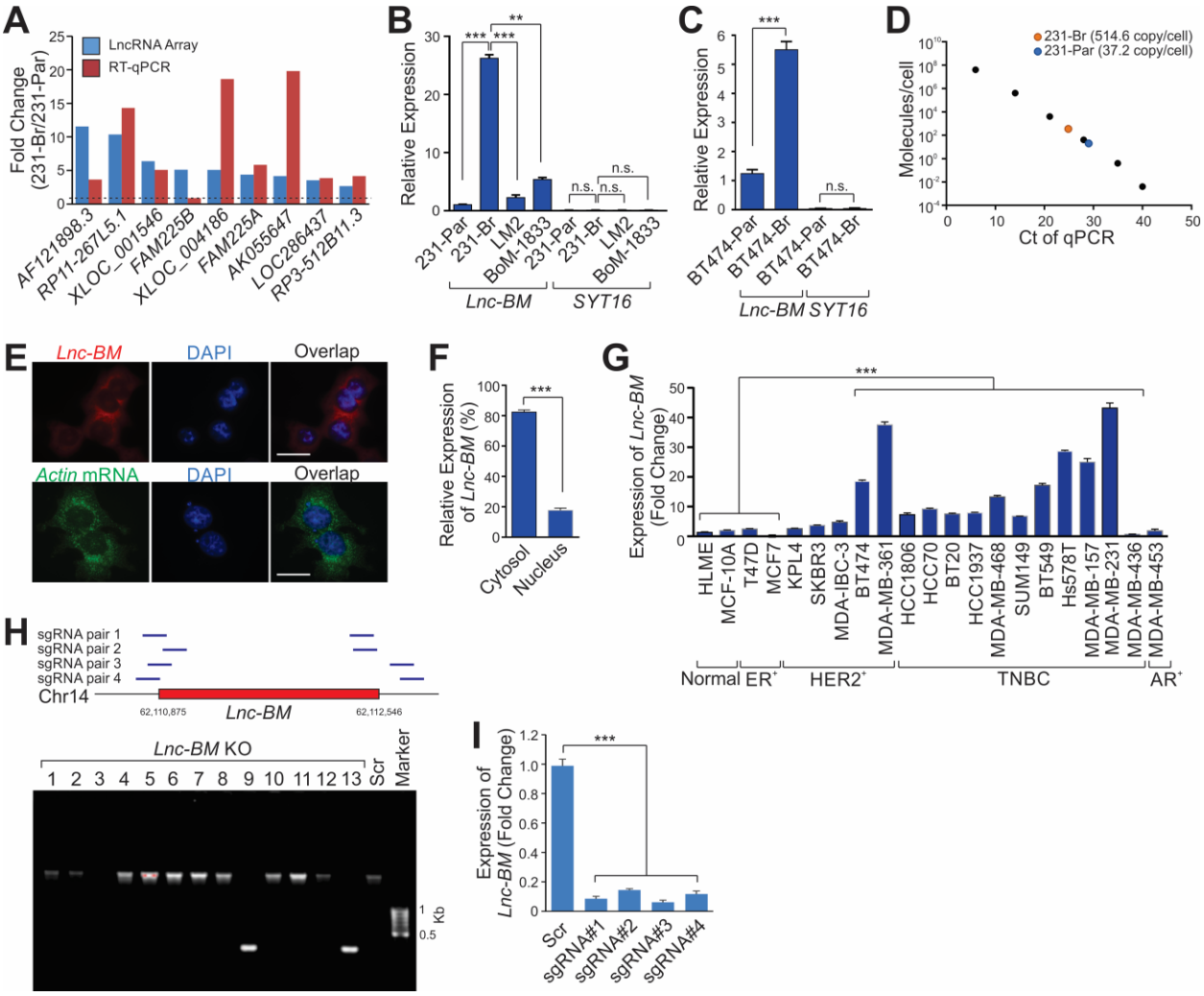
SUPPLEMENTAL INFORMATION

Supplemental Figure Legends:



Supplemental Figure 1, related to Figure 1. Lnc-BM expression in breast cancer cells and characterization of Lnc-BM.

(A) Fold changes of the expression of the top 9 lncRNAs (231-Br/231-Par) from the lncRNA array or detection by RT-qPCR. Black dashed line: fold change = 1.

(B-C) RT-qPCR detection of Lnc-BM expression in MDA-MB-231 derived brain seeking cells (231-Br), lung metastatic cells (LM2), and bone metastatic cells (BoM-1833) (B), or in BT474

brain seeking (Br) cells and paired parental cells (C) (n = 3 independent experiments, paired Student's t-test).

(D) Calculation of Lnc-BM copy numbers in 231-Br or 231-Par cells.

(E) Cytoplasmic localization of Lnc-BM detected by RNA FISH. Actin mRNA serves as specificity control (n = 3 independent experiments), scale bar = 50 μm.

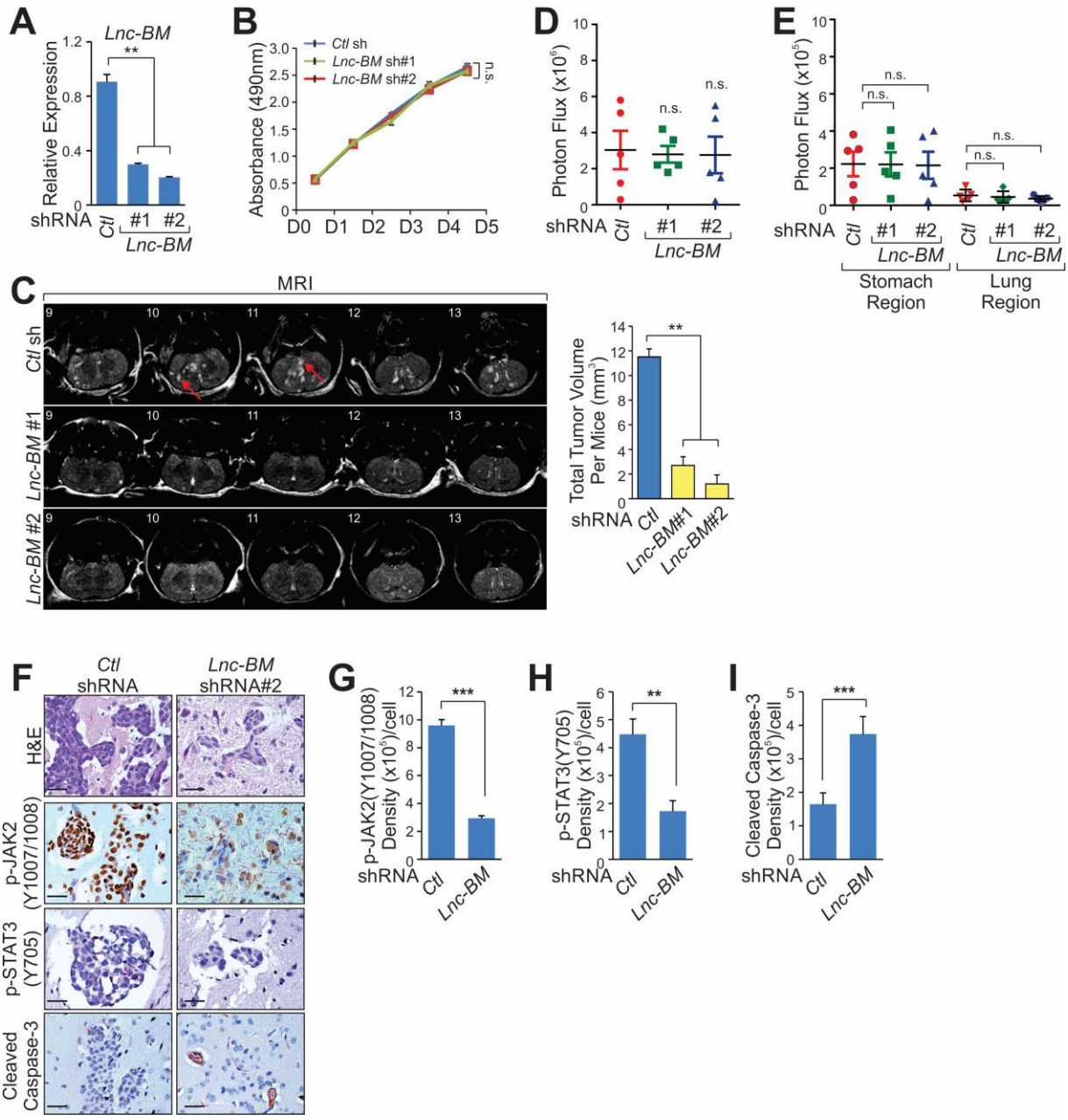
(F) Cytoplasmic localization of Lnc-BM detected by RT-qPCR (n = 3 independent experiments, paired Student's t-test).

