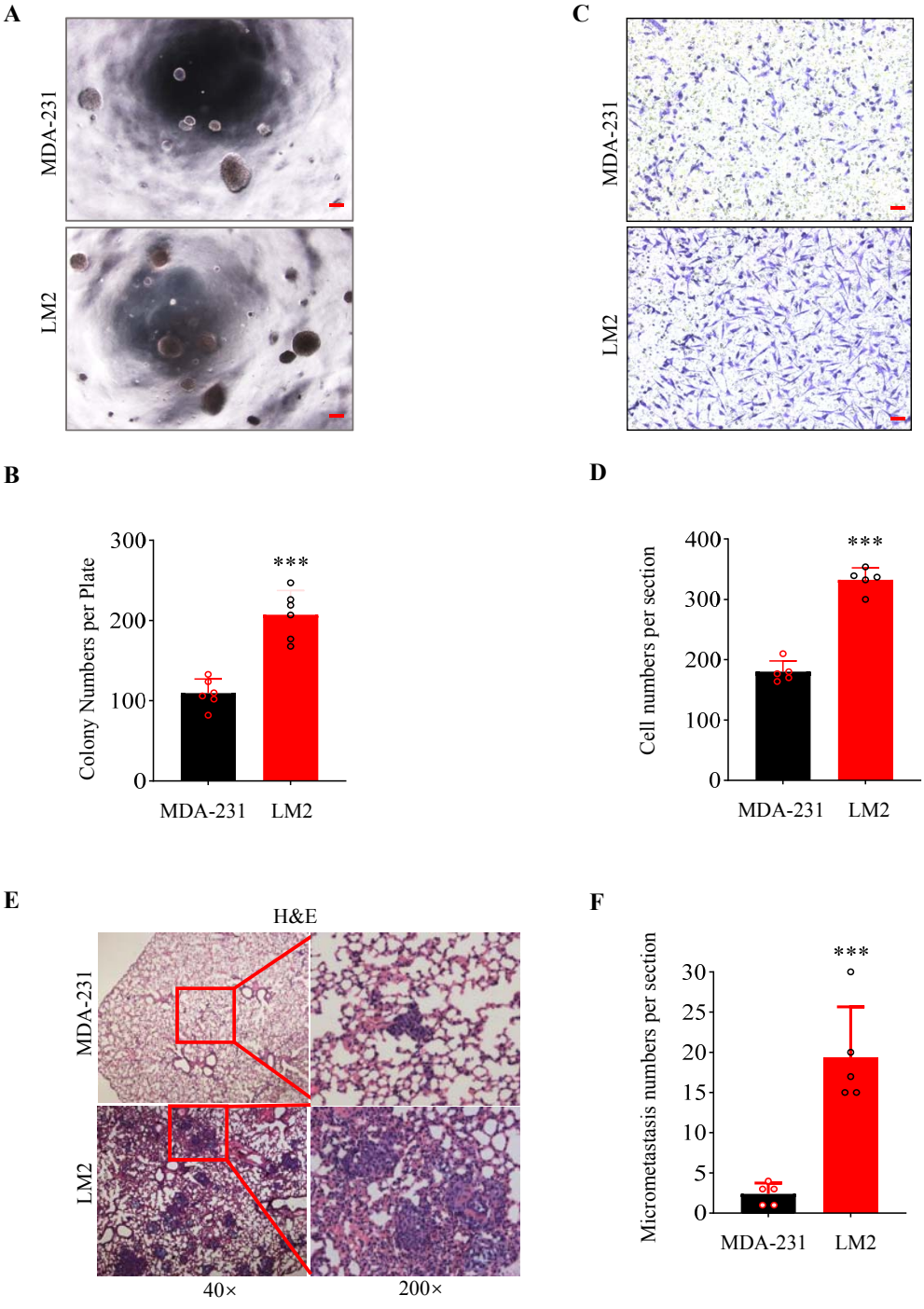
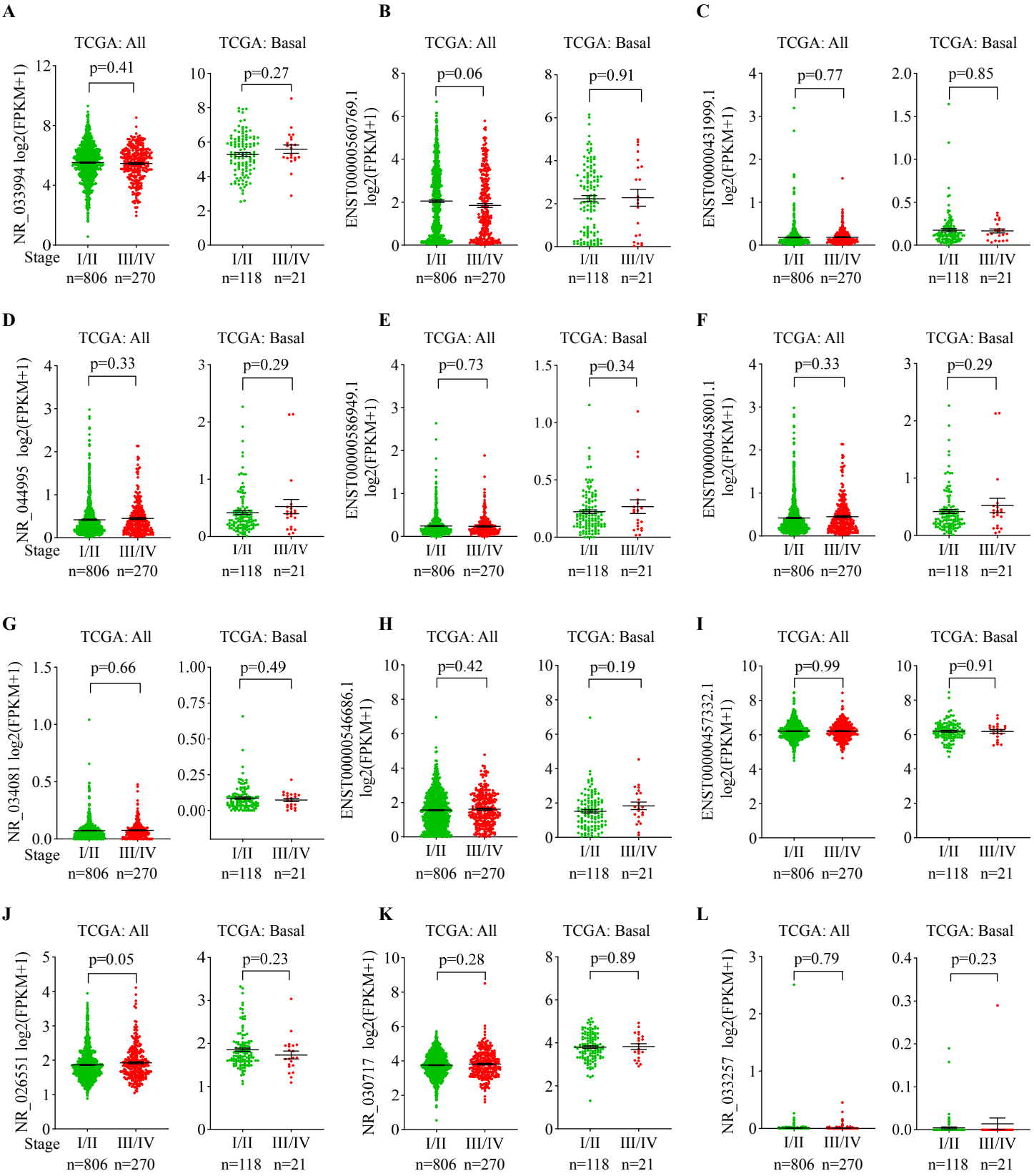


