MRPS18-2 protein: functions and possibility of vaccine targeting

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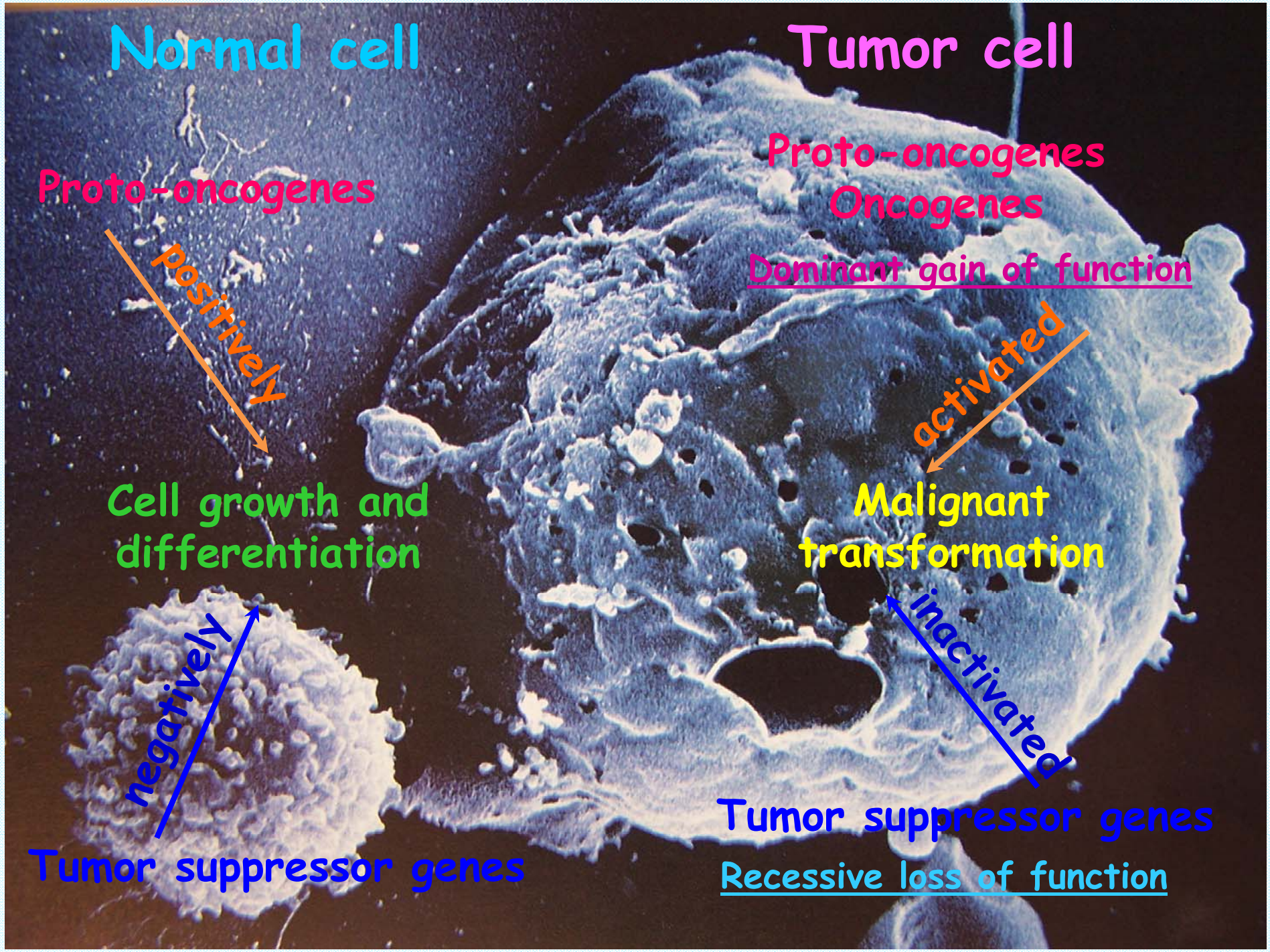
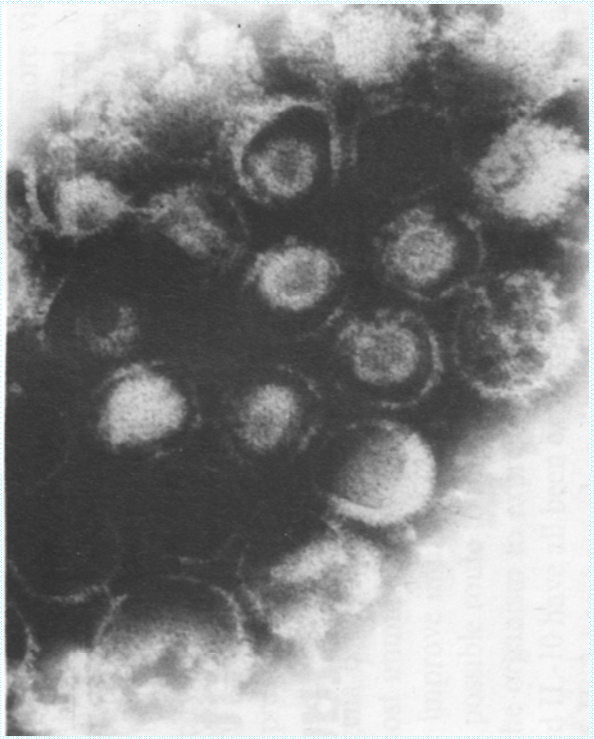
Leading Scientist

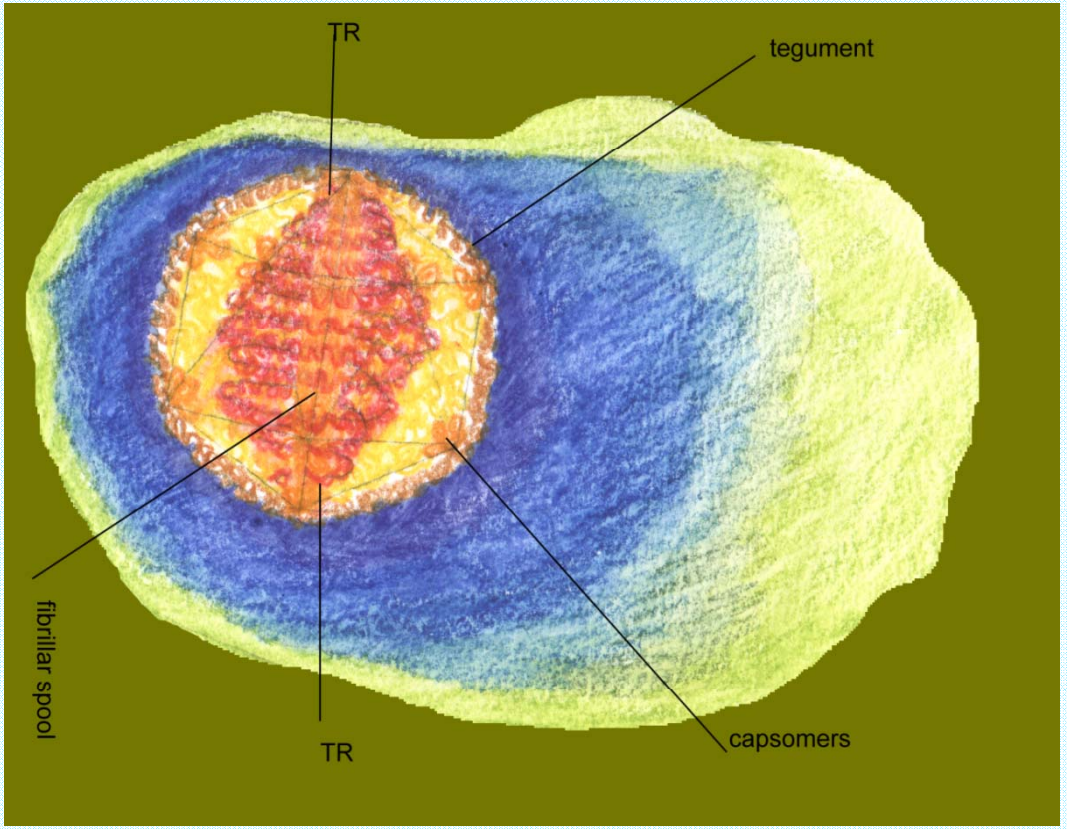
RE Kavetsky IEPOR of NASU

Normal cell	Tumor cell
- Recognize others of the same type Stick close to each other	- Loners
- Differentiated (specialized)	Loss of differentiation— become anaplastic
- Display contact inhibition-	Loss of contact inhibition
- Survive relatively short time, even in culture	Immortal (overcome Hayflick limit)


Normal cell
Tumor cell
Proto-oncogenes
positively
Cell growth and differentiation
Proto-oncogenes
Oncogenes
Dominant gain of function
activated
Malignant transformation
negatively
Tumor suppressor genes
inactivated
Tumor suppressor genes
Recessive loss of function

