Supplementary data

Chemotherapeutic drugs inhibit ribosome biogenesis at various levels

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Fig. S1. Labeling kinetics of cells. *A*. Cells were pulse labeled with ^{32}P-orthophosphate for 1h and chased for different periods of time (30min to 6h). *B*. Sufficient labeling of mature and immature rRNAs was achieved after a 3h chase. Depletion of phosphate from medium and serum improved the labeling efficiency of rRNA several-fold, but did not influence the pattern of labeled rRNAs (data not shown).

Fig. S2. Inhibition of transcription and processing of rRNA by cytostatic drugs. *A-G*. Alkylating agents Busulfan, Chlorambucil, Melphalan, Nimustine, Cyclophosphamide, Dacarbazine, and Thio-TEPA did not affect rRNA production. Only Melphalan inhibited rRNA gene transcription at 100μM. *H*. The proteasome inhibitor Bortezomib and the translation inhibitor *I*. Cyclohexemide showed an inhibitory activity on rRNA processing. *J*. The intercalating agent Actinomycin D strongly inhibited rRNA transcription, *K,L* kinase inhibitors Rapamycin and Sorafenib showed little or no inhibition. *M-P*. The alkaloids Etoposide, Vinblastine and Irinotecan were not inhibitory, while Camptotecin at low concentrations inhibited rRNA processing, and at higher concentrations also rRNA transcription. *Q-U*. The antimetabolics 6-Mercaptopurine, 6-Thioguanine, Fludarabine, Cytarabine, and Hydroxyurea, *V-W*. the HDAC inhibitors Vorinostat and Trichostatin A, and *X*. the mitosis inhibitor Paclitaxel did not inhibit rRNA synthesis and processing. For experimental design see Fig. 2.

Fig. S3. *A*. Cells were pulse-labeled for 15min, 30min, 45min, and 60min with ^{32}P-orthophosphate and chased as indicated. B. Autoradiography of labeled rRNAs. EtBr.-stained 28S rRNA.

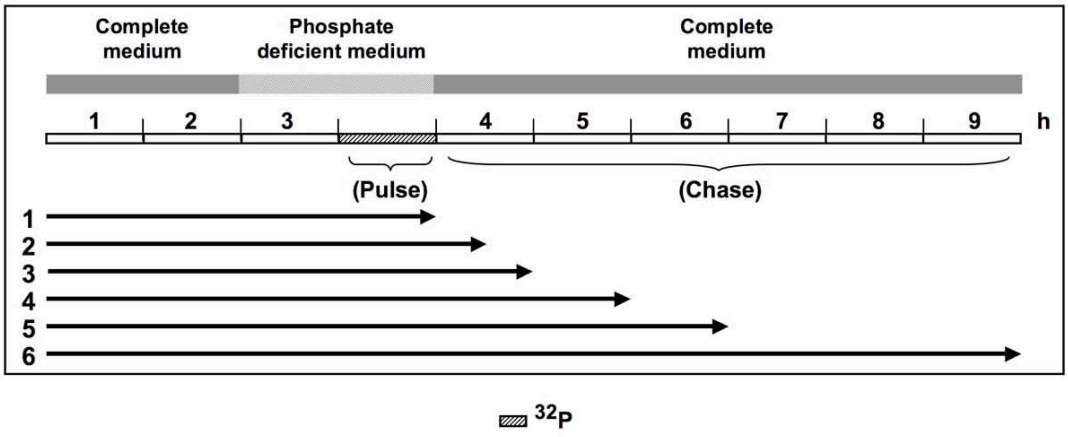
Fig. S4. *A*. Schematic of the labelling protocol. *B*. Autoradigraphy of labelled rRNAs and 5S RNA. EtBr.-stained 28S rRNA. *C*. Signals of control cells were set as 100%. Relative signal intensities for drug-treated cells were calculated.

Fig. S5**.** *A*. Mitomycin C inhibits transcription of rRNA genes and induces nucleoplasmic translocation of NPM. *B*. Homoharringtonin inhibits maturation of the 18S and 28S rRNA without translocation of NPM to the nucleoplasm. For details see Fig. 4.

Fig. S6. Methotrexate, Actinomycin D, Mitomycin C, Etoposide, Flavopiridol, and Camptothecin affect the integrity of the nucleolus and induce nucleoplamic translocation of NPM. Vinblastine, 5-Fluorouracil, and Cycloheximide show no nucleolar phenotype. For experimental details see Fig. 5.

Fig. S7. *A-Q*. These substances did not induce translocation of NPM to the nucleoplasm at the indicated concentrations. For details see Fig. 5.

A


Complete medium
Phosphate deficient medium
Complete medium
1
2
3
4
5
6
7
8
9
h
(Pulse)
(Chase)
1
2
3
4
5
6
32P

