# Supplemental Materials

***Molecular Biology of the Cell***

Chen et al.

**Figure Legends**

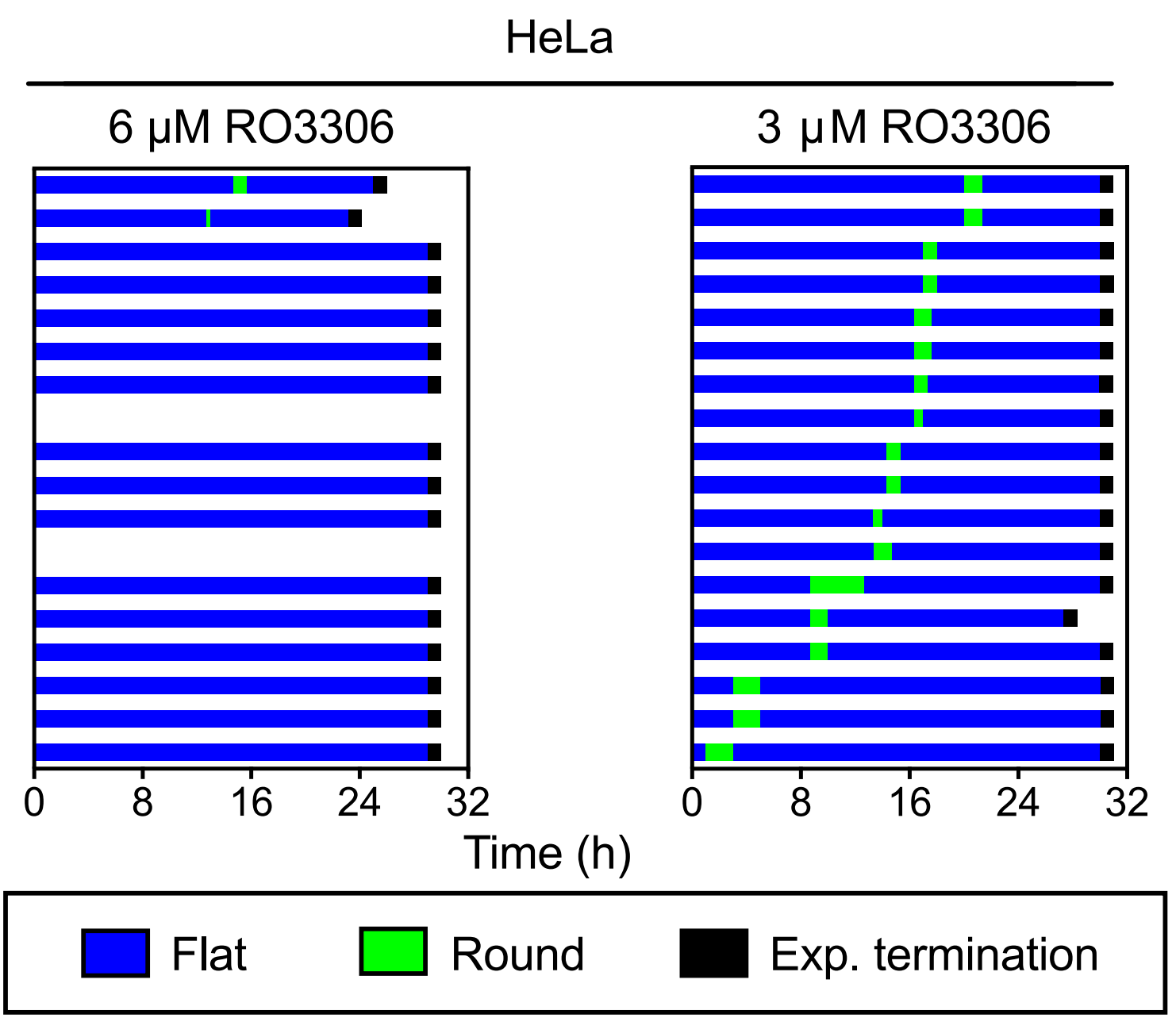
**Supplementary Figure 1**. The concentration of RO3306 added to HeLa cells alters endocycle vs. endomitosis. Cell fate profiles of HeLa cells exposed to the indicated treatment in which t=0 corresponds to the time of drug addition.

