Online resources

Cyclin A2, a novel regulator of EMT, Cellular and Molecular Life Sciences

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Online resource 1: Cyclin A2 depletion in NMuMG cells leads to a slight accumulation in S phase and reduced CDK1 and CDK2 activities.

a. Cell cycle analysis by FACS of sh CycA2 NMuMG cells by comparison to sh Luc NMuMG cells. Even if we do not detect much difference in cell growth, shCycA2 cells present a slight accumulation in S phase (n=3, *P=0.05) and subsequent reduction of the G2M fraction (n=3, *P=0.05). **b.** Quantification of the FACS analysis shown as percentage of cells in each phase for both genotypes. **c.** CDK1 and CDK2 kinase activities shown as counts per minutes (cpm). Sh CycA2 cells show decreased CDK2 kinase activity (n=3, *P=0.0127) which correlates with the accumulation of the cells in S phase. They also show decreased CDK1 activity (n=3, **P=0.0014) which could be due to the decreased proportion of cells in G2M phase. Data are represented as mean ± SEM.

Online resource 2: cell growth and morphology following Cyclin A2 depletion in epithelial cells.

a, Growth of NMuMG cells following Cyclin A2 depletion was measured every day for a week (n=3). **b,** Light microscopy of epithelial cells after Cyclin A2 knockdown. Cyclin A2 inactivation induces a spindle-like morphology in NMuMG cells cultivated in collagen, i.e. 3D matrix or 2D monolayer in BMEL and 67NR cells. Scale bars: 100μm.

Online resource 3: Mesenchymal traits induced by Cyclin A2 inactivation are rescued by Wt-Cyclin A2.

a. Immunofluorescence analysis of E-Cadherin and p120 Catenin cellular localization which are disturbed in sh CycA2+PMSCV cells by comparison to sh Luc+PMSCV, and restored in sh CycA2+Wt-CycA2 Flag NMuMG cells. Scale bars: 100μm. **b**. Western blot analysis (left panel) and quantification (right panel) of the non phosphorylated active β Catenin isoform. Active β Catenin is increased in absence of Cyclin A2 (n=3, ***P<0.0001) and returns to control levels following reintroduction of Wt-Cyclin A2 (n=3,** P=0.0019). **c.** The increased invasion induced by Cyclin A2 inactivation (n=3,**P=0.005) is rescued by Wt-Cyclin A2 (n=3,* P=0.0196). Data are represented as mean ± SEM.

Online resource 4: Cyclin A2 depletion in NMuMG cells leads to increased invasion and RhoC activity.

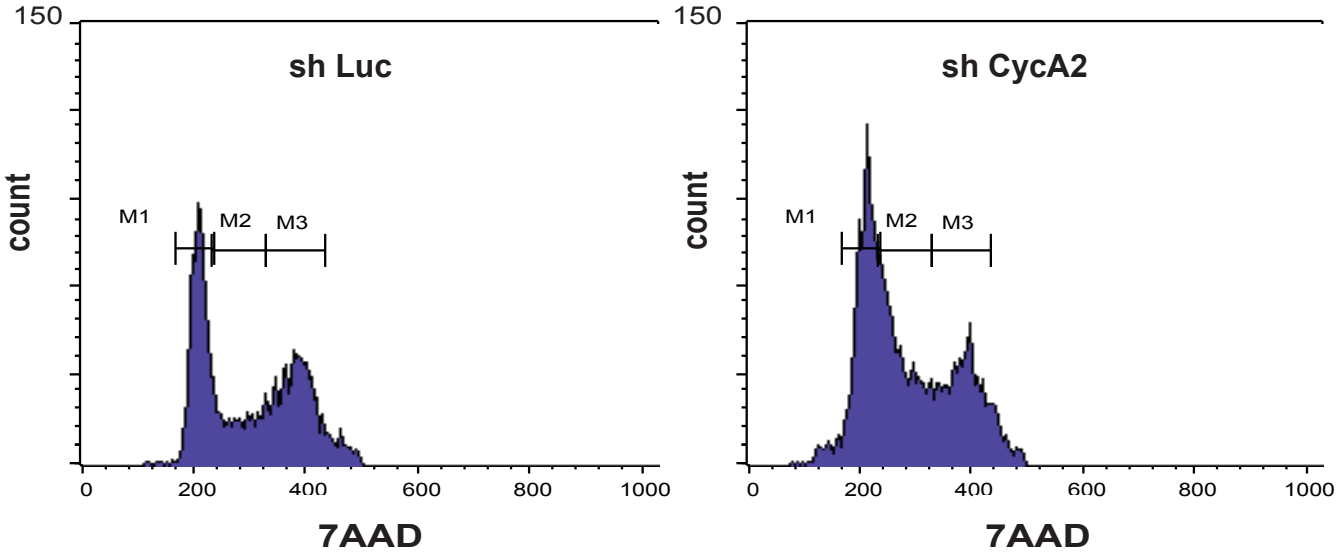
a, Invasion of sh Luc and sh CycA2 NMuMGRasV12 cells in collagen was performed as mentioned above and measured 24h after plating the cells (n=3, ***P=0.0004). **b,** RhoC activity was measured by quantifying the RhoGTP active forms pulled down by Rhotekin-beads in cell extracts corresponding to the different genotypes (n=3, *P=0.03). Data are represented as mean ± SEM.

