SUPPLEMENTARY MATERIALS

Antisense sequences:

Antisense	Sequence
DICER1 as	5'-GCUGACCTTTTTGCTUCUCA-3'
Ctrl as (of *DICER1* as)	5'-TTGGTACGCATACGTGTTGACTGTGA-3'
Alu as	5'- CCCGGGTTCACGCCATTCTCCTGCCTCAGCCTCACGATAGCTG GGACTACAGGCGCCCGACACCACTCCCGGCTAATTTTTTGTATT TTT-3'
Ctrl as (of *Alu* as)	5'- GCATGGCCAGTCCATTGATCTTGCACGCTTGCCTAGTACGCTC CTCAACCTATCCTCCTAGCCCGTTACTTGGTGCCACCGGCG-3'

QPCR Primers:

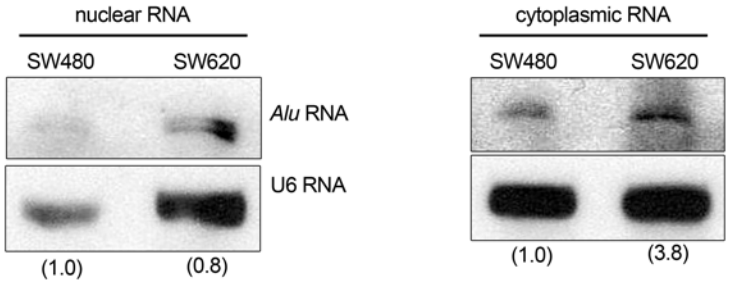
Primer	5'-Sequence-3'
hDICER1 F	CCCGGCTGAGAGAACTTACG
hDICER1 R	CTGTAACTTCGACCAACACCTTTAAA
h18S F	CGCAGCTAGGAATAATGGAATAGG
h18S R	GCCTCAGTTCCGAAAACCAA
Alu F	CAACATAGTGAAACCCCGTCTCT
Alu R	GCCTCAGCCTCCCGAGTAG
hFibronectin F	CAGTGGGAGACCTCGAGAAG
hFibronectin R	GTCCCTCGGAACATCAGAAA
hE-cadherin F	GAACGCATTGCCACATACAC
hE-cadherin R	ATTCGGGCTTGTTGTCATTC
hN-cadherin F	GGAGATGGGGGAAATTTGTT
hN-cadherin R	GGTCAAGGTGAAGGTTGGAA
hVimentin F	TGCCCTTAAAGGAACCAATG
hVimentin R	CTCAATGTCAAGGGCCATCT
hZEB1 F	ACTGCTGGGAGGATGACAGA
hZEB1 R	ATCCTGCTTCATCTGCCTGA
hTWIST1 F	AGCTACGCCTTCTCGGTCT
hTWIST1 R	CCTTCTCTGGAAACAATGACAT
hVHL F	CCCAGGTCATCTTCTGCAAT
hVHL R	GTGTGTCCCTGCATCTCTGA
hSnail F	CTTCCAGCAGCCCTACGAC
hSnail R	CGGTGGGGTTGAGGATCT
hSlug F	TTCGGACCCACACATTACCT
hSlug R	GCAGTGAGGGCAAGAAAAA
hZEB2 F	CAAGAGGCGCAAACAAGC
hZEB2 R	GGTTGGCAATACCGTCAT

Antibodies:

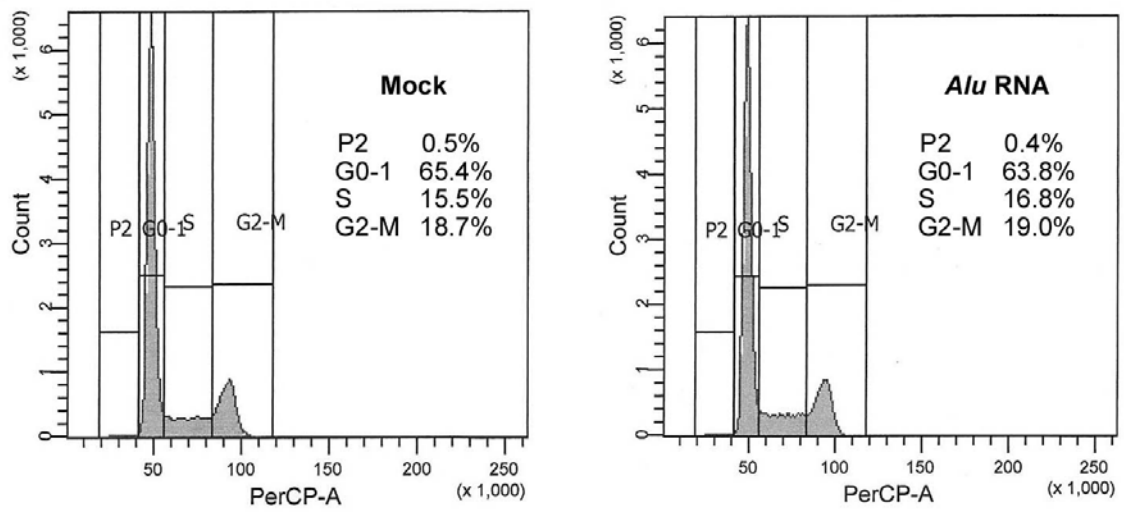
Antigen	Vendor	Catalog Number
DICER1	Bethyl	A301-937A
Fibronectin	Sigma-Aldrich	F3648
Vimentin	Cell Signaling	5741
E-cadherin	Cell Signaling	5296
Zeb1	Atlas Antibodies	HPA027524
TWIST1	Santa Cruz	sc-1593 (H-25)
β-Tubulin	Santa Cruz	sc-9104 (H-235)

