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.

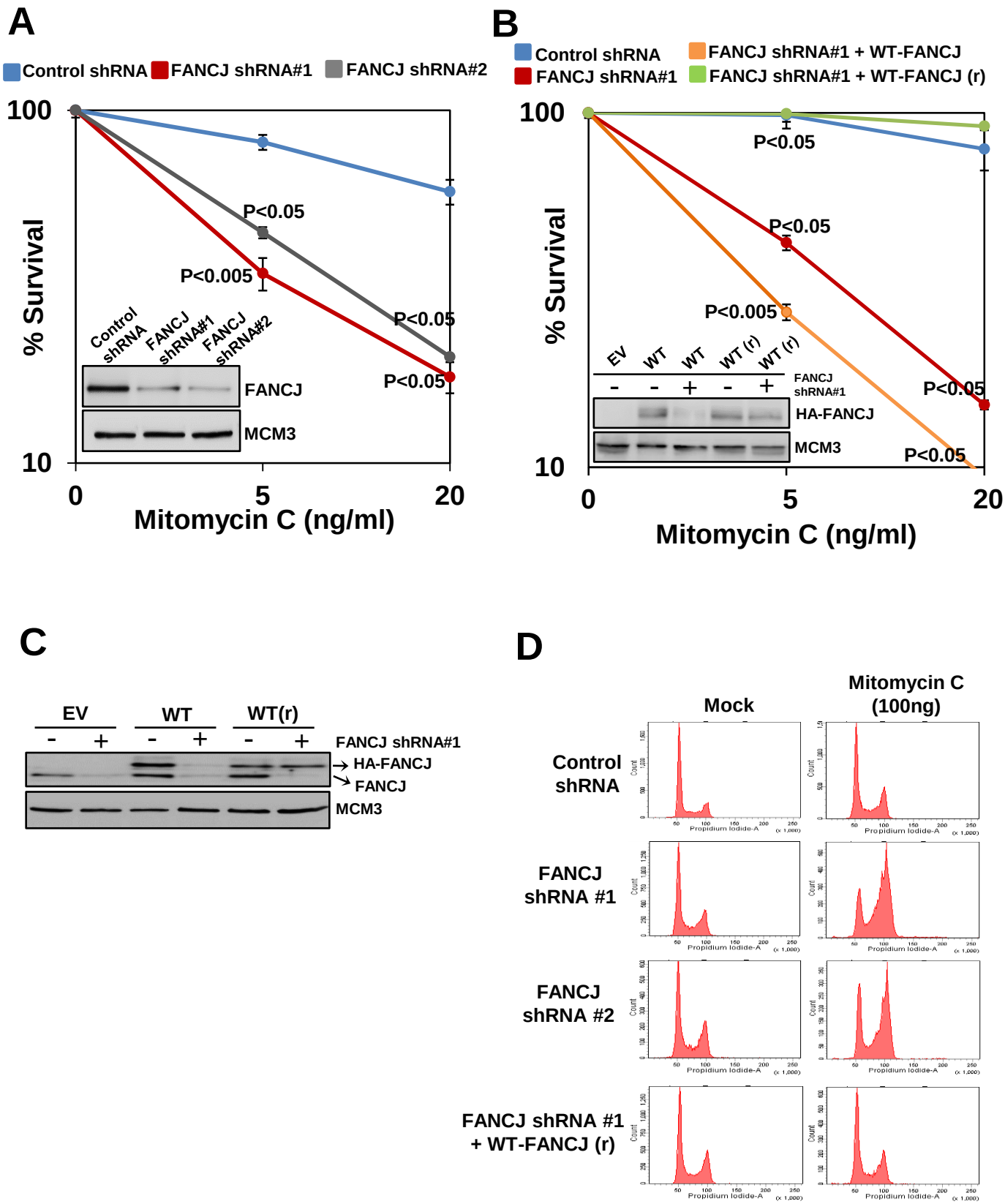
Supplementary Figure 7. Analysis of Fe-S cluster mutants of FANCJ helicase. (A) Schematic diagram of FANCJ depicting the conserved helicase domains and Fe-S cluster. Cysteine 350 and A349P missense mutation identified in FA patient are indicated. (B) Frequency of I-SceI induced GFP^+ cells in U2OS SCR18 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.

