Expanded View Figures

Figure EV1. SCAI-deficient cells display major hallmarks of FA gene dysfunction (related to Figs 1 and 2).

A GO term analysis of significant hits (NormZ < −3) in CRISPR-Cas9 screen in Fig 1A, using Reactome pathways from PANTHER16.0.
B Clonogenic survival of U2OS WT and U2OS/SCAI KO cells subjected to indicated doses of MMC for 24 h (mean ± SEM; $n = 3$ independent experiments).
C As in (B), except that cells were treated with indicated doses of cisplatin for 24 h (mean ± SEM; $n = 3$ independent experiments).
D Scatter plot showing cell cycle distribution of cells in Fig 1F. Light grey, G1 phase; dark grey, S phase; red: G2/M phase. Proportion of cells in G2/M phase is indicated.
E U2OS WT, U2OS/SCAI KO, and U2OS/SCAI KO/Strep-HA-SCAI cells were treated or not with MMC (90 nM) for 1 h, fixed 24 h later, and co-stained with 53BP1 antibody and DAPI. 53BP1 foci were quantified by QIBC (≥ 3,000 cells analyzed per condition; mean ± SD; $n = 3$ independent experiments; $^*P < 0.05$; two-tailed paired *t*-test).
F As in (E), except that cells were stained with RAD51 antibody (≥ 3,000 cells analyzed per condition; mean ± SD; $n = 3$ independent experiments; $^*P < 0.05$; ns, not significant, two-tailed paired *t*-test).
G Representative images of the experiments in Fig 1G and H. Scale bar, 10 μm.
H Representative images of the experiments in (E) and (F). Scale bar, 10 μm.
I Immunoblot analysis of FANCD2 siRNA knockdown efficiency in U2OS cells.
J Clonogenic survival of U2OS and U2OS/SCAI KO cells transfected with non-targeting control (CTRL) or FANCD2 siRNAs and subjected to indicated doses of MMC for 24 h (mean ± SEM; $n = 3$ independent experiments).
K Immunoblot analysis of U2OS WT and U2OS/SCAI KO cells harvested at the indicated times after exposure to MMC (0.5 μM).

