Developmental Cell, Volume *47*

Supplemental Information

Oxidative Stress in Cells with Extra Centrosomes Drives Non-Cell-Autonomous Invasion

Teresa Arnandis, Pedro Monteiro, Sophie D. Adams, Victoria Louise Bridgeman, Vinothini Rajeeve, Emanuela Gadaleta, Jacek Marzec, Claude Chelala, Ilaria Malanchi, Pedro R. Cutillas, and Susana A. Godinho

Figure S1

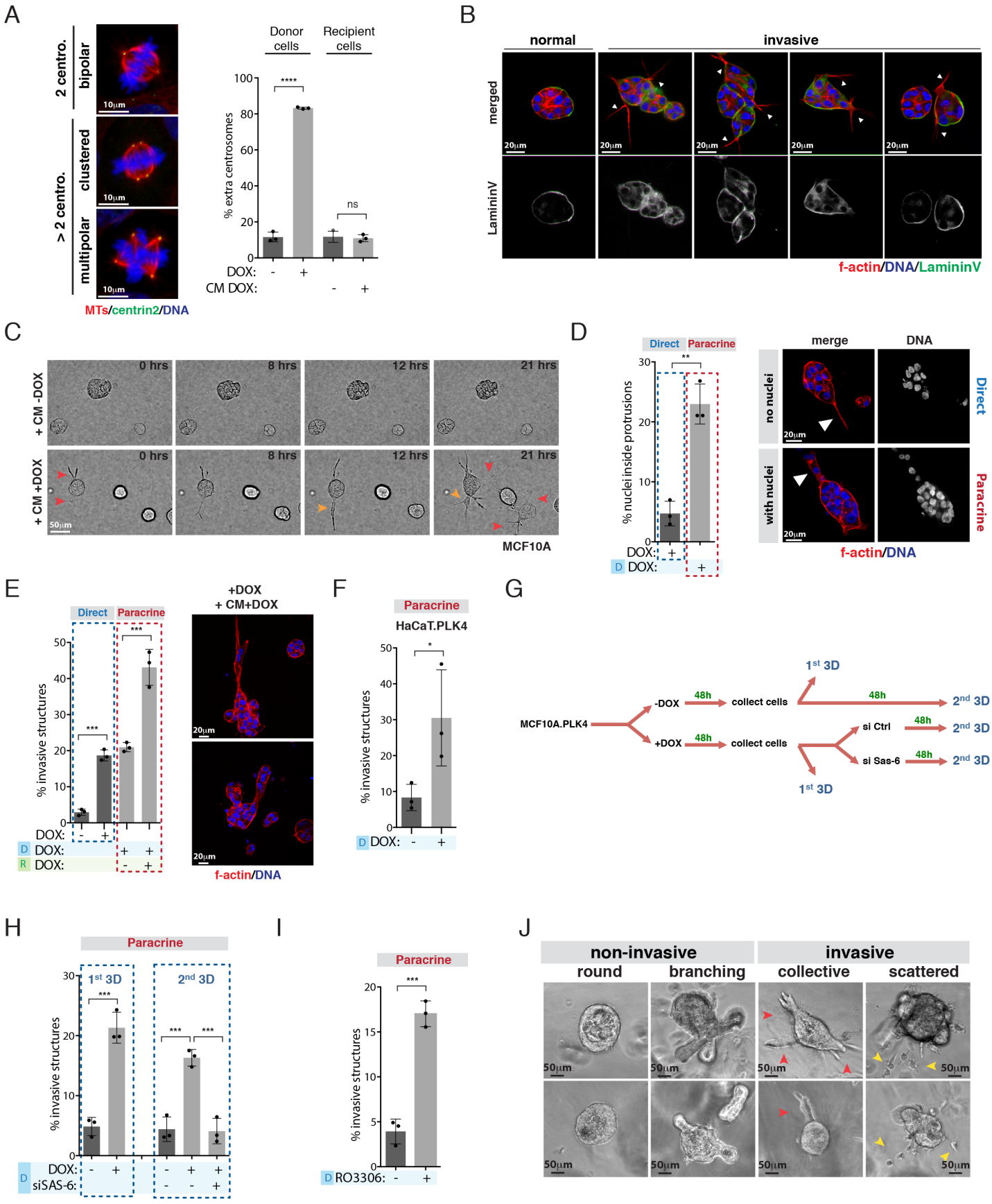
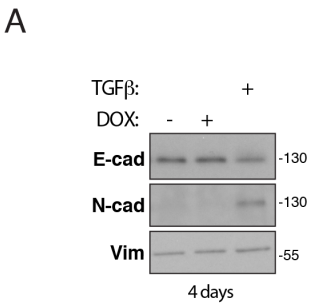
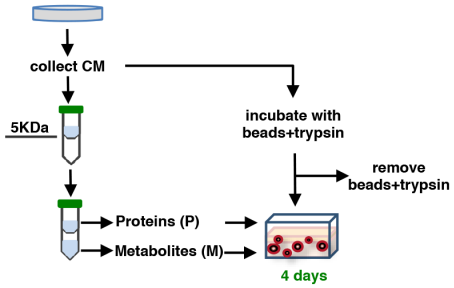
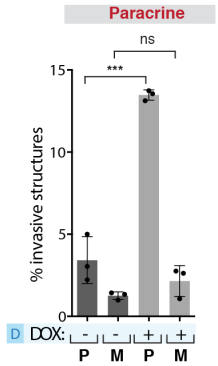

A
2 centro.
bipolar
> 2 centro.
clustered
multipolar
10µm
MTs/centrin2/DNA
Donor cells
Recipient cells
% extra centrosomes
****
ns
DOX:
CM DOX:
B
normal
invasive
merged
LamininV
20µm
f-actin/DNA/LamininV
C
+ CM -DOX
+ CM +DOX
0 hrs
8 hrs
12 hrs
21 hrs
50µm
MCF10A
D
Direct
Paracrine
% nuclei inside protrusions
DOX:
D DOX:
merge
DNA
no nuclei
with nuclei
Direct
Paracrine
f-actin/DNA
E
Direct
Paracrine
% invasive structures
D DOX:
R DOX:
+DOX
+ CM+DOX
f-actin/DNA
F
Paracrine
HaCaT.PLK4
% invasive structures
D DOX:
G
MCF10A.PLK4
-DOX
+DOX
48h
collect cells
1st 3D
2nd 3D
si Ctrl
si Sas-6
H
Paracrine
1st 3D
2nd 3D
% invasive structures
D DOX:
siSAS-6:
I
Paracrine
% invasive structures
D RO3306:
J
non-invasive
invasive
round
branching
collective
scattered
50µm

