Supplemental figure legends

Figure S1.

SCAI is enriched on DNA damage-containing chromatin and interacts with 53BP1.

a. Protein intensity of SCAI on psoralen (PSO)-crosslinked (red) or undamaged chromatin (blue) plotted against time. Data was replotted from [1].

b. Protein intensity of SCAI on psoralen-crosslinked chromatin in the absence (red) or presence of the replication inhibitor geminin (GEM) (black) plotted against time. Data was replotted from [1].

c. Soluble and chromatin-enriched (CHR) fractions of lysates from WT and SCAI-deleted (KO) U2OS cells were analyzed by immunoblotting with the indicated antibodies.

d. U2OS cells were treated with doxycycline (DOX) to induce expression of Strep-HA-tagged SCAI, and SCAI-containing complexes were isolated by Strep-tactin pull-down from total cell lysates. Associated proteins were analyzed by immunoblotting with antibodies to 53BP1 and HA.

e. U2OS cells stably expressing GFP-SCAI transfected with control (CTRL) or 53BP1 siRNAs were subjected to laser micro-irradiation, fixed 1 h later and immunostained with γ-H2AX antibody.

f. U2OS cells stably expressing GFP-SCAI were transfected with control (CTRL) or 53BP1 siRNA, exposed to ionizing radiation (IR, 5 Gy) as indicated and fixed 4 h later.

g. U2OS cells were transfected with control (CTRL) or one of two independent SCAI siRNAs (si#9, si#10). Chromatin-enriched fractions of the cells were analyzed by immunoblotting with antibodies against SCAI and MCM6.

h. U2OS cells stably expressing GFP-SCAI (U2OS/GFP-SCAI) were treated with proteasome inhibitor MG132 for 3 h and subjected to laser micro-irradiation. Cells were fixed after 1 h and immunostained with γ-H2AX antibody.

i. Cells from (g) were exposed to ionizing radiation (IR, 5 Gy) as indicated, fixed 1 h later and immunostained with antibodies against 53BP1 and γ-H2AX. All scale bars, 10 μm. Uncropped blots (**c,d,g**) are shown in Figure S6

Figure S2.

53BP1-dependent and -independent recruitment of SCAI to DNA damage sites.

a. Schematic representation of human 53BP1 constructs used in the complementation assay in (Figure 2a). WT; wild type, ΔBRCT; deletion of tandem BRCT domains; ΔN-term; deletion of N-terminus, S28A; alanine substitution of all putative N-terminal ATM phosphorylation sites. Ability to support SCAI recruitment when transfected into *53BP1*$^{-/-}$ MEFs is indicated for each construct on the right.

b. U2OS cells transfected with control (CTRL) or siRNA targeting SCAI or 53BP1 were subjected to laser micro-irradiation, fixed 1 h later and immunostained with antibodies against RIF1 and γ-H2AX.

c. U2OS/GFP-SCAI cells were transfected with control (CTRL), 53BP1 or RNF8 siRNAs, subjected to laser micro-irradiation, and pre-extracted and fixed 1 h later. Inserts show larger magnifications of the highlighted nuclear regions, revealing accumulation of SCAI in the DSB-surrounding chromatin compartment dependent on 53BP1 and RNF8, and in the ssDNA compartment, independently of 53BP1 and RNF8. Lower panel: Schematic of compartmentalization of IRIF. “Chromatin compartment” denotes regions of DDR-modified chromatin, into which 53BP1

accumulates and recruits SCAI. “ssDNA compartment” denotes areas of resected DNA to which SCAI is recruited by a 53BP1-independent mechanism.

d. U2OS/GFP-SCAI cells were transfected with control (CTRL) or siRNAs targeting BRCA1 or BRCA2, and treated with proteasome inhibitor MG132 for 3 h to interfere with ubiquitin-dependent recruitment of SCAI and 53BP1 to chromatin regions. Cells were the subjected to laser micro-irradiation, pre-extracted and fixed 1 h later and immunostained with γ-H2AX antibody. All scale bars, 10 μm.

e. SCAI interacts with ssDNA. Untagged or Biotinylated ssDNA oligos were incubated with purified GST or GST-SCAI as indicated and subjected to pull-down (PD) with Streptavidin beads. Beads were washed extensively and resolved on SDS-PAGE along with input (5%) samples. Uncropped blots (**e**) are shown in Figure S6.

Figure S3.

SCAI is required for optimal DSB repair but is dispensable for accrual of HR factors.

a. U2OS cells (WT) or derivate lines with targeted knockout of SCAI (SCAI KO) and reconstituted expression of ectopic SCAI (rescue) were incubated in the presence of EdU, exposed to IR (1Gy) and fixed at the indicated time points. Cell cycle position of individual cells was determined by detection of EdU and DAPI signal.

b. U2OS cells lacking SCAI (SCAI KO) were obtained by CRISPR-mediated knockout and used to generate a derivative cell line in which expression of SCAI was stably reconstituted (‘rescue’). Chromatin-enriched fractions of these cell lines were analyzed by immunoblotting with SCAI and MCM6 antibodies.

c. U2OS cells deleted for 53BP1 were generated by CRISPR-mediated knockout. Lysates from parental and 53BP1 KO derivatives of U2OS cells were analyzed by immunoblotting with 53BP1 and MCM6 antibodies.

d. U2OS cells and derivative cell lines from Figure S3b were fixed at indicated times after exposure to IR (1 Gy) and stained with 53BP1 antibody. The number of foci per cell was measured by high content microscopy. Centre indicates the median and whiskers the borders of the 95% quantiles. 1000 cells (n= 1000 independent measurements) were measured per condition and p-values were calculated from a non-parametric two-tailed Mann-Whitney U test.

e. Parental U2OS cells and two independently derived derivative lines with targeted *SCAI* knockout were co-transfected with either circular (negative control) or linearized GFP-NHEJ reporter plasmid and construct encoding RFP. Cells were analyzed by flow cytometry for GFP and RFP positivity 48 h later, and the proportion of GFP-/RFP-positive cells compared to RFP positive cells only cells was determined. The NHEJ efficiency of WT cells was set to 1. Values indicate the mean and error bars the standard deviation from 3 independent experiments (n=3). P-values were calculated from a two-tailed t-test.

f. Cells from Figure 3b were treated and analyzed as in (e), expect cells were transfected with either circular or linearized GFP-HR reporter plasmid. The HR efficiency of WT cells was set to 1. Error bars indicate the standard deviation from 3 independent experiments (n=3) and P-values were calculated from a two-tailed t-test.

g. U2OS WT and SCAI KO cells were exposed to IR, fixed 6 h later and immunostained with antibodies against RAD51 and γ-H2AX.

h. As in (g), except that WT or *SCAI*$^{-/-}$ MEFs and the indicated dose of IR were used. All scale bars, 10 μm.

i. Analysis of RAD51 foci in WT and *SCAI*$^{-/-}$ primary MEFs at different cell cycle stages. Cells were incubated in the presence of EdU for 1 h, exposed to IR and

fixed after another 3.5 h, then immunostained with RAD51 antibody and counterstained for EdU and DAPI. Cells were analyzed by automated high-content microscopy using EdU positivity to identify S phase cells and DAPI intensity to discriminate between G1 and G2 phase in EdU-negative cells. Four individual MEF preparations were analyzed for each genotype (n=4 biologically independent samples), and 1500 cells were analyzed per sample. Values indicate the mean and error bars are the standard deviation. Uncropped blots (**b,c**) are shown in Figure S6.

