



STRUCTURAL
BIOLOGY

Volume 79 (2023)

Supporting information for article:

Crystal structures of the DExH-box RNA helicase DHX9

Young-Tae Lee, E. Allen Sickmier, Simina Grigoriu, Jennifer Castro and P. Ann Boriack-Sjodin

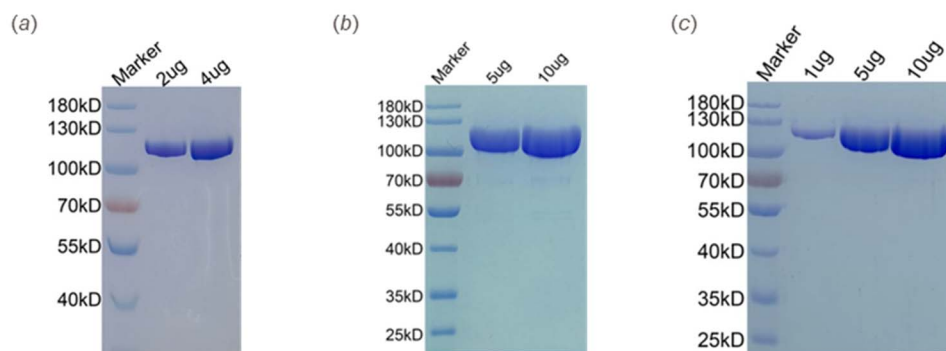
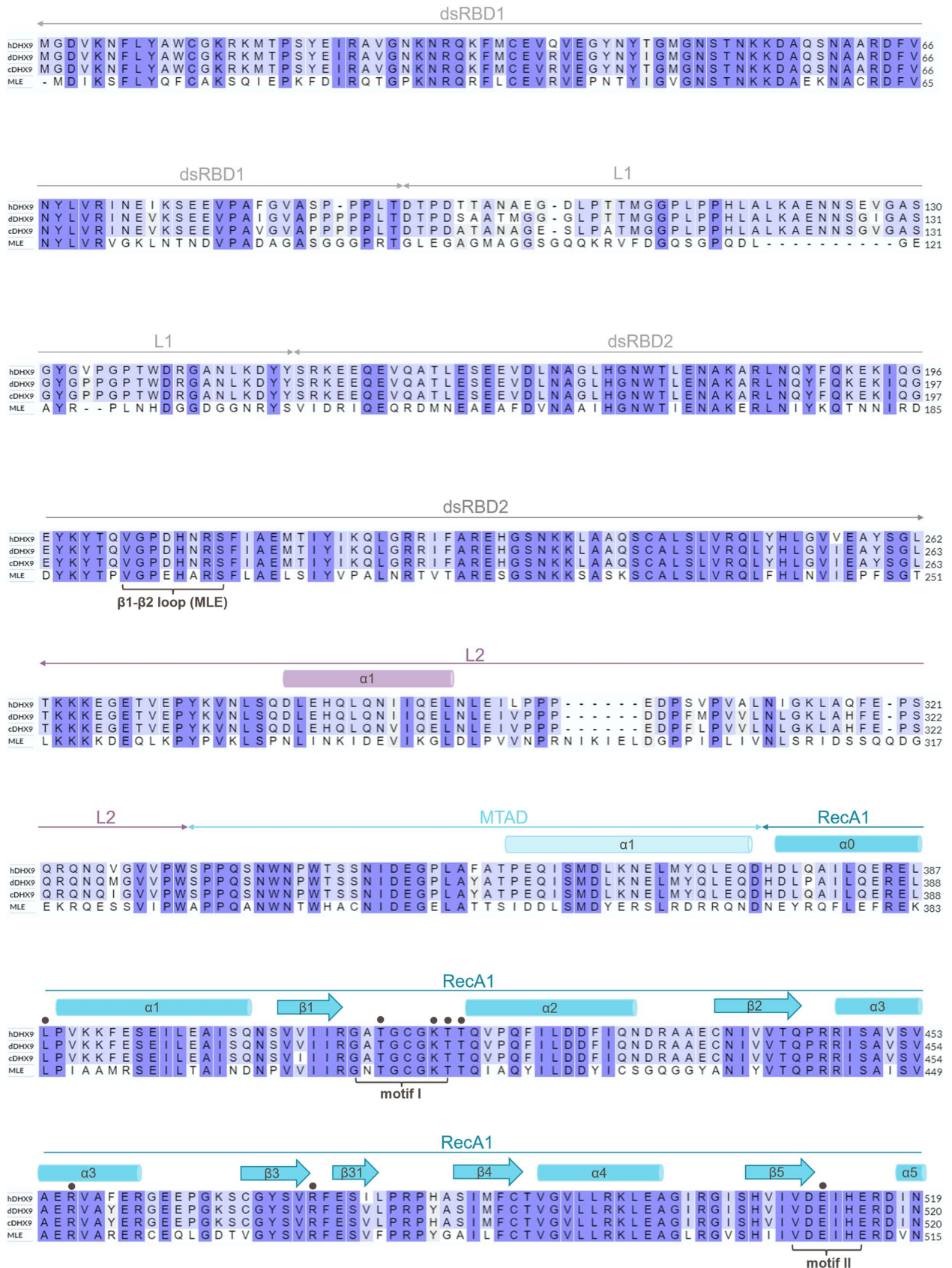
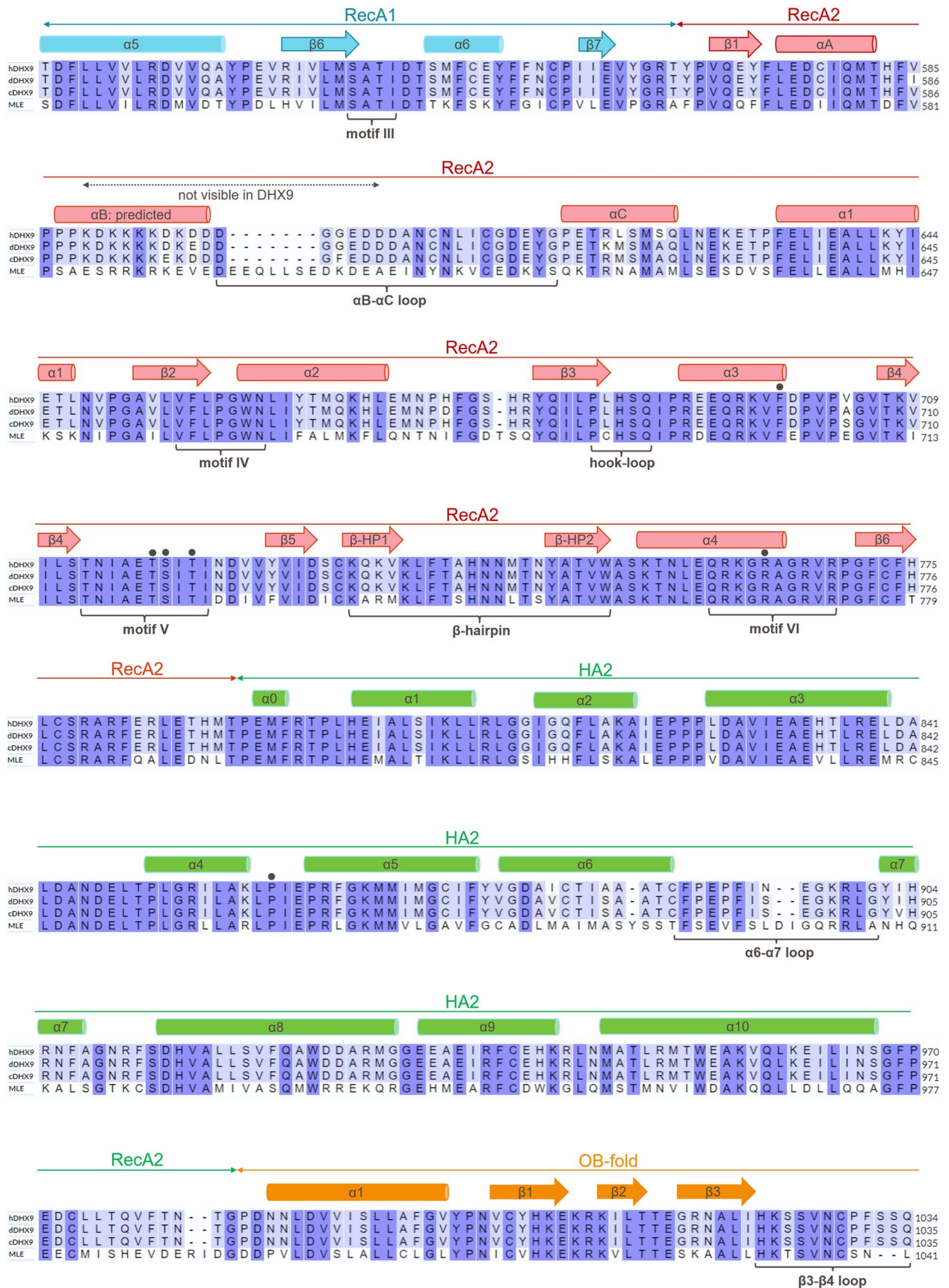