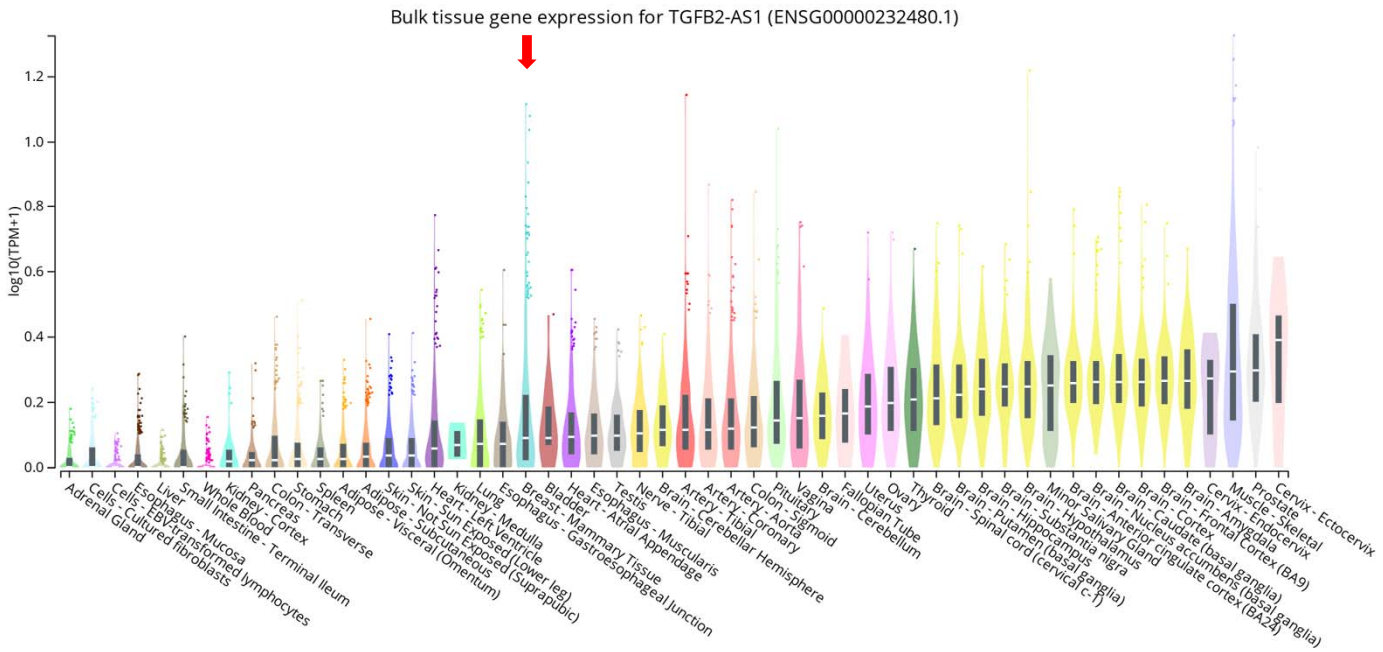
Zhou CX et al, Fig. *S1*

Figure S1. Verification of human TNBC MDA-MB-231 cells (shortly, named MDA-231 cells) and its lung-metastatic subclone MDA-MB-231-LM2 cells (shortly, named LM2 cells)**.** (A) Soft agar colony formation assay was photographed (scale bar 200μm) and (B) colony quantities statistics analysis in MDA-231 and LM2 cells. (C) Representative images (scale bar 100μm) and (D) statistics analysis of transwell invasion assay in MDA-231 and LM2 cells. Representative images (E) and statistics analysis (F) of Haematoxylin-eosin staining in the lung metastatic foci of MDA-231 and LM2 cells. Error bars represent mean ± SD ($n \geq 3$). Statistical significance was assessed using 2-tailed Student's t test. ***$P < 0.001$.



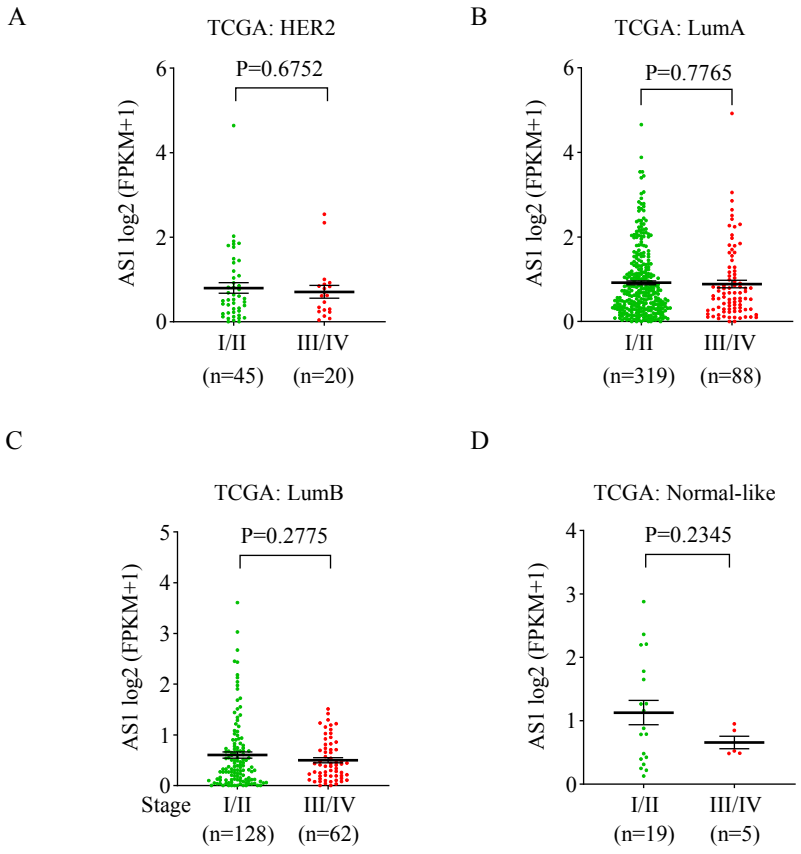
Zhou CX et al, Fig. *S2*

Figure S2. Screening of the candidate LncRNAs associated with breast cancer. Comparing the expression of (A)NR_033994, (B)ENST00000560769.1, (C)ENST00000431999.1, (D)NR_044995, (E)ENST00000586949.1, (F)ENST00000458001.1, (G)NR_034081, (H)ENST00000546686.1, (I)ENST00000457332.1, (J)NR_026551, (K)NR_030717 and (L)NR_033257 between stage I/II (n = 806) and stage III/IV (n = 270) in all breast cancer tissues (left) and in basal-like breast cancer tissues (right) between stage I/II (n = 118) and stage III/IV (n = 21) using the TCGA database. Statistical significance was assessed using 2-tailed Student's t test.



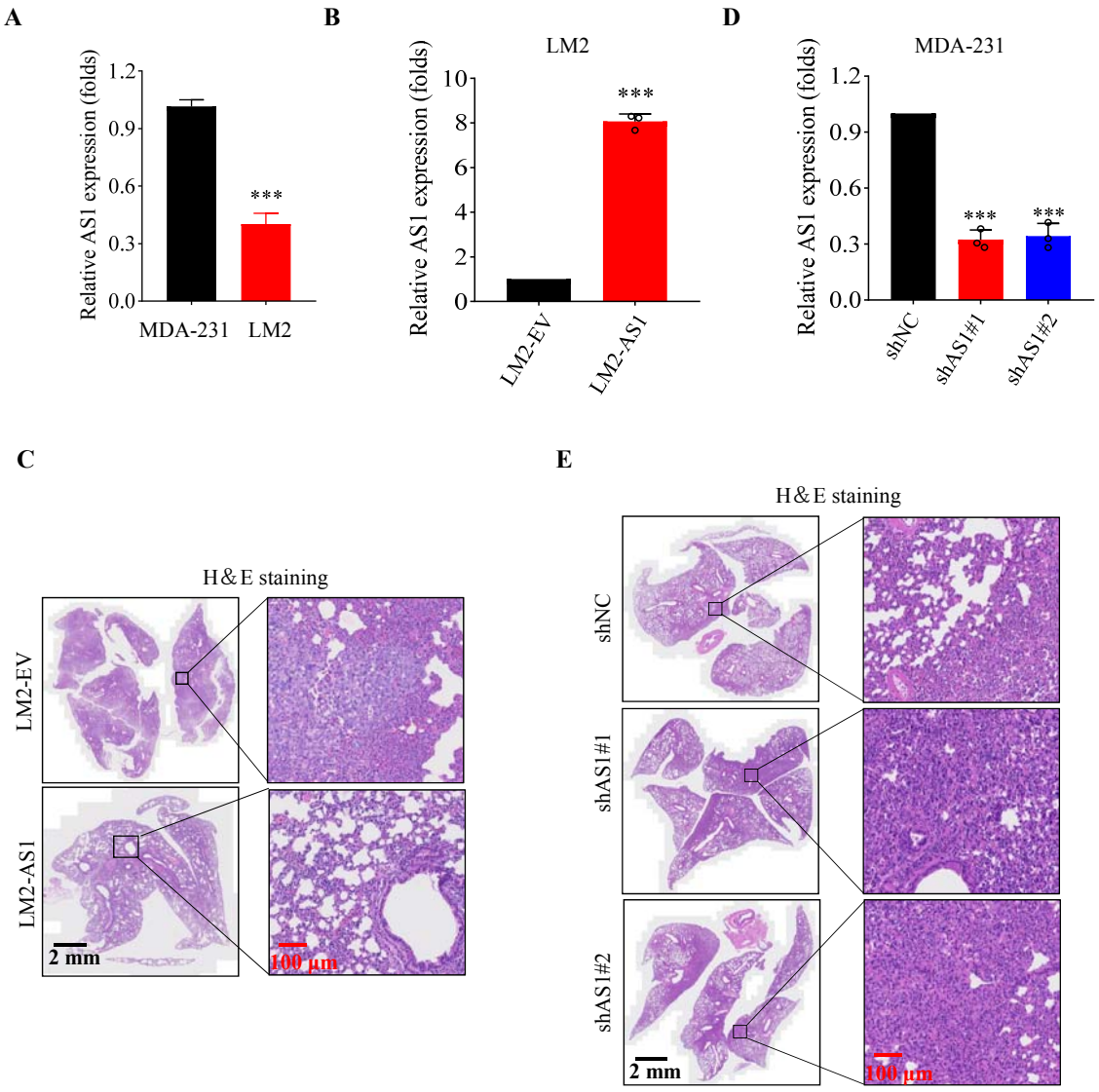
Zhou CX et al, Fig. *S3* ⇧

Figure S3. The expression level of TGFB2-AS1 from GTEx database. TGFB2-AS1 is widely expressed in various normal tissues. The expression amount of TGFB2-AS1 in each normal tissue is arranged from small to large, and the red arrow indicates the expression of TGFB2-AS1 in normal breast and mammary tissue.



