

Supplementary Materials for

Fanconi anemia A protein participates in nucleolar homeostasis maintenance and ribosome biogenesis

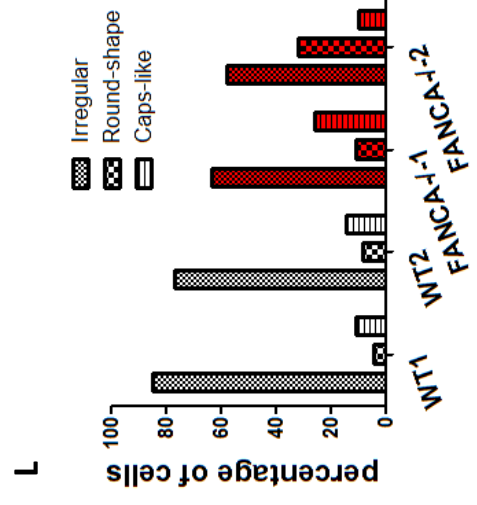
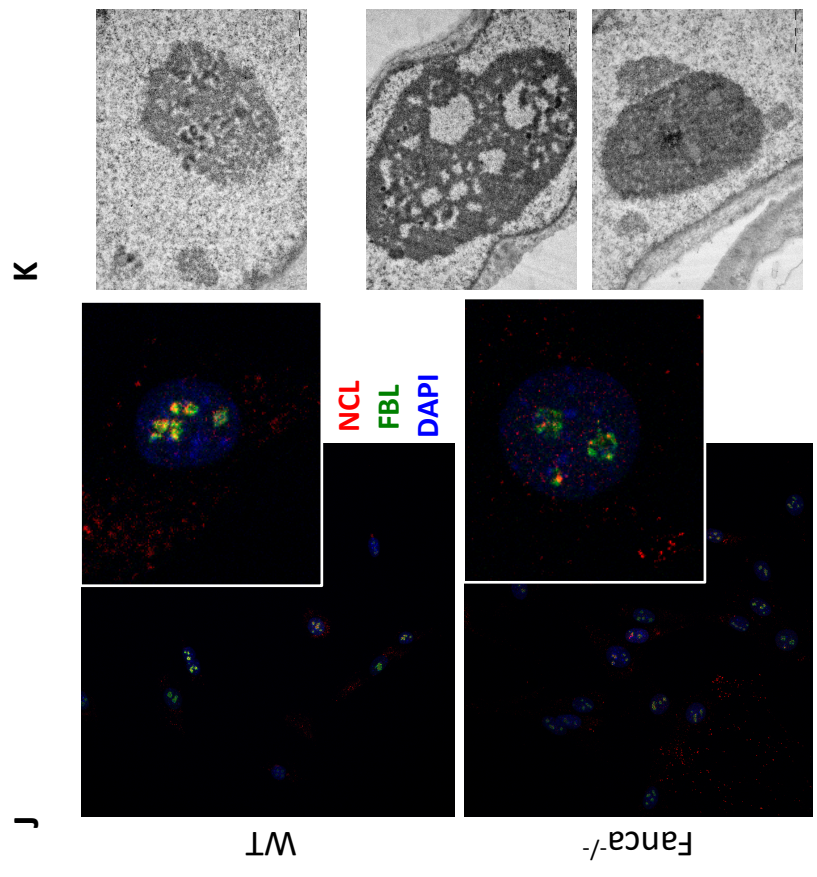
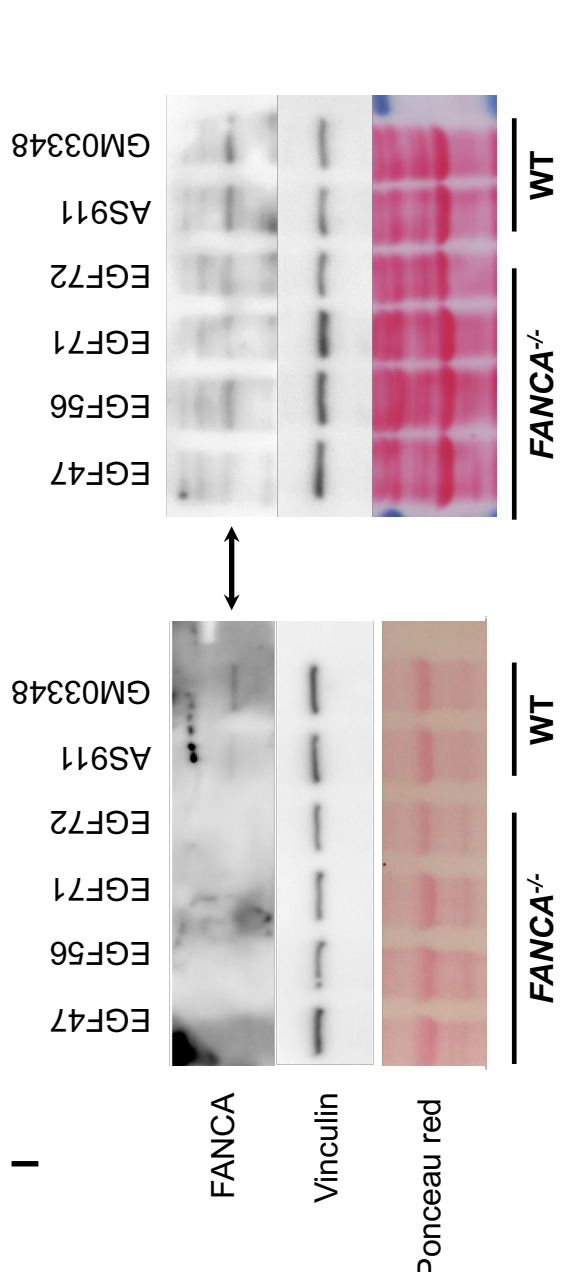
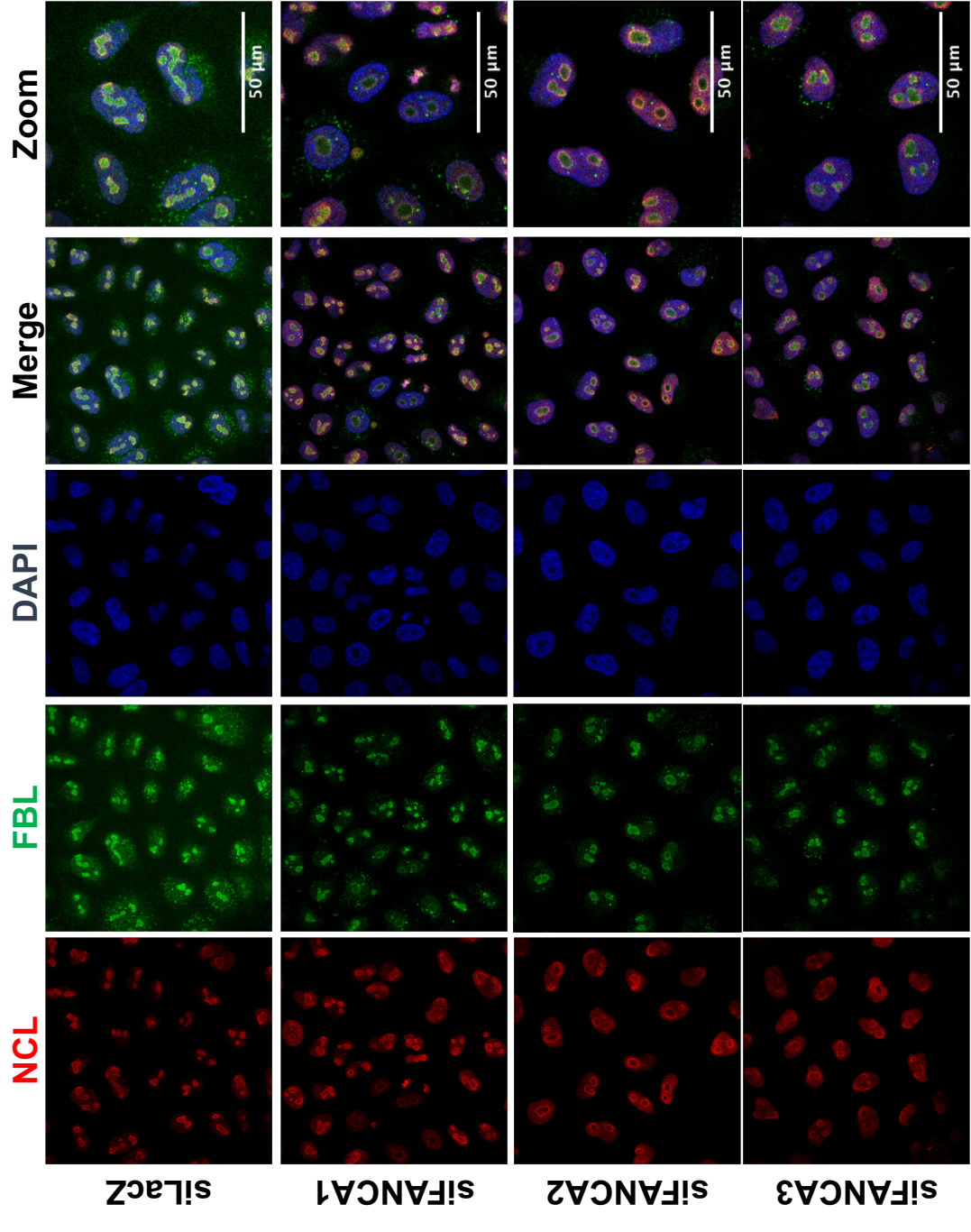
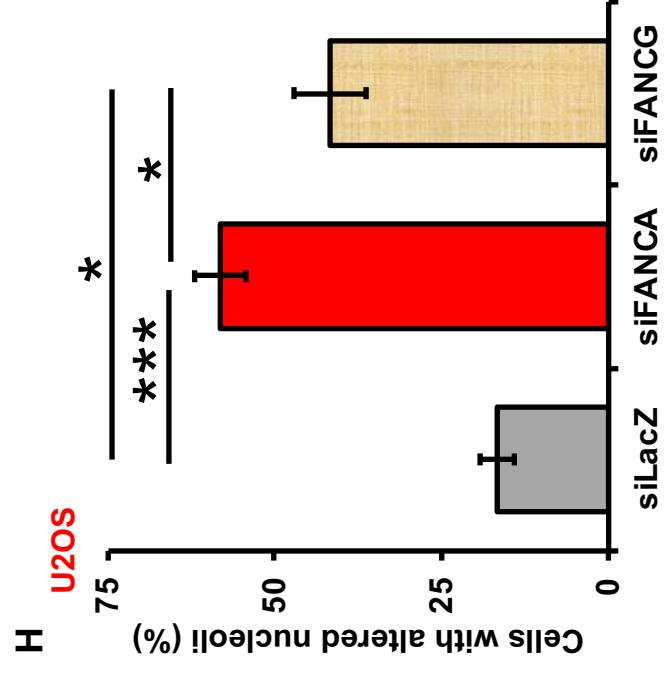
