

Supplementary Figure Legends

Supplementary Figure 1. FANCJ depleted U2OS cells exhibit sensitivity to MMC (A) that can be rescued with expression of shRNA resistant wtFANCJ (B). Inside panel A; FANCJ protein levels were assessed after depletion with two shRNA constructs using FANCJ specific antibodies. Inside panel B; Ectopic expression of HA-6xHis-FANCJ was measured by HA specific antibody. (C) Relative protein levels of endogenous FANCJ and HA-6xHis-FANCJ. Western blotting was carried out using FANCJ specific antibody. (D) FANCJ depleted cells accumulate in G2/M phase of the cell cycle in response to MMC induced damage.

Supplementary Figure 2. FACS analysis of control and FANCJ depleted U2OS SCR35S cells after transfection with control plasmid (spontaneous) or I-SceI plasmid. Frequency of I-SceI induced GFP⁺ cells (A) and absolute frequency of I-SceI induced GFP⁺ RFP⁺ cells (B) from control and FANCJ depleted U2OS SCR35S cells. (C) Ratio of I-SceI-induced RFP⁺GFP⁺/Total GFP⁺ frequencies (LTGC/overall GC, expressed as a percentage) from the experiment whose results are shown in panels A and B.

Supplementary Figure 3. Protein sequence alignment of FANCJ helicase from human (*Homo sapiens*), mouse (*Mus musculus*) and Chinese hamster (*Cricetulus griseus*) using ClustalW2. Red boxes indicate conserved helicase signature motifs. NLS motif is indicated by green box. The four conserved cysteine residues that form Fe-S domain are shown in pink boxes. The C-terminal BRCA1 interacting domain is indicated in blue dotted box. The S990 residues responsible for FANCJ interaction with BRCA1 upon phosphorylation is shown in orange box.

Supplementary Figure 4. Hamster FANCJ controls SCR and suppresses LTGC. (A) FANCJ deficiency cause MMC induced sensitivity in hamster cells. Western blot of FANCJ

depletion is shown inside the graph. (B) I-SceI induced GFP⁺ frequencies in control and FANCJ depleted V79B SCR55 cells. (C) I-SceI induced BsdR⁺ frequencies for the same experiment shown in panel A. (D) Ratio of I-SceI-induced LTGC/overall GC (expressed as a percentage) from the experiment whose results are shown in panels B and C.

Supplementary Figure 5. (A) I-SceI induced GFP⁺ frequencies in control and FANCJ depleted U2OS SCR18 cells. (B) Frequencies of I-SceI induced BsdR⁺ colonies for the same experiment shown in panel A. (C) Ratio of I-SceI-induced BsdR⁺/GFP⁺ frequencies (LTGC/overall GC, expressed as a percentage) from the experiment whose results are shown in panels A and B.

Supplementary Figure 6. Southern blot analysis of I-SceI induced BsdR⁺GFP⁺ colonies from control and FANCJ depleted U2OS SCR18 cells. Genomic DNA was isolated and 5 µg of DNA was digested with EcoRI (A), SacI (B) and PstI (C) and DNA samples were resolved on 0.8% agarose gel and subjected to Southern hybridization using *GFP* cDNA specific probe. “L” and “R” indicate left and right arms of the reporter, respectively.

