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Supplemental Material to:

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HDAC isoenzyme expression is deregulated in chronic lymphocytic leukemia B-cells and has a complex prognostic significance

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ONLINE SUPPLEMENTAL APPENDIX

Supplemental Data Text 1

ZAP70 and LPL assessment by real-time PCR analysis

We used 25 ng of cDNA (produced by standard reverse transcription) in a qPCR assay with SYBR® Green PCR Master Mix (Applied Biosystems) and 0.32 mol/L of gene-specific forward and reverse primers (Invitrogen). We standardized all of the results using cyclophilin A (PPI) gene expression. The primer sequences used to amplify ZAP70, PPI and LPL are listed in the table below. Standard real-time PCR was performed with an ABI Prism 7900 HT (Applied Biosystems). A calibrator sample (cDNA from the Namalwa cell line, a human B-lymphoid leukemia cell line that expresses ZAP70 at a low level; ATCC) was included as a control in each experiment. In all cases, we created dissociation curves to confirm PCR specificity. Data were analyzed using the comparative ΔΔCt method.

Symbo	Gene	Forward primer	Reverse
ZAP70	**zeta associated protein**	GTT GAC TCA TCC TCA GAG ACG	AGG TTA TCG CGC TTC AGG
LP	**lipoprotein**	CCGCCGACCAAAGAAGAGA	TTCCTGTTACCGTCCAGCCA
PP	**Cyclophilin**	GCTCGTGCCGTTTTGC	GCAAACAGCTCAAAGGAGAC

CD38 assessment by flow cytometry (FC)

We evaluated the cell surface expression of CD38 by FC in a CD19+ gate with a panel of fluorochrome-labeled monoclonal antibodies (phycoerythrin-conjugated CD38, cyanine-5-conjugated CD19, Immunotech). CD38 expression was deemed positive if 7% of the cells stained positive in a standard 3-color FC analysis. This cut-off was calculated using ROC curve analysis, maximizing the concordance with IgVH mutational status.

sCD23 and β2-microglobulin ELISAs

sCD23 and β2-microglobulin serum levels were determined using commercial immunoassay kits. Standards were used to fully quantify the sCD23 or β2-microglobulin level, and the provided controls were included in each experiment to monitor the assay performance and the inter-assay variability.

Lymphocyte doubling time assessment

Lymphocyte doubling time was determined as described by Montserrat et al. and is defined as the time needed to double the peripheral lymphocyte count.

Cytogenetic abnormality assessment

For conventional cytogenetic analysis, culture conditions, harvesting, slide preparation, and G-banding were carried out as described previously. Additional cytogenetic abnormalities were investigated with the Chromoprobe Multiprobe® - CLL System. Fresh or frozen CLL cells were washed twice with PBS and incubated in KCl (0.075 M, pH 7) for 10 min. Cells were then fixed with Carnoy's fixative (3:1 methanol:glacial acetic acid). Hybridization was performed according to the manufacturer's recommendation. The cells (100 to 200) were counted to generate representative results. A CLL FISH panel allowed for the detection of trisomy of 12, deletions in 13q14 ATM (11q22.3), TP53 (17p13.1) and MYB (6q23.3) and translocation involving IGH fission (14q32), IGH/CCND1 (14q32/11q13.3) and IGH/BCL2 (14q32/18q21.3).

IgVH gene mutational analysis

IgVH gene mutational analysis was performed as previously described, and the sequences were aligned with those in the international ImMunoGeneTics information system database (http://imgt.cines.fr). Sequences with ≤2% deviation from any germline IgVH sequence were considered unmutated.

Supplemental Data Text 2

HDAC score 5-fold cross-validation

Cross-validation is the statistical practice of partitioning a sample of data into subsets such that the analysis is initially performed on a single subset, while the other subsets are retained for subsequent use in confirming and validating the initial analysis (http://en.wikipedia.org/wiki/Cross-validation). The initial subset of data is called the training set; the other subsets are called the validation sets (Supplemental Data Figure 4A).

In a 5-fold cross-validation, the original sample is partitioned into 5 subsamples (5 subsamples of 40 patients in our study). Of the 5 subsamples, a single subsample is retained as the validation data for testing the model, and the remaining 4 subsamples are used as training data. The cross-validation process is then repeated 5 times (see figure below), with each of the 5 subsamples used exactly once as the validation data. The 5 results from the folds are then combined to produce a single estimation and correlated to survival data (TFS and OS in our study). The advantage of this method over repeated random sub-sampling is that all observations are used for both training and validation, and each observation is used for validation exactly once. The 5-fold cross-validation method is commonly used to estimate the prediction accuracy of a classification model.

Supplemental Data Figures 4B and 4E show the variation of the cut-off in the 5 subsamples of the 5-fold cross-validation. These scores were stable when they lost 20% of the population (70.5 and 79.5% for TFS and OS, respectively), as shown in the confusion tables (Supplemental Data Figures 4C and 4F). The score computed by the cross-validation model remained significant to predict TFS and OS (Supplemental Data Figures 4D and 4G).

