

SUPPLEMENTARY MATERIALS

Identification of the initial nucleocapsid recognition element in the HIV-1 RNA packaging signal

Pengfei Ding^{a,b}, Siarhei Kharytonchyk^c, Alexis Waller^a, Ugonna Mbaekwe^a, Sapna Basappa^a, Nansen Kuo^a, Heather M. Frank^a, Christina Quasney^a, Aaron Kidane^a, Canessa Swanson^a, Verna Van^a, Mitali Sarkar^a, Emily Cannistraci^a, Ridhi Chaudhary^a, Hana Flores^a, Alice Telesnitsky^{c,1}, Michael F. Summers^{a,b,1}

^aDepartment of Chemistry and Biochemistry, University of Maryland Baltimore County, Baltimore, MD 21250;

^bHoward Hughes Medical Institute, University of Maryland Baltimore County, Baltimore, MD 21250;

^cDepartment of Microbiology and Immunology, University of Michigan Medical School, Ann Arbor, MI 48109

¹To whom correspondence may be addressed. Email: summers@hhmi.umbc.edu or ateles@umich.edu

CONTENTS:

CONTAINS SIX FIGURES AND FIGURE CAPTIONS

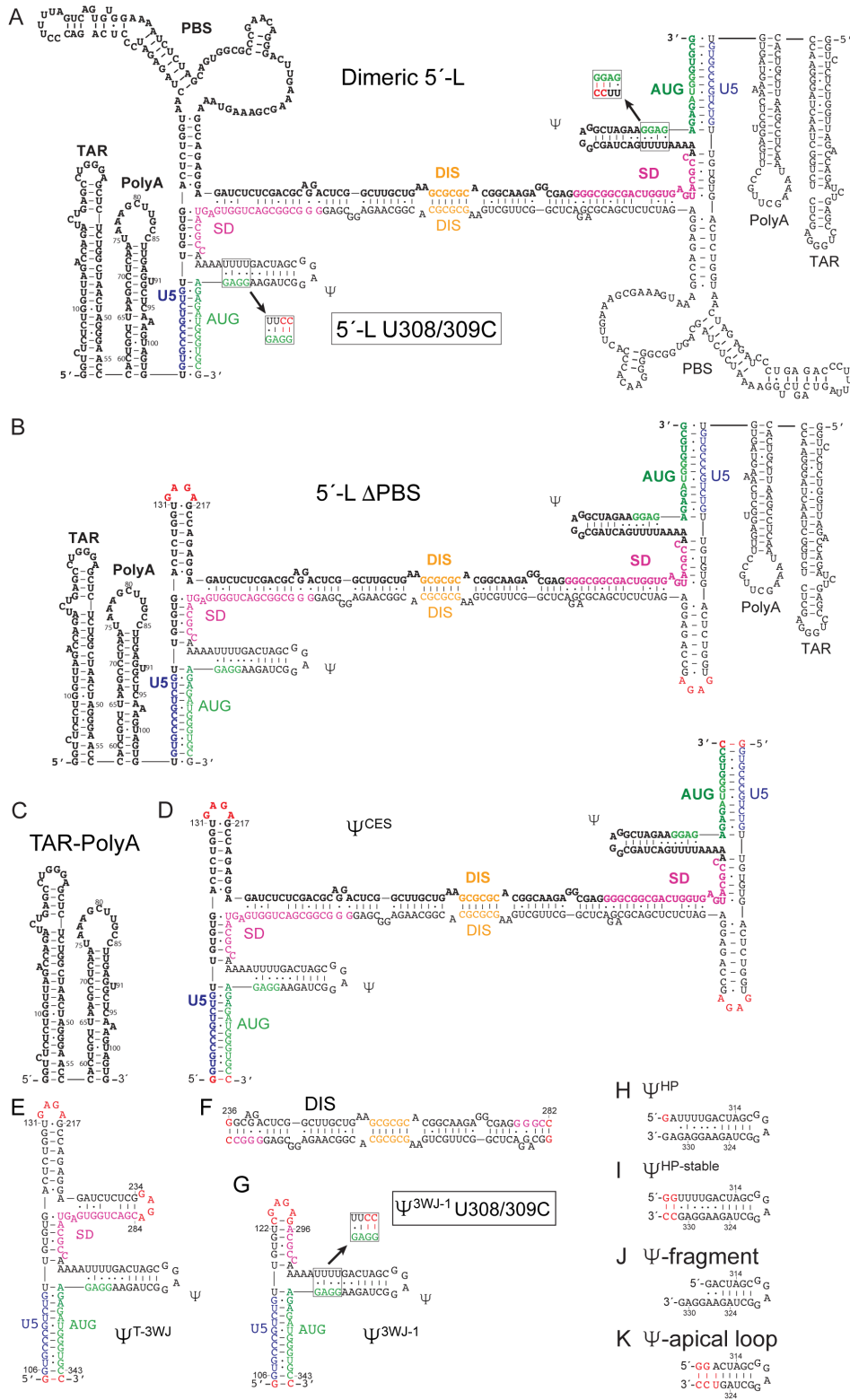


Fig. S1. Summary of all RNA constructs used in this study. Non-native residues are shown in red.