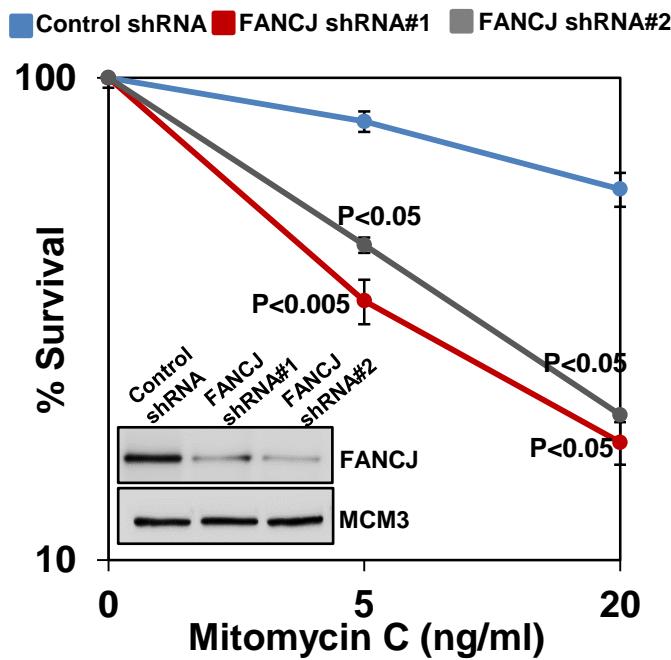
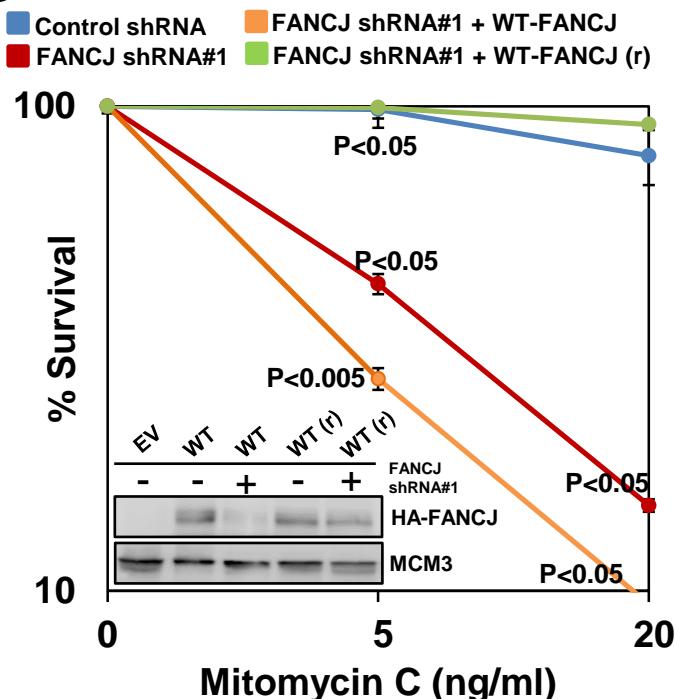
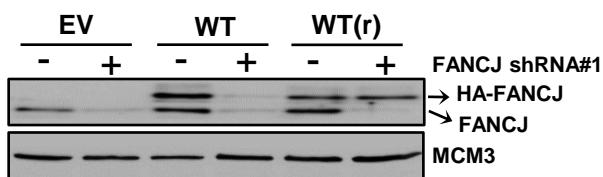
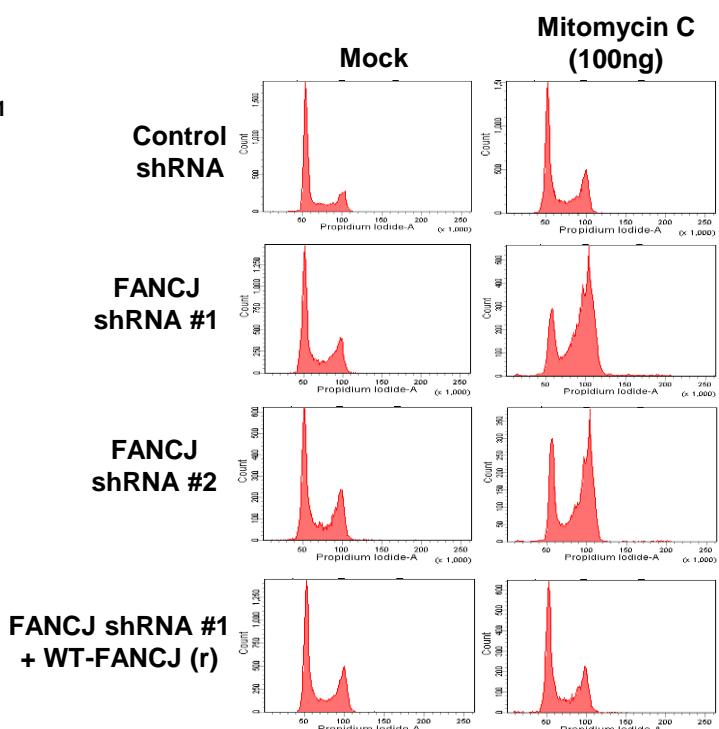
Supplementary Figure 7. Analysis of Fe-S cluster mutants of FANCJ helicase. (A) Schematic diagram of FANCJ depicting the conserved helicase domains and Fe-S cluster. Cysteine 350 and A349P missense mutation identified in FA patient are indicated. (B) Frequency of I-SceI induced GFP⁺ cells in U2OS SCR18 clone transfected with empty vector (EV) and the plasmids that express wtFANCJ and its indicated mutant proteins. (C) Absolute frequencies of BsdR⁺ colonies for the experiment shown in panel B. (D) Ratio of LTGC:Overall GC obtained from the data shown in panels B and C. (E) Interaction of FANCJ Fe-S cluster mutants with BRCA1.

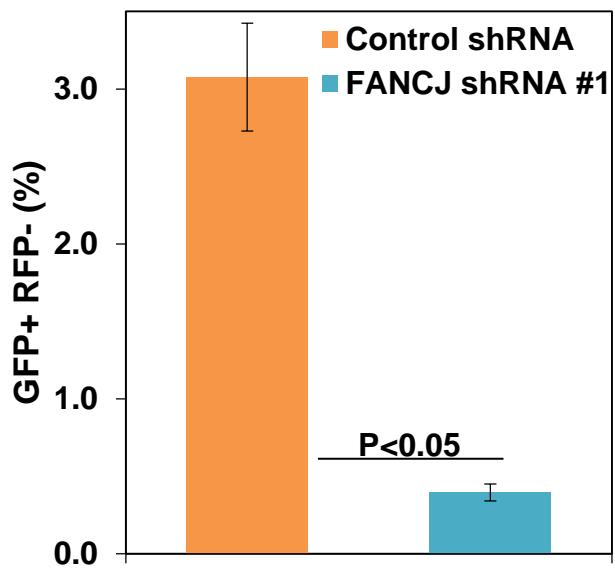
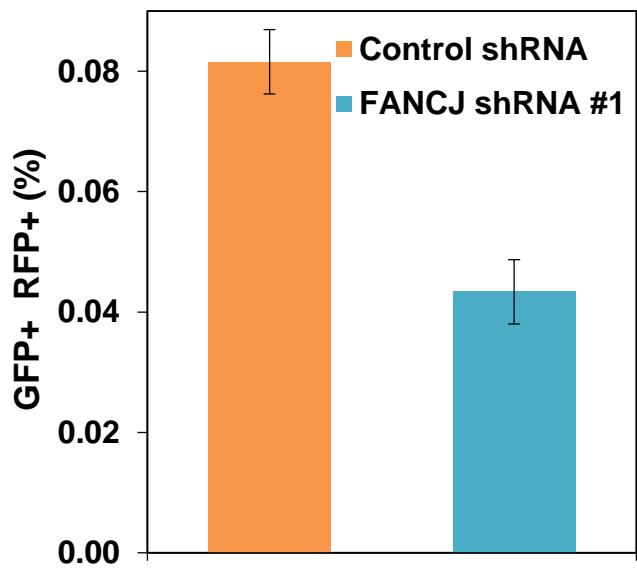
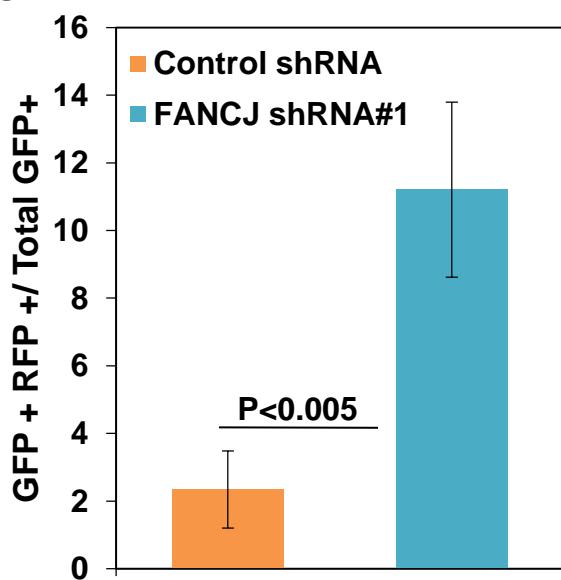
Supplementary Table 1. Oligo sequences used for generating shRNA constructs for depleting FANCJ, FANCM, BRCA1 and also for generating shRNA resistant wt and FANCJ mutants. The sequences corresponding to the mRNAs of respective genes are underlined. Modified bases that were designed for constructing shRNA resistant FANCJ are in bold and underlined.

