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GPR35/CXCR8 IS THE RECEPTOR OF THE MUCOSAL CHEMOKINE CXCL17

José L. Maravillas-Montero[*,1], **Amanda M. Burkhardt**[*,1], **Peter A. Hevezi**[*], **Christina D. Carnevale**[*], **Martine J. Smit**[†], and **Albert Zlotnik**[*]

[*]Department of Physiology and Biophysics and Institute for Immunology, University of California, Irvine, Irvine, CA 92697 [†]Division of Medicinal Chemistry, VU University Amsterdam, De Boelelaan 1083, 1081 HV Amsterdam, The Netherlands

Abstract

Chemokines are chemotactic cytokines that direct the traffic of leukocytes and other cells in the body. Chemokines bind to G protein-coupled receptors (GPCRs) expressed on target cells to initiate signaling cascades and induce chemotaxis. Although the cognate receptors of most chemokines have been identified, the receptor for the mucosal chemokine CXCL17 is still undefined. Here we show that GPR35 is the receptor of CXCL17. GPR35 is expressed in mucosal tissues, in CXCL17-responsive monocytes, and in the THP-1 monocytoid cell line. Transfection of GPR35 into Ba/F3 cells rendered them responsive to CXCL17 as measured by calcium mobilization assays. Furthermore, GPR35 expression is downregulated in the lungs of *Cxcl17*$^{-/-}$ mice, which exhibit defects in macrophage recruitment to the lungs. We conclude that GPR35 is a novel chemokine receptor, and suggest that it should be named chemokine (C-X-C motif) receptor 8 (CXCR8).

INTRODUCTION

Chemokines (chemotactic cytokines) are small-secreted proteins that direct the migration of various cell types (1). They have been classified as homeostatic or inflammatory depending on the stimuli that regulate their production (2). Chemokines bind receptors that belong to the Class A G-protein–coupled receptor (GPCR) superfamily. Binding these receptors trigger signaling cascades that promote multiple cellular functions (3) through the coupling of G proteins. The human chemokine superfamily currently includes 48 ligands and 19 receptors. The receptors for most of the ligands have been identified (2), and only two chemokine ligands remain orphan, that is, their receptors have not been identified (CXCL14 and CXCL17). CXCL17 was the last chemokine described (4), and its expression pattern is closely associated with mucosal tissues (5-6). Few reports exist on CXCL17, but it is known to chemoattract macrophages both *in vitro* (4, 6), and *in vivo* (7). CXCL17 is also known to promote angiogenesis (6).

Correspondence to: Albert Zlotnik. Department of Physiology and Biophysics and Institute for Immunology, University of California, Irvine, Irvine, CA 92697. Phone: (949) 824-0876; Fax: (949) 824-8540; azlotnik@uci.edu.

[1]These authors contributed equally to this work.

Here, we show that CXCL17 signals through the orphan G-protein coupled receptor GPR35. This receptor is not currently known to bind chemokines (8), however, like CXCL17, it also exhibits a mucosal expression pattern (9). Partly because of this, it has attracted attention as a potential therapeutic target (9). Since our findings indicate that it represents a novel chemokine receptor, we suggest it should be named chemokine (C-X-C motif) receptor 8 (CXCR8).

MATERIALS AND METHODS

Cells and reagents

THP-1 leukemia cells and the pro-B-cell line Ba/F3 were maintained in RPMI. Antibodies used include rabbit IgG (Jackson ImmunoResearch, West Grove, PA) and polyclonal rabbit anti-human GPR35 (Cayman Chemicals, Ann Arbor, MI). A clone encoding human GPR35 was obtained from The Missouri S&T cDNA Resource Center, under Gene bank accession number AY275467 (http://www.ncbi.nlm.nih.gov/nuccore/AY275467).

BIGE database

The BIGE (Body Index of Gene Expression) is a comprehensive database of human gene expression (5, 10). Data from a probeset (210264_at) corresponding to GPR35 were used to determine its expression in the database.

Quantitative real-time PCR analysis

Quantitative real-time PCR (qRT-PCR) data were generated with a Lightcycler 480 (Roche). cDNA was obtained from total RNA extracted from THP-1 cells using Qiagen kits. Gene-specific primers and corresponding Universal Probes were used to quantify GPR35 or control gene transcripts.

