Supplemental figure legends

Figure S1: Prolonged mitotic arrest causes G1 arrest irrespective of whether or not chromosome mis-segregation occurred during the prolonged mitosis (Related to Figure 1).
(A) Experimental set up to examine the consequences of monastrol treatment on cell cycle progression.

(B) Representative images of RPE1 cells co-expressing PCNA::GFP and RFP::H2B were acquired with a Yokagawa CV1000 confocal microscope using a 20x objective. Images shown are of DMSO- and monastrol-treated mother cell anaphases and the following G1, S, and G2 for one daughter cell. In this experiment unsynchronized cells were treated with DMSO or 100 μM monastrol and immediately filmed every 10 min for 6 hrs to capture mitotic entry. The plate was then removed, monastrol washed out, and the plate replaced. Cells were then immediately filmed every 10 min for 2 hrs to capture mitotic exit and then filmed every 20 min for 42 hrs to capture daughter cell cell cycle progression. In this way cell cycle progression in trios of mother and daughter cells was followed to correlate mother cell mitotic timing and mis-segregation with daughter cell cell cycle progression.

(C) Daughter cell fate in monastrol-treated RPE1 cells co-expressing PCNA::GFP and RFP::H2B. Each bar represents a measurement from individual daughter cells. Height of the bar represents mother cell mitotic timing from NEBD to anaphase, and color indicates arrest (blue) or division (grey). Red asterisks indicate chromosome mis-segregation in the mother cell as measured by lagging chromosomes and/or micronucleus formation. Daughter cells arrest when mother cells spent more than ~116 min in mitosis, as indicated by the horizontal dashed black line. Data are combined from 3 replicate experiments. Note, mis-segregation is rare in monastrol-treated cells with short mitoses (<100min). Thus, to obtain sufficient mis-segregation events in this group of mother cells, a significant number of cells with short mitoses was imaged but the analysis enriched for cells with a visible mis-segregation event. Thus the frequency of mis-segregation in cells with short mitosis is artificially inflated in this graph.

(D) Daughter cell fate is presented in four categories to determine its correlation with mother cell mitotic timing or mother cell chromosome mis-segregation. The categories include daughter cells with no mother cell mis-segregation and short mitosis (< 116 min as determined in Fig. S1C), mother cell mis-segregation and short mitosis, no mother cell mis-segregation and long mitosis (> 116 min), and mother cell mis-segregation and long mitosis. Cell fate is presented as % G1 arrest per category, as indicated by no G1 arrest (grey) and G1 arrest (blue.) Data were combined from 3 replicate experiments.

Figure S2: Inhibition of Mps1 causes chromosome mis-segregation (Related to Figure 1).

(A) Mitotic duration in DMSO and NMS-P715 (1μM) treated hTERT RPE-1 cells co-expressing PCNA::GFP and RFP::H2B. Cells were immediately filmed after drug addition with a Yokagawa CV7000 spinning disk confocal microscope using a 20x objective. Images were acquired every 10 min for 8 hrs to capture mother cell mitoses and then every 15 min for 2 days to capture daughter cell fate. Mitotic duration (nuclear envelope break-down [NEBD] to anaphase onset; mean +/- S.D.) of 100 mother cells is plotted per condition and indicates accelerated mitosis in the presence of NMS-P715.

(B) Representative anaphase images of DMSO-treated and NMS-P715-treated hTERT RPE-1 cells co-expressing PCNA::GFP and RFP::H2B.

(C) Representative images of hTERT RPE-1 cells co-expressing PCNA::GFP and RFP::H2B were acquired on a Yokagawa CQ1 confocal microscope using a 40x objective. Unsynchronized cells were treated with DMSO, 0.5 μM, or 2 μM reversine and then immediately filmed for 48h. Cells were filmed every 5 min for 6 hrs to capture mitotic mis-segregation events and then every 20 min for 42 hrs to capture daughter cell S phase duration (estimated as the time between PCNA focus appearance and disappearance). Representative images are shown for DMSO- and reversine-treated mother cell anaphases and G1, S, and G2 of one of their daughter cells.

(D - F) RPE1 cells were treated with DMSO or 1 μM NMS-P715 for 24 hrs, and then fixed and stained with Hoechst and centromeric FISH probes for chromosome 6 (red; D) or chromosome 11 (green; E). Nuclei were imaged on a Deltavision microscope with a 40x objective. The number of foci per nucleus was counted. Bars represent the percentage of cells with that number of chromosomes. Mis-segregation is reported as percent of cells with greater or less than 2 FISH signals. Representative images are shown in (F).

Figure S3: Chromosome mis-segregation causes DNA damage (Related to Figure 2).

(A) RPE-1 cells were synchronized at the G1/S transition by thymidine treatment. After thymidine wash-out, cells were treated with 0.5 μM reversine or DMSO (vehicle control). Time points were taken 15, 18, 21, 24 hours after release from the thymidine block (which corresponds to about 3, 6, 9, 12 hours after mitosis). Levels of γ-H2AX were determined by Western blot analysis at the indicated time points after mitosis. Treatment with doxorubicin (100 ng/ml, 24 hours) was performed as a positive control for DNA damage.

(B) Schematic representation of experimental setup used to generate aneuploid cells. RPE-1 cells were synchronized at the G1/S transition by thymidine treatment for 24 hours. Six hours after thymidine release, cells were treated with control vehicle or 0.5 μM reversine for 12 hours allowing for the progression of cells trough mitosis in the presence of an Mps1 inhibitor (or control vehicle).

