Supporting information

Experimental Methods

Synthesis of DV1 and FITC-DV1

The DV1 and FITC-DV1 were synthesized manually using Fmoc (N-9-fluorenylmethoxy carbonyl) chemistry. TentaGel S RAM resin (loading 0.24 mmol/g; Fisher Scientific) was used for the in-house synthesis. We used 5-fold excess of the raw material Nα-Fmoc-amino acid, hydroxybenzotriazole (HOBt), and the condensation reagent diisopropylcarbodiimide (DIC) in each coupling step. The Fmoc group deprotection at each step was conducted using 20% piperidine in dimethylformamide (DMF). The removal of the dde (N-[1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl]) group was achieved by incubation with 2% hydrazine in DMF twice for 3 minutes at room temperature. The DV1 was synthesized by regular fmoc solid phase peptide synthesis with all $_{D}$-amino acids. The synthesis of FITC-DV1 was initiated by coupling sequentially aminocaproic acid (Ahx) and whole sequence of DV1 from C-terminal to N-terminal to the dde group protected Fmoc-lysine already bound to resin. The last coupled D-amino acid leucine was protected by a Boc group. The dde group was then removed and one FITC group was coupled onto the ε-amino of the C-terminal lysine through fluorescein isothiocyanate (FITC) (FITC isomer I, Acros Organics). The peptide resin was cleaved by incubation with a cocktail cleavage solution composed of trifluoroacetic acid (TFA) (90%, vol/vol), water (5%, vol/vol), and thiophenol (5%, vol/vol), at room temperature for 2 hours. Cold diethyl ether was added and the crude peptides were washed twice with cold diethyl ether, dissolved in 10% acetonitrile in water, and then cooled down in dry ice before being dried in a lyophilizer (*1*). The crude peptides were then purified by semi-preparative RP-HPLC (Waters)

(*2*). The fractions corresponding to different peaks were collected separately and lyophilized. The final peptide products were characterized by MALDI-TOF-MS (Autoflex III Smartbeam, Bruker). The overall yields of DV1 and FITC-DV1 were 15.6% and 7%, respectively. The purity of both peptides was above 95%. The mass data are shown in Figures S1 and S2.

The saturation curve of FITC-DV1

CXCR4 overexpression chinese hamster ovary (CHO) (CHO-CXCR4) cell line was established according to the methods published previously by our group (*3, 4*). CHO-CXCR4 and CHO cells were cultured in RPMI1640 media supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin. CHO cells expressing CXCR4 (CHO-CXCR4) were selected using G418 sulfate. One hundred microliters of 5×10^6 cells/mL in 1% BSA/PBS were added to each well of a v-shaped 96-well plate. After centrifugation, 100 μL of FITC-DV1 at a series of concentration (5 nM, 10 nM, 50 nM, 100 nM, 200 nM, 400 nM, 800 nM) in 1% BSA/PBS were added to the wells. After sufficient mixture of each well, the plate was covered with an opaque sealing tape and made incubation for 50 minutes on a shaker in the dark at 4 ºC. The cells were then washed three times and resuspended in 100 μL 1% BSA/PBS. The fluorescence intensity ($485_{EX}/528_{EM}$, sensitivity: 90) was determined using a Synergy 2 microplate reader (BioTek). The FITC-DV1 values represented total binding capacity. Nonspecific binding was derived from the binding activity of FITC-DV1 to CHO cells under the same conditions used for the CHO-CXCR4 cells. Specific binding of FITC-DV1 to CXCR4 was calculated by subtracting nonspecific binding from the total binding.

CXCR4-12G5 antibody competitive binding assay

Cultured CHO-CXCR4 cells were diluted into 5×10^6 cells/mL in 0.5% BSA/PBS. One hundred microliters of above cell solutions were added to each well of a v-shaped 96-well plate. After two washes with 0.5% bovine serum albumin (BSA)/phosphate-buffered saline (PBS), the cells were incubated with 100 μL primary 12G5 Ab (1:2000, mouse anti-human CD184 antibody) in 0.5% BSA/PBS, in the presence or absence of different concentrations of DV1, SDF-1α (SDF-1α Human, ProSpec), AMD3100 (Plerixafor 8HCl, Selleckchem), or IT1t (EMD Millipore), for 40 minutes on a shaker at 4 ºC. Following two washes with 0.5% BSA/PBS, the cells were incubated with a 100 μL of secondary antibody in 0.5% BSA/PBS (1:250, FITC conjugated anti-mouse IgG, Sigma-Aldrich) for 30 minutes on a shaker in the dark at 4 ºC and washed twice with 0.5% BSA/PBS. The cells were then resuspended in 100 μL 0.5% BSA/PBS and the fluorescence intensity ($485_{EX}/528_{EM}$, sensitivity: 55) was determined using a microplate reader (Synergy 2, BioTek) (*5, 6, 7*).

