Supplementary Information:

Genetic Subtypes of Smoldering Multiple Myeloma are associated with Distinct Pathogenic Phenotypes and Clinical Outcomes

Tables and Figures with legends:

Supplementary Table 1A: Baseline demographics and clinical characteristics of the SMM patients in the primary cohort (n=214). Numerical Variables are described as medians and the range of values, while categorical variables are presented as numbers and percentages.

	Total
	n = 214 (%)
Age	
Median (range)	62 (34 - 85)
Sex	
Female	115 (54)
Male	99 (46)
Race	
Black or African American	10 (5)
White	204 (95)
BM % involvement	
Median (range)	0.250 (0.028 - 0.800)
M-spike	
Median (range)	1.80 (0.00 - 5.17)
LDH	
Median (range)	174.5 (92.0 - 562.0)
Missing	*24 (11)*
β2M	
Median (range)	2.40 (0.80 - 9.10)
Mayo, 2008	
Low	48 (22)
Intermediate	136 (64)
High	30 (14)
20-2-20 Risk score	
Low	53(25)
Intermediate	63(29)
High	98(46)

Supplementary Table 1B: Significant genetic features in the six identified clusters that were positively associated with each cluster in the primary cohort (n=214). The features were determined by a one-sided Fisher test and ranked by significance (Benjamini-Hochberg adjusted p value < 0.1).

Cluster	Genetic feature	P value	Adjusted P value
HL1	NRAS	3.23E-05	1.58E-03
	TRAF3	8.11E-05	1.99E-03
	MAX	1.27E-03	1.55E-02
	FAM46C	8.40E-03	8.23E-02
HL2	16q_del	6.26E-09	3.07E-07
	t(14;20)	6.60E-07	1.62E-05
	6q_del	1.87E-06	3.05E-05
	MAFB	1.19E-05	1.46E-04
	BRAF	3.12E-04	3.06E-03
	del_20q	7.52E-04	6.14E-03
	del_18q	1.27E-03	8.88E-03
	NRAS	1.64E-03	1.00E-02
	DUSP2	3.71E-03	1.82E-02
	1p_del	5.19E-03	2.12E-02
	4q_del	4.47E-03	1.99E-02
	TP53	8.40E-03	2.74E-02
	ATM	8.40E-03	2.74E-02
	NFKB2	8.40E-03	2.74E-02
	17p_del	1.46E-02	4.46E-02
	LTB	1.62E-02	4.67E-02
HL3	KRAS	6.38E-07	3.13E-05
	NFKBIA	3.71E-03	6.06E-02
	MYC translocation	3.71E-03	6.06E-02
HL4	2p_amp	2.36E-06	1.16E-04
	NFKB2	3.13E-04	7.67E-03

Cluster	Genetic feature	P value	Adjusted P value
TL1	t(14;16)	6.29E-08	2.87E-06
	t(4;14)	1.17E-07	2.87E-06
	14q_del	1.87E-06	3.05E-05
	DIS3	1.10E-04	7.54E-04
	MAF	8.11E-05	7.54E-04
	17p_del	1.39E-04	7.54E-04
	12p_del	1.10E-04	7.54E-04
	8p_del	1.33E-04	7.54E-04
	10p_del	1.10E-04	7.54E-04
	HIST1H1E	9.01E-04	4.01E-03
	ZNF292	1.27E-03	4.78E-03
	SETD2	1.27E-03	4.78E-03
	PRKD2	3.71E-03	1.07E-02
	22q_del	3.31E-03	1.07E-02
	9q_amp	3.71E-03	1.07E-02
	ATM	8.40E-03	2.06E-02
	EGR1	8.40E-03	2.06E-02
TL2	t(11;14)	1.54E-19	7.55E-18
	11q_amp	3.12E-04	7.65E-03

Supplementary Table 2: Baseline demographics and clinical characteristics of the 87 untreated SMM patients. These patients were followed for disease progression and their clinical stratification was according to the 20/2/20 clinical risk model. Variables in each group are described as medians with the range of values. Comparison between the different risk groups is done using Cuzick's trend test.

