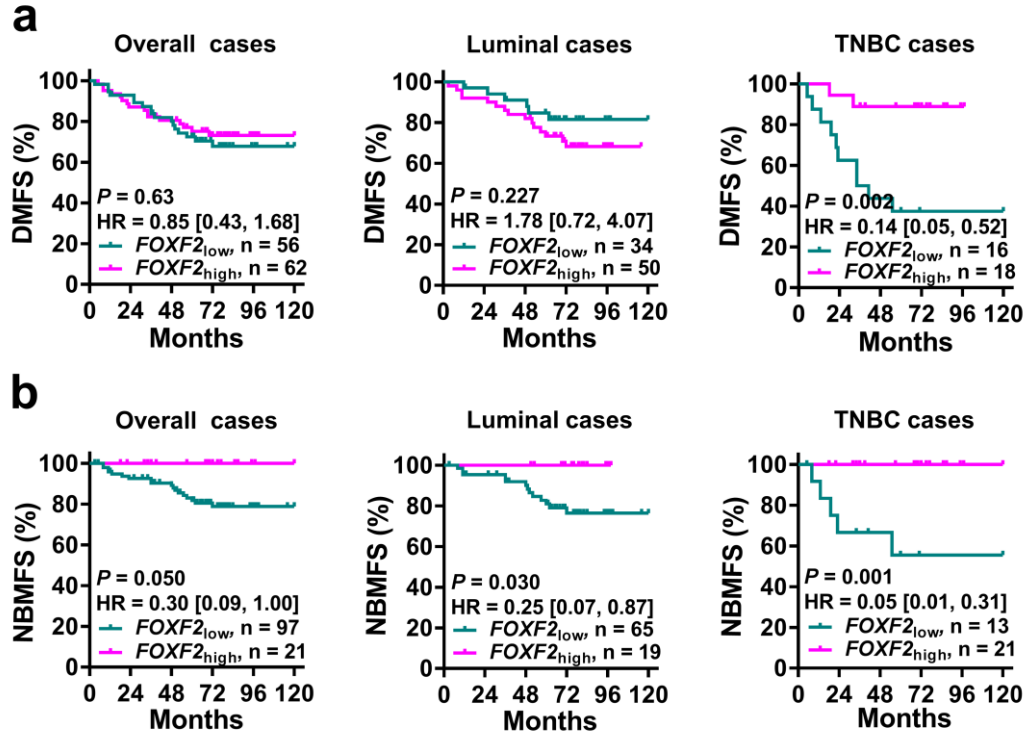


Supplementary Information

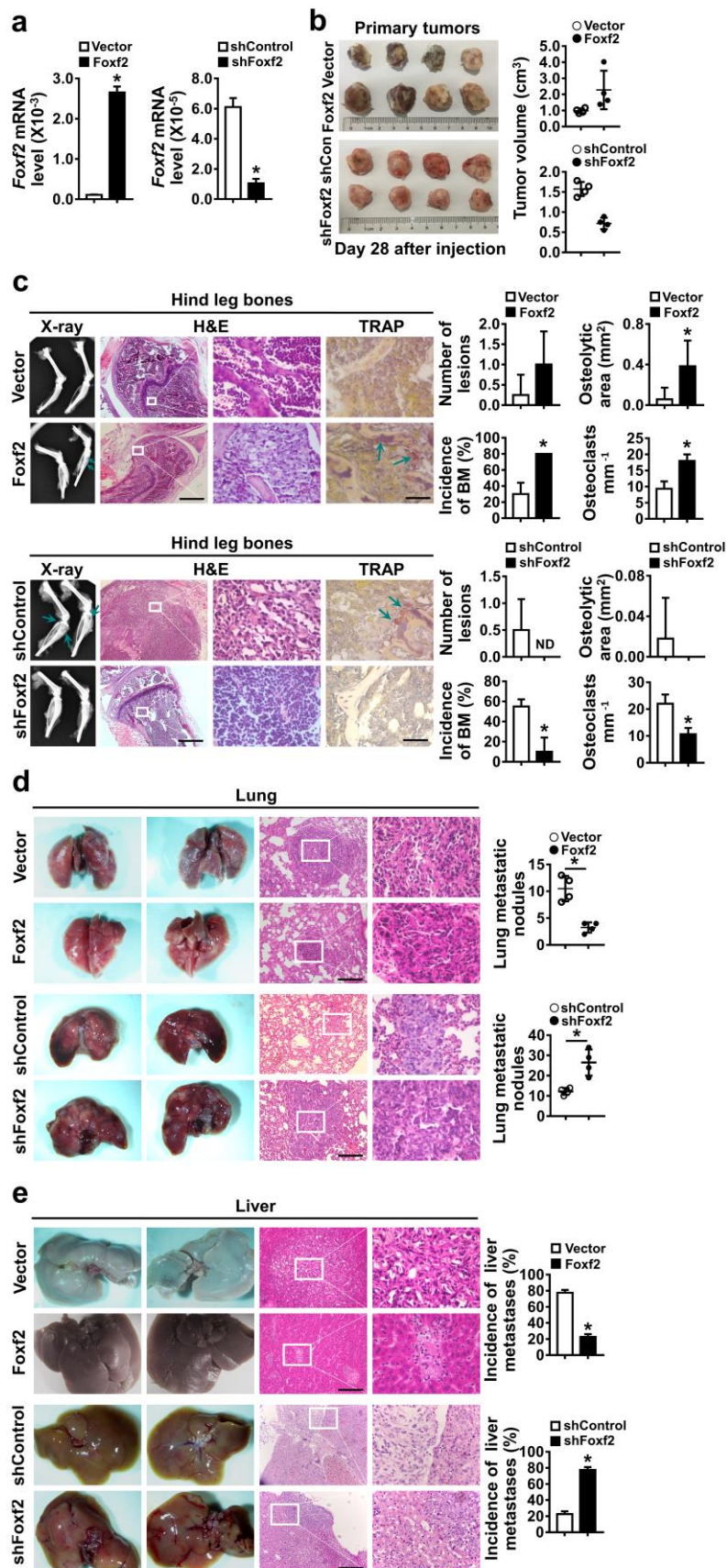
FOXF2 reprograms breast cancer cells into bone metastasis seeds

Wang et al.