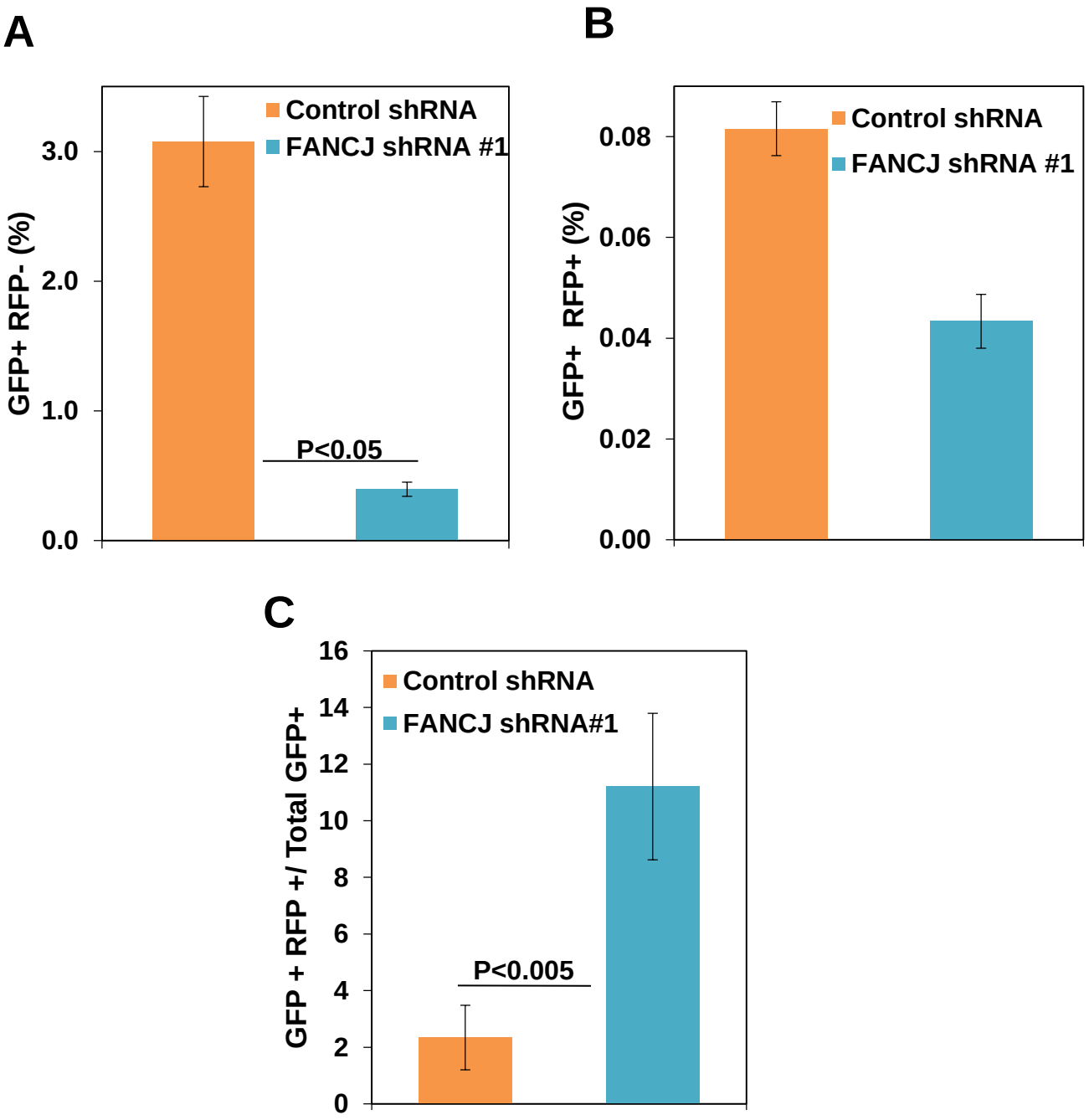
shRNA		**Sequence (5′→ 3′)**
FANCJ #1	Oligo 1	GATCCGTACAGTACCCCACCTTATTTCAAGAGA ATAAGGTGGGGTACTGTACTTTTTA
	Oligo 2	AGCTTAAAAAGTACAGTACCCCACCTTATTCTCTTGAA ATAAGGTGGGGTACTGTACG
FANCJ #2	Oligo 1	GATCCAGCTTACCCGTCACATTCAAGAGA TGTGACGGGTAAGCTTTTTTA
	Oligo 2	AGCTTAAAAA AGCTTACCCGTCACATCTCTTGAA TGTGACGGGTAAGCTG