B

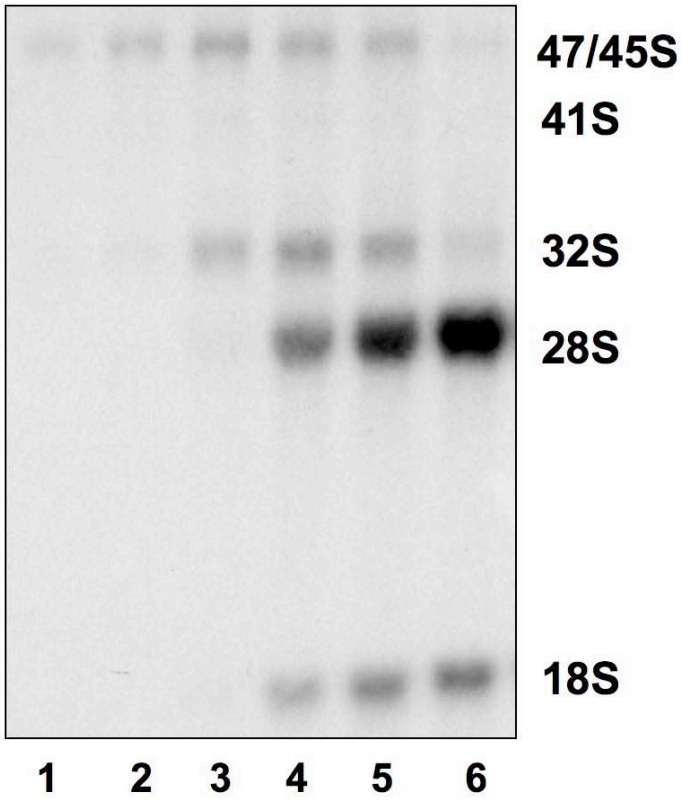

47/45S
41S
32S
28S
18S
1
2
3
4
5
6

Figure S1

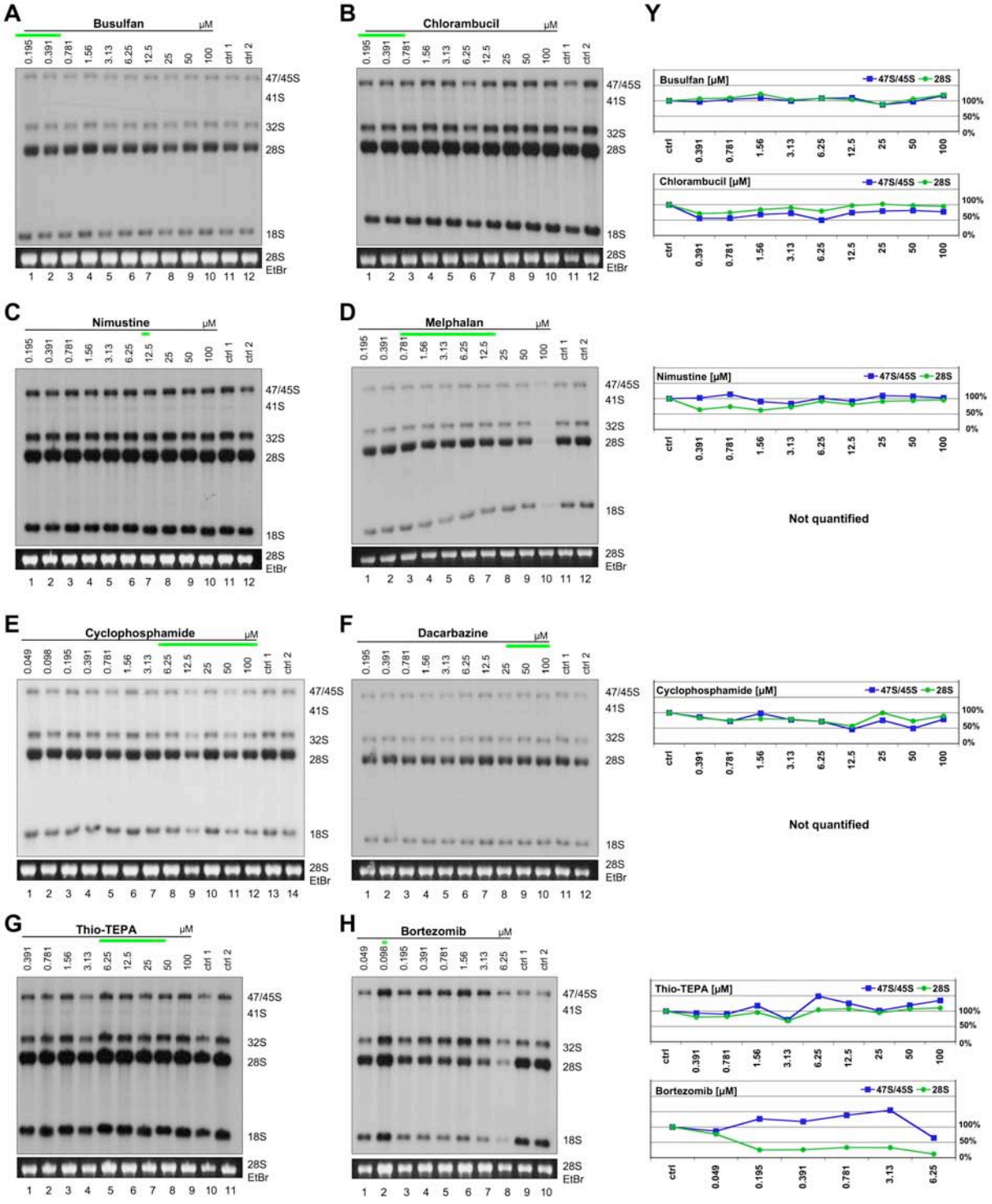

A
Busulfan
µM
0.195 0.391 0.781 1.56 3.13 6.25 12.5 25 50 100 ctrl 1 ctrl 2
47/45S
41S
32S
28S
18S
28S
EtBr
1 2 3 4 5 6 7 8 9 10 11 12
B
Chlorambucil
µM
0.195 0.391 0.781 1.56 3.13 6.25 12.5 25 50 100 ctrl 1 ctrl 2
47/45S
41S
32S
28S
18S
28S
EtBr
1 2 3 4 5 6 7 8 9 10 11 12
Y
Busulfan [µM]
47S/45S
28S
100%
50%
0%
ctrl 0.391 0.781 1.56 3.13 6.25 12.5 25 50 100
Chlorambucil [µM]
47S/45S
28S
100%
50%
0%
ctrl 0.391 0.781 1.56 3.13 6.25 12.5 25 50 100
C
Nimustine
µM
0.195 0.391 0.781 1.56 3.13 6.25 12.5 25 50 100 ctrl 1 ctrl 2
47/45S
41S
32S
28S
18S
28S
EtBr
1 2 3 4 5 6 7 8 9 10 11 12
D
Melphalan
µM
0.195 0.391 0.781 1.56 3.13 6.25 12.5 25 50 100 ctrl 1 ctrl 2
47/45S
41S
32S
28S
18S
28S
EtBr
1 2 3 4 5 6 7 8 9 10 11 12
Nimustine [µM]
47S/45S
28S
100%
50%
0%
ctrl 0.391 0.781 1.56 3.13 6.25 12.5 25 50 100
Not quantified
E
Cyclophosphamide
µM
0.049 0.098 0.195 0.391 0.781 1.56 3.13 6.25 12.5 25 50 100 ctrl 1 ctrl 2
47/45S
41S
32S
28S
18S
28S
EtBr
1 2 3 4 5 6 7 8 9 10 11 12 13 14
F
Dacarbazine
µM
0.195 0.391 0.781 1.56 3.13 6.25 12.5 25 50 100 ctrl 1 ctrl 2
47/45S
41S
32S
28S
18S
28S
EtBr
1 2 3 4 5 6 7 8 9 10 11 12
Cyclophosphamide [µM]
47S/45S
28S
100%
50%
0%
ctrl 0.391 0.781 1.56 3.13 6.25 12.5 25 50 100
Not quantified
G
Thio-TEPA
µM
0.391 0.781 1.56 3.13 6.25 12.5 25 50 100 ctrl 1 ctrl 2
47/45S
41S
32S
28S
18S
28S
EtBr
1 2 3 4 5 6 7 8 9 10 11
H
Bortezomib
µM
0.049 0.098 0.195 0.391 0.781 1.56 3.13 6.25 ctrl 1 ctrl 2
47/45S
41S
32S
28S
18S
28S
EtBr
1 2 3 4 5 6 7 8 9 10
Thio-TEPA [µM]
47S/45S
28S
100%
50%
0%
ctrl 0.391 0.781 1.56 3.13 6.25 12.5 25 50 100
Bortezomib [µM]
47S/45S
28S
100%
50%
0%
ctrl 0.049 0.195 0.391 0.781 3.13 6.25