Epstein-Barr virus - EBV - Everybody virus





200 nm Enveloped

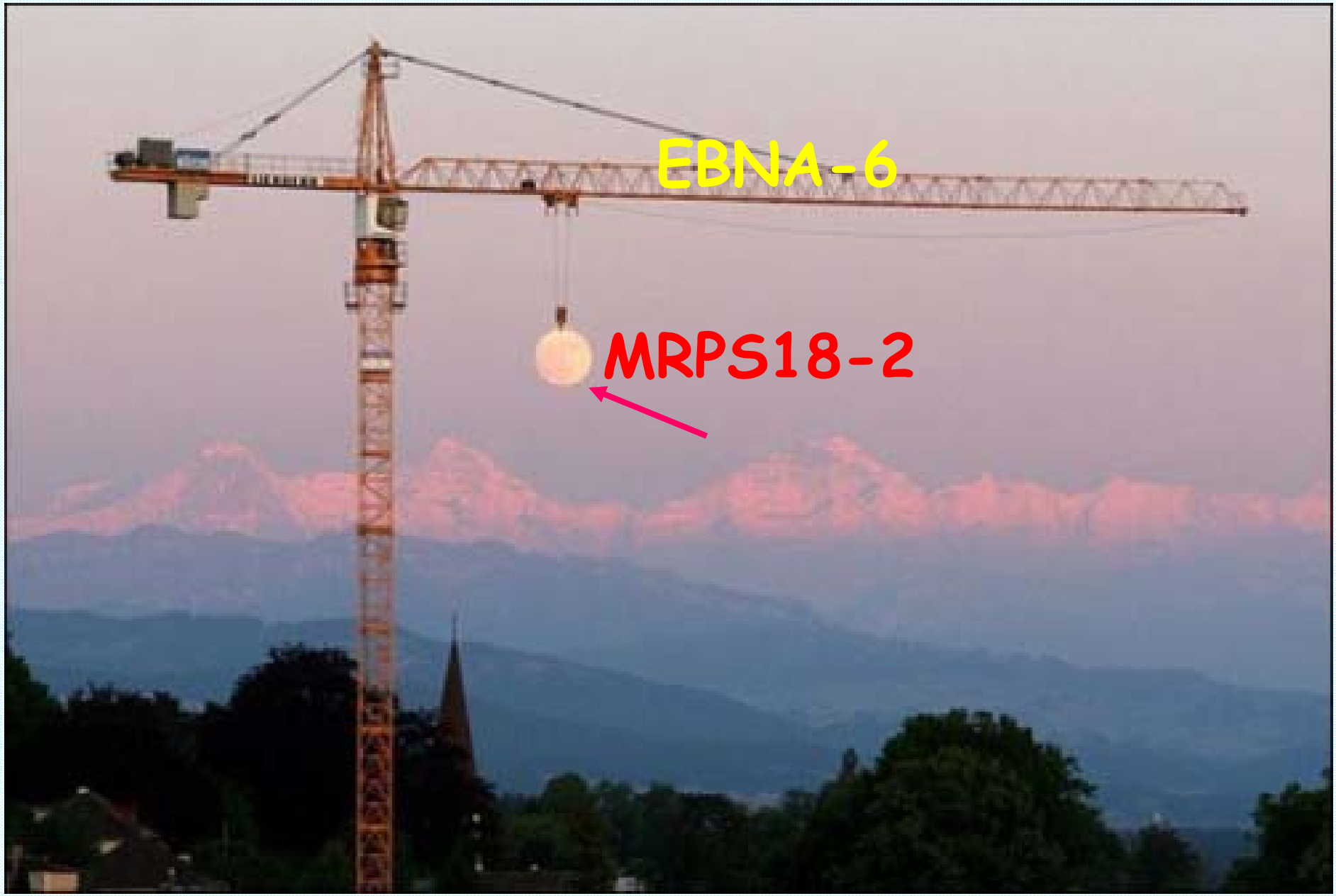
Double-stranded linear DNA 172274 bp

82 open reading frames

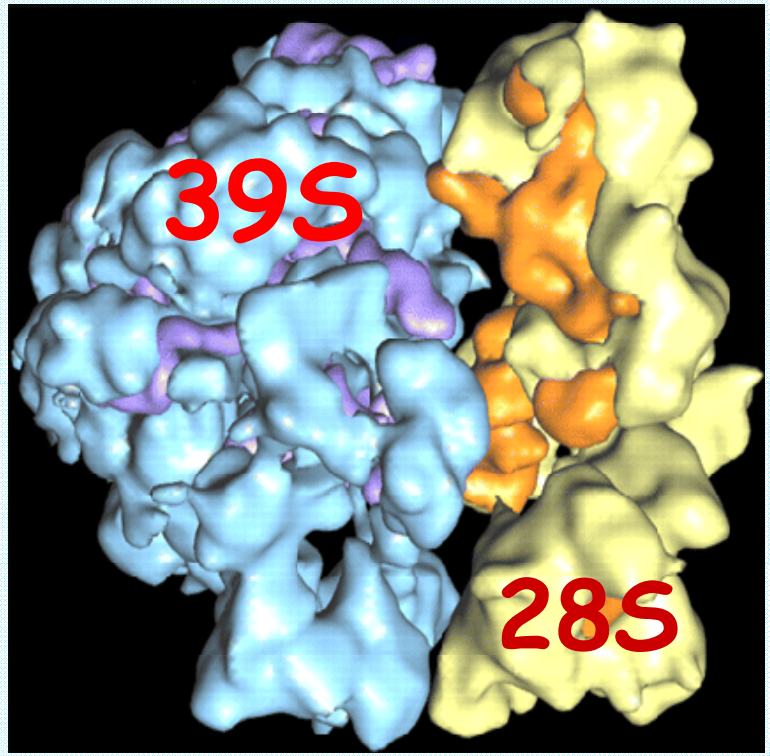
12 genes used in control of latency

71 genes used in virus replication

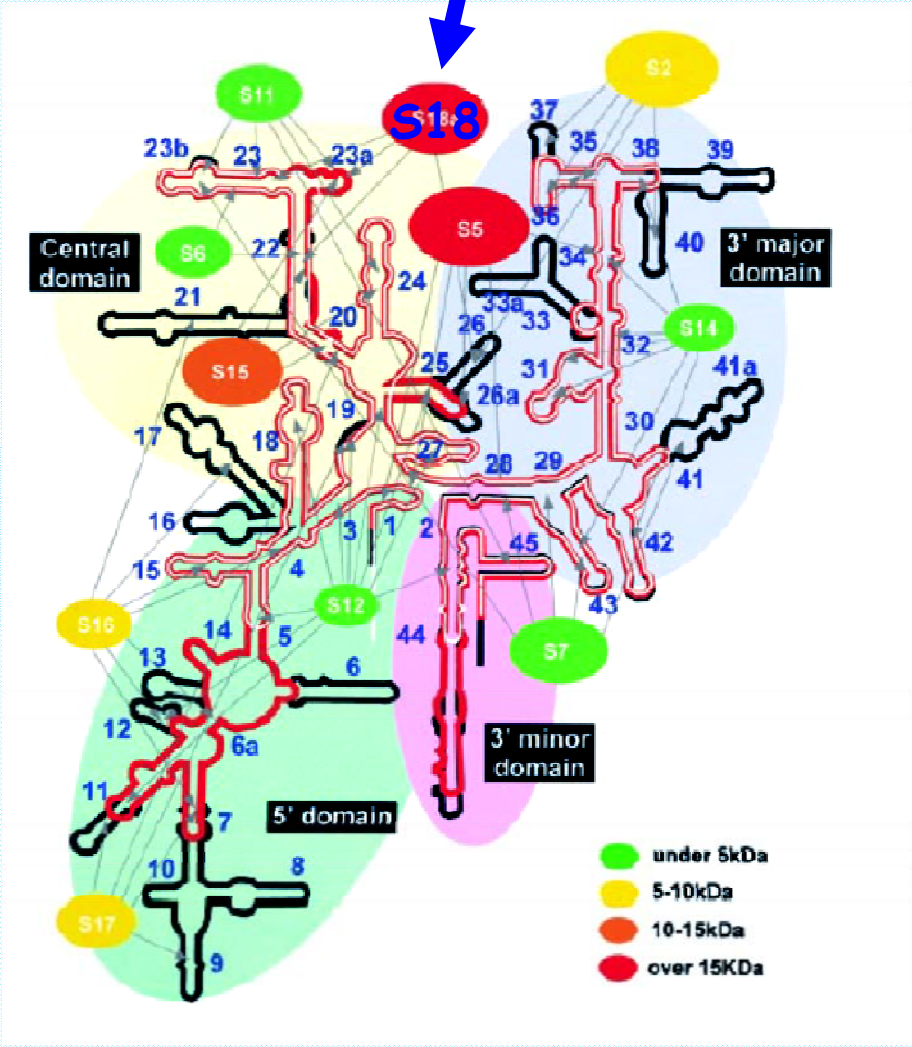
Yeast two-hybrid screening of cDNA library (LCL)



Mitochondrial ribosome:



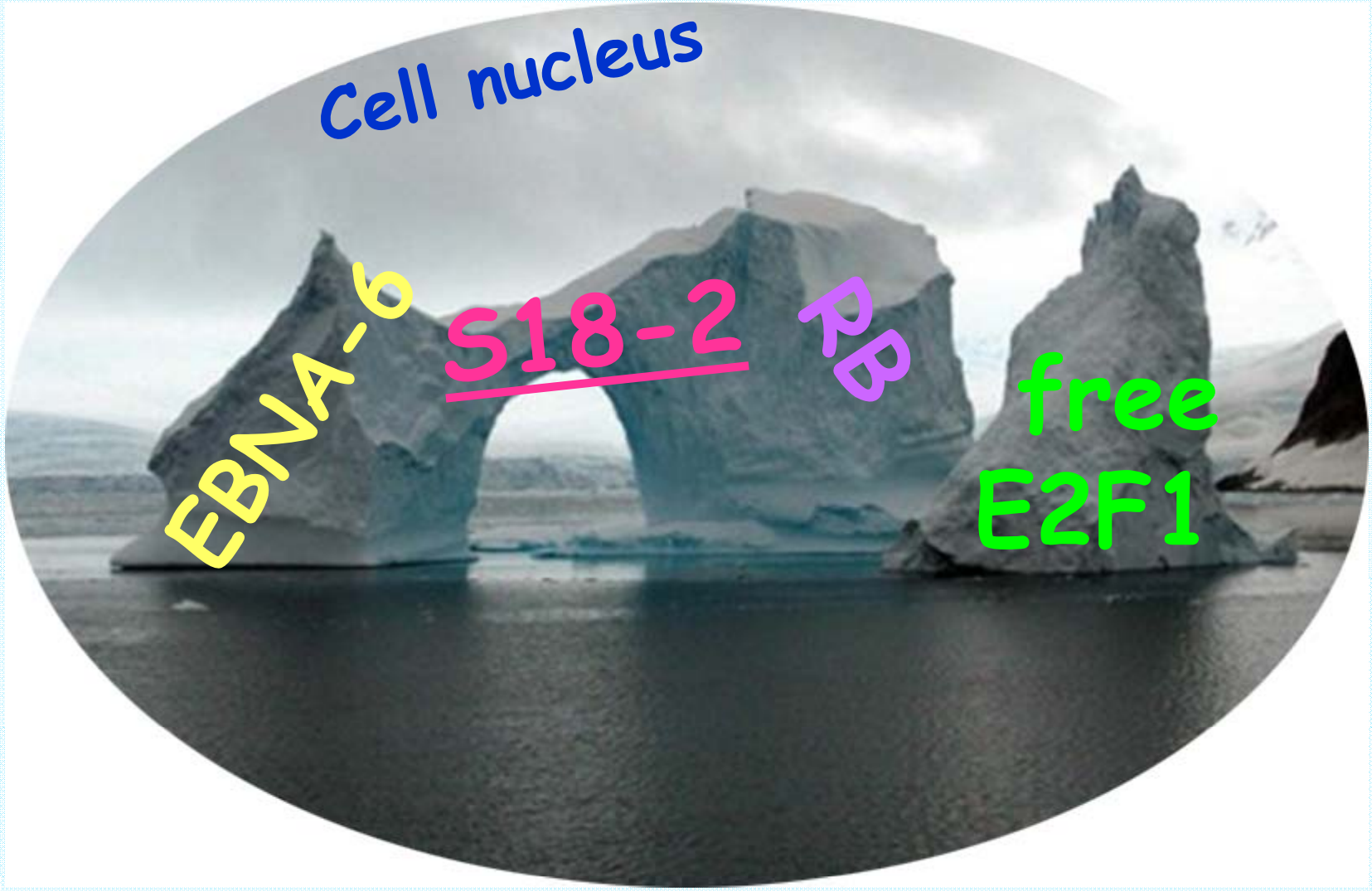
28S small subunit:



In 28S small subunit, three proteins:

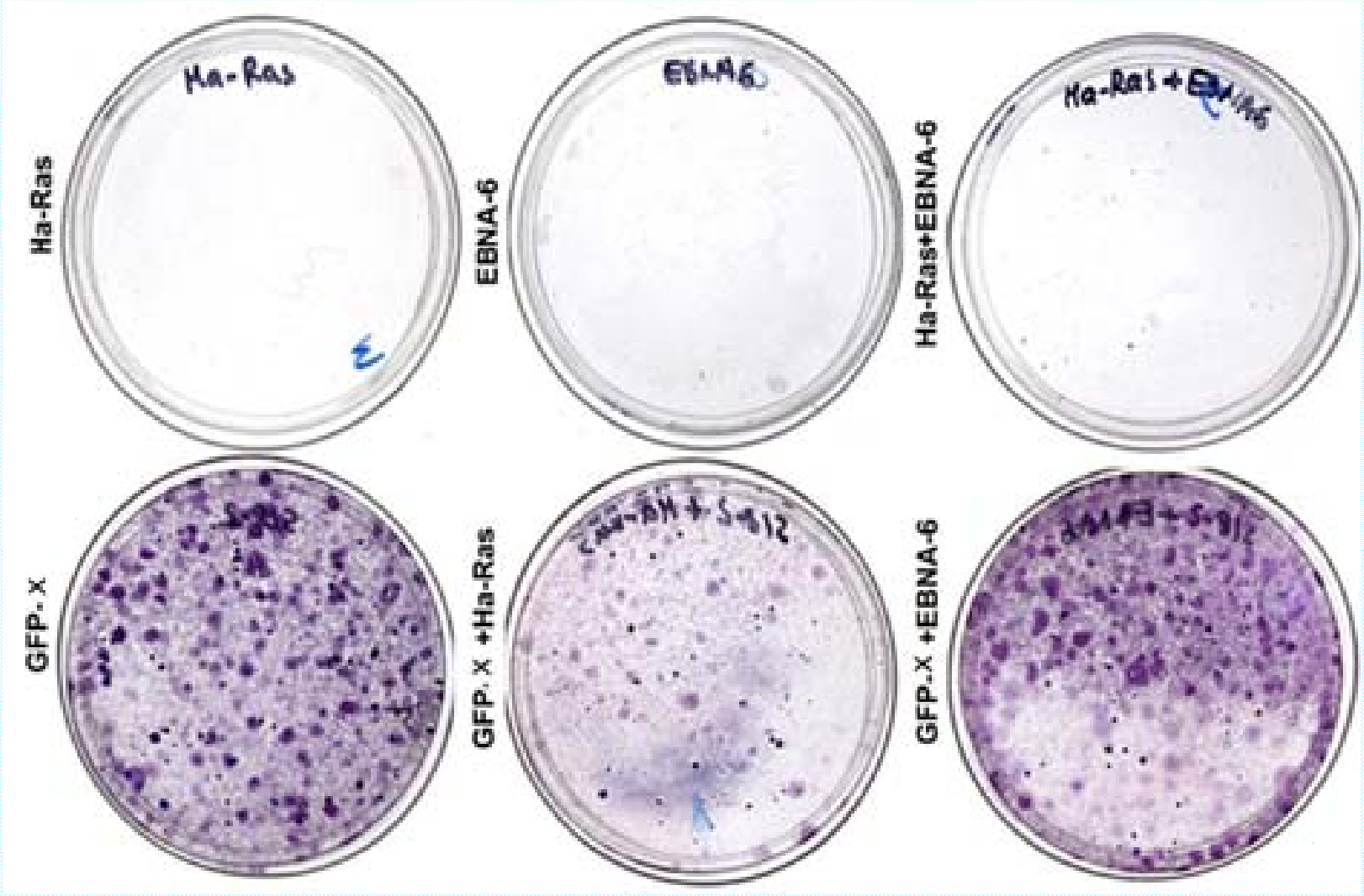
MRPS18-1 142 a.a.
MRPS18-2 258 a.a.
MRPS18-3 196 a.a.

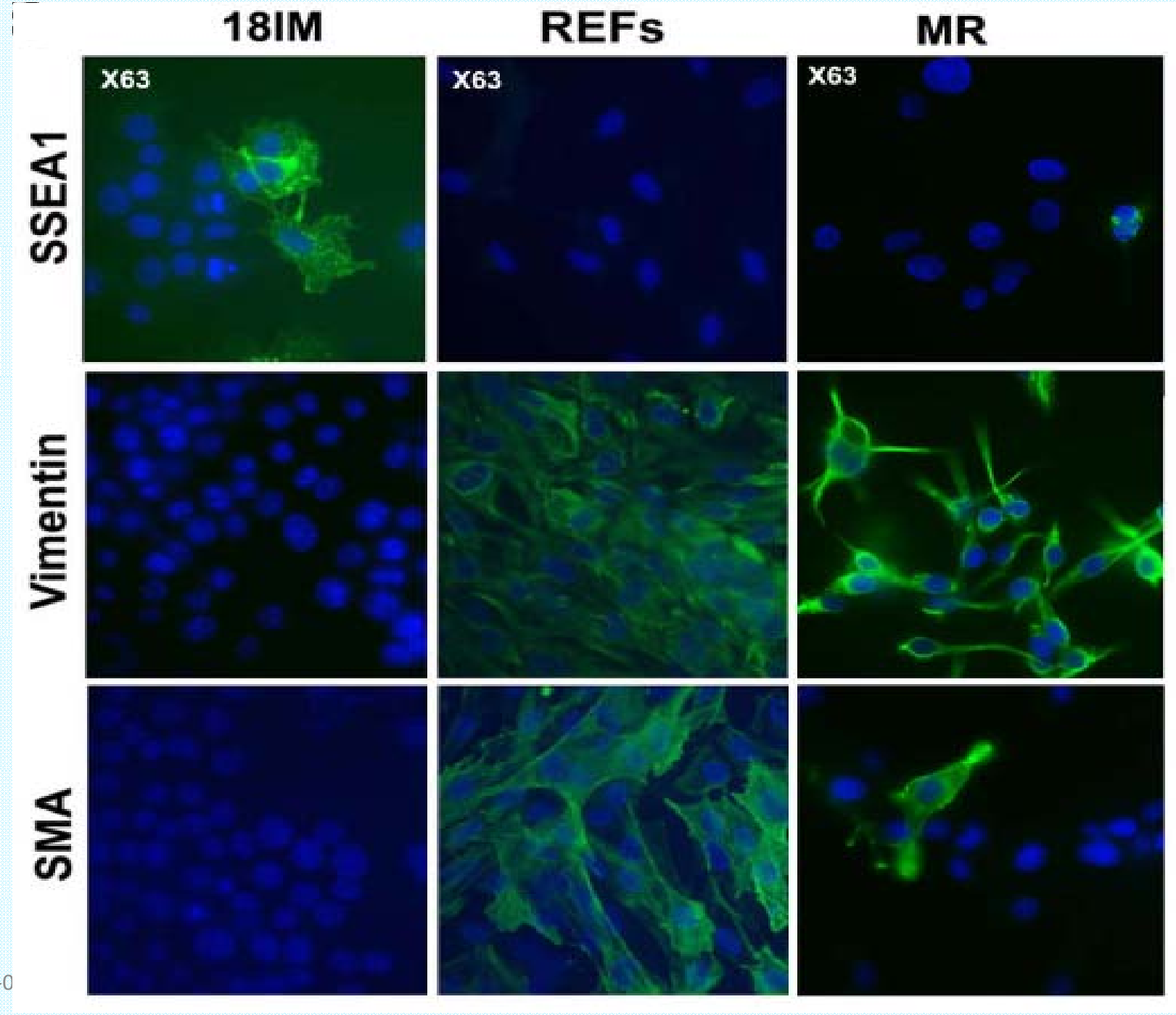
Elevated expression of S18-2 results
in enhanced S-phase



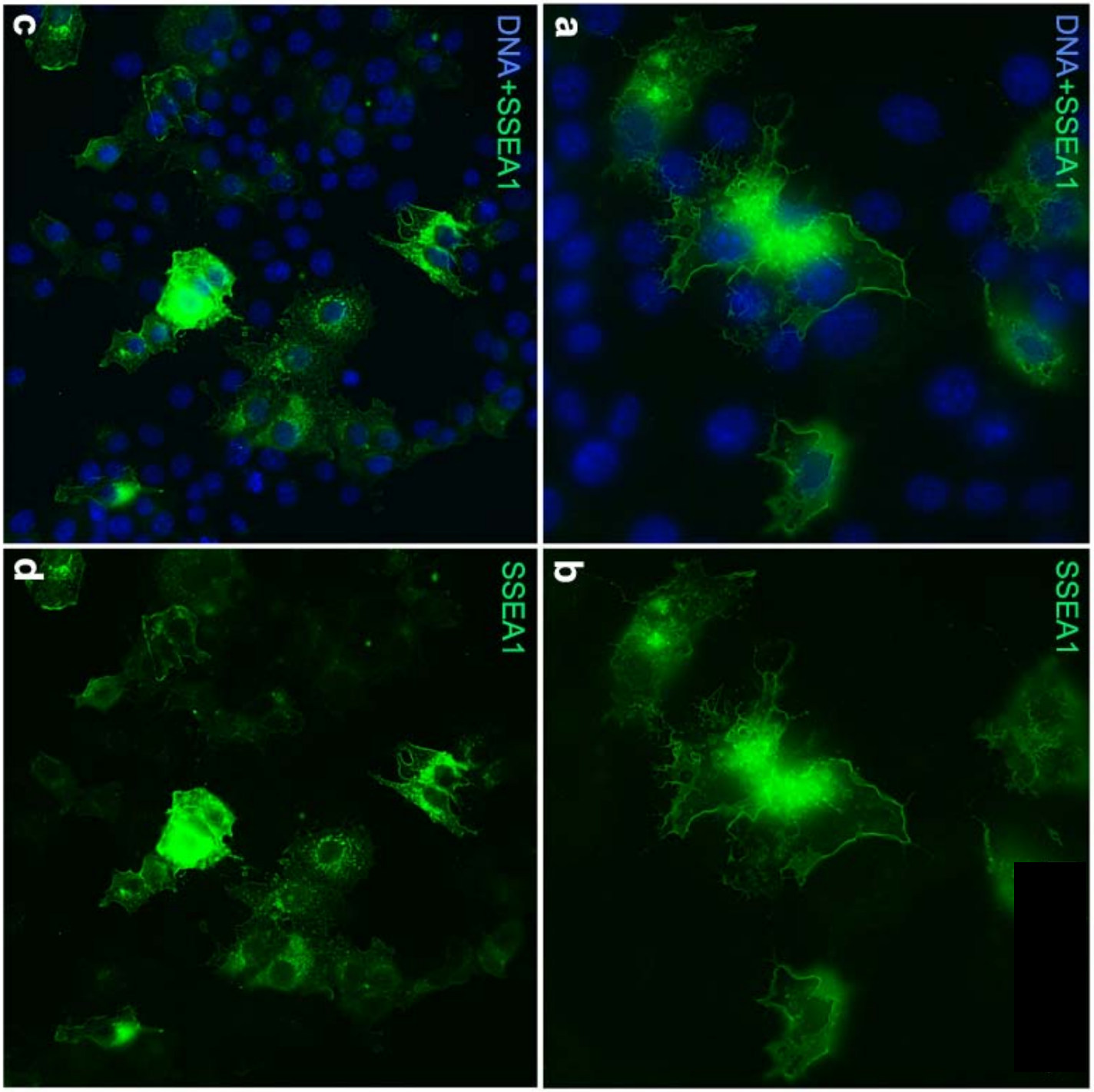
By other words, cell got a permanent stimulus to divide!

