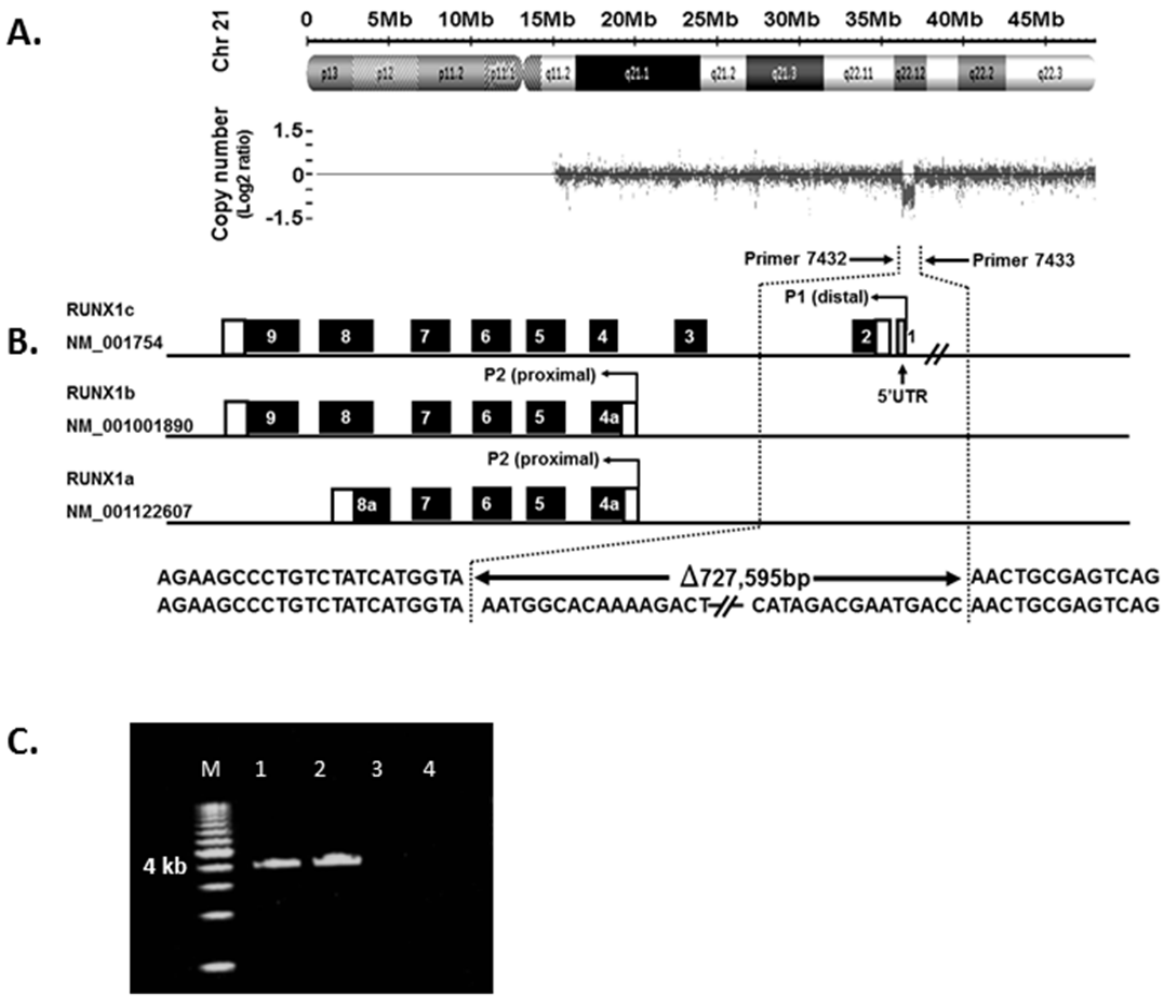
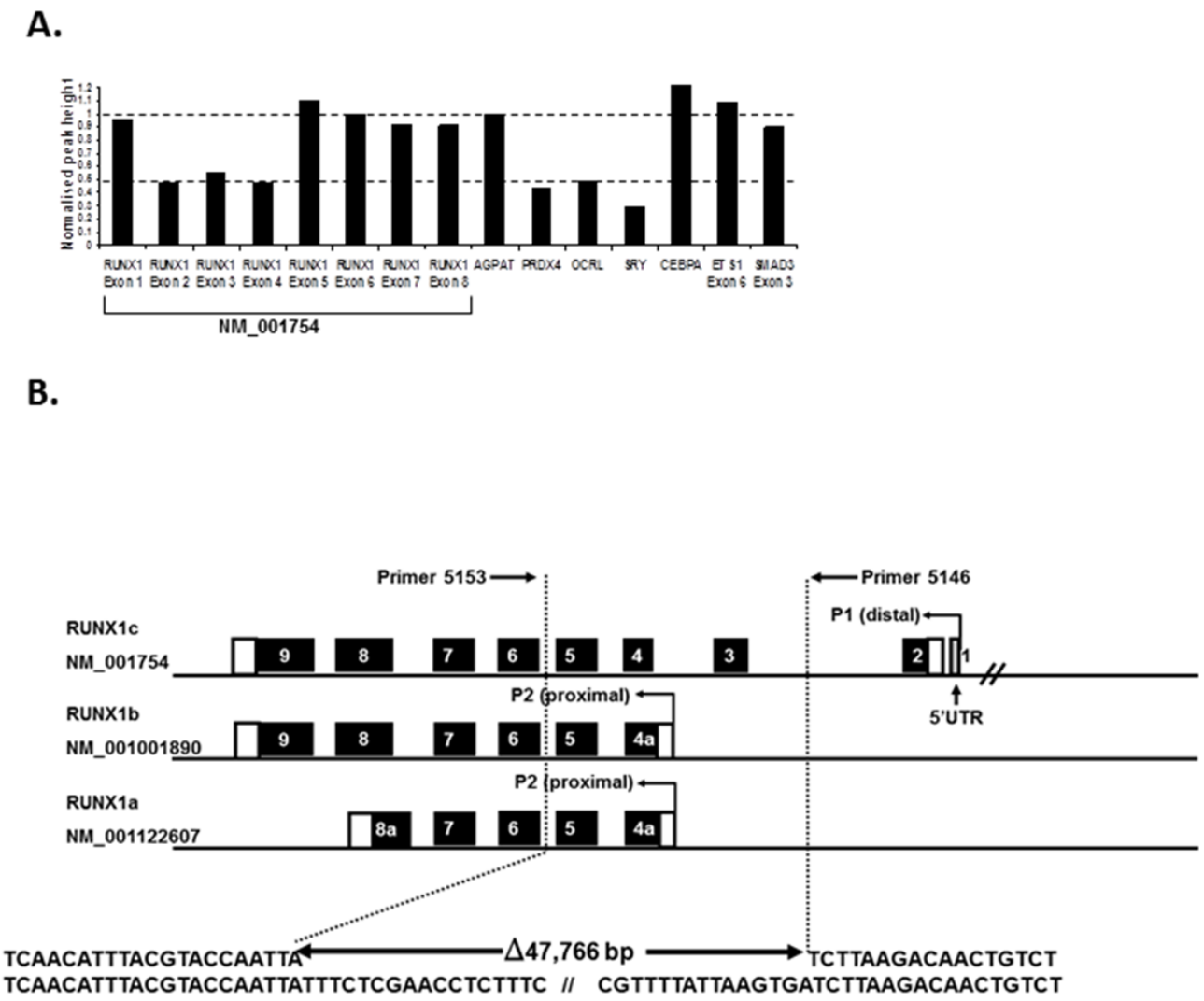
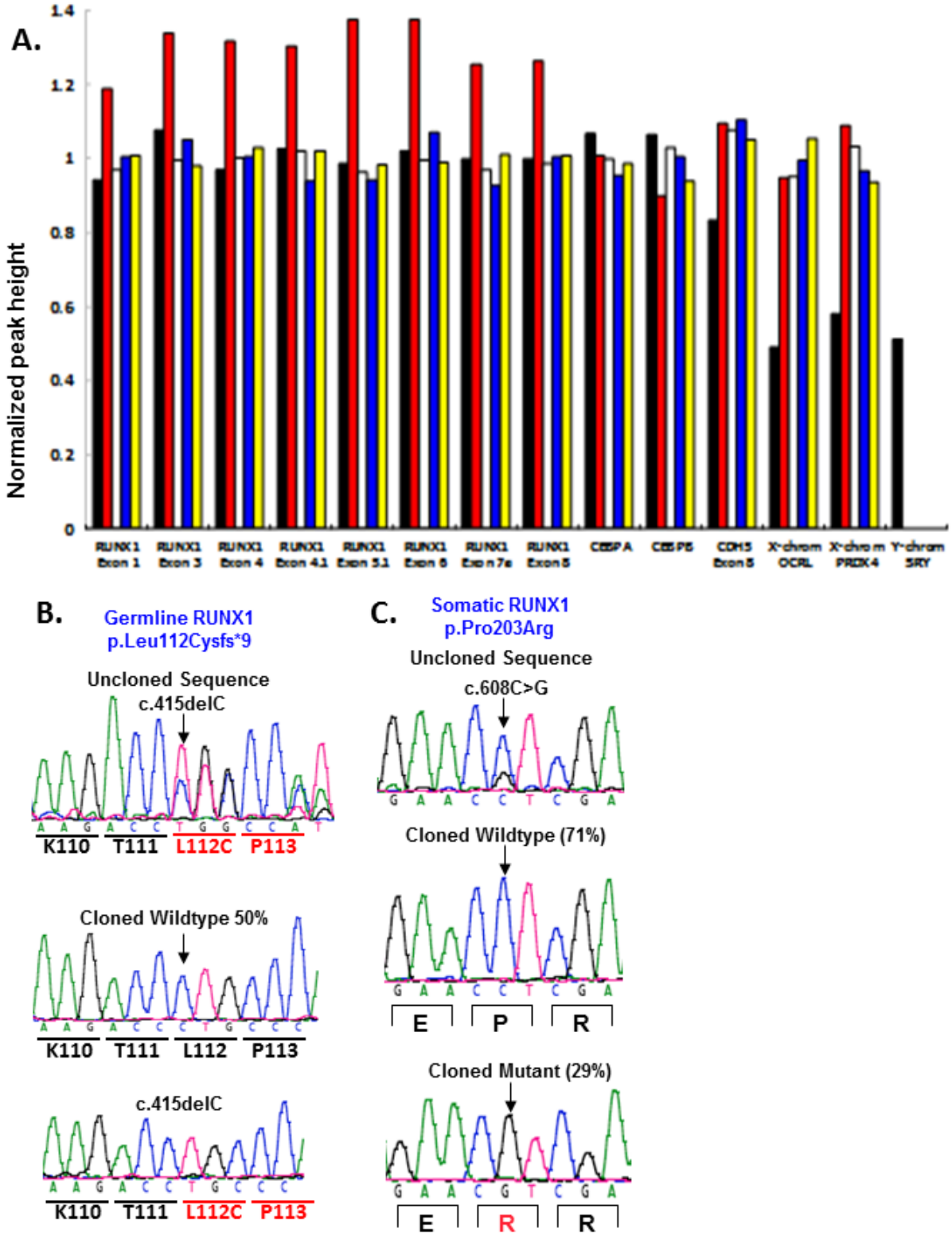
Supplemental Figure 1



