Validation of a Prognostic Model and the Impact of Mutations in 288 Patients with Lower Risk Myelodysplastic Syndromes

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Supplementary Materials

Supplemental Methods

MDS Patient Sample Cohort

Samples were obtained from the MD Anderson Cancer Center, Rush University Medical Center, and the University of Massachusetts Medical Center from adult patients who provided written informed consent according to protocols approved by institutional review boards at each institution. Patient samples with sufficient DNA for analysis and available clinical information where selected for inclusion. The characteristics of these MDS patients are comparable to those used to establish the LR-PSS with the exception that we did not include patients with CMML. Patients were determined to have died if this fact was noted in their medical record or if they appeared in the Social Security Death Index. Patients not determined to have died were censored at the time of their last known follow up.

Statistical Methods

An exact binomial test was used to assess co-occurrence between mutations. The Fisher exact test was used to test for differences between categorical variables, while ordered categories were assessed with a Jonckheere-Terpstra test. Differences between groups for continuous measures were assessed with a Wilcoxon rank sum test. Cox proportional hazard regression models were constructed for individual mutation status and adjusted for the LR-PSS and IPSS risk classifications.SAS version 9.2 and R version 2.11.1 were used in all analysis.

Supplementary Table 1: Patient Characteristics

	N (%)
N	288
Age at Time of BM Sample (yrs.), median (range)	69 (15, 90)
Sex	
Female	85 (30)
Male	203 (70)
FAB	
RA	173 (60)
RARS	41 (14)
RAEB	71 (25)
RAEB-T†	3 (1)
IPSS	
Low	106 (37)
Intermediate-1	182 (63)
Red Blood Cell Transfusion	
Yes	131 (45)
No	111 (39)
Unknown	46 (16)
Platelet Transfusion	
Yes	39 (14)
No	200 (69)
Unknown	49 (17)
Karyotype	
-7/del(7q) isolated or +1	1 (<1)
Del(20q) isolated	14 (5)
Del(5q) isolated	18 (6)
+8 isolated	13 (5)
Complex	6 (2)
Normal	206 (72)
Other	30 (10)
Blast %, median (range)	0 (0, 10)
< 4 %	217 (75)
4 – 10 %	71 (25)
Hemoglobin, median (range)	10.0 (6.1, 17.0)
< 8.0 (gm/dl)	30 (10)
8.0 – 9.99 (gm/dl)	114 (40)
10.0 – 11.99 (gm/dl)	96 (33)
≥12.0 (gm/dl)	48 (17)

Absolute Neutrophil Count (ANC), median (range)	1,887 (19.8, 25,830)
< 500 (cells/mm^3)	26 (9)
500 – 1,499 (cells/mm^3)	87 (30)
1,500 - 9,999 (cells/mm^3)	155 (54)
≥10,000 (cells/mm^3)	6 (2)
Unknown	14 (5)
Platelets, median (range)	105 (3, 915)
< 50 ($x10^9$/L)	72 (25)
50 – 200 ($x10^9$/L)	136 (47)
> 200 ($x10^9$/L)	80 (28)

† The 3 patients with RAEB-T had circulating blasts but ≤10% blasts in the bone marrow

Supplementary Table 2:

Assignments from IPSS Lower Risk Groups to LR-PSS Risk Categories

A) Mapping of IPSS Lower Risk Groups (n=288)

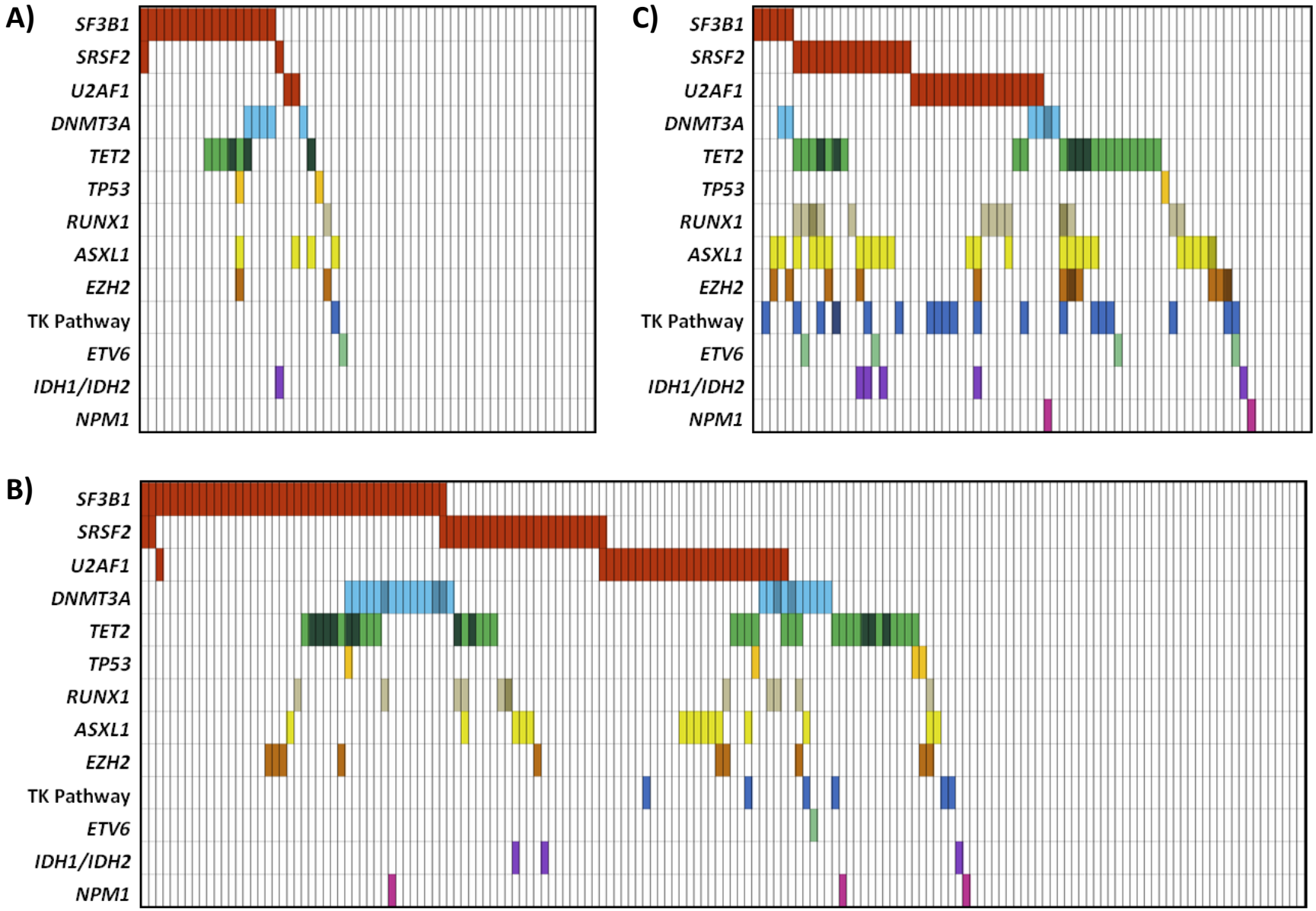
		LR-PSS Risk Category		
	N	**1**	**2**	**3**
IPSS Risk Group	**Low**	38	67	1
	Intermediate-1	19	93	70

B) Mapping of IPSS Lower Risk Scores (n=283)*

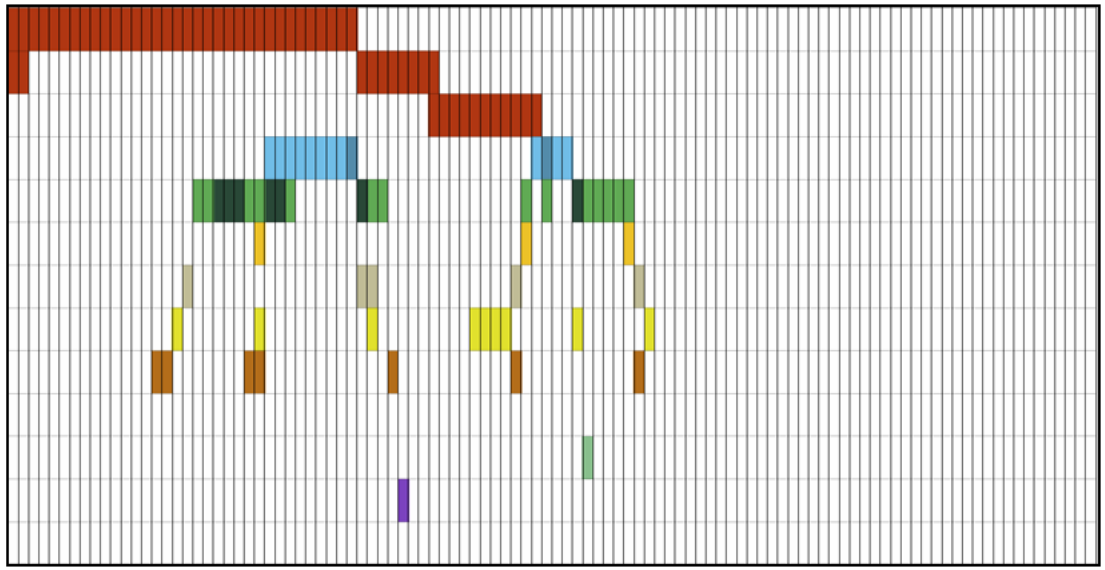
		LR-PSS Risk Category		
	N	**1**	**2**	**3**
Total IPSS Score	**0**	38	67	1
	0.5	13	70	27
	1.0	6	19	42

* 5 patients with Intermediate-1 risk were excluded from this table because they had missing clinical information making it unclear if their total IPSS score was 0.5 or 1.0.

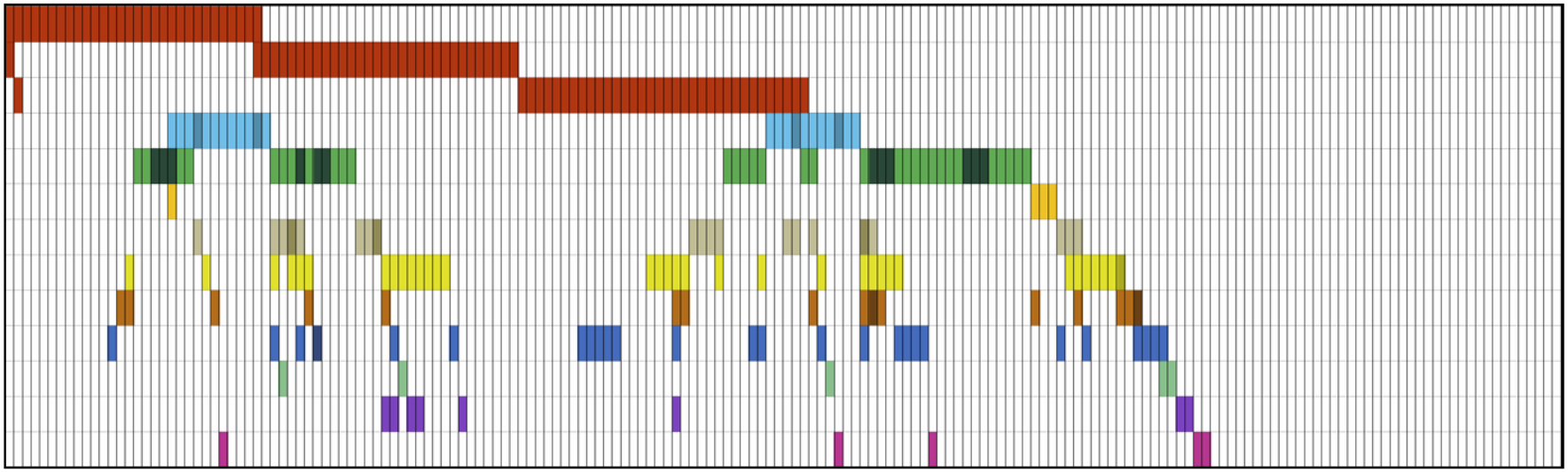
Supplementary Figure 1: Distribution of Mutations in Each LR-PSS Risk Category. A) Category 1 - 46% of samples have one or more mutations. **B) Category 2** - 72% of samples have one or more mutations. **C) Category 3** - 90% of samples have one or more mutations. **D) IPSS Low** risk patients - 59% of samples have one or more mutations. **E) IPSS Intermediate-1** risk patients - 77% of samples have one or more mutations. Tyrosine Kinase (TK) pathway genes include *NRAS*, *KRAS*, *BRAF*, *CBL*, and *JAK2*.



