Supporting Information

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SI Materials and Methods

Gene Constructs. The DREADD (designer receptor exclusively activated by a designer drug) constructs hM3Dq, hM3(M2i3)Di, and hM4Di were a generous gift from Bryan Roth (University of North Carolina Medical School, Chapel Hill, NC) (1). We refer to these constructs as Dq, Di3, and Di, respectively, in this manuscript. More information about these constructs can be found on Bryan Roth's online DREADD wiki resource at http://pdspit3.mml.unc.edu/projects/dreadd/wiki/WikiStart. The enhanced firefly luciferase gene (effLuc) was a generous gift from Brian Rabinovich (M. D. Anderson Cancer Center, Houston) (2). Prolactin signal sequence–mCherry fusions to the DREADD genes were generated using PCR and a combinatorial cloning strategy based on the type II restriction enzyme AarI developed by Peisajovich et al. and ligated into compatible pcDNA3.1(+), pHR'SIN:CSW lentiviral, and pMSCV retroviral plasmid backbones (3). The aforementioned signal sequence, which is removed by proteolysis during receptor processing, was previously described for improving RASSL (receptor activated solely by a synthetic ligand) surface expression in Coward et al. (4). Similarly, an mCherry–Di fusion, an IRES2 element, and effLuc genes were inserted into the pMSCV retroviral plasmid backbone using the combinatorial cloning strategy.

Cell Culture. HL-60 cells were cultured in suspension in RPMI 1640 plus 10% (vol/vol) FBS at a density of 0.15e6 to 1.5e6 cells/mL. For migration experiments, cells were differentiated into neutrophil-like cells by seeding at 0.15e6 cells/mL and treating with 1.3% (vol/vol) DMSO (Sigma) for 5–6 d.

Human peripheral blood mononuclear cells were collected from normal donors and acquired as cell suspensions from flushed TRIMA leukoreduction chambers (Blood Centers of the Pacific, San Francisco, CA). Primary $CD4^+$ T lymphocytes were purified by negative selection and Ficoll-Paque PLUS density medium separation (RosetteSep; Stem Cell Technologies). Purified cells were cryopreserved and placed in liquid nitrogen storage. Cells were grown in the following growth medium: X-VIVO 15 (Lonza) plus 5% (vol/vol) human AB serum plus 10 mM *N*-acetylcysteine plus 1× beta-mercaptoethanol plus 1× Primocin supplemented every 2 d with 30 U/mL IL-2.

Mouse $CD8^+$ T lymphocytes were isolated using negative magnetic selection beads (Stem Cell Technologies) from spleens harvested from C57BL/6J mice. Cells were grown in the following growth medium: X-VIVO 15 (Lonza) plus 5% (vol/vol) FBS plus 10 mM *N*-acetylcysteine plus 1× beta-mercaptoethanol plus 1× Primocin supplemented every 2 d with 30 U/mL human IL-2.

Neonatal human epidermal keratinocytes (Invitrogen) were cultured in EpiLife medium plus HKGS supplement according to vendor recommendations.

Human umbilical vein endothelial cells (HUVECs) (Lonza) were cultured in EGM-2 media according to vendor recommendations on BD Biocoat Collagen I-coated tissue culture-treated plasticware.

Viral Supernatant Generation. Replication-incompetent retroviral particles were prepared in the Plat-E cell line (a 293T-based ecotropic retrovirus packaging cell line) (Cell Biolabs). Briefly, constructs of interest were cloned into the transfer vector pMSCV using standard molecular biology techniques and then cotransfected into the Plat-E cell line along with the packaging plasmid pCL-Eco using the transfection reagent FuGENE HD (Promega). Ecotropic envelope pseudotyped retroviral particles in the supernatant were collected 48 h later.

Replication-incompetent lentiviral particles were prepared in 293T cells by standard methods. Briefly, constructs of interest were cloned into the transfer vector pHR′SIN:CSW using standard molecular biology techniques and then cotransfected into 293T cells along with the viral packaging plasmids pCMVdR8.91 and pMD2.G using the transfection reagent FuGENE HD (Promega). Amphotropic VSV-G pseudotyped lentiviral particles in the supernatant were collected 48 h later.

Viral supernatants were titered by transducing 3T3 mouse fibroblast cells with serial dilutions of virus and determining the percentage of transduced cells via fluorescence microscopy and flow cytometry.

Transduction and Stable Cell Line Generation. Cells were transduced with viral vectors for stable expression of gene constructs of interest. HL-60 cells were incubated with amphotropic lentiviral supernatant at a multiplicity of infection (MOI) of 1–10 overnight and then washed and resuspended in normal growth medium. Human T lymphocytes were transduced with the same protocol except that the media was supplemented with 100 U/mL hIL-2 and the cells were activated 24 h before transduction using CD3/CD28 Dynabeads (Invitrogen) following manufacturer recommendations. Human epidermal keratinocytes were transduced at an MOI of 10, and HUVECs were transduced at an MOI of 3 overnight by addition of lentiviral supernatants to cultures overnight before washing and resuspending in normal growth medium the next day.

Mouse T lymphocytes were transduced with ecotropic envelope-pseudotyped MSCV retrovirus by Retronectin binding and spinfection. Non–tissue culture-treated plates were coated with Retronectin (Takara/Clontech) at a concentration of 32 μg/mL in PBS for 2 h. Following a 30-min block in PBS plus 2% (wt/vol) BSA and a wash with PBS, retroviral supernatant was added to each well and the plate was spun in a swinging bucket centrifuge for 1.5 h at 1,200 × *g*. Following one additional rinse with PBS, T lymphocytes in growth medium supplemented with 100 U/mL hIL-2 were added to the wells, and the plate was spun for an additional hour at 1,200 × *g* at 32 °C. T lymphocytes transduced in this way were activated 24 h before spinfection using CD3/CD28 Dynabeads (Invitrogen) according to manufacturer recommendations.

