Supplementary file

Supplementary Materials and Methods

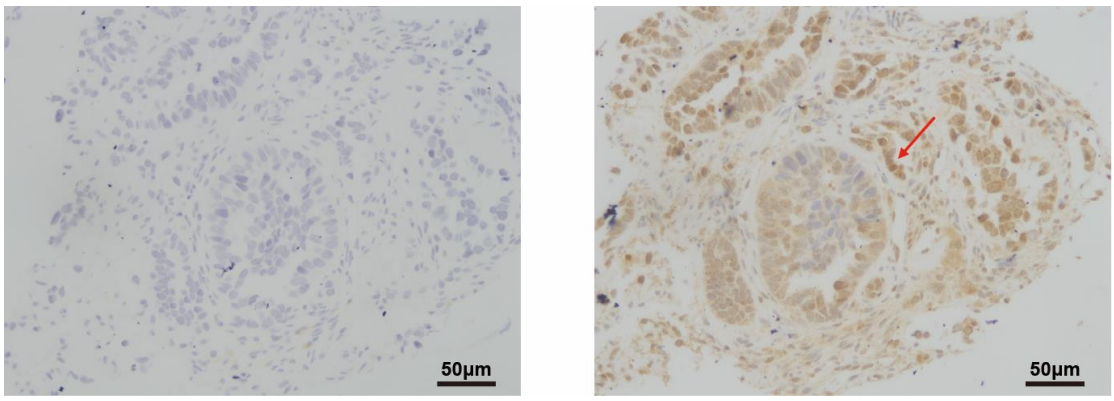
Immunohistochemistry

The paraffin sections of spinal metastatic breast cancer were stained to detect CX3CR1 using rabbit anti-CX3CR1 antibody (Abcam, Cambridge, UK). After heat-mediated antigen retrieval, the sections were incubated with primary antibody at a 1:100 dilution overnight at 4°C. The sections were incubated with peroxidase-labeled anti-rabbit antibody for 60 mins at 37°C and then developed with diaminobenzidine. All sections were counterstained with hematoxylin.

