Supplementary video legends

Supplementary video 1: Timelapse phase contrast imaging of acini lacking centrosome amplification (-Dox). 3-D culture was for 4 days prior to initiating imaging. Images were acquired over 20hrs with images acquired every 4 minutes.

Supplementary video 2: Timelapse imaging of an acinus with centrosome amplification as in Supplementary Movie 1. Note that invasive protrusions are dynamic.

Supplementary video 3: Multiple cells can migrate into an invasive protrusion from acini with multiple centrosomes. Imaging was as in Supplementary Movie S2 except that images were acquired for 8hrs and nuclei were visualized by fluorescence imaging every 4 minutes to detect nuclei marked by H2B-GFP. Phase contrast images were acquired at each timepoint with the fluorescence images.

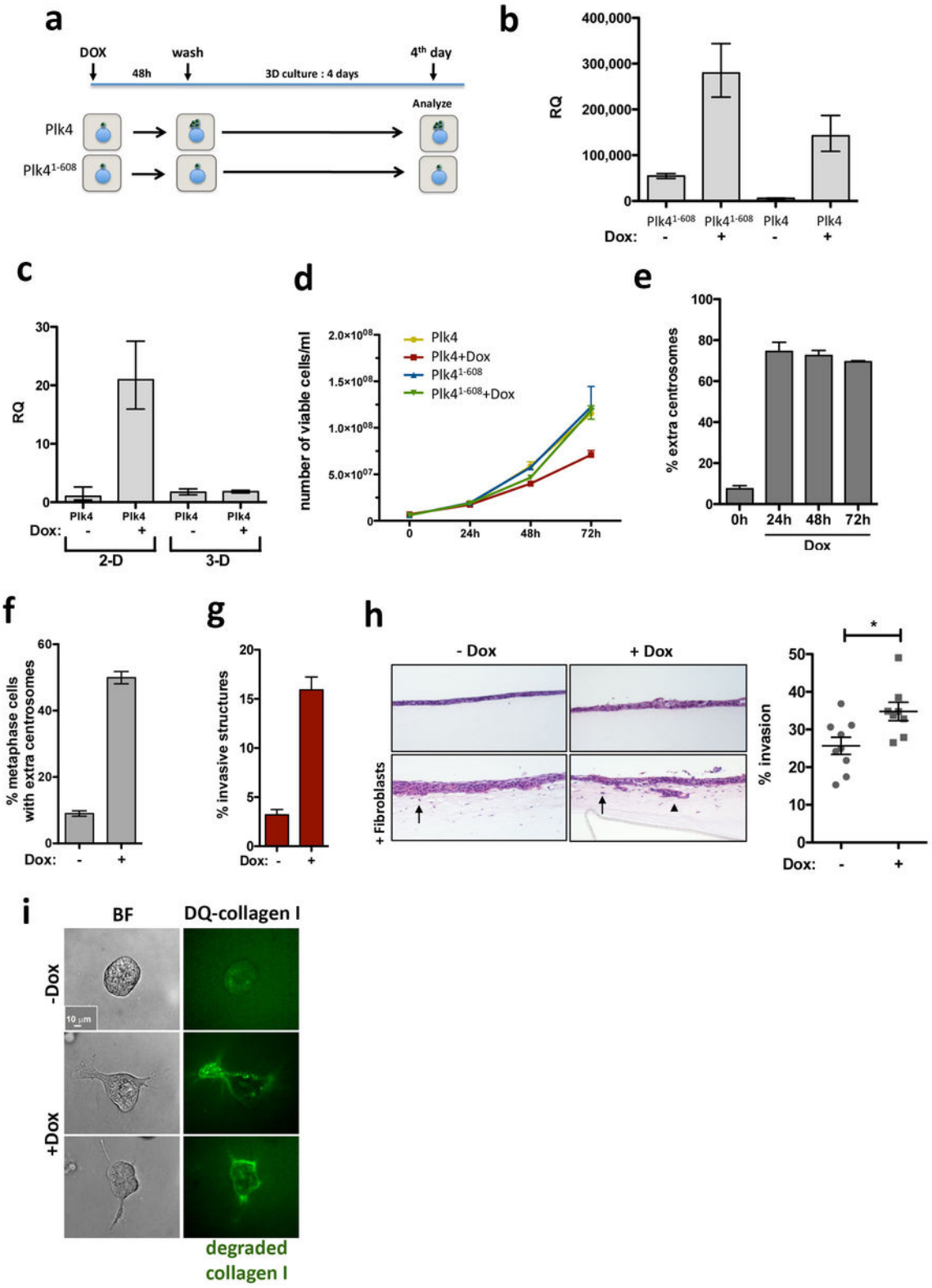
Supplementary video 4: Time lapse imaging of dividing single cells in 2-D substrates that stay together after mitosis. Images were acquired for 15hrs and nuclei were visualized by fluorescence imaging every 4 minutes to detect nuclei marked by H2B-GFP. Phase contrast images were acquired at each timepoint with the fluorescence images. Note that this time lapse represents only 5rs of the original 15hrs movie.

