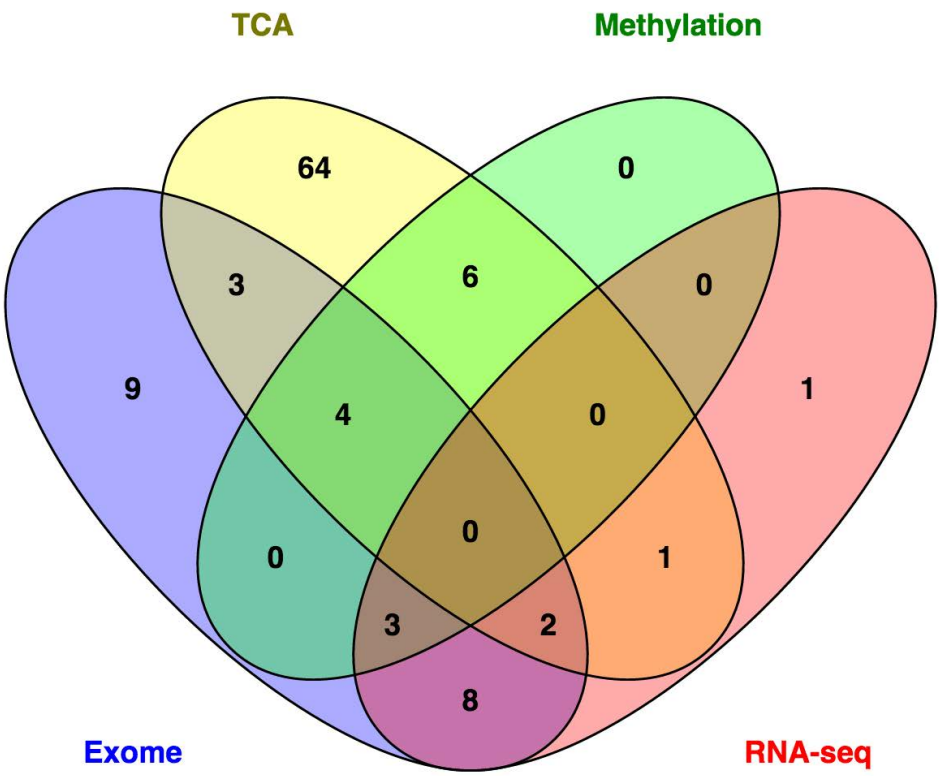
The Genomic Landscape of Juvenile Myelomonocytic Leukemia

Supplementary Information

Elliot Stieglitz[1], Amaro N. Taylor-Weiner[2], Tiffany Y. Chang[1], Laura C. Gelston[1], Yong-Dong Wang[3], Tali Mazor[4], Emilio Esquivel[1], Ariel Yu[1], Sara Seepo[2], Scott Olsen[5], Mara Rosenberg[2], Sophie L. Archambeault[1], Ghada Abusin[6], Kyle Beckman[1], Patrick A. Brown[7], Michael Briones[8], Benjamin Carcamo[9], Todd Cooper[10], Gary V. Dahl[11], Peter D. Emanuel[12], Mark N. Fluchel[13], Rakesh K. Goyal[14], Robert J. Hayashi[15], Johann Hitzler[16], Christopher Hugge[17], Y. Lucy Liu[12], Yoav H. Messinger[18], Donald H. Mahoney Jr[19], Philip Monteleone[20], Eneida R. Nemecek[21],, Philip A. Roehrs[22], Reuven J. Schore[23], Kimo C. Stine[24], Clifford M. Takemoto[7], Jeffrey A. Toretsky[25,26], Joseph F. Costello[4], Adam B. Olshen[27,28], Chip Stewart[2], Yongjin Li[3], Jing Ma[29], Robert B. Gerbing[30], Todd A. Alonzo[31], Gad Getz[2,32,33], Tanja Gruber[29,34], Todd Golub[2,35,36], Kimberly Stegmaier[2,35,36] and Mignon L. Loh[1,37]

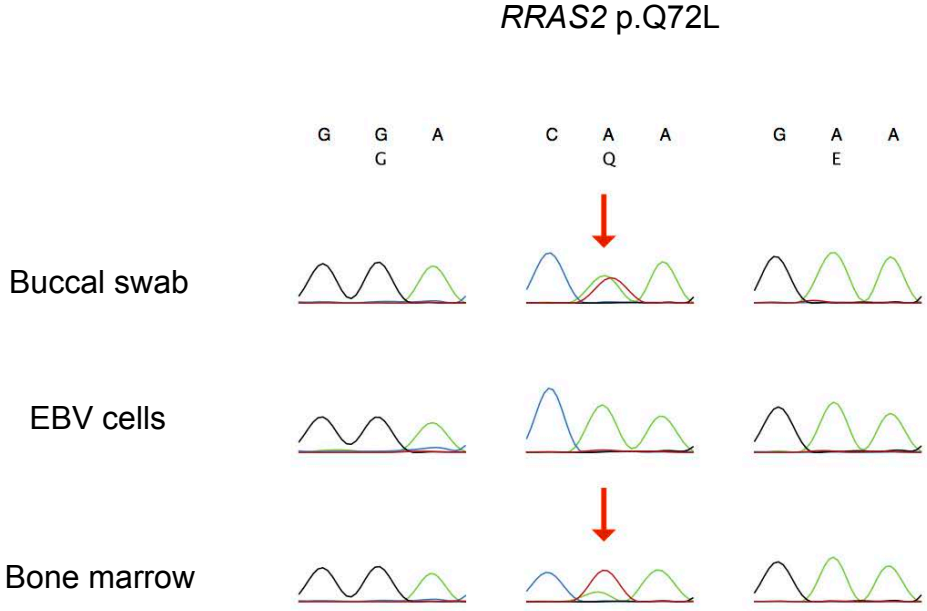
[1]Department of Pediatrics, Benioff Children's Hospital, University of California, San Francisco, San Francisco, CA;
[2]Broad Institute of MIT and Harvard, Cambridge, MA;
[3]Department of Computational Biology, St. Jude Children's Research Hospital, Memphis, TN;
[4]Department of Neurological Surgery, University of California, San Francisco, CA;
[5]Hartwell Center for Bioinformatics and Biotechnology, St. Jude Children's Research Hospital, Memphis, TN;
[6]Stead Family Department of Pediatrics, University of Iowa Carver College of Medicine, Iowa City, IA;
[7]Department of Pediatrics, The Johns Hopkins Hospital, Baltimore, MA;
[8]Department of Pediatrics, Emory University School of Medicine, Aflac Cancer and Blood Disorder Center, Atlanta, GA;
[9]Department of Pediatrics, Texas Tech University, El Paso, TX;
[10]Department of Pediatrics, Seattle Children's Hospital, Seattle, WA
[11]Department of Pediatrics, Stanford School of Medicine, Stanford, CA;
[12]Winthrop P. Rockefeller Cancer Institute, University of Arkansas for Medical Sciences, Little Rock, AR;
[13]Department of Pediatric Hematology Oncology, University of Utah, Salt Lake City, UT;
[14]Department of Pediatrics, Children's Hospital of Pittsburgh of UPMC, Pittsburgh, PA;
[15]Department of Pediatrics, Washington University School of Medicine, St. Louis, MO;
[16]Division of Hematology/Oncology, The Hospital for Sick Children. Toronto, Ontario, Canada;
[17]Pediatric Hematology Oncology, SSM Cardinal Glennon Children's Medical Center, Saint Louis, MO;
[18]Division of Pediatric Hematology Oncology, Children's Hospitals and Clinics of Minnesota, Minneapolis, MN;
[19]Department of Pediatrics, Texas Children's Hospital, Baylor College of Medicine, Houston, TX;
[20]Pediatric Hematology Oncology, Pediatric Specialists of Lehigh Valley Hospital, Bethlehem, PA;
[21]Pediatric Bone Marrow Transplant Program, Oregon Health & Science University, Portland, OR;
[22]Department of Pediatrics, University of North Carolina at Chapel Hill, NC;
[23]Division of Pediatric Oncology, Children's National Medical Center, Washington, DC;
[24]Department of Pediatrics, University of Arkansas for Medical Sciences, Little Rock, AR;
[25]Department of Pediatrics, Georgetown University, Washington, DC;
[26]Department of Oncology, Georgetown University, Washington, DC;
[27]Helen Diller Family Comprehensive Cancer Center, University of California, San Francisco, CA;
[28]Department of Epidemiology and Biostatistics, University of California, San Francisco, CA;
[29]Department of Pathology, St. Jude Children's Research Hospital, Memphis, TN;
[30]Department of Statistics, Children's Oncology Group, Monrovia, CA;
[31]Keck School of Medicine, University of Southern California, Los Angeles, CA;
[32]Harvard Medical School, Boston, MA;
[33]Department of Pathology and Cancer Center, Massachusetts General Hospital, Boston, MA;
[34]Department of Oncology, St. Jude Children's Research Hospital, Memphis, TN;
[35]Department of Pediatric Oncology, Dana-Farber Cancer Institute, Boston, MA;
[36]Division of Hematology/Oncology, Boston Children's Hospital and Harvard Medical School, Boston, MA;
[37]Department of Pediatrics, Benioff Children's Hospital, Helen Diller Family Comprehensive Cancer Center, University of California, San Francisco, San Francisco, CA;

Elliot Stieglitz and Amaro Taylor-Weiner contributed equally.

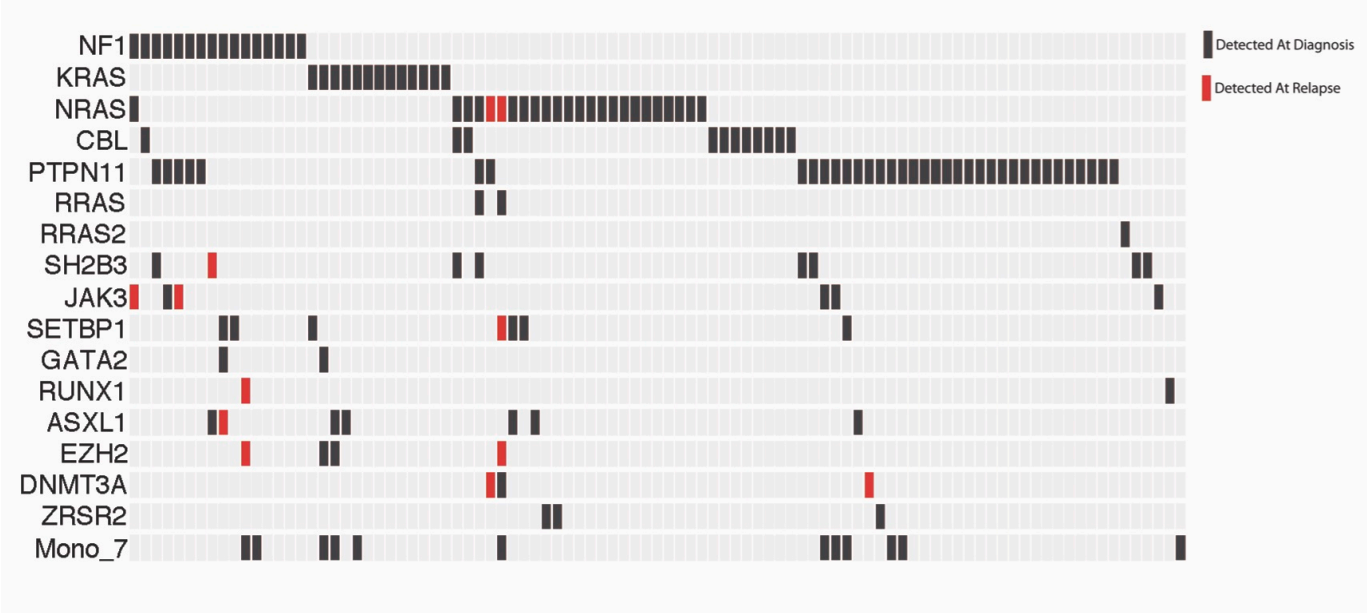


Built using Venny 2.0. Oliveros, J.C. (2007-2015) Venny. An interactive tool for comparing lists with Venn's diagrams. http://bioinfogp.cnb.csic.es/tools/venny/index.html.

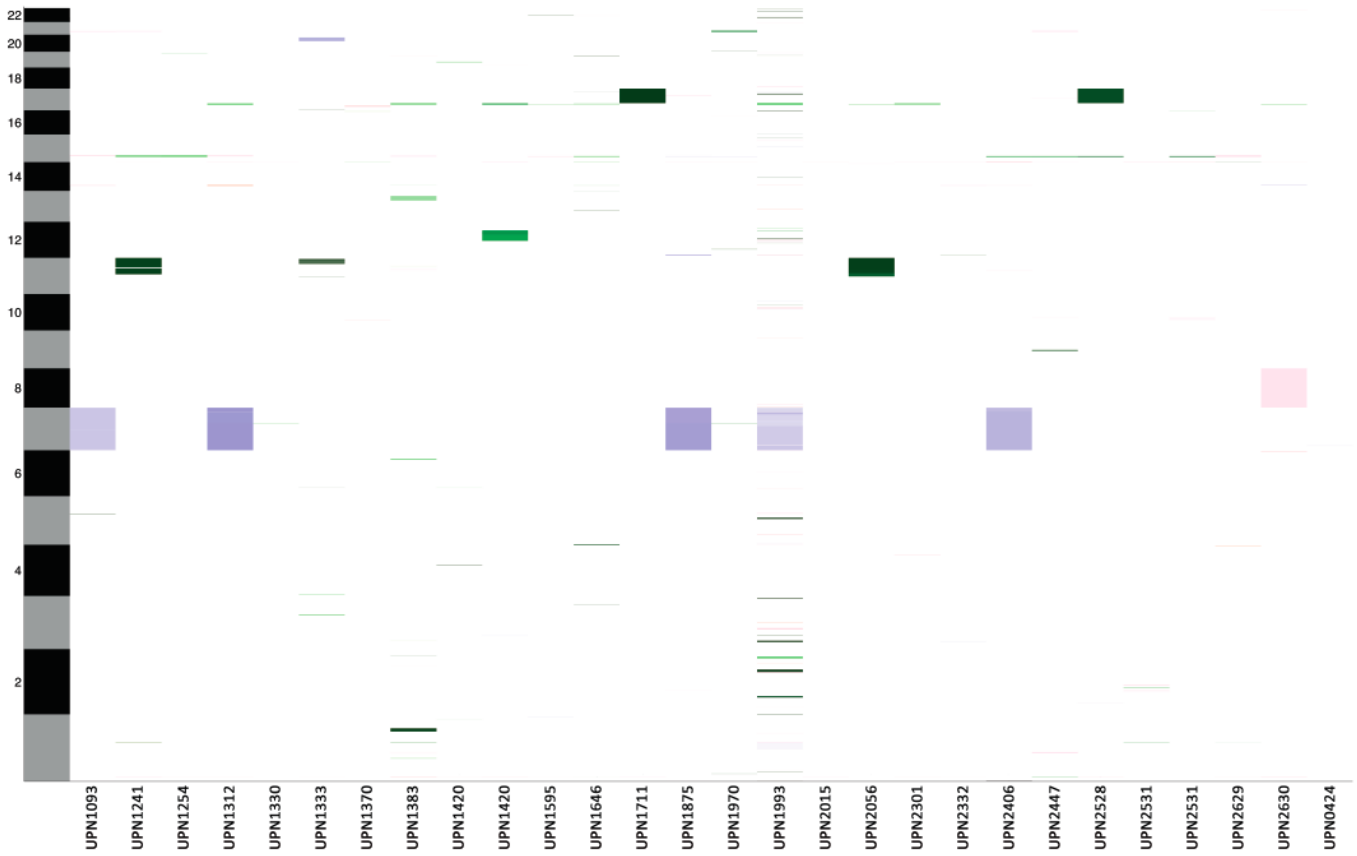
Supplementary Figure 1. Venn diagram demonstrating overlap of various assays. Patient samples were subjected to four assays including whole exome sequencing, targeted resequencing using a TruSeq Custom Amplicon (TCA) approach, genome wide DNA methylation analysis and RNA-seq. Numbers represent the patients subjected to each assay.



