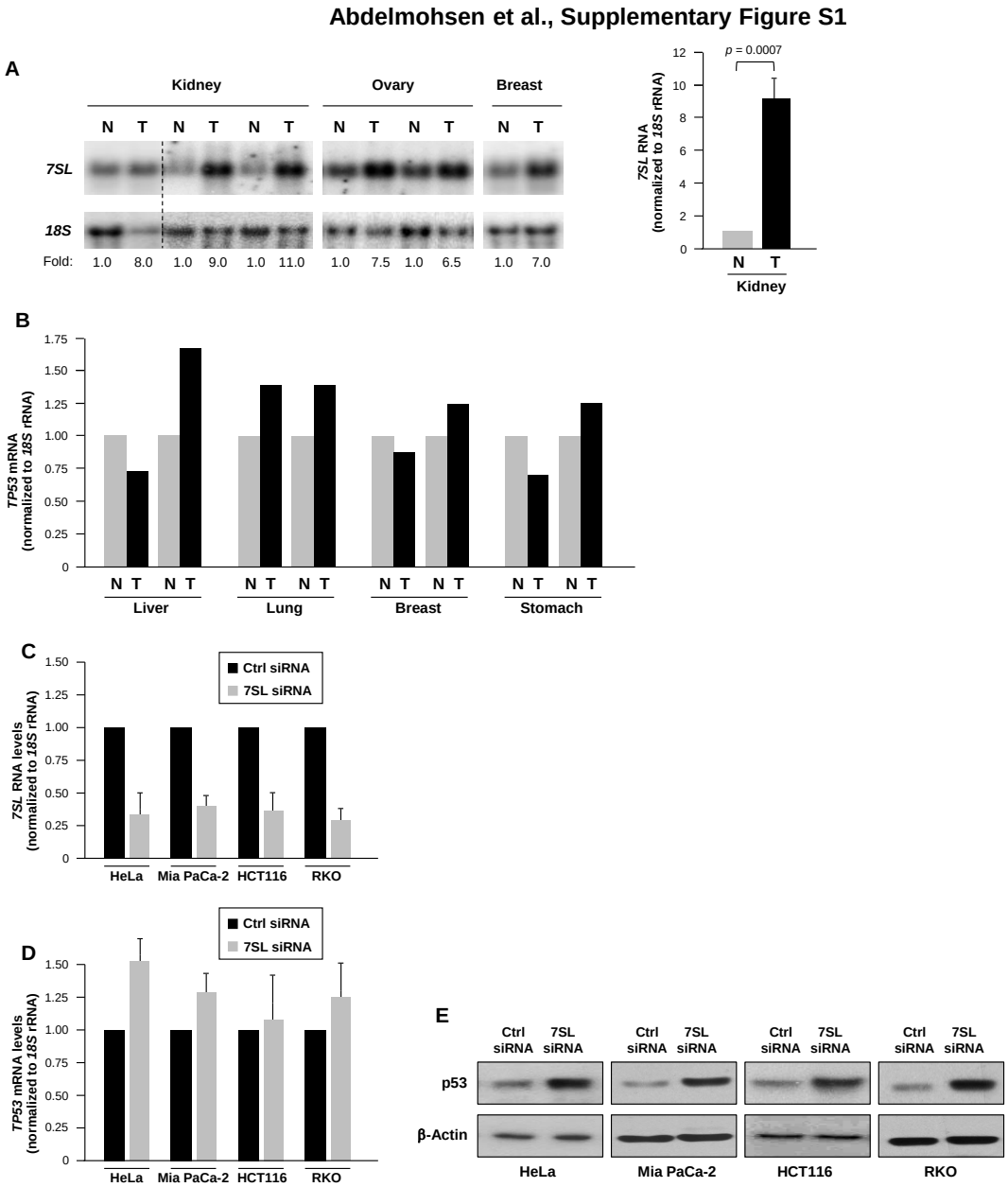
SUPPLEMENTARY RESULTS

Effect of *7SL* silencing on cell toxicity

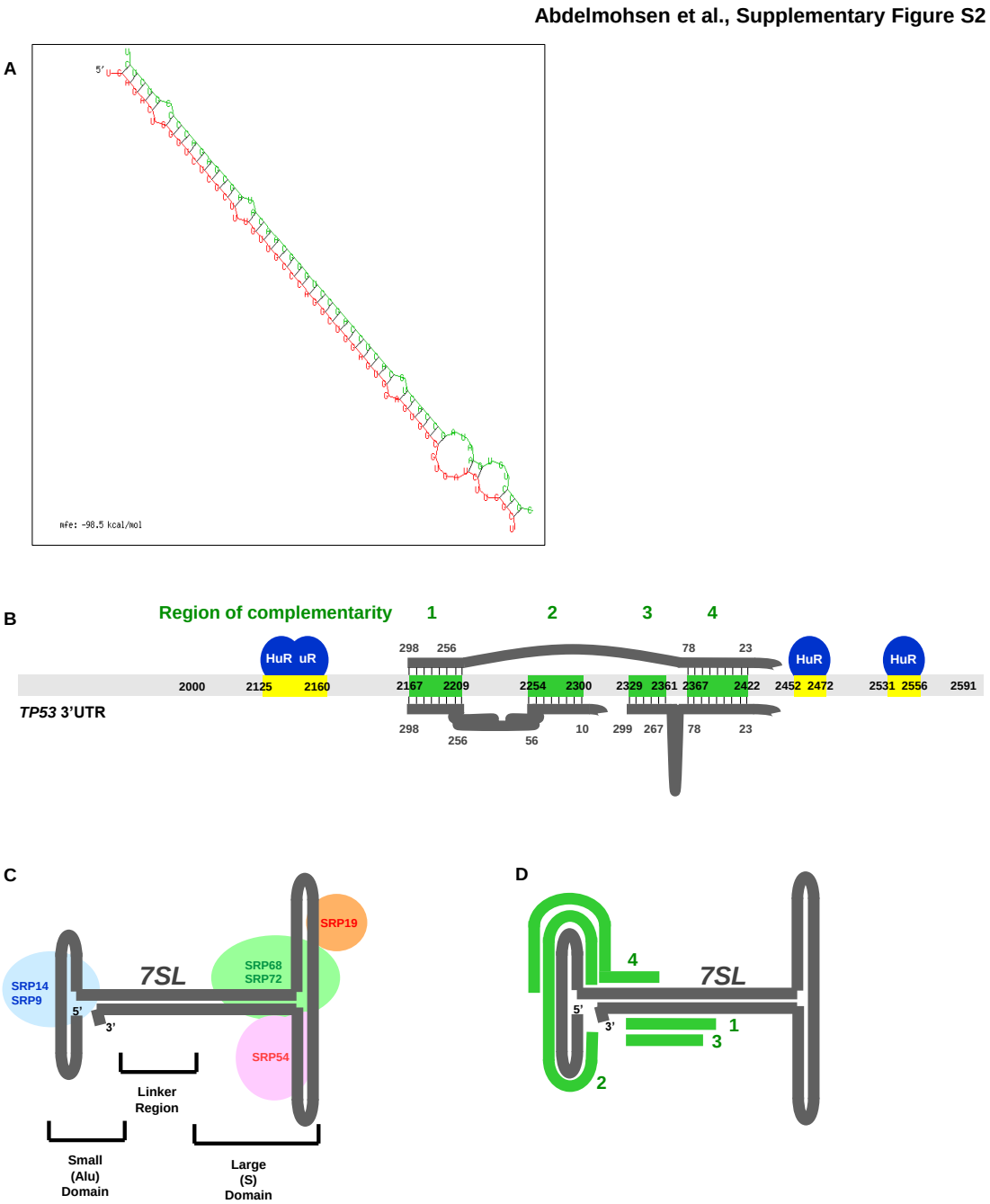
Since prolonged silencing of *7SL* causes cell death, we tested the possibility that the upregulation of p53 was due to cell toxicity caused by lowering *7SL*. At earlier time points after transfection of 7SL siRNA, *7SL* abundance was quantified by RT-qPCR (Fig. S3A), HuR binding to *TP53* mRNA was assessed by RIP analysis (Fig. S3B), and cell viability was evaluated by counting live cells (Fig. S3C). As shown, toxicity was negligible at 12 and 24 h after transfection (Fig. S3C), even though *7SL* abundance was already reduced to ~50% of the initial levels (Fig. S3A), and HuR binding to *TP53* mRNA had already more than doubled (Fig. S3B). These findings support the view that the increase in p53 levels was not indirectly due to toxicity derived from lowering *7SL* levels, since the decline in *7SL* amply precedes HeLa cell toxicity.