Supplemental Figure 2

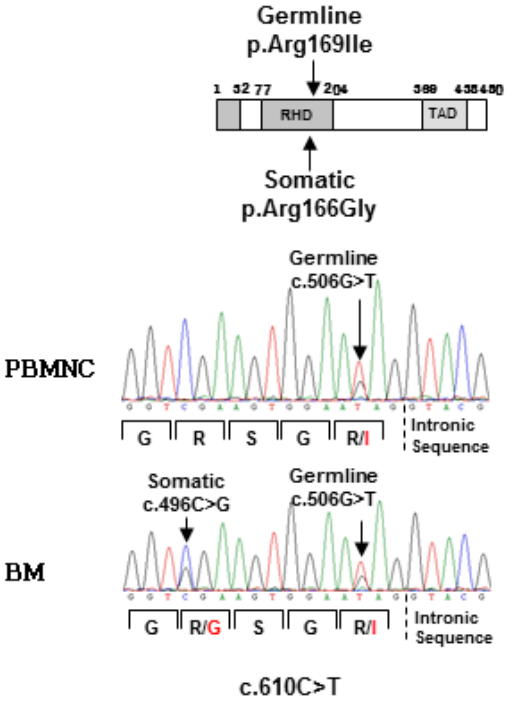


Supplemental Figure 3



Supplemental Figure 4

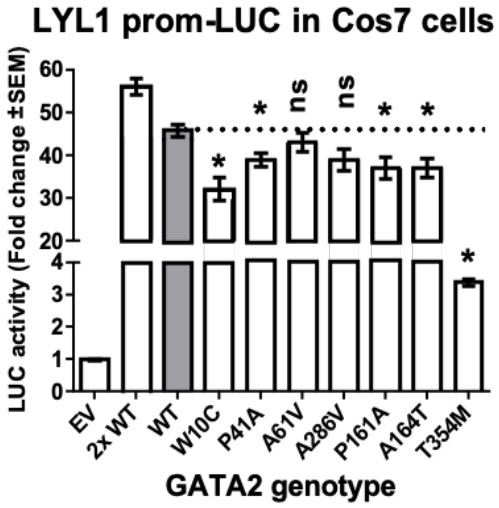
A.



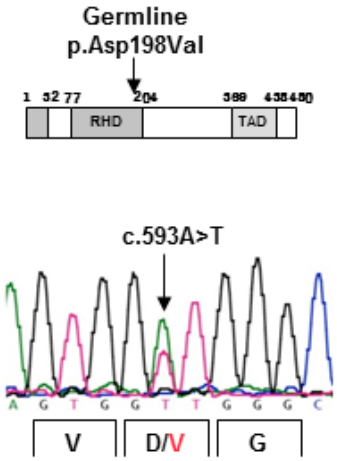
B.

Gene	Mutation	Cancer type	Number	Source
RUNX1	R169K	Breast carcinoma	2	COSMIC
RUNX1	R169S	MDS	1	COSMIC
RUNX1	R169Kfs*44	Breast carcinoma	3	TCGA
RUNX1	R169Kfs*8	AML	1	Gaidzik, 2016
RUNX1	R169Qfs*10	AML	1	Gaidzik, 2016

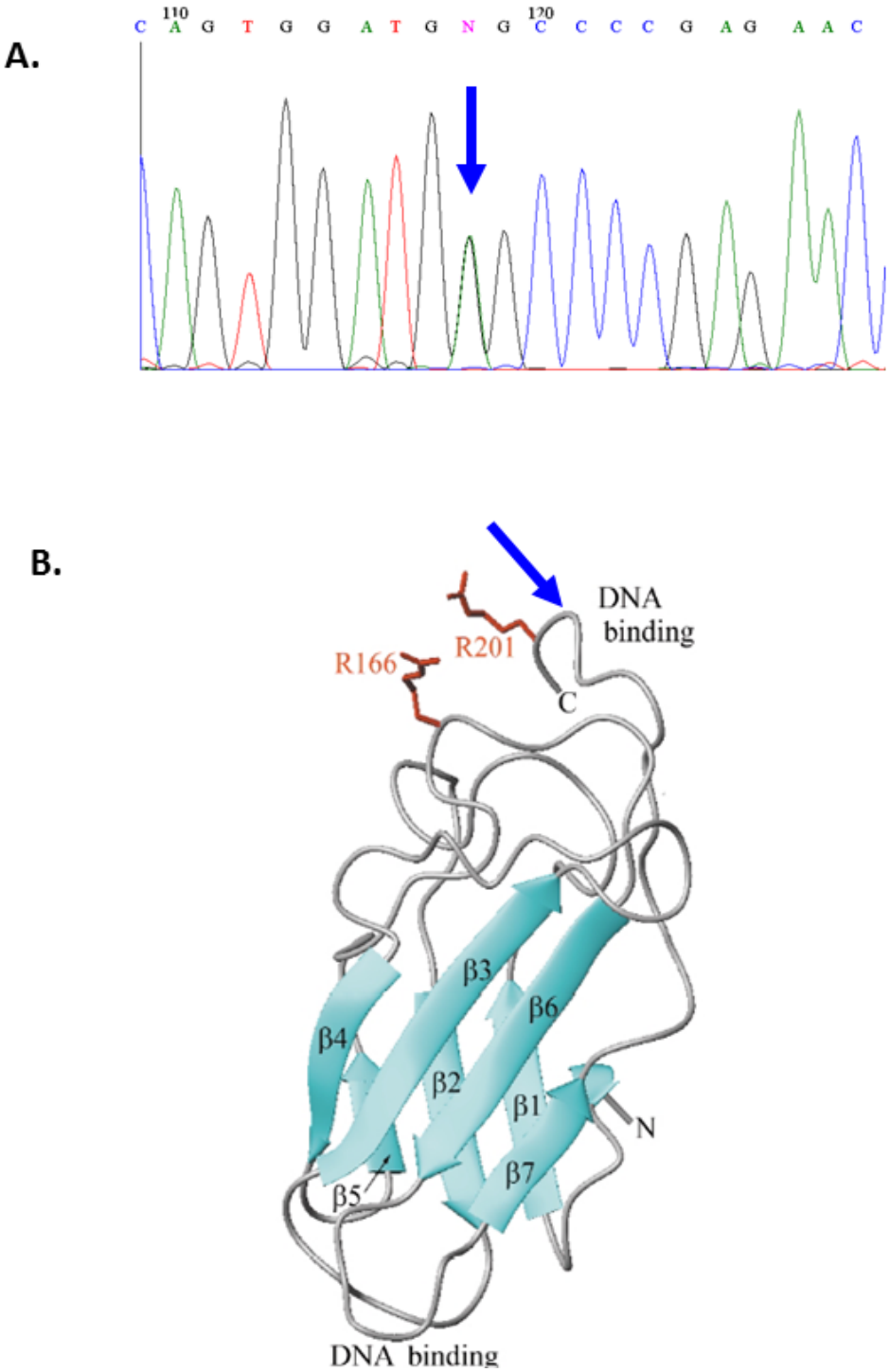
C.