FANCM	Oligo 1	GATCCAGACATCGCTGAATTTAAATTCAAGAGA TTTAAATTCAGCGATGTCTTTTTTA
	Oligo2	AGCTTAAAAA AGACATCGCTGAATTTAAATCTCTTGAA TTTAAATTCAGCGATGTCTG
BRCA1	Oligo 1	GATCC GTGTGCAGCTGAGAGGCATTCAAGAGA TGCCTCTCAGCTGCACACTTTTTA
	Oligo 2	AGCTTAAAAAGTGTGCAGCTGAGAGGCATCTCTTGAA TGCCTCTCAGCTGCACACG
FANCJ shRNA#1 resistant primers	Oligo 1	ACCTCTTTAAA **A**TA**T**AG**C**AC**A**TC**C**CC**C**TA**C**TTACTGGAA
	Oligo 2	TTCCAGTAA **G**TA**G**GG**G**GA**T**GT**G**CT**A**TA**T**TTTAAAGAGGT

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.

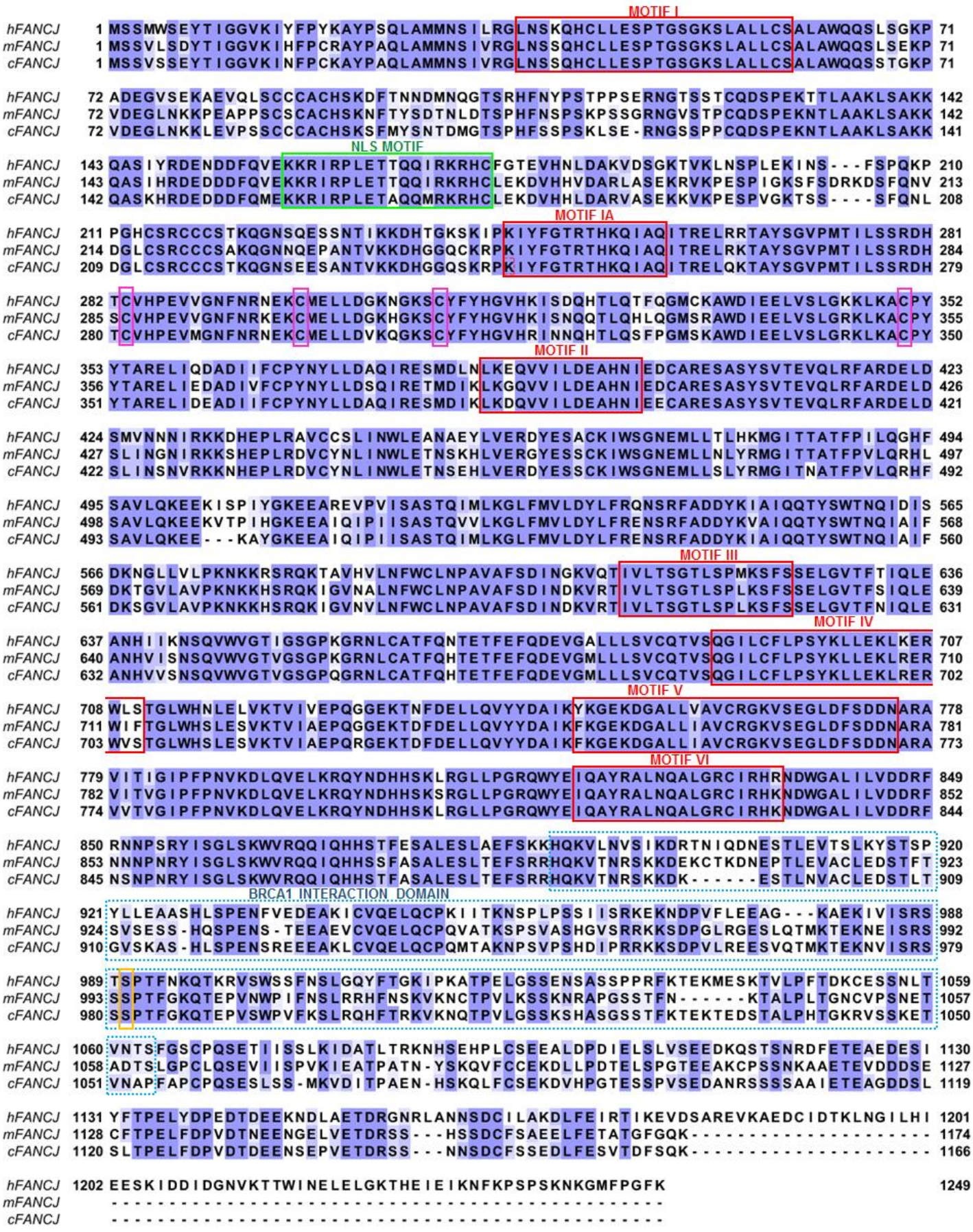
Primer name		**Sequence (5'→3')**
WT-FANCJ	Forward	ATAGA**GATATC**ATGTCTTCAATGTGGTCT