Figure S1

a



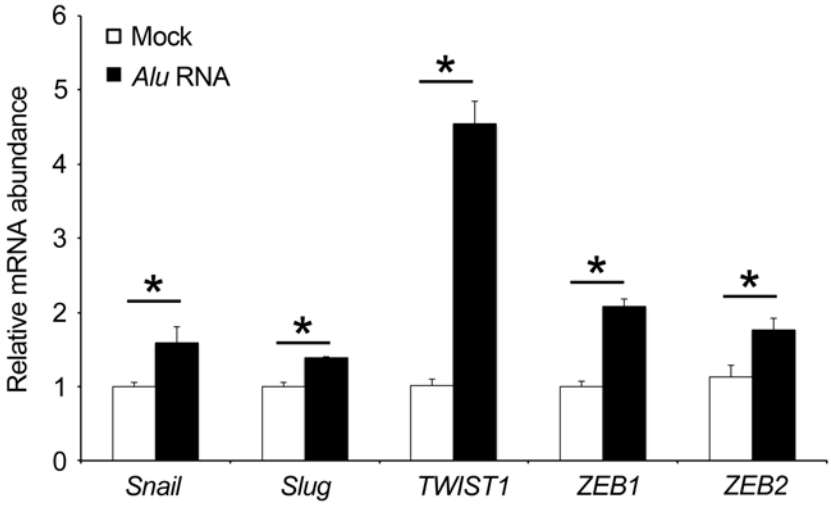
b



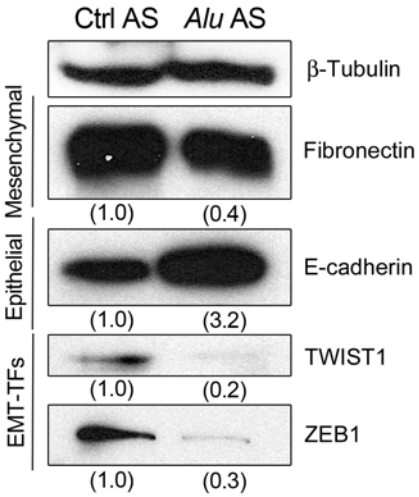
a) Northern blot analysis shows *Alu* RNA abundance in nucleus and cytoplasm of SW480 and SW620 cells. Densitometric values normalized against U6 snRNA are shown in parentheses. **b**) *Alu* RNA does not affect SW480 cell cycle. Cell cycle profiles were analyzed by propidium iodide staining and flow cytometry. Tables indicate the cells in the G0/G1, S and G2/M phases of the cell cycle, respectively.

Figure S2

a



b

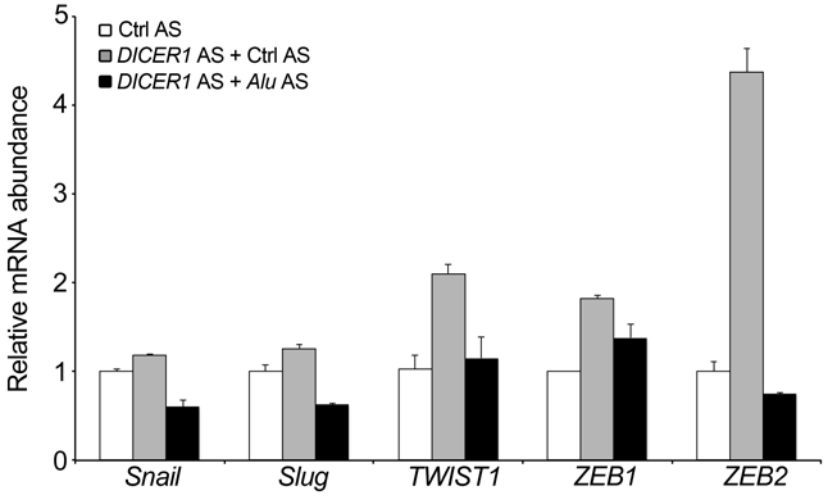


a) *Alu* RNA induces the expression of *Snail, Slug, TWIST1*, *ZEB1 and ZEB2* mRNAs as evaluated by qRT-PCR and normalized against 18S rRNA. **b**) *Alu* RNA knockdown obtained transfecting an antisense oligonucleotide (*Alu* AS), decreases the expression of Fibronectin and EMT-TFs (TWIST1 and ZEB1) and increases that of E-cadherin compared

