

Table S1. SNPs and VNTRs analyzed for haplotype determination and the corresponding primers used to amplify each region

SNP/VNTR	Position	Fragm. (bp)	Primers
rs1800285	c.596+74G/A	384	F: 5'GCCCAAAGGCCAGGGAGTTT 3' R: 5'AGCCAGAAATCAAACCCGTCTGA 3'
rs1800286	c.710-12G/A	294	F: 5'CCCAAGCCATATGCTCACC 3' R: 5'GGTACAAACAGCACGTTTCAA 3'
rs7190823	c.796G/A	189	F: 5'GCAGGTATCACACAAATTACAG 3' R: 5'GCACAGTGAAACATACAGAGG 3'
rs2239359	c.1501G/A	247	F: 5'TGACTGTAAAGGGCTGTGTTTC 3' R: 5'GTGAGGAGTGGGCATGGA 3'
rs1800335	c.1715+82T/C	316	F: 5'GGCCTTCACTGCTCAGTCTT 3' R: 5'TGGGACACATTCCAAAGAGC 3'
rs1800340	c.2151+8T/C	328	F: 5'AGCAGGATCCGTGGAATCGTAC 3' R: 5'GAGAAGGCTCCATGCGTCTAATG 3'
rs7195066	c.2426G/A	444	F: 5'GAGGGCCAGGCTGCTACTT 3' R: 5'GACAGATAAAATTCTGGAAGG 3'
rs1800345	c.3240-42 G/A	390	F: 5'GACACAGGCCAAGGCTCTG 3' R: 5'GGCATTCCAGACACTGTTCC 3'
rs1061646	c.3935-16T/C	314	F: 5'CCAGCTGCTGACAGGTACC 3' R: 5'GGACCCAGAAGTGCTGAGATG 3'
rs1800359	c.4260+29T/C	275	F: 5'GACTATGGGGGACACACCAG 3' R: 5'GGCTCTGGCAGAAATAGTCG 3'
d16s3016	3' FANCA	178–210	F: 5'CTCCCTGAGCAACAAACACC 3' R: 5'GGTCATTTATATGCGCCTGA 3'
d16s3121	3' FANCA	79–85	F: 5'CATGTTGTACATCGTGATGC 3' R: 5'AGCTTTTATTTCCCAGGGGT 3'
d16s3407	5' FANCA	195–205	F: 5'CCAACAATGCCAATTTCTAG 3' R: 5'CTCAGCCACACAGATAAAC 3'
d16s303	5' FANCA	101–121	F: 5'GATCAGTGCTCGTTTTTTTTGGTTTGG 3' R: 5'CAACAAGAGCGAAACTCGGTCTCAA 3'

Table S2. Analysis of hemizygosity of SNPs for breakpoint characterization of ex1-20del in *FANCA*

Size of amplified regions, primers used, and RT-PCR amplification conditions are shown.

