Science Advances
AAAS

Supplementary Materials for

XPF interacts with TOP2B for R-loop processing and DNA looping on actively transcribed genes

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Sci. Adv. **9**, eadi2095 (2023)
DOI: 10.1126/sciadv.adi2095

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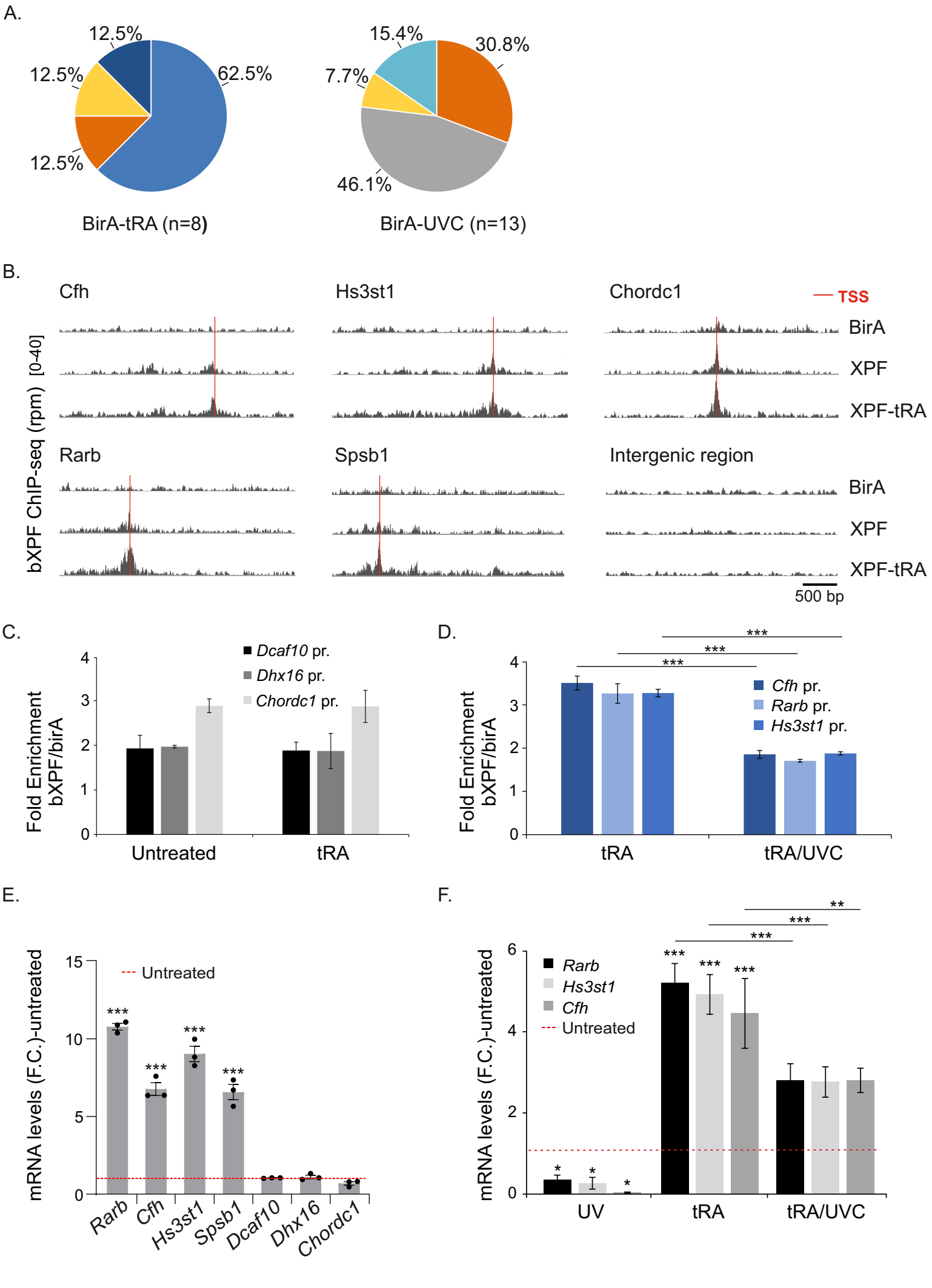
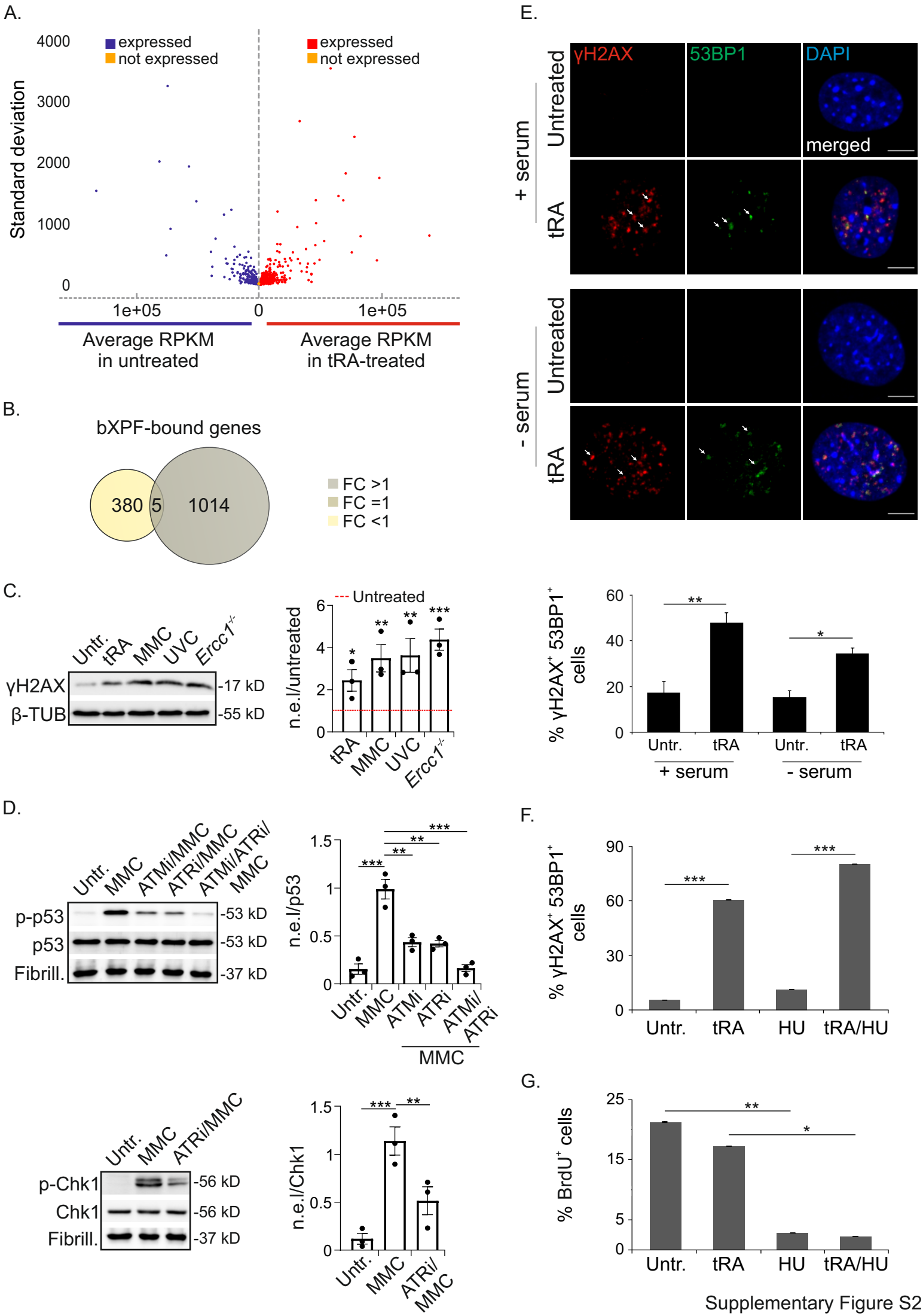
Figs. S1 to S8



