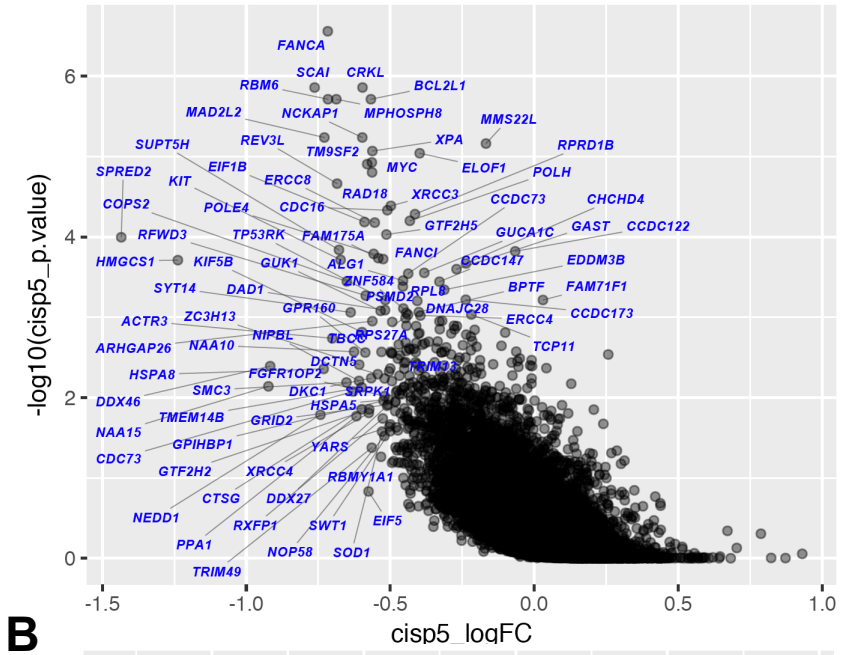
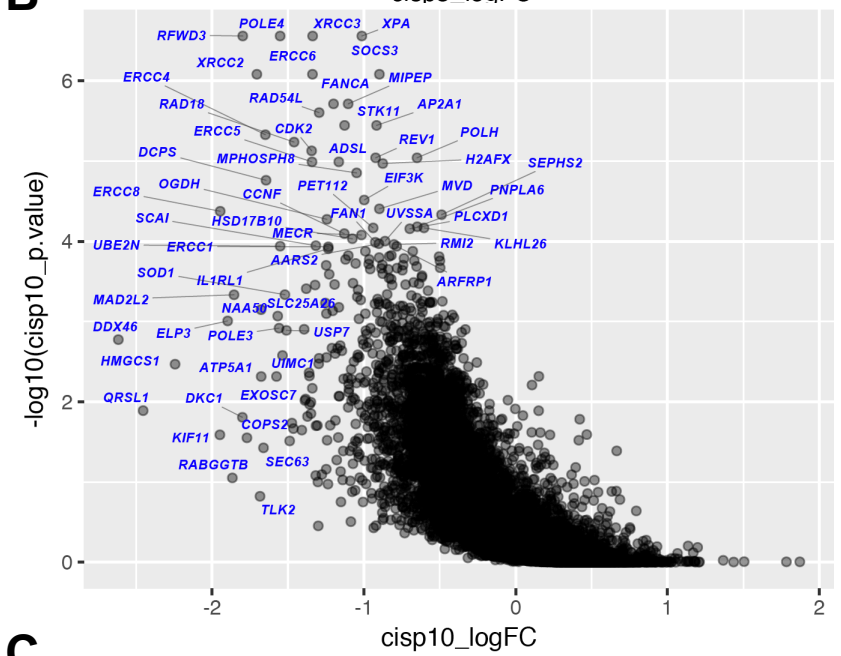
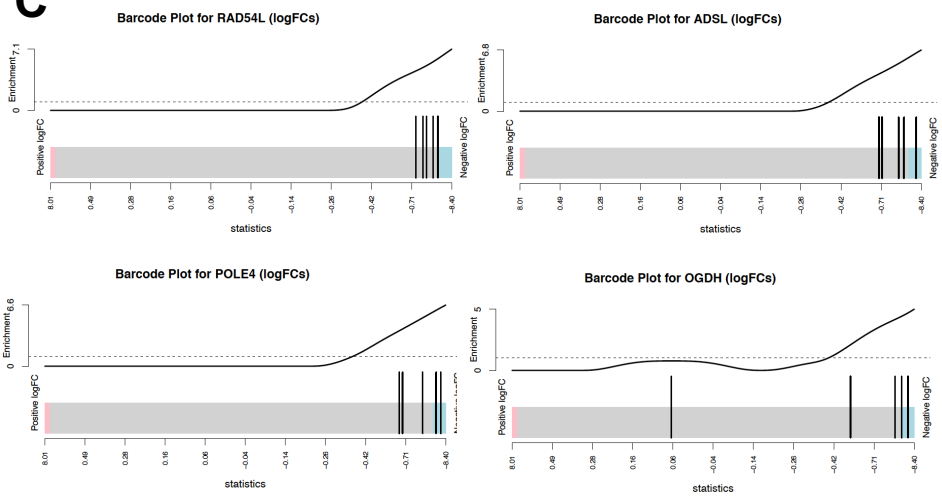
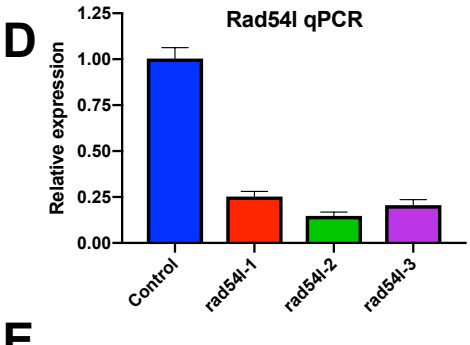
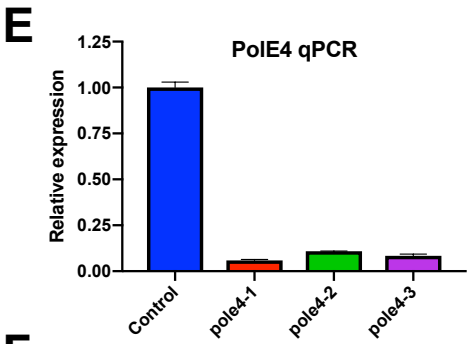
Figure S1

