Supplementary Tables

Table S1. Primers for genes used in this study

Gene	Sequence (5′ to 3′)
FN1	Forward: GCTTCCTGGCACTTCTGGTC
	Reverse: CTTCTTGTCCTACATTCGGCG
LOX	Forward: GCCGACCAAGATATTCCTGGG
	Reverse: GCAGGTCATAGTGGCTAAACTC
ABCC3	Forward: ATTCCACTCAACGGAGCTGTG
	Reverse: GCGCGAGTCCTTCAATTTCAT
ICAM-1	Forward: ATGCCCAGACATCTGTGTCC
	Reverse: GGGGTCTCTATGCCCAACAA
MUC1	Forward: TGCCGCCGAAAGAACTACG
	Reverse: TGGGGTACTCGCTCATAGGAT
SELPLG	Forward: CCTGAGTCTACCACTGTGGAG
	Reverse: GCTGCTGAATCCGTGGACA
TGFBR2	Forward: GTAGCTCTGATGAGTGCAATGAC
	Reverse: CAGATATGGCAACTCCCAGTG
TGFB1	Forward: CAATTCCTGGCGATACCTCAG
	Reverse: GCACAACTCCGGTGACATCAA
PTGS2	Forward: ATGCTGACTATGGCTACAAAAGC
	Reverse: TCGGGCAATCATCAGGCAC
GAPDH	Forward: GGATTTGGTCGTATTGGG
	Reverse: GCTCCTGGAAGATGGTGAT
NFAT2	Forward: CACCGCATCACAGGGAAGAC
	Reverse: GCACAGTCAATGACGGCTC
NFAT1	Forward: CGATTCGGAGAGCCGGATAG
	Reverse: TGGGACGGAGTGATCTCGAT

Table S2. Sequences of siRNA

	Sequence(5′-3′)
si-COX-2#1	Forward: CCU CCU GUG CCU GAU GAU UTT
	Reverse: AAU CAU CAG GCA CAG GAG GTT
si-COX-2#2	Forward: GCA ACA CUU GAG UGG CUA UTT
	Reverse: AUA GCC ACU CAA GUG UUG CTT
si-Ctrl	Forward: UUC UCC GAA CGU GUC ACG UTT
	Reverse: ACG UGA CAC GUU CGG AGA ATT

