Supplementary Materials

EMT and cell cycle control invadopodia and metastasis in breast cancer via Filip1L

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Supplementary Figures

Supplementary Figure 1. Induced overexpression of Slug leads to increases in invadopodia-mediated ECM degradation and in the number of G1 cells.

A. Binarized images of invadopodia degradation (black puncta) in 4T1-FUCCI-TetOn-Slug cells cultured on fluorescent gelatin for 16 h, synchronized with DMSO, Lov, or MitC. Prior to plating on gelatin, cells were exposed to -Dox or +Dox for 3 days. Nuclei were stained with DRAQ5 (magenta). Scale bar, 20 µm.
B. Fluorescence overlay images of 4T1-FUCCI-TetOn-Slug cells in G1 (red) and G2 (green), imaged on day 3 after addition of doxycycline. Scale bar, 100 µm.
C. Quantification of cell cycle distribution in -Dox d3 and +Dox d3 cells from panel B. Data represent mean ± SD. N=3, n=3, 9 FOVs per n per condition. Data for G1 and G2 cells have passed Shapiro-Wilk test for Gaussian distribution. Statistical significance was determined using unpaired two-tailed t-test; G1 cells (-Dox d3 vs +Dox d3): P = 0.0036. G2 cells (-Dox d3 vs +Dox d3): P = 0.0034. Mann-Whitney test was used for Early S (-Dox d3 vs +Dox d3): P = 0.2084, ns.

Supplementary Figure 2. MCF10A cells transition from E to early E/M when treated with TGFβ1.

A. Cell transition from E to early E/M upon TGFβ1 treatment. Created in BioRender.
B. Fluorescent gelatin layer (left) and corresponding phase-contrast images of MCF10A cells, with or without TGFβ1 treatment, and with lovastatin-driven synchronization to G1 phase. Scale bar, 50 µm.
C. Quantification of matrix degradation area per cell corresponding to (B). Dotted lines represent quartiles; the dashed line represents the median. Statistical significance was determined using the unpaired Mann–Whitney test. ***P < 0.001; ****P < 0.0001. N=2, n=1, 9-10 FOVs per n per condition.
D. Western blot analysis of E and M marker expression in MCF10A cells before and after TGFβ1 treatment.

Supplementary Figure 3. Differential expression of matrix remodeling genes across cell cycle phases in early- and late E/M states.

A. Heatmap showing the relative expression levels of statistically significant matrix remodeling and invadopodia- related genes in early E/M cells, G1 versus G2 cell cycle phases.
B. Heatmap showing the relative expression levels of statistically significant matrix remodeling and invadopodia- related genes in late E/M cells, G1 versus G2 cell cycle phases. The color scale represents Z-score normalized expression values across conditions.

Supplementary Figure 4. Differential expression of matrix remodeling genes between early- and late E/M cells in the same cell cycle phases.

A. Heatmap showing the relative expression levels of statistically significant matrix remodeling and invadopodia- related genes in G1 phase: early- vs late E/M cells.
B. Heatmap showing the relative expression levels of statistically significant matrix remodeling and invadopodia- related genes in G2 phase: early- vs late E/M cells. The color scale represents Z-score normalized expression values across conditions.

Supplementary Figure 5. High FILIP1L expression is associated with increased expression of ECM-degrading proteases.

A-F. Violin plots showing lo G2 mRNA expression of MMPs (**a**-**b**), invadopodia components (**c**), ADAMs (**d**), ADAM, ADAMEC and ADAMTS family proteases (**e**-**f**) in breast cancer patient samples stratified by FILIP1L expression (low: Group A, blue; high: Group B, pink). Data was retrieved from publicly available breast cancer TCGA PanCancer Atlas dataset. Dotted lines represent quartiles; the dashed line represents the median. Significance was determined using the unpaired Mann-Whitney test. P values: P>0.05 (ns), $P < 0.05$ (*), $P < 0.01$ (**), $P < 0.001$ (***), $P < 0.0001$ (****).

Supplementary Figure 6. FILIP1L colocalizes with Tks5 and cortactin in MDA-MB-231 cells

A. In MDA-MB-231 cells labeled by DAPI (blue), Tks5 (green) and FILIP1L (red), white arrows point to invadopodia. Line (magenta) was drawn through one of the invadopodia punctae. Right panel: Profile of FILIP1L (red) and Tks5 (green) intensities along the magenta line Scale bar: 20 µm.