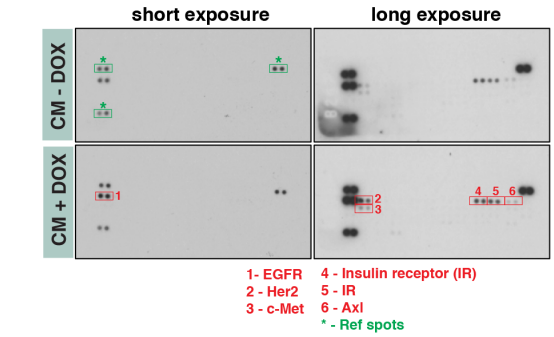
Figure S1. Centrosome amplification induces paracrine invasion. Related to Figure 1, Table S1, Videos S3 and S4. (A) Left, Cells in mitosis with normal and amplified centrosomes (>2 centro.; clustered + multipolar) were stained for microtubules (MTs) (α-Tubulin, red), centrioles (centrin2, green) and DNA (blue). Scale bar 10μm. Right, Centrosome amplification in cells with extra centrosomes (+DOX) or incubated with CM collected from cells with extra centrosomes (CM+DOX). (B) Normal and invasive 3D acini stained for f-actin (phalloidin, red), laminin V (green) and DNA (blue). White arrowheads indicate invasive protrusions. Scale bar 20μm. (C) Still images from live cell imaging of acini incubated with CM (CM-/+DOX). Red arrowheads indicate invasive acini and orange arrowhead indicates a cell/nuclei moving through the invasive protrusion. Scale bar 20μm (D) Left, Quantification of acini with invasive protrusions containing nuclei in cells with extra centrosomes (+DOX) or incubated with CM (CM-/+DOX). Right, Invasive acini stained for f-actin (phalloidin, red) and DNA (blue). Arrowheads indicate invasive protrusions. Scale bar 20μm. (E) Left, Quantification of invasive structures in acini with extra centrosomes (+DOX) and incubated with CM+DOX. Right, Highly invasive/disrupted acini observed in cells with extra centrosomes treated with CM+DOX. Cells were stained for f-actin (phalloidin, red) and DNA (blue). Scale bar 20μm. (F) Quantification of invasive acini incubated with CM from HaCaT.PLK4 cells. (G) Schematic representation of SAS-6 depletion to revert centrosome amplification. (H) Quantification of invasive acini incubated with CM from cells where centrosome amplification was lost (SAS-6 siRNA). (I) Invasive acini in cells incubated with CM collected from cells treated with the CDK1 inhibitor RO-3306 (5μM, 40 hrs). (J) Bright field images of non-invasive and invasive mouse mammary organoids. Scale bar 50μm. For all graphics error bars represent mean +/- SD from three independent experiments. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, $****P < 0.0001$, ns not significant.

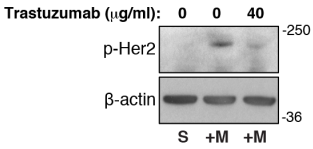
Figure S2

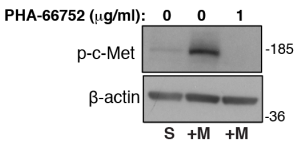


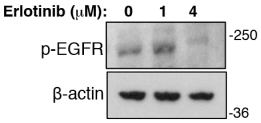


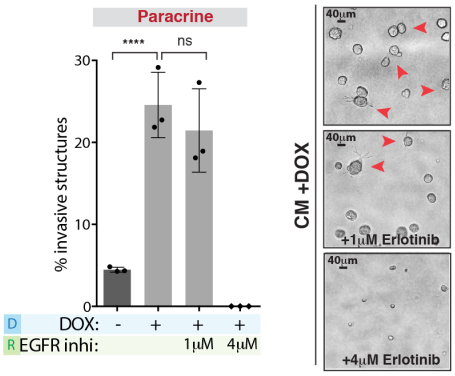












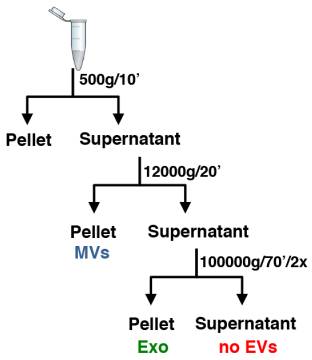
I

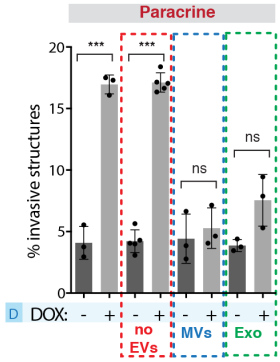
MCF10A.PLK4 - DOX

sample	Initial cell #	Final cell #	Protein (mg/ml)	LDH (fold)	% invasion
1	$33.5e10^5$	$26e10^6$	16.58	1.0	3.36
2	$33.5e10^5$	$26e10^6$	16.01	1.0	3.24
3	$33.5e10^5$	$26e10^6$	17.86	0.9	4.79

MCF10A.PLK4 + DOX

sample	Initial cell #	Final cell #	Protein (mg/ml)]	LDH (fold)	% invasion
1	$45.2e10^5$	$20e10^6$	19.11	1.1	15.91
2	$45.2e10^5$	$20e10^6$	19.24	1.2	17.45
3	$45.2e10^5$	$20e10^6$	20.63	1.3	17.67





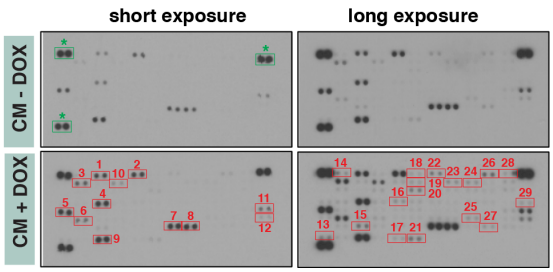


Figure S2. Proteins secreted by cells with extra centrosomes promote paracrine invasion via RTK activation. Related to Figure 2. (A) Levels of E-cadherin, N-cadherin and Vimentin in cells incubated with CM for 4 days. TGF-β treatment (5 ng/ml, 4 days) was used as positive control. (B) Schematic representation of Vivaspin filtration and tryspin incubation. (C) Quantification of invasive acini cells incubated with CM after Vivaspin filtration. P: proteins, M: metabolites. (D) phospho-RTK array obtained from cells incubated with CM. (E) phospho-Her2 levels in cells treated with 40 μg/ml of Trastuzumab (Her2 inhibitor) for 1 hr. S, serum starved; +M, plus fresh medium. (F) phospho-c-Met levels in cells treated with 1 μM PHA-66752 (c-Met inhibitor) for 1 hr. S, serum starved; +M, plus fresh medium. (G) phospho-EGFR levels in cells treated with different concentrations of Erlotinib (1 and 4 μM, 24 hrs). (H) Left, Quantification of invasive acini cells treated with EGFR inhibitor (Erlotinib, 1 and 4 μM) and incubated with CM. Right, Acinar structures. Red arrowheads indicate invasive acini. Scale bar 40μm. (I) Table summarising the conditions used to prepare CM for proteomic analysis. Cells with (+DOX) or without extra centrosomes (-DOX) were plated at different densities to ensure a similar cell number by the time of CM collection. Protein concentration and invasive capacity of the CM was assessed in all samples. (J) Left, Schematic representation of the ultracentrifugation method to isolate MVs and Exo. Right, Quantification of invasive acini in cells incubated with CM with and without MVs/Exo. (K) Human XL Oncology Array incubated with CM collected from cells with (CM+DOX) and without (CM-DOX) extra centrosomes. For all graphics error bars represent mean +/- SD from three independent experiments. ****P < 0.001, ****P < 0.0001,* ns not significant.