Supplementary Figures

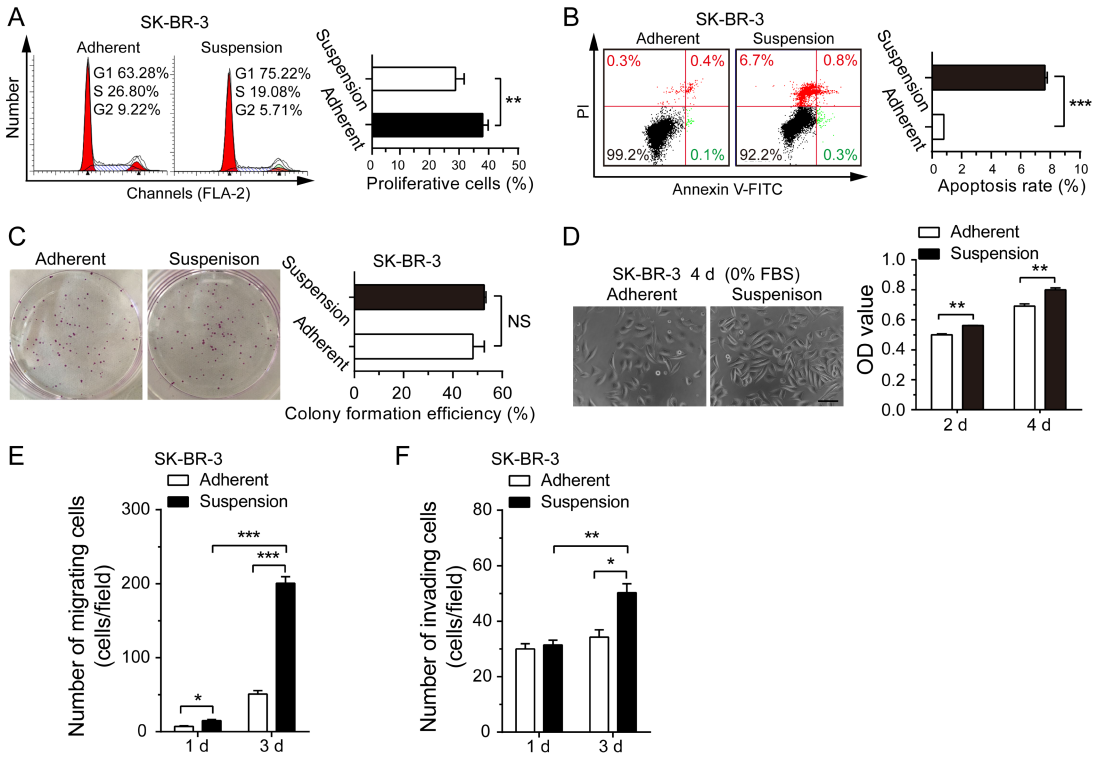

Figure S1. Effects of suspension state on proliferation, apoptosis, colony formation, survival, migration and invasion ability of SK-BR-3 cells. SK-BR-3 cells were cultured under suspension state or conventional adherent condition for 3 d. **(A)** Cell cycle analysis and **(B)** cell apoptosis analysis by flow cytometry. Representative plots are displayed. **(C)** Adherent or suspension cultured SK-BR-3 cells were seeded at density of 400 cells/well for clone formation assays. Representative crystal violet staining of clone formation is shown. **(D)** SK-BR-3 cells from adherent culture and suspension culture were reattached and cultured in DMEM medium without FBS. Phase contrast micrographs of SK-BR-3 cells cultured for 4 d without FBS are given (scale bar = 50 μm). MTS assays were used to measure cell viability. **(E)** Migration assays and **(F)** invasion assays of SK-BR-3 cells after adherent or suspension culture for 1 d and 3 d. Values are presented as means ± SE (n=3). *P< 0.05; **P< 0.01; ***P< 0.001; NS, not significant.

