

Supporting Information

for

Peptide-binding Induced Inhibition of Chemokine CXCL12

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1. Binding affinity between W4 and CXCL12

We utilized the SPR assay to identify the interaction between CXCL12 and W4. W4 was immobilized on sensor chips. When CXCL12 was injected into the biosensor, a typical increase in SPR response corresponding to the association phase was observed in a dose-dependent manner. Upon displacement of the chemokine by the running buffer, a decrease in the SPR response signal emerged, consistent with the dissociation phase (Fig. S1). The binding curve was fitted and demonstrated a multivalent binding mode with a clear K_D of 3.0×10^{-9} M for anti-CXCL12 and 5.7×10^{-8} M for W4.

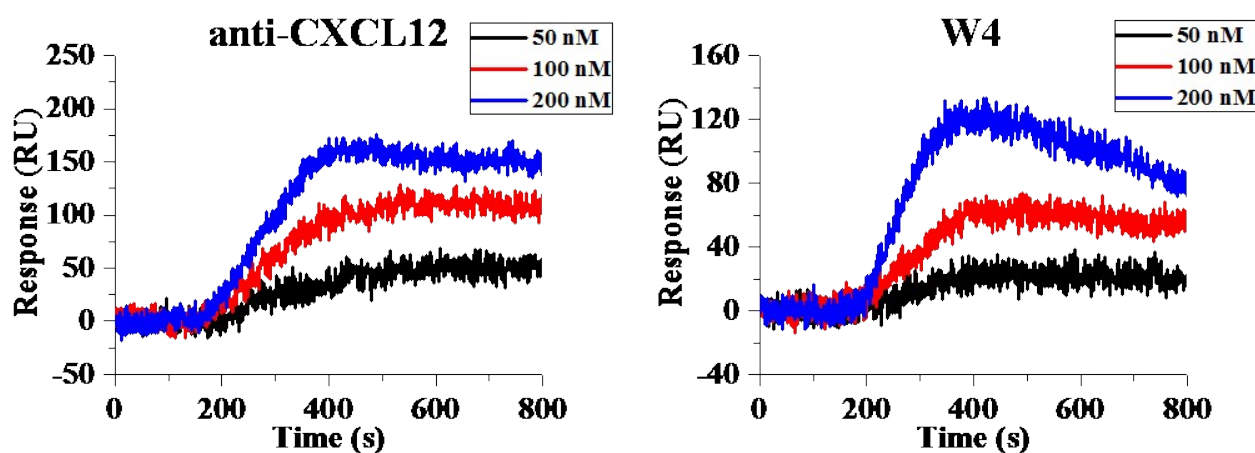


Fig. S1 SPR response on biosensor of anti-CXCL12 and W4 reference to CXCL12. The three curves correspond to three different concentrations of CXCL12 of 50, 100 and 200 nM.

2. The CXCR4 expression level of cancer cells

The MCF-7, MDA-MB-231, HL-60 and U937 cells were washed 3 times and then resuspended with washing buffer to 5×10^6 cells/mL. 50 μ L of the cell suspension was added into the tube and centrifuged. Then the supernatant was removed carefully. 60 μ L of monoclonal anti-human FITC labeled CXCR4 antibody (MBL, Japan) in the washing buffer was added at 10 μ g /mL. The cells were incubated for 30 minutes at room temperature. 1 mL of washing buffer was added followed by centrifugation. Then the supernatant was removed and cells were resuspended with 500 μ L washing buffer. 1×10^4 cells were analyzed. FITC-labeled IgG was chosen as the control.

Four cell lines including MCF-7, MDA-MB-231, HL-60 and U937 were chosen as cell models because the cells express different levels of CXCR4 in the surface. The CXCR4 levels for MCF-7, MDA-MB-231, HL-60 and U937 were detected 61.4%, 88.1%, 97.6% and 92.5% respectively. Results were shown in Fig. S2.