Colony formation assay




18IM
REFs
MR
X63
X63
X63
SSEA1
Vimentin
SMA

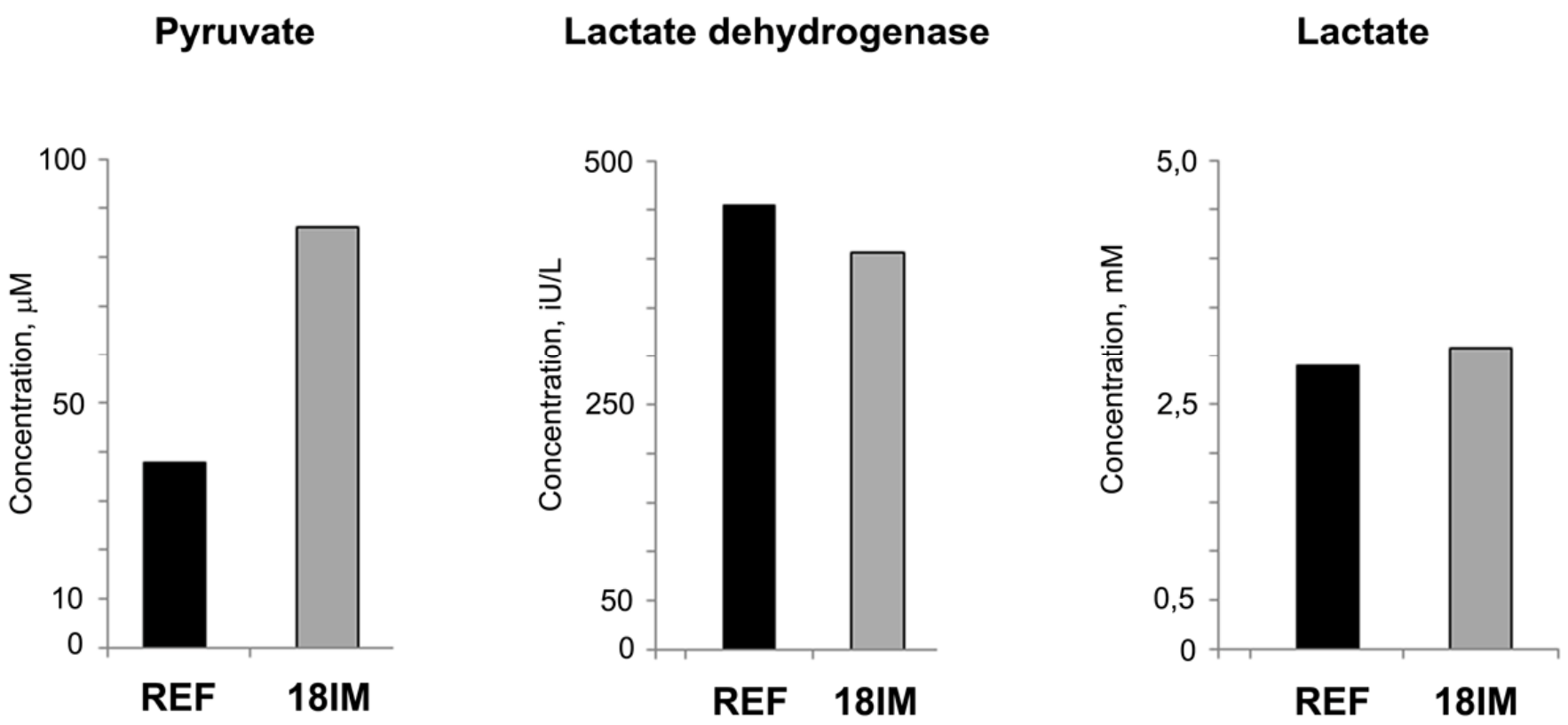
Growth for 2 years in a glass bottle



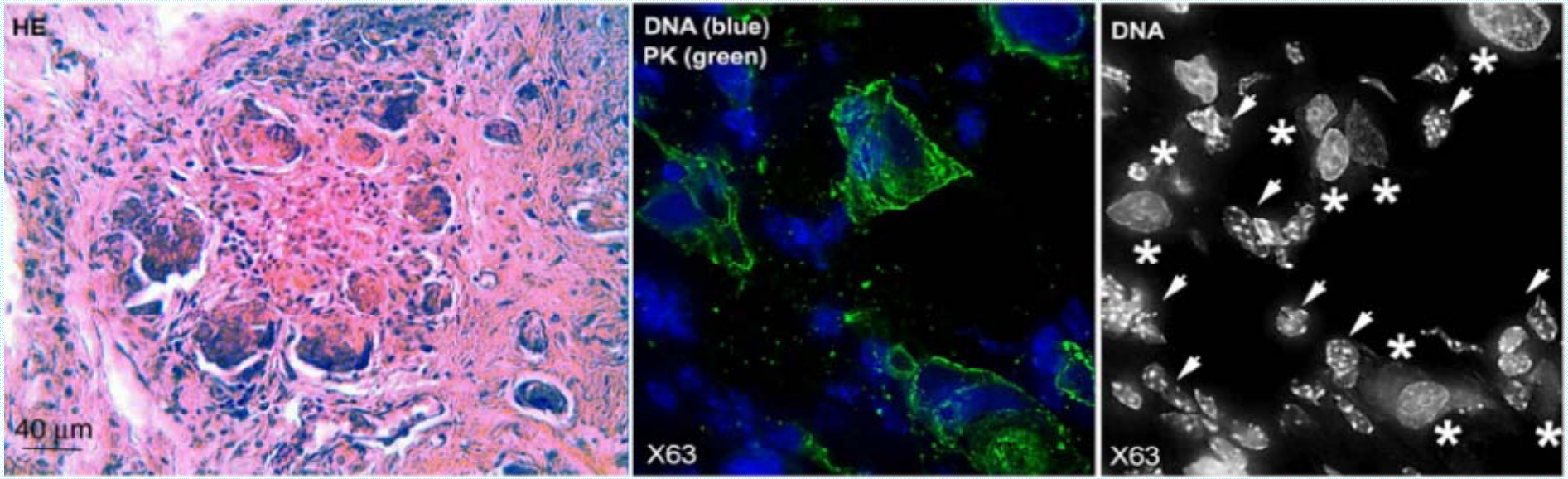
Still 30% of cells express SSEA-1

18IM cells proliferate very fast . . .

Figure S1



...and yet they do not grow as a tumor in SCID mice



Why?

18IM cells behave like stem cells - they differentiate *in vitro*

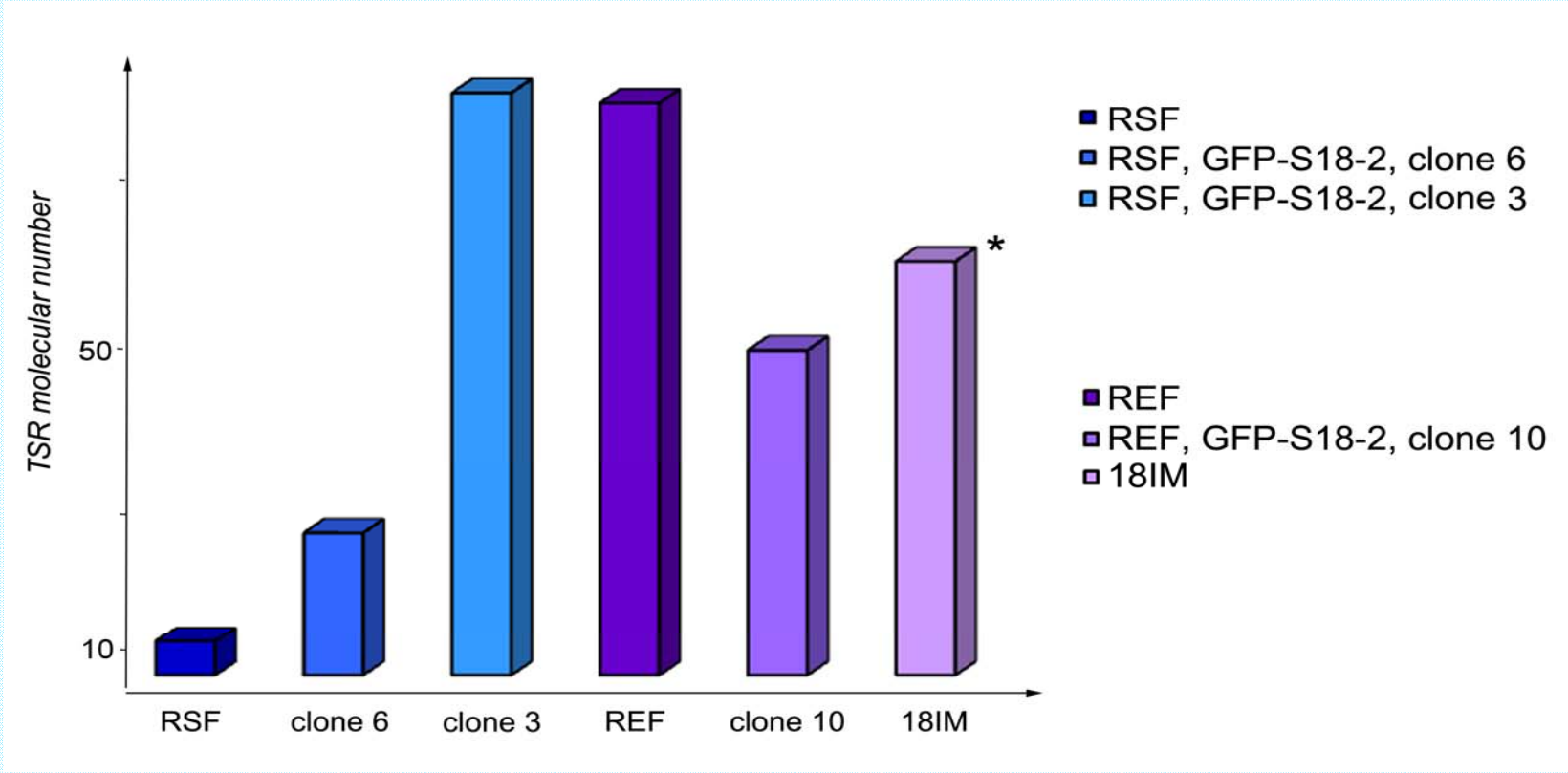
- To evoke osteogenic differentiation, cells were cultured in the medium, which contained ascorbic acid-2-phosphate, glycerol, and dexamethasone.

- To induce chondrogenesis, 18IM cells were grown in a medium supplemented with H-89.