(G) RT-qPCR detection of Lnc-BM expression in a panel of breast cell lines (n = 3 independent experiments, one-way ANOVA).

(H) Top panel: graphic illustration of sgRNAs of *Lnc-BM*. Bottom panel: agarose gel detection of the depletion of *Lnc-BM* gene.

(I) RT-qPCR detection of Lnc-BM expression in the presence of indicated sgRNAs (n = 3 independent experiments, one-way ANOVA).

Data are mean ± S.E.M, n.s. $p > 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.



Supplemental Figure 2, related to Figure 2. Expression of Lnc-BM and its associated proteins in mice brain metastatic lesions.

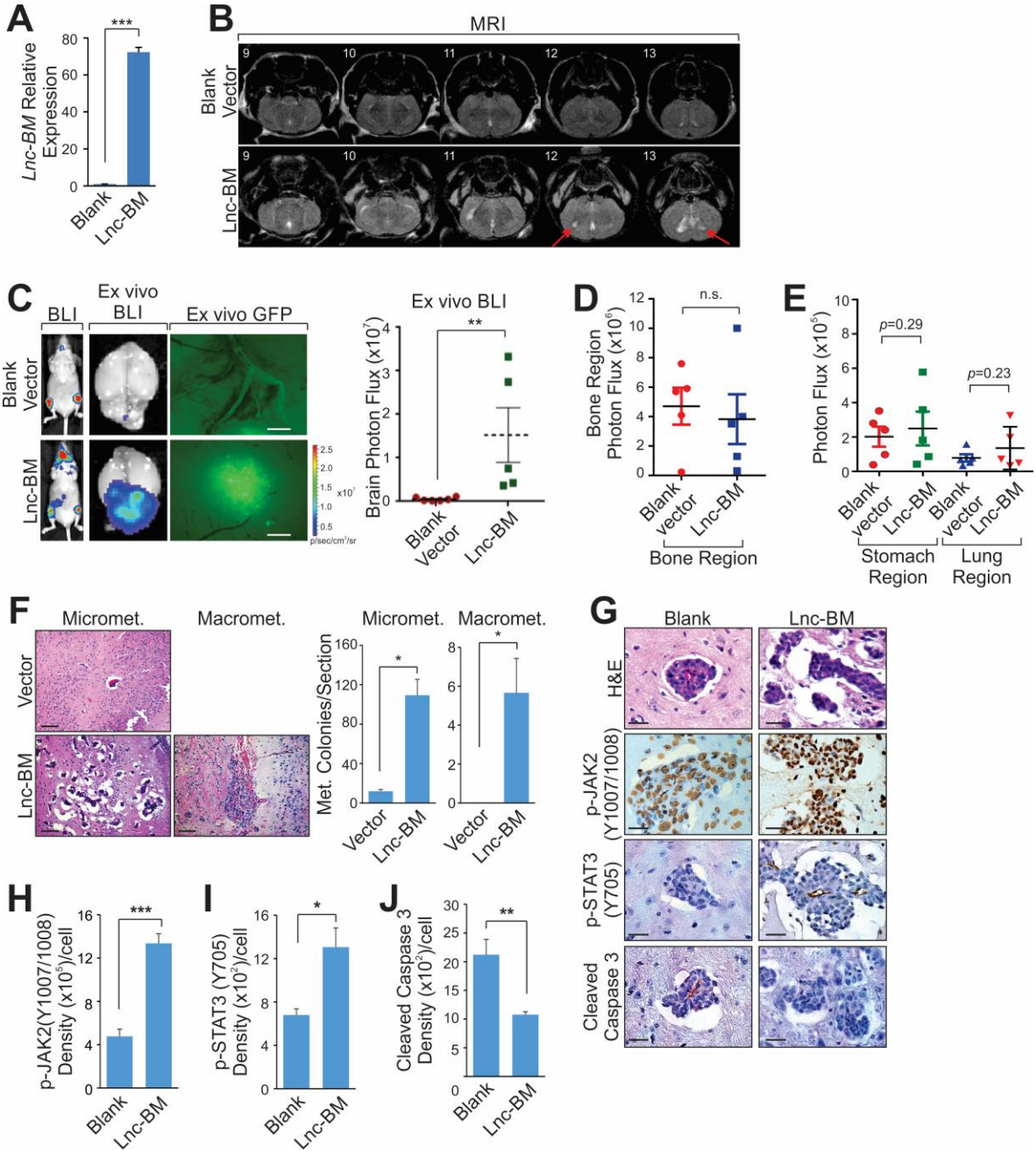
(A) RT-qPCR detection of Lnc-BM expression in 231-Br cells stably expressing indicated shRNAs (n = 3 independent experiments, unpaired Student's t-test).

(B) Cell proliferation rate was assessed by OD density (490 nm) in 231-Br cells stably expressing indicated shRNAs (n = 6 independent experiments, one-way ANOVA).

(C) MRI imaging or statistical analysis of mouse, 4 weeks post intracardiac injection of 231-Br cells harboring indicated shRNAs (*n* = 3 animals per group, one-way ANOVA). Red arrows: metastatic lesions. 9-13: sections numbers of MRI images from top to bottom of the mouse brain.

(D-E) Quantification of BLI of the tumor lesions in bone (D) or stomach and lung region (E), related to Figure 2C (n = 5 animals per group, one-way ANOVA).

(F-I) Representative images (F) or statistical analysis of p-JAK2 (G), p-STAT3 (H), or cleaved Caspase 3 (I) staining intensity in brain metastasis-bearing mice inoculated with 231-Br cells harboring indicated shRNAs (*n* = 3 animals *per* group, paired Student's t-test), scale bar = 100 μm. Data are mean ± S.E.M, n.s. $p > 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.



Supplemental Figure 3, related to Figure 2. Overexpression of Lnc-BM in 231-Par cells promotes BCBM in vivo

(A) RT-qPCR detection of Lnc-BM expression in 231-Par cells stably expressing exogenous Lnc-BM (n = 3 independent experiments, paired Student's t-test).