	Reverse	ATAGA**CTCGAG**TCAGTGATGGTGGTGATGGTGTGCAT AGTCGGGGACGTCATAGGGGTACTTAAAACCAGGAAA
S990A-FANCJ	Forward	TCCAGATCCACA**GCC**CCAACTTTCAAC
	Reverse	GTTGAAAGTTGG**GGC**TGTGGATCTGGA
S990E-FANCJ	Forward	TCCAGATCCACA**GAG**CCAACTTTCAAC
	Reverse	GTTGAAAGTTGG**CTC**TGTGGATCTGGA
K52A-FANCJ	Forward	ACAGGAAGTGGA**GCA**AGCTTAGCCTTA
	Reverse	TAAGGCTAAGCT**TGC**TCCACTTCCTGT
K52R-FANCJ	Forward	ACAGGAAGTGGA**CGA**AGCTTAGCCTTA
	Reverse	TAAGGCTAAGCT**TCG**TCCACTTCCTGT
C350A-FANCJ	Forward	AAACTAAAGGCC**AGT**CCATATTACACA
	Reverse	TGTGTAATATGG**ACT**GGCCTTTAGTTT
C350S-FANCJ	Forward	AAACTAAAGGCC**GCT**CCATATTACACA
	Reverse	TGTGTAATATGG**AGC**GGCCTTTAGTTT
A349P-FANCJ	Forward	AAGAAACTAAAG**CCC**TGTCCATATTAC
	Reverse	GTAATATGGACA**GGG**CTTTAGTTTCTT