Chemotaxis assays

Chemotaxis assays were performed for 18-20 h using 5.0 μm-24 transwell migration plates (Corning, NY), using 200 ng/mL chemokine (R&D Systems) in 600 μl of incomplete RPMI added to the bottom chambers; 0.5-1.0 × 10^6 cells per well. Where noted, cells were pre-treated with 200 ng/mL of *Bordetella pertussis* toxin (PTX) (Sigma, St. Louis, MO) or 10 μM prostaglandin E_2 (PGE_2) (Sigma) for 24 hours.

Quantitation of chemotaxis by flow cytometry

This protocol was adapted from Proudfoot et.al (11). Briefly, the chemotaxed cells were resuspended in 200 μL of 1X PBS. Standards were generated through 10-fold dilutions ranging from 10^6 to 10^2 cells/200 μL. The cell counts were number of events in 30 seconds recorded in a FACSCalibur (Becton Dickinson).

GPR35 transfection asssays

2 × 10^7 cells/ml Ba/F3 cells were resuspended in 500 μL of cytomix (12) and transferred to a 0.4 cm electroporation cuvette (USA Scientific). Then, 20 μg of pcDNA3.1+/GPR35 DNA

were added prior to electroporation using a Bio-Rad system (300 V, 960 μF). Cells were cultured in RPMI at 37°C for 48 h before performing assays.

Calcium mobilization assays

5×10^7 THP-1 or Ba/F3 cells/mL, were loaded with Calcium green-1-AM and Fura-red-AM (Life Technologies, Carlsbad, CA) at 10 μmol/L for 30 minutes at 37°C. After 30 seconds of onset of data acquisition, cells were stimulated by human CXCL17 (R&D Systems), or 100 μM Ionomycin (Sigma) (as positive control). The Calcium-green versus Fura-red fluorescence ratio was measured in a FACSCalibur before and after the addition of activators and analyzed with FlowJo software.

Wild Type and *Cxcl17*$^{-/-}$ Mice

Lung tissue was collected from wild type (WT) C57Bl/6 or *Cxcl17*$^{-/-}$ mice which were obtained as described (13). All mouse studies were performed under an approved IACUC protocol.

RESULTS AND DISCUSSION

We report a novel chemokine receptor, GPR35, which we have identified as the receptor of CXCL17, a chemokine first reported in 2006 (4). CXCL17 is a mucosal chemokine that has been associated with human diseases including IPF (5) and cancer (14-15). Recently, we reported that a *Cxcl17*$^{-/-}$ mouse exhibits a paucity of macrophages in the lungs (7), and conversely, injection of Cxcl17 i.p. induces strong macrophage recruitment *in vivo* (7). In vitro, macrophages (including THP-1 cells) chemotax to CXCL17 as well as to CCL2 (data not shown), a well-known macrophage chemoattractant (16). These data indicate that CXCL17 is a major macrophage chemoattractant.

CXCL17 induces chemotactic responses in the THP-1 human monocyte cell line (Fig. 1A), indicating that THP-1 expresses the CXCL17 receptor. The chemotactic response of THP-1 to CXCL17 was amplified by pre-treatment with PGE_2 (Fig. 1A), as has been shown for other chemokines (17). These responses are also sensitive to pertussis toxin (PTX) (Fig 1A), which inhibits $G_{\alpha i/o}$ protein signaling pathways, as observed in other chemokine receptors (18).

The binding of chemokines to their receptors induces an increase in cytosolic calcium (18). As shown in Figure 1B, CXCL17 induces a Ca^{+2} flux in both resting and PGE_2-treated cells, however, as observed with chemotactic responses (Fig. 1A), the Ca^{+2} fluxes observed in PGE_2-treated THP-1 cells in response to CXCL17 were stronger (Fig. 1B). Both CCL2 and CXCL17 induced calcium fluxes in THP-1 cells, while CXCL14 did not (data not Shown).

Chemokine receptors can be desensitized for a certain period of time following activation (19). As shown in Figure 1D, CXCL17 desensitizes itself in THP-1 cells, but it does not desensitize the Ca^{+2} flux induced by CCL2 (a chemokine that signals through CCR2) (Fig 1C)(20). Conversely, CCL2 did not desensitize CXCL17-mediated responses, indicating that these two chemokines signal through different receptors (19).

Before searching for the CXCL17 receptor, we tested whether CXCL17 bound or activated other known chemokine receptors. We confirmed that CXCL17 does not bind or signal to CCR2, CCR5, CXCR2, CXCR3, CXCR4 and CXCR7 (supplementary tables). Therefore, we decided to search for orphan GPCRs expressed in macrophages as potential candidates to be the CXCL17 receptor.