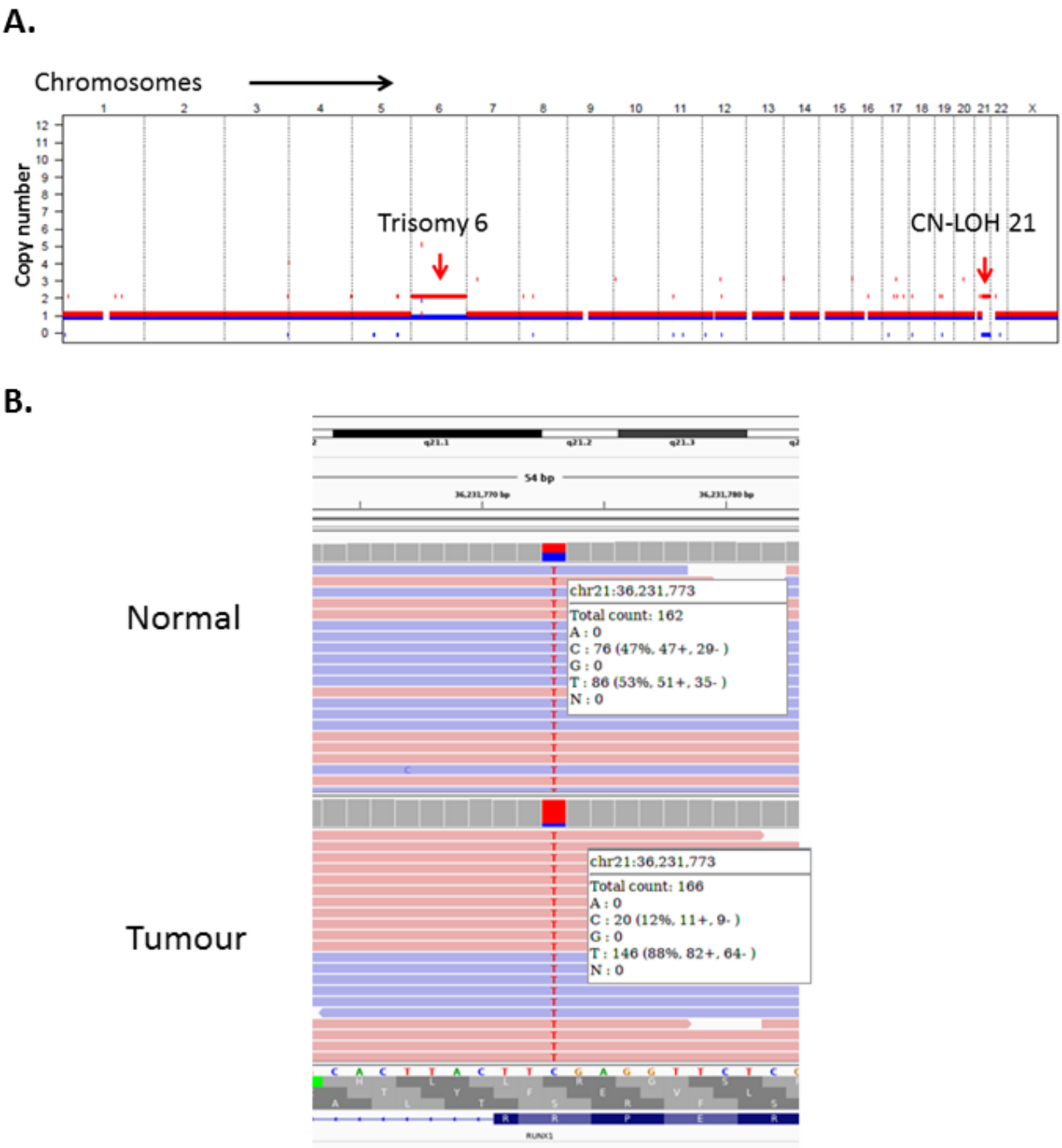
Supplemental Figure 5



Supplemental Figure 6

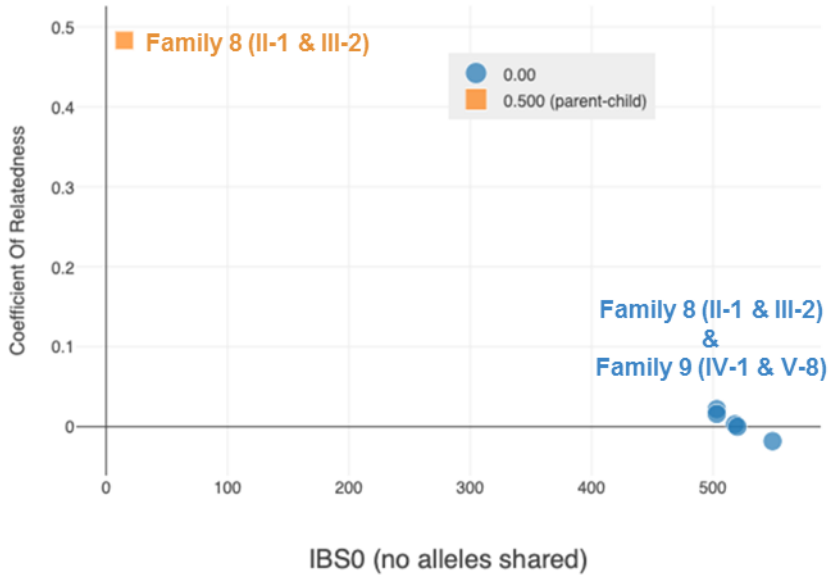


Supplemental Figure 7

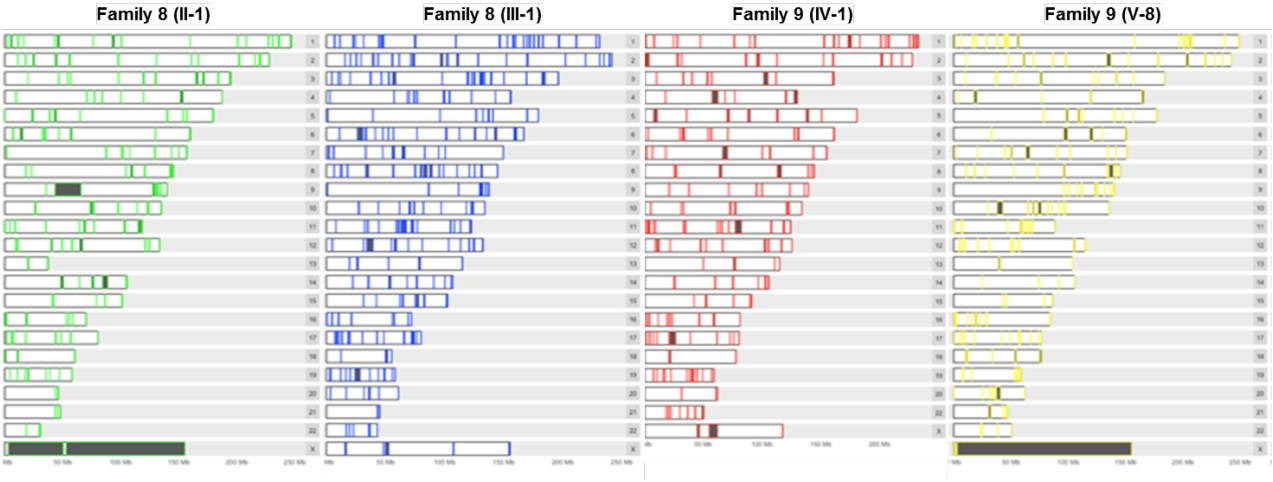


Supplemental Figure 8

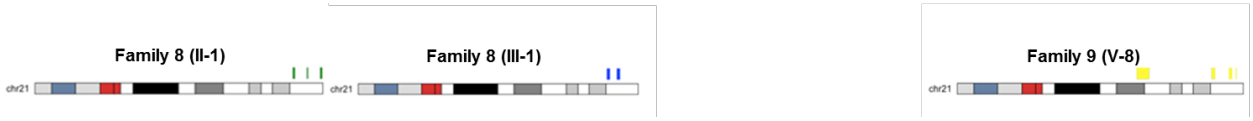
A.



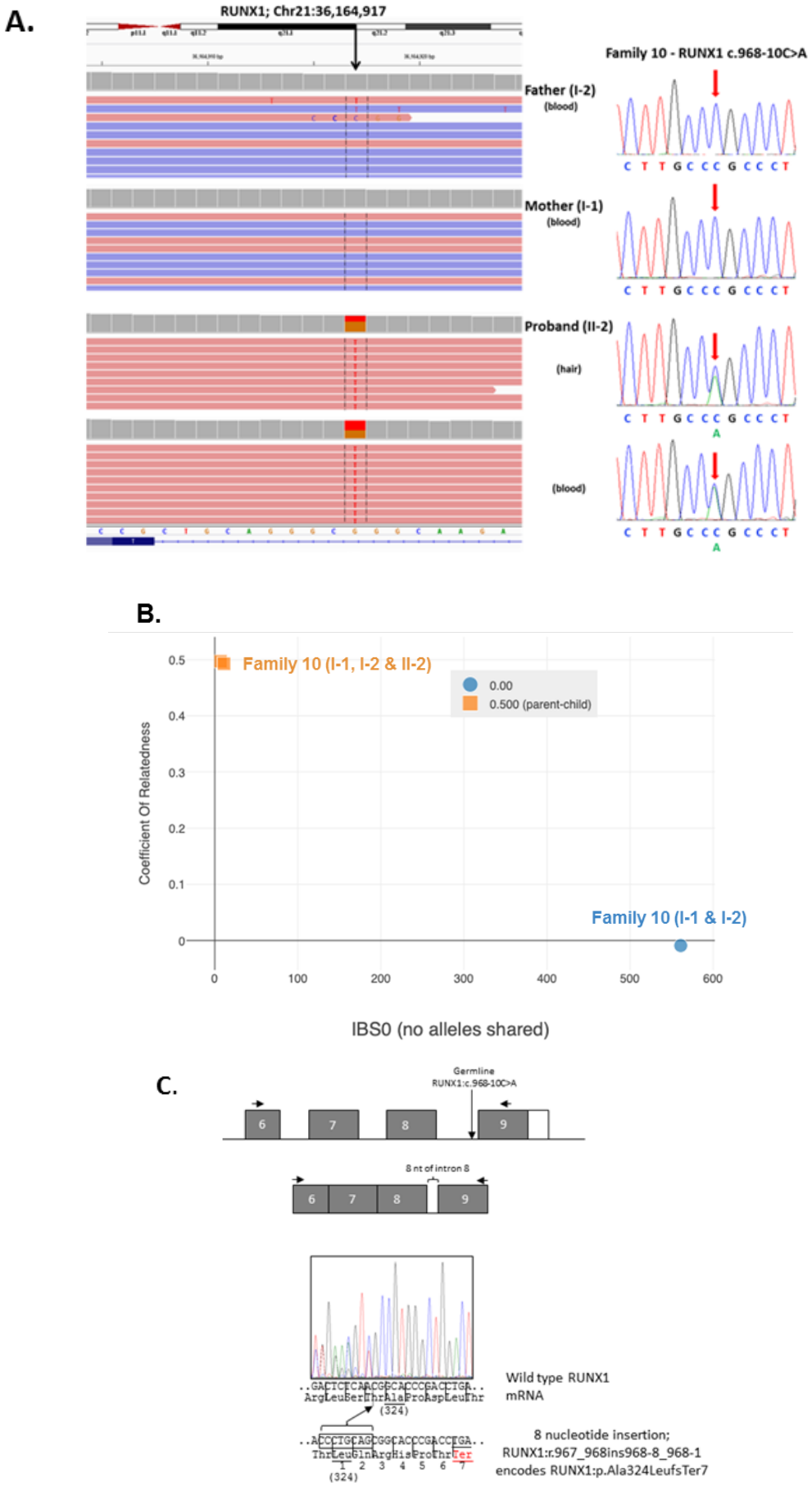
B.



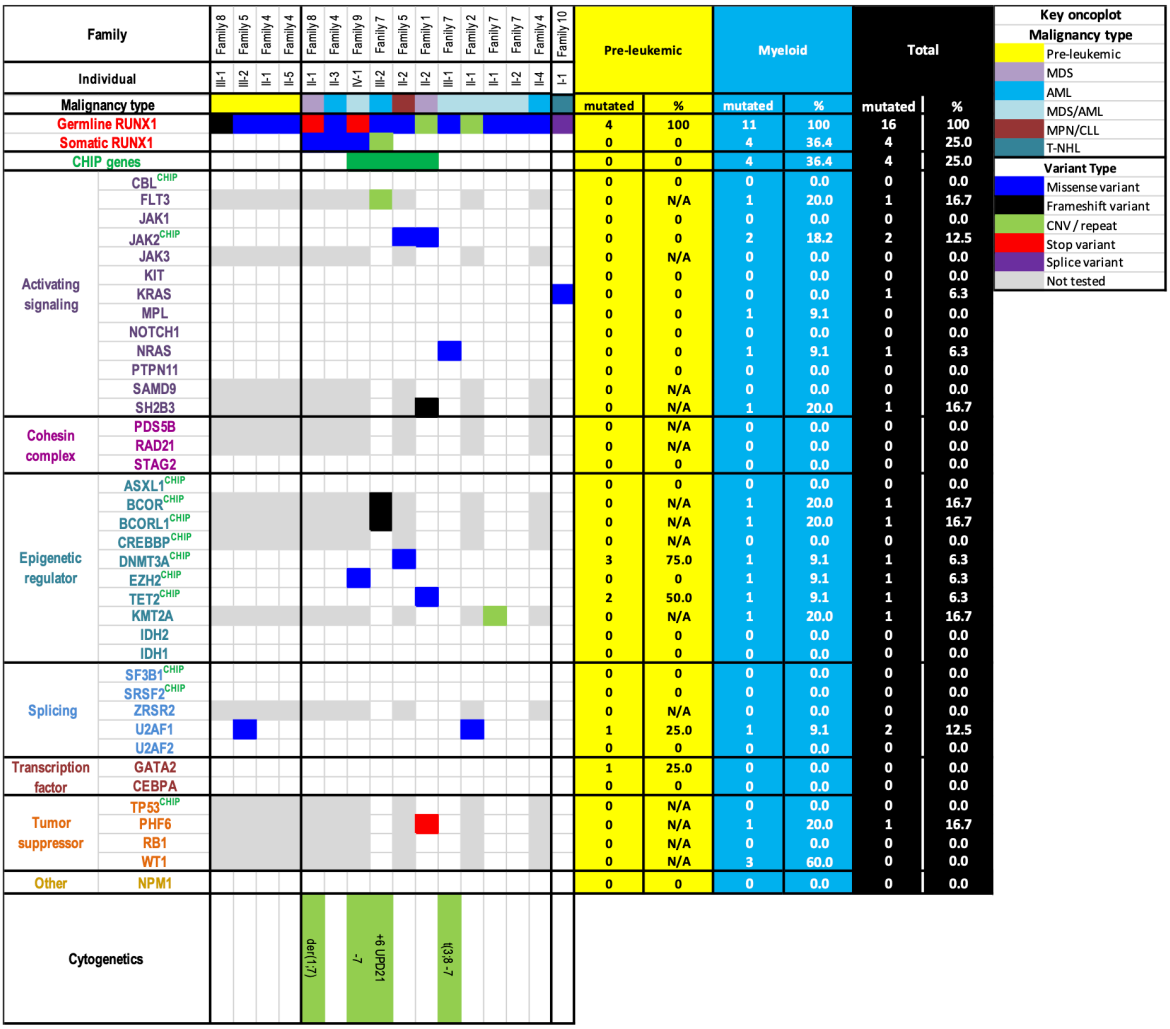
C.