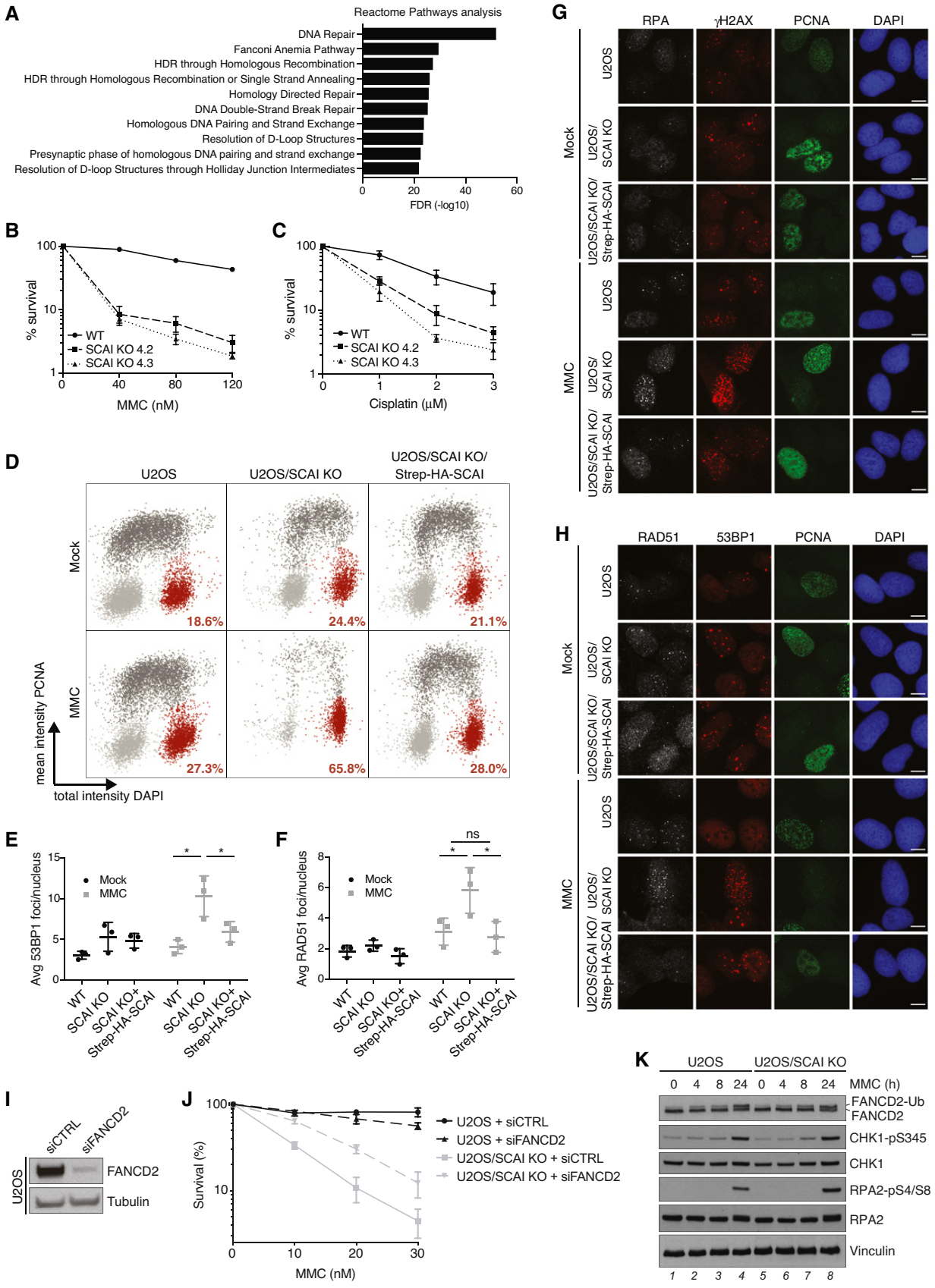

Figure EV1.

Figure EV2. SCAI promotes replication-coupled ICL repair (related to Fig 3).

A Samples in Fig 3B were replicated in the presence of [α-^{32}P]dATP and analyzed by native agarose gel electrophoresis. RI, replication intermediates; OC, open circular; SC, supercoiled.

B SCAI-C immunoblot analysis of *Xenopus* egg extracts (NPE) subjected to immunodepletion with SCAI-C, SCAI-N, or an IgG control antibody.

C SCAI-N immunoblot analysis of samples from (B).

D Immunoblot analysis of *Xenopus* egg extracts subjected to immunodepletion with antibodies targeting the C-terminal (SCAI-C) or N-terminal (SCAI-N) region of SCAI or an IgG control. Asterisks denote antibody cross-reactivity. Note that SCAI-N but not SCAI-C immunodepletion also co-depletes CtIP.

E Schematic of the products generated by HincII or HincII-SapI digest of pICL^{Pt}.

F, G pICL^{Pt} was replicated in mock- or SCAI-depleted extracts and samples were digested with HincII (F) or HincII and SapI (G) and analyzed by agarose gel electrophoresis. DSB repair by HR restores the SapI site (Long *et al*, 2011). pQuant is used as a recovery control (Knipscheer *et al*, 2012) and the quantification of this experiment is shown in Fig 3H.

H pICL^{Pt} was replicated in mock- or SCAI-N-depleted egg extracts in the presence of [α-^{32}P]dATP. Samples were digested with AflIII and analyzed on a denaturing polyacrylamide gel. Stalling points relative to the ICL site are indicated. See Fig 3I for schematic of intermediates and extension products generated by AflIII digest of pICL^{Pt}.

I Samples from (H) were digested with PsiI and XhoI and analyzed on a denaturing polyacrylamide gel. Stalling points relative to the ICL site are indicated. See Fig 3I for schematic of intermediates and extension products generated by PsiI and XhoI double digest of pICL^{Pt}.

