

Supplementary Table 1. Primers and oligonucleotides used in this study.

Name	Sequence (5' to 3')	Nt	Description
Phospho-loop forward primer	GTTACAACCTCTGCTGCCAACGAGGCTTTCT GTCTGGAGATTC	44	Used to linearize the pACEBac FANCI ^{WT} and insert gBlocks encoding mutations in the phospho-loop region
Phospho-loop reverse primer	AAGTTACGCAGCAGCAGCAGAAAACCGG CTACAG	44	Used to linearize the pACEBac FANCI ^{WT}
gBlock FANCI ^{3D}	GTTTTCTGCTGCTGCTGCGTAACTTCAAGA TCCTGGGTTCTCTGACCTCTGATCAGTGTG ATCAGGCAATCGGTGCTGATCAGGTTTCAG GCAGACGTTACGCTTGTTACAACCTCTGCT GCCAACGAGGC	130	gBlock encoding S558D, S561D and T567D mutations that was inserted by Gibson cloning into linearized pACEBac FANCI ^{WT}
gBlock FANCI ^{3A}	GTTTTCTGCTGCTGCTGCGTAACTTCAAGA TCCTGGGTTCTCTGACCTCTGCCAGTGTG CACAGGCAATCGGTGCTGCCAGGTTTCAG GCAGACGTTACGCTTGTTAC AACTCTGCTGCCAACGAGGC	130	gBlock encoding S558A, S561A and T567A mutations that was inserted by Gibson cloning into linearized pACEBac FANCI ^{WT}
gBlock FANCI ^{3K}	GTTTTCTGCTGCTGCTGCGTAACTTCAAGA TCCTGGGTTCTCTGACCTCTAAACAGTGTG AACAGGCAATCGGTGCTAAACAGGTTTCAG GCAGACGTTACGCTTGTTACAACCTCT GCTGCCAACGAGGC	130	gBlock encoding S558K, S561K and T567K mutations that was inserted by Gibson cloning into linearized pACEBac FANCI ^{WT}
gBlock FANCI ^{Dxx}	GTTTTCTGCTGCTGCTGCGTAACTTCAAGA TCCTGGGTTCTCTGACCTCTGATCAGTGTG CTCAGGCAATCGGTGCTACTCAGGTTTCAG GCAGACGTTACGCTTGTTACAACCTCTGCT GCCAACGAGGC	130	gBlock encoding S558D mutation that was inserted by Gibson cloning into linearized pACEBac FANCI ^{WT}
gBlock FANCI ^{DxD}	GTTTTCTGCTGCTGCTGCGTAACTTCAAGA TCCTGGGTTCTCTGACCTCTTCCAGTGTG ATCAGGCAATCGGTGCTACTCAGGTTTCAG GCAGACGTTACGCTTGTTACAACCTCTGCT GCCAACGAGGC	130	gBlock encoding S561D mutation that was inserted by Gibson cloning into linearized pACEBac FANCI ^{WT}
gBlock FANCI ^{DxD}	GTTTTCTGCTGCTGCTGCGTAACTTCAAGA TCCTGGGTTCTCTGACCTCTTCCAGTGTG CTCAGGCAATCGGTGCTGATCAGGTTTCAG GCAGACGTTACGCTTGTTACAACCTCTGCT GCCAACGAGGC	130	gBlock encoding T567D mutation that was inserted by Gibson cloning into linearized pACEBac FANCI ^{WT}
gBlock FANCI ^{DDx}	GTTTTCTGCTGCTGCTGCGTAACTTCAAGA TCCTGGGTTCTCTGACCTCTGATCAGTGTG ATCAGGCAATCGGTGCTACTCAGGTTTCAG GCAGACGTTACGCTTGTTACAACCTCTGCT GCCAACGAGGC	130	gBlock encoding S558D and S561D mutations that was inserted by Gibson cloning into linearized pACEBac FANCI ^{WT}
gBlock FANCI ^{DxD}	GTTTTCTGCTGCTGCTGCGTAACTTCAAGA TCCTGGGTTCTCTGACCTCTGATCAGTGTG CTCAGGCAATCGGTGCTGATCAGGTTTCAG GCAGACGTTACGCTTGTTACAACCTCTGCT GCCAACGAGGC	130	gBlock encoding S558D and T567D mutations that was inserted by Gibson cloning into linearized pACEBac FANCI ^{WT}
gBlock FANCI ^{DDD}	GTTTTCTGCTGCTGCTGCGTAACTTCAAGA TCCTGGGTTCTCTGACCTCTTCCAGTGTG ATCAGGCAATCGGTGCTGATCAGGTTTCAG	130	gBlock encoding S561D and T567D mutations that was inserted by Gibson cloning

	GCAGACGTTACGCTTGTTACAACCTCTGCT GCCAACGAGGC		into linearized pACEBac FANCI ^{WT}
Loop deletion forward primer	GAGGCTTTCTGTCTGGAGATTCTGGGCTCC CTGCG	35	Primer used to delete residues 555-582 of FANCI from pACEBac FANCI ^{WT}
Loop deletion reverse primer	AGAACCCAGGATCTTGAAGTTACGCAGCA GCAGCAG	36	Primer used to delete residues 555-582 of FANCI from pACEBac FANCI ^{WT}
44-P1	ATCGATGTCTCTAGACAGCTGCTCAGGAT TGATCTGTAATGGCC	44	Annealed with 44-P7 and used in assays
44-P1/36-FAM/	ATCGATGTCTCTAGACAGCTGCTCAGGAT TGATCTGTAATGGCC/36-FAM/	44	Annealed with 44-P7 and used in all assays and for structural analysis
44-P7	GGCCATTACAGATCAATCCTGAGCAGCTG TCTAGAGACATCGAT	44	Annealed with 44-P1 or 44- P1/36-FAM/ and used in all assays and for structural analysis
Nano-lever A (NLA)	TAGTGCTGTAGGAGAATATACGGGCTGCT CGTGTTGACAAGTACTGAT	48	Tethered to the SwitchSENSE chip
Nano-lever B (NLB)	TAGTCGTAAGCTGATATGGCTGATTAGTC GGAAGCATCGAACGCTGAT	48	Tethered to the SwitchSENSE chip
Complementary NLA-stem16 (cNLA-stem16)	GCGGCATACAACACTCACAACCGCATCAG TATGTCAACACGAGCAGCCCGTATATTCC TCTACAGCACTA	72	Annealed to NLA tethered to the SwitchSENSE chip
Complementary NLB-stem16 (cNLB-stem16)	GCGGCATACAACACTCACAACCGCATCAG CGTTCGATGCTTCCGACTAATCAGCCATAT CAGCTTACGACTA	72	Annealed to NLB tethered to the SwitchSENSE chip