**SUPPLEMENTARY TABLE 1A**
**Oligonucletides used for DNA binding substrates**

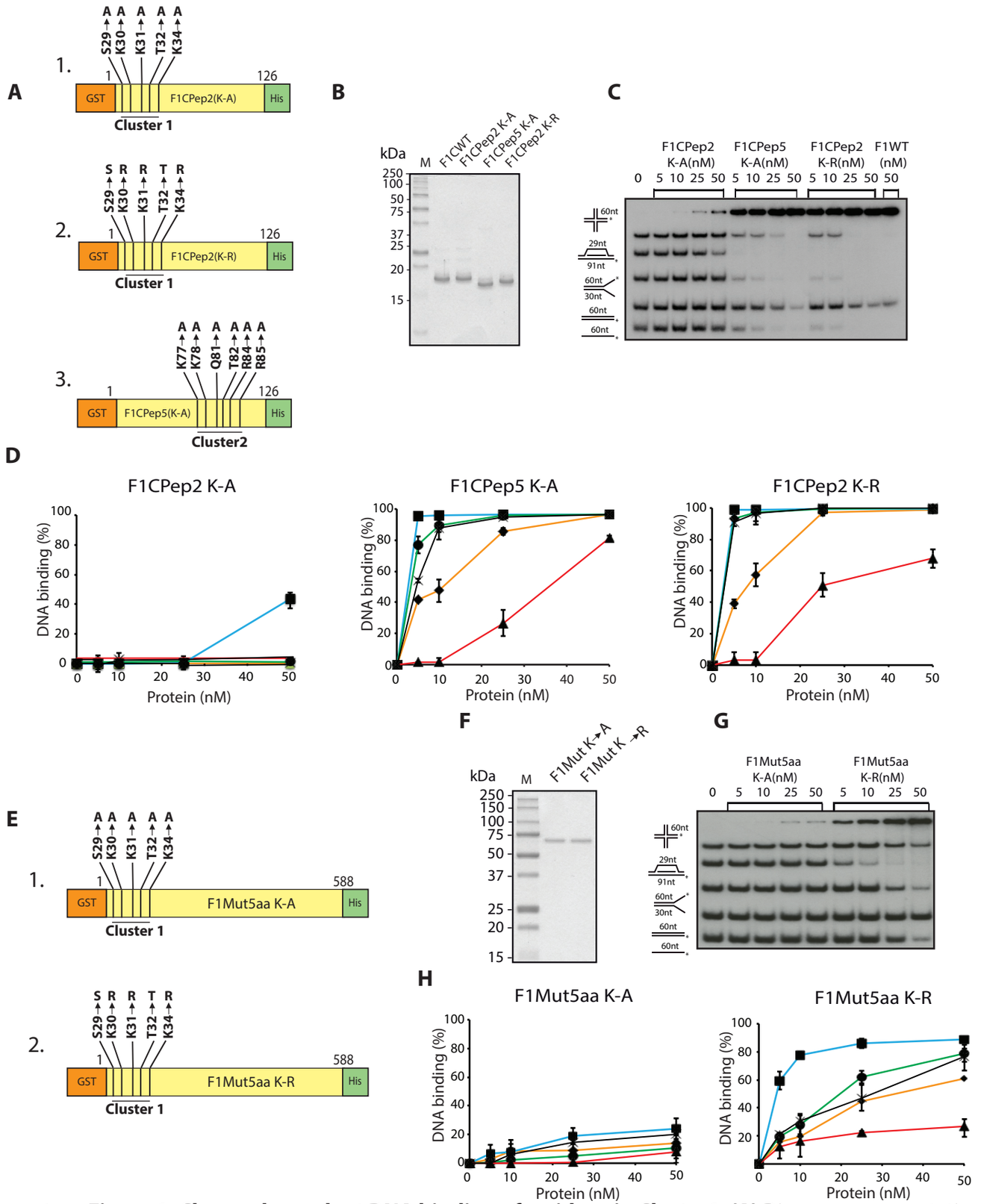
| Oligonucleotide name | DNA sequences (5′ to 3′) |
|---|---|
| **JYM925** | GGGTGAACCT GCAGGTGGGC AAAGATGTCC TAGCAATGTA ATCGTCAAGC TTTATGCCGT |
| **JYM926** | ACGCTGCCGA ATTCTACCAG TGCCAGCGAC GGACATCTTT GCCCACCTGC AGGTTCACCC |
| **JYM927** | ACGGCATAAA GCTTGACGATT ACATTGCTAC ATGGAGCTGT CTAGAGGATC CGACTATCG |
| **JYM928** | CGATAGTCGG ATCCTCTAGA CAGCTCCATGG TCGCTGGCAC TGGTAGAATT CGGCAGCGT |
| **JYM945** | ACGGCATAAA GCTTGACGAT TACATTGCTA GGACATCTTT GCCCACCTGC AGGTTCACCC |
| **JYM1395** | GCCAGGGACG GGGTGAACCT GCAGGTGGGC GGCTGCTCAT CGTAGGTTAG TATCGACCT ATTGGTAGAAT TCGGCAGCGTC ATGCGACGGC |
| **JYM1396** | GCCGTCGCAT GACGCTGCCG AATTCTACCA CGCTACTAGG GTGCCTTGCT AGGACATCTT TGCCCACCTG CAGGTTCACC CCGTCCCTGGC |