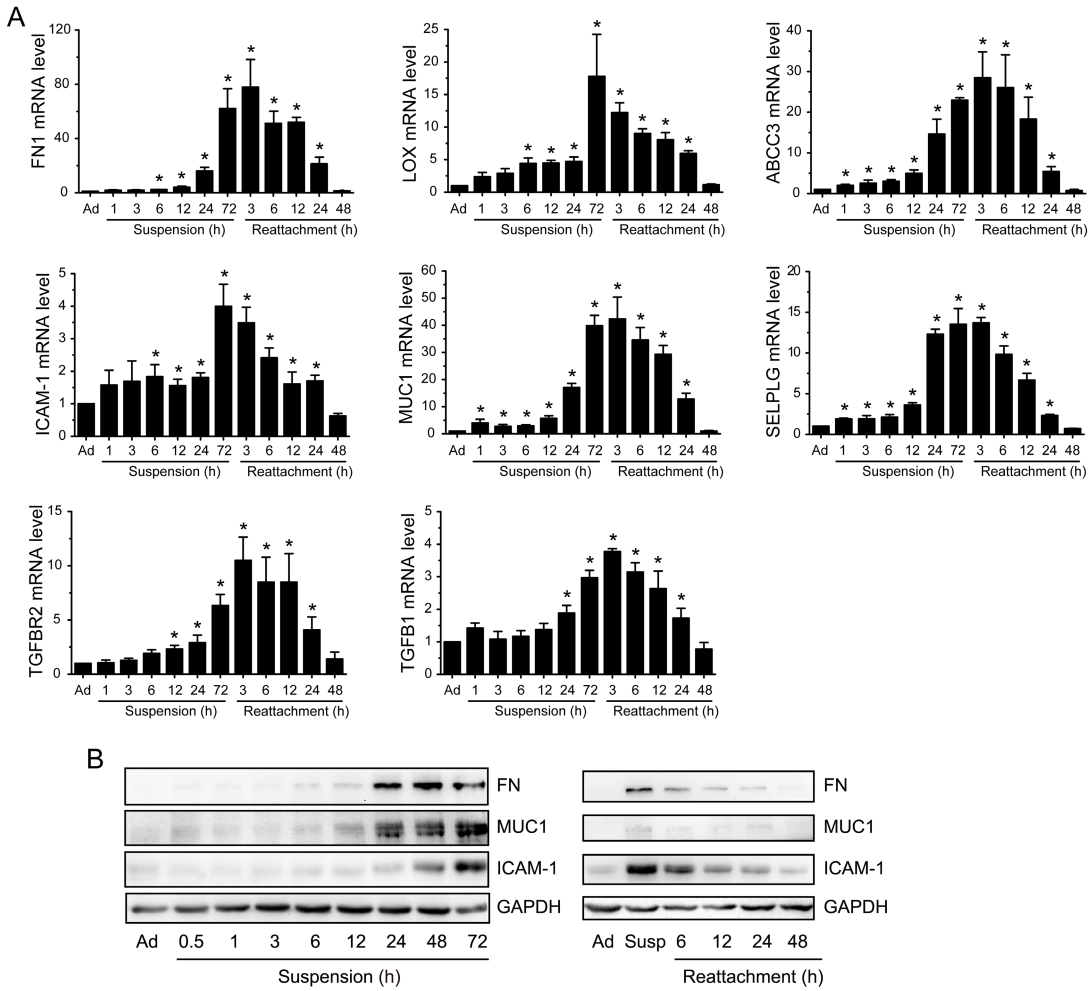

Figure S2. Suspension state induced expression of pro-metastasis genes in MDA-MB-231 cells. (A) Real-time RT-PCR of pro-metastasis genes after suspension and reattachment culture for a seris of time. Fold changes are relative to adherent cultured tumor cells. Values are presented as means ± SE (n=3). *P< 0.05 (t-test). **(B)** Protein level changes of pro-metastasis genes after tumor cells suspension and reattachment culture for a seris of time. Ad: adherent, Susp: suspension.

