Supplementary material

Figure S1.

Interaction between UBL5 and FANCI

A. List of selected proteins with high SILAC (H/L) ratios identified in mass spectrometry (MS)-based analysis of UBL5-interacting proteins, as described previously [1]. HeLa cells transfected with Strep-HA-UBL5 plasmid or empty vector were cultured in heavy (H) or light (L) SILAC medium, respectively. Cell lysates were incubated with Strep-Tactin Sepharose. Bound complexes were washed extensively and analyzed by MS.

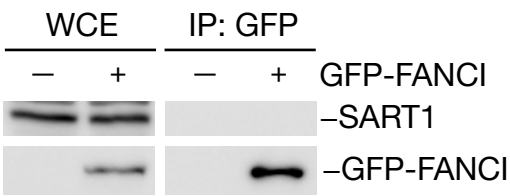
B. Whole cell extracts (WCE) of U2OS cells transfected with cDNA encoding GFP-FANCI were subjected to GFP immunoprecipitation followed by immunoblotting with SART1 and GFP antibodies.

C. U2OS cells transfected with cDNA encoding Strep-HA-tagged UBL5 were treated or not with 1 μM mitomycin C (MMC) for 18 h. Whole cell extracts (WCE) were subjected to Strep-Tactin pull-down followed by immunoblotting with FANCI and HA antibodies. *, non-specific band.

A

Protein symbol	SILAC ratio (H/L)
SART1	9.21
*UBL5	7.28
MFAP1	5.87
WBP11	4.79
NPW38	3.76
PRPF38A	3.70
FANCI	3.09
EFTUD2	2.97
SNRPD1	2.75
PRPF8	2.69
SNRPD3	2.66

B



C

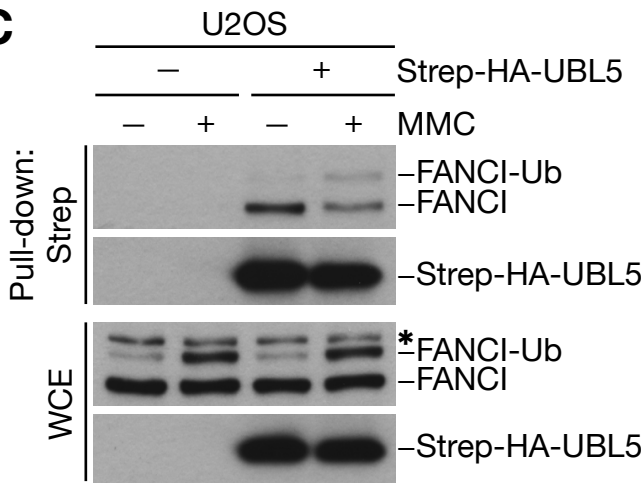


Figure S2.

UBL5 is required for the integrity of the FA pathway

A. Extracts of HeLa cells transfected with UBL5 siRNAs were analyzed by immunoblotting with indicated antibodies.

B. Extracts of HeLa cells transfected with non-targeting (CTRL) or FANCI siRNAs were analyzed by immunoblotting with indicated antibodies.

C. U2OS cells were transfected with non-targeting control (CTRL) or UBL5 siRNA, subsequently transfected with a construct encoding HA-FANCI and then treated with 0.2 μM MMC for 24 h. Cells were fixed and immunostained with HA antibody. Representative images are shown. Arrows indicate cells transiently expressing HA-FANCI. Scale bar, 10 μm. For quantification, 200 cells were counted in each experiment. Mean values (±SD) from three independent experiments are shown.

