Supplementary data

Supplementary Figures.

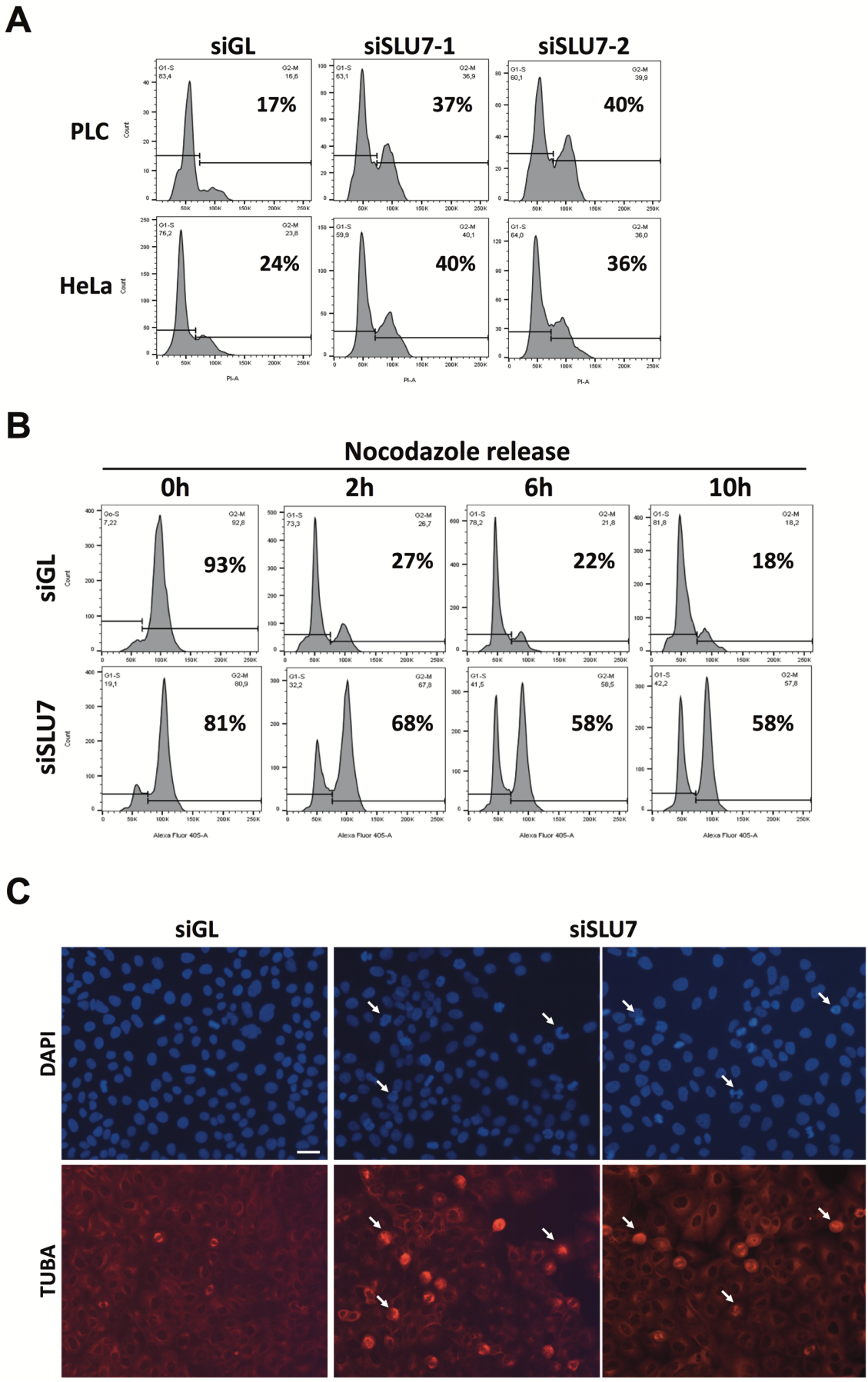

Figure S1. (**A**) Cell cycle analysis by FACS of PLC/PRF/5 and HeLa cells 48 hours after transfection with two different siRNAs specific for SLU7 and an irrelevant control siRNA siGL. The percentage of cells in G2/M is indicated. (**B**) Cell cycle analysis by FACS of PLC/PRF/5 cells 48 hours after transfection with siSLU7 or siGL and overnight treatment with nocodazole (0h) or 2, 6 and 10 hours after release from nocodazole arrest. The percentage of cells in G2/M is indicated. (**C**) Immunocytochemical staining of alpha-tubulin (TUBA) in PLC/PRF/5 cells 48 hours after transfection with the siRNA control siGL or siSLU7. DNA was stained with DAPI. Accumulation of cells in prometaphase and aberrant metaphases (pointed with arrows) are observed in siSLU7 cells. Scale bar: 10 µm. All experiments were performed at least three times with biological duplicates per condition.

