OMTN, Volume *27*

Supplemental information

Promotion of tumor progression by exosome transmission of circular RNA circSKA3

William W. Du, Xiangmin Li, Jian Ma, Ling Fang, Nan Wu, Feiya Li, Preet Dhaliwal, Weining Yang, Albert J. Yee, and Burton B. Yang

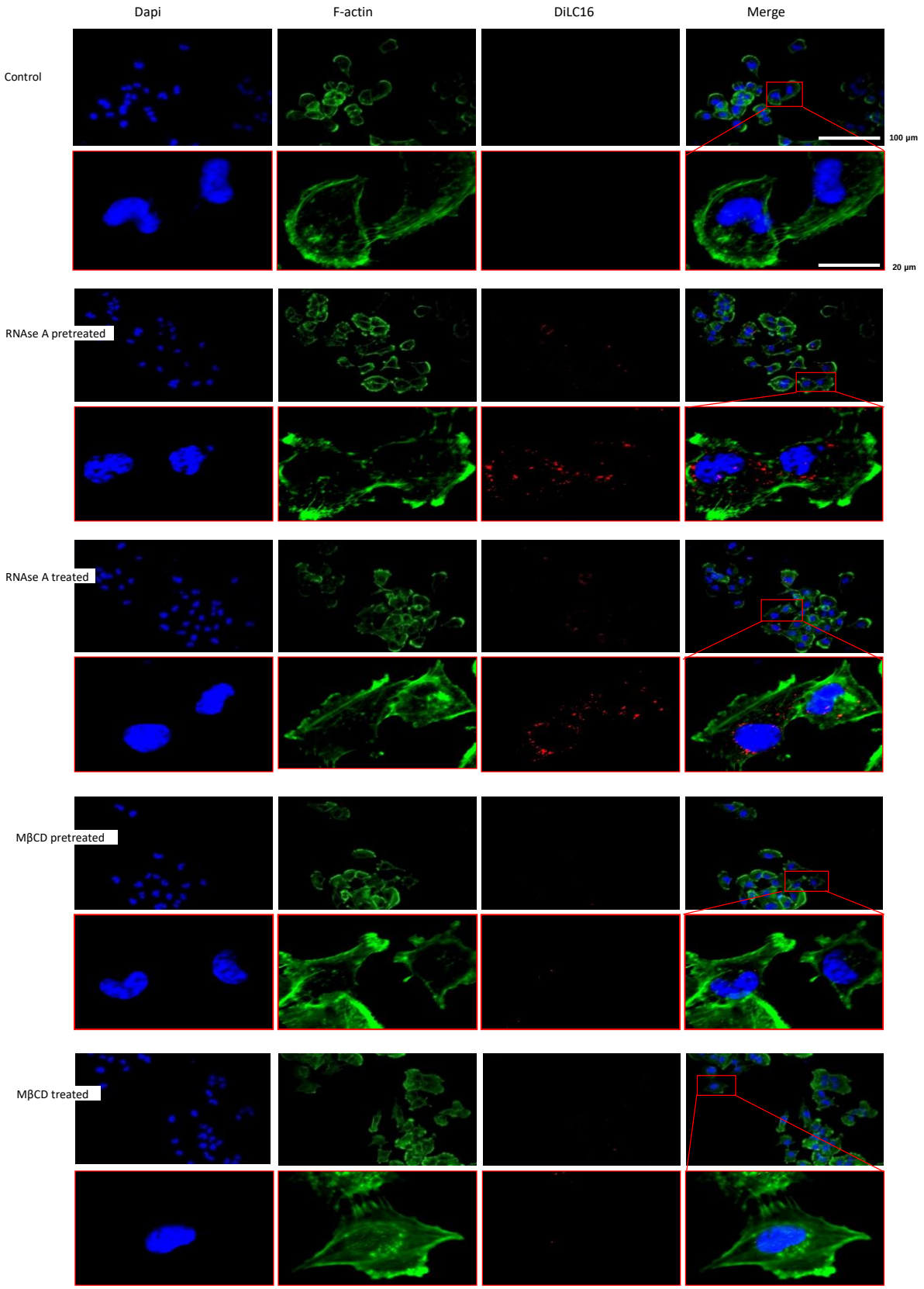
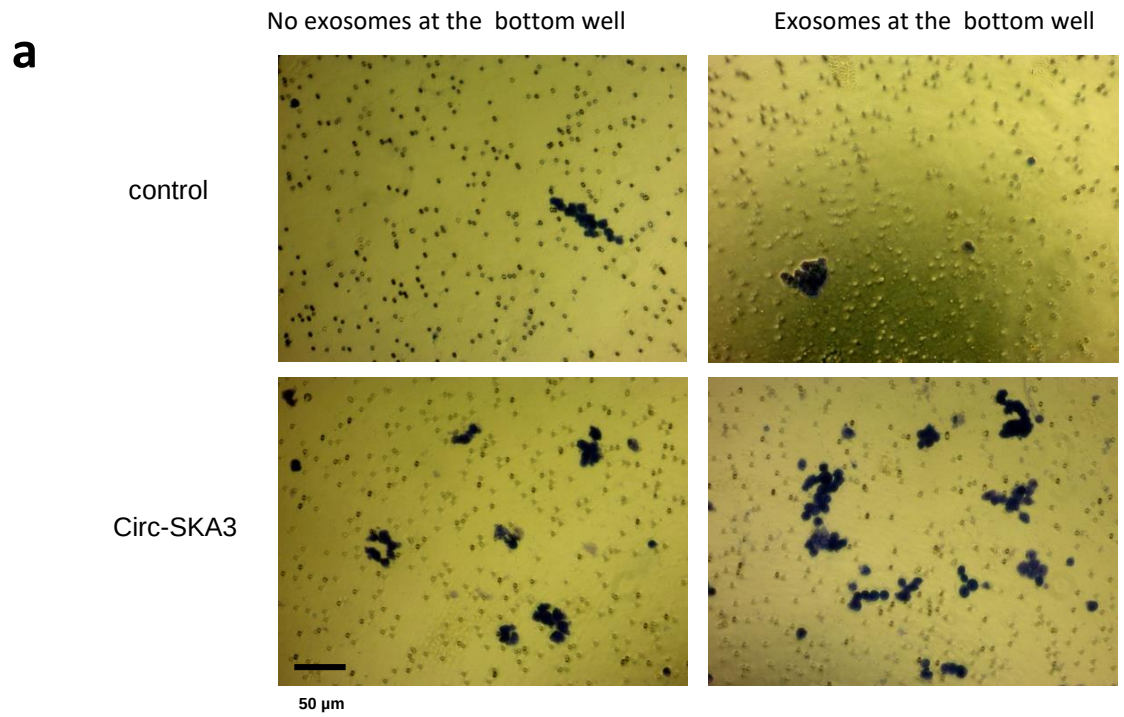


Fig S1. MB-231 cell derived exosomes entered MB-231 cells *in vitro*. Exosomes that were isolated from the medium of DiIC16-labed MB-231 cells (exosome) or, the DiIC16-containing medium without cells (control) and applied to culture MB-231 cells for 24 before detection for fluorescent DiIC16. The RNAse A or MβCD pretreated samples were treated with RNAse A or MβCD during DiIC16 staining, while the RNAse A or MβCD treated cells were cultured with conditioned medium with RNAse A or MβCD for 24 h. Cells were stained with DAPI (blue) for nucleus, green fluorescence showing F-actin, red fluorescence showing DiIC16 (exosome).



