Supplemental material

A gene signature that distinguishes conventional and leukemic nonnodal mantle cell lymphoma helps predict outcome

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SUPPLEMENTAL TABLES

Table S1. Clinico-biological characteristics of the MCL patients in the training cohort according to the MCL subtype.

Variable	Total	MCL subtype		*P* value
		cMCL	nnMCL	
Number of cases (%)	19	12 (63)	7 (37)	
Clinical data (at diagnosis)				
Male/Female, n	17/2	11/1	6/1	1
Nodal presentation*, n (%)	8/19 (42)	8/12 (67)	0/7 (0)	.013
Splenomegaly, n (%)	8/19 (42)	6/12 (50)	2/7 (29)	.633
LDH (>ULN), n (%)	6/15 (40)	6/9 (67)	0/6 (0)	.028
MIPI high risk, n (%)	7/9 (78)	6/6 (100)	1/3 (33)	.083
ECOG (≥2), n (%)	6/10 (60)	5/7 (71)	1/3 (33)	.5
Lymphocytosis (L/mm3), median (range)	11 570 (1 300-45 000)	19 000 (1 300-45 000)	8 378 (1 624-11 055)	.024
Pathological and molecular data (at sampling)				
CCND1 expression, mean (range)	14.0 (11.2-15.8)	14.5 (13.3-15.8)	13.1 (11.2-13.6)	.004
IGHV (<97%), n (%)	8/19 (42)	2/12 (17)	6/7 (86)	.006
17p/*TP53* alteration, n (%)	6/19 (32)	5/12 (42)	1/7 (14)	.333
9p/*CDKN2A* deletion, n (%)	2/19 (11)	2/12 (17)	0/7 (0)	.509
11q deletion, n (%)	4/19 (21)	4/12 (33)	0/7 (0)	.245
CNA, median (range)	3 (0-31)	7.5 (1-31)	1 (0-2)	.001
Treatment at diagnosis, n (%)†				<.001
High-dose therapy	2/19 (11)	2/12 (17)	0/7 (0)	
Immunochemotherapy	9/19 (47)	9/12 (75)	0/7 (0)	
Low-dose chemotherapy	1/19 (5)	1/12 (8)	0/7 (0)	
Observation	7/19 (37)	0/12 (0)	7/7 (100)	
Follow-up data				
Median follow-up, mo	53	53	61	.542
Mean time from diagnosis to sample (range), mo	18.6 (0-147)	1.6 (0-10)	47.8 (0-147)	.047
Dead patients, n (%)	10/19 (53)	9/12 (75)	1/7 (14)	.02
Treated at 3 y from diagnosis, % (95% CI)	64 (34-81)	100 (100-100)	0 (0-0)	<.001
Treated at 3 y from sampling, % (95% CI)	76 (43-90)	100 (100-100)	36 (0-66)	<.001
3-y OS, diagnosis, % (95% CI)	45 (26-78)	11 (2-70)	100 (100-100)	.002
3-y OS, sampling, % (95% CI)	34 (15-76)	12 (2-75)	86 (63-100)	.044

Abbreviations: CI, confidence interval; cMCL, conventional mantle cell lymphoma; CNA, copy number alterations; ECOG, Eastern Cooperative Oncology Group; IGHV, immunoglobulin heavy chain genes; LDH, lactate dehydrogenase; MIPI, mantle cell lymphoma International Prognostic Index; nnMCL, non-nodal mantle cell lymphoma; OS, overall survival; R-CHOP, rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone; ULN, upper level of normal.

* Nodal presentation was considered when the lymph nodes were <1 cm.

† High-dose therapy includes Cytarabine-based immunochemotherapy and/or autologous stem-cell transplantation; Immunochemotherapy includes R-CHOP-like regimens; and Low-dose therapy includes Alkylating agents alone or in combination.

Table S2. Differentially expressed genes selected to include in the pilot NanoString code set.

