Supplemental Table and Figures for the manuscript:

Spontaneous abrogation of a DNA damage checkpoint has clinical benefit but promotes leukemogenesis in Fanconi anemia patients

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Patient ID	Gene	FANC germline mutations (one line for one allele)	Reported as FA mutation database	Age at diagnosis	Age at evaluation	Extent of malformation	Bone marrow failure; Cancer	Fibroblast phenotype	Somatic genomic abnormalities in PBL
Two large deletions and/or non sense mutations									
EGF037	*FANCA*	c.[718C>T]p.[Gln240X] c.[3696delT]p.[Phe1232LeufsX15]	No No	6	15	L	Moderate BMF, then AML	Massive G2 arrest	1pter-, 2p+, 6q-
EGF165	*FANCA*	[deletion exons 18-21] c.[1115_1118del4]p.[Val372AlafsX42]	Yes Yes	14	24	E	No	Massive G2 arrest	No abn detected
EGF036	*FANCA*	[deletion exon 15] [deletion exon 15]	Yes Yes	27	36	L	No, late onset of MDS	Massive G2 arrest	1q+, 2qter-, 3q+, 11q-, 16q+, 21q+
EGF089	*FANCA*	[deletion exons 11-20] [deletion exon 16]	No Yes	26	28	L	Moderate stable BMF	Massive G2 arrest	1q+
EGF164	*FANCA*	[deletion exon 0-6] [deletion exon 16-17]	Yes Yes	29	29	L	No	Massive G2 arrest	1q+
EGF199	*FANCD2*	c.[782 A>T] (c.[696_783del88])p.[Ser232argfsX6] c.[4336_4337dupAG]p.[Ser1446argfsX28]	Yes No	3	6	E	BMF with severe thrombocytopenia	Massive G2 arrest	Not done
At least one splice site or missense mutation									
EGF117	*FANCG*	c.[1182_1192del11insC]p.[Glu395_Leu398>LeufsX5] [IVS09-1G>T]	Yes Yes	11	22	L	Moderate BMF, then AML	Massive G2 arrest	1q+, 3q+, 10q-, UPD17q
EGF167	*FANCA*	c.[3788_3790delTCT]p.[Phe1263del] c.[3788_3790delTCT]p.[Phe1263del]	Yes Yes	23	25	E	Moderate BMF	Massive G2 arrest	1q+, 6p-
EGF065	*FANCA*	c.[2T>C]p.[Met1?] c.[3391A>G]p.[Thr1131Ala]	Yes Yes	50	50	L	No, late onset of AML	Massive G2 arrest	1q+, +11
EGF142	*FANCA*	[deletion exons 22-28] [IVS28+1G>T]	Yes No	9	31	L	Moderate BMF	Massive G2 arrest	Not done
EGF078	*FANCA*	[IVS39+2T>C] [IVS39+2T>C]	No No	5	5	NA	Moderate BMF	Not done	Not done
At least one variant of unknown significance									
EGF042[†]	*FANCA*	c.[3788_3790delTCT]p.[Phe1263del] [IVS07+5G>A]	Yes Yes	17	36	L	No	Massive G2 arrest	No abn detected
EGF136[†]	*FANCA*	[deletion exons 04-05] [IVS21-123C>T]; [IVS30+88C>A]; c.[3386A>T]p.[Asp1129Val]	Yes No	46	56	L	No; two solid cancers (mouth and endometrial)	Massive G2 arrest	No abn detected
EGF017[†]	*FANCA*	[IVS16+68C>A]; c.[683C>G]p.[Ala228Gly] [IVS16+68C>A]; c.[683C>G]p.[Ala228Gly]	No No	5	26	L	No	Massive G2 arrest	1q+, 2p-
EGF166	*FANCA*	[IVS33+1G>A] c.[2513C>G]p.[Thr838Arg]	No No	26	26	L	Moderate BMF, then MDS	Massive G2 arrest	3q+, 7p-, 13q-
EGF045[†,#]	*FANCA*	c.[1153C>T]p.[His385Tyr] c.[3599T>C]p.[Leu1200Pro] c.[3611G>C]p.[Arg1204Pro]	No No No	30	36	E	Moderate BMF	Mild G2 arrest	No abn detected
EGF152*	*FANCA*	c.[3788_3790delTCT]p.[Phe1263del] c.[2513C>G]p.[Thr838Arg]	Yes No	15	43	E	BMF with severe thrombocytopenia and worsening neutropenia	Mild G2 arrest	No abn detected