Figure S2 A-H

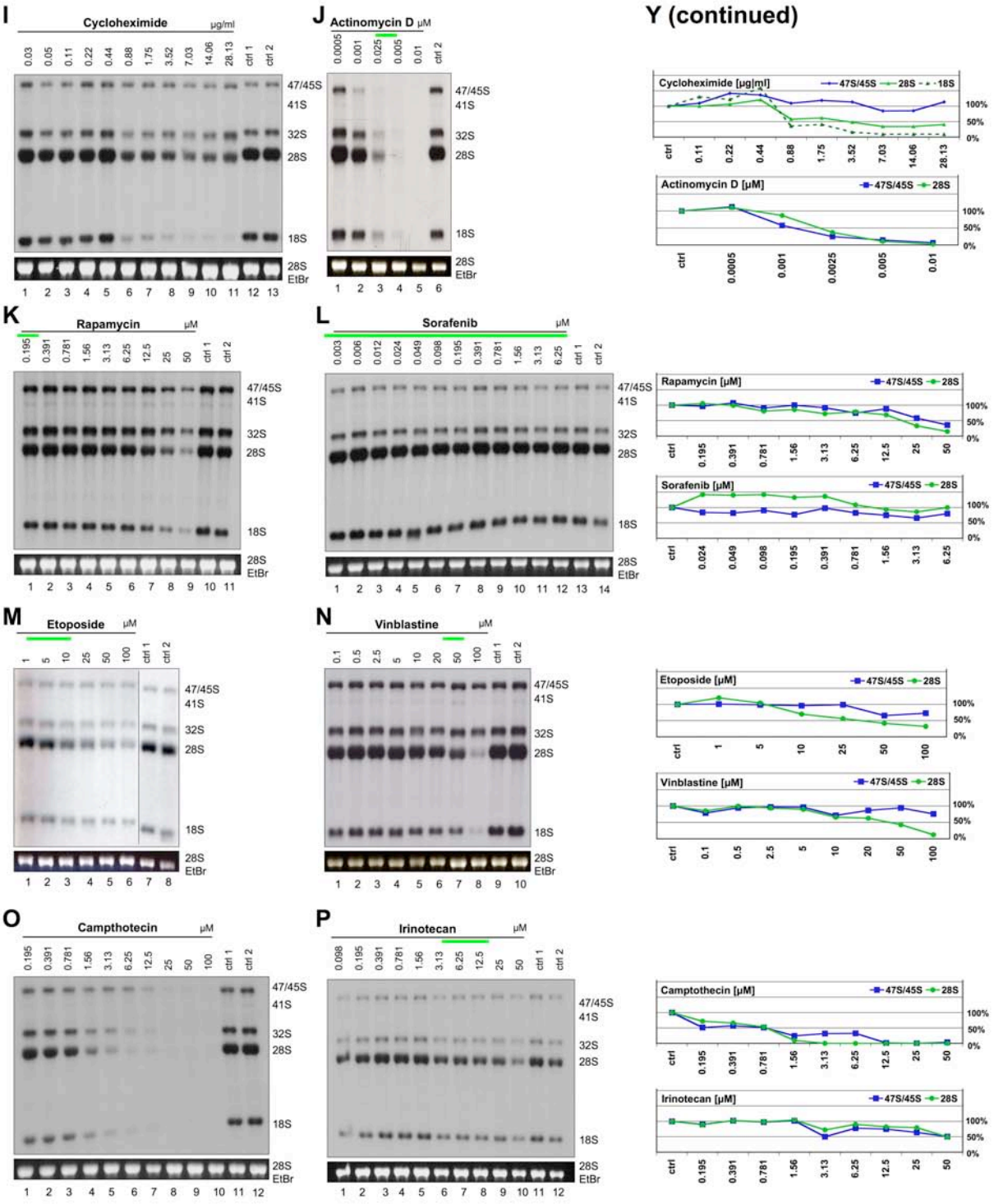

I
Cycloheximide µg/ml
0.03 0.05 0.11 0.22 0.44 0.88 1.75 3.52 7.03 14.06 28.13 ctrl 1 ctrl 2
47/45S
41S
32S
28S
18S
28S EtBr
1 2 3 4 5 6 7 8 9 10 11 12 13
J
Actinomycin D µM
0.0005 0.001 0.025 0.005 0.01 ctrl 2
47/45S
41S
32S
28S
18S
28S EtBr
1 2 3 4 5 6
Y (continued)
Cycloheximide [µg/ml] 47S/45S 28S 18S
100%
50%
0%
ctrl 0.11 0.22 0.44 0.88 1.75 3.52 7.03 14.06 28.13
Actinomycin D [µM] 47S/45S 28S
ctrl 0.0005 0.001 0.0025 0.005 0.01
K
Rapamycin µM
0.195 0.391 0.781 1.56 3.13 6.25 12.5 25 50 ctrl 1 ctrl 2
47/45S
41S
32S
28S
18S
28S EtBr
1 2 3 4 5 6 7 8 9 10 11
L
Sorafenib µM
0.003 0.006 0.012 0.024 0.049 0.098 0.195 0.391 0.781 1.56 3.13 6.25 ctrl 1 ctrl 2
47/45S
41S
32S
28S
18S
28S EtBr
1 2 3 4 5 6 7 8 9 10 11 12 13 14
Rapamycin [µM] 47S/45S 28S
ctrl 0.195 0.391 0.781 1.56 3.13 6.25 12.5 25 50
Sorafenib [µM] 47S/45S 28S
ctrl 0.024 0.049 0.098 0.195 0.391 0.781 1.56 3.13 6.25
M
Etoposide µM
1 5 10 25 50 100 ctrl 1 ctrl 2
47/45S
41S
32S
28S
18S
28S EtBr
1 2 3 4 5 6 7 8
N
Vinblastine µM
0.1 0.5 2.5 5 10 20 50 100 ctrl 1 ctrl 2
47/45S
41S
32S
28S
18S
28S EtBr
1 2 3 4 5 6 7 8 9 10
Etoposide [µM] 47S/45S 28S
ctrl 1 5 10 25 50 100
Vinblastine [µM] 47S/45S 28S
ctrl 0.1 0.5 2.5 5 10 20 50 100
O
Campthotecin µM
0.195 0.391 0.781 1.56 3.13 6.25 12.5 25 50 100 ctrl 1 ctrl 2
47/45S
41S
32S
28S
18S
28S EtBr
1 2 3 4 5 6 7 8 9 10 11 12
P
Irinotecan µM
0.098 0.195 0.391 0.781 1.56 3.13 6.25 12.5 25 50 ctrl 1 ctrl 2
47/45S
41S
32S
28S
18S
28S EtBr
1 2 3 4 5 6 7 8 9 10 11 12
Camptothecin [µM] 47S/45S 28S
ctrl 0.195 0.391 0.781 1.56 3.13 6.25 12.5 25 50
Irinotecan [µM] 47S/45S 28S
ctrl 0.195 0.391 0.781 1.56 3.13 6.25 12.5 25 50