**SUPPLEMENTARY TABLE 1B**
**DNA binding substrates**

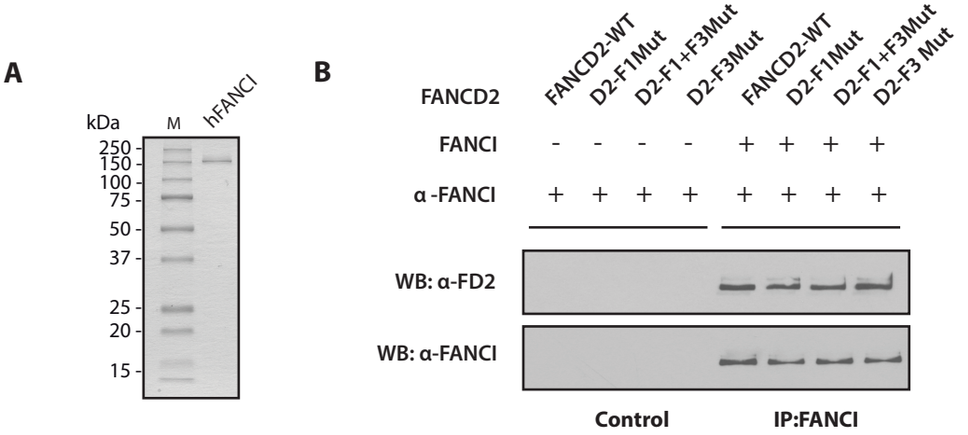
| Structures | Oligonucletide used |
|---|---|
| SS DNA | JYM 925 |
| DS DNA | JYM 925, JYM 945 |
| SA | JYM 925, JYM 926 |
| D-loop | JYM 1745, JYM 1395 and JYM 1396 |
| HJ | JYM 925, JYM 926, JYM 927 and JYM 928 |