	Patients		Controls				fold change compared to			
	CLL Ct (n=200)		PB B cells (n=20)		UCB B cells (n=20)		PB B cells		UCB B cells	
	mean	*SEM*	*mean*	*SEM*	*mean*	*SEM*	*fold*	*P value*	*fold*	*P value*
HDAC1	25.80	0.06	26.06	0.12	27.40	0.16	1.1	0.7752	1.9	**P<0.0001**
HDAC2	28.50	0.10	27.96	0.15	29.59	0.18	-1.5	**P<0.0001**	1.4	**0.0301**
HDAC3	26.82	0.05	27.18	0.10	28.02	0.15	1.1	0.7030	1.5	**0.0041**
HDAC4	30.02	0.11	29.79	0.11	30.07	0.22	-1.2	0.0552	-1.4	**0.0070**
HDAC5	29.50	0.10	29.95	0.13	31.56	0.29	1.3	0.4103	2.3	**P<0.0001**
HDAC6	28.47	0.06	29.62	0.14	29.96	0.17	1.8	**P<0.0001**	1.8	**0.0002**
HDAC7	26.01	0.07	27.77	0.13	27.62	0.19	3.0	**P<0.0001**	2.0	**0.0002**
HDAC8	30.04	0.10	29.91	0.14	31.82	0.28	-1.2	0.0625	2.1	**P<0.0001**
HDAC9	27.43	0.17	27.15	0.14	28.22	0.24	1.2	0.7388	1.7	**0.0407**
HDAC10	29.23	0.08	29.37	0.11	30.14	0.22	-1.0	0.5970	1.2	0.4713
HDAC11	33.11	0.11	34.52	0.32	36.78	0.58	2.6	**0.0002**	4.1	**P<0.0001**
SIRT1	26.61	0.07	27.11	0.16	27.85	0.16	1.4	0.3541	1.7	**0.0319**
SIRT2	27.94	0.07	28.27	0.12	29.18	0.21	1.0	0.8785	1.4	**0.0120**
SIRT3	28.99	0.07	29.89	0.13	31.02	0.19	1.7	**0.0049**	2.6	**P<0.0001**
SIRT4	35.19	0.20	34.01	0.18	38.87	0.46	-1.6	**0.0134**	4.6	**P<0.0001**
SIRT5	33.30	0.12	33.32	0.22	35.53	0.46	1.1	0.5396	2.5	**P<0.0001**
SIRT6	28.85	0.06	29.52	0.13	30.33	0.19	1.3	**0.0448**	1.7	**0.0016**
SIRT7	28.24	0.08	29.04	0.12	31.13	0.36	1.5	**0.0022**	3.6	**P<0.0001**
PPIA	21.19	0.07	21.47	0.16	21.93	0.16				

Supplemental Data Table S1. HDAC expression in CLL, PB and UCB B cells.

(Supplemental data Table S2 – part I)

			HDAC1		HDAC2		HDAC3		HDAC4		HDAC5		HDAC6		HDAC7		HDAC8		HDAC9	
	n	%	mean	SEM	mean	SEM	mean	SEM	mean	SEM	mean	SEM	mean	SEM	mean	SEM	mean	SEM	mean	SEM
Patients																				
Male	111	56	46.57	1.98	7.80	0.54	23.76	1.33	3.13	0.23	4.23	0.36	7.76	0.50	48.06	4.63	2.88	0.22	22.54	1.84
Female	89	45	45.28	2.33	8.34	0.88	23.02	1.36	3.01	0.26	4.33	0.42	7.66	0.50	45.14	3.78	2.69	0.19	28.50	3.38
Pvalue			0.4329		0.5844		0.5635		0.8537		0.9637		0.8605		0.5768		0.8354		0.1604	
Binet	200																			
Stage A	148	74	45.37	1.76	7.51	0.51	22.66	1.05	2.94	0.20	4.26	0.32	7.66	0.44	46.63	4.01	2.79	0.17	25.67	2.27
Stage B-C	52	26	47.79	2.94	9.55	1.19	25.60	2.13	3.47	0.37	4.32	0.51	7.85	0.55	47.14	3.01	2.80	0.27	23.81	2.79
Pvalue			0.3214		0.0314		0.0986		0.1322		0.5345		0.2147		0.0204		0.6609		0.9445	
Mutational status[a]	135																			
IgVH - Unmutated	76	56	46.75	2.49	7.91	0.87	21.93	1.22	3.07	0.26	4.19	0.39	7.38	0.52	45.85	4.73	2.78	0.21	28.39	3.92
IgVH - Mutated	59	44	42.80	2.35	7.94	0.96	22.62	2.07	2.74	0.31	4.07	0.40	6.63	0.43	52.13	7.45	2.54	0.17	18.64	1.93
Pvalue			0.2200		0.8072		0.2389		0.1899		0.9417		0.4959		0.1564		0.7918		0.0666	
ZAP-70[b]	200																			
>139 (positive)	108	54	46.85	2.20	8.27	0.70	24.12	1.20	3.18	0.23	4.26	0.40	8.04	0.51	48.86	5.14	2.85	0.20	29.06	2.99
<139 (negative)	92	46	44.99	2.03	7.78	0.69	22.61	1.52	2.95	0.26	4.29	0.37	7.32	0.48	44.30	2.85	2.73	0.22	20.65	1.76
