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Supplemental Material to:

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Supplementary data

4-thiouridine inhibits ribosomal RNA synthesis and causes a nucleolar stress response*

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*Running title: Impact of 4sU on rRNA metabolism

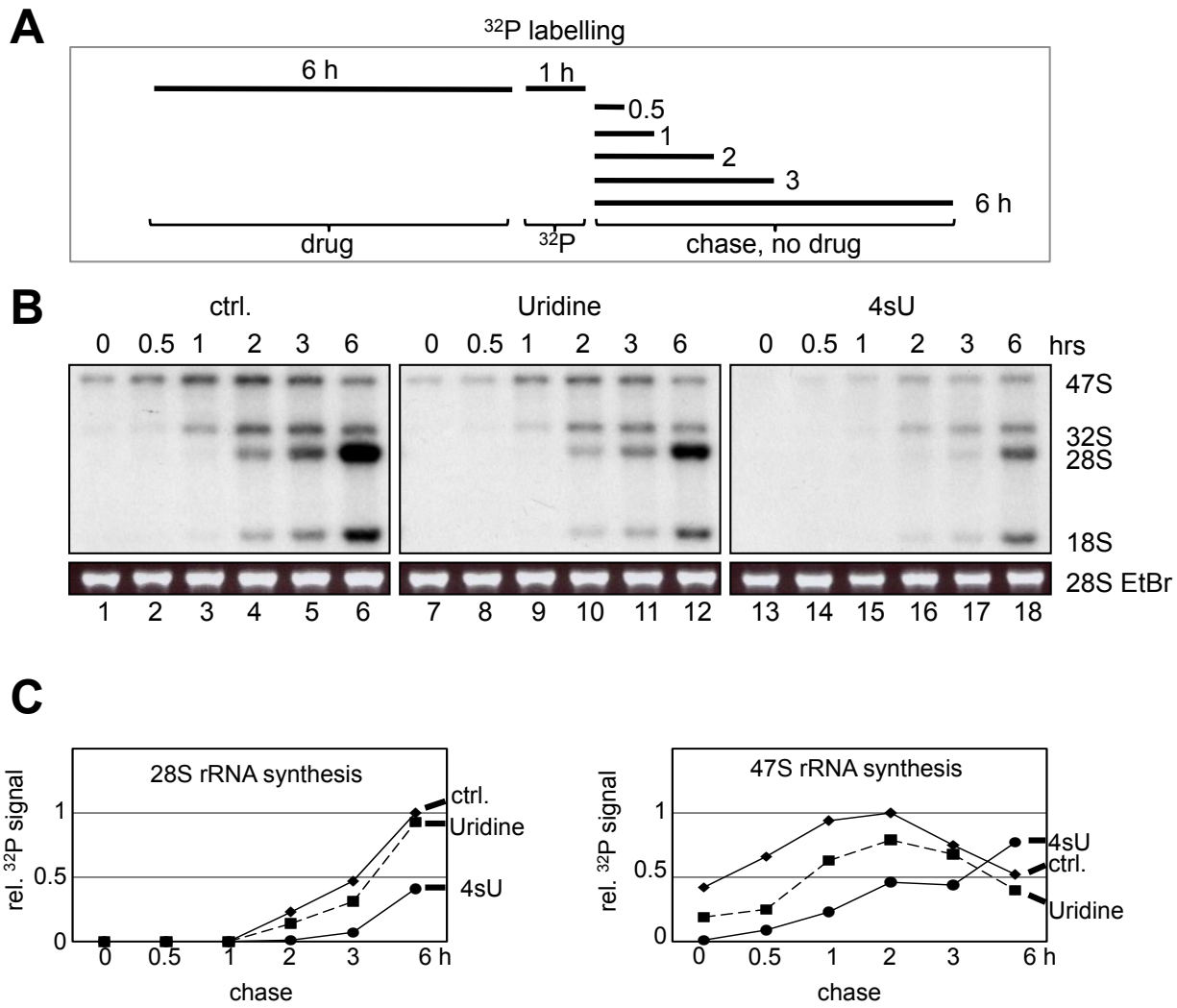

Figure S1. Inhibition of rRNA production and processing by 4sU. A Scheme of ^{32}P *in vivo* metabolic labelling workflow. U2OS cells were incubated with uridine (100 µM) or 4sU (100 µM) for six hours. ^{32}P was added for one hour (pulse) and cells were incubated for different chase times as indicated. *B* Inhibition of rRNA synthesis by 4sU. rRNA synthesis was analysed as described in *Materials and Methods*. ctrl.: 0.1 % DMSO. *C* Quantitation of rRNA signals from *B*. Signals were measured by a PhosphorImager and plotted as rRNA signals relative to 47S (lane 4) or 28S (lane 6) rRNA signals from control cells (0.1 % DMSO) set as one. (Drug: uridine or 4sU)

