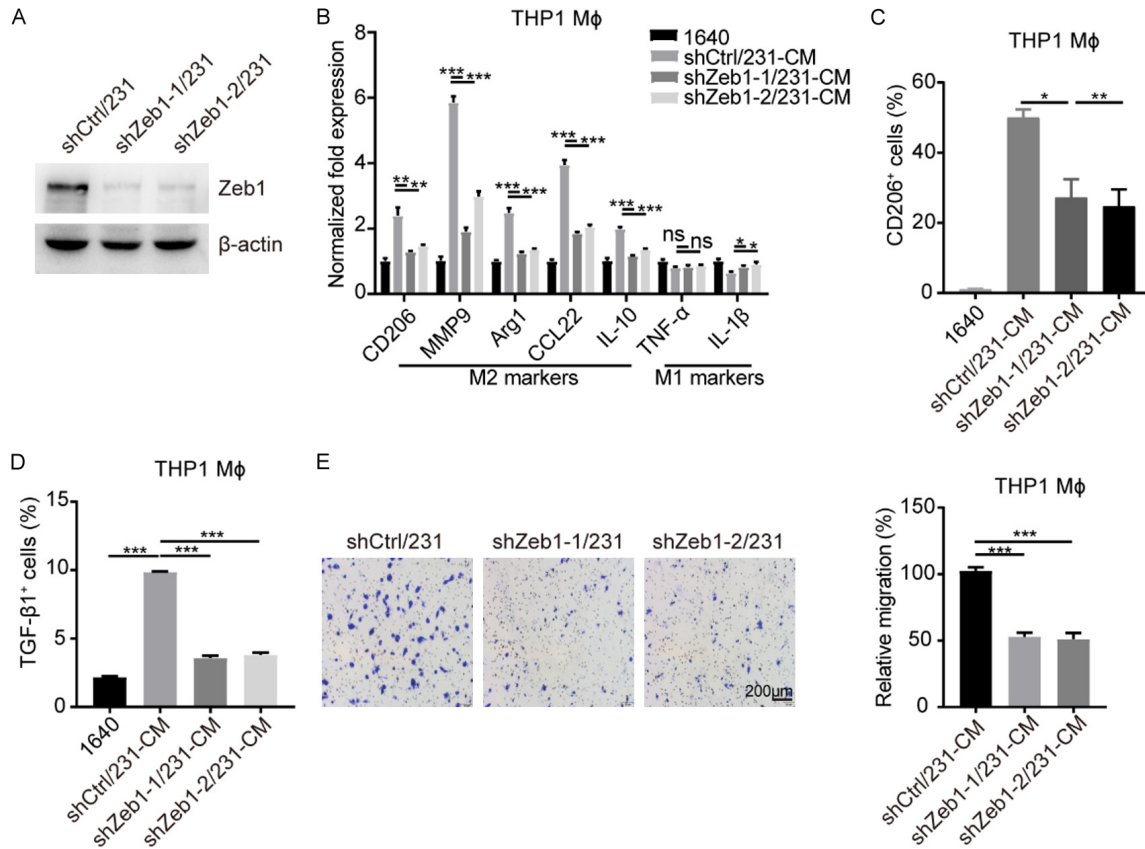


## Involvement of Zeb1-Cxcl1 axis in breast cancer immune escape

**Table S1.** Primer sequences and shRNA used in this study

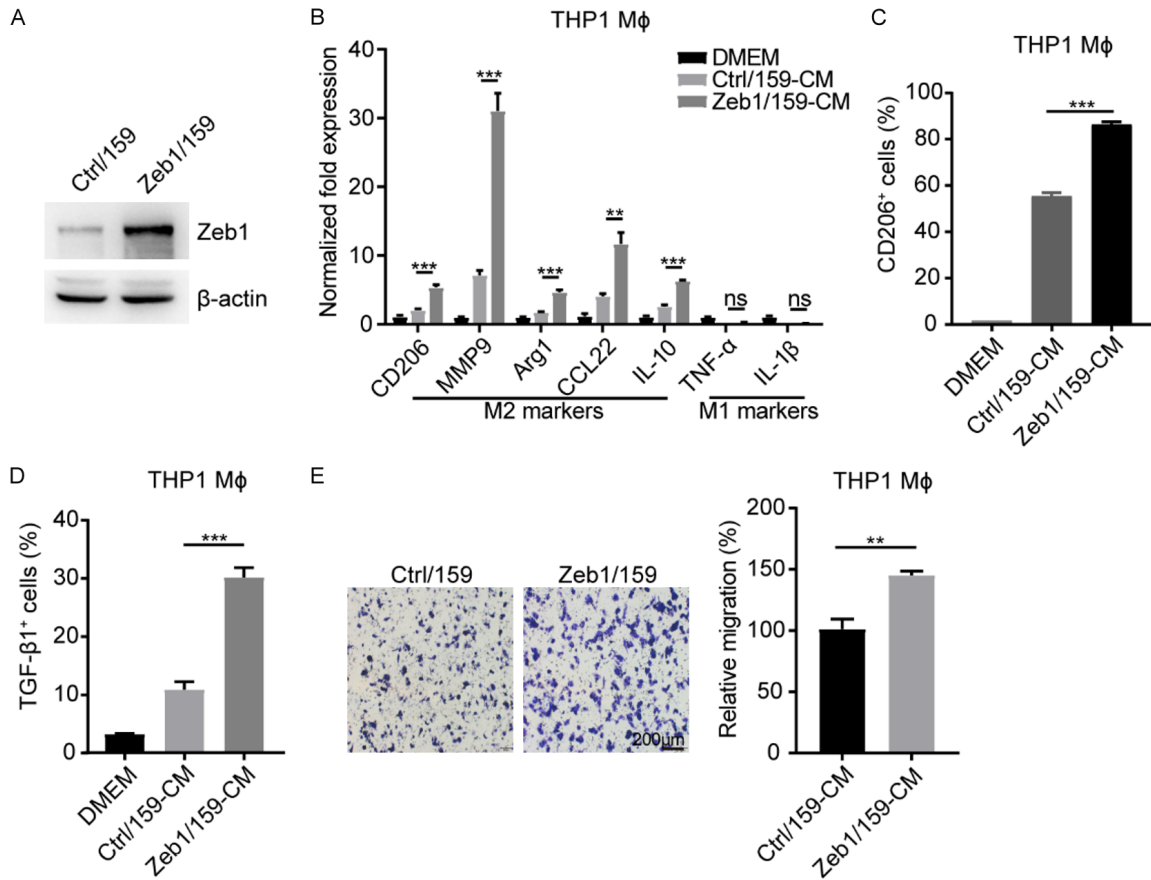
Gene	Primer (5'-3')	
mCD206	Forward	ACGCAGTGGTTGGCAGTGGG
	Reverse	TTGCCAGGTCCCCACCCTCC
mArg1	Forward	CAGTCTGGCAGTTGGAAGC
	Reverse	TTGGCAGATATGCAGGGAG
mCCL22	Forward	CAGGCAGGTCTGGGTGAA
	Reverse	TAAAGGTGGCGTCGTTGG
mIL10	Forward	CAACATACTGCTAACCGACTC
	Reverse	CATGGCCTGTAGACACCT
mTNF $\alpha$	Forward	CCTCTTCTCATTCTGCTTG
	Reverse	CACTTGGTGGTTTGCTACG
mIL1 $\beta$	Forward	ATCCAGCTTCAATCTCGC
	Reverse	ATCTCGGAGCCTGTAGTGC
hCD206	Forward	GGGTTGCTATCACTCTCTATGC
	Reverse	TTTCTTGCTGTTGCCGTAGTT
hMMP9	Forward	ATGCGTGGAGAGTCGAAATC
	Reverse	TACACGCGAGTGAAGGTGAG
hIL10	Forward	GGTTGCCAAGCCTTGCTGA