Pvalue			0.8064		0.9005		0.0741		0.3027		0.6327		0.4886		0.3096		0.7818		0.0415	
LPL[b]	200																			
>13.5 (positive)	118	59	46.03	2.08	7.94	0.71	22.62	1.15	2.99	0.22	4.01	0.32	7.47	0.43	43.65	4.52	2.58	0.14	26.62	2.79
<13.5 (negative)	82	41	45.95	2.15	8.19	0.62	24.59	1.63	3.20	0.28	4.65	0.48	8.07	0.61	51.23	3.67	3.10	0.29	23.13	1.95
Pvalue			0.7895		0.4260		0.3408		0.5096		0.2951		0.5273		0.0009		0.4398		0.9535	
CD38[b]	190																			
>7% (positive)	108	57	46.45	2.20	7.86	0.64	23.27	1.23	2.92	0.25	4.45	0.40	7.65	0.53	48.20	5.05	2.84	0.20	27.01	2.88
<7% (negative)	82	43	45.70	2.08	8.44	0.83	23.14	1.53	3.27	0.26	4.00	0.34	7.61	0.44	46.25	3.29	2.71	0.21	22.76	2.18
Pvalue			0.7818		0.4623		0.8741		0.1286		0.7644		0.2801		0.0995		0.5516		0.4344	
Cytogenetic abnormalities[c]	144																			
del(17p), (11q), (6q), +12, complex	83	58	48.52	2.50	8.29	0.78	22.89	1.34	3.12	0.30	4.44	0.42	7.48	0.52	47.25	4.50	2.71	0.19	25.54	3.48
normal,del(13q), other	61	42	44.76	2.75	8.48	1.02	24.28	2.11	2.96	0.23	4.07	0.40	7.45	0.53	45.19	3.65	2.65	0.22	20.02	2.16
Pvalue			0.2534		0.9984		0.9356		0.2988		0.9758		0.7402		0.4281		0.9003		0.3903	
LDT	172																			
<1year	123	72	46.49	2.02	8.13	0.63	23.36	1.26	3.23	0.23	4.37	0.36	7.92	0.50	47.86	4.60	3.00	0.19	28.48	2.66
>1year	49	28	41.13	2.37	7.14	0.65	20.50	1.10	2.55	0.24	3.60	0.30	6.74	0.50	39.69	2.62	2.24	0.22	18.19	1.96
Pvalue			0.1124		0.5505		0.8361		0.1927		0.6666		0.3244		0.6445		0.0090		0.0124	
soluble CD23[b]	139																			
>120 U (positive)	72	52	43.49	2.41	7.41	0.83	21.56	1.50	2.73	0.25	3.94	0.35	6.69	0.43	44.54	4.37	2.67	0.21	25.80	4.03
<120 U (negative)	67	48	44.99	2.71	7.32	0.92	22.16	1.69	3.06	0.29	4.22	0.43	7.04	0.47	41.59	2.59	2.63	0.19	22.92	2.55
Pvalue			0.6429		0.4907		0.8314		0.1971		0.4633		0.4016		0.3397		0.7249		0.9681	
b2-microglubulin[b]	163																			
>2.77 µg (positive)	83	51	46.19	2.25	7.65	0.61	22.01	1.22	3.23	0.30	4.52	0.44	7.56	0.59	45.83	3.92	2.95	0.23	27.22	3.57
<2.77 µg (negative)	80	49	43.51	2.32	8.18	0.97	22.89	1.74	2.85	0.25	3.77	0.34	6.95	0.40	39.97	2.75	2.59	0.21	21.78	2.35
Pvalue			0.2238		0.9009		0.5953		0.5976		0.2460		0.9828		0.6150		0.1003		0.2018	
	200																			
Patients not requiring treatment	102	51	48.35	2.37	8.33	0.69	24.37	1.38	3.26	0.26	4.50	0.44	8.38	0.62	51.44	5.48	2.80	0.22	26.05	2.89
Patients requiring treatment	98	49	43.54	1.82	7.74	0.71	22.45	1.31	2.89	0.23	4.04	0.31	7.02	0.32	41.89	2.53	2.78	0.19	24.29	2.22
Pvalue			0.2487		0.2442		0.413		0.5874		0.6285		0.8546		0.8671		0.6328		0.6814	
	200																			
Patients still alive	159	79.5	47.80	1.75	8.03	0.58	24.48	1.11	3.09	0.20	4.49	0.33	8.13	0.43	49.15	3.78	2.82	0.17	26.21	2.08
Patients died during the study	41	20.5	39.00	2.58	8.07	0.89	19.36	1.63	3.02	0.35	3.42	0.29	6.11	0.43	37.49	2.60	2.70	0.29	21.22	3.75
Pvalue			0.016		0.641		0.010		0.855		0.513		0.027		0.574		0.925		0.058	

