Overall cancer risk in people with deleterious germline *DDX41* variants

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Supplementary Materials

Supplementary Methods

Patients

All individuals signed written informed consent to participate in research approved by Institutional Review Boards at the University of Chicago and Northwestern University, conducted in accordance with the Declaration of Helsinki, and protected by National Institutes of Health Certificates of Confidentiality.

Germline Sequencing

Individuals with personal and/or family histories consistent with a deleterious germline *DDX41* variant or those with such alleles identified via tumor profiling[1] underwent clinical germline genetic testing (Supplementary Table 1). DNA was sequenced using an augmented whole exome sequencing platform[2] in the University of Chicago Genetic Services Laboratory (https://genes.uchicago.edu/clinical-genetics). DNA variants in 139 cancer-predisposing genes (Supplementary Table 2) were analyzed, including the 5'UTRs of *ANKRD26*, *DKC1*, *TERC,* and *TERT*, two *RTEL1* intronic regions (c.3724+78 and c.3724+139; NM_032957.4), and one *GATA2* intronic region (c.1017+572; NM_032638.4). DNA sequence reads were aligned using the UCSC human genome build Hg19 as a reference, and a custom bioinformatic pipeline capable of detecting single nucleotide variants and copy number variants was used to identify potential predisposition alleles (https://github.com/LucyGodley/Pipeline/blob/main/Variant_Calling/WES/hg/Automated/WES_Pipeline.sh).[3] Variants were curated according to the American College of Medical Genetics and Genomics/Association of Molecular Pathology.[4] Deleterious variants in cancer-causing genes were confirmed by Sanger sequencing.

Somatic Solid Tumor Sequencing

DNA derived from formalin-fixed, paraffin-embedded solid tumor tissue derived from eight patients with germline *DDX41*^{LOF} variants was sequenced via the OncoPlus next-generation panel, which includes *DDX41*.[5] Additional tumor-derived sequencing data from The Cancer Genome Atlas (TCGA; https://portal.gdc.cancer.gov/) were acquired for eleven additional patients with truncating *DDX41* alleles that are likely to be germline based on the frequency with which such alleles are inherited.[6]

LCL Preparation

Lymphoblastoid cell lines (LCLs) were derived from peripheral blood B-cells from individuals with deleterious germline *DDX41*^{LOF} variants (*DDX41*$^{var/+}$), which were transformed using Epstein-Barr Virus cultured in standard LCL growth media (Roswell Park Memorial Institute (RPMI) 1640 Medium + 20% FBS + 1% penicillin/streptomycin + 1X GlutaMAX). *DDX41*^{WT} LCLs were purchased from the Coriell Institute for Medical Research (https://www.coriell.org/), which were derived using a virtually identical transformation protocol. *DDX41*^{WT} LCLs were derived from three individuals: a 44yo man; a 25yo man; and a 42yo woman.

Protein Isolation and Western Blotting

Whole-cell protein lysates were prepared from *DDX41*^{WT} and *DDX41*$^{var/+}$ LCLs two days after passaging using RIPA buffer (150mM NaCl; 5mM EDTA, pH8.0; 20mM Tris, pH 7.5; 1.0% NP-40; 1% sodium deoxycholate; 0.1% SDS). Nuclear and cytoplasmic fractions were prepared from *DDX41*^{WT} and *DDX41*$^{var/+}$ LCLs two days after passaging using the Pierce "NE-PER Nuclear and Cytoplasmic Extraction Reagents" kit (Thermo Fisher Scientific). A standard SDS-PAGE Western blotting protocol was performed to quantify total DDX41 (cs-15076; Cell Signaling Technology) in whole-cell lysates and NF-κB (p65 subunit, cs-8242; Cell Signaling Technology) in nuclear and cytoplasmic fractions.

RNA Sequencing

RNA-sequencing was performed at the University of Chicago Functional Genomics Laboratory, and data was analyzed using the Cufflinks pipeline (https://cole-trapnell-lab.github.io/cufflinks/manual/; Supplementary Figure 1). Genes of interest were validated using real-time qualitative reverse transcriptase polymerase chain reaction (qRT-PCR).

Measurement of cytokine levels

Quantification of 105 unique cytokines from conditioned LCL growth medium was performed using the "Proteome Profiler Human XL Cytokine Array Kit" (R&D Systems). Quantification of 65 unique cytokines (43 of which were also assessed in the cytokine arrays; Supplementary Figure 2) from conditioned LCL growth medium was performed using the "Human Magnetic Luminex Multiplex Cytokine/Chemokine Array Kit-65 Plex" (Creative

Biolabs). Quantification of transforming growth factor-β (TGF-β) was performed using the "Human/Mouse/Rat/Porcine/Canine TGF-beta 1 Quantikine ELISA" (R&D Systems). Levels of ANG, CXCL13, CXCL8, and IL-9 were confirmed using a custom "ProcartaPlex" Luminex panel (Thermo Fisher Scientific) and normalized to a GDF-15 internal control. Conditioned LCL growth media from *DDX41*^{WT} and *DDX41*$^{var/+}$ LCLs was 8X concentrated for all assays.

UK Biobank Proteomics Analysis

We compared proteomics data from blood plasma in a cohort of 49 individuals with deleterious, likely germline *DDX41* variants (cases) to 98 age and sex-matched controls available in the UK Biobank (https://biobank.ndph.ox.ac.uk/ukb/field.cgi?id=30900, Project ID 83200).[7] To ensure none of the selected participants (neither cases nor controls) had cancer, we used national cancer registry data (https://biobank.ndph.ox.ac.uk/ukb/label.cgi?id=100092) and "summary diagnosis" (ICD10) from health-related outcomes data (https://biobank.ndph.ox.ac.uk/ukb/field.cgi?id=41270) in the UK Biobank. Therefore, at the time their peripheral blood was collected, none of the individuals included had been diagnosed with cancer. Normalized Protein Expression (NPX) values from 2922 proteins were obtained from the UK Biobank (https://biobank.ndph.ox.ac.uk/ukb/coding.cgi?id=143&nl=1). While preprocessing, missing NPX values (n=45244/384290, 11.78%) were imputed using K Nearest Neighbor. Differential expression analysis was conducted using the limma package in R with Olink's protein NPX values as the outcome, and group (case vs. control), age, and sex as predictors (https://academic.oup.com/braincomms/article/4/4/fcac155/6608340?login=true#366642284). After multiple test correction using the Benjamini Hochberg method, no proteins passed the threshold of FDR-adjusted p value with 95% confidence. Differential expression analysis plots were generated using ggplot2 and the EnhancedVolcano package in R (https://bioconductor.org/packages/devel/bioc/vignettes/EnhancedVolcano/inst/doc/EnhancedVolcano.html). Protein interaction analysis was performed using STRING (https://string-db.org/) with the minimum required interaction score set to "high confidence" (0.700). Pathway enrichment analysis was performed using the STRING database, the Kyoto Encyclopedia of Genes and Genomes (KEGG,

https://www.genome.jp/kegg/pathway.html), and the DISEASES database (https://diseases.jensenlab.org/Search).

Supplementary Table 1. Comprehensive cohort of patients with deleterious germline *DDX41* variants

Family	Relationship to Proband	Pedigree ID	Age (y)	Sex	Diagnosis (Age of Diagnosis)	*DDX41* Germline Variant [NM_016222.4]	DDX41 Encoded Protein Variant [NP_057306.2]	*DDX41* Germline Variant Classification	Second Germline Variant Gene	Second Germline Variant*	Encoded Protein Variant†	Second Germline Variant Classification
1	Proband	III-13	67	F	AML (67)	c.3G>A	p.Met1?	P				
	Daughter	IV-13	45	F	none	c.3G>A	p.Met1?	P				
	Granddaughter	V-6	18	F	none	c.3G>A	p.Met1?	P				
	Granddaughter	V-5	21	F	none	c.3G>A	p.Met1?	P				
	Niece	IV-10	35	F	none	c.3G>A	p.Met1?	P				
	Grandniece	V-3	17	F	none	c.3G>A	p.Met1?	P				
	Grandniece	V-4	11	F	none	c.3G>A	p.Met1?	P				
2	Proband	III-2	47	M	none	c.3G>A	p.Met1?	P				
	Father	II-5	73	M	MDS (71)	c.3G>A	p.Met1?	P				
3	Proband	III-10	74	M	AML (73)	c.3G>A	p.Met1?	P				
	Son	IV-16	47	M	none	c.3G>A	p.Met1?	P				
	Son	IV-18	42	M	none	c.3G>A	p.Met1?	P				
	Niece	IV-5	58	F	none	c.3G>A	p.Met1?	P				
	Grandniece	V-2	37	F	none	c.3G>A	p.Met1?	P				
4	Proband	III-1	65	M	AML (65)	c.3G>A	p.Met1?	P				
5	Proband	II-12	52	M	AML (52)	c.3G>A	p.Met1?	P				
6	Proband	III-6	65	M	AML (65)	c.3G>A	p.Met1?	P				
	Brother	III-4	59	M	none	c.3G>A	p.Met1?	P				
	Sister	III-5	63	F	none	c.3G>A	p.Met1?	P				
7	Proband	III-1	73	M	MDS (73)	c.121C>T	p.Gln41*	P				
8	Proband	III-2	49	F	Basal cell carcinoma (30s)	c.121C>T	p.Gln41*	P				
	Mother	II-3	69	F	AML (63), Lung (69)	c.121C>T	p.Gln41*	P				
	Maternal Aunt	II-6	69	F	none	c.121C>T	p.Gln41*	P				
	Maternal Aunt	II-4	55	F	Ovarian, Vulvar, AML (55)	c.121C>T	p.Gln41*	P				
	Maternal Cousin	III-3	47	M	AML (47)	c.121C>T	p.Gln41*	P				
9	Proband	III-1	58	M	Pancytopenia cirrhosis	c.142C>T	p.Gln48*	P	*PALB2*	c.2938del	p.Ser980Alafs*10	P
						c.566C>T	p.Pro189Leu	VUS				
10	Proband	IV-14	57	F	CML (51), AML (54)	c.232_233insAA	p.Pro78Glnfs*3	P				
	Paternal Uncle	III-19	81	M	MDS (80), AML (80)	c.232_233insAA	p.Pro78Glnfs*3	P				
	Paternal Cousin	IV-20	46	M	HL	c.232_233insAA	p.Pro78Glnfs*3	P				
	Brother	IV-15	56	M	none	c.232_233insAA	p.Pro78Glnfs*3	P				
	Paternal Cousin	IV-8	?	M	none	c.232_233insAA	p.Pro78Glnfs*3	P				
	Paternal Cousin	IV-22	47	M	none	c.232_233insAA	p.Pro78Glnfs*3	P				
11	Proband	III-1	65	M	AML (64)	c.268C>T	p.Gln90*	P	*ATRX*	c.7219C>T	p.Arg2407*	P
12	Proband	II-7	62	M	AML (60)	c.323del	p.Lys108Serfs*3	P				
	Brother	II-4	66	M	AML (65)	c.323del	p.Lys108Serfs*3	P				
	Sister	II-1	68	F	none	c.323del	p.Lys108Serfs*3	P				
	Brother	II-3	55	M	none	c.323del	p.Lys108Serfs*3	P				
	Mother	I-3	90	F	Colon (70), Breast (75), AML (89)	c.323del	p.Lys108Serfs*3	P				
	Nephew	III-7	36	M	none	c.323del	p.Lys108Serfs*3	P				
13	Proband	III-1	72	F	Gastric (70), t-AML (72)	c.415_418dupGATG	p.Asp140Glyfs*2	P				
14	Proband	III-17	76	M	AML (75)	c.415_418dupGATG	p.Asp140Glyfs*2	P				
	Sister	III-10	84	F	Melanoma (78)	c.415_418dupGATG	p.Asp140Glyfs*2	P				
	Niece	IV-4	62	F	none	c.415_418dupGATG	p.Asp140Glyfs*2	P				
	Nephew	IV-12	57	M	AML (56)	c.415_418dupGATG	p.Asp140Glyfs*2	P				
	Nephew	IV-13	57	M	Melanoma	c.415_418dupGATG	p.Asp140Glyfs*2	P				
	Daughter	IV-16	54	F	none	c.415_418dupGATG	p.Asp140Glyfs*2	P				
	Grandniece	V-14	19	F	none	c.415_418dupGATG	p.Asp140Glyfs*2	P				
	Brother	III-19	73	M	none	c.415_418dupGATG	p.Asp140Glyfs*2	P				
	Brother	III-12	85	M	Melanoma	c.415_418dupGATG	p.Asp140Glyfs*2	P				
	Nephew	IV-6	65	M	none	c.415_418dupGATG	p.Asp140Glyfs*2	P				
15	Proband	III-2	75	F	CN-AML (70s)	c.415_418dupGATG	p.Asp140Glyfs*2	P				
16	Proband	III-4	75	M	Prostate (64), AML with MDS changes (74)	c.415_418dupGATG	p.Asp140Glyfs*2	P				
17	Proband	III-3	63	M	AML (63)	c.415_418dupGATG	p.Asp140Glyfs*2	P				
18	Proband	III-1	64	M	MDS (63)	c.946_947del	p.Met316Asp*31	P				
	Son	IV-1	32	M	none	c.946_947del	p.Met316Asp*31	P				
	Brother	III-2	63	M	none	c.946_947del	p.Met316Asp*31	P				
19	Proband	II-2	80	M	Renal (68), MDS (75), AML (80)	c.1141A>T	p.Lys381*	P				
20	Proband	II-3	79	M	MDS (75)	c.1285C>T	p.Gln429*	P				
21	Proband	II-2	65	F	MDS (63)	c.1496dup	p.Ala500Cysfs*9	P				
22	Proband	III-1	54	M	none	c.108T>A	p.Tyr36*	LP				
	Father	II-1	?	M	MDS	c.108T>A	p.Tyr36*	LP				
	Brother	III-2	61	M	MDS (61)	c.108T>A	p.Tyr36*	LP				
23	Proband	III-1	69	M	Colon (69)	c.386dup	p.Lys130Glufs*5	LP				
24	Proband	III-1	67	M	CN-MDS (67)	c.435-2_435-1delinsCA	p.?	LP	*CHEK2*	c.470T>C	p.Ile200Thr	P
25	Proband	III-1	81	F	Mesothelioma (76)	c. 490C>T	p.Arg164Trp	LP				
26	Proband	III-6	76	F	Basal cell carcinoma (68), Colon (68), MPN/MDS overlap syndrome (70)	c. 490C>T	p.Arg164Trp	LP	*APC*	c.3920T>A	p.Ile1307Lys	LP
						c.38C>T	p.Thr13Ile	VUS				
27	Proband	III-6	69	F	Breast (54), AML (67)	c. 490C>T	p.Arg164Trp	LP				
	Brother	III-7	67	M	Head and neck (54)	c. 490C>T	p.Arg164Trp	LP				
	Son	IV-1	?	M	none	c. 490C>T	p.Arg164Trp	LP				
	Nephew	IV-3	31	M	none	c. 490C>T	p.Arg164Trp	LP				
28	Proband	III-6	58	F	Ovarian (53)	c. 490C>T	p.Arg164Trp	LP	*BRCA1*	c.68_69delAG	p.Glu23Valfs*17	P
29	Proband	III-4	66	M	Tonsillar (64), t-AML (66)	c.653G>A	p.Gly218Asp	LP				
30	Proband	III-1	37	F	Neuroendocrine carcinoma (31), CMML-2 (37)	c.653G>A	p.Gly218Asp	LP	*ATM*	c.2921+1G>A	p.?	P
31	Proband	III-4	67	M	Prostate (62), Basal Cell Carcinoma (66), MDS (67)	c.766G>A	p.Glu256Lys	LP	*CDKN2A*	c.9_32dup	p.Ala4_Pro11dup	LP
32	Proband	III-2	66	M	Prostate (57), MDS-EB-2 (60)	c.773C>T	p.Pro258Leu	LP				
33	Proband	III-3	56	F	MDS (54)	c.847delC	p.Leu283Cysfs*21	LP				
34	Proband	III-5	65	M	MDS, AML	c.1013G>A	p.Cys338Tyr	LP				
35	Proband	III-26	54	M	MDS (50)	c.1016G>T	p.Arg339Leu	LP				
	Brother	III-27	52	M	MDS (51)	c.1016G>T	p.Arg339Leu	LP				
	Paternal Aunt	II-7	87	F	MDS (87)	c.1016G>T	p.Arg339Leu	LP				
	Paternal Uncle	II-10	83	M	none	c.1016G>T	p.Arg339Leu	LP				
36	Proband	III-2	65	M	MDS and LGL (63)	c.1105C>G	p.Arg369Gly	LP				
	Sister	III-3	62	F	AML (62)	c.1105C>G	p.Arg369Gly	LP				
37	Proband	II-3	65	F	AML (65)	c.1118T>C	p.Leu373Pro	LP				
38	Proband	III-1	41	F	Breast (33)	c.1187T>C	p.Ile396Thr	LP	*BRCA2*	c.6174delT	p.Phe2058LeufsTer12	P
	Mother	II-4	52	F	NHL (38)	c.1187T>C	p.Ile396Thr	LP				
	Maternal Uncle	II-7	65	M	none	c.1187T>C	p.Ile396Thr	LP				
39	Proband	IV-1	70	F	Endometrial (68)	c.1187T>C	p.Ile396Thr	LP				
40	Proband	IV-I	17	F	Aplastic anemia (13)	c.1283T>C	p.Leu428Pro	LP				
41	Proband	III-3	73	M	AML (71)	c.1474dup	p.Ala492Glyfs*17	LP				
42	Proband	III-1	69	M	MDS (68)	c.1721del	p.Leu574Arg*fs143	LP				
43	Proband	III-4	74	M	MDS (73)	c.?	Del. Exons 12-17	LP				
	Daughter	IV-1	46	F	none	c.?	Del. Exons 12-17	LP				
44	Proband	III-1	63	M	CML (62), chronic phase PMF (62)	c.6G>T	p.Glu2Asp	VUS				
45	Proband	II-3	62	F	AML (62)	c.27+9G>A	p.?	VUS	*CHEK2*	c.1283C>T	p.Ser428Phe	P
46	Proband	II-3	62	M	AML (62)	c.138+5G>A	p.Gly?Ala	VUS				
47	Proband	I-1	75	M	Kidney, Prostate, MDS	c.301C>T	p.Arg101Cys	VUS				
48	Proband	III-1	73	M	Systemic mastocytosis (72), Melanoma (73)	c.465G>A	p.Met155Ile	VUS				
49	Proband	III-5	55	F	HL (54)	c.465G>A	p.Met155Ile	VUS				
50	Proband	II-2	73	M	AML	c.511G>C	p.Val171Leu	VUS				
51	Proband	III-2	77	M	NHL (67), Small bowel (68), Prostate (72)	c.511G>C	p.Val171Leu	VUS				
52	Proband	III-2	59	F	Fallopian tube (58), Ovarian (67)	c.926C>T	p.Thr309Ile	VUS				