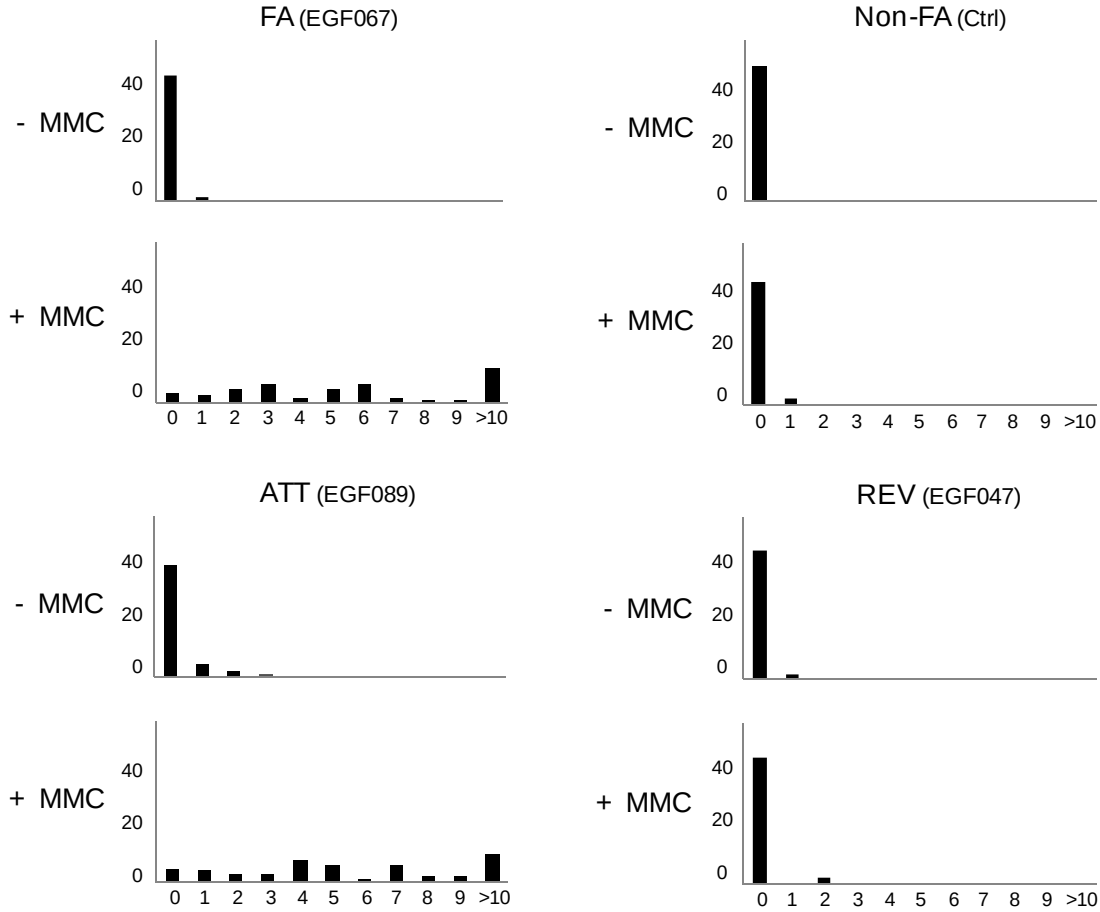
Symbols, notes and abbreviations

ID, identification number; abn, abnormality; UPD, uniparental disomy; E, extensive physical FA malformations (see Guardiola et al., Blood 2000) and L, limited; NA, information not available; BMF, bone marrow failure.

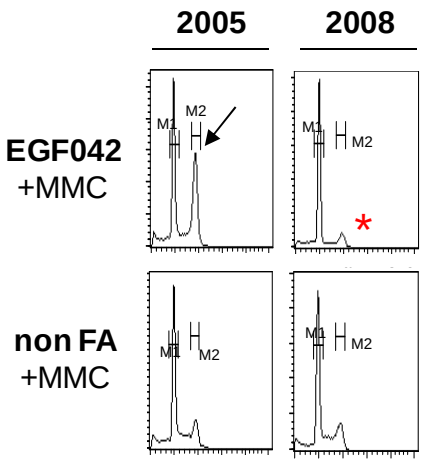
† FA group A was determined by retroviral complementation; # the relative allelic assignment of each variant could not be realized; * Patients with a mild G2 arrest in fibroblast and further considered as hypomorphic.