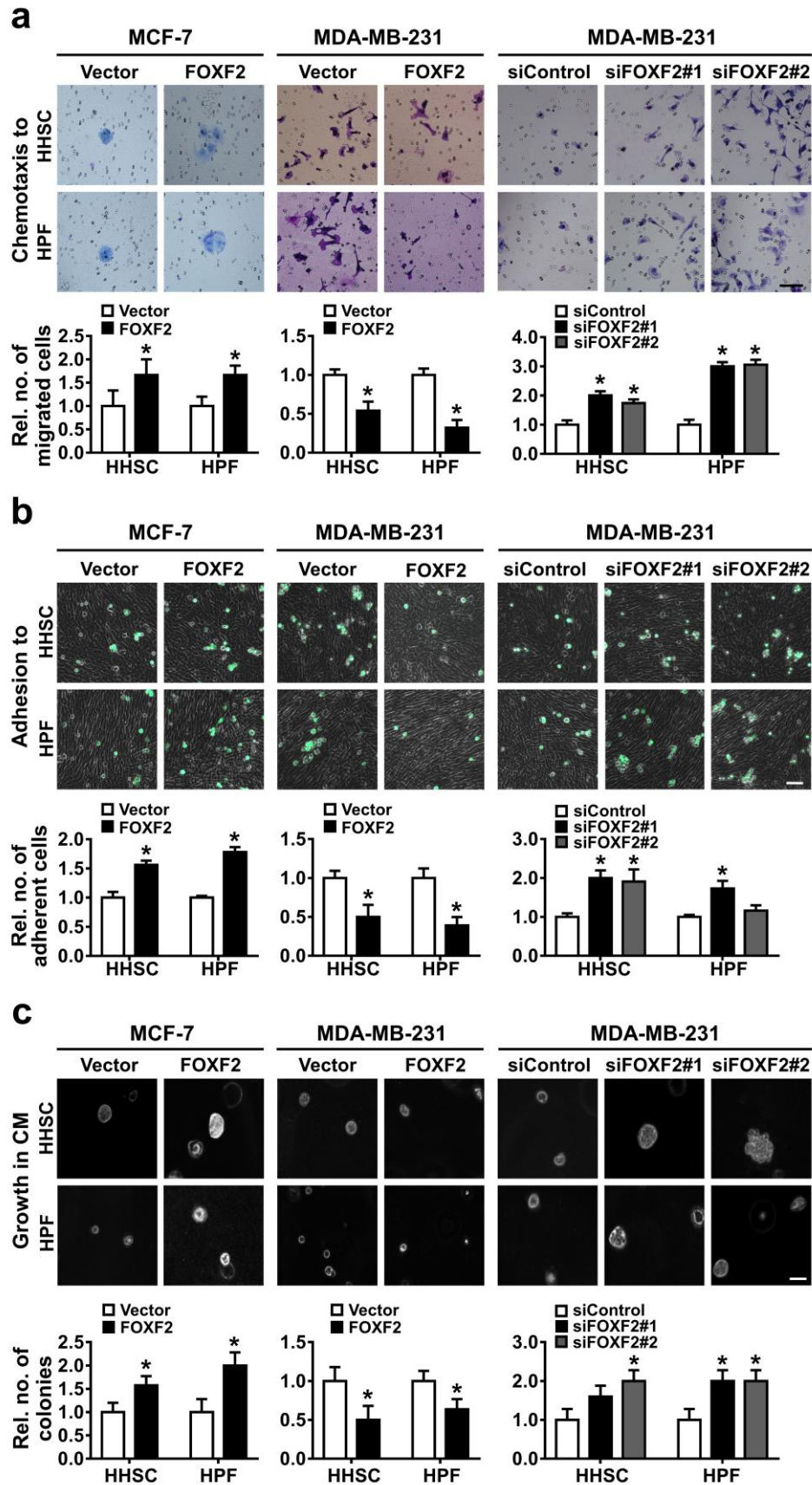
Supplementary Figures



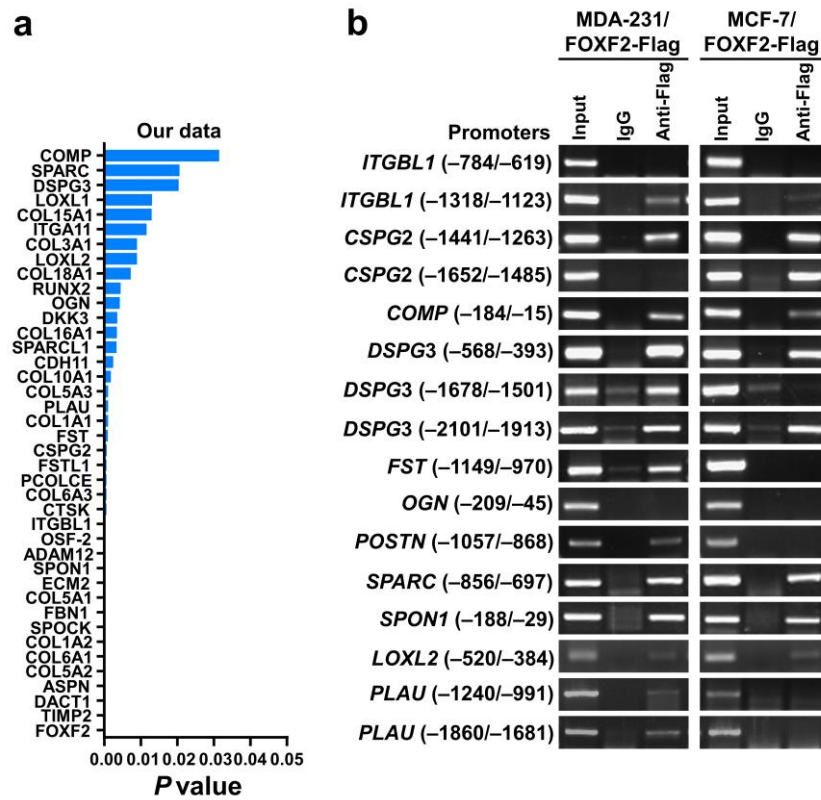
Supplementary Figure 1 | *FOXF2* expression negatively correlated with non-bone/visceral metastasis. *FOXF2* mRNA levels in primary breast cancer tissues (n = 118) were detected by RT-qPCR. The Kaplan-Meier survival curve shows the DMFS (a) and NBMFS (b) of patients in the *FOXF2*_{high} and *FOXF2*_{low} groups. The overall, luminal subtype and TNBC subtype cases were analyzed separately.