B. In MDA-MB-231 cells labeled by DAPI (blue), cortactin (green) and FILIP1L (red), white arrows point to invadopodia. Line (magenta) was drawn through one of the invadopodia punctae. Right panel: Profile of FILIP1L (red) and cortactin (green) intensities along the magenta line. Scale bar: 20 µm.

Supplementary Tables

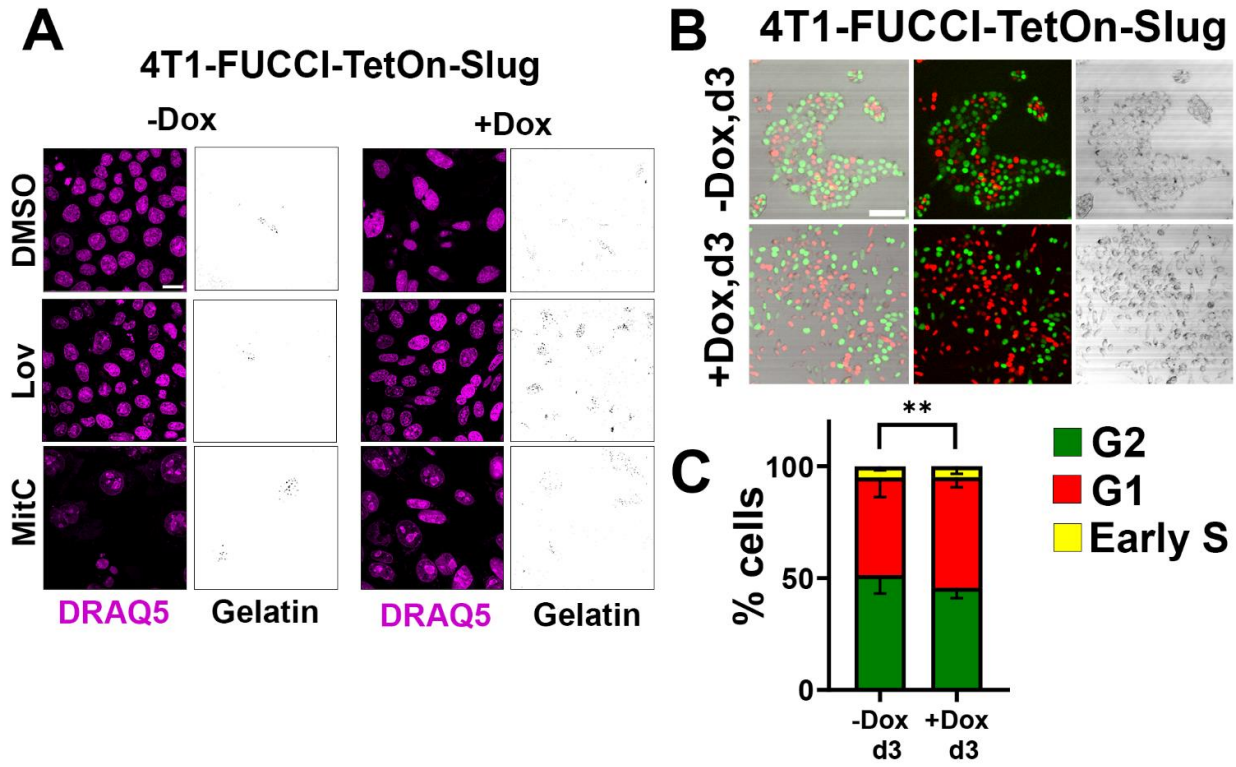
Supplementary Table 1. DESeq2 design setup