Gene	SNPs	Fragm. (bp)	Primers	Amp. Cond.*
<i>SNPs region 1</i>				
SPIRE2	rs9928396, rs9935541	389	F: 5'TGCTCTCTGCATGGGAAGGG 3' R: 5'GGATGTCATGGGTGTGTGGC 3'	Hyb. T: 60°C Ext time: 45"
FLJ20286	rs12596872, rs8049897, rs8051733	476	F: 5'GAGGGTCTAGGCTAATAGGG 3' R: 5'GTTTCCTTCTCTGGGTGAGG 3'	Hyb. T: 58°C Ext. time: 45"
GAS8	rs3743829, rs3743827, rs3743826	406	F: 5'CCGCTGATGGGAATTATGGG 3' R: 5'TGTGTGTGGTAAAGGGTGGG 3'	Hyb. T: 60°C Ext. time: 45"
<i>SNPs region 2</i>				
TCF25 (1)	rs1109334	701	F: 5'ACACGCATGCTAGAAGCTGG 3' R: 5'TCTTCTGTCTGGGATGTGCG 3'	Hyb. T: 56°C Ext. time: 60"
TCF25 (2)	rs11866200, rs2270461, rs2270460	416	F: 5'CGGCTTCCTTGATCTCAGAG 3' R: 5'AGCAGTGACGATGGTGACAC 3'	Hyb. T: 58°C Ext. time: 45"
MC1R	rs3212359, rs3212361, rs3212363	706	F: 5'AAGCAGGAAGGAGTCGTTGG 3' R: 5'TGCTTTGGCTGAGAGCAGAG 3'	Hyb. T: 58°C Ext. time: 60"
<i>SNPs region 3</i>				
TCF25 (3)	rs7199642, rs7184225, rs12919894	1628	F: 5'GCCTAGTTTTGCTTCTTCAG 3' R: 5'CTGGCCTAGAATCTTTTCTG 3'	Hyb. T: 54°C Ext. time: 120"
TCF25 (4)	rs12930056, rs12930606, rs17784386	950	F: 5'CAAGGATACGATTCAGGAGC 3' R: 5'CCATTGAAGTTCAGCTTGGC 3'	Hyb. T: 56°C Ext. time: 60"
TCF25 (5)	rs9806913, rs9922515	548	F: 5'TCCCTCAAGGGTAAACTAGC 3' R: 5'AATCGCTGTCCCTACTTCAG 3'	Hyb. T: 56°C Ext. time: 45"
TCF25 (6)	rs7184960, rs2293586	967	F: 5'AGCTGCACTCACCTGTTCTC 3' R: 5'ACCTCGTAGGTAAGGCAGAG 3'	Hyb. T: 58°C Ext. time: 60"
TCF25 (7)	rs11646448	328	F: 5'CCTTCTCAGCCTATGTCAC 3' R: 5'CAGCTCTTGACTTTCTCTGC 3'	Hyb. T: 56°C Ext. time: 45"
TCF25 (8)	rs10153210, rs10153196	418	F: 5'CACTGGTACAATCCAATGCC 3' R: 5'TTCCACTGGCGTATTTCTCTG 3'	Hyb. T: 54°C Ext. time: 45"

* Amp. Cond.: "Amplification conditions". Hyb. T.: "Hybridization temperature" and Ext. time.: "Extension time".

Table S3. Genetic dosage analysis for breakpoint characterization of ex1-20del in *FANCA*
The size of amplified regions, primers used, and RT-PCR amplification conditions are shown.

Region	Fragm. (bp)	Primers*	Amp. Cond.
SP1	154	F: 5'GAAGTCAAGAGTCCTGGTGT 3' R: 5'CTGTGTCCTGAAGGATGTCT 3'	Hyb. T: 58°C Ext time: 11"
TC1	202	F: 5'TGTCATCTCCATCCGTCTTG 3' R: 5'ATCTCAGCATGAGCGTTCAC 3'	Hyb. T: 58°C Ext time: 11"
TC2	252	F: 5'TGTTGTTTCGGCTCCATAGAC 3' R: 5'CACTCAGGTCAAGTTTGTCG 3'	Hyb. T: 58°C Ext time: 11"
TC3	192	F: 5'GAGTAGATTGTGTCCGAAGG 3' R: 5'TCAGACTCTGTGGCTATCTC 3'	Hyb. T: 58°C or 62°C Ext time: 11"
TC4	256	F: 5'TGCTGTCCTCAATCCTCTCT 3' R: 5'GATTCTGTCTATGCTGAGCG 3'	Hyb. T: 62°C Ext time: 11"
TC5	262	F: 5'TGGATTCAAGTGTCTGGGGA 3' R: 5'ACACAGTGCTCTTGACACA 3'	Hyb. T: 62°C Ext time: 11"
TC6	207	F: 5'TTTAGGGGTGGTCAGCCAT 3' R: 5'CATCCTGTCCTTAATGCGTC 3'	Hyb. T: 62°C Ext time: 11"

*Primers are all 30nt long and % of GC is 50.

Amp. Cond.: "Amplification conditions". Hyb. T.: "Hybridization temperature" and Ext. time.: "Extension time".