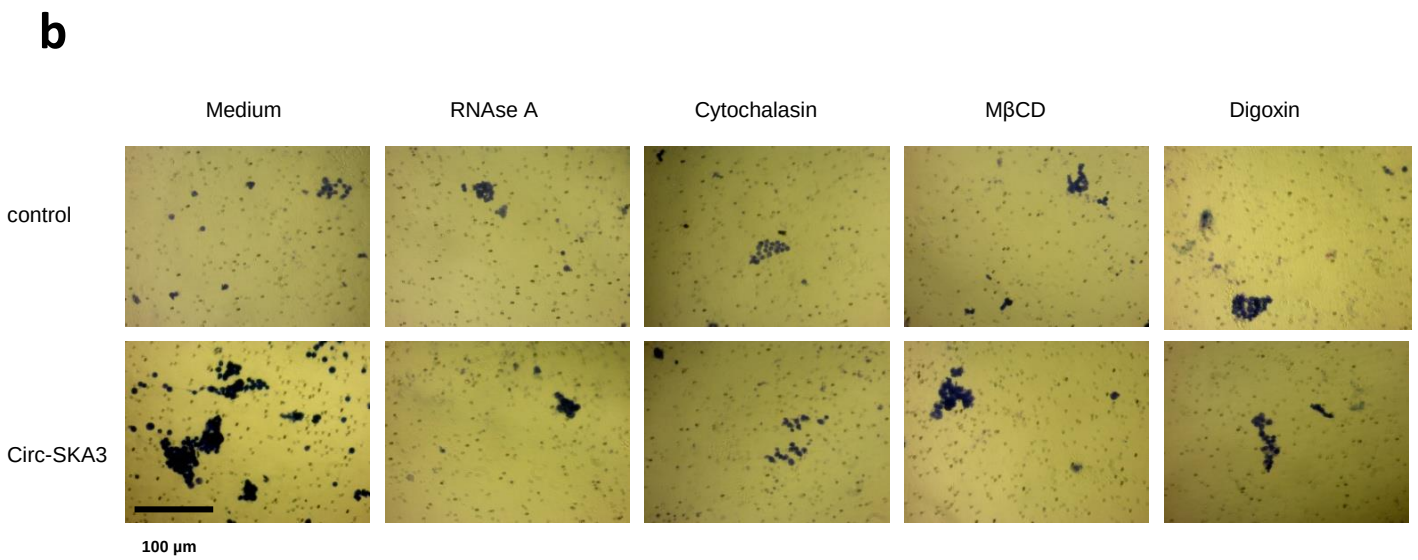
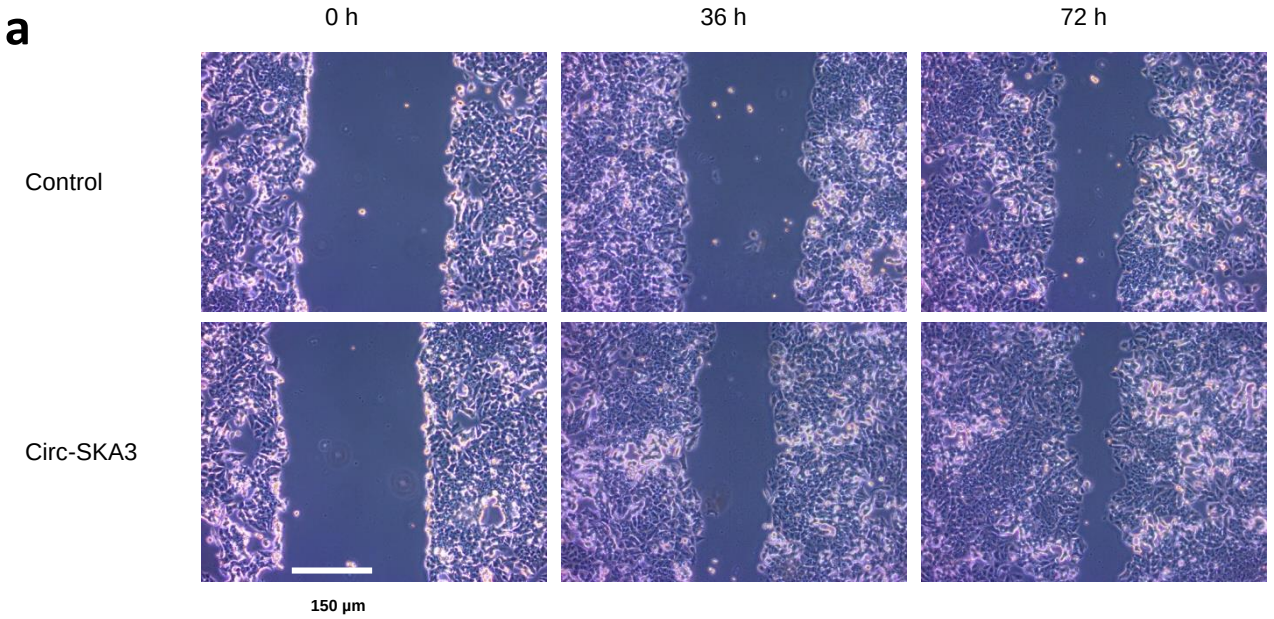


Fig S2. CircSKA3 enhanced cell invasion.
(a) Addition of exosomes containing circSKA3 enhanced MCF-7 cell invasion.
(b) Enhanced invasion of MCF-7 cells was inhibited by RNAse-A, Cytochalasin D, Digoxin and MβCD.



