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Supplemental Material to:

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MiR-93 enhances angiogenesis and metastasis by targeting LATS2

2012; 11(23)
http://dx.doi.org/10.4161/cc.22670

http://www.landesbioscience.com/journals/cc/article/22670

Supplementary Figure Legends

Fig S1. Co-culture experiments. Mock- and miR-93-transfected cells were co-cultured with endothelial cells Ypen (a) or EOMA (b) at the ratio of 2:1. After 2 days, the co-cultured cells were photographed. The miR-93 cells displayed higher capacities in expansion than the vector-transfected cells. As a result, the endothelial cells were squeezed into small islands by the miR-93 cells. The mock- and miR-93-transfected cells were also co-cultured with lung cells BEAS-2B. The miR-93 cells could mix well with the lung cells as compared with the mock cells (c).

Fig S2. The role of miR-93 in cell adhesion, tube formation and metastasis.

(a) MT-1 cells stably transfected with miR-93 or mock were seeded on tissue culture plates at a cell density of $1.5x10^5$ cells/well on 6-well plates. After overnight culture, endothelial cells Ypen were inoculated on top of the existing cultures ($6x10^4$ cells/well). Cell-cell interaction was examined and photographed.

(b) The miR-93- and mock-transfected cells were mixed with Ypen cells and inoculated in Matrigel, followed by examination of tube formation. The Ypen cells formed larger complexes and longer tubes when mixed with the miR-93 expressing cells compared with the mock-transfected cells.

(c) DNA was isolated from lung tissues and subjected to PCR to amplify the CMV promoter to indicate metastasis of the tissues. Expression of miR-93 promoted metastasis.

Fig S3. The role of miR-93 in cell survival and invasion.

(a) The MT-1 cells transfected with miR-93 or mock (2.5×10^5 cells/ml) were seeded onto Petri dishes and tissue culture dishes and were maintained in a serum-free medium at 37°C and 5% CO_2 for 4 days. The cells were counted at the end of seventh. The miR-93 transfected cells were seen to survive better.

(b) The miR-93 and mock cells ($1x10^5$) suspended in 100 µl serum-free DMEM medium were loaded into the insert and incubated at 37°C for 48 h. The invasive cells were stained blue.

(c) MT-1 cells were transiently transfected with anti-miR-93 oligos or control oligos with random sequence. The cultures were maintained in tissue culture dishes in serum-free DMEM for 5 days, followed by microscopic examination and photographed.

(d) MT-1 cells were transiently transfected with anti-miR-93 oligos or control oligos with random sequence, or with anti-miR-93 plasmid or control vector. The cells ($1x10^5$) were subjected to invasion assays at 37°C for 48 h. Transfection with anti-miR-93 inhibited cell invasion.

Fig S4. Generation of luciferase constructs. Fragments of LATS2 3'UTRs were inserted into the luciferase report vector pMir-Report producing constructs Luc-Lats-3955 and Luc-Lats-4058. The potential miR-93 target sites were labeled in blue. Mutations labeled in red were generated in the miR-93 target sites producing mutant constructs Luc-Lats-3955-mut and Luc-Lats-4058-mut.

Fig S5. Expression of LATS2 in human breast cancer and mouse cell lines.

(a) Sections from human breast carcinoma and normal tissues (N-) were subjected to immunohistochemistry for LATS2 expression. LATS2 was detected in the ductal structures but not in the tumor mass.

(b) Left, cell lysates prepared from different mouse breast cancer cell lines were subjected to western blot analysis probed with anti-LATS2 and anti-actin antibodies. Right, The levels of miR-93 were analyzed by real-time PCR.

Fig S6. Confirmation of LATS2 functions.

(a) The MT-1 cells ($1x10^5$ cells/ml/well) were transfected with siRNA oligos targeting LATS2 or a control oligo. The cells were seeded onto a 12-well plate and incubated in a serum-free medium at 37°C for 7 days. The survived cells were counted. Transfection with siRNAs increased cell survival.

(b) The MT-1 cells transfected with siRNA oligos targeting LATS2 or a control oligo were subjected to invasion assays at 37°C for 48 h. Transfection with siRNAs increased invasion.