Supplementary Table 2. Result of Multivariate DESeq2 analysis

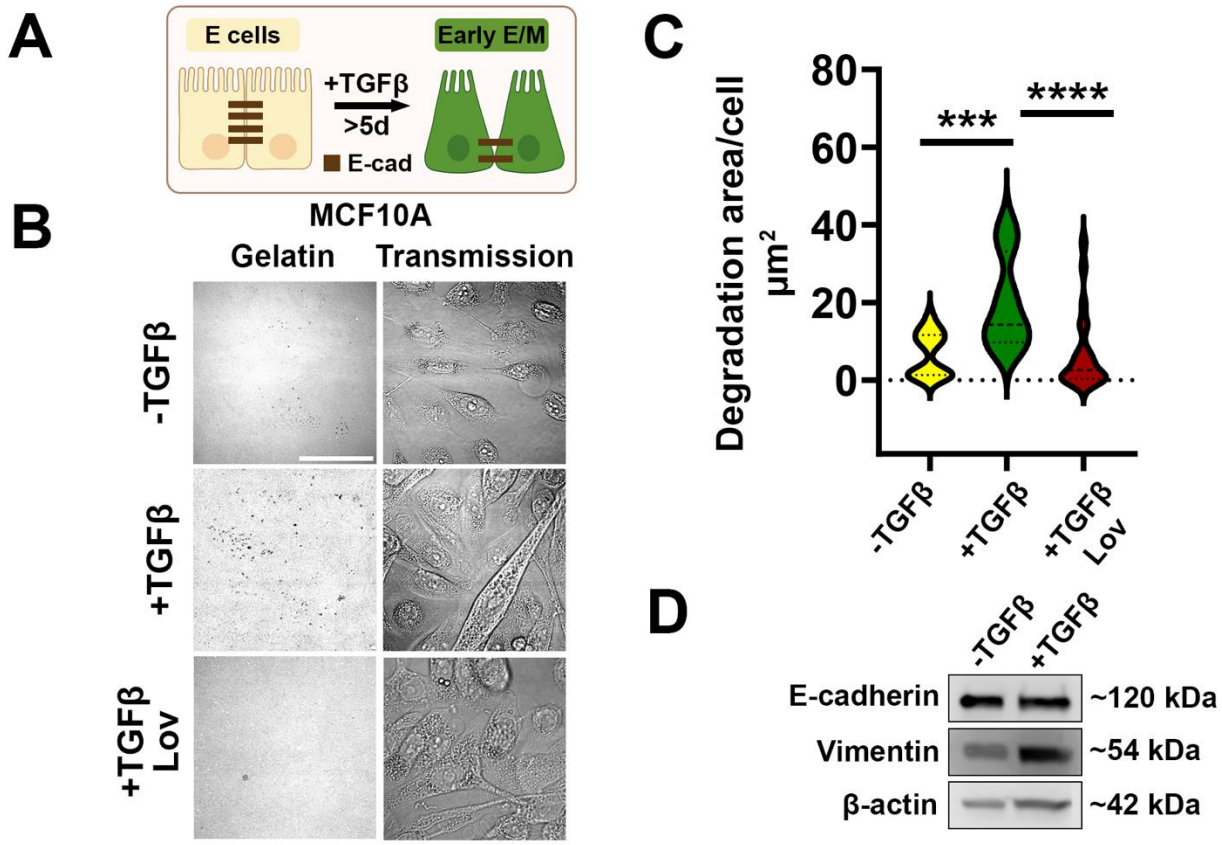
Supplementary Table 3. Materials for immunofluorescence