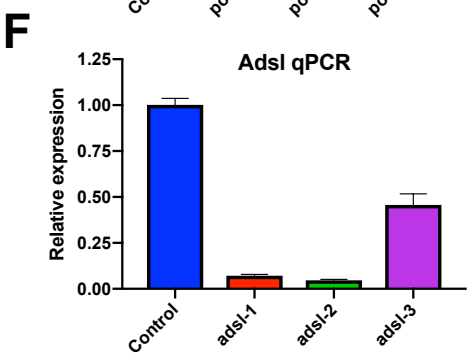

A
-log10(cisp5_p.value)
cisp5_logFC
FANCA
SCAI
CRKL
RBM6
BCL2L1
MAD2L2
NCKAP1
MPHOSPH8
MMS22L
SUPT5H
REV3L
XPA
RPRD1B
TM9SF2
SPRED2
EIF1B
MYC
ELOF1
POLH
KIT
ERCC8
RAD18
XRCC3
CCDC73
CHCHD4
COPS2
POLE4
CDC16
GTF2H5
RFWD3
TP53RK
FAM175A
GUCA1C
GAST
CCDC122
FANCI
HMGCS1
KIF5B
GUK1
ALG1
CCDC147
EDDM3B
ZNF584
SYT14
DAD1
PSMD2
RPLP0
BPTF
FAM71F1
GPR160
DNAJC28
ACTR3
ZC3H13
ERCC4
CCDC173
NIPBL
TBCC
PSMA7
ARHGAP26
NAA10
TCP11
DCTN5
RPA3
HSPA8
FGFR1OP2
SMC3
DKC1
STRAP
DDX46
HSPA5
TMEM14B
GRID2
NAA15
GPIHBP1
YARS
CDC73
GTF2H2
XRCC4
RBMY1A1
CTSG
DDX27
NEDD1
RXFP1
SWT1
EIF5
PPA1
NOP58
SOD1
TRIM49

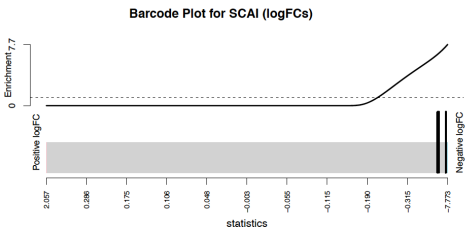

B
-log10(cisp10_p.value)
cisp10_logFC
POLE4
XRCC3
XPA
RFWD3
SOCS3
XRCC2
ERCC6
ERCC4
MIPEP
FANCA
RAD18
RAD54L
STK11
AP2A1
ERCC5
CDK2
REV1
POLH
DCPS
ADSL
MPHOSPH8
H2AFX
SEPHS2
ERCC8
OGDH
EIF3K
MVD
PNPLA6
CCNF
PET112
SCAI
HSD17B10
FAN1
UVSSA
PLCXD1
MECR
UBE2N
ERCC1
RMI2
KLHL26
AARS2
SOD1
IL1RL1
ARFRP1
MAD2L2
NAA50
SLC25A26
DDX46
ELP3
POLE3
USP7
HMGCS1
ATP5A1
UIMC1
QRSL1
DKC1
EXOSC7
COPS2
KIF11
RABGGTB
SEC63
TLK2


C
Barcode Plot for RAD54L (logFCs)
Barcode Plot for ADSL (logFCs)
Barcode Plot for POLE4 (logFCs)
Barcode Plot for OGDH (logFCs)
Enrichment
Positive logFC
Negative logFC
statistics


D
Rad54l qPCR
Relative expression
Control
rad54l-1
rad54l-2
rad54l-3


E
PolE4 qPCR
Relative expression
Control
pole4-1
pole4-2
pole4-3


F
Adsl qPCR
Relative expression
Control
adsl-1
adsl-2
adsl-3


G
Barcode Plot for SCAI (logFCs)
Enrichment
Positive logFC
Negative logFC
statistics

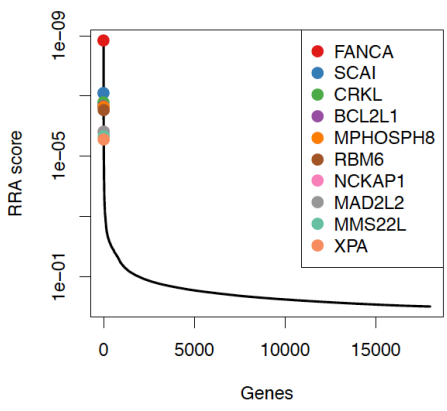

RRA score
Genes
FANCA
SCAI
CRKL
BCL2L1
MPHOSPH8
RBM6
NCKAP1
MAD2L2
MMS22L
XPA

Figure S1. Genome-wide cisplatin sensitivity screen, related to Figure 1

(A & B). Scatter plots of the PD5 (A) and PD10 (B) cisp screens showing log fold changes (on the x-axes) plotted against negative log of the p-values (y-axes) generated by MAGeCK. A few of the top scoring genes are indicated. **(C)** EdgeR camera plots showing the distribution of guide RNAs. Statistics indicates the log fold changes from positive (on the left) to negative (on the right) of the plots. **(D-F)** qPCR depletion analysis of cells expressing shRNAs against the indicated proteins. **(G)** Top panel shows an EdgeR camera plot of the distribution of gRNAs against *SCAI* at PD5. Bottom panel shows MAGeCK scores of the top 10 scoring genes at PD5.