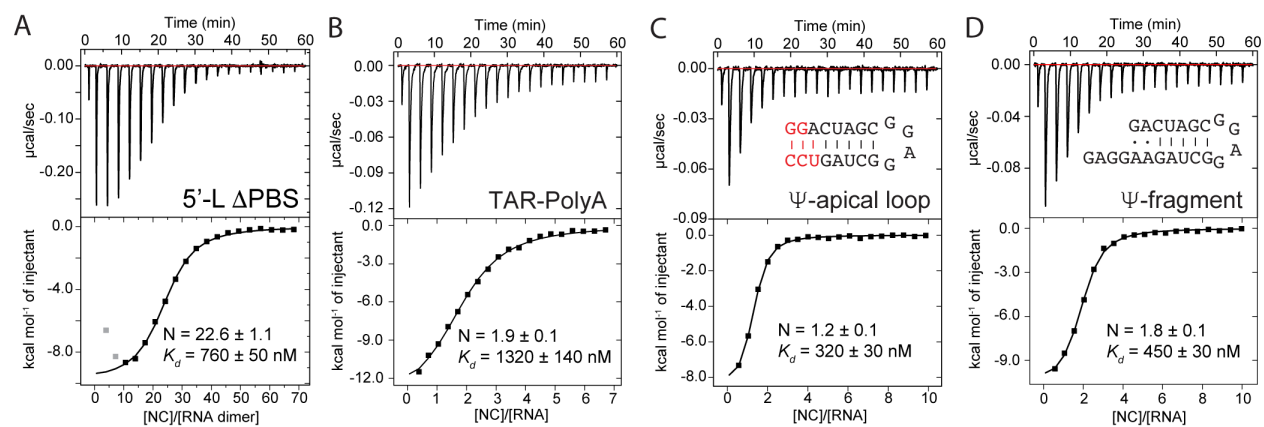


Fig. S2. ITC data of HIV-1 5'-L constructs. (A) 5'-L with the PBS region deleted. The gray data points due to endothermic binding were not included in data fitting. (B) TAR and PolyA hairpins; (C) the upper stem region and the apical loop from the Ψ-stem loop; (D) A construct with Ψ-apical loop and a single stranded “GGAG” region.