Supplementary Table 4. Materials for Western blots

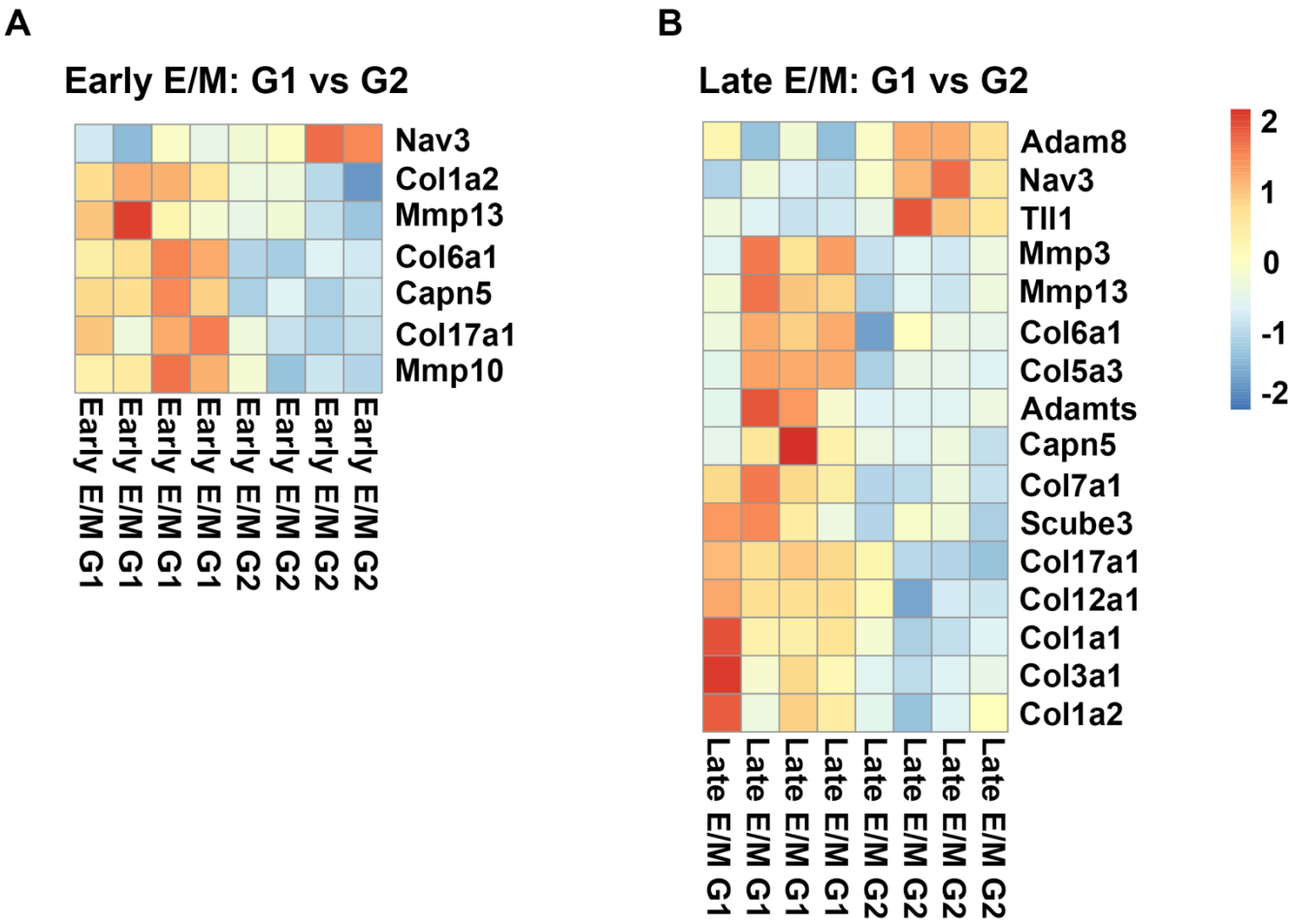
Supplementary Figure 1.