Figure S2 I-P

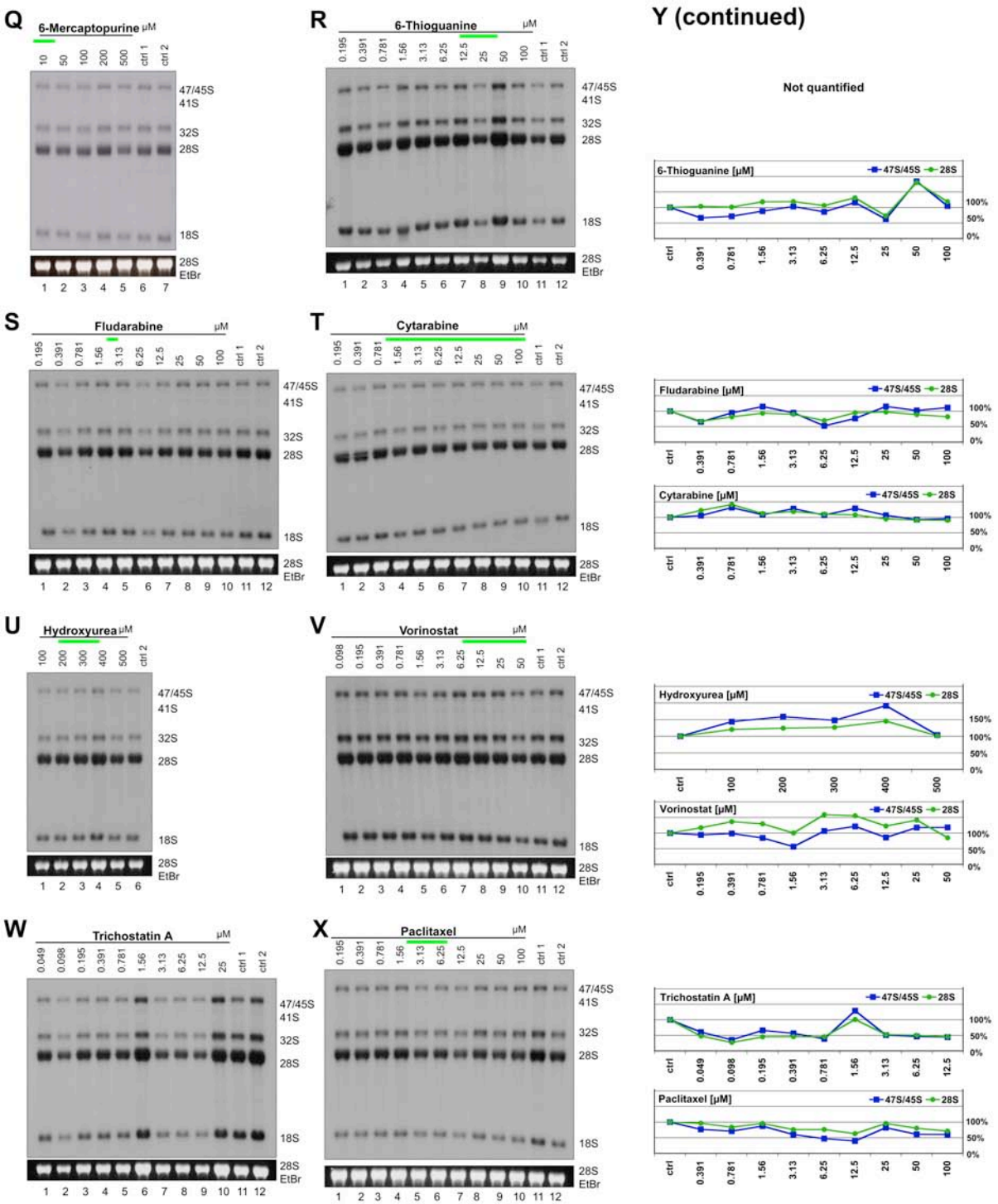
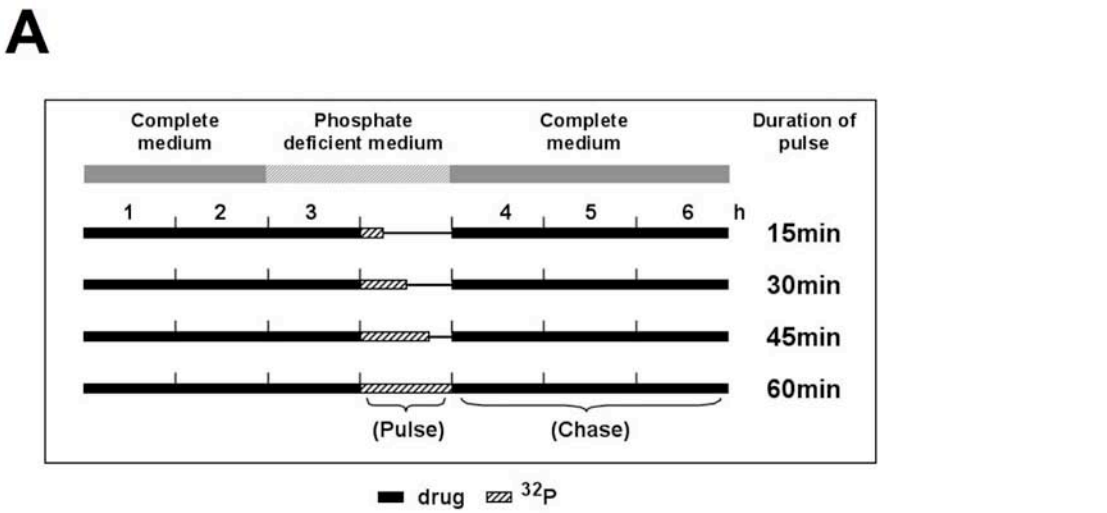