D)


SF3B1
SRSF2
U2AF1
DNMT3A
TET2
TP53
RUNX1
ASXL1
EZH2
TK Pathway
ETV6
IDH1/IDH2
NPM1

E)


SF3B1
SRSF2
U2AF1
DNMT3A
TET2
TP53
RUNX1
ASXL1
EZH2
TK Pathway
ETV6
IDH1/IDH2
NPM1

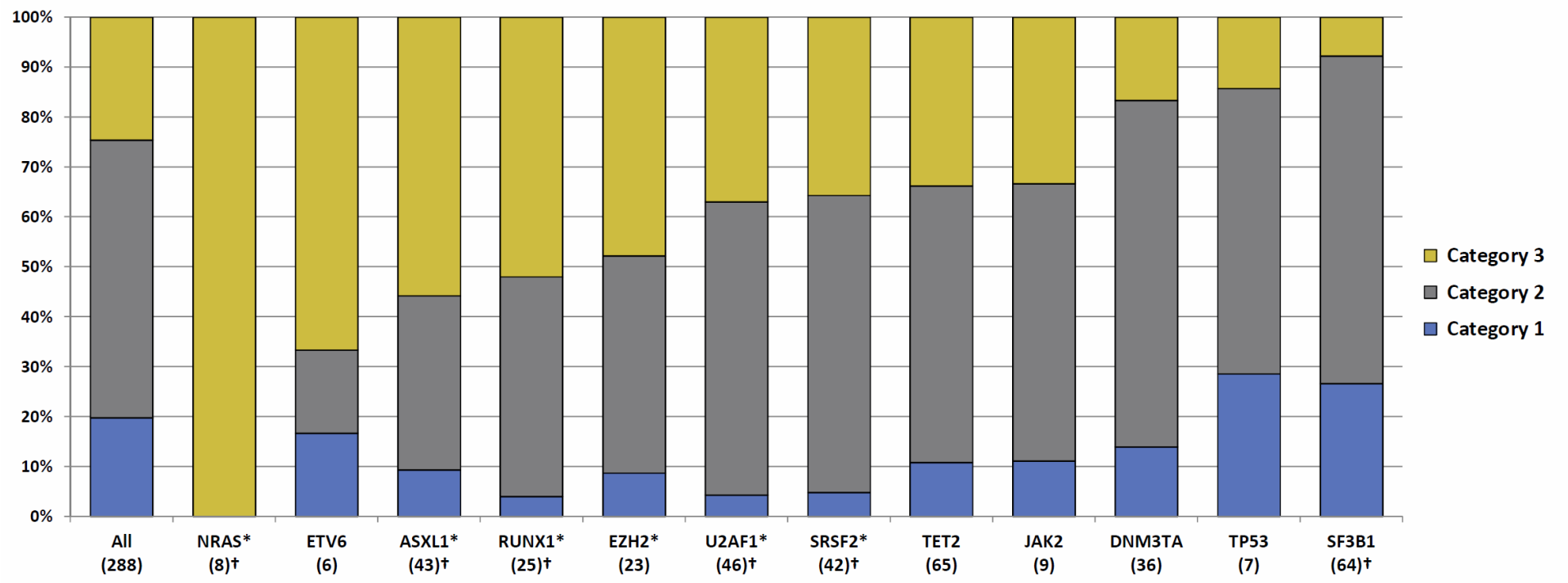
Supplementary Figure 2: Distribution of Mutated Genes in the A) LR-PSS and B) IPSS Risk Groups.

(n) - Number of samples with a given mutation

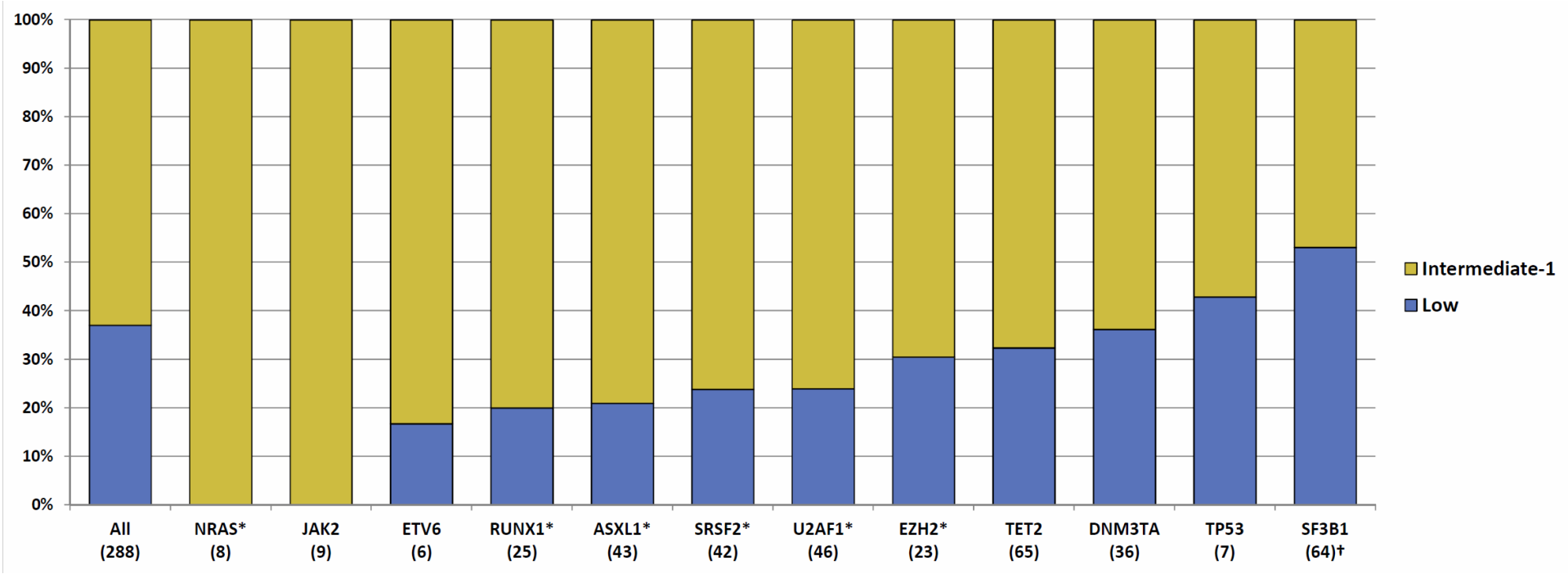
* - Mutated genes that are univariately associated with a poor prognosis (p <0.05)

† - Mutated genes with a risk group distribution significantly different from unmutated cases (p <0.01)

A)

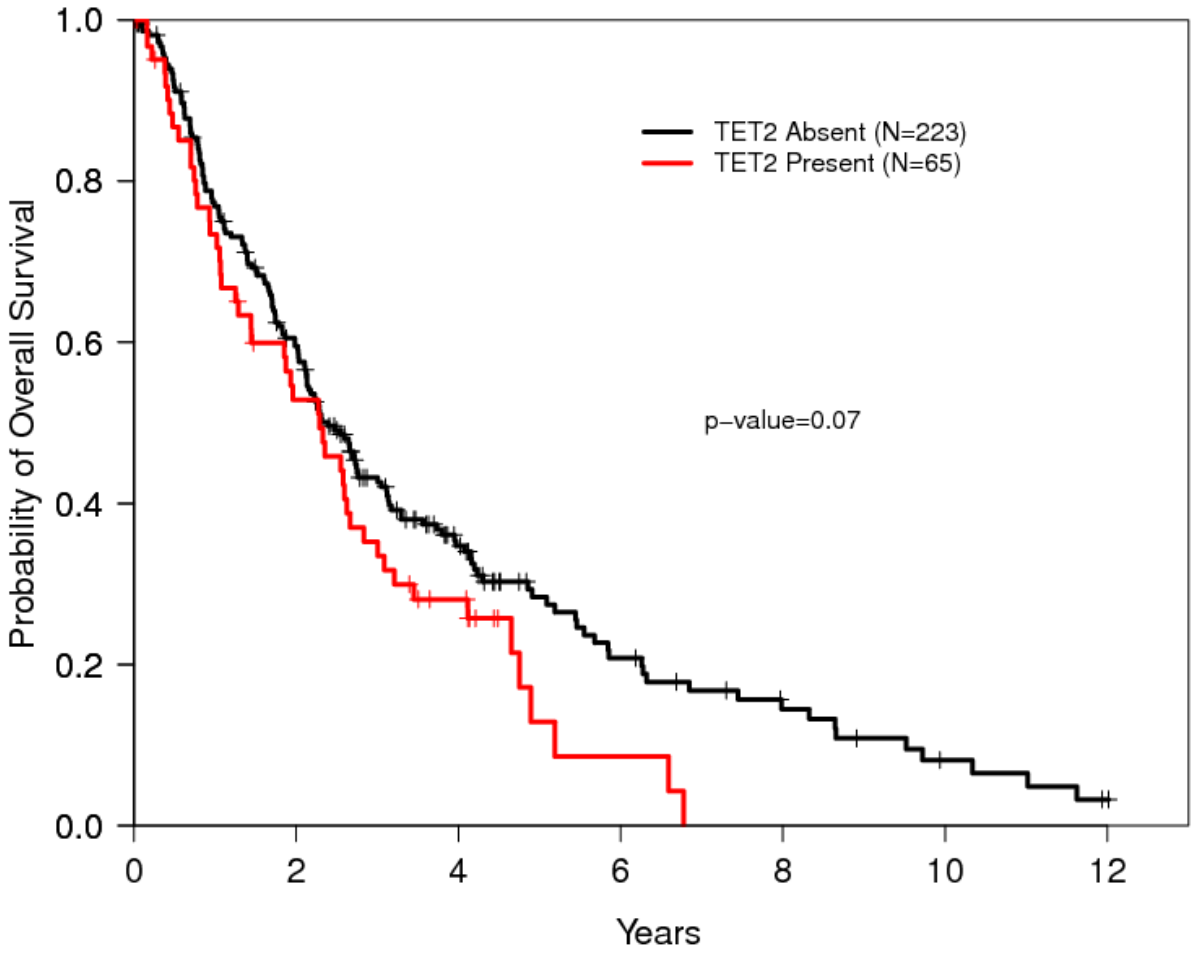


B)