FANC constitutional mutations were searched for in primary fibroblasts for all patients by exons and exon-intron junctions direct sequencing, and large deletions by MLPA using the MRC Holland FANCA kit. Somatic chromosomal abnormalities were evaluated by array-CGH or SNP-array (see Methods)

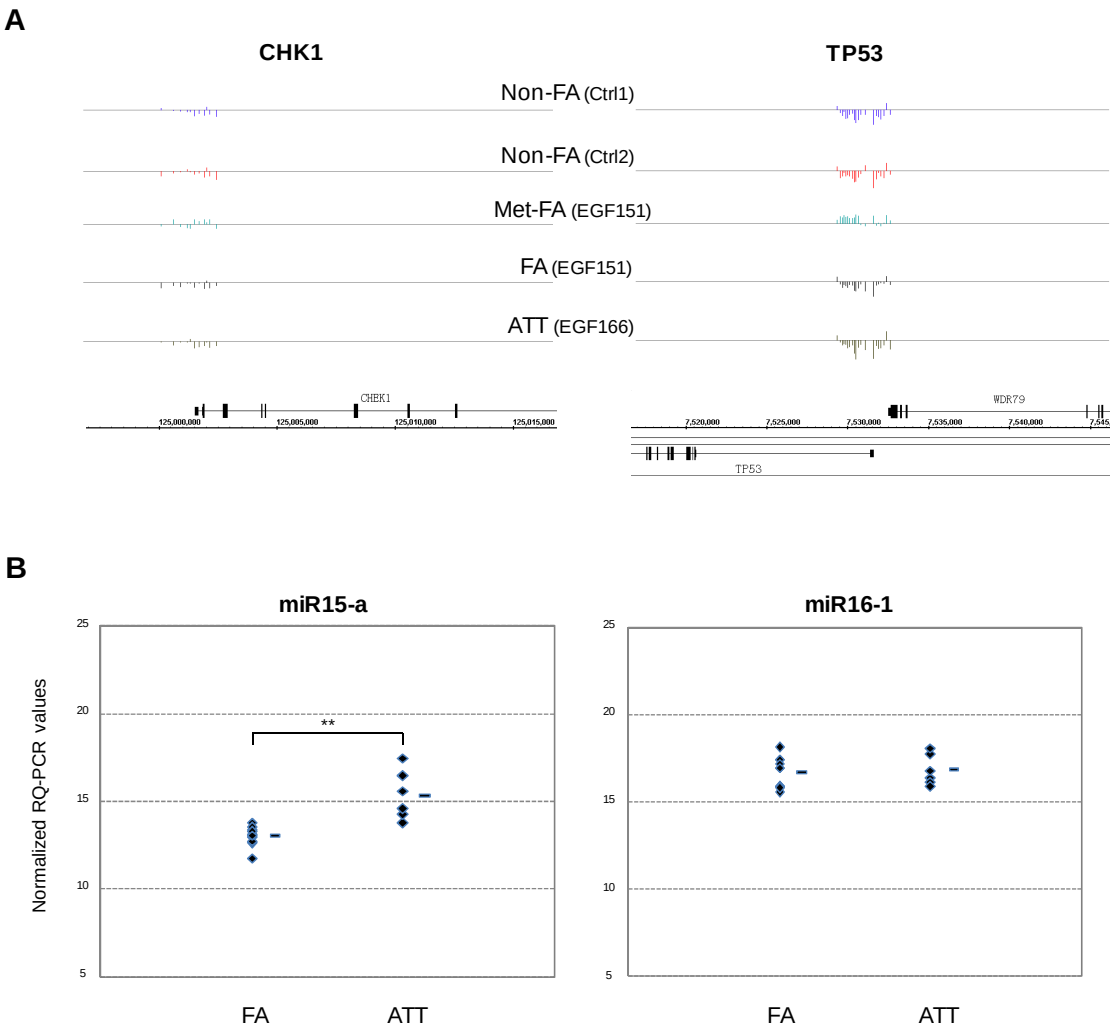
Supplemental Table 1. Germline *FANC* mutations and clinical data in the FA patients with attenuation.