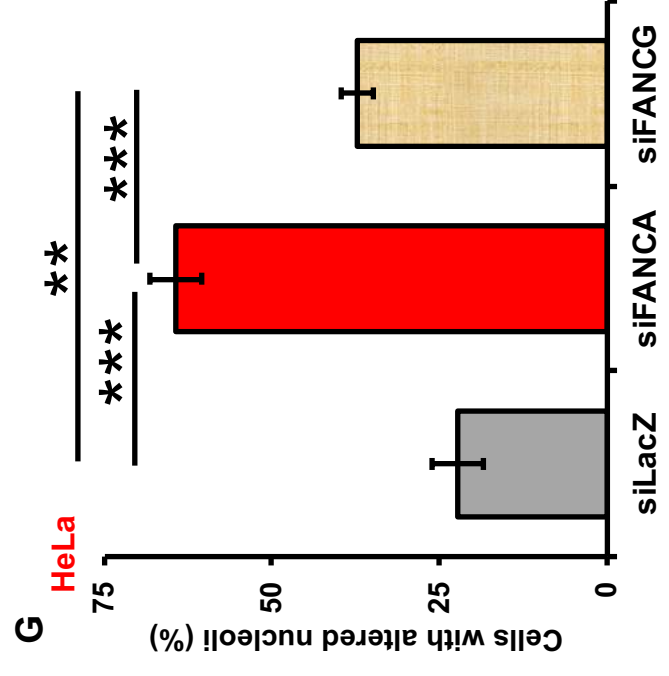
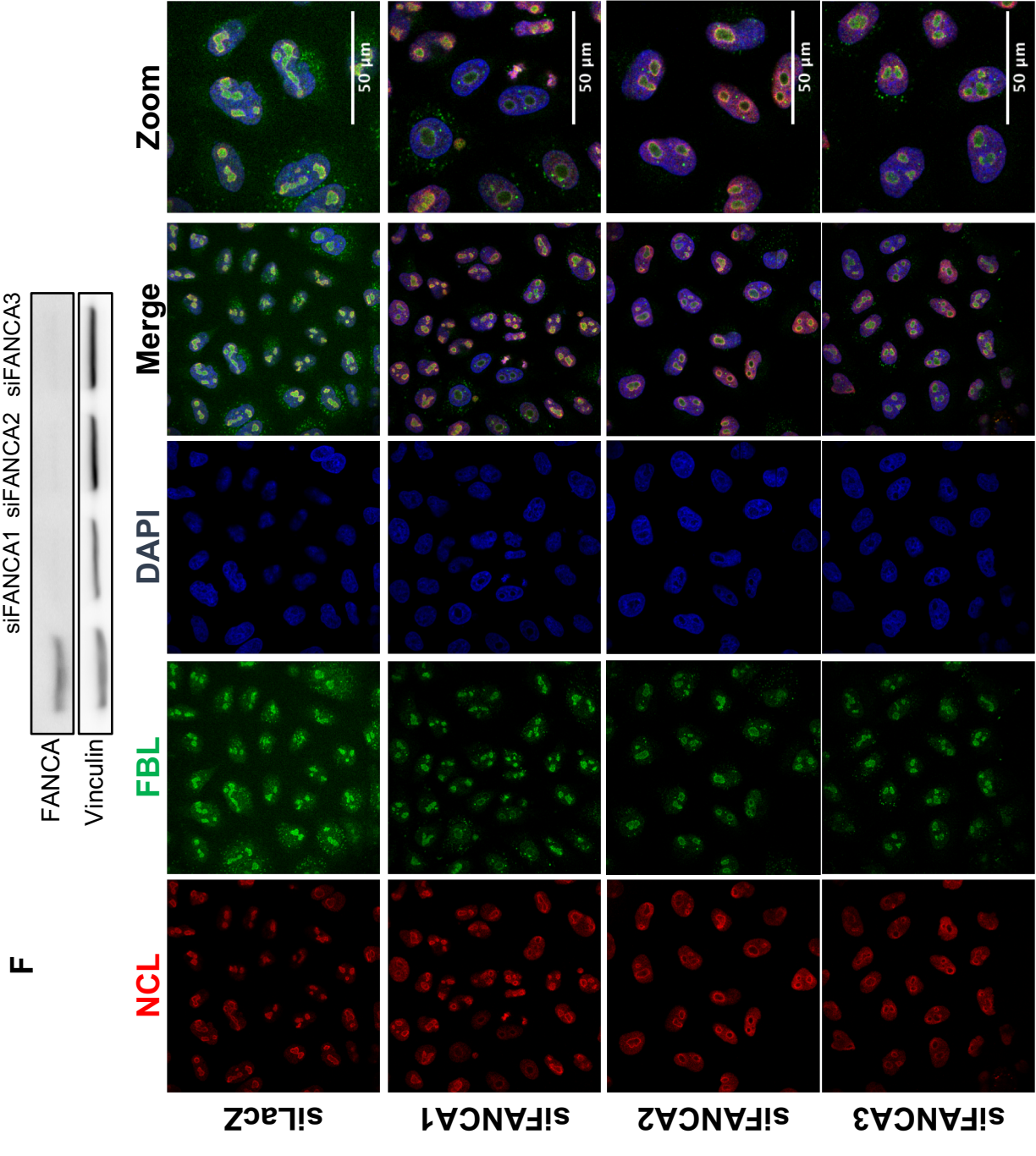
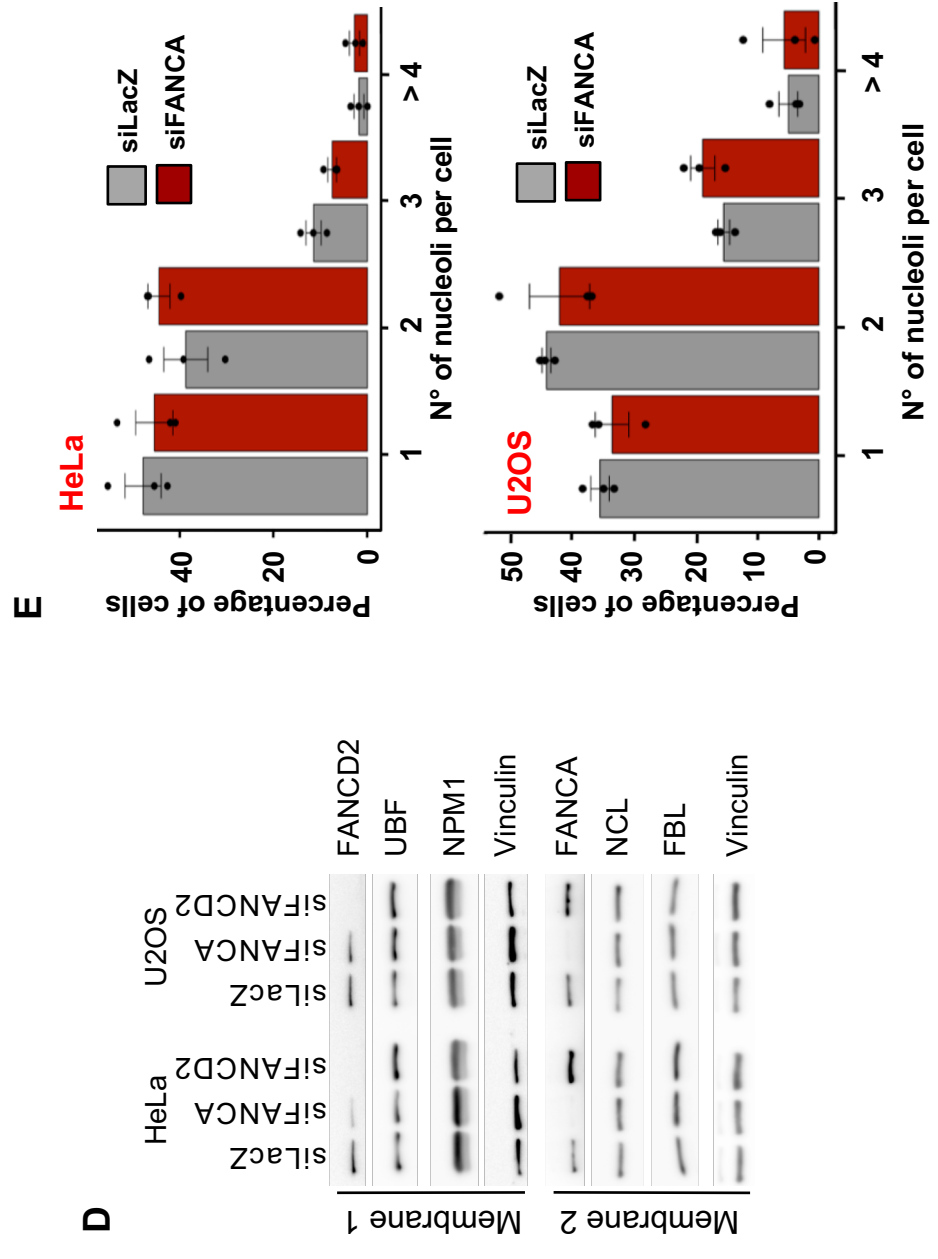
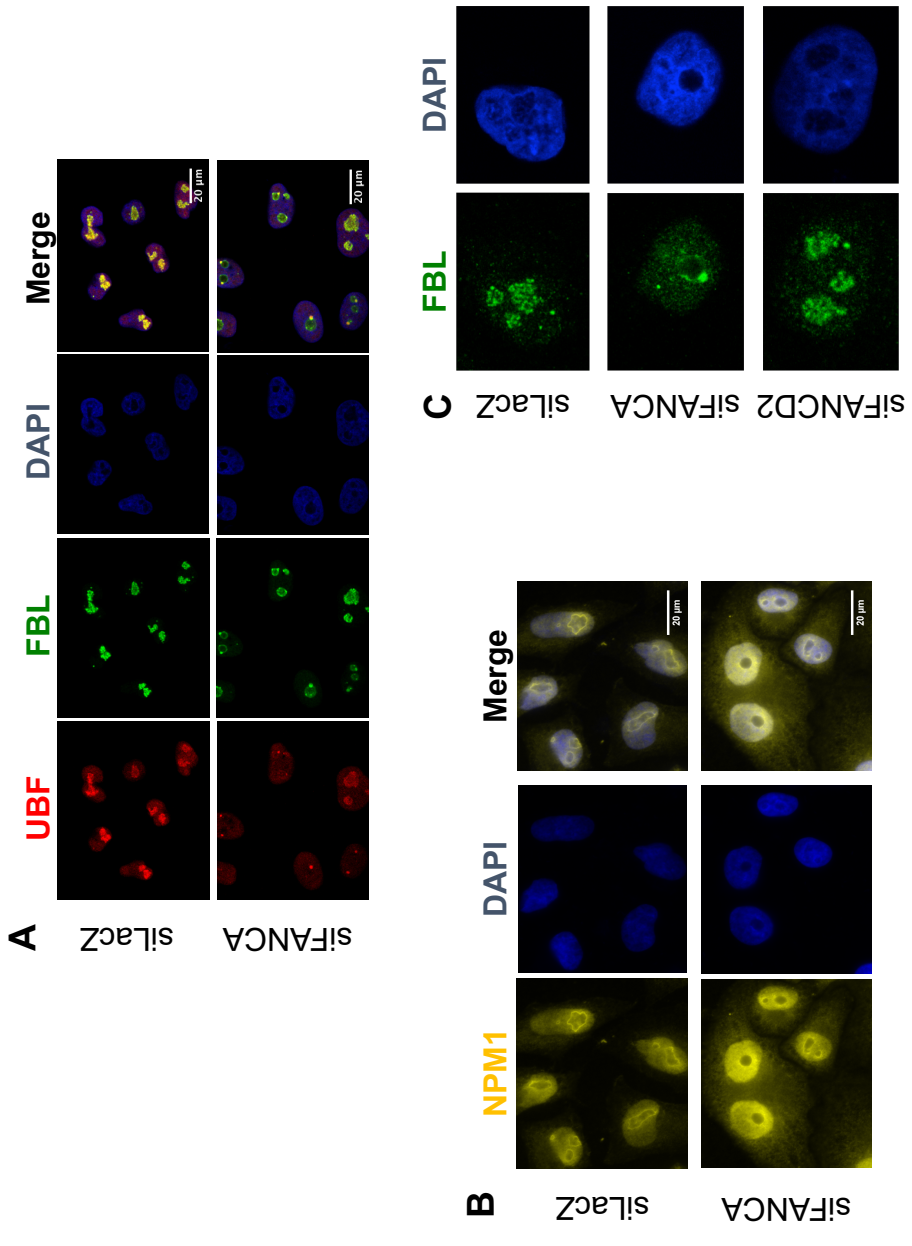
Anna Gueiderikh, Frédérique Maczkowiak-Chartois, Guillaume Rouvet, Sylvie Souquère-Besse, Sébastien Apcher, Jean-Jacques Diaz, Filippo Rosselli*

