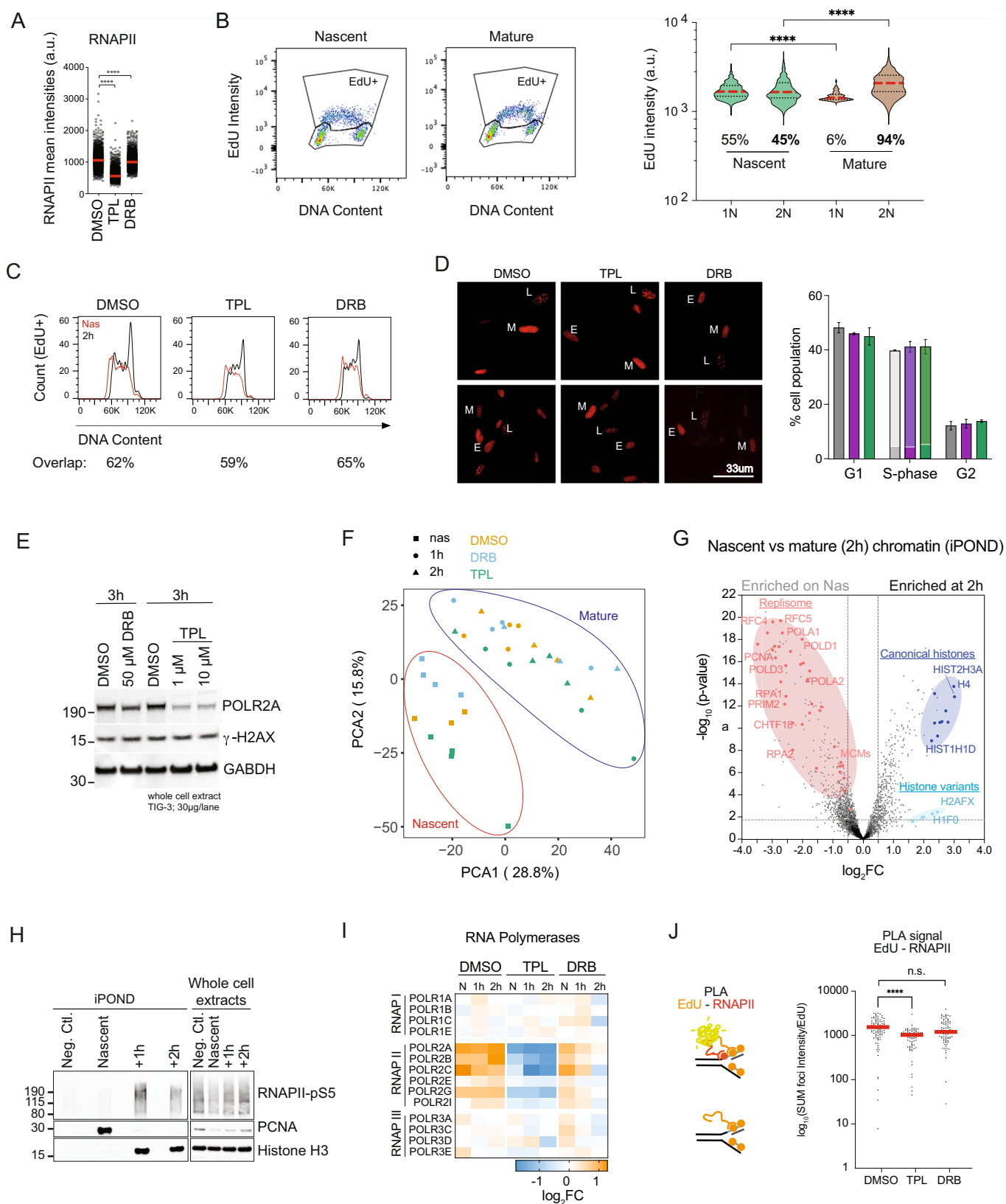
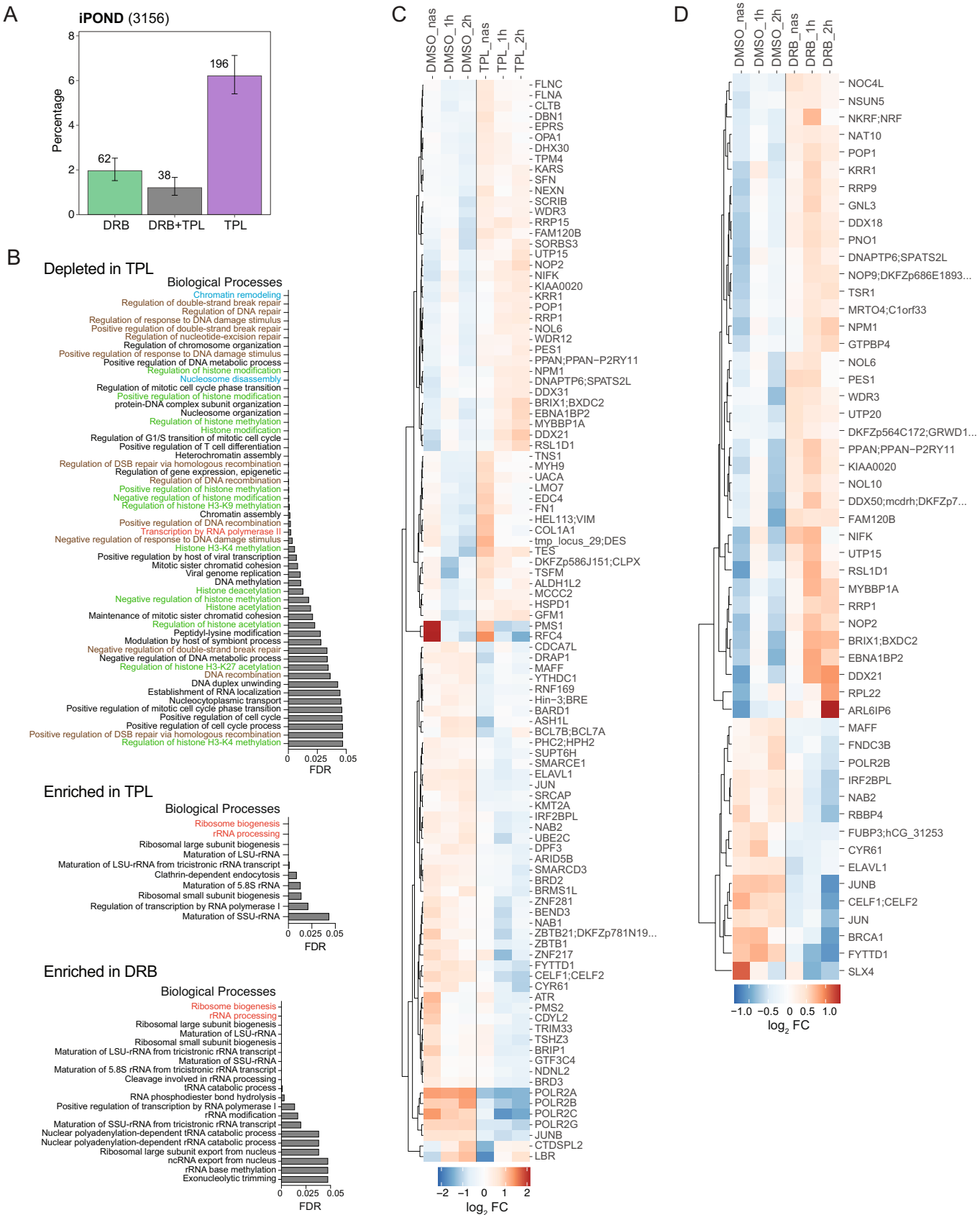


Expanded View Figures

Figure EV1. Proteomic profiling of chromatin behind replisomes upon transcription inhibition. Related to Fig. 1.

