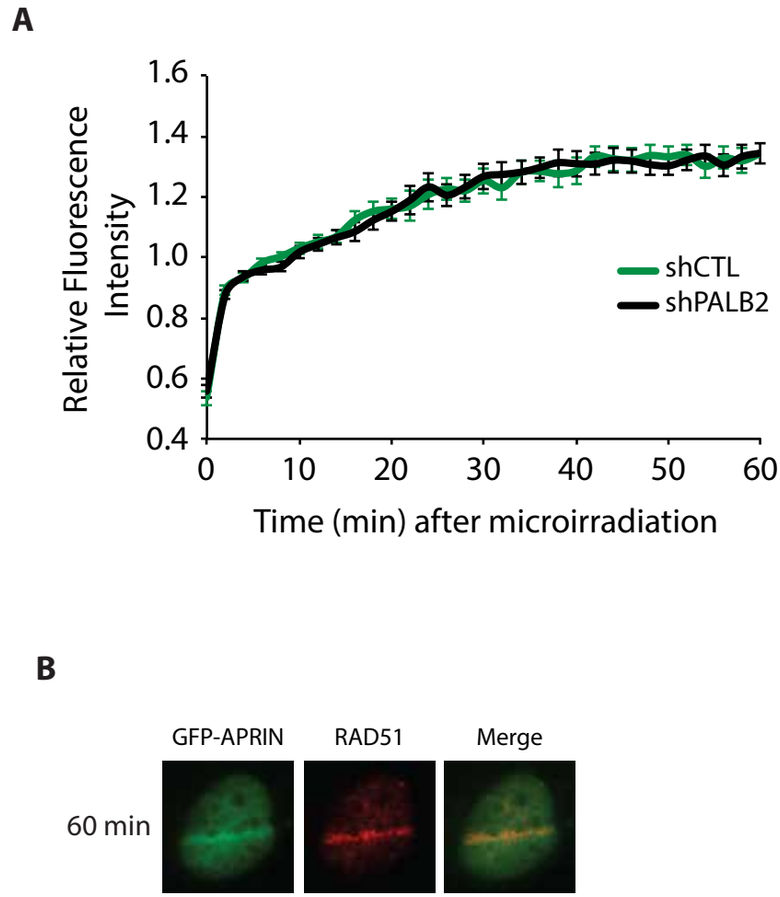
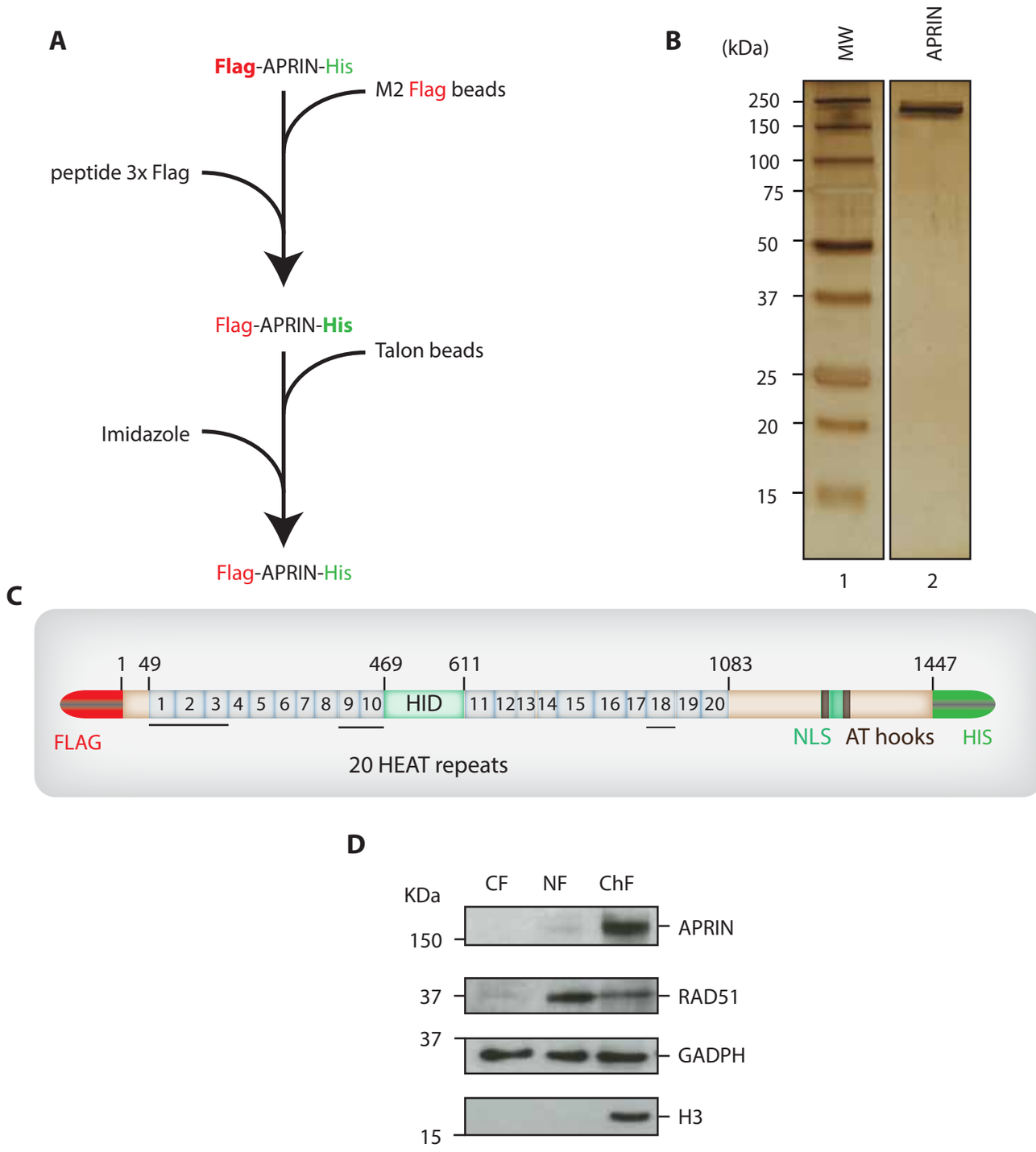
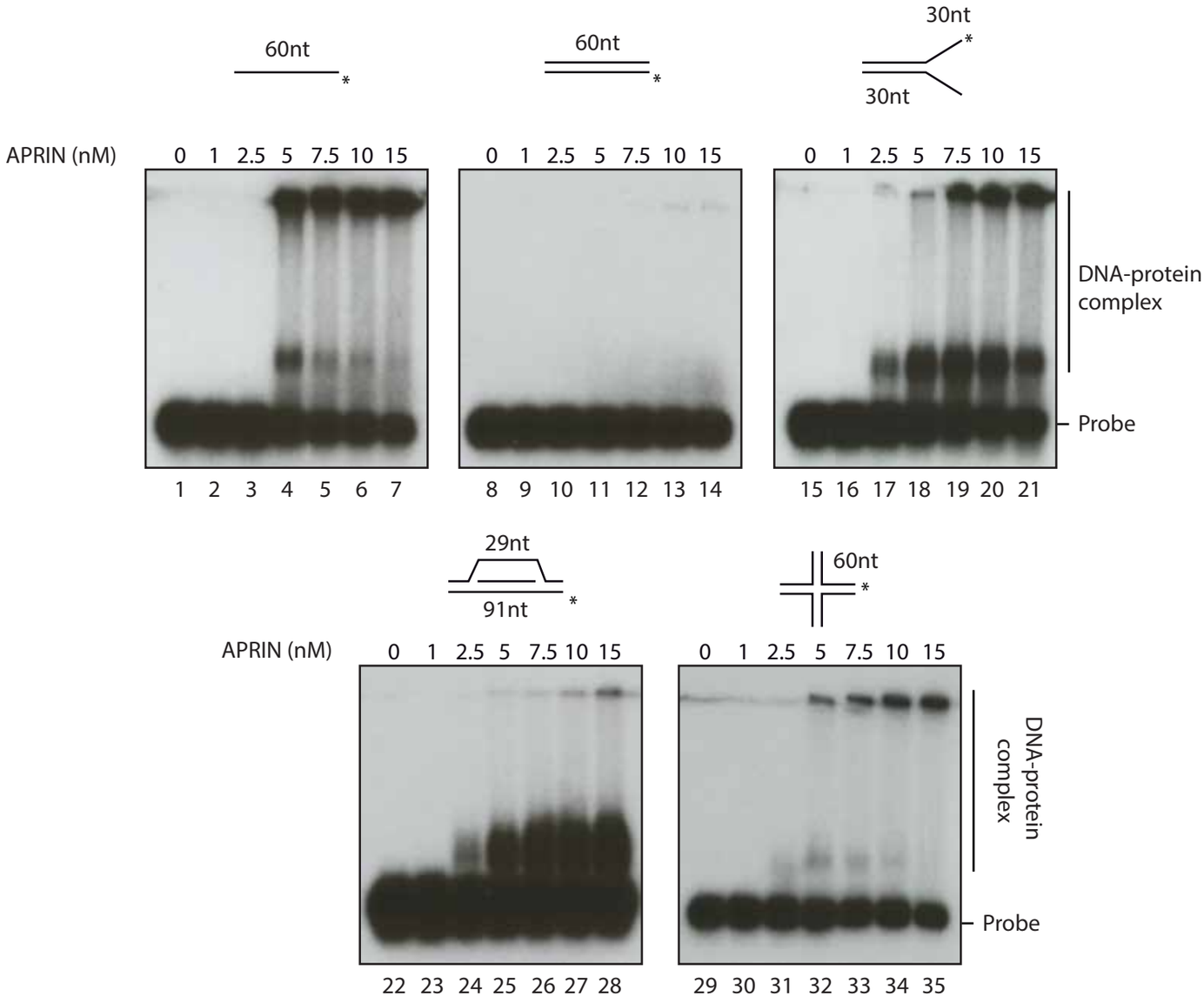


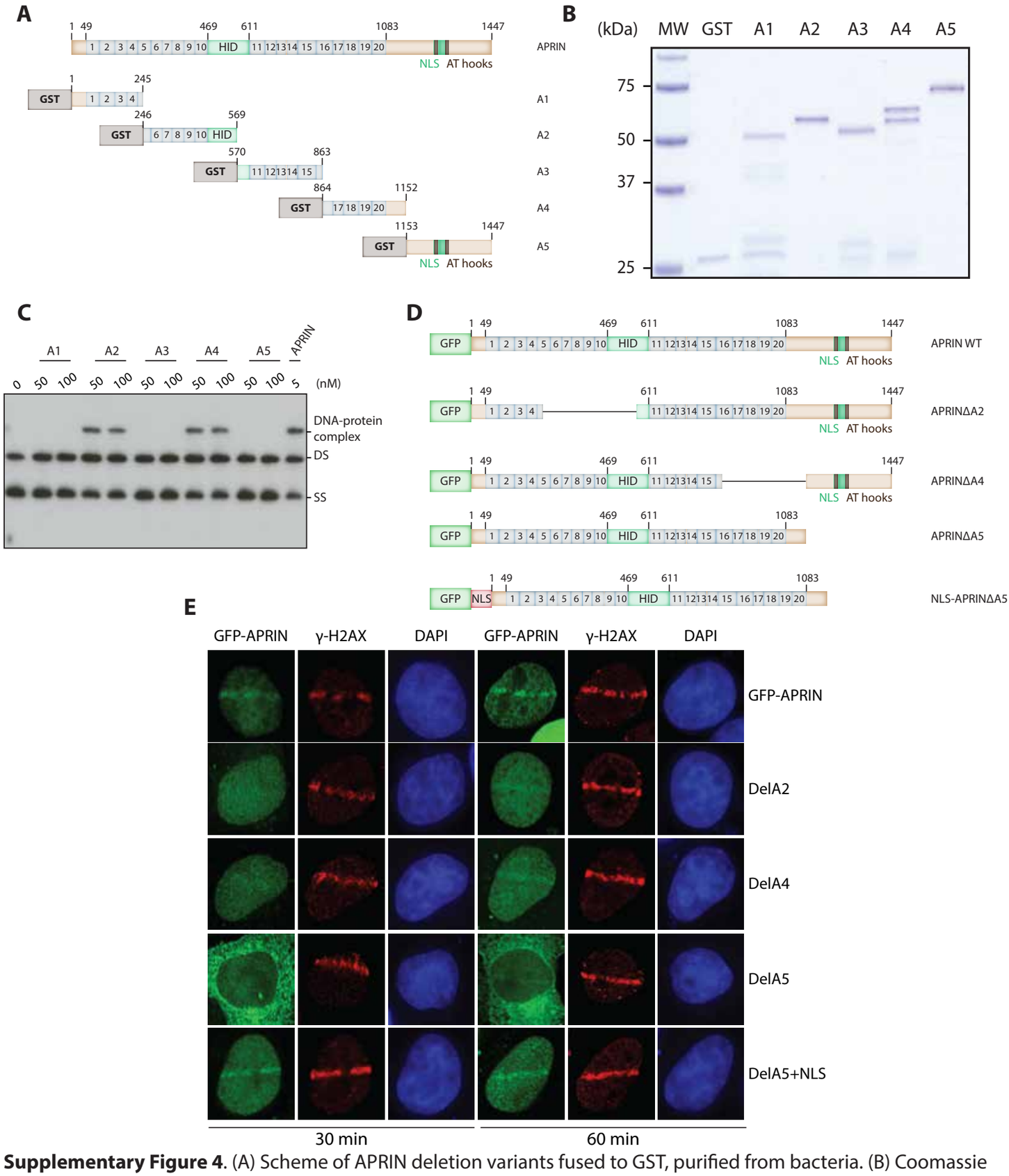
Supplementary Figure 1. (A) Recruitment of GFP-APRIN in shCTL and shPALB2 Hela cells. (B) Representative images of laser-irradiated SKOV3 GFP-APRIN (green) cells and subjected to RAD51 immunofluorescence staining, 60 min post-DNA damage.



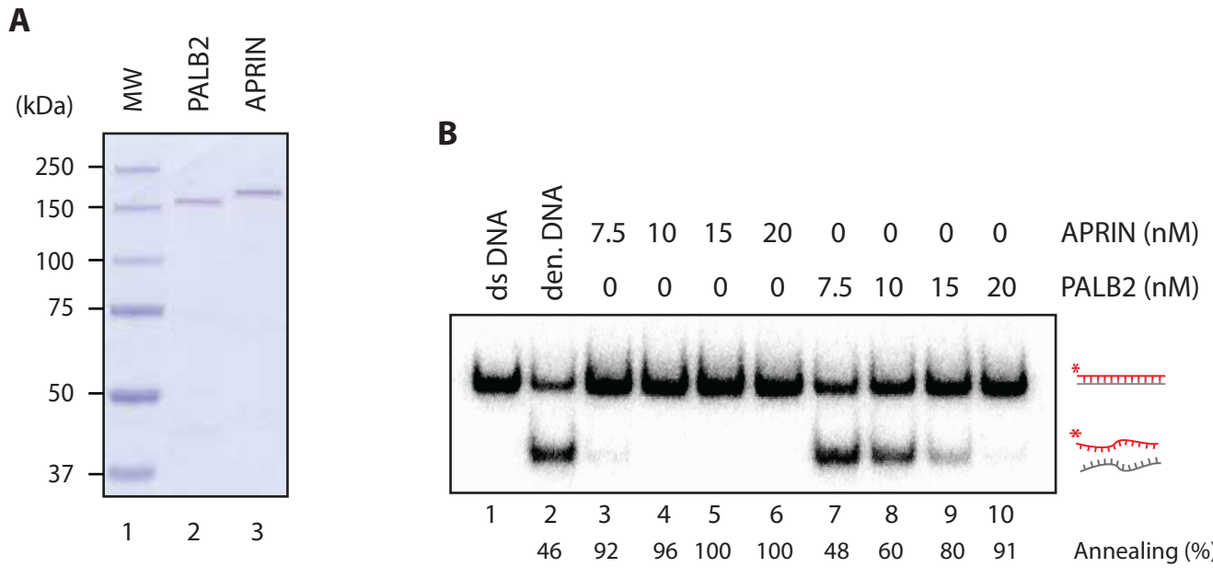