Probe set	Gene	Average Expression	Fold-change	Modified t-statistic	Adjusted *P* value	Up/Down-regulated in cMCL
209524_at	*HDGFRP3*	7.15	36.89	18.17	3.78E-09	Up
230441_at	*PLEKHG4B*	6.53	7.74	11.82	2.34E-06	Up
239246_at	*FARP1*	6.44	9.22	10.64	9.96E-06	Up
201876_at	*PON2*	7.79	19.54	10.38	1.20E-05	Up
202806_at	*DBN1*	6.73	10.26	8.94	1.07E-04	Up
204914_s_at	*SOX11*	8.60	41.53	8.14	3.33E-04	Up
207705_s_at	*NINL*	7.08	6.21	8.07	3.36E-04	Up
222101_s_at	*DCHS1*	6.76	8.65	8.02	3.36E-04	Up
206181_at	*SLAMF1*	5.74	0.13	-7.84	4.22E-04	Down
201310_s_at	*NREP*	7.85	20.73	7.48	5.42E-04	Up
215017_s_at	*FNBP1L*	6.30	12.05	7.15	8.92E-04	Up
215001_s_at	*GLUL*	9.67	6.08	6.64	0.001697	Up
201445_at	*CNN3*	6.70	9.80	6.60	0.001697	Up
1560225_at	*CNR1*	8.36	10.39	6.44	0.002159	Up
215807_s_at	*PLXNB1*	6.40	2.61	6.42	0.002218	Up
236226_at	*BTLA*	6.57	0.14	-6.20	0.002995	Down
209583_s_at	*CD200*	5.50	0.11	-5.42	0.007941	Down
201540_at	*FHL1*	8.70	7.00	5.28	0.009019	Up

The genes included in the pilot NanoString code set were selected among the 109 probe sets with an adjusted *P* value < 0.01 (limma). Probe sets with high fold-change (or low in case of down-regulation) and confirmed gene annotation were prioritized.