Supplemental Figure 9

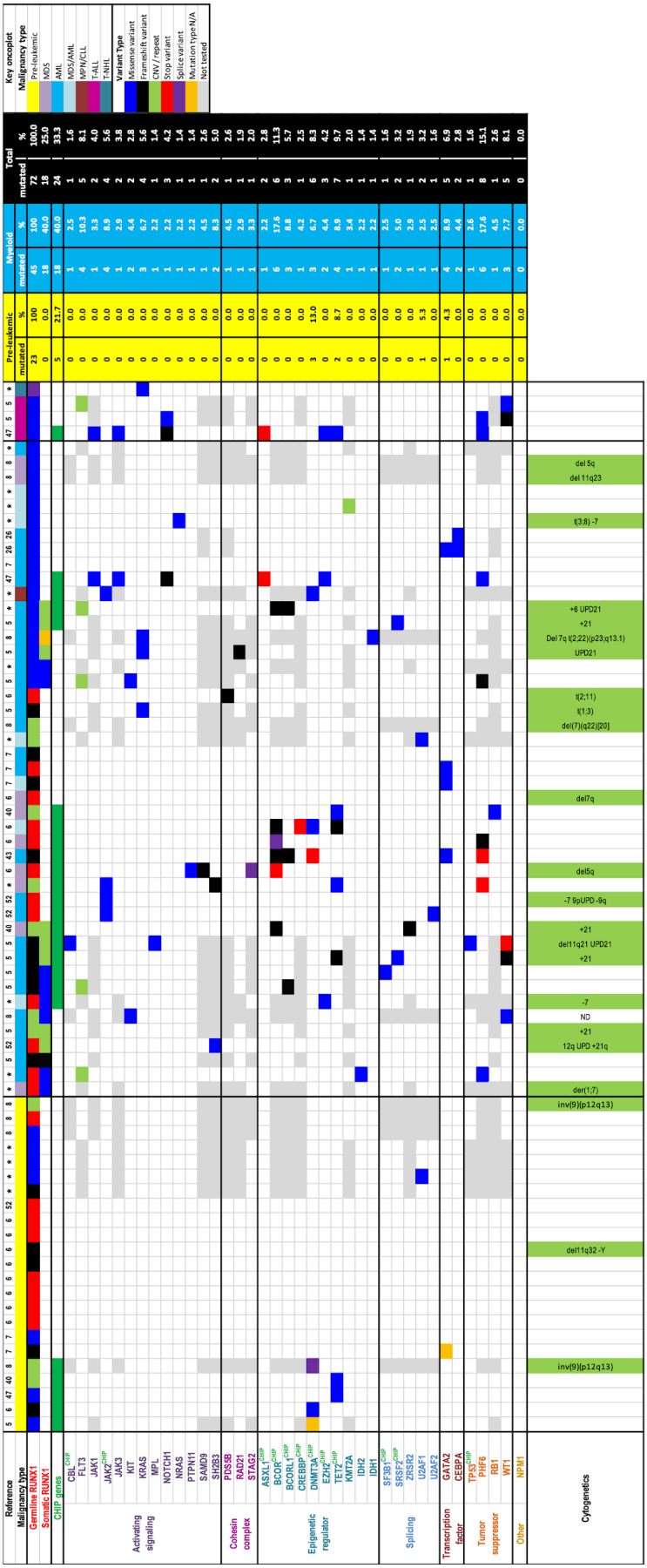


Supplemental Figure 10

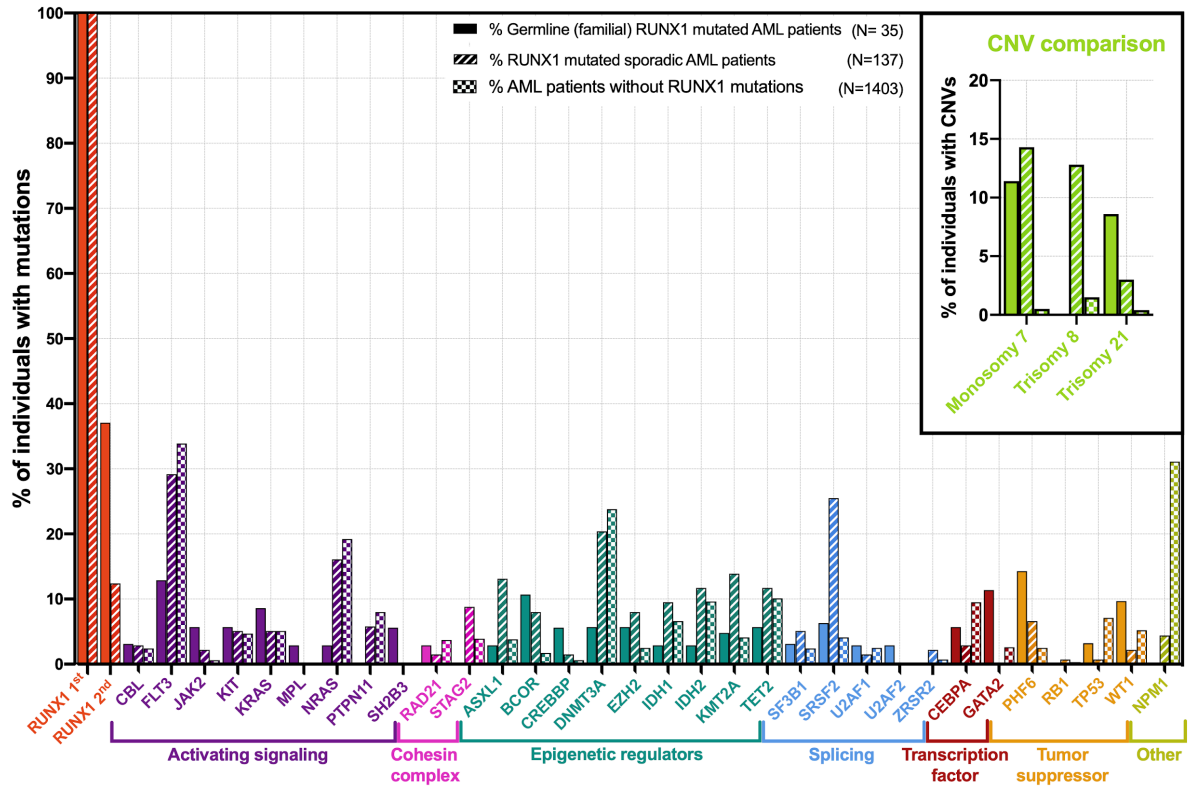


Family	Individual	Malignancy type	Pre-leukemic mutated	Pre-leukemic %	Myeloid mutated	Myeloid %	Total mutated	Total %
	Family 8 III-1							
	Family 5 III-2							
	Family 4 II-1							
	Family 4 II-5							
	Family 8 II-1							
	Family 4 II-3							
	Family 9 IV-1							
	Family 7 III-2							
	Family 5 II-2							
	Family 1 II-2							
	Family 7 III-1							
	Family 2 II-1							
	Family 7 II-1							
	Family 7 II-2							
	Family 4 II-4							
	Family 10 I-1							
Germline RUNX1			4	100	11	100	16	100
Somatic RUNX1			0	0	4	36.4	4	25.0
CHIP genes			0	0	4	36.4	4	25.0
Activating signaling	CBL^{CHIP}		0	0	0	0.0	0	0.0
	FLT3		0	N/A	1	20.0	1	16.7
	JAK1		0	0	0	0.0	0	0.0
	JAK2^{CHIP}		0	0	2	18.2	2	12.5
	JAK3		0	N/A	0	0.0	0	0.0
	KIT		0	0	0	0.0	0	0.0
	KRAS		0	0	0	0.0	1	6.3
	MPL		0	0	1	9.1	0	0.0
	NOTCH1		0	0	0	0.0	0	0.0
	NRAS		0	0	1	9.1	1	6.3
	PTPN11		0	0	0	0.0	0	0.0
	SAMD9		0	N/A	0	0.0	0	0.0
	SH2B3		0	N/A	1	20.0	1	16.7
Cohesin complex	PDS5B		0	N/A	0	0.0	0	0.0
	RAD21		0	N/A	0	0.0	0	0.0
	STAG2		0	0	0	0.0	0	0.0
Epigenetic regulator	ASXL1^{CHIP}		0	0	0	0.0	0	0.0
	BCOR^{CHIP}		0	N/A	1	20.0	1	16.7
	BCORL1^{CHIP}		0	N/A	1	20.0	1	16.7
	CREBBP^{CHIP}		0	N/A	0	0.0	0	0.0
	DNMT3A^{CHIP}		3	75.0	1	9.1	1	6.3
	EZH2^{CHIP}		0	0	1	9.1	1	6.3
	TET2^{CHIP}		2	50.0	1	9.1	1	6.3
	KMT2A		0	N/A	1	20.0	1	16.7
	IDH2		0	0	0	0.0	0	0.0
	IDH1		0	0	0	0.0	0	0.0
Splicing	SF3B1^{CHIP}		0	0	0	0.0	0	0.0
	SRSF2^{CHIP}		0	0	0	0.0	0	0.0
	ZRSR2		0	N/A	0	0.0	0	0.0
	U2AF1		1	25.0	1	9.1	2	12.5
	U2AF2		0	0	0	0.0	0	0.0
Transcription factor	GATA2		1	25.0	0	0.0	0	0.0
	CEBPA		0	0	0	0.0	0	0.0
Tumor suppressor	TP53^{CHIP}		0	N/A	0	0.0	0	0.0
	PHF6		0	N/A	1	20.0	1	16.7
	RB1		0	N/A	0	0.0	0	0.0
	WT1		0	N/A	3	60.0	0	0.0
Other	NPM1		0	0	0	0.0	0	0.0
Cytogenetics		der(1;7)						
		+6 UPD21 -7						
		t(3;8) -7						

Supplemental Figure 11



Supplemental Figure 12



Supplemental Figure Legends

Supplemental Figure 1. Identification of a Novel *RUNX1* deletion in Family 1

A. SNP arrays were used to detect a large deletion in the 21q22.12 region. **B**. The breakpoints were determined by PCR amplification with primers 7432 and 7433 (7432–GCTCGTCCACCTTCAGACTC; 7433 – GGCTACAGTAGGCTCAGACATTC) on DNA derived from peripheral blood mononuclear cells of proband resulting in a product of ~4 kb. Sanger sequencing of the PCR product identified a deleted region of 727,595 bp from 36,319,974 to 37,047,568 (GRCh37/hg19; Chr21) leading to the removal of the promoter (P1), 5' untranslated region (UTR) and exon 1-2 for *RUNX1* (NM_001754). Exon numbering is per LRG annotations for RUNX1c. Exons 4a and 8a indicate exons in RUNX1a/RUNX1b as marked that are overlapping with RUNX1c but not identical. **C.** Heritability of the *RUNX1* deletion was confirmed through PCR amplification across the *RUNX1* deletion with primers 7432 and 7433 on DNA

derived from peripheral blood mononuclear cells for proband (lane 1) and from hair follicles for her father and sister (lane 2 and 3, respectively). A 4 kb product indicates deletion carrier. No template control (lane 4). M, 1 kb marker.