(Supplemental Data Table S2 – Part II)

	n	%	HDAC10 mean	HDAC10 SEM	HDAC11 mean	HDAC11 SEM	SIRT1 mean	SIRT1 SEM	SIRT2 mean	SIRT2 SEM	SIRT3 mean	SIRT3 SEM	SIRT4 mean	SIRT4 SEM	SIRT5 mean	SIRT5 SEM	SIRT6 mean	SIRT6 SEM	SIRT7 mean	SIRT7 SEM
Patients																				
Male	111	56	4.73	0.34	0.40	0.04	32.99	3.16	10.81	0.67	5.69	0.50	0.13	0.01	0.34	0.06	5.97	0.35	9.32	0.56
Female	89	45	4.78	0.30	0.37	0.04	32.02	2.98	10.71	0.55	6.11	0.55	0.14	0.01	0.32	0.03	5.97	0.47	8.78	0.60
Pvalue			0.5307		0.5963		0.8508		0.31		0.5156		0.6343		0.5929		0.7418		0.5291	
Binet	200																			
Stage A	148	74	4.56	0.25	0.37	0.03	30.62	2.60	10.74	0.51	6.01	0.47	0.13	0.01	0.33	0.05	6.09	0.37	8.93	0.50
Stage B-C	52	26	5.31	0.54	0.44	0.07	38.09	4.00	10.83	0.89	5.51	0.50	0.13	0.02	0.34	0.03	5.63	0.35	9.50	0.71
Pvalue			0.0827		0.3147		0.0030		0.7215		0.5558		0.5755		0.1444		0.7007		0.1608	
Mutational status[a]	135																			
IgVH - Unmutated	76	56	4.38	0.29	0.40	0.04	29.64	3.00	10.24	0.51	5.11	0.35	0.13	0.01	0.29	0.03	6.03	0.51	8.73	0.60
IgVH - Mutated	59	44	4.45	0.30	0.35	0.03	30.08	3.64	9.49	0.70	4.75	0.41	0.16	0.02	0.39	0.11	5.23	0.44	8.51	0.54
Pvalue			0.6589		0.7884		0.8819		0.0534		0.3097		0.1899		0.4779		0.1796		0.9629	
ZAP-70[b]	200																			
≥139 (positive)	108	54	4.76	0.35	0.41	0.05	33.75	3.21	11.02	0.59	5.90	0.43	0.12	0.01	0.36	0.06	6.25	0.42	9.10	0.53
<139 (negative)	92	46	4.75	0.29	0.36	0.03	31.16	2.93	10.46	0.66	5.85	0.63	0.15	0.01	0.30	0.02	5.64	0.38	9.06	0.64
Pvalue			0.5605		0.9336		0.3510		0.3522		0.2975		0.1138		0.6414		0.2908		0.7658	
LPL[b]	200																			
≥13.5 (positive)	118	59	4.16	0.22	0.39	0.04	31.23	2.62	10.51	0.51	5.21	0.31	0.13	0.01	0.29	0.02	5.91	0.40	8.49	0.48
<13.5 (negative)	82	41	5.62	0.45	0.38	0.03	34.47	3.80	11.12	0.79	6.84	0.77	0.14	0.01	0.39	0.08	6.06	0.41	9.93	0.73
Pvalue			0.0052		0.4867		0.5712		0.9980		0.3639		0.7039		0.1319		0.3786		0.1217	
CD38[b]	190																			
>7% (positive)	108	57	4.42	0.28	0.40	0.04	34.02	3.34	11.04	0.69	6.06	0.56	0.14	0.01	0.31	0.02	6.05	0.45	9.01	0.52
<7% (negative)	82	43	4.89	0.30	0.38	0.04	30.86	3.00	10.32	0.55	5.76	0.52	0.14	0.01	0.38	0.08	5.99	0.35	9.12	0.67
Pvalue			0.1023		0.5777		0.9607		0.8626		0.4811		0.9342		0.2650		0.2940		0.9649	
Cytogenetic abnormalities[c]	144																			
del(17p), (11q), (6q), +12, complex	83	58	4.56	0.39	0.44	0.05	31.11	3.19	10.10	0.55	5.74	0.48	0.15	0.01	0.30	0.02	5.79	0.42	9.27	0.69
normal,del(13q), other	61	42	4.71	0.33	0.32	0.04	32.37	4.47	10.53	0.77	5.09	0.49	0.13	0.01	0.44	0.11	6.14	0.56	8.54	0.44
Pvalue			0.3847		0.0392		0.6979		0.8987		0.2306		0.8747		0.3545		0.3279		0.5769	
LDT	172																			
<1year	123	72	4.60	0.27	0.38	0.03	31.47	2.99	10.85	0.57	6.02	0.52	0.14	0.01	0.36	0.06	6.15	0.42	8.99	0.53
>1year	49	28	4.61	0.32	0.35	0.04	30.88	3.05	9.67	0.66	4.67	0.42	0.11	0.01	0.31	0.03	5.11	0.31	8.13	0.52
Pvalue			0.4996		0.7874		0.4352		0.2702		0.1159		0.2118		0.7103		0.6814		0.6518	
soluble CD23[b]	139																			
≥120 U (positive)	72	52	3.95	0.33	0.37	0.06	27.99	3.65	9.32	0.53	4.41	0.31	0.13	0.01	0.37	0.09	5.23	0.35	8.34	0.60
<120 U (negative)	67	48	4.71	0.27	0.39	0.04	29.77	2.86	9.29	0.49	4.89	0.45	0.14	0.02	0.31	0.03	5.42	0.50	8.54	0.49
Pvalue			0.0073		0.2542		0.1899		0.7312		0.6249		0.6595		0.7631		0.8595		0.4075	
b2-microglubulin[b]	163																			
≥2.77 µg (positive)	83	51	4.71	0.34	0.39	0.04	28.89	3.01	10.55	0.61	6.04	0.69	0.15	0.01	0.40	0.08	5.92	0.42	9.16	0.69
<2.77 µg (negative)	80	49	4.16	0.26	0.35	0.05	33.25	4.06	9.36	0.56	4.65	0.40	0.12	0.01	0.27	0.02	5.07	0.30	8.03	0.46
Pvalue			0.4920		0.2904		0.5196		0.1497		0.0642		0.0714		0.0914		0.2048		0.5261	
	200																			
Patients not requiring treatment	102	51	4.54	0.30	0.38	0.04	37.64	3.79	11.35	0.74	6.66	0.65	0.13	0.01	0.31	0.02	6.47	0.51	9.34	0.68
Patients requiring treatment	98	49	4.98	0.35	0.40	0.04	27.27	2.01	10.15	0.47	5.06	0.33	0.14	0.01	0.36	0.07	5.44	0.25	8.82	0.46
Pvalue			0.1614		0.7787		0.1882		0.668		0.2467		0.7433		0.7194		0.9105		0.5716	
	200																			
Patients still alive	159	79.5	4.88	0.27	0.40	0.03	33.83	2.61	11.45	0.53	6.25	0.44	0.13	0.01	0.34	0.04	6.34	0.34	9.46	0.50
Patients died during the study	41	20.5	4.30	0.38	0.33	0.04	27.62	3.40	8.09	0.43	4.43	0.51	0.14	0.02	0.30	0.03	4.54	0.36	7.61	0.51
Pvalue			0.4677		0.5046		0.5964		0.007		0.0177		0.8929		0.4883		0.0043		0.1563	

[a] Mutational status is based on a 98% cut-off value.

[b] The cut-off determined using ROC curve analysis maximising the concordance with the IgVH status

[c] Among patients with unfavorable cytogenetic abnormalities (n=61), we found 11 patients with a del(17p) (7.6%), 16 with a del(11q) (11.1%), 10 with del(6q) (6.9%), and 22 with a trisomy-12 (15.3%). Furthermore, 2 patient presents a complex karyotype associated with poor prognosis (1.4%). Among patients with favorable cytogenetic abnormalities (n=83), we found 38 patients with del(13q) (26.4%) and 7 patients with other(s) abnormalities (del(16q), translocation t(13,14), tri7 or tri18) (4.9%). 38 patients had a normal karyotype (26.4%).

Supplemental Data Table S2. Patient characteristics and HDAC expression in different prognostic subgroups.