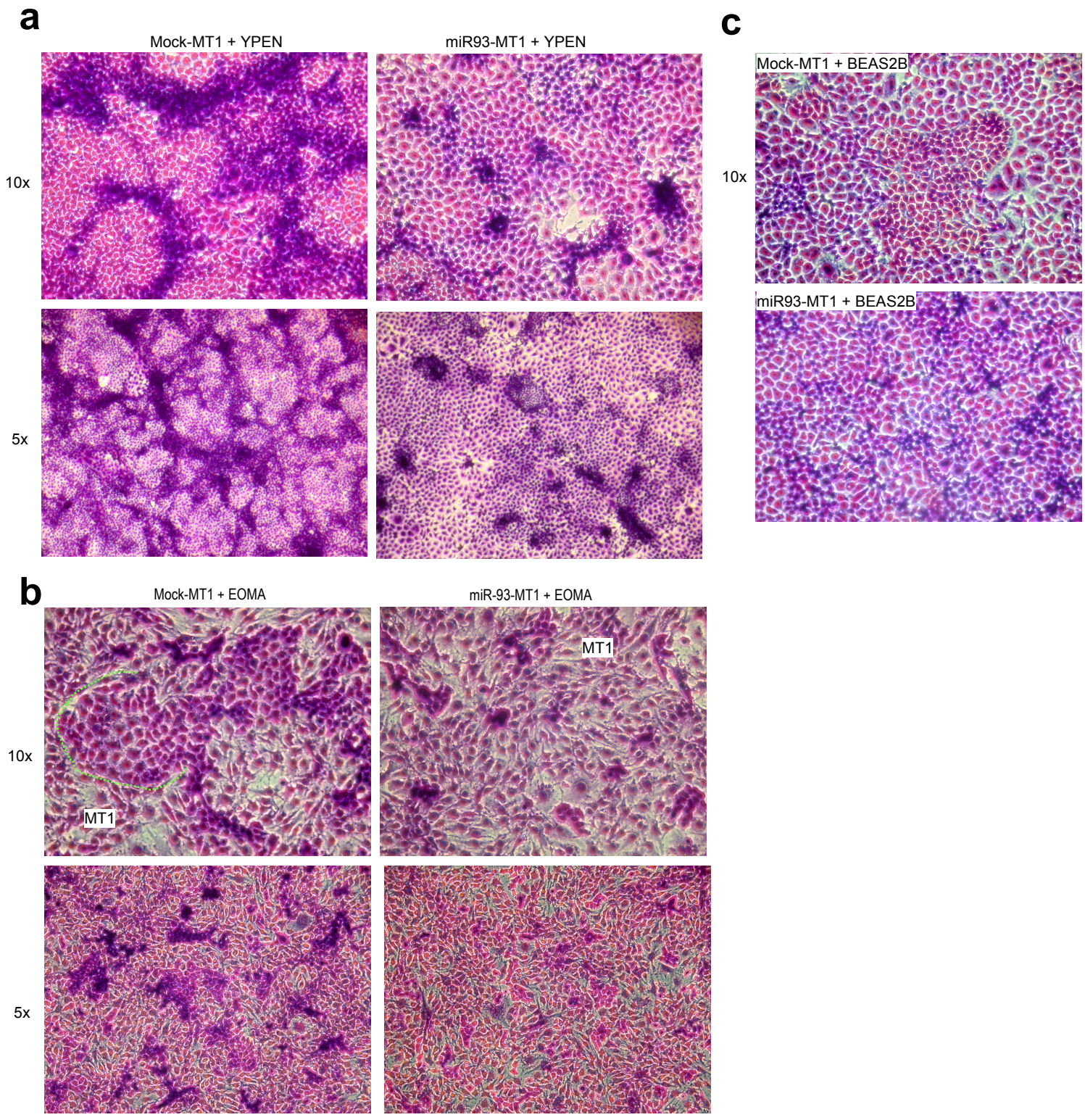
(c) Cell lysates prepared from MT-1 cells transfected with siRNA-2439 or a control oligo were subjected to Western blot analysis for LATS2 levels. As expected, transfection with siRNA-2439 silenced its expression.