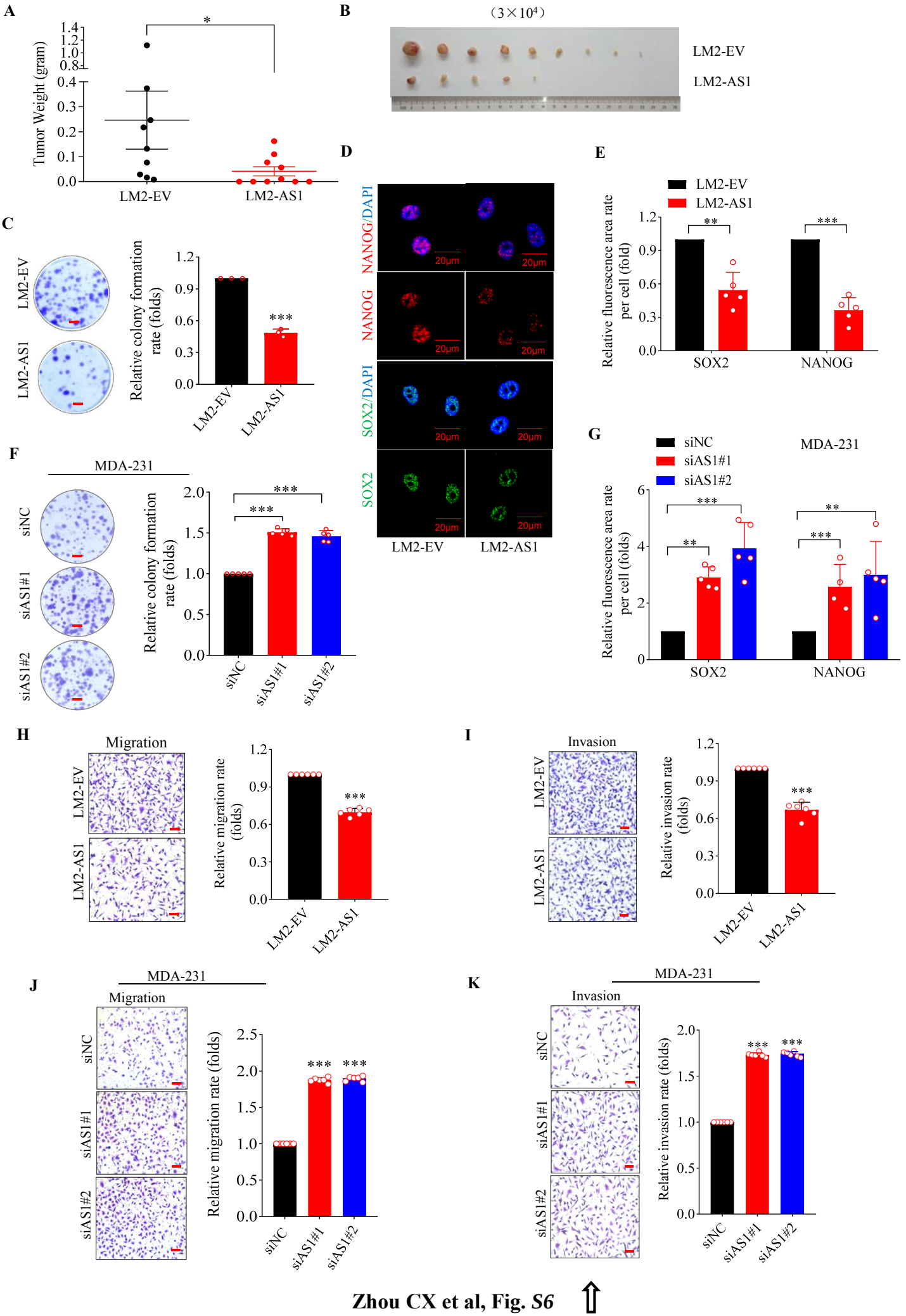
Zhou CX et al, Fig. *S4* ⇧

Figure S4. Analyzing the expression levels of TGFB-AS1 across breast cancer subtypes. Scatter plots comparing TGFB2-AS1 expression between stage I/II and stage III/IV in breast tumour samples with different subtypes as indicted including (A) HER2 (n=65), (B) LumA (n=407), (C) LumB (n=190) and (D) Normal-like (n=24) using the TCGA database. Statistical significance was assessed using 2-tailed Student's t test.



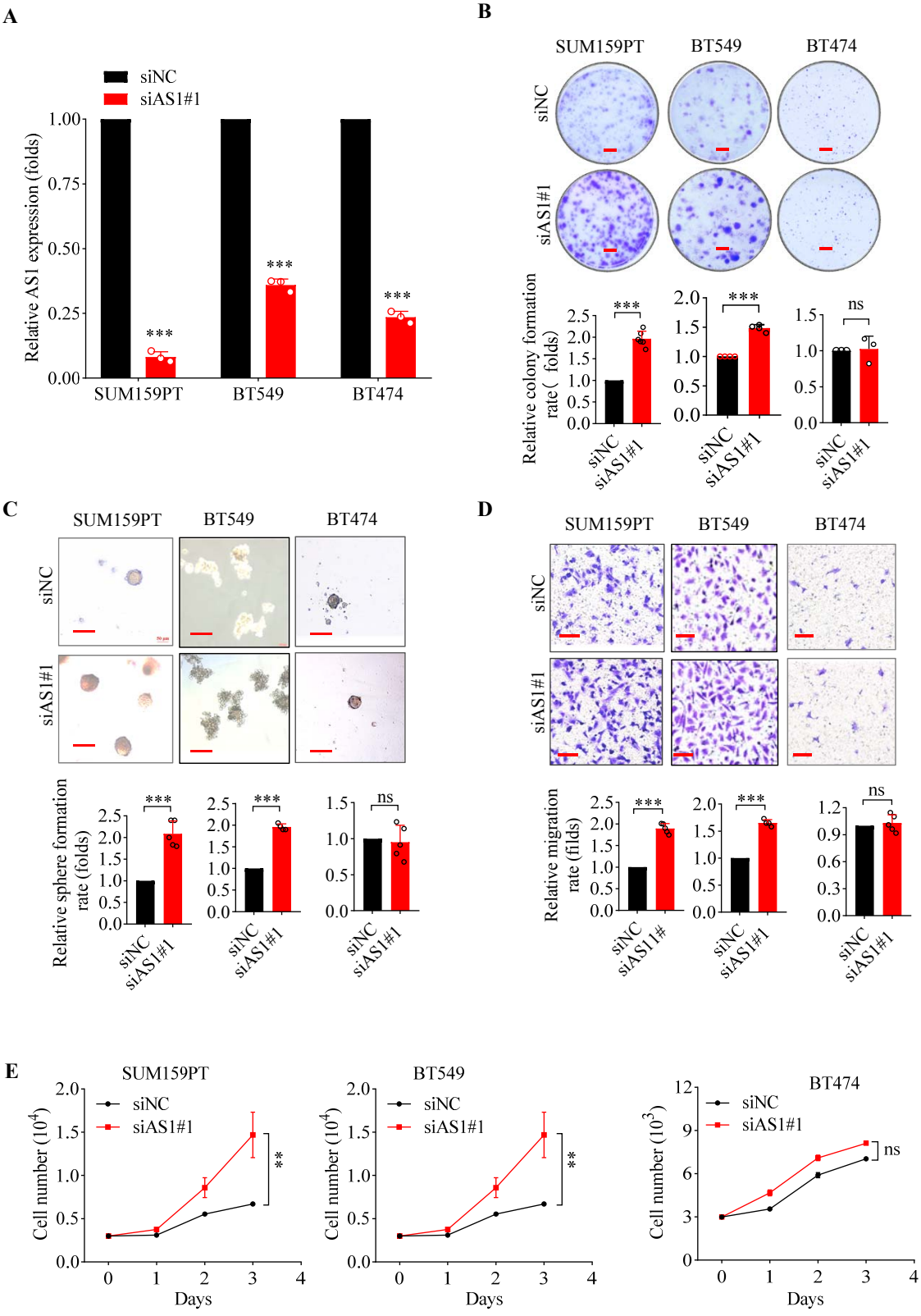
Zhou CX et al, Fig. *S5*

Figure S5. TGFB2-AS1 is a key inhibitor of breast cancer metastases. (A) Validating the expression of TGFB2-AS1 by Q-PCR in MDA-231 and LM2 cells. (B) Q-PCR analyzing the overexpression efficiency of TGFB2-AS1 in LM2-AS1 and the control LM2-EV cells. (C) Representative images of Haematoxylin-eosin staining in the lung metastatic foci of LM2-AS1 group and LM2-EV group. (D) Q-PCR analyzing the knockdown efficiency of TGFB2-AS1 in MDA-231 cells. (E) Representative images of Haematoxylin-eosin staining in the lung metastatic foci of in MDA-231 cells with TGFB2-AS1 knockdown group and control group. Statistical significance was assessed using 2-tailed Student's t test. ***P < 0.001. Error bars represent mean ± SD (n=3).