We screened the BIGE database of human gene expression (10) to identify GPCRs expressed by monocytes. We narrowed the list of possible candidates by selecting orphan GPCRs that exhibit structural characteristics of chemokine receptors and a tissue expression pattern similar to CXCL17. The orphan receptor GPR35 is known to be expressed in mucosal tissues including the gastrointestinal tract (21), and adult lung (22), an expression profile that strongly correlates with the expression of CXCL17. Importantly, it is also expressed by monocytes (23). The ImmGen database (www.immgen.org) indicates that GPR35 is expressed by dendritic cells, macrophages and granulocytes, all of which chemotax to CXCL17 (4, 24).

To explore the possible ability of CXCL17 to signal through GPR35, we sought to induce a CXCL17-mediated calcium flux in previously non-responsive cells by transfecting this receptor into a GPR35 null cell line. We transfected the mouse pro-B cell line Ba/F3 (which does not express GPR35) (data not shown and (25)). CXCL17 induced a robust calcium flux in GPR35-transfected BA/F3 cells (Fig. 2A), and exhibited a dose-response pattern with increasing CXCL17 concentrations (Fig. 2B). Furthermore, transfection of HEK293 cells also induced a CXCL17-mediated calcium flux (Fig 2C). GPR35 is located in human chromosome 2 near CXCR7, and a phylogenetic analysis of chemokine receptors indicates that it is closest to CXCR7, suggesting that these genes may share common ancestor (Supplementary Figure 1). GPR35 exhibits a DRY box (Fig. 2D), a critical motif shared by all signaling chemokine receptors that is required for G protein coupling (26). GPR35 also exhibits a conserved Asp residue, a TxP (Thr-Xaa-Pro) and CWxP (Cys-Trp-Xaa-Pro) motifs (27). These regions are highly conserved in chemokine receptors (28-29). Additionally, some specific residues known as “micro-switches”, which are also highly conserved in chemokine receptors, are also present in GPR35 (27). Taken together, these structural characteristics are consistent with the conclusion that GPCR35 is a signaling receptor for CXCL17.

Kynurenic acid, 2-Acyl lysophosphatidic acid (2-acyl-LPA), and Zaprinast have been reported to be GPR35 agonists (30). However, the responses observed with these ligands are in the micromolar range. In contrast, CXCL17 induces calcium fluxes in the nanomolar range (Fig. 2B), within physiologic concentrations of this chemokine *in vivo* (31).

We were able to increase the responsiveness of THP-1 cells to CXCL17 through pretreatment with PGE_2 (Figures 1A and 1B) suggesting that PGE_2 induces the expression of the CXCL17 receptor in THP-1 cells. Using qRT-PCR, we confirmed that GPR35 expression is significantly elevated in PGE_2 treated THP-1 cells (Figure 3A). We recently reported that a *Cxcl17*$^{-/-}$ mouse exhibits a paucity of macrophages in the lungs (7), and Cxcl17 chemoattracts macrophages from lungs of WT, but not from *Cxcl17*$^{-/-}$ mice (7). These observations indicate that CXCL17 is a physiologic signal that recruits certain

macrophage subsets to the lungs, which should express the CXCL17 receptor. This model predicts that cells expressing the CXCL17 receptor would not be recruited into the lungs of *Cxcl17*$^{-/-}$ mice. To test this prediction, we measured the expression of GPR35 in the lungs of wild type or *Cxcl17*$^{-/-}$ mice. As shown in Figure 3B, GPR35 is robustly expressed in WT mouse lungs but strongly down-regulated in *Cxcl17*$^{-/-}$ mouse lungs. This effect is macrophage specific, because there is a concurrent decrease in the expression of two lung macrophage specific markers in the lungs of *Cxcl17*$^{-/-}$ mice (7). Taken together, these observations strongly support the conclusion that GPR35 is the receptor of CXCL17. We therefore propose renaming GPR35 as chemokine (C-X-C motif) receptor 8 (CXCR8) in accordance with the chemokine receptor nomenclature (32).