*Corresponding author. Email: filippo.rosselli@gustaveroussy.fr

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This PDF file includes:

Figs. S1 to S3
Tables S1 to S5
References



Supplementary Figure S1: Nucleolar abnormalities in FANCA pathway-deficient cells.

A. Confocal microscopy images showing wide fields of HeLa cells after transfection with untargeted (siLacZ) or FANCA-targeted siRNAs stained with antibodies against the nucleolar proteins UBF (Red) and FBL (Green) and counterstained with DAPI to visualize DNA

B. Confocal microscopy images showing wide fields of HeLa cells after transfection with untargeted (siLacZ) or FANCA-targeted siRNAs stained with antibodies against the nucleolar protein NPM1 (yellow) counterstained with DAPI to visualize DNA.

C. Confocal microscopy images showing single HeLa cells after transfection with untargeted (siLacZ), FANCA- or FANCD2-targeted siRNAs stained with antibodies against the nucleolar protein FBL (Green) and counterstained with DAPI to visualize DNA.

D. Western blot showing the consequence of FANCA or FANCD2 siRNA-mediated depletion in HeLa or U2OS cells 72 h after transfection on FANCA, FANCD2, NPM1, NCL and FBL expression. Vinculin was utilized as a loading control.

E. Percentage of cells showing 1, 2 or more nucleoli as a function of FANCA expression in HeLa (top panel) or U2OS (bottom panel) cells. Bars represent the means of 3 independent experiments +/- sem.

F. Immunofluorescence microscopy images showing wide fields of HeLa cells 72 h after transfection with untargeted (siLacZ) or one of the three siFANCA1 to siFANCA3 siRNAs that were pooled are shown in Fig. 1a. Cells were stained with antibodies against the nucleolar proteins NCL (Red) and FBL (Green) and counterstained with DAPI to visualize DNA. Top : WB illustrating the shutdown of FANCA expression induced by each single siRNA is shown. Vinculin was utilized as a loading control. Bars represent the means of 3 independent experiments +/- sem.

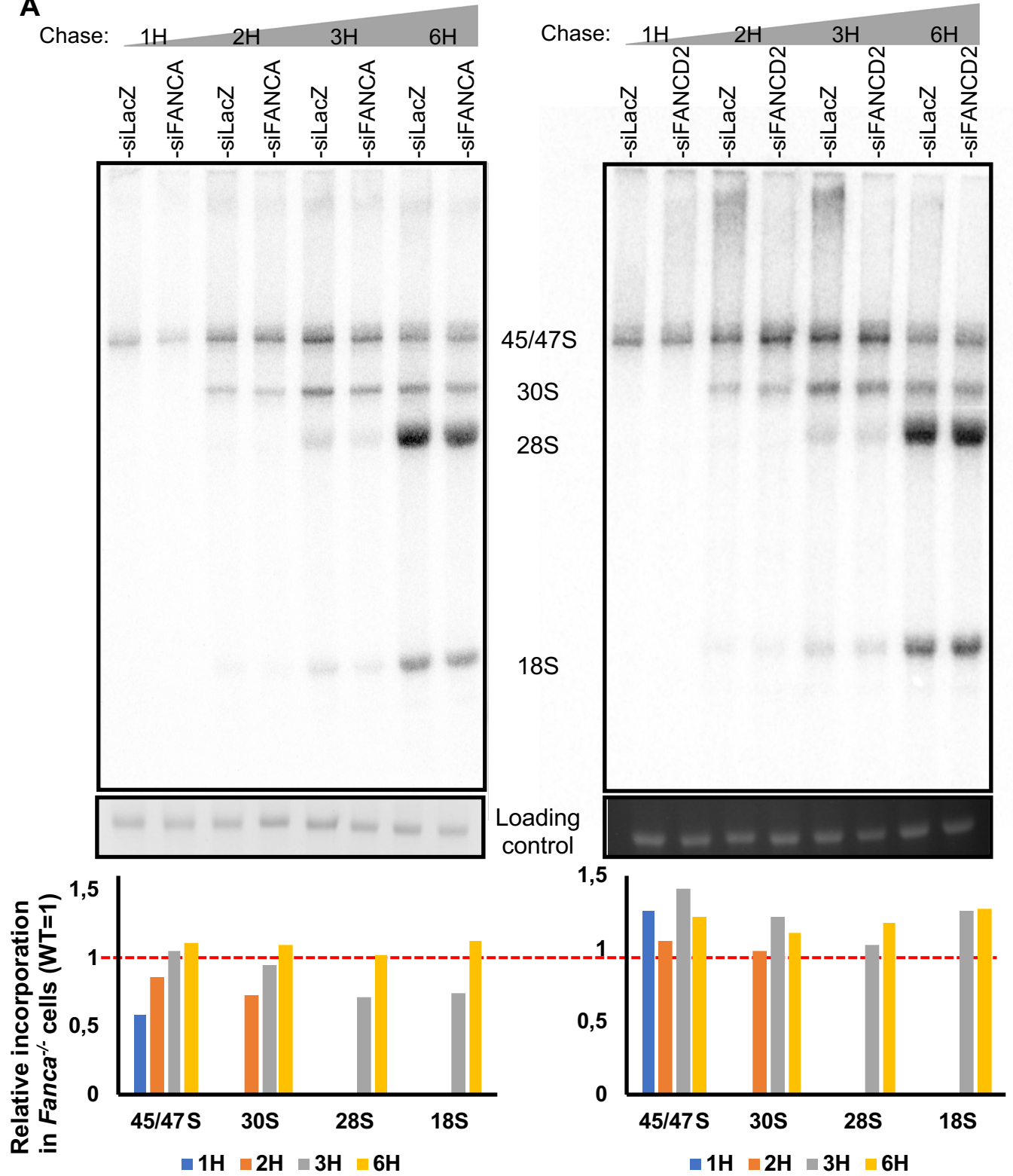
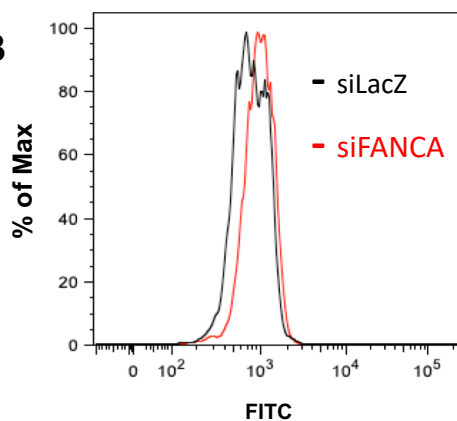
G and H. Percentage of HeLa (G) or U2OS (H) cells presenting nucleolar abnormalities (a round or cap-like shape) following transfection with the indicated siRNA. The presented data originate from an independent set of experiments that are presented in Figure 1.