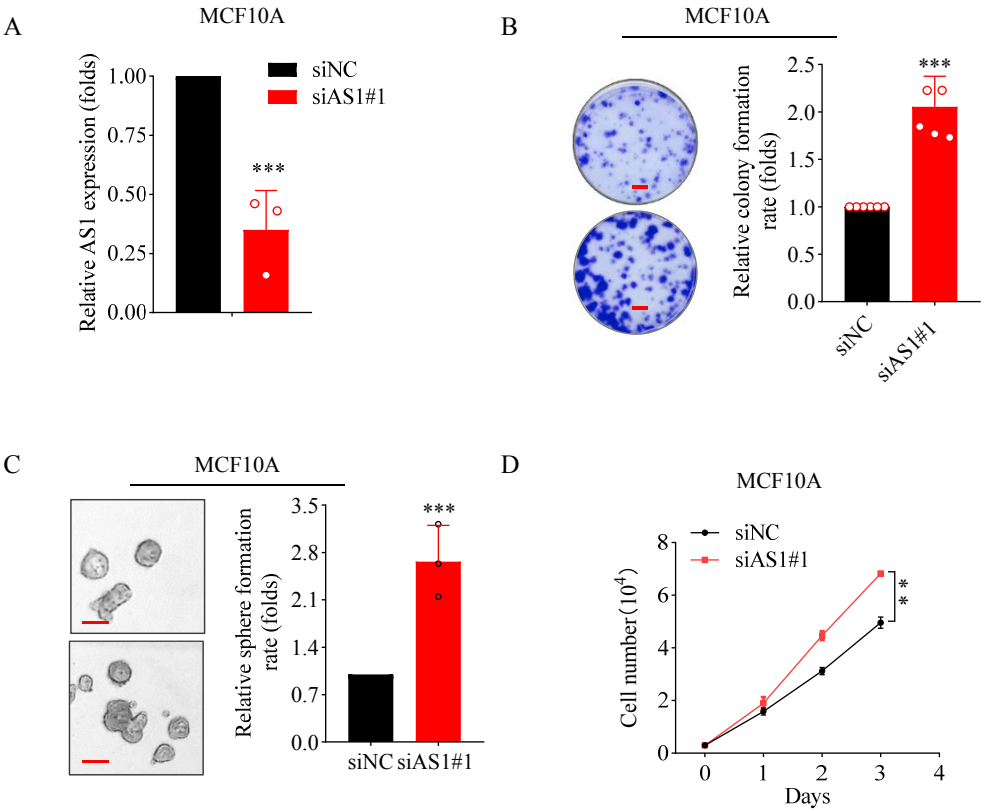
Zhou CX et al, Fig. *S6*

Figure S6. TGFB2-AS1 impairs TNBC cells malignant characteristics. 3×10^4 LM2-AS1 cells and LM2-EV cells were inoculated subcutaneously into female BALB/c nude mice respectively, (A) tumor weight and (B) tumor morphology were shown. Data are presented as means ± SEM (n = 9 and 10 respectively). (C) Plate colony formation assay in LM2-EV and LM2-AS1 cells (scale bar 0.5cm). (D) The representative images of immunofluorescence staining for Nanog and SOX2 (scale bar 20μm) and (E) Quantitative statistical histogram of fluorescence intensity in LM2-EV and LM2-AS1 cells. (F) Plate colony formation assay (scale bar 0.5cm) in MDA-231 cells with TGFB2-AS1 knockdown (MDA-231-siAS1#1, MDA-231-siAS1#2). The images are showing in the left and statistical histogram are showing in the right. (G) Quantitative statistical histogram of fluorescence intensity for SOX2 and Nanog staining in MDA-231-siAS1#1, MDA-231-siAS1#2 and MDA-231-siNC cells. (H) Transwell migration assay and (I) Transwell invasion assay were performed between LM2-EV and LM2-AS1 cells. The representative images (scale bar 100μm) are showing in the left and statistical histogram are showing in the right. (J) Transwell migration assay and (K)Transwell invasion assay were performed between MDA-231-siAS1#1, MDA-231-siAS1#2 and MDA-231-siNC cells. The representative images(scale bar 100μm) are showing in the left and statistical histogram are showing in the right. Fluorescence intensity was analysed using ImageJ software. Statistical significance was assessed using 2-tailed Student's t test. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. Error bars represent mean ± SD ($n\geq3$).



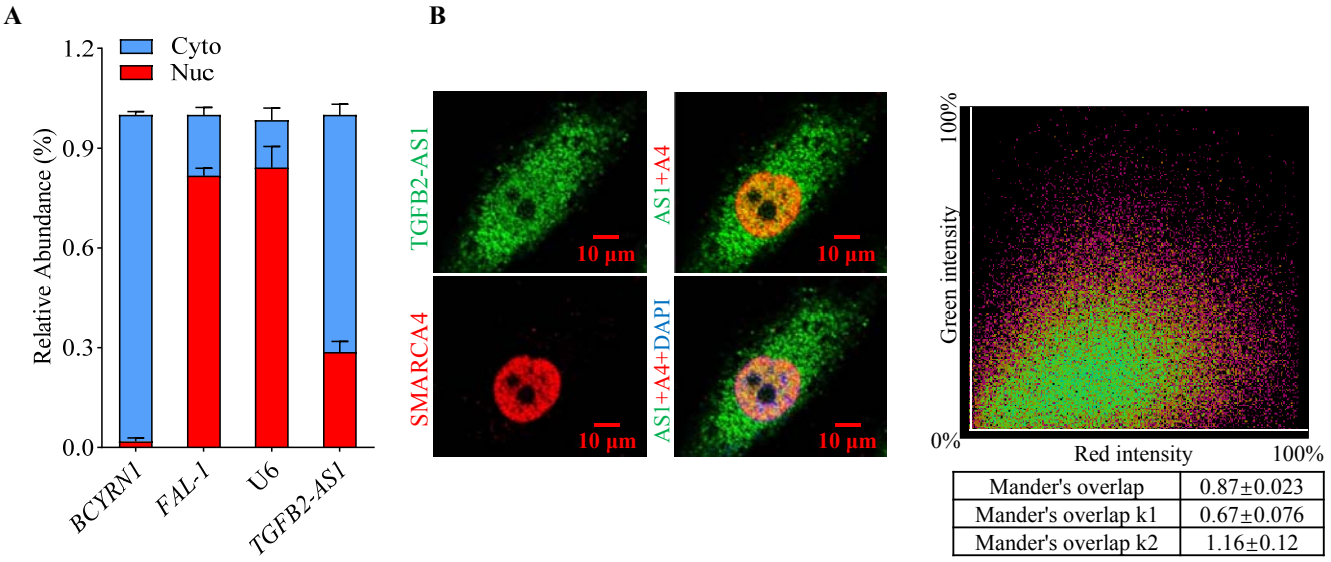
Zhou CX et al, Fig. *S7*

Figure S7. TGFB2-AS1 impairs malignant characteristics in various TNBC cells. (A) Q-PCR analyzing the knockdown efficiency of siTGFB2-AS1 in different breast cancer cell lines as indicated. (B) Representative images (top, scale bar 0.5cm) and values (bottom) of plate colony formation assay. (C) Representative images (top, scale bar 50μm) and values (bottom) of mammosphere assay. (D) Representative images (top, scale bar 100μm) and values (bottom) of Transwell migration assay in different breast cancer cell lines with TGFB2-AS1 knockdown. (E) Cell proliferation assays using CCK-8 in different breast cancer cell lines with TGFB2-AS1 knockdown. Error bars represent mean ± SD ($n \geq 3$). Statistical significance was assessed using 2-tailed Student's t test. **P < 0.01, ***P < 0.001.



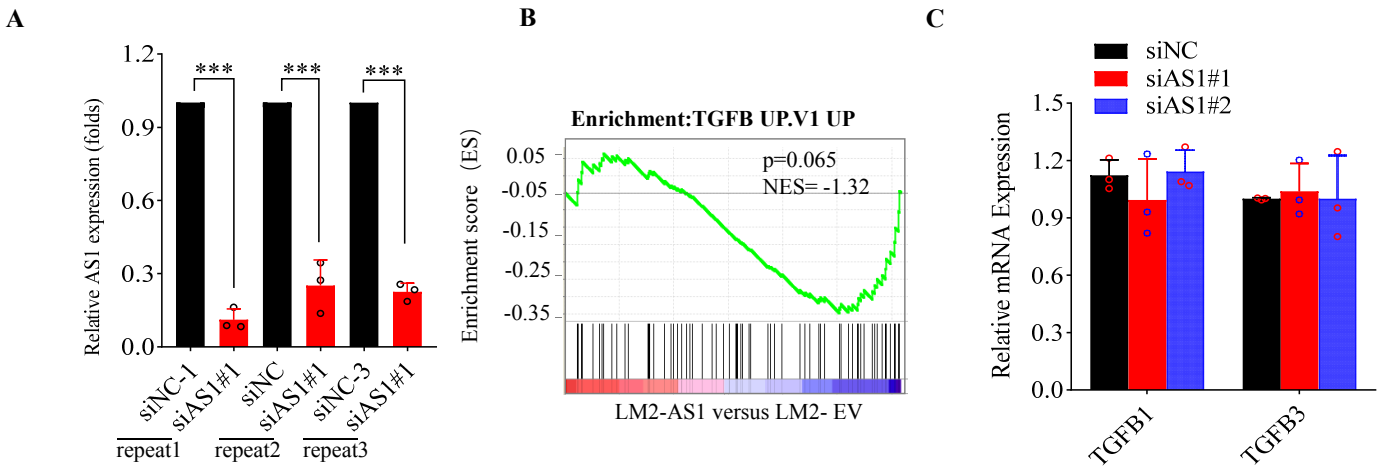
Zhou CX et al, Fig. *S8* ⇧

Figure S8. TGFB2-AS1 suppresses the proliferation, plate colony formation and mammosphere formation of MCF10A cells. (A) Q-PCR analyzing the knockdown efficiency of siTGFB2-AS1 in MCF10A cells. (B) Representative images (left, scale bar 0.5cm) and values (right) of plate colony formation assay. (C) Representative images (left, scale bar 50μm) and values (right) of mammosphere assay in MCF10A cells with TGFB2-AS1 knockdown. (D) Cell proliferation assays using CCK-8 in MCF10A cells with TGFB2-AS1 knockdown. Error bars represent mean ± SD ($n \geq 3$). Statistical significance was assessed using 2-tailed Student's t test. $^{**}P < 0.01$, $^{***}P < 0.001$.