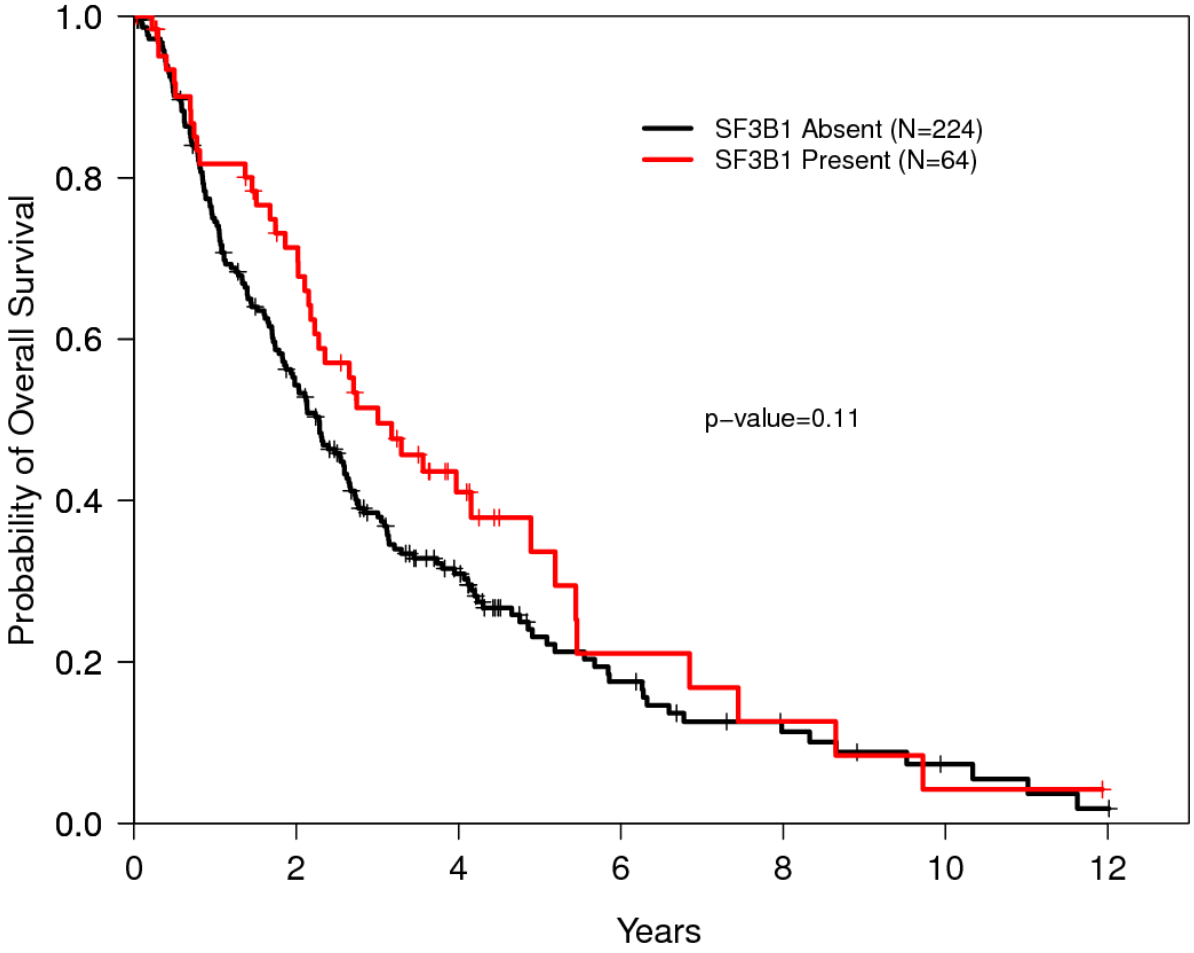

100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
All (288)
NRAS* (8)
JAK2 (9)
ETV6 (6)
RUNX1* (25)
ASXL1* (43)
SRSF2* (42)
U2AF1* (46)
EZH2* (23)
TET2 (65)
DNM3TA (36)
TP53 (7)
SF3B1 (64)†
Intermediate-1
Low

Supplementary Figure 3: Kaplan-Meier Overall Survival Curves for MDS Patients With and Without Mutations in the 13 most Frequently Mutated Genes.

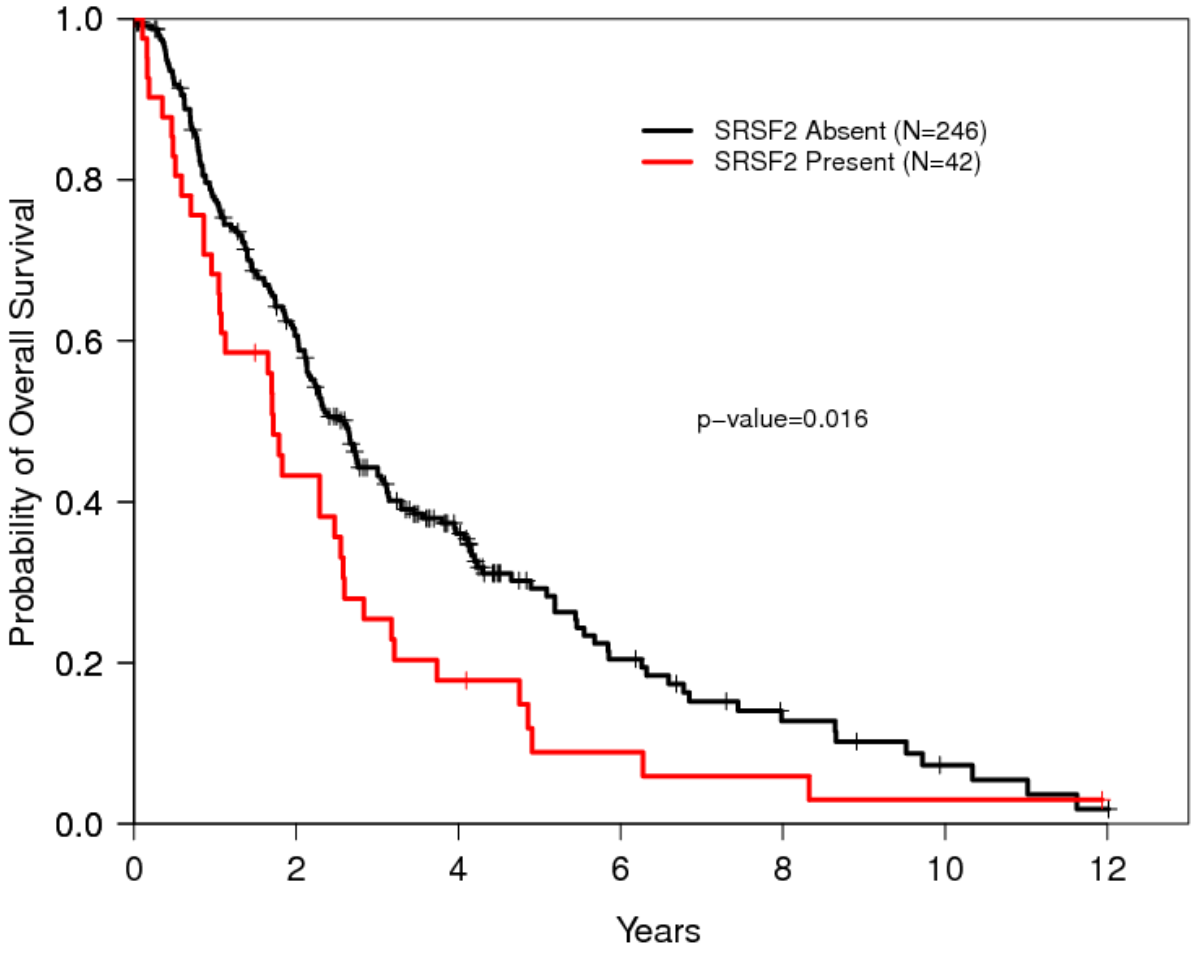
A) *TET2* mutations:



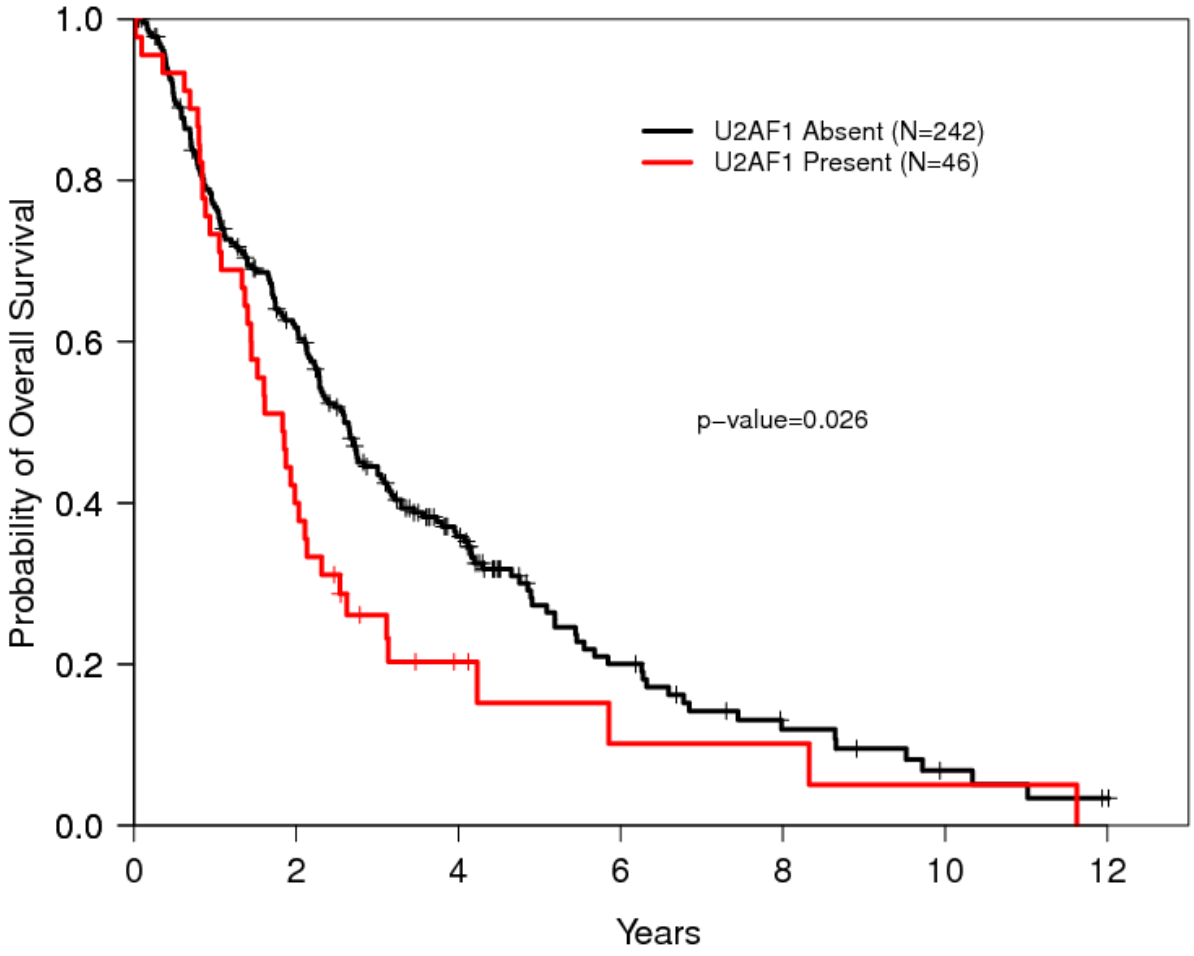
B) *SF3B1* mutations:



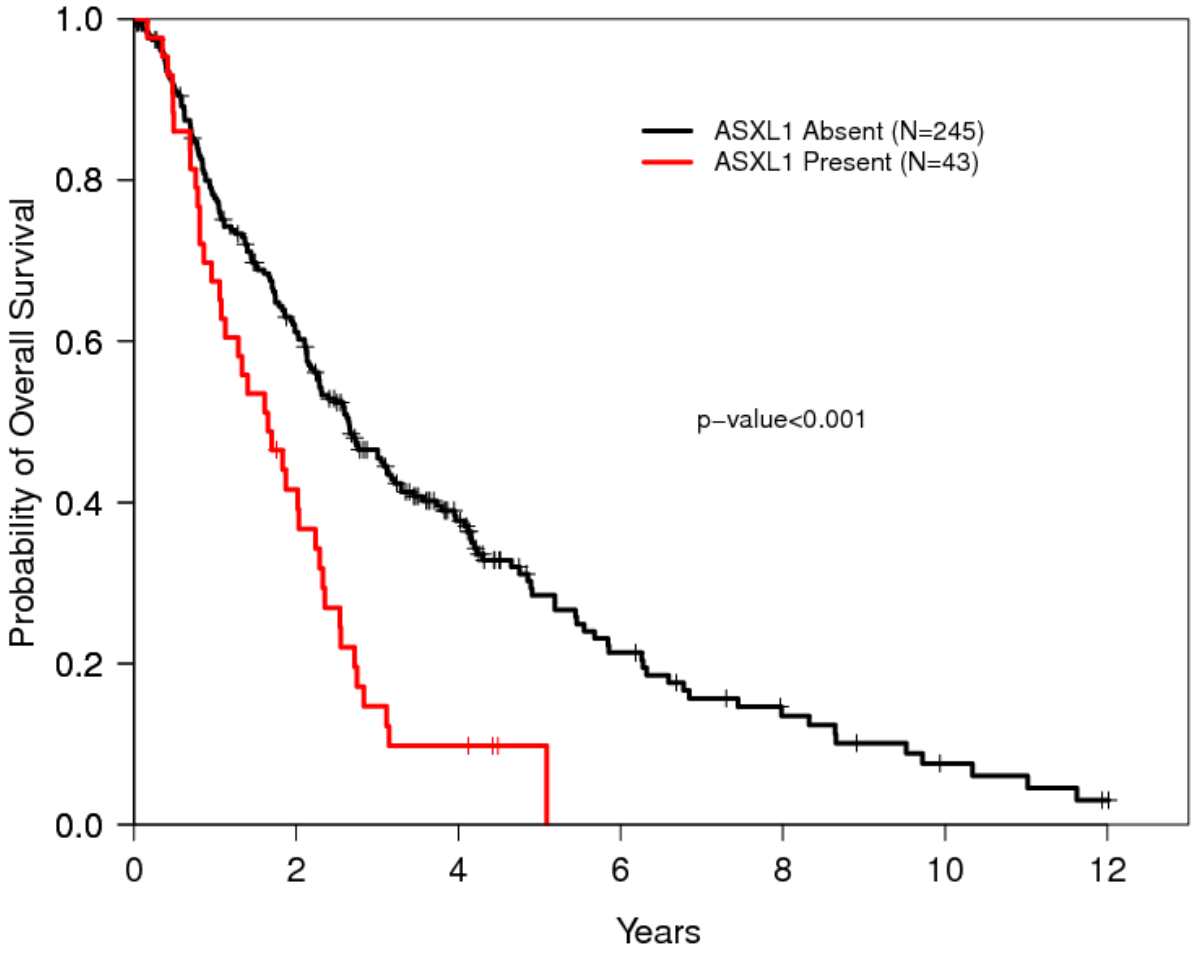
C) *SRSF2* mutations:



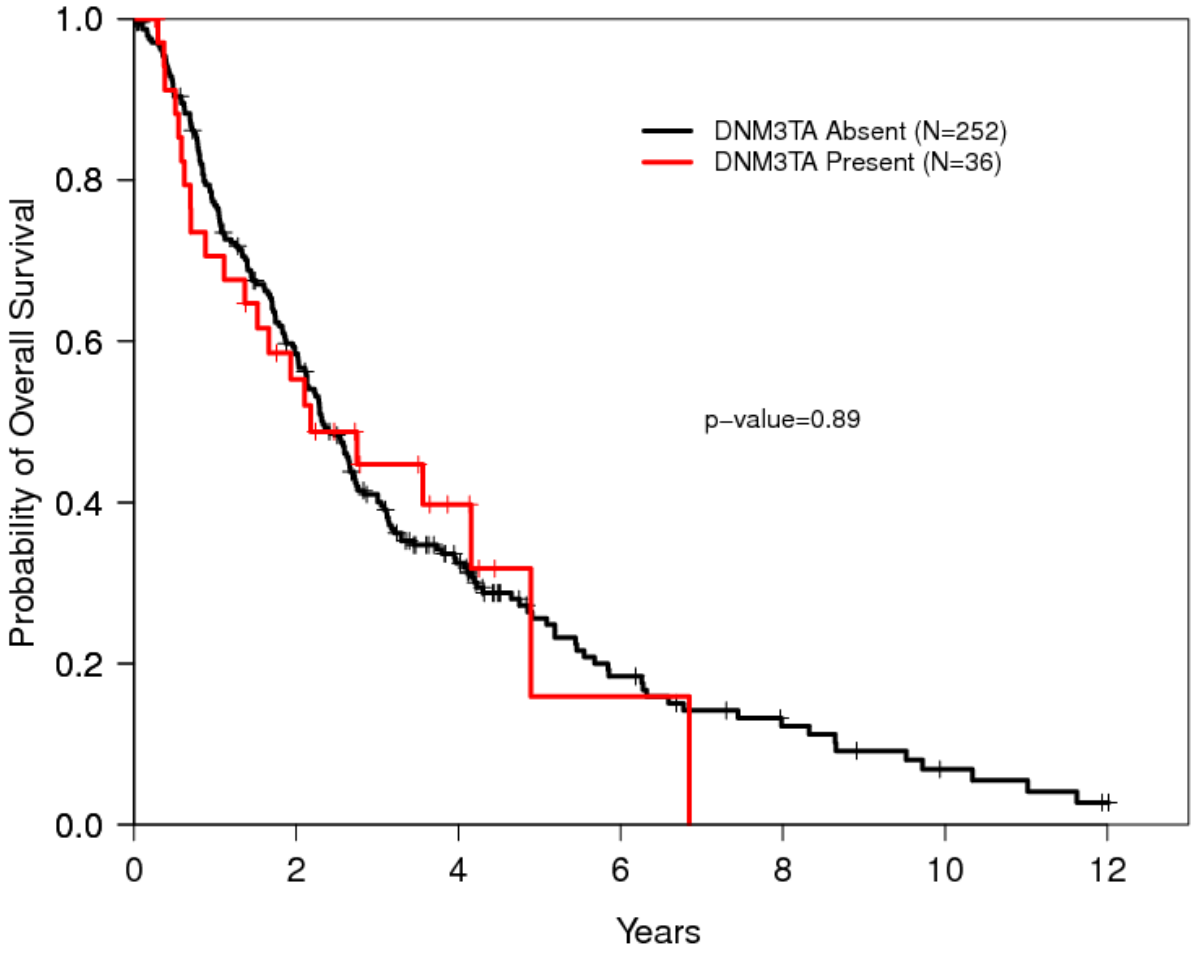
D) *U2AF1* mutations:



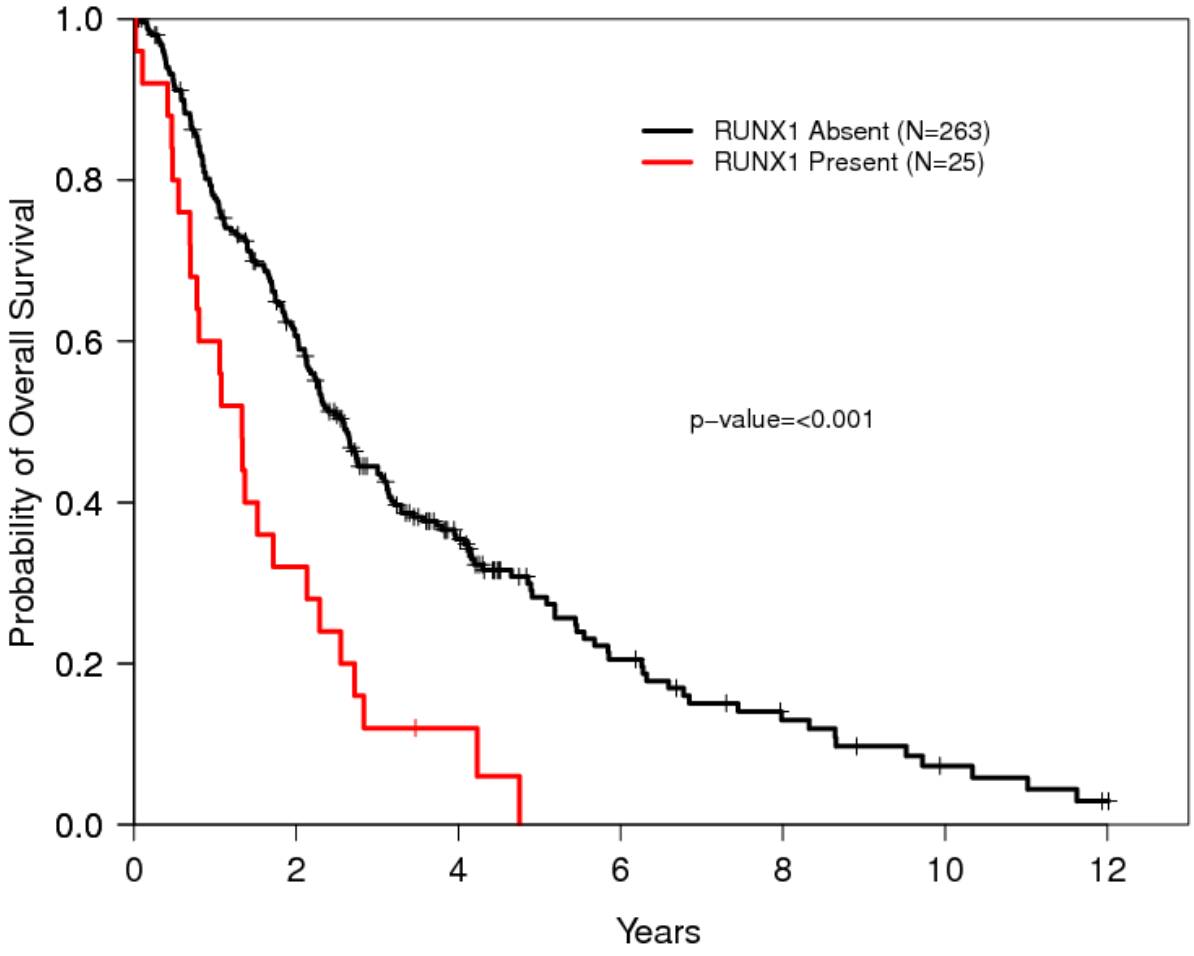
E) *ASXL1* mutations:



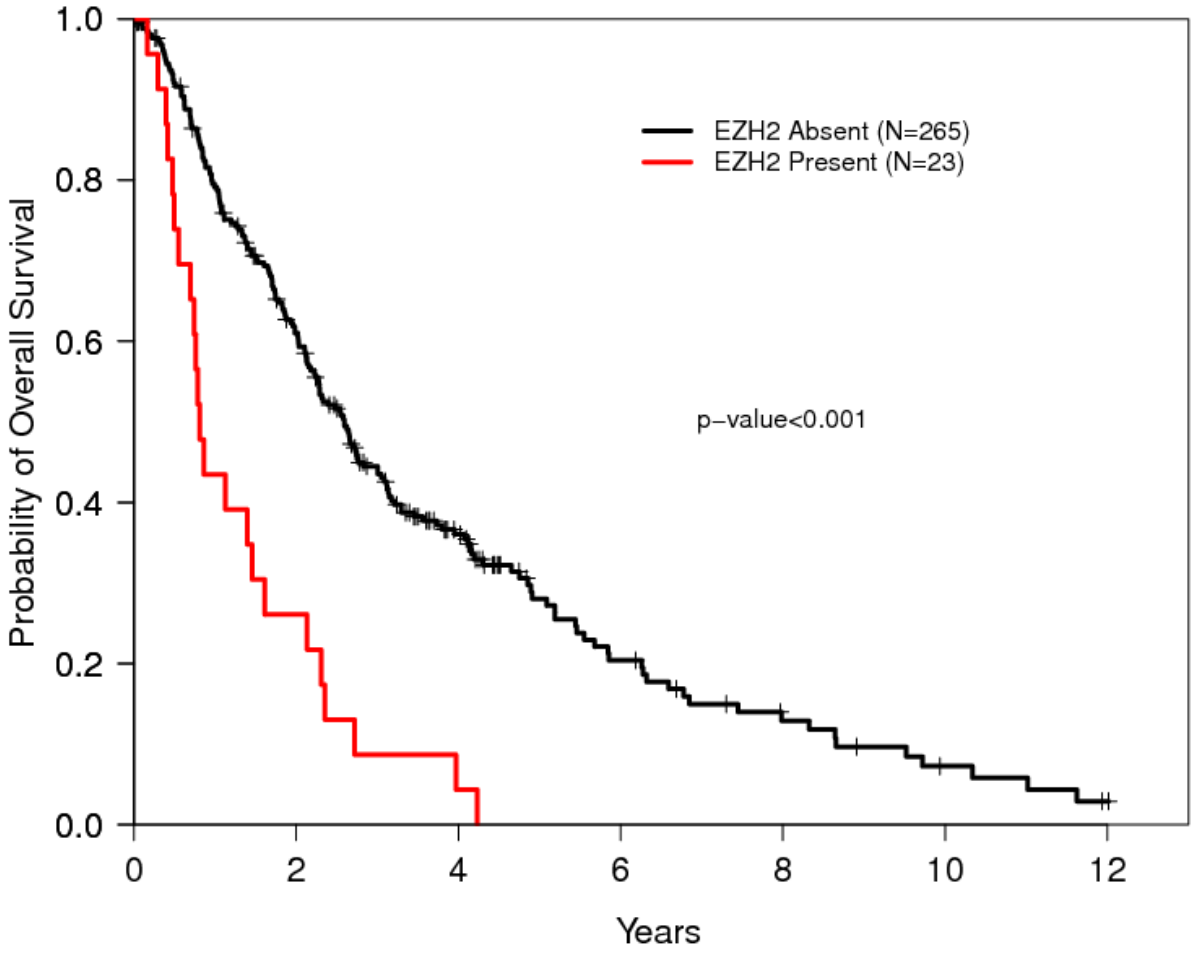
F) *DNMT3A* mutations:



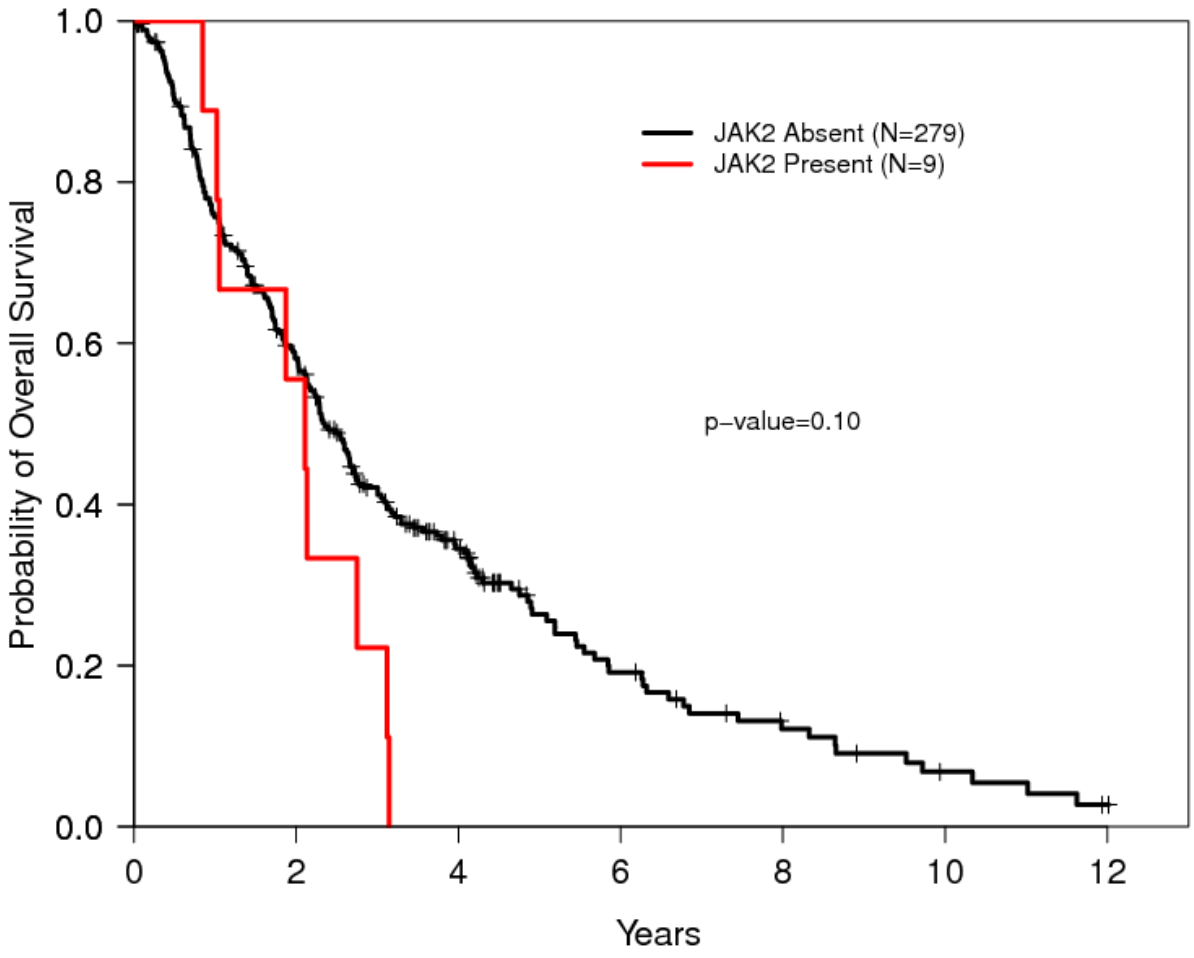
G) *RUNX1* mutations:



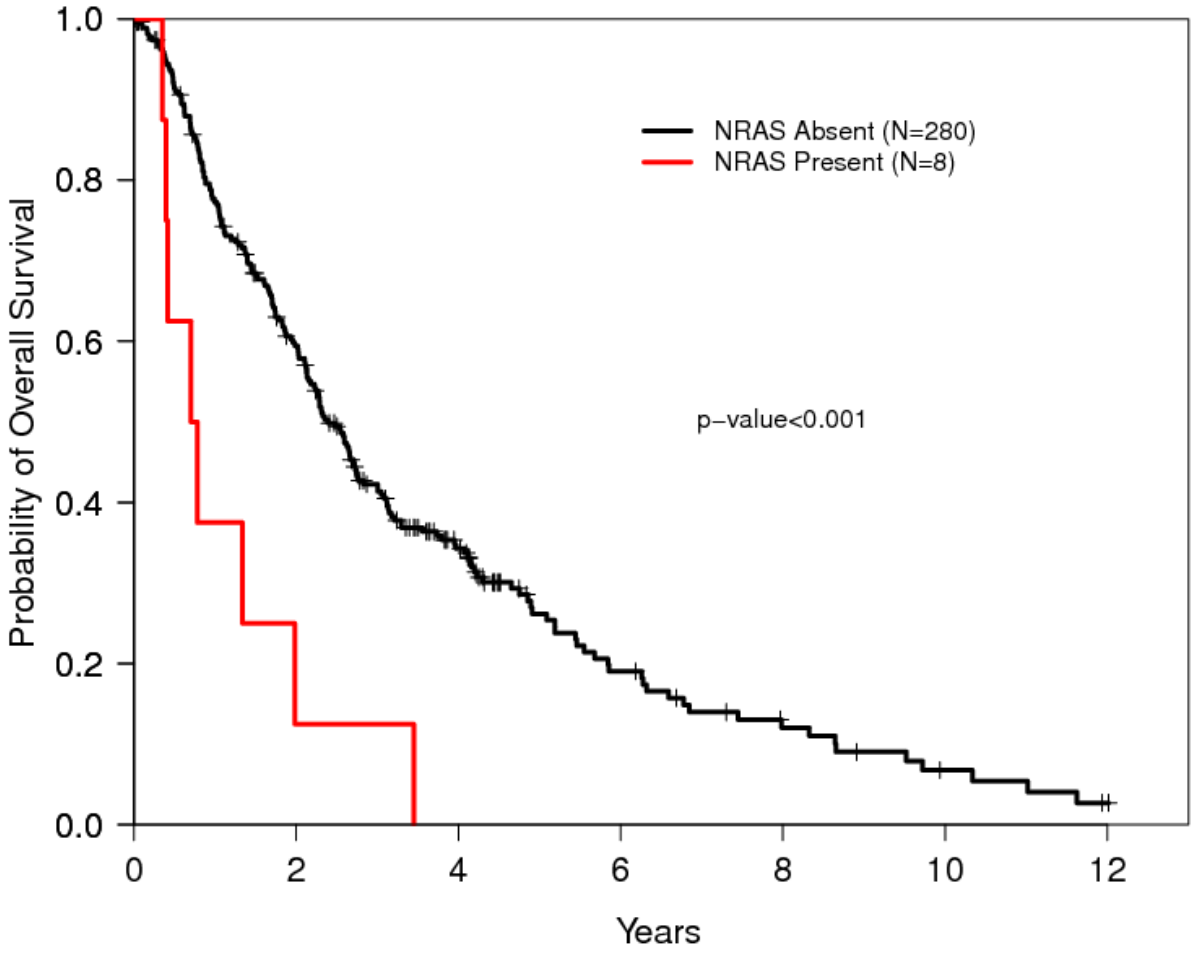
H) *EZH2* mutations:



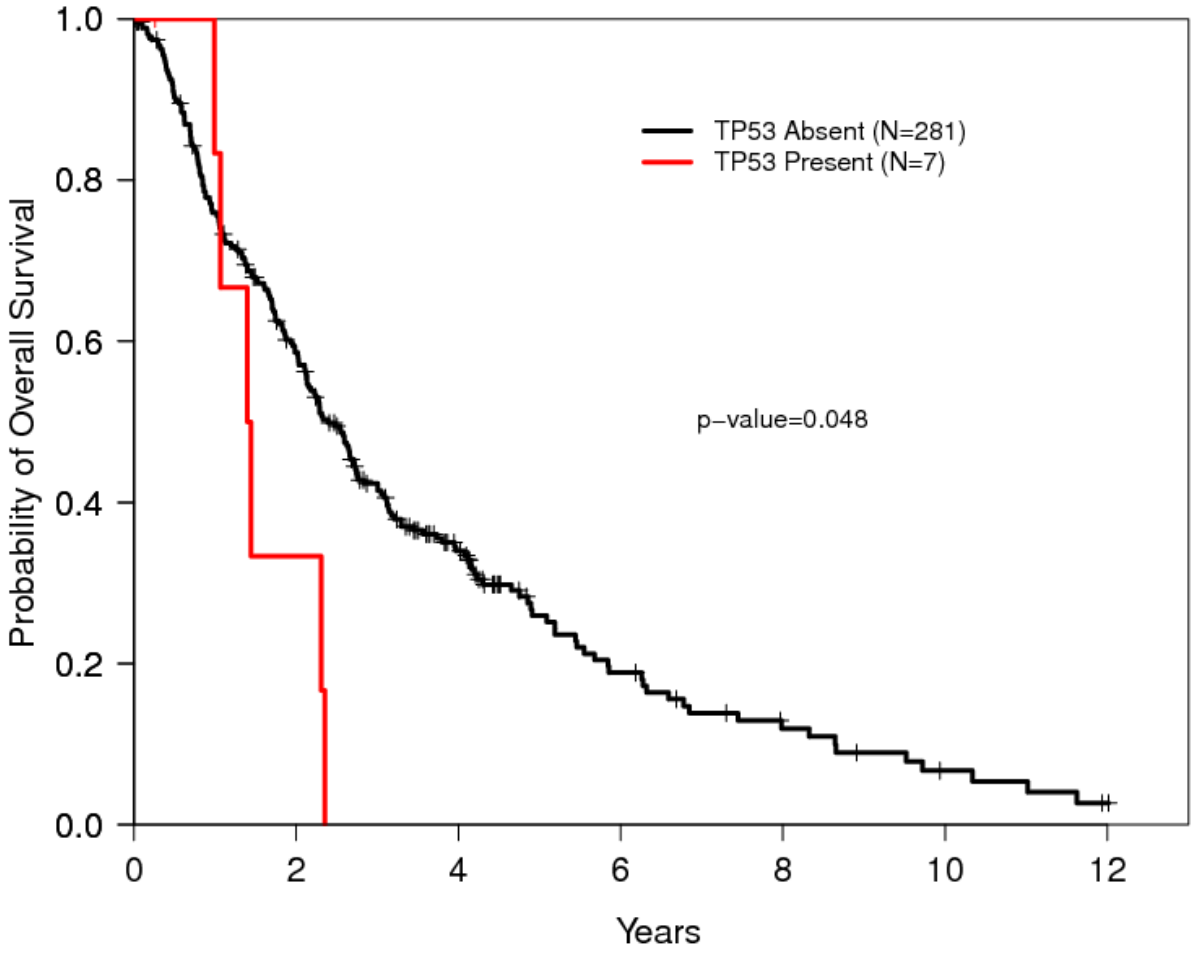
I) *JAK2* mutations:



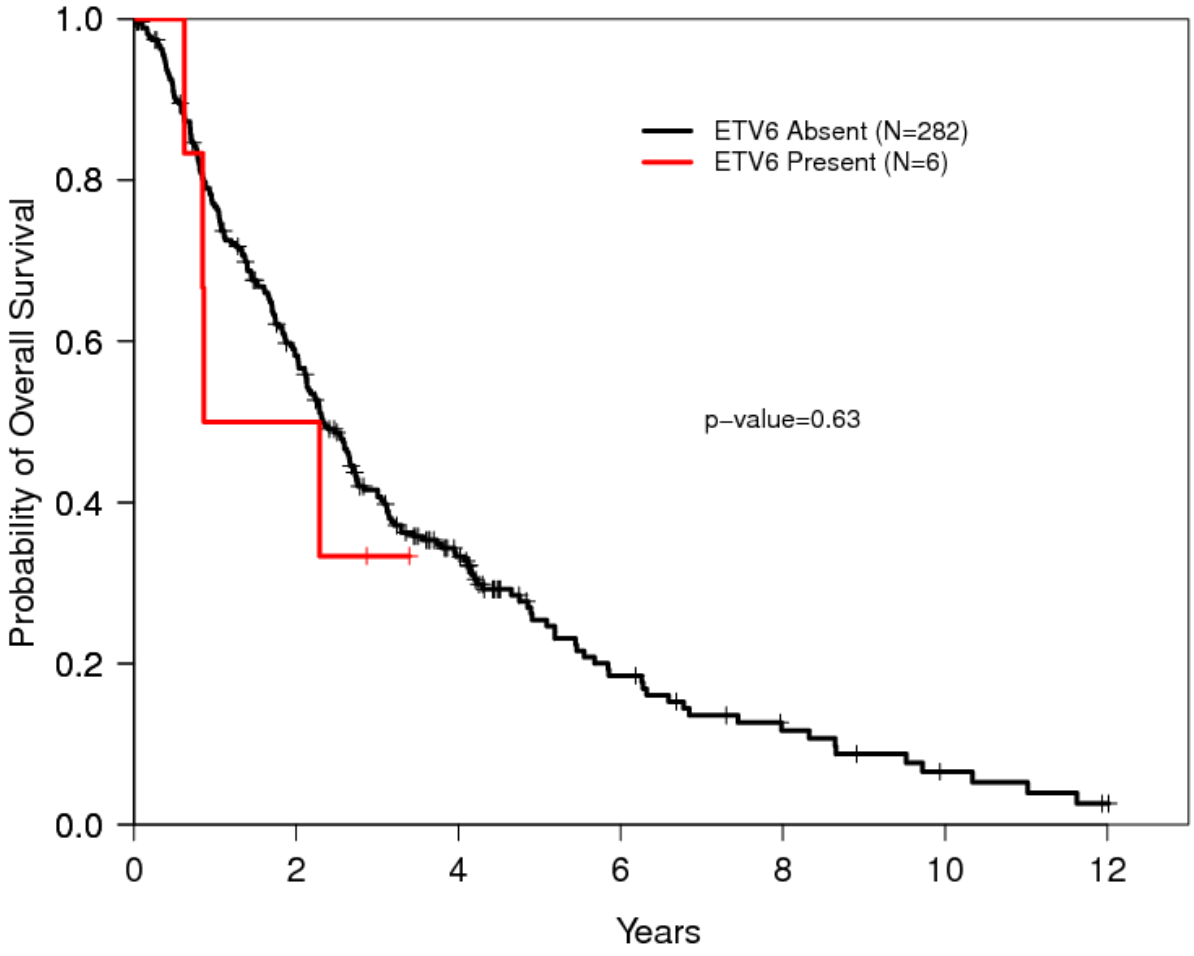
J) *NRAS* mutations:



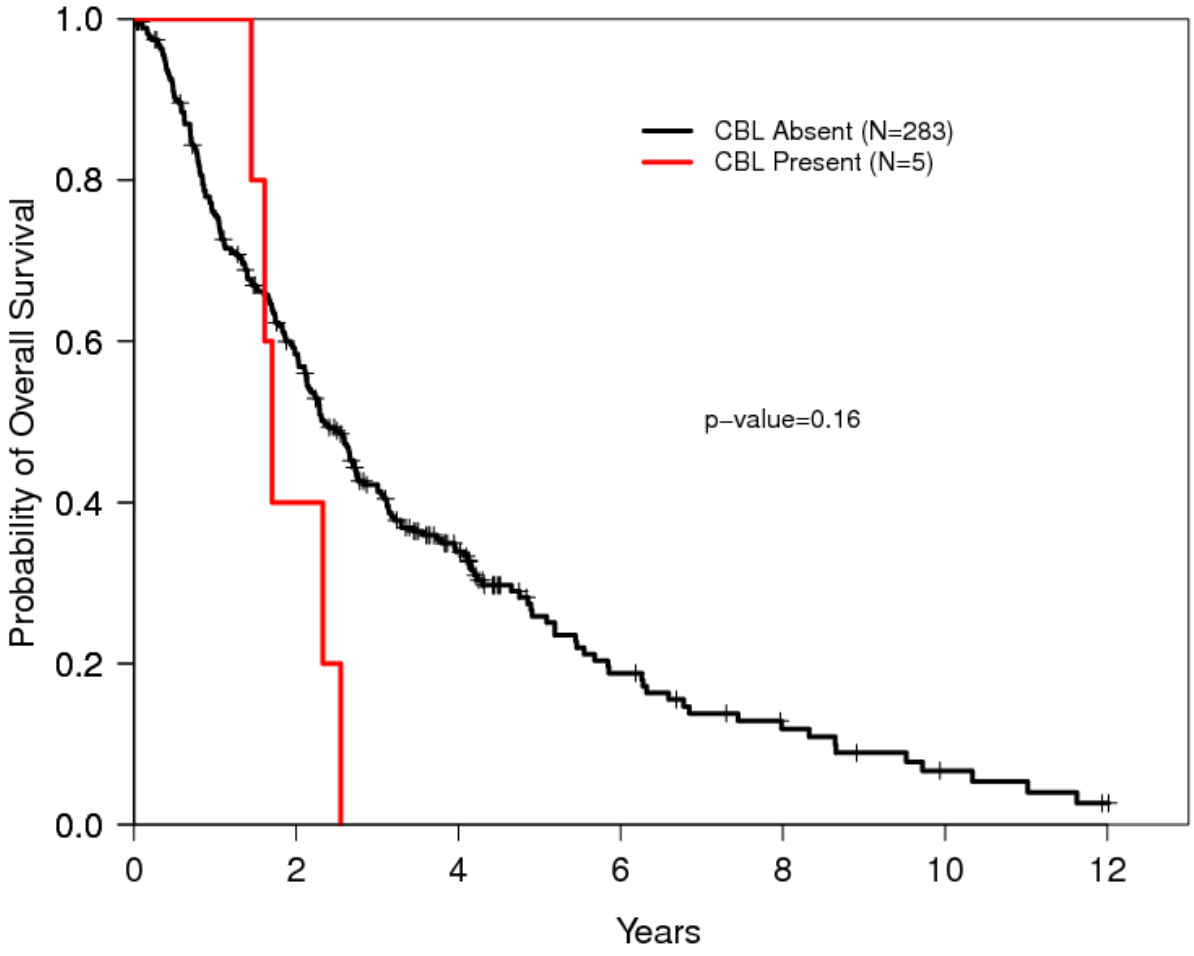
K) *TP53* mutations:



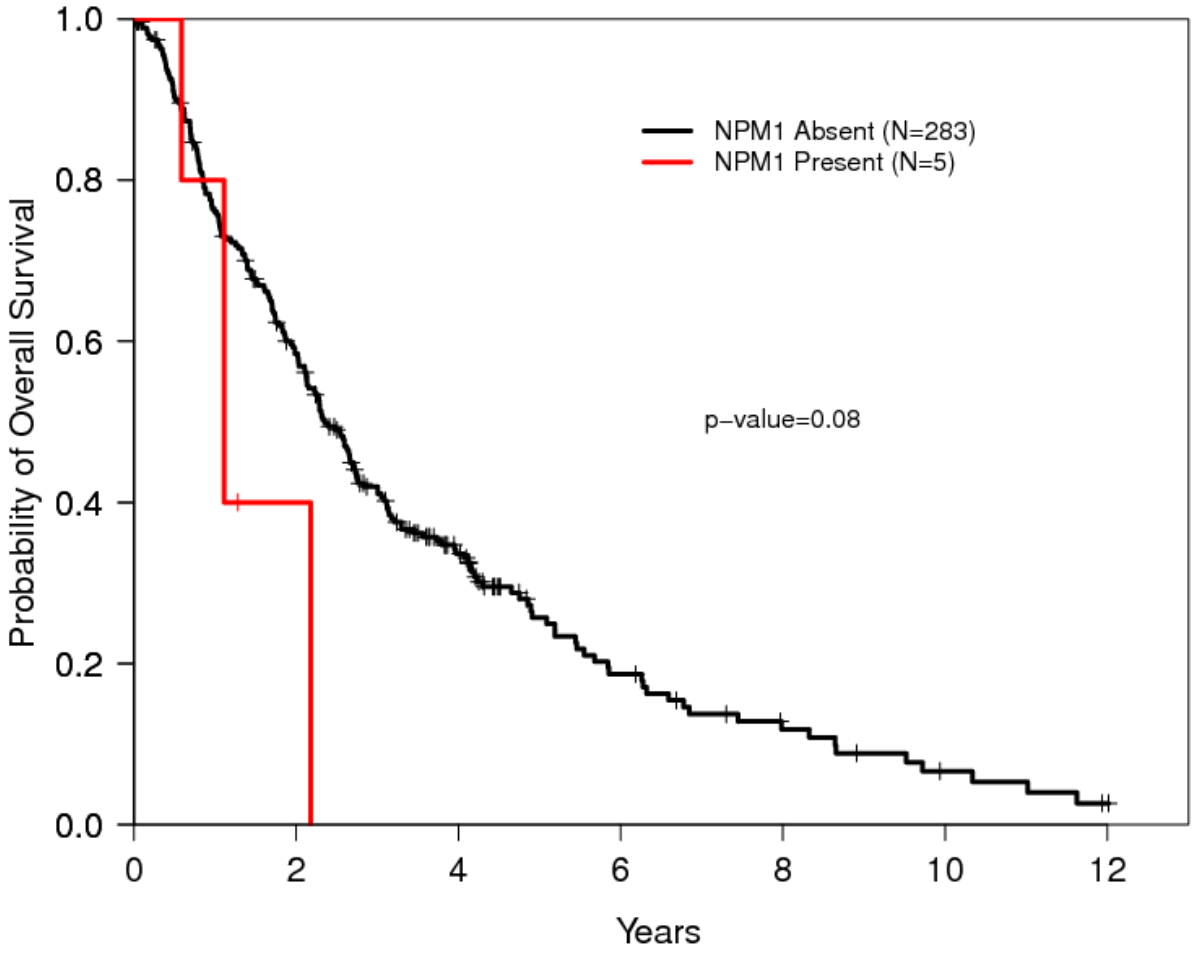
L) *ETV6* mutations:



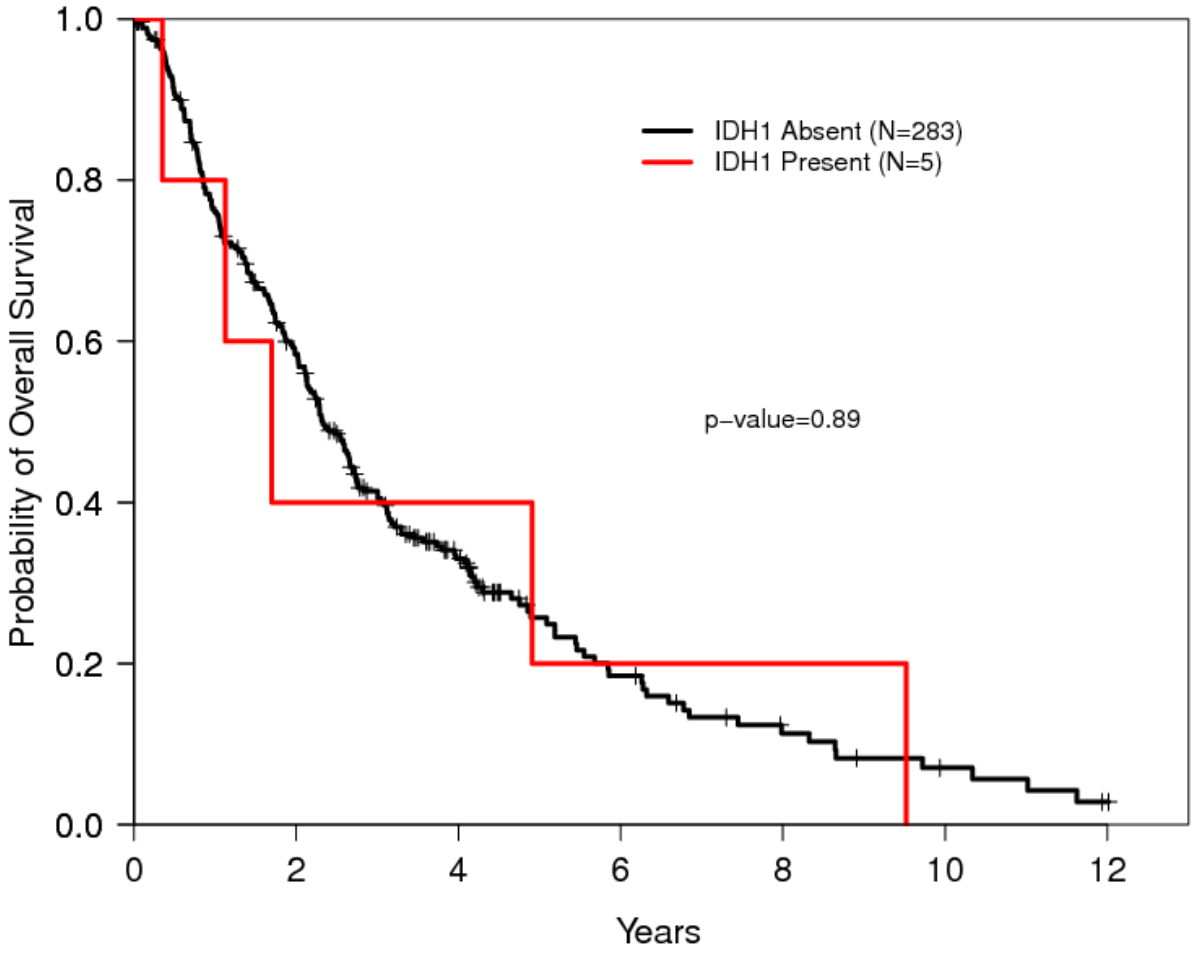
M) *CBL* mutations:



N) *NPM1* mutations:

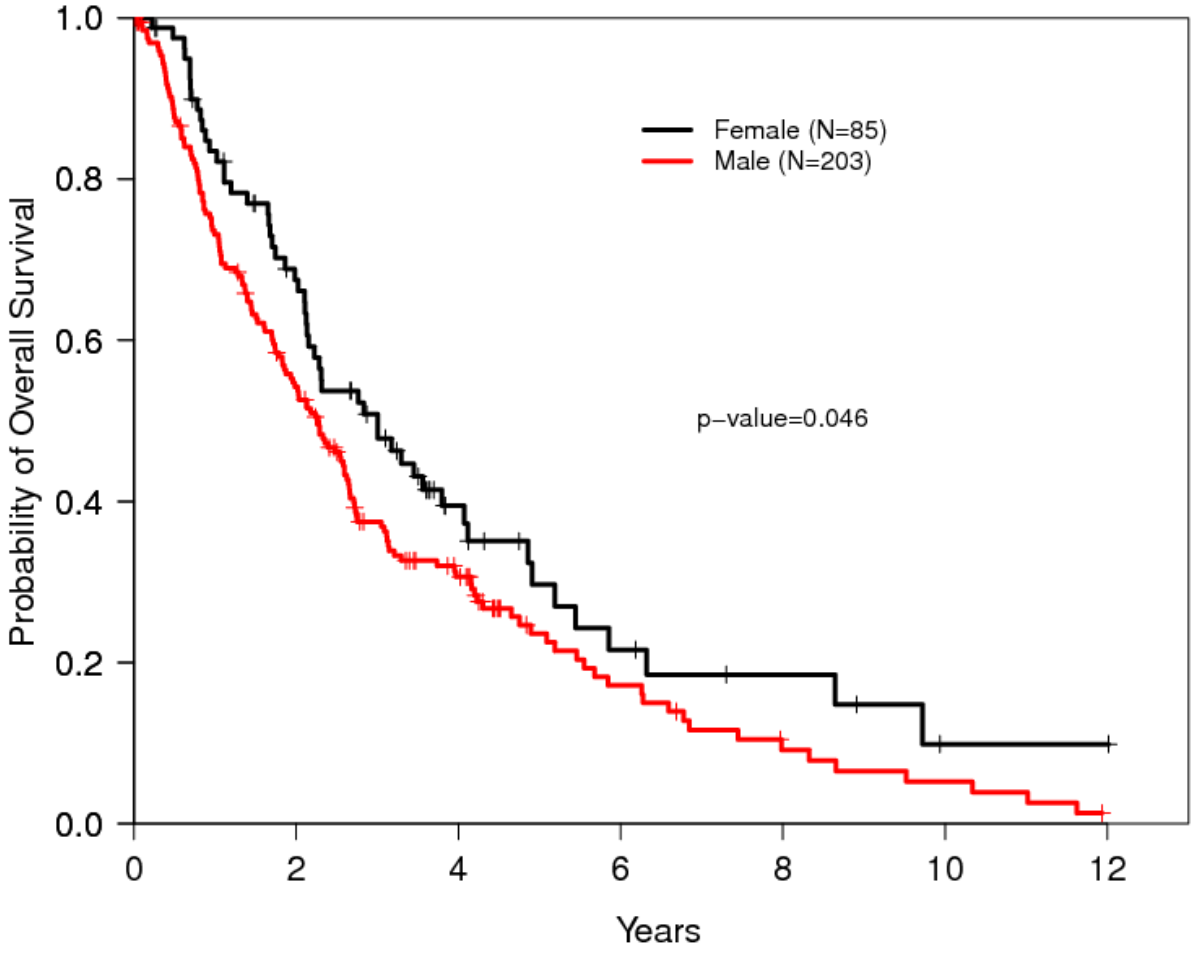


O) *IDH1* mutations:

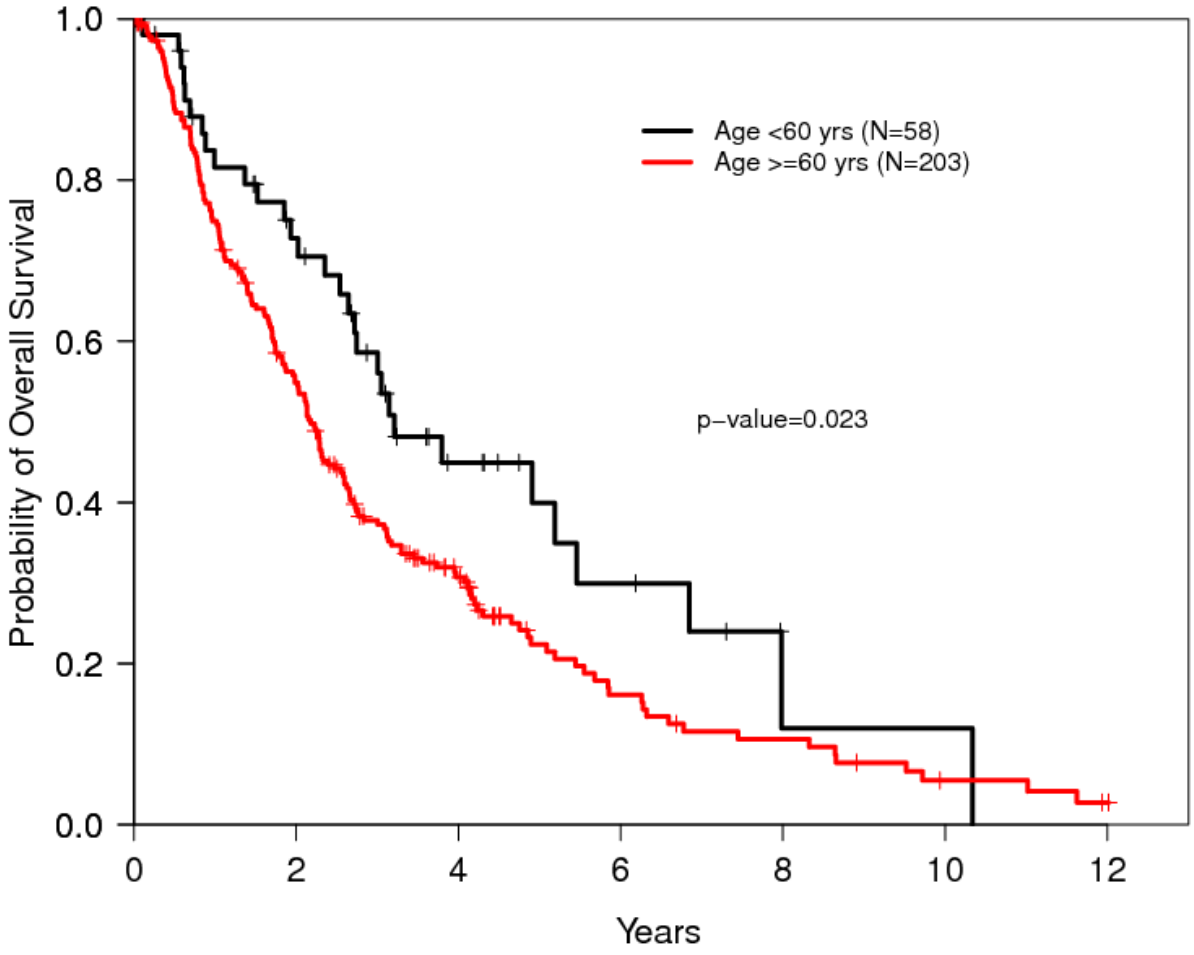


Supplementary Figure 4: Kaplan-Meier Overall Survival According to Clinical Features.