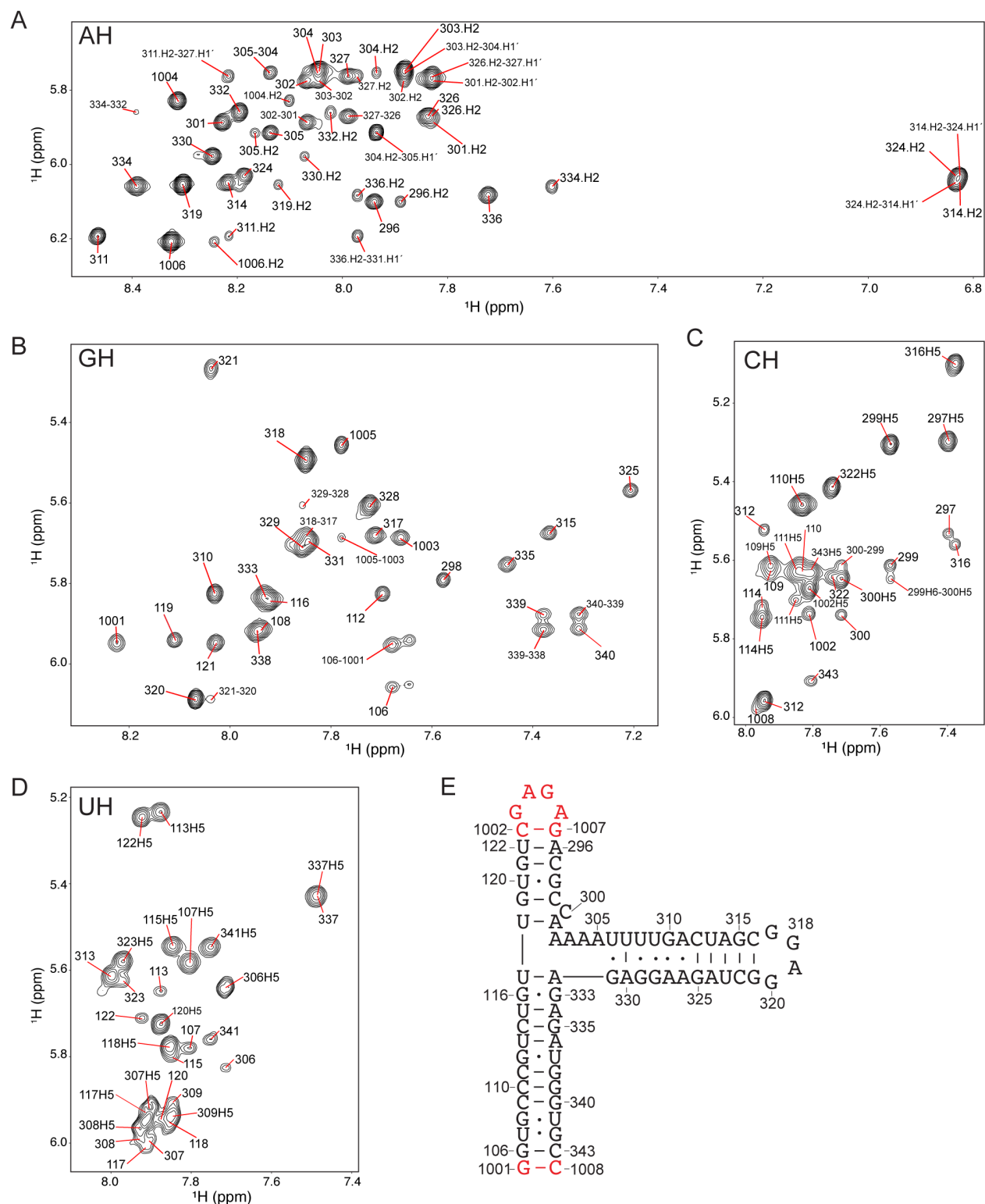


Fig. S3. Chemical shift assignments for Ψ^{3WJ-1} . For A^H - (A) and G^H - (B) spectra, intra-residue H8-H1' cross-peaks are denoted by residue number; inter-residue H8-H1' are indicated by (residue number of H8)-(residue number of H1'); Intra-residue H2-H1' are

shown by (residue number).H2; Inter-residue H2-H1' are labeled by (residue number of H2).H2-(residue number of H1').H1'. For C^H- (C) and U^H- (D) spectra, intra-residue H6-H1' cross-peaks are denoted by residue number; inter-residue H6-H1' are indicated by (residue number of H6)-(residue number of H1'); Intra-residue H6-H5 cross-peaks are labeled by (residue number).H5; Inter-residue H6-H5 cross-peaks are indicated by (residue number of H6).H6-(residue number of H5).H5. (E) Secondary structure of Ψ^{3WJ-1} . Non-native residues are shown in red and numbered 1001-1008.