Q
6-Mercaptopurine µM
10 50 100 200 500 ctrl 1 ctrl 2
47/45S
41S
32S
28S
18S
28S EtBr
1 2 3 4 5 6 7
R
6-Thioguanine µM
0.195 0.391 0.781 1.56 3.13 6.25 12.5 25 50 100 ctrl 1 ctrl 2
47/45S
41S
32S
28S
18S
28S EtBr
1 2 3 4 5 6 7 8 9 10 11 12
Y (continued)
Not quantified
6-Thioguanine [µM]
47S/45S
28S
100%
50%
0%
ctrl 0.391 0.781 1.56 3.13 6.25 12.5 25 50 100
S
Fludarabine µM
0.195 0.391 0.781 1.56 3.13 6.25 12.5 25 50 100 ctrl 1 ctrl 2
47/45S
41S
32S
28S
18S
28S EtBr
1 2 3 4 5 6 7 8 9 10 11 12
T
Cytarabine µM
0.195 0.391 0.781 1.56 3.13 6.25 12.5 25 50 100 ctrl 1 ctrl 2
47/45S
41S
32S
28S
18S
28S EtBr
1 2 3 4 5 6 7 8 9 10 11 12
Fludarabine [µM]
47S/45S
28S
100%
50%
0%
ctrl 0.391 0.781 1.56 3.13 6.25 12.5 25 50 100
Cytarabine [µM]
47S/45S
28S
100%
50%
0%
ctrl 0.391 0.781 1.56 3.13 6.25 12.5 25 50 100
U
Hydroxyurea µM
100 200 300 400 500 ctrl 2
47/45S
41S
32S
28S
18S
28S EtBr
1 2 3 4 5 6
V
Vorinostat µM
0.098 0.195 0.391 0.781 1.56 3.13 6.25 12.5 25 50 ctrl 1 ctrl 2
47/45S
41S
32S
28S
18S
28S EtBr
1 2 3 4 5 6 7 8 9 10 11 12
Hydroxyurea [µM]
47S/45S
28S
150%
100%
50%
0%
ctrl 100 200 300 400 500
Vorinostat [µM]
47S/45S
28S
100%
50%
0%
ctrl 0.195 0.391 0.781 1.56 3.13 6.25 12.5 25 50
W
Trichostatin A µM
0.049 0.098 0.195 0.391 0.781 1.56 3.13 6.25 12.5 25 ctrl 1 ctrl 2
47/45S
41S
32S
28S
18S
28S EtBr
1 2 3 4 5 6 7 8 9 10 11 12
X
Paclitaxel µM
0.195 0.391 0.781 1.56 3.13 6.25 12.5 25 50 100 ctrl 1 ctrl 2
47/45S
41S
32S
28S
18S
28S EtBr
1 2 3 4 5 6 7 8 9 10 11 12
Trichostatin A [µM]
47S/45S
28S
100%
50%
0%
ctrl 0.049 0.098 0.195 0.391 0.781 1.56 3.13 6.25 12.5
Paclitaxel [µM]
47S/45S
28S
100%
50%
0%
ctrl 0.391 0.781 1.56 3.13 6.25 12.5 25 50 100

Figure S2 Q-X


A
Complete medium
Phosphate deficient medium
Complete medium
Duration of pulse
1
2
3
4
5
6
h
15min
30min
45min
60min
(Pulse)
(Chase)
drug
32P

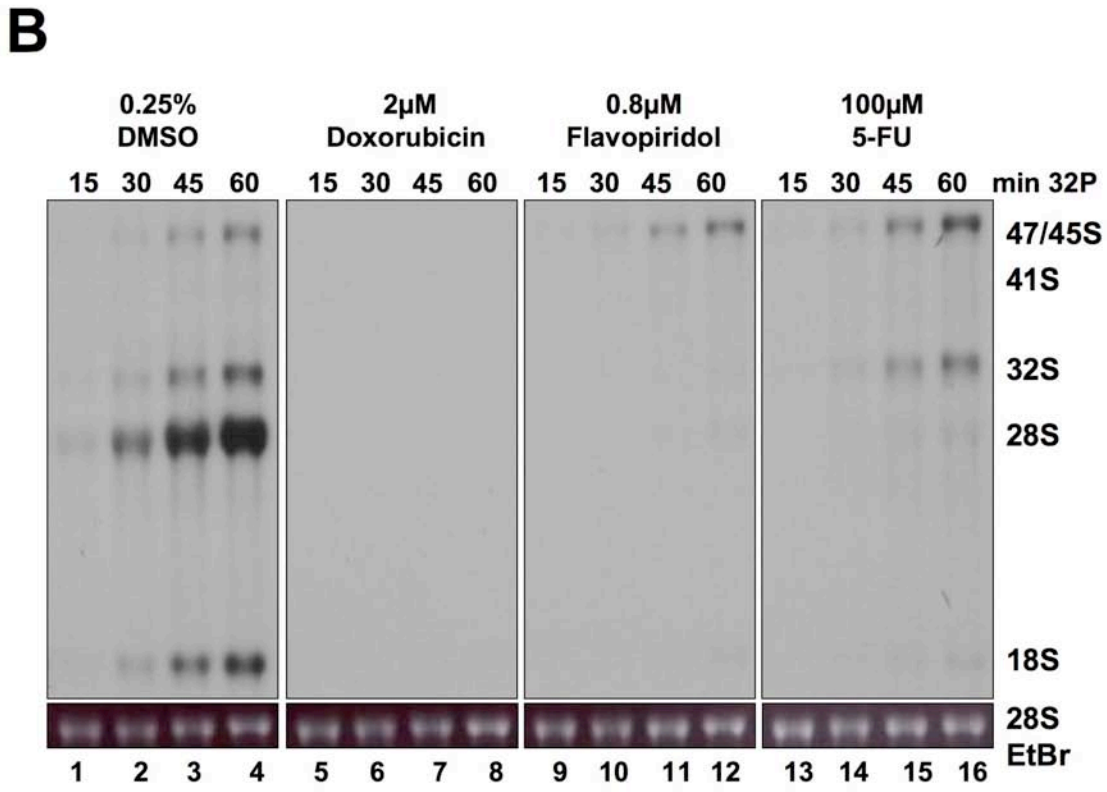

B
0.25% DMSO
2μM Doxorubicin
0.8μM Flavopiridol
100μM 5-FU
15 30 45 60
min 32P
47/45S
41S
32S
28S
18S
28S
EtBr
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16