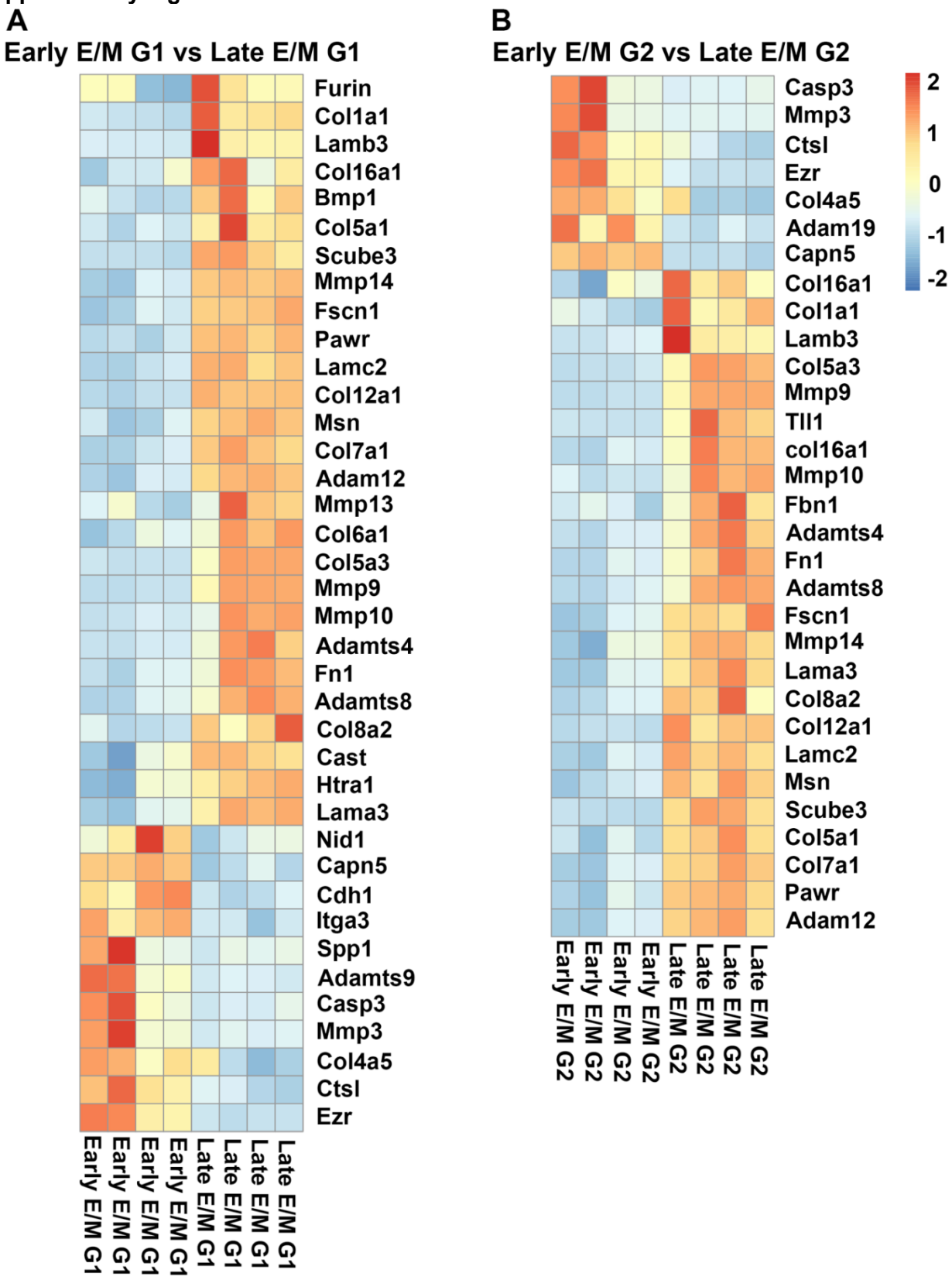
Supplementary Figure 2.



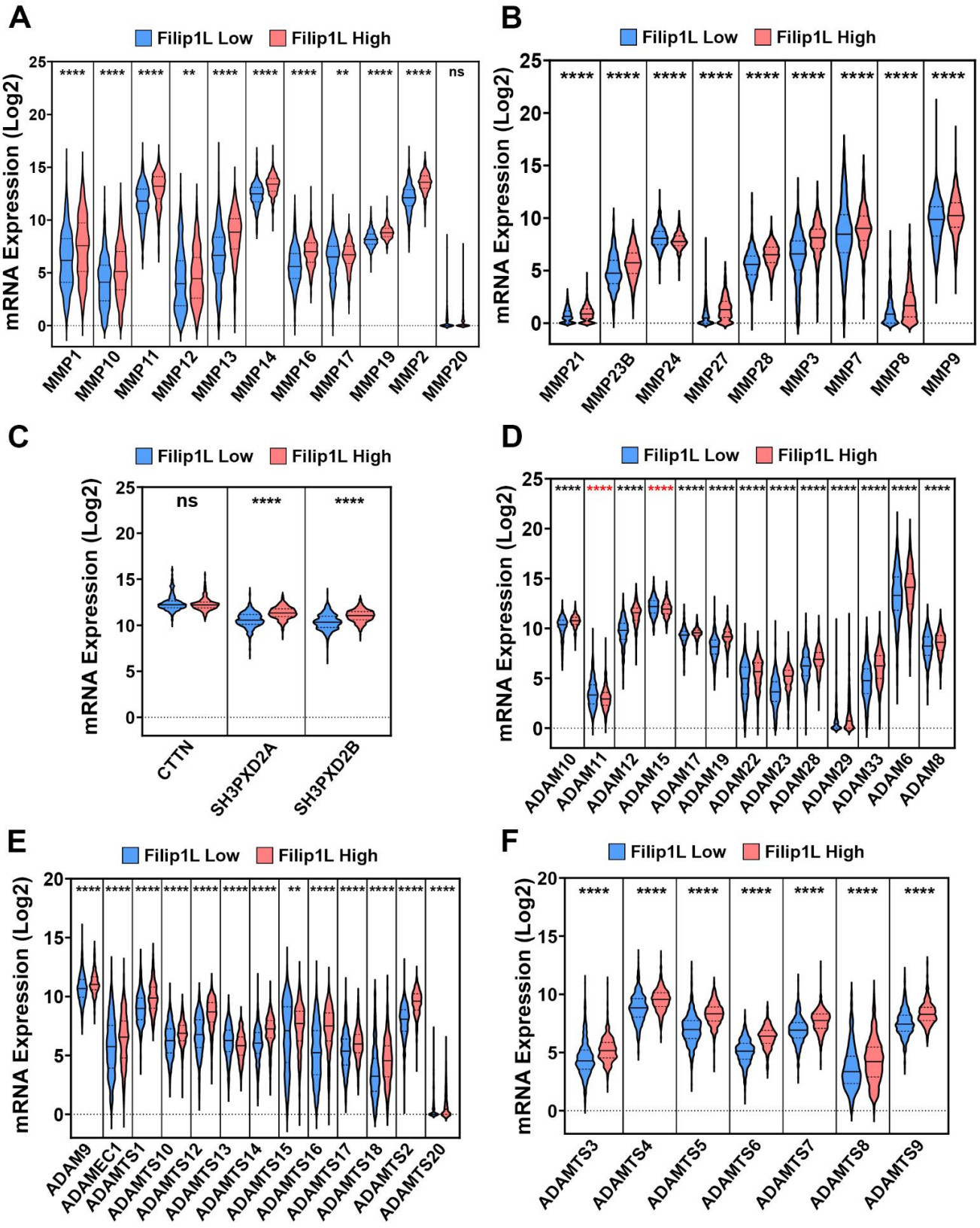
Supplementary Figure 3.