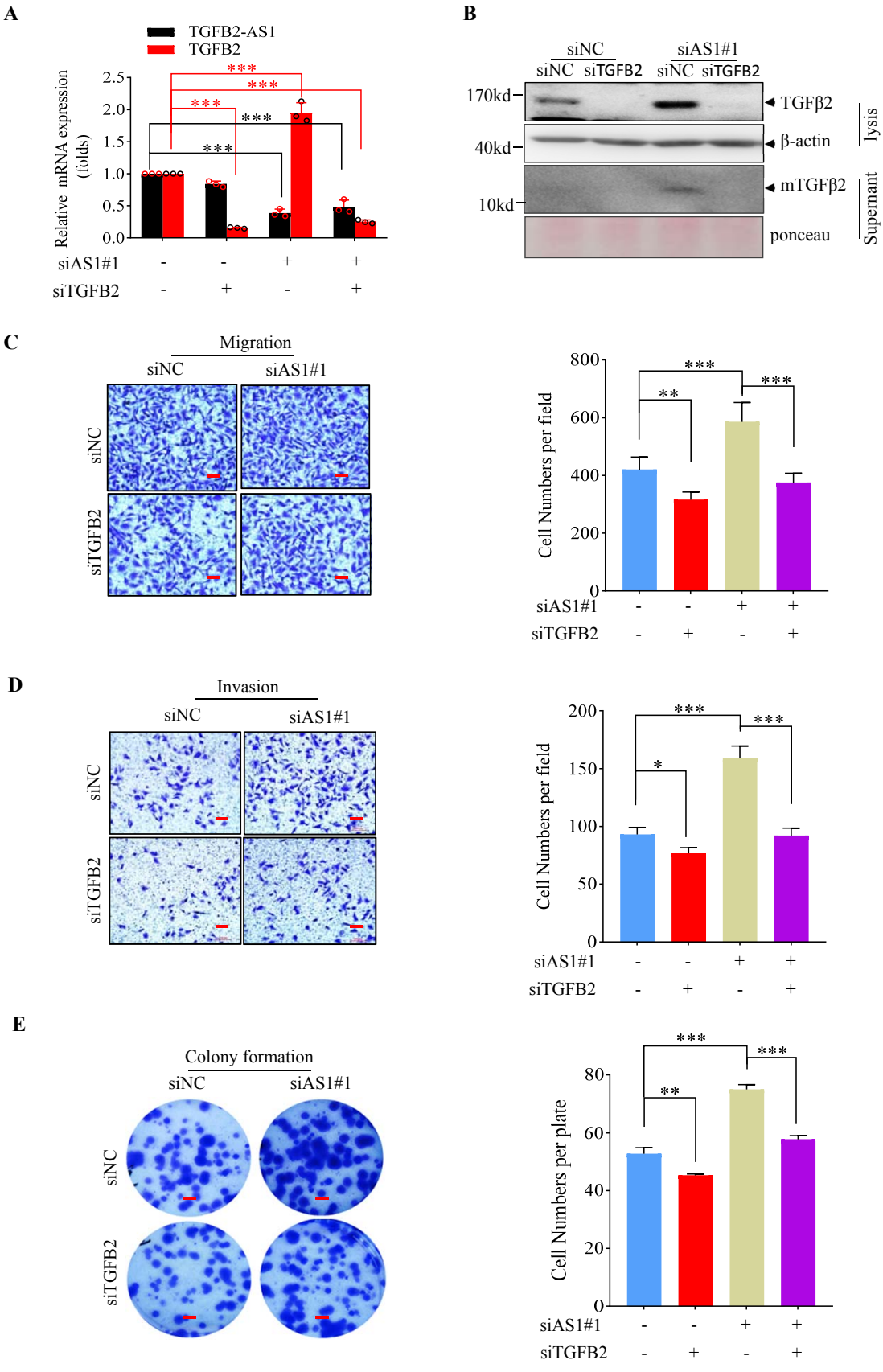
Zhou CX et al, Fig. *S9*

Figure S9. TGFB2-AS1 interacting with SMARCA4 in the nucleus. (A) Q-PCR analyzing TGFB2-AS1 expression levels in different subcellular fractions of MDA-231 cells. Red indicates the nucleus fraction (Nuc), and blue indicates the cytoplasmic (Cyto) fraction. BCYRN1 served as positive control of the cytoplasmic fraction, FAL-1 and U6 served as positive control of the nuclear fraction. (B) Representative confocal microscopy image of TGFB2-AS1 FISH and SMARCA4 immunofluorescence are shown (left). DAPI (blue), used for nucleus counterstaining. Scale bars, 10μm. Images are representative of 3 independent experiments. The co-localization analysis of TGFB2-AS1 and SMARCA4 in the MDA-231 cell nucleus were estimated via the Image J software with coloc2 plug-in (right). (C) Western blot showing BAF170 and SNF5 immunoprecipitated with SMARCA4 in both siTGFB2-AS1 transfected MDA-231 cells and its control cells. (D) The secondary structure prediction of TGFB2-AS1 on website (http://rna.tbi.univie.ac.at/). Color scale indicates the confidence of the prediction for each base with shades of red indicating strong confidence.



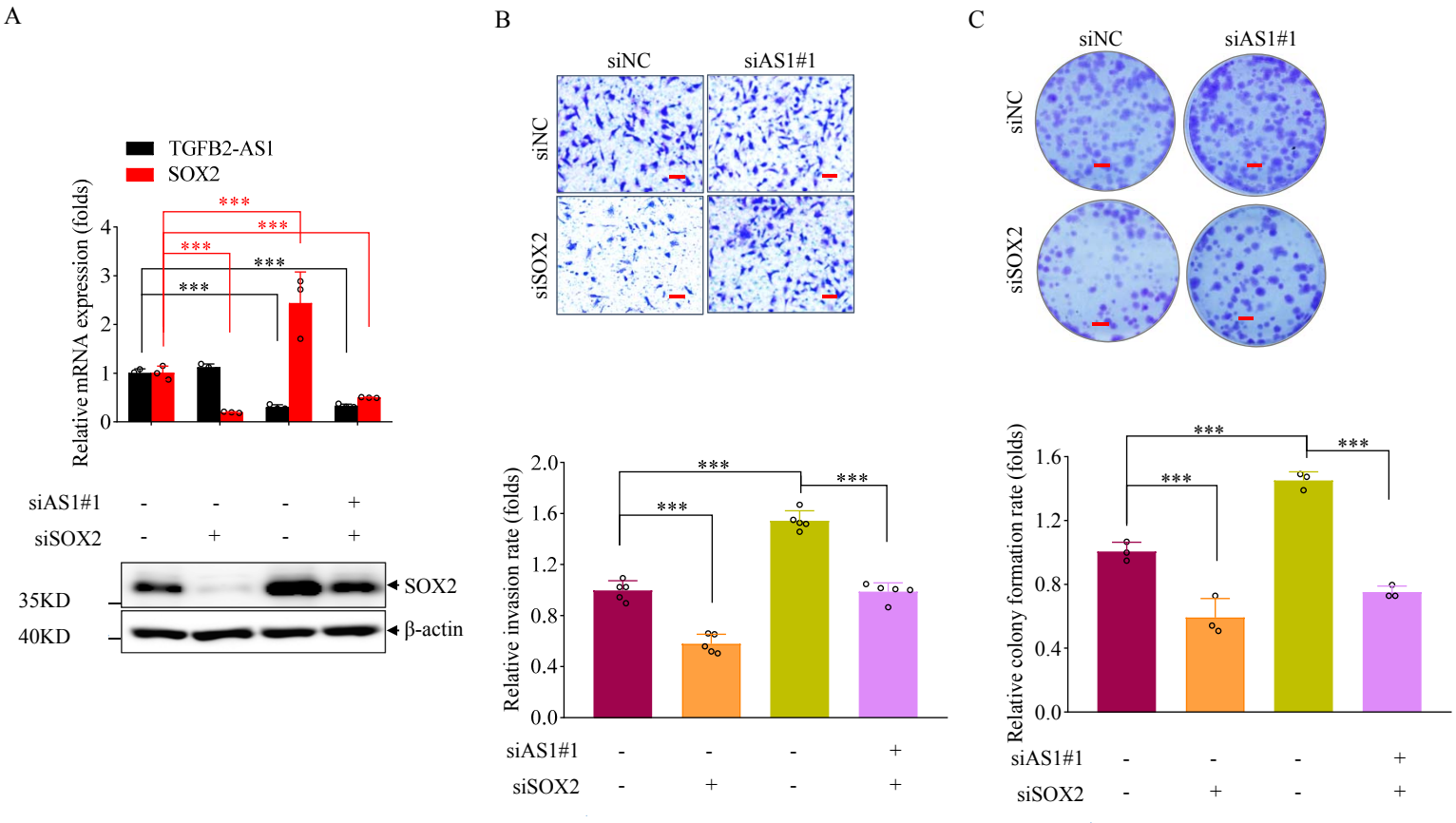
Zhou CX et al, Fig. *S10* ⇧

Figure S10. TGFB2-AS1 suppresses TGFβ2 signal pathway *in vitro*. (A) Q-PCR measuring the knockdown efficiency of siTGFB2-AS1 in MDA-231 cells (MDA-231-siAS1). (B) GSEA enrichment focused on inhibiting of TGFβ signal pathway from mRNA expression profiles in LM2-AS1 and LM2-EV cells. (C) Q-PCR detecting TGFB1 and TGFB3 mRNA expression in MDA-231 cells after transfected with two TGFB2-AS1 siRNAs respectively. Error bars represent mean ± SD (n=3). Statistical significance was assessed using 2-tailed Student's t test. ***P < 0.001.