Table S4. Spanish FA-A patients and corresponding mutations

Pat. ID	Mutation 1		Mutation 2	
	DNA	Protein	DNA	Protein
FA5	c.1115-1118delTTGG	V372AfsX42	c.4198C>T	R1800C
FA13	c.3788_3790delTCT	F1263del	c.3788_3790delTCT	F1263del
FA16	c.295C>T	Q99X	c.295C>T	Q99X
FA27	c.3788_3790delTCT	F1263del	c.3788_3790delTCT	F1263del
FA40	c.1475A>G	H492R	c.1475A>G	H492R
FA46	c.3788_3790delTCT	F1263del	c.3788_3790delTCT	F1263del
FA47	c.3788_3790delTCT	F1263del	c.3788_3790delTCT	F1263del
FA48	c.3164G>T	R1055L	c.3788_3790delTCT	F1263del
FA49	ex21-29del	No Protein	ND*	
FA50	ex21-29del	No Protein	ND	
FA52	c.3558insG	R1187E fsX28	c.710-5T>C	Altered splicing
FA53	c.2851C>T	R951W	c.3913C>T	L1305F
FA54	ex6-14del	No Protein	c.3558insG	R1187EfsX28
FA55	c.295C>T	Q99X	c.295C>T	Q99X
FA56	c.233_236delTTGA	I78TfsX16	c.3913C>T	L1305F
FA58	ex1-20del	No Protein	c.821C>T	L274P
FA74	ex1-3del	No Protein	c.893+1G>T	Exon 10 skipping
FA75	c.3788_3790delTCT	F1263del	c.2639G>A	R880Q
FA82	c.709+5G>T	D237G+238ins10 (AFMTRCGFLD)	c.3788_3790delTCT	F1263del
FA84	c.3788_3790delTCT	F1263del	c.1771C>T	R591X
FA87	c.295C>T	Q99X	c.295C>T	Q99X
FA88	c.1147delG	E383RfsX32	c.709+5 G>A	D237G+238ins10 (AFMTRCGFLD)
FA91	ex21-29del	No Protein	c.2224C>A	Q742K
FA94	c.1470+1G>T	Exon 15 skipping	c.2641 C>T	Q881X
FA102	ex21-22del	No Protein	c.1115-1118delTTGG	V372AfsX42
FA103	c.295C>T	Q99X	c.295C>T	Q99X
FA105	ex4-5del	No Protein	c.1115-1118delTTGG	V372AfsX42
FA106	ex15-43del	No Protein	c.1115-1118delTTGG	V372AfsX42
FA108	c.295C>T	Q99X	c.295C>T	Q99X
FA109	c.295C>T	Q99X	c.295C>T	Q99X
FA110	c.3558insG	R1187E fsX28	c.987_990delTCAC	H330AfsX4
FA112	c.1771C>T	R591X	c.1771C>T	R591X
FA113	c.295C>T	Q99X	c.295C>T	Q99X
FA117	c.3558insG	R1187E fsX28	c.3788_3790delTCT	F1263del
FA122	c.295C>T	Q99X	c.295C>T	Q99X
FA123	c.295C>T	Q99X	c.295C>T	Q99X
FA125	c.65G>A	W22X	c.1115-1118delTTGG	V372AfsX42
FA126	c.65G>A	W22X	c.1115-1118delTTGG	V372AfsX42
FA145	ex1-20del	No Protein	c.893+920 C>A	Altered splicing
FA153	c.3558insG	R1187E fsX28	c.4123_4139del17	T1375Sfs
FA155	c.3788_3790delTCT	F1263del	c.3348+5 G>A and 3335 T>G	Exon 33 skipping
FA157	ex4-5del	No Protein	c.3788_3790delTCT	F1263del
FA163	ex3del	No Protein	ex3del	No Protein
FA164	c.4130C>G	S1377X	c.3788_3790delTCT	F1263del
FA166	c.3558insG	R1187E fsX28	ND	
FA170	ex1-6del	No Protein	c.3788_3790delTCT	F1263del
FA178	c.3558insG	R1187E fsX28	c.3558insG	R1187E fsX28
FA183	c.2535_2536delCT	L845fs	c.1703delT	V568AfsX
FA184	c.2535_2536delCT	L845fs	c.1703delT	V568AfsX
FA192	c.3788-3790delTCT	F1263del	c.3828+1G>T	Exon 38 skipping
FA203	c.718C>T	Q240X	c.1115-1118delTTGG	V372AfsX42
FA311	c.3788_3790delTCT	F1263del	c.3913C>T	L1305F
FA300	c.295C>T	Q99X	c.295C>T	Q99X
FA304	c.295C>T	Q99X	c.295C>T	Q99X
FA306	c.233_236delTTGA	T78IfsX16	c.3239G>A	R1080Q
FA328	c.2303T>C	L768P	c.2303T>C	L768P
FA330	c.238delT	C80VfsX	c.238delT	C80VfsX
FA334	ex19-21del	No Protein	c.1115-1118delTTGG	V372AfsX42
FA337	c.284delG	G95AfsX	c.4124_4125delCA	T1375SfsX
FA345	ex16-17del	No Protein	c.1115-1118delTTGG	V372AfsX42
FA360	c.3788_3790delTCT	F1263del	c.4198C>T	R1800C
FA363	ex4-6del	No Protein	ex4-6del	No Protein
FA378	ex18-20del	No Protein	c.1873G>C	C625S
FA379	c.295C>T	Q99X	c.295C>T	Q99X
FA387	ex28-29del	No Protein	c.50delG	G17AfsX17
FA388	c.1115_1118delTTGG	V372AfsX42		
FA407	c.3788_3790delTCT	F1263del	c.3788_3790delTCT	F1263del