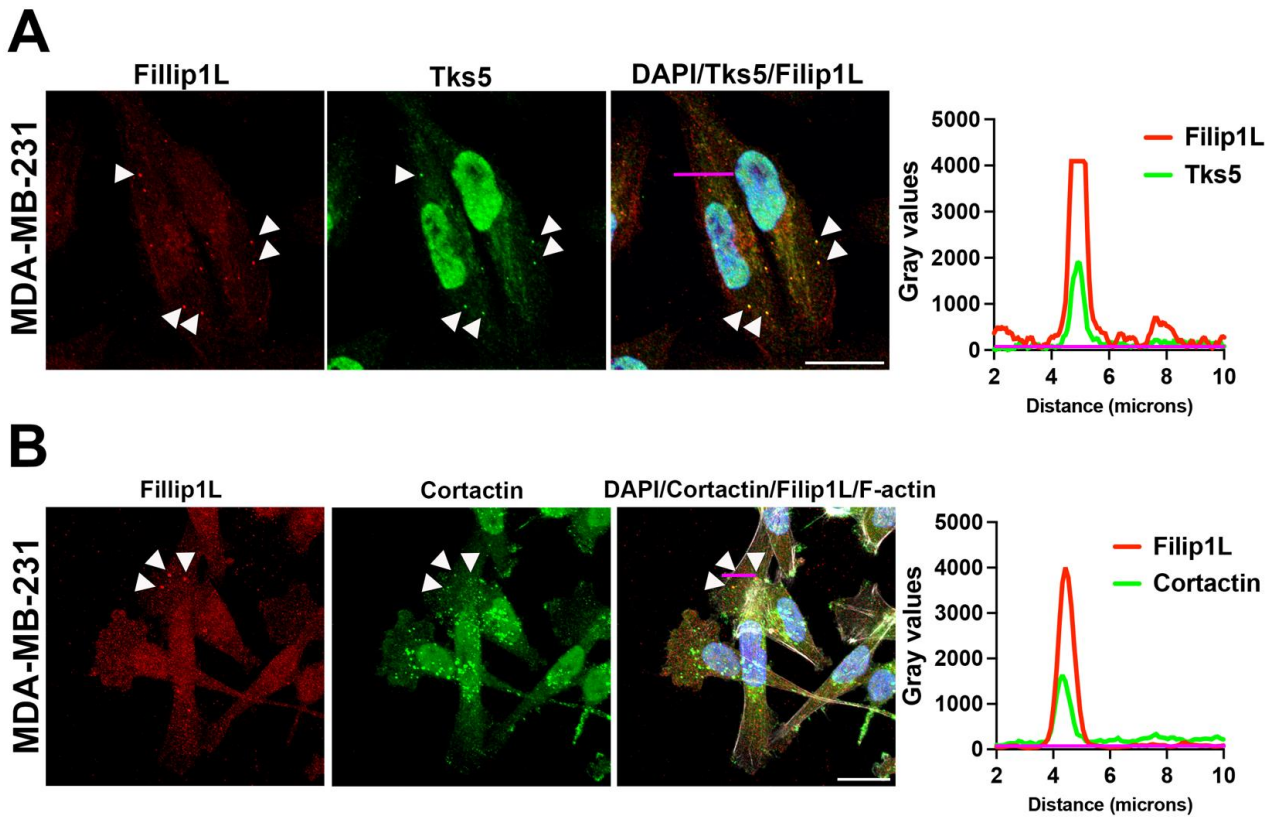
Supplementary Figure 4.