Abbreviations used: AML, acute myeloid leukemia; CML, chronic myeloid leukemia; CN-AML, cytogenically normal acute myeloid leukemia; F, female; M, male; ID, identification; LGL, large granular lymphocyte; LP, likely-pathogenic; MDS, myelodysplastic syndrome; MDS-EB-2, myelodysplastic syndrome with excess blasts; MPN, myeloproliferative neoplasm; NHL, non-Hodgkin's lymphoma; P, Pathogenic; P#, pedigree number; VUS, variant of uncertain significance; y, years

*These numberings are given according to: *APC* (NM_000038.6), *ATM* (NM_000051.4), *ATRX* (NM_000489.6), *BRCA1* (NM_007294.4), *BRCA2* (NM_000059.4), *CDKN2A* (NM_000077.5), *CHEK2* (NM_007194.4), *PALB2* (NM_024675.3)

†These numberings are given according to: APC (NP_000029.2), ATRX (NP_000480.3), BRCA1 (NP_009225.1), BRCA2 (NP_000050.3) CDKN2A (NP_000068.1), CHK2 (NP_009125.1), PALB2 (NP_078951.2)

Supplementary Table 2. Genes assessed using augmented whole exome sequencing

AIP	*GPC3*	*RBBP6*
ALK	*GREM1*	*RBM8A*
ANKRD26	*GSN*	*RECQL4*
APC	*HOXB13*	*RET*
APOA1	*HRAS*	*RTEL1*
APOA2	*IKZF1*	*RUNX1*
ARID1A	*ITK*	*SAMD9*
ATM	*JAK2*	*SAMD9L*
AXIN2	*KDM1A*	*SDHA*
BAP1	*KIT*	*SDHAF2*
BARD1	*LYZ*	*SDHB*
BLM	*MAGT1*	*SDHC*
BMPR1A	*MAX*	*SDHD*
BRCA1	*MBD4*	*SH2B3*
BRCA2	*MECOM*	*SMAD4*
BRIP2	*MEN1*	*SMARCA4*
BTK	*MET MITF*	*SMARCB1*
CARD11	*MLH1*	*SMARCE1*
CASP10	*MPL*	*SRP72*
CASR	*MRTFA*	*STAT3*
CBL	*MSH2*	*STK1*
CD27	*MSH3*	*SUFU*
CD40LG	*MSH6*	*TERC*
CD70	*MUTYH*	*TERT*
CDC73	*NAF1*	*TET2*
CDH1	*NBN*	*TMEM127*
CDK4	*NF1*	*TNFRSF9*
CDKN1B	*NF2*	*TP53*
CDKN1C	*NPAT*	*TSC1*
CDKN2A	*NPM1*	*TSC2*
CEBPA	*NTHL1*	*TTR*
CHEK2	*PAB2*	*UNC13D*
CSF3R	*PAX5*	*UP45*
CST3	*PDGFRA*	*VHL*
CTLA4	*PGM3*	*WAS*
CTNNA1	*PHOX2B*	*WRN*
CTPS1	*PIK3CD*	*WT1*
DDX41	*PMS2*	*ZNF431*
DICER1	*POLD1*	
DIS3	*POLE*	
DIS3L2	*POT1*	
DOCK8	*PRKAR1A*	
EGFR	*PTCH1*	
EPCAM	*PTEN*	
ERCC6L2	*PTPN11*	
ETV6	*RAD50*	
FGA	*RAD51C*	
FH	*RAD51D*	
FLCN	*RASGRP1*	
GATA2	*RB1*	

Supplementary Table 3. Pathologic descriptions of blood and BM biopsies in individuals with germline deleterious *DDX41* variants at baseline or with HMs