Figure S1 SDS-PAGE of purified mammalian DHX9 proteins used for crystallization. (a) Human DHX9 (residues 150-1150, 114.2 kDa), (b) Dog DHX9 (residues 151-1151, 114.4 kDa), (c) cat DHX9 (residues 151-1151, 114.5 kDa).





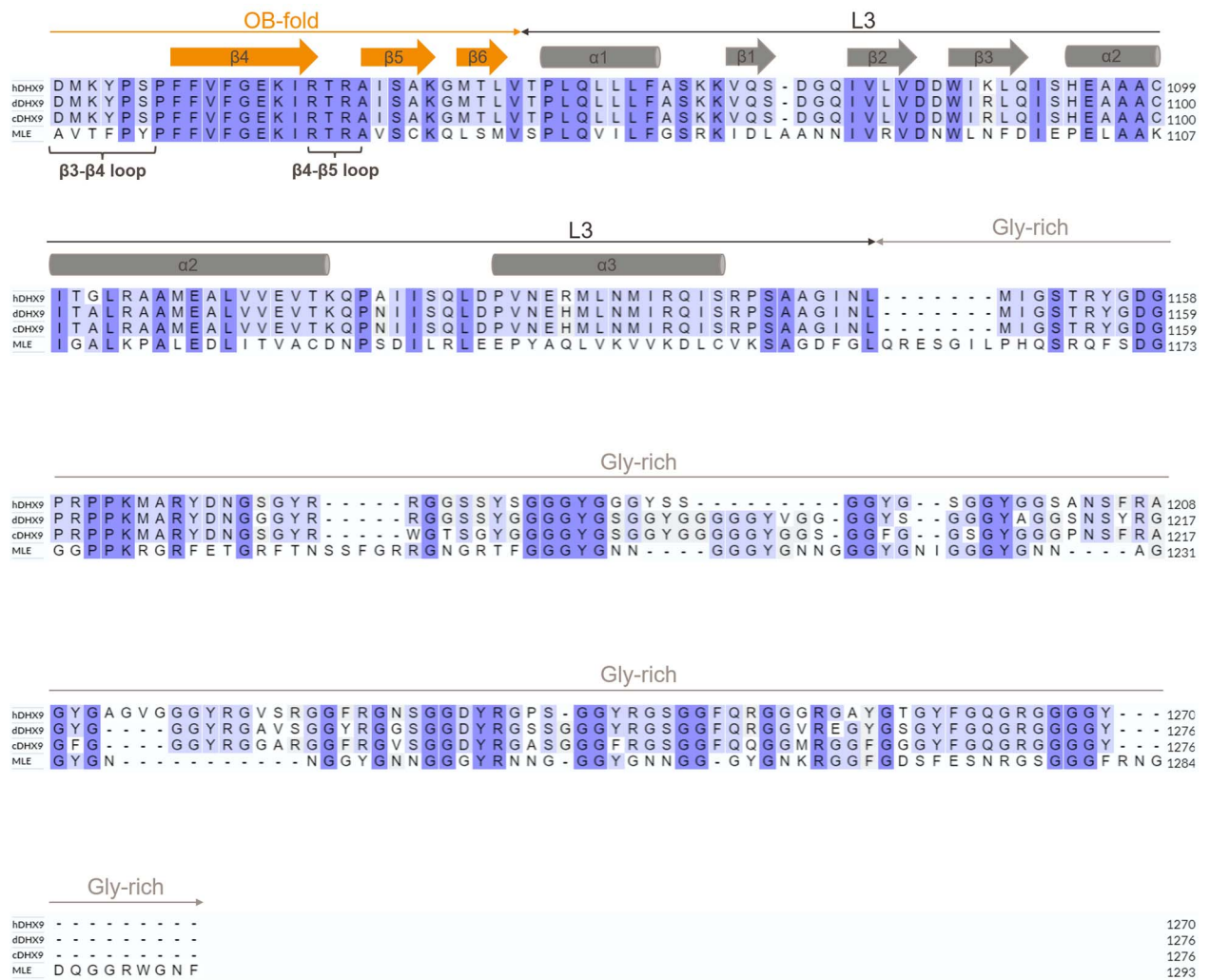


Figure S2 Sequence alignment of DHX9 sequences from human (hDHX9), dog (dDHX9), cat (cDHX9) and *Drosophila* MLE. Sequence conservation levels are indicated by color: white = low conservation, light blue = moderate conservation, blue = sequence identity. Secondary structure elements of human DHX9 (cylinder, α -helix; arrow, β -strand) are shown and mirror