shRNA		Sequence (5' → 3')
FANCJ #1	Oligo 1	GATCC <u>GTACAGTACCCCACCTTATT</u> CAAGAGA ATAAGGTGGGTACTGTACTTTTA
	Oligo 2	AGCTTAAAA <u>AGTACAGTACCCCACCTTATT</u> CTCTTGAA ATAAGGTGGGTACTGTACG
FANCJ #2	Oligo 1	GATCC <u>AGCTTACCCGTACAT</u> TCAGAGA TGTGACGGGTAA <u>GCTTACCCGTACAT</u> TCTCTTGAA
	Oligo 2	AGCTTAAAAAA <u>AGCTTACCCGTACAT</u> TCTCTTGAA TGTGACGGGTAA <u>GCTG</u>
FANCM	Oligo 1	GATCC <u>AGACATCGCTGAATTAAATT</u> CAAGAGA TTTAAATT <u>CAGCGATGTCTTTTA</u>
	Oligo2	AGCTTAAAAAA <u>AGACATCGCTGAATTAAAT</u> CTCTTGAA TTTAAATT <u>CAGCGATGTCTG</u>
BRCA1	Oligo 1	GATCC GTGTGCAGCTGAGAGGCATT CAAGAGA TGCCTCTCAGCTGCACACTTTTA
	Oligo 2	AGCTTAAAA <u>AGTGTGCAGCTGAGAGGCAT</u> CTCTTGAA TGCCTCTCAGCTGCACACG
FANCJ shRNA#1 resistant primers	Oligo 1	ACCTTTAAA <u>ATATAGCACATCCCCCTACTTACTGGAA</u>
	Oligo 2	TTCCAGTAA <u>GTAGGGGGATGTGCTATATT</u> AAAGAGGT

Supplementary Table 2. Primer sequences that were used for generating wt and mutant FANCJ constructs. The EcoRV and XhoI restriction site in the forward and reverse primers are bolded and underlined, respectively. The triplet sequences corresponding to the respective FANCJ mutants are in bold and underlined.

Primer name		Sequence (5'→3')
WT-FANCJ	Forward	ATAG <u>A</u> GATATCATGTCTTCAATGTGGTCT
	Reverse	ATAG <u>A</u> CTCGAGTCAGTGATGGTGGTATGGTGTGCAT AGTCGGGGACGTCATAAGGGTACTTAAAACCAGGAAA
S990A-FANCJ	Forward	TCCAGATCCACAG <u>CCC</u> AACTTCAAC
	Reverse	GTTGAAAGTTGG <u>GG</u> CTGTGGATCTGGA
S990E-FANCJ	Forward	TCCAGATCCACAG <u>GAG</u> CCAACTTCAAC
	Reverse	GTTGAAAGTTGG <u>CTC</u> TGTGGATCTGGA
K52A-FANCJ	Forward	ACAGGAAGTGG <u>AGCA</u> AGCTTAGCCTTA
	Reverse	TAAGGCTAAC <u>GCTTG</u> CTCCACTTCCTGT
K52R-FANCJ	Forward	ACAGGAAGTGG <u>ACGA</u> AGCTTAGCCTTA
	Reverse	TAAGGCTAAC <u>GCTCG</u> TCCACTTCCTGT
C350A-FANCJ	Forward	AAACTAAAGGCC <u>AGT</u> CCATATTACACA
	Reverse	TGTGTAATATGG <u>ACT</u> GGCCTTAGTT
C350S-FANCJ	Forward	AAACTAAAGGCC <u>GCT</u> CCATATTACACA
	Reverse	TGTGTAATATGG <u>AGC</u> GGCCTTAGTT
A349P-FANCJ	Forward	AAGAAACTAAAG <u>CC</u> CTGTCCATATTAC
	Reverse	GTAATATGGAC <u>GGG</u> CTTAGTTCTT
P47A-FANCJ	Forward	TTGTTGGAGAGT <u>GCC</u> CACAGGAAGTGGA
	Reverse	TCCACTCCTGT <u>GGC</u> ACTCTCCAACAA
Q944E-FANCJ	Forward	AAGATATGTGTC <u>GAGG</u> AACTACAGTGT
	Reverse	ACACTGTAGTT <u>CTC</u> CGACACATATCTT