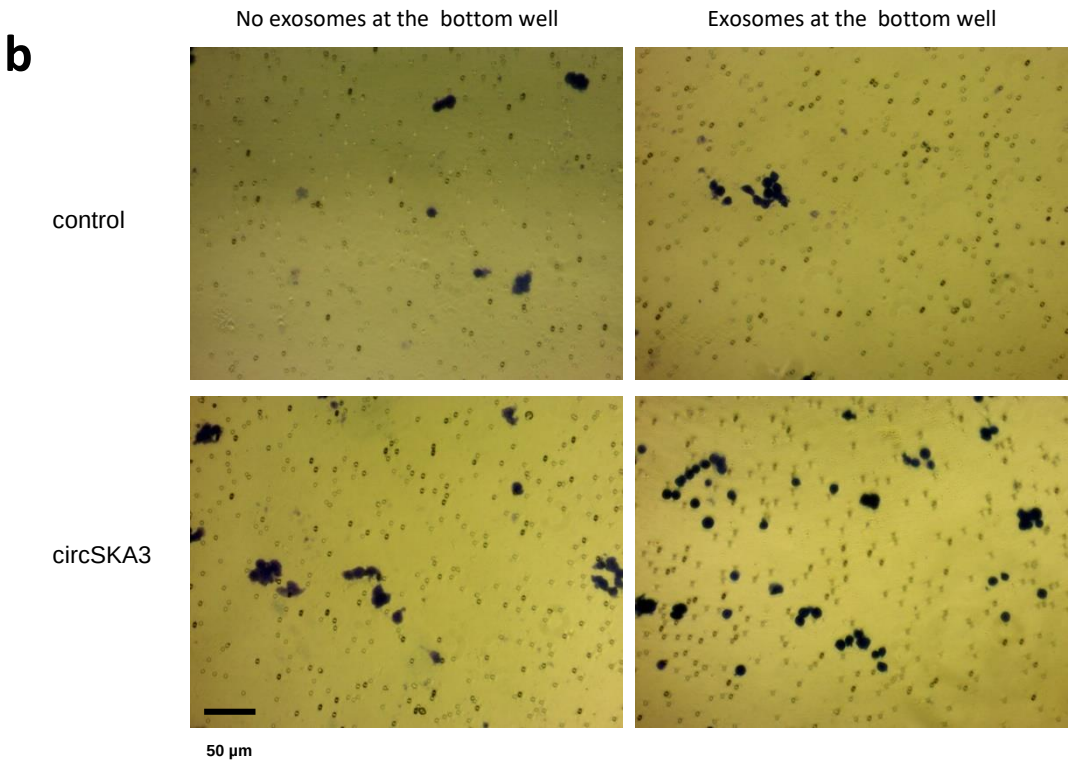


Fig S3. CircSKA3 enhanced cell migration.

(a) MCF-7 cells were culture in basal medium with 100 μg/ml exosomes, and processed to wound healing assays for 3 days. circSKA3-packed exosomes enhanced cell migration.

(b) MCF-7 cells were cultured in basal medium with 100 μg/ml exosomes harvested from vector-, or circSKA3-transfected cells for 3 days. In chamber migration assay, the circSKA3-packed exosomes were placed at the bottom wells. After 3 days, circSKA3-packed exosomes enhanced MCF-7 cell migration.

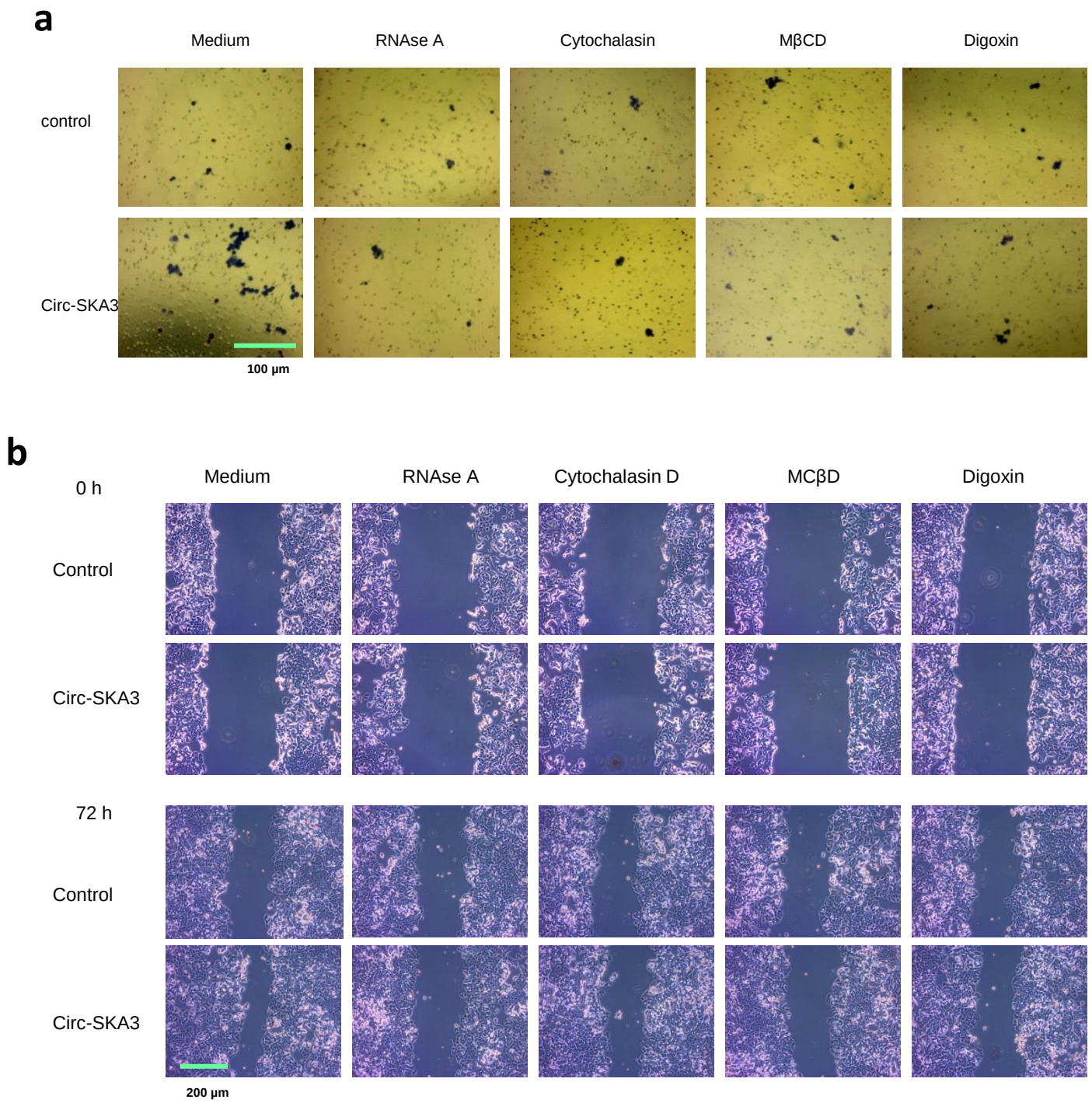


Fig S4. CircSKA3 enhanced cell migration.

(a) MCF-7 cells were culture in basal medium with 100 μg/ml containing vector-, or circSKA3-packed exosomes, to which the chemicals (RNAse-A, Cytochalasin D, Digoxin or MβCD) were added and incubated for 3 days. In the wound healing assays, circSKA3-packed exosomes enhanced cell migration, which could be inhibited by RNAse, Cytochalasin D, Digoxin and MβCD.

(b) In chamber migration assays, enhanced cell migration by circSKA3 was inhibited by RNAse-A, Cytochalasin D, Digoxin and MβCD.