the domain color scheme from Figure 1. Structural features and motifs discussed in the text are indicated with brackets; Residues highlighted in the text are denoted with a black mark.

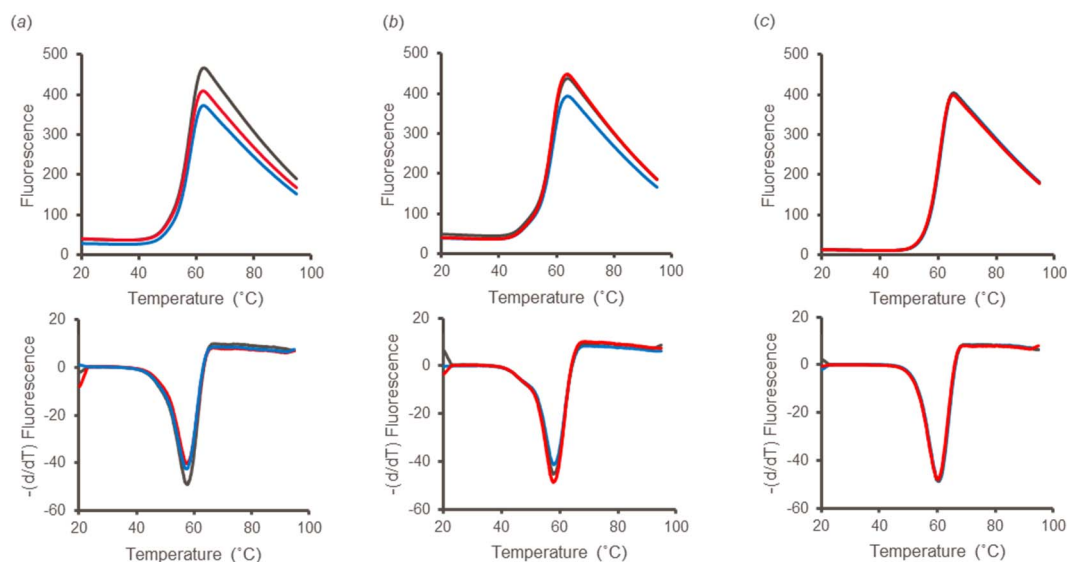


Figure S3 Melting temperature measurements of mammalian DHX9 proteins used for crystallization. Melting curves and the first derivatives plots are shown in the top and the bottom panels, respectively. (a) Human DHX9 (residues 150-1150); melting temperature = 57.4 °C. (b) Dog DHX9 (residues 151-1151); melting temperature = 58.7 °C. (c) Cat DHX9 (residues 151-1151); melting temperature = 60.6 °C.