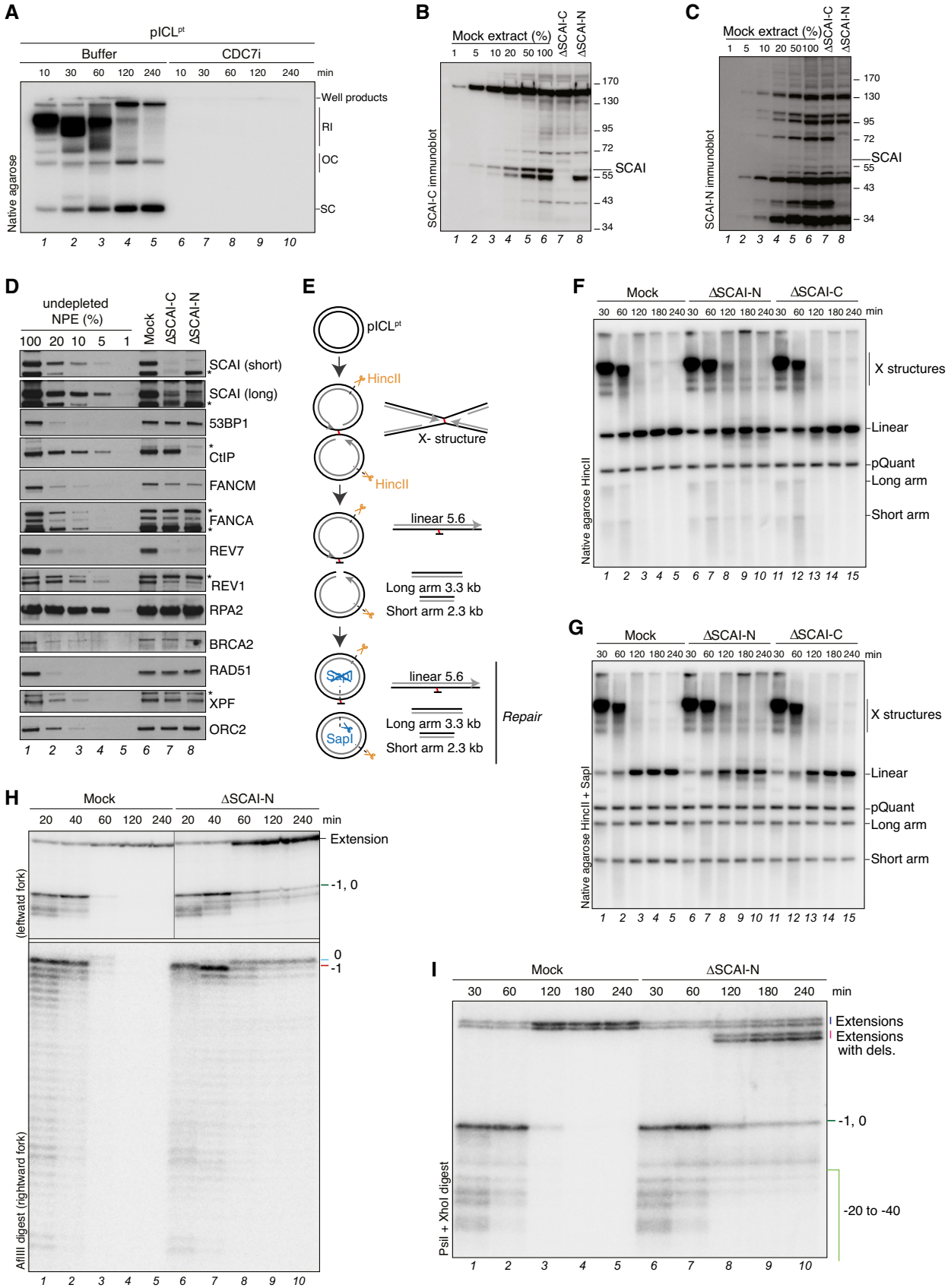

Figure EV2.