					TFS			OS	
	Cut-off[a]		n	%	median	P value	χ^2	median	P value	χ^2
HDAC 1	***<35.75***	-	70	35.0	57	0.4560	0.5556	183.03	0.0829	3.007
	>35.75	+	130	65.0	88.07			>360		
HDAC 2	***<9.317***	-	151	75.5	75.7	0.4269	0.6312	>360	0.5630	0.3345
	>9.317	+	49	24.5	89.33			241.87		
HDAC 3	***<20.56***	-	110	55.0	62.63	0.0824	3.017	183.03	**0.0116**	6.363
	>20.56	+	90	45.0	88.07			>360		
HDAC 4	***<3.053***	-	128	64.0	57.3	0.0941	2.804	2373.1	0.5124	0.4292
	>3.053	+	72	36.0	97			>360		
HDAC 5	***<3.133***	-	94	47.0	89.33	0.6716	0.1797	>360	0.6170	0.2501
	>3.133	+	106	53.0	80.47			237.07		
HDAC 6	***<10.1***	-	161	80.5	75.7	**0.0148**	5.945	237.07	0.2593	1.273
	>10.1	+	39	19.5	>244			>360		
HDAC 7	***<25.88***	-	61	30.5	129.47	0.0971	2.753	>360	0.2791	1.171
	>25.88	+	139	69.5	62.63			237.07		
HDAC 8	***<3.038***	-	135	67.5	84	0.9570	0.003	241.87	0.5412	0.3733
	>3.038	+	65	32.5	85			237.07		
HDAC 9	***<22.49***	-	119	59.5	80.47	0.5634	0.3339	241.87	0.1181	2.442
	>22.49	+	81	40.5	87.2			>360		
HDAC 10	***<3.22***	-	76	38.0	97	0.1066	2.604	237.07	0.6464	0.2105
	>3.22	+	124	62.0	60.9			>360		
HDAC 11	***<0.1815***	-	69	34.5	62.63	0.6749	0.176	241.87	0.3549	0.8559
	>0.1815	+	131	65.5	87.2			>360		
SIRT 1	***<14.01***	-	43	21.5	80.47	0.4976	0.4601	168.4	0.5274	0.3994
	>14.01	+	157	78.5	85			241.87		
SIRT 2	***<10.11***	-	115	57.5	75.7	0.2205	1.0501	183.03	**0.0098**	6.674
	>10.11	+	85	42.5	88.07			>360		
SIRT 3	***<3.46***	-	69	34.5	44.71	0.0551	3.678	159	**0.0164**	5.755
	>3.46	+	131	65.5	89.93			>360		
SIRT 4	***<0.0085***	-	42	21.0	97	0.5112	0.4315	>360	0.2633	1.251
	>0.0085	+	158	79.0	75.7			237.07		
SIRT 5	***<0.2665***	-	113	56.5	87.2	0.4845	0.4888	241.87	0.0552	3.677
	>0.2665	+	87	43.5	75.7			>360		
SIRT 6	***<5.203***	-	110	55.0	89.33	0.8442	0.0386	168.4	**0.0082**	6.981
	>5.203	+	90	45.0	85			>360		
SIRT 7	***<8.789***	-	126	63.0	60.9	0.1699	1.884	183.03	0.1158	2.473
	>8.789	+	74	37.0	89.93			>360		

[a] Cut-off were calculated using ROC curve maximising the concordance between HDAC expression and ZAP70 status and minimising the number of false negative

Supplemental Data Table S3. Prognostic power of HDAC expression

	n	%	TFS median	TFS P value	TFS χ^2	OS median	OS P value	OS χ^2
Binet	200							
Stage A	148	74.0	109.0	P<0.0001	31.91	>360	0.0005	12.08
Stage B-C	52	26.0	28.6			136.6		
Mutational status[a]	135							
IgVH - Unmutated	76	56.3	32.0	P<0.0001	34.55	152.5	0.0005	12.19
IgVH - Mutated	59	43.7	126.0			>360		
ZAP-70[b]	200							
≥139 (positive)	92	46.0	37.4	P<0.0001	45.87	137.2	P<0.0001	17.92
<139 (negative)	108	54.0	155.1			>360		
LPL[b]	200							
≥13.5 (positive)	82	41.0	41.6	P<0.0001	23.43	152.1	0.0139	6.05
<13.5 (negative)	118	59.0	129.5			>360		
CD38[b]	190							
≥7% (positive)	82	43.2	41.6	P<0.0001	19.68	136.6	0.0008	11.23
<7% (negative)	108	56.8	129.5			241.9		
Cytogenetic abnormalities[c]	144							
del(17p), (11q), (6q), +12, complex	61	42.4	39.0	P<0.0001	17.53	159.0	0.0094	6.745
normal,del(13q), other	83	57.6	111.0			>360		
LDT	172							
<1year	49	28.5	29.3	P<0.0001	35.18	136.6	0.0028	8.917
>1year	123	71.5	109.0			241.9		
soluble CD23[b]	139							
≥120 U (positive)	67	48.2	37.3	P<0.0001	24.64	136.6	0.0010	10.9
<120 U (negative)	72	51.8	178.4			>360		
b2-microglubulin[b]	163							
≥2.77 µg (positive)	80	49.1	47.0	0.0015	10.09	159.0	0.0487	3.885
<2.77 µg (negative)	83	50.9	109.0			>360		

[a] Mutational status is based on a 98% cut-off value.

[b] The cut-off determined using ROC curve analysis maximising the concordance with the IgVH status

[c] Among patients with unfavorable cytogenetic abnormalities (n=61), we found 11 patients with a del(17p) (7.6%), 16 with a del(11q) (11.1%), 10 with del(6q) (6.9%), and 22 with a trisomy-12 (15.3%). Furthermore, 2 patient presents a complex karyotype associated with poor prognosis (1.4%). Among patients with favorable cytogenetic abnormalities (n=83), we found 38 patients with del(13q) (26.4%) and 7 patients with other(s) abnormalities (del(16q), translocation t(13,14), tri7 or tri18) (4.9%). 38 patients had a normal karyotype (26.4%).