Figure S4.

Characterization of *SCAI*$^{-/-}$ mice and MEFs.

a. Proliferation of MEFs. Primary WT (n = 9 biologically independent samples from 3 cell lines) and *SCAI*$^{-/-}$ (n = 12 biologically independent samples from 4 cell lines) MEFs were plated at passages 2, 3, 4 and 5, and trypsinized, counted and replated every second day. Values indicate the mean and error bars are the standard deviation.

b. Overall DSB repair proficiency of 3 immortalized pairs of WT and *SCAI*$^{-/-}$ MEF cell lines was evaluated by enumeration of residual 53BP1 foci in G2 phase (H3-pS10-positive) cells at the indicated times after exposure to IR (2 Gy). Values indicate the mean and error bars the standard deviation across 3 independent experiments. P-value was calculated from a one-tailed t-test using Welch correction (n = 9 independent measurements across 3 MEF lines). Bars indicate mean ± SD. IRIF: Ionizing Radiation Induced Foci.

c. Examples of cells from the experiment in (b). MEFs were fixed 8 h after exposure to IR (2 Gy), immunostained with antibodies to 53BP1 and H3-pS10 and counterstained for nuclear content with DAPI.

d. 12 WT and 16 *SCAI*$^{-/-}$ age-matched male mice were subjected to whole-body gamma-irradiation with a one-time dose of 8 Gy. Survival was monitored over a 28-day period, after which remaining animals were euthanized.

e. As in (d) but with female mice . 7 WT and 6 *SCAI*$^{-/-}$ mice were used. Data from (d) and (e) have been combined in Figure 3b.

f. Primary B cells isolated from WT and *SCAI*$^{-/-}$ mice were labeled with CellTrace Violet and analyzed by flow cytometry to monitor cell proliferation by dye dilution.

g. Flow cytometric quantification of splenic B220+IgM+ B cell populations from one WT and one *SCAI*$^{-/-}$ mouse. A representative of multiple mice.

h. Graphical representation of B220+CD19+ total B cell number from 6 WT and 6 *SCAI*$^{-/-}$ mice.

i. B cells isolated from 6 WT and 6 *SCAI*$^{-/-}$ mice were stimulated for 4 days to undergo IgH class-switching and IgG1 (left) and IgG3 (right) frequencies were measured by flow cytometry.

j. Serum levels of IgG1, IgG3, and IgM immunoglobulins in 6 WT and 6 *SCAI*$^{-/-}$ mice as measured by ELISA. Each dot in the quantifications in (h), (i) and (j) represents data from individual mice and all lines indicate the mean. For each genotype, 3 males and 3 females were analyzed.

k. Breeding experiment of WT (male n=19 and female n=15 animals) and *SCAI*$^{-/-}$ (male n=7 and female n=4 animals) mice. Quantification of litter sizes. Bars indicate mean ± SD and P-value was calculated with a two-tailed t test. Source data in Table S2.

l. Spermatocyte spreads from WT and *SCAI*$^{-/-}$ mice were stained with SYCP3 and DMC1 antibodies. Representative pachynema spermatocytes are shown. Quantified in Figure 3j.

m. Examples of the types of chromosomal aberrations observed in *SCAI*$^{-/-}$ B cells treated with PARP inhibitor and quantified in Figure 3m.

Figure S5.

SCAI promotes DSB repair in heterochromatin.

a. GFP-SCAI was affinity-purified on GFP-Trap beads from chromatin-enriched fractions of U2OS and U2OS/GFP-SCAI cells, and co-purifying proteins and input lysates were analyzed by immunoblotting with antibodies against HP1γ, GFP and Tubulin.

b. As in (a), except that co-purifying proteins and input lysates were analyzed with antibodies against HP1β, GFP and Tubulin.

c. Extended version of Figure 4a. Independent and immortalized WT and *SCAI*$^{-/-}$ MEF cell lines were arrested in G0/G1 by growing to full confluency. Cultures were mock-treated or exposed to IR (2 Gy), fixed either 0.5 or 24 h later and stained with 53BP1 antibody. Images were acquired as Z-stacks and the number of 53BP1 foci per cell was counted through the entire nuclear volume. Bars indicate mean ± SD (n = 9 independent measurements across 3 MEF lines). IRIF: Ionizing Radiation Induced Foci

d. Extended version of (Figure 4b). Immortalized WT and *SCAI*$^{-/-}$ MEFs were grown to full confluency while transfecting with 53BP1 siRNA for 72 h or incubating with ATM inhibitor for 1 h prior to irradiation. Cells were treated and analyzed as in (c), except that they were immunostained for γ-H2AX as a marker of unrepaired DSB. (n = 3 biologically independent samples).

e. Immortalized WT and *SCAI*$^{-/-}$ MEFs were treated and analyzed as in (c), except that cells were incubated with ATM inhibitor and co-stained with antibodies to γ-H2AX and the heterochromatin marker H3K9me3 to determine chromatin context (n = 3 biologically independent samples). HC (heterochromatin).

f. Representative images from the analysis in (Figure 4e,f).

g. Mouse NIH-3T3 fibroblasts were transfected with GFP-SCAI, CAS9 and guide RNA (gRNA) targeting the major satellite repeats in heterochromatin containing chromocenters. Cells were fixed after 16 h and immunostained with 53BP1 antibodies and DNA stain DAPI.

h. Immortalized WT and *SCAI*$^{-/-}$ MEFs were transfected with Cas9-GFP and gRNAs targeting the major satellite repeats to induce CRISPR-mediated DSBs in heterochromatin-containing chromocenters. After 8 h cells were fixed and immunostained with antibodies against H3K9me3. Cells were analyzed by high content microscopy using DAPI signal as a mask for chromocenters. 220 cells (n=220 independent measurements) were measured per condition and P-values were calculated from two-tailed t-tests using Welch correction. Centre indicates the median and whiskers the borders of the 95% quantiles. Y-axis on the left side corresponds to the non-transfected conditions, while y-axis on the right side corresponds to the transfected conditions.

i. As in (h), except cells were immunostained with HP1β antibodies. (n=120 independent measurements)

j. As in (h), except cells were immunostained with HP1γ antibodies. (n=130 independent measurements)

k. As in (h), except cells were only stained with DAPI. (n=320 independent measurements)

l. Cells from (g) were treated with ATM inhibitor and transfected as in (g) to induce heterochromatin associated DSBs by CRISPR. Lysates were analyzed for markers of ATM signaling by immunoblotting with the indicated antibodies.

m. WT and *SCAI*$^{-/-}$ MEFs were exposed to ionizing radiation (IR, 1Gy) to induce DSBs predominantly in euchromatin. Lysates were collected at the indicated time points and analyzed for markers of ATM signaling by immunoblotting with the indicated antibodies. All scale bars, 10 μm. Uncropped blots (**a,b,l,m**) are shown in Figure S6.