D. Extracts of U2OS cells transfected with indicated siRNAs were analyzed by immunoblotting. See also Fig. 2F.

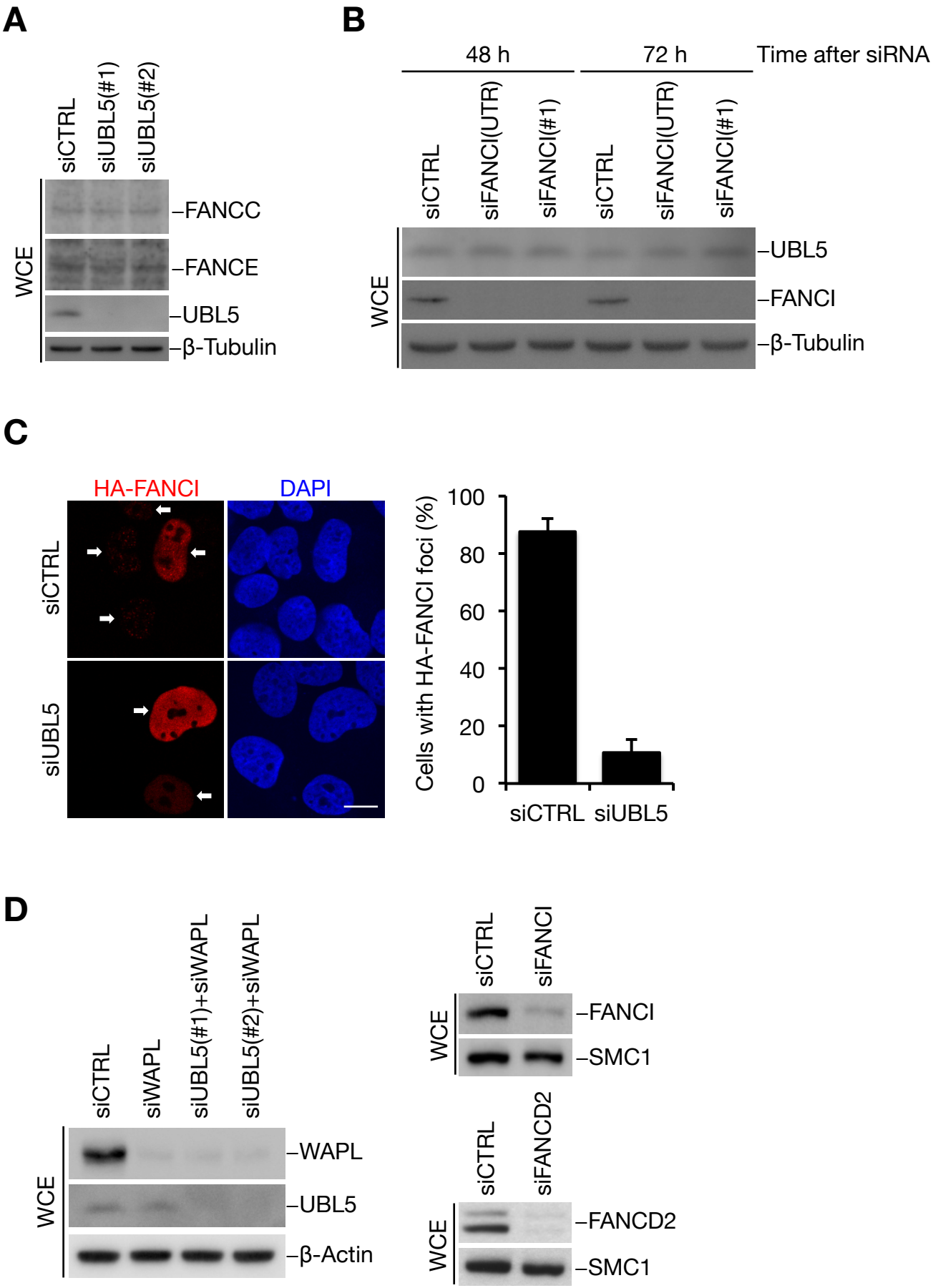

A
siCTRL
siUBL5(#1)
siUBL5(#2)
WCE
FANCC
FANCE
UBL5
β-Tubulin
B
48 h
72 h
Time after siRNA
siCTRL
siFANCI(UTR)
siFANCI(#1)
siCTRL
siFANCI(UTR)
siFANCI(#1)
WCE
UBL5
FANCI
β-Tubulin
C
HA-FANCI
DAPI
siCTRL
siUBL5
Cells with HA-FANCI foci (%)
100
80
60
40
20
0
siCTRL
siUBL5
D
siCTRL
siWAPL
siUBL5(#1)+siWAPL
siUBL5(#2)+siWAPL
WCE
WAPL
UBL5
β-Actin
siCTRL
siFANCI
WCE
FANCI
SMC1
siCTRL
siFANCD2
WCE
FANCD2
SMC1

Figure S3.

UBL5 has a direct role uncoupled from its pre-mRNA splicing involvement in promoting the FA pathway

A. HeLa cells were transfected with non-targeting control (CTRL), UBL5 or SART1 siRNAs. Total mRNAs were extracted and levels of FANCI mRNA were measured by reverse transcription-qPCR, normalized to the expression of β-Actin.

B. U2OS cells stably expressing GFP-tagged FANCI and transfected with non-targeting control (CTRL) or UBL5 siRNAs were harvested following addition of MG132. Cell lysates were immunoblotted with GFP, UBL5 and β-Tubulin antibodies.

C. Whole cell extracts (WCE) of HeLa cells transfected with indicated Strep-HA-UBL5 constructs were subjected to Strep-Tactin pull-down followed by immunoblotting with FANCI and HA antibodies.

D. U2OS cells stably expressing siRNA-resistant (si^R) forms of Strep-HA-UBL5 WT or D64A mutant were induced or not with doxycycline, transfected with non-targeting control (CTRL) or UBL5 siRNAs and treated with MMC for 24 h. Cell extracts were analyzed by immunoblotting with indicated antibodies.

