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Supplemental Information

DNA Structure-Specific Cleavage of DNA-Protein Crosslinks by the SPRTN Protease

Hannah K. Reinking, Hyun-Seo Kang, Maximilian J. Götz, Hao-Yi Li, Anja Kieser, Shubo Zhao, Aleida C. Acampora, Pedro Weickert, Evelyn Fessler, Lucas T. Jae, Michael Sattler, and Julian Stingele

Figure S1 (related to Figure 1)

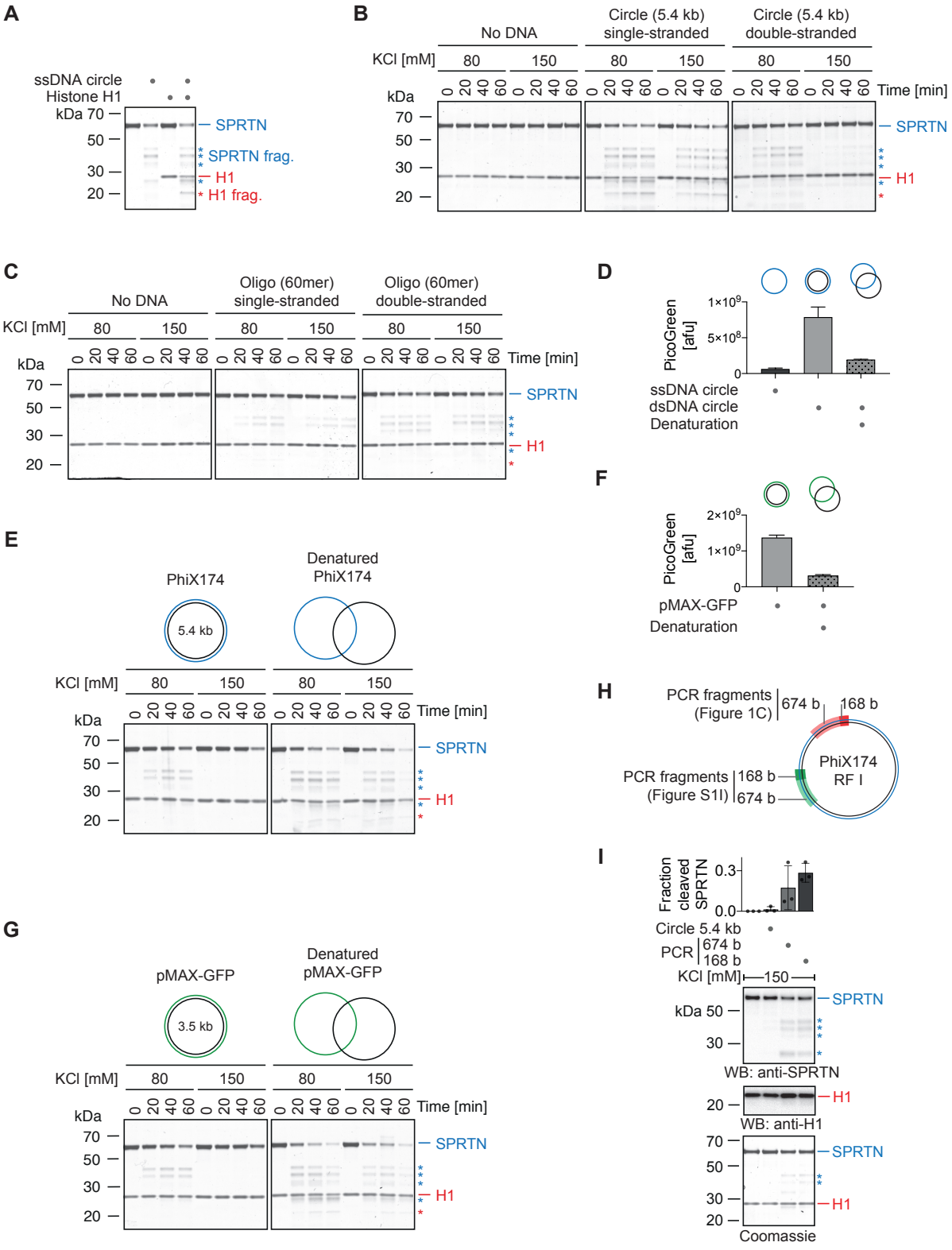

Figure S1. DNA length determines activation of SPRTN by double-stranded DNA, Related to Figure 1.