Figure S6.

Uncropped western blots from all main and supplementary figures

Supplementary table legends

Table S1

Breeding of *SCAI*$^{-/-}$ mice

Table showing the expected and observed genotypes of 3-week old pups from *SCAI*$^{+/-}$ intercross breedings.

Table S2

Fertility measurements of *SCAI*$^{-/-}$ mice

Number of live-born pups from breeding cages containing one male and one female mouse of the indicated genotypes. Four breeding cages were set up to test fertility of *SCAI*$^{-/-}$ males along with four additional breeding cages using WT littermates as the control group.

Similar experimental and control groups were setup to test fertility of *SCAI*$^{-/-}$ females. All mating experiments lasted at least six months.

Table S2

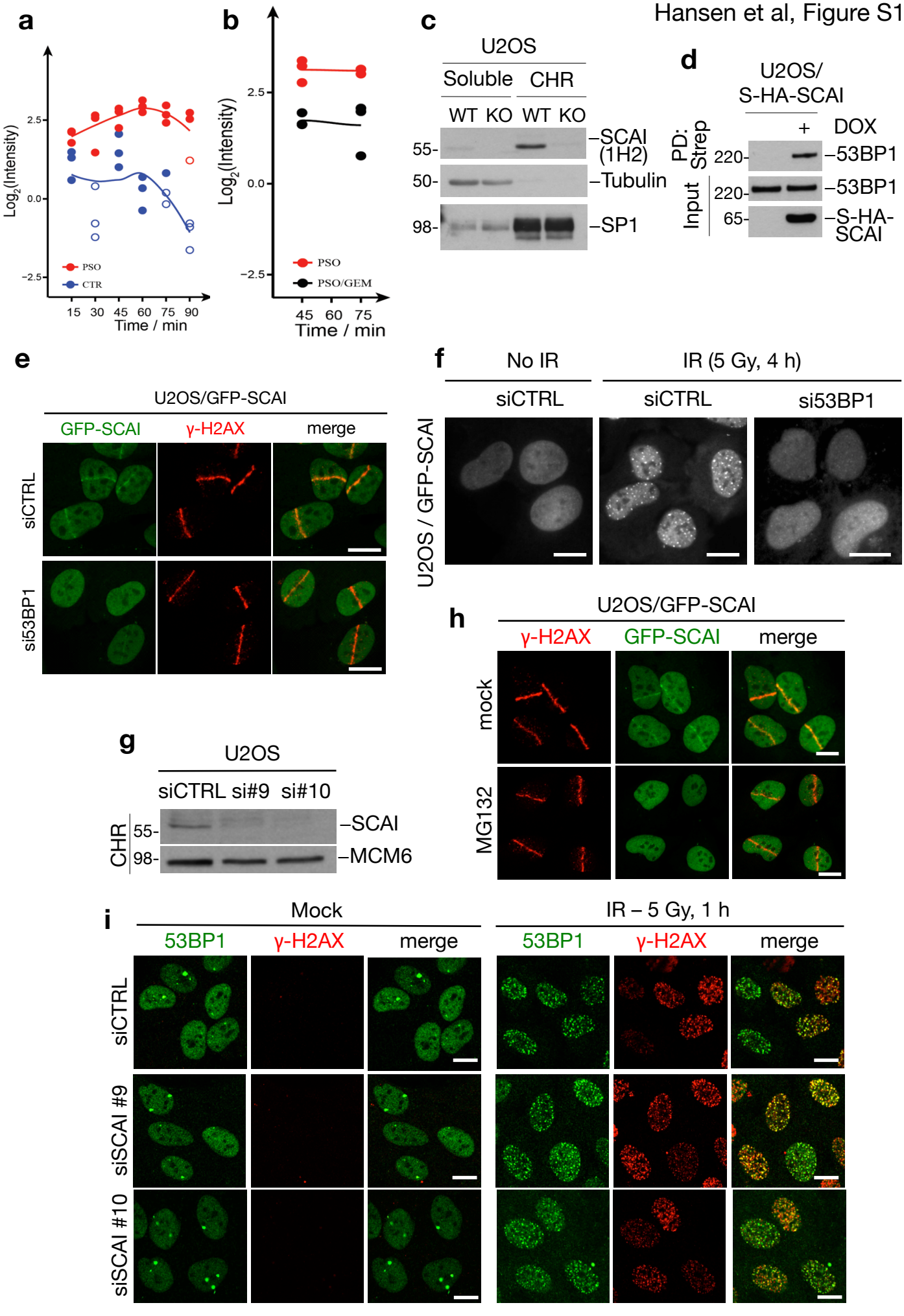
Genomic instability in *SCAI*$^{-/-}$ B cells

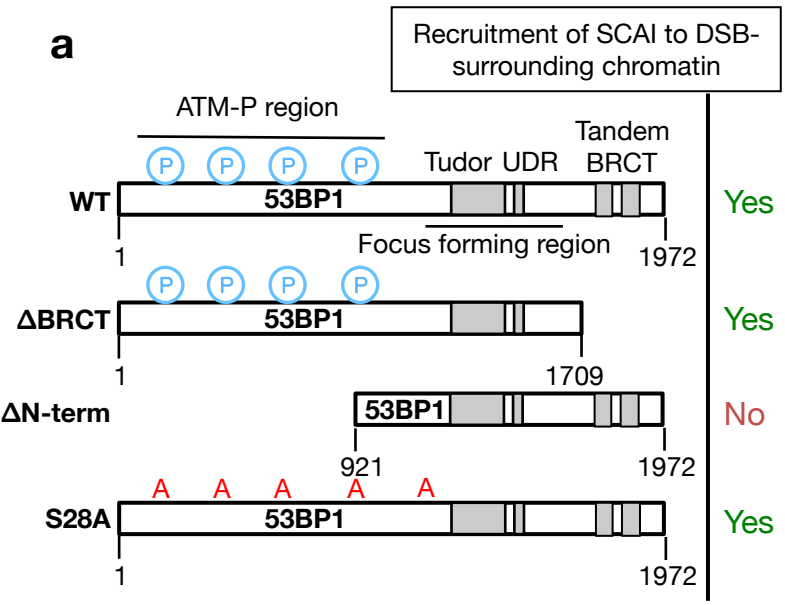
Frequencies of chromosomal aberrations in primary murine B cells, stimulated with LPS/IL4/RP105 to undergo IgG1 class switching for 72 hours, and treated with DMSO or PARP inhibitor olaparib (2 μM) for 16 h. Quantifications and classifications are displayed graphically in (Fig. 3m; Fig. S4m).