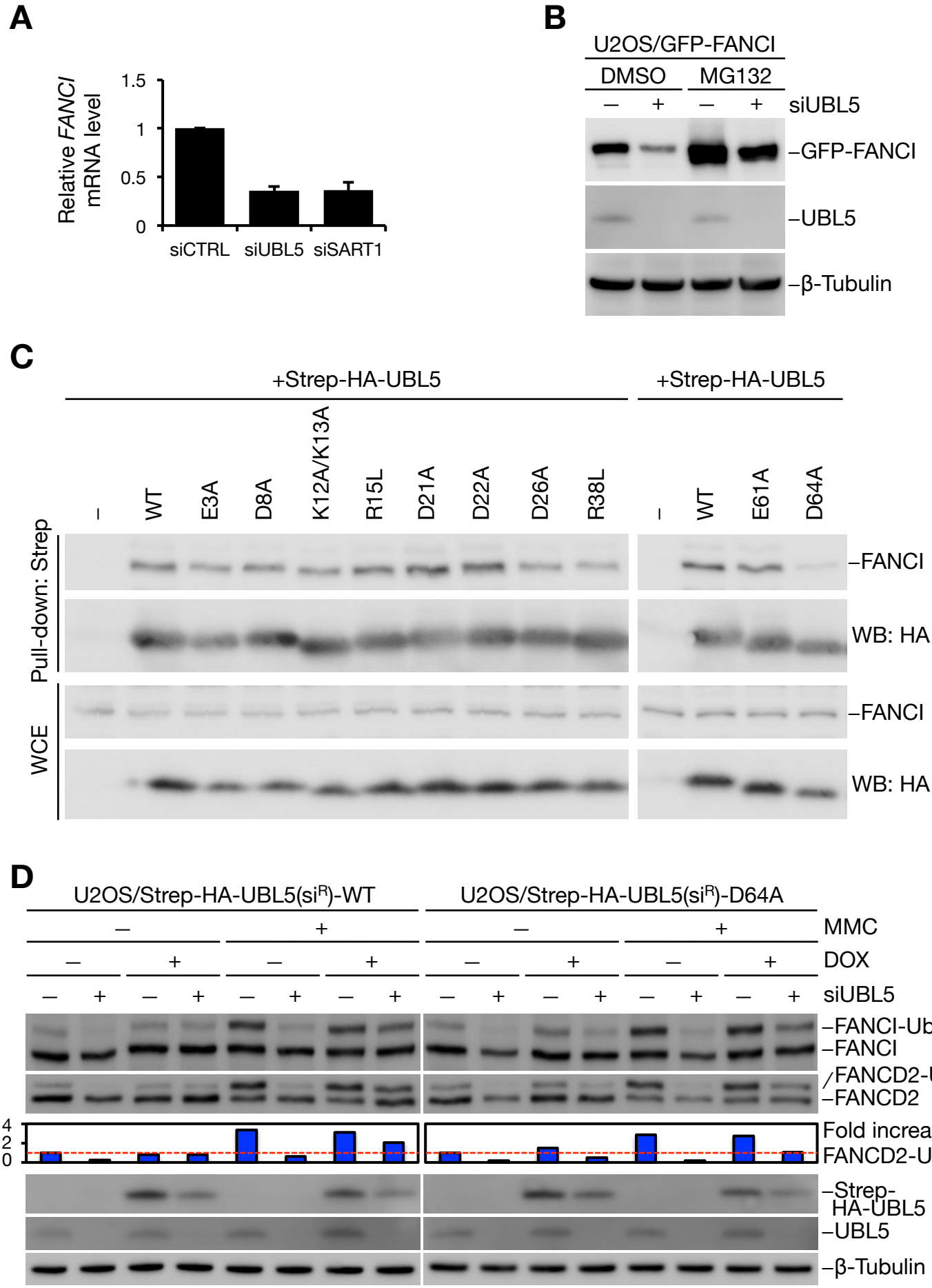

A
Relative FANCI mRNA level
1.5
1
0.5
0
siCTRL
siUBL5
siSART1
B
U2OS/GFP-FANCI
DMSO
MG132
– + – +
siUBL5
–GFP-FANCI
–UBL5
–β-Tubulin
C
+Strep-HA-UBL5
+Strep-HA-UBL5
– WT E3A D8A K12A/K13A R15L D21A D22A D26A R38L
– WT E61A D64A
Pull-down: Strep
WCE
–FANCI
WB: HA
–FANCI
WB: HA
D
U2OS/Strep-HA-UBL5(siR)-WT
U2OS/Strep-HA-UBL5(siR)-D64A
MMC
DOX
siUBL5
–FANCI-Ub
–FANCI
/FANCD2-Ub
–FANCD2
4
2
0
Fold increase of FANCD2-Ub
–Strep-HA-UBL5
–UBL5
–β-Tubulin

Figure S4.

Binding of FANCI fragments to FANCD2

Whole cell extracts (WCE) of HEK293T cells transfected with indicated constructs were subjected to GFP immunoprecipitation followed by immunoblotting with FANCD2 and GFP antibodies.