(d) Cell lysates prepared from miR-93-cells transfected with LATS2 or a control vector were subjected to Western blot analysis for expression of LATS2. As expected, transfection with

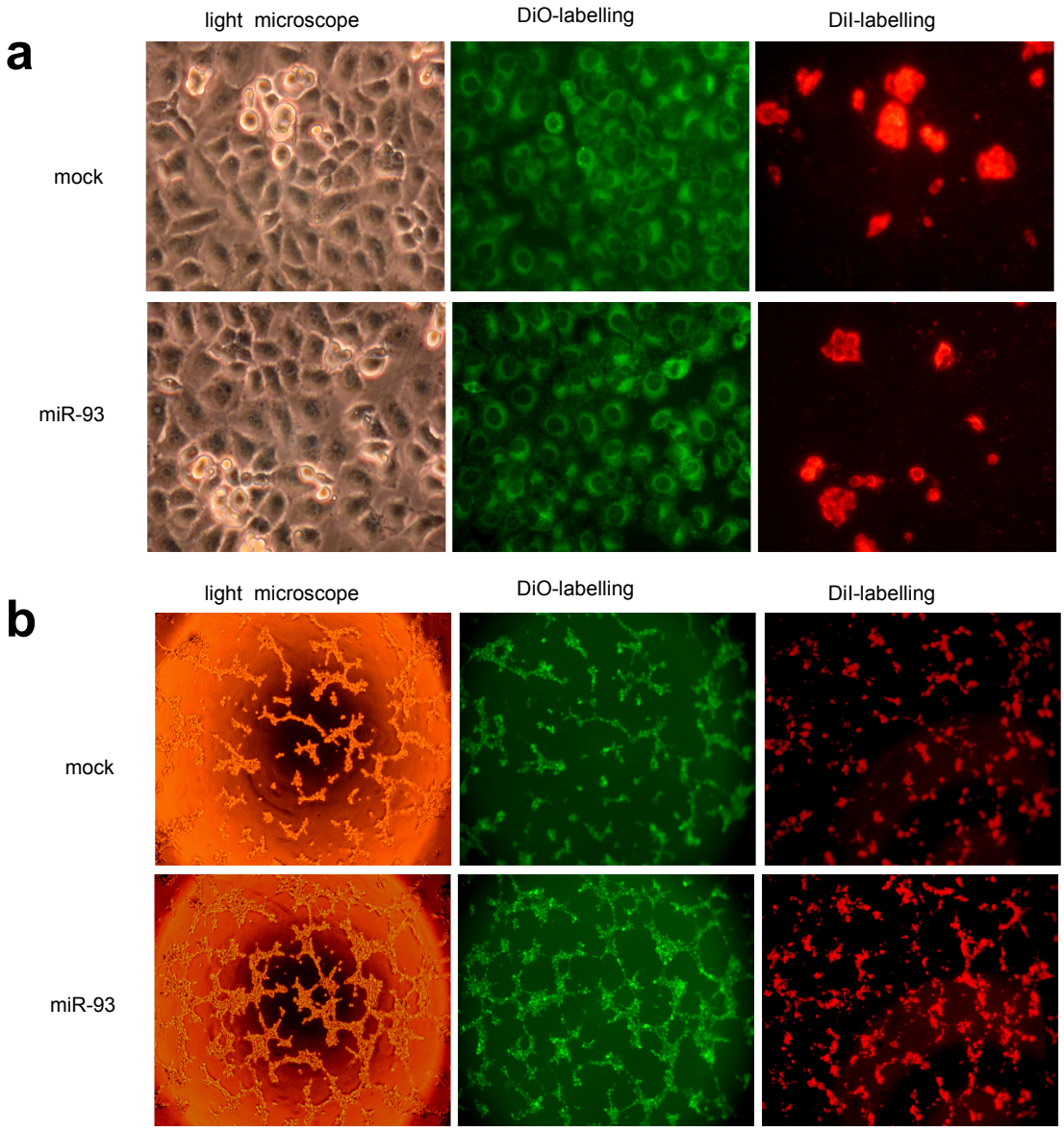
LATS2 increased its expression.

(e) Ectopic expression of LATS2 in the miR-93 cells reversed miR-93 effect on cell survival.

(f) Ectopic expression of LATS2 in the miR-93 cells reversed miR-93 effect on cell invasion.