Figure S3

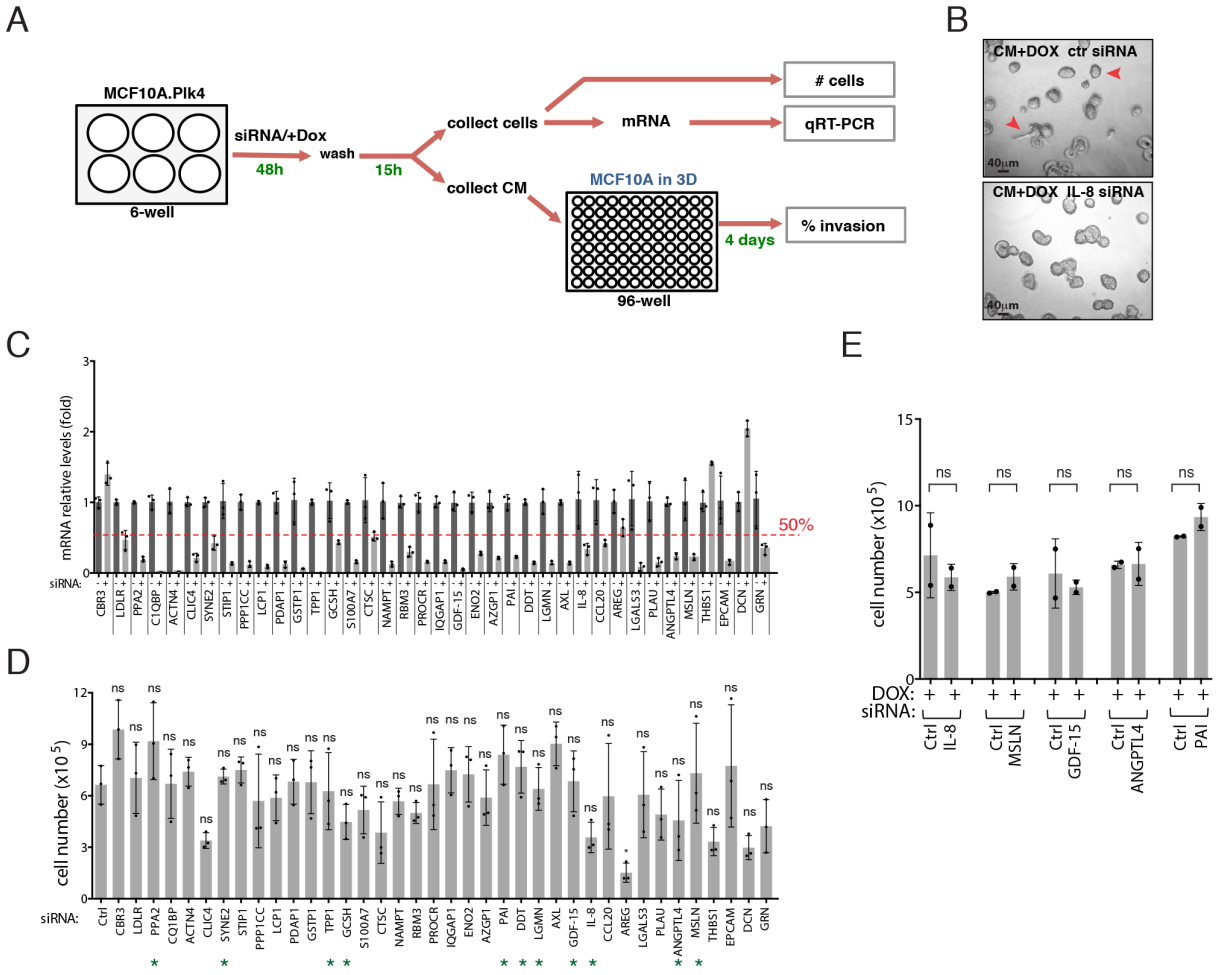

A
MCF10A.Plk4
6-well
siRNA/+Dox
48h
wash
15h
collect cells
mRNA
qRT-PCR
cells
collect CM
MCF10A in 3D
96-well
4 days
% invasion
B
CM+DOX ctr siRNA
CM+DOX IL-8 siRNA
40µm
C
mRNA relative levels (fold)
50%
siRNA:
D
cell number (x10 5)
siRNA:
Ctrl
CBR3
LDLR
PPA2
CQ1BP
ACTN4
CLIC4
SYNE2
STIP1
PPP1CC
LCP1
PDAP1
GSTP1
TPP1
GCSH
S100A7
CTSC
NAMPT
RBM3
PROCR
IQGAP1
ENO2
AZGP1
PAI
DDT
LGMN
AXL
GDF-15
IL-8
CCL20
AREG
LGALS3
PLAU
ANGPTL4
MSLN
THBS1
EPCAM
DCN
GRN
E
cell number (x10 5)
ns
DOX:
siRNA:
Ctrl
IL-8
MSLN
GDF-15
ANGPTL4
PAI

Figure S3. siRNA screen identified secreted pro-invasive factors in MCF10A cells with extra centrosomes. Related to Figure 3 and Table S1. (A) Schematic representation of the siRNA screen set-up. (B) Acini images from 96-well plate siRNA screen. Scale bar 40μm. (C) mRNA levels for all the siRNA conditions to assess depletion efficiency. Red dashed line represents 50% reduction of mRNA levels. (D) Cell number upon siRNA treatment to assess the impact of the different conditions on cell proliferation/viability. Green asterisks depict the positive screen hits shown in Figure 3F. (E) Cell number in cells with extra centrosomes (+DOX) upon siRNA depletion of some of our positive hits. For all graphics error bars represent mean +/- SD from three independent experiments. *$P < 0.05$, ns not significant.