Figure S3

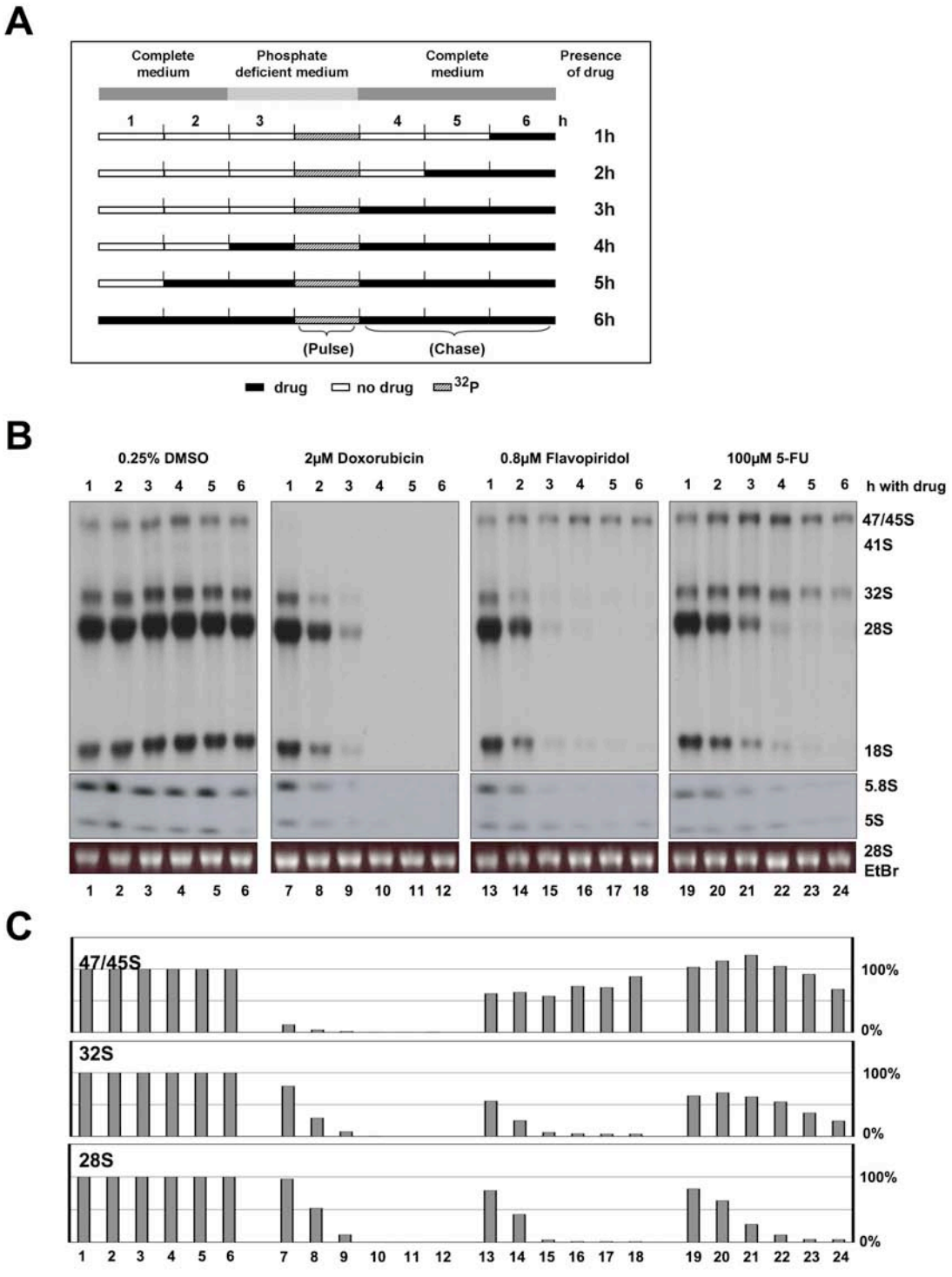

A
Complete medium
Phosphate deficient medium
Complete medium
Presence of drug
1 2 3 4 5 6 h
1h
2h
3h
4h
5h
6h
(Pulse)
(Chase)
drug
no drug
32P
B
0.25% DMSO
2μM Doxorubicin
0.8μM Flavopiridol
100μM 5-FU
h with drug
47/45S
41S
32S
28S
18S
5.8S
5S
28S EtBr
C
47/45S
32S
28S
100%
0%