(C) RPE-1 cells were synchronized at the G1/S transition by thymidine treatment. After thymidine wash-out, cells were treated with 0.5 μM reversine or DMSO (vehicle control). Time points were taken 15, 18, 21, 24 hours after release from the thymidine block (which corresponds to about 3, 6, 9, 12 hours after mitosis). Representative images of cells harboring γ-H2AX foci are shown. CREST serum was used to identify centromeric regions. γ-H2AX in green, CREST in red, DNA in blue.

(D) Representative images of RPE-1 cells scored as EdU positive (red star) or negative (green star). EdU in red, DNA in blue.

Figure S4: Chromosome mis-segregation causes genomic instability (Related to Figure 4).
(A) RPE-1 cells stably expressing H2B-GFP were synchronized in G0 by serum starvation for 48 hours. Eighteen hours after serum addition cells were treated with control vehicle (euploid) or 0.5 μM reversine (aneuploid) for 12 hours to induce chromosome mis-segregation. Reversine was then washed out and cells were filmed (5' frames). The percentage of cells with lagging chromosomes or micronuclei during the mitosis following reversine wash out was determined (mean ± SEM).
(B) Asynchronous cells were exposed to control vehicle (euploid) or 0.5 μM reversine (aneuploid) for 24 hours. Reversine was then washed out and cells were filmed (5' frames). The percentage of cells with lagging chromosomes or micronuclei during the mitosis following reversine wash out was determined (mean ± SEM).

Figure S5: Chromosome mis-segregation leads to the generation of cells with complex karyotypes (Related to Figure 5).
Schematic representation of the experimental method used for karyotype determination after the first and second cell cycle following chromosome mis-segregation. RPE-1 cells were synchronized at the G1/S transition by thymidine treatment. Six hours after Thymidine release, cells were treated with DMSO or 0.5 μM reversine for 12 hours. To determine the karyotype of the resulting aneuploid cells, cells were treated with the CDK1 inhibitor RO-3306 for 12 hours, 12 hours after drug wash-out. Cells were then released into fresh medium containing colchicine and metaphase spreads were prepared ("First cell cycle" in (A)). To analyze the karyotype of aneuploid cells in the subsequent cell cycle, aneuploid cells generated by reversine treatment (as described above) were placed into fresh medium for 30 hours after drug wash-out. Then, cells were exposed to RO-3306 for 12 hours, released into fresh medium containing colchicine and metaphase spreads were prepared ("Second cell cycle" in (B)).

Figure S6: Arrested cells with complex karyotypes exhibit a senescence gene expression signature (Related to Figure 6).
(A) Schematic representation of experimental method used to isolate cells that arrest due to complex karyotypes for RNA-seq analysis. RPE-1 cells were synchronized at the G1/S transition by thymidine treatment. Six hours after thymidine release, cells were treated with DMSO or 0.5 μM reversine for 12 hours. After drug wash-out, cells were

placed into fresh medium and either harvested 66 hours later (euploid and aneuploid cycling cells) or exposed to nocodazole. 12 hours later, mitotic cells were removed by shake-off and the remaining cells were placed into fresh medium containing nocodazole. This procedure was repeated three times to remove all dividing cells and to enrich for cells that had ceased to divide.

(B, C) Heat map of differentially expressed genes (absolute $\log_2$ fold change > 1, adjusted p-value < 0.05) in euploid (euploid), aneuploid cycling (cycling) and arrested cells with complex karyotypes (arrested). Expression of 358 genes is elevated in euploid cells relative to arrested cells with complex karyotypes, Expression of 192 genes is elevated in arrested cells with complex karyotypes relative to euploid cells (B). Canonical pathway gene set (c2cp of msigdb) enrichment for down-regulated genes is shown for arrested cells with complex karyotypes relative to euploid ones (C). The graph shows normalized enrichment score for the top 7 down-regulated gene sets. p values indicate the significance of this enrichment.

(D) Gene-set enrichment analysis (GSEA) of euploid and arrested cells with complex karyotypes for the custom gene list “SASP”. The SASP gene set is significantly enriched in arrested cells with complex karyotypes relative to euploid cells with a normalized enrichment score of 1.88.

(E) Gene-set enrichment analysis (GSEA) of wild-type MEFs and MEFs trisomic for chromosome 1, 13, 16, 19 for the custom gene list “SASP”. The SASP gene set is significantly enriched in trisomic MEFs relative to wild type with a normalized enrichment score of 1.5.

(F) MEFs, either wild type or trisomic for chromosome 13 or 19, were fixed at passage 2 (trisomy 13 and 19) or passage 6 (trisomy 13) and stained for γ–H2AX to determine the extent of DNA damage in cells. The graph shows number of γH2AX foci per cell. Cells treated with Doxorubicin provide a positive control for DNA damage. Graph shows mean ± SEM.

(G) Gene-set enrichment analysis (GSEA) of euploid cells and arrested cells with complex karyotypes for the custom gene list “STING_ISG” (Stimulator of interferon genes/Interferon stimulated genes). The STING_ISG gene set is significantly enriched in arrested cells with complex karyotypes relative to euploid cells with a normalized enrichment score of 1.5.

Figure S7: Arrested cells with complex karyotypes show increased expression of Nectin-2/CD112 and ULBP1 (Related to Figure 7).
(A) Gating to obtain cell population of the same size (dashed rectangle) is shown for MICA/B staining analysis.