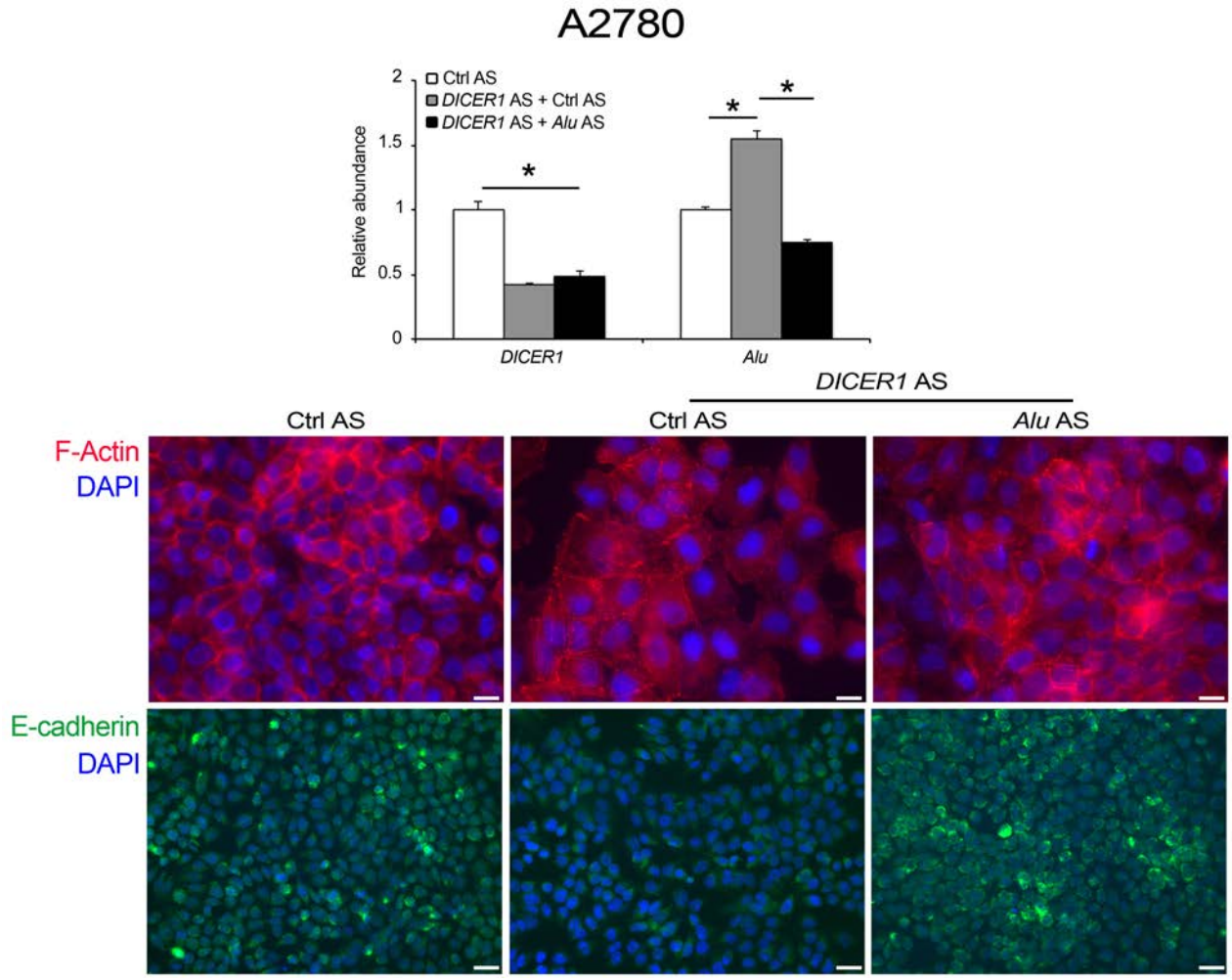
to cell transfected with a scramble oligonucleotide (Ctrl AS), as evaluated by western blot. Densitometric values normalized against β-Tubulin are shown in parentheses. Data represent as mean +SEM (error bars) (n=3), $p<0.05$.