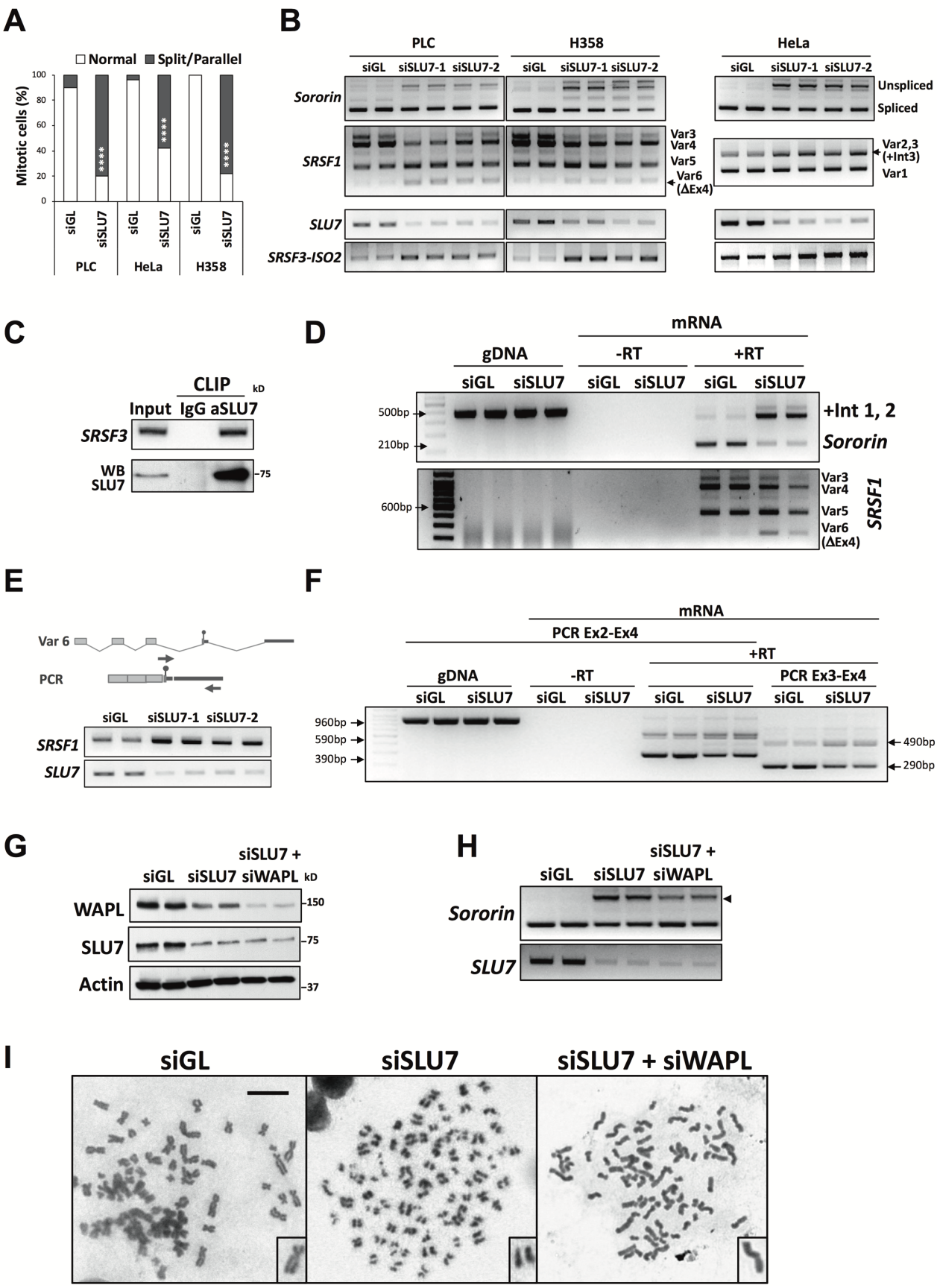
A
Normal
Split/Parallel
Mitotic cells (%)
siGL
siSLU7
PLC
HeLa
H358
B
PLC
H358
HeLa
siGL
siSLU7-1
siSLU7-2
Sororin
SRSF1
SLU7
SRSF3-ISO2
Unspliced
Spliced
Var3
Var4
Var5
Var6
(ΔEx4)
Var2,3
(+Int3)
Var1
C
CLIP
kD
Input
IgG
aSLU7
SRSF3
WB
SLU7
75
D
mRNA
gDNA
-RT
+RT
siGL
siSLU7
500bp
210bp
600bp
+Int 1, 2
Sororin
SRSF1
Var3
Var4
Var5
Var6
(ΔEx4)
E
Var 6
PCR
siGL
siSLU7-1
siSLU7-2
SRSF1
SLU7
F
mRNA
PCR Ex2-Ex4
+RT
gDNA
-RT
PCR Ex3-Ex4
siGL
siSLU7
960bp
590bp
390bp
490bp
290bp
G
siSLU7 +
siGL
siSLU7
siWAPL
kD
WAPL
SLU7
Actin
150
75
37
H
siSLU7 +
siGL
siSLU7
siWAPL
Sororin
SLU7
I
siGL
siSLU7
siSLU7 + siWAPL

Figure S2. (**A**) Quantification of the percentage of mitoses with normal or split/parallel chromosomes in chromosome spreads prepared from metaphases of PLC/PRF/5, HeLa and H358 cells 48 hours after transfection with siGL or siSLU7. ****P<0.0001. (**B**) PCR analysis of *sororin, SRSF1, SLU7* and *SRSF3-ISO2* expression in PLC/PRF/5, HeLa and H358 cells 48 hours after transfection with control siRNA (siGL) or two different siRNAs specific for SLU7. (**C**) RNA-CLIP assay demonstrates the binding of SLU7 to endogenous SRSF3 mRNA in PLC/PRF/5 cells. Upper panel: PCR analyses of SRSF3 cDNA after RNA purification and retrotranscription from the input and in the