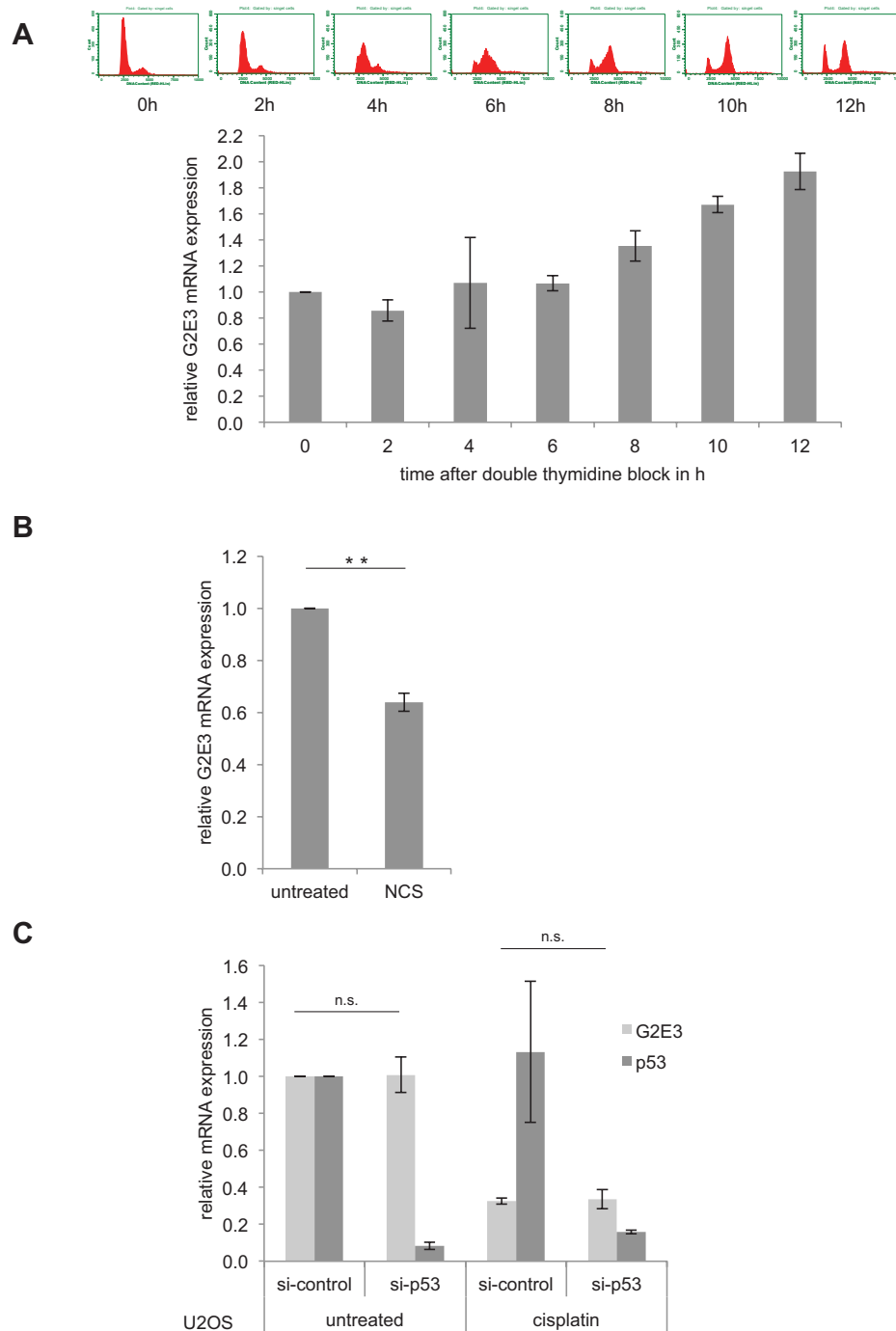


SUPPLEMENTARY FIGURE AND TABLE



Supplementary Figure S7: Regulation of G2E3 mRNA levels. (A) G2E3 levels are at their maximum in the G2/M phase of the cell cycle. U2OS cells were arrested by a double thymidine block and then released. Cell cycle progression was monitored by flow cytometry of propidium iodide stained cells for 12 h after release from the block. The results in the upper panel are representative of three independent replicates. G2E3 mRNA levels were analyzed by quantitative RT-PCR at each time point. Results were normalized to the reference gene GAPDH. Data are represented as mean. Error bars represent the standard deviation (SD, $n = 3$). (B) G2E3 mRNA levels are decreased after neocarzinostatin treatment. U2OS cells were transfected with control-siRNA and either left untreated or treated with 150 ng/ml neocarzinostatin (NCS) for 2 h. Cells were harvested and G2E3 mRNA levels were analyzed by quantitative RT-PCR and normalized to the reference gene GAPDH. Data are represented as mean. Error bars represent the standard deviation (SD, $n = 3$). $**p < 0.01$, (Student's t -test). (C) Endogenous G2E3 mRNA levels are decreased after DNA damage, independent of p53. U2OS cells were transfected with control- and p53-siRNA and treated with 30 μ M cisplatin for 16 h. The cells were harvested and G2E3 and p53 mRNA levels were analyzed by quantitative RT-PCR as in B. Error bars represent the standard error of the mean (SEM, $n = 3$). n.s. = not significant (Student's t -test).

Supplementary Table S1: Results from the siRNA screen to identify modulators of the cellular response to cisplatin. U2OS cells were transfected with a library containing siRNAs against 327 human ubiquitin ligases and 92 deubiquitinating enzymes with three different siRNAs per target gene. The cells were treated with 30 μ M cisplatin for 16 h, fixed and stained for γ H2AX. Automated microscopy and image analysis was performed using the BD Pathway System. A robust z-score was assigned to each siRNA as a measure of H2AX phosphorylation. Genes are sorted by the average robust z-score of the three siRNAs. PC = positive control.