The identification of CXCR8 is an important addition to the chemokine field (33). Importantly, GPR35 has already been identified as a target for gastrointestinal diseases (21). Genome wide association studies (GWAS) have linked *GPR35* to primary sclerosing cholangitis and ulcerative colitis (34). In the respiratory system, CXCL17 is upregulated in IPF (5). Given the importance of macrophages in inflammation, and the strong expression of both CXCL17 (5) and GPR35/CXCR8 in mucosal tissues (21-22), we hypothesize that the CXCL17/CXCR8 axis is an important player in mucosal inflammation and in the pathology of various human diseases.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Campbell JJ, Butcher EC. Chemokines in tissue-specific and microenvironment-specific lymphocyte homing. Curr Opin Immunol. 2000; 12:336–341. [PubMed: 10781407]
2. Zlotnik A, Yoshie O. The chemokine superfamily revisited. Immunity. 2012; 36:705–716. [PubMed: 22633458]
3. Vassilatis DK, Hohmann JG, Zeng H, Li F, Ranchalis JE, Mortrud MT, Brown A, Rodriguez SS, Weller JR, Wright AC, Bergmann JE, Gaitanaris GA. The G protein-coupled receptor repertoires of human and mouse. Proc Natl Acad Sci U S A. 2003; 100:4903–4908. [PubMed: 12679517]
4. Pisabarro MT, Leung B, Kwong M, Corpuz R, Frantz GD, Chiang N, Vandlen R, Diehl LJ, Skelton N, Kim HS, Eaton D, Schmidt KN. Cutting edge: novel human dendritic cell- and monocyte-attracting chemokine-like protein identified by fold recognition methods. J Immunol. 2006; 176:2069–2073. [PubMed: 16455961]
5. Burkhardt AM, Tai KP, Flores-Guiterrez JP, Vilches-Cisneros N, Kamdar K, Barbosa-Quintana O, Valle-Rios R, Hevezi PA, Zuniga J, Selman M, Ouellette AJ, Zlotnik A. CXCL17 is a mucosal chemokine elevated in idiopathic pulmonary fibrosis that exhibits broad antimicrobial activity. J Immunol. 2012; 188:6399–6406. [PubMed: 22611239]