CLIP fractions after immunoprecipitation with control IgG and anti-SLU7 antibody. Lower panel: Western blot for SLU7 in the input fraction and after CLIP with control IgG and anti-SLU7 antibody (aSLU7). (**D**) PCR analysis in PLC/PRF/5 cells 48 hours after transfection with siGL or siSLU7 of sororin (PCR3 from Fig 2D) in the upper panel and SRSF1 (PCR2 from Fig 4A) in the lower panel. PCRs were performed on genomic DNA (gDNA) and on mRNA treated twice with DNAse and converted to cDNA by retro-transcription (+RT) or the same mRNA treated without adding the enzyme (-RT), to verify the absence of contaminating gDNA in the cDNA preparations. (**E**) Scheme of SRSF1 variant 6 from figure 2D and the processed mRNA. The position of the primers used to detect the expression of this isoform specifically is indicated. The lower panel confirm the induction of the variant 6 of SRSF1 defective in exon 4 in PLC/PRF/5 cells in parallel with the downregulation of SLU7 after transfection with two different siRNAs for SLU7. (**F**) Control PCRs performed to confirm the specific inclusion of intron 3 into the mRNA of *SRSF1* after SLU7 knockdown in HeLa cells. PCRs to amplify *SRSF1* were performed with primers located on exon2-exon4 or exon3-exon4 (PCR1 in Fig 2D). gDNA and mRNA samples from HeLa cells were treated as