Figure S3

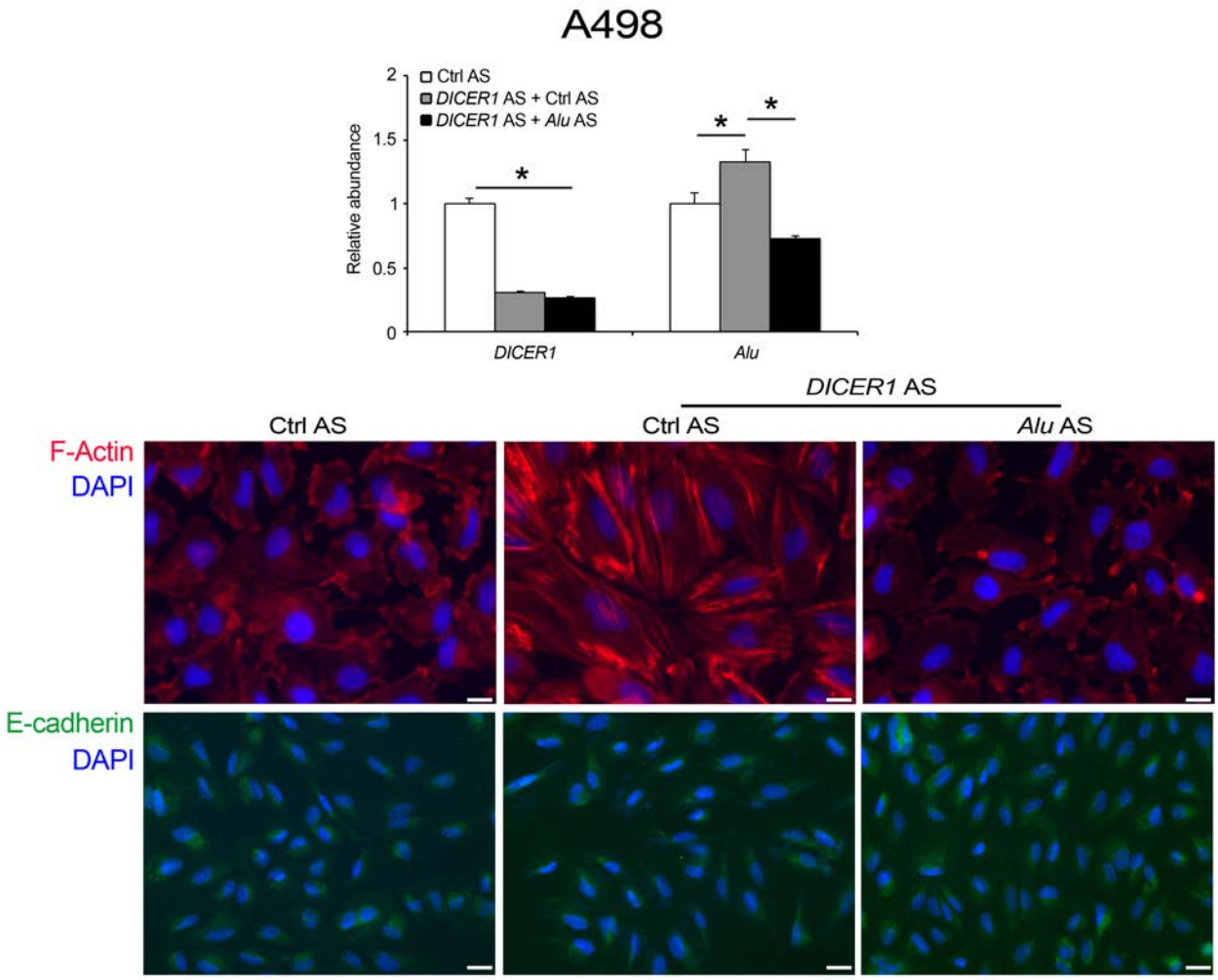
a


Ctrl AS
DICER1 AS + Ctrl AS
DICER1 AS + Alu AS
Relative mRNA abundance
0
1
2
3
4
5
Snail
Slug
TWIST1
ZEB1
ZEB2