Supplementary Figure S1

Fig. S1.

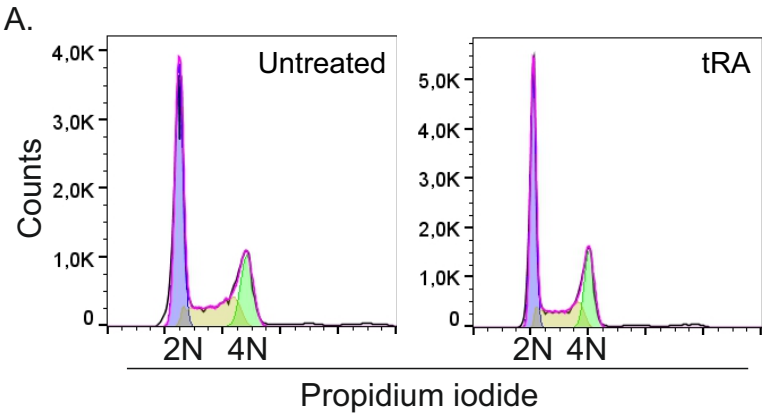
bXPF ChIP signals on promoters and gene expression levels. (**A**). Genomic distribution of binding sites in tRA- and UVC-treated BirA MEFs. (**B**). Genome browser views depicting bXPF ChIP-Seq signals on ±10kb genomic regions flanking the TSS of representative tRA-responsive (e.g. *Cfh*, *Hs3st1*, *Rarb*, *Spsb1*), tRA non-responsive gene (*e.g. Chordc1*) and the non-transcribed genomic region (intergenic region) in untreated (bXPF), tRA-treated (bXPF-tRA) and BirA MEFs. The black line sets the scale at 500bp. (**C**). bXPF ChIP signals on the promoters of *Chordc1, Dcaf10* and *Dhx16* genes. (**D**). bXPF ChIP signals on the promoters of *Cfh*, *Rarb*, and *Hs3st1* genes in tRA- and UVC-irradiated, tRA-treated (UVC/tRA) MEFs. (**E**). mRNA levels of *Rarb, Cfh*, *Hs3st1, Spsb1, Dcaf10, Dhx16* and *Chordc1* genes in untreated (red dotted line) and tRA-treated MEFs. (**F**). mRNA levels of *Cfh*, *Rarb* and *Hs3st1* genes in untreated (red dotted line), UVC-irradiated, tRA-treated and UVC-irradiated/tRA-treated MEFs. F.C.: Fold Change.

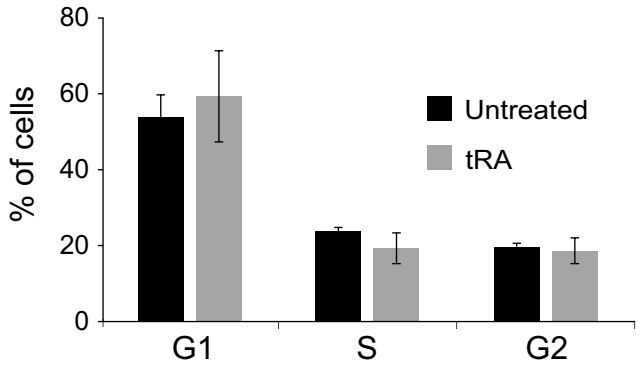


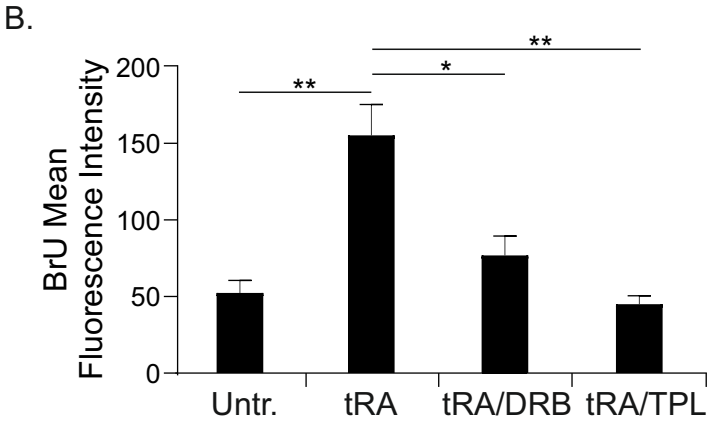
Supplementary Figure S2

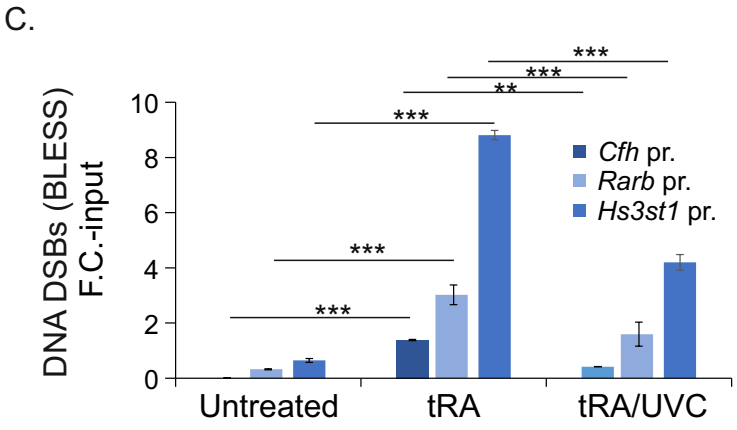
Fig. S2.