		20-2-20 clinical model			
	Total n = 87 (%)	**Low** n = 23 (27)	**Intermediate** n = 22 (26)	**High** n = 42 (55)	***p-value***
BM % involvement					
Median (range)	0.30 (0.10 - 0.80)	0.15 (0.10 - 0.20)	0.20 (0.10 - 0.55)	0.40 (0.20 - 0.80)	*< 0.001*†
M-spike					
Median (range)	1.69 (0.00 - 5.17)	1.18 (0.00 - 1.87)	1.34 (0.00 - 2.30)	2.16 (0.00 - 5.17)	*< 0.001*†
FLCr, Involved/uninvolved ratio					
Median (range)	19.3 (1.1 - 325.3)	2.9 (1.1 - 15.7)	11.1 (1.6 - 255.9)	48.6 (2.3 - 325.3)	*< 0.001*†
β2M					
Median (range)	2.50 (1.40 - 6.40)	2.50 (1.50 - 6.40)	2.35 (1.50 - 3.80)	2.50 (1.40 - 5.90)	*0.95*†
Hemoglobin					
Median (range)	12.6 (8.3 - 16.2)	12.7 (8.3 - 15.4)	13.2 (11.0 - 16.2)	12.4 (10.3 - 15.2)	*0.14*†
LDH					
Median (range)	156 (92 - 422)	147 (92 - 422)	172 (120 - 262)	160 (104 - 364)	*0.21*†
Missing	*15 (18)*	*6 (26)*	*7 (32)*	*2 (5)*	
Calcium					
Median (range)	9.60 (8.70 - 10.50)	9.60 (8.70 - 10.50)	9.45 (8.80 - 10.00)	9.60 (9.00 - 10.10)	*0.82*†
Creatinine					
Median (range)	0.90 (0.60 - 1.60)	0.90 (0.69 - 1.40)	0.99 (0.60 - 1.60)	0.90 (0.60 - 1.40)	*0.12*†
Missing	*1 (1)*	-	*1 (5)*	-	
Total protein					
Median (range)	8.1 (5.5 - 11.0)	7.7 (6.8 - 8.4)	7.8 (5.5 - 8.8)	8.6 (5.5 - 11.0)	*< 0.001*†
Albumin					
Median (range)	4.1 (2.9 - 5.0)	4.2 (3.5 - 5.0)	4.2 (3.5 - 4.8)	4.0 (2.9 - 4.8)	*0.10*†
Missing	*1 (1)*	*1 (4)*	-	-	
Globulin					
Median (range)	3.8 (0.3 - 7.8)	3.7 (2.2 - 4.6)	3.5 (2.0 - 4.6)	4.2 (0.3 - 7.8)	*0.003*†
Missing	*1 (1)*	*1 (4)*	-	-	
Albumin/Globulin ratio					
Median (range)	1.1 (0.4 - 13.0)	1.1 (0.8 - 2.3)	1.2 (0.9 - 1.8)	0.9 (0.4 - 13.0)	*0.005*†
Missing	*1 (1)*	*1 (4)*	-	-	

Supplementary Table 3: List of genetic alterations that were significantly enriched in High-risk genetic subgroups (HL2, TL1, HL3) in the primary cohort (n=214). The features were determined by a two-sided Fisher test and ranked by significance (Benjamini-Hochberg adjusted p value < 0.1).

Genetic alteration	P value	Adjusted P value
16q_del	3.92133701244461E-19	1.88224176597341E-17
6q_del	1.30661737533785E-16	3.13588170081083E-15
del_22q	3.46052641707861E-15	5.53684226732578E-14
del_20q	9.81983940020603E-14	1.17838072802472E-12
KRAS	5.74309437412292E-12	5.09021145715801E-11
1p_del	6.36276432144751E-12	5.09021145715801E-11
del_8p	1.076934966262E-11	7.38469691151089E-11
17p_del	2.66210661752379E-10	1.59726397051427E-09
t(4;14)	9.59521520668869E-10	5.11744811023397E-09
14q_del	7.16829126182379E-09	3.44077980567542E-08
del_4q	1.03923060318069E-08	4.534824450243E-08
ATM	6.28534662885084E-08	2.32074337065262E-07
t(14;16)	6.28534662885084E-08	2.32074337065262E-07
DIS3	7.72727909076587E-08	2.64935283111973E-07
BRAF	5.07514260624449E-07	1.52254278187335E-06
LTB	6.5996139602957E-07	1.6672708952326E-06
HIST1H1E	6.5996139602957E-07	1.6672708952326E-06
t(14;20)	6.5996139602957E-07	1.6672708952326E-06
NFKBIA	7.64717173177041E-07	1.8353212156249E-06
del_10p	3.75087303233623E-06	8.18372297964268E-06
TP53	6.63104069343861E-06	1.32620813868772E-05
ZNF292	1.19077388394712E-05	1.97093608377455E-05
MAFB	1.19077388394712E-05	1.97093608377455E-05
1q_gain	3.91221254141483E-05	6.25954006626372E-05
FAM46C	4.98897812477866E-05	7.72486935449599E-05
MAF	8.10600203567709E-05	0.00011116802791785700

Supplementary Table 4: Performance of the Clinical Models only and with adding the Genetic Model (based on the genetic risk groups). Improvement in goodness of fit was assessed with a likelihood ratio test. The genetic model significantly improved the fit of the clinical-only models. A global assessment of each model was also assessed using a C-statistic for censored survival data. The statistic for each time-to-event model is reported with a 95% CI. Values range from 0.5 to 1, which indicates a poor to perfect model.