	Reverse	GGGAGTTCACATGCGCCT
hArg1	Forward	ACCATAGGGATTATTGGAGC
	Reverse	TGTCATTAGGGATGTCAGCA
hCCL22	Forward	AGCCAATGAAGAGCCTAC
	Reverse	GCAGAGGATGGGTTAGAG
hTNF $\alpha$	Forward	CGAGTGACAAGCCTGTAGCC
	Reverse	TGAAGAGGACCTGGGAGTAGAT
hIL1 $\beta$	Forward	GCTTATTACAGTGGCAATGAGGAT
	Reverse	CCTCGTTATCCCATGTGTCG
mCxcl1	Forward	GCACCCAAACCGAAGTCA
	Reverse	AAGCCAGCGTTCACCAGA
hCxcl1	Forward	CCAAACCGAAGTCATAGCC
	Reverse	TTCTCTCCCTTCTGGTC
mIFN $\gamma$	Forward	AGCAACAACATAAGCGTCAT
	Reverse	CCTCAAACCTTGGCAATACTC
GAPDH	Forward	GGAGCGAGATCCCTCCAAAAT
	Reverse	GGCTGTTGTCATACTTCTCATGG
hCxcl1-E <sub>2</sub> box1	Forward	GGGGTAGAAACGGAGAGGCT
	Reverse	GCCCAGCTCAATAGGTAAGA
hCxcl1-E <sub>2</sub> box2	Forward	CCTTCTCCGTTCCAGCCCC
	Reverse	CGCCTTCTGCCCCAGATCCC
shZeb1-1	CGGCGCAATAACGTTACAAAT	
shZeb1-2	GGCGCAATAACGTTACAAA	

