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Supplemental Information

NHEJ-Mediated Repair of CRISPR-Cas9-Induced DNA Breaks Efficiently Corrects Mutations in HSPCs from Patients with Fanconi Anemia

Francisco José Román-Rodríguez, Laura Ugalde, Lara Álvarez, Begoña Díez, María José Ramírez, Cristina Risueño, Marta Cortón, Massimo Bogliolo, Sara Bernal, Francesca March, Carmen Ayuso, Helmut Hanenberg, Julián Sevilla, Sandra Rodríguez-Perales, Raúl Torres-Ruiz, Jordi Surrallés, Juan Antonio Bueren, and Paula Río

SUPPLEMENTAL TABLES

Table S1. Designed sgRNAs used in this study, Related to STAR Methods.

Gene	Target mutation	sgRNA name	Sequence (5' to 3')	Orientation
FANCA	c.3558insG	gINS8	CTCCACCGGCAGAGCAGCAC	Antisense
		gINS11	ACTGCCAGAGCCCGCTGCCC	Sense
	c.295C>T	gGM4	CACGGGAACCCCCAGCCTTG	Antisense
		gGM10	AGGATCAAGCCTCAAGGCTG	Sense
		gGM0	TGTTTAAGGCTCTGCTTTGT	Sense
FANCB	c.1815_1816delAA	gP2	TACAAATTATGGAGAGAGAG	Sense
FANCC	c.67delC	gP3	AGAAGCTTTCTGTATGGATC	Sense
FANCD1/BRCA2	c.1596delA	gFA62	TATTTCCAGTCCACTTTCAG	Antisense
FANCD2	c.718delT	gFA344	AGTGAGTATTCTCTATCAGT	Antisense

Table S2. FA lymphoblastic cell lines (LCLs) used in this study, Related to STAR Methods.

Gene	LCL	Target mutation	Genotype
FANCA	FA-178	c.3558insG	Homozygous
	FA-55	c.295C>T	Homozygous
FANCB	P2	c.1815_1816delAA	Homozygous
FANCC	P3	c.67delC	Homozygous
FANCD2	FA-344	c.718delT	Heterozygous (c.3707 G>A)
FANCD1/BRCA2	FA-62	c.1596delA	Heterozygous (ex15-16del)

Table S3. Editing events described in Venn Diagram, Related to Figure 5D.

Code	Indels
A	c.311delG
	c.304_311del8
	c.306_312del7
	c.312_314del3
	c.311insG
B	c.310insA
	c.311_316del6
	c.309delA
C	c.311_314del4
D	c.296_309del14
E	c.311_313del3
	c.290_310del21
	c.296_310del15
	c.294_311del18
	c.297_317del21
	c.309_311del3
	c.312_323del12
F	c.311_312del2
	c.300_306del7
G	c.311_315del5
H	c.310insAA
	c.312ins3
	c.311_319del9
	c.293_325del33
I	c.300_320del21
	c.310_312del3
	c.307_311del5
J	c.308delC, c.309A>T
	c.311_324del14
	c.307delT, c.310_312del3
	c.299_310del12
	c.305_310del6
K	c.312insT
	c.311_318del8
	c.311_317del7
L	c.312_313del2
M	c.311insT

Code	Indels
N	c.307_309del3, c.310A>C
	c.307_309del3
	c.289_318del30
O	c.293_310del18
	c.292_315del24
	c.308ins3
	c.311G>A, c.314ins3, c.314T>A, c.315G>A, c.316G>A
	c.312ins5
P	c.310_317del8
	c.304_317del14
	c.292_323del32
	c.308_310del3
Q	c.307T>C, c.309_311del3
	c.293_319del27
	c.315delG
R	c.307_320del14
	c.305_321del17
	c.312insGG
	c.309_310del2
	c.306_310del5
	c.306_335del30

Code	Indels
S	c.311delG, c.308C>T
	c.299_302del4, c.309ins6, c.310_317del8
	c.306_326del21
	c.313ins2, c.314_321del8
	c.300_312del13
	c.308_324del17
	c.296_311del16
	c.304_319del16
	c.309_320del12
	c.313ins2
	c.297_316del20
	c.311G>A
	c.312_314del3, c.315T>G
	c.301_315del15
	c.289_317del29
	c.313ins23
	c.298_310del13
	c.311delG, c.312G>A
	c.284-1_317del35
	c.311G>A, c.312G>C, c.313insA
	c.303_330del28
	c.311delG, c.313C>T
	c.312_320del9
	c.312ins2, c.316ins7
	c.312insC, c.315_316del2
	c.311_312del2, C.316delG
	c.307_319del13
	c.309delA, c.310A>G
	c.307_318del12
	c.314_328del15
	c.313ins6
	c.310_325del16
	c.298_320del23
	c.307T>C, c.310_312del3

Code	Indels
T	c.300_325del26
	c.310_311del2
	c.308ins9
	c.297_314del18
	c.308ins23
	c.284-1_322del40
	c.311ins2
	c.309_314del6
	c.302_310del9
	c.307_317del11
	c.293_315del23
	c.297_311del15
	c.293_313del21
	c.312insG
	c.301_323del23
	c.286_310del35
	c.295_321del27
	c.311ins4
	c.284-4_308del28
	c.300_314del15
	c.293_308del16, c.311_315del5
	c.313ins39
U	c.309_311del3, c.316delG
	c.309A>G, c.311insG
	c.315C>T
	c.312insC
	c.284-1_24del42
	c.312insA
	c.284-14ins35, c.304G>C, c.307_312del5, c.313GZA
	c.295_322del28
	c.311G>T,312G>A,313insC
	c.299_308del8
	c.311ins9
	c.295_309del15
	c.307_310del4, c.311G>C

V	c.308C>T, c.311_313del3
	c.311_313del3, c.314T>C
	c.310A>T
	c.308C>T
	c.308C>G
	c.306_333del28
	c.284-9_318del44
	c.313insG, c.316G>A
	c.300_334del35
	c.311_313del3, c.315G>A
	c.303_308del6
	c.311insA
	c.311ins3
	c.309delA, c.310A>C
	c.309_312del3, c.316delG
	c.310delA
	c.313C>G, c.314insT
	c.313insA
	c.297_323del27
	c.309_311del3, c.313C>A
	c.312G>A
	c.307_329del23
	c.315G>A
	c.31_316del6
	c.311insAA c.310_318del9
	c.314T>A
	c.307T>C, c.313insG
	c.312G>C, c.313C>T, c.314T>G
	c.309A>G, c.313insG
	c.311ins37
	c.307T>G
	c.307T>A, c,308C>A, c.309A>G, c.310A>C, c.311ins3
	c.284-3_308del28
	c.308_310del3, c.311G>A
	c.301_310del10
	c.310_312del3, c.313C>G
	c.309A>G
	c.313insG, c.315T>C
	c.316G>T
	c.311G>A, c.313ins3, c.314T>A, c.315G>A, c.316G>A

Table S4. Diseases susceptible to be treated by NHEJ-based gene editing, Related to Figure 3.