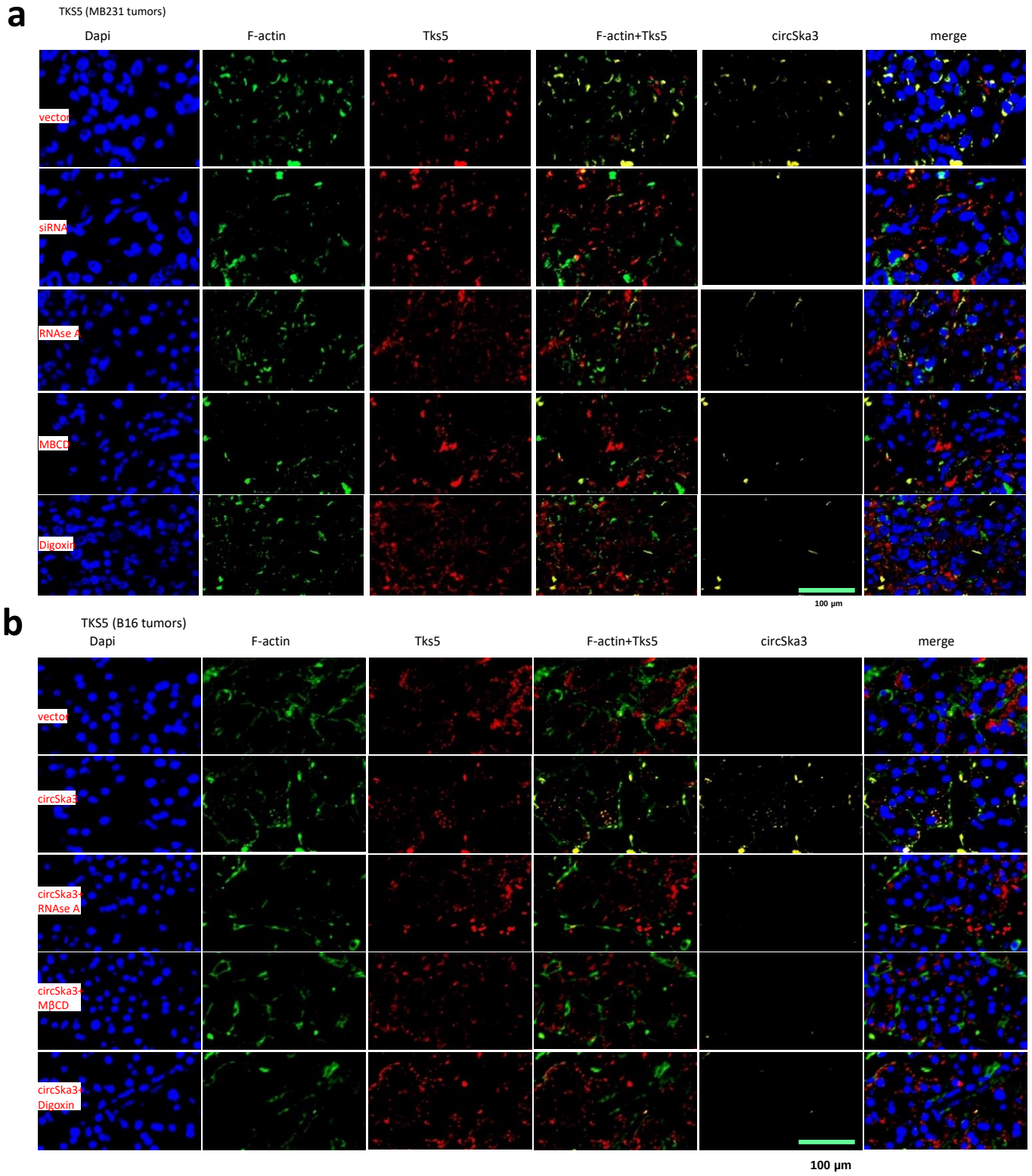
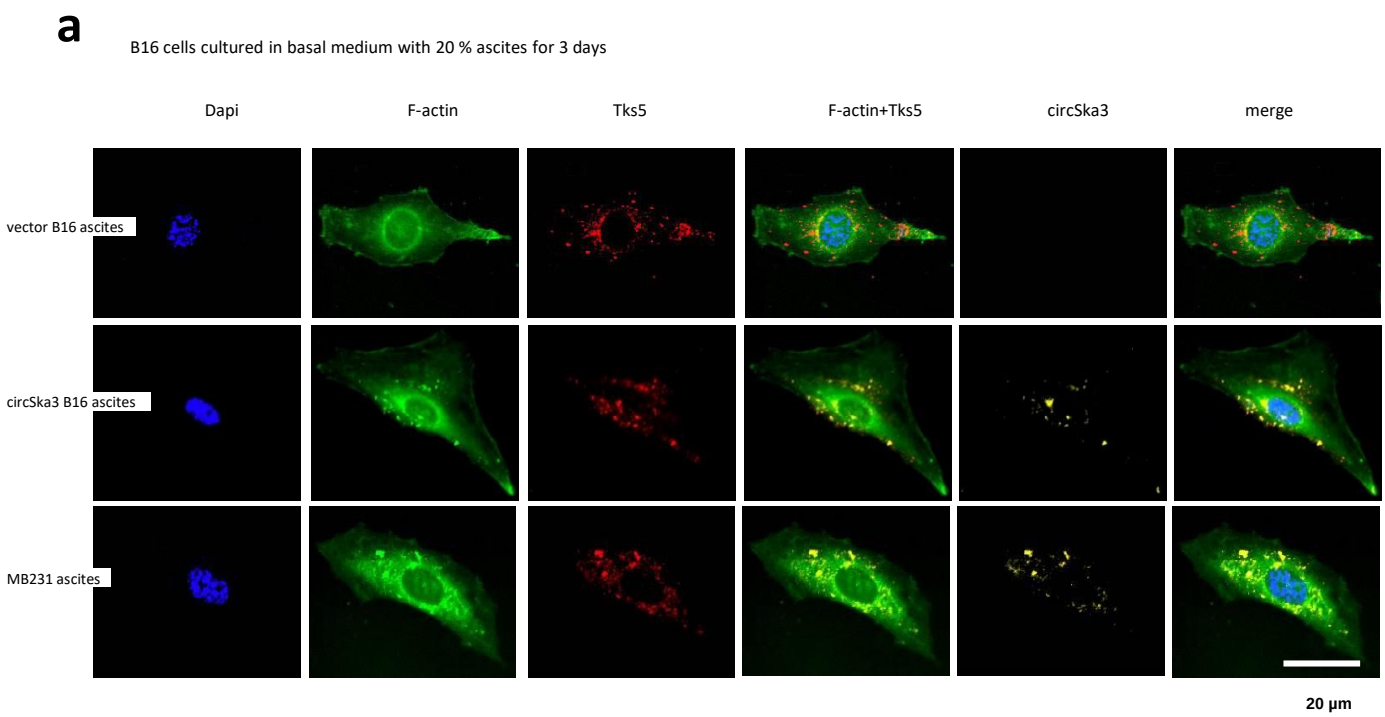


Fig S5. *In vivo* inhibition of exosome transfer.