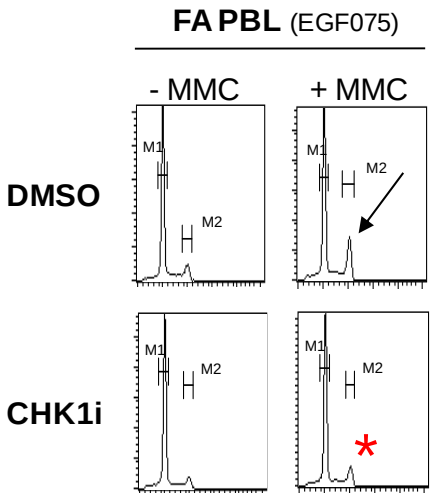
Supplemental Figure 1. Detailed breakage test data in the patients shown in Figure 1. Standard chromosome breakage tests on PHA-stimulated PBL were realized, with 50 mitoses being scored for each condition. The number of chromosomal breaks by cell is indicated on the x-axis and the number of cells in each category on the y-axis.



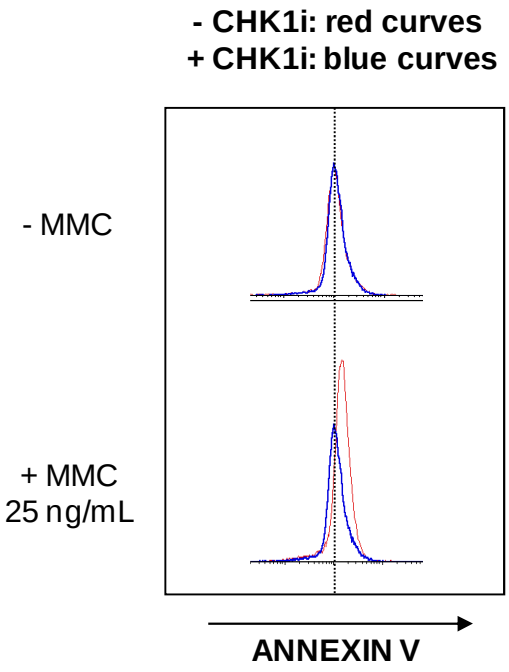
Supplemental Figure 2. Attenuation is an acquired phenotype in PBL. FA Patient EGF042 demonstrated a change from a typical G2 arrest (arrow) to an attenuated phenotype (star) at two distant evaluations (April 2005 and April 2008, top panel). Non-FA controls performed in parallel are shown on the bottom panel, whereas FA controls had typical G2 arrest (not shown). The attenuated phenotype was conserved on an additional subsequent evaluation (not shown).



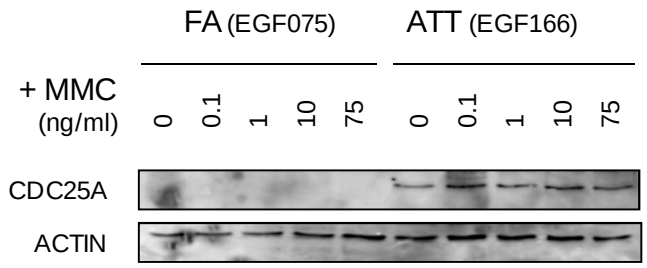
Supplemental Figure 3. No change of the DNA methylation status of the *CHK1* and *TP53* genes but overexpression of miR15-a in the attenuated FA cells. (A) Global DNA Methylation at *CHK1* and *TP53* was unchanged in PHA-stimulated PBL from attenuated compared to classical FA and non-FA healthy controls. As a positive control, the DNA of the classical FA patient EGF151 sample was in-vitro methylated (Met-FA). Highly methylated regions have log ratios above zero while less methylated regions have log ratios below zero. The 5' region of the *CHK1* and *TP53* genes are shown (left and right panel, respectively). Of note, we could not find any changes either in the methylation status of the other DNA damage response and cell cycle genes, including *ATR, RAD17, MDC1, 53BP1, ATM*, and *BRCA1* (data not shown). **(B)** Overexpression of miR15-a, but not miR16-1, in PHA-stimulated PBL from attenuated patients (n=6) compared to classical FA patients (n=10); ** P value < 0.001. These two miRNA were selected based on the analysis of the 3'UTR of *CHK1* using the softwares Target scan, PICTAR, microRNA.org and RNA22 (www.targetscan.org; pictar.mdc-berlin.de; microRNA.org; cbcsrv.watson. ibm.com).