I. Western blots showing the expression of FANCA in human primary fibroblasts.

J. Immunofluorescence microscopy images showing single nuclei of mouse primary fibroblasts from 3- to 5-month-old WT or *Fanca*^{-/-} mice stained as described above.

K. Electronic micrographs showing nucleolar morphology as observed in mouse primary fibroblasts from WT (i) or *Fanca*^{-/-} mouse fibroblasts (ii and iii).

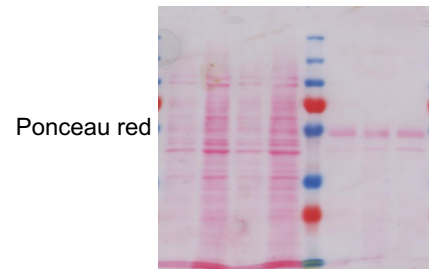
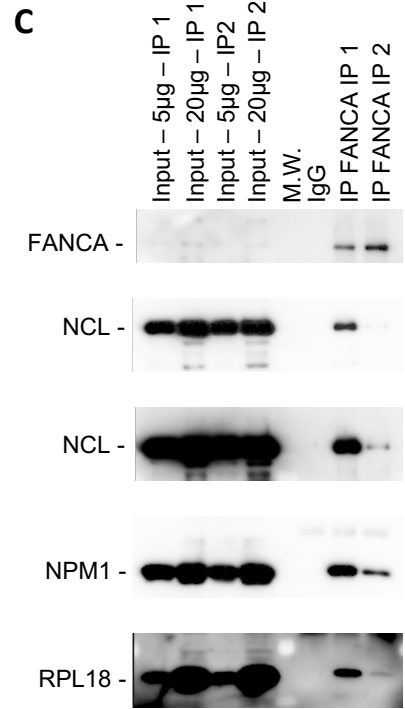
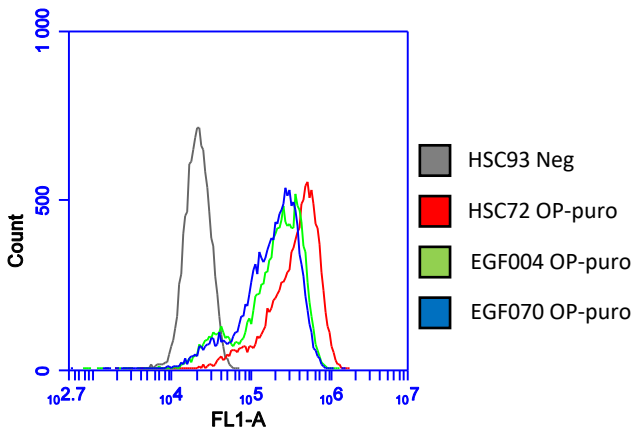
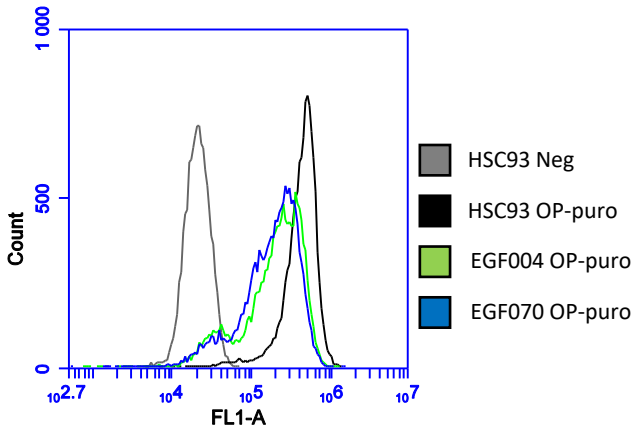
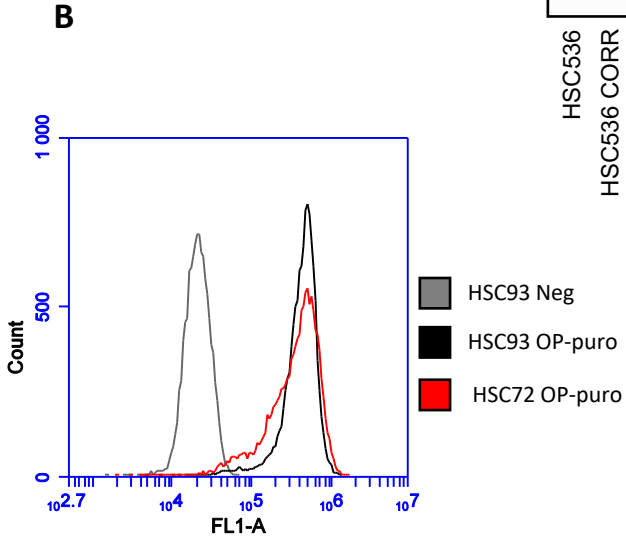
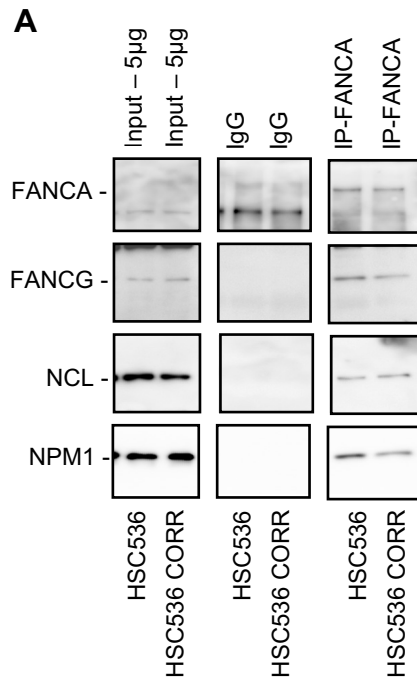
L. Percentage of mouse primary fibroblasts presenting canonical nucleolar morphology (irregular shape) or a round or cap-like shape. Analyses of fibroblasts isolated from two WT and two *Fanca*^{-/-} mice are reported.