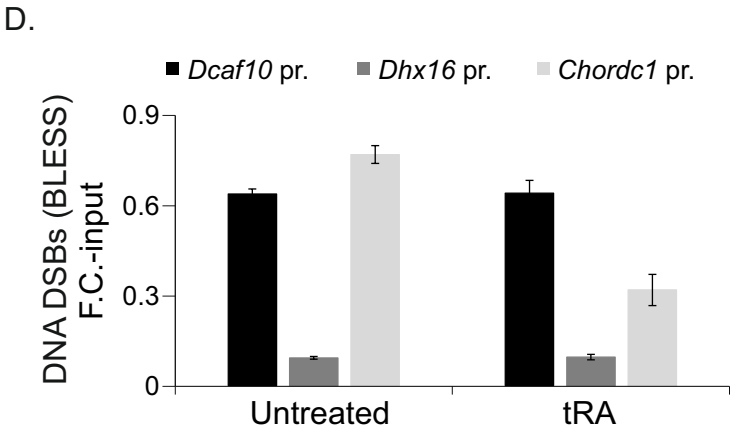
Transcription activation triggers a DNA Damage Response. (**A**). Volcano plot of RNA-seq FPKM counts, over standard deviation (n = 3) for bXPF-bound genes in untreated and tRA-treated MEFs. (**B**). Venn diagram depicting the fold change (FC) of tRA/untreated RNA-seq FPKM counts for bXPF-bound genes (5'UTR, promoter-TSS, exon, intron, TTS, 3'UTR). FC>1: 1014 genes, FC=1: 5 genes, FC<1: 380 genes. (**C**). Western blot with anti-γH2AX in whole cell extracts derived from wt MEFs exposed to 10μM tRA for 16h or 10μg/ml MMC for 4h, or UVC irradiation or from *Ercc1*$^{-/-}$ primary cells. The graph represents the β-tubulin (β-TUB)–normalized protein expression levels (n.e.l.) in tRA-, MMC-, UVC-treated and *Ercc1*$^{-/-}$ MEFs compared to wt controls (red-dotted line; n = 3 per group). (**D**). Western blots with anti-phospho Chk1, Chk1, phospho p53, p53 and fibrillarin in whole cell extracts derived from MMC-treated (10μg/ml, 4h) wt MEFs pre-cultured for 1h in the presence of 10μM ATM (ATMi) and/or ATR (ATRi) inhibitors. The graph represents the fibrillarin- and Chk1- or p53-normalized protein expression levels (n.e.l.) (n = 3 per group). (**E**). Detection of γH2AX and 53BP1 in untreated, and tRA-treated wt MEFs cultured in the presence (+ serum) or absence (-serum) of serum. The graph represents the number of γH2AX^{+}/53BP1^{+} cells for the treatments shown in the x-axis. Grey line is set at 10μm scale. (**F**). Immunofluorescence detection of γH2AX and 53BP1 in primary untreated, tRA-treated wt MEFs and tRA-treated wt MEFs also exposed to hydroxyurea or in hydroxyurea-treated MEFs. (**G**). Immunofluorescence detection of BrdU in primary untreated, tRA-treated wt MEFs and tRA-treated wt MEFs also exposed to hydroxyurea or in hydroxyurea-treated MEFs (as indicated).