(A) SPRTN protease assay in the absence and presence of histone H1 to reveal H1 proteolytic fragments. Recombinant SPRTN (500 nM) - with and without histone H1 (500 nM) - was incubated alone or in the presence of ssDNA circles (ΦX174 virion) for 2 h at 25°C. Reactions were analysed by SDS-PAGE followed by Coomassie staining. Proteolytic fragments of SPRTN and H1 are indicated by asterisks.

(B-C) Kinetics of enzymatic reactions shown in Figure 1A. Recombinant SPRTN (500 nM) and histone H1 (500 nM) were incubated alone or in the presence of DNA (5.4 kb circles (B) or 60mer oligonucleotides (C), each either single- or double-stranded for the indicated amount of time at 25°C. DNA concentrations were 1 μM for 60mer oligonucleotides or the corresponding amount of circular DNA (11.4 nM). Reactions were analysed by SDS-PAGE followed by Coomassie staining. Cleaved fragments of SPRTN and H1 are indicated by asterisks.

(D) Denaturation of circular dsDNA (ΦX174 RFI) monitored by PicoGreen fluorescence. dsDNA was melted at 95°C prior to snap-cooling on ice. Denaturation was assessed using PicoGreen, a fluorescent dye specific for dsDNA.

(E) Kinetics of enzymatic reactions shown in Figure 1B. SPRTN (500 nM) and histone H1 (500 nM) were incubated in the presence of double- or denatured dsDNA (ΦX174 RFI) for the indicated amount of time at 25°C. Reactions were analysed by SDS-PAGE followed by Coomassie staining. Cleaved fragments of SPRTN and H1 are indicated by asterisks.

(F) Denaturation of circular dsDNA (pMAX-GFP) monitored by PicoGreen fluorescence. dsDNA was melted at 95°C prior to snap-cooling on ice. Denaturation was assessed using PicoGreen, a fluorescent dye specific for dsDNA.

(G) SPRTN (500 nM) and histone H1 (500 nM) were incubated in the presence of ds- or denatured dsDNA (pMAXGFP plasmid) for the indicated amount of time at 25°C. Reactions were analysed by SDS-PAGE followed by Coomassie staining. Cleaved fragments of SPRTN and H1 are indicated by asterisks.

(H) Schematic of the ΦX174 RFI regions amplified by PCR for use in Figure 1C and **I**.

(I) Fragment length determines activation of SPRTN by PCR-generated double-stranded DNA (as Figure 1C but using different DNA fragments). SPRTN (500 nM) and histone H1 (500 nM) were incubated in the presence of the indicated types of DNA for 2 h at 25°C. Cleaved fragments of SPRTN and H1 are indicated by asterisks. Quantification of Western blot results of SPRTN and histone H1 cleavage: values represent the mean ± SD of three independent experiments.

Figure S2 (related to Figure 2)