Amaxa Transient Electroporation. Differentiated HL-60 cells were electroporated with 2 μg of plasmid DNA using program Y-001 in the Amaxa transfection solution Kit V (Lonza) and allowed to recover for 6 h in IMDM (Gibco) plus 20% (vol/vol) FBS plus 2 mM glutamine at 37 °C before use in experiments.

xCELLigence Impedance Array-Based Cell-Spreading Assay. We used the xCELLigence RTCA MP impedance array assay platform (ACEA Biosciences/Roche) as a screen to monitor cytoskeletal changes (adhesion/spreading) of HL-60 neutrophils in response to agonist. Wells were coated in human fibronectin (Sigma) at 100 μg/mL in calcium/magnesium-free D-PBS for 1 h and then blocked with mGey's buffer plus 1% BSA (low endotoxin; Sigma) for 20 min. After washing the wells twice with mGey's buffer plus 1% BSA, cells were plated and allowed to attach for 3 h before stimulation. Cell-index (impedance) measurements were taken in real-time during and after stimulation with 100 nM clozapine-*N*-oxide (CNO) drug or positive control chemoattractant [100 nM formyl-Met-Leu-Phe (fMLP)]. Measurement values were normalized using RTCA software to a time point just before drug stimulation.

Boyden-Chamber Assay. We used Boyden-chamber assays to test the migration of cells through porous membranes. For HL-60 neutrophils (3e5 cells per insert), 24-well format BD Cell Culture Inserts (3-μm pore size) or equivalent Fluoroblok format inserts were used. For T lymphocytes (1e5 cells per insert), 96-well format polycarbonate Transwells (5-μm pore size; Corning) were used. For keratinocytes (4e4 cells per insert), 24-well format polycarbonate Transwells (8-μm pore size; Corning) were used. For endothelial cells, 24-well HTS format BD Fluoroblok Cell Culture inserts were used. Incubation times were 1 h, 2 h, 16 h, and 16 h, respectively. Migrating cells were quantitated by flow cytometry using Absolute Count standard beads (Bangs Laboratories), luciferase assay (with luciferase-expressing cells), or fluorescence microscopy [imaging mCherry-expressing cells or staining cells postexperiment with Calcein AM (Molecular Probes)]. The migration index was calculated by dividing the amount of cell migration in the sample well by the amount of cell migration in a well with no chemoattractant (basal level of cell migration).

Transendothelial Migration Boyden-Chamber Assay. HUVECs were seeded at 200,000 per well in EGM-2 medium into BD fibronectin-coated 3.0-μm pore size, 24-well HTS format Fluoroblok cell-culture inserts. Endothelial monolayer integrity was measured by two methods: transendothelial electrical resistance (TEER) measurements using an EVOM2 Voltohmmeter and STX100F electrode (World Precision Instruments) and via a standard FITC-dextran permeability assay. TEER measurements were performed daily according to manufacturer instructions. The permeability assay was performed as follows. FITC-dextran (MW 40,000) (Sigma) was added to the top insert of a HUVEC endothelial monolayer at a final concentration of 1 mg/mL. Every 30 min for 2 h, 100-μL samples were taken from the bottom well for measurement and replaced with 100 μL of medium. The FITC content of samples was quantitated using a FlexStation 3 plate reader (Molecular Devices), 492 nm/525 nm excitation/emission wavelength with 515-nm cutoff filter.

Thereafter, the experiment was conducted in the same manner as for standard Boyden-Chamber assays (without endothelial monolayers), as described above (*SI Materials and Methods, Boyden-Chamber Assay*). Migration index was calculated in the same manner.

HL-60 Stimulation, Lysis, and Western Blot. For assaying protein phosphorylation in stimulated cells, we used a modification of the trichloroacetic acid precipitation (TCA) protocol (5). Differentiated HL-60 cells were starved for 1 h in modified Hanks' buffered saline solution (mHBSS) [150 mM sodium chloride, 4 mM potassium chloride, 1 mM magnesium chloride, 10 mM glucose, 20 mM Hepes (pH 7.4)] containing 0.2% HSA, washed into calcium-free Gey's buffer containing 1 mM diisopropylfluorophosphate at 1–2e6 cells per mL, equilibrated for 10 min at 37 °C, and stimulated with 1/10 volume of 10× chemoattractant, and the reaction was stopped and proteins were precipitated by adding an equal volume of ice-cold 20% TCA containing 40 mM NaF and 20 mM β-glycerophosphate. Following TCA disruption, cells were incubated on ice for 20 min, spun at 14,000 × *g* in a 4C microfuge for 15 min, washed with chilled 0.5% TCA containing NaF and β-glycerophosphate, and resuspended in SDS sample buffer and boiled for 5 min.

For Western blots, samples were separated on Novex 4–12% gradient gels, transferred to pure nitrocellulose, blocked in Odyssey blocking buffer, incubated with 1:1,000 primary antibodies (Cell Signaling) overnight at 4 °C in Odyssey blocking buffer plus 0.1% Tween 20, washed in TBST, incubated for 45 min in 1:20,000 DyLight 680 and DyLight 800 secondary antibodies, washed in TBS plus 0.1% Tween 20, then TBS, and imaged on an Odyssey infrared imaging system.

Micropipet Gradient Chemotaxis Assay. Micropipet gradient chemotaxis assays were performed as described in Millius et al. (6). Briefly, glass capillaries were pulled on a Sutter Model P-97 and backfilled with sterile-filtered chemoattractant solution mixed with 1μM Alexa 594 hydrazide dye (Molecular Probes/Invitrogen) as a fluorescent tracer. A micromanipulator (Narishige MM-89) was used to control flow rate by adjusting balance pressure. HL-60 neutrophils were plated on fibronectin-coated no. 1.5 German glass and visualized with time-lapse microscopy.

Assay for HL-60 Neutrophil Symmetry Breaking and Random Migration. HL-60 neutrophils were starved for 45 min in modified Hanks' buffered saline solution (mHBSS) [150 mM sodium chloride, 4 mM potassium chloride, 1 mM magnesium chloride, 10 mM glucose, 20 mM Hepes (pH 7.4)] and then plated on fibronectin-coated no. 1.5 German glass. Cells were stimulated with 200 nM CNO while being visualized with time-lapse microscopy to assess for morphological changes and cell motility.

