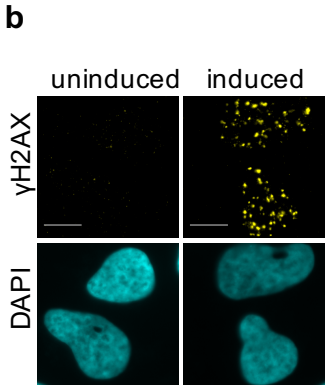
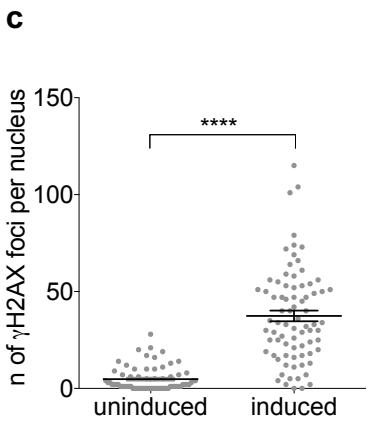
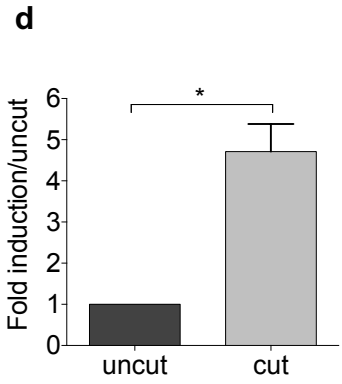
Supplementary Figure 1

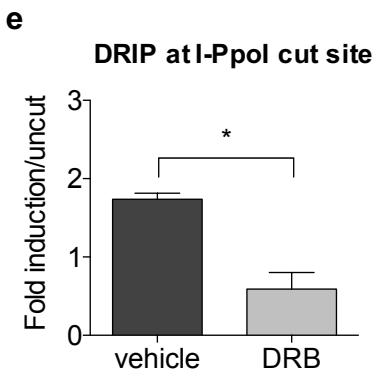
a

Site	Genome	Position	Annotation	Gene	Upstream Gene	Upstream Gene Distance (bp)	Downstream Gene	Downstream Gene Distance (bp)
I-PpoI genic	Human GRCh37/hg19	chr1:58,453,282-58,453,296	Intronic	DAB1	DAB1	0	DAB1	0
I-PpoI non-genic	Human GRCh37/hg19	chr2:133,037,429-133,037,443	Intergenic	-	ANKRD30BL	21888	ZNF806	27274
AsiSI non-genic	Human GRCh37/hg19	chr20:37,360,268-37,360,276	Intergenic	-	SLC32A1	2254	ACTR5	16281