	Family	Relationship to Proband (ID)	Age at biopsy (y)	Diagnosis at biopsy	Peripheral Blood	Core Biopsy	Aspirate Smear	Staining	
								Reticulin	Iron
Baseline	1	Niece (IV-10)	36	Baseline	- Slight left shift of granulocytes - Rare circulating bands - Extremely rare metamyelocytes	Normocellular (60%)	- Slight left shift towards immature forms - Megakaryocytes are smaller and hypolobated - Plasma cells are slightly increased (5.6%) with extremely rare small cells and possible Dutcher body inclusions in the nuclei	Normal	Slightly decreased histiocytic iron
	1	Grandniece (V-3)	17	Baseline	No data	No data	- Mild dysplastic changes in erythroid and megakaryocytic lineages	No data	No data
	3	Son (IV-16)	51	Baseline	- Occasional neutrophils show nuclear excrescences or chromatin hypercondensation - Minimal anisocytosis - Occasional large hypogranular platelets	Normocellular (40-50%)	- Focal shift towards immaturity and focal mild megaloblastoid feature - Some hypolobated/immature megakaryocytes	Normal	Normal
	8	Proband (III-2)	49	Baseline	- Platelets are slightly increased	Normocellular	- Some small hypolobated megakaryocytes	Normal	Increased histiocytic iron
	10	Brother (IV-15)	55	Baseline	Normal	Normocellular (40%)	- Some (<10%) smaller and hypolobated megakaryocytes	Normal	Decreased histiocytic iron
	28	Proband (III-6)	58	Baseline	- Myelocytes, metamyelocytes and band forms - Red cells have Howell-Jolly bodies and poikilocytosis due to prior surgical removal of spleen	Normocellular (40-50%)	- Rare cells with erythrophagocytosis	Normal	Normal
	38	Maternal Uncle (II-7)	65	Baseline	- Some large platelets	- Normocellular (30%) - Subcortical bone	Normal	Normal	Normal
	43	Daughter (IV-1)	46	Baseline	- Occasional neutrophils show nuclear excrescences or chromatin hypercondensation - Occasional reactive and large granular lymphocytes - Focal mild macrocytic erythrocytes with shift towards immaturity - Some dyspoietic megakaryocytes with immature nuclei with multiple separate nuclear lobes, hyperchromatic nuclei or small hypolobated/immature forms	Normocellular (40-50%)	- Focal mild macrocytic erythrocytes with shift towards immaturity, occasional irregular nuclear outlines consistent with "stress" dyserythropoiesis - Small, dyspoietic, hypolobated/immature megakaryocytes	Focally mild (grade 1 of 3) increase in reticulin fibrosis	Decreased storage iron
Malignant	4	Proband (III-1)	69	AML	- Pancytopenia - Macrocytic anemia with significant anisopoikilocytosis including fragmented RBCs and tear-drop cells. Significant RBC polychromasia. - Megakaryocytes increased with dysplasia - Platelets are reduced with hypogranulation - Increased monocytes	Hypocellular (~25%)	- Erythroid and megakaryocytic hyperplasia - Granulocytic hypoplasia	Moderately increased (MF-2, ~25%)	Could not be assessed
	6	Proband (III-6)	65	AML	- Red cells show moderate anisopoikilocytosis with scattered stubby elliptocytes, teardrop cells, and macrocytes with polychromasia - Rare circulating blasts	Hypocellular (0-30%, overall 20%)	- Dysplastic, small, hypolobated megakaryocytes - Megaloblastic erythroid precursors with dysplastic changes - Decreased maturing myeloid component including neutrophils. Instead, several pockes within interstitium comprise blastic cells or maturing myeloid precursors. - Clusters of lymphocytes and plasma cells with edematous stroma	Normal	Rare sideroblasts are present
	7	Proband (III-1)	73	MDS	- Pancytopenia severe neutropenia (ANC 0.5 K/uL), moderate anemia (HGB 9.8 g/dL), severe thrombocytopenia - Mild dyspoietic neutrophils in the form of abnormal nuclear segmentation, chromatin hypercondensation, hypogranular cytoplasm, and some toxic granulation. - Red cells are mildly hypochromic and show moderate anisopoikilocytosis, including ovalocytes, elliptocytes, teardrop cells and occasional fragments. - Mild polychromasia and occasional NRBCs - Some large and occasional giant platelets	Variably cellular (<5-50%, overall 30-40%)	- Increase in blasts, scattered interstitially with variable distribution (10-20% to focally 20-30%) - Immature and maturing erythroid precursors with megaloblastoid features and evidence of dyserythropoiesis in the form of irregular nuclear outline, nuclear budding, cytoplasmic vacuolization - Granulopoiesis reduced with shift towards immaturity and reduced dyspoietic maturation - Megakaryocytes are markedly increased and dysplastic with clustering, mostly small hypolobated/immature	Mild to moderate (grade 1-2 of 3) increase in reticulin fibrosis	No data
	9	Proband (III-1)	56	Baseline, Pancytopenia Cirrhosis	- Left-shifted myelopoiesis - Erythroid hyperplasia with many vacuolated erythroid precursors - Megakaryocytic dysplasia - Mild polyclonal plasmacytosis **[often associated with inflammatory diseases such as autoimmune or infections]**	Normocellular (~50%)	- Marked inflammatory changes in the bone marrow - Mild dyserythropoiesis and dysmegakaryopoiesis	No data	Reduced storage iron
	10	Proband (IV-14)	52	CML, AML	- Increased blasts - Reduced megakaryocytes - Reduced erythropoiesis	Hypocellular (10-15%)	- 10-15% blasts - Shift towards immaturity in granulocytic lineage - Reduced megakaryocytes	No data	No data
	10	Paternal uncle (III-19)	80	AML	- Red cells show mild anisopoikilocytosis with a few microcytes, macrocytes, polychromasia, occasional elliptocytes, and rare teardrop cells	Hypercellular (40%)	- 21% blasts - Megaloblastoid erythroid precursors	No data	No stainable or sideroblastic iron
	12	Proband (II-7)	60	AML	- Leukopenia - Thrombocytopenia - Neutropenia - Red cells are macrocytic with moderate anisopoikilocytosis - Marked proliferation of erythroid precursors - A few small, hypolobated megakaryocytes	Normocellular (50%)	- Erythroid precursors with dyspoietic and megaloblastoid features	No data	Rare ring sideroblasts are present
	13	Proband (III-1)	72	t-MDS RAEB-2 (history of chemo)	- Pancytopenia - Reduced leukocytes - Some neutrophils with toxic granulation, and some with other dysplastic changes including hypogranulation, abnormal nuclear segmentation and chromatin patterns - Mild normocytic anemia - RBCs show mild anisocytosis with occasional spherocytes and rare teardrop cells	Hypocellular (~15%)	- Increased blasts (~14%) - Increased erythropoiesis with full spectrum maturation and significant dysplasia including megaloblastoid changes, nuclear irregularities and rare multinucleated forms - Decreased megakaryocytes with dysplasia including hypolobated nuclei widely separate nuclear lobes and occasional micromegakaryocytes	Normal	Adequate storage iron
	15	Proband (III-2)	69	CN-MDS	- Leukopenia - Absolute neutropenia - Mild anisocytosis and increased polychromasia - Occational circulating blasts	Hypercellular (variable from <5% to 20-30%)	- 11% blasts: an interstitial infiltrate of small blasts in clusters between islands of erythroid precursors - Granulopoiesis is reduced, shifted toward immaturity	No data	No data
	16	Proband (III-4)	73	AML	- Leukopenia with absolute neutropenia - Macrocytic anemia - Moderate thrombocytopenia - Blasts have round/oval nuclei, dispersed chromatin, and scant blue agranular cytoplasm - RBCs show anisopoikilocytosis with macro-ovalocytes, rare dacryocytes, fragmented forms, and some polychromasia	Variable cellularity (20-40%)	- Occasional small, dyspoietic megakaryocytes - Erythropoiesis shows dyspoietic features including nuclear-cytoplasmic dyssynchrony, irregular nuclear borders, and rare nuclear budding	No data	Normal
	17	Proband (III-3)	63	AML	- Circulating blasts - Granulocytic dysplasia characterized by abnormal chromatin clumping and abnormal nuclear condensation - Normocytic anemia with mild anisopoikilocytosis characterized by macroovalocytes, and occasional microcytes - Mild RBC polychromasia	Normocellular (~30%)	- Marginally increased blasts (4.4%) - Many small hypolobated megakaryocytes indicating significant dysplasia - Significant left-shift in granulocytes with markedly increased myelocytes - Mild erythroid hyperpasia with megaloblastoid maturation, occasional nuclear irregularities and rare bi-nucleation	Focal mild increase in reticulin fibrosis, MF-1	Increased storage iron
	21	Proband (II-2)	64	MDS	- Numerous macroovalocytes - Circulating blasts	Normocellular (30%)	- 12-15% blasts - Erythroid precursors appear megaloblastoid - Small hypolobated dysplastic megakaryocytes present	No data	No data
	24	Proband (III-1)	67	MDS REAB-2	- Mild neutropenia, some neutrophils show toxic granulation, are pale, larger, and hypogranular with hyper-condensed chromatin - Occasional circulating blasts - Polychromatophilic RBCs	Hypocellular (15%)	- Both erythroid and myeloid lineages show shift towards immaturity - Erythropoiesis appears megaloblastoid as judged from the pronormoblasts - Megakaryocytes are reduced, and some are dysplastic with widely separated nuclear lobes	Patchy increase in reticulin fibrosis (grade 1/3)	- Increase in storage iron - Granular appearance
	26	Proband (III-6)	75	MDS/MPN	- Normocytic anemia (11.0 g/dL) - Thrombocytosis (613 K/uL)	Hypercellular (45%)	- Granulocytes show abnormal nuclear segmentation, numerous pseudo-Pelger-Huet forms, nuclear excrescencies, and some forms with hypogranulated cytoplasm - Erythroid cells show significant dysplasia including megaloblastoid changes, nuclear irregularities and occasional multinucleated forms - Increased megakaryocytes with numerous small, mono-lobated and some hypolobated forms indicative of dysmegakaryopoiesis	Mild focal increase in reticulin fibrosis, MF-0-1	Adequate storage iron
	29	Proband (III-4)	65	t-AML (TP53 mut and complex karyotype), low-grade B-cell lymphoproliferative disorder	- Pancytopenia - Occasional (~4%) circulating blasts - Blasts have irregular nuclear contours, fine chromatin, distinct nucleoli, and a small amount of cytoplasm - Rare segmented neutrophils - Reduced RBCs, some with anisocytosis, some elliptocytes and teardrop cells	Hypercellular (~55%)	- Decreased erythroid cells, some show dyserythropoiesis - Decreased megakaryocytes, some small hypolobated	Moderate reticulin fibrosis, MF-2	Increased storage iron
	30	Proband (III-1)	36	t-MN (history of chemo, t(11;16)(q23;p13) translocation)	- Monocytosis (64%) - Macrocytic anemia - RBCs show marked anisopoikilocytosis including occasional tear-drop cells - Thrombocytopenia - Granulocytic dysplasia	Hypocellular (~30%)	- Mature neutrophils markedly reduced and show significant granulocytic dysplasia - Mild to moderate erythroid dysplasia including megaloblastoid changes, nuclear irregularities and occasional bi-nucleated forms - Marked megakaryocytic dysplasia, some are small hypolobated	Mild to moderate reticulin fibrosis, MF-1-2	Could not be assessed
	31	Proband (III-4)	67	t-MDS (morphology consistent with therapy-related)	- Increased megakaryocytes, many are small dyspoietic hypolobated or with separated nuclear lobes, occasional micromegakaryocytes - Mild leukopenia with moderate neutropenia and mild lymphopenia - Rare circulating blasts - Moderate anemia, RBCs are macrocytic and show moderate anisopoikilocytosis, including macro-ovalocytes, microcytes, elliptocytes, and occasional spherocytes	Normocellular (30-40%)	- Predominance of erythropoiesis with megaloblastoid features, focal shift towards immaturity, evidence of dyserythropoiesis in the form of irregular nuclear outlines, binucleation and cytoplasmic vacuolization - Megakaryocytes are increased with many small dyspoietic hypolobated/immature forms or with separated nuclear lobes, occasional micromegakaryocytes	- Mild to focally moderate (grade 1-2 of 3) increase in reticulin fibrosis	- Increased iron - Occasional ring sideroblasts
	32	Proband (III-2)	61	t-MDS-EB-2	- Occasional dysplastic neutrophils with hypogranulation and abnormal nuclear segmentation, and pseudo-Pelger-Huet nuclei - Anemia, RBCs show marked anisocytosis with macro-ovalocytes, occasional spherocytes, elliptocytes, and teardrop cells - Increased polychromasia	Hypocellular (~10%)	- Increased blasts (~15%) - Occasional granulocytes show dysplastic changes including hyposegmented nuclei, abnormal chromatin patterns and rare hypogranulation - Erythroid cells show dysplastic changes including megaloblastoid maturation, occasional nuclear irregularities and bi-/multi-nucleation - Megakaryocytes are reduced, with dysplasia including hypolobated nuclei, widely separate nuclear lobes and micromegakaryocytes	Moderate increase in reticulin fibrosis, MF-2	Could not be assessed
	34	Proband (III-5)	62	t-MDS (t(11;16)(q23;p13) translocation)	- Moderate macrocytic anemia - Dyspoietic neutrophils in the form of abnormal nuclear segmentation with pseudo Pelgar-Huet change, chromatin hypercondensation, nuclear excrescences, and hypogranular cytoplasm. Some show toxic granulation. - Mild lymphopenia - RBCs are macrocytic and show moderate anisocytosis, including macro-ovalocytes, microcytes, and occasional red cell fragments - Mild thrombocytopenia, platelets show anisocytosis, including some large and occasional giant platelets	Hypocellular (10-20%)	- Dysplastic megakaryocytes including some with separated nuclear lobes, many hypolobated forms, and micromegakaryocytes with frequent clustering - Erythropoiesis with megaloblastoid features - Decreased granulopoiesis with dysplastic maturation - Evidence of Intrasinusoidal hematopoiesis	- Mild (grade 1 of 3) increase in reticulin fibrosis	No data
	41	Proband (III-3)	71	AML	- Significant pancytopenia - Acrocytic hypochromic anemia - Mild anisopoikilocytosis - Reduced platelets - Rare circulating blasts	Hypocellular (5-10%)	- Increased blasts, some with irregular nuclear membranes, high nuclear cytoplasmic ratio, prominent nucleoli and scant cytoplasm - Left shifted granulopoiesis - Rare blasts, granulocyte precursors, and erythroid precursors present	- No increase in reticulin fibrosis	- No marrow stroma or spicules to assess for storage iron - Too few erythroid precursors to assess for ring sideroblasts
	43	Proband (III-4)	73	MDS-MLD	No data	No data	- Significant dysgranulopoiesis - 4.6% blasts - Significant dyserythropoiesis - Significant dysmegakaryopoiesis	No data	No data

Abbreviations used: ANC, absolute neutrophil count; BM, bone marrow; HGB, hemoglobin; ID, identification; MF, marrow fibrosis; NRBCs, nucleated red blood cells; RBCs, red blood cells; y, years

Supplementary Table 4. Individuals with germline *DDX41*^{LoF} and other cancer-risk alleles

Relationship to Proband	Pedigree ID	Sex	Age, y	Diagnosis (Age of Diagnosis)	Second Germline Variant Gene	Second Germline Variant*	Encoded Protein Variant†	Classification
***DDX41* P/LP**								
Proband	F9-III-1	M	58	Thrombocytopenia	*PALB2*	c.2938del	p.Ser980Alafs*10	P
Proband	F11-III-1	M	65	AML (64)	*ATRX*	c.7219C>T	p.Arg2407*	LP
Proband	F24-III-1	M	67	CN-MDS (67)	*CHEK2*	c.470T>C	p.Ile200Thr	P
Proband	F26-III-6	F	76	Basal cell carcinoma (68), MPN/MDS overlap syndrome (70)	*APC*	c.3920T>A	p.Ile1307Lys	LP
Proband	F28-III-6	F	58	Ovarian (53)	*BRCA1*	c.68_69delAG	p.Glu23Valfs*17	P
Proband	F30-III-1	F	37	Neuroendocrine carcinoma (31), t-AML (37)	*ATM*	c.2921+1G>A	p.?	P
Proband	F31-III-4	M	67	Prostate (62), MDS (67)	*CDKN2A*	c.9_32dup	p.Ala4_Pro11dup	LP
Proband	F38-III-1	F	41	Breast (33)	*BRCA2*	c.6174delT	p.Phe2058LeufsTer12	P
***DDX41* VUS**								
Proband	F45-II-3	F	62	AML (62)	*CHEK2*	c.1283C>T	p.Ser428Phe	P