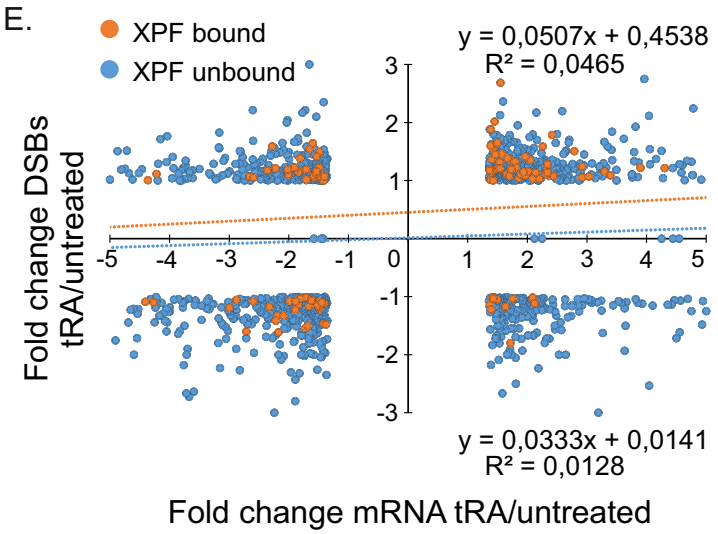


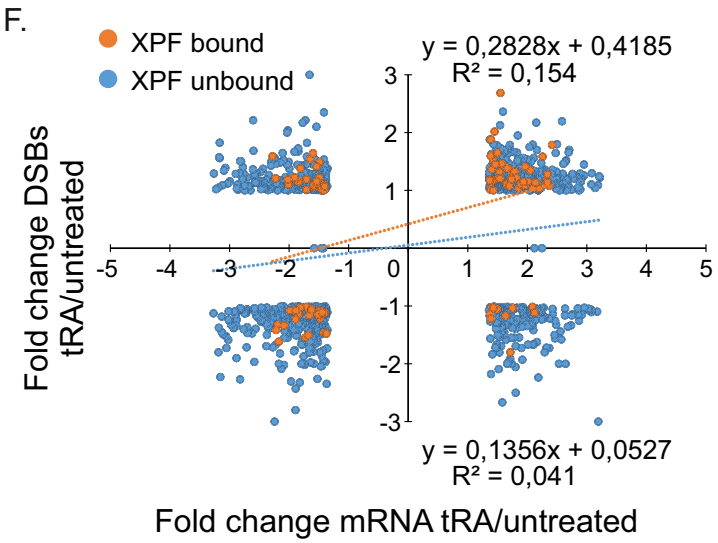


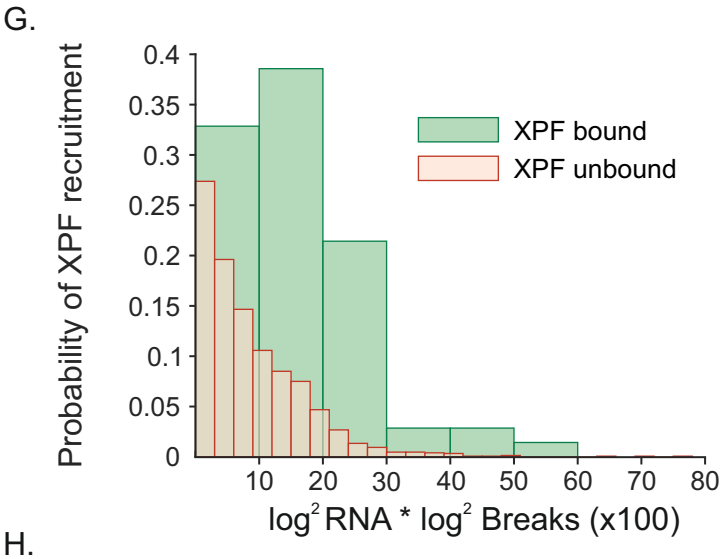


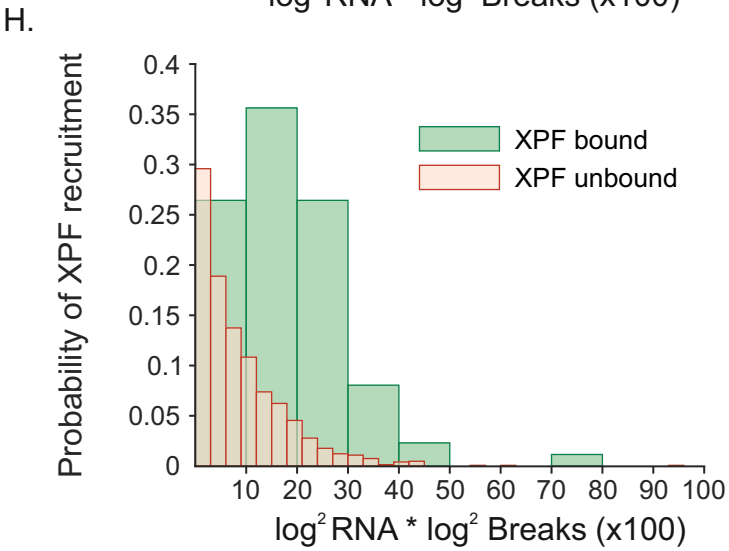








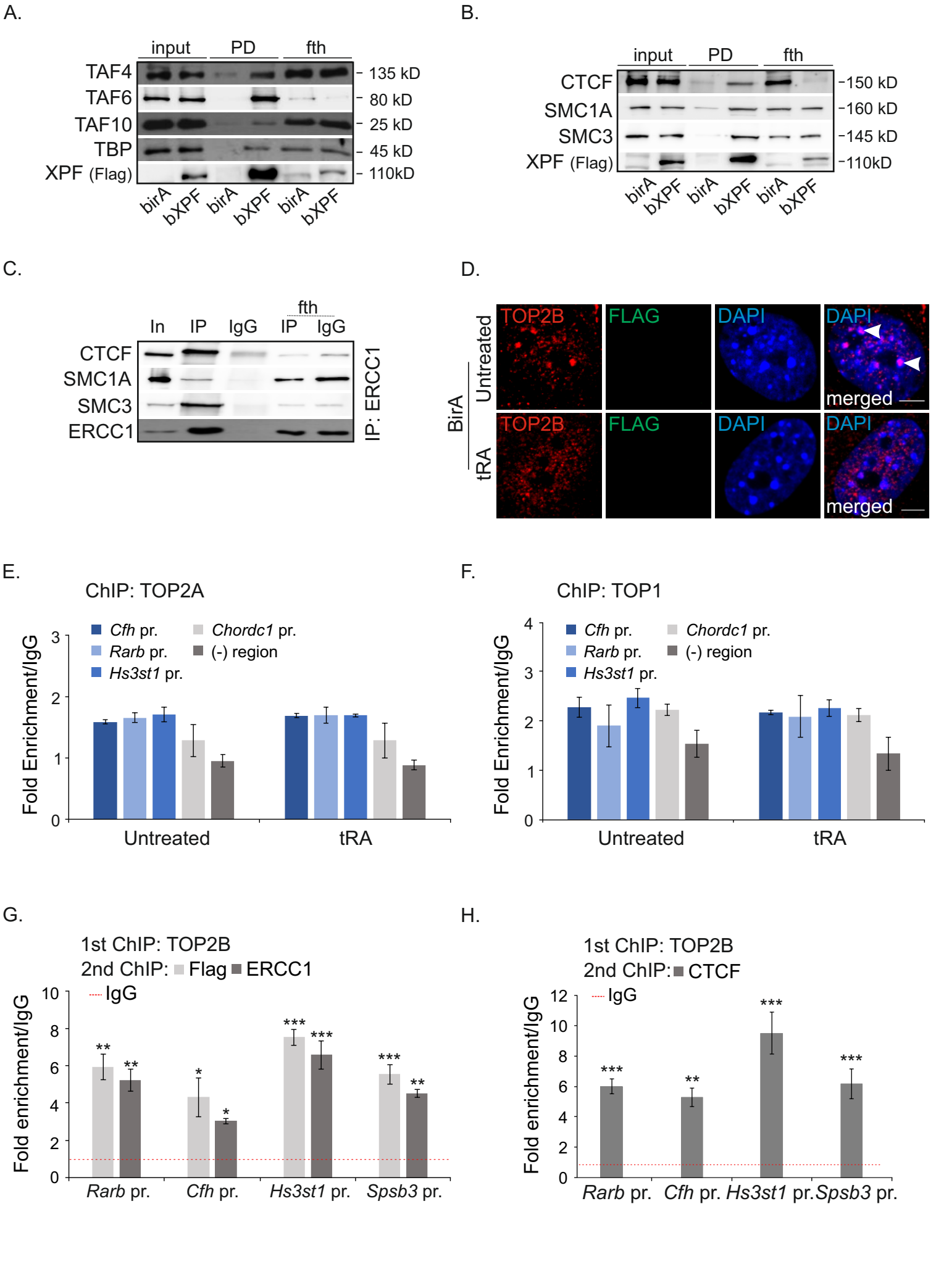




Supplementary Figure S3

Fig. S3.