**SUPPLEMENTARY TABLE 2**
**Summary of DNA binding screen**

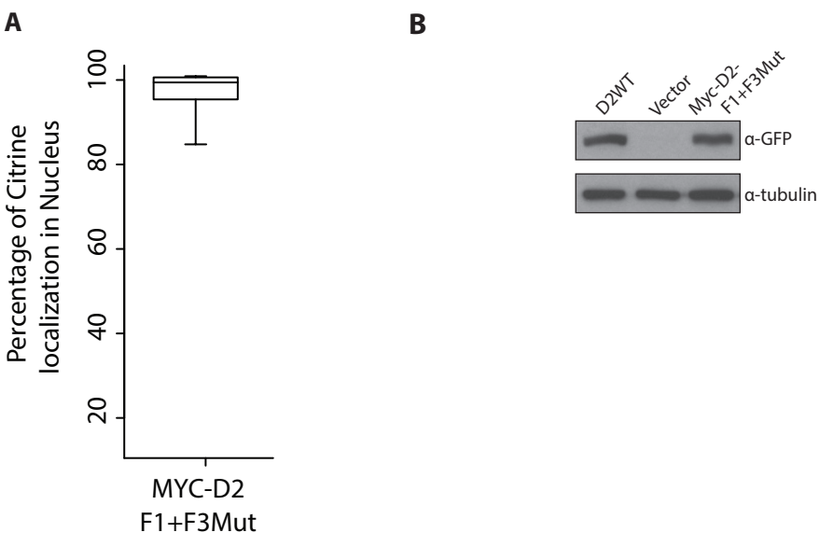
| Peptide | Number of Substituent Mutations | Residues Substituted | Effect on DNA binding |
|---|---|---|---|
| F1-C (aa1 to 126) | - | - | ++ |
| F1-E (aa 1 to 49) | - | - | ++ |
| F1-F (aa 50 to 126) | - | - | + |
| F1-E peptide 2 mutated | 8 aa | R24A ; K25A ; Q26A ; S29A ; K30A ; K31A ; T32A ; K34A | - |
| F1-C Mut Pep2 8aa | 8 aa | R24A ; K25A ; Q26A ; S29A ; K30A ; K31A ; T32A ; K34A | - |
| F1-C : Peptide 5 | 6 aa | K77A; K78A ; Q81A ; T82A ; R84A ; R85A | + |
| F1-C Mut Pep2+Pep5 | 14 aa | R24A; K25A; Q26A; S29A; K30A; K31A; T32A; K34A K77A; K78A; Q81A; T82A; R84A ; R85A | - |
| F1 peptide 2 mutated | 4 aa | S29A; K30A; K31A; T32A | + |
| F3-A (aa 841 to 1004) | - | - | ++ |
| F3-B (aa 1005 to 1178) | - | - | + |
| F3: Pep2+Pep10 mutated | 11 aa | K861A; R863A; K864A; K865A; K867A, K992A; N993A; K994A; S996A; R997A; N998A | - |
| F3 : peptide 2 | 5 aa | K861A; R863A; K864A; K865A; K867A | - |
| F3 : peptide 2 | 3 aa | R863A; K864A; K865A | - |
| F3 : peptide 2 | 1 aa | K865A | - |
| F3 : peptide 10 | 6 aa | K992A; N993A; K994A; S996A; R997A; N998A | + |




**Supplementary Figure 1. Charge dependant DNA binding of residues in Cluster1. (A)** Diagram representation indicating point mutations generated by site-specific mutagenesis. Cluster2 represents the mutations done in F1Cpep5 K-A. (**B**) Coomassie blue stained gel of purified F1CWT (18 kDa), F1Cpep2 K-A , F1Cpep5 K-A and F1Cpep2 K-R proteins. M, Molecular mass marker. (**C**) DNA binding activity of F1CWT, F1Cpep2 K-A , F1Cpep5 K-A and F1Cpep2 K-R to various recombinant DNA substrates. The percentage of DNA probes shifted were quantified by phosphorimaging and shown in (**D**). (**E**) Diagram representation of F1Mut5aa K-A (1) and F1Mut5aa K-R (2) mutants indicating point mutations generated by site-specific mutagenesis. Bold characters represent the substitutive mutations. (**F**) Purified F1Mut5aa K-A(66 kDa), F1Mut5aa K-R (66 kDa) proteins. M, Molecular mass marker. (**G**) DNA binding activity of F1Mut5aa K-A and F1Mut5aa K-R to various recombinant DNA substrates and DNA binding quantification (**H**) Error bars indicate S.E from three independently performed experiments.




**Supplementary Figure 2: Interaction of FANCD2 WT and mutant proteins with FANCI.** (**A**) The Coomassie blue stained gel of purified His10-tagged FANCI protein. M, Molecular mass marker. (**B**) Purified Human FANCI and equimolar concentrations of purified full-length FANCD2WT, D2-F1Mut, D2-F3Mut, or D2-F1+F3Mut were subjected to immunoprecipitation analysis. Immunoprecipitations with anti-FANCI antibody was performed and immune complexes were immunobloted with anti-FANCD2 and anti-Histidine antibody, as indicated.