Abbreviations used: AML, acute myeloid leukemia; CN-MDS, cytogenetically normal myelodysplastic syndrome; F, family; ID, identification; LP, likely pathogenic; MDS, myelodysplastic syndrome; MPN, myeloproliferative neoplasm; P, pathogenic; P#, pedigree number; VUS, variant of uncertain significance; y, years

*These numberings are given according to: *APC* (NM_000038.6), *ATM* (NM_000051.4), *ATRX* (NM_000489.6), *BRCA1* (NM_007294.4), *BRCA2* (NM_000059.4), *CDKN2A* (NM_000077.5), *CHEK2* (NM_007194.4), *PALB2* (NM_024675.3)

†These numberings are given according to: APC (NP_000029.2), ATRX (NP_000480.3), BRCA1 (NP_009225.1), BRCA2 (NP_000050.3), CDKN2A (NP_000068.1), CHEK2 (NP_009125.1), GATA2 (NP_116027.2), PALB2 (NP_078951.2)

Supplementary Table 5. Classifications of second cancer-risk alleles

Pedigree ID	Second Germline Variant Gene	Second Germline Variant*	Encoded Protein Variant†	Classification	DNA Source	Test Type	VAF; Germline confirmation	Justification
F9-III-1	*PALB2*	c.2938del	p.Ser980Alafs*10	P	Bone marrow	OncoPlus large tumor panel (NGS)	Confirmed germline in cultured skin fibroblasts	P in ClinVar; Clinical report
F11-III-1	*ATRX*	c.7219C>T	p.Arg2407*	LP			Confirmed germline	LP in ClinVar
F24-III-1	*CHEK2*	c.470T>C	p.Ile200Thr	P			Confirmed germline	P in ClinVar
F26-III-6	*APC*	c.3920T>A	p.Ile1307Lys	LP	Peripheral blood	OncoPlus large tumor panel (NGS)	48%; Confirmed germline	LP in ClinVar; Clinical report
F28-III-6	*BRCA1*	c.68_69delAG	p.Glu23Valfs*17	P	Bone marrow	OncoPlus large tumor panel (NGS)	46%; Confirmed germline	P in ClinVar; Reviewed by expert panel
F30-III-1	*ATM*	c.2921+1G>A	p.?	P	Bone marrow	OncoPlus large tumor panel (NGS)	49%; Confirmed germline	P in ClinVar; Clinical report
F31-III-4	*CDKN2A*	c.9_32dup	p.Ala4_Pro11dup	LP	Skin biopsy	Hereditary Leukemia and Breast Cancer Panel (NGS)	Confirmed germline	LP in Clinical report; P/LP in ClinVar
F38-III-1	*BRCA2*	c.6174delT	p.Phe2058LeufsTer12	P	Right pleura; formalin-fixed, paraffin-embedded	OncoPlus large tumor panel (NGS)	56%; Confirmed germline	P in ClinVar; Reviewed by expert panel
F45-II-3	*CHEK2*	c.1283C>T	p.Ser428Phe	P				P in ClinVar

Abbreviations used: F, family; P, pathogenic; LP, likely pathogenic; VAF, variant allele frequency

*These numberings are given according to: *APC* (NM_000038.6), *ATM* (NM_000051.4), *ATRX* (NM_000489.6), *BRCA1* (NM_007294.4), *BRCA2* (NM_000059.4), *CDKN2A* (NM_000077.5), *CHEK2* (NM_007194.4), *PALB2* (NM_024675.3)

†These numberings are given according to: APC (NP_000029.2), ATRX (NP_000480.3), BRCA1 (NP_009225.1), BRCA2 (NP_000050.3), CDKN2A (NP_000068.1), CHEK2 (NP_009125.1), GATA2 (NP_116027.2), PALB2 (NP_078951.2)

Supplementary Table 6. FPKM RNA-sequencing values indicating gene expression in *DDX41*$^{var/+}$ and *DDX41*^{WT} LCLs for proteins of interest

Gene	*DDX41* ^{WT}			*DDX41* $^{var/+}$					P value*	Significant† (yes/no)	Validation with qRT-PCR (fold change from WT)
	WT #1	WT #2	WT #3	M1?	P258L	A492G*17	A500C*9	del ex. 12-17			
CD244	0.273343	0.269191	0.285173	11.0895	1.66513	0.774687	0.953599	0.685519	5.00E-05	yes	1.531
CD9	2.25758	7.70813	1.90579	69.2145	3.17566	12.9506	14.8167	5.7882	5.00E-05	yes	2.886
CDC14B	5.85656	2.67411	2.26175	7.17762	86.3545	6.16464	85.5129	5.57933	5.00E-05	yes	1.702
IL1R1	0.438136	0.581984	1.52523	49.0788	1.66037	2.10244	9.29089	6.67852	5.00E-05	yes	2.685
IL23R	0.0295062	1.05441	0.335415	9.57457	0.513941	0.320671	0.74782	3.37672	5.00E-05	yes	5.443
IL32	13.369	43.0563	14.4911	139.772	13.4532	26.6972	7.69567	249.089	1.00E-04	yes	1.915
LTBR	0.481455	0.718212	1.19857	23.8029	3.93722	2.87089	15.654	2.87813	5.00E-05	yes	9.553
PTPN14	0.107992	0.254362	0.203035	0.0191269	1.74213	2.31123	0.844935	2.86304	0.00085	yes	7.788
ANG	0.0766067	0	0.0800762	0.136627	0.138466	0.13552	0	0	1	no	
CXCL13	0	0	0	0	0	0	0.0748012	0.0771039	1	no	
CXCL8	0	0.309606	0	3.54681	0.177275	0.276612	0.050492	0.453417	0.24035	no	
DDX41	91.4954	101.097	97.9534	66.87	70.5655	51.5405	45.3021	84.5139	0.0575	no	
IL9	0	0	0	0	0	0	0	0	1	no	
NFKB1	61.2399	69.3384	66.4465	136.892	66.4874	88.2016	73.7998	95.4516	0.0543	no	
NFKB2	70.8146	116.882	80.716	205.606	63.6811	103.725	140.523	106.256	0.10435	no	
REL	5.93962	4.56755	5.27166	16.9442	3.532	10.2362	4.44955	8.30849	0.10055	no	
RELA	71.8171	69.5421	68.9387	76.3011	85.5456	79.914	76.0734	97.7434	0.4517	no	
RELB	13.8668	16.8349	11.4696	26.1377	12.8202	20.1112	23.1998	23.5245	0.0563	no	

Abbreviations used: var, variant

*P values were determined using a Pearson's correlation

†Confidence interval=95%

Supplementary Table 7. UK Biobank participants used in proteomics analysis

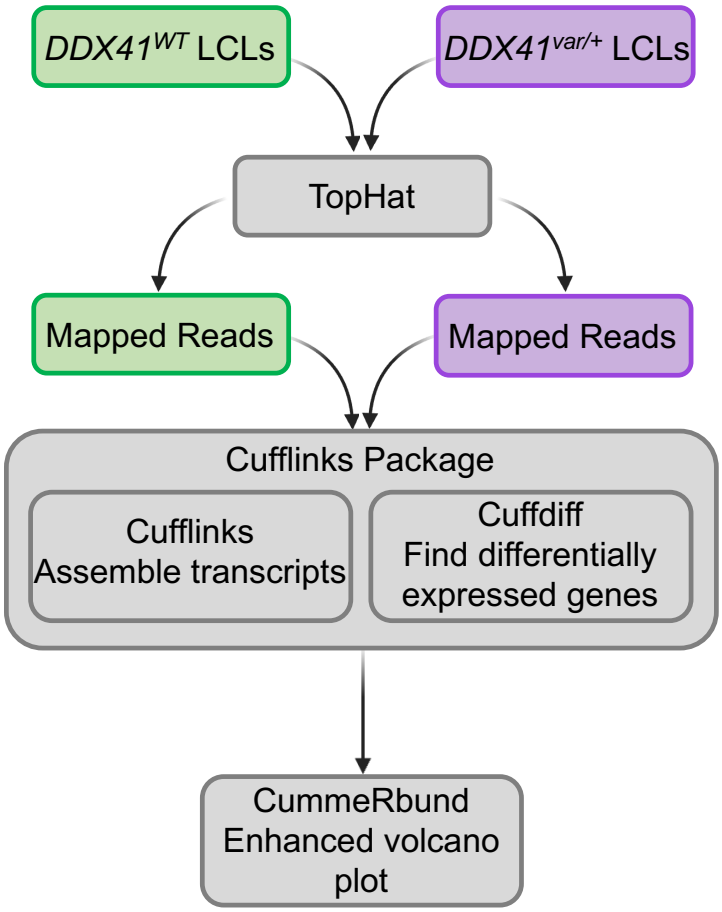
Individuals with Likely Germline P/LP *DDX41* Variants					Corresponding WT Controls		
Case #	Age at Recruitment	Sex	*DDX41* Likely Germline Variant [NM_016222.4]	DDX41 Encoded Protein Variant [NP_057306.2]	Control #	Age at Recruitment	Sex
1	40	Female	c.415_418dupGATG	p.Asp140Glyfs*2	1	40	Female
					2	40	Female
2	41	Male	c.415_418dupGATG	p.Asp140Glyfs*2	3	41	Male
					4	41	Male
3	41	Female	c.3G>A	p.Met1?	5	41	Female
					6	41	Female
4	43	Male	c.415_418dupGATG	p.Asp140Glyfs*2	7	43	Male
					8	43	Male
5	44	Male	c.1187T>C	p.Ile396Thr	9	44	Male
					10	44	Male
6	45	Female	c.3G>A	p.Met1?	11	45	Female
					12	45	Female
7	46	Male	c.1187T>C	p.Ile396Thr	13	46	Male
					14	46	Male
8	46	Female	c.415_418dupGATG	p.Asp140Glyfs*2	15	46	Female
					16	46	Female
9	47	Female	c.946_947del	p.Met316Asp*31	17	47	Female
					18	47	Female
10	47	Male	c.3G>A	p.Met1?	19	47	Male
					20	47	Male
11	48	Male	c.3G>A	p.Met1?	21	48	Male
					22	48	Male
12	48	Female	c.415_418dupGATG	p.Asp140Glyfs*2	23	48	Female
					24	48	Female
13	48	Female	c.3G>A	p.Met1?	25	48	Female
					26	48	Female
14	50	Female	c.3G>A	p.Met1?	27	50	Female
					28	50	Female
15	50	Female	c.121C>T	p.Gln41*	29	50	Female
					30	50	Female
16	52	Female	c.3G>A	p.Met1?	31	52	Female
					32	52	Female
17	52	Male	c.946_947del	p.Met316Asp*31	33	52	Male
					34	52	Male
18	53	Male	c.3G>A	p.Met1?	35	53	Male
					36	53	Male
19	54	Male	c.1586_1587del	p.Thr529Argfs*12	37	54	Male
					38	54	Male
20	54	Male	c.415_418dupGATG	p.Asp140Glyfs*2	39	54	Male
					40	54	Male
21	55	Female	c.1187T>C	p.Ile396Thr	41	55	Female
					42	55	Female
22	56	Female	c.415_418dupGATG	p.Asp140Glyfs*2	43	56	Female
					44	56	Female
23	56	Male	c.157G>A	p.Gly173Arg	45	56	Male
					46	56	Male
24	56	Male	c.415_418dupGATG	p.Asp140Glyfs*2	47	56	Male
					48	56	Male
25	57	Female	c.3G>A	p.Met1?	49	57	Female
					50	57	Female
26	58	Female	c.3G>A	p.Met1?	51	58	Female
					52	58	Female
27	58	Male	c.415_418dupGATG	p.Asp140Glyfs*2	53	58	Male
					54	58	Male
28	58	Male	c.3G>A	p.Met1?	55	58	Male
					56	58	Male
29	59	Male	c.3G>A	p.Met1?	57	59	Male
					58	59	Male
30	59	Female	c.121C>T	p.Gln41*	59	59	Female
					60	59	Female
31	60	Male	c.157G>A	p.Gly173Arg	61	60	Male
					62	60	Male
32	60	Female	c.415_418dupGATG	p.Asp140Glyfs*2	63	60	Female
					64	60	Female
33	60	Male	c.121C>T	p.Gln41*	65	60	Male
					66	60	Male
34	61	Male	c.3G>A	p.Met1?	67	61	Male
					68	61	Male
35	61	Male	c.3G>A	p.Met1?	69	61	Male
					70	61	Male
36	61	Male	c.3G>A	p.Met1?	71	61	Male
					72	61	Male
37	63	Male	c.415_418dupGATG	p.Asp140Glyfs*2	73	63	Male
					74	63	Male
38	64	Female	c.415_418dupGATG	p.Asp140Glyfs*2	75	64	Female
					76	64	Female
39	64	Female	c.415_418dupGATG	p.Asp140Glyfs*2	77	64	Female
					78	64	Female
40	64	Female	c.415_418dupGATG	p.Asp140Glyfs*2	79	64	Female
					80	64	Female
41	65	Female	c.3G>A	p.Met1?	81	65	Female
					82	65	Female
42	65	Female	c.415_418dupGATG	p.Asp140Glyfs*2	83	65	Female
					84	65	Female
43	67	Male	c.415_418dupGATG	p.Asp140Glyfs*2	85	67	Male
					86	67	Male
44	67	Female	c.3G>A	p.Met1?	87	67	Female
					88	67	Female
45	67	Female	c.415_418dupGATG	p.Asp140Glyfs*2	89	67	Female
					90	67	Female
46	67	Male	c.157G>A	p.Gly173Arg	91	67	Male
					92	67	Male
47	68	Female	c.946_947del	p.Met316Asp*31	93	68	Female
					94	68	Female
48	68	Female	c.415_418dupGATG	p.Asp140Glyfs*2	95	68	Female
					96	68	Female
49	69	Male	c.3G>A	p.Met1?	97	69	Male
					98	69	Male