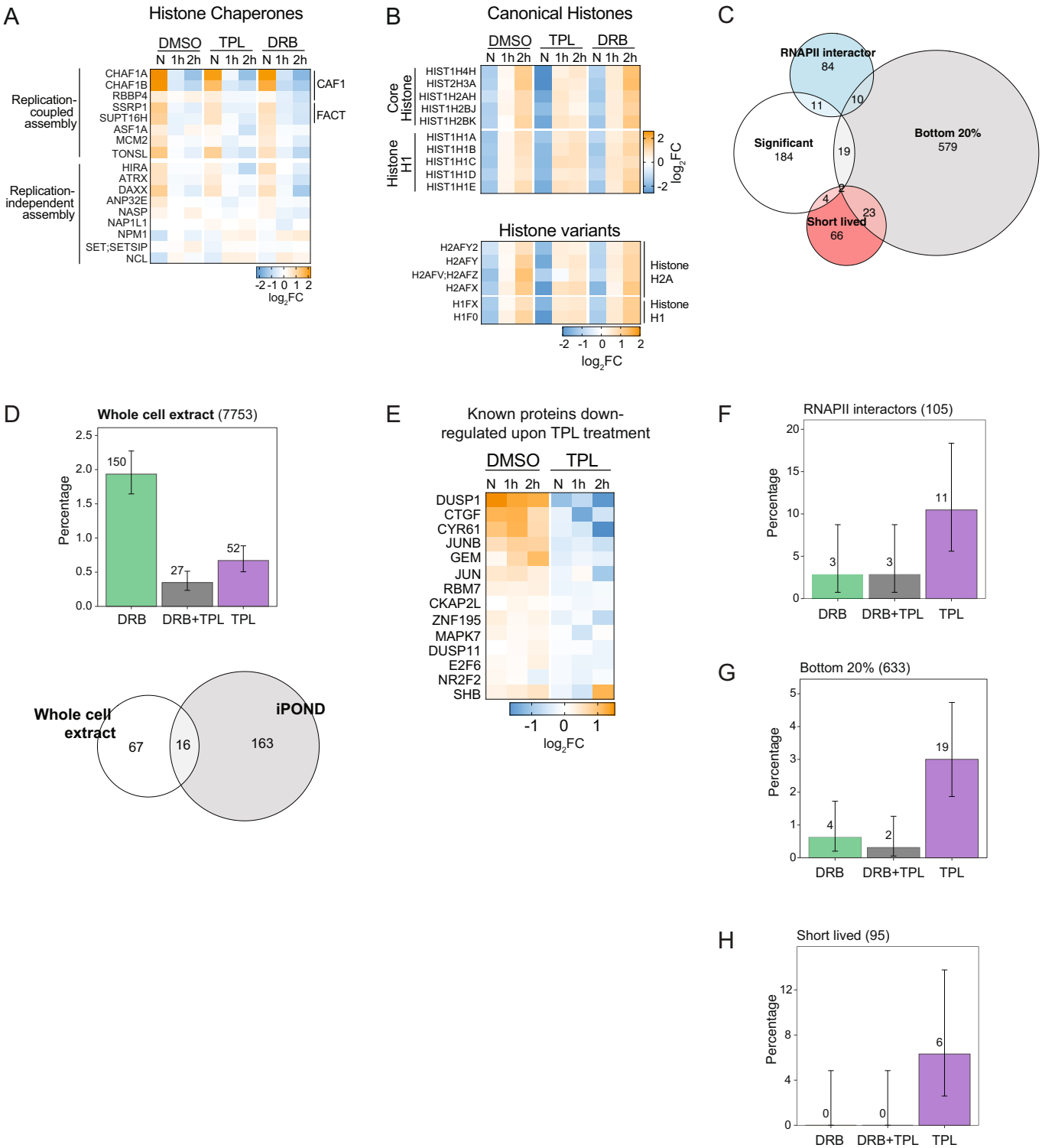
(A) QIBC-based analysis of RNAPII level in DMSO, TPL and DRB treated cells. Graphs show the mean intensity per nuclei, > 539 nuclei were analysed per sample. Red line: median. Unpaired Mann-Whitney *t* test; *****P* value < 0.0001. *N* = 3 biological replicates, one representative experiment is shown. (B) EdU pulse chase quantification by flow cytometry. Cells were pulsed for 11 min with EdU (20 μ M) (Nascent sample) and chased 2 h by 20 μ M of thymidine (Mature sample). Left, the gating for EdU-positive and negative cells is shown. Right, quantification of cell density (expressed as %) and EdU intensities (a.u.) for 1 N and 2 N populations. (C) Flow cytometry plots of asynchronous cells treated with DMSO, TPL and DRB. Cells were EdU pulsed and chased as described in (B). Nas, Nascent sample, red; 2 h, Mature sample, black. The percentage of overlapping area between Nas and 2 h is indicated. (D) Cells were treated for 2 h with DMSO, TPL and DRB, followed by a 20 min EdU pulse and analysed by microscopy. Left: Representative images of Early/Mid and Late S-phase patterns. Early and Mid-S-phase cells have a pattern of replication foci distributed throughout the nucleus. Late S-phase cells would have a small number of large foci within the nucleus. Right: Distribution of cells in G1 and G2 phase based on EdU and DAPI intensities. Distribution of cells in Early/Mid (top section) and late S phase (bottom section) based on the distinct EdU patterns shown on the left. The mean and standard deviation (S.D.) of >1230 nuclei are shown. *N* = 2 biological replicates. One representative experiment is shown. (E) Western plot showing the γ H2AX level in cells treated with DMSO, TPL or DRB. Cells were treated with inhibitors for 3 h according to the concentrations indicated. Total cell extracts were collected and GAPDH used as a loading