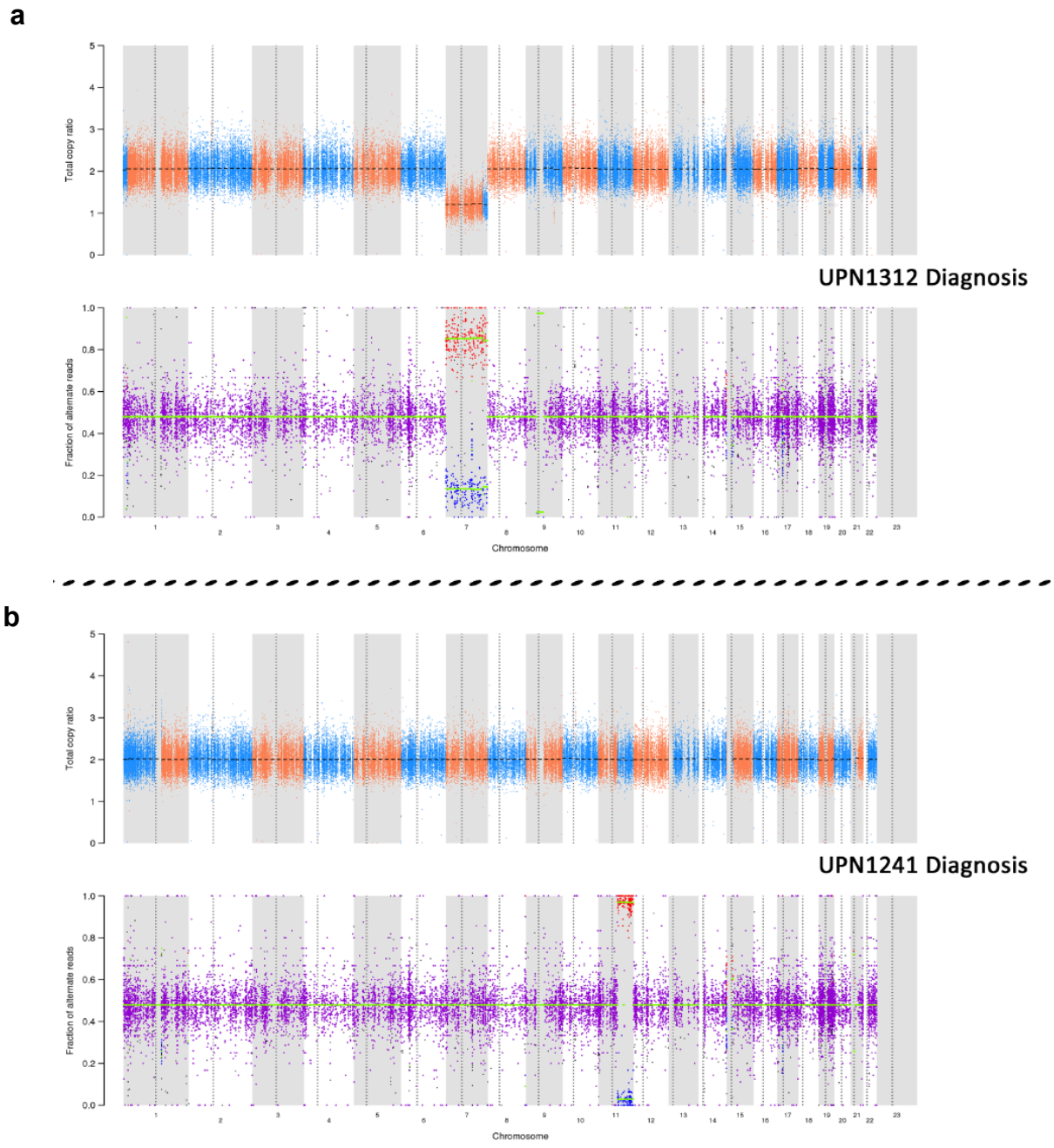
Supplementary Figure 2. Sanger sequencing from multiple germline tissue types in one patient. Patient UPN2447 had both a buccal swab and an EBV immortalized cell line available for analysis. Sequencing of the buccal swab implied germline inheritence of the *RRAS2* p.Q72L mutations. However, Sanger sequencing of the EBV cells revealed a wild type sequence indicating the buccal sample was contaminated with tumor and the mutation was in fact somatic in origin.



Supplementary Figure 3. Landscape plot of mutations detected from exome and targeted resequencing. Genes are plotted on the Y-axis with all 100 patients from the combined cohorts on the X-axis. All pathogenic mutations listed in supplemental tables 2 and 3 are depicted as a vertical bars. Mutations detected at diagnosis are in black and mutations only detected at relapse are in red. As noted in supplemental tables 2 and 3, mutations detected at diagnosis were present at relapse.



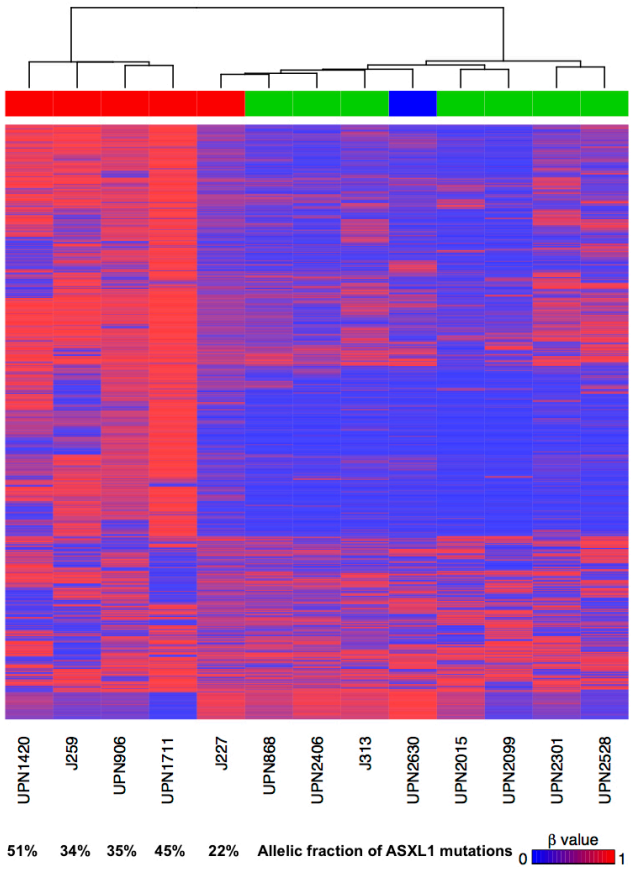
Supplementary Figure 4. Segmented allelic copy number heat map. Amplifications are depicted in red, deletions in blue and copy neutral loss of heterozygosity in green. Monosomy 7, copy neutral loss of 11q and 17q are the only recurring structural alterations.



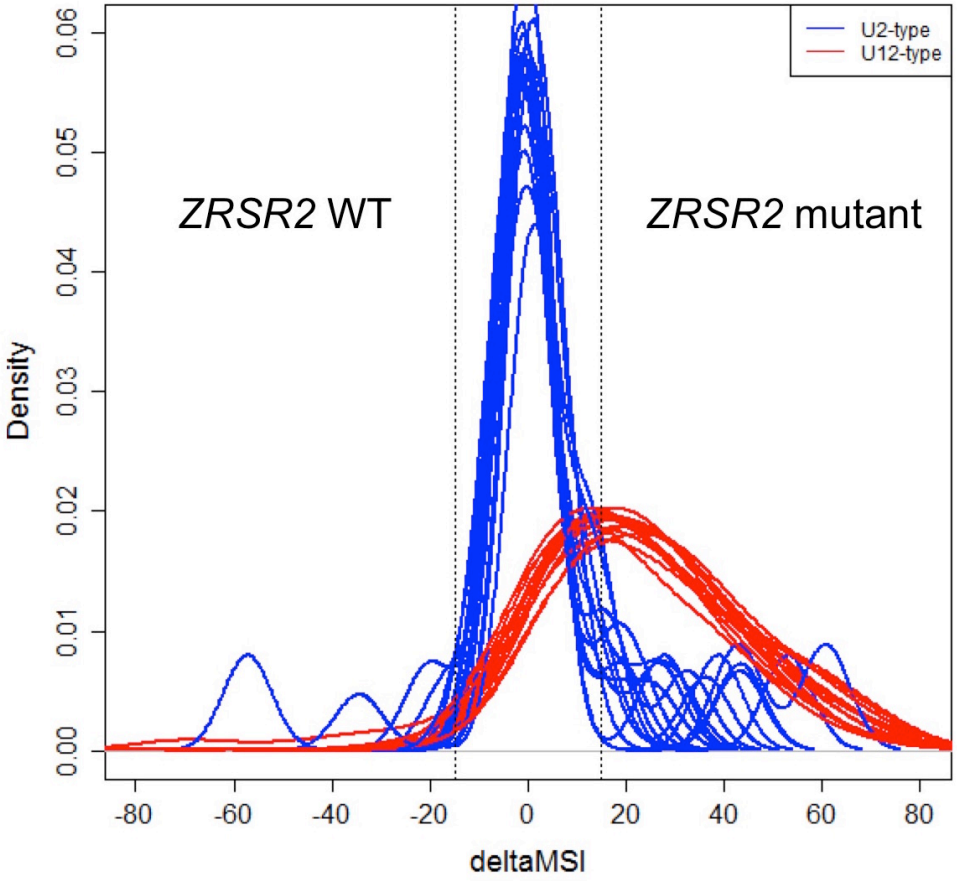
Supplementary Figure 5. Illustrative examples showing copy ratio and allelic balance in genomes with recurrent structural alterations. **(A)** Patient UPN1312 had monosomy 7 at diagnosis shown by matched lower copy ratio and allelic imbalance at chromosome 7. **(B)** Patient UPN1241 had cnLOH at chromosome 11 where *CBL* resides. This patient was born with a heterozygous *CBL* mutation that underwent uniparental isodisomy.

Samples included in methylation analysis:

	Sample ID	Mutations		
Subject	UPN0906	*PTPN11*	*ASXL1*	
Control	UPN0868	*PTPN11*		
Control	UPN2406	*PTPN11*		
Subject	J227	*NRAS*	*ASXL1*	
Control	UPN2099	*NRAS*		
Control	UPN2015	*NRAS*		
Subject	J259	*NRAS*	*SETBP1*	*ASXL1*
Control	UPN2301	*KRAS*	*SETBP1*	
Control	J313	*NRAS*	*SETBP1*	
Subject	UPN1711	*NF1*	*SETBP1*	*ASXL1*
Subject	UPN1420	*NF1*	*ASXL1*	
Control	UPN2528	*NF1*		
Subject	UPN2630	*RRAS*	*DNMT3A*	

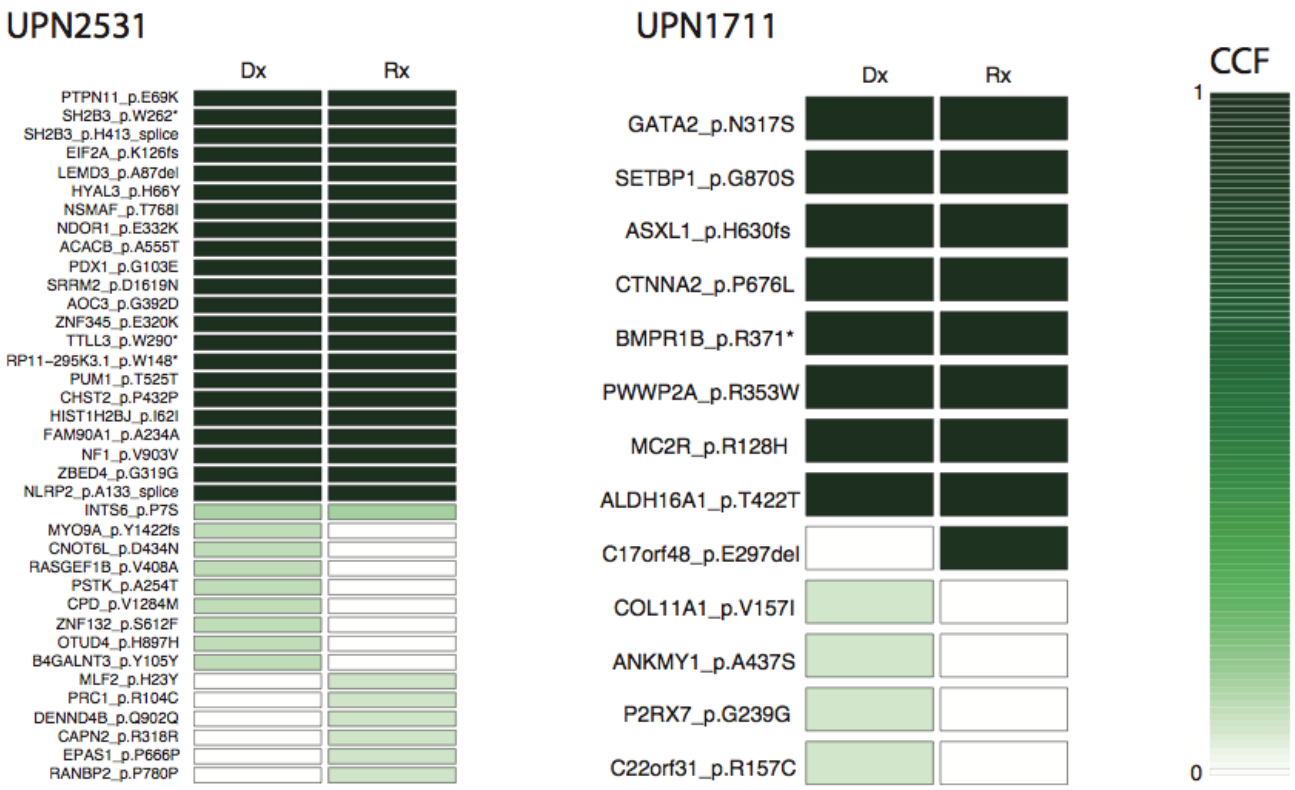


Supplementary Figure 6. Genome wide DNA methylation analysis in patients with *ASXL1* mutations. Four subjects with *ASXL1* mutations (red) and appropriate controls (green) were analyzed on the Illumina 450k Bead Chip platform. One patient with a *DNMT3A* mutation (blue) was included for comparison.