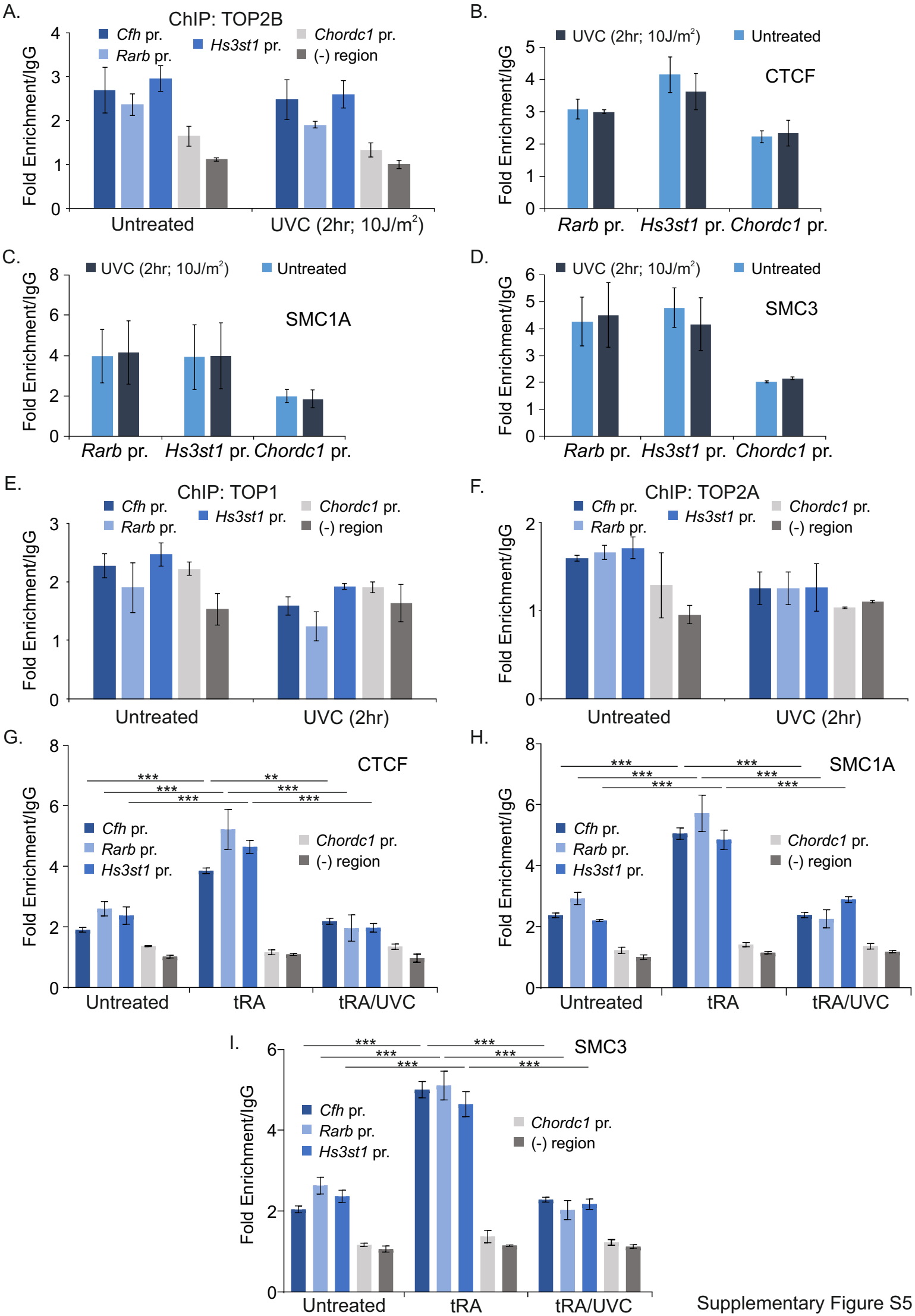
Genome-wide probability of XPF recruitment and XPF-CTCF interaction. (**A**). Cell cycle analysis through propidium iodide staining in untreated and tRA-treated wt MEFs. (**B**). Mean fluorescence intensity (MFI) of BrU incorporation in untreated, tRA, tRA/DRB and tRA/TPL-treated MEFs. (**C**). BLESS signals quantified by qPCR on the tRA-inducible *Cfh*, *Rarb* and *Hs3st1* gene promoters in untreated MEFs, tRA-treated and UVC-irradiated (UVC/tRA), tRA-treated (tRA) MEFs. (**D**). BLESS signals quantified by qPCR on the tRA non-inducible *Dcaf10*, *Dhx16* and *Chordc1* gene promoters in untreated MEFs, tRA-treated MEFs. (**E**). Focused view of scatter plot of transcription (tRA/untreated fold change) and DSBs (tRA/untreated fold change) levels, for XPF-bound (orange) and XPF-non-bound (blue) genes. (**F**). Focused view of scatter plot of transcription (tRA/untreated fold change) and DSBs (tRA/untreated fold change) levels, for XPF-bound (orange) and XPF-non-bound (blue) genes without outliers (80^{th} percentile from the mean). (**G**). Distribution of XPF-bound (light green-colored) and XPF-non-bound (pink-colored) sites in untreated and (**H**). tRA-activated wt MEFs, as predicted by JAD Bio.



Supplementary Figure S4

Fig. S4.