*ND: Not detected

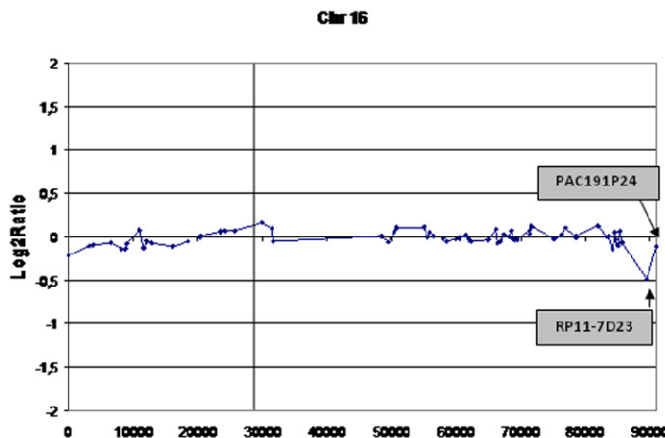
Figure S1. 5' breakpoint characterization of ex1-20del in patient FA145 by microarray CGH

(A) Localization and Log_2 ratio obtained for the BACs in *FANCA* region. *FANCA* position is also provided (Human version NCBI36). (B) Graph showing mean log_2 ratio values of all BACs in chromosome 16 (Microarray 1.4Mb). The BACs relevant for this study are highlighted. (C) Scheme of studied region showing gene and BAC positions. The region previously known to be deleted by MLPA is shown in red. The region in which the breakpoint should be located is marked in purple.

a

BAC	From(Kb)	To (Kb)	Log_2 ratio	$SD_{\text{Log}_2\text{rat}}$
<i>Array 1.4Mb USCF</i>				
RP11-7D23	88297	88466	-0,48764	0,0153
PAC191P24	88827	88830	-0,11170	0,0067
<i>Tiling resolution Array (32K) (BCCRC)</i>				
RP11354M24	88290	88417	0,14897	0,0117
RP11665E03	88469	88686	-0,08293	0,0035
<i>FANCA</i>	88331	88410		