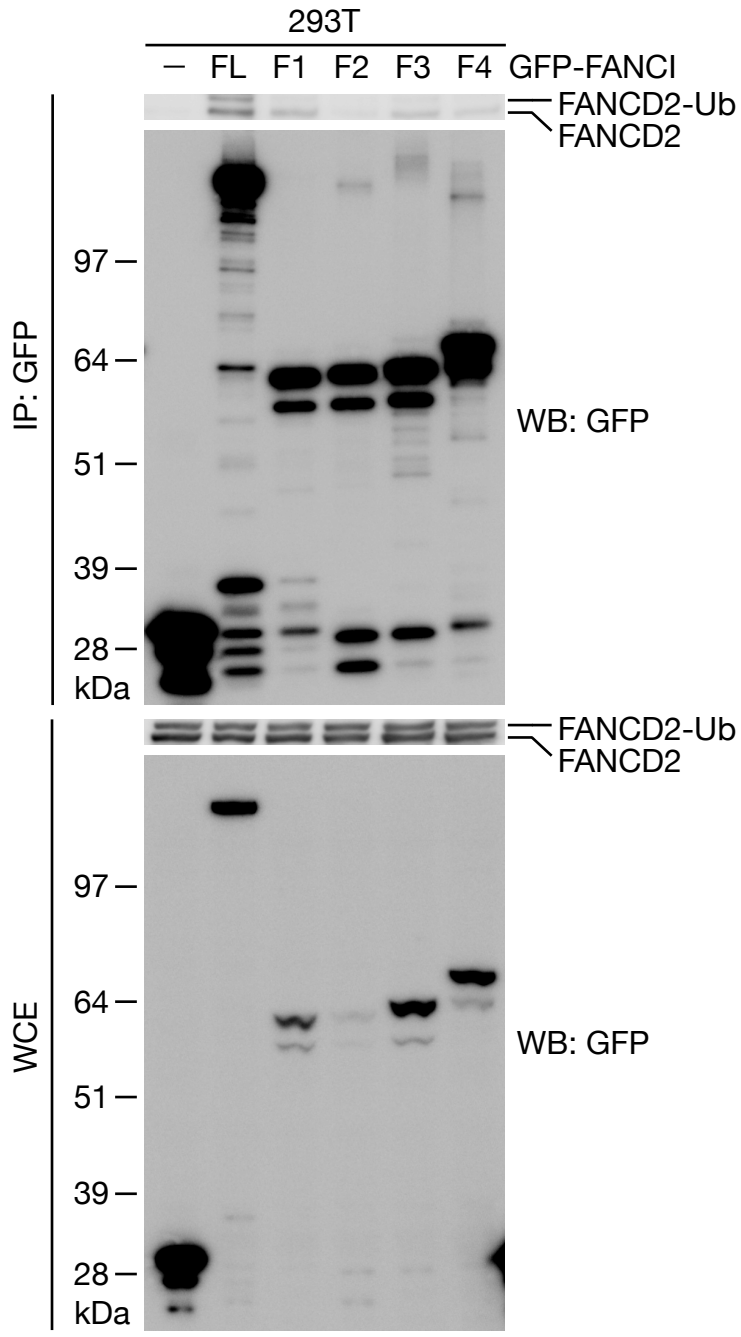

293T
–
FL
F1
F2
F3
F4
GFP-FANCI
FANCD2-Ub
FANCD2
IP: GFP
97
64
51
39
28
kDa
WB: GFP
FANCD2-Ub
FANCD2
WCE
97
64
51
39
28
kDa
WB: GFP

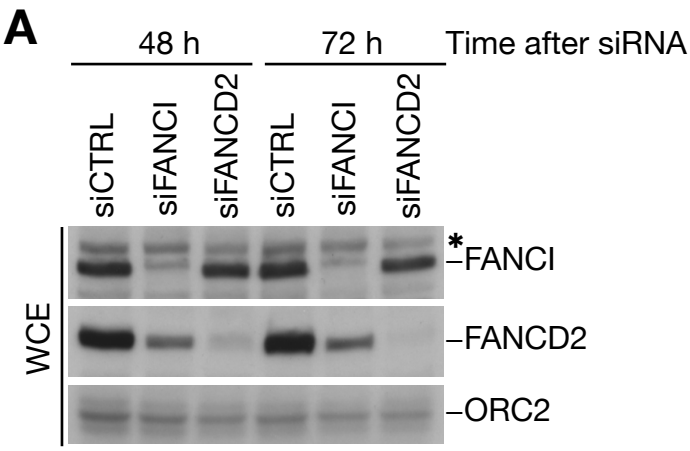
Figure S5.