## Involvement of Zeb1-Cxcl1 axis in breast cancer immune escape



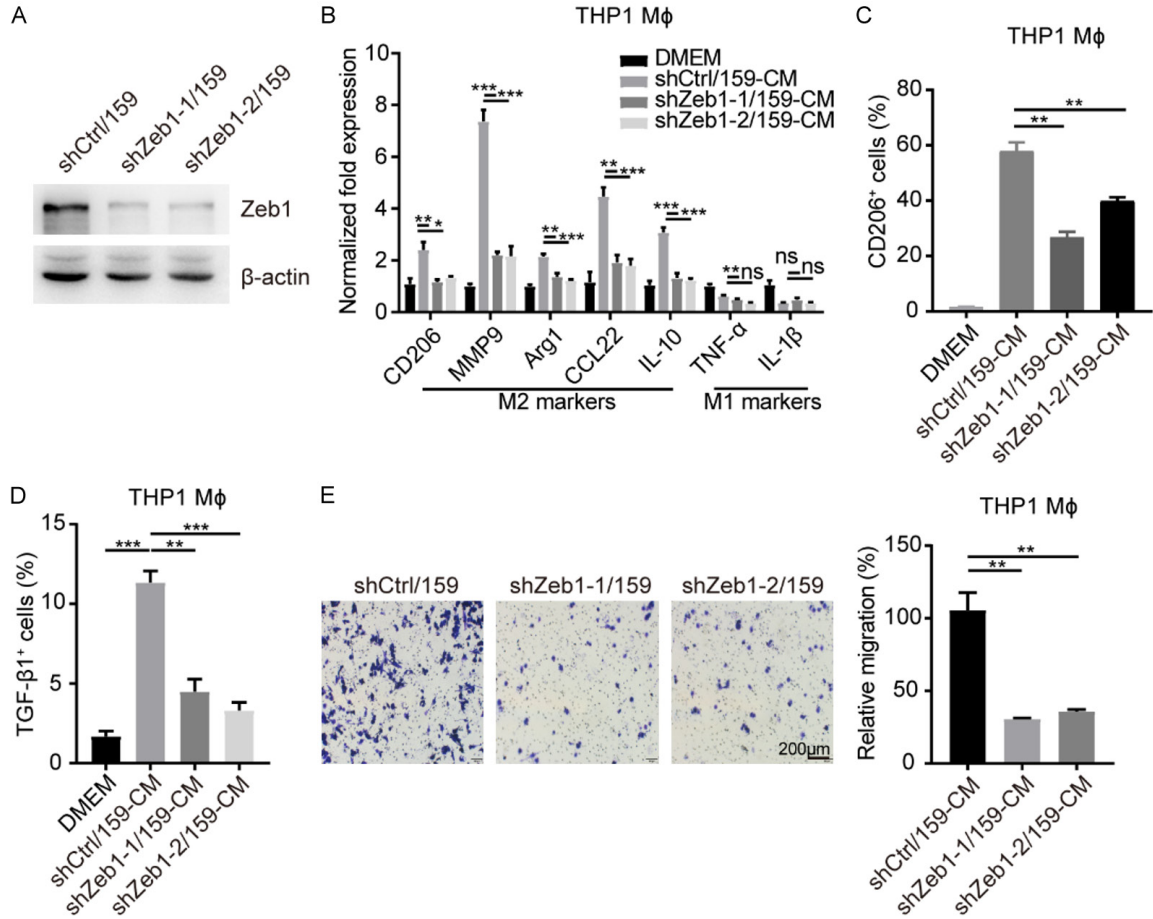
**Figure S1.** (A) Western blotting of Zeb1 expression in Zeb1-interfered MDA-MB-231 cells. (B) Relative mRNA levels of M1- and M2-TAM markers in THP1 macrophages treated with CM from Zeb1-interfered MDA-MB-231 cells. (C, D) Flow cytometry analysis of CD206<sup>+</sup> (C) and TGF-β1<sup>+</sup> (D) cells in THP1 macrophages treated with CM from Zeb1-interfered MDA-MB-231 cells. (E) Transwell migration assay in THP1 macrophages treated with CM from Zeb1-interfered MDA-MB-231 cells. Indicated *P*-values were calculated using two-tailed unpaired Student's *t*-test. Data are presented as mean ± SEM in (A-E). Data are representative of three (A-E) independent experiments. Source data are provided as a Source Data file.