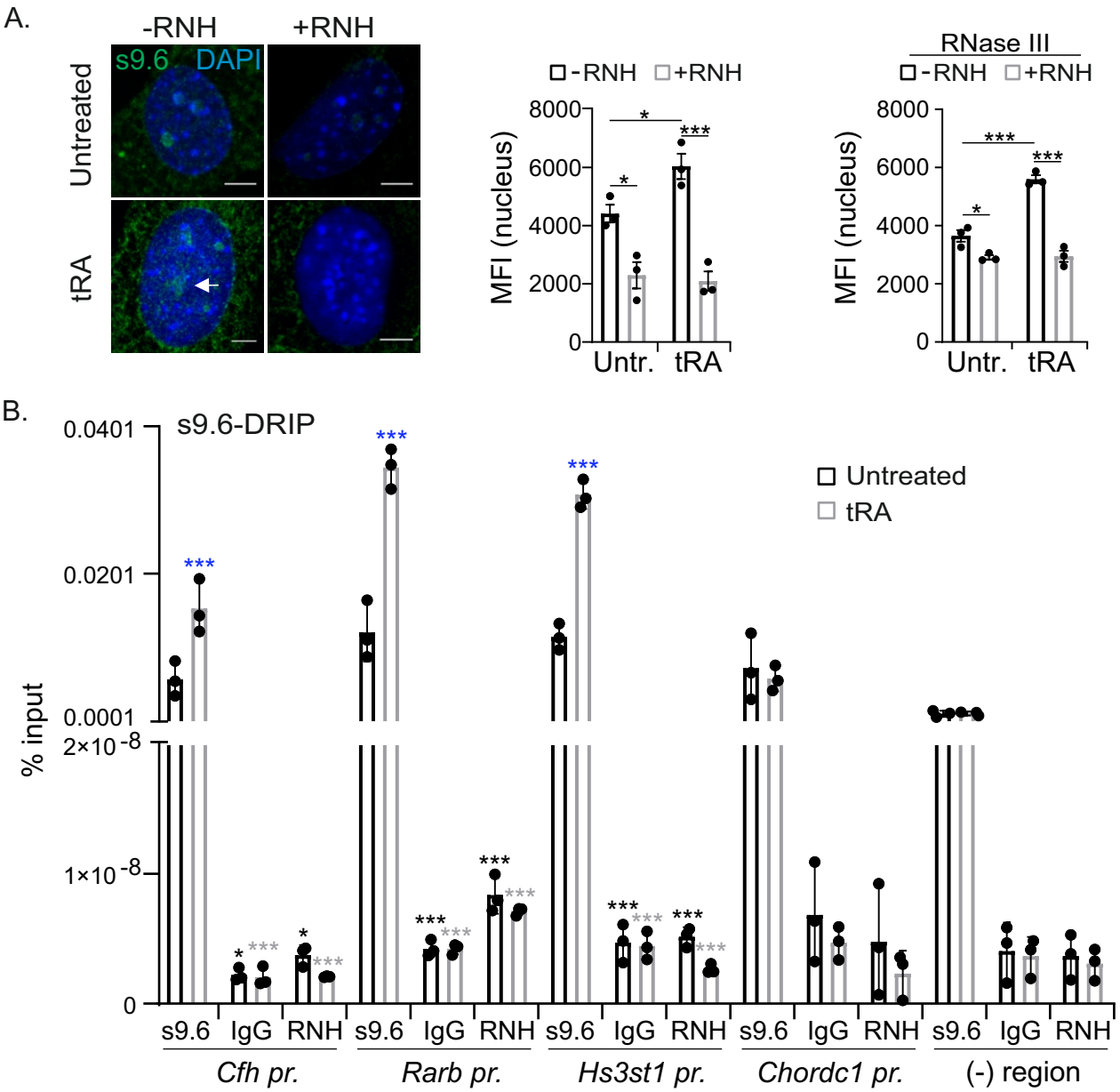
TOP1, 2A, 2B, CTCF, SMC1A and SMC3 ChIP signals on promoters. (**A**). Streptavidin pull-downs (PD) in nuclear extracts (benzonase and RNase-treated) under native conditions derived from primary bXPF MEFs expressing the BirA transgene or the BirA transgenic animals (as indicated) and analyzed by Western blotting for anti-TAF4, TAF6, TAF10, TBP and (**B**). CTCF, SMC1A and SMC3. (**C**). Co-immunoprecipitation experiments using anti-ERCC1 in nuclear extracts from wt MEFs analyzed by western blotting for CTCF, SMC1A and SMC3 (as indicated). (**D**). Immunofluorescence detection of TOP2B (red) and bXPF (FLAG, green) in untreated and tRA-treated BirA transgenic MEFs. White arrowheads point to the co-localization of TOP2B and DAPI-stained heterochromatin. Grey line is set at 5μm scale. (**E**). TOP2A ChIP signals on the promoters of tRA-induced *Cfh*, *Rarb*, and *Hs3st1* gene, of the tRA-non induced *Chordc1* gene and on an intergenic non-transcribed (-) region (as indicated). (**F**). TOP1 ChIP signals on the promoters of tRA-induced *Cfh*, *Rarb*, and *Hs3st1* genes, the tRA-non induced *Chordc1* gene and on an intergenic non-transcribed (-) region (as indicated). (**G**). qPCR quantification of ChIP re-ChIP with antibodies raised against TOP2β followed by re-ChIP with antibodies raised against ERCC1 or Flag-tagged XPF on *Cfh*, *Rarb*, *Hs3st1* and *Spsb3* gene promoters (as indicated). (**H**). qPCR quantification of ChIP re-ChIP with antibodies raised against TOP2B followed by re-ChIP with an antibody raised against CTCF on *Cfh*, *Rarb*, *Hs3st1* and *Spsb3* gene promoters (as indicated).



Supplementary Figure S5

Fig. S5.