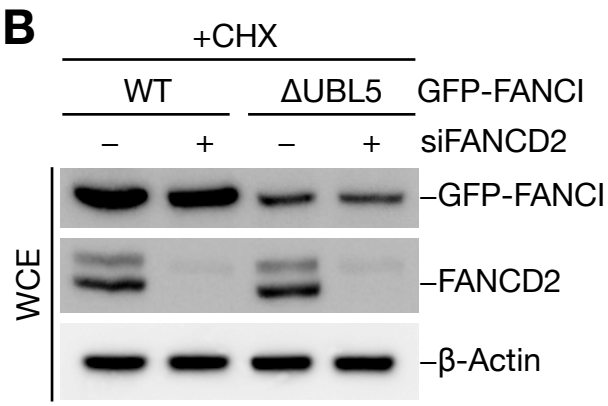
Loss of UBL5 binding impairs the stability and activation of FANCI, while FANCD2 knockdown does not affect FANCI stability.

A. Extracts of HeLa cells transfected with non-targeting control (CTRL), FANCI or FANCD2 siRNAs for 48 or 72 h were analyzed by immunoblotting with antibodies to FANCI, FANCD2 or ORC2 (loading control).

B. HeLa cells transfected with non-targeting control or FANCD2 siRNA and subsequently transfected with GFP-tagged FANCI WT or ΔUBL5 were treated with cycloheximide (CHX) for 3 h. Cell extracts were analyzed by immunoblotting with indicated antibodies.

C. U2OS cells stably expressing HA-tagged FANCI WT or ΔUBL5 were treated or not with 30 μM ML323 for 6 h. Cell extracts were analyzed by immunoblotting with HA and SMC1 antibodies.


A
48 h
72 h
Time after siRNA
siCTRL
siFANCI
siFANCD2
siCTRL
siFANCI
siFANCD2
WCE
*
–FANCI
–FANCD2
–ORC2