Cohort	Model	Likelihood Ratio Test Statistic	χ2 *P*	Model C-statistic (95% CI)	AIC/BIC
Primary (DFCI, UCL, Greece) (n=87)	Clinical	14.7	< 0.001	0.71 (0.64 - 0.78)	406/411
	Clinical + Genetic			0.76 (0.68 - 0.83)	396/404
Validation (UAMS) (n=75)	Clinical	12.1	0.002	0.65 (0.49 - 0.80)	165/168
	Clinical + Genetic			0.76 (0.62 - 0.90)	157/162
Validation (UAMS, Mayo) (n=142)	Clinical	13.53	0.001	0.66 (0.59 - 0.74)	567/571
	Clinical + Genetic			0.71 (0.64 - 0.78)	560/569
Combined (Primary, UAMS, Mayo) (n=229)	Clinical	29.84	< 0.001	0.69 (0.64 - 0.73)	1146/1151
	Clinical + Genetic			0.74 (0.69 - 0.78)	1123/1135

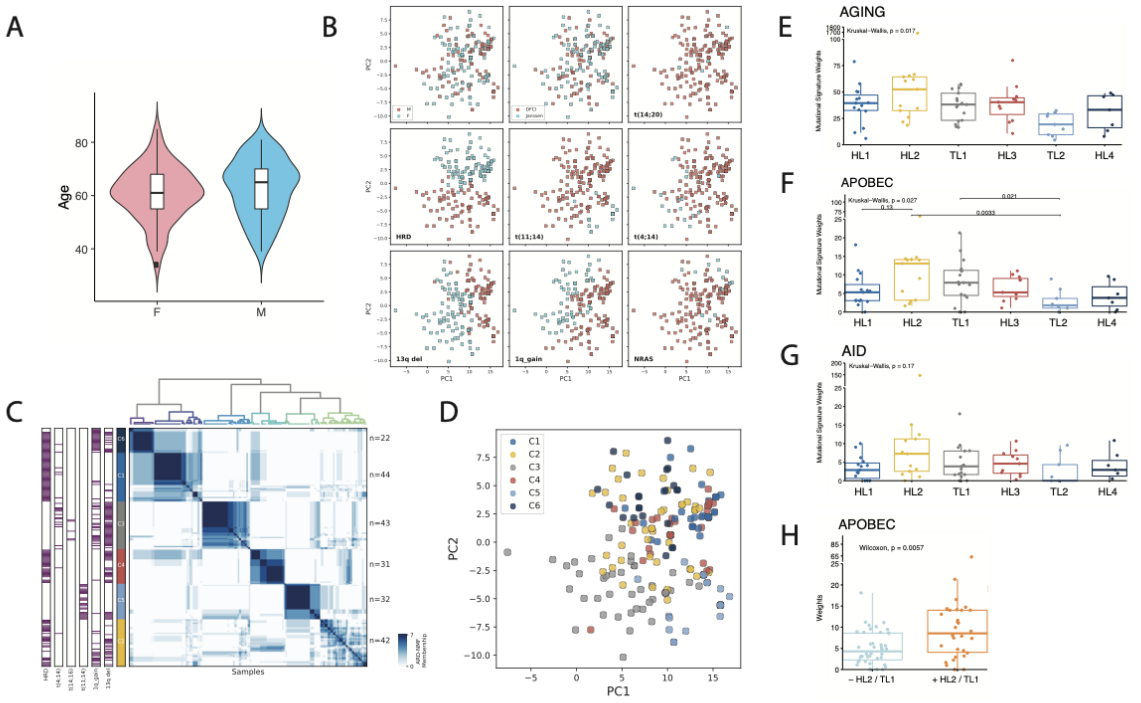
Supplementary Table 5: Univariate Cox PFS regression of the high-risk genetic features in the primary cohort (n = 87). Number of patients with event and percentages from the total number of patients evaluable, HR hazards ratio, error bars indicate 95% CI. All p values are two-sided. Naïve and corrected p-values are presented. Multiple testing correction was done by Benjamini-Hochberg method with p value < 0.1.