Online resource 5: Cooperation of Cyclin A2 inactivation and RasV12 expression in the induction of some mesenchymal traits. Analysis of E-cadherin, N-Cadherin, Slug, MMP3, Nanog and Oct 4 mRNA levels in sh CycA2 NMuMGRasV12 cells over PMSCV sh CycA2 NMuMG cells.

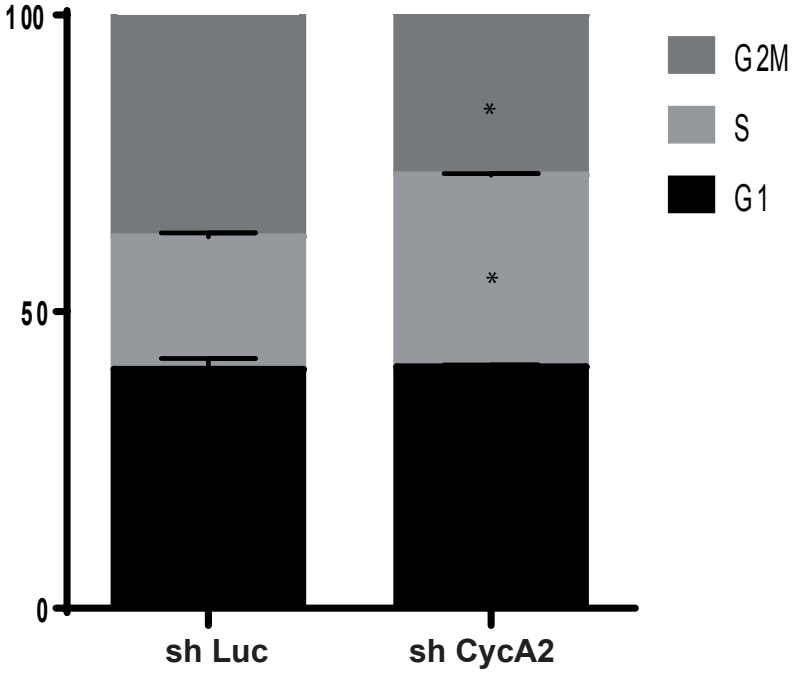
Online resource 6: Increased resistance to anoïkis of sh CycA2 NMuMGRasV12 cells.

Upper panel: Cell death of sh Luc and sh CycA2 NMuMGRasV12 cells was measured by FACS analysis following 7AAD staining and after 12h of culture in non-adherent conditions. **Lower panel**: Quantification of cell death expressed as percentage of dead cells detected by 7AAD (n=3, *P=0.05).