CellASIC Microfluidic Chemotaxis Assay. The ONIX microfluidic platform with M04G gradient generator plate (CellASIC/EMD Biosciences) was used to study HL-60 neutrophil migration.

Culture chambers were coated with 100 μg/mL fibronectin in D-PBS (calcium/magnesium-free) for 30 min and then blocked in migration buffer [mHBSS plus 0.25% BSA (low endotoxin)] for 30 min. HL-60 neutrophils at a density of 0.8e6/mL were flowed into the chamber and allowed to adhere for 20 min. Migration buffer was flowed through the chamber to wash away nonadherent and dead cells. A chemoattractant gradient was generated according to vendor recommendations, with a 1:10,000 dilution of Alexa594 hydrazide dye (Molecular Probes/Invitrogen) diluted into chemoattractant solutions as a fluorescent tracer. Time-lapse imaging was performed on a Nikon Ti-E inverted fluorescence microscope. Volocity software (Perkin-Elmer) was used to track cells, generate cell traces, and calculate migration metrics. Track velocity is defined as total track length divided by time. Displacement rate is defined as track end-to-end distance divided by time. Directionality is defined as displacement divided by total track length.

PLGA Bead Fabrication. Biodegradable microspheres loaded with CNO were generated using a standard oil-in-water emulsion method (7) in a sterile environment using depyrogenated glassware, equipment, and water. Ester-terminated 50:50 Poly(DL-lactide-coglycolide) (PLGA) (LACTEL) was dissolved in dichloromethane to make a 10% polymer solution. A 10 mg/mL solution of CNO dissolved in methanol or methanol alone (vehicle control) was mixed with the PLGA polymer solution at a final concentration of 1 mg/mL The mixture was then slowly dispensed into a 0.5% polyvinyl alcohol (PVA) (Sigma) solution via glass syringe (Hamilton) with continuous homogenization at 4500 rpm (IKA Model S25N-18G) for 5 min. An additional 0.5% PVA was added to the beaker, and the mixture was continuously stirred at 300 rpm using an overhead propeller blade stirrer for 4 h. Then, the microspheres were collected by centrifuging at 1,000 × *g* for 10 min, washed twice in pyrogen-free water, flash frozen in liquid nitrogen, and lyophilized overnight.

Total drug encapsulation was determined by dissolving a preweighed quantity of microspheres overnight by agitating at 275 rpm at 37 °C in 0.1 M NaOH to accelerate hydrolysis. A time course of the release kinetics of the CNO-loaded microspheres in PBS was taken by suspending a preweighed quantity of microspheres in PBS, incubating with agitation at 275 rpm at 37 °C, removing aliquots at predetermined time intervals, and replacing the buffer with an equivalent volume. The amount of CNO in solution was measured by two methods: liquid chromatography-tandem mass spectrometry (LC-MS/MS) [University of California, San Francisco (UCSF) Drug Studies Unit] and by functional assay (quantitation of stable Di-expressing HL-60 neutrophil transwell migration to

serial dilutions of CNO drug released into PBS solution by PLGA microspheres compared with migration to serial dilutions of a known CNO standard).

Mouse T Lymphocyte Homing Study. Animal studies were conducted with the UCSF Preclinical Therapeutics Core under a protocol approved by the UCSF Institutional Animal Care and Use Committee. PLGA microspheres were weighed, resuspended in PBS, vortexed, and bath sonicated for 30 s. The microspheres were injected s.c. (20 μL) into the shaved flanks of albino C57BL/6J mice (The Jackson Laboratory). Retrovirally transduced C57BL/6J mouse T lymphocytes expressing mCherry-tagged Di and an enhanced firefly luciferase (effLuc) were washed twice and resuspended in PBS before injection via the lateral tail vein (2e6 per mouse). Bioluminescent imaging was performed using the IVIS 100 (Xenogen) preclinical imaging system at predetermined time points, with images taken 10 min following i.p. injection of 150 mg/kg D-luciferin (Gold Biotechnology). Quantitation was performed using Living Image software by drawing regions of interest over areas of microsphere injections on flanks and summing total photon flux.

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4. Coward P, et al. (1998) Controlling signaling with a specifically designed Gi-coupled receptor. *Proc Natl Acad Sci USA* 95(1):352–357.
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6. Millius A, Dandekar SN, Houk AR, Weiner OD (2009) Neutrophils establish rapid and robust WAVE complex polarity in an actin-dependent fashion. *Curr Biol* 19(3):253–259.
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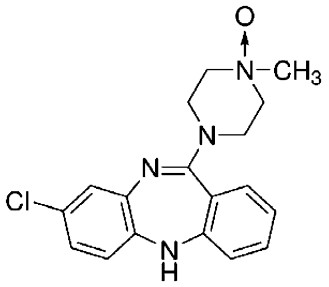


Fig. S1. Chemical structure of clozapine-*N*-oxide (CNO). CNO is an inert metabolite of the FDA-approved antipsychotic drug clozapine.

