Supporting Information

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SI Materials and Methods

Mobilization of Hematopoietic Stem and Progenitor Cells. Mobilization of hematopoietic stem and progenitor cells (HSC/HPCs) in peripheral blood after treatment with soluble and virally delivered chemokine receptor CXCR4 antagonists was determined on days 4 and 8 by flow cytometry using the following mAbs: anti–c-kit-PE (Abcam), anti–Sca-1-PE-Cy7 (eBioscience), and lineage markers conjugated to FITC [CD8α, T-cell receptor (TCR-β), CD11b, Gr1, NK1.1, B220, CD3ε, Ter119, CD11c, CD127, and CD4] (eBioscience). All evaluations were performed on FACS-calibur flow cytometer (Becton Dickinson). After gating on forward and side-scatter parameters, at least 10,000 gated events were routinely acquired and analyzed using CellQuest software (Becton Dickinson Immunocytometry System).

Complement-Dependent Cytotoxicity and Antibody-Dependent Cell-Mediated Cytotoxicity. For the complement-dependent cytoxicity (CDC) assay, ^{51}Cr-labeled 4T1 cells (10^4/well) were preincubated with various concentrations of CXCR4-A-mFc fusion protein and 1:10 dilution of rabbit complement (Cedarlane Laboratories). The murine mAb OKT3 was included as an isotype control. The antibody-dependent cell-mediated cytoxicity (ADCC) against 4T1 cells (10^4/well) was performed using the same concentrations of the CXCR4-A-mFc antagonist and OKT3 mAb by a standard ^{51}Cr-release assay. The cells were mixed with NK cell-enriched splenocytes isolated from tumor-free BALB/c mice at the effector-to-target ratio of 100:1. NK cells were obtained from pooled splenocytes by centrifugation over a discontinuous density gradient consisting of 70%, 65%, 60%, 57%, 55%, and 50% Percoll (Amersham Biosciences), and recovered in the lower density fractions. Spontaneous release for ADCC and CDC was calculated based on the chromium released by target cells incubated with NK cells or complement alone, respectively. Maximum radioactivity release was determined from supernatants of cells that were lysed by the addition of 5% Triton X-100. The percent of specific lysis was calculated as: [(cpm experimental release/cpm spontaneous release)/(cpm maximum release/cpm spontaneous release)] × 100.

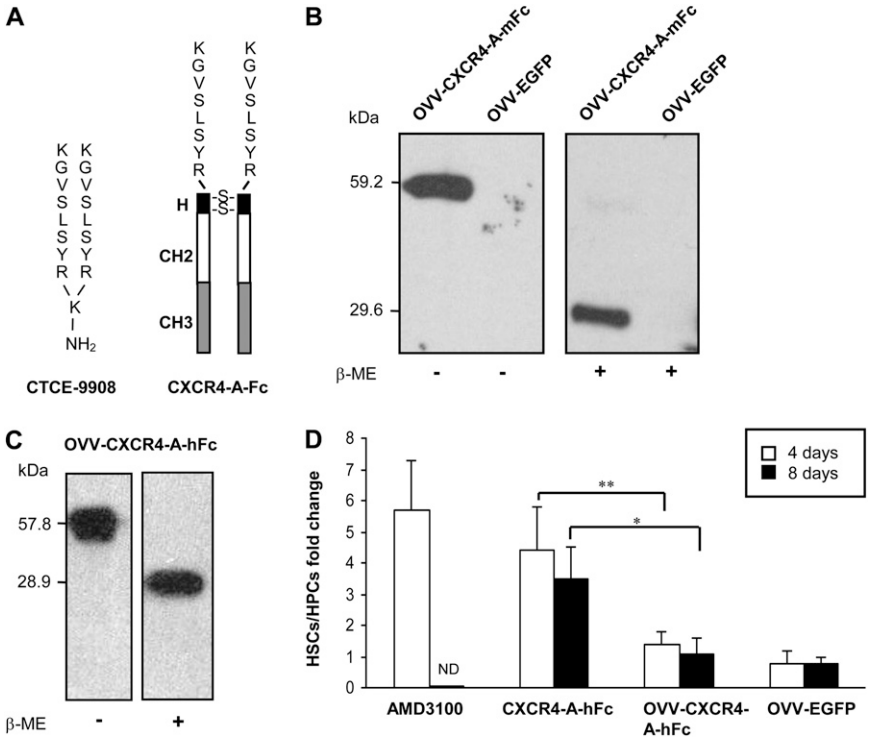

Fig. S1. Characterization of CXCR4-A-Fc fusion protein and its effect on mobilization of HSCs/HPCs. (*A*) In the fusion protein construct, the first eight amino acids (KGVSLSYR) of CTCE-9908 were expressed in the context of murine (mFc) or human (hFc) fragment of IgG with disulfide bonds in a hinge region for preservation of its dimeric structure. (*B* and *C*) Western blotting of CXCR4-A-mFc and CXCR4-A-hFc isolated from supernatants of virally infected cells under nonreducing and reducing conditions. Control samples included supernatants for oncolytic vaccinia virus (OVV)-EGFP–infected cultures. (*D*) Effect of soluble and virally delivered CXCR4-A-mFc antagonists on mobilization of HSCs/HPCs. The 4T1-bearing mice were injected intravenously with 10^8 PFU of OVV-CXCR4-A-hFc, OVV-EGFP, or 25 mg/kg of soluble CXCR4-A-hFc protein was delivered for 7 d. The blood analysis was carried out on days 4 and 8. A single injection of AMD3100-a bicyclam (5 mg/kg) was used as a control antagonist and blood was analyzed 1 h later. Data are presented as the means ± SD of three independent experiments. $*P < 0.05$, $**P < 0.01$.

