

## **Supplementary figures**

### **Supplementary Figure 1 Bcl-3 knockdown strategies**

(a) Bcl-3 knockdown strategy, an inducible lentivirus pTRIPZ plasmid invented by OpenBiosystems was used.

(b) Two shRNA sequences for human Bcl-3 knockdown. White indicates the enzyme, red and blue indicate the knockdown sequence and its reverse complementary sequence. Green indicates the loop.

(c) The effect of Bcl-3 knockdown with two shRNA sequences in different breast cancer cells.

### **Supplementary Figure 2 Tumor volume of WT and Bcl-3 knockout MMTV-**

#### **PyMT mice**

(a) Tumor volume of Bcl-3-sufficient or Bcl-3-deficient MMTV-PyMT mice. The tumor burden had no changes between the groups after 3.5 months.

(b) WT bone marrow cells were transferred into lethally irradiated Bcl-3-sufficient or Bcl-3-deficient MMTV-PyMT mice. Tumor volumes were measured.

### **Supplementary Figure 3 Bcl-3 regulates TGF $\beta$ signaling by stabilizing Smad3**

#### **protein**

(a) qRT-PCR analysis of metastasis-related genes in Bcl-3 knockdown MDA-MB-231 cells after TGF $\beta$  stimulation.

(b) qRT-PCR analysis of TGF $\beta$ -1 expression in MDA-MB-231 and LM2 cells.

(c, d) Immunoblots for Smad4 in MDA-MB-231 (c) and MCF-7 (d) cells under TGF $\beta$  stimulation.

(e, f) Immunoblots for Smad2, phospho-Smad2, Smad3 and phos-Smad3 of LM2 (e) and MCF-7 (f) cells under TGF $\beta$  stimulation.

(g) Immunoblots for Smad3 and Smad2 of 4T1 cells after RNAi Bcl-3 under TGF $\beta$  stimulation.

(h) Luciferase activity of MCF-7 cells transfected with Smad3 luciferase-reporter plasmids and Renilla luciferase plasmids after TGF $\beta$  stimulation.

(i) qRT-PCR analysis of BTRC ( $\beta$ -TrCP) in BTRC knockdown MDA-MB-231 cells.

(j) Immunoblots for Smad3 in MDA-MB-231 cells after MG132 and TGF $\beta$  stimulation to detect the ubiquitinated Smad3.

#### **Supplementary Figure 4 Bcl-3 regulation of TGF $\beta$ signaling is not correlated with the phosphorylation of Smad3**

(a and b) Immunoblots for phospho-Smad3 with different phospho-antibodies in LM2 cell (a) and MDA-MB-231 cell (b) under TGF $\beta$  stimulation or untreated control cells.

(c) Immunoblots for Bcl-3 and Flag tagged Smad3 mutants in MDA-MB-231 cells after TGF $\beta$  stimulation.

(d) Fluorescence microscopy analysis of F-actin expression in MDA-MB-231 cells.

(e) Morph-Change of 4T1 cell after Bcl-3 was knocked down.

#### **Supplementary Figure 5 Cellular localization of Bcl-3 and Smad3 in TGF $\beta$ treated or untreated MDA-MB-231 cells**

Fluorescence microscopy analysis of Bcl-3 and Smad3 expression in MDA-MB-231 cells treated with TGF $\beta$  or untreated control cells. Blue, nucleus stain.

#### **Supplementary Figure 6 Loss of Bcl-3 inhibits the metastasis of breast cancer**

**cells**

(a) The knockdown effect of Bcl-3 in MDA-MB-231 and LM2 cells detected by Western Blot.

(b) Wound-healing assay in Bcl-3 knockdown LM2 cells.

(c,d) Cell migration and Matrigel-Transwell invasion analysis of MDA-MB-231 cells

(c) and LM2 cells (d), scale bar = 50 $\mu$ m. \* represents  $p < 0.05$  and \*\* represents  $p < 0.01$  as determined by Student's *t*-test.

**Q-RT-PCR primers (5'-3')**

TGF- $\beta$ -1-F	GGCCAGATCCTGTCCAAGC
TGF- $\beta$ -1-R	GTGGGTTTCCACCATTAGCAC
GAPDH-F	TGCACCACCAACTGCTTAGC
GAPDH-R	GGCATGGACTGTGGTCATGAG
ANGPTL4-F	TCTCCGTACCCTTCTCCACT
ANGPTL4-R	AGTACTGGCCGTTGAGGTTG
CTGF-F	TTGCGAAGCTGACCTGGAAGAGAA
CTGF-R	AGCTCGGTATGTCTTCATGCTGGT
PAI-1-F	TCTTTGGTGAAGGGTCTGCT
PAI-1-R	CTGGGTTTCTCCTCCTGTTG
IL-11-F	ACTGCTGCTGCTGAAGACTC
IL-11-R	CCACCCCTGCTCCTGAAATA
PTHrP-F	ACCTCGGAGGTGTCCCCTAAC
PTHrP-R	TCAGACCCAAATCGGACG
ID1-F	GGCTGTTACTCACGCCTCAAG
ID1-R	CCAAGTGAAGGTCCTGATGTAG
COX2-F	ACAACA TTC CTT CCT TC
COX2-R	CCTTATTTCTTTTCACACC
ID3-F	TGAGCTTGCTGGACGACATG
ID3-R	GATGACGCGCTGTAGGATTTT
MMP1-F	GAGCAAACACATCTGACCTACAGGA
MMP1-R	TTGTCCCGATGATCTCCCCTGACA
BTRC-F	ACCAACATGGGCACATAAACTC
BTRC-R	TGGCATCCAGGTATGACAGAAT

shBcl-3-1 (5'-3'):

GAGCTCGAACCAACCTAAAGAAAACATTATCACTTCGGTGTCTACATAATGTTTTCTTT  
AGGTTGGTTTCTTAAG

shBcl-3-2 (5'-3'):

GAGCTCTGGCCCTCGAGCTGTAGATGTTATCACTTCGGTGTCTACATAACATCTACAGC  
TCGAGGGCCG CTTAAG

shBcl-3-3(5'-3'):

GATCCGTCGACGCAGTGGACATTAATTCAAGAGATTAATGTCCACTGCGTCGATTTTTT  
G

RBX1 siRNA: UCCAUA AUGUGGUUCCUGC

BTRC siRNA: AAGUGGAAUUUGUGGAACAUC