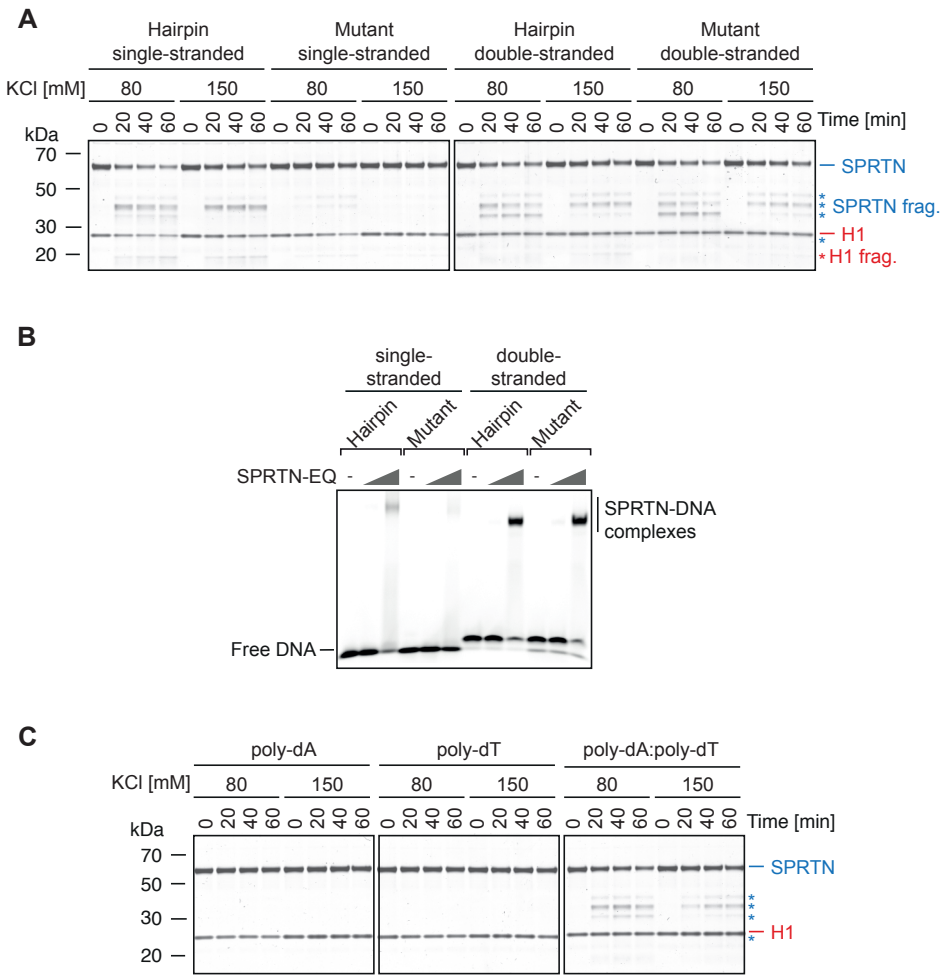

Figure S2. SPRTN is not activated by strictly single-stranded DNA, Related to Figure 2.

(A) Kinetics of enzymatic reactions shown in Figure 2F. Recombinant SPRTN (500 nM) and histone H1 (500 nM) were incubated alone or in the presence of the indicated DNAs (4 μM) for the indicated amount of time at 25°C. Reactions were analysed by SDS-PAGE followed by Coomassie staining. Cleaved fragments of SPRTN and H1 are indicated by asterisks.

(B) SPRTN binds to a DNA hairpin. EMSA assays were used to asses binding of catalytically inactive SPRTN E112Q (500 nM and 4 μM) to the indicated fluorescently-labelled oligonucleotides (4 μM).

(C) Kinetics of enzymatic reactions shown in Figure 2H. Reactions were incubated for the indicated amount of time at 25°C. Reactions were analysed by SDS-PAGE followed by Coomassie staining. Cleaved fragments of SPRTN and H1 are indicated by asterisks.