Figure S4

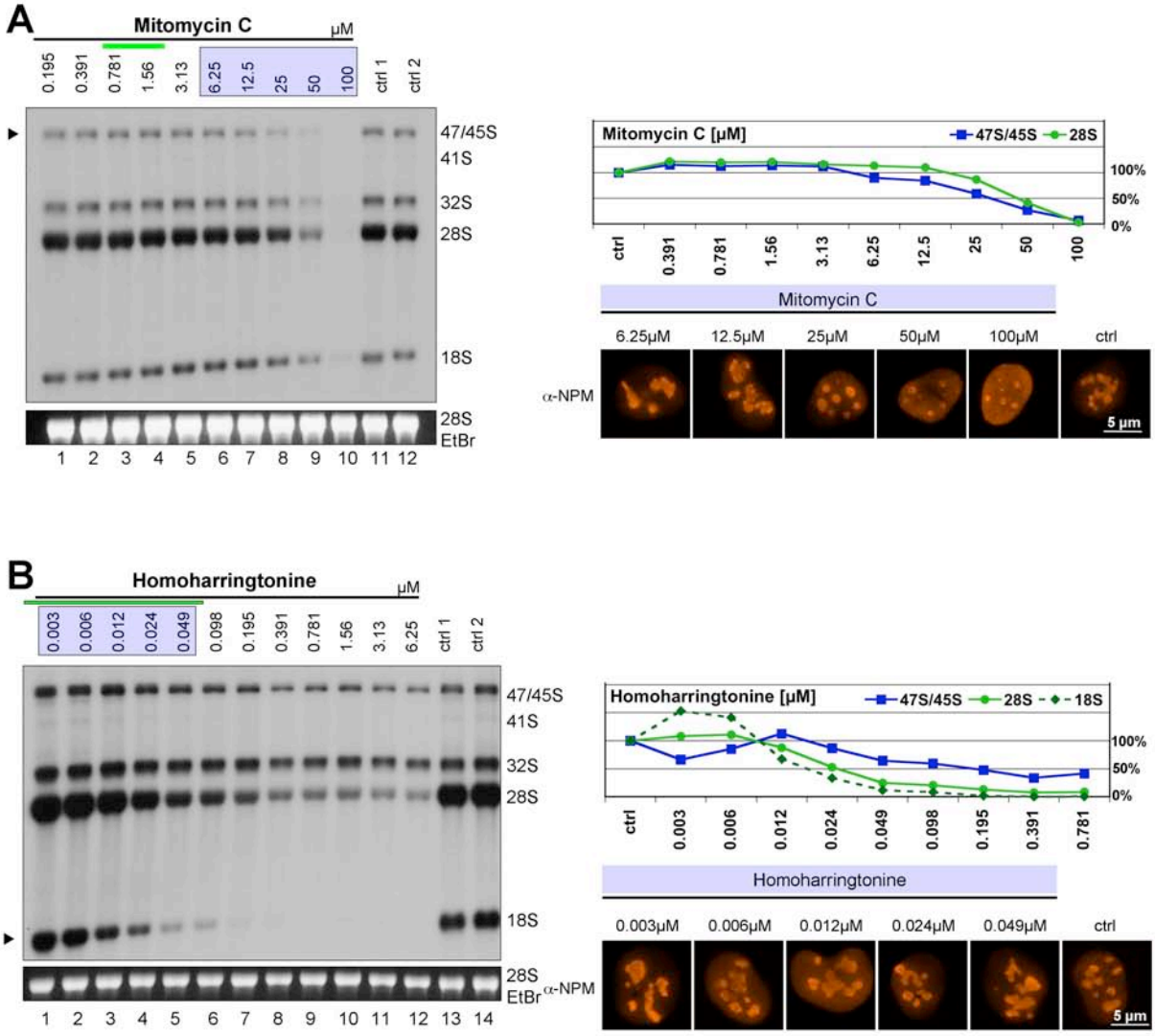

A
Mitomycin C
µM
0.195
0.391
0.781
1.56
3.13
6.25
12.5
25
50
100
ctrl 1
ctrl 2
47/45S
41S
32S
28S
18S
28S
EtBr
1 2 3 4 5 6 7 8 9 10 11 12
Mitomycin C [µM]
47S/45S
28S
100%
50%
0%
ctrl
0.391
0.781
1.56
3.13
6.25
12.5
25
50
100
Mitomycin C
6.25µM
12.5µM
25µM
50µM
100µM
ctrl
α-NPM
5 µm
B
Homoharringtonine
µM
0.003
0.006
0.012
0.024
0.049
0.098
0.195
0.391
0.781
1.56
3.13
6.25
ctrl 1
ctrl 2
47/45S
41S
32S
28S
18S
28S
EtBr
α-NPM
1 2 3 4 5 6 7 8 9 10 11 12 13 14
Homoharringtonine [µM]
47S/45S
28S
18S
100%
50%
0%
ctrl
0.003
0.006
0.012
0.024
0.049
0.098
0.195
0.391
0.781
Homoharringtonine
0.003µM
0.006µM
0.012µM
0.024µM
0.049µM
ctrl
5 µm