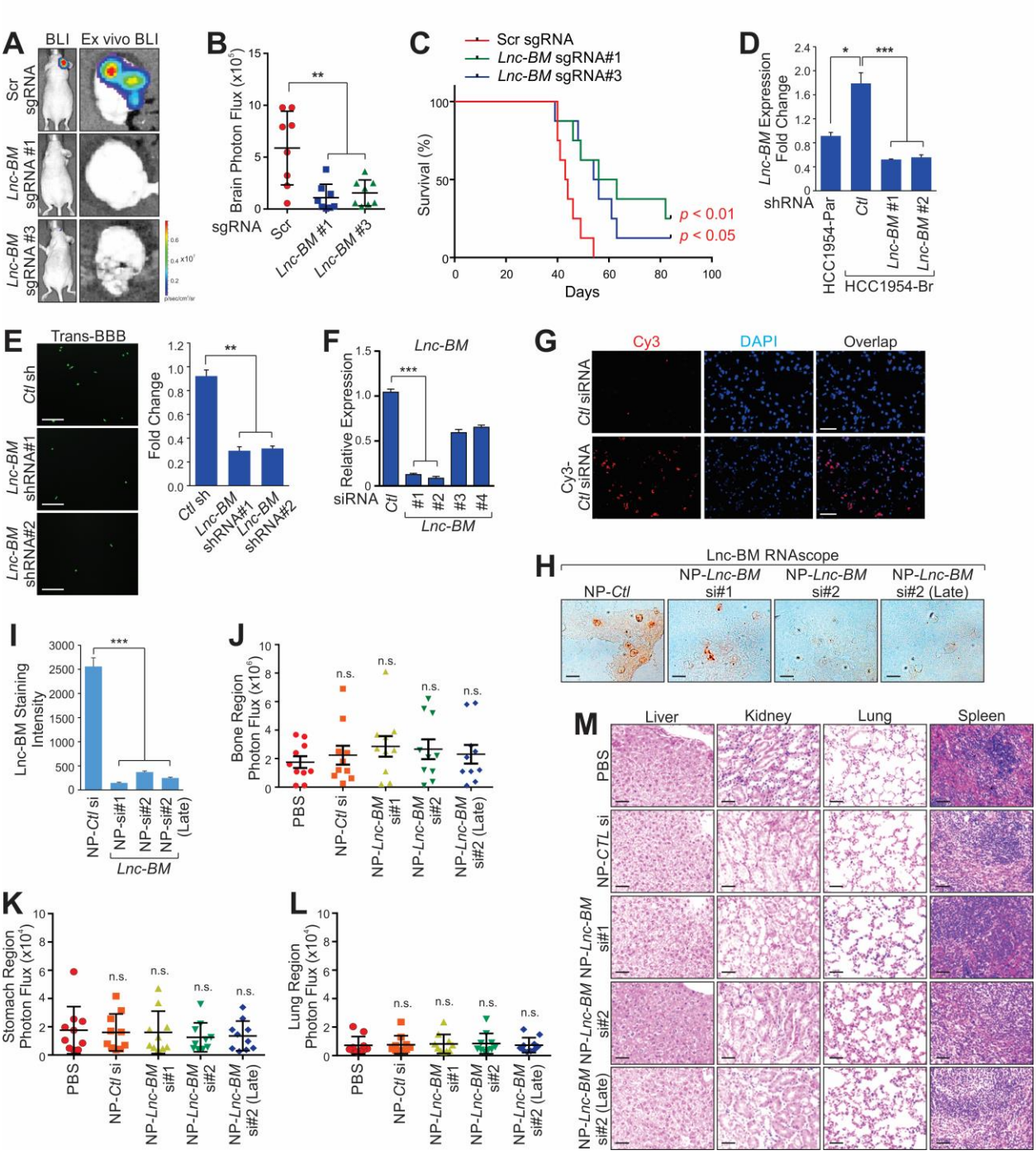
(B-C) Representative images of MRI (*n* = 3 animals per group) (B) and BLI (*n* = 5 animals per group), brain ex vivo BLI and brain ex vivo GFP (C, left panel) 5 weeks after intracardiac injection of 231-Par cells stably expressing indicated expression vector (one-way ANOVA). Scale bars: 200 μM. Red arrows (B): metastatic lesions. 9-13: sections numbers of MRI images from top to bottom of the mouse brain.

(D-E) Quantification of BLI of the tumor lesions in bone (D) or stomach and lung regions (E), related to Supplemental Figure 3C (n = 5 animals per group, one-way ANOVA).

(F) Representative images (left panels) and statistical analyses (middle and right panels) of micro-metastasis (Micromets) and macro-metastasis (Macromets) in brain metastasis-bearing mice inoculated with 231-Par cells expressing blank vector or Lnc-BM (*n* = 3 animals per group, paired Student's t-test), scale bar = 200 μm.

(G-J) Representative images (G) or statistical analysis of p-JAK2 (H), p-STAT3 (I), or cleaved Caspase 3 (J) staining intensity in brain metastasis-bearing mice inoculated with 231-Par cells expressing blank vector or Lnc-BM (*n* = 3 animals per group, paired Student's t-test), scale bar = 100 μm.

Data are mean ± S.E.M, n.s. $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.



Supplemental Figure 4, related to Figure 2 and 3. Lnc-BM is required for the development of brain metastasis.

(A-B) BLI and ex vivo BLI imaging (A) and quantification of ex vivo BLI imaging (B) of mouse, 4 weeks post intra-arterial injection of 231-Br cells harboring indicated sgRNAs (n = 8 animals *per* group, one-way ANOVA).

(C) Kaplan-Meier plot of survival of animals subjected to intra-arterial injection of 231-Br cells harboring indicated sgRNAs (n = 8 animals *per* group, Log rank test).

(D) RT-qPCR detection of Lnc-BM in HCC1954-Par, HCC1954-Br cells harboring indicated shRNAs (n = 3 independent experiments, paired Student's t-test).

(E) In vitro BBB transmigration activity of the HCC1954-Br cells harboring control or Lnc-BM shRNAs. The number of transmigrated cells relative to the control cells is plotted (n = 3 independent experiments, unpaired Student's t-test). Scale bars = 100 μm.

(F) RT-qPCR detection of Lnc-BM in 231-Br cells harboring indicated siRNAs (n = 3 independent experiments, unpaired Student's t-test).

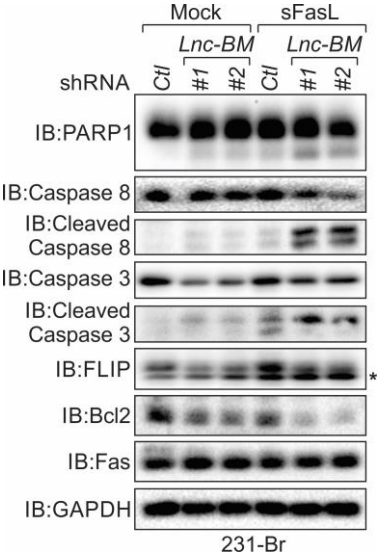
(G) Fluorescence microscopy detection of nanoparticle-coated control siRNA (NP-siRNA) labelled with or without Cy3 in the mice brain 48 hr post-intravenous injection of NP-siRNAs (*n* = 3 animals *per* group), scale bar = 200 μm.

(H and I) Representative images (H) and quantification analysis of Lnc-BM expression using staining density of RNAscope (I) in the brain metastatic lesion. Randomly selected mice were examined after 4th injection of NP-siRNAs (*n* = 3 animals per group, unpaired Student's t-test). Scale bar = 100 μm.

(J-L) Quantification of BLI of the tumor lesions in bone (J), stomach (K) or lung regions (L), related to Figure 3B (n = 10 animals *per* group, one-way ANOVA).