6. Lee WY, Wang CJ, Lin TY, Hsiao CL, Luo CW. CXCL17, an orphan chemokine, acts as a novel angiogenic and anti-inflammatory factor. Am J Physiol Endocrinol Metab. 2013; 304:E32–40. [PubMed: 23115081]
7. Burkhardt AM, Maravillas-Montero JL, Carnevale CD, Vilches-Cisneros N, Flores JP, Hevezi PA, Zlotnik A. CXCL17 Is a Major Chemotactic Factor for Lung Macrophages. J Immunol. 2014; 193:1468–1474. [PubMed: 24973458]
8. Milligan G. Orthologue selectivity and ligand bias: translating the pharmacology of GPR35. Trends Pharmacol Sci. 2011; 32:317–325. [PubMed: 21392828]
9. Mackenzie AE, Lappin JE, Taylor DL, Nicklin SA, Milligan G. GPR35 as a Novel Therapeutic Target. Front Endocrinol (Lausanne). 2011; 2:68. [PubMed: 22654822]
10. Roth RB, Hevezi P, Lee J, Willhite D, Lechner SM, Foster AC, Zlotnik A. Gene expression analyses reveal molecular relationships among 20 regions of the human CNS. Neurogenetics. 2006; 7:67–80. [PubMed: 16572319]
11. Proudfoot AE, Power CA, Church DJ, Soler D, Mack M. Cellular assays of chemokine receptor activation. Curr Protoc Pharmacol. 2001; Chapter 12(Unit12):14.
12. Maravillas-Montero JL, Gillespie PG, Patino-Lopez G, Shaw S, Santos-Argumedo L. Myosin 1c participates in B cell cytoskeleton rearrangements, is recruited to the immunologic synapse, and contributes to antigen presentation. J Immunol. 2011; 187:3053–3063. [PubMed: 21841128]
13. Tang T, Li L, Tang J, Li Y, Lin WY, Martin F, Grant D, Solloway M, Parker L, Ye W, Forrest W, Ghilardi N, Oravecz T, Platt KA, Rice DS, Hansen GM, Abuin A, Eberhart DE, Godowski P, Holt KH, Peterson A, Zambrowicz BP, de Sauvage FJ. A mouse knockout library for secreted and transmembrane proteins. Nat Biotechnol. 2010; 28:749–755. [PubMed: 20562862]
14. Weinstein EJ, Head R, Griggs DW, Sun D, Evans RJ, Swearingen ML, Westlin MM, Mazzarella R. VCC-1, a novel chemokine, promotes tumor growth. Biochem Biophys Res Commun. 2006; 350:74–81. [PubMed: 16989774]
15. Mu X, Chen Y, Wang S, Huang X, Pan H, Li M. Overexpression of VCC-1 gene in human hepatocellular carcinoma cells promotes cell proliferation and invasion. Acta Biochim Biophys Sin (Shanghai). 2009; 41:631–637. [PubMed: 19657564]
16. Conti I, Rollins BJ. CCL2 (monocyte chemoattractant protein-1) and cancer. Semin Cancer Biol. 2004; 14:149–154. [PubMed: 15246049]
17. Kurth I, Willimann K, Schaerli P, Hunziker T, Clark-Lewis I, Moser B. Monocyte selectivity and tissue localization suggests a role for breast and kidney-expressed chemokine (BRAK) in macrophage development. J Exp Med. 2001; 194:855–861. [PubMed: 11561000]
18. Murphy PM. Chemokine receptors: structure, function and role in microbial pathogenesis. Cytokine Growth Factor Rev. 1996; 7:47–64. [PubMed: 8864354]
19. Wang W, Soto H, Oldham ER, Buchanan ME, Homey B, Catron D, Jenkins N, Copeland NG, Gilbert DJ, Nguyen N, Abrams J, Kershenovich D, Smith K, McClanahan T, Vicari AP, Zlotnik A. Identification of a novel chemokine (CCL28), which binds CCR10 (GPR2). J Biol Chem. 2000; 275:22313–22323. [PubMed: 10781587]
20. Gouwy M, Struyf S, Noppen S, Schutyser E, Springael JY, Parmentier M, Proost P, Van Damme J. Synergy between coproduced CC and CXC chemokines in monocyte chemotaxis through receptor-mediated events. Mol Pharmacol. 2008; 74:485–495. [PubMed: 18469140]
21. Okumura S, Baba H, Kumada T, Nanmoku K, Nakajima H, Nakane Y, Hioki K, Ikenaka K. Cloning of a G-protein-coupled receptor that shows an activity to transform NIH3T3 cells and is expressed in gastric cancer cells. Cancer Sci. 2004; 95:131–135. [PubMed: 14965362]
22. Horikawa Y, Oda N, Cox NJ, Li X, Orho-Melander M, Hara M, Hinokio Y, Lindner TH, Mashima H, Schwarz PE, del Bosque-Plata L, Oda Y, Yoshiuchi I, Colilla S, Polonsky KS, Wei S, Concannon P, Iwasaki N, Schulze J, Baier LJ, Bogardus C, Groop L, Boerwinkle E, Hanis CL, Bell GI. Genetic variation in the gene encoding calpain-10 is associated with type 2 diabetes mellitus. Nat Genet. 2000; 26:163–175. [PubMed: 11017071]
23. Barth MC, Ahluwalia N, Anderson TJ, Hardy GJ, Sinha S, Alvarez-Cardona JA, Pruitt IE, Rhee EP, Colvin RA, Gerszten RE. Kynurenic acid triggers firm arrest of leukocytes to vascular endothelium under flow conditions. J Biol Chem. 2009; 284:19189–19195. [PubMed: 19473985]