described in (C). (**G**) Western blot analysis of WAPL, SLU7 and Actin, as loading control, in PLC/PRF/5 cells 48 hours after transfection with siGL, siSLU7 or siSLU7+ siWAPL. (**H**) PCR analysis of *sororin* and *SLU7* expression in PLC/PRF/5 cells 48 hours after transfection with siGL, siSLU7 and siSLU7+ siWAPL. Arrowhead indicates the aberrant unspliced isoform. (**I**) Chromosome spreads of PLC/PRF/5 cells 48 hours after transfection with siGL, siSLU7 and siSLU7+ siWAPL. Scale bar: 10 µm. All experiments were performed at least three times with biological duplicates per condition.

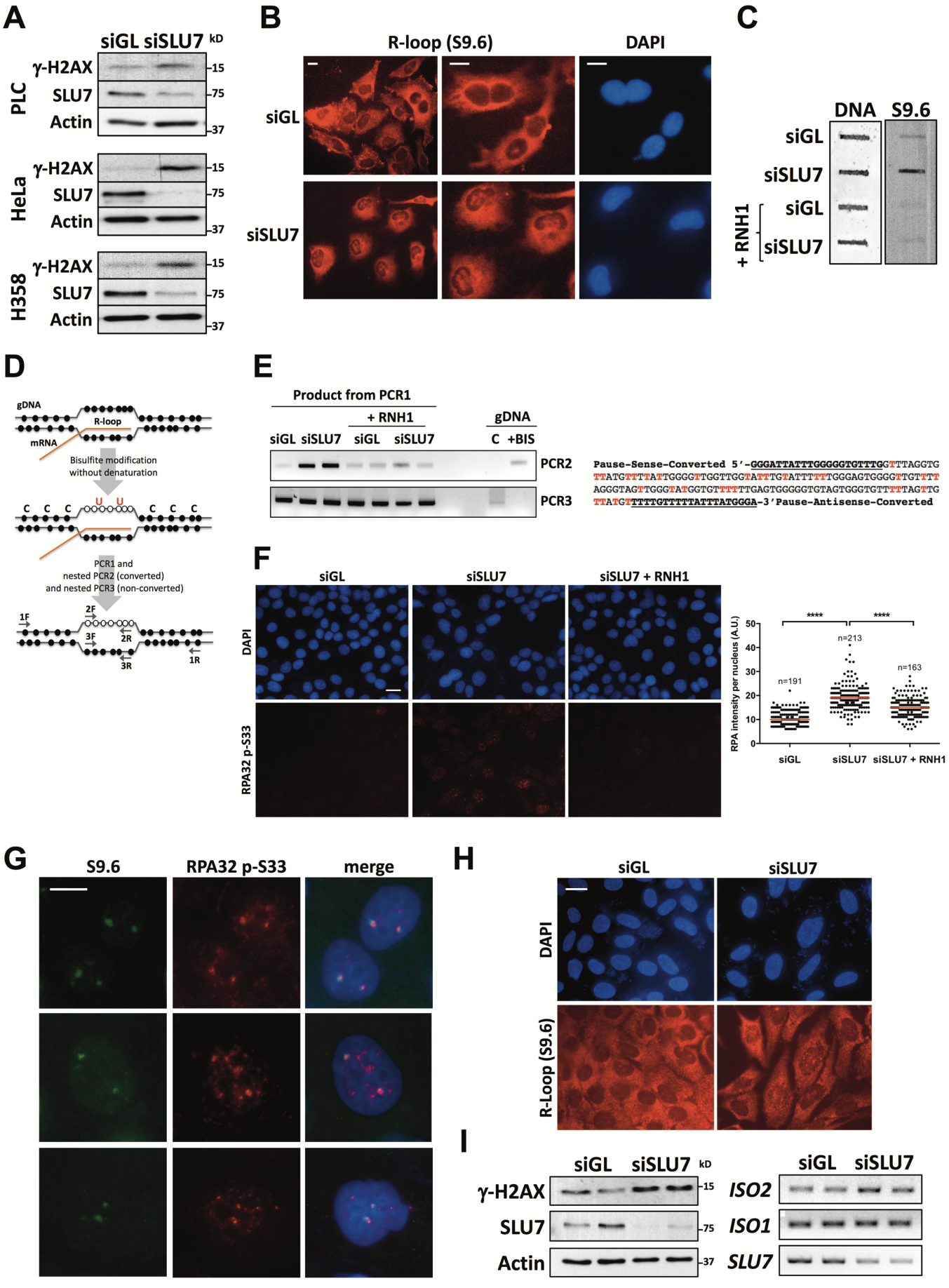
A
siGL siSLU7 kD
PLC
γ-H2AX
SLU7
Actin
HeLa
γ-H2AX
SLU7
Actin
H358
γ-H2AX
SLU7
Actin
15
75
37
B
R-loop (S9.6)
DAPI
siGL
siSLU7
C
DNA
S9.6
siGL
siSLU7
+ RNH1
siGL
siSLU7
D
gDNA
R-loop
mRNA
Bisulfite modification
without denaturation
PCR1 and
nested PCR2 (converted)
and nested PCR3 (non-converted)
1F
2F
3F
2R
3R
1R
E
Product from PCR1
+ RNH1
gDNA
siGL siSLU7 siGL siSLU7
C +BIS
PCR2
PCR3
Pause-Sense-Converted 5'-GGGATTATTTGGGGGTGTTTGCTTTAGGTG
TTATGTTTTATTGGGGTTGGTTGGTATTTGTATTTTTTGGGAGTGGGGTTGTTTT
AGGGTAGTTGGGTATGGTGTTTTTTGAGTGGGGGTGTAGTGGGTGTTTTTAGTTG
TTATGTTTTTGTTTTTATTTATGGGA-3'Pause-Antisense-Converted
F
siGL
siSLU7
siSLU7 + RNH1
DAPI
RPA32 p-S33
RPA intensity per nucleus (A.U.)