control. (F) Principal-component analysis of the four biological replicates based on the proteins identified. (G) Volcano plot generated using the iPOND-TMT time course data showing protein fold changes based on the full model for the 2 hr mature time point. *N* = 4 biological replicates, Limma *t* test, significantly changing proteins with a magnitude fold change ≥ 0.5 (*x* axis) and an FDR ≤ 0.05 (*y* axis) are highlighted. Replisome, red; Canonical Histones, blue; Histone variants, light blue. The dashed horizontal line shows the *P* value cut-off, and the two vertical dashed lines indicate proteins enriched or depleted from 2 hr mature chromatin. (H) Western blot analysis of iPOND samples. The western blot was probed with antibodies against RNAPII-pS5, PCNA, and Histone H3 (indicated on the right). Sample labelling shown on top. (I) Heatmap of RNA Polymerase I, II, and III. The log₂-fold change of batch-corrected abundance with respect to the protein mean is shown (*n* = 4 biological replicates). Each column represents a time point (N: Nascent, 1 h, and 2 h) and each row corresponds to the protein indicated on the left. Colour scale is indicated. (J) Left: Scheme of the PLA analysis between EdU and protein of interest by QIBC. TIG-3 cells are EdU-labelled, and the PLA signal between EdU and the protein of interest on nascent chromatin analysed (see details in 'Methods'). Right: Single-cell PLA signal of EdU-RNAPII interaction shown for nascent chromatin in DMSO, TPL and DRB treated cells. Cells with a similar EdU signal were chosen and the PLA signal was calculated as the SUM of the total intensity of PLA foci per nucleus. >78 nuclei were analysed per sample. Red line, median; Unpaired Mann-Whitney *t* test; *****P* value < 0.0001; n.s., non-significant. *N* = 2 biological replicates, one representative experiment is shown. Source data are available online for this figure.





