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A SAM key domain required for activation of catalytic activity of the Fun30 nucleosome remodeler

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Fun30 is the prototype of the Fun30-SMARCAD1-ETL sub-family of nucleosome remodelers involved in DNA repair and gene silencing. These appear to act as single subunit nucleosome remodelers, but their molecular mechanisms are at this point poorly understood. Using multiple sequence alignment and structure prediction we identify an evolutionary conserved domain that is modeled to contain a SAM-like fold with one long, protruding helix, which we term SAM key. Deletion of the SAM key leads to a defect in DNA repair and gene silencing similar to that of the *fun30Δ* mutant. *In vitro,* Fun30 protein lacking the SAM key is able to bind nucleosomes, but is deficient in DNA-stimulated ATPase activity as well as nucleosome sliding and eviction. A structural model based on prediction by AlphaFold2 and verified by crosslinking-MS indicates an interaction of the long SAM key helix with protrusion I, which is located between the two ATPase lobes. Mutation of the interaction interface phenocopies the domain deletion with a lack of DNA-stimulated ATPase activation and a nucleosome remodeling defect, thereby confirming a role of the SAM key helix in regulating ATPase activity. These data suggest a model by which the SAM key is involved in allosteric activation of Fun30 and thereby acts similar to unrelated elements in other nucleosome remodelers highlighting the importance of allosteric activation for this class of enzymes.