Supplementary Information

Structural and biochemical characterization of an RNA/DNA binding motif in the N-terminal domain of RecQ4 helicases.

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Supplementary Table S1.

Oligonucleotides used in this study (DNA, upper case; RNA, lower case).

Name	Sequence 5'-3'
A1	GTAGTGCATGTACACCACACTCTTTTTTTTTTTTTTT
A2	TTTTTTTTTTTTTTTGAGTGTGGTGTACATGCACTAC
A3	guagugcauguacaccacacucuuuuuuuuuuuuuuu
A4	uuuuuuuuuuuuuuugagugugguguacaugcacuac

Supplementary Figure S1

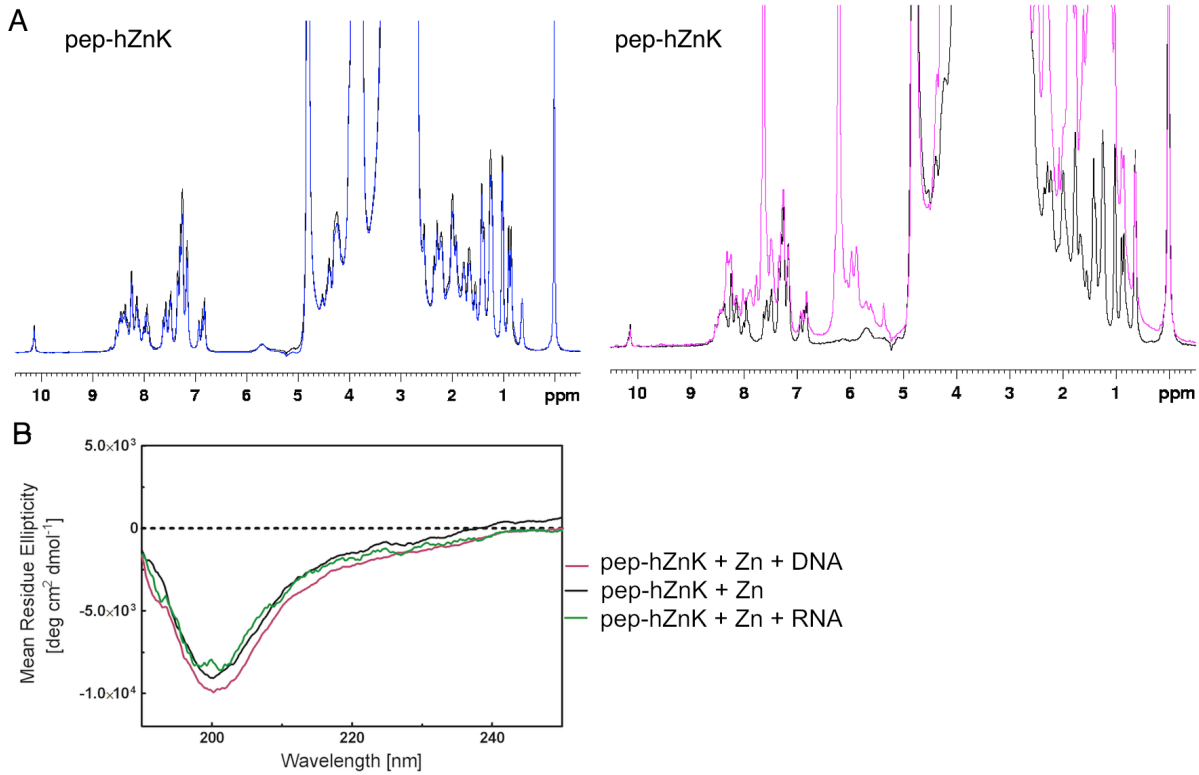

Figure S1. Additional spectroscopic studies of pep-hZnK

(**A**) Left: NMR 1D ^{1}H spectrum of pep-hZnK in HEPES buffer without (black) and with (blue) zinc. Right: NMR 1D ^{1}H spectrum of pep-hZnK in presence (pink) or absence (black) of Zn^{2+} and ssDNA. (**B**) CD spectra of pep-hZnK: no secondary structure appears after the addition of Zn^{2+} (black) or Zn^{2+} plus ssDNA (pink) or ssRNA (green).