Supplemental Data Table S4. Prognostic power of classical prognostic factors

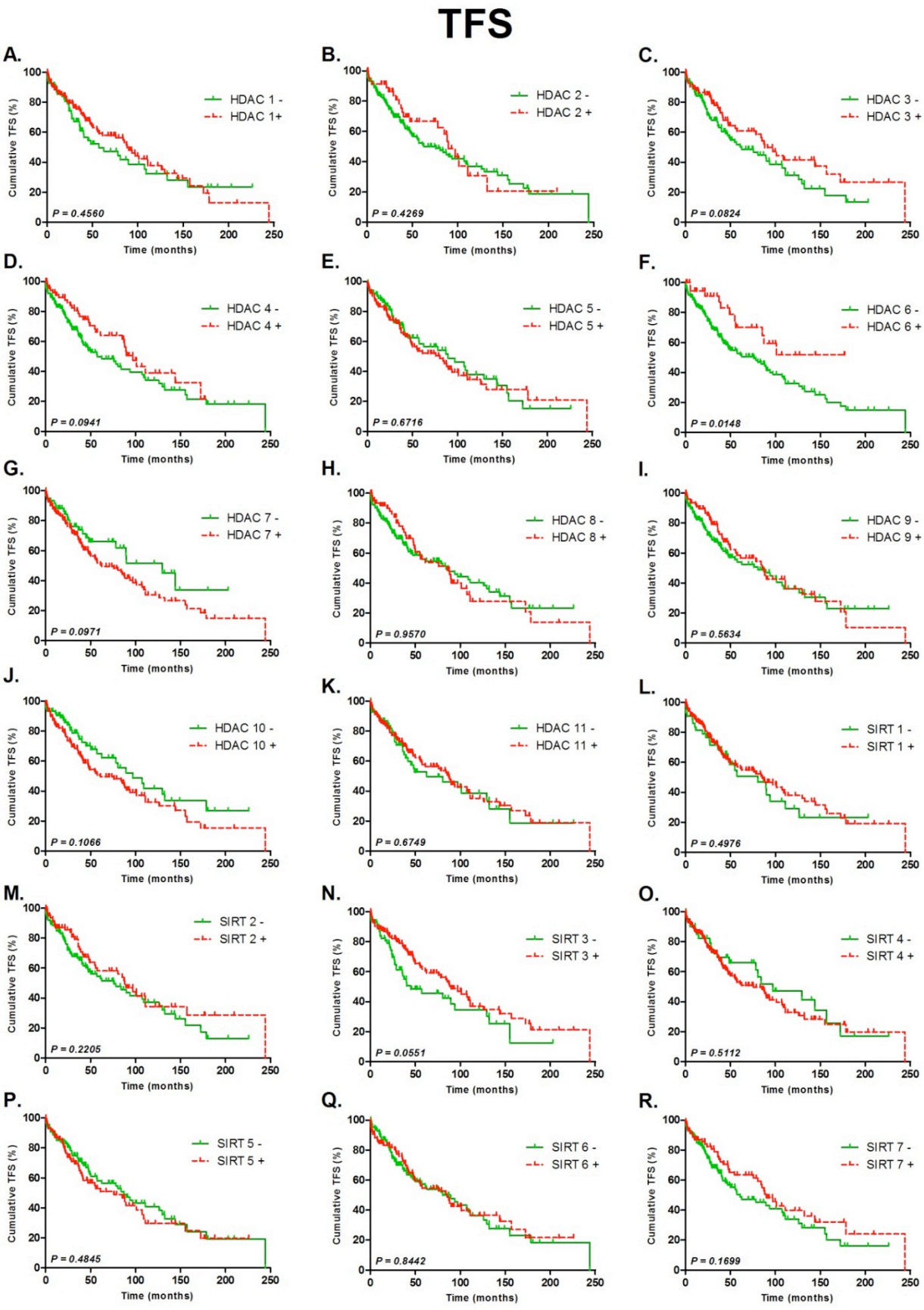
Supplemental Data Figure Legend

Supplemental Data Figure S1. Prognostic power of HDAC expression in terms of TFS. TFS values for HDAC1 (A), HDAC2 (B), HDAC3 (C), HDAC4 (D), HDAC5 (E), HDAC6 (F), HDAC7 (G), HDAC8 (H), HDAC9 (I), HDAC10 (J), HDAC11 (K), SIRT1 (L), SIRT2 (M), SIRT3 (N), SIRT4 (O), SIRT5 (P), SIRT6 (Q), and SIRT7 (R) were plotted using Kaplan-Meier estimates. ROC curves were used to determine HDAC cut-off values that best distinguished ZAP70+ and ZAP70- cases and minimized the number of false negatives. Significant differences between curves were calculated using the log-rank test. Statistical details can be found in Supplemental Data Table S3.