P47A-FANCJ	Forward	TTGTTGGAGAGT**GCC**ACAGGAAGTGGA
	Reverse	TCCACTTCCTGT**GGC**ACTCTCCAACAA
Q944E-FANCJ	Forward	AAGATATGTGTC**GAG**GAACTACAGTGT
	Reverse	ACACTGTAGTTC**CTC**GACACATATCTT



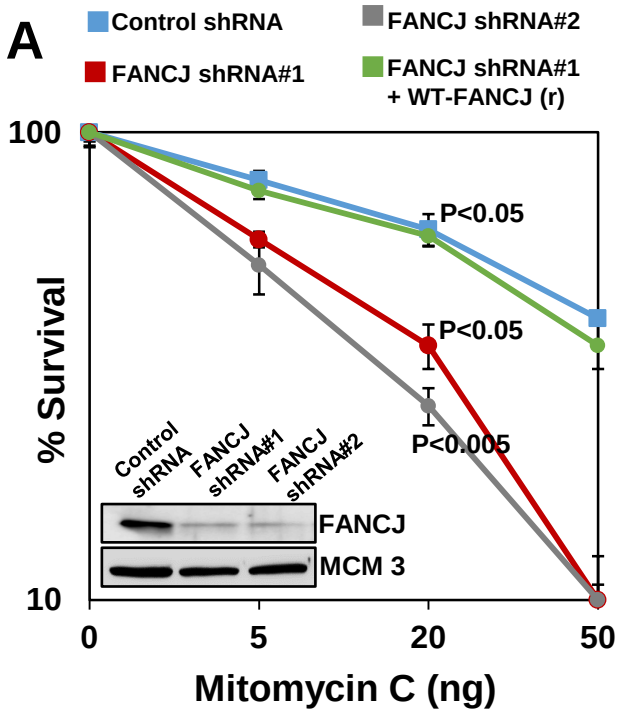
Supplementary Figure 1

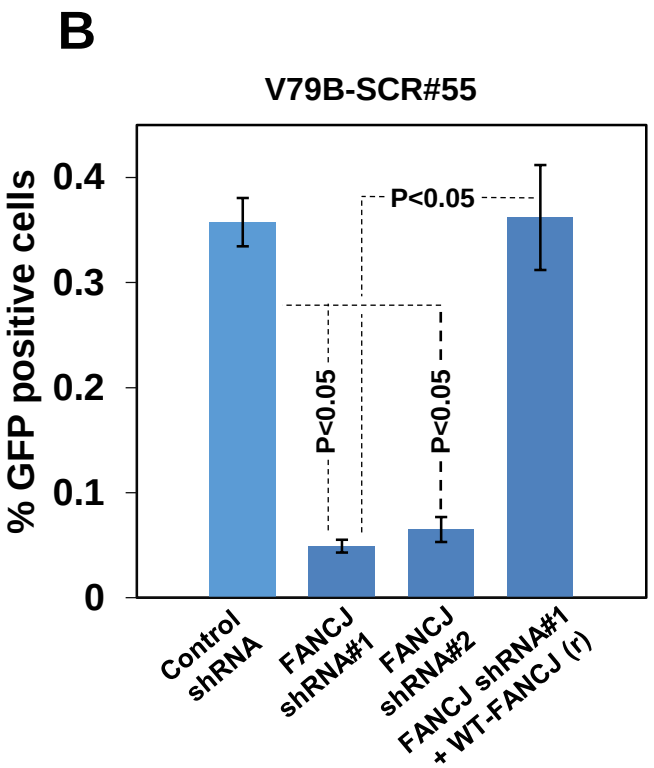


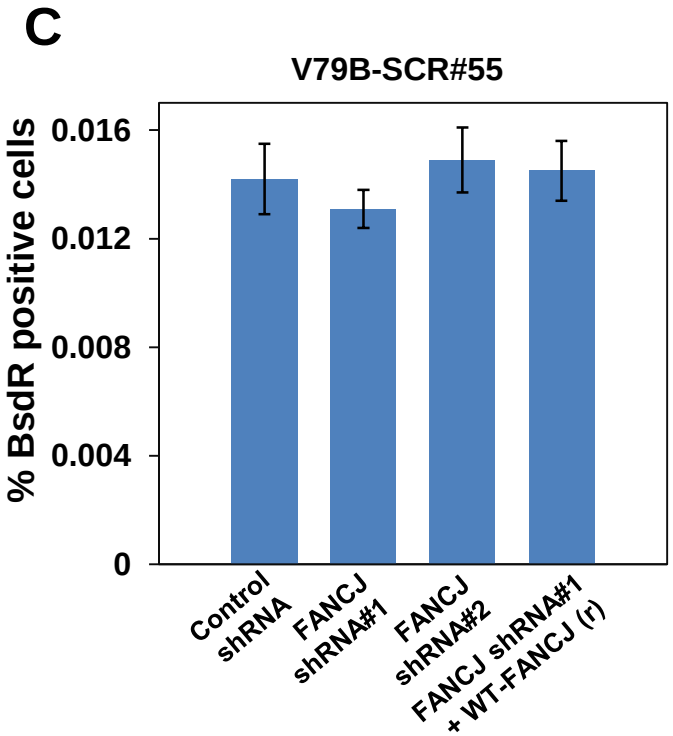