Supplementary Fig S1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DiI-labelling
mock
miR-93
b
light microscope
DiO-labelling
DiI-labelling
mock
miR-93
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miR-93
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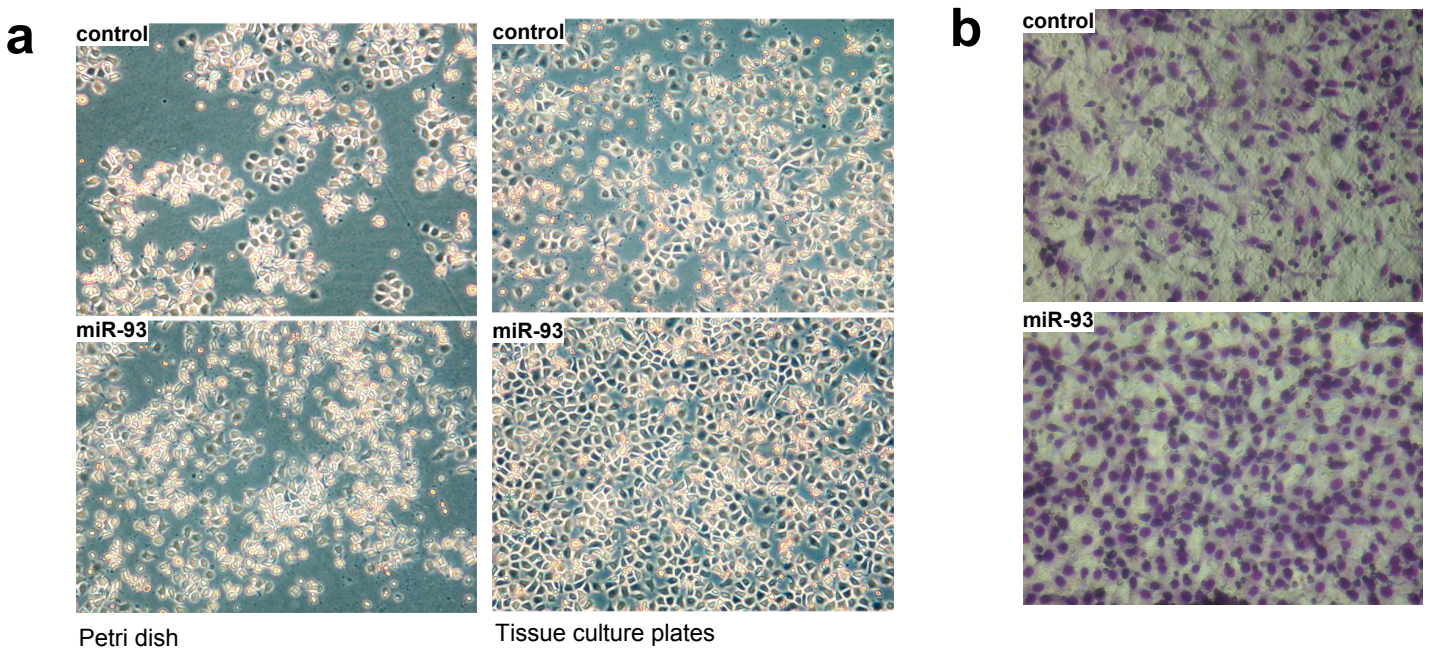
Fig S2.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miR-93
control
miR-93
Petri dish
Tissue culture plates
b
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miR-93
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anti-miR-93
d
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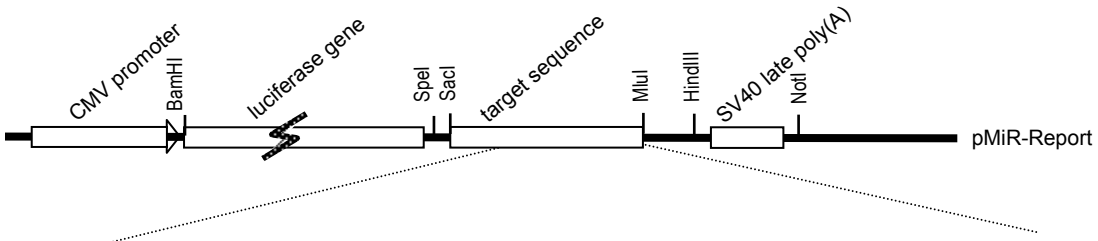
anti-miR-93
control
anti-miR-93
oligo
plasmid

Fig S3.