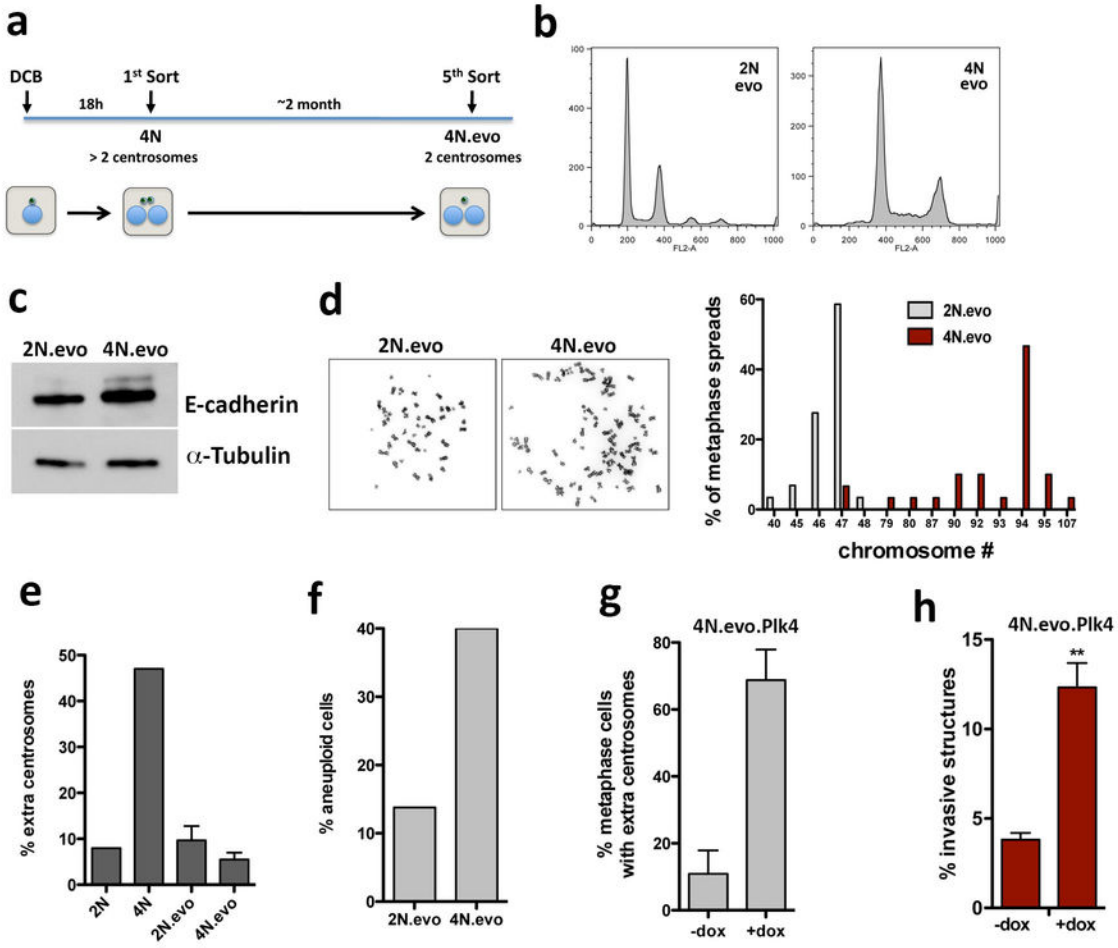
Supplementary video 5: Time lapse imaging of dividing single cells on 2-D substrates that move apart after mitosis. Images were acquired for 15hrs and nuclei were visualized by fluorescence imaging every 4 minutes to detect nuclei marked by H2B-GFP. Phase contrast images were acquired at each timepoint with the fluorescence images. Note that this time lapse represents only 5rs of the original 15hrs movie.

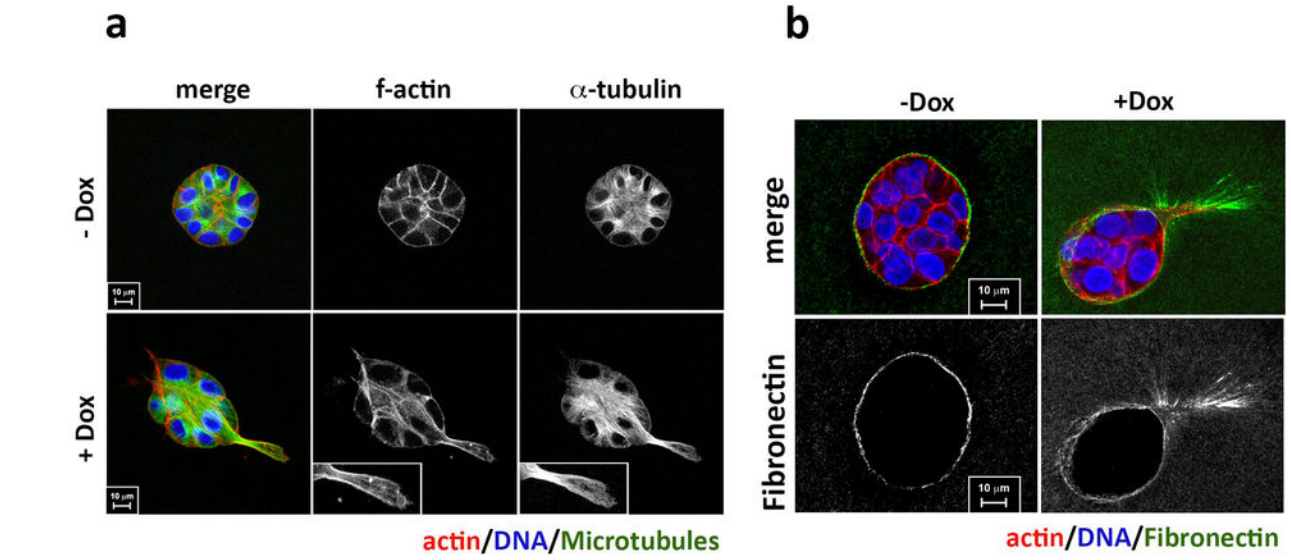
Supplementary video 6: Time lapse imaging of a monolayer of MCF10A cells expressing Raichu-Rac to visualize cell-cell boundaries. Images were acquired every