Recessive or X linked blood disorders	Gene	Nonsense (*)	Small deletions	Small insertions	Small indels	Total targetable mutations	Total mutations described	Mutations targetable by NHEJ (%)
X1-SCID	*IL2RG*	44	49	16	7	116	256	45.3
Adenosin deaminase deficiency	*ADA*	8	11	2	2	23	96	24.0
β-thalassemia	*HBB*	23	133	48	22	226	884	25.6
Wiskot-Aldrich syndrome	*WAS*	45	132	52	7	236	443	53.3
Chronic granulomatous disease	*CYBB*	106	160	57	29	352	790	44.6
Fanconi anemia complementation group A	*FANCA*	78	98	43	4	223	693	32.2
Fanconi anemia complementation group B	*FANCB*	5	5	1	0	11	22	50.0
Fanconi anemia complementation group C	*FANCC*	12	12	6	2	32	66	48.5
Fanconi anemia complementation group D2	*FANCD2*	7	9	2	2	20	65	30.8
Fanconi anemia complementation group E	*FANCE*	3	3	2	0	8	18	44.4
Fanconi anemia complementation group F	*FANCF*	4	5	0	0	9	16	56.3
Fanconi anemia complementation group G	*FANCG*	15	25	12	4	56	92	60.9
Fanconi anemia complementation group I	*FANCI*	9	11	2	0	22	45	48.9
Shwachman diamond syndrome	*SBDS*	4	9	1	5	19	90	21.1
Severe congenital neutropenia	*HAX1*	4	4	5	0	13	21	61.9
Dyskeratosis congenita	*DKC1*	0	4	1	4	9	75	12.0
Bernard Soulier Syndrome type A	*GP1BA*	13	16	11	1	41	78	52.6
Bernard Soulier Syndrome type B	*GP1BB*	6	5	3	1	15	53	28.3
Bernard Soulier Syndrome type C	*GP9*	4	3	1	0	8	42	19.0
Hemophilia A	*F8*	327	539	189	42	1097	3246	33.8
Hemophilia B	*F9*	100	174	59	19	352	1288	27.3
Leucocyte adhesion deficiency type 1	*ITGB2*	18	17	5	3	43	119	36.1
Diamond-Blackfan Anemia	*RPS19*	15	38	27	3	83	175	47.4
Diamond-Blackfan Anemia	*RPS26*	1	6	3	0	10	34	29.4
Diamond-Blackfan Anemia	*RPS24*	2	2	0	1	5	10	50.0
Diamond-Blackfan Anemia	*RPS17*	1	3	0	1	5	19	26.3

Diamond-Blackfan Anemia	*RPL5*	19	27	16	4	66	98	67.3
Diamond-Blackfan Anemia	*RPL11*	5	19	12	1	37	56	66.1
Diamond-Blackfan Anemia	*RPL35A*	1	2	0	0	3	19	15.8
Diamond-Blackfan Anemia	*RPL15*	3	0	1	0	4	9	44.4
Diamond-Blackfan Anemia	*GATA1*	0	1	0	1	2	16	12.5
Diamond-Blackfan Anemia	*RPS10*	1	0	4	0	5	7	71.4
Diamond-Blackfan Anemia	*RPS7*	0	1	0	0	1	10	10.0
								Mean: 39.3

* Estimated value considering that missense and nonsense are shown together in HGMD database.

FA genes involved in cancer have not been included since many of the mutations described in the data base are not associated to FA.

Diamond-Blackfan anemia: the most frequently described genes associated to the disease have been included.

Table S5. Primers used for sgRNA cloning in pX330-U6-Chimeric_BB-CBh-hSpCas9 plasmid (A) and for sgRNA *in vitro* transcription (B), Related to STAR Methods.

gRNA	Primer	Sequence (5' to 3')
A. Primers used for sgRNA cloning in pX330-U6-Chimeric_BB-CBh-hSpCas9 plasmid		
gINS8	Fw	CACCGCTCCACCGGCAGAGCAGCAC
	Rv	AAACGTGCTGCTCTGCCGGTGGAGC
gINS11	Fw	CACCGACTGCCAGAGCCCGCTGCCC
	Rv	AAACGGGCAGCGGGCTCTGGCAGTC
gGM4	Fw	CACCGCACGGGAACCCCCAGCCTTG
	Rv	AAACCAAGGCTGGGGGTTCCCGTGC
gGM10	Fw	CACCGAGGATCAAGCCTCAAGGCTG
	Rv	AAACCAGCCTTGAGGCTTGATCCTC
B. Primers used for sgRNA *in vitro* transcription		
gINS11	Fw	GAAATTAATACGACTCACTATAGACTGCCAGAGCCCGCTGCCC
	Rv	AAAAGCACCGACTCGGTGCC
gGM4	Fw	GAAATTAATACGACTCACTATAGCACGGGAACCCCCAGCCTTG
	Rv	AAAAGCACCGACTCGGTGCC

Table S6. Primers used for Sanger sequencing and *ICE* analyses (A) and for NGS analyses (B), Related to STAR Methods.

Gene	Mutation	Primer	Sequence (5' to 3')	Tm (°C)	PCR product size (bp)
A. Primers used for Sanger sequencing and ICE analyses					
FANCA	c.3558insG	Fw	TGTAGTGGCCTGTAGGAGCA	60	394
		Rv	CCCAGTAGTTGGGATTACAG		
	c.295C>T	Fw	TGCTCCTTTTGTGTCATGGGA	60	422
		Rv	TGCTGGTGTCTTACTCTCTGC		
FANCB	c.1815_1816delAA	Fw	ACGTTGACCCCTGATAGCAA	60	236
		Rv	ACTTCCCAGTTGAAAGATCTTCT		
FANCC	c.67delC	Fw	GGGACATCACCTTTTCGCTT	60	194
		Rv	ACCATCTCTTTCAAGGCTTCA		
FANCD1/ BRCA2	c.1596delA	Fw	AGTGGCTTCTTCATTTCAGGGT	60	232
		Rv	AATTCTGTGTGGTGGTGGCT		
FANCD2	c.718delT	Fw	CTGCCCAGCTCTGTTCAAAC	60	226
		Rv	ACTCCCAAGGCAATGACTGA		
B. Primers used for NSG analyses					
FANCA	c.3558insG	Fw	**ATCTCAGCCACCCTCATCTG**	60	176
		Rv	**ATCTCACCACCCACACGTAC**		
	c.295C>T	Fw-StV	**CCTTTGCATCTATTCTCCCCGT**	60	234
		Rv-StV	**TGCAGATCTGTCCCACGCTA**		
		Fw-Gwz	ACACTCTTTCCCTACACGACGCTCTTCCGATCT**CCTTTGCATCTATTCTCCCCGT**	60	299
		Rv-Gwz	GACTGGAGTTCAGACGTGTGCTCTTCCGATCT**TGCAGATCTGTCCCACGCTA**		
		Fw-UAB	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**CCTTTGCATCTATTCTCCCCGT**	60	301
		Rv-UAB	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**TGCAGATCTGTCCCACGCTA**		
FANCB	c.1815_1816delAA	Fw	ACACTCTTTCCCTACACGACGCTCTTCCGATCT**ACGTTGACCCCTGATAGCAA**	60	301
		Rv	GACTGGAGTTCAGACGTGTGCTCTTCCGATCT**ACTTCCCAGTTGAAAGATCTTCT**		
FANCC	c.67delC	Fw	ACACTCTTTCCCTACACGACGCTCTTCCGATCT**GGGACATCACCTTTTCGCTT**	60	259
		Rv	GACTGGAGTTCAGACGTGTGCTCTTCCGATCT**ACCATCTCTTTCAAGGCTTCA**		
FANCD1/ BRCA2	c.1596delA	Fw	ACACTCTTTCCCTACACGACGCTCTTCCGATCT**AGTGGCTTCTTCATTTCAGGGT**	60	297
		Rv	GACTGGAGTTCAGACGTGTGCTCTTCCGATCT**AATTCTGTGTGGTGGTGGCT**		
FANCD2	c.718delT	Fw	ACACTCTTTCCCTACACGACGCTCTTCCGATCT**CTGCCCAGCTCTGTTCAAAC**	60	291
		Rv	GACTGGAGTTCAGACGTGTGCTCTTCCGATCT**ACTCCCAAGGCAATGACTGA**		