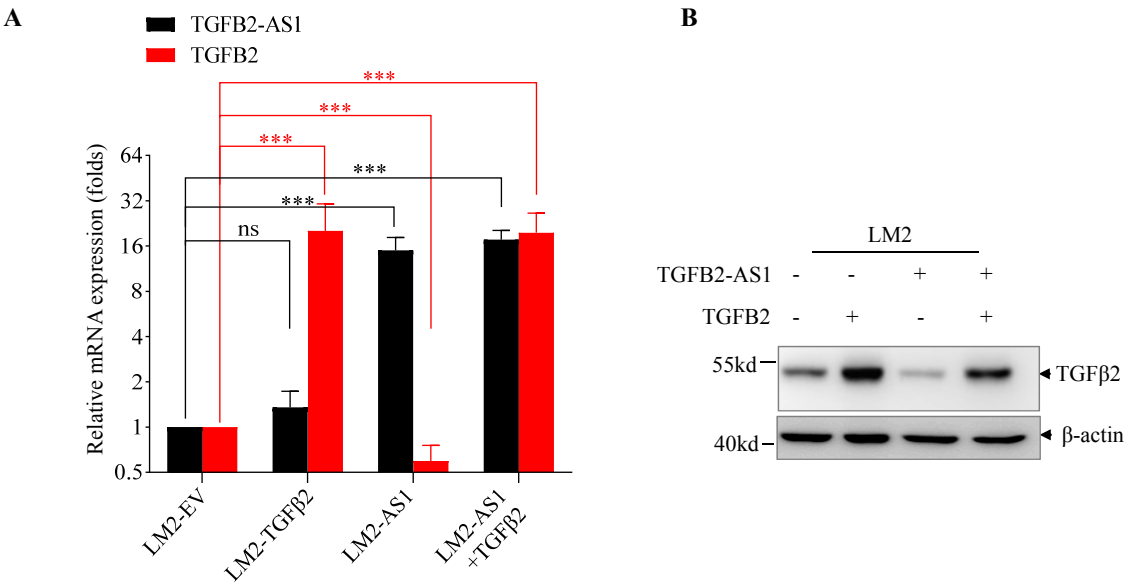
Zhou CX et al, Fig. *S11*

Figure S11. TGFB2-AS1 impairs the malignant characters of TNBC cells via suppressing TGFβ2 signal pathway *in vitro*. (A) Q-PCR analysis of TGFB2-AS1 and TGFB2 RNA expression levels (B)Western blot analysis of TGFβ2 and mTGFB2 (mature TGFβ2) protein expression levels in MDA-231 cells transfected with siAS1 or/and siTGFB2. (C-E) Representative images (left) and quantitative statistical histogram (right) of migration assay C (scale bar 100μm), invasion assay D (scale bar 100μm) and plate colony formation assay E (scale bar 0.5cm) in MDA-231 cells transfected with siAS1 or/and siTGFB2. Error bars represent mean ± SD (n≥3). Statistical significance was assessed using 2-tailed Student's t test. *P < 0.05. **P < 0.01. ***P < 0.001.



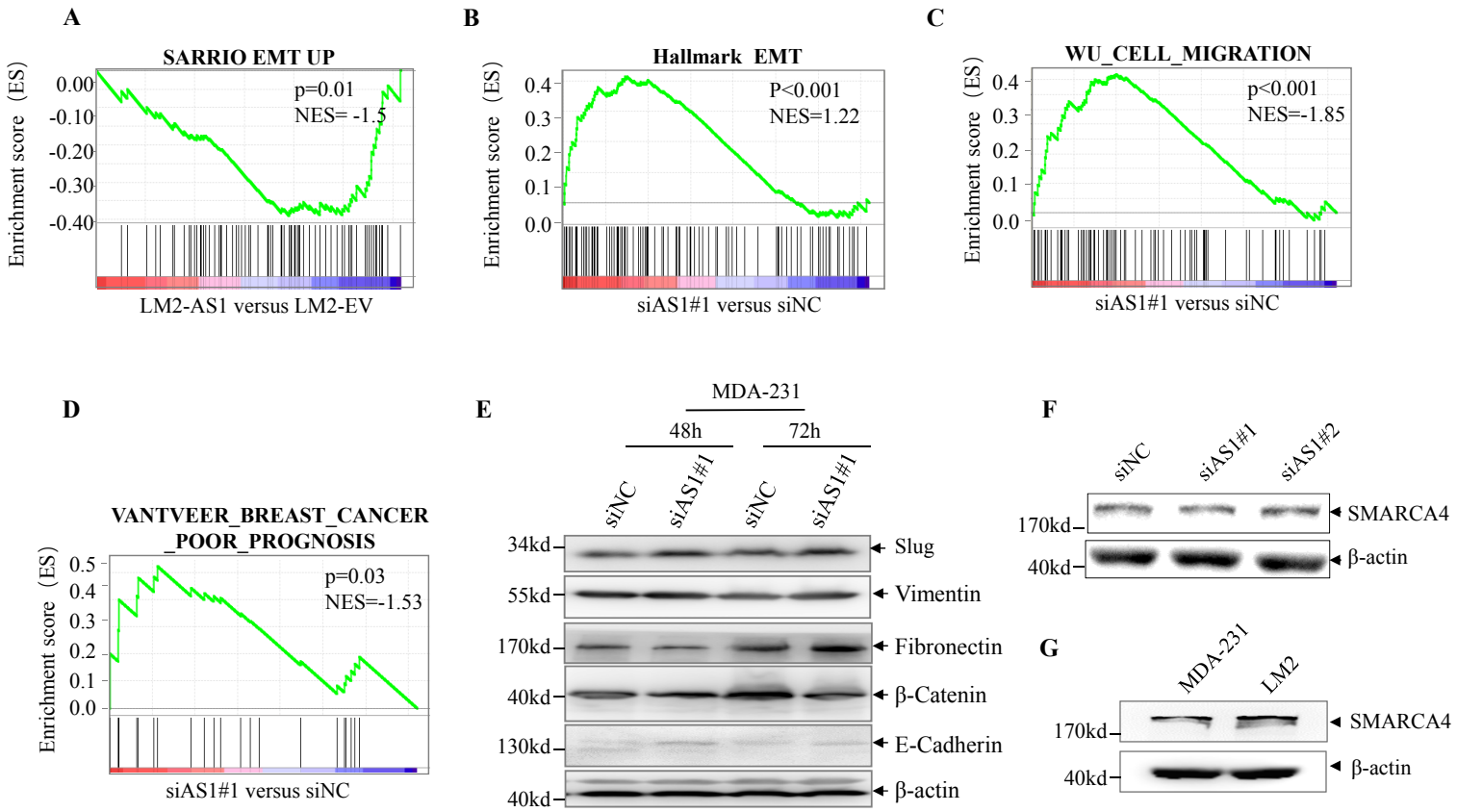
Zhou CX et al, Fig. *S12* ⇧

Figure S12. TGFB2-AS1 impairs the malignant characters of TNBC cells via suppressing SOX2 *in vitro*. (A) Q-PCR (top) and Western blot (bottom) analysis of TGFB2-AS1 and SOX2 expression in MDA-231 cells transfected with siAS1 or/and siSOX2. (B-C) Representative images (top) and quantitative statistical histogram (bottom) of invasion assay B (scale bar 100μm) and plate colony formation assay C (scale bar 0.5cm) in MDA-231 cells transfected with siAS1 or/and siSOX2. Error bars represent mean ± SD (n≥3). Statistical significance was assessed using 2-tailed Student's t test. ***P < 0.001.



Zhou CX et al, Fig. *S13*

Figure S13. TGFB2-AS1 suppresses the functions of TGFβ2 to restraint TNBC development. (A) Q-PCR analysis of TGFB2-AS1 and TGFB2 mRNA expression, (B) Western blot analysis of TGFβ2 protein expression in LM2 cells transfected with TGFB2-AS1 or/and TGFB2 overexpression plasmids. Error bars represent mean ± SD (n=3). Statistical significance was assessed using 2-tailed Student's t test. ***P < 0.001.



Zhou CX et al, Fig. *S14*

Figure S14. Knockdown of TGFB2-AS1 facilitates breast cancer malignancy and poor prognosis. (A) GSEA enrichment focused on inhibiting EMT from mRNA expression profiles assay in LM2-AS1 and LM2-EV cells. (B) GSEA enrichment focused on activating EMT from mRNA expression profiles assay in MDA-231-siAS1#1 and MDA-231-siNC cells. (C-D) GSEA enrichment focused on activating cell migration (C) poor clinical outcome (D) from mRNA expression profiles assay in MDA-231-siAS1#1 and MDA-231-siNC cells. (E) Western blot analysis of a set of EMT markers in MDA-231-siAS1#1 and MDA-231-siNC cells. Error bars represent mean ± SD (n=3). Statistical significance was assessed using 2-tailed Student's t test. ***P < 0.001. (F) Western blot analyzing the expression of SMARCA4 in MDA-231-siAS1 cells and its control cells. (G) Western blot analyzing the expression of SMARCA4 in MDA-231 and LM2 cells.

Supplementary Materials and methods

Cell lines and cell culture. Human breast cancer cell lines MDA-MB-231, kindly provided by Prof. MingYao Liu (East China Normal University), were cultured in Leibovitz L-15 medium (11415–064, Gibco) with 10% fetal bovine serum (FBS, Sigma, F2442). LM2 were kindly provided by Prof. Guohong Hu (Shanghai Institute of Nutrition and Health) is a luciferase expressing cell line that was derived from parental MDA-MB-231 cells. Cell lines BT-549 were cultured in RPMI 1640 (SH30027.01, Hyclone) with 10% FBS. The immortalized human embryonic kidney cell line HEK293T were cultured in Dulbecco's modified Eagle's medium with 10% FBS. All cell lines were fostered in a humidified atmosphere of 5% CO2 and 95% air except for MDA-MB-231, which were fostered at 37°C in a humidified atmosphere containing 100% air. All cell lines were authenticated by short tandem repeats (STR) profiling.

Plasmids, shRNA and Retroviruses. All information about the plasmids (SI Appendix Table S3), shRNA sequences (SI Appendix Table S4) are provided, in which full length SMARCA4 CDS expressing plasmid which was later cloned into pcDNA3.1(+) was a gift from Xiang Huo He (Fudan University Shanghai Cancer Center and Institutes of Biomedical Sciences). Retrovirus was produced by co-transfecting 293T cells with the retroviral construct pSIREN-RetroQ plasmid containing the genes, and the pCMV-Gag-Pol packaging plasmid containing the gag and pol, and the pVSV-G envelope-expressing plasmid, using Lipofectamine 2000 Transfection Reagent (Thermo Fisher Scientific). Viral supernatant was harvested at 72 hrs post-transfection, filtered through a 0.45μm PVDF membrane, contained 8μg ml−1 polybrene, and used to infect the target cells at 40% confluence.

