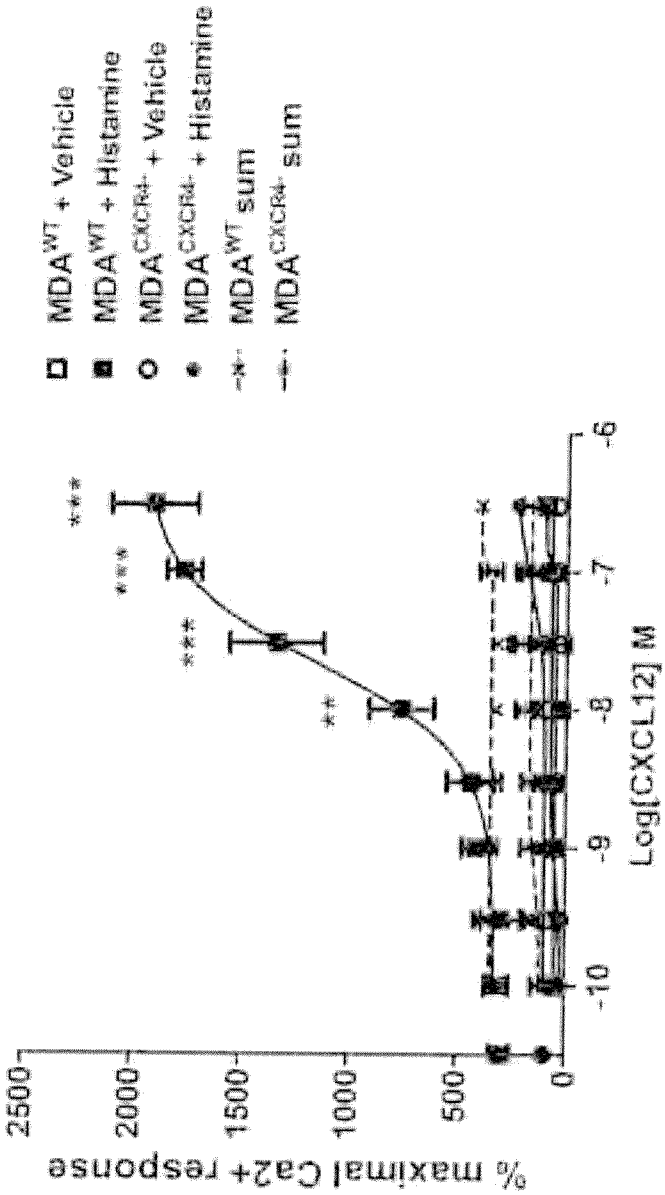
	EC_{50} (nM)		E_{max} (% maximal)	
MDA^{CXCR4-} sum	843.6 ± 164.2	**	104.4 ± 5.2	*
MDA^{CXCR4+} + CXCL12	246.8 ± 43.6		121.7 ± 8.1	
$MDA^{CXCR4-, HRH1-}$ sum	420.9 ± 171	ns	6.2 ± 0.4	ns
$MDA^{CXCR4-, HRH1-}$ + CXCL12	398.4 ± 346.9		6.4 ± 0.7	

[FIG. 27b]



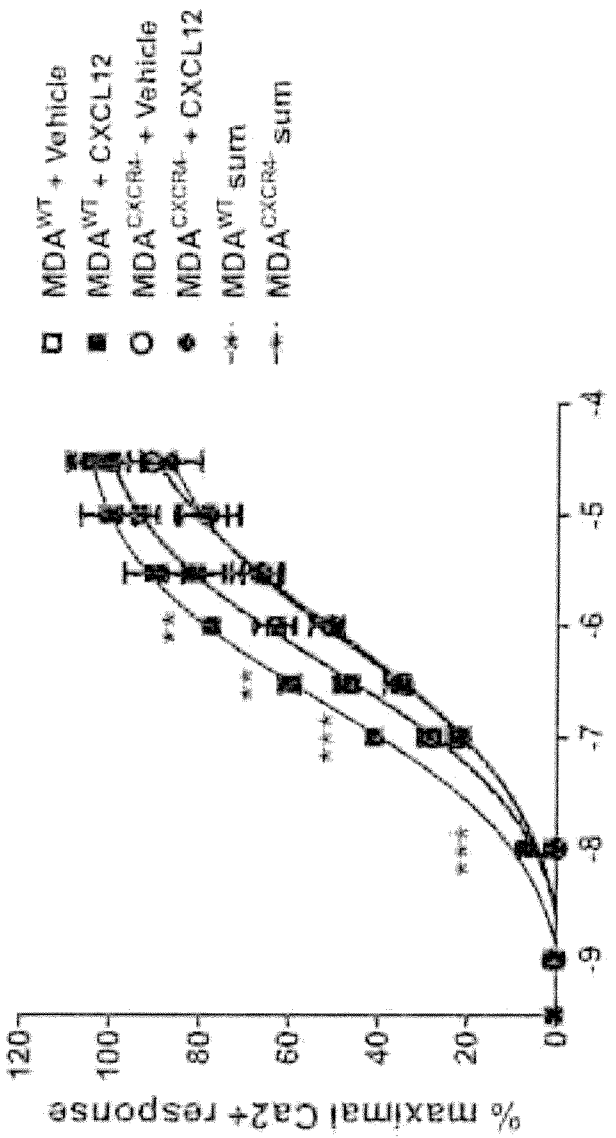
	EC50 (nM)		Emax (% maximal)	
MDA^CXCR4+ sum	40.7 ± 14.9	**	114.9 ± 13.7	***
MDA^CXCR4+ + Histamine	5.86 ± 0.1		456.6 ± 32.1	
MDA^CXCR4+, HRH1- sum	41.2 ± 25.7	ns	116.1 ± 18.9	ns
MDA^CXCR4+, HRH1- + Histamine	60.9 ± 29.8		138.7 ± 12.9	