(a) Invadopodia formation was repressed in the tumor tissues when the mice were injected with circSKA3 siRNA, RNAse A, Methyl-β-cyclodextrin (MβCD), or Digoxin. MB-231 cell formed tumor tissues were stained with DAPI (blue) for nucleus, green fluorescence showing F-actin, red fluorescence showing Tks5, and yellow fluorescence showing circSKA3.

(b) B16 cell formed tumor tissues were stained with DAPI (blue) for nucleus, green fluorescence showing F-actin, red fluorescence showing Tks5, and yellow fluorescence showing circSKA3.



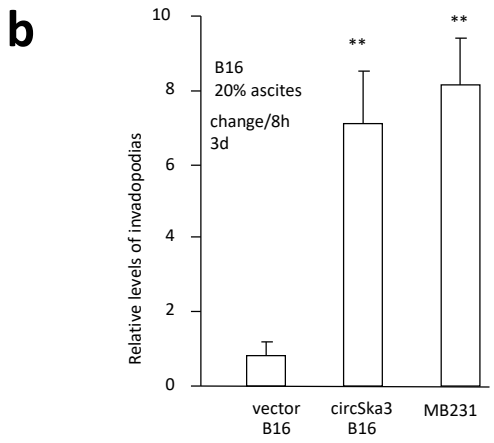


Fig S6. *In vivo* inhibition of exosome transfer.

(a) B16 cells were incubated with basal medium with 20% ascites from B16 cells injected mice, circSKA3 transfected B16 injected mice, or MB-231 cells injected mice for 3 days. Cells were stained with DAPI (blue) for nucleus, green fluorescence showing F-actin, red fluorescence showing Tks5, and yellow fluorescence showing circSKA3. Ascites from circSKA3 transfected B16 or MB-231 cells injected mice enhanced expression of circSKA3 and invadopodia formation.

(b) Image J analysis showed that ascites from mice injected with circSKA3-transfected B16 cells or MB-231 cells promoted invadopodia formation in B16 cells.

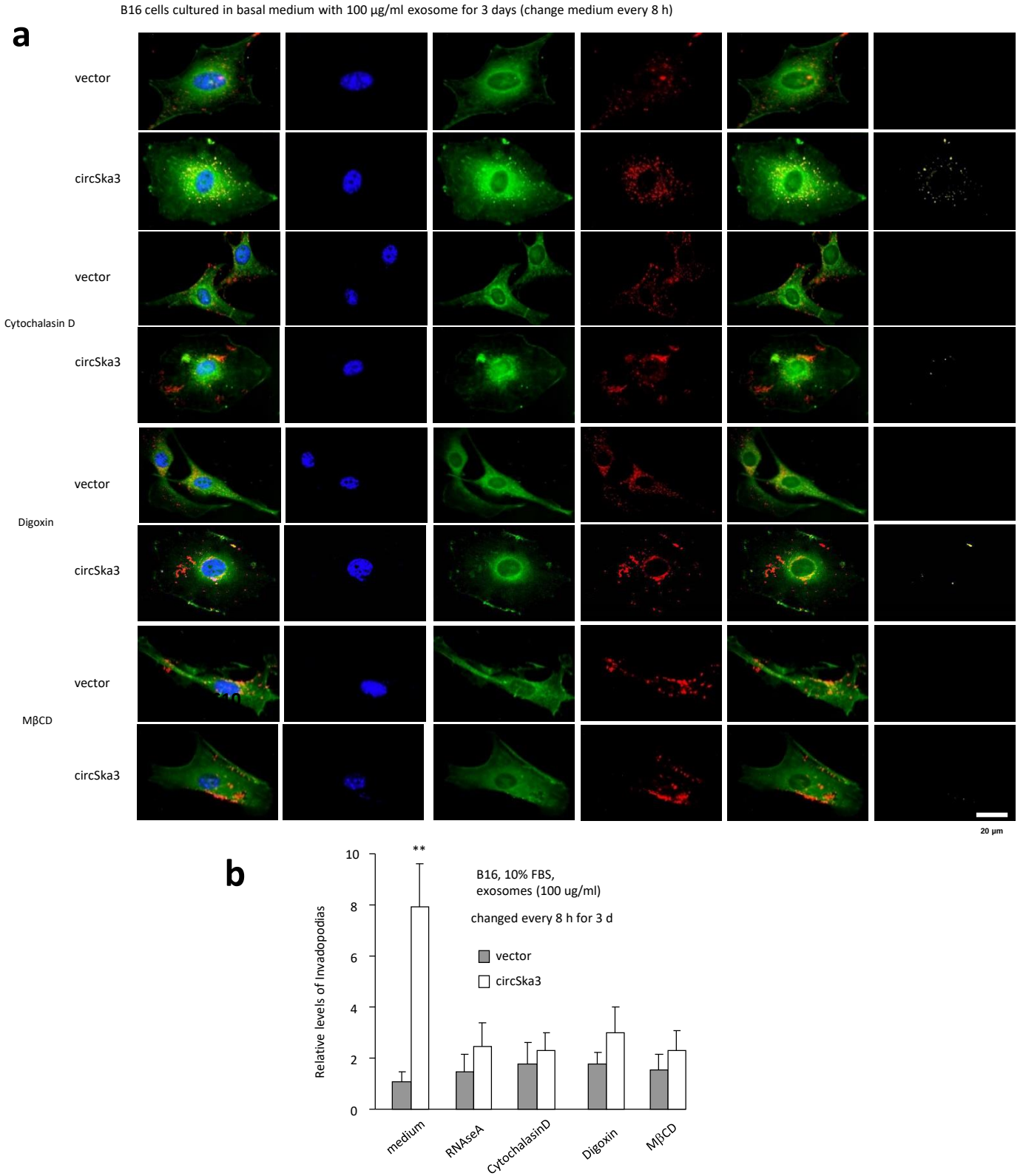


Fig S7. Identification of fractions containing circSKA3 complex.

(a) B16 cells were incubated with 100 μg/ml control vector-, or circSKA3 packed exosomes for 3 days. Cells were stained with DAPI (blue) for nucleus, green fluorescence showing F-actin, red fluorescence showing Tks5, and yellow fluorescence showing circSKA3. circSKA3 packed exosomes enhanced expression of circSKA3 and invadopodia formation in cells, which could be prevented by RNAse, Cytochalasin D, Digoxin and MβCD.

(b) Image J showed that circSKA3-containing exosomes promoted invadopodia formation in B16 cells, which could be prevented by RNAse, Cytochalasin D, Digoxin and MβCD.