Figure S2

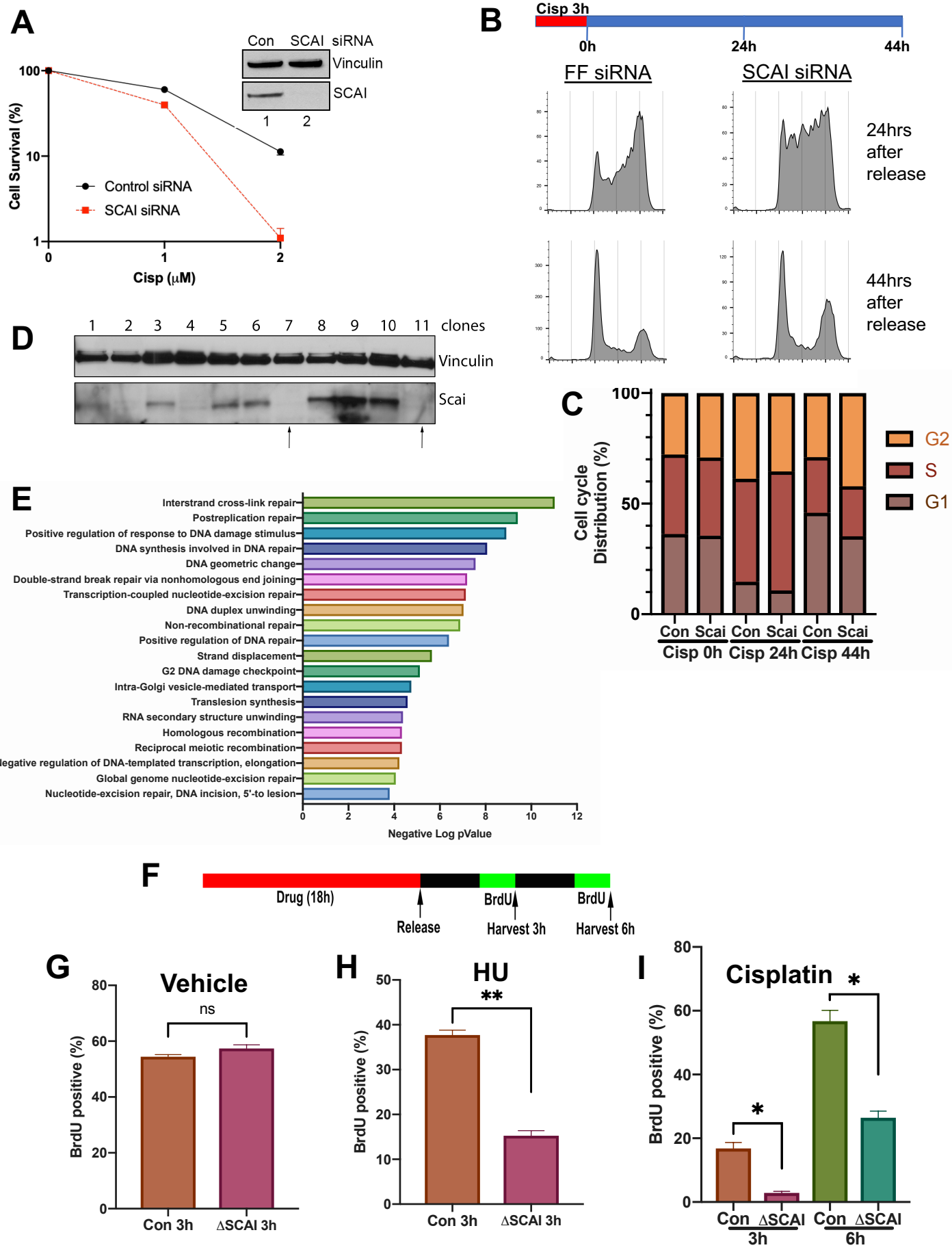

A
Con SCAI siRNA
Vinculin
SCAI
1 2
100
10
1
Cell Survival (%)
Control siRNA
SCAI siRNA
0 1 2
Cisp (μM)
B
Cisp 3h
0h 24h 44h
FF siRNA
SCAI siRNA
24hrs after release
44hrs after release
C
100
50
0
Cell cycle Distribution (%)
G2
S
G1
Con Scai Con Scai Con Scai
Cisp 0h Cisp 24h Cisp 44h
D
1 2 3 4 5 6 7 8 9 10 11 clones
Vinculin
Scai
E
Interstrand cross-link repair
Postreplication repair
Positive regulation of response to DNA damage stimulus
DNA synthesis involved in DNA repair
DNA geometric change
Double-strand break repair via nonhomologous end joining
Transcription-coupled nucleotide-excision repair
DNA duplex unwinding
Non-recombinational repair
Positive regulation of DNA repair
Strand displacement
G2 DNA damage checkpoint
Intra-Golgi vesicle-mediated transport
Translesion synthesis
RNA secondary structure unwinding
Homologous recombination
Reciprocal meiotic recombination
Negative regulation of DNA-templated transcription, elongation
Global genome nucleotide-excision repair
Nucleotide-excision repair, DNA incision, 5'-to lesion
0 2 4 6 8 10 12
Negative Log pValue
F
Drug (18h)
BrdU
BrdU
Release
Harvest 3h
Harvest 6h
G
Vehicle
ns
BrdU positive (%)
80 60 40 20 0
Con 3h
ΔSCAI 3h
H
HU
**
BrdU positive (%)
50 40 30 20 10 0
Con 3h
ΔSCAI 3h
I
Cisplatin
*
*
BrdU positive (%)
80 60 40 20 0
Con ΔSCAI Con ΔSCAI
3h 6h

Figure S2. SCAI is important for proper repair of ICLs, related to Figures 1 & 2.

(A) CSAs showing survival of control or *SCAI* siRNA-treated HeLa cells upon treatment with the indicated doses of cisp. Data are normalized to untreated cells for each siRNA treatment condition. Mean ± SEM survival of two independent experiments are shown. Western blots showing SCAI knockdown shown on right. **(B) Top.** Schematic showing details of the cell cycle arrest experiment. Cisp or vehicle alone was added for 3h and then washed out. **Bottom.** Cell cycle distribution showing increased accumulation of *SCAI* siRNA treated cells in S and G2 phases following treatment with 2 μM cisp. **(C)** Cell cycle profile of experiment in (B). **(D)** Western blot analyses showing *SCAI* expression in various *SCAI* knockout clones. **(E)** GO term enrichment analyses showing the enriched pathways among the high scoring hits from Figure 1C. **(F).** Schematic for the experiments in G-I showing the BrdU incorporation assay protocol. Cells were treated with the indicated agent for 18 h, washed and released for 2.5 or 5.5 h and labeled for 30 m with BrdU. **(G - I).** WT and *SCAI* null U2OS cells were treated with vehicle (G), or HU (H) or cisp (I) and analyzed for BrdU incorporation by flow cytometry 3 h or 6 h after release as indicated. Data in G-I shows mean and SEM of two independent experiments. Student t-test, ns= $p>0.05$, *= $p<0.05$, **= $p<0.01$. Con represents WT cells.