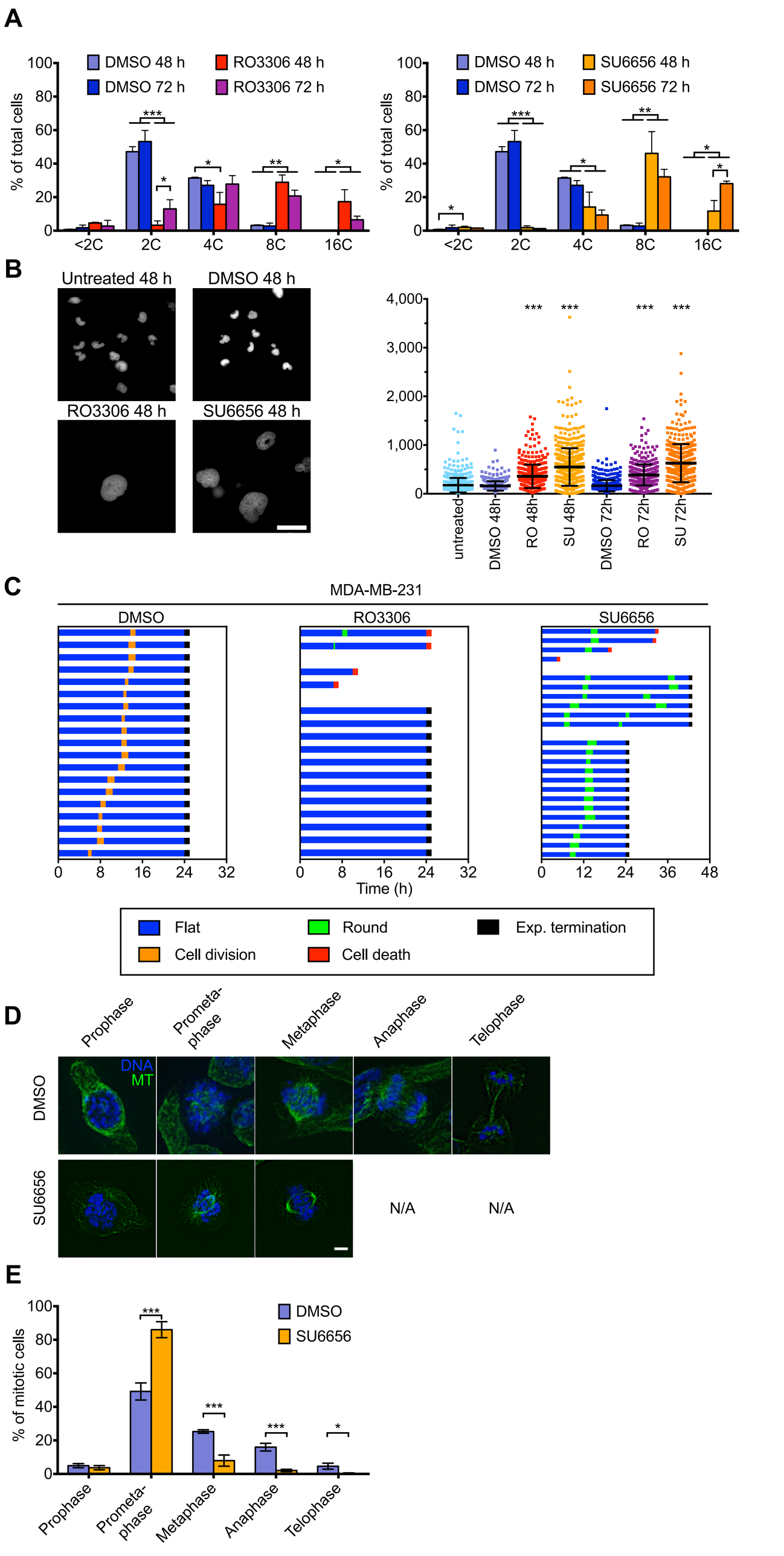
**Supplementary Figure 2.** MDA-MB-231 cells behave similar to HeLa cells upon endoreplication. (A) Quantification of FACS data from three independent experiments showing the percentage of MDA-MB-231 cells with different ploidy under the indicated treatments. For each experiment, 10,000 cells were measured for DNA content, and the mean ± standard deviation (SD) is graphed. (B) (Left) Micrographs of MDA-MB-231 cells stained with Hoechst for DNA after the indicated treatment. Scale bar, 20 μm. (Right) Dot plots showing the quantification of the nuclear size from three independent experiments in which at least 300 cells were scored per experiment. Bar and whiskers indicate the mean and SD. (C) Cell fate profile of MDA-MB-231 cells exposed to indicated treatment in which t=0 corresponds to the time of drug addition. (D) DMSO control or SU6656 treated MDA-MB-231 cells were fixed at ~14 h post-drug addition and processed for immunofluorescence to visualize MTs (green) and DNA (blue). Scale bar, 5 μm. (E) Quantification of the percentage of mitotic cells at different mitotic stages. Data are mean ± SD from three independent experiments in which 900 cells were scored per condition. * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