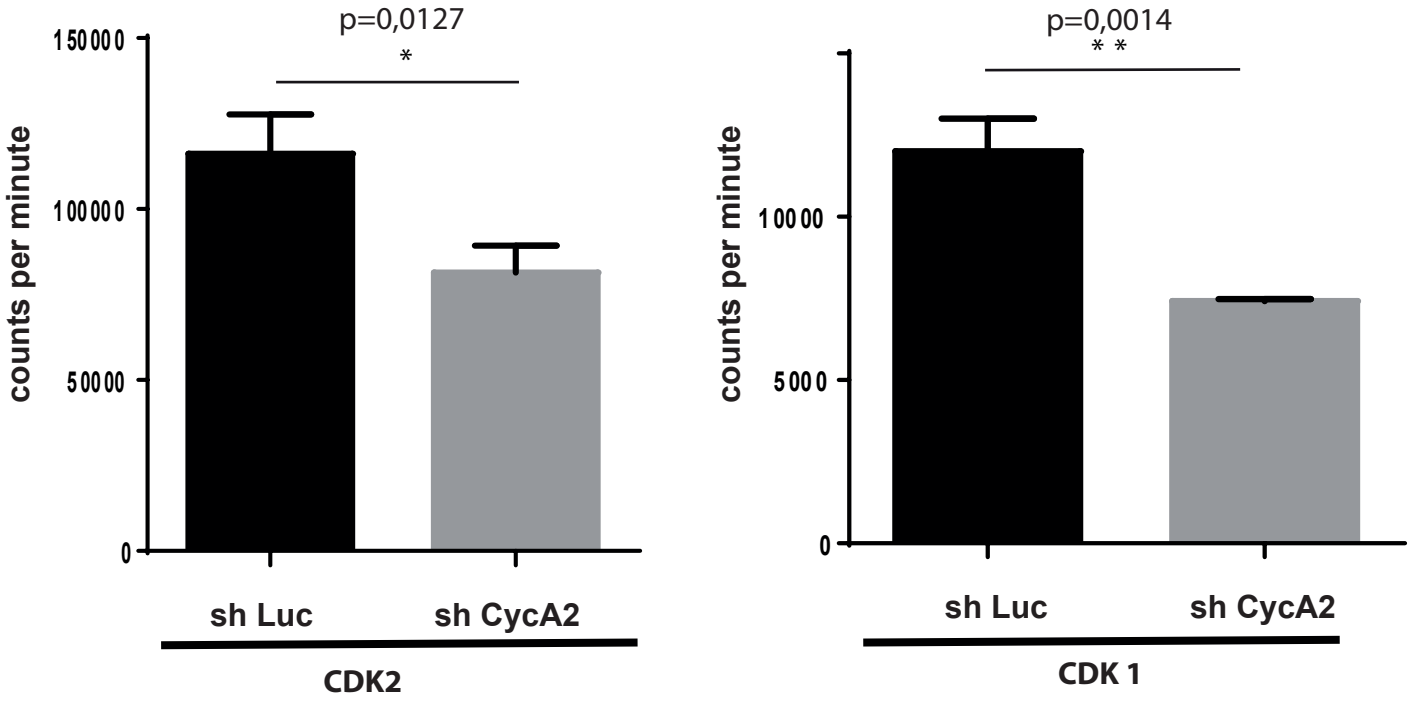
a.


150
sh Luc
count
M1
M2
M3
0
200
400
600
800
1000
7AAD
150
sh CycA2
count
M1
M2
M3
0
200
400
600
800
1000
7AAD