Figure S3

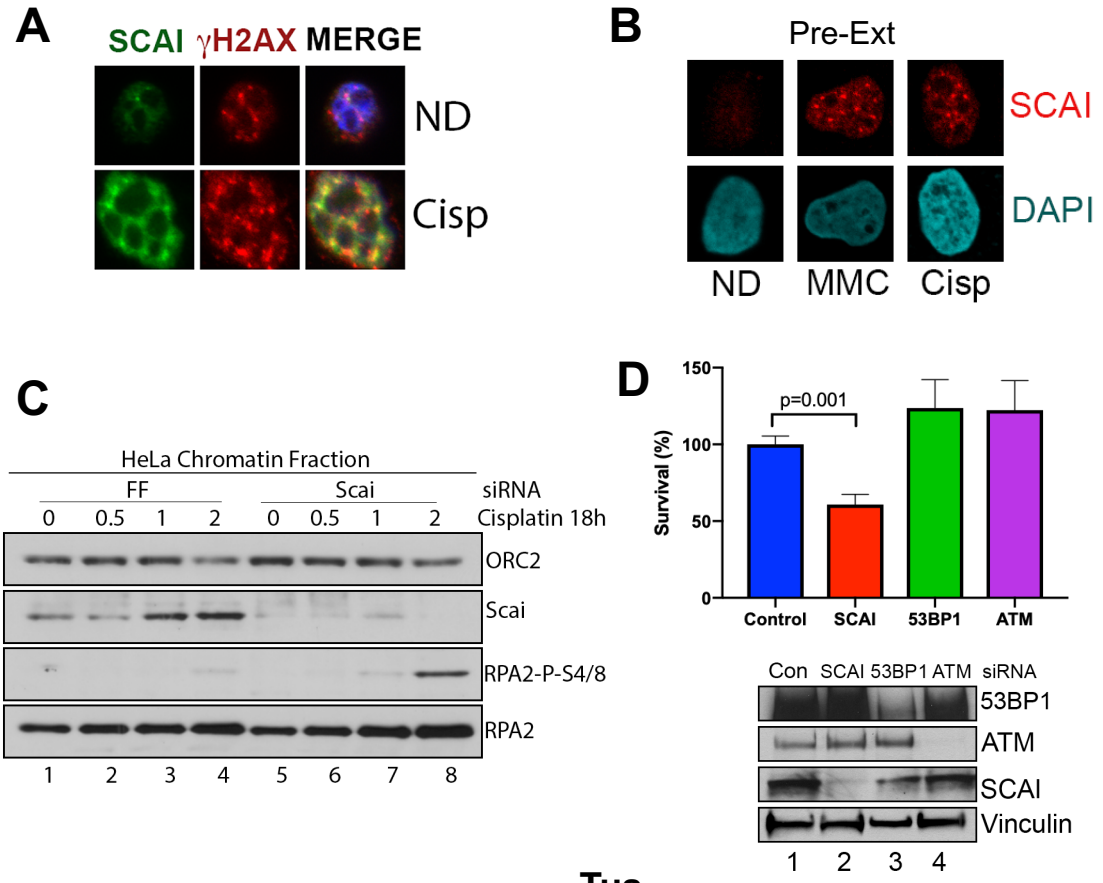

A
SCAI γH2AX MERGE
ND
Cisp
B
Pre-Ext
SCAI
DAPI
ND
MMC
Cisp
C
HeLa Chromatin Fraction
FF
Scai
siRNA
0 0.5 1 2 0 0.5 1 2
Cisplatin 18h
ORC2
Scai
RPA2-P-S4/8
RPA2
1 2 3 4 5 6 7 8
D
p=0.001
Survival (%)
150
100
50
0
Control
SCAI
53BP1
ATM
Con SCAI 53BP1 ATM siRNA
53BP1
ATM
SCAI
Vinculin
1 2 3 4
Tus
E
SCAI experiment
STGC
LTGC
GFP+ RFP− (%)
GFP+ RFP+ (%)
0.4
0.3
0.2
0.1
0.0
0.04
0.03
0.02
0.01
0.00
***
***
siLUC
siSCAI-1
siSCAI-2
F
REV3 experiment
STGC
LTGC
****
*
siLUC
siREV3

Figure S3. SCAI is recruited to ICL sites and promotes HR, related to Figure 3.

(A) IF images showing recruitment of U2OS cells expressing GFP-tagged *SCAI* to foci that colocalize with H2AX after treatment with 1 μM Cisp for 2 h. ND – no drug **(B)** U2OS cells expressing FLAG-*SCAI* pre-extracted as described in experimental procedures, treated with 50 nM MMC or 1 μM Cisp for 8 h before IF analyses. ND-no drug. **(C)** HeLa cells were transfected with either firefly control (FF) or *SCAI* siRNA for 48 h then treated with vehicle or the indicated doses of cisp for 18 h prior to chromatin fractionation and immunoblotting against the indicated proteins. **(D)** U2OS cells were transfected with control or siRNAs against the indicated genes for 48 h, then treated with vehicle or 3 μM cisp for 24 h and analyzed using cell titer viability assays 48 h afterwards. Data is mean ± SEM of two independent experiments. Student t-test. Bottom panel shows western blot analysis of lysates from the experiment. **(E)** STGC (left panel) and LTGC (right panel) frequencies of Tus-stalled forks in mouse 6xTer/HR cells co-transfected with Tus and either control siRNA or two different mouse siRNAs against *SCAI*. Data represent the mean ± SEM. Welch's test, ***=$p<0.001$. **(F)** STGC (left panel) and LTGC (right panel) frequencies of Tus-stalled forks in mouse 6xTer/HR cells co-transfected with Tus and either control siRNA or siRNAs against mouse *REV3*. Data represent the mean ± SEM. Welch's test, *=$p<0.05$, ****=$p<0.0001$.