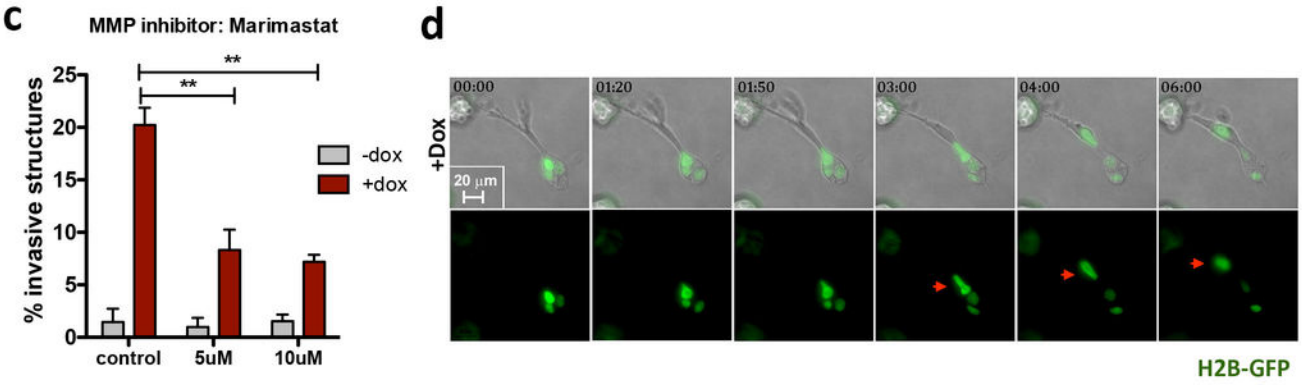
5 minutes for 12.4 hrs to monitor cell-cell edges interactions marked by Raichu-Rac-CFP. Scale bar :10μm

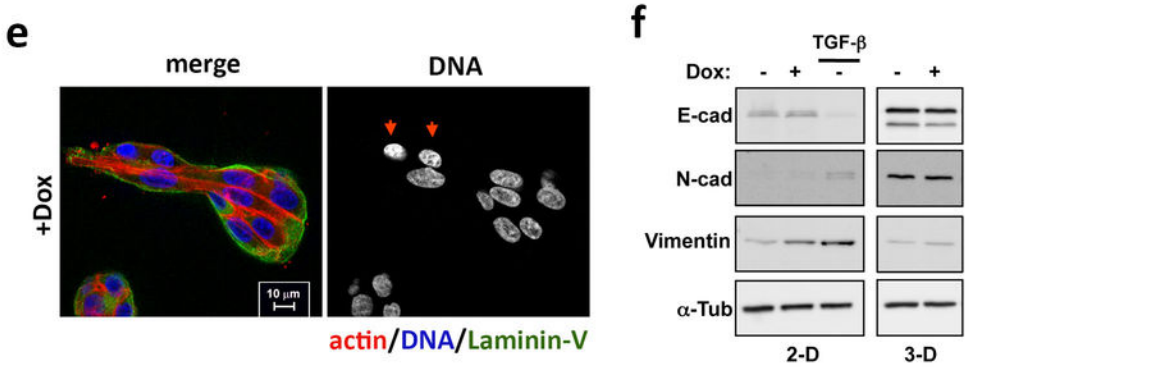
Supplementary video 7: Time lapse imaging of a monolayer of MCF10A cells with centrosome amplification (+Dox) expressing Raichu-Rac-CFP as in Supplementary Movie 6. Overlapping regions between adjacent cells are marked by the white arrows. Scale bar :10μm