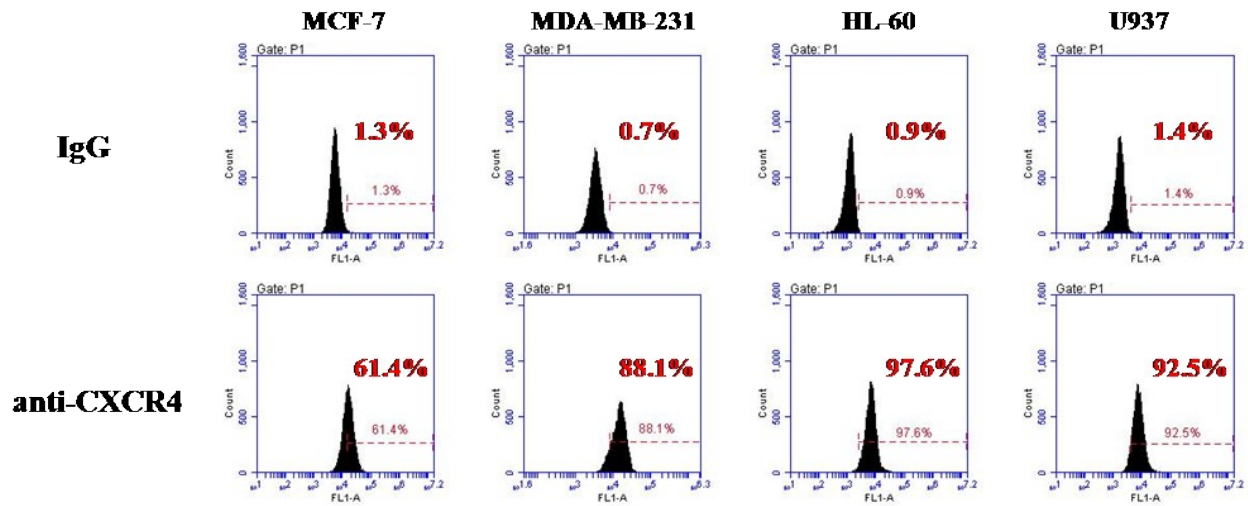


Fig. S2 Expression levels of CXCR4 in MCF-7, MDA-MB-231, HL-60 and U937 cells. Expression level of IgG was used as control. The typical results representative of three repeated experiments were shown.

3. Inhibition of CXCL12-induced migration of both breast cancer cells and acute myelocytic leukemia cells by W4

As shown in Fig. S3 and Fig. S4, with the increase of the mole ratio of W4 and CXCL12 to 5:1, the inhibitory effects detected on the four cell lines were all slightly decreased.