Adapter sequences are indicated in regular letters, while primer sequences are marked in bold.

Table S7. Primers for off-target loci NGS Analysis, Related to STAR Methods.

Off-target	Primer	Sequence (5' to 3')	Tm (°C)	PCR size (bp)
OT1	Fw	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**GCAAGTGGGAAAACACAGGT**	62	299
	Rv	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**AGACCCCTCTTCAGGAAGTC**		
OT2	Fw	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**TCCAGGGCTGGAATGTAGTC**	62	260
	Rv	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**GCTTCTGAGCTGCAAGGTCT**		
OT3	Fw	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**AGTGCCACCAGAGGTTAGGT**	62	228
	Rv	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**CTCACCCTCCATATGGGACA**		
OT4	Fw	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**CGCTTCCTGACATGATGGT**	62	235
	Rv	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**AGGCACAGGTGCTTTACCTC**		
OT5	Fw	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**GGGAACAGCCAGTTCTCATC**	62	250
	Rv	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**TGCATGTGGAGGTGGTACAT**		
OT6	Fw	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**CATTCCCTTGGGAAATCCTT**	60	259
	Rv	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**AAAGCCCCTGTGTAGGGAAG**		
OT7	Fw	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**GGGCTCTGTGTGCTAAAGGA**	60	279
	Rv	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**AGAAACTGCCTGGAGCTGAG**		
OT8	Fw	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**CAAGTGCCCTCTTGGACTTC**	60	245
	Rv	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**GAAGCATGGAGCCCATAGTC**		
OT9	Fw	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**CAGCAGAGACCATTAGCCTTC**	64	310
	Rv	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**CGTTTCAGATGCCATGACTG**		
OT10	Fw	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**TAAGGAAGAGCAGGGCACTC**	60	273
	Rv	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**AGAGGGCATAAAGGGACCTC**		
OT11	Fw	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**CCCCAAGCTAGCAACACAAT**	60	245
	Rv	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**CAGAGTTGTTGGCTCCTGTG**		
OT12	Fw	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**TTGATGCCTGCAAGTCTCTG**	64	316
	Rv	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**CTGGGTTGGGTTATCACTGG**		
OT13	Fw	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**AAGCCACTGTCCCTCTCCTT**	60	245
	Rv	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**CACAAATGCCGCTGATGTC**		
OT14	Fw	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**TGAGCTTCACCTTGTGGTTG**	56	295
	Rv	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**CCAGAAGCAGGTCATGTTGA**		
OT15	Fw	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**CCTCAGCTGCCACTGTGATA**	64	242
	Rv	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**TGGGGCATCATCTCCTAAAG**		
OT16	Fw	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**TGTACGTGCAGCCAAGAAAG**	64	252
	Rv	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**GAAGGGCATGTGTCCTGAAT**		
OT17	Fw	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**CAGTGTGGCATGTCAAATCC**	64	300
	Rv	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**GTCCATGGAGAAATGGAAGC**		
OT18	Fw	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**ATTTCCTCGGTTTCTGAGCA**	60	226
	Rv	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**AGGAAACTCTTCCCGAGTCC**		
OT19	Fw	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**CCACATCACCAAACTGACCA**	64	222
	Rv	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**CGTTAAGCTTTGCCCTTCAG**		
OT20	Fw	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**AGAGGGAGAGTGAAGGCTGA**	64	306
	Rv	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**GACACTACGTGGGGAAATGG**		

Adapter sequences are indicated in regular letters, while primers sequences are marked in bold.