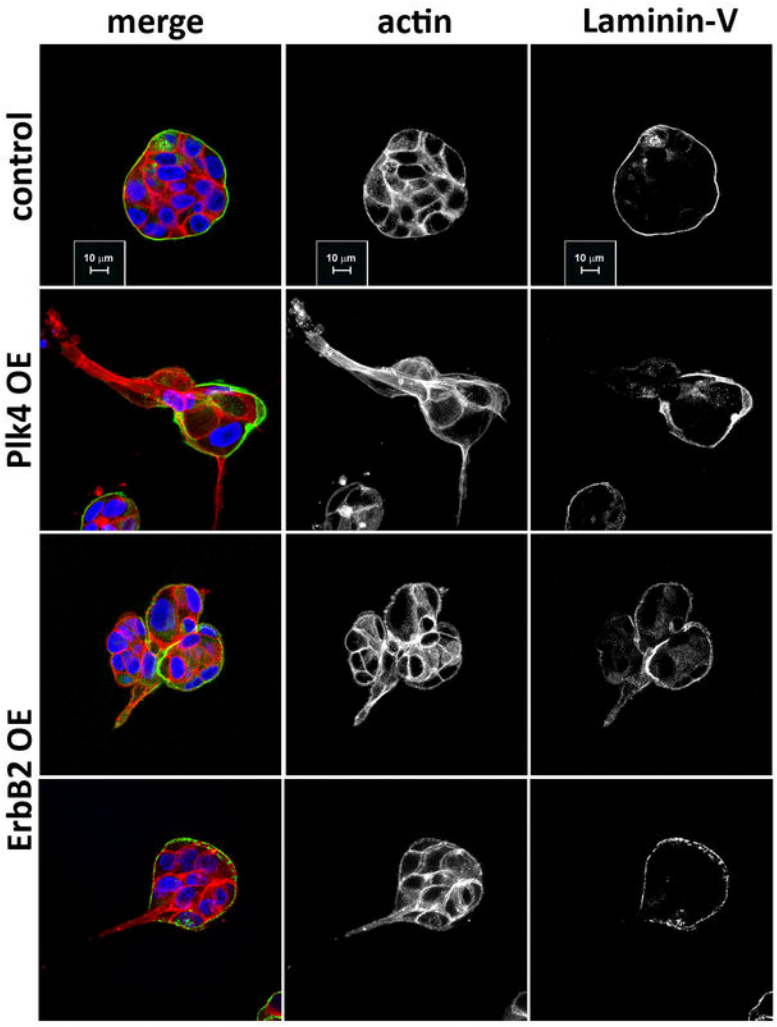

a
DOX
wash
4th day
48h
3D culture : 4 days
Analyze
Plk4
Plk4 1-608
b
RQ
400,000
300,000
200,000
100,000
0
Plk4 1-608
Plk4 1-608
Plk4
Plk4
Dox: - + - +
c
RQ
30
20
10
0
Plk4
Plk4
Plk4
Plk4
Dox: - + - +
2-D
3-D
d
number of viable cells/ml
2.0×10 08
1.5×10 08
1.0×10 08
5.0×10 07
0
Plk4
Plk4+Dox
Plk4 1-608
Plk4 1-608+Dox
0
24h
48h
72h
e
% extra centrosomes
100
80
60
40
20
0
0h
24h
48h
72h
Dox
f
% metaphase cells with extra centrosomes
60
40
20
0
Dox: - +
g
% invasive structures
20
15
10
5
0
Dox: - +
h
- Dox
+ Dox
+ Fibroblasts
% invasion
*
50
40
30
20
10
0
Dox: - +
i
BF
DQ-collagen I
-Dox
+Dox
10 µm
degraded collagen I

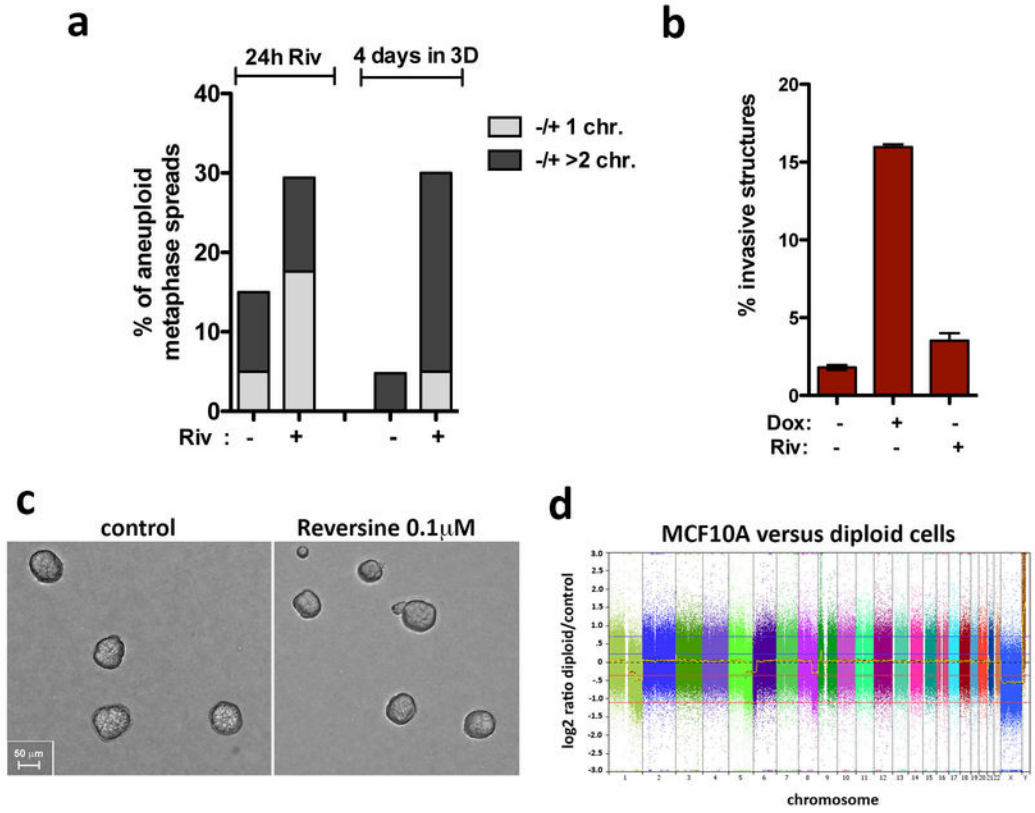

a
DCB
1st Sort
18h
~2 month
5th Sort
4N
> 2 centrosomes
4N.evo
2 centrosomes
b
2N evo
4N evo
FL2-A
c
2N.evo 4N.evo
E-cadherin
α-Tubulin
d
2N.evo
4N.evo
% of metaphase spreads
chromosome #
e
% extra centrosomes
2N
4N
2N.evo
4N.evo
f
% aneuploid cells
2N.evo 4N.evo
g
4N.evo.Plk4
% metaphase cells with extra centrosomes
-dox
+dox
h
4N.evo.Plk4
% invasive structures
**
-dox
+dox