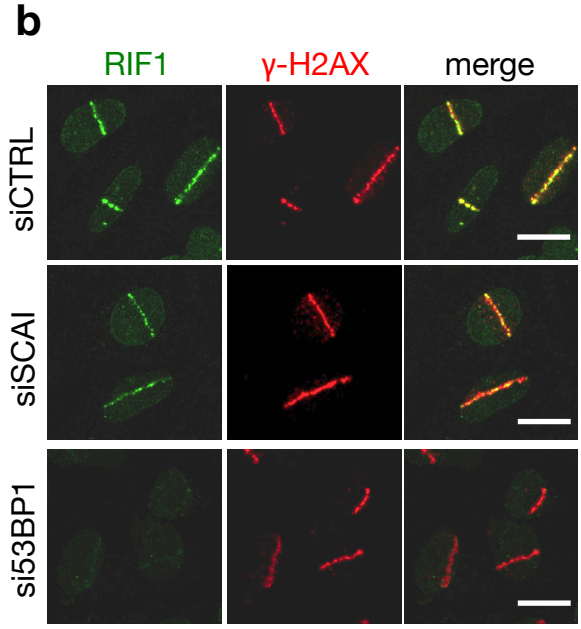
References

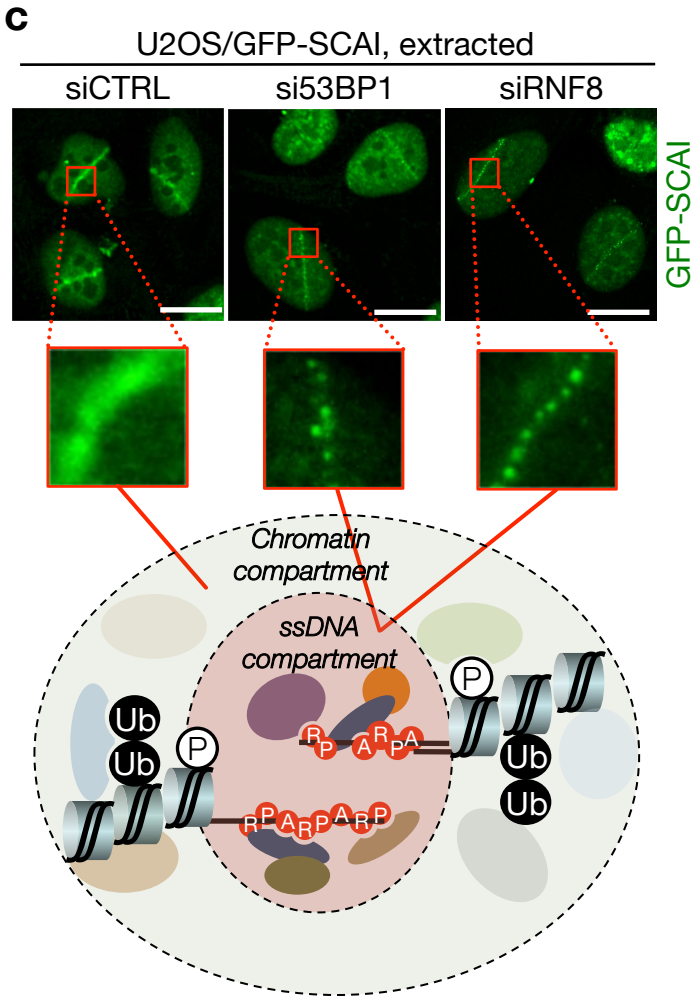
1 Raschle, M. *et al.* DNA repair. Proteomics reveals dynamic assembly of repair complexes during bypass of DNA cross-links. *Science* **348**, 1253671, doi:10.1126/science.1253671 (2015).