Supplementary Figure S2

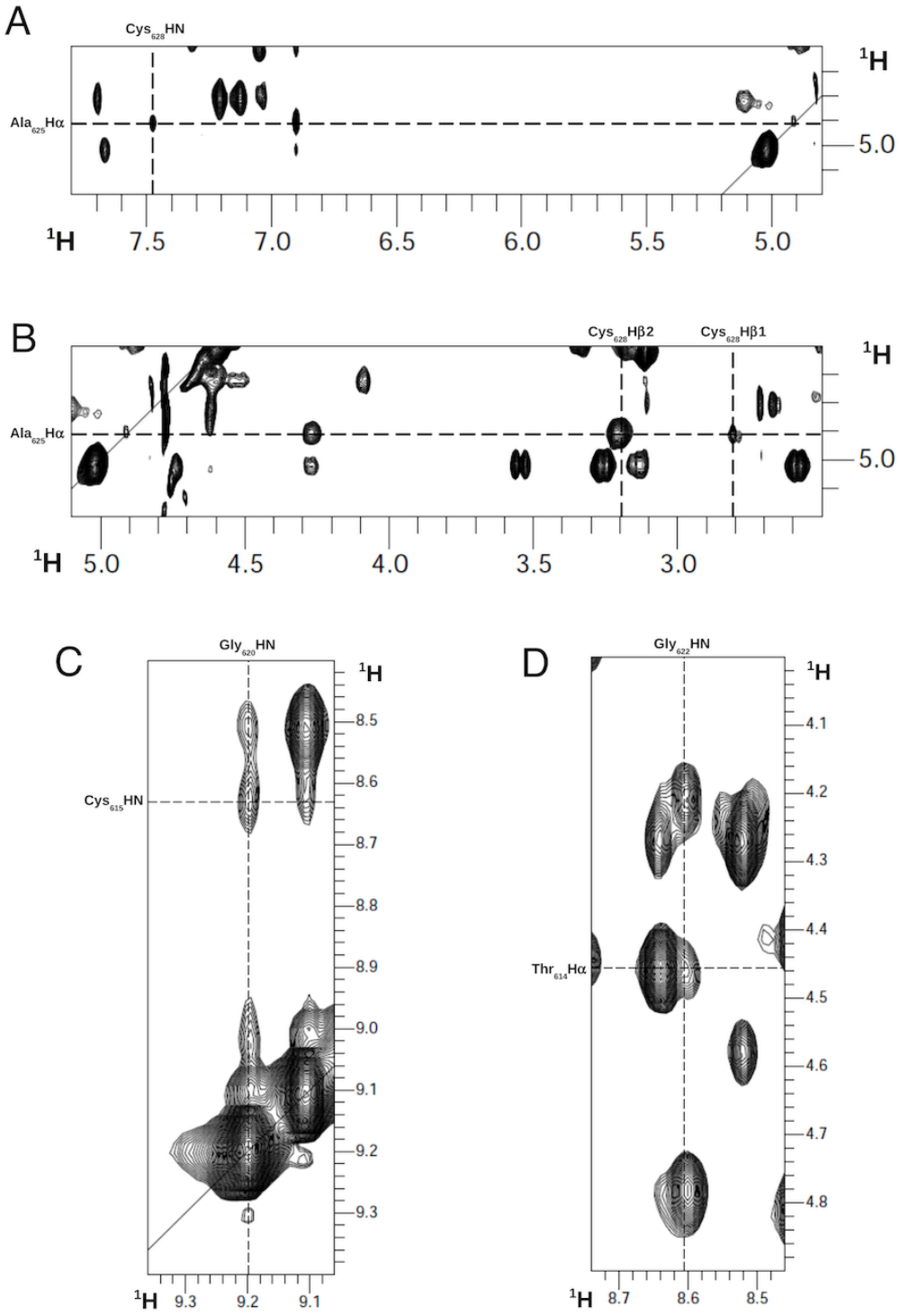

Figure S2. 1H-2D NOESY spectrum of pep-xZnK.