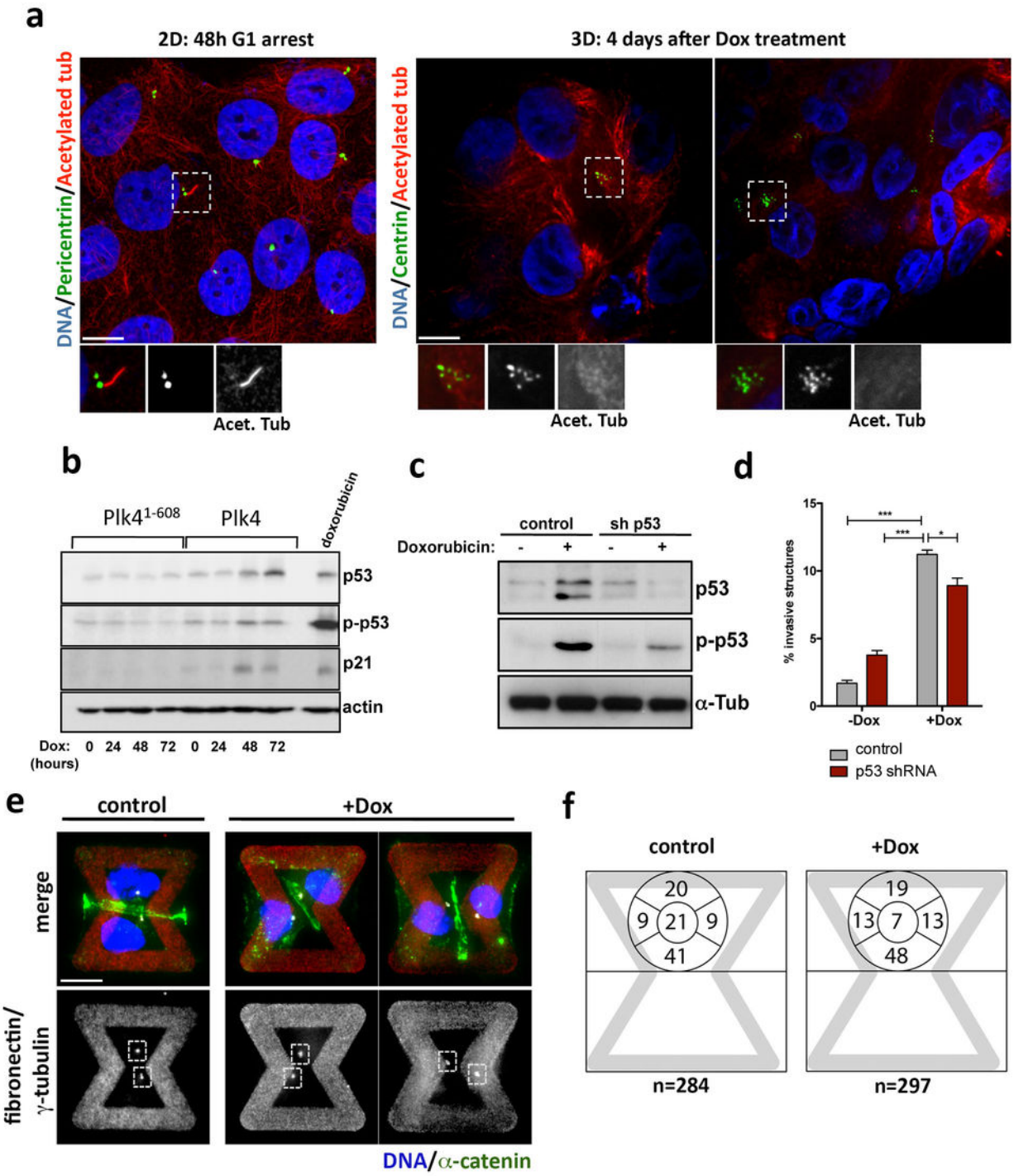

a
merge
f-actin
α-tubulin
- Dox
+ Dox
actin/DNA/Microtubules
b
-Dox
+Dox
merge
Fibronectin
actin/DNA/Fibronectin