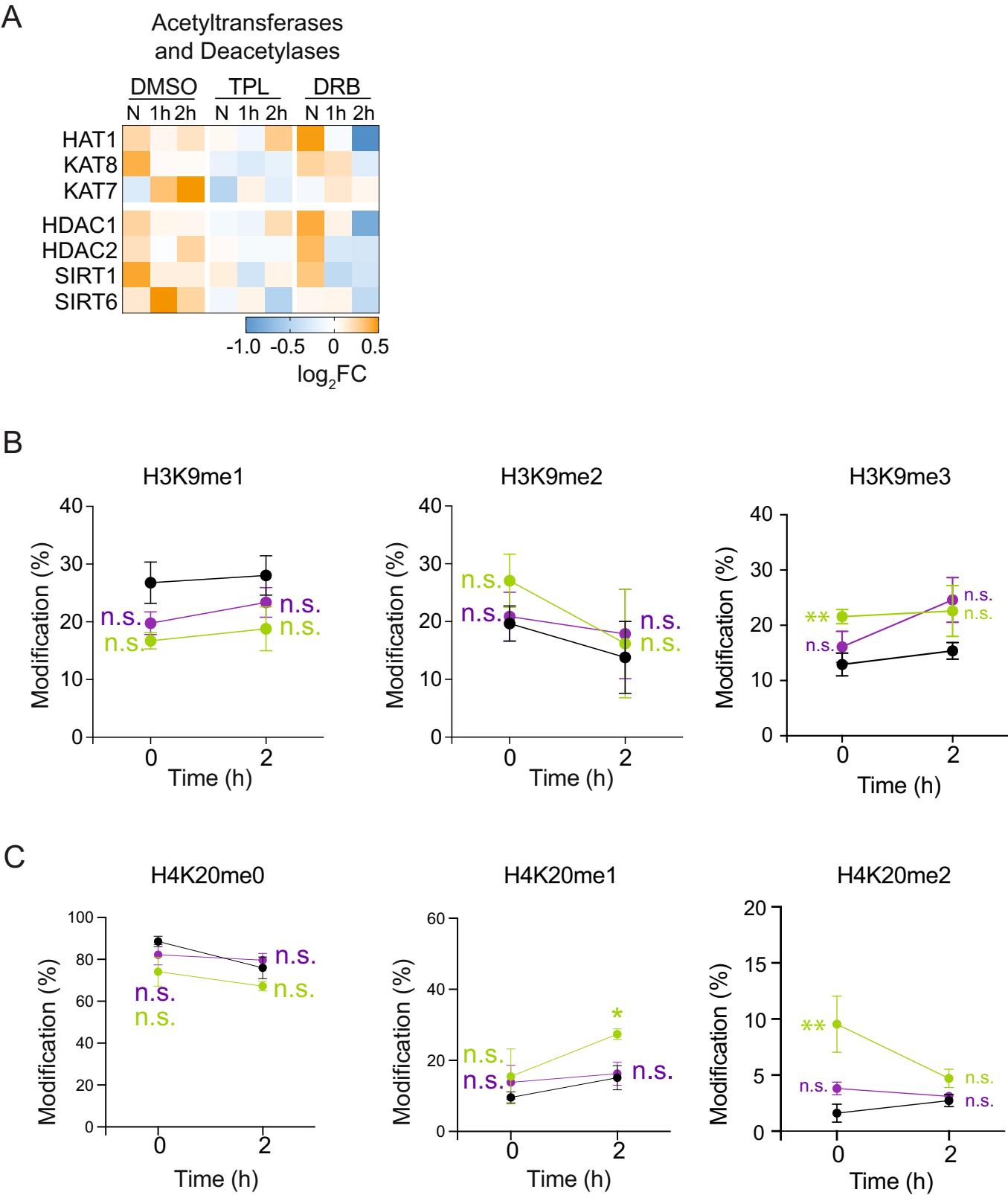
◀ Figure EV2. Proteomic profiling of chromatin behind replisomes upon transcription inhibition. Related to Fig. 1.

(A) Histogram showing the number of factors that had a significant fold change based on the full model for TPL and DRB treatment shown in Fig. 1F,G. TPL + DRB: common factors between TPL and DRB treatment. $N = 4$ biological replicates. 95% confidence interval of a proportion is shown. (B) GO term analysis using STRING (Szklarczyk et al, 2015) with the significantly changing proteins based on the full model for TPL and DRB treatment shown in Fig. 1F,G. GO terms were filtered for a background gene count <400, chromatin related biological processes and an FDR below 0.05. The proteins were categorised into three groups: Depleted in TPL, enriched in TPL, and enriched in DRB. The GO terms have been colour coded based on four categories: Chromatin remodelling, blue; DNA repair, brown; Histone modification, green; Transcription, red. (C, D) The rows in the heatmaps were clustered using complete-linkage hierarchical clustering with Euclidean distance metric (see material and method for details). Source data are available online for this figure.