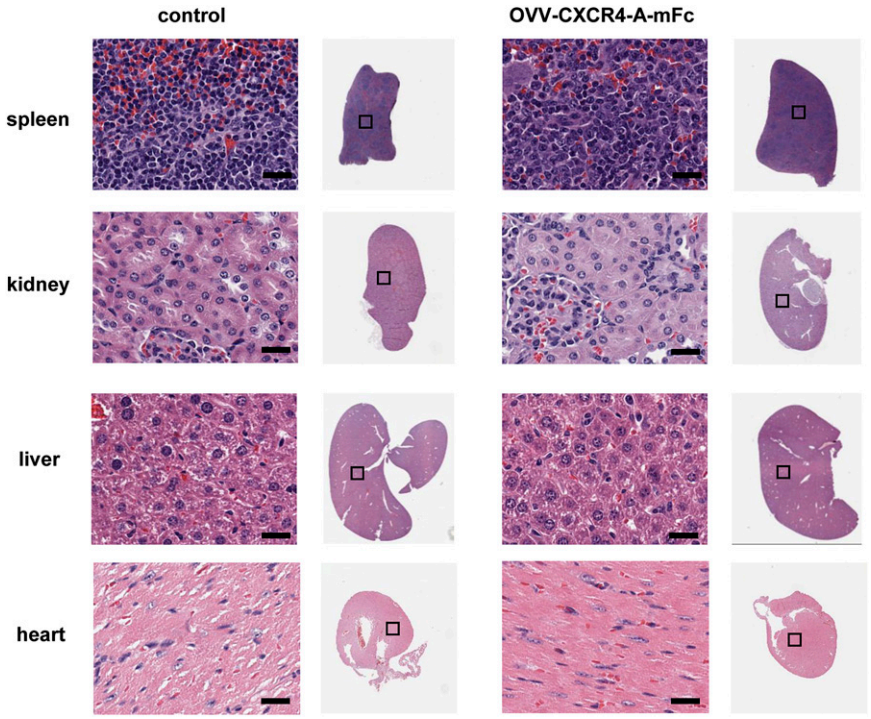

Fig. S2. Representative histograms of spleen, kidney, liver and heart isolated from control (*Left*) and OVV-CXCR4-A-mFc–treated mice (*Right*). BALB/c mice with orthotopic 4T1 tumors were treated with PBS (control) or 10^8 PFU of OVV-CXCR4-A-mFc. Four days after the treatment and at the peak of viral replication, spleen, kidney, liver, and heart were harvested for histology. Organs were fixed, paraffin embedded, and sectioned. Five-micrometer-thick sections were stained with H&E. (Scale bars, 25 μm.)

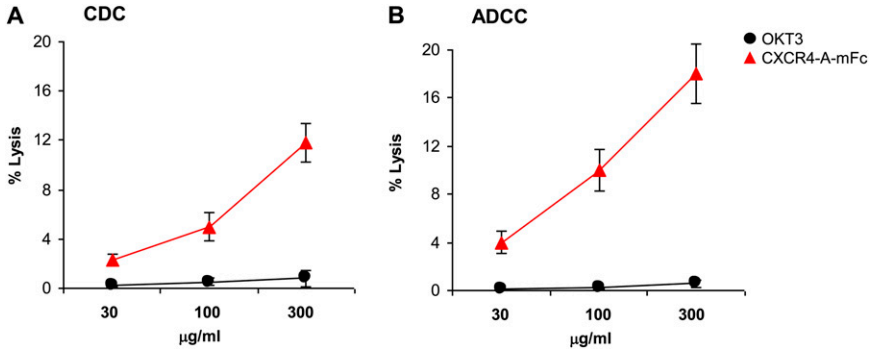

Fig. S3. CDC and ADCC against CXCR4-A-mFc protein-coated 4T1 cells. (*A*) Complement-mediated lysis of tumor cells was assayed with ^{51}Cr-labeled 4T1 cells coated with the indicated concentrations of CXCR4-A-mFc fusion protein (red triangle) or murine OKT3 mAb used as an isotype (IgG2a) control (black circle), followed by incubation with the rabbit complement (1:10 dilution). (*B*) For ADCC, ^{51}Cr-labeled 4T1 cells were preincubated with different concentrations of CXCR4-A-mFc fusion protein (red triangle) or murine OKT3 mAb (black circle), and mixed with NK cell-enriched splenocytes at the effector-to-target ratio of 100:1. All determinations were made in triplicate samples, and the SD was <10%. Results are presented as the means ± SD of two independent experiments.