		Estimates		**p-value**	
Variable	**No. of patients (%)**	**HR**	**95% CI**	**naive**	**corrected**
MYC alterations	7 (9)	7.58	3.02 - 19.03	< 0.001	< 0.001
KRAS	12 (14)	4.13	2.10 - 8.12	< 0.001	< 0.001
del(1p)	7 (8)	4.24	1.89 - 9.51	< 0.001	0.003
del(14q)	10 (12)	3.06	1.47 - 6.34	0.003	0.010
del(8p)	8 (9)	3.43	1.53 - 7.70	0.003	0.010
TP53	5 (6)	3.89	1.50 - 10.10	0.005	0.017
NRAS	5 (6)	3.23	1.27 - 8.21	0.014	0.035
del(6q)	11 (13)	2.46	1.18 - 5.11	0.016	0.035
del(22q)	7 (8)	2.97	1.24 - 7.09	0.014	0.035
t(4;14)	5 (6)	2.69	1.05 - 6.89	0.039	0.072
del(16q)	18 (21)	1.66	0.89 - 3.08	0.11	0.06
del(4q)	5 (6)	1.69	0.61 - 4.69	0.32	0.41
del(13q)	42 (49)	1.26	0.73 - 2.17	0.41	0.50
gain(1q)	21 (25)	1.6	0.90 - 2.83	0.47	0.12

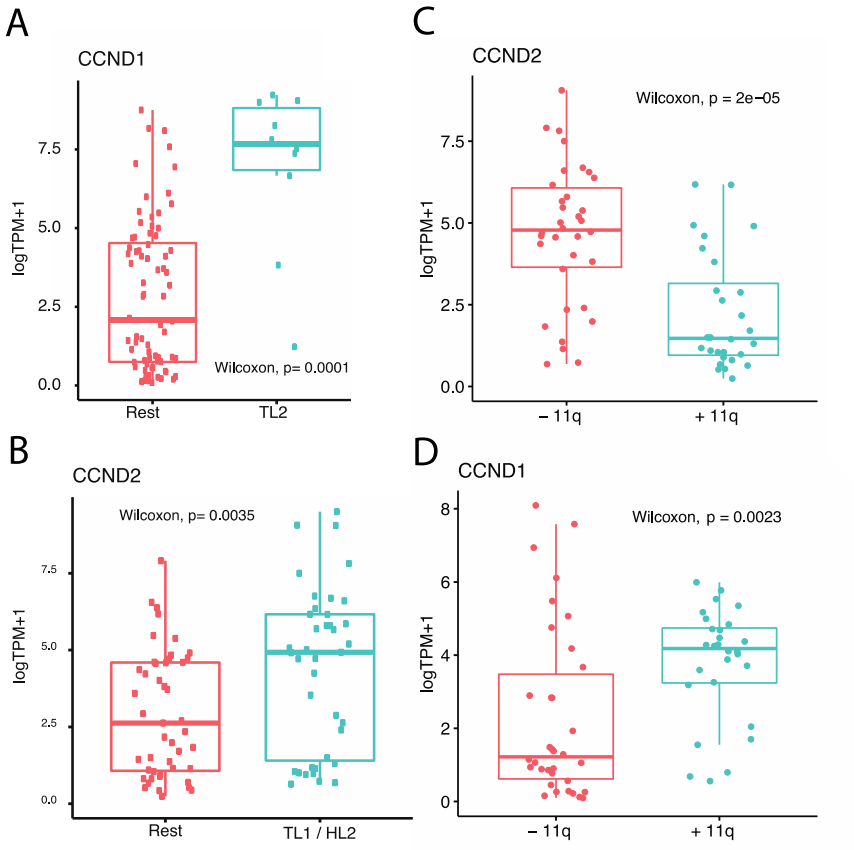
Supplementary Table 6: Univariate Cox PFS regression of the high-risk genetic features in the combined cohorts (n = 229). Number of patients with event and percentages from the total number of patients evaluable, HR hazards ratio, error bars indicate 95% CI. All p values are two-sided. Naïve and corrected p-values are presented. Multiple testing correction was done by Benjamini-Hochberg method with p value < 0.1.