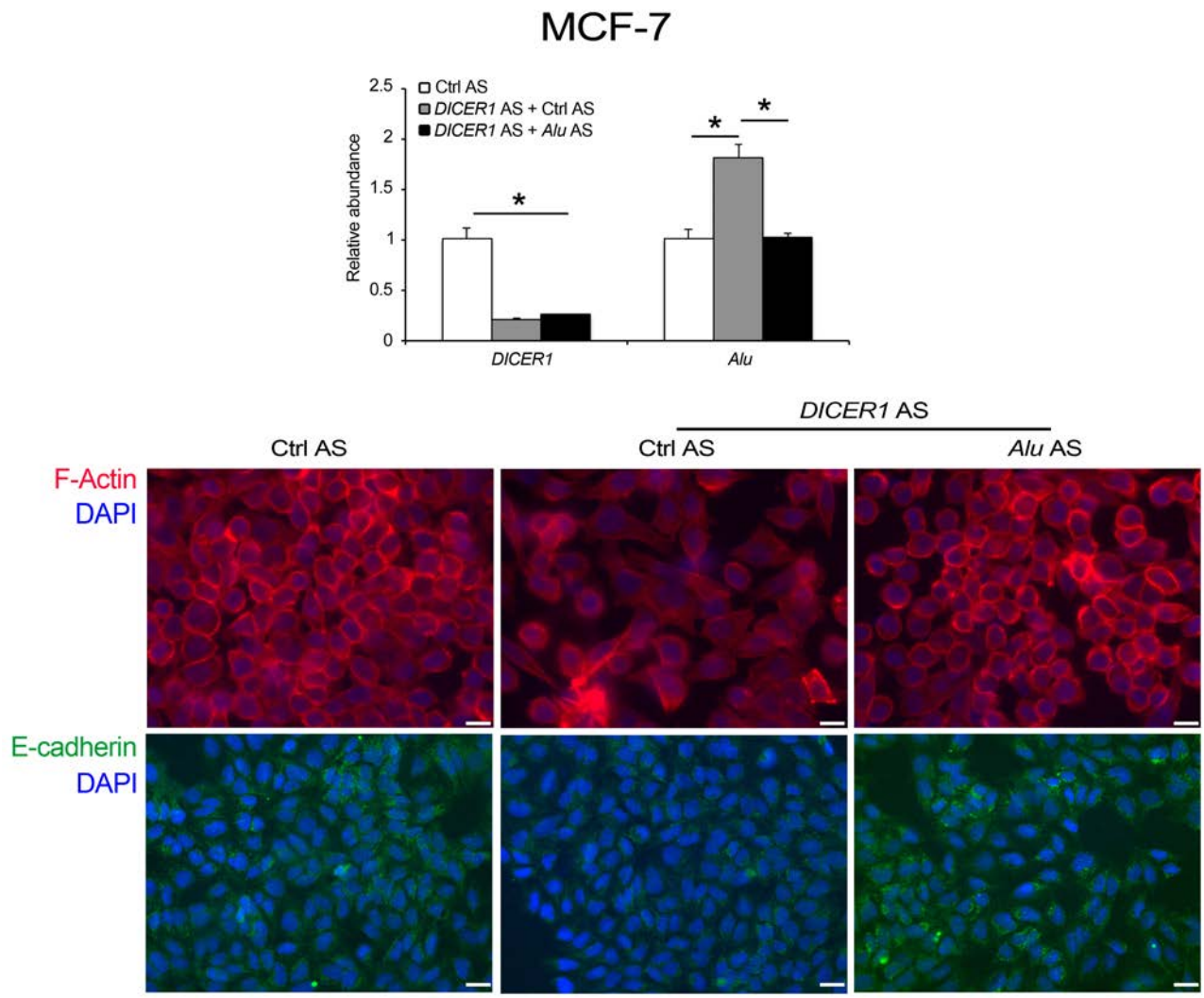
b


A2780
Ctrl AS
DICER1 AS + Ctrl AS
DICER1 AS + Alu AS
Relative abundance
0
0.5
1
1.5
2
*
*
*
DICER1
Alu
DICER1 AS
Ctrl AS
Ctrl AS
Alu AS
F-Actin
DAPI
E-cadherin
DAPI

c


A498
Ctrl AS
DICER1 AS + Ctrl AS
DICER1 AS + Alu AS
Relative abundance
2
1.5
1
0.5
0
*
*
*
DICER1
Alu
DICER1 AS
Ctrl AS
Ctrl AS
Alu AS
F-Actin
DAPI
E-cadherin
DAPI

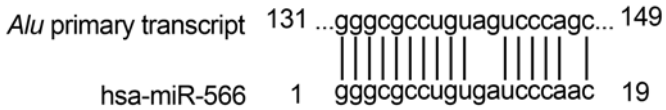
d



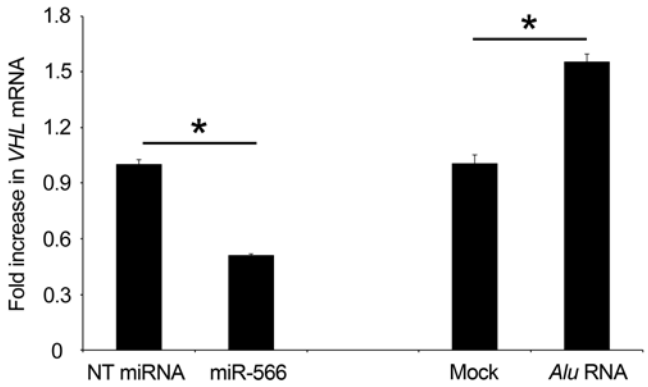
***a**) DICER1* AS increases the expression of *Snail, Slug, TWIST1*, *ZEB1 and ZEB2* mRNAs and the co-transfection of *Alu* AS reduces the expression as evaluated by qRT-PCR and normalized against 18S rRNA. Data represent as mean ±SEM (error bars). *Alu* RNA accumulate as consequence of DICER1 deficit in A2780 (**b**), A498 (**c**) and MCF-7 (**d**) cells and the co-transfection of *Alu* AS is able to inhibit their accumulation and the DICER1-induced EMT as showed by F-Actin and E-cadherin staining. Representative images of F-actin (red) and E-cadherin (green) staining. Nuclei are counterstained with DAPI (blue). Scale bar: 75 μm.

