Supplementary Figure Legends

Supplementary Figure 1

Analysis of cell length after growth in EdU.

A. *rad3*$^{+}$ (*adh1:tk adh1:hENT1* (P2470)) and *rad3Δ* (*adh1:tk adh1:hENT1 rad3Δ* (P2472)) cells were arrested in G1 by growing in EMM-N for 16 h at 26°C, then released from the cell cycle block in EMM+N medium at 32°C in the presence (1 μM) or absence of EdU for 10 hours and imaged following DAPI staining and EdU detection. Bar=10μm. Mean cell length shown in the EdU panels (μm) was calculated by measuring at least 100 cells and standard deviation is shown.

B. As (A) except septa were also stained with 50 μg/ml methyl blue and only cells with EdU treatment are shown. *rad3Δ* cells grown in EdU execute mitosis (see also Fig. 2E) and form septa, but a high proportion of cells fail to carry out later stages of cytokinesis and arrest as septated binuclear cells.

Arrows show defective mitoses in *rad3Δ* cells grown in EdU.

Supplementary Figure 2

Effect of EdU on division of *rad3Δ* cells.

rad3Δ (*adh1:tk adh1:hENT1 rad3Δ* (P2472)) cells were arrested in G1 by growing in EMM-N for 16 h at 26°C, then released in EMM+N medium at 32°C in the presence (1 μM) or absence of EdU and cell concentrations were monitored. *rad3Δ* cells appear to divide more slowly compared to *rad3*$^{+}$ after release from a minus nitrogen arrest in the absence of EdU (compare to Fig. 2D). There is little difference in cell division comparing *rad3*$^{+}$ and *rad3Δ* strains after growth in EdU as cells arrest either before mitosis (*rad3*$^{+}$) or as mostly as binucleated septated cells (*rad3Δ,* see Fig. S1).

Supplementary Figure 3

Effect of EdU on kinetics of DNA replication.

rad3^{+} (*adh1:tk adh1:hENT1* (P2470)) cells were arrested in G1 by growing in

EMM-N for 16 h at 26°C, then released from the cell cycle block in EMM+N medium at 32°C in the presence (1 µM) of EdU. Cells were processed for analysis of DNA content after SYTOX Green staining by flow cytometry in the absence of conjugation to fluorescent azide. This figure is a longer time course of the experiment shown in Fig. 3D. A similar result was shown with *rad3Δ cells* (data not shown).

Supplementary Figure 4

Flow cytometric analysis of DNA content of cells grown in the presence or absence of BrdU, showing that cells with incorporated BrdU show reduced SYTOX Green fluorescence.

*adh1:tk adh1:hENT1 (*P2470) cells were nitrogen starved to arrest cells predominantly in G1 by growing in EMM lacking nitrogen for 16 h at 26°C, then released from the block at 32°C in EMM and cells were processed for SYTOX Green staining at the time points shown. (A) shows cells released in the absence of BrdU, (B) shows cells released in the presence of 1 µM BrdU. The shoulder on the right of the 2C peaks at t=4,5 h in (B) represent G2 cells in the nitrogen-starved population that have not carried out S phase at this stage. Similar results were obtained using 0.5 and 10 µM BrdU (data not shown).

Supplementary Figure 5

Cells from the +HU,-EdU culture in Fig. 4, analyzed for DNA content by flow cytometry after SYTOX Green staining, confirming arrest of DNA replication.