		Estimates	**p-value**	
Variable	**No. of patients (%)**	**HR (95% CI)**	**naive**	**corrected**
MYC aberrations	48 (21)	1.9 (1.2-2.8)	0.0022	0.0055
KRAS	43 (19)	2.7 (1.9-4)	3.50E-07	<0.0001
gain_1q	60 (26)	1.5 (1-2.1)	0.047	0.0588
del_16q	38 (17)	1.4 (0.91-2.2)	0.12	0.1385
del_1p	24 (11)	2 (1.3-3.3)	0.0027	0.0058
del_8p	20 (9)	3.2 (1.9-5.4)	1.10E-05	0.0001
del_6q	29 (13)	2.6 (1.7-4.1)	2.10E-05	0.0001
t.4.14.	16 (7)	2.6 (1.5-4.6)	0.00082	0.0025
TP53_aberrations	14 (6)	2.6 (1.4-4.8)	0.0035	0.0066
DIS3	13 (6)	2.3 (1.2-4.3)	0.0082	0.0123
FAM46C	9 (4)	2.7 (1.3-5.7)	0.0067	0.0112
del_22q	22 (10)	1.7 (1-2.9)	0.039	0.0532
del_14q	23 (10)	2.6 (1.6-4.3)	0.00011	0.0004
t.14.16.	6 (3)	0.98 (0.35-2.7)	0.97	0.9700
ATM	6 (3)	2.1 (0.75-5.6)	0.16	0.1714

Supplementary Figure 1: Outline and results of the binary matrix factorization and consensus clustering. A) Age and sex distribution of primary cohort (n=214). B) Logistic principal components analysis (PCA) projection of binarized dataset (n=214). Green color denotes the presence of the variable of interest while red color indicates the rest of the dataset. C) Sample-sample consensus matrix of binary matrix factorization results (K=2-10) with translocations and copy number (left). D) LogisticPCA colored by subtypes. Mutational signature abundance per molecular subtype for E) Aging , F) APOBEC, and G) AID signatures. H) APOBEC mutational signature abundance in HL2/TL1 vs the other clusters. Two-sided p-value was calculated using the Wilcoxon rank-sum test. Boxplots representing median, and interquartile range, whiskers representing first, and fourth quartile



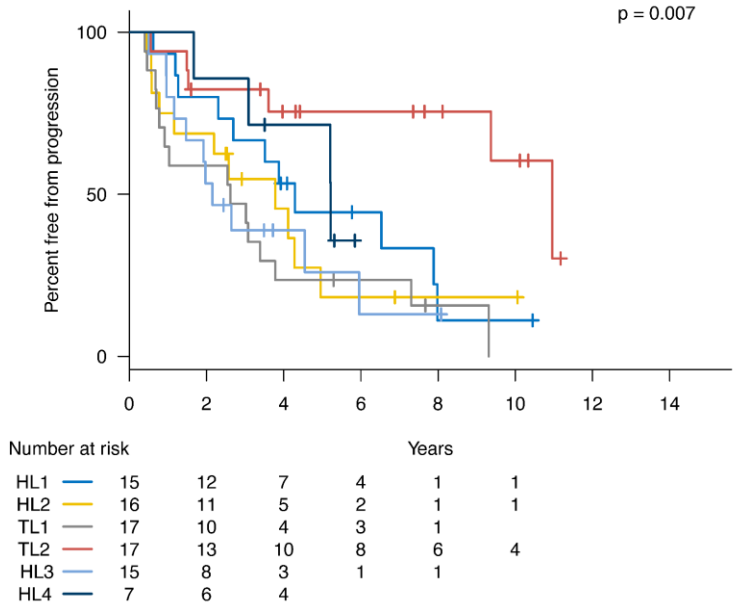
Supplementary Figure 2: Additional gene expression (log TPM + 1) comparisons (n=89). A) *CCND1* gene in TL2 tumors vs. the other tumors; B) *CCND2* gene in TL1 & HL2 tumors vs. the rest. C) *CCND2* expression in HL1-4 (Hyperdiploid) subtypes with and without 11q gain. D) *CCND1* expression in HL1-4 subtypes with and without 11q gain. Two-sided p-value was calculated using the Wilcoxon rank-sum test. Expression is measured by the log2 value of transcript per million of each gene (log2 TPM +1). Boxplots representing median, and interquartile range, whiskers representing first, and fourth quartile. Two-sided p-value was calculated using the Wilcoxon rank-sum test.