Figure S1

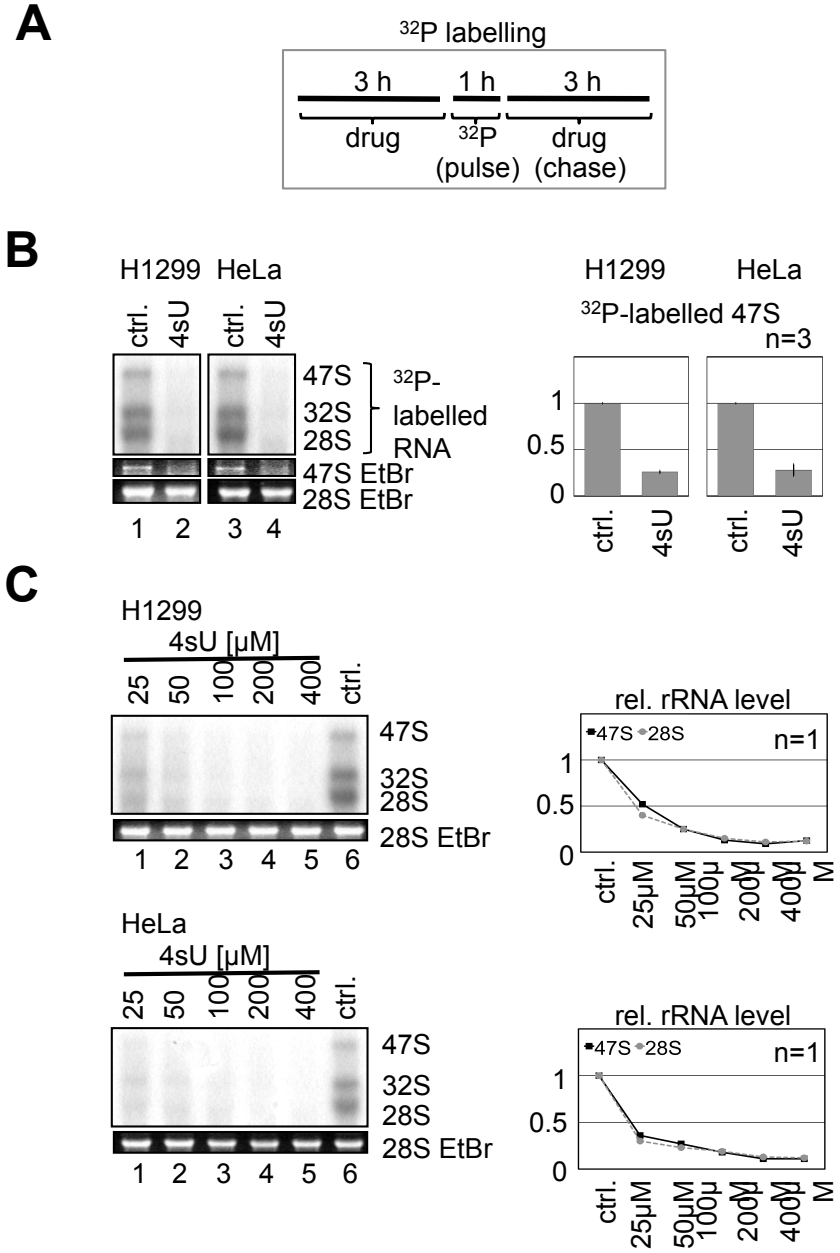

Figure S2. Inhibition of rRNA production and processing by 4sU treatment. (**A**) H1299 or HeLa cells were treated with 4sU (100 µM) and nascent RNA was labelled by [^{32}P]-ortho-phosphate as indicated. (**B**) ^{32}P-labelled RNA was visualised by autoradiography, total RNA was visualised by ethidium bromide (EtBr) staining under UV-light. ^{32}P-labelled rRNA signals were measured by a PhosphorImager and AIDA and ratios were plotted relative to signals from control cells (0.1 % DMSO). (**C**) H1299 or HeLa cells were incubated with increasing concentrations of 4sU as indicated. Signals were quantified by a PhosphorImager and plotted as relative rRNA levels compared to control cells. (Drug: 4sU)

Figure S2

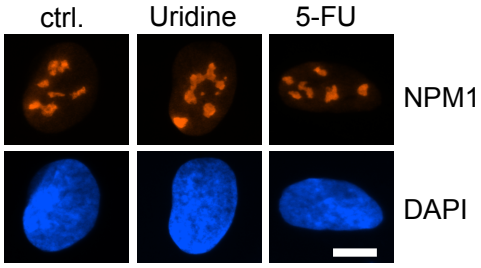