Figure S4

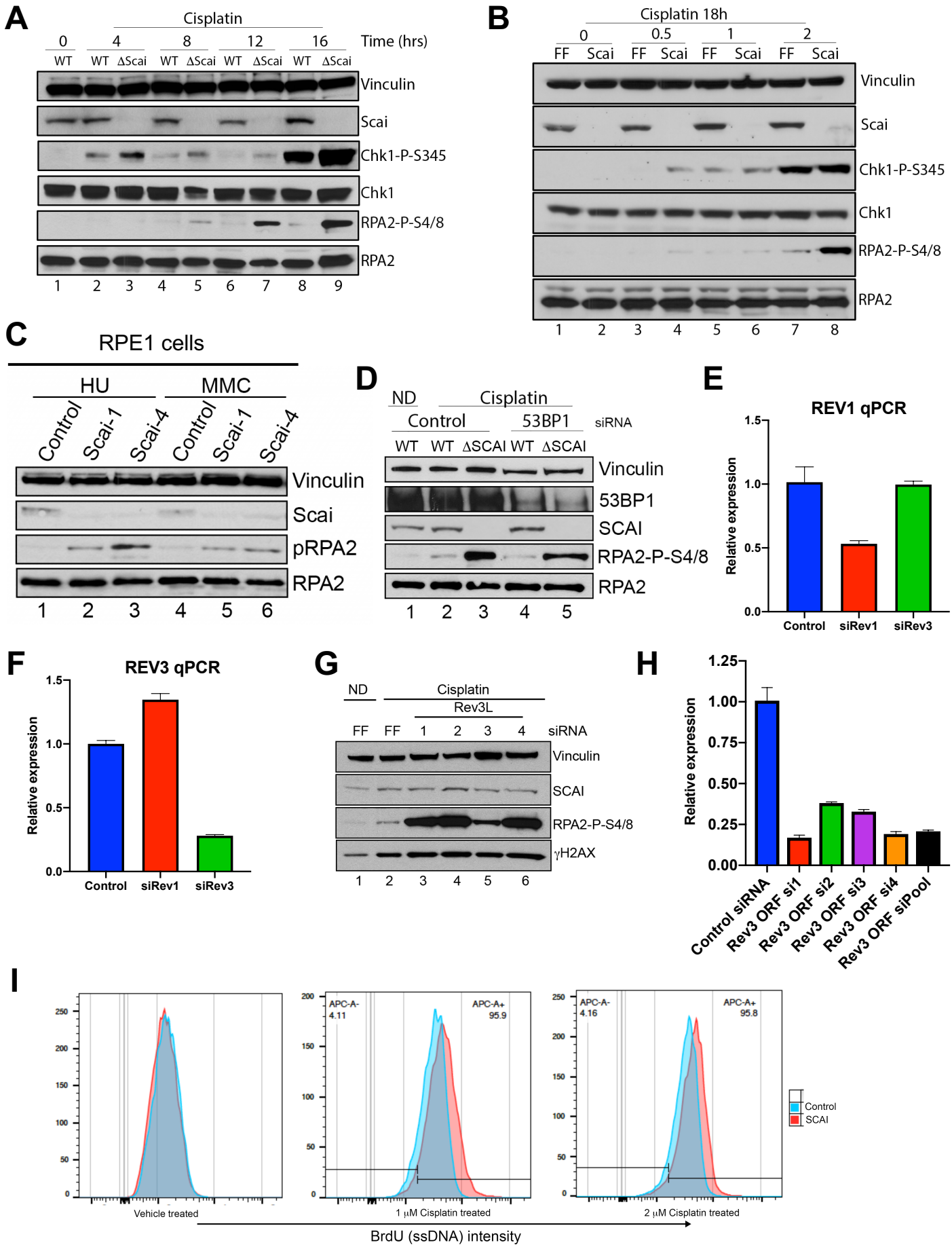

A
Cisplatin
0
4
8
12
16
Time (hrs)
WT
WT ΔScai
WT ΔScai
WT ΔScai
WT ΔScai
Vinculin
Scai
Chk1-P-S345
Chk1
RPA2-P-S4/8
RPA2
1 2 3 4 5 6 7 8 9
B
Cisplatin 18h
0
0.5
1
2
FF Scai FF Scai FF Scai FF Scai
Vinculin
Scai
Chk1-P-S345
Chk1
RPA2-P-S4/8
RPA2
1 2 3 4 5 6 7 8
C
RPE1 cells
HU
MMC
Control
Scai-1
Scai-4
Control
Scai-1
Scai-4
Vinculin
Scai
pRPA2
RPA2
1 2 3 4 5 6
D
ND
Cisplatin
Control
53BP1
siRNA
WT WT ΔSCAI WT ΔSCAI
Vinculin
53BP1
SCAI
RPA2-P-S4/8
RPA2
1 2 3 4 5
E
REV1 qPCR
Relative expression
1.5
1.0
0.5
0.0
Control
siRev1
siRev3
F
REV3 qPCR
Relative expression
1.5
1.0
0.5
0.0
Control
siRev1
siRev3
G
ND
Cisplatin
Rev3L
FF FF 1 2 3 4 siRNA
Vinculin
SCAI
RPA2-P-S4/8
γH2AX
1 2 3 4 5 6
H
Relative expression
1.25
1.00
0.75
0.50
0.25
0.00
Control siRNA
Rev3 ORF si1
Rev3 ORF si2
Rev3 ORF si3
Rev3 ORF si4
Rev3 ORF siPool
I
APC-A- 4.11
APC-A+ 95.9
APC-A- 4.16
APC-A+ 95.8
Control
SCAI
Vehicle treated
1 μM Cisplatin treated
2 μM Cisplatin treated
BrdU (ssDNA) intensity

Figure S4. Protexin limits ssDNA accumulation, related to Figure 4.

(A) Time course of pRPA. Control WT U2OS cells or *SCAI* null U2OS cells were treated with 2 μM of cisp for the indicated duration. Cells were then processed for western blotting against the indicated antibodies. **(B)** U2OS cells were transfected with control (FF) or *SCAI* siRNAs for 48 h, then treated with vehicle or the indicated doses of cisp for 18 h prior to western blot analyses using antibodies against the indicated proteins. **(C)** RPE1 cells were treated with control or indicated siRNAs against SCAI for 60 h. Cells were then treated with 1 mM HU or 1 μM MMC for 18 h before immunoblotting. **(D)** WT U2OS or *SCAI* null U2OS cells were transfected with control siRNAs or siRNA to 53BP1 for 72 h. Cells were then treated with 2 μM Cisp for 18 h, harvested and processed as above. **(E & F)** qPCR showing knockdown of REV1 and REV3 respectively. **(G)** U2OS cells were transfected with firefly (FF) siRNAs or the indicated siRNAs to *REV3* for 72 h. Cells were harvested and processed for immunoblotting against the indicated proteins. **(H)** qPCR of RNA samples from the experiment in (G). **(I)** Control or SCAI siRNA treated cells were stained with BrdU for 10 h followed by treatment with vehicle or indicated doses of cisp for 6 h. BrdU intensity was assayed using flow cytometry under non-denaturing conditions.