(B, C) ULBP1 cell surface levels in euploid cells and arrested cells with complex karyotypes (obtained as described in Figure 6B). Doxorubicin (100 ng/ml, 48 hours) treated cells were used as positive control. Graph (B) shows fluorescence intensity of cells gated as shown in (C).
(D) Gating to obtain cell population of the same size (dashed rectangle) are shown for ULBP2 staining analysis.
(E, F) Nectin-2/CD112 cell surface levels in euploid cells and arrested cells with complex karyotypes (obtained as described in Figure 6B). Doxorubicin (100 ng/ml, 48 hours) treated cells were used as positive control. Graph (E) shows fluorescence intensity of cells gated as shown in (F).
(G) Gating to obtain cell population of the same size (dashed rectangle) are shown for CD155/PVR staining analysis.
(H) $10^{\wedge 4}$ aneuploid cells with complex karyotypes were seeded in a 12 well plate and allowed to attach for 12 hours. Cell number was then determined by cell counting at the indicated times after gentle shake-off, as performed when aneuploidy cells were co-cultured with NK92 cells. Graph shows cell number ($*10^{\wedge 4}$).

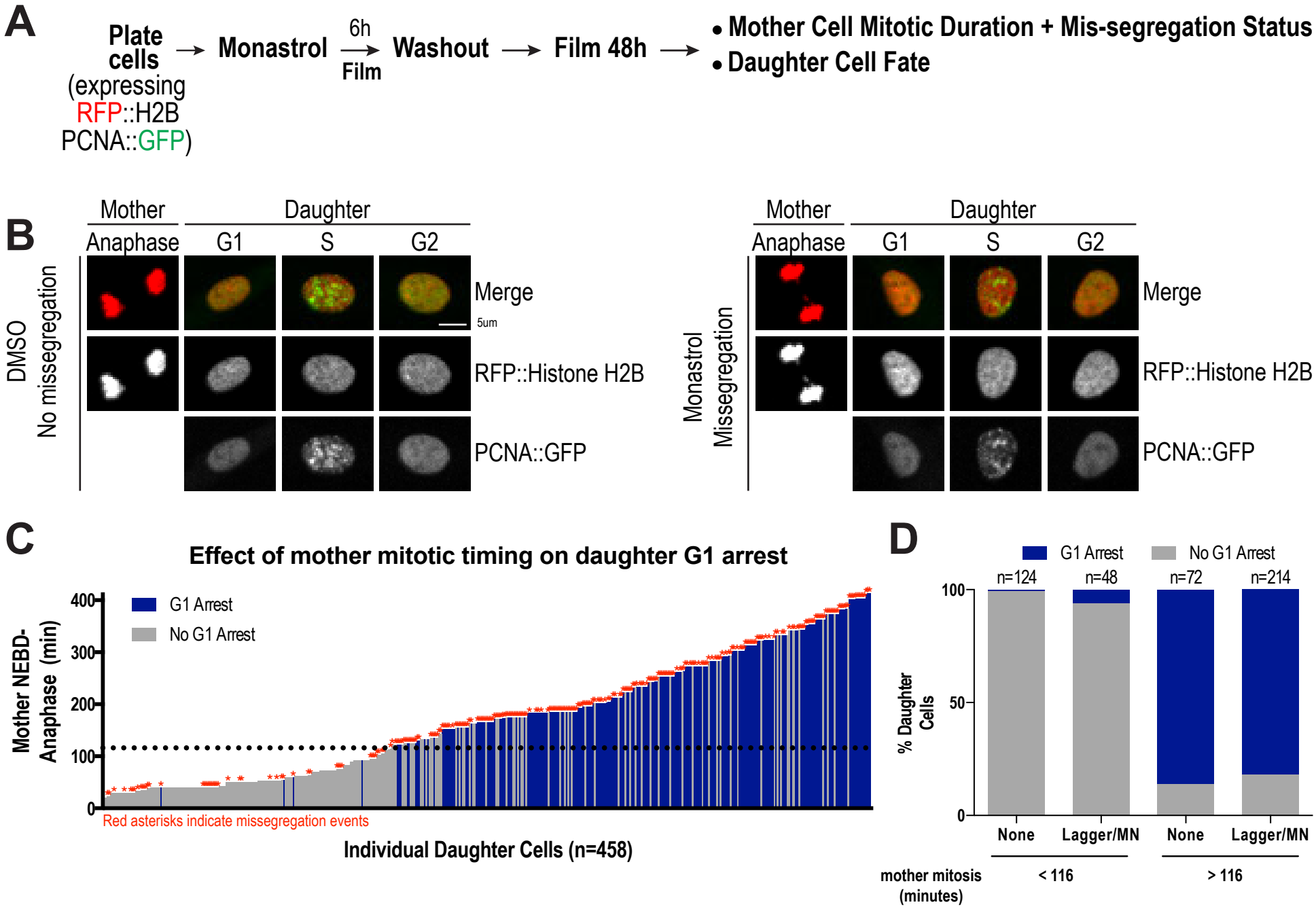

A
Plate cells (expressing RFP::H2B PCNA::GFP)
Monastrol
6h
Film
Washout
Film 48h
• Mother Cell Mitotic Duration + Mis-segregation Status
• Daughter Cell Fate
B
Mother
Daughter
Anaphase
G1
S
G2
DMSO
No missegregation
Merge
5um
RFP::Histone H2B
PCNA::GFP
Monastrol
Missegregation
C
Effect of mother mitotic timing on daughter G1 arrest
Mother NEBD-Anaphase (min)
G1 Arrest
No G1 Arrest
Red asterisks indicate missegregation events
Individual Daughter Cells (n=458)
D
G1 Arrest
No G1 Arrest
n=124
n=48
n=72
n=214
% Daughter Cells
None
Lagger/MN
None
Lagger/MN
mother mitosis (minutes)
< 116
> 116