Figure S3 (related to Figure 3)

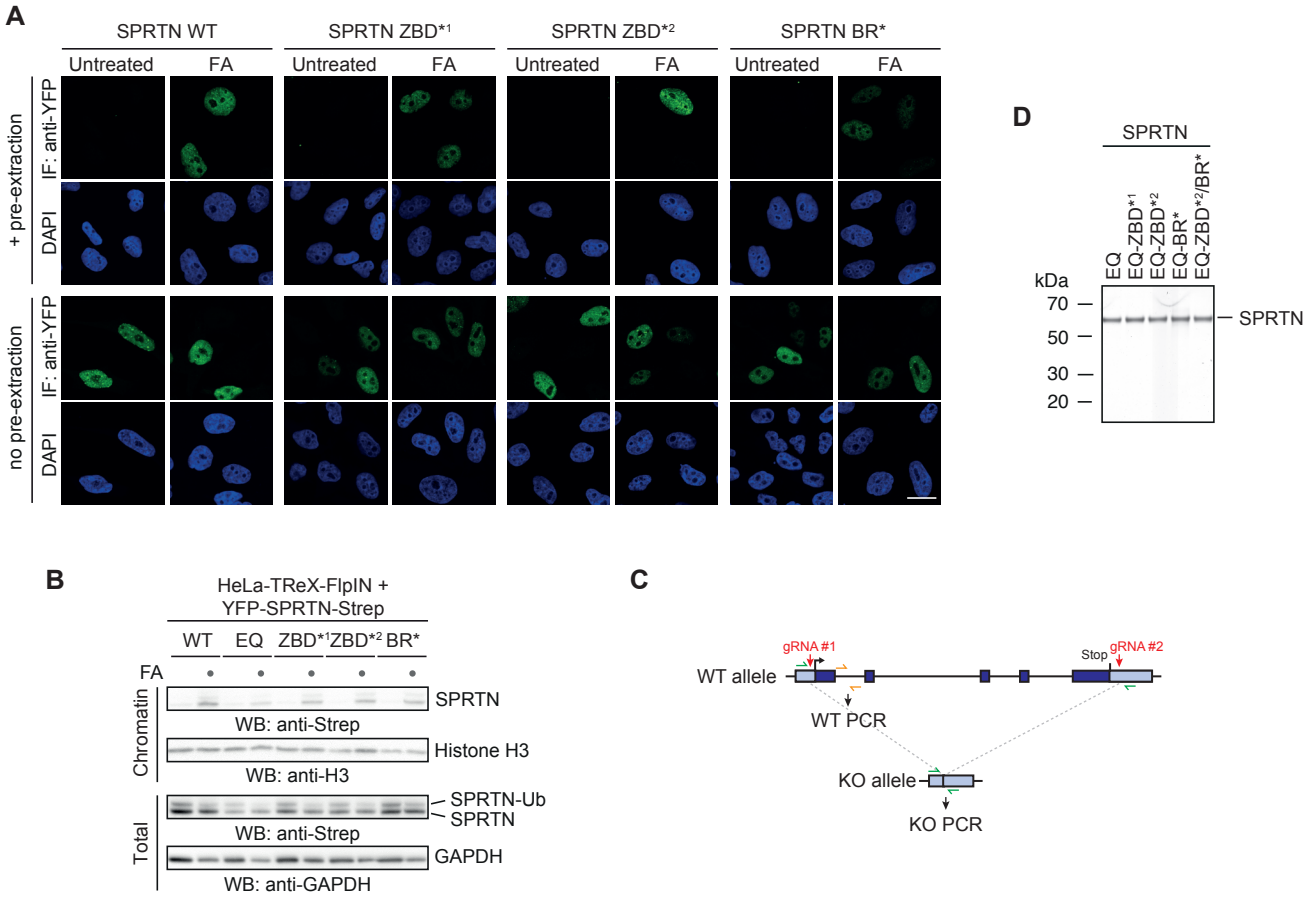

Figure S3. The DNA-dependent induction of SPRTN's protease activity requires two distinct DNA binding domains, Related to Figure 3.

(A) Recruitment of SPRTN to chromatin upon DPC induction is unaltered in ZBD and BR mutant variants. Doxycycline-inducible YFP-SPRTN-Strep HeLa Flp-In TRex cells expressing the indicated SPRTN variants were treated with 500 μM formaldehyde (FA) for 2 h prior to immunofluorescence-staining with or without pre-extraction prior to fixation. Scale bar represents 20 μm.