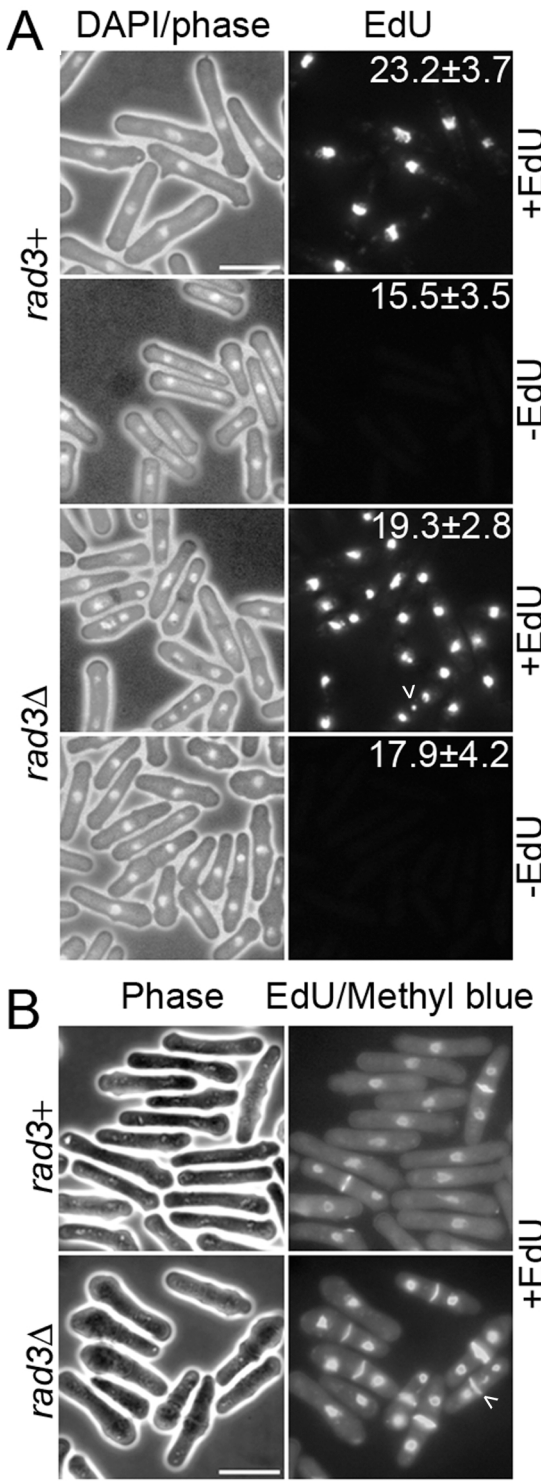
A
DAPI/phase
EdU
23.2±3.7
+EdU
rad3+
15.5±3.5
-EdU
19.3±2.8
+EdU
rad3Δ
17.9±4.2
-EdU
B
Phase
EdU/Methyl blue
rad3+
+EdU
rad3Δ

Fig S1

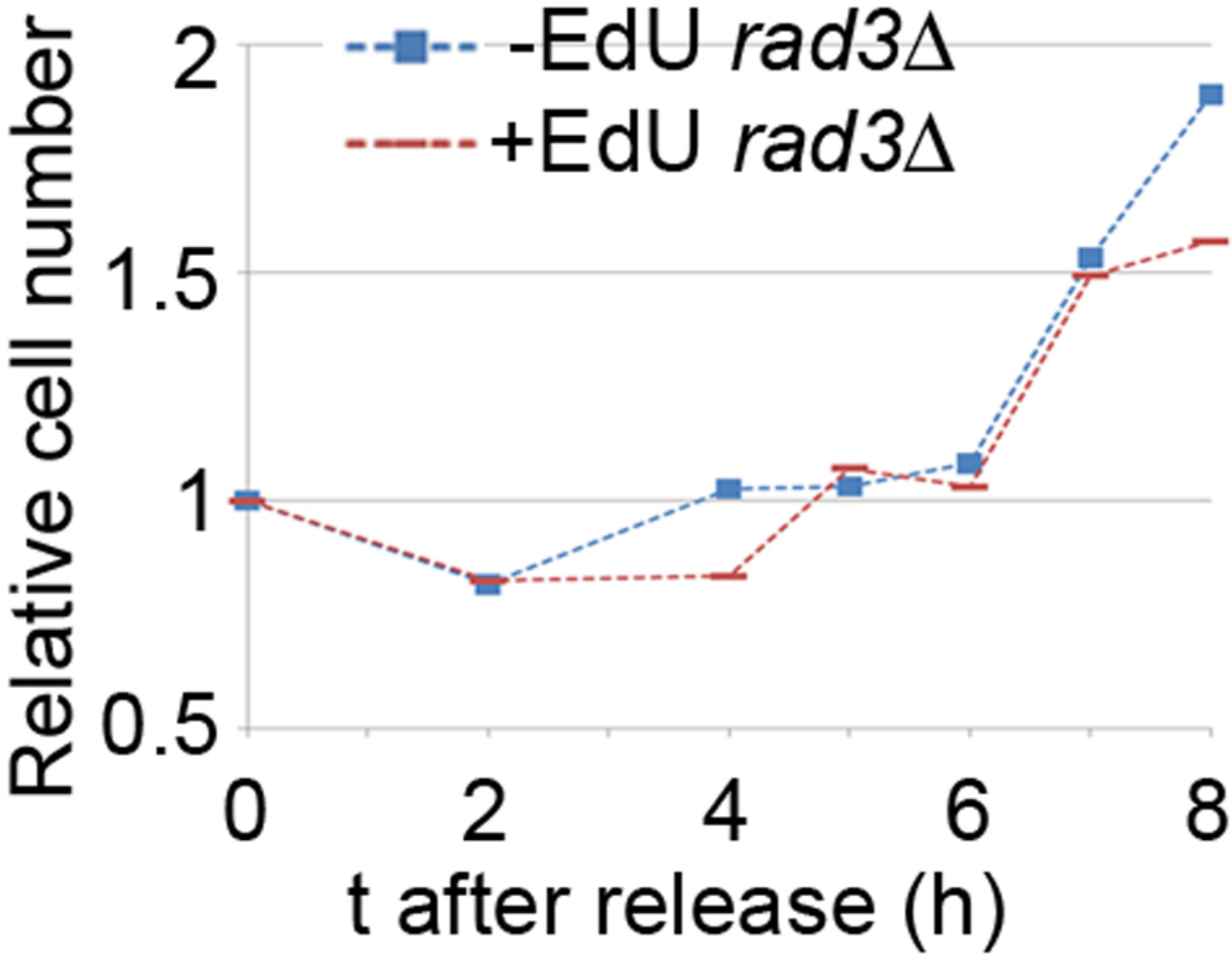
-EdU rad3Δ
+EdU rad3Δ
Relative cell number
2
1.5
1
0.5
0
2
4
6
8
t after release (h)