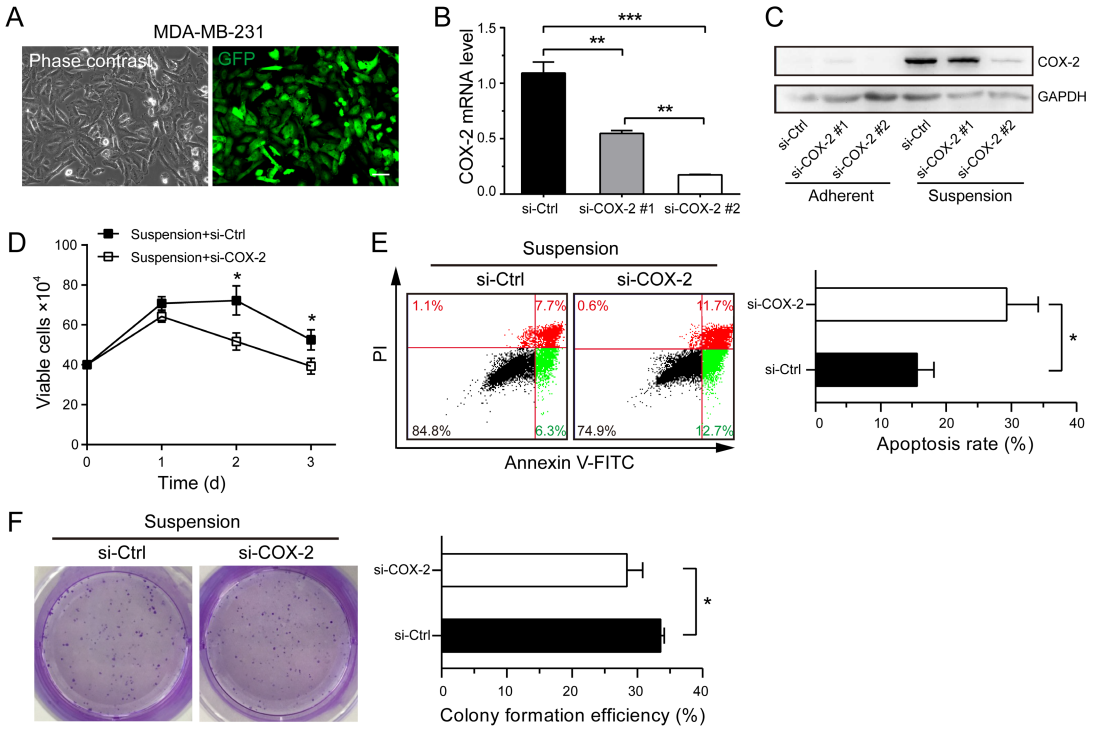

Figure S3. Knockdown COX-2 promoted apoptosis of suspension MDA-MB-231 cells. (A) Representative fluorescent images of GFP-MDA-MB-231 cells (scale bar = 20 μm). **(B)** COX-2 mRNA levels in adherent MDA-MB-231 cells were detected by real-time RT-PCR after knockdown with siRNA. **(C)** COX-2 protein levels in adherent or suspension MDA-MB-231 cells were detected by western blotting after knockdown with siRNA. #1 and #2 represented two siRNA sequences. **(D)** Counting of viable cells in suspension culture conditmoion after COX-2 knockdown or not. **(E)** Cell apoptosis analysis by flow cytometry of suspension cultured MDA-MB-231 cells for 3 d with COX-2 knockdown or not. **(F)** Clone formation assays of suspension cultured MDA-MB-231 cells with COX-2 knockdown or not. The left panels are crystal violet staining. Values are presented as means ± SE (n=3). *P< 0.05; **P< 0.01; ***P< 0.001 (t-test).

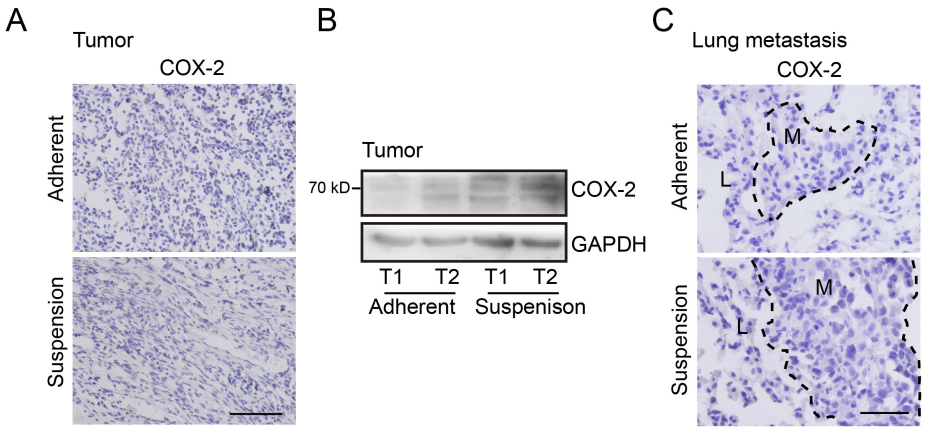

Figure S4. COX-2 up-expression induced by suspension state was reversible *in vivo*. (A) Representative images COX-2 stained sections of xenograft tumors from adherent or suspension cultured MDA-MB-231 cells (scale bar = 100 μm). **(B)** Western blotting of COX-2 in xenograft tumors. T1 and T2 represented tumor tissue code. **(C)** Representative images of COX-2 stained sections of lung metastases driven by tail vein injection with adherent or suspension MDA-MB-231 cells (scale bar = 50 μm). M: metastasis, L: lung.