## Involvement of Zeb1-Cxcl1 axis in breast cancer immune escape

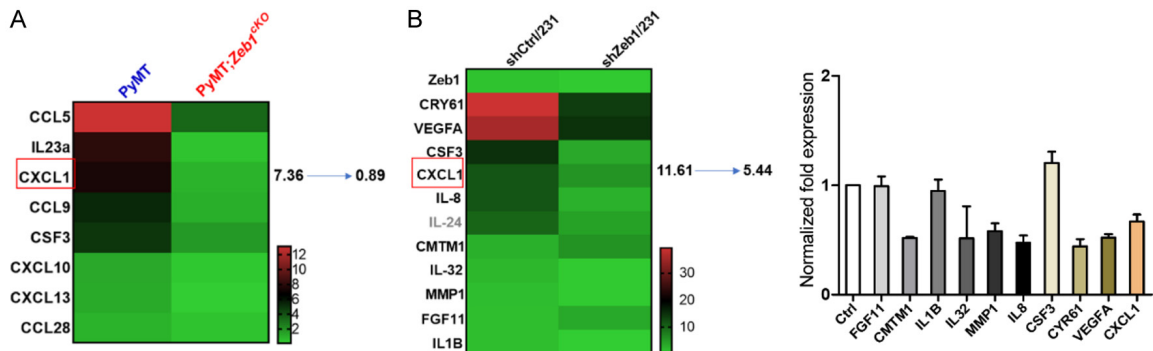


**Figure S2.** (A) Western blotting of Zeb1 expression in Zeb1-expressing SUM-159 cells. (B) Relative mRNA levels of M1- and M2-TAM markers in THP1 macrophages treated with CM from Zeb1-expressing SUM-159 cells. (C, D) Flow cytometry analysis of (C) CD206<sup>+</sup> and (D) TGF- $\beta$ 1<sup>+</sup> cells in THP1 macrophages treated with CM from Zeb1-expressing SUM-159 cells. (E) Transwell migration assay in THP1 macrophages treated with CM from Zeb1-expressing SUM-159 cells. Indicated *P*-values were calculated using two-tailed unpaired Student's *t*-test. Data are presented as mean  $\pm$  SEM in (A-E). Data are representative of three (A-E) independent experiments. Source data are provided as a Source Data file.

## Involvement of Zeb1-Cxcl1 axis in breast cancer immune escape

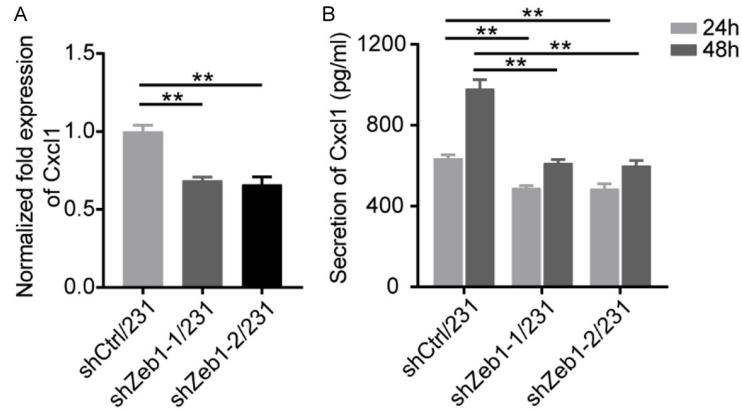


**Figure S3.** (A) Western blotting of Zeb1 expression in Zeb1-interfered SUM-159 cells. (B) Relative mRNA levels of M1- and M2-TAM markers in THP1 macrophages treated with CM from Zeb1-interfered SUM-159 cells. (C, D) Flow cytometry analysis of (C) CD206<sup>+</sup> and (D) TGF-β1<sup>+</sup> cells in THP1 macrophages treated with CM from Zeb1-interfered SUM-159 cells. (E) Transwell migration assay in THP1 macrophages treated with CM from Zeb1-interfered SUM-159 cells. Indicated *P*-values were calculated using two-tailed unpaired Student's *t*-test. Data are presented as mean ± SEM in (A-E). Data are representative of three (A-E) independent experiments. Source data are provided as a Source Data file.