Figure S5

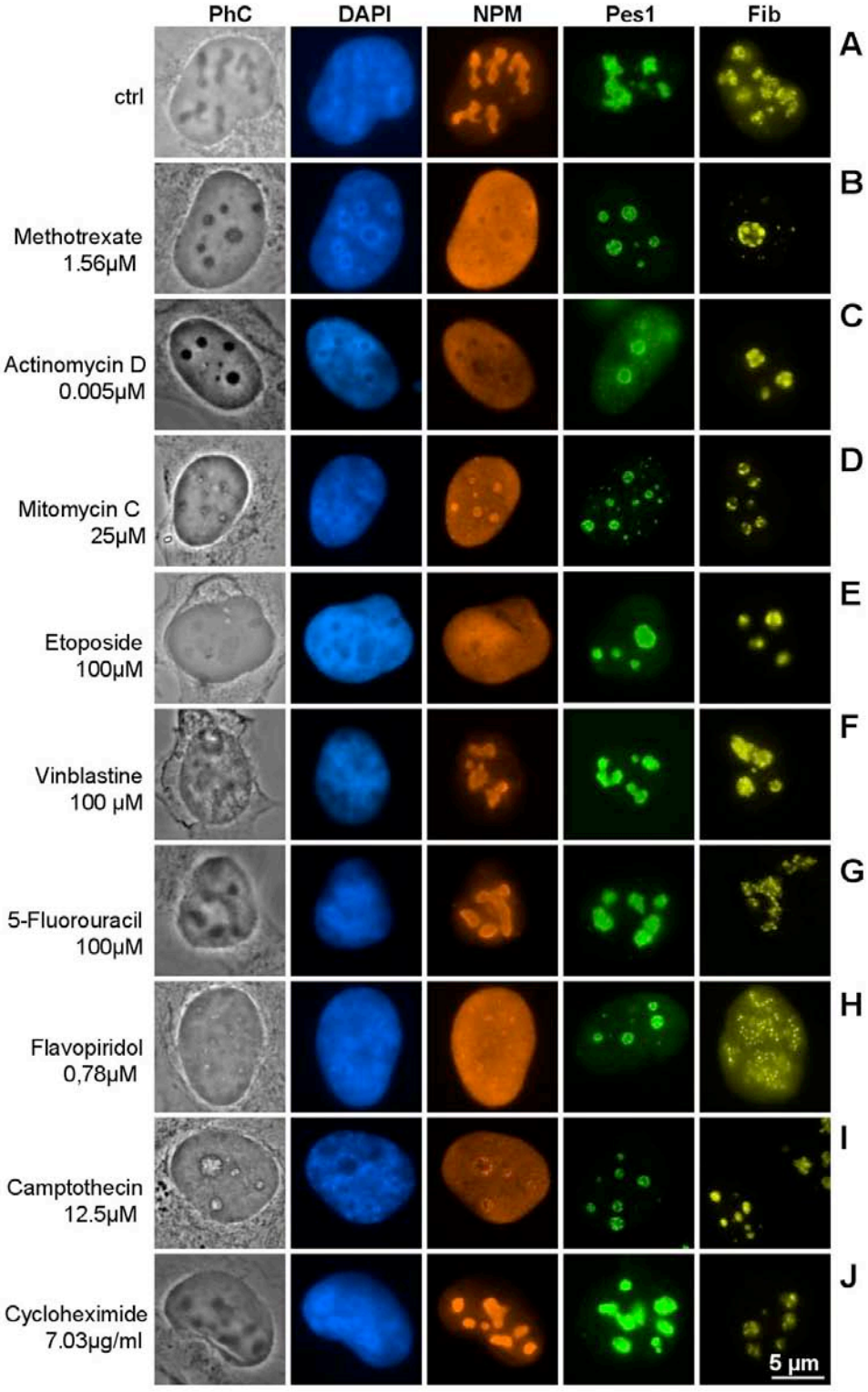

PhC
DAPI
NPM
Pes1
Fib
ctrl
A
Methotrexate
1.56μM
B
Actinomycin D
0.005μM
C
Mitomycin C
25μM
D
Etoposide
100μM
E
Vinblastine
100 μM
F
5-Fluorouracil
100μM
G
Flavopiridol
0,78μM
H
Camptothecin
12.5μM
I
Cycloheximide
7.03μg/ml
J
5 μm

Figure S6

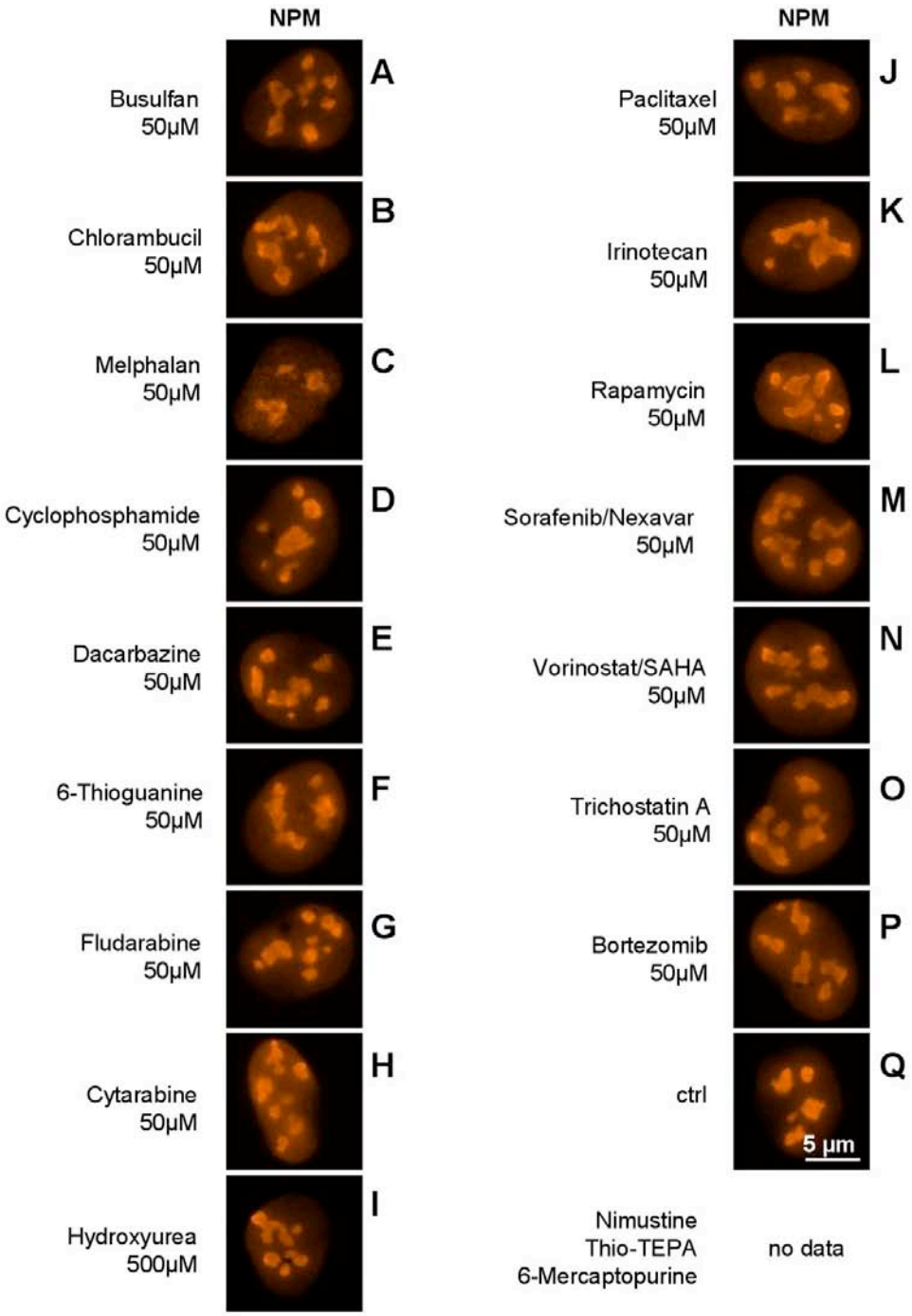

NPM
Busulfan 50µM
A
Chlorambucil 50µM
B
Melphalan 50µM
C
Cyclophosphamide 50µM
D
Dacarbazine 50µM
E
6-Thioguanine 50µM
F
Fludarabine 50µM
G
Cytarabine 50µM
H
Hydroxyurea 500µM
I
NPM
Paclitaxel 50µM
J
Irinotecan 50µM
K
Rapamycin 50µM
L
Sorafenib/Nexavar 50µM
M
Vorinostat/SAHA 50µM
N
Trichostatin A 50µM
O
Bortezomib 50µM
P
ctrl
Q
5 µm
Nimustine
Thio-TEPA
6-Mercaptopurine
no data

Figure S7