****
****
n=191
n=213
n=163
siGL
siSLU7
siSLU7 + RNH1
G
S9.6
RPA32 p-S33
merge
H
siGL
siSLU7
DAPI
R-Loop (S9.6)
I
siGL siSLU7 kD
γ-H2AX
SLU7
Actin
15
75
37
siGL siSLU7
ISO2
ISO1
SLU7

Figure S3. (**A**) Western blot analysis of γH2AX, SLU7 an Actin, as loading control, in PLC/PRF/5, HeLa and H358 cells 48 hours after transfection with siGL or siSLU7. (**B**) Immunofluorescence detection of R-loops with S9.6 antibody in HeLa cells 48 hours after transfection with siGL or siSLU7. Nuclei were stained with DAPI. Scale bar: 10 µm. (**C**) Slot-blot detection of RNA-DNA hybrids (R-loops) in genomic DNA from HeLa cells 48 hours after transfection with siGL or siSLU7 using S9.6 antibody. Aliquots of genomic DNA were treated with RNAse H1 as control. The DNA fixed in the membrane was stained with methylene blue as loading control. (**D**) Scheme of R-loops detection by non-denaturing bisulfite nested PCR (see Materials and Methods). Briefly, in non-denatured gDNA from siGL and siSLU7 PLC/PRF/5 cells, cytosines (C, black dots) can be converted to uracil (U, white dots) by bisulfite treatment only in the non-protected ssDNA in the R-loop. The position of the primers used to detect R-loop formation on the 3'UTR region of the beta-actin gene is indicated. PCR1 was performed using primers outside the R-loop. Nested PCR3 was performed using primers described to perform DRIP (Hatchi et al. Mol Cell 2015). We modified the sequence of those primers to detect the converted ssDNA in PCR2. (**E**) PCR2 confirmed the presence of R-loops in PLC/PRF/5 cells 48 hours after transfection with siSLU7. gDNA (C) and gDNA converted after bisulfite treatment (+BIS) were used as controls. The right panel shows the sequence of the PCR product amplified with PCR2 in siSLU7 samples. In red are marked the C to T bisulfite conversions confirming the amplification of the non-protected DNA strand in the R-loop. (**F**) Immunofluorescence detection of RPA32 P-S33 in PLC/PRF/5 cells 48 hours after transfection with siGL, siSLU7 and siSLU7 + RNH1 plasmid. Scale bar: 10 µm. The right panel shows the quantification of RPA32 P-S33 staining per nucleus with ImageJ software. The DAPI signal was used to create a mask of the nucleus. The number of nucleus analyzed per condition is indicated. The plot was represented using GraphPad Prism. Red bars represent the median. ****P<0.0001 (Mann-Whitney U-test). (**G**) Colocalization by immunofluorescence of R-loops (S9.6 antibody) and phospho-RPA32 (RPA32 P-S33 antibody) in PLC/PRF/5 cells 48 hours after transfection with siSLU7. Nuclei were stained with DAPI. Scale bar: 10 µm. (**H**) Immunofluorescence detection of R-loops with S9.6 antibody in HepaRG cells 48 hours after transfection with siGL or siSLU7. Nuclei were stained with DAPI. Scale bar: 10 µm. (**I**) Left panel: PCR detection of SRSF3 isoforms ISO2 and ISO1 and SLU7 in cells described in (H). Right panel: detection