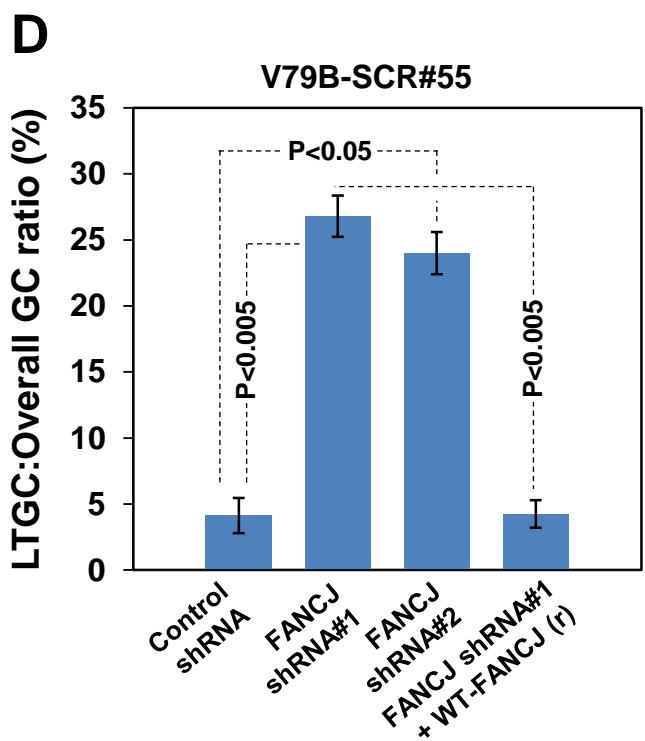
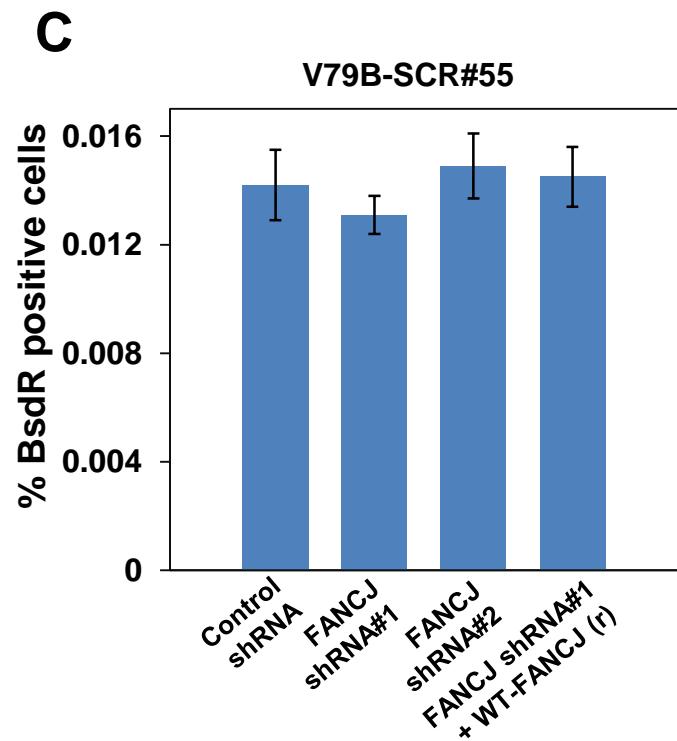
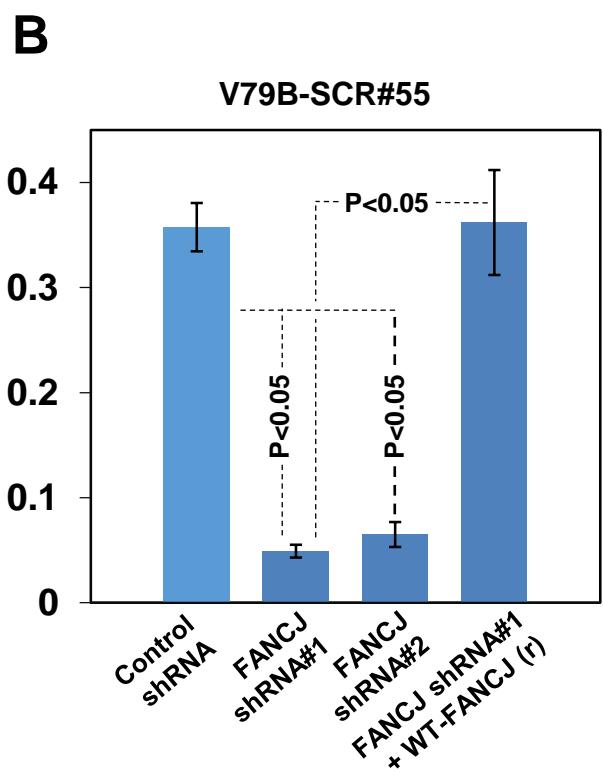
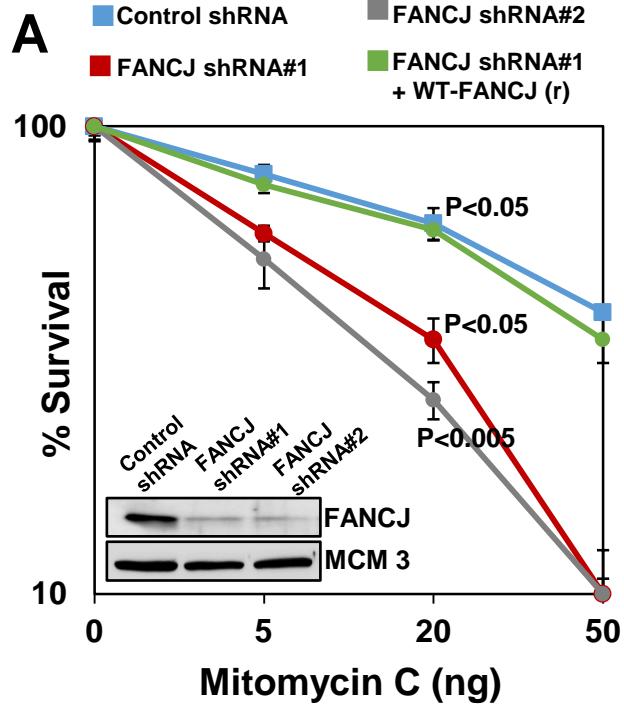
A**B****C****D****Supplementary Figure 1**