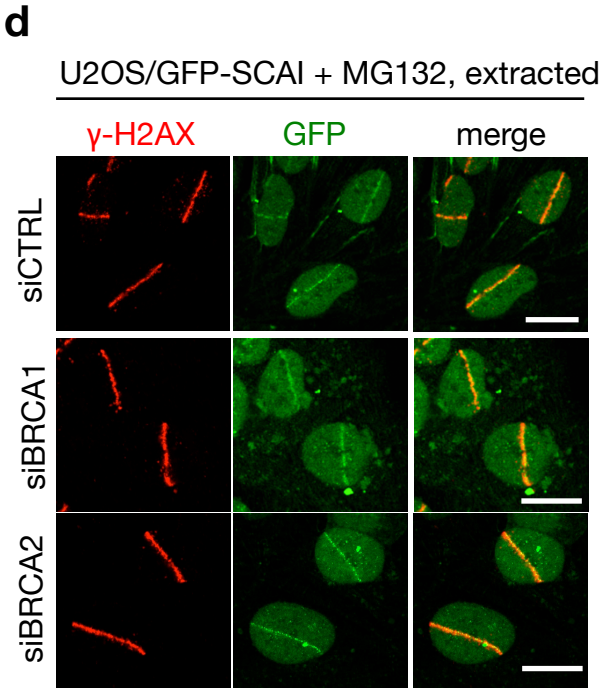
Hansen et al, Figure S1

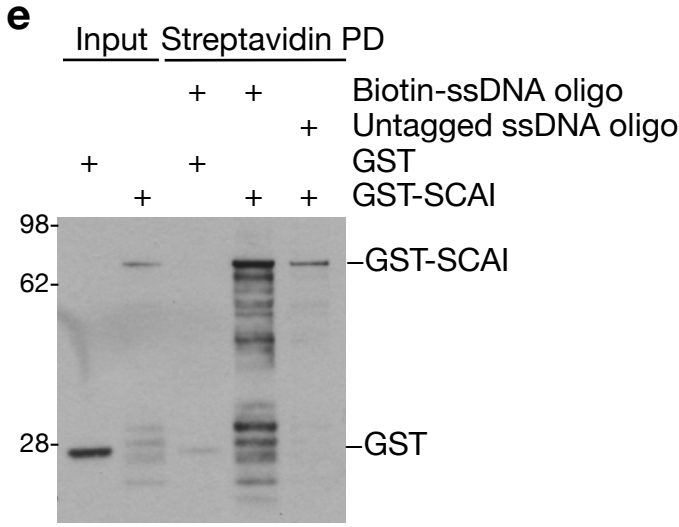


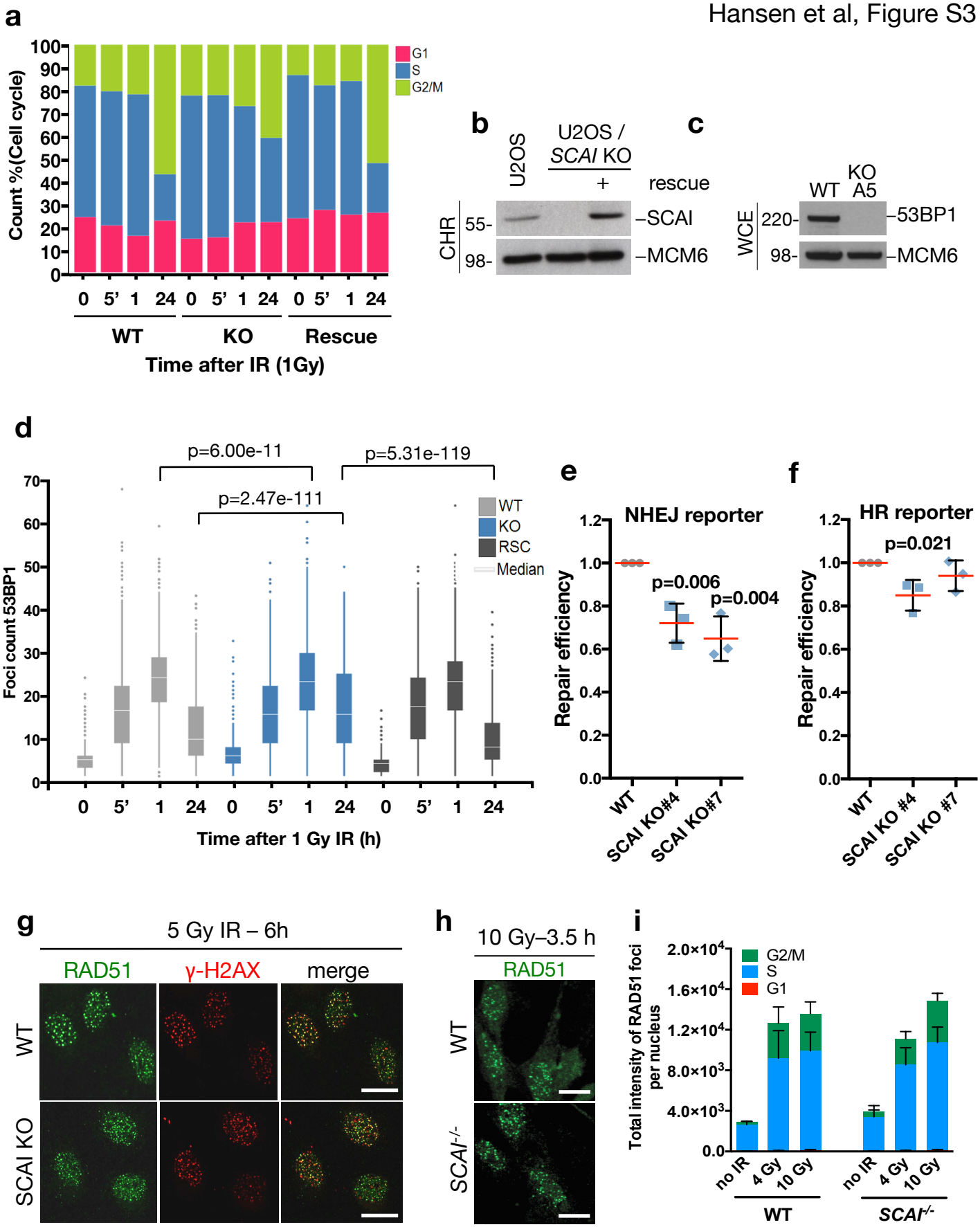

a
Recruitment of SCAI to DSB-surrounding chromatin
ATM-P region
Tandem
Tudor UDR
BRCT
WT
53BP1
Yes
1
1972
Focus forming region
ΔBRCT
53BP1
Yes
1
1709
ΔN-term
53BP1
No
921
1972
S28A
53BP1
Yes
1
1972

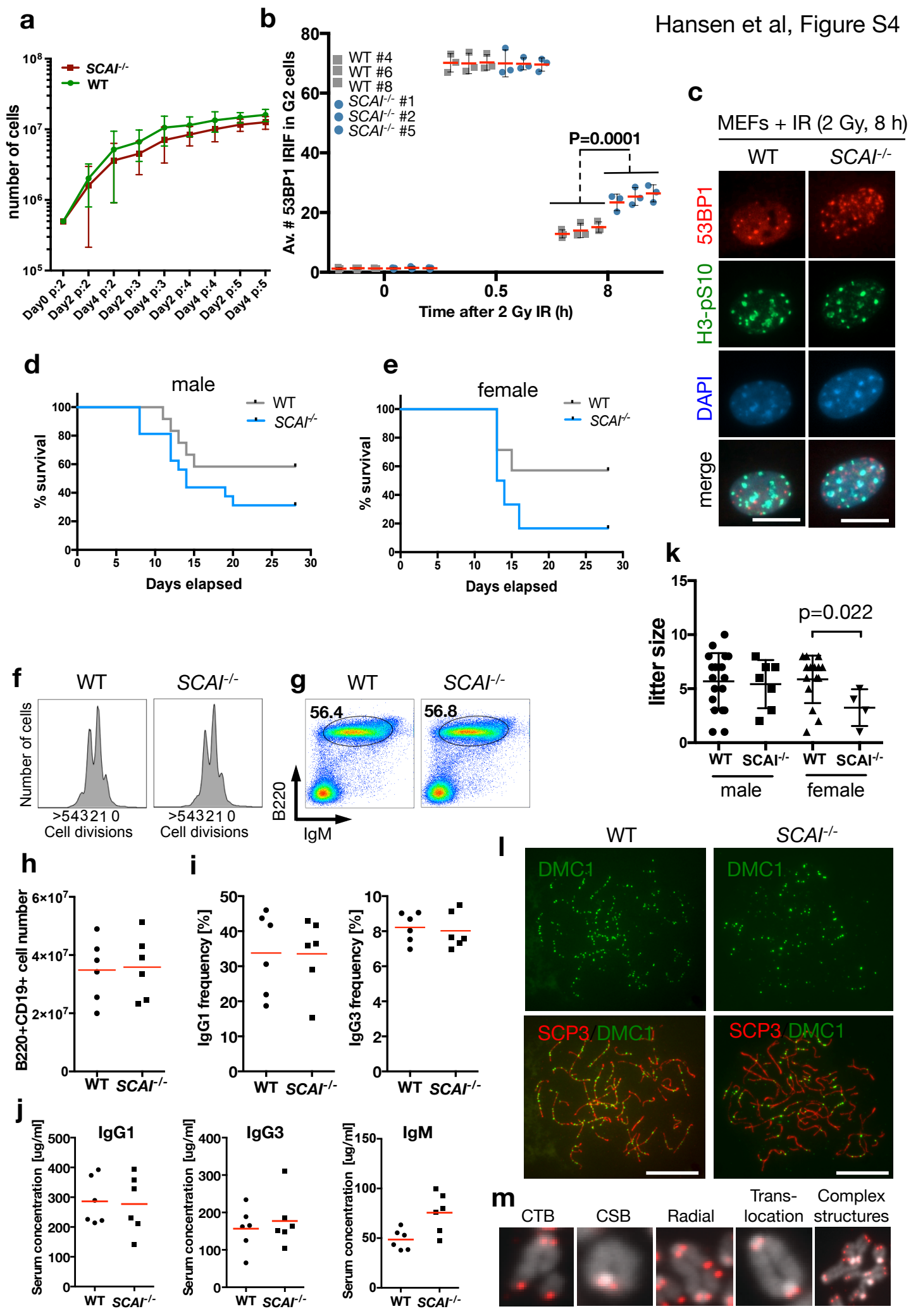

b
RIF1
γ-H2AX
merge
siCTRL
siSCAI
si53BP1


c
U2OS/GFP-SCAI, extracted
siCTRL
si53BP1
siRNF8
GFP-SCAI
Chromatin compartment
ssDNA compartment
Ub
P
R
A


d
U2OS/GFP-SCAI + MG132, extracted
γ-H2AX
GFP
merge
siCTRL
siBRCA1
siBRCA2


e
Input
Streptavidin PD
Biotin-ssDNA oligo
Untagged ssDNA oligo
GST
GST-SCAI
98
62
28
GST-SCAI
GST