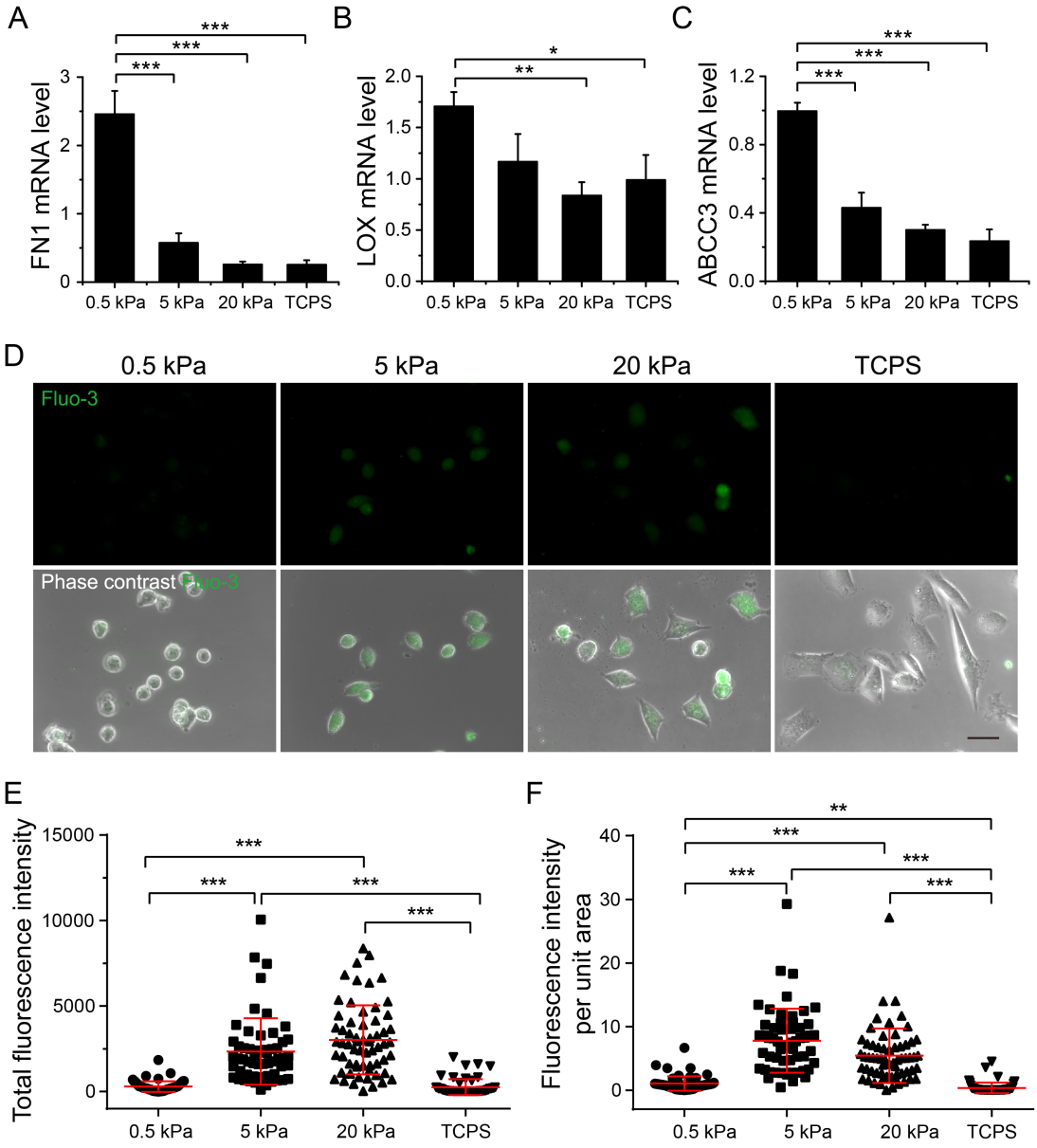

Figure S5. Effects of matrix stiffness on expression of pro-metastatic genes and cytosolic Ca^{2+} level in MDA-MB-231 cells. (A-C) Detection of *FN1*, *LOX*, *ABCC3* mRNA levels in MDA-MB-231 cells cultured on different matrix stiffness. Values are presented as means ± SE (n=3). *P< 0.05; **P< 0.01; ***P< 0.001 (Tukey test). **(D)** Cytosolic Ca^{2+} levels were estimated in MDA-MB-231 cells cultured on different matrix stiffness (scale bar = 25 μm). **(E-F)** Analysis of total fluorescence intensity and fluorescence intensity per unit area. Values are presented as means ± SE (n=61, 56, 62, 54 from three separate experiments). **P< 0.01; ***P< 0.001 (Dunn's multiple comparisons test).