Fig S2

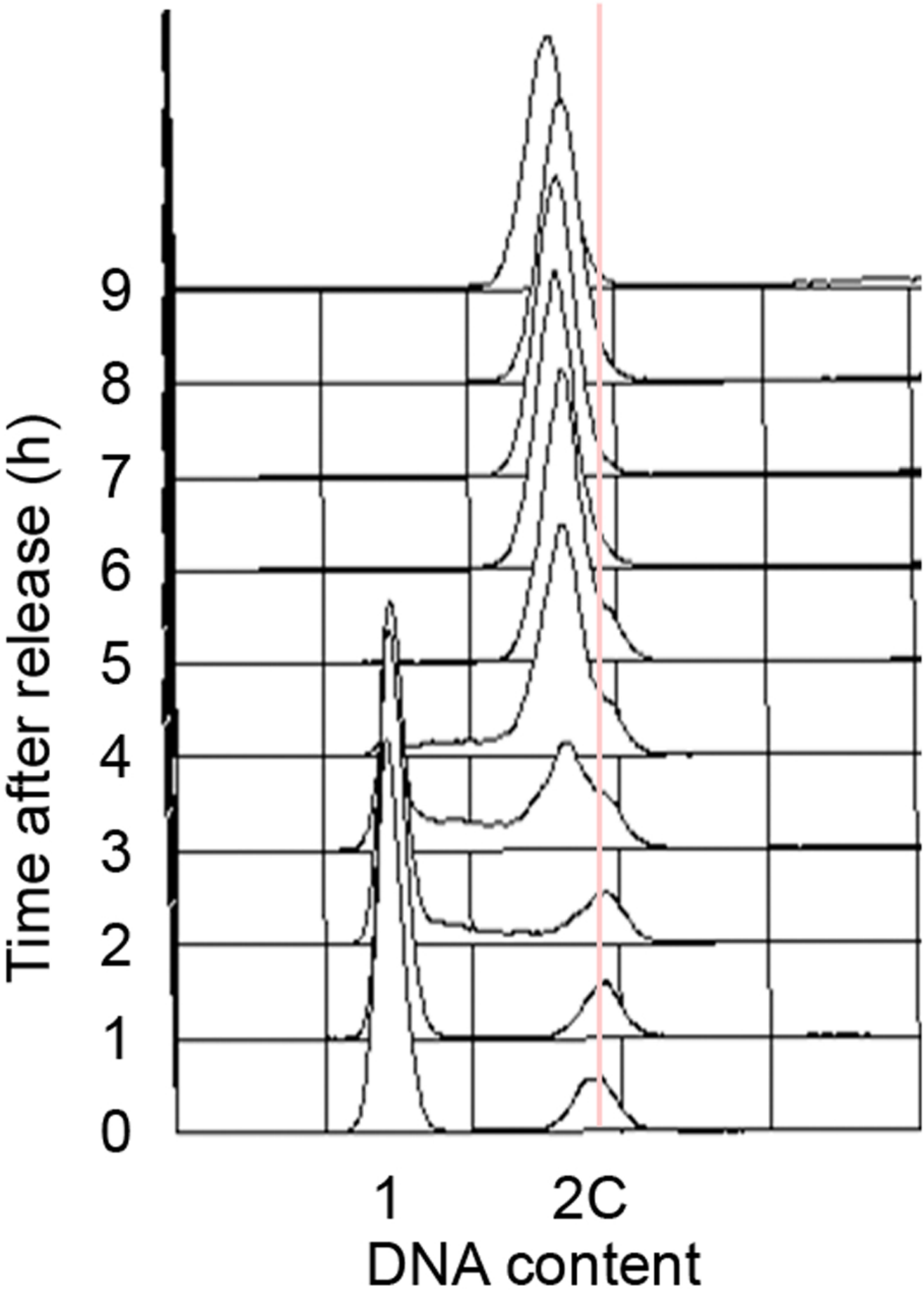

Fig S3

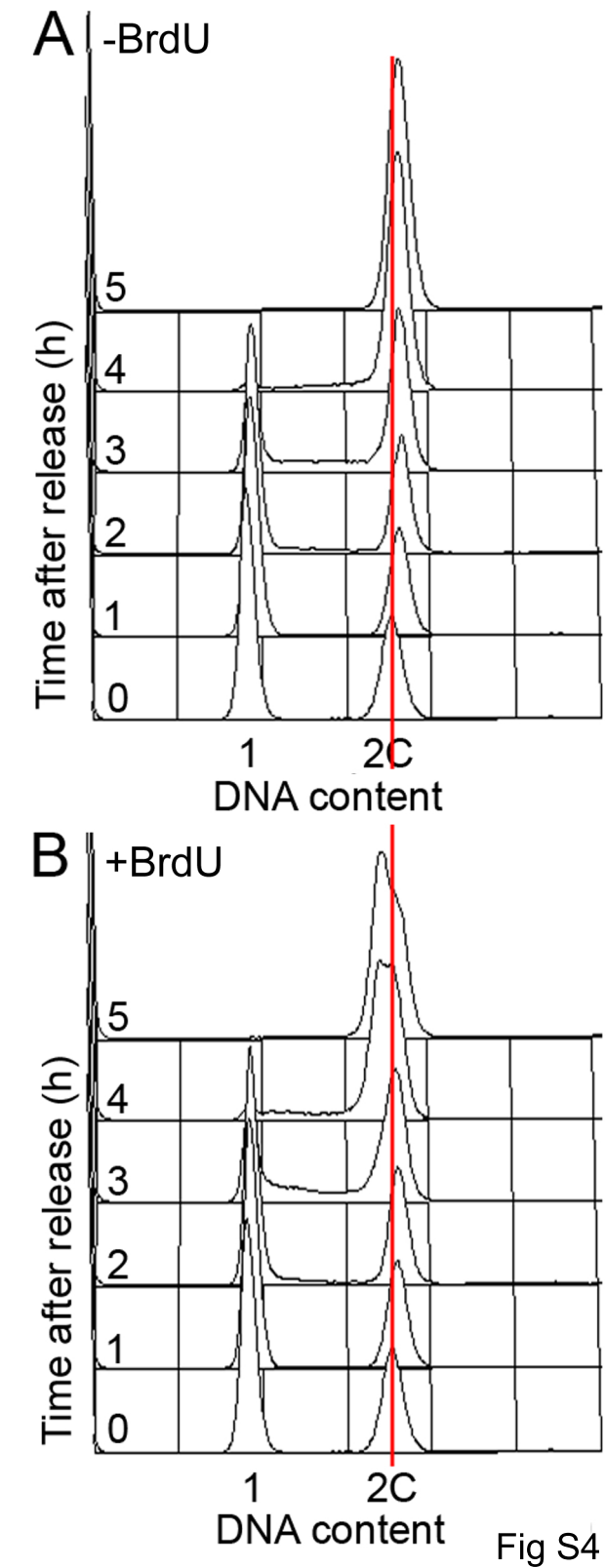
A
-BrdU
5
4
3
2
1
0
Time after release (h)
1
2C
DNA content
B
+BrdU
5
4
3
2
1
0
Time after release (h)
1
2C
DNA content