by Western blot of γ-H2AX, SLU7 and Actin as loading control in cells described in (H). All experiments were performed at least three times with biological duplicates per condition.

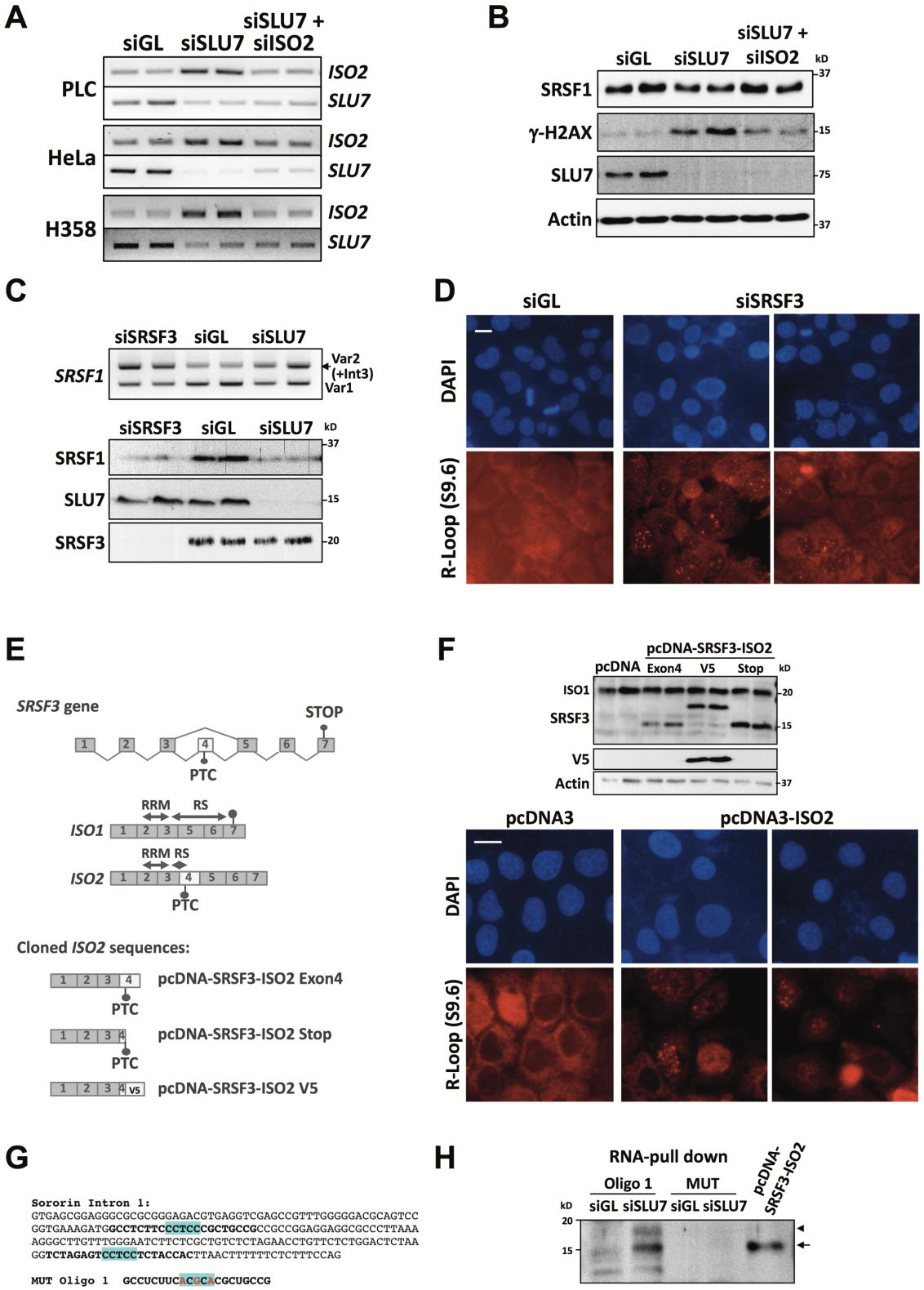

Figure S4. (**A**) Detection of *SRSF3-ISO2* and *SLU7* expression by PCR in PLC/PRF/5, HeLa and H358 cells 48 hours after transfection with siGL, siSLU7 or siSLU7 + siISO2. (**B**) Western blot analysis of SRSF1, γ-H2AX, SLU7 an Actin, as loading control, in HeLa cells 48 hours after transfection with siGL, siSLU7 or siSLU7 + siISO2. (**C**) PCR detection of *SRSF1* variants in HeLa cells after SRSF3 or SLU7 knockdown. Western blot analysis of SRSF1, SLU7 and SRSF3 proteins are shown in the lower panels. (**D**) Immunofluorescence detection of R-loops with S9.6 antibody in PLC/PRF/5 cells 48 hours after transfection with siGL or siSRSF3. Nuclei were stained with DAPI. Scale bar: 10 µm. (**E**) Schematic representation of *SRSF3* gene, the protein isoforms and the pcDNA3 constructs generated. (**F**) PLC/PRF/5 cells were transfected with the control plasmid (pcDNA) and the three constructs represented in E (Exon4, V5 and Stop) to overexpress *SRSF3-ISO2* mRNA. SRSF3 proteins were detected by Western blot. In the lower panel representative images of the immunofluorescence detection of R-loops with S9.6 antibody in PLC/PRF/5 cells transfected with control pcDNA and pcDNA3-ISO2 are shown. Nuclei were stained with DAPI. Scale bar: 10 µm. (**G**) Sequence of human *sororin* intron 1. The two predicted SRSF3 binding motifs are highlighted in blue and the sequence corresponding to the biotinylated RNA oligos used in the RNA-pull down assay is indicated in bold. The sequence of the mutated oligo 1 used as negative control is indicated. Red nucleotides indicate the changes in the binding motif. (**H**) Western blot analysis of SRSF3 after RNA-pull down with biotinylated RNA oligo O1 from sororin intron 1 and the corresponding mutated oligo 1 (MUT) described in (G), using extracts from PLC/PRF/5 cells transfected with control siGL or siSLU7. The truncated isoform expressed from the pcDNA-SRSF3-ISO2 Stop construct is shown as control (arrow). The two truncated SRSF3 isoforms induced upon SLU7 knockdown (arrow and arrowhead) are able to bind only oligo 1. All experiments were performed at least three times with biological duplicates per condition.