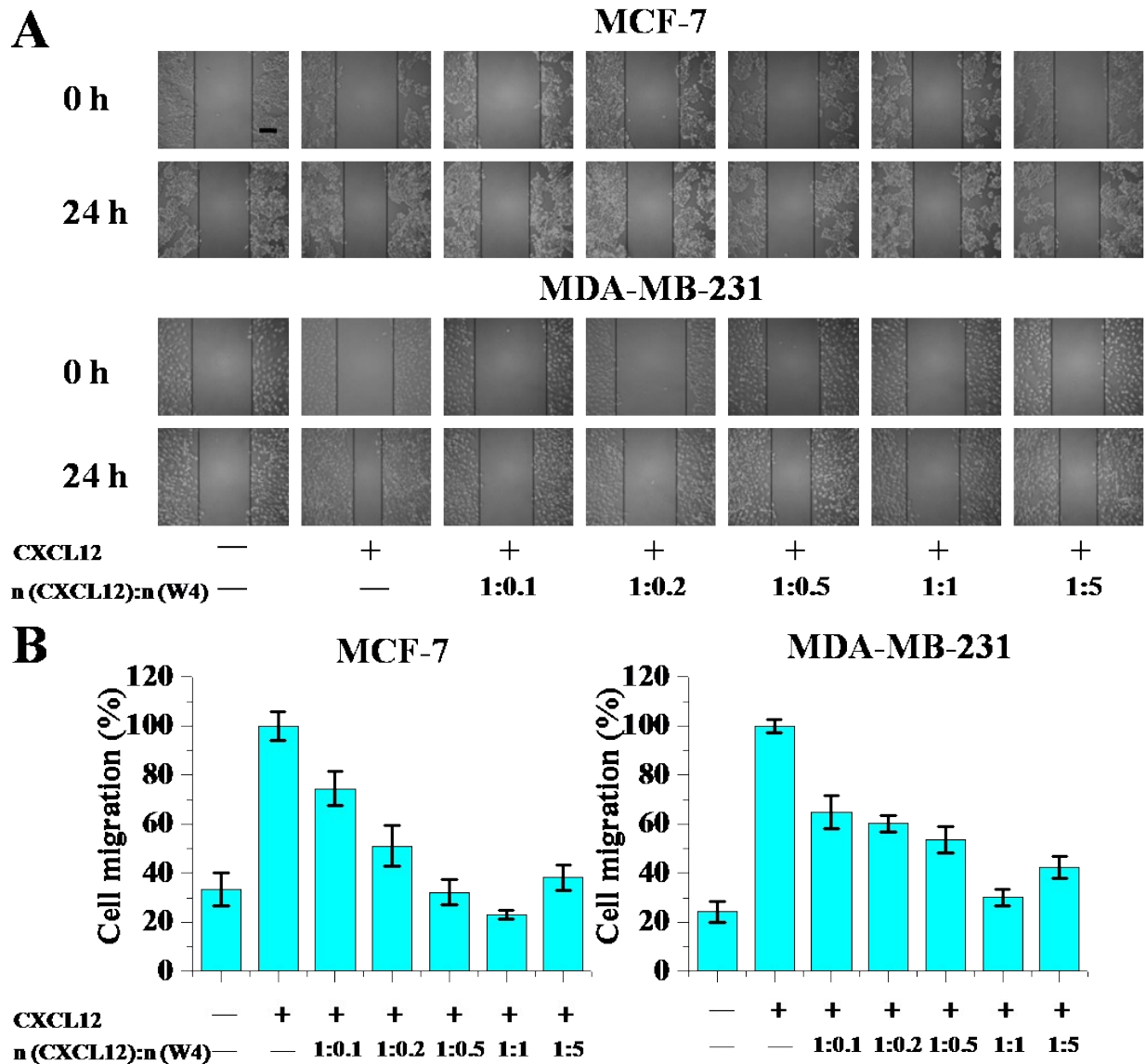


Fig. S3 Wound healing assay showed the inhibitory effect of W4 on cell migration. (A) Representative images showed the migration of two adhesive cancer cells before (0 h) and after (24 h) W4 treatment in the presence or absence of CXCL12. Scale bar represents 200 μ m. (B) The areas of migrated cells were counted at the three selected sites. The CXCL12 supplemented one without W4 was set as 100% as control. Error bars represent the standard deviation (n=3).

