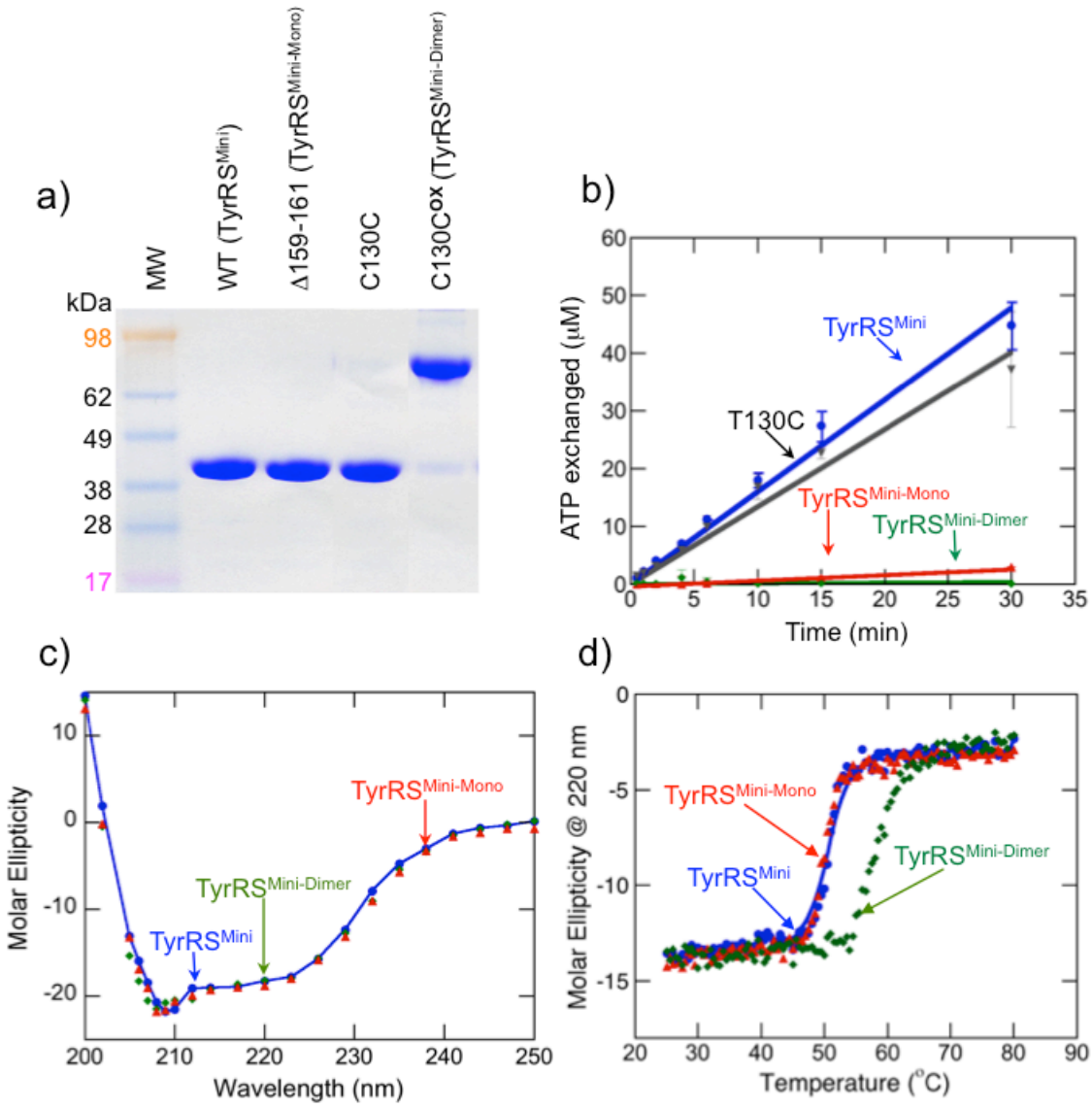
Supplemental Information

Supplemental Figure Legend. Characterization of the Δ159-161 and T130C^{ox} variants. (a) Non-reducing SDS-PAGE analysis showing Δ159-161 (named TyrRS$^{Mini-Mono}$) and T130C^{ox} (named TyrRS$^{Mini-Dimer}$) electrophoresis as monomer and dimer, respectively. (b) Amino acid activation assay comparing activity of WT TyrRS^{Mini}, TyrRS$^{Mini-Mono}$ and TyrRS$^{Mini-Dimer}$. (c) Far-UV CD spectra of WT TyrRS^{Mini} (●), TyrRS$^{Mini-Mono}$ (▲) and TyrRS$^{Mini-Dimer}$ (■). (d) Thermal melting CD spectra of WT TyrRS^{Mini} (●), TyrRS$^{Mini-Mono}$ (▲) and TyrRS$^{Mini-Dimer}$ (■