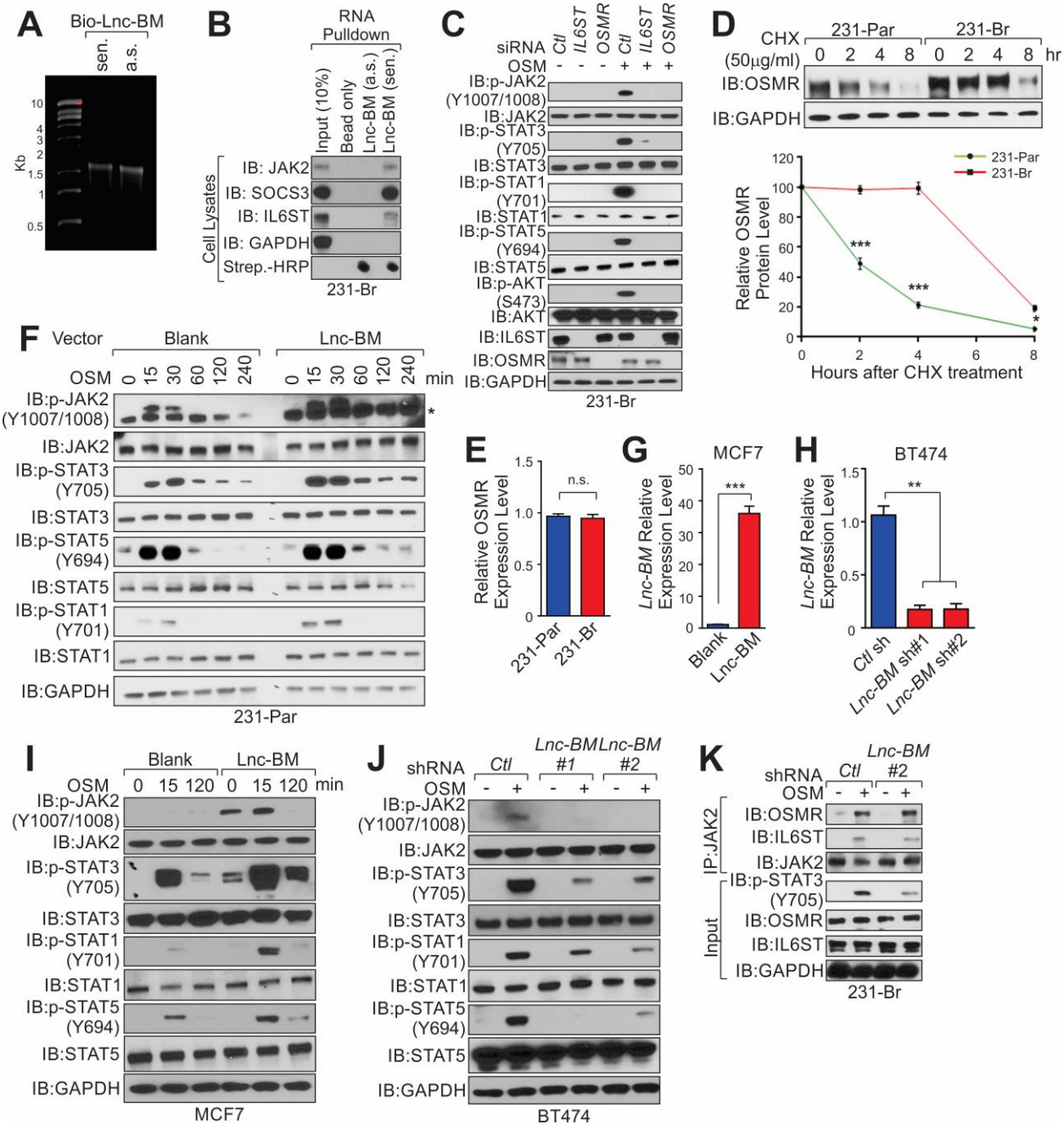
(M) H&E staining of indicated organs in brain metastasis-bearing mice inoculated with 231-Br cells followed by administration of indicated NP-siRNAs, related to Figure 3B (*n* = 3 animals per group), scale bar = 200 μm.

Data are mean ± S.E.M, n.s. $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.



Supplemental Figure 5, related to Figure 6, Lnc-BM attenuates FasL-induced apoptosis.

IB detection of MDA-MB-231 cells harboring indicated shRNAs following by sFASL (200 ng/ml) treatment for 24 h. *, unspecific band.



Supplemental Figure 6, related to Figure 7. Lnc-BM regulates OSM-triggered JAK2/STATs signaling.

(A) Denaturing agarose gel electrophoresis of *in vitro* transcribed biotinylated Lnc-BM sense (sen.) and antisense (a.s.) transcripts.

(B) IB detection using indicated antibodies to detect the proteins retrieved from RNA pulldown using *in vitro* transcribed biotinylated Lnc-BM from 231-Br cell lysates. Streptavidin-HRP indicates the presence of equal amounts of biotinylated RNA transcripts.

(C) IB detection using indicated antibodies in 231-Br cells transfected with indicated siRNAs followed by OSM (50 ng/ml, 30 min) treatment.

(D) Upper panel, IB detection of OSMR protein levels in 231-Par and 231-Br cells treated with 50 μg/ml cycloheximide (CHX) for indicated times. Bottom panel, quantification of OSMR protein levels (n = 3 independent experiments, paired Student's t-test).

(E) RT-qPCR detection of OSMR expression in 231-Par and 231-Br cells (n = 3 independent experiments, paired Student's t-test).

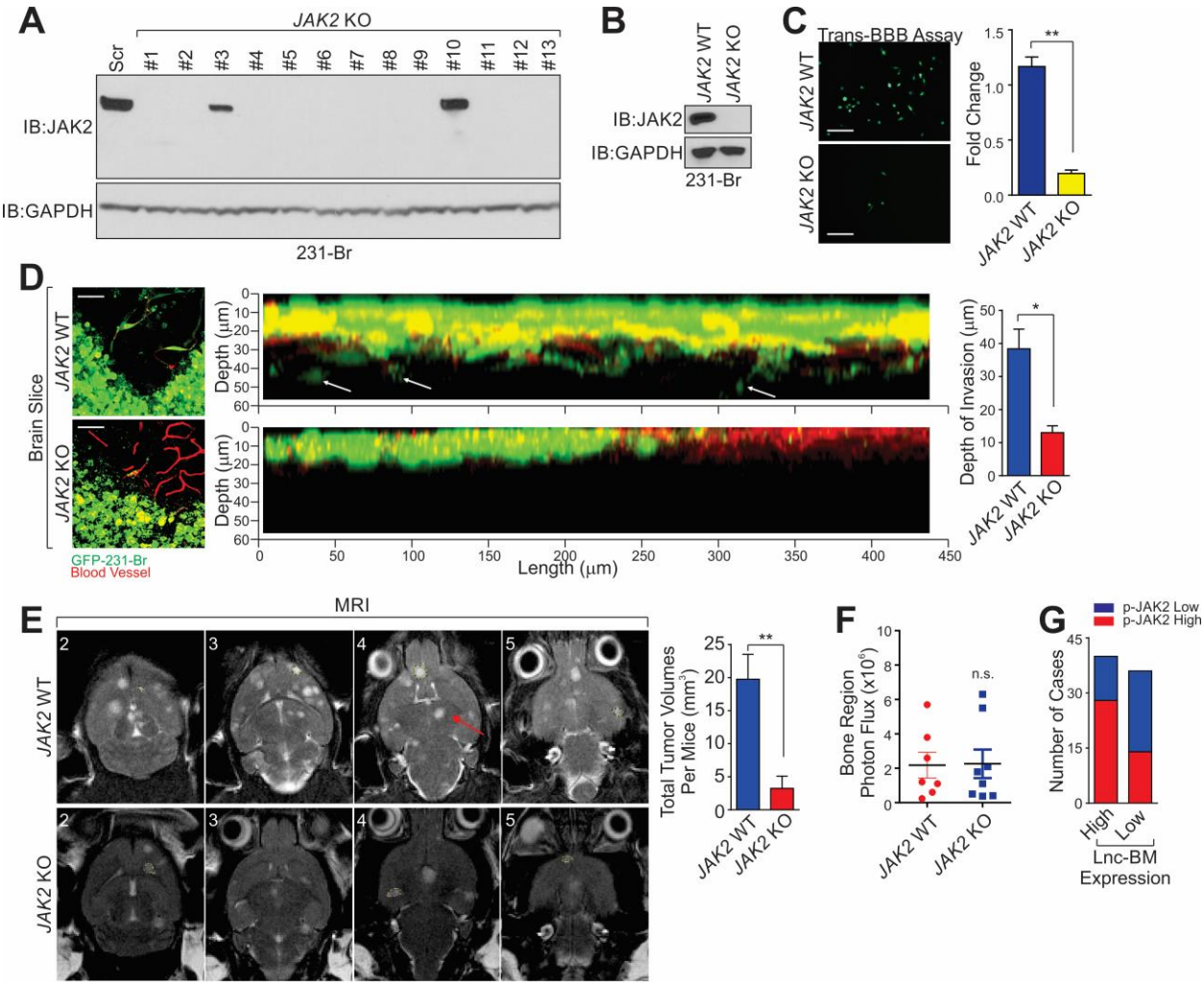
(F) IB detection using indicated antibodies in 231-Par cells with stable overexpression of Lnc-BM that were treated with OSM (50 ng/ml, 30 min) for indicated times. *: unspecific band.