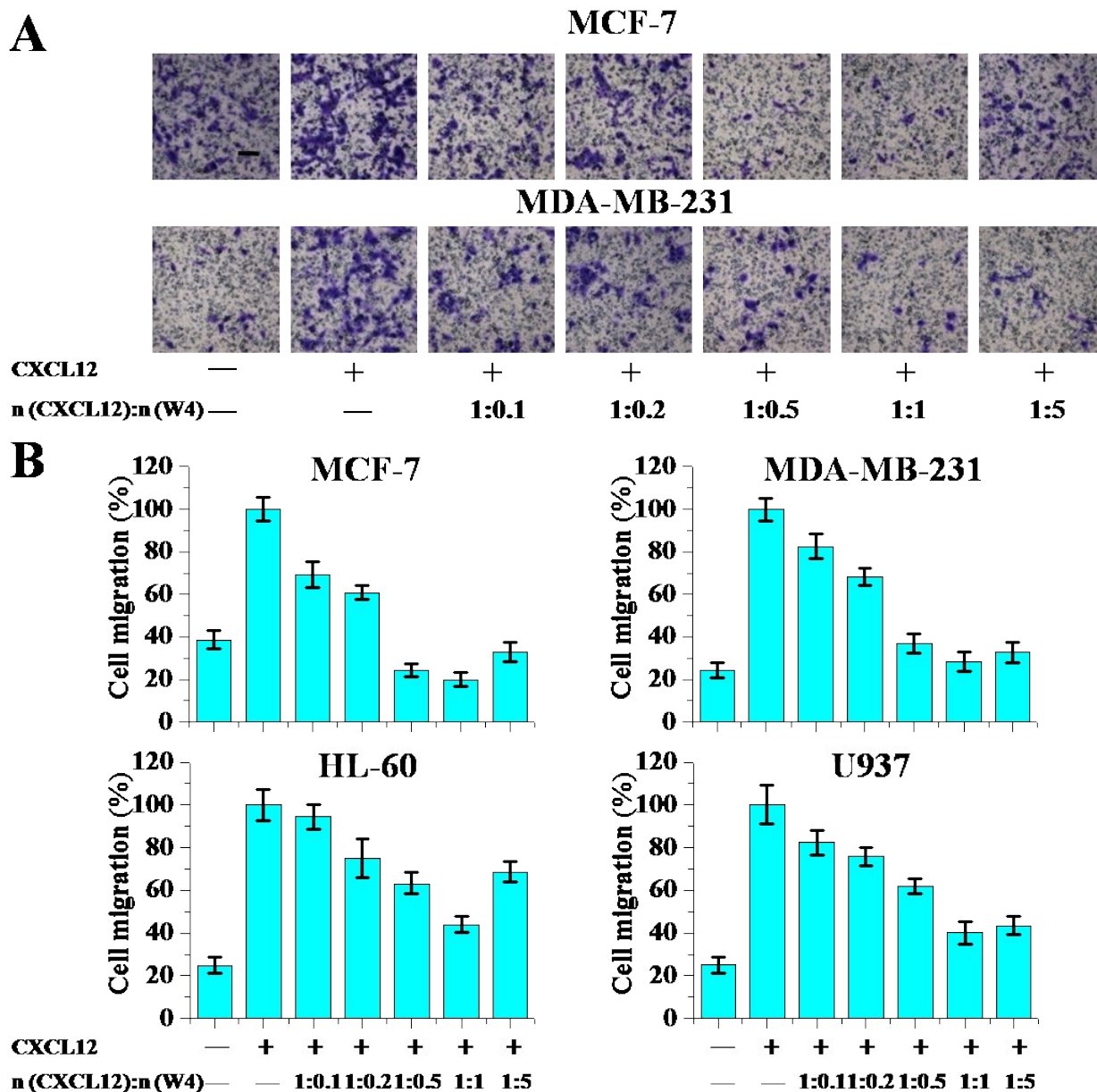


Fig. S4 Transwell assay showed the inhibitory effect of W4 on cell migration. (A) Representative images showed the migration of two adhesive cancer cells after W4 treatment for 24 h in the presence or absence of CXCL12. Scale bar represents 100 μ m. (B) The migrated cells were visualized and counted in five randomly selected fields using a microscope. The CXCL12 supplemented one without W4 was set as 100% as control. Error bars represent the standard deviation (n=3).

4. Analysis of the particle diameter of W4 by dynamic light scattering

The particle diameters of W4 of different concentrations in PBS were determined using dynamic light scattering (Zetasizer NanoZS, Malvern, UK). After 24 h, the solutions were tested again. As shown in Fig. S5, at 0 h, the dynamic size of W4 was associated with the concentration. After 24 h, the diameters of W4 increased compared with the very beginning and had larger growing trend at the higher concentration, which indicated that W4 had a mild tendency of aggregation at higher concentrations.

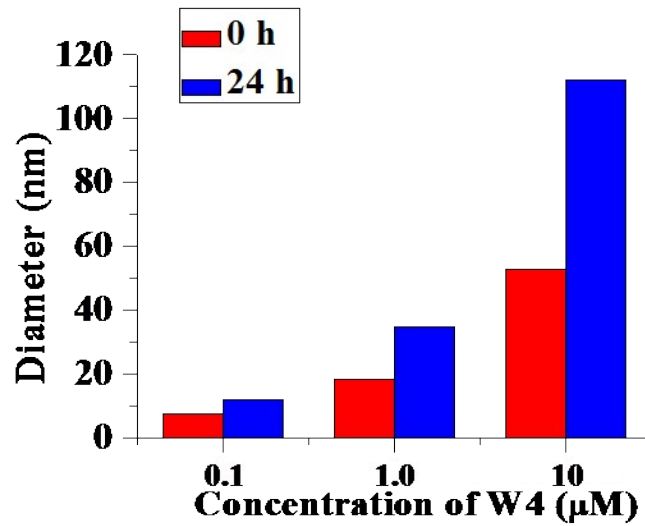


Fig. S5 The particle diameters of W4 at different concentrations at 0 h and 24 h.

5. Confocal microscopic images of labeled W4 bound MDA-MB-231 cells

As shown in Fig. S6, when cells were incubated in medium containing FITC-W4, the green fluorescence on the cell membrane was faint in both cells. After treatment with CXCL12, intensity on the cell surface was attenuated and even negligible for the green fluorescence, which proved that W4 didn't bind to the cell surface. In order to illustrate the amounts of W4 bound to cell surface, we performed FCM assay. Results showed that lower MFI of FITC was observed after interaction of CXCL12 and W4.