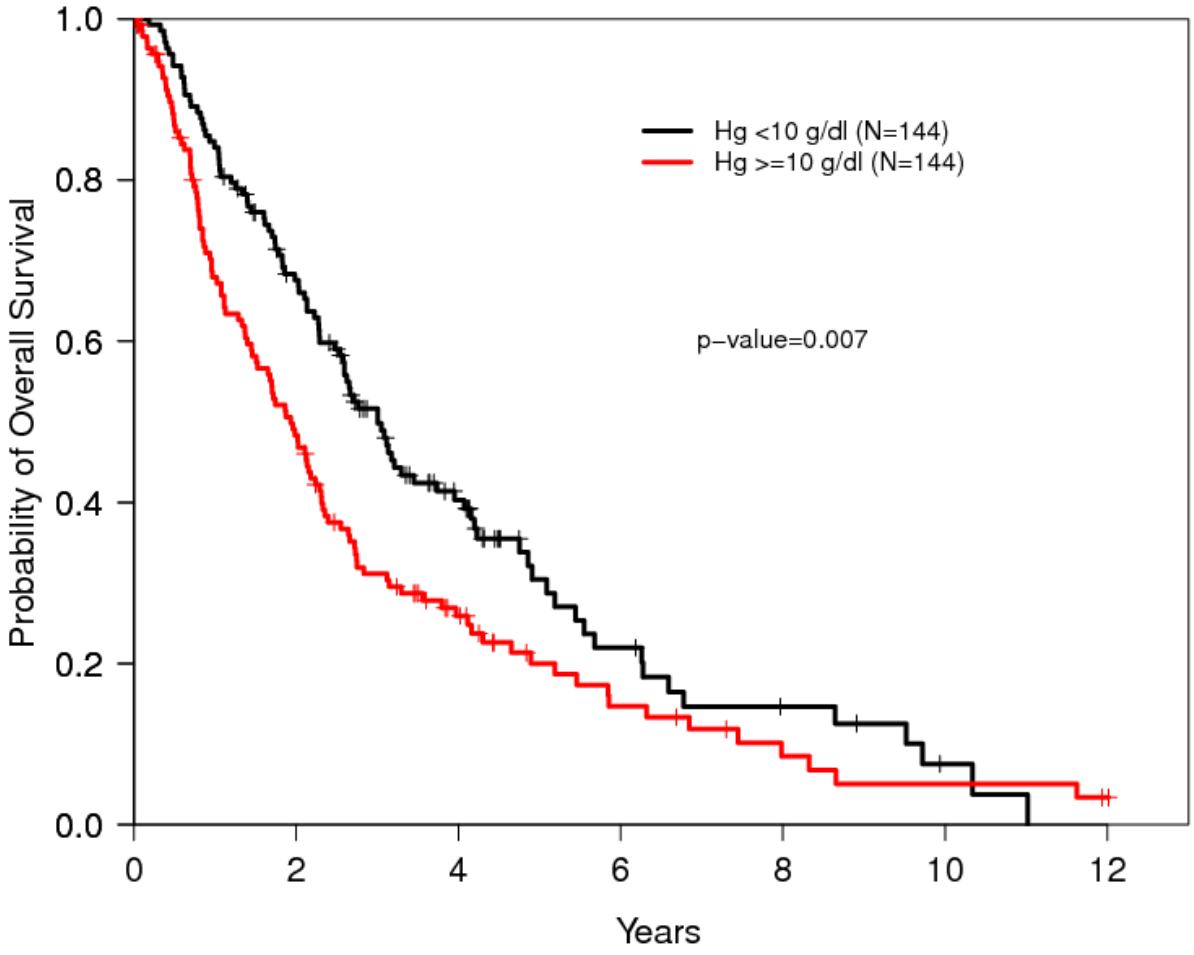
A) Patient sex



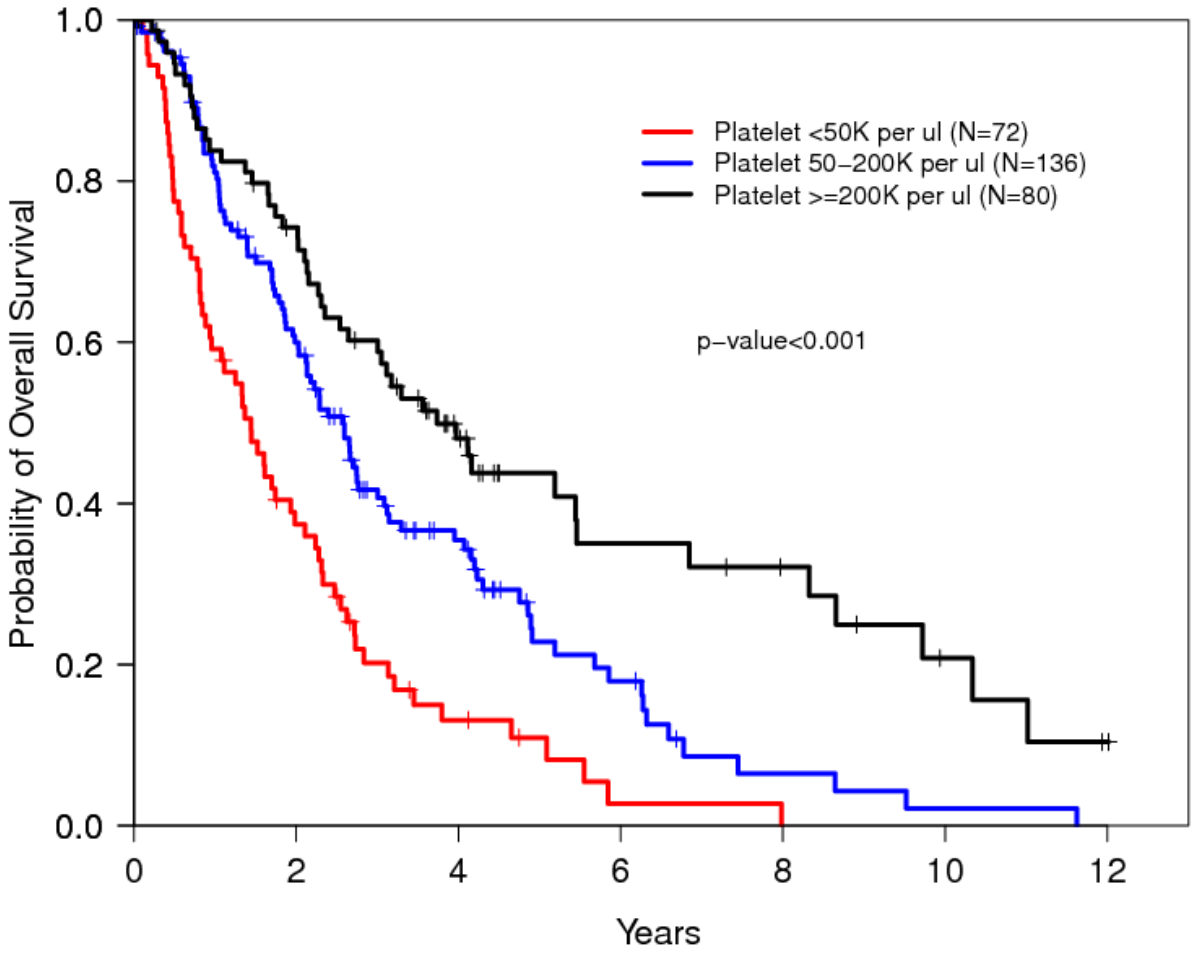
B) Patient age



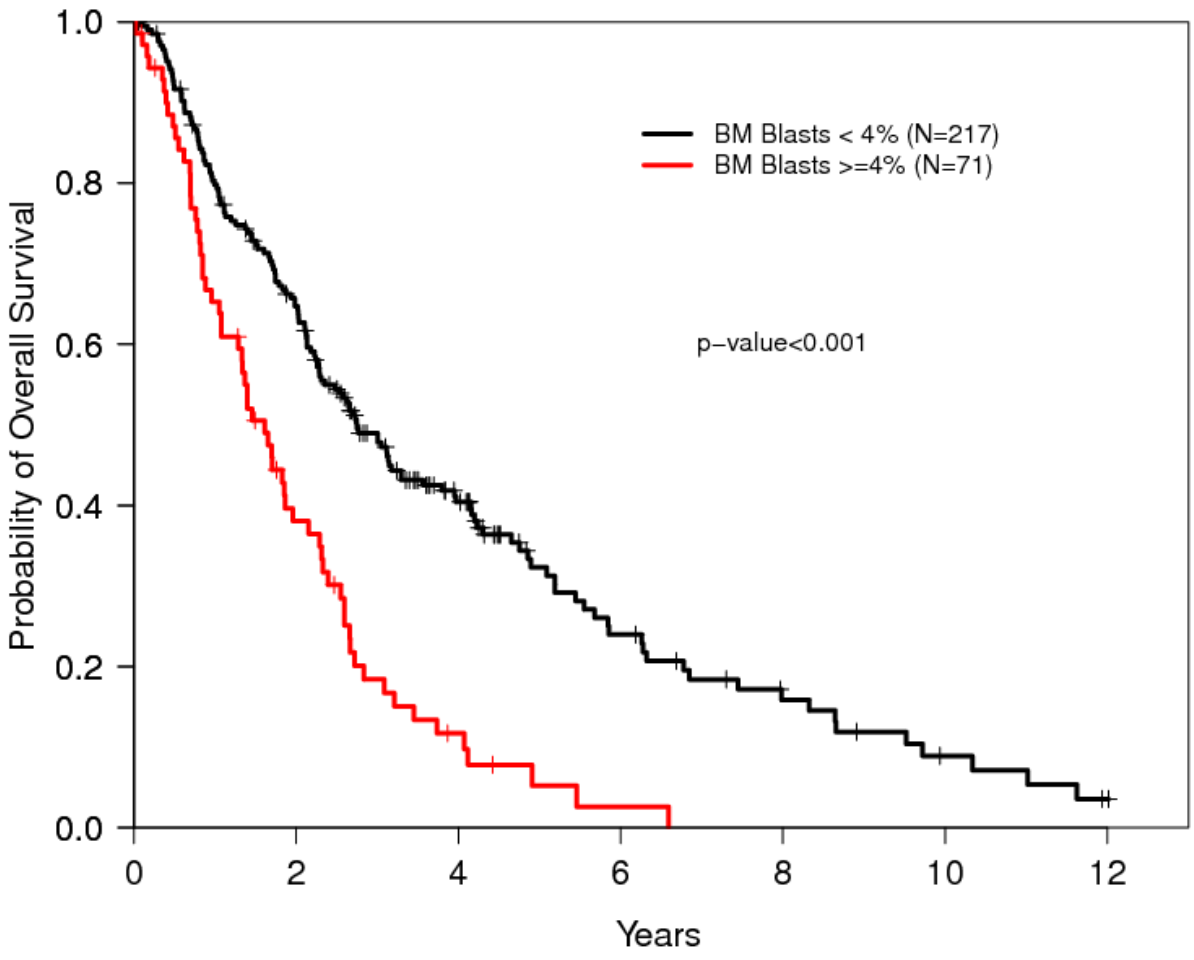
C) Hemoglobin level



D) Platelet count



E) Bone marrow blast percentage



Supplementary Figure 5: Overall Survival of MDS Patients with *DNMT3A* Mutations Stratified by their *SF3B1* mutations status.