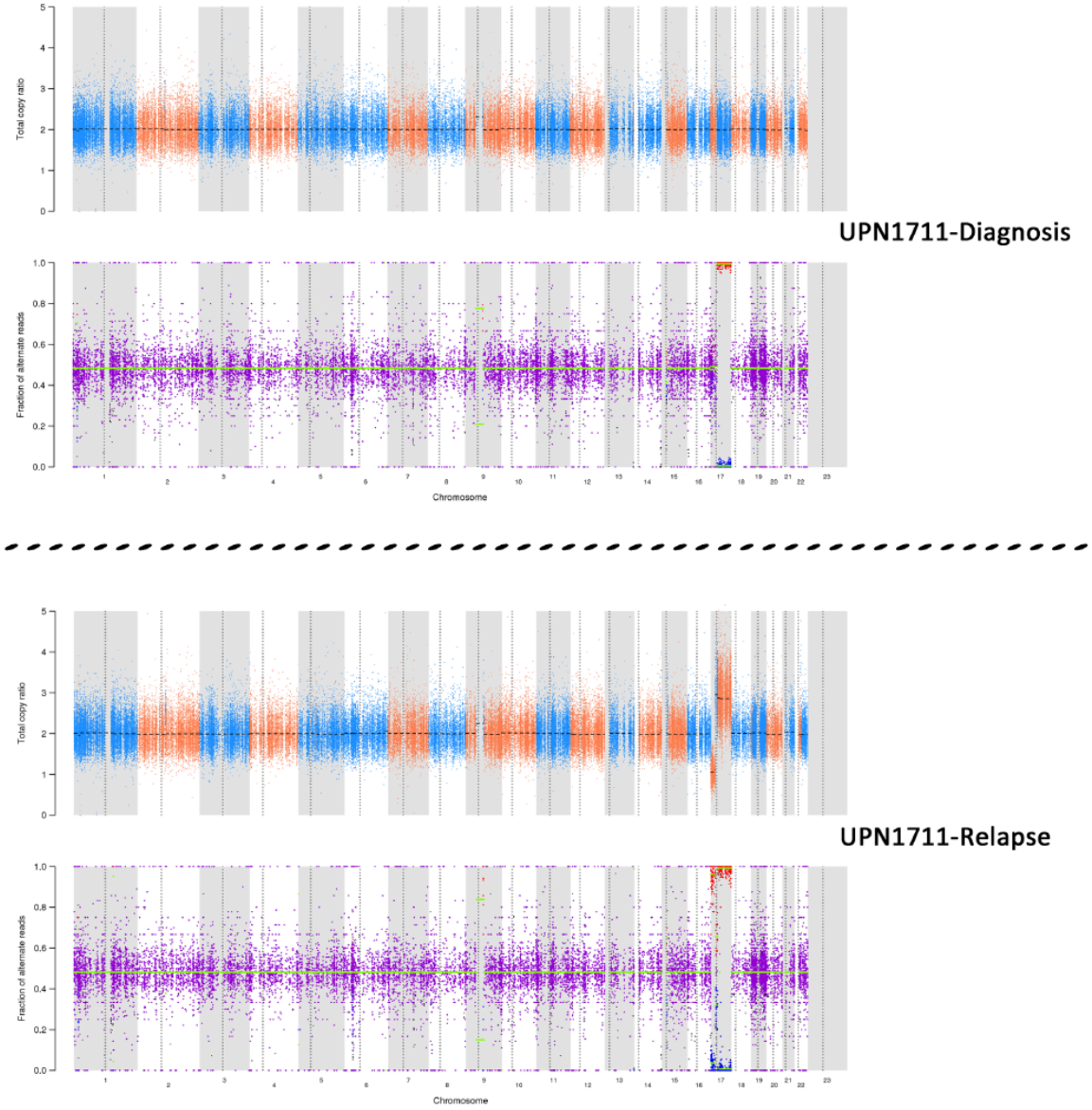


Supplementary Figure 7. U12-type intron retention in *ZRSR2* mutated JMML. Density plot showing ΔMSI distribution from comparisons of *ZRSR2* mutant versus each of the 14 *ZRSR2* WT (wild type) patients. Each curve represents a comparison for U12-type (red) and U2-type (blue) intron. There is increased retention of a large majority of U12-type introns in the *ZRSR2* mutated sample. Dotted vertical lines represent ΔMSI cutoff of 15 or -15.

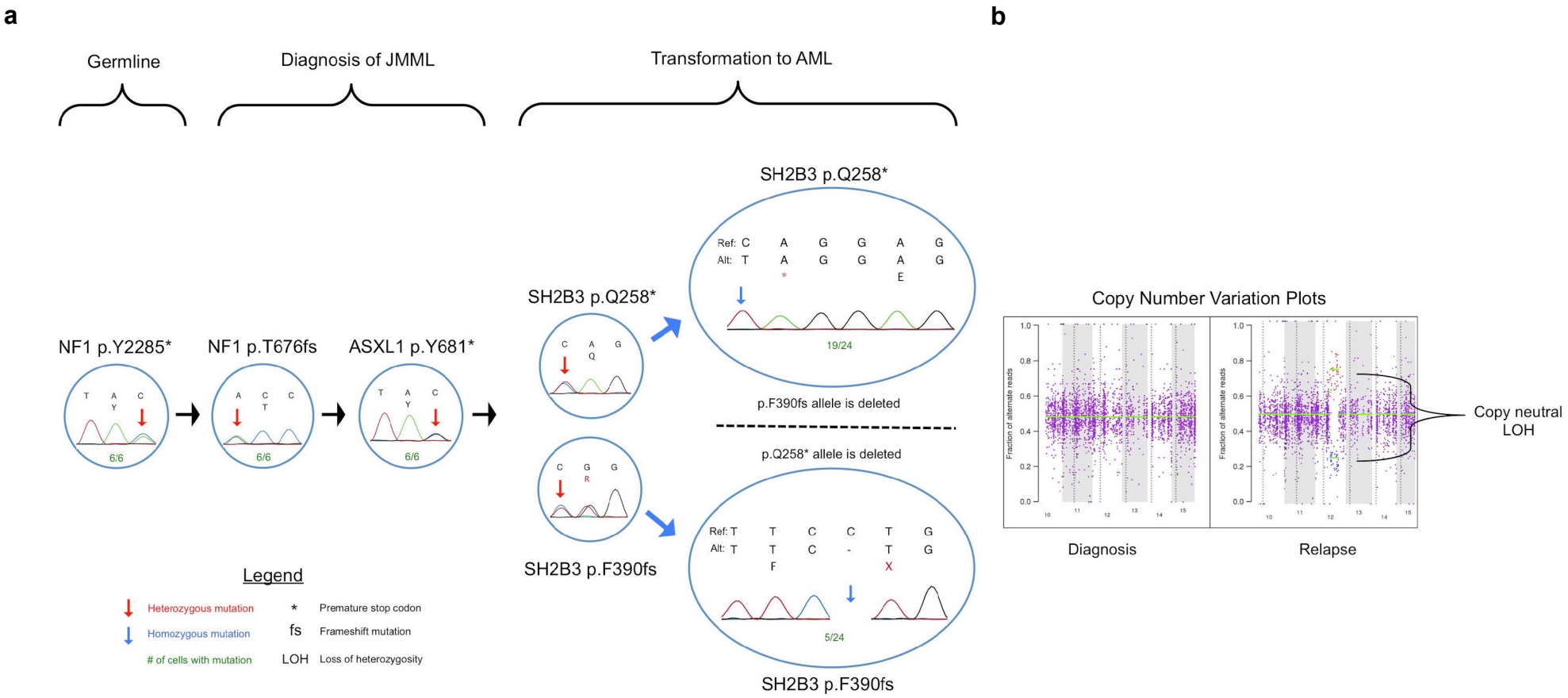
a


UPN2531
Dx
Rx
PTPN11_p.E69K
SH2B3_p.W262*
SH2B3_p.H413_splice
EIF2A_p.K126fs
LEMD3_p.A87del
HYAL3_p.H66Y
NSMAF_p.T768I
NDOR1_p.E332K
ACACB_p.A555T
PDX1_p.G103E
SRRM2_p.D1619N
AOC3_p.G392D
ZNF345_p.E320K
TTLL3_p.W290*
RP11−295K3.1_p.W148*
PUM1_p.T525T
CHST2_p.P432P
HIST1H2BJ_p.I62I
FAM90A1_p.A234A
NF1_p.V903V
ZBED4_p.G319G
NLRP2_p.A133_splice
INTS6_p.P7S
MYO9A_p.Y1422fs
CNOT6L_p.D434N
RASGEF1B_p.V408A
PSTK_p.A254T
CPD_p.V1284M
ZNF132_p.S612F
OTUD4_p.H897H
B4GALNT3_p.Y105Y
MLF2_p.H23Y
PRC1_p.R104C
DENND4B_p.Q902Q
CAPN2_p.R318R
EPAS1_p.P666P
RANBP2_p.P780P
UPN1711
Dx
Rx
GATA2_p.N317S
SETBP1_p.G870S
ASXL1_p.H630fs
CTNNA2_p.P676L
BMPR1B_p.R371*
PWWP2A_p.R353W
MC2R_p.R128H
ALDH16A1_p.T422T
C17orf48_p.E297del
COL11A1_p.V157I
ANKMY1_p.A437S
P2RX7_p.G239G
C22orf31_p.R157C
CCF
1
0

b

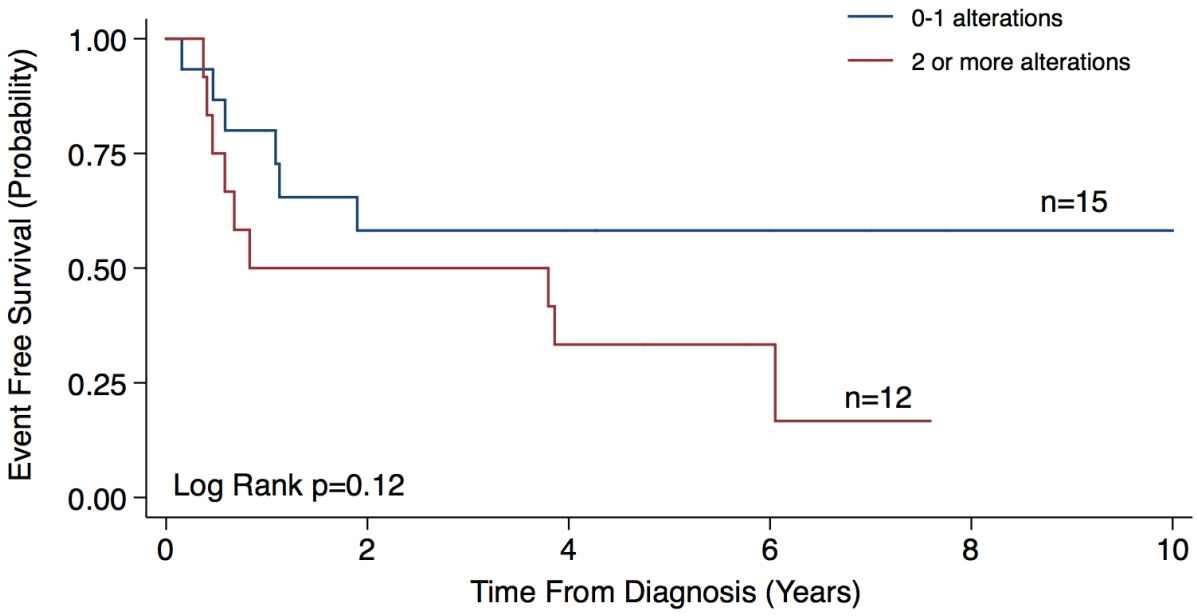


Supplementary Figure 8. Evidence of a linear model of disease progression via exome sequencing. **(A)** For each mutation we used ABSOLUTE to computationally estimate the fraction of cancer cells or cancer cell fraction (CCF) that harbor a given somatic variant. Shown are the CCFs for each somatic variant in both respective samples with diagnosis on the left and relapse on the right. The CCF for all putative driver events detected approach 100% at relapse, implying that these mutations are present in the same dominant clone. **(B)** Patient UPN1711 had two copy number events, one cnLOH at diagnosis and one unbalanced amplification/deletion at relapse.

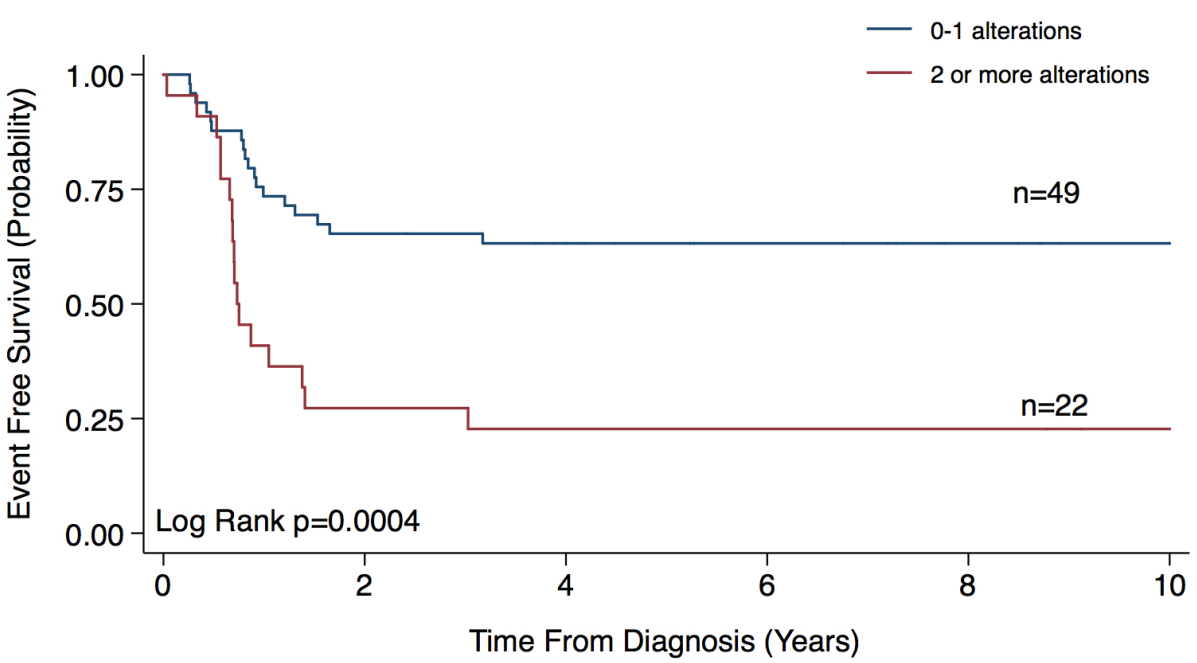


Supplementary Figure 9. Single colony analysis demonstrated progression of disease in UPN1420. **(A)** At diagnosis of JMML, UPN1420 possessed compound heterozygous mutations in NF1 as well as ASXL1 in all six single colonies analyzed. Upon transformation to AML, the patient acquired compound heterozygous mutations in SH2B3 as detected in unsorted mononuclear cells. Analysis of single colonies at relapse demonstrated three patterns: (1) colonies with heterozygous SH2B3 lesions, (2) colonies homozygous for SH2B3 p.Q258* mutation and (3) colonies homozygous for SH2B3 p.F390fs mutation. The colony containing compound heterozygous mutations was therefore first to arise. **(B)** Copy number data comparing the diagnostic and relapsed timepoints established that a copy number alteration led to two distinct clones arising with colonies homozygous for each of the respective SH2B3 lesions. .