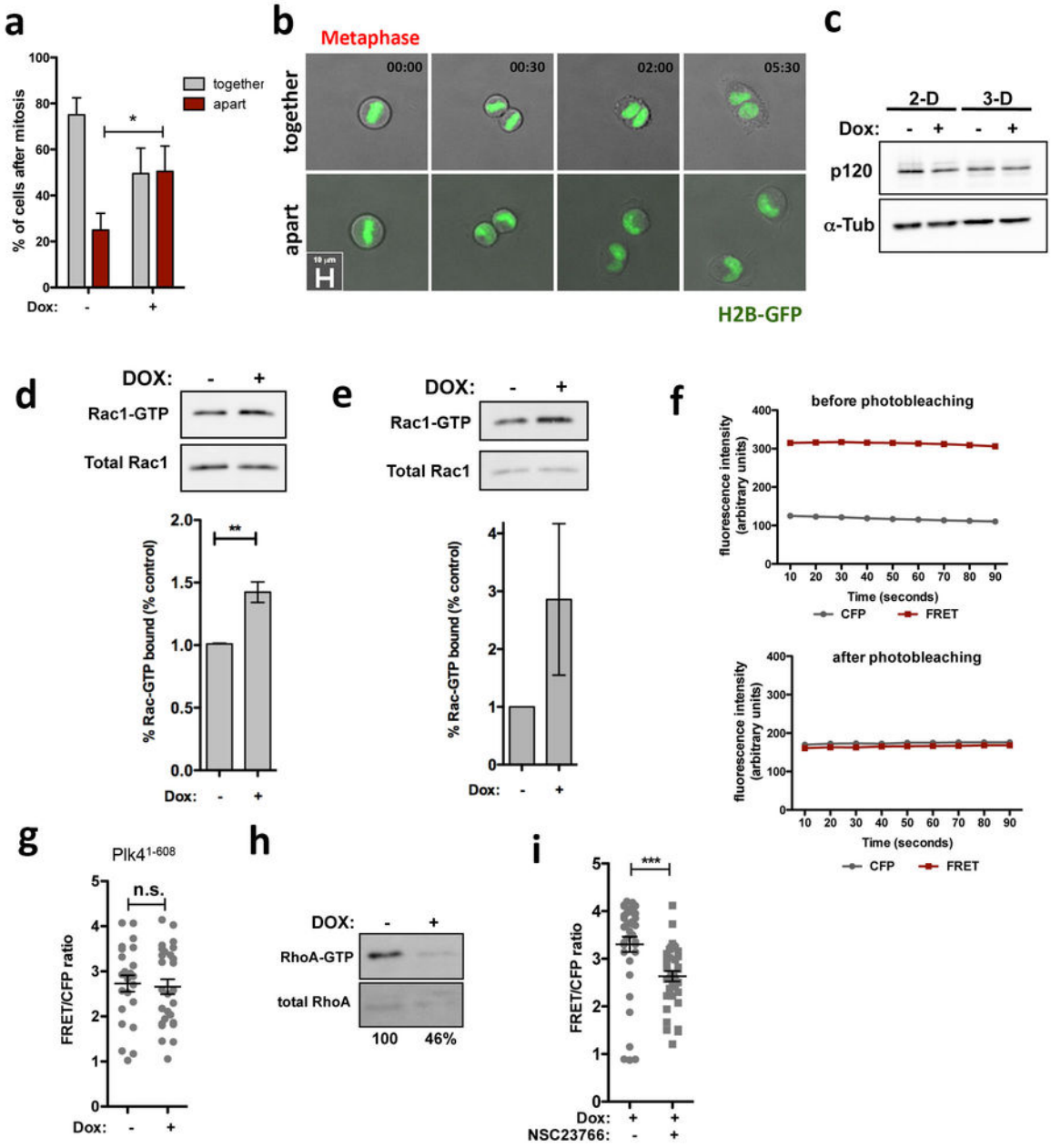

c
MMP inhibitor: Marimastat
% invasive structures
-dox
+dox
control
5uM
10uM
d
+Dox
00:00
01:20
01:50
03:00
04:00
06:00
H2B-GFP

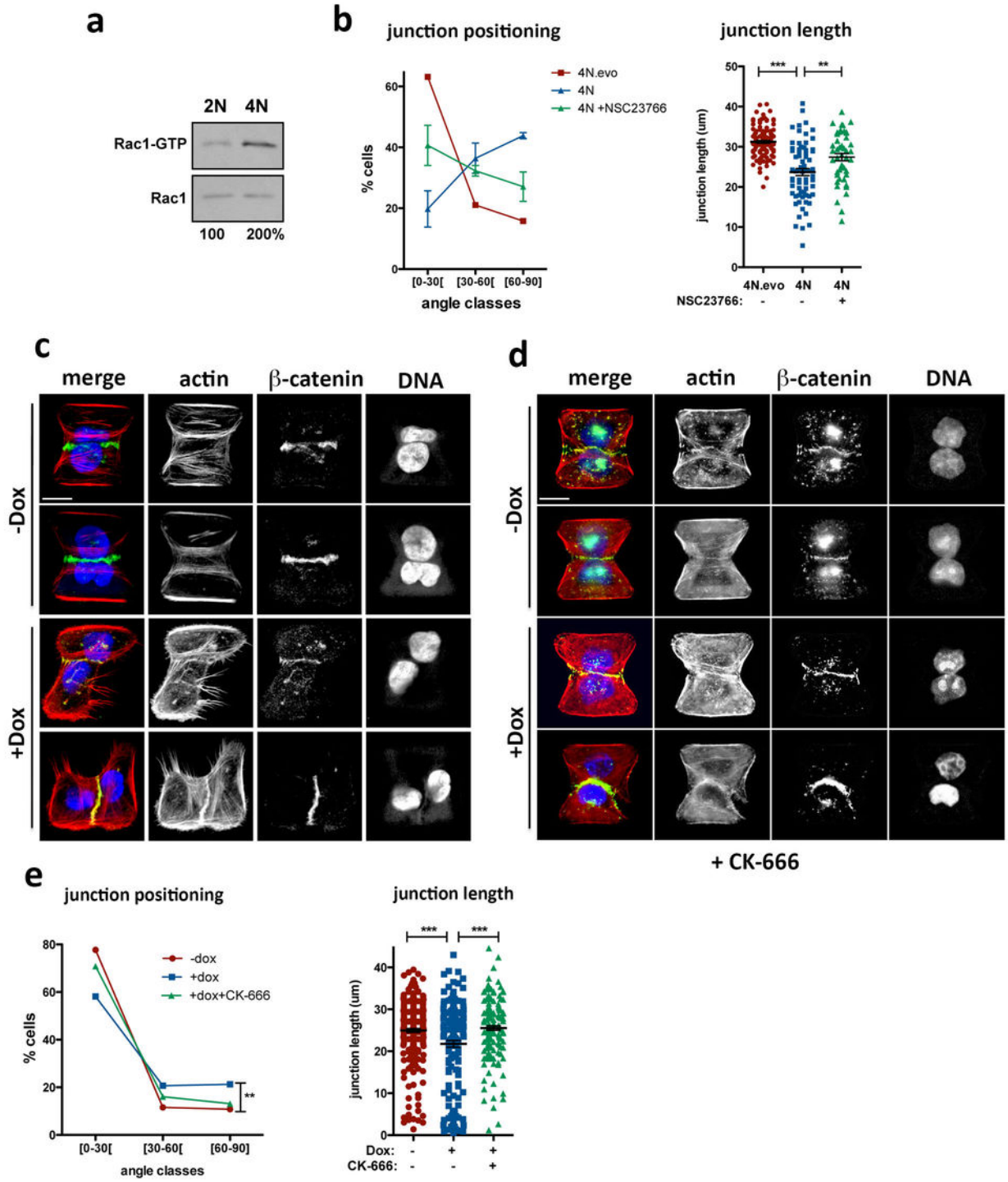

e
merge
DNA
+Dox
actin/DNA/Laminin-V
f
TGF-β
Dox:
E-cad
N-cad
Vimentin
α-Tub
2-D
3-D