a
Count %(Cell cycle)
G1
S
G2/M
0 5' 1 24 0 5' 1 24 0 5' 1 24
WT
KO
Rescue
Time after IR (1Gy)
b
U2OS
U2OS / SCAI KO
+
rescue
CHR
55-
98-
-SCAI
-MCM6
c
WT
KO A5
WCE
220-
98-
-53BP1
-MCM6
d
p=6.00e-11
p=5.31e-119
p=2.47e-111
WT
KO
RSC
Median
Foci count 53BP1
0 5' 1 24 0 5' 1 24 0 5' 1 24
Time after 1 Gy IR (h)
e
NHEJ reporter
p=0.006
p=0.004
Repair efficiency
WT
SCAI KO#4
SCAI KO#7
f
HR reporter
p=0.021
Repair efficiency
WT
SCAI KO #4
SCAI KO #7
g
5 Gy IR – 6h
RAD51
γ-H2AX
merge
WT
SCAI KO
h
10 Gy–3.5 h
RAD51
WT
SCAI-/-
i
G2/M
S
G1
Total intensity of RAD51 foci per nucleus
no IR
4 Gy
10 Gy
WT
SCAI-/-


a
number of cells
SCAI-/-
WT
Day0 p:2
Day2 p:2
Day4 p:2
Day2 p:3
Day4 p:3
Day2 p:4
Day4 p:4
Day2 p:5
Day4 p:5
b
Av. # 53BP1 IRIF in G2 cells
WT #4
WT #6
WT #8
SCAI-/- #1
SCAI-/- #2
SCAI-/- #5
P=0.0001
Time after 2 Gy IR (h)
c
MEFs + IR (2 Gy, 8 h)
WT
SCAI-/-
53BP1
H3-pS10
DAPI
merge
d
male
% survival
Days elapsed
e
female
% survival
Days elapsed
k
litter size
p=0.022
male
female
f
WT
SCAI-/-
Number of cells
Cell divisions
g
WT
SCAI-/-
56.4
56.8
B220
IgM
h
B220+CD19+ cell number
i
IgG1 frequency [%]
IgG3 frequency [%]
j
IgG1
IgG3
IgM
Serum concentration [ug/ml]
l
WT
SCAI-/-
DMC1
SCP3 DMC1
m
CTB
CSB
Radial
Trans-location
Complex structures