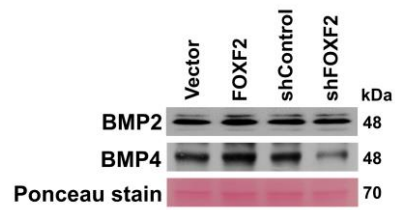
Supplementary Figure 2 | Foxf2 deficiency suppresses the bone-specific metastasis but promotes the visceral metastasis of breast cancer cells *in vivo*. 4T1 cells were infected with LV-Foxf2-EGFP (Foxf2), LV-shFoxf2-EGFP (shFoxf2) or their controls, and 5×10^6 cells were injected into the fat pad of female BALB/c mice (n = 4 per group). The mice were killed by cervical dislocation on day 28 after cell injection. **(a)** *Foxf2* mRNA levels in the indicated cells were detected by RT-qPCR. **(b)** The volume of xenograft tumor was calculated. **(c)** The osteolytic lesions and metastases in bones were observed by X-ray, H&E staining and TRAP staining. The number of osteolytic lesions, osteolytic area and $\log_2$ signal intensity of hind leg bones detected by X-ray were calculated. Scale bars, 500 μm for H&E staining and 50 μm for TRAP staining. Arrows point to osteolytic lesions or TRAP⁺ cells. **(d-e)** Metastatic nodules on two sides of the lung (d) and liver (e) surface were photographed, identified by H&E staining and statistically analyzed. The metastatic nodules were counted, or the incidence of metastasis was calculated. Scale bars, 200 μm . * $P < 0.05$ by student's *t*-test. Error bars are defined as s.d.



Supplementary Figure 3 | FOXF2 enhances the bone-specific metastasis potential of breast cancer cells. MCF-7 and MDA-MB-231 cells were treated as indicated. **(a)** Chemotactic migration of cancer cells toward HPFs or HHSCs was assessed by transwell assays. **(b)** Adhesion of cancer cells to HPFs or HHSCs was assessed by adding cancer cells on HPFs or HHSCs at 100% saturation, followed by incubation for 30 min. **(c)** Anchorage-independent growth of cancer cells in CM from HPFs or HHSCs was assessed by soft agar colony formation assays. Scale bars, 100 μ m. * $P < 0.05$ by student's t -test. Error bars are defined as s.d.