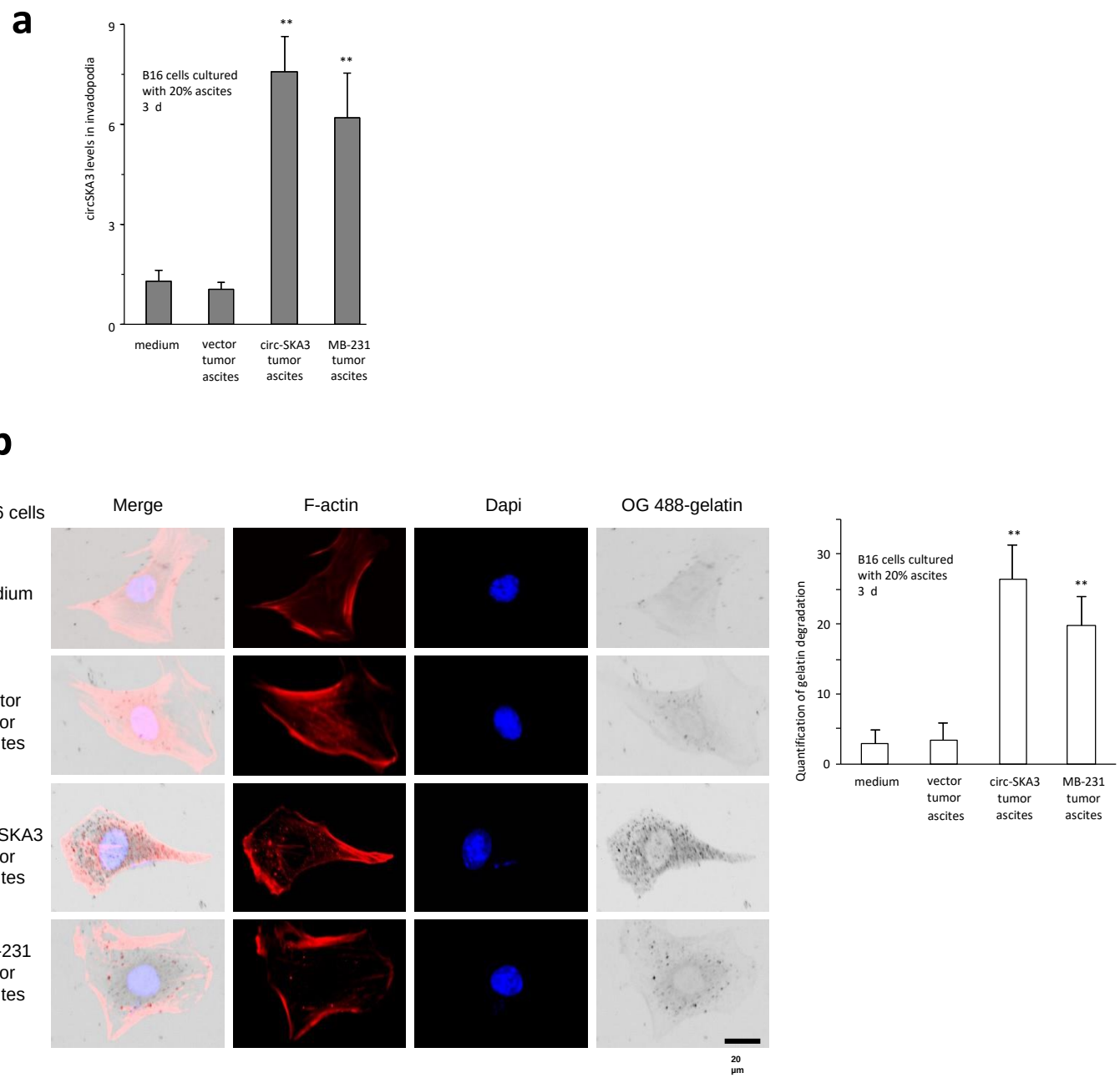
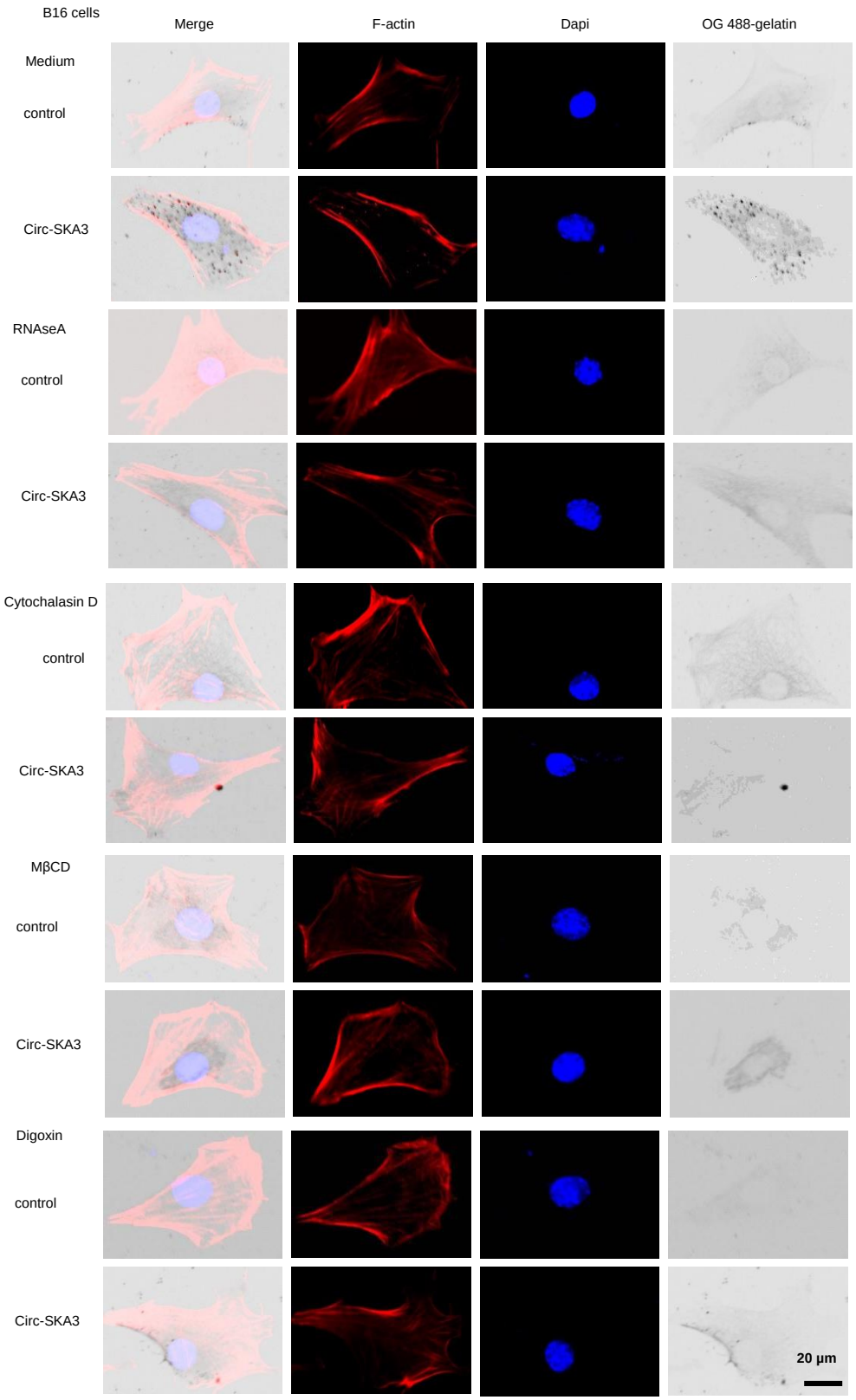