Figure S4

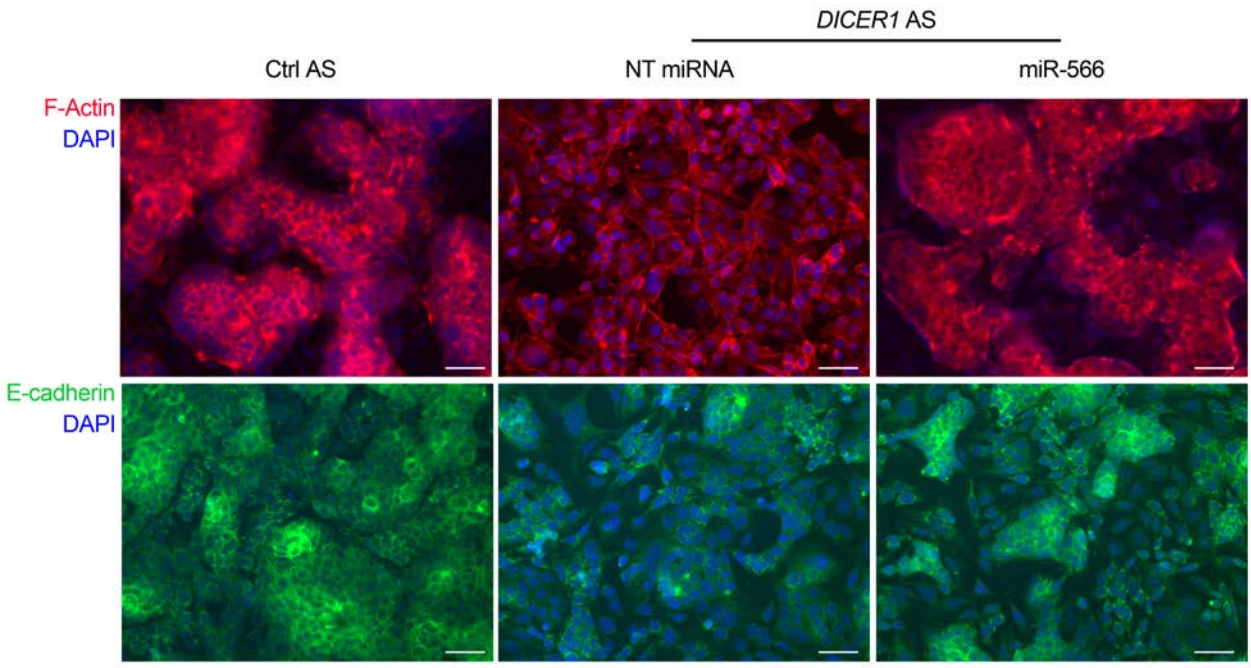
a



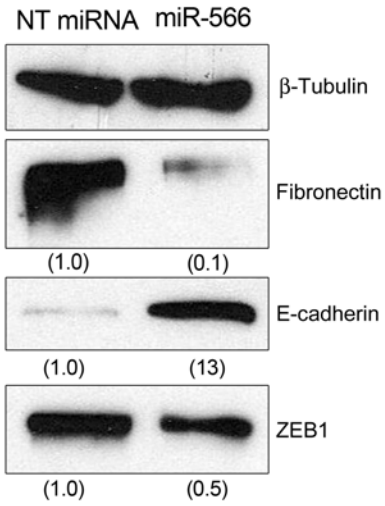
b



c



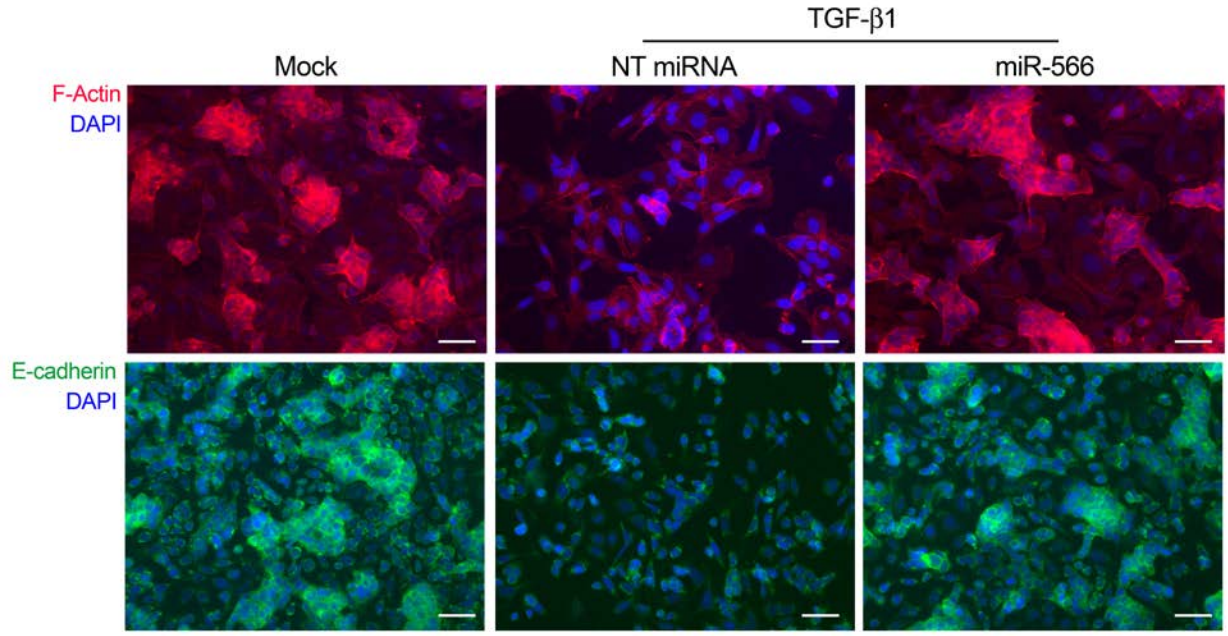
d



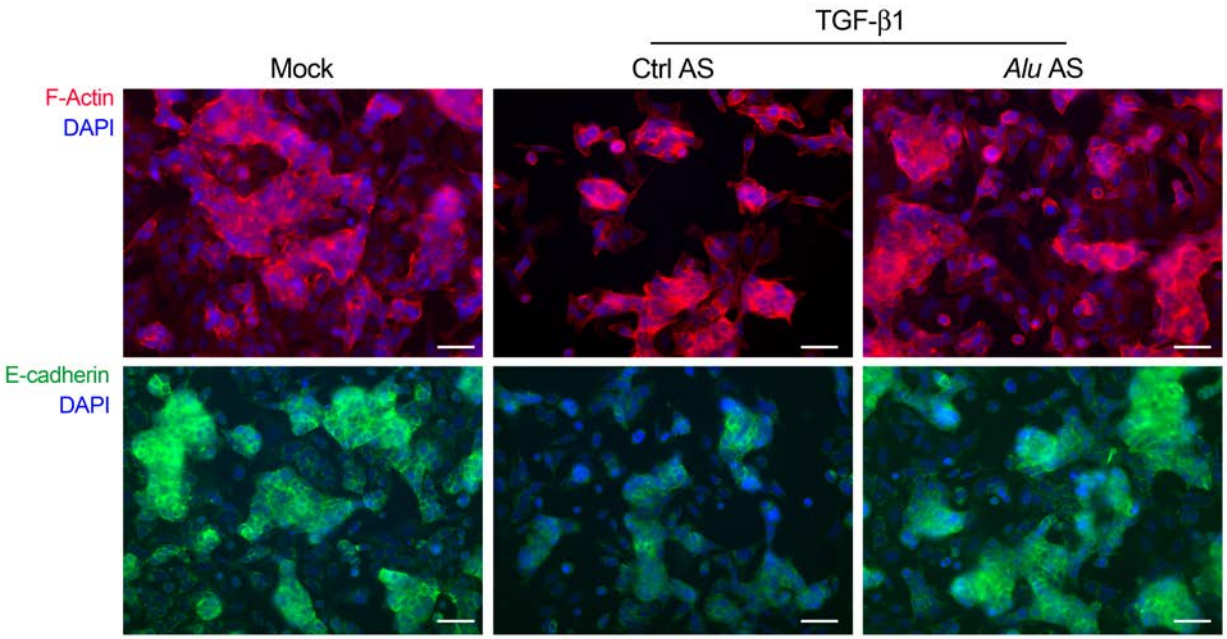
(**a**) Base-pairing between human *Alu* primary transcript (GenBank: U67825; clone TS 103) and miR-566 (**b**) miR-566 reduces and *Alu* RNA increases the abundance of *VHL* mRNA as evaluated by qRT-PCR and normalized against 18S rRNA. Data represent as mean +SEM (error bars) (n=3), $p<0.05$. (**c**) miR-566 recues the DICER1 deficit-induced EMT. Representative images of F-actin (red) and E-cadherin (green) staining. Nuclei are counterstained with DAPI (blue). Scale bar: 75 μm. (**d**) miR-566 transfection in SW620 decreases the protein abundance of Fibronectin and ZEB1 and increases E-cadherin level as evaluated by western blot. Densitometric values normalized against β-Tubulin are shown in parentheses. For all panels: n=3; *$p<0.05$. Error bars denote SEM.