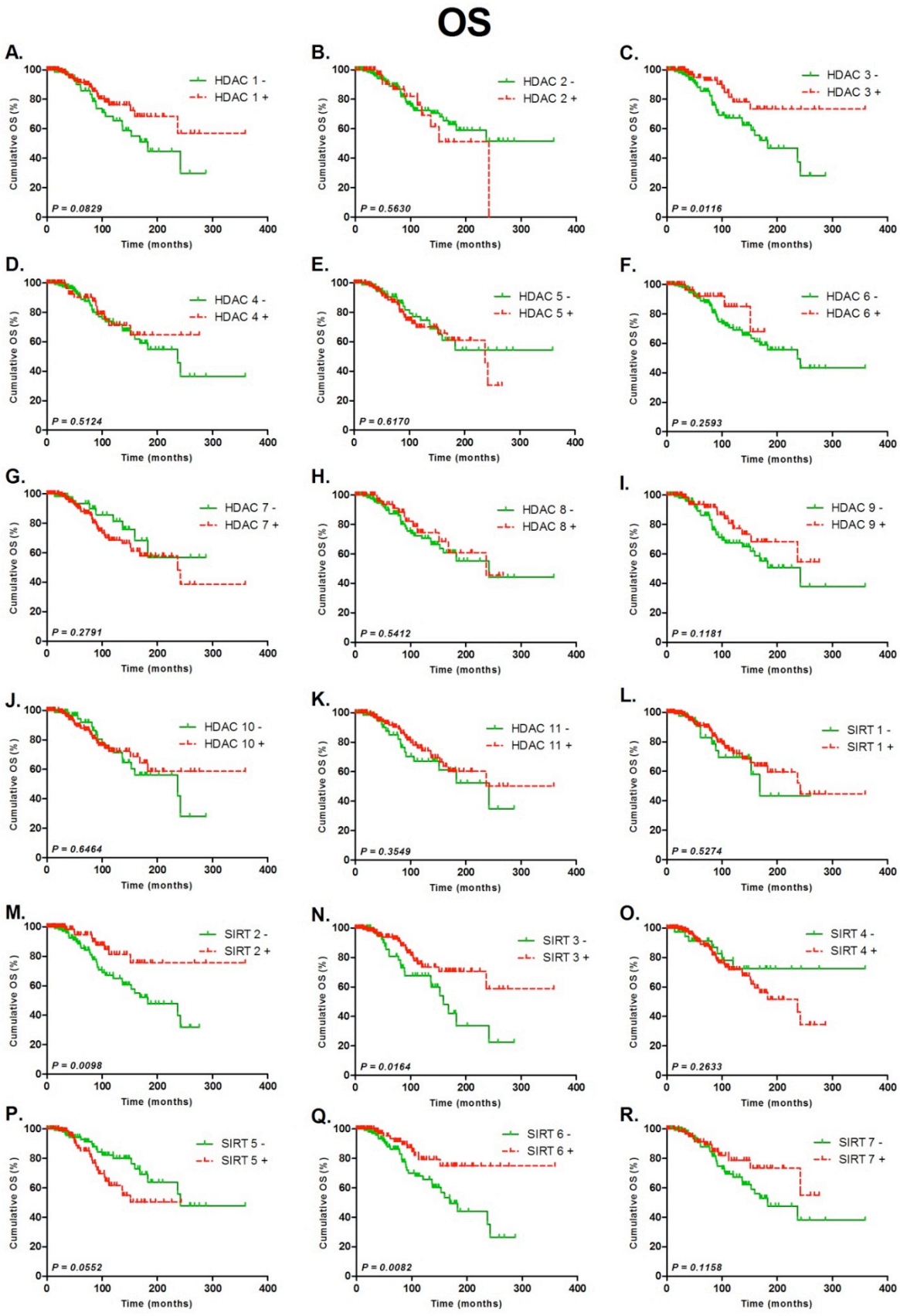
Supplemental Data Figure S2. Prognostic power of HDAC expression in terms of OS. OS values for HDAC1 (A), HDAC2 (B), HDAC3 (C), HDAC4 (D), HDAC5 (E), HDAC6 (F), HDAC7 (G), HDAC8 (H), HDAC9 (I), HDAC10 (J), HDAC11 (K), SIRT1 (L), SIRT2 (M), SIRT3 (N), SIRT4 (O), SIRT5 (P), SIRT6 (Q), and SIRT7 (R) were plotted using Kaplan-Meier estimates. ROC curves were used to determine HDAC cut-off values that best distinguished ZAP70+ and ZAP70- cases and minimized the number of false negatives. Significant differences between curves were calculated using univariate Cox regression analysis. Statistical details can be found in Supplemental Data Table S3.

Supplemental Data Figure S3. Five-fold cross-validation study of TFS and OS score. (A) Five-fold cross-validation model. (B) and (E) present the cut-off variation for the 5 parts of the cross-validation for each element of the TFS and OS score, respectively. (C) and (F) show the concordance between the real score and the cross-validated score for TFS and OS, respectively. (D) and (G) show Kaplan-Meier estimates of the cross-validated score for TFS and OS, respectively. Significant differences between curves were calculated using univariate Cox regression analysis.

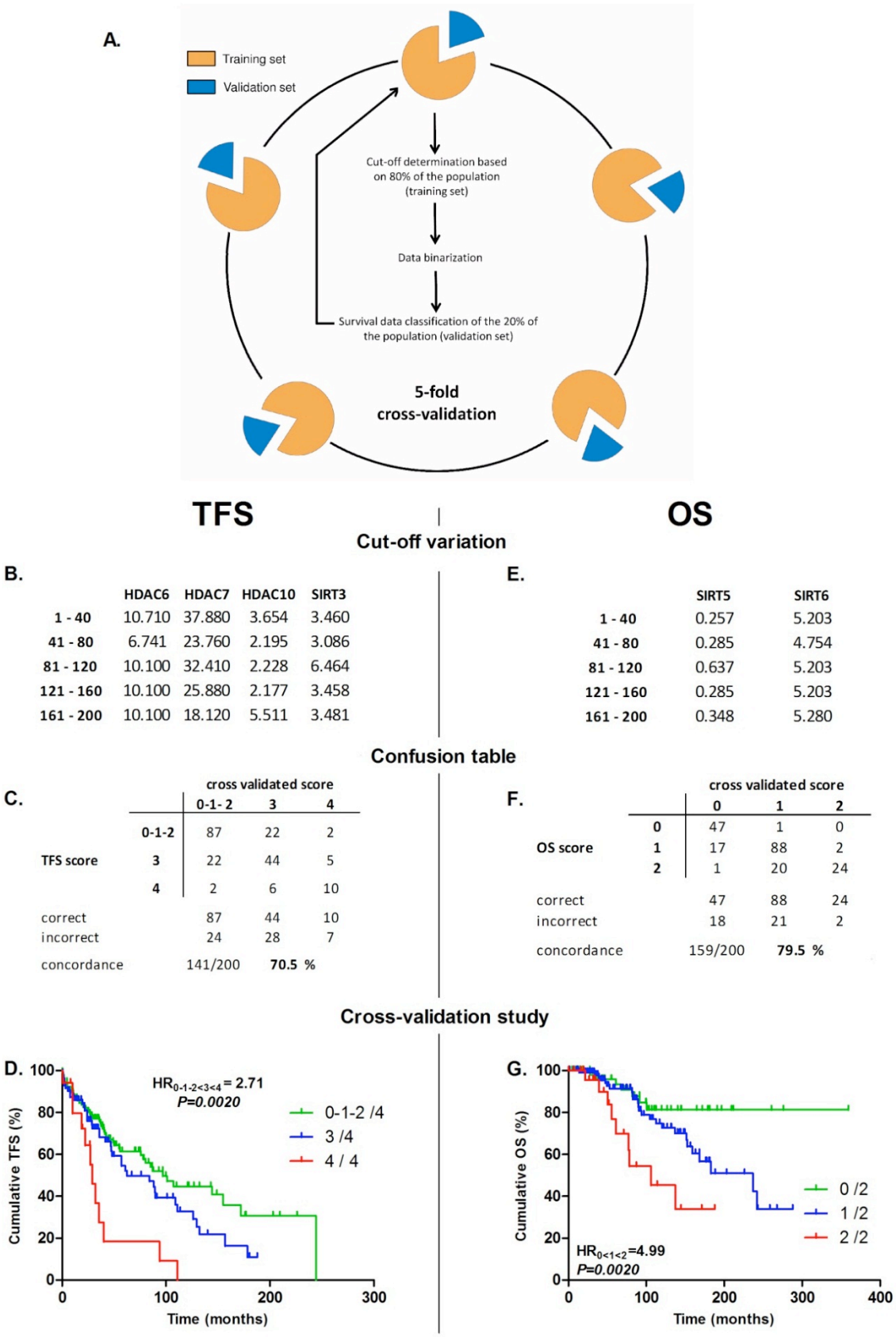
Supplemental Data Figure S4. Prognostic power of classical prognostic factors. (A and J) TFS and OS curves for Binet stage A vs. B-C (n=200); (B and K) IgVH mutational status (n=135); (C and L) LDT (n=172); (D and M) ZAP70 by qPCR (n=200); (E and N) LPL by qPCR (n=200); (F and O) CD38 (n=190); (G and P) cytogenetic abnormalities detected by classical karyotype analysis or by FISH (normal/del(13q)/other) vs. (del(17p)/(11q)/(6q)/+12/complex) (n=144); (H and Q) sCD23 (n=139); (I and R) β2-M (n=163). ROC curves were used to determine the ZAP70, LPL, CD38, miR-29c, miR-223, sCD23 and β2-M expression cut-off values that best distinguished mutated and unmutated cases. IgHV mutational status is based on a 98% cut-off value. Significant differences between curves were calculated using the log-rank test. Statistical details can be found in Supplemental Data Table S4.