A**B****C**

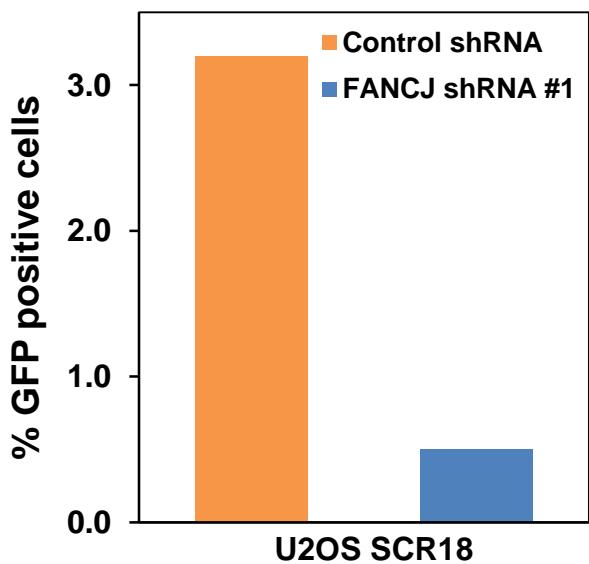
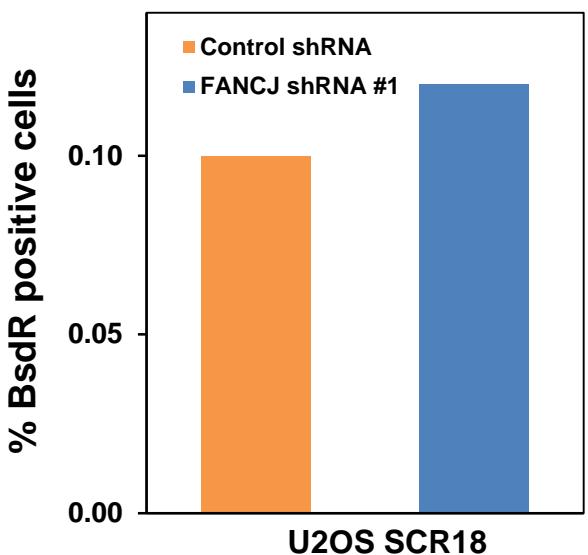
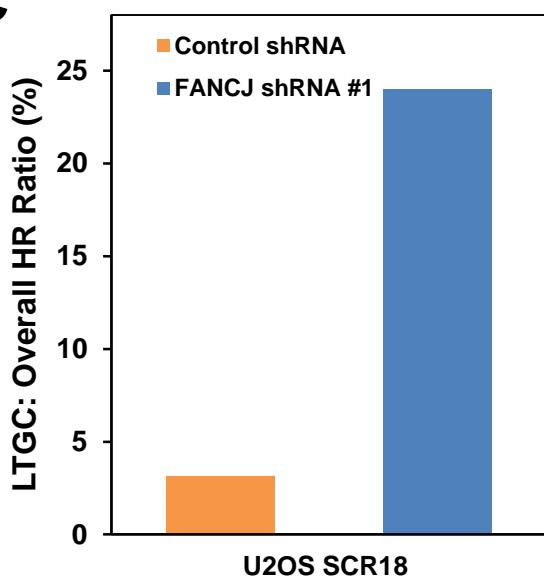
Supplementary Figure 2

