Generation of MHF and MID-MHF complexes for crystallization



**Figure S1. (A)** Domain architecture of FANCM (top) showing the N-terminal helicase domain, followed by the MHF-Interaction Domain (MID) and the C-terminal ERCC4 domain. MHF (below) is composed of MHF1 and MHF2 with their histone-fold domains shown as red helices (labeled  $\alpha$ 1-  $\alpha$ 4 for MHF1 and  $\alpha$ 1-  $\alpha$ 3 for MHF2). **(B)** Coomassie blue-stained SDS-PAGE gels showing the purified MHF and MID-MHF complexes from *E.coli*. **(C)** Coomassie blue-stained SDS-PAGE gels showing the trypsinized MHF and MID-MHF complexes used for generating the crystal structures. Since the purified MHF and MID-MHF complexes with full length MHF1 could not be crystalized, we used trypsin-limited proteolysis to generate crystallizable constructs, which include the full-length MHF2, C-terminal truncated MHF1 (1-110), and MID (669-800).

(D) Crystal structure of the MHF complex with orthogonal orientations (MHF1 - light gray and MHF2 – salmon as indicated). The individual MHF1/MHF2 heterodimers are labeled [AB] or [CD] to denote the chains relating to the deposited crystal structure (PDB ID: 4e44), and the secondary structure is labeled accordingly (MHF1, black,  $\alpha$ 1-  $\alpha$ 4; MHF2, red,  $\alpha$ 1-  $\alpha$ 3).