SUPPLEMENTAL FIGURES

Figure S1

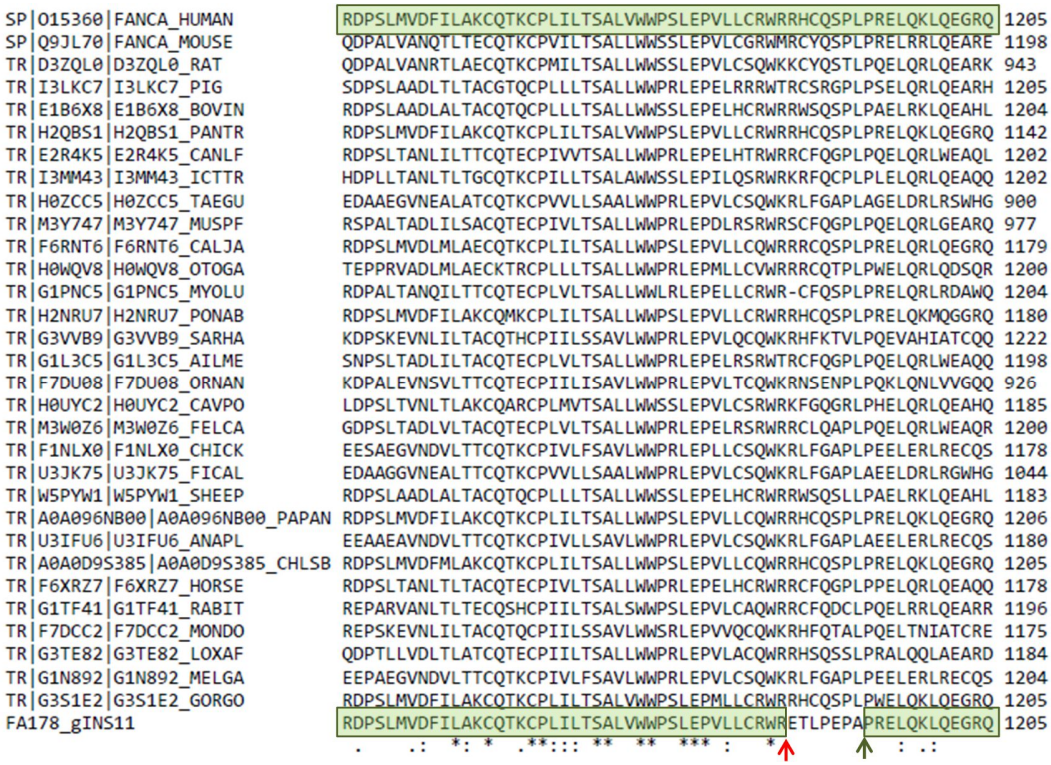


Figure S1. FANCA protein BLAST analysis suggests that exon 36 is a highly conserved domain, Related to Figure 1. A protein BLAST analysis was conducted using the bioinformatic tool available in the Uniprot webpage (https://www.uniprot.org/). The FANCA protein amino acid sequences available of FANCA proteins from different vertebrates were compared in order to identify the most conserved regions. The results obtained evidenced that exon 36 sequence is one of the most conserved, which implies that very few variations could be admitted without compromising the functionality of the protein. This fact perfectly explains why the deletion of the G next to the Cas9 cutting site is the only event that became more frequent over time among the wide variations of corrective NHEJ-repair events obtained in gene edited FA-178 LCL, as the sequence comprised between the c.3558insG mutation (where the frameshift starts –red arrow–) and the c.3579delG generated by NHEJ after the DSB (where the ORF is restored –green arrow–) is not conserved. Conversely, as the other potentially corrective indels implied higher deletions, a conserved domain was affected and the functionality of the protein was impaired. According to Uniprot protein BLAST analysis tool: "*****" (asterisk) indicates positions which have a single, fully conserved residue; "**:**" (colon) indicates conservation between groups of strongly similar properties - scoring > 0.5 in the Gonnet PAM 250 matrix and "**.**" (period) indicates conservation between groups of weakly similar properties - scoring =< 0.5 in the Gonnet PAM 250 matrix.

Figure S2

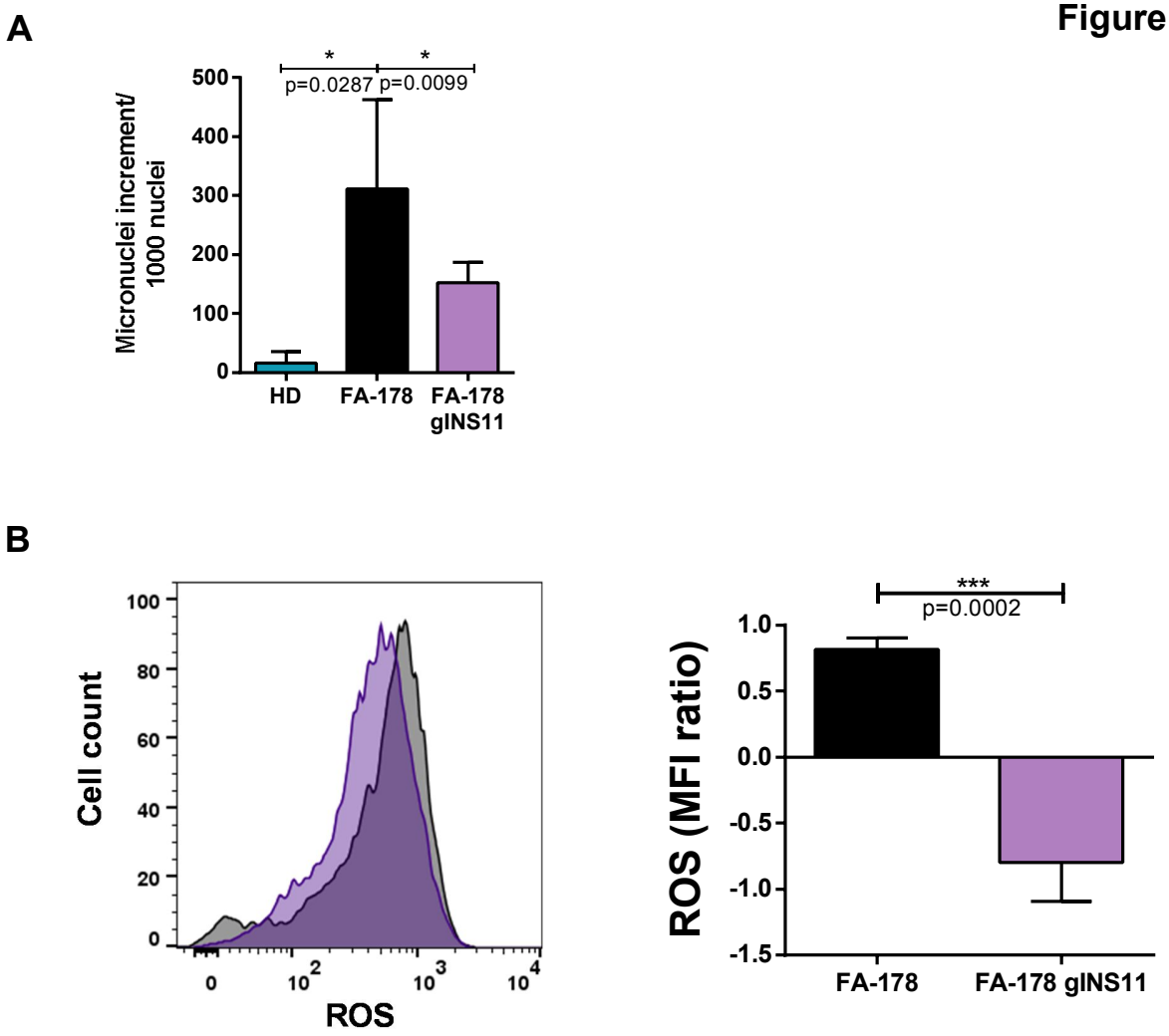
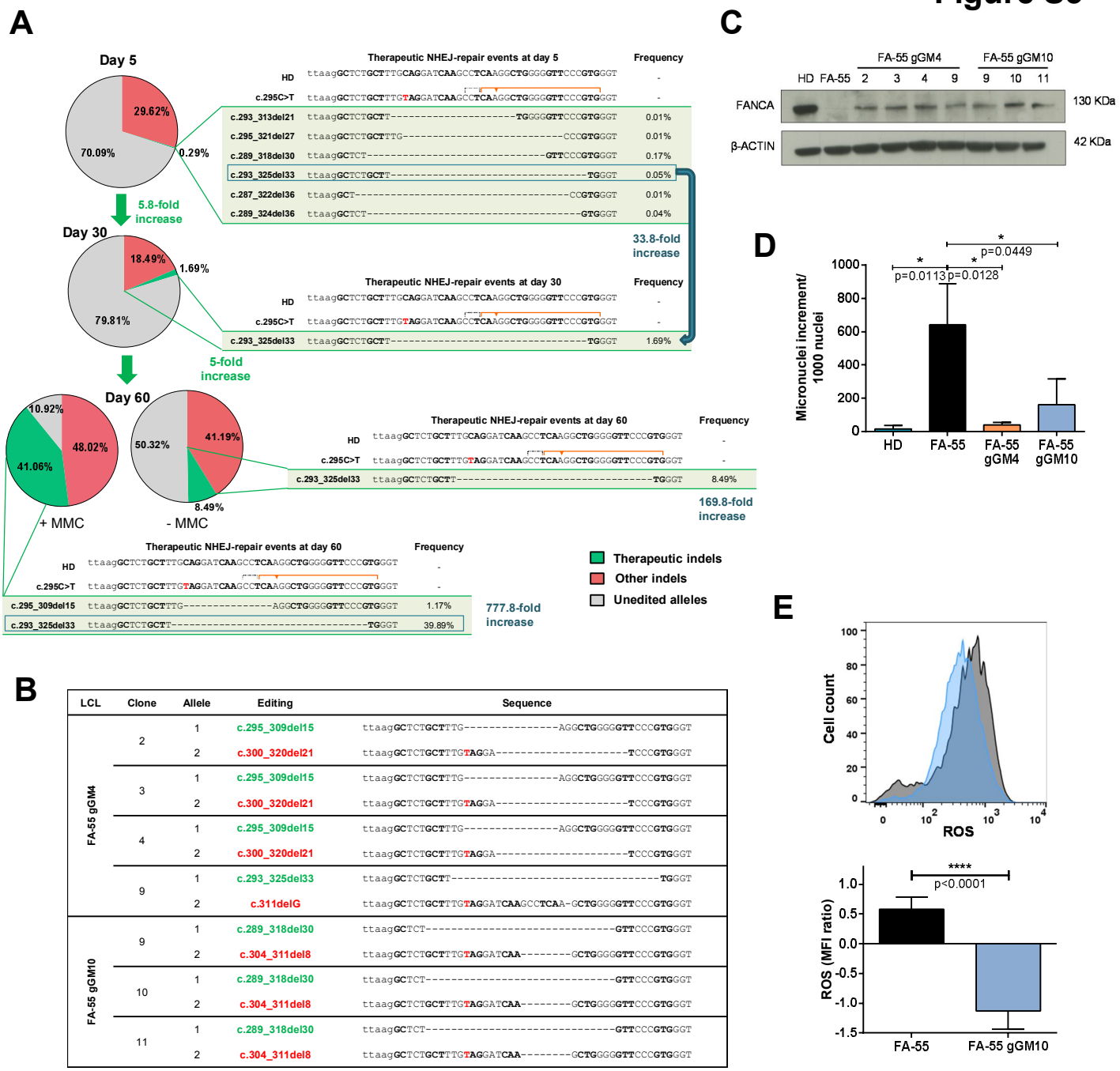