LncRNA-seq. Total RNA from MDA-MB-231 and LM2 cell lines were extracted

using TRIzol reagent (Invirogen) according to manufacturer's instructions. RNA integrity and purity were verified by formaldehyde agarose gel electrophoresis and Bioanalyzer 2100 (Agilent, Santa Clara, CA, USA). Total RNA were quantified by Nanodrop 2000 spectrophotometer with OD260/280 between 1.8 to 2.0. Before the RNA libraries were constructed, ribosomal RNA (rRNA) was depleted from DNA-free RNA by an Epicentre Ribo-Zero Gold Kit (Illumina, San Diego, CA, USA). Then rRNA-free RNA was reverse-transcribed by random primers to create the final complementary DNA (cDNA) libraries. Finally, the libraries were paired-end sequenced on an Illumina HiSeq 2500 (Illumina, San Diego, CA, USA) at Guangzhou RiboBio Co., Ltd. (Guangzhou, China). Cutadapt and FastQC were used to remove reads containing adaptors and low-quality bases, and to verify sequence quality. Then clean data were mapped to Human Genome Assembly GRC38. The whole samples expression levels were presented as RPKM. Significant differentially expressed lncRNAs were chosen according to two criteria: (1) a significance level $p < 0.05$; and (2) RPKM ≥ 1.

Cell transfection and Oligo RNA transfection. The sequences of small interfering RNA (siRNA) oligonucleotides are provided in Supplementary Data Table S1, and they were purchased from Shanghai GenePharma Co. Ltd. Oligo RNA transfection (100 nM) were performed using Lipofectamine 2000 according with Reverse Transfection Protocol. For plasmids transfection, DNA (μg): Lipofectamine 2000 (μl) ratios 1:2, plasmid and reagent were diluted in Opti-mum respectively. DNA-liposome complexes forming and cells transfecting were performed as Lipofectamine 2000 manual.

RNA extraction, Reverse transcription PCR and Quantitative real-time PCR. Total RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA) according

to the manufacturer's instructions. First-strand cDNA was synthesized using Reverse Transcriptase kit (Tiangen, China). Quantitative Real-Time PCR (qPCR) using the SYBR Green (Applied Biosystems, USA) method on the 7900 Real-Time PCR System with the SDS 2.4 software sequence detection system (Applied Biosystems, USA). The sequences for the gene-specific primers used are listed in Supplementary Data Table S3. β-actin was employed as an internal control to quantify TGFB2-AS1 and the mRNA levels of other genes. The relative levels of RNA were calculated using the comparative CT (2−ΔΔCT) method.

***In vivo* assays.** Female NOD/SCID mice or female BALB/c nude mice were purchased from Shanghai Slack Laboratory animal Co., Ltd and housed under SPF conditions. For *in vivo* tumorigenesis assays, the indicated number of LM2 cells suspended in 75μl phosphate-buffered saline containing 37.5μl matrigel were injected subcutaneously into 6 weeks old BALB/c female nude mice. For xenograft models, 3×10^5 LM2 cells were orthotopically injected directly into the mammary fat pads of female NOD/SCID mice in 100 μl phosphate-buffered saline containing 25% matrigel. Tumor growth rate was monitored by measuring tumor diameters (long and short diameters) every 4 days since indicated post injection date. The tumor volume was calculated as long diameter × (short diameter)^2/2. Before mice were killed, mice were given intraperitoneal injection of d-luciferin (150 μg/g prepared in phosphate buffered saline). After 10 mins, the mice were killed under anesthesia and tumors were isolated and weighed. Lungs were dissected and monitored by bioluminescence imaging (BLI) with IVIS system (Xenogen). After that, lung were fixed in 4% polyoxymethylene for H&E staining analysis.

***In vitro* translation assay**. *In vitro* translation of *TGFB2-AS1* was conducted using a TnT Quick Coupled Translation Kit (Promega, USA). Briefly, To use cell-free

expression systems, 2.0 μg of circular plasmid DNA containing a T7 promoter, is added to an aliquot of the TnT Quick Master Mix and incubated in a 50 μl reaction volume for 90 min at 30°C. After SDS-PAGE and electro-blotting, the biotinylated proteins can be visualized via binding of streptavidin-horseradish peroxidase (Streptavidin-HRP), followed by chemiluminescence detection. LncRNA TGFB2-AS1 was cloned into pcDNA3.1(+) downstream of its T7 promoter.

Transwell migration and invasion assays. Cell migration and invasion ability were determined by Corning transwell insert chambers (8μm pore size; Corning) and BD BioCoat Matrigel Invasion Chamber (BD Biosciences, Bedford, MA), respectively. The chemoattractant was 500ml or 600ml medium containing 10% FBS which were added into the lower well of each chamber. About 4×10^4 (migration assay) or 8×10^4 (invasion assay) prepared cells were added into the chamber and incubated for 18-20 hrs 37°C.

Soft Agar Colony-Forming Units Assay, Plate Colony-Forming Units Assay, Mammosphere Assay, 3D cell culture and Cell proliferation assay. For soft agar colony formation assay, 3.5cm cell culture dish was paved with 1.5ml 0.8% melted low melt agar solution. After solidifying, top agar (1ml 0.4%) with 2500 cells were added and placed into a 37°C humidified cell culture incubator. Cultured two months continuously and prevented desiccation. For plate colony-forming unit assay, cells were seeded into 3.5cm culture dishes at 800 cells. Cell continuously incubated for 12 days and the medium was changed every 3 days. The colonies were stained by crystal violet (1.5%, w/v; Sigma, St Louis, MO, USA). For the mammosphere assay, according to the manufacturer's protocol, MammoCult™ Human Medium Kit (STEMCELL 05620) and 24-well Ultra-low Adherent plate were used. The cells were trypsinized, filtered through a 40 μm Strainer and seeded 1250 cells per well, cultured in incubater for 7

days. For 3D cell culture, Cells were cultured in 2% matrigel diluted in completed medium. The bottom of U-wells of uncoated Angiogenesis μ-slides (Ibidi Gmbh, Germany) were filled with 10 ml of Matrigel and polymerized at 37°C for 30 min. Cell culture medium was changed every second day. For CCK-8 assay, the cells were embedded in 96-well plates (3×10^3cells per well) and incubated lasting 4 days at 37°C. The changes of cell proliferation were monitored every day using CCK-8 reagent (Dojindo, Kumamoto, Japan) and the absorbance values were measured at 450 nm via a Hybrid Reader (BioTek Laboratory Instrument).

Microarray Expression Profiling. Total RNA of MDA-MB-231 cells from TGFB2-AS1 knockdown group and control group were extracted using TRIzol reagent (Invirogen) according to manufacturer's instructions. The whole genomic expression profile microarray (Agilent, Human GE 4x44k v2) was done by KangCheng Bio-tech, Shanghai, China. The data extracted from Agilent Feature Extraction software (version 11.0.1.1) were quantile normalized and analyzed by the GeneSpring GX v12.1 software package (Agilent Technologies). After quantile normalization of the raw data, genes that at least 3 out of 6 samples have flags in Detected ("All Targets Value") were chosen for further data analysis. Differentially expressed genes with statistical significance between the two groups were identified through Volcano Plot filtering. Differentially expressed genes between the two samples were identified through Fold Change filtering.

ChIP-qRT-PCR. ChIP (Chromatin immunoprecipitation) was performed with 4 x 10^6 cells per reaction. Cells were cross-linked with a final concentration of 1% formaldehyde in growth medium for 10 min at RT, and cross-linking was quenched by the addition of glycine to a final concentration of 0.125M and incubation for 5 min at RT. Cells were rinsed twice with cold PBS supplemented with 1mM PMSF (Roche) and 1 x protease inhibitor cocktail (CST). Cells were then lysed by ChIP lysis buffer

(CST) and socinated to shear the chromatin to yield DNA fragment sizes of 0.2 to 1 kb. A portion of the sample was used as input DNA. Then, 10 μg of SMARCA4 (CST，D1Q7F) antibody or rabbit normal immunoglobulin (IgG) was added to the remainder of the samples and incubated on a rotator for overnight at 4°C. Next day, ChIP-Grade Protein G Magnetic Beads (CST，#9006) were added, and the mixture was incubated for 4 hr at 4°C. Beads were washed three times with 1x ChIP buffer（CST, #7008）, one time with 1ml lysis buffer with 1x ChIP buffer and 0.5 MNaCl by magnetic separation rack. The chromatin was eluted in ChIP Elution Buffer (CST，#7009) followed by reverse crosslinking at 65°C for at least 4 hrs. ChIP DNA was treated with 5g/ml RNase A and 0.2mg/ml protease K, and purified using NucleoSpin Gel and PCR Clean-up (MACHEREY-NAGEL). The purified ChIP DNA was quantified by qRT-PCR. The primers (SI Appendix Table S5) used are listed in Supplementary Table .

Conditional medium stimulation assay. After 60 hours of MDA-MB-231cells transfected with siAS1 or siNC, the cell culture medium was changed to fresh medium without FBS. Re-incubating for 24 hours, the medium was harvested, filtered through 0.45μm filter membrane and used for conditional medium. MDA-MB-231 or LM2 cells were plated in completed culture medium. After 24 hours, serum-free medium was replaced and incubated for 24 hrs. FBS free culture medium was withdrawn and changed to the conditional medium with 1% FBS. Cells re-incubated at 37°C.