Figure S1

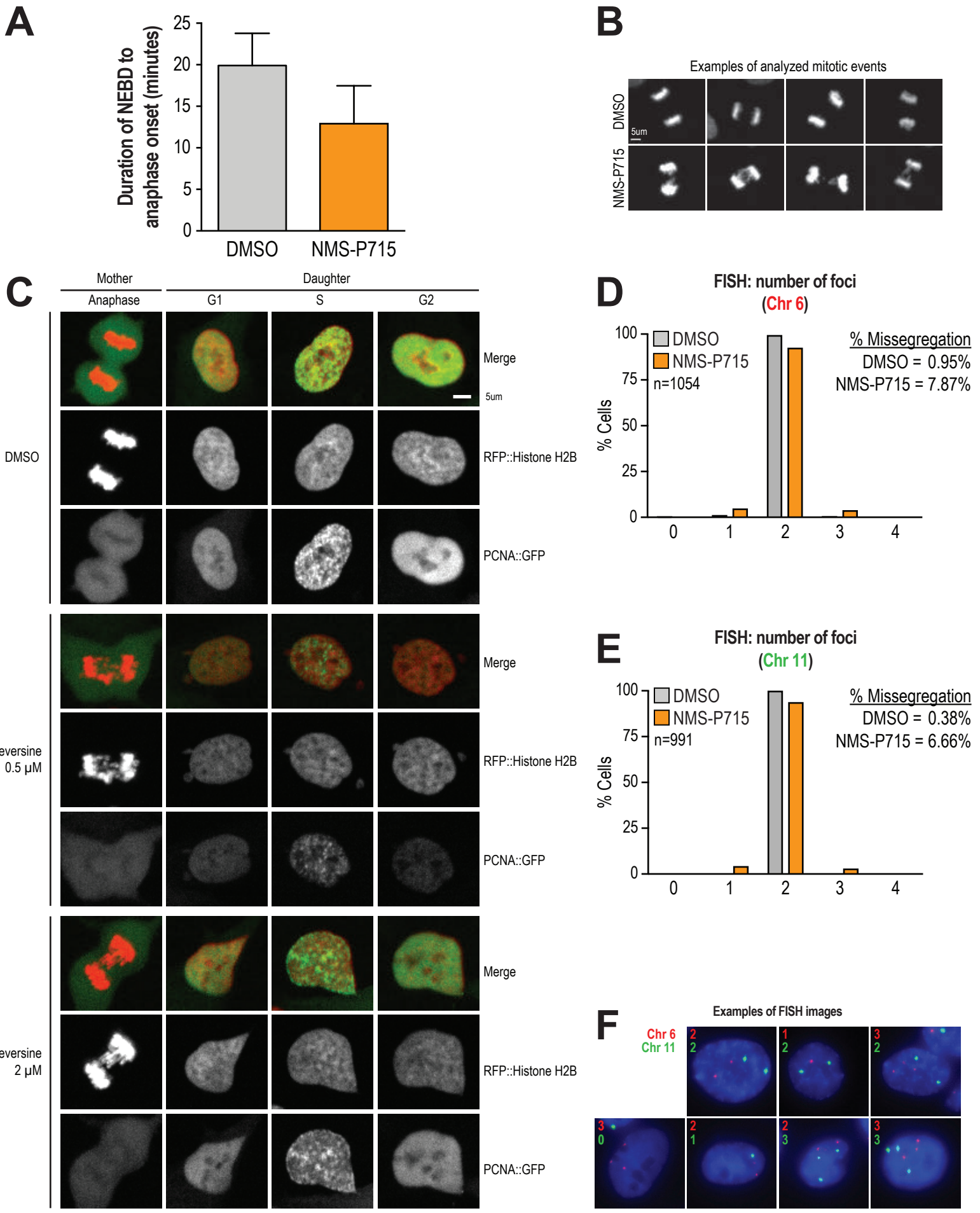

A
Duration of NEBD to anaphase onset (minutes)
25
20
15
10
5
0
DMSO
NMS-P715
B
Examples of analyzed mitotic events
DMSO
NMS-P715
5um
C
Mother
Daughter
Anaphase
G1
S
G2
Merge
5um
DMSO
RFP::Histone H2B
PCNA::GFP
Reversine 0.5 µM
Merge
RFP::Histone H2B
PCNA::GFP
Reversine 2 µM
Merge
RFP::Histone H2B
PCNA::GFP
D
FISH: number of foci
(Chr 6)
100
75
50
25
0
% Cells
DMSO
NMS-P715
n=1054
% Missegregation
DMSO = 0.95%
NMS-P715 = 7.87%
0
1
2
3
4
E
FISH: number of foci
(Chr 11)
100
75
50
25
0
% Cells
DMSO
NMS-P715
n=991
% Missegregation
DMSO = 0.38%
NMS-P715 = 6.66%
0
1
2
3
4
F
Examples of FISH images
Chr 6
Chr 11
2 2
1 2
3 2
3 0
2 1
2 3
3 3