Figure S4

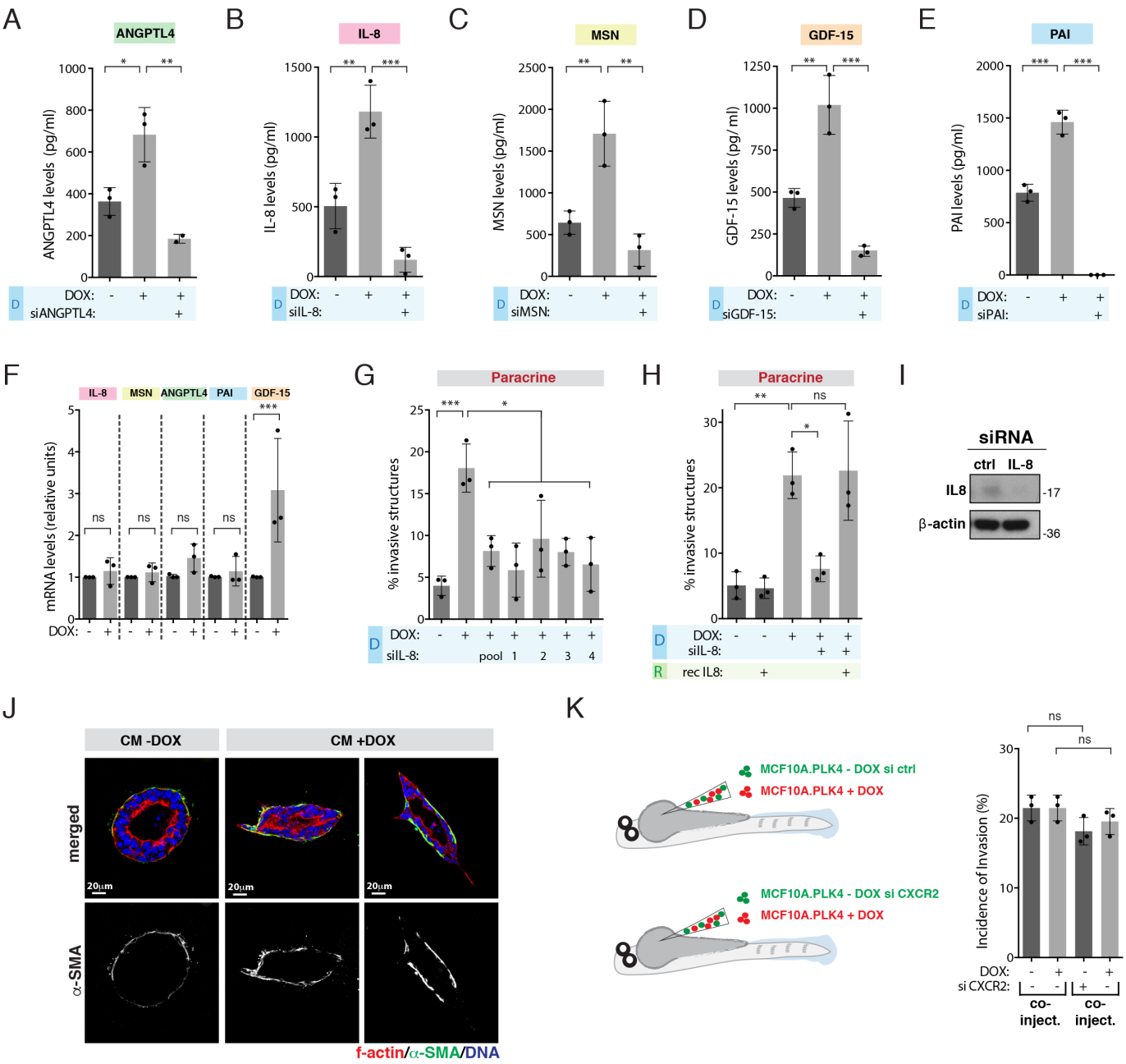

A
ANGPTL4
ANGPTL4 levels (pg/ml)
DOX:
siANGPTL4:
B
IL-8
IL-8 levels (pg/ml)
DOX:
siIL-8:
C
MSN
MSN levels (pg/ml)
DOX:
siMSN:
D
GDF-15
GDF-15 levels (pg/ml)
DOX:
siGDF-15:
E
PAI
PAI levels (pg/ml)
DOX:
siPAI:
F
IL-8
MSN
ANGPTL4
PAI
GDF-15
mRNA levels (relative units)
ns
DOX:
G
Paracrine
% invasive structures
DOX:
siIL-8:
pool 1 2 3 4
H
Paracrine
% invasive structures
DOX:
siIL-8:
rec IL8:
I
siRNA
ctrl IL-8
IL8
β-actin
-17
-36
J
CM -DOX
CM +DOX
merged
α-SMA
20μm
f-actin/α-SMA/DNA
K
MCF10A.PLK4 - DOX si ctrl
MCF10A.PLK4 + DOX
MCF10A.PLK4 - DOX si CXCR2
MCF10A.PLK4 + DOX
Incidence of Invasion (%)
DOX:
si CXCR2:
co-inject.
co-inject.

Figure S4. Validation of the positive hits identified in the siRNA screen. Related to Figure 4. (A) Levels of secreted ANGPTL4. (B) Levels of secreted IL-8. (C) Levels of secreted MSN. (D) Levels of secreted GDF-15. (E) Levels of secreted PAI. (F) mRNA levels of the different pro-invasive factors after induction of centrosome amplification (48 hrs). (G) Quantification of invasive structures in acini incubated with CM collected from cells depleted of IL-8 (siRNA pool and individual sequences). (H) Quantification of invasive structures in acini incubated with CM collected from cells depleted of IL-8 and supplemented with recombinant IL-8 (0.5 μg/ml). (I) IL-8 levels after siRNA depletion. (J) Non-invasive and invasive mammary organoids from WT mice stained for f-actin (phalloidin, red), α-SMA (green) and DNA (blue). Scale bar 20μm. (K) Left, Schematic representation of the zebrafish co-injections. Right, Quantification of the incidence of invasion in the co-injection experiments (-DOX/+DOX) when CXCR2 is depleted in cells with normal centrosomes (-DOX). Number of injected fish co-injection control siRNA=71; co-injection CXCR2 siRNA=121. For all graphics error bars represent mean +/- SD from three independent experiments. *$P < 0.05$, **$P < 0.01$, ***$P < 0.$001, ns not significant.