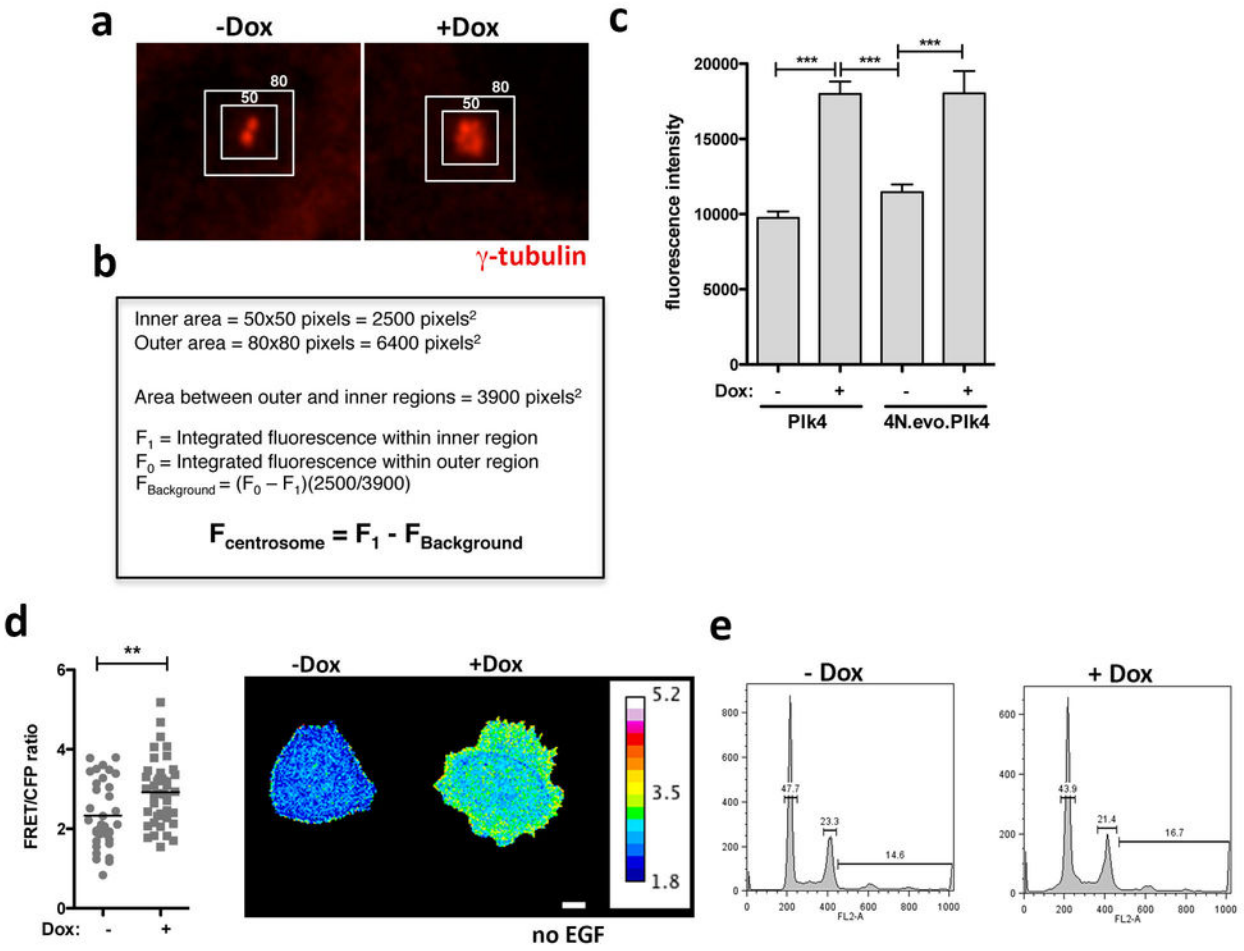

merge
actin
Laminin-V
control
Plk4 OE
ErbB2 OE
10 µm
10 µm
10 µm

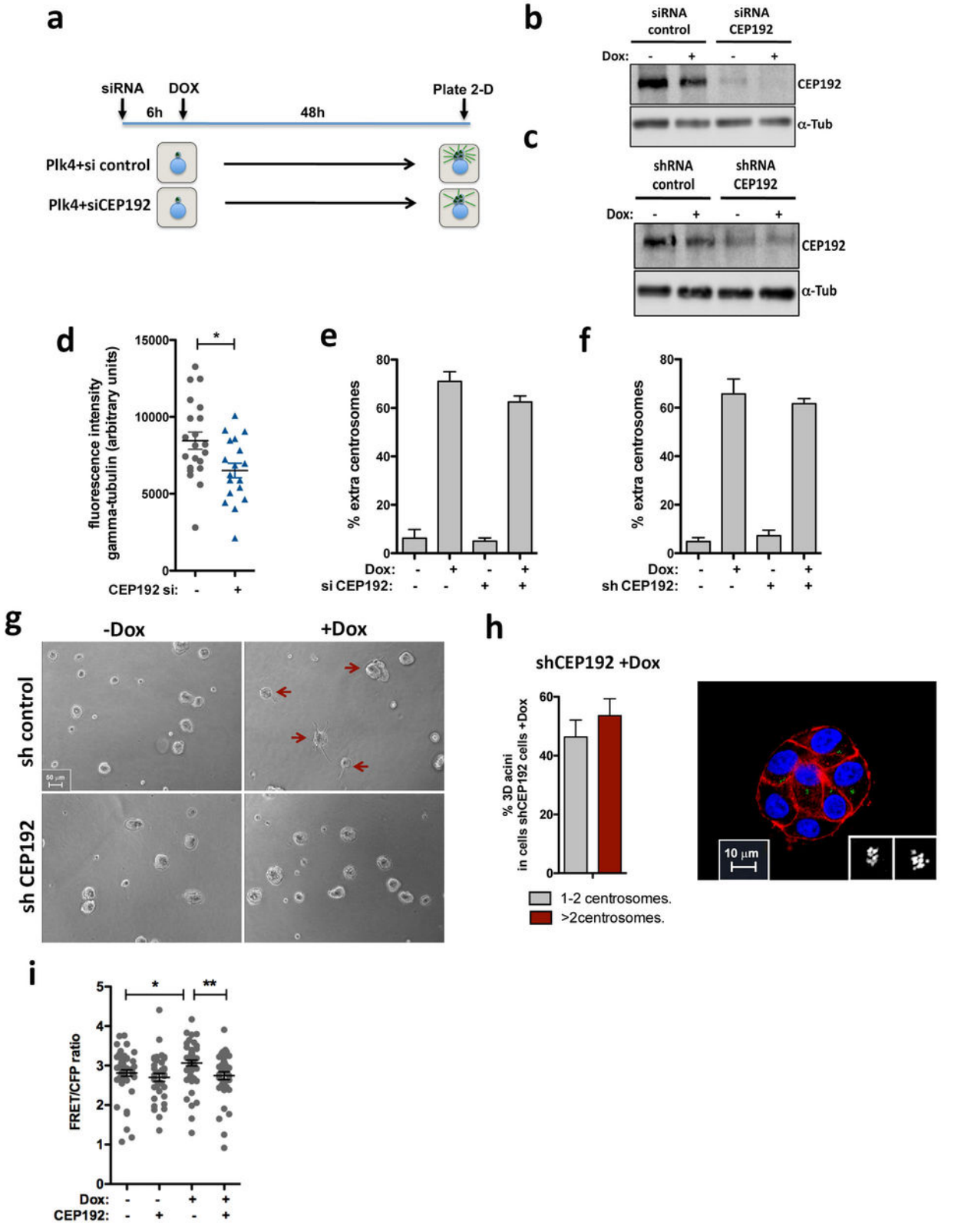

a
24h Riv
4 days in 3D
% of aneuploid metaphase spreads
40
30
20
10
0
-/+ 1 chr.
-/+ >2 chr.
Riv : - + - +
b
% invasive structures
20
15
10
5
0
Dox: - + -
Riv: - - +
c
control
Reversine 0.1μM
50 μm
d
MCF10A versus diploid cells
log2 ratio diploid/control
chromosome


a
2D: 48h G1 arrest
3D: 4 days after Dox treatment
DNA/Pericentrin/Acetylated tub
DNA/Centrin/Acetylated tub
Acet. Tub
Acet. Tub
Acet. Tub
b
Plk4 1-608
Plk4
doxorubicin
p53
p-p53
p21
actin
Dox: 0 24 48 72 0 24 48 72
(hours)
c
control
sh p53
Doxorubicin: - + - +
p53
p-p53
α-Tub
d
% invasive structures
15
10
5
0
***
***
*
-Dox
+Dox
control
p53 shRNA
e
control
+Dox
merge
fibronectin/
γ-tubulin
DNA/α-catenin
f
control
+Dox
20
9
21
9
41
19
13
7
13
48
n=284
n=297


a
% of cells after mitosis
together
apart
*
Dox: - +
b
Metaphase
together
apart
00:00
00:30
02:00
05:30
H2B-GFP
c
2-D
3-D
Dox: - + - +
p120
α-Tub
d
DOX: - +
Rac1-GTP
Total Rac1
% Rac-GTP bound (% control)
**
Dox: - +
e
DOX: - +
Rac1-GTP
Total Rac1
% Rac-GTP bound (% control)
Dox: - +
f
before photobleaching
fluorescence intensity (arbitrary units)
Time (seconds)
CFP
FRET
after photobleaching
fluorescence intensity (arbitrary units)
Time (seconds)
CFP
FRET
g
Plk4 1-608
n.s.
FRET/CFP ratio
Dox: - +
h
DOX: - +
RhoA-GTP
total RhoA
100 46%
i
***
FRET/CFP ratio
Dox: + +
NSC23766: - +


a
2N
4N
Rac1-GTP
Rac1
100
200%
b