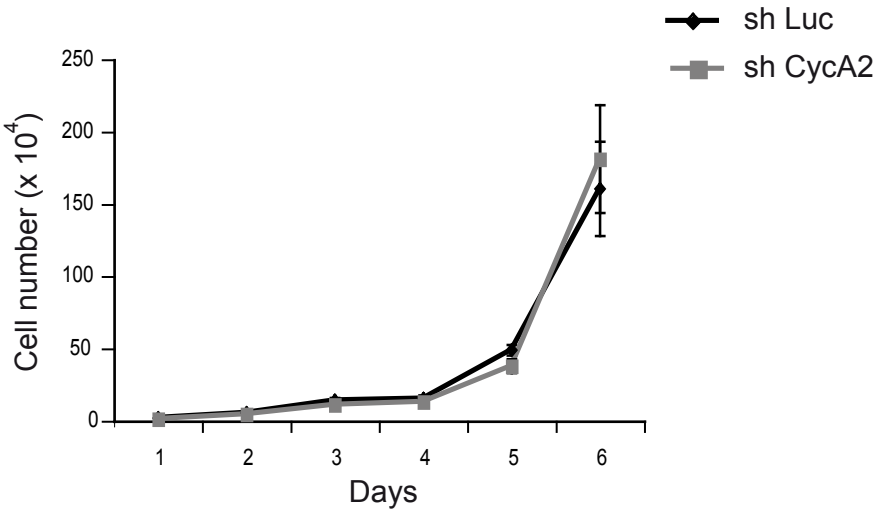
b.


100
50
0
*
*
G2M
S