Supplementary Figure 5.



Supplementary Figure 6.



Supplementary Table 1. DESeq2 design setup

Samples	Treatment	Cell cycle phase	Treatment_color	Invasiveness
Early E/M G2 #1	-TGFβ-1 (Ctl)	G2 (Green)	Ctl_G	Invasive
Early E/M G1 #1		G1 (Red)	Ctl_R	Noninvasive
Early E/M G2 #2		G2 (G)	Ctl_G	Invasive
Early E/M G1 #2		G1 (R)	Ctl_R	Noninvasive
Early E/M G2 #3		G2 (G)	Ctl_G	Invasive
Early E/M G1 #3		G1 (R)	Ctl_R	Noninvasive
Early E/M G2 #4		G2 (G)	Ctl_G	Invasive
Early E/M G1 #4		G1 (R)	Ctl_R	Noninvasive
Late E/M G2 #1	+TGFβ-1	G2 (G)	Growth_G	Noninvasive
Late E/M G1 #1		G1 (R)	Growth_R	Invasive
Late E/M G2 #2		G2 (G)	Growth_G	Noninvasive
Late E/M G1 #2		G1 (R)	Growth_R	Invasive
Late E/M G2 #3		G2 (G)	Growth_G	Noninvasive
Late E/M G1 #3		G1 (R)	Growth_R	Invasive
Late E/M G2 #4		G2 (G)	Growth_G	Noninvasive
Late E/M G1 #4		G1 (R)	Growth_R	Invasive

Supplementary Table 2. Result of Multivariate DESeq2 analysis

Gene ID	baseMean	lo G2 FoldChange	p-value	padj	Symbol
ENSMUSG00000020897	2090.73	-0.304	5.20E-08	0.0013	Aurkb
ENSMUSG00000043336	1743.81	0.278	1.47E-07	0.0018	FILIP1L

Supplementary Table 3. Materials for immunofluorescence

Antibody and dyes	Cat. no	Dilution
Anti-Tks5	Millipore, MABT336	1:200
Anti-E-cadherin	Thermo Fisher Scientific, 131900	1:200
Anti-FILIP1L	Abcam, ab151331	1:200
Vimentin	Abcam, ab92547	1:200
Phalloidin	Life Technologies A22283, A22284	1:250

Supplementary Table 4. Materials for Western blots

I Antibody	Cat. #	Host	I Antibody Dilution	II Antibody Dilution	µg of protein/lane	Chemiluminescent reagent
Vimentin	Abcam, ab92547	Rabbit	1:1000	1:4000	10	WesternBright
FILIP1L	Abcam, ab151331	Rabbit	1:1000	1:4000	10	WesternBright
FILIP1L Thermo	PA5-60251	Rabbit	1:1000	1:5000	25	WesternBright
Slug (C19G7)	Cell Signaling Technology 9585T	Rabbit	1:1000	1:5000	20	WesternBright
E-cadherin BD	BD Biosciences, 610181	Mouse	1:1000	1:4000	10	WesternBright
β-actin Loading Control Monoclonal Antibody (BA3R)	Thermo Scientific MA5-15739	Mouse	1:1000	1:5000	10	WesternBright
ZO1	Thermo Fisher, 21773-1-AP	Rabbit	1:1000	1:5000	10	WesternBright
Tks5	MABT336	Mouse	1:100	1:5000	10	Supersignal
Twist1	Sigma, T6451-25UL	Rabbit	1:1000	1:4000	10	Super signal