A**B**

Supplementary Figure S2: rDNA transcription and rRNA processing in FANC pathway-deficient cells.

A. Representative experiments showing precursor and mature rRNAs. After 72 h of siRNA transfection, HeLa cells were labelled for 20 min with ^{32}P -orthophosphate and chased with cold orthophosphate for 1, 2, 3 and 6 h. The EtBr-stained gel is shown at the bottom as a loading control. Bottom, relative level of different rRNA forms in siFANCA- or FANCD2-transfected cells normalized vs the FANC proficient cells settled to 1 each time.

B. Example of FACS analysis measuring the level of EU incorporation into cells following 15 min of incubation. The black line represents the profile of HeLa cells transfected with an untargeted siRNA, and the red line shows the profile of HeLa cells 72 h following transfection with siRNAs targeting FANCA.



Supplementary Figure S3: FANCA partnership in ribosome biogenesis.

A. Western blot illustrating that FANCG and NCL proteins coimmunoprecipitate with FANCA in FANCC-deficient and FANCC-proficient human lymphoblasts issued from an FA patient.

B. Example FACS analysis measuring the level of OP-puro incorporation into cells following 30 min of incubation. The black line on the left represents the profile of unstained FANCC proficient (HSC93) cells.

C. Western blot illustrating that FANCA coimmunoprecipitates NCL, NPM1 and RPL18 in HSC93 FANCC pathway-proficient lymphoblasts. Two different IPs (IP1 and IP2) realized at one week intervals are reported.

Supplementary Table 1: list of the cell lines used.

Cell line	Cell type	FANC pathway status	Origin	Research Resource Identifier (RRID)
HeLa	Human papillomavirus-adenocarcinoma	WT	In house and ATCC	CVCL_0030
U2OS	Osteosarcoma	WT	In house and ATCC	CVCL_0042
HEK293	Human embryonic kidney	WT	In house	CVCL_0045
HSC93	EBV-immortalized lymphoblasts	WT	Gift of M. Buchwald lab, received in the 90s	CVCL_G049
HSC72	EBV-immortalized lymphoblasts	<i>FANCA</i> ^{-/-} Homozygous Ex18-28del	Gift of M. Buchwald lab, received in the 90s & Coriell Repository	CVCL_AK37
HSC99	EBV-immortalized lymphoblasts	Not determined	Gift of M. Buchwald lab, received in the 90s	CVCL_G050)
HSC536	EBV-immortalized lymphoblasts	<i>FANCC</i> ^{-/-} p.Leu554Pro (c.1661T>C); ?	Gift of M. Buchwald lab, received in the 90s & Coriell Repository	CVCL_G045
HSC72CORR	EBV-immortalized lymphoblasts	<i>FANCA</i> ^{-/-} + <i>FANCA</i>	Gift of M. Buchwald lab, received in the 90s	#
HSC536CORR	EBV-immortalized lymphoblasts	<i>FANCC</i> ^{-/-} + <i>FANCC</i>	Gift of M. Buchwald lab, received in the 90s	#
EGF004	EBV-immortalized lymphoblasts	<i>FANCG</i> ^{-/-}	Gift of J Soulier lab	#
EGF070	EBV-immortalized lymphoblasts	<i>FANCG</i> ^{-/-}	Gift of J Soulier lab	#
MRC5	Human primary fibroblasts	WT	In house	CVCL_0440
MRC5-SV	SV40-immortalized fibroblasts	WT	In house	#
GM03657	EBV-immortalized lymphoblasts	WT	Coriell Repository	CVCL_7398
GM03348	Human primary fibroblasts	WT	Coriell Repository	CVCL_7382
GM03652	Human primary fibroblasts	WT	Coriell Repository	CVCL_7397
GM05757	Human primary fibroblasts	WT	Coriell Repository	CVCL_7437
GM16754	Human primary fibroblasts	<i>FANCC</i> ^{-/-}	Coriell Repository	CVCL_AK43
GM00449	Human primary fibroblasts	<i>FANCC</i> ^{-/-} Homozygous c.456+4A>T (IVS4+4A>T)	Coriell Repository	CVCL_F125
GM02361	Human primary fibroblasts	<i>FANCG</i> ^{-/-}	Coriell Repository	CVCL_AK22
GM13136	Human GM00449-SV40-immortalized fibroblasts	<i>FANCC</i> ^{-/-} Homozygous c.456+4A>T (IVS4+4A>T)	Coriell Repository	CVCL_F126
GM16635	Human GM00449-SV40-immortalized fibroblasts	<i>FANCG</i> ^{-/-}	Coriell Repository	CVCL_F634
AS911	Human primary fibroblasts	WT	Gift of A. Sarasin lab	#
PD352	Human primary fibroblasts	<i>FANCG</i> ^{-/-}	In house	#
EGF47	Human primary fibroblasts	<i>FANCA</i> ^{-/-} 1263-1264del; ?	Gift of J Soulier lab	#
EGF47CORR	Human primary fibroblasts	<i>FANCA</i> ^{-/-} + <i>FANCA</i>	Gift of J Soulier lab	#
EGF56	Human primary fibroblasts	<i>FANCA</i> ^{-/-} L1305D; V372fs	Gift of J Soulier lab	#
EGF56CORR	Human primary fibroblasts	<i>FANCA</i> ^{-/-} + <i>FANCA</i>	Gift of J Soulier lab	#
EGF71	Human primary fibroblasts	<i>FANCA</i> ^{-/-} S725fs; F1306fs	Gift of J Soulier lab	#
EGF71CORR	Human primary fibroblasts	<i>FANCA</i> ^{-/-} + <i>FANCA</i>	Gift of J Soulier lab	#
EGF72	Human primary fibroblasts	<i>FANCA</i> ^{-/-} S1187fs; ?	Gift of J Soulier lab	#
EGF72CORR	Human primary fibroblasts	<i>FANCA</i> ^{-/-} + <i>FANCA</i>	Gift of J Soulier lab	#
Mouse Primary Fibroblasts			In house	#