		MOTIF I	
hFANCJ	1	MSSMWSEY蒂GGVKIYFPYKAYPSQLAMMNSILRG	LNSKQHCLLESPTGSGKSLALLCSALAWQQSLSGKP 71
mFANCJ	1	MSSVLSDY蒂GGVKIHFPCKRAYPAQLAMMNSIVRGL	NSSQHCLLESPTGSGKSLALLCSALAWQQSLSEKP 71
cFANCJ	1	MSSSSEY蒂GGVKINFPCKAYPAQLAMMNSIVRGL	NSSQHCLLESPTGSGKSLALLCSALAWQQSTGKP 71
hFANCJ	72	ADEGVSEKAEVQLSCCCACHSKDFTNNDMNQGTSRHF	YPSTPPSERNGTSSCTQDSPEKTTLAAKLSAKK 142
mFANCJ	72	VDEGLNKKPEAPPSCSACHSKNFTYSNDLTDSPHFN	SPSKPSSGRNGVSTPCQDSPEKNTLAAKLSAKK 142
cFANCJ	72	VDEGLNKKLEVPSCCCCACHSKSFMYNSNDMGTSPHF	SSPKLSE-RNGSSPPCQDSPEKNTLAAKLSAKK 141
		NLS MOTIF	
hFANCJ	143	QASIYRDENDDFQVEKKRIRPLETTQQIRKRHCF	GTEVHNLDANKVDSGKTVKLNSPLEKINS---FSPQKP 210
mFANCJ	143	QASIHRDDEDDDFQVEKKRIRPLETTQQIRKRHCLE	KDVHHVDARLASEKRVKPESPIGKSFSDRKDSFQNV 213
cFANCJ	142	QASKHRDDEDDDFQMEKKRIRPLETAQQMRKRHCLE	KDVHHLDARVASEKKVVKPESPVGKTSS---SFQNL 208
		MOTIF IA	
hFANCJ	211	PGHCSRCCSTKQGNSQESSNTIKKDHTGKSKIPK	IYFGTRTHKQIAQITRELRRTAYSGVPMTILSSRDH 281
mFANCJ	214	DGLCSRCCCSAKQGNNQEPAINTVKKDHGGQCKRP	KIYFGTRTHKQIAQITRELRKTAISGVPMTILSSRDH 284
cFANCJ	209	DGLCSRCCSTKQGNSEESANTVKKDHGGQSKRP	KIYFGTRTHKQIAQITRELQKTAISGVPMTILSSRDH 279
hFANCJ	282	TCVHPPEVVGNFNRNEKCME	LLDGKNGKSCYFYHGVKISDQHTLQTFFQGMCKAWDIEELVSLGKKLKACPY 352
mFANCJ	285	CVHPEVVGNFNRKEKCME	LLDGKHKGKSCYFYHGVKISNQTLQHLQGMSRAWDIEELVSLGRKLKACPY 355
cFANCJ	280	TCVHPPEVMGNFNRNEKCME	LLDVVKQGKSCYFYHGVRIINNQHTLQSFPGMMSKAWDIEELVSLGRKLKACPY 350
		MOTIF II	
hFANCJ	353	YTARELIQDADIIFCP	CPNYLLDAQIRESMIDLNLKEQVVILDEAHNIEDCARESASYSVTEVQLRFARDELD 423
mFANCJ	356	YTARELIEDADIVFC	CPNYLLDSQIRETMIDLKGQVVLDEAHNIEDCARESASYSVTEVQLRFARDELD 426
cFANCJ	351	YTARELIDEADIIFCP	CPNYLLDAQIRESMIDLKDQVVLDEAHNIEECARESASYSVTEVQLRFARDELD 421
hFANCJ	424	SMVNNTIRKKDHEPLRAVCCSILNWLE	ANAEYLVERDYESACKIWSGNEMLLTLHKMGITTATFPILQGHF 494
mFANCJ	427	SLINGNIRKKSH	EPLRVCYNLINWLETNSKHLVERGYESSCKIWSGNEMLLNLYRMGITTATFPVLQRHL 497
cFANCJ	422	SLINSNVRKKNHE	PLRVCYNLINWLETNSEHLVERDYESSCKIWSGNEMLLSLYRMGITNATFPVLQRHF 492
hFANCJ	495	SAVLQKEEKISPIYGKEEAREVPVISASTQIMLKGLFMVLDYLFRQNSRFADDYKIAI	QQTYSWTNQIDIS 565
mFANCJ	498	SAVLQKEEKVTPIHGKEEAIQIPII	SASTQVVLKGLFMVLDYLFRENSRFADDYKVAIQQTYSWTNQIAIF 568
cFANCJ	493	SAVLQKEE---	KAYGKEEAIQIPIIISASTQIMLKGLFMVLDYLFRENSRFADDYKIAIQQTYSWTNQIAIF 560
		MOTIF III	
hFANCJ	566	DKNGLLVLPKNNKRSRQKTAHV	LNFCLNPAAVAFSDINGKVQITIVLTSGTLSPMKSFSELGVFTTIQLE 636
mFANCJ	569	DKTGVLA	VPKNNKHSRQKIGVNALNFCLNPAAVAFSDINDKVRTIVLTSGTLSPKSFSELGVFTSIQLE 639
