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Supplemental Information

Functional Analysis of the Replication Fork Proteome Identifies BET Proteins as PCNA Regulators

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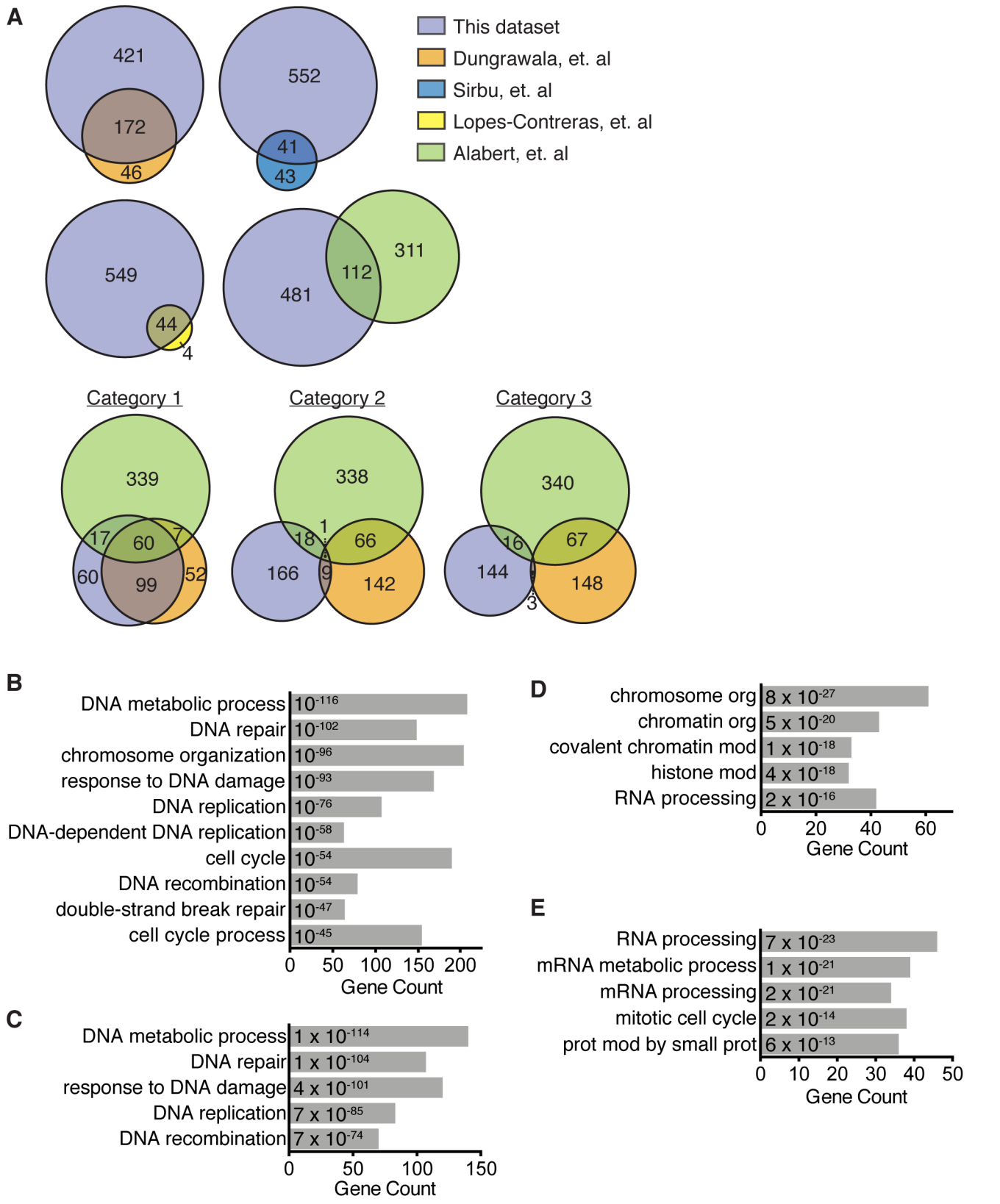

Figure S1. Summary of fork proteome dataset (related to Figure 1). (A) Venn diagrams depicting the overlaps between the 593 nascent DNA associated proteins in this dataset and other reported replication fork proteomes. (B) GO analysis (ToppGene) of proteins enriched on nascent DNA. Top ten enriched biological pathways are shown. The p-value for each pathway is indicated within each bar. Top five GO biological processes enriched in (C) category 1, (D) category 2, and (E) category 3.