G1
sh Luc
sh CycA2

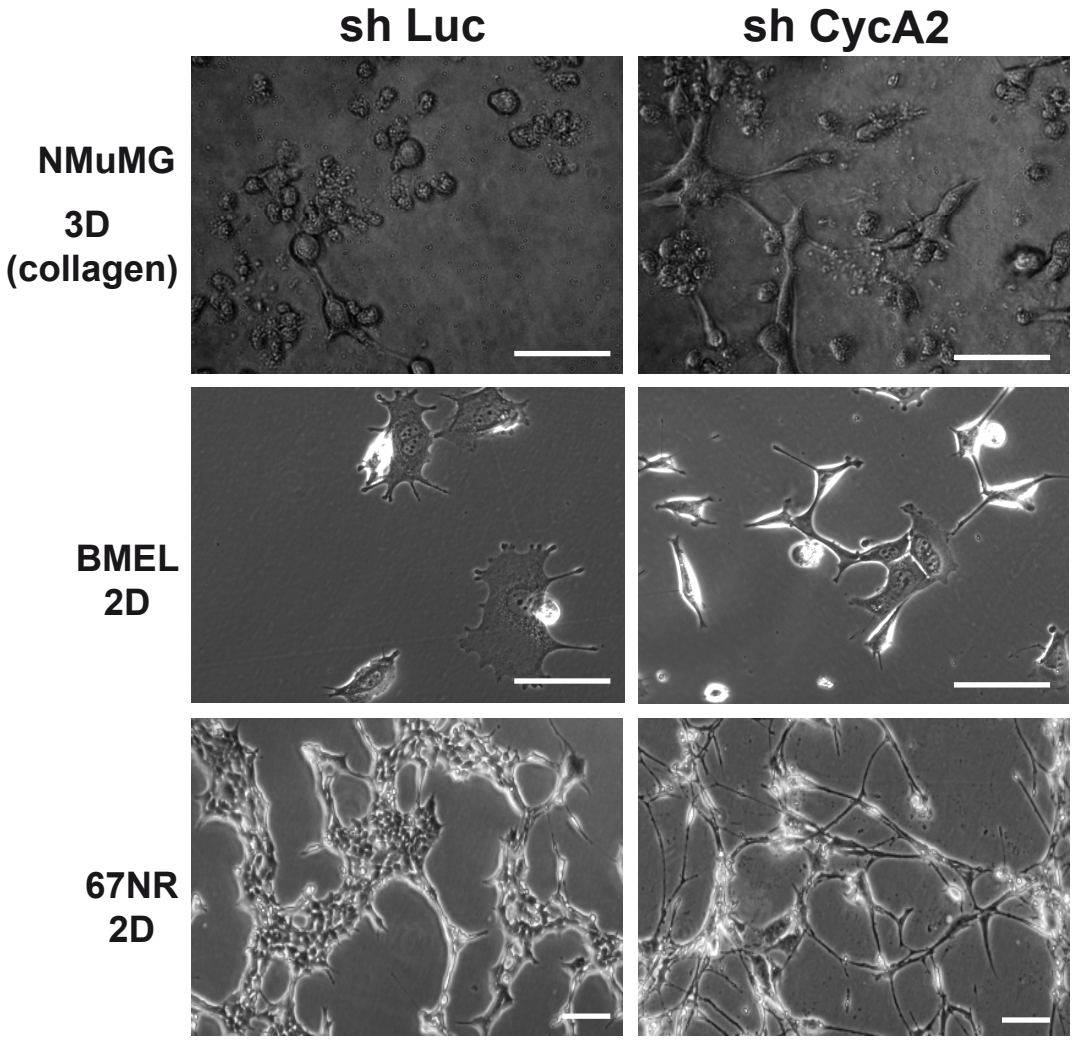
c.