Figure S5

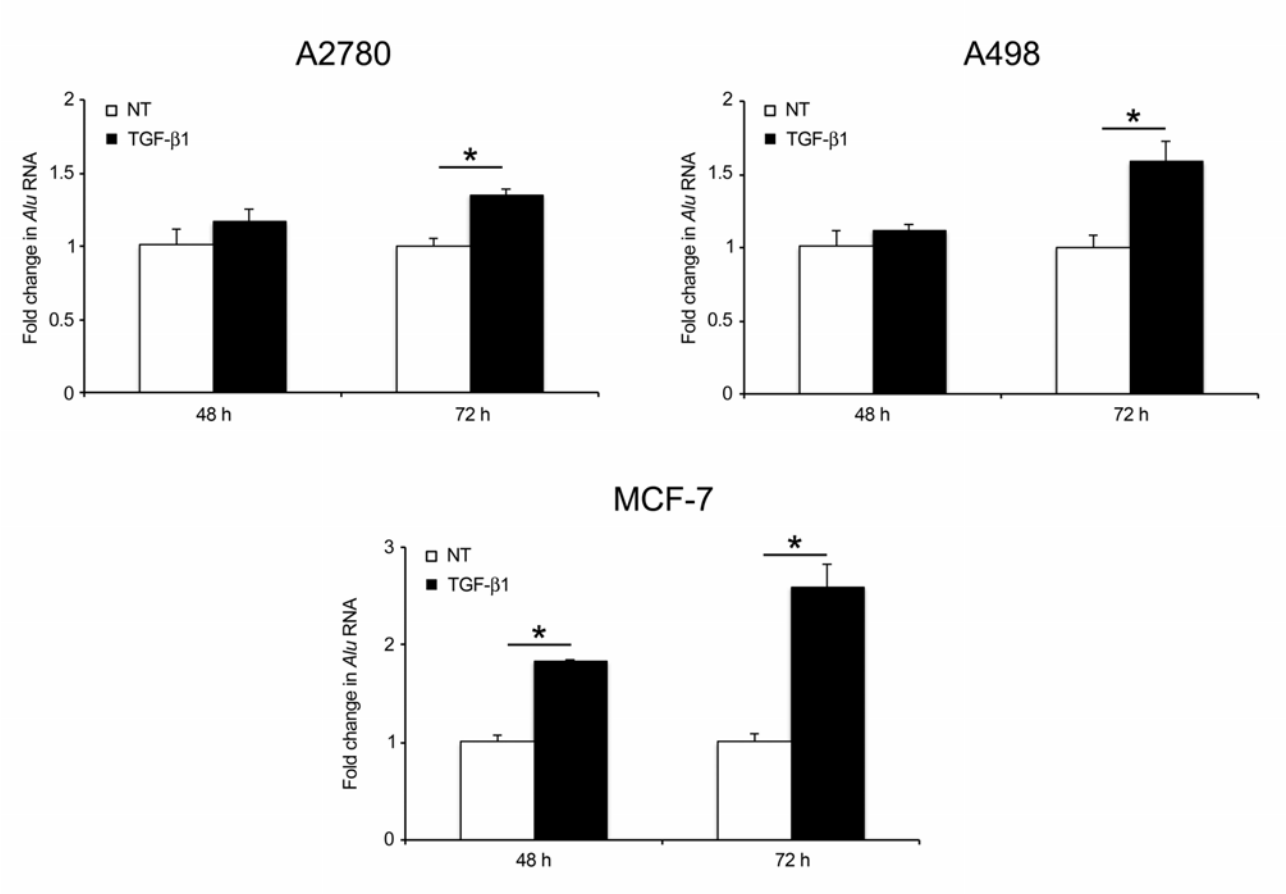
a


TGF-β1
Mock
NT miRNA
miR-566
F-Actin
DAPI
E-cadherin
DAPI

b


TGF-β1
Mock
Ctrl AS
Alu AS
F-Actin
DAPI
E-cadherin
DAPI

c



Representative images of F-Actin (red) and E-cadherin (green) staining of SW480 cells treated with TGF-β1 and then transfected with **a**) NT miRNA or miR-566 and **b**) Ctrl AS or *Alu* AS. Nuclei are counterstained with DAPI (blue). Scale bar: 75 μm. **c**) TGF-β1 increases the abundance of *Alu* Pol III-derived transcripts as evaluated by qRT-PCR analysis in A2780, in A498 and in MCF-7. Data represent as mean +SEM (error bars) (n=3), $p<0.05$.