Abbreviations used: LP, likely-pathogenic; P, pathogenic

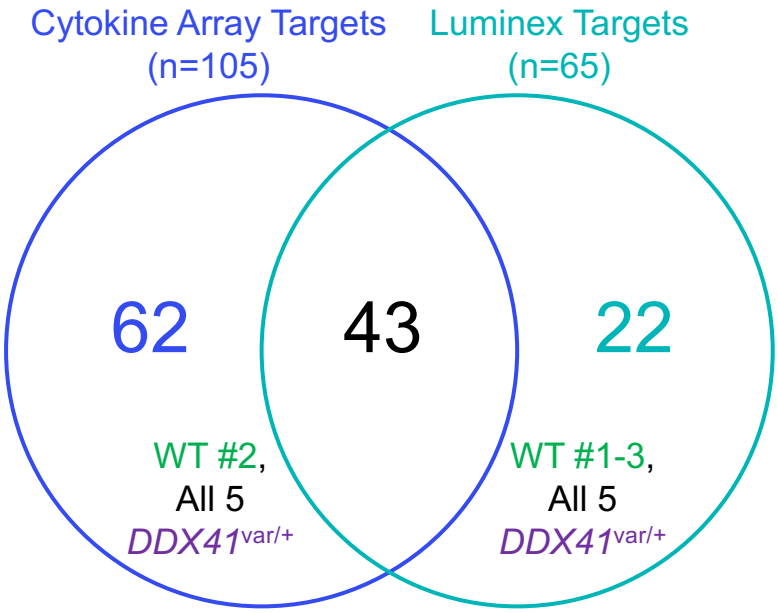
Supplementary Table 8. Summary of UK Biobank participants with likely germline *DDX41*^{LoF} variants

DDX41 Likely Germline Variant [NM_016222.4]	DDX41 Encoded Protein Variant [NP_057306.2]	*DDX41* Germline Variant Classification	Number of UK Biobank Participants
c.3G>A	p.Met1?	P	8
c.121C>T	p.Gln41*	P	3
c.415_418dupGATG	p.Asp140Glyfs*2	P	8
c.157G>A	p.Gly173Arg	P/LP	3
c.946_947del	p.Met316Asp*31	P	3
c.1187T>C	p.Ile396Thr	LP	3
c.1586_1587del	p.Thr529Argfs*12	P	1

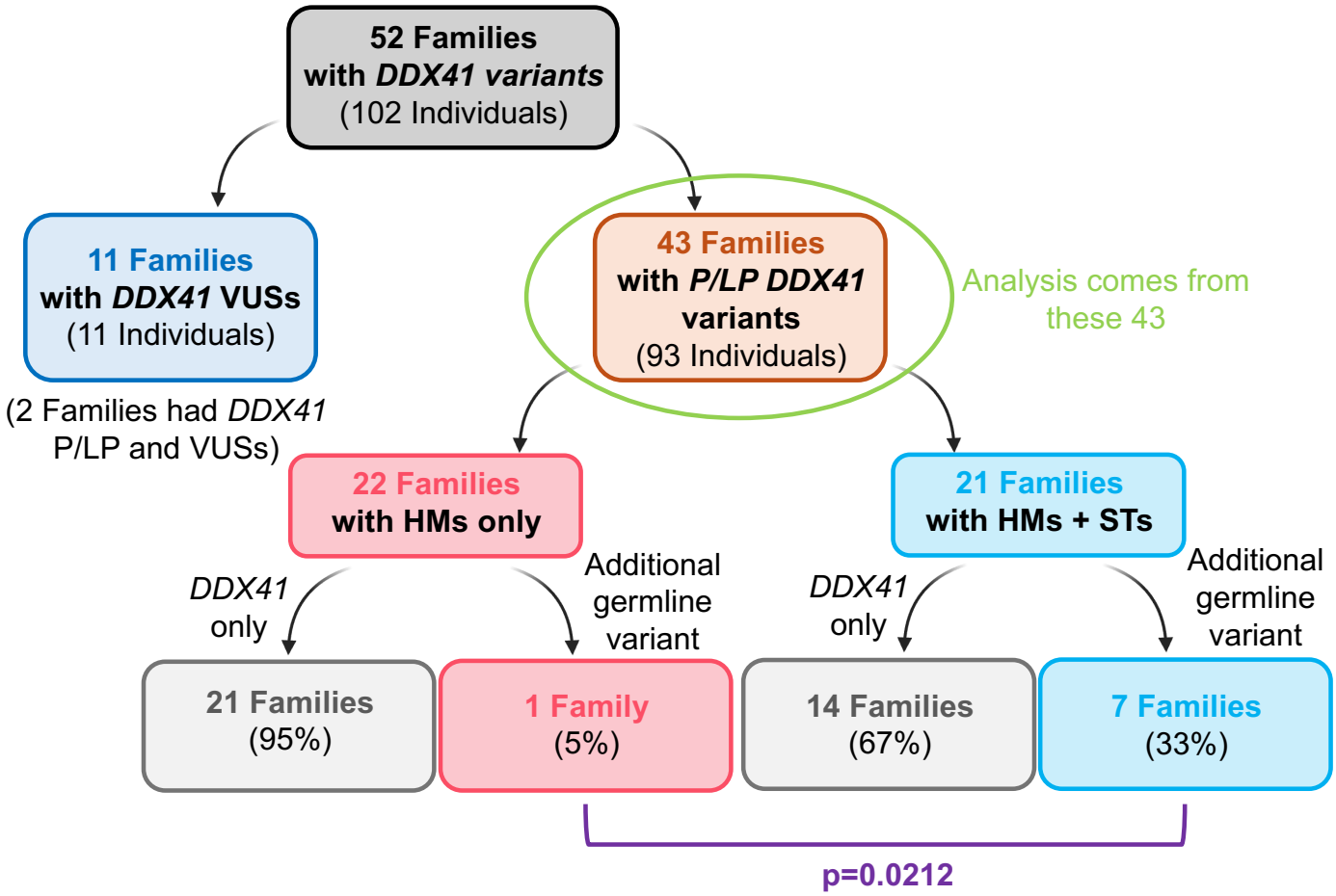
Abbreviations used: LP, likely-pathogenic; P, pathogenic



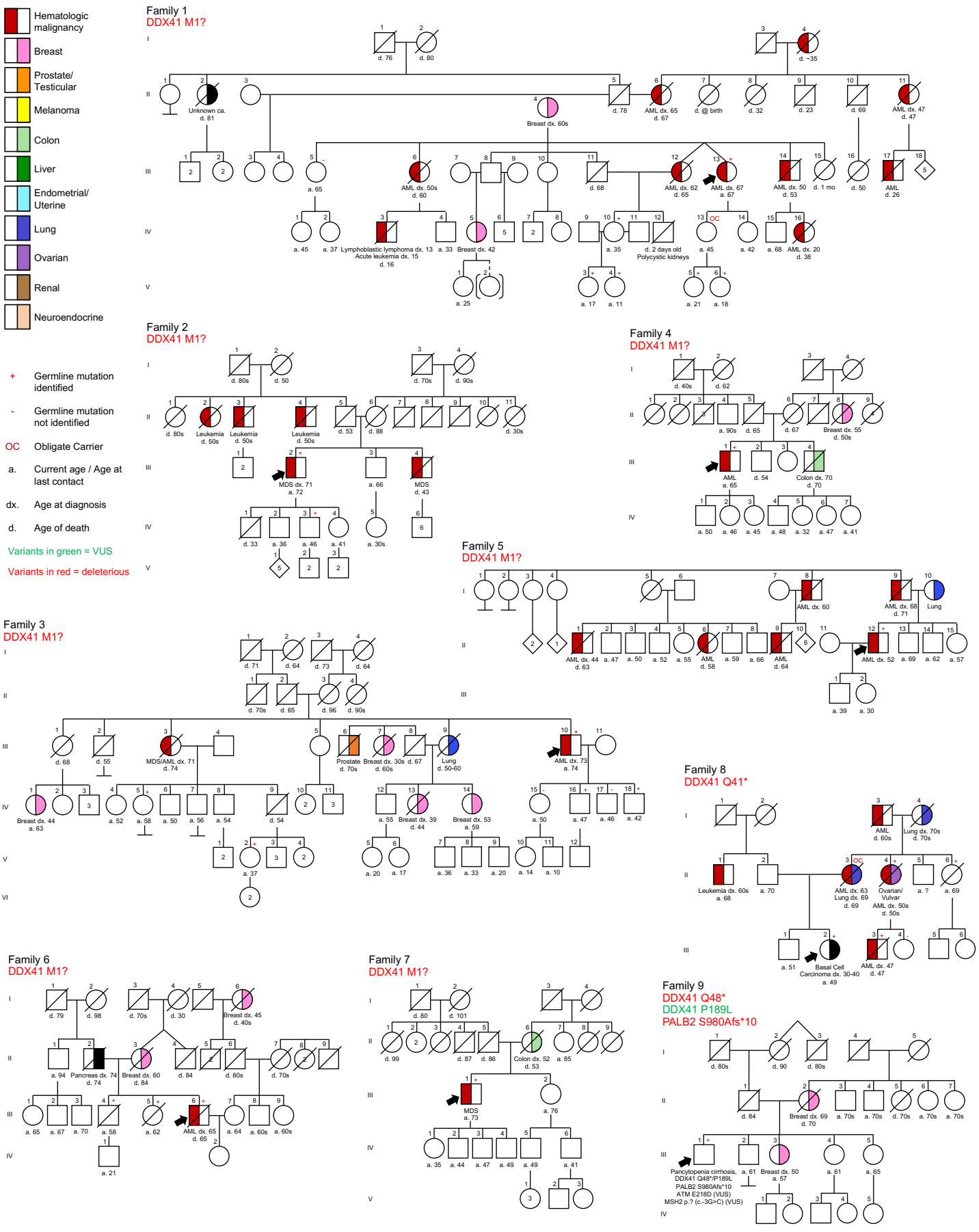
Supplementary Figure 1. Cufflinks pipeline used to analyze RNA-sequencing data. Data from *DDX41*^{WT} LCLs (green) and from patient-derived *DDX41*$^{var/+}$ LCLs (purple) is shown. Packages used to input data are indicated in gray.

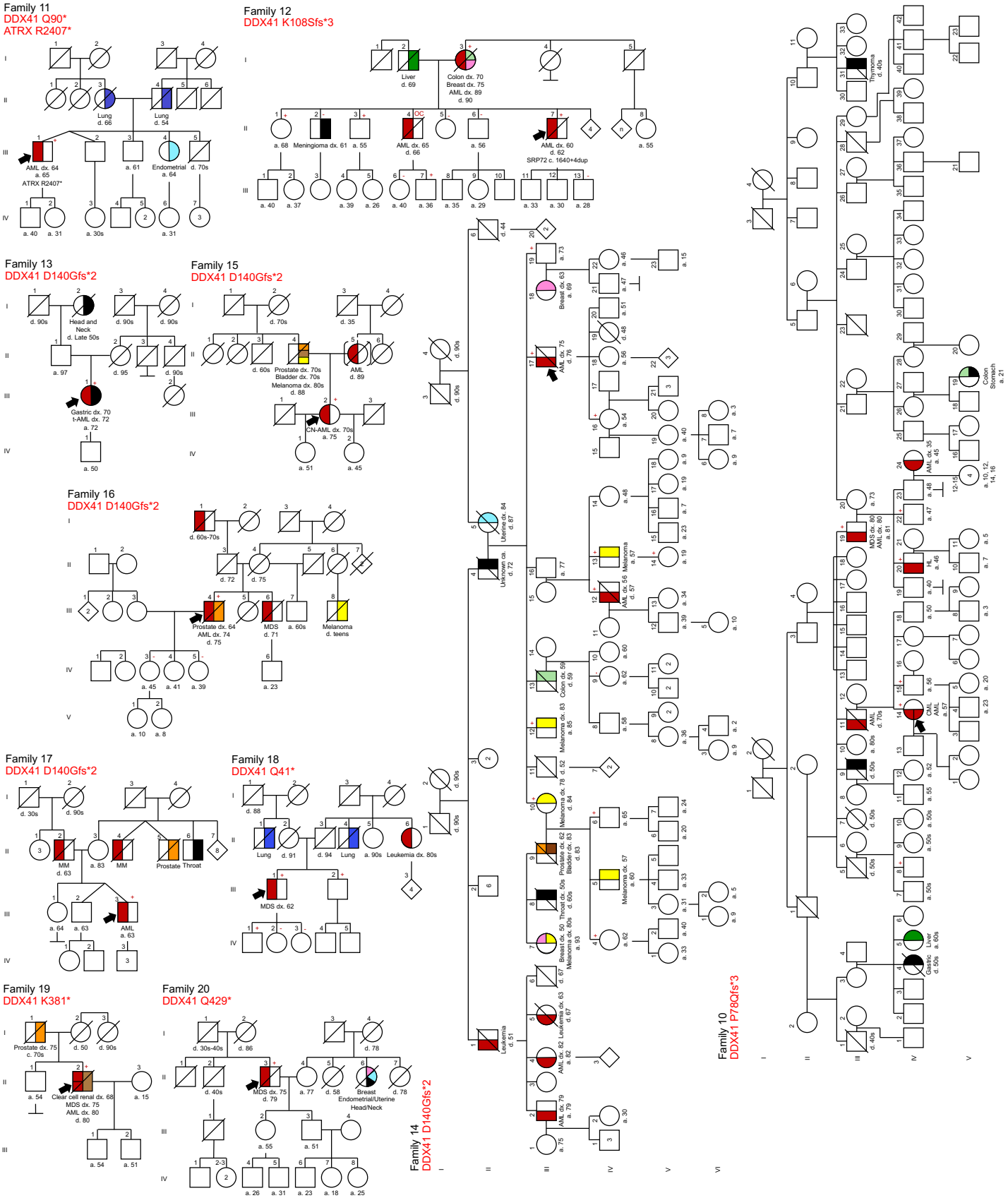


Supplementary Figure 2. Venn diagram of inflammatory cytokines assessed by cytokine arrays and Luminex. The number of inflammatory cytokines assessed by cytokine arrays only (blue), Luminex only (teal), and by both cytokine arrays and Luminex (black) are shown. Levels of inflammatory cytokines were measured in conditioned media from *DDX41*^{WT} (green) and patient-derived *DDX41*$^{var/+}$ (purple) LCLs.