b



c

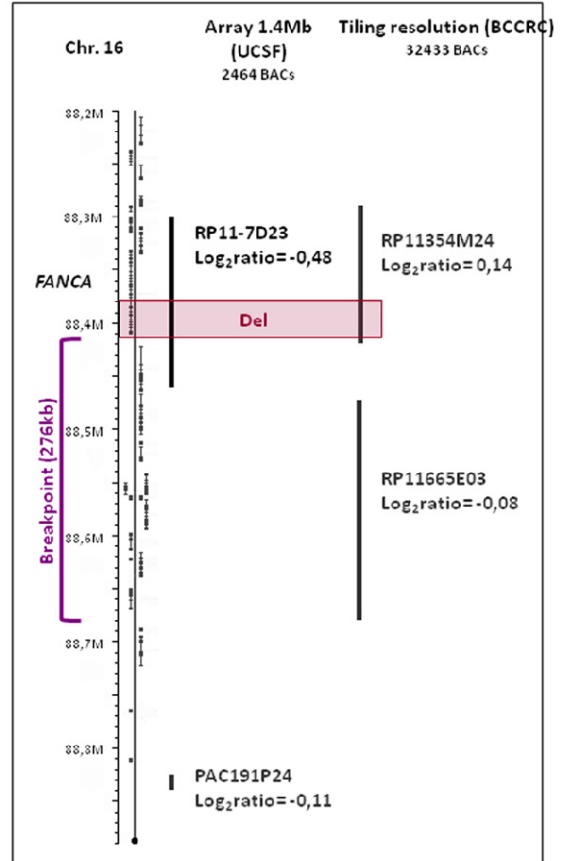


Figure S2. 5' breakpoint characterization of ex1-20del in patient FA145 by analysis of homozygosity/hemizyosity of SNPs in patient FA145 and progenitors

FA145m (mother) is the carrier of ex1-20del and is indicated with an asterisk (*). SNP analysis was conducted in 3 rounds. In each round, the region containing the breakpoint was narrowed down as indicated. The key color used to indicate alleles found for each SNP is shown in the lower-right corner of the figure.