Supplemental Figure 2. Identification of a novel *RUNX1* deletion in Family 2. A. Identification of deletion of *RUNX1* exons 2, 3 and 4 (NM_001754) in patient III-1 using MLPA. Of the control genes, *PRDX4* and *OCRL* are on ChrX and *SRY* on ChrY. **B.** Deletion breakpoints defined by PCR amplification with primers 5146 and 5153 (5146 - AGGAAATGCAGTGAGAGAAACCAC; 5153 - CCCTTGGAGAATCCATCAGA) on germline DNA from individual III-3 yielding a product of size 1,322 bp rather than the expected 49,088 bp. Sequence analysis defined a 47,766 bp deletion from 36,298,116 to 36,250,351 (GRCh37/hg19 Assembly; Chr21) inclusive, removing Exon 3, 4 and 5 for NM_001754 (RUNX1c) and the promoter (P2), exon 4a and 5 for RUNX1a/b. Exon numbering is per LRG annotations for RUNX1c. Exons 4a and 8a indicate exons in RUNX1a/b as marked that are overlapping with RUNX1c but not identical.

Supplemental Figure 3. Family 3 Supplemental information (Individual III-3). A. MLPA analysis of *RUNX1* exons with *CEBPA*, *CEBPB* and autosomal and sex chromosome control genes shows somatic amplification of the entire *RUNX1* gene in BMMNC from patient III-3 (red bars), relative to controls **B.** Determination of the numbers of cloned RUNX1 PCR products amplified from buccal swab DNA revealed the expected 50% for a germline mutation. **C.** A novel p.Pro203Arg somatic mutation is seen at 29% in cloned *RUNX1* PCR products amplified from BMMNC.

Supplemental Figure 4. Family 4 Supplemental Information. A. Sanger confirmation of germline and somatic *RUNX1* mutations in individual II-3. **B.** other mutations found at this site in *RUNX1* from somatic sources. **C.** Luciferase assay (see supplementary methods) showing reduced GATA2 activity of potentially modifying variants in GATA2.

Supplemental Figure 5. Family 5 Supplemental information. Sanger confirmation of the germline (inherited) c.593A>T;D198V *RUNX1* mutation in Individual II-2 (PBMNC)

Supplemental Figure 6. Family 6 supplemental information. A. Sanger confirmation of the c.596G>A RUNX1 G199E germline mutation in individual III-1. **B.** location of G199 within a DNA binding region of RUNX1.

Supplemental Figure 7. Extended analysis on tumour-normal WES of Individual III-2 from Family 7. A. Analysis of matched tumour-normal (saliva) WES using Sequenza combined VAF and copy number analysis. Changes were observed with duplication of chromosome 6 (trisomy) and chromosome 21 copy number neutral loss of heterozygosity (CN-LOH) specific to the tumour. **B.** visualisation of the inherited *RUNX1* mutation using IGV. The differential VAF of the mutation in tumour (88%) compared to normal (53%), indicates that somatic CN-LOH of chromosome 21 in tumour has duplicated the *RUNX1* mutated chromosome.

Supplemental Figure 8. Relatedness analysis of families 8 and 9 who share the same RUNX1 R320* germline mutation. **A.** Scatterplots showing coefficient of relatedness (Y-axis) based on number of SNPs with zero shared alleles (X-axis) for each paired sample. Parent-offspring (squares), unrelated pairs (circles) are represented as clusters on the scatterplot. **(B-C).** Runs of homozygosity from multiple individuals sharing the RUNX1 genotype across all chromosomes **(B)** or chromosome 21 **(C)**. Lines and shaded boxes highlight runs of homozygosity (RoH) predicted by BCFtools (Narasimhan et al., 2016) in exome data. RoH can infer regions of autozygosity, chromosomal segments identical by descent, among the samples tested. Karyograms were generated using the autoplot function from ggbio (Yin et al., 2012) and RoH with high confidence (phred score = 20). Ideograms were generated using karyoploteR and RoH (Gel, B & Serra, E 2017). Family 9 (IV-1) had no RoH across chromosome 21, therefore is not represented in (B).

Supplemental Figure 9. Family 10 supplemental information. A. WES analysis of the proband (II-2) and parents (I-1 and I-2) of Family 10 revealed the germline c.968-10C>A variant in the proband (II-2) in both hair and blood samples which was confirmed by Sanger sequencing. WES analysis of the father's blood showed the variant at 2% allele load which was not detectable via sanger sequencing. **B.** Scatterplots showing coefficient of relatedness (Y-axis) based on number of SNPs with zero shared alleles (X-axis) for each paired sample. Parent-offspring (squares) and unrelated pairs are represented as clusters on the scatterplot. Relatedness between samples from Pedigree 10 with *RUNX1* genotype, confirms parentage and *de novo* status of the *RUNX1* c.968-10C>A variant **C.** RNA studies of the proband (RT-PCR), identified missplicing associated with the germline variant leading to RNA encoding a truncated protein (p.Ala324Leufs*7).

Supplemental Figure 10. Oncoplot of somatic mutations identified in the available samples of the families reported in this study. Samples are grouped by disease status; 4 pre-leukemic individuals are separated from the 11 patient samples with myeloid malignancies and one sample with T-cell Non-Hodgkin's Lymphoma (T-NHL). Further abbreviations used: AML = Acute Myeloid Leukemia; CLL = Chronic lymphocytic Leukemia; CNV = Copy Number Variant; MDS = Myelodysplastic Syndrome; MPN = Myeloproliferative Neoplasm.

Supplemental Figure 11. Oncoplot of somatic mutations of the samples in this study (supplementary figure 10) combined with other published somatic mutations in individuals with germline *RUNX1* mutations. Samples are grouped based on disease phenotypes: The first group contains 23 pre-leukemic samples, the second group 45 samples with myeloid malignancies (AML, MDS and MPN), and the last group of 4 includes 3 T-ALL samples and 1 T-NHL. Variant percentages were calculated based on the number of samples for which that gene was analysed in case of gene panels. Reference numbers match main reference list for relevant published study. New data from the families described in this study are indicated with *. Abbreviations used: AML = Acute Myeloid Leukemia; CLL = Chronic lymphocytic Leukemia; CNV = Copy Number Variant; MDS = Myelodysplastic Syndrome; MPN = Myeloproliferative Neoplasm; T-ALL = T-cell Acute Lymphoblastic Leukemia; T-NHL = T-cell Non Hodgkin's Lymphoma.

Supplemental Figure 12. Side by side comparison of somatic mutations and copy number variants reported in 3 groups based on their RUNX1 mutation. **1** AML patients with germline RUNX1 mutations (N=35) **2** AML patients with somatic RUNX1 mutations (N=137) and **3** AML patients without RUNX1 mutations (N=1403).

Supplemental Clinical Information

Family 1, (Figure 1A) is a family with a proband diagnosed with MDS (RAEB-2) at 21 years of age. Her father suffered from thrombocytopenia and there was a family history of AML in the sister and niece of the proband's father. SNP microarray identified a deletion at the *RUNX1* locus (also confirmed by both MLPA and copy number assessment of WES Supplemental Figure 1B,C). PCR and Sanger sequencing identified the breakpoints of the 727,585bp deletion that removes the P1 promoter and exons 1 and 2 of the RUNX1c isoform (Supplemental Figure 1B). This deletion was present in both the affected father and daughter but not in the asymptomatic sister (Supplemental Figure 1C).

Family 2 (Figure 1B) is a four-generation family of Caucasian background. The proband (II-1) was diagnosed with thrombocytopenia in her 40s and was subsequently diagnosed with breast cancer at age 64, and MDS at age 65 that progressed to AML (age 74). Her son (III-1) also had thrombocytopenia and developed MDS/AML at the age of 49 years. One daughter (III-3) developed breast cancer at age 42, without haematological abnormalities; another daughter (III-4) has thrombocytopenia. Analysis of the DNA from III-1 by MLPA and SNP microarray identified a novel 47,766 bp deletion in *RUNX1* which removes exon 3, 4 and 5 of the RUNX1c isoform (Supplemental Figure 2). His mother (II-1 the proband) and her thrombocytopenic daughter (III-4) were also found to carry the deletion. This deletion predicts altered splicing of exon 1 to exon 5 which would result in an in-frame p.E20-G170 deletion removing almost the entire runt homology domain (RHD) for variant 1 (i.e. NP_001745; isoform c), thereby preventing DNA binding, while retaining the transactivation domain (TAD).

Family 3 (Figure 1C) consists of seven affected individuals of whom four were thrombocytopenic and five had leukemia; two with thrombocytopenia developed AML. The proband (III-3) suffered from a seizure disorder and thrombocytopenia ($100x10^9$/L) since adolescence. At the age of 49 years, her platelets dropped to $10x10^9$/L, she was diagnosed with MDS with trisomy 21 and underwent allogeneic bone marrow transplant from her HLA-matched sister (III-2). Her son (IV-5) had thrombocytopenia from birth and died from AML at the age of three years. Her nephew (IV-2) also presented with thrombocytopenia from birth and later developed AML with acquired trisomy 1. Sanger sequencing of *RUNX1* in the proband identified a novel germline heterozygous single nucleotide deletion (c.415delC; 50% VAF in buccal DNA, Supplemental Figure 3B), which leads to a frameshift p.L112fs*9 and premature termination after amino acid 120. This results in a truncated protein lacking most of the RHD and the entire TAD.

Family 4 (Figure 1D) consists of one affected parent and four affected siblings. The proband (II-4), was found to be thrombocytopenic at age 38 and was subsequently diagnosed with AML (FAB M2) with a normal karyotype. She was initially treated successfully with induction chemotherapy but relapsed and subsequently received a bone marrow transplant from her HLA-identical brother (II-1), who was reported to be thrombocytopenic. In addition to II-1, two other siblings were also reported to be thrombocytopenic, with one also developing AML (II-3). The proband's mother (I-2) had also been diagnosed with AML in her 40s, from which she died. Sanger sequencing identified a germline heterozygous single nucleotide substitution *RUNX1* (c.506G>T), leading to a missense p.R169I mutation. Recurrent somatic missense and frameshift mutations are found at this residue p.R169 in both MDS/AML and Breast carcinoma (Supplemental Figure 4B) but this

is the first description of a germline alteration at this position leading to FPD-MM. The mutation was identified in all four siblings with thrombocytopenia and/or AML, (including the sibling that acted as a BMT donor) and was absent from II-2 who was unaffected.