Supplementary Figure 3:

A) Kaplan-Meier curves for analysis of TTP according to the six genetic subtypes from the primary cohort. **B)** Cox proportional hazards analysis of the six genetic subtypes and in the primary cohort**. C)** Kaplan-Meier curves for analysis of TTP in patients from the primary cohort belonging to high vs intermediate and low-risk genetic subtypes in the clinically high-risk group by the 20-2-20 model. All p values are two-sided. Differences in survival curves and subsequent two-sided p values were calculated using the log-rank test. Forest plots are used to visualize the multivariate analysis. N: number of patients with event and percentages from the total number of patients evaluable, HR hazards ratio, error bars indicate 95% CI.

A)



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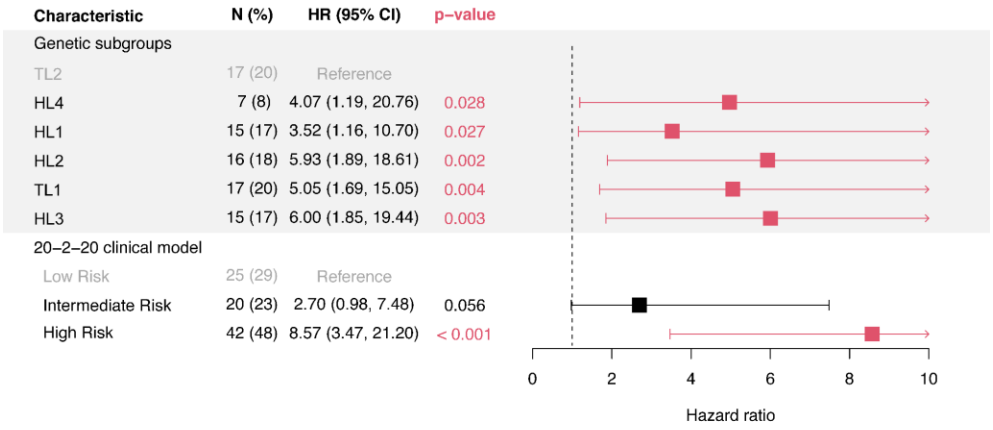
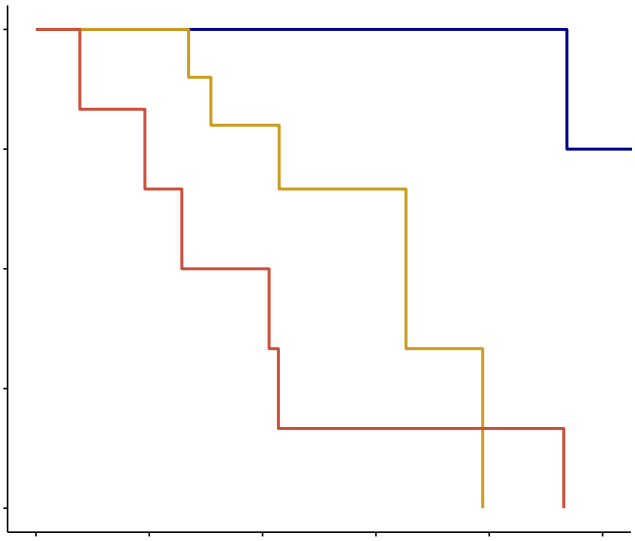


C)