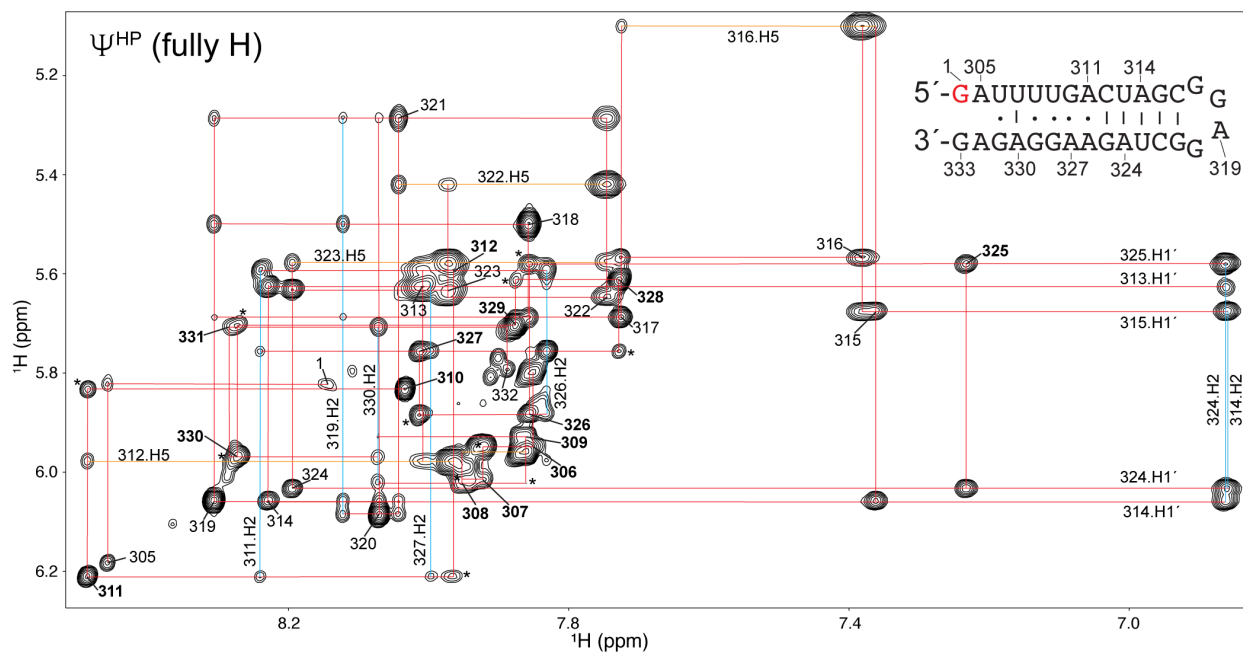


Fig. S4. Chemical shift assignments for Ψ^{HP} . Intra-residue H8/H6-H1' cross-peaks are denoted by residue number. Adenosine H2 signals are indicated by blue lines; Pyrimidine H5 signals are labeled by yellow lines. Sequential H8/H6-H1' cross-peaks for the [UUUUGAC]:[GAAGGAG] region are marked by stars.

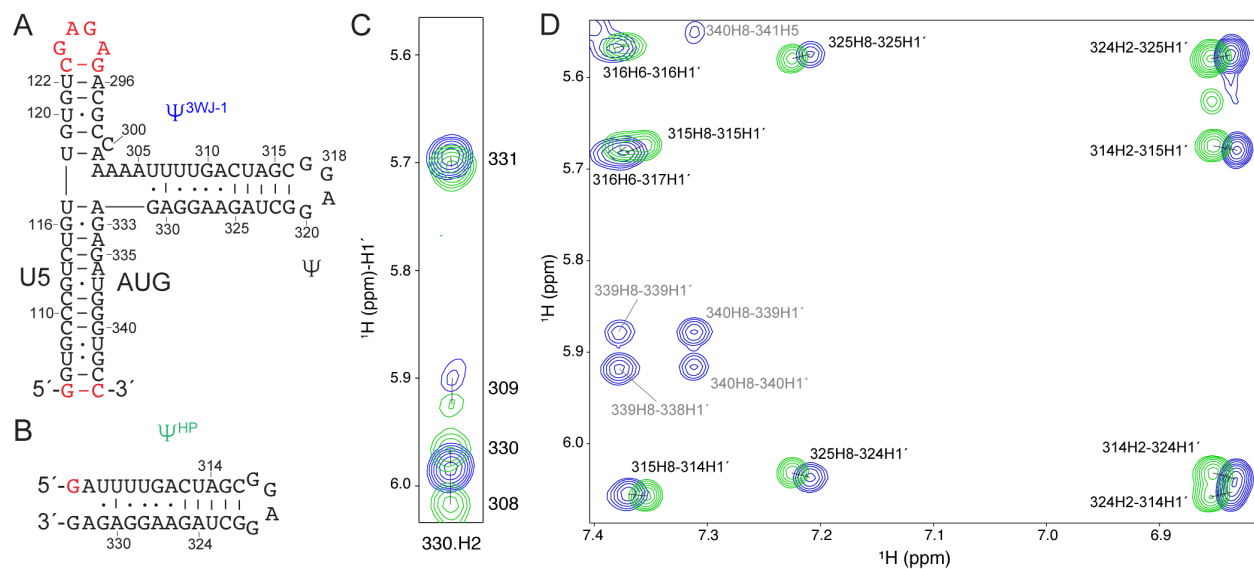


Fig. S5. Ψ^{HP} is structurally similar to the same region in Ψ^{3WJ-1} . Secondary structure of Ψ^{3WJ-1} (A) and Ψ^{HP} (B). Non-native residues are shown in red. (C) 2D NOESY spectra overly of fully protonated Ψ^{HP} (green) and $A^{2r}G^rU^r$ -labeled Ψ^{3WJ-1} (blue). (D) 2D NOESY spectra overly of fully protonated Ψ^{HP} (green) and fully protonated Ψ^{3WJ-1} (blue).