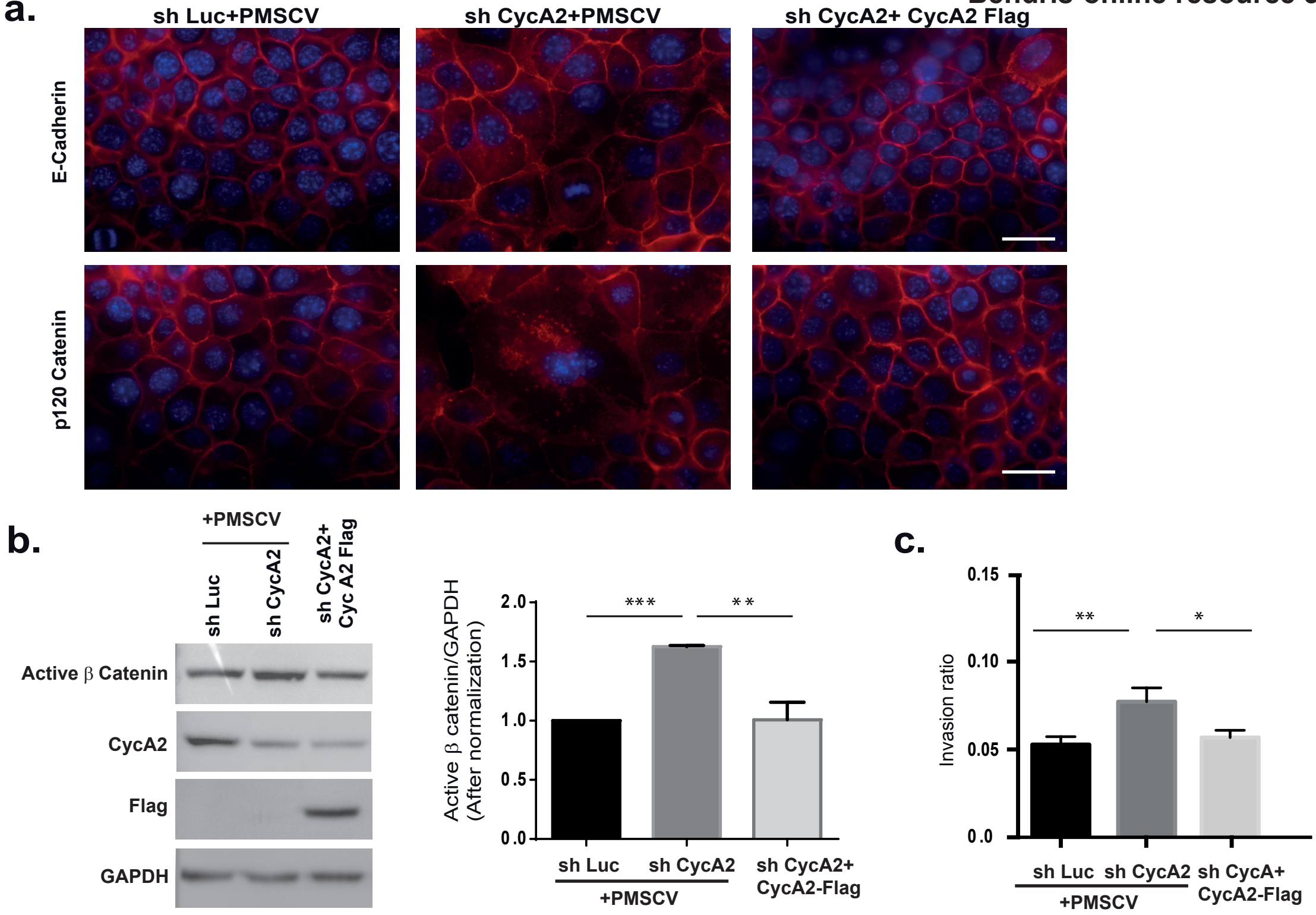

p=0,0127
*
150000
100000
50000
0
counts per minute
sh Luc
sh CycA2
CDK2
p=0,0014
* *
10000
5000
0
counts per minute
sh Luc
sh CycA2
CDK 1

a.

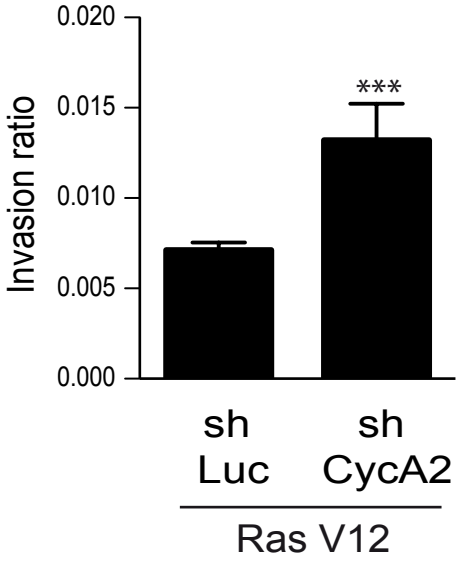

sh Luc
sh CycA2
Cell number (x 10^4)
250
200
150
100
50
0
1
2
3
4
5
6
Days

b.