24. Matsui A, Yokoo H, Negishi Y, Endo-Takahashi Y, Chun NA, Kadouchi I, Suzuki R, Maruyama K, Aramaki Y, Semba K, Kobayashi E, Takahashi M, Murakami T. CXCL17 expression by tumor cells recruits CD11b+Gr1 high F4/80-cells and promotes tumor progression. PLoS One. 2012; 7:e44080. [PubMed: 22952881]
25. Wu C, Orozco C, Boyer J, Leglise M, Goodale J, Batalov S, Hodge CL, Haase J, Janes J, Huss JW 3rd, Su AI. BioGPS: an extensible and customizable portal for querying and organizing gene annotation resources. Genome Biol. 2009; 10:R130. [PubMed: 19919682]
26. Daiyasu H, Nemoto W, Toh H. Evolutionary Analysis of Functional Divergence among Chemokine Receptors, Decoy Receptors, and Viral Receptors. Front Microbiol. 2012; 3:264. [PubMed: 22855685]
27. Nygaard R, Frimurer TM, Holst B, Rosenkilde MM, Schwartz TW. Ligand binding and micro-switches in 7TM receptor structures. Trends Pharmacol Sci. 2009; 30:249–259. [PubMed: 19375807]
28. Govaerts C, Blanpain C, Deupi X, Ballet S, Ballesteros JA, Wodak SJ, Vassart G, Pardo L, Parmentier M. The TXP motif in the second transmembrane helix of CCR5. A structural determinant of chemokine-induced activation. J Biol Chem. 2001; 276:13217–13225. [PubMed: 11278962]
29. Cancellieri C, Caronni N, Vacchini A, Savino B, Borroni EM, Locati M, Bonecchi R. Review: Structure-function and biological properties of the atypical chemokine receptor D6. Mol Immunol. 2013; 55:87–93. [PubMed: 22939232]
30. Taniguchi Y, Tonai-Kachi H, Shinjo K. Zaprinast, a well-known cyclic guanosine monophosphate-specific phosphodiesterase inhibitor, is an agonist for GPR35. FEBS Lett. 2006; 580:5003–5008. [PubMed: 16934253]
31. Yung SC, Murphy PM. Antimicrobial chemokines. Frontiers in immunology. 2012; 3:276. [PubMed: 22973274]
32. Bachelerie F, Ben-Baruch A, Burkhardt AM, Combadiere C, Farber JM, Graham GJ, Horuk R, Sparre-Ulrich AH, Locati M, Luster AD, Mantovani A, Matsushima K, Murphy PM, Nibbs R, Nomiyama H, Power CA, Proudfoot AE, Rosenkilde MM, Rot A, Sozzani S, Thelen M, Yoshie O, Zlotnik A. International Union of Basic and Clinical Pharmacology. [corrected]. LXXXIX. Update on the extended family of chemokine receptors and introducing a new nomenclature for atypical chemokine receptors. Pharmacol Rev. 2014; 66:1–79. [PubMed: 24218476]
33. Han DK, Jeong SY, Kim YH, Min BG. Surface characteristics and blood compatibility of polyurethanes grafted by perfluoroalkyl chains. J Biomater Sci Polym Ed. 1992; 3:229–241. [PubMed: 1610733]
34. Ellinghaus D, Folseraas T, Holm K, Ellinghaus E, Melum E, Balschun T, Laerdahl JK, Shiryaev A, Gotthardt DN, Weismuller TJ, Schramm C, Wittig M, Bergquist A, Bjornsson E, Marschall HU, Vatn M, Teufel A, Rust C, Gieger C, Wichmann HE, Runz H, Sterneck M, Rupp C, Braun F, Weersma RK, Wijmenga C, Ponsioen CY, Mathew CG, Rutgeerts P, Vermeire S, Schrumpf E, Hov JR, Manns MP, Boberg KM, Schreiber S, Franke A, Karlsen TH. Genome-wide association analysis in primary sclerosing cholangitis and ulcerative colitis identifies risk loci at GPR35 and TCF4. Hepatology. 2013; 58:1074–1083. [PubMed: 22821403]

Abbreviations

GPCR	G-coupled protein receptor
7TM	7 transmembrane
IPF	idiopathic pulmonary fibrosis
PTX	pertussis toxin
SPF	specific pathogen free
PGE_2	prostaglandin E2