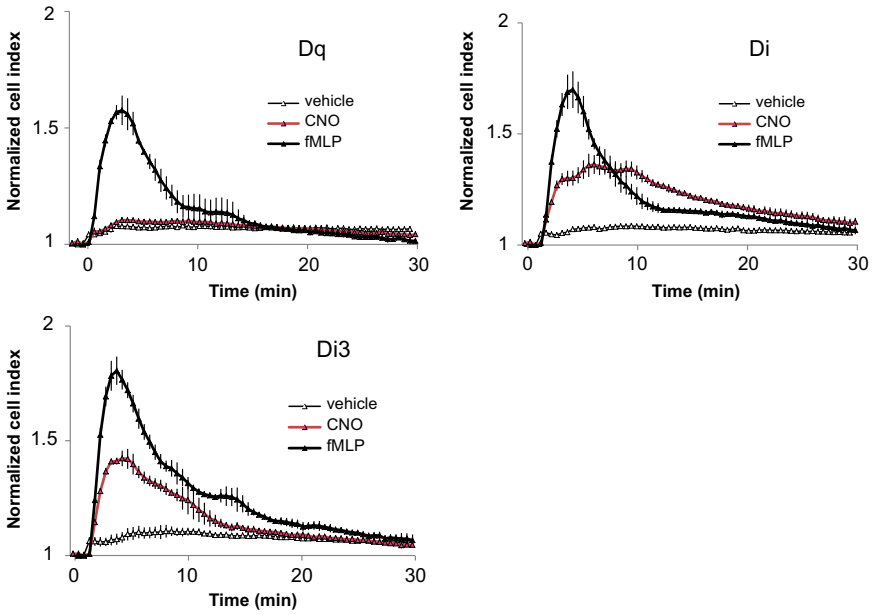


Fig. S2. CNO induces adhesion/spreading in engineered Gαi-coupled GPCR-expressing HL-60 neutrophils, as determined using a real-time impedance-based assay. Changes in electrical impedance as a result from cell spreading in response to drug or ligand are detected real-time by an electrode array. Engineered GPCRs were expressed in HL-60 neutrophils, and response to vehicle control, positive control chemoattractant fMLP, or CNO was monitored by real-time impedance measurements. All cells responded to fMLP whereas only cells expressing Di3 or Di responded to CNO. Mean ± SD for $n = 3$ replicates is shown. Peak responses in this figure correspond to bar graph shown in Fig. 1*C*.

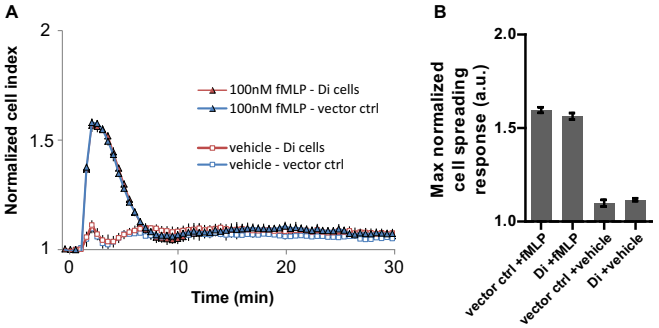


Fig. S3. fMLP induces similar extent of spreading response in engineered Di receptor vs. vector control transfected HL-60 neutrophils. (*A*) Changes in electrical impedance as a result from cell spreading in response to drug or ligand are detected real-time by an electrode array. HL-60 neutrophils transfected with either vector control plasmid (blue curves) or Di receptor plasmid (red curves) were assayed for their response to the endogenous chemoattractant fMLP (filled triangles) or vehicle control (open squares). Vector control and Di cells responded to a similar extent to 100 nM fMLP (blue triangles and red triangles, respectively). Mean ± SD for $n = 3$ replicates is shown. (*B*) Peak responses from *A* shown in bar graph format. Plasmid constructs and treatment condition are listed on the *x* axis.

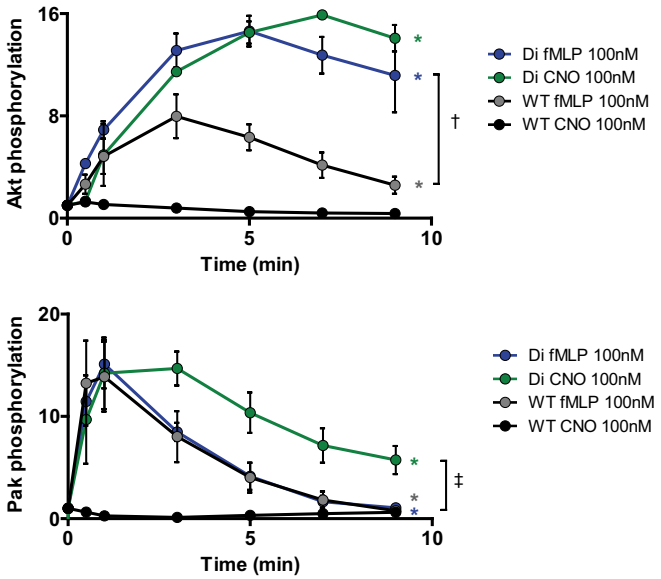


Fig. S4. Engineered Di receptor-expressing HL-60 neutrophils activate Rac and PI3K in response to CNO. Control or Di receptor-expressing HL-60 neutrophils were stimulated in suspension with 100 nM fMLP or 100 nM CNO. TCA was used to stop the reaction and precipitate proteins for immunoblotting at the specified time points. Western blots were quantitated using fluorescent secondary antibodies and the Odyssey system. The *y* axis indicates integrated (background-subtracted) fluorescence of secondary antibody on blots in arbitrary units, normalized to total Akt and to phosphorylation level before cell stimulation. Both control and Di-expressing cells activated PI3K (Akt phosphorylation) and Rac (Pak phosphorylation) signaling in response to fMLP whereas only Di cells activated PI3K and Rac signaling in response to CNO (*$P < 0.01$). Di cells exhibited a longer duration of Akt phosphorylation in response to fMLP (as well as CNO) compared with control cell response to fMLP (†, $P < 0.05$ at 5, 7, and 9 min time points). Akt phosphorylation in response to fMLP was greater than response to CNO in Di cells at 0.5 min ($P < 0.01$), but comparable at later time points. Pak phosphorylation in Di cells in response to CNO stimulation was more prolonged than corresponding phosphorylation of either control or Di cells in response to fMLP (‡, $P < 0.05$ for 3, 5, 7, and 9 min time points for Di cells, $P < 0.05$ for 5, 7, and 9 min time points for control cells). Drug concentrations used: 100 nM CNO, 100 nM fMLP. Three (CNO stimulation) or four (fMLP stimulation) independent experiments were performed and mean ± SEM are shown. Peak responses in this figure correspond to the bar graph shown in Fig. 1*E*.