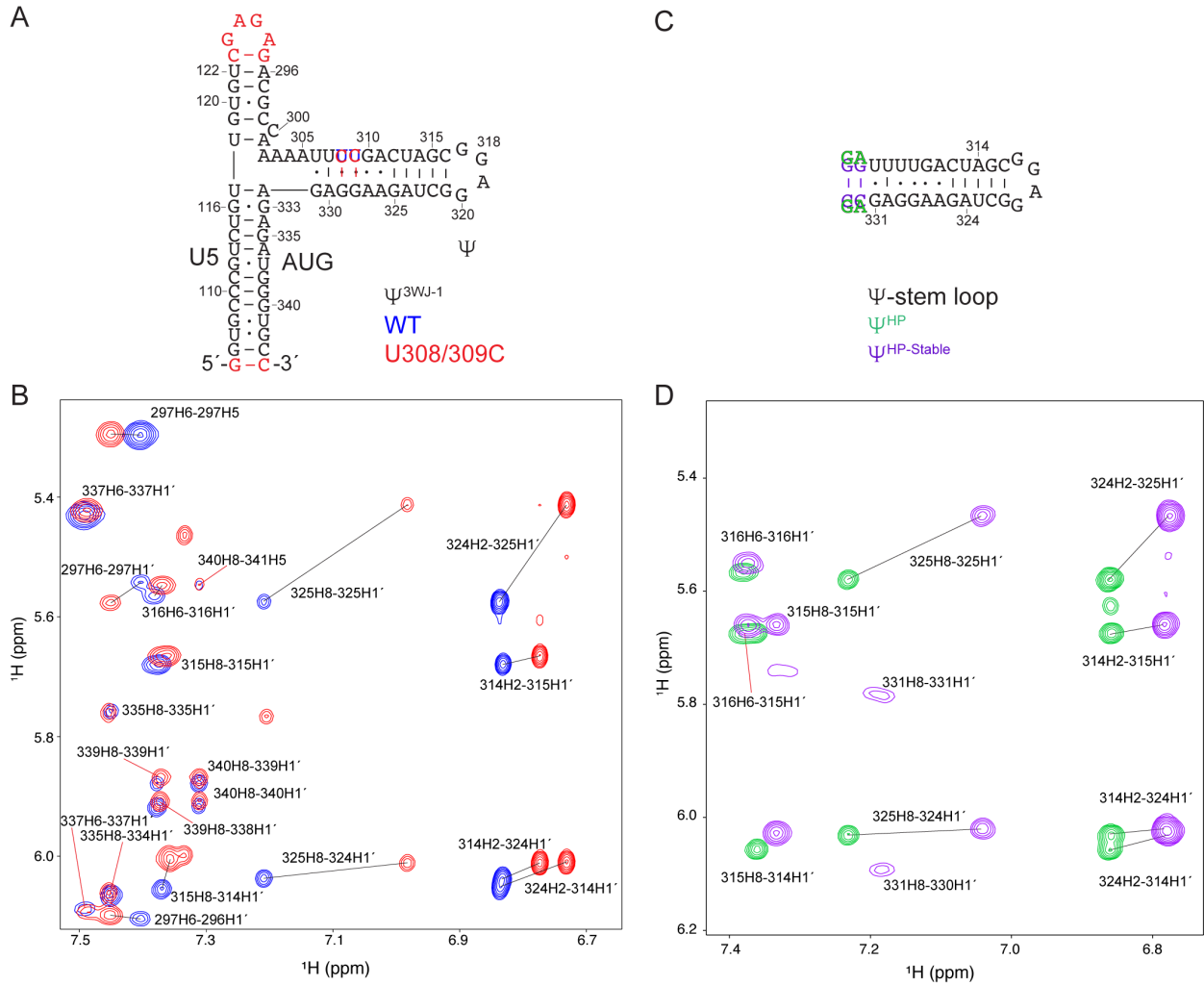


Fig. S6. Structural effects of stabilizing the [UUUU]:[GGAG] region. (A) Secondary structure of wild-type and G:U to G-C mutant of Ψ^{3WJ-1} . Non-native residues are shown in red. (B) 2D NOESY spectra overlay of fully protonated wild-type (blue) and G:U to G-C mutant (red) Ψ^{3WJ-1} . (C) Secondary structure of Ψ^{HP} and $\Psi^{HP-stable}$. (D) 2D NOESY spectra overlay of fully protonated Ψ^{HP} (green) and $\Psi^{HP-stable}$ (purple).