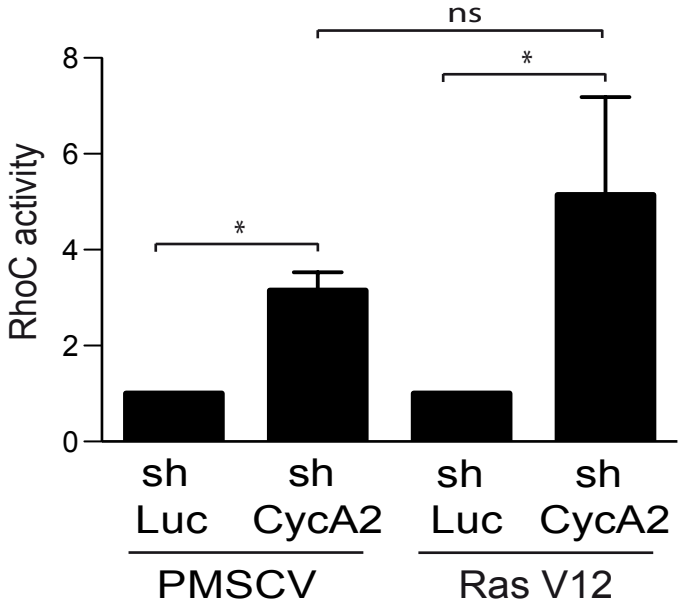

sh Luc
sh CycA2
NMuMG
3D
(collagen)
BMEL
2D
67NR
2D

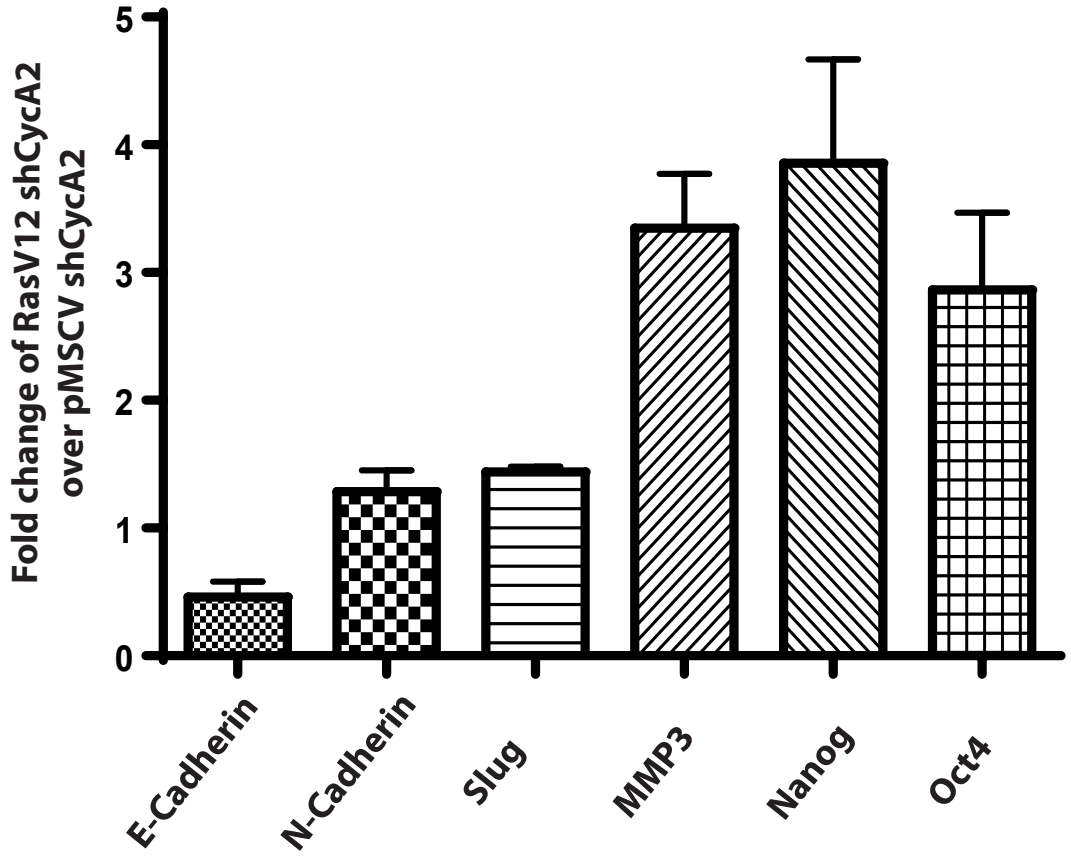

a.
sh Luc+PMSCV
sh CycA2+PMSCV
sh CycA2+ CycA2 Flag
E-Cadherin
p120 Catenin
b.
+PMSCV
sh Luc
sh CycA2
sh CycA2+ Cyc A2 Flag
Active β Catenin
CycA2
Flag
GAPDH
Active β catenin/GAPDH (After normalization)
2.0
1.5
1.0
0.5
0.0
***
**
sh Luc
sh CycA2
+PMSCV
sh CycA2+ CycA2-Flag
c.
Invasion ratio
0.15
0.10
0.05
0.0
**
*
sh Luc
sh CycA2
+PMSCV
sh CycA+ CycA2-Flag