[FIG. 28a]



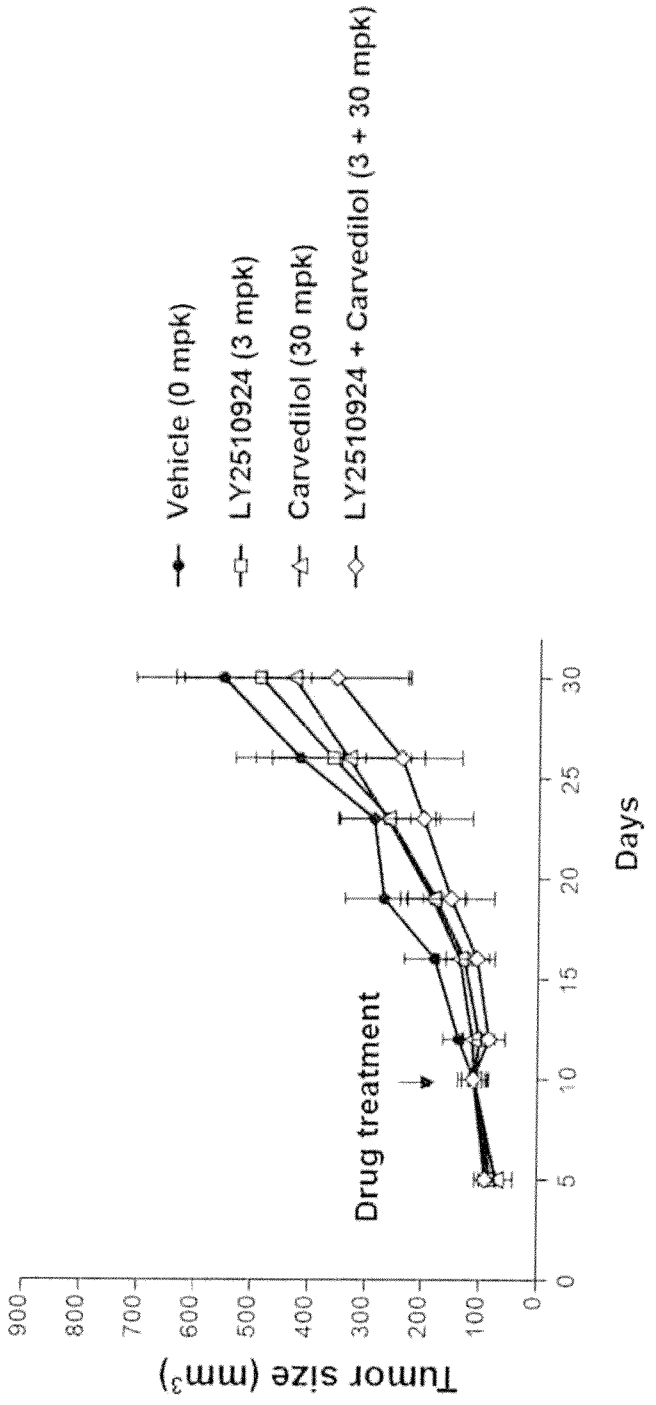
	EC_{50} (nM)	E_{max} (% maximal)	
MDA^{WT} sum	-	396.9 ± 10.8	***
MDA^{WT} + Histamine	21.4 ± 6.2	1902 ± 199.6	
MDA^{CXCR4-} sum	-	142.1 ± 66.6	ns
MDA^{CXCR4-} + Histamine	-	230.6 ± 30.5	