Supplemental Figure 4. CHK1 inhibition attenuated the G2 arrest in PBL-PHA fresh FA cells. A representative experiment is shown; consistent data were found in 3 unrelated FA patients. The arrow shows the G2 arrest and the star its abrogation.



Supplemental Figure 5. Inhibition of the DNA damage-induced apoptosis by CHK1 inhibitor in FA cells in short term culture. MMC-induced apoptosis of FA primary fibroblasts (EGF177), incubated with and without CHK1i (red and blue curves, respectively), was revealed by Annexin V staining. Notably, a fraction of the sub-G1 cells as shown in Figure 5 were Annexin V negative, suggesting multiple response pathways of FA cells to DNA damage.



Supplemental Figure 6. CDC25A protein level increase in attenuated FA cells. CDC25 protein levels were analyzed in the PHA-PBL extracts from attenuated and classical FA cells with increasing concentrations of MMC. CDC25A expression was dramatically higher in the attenuated cells suggesting a lack of repression due to the absence of CHK1 in these cells, see Figure 3A. Therefore CHK1 low expression in the attenuated cells could add to the overall genetic instability through an acceleration of the CDC25A-dependent cell cycle transition (38).

A

gene	primers	genomic primers	cDNA primers
MPP1	F	5' TTCATGCCTGTTCTAGTTGAG 3'	5' GAAGCGTAGTCGGCCAG 3'
	R	5' AAAGTCTCTTGGCACACTCAC 3'	5' TCTGCAGCTGATCCACTGAAT 3'
FHL1	F	5' CTTCTGGAAGCTTAACAAAACTA 3'	5' TGTTTCAGAGGAACATCGTC 3'
	R	5' CGGTAGGTGGAAATCCAGATT 3'	5' GACTTTGCAGTCCTCATTAAC 3'
BTK	F	5' TTTACTCCCTGGGGAAGATGC 3'	5' GAGATTTACTAACAGTGAGACT 3'
	R	5' TGTGCAGCTATCAGTCTTTGGT 3'	5' AGAACCAAGAAGCTTATTGGC 3'

B

CHK1 exons	primers F	primers R
1	5'-ATACCGCTCCCTATATCCTCT-3'	5'-TATCCAAGTCTTTCAACCACG-3'
2	5'-TAGAAGGGGAAGGCAAGAGC-3'	5'-CTCAGAAAACGAAGGCAAGC-3'
3	5'-GAGGTAAAATCGTTTTGGATGAG-3'	5'-GCAATTTTGAAAGGACAACG-3'
4-5	5'-GAAGCTATGTGGTTGCTACCTG-3'	5'-GACTTGATTTTGCCTTGTATGG-3'
6	5'-TTGCAAAACATTTTTATTCAGTGTC-3'	5'-TGACTTTTTATAGGAGTTTTACCATGA-3'
7	5'-CTGCCATGCCTATCCTGATT-3'	5'-AAAATTCAAATCGCACAAGACTTC-3'
8	5'-CCTCAAGCCATAGGCTTCTC-3'	5'-GCCTGCCTAGCTTCCCTTTA-3'
9	5'-GCATAGAAGACTTGAAAGCATTTG-3'	5'-CAGGCCTTTCTTATATCACACACA-3'
10	5'-AAGCATGAGAACTTGTGTGTGA-3'	5'-AAATAAAGAGCTGCCATTACTTTTA-3'
11	5'-TGGATTTATTCATTTGTCTTCTGTTT-3'	5'-AGGTGTGAGCCACAGCCTAT-3'
12	5'-CACCCATGTGGCTTAACCTT-3'	5'-CAAGTAACCTATTTACAAATGCCACA-3'
13	5'-GACCGAAAAGAAAATGGTAGC-3'	5'-TTCTATTCATCCTTTCCCCAAA-3'

Supplemental Figure 7. Primer sequences. (**A**) sequence of the primers which were used on genomic DNA and cDNA for evaluation of clonal X-linked inactivation. (**B**) primers used for analysis of the 13 exons of the *CHK1* gene on genomic DNA.