(G-H) RT-qPCR detection of Lnc-BM expression in MCF7 cells stably overexpressing Lnc-BM (G) (n = 3 independent experiments, paired Student's t-test) or BT474 cells with stable knockdown of *Lnc-BM* (H) (n = 3 independent experiments, one-way ANOVA).

(I-J) IB detection using indicated antibodies in MCF7 cells stably overexpressing Lnc-BM (I) or BT474 cells with stable knockdown of *Lnc-BM* (J) treated with OSM.

(K) IP using JAK2 antibody followed by IB detection using indicated antibodies in *Lnc-BM* deficient 231-Br cells treated with OSM.

Data are mean ± S.E.M, n.s. $p > 0.05$, ** $p < 0.01$, *** $p < 0.001$.



Supplemental Figure 7, related to Figure 8. JAK2 is required for BCBM.

(A-B) IB detection using indicated antibodies in 13 single clones (A) or pool of 11 positive single clones (#1/2/4/5/6/7/8/9/11/12/13) (B) selected from CRISPR-Cas9 mediated JAK2 knockout 231-Br cells.

(C) Trans-BBB assay was performed in JAK2 WT or KO 231-Br cells. Left panel, representative images; right panel, quantification of transmigrated cells relative to control samples (*n* = 3 independent experiments, at least 6 different fields in each sample were calculated, paired Student's t-test).. Scale bars = 100 μm.

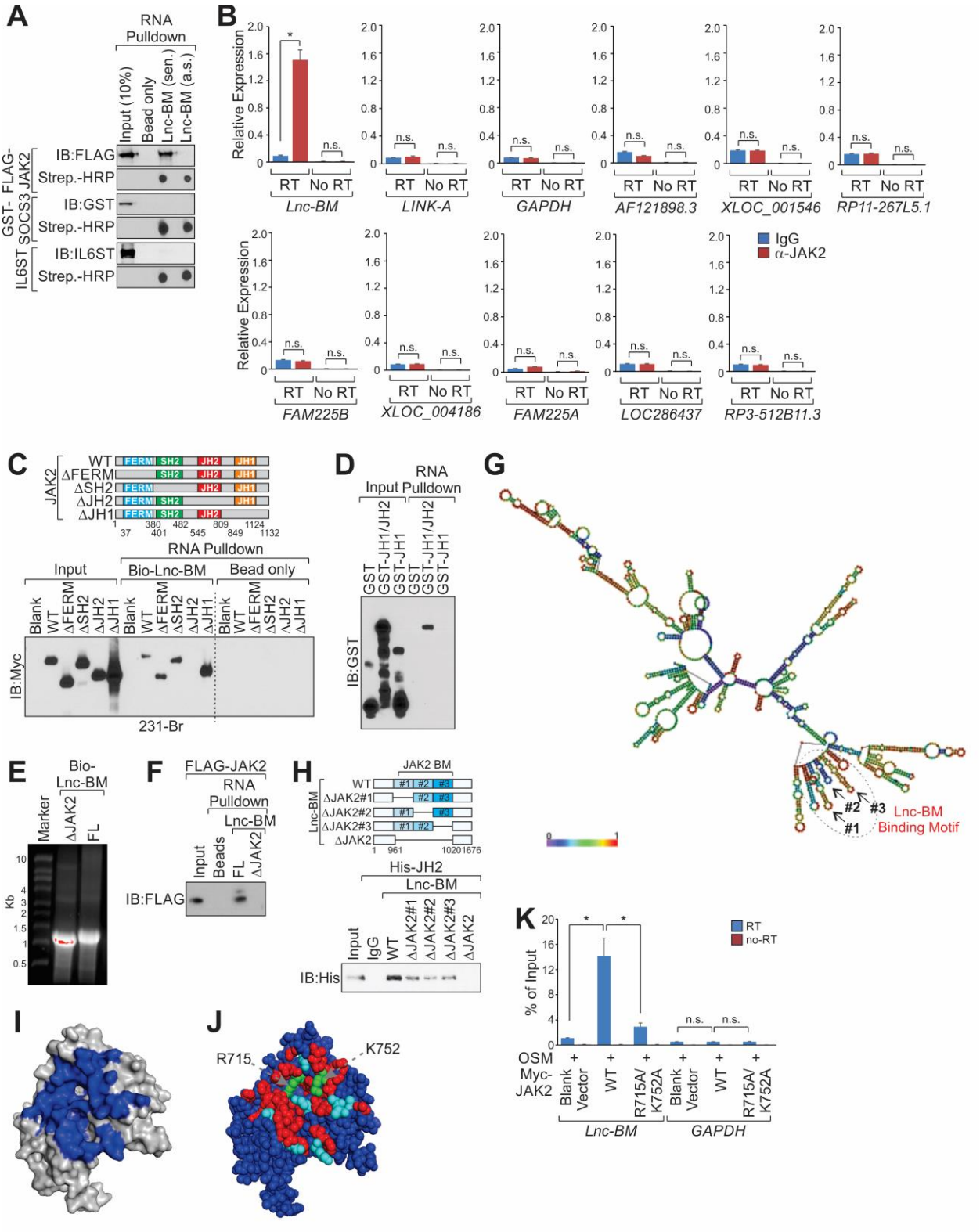
(D) Representative images and quantification of infiltrated depth of brain slices co-cultured with indicated 231-Br cells (n = 3 independent experiments, paired Student's t-test). Scale bars = 50 μm, white arrows: infiltrated cells.

(E) MRI detection (n = 3 animals, paired Student's t-test) of mice 4 weeks post-intracardiac injection of indicated cells. Red arrows: metastatic lesions; 2-5: sections numbers of MRI images from dorsal to ventral of the mouse brain.

(F) Quantification of BLI of the tumor lesions in bone, related to Figure 8A (n = 7, and 7 animals respectively, one-way ANOVA).

(G) High or low expression of Lnc-BM (related to Figure 1I) and p-JAK2 (related to Figure 8C) were plotted for correlation in breast cancer tissues (Fisher's exact test).