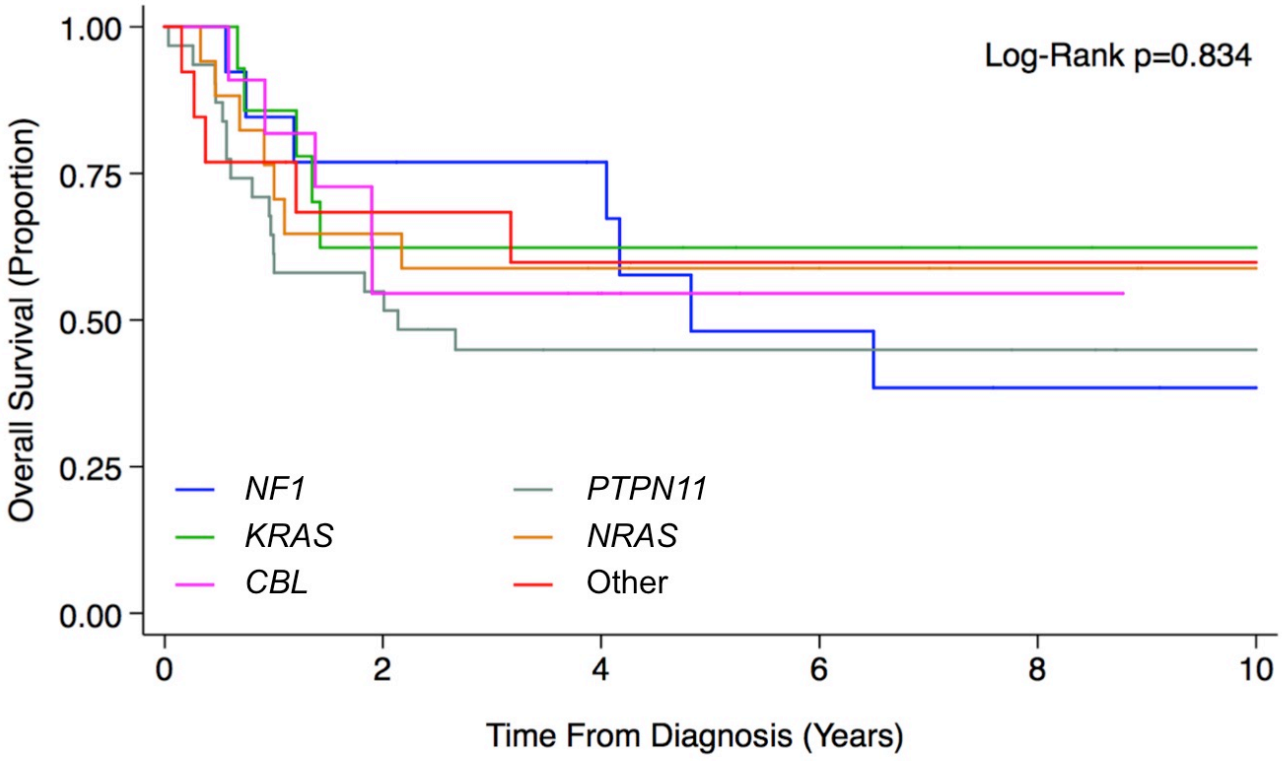
a



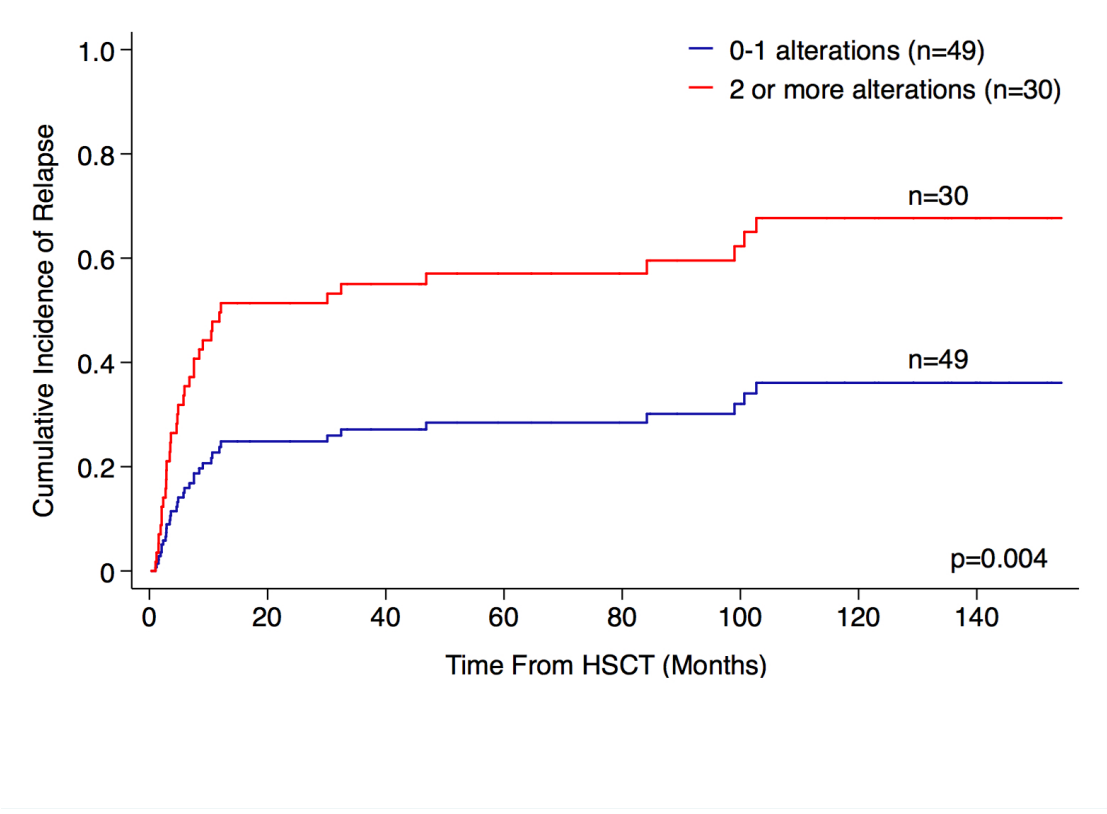
b



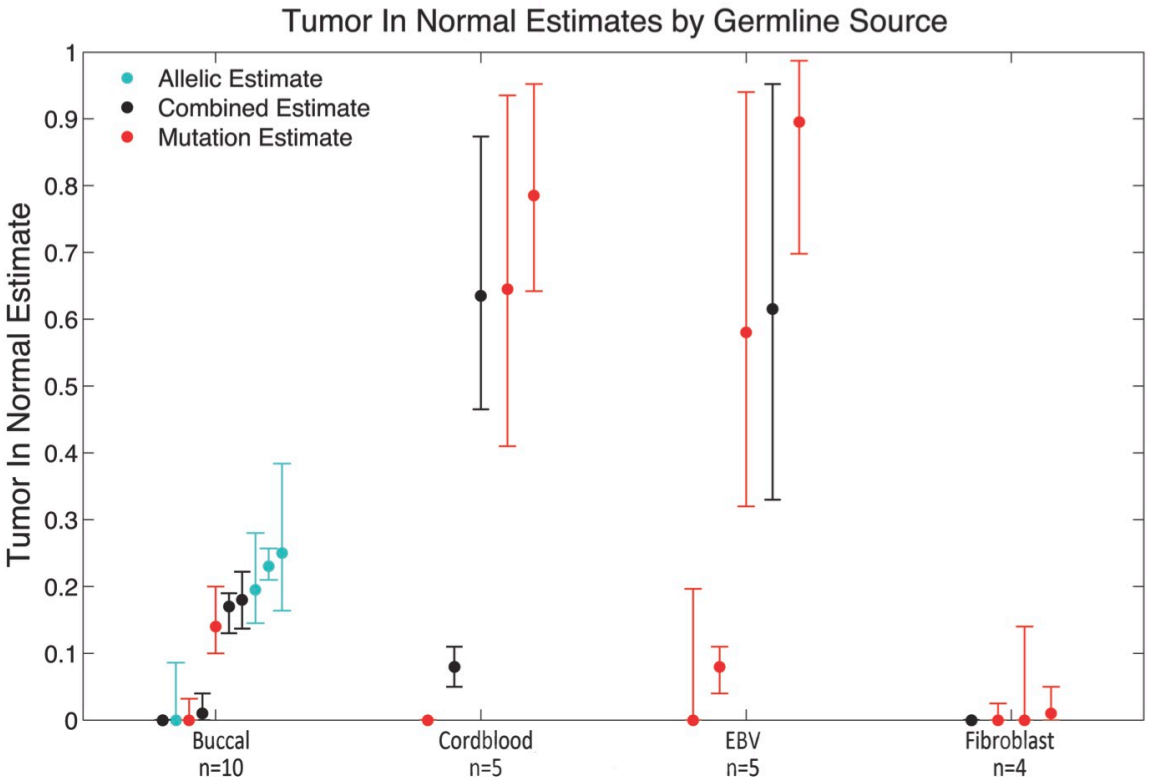
Supplementary Figure 10. Event free survival (EFS) based on number of somatic events. **(A)** EFS is displayed for all 27 patients in the exome cohort (two patients with Noonan's syndrome were excluded) **(B)** EFS is displayed for 71 distinct patients from our validation cohort.



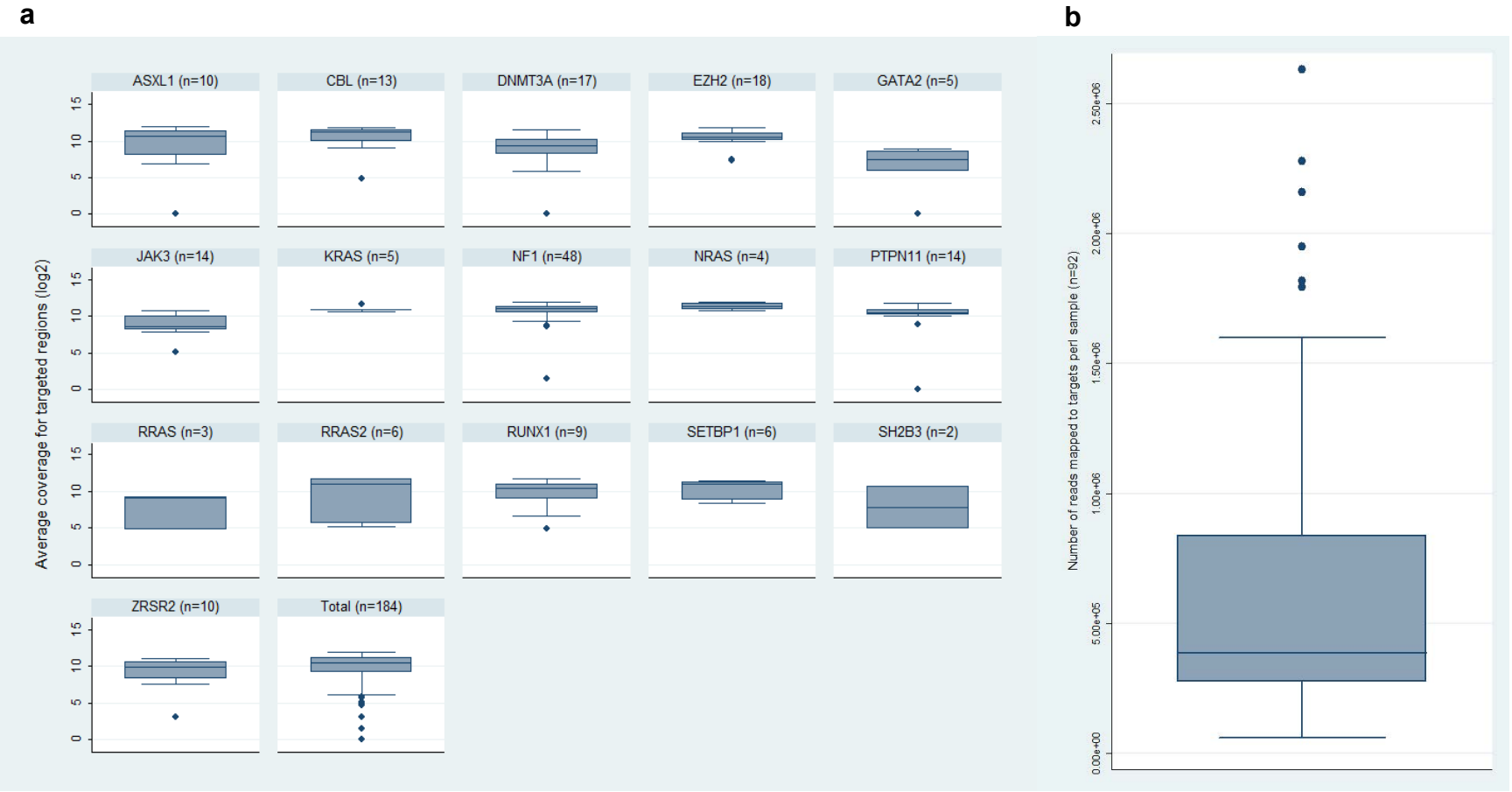
Supplementary Figure 11. Overall Survival (OS) based on canonical mutation. Individual canonical driver mutations were not associated with outcome (Log-rank p=0.834).



Supplementary Figure 12. Cumulative incidence of relapse (RI) based on mutation status. Data displayed reflects the number of months from hematopoietic stem cell transplant according to number of somatic alterations at diagnosis (log-rank p=0.004).

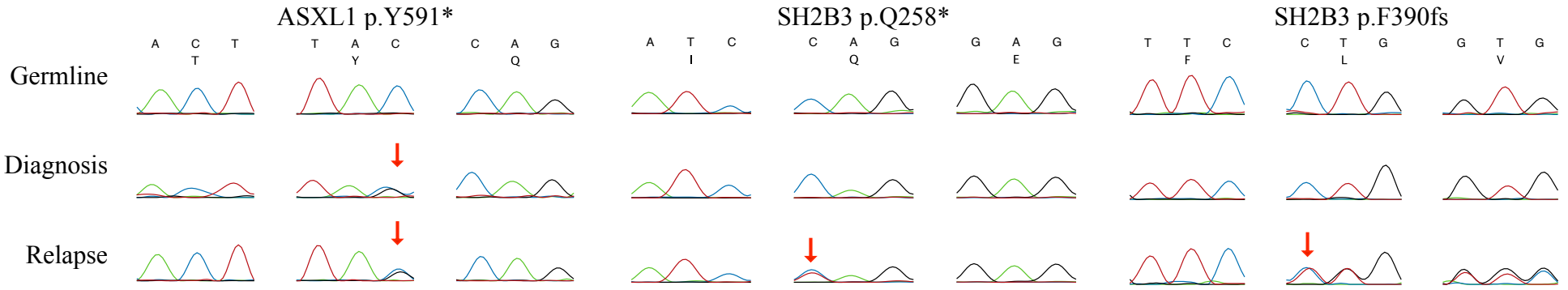


Supplementary Figure 13. Tumor in normal content (TiN) by germline tissue source. Each patient is represented by a single estimate of TiN using the diagnostic tumor sample. Germline samples are grouped by tissue type on the x-axis and TiN content is plotted on the y-axis, with 95% confidence intervals displayed on each estimate. TiN content was estimated by detecting somatic mutation estimates (shown in red) or allelic imbalances (shown in blue) in the putative germline material. A combined estimate
(shown in black) is displayed if a germline sample had evidence of both a somatic mutation and an allelic imbalance (Supplementary Table 10).