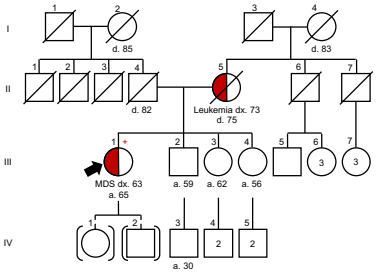


Supplementary Figure 3. Summary of our 52-family cohort. Our cohort consists of 52 families with germline *DDX41* variants of any classification (dark grey). Eleven of these families had germline *DDX41* variants of uncertain significance (VUSs, dark blue). Forty-three of these families had deleterious (P/LP) germline *DDX41* variants (orange) and were used for most of our analyses (light green). Twenty-two of those 43 families had hematopoietic malignancies (HMs) only (pink). The rest (21) had HMs and solid tumors (STs, light blue), defined as those with a history of solid tumors in ≥15% of primary relatives of the proband including the proband. Families with HMs and STs were significantly more likely to have additional germline variants in other cancer-associated genes (p=0.0212).

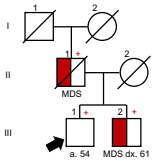

Hematologic malignancy
Breast
Prostate/ Testicular
Melanoma
Colon
Liver
Endometrial/ Uterine
Lung
Ovarian
Renal
Neuroendocrine
+ Germline mutation identified
- Germline mutation not identified
OC Obligate Carrier
a. Current age / Age at last contact
dx. Age at diagnosis
d. Age of death
Variants in green = VUS
Variants in red = deleterious
Family 1
DDX41 M1?
Family 2
DDX41 M1?
Family 3
DDX41 M1?
Family 4
DDX41 M1?
Family 5
DDX41 M1?
Family 6
DDX41 M1?
Family 7
DDX41 M1?
Family 8
DDX41 Q41*
Family 9
DDX41 Q48*
DDX41 P189L
PALB2 S980Afs*10


Family 11
DDX41 Q90*
ATRX R2407*
Lung d. 66
Lung d. 54
AML dx. 64
a. 65
ATRX R2407*
a. 61
Endometrial
a. 64
d. 70s
a. 40
a. 31
a. 30s
a. 31
Family 12
DDX41 K108Sfs*3
Liver d. 69
Colon dx. 70
Breast dx. 75
AML dx. 89
d. 90
a. 68
Meningioma dx. 61
a. 55
AML dx. 65
d. 66
a. 56
AML dx. 60
d. 62
SRP72 c. 1640+4dup
a. 55
a. 40
a. 37
a. 39
a. 26
a. 40
a. 36
a. 35
a. 29
a. 33
a. 30
a. 28
Family 13
DDX41 D140Gfs*2
d. 90s
Head and Neck
d. Late 50s
a. 97
d. 95
d. 90s
Gastric dx. 70
t-AML dx. 72
a. 72
a. 50
Family 15
DDX41 D140Gfs*2
d. 70s
a. 35
d. 60s
Prostate dx. 70s
Bladder dx. 70s
Melanoma dx. 80s
d. 88
AML
d. 89
CN-AML dx. 70s
a. 75
a. 51
a. 45
d. 90s
Family 16
DDX41 D140Gfs*2
d. 60s-70s
d. 72
d. 75
Prostate dx. 64
AML dx. 74
d. 75
MDS
d. 71
a. 60s
Melanoma
d. teens
a. 45
a. 41
a. 39
a. 23
a. 10
a. 8
Family 17
DDX41 D140Gfs*2
d. 30s
d. 90s
MM
d. 63
a. 83
MM
Prostate
Throat
a. 64
a. 63
AML
a. 63
Family 18
DDX41 Q41*
d. 88
Lung
d. 91
d. 94
Lung
a. 90s
Leukemia dx. 80s
MDS dx. 62
Family 19
DDX41 K381*
Prostate dx. 75
c. 70s
d. 50
d. 90s
a. 54
Clear cell renal dx. 68
MDS dx. 75
AML dx. 80
d. 80
a. 15
a. 54
a. 51
Family 20
DDX41 Q429*
d. 30s-40s
d. 86
d. 78
d. 40s
MDS dx. 75
d. 79
a. 77
d. 58
Breast
Endometrial/Uterine
Head/Neck
d. 78
a. 55
a. 51
a. 26
a. 31
a. 23
a. 18
a. 25
Family 14
DDX41 D140Gfs*2
d. 44
Uterine dx. 84
d. 87
Unknown ca.
d. 72
Leukemia
d. 51
Breast dx. 73
Breast dx. 69
a. 69
AML dx. 75
d. 76
a. 54
Melanoma
a. 57
AML dx. 56
d. 57
Colon dx. 59
d. 59
Melanoma dx. 83
a. 85
Melanoma dx. 78
d. 84
d. 52
Prostate dx. 62
Bladder dx. 83
Throat dx. 50s
d. 60s
Breast dx. 50
Melanoma dx. 80s
a. 93
Melanoma dx. 67
a. 60
Leukemia dx. 63
d. 67
AML dx. 82
a. 82
AML dx. 79
a. 79
a. 75
a. 30
Family 10
DDX41 P78Qfs*3
Thymoma
d. 40s
MDS dx. 80
AML dx. 80
a. 81
HL
a. 46
AML
d. 70s
CML
AML
a. 57
Colon
Stomach
a. 21
AML dx. 35
a. 45
a. 10, 12,
14, 16
Liver
a. 60s
Gastric
d. 50s

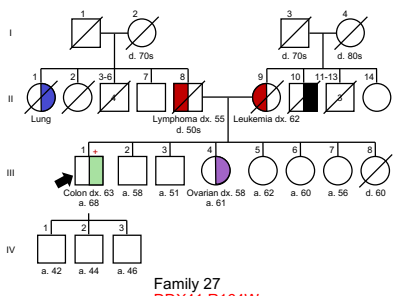
Family 21
DDX41 A500Cfs*9



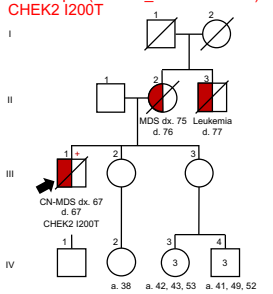
Family 22
DDX41 Y36*



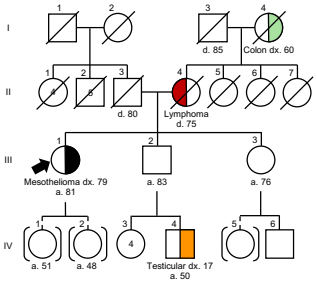
Family 23
DDX41 K130Efs*5



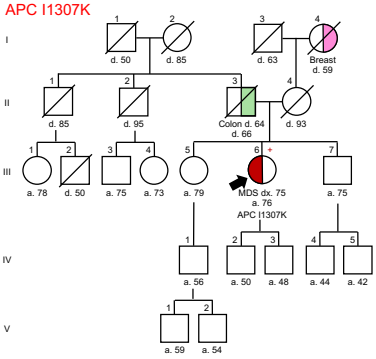
Family 24
DDX41 p.? (c.435-2_435-1delinsCA)
CHEK2 I200T



Family 25
DDX41 R164W



Family 26
DDX41 R164W
APC I1307K



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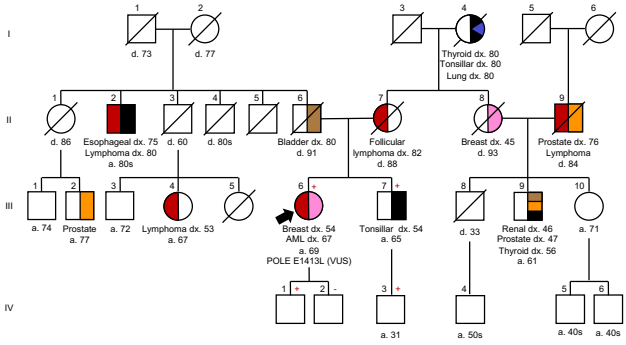
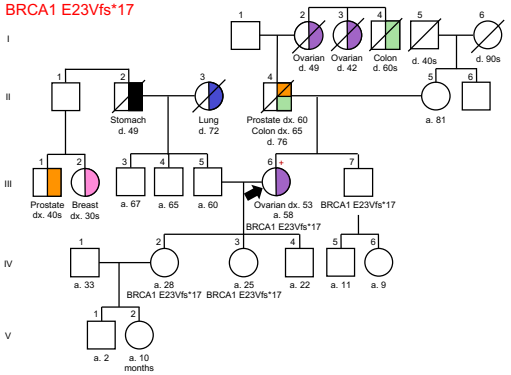
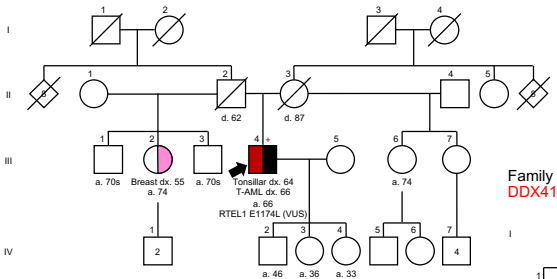
DDX41 R164W



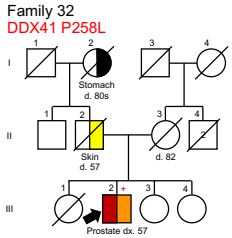
Family 28
DDX41 R164W
BRCA1 E23Vfs*17



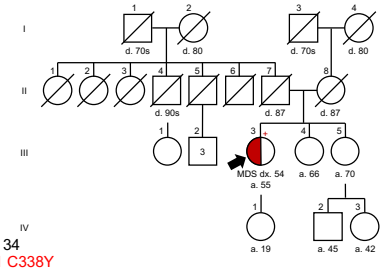
Family 29
DDX41 G218D



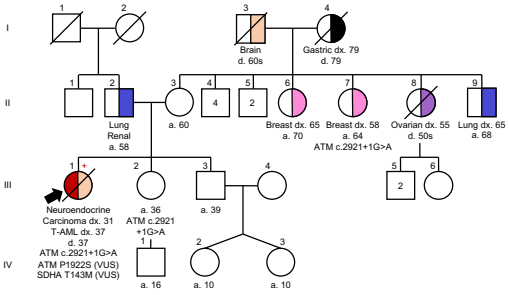
Family 32
DDX41 P258L



Family 33
DDX41 L283Cfs*21



Family 30
DDX41 G218D
ATM p.? (c.2921+1G>A)



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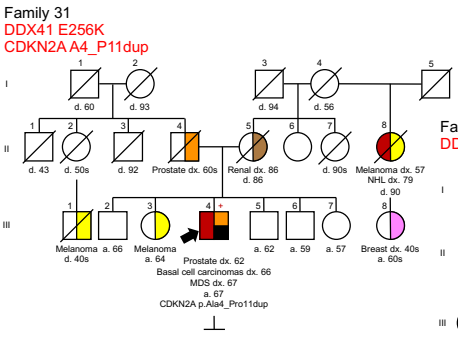
DDX41 E256K
CDKN2A A4_P11dup



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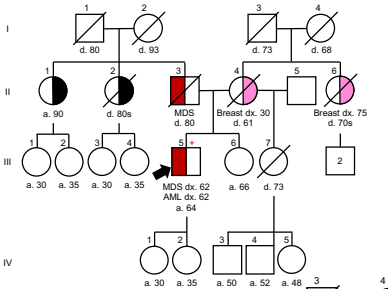
DDX41 C338Y