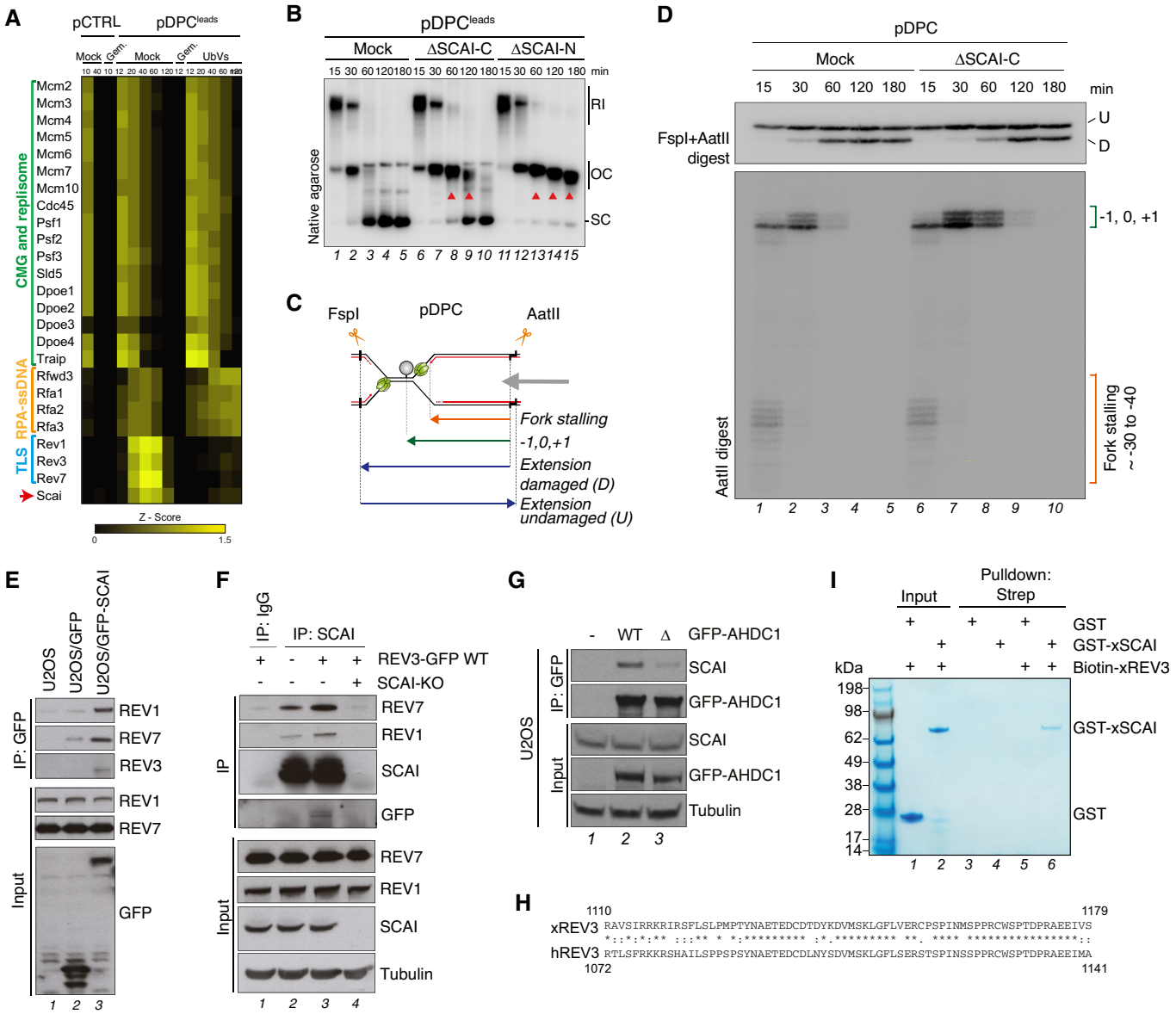

Figure EV3. SCAI interacts with Polζ (related to Fig 4).

A Heat map depicting the mean of the Z-scored Log2 label-free quantification LFQ intensity from four biochemical replicates of pCTRL and pDPC^{Leads} replication in *Xenopus* egg extracts (data originally published in Larsen *et al* (2019)). Where indicated, Geminin was added to block DNA replication, and ubiquitin-vinyl sulfone (Ub-VS) was added to deplete the pool of free ubiquitin from the extracts.

B pDPC^{Leads} was replicated in mock-, SCAI-C-, and SCAI-N-depleted egg extracts in the presence of [α-^{32}P]dATP. Reactions were analyzed by native agarose gel electrophoresis. Red arrowheads indicate accumulation of open circular (OC) molecules caused by a TLS defect.

C Nascent leading strand and extension products generated upon FspI+AatII digest of pDPC. Double digestion generates shorter damaged and longer undamaged extension products. The CMG helicase is depicted in green and the crosslinked M.HpaII protein in grey.

D pDPC was replicated in mock- or SCAI-depleted *Xenopus* egg extract in the presence of [α-^{32}P]dATP. Samples were digested with FspI+AatII and analyzed on a denaturing polyacrylamide gel. Stalling points relative to the DPC site are indicated. U, undamaged; D, damaged extension product.

E U2OS cells or derivative lines stably expressing GFP or GFP-SCAI were subjected to GFP IP followed by immunoblotting with indicated antibodies.

F U2OS WT or SCAI KO cells that were transfected with REV3-GFP where indicated were subjected to IP with IgG or SCAI antibody followed by immunoblotting.

G GFP IPs from U2OS cells transfected with indicated GFP-AHDC1 expression constructs were analyzed by immunoblotting.

H Alignment of the SCAI-interacting regions of human and *Xenopus* REV3 proteins. Asterisk indicates positions with a conserved amino acid; colon indicates conservation between amino acids with strongly similar properties; period indicates conservation between amino acids with weakly similar properties.

I Recombinant GST or GST-xSCAI proteins incubated or not with Biotin-xREV3 peptide (aa 1110–1179) were subjected to streptavidin pull down and analyzed by Coomassie staining.