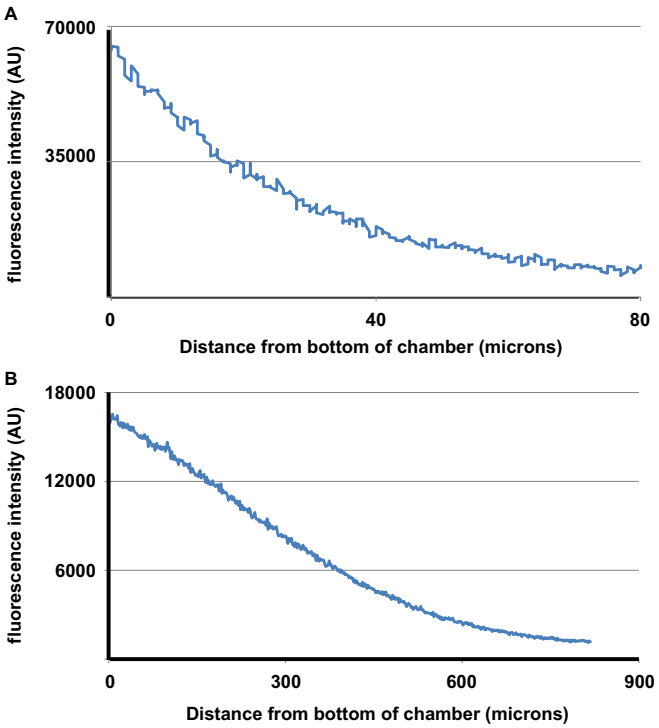


Fig. S5. Intensity profile of gradient generated by micropipet and by microfluidic chemotaxis device, as measured by quantitation of fluorescent dye intensity. (*A*) Fluorescence microscopy was used to quantitatively analyze the diffusion of an Alexa 594 fluorescent dye tracer from the tip of a micropipet, as used in micropipet assays such as those shown in Fig. 2*A* and Movies S2 and S3. An intensity profile plot is shown for a radial line drawn pointing away from the source of the micropipette-generated gradient. (*B*) Fluorescence microscopy was used to quantitatively analyze the diffusion of an Alexa 594 fluorescent dye tracer used to examine the gradient generated by the M04G microfluidic chemotaxis assay device. An intensity profile plot is shown for a vertical line drawn in the center of the field of view (see Fig. 2*B* and Movies S5 and S6 for images from microfluidic assays).

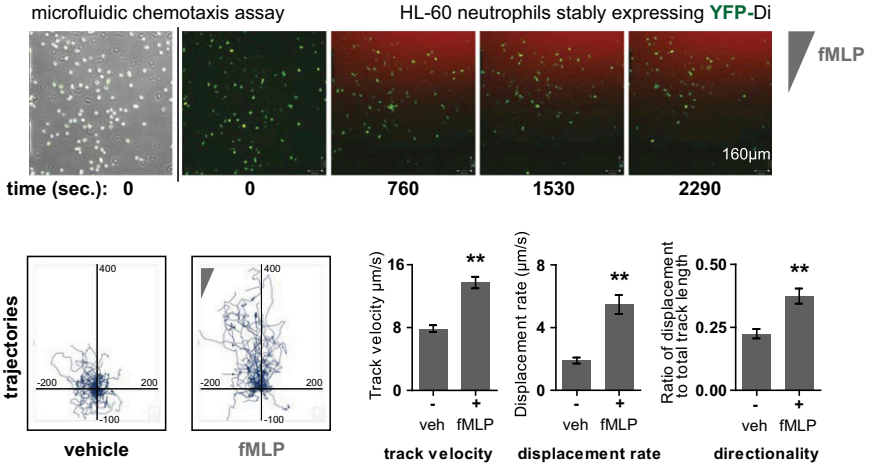


Fig. S6. HL-60 neutrophils migrate up a microfluidic device-generated fMLP gradient. HL-60 neutrophils stably expressing Di were placed in the fibronectin-coated viewing area of a microfluidic chemotaxis assay device capable of generating a smooth, stable gradient of fMLP. Time-lapse microscopy was used to track cell migration, and cell-tracking software was used to quantitate various migration metrics. Cells migrated up the fMLP gradient (trajectories plotted with cell start locations at origin) and exhibited increased track velocity, displacement rate, and directionality compared with basal motility in the presence of vehicle control. Drug concentration used (at source): 200 nM fMLP. Mean ± SEM is shown for $n = 41$ cells tracked (**$P < 0.0001$ by Student t test). See Movie S6 for full movie.

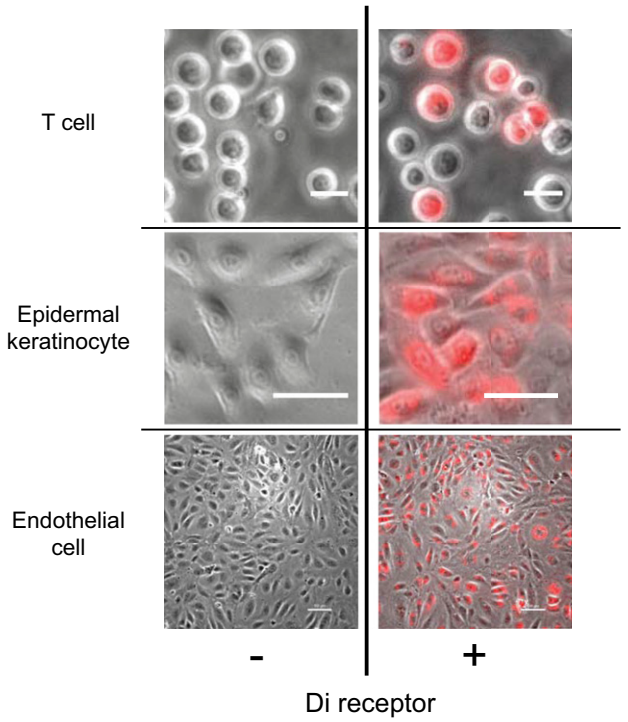


Fig. S7. Expression of Di receptor does not cause alterations in gross cellular morphology in the absence of CNO. Cells transduced with lentivirus to express mCherry-tagged Di receptor (as well as non–receptor-expressing controls) were visualized by phase contrast and fluorescence microscopy. Di-expressing and nonexpressing cells appear morphologically indistinct. mCherry (fused to Di receptor construct) is shown pseudocolored in red. [Scale bars: 25 μm (T cell), 50 μm (epidermal keratinocyte), and 100 μm (endothelial cell).]