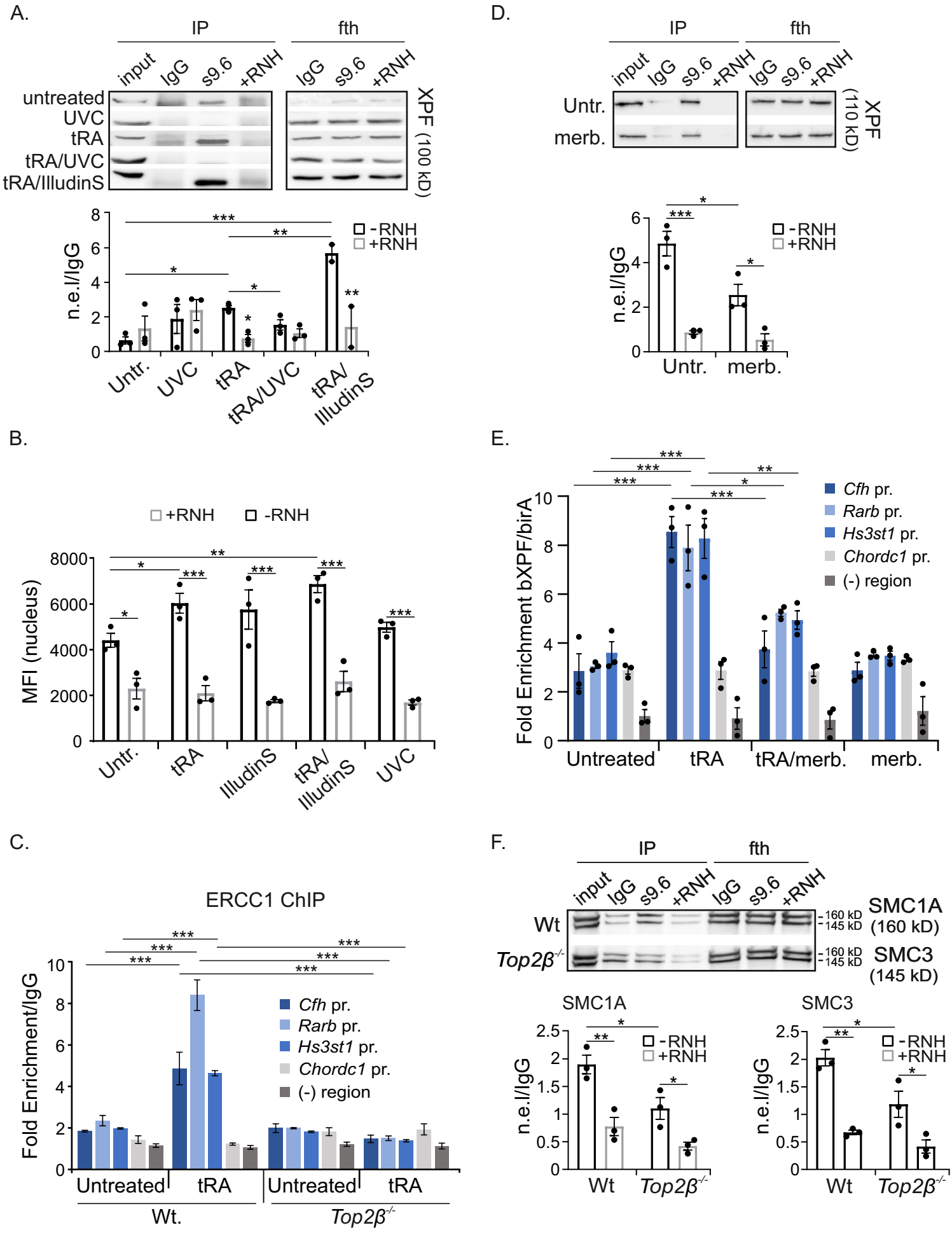
Effect of UVC-irradiation on the recruitment of TOP2B, CTCF, SMC1A, SMC3, TOP1 and TOP2A. (**A**). TOP2B, (**B**). CTCF, (**C**). SMC1A, (**D**). SMC3, (**E**). TOP1 and (**F**). TOP2A ChIP signals on the promoters of tRA-induced *Cfh*, *Rarb*, and *Hs3st1* genes, the tRA-non induced *Chordc1* gene and on an intergenic non-transcribed (-) region in UVC-irradiated MEFs. (**G**). CTCF ChIP signals on the promoters of tRA-induced *Cfh*, *Rarb*, and *Hs3st1* genes, the tRA-non induced *Chordc1* gene and on an intergenic non-transcribed (-) region in untreated, tRA- and tRA/UVC-treated MEFs (as indicated). (**H**). SMC1A ChIP signals on the promoters of tRA-induced *Cfh*, *Rarb*, and *Hs3st1* genes, the tRA-non induced *Chordc1* gene and on an intergenic non-transcribed (-) region in untreated, tRA- and tRA/UVC-treated MEFs (as indicated). (**I**). SMC3 ChIP signals on the promoters of tRA-induced *Cfh*, *Rarb*, and *Hs3st1* genes, the tRA-non induced *Chordc1* gene and on an intergenic non-transcribed (-) region in untreated, tRA- and tRA/UVC-treated MEFs (as indicated). ChIP signals are shown as fold enrichment of % input antibody (Ab) over % input control antibody (IgG).



Supplementary Figure S6

Fig. S6.