Data are mean ± S.E.M, n.s. $p > 0.05$, * $p < 0.05$, ** $p < 0.01$.



Supplemental Figure 8, related to Figure 9. Characterization of Lnc-BM-JAK2 interaction.

(A) IB detection using indicated antibodies to detect proteins retrieved from RNA pulldown using *in vitro* transcribed biotinylated Lnc-BM and indicated recombinant proteins. Streptavidin-HRP indicated the presence of equal amounts of biotinylated RNA transcripts.

(B) RT-qPCR detection of RIP assay using anti-JAK2 antibody in 231-Br cells. The RNA targets detected were shown (n = 3 independent experiments, paired Student's t-test).

(C-D) Graphic illustration of the domain structure of JAK2 (upper panel of C). IB detection of Myc-tagged JAK2 (low panel of C) or indicated recombinant proteins (D) pull downed by *in vitro* transcribed biotinylated Lnc-BM.

(E) Denaturing agarose gel electrophoresis of *in vitro* transcribed biotinylated Lnc-BM full length (FL) and ΔJAK2 transcripts.

(F) RNA pulldown followed by IB detection of FLAG-JAK2 retrieved from RNA pulldown assay using *in vitro* transcribed biotinylated Lnc-BM FL or ΔJAK2 and FLAG-tagged JAK2 recombinant protein.

(G) Predicted secondary structure of Lnc-BM. A segment of Lnc-BM (911~1050 nt) is indicated by dash circle. Black arrows: potential stem-loop structures.

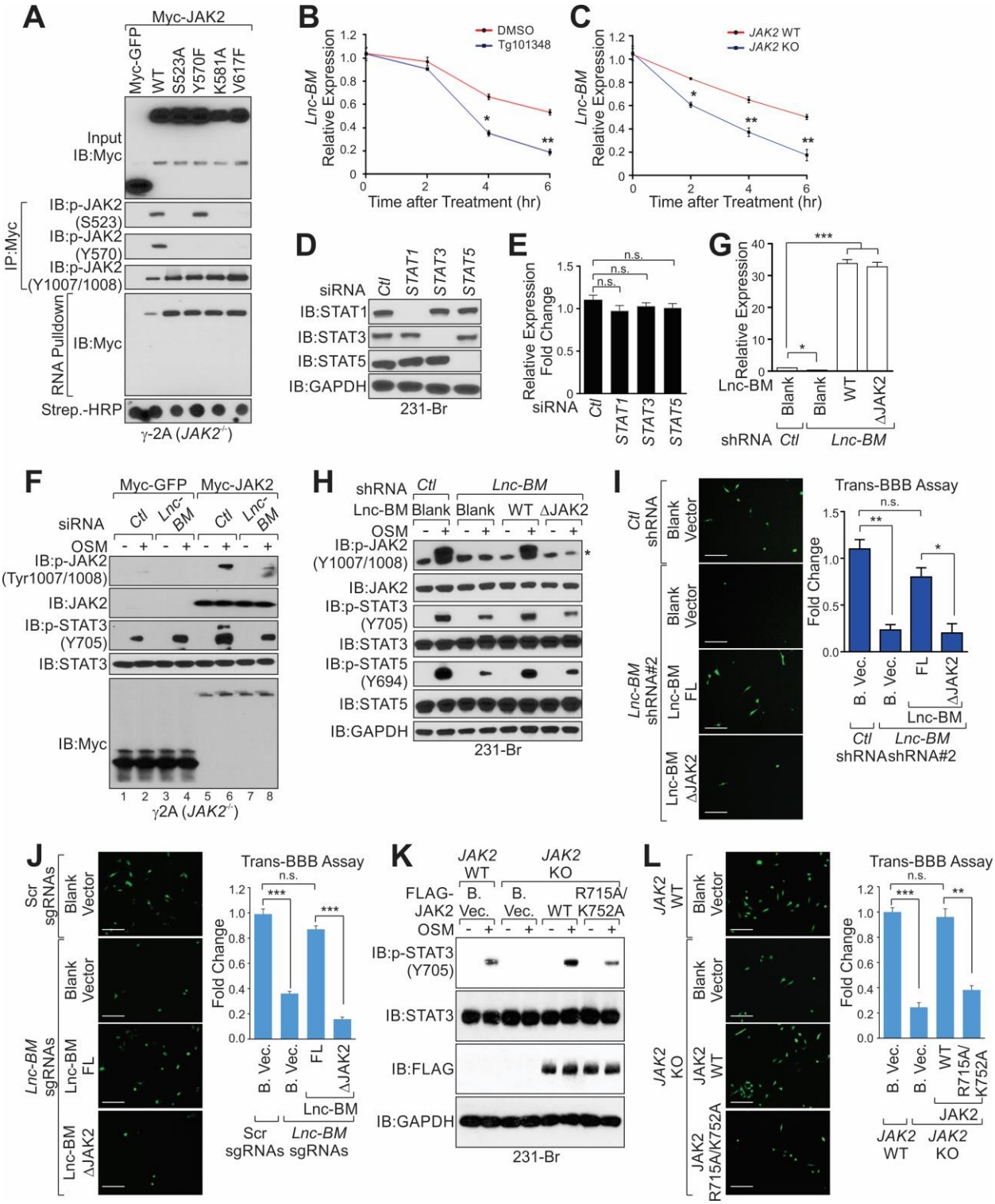
(H) Top panel : graphic illustration of the JAK2 binding-domain (JAK2 BM) of Lnc-BM. Bottom panel : IB detection of His-tagged JAK2 pull downed by *in vitro* transcribed biotinylated Lnc-BM FL or deletion mutants.

(I) The largest positive electrostatic patch (blue) calculated by Bindup that contains 10 LYS and 1 ARG with the input of structure (pdbID: 4fvp).

(J) Illustration of JH2 domain. The blue are the predicted non-binding resides, the red are predicted binding residues, the cyan are 9 validated non-binding residues, and the green are two validated binding residues (R715 and K752).

(K) RIP assay and RT-PCR detection of Lnc-BM in 231-Br *JAK2* KO cells transfected with indicated expression constructs followed by OSM stimulation (50 ng/ml, 30 min) (n = 3 independent experiments, paired Student's t-test).

Data are mean ± S.E.M, n.s. $p > 0.05$, *$p < 0.05$.



Supplemental Figure 9, related to Figure 10. Lnc-BM-JAK2 interaction stabilized Lnc-BM.

(A) IP using anti-Myc antibody and RNA pulldown using *in vitro* transcribed biotinylated Lnc-BM was performed using cell lysates extracted from γ-2A cells transfected with the indicated

expression vectors. The precipitated proteins were subjected to IB detection using the indicated antibodies. Streptavidin-HRP indicated the presence of equal amounts of biotinylated RNA transcripts.

(B-C) The stability of the Lnc-BM transcript over time was measured by RT-qPCR relative to time 0 after blocking new RNA synthesis with Actinomycin D (2 μg/ml) in 231-Br cells treated with a JAK2 inhibitor (TG101348) (B) or in *JAK2* WT or KO 231-Br cells (C). The expression of Lnc-BM was normalized to GAPDH (*n* = 3 independent experiments, paired Student's t-test).

(D) IB detection of indicated proteins in 231-Br cells transfected with indicated siRNAs.

(E) RT-qPCR analysis of Lnc-BM expression in 231-Br cells transfected with indicated siRNAs (*n* = 3 independent experiments, paired Student's t-test).

(F) IB detection using indicated antibodies in γ-2A cells transfected with Myc-GFP or Myc-JAK2 expression vectors followed by transfection of indicated siRNAs.