**Supplementary Figure 3:** (**A**) **Localisation of MYC-D2-F1+F3Mut.** A total of seventy-five FA-D2 cells expressing MYC-D2-F1+f3Mut were scored and box plotted for percentage of citrine expressed in the nucleus. (**B**) **Western blot showing comparable expression of D2WT and MYC-D2-F1+F3Mut in PD20 cells employed in Fig. 8*E*, *F*, and *G*.**

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H. sapiens      1-MVSKRRLSKSEDK-ESLTEDASKTRKQPL---SKKTKKSHIANEVEEND
B. taurus       1-MVSKRRLSKSEDK-ESLTEDASKTRKQPL---SKKTKKSHIANEVEEND
P. troglodytes  1-MISKRKLSVSENK-ESLTEDASEARKQPL---SKKTKKSHVHNEVEEND
M. musculus     1-MISKRRRLDSEDK-ENLTEDASK--TMPL---SKLAKKSHNSHEVEENG
G. gallus       1-MVSKRKLSKIDAAEESSKTDLQS--RCPETKRSRISDKRAPSQGGLENE
X. laevis       1-MVAKRKLSRSDDREESFTADTSKNKKCRT---SSKKSKALPQDGVVEND

H. sapiens      835-DYVPPLGNFDVETLDITPHTVTAISAK-IRKKGKIERKQKTDGSKTS
B. taurus       835-DYVPPLGNFDVETLDITPHTVTAISAK-IRKKGKIERKQKTDGSKTS
P. troglodytes  835-DYVPPLANFDLETLEETPHTSTAVAKI-RMKGKTGGKKRKADGGKTS
M. musculus     832-DYVPPFASVDLDTLDMMPRSSSAVAAKNRNKGKTGGKKQKADSNKAS
G. gallus       837-GYVPPPATFDSEAPEGVPSINAGG----PVRKK-NGKKRKSDSSKAC
X. laevis       834-GYIPSSAHFDSEPQEVLPSAIAPA----PAKKAKKGKTPKSAGSKNA
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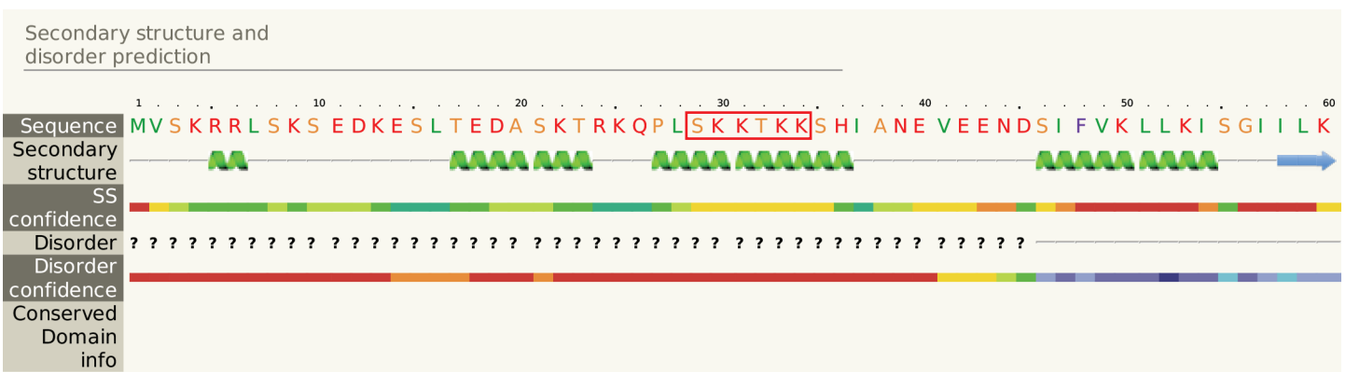
**Supplementary Figure 4: DNA binding residues are evolutionary conserved.** A ClustalW multiple alignment of the DNA binding residues and the NLS of FANCD2 exhibits evolutionary semiconserved pattern, indicated by red and green colour bold letters.




**Supplementary Figure 5: The N-terminal of FANCD2 has tendency to form helical structure.** A Phyre2 protein secondary structure prediction algorithm of the N-terminal residues in FANCD2 indicates its propensity to form the α-helical secondary structure. DNA binding/NLS residues are depicted by the red box.