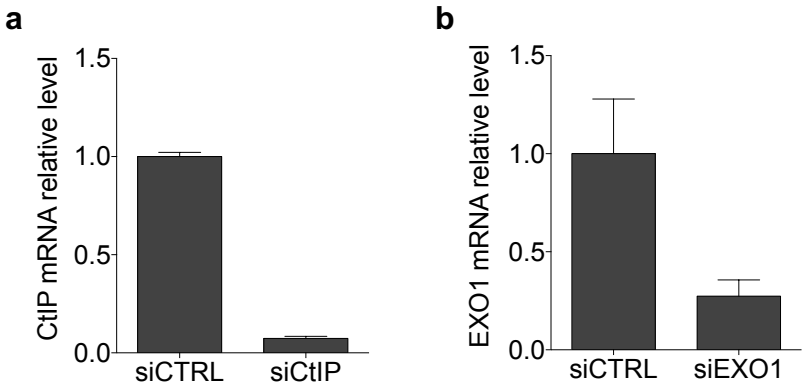




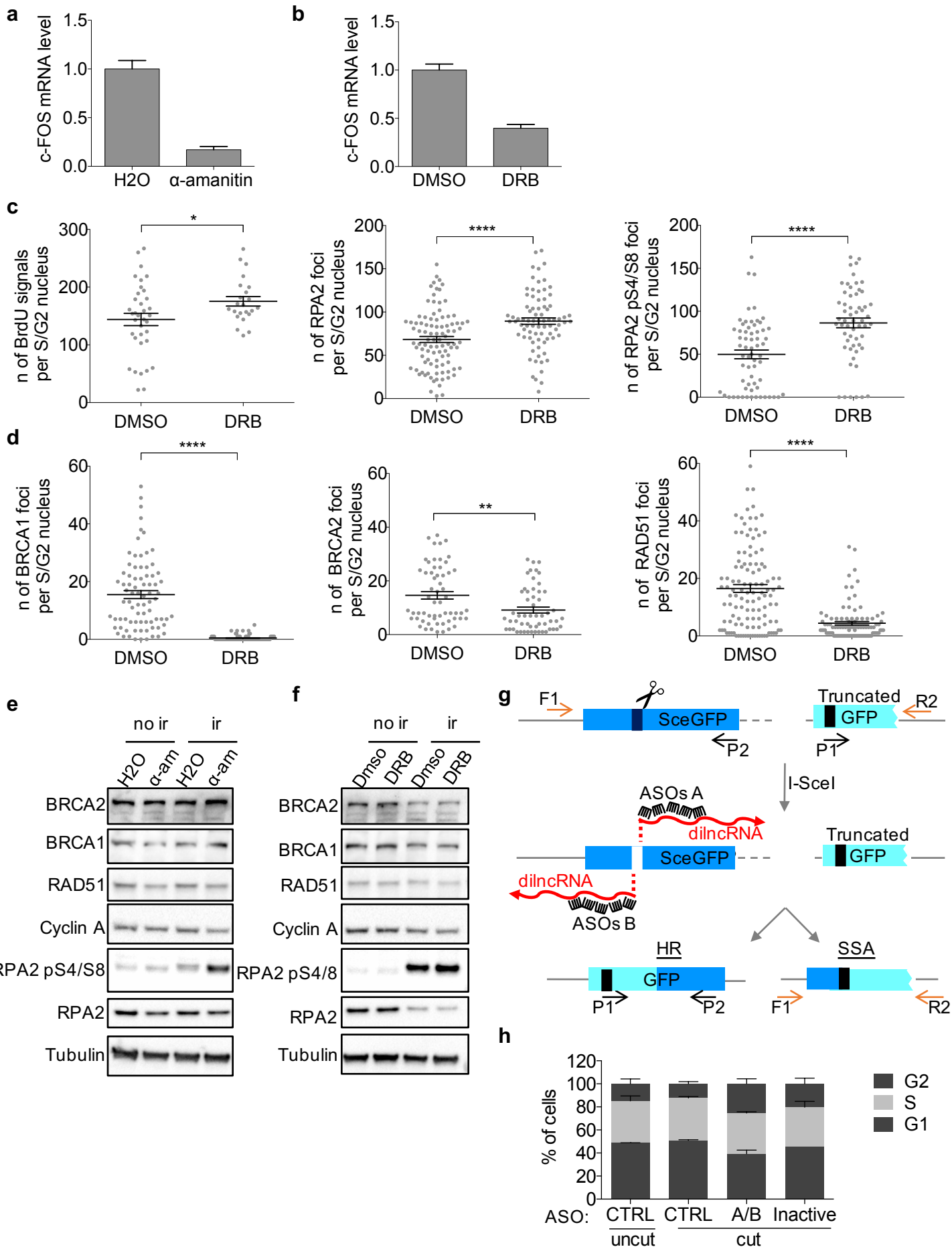




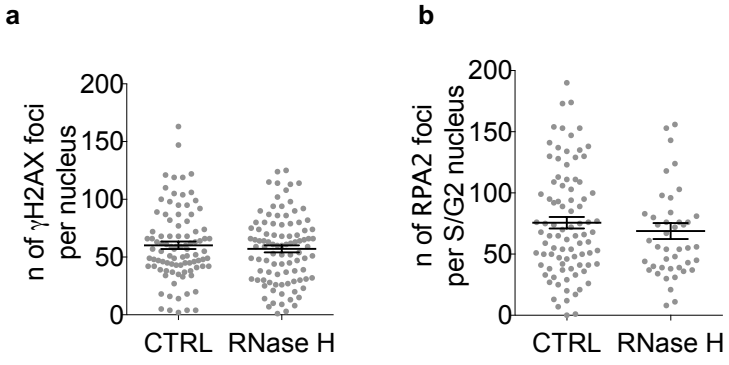
Supplementary Figure 2



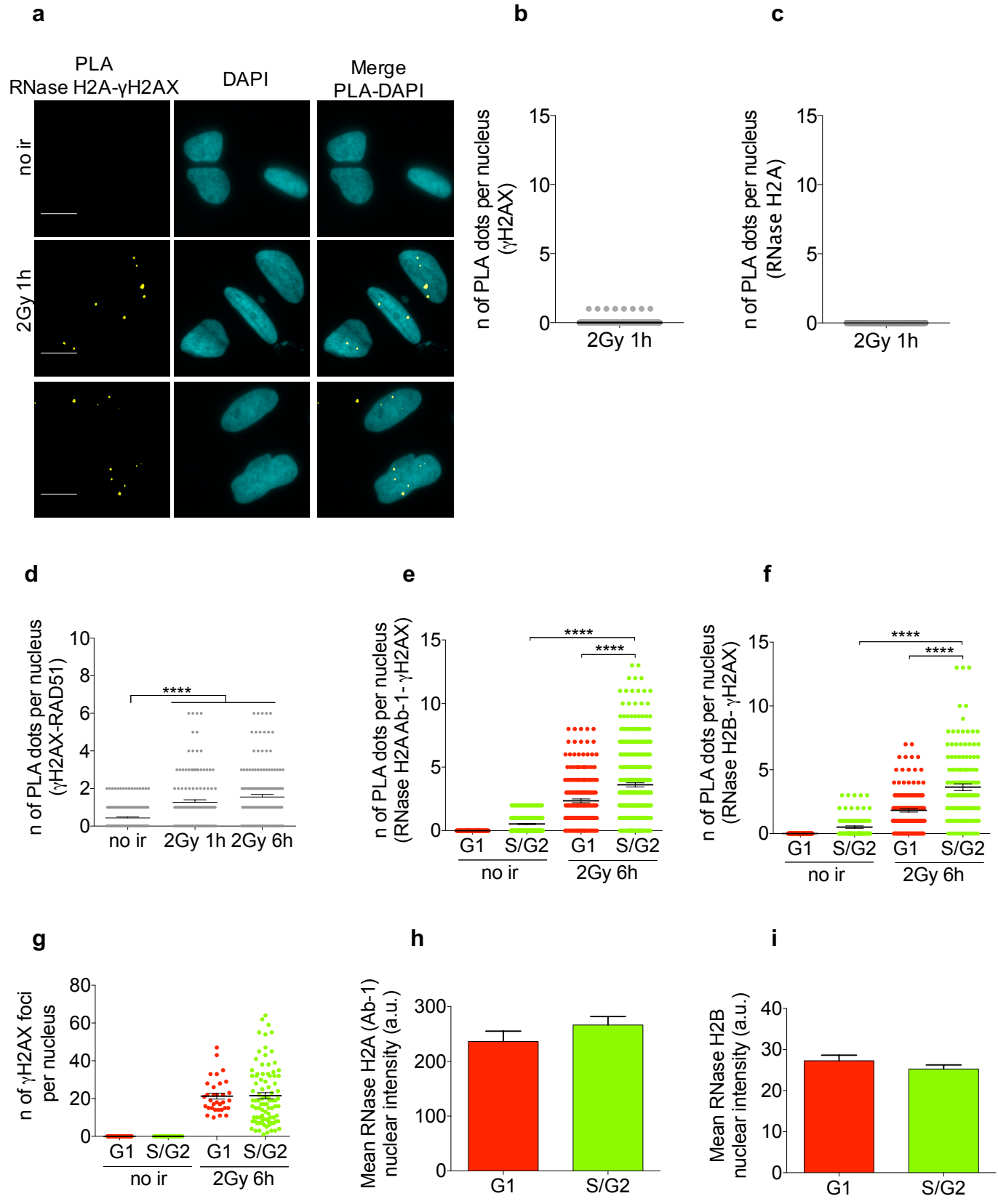
Supplementary Figure 3



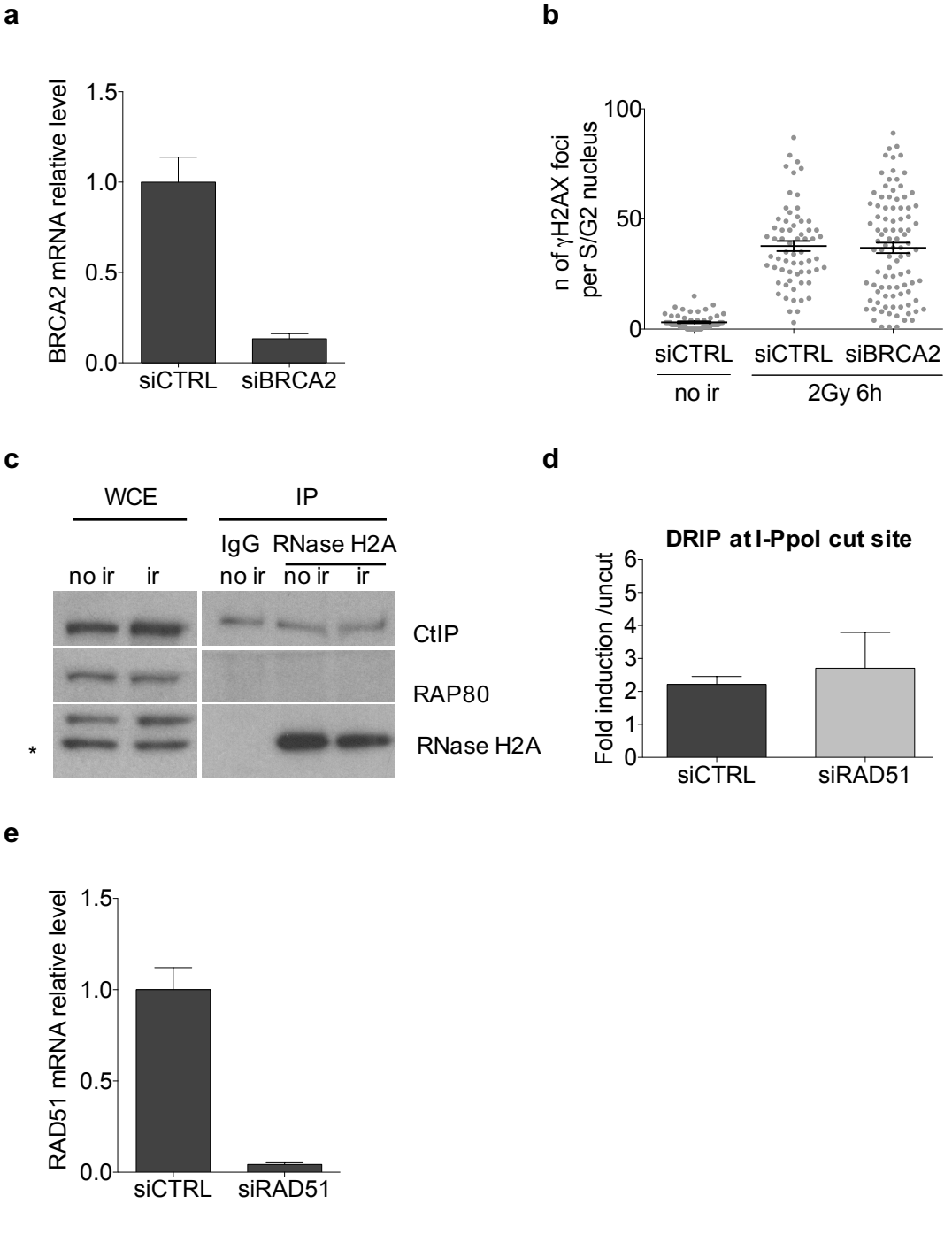
Supplementary Figure 4



Supplementary Figure 5



Supplementary Figure 6



Supplementary figure legends:

Supplementary Fig. 1: DNA:RNA hybrids formation at DBSs

(**a**) Table indicating the coordinates and the genomic context of the restriction enzymes-induced DSBs in the study (**b**) Representative images of γH2AX staining in DIvA cells before and after AsiSI induction with 4-OHT. Scale bar: 10 μm. (**c**) Dot plot shows the number of foci in (**b**). Pooled data from $n = 2$ independent experiments are shown. Lines depict the mean±s.e.m. (**d**) dilncRNAs induction at 0.5 Kb from the AsiSI