Figure S2

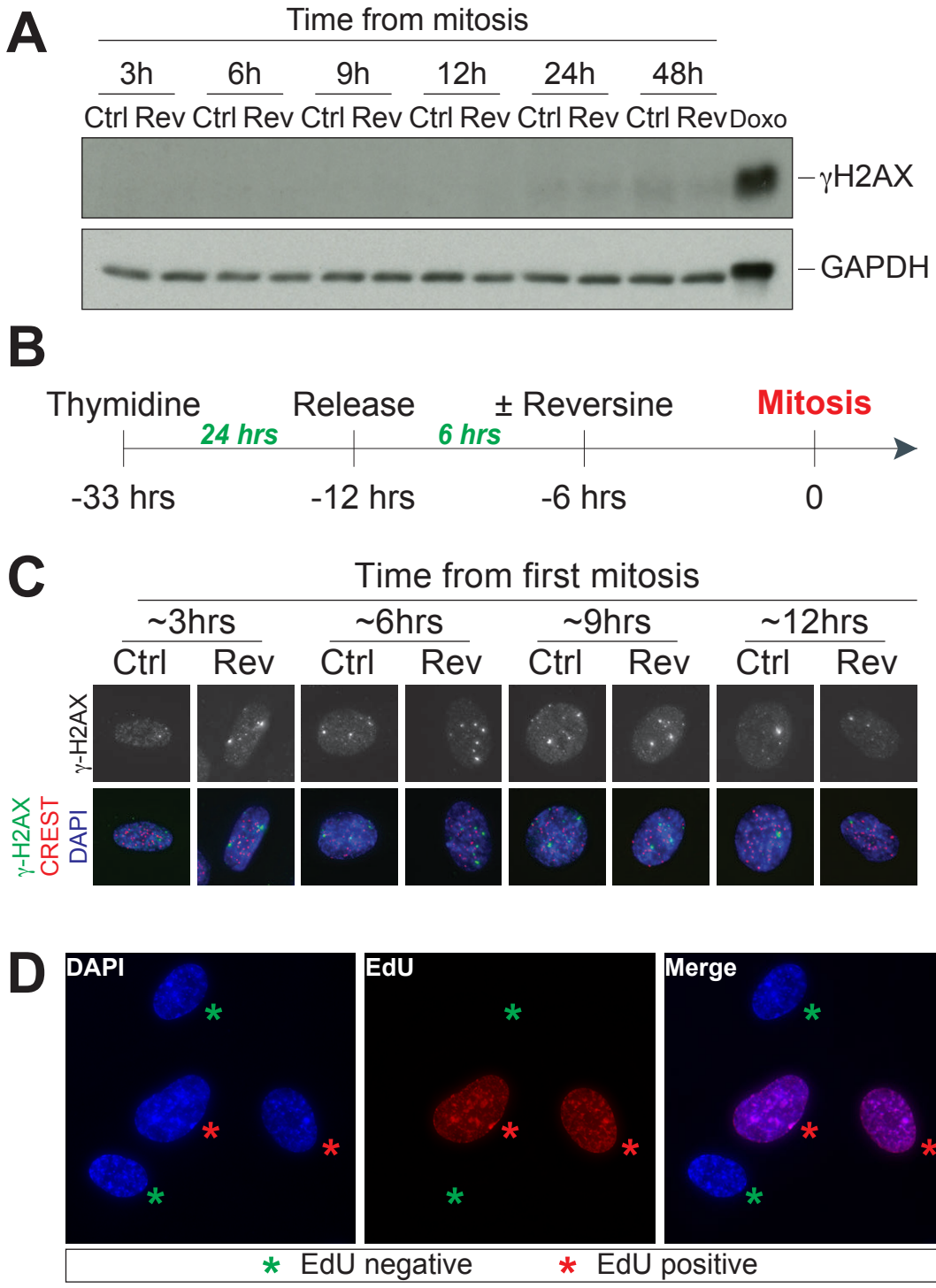

A
Time from mitosis
3h
6h
9h
12h
24h
48h
Ctrl Rev Ctrl Rev Ctrl Rev Ctrl Rev Ctrl Rev Ctrl Rev Doxo
γH2AX
GAPDH
B
Thymidine
Release
± Reversine
Mitosis
24 hrs
6 hrs
-33 hrs
-12 hrs
-6 hrs
0
C
Time from first mitosis
~3hrs
~6hrs
~9hrs
~12hrs
Ctrl Rev Ctrl Rev Ctrl Rev Ctrl Rev
γ-H2AX
γ-H2AX CREST DAPI
D
DAPI
EdU
Merge
EdU negative
EdU positive