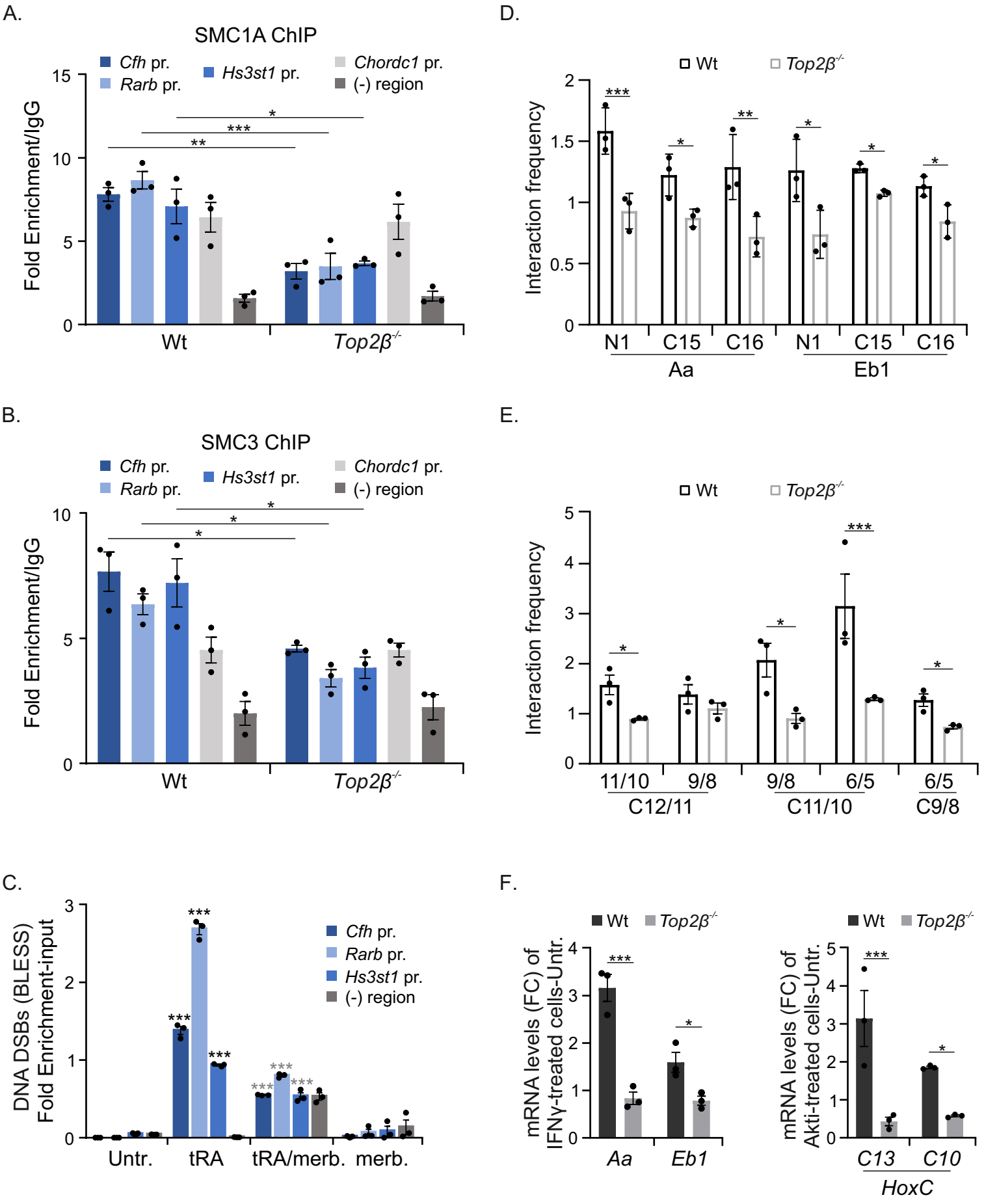
XPF and TOP2B are recruited on transcription-induced R loops. (**A**). Immunofluorescence detection of R loops, by means of s9.6 antibody staining, with or without RNase H (RNH) treatment in untreated and tRA-treated wt MEFs. The graphs depict the mean S9.6 fluorescence intensity per nucleus in RNase T1- (left) or RNase T1/RNase III-treated (right) MEFs. Grey line is set at 5μm scale. (**B**). DRIP analysis of *Cfh, Rarb, Hs3st1* and *Chordc1* gene promoters and of an intergenic non-transcribed (-) region with or without RNase H (RNH) in untreated and tRA-treated MEF. The p-values are depicted as blue: between untr s9.6-tRA s9.6, black: between untr s9.6-untr IgG or RNH, grey: tRA s9.6-tRA IgG or RNH conditions. (**C**). Representative immunofluorescence images and quantification of RNA-DNA hybrids in *Top2β*$^{-/-}$ and wt MEFs with or without RNH treatment. The graphs depict the mean S9.6 fluorescence intensity per nucleus in RNase T1- (left) or RNase T1/RNase III-treated (right) MEFs. Grey line is set at 5μm scale. (**D**). mRNA levels of *Cfh*, *Rarb* and *Hs3st1* genes in tRA-treated *Top2β*$^{-/-}$ and wt control MEFs over the untreated cells (red dotted line).



Supplementary Figure S7

Fig. S7.

TOP2B-dependent recruitment of CTCF/cohesin on R-loops. (**A**). S9.6 immunoprecipitation (DRIP) followed by Western blotting for XPF in untreated, UVC-, tRA-, tRA/UVC- and tRA/IlludinS-treated MEFs with or without RNH treatment. (**B**). R loops, by means of s9.6 antibody staining, with or without RNH treatment in untreated, tRA-, IlludinS-, tRA/IlludinS- and UVC-treated MEFs. (**C**). ERCC1 ChIP signals on the promoters of tRA-induced *Cfh*, *Rarb*, and *Hs3st1* genes, the tRA-non induced *Chordc1* gene and on an intergenic non-transcribed (-) region in untreated and tRA-treated *Top2β*$^{-/-}$ and wt MEFs. (**D**). DRIP followed by Western blotting for XPF in wt and merbarone-treated MEFs with or without RNH treatment. (**E**). bXPF ChIP signals on the promoters of the tRA-induced *Cfh*, *Rarb*, or *Hs3st1* genes, of the tRA-non induced *Chordc1* gene and on an intergenic non-transcribed (-) region in untreated, tRA-treated and tRA-treated MEFs also exposed to merbarone or in merbarone-treated MEFs. (**F**). DRIP followed by Western blotting for SMC1A and SMC3 in wt and *Top2β*$^{-/-}$ MEFs with or without RNase H (RNH) treatment (n.e.l. over IgG).



Supplementary Figure S8

Fig. S8.

TOP2B-dependent CTCF-mediated DNA looping. (**A**). SMC1A and (**B**). SMC3 ChIP signals on the promoters of *Cfh*, *Rarb*, *Hs3st1* and *Chordc1* genes and on an intergenic non-transcribed (-) region in *Top2β*$^{-/-}$ and wt MEFs. (**C**). BLESS signals quantified by qPCR on the tRA-inducible *Cfh*, *Rarb* and *Hs3st1* gene promoters and on a non-transcribed intergenic region (-) region in untreated MEFs (Untr.) or MEFs treated with tRA (tRA) or tRA and merbarone (tRA/merb) or merbarone (merb). Grey asterisks: tRA vs tRA/merb., black asterisks: untr vs trA. (**D**). Interaction frequencies, quantified by 3C-qPCR, between the MHC-II *Aa* or *Eb1* gene promoters and the *IA/IE-SE* super enhancer N1, C15 and C16 sites, in IFNγ-treated wt and *Top2β*$^{-/-}$ MEFs. The results from three biological replicates were averaged after normalization for untreated cells. (**E**). Interaction frequencies, quantified by 3C-qPCR, between the HoxC CTCF binding sites in wt and *Top2β*$^{-/-}$ MEFs, treated with an Akt inhibitor. The assessed interactions shown are between the C12|11 - C11|10, C12|11 – C9|8, C11|10 – C9|8, C9|8 – C6|5 sites. The results from three biological replicates were averaged after normalization for untreated cells. (**F**). mRNA levels of the MHC-II *Aa* and *Eb1* genes in IFNγ-treated wt and *Top2β*$^{-/-}$ MEFs (left) and of the Hox*C10* and *C13* genes in wt and *Top2β*$^{-/-}$ MEFs treated with an Akt inhibitor (right).