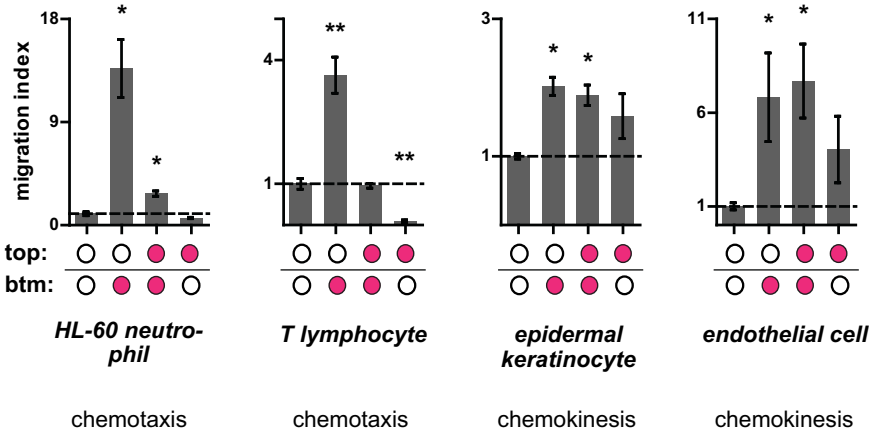


Fig. S8. Checkerboard control Transwell experiments distinguish CNO-mediated chemotaxis from chemokinesis. HL-60 neutrophils, primary human T lymphocytes, primary human epidermal keratinocytes, and primary human umbilical vein endothelial cells were transduced with lentivirus to express the Di receptor and assayed for migration through porous membranes for response to vehicle control (white circles) and/or CNO (magenta circles) placed in the top and bottom chambers of the Transwell setup. HL-60 neutrophils and T lymphocytes exhibited chemotaxis to CNO whereas keratinocytes and endothelial cells exhibited chemokinesis. Height of bars indicates migration index, which quantitates the number of cells that migrated to the bottom of the filter normalized to the basal level of cell migration in response to no chemoattractant. CNO dose used was 100 nM except for T lymphocyte experiments, where it was 25 nM. Mean ± SEM is shown for three repeats (*$P < 0.05$, **$P < 0.01$ by Student t test).

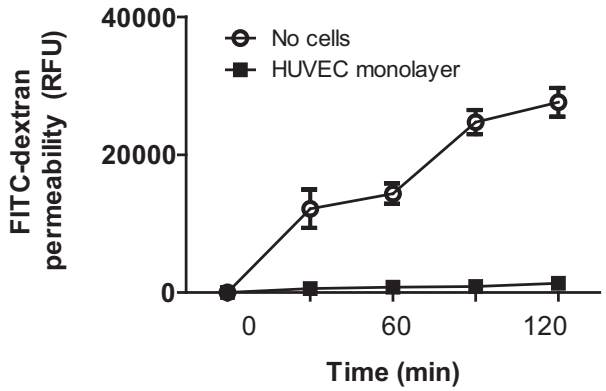


Fig. S9. Integrity of tight endothelial cell monolayers grown on porous inserts is demonstrated by blockage of FITC-dextran diffusion in a permeability assay. Human umbilical vein endothelial cells were seeded on fibronectin-coated porous transwell inserts to generate a tight monolayer, and monolayer integrity was assessed in a standard FITC-dextran permeability assay. Fluorescent dye diffusing through the porous inserts with or without endothelial-cell monolayers was quantitated at 30-min time points. Mean ± SEM for $n = 4$ replicates is shown.

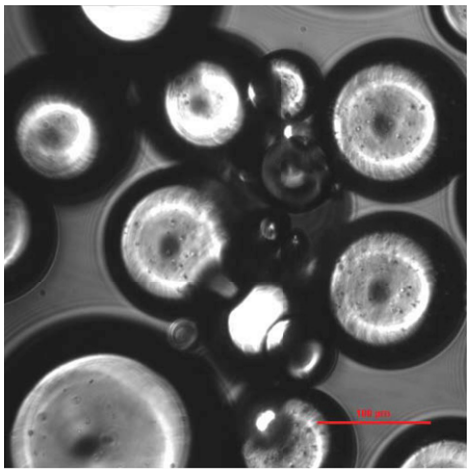


Fig. S10. Fabricated PLGA microspheres show typical morphology and size distribution suitable for s.c. injection. Biodegradable PLGA microspheres loaded with CNO were generated using a standard oil-in-water emulsion method and visualized by standard bright field light microscopy.