TxP	Thr-Xaa-Pro
qRT-PCR	quantitative real-time PCR

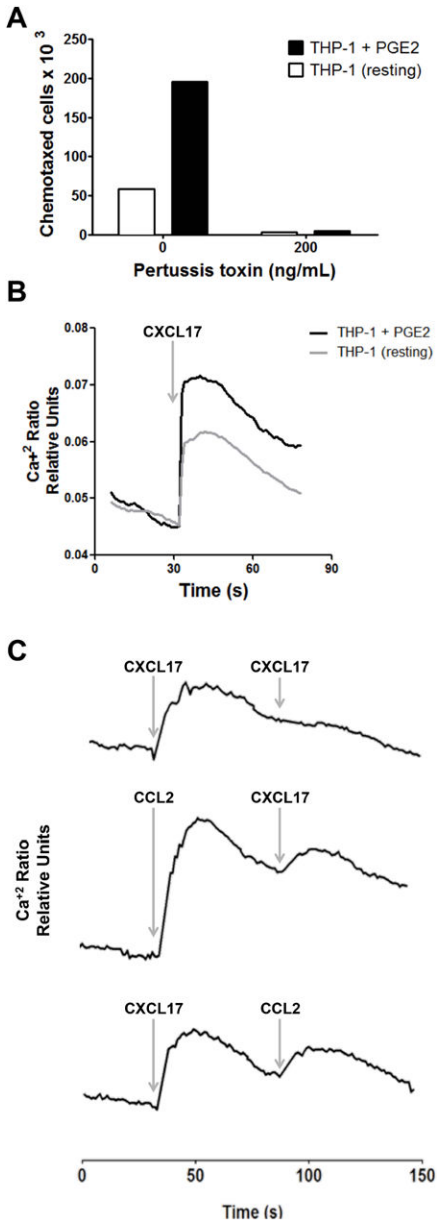

Figure 1. THP-1 cells are responsive to CXCL17

A, THP-1 cells were tested in CXCL17-directed chemotaxis transwell assays, both under control or PGE_2 pre-treated conditions; additionally, these cells were also tested after pre-treatment with PTX (200ng/ml). The bars show the total number of recovered cells (chemotaxed) in the lower chamber. B, representative calcium flux response of THP-1 cells (loaded with Ca^{+2} sensitive dyes), either resting or PGE_2 pre-treated, when stimulated with 100 nM of CXCL17. n=2. C, Desensitization of the CXCL17 receptor expressed by the THP-1 cells, 100 nM of CXCL17 or CCL2 were alternatively added at the indicated time points to induce THP-1 calcium flux responses. Representative graphs shown, n=3.