Figure S2. Fanconi anemia phenotypic correction in edited FA-178 LCLs, Related to Figure 1. (A) Chromosomal fragility measured by the reduction in the number of micronuclei in edited FA-178 LCL (purple) when exposed to DEB in comparison to untreated FA-178 LCL (black). Bars represent mean ± SD of three different analyses. An unpaired t-test was conducted. (B) Edited FA-178 LCL showed a reduction in ROS production. ROS production was measured in FA-178 LCL after NHEJ-mediated editing (purple) and compared with untreated FA-178 control LCL (black). Left panel: ROS mean fluorescence intensity (MFI) representative histogram. Right panel: decrease in ROS production calculated as the MFI ratio of ROS in edited cells compared to untreated ones. Bars represent mean ± SD of three different analyses. An unpaired t-test was conducted.

Figure S3



B

LCL	Clone	Allele	Editing	Sequence
FA-55 gGM4	2	1	c.295_309del15	ttaagGCTCTGCTTTG----------AGGCTGGGGGTTCCCGTGGGT
		2	c.300_320del21	ttaagGCTCTGCTTTGTAGGA----------TCCCGTGGGT
	3	1	c.295_309del15	ttaagGCTCTGCTTTG----------AGGCTGGGGGTTCCCGTGGGT
		2	c.300_320del21	ttaagGCTCTGCTTTGTAGGA----------TCCCGTGGGT
	4	1	c.295_309del15	ttaagGCTCTGCTTTG----------AGGCTGGGGGTTCCCGTGGGT
		2	c.300_320del21	ttaagGCTCTGCTTTGTAGGA----------TCCCGTGGGT
	9	1	c.293_325del33	ttaagGCTCTGCTT----------TGGGT
		2	c.311delG	ttaagGCTCTGCTTTGTAGGATCAAGCCTCAA-GCTGGGGGTTCCCGTGGGT
FA-55 gGM10	9	1	c.289_318del30	ttaagGCTCT----------GTTCCCGTGGGT
		2	c.304_311del8	ttaagGCTCTGCTTTGTAGGATCAA--------GCTGGGGGTTCCCGTGGGT
	10	1	c.289_318del30	ttaagGCTCT----------GTTCCCGTGGGT
		2	c.304_311del8	ttaagGCTCTGCTTTGTAGGATCAA--------GCTGGGGGTTCCCGTGGGT
	11	1	c.289_318del30	ttaagGCTCT----------GTTCCCGTGGGT
		2	c.304_311del8	ttaagGCTCTGCTTTGTAGGATCAA--------GCTGGGGGTTCCCGTGGGT

F

Therapeutic NHEJ-repair events at day 5

		Frequency
HD	ttttgtgtttaagGCTCTGCTTTGCAGGATCAAGCCTCAAGGCTGGGGGTTC	-
c.295C>T	ttttgtgtttaagGCTCTGCTTTGTAGGATCAAGCCTCAAGGCTGGGGGTTC	-
c.285_305del21	ttttgtgtttaagG----------CTCAAGGCTGGGGGTTC	1.53%
c.289_303del15	ttttgtgtttaagGCTCT----------GCCTCAAGGCTGGGGGTTC	0.9%
c.293_298del6	ttttgtgtttaagGCTCTGCTT------ATCAAGCCTCAAGGCTGGGGGTTC	0.73%
c.292_306del15	ttttgtgtttaagGCTCTGCT----------TCAAGGCTGGGGGTTC	0.45%
c.293_302del9	ttttgtgtttaagGCTCTGCTT---------AAGCCTCAAGGCTGGGGGTTC	0.27%
c.291_299del9	ttttgtgtttaagGCTCTGC---------TCAAGCCTCAAGGCTGGGGGTTC	0.24%
c.293_304del12	ttttgtgtttaagGCTCTGCTT----------CCTCAAGGCTGGGGGTTC	0.08%
c.291_302del12	ttttgtgtttaagGCTCTGC----------AGCCTCAAGGCTGGGGGTTC	0.06%

G

Therapeutic NHEJ-repair events after MMC treatment

		Frequency
HD	ttttgtgtttaagGCTCTGCTTTGCAGGATCAAGCCTCAAGGCTGGGGGTTC	-
c.295C>T	ttttgtgtttaagGCTCTGCTTTGTAGGATCAAGCCTCAAGGCTGGGGGTTC	-
c.289_303del15	ttttgtgtttaagGCTCT----------GCCTCAAGGCTGGGGGTTC	18.77%
c.285_305del21	ttttgtgtttaagG----------CTCAAGGCTGGGGGTTC	12.08%
c.288_302del15	ttttgtgtttaagGCTC----------AGCCTCAAGGCTGGGGGTTC	5.27%
c.292_306del15	ttttgtgtttaagGCTCTGCT----------TCAAGGCTGGGGGTTC	4.46%
285_296del12	ttttgtgtttaagG----------GGATCAAGCCTCAAGGCTGGGGGTTC	3.38%
c.293_298del6	ttttgtgtttaagGCTCTGCTT------ATCAAGCCTCAAGGCTGGGGGTTC	2.31%
c.291_299del9	ttttgtgtttaagGCTCTGC---------TCAAGCCTCAAGGCTGGGGGTTC	1.87%
c.291_302del12	ttttgtgtttaagGCTCTGC----------AGCCTCAAGGCTGGGGGTTC	0.59%

Figure S3. Fanconi anemia phenotypic correction in edited FA-55 LCL and improvement of potentially corrective indels by using a more specific MS-RNP (gGM0), Related to Figure 2. (A) Percentage of indels identified by Next Generation Sequencing 5, 30 and 60 days after editing with gGM4 before and after MMC selection. (B) Sanger sequencing of FA-55 edited clones. NHEJ-corrected alleles are marked in green while non-corrected ones are marked in red. (C) FANCA protein expression was restored in isolated edited FA-55 LCL clones. Panel shows FANCA Western-blot in different isolated clones. β-Actin was used as a loading control. (D) Edited FA-55 LCL (orange and light blue) exhibited a decrease in the chromosomal fragility, measured by the number of micronuclei, when exposed to DEB in comparison to untreated FA-55 LCL (black), acquiring levels similar to a HD LCL (dark blue). Bars represent mean ± SD of three different analyses. An unpaired t-test was conducted. (E) Reduced ROS MFI in NHEJ-edited FA-55 LCL. ROS production was measured in FA-55 LCL after NHEJ-mediated editing (blue) and compared with untreated FA-55 control LCL (black). Upper panel: representative histogram of ROS MFI. Lower panel: decrease in ROS production calculated as the MFI ratio of ROS in edited cells compared to untreated ones. Bars represent mean ± SD of three different analyses. An unpaired t-test was conducted. (F) The design of a more specific, chemically modified sgRNA (gGM0) and its use as a ribonucleoprotein (MS-RNP0) increases the correction efficacy. The most frequent potentially corrective NHEJ-repair events at 5 days after electroporation (F) and after MMC treatment (G) and their frequencies are displayed.

The sequences obtained in the NGS were classified as in Figure 1.

Figure S4

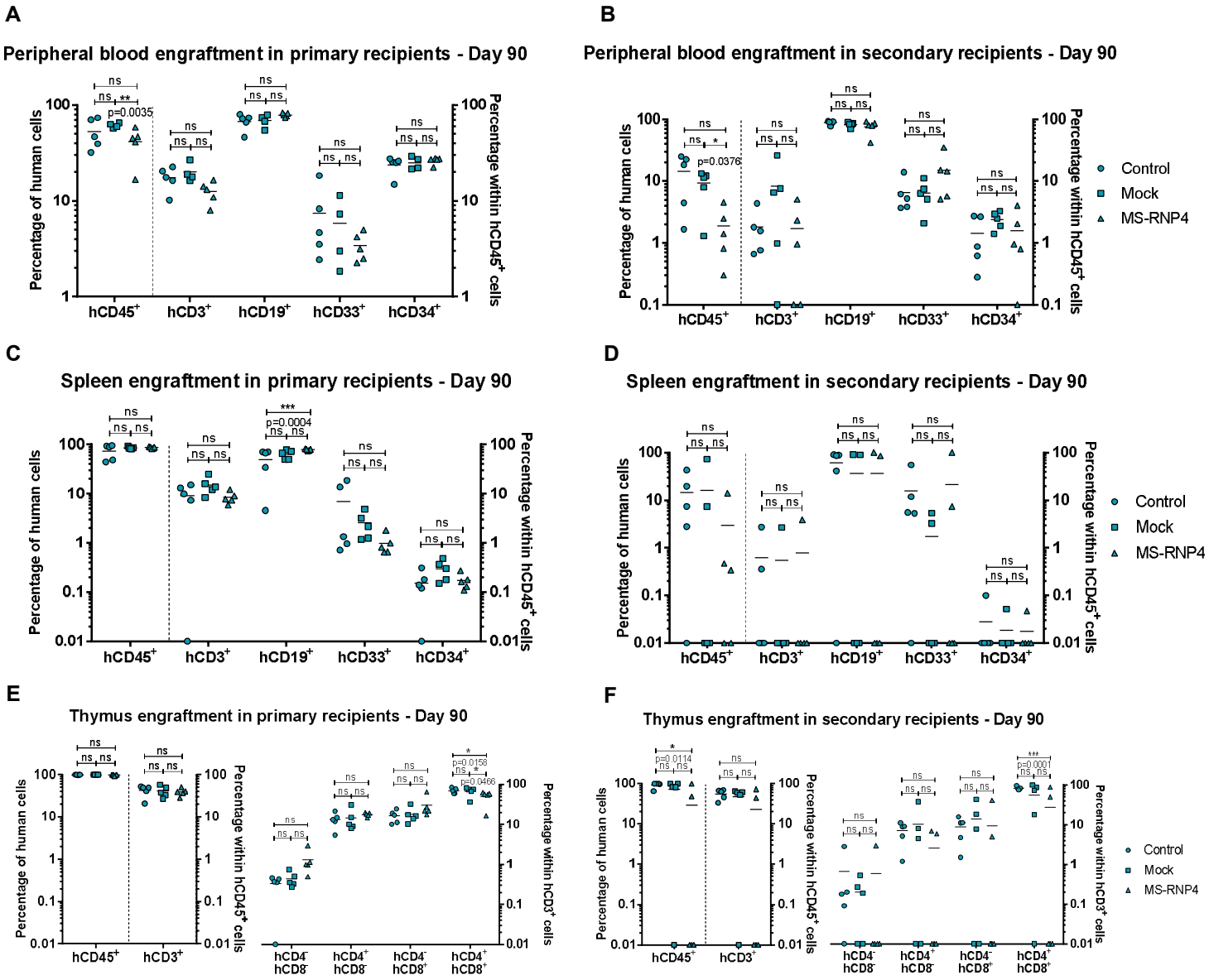


Figure S4. Multi-lineage engraftment of human HSPCs in primary and secondary recipients three months post-transplantation, Related to Figure 4. Peripheral blood reconstitution was evaluated in primary (A) and secondary recipients (B) using antibodies against hCD3 for T cells, hCD19 for B cells, hCD33 for myeloid cells and hCD34 for HSPCs. A two-way ANOVA was performed followed by Tukey's post hoc test in all analyses. Spleen multi-lineage reconstitution was evaluated in primary (C) and secondary recipients (D) using antibodies against hCD3 for T cells, hCD19 for B cells, hCD33 for myeloid cells and hCD34 for HSPCs. Thymus multi-lineage reconstitution was evaluated in primary (E) and secondary recipients (F) using antibodies against hCD3 for T cells. The distinction between cytotoxic and helper T cells was made according to the presence of hCD8 and hCD4 antibodies respectively. Data are represented as mean ± SD.

Figure S5

A

FA-807 BM CD34⁺ CFCs (0 nM MMC)

Allele	Editing	Sequence	Frequency	Phenotype
1	c.288_338del51	ttaag**GC**TC----------A**GCCGG**	3.8% (1/26)	Healthy 50% (13/26)
2	NE	ttaag**GC**TCT**GCTTTG**T**AG**GAT**CAAGCCTCAAGG**CTGGGGGTTCCCGTGGGTATTCTCTCAGCCGG**		
1	c.289_318del30	ttaag**GC**TCT----------**GTTCCCGTGGGTATTCTCTCAGCCGG**	30.8% (8/26)	
2	NE	ttaag**GC**TCT**GCTTTG**T**AG**GAT**CAAGCCTCAAGG**CTGGGGGTTCCCGTGGGTATTCTCTCAGCCGG**		
1	c.289_318del30	ttaag**GC**TCT----------**GTTCCCGTGGGTATTCTCTCAGCCGG**	11.5% (3/26)	
2	c.304_311del8	ttaag**GC**TCT**GCTTTG**T**AG**GAT**CAA**--------**GCTGGGGGTTCCCGTGGGTATTCTCTCAGCCGG**		
1	c.289_318del30	ttaag**GC**TCT----------**GTTCCCGTGGGTATTCTCTCAGCCGG**	3.8% (1/26)	
2	c.286_320del35	ttaag**GC**----------**TCCCGTGGGTATTCTCTCAGCCGG**		
1	c.304_317del14	ttaag**GC**TCT**GCTTTG**T**AG**GAT**CAA**----------**GGTTCCCGTGGGTATTCTCTCAGCCGG**	3.8% (1/26)	FA 50% (13/26)
2	NE	ttaag**GC**TCT**GCTTTG**T**AG**GAT**CAAGCCTCAAGG**CTGGGGGTTCCCGTGGGTATTCTCTCAGCCGG**		
1	c.311delG	ttaagGCTCT**GCTTTG**T**AG**GAT**CAAGCCTCA**-**GCTGGGGGTTCCCGTGGGTATTCTCTCAGCCGG**	7.7% (2/26)	
2	NE	ttaag**GC**TCT**GCTTTG**T**AG**GAT**CAAGCCTCAAGG**CTGGGGGTTCCCGTGGGTATTCTCTCAGCCGG**		
1	c.310delA	ttaagGCTCT**GCTTTG**T**AG**GAT**CAAGCCTCA**-**GGCTGGGGGTTCCCGTGGGTATTCTCTCAGCCGG**	3.8% (1/26)	
2	NE	ttaag**GC**TCT**GCTTTG**T**AG**GAT**CAAGCCTCAAGG**CTGGGGGTTCCCGTGGGTATTCTCTCAGCCGG**		
1	NE	ttaag**GC**TCT**GCTTTG**T**AG**GAT**CAAGCCTCAAGG**CTGGGGGTTCCCGTGGGTATTCTCTCAGCCGG**	34.6% (9/26)	
2	NE	ttaag**GC**TCT**GCTTTG**T**AG**GAT**CAAGCCTCAAGG**CTGGGGGTTCCCGTGGGTATTCTCTCAGCCGG**		

B

FA-807 BM CD34⁺ CFCs (3 nM MMC)

Allele	Editing	Sequence	Frequency	Phenotype
1	c.289_318del30	ttaag**GC**TCT----------**GTTCCCGTGGGTATTCTCTCAGCCGG**	14.3% (1/7)	Healthy 85.7% (6/7)
2	NE	ttaag**GC**TCT**GCTTTG**T**AG**GAT**CAAGCCTCAAGG**CTGGGGGTTCCCGTGGGTATTCTCTCAGCCGG**		
1	c.289_318del30	ttaag**GC**TCT----------**GTTCCCGTGGGTATTCTCTCAGCCGG**	42.9% (3/7)	
2	c.304_311del8	ttaag**GC**TCT**GCTTTG**T**AG**GAT**CAA**--------**GCTGGGGGTTCCCGTGGGTATTCTCTCAGCCGG**		
1	c.289_318del30	ttaag**GC**TCT----------**GTTCCCGTGGGTATTCTCTCAGCCGG**	14.3% (1/7)	
2	c.311delG	ttaagGCTCT**GCTTTG**T**AG**GAT**CAAGCCTCA**-**GCTGGGGGTTCCCGTGGGTATTCTCTCAGCCGG**		
1	c.289_318del30	ttaag**GC**TCT----------**GTTCCCGTGGGTATTCTCTCAGCCGG**	14.3% (1/7)	
2	c.310_311del2	ttaag**GC**TCT**GCTTTG**T**AG**GAT**CAAGCCTCA**--**GCTGGGGGTTCCCGTGGGTATTCTCTCAGCCGG**		
1	NE	ttaag**GC**TCT**GCTTTG**T**AG**GAT**CAAGCCTCAAGG**CTGGGGGTTCCCGTGGGTATTCTCTCAGCCGG**	14.3% (1/7)	FA 14.3% (1/7)
2	NE	ttaag**GC**TCT**GCTTTG**T**AG**GAT**CAAGCCTCAAGG**CTGGGGGTTCCCGTGGGTATTCTCTCAGCCGG**		

C

FA-807 BM CD34⁺ CFCs (10 nM MMC)

Allele	Editing	Sequence	Frequency	Phenotype
1	c.289_318del30	ttaag**GC**TCT----------**GTTCCCGTGGGTATTCTCTCAGCCGG**	33.3% (2/6)	Healthy 100% (6/6)
2	NE	ttaag**GC**TCT**GCTTTG**T**AG**GAT**CAAGCCTCAAGG**CTGGGGGTTCCCGTGGGTATTCTCTCAGCCGG**		
1	c.289_318del30	ttaag**GC**TCT----------**GTTCCCGTGGGTATTCTCTCAGCCGG**	33.3% (2/6)	
2	c.304_311del8	ttaag**GC**TCT**GCTTTG**T**AG**GAT**CAA**--------**GCTGGGGGTTCCCGTGGGTATTCTCTCAGCCGG**		
1	c.289_318del30	ttaag**GC**TCT----------**GTTCCCGTGGGTATTCTCTCAGCCGG**	16.7% (1/6)	
2	c.311delG	ttaagGCTCT**GCTTTG**T**AG**GAT**CAAGCCTCA**-**GCTGGGGGTTCCCGTGGGTATTCTCTCAGCCGG**		
1	c.289_318del30	ttaag**GC**TCT----------**GTTCCCGTGGGTATTCTCTCAGCCGG**	16.7% (1/6)	
2	c.302_321del20	ttaag**GC**TCT**GCTTTG**C**AG**GATC----------**CCCGTGGGTATTCTCTCAGCCGG**		

Figure S5. Sanger sequencing of the *FANCA* exon 4 targeted locus in BM FA-807 hematopoietic CFCs, Related to Figure 6A. The frequency and phenotype of the different allele combination detected in the CFCs are indicated. (A) Colonies obtained in the absence of MMC. (B) Colonies obtained at 3 nM MMC. (C) Colonies obtained at 10 nM MMC. NE = not edited. The c.295C>T mutation is signalled in red. Corrective NHEJ-repair events are marked in green while non-corrective ones are shown in red. Codons are represented by alternating bold and normal upper-case letters and intronic sequence is shown in lower-case letters. The symbol "-" stands for deletion.

Figure S6

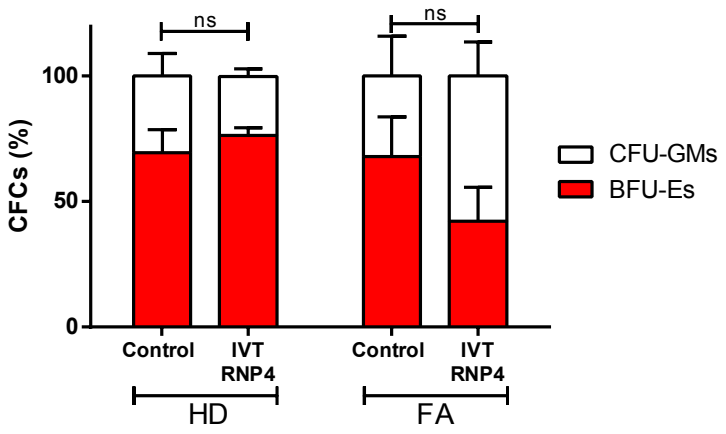


Figure S6. *In vitro* differentiation capacity of edited FA HSPCs, Related to Figures 4, 5 and 6. Similar percentage of erythroid (BFU-Es) and myeloid (CFU-GMs) progenitors were observed *in vitro* in edited FA-HSPCs (N=4) versus unedited or HD cells (N=6). Bars represent mean ± SD. A two-way ANOVA was performed followed by Tukey's post hoc test.

Figure S7

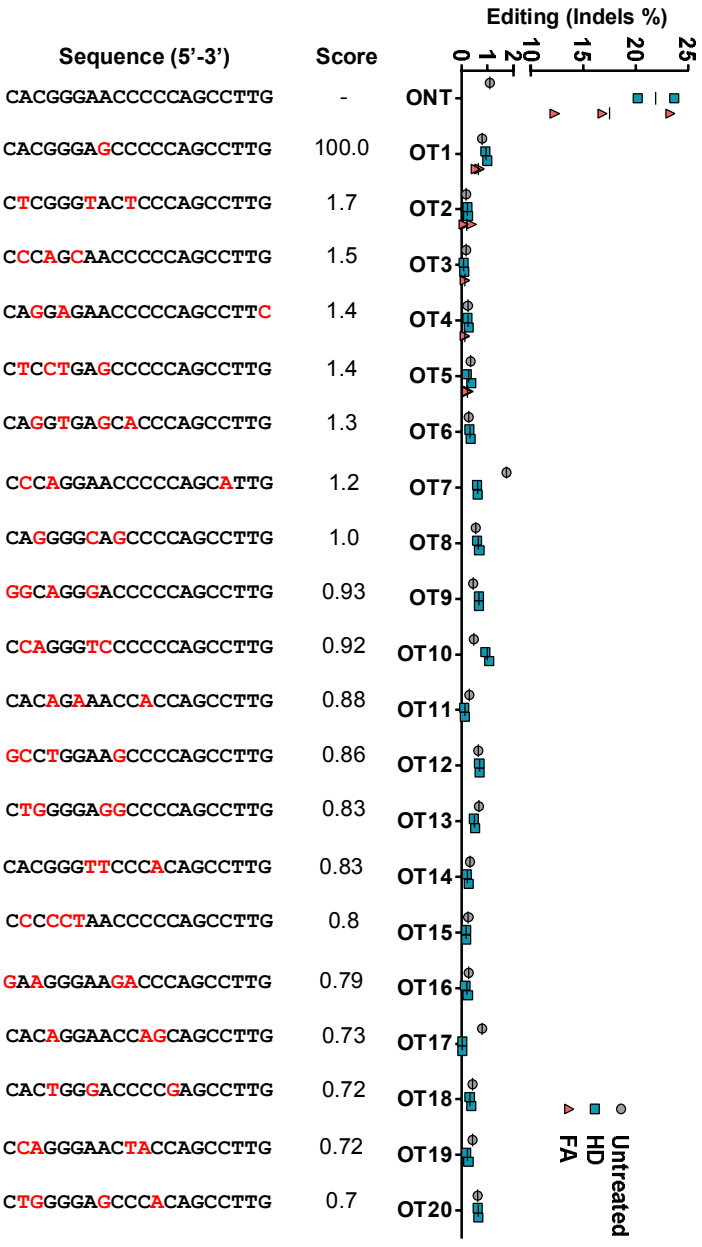


Figure S7. NGS analysis of the top-20 *in silico* predicted off-target loci for gGM4, Related to Figures 4, 6 and 7. The top-5 loci were analysed by NGS in three different samples from FA-A patients bearing the c.295C>T mutation (red triangles) after NHEJ-mediated gene editing using the IVT-RNP4. The top-20 loci were analysed in two edited hCD34^{+} cell samples from healthy donors after editing using IVT-RNP4 (blue squares). Unedited hCD34^{+} cells from a healthy donor were also sequenced control (grey circle). The probability score calculated by the *CRISPR Design Tool* is shown. The mismatches are signalled in red.