Hansen et al, Figure S5

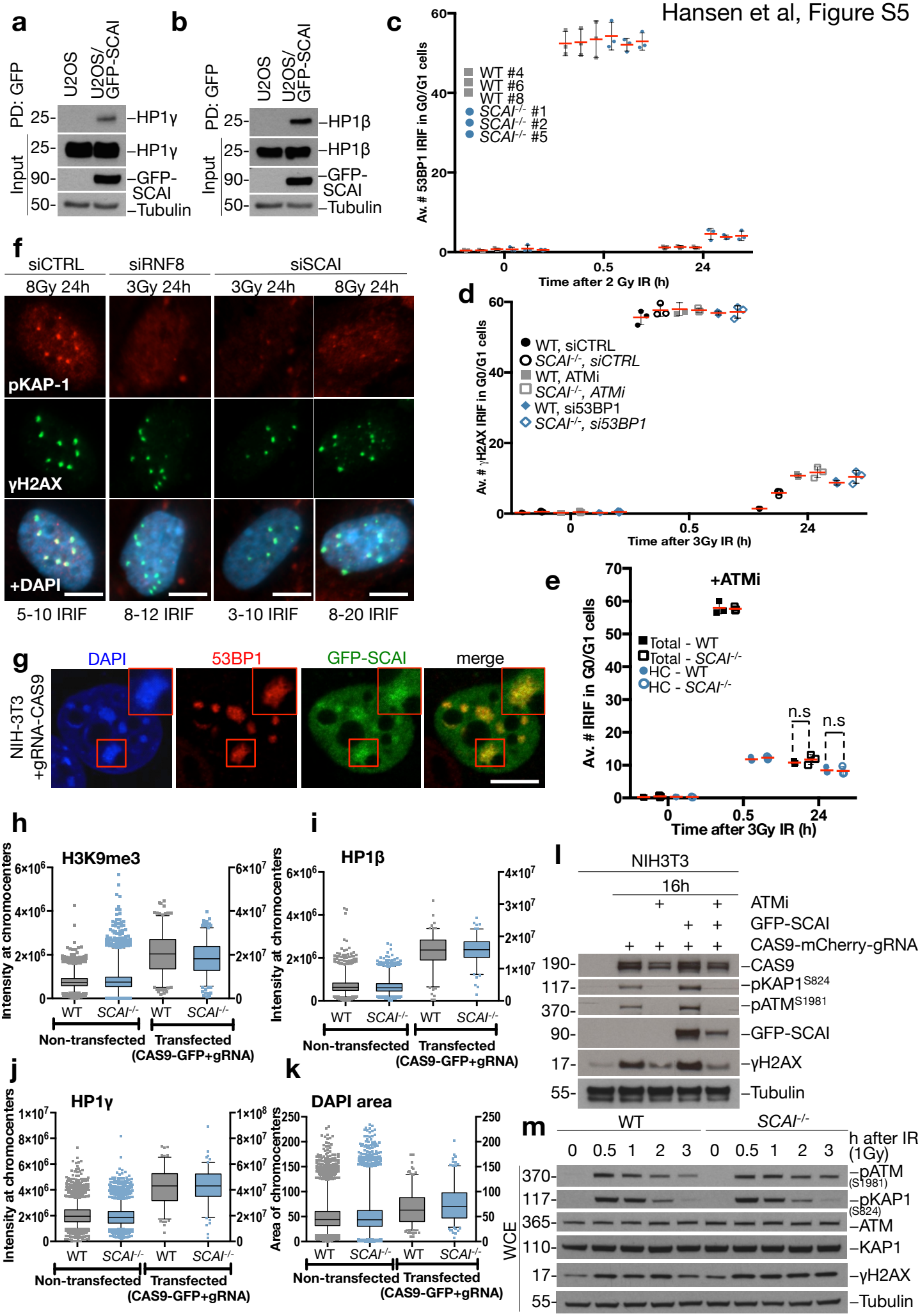


Figure 1e

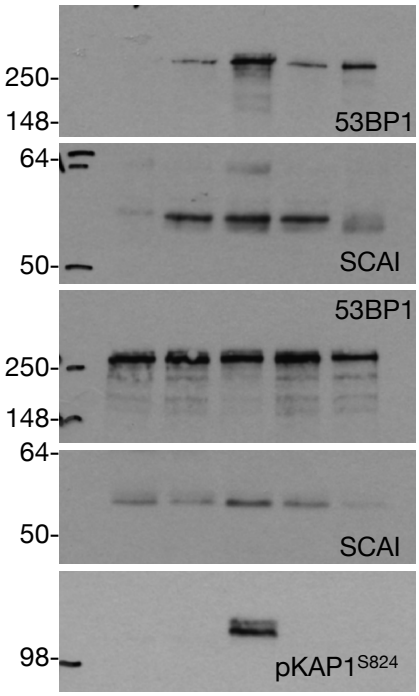
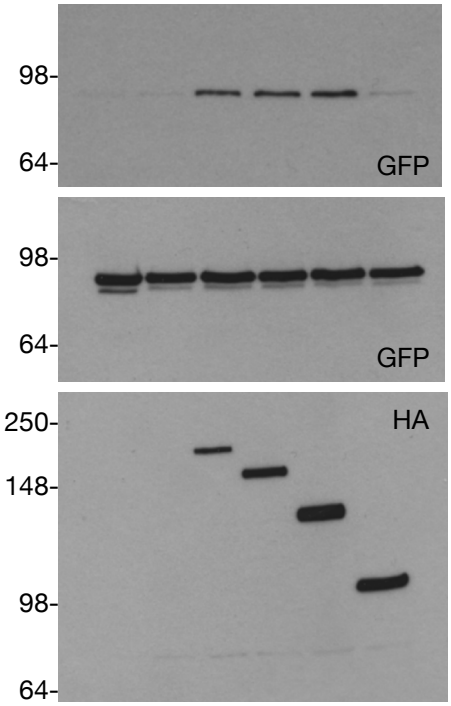


Figure 1i



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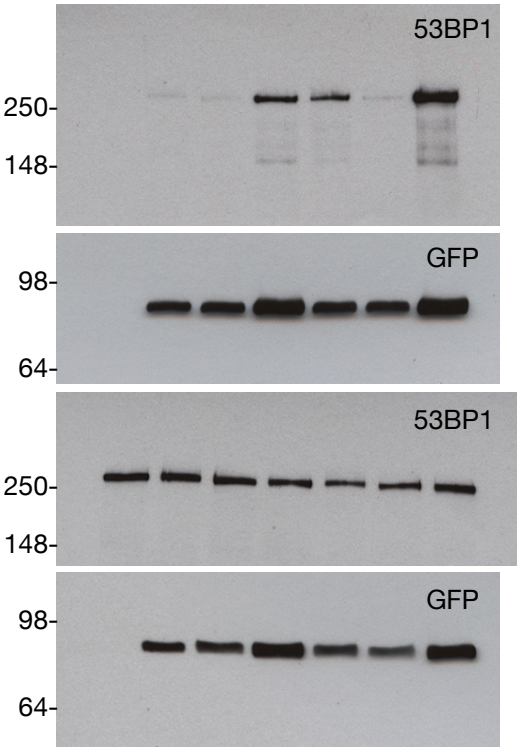

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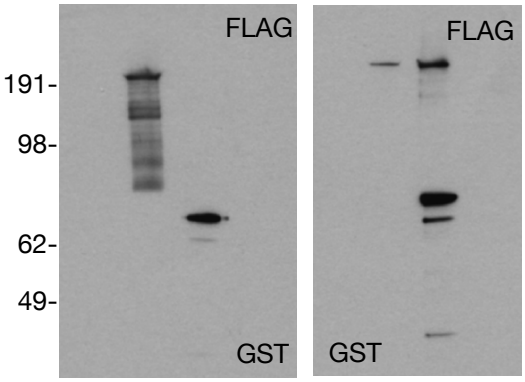


Figure 3a

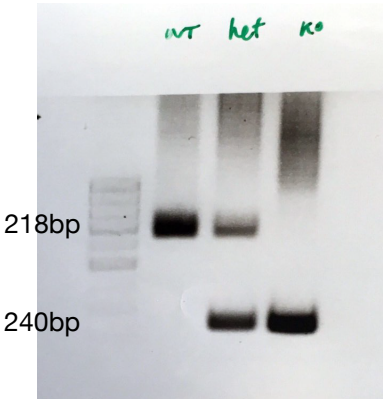
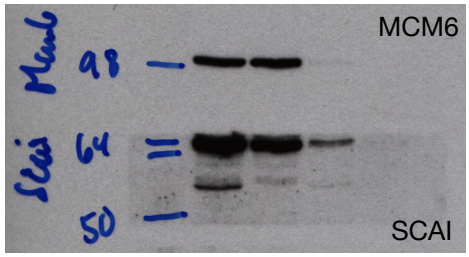


Figure 3a



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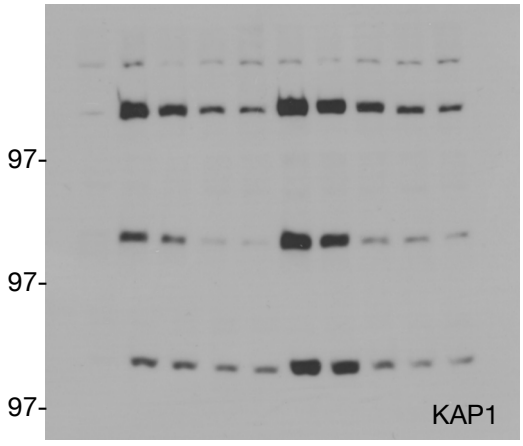


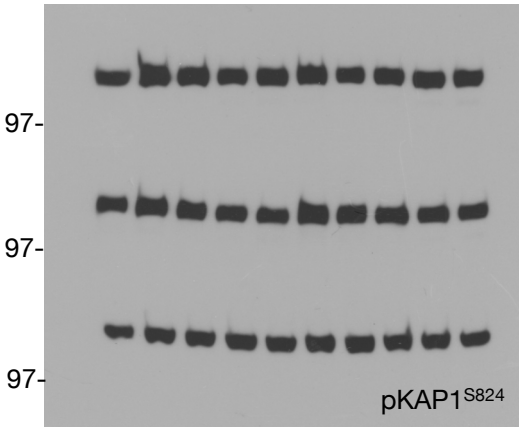


Figure 5f

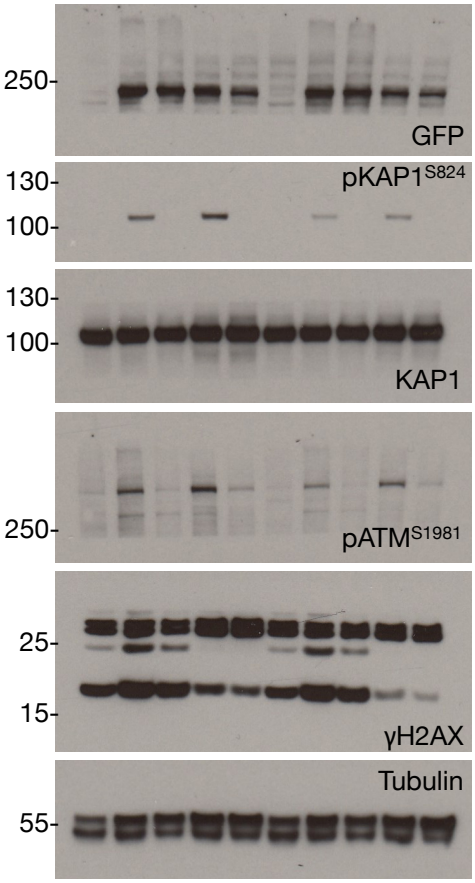
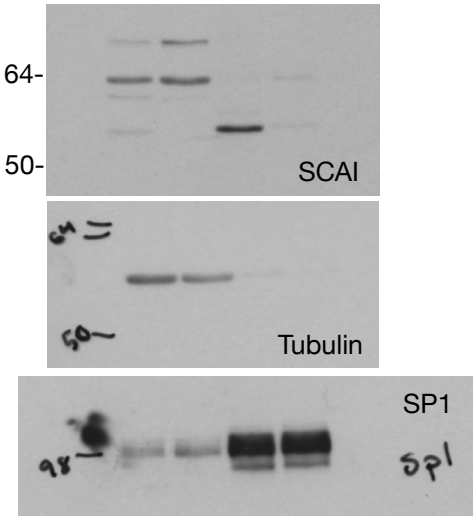