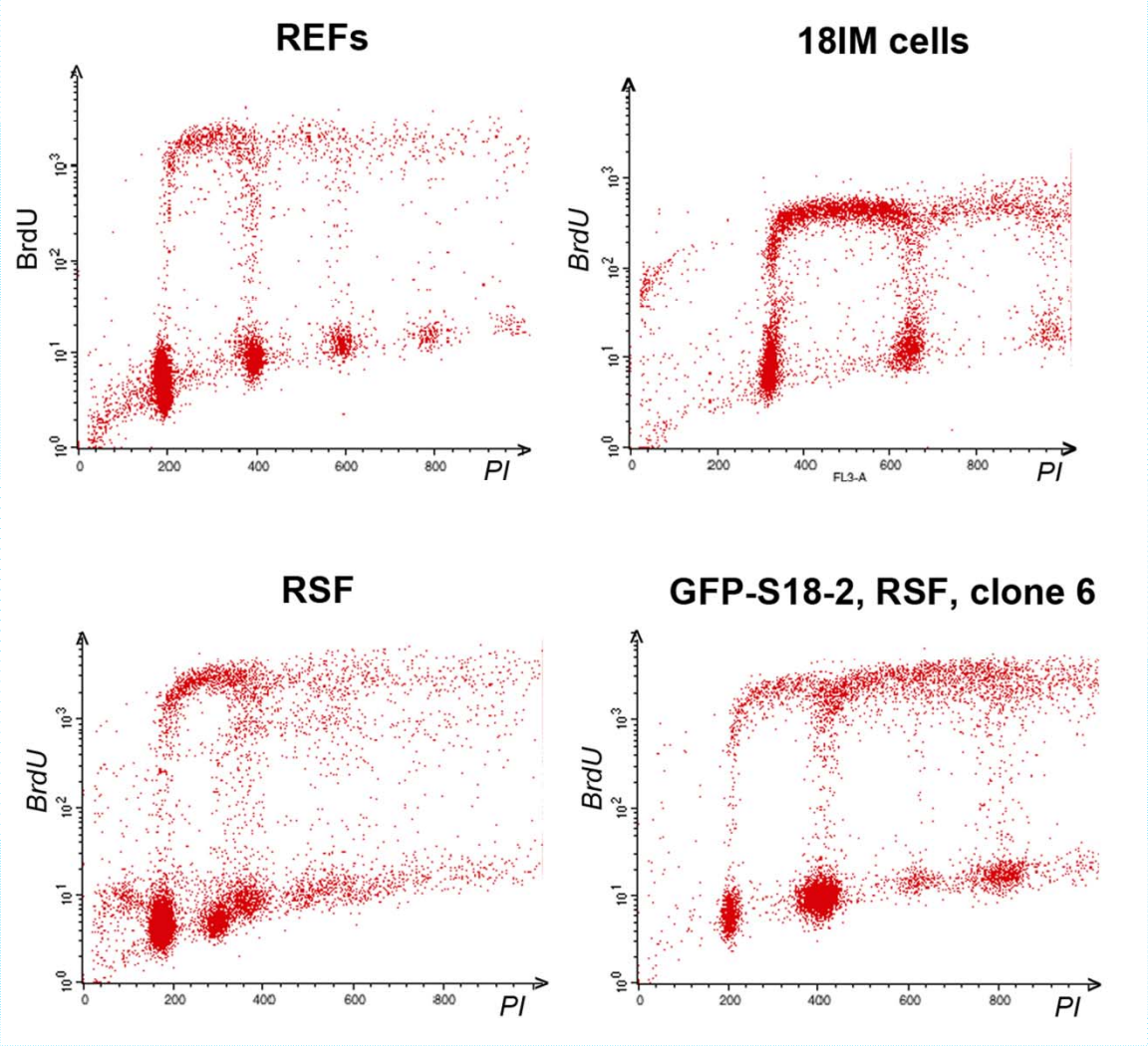
- RT^2 profiler assay was performed, using RNA isolated from 18IM cells and REFs for comparison.

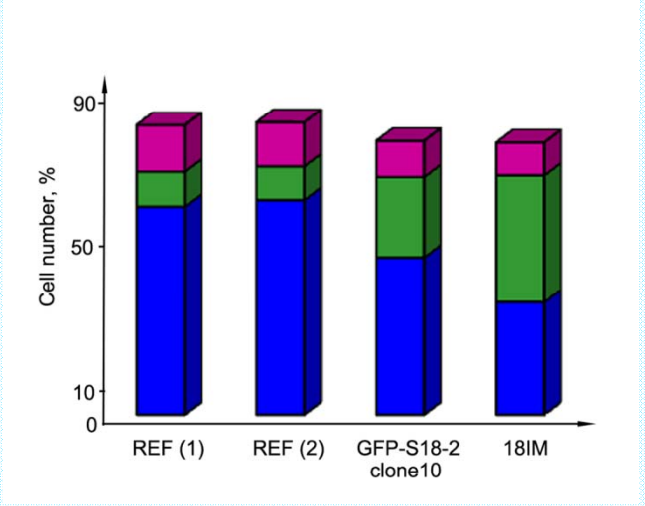
- 26 genes out of 84 analyzed were expressed in 18IM at the higher levels compared with REF. All of these genes encode proteins that induce pro-inflammatory effects.

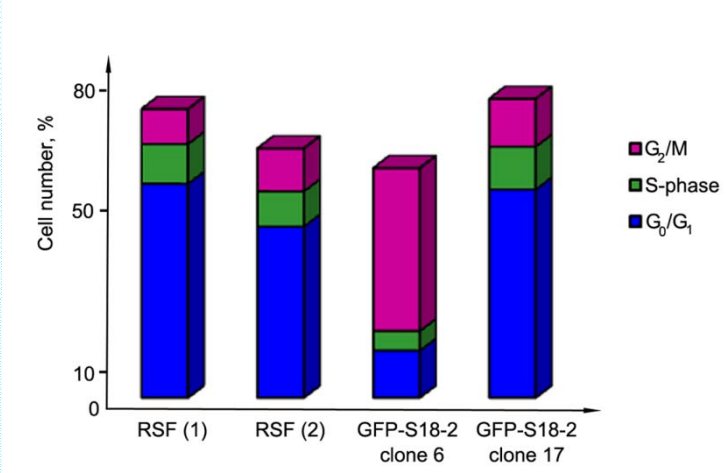
Transformed and immortalized cells show a high telomerase activity, compared to primary cells



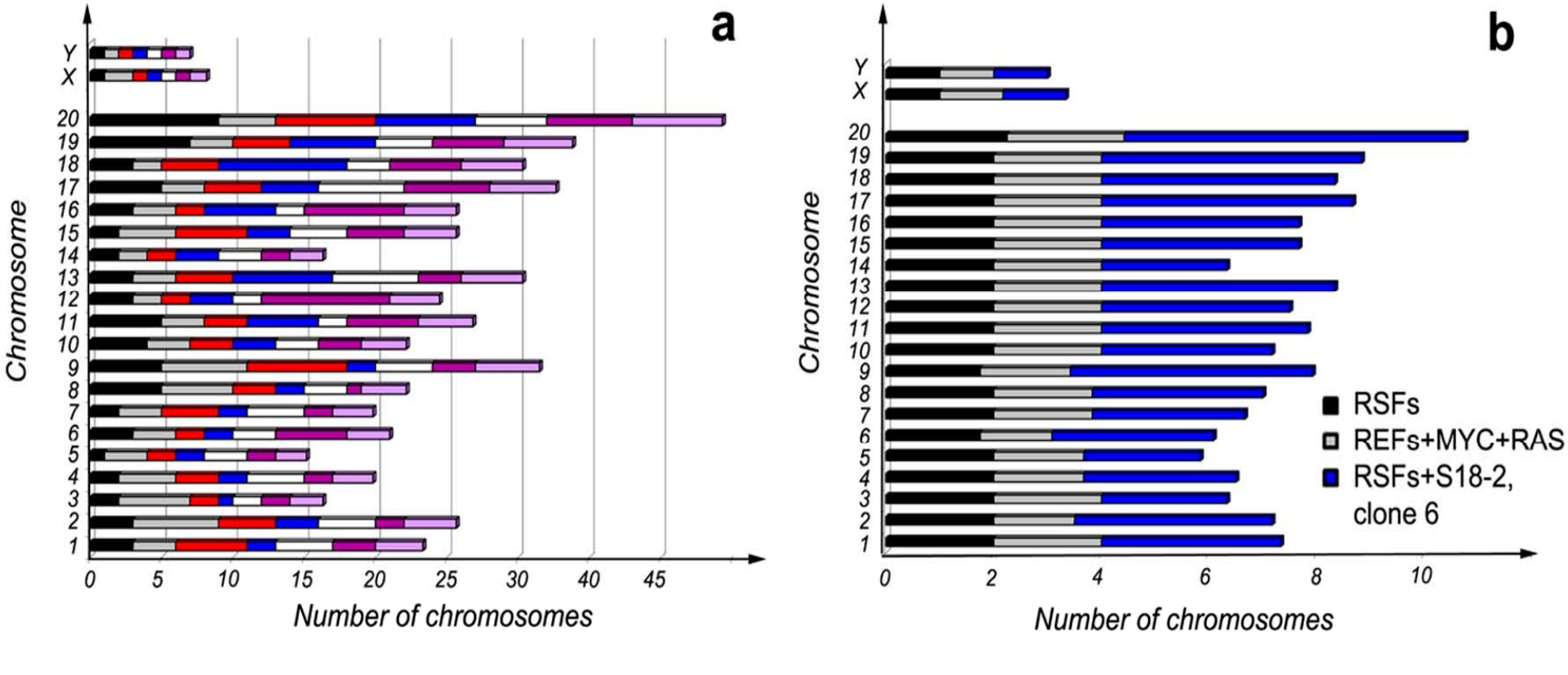
An elevated expression of S18-2 leads to disturbance in cell cycle