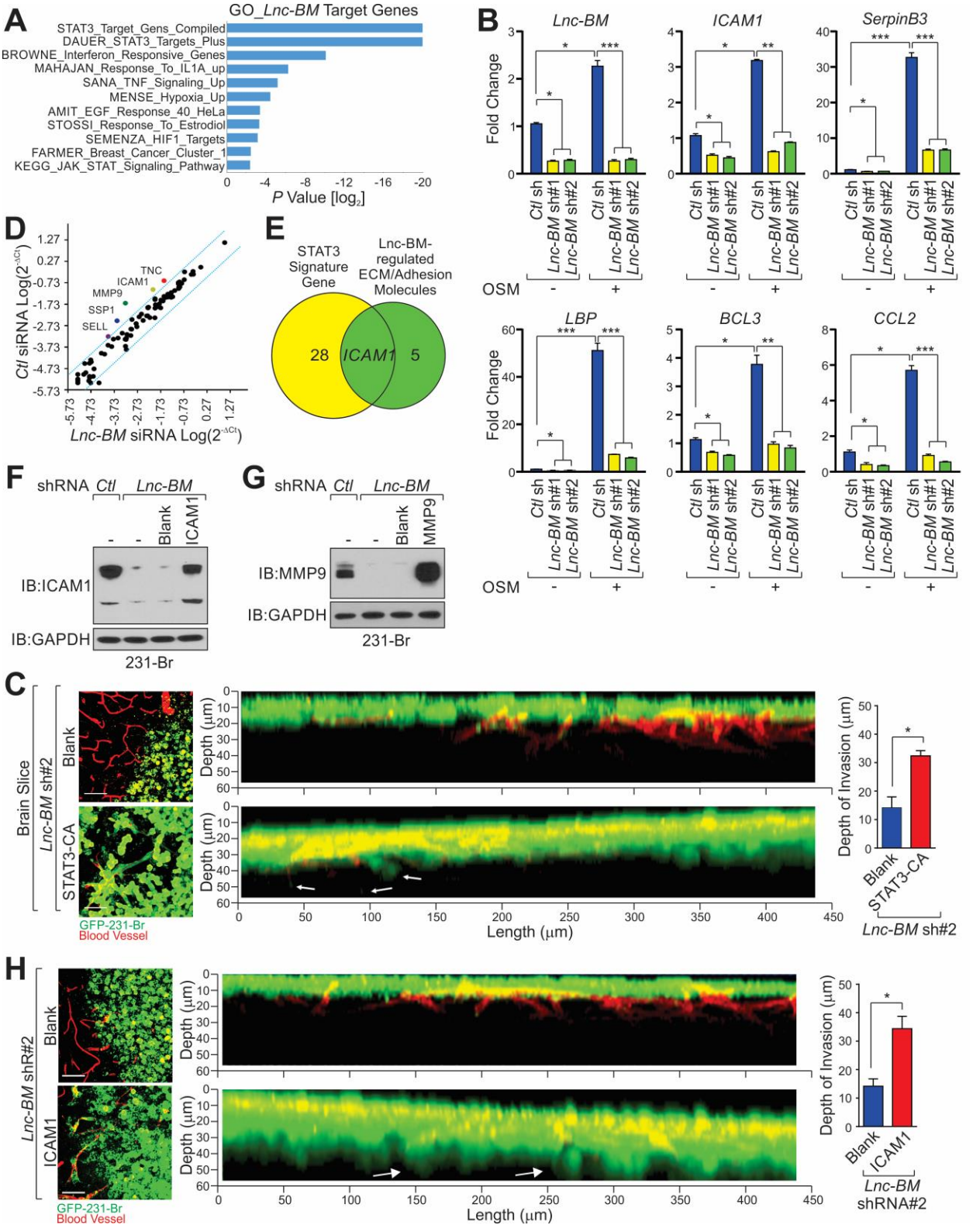
(G) RT-qPCR detection of Lnc-BM expression in *Lnc-BM* deficient cells transfected with shRNA-resistant Lnc-BM FL or ΔJAK2 expression vectors (*n* = 3 independent experiments, one-way ANOVA).

(H-J) IB detection (H) or Trans-BBB assay was performed in *Lnc-BM* knockdown (I), or *Lnc-BM* deficient 231-Br cells (J) followed by overexpression of the indicated expression vectors. Left panel (I and J), representative images, scale bars = 100 μm; right panel (I and J), quantification of transmigration cancer cells relative to control sample (*n* = 3 independent experiments, paired Student's t-test). * : unspecific band.

(K and L) IB detection (K) or Trans-BBB assay (L) was performed in *JAK2* deficient 231-Br cells followed by overexpression of the indicated expression vectors. Left panel (L), representative

images, scale bars = 100 μm; right panel (L), quantification of transmigration cancer cells relative to control sample (n = 3 independent experiments, paired Student's t-test).

Data are mean ± S.E.M, n.s. $p > 0.05$, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.



Supplemental Figure 10, related to Figure 11. STAT3 activation is required for Lnc-BM-dependent BCBM.

(A) Gene Ontology (GO) analysis showing the signaling pathways that are regulated by Lnc-BM in 231-Br cells induced by OSM over the vehicle.

(B) RT-qPCR detection of STAT3-target genes expression in 231-Br cells harboring indicated shRNAs treated with OSM (n = 3 independent experiments, one-way ANOVA).

(C) Representative confocal images (X-Y facet, left panel), Z-stacking (Y-Z facet, middle panel), and quantification of infiltrated depth (right panel) of brain slices co-cultured with Lnc-BM deficient 231-Br cells transduced with STAT3-constitutively active (CA) or control lentivirus (n = 3 brain slices, scoring at least three fields per slice, paired Student's t-test). Scale bars = 50 μm, white arrow: infiltrated cells.