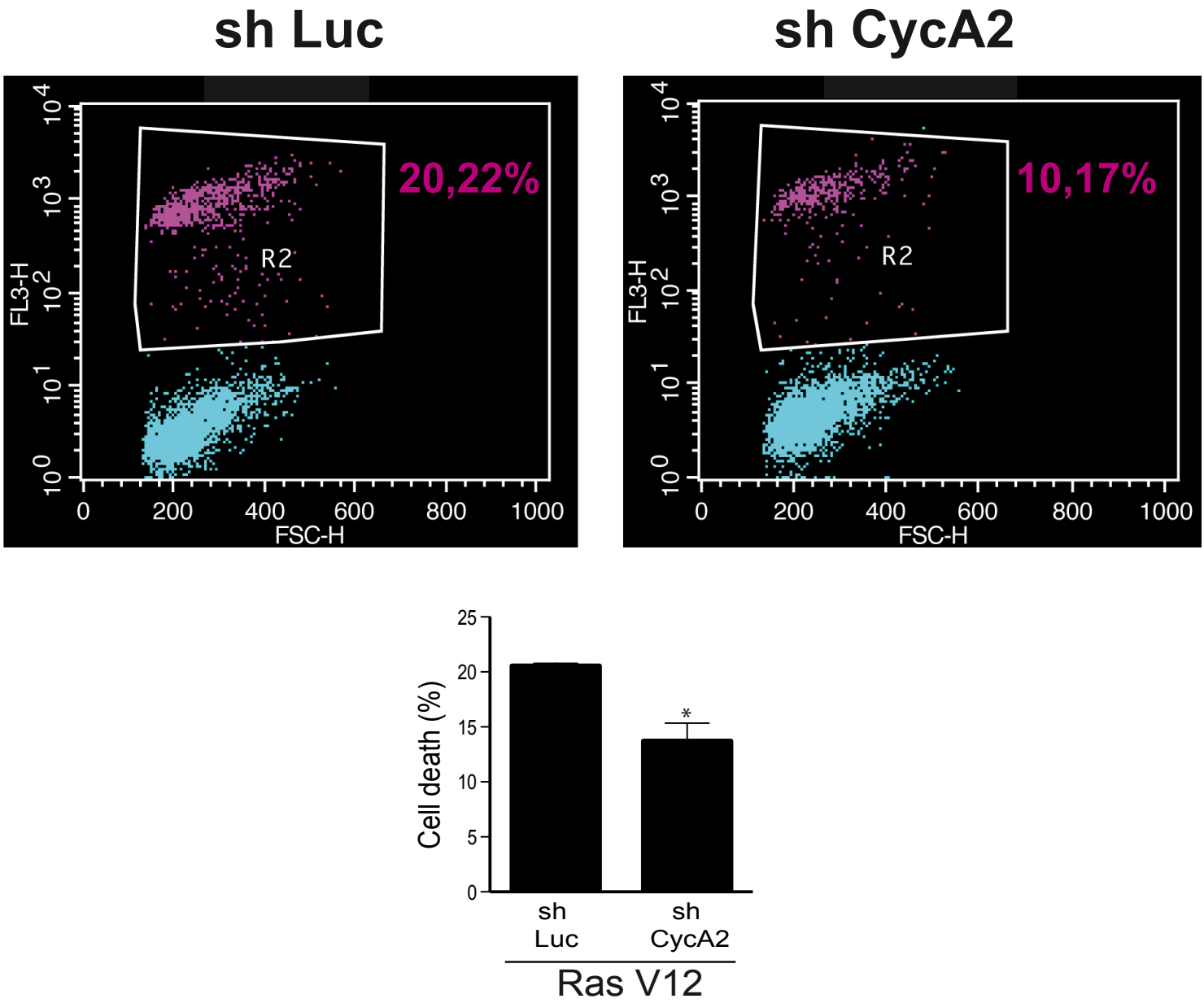
a.


0.020
0.015
0.010
0.005
0.000
Invasion ratio
***
sh Luc
sh CycA2
Ras V12

b.


ns
*
*
8
6
4
2
0
RhoC activity
sh Luc
sh CycA2
sh Luc
sh CycA2
PMSCV
Ras V12


Fold change of RasV12 shCycA2 over pMSCV shCycA2
5
4
3
2
1
0
E-Cadherin
N-Cadherin
Slug
MMP3
Nanog
Oct4


sh Luc
sh CycA2
20,22%
10,17%
R2
FL3-H
FSC-H
0
200
400
600
800
1000
Cell death (%)
25
20
15
10
5
0
*
sh Luc
sh CycA2
Ras V12