Figure EV4. SCAI is recruited to UV-induced DNA lesions (related to Fig 4).

A Sperm chromatin was left untreated or exposed to UV-C (2,000 J/m^2), incubated in HSS/NPE mix in the presence of [α-^{32}P]dATP and nucleotide incorporation into chromatin was analyzed at the indicated time points via native agarose gel electrophoresis.

B Quantification of data in (A) (mean ± SEM; $n = 3$ independent experiments).

C Schematic outline of CHROMASS workflow to analyze protein recruitment to UV-damaged chromatin. Sperm chromatin left untreated or irradiated with UV-C (2,000 J/m^2) was incubated in *Xenopus* egg extract for 30 min, isolated by sucrose cushion centrifugation, and analyzed by label-free mass spectrometry (MS).

D Protein recruitment to UV-damaged chromatin compared to an undamaged control. Volcano plot shows enrichment of individual proteins (UV/mock ratio) plotted against the *P*-value ($n = 4$ independent experiments; FDR < 0.05, $s_0 = 0.5$).

E Term enrichment analysis showing GO terms corresponding to proteins significantly recruited to UV-C-damaged chromatin (Dataset EV5). All displayed terms were significant with $P < 0.02$, as determined through Fisher exact testing with Benjamini–Hochberg correction.

F Sperm chromatin left untreated or irradiated with UV-C (2,000 J/m^2) was isolated at the indicated time points and analyzed by immunoblotting.

G Representative images of GFP-SCAI and GFP-Polη recruitment to UV-C laser micro-irradiation in U2OS cells at indicated time points. Scale bar, 10 μm.

H U2OS cells stably expressing GFP-SCAI or transiently transfected with GFP-Polη were subjected to UV-C laser micro-irradiation. At indicated time points, GFP fluorescence intensities at damage sites over the nuclear background were quantified. Recruitment was normalized to the time point of maximal recruitment (mean ± SEM; $n = 3$ independent experiments; at least 30 cells analyzed per condition).

I Clonogenic survival of U2OS cells subjected to indicated doses of UV-C (mean ± SEM; $n = 3$ independent experiments).