Experimental Procedures

Receptor internalization assay. HEK 293 cells that stably express CXCR1 or CXCR2 (a gift from Dr. Adit Ben-Baruch at Tel Aviv University) were trypsinized and washed two times with BSA medium (RPMI 1640 containing 1% bovine serum albumin and 25 mM HEPES). Aliquots of one million cells were treated with 100 nM WT $TyrRS^{Mini}$, $TyrRS^{Mini}$ variants or IL-8 at 37 °C for 2 hours and placed on ice for an additional 10 minutes. The cells were then washed once with BSA medium and once with FACS buffer (PBS containing 1 mM EDTA, 1 % fetal calf serum (FBS), 0.05 % NaN_3, and 25 mM HEPES) prior to staining with antibodies. Fluorescein-conjugated human α-CXCR1 or α-CXCR2 specific antibodies (Biolegend, San Diego, CA) were added to CXCR1-293 or CXCR2-293 cells, respectively, and incubated at 4°C for 1 hour. The cells were washed three times with FACS buffer then analyzed using a BD LSR II flow cytometer. Baseline control cells were stained with PBS instead of antibodies to CXCR1 or CXCR2.

Plasmid construction, Expression, and purification of $TyrRS^{Mini}$ variants. The plasmid encoding wild-type (WT) human $TyrRS^{Mini}$ has been cloned previously (4). This plasmid template was used to generate $TyrRS^{Mini}$ variants by site-directed mutagenesis using the QuikChange™ mutagenesis kit from Stratagene (La Jolla, CA). Primers were purchased from Integrated DNA Technology, Inc. (Coralville, Iowa). Recombinant proteins were expressed and purified as previously described (24). To perform I_2 oxidation, T130C $TyrRS^{Mini}$ (1 mg/ml) was incubated with I_2 (0.5 mM) in phosphate buffer saline (PBS) for 15 min at 37 °C, followed by extensive dialysis overnight against PBS at 4 °C to remove I_2. Endotoxin was removed by running the sample through an EndoTrap Red column (Lonza Walkersville, Inc., Walkerville, MD) twice.

Circular dichroism analysis. Circular dichroism (CD) spectra were obtained with a CD Spectrometer 400 (Aviv Biomedical, Inc. Lakewood, NJ). Prior to CD measurements, protein samples were extensively dialyzed overnight against PBS. The measurements were performed using 250 μL of each protein sample at 10 μM for far-UV spectra and 500 μL at 50 μM for near-UV spectra. For thermal melting scans, protein samples were monitored at a fixed wavelength of 220 nm as a function of temperature.

Analytical gel chromatography. Each protein sample (200 μl of 10 μM) was injected onto a Superdex 200 chromatography column (GE Healthcare, 10/300GL) in PBS containing 5 mM β-mercaptoethanol and protein absorbance at 280 nm was monitored. The column was calibrated using standard proteins. To estimate the apparent monomer-dimer equilibrium dissociation constants of WT $TyrRS^{Mini}$ and $TyrRS^{Mini-Mono}$, 200 μL of WT $TyrRS^{Mini}$ (ranging from 25 nM to 1.8 μM) or of $TyrRS^{Mini-Mono}$ (ranging from 10 μM to 1000 μM) were loaded onto a Superdex 200 chromatography column. The elution volume peak of each concentration of $TyrRS^{Mini-Mono}$ was monitored by its absorbance at 280 nm. Due to undetectable absorbance of WT $TyrRS^{Mini}$ at low concentrations, 0.5 mL fractions from elution volume ranges of 12 mL to 16 mL were collected and then subjected to SDS-PAGE and immunoblotted with anti-TyrRS antibodies to identify the elution volume peak at each concentration. Elution volume shift was defined as the fractional distance between the monomer and dimer peaks. This difference was plotted *versus* concentration. The apparent K_d was taken as the concentration at which the volume shift is half-way between the monomer and dimer peaks.