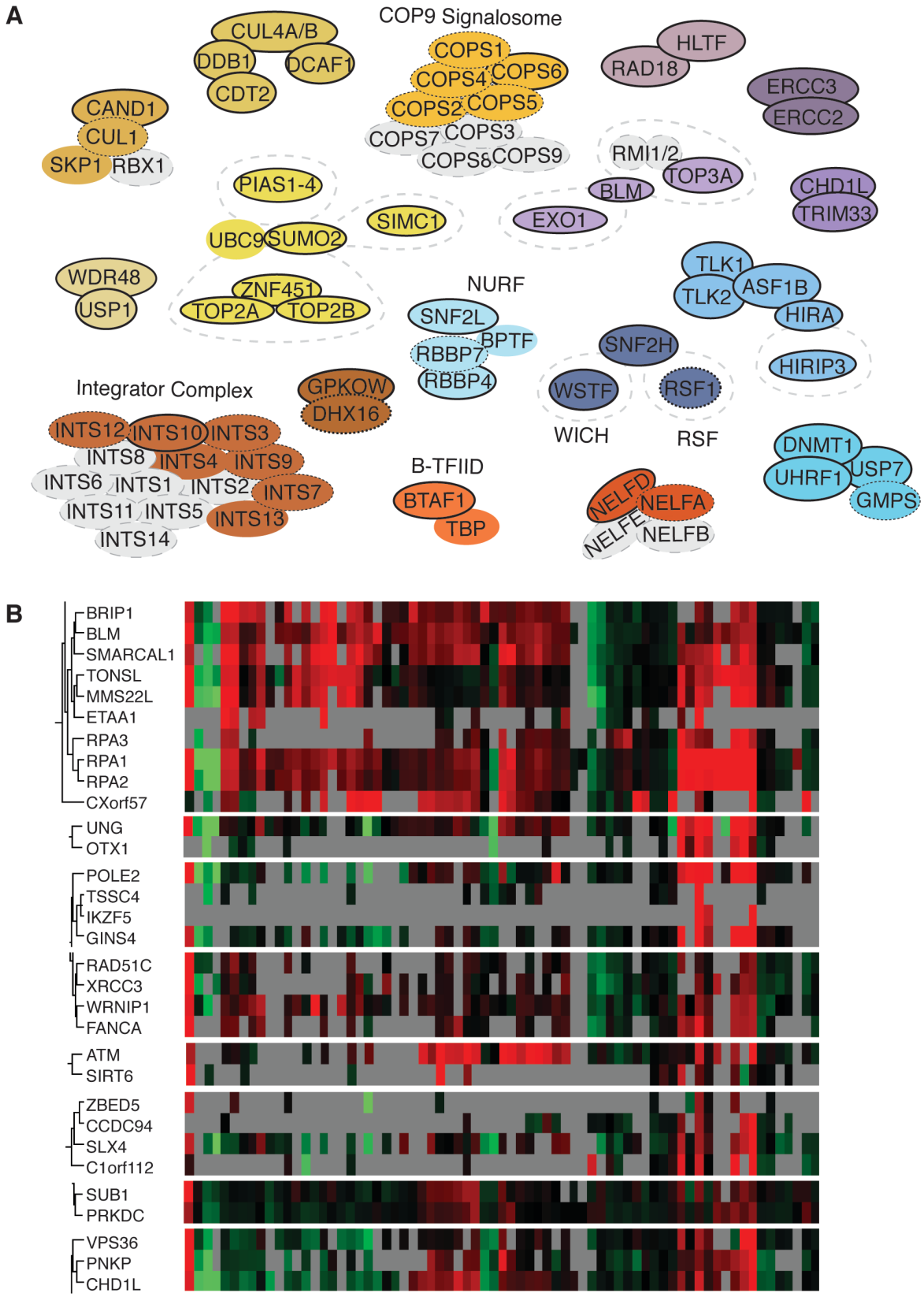

Figure S2. Analysis of protein complexes by unsupervised hierarchical clustering (related to Figure 2). (A) Selected protein complexes in the 593 nascent DNA proteome are depicted. Subunits in gray were not significantly enriched or were not observed, those outlined with a solid line are category 1, dotted outline are category 2, and no outline are category 3. (B) Hierarchical clustering of the 593 proteins enriched on nascent DNA with the potential false negatives identifies putative functional or physical interactions. Selected portions of the heat map that depict the abundance of proteins (row) in each experiment (column) with black being unchanged are shown. Red and green indicate an increase or decrease in abundance and gray indicates the protein was not observed.