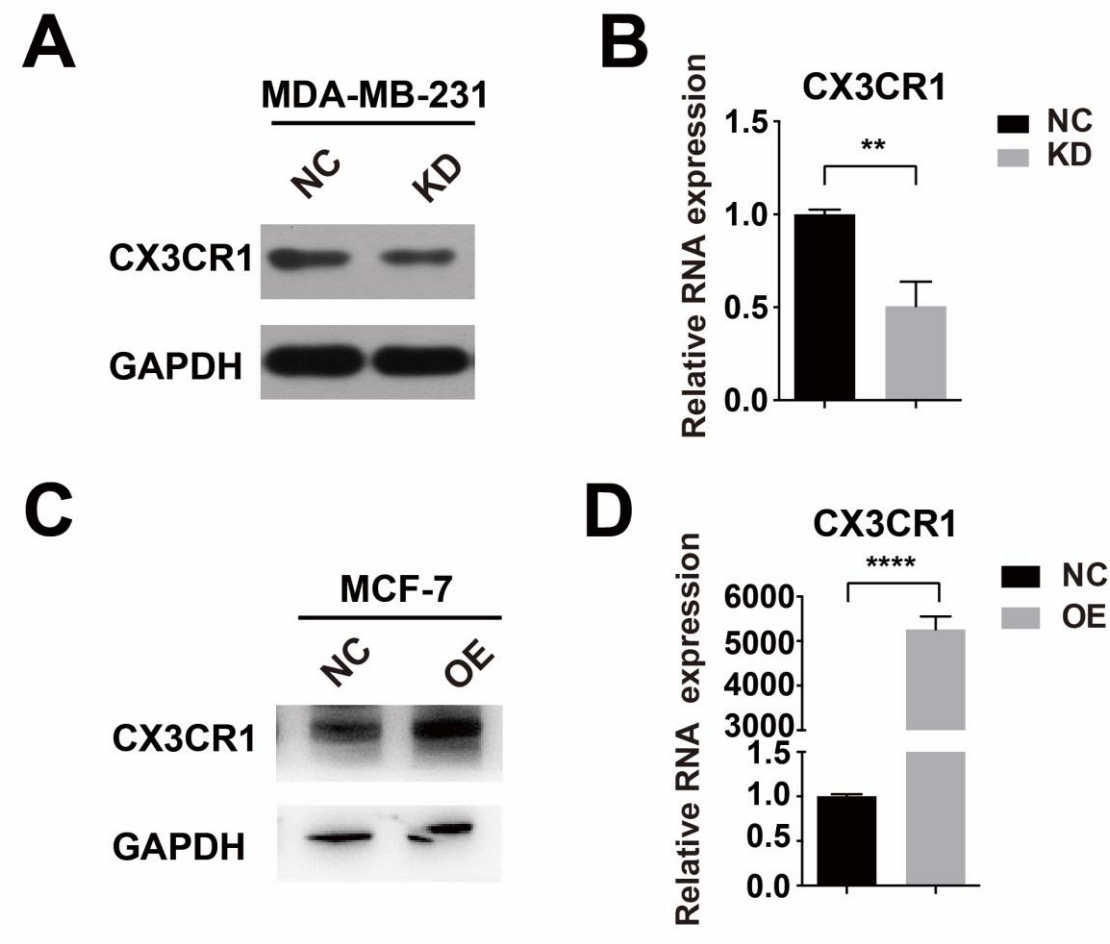
Lentivirus transfection

MDA-MB-231 and MCF-7 cells were grown on a 6-well plate at 2×10^5 cell density per well. Next day, 20 µl of control (NC), CX3CR1-overexpressing and CX3CR1-knockdown lentivirus (Genechem Co. Ltd. Shanghai, China) with a titer of 1×10^8 TU/ml was added to the corresponding well. When grown to 90% confluence, the cells were sub-cultured and screen with 2.5 µg/ml of puromycin for successfully transfected cells. Western blot and PCR were used to verify the transfection was successful or not.

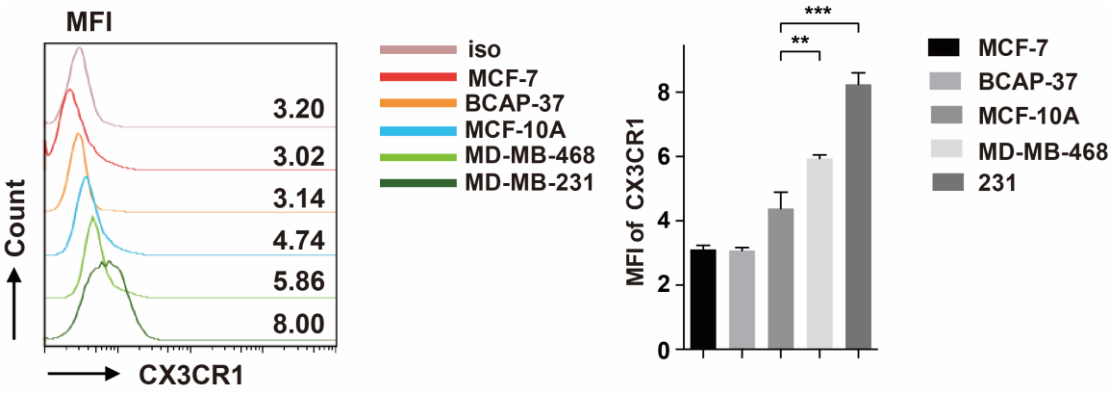
Supplementary figure



Supplementary figure 1 The expression of CX3CR1 in the metastatic tumor tissue (Right). Negative control (Left). Original magnification × 200 (scale bar = 50 µm).



Supplementary Figure 2 The expression of MDA-MB-231 CX3CR1-knockdown (KD) cells and MCF-7 CX3CR1-overexpressing (OE) cells. (A-B) Protein and RNA levels of MDA-MB-231 (KD) cells. (C-D) Protein and RNA levels of MCF-7 (OE) cells. NC: Negative control, KD: knockdown, OE: Overexpressing **P< 0.001, ****P< 0.0001.



Supplementary Figure 3 The expression of CX3CR1 in cell lines. FACS analysis of

CX3CR1 level in breast cancer cell lines. **P< 0.01, ***P< 0.001.