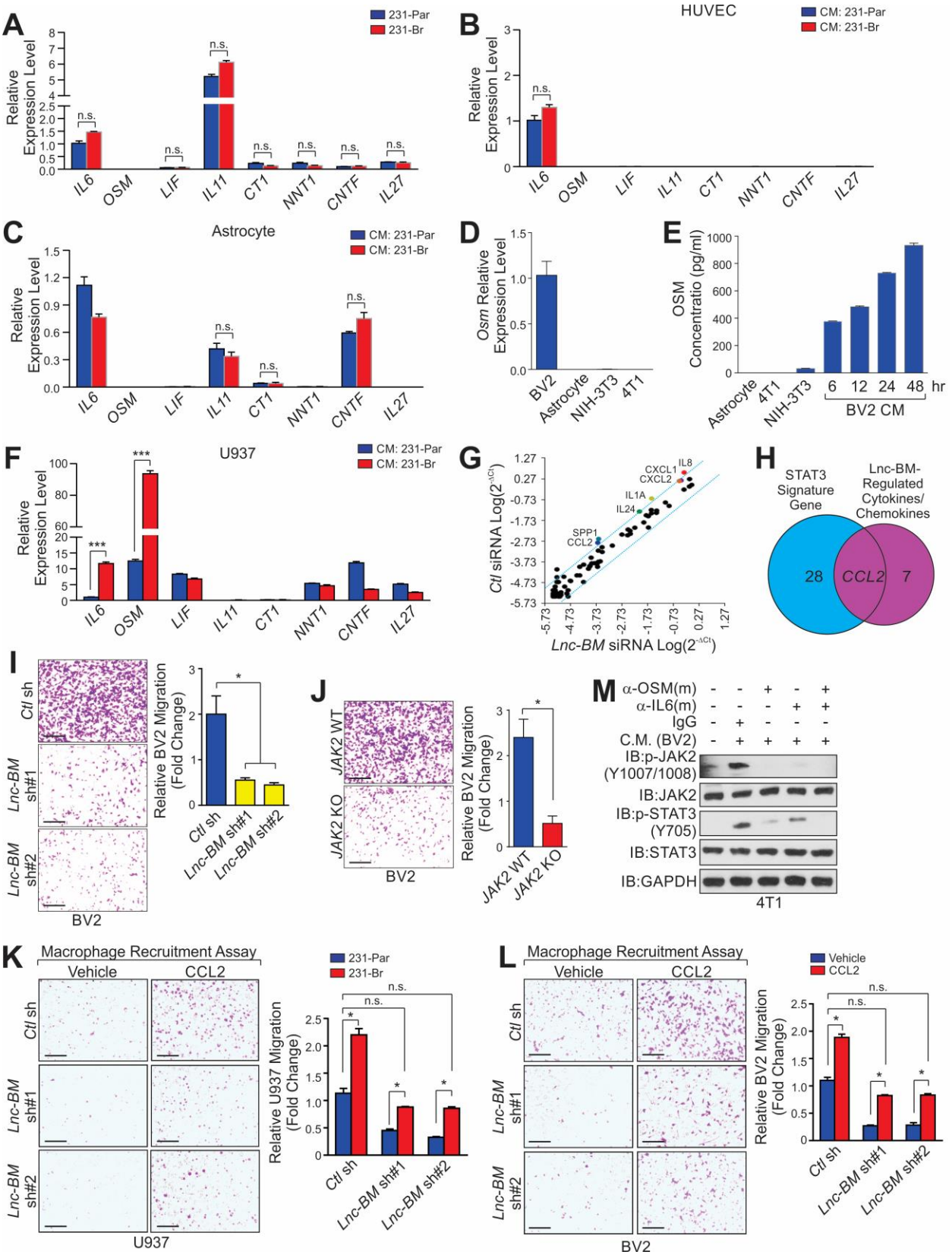
(D) RT-qPCR analysis of ECM & adhesion molecules expression (n = 84) in 231-Br cells transfected with indicated siRNAs. Blue dashed lines, fold changes = 4.

(E) Overlap between Lnc-BM regulated ECM/adhesion molecules (Supplemental Figure 10D, n = 5, fold change > 4) and STAT3 target genes (Figure 11A, n = 28).

(F-G) IB detection using indicated antibodies in Lnc-BM deficient 231-Br cells stably overexpressing ICAM1 (F) or MMP9 (G).

(H) Representative confocal images and quantification of infiltrated depth of brain slices co-cultured with *Lnc-BM* deficient 231-Br cells overexpressing ICAM1 (n = 3 independent experiments, paired Student's t-test).

Data are mean ± S.E.M, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.



Supplemental Figure 11, related to Figure 12. Lnc-BM modulates recruitment of

macrophages to trigger JAK2-STAT3 signaling in cancer cells.

(A-C) RT-qPCR analysis of IL6ST-associated cytokines expression in 231-Par cells and 231-Br cells (A); in HUVECs treated with conditioned medium (CM) from 231-Par or 231-Br cells (B); or in astrocytes treated with conditioned medium (CM) from 231-Par or 231-Br cells (C) (*n* = 3 independent experiments, paired Student's t-test).

(D-E) RT-qPCR detection of *Osm* mRNA (D) or ELISA assay detection of OSM protein (E) in indicated mouse cell lines. The OSM concentration in conditioned medium of BV2 cells at various time points were shown (E).

(F) RT-qPCR analysis of IL6ST-associated cytokines expression in U937 cells pre-primed with 5 nM PMA for 48 h, followed by treatment with conditioned medium (CM) from 231-Par or 231-Br cells (4 hr) (*n* = 3 independent experiments, paired Student's t-test).

(G) RT-qPCR analysis of human cytokines & chemokines expression (n = 84) in 231-Br cells transfected with indicated siRNAs. Blue dashed lines, fold changes = 4.

(H) Overlap between Lnc-BM regulated cytokines & chemokines (Suplemental Figure 11G, *n* = 7, fold change > 4) and STAT3 target genes (Figure 11A, *n* = 28).

(I-J) Macrophage recruitment assay examining the directional migration of BV2 cells regulated by 231-Br cells harboring indicated shRNAs (I), or by JAK2 wild type (*JAK2* WT) or deficient (*JAK2* KO) 231-Br cells (J). Left panel, representative images, scale bar = 200 μm; right panel, quantification of BV2 cell migration relative to respective control groups (*n* = 3 independent experiments, at least 3 different fields in each sample were calculated, paired Student's t-test).

(K-L) Macrophage recruitment assay examining the migration of pre-primed U937 cells (K) or BV2 cells (L) regulated by conditioned medium from 231-Br cells harboring indicated shRNAs with or without additional recombinant CCL2 (400 pg/ml). Left panel, representative images, scale

bar = 200 μm; right panel, quantification of cell migration relative to control groups (n = 3 independent experiments, at least 3 different fields in each sample were calculated, paired Student's t-test).

(M) IB analysis using indicated antibodies in 4T1 cells treated with conditioned medium (CM) from BV2 cells in the presence of indicated mouse antibodies.

Data are mean ± S.E.M, n.s. $p > 0.05$, *$p < 0.05$, ***$p < 0.001$.

Supplemental Figure 12, Full unedited gel for figures with gel and blot images.

Supplemental Table Legends

Supplemental Table 1: Clinicopathological parameters of breast cancer tissue microarrays and cDNA arrays used in this study, related to Figures 1, 6 and 7.

This table lists the clinicopathological parameters of 3 breast cancer tissue/cDNA Microarrays. Yixing Cohort: Frozen breast cancer tissue array with adjacent breast tissue as control, 20 cases/ 40 cores, with IHC results of Her-2, ER, and PR; Duke Cohort:161 fresh frozen breast tumor tissues with metastasis and recurrence information; these samples were subjected to RNA isolation and tissue microarray preparation. RNAs were isolated from 121 samples and used for RT-qPCR analysis of Lnc-BM expression and 92 and 85 tissue samples were presented in the final tissue microarray staining for OSMR and phospho-JAK2 respectively; TMA-007: Breast cancer tissue array including TNM, clinical stage and pathology information, 40 cases/ 40 cores. Breast Cancer TissueScan™ Cancer and Normal Tissue cDNA Arrays I-IV: (I) 48 samples containing 7 normal, 10 Stage I, 13 IIA, 7 IIB, 8 IIIA, and 3 IIIC with ER, PR, and HER2 status; (II) 48 samples containing 5 normal, 11 Stage I, 8 IIA, 6 IIB, 8 IIIA, 2 IIIB, 4 IIIC, and 4 IV with ER, PR, and HER2 status; (III) 48 samples containing 12 Stage I, 6 IIA, 10 IIB, 7 IIIA, 3 IIIB, 5 IIIC, and 5 IV with ER, PR, and HER2 status; (IV) 48 samples containing 4 normal, 2 Stage I, 15 IIA, 9 IIB, 7 IIIA, IIIB, 6 IIIC, and 1 IV with ER, PR, and HER2 status.

Supplemental Table 2. LC-MS/MS protein identification results for biotinylated Lnc-BM RNA pulldown experiment, related to Figure 6.

Complete mass spectrometry results for biotinylated Lnc-BM-associated proteins pulled down from 231-Br cell lysates. This file has 3 tables, listing the number of unique and shared peptides identified for proteins associated with monoavidin magnetic beads and sense and antisense Lnc-BM.

Supplemental Table 3. Oligonucleotides used in this study.

Supplemental Table 4. Antibodies used in this study.