Figure S3

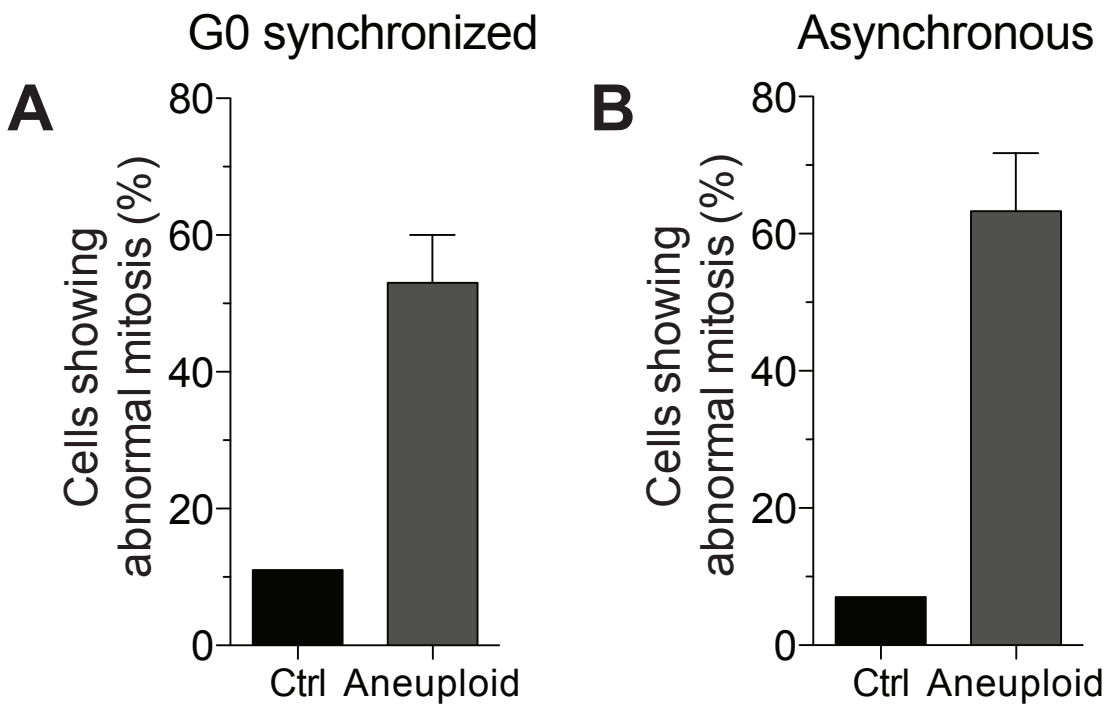

G0 synchronized
Asynchronous
A
B
Cells showing abnormal mitosis (%)
80
60
40
20
0
Ctrl
Aneuploid

Figure S4

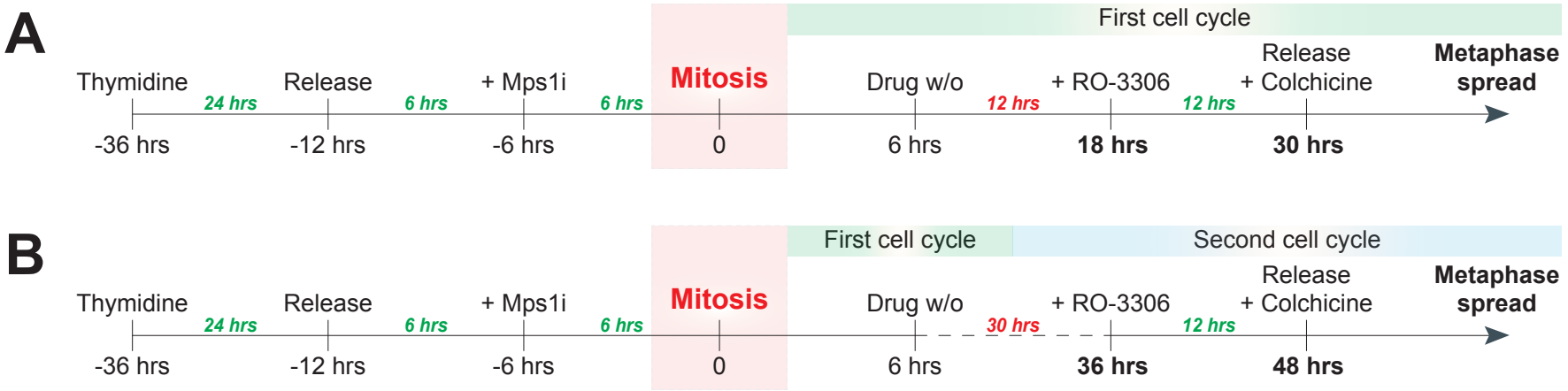
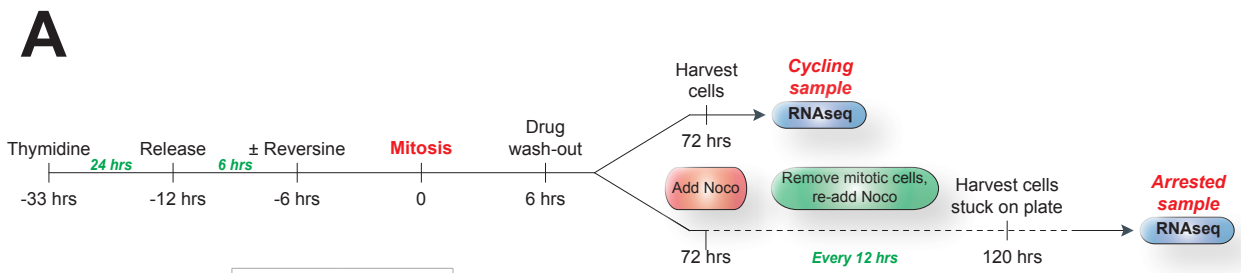
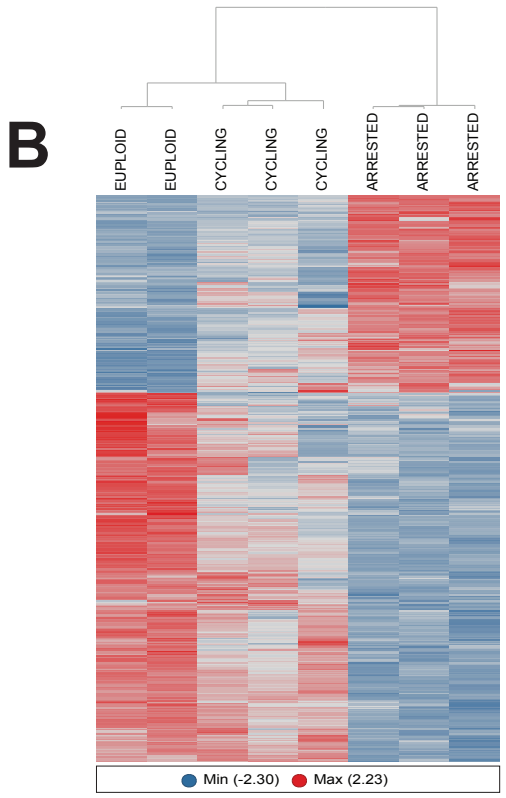
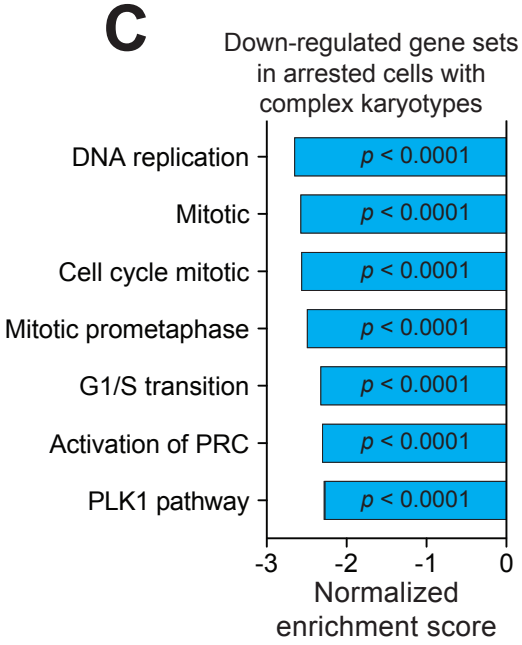
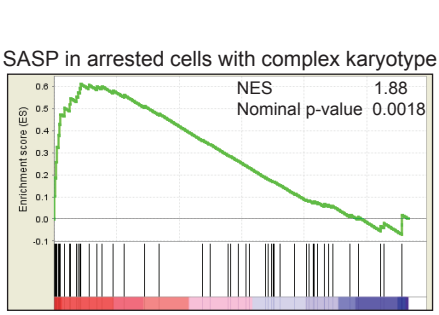