junction positioning
4N.evo
4N
4N +NSC23766
% cells
[0-30[
[30-60[
[60-90]
angle classes
junction length
junction length (um)
***
**
4N.evo
4N
4N
NSC23766:
c
merge
actin
β-catenin
DNA
-Dox
+Dox
d
merge
actin
β-catenin
DNA
-Dox
+Dox
+ CK-666
e
junction positioning
-dox
+dox
+dox+CK-666
% cells
**
[0-30[
[30-60[
[60-90]
angle classes
junction length
junction length (um)
***
***
Dox:
CK-666:


a
-Dox
+Dox
80
50
γ-tubulin
b
Inner area = 50x50 pixels = 2500 pixels²
Outer area = 80x80 pixels = 6400 pixels²
Area between outer and inner regions = 3900 pixels²
F1 = Integrated fluorescence within inner region
F0 = Integrated fluorescence within outer region
FBackground = (F0 – F1)(2500/3900)
Fcentrosome = F1 - FBackground
c
fluorescence intensity
20000
15000
10000
5000
0
***
Dox: - + - +
Plk4
4N.evo.Plk4
d
FRET/CFP ratio
**
Dox: - +
-Dox
+Dox
5.2
3.5
1.8
no EGF
e
- Dox
+ Dox
47.7
23.3
14.6
43.9
21.4
16.7
FL2-A


a
siRNA
DOX
6h
48h
Plate 2-D
Plk4+si control
Plk4+siCEP192
b
siRNA control
siRNA CEP192
Dox: - + - +
CEP192
α-Tub
c
shRNA control
shRNA CEP192
Dox: - + - +
CEP192
α-Tub
d
fluorescence intensity gamma-tubulin (arbitrary units)
15000
10000
5000
0
*
CEP192 si: - +
e
% extra centrosomes
80
60
40
20
0
Dox: - + - +
si CEP192: - - + +
f
% extra centrosomes
80
60
40
20
0
Dox: - + - +
sh CEP192: - - + +
g
-Dox
+Dox
sh control
sh CEP192
50 μm
h
shCEP192 +Dox
% 3D acini in cells shCEP192 cells +Dox
60
40
20
0
1-2 centrosomes.
>2centrosomes.
10 μm
i
FRET/CFP ratio
5
4
3
2
1
0
*
**
Dox: - - + +
CEP192: - + - +