Figure S5

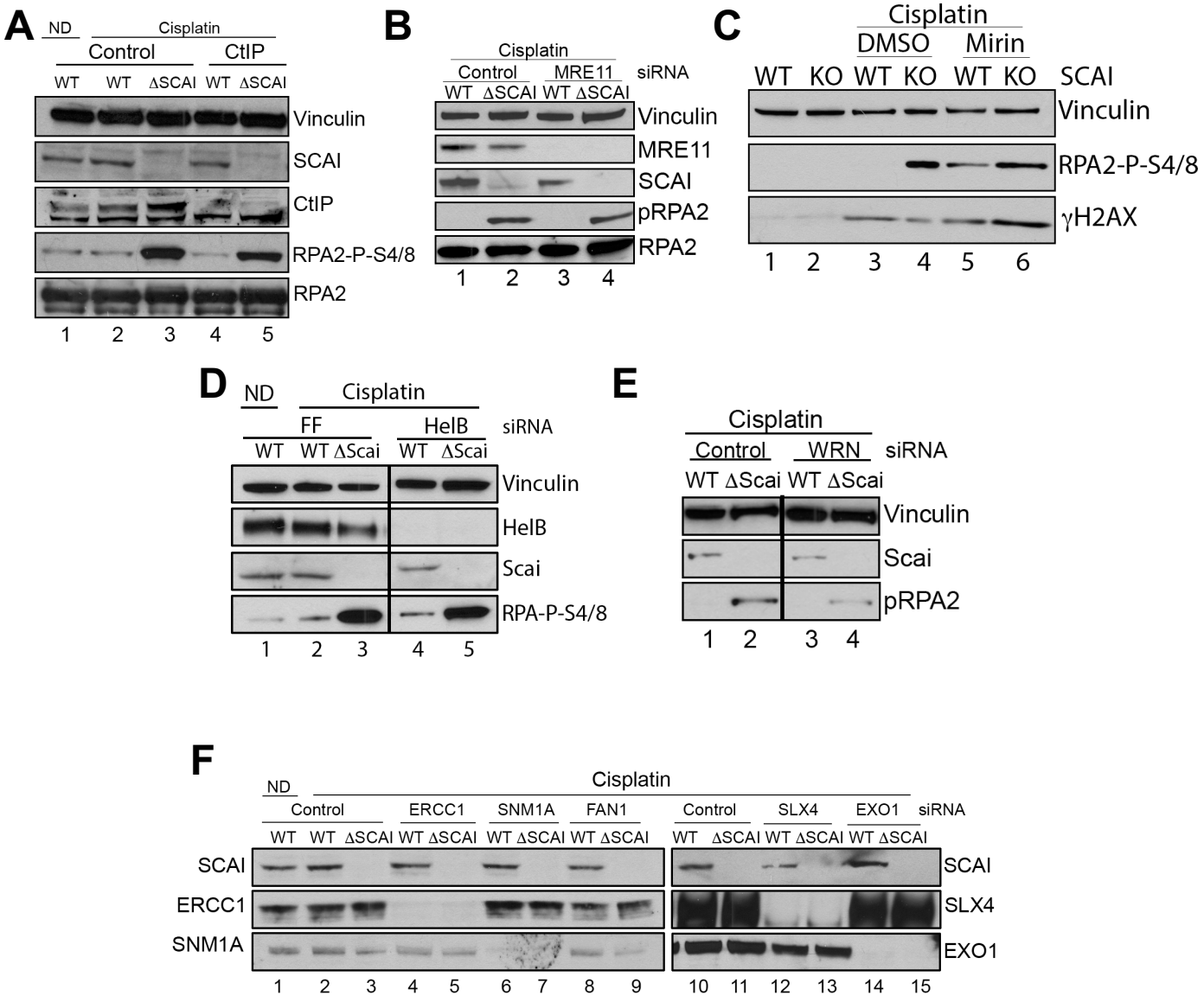

A
ND
Cisplatin
Control
CtIP
WT WT ΔSCAI WT ΔSCAI
Vinculin
SCAI
CtIP
RPA2-P-S4/8
RPA2
1 2 3 4 5
B
Cisplatin
Control MRE11 siRNA
WT ΔSCAI WT ΔSCAI
Vinculin
MRE11
SCAI
pRPA2
RPA2
1 2 3 4
C
Cisplatin
DMSO Mirin
WT KO WT KO WT KO SCAI
Vinculin
RPA2-P-S4/8
γH2AX
1 2 3 4 5 6
D
ND
Cisplatin
FF HelB siRNA
WT WT ΔScai WT ΔScai
Vinculin
HelB
Scai
RPA-P-S4/8
1 2 3 4 5
E
Cisplatin
Control WRN siRNA
WT ΔScai WT ΔScai
Vinculin
Scai
pRPA2
1 2 3 4
F
ND
Cisplatin
Control ERCC1 SNM1A FAN1 Control SLX4 EXO1 siRNA
WT WT ΔSCAI WT ΔSCAI WT ΔSCAI WT ΔSCAI WT ΔSCAI WT ΔSCAI
SCAI
ERCC1
SNM1A
SCAI
SLX4
EXO1
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

Figure S5. Protexin protects replication forks from nucleases, related to Figure 5.
(A) WT and *SCAI*-null U2OS cells were treated with control or CtIP siRNAs for 72 h. Cells were then treated with vehicle or 1.5 μM cisp for 18 h before immunoblotting. **(B)** WT and *SCAI*-null U2OS cells were treated with control or MRE11 siRNAs for 72 h. Cells were then treated with vehicle or 1.5 μM cisp for 18 h before immunoblotting. **(C)** WT and *SCAI*-null U2OS cells were treated with cisp for 8 h and then treated with vehicle (DMSO) or 10 μM mirin for 10 h prior to harvest. Cisp was left on throughout the mirin (or DMSO) treatment. Cells were harvested and processed for immunoblotting as above. **(D)** WT U2OS or *SCAI*-null U2OS cells were transfected with control siRNAs or siRNA to HELB for 72 h. Cells were then treated with 2 μM Cisp for 18 h, harvested and processed for immunoblotting. All lanes are from the same blots – irrelevant lanes were cropped out. **(E)** WT U2OS or *SCAI*-null U2OS cells were transfected with control siRNAs or siRNA to *WRN* for 60 h. Cells were then treated with 2 μM Cisp for 18 h, harvested and processed for immunoblotting. All lanes are from the same blots – irrelevant lanes were cropped out. **(F)** Additional blots against the indicated proteins from the experiment in Figure 5E.