◀ **Figure EV3. Proteomic profiling of chromatin behind replisomes upon transcription inhibition. Related to Fig. 1.**

(A, B) Heatmaps of Histone Chaperones (A) and Canonical Histones/Histone variants (B). The log₂-fold change of batch-corrected abundance with respect to the protein mean is shown ($n = 4$ biological replicates). Each column represents a time point (N: Nascent, 1 h, and 2 h) and each row corresponds to the protein indicated on the left. Colour scale is indicated. (C) Venn diagram between proteins with a significant fold change based on the full model for TPL and DRB treatment (white, Fig. 1F/G), identified RNAPII interactor (blue), identified short-lived proteins (red), and the 20% lowest abundant proteins identified (light purple). The lists of RNAPII interactor and short-lived proteins were generated based on (Ebmeier et al, 2017) and (Li et al, 2021), respectively. (D) Top: Same as in Fig. EV2A for significantly changing proteins in the whole-cell extract upon TPL or DRB treatment (shown in Fig. 1H,I). 95% confidence interval of a proportion is shown. Bottom: Venn diagram between significantly changing proteins (FDR < 0.05, full model for TPL and DRB treatment) from the whole-cell extract and the iPOND-TMT time course experiments. $N = 4$ biological replicates. (E) Same as in (A, B) for reported proteins by (Vispe et al, 2009) downregulated upon TPL treatment. $N = 4$ biological replicates. (F–H) Same as in Fig. EV2A for identified RNAPII interactor (F), the 20% lowest abundant proteins (G) and the identified short-lived proteins (H). $N = 4$ biological replicates. 95% confidence interval of a proportion is shown.



◀ Figure EV4. Transcription promotes H3.3K36me2 re-establishment on replicated chromatin. Related to Fig. 2.

(A) Heatmap of Histone Acetyltransferases and Deacetylases ($n = 4$ biological replicates). The log₂-fold change of batch-corrected abundance with respect to the protein mean is shown ($n = 4$ biological replicates). Each column represents a time point (N: Nascent, 1 h, and 2 h) and each row corresponds to the protein indicated on the left. Colour scale is indicated. (B) Proportion of H3K9me1, H3K9me2, and H3K9me3 (left to right) on newly replicated chromatin for each time point in DMSO (black), DRB (green) and TPL (purple) treated cells. Standard error of the mean is shown. $N = 3$ biological replicates. Paired t test, ** P value < 0.01 ; n.s., non-significant. (C) Same as in (B) for H4K20me0, H4K20me1, and H4K20me2 (left to right).