(B) Recruitment of SPRTN to chromatin upon DPC induction is unaltered in ZBD and BR mutant variants. Doxycycline-inducible YFP-SPRTN-Strep HeLa Flp-In TRex cells expressing the indicated SPRTN variants were treated with 500 μM formaldehyde (FA) for 2 h prior to chromatin fractionation and Western blotting against Strep-tag, histone H3 and GAPDH.

(C) Schematic depiction of the knock-out and genotyping strategy used in Figure 3E. NLS-Cas9/gRNA complexes targeting the UTRs of the endogenous SPRTN allele were transfected in HAP1 cells complemented with empty vector or different SPRTN variants. The abundance of the resulting SPRTN KO allele was then monitored over time using qPCR with the indicated primers.

(D) Coomassie-stained SDS-PAGE showing purified SPRTN-E112Q variants used in electrophoretic mobility shift assays (Figure 3I-K).

Figure S4 (related to Figure 4)

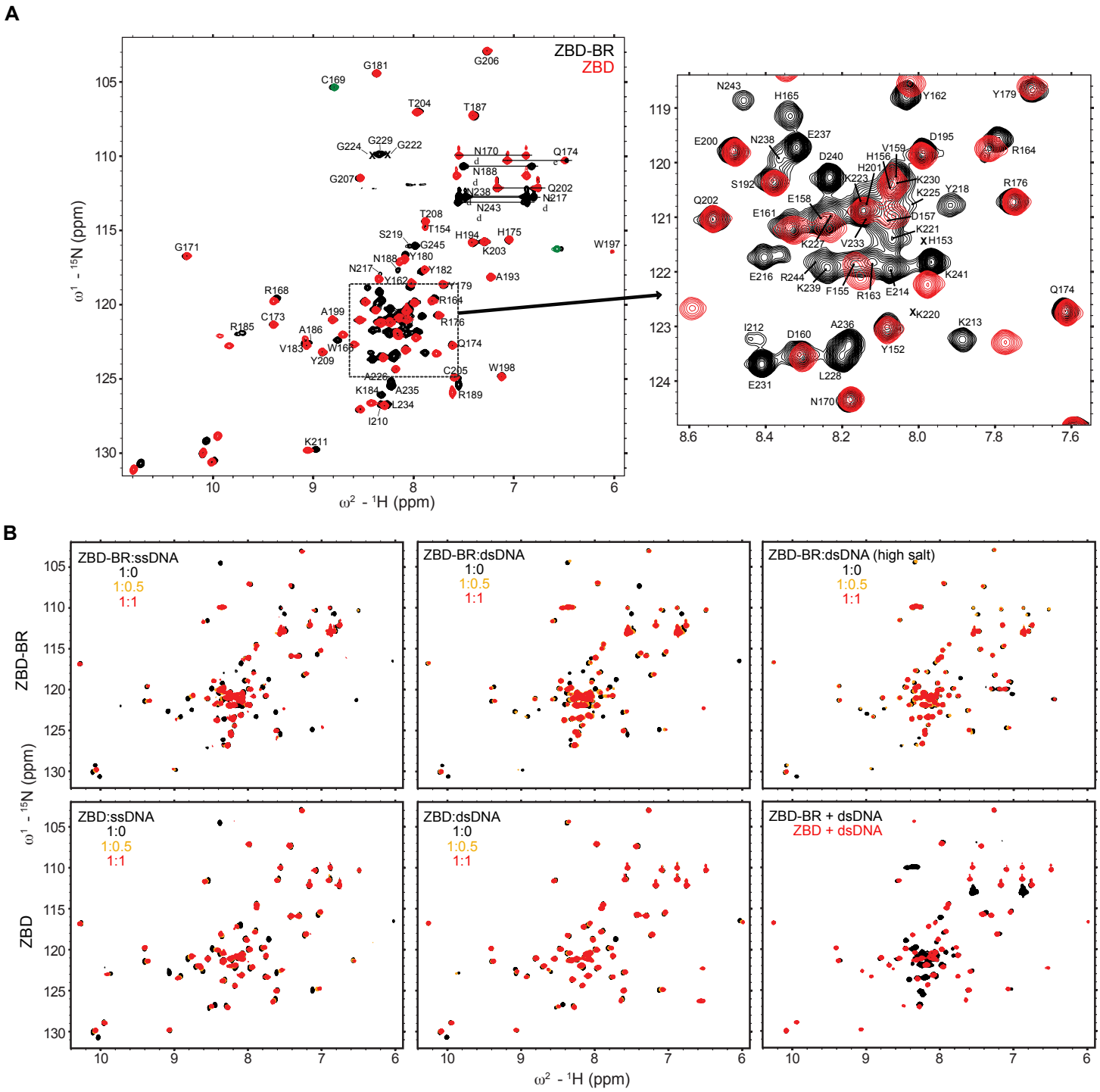

Figure S4. NMR analysis reveals a bipartite recognition of DNA structures by SPRTN, Related to Figure 4.

(A) Chemical shift assignments of amide signals in a ^{1}H, ^{15}N correlation spectrum (HSQC) of ZBD-BR (black). The spectrum of ZBD only (red) is superimposed. Both samples were measured at 100 μM concentration in 20 mM sodium chloride, 50 mM sodium phosphate pH 6.5, 2 mM TCEP at 298 K on a 600 MHz spectrometer.