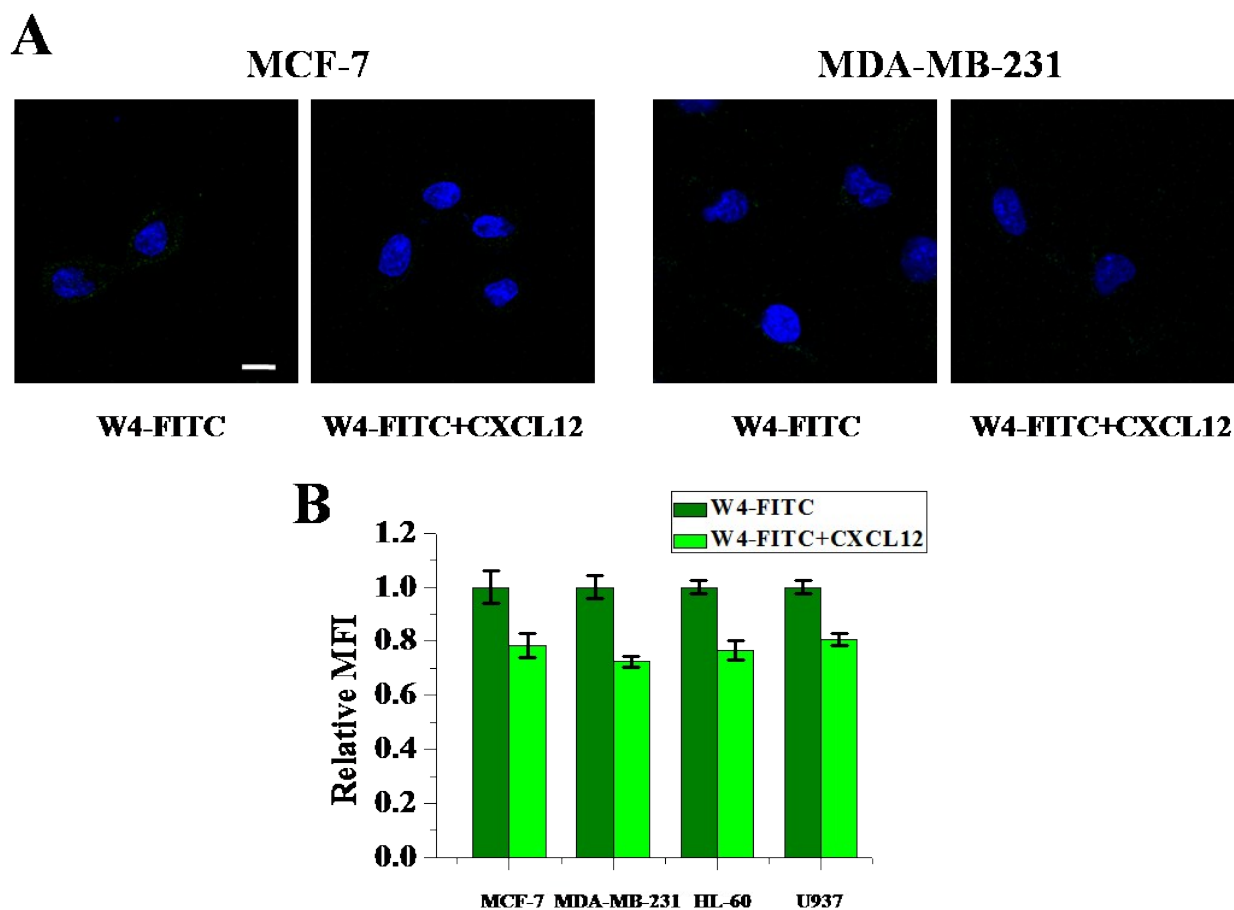


Fig. S6 The amounts of W4 bound to cells. (A) Confocal microscopic images of W4 (left line) and W4 with CXCL12 (right line) bound to two adhesive cancer cells were obtained using FITC labeled CXCL12, and the nucleus was revealed with Hoechst.33342 (blue fluorescence). Scale bar represents 10 μ m. (B) The amounts of W4 on cell surface after bound with CXCL12 were measured by FCM analysis. The W4 treated group was set as 1. Error bars represent the standard deviation (n=3). The mole ratio of W4 to CXCL12 was 1:1.