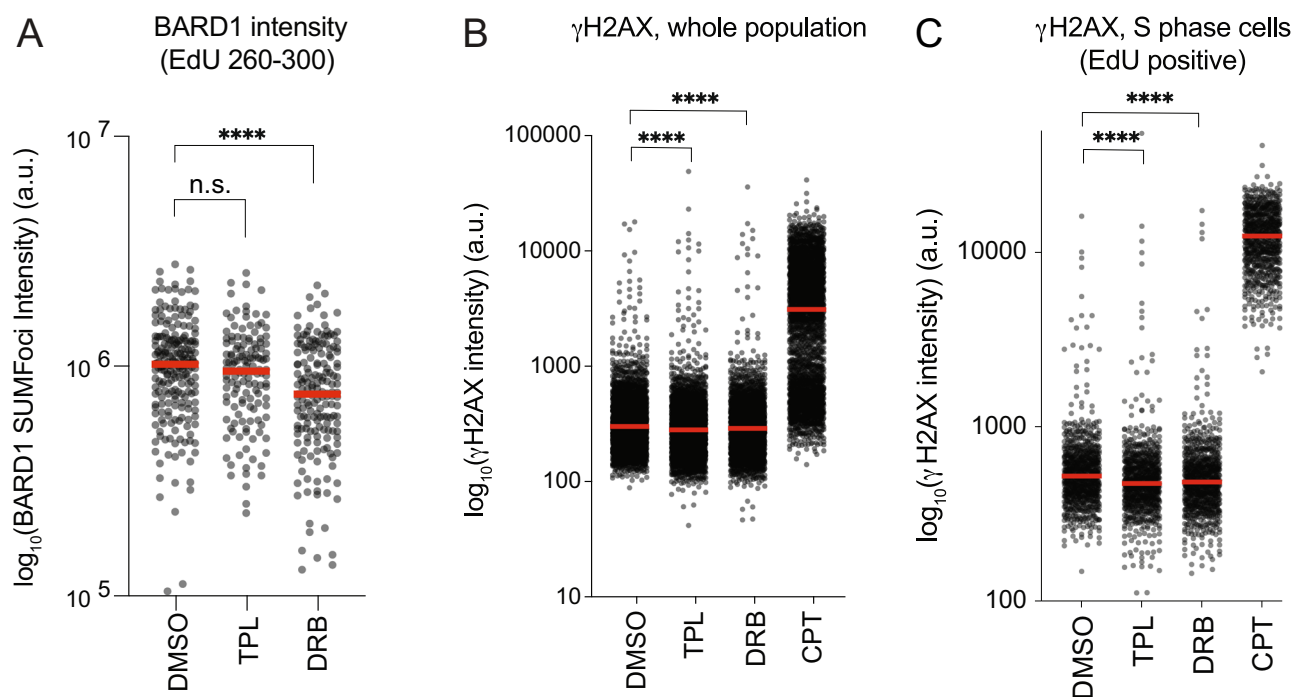
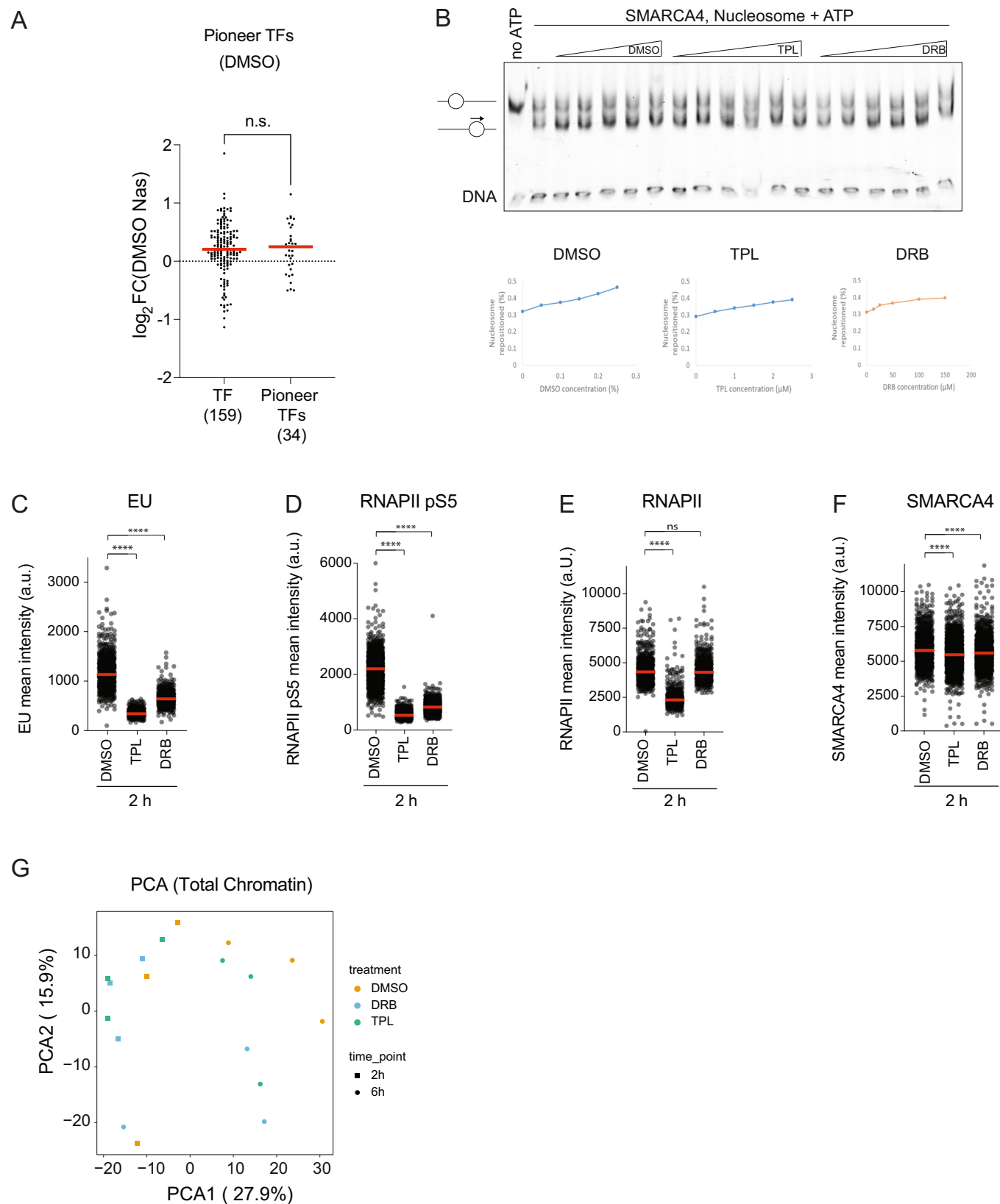


Figure EV5. Blocking transcription reduced the recruitment of DNA repair proteins on replicated chromatin. Related to Fig. 3.

(A) Single-cell PLA signal of EdU-BARD1 interaction shown for nascent chromatin in DMSO, TPL and DRB treated cells. Cells with a similar EdU signal were chosen and the PLA signal was calculated as the SUM of the total intensity of PLA foci per nucleus. >138 nuclei were analysed per sample. Red line, median; Unpaired Mann-Whitney *t* test; *****P* value < 0.0001; n.s., non-significant. *N* = 2 biological replicates, one representative experiment is shown. (B, C) Quantification of γ H2AX signal in the whole single-cell population (B) and EdU-positive cells only (C). Quantification of γ H2AX fluorescence signals in individual cells was measured by QIBC. Cells were treated with TPL and DRB according to conditions used in the study and labelled with EdU for the last 20 min. Camptothecin treatment is used as a positive control for DNA damage. >600 nuclei were analysed per condition. Red line, median; Unpaired Mann-Whitney *t* test; *****P* value < 0.0001. *N* = 2 biological replicates, one representative experiment is shown. Source data are available online for this figure.



**Figure EV6. Analysis of TF and remodellers on replicated chromatin and of steady state chromatin. Related to Figs. 4, 5 and 6.**

(A) Log₂ fold change of batch-corrected abundance with respect to the protein mean for the nascent time point is shown ($n = 4$ biological replicates). TFs are divided into two groups, pioneer factors and non-pioneer (Sherwood et al, 2014). Red line, median; Unpaired Mann-Whitney t test; n.s., non-significant. (B) Nucleosome sliding in vitro assay (left) and its quantification (right). (C–F) QIBC analysis of chromatin-bound intensities of EU, RNAPII-pS5, RNAPII and SMARCA4 in DMSO, TPL or DRB treated cells. Graphs show the mean intensity per nuclei, >291 nuclei were analysed per sample. Red line, median; Unpaired Mann-Whitney t test; **** P value < 0.0001; n.s., non-significant. $N = 3$ biological replicates, one representative experiment is shown. (G) Principal-component analysis using the identified proteins from the iPOND-TMT chase experiments. Source data are available online for this figure.