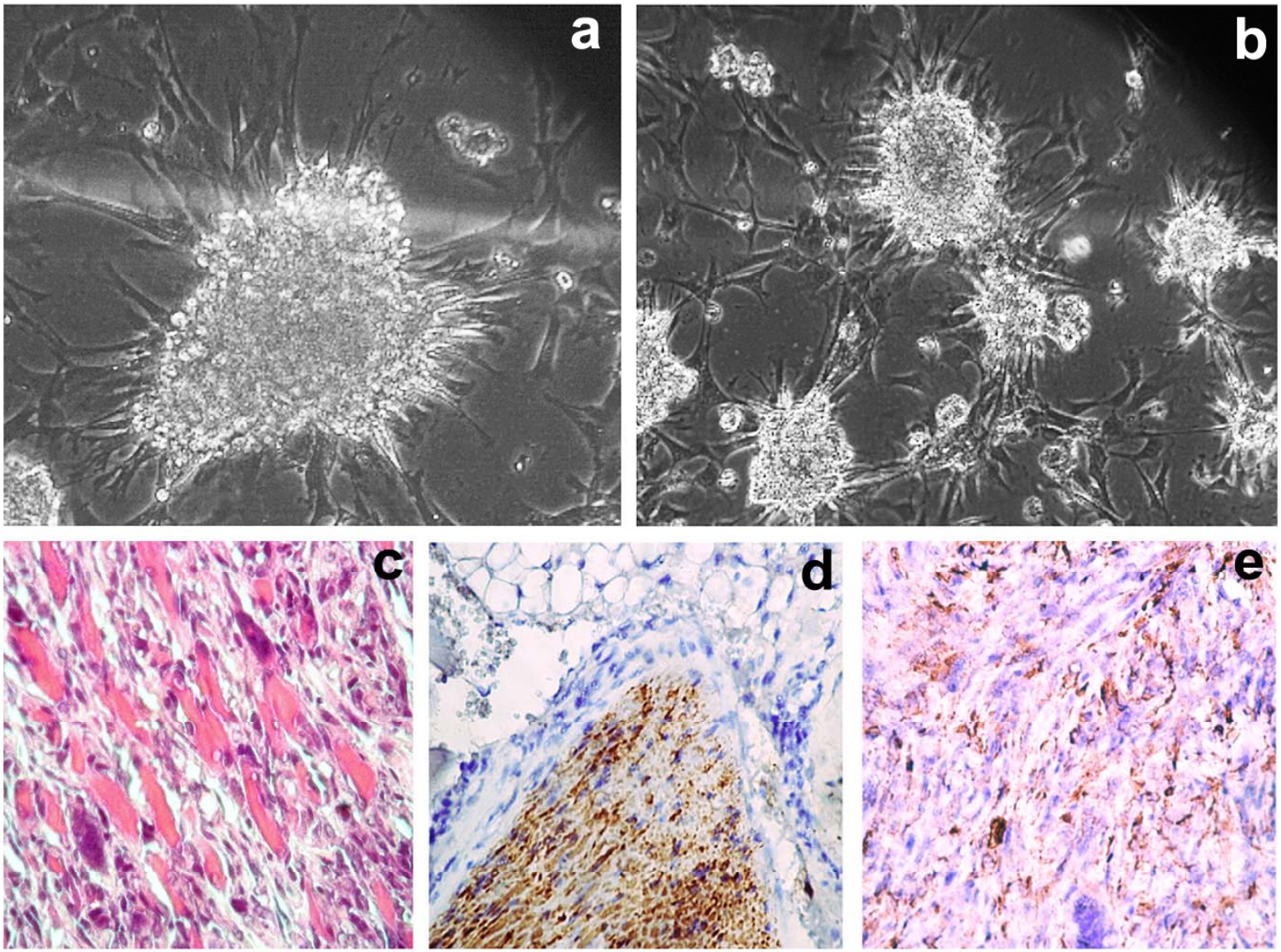




High levels of S18-2 expression caused chromosomal instability



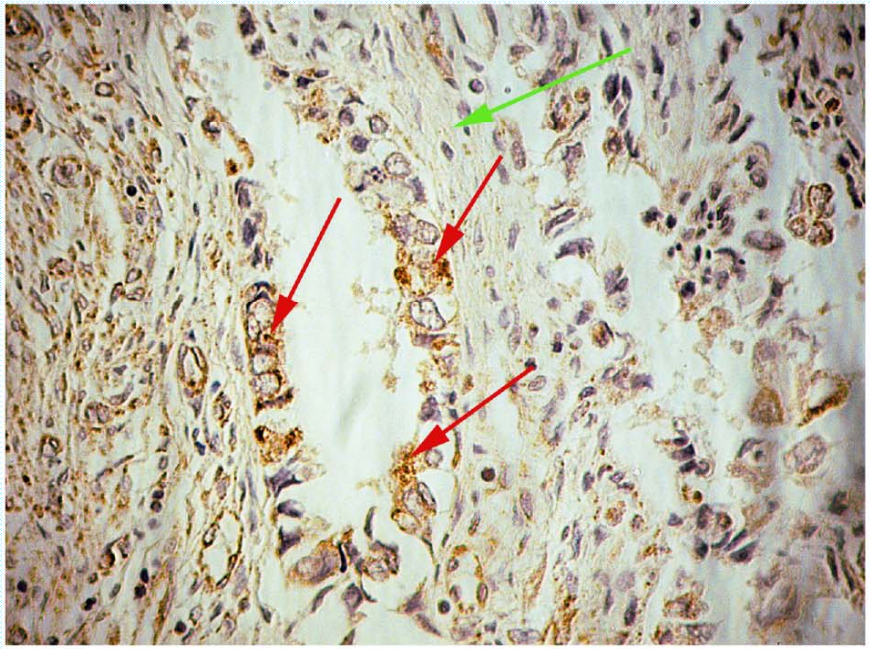
Transformed cells showed anability to grow as tumors in SCID mice



Endometrial cancer

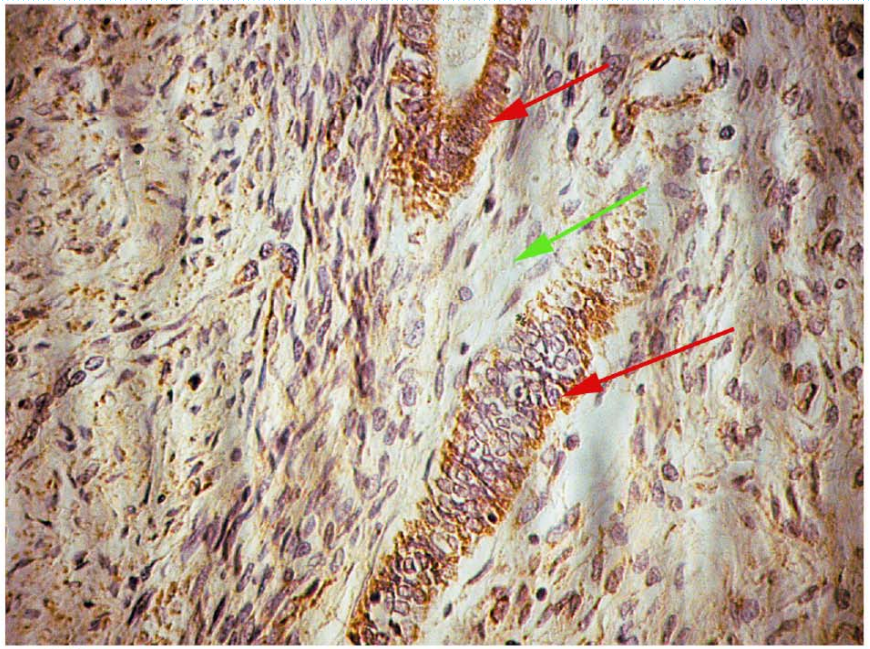
- Worldwide, more than 287,000 women were diagnosed with the disease in 2011.
- Approximately 6% of new cancer cases is EC.
- An incidence rate is about 20 per 100 000 women;

	Type I (85-90%)	**Type II (5-10%)**
Grade	Low	High
Histology	Endometrioid	Serous or clear cell
Stage at diagnosis	I or II	III or IV
Molecular alterations	Mut PTEN, MSI, β-catenin, high p53 (up to 90%)	Mut HER2/neu, mut p53 (90%)



x40

C



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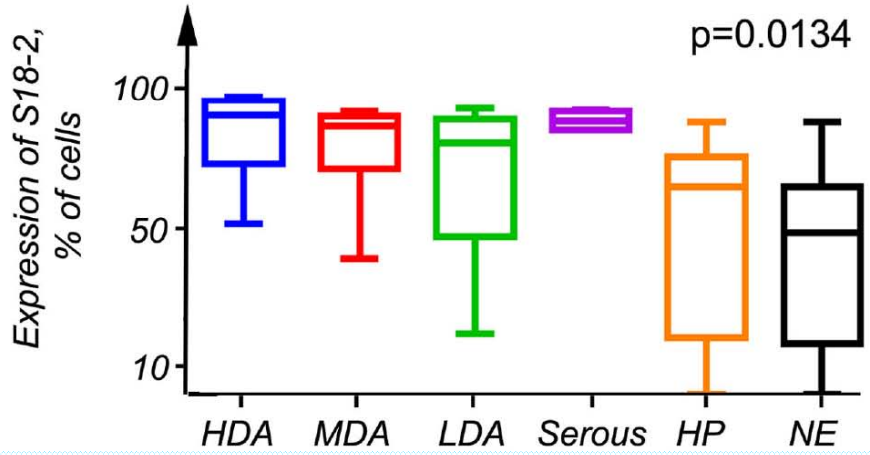
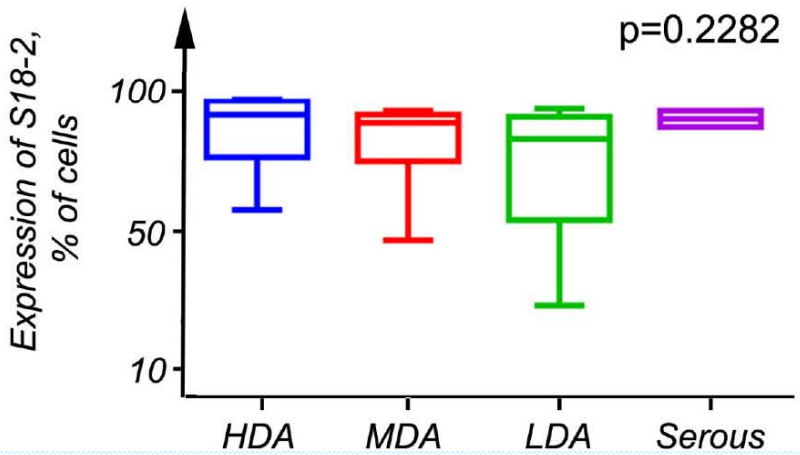
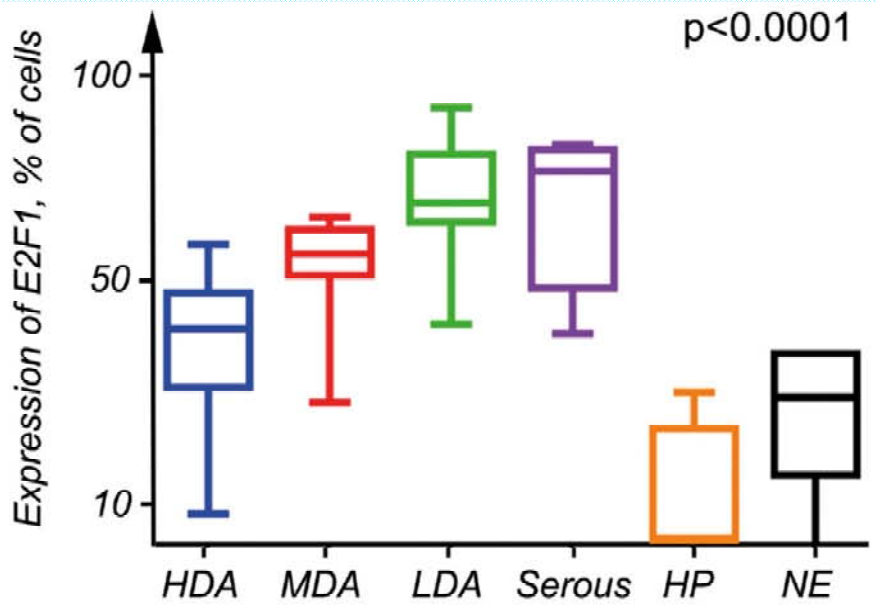
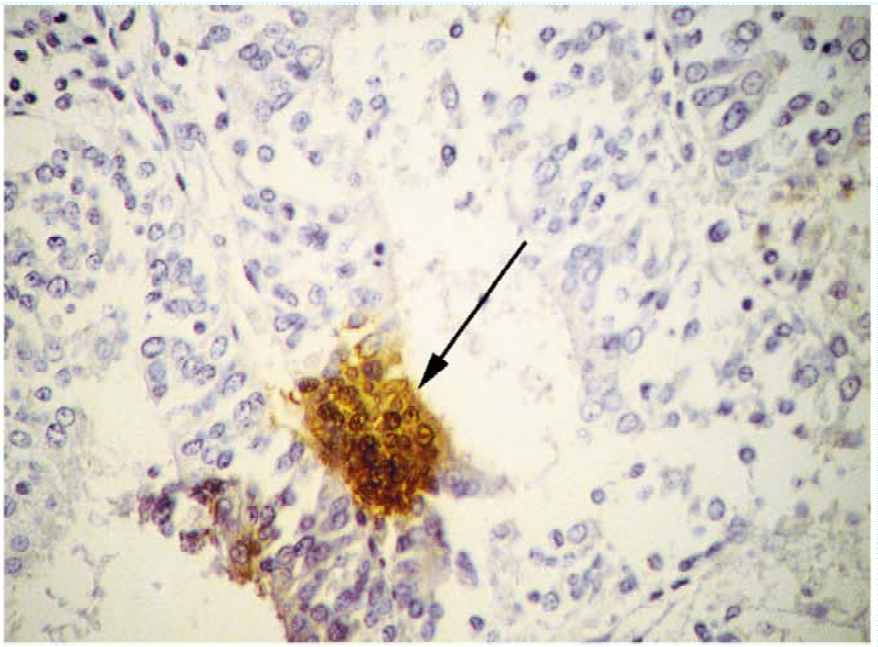