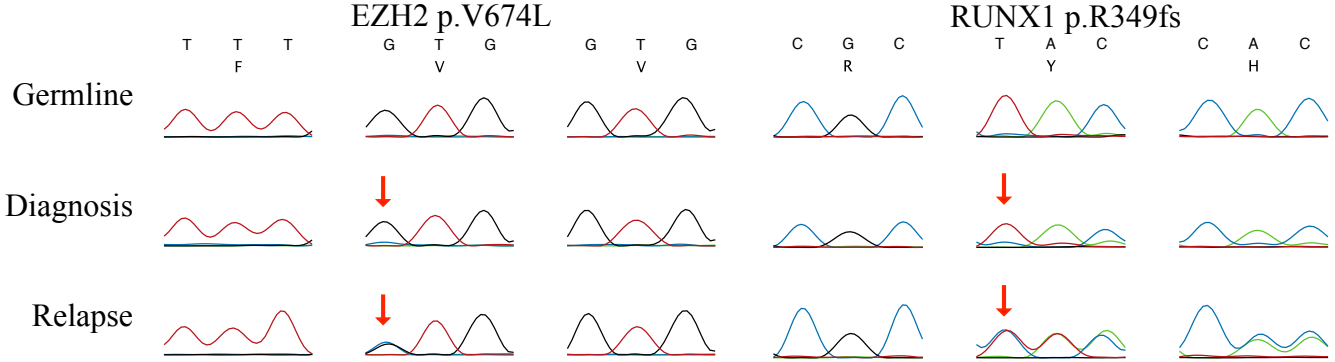


Supplementary Figure 14. Targeted resequencing coverage and mapped reads**.** Ninety-two samples were sequenced for 16 genes. **(A)** Average coverage for each gene is depicted on a log2 scale. **(B)** Number of reads mapped to targets per sample.

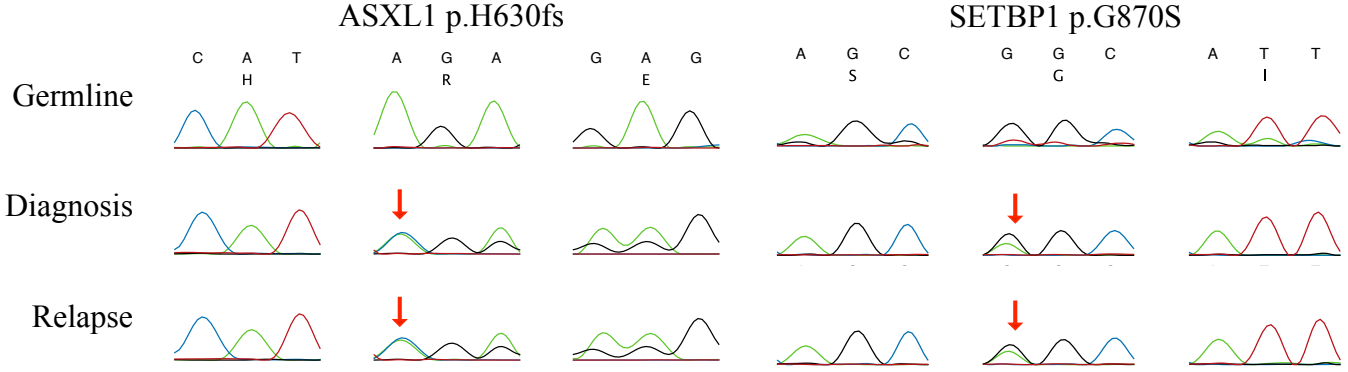
UPN1420



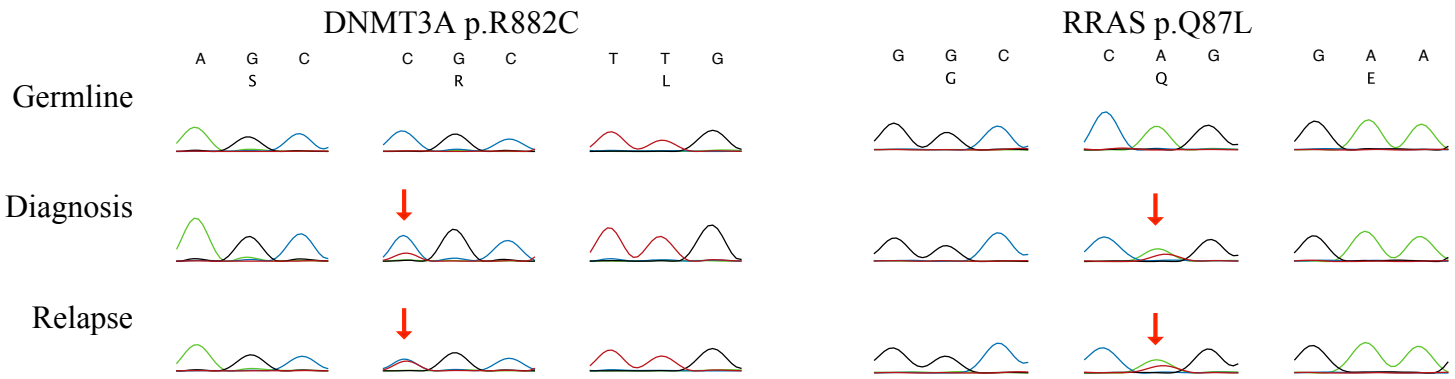
UPN1993



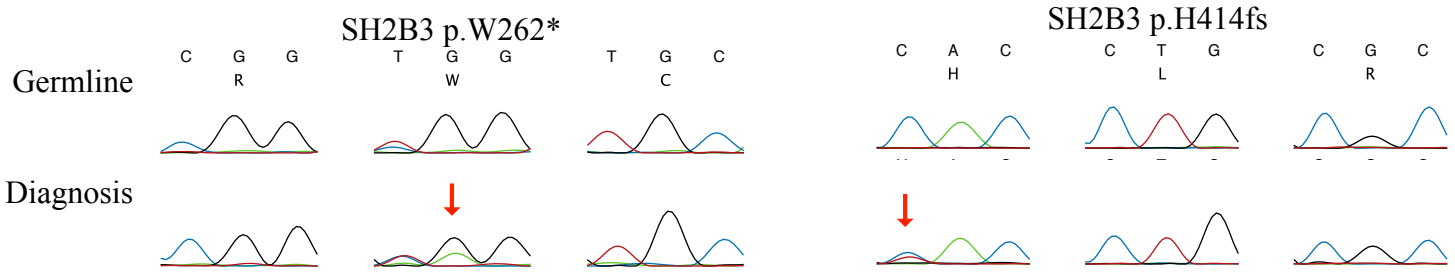
UPN1711



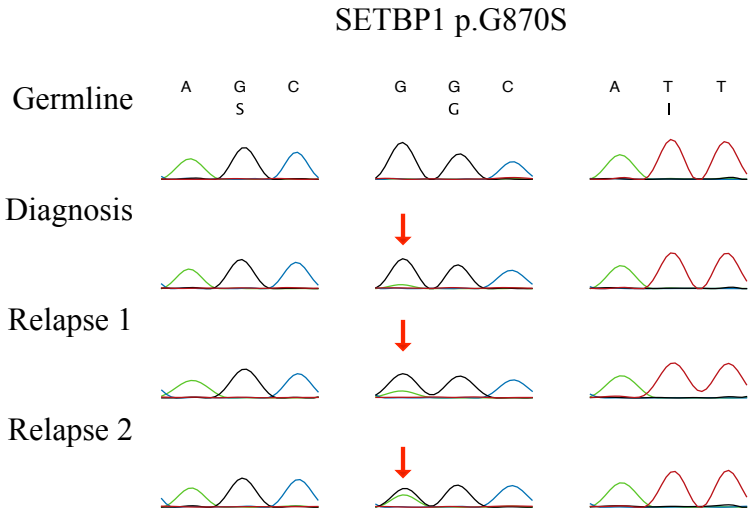
UPN2630



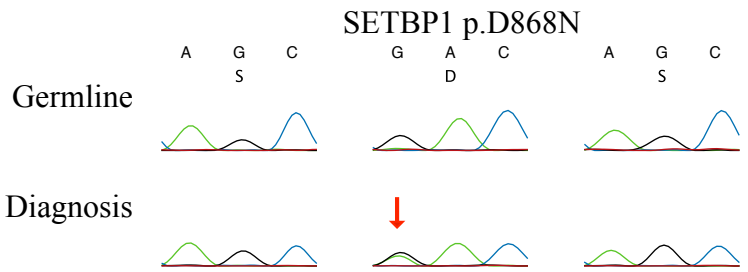
UPN2531



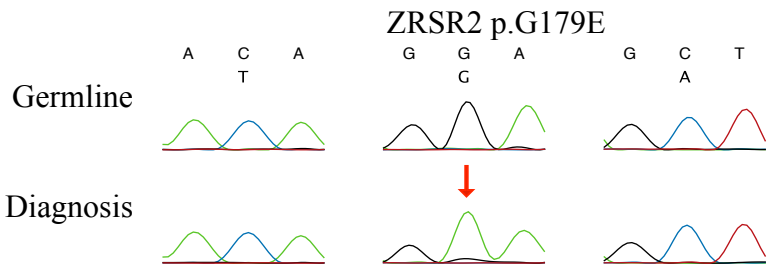
UPN1383



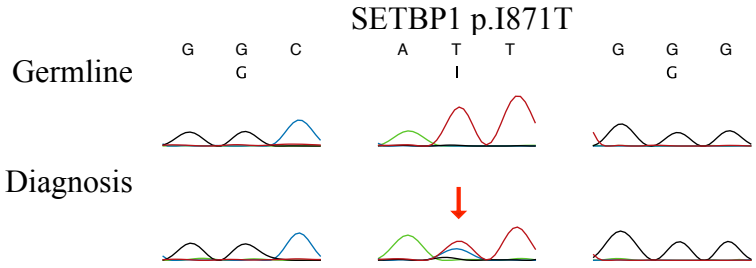
UPN2301



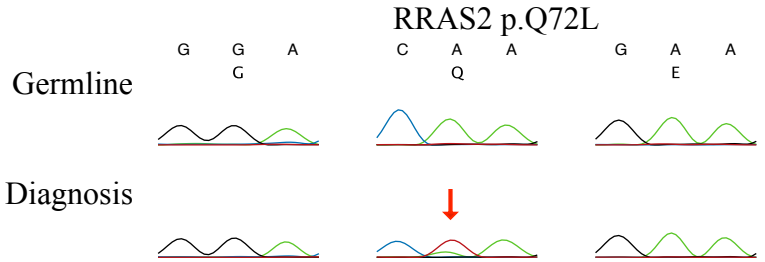
UPN1646



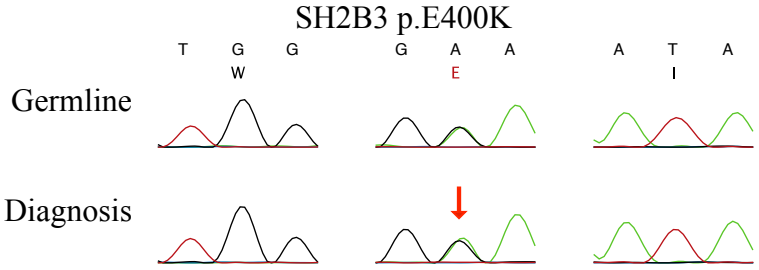
UPN1875



UPN2447



UPN1970



Supplementary Figure 15. Validation of exome findings via Sanger sequencing. Each mutation was validated by Sanger sequencing of germline, diagnostic and relapse samples when available.

Supplementary Table 1. Whole exome sequencing coverage.

Patient	Time point	Tissue	Mean Coverage	Bases > 10x	Bases > 20x	Reads Aligned	Total Reads
UPN0424	Diagnosis	Bone marrow	86.2	93%	88%	91%	85581456
UPN0424	Germline	Cord blood	104.9	93%	89%	91%	114393386
UPN1093	Diagnosis	Blood	101.3	93%	89%	92%	104037884
UPN1093	Germline	Cord blood	100.4	93%	89%	92%	104439430
UPN1125	Diagnosis	Blood	106.8	94%	90%	92%	101492080
UPN1125	Relapse	Blood	115.4	94%	91%	92%	134265566
UPN0969	Diagnosis	Blood	89.2	93%	87%	91%	92727402
UPN0969	Germline	Buccal	94.9	93%	88%	91%	101177836
UPN0969	Relapse	Blood	78.5	92%	86%	91%	80522016
UPN1241	Germline	Cord blood	93.9	94%	90%	91%	106713182
UPN1254	Diagnosis	Blood	82.5	92%	87%	91%	84966374
UPN1254	Germline	Buccal	66.5	91%	84%	91%	69912740
UPN1312	Diagnosis	Bone marrow	93.5	93%	88%	92%	94211390
UPN1312	Germline	EBV	74.8	92%	85%	91%	74232218
UPN1330	Germline	EBV	106.2	94%	88%	91%	108088672
UPN1330	Diagnosis	Bone marrow	78.9	92%	87%	92%	78230552
UPN1333	Relapse	Blood	92.2	93%	88%	92%	84049626
UPN1333	Germline	Buccal	40.8	83%	69%	82%	78519716
UPN1370	Diagnosis	Blood	124.1	95%	91%	92%	140490158
UPN1370	Germline	Buccal	49.5	92%	84%	90%	86205028
UPN1383	Diagnosis	Bone marrow	77.7	92%	86%	92%	79915454
UPN1383	Germline	EBV	22.0	78%	49%	82%	80153106
UPN1383	Relapse	Blood	169.2	95%	93%	91%	193077954
UPN1420	Germline	Buccal	72.1	91%	85%	92%	72076496
UPN1420	Diagnosis	Bone marrow	79.1	92%	86%	91%	81360698
UPN1420	Relapse	Bone marrow	87.8	93%	88%	91%	89176740
UPN1595	Diagnosis	Blood	127.8	95%	91%	91%	136397096
UPN1595	cordblood	Blood	79.4	92%	86%	92%	85592194
UPN1646	Diagnosis	Blood	78.9	93%	88%	92%	85475120
UPN1646	Germline	Buccal	88.0	83%	70%	90%	94052612
UPN1711	Diagnosis	Bone marrow	107.3	94%	90%	92%	109819352
UPN1711	Relapse	Bone marrow	108.7	93%	89%	92%	114766492
UPN1711	Germline	Fibroblast	177.2	96%	93%	92%	281794224
UPN1875	Diagnosis	Blood	95.5	94%	89%	92%	101462930
UPN1875	Germline	Buccal	52.3	91%	84%	91%	83773946

Supplementary Table 1. continued

Patient	Time point	Tissue	Mean Coverage	Bases > 10x	Bases > 20x	Reads Aligned	Total Reads
UPN1970	Diagnosis	Blood	73.1	93%	88%	89%	104665668
UPN1970	Germline	Buccal	70.3	92%	85%	91%	76427786
UPN1974	Diagnosis	Blood	93.3	94%	90%	92%	105527206
UPN1974	Germline	Buccal	70.6	92%	86%	91%	70385390
UPN1993	Diagnosis	Blood	78.8	92%	85%	92%	81559166
UPN1993	Relapse	Bone marrow	40.6	87%	72%	92%	35105070
UPN1993	Germline	Buccal	135.7	94%	90%	92%	138958392
UPN2015	Diagnosis	Blood	73.9	92%	86%	92%	74123848
UPN2015	cordblood	Blood	35.3	88%	74%	90%	81367344
UPN2056	Diagnosis	Blood	106.8	94%	90%	91%	140418478
UPN2056	Germline	Buccal	130.2	94%	90%	90%	143806736
UPN2301	Diagnosis	Bone marrow	89.3	93%	88%	92%	87875804
UPN2301	Germline	EBV	81.8	92%	87%	92%	84483486
UPN2332	Diagnosis	Blood	90.7	93%	88%	91%	93135036
UPN2332	Germline	EBV	85.9	93%	87%	92%	86434600
UPN2406	Diagnosis	Bone marrow	94.7	93%	89%	92%	100087240
UPN2406	Germline	Buccal	74.1	93%	87%	88%	105122746
UPN2447	Diagnosis	Bone marrow	100.9	93%	88%	92%	90256144
UPN2447	Germline	Buccal	85.4	92%	86%	92%	75665362
UPN2528	Diagnosis	Blood	100.5	94%	89%	91%	147121284
UPN2528	Germline	Buccal	100.8	94%	89%	90%	106305846
UPN2531	Diagnosis	Bone marrow	116.7	95%	91%	91%	141181506
UPN2531	Germline	Fibroblast	137.8	95%	92%	92%	140458118
UPN2531	Relapse	Bone marrow	103.5	94%	90%	92%	112593940
UPN2629	Germline	Fibroblast	125.3	94%	90%	92%	127146016
UPN2629	Diagnosis	Bone marrow	140.9	95%	91%	92%	145427856
UPN2630	Germline	Fibroblast	188.8	96%	93%	91%	278772638
UPN2630	Diagnosis	Blood	162.1	97%	94%	90%	337688368

Supplementary Table 2. Whole exome sequencing mutations.