A
First cell cycle
Thymidine
24 hrs
Release
6 hrs
+ Mps1i
6 hrs
Mitosis
Drug w/o
12 hrs
+ RO-3306
12 hrs
Release
+ Colchicine
Metaphase
spread
-36 hrs
-12 hrs
-6 hrs
0
6 hrs
18 hrs
30 hrs
B
First cell cycle
Second cell cycle
Thymidine
24 hrs
Release
6 hrs
+ Mps1i
6 hrs
Mitosis
Drug w/o
30 hrs
+ RO-3306
12 hrs
Release
+ Colchicine
Metaphase
spread
-36 hrs
-12 hrs
-6 hrs
0
6 hrs
36 hrs
48 hrs

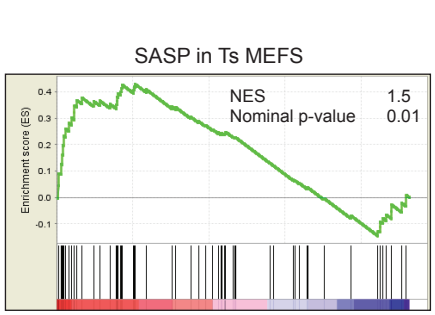
Figure S5

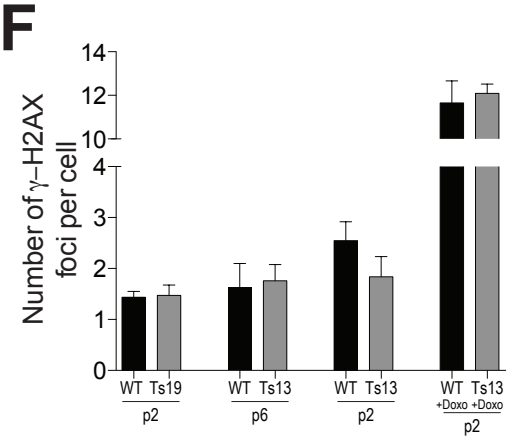

A
Thymidine
24 hrs
Release
6 hrs
± Reversine
Mitosis
Drug wash-out
-33 hrs
-12 hrs
-6 hrs
0
6 hrs
Harvest cells
72 hrs
Cycling sample
RNAseq
Add Noco
Remove mitotic cells, re-add Noco
Every 12 hrs
Harvest cells stuck on plate
120 hrs
72 hrs
Arrested sample
RNAseq


B
EUPLOID
EUPLOID
CYCLING
CYCLING
CYCLING
ARRESTED
ARRESTED
ARRESTED
Min (-2.30)
Max (2.23)


C
Down-regulated gene sets in arrested cells with complex karyotypes
DNA replication
Mitotic
Cell cycle mitotic
Mitotic prometaphase
G1/S transition
Activation of PRC
PLK1 pathway
p < 0.0001
-3
-2
-1
0
Normalized enrichment score