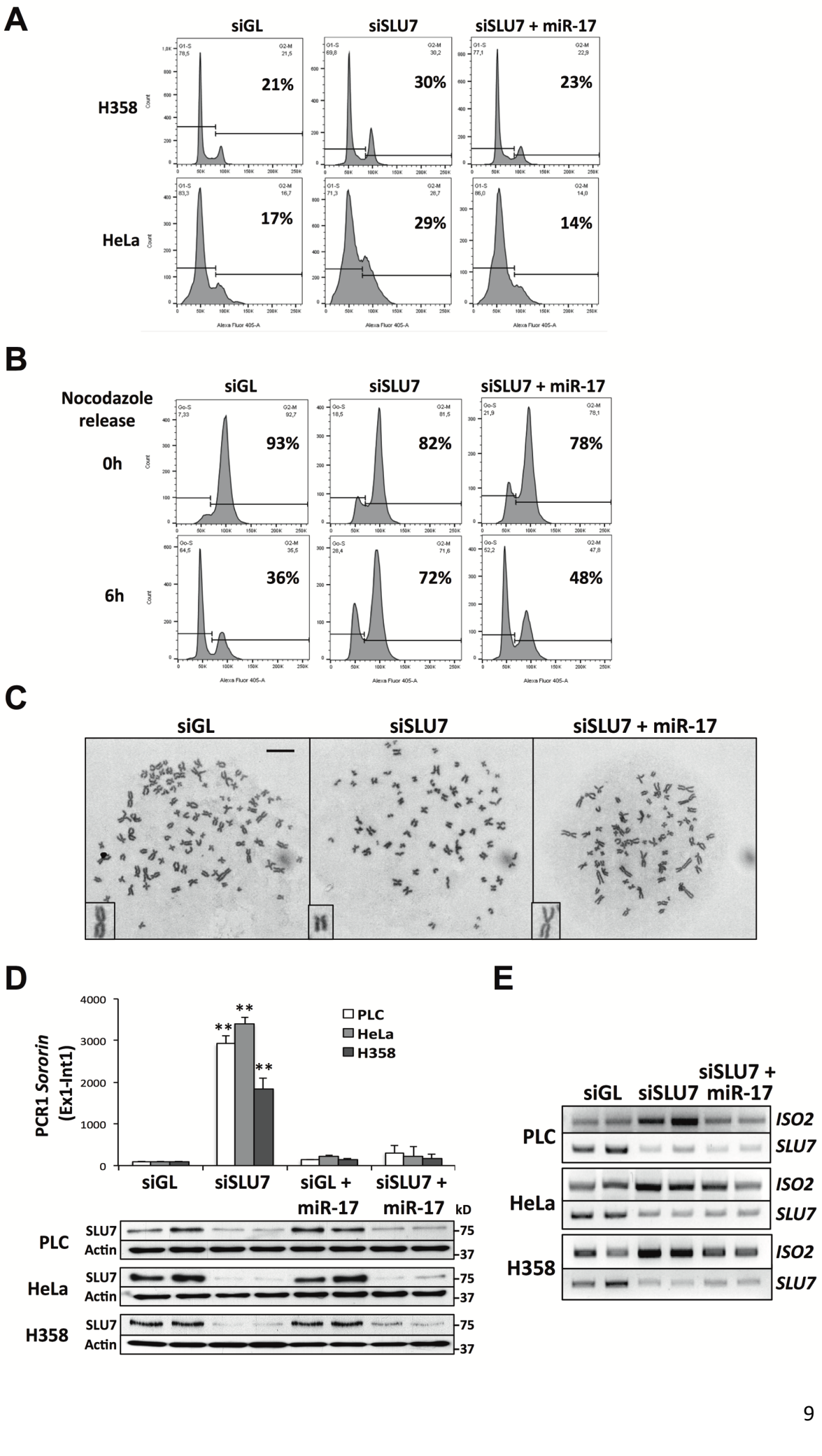
A
siGL
siSLU7
siSLU7 + miR-17
H358
HeLa
21%
30%
23%
17%
29%
14%
B
Nocodazole release
0h
6h
93%
82%
78%
36%
72%
48%
C
siGL
siSLU7
siSLU7 + miR-17
D
PCR1 Sororin (Ex1-Int1)
PLC
HeLa
H358
siGL
siSLU7
siGL + miR-17
siSLU7 + miR-17
kD
SLU7
Actin
75
37
E
siGL
siSLU7
siSLU7 + miR-17
ISO2
SLU7

Figure S5. (**A**) Cell cycle analysis by FACS of H358 and HeLa cells 48 hours after transfection with siGL, siSLU7 or siSLU7 + miR-17. The percentage of cells in G2/M is indicated. (**B**) Cell cycle analysis by FACS of PLC/PRF/5 cells 48 hours after transfection with siGL, siSLU7 or siSLU7 + miR-17 and overnight treatment with nocodazole (0h) or 6 hours after release from nocodazole arrest. The percentage of cells in G2/M is indicated. (**C**) Representative images from metaphase chromosome spreads of HeLa cells 48 hours after transfection with siGL, siSLU7 or siSLU7 + miR-17. Scale bar: 10 µm. (**D**) Real time PCR analysis of sororin intron 1 expression (PCR1) in PLC/PRF/5, HeLa and H358 cells 48 hours after transfection with siGL, siSLU7 or siSLU7 + miR-17. Western blot analysis of SLU7 and Actin, as loading control is shown. ***P*<0.01. (**E**) PCR analysis of *SRSF3-ISO2* and *SLU7* expression in PLC/PRF/5, HeLa and H358 cells 48 hours after transfection with siGL, siSLU7 or siSLU7 + miR-17. All experiments were performed at least three times with biological duplicates per condition.