Amino acid activation assay. Amino acid activation was performed at 25 °C as previously described (31), with some modifications, in a 96-well format. Briefly, each reaction mixture contained 100 mM Tris-HCl, pH 7.4, 10 mM $MgCl_2$, 1 mM ATP, 0.1 mg/ml bovine serum albumin, 0.5 mM sodium pyrophosphate including [^{32}P]-pyrophosphate (Perkin Elmer; with specific activity of 37 TBq/mml), 5 mM β-mercaptoethanol, 2 mM tyrosine, and 100 nM of WT $TyrRS^{Mini}$ or its variants. Aliquots were quenched at different time points in a 96-well Multiscreen filter plate (Millipore, Billerica, MA) containing 200 mM sodium pyrophosphate, 1 M HCl and 4% (w/v) activated charcoal. After washing four times with a solution of 200 mM sodium pyrophosphate and 1 M HCl, charcoal-absorbed [^{32}P]-ATP samples were punched from the plate and quantified by scintillation counting.

Receptor binding assay. For immunofluorescence assays, HeLa cells were grown on coverslips and transiently transfected with pcDNA3.1-CXCR1 or –CXCR2 that encodes a V5-CXCR1 or –CXCR2

receptor. Twenty-four hours after cells were transfected, they were treated with purified His_6-tagged $TyrRS^{Mini}$ for 1 hour at 4°C. After treatment, cells were washed twice with PBS and then fixed with 2 % formaldehyde-PBS for 20 minutes. Cells were subsequently incubated with rabbit anti-His_6- (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) and mouse anti-V5- (Invitrogen Corporation, Carlsbad, CA) antibodies, and then with goat anti-rabbit IgG-Alexa Fluor 448 (Invitrogen Corporation, Carlsbad, CA) and goat anti-mouse IgG-Texas Red (Invitrogen Corporation, Carlsbad, CA) to detect $TyrRS^{Mini}$ (Green) and CXCR1/2 (Red), respectively. Coverslips containing immunofluorescence-stained cells were then mounted on slides and viewed under a Zeiss confocal fluorescence microscope. For immunobloting assays, CXCR1- and CXCR2-transfected HEK 293 cells were incubated with 100 nM purified His_6-tagged WT $TyrRS^{Mini}$ or $TyrRS^{Mini\text{-}Mono}$ for 1 hr at 4 °C. Cells were then washed twice with PBS, lysed in M-PER buffer (Thermo Scientific, Rockford, IL) and then analyzed by immunoblotting using anti-His_6 (Immunology Consultants Laboratory, Inc. Newberg, OR) and anti-actin (Sigma-Aldrich, St. Louis, MO) antibodies to detect $TyrRS^{Mini}$ binding and relative number of cells, respectively. For competition experiments, CXCR1-293 or CXCR2-293 cells were incubated with 100 nM WT $TyrRS^{Mini}$ alone or together with progressivly increasing in concentration of $TyrRS^{Mini\text{-}Dimer}$ (100 nM to 2 μM), and then processed and analyzed as above. The intensity of the WT $TyrRS^{Mini}$ was scanned, normalized to the amount of actin and plotted as a function of $TyrRS^{Mini\text{-}Dimer}$ concentration.

Transwell cell migration assay. Human PMN cells were prepared from heparin-treated whole blood obtained from healthy volunteers using the RosetteSep Human Granulocyte Enrichment Kit (StemCell Technologies, Vancouver, BC, Canada). Isolated PMN cells were resuspended in cell migration medium containing RPMI 1640 (American Type Culture Collection, Manassas, VA) and 0.5% heat-inactivated fetal bovine serum (FBS) (Invitrogen Corporation, Carlsbad, CA) and incubated with Calcein AM (Invitrogen Corporation, Carlsbad, CA) at 37 °C in a 5% CO_2 incubator for 30 minutes. After the incubation, cells were collected and washed two times with RPMI 1640 containing 0.5% FBS then resuspended in cell migration medium at a concentration of 10×10^6 cells/ml. Cell migration was performed using a 24-well transwell permeable support with 5 μm pores (Corning Inc., Lowell, MA). Briefly, Calcein-labeled cells (at a concentration of 10×10^6 cells/ml in 100 μL) were plated in an upper transwell insert and 600 μL of proteins at the indicated concentrations was added to the lower chamber. For competition assays, cells (in the upper chamber) were pre-incubated with the indicated concentrations of $TyrRS^{Mini\text{-}Mono}$ or $TyrRS^{Mini\text{-}Dimer}$ for 30 minutes prior to adding 10 nM WT $TyrRS^{Mini}$ to the lower chamber. Cells were allowed to migrate at 37 °C for 45 minutes before being collected, and the fluorescence intensity of migrated cells was measured using a Synergy HT Microplate reader reader with filters set to 485 nm for excitation and 520 nm for emission (BioTek Instruments, Inc, Vinooski, VT). Fold migration over control was calculated as the fluorescence intensity of cells migrating toward the proteins divided by the fluorescence intensity of cells migrating to medium.