Figure S8. (a) B16 cells were loaded on gelatin coated and crosslinked culture dishes and incubated with basal medium and 20% mouse ascites for 3 days, followed by invadopodia collection and RNA extraction. Ascites from tumor-bearing mice injected with circSKA3-transfected B16 cells or MB-231 cells increased circSKA3 levels in invadopodia of the cultured B16 cells. **, $p<0.01$. Error bars, SD ($n=6$).

(b) B16 cells were incubated with basal medium and 20% mouse ascites for 3 days, followed by gelatin degradation assays (left) and Image-J analysis (right). Ascites from tumor-bearing mice injected with circSKA3-transfected B16 cells or MB-231 cells showed enhanced gelatin degradation. **, $p<0.01$. Error bars, SD ($n=6$).



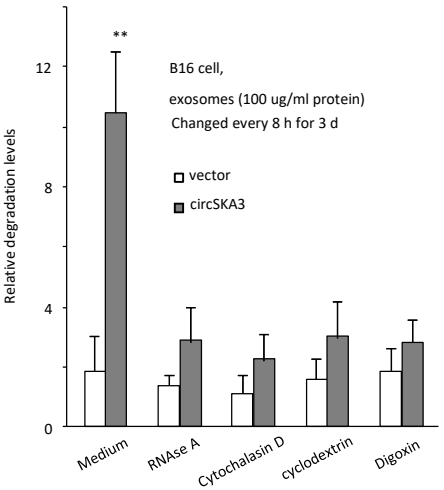
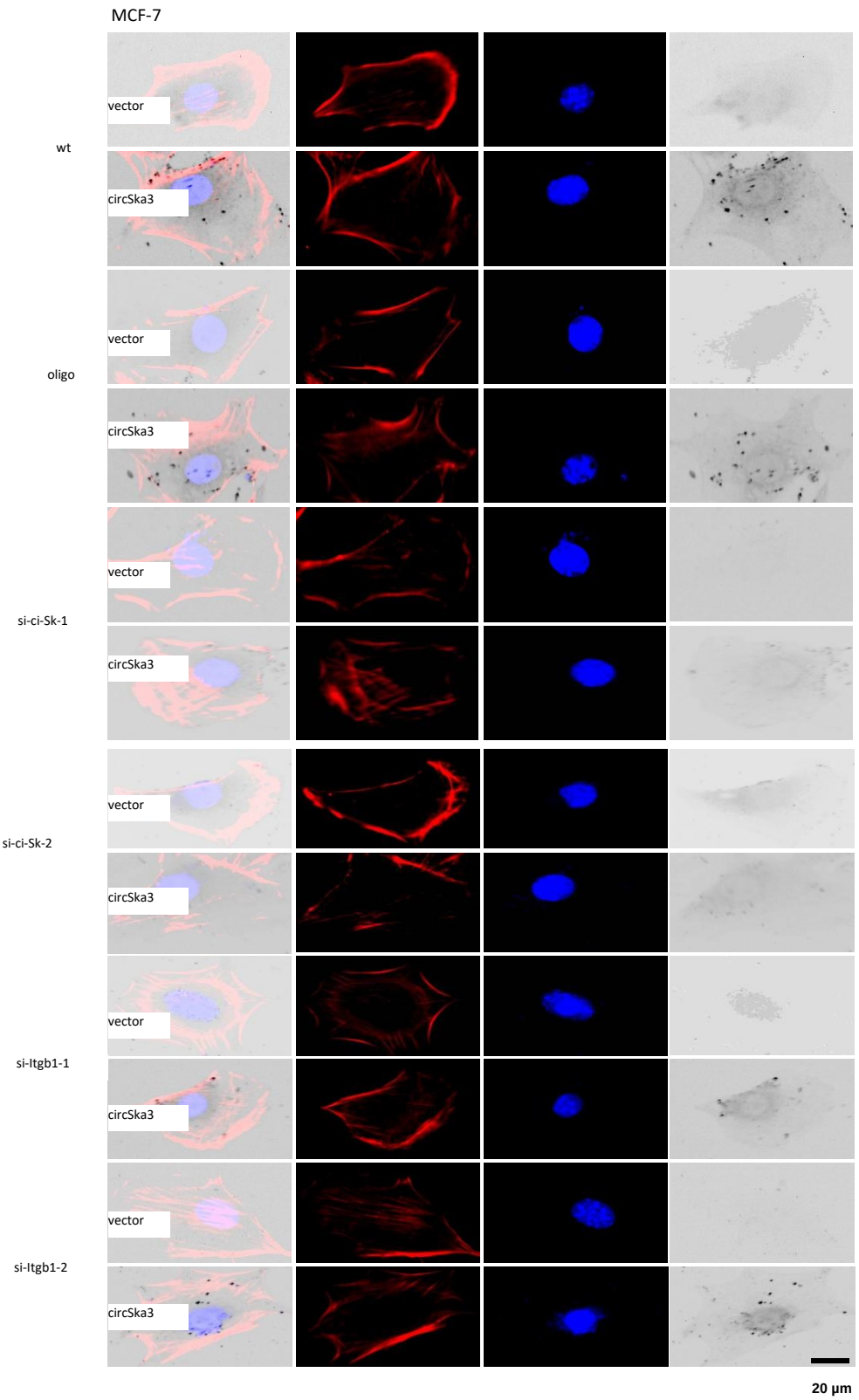


Fig S9. B16 cells were cultured in basal medium with 100 μg/ml control vector-, or circSKA3-packed exosomes and chemicals including RNAseA, Cytochalasin D, Digoxin or MβCD for 3 days, and processed to gelatin degradation assays (left) and Image-J analysis (right). Ascites from tumor-bearing mice injected with circSKA3-transfected B16 cells showed enhanced gelatin degradation, which could be prevented by RNAseA, Cytochalasin D, Digoxin or MβCD treatment. **, $p<0.01$. Error bars, SD ($n=5$).