CXCR4-FITC-DV1 competitive binding assay

Various concentrations of DV1, SDF-1α, AMD3100, and IT1t were prepared in advance with 1% BSA/PBS. The CHO-CXCR4 cells were added at 5×10^5 cells/100 μL/well to a v-shaped 96-well plate and pre-cultured in 1% BSA/PBS for 20 minutes. After centrifugation, 100 μL of 800 nM FITC-DV1 in 1% BSA/PBS were added to each well (except for the negative control group where 100 μL of 800 nM FITC was added). Then, different concentrations of the above pre-prepared compounds solution were added to each corresponding well. After mixing the contents of each well, the plate was covered with the opaque sealing tape and then incubated for 50 minutes on a shaker in the dark at 4 ºC. The cells were then washed twice and resuspended in 100 μL 1% BSA/PBS. The fluorescence intensity ($485_{EX}/528_{EM}$, sensitivity: 90) was determined

using a Synergy 2 microplate reader (BioTek). The value determined for non-specific binding of FITC (negative group) wassubtracted from every FITC-DV1 group.

Binding of FITC-DV1 to CCR5, CCR3, and CXCR7

The $CCR5^+$ MOLT-4 cells (NIH AIDS Reagent Program) were cultured in RPMI-1640 media supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin. The $CCR3^+$ 3T3.T4 cells (NIH AIDS Reagent Program) were cultured in DMEM media with the same supplemental components as for $CCR5^+$ MOLT-4. A 100 μL volume containing 5×10^6 cells/mL in 1% BSA/PBS was added to each well of v-shaped 96-well plate. After centrifugation, different concentrations of FITC-DV1 were incubated with anti-CCR5 [1:1000, mouse anti-human CD195 MAb, NIH AIDS Reagent Program] or anti-CCR3 [1:1000, rat anti-human CCR3 MAb, NIH AIDS Reagent Program] antibodies. After incubation for 40 minutes on a shaker in the dark at 4 ºC, the cells were washed twice. Following this, 100 μL 1% BSA/PBS containing peroxidase-conjugated secondary antibody against CCR5 [1:4000, Anti-Mouse IgG, Sigma-Aldrich] or CCR3 [1:4000, Anti-Rat IgG, Sigma-Aldrich] was added to each well. After incubating for 30 minutes on a shaker in the dark at 4 ºC, the cells were washed twice and 100 μL ADHP substrate solutions (Violabs) containing 0.006% hydrogen peroxide (Sigma-Aldrich) was added and incubated for 20 minutes in the dark at room temperature. The fluorescence intensity ($530_{EX}/590_{EM}$, sensitivity: 55) was then measured using a microplate reader (Synergy H1, BioTek) (*8, 9, 10, 11, 12*).

The CHO-CXCR7 cells were added at 5×10^5 cells/100 μL/well in 1% BSA/PBS to a v-shaped 96-well plate. After centrifugation, different concentrations of FITC-DV1 in 1% BSA/PBS were

added into corresponding wells together with PE (phycoerythrin) anti-human/mouse CXCR7 Antibody (Biolegend). After incubation for 40 minutes on a shaker in the dark at 4 ºC, the cells were washed three times and resuspended in 100 μL 1% BSA/PBS. The fluorescence intensity ($496_{EX}/578_{EM}$, sensitivity: 80) was then measured using a microplate reader (Synergy H1, BioTek).

Statistical analysis

The experiments were performed at least three times and data were analyzed in Microsoft® Excel and plotted in GraphPad Prism 4. Average values were expressed as mean ± SD. The statistical analysis was performed using the Student's *t*-test. *P* values of <0.5 were considered statistically significant.