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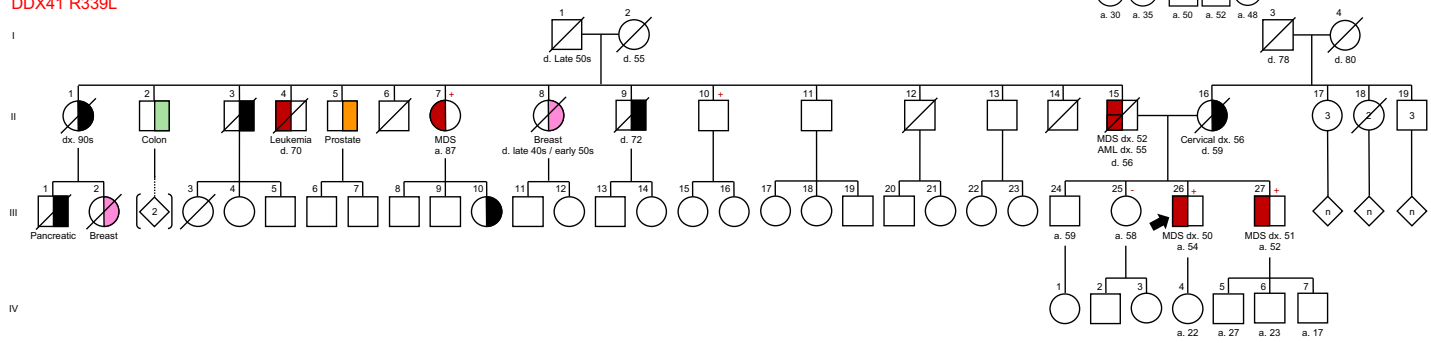
DDX41 R339L



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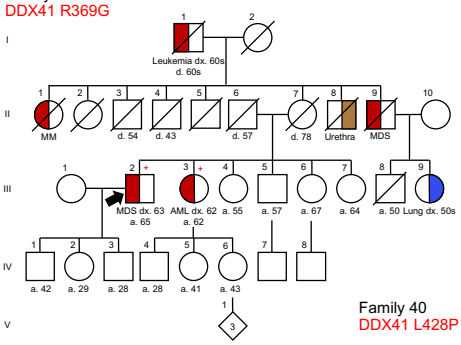
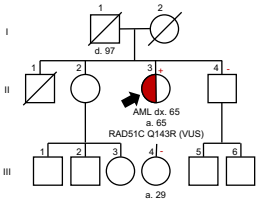
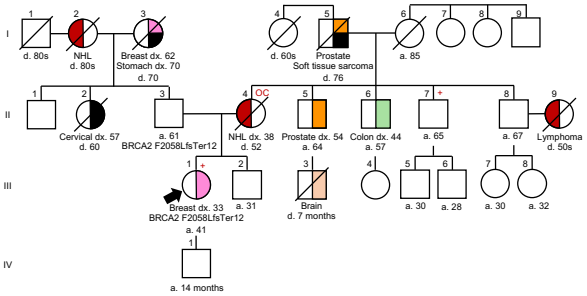
DDX41 R369G



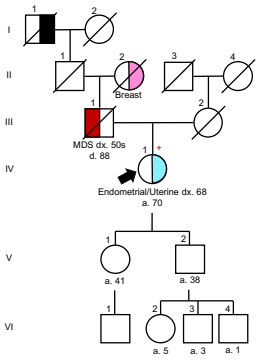
Family 37
DDX41 L373P



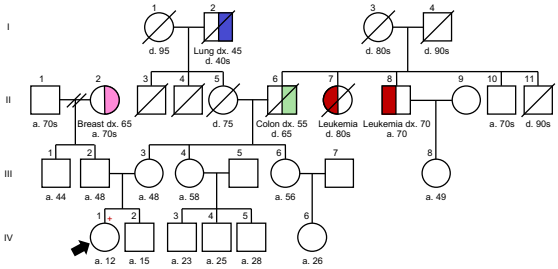
Family 38
DDX41 I396T
BRCA2 F2058LfsTer12



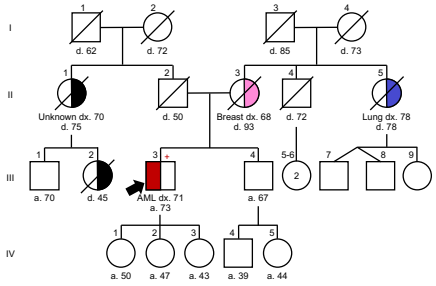
Family 39
DDX41 I396T



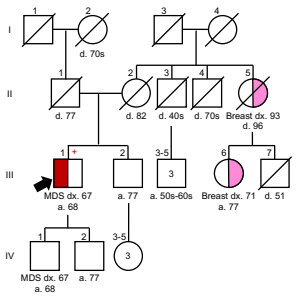
Family 40
DDX41 L428P



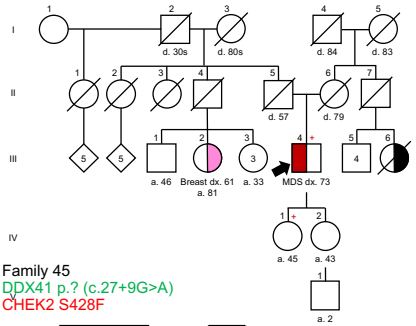
Family 41
DDX41 A492Gfs*17



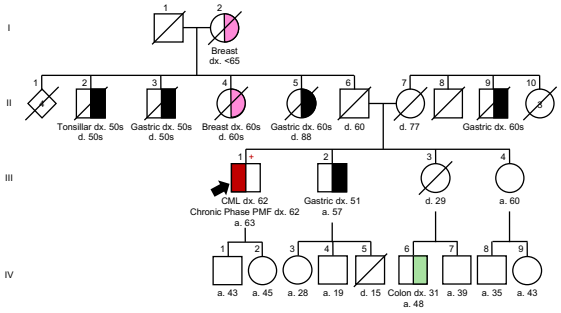
Family 42
DDX41 L574Rfs*143



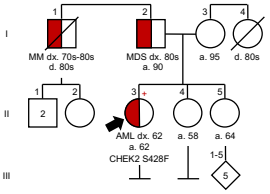
Family 43
Deletion exons 12-17



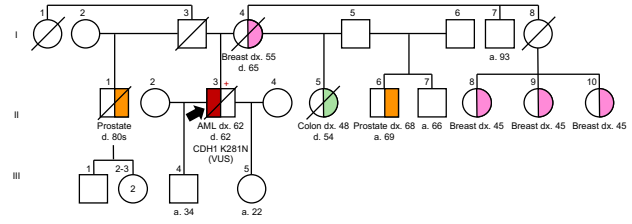
Family 44
DDX41 E2D



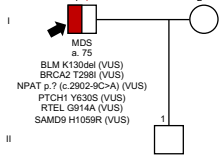
Family 45
DDX41 p.? (c.27+9G>A)
CHEK2 S428F



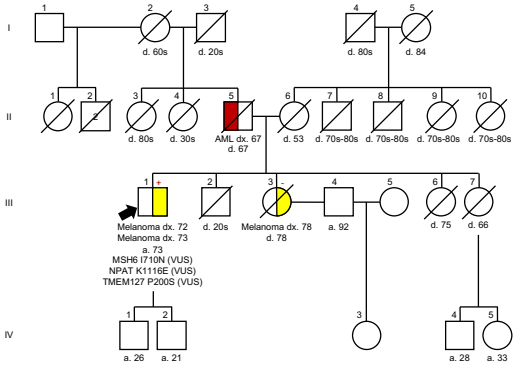
Family 46
DDX41 p.? (c.138+5G>A)