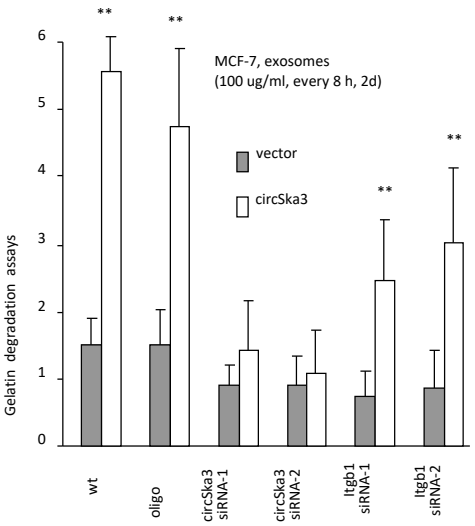


Fig S10. **Dissociation of the complex decreased gelatin invasion.**

(b) MCF-7 cells were transfected with control oligo, circSKA3 siRNA or integrin beta1 siRNA; incubated in vector or circSKA3 packed exosomes for 2 days and proceeded to gelatin degradation assay. Image J analysis showed that delivering circSKA3 packed exosomes enhanced invadopodia formation which could be blocked by silencing circSKA3 but not integrin beta1. ** $p<0.01$, Error bar, SD, (n=6)

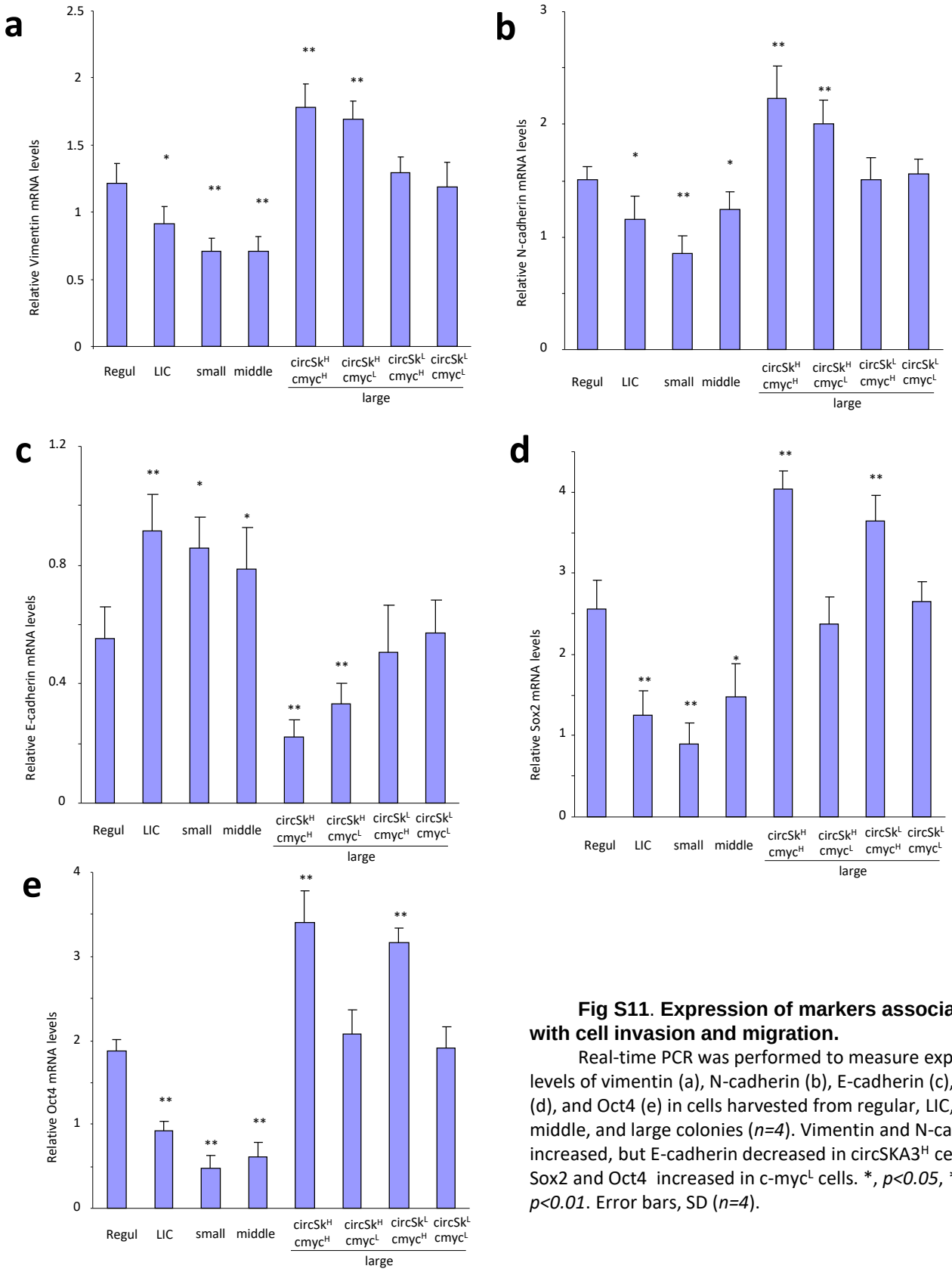


Fig S11. Expression of markers associated with cell invasion and migration.

Real-time PCR was performed to measure expression levels of vimentin (a), N-cadherin (b), E-cadherin (c), Sox2 (d), and Oct4 (e) in cells harvested from regular, LIC, small, middle, and large colonies (*n=4*). Vimentin and N-cadherin increased, but E-cadherin decreased in circSKA3^{H} cells. Sox2 and Oct4 increased in c-myc^{L} cells. *, *p<0.05*, **, *p<0.01*. Error bars, SD (*n=4*).

Supplementary Table S1. Primers used for PCR

29.70.H-coding-Ska3-F	5' gaaggcattgatttcataaaggca
29.12.H-coding.SKA3-R	5’ cag aca gat cat ctttc aca tcag
31.15.hu.Cir.SKA3-R2	5’ cacaattagacaactctgggtcag
31.16.hu.Cir.SKA3-F2	5’ cacaatgggacttaaaaatgcgag
39.41.hu.Vimentin-F.	5’ cttctccgggagccagtccg
39.42.hu.Vimentin-R.	5’ cctgcggtaggaggacgagg
39.43.hu.NCadherin-F.	5’ gcgaatgatcttaggattggg
39.44.hu.NCadherin-R.	5’ gggaattcagcacccgcctc
22-64.Hu-E-cadherin-601F	5’ tcccatcagc tgcccagaaa atga
22-65.Hu-E-cadherin-720R	5’ gtgt ca gc tcctt ggcc ag tg atg
19-71.Hom-Sox2-421F	5’ cgcccgcatg tacaacatga tgg
19-72.Hom-Sox2-660R	5’ tc ggc gccc agg cgc ttg ct gatc
19-73.Hom-c-myc-64F	5’ cgggtag tggaaaacca ggtaagc
19-74.Hom-c-myc-299R	5’ tttccc tct gcc ttc tcct ctccc
19-67.Hom-Oct4-241F	5’ gcgccttcct tccccatggc ggg
19-68.Hom-Oct4-480R	5’ gt ac gcc at cccccc aca taa ctc