The immunohistochemistry (IHC). Paraffin-embedded tissue slides were de-paraffinized at 60°C, followed by treatment with xylene and a graded alcohol series. The endogenous peroxidase activity was blocked by incubation in a 3% hydrogen peroxide solution for 15 min. After antigen retrieved, the slides were rinsed and incubated with 5% BSA to block no-specific staining. Then, the primary antibody was incubated with overnight at 4°C in a humid chamber. The slides were visualized by

standard avidin-biotinylated peroxidase complex method. Finally, hematoxylin was used for counterstaining. All of the ISH and IHC staining were assessed by two pathologists blinded to the origination of the samples and subject outcome. The staining intensity was graded as follows: 1, no staining; 2, weak staining (light purple for ISH, light yellow for IHC); 3, moderate staining (moderate purple for ISH, yellow brown for IHC); and 4, strong staining (strong purple for ISH, brown for IHC). The proportion of tumor cells with positive staining was designated as follows: 0, no positive tumor cells; 1, 0%–10% positive tumor cells; 2, 10%–30% positive tumor cells; 3, 30%–70% positive tumor cells; and 4, more than 70% positive tumor cells. The staining index (SI) was calculated by multiplying the proportion of positive tumor cells and the staining intensity score, with a final score ranging from 0 to 4.

Immunoblotting. Cell or tissue lysates were lysed for 15 min with 1xSDS lysis buffer and the total protein was separated by SDS PAGE and transferred to nitrocellulose membrane (Axygen, Union City, CA). After blocking with 5% non-fat milk, the membrane was incubated with primary antibodies overnight at 4℃ and then incubated with horseradish peroxidaseconjugated secondary antibody. The signal was detected in a sensitive digital imaging equipment (ImageQuant LAS 4000 mini, GE Healthcare, Piscataway, NJ, USA) using the ECL detection kit (Millipore).

Gene set enrichment analysis (GSEA). We used GSEA v3.0 to perform GSEA on various gene signatures. Gene sets were either obtained from the MSigDB database v7.0 or from published gene signatures (https://www.gsea-msigdb.org/gsea/msigdb/index.jsp). Statistical significance was assessed by comparing the enrichment score to enrichment results generated from 1,000 random permutations of the gene set to obtain p values (nominal p value).

Ingenuity Pathways Analysis (IPA). The Ingenuity pathway analysis (IPA,

Qiagen, Redwood City, http://www.ingenuity.com/) tool was used for pathway analysis and upstream regulator prediction.

Fluorescence co-location analysis. Microscopy images from MDA-231 cells with TGFB2-AS1 and SMARCA4 stained via FISH and Immunofluorescence respectively were scanned by Confocal microscope. Nucleus selected as region of interest (ROI), an overlap coefficient according to Mander's, and overlap coefficients k1 and k2 were examined using ImageJ with coloc2 plug-in.

RNA FISH and RNA ISH. TGFB2-AS1 expression was examined by LNA probes (Qiagen, 339500) which were designed using Primer 3 and checked for self-complementarity and secondary structure using Qiagen's LNA Oligo Optimizer tool (www.qiagen.com). Probe sequence against all known human sequences in NCBI using BLAST to ensure it is unique to the target RNA. LNAs are available with a DIG label at the 5′ and 3′ends. For RNA FISH, cells were washed shortly with phosphate-buffered saline (PBS), fixed with 4% formaldehyde for 15 min, treated with pepsin (0.1% in 10 mM HCl) for 10 min at room temperature, dehydrated through 70%-, 90%- and 100% ethanol and air dried. Cells were hybridized with denature probe (90°C for 4mim) and diluted probe (40nM in formamide hybridization buffer) for 3 hr in a humid chamber at 42°C for 3 hr. Then, washed 2 x 5 min with 2xSSC, 0.1% Tween-20; washed 5 min with 1xSSC, washed 3 x 5 min with 0.1xSSC at 42°C. For In situ hybridization (ISH), paraffin sections were dewaxed in xylene and rehydrated through ethanol graded solutions to PBS following the traditional sequence. Pepsin treatment in 3% citrate buffer at room temperature for 10 min was performed. sections were subjected to incubation with denatured Dig-labeled probes (40 nM) in formamide hybridization buffer at 42°C for 3 hr. After hybridization, sections were blocked with blocking solution (2% sheep serum and 1% bovine serum albumin in PBST) at room temperature

15 min. Alkaline phosphatase (AP)-conjugated anti-DIG (11 093274 910 Roche) was diluted 1:1000 in the blocking solution and incubated for 60 min at room temperature. BCIP/NBT and levamisole was diluted in AP-buffer (1M NaCl, 50mM MgCl2, 1M Tris .Cl PH 9.5).The enzymatic reaction was performed in the humidifying chambers at 30°C overnight to develop the dark-blue NBT-formazan precipitate. Slides were stained with nuclear fast red at room temperature for 2 min. Then samples were washed, dehydrated and mounted. Results were analyze by light microscopy on one of the subsequent days.

RNA immunoprecipitation assay. MDA-MB-231 cells at a concentration of 2 x 10^6 cells/mL were treated with 0.3% formaldehyde in medium for 10 min at 37°C. 1.25 M glycine dissolved in PBS was added to a concentration of 0.125 M, and the sample was incubated for 5 min at room temperature. Cells were then washed twice in cold PBS and pelleted. The pellet was resuspended in 1 ml of RIPA lysis buffer (100 mM Tris, pH 7.4, 150 mM NaCl, 25 mM ethylenediaminetetraacetic acid [EDTA], 25 mM MgCl2, 0.05% Nonidet P-40, 1 mM dithiothreitol [DTT] and 1 mM phenylmethylsulfonyl fluoride mixture treated with RNase free water), incubated on ice with frequent vortex for 30 minutes, and the lysate was obtained by centrifugation at 2000g for 5 min. Antibodies were added and samples were incubated for 4 hrs at 4℃. Samples were washed two times in RIPA buffer, four times in 1M RIPA buffer (100 mM Tris, pH 7.4, 150 mM NaCl, 2 mM EDTA, 0.05% NP-40, and 1mM DTT treated by RNase free water). The beads were resuspended in RIPA buffer and treated with proteinase K at 45°C for 45 min. RNA samples were extracted with 1ml Trizol. Proteins isolated before proteinase K treatment from the beads were detected by western blot analysis.

RNA pull-down assay. 4×10^7 MDA-MB-231 cells were transfected with

pcDNA3.1-4×S1m or pcDNA3.1-4×S1m-TGFb2-AS1. After 48 hr, cells were washed twice with PBS and harvested and solubilized in 5ml ice-cold SA-RNP lysis buffer [20mM Tris-HCl (pH 7.5), 150mM NaCl, 1.5mM MgCl2, 2mM DTT, 1% Triton-100, 500u/ml RNase inhibitor (TaKaRa,2313A), 1 tablet/10 ml Mini Complete Protease Inhibitors, EDTA-free (MCN, HY-K0010)]. Incubating on an end-over-end rotator for 30 min at 4°C, sonication for 6×10 sec with an interval of 1 min on ice， cell debris was removed by centrifugation for 15 mins at 14000 rpm at 4°C, supernatant was transferred to a fresh tube, 100μl was saved as protein input. Lysates were first incubated with 50 μg/ml tRNA (Invitrogen, AM7119) for 20 min at 4°C before add 20μl Streptavidin Magnetic Beads (Bio lab, S1402s) for 3 hrs at 4°C under rotation. SA-RNP was washed five times for 10 min at 4°C with wash buffer [20 mM Tris-HCl (pH 7.5), 300Mm NaCl, 5mM MgCl2, 2 mM DTT, 1% Triton -100]. Protein was eluted from the beads by 80 μl 1×SDS loading buffer for 10 min at 95°C. The eluate was separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis and Coomassie staining before liquid chromatography tandem mass spectrometry analysis.

***In vitro* RNP purification.** Six RNAs were synthesized by *in vitro* transcription according to the instructions (Large Scale RNA Production Systems, promega, P1280 and P1300): 4×S1m as negative control RNA, 4×S1m-TGFb2-AS1 attaching the 4×S1m aptamer to full length of TGFb2-AS1, 4×S1m-TGFb2-AS1-ex1 containing the first exon, 4×S1m-TGFb2-AS1-ex1/2 containing the first exon and the second exon, 4×S1m-TGFb2-AS1-ex2/3 containing the second exon and the third exon, 4×S1m-TGFb2-AS1-ex3 containing the third exon, respectively. The synthesized RNAs were isolated by TRIZOLl regent (Invitrogen, 15596-026). For the following procedures, 6×10^7 MDA-MB-231 cells were solubilized in 8ml ice-cold SA-RNP lysis buffer with 1% Triton-100, followed by 6×10 sec sonication with an interval of 1 min on ice. After

centrifuging at 14,000 rpm for 10 min at 4°C, the supernatant was pre-cleared with 30 µl Streptavidin Magnetic Beads (Bio lab, S1402s) beads for 30 min at 4°C. 100 µl was saved as protein input, the rest was divided into six aliquot parts. 2 µg of the *in vitro* transcribed RNAs was in 50 µl SA-RNP lysis buffer by heating at 56°C for 5 min, 10 min at 37°C and incubation at room temperature for several minutes to refold RNA structures. 2 µg renatured RNA further incubated with lysate for 3 hrs at 4°C on rotator. Then, the beads were washed with washing buffer for 6×10 min at 4°C on rotator. The RNA binding proteins were eluted from the beads by 80µl 1×SDS loading buffer for 10 min at 95°C. LncTGFb2-AS1 associated proteins were identify by Western blot.

Luciferase reporter assay. For the TGFB2 promoter reporter assay, plasmid was constructed by inserting 3kb human TGFB2 promoter into the chromatinized episomal pREP4-Luc plasmid. Plasmids were transfected into MDA-MB-231 cells using lipofectamine 2000 (Invitrogen). The cells were cultured for 48 hrs and harvested for luciferase assay using the dual luciferase assay kit (Promega) on a Berthold AutoLumat LB9507 rack luminometer.