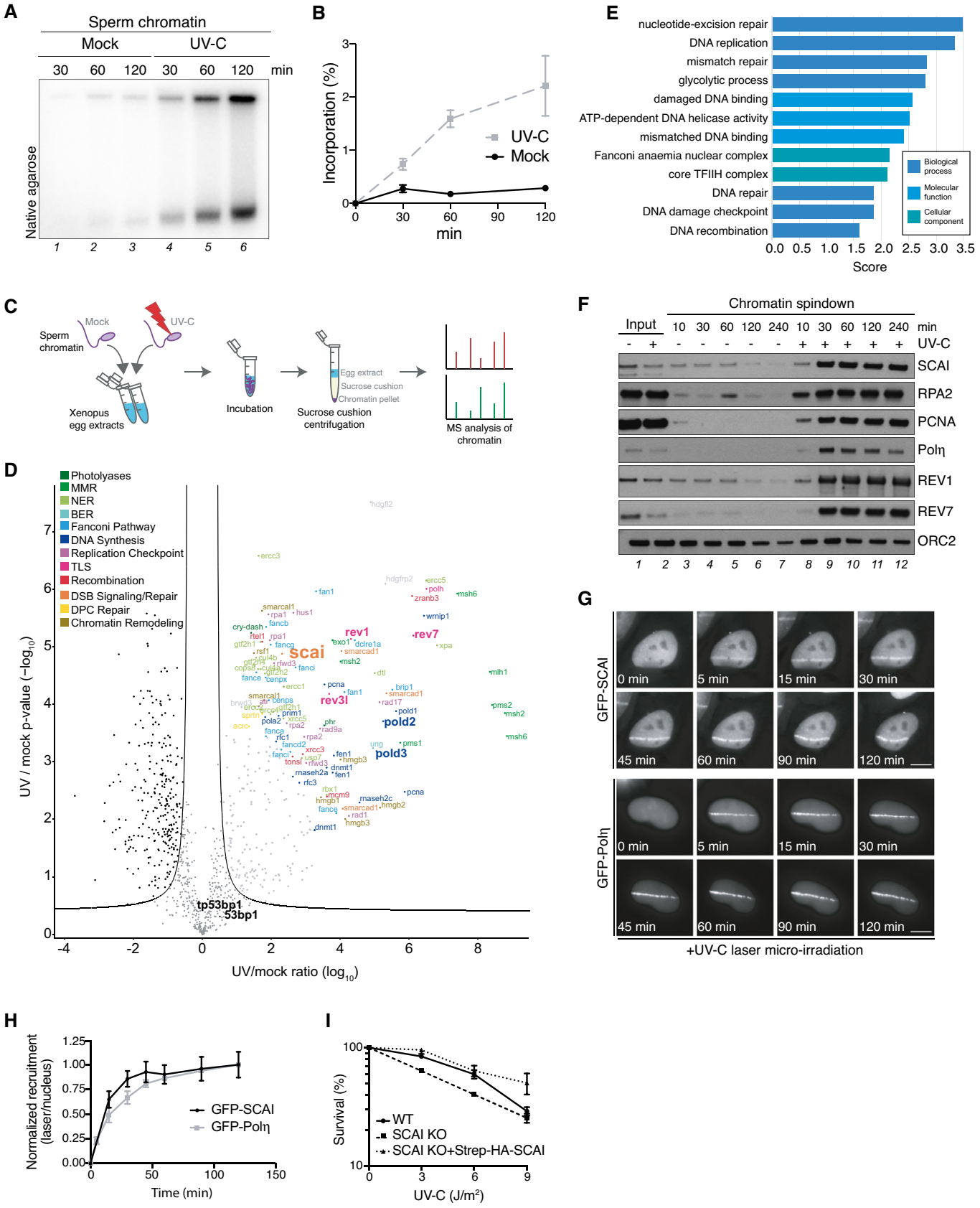
A
Sperm chromatin
Mock
UV-C
30 60 120 30 60 120 min
Native agarose
1 2 3 4 5 6
B
Incorporation (%)
UV-C
Mock
min
E
nucleotide-excision repair
DNA replication
mismatch repair
glycolytic process
damaged DNA binding
ATP-dependent DNA helicase activity
mismatched DNA binding
Fanconi anaemia nuclear complex
core TFIIH complex
DNA repair
DNA damage checkpoint
DNA recombination
Biological process
Molecular function
Cellular component
Score
C
Mock
UV-C
Sperm chromatin
Xenopus egg extracts
Incubation
Egg extract
Sucrose cushion
Chromatin pellet
Sucrose cushion centrifugation
MS analysis of chromatin
F
Chromatin spindown
Input
10 30 60 120 240 10 30 60 120 240 min
UV-C
SCAI
RPA2
PCNA
Polη
REV1
REV7
ORC2
1 2 3 4 5 6 7 8 9 10 11 12
D
Photolyases
MMR
NER
BER
Fanconi Pathway
DNA Synthesis
Replication Checkpoint
TLS
Recombination
DSB Signaling/Repair
DPC Repair
Chromatin Remodeling
UV / mock p-value (−log10)
UV/mock ratio (log10)
scai
rev1
rev7
rev3l
pold2
pold3
tp53bp1
53bp1
G
GFP-SCAI
GFP-Polη
0 min
5 min
15 min
30 min
45 min
60 min
90 min
120 min
+UV-C laser micro-irradiation
H
Normalized recruitment (laser/nucleus)
GFP-SCAI
GFP-Polη
Time (min)
I
Survival (%)
WT
SCAI KO
SCAI KO+Strep-HA-SCAI
UV-C (J/m2)

Figure EV4.

Figure EV5. SCAI prevents Polθ-mediated MMEJ during ICL repair (related to Fig 5).

A Whole cell extracts of U2OS and U2OS/SCAI KO cells were analyzed by immunoblotting with indicated antibodies.
B Mock-, 53BP1-, or SCAI-C-depleted egg extracts were analyzed by immunoblotting with indicated antibodies.
C $pICL^{pt}$ was replicated in extracts immunodepleted in mock-, 53BP1-, or SCAI-C-depleted egg extracts digested with PsiI+XhoI and resolved on a denaturing polyacrylamide gel.
D $pICL^{pt}$ was replicated in SCAI-C-depleted extracts that were also depleted of BRCA2, or supplemented with DNA-PKcs inhibitor (NU7441; 100 μM) where indicated. Reaction samples were digested with PsiI+XhoI and analyzed on a denaturing polyacrylamide gel.
E Mock- or BRCA2-depleted egg extracts were analyzed by immunoblotting with indicated antibodies.
F Most frequent deletion products in sequencing data in Fig 5A. In red, crosslinked guanines; highlighted in grey, deletions; underlines, microhomology regions.
G Schematic representation of DNA synthesis occurring across the adducted base following DNA incisions on either the top or bottom strand. The denoted nucleotide position 1,548 corresponds to the insertion 0 position when incisions occur in the bottom strand or to the −1 position when incisions occur in the top strand. Sequencing products of correct length from Fig 5A were analyzed for their nucleotide misincorporation distribution (bottom graphs). Misincorporation is based on the bottom strand read.
H Samples in Fig 5C were analyzed by native agarose gel electrophoresis.
I $pICL^{pt}$ was replicated in mock- or SCAI-depleted extracts treated or not with Novobiocin (NVB; 150 μM) in the presence of [α-^{32}P]dATP, and reactions were analyzed by native agarose gel electrophoresis. RI, replication intermediates; OC, open circular; SC, supercoiled.
J Samples in (I) were digested with PsiI+XhoI or AflIII and analyzed on a denaturing polyacrylamide gel.
K Clonogenic survival of U2OS and U2OS/SCAI KO cells treated or not with Novobiocin (NVB; 50 μM) and subjected to various doses of MMC for 24 h (mean ± SEM; $n = 3$ independent experiments).