Figure S6

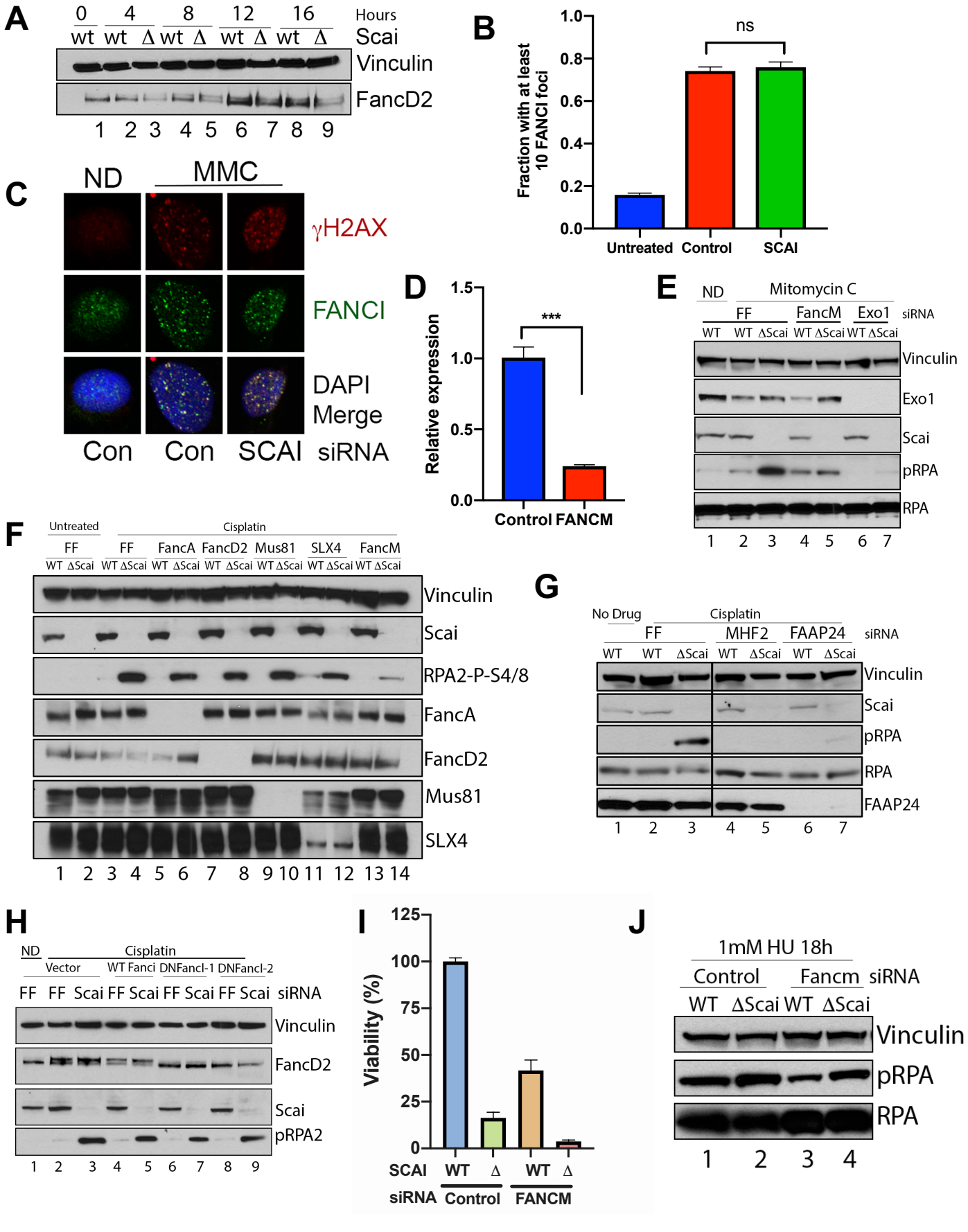

A
0 4 8 12 16 Hours
wt wt Δ wt Δ wt Δ wt Δ
Scai
Vinculin
FancD2
1 2 3 4 5 6 7 8 9
B
Fraction with at least 10 FANCI foci
1.0
0.8
0.6
0.4
0.2
0.0
ns
Untreated Control SCAI
C
ND MMC
γH2AX
FANCI
DAPI
Merge
Con Con SCAI siRNA
D
Relative expression
1.5
1.0
0.5
0.0
***
Control FANCM
E
ND Mitomycin C
FF FancM Exo1 siRNA
WT WT ΔScai WT ΔScai WT ΔScai
Vinculin
Exo1
Scai
pRPA
RPA
1 2 3 4 5 6 7
F
Untreated Cisplatin
FF FF FancA FancD2 Mus81 SLX4 FancM
WT ΔScai WT ΔScai WT ΔScai WT ΔScai WT ΔScai WT ΔScai
Vinculin
Scai
RPA2-P-S4/8
FancA
FancD2
Mus81
SLX4
1 2 3 4 5 6 7 8 9 10 11 12 13 14
G
No Drug Cisplatin
FF MHF2 FAAP24 siRNA
WT WT ΔScai WT ΔScai WT ΔScai
Vinculin
Scai
pRPA
RPA
FAAP24
1 2 3 4 5 6 7
H
ND Cisplatin
Vector WT Fanci DNFancI-1 DNFancI-2
FF FF Scai FF Scai FF Scai FF Scai siRNA
Vinculin
FancD2
Scai
pRPA2
1 2 3 4 5 6 7 8 9
I
Viability (%)
125
100
75
50
25
0
SCAI WT Δ WT Δ
siRNA Control FANCM
J
1mM HU 18h
Control Fancm siRNA
WT ΔScai WT ΔScai
Vinculin
pRPA
RPA
1 2 3 4

Figure S6. FANCM drives ssDNA accumulation following Protexin loss independently of FA pathway activation, related to Figures 5 & 6.

(A) Western blot analyses showing time course of FANCD2 ubiquitination following 2 μM cisp treatment in WT and *SCAI*-null cells. **(B & C)** U2OS cells transfected with the indicated siRNAs for 60 h were treated with vehicle or 1 μM MMC for 6 h prior to IF analyses for FANCI and γ-H2AX foci. Quantification of the experiment is shown in B. At least 50 cells were counted for each condition. Analysis by student t-test, ns – p-value < 0.05. **(D)** U2OS cells were transfected with control or *FANCM* siRNAs for 72 h and processed for qPCR analysis. **(E)** WT and *SCAI* null cells were treated with siRNA against the indicated proteins and control (FF) for 72 h. Cells were then treated with vehicle (ND) or 100 nM MMC for 16 h and analyzed by immunoblotting. **(F)** WT and *SCAI*-null U2OS cells were treated with control (FF) or siRNA against the indicated proteins for 72 h. Cells were then treated with vehicle or 1.5 μM cisp for 18 h before immunoblotting. **(G)** WT or *SCAI*-null U2OS cells were transfected with control (FF), MHF2 or FAAP24 siRNAs for 72 h. Cells were then treated with 2 μM Cisp for 18 h, harvested and processed for immunoblotting. All lanes are from the same blots – irrelevant lanes were cropped out. **(H)** U2OS cells expressing vector or the indicated ORFs were treated with control (FF) or *SCAI* siRNA for 72 h before treatment with vehicle (ND) or 1.5 μM cisp for 18 h and analysis by immunoblotting. **(I)** WT and *SCAI*-null U2OS cells were treated with control or siRNAs to FANCM for 48 h before 16 h treatment with vehicle or 2 μM cisp. CSAs showing dependence on FANCM for survival. Viability is relative to vehicle-treated cells. Mean and SEM of two independent experiments are shown. **(J)** WT or *SCAI*-null U2OS cells were transfected with control or FANCM siRNAs for 72 h. Cells were then treated with 1mM HU for 18 h, harvested and processed for immunoblotting.