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Luc-Lats-3955-mut

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Luc-Lats-4058-mut

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ACGCGT

Luc-Ctrl

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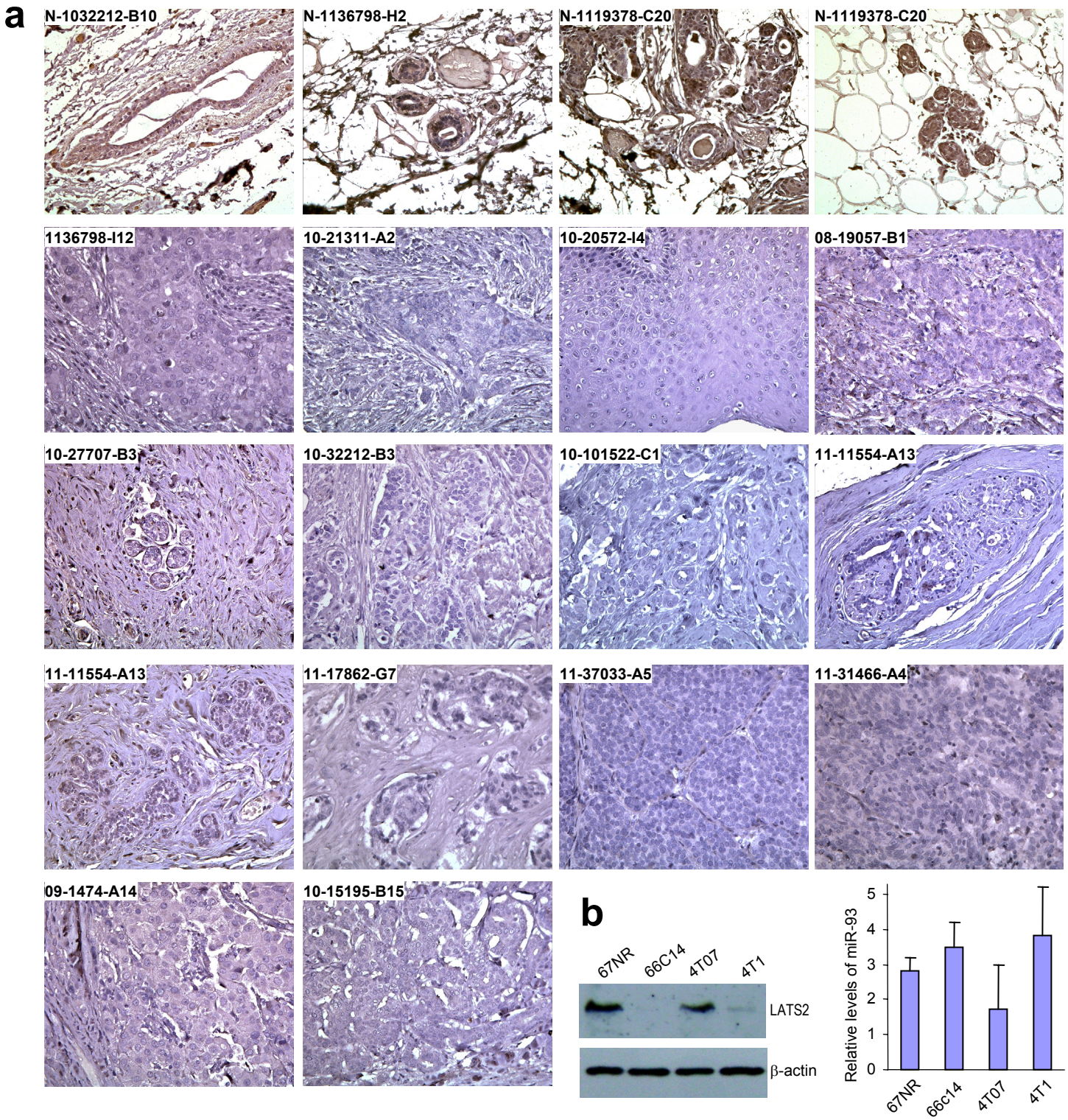
Fig S4.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66C14