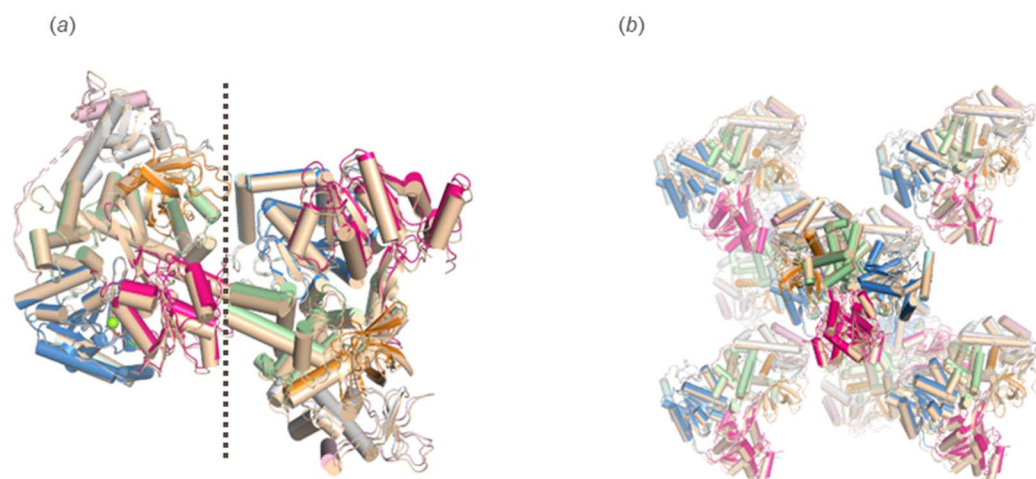


Figure S4 Packing interactions are similar between human DHX9 (space group $C222_1$) and cat DHX9 (space group $P4_32_12$). (a) The human DHX9 asymmetric unit (DHX9 domains colored as in Fig. 1, chain A on the left, chain B on the right) is recapitulated by cat DHX9 (wheat, left) by including one symmetry molecule (wheat, right). (b) Symmetry molecules for human and cat DHX9 overlap well in the extended unit cell. The same color schemes are used as in (a).

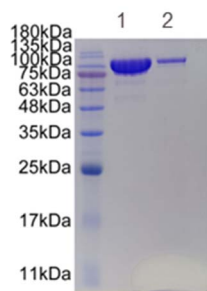


Figure S5 SDS-PAGE of human DHX9 (114.2 kDa) shows intact protein before and after crystallization. Lane 1, protein sample used for human DHX9 crystallization. Lane 2, dissolved crystals of human DHX9.

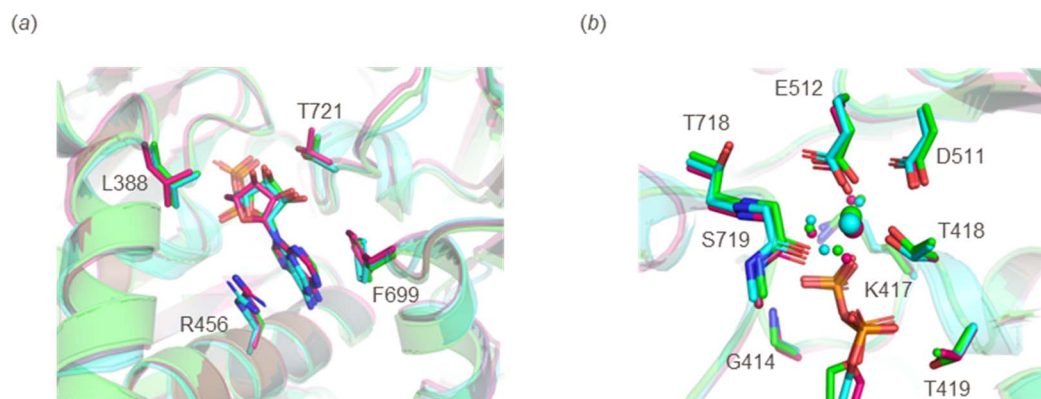


Figure S6 The nucleotide binding site is conserved across human (green), dog (cyan) and cat (magenta) DHX9. (a) and (b) correspond to the perspectives used in Figures 3d and 3e, respectively. Human residue numbers are shown.

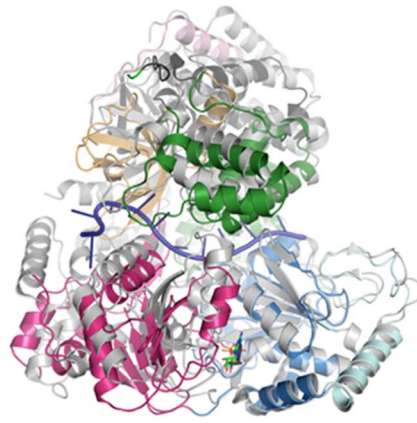


Figure S7 Superposition of DHX9 and MLE relative to the RecA1 domain. The color scheme is same as in Fig. 4a.