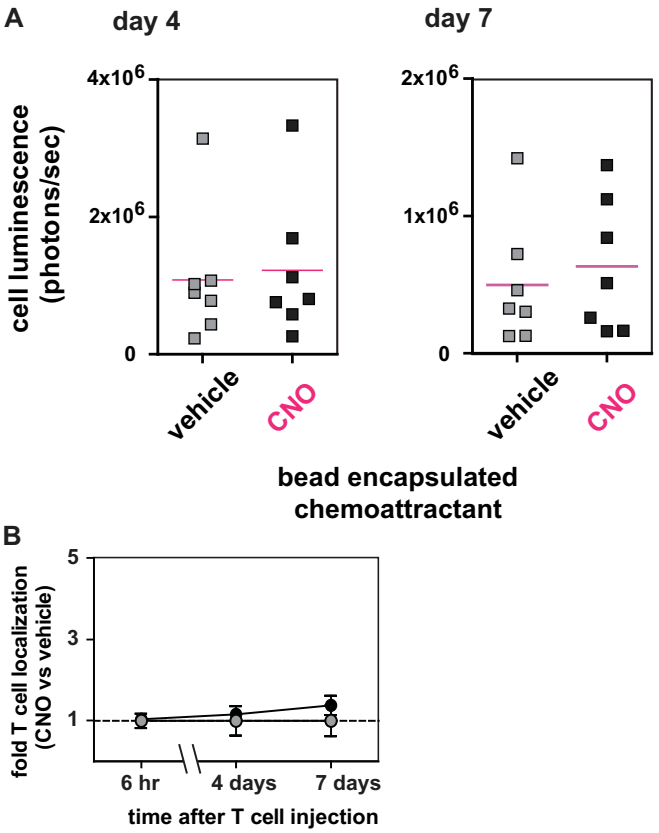
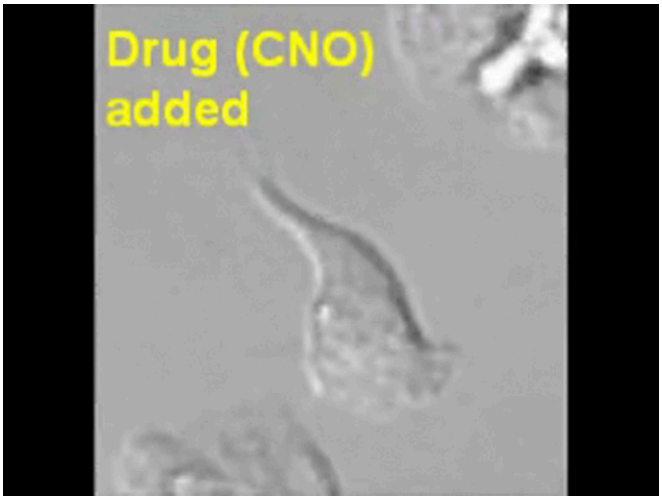
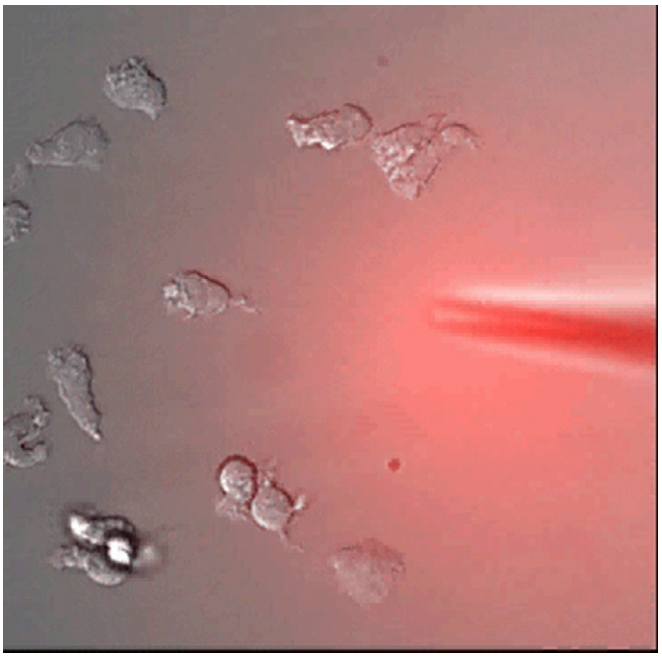


Fig. S11. Intravenously administered mouse T cells expressing luciferase (but not Di receptor) do not show preferential localization to CNO slow-release microspheres. CNO-releasing (and vehicle control) biodegradable PLGA microspheres (2 mg per injection) were implanted s.c. in the rear flanks of albino mice, and retrovirally transduced mouse T lymphocytes expressing firefly luciferase were systemically administered via tail-vein injection. In vivo bioluminescent imaging and quantitative analysis were performed. (*A*) Quantitation of bioluminescent imaging shown at 4 and 7 d postinjection of T lymphocytes shows no specific localization to CNO-injected flanks (black squares) versus vehicle control-injected flanks (gray squares) through 7 d postinjection. Mean shown for $n = 7$ mice ($P = 0.17$ by Student t test at both 4 and 7 d). (*B*) No fold difference was observed in T lymphocyte luminescent signal in CNO microsphere-injected flanks (black circles) versus vehicle microsphere-injected flanks (gray circles) at 6 h, 4 d, and 7 d after T lymphocyte injection. Mean ± SEM shown for $n = 7$ mice. ($P = 0.74$, 0.76, and 0.41 at 6 h, 4 d, and 7 d after injection, respectively).



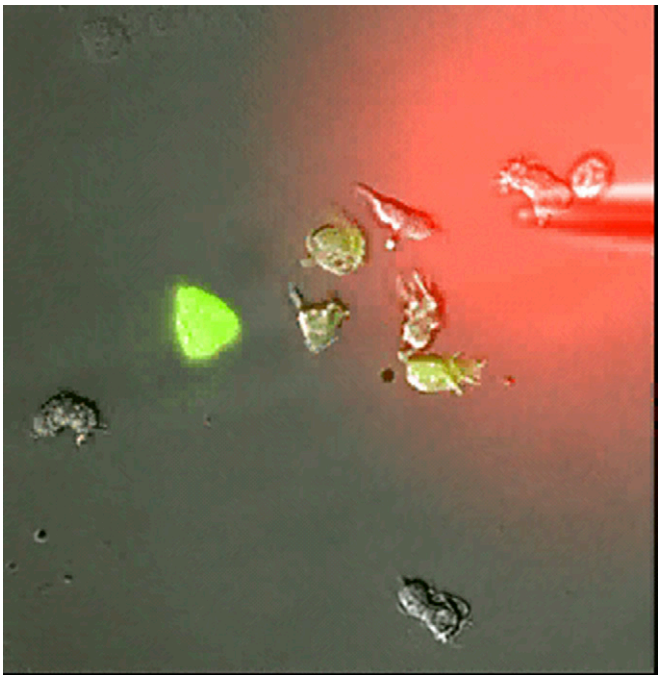
Movie S1. CNO induces symmetry breaking and motility in Di-expressing HL-60 neutrophils. HL-60 neutrophils were serum-starved for 45 min, plated on fibronectin-coated glass, and then stimulated with 200 nM CNO while being visualized by time-lapse microscopy. Cells polarize and exhibit motility in response to CNO treatment. Duration of each individual cell movie (in real time) was 10 min. Cells were stimulated with CNO at t = 3 min. Five representative cell movies shown.