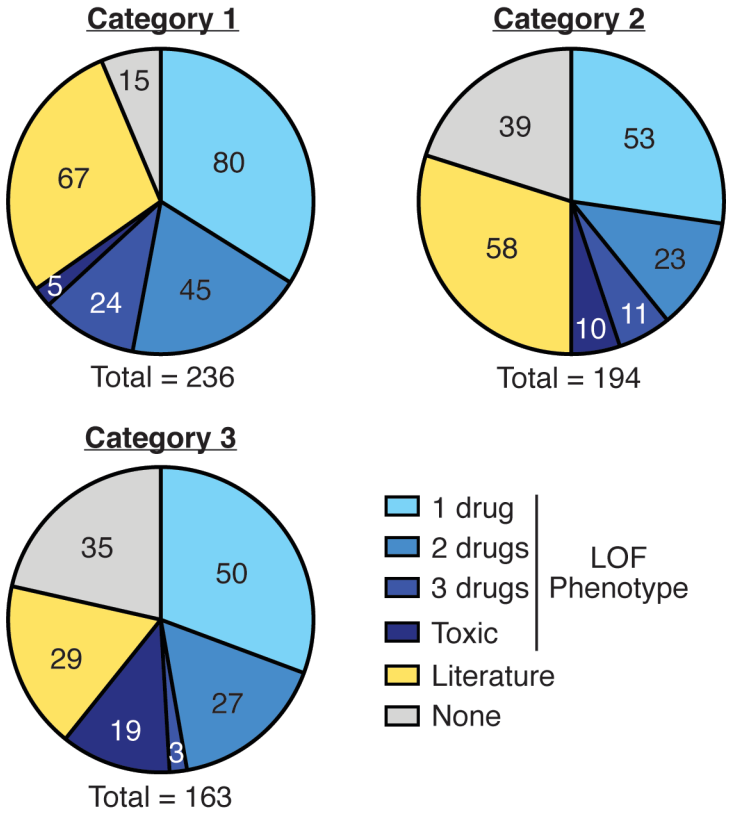

Figure S3. Summary of siRNA screens (related to Figures 3 and 4). Pie charts depicting phenotypes and functions observed from drug sensitivity screens and existing literature. Loss of function (LOF) phenotype includes sensitivity, resistance, and toxicity. Literature indicates published evidence for a function in DNA replication, repair, or related process.