Figure S5

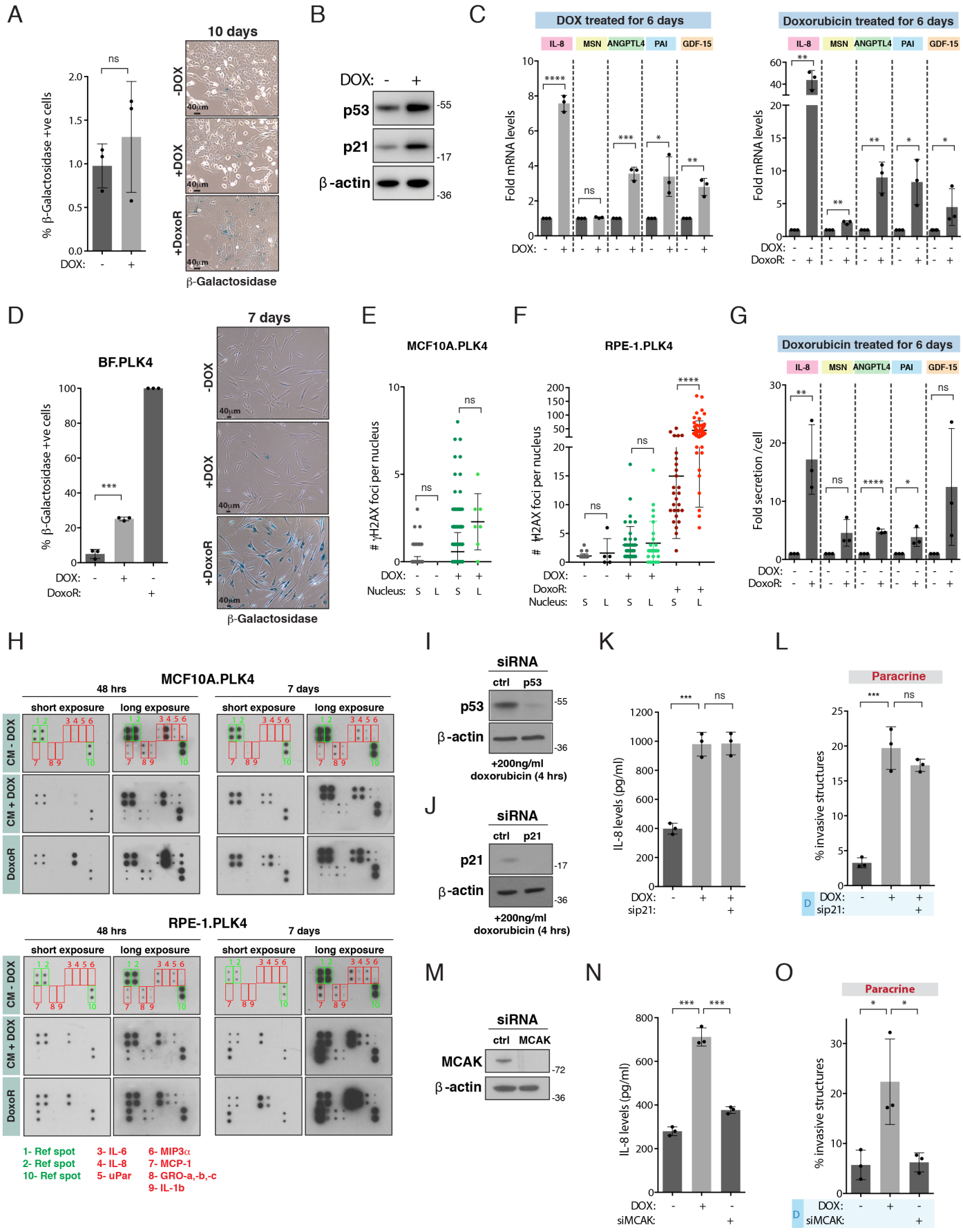

A
10 days
% β-Galactosidase +ve cells
DOX:
-DOX
+DOX
+DoxoR
β-Galactosidase
B
DOX:
p53
p21
β-actin
C
DOX treated for 6 days
Doxorubicin treated for 6 days
IL-8
MSN
ANGPTL4
PAI
GDF-15
Fold mRNA levels
DoxoR:
D
7 days
BF.PLK4
E
MCF10A.PLK4
γH2AX foci per nucleus
Nucleus:
F
RPE-1.PLK4
G
Fold secretion /cell
H
MCF10A.PLK4
48 hrs
7 days
short exposure
long exposure
CM - DOX
CM + DOX
DoxoR
RPE-1.PLK4
1- Ref spot
2- Ref spot
10- Ref spot
3- IL-6
4- IL-8
5- uPar
6- MIP3α
7- MCP-1
8- GRO-a,-b,-c
9- IL-1b
I
siRNA
ctrl
p53
+200ng/ml
doxorubicin (4 hrs)
J
p21
K
IL-8 levels (pg/ml)
sip21:
L
Paracrine
% invasive structures
M
MCAK
N
siMCAK:
O

Figure S5. Secretion of pro-invasive factors in cells with extra centrosomes does not require a full senescence response and is not a consequence of aneuploidy. Related to Figure 5 and Table S1. (A) Left, Quantification of β-galactosidase positive MCF10A.PLK4 cells after induction of centrosome amplification for 10 days. Right, Images representing cells stained for β-galactosidase (blue). Scale bar 40μm. (B) Levels of p53 and p21 after induction of extra centrosomes (48 hrs). (C) mRNA levels of the different pro-invasive factors 6 days after induction of centrosome amplification (Left) or Doxorubicin treatment (Right). (D) Left, Quantification of β-galactosidase positive BF.PLK4 cells 7 days after induction of centrosome amplification or doxorubicin treatment (100 ng/ml). Right, Cells stained for β-galactosidase (blue). Scale bar 40μm. (E) Quantification of γH2AX foci in MCF10A cells with extra centrosomes after 7days. (F) Quantification of γH2AX foci in RPE-1 cells with extra centrosomes after 7days. DoxoR-induced senescent cells were used as positive control. L, large nuclei; S, small nuclei. Data as seen in Fig. 5f. (G) Quantification of secreted pro-invasive factors in senescent cells treated with DoxoR after 6 days. (H) SASP array incubated with CM collected from cells with (CM+DOX) and without (CM-DOX) extra centrosomes. DoxoR-induced senescent cells were used as positive control. (I) Levels of p53 after p53 siRNA. (J) Levels of p21 after p21 siRNA. (K) Levels of secreted IL-8 in cells depleted of p21. (L) Quantification of invasive structures in acini incubated with CM collected from cells depleted of p21. (M) Levels of MCAK after MCAK siRNA depletion. (N) Levels of secreted IL-8 in cells depleted of MCAK. (O) Quantification of invasive structures in acini incubated with CM collected from cells depleted of MCAK. For all graphics error bars represent mean +/- SD from three independent experiments. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$, ns not significant.