[FIG. 28b]

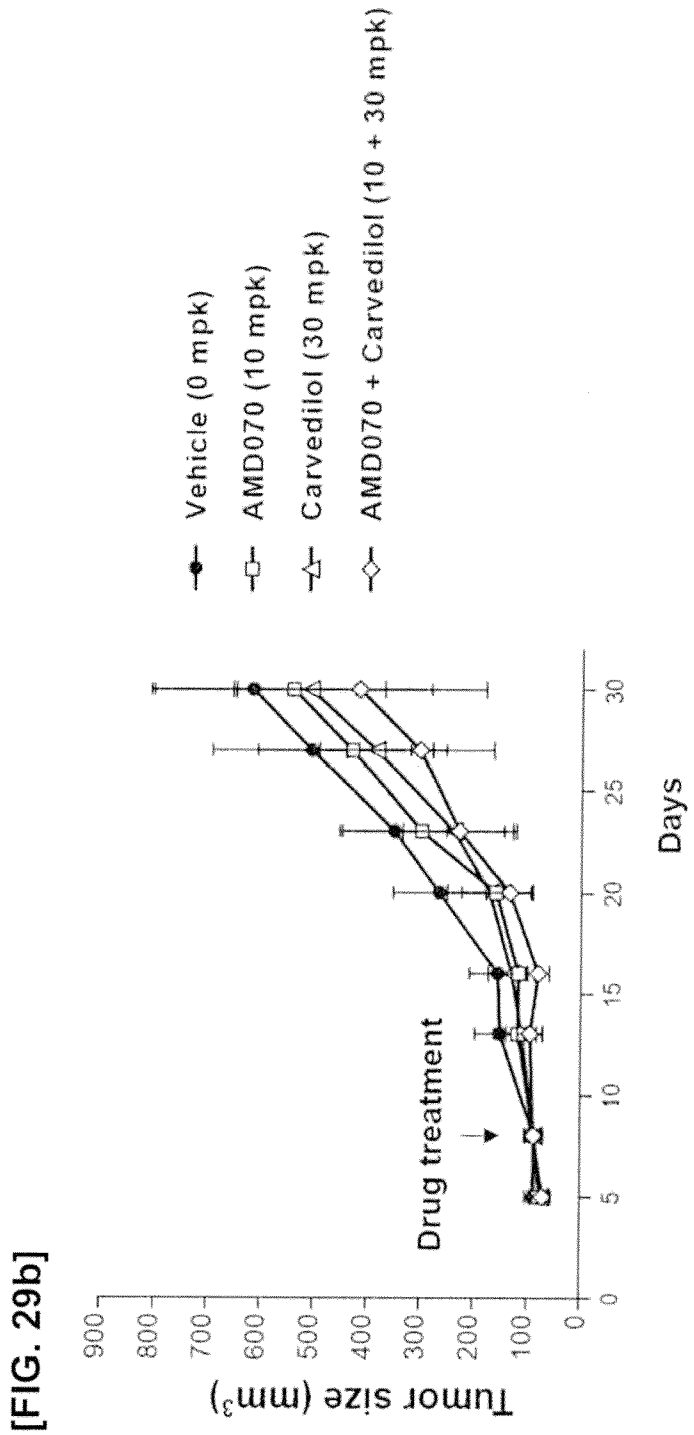


	EC_{50} (nM)		E_{max} (% maximal)	
MDA^{WT} sum	433.4 ± 27.1	***	100.4 ± 8.1	ns
MDA^{WT} + CXCL12	205.3 ± 34.8		104.8 ± 5.0	
MDA^{CXCR4-} sum	992.7 ± 341.2	ns	91.29 ± 5.1	ns
MDA^{CXCR4-} + CXCL12	630.8 ± 109.8		86.63 ± 6.7	

[FIG. 29a]


Vehicle (0 mpk)
LY2510924 (3 mpk)
Carvedilol (30 mpk)
LY2510924 + Carvedilol (3 + 30 mpk)
Tumor size (mm³)
900
800
700
600
500
400
300
200
100
0
Drug treatment
0
5
10
15
20
25
30
Days

[FIG. 29b]


Vehicle (0 mpk)
AMD070 (10 mpk)
Carvedilol (30 mpk)
AMD070 + Carvedilol (10 + 30 mpk)
Tumor size (mm³)
900
800
700
600
500
400
300
200
100
0
Drug treatment
0
5
10
15
20
25
30
Days

SEQUENCE LISTING

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