SUPPLEMENTARY TABLE LEGEND

Supplementary Table 1. The human transcriptome was examined to identify mRNAs showing at least 30 nucleotide-long matches with *7SL*. The positions of *7SL* and the target mRNA in these putative hybrids are indicated; the location of the coding region and 3'UTR for each mRNA, as well as the location of HuR PAR-CLIP sites are also indicated. These interactions will be investigated separately.

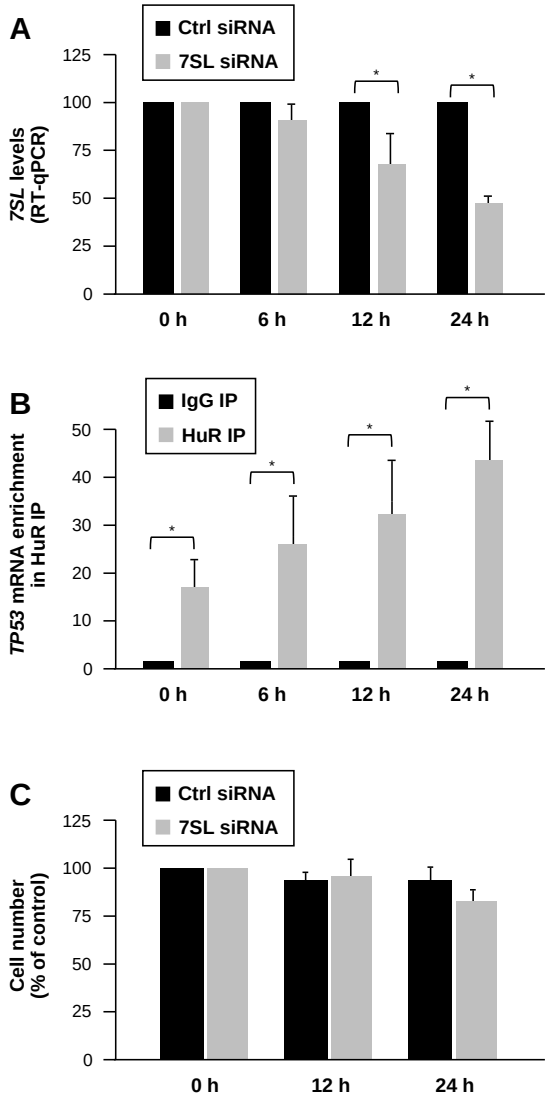


Supplementary Figure S1. (A) The abundance of *7SL* in the tumor tissues (T) indicated (kidney, ovary, breast) relative to adjacent normal tissues (N) was measured by Northern blot analysis and normalized to *18S* rRNA levels (right). Samples were obtained from BioChain Institute, Inc. (Breast and first pair of N and T Kidney tissues) or from the University of Maryland tissue bank (Ovary and remaining Kidney tissues). *Right*, for kidney, three pairs of (N,T) samples were available, and therefore the means and standard deviation (S.D.) were calculated and plotted; *p*-value is indicated. **(B)** The abundance of *TP53* mRNA in (T) tissues (liver, lung, breast, stomach) and adjacent normal tissues (N) was measured by RT-qPCR analysis and normalized to *18S* rRNA levels. **(C-E)** Forty-eight h after Ctrl siRNA or 7SL siRNA transfection of HeLa, Mia PaCa-2, HCT116, or RKO cells, the levels of *7SL* RNA (C) and *TP53* mRNA (D) were assessed by RT-qPCR, normalized to *18S* rRNA, and plotted, and p53 levels were assessed by Western blot analysis (E). Data in A,C,D are the means ±S.D. from three independent experiments.