Supplementary Fig. 2: (A) Schematic representation of the double affinity purification of APRIN. Two-step affinity purification of Flag-APRIN-His from Sf9 soluble cell lysate: Flag binding, followed by elution of the protein with Flag peptides 3X, TALON-binding and elution with imidazole. (B) Silver-stained SDS-PAGE of APRIN. (C) Scheme of APRIN tagged Flag (red)/His (green). APRIN bears Heat repeats and AT hooks domains. (D) Fractionation from 293T cells. Endogenous APRIN was detected in the chromatin fraction (ChF). RAD51 is a control for the Nuclear soluble Fraction (NF), Histone H3 of the ChF fraction and GAPDH of the cytoplasmic Fraction (CF).



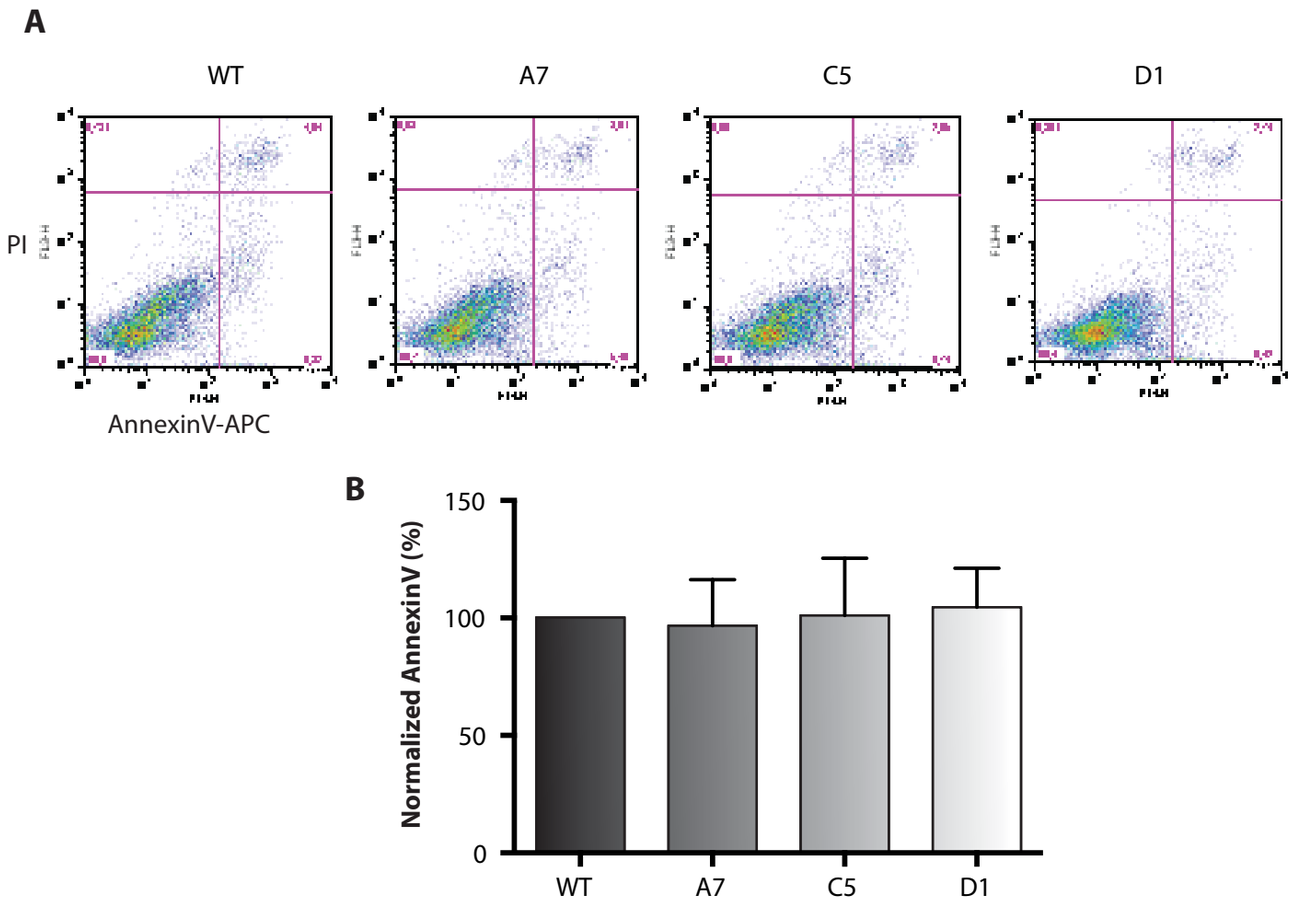
Supplementary Fig. 3: EMSA were performed with APRIN (0-15 nM) and ssDNA (lanes 1-7), or dsDNA (lanes 8-14), or Splayed Arms (lanes 15-21), or D-loop (lanes 22-28), or Holliday Junction substrates (lanes 29-35).



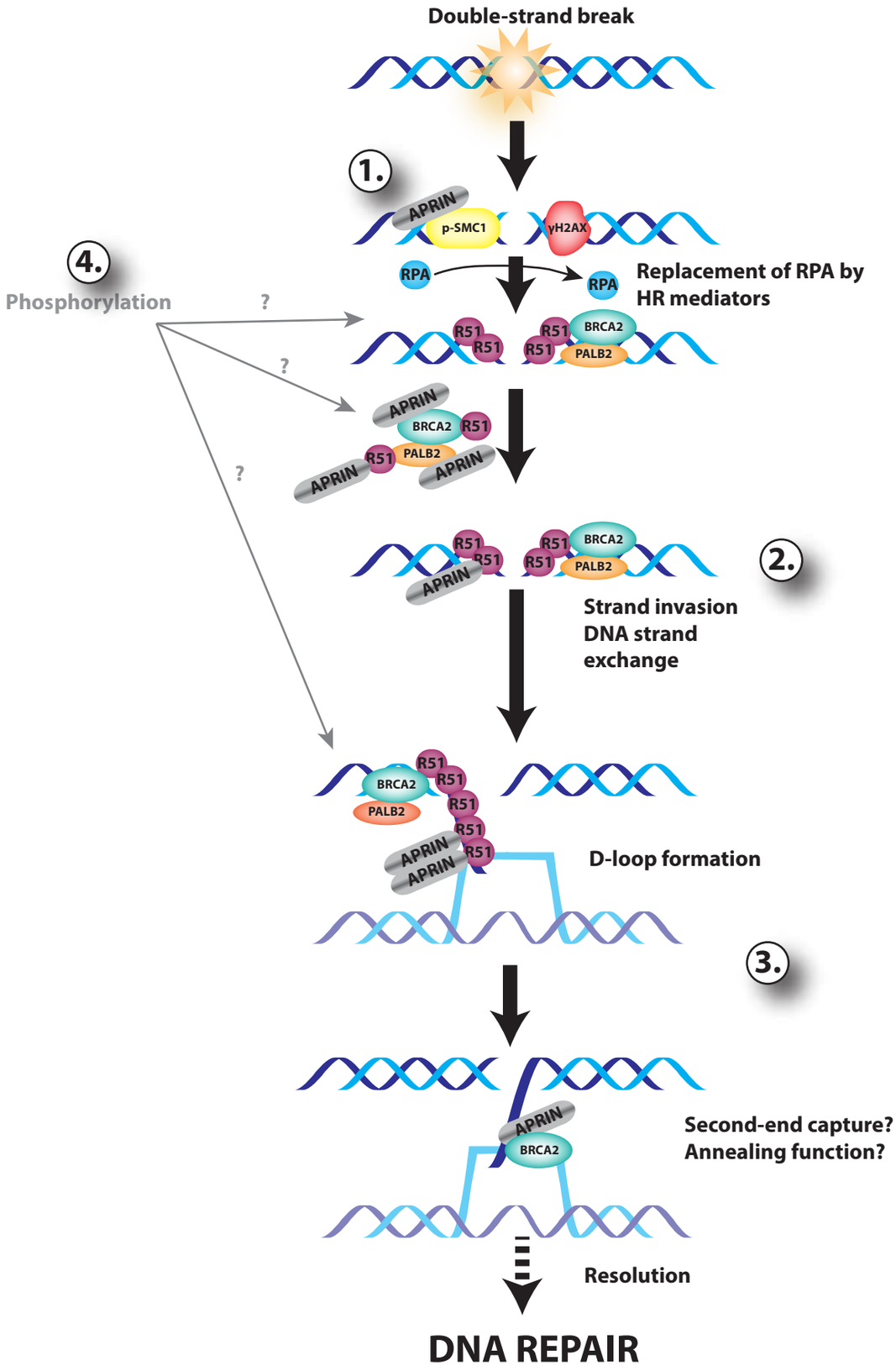
