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SUPPLEMENTARY INFORMATION

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Use of the iNo score to discriminate normal from altered nucleolar morphology, with applications in basic cell biology and potential in human disease diagnostics

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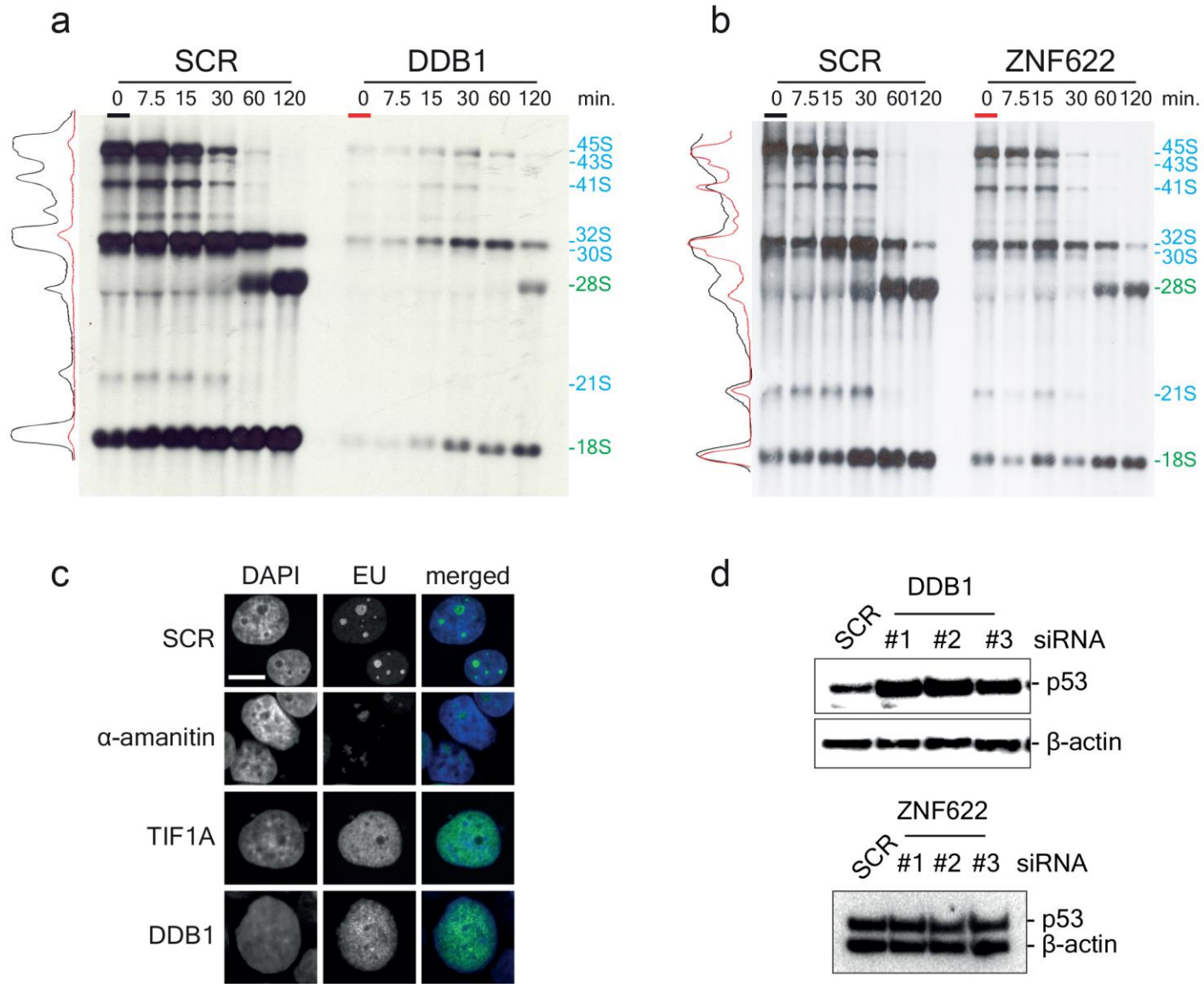

Supplementary Figure 1

Analysis of the role of DDB1 and ZNF622 in pre-rRNA synthesis, pre-rRNA processing, and nucleolar stress activation

a, b. Dynamic analysis of pre-rRNA synthesis and processing by metabolic pulse-chase labeling. Cells treated for 3 days with an siRNA targeting DDB1 (**a**), or ZNF622 (**b**) , or with a non-targeting SCR siRNA control were labeled with tritiated methionine for 30 min and the label was then "chased", by incubating cells with non-radioactive methionine, for the indicated times. At each time point, total RNA was extracted, resolved by denaturing agarose-gel electrophoresis and analyzed by fluorography. In order to compare RNA synthesis under different conditions, the signal was quantitated with a phosphor imager at the 0-min time point (black profile for cells treated with SCR siRNA, red profiles for DDB1-depleted and ZNF622-depleted cells). Pre-rRNA intermediates (labelled in cyan) and mature rRNAs (the 18S and 28S rRNAs, in green) are indicated. Depletion of DDB1 expression strongly influences the accumulation of the high-molecular-weight RNA species (45S, 43S, 41S). This observation confirms the PCA-based prediction that this protein is required for RNA synthesis (see also flat profile in red). By comparison, ZNF622 expression depletion influences RNA synthesis only marginally. Pre-rRNA processing remains active after

depletion of either proteins (the mature rRNAs 18S and 28S are produced) but it is severely delayed after depletion of DDB1. **c,** *In situ* analysis of RNA synthesis by metabolic labeling. Cells treated for 3 days with an siRNA targeting TIF1A or DDB1 or with a non-targeting SCR siRNA control were incubated for 1 h with 5-ethynyl uridine (EU). EU-labeled RNAs were detected by chemoselective ligation ('click' chemistry). In cells treated with SCR, intense EU signals are detected in the DAPI-counterstained nucleoli (here the signal corresponds to robust RNA Pol I activity) and the DAPI-stained nucleoplasm (where the signal corresponds largely to transcription by RNA Pol II). In cells depleted of TIF1A or DDB1, the nucleolar signal is lost and only the nucleoplasm displays an EU signal. In control cells incubated for 2 h with α-amanitin, which inhibits RNA Pol II, the nucleoplasmic signal is lost while the nucleolar staining remains. DNA was labeled with DAPI. Scale bar: 10 μm. **d**, nucleolar stress activation assessed by western-blot detection of the p53 steady-state level. Three independent siRNAs (#1, #2, #3) were used to deplete the expression of target proteins DDB1 or ZNF622 for 3 days. DDB1 depletion, which severely inhibits rRNA synthesis (see panels a and c), leads to a marked activation of nucleolar stress (p53 accumulation). By contrast, ZNF622 depletion, which only marginally affects pre-rRNA synthesis and processing (panel b), does not activate nucleolar stress. As loading controls, blots were probed for β-actin.