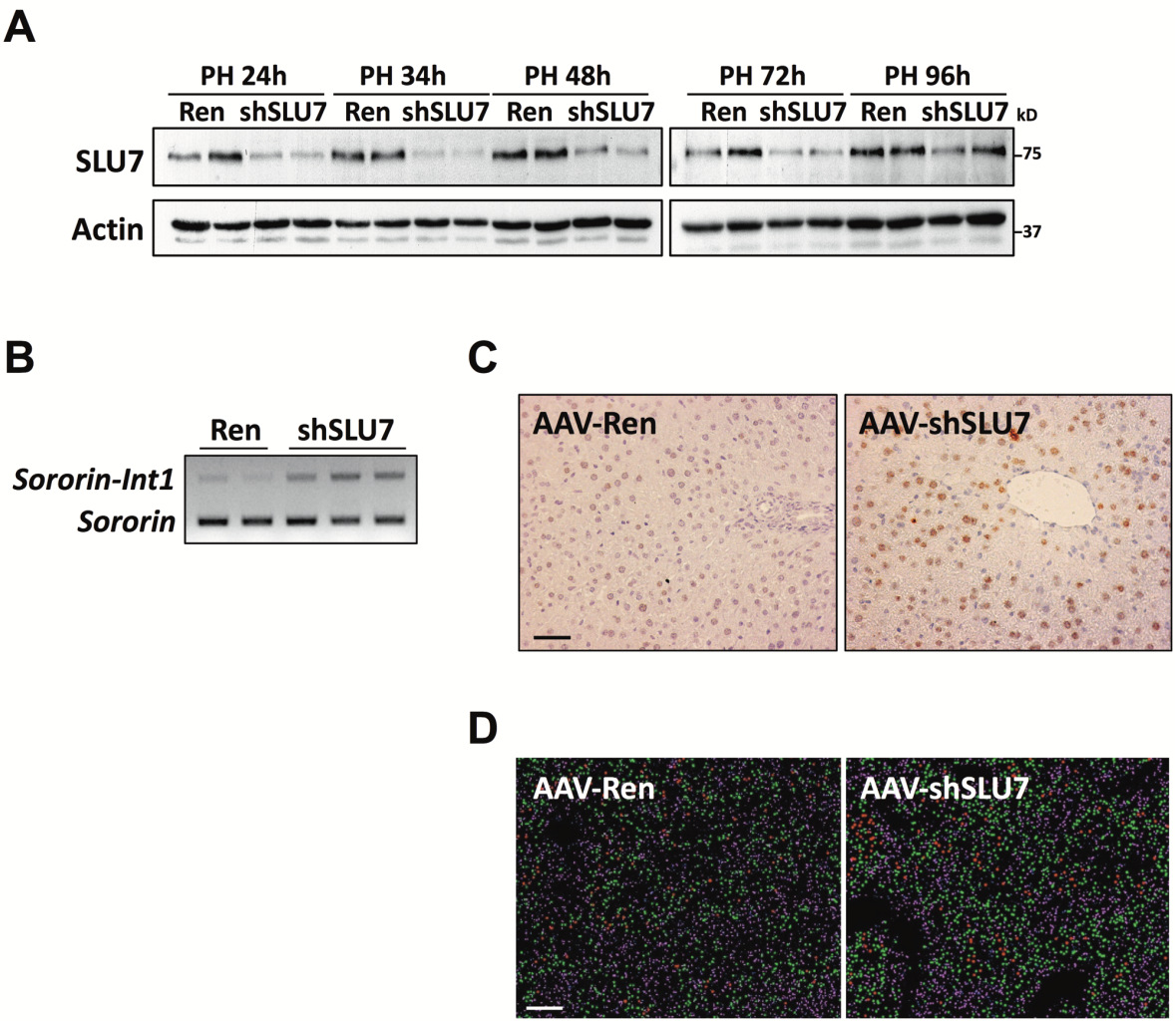

Figure S6. (**A**) **Detection by Western blot of SLU7 and Actin as loading control in the liver of mice injected with AAV-Ren and AAV-shSLU7 viruses 24, 34, 48, 72 and 96 hours after partial hepatectomy (PH).** (**B**) PCR analysis of *sororin* transcripts (PCR3 according to the scheme in Figure 2D) to evaluate the incorporation of intron 1 and 2 in the liver of AAV-Ren and AAV-shSLU7 mice 24 hours after PH. (**C**) Detection of γ-H2AX by immunohistochemistry in the livers of AAV-Ren and AAV-shSLU7 mice. Scale bar: 30 µm. (**D**) Ploidy detection in the liver of AAV-Ren and AAV-shSLU7. 2n nuclei are purple, 4n nuclei are green and 8n nuclei are red. Scale bar: 150 µm.