Family 5 (**Figure 1E**) has 4 generations reported to have a constitutional bleeding disorder with thrombocytopenia and abnormal platelets. The proband (II-2), in addition to his constitutional thrombocytopenia, developed JAK2-V617F positive MPN at the age of 62 as well as a lymphoproliferative disorder at age 63. The son of the proband (III-1) developed an acute myelomonocytic leukaemia at the age of 12 years, from which he died. Sequencing of DNA from the proband identified a heterozygous c.593A>T substitution in *RUNX1* that leads to a missense p.D198V mutation (Figure 1E, Supplemental Figure 5). The thrombocytopenic daughter of the proband (III-2) was also a carrier of the variant.

Family 6 (**Figure 1F**) is a three generation family, with a history of thrombocytopenia and early onset AML. The proband (III-1) presented with thrombocytopenia at 2 months of age and genetic investigation identified a c.596G>A; p.Gly199Glu variant in RUNX1, which was also shared by the father and likely associated with the early age of AML onset with the grandmother (I-II), although not able to be confirmed. This variant has been reported previously in the context of inherited thrombocytopenia but not associated with a family history of leukemia.[7]

Family 7 (**Figure 1G**), is a complex family with thrombocytopenia, multiple cases of MDS and AML as well as a case of T-ALL. The proband (III-2), had suffered from thrombocytopenia since childhood, but at the age of 28 developed symptoms of acute leukemia within days, with no documented pre-leukemic or MDS phase. The acute undifferentiated leukaemia was FLT3-ITD positive with trisomy 6 in tumour cells. She was treated with a MUD-SCT and is currently well. The sister, father and uncle of the proband all developed acute leukemia and were also treated with MUD-SCT. The father who suffered MDS/AML, as well as a psoriatic skin condition is currently well. The sister (III-1), was diagnosed with ALL aged 22, and developed therapy related MDS which progressed to AML. She received two SCT from the same matched unrelated donor (MUD), but died from treatment related mortality (aged 25). The uncle (II-2) who also developed AML, was treated with MUD-SCT and also died from treatment related mortality. Molecular analysis of material from the proband identified a *RUNX1* single nucleotide substitution (c.611G>T), leading to a p.R204Q missense variant. Further screening identified that all four affected family members were carriers of this mutation (Figure 1G). The germline mutation affecting p.R204 (Family 7) is located in the RUNT domain. Somatic mutations (including somatic R204Q) have frequently been

reported in AML.

Family 8 (Figure 1H), is a family with a history of both solid tumor and hematological malignancies. The father of the proband suffered from long standing thrombocytopenia that progressed to MDS (RAEB-1) at age 58. At this time cytogenetics showed an unbalanced translocation (der(1;7)(q10;p10)) resulting in trisomy of 1q and monosomy of 7q. Subsequently his platelets dropped to almost undetectable levels and he passed away due to bleeding complications at age 58. At this time, the proband was also diagnosed with thrombocytopenia and sequencing of a 29 gene myeloid NGS panel revealed a germline *RUNX1* p.R320* variant in DNA of the proband III-1 and father II-1. (Figure 1H).

Family 9 (Figure 1I) is a large family with a 4 generation history including a complex range of haematological malignancies, solid tumours and a prevalent psoriatic skin disorder. The proband of this family (IV-1), which is not known to be related to Family 8 (Supplemental Figure 8), had thrombocytopenia and was diagnosed with MDS, with dysplastic erythropoiesis and monosomy 7, at age 54. Within 3 months this rapidly progressed to AML with 24% myeloblasts and a platelet count of 13,000. She was treated with induction chemotherapy and MUD SCT but relapsed with the original monosomy 7 disease two years later and passed away due to complications from a second MUD SCT. Her sibling (IV-2) and mother (III-1) also had diagnoses of AML. The proband (IV-1) and her brother (IV-2) were both found to be carriers of a germline RUNX1 c.958C>T; p.R320* variant. Individual V-5 was found to carry the same RUNX1 variant and by extension conferred obligate carrier status on both the father (IV-5) and the grandmother of V-5 (III-3). Other second cousins of the proband that carry the RUNX1 variant (IV-7, IV-8), have had long standing mild thrombocytopenia, but have no reported hematological malignancies in themselves, a child (V-7) or their mother (III-5) who are also either obligate carriers or known carriers of the RUNX1 c.958C>T; p.R320* variant. Of these carriers, the child (V-7) was diagnosed with ALL at age 31. In contrast to multiple cases of AML observed in the proband and first-degree relatives, a second cousin of the proband (IV-5) was diagnosed with non-Hodgkin lymphoma at age 38 and ALL at age 41 and his daughter (V-5) T-ALL at age 3. Both were carriers of the same RUNX1 c.958C>T; p.R320* variant.

Family 10 (Figure 1J) was recruited due to a proband with a personal history of thrombocytopenia and T-NHL. He had no ancestral family history of thrombocytopenia or haematological malignancies; however both daughters were also thrombocytopenic suggesting an inherited condition. Upon diagnosis of T-cell Non-Hodgkin's Lymphoma (T-NHL) at age 30, the proband

underwent chemotherapy with supportive autologous stem cell transplant. Investigations 9 months later revealed massive hepatosplenomegaly and accordingly a splenectomy was performed. Persistent thrombocytopenia seen in bone marrow aspirate and trephine post-splenectomy indicated a concern for evolving therapy related MDS (tMDS) and this was confirmed approximately 8 months later following bone marrow biopsy, with 6% blasts seen along with cytogenetic abnormalities (46,XY,del(20) (q11.2q13.1)/47, indem,+1,del(1) (p32)). Induction chemotherapy and MUD SCT was performed soon after to treat the tMDS and results indicated hematologic and cytogenetic remission. WES on blood from the proband identified a *RUNX1* c.968-10C>A variant with a predicted effect on splicing, that was confirmed by RNA studies to generate a transcript that encodes a frameshift RUNX1 protein, p.Ala324Leufs*7. Consistent with the family phenotypes, Sanger sequencing showed that both daughters but neither parent were carriers of the variant, suggesting it was *de novo* in the proband. Peddy analysis of WES of the parents confirmed parentage,[11] and additionally WES identified 2 *RUNX1* variant reads in the blood of the father suggesting that he may be a mosaic carrier of the mutation.

Supplemental Methods

Ethics

Clinical information and samples were collected with informed consent from all subjects and approval through institutional human ethics review board-approved protocols from the Australian Familial Haematological Cancer Study (AFHCS) (Royal Adelaide Hospital (RAH) #091203 and #100702, and Children, Youth and Women's Health Service #REC1542/12/12, Adelaide, South Australia, Australia), and conducted in accordance with the Declaration of Helsinki. Ethics for families identified through non-Australian co-authors were held by the local diagnosing center.

DNA isolation, Oligonucleotides, PCR and Sanger sequencing

DNA was isolated using the Qiagen DNA mini kit according to the supplied protocol. The primers for the coding regions of all exons of *RUNX1* were used for PCR amplification and Sanger sequencing as previously described.[1]

Multiplex ligation-dependent probe amplification (MLPA) analysis

All reagents for MLPA were purchased from MRC-Holland with the exception of the oligonucleotides (Sigma). The reaction was carried out as described.[2] Products were separated by capillary electrophoresis on an ABI 3730 (Applied Biosystems). Data was analysed as described,[2] and a ratio of 0.8 to 1.2 was taken as representative of two copies of the exon present.

SNP array copy number analysis

SNP array copy number analysis was performed using a high density SNP array (Illumina 850K assay chip) as previously described.[3]

Relatedness and haplotype analysis

Genetic relatedness testing was performed on WES data from individuals to compare Families 8 and 9, as well as confirm relationships between individuals in Family 10. Relatedness plots were generated from Peddy,[4] using a VCF and associated PED file. For WES samples from individuals in Families 8 and 9, runs of homozygosity (RoH) on chromosome 21 were predicted by BCFtools,[5] in exome data. RoH can infer regions of autozygosity, chromosomal segments identical by descent, among the samples tested. Karyograms were generated using the autoplot function from ggbio[6] and RoH with high confidence (phred score = 20). Ideograms were generated using karyoploteR and RoH (Gel, B & Serra, E 2017).[7] Family 9 (IV-1) had no RoH across chromosome 21, therefore is not represented in (B).

Identification of potential germline modifier mutations in FPD-MM.

In addition to characterisation of the causative germline RUNX1 mutation in our families, we also explored germline variants in other relevant genes associated with leukaemia development, that could potentially act as phenotype modifiers in combination with the germline RUNX1 mutation.[8] To identify potential modifier variants the following filtering was performed: VAF of the variant greater than 40%, population MAF in gnomAD is less than 5% (based on carrier/allele frequencies of damaging alleles in autosomal recessive disorders per ACMG guidelines),[9,10] a sequence variant predicted to change the amino acid sequence of the protein. This was applied to all genes if sequence data was from the myeloid panel and for WES a gene list filter, with all genes known to be mutated in FPD-MM tumors was applied (See supplemental Figure 11).

Aggregation of FPD-MM associated germline and somatic mutations from the literature

We collated germline *RUNX1* variants from the literature and where possible converted all variants to current LRG RUNX1 nomenclature. Information on malignancy and HM phenotype was also collated. For studies reporting somatic mutations, we used the information as reported (gene level, not all studies reported variant level) and collated phenotype and age of HM diagnosis. Reference numbers for studies with somatic mutations are shown per sample in Figure 3 and Supplemental Figure 11.

Comparing familial *RUNX1* to somatic *RUNX1* mutation positive AML cases

In addition to the 8 AML patients from our families (figure 1, supplementary table 2) , we have further analysed 27 patients with *RUNX1* germline mutations that have been reported to develop AML (Figure 3 (oncoplot). Somatic variants of this combined group of 35 patients with familial AML was compared to 137 published sporadic AML patients that had least 1 *RUNX1* mutation revealed the difference in somatic signature between these cohorts. [11] The somatic CN-LOH detected in 3 of our germline R*UNX1* cases were excluded from analysis since CN-LOH was not reported in the sporadic AML dataset.