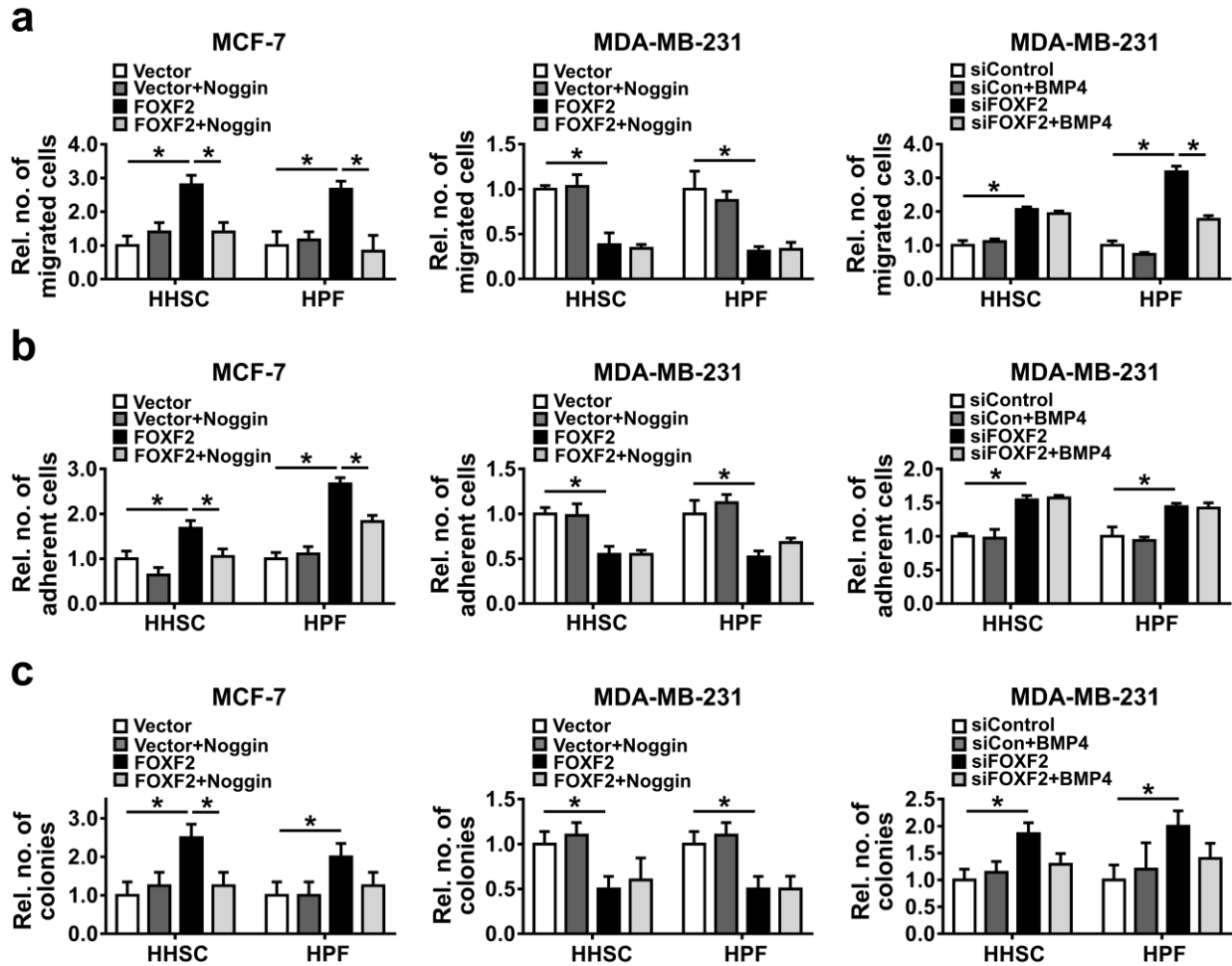


Supplementary Figure 4 | FOXF2 endows breast cancer cells with osteomimetic features that facilitate bone metastasis. (a) The *P* values of genes coexpressed with FOXF2 in primary breast cancer tissues (*n* = 49) were calculated by Pearson's correlation analysis. (b) The binding of FOXF2 to promoter regions of BRGs containing candidate binding sites in the indicated cells was determined by ChIP-PCR assay.

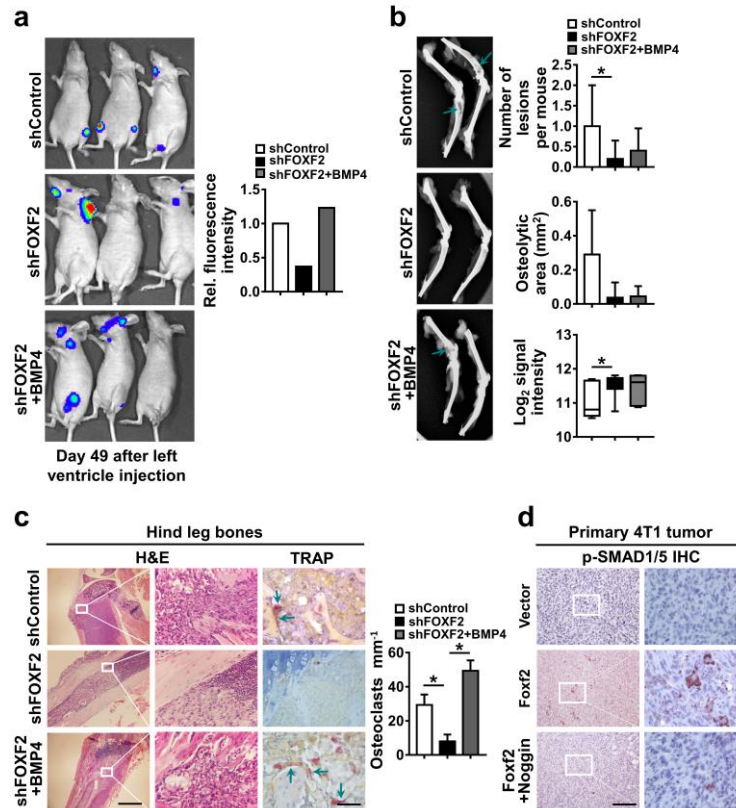


Supplementary Figure 5 | FOXF2 positively regulates BMP4 secretion in breast cancer cells.

Secreted BMP2 and BMP4 protein levels in the CM from MDA-MB-231 cells treated as indicated were detected by immunoblot. Ponceau S staining served as a loading control.