Figure S6

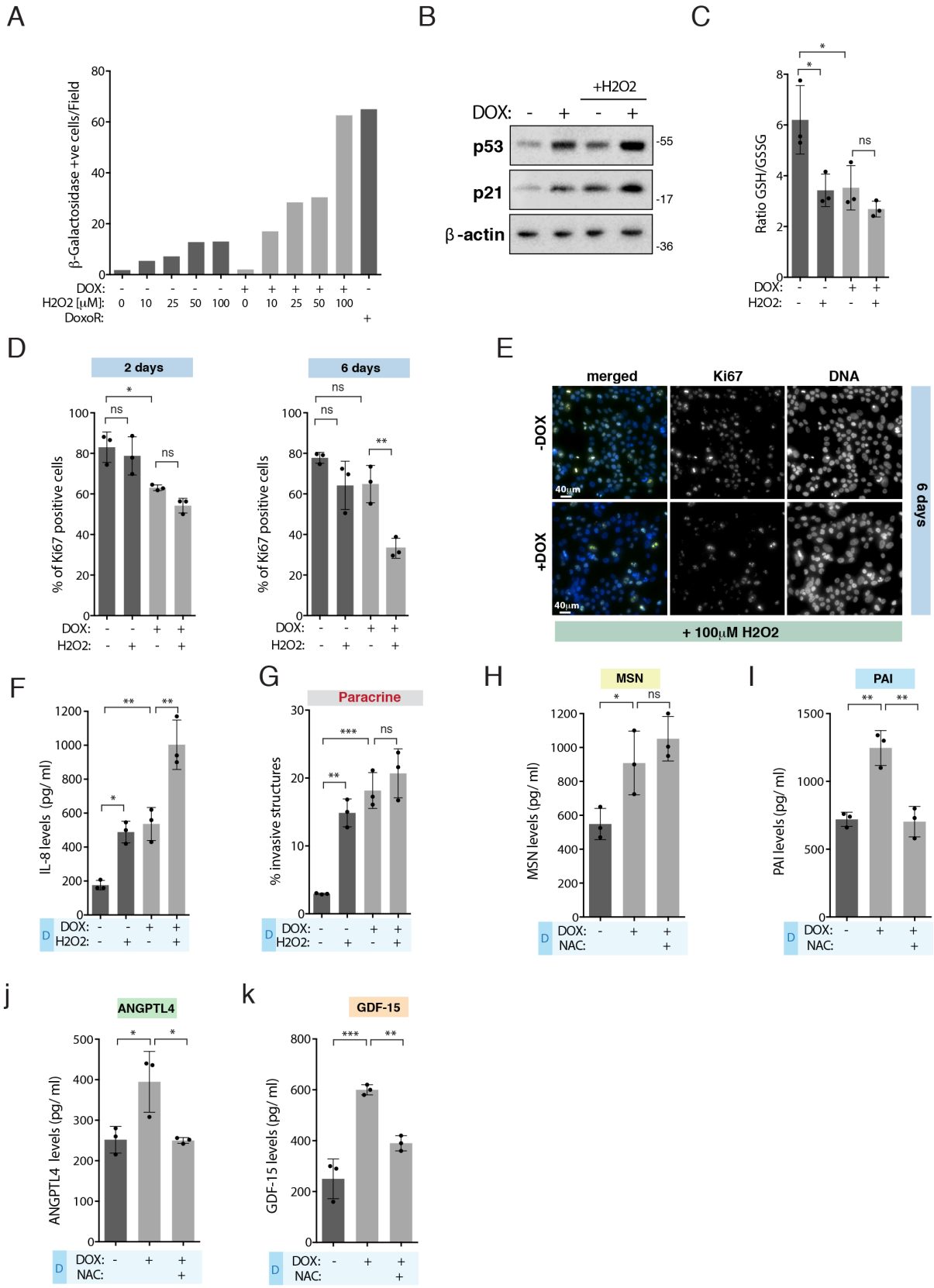

A
β-Galactosidase +ve cells/Field
DOX:
H2O2 [μM]: 0 10 25 50 100 0 10 25 50 100
DoxoR:
B
+H2O2
DOX: - + - +
p53
p21
β-actin
-55
-17
-36
C
Ratio GSH/GSSG
DOX:
H2O2:
ns
D
2 days
6 days
% of Ki67 positive cells
ns
E
merged
Ki67
DNA
-DOX
+DOX
40μm
6 days
+ 100μM H2O2
F
IL-8 levels (pg/ ml)
G
Paracrine
% invasive structures
H
MSN
MSN levels (pg/ ml)
I
PAI
PAI levels (pg/ ml)
NAC:
j
ANGPTL4
ANGPTL4 levels (pg/ ml)
k
GDF-15
GDF-15 levels (pg/ ml)

Figure S6. Increased ROS levels in cells with extra centrosomes promote secretion and paracrine invasion. Related to Figure 6 and Table S1. (A) Quantification of β-galactosidase positive MCF10A.PLK4 cells after 6 days, before and after 48 hrs treatment with DOX and treated with different doses of H2O2 for 48 hrs. (B) Levels of p53 and p21 in cells with (+DOX) and without (-DOX) extra centrosomes treated with H2O2 (100 μM). (C) Ratio of GSH/GSSG in cells after induction of extra centrosomes (48 hrs) and H2O2 (100 μM) treatment. (D) Quantification of Ki67 positive cells 2 and 6 days after induction of centrosome amplification and H2O2 (100 μM) treatment. (E) Cells stained for Ki67 (green) and DNA (blue). Scale bar 40μm. (F) Levels of secreted IL-8 in cells with (+DOX) and without (-DOX) extra centrosomes treated with H2O2 (100 μM, 48 hrs). (G) Quantification of invasive structures in acini incubated with CM collected from cells with (+DOX) and without (-DOX) extra centrosomes treated with H2O2 (100 μM). (H) Levels of secreted MSN after NAC treatment (5 mM, 48 hrs). (I) Levels of secreted PAI after NAC treatment (5 mM, 48 hrs). (J) Levels of secreted ANGPTL4 after NAC treatment (5 mM, 48 hrs). (K) Levels of secreted GDF-15 after NAC treatment (5 mM, 48 hrs). For all graphics error bars represent mean +/- SD from three independent experiments. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ns not significant.