cFANCJ	561	DKSGVLA	VPKNNKHSRQKIGVNVLNFCLNPAAVAFSDINDKVRTIVLTSGTLSPKSFSELGVFTNIQLE 631
hFANCJ	637	ANHIIKNSQVWVGTIGSGPKGRNL	CATFQNTETFEFQDEVGALLSVCQTVSQGILCFLPSYKLEKLKER 707
mFANCJ	640	ANHVISNSQVWVGTVGSGPKGRNL	CATFQHETFEFQDEVGMLL SVCQTVSQGILCFLPSYKLEKLRRER 710
cFANCJ	632	ANHVVNSNSQVWVGTVGSGPQGRNL	CATFQHETFEFQDEVGMLL SVCQTVSQGILCFLPSYKLEKLRRER 702
		MOTIF V	
hFANCJ	708	WLS	TGLWHNLELVKTVI
mFANCJ	711	WIFTGLWHSLES	VKTVIAEPQGGEKTNFDELLQVYYDAIKYKGEKGALLVAVCRGKVSEGLDFSDDNARA 778
cFANCJ	703	WVSTGLWHSLES	VKTVIAEPQRGEKTFDDELLQVYYDAIKFKGEKGALLIAVCRGKVSEGLDFSDDNARA 773
		MOTIF VI	
hFANCJ	779	VITIGIPFPNVKDLQVELKRQYNDHHSKLRGLLPGRQWYE	IQAYRALNQALGRCIRHNDWGALILVDDRF 849
mFANCJ	782	VITVGIPFPNVKDLQVELKRQYNDHHSKSRGLLPGRQWYE	IQAYRALNQALGRCIRHNDWGALILVDDRF 852
cFANCJ	774	VVTVGIPFPNVKDLQVELKRQYNDHHSKLRGLLPGRQWYE	IQAYRALNQALGRCIRHNDWGALILVDDRF 844
hFANCJ	850	RNNPSRYISGLSKWVRQQI	QHHSTFESALESIAEFSKKHQVLNVSIKDRTNIQDNESTLEVTSKYSTSP 920
mFANCJ	853	NNNPNRYSISGLSKWVRQQI	QHHSSFASALESLTEFSRRHQVTKNRSKKDEKCTKDNEPTLEVACLEDSTFT 923
cFANCJ	845	NSNPNRYSISGLSKWVRQQI	QHHSTFASALESLTEFSRRHQVTKNRSKKDK-----ESTLNACLEDSTLT 909
		BRCA1 INTERACTION DOMAIN	
hFANCJ	921	YLLEAASHLSPENFVEDEAKICVQELQCPKII	TNSPLPSSIISRKEKNDPVFLEEAG--KAEKIVISRS 988
mFANCJ	924	SVSESS-HQSPENS-TEEAEV	CVQELQCPQVATKSPSVASHGVSRKKSDPGLRGESLQTMKTEKNEISRS 992
cFANCJ	910	GVSKAS-HLSPENS	REEEAKLCVQELQCPQMTAKNPSVPSHDIPRRKKSDPVLREESVQTMKTEKNVISRS 979
hFANCJ	989	TSPTFNQTKRVSWSFN	SLGQYFTGKIPKATPELGSSSENSASSPPRFKTEKMESKTVLPFTDKCESSNLT 1059
mFANCJ	993	SSPTFGKQTEPVNWP	I FNLSRRHFNSKVKNCTPVLKSSKNRAPGSSTFN-----KTA
cFANCJ	980	SSPTFGKQTEPV	WVPSFKSLRQHTRKVKNQTPVLGSSKSHASGSSTFKTEKTEDSTALPHGTGKRVSSKET 1050
hFANCJ	1060	VNTSFGSCPQSETI	SSLKIDATLTRKNHSEHPLCSEEALDPDI
mFANCJ	1058	ADTSLGPC	LQSEVIISPVKIEATPATN-YSKQVFCCEDL
cFANCJ	1051	QSESLSS-MKVDITPAEN-HSKQLFCSE	KDLPDTELSPGTEEAKCPSNKAAETEVDDDSE 1127
hFANCJ	1131	YFTPELYDPEDTDEEKNDLAETDRGNRLANN	SDCILAKDLFEIRTKEVDSAREVKAEDCIDTKLNGILHI 1201
mFANCJ	1128	CFTPELFDPVDTNEENGELVETDRSS	--HSSDCFSAEELFETATGFGQK----- 1174
cFANCJ	1120	SLTPELFDPVDTDEENSEP	VETDRSS--NNSDCFSSEDLFESVTDFSQK----- 1166
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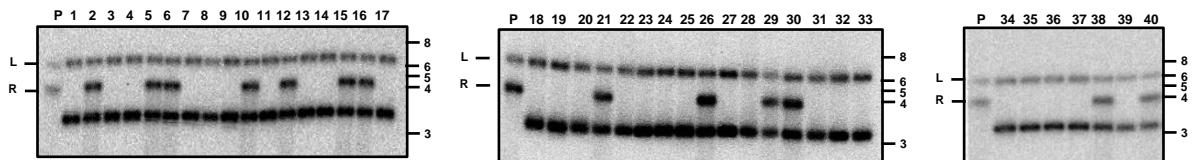
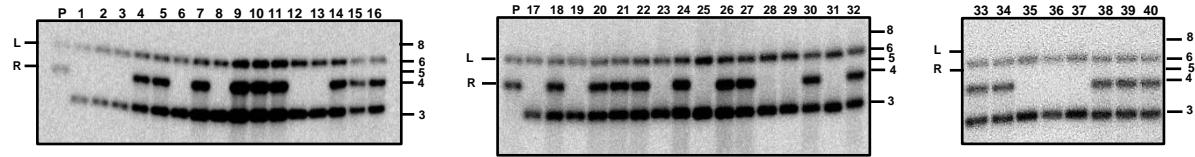
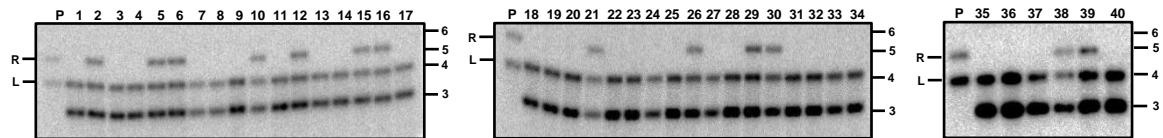
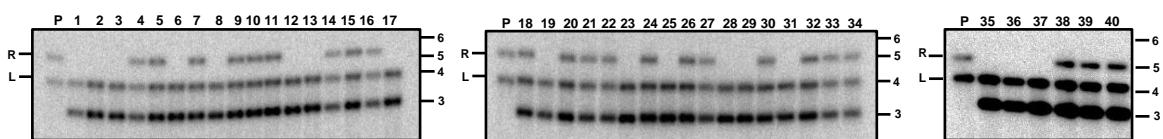
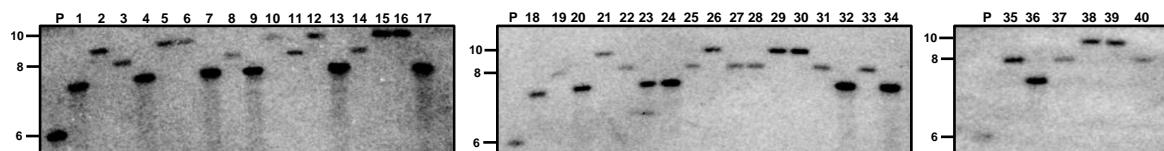
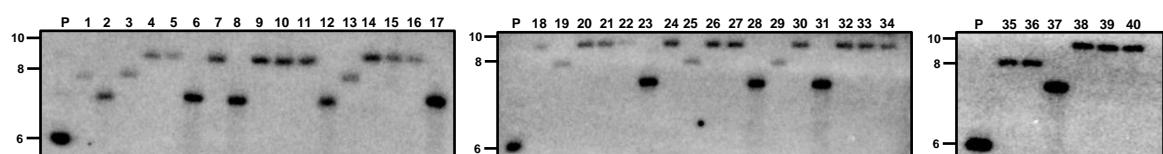
Supplementary Figure 3