Supplementary Figure 6 | FOXF2 enhances the bone-specific metastasis of breast cancer cells through directly activating the BMP/SMAD signaling pathway. MCF-7 and MDA-MB-231 cells were treated as indicated. **(a)** Chemotactic migration of cancer cells toward HPFs or HHSCs was assessed by transwell assays. **(b)** Adhesion of cancer cells to HPFs or HHSCs was assessed by adding cancer cells to HPFs or HHSCs at 100% saturation, followed by incubation for 30 min. **(c)** Anchorage-independent growth of cancer cells in CM from HPFs or HHSCs was assessed by soft agar colony formation assays. * $P < 0.05$ by student's t -test. Error bars are defined as s.d.



Supplementary Figure 7 | FOXF2 enhances the bone-specific metastasis of breast cancer cells through activating the BMP/SMAD signaling pathway. A total of 2×10^5 231-Luc-BM cells infected with LV-shFOXF2 (shFOXF2) or LV-shControl (shControl) were injected into the left ventricle of female nude mice ($n = 5$ per group). The mice bearing FOXF2-silenced tumors were treated with 40 ng BMP4 by intraperitoneal injection three times weekly for four weeks. **(a-b)** Bioluminescence (a) and X-ray imaging (b) of mice injected with MDA-MB-231 cells treated as indicated were analyzed. Arrows point to osteolytic lesions. **(c)** The metastases and osteoclasts in the hind leg bones of mice treated as indicated were visualized by H&E and TRAP staining. Arrows point to TRAP⁺ cells. **(d)** Phospho-SMAD1/5 protein expression in the indicated primary 4T1 tumors was detected by immunohistochemistry. Scale bars, 500 μ m for H&E staining, 50 μ m for TRAP staining and 200 μ m for immunohistochemistry. * $P < 0.05$ by student's t -test. Error bars are defined as s.d.

Supplementary Tables

Table S1. Primers and probes for RT-qPCR assays

Genes	Primer and probe sequences	Reaction system
<i>Gapdh</i>	Forward: ATTGTCAGCAATGCATCCTG Reverse: ATGGACTGTGGTCATGAGCC	SYBR [®] Premix Ex Taq [™]
<i>Foxf2</i>	Forward: CCCAGCATGTCTTCCTACTCG Reverse: CGAAATCTTTCCTGTCGCACAC	
<i>CTSK</i>	Forward: GCAATAGCGATAATCTGAACCAT Reverse: CGTTGTTCTTATTTTCGAGCCAT	Platinum [®] Quantitative PCR System
<i>GAPDH</i>	Forward: GAAGGTGAAGGTCGGAGTC Reverse: GAAGATGGTGATGGGATTTC Probe: CAAGCTTCCCGTTCTCAGCC	
<i>FOXF2</i>	Forward: TGCACTCCAGCATGTCCTCCTA Reverse: CGCTAGCTGAGGGATGGAAAGA Probe: ACCTCTCAGTGGGACTGCCCCGTTA	

Table S2. Antibodies used in immunoblot

Name	Catalogue number	Dilution	Company
Primary antibody			
β -actin	A1978	1:5000	Sigma-Aldrich
SMAD1	6944	1:1000	Cell signaling technology
Phospho-SMAD1	5753	1:500	Cell signaling technology
SMAD2	3122	1:1000	Cell signaling technology
Phospho-SMAD2	8828	1:500	Cell signaling technology
SMAD3	9523	1:1000	Cell signaling technology
Phospho-SMAD3	9520	1:500	Cell signaling technology
SMAD4	9515	1:1000	Cell signaling technology
SMAD5	12534	1:1000	Cell signaling technology
SMAD6	13727	1:2000	Abcam
SMAD7	365846	1:1000	Santa Cruz
SMAD9	115900	1:1000	Abcam
Phospho-SMAD1/5	9516	1:1000	Cell signaling technology
BMP2	MAB3551	1:1000	R&D systems
BMP4	MAB757	1:1000	R&D systems
RUNX2	10758	1:1000	Santa Cruz
ITGBL1	HPA005676	1:500	Sigma-Aldrich
CDH11	4442	1:1000	Cell signaling technology
POSTN	14041	1:2000	Abcam
SPON1	40797	1:1000	Abcam
SPARC	AF941	1:1000	R&D systems
CTSK	48353	1:1000	Santa Cruz
FOXF2	H00002295-M04	1:1000	Abnova
Flag	F3165	1:2000	Sigma-Aldrich
Secondary antibody			
Anti-Mouse	7076	1:2000	Cell signaling technology
Anti-Rabbit	7074	1:2000	Cell signaling technology
Anti-Goat	HAF109	1:2000	R&D systems