Movie S1



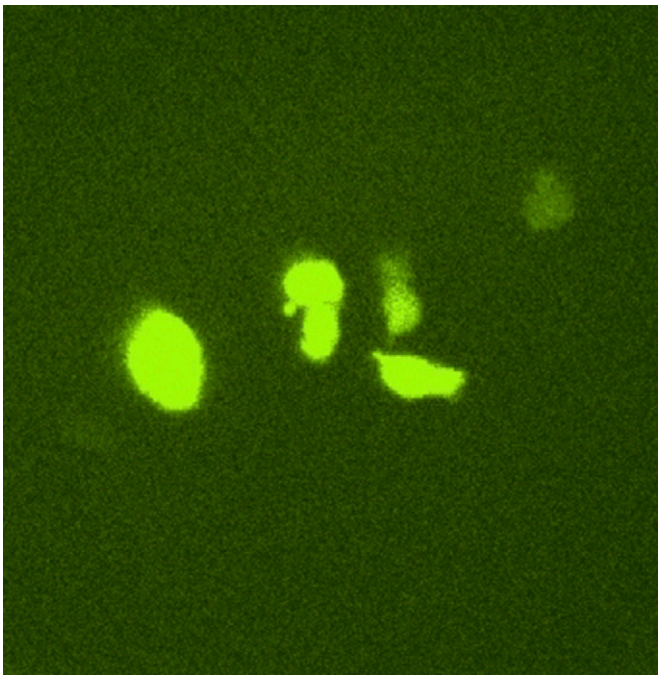
Movie S2. Direct visualization of engineered HL-60 neutrophil migration up a steep CNO gradient in a micropipet assay. In Movies S2 and S3, shown are cells visualized by differential interference contrast and fluorescence microscopy. CNO gradient is shown in red; Alexa594 dye tracer is mixed into 1 μM CNO solution. These movies correspond to the still frames shown in Fig. 2*A*. HL-60 neutrophils transfected with vector control do not respond to CNO gradient.

Movie S2



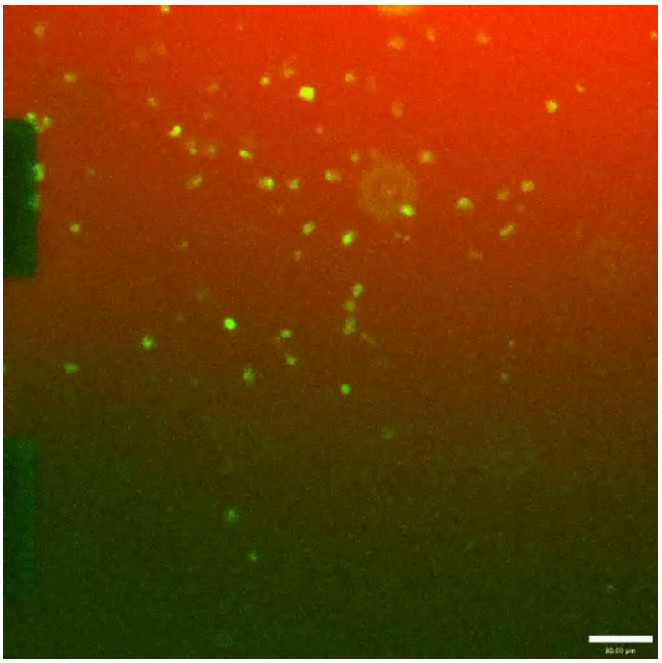
Movie S3. Direct visualization of engineered HL-60 neutrophil migration up a steep CNO gradient in a micropipet assay. In Movies S2 and S3, shown are cells visualized by differential interference contrast and fluorescence microscopy. CNO gradient is shown in red; Alexa594 dye tracer is mixed into 1 μM CNO solution. These movies correspond to the still frames shown in Fig. 2*A*. HL-60 neutrophils transfected with Di and GFP migrate up CNO gradient.

Movie S3



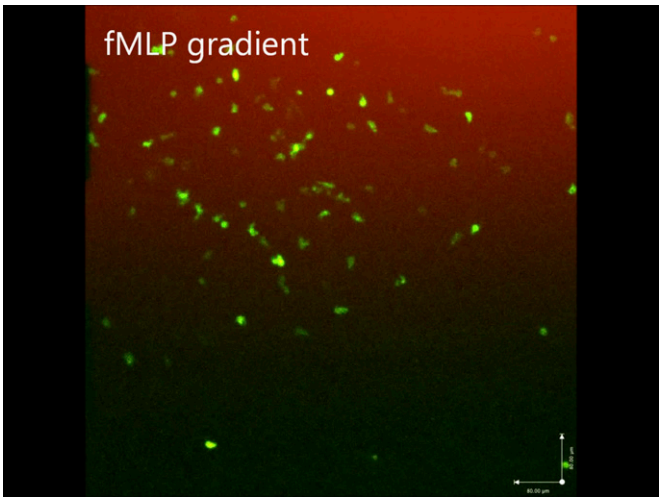
Movie S4. Direct visualization of engineered HL-60 neutrophil migration up a steep CNO gradient in a micropipet assay. This is the same movie as Movie S3 but with only green channel shown for better visualization of GFP^+ cells.

Movie S4



Movie S5. Direct visualization of engineered HL-60 neutrophil migration up a precisely generated CNO gradient in a microfluidic chemotaxis assay device. Stable YFP and Di-expressing HL-60 neutrophils visualized by fluorescence microscopy (cells shown in green) migrate in a microfluidic chemotaxis assay device. Nonadherent cells are washed away at the beginning of the movie. The CNO gradient is visualized by the presence of a red fluorescent Alexa594 dye tracer mixed into a 200-nM CNO chemoattractant solution. This movie corresponds to the still frame images, tracks, and quantitation in Fig. 2*B*.

Movie S5



Movie S6. Direct visualization of engineered HL-60 neutrophil migration up a precisely generated fMLP gradient in a microfluidic chemotaxis assay device. Stable YFP and Di-expressing HL-60 neutrophils visualized by fluorescence microscopy (cells shown in green) migrate in a microfluidic chemotaxis assay device. Nonadherent cells are washed away at the beginning of the movie. The fMLP gradient is visualized by the presence of a red fluorescent Alexa594 dye tracer mixed into a 200-nM fMLP chemoattractant solution. This movie corresponds to the still frame images, tracks, and quantitation in Fig. S5.

Movie S6