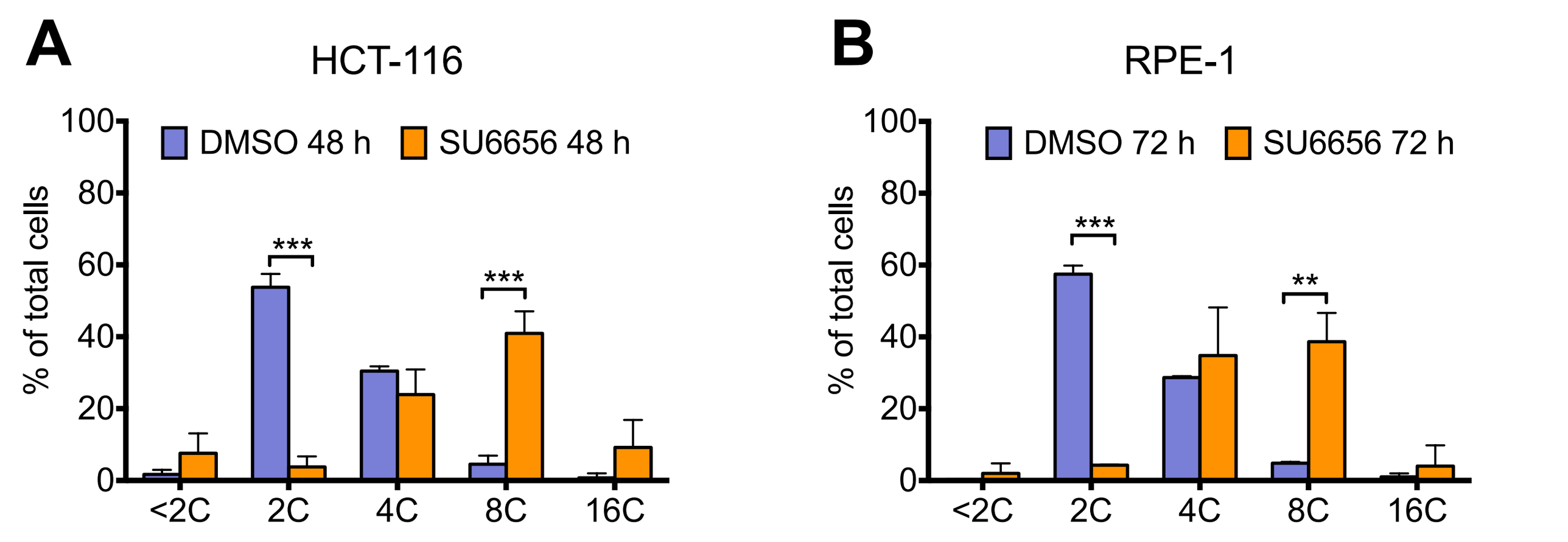
**Supplementary Figure 3.** HCT-116 and RPE-1 increase ploidy after iEC inducation. (A, B) Quantification of FACS data from three independent experiments showing the percentage of HCT-116 cells at 48 h (A) and RPE-1 cells at 72 h (B) with different ploidy under the indicated treatments. For each experiment, 10,000 cells were measured for DNA content, and the mean ± SD is graphed. ** $p<0.01$, *** $p<0.001$.