Supplementary Figure 4. (A) Scheme of APRIN deletion variants fused to GST, purified from bacteria. (B) Coomassie Blue stained SDS-PAGE gel of APRIN deletion variants before GST cleavage. (C) EMSAs were performed with both a ssDNA (ss) and dsDNA (ds) and APRIN fragments (50 or 100 nM): A1 (lanes 2-3), A2 (lanes 4-5), A3 (lanes 6-7), A4 (lanes 8-9), A5 (lanes 10-11), or full length APRIN (7.5 nM) (lane 12). (D) Scheme of APRIN deletion variants fused to GFP. (E) Representative images of laser-irradiated Hela cells expressing different deletions of APRIN (green – variants are mentioned on the right) and immunostained for γ-H2AX (red), 30 minutes or 60 minutes after DNA damage. DAPI (blue) represents the nucleus.



Supplementary Figure 5. (A) SDS-PAGE gel of purified His/Flag APRIN and PALB2 stained with Coomassie blue. Lane 1, Molecular weight markers; Lane 2, PALB2 purified protein (250 ng); Lane 3, APRIN purified protein (250 ng). (B) APRIN and PALB2 promote single-strand annealing. Lane 1, purified 60-bp duplex DNA (dsDNA). Reactions contained denatured 60-bp duplex DNA in buffer with no protein (lane 2; den. DNA: denatured DNA) or with purified APRIN (1–10 nM), lanes 3–6; or with purified PALB2 (1–10 nM), lanes 7–10. Percentage of annealing is depicted under the figure.



Supplementary Figure 6. (A) AnnexinV staining of Mouse B-cell line CH12F3 depleted from APRIN. WT: wild-type CH12F3. A7 clone represents a deletion in exon 3. C5 and D1 clones represent deletion of exon 5 and a part of exon 6. (B) Quantification of the result.



Supplementary Figure 7. Model for the specific roles of APRIN in homologous recombination (HR). 1. APRIN is rapidly recruited after the DNA damage. 2. APRIN interacts with different proteins implicated in HR. 3. APRIN enhances different steps of HR. 4. APRIN activity could be modulated by phosphorylation.