B
+CHX
WT
ΔUBL5
GFP-FANCI
–
+
–
+
siFANCD2
WCE
–GFP-FANCI
–FANCD2
–β-Actin

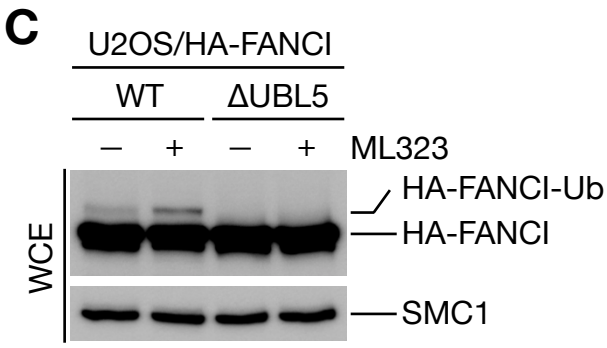

C
U2OS/HA-FANCI
WT
ΔUBL5
–
+
–
+
ML323
WCE
HA-FANCI-Ub
HA-FANCI
SMC1

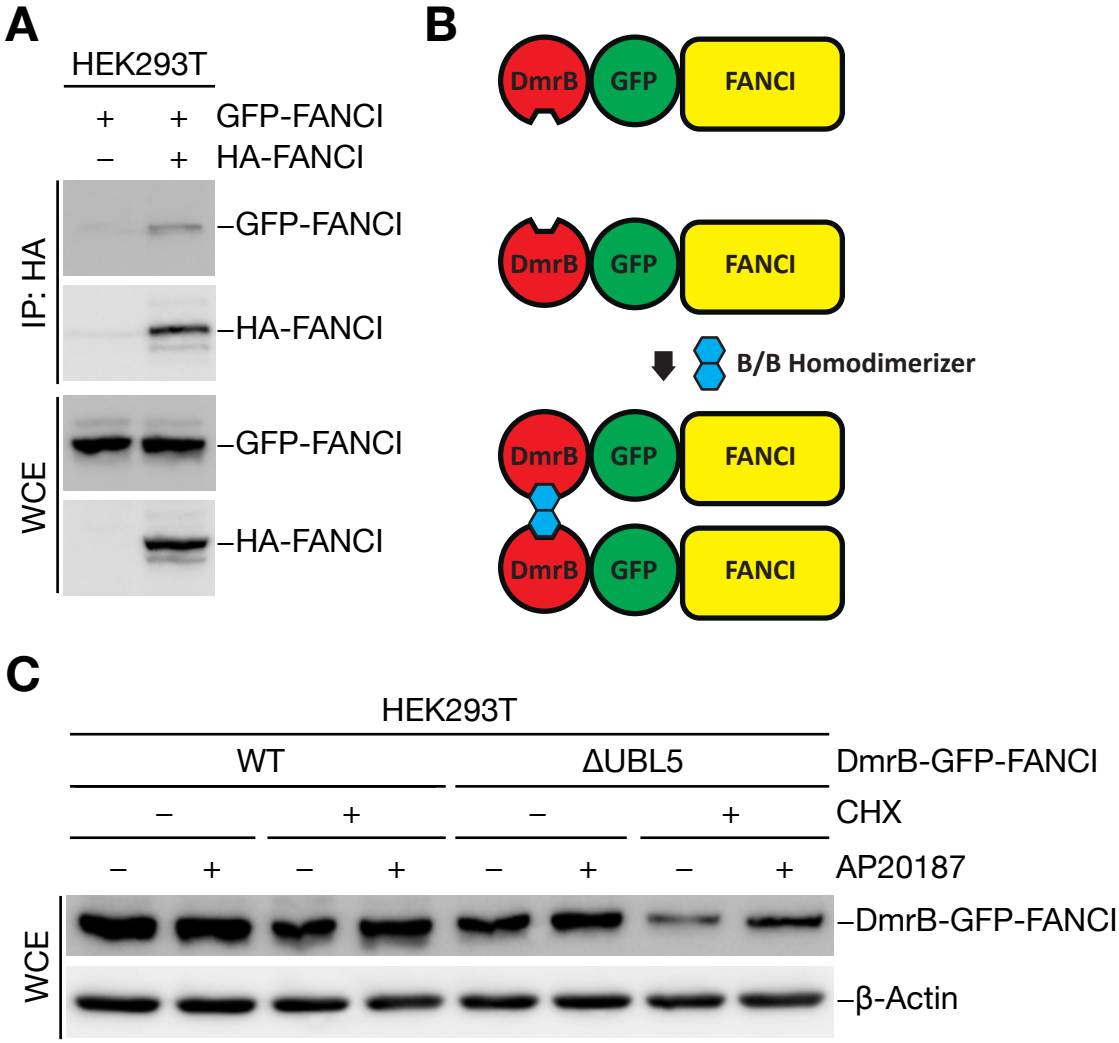
Figure S6.

UBL5 promotes FANCI stability by facilitating its homodimerization

A. Extracts of HEK293T cells co-transfected with GFP-FANCI and HA-FANCI constructs as indicated were subjected to HA immunoprecipitation followed by immunoblotting with GFP and HA antibodies.

B. Schematic diagram of enforced homodimerization of FANCI by means of the DmrB system.

C. HEK293T cells transfected with plasmids encoding DmrB-GFP-FANCI WT or ΔUBL5 were treated with 100 nM AP20187 (B/B Homodimerizer) or left untreated, and then treated with cycloheximide (CHX). Cell extracts were analyzed by immunoblotting with GFP and β-Actin antibodies.


A
HEK293T
+ + GFP-FANCI
– + HA-FANCI
IP: HA
–GFP-FANCI
–HA-FANCI
WCE
–GFP-FANCI
–HA-FANCI
B
DmrB
GFP
FANCI
B/B Homodimerizer
C
HEK293T
WT
ΔUBL5
DmrB-GFP-FANCI
CHX
AP20187
WCE
–DmrB-GFP-FANCI
–β-Actin

Supplementary reference

1. Oka, Y. et al. UBL5 is essential for pre-mRNA splicing and sister chromatid cohesion in human cells. EMBO Rep 15, 956-64 (2014).