Figure S1. The MALDI-MS spectra of DV1

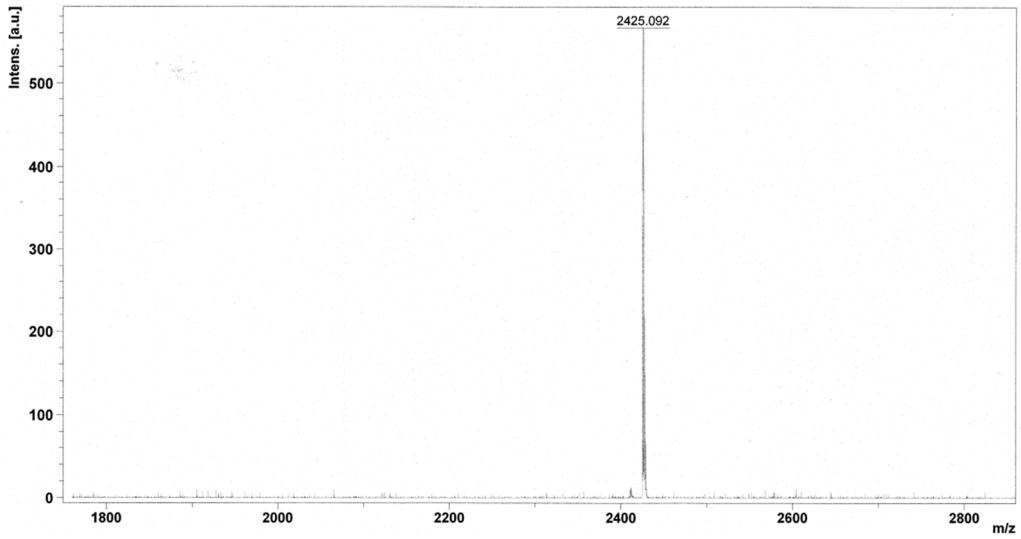


Figure S2. The MALDI-MS spectra of FITC-DV1

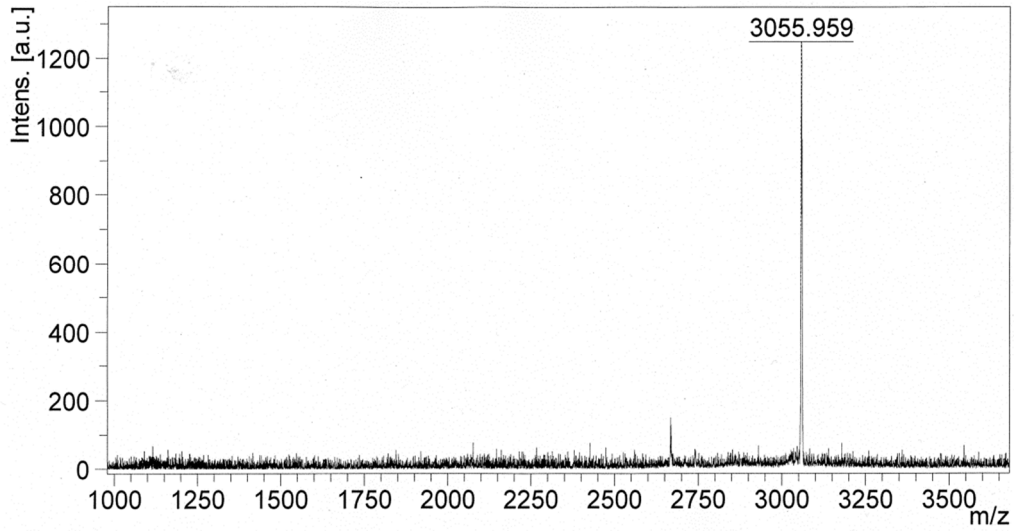
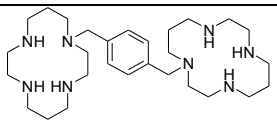
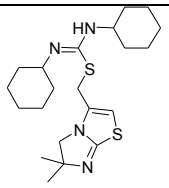


Table S1. A list of compounds tested in CXCR4 binding assay (Bold sequences are $_{D}$-amino acids).

Name	Amino acid sequences or chemical structures
DV1	**LGASWHRPDKCCLGYQKRPLP** [a,b]
FITC-DV1	**LGASWHRPDKCCLGYQKRPLP-**Ahx-K-FITC [a]
SDF-1α	KPVSLSYRCPCRFFESHVARANVKHLKILNTPNCALQIVARLKNNNRQV CIDPKLKWIQEYLEKALNK [c,d]
AMD3100[d]	
IT1t [e]	

a. Bold sequences are $_{D}$-amino acids. b. Reference (*3*). c. Reference (*13*). d. Reference (*14*). e. Reference (*15*).

Table S2. Comparison of IC_{50} values of different CXCR4 ligands in FITC-DV1 and 12G5 Ab based CXCR4 binding assays.

Compound	IC_{50} (nM)	
	12G5 Ab Inhibition	FITC-DV1 Inhibition
DV1	255	252
SDF-1α	73	71
AMD3100	58	63
IT1t	4	6

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