Figure S1c



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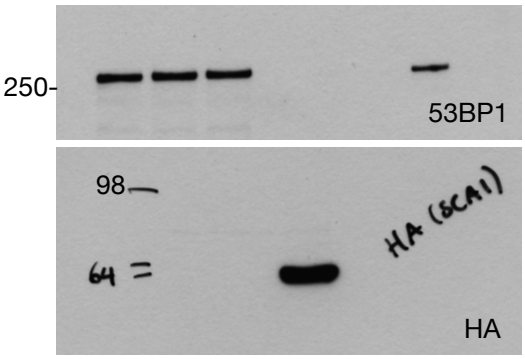

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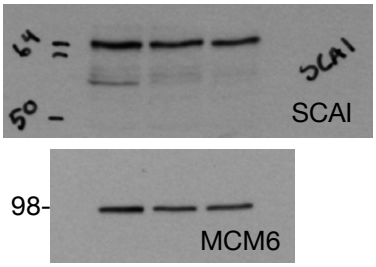

Figure S2f

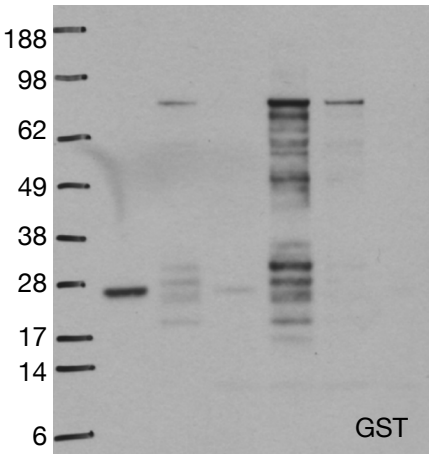

Figure S3b

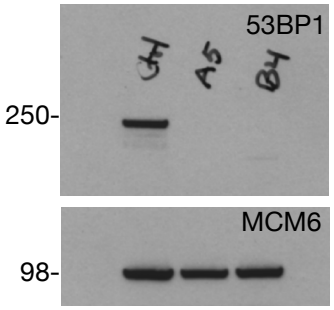

Figure S3c

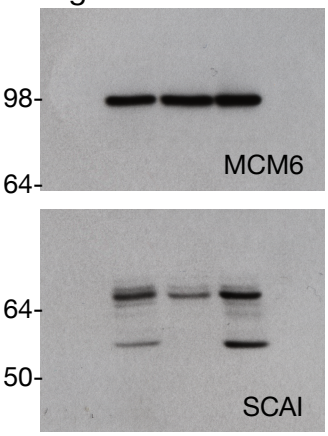

Figure S5a

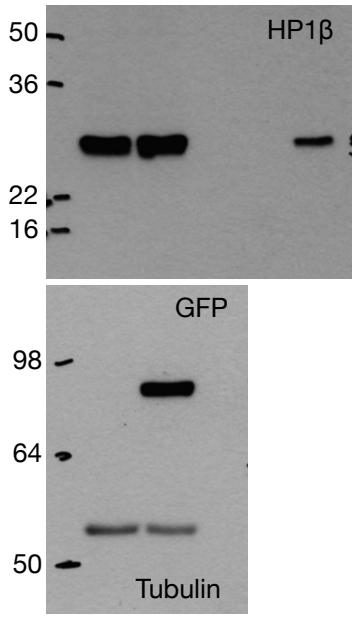

Figure S5b

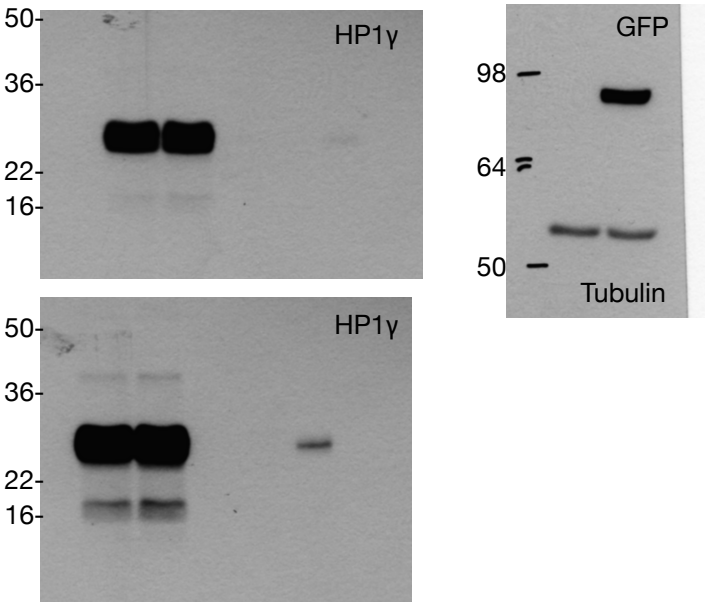

Figure S5l

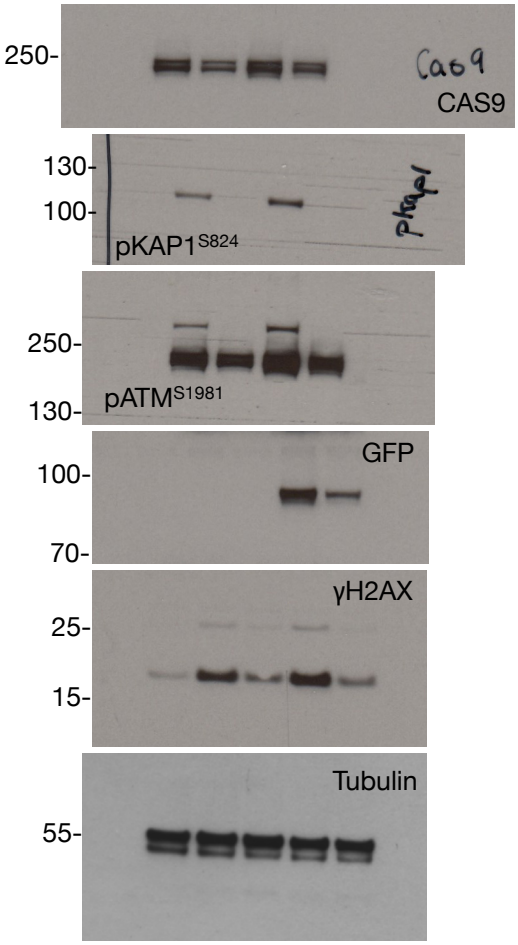

Figure S5m