Luciferase assays for GATA2 WT and mutant proteins

The luciferase assays for *GATA2* WT or mutants (among which P161A) were performed as described previously.[12] In brief, expression plasmids were co-transfected into Cos-7 cells, combined with a LUC reporter construct driven by a *LYL1* promoter. After 20 hours, the cells were harvested and measured using a Dual-Luciferase Reporter Assay System (Promega).[13] In Supplemental Figure 4C, we show the fold increase in normalized luciferase activity of GATA2 WT and mutant proteins on *LYL1* promoter expression. ***** $p<0.05$ (Fisher's exact test)

Supplemental references

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Supplemental Table 1 - Published germline *RUNX1* mutations – 130 annotated families

NM_001754.4 1c	NP_001745.2	gnomAD (%)	No. of families	mutation type	HM type, nd= not determined, na= not applicable (no HM)	References*
c.97G>A	p.D33N	0	1	missense	na	Johnson 2016 [17]
c.163dup	p.A55Gfs*83	0	1	frameshift	MDS/AML/DLBCL/SCC	Owen 2008 [21]
c.171_198dup	p.L67Afs*80	0	1	frameshift	MDS	Pastor 2017 [22]
c.308dup	p.T104Yfs*34	0	1	frameshift	na	Kanagal-Shamanna 2017 [8]
c.317G>A	p.W106*	0	1	stopgain	na	Stockley 2013 [23] ,Johnson 2016 [17]
c.320G>A	p.R107H	0	1	missense	AML	Latger-Cannard 2016 [24]
c.328A>G	p.K110E	0	1	missense	AML	Michaud 2002 [25]
nd	p.K110Q	0	1	missense	AML	Simon 2017 [26]
c.334delC	p.L112Cfs*10	0	1	frameshift	MDS/AML	This study
c.351+1G>A		0	1	splicing	MDS/AML	Owen 2008 [21]
c.351+1G>T		0	5	splicing	na	Stockley 2013 [23], Johnson 2016 [17] , De Rocco 2017 [27], Perez- Botero 2017 [28]
c.352-1G>T	p.V118Gfs*11	0	1	splicing	AML	Song 1999 [2]
c.352-1G>A	p.V118Gfs*11	0	3	splicing	Na/MDS/AML	Sun 2004 [29], Ouchi-Uchiyama 2015 [30], Guidugli 2017 [31]
c.367G>C	p.D123H	0	1	missense	MDS/AML	Owen 2008 [21]
c.400G>C	p.A134P	0	1	missense	AML	Walker 2002 [32]
nd	p.G135V	0	2	missense	T-ALL	Antony-Debre 2016 [5]
nd	p.S141A	0.0007	1	missense	AML	Simon 2017 [26]
c.425C>A	p.A142D	0	1	missense	MDS	Schwartz 2017 [33]
c.442_449del	p.T148Hfs*9	0	1	frameshift	AML	Beri-Dexheimer 2008 [34], Latger-Cannard 2016 [24], Antony-Debre 2016 [5]
c.460del	p.Q154Rfs*22	0	1	frameshift	NHL	Marneth 2017 [35]
c.467C>A	p.A156E	0	1	missense	AML	Preudhomme 2009 [18], Latger-Cannard 2016 [24]
c.508+3delA	p.R162fs	0	1	frameshift	AML	Michaud 2002 [25]
nd	p.R166Q	0	2	missense	MDS	Song 1999 [2], Ouchi-Uchiyama 2015 [30]
c.496C>T	p.R166*	0	3	stopgain	AML	DiNardo 2016 [36], Kanagal-Shamanna 2017 [8], Bluteau 2011 [37], Latger-Cannard 2016 [24], Antony-Debre 2016 [5], Haslam 2015 [38]
c.500G>A	p.S167N	0	2	missense	na	Obata 2015 [39], Yoshimi 2014 [7]
nd	p.S167N	0	1	missense	MDS/AML	Yoshimi 2014 [7]
c.506G>T	p.R169I	0	1	missense	AML	This study
nd	p.G143W	0	1	missense	MDS/AML	Sakurai 2016 [40] , Chin 2016 [41]
nd	p.G170fs	0	1	frameshift	AML	Antony-Debre 2016 [5]
c.508G>A	p.G170R	0	1	missense	MDS/AML	Buijs 2012 [42]
c.508+1G>T	nd	0	2	splicing	na	Stockley 2013 [23], Johnson 2016 [17]
nd	p.S172G	0	1	missense	AML	Simon 2017 [26]
c.554_560del	p.Q185Pfs*24	0	1	frameshift	AML	Ng 2018 [43]
c.557T>A	p.V186D	0	1	missense	BMF	Guidugli 2017 [31]
c.567C>G	p.Y189*	0	2	stopgain	MDS	Zhang 2015 [44], Chisholm 2019 [45]
c.578T>A	p.I193N	0	1	missense	na	De Rocco 2017 [27] [26]
c.582A>C	p.K194N	0	1	missense	MDS/NHL	DiNardo 2016 [36], Kanagal-Shamanna 2017 [8]
c.583dup	p.I195Nfs*18	0	1	frameshift	na	Badin 2017 [46]
c.587C>G	p.T196R	0	1	missense	MDS/AML	Antony-Debre 2016 [5], Latger-Cannard 2016 [24]
nd	p.T196fs	0	1	frameshift	MDS/AML	Langabeer 2010 [48]

c.586A>G	p.T196A	0	1	missense	na	Johnson 2016 [17]
nd	p.D198Y	0	1	missense	MDS/AML	Buijs 2001 [49]
c.593A>T	p.D198V	0	2	missense	AML/MPN/CLL/SLL	Johnson 2016 [17], This study
c.592G>A	p.D198N	0	1	missense	na	Ouchi-Uchiyama 2015 [30]
c.596G>A	p.G199E	0	2	missense	na/AML	Yoshimi 2014 [7] , Sakurai 2016 [40], This study
c.601C>T	p.R201*	0	5	stopgain	AML	Song 1999 [2], Ripperger 2009 [50], Yoshimi 2014 [7], Nishimoto 2010 [51], Tawana 2017 [52], Chisholm 2019 [45]
c.602G>A	p.R201Q	0	2	missense	AML	Song 1999 [2], Holme 2012 [53], Bluteau 2011 [37], Latger-Cannard 2016 [24], Manchev 2017 [47]
c.601dup	p.R201Pfs*12	0	1	frameshift	na	Ouchi-Uchiyama 2015 [30]
c.611G>A	p.R204Q	0	3	stopgain	AML/T-ALL	Preudhomme 2009 [18], Antony-Debre 2016 [5], Latger-Cannard 2016 [24], Johnson 2016 [17], This study
c.610C>T	p.R204*	0	2	stopgain	AML	Song 1999 [2], Churpek 2015 [6]
c.649G>A	p.G217R	0	1	missense	B-ALL	Linden 2010 [54]
c.719delC	p.P240Hfs*14	0	1	frameshift	MDS/AML/NHL	DiNardo 2016 [36], Kanagal-Shamanna 2017 [8]
nd	p.P245S	0	1	missense	na	Antony-Debre 2016 [5]
delC	p.T246Rfs*8	0	1	frameshift	CMMoL, AML	Heller 2005 [55], Antony-Debre [5] 2016
nd	p.N260fs	0	1	frameshift	MDS/AML	Sakurai 2016 [40]
c.784C>T	p.Q262*	0	1	stopgain	MDS/AML	Buijs 2012 [42]
c.788delC	p.P263Lfs*48	0	1	frameshift	AML	Appelmann 2009[56]
c.837G>A	p.W279*	0	2	stopgain	MDS	Schmit 2015 [57], Churpek 2015 [6], DiNardo 2016 [36], Ok 2016 [58], Kanagal-Shamanna 2017 [8]
nd	p.Y287*	0	1	stopgain	MDS/AML	Michaud 2002 [25], Churpek 2015 [6], Chisholm 2019 [45]
nd	p.G289fs	0	1	frameshift	CMML/MDS	Shiba 2012 [59], Yoshimi 2014 [7]
c.866delG	p.G289Dfs*22	0	1	frameshift	MDS/AML	Kozubík 2018 [60]
c.967+2_5 del	p.A297fs	0	1	frameshift	AML	De Rocco 2017 [27]
c.958C>T	p.R320*	0	3	stopgain	MDS/AML	Owen 2008 [21], This study
c.968-10C>A	p.A324Lfs*7	0	1	splicing	T-NHL/tMDS	This study
c.987delG	p.F330Sfs*264	0	1	frameshift	MF/MDS/AML	Yoshimi 2014 [7]
nd	p.D332Tfs*262	0	1	frameshift	AML	Antony-Debre 2016 [5]
c.999_1003dup	p.Q335Rfs*261	0	1	frameshift	AML/T-ALL/CLL	Preudhomme 2009 [18], Latger-Cannard 2016 [24], Antony-Debre 2016 [5]
c.1088_1094del	p.G363Afs*229	0	1	frameshift	MDS/AML/T-ALL	Owen 2008 [21]
c.1092del	p.I364Mfs*230	0	1	frameshift	na	Latger-Cannard 2016 [24]
c.1098_1103dup	p.I366_G367dup	0.01	1	duplication	na	DiNardo 2016 [36]
c.1160G>C	p.G387A	0.0006	1	missense	MPN	DiNardo 2016 [36]
c.1163C>A	p.S388*	0	1	stopgain	MDS/AML	Churpek 2010 [61], Churpek 2015[6]
c.1208_1322del	Y403Cfs*153	0	1	frameshift	na	Chisholm 2019 [45] [31]
nd	p.H404Pfs*43	0	1	frameshift	AML	Antony-Debre 2016 [5]
nd	p.Y414*	0	2	stopgain	MDS/AML	Chisholm 2019 [45]
nd	p.N465K	0	1	missense	MDS	Yoshimi 2016 [62]
c.1415T>C	p.L472P	0.02	1	missense	AML/HCL	Yoshimi 2014 [7], Yoshimi 2016 [62]
c.1413_1414insGC	p.L472Afs*123	0	1	frameshift	AML/MDS/ALL	Sorrell 2012 [63], Churpek 2015 [6]
del P1 ex1-2			1	deletion	MDS	This study
del ex 1-2			1	deletion	AML/T-NHL	Cavalcante de Andrade Silva 2018 [64]
del ex 1-2			1	deletion	BMF	Guidugli 2017 [31]
del ex 1-2			1	deletion	MDS/AML/ lymphoma	Sakurai 2016 [40]

del ex 1-6			1	deletion	AML	DiNardo 2016 [36], Kanagal-Shamanna 2017 [8]
del ex 3-9			1	deletion	AML/lymphoma/ lymphosarcoma	Song 1999 [2]
del ex 3-5	p.E20-G170del		1	deletion	MDS/AML/MPN/ALL	This study
del ex 6			1	deletion	MDS/AML	Jongmans 2010 [65]
tandem dup ex 3-7			1	duplication	AML/NHL	Jongmans 2010 [65], Marneth 2017 [35]
whole gene			1	deletion	na	Chisholm 2019 [45]
whole gene			1	deletion	MDS/AML	Shinawi 2008 [66]
whole gene			1	deletion	AML	Preudhomme 2009 [18]
whole gene			1	deletion	MDS	Van der crabben 2010 [67]
whole gene			1	deletion	na	Hoyer 2007 [68]
whole gene			1	deletion	na	Beri-Dexheimer 2008 [34], Antony-Debre 2016 [5], Latger-Cannard 2016 [24]
whole gene			1	deletion	na	Shinawi 2008 [66]
1.81Mb deletion			1	deletion	na	Shinawi 2008 [66]
whole gene			1	deletion	na	Lyle 2009 [69]
whole gene			1	deletion	MDS	Pastor 2017 [22]
whole gene			1	deletion	RCC	Pastor 2017 [22]
4.47 Mb deletion			1	deletion	na	Katzaki 2010 [70]
2.9 Mb deletion			1	deletion	na	Katzaki 2010 [70]
4.2 Mb deletion			1	deletion	na	Katzaki 2010 [70]
2.16 Mb deletion			1	deletion	MDS/AML	Latger-Cannard 2016 [24]
2 Mb deletion			1	deletion	MDS/AML	Sakurai 2016 [40]
t(16;21)			1	translocation	AML	Buijs 2012 [42]