Patient	Gene	Chr.	Position	Protein Change	Nucleotide Change	Germline Alternate Reads	Germline Reference Reads	Diagnosis Alternate Reads	Diagnosis Reference Reads	Relapse Alternate Reads	Relapse Reference Reads
UPN0424	PTPN11	12	112888198	p.A72T	c.214G>A	73	109	59	57		
UPN0969	NRAS	1	115258747	p.G12V	c.35G>T	0	149	51	78	32	62
UPN0969	NF1	17	29661956	p.T1972fs	c.5913_5914insA	0	93	85	11	62	5
UPN1093	NF1	17	29586048	p.R416	c.e33	12	8	9	5		
UPN1093	NF1	17	29528489	p.R440*	c.1246C>T	3	158	43	146		
UPN1125	CBL	11	119148891	p.Y371H	c.1111T>C	0	2	54	3	76	5
UPN1241	CBL	11	119148891	p.Y371H	c.1111T>C	26	26	44	5		
UPN1254	NF1	17	29533315	p.R440*	c.1318C>T	40	44	56	68		
UPN1312	KRAS	12	25398284	p.G12D	c.35G>A	7	19	8	11		
UPN1330	NRAS	1	115258748	p.G12S	c.34G>A	58	83	40	48		
UPN1333	CBL	11	119148891	p.Y371H	c.1111T>C	3	4	29	10		
UPN1370	NRAS	1	115258747	p.G12V	c.35G>T	0	51	93	106		
UPN1383	NF1	17	29546175	p.R681*	c.2041C>T	5	0	33	23	52	60
UPN1383	SETBP1	18	42531913	p.G870S	c.2608G>A	0	12	4	33	40	55
UPN1420	NF1	17	29553477	p.T676fs	c.2026insC	4	49	33	55	24	63
UPN1420	NF1	17	29665757	p.Y2285*	c.6855C>A	40	46	29	57	46	58
UPN1420	ASXL1	20	31022288	p.Y591*	c.1773C>G	5	65	46	44	47	52
UPN1420	SH2B3	12	111884596	p.Q258*	c.772C>T	0	19	0	36	5	20
UPN1420	SH2B3	12	111885279	p.F390fs	c.1170delC	0	110	0	95	50	68
UPN1595	NRAS	1	115258744	p.G13D	c.38G>A	25	85	80	145		
UPN1646	NRAS	1	115258747	p.G12D	c.35G>A	11	66	43	55		
UPN1646	ZRSR2	X	15827420	p.G179E	c.536G>A	2	16	38	4		
UPN1711	NF1	17	29527569	p.N339fs	c.1015delCT	100	122	120	1	181	5

Supplementary Table 2, continued

Patient	Gene	Chr.	Position	Protein Change	Nucleotide Change	Germline Alternate Reads	Germline Reference Reads	Diagnosis Alternate Reads	Diagnosis Reference Reads	Relapse Alternate Reads	Relapse Reference Reads
UPN1711	SETBP1	18	42531913	p.G870S	c.2608G>A	0	84	36	28	23	24
UPN1711	ASXL1	20	31022403	p.H630fs	c.1888_1910del	0	55	24	29	24	34
UPN1711	GATA2	3	128202770	p.N317S	c.950A>G	0	122	48	35	32	36
UPN1875	PTPN11	12	112888210	p.E76K	c.226G>A	0	74	52	97		
UPN1875	SETBP1	18	42531917	p.I871T	c.2612T>C	0	29	13	25		
UPN1970	SH2B3	12	111885310	p.E400K	c.1198G>A	30	42	31	43		
UPN1974	None										
UPN1993	NF1	17	29562746	p.R1276*	c.3826C>T	11	337	39	78	72	173
UPN1993	NF1	17	29533330	p.E445*	c.1333G>T	119	146	28	36	63	64
UPN1993	EZH2	7	148506477	p.V674L	c.2035G>C	11	168	18	83	26	11
UPN1993	RUNX1	21	36164746	p.R349fs	c.1047_1048insC	0	8	0	9	3	5
UPN2015	NRAS	1	115258748	p.G12S	c.34G>A	11	31	40	60		
UPN2056	CBL	11	119148532	p.Q358fs	c.1076_1087del12	2	88	15	1		
UPN2301	KRAS	12	25398281	p.G13D	c.38G>A	1	23	7	29		
UPN2301	SETBP1	18	42531907	p.D868N	c.2602G>A	9	40	8	20		
UPN2332	NRAS	1	115258748	p.G12S	c.34G>A	46	68	50	64		
UPN2406	PTPN11	12	112926885	p.S502L	c.1505C>T	80	77	101	110		
UPN2447	RRAS2	11	14316390	p.Q72L	c.233A>T	2	98	31	44		
UPN2528	NF1	17	29553477	p.T676fs	c.2026_2027insC	8	79	54	65		
UPN2531	PTPN11	12	112888189	p.E69K	c.205G>A	0	197	62	97	55	78
UPN2531	SH2B3	12	111884609	p.W262*	c.785G>A	0	61	15	28	16	22

Supplementary Table 2, continued

Patient	Gene	Chr.	Position	Protein Change	Nucleotide Change	Germline Alternate Reads	Germline Reference Reads	Diagnosis Alternate Reads	Diagnosis Reference Reads	Relapse Alternate Reads	Relapse Reference Reads
UPN2531	SH2B3	12	111885460	p.H414fs	c.1236_splice	0	87	34	50	28	49
UPN2629	KRAS	12	25398284	p.G12V	c.35G>T	1	158	21	102		
UPN2630	RRAS	19	50140165	p.Q87L	c.260A>T	1	108	42	43		
UPN2630	DNMT3A	2	25457243	p.R882C	c.2644C>T	0	167	29	96		

Supplementary Table 3. Mutations detected on targeted resequencing.

Patient	Gene	Chromsome	Position	Protein Change	Nucleotide Change	Diagnosis Alternate Reads	Diagnosis Reference Reads	Relapse Alternate Reads	Relapse Reference Reads
UPN0585	NRAS	chr1	115258744	p.G13D	c.G38A	355	769		
UPN0585	SETBP1	chr18	42531907	p.D868N	c.G2602A	229	476		
UPN0868	PTPN11	chr12	112888210	p.E76K	c.G226A	444	1141		
UPN0906	PTPN11	chr12	112888210	p.E76K	c.G226A	42	32		
UPN0906	ASXL1	chr20	31022694	p.E727*	c.G2179T	522	976		
UPN0969	NRAS	chr1	115258747	p.G12V	c.G35T			1744	2391
UPN0969	NF1	chr17	29661957	p.T1972fs	c.5914insA			3772	132
UPN0969	JAK3	chr19	17945970	p.R657W	c.C1969T			24	256
UPN1043	PTPN11	chr12	112888210	p.E76K	c.G226A	211	249	187	187
UPN1142	PTPN11	chr12	112888210	p.E76K	c.G226A	538	901		
UPN1188	NRAS	chr1	115258744	p.G13D	c.G38A	386	423		
UPN1199	KRAS	chr12	25398284	p.G12D	c.G35A	205	190		
UPN1254	NF1	chr17	29533315	p.R440*	c.C1318T	1101	1024		
UPN1348	KRAS	chr12	25380276	p.Q61L	c.A182T	601	1332		
UPN1420	NF1	chr17	29553478	pT676fs	c.2027insC			50	85
UPN1420	NF1	chr17	29665757	p.Y2285*	c.C6855A			1766	1760
UPN1420	ASXL1	chr20	31022288	p.Y591*	c.C1773G			925	1068
UPN1420	SH2B3	chr12	111885279	p.L390fs	c.1168delC			968	1033
UPN1420	SH2B3	chr12	111884596	p.Q258*	c.C772T			1006	1261
UPN1447	PTPN11	chr12	112888210	p.E76K	c.G226A	308	712	145	65
UPN1447	DNMT3A	chr2	25463562	p.G707fs	c.2119insG			11	58
UPN1484	NF1	chr17	29553478	p.T676fs	c.2027insC	82	82		
UPN1484	NF1	chr17	29588751	p.R1534*	c.C4600T	97	1595		

Supplementary Table 3, continued

Patient	Gene	Chromsome	Position	Protein Change	Nucleotide Change	Diagnosis Alternate Reads	Diagnosis Reference Reads	Relapse Alternate Reads	Relapse Reference Reads
UPN1484	PTPN11	chr12	112888162	p.G60R	c.G178C	613	645		
UPN1597	NRAS	chr1	115256536	p.A59T	c.G175A			58	414
UPN1597	PTPN11	chr12	112888210	p.E76K	c.G226A	344	466	141	167
UPN1597	DNMT3A	chr2	25458620	p.F851fs	c.2553delC			2	15
UPN1711	NF1	chr17	29527568	p.N339fs	c.1017_1018delCT			2771	88
UPN1711	SETBP1	chr18	42531913	p.G870S	c.G2608A			1064	877
UPN1711	ASXL1	20	31022403	p.H630fs	c.1888_1910del			5	6
UPN1711	GATA2	chr3	128202770	p.N317S	c.A950G			110	235
UPN1740	NRAS	chr1	115256530	p.Q61K	c.C181A	399	536		
UPN1778	CBL	chr11	119148891	p.Y371N	c.T1111A	623	62		
UPN1941	PTPN11	chr12	112888210	p.E76K	c.G226A	222	298		
UPN1970	SH2B3	chr12	111885310	p.E400K	c.G1198A	183	245		
UPN1974	None								
UPN1993	NF1	chr17	29562746	p.R1276*	c.C3826T			325	1940
UPN1993	NF1	chr17	29533330	p.E445*	c.G1333T			464	2340
UPN1993	EZH2	chr7	148506477	p.V674L	c.G2020C			846	2233
UPN1993	RUNX1	chr21	36164747	p.R349fs	c.1046insC			11	33
UPN2025	CBL	chr11	119148891	p.Y371H	c.T1111C	468	133		
UPN2026	PTPN11	chr12	112888189	p.E69K	c.G205A	1525	1618		
UPN2099	NRAS	chr1	115258747	p.G12D	c.G35A	306	366		
UPN2178	CBL	chr11	119148891	p.Y371H	c.T1111C	994	30		
UPN2309	CBL	chr11	119148891	p.Y371H	c.T1111C	165	12		
UPN2492	PTPN11	chr12	112888199	p.A72V	c.C215T			416	453
UPN2492	NF1	chr17	29553478	p.T676fs	c.2027insC			31	41
UPN2492	NF1	chr17	29679283	p.K2489R	c.A7466G			181	10
UPN2492	JAK3	chr19	17943472	p.D846N	c.G2536A			378	634

Supplementary Table 3, continued

Patient	Gene	Chromsome	Position	Protein Change	Nucleotide Change	Diagnosis Alternate Reads	Diagnosis Reference Reads	Relapse Alternate Reads	Relapse Reference Reads
UPN2497	PTPN11	chr12	112888210	p.E76Q	c.G226C	691	715		
UPN2528	NF1	chr17	29553478	p.T676fs	c.2027insC	38	13		
UPN2613	KRAS	chr12	25398281	p.G13D	c.G38A	162	204	53	110
UPN2630	RRAS	chr19	50140165	p.Q87L	c.A260T			1	4
UPN2630	DNMT3A	chr2	25457243	p.R882C	c.C2644T			211	207
UPN2630	SETBP1	chr18	42531907	P.D868N	c.G2602A			413	666
UPN2630	EZH2	chr7	148523590	p.R288Q	c.G863A			586	55
UPN2630	NRAS	chr1	115258747	p.G12D	c.G35A			1075	317
J212	PTPN11	chr12	112888210	p.E76K	c.G226A	299	1249		
J217	PTPN11	chr12	112888210	p.E76K	c.G226A	1037	1025		
J221	None								
J222	RUNX1	chr21	36231770	Splicing	c.613+1G>-	277	266		
J227	NRAS	chr1	115256528	p.Q61H	c.A183C	116	722		
J227	ASXL1	chr20	31022403	p.H630fs	c.1888_1910del	60	207		
J246	CBL	chr11	119148930	p.C384R	c.T1150C	34	6		
J246	NF1	chr17	29585473	p.K1429fs	c.4285insA	10	23		
J250	PTPN11	chr12	112888210	p.E76K	c.G226A	65	66		
J257	KRAS	chr12	25398281	p.G13D	c.G38A	628	917		
J258	JAK3	chr19	17943348	p.R887H	c.G2660A	322	373		
J259	NRAS	chr1	115258747	p.G12V	c.G35T	249	252	146	179
J259	SETBP1	chr18	42531907	p.D868N	c.G2602A	164	202	809	932
J259	ASXL1	chr20	31022403	p.H630fs	c.1888_1910del	79	108	387	738
J264	PTPN11	chr12	112888210	p.E76K	c.G226A	260	913		
J264	JAK3	chr19	17945696	p.V722I	c.G2164A	188	205		
J269	NF1	chr17	29665110	p.R2258*	c.C6772T	153	169		
J270	KRAS	chr12	25380276	p.Q61L	c.A182T	112	38		

Supplementary Table 3, continued

Patient	Gene	Chromsome	Position	Protein Change	Nucleotide Change	Diagnosis Alternate Reads	Diagnosis Reference Reads	Relapse Alternate Reads	Relapse Reference Reads
J270	EZH2	chr7	148523591	p.R288*	c.C862T	157	37		
J270	ASXL1	chr20	31022403	p.H630fs	c.1888_1910del	147	142		
J271	PTPN11	chr12	112888210	p.E76K	c.G226A	23	544		
J275	PTPN11	chr12	112888202	p.T73I	c.C218T	215	204		
J276	NF1	chr17	29553478	p.T676fs	c.2027insC	24	58	116	134
J277	None								
J278	PTPN11	chr12	112888211	p.E76G	c.A227G	214	265		
J279	PTPN11	chr12	112888199	p.A72G	c.C215G	90	114		
J281	NRAS	chr1	115258744	p.G13D	c.G38A	546	566		
J282	PTPN11	chr12	112888210	p.E76K	c.G226A	87	129		
J283	NRAS	chr1	115256528	p.Q61H	c.A183T	252	277		
J283	ZRSR2	chrX	15841231	p.439_442del	c.1315_1326del	105	2		
J286	PTPN11	chr12	112888166	p.D61V	c.A182T	81	79		
J286	ZRSR2	chrX	15809055	Splicing	c.42-2A>T	10	31		
J289	NRAS	chr1	115258747	p.G12D	c.G35A	99	78		
J295	PTPN11	chr12	112888210	p.E76K	c.G226A	139	166		
J295	SH2B3	chr12	111885480	p.T419fs	c.1257_1258delAG	67	49		
J300	None								
J309	KRAS	chr12	25398285	p.G12R	c.G34C	151	133		
J316	NRAS	chr1	115258747	p.G12D	c.G35A	33	261		
J316	PTPN11	chr12	112926888	p.G503A	c.G1508C	98	57		
J316	SH2B3	chr12	111885514	p.F431fs	c.1291delT	78	153		
J316	RRAS	chr19	50139934	p.R132H	c.G395A	88	103		