6. Cell viability of W4

A Cell Counting Kit-8 (Fanbo Biochemicals, China) assay was used to measure the cell viability of W4 on MCF-7, MDA-MB-231, HL-60 and U937 cells. Cells (1×10^5 cells/mL) were grown in 96-well plates for 24 h and treated with W4 over a range of concentrations (0 [control], 0.01, 0.1, 1, 10, 20, 30, 40, 50, 60 and 70 μ M). After 48 h, 10 μ L CCK-8 solution was added into each well, followed by incubation for 2 h. The absorbance at 450 nm was determined by a plate reader. Cell viability was expressed as a percentage of that of the control cells.

Results from the CCK-8 assay showed that W4 induced the cytotoxicity of cells in a concentration dependent manner and W4 didn't affect the viability of the four cell lines until the concentration reached 30 μ M (Fig. S7).

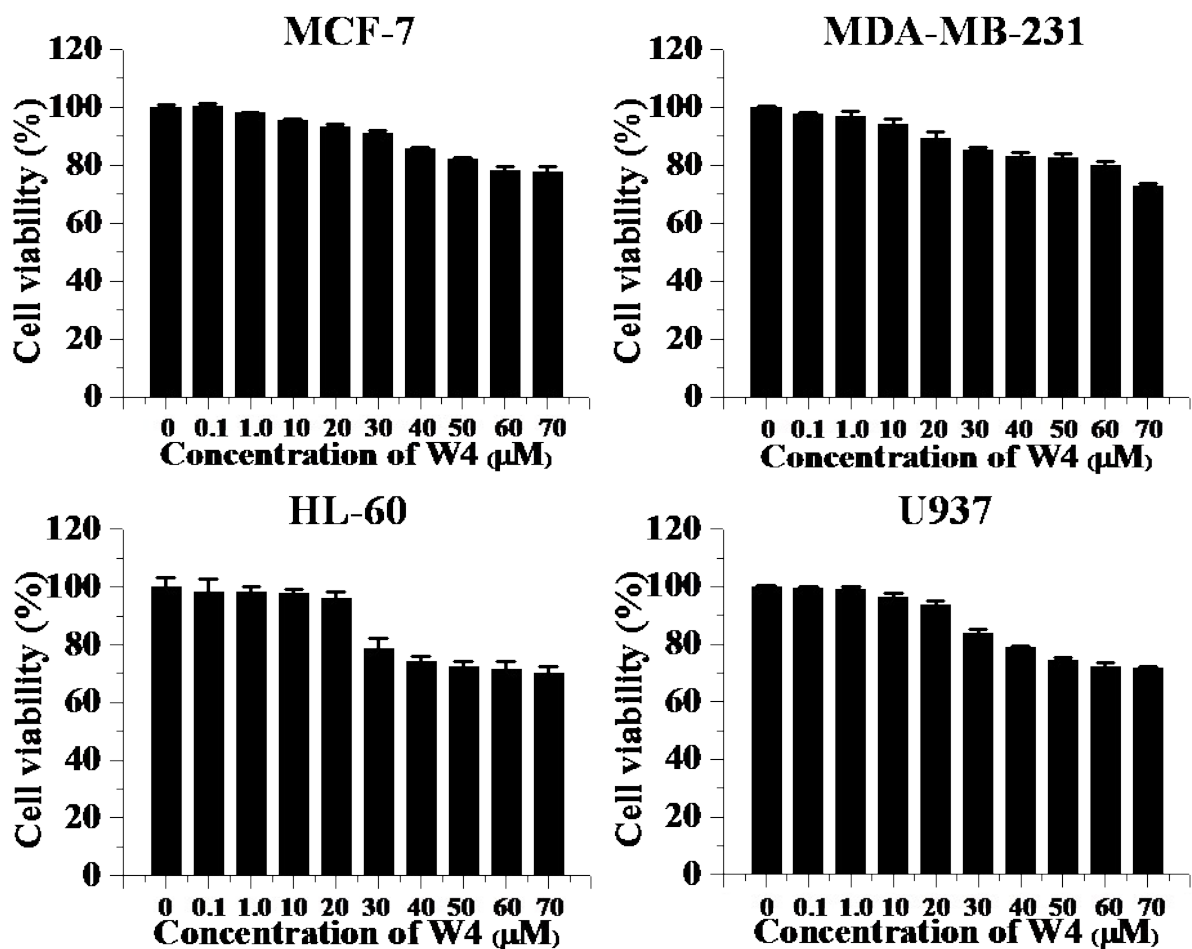


Fig. S7 Cell viability of MCF-7, MDA-MB-231, HL-60 and U937 cells at added W4 concentration. Error bars represent the standard deviation (n=3).