Figure S7

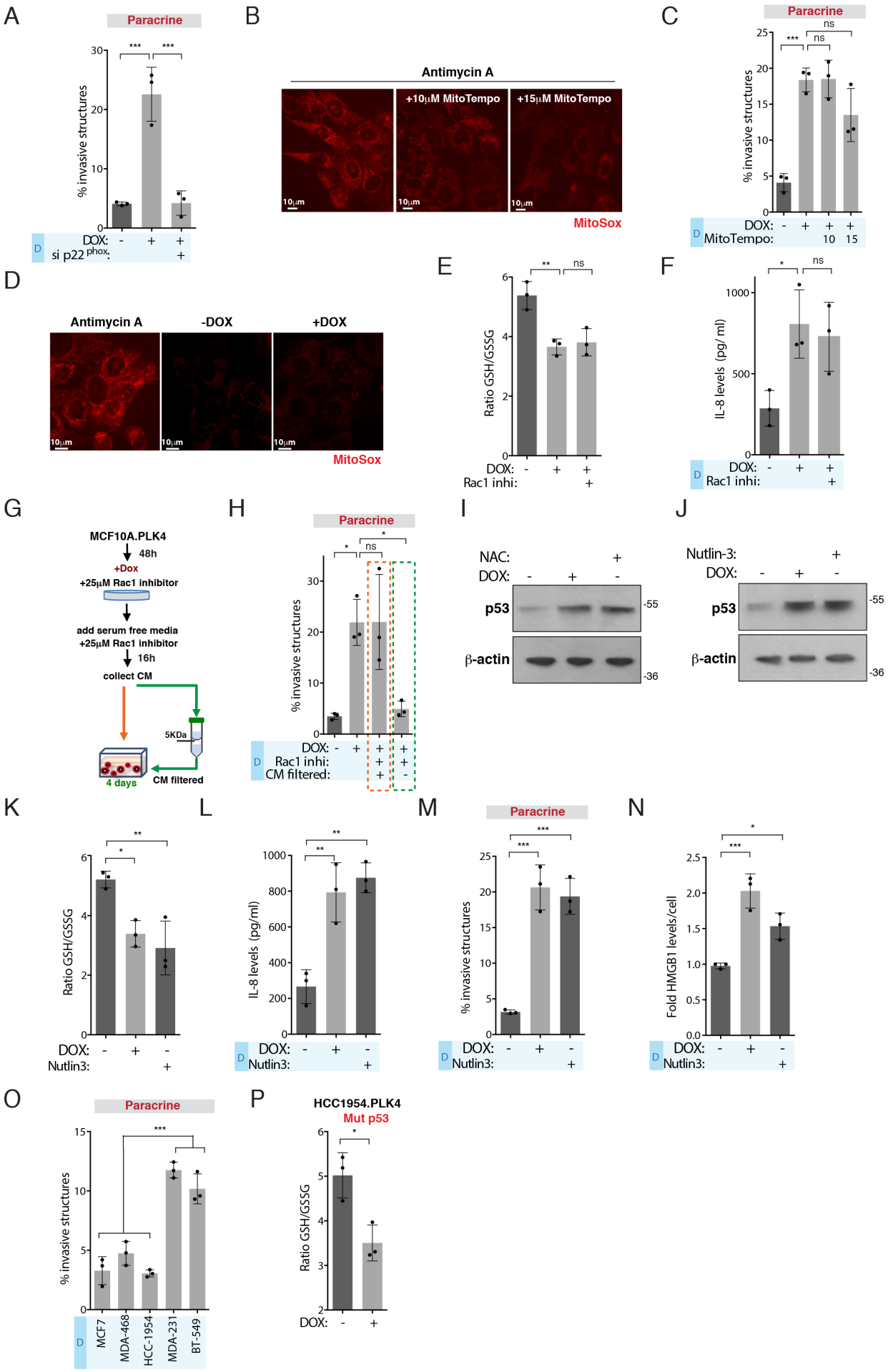

A
Paracrine
% invasive structures
DOX:
si p22 phox:
B
Antimycin A
+10μM MitoTempo
+15μM MitoTempo
MitoSox
C
Paracrine
% invasive structures
DOX:
MitoTempo:
D
Antimycin A
-DOX
+DOX
MitoSox
E
Ratio GSH/GSSG
DOX:
Rac1 inhi:
F
IL-8 levels (pg/ ml)
DOX:
Rac1 inhi:
G
MCF10A.PLK4
48h
+Dox
+25μM Rac1 inhibitor
add serum free media
+25μM Rac1 inhibitor
16h
collect CM
5KDa
4 days
CM filtered
H
Paracrine
% invasive structures
DOX:
Rac1 inhi:
CM filtered:
I
NAC:
DOX:
p53
β-actin
-55
-36
J
Nutlin-3:
DOX:
p53
β-actin
-55
-36
K
Ratio GSH/GSSG
DOX:
Nutlin3:
L
IL-8 levels (pg/ml)
DOX:
Nutlin3:
M
Paracrine
% invasive structures
DOX:
Nutlin3:
N
Fold HMGB1 levels/cell
DOX:
Nutlin3:
O
Paracrine
% invasive structures
MCF7
MDA-468
HCC-1954
MDA-231
BT-549
P
HCC1954.PLK4
Mut p53
Ratio GSH/GSSG
DOX:

Figure S7. p53-mediated ROS production promotes secretion and paracrine invasion in cells with extra centrosomes. Related to Figure 7 and Table S1. (A) Quantification of invasive structures in acini incubated with CM collected from cells depleted of $p22^{phox}$. (B) Cells treated with Antimycin A (35 μM) to increase mitochondrial ROS and stained with fluorogenic dye Mitosox (red). MitoTempo was used to inhibit mitochondrial ROS (10 and 15 μM, 48 hrs). Scale bar 10μm. (C) Quantification of invasive structures in acini incubated with CM collected from cells with extra centrosomes treated with MitoTempo (10 and 15 μM, 48 hrs). (D) Cells were stained with MitoSox (red). Scale bar 10μm. (E) Ratio of GSH/GSSG in cells after induction of extra centrosomes (48 hrs) and treated with RAC1 inhibitor (NSC23766, 25 μM, 48 hrs). (F) Levels of secreted IL-8 in cells with (+DOX) and without (-DOX) extra centrosomes treated with RAC1 inhibitor (25 μM, 48 hrs). (G) Schematic representation of the experimental setting to remove RAC1 inhibitor from the CM before adding on 3D cultures. (H) Quantification of invasive structures in acini incubated with CM collected from cells treated with RAC1 inhibitor (25 μM) before and after removal of the drug (CM filtered). (I) p53 levels in cells with extra centrosomes (+DOX) and treated with NAC (5 mM, 48 hrs). (J) Western blot analysis of p53 levels in cells treated with Nutlin-3 (5 mM, 48 hrs). (K) Ratio of GSH/GSSG in cells treated with Nutlin-3 (5 mM, 48 hrs). (L) Levels of secreted IL-8 in cells treated with Nutlin-3 (5 mM, 48 hrs). (M) Quantification of invasive structures in acini incubated with CM collected from cells treated Nutlin-3 (5 mM, 48 hrs). (N) Levels of secreted HMGB1 in cells treated with Nutlin-3 (5 mM, 48 hrs). (O) Quantification of invasive structures in acini incubated with CM collected from breast cancer cell lines with low and high centrosome amplification. (P) Ratio of GSH/GSSG in HCC1954.PLK4 cells after induction of extra centrosomes (+DOX) for 48 hrs. For all graphics error bars represent mean +/- SD from three independent experiments. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, ns not significant.

Table S1: Quantification of the percentage of extra centrosomes in the different conditions and cell lines. Related to Figures 1, 3, 5, 6, 7, S1, S3, S5, S6 and S7.

Cell line	Treatment(s)	% Centrosome amplification
MCF10A.PLK4	n/a*	12
MCF10A.PLK4	DOX	87
MCF10A.PLK4-608	DOX	11
MCF10A.PLK4	after DOX + siRNA ctr	42
MCF10A.PLK4	after DOX + siRNA SAS-6	18
HaCat.PLK4	n/a*	21
HaCaT.PLK4	DOX	73
MCF10A	RO-3306	58
MCF10A.PLK4	DOX + siRNA IL-8	66
MCF10A.PLK4	DOX + siRNA ANGPTL4	78
MCF10A.PLK4	DOX + siRNA GDF15	65
MCF10A.PLK4	DOX + siRNA PAI	71
MCF10A.PLK4	DOX + siRNA MSN	61
MCF10A.PLK4	DOX + siRNA p53	64
MCF10A.PLK4	DOX + siRNA p21	65
MCF10A.PLK4	DOX 6 days	39
MCF10A.PLK4	DOX + NAC	87
MCF10A.PLK4	DOX + Apocynin	93
MCF10A	Nutlin-3	8
MCF10A.PLK4	DOX + Rac inhibitor	82
MCF10A.PLK4	H2O2	8
MCF10A.PLK4	DOX + H2O2	46
MCF7. PLk4	n/a*	18
MCF7. PLk4	DOX	57
HCC1954.PLK4	n/a*	16
HCC1954. PLK4	DOX	82
BF.PLK4	n/a*	1
BF.PLK4	DOX	44
RPE.PLK4	n/a*	15
RPE.PLK4	DOX	80

* n/a: not applicable, no treatment performed

Supplementary Table 6: References for all siRNAs used. Related to Figures 3 and S3.