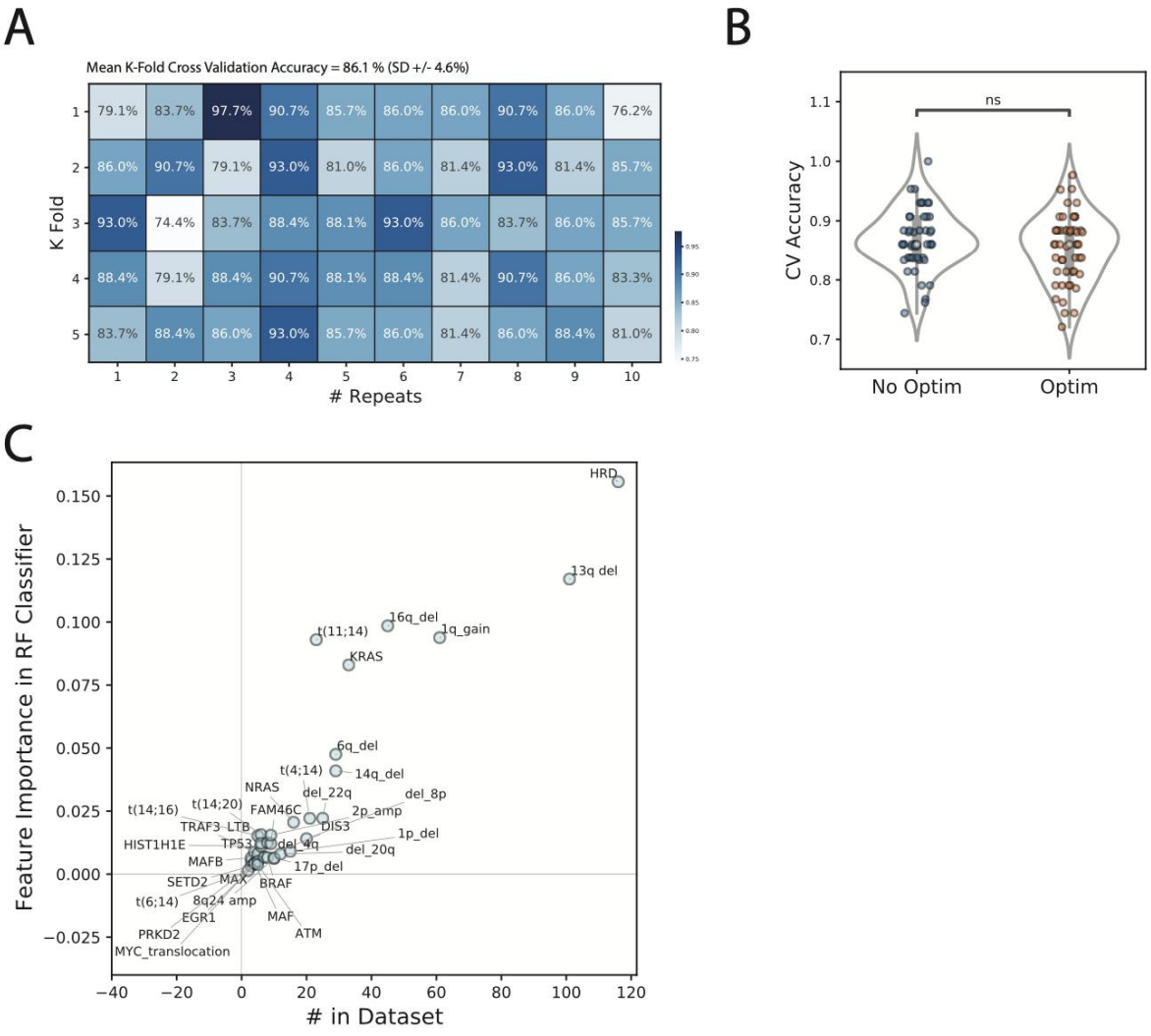


Low (TL2)

(HL1, HL4)

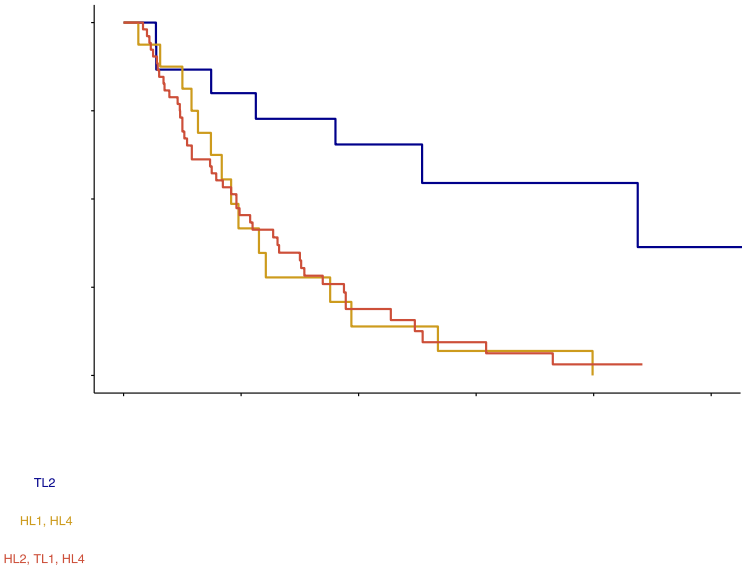
High (HL2, TL1, HL3)

Supplementary Figure 4: **A)** Random Forest K-fold cross validation accuracy over 10 repeats with mean accuracy and standard deviation reported. **B)** Grid search to optimize parameters did not improve over initial random forest parameters. **C)** The prevalence of each feature in the dataset plotted by feature importance in classifying genetic subtypes.