cut site in DivA cells measured by strand-specific RT–qPCR. The bar graph shows the average fold induction of cut samples relative to uncut from $n = 2$ independent experiments. Error bars represent s.e.m. (**e**) DRIP-qPCR at 1.5 Kb on the right from the I-PpoI cut site within *DAB1* gene in HeLa cells treated with DMSO or DRB for 2 hours prior to DSB induction. The bar graph shows the average fold induction of cut samples relative to uncut ($n = 3$). Error bars represent s.e.m. $*P < 0.05$, $****P < 0.0001$ (two-tailed Student's t test).

Supplementary Fig. 2: CtIP and EXO1 knock-down efficiency

(**a**,**b**) CtIP or EXO1 knock-down efficiency monitored by RT-qPCR. One representative experiment is shown. Error bars represent s.d. of the RT-qPCR technical replicates.

Supplementary Fig. 3: Transcriptional inhibition does not significantly alter HR protein levels

(**a**,**b**) Efficiency of α-amanitin (**a**) or DRB (**b**) treatment monitored by RT-qPCR analysis of the mRNA levels of *c-FOS*, a short-lived RNA pol II transcript. One representative experiment is shown. Error bars represent s.d. of the RT-qPCR technical replicates. (**c**,**d**) Dot plots show the number of signals/foci per S/G2 nucleus of the indicated DNA-end resection (**c**) or HR (**d**) markers. Pooled data from $n = 2$ independent experiments are shown. Lines depict the mean±s.e.m. (**e**,**f**) Representative immunoblots of not irradiated (no ir) or irradiated 5Gy (ir) HeLa cells treated with α-amanitin (**e**) or DRB (**f**) or vehicles. These experiments were repeated twice independently with similar results. (**g**) Schematic representation of the ASOs used to inhibit dilncRNAs and the primers

used to detect homologous recombination (HR) and single strand annealing (SSA). Upon HR, the correct GFP sequence containing the 5' portion of the truncated GFP and the 3' of the sceGFP is generated. The HR product can be amplified by PCR using one primer matching the 5' part of the truncated GFP (P1) and one primer matching the 3' part of the sceGFP (P2). Over-resection of the exposed DNA ends results in SSA and generates a 0.8 Kb amplicon when a primer matching a region upstream to the sceGFP (F1) and a primer matching a region downstream of the truncated GFP (R2) are used for PCR. (**h**) FACS analysis of the cell-cycle profile of cells treated with control ASO (CTRL), ASOs matching dilncRNAs (A/B), or inactive ASOs A/B. Bar graphs represent mean values from n = 2 independent experiments. Error bars represent s.e.m. *$P < 0.05$, **$P < 0.005$, ****$P < 0.0001$ (two-tailed Student's t test).