p=0.0134
Expression of S18-2, % of cells
100
50
10
HDA
MDA
LDA
Serous
HP
NE

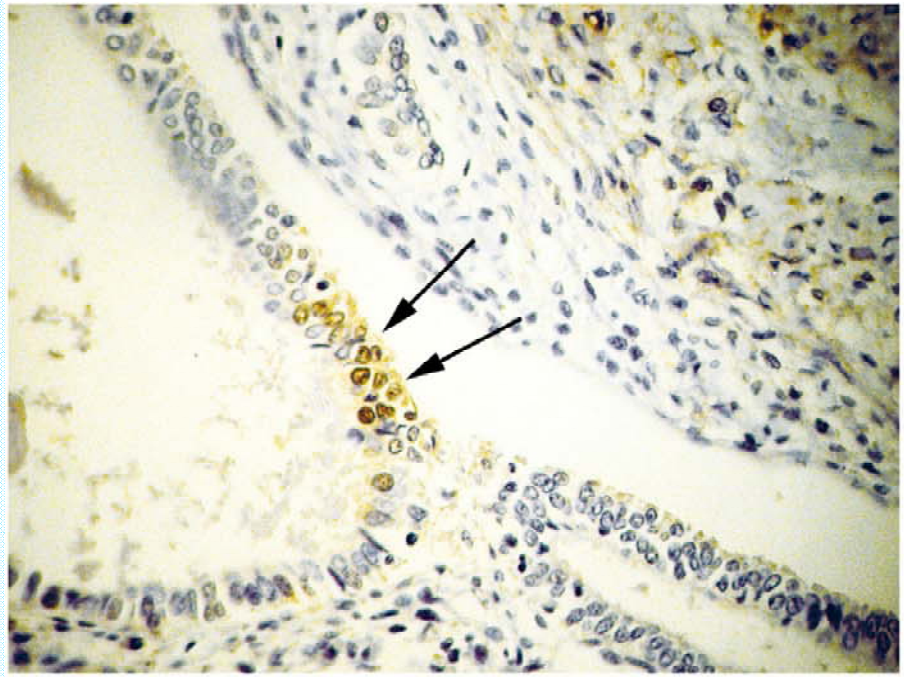

p=0.2282
Expression of S18-2, % of cells
100
50
10
HDA
MDA
LDA
Serous


p<0.0001
Expression of E2F1, % of cells
100
50
10
HDA
MDA
LDA
Serous
HP
NE



x40

C



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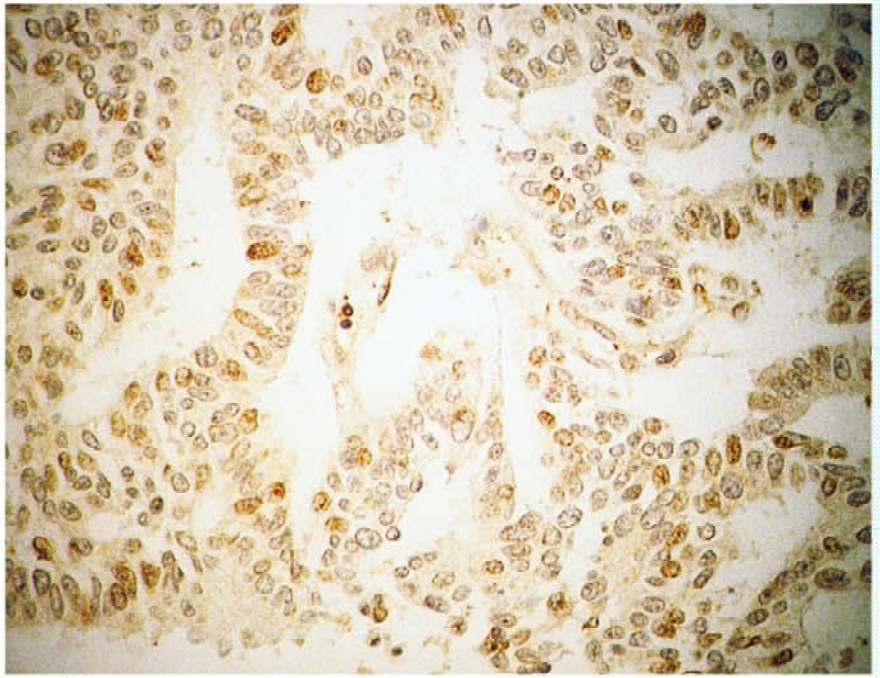