(B) Superimposed HSQC of ZBD-BR and ZBD, and ZBD-BR at high salt at three different molar ratios of protein to ssDNA (left) or dsDNA (middle) (1:0, black; 1:0.5, orange; 1:1, red). ZBD-BR titrations with dsDNA at high salt concentration is shown on right, top. Superimposed spectra of ZBD-BR (black) and ZBD (red) with equimolar dsDNA are shown on right, bottom. All the samples (protein concentration 100 μM) were measured in 100 mM (500 mM for high salt) potassium chloride, 50 mM HEPES pH 7.5, 2 mM TCEP at 298 K at 600 MHz (ssDNA, dsDNA at high salt) or 900 MHz ^{1}H Larmor frequency (dsDNA).

(C) Chemical shift perturbations (CSPs) and intensity differences (line-broadening) of backbone amides in ZBD upon addition of an equimolar ratio of ssDNA (grey) and dsDNA (red). Errors for intensity ratios upon DNA-binding were estimated from error propagation of peak height uncertainties based on average noise levels (six randomly chosen positions in each NMR spectra). No boxes are shown for proline, unassigned, ambiguous (overlapped) residues in both plots.

Figure S5 (related to Figure 5)

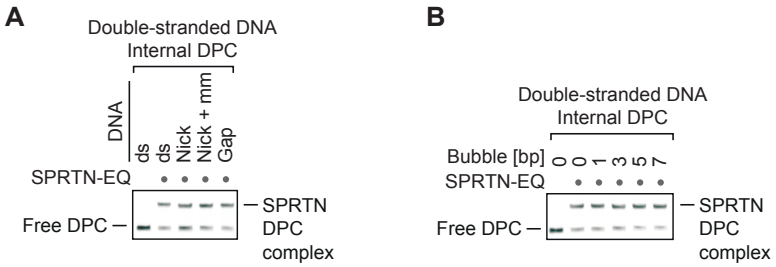

Figure S5. Cleavage by SPRTN requires disruptions within duplex DNA in close proximity to the DNA-protein crosslink, Related to Figure 5.

(A-B) SPRTN binds similarly to the model DPCs used in Figure 5A and 5D. Binding of catalytically inactive SPRTN E112Q (EQ, 25 nM) to the indicated model DPCs (25 nM) was assessed using EMSA assays.