Gene symbol	Reference	Company
CXCL8	M-004756-00	Dharmacon
CCL20	M-007832-01	Dharmacon
AREG	M-017435-00	Dharmacon
LGALS3	M-010606-02	Dharmacon
PLAU	M-006000-02	Dharmacon
ANGPTL4	M-007807-02	Dharmacon
MSLN	M-006346-02	Dharmacon
THBS1	M-019743-01	Dharmacon
EPCAM	M-004568-03	Dharmacon
DCN	M-021491-00	Dharmacon
GRN	M-009285-02	Dharmacon
SYNE2	D-019259-01	Dharmacon
CBR3	L-008597-00	Dharmacon
LDLR	L-011073-00	Dharmacon
PPA2	L-012348-00	Dharmacon
C1QBP	L-011225-01	Dharmacon
ACTN4	L-011988-00	Dharmacon
CLIC4	L-013553-00	Dharmacon
STIP1	L-019802-00	Dharmacon
PPP1CC	L-006827-00	Dharmacon
LCP1	L-011716-00	Dharmacon
PDAP1	L-017675-00	Dharmacon
GSTP1	L-011179-00	Dharmacon
TPP1	L-005810-00	Dharmacon
GCSH	L-017907-00	Dharmacon
S100A7	L-011769-02	Dharmacon
CTSC	L-005835-00	Dharmacon
NAMPT	L-004581-00	Dharmacon
RBM3	L-018969-01	Dharmacon
PROCR	L-017326-00	Dharmacon
IQGAP1	L-004694-00	Dharmacon
GDF15	L-019875-00	Dharmacon
ENO2	L-009777-00	Dharmacon
AZGP1	L-012567-01	Dharmacon
SERPINE1	L-019376-01	Dharmacon
DDT	L-012201-01	Dharmacon
LGMN	L-005924-00	Dharmacon
AXL	L-003104-00	Dharmacon
CEP192	L-032250-01	Dharmacon
P21	L-003471-00	Dharmacon
SAS6	L-019156-01	Dharmacon
P53	L-003329-00	Dharmacon
CXCR2	L-005647-00	Dharmacon
CYBA	L-011020-02	Dharmacon
CONTROL	1027310	Qiagen

Supplementary Table 7: Primer sequences used in the RT-qPCR. Related to Figures S3, S4 and S5.

Gene symbol	Primer Forward 5'-3'	Primer Reverse 5'-3'
GAPDH	TTAAAAGCAGCCCTGGTGAC	CTCTGCTCCTCCTGTTCGAC
CXCL8	GTTTTTGAAGAGGGCTGAG	TTTGCTTGAAGTTTCACTGG
CCL20	TATATTGTGCGTCTCCTCAG	GCTATGTCCAATTCCATTCC
AREG	AAAGAAAGAAAAAGGGAGGC	CATTTGCATGTTACTGCTTC
LGALS3	AGATTTCCAAAGAGGGAATG	AAGTGCAAACAATGACTCTC
PLAU	GAAAACCTCATCCTACACAAG	ATTCTCTTTTCCAAAGCCAG
ANGPTL4	AGGCAGAGTGGACTATTTG	CCTCCATCTGAGGTCATC
MSLN	GACGTCCTAAAGCATAAACTG	TCATCTTGAGGAAGAGGTAG
THBS1	GTGACTGAAGAGAACAAAGAG	CAGCTATCAACAGTCCATTC
EPCAM	GTCTGAGAAGGCTGAGATAAAG	CTTCAAAGATGTCTTCGTCC
DCN	TTCACGCATTGATTCTTGTC	GCTGATTCTTGGACAGATAAAG
GRN	GACCTGATCCAGAGTAAGTG	CATGTCACATTTCACATCCC
SYNE2	GAGAAGATAGAAGAAGCACTC	TCTTATAGGTTTTCTGCTGC
CBR3	AGTGAGGTGCTAGTTTCCAAGG	CTGCACATTCTTGTGGACCC
LDLR	GAGGACAAAGTATTTTGGACAG	GTAGGTTTTCAGCCAACAAG
PPA2	CTGGAAGCTACTCTTAATTGG	GCCTTGTTTTTGAATTCTCC
C1QBP	AACATTAACAACAGCATCCC	TCATCCTCTGGATAATGACAG
ACTN4	AGTATGACAAGCTGAGGAAG	CTGAAAAGGCATGGTAGAAG
CLIC4	CCCAGAATCAAATCATGCTG	TCAATTTCATCAGGGAGAGG
STIP1	AACGAGTGTTTTCAGAAAGG	TATAACCCTTGATGAAGGTCG
PPP1CC	GAGGTTTATCACCAGATCTTC	CAGCCTAAGACATGTTTATCG
LCP1	AAGCTCTGATTGCTCTTTTG	GAAGTTGCCAATTTTGTTGC
PDAP1	AAGATGACTACCAGCAAAAG	CTGCTTCTCAATCTCTTCTC
GSTP1	GCAAATACATCTCCCTCATC	GTCTCAAAAGGCTTCAGTTG
TPP1	AATAACCTGACCCTAGAGAATG	CCACATAGTGATGAAACTCAG
GCSH	TTGTTATGAAGATGGTTGGC	TCTAAGTCTTCTATCCACCAC
S100A7	TTAGTGCCTGTGACAAAAAG	GTAGTCTGTGGCTATGTCTC
CTSC	AACAAACTGGCCATGAAC	ATAAAGACTCCAGAAGGGAC
NAMPT	CTAATGGCCTTGGGATTAAC	TCCAGTGTAACAAAATTCCC
RBM3	AGTGGCAGGTATTATGACAG	TCTGCCATTATAGTCTCTGG
PROCR	CATATGAAGTCTTTGGAGGC	CATATGGAAGTCTTTGGAGGC
IQGAP1	ATGCCTTTGACATCATTGAC	TCTAGGTTTCTGGTAGGACTG
GDF15	CGAAGACTCCAGATTCCG	ACTTCTGGCGTGAGTATC
ENO2	ATGTCCATAGAGAAGATCTGG	GACACCTTTGCCTAAGTAAC
AZGP1	ACAGAAATCACAGTCAATGG	TCCAAGTCTACTCAAGACAG
SERPINE1	ATCCACAGCTGTCATAGTC	CACTTGGCCCATGAAAAG
DDT	CCCACTTCTTTGAGTTTCTC	ATCTCTCTGGAAGAAGCAG
LGMN	ACTATGATGAGAAGAGGTCC	GGTGGAGATTGTTTTGTTTC
AXL	CATGAAACATGGAGACCTAC	ATCTCTTGGTACTCAGATACTC