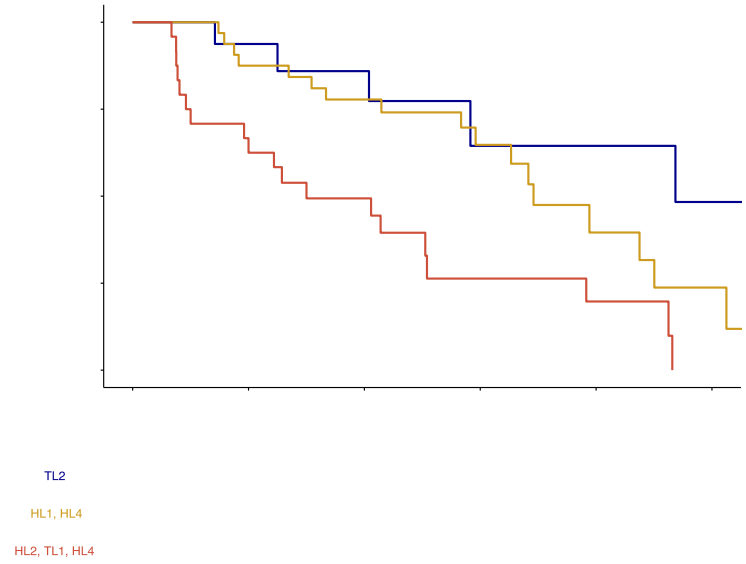


Supplementary Figure 5: Kaplan-Meier curves for analysis of TTP in the combined cohort (n=229) belonging to high vs intermediate and low-risk genetic subtypes in the clinically high(A) and intermediate(B)groups by the 20-2-20 model in the combined cohort. All p values are two-sided. Differences in survival curves and subsequent two-sided p values were calculated using the log-rank test.

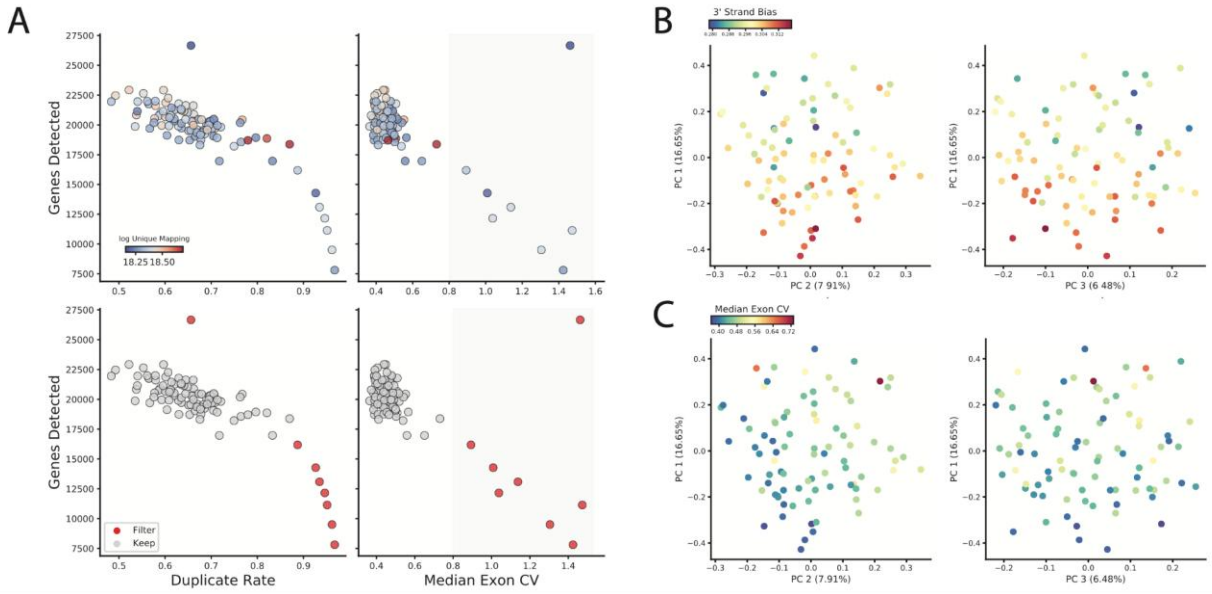
A)



B)



Supplementary Figure 6: RNA-SeQC quality control metrics for NGS RNA samples A) general metrics with filtered samples (n=13) for a cutoff of 0.8 median exon CV. B) PCA of filtered transcriptome colored by 3’ Strand Bias C) Median Exon CV



Supplementary Figure 7: Down sampling analysis of dataset for binary matrix factorization; A-D) random down-sample runs (n=100) for a range of sample sizes (n=25-214) showing explained variance (A) K-L divergence (B) Residuals (C) and Silhouette Score (D); E) Heatmap of mean scores for each down-sample run.