Supplementary Table 3, continued

Patient	Gene	Chromsome	Position	Protein Change	Nucleotide Change	Diagnosis Alternate Reads	Diagnosis Reference Reads	Relapse Alternate Reads	Relapse Reference Reads
J320	KRAS	chr12	25398285	p.G12C	c.G34T	163	169		
J320	ASXL1	chr20	31022330	p.C605fs	c.1815insC	12	182		
J322	PTPN11	chr12	112888166	p.D61V	c.A182T	246	229		
J322	NF1	chr17	29533315	p.R440*	c.C1318T	97	309		
J322	NF1	chr17	29562981	p.R1306*	c.C3916T	75	496		
J322	SH2B3	chr12	111884605	p.R261W	c.C781T	50	435		
J325	SH2B3	chr12	111856165	p.Q72H	c.G216C	14	65		
J336	PTPN11	chr12	112888210	p.E76K	c.G226A	225	236		
J336	JAK3	chr19	17945969	p.R657Q	c.G1970A	10	147		
J339	KRAS	chr12	25398281	p.G13D	c.G38A	270	370		
J342	PTPN11	chr12	112888210	p.E76K	c.G226A	83	79		
J346	PTPN11	chr12	112888210	p.E76K	c.G226A	118	160		
J348	NRAS	chr1	115258745	p.G13R	c.G37C	154	248		
J351	PTPN11	chr12	112888210	p.E76K	c.G226A	6	3		
J366	PTPN11	chr12	112891083	p.E139D	c.G417C	55	46		
J374	None								
J384	KRAS	chr12	25398284	p.G12D	c.G35A	22	121		
J384	GATA2	chr3	128202732	p.R330*	c.C988T	23	13		
J384	GATA2	chr3	128202758	p.L321R	c.T962G	13	24		
J384	EZH2	chr7	148515006	p.K400fs	c.1200_1203del	75	76		
J397	None								
J402	NF1	chr17	29553478	p.T676fs	c.2027insC	133	39		
J402	PTPN11	chr12	112888198	p.A72T	c.G214A	656	571		
J403	NRAS	chr1	115258744	p.G13D	c.G38A	307	696		
J403	NRAS	chr1	115256530	p.Q61K	c.C181A	163	1138		

Supplementary Table 3, continued

Patient	Gene	Chromsome	Position	Protein Change	Nucleotide Change	Diagnosis Alternate Reads	Diagnosis Reference Reads	Relapse Alternate Reads	Relapse Reference Reads
J404	CBL	chr11	119148892	p.Y371S	c.A1112C	108	34		
J404	NRAS	chr1	115258747	p.G12D	c.G35A	8	78		
J404	SH2B3	chr12	111885350	Splicing	c.1236+2T>C	8	182		
J405	NRAS	chr1	115258744	p.G13D	c.G38A	212	220		
J413	CBL	chr11	119148892	p.Y371S	c.A1112C	70	17		
J413	NRAS	chr1	115258747	p.G12D	c.G35A	10	217		
J420	PTPN11	chr12	112888199	p.A72V	c.C215T	161	151		
J426	PTPN11	chr12	112888198	p.A72T	c.G214A	293	293		
J426	NF1	chr17	29553478	p.T676fs	c.2027insC	38	52		
J426	JAK3	chr19	17945969	p.R657Q	c.G1970A	106	103		
J430	KRAS	chr12	25398281	p.G13D	c.G38A	498	630		

Supplementary Table 4. RNA-seq validation of pathogenic variants identified on exome sequencing.

	NRAS p.G13D	NRAS p.G12D	NRAS p.G12S	CBL p.Y371H	RRAS2 p.Q72L	SH2B3 p.W262*	SH2B3 p.H414fs	PTPN11 p.E69K	PTPN11 p.S502L	KRAS p.G13D	NF1 p.R440*	SETBP1 p.D868N	ZRSR2 p.G179E
UPN1125	0|290	0|292	0|288	30|3	0|134	0|265	1|443	0|139	0|106	0|0	0|27	0|9	0|170
UPN2447	0|28	0|29	0|29	0|9	9|13	0|9	0|20	0|51	0|4	0|0	0|1	0|0	0|31
UPN1254	0|51	0|51	0|50	0|25	0|149	0|22	0|40	2|70	0|5	0|0	2|2	0|1	0|99
UPN1330	0|84	0|83	48|37	0|14	0|64	1|48	1|134	0|79	0|19	0|0	0|12	0|3	0|100
UPN1595	5|6	0|11	0|9	0|10	0|28	0|2	0|7	0|19	0|0	0|0	0|2	0|3	0|24
UPN1646	0|76	43|34	0|76	0|61	0|10	0|80	0|151	0|95	0|30	0|0	0|7	0|13	89|7
UPN1897	0|164	0|168	0|169	0|27	0|98	0|239	0|355	0|83	0|27	0|0	0|9	0|9	0|72
UPN1974	0|166	1|171	0|173	0|30	0|111	0|154	1|277	0|97	0|63	0|0	0|9	0|9	0|138
UPN2015	0|228	0|233	115|116	0|78	0|88	0|382	0|567	1|113	0|104	0|0	0|19	0|9	0|117
UPN2026-D	0|66	0|64	0|65	0|29	0|83	0|61	0|98	26|18	0|20	0|0	0|7	0|6	0|117
UPN2026-R	0|28	0|31	0|30	0|2	0|19	0|9	0|22	3|0	0|6	0|0	0|10	0|3	0|52
UPN2301	0|165	0|164	0|164	0|30	0|116	0|286	0|450	0|82	0|85	75|68	0|24	1|0	0|49
UPN2332	0|344	0|349	196|155	0|187	0|13	0|404	0|617	0|262	0|180	0|0	0|14	0|4	1|179
UPN2406	0|244	0|241	0|239	0|56	0|156	1|311	1|564	3|197	113|103	0|0	0|24	0|8	0|111
UPN2531	0|89	0|90	0|87	0|15	0|70	18|36	48|56	20|26	0|16	0|0	0|19	0|5	0|88

Mutant allele | Wild type allele

Variant detected on exome sequencing

Supplementary Table 5. Single colony analysis in patients with compound Ras pathway mutations.

Patient	Time of sample	Number of colonies	Mutation A	Percent of colonies with mutation A	Mutation B	Percent of colonies with mutation B
UPN0969	Diagnosis	22	NF1 p.T1972fs	100.0	NRAS p.G12V	81.8
UPN0969	Relapse	56	NF1 p.T1972fs	100.0	NRAS p.G12V	21.4
J316	Diagnosis	55	PTPN11 p.G503A	92.7	NRAS p.G12D	16.4

Supplementary Table 6. Subclonal Mutations in *SETBP1* Detected Only by ddPCR**.**

Patient ID	Mutation
J222	G870S
J264	G870D
J276	D868N
J286	G870D
J295	D868N
J322	D868N
J342	D868N
J397	D868N
J403	D868N
J404	G870D
J405	D868N
UPN0424	D868N
UPN1254	D868N
UPN1348	D868N
UPN1142	G870S
UPN1484	G870D
UPN2015	D868N

Supplementary Table 7. Single colony analysis for UPN1420 at diagnosis and relapse.

Diagnostic Sample

Colony Pick	**NF1 p.Y2285***	**NF1 p.T676fs**	**ASXL1 p.Y591***
10	Heterozygous	Heterozygous	Heterozygous
14	Heterozygous	Heterozygous	Heterozygous
17	Heterozygous	Heterozygous	Heterozygous
20	Heterozygous	Heterozygous	Heterozygous
23	Heterozygous	Heterozygous	Homozygous
24	Heterozygous	Heterozygous	Homozygous

Relapse Sample

Colony Pick	**SH2B3 p.Q258***	**SH2B3 p.F390fs**
15	Heterozygous	Heterozygous
20	Heterozygous	Heterozygous
4	Homozygous	Wild type
5	Homozygous	Wild type
6	Homozygous	Wild type
7	Homozygous	Wild type
10	Homozygous	Wild type
11	Homozygous	Wild type
14	Homozygous	Wild type
16	Homozygous	Wild type
18	Homozygous	Wild type
19	Homozygous	Wild type
22	Homozygous	Wild type
24	Homozygous	Wild type
25	Homozygous	Wild type
26	Homozygous	Wild type
28	Homozygous	Wild type
29	Homozygous	Wild type
30	Homozygous	Wild type
31	Homozygous	Wild type
32	Homozygous	Wild type
9	Wild type	Homozygous
13	Wild type	Homozygous
17	Wild type	Homozygous
21	Wild type	Homozygous
23	Wild type	Homozygous

Supplementary Table 8. Characteristics of patient cohort.

Number of patients in cohort with follow up data	98
Gender, Male/Female (%)	68 (69.4) / 30 (30.6)
Median age at diagnosis, months (range)	18.1 (1-120)
Median WBC count at diagnosis $x10^9$ (range)*	34 (0.5-196)
Median monocyte count at diagnosis $x10^9$ (range)**	6.2 (0.4-48.8)
Median platelet count at diagnosis $x10^9$ (range)***	52 (8-504)
Median HbF level % at diagnosis (range) ξ	10.8 (0-74)
Monosomy 7 (%) ψ	12 (12.2)
Clinical NF1 or germline NF1 mutation (%)	10 (10.2)
SM at diagnosis (%) ζ	80 (81.6)
HM at diagnosis (%) ϕ	65 (66.3)
GM-CSF Hypersensitivity (%) ω	71 (72.5)
Number of mutations at diagnosis 0 1 2 3 4	 7 (7.1) 57 (58.2) 21 (21.4) 9 (9.2) 4 (4.1)
Canonical mutation at diagnosis *NF1* *PTPN11* *KRAS* *NRAS* *CBL* None Other λ	 13 (13.3) 31 (31.6) 14 (14.3) 17 (17.4) 11 (11.2) 6 (6.1) 6 (6.1)
Treatment ∞ HSCT Chemotherapy None	 82 (82.8) 4 (4.0) 7 (7.1)

* 11 unknown ξ 5 unknown ϕ 12 unknown ∞ 5 unknown
** 13 unknown ψ 1 unknown ω 10 unknown
*** 11 unknown ζ 12 unknown
λ Other: SH2B3 (2), RRAS2 (1), RRAS (1), JAK3 (1), RUNX1 (1)

WBC, white blood cell count

GM-CSF, granulocyte macrophage- colony stimulating factor

Supplementary Table 9. Clinical and biologic characteristics by number of somatic alterations at diagnosis.

Characteristic	0-1 alterations	2 or more alterations	p-value
Mean age at diagnosis, months	17.1	40.0	**<0.001**
Age at diagnosis <24 months (%) ≥ 24 months (%)	 46 (72) 18 (28)	 12 (35) 23 (65)	**<0.001**
Gender (%) Male Female	 37 (58) 27 (42)	 31 (89) 3 (9)	**0.001**
WBC count at diagnosis $x10^9$ <35 (%) ≥35 (%)	 29 (45) 27 (42)	 16 (47) 15 (44)	0.860
Monocyte count at diagnosis $x10^9$ <6 (%) ≥6 (%)	 24 (38) 30 (47)	 14 (41) 17 (50)	0.639
Platelet count at diagnosis $x10^9$ <40 (%) ≥40 (%)	 17 (27) 39 (61)	 10 (29) 21 (62)	0.846
HbF level at diagnosis Not elevated for age (%) Elevated for age (%)	 33 (52) 26 (41)	 14 (41) 20 (59)	0.094
Splenomegaly (%) No Yes	 3 (5) 53 (83)	 3 (9) 27 (79)	0.712
Monosomy7 (%) No Yes	 62 (97) 2(3)	 23 (68) 10 (29)	**<0.001**
Clinical NF1 or Germline *NF1* mutation (%) No Yes	 62 (97) 2 (3)	 26 (76) 8 (24)	**0.003**
Somatic *PTPN11* Mutation (%) No Yes	 43 (67) 21 (33)	 24 (71) 10 (29)	0.730
Somatic *NF1* Mutation (%) No Yes	 61 (95) 3 (5)	 22 (65) 12 (35)	**<0.001**
Clonal/Subclonal *SETBP1* Mutation (%) No Yes	 46 (79) 12 (21)	 21 (64) 12 (36)	0.138

Supplementary Table 10. Univariate analysis.

Univariate Cox Analysis	EFS from date of diagnosis					OS from date of diagnosis		
Age at diagnosis (months)	**N**	**HR**	**95% CI**	**p**		**HR**	**95% CI**	**p**
<24	58	1				1		
>24	40	2.09	1.20-3.62	**0.009**		2.03	1.13-3.66	**0.018**
Gender								
Male	68	1				1		
Female	30	1.59	0.85-2.99	0.148		1.56	0.79-3.09	0.197
WBC count at diagnosis $x10^9$								
<35	45	1				1		
≥40	42	1.37	0.77-2.44	0.288		1.29	0.69 -2.40	0.420
Platelet count at diagnosis $x10^9$								
<40	27	1				1		
≥40	60	1.46	0.80-2.66	0.214		1.71	0.90-3.20	0.099
Fetal hemoglobin at diagnosis								
Not elevated for age	47	1				1		
Elevated for age	46	2.14	1.19 - 3.84	0.110		1.90	1.02-3.55	**0.043**
Monosomy 7								
No	85	1				1		
Yes	12	1.62	0.76-3.46	0.211		1.61	0.72 - 3.62	0.250
Clinical NF1/Germline *NF1* mutation								
No	88	1				1		
Yes	10	1.63	0.77-3.48	0.203		1.25	0.53-2.95	0.612
Somatic *PTPN11* mutation								
No	67	1				1		
Yes	31	1.40	0.79-2.50	0.247		1.55	0.85-2.83	0.157
Somatic *NF1* mutation								
No	83	1				1		
Yes	15	1.79	0.94-3.43	0.077		1.26	0.62-2.69	0.488
Clonal/Subclonal *SETBP1* mutation								
No	67	1				1		
Yes	24	1.30	0.69 -3.24	0.413		1.16	0.59-2.28	0.670
Somatic alterations at diagnosis								
0-1	64	1				1		
2 or more	34	2.67	1.53-4.64	**0.001**		2.44	1.35-4.38	**0.003**

Supplementary Table 11. Estimates of tumor content in germline tissue.

Patient	Combined TiN	Tissue Type	deTiN Based on Mutations	deTiN Based on SCNAs
UPN0424	0.785	Cord Blood	0.785	N/A
UPN0969	0	Buccal	0	0
UPN1093	0.08	Cord Blood	0.08	0.08
UPN1125	N/A	EBV	N/A	N/A
UPN1241	0	Cord Blood	0.05	0
UPN1312	0.615	EBV	0.615	N/A
UPN1330	0.895	EBV	0.895	N/A
UPN1333	0	Buccal	N/A	0
UPN1370	0.195	Buccal	N/A	N/A
UPN1383	0	EBV	0	N/A
UPN1420	0.13	Buccal	0.145	0.12
UPN1595	0.635	Cord Blood	0.635	N/A
UPN1646	0.25	Buccal	0.25	N/A
UPN1711	0	Fibroblast	0	0
UPN1875	0.01	Buccal	0.01	0.04
UPN1970	N/A	Buccal	N/A	N/A
UPN1974	N/A	Buccal	N/A	N/A
UPN1993	0.1	Buccal	0.085	0.21
UPN2015	0.645	Cord Blood	0.645	N/A
UPN2056	0.23	Buccal	N/A	0.23
UPN2301	0.22	EBV	N/A	N/A
UPN2406	0.18	Buccal	N/A	0.18
UPN2447	0.07	EBV	0.09	0.05
UPN2528	0	Buccal	N/A	0
UPN2531	0	Fibroblast	0	N/A
UPN2629	0	Fibroblast	0	N/A
UPN2630	0.01	Fibroblast	0.01	N/A

Supplementary Table 12. Targeted resequencing coverage and mapped reads.