*Refer to reference

list in main document for reference numbering shown here

Supplemental table 2

Family	Individual	Malignancy type	Sample type	Sequencing type	Germline/ somatic	Gene name	cDNA change	AA change	VAF	GnomAD allele count	GnomAD %	CADD (V1.3)	ClinVar	Cosmic count
Family 1	III-2	MDS	PBMNC	WES	Germline	RUNX1	Δex1-2	Δex1-2	N/A	0	0	N/A	N/A	N/A
					Somatic	JAK2	c.1849G>T	p.V617F	38%	97	0.0344	33	14 submissions (P)	41459
					Somatic	SH2B3	c.832delA	p.K278fs	33%	0	0	N/A	absent	absent
					Somatic	TET2	c.1584C>A	p.N528K	49%	0	0	4.7	absent	absent
					Somatic	PHF6	c.820C>T	p.R274*	54%	0	0	46	1 submission (P)	14
Family 2	II-1	MDS/AML	PBMNC	Gene panel	Germline	RUNX1	Δex3-5	Δex3-5	N/A	N/A	N/A	N/A	N/A	N/A
					Somatic	U2AF1	c.470A>G	p.Q157R	41%	6	0.002	29.1	1 submission (LP)	35
Family 3	III-3	MDS	Buccal/ BMMNC	Sanger/ Gene panel/ MLPA	Germline	RUNX1	c.334delC	p.L112Cfs*10	50% (buccal) 66% (tumor, +21)	0	0	N/A	absent	absent
			BMMNC	Sanger	Somatic	RUNX1	c.608C>G	p.P203R	29%	0	0	31	absent	absent
			BMMNC	Sanger	Unknown	MEIS1	c.490G>A	p.E164K	nd	0	0	28.3	absent	1
Family 4	II-1	Pre	PBMNC	Gene panel	Germline	RUNX1	c.506G>T	p.R169I	42%	0	0	34	absent	absent
	II-3	AML	PBMNC	Gene panel	Germline	RUNX1	c.506G>T	p.R169I	40%	0	0	34	absent	absent
					Germline	ASXL1	c.3306G>T	p.E1102D	53%	2754	0.97	23.5	2 submissions (LB)	22
					Somatic	RUNX1	c.496C>G	p.R166G	12%	0	0	27.4	absent	12
	II-4	AML	PBMNC	Gene panel/ Sanger	Germline	RUNX1	c.506G>T	p.R169I	43%	0	0	34	absent	absent
					Germline	ASXL1	c.3306G>T	p.E1102D	50%	2754	0.97	23.5	2 submissions (LB)	22
	II-5	Pre	PBMNC	Gene panel	Germline	RUNX1	c.506G>T	p.R169I	44%	0	0	34	absent	absent
					Germline	GATA2	c.481C>G	p.P161A	38%	2229	0.82	17.3	5 submissions (B/LB)	2
Family 5	II-2	MPN/CLL	PBMNC	Gene panel /Sanger	Germline	RUNX1	c.593A>T	p.D198V	53%	0	0	31	absent	5
					Germline	CEBPA	c.584_589dup	p.H195_P196dup	50%	1206	3.25	N/A	2 submissions (B/LB)	11
					Germline	JAK2	rs12340895 GG	n/a	50%	7962	25.4	N/A	absent	absent
					Somatic	JAK2	c.1849G>T	p.V617F	40%	97	0.03	33	14 submissions (P)	41459
					Somatic	DNMT3A	c.1904G>A	p.R635Q	43%	0	0	35	absent	4
	III-2	Pre	PBMNC	gene panel	Germline	RUNX1	c.593A>T	p.D198V	51%	0	0	31	absent	5
					Somatic	U2AF1	c.122C>T	p.T41M	25%	0	0	33	absent	absent
Family 6	II-2	Pre	nd	Sanger	Germline	RUNX1	c.596G>A	p.G199E	nd	0	0	33	absent	absent
	III-1	Pre	nd	Sanger	Germline	RUNX1	c.596G>A	p.G199E	nd	0	0	33	absent	absent

Supplemental table 2 (continued)

Family	Individual	Malignancy type	Sample type	Sequencing type	Germline/ somatic	Gene name	cDNA change	AA change	VAF	GnomAD allele count	GnomAD %	CADD (V1.3)	ClinVar	Cosmic count
Family 7	II-1	MDS/AML	nd	nd	Germline	RUNX1	c.611G>A	p.R204Q	nd	0	0	35	1 submission (P)	24
					Somatic	KMT2A	nd	PTD	nd	nd	nd	N/A		
	II-2	MDS/AML	nd	nd	Germline	RUNX1	c.611G>A	p.R204Q	nd	0	0	35	1 submission (P)	24
	III-1	MDS/AML	nd	nd	Germline	RUNX1	c.611G>A	p.R204Q	nd	0	0	35	1 submission (P)	24
					Somatic	NRAS	c.182A>G	p.Q61R	nd	0	0	23.1	25 submissions (P)	1,722
	III-2	AML	Tumor / saliva	WES	Germline	RUNX1	c.611G>A	p.R204Q	50% (saliva) 88% (tumor, CN-LOH)	0	0	35	1 submission (P)	24
					Somatic	FLT3	nd	ITD	19%	0	0	N/A	overlapping	overlapping
					Somatic	BCOR	c.4074delC	p.K1360fs	31%	0	0	N/A	absent	absent
					Somatic	BCORL1	c.3179_1788insGATGGATT	p.F1060fs	36%	0	0	N/A	absent	absent
Family 8	II-1	MDS	PBMNC	Gene panel	Germline	RUNX1	c.958C>T	p.R320*	39%	0	0	48	absent	17
					Germline	IDH1	c.548A>G	p.Y183C	58%	2839	1.00	27.2	1 submission (not prov)	absent
					Somatic	RUNX1	c.322T>C	p.C108R	26%	0	0	27.4	absent	1
	III-1	Pre	PBMNC	Gene panel	Germline	RUNX1	c.958C>T	p.R320*	44%	0	0	48	absent	17
Family 9	IV-1	MDS/AML	BMMNC/ PBMNC	Gene panel /Sanger	Germline	RUNX1	c.958C>T	p.R320*	47%	0	0	48	absent	17
					Germline	CEBPA	c.584_589dup	p.H195_P196dup	50%	1206	3.25	N/A	2 submissions (B/LB)	11
					Somatic	RUNX1	c.491T>A	p.V164D	12%	0	0	29.9	absent	2
					Somatic	EZH2	c.433T>G	p.F145V	28%	0	0	23.8	absent	absent
	V-5	ALL (remission)	PBMNC	Gene panel	Germline	RUNX1	c.958C>T	p.R320*	49%	0	0	48	absent	17
Family 10	II-2	T-NHL/ tMDS	PBMNC/ hair	WES	Germline	RUNX1	c.968-10C>A	p.A324Lfs*7	51%	0	0	N/A	absent	1
					Somatic	KRAS	c.35G>A	p.G12D	41%	0	0	25.3	24 submissions (P)	15,022

Supplemental Table 3 - ACMG classifications with ASH- ClinGen Myeloid Malignancy Expert Panel *RUNX1*

Family	RUNX1 variant	ACMG – ASH-ClinGen classification	Rules applied
Family 1	c.-626163_59-54714del - Deletion P1Ex1-2 (p.?)	Likely Pathogenic	Absent from controls (PM2), Deletion specific to RUNX1c isoform (PVS1_moderate), 3 probands meeting RUNX1-phenotypic criteria (PS4_moderate, see also PMID: 29666006, PMID: 26849013).
Family 2	c.59-32,857_508+2,502del (p.E20-G170 del)	Likely Pathogenic	Absent from controls (PM2), Null variant in a gene where LOF is a known mechanism of disease (PVS1_strong), Segregation in 3 affected family members (PP1 supporting), 1 proband meeting RUNX1-phenotypic criteria (PS4_supporting)
Family 3	c.334delC (p.L112Cfs*10)	Pathogenic	Absent from controls (PM2), Null variant in a gene where LOF is a known mechanism of disease (PVS1), 1 proband meeting RUNX1-phenotypic criteria (PS4_supporting)
Family 4	c.506G>T (p.R169I)	Likely Pathogenic	Absent from controls (PM2), Computational evidence supports deleterious effect (PP3), Variant affecting hotspot residue (PM1), Segregation in 4 affected family members (PP1 supporting), 1 proband meeting RUNX1-phenotypic criteria (PS4_supporting)
Family 5	c.593A>T (p.D198V)	Likely Pathogenic	Absent from controls (PM2), Computational evidence supports deleterious effect (PP3). Variant affecting hotspot residue (PM1), 1 proband meeting RUNX1-phenotypic criteria (PS4_supporting)
Family 6	c.596G>A (p.G199Q)	Likely Pathogenic	Absent from controls (PM2), Computational evidence supports deleterious effect (PP3), Variant affecting one of the other non-hotspot AA residues 105-204 within the RHD (PM1_Supporting); data from a secondary assay demonstrating altered function (PS3 not able to be applied, see PMID: 24732596); 3 or 4 meioses observed within one or across multiple families (PP1_Supporting); 2-3 probands meeting RUNX1-phenotypic criteria (PS4_Moderate, see also PMID: 25159113; PMID: 26884589; PMID: 24732596).
Family 7	c.611G>A (p.R204Q)	Pathogenic	Absent from controls (PM2), Computational evidence supports deleterious effect (PP3), Variant affecting hotspot residue (PM1), ≥ 7 meioses observed within one or across multiple families (PP1_Strong), 3 probands meeting RUNX1-phenotypic criteria (PS4_ moderate, see also PMID: 19357396 ; PMID: 27112265; PMID: 26316320; PMID: 27479822)
Family 8 & 9	c.958C>T (p.R320*)	Pathogenic	Absent from controls (PM2), Null variant in a gene where LOF is a known mechanism of disease (PVS1_strong), ≥ 7 meioses observed within one or across multiple families (PP1_Strong), 3 probands meeting RUNX1-phenotypic criteria (PS4_moderate, see also PMID: 18723428)
Family 10	c.968-10C>A (p.A324Lfs*7)	Pathogenic	Absent from controls (PM2), Predicted null variant in a gene where LOF is a known mechanism of disease (PVS1_Strong applied due to RNA studies, therefore PS3 not applied), Computational evidence supports deleterious effect (PP3), De novo (maternity and paternity confirmed) in a patient with the disease and no ancestral family history (PS2_supporting), 3 or 4 meioses observed within one or across multiple families (PP1_Supporting), 1 proband meeting RUNX1-phenotypic criteria (PS4_supporting).