Selected regions of the 1H-2D NOESY spectrum showing the NOE cross-peaks between (**A**) Cys628HN and Ala625HA, (**B**) Ala625Hα/Cys628Hβ1/Hβ, (**C**) Gly620HN/Cys615HN, (**D**) Gly622HN/Thr614Hα

Supplementary Figure S3

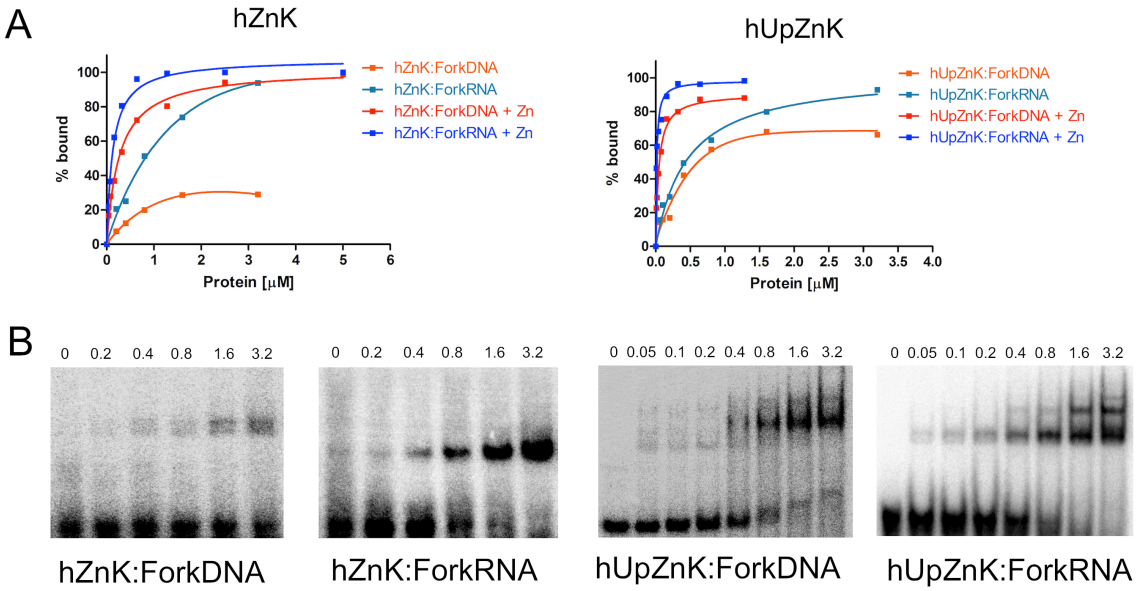

Figure S3. The effect of the presence of Zn^{2+} ions in the binding buffer on the interaction between the human RecQ4 fragments and forked substrates.

(**A**) The plots compare the binding of forked DNA and RNA substrates in the presence of 5mM $ZnCl_2$ (red and blue curves respectively), as reported in Figure 3, and the binding in the absence of Zn^{2+} ions in the binding buffer (orange and teal curves, respectively). (**B**) Example of gel shifts assays: the experiments were carried out with increasing concentrations of His-GST-tagged recombinant proteins (0-3.2 μM). Each experiment was repeated at least three times to plot the binding curves. Errors were very small: for the sake of clarity error bars are not shown on the plots. The proteins do bind nucleic acids also in the absence of Zn^{2+} ions, but the presence of Zn^{2+} in the buffer significantly improves binding to both DNA and RNA substrates, indicating that Zn^{2+} plays a relevant role in the interaction, and suggesting that the folding of the Zn knuckle is indeed important for the interaction.