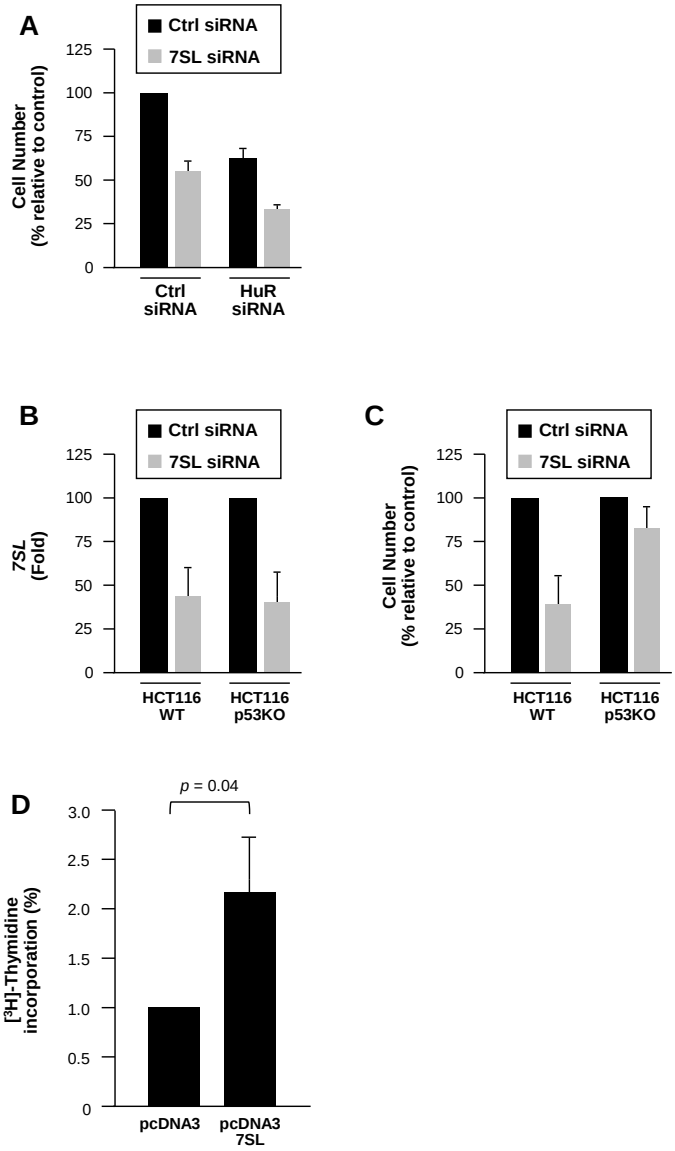
Supplementary Figure S2. (A) The putative association between *7SL* and *TP53* 3'UTR was tested using RNA hybrid (http://bibiserv.techfak.uni-bielefeld.de/rnahybrid). Red, *TP53* mRNA; green, *7SL*. **(B)** Shown are the regions of complementarity (numbered 1-4) between *7SL* (dark gray) and *TP53* 3'UTR. **(C)** The main areas of association of SRP proteins with *7SL*, as well as the main *7SL* regions ['Small (Alu) Domain', 'Linker Region', and 'Large (S) Domain'] are indicated in the schematic. **(D)** The interaction of TP53 3'UTR regions 1-4 (panel A) with *7SL* are indicated schematically.

Abdelmohsen et al., Supplementary Figure S3



Supplementary Figure S3. (A) HeLa cells were transfected with Ctrl siRNA or 7SL siRNA and the levels of *7SL* remaining at the times indicated (0, 6, 12, 24 h after transfection) were measured by RT-qPCR, normalized to *U6* RNA levels, and plotted relative (%) to *7SL* levels in the Ctrl siRNA transfection group. **(B)** RIP analysis of *TP53* mRNA levels in HuR IP (relative to IgG IP) at the times indicated after transfection of either Ctrl siRNA or 7SL siRNA. **(C)** Percentage of cells remaining in the 7SL siRNA transfection group 12 and 24 h after transfection, compared with the cells remaining in the Ctrl siRNA transfection group. Data in (A-C) are shown as the means and S.D. of three independent experiments; * denotes $p < 0.05$.

Abdelmohsen et al., Supplementary Figure S4



Supplementary Figure S4. (A) Forty-eight h after transfection of HeLa cells with indicated siRNAs, cell numbers were measured using a hemocytometer and represented as percentage of cells relative to the Ctrl siRNA group. **(B)** RT-qPCR analysis of *7SL* levels two days after transfection of HCT116 (WT or p53KO) cells either with Ctrl siRNA or 7SL siRNA. **(C)** Forty-eight hours after the transfection of HCT116 (WT or p53KO) cells with Ctrl siRNA or 7SL siRNA, cell numbers were determined by direct cell counts using a hemocytometer. **(D)** Forty-eight h after transfecting HeLa cells with plasmids pcDNA3 or pcDNA3-7SL (as described in the main Figure 2E), [^{3}H]-thymidine incorporation was measured. Data in A-D represent the means and ± SD from 3 independent experiments.