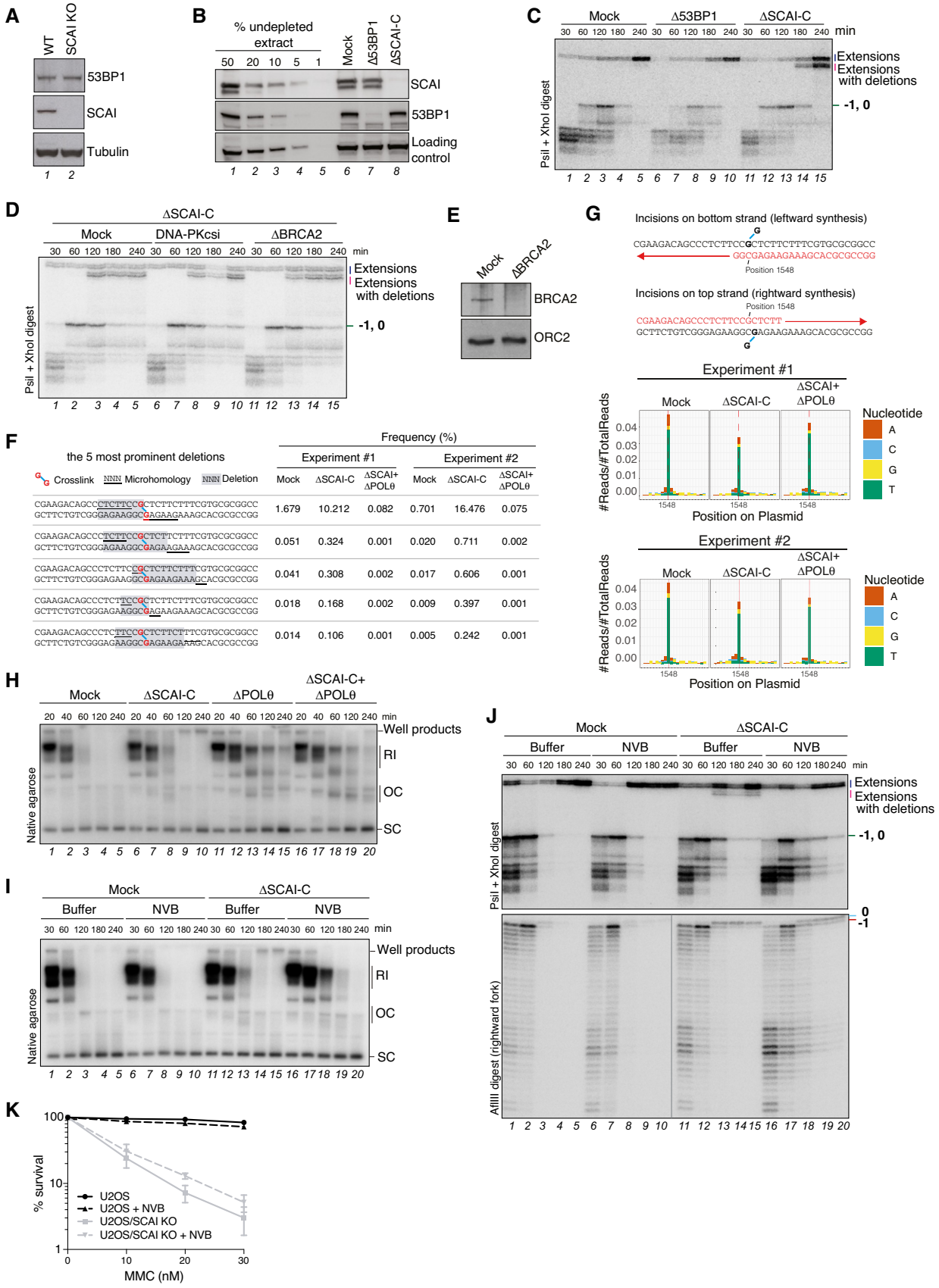
A
WT
SCAI KO
53BP1
SCAI
Tubulin
1 2
B
% undepleted extract
50 20 10 5 1
Mock
Δ53BP1
ΔSCAI-C
SCAI
53BP1
Loading control
1 2 3 4 5 6 7 8
C
Mock
Δ53BP1
ΔSCAI-C
30 60 120 180 240 30 60 120 180 240 30 60 120 180 240 min
Psi1 + XhoI digest
Extensions
Extensions with deletions
-1, 0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
D
ΔSCAI-C
Mock
DNA-PKcsi
ΔBRCA2
30 60 120 180 240 30 60 120 180 240 30 60 120 180 240 min
Psi1 + XhoI digest
Extensions
Extensions with deletions
-1, 0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
E
Mock
ΔBRCA2
BRCA2
ORC2
F
the 5 most prominent deletions
Crosslink NNN Microhomology NNN Deletion
Frequency (%)
Experiment #1
Experiment #2
Mock
ΔSCAI-C
ΔSCAI+ ΔPOLθ
CGAAGACAGCCCTCTTCCGCTCTTCTTTCGTGCGCGGCC
GCTTCTGTCGGGAGAAGGCGAGAAGAAAGCACGCGCCGG
1.679 10.212 0.082 0.701 16.476 0.075
CGAAGACAGCCCTCTTCCGCTCTTCTTTCGTGCGCGGCC
GCTTCTGTCGGGAGAAGGCGAGAAGAAAGCACGCGCCGG
0.051 0.324 0.001 0.020 0.711 0.002
CGAAGACAGCCCTCTTCCGCTCTTCTTTCGTGCGCGGCC
GCTTCTGTCGGGAGAAGGCGAGAAGAAAGCACGCGCCGG
0.041 0.308 0.002 0.017 0.606 0.001
CGAAGACAGCCCTCTTCCGCTCTTCTTTCGTGCGCGGCC
GCTTCTGTCGGGAGAAGGCGAGAAGAAAGCACGCGCCGG
0.018 0.168 0.002 0.009 0.397 0.001
CGAAGACAGCCCTCTTCCGCTCTTCTTTCGTGCGCGGCC