Supplementary Figure 2

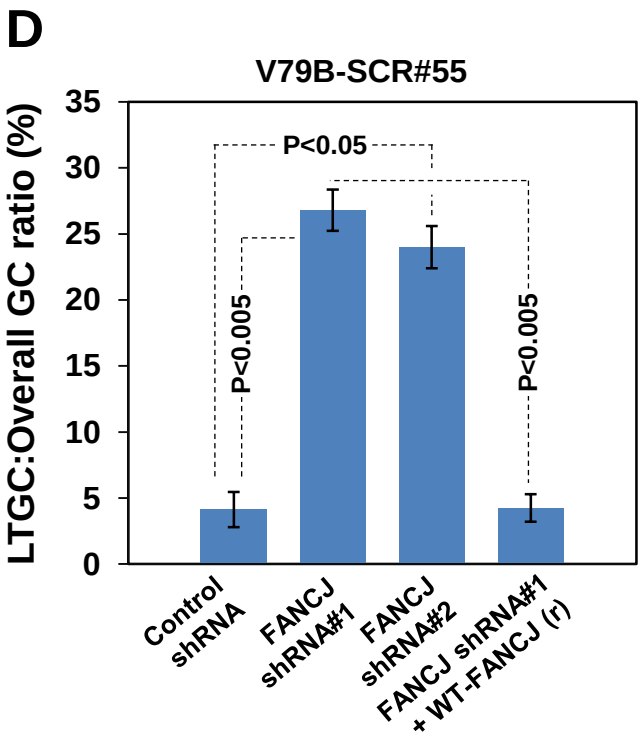


Supplementary Figure 3









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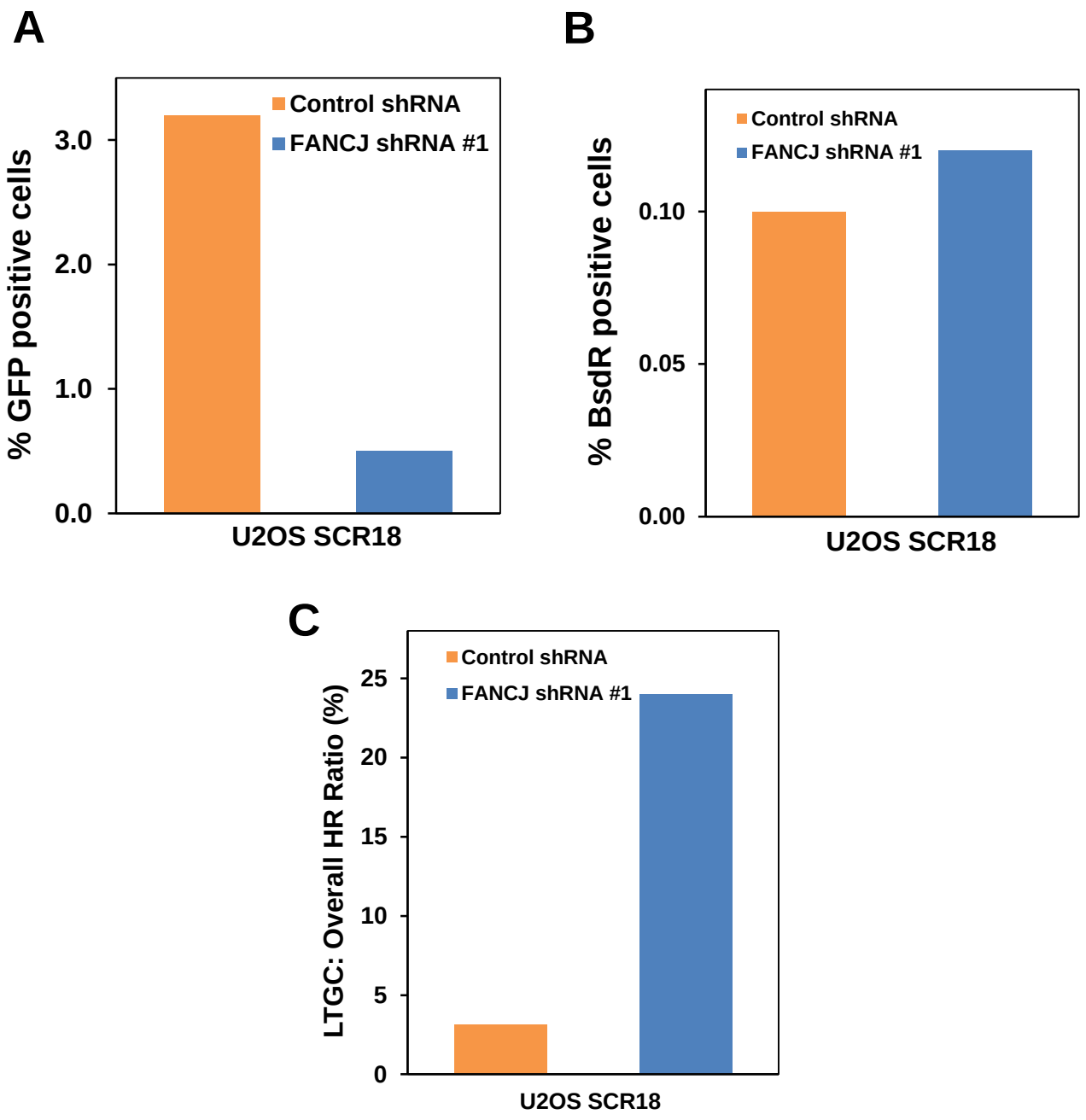
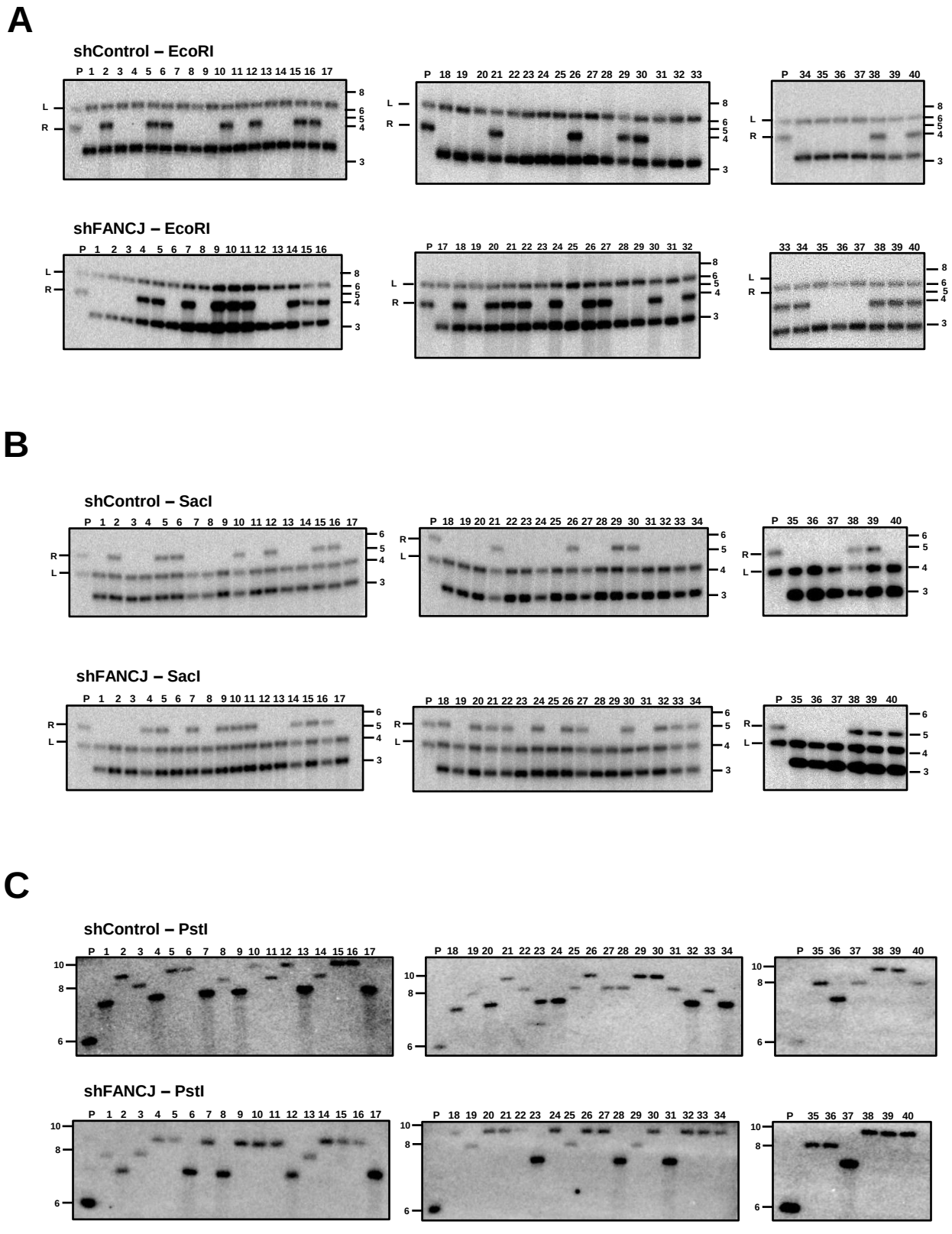
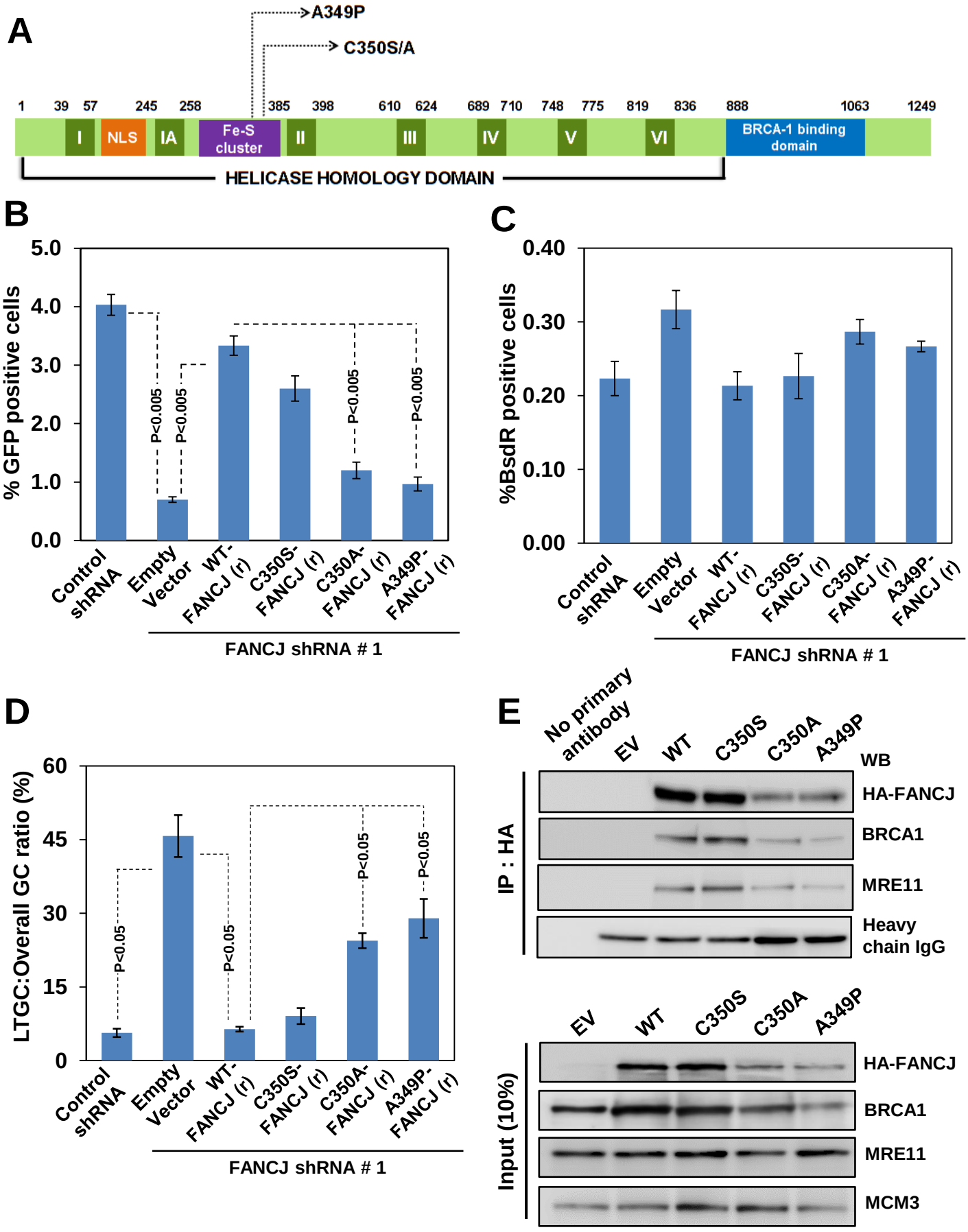


Supplementary Figure 5



Supplementary Figure 6



Supplementary Figure 7