Supplementary Table 2: list of used siRNA.

GENE	SEQUENCE	SEQUENCE CODE
<i>LACZ</i>	CGUCGACGGAAUACUUCGA	#
<i>FANCA</i>	GUACAGCAGCAAUUUCUUA GAUCGUGGCUCUUCAGGAA GGACAUCACUGCCCACUUC	FANCA1 FANCA2 FANCA3
<i>FANCD2</i>	GGAGAUUGAUGGUCUACUA AACAGCCAUGGAUACACUUGATT CAGAGUUUGCUUCACUCUCUA	FANCD2_1 FANCD2_2 FANCD2_3
<i>FANCC</i>	GAGAGAAUCAUCUUAUUGG GGAAUCGUCUUGGCAUUGA GGUAUGCACCUAUAGAUUA	FANCC_1 FANCC_2 FANCC_3
<i>FANCG</i>	UGCUUCACCAUCAUUAGGAAU CCUGUGAAAUUUGCCCUAGUU	FANCG_1 FANCG_2
<i>ATM</i>	ATM1-492 (Invitrogen)	#
<i>ATR</i>	HSS100876, HSS10877, HSS878 (Invitrogen)	#

Supplementary Table 3: list of antibodies used:

Antibody target	Supplier	Code N°	Specie	WB dilution	IF dilution	CHIP	IP
Alexa-Fluor Secondary Antibodies	Life Technologies		D		1:1000		
FANCA	Bethyl	A301-980A	R	1:1000			X
FANCA	FARF	FANCA-1	R	1:1000			
FANCA	Abcam	ab97578	R	1:1000			X
FANCA	R&D	AF6026	G	1 :500			
FANCA	Cell Signalling	#14657	R	1 :1000			X
FANCC	FARF	FANCC-2	R	1:1000			
FANCG	Santa Cruz	sc-393382	M	1 :500			X
FANCD2	Novus	NB100-182	R			4µg	
FANCD2	Abcam	Ab2187	R	1:1000			
FANCD2 (F117)	Santa Cruz	sc-20022	M	1:1000			
Fibrillarin	Abcam	ab5821	R	1:1000	1:2000		
G4s- (clone1H6)	Merck	MABE	M		1:200		
H2AX	Abcam	ab11175	R	1:1000			
γH2AX (JBW301)	Millipore	05-636	M	1:1000	1:2000		
H3K9me3 (6F12-H4)	Millipore	05-12432	M			4µg	
H3K4me3	Active motif	#61379	M			4µg	
Lamin A/C	Santa Cruz	sc-7292	M	1:1000			
NPM1 (FC61991)	Thermo Fisher	32-5200	M	1:1000	1:500		X
Nucleolin (4E2)	Abcam	ab13541	M		1:4000		X
RNAPolII	Abcam	ab101977	R			4µg	
S9.6	P. Pasero's team		M		1:200		
UBF (F9)	Santa Cruz	sc-13125	M	1:1000	1:250		
Vinculin (spm227)	Abcam	ab18058	M	1:4000			
EIF4E2	Abcam	ab238519	R	1:1000			
EIF6	Abcam	ab245532	R	1:1000			
RPL18	Novus	NBP2-13251	R	1 :500			
RPL5	Bethyl	A303-933AM	R	1:1000			
IgG1	Dako	X0931	M			4µg	X
IGg	Dako	X0903	R				X
IgG	Cell Signalling	#3900	R				X

FARF: Fanconi Anemia Research Found; R: Rat; M: Mouse; D: Donkey; G: Goat

Supplementary Table 4: Northern blot probe used.

Name	Sequence	Position in gene Genbank accession #U13369
5'ETS1b	5'-AGACGAGAACGCCTGACACGCACGGCAC-3'	297 to 324

Supplementary Table 5: list of the ChIP-qPCR primers used.

Name	Forward	Reverse	Efficiency ($10^{(-1/\text{slope})-1}$)	Elongation T°	Position
H1	GGCGGTTTGAGTGAGACGAGA	ACGTGCGCTCA2CCGAGAGCAG	0,95	58°	952-1030
H8	AGTCGGGTTGCTTGGGAATGC	CCCTTACGGTACTTGTTGACT	0,90	58°	8204-8300
H27	CCTTCCACGAGAGTGAGAAGCG	CTCGACCTCCCGAAATCGTACA	1,11	58°	27366-27477
H42.9	CCCGGGGAGGTATATCTTT	CCAACCTCTCCGACGACA	1,05	58°	42943 to 33

Primers pairs were as in (64).

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