Fig S4

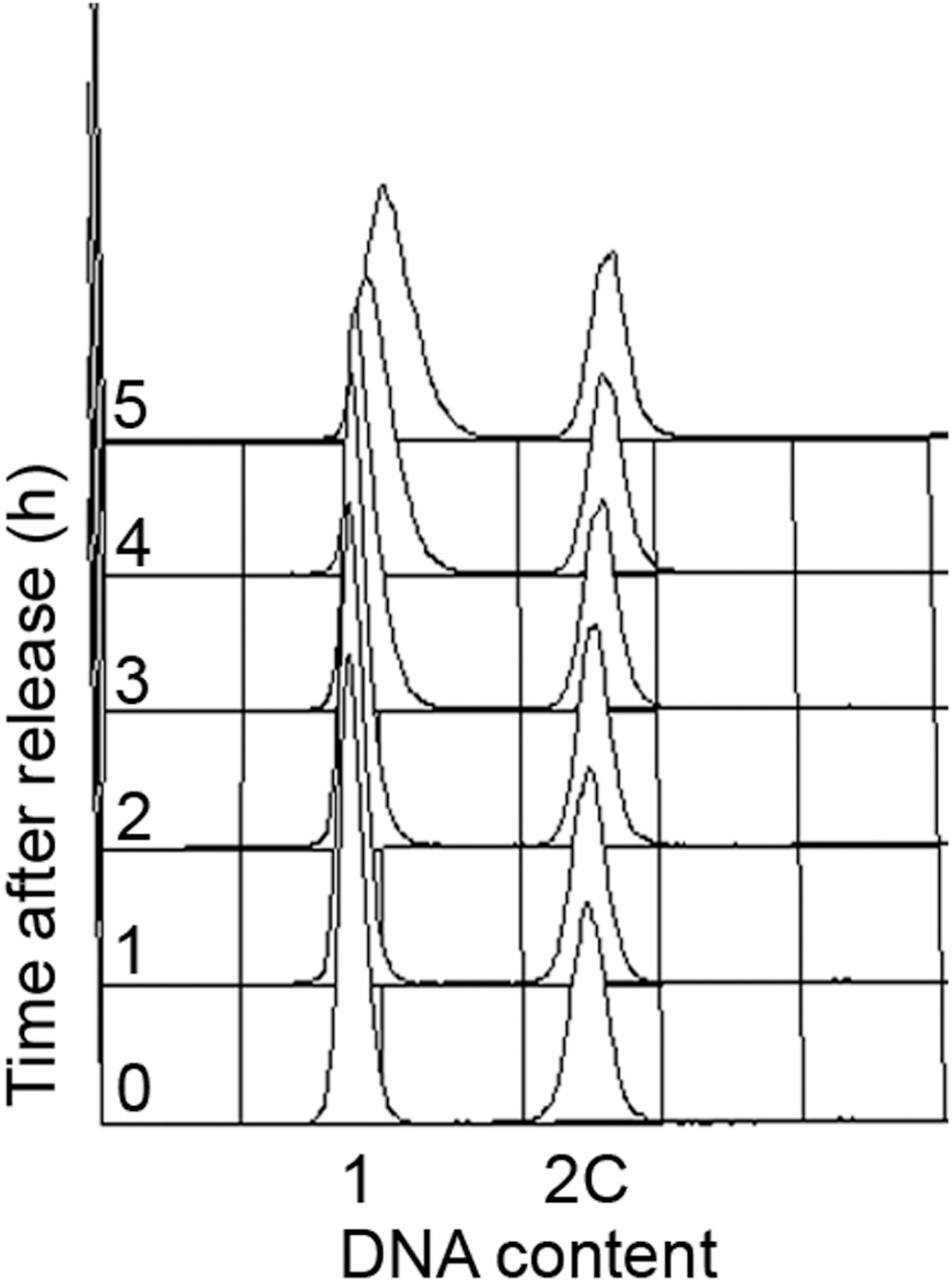

Fig S5