Figure S7

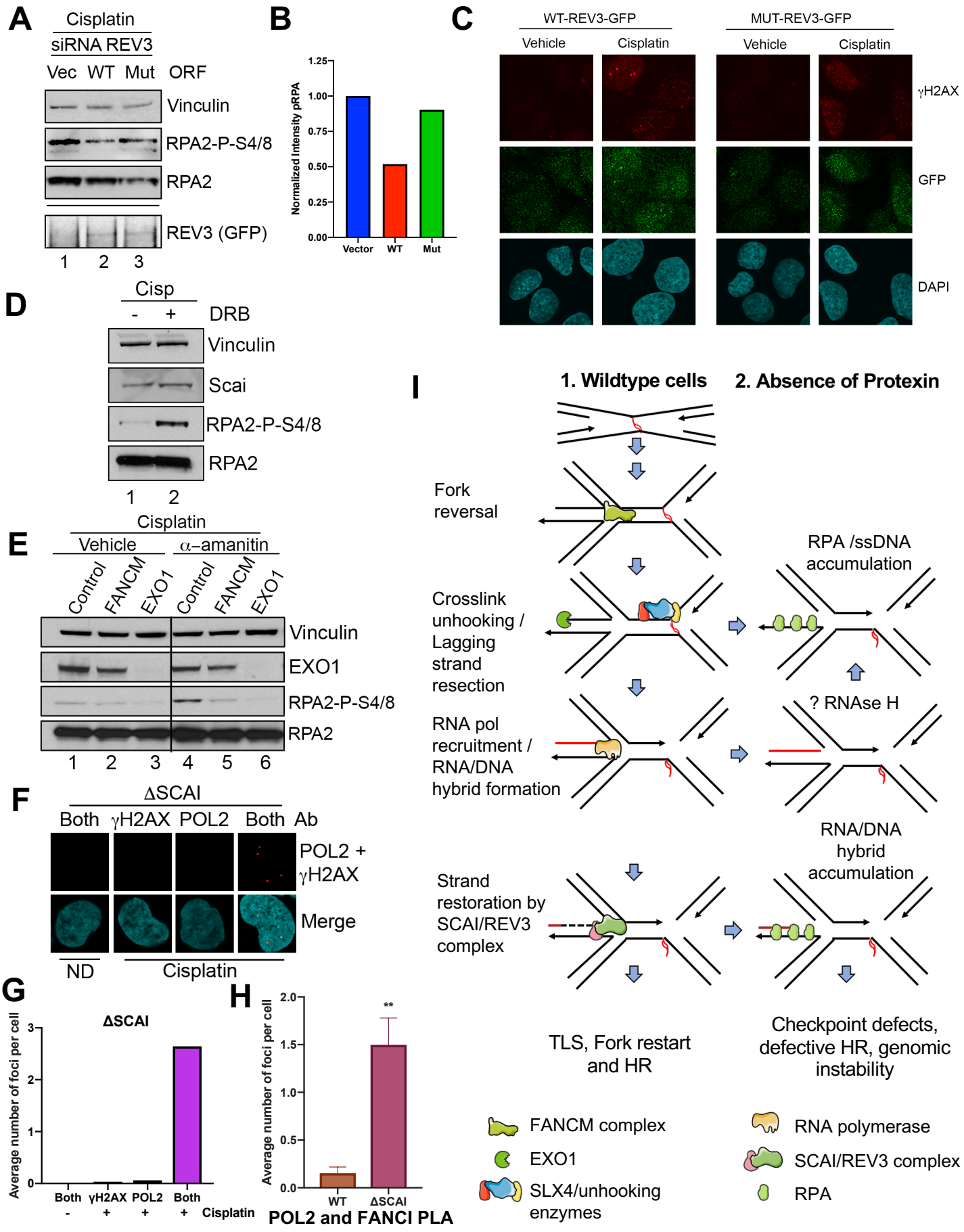

A
Cisplatin
siRNA REV3
Vec WT Mut ORF
Vinculin
RPA2-P-S4/8
RPA2
REV3 (GFP)
1 2 3
B
Normalized Intensity pRPA
1.25
1.00
0.75
0.50
0.25
0.00
Vector WT Mut
C
WT-REV3-GFP
MUT-REV3-GFP
Vehicle Cisplatin
Vehicle Cisplatin
γH2AX
GFP
DAPI
D
Cisp
- +
DRB
Vinculin
Scai
RPA2-P-S4/8
RPA2
1 2
E
Cisplatin
Vehicle
α–amanitin
Control FANCM EXO1 Control FANCM EXO1
Vinculin
EXO1
RPA2-P-S4/8
RPA2
1 2 3 4 5 6
F
ΔSCAI
Both γH2AX POL2 Both Ab
POL2 + γH2AX
Merge
ND
Cisplatin
G
ΔSCAI
Average number of foci per cell
3
2
1
0
Both γH2AX POL2 Both
- + + +
Cisplatin
H
Average number of foci per cell
2.0
1.5
1.0
0.5
0.0
**
WT ΔSCAI
POL2 and FANCI PLA
I
1. Wildtype cells
2. Absence of Protexin
Fork reversal
Crosslink unhooking / Lagging strand resection
RPA /ssDNA accumulation
RNA pol recruitment / RNA/DNA hybrid formation
? RNAse H
Strand restoration by SCAI/REV3 complex
RNA/DNA hybrid accumulation
TLS, Fork restart and HR
Checkpoint defects, defective HR, genomic instability
FANCM complex
EXO1
SLX4/unhooking enzymes
RNA polymerase
SCAI/REV3 complex
RPA

Figure S7. REV3 and POL2 polymerase roles in limiting ssDNA, related to Figure 7. **(A)** Top panel: U2OS cells stably expressing vector control, GFP-tagged WT REV3 or a REV3 D2781A/D2783A mutant that lacks polymerase activity were treated with control siRNA or an siRNA to the 3' UTR of endogenous REV3 as indicated for 48 h. Cells were then treated with cisp for 18 h and assayed for western blotting. In parallel, U2OS cells expressing vector control, GFP-tagged WT-REV3 or D2781A/D2783A mutant REV3 were immunoprecipitated and blotted for GFP expression (bottom panel). **(B)** Quantification of the pRPA intensity compared to total RPA amounts from the experiment in (A). **(C)** Cells expressing GFP-tagged WT- REV3 or mutant were treated with cisp for 16 h and processed for IF against GFP and γH2AX. Representative images are shown. **(D)** WT U2OS cells were treated with cisp for 12 h followed by vehicle or treatment with 100 μM DRB for 3 h. Cells were blotted against indicated proteins. **(E)** WT U2OS cells were transfected with control siRNAs or siRNA to *FANCM or EXO1* for 60 h before treatment with 2 μM Cisp for 10 h. Cells were then treated with 10 μg/ml α-amanitin for 6 h and processed for western blotting as above. **(F)** *SCAI*-null U2OS cells were treated with vehicle or 1 μM cisp for 8 h and processed for PLAs using individual or both mouse γH2AX and rabbit POL2 antibodies as indicated. Representative images are shown. **(G)** Quantification of the experiment in (F). **(H)** WT and *SCAI*-null U2OS were treated with 1 μM cisp for 8 h and processed for PLAs using antibodies against mouse POL2 and rabbit FANCI. Mann Whitney test, **=$p<0.01$. **(I)** Model showing potential roles of the Protexin complex in resection control and repair at an ICL stalled fork.