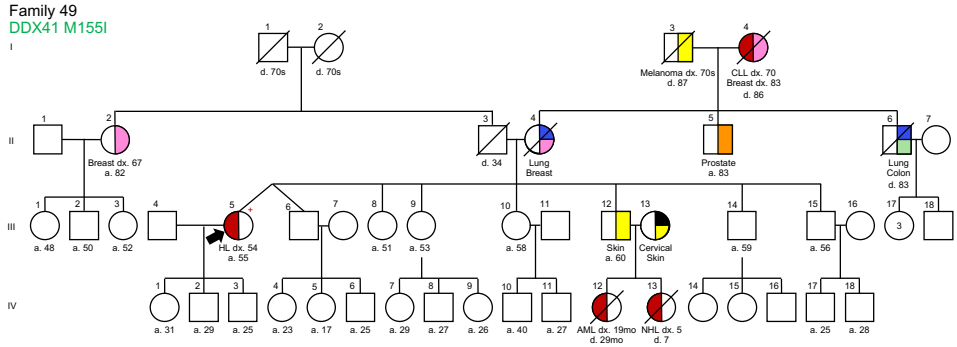
Family 47
DDX41 R101C



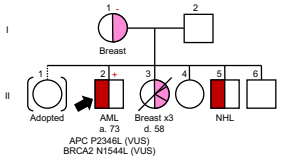
Family 48
DDX41 M155I

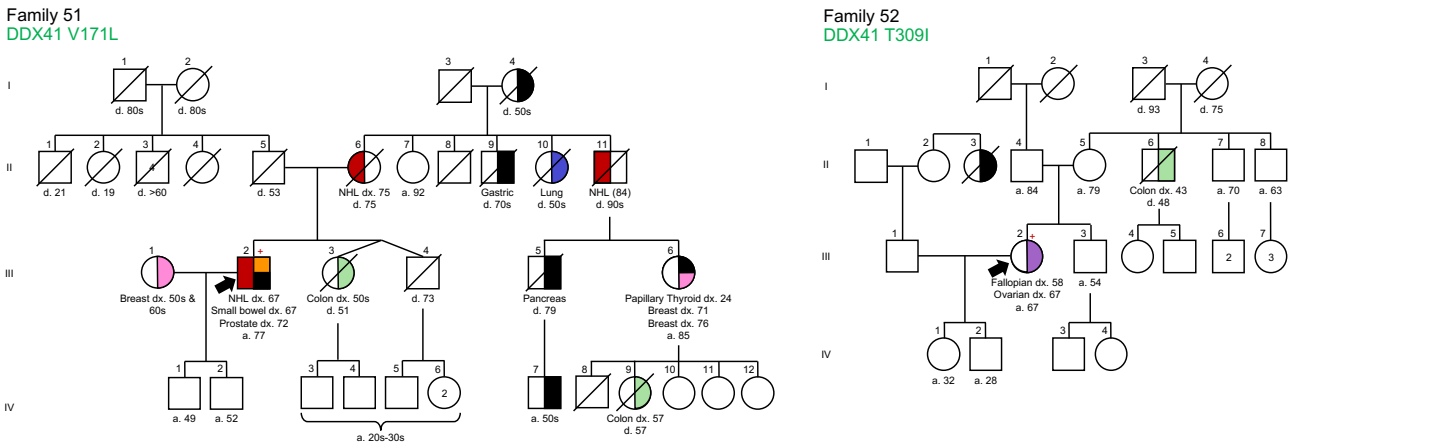


Family 49
DDX41 M155I

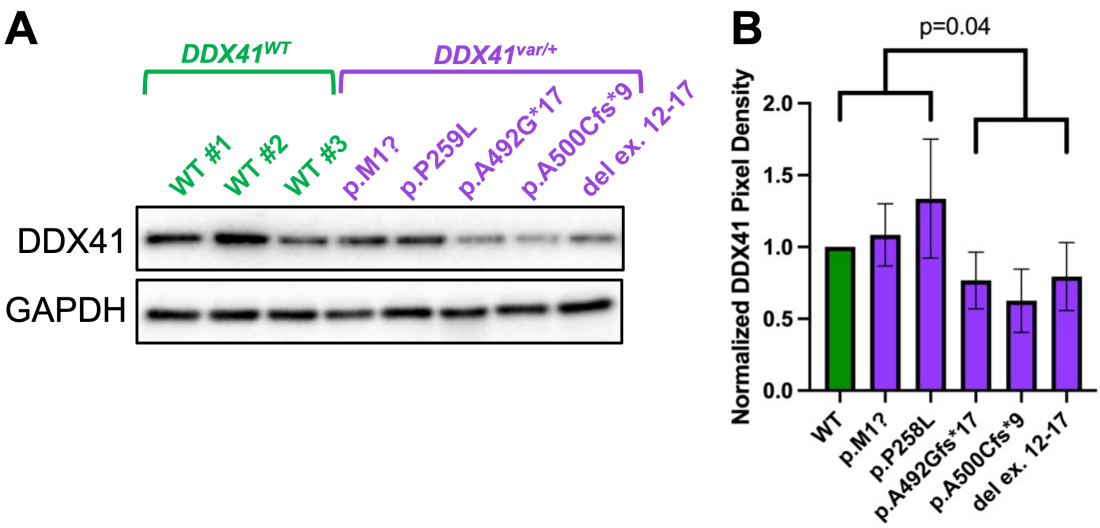


Family 50
DDX41 V171L

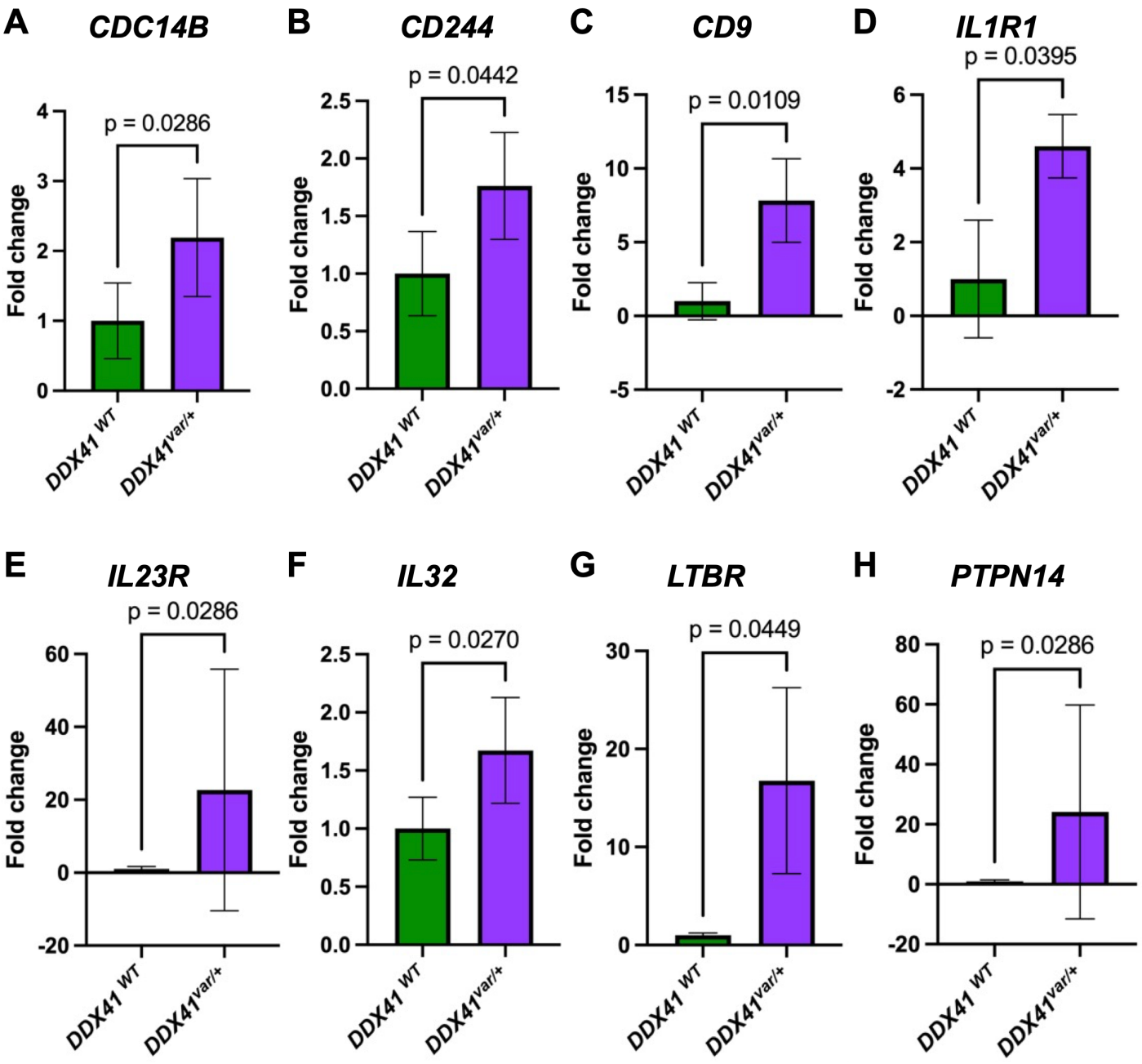




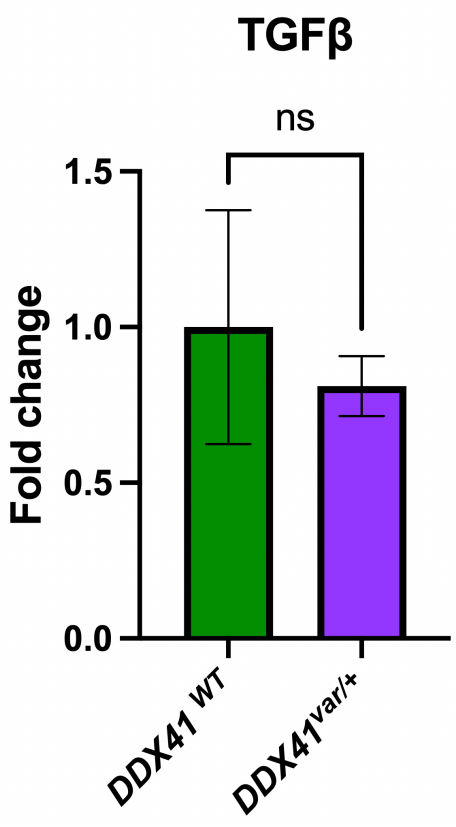
Supplementary Figure 4. Family pedigrees representing our comprehensive cohort of families with germline *DDX41*^{LoF} alleles. Squares represent males, circles represent females, and diamonds indicate that the sex is unknown. All family members that we have knowledge of are shown, regardless of genotype or presence of disease. A "+" sign indicates an individual who has tested positive for the familial DDX41 variant, whereas a "-" sign indicates an individual who has tested negative for the familial DDX41 variant. "OC" indicates that someone is an obligate carrier of the familial variant. "a." indicates the individual's age, and "d." and a strikethrough indicates that the individual is deceased, with the age at time of death indicated. Dark red denotes individuals with HM(s). Solid tumors such as breast (pink), prostate (orange), melanoma (yellow), colon (light green), liver (dark green), endometrial (light blue), lung (dark blue), ovarian (purple), renal (brown), and neuroendocrine (peach) are shown. Age of diagnosis is given after "dx." if it is known.



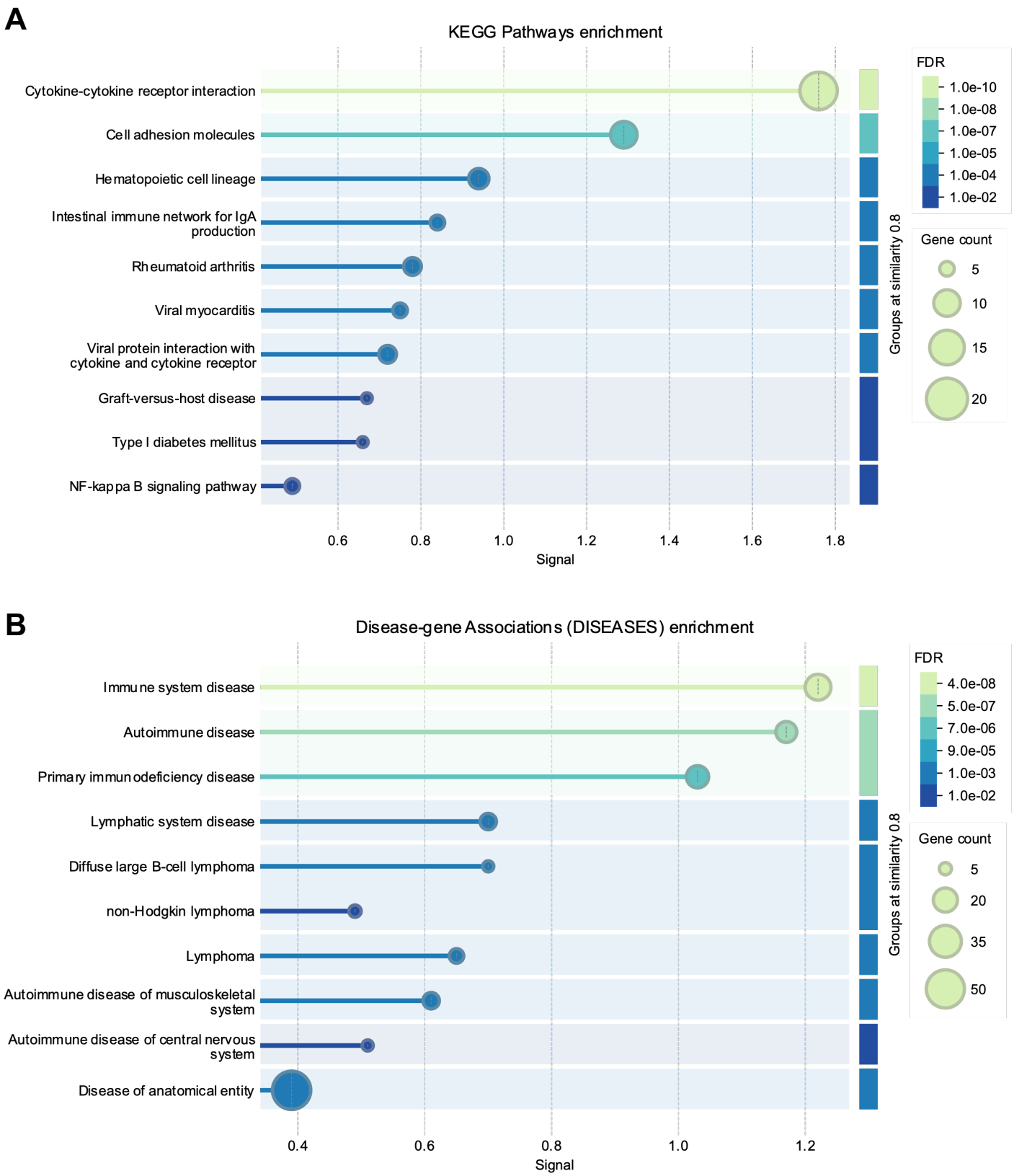
Supplementary Figure 5. DDX41 protein levels in patient-derived LCLs with different *DDX41* alleles. (A) Western blots for total DDX41 in whole cells lysates from *DDX41*^{WT} (n=3, green) and *DDX41*$^{var/+}$ (n=5, purple) patient-derived LCLs. GAPDH was used as a loading control. **(B)** Bar plot of average normalized DDX41 pixel densities in *DDX41*^{WT} (n=3, green) and *DDX41*$^{var/+}$ (n=5, purple) patient-derived LCLs. DDX41 levels were lower in LCLs with frameshift deletions (A492Gfs*17, A500Cfs*9, and del ex. 12-17) in *DDX41* than in those with other mutations (M1?, and P258L) or those with wild type *DDX41* alleles (p=0.04).



Supplementary Figure 6. RT-qPCR results to validate RNA-sequencing findings. (A-H) Fold changes in gene expression are shown in *DDX41*^{WT} (green) and *DDX41*$^{var/+}$ (purple) patient-derived LCLs. P-values were determined using two-tailed t-tests with Welch's correction and confirm increased expression of **(A)** *CDC14B*, **(B)** *CD244*, **(C)** *CD9*, **(D)** *IL1R1*, **(E)** *IL23R*, **(F)** *IL32*, **(G)** *LTBR*, and **(H)** *PTPN14* in *DDX41*$^{var/+}$ LCLs.



Supplementary Figure 7. Quantification of TGF-β by ELISA. (A) Average concentrations of TGF-β (pg/mL) in conditioned media from *DDX41*^{WT} LCLs (n=3, green) and *DDX41*$^{var/+}$ LCLs (n=5, purple). No significant change in TGF-β levels was detected as determined by a two-tailed t-test with Welch's correction (p=0.38).



Supplementary Figure 8. Associated pathways and diseases of proteins found to decrease in individuals with likely germline *DDX41*^{LoF} variants. (A) Results of KEGG pathway enrichment analysis and **(B)** disease-gene association analysis (based on the DISEASES database) of 114 proteins found to decrease in the context of likely germline *DDX41*^{LoF} alleles compared to WT controls. **(A-B)** Plots were generated using STRING (https://version11.string-db.org/).

Supplementary References

1. Kraft IL, Godley LA. Identifying potential germline variants from sequencing hematopoietic malignancies. *Hematology*. 2020;2020(1):219-227. doi:10.1182/hematology.2020006910

2. Guidugli L, Johnson AK, Alkorta-Aranburu G, et al. Clinical utility of gene panel-based testing for hereditary myelodysplastic syndrome/acute leukemia predisposition syndromes. *Leukemia*. 2017;31(5):1226-1229. doi:10.1038/leu.2017.28

3. Feurstein S, Trottier AM, Estrada-Merly N, et al. Germ line predisposition variants occur in myelodysplastic syndrome patients of all ages. *Blood*. 2022;140(24):2533-2548. doi:10.1182/blood.2022015790

4. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*. 2015;17(5):405-424. doi:10.1038/gim.2015.30

5. Kadri S, Long BC, Mujacic I, et al. Clinical Validation of a Next-Generation Sequencing Genomic Oncology Panel via Cross-Platform Benchmarking against Established Amplicon Sequencing Assays. *J Mol Diagn*. 2017;19(1):43-56. doi:10.1016/j.jmoldx.2016.07.012

6. Makishima H, Saiki R, Nannya Y, et al. Germline DDX41 mutations define a unique subtype of myeloid neoplasms. *Blood*. Published online November 2, 2022:blood.2022018221. doi:10.1182/blood.2022018221

7. Sun B B, Chiou J, Traylor M, et al. Plasma proteomic associations with genetics and health in the UK Biobank. *Nature.* 2023;622(7982);329-338. Doi: 10.1038/s41586-023-06592-6