Sample ID	Time Point	Mean coverage	Bases > 3x	Bases > 20x	Bases > 50x	Bases > 100x	Bases > 200x	Reads aligned	Total reads
J212	Diagnosis	2698	98.5%	96.2%	93.8%	89.8%	86.5%	98.9%	1,359,650
J217	Diagnosis	3206	97.8%	92.7%	90.0%	86.3%	80.8%	98.8%	1,858,173
J221	Diagnosis	746	95.7%	89.0%	82.6%	76.8%	70.4%	99.2%	356,464
J222	Diagnosis	1634	97.8%	93.2%	89.1%	85.0%	79.2%	98.6%	801,960
J227	Diagnosis	913	95.8%	89.2%	83.7%	77.8%	71.0%	99.2%	438,433
J246	Diagnosis	238	96.4%	90.3%	79.1%	64.6%	38.7%	96.0%	118,785
J250	Diagnosis	646	93.3%	80.9%	74.2%	66.6%	57.6%	99.8%	296,715
J257	Diagnosis	2593	96.0%	88.5%	83.7%	79.7%	74.9%	98.2%	1,379,734
J258	Diagnosis	568	98.4%	96.4%	93.0%	85.9%	72.7%	99.4%	263,520
J259	Diagnosis	642	97.4%	93.2%	85.7%	78.0%	69.8%	99.6%	304,936
J259	Diagnosis	1017	90.5%	81.0%	73.4%	65.5%	56.0%	99.8%	458,409
J264	Diagnosis	2304	96.6%	91.0%	86.8%	82.0%	76.8%	98.4%	1,152,097
J269	Diagnosis	908	96.7%	89.6%	84.9%	78.4%	72.3%	98.7%	440,797
J270	Diagnosis	719	97.9%	95.3%	92.0%	85.7%	75.6%	98.9%	338,408
J271	Diagnosis	1021	97.3%	93.0%	88.1%	82.5%	74.6%	98.5%	501,311
J275	Diagnosis	745	96.8%	89.9%	83.3%	77.0%	70.2%	98.7%	362,456
J276	Diagnosis	1275	96.3%	87.6%	81.5%	76.9%	72.1%	98.4%	627,956
J276	Relapse	2433	97.2%	92.9%	88.4%	84.1%	78.5%	99.8%	1,250,976
J277	Diagnosis	657	96.6%	88.8%	83.8%	76.5%	69.4%	99.0%	319,662
J278	Diagnosis	917	95.7%	88.7%	83.3%	75.9%	69.6%	99.2%	446,882
J279	Diagnosis	613	96.5%	94.2%	88.9%	83.0%	73.7%	99.8%	292,136
J281	Diagnosis	1564	97.3%	93.5%	88.1%	83.4%	76.5%	98.3%	777,260
J282	Diagnosis	504	96.4%	91.8%	85.9%	79.4%	69.3%	99.6%	240,272
J283	Diagnosis	661	97.5%	93.7%	90.4%	83.4%	73.4%	99.5%	323,278
J286	Diagnosis	528	94.4%	91.3%	87.6%	81.1%	69.3%	99.1%	254,026

Supplementary Table 12, continued

Sample ID	Time Point	Mean coverage	Bases > 3x	Bases > 20x	Bases > 50x	Bases > 100x	Bases > 200x	Reads aligned	Total reads
J289	Diagnosis	549	94.9%	91.8%	88.3%	82.6%	70.8%	99.5%	257,666
J295	Diagnosis	514	96.5%	87.3%	79.7%	69.0%	57.8%	99.7%	258,928
J300	Diagnosis	96	32.7%	17.3%	10.3%	7.1%	6.2%	98.0%	70,519
J309	Diagnosis	737	98.3%	95.8%	93.5%	90.3%	81.8%	99.0%	354,062
J313	Diagnosis	1756	97.7%	95.3%	93.1%	87.8%	83.8%	99.4%	847,001
J313	Relapse	3010	97.3%	93.9%	90.2%	87.0%	81.5%	99.8%	1,704,180
J316	Diagnosis	561	96.8%	92.1%	85.5%	79.3%	69.7%	99.6%	269,770
J320	Diagnosis	681	96.8%	94.1%	90.1%	84.4%	75.5%	99.2%	321,689
J322	Diagnosis	928	95.9%	88.8%	81.5%	74.9%	69.5%	98.4%	456,768
J325	Diagnosis	632	96.5%	94.5%	91.8%	85.3%	75.4%	99.0%	301,675
J336	Diagnosis	723	97.4%	94.0%	88.1%	82.6%	69.9%	99.0%	353,171
J339	Diagnosis	1692	97.7%	94.8%	92.6%	88.3%	83.2%	99.2%	810,505
J342	Diagnosis	1228	95.6%	88.2%	81.8%	76.1%	66.8%	99.8%	572,026
J346	Diagnosis	601	96.6%	91.8%	85.4%	78.5%	69.6%	99.1%	287,129
J348	Diagnosis	876	96.1%	94.6%	91.8%	86.1%	76.7%	98.9%	409,938
J351	Diagnosis	701	86.7%	73.7%	62.7%	54.8%	44.4%	99.8%	301,850
J366	Diagnosis	767	97.3%	93.1%	86.5%	78.7%	68.9%	99.8%	362,826
J374	Diagnosis	853	96.1%	89.1%	84.7%	79.1%	72.8%	98.6%	412,154
J384	Diagnosis	590	96.9%	93.5%	85.4%	73.5%	63.4%	99.0%	297,186
J384	Relapse	2332	97.4%	92.6%	88.4%	82.9%	79.1%	99.8%	1,152,136
J397	Diagnosis	651	97.2%	94.4%	91.0%	84.0%	70.3%	99.4%	315,603
J402	Diagnosis	2503	97.7%	94.7%	90.7%	87.1%	81.6%	99.4%	1,253,390
J403	Diagnosis	1520	96.5%	91.9%	87.4%	83.1%	77.0%	99.3%	735,164
J404	Diagnosis	333	95.9%	86.7%	74.4%	57.7%	33.0%	99.6%	356,872

Supplementary Table 12, continued

Sample ID	Time Point	Mean coverage	Bases > 3x	Bases > 20x	Bases > 50x	Bases > 100x	Bases > 200x	Reads aligned	Total reads
J405	Diagnosis	609	96.0%	88.8%	83.1%	75.3%	66.7%	99.7%	292,605
J413	Diagnosis	686	89.5%	78.1%	69.1%	60.0%	48.8%	99.8%	296,282
J420	Diagnosis	667	96.2%	92.1%	86.2%	80.4%	71.0%	99.8%	312,462
J426	Diagnosis	1230	96.0%	90.4%	85.6%	79.8%	74.7%	99.3%	586,339
J430	Diagnosis	2311	97.1%	91.6%	87.7%	83.3%	78.5%	99.0%	1,142,899
UPN0585	Diagnosis	1512	95.0%	89.3%	83.7%	80.1%	74.6%	99.5%	720,878
UPN0868	Diagnosis	2998	96.1%	90.1%	84.9%	81.3%	76.3%	99.0%	1,781,159
UPN0906	Diagnosis	1107	94.8%	86.1%	79.2%	72.9%	62.7%	99.8%	495,621
UPN0906	Relapse	922	96.7%	92.0%	86.7%	81.6%	72.7%	99.8%	432,863
UPN0969	Relapse	3706	97.4%	92.0%	89.3%	85.6%	81.1%	98.2%	2,662,590
UPN1043	Diagnosis	1064	96.2%	91.2%	85.8%	81.7%	75.1%	99.4%	505,482
UPN1043	Relapse	850	96.9%	93.3%	88.2%	82.4%	75.1%	99.5%	400,511
UPN1142	Diagnosis	2760	97.9%	95.1%	91.7%	87.9%	83.2%	99.4%	1,464,305
UPN1188	Diagnosis	1201	96.5%	92.2%	89.2%	84.6%	78.9%	99.8%	562,991
UPN1199	Diagnosis	1059	96.6%	91.0%	85.5%	81.1%	74.0%	99.6%	498,920
UPN1254	Diagnosis	3299	97.2%	92.1%	88.8%	84.0%	79.1%	98.1%	2,095,048
UPN1348	Diagnosis	2607	98.5%	96.0%	93.7%	90.2%	85.1%	98.3%	1,332,228
UPN1420	Relapse	3345	95.0%	88.6%	83.1%	79.2%	74.1%	98.7%	2,277,045
UPN1447	Diagnosis	2665	98.0%	95.8%	93.1%	90.4%	85.8%	99.0%	1,359,303
UPN1447	Relapse 1	1067	94.8%	89.8%	84.5%	79.9%	74.1%	98.6%	524,212
UPN1447	Relapse 2	153	89.5%	81.9%	63.4%	46.2%	26.9%	94.0%	82,125
UPN1484	Diagnosis	2388	96.8%	91.1%	87.7%	82.6%	78.0%	98.3%	1,198,358
UPN1597	Diagnosis	1879	97.4%	92.0%	88.2%	83.2%	78.2%	98.3%	925,057
UPN1597	Relapse	753	96.8%	90.6%	85.6%	79.6%	72.7%	99.3%	361,742

Supplementary Table 12, continued

Sample ID	Time Point	Mean coverage	Bases > 3x	Bases > 20x	Bases > 50x	Bases > 100x	Bases > 200x	Reads aligned	Total reads
UPN1711	Relapse	3717	96.9%	91.0%	85.6%	82.7%	78.4%	99.0%	3,072,802
UPN1740	Diagnosis	989	94.8%	85.7%	80.1%	75.1%	68.9%	98.9%	486,291
UPN1778	Diagnosis	996	95.6%	89.7%	84.2%	78.3%	73.0%	98.8%	482,210
UPN1941	Diagnosis	915	92.4%	84.2%	78.3%	72.1%	65.9%	98.4%	448,254
UPN1970	Diagnosis	947	92.4%	84.0%	79.3%	73.9%	69.5%	99.0%	448,736
UPN1974	Diagnosis	593	89.2%	79.8%	73.4%	68.3%	60.1%	99.6%	281,857
UPN1993	Relapse	3707	97.4%	93.0%	90.0%	86.9%	82.8%	98.3%	2,523,767
UPN1711	Relapse	3717	96.9%	91.0%	85.6%	82.7%	78.4%	99.0%	3,072,802
UPN1740	Diagnosis	989	94.8%	85.7%	80.1%	75.1%	68.9%	98.9%	486,291
UPN1778	Diagnosis	996	95.6%	89.7%	84.2%	78.3%	73.0%	98.8%	482,210
UPN2025	Diagnosis	867	96.2%	88.2%	81.9%	75.7%	69.9%	99.0%	420,823
UPN2026	Diagnosis	2969	96.3%	91.3%	86.6%	82.8%	78.0%	99.1%	1,653,786
UPN2026	Relapse	3511	98.2%	96.3%	93.4%	91.4%	87.2%	99.0%	2,125,521
UPN2099	Diagnosis	851	94.5%	86.9%	80.1%	75.0%	69.0%	99.1%	408,427
UPN2178	Diagnosis	1362	94.1%	85.9%	81.5%	76.1%	69.7%	99.1%	660,846
UPN2309	Diagnosis	727	91.7%	78.6%	71.2%	63.6%	53.4%	99.8%	329,185
UPN2492	Relapse	1670	96.4%	91.1%	86.3%	79.5%	73.8%	98.0%	832,939
UPN2497	Diagnosis	3344	98.1%	97.0%	94.5%	92.1%	88.8%	99.6%	1,868,654
UPN2528	Diagnosis	802	89.8%	81.1%	75.2%	70.6%	64.0%	99.1%	388,406
UPN2613	Diagnosis	1022	95.0%	89.1%	81.8%	77.1%	70.3%	99.7%	483,553
UPN2613	Relapse	732	95.1%	87.7%	79.6%	73.3%	66.1%	99.8%	337,881
UPN2630	Relapse	2095	96.9%	91.2%	86.3%	82.2%	77.8%	98.7%	1,034,299

Supplementary Table 13. Primer sequences.

Gene	Nucleotide Change	Protein Change	Forward Primer (5'--3')	Reverse Primer (5'--3')
ASXL1	c.1773C>G	p.Y591*	ACAGCCCTTGAGCAGAATCT	GCATCTCCTAGCCCATCTGT
ASXL1	c.1888_1910del	p.H630fs	ACAGCCCTTGAGCAGAATCT	GCATCTCCTAGCCCATCTGT
DNMT3A	c.2644C>T	p.R882C	ACAGAAAACCCCTCTGAAAAGAGT	CCCTGCCCTCTCTGCCTTTTC
EZH2	c.2035G>C	p.V674L	TTCACATAACAAACAACTATCCCAGAA	ATGTGAAAATGCCTATTCGTGATGT
GATA2	c.950A>G	p.N317S	AAGACGGGGTGGGGCAGACACAGTTG	GGCCCCCAAAGCAGGGAACGATTTAA
PTPN11	c.205G>A	p.E69K	CGACGTGGAAGATGAGATCTGA	CAGTCACAAGCCTTTGGAGTCAG
RRAS	c.260A>T	p.Q87L	GAGTGAAGCCGGAGGCATGA	TTAGAGAGAGAGGGACAGAGACTG
RRAS2	c.233A>T	p.Q72L	GCAAGTCTCATGCCCCAATTAGTA	CCTGACTCCATCTGATTAAATGTGTT
RUNX1	c.1047_1048insC	p.R349fs	GCGGCAGGTAGGTGTGGTAG	CGACCTCCTGGGCATAGCATCA
SETBP1	c.2608G>A	p.G870S	CTTCACCAGCAGCTATGCAC	CGGTGGGAGATTCTGAACAC
SETBP1	c.2608G>A	p.G870S	CTTCACCAGCAGCTATGCAC	CGGTGGGAGATTCTGAACAC
SETBP1	c.2612T>C	p.I871T	CTTCACCAGCAGCTATGCAC	CGGTGGGAGATTCTGAACAC
SETBP1	c.2602G>A	p.D868N	CTTCACCAGCAGCTATGCAC	CGGTGGGAGATTCTGAACAC
SH2B3	c.772C>T	p.Q258*	GACTATAGACAAACTCAGGCCTGGCT	TAGGCTCTAGGGCTGAGGGAATAT
SH2B3	c.1170delC	p.F390fs	GGCCATTGTCTTCTGGGTACG	ATGTCCACGACCGAGGGAAAG
SH2B3	c.1198G>A	p.E400K	GGCCATTGTCTTCTGGGTACG	ATGTCCACGACCGAGGGAAAG
SH2B3	c.785G>A	p.W262*	GACTATAGACAAACTCAGGCCTGGCT	TAGGCTCTAGGGCTGAGGGAATAT
SH2B3	c.1236_splice	p.H414fs	GGGAATACGTGCTCACTTTCAAC	AGTGAGGAAGGGAGAAAGGGAAGAG
ZRSR2	c.536G>A	p.G179E	CCTGTTCCAACTTAAATGTTTGCAT	AGTGGCTTCCTAAAGATGCAGAA