Table S3. Primers for ChIP-PCR assays and the constructions of luciferase reporters

Genes	Region on promoters	Forward primers	Reverse primers
Primers for ChIP-PCR assays			
<i>BMP4</i>	−938/−757	AAAACAGACCAAGGGGCATC	AAGCCGTCTTAAGAACCGACA
<i>SMAD1</i>	−400/−230	AGGTAAGTGTCAACATGCACT	CGTTCAGGCTTTCCTAACCC
<i>CTSK</i>	−647/−497	AAGCATTTGGAGAAGCTCAT	GACAGCACTTGAATCAATGCC
	−821/−646	ATGGAATCCAGCTAGAACTGA	TTCCCCAACATCCAAGCAT
	−1733/−1534	ATCTGTGCTAAGCTTTACAGT	GTGCCTTATGTTTGTATGTCT
<i>ITGBL1</i>	−784/−619	TGTCAATCTACGGATCCAACAAT	AACACCACCTGAATCAGACCTC
	−1318/−1123	GCTCAGGCATTGTACTAAGTACCC	GGAACCTAATATTGGCATGGCTTTAAT
<i>CSPG2</i>	−1441/−1263	ACTGCAGAGGGCTGATAAATAACA	ACTTAGACTCCTGTACATGCGCA
	−1652/−1485	TGAAGGTTGAAGTCTGATTCTGCT	TGTTATTTATCAGCCCTCTGCAGTAAT
<i>COMP</i>	−184/−15	TTTCCCCACCATGTCCGATT	CCGCGGGGCCTATTTATCC
<i>DSPG3</i>	−568/−393	CTCCCCAAAACACGCAGATCA	AAATGTTGCAGAAACCTTACCTGTTT
	−1678/−1501	TGAGGGAAAAGGAAGAAAGTTGG	GAGTCTGTCTGAGGGATACGTG
	−2101/−1913	GGTCCTCGAGTGATTGTAACC	TTGTGTAGCATTGCAAGCAG
<i>FST</i>	−1149/−970	GTGACCTGCGAATGTCCAAG	GCACCGCTGAATGAAACTGA
<i>OGN</i>	−209/−45	AGCAGATTGTTTGATCTCCTGGGA	ACTGCTGCACTCAGTCTGCT
<i>POSTN</i>	−1057/−868	TGACATTACAGAGGGAGCATCT	GCATGGAGTAGTAAGAACGCAC
<i>SPARC</i>	−856/−697	TTTTTGAGGACAAGGACCAGGT	CAAAAAGTGGTATGAGAGGGCT
<i>SPON1</i>	−188/−29	CAGAGGGTCCGGAGTGTAG	TTTCCTCCGAACTGGCGAT
<i>LOXL2</i>	−520/−384	CCAGAGGTCACGGAATCCAA	GAAAACCGATCCTGAACCAGT
<i>PLAU</i>	−1240/−991	TATGTTCCCTCCAAGTGTGC	CTCTGTATAGCCCATTCCA
	−1860/−1681	ATGAATCATGACGGTCCCT	CTTCAGAGCCAACCTTGCTAC
Primers for the constructions of luciferase reporters			
<i>BMP4</i>	−646/+146	GGGGTACCGGGTCTACCTCAGGGTCATCAC	CCGCTCGAGCAATCTTGAACAAACTTGCTGGA
	−897/+146	GGGGTACCTAAAAGGTTACTGCTTCTGTG	CCGCTCGAGCAATCTTGAACAAACTTGCTGGA
<i>SMAD1</i>	−99/+258	GGTACCATTGAGAGGATCCCTGGTCGCGC	CCGCTCGAGAAGTAACCCAGTCAGCA CCG
	−343/+258	GGTACCAGCGTTAATGAACATTCAGAAAAT	CCGCTCGAGAAGTAACCCAGTCAGCA CCG
<i>CTSK</i>	−532/+242	GGGGTACCTCCATCAGAACTTACGGCAT	GAAGATCTGTTGCAAACGTTACCTGCT
	−607/+242	GGGGTACCAGATTGGGGATTTTAATTGAGAC	GAAGATCTGTTGCAAACGTTACCTGCT
	−1548/+242	GGGGTACCCAGACATACAAACATAAGGCA	GAAGATCTGTTGCAAACGTTACCTGCT