Figure S3. Analysis of nucleophosmin (NPM1) localisation in presence of uridine or 5-FU. U2OS cells were treated with uridine (100 µM) or 5-FU (100 µM) for six hours. NPM1 localisation was analysed by immunofluorescence analysis using a specific antibody. Nuclei were stained with DAPI. Scale bar: 1 µm.

Figure S3

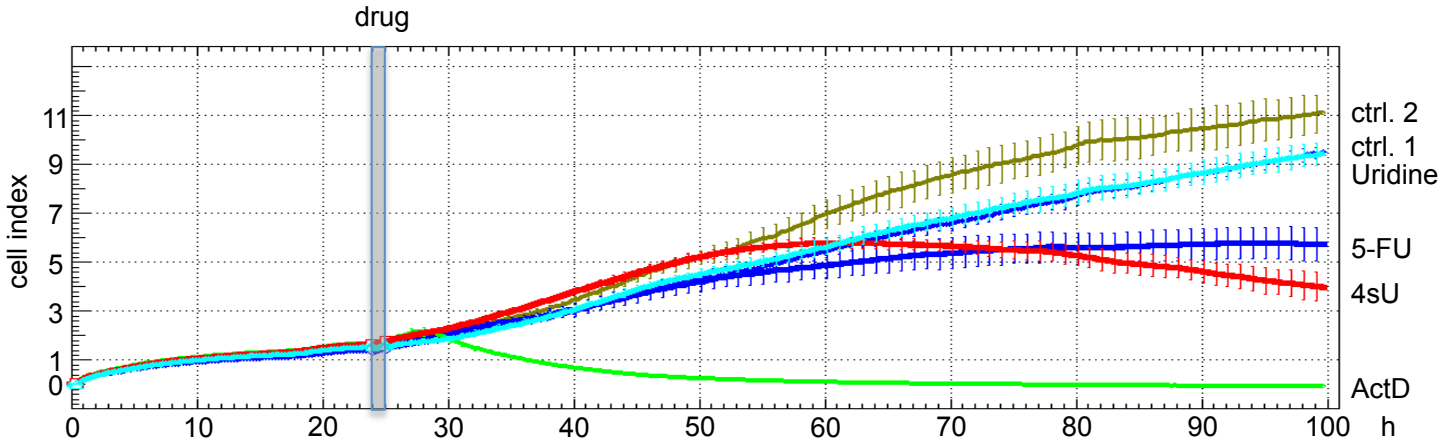

Figure S4. Analysis of proliferation upon 4sU treatment. U2OS cells were seeded and cultured over night. Drugs (uridine, 100 µM; 4sU 100 µM; 5-FU 100 µM; ActD 1 µM) were added for one hour (grey box), medium was replaced and cells were cultured for 100 hours. Cell number was measured in real time and compared to control cells (DMEM, ctrl. 1; 0.1% DMSO, ctrl. 2). The cell number correlates to changes in impedance, which is termed 'Cell index' (see details in *Material and Methods*).

Figure S4