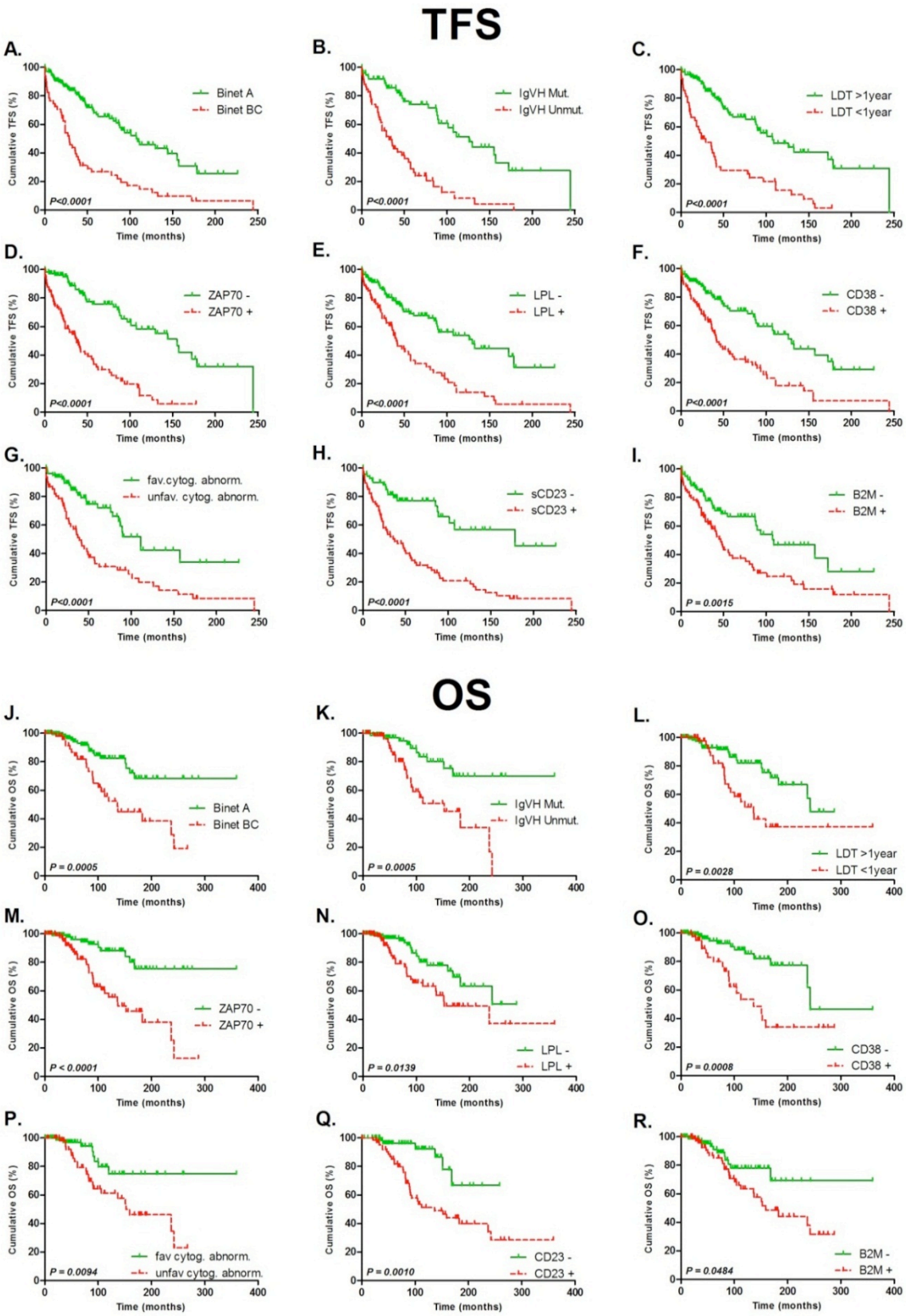
Supplemental Data Figure S1. Prognostic power of HDAC expression in terms of TFS



Supplemental Data Figure S2. Prognostic power of HDAC expression in terms of OS



Supplemental Data Figure S3. Five-fold cross-validation study of TFS and OS score



Supplemental Data Figure S4. Prognostic power of classical prognostic factors