D
SASP in arrested cells with complex karyotype
NES 1.88
Nominal p-value 0.0018
Enrichment score (ES)


E
SASP in Ts MEFS
NES 1.5
Nominal p-value 0.01
Enrichment score (ES)


F
Number of γ–H2AX foci per cell
WT Ts19
p2
WT Ts13
p6
WT Ts13
p2
WT Ts13
+Doxo +Doxo
p2

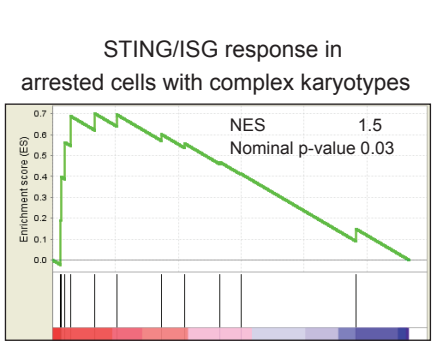

G
STING/ISG response in arrested cells with complex karyotypes
NES 1.5
Nominal p-value 0.03
Enrichment score (ES)

Figure S6

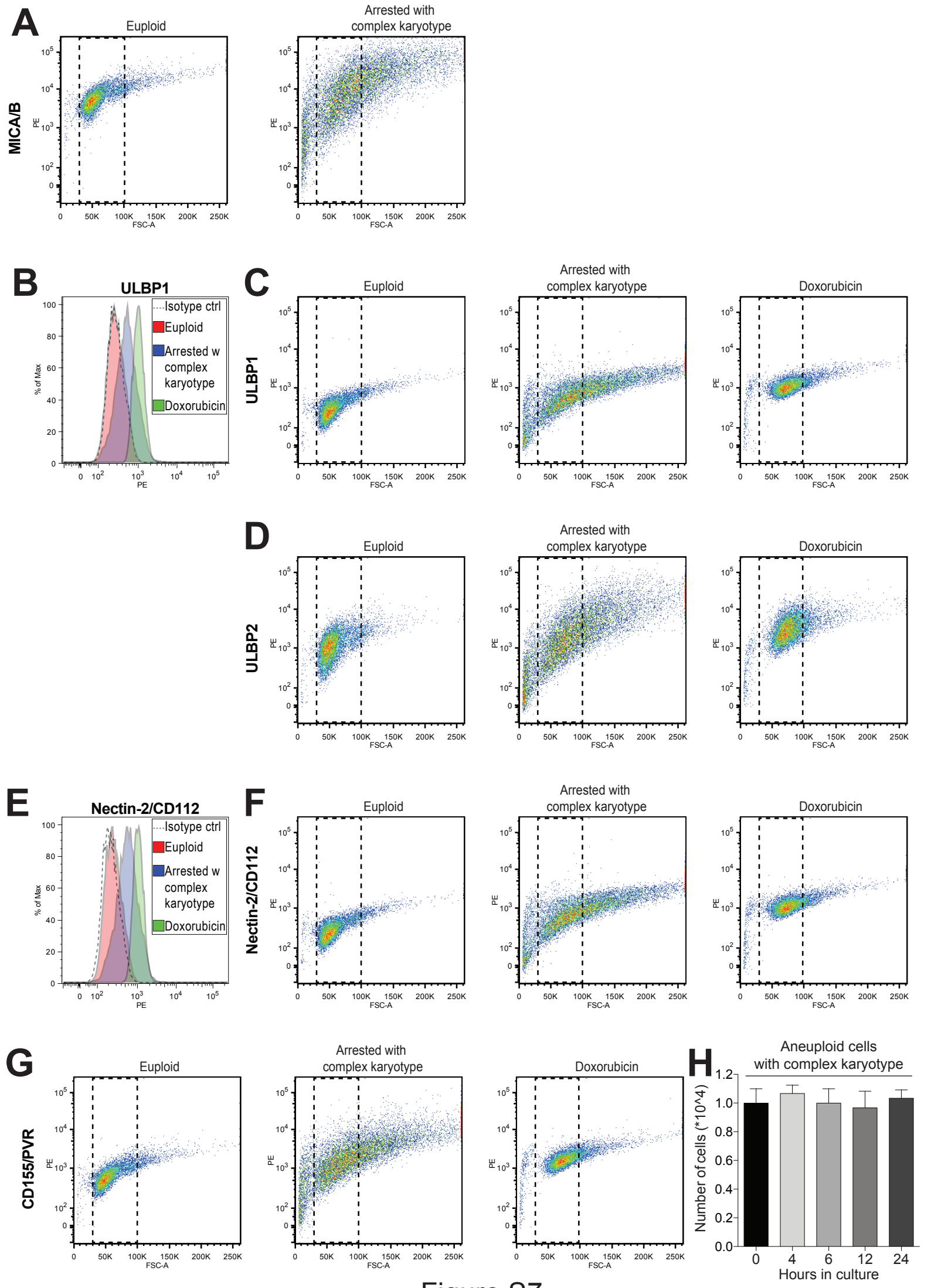

A
Euploid
Arrested with complex karyotype
MICA/B
PE
FSC-A
B
ULBP1
Isotype ctrl
Euploid
Arrested w complex karyotype
Doxorubicin
% of Max
PE
C
Euploid
Arrested with complex karyotype
Doxorubicin
ULBP1
D
ULBP2
E
Nectin-2/CD112
F
Nectin-2/CD112
G
CD155/PVR
H
Aneuploid cells with complex karyotype
Number of cells (*10^4)
Hours in culture

Figure S7