GCTTCTGTCGGGAGAAGGCGAGAAGAAAGCACGCGCCGG
0.014 0.106 0.001 0.005 0.242 0.001
G
Incisions on bottom strand (leftward synthesis)
CGAAGACAGCCCTCTTCCGCTCTTCTTTCGTGCGCGGCC
GGCGAGAAGAAAGCACGCGCCGG
Position 1548
Incisions on top strand (rightward synthesis)
Position 1548
CGAAGACAGCCCTCTTCCGCTCTT
GCTTCTGTCGGGAGAAGGCGAGAAGAAAGCACGCGCCGG
Experiment #1
Mock
ΔSCAI-C
ΔSCAI+ ΔPOLθ
#Reads/#TotalReads
0.04 0.03 0.02 0.01 0.00
1548
Position on Plasmid
Nucleotide
A
C
G
T
Experiment #2
H
Mock
ΔSCAI-C
ΔPOLθ
ΔSCAI-C+ ΔPOLθ
20 40 60 120 240 20 40 60 120 240 20 40 60 120 240 20 40 60 120 240 min
Native agarose
Well products
RI
OC
SC
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
I
Mock
ΔSCAI-C
Buffer
NVB
30 60 120 180 240 30 60 120 180 240 30 60 120 180 240 30 60 120 180 240 min
Native agarose
Well products
RI
OC
SC
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
J
Mock
ΔSCAI-C
Buffer
NVB
30 60 120 180 240 30 60 120 180 240 30 60 120 180 240 30 60 120 180 240 min
Psi1 + XhoI digest
Extensions
Extensions with deletions
-1, 0
0
-1
AflIII digest (rightward fork)
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
K
% survival
100
10
1
0 10 20 30
MMC (nM)
U2OS
U2OS + NVB
U2OS/SCAI KO
U2OS/SCAI KO + NVB

Figure EV5.