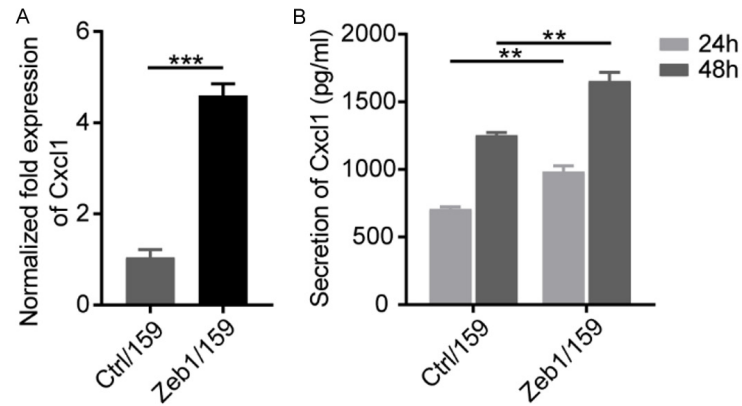


**Figure S4.** (A, B) RNA sequencing analysis of paracrine factors expressed in the indicated breast cancer tissues (A) and Zeb1-interfered MDA-MB-231 cells (B). Source data are provided as a Source Data file.

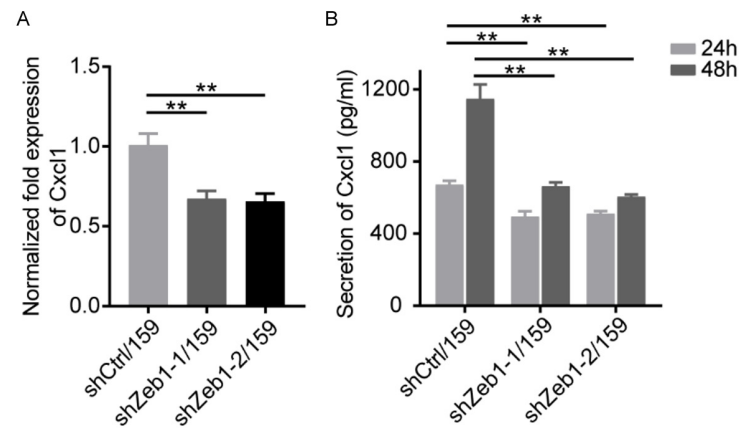
## Involvement of Zeb1-Cxcl1 axis in breast cancer immune escape



**Figure S5.** (A) Relative mRNA levels of Cxcl1 in Zeb1-interfered MDA-MB-231 cells. (B) ELISA analysis of Cxcl1 concentration in CM from Zeb1-interfered MDA-MB-231 cells. Indicated *P*-values were calculated using two-tailed unpaired Student's *t*-test. Data are presented as mean  $\pm$  SEM in (A, B). Data are representative of three (A, B) independent experiments. Source data are provided as a Source Data file.

