Supplementary Materials

Methods

Yeast two-hybrid analysis

The yeast two-hybrid assay protocol used to study the interaction between the Rev1-CT domain and PolD3 is described in detail in our previous characterization of the Rev1-CT - polη interaction.[S1] The Rev1-CT domain and various constructs of encoding PolD3 fragments (WT and FF238/239AA 147-end, 203-end, 147-300, and 219-255) were subcloned into the vectors pGAD-C1 (*GAL4* activation domain) and pGBD-C1 (*GAL4* binding domain) marked with leucine and tryptophan, respectively. Yeast strain PJ69-4A,[S2] containing *ADE2*, *HIS3*, and *lacZ* under control of the *GAL1*, *GAL2*, and *GAL7* promoters, respectively, was sequentially transformed with the two complementary plasmids as appropriate. Transformed yeast was grown at 30 °C in medium lacking leucine and tryptophan and then spotted on selective plates lacking leucine and tryptophan (-LW) and on plates also lacking histidine or lacking histidine and adenine (-HLW and -AHLW) to score the interaction. Interactions were assessed after 3 days of growth at 30 °C.

Figures

Figure S1. Yeast two-hybrid analysis of hRev1-CT – PolD3 interaction. Yeast strain PJ69-4A was transformed with plasmids encoding for fusions of (**A**) the Rev1-CT domain and PolD3 (WT & FF238/239AA) with the *GAL4* activation domain (AD) and *GAL4* binding domain (BD), respectively, and (**B**) the Rev1-CT domain and PolD3 with the *GAL4* BD and *GAL4* AD, respectively. Transformed yeast strains were selected on plates lacking leucine and tryptophan (-LW), and the Rev1-CT – PolD3 interaction was scored on plates lacking histidine, leucine, and tryptophan (-HLW), or lacking adenine, histidine, leucine, and tryptophan (-AHLW). (**C**) Control experiment showing that transformation of the hRev1-CT domain fused to the *GAL4* BD into a strain carrying the AD only (empty vector) is able to activate the less stringent *HIS3* gene (false positive) under control of the *GAL1* promoter as shown in (B).

Figure S2. ^{1}H-^{15}N HSQC spectrum of the Rev1-CT domain in the absence (blue) and presence (magenta) of the peptide including Spartan RIR motif (DKTVFDN**FF**IKKEQIK; residues 413-428).

Figure S3. (**A**) Selected strips from ^{13}C-edited, ^{13}C/^{15}N-filtered NOESY-HSQC spectrum of the ^{15}N/^{13}C labeled Rev1-CT domain in complex with the unlabeled PolD3-RIR peptide showing intermolecular NOE correlations between the domain and the peptide. Only those cross-peaks that can be unambiguously assigned to protein-peptide ^{1}H-^{1}H pairs were used for structure calculation of the complex; some strips displayed additional cross-peaks to water protons at 4.7 ppm (^{1}H) and/or low intensity filtering artifacts. (**B**) Mapping of NOE-derived intermolecular ^{1}H-^{1}H distance restraints (yellow lines) onto the structure of the Rev1-CT/PolD3-RIR complex.