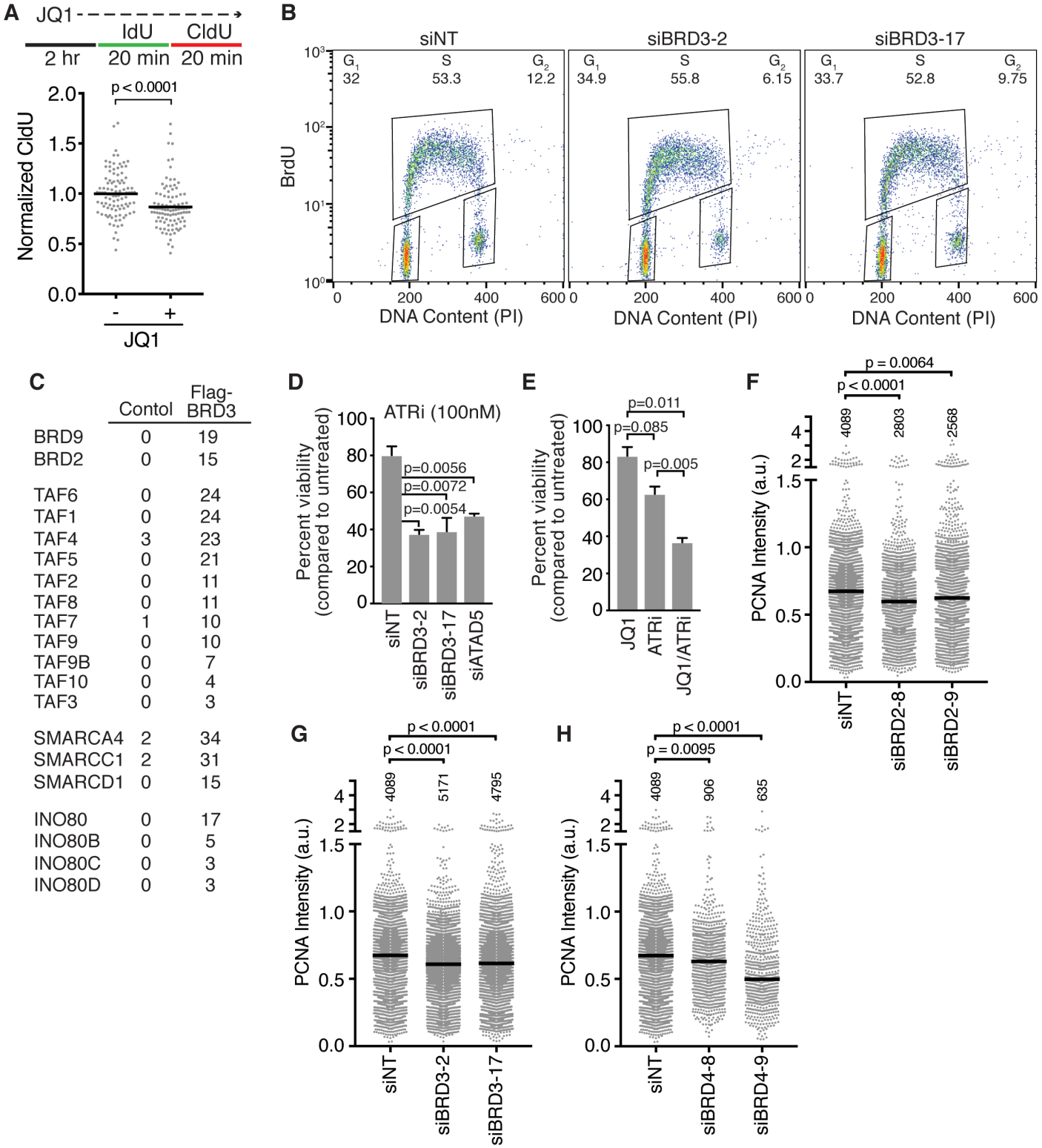

Figure S4. BET family proteins regulate PCNA by interacting with ATAD5 (related to Figures 5, 6, and 7). (A) Kasumi-1 cells were treated with 1μM JQ1 for 2 hours and then labeled with IdU followed by CldU (mean, two-tailed t-test). (B) U2OS cells were labeled with BrdU for 30 minutes and analyzed for BrdU and DNA content by flow cytometry 72 hours after siRNA transfection. (C) Expansion of table presented in Figure 6A. (D) Clonogenic survival assay of U2OS cells transfected with non-targeting (siNT), BRD3, or ATAD5 siRNA and treated with 100nM ATRi (VX-970) for 24 hours (mean+/-SD, n=3, ANOVA with Dunnett post-test). (E) Clonogenic survival assay of S-phase synchronized U2OS cells treated with the indicated combinations of 1μM JQ1 (4hrs) and 100nM ATRi (2hrs). All viability assays show mean+/-SD, n=3, ANOVA with Dunnett post-test. (F-H) Intensity of detergent-resistant PCNA immunofluorescence in S-phase cells transfected with the indicated siRNAs. Number of cells analyzed in each column is indicated (median indicated by bar, ANOVA with Dunnett post-test).

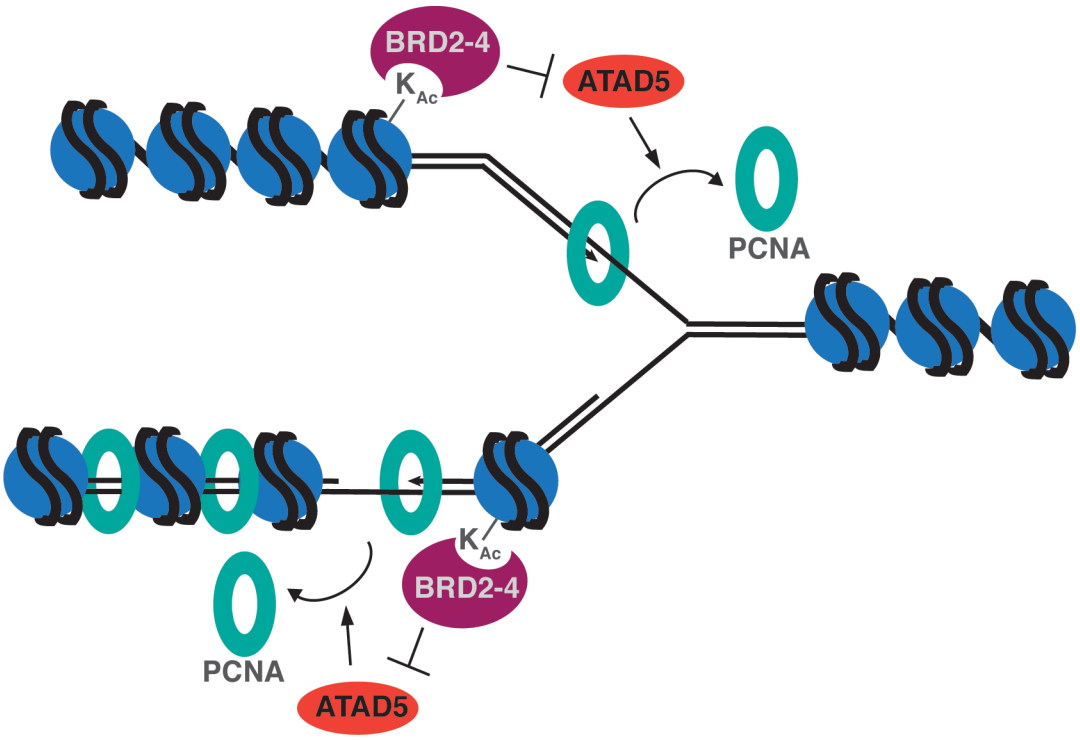

Figure S5. Model for the regulation of ATAD5-mediated PCNA unloading by BET family proteins (related to Figures 5, 6, and 7).