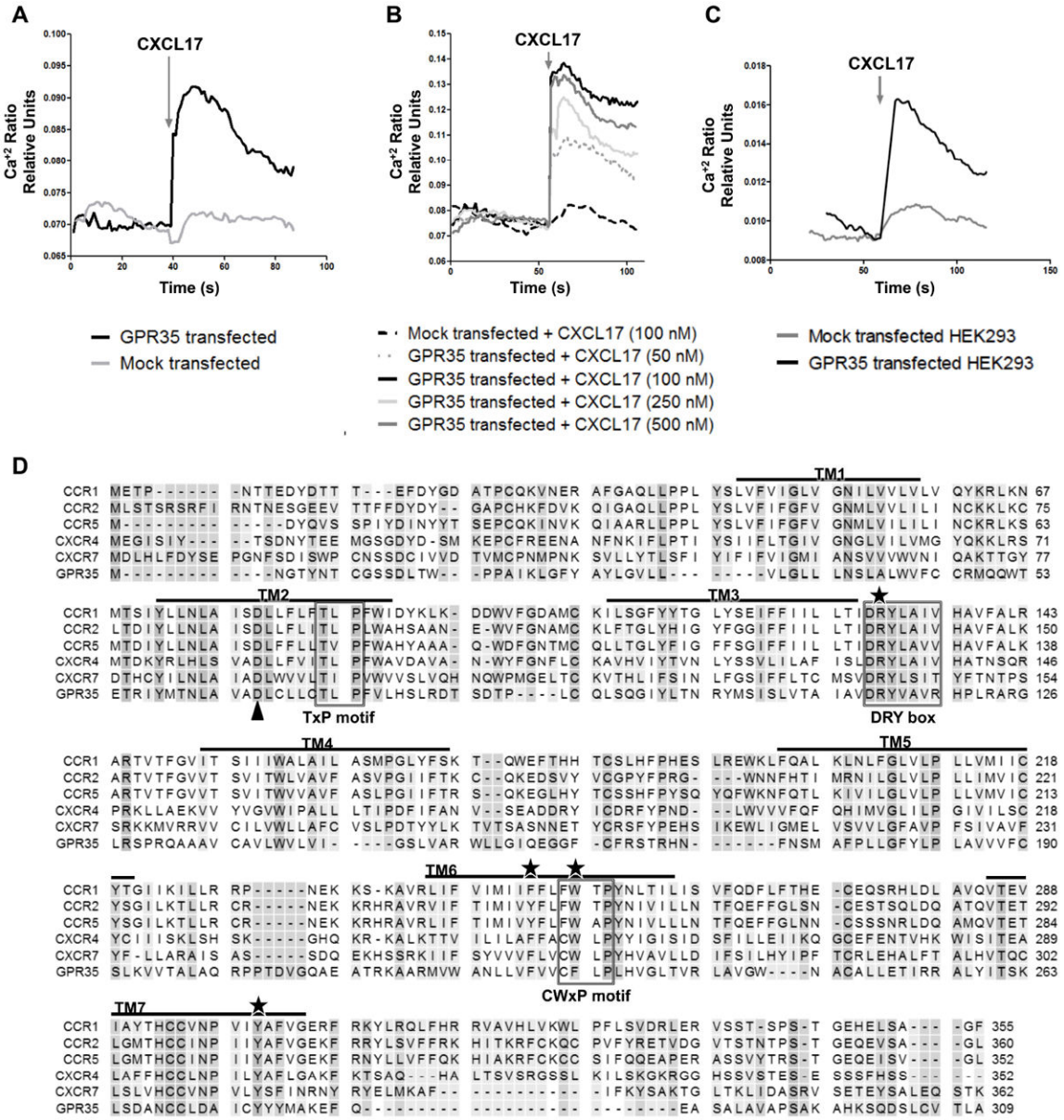

Figure 2. GPR35 is the CXCL17 chemokine receptor

A. CXCL17 (100 nM) induces a calcium flux in GPR35-transfected BA/F3 cells loaded with Ca2+ sensitive dyes. Representative experiment (N=6). B. Dose response of CXCL17-induced calcium flux in GPR35-transfected cells. Representative Experiment N=2. C. CXCL17 (100nM) induces a calcium flux in GPR35-transfected HEK293 cells. (Representative experiment n=2). D. GPR35 exhibits structural characteristics typical of chemokine receptors. Alignment of protein sequences of CCR1, CCR2, CCR5, CXCR4, CXCR7 and GPR35. Conserved residues are shown as darker gray shades in the background of each amino acid. The seven transmembrane (TM) domains are shown. The boxes highlight the DRY box, the TxP and CWxP motifs; the arrow heads indicate the conserved aspartic acid at the second TM region and the conserved leucine/phenylalanine at the fifth TM region; the stars indicate the position of the conserved micro-switches.

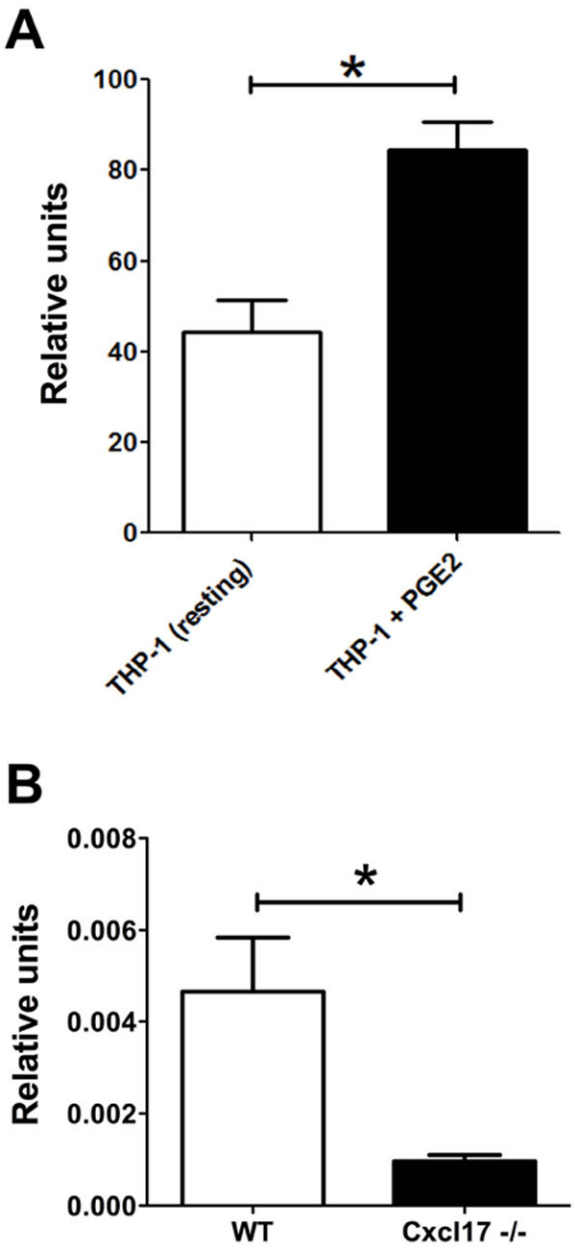

Figure 3. GPR35 expression in macrophages

A. GPR35 expression is induced by PGE_2 treatment in THP-1 cells. THP-1 cells were treated with PGE_2 for 24 h and then tested for GPR35 expression by qRT-PCR. The data were normalized with the relative expression of GAPDH in the samples. Representative experiment (n=3). B. GPR35 expression is reduced in lungs of *CXCL17*$^{-/-}$ mice. Lungs were obtained from either WT or *CXCL17*$^{-/-}$ mice and GPR35 expression was measured by qRT-PCR and normalized to GAPDH. (Mean+/-SD of lungs from 4 mice per group)($p<0.04$).