Supplementary Figure S4

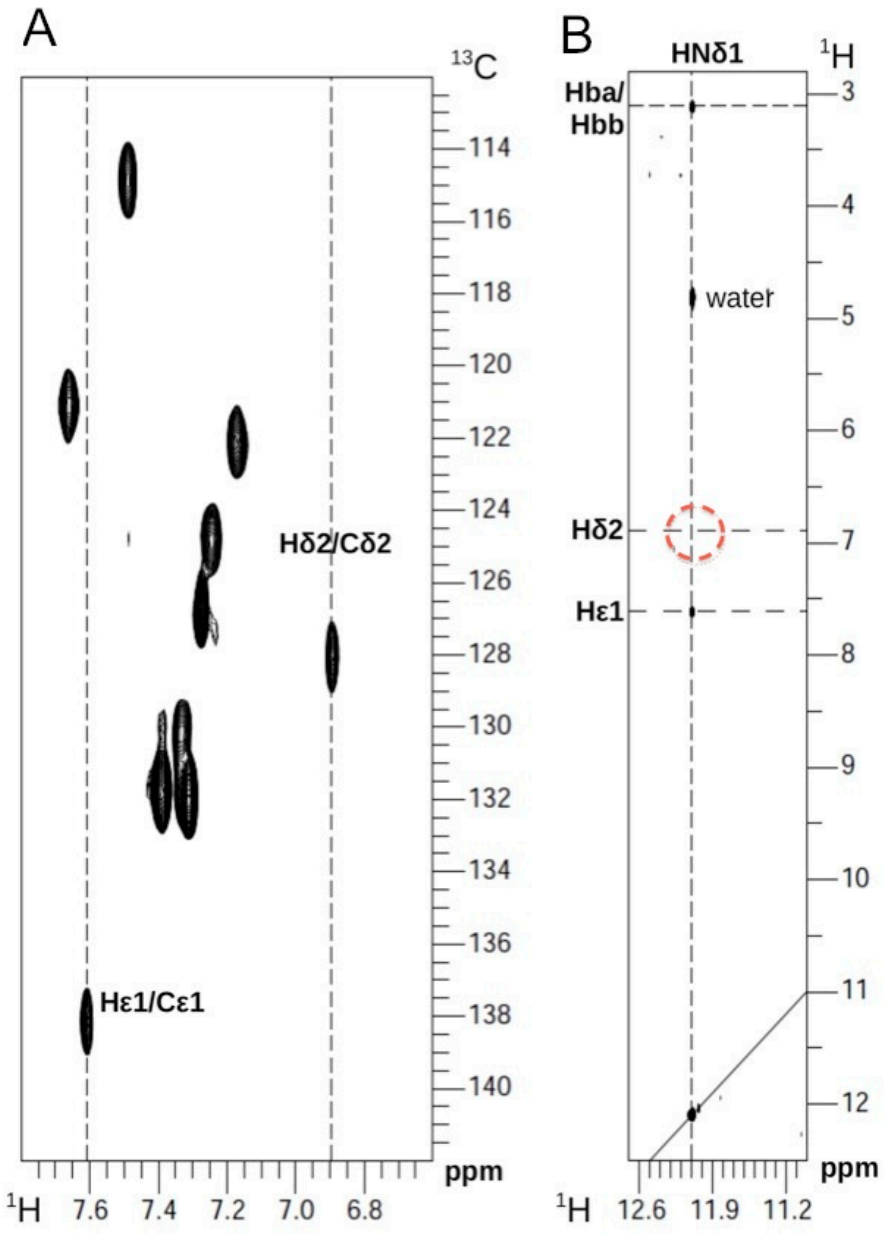

Figure S4.

(A) 13C-HSQC experiment (natural abundance) used to assign the aromatic resonances (1H and 13C) of His623. **(B)** Zoom on the NOESY spectrum (tmix=100ms) showing the NOEs between HNδ1 and i. its own beta-protons (Hba and Hbb are isochronous) and ii. Hε2. The presence of these NOEs and the absence of the NOE (red dotted circle) with Hδ2 support Zn2+ coordination via Nε2.