**Supplementary Figure 4.** Cells overexpressing GFP-HSET return to mitosis after drug washout. (A) Western blot of HeLa cells treated with the indicated conditions and probed with anti-HSET (top) or anti-tubulin (bottom) antibodies. (B) Representative images of HeLa cells overexpressing GFP-HSET after SU6656 washout (t=0). Time post-washout is marked in the upper left corner of each panel. The orange arrow represents one cell that divides into two cells (yellow and red arrows). Scale Bar, 20 μm. (C) HeLa cells were synchronized, treated with SU6656 for 48 h and then imaged at 5 min intervals starting from 7 to 16 h post drug washout. A total of 1500 cells were analyzed of which 749 entered mitosis. The number in parentheses represents the total number of mitotic cells with the indicated cell fate. (D, E) GFP-HSET overexpressing HeLa cells were synchronized, treated with DMSO (D), or SU6656 (E) for 48 h and

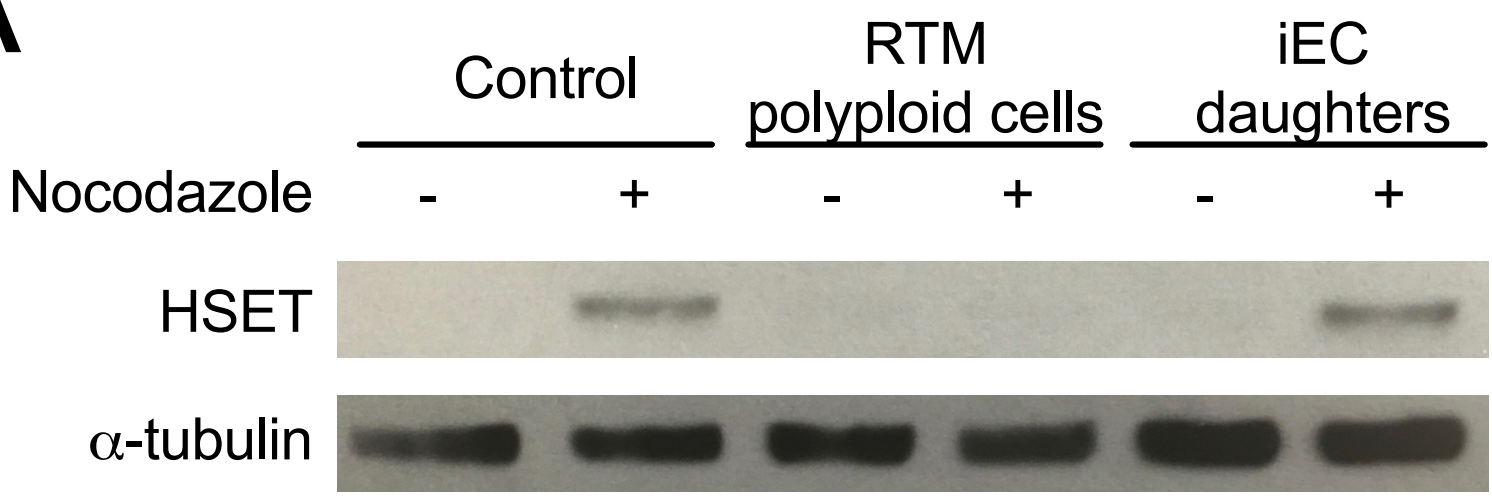
cultured in drug-free media for 20 h. Cells were then fixed and stained with probes specific for centrometric satellite DNA of chromosome 2 or 7 and stained with Hoechst to visualize DNA. Scale bar, 20 μm.