SUPPLEMENTAL FIGURES

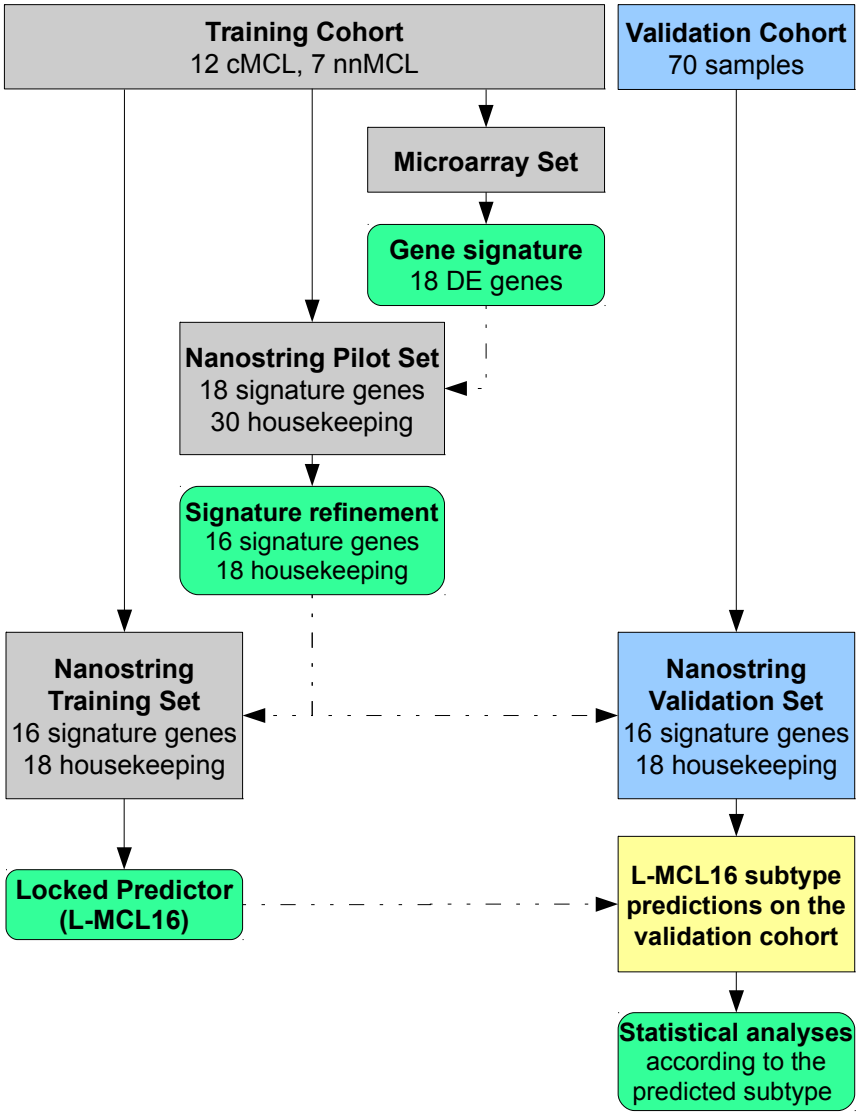

Figure S1. Cohorts or sets of MCL patients related to training samples are represented in gray, cohorts or sets related to validation samples in blue, statistical analyses in green, and predictor outcome in yellow. DE, differentially expressed.

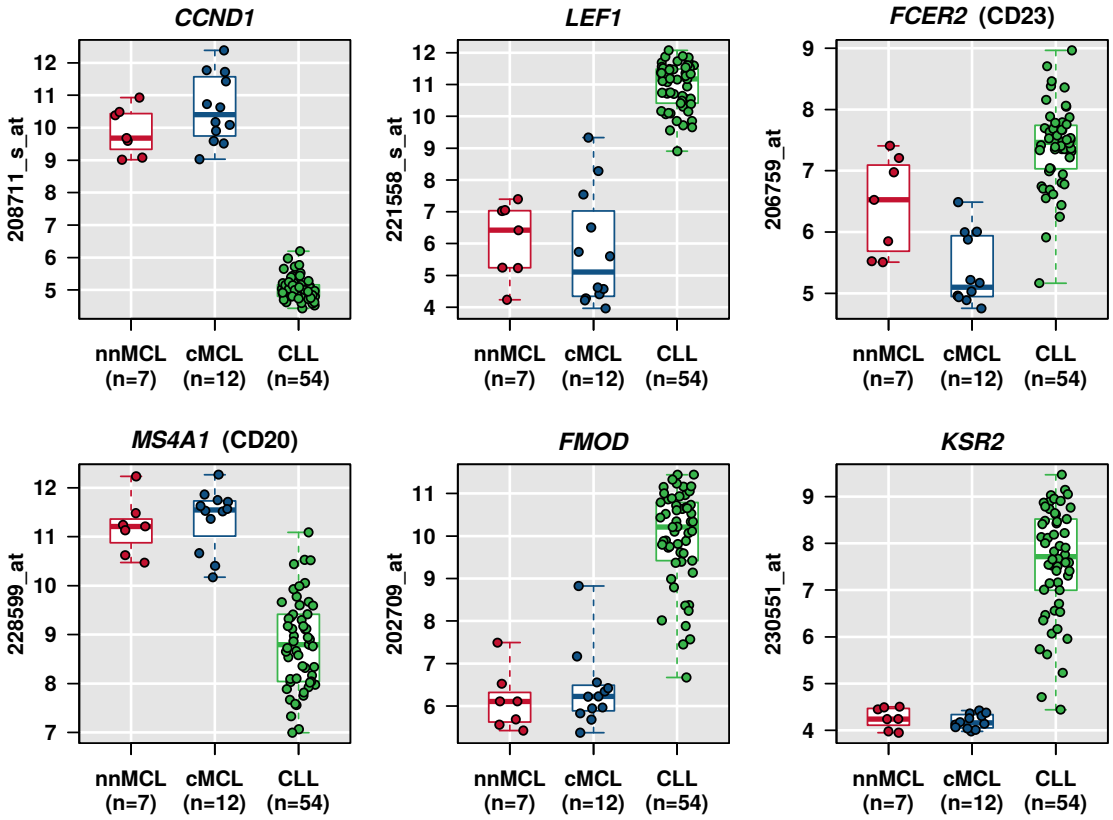

Figure S2. Microarray expression