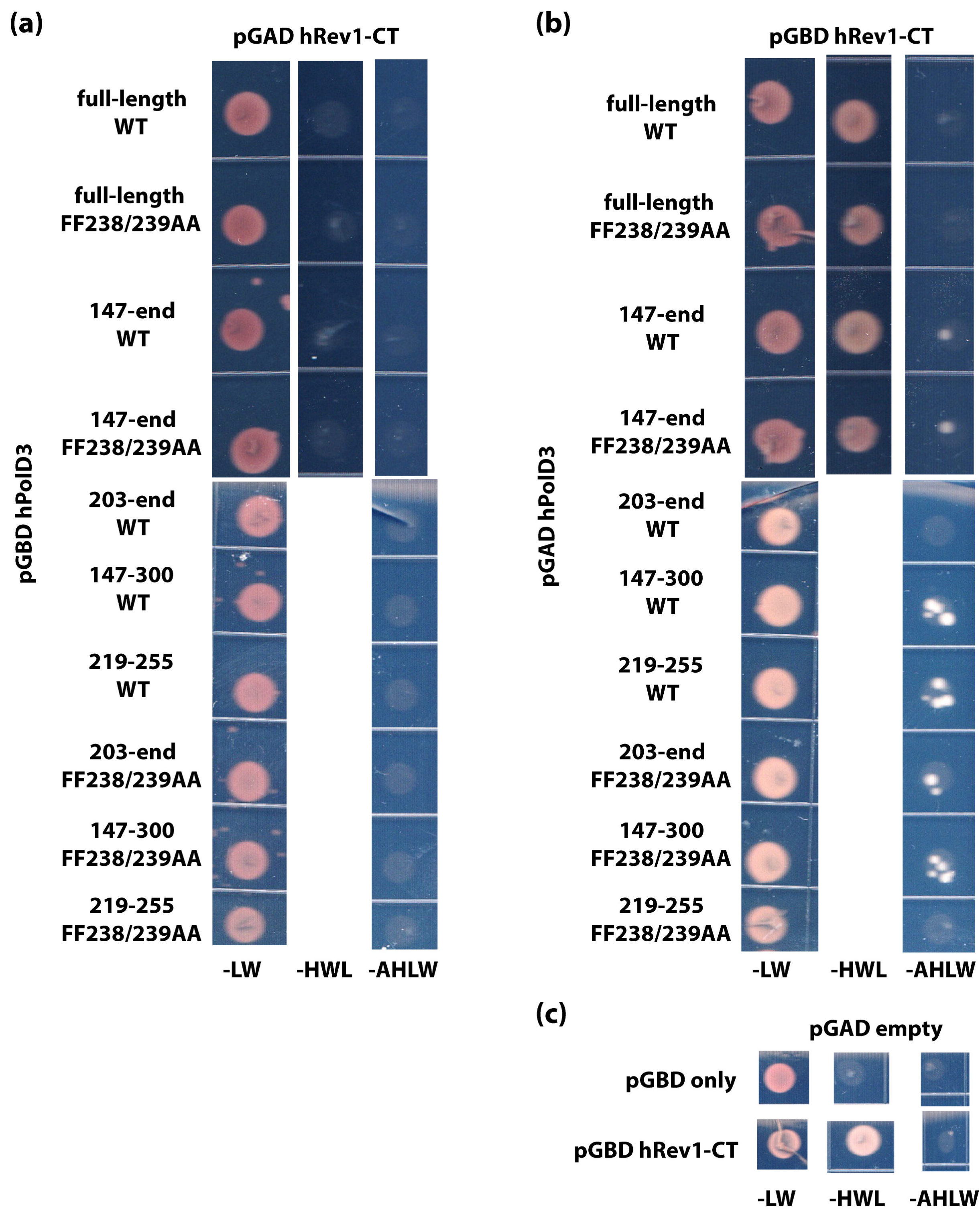

Figure S1

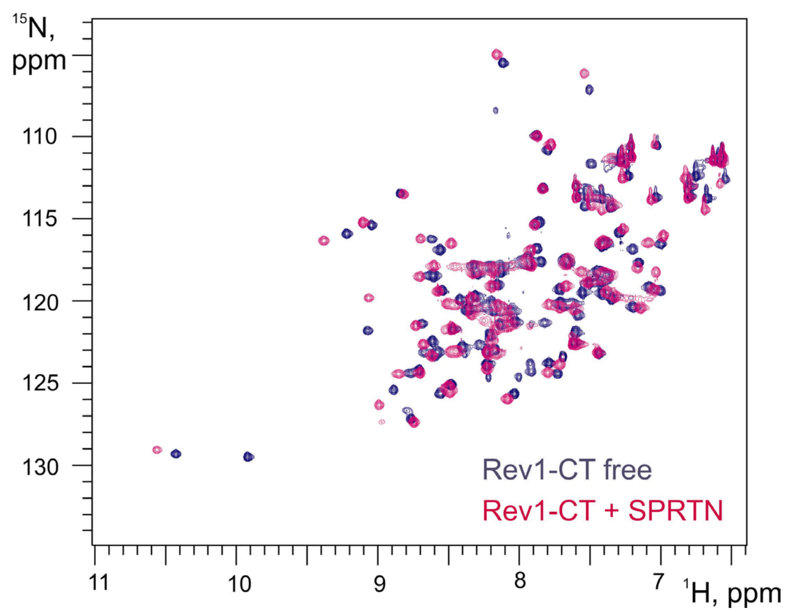

Figure S2

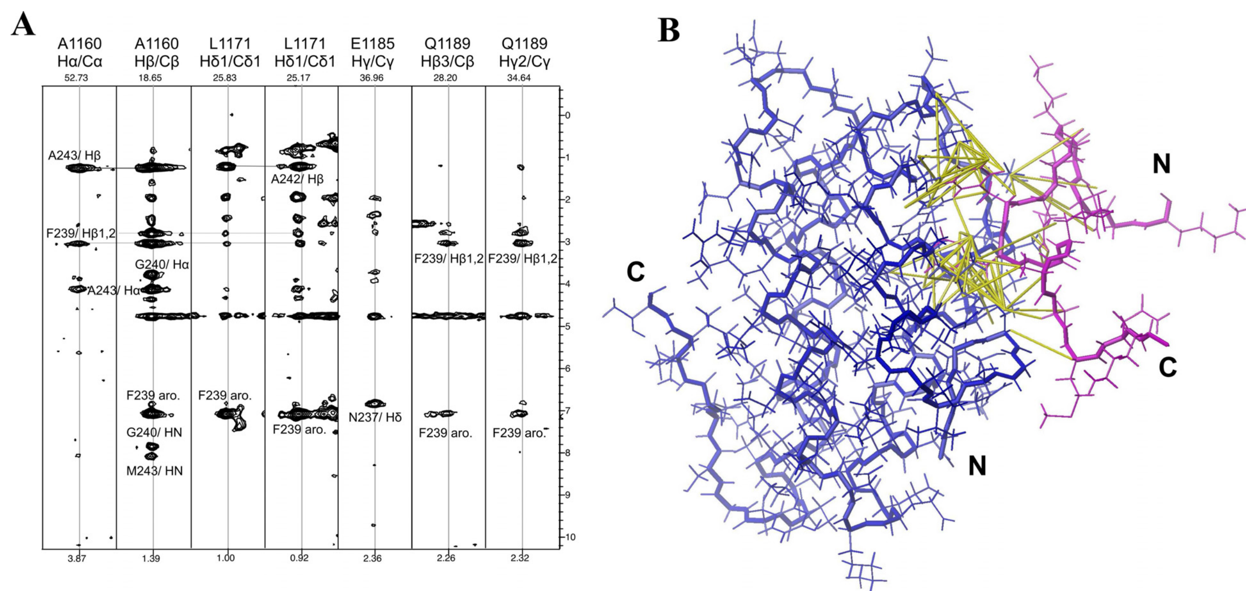

Figure S3

References

S1. Pozhidaeva, A., Pustovalova, Y., D'Souza, S., Bezsonova, I., Walker, G.C. and Korzhnev, D.M. (2012). NMR structure and dynamics of the C-terminal domain from human Rev1 and its complex with Rev1 interacting region of DNA polymerase eta. *Biochemistry* **51**, 5506-5520.

S2. James, P., Halladay, J., and Craig, E.A. (1996) Genomic libraries and a host strain designed for highly efficient two-hybrid selection in yeast. *Genetics* **144**, 1425−1436.