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4T1
LATS2
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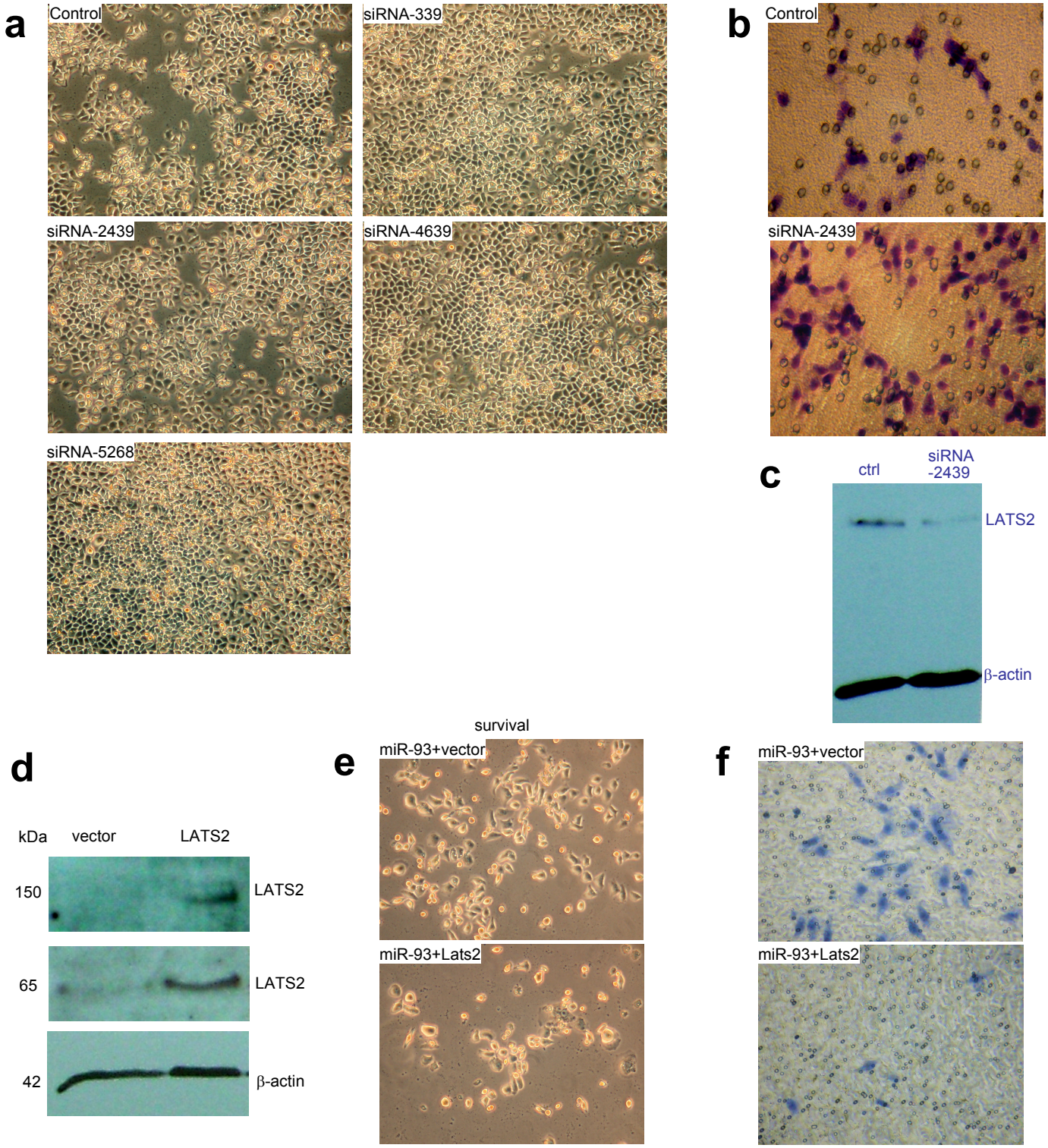
66c14
4T07
4T1

Fig S5.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d
kDa
vector
LATS2
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65
LATS2
42
β-actin
e
survival
miR-93+vector
miR-93+Lats2
f
miR-93+vector
miR-93+Lats2

Fig S6.