Supplementary Fig. 4: RNase H treatment has no impact on γH2AX foci or DNA end resection signals

(**a**) Dot plot shows γH2AX foci in irradiated (2Gy) U2OS cells treated with RNase H prior to fixation. (**b**) Dot plots shows RPA2 foci co-stained with cyclin A, as S/G2-phase marker, in irradiated (2Gy) U2OS cells treated with RNase H prior to fixation. Pooled data from n = 2 independent experiments are shown. Lines depict the mean±s.e.m.

Supplementary Fig. 5: Specificity controls of PLA

(**a**) Representative images of PLA between RNase H2A and γH2AX in not irradiated (no ir) or irradiated (2Gy) U2OS cells. Scale bar: 10 μm. (**b**,**c**) Dot plots show the number of signals per nucleus of PLA when only γH2AX (**b**) or RNase H2A (**c**) antibody is used in irradiated (2Gy) U2OS cells. Pooled data from n = 3 independent experiments are shown. Lines depict the mean±s.e.m. (**d**) Dot plot shows the number of signals per nucleus of PLA between γH2AX and RAD51 in irradiated (2Gy) or not irradiated (no ir) U2OS cells. Pooled data from n = 3 independent experiments are shown. Lines depict the mean±s.e.m. (**e**,**f**) Dot plots show the number of signals per

nucleus of PLA between γH2AX and RNase H2A detected with a different antibody (Ab-1) (**e**) or γH2AX and RNase H2B (**f**) in not irradiated (no ir) or irradiated (2Gy) G1- and S/G2-phase HeLa-FUCCI cells. Pooled data from $n = 5$ **(e)** and $n = 2$ **(f)** independent experiments are shown. Lines depict mean±s.e.m**.** Error bars represent s.e.m. **(g)** Dot plot shows the number of γH2AX foci per nucleus in not irradiated (no ir) or irradiated (2Gy) G1- and S/G2-phase HeLa-FUCCI cells. Pooled data from $n = 2$ independent experiments are shown. Lines depict mean±s.e.m. **(h,i)** Bar graphs represent the average intensity of RNase H2A (**h**) or RNase H2B (**i**) signals in G1- or S/G2-phase HeLa-FUCCI cells from $n = 2$ independent experiments. Error bars represent s.e.m. $****P < 0.0001$ (two-tailed Student's *t* test).

Supplementary Fig. 6: RAD51 knock-down does not affect DNA:RNA hybrids formation at DSBs

(**a)** BRCA2 knock-down efficiency monitored by RT-qPCR**.** One representative experiment is shown. Error bars represent s.d. of the RT-qPCR technical replicates**. (b)** Dot plot shows the number of γH2AX foci in S/G2-phase HeLa-FUCCI cells knocked-down for BRCA2. Pooled data from $n = 2$ independent experiments are shown. Lines depict mean±s.e.m. (**c**) Co-immunoprecipitation of endogenous RNase H2A from not irradiated (no ir) or irradiated 5Gy (ir) HEK293T cell extract in the presence of benzonase. Asterisk indicates specific band. This experiment was repeated three times independently with similar results**. (d)** DRIP-qPCR at 1.5 Kb on the right from the I-PpoI cut site within *DAB1* gene in RAD51 knocked-down S/G2-phase-sorted HeLa-FUCCI cells transfected with the I-PpoI nuclease. The bar graph shows the average fold induction of cut samples relative to uncut from $n = 3$ independent experiments**.** Error bars represent s.e.m. (**e**) RAD51 knock-down efficiency monitored by RT-qPCR**.** One representative experiment is shown. Error bars represent s.d. of the RT-qPCR technical replicates**.**