HeLa
6 μM RO3306
3 μM RO3306
0
8
16
24
32
Time (h)
Flat
Round
Exp. termination

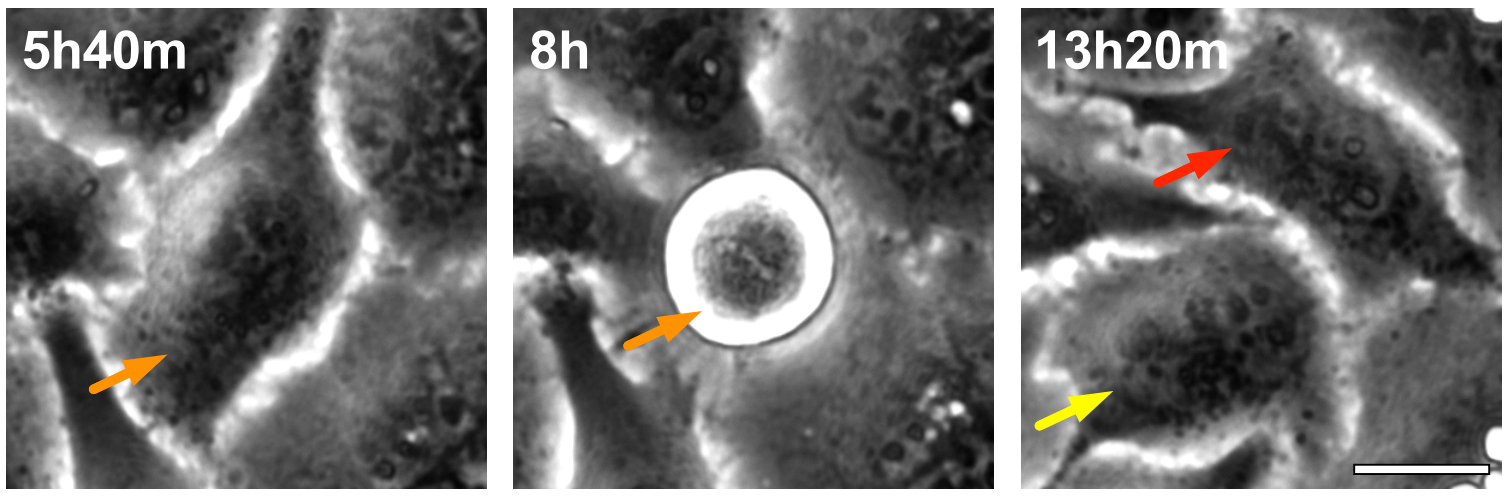

A
DMSO 48 h
DMSO 72 h
RO3306 48 h
RO3306 72 h
% of total cells
<2C
2C
4C
8C
16C
SU6656 48 h
SU6656 72 h
B
Untreated 48 h
DMSO 48 h
RO3306 48 h
SU6656 48 h
untreated
DMSO 48h
RO 48h
SU 48h
DMSO 72h
RO 72h
SU 72h
C
MDA-MB-231
DMSO
RO3306
SU6656
Time (h)
Flat
Round
Exp. termination
Cell division
Cell death
D
Prophase
Prometa-phase
Metaphase
Anaphase
Telophase
DNA
MT
DMSO
SU6656
N/A
E
% of mitotic cells
DMSO
SU6656


A
HCT-116
DMSO 48 h
SU6656 48 h
% of total cells
100
80
60
40
20
0
***
***
<2C
2C
4C
8C
16C
B
RPE-1
DMSO 72 h
SU6656 72 h
% of total cells
100
80
60
40
20
0
***
**
<2C
2C
4C
8C
16C

**A**




**B**




**C**

| Observed phenotype | HeLa GFP-HSET(1500 cells) |
|---|---|
| **Successful P1 polyploid mitosis** | 14.0% of total cells |
| 2 daughter cells | 85.4% (182) |
| 3 daughter cells | 14.6% (31) |
| **Failed P1 polyploid mitosis** | 34.9% of total cells |
| Mitotic failure | 68.7% (368) |
| Mitotic arrest | 9.5% (51) |
| Cell death | 21.8% (117) |

**D**

**E**