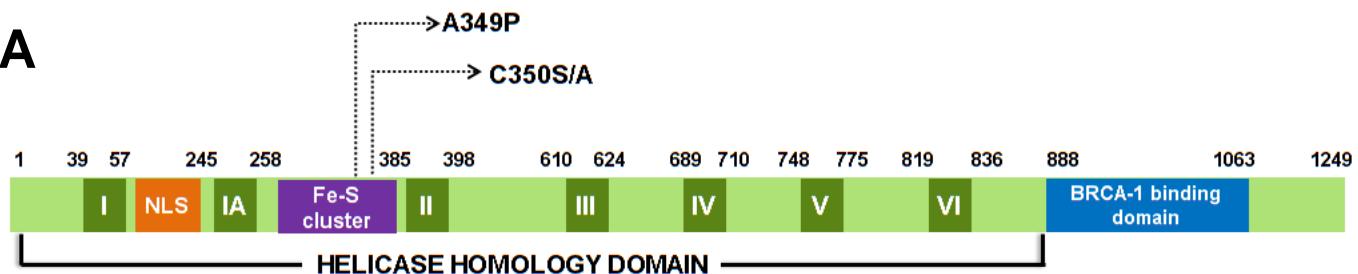
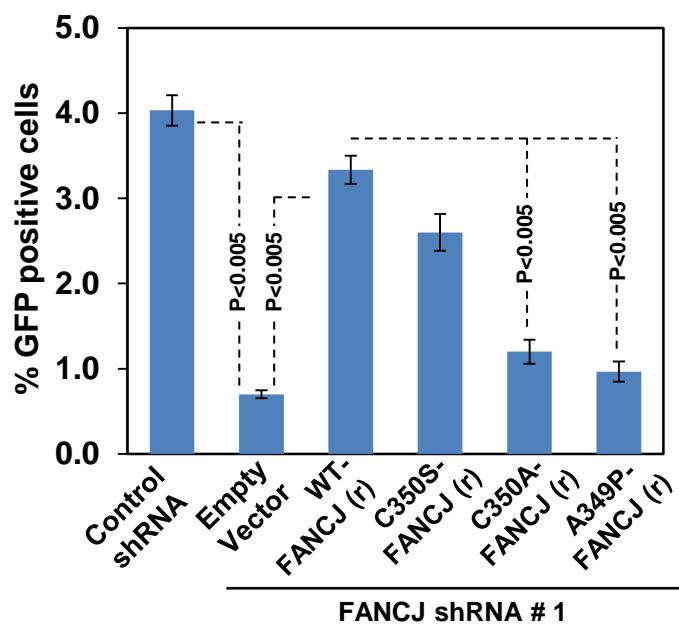
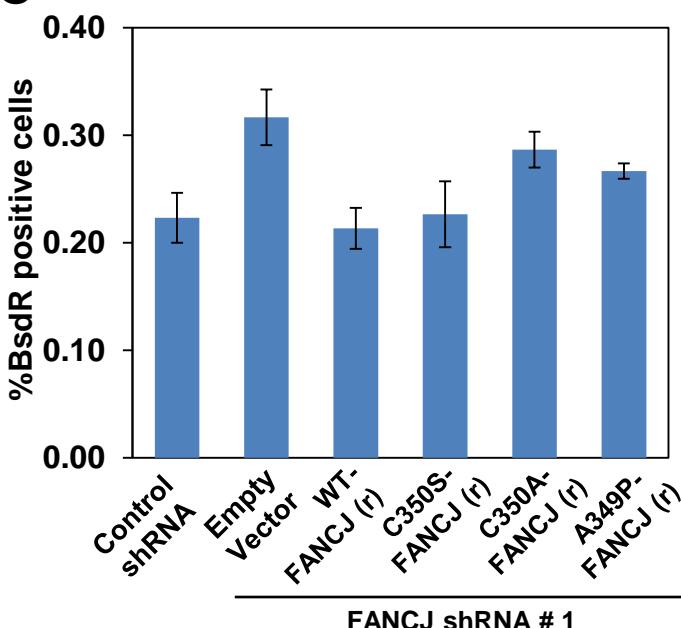
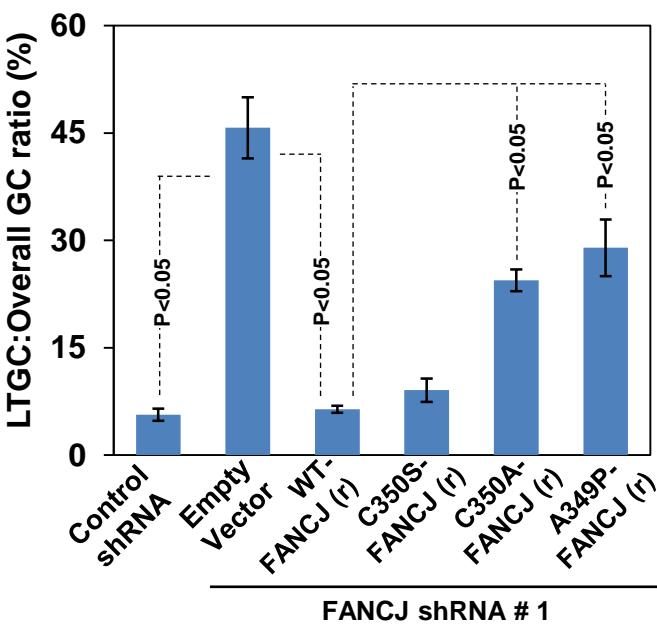
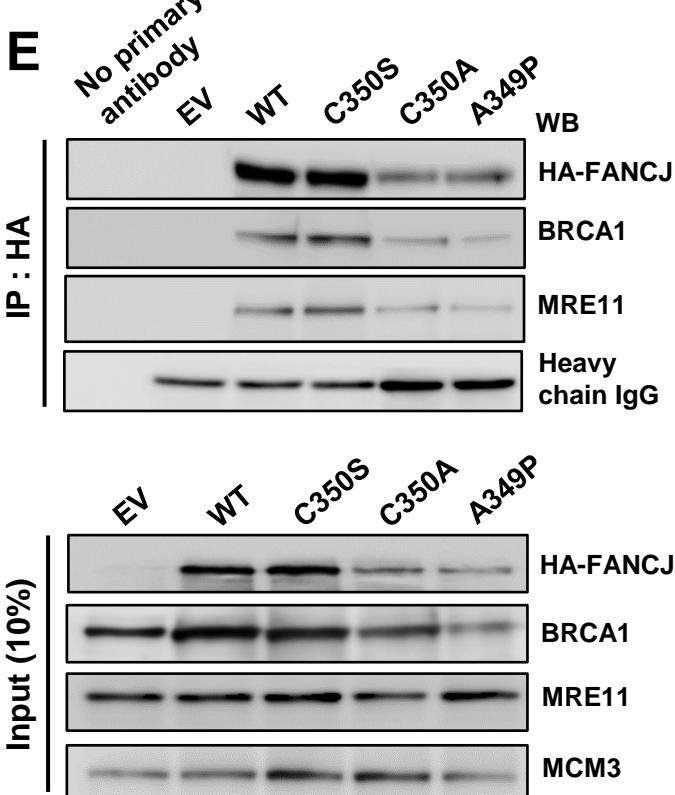


Supplementary Figure 4

A**B****C**

Supplementary Figure 5

A**shControl – EcoRI****shFANCJ – EcoRI****B****shControl – SacI****shFANCJ – SacI****C****shControl – PstI****shFANCJ – PstI****Supplementary Figure 6**

A**B****C****D****E****Supplementary Figure 7**