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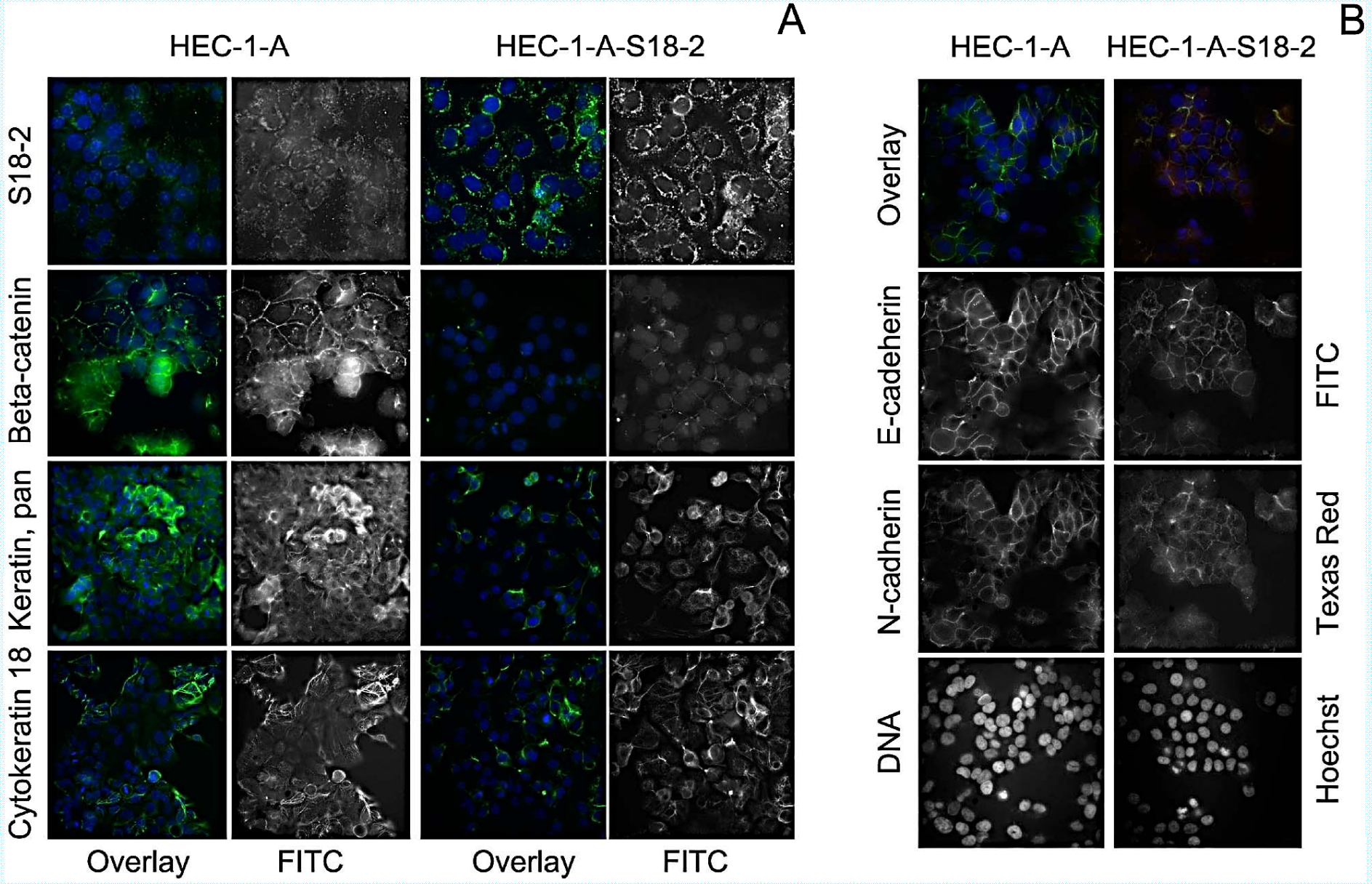
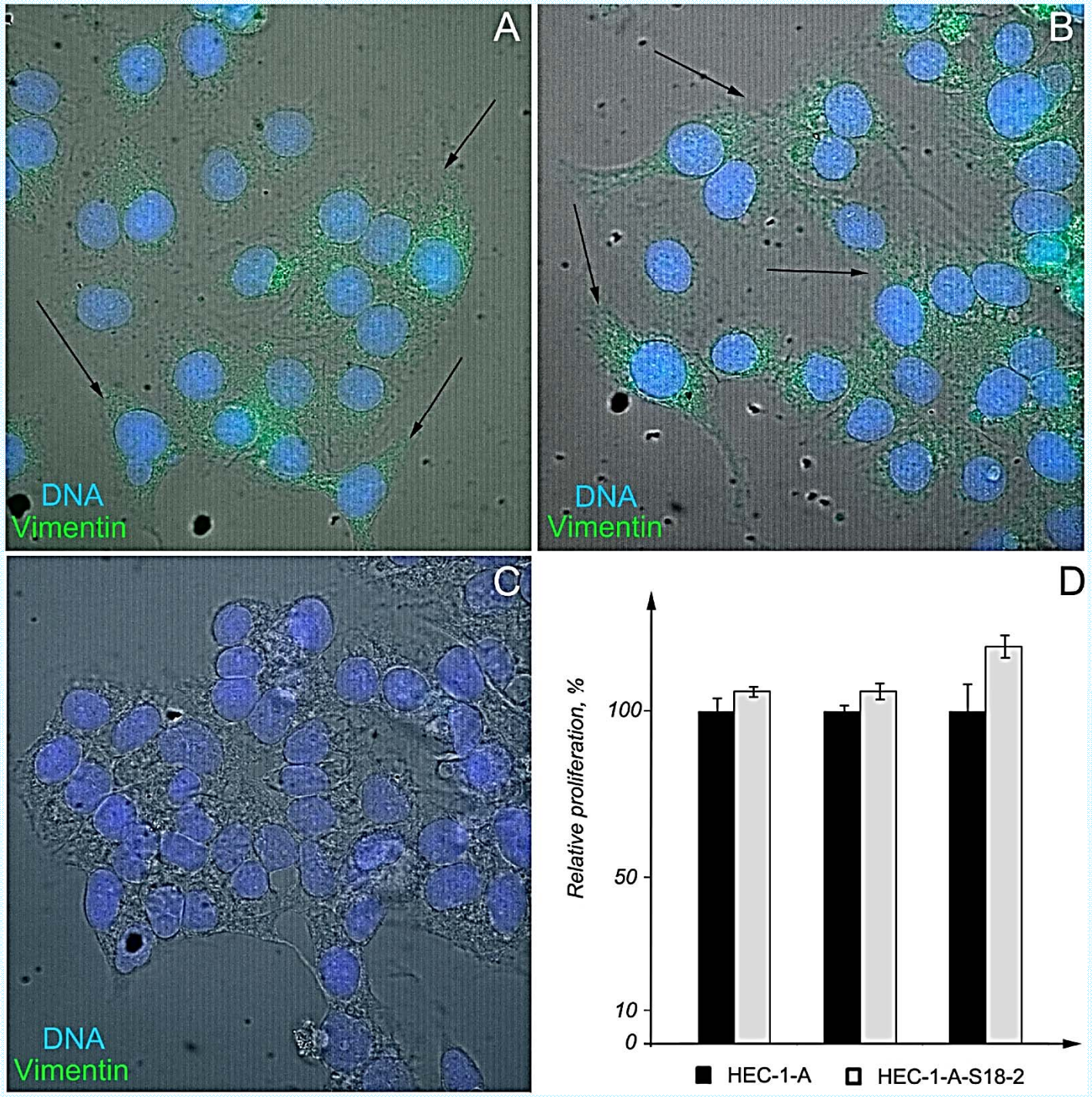
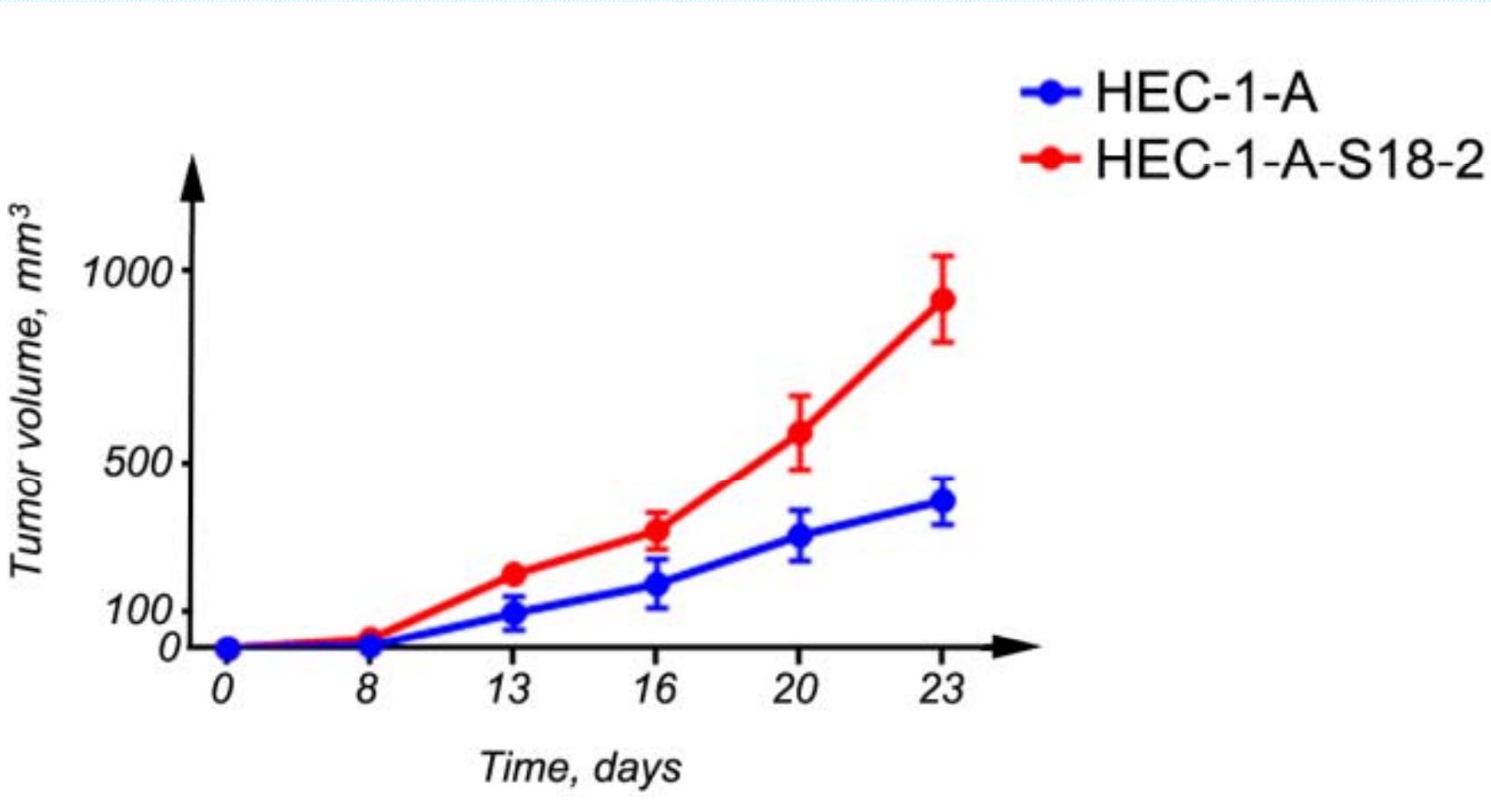
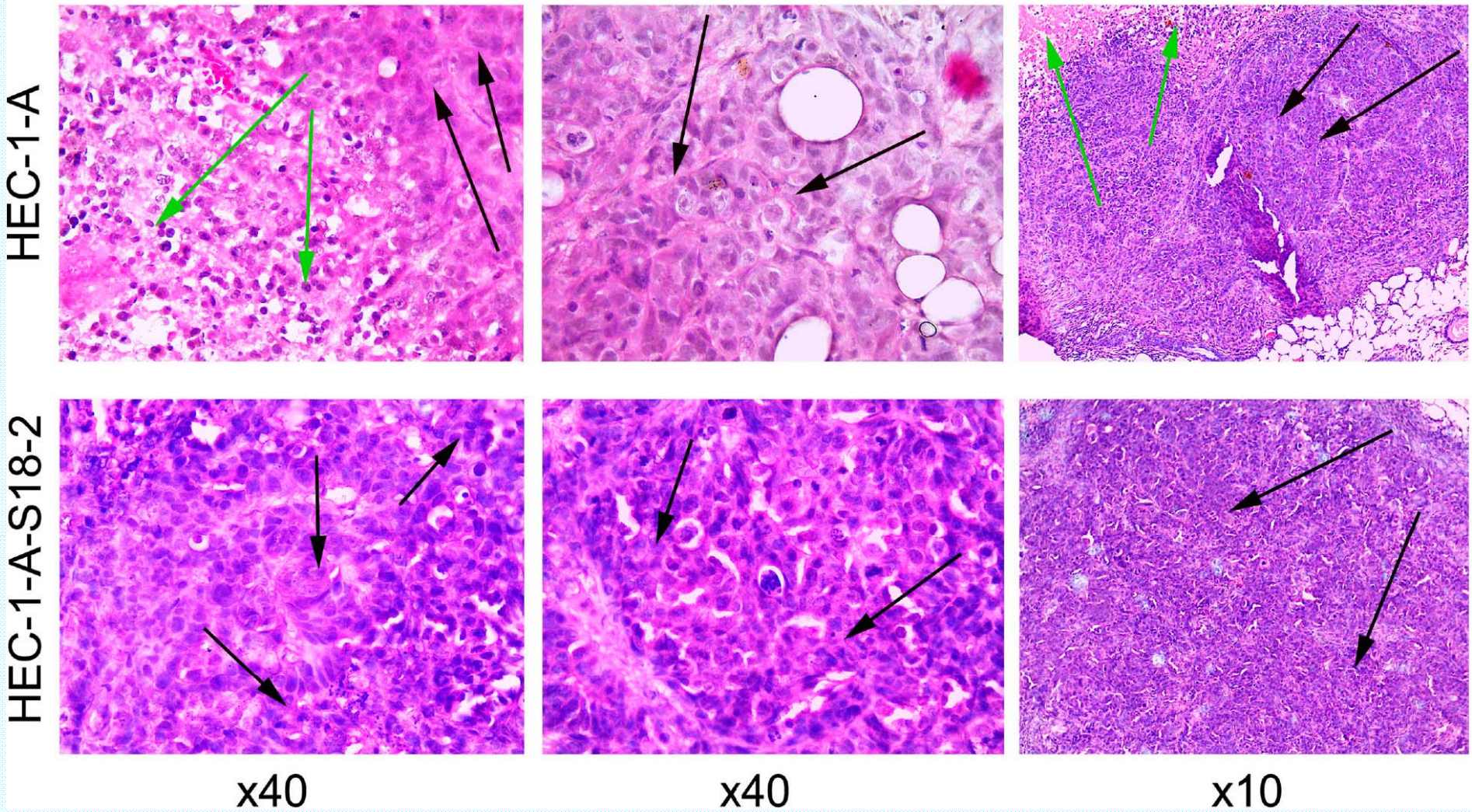

A
HEC-1-A
HEC-1-A-S18-2
S18-2
Beta-catenin
Keratin, pan
Cytokeratin 18
Overlay
FITC
Overlay
FITC
B
HEC-1-A
HEC-1-A-S18-2
Overlay
E-cadeherin
N-cadherin
DNA
FITC
Texas Red
Hoechst


A
DNA
Vimentin
B
DNA
Vimentin
C
DNA
Vimentin
D
Relative proliferation, %
100
50
10
0
HEC-1-A
HEC-1-A-S18-2


HEC-1-A
HEC-1-A-S18-2
Tumor volume, mm3
1000
500
100
0
0
8
13
16
20
23
Time, days


HEC-1-A
HEC-1-A-S18-2
x40
x40
x10

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