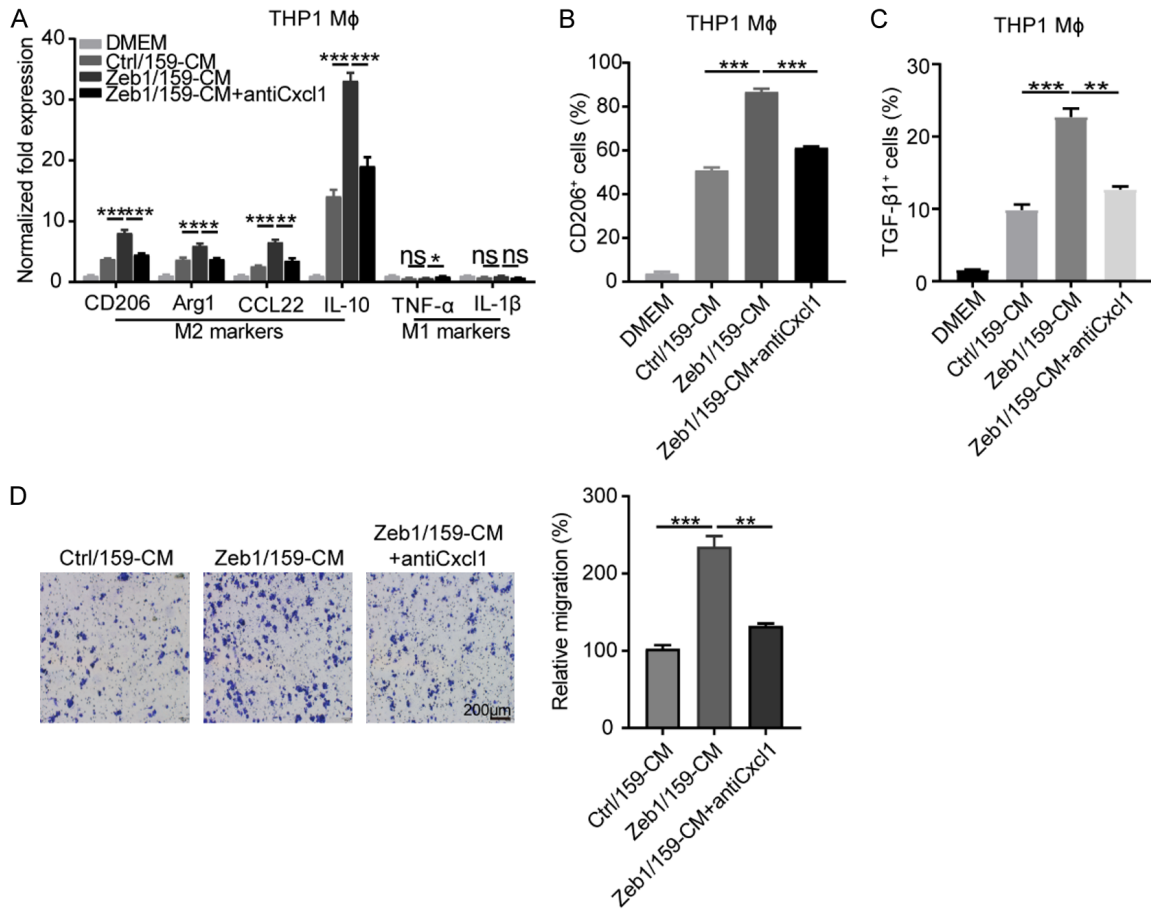


**Figure S6.** (A) Relative mRNA levels of Cxcl1 in Zeb1-expressing SUM-159 cells. (B) ELISA analysis of Cxcl1 concentration in CM from Zeb1-expressing SUM-159 cells. Indicated *P*-values were calculated using two-tailed unpaired Student's *t*-test. Data are presented as mean  $\pm$  SEM in (A, B). Data are representative of three (A, B) independent experiments. Source data are provided as a Source Data file.



**Figure S7.** (A) Relative mRNA levels of Cxcl1 in Zeb1-interfered SUM-159 cells. (B) ELISA analysis of Cxcl1 concentration in CM from Zeb1-interfered SUM-159 cells. Indicated *P*-values were calculated using two-tailed unpaired Student's *t*-test. Data are presented as mean  $\pm$  SEM in (A, B). Data are representative of three (A, B) independent experiments. Source data are provided as a Source Data file.

## Involvement of Zeb1-Cxcl1 axis in breast cancer immune escape



**Figure S8.** (A) Relative mRNA levels of M1- and M2-TAM markers in THP1 macrophages treated with CM from Zeb1-expressing SUM-159 cells in the presence of a Cxcl1 neutralizing antibody. (B, C) Flow cytometry analysis of CD206<sup>+</sup> (B) and TGF- $\beta$ 1<sup>+</sup> (C) cells in THP1 macrophages treated with CM from Zeb1-expressing SUM-159 cells in the presence of a Cxcl1 neutralizing antibody. (D) Transwell migration assay in THP1 macrophages treated with CM from Zeb1-expressing SUM-159 cells in the presence of a Cxcl1 neutralizing antibody. Indicated *P*-values were calculated using two-tailed unpaired Student's *t*-test. Data are presented as mean  $\pm$  SEM in (A-D). Data are representative of three (A-D) independent experiments. Source data are provided as a Source Data file.