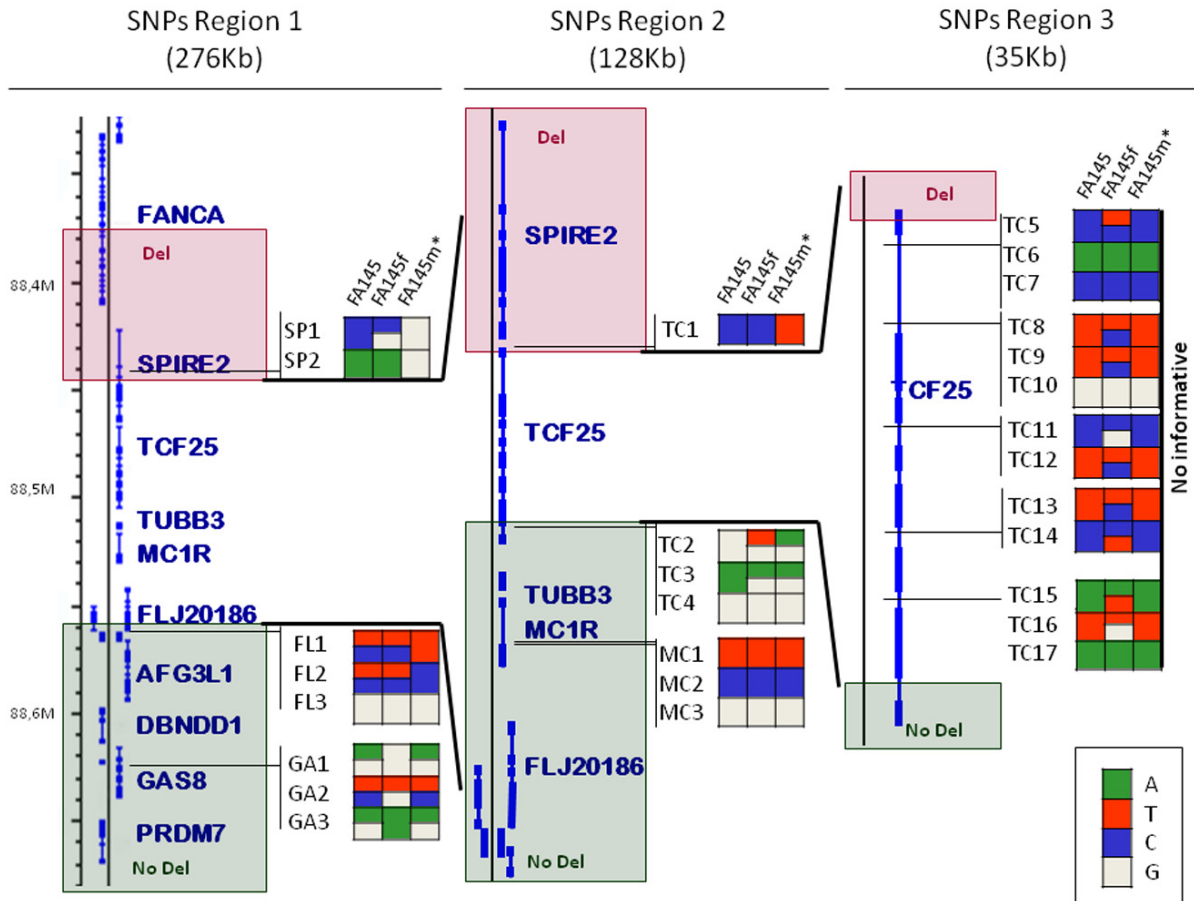
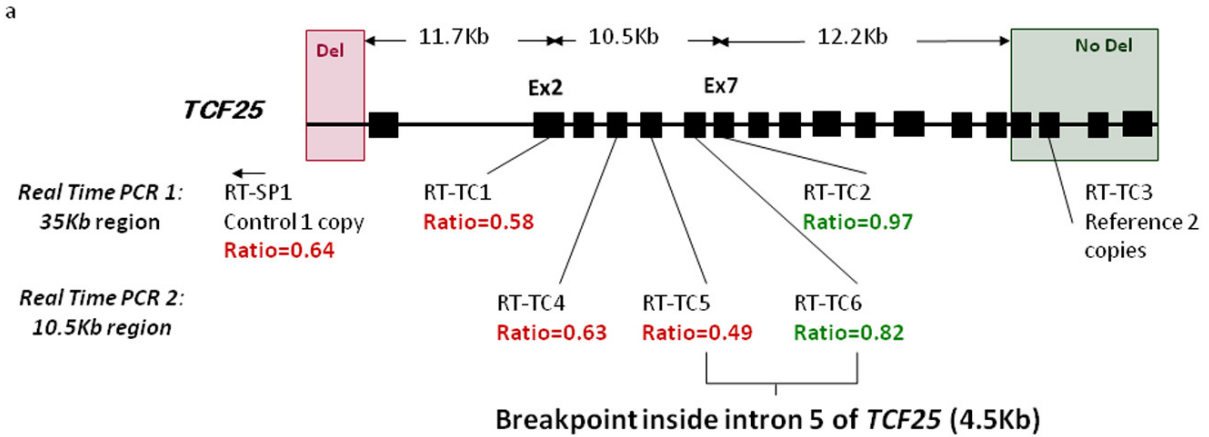


Figure S3. 5' breakpoint characterization of ex1-20del in patient FA145 by gene-dosage quantification by Real Time PCR

(A) Scheme of the amplified regions in *TCF25* gene and the relative copy number value obtained. (B) Results obtained in patient (FA145) and reference (FA145f), including the normalization of results (using control region RT-TC3), and ratio calculation.



b

Real Time PCR 1				Real Time PCR 2					
	RT_TC3	RT_SP1	RT_TC1	RT_TC2		RT_TC3	RT_TC4	RT_TC5	RT_TC6
FA145f	1	14,44	41,59	2,08	FA145f	1	4,67	2,87	1,51
FA145	1,04	9,72	25,33	2,10	FA145	1,24	3,70	1,76	1,54
FA145_Norm		9,34	24,35	2,02	FA145_Norm		2,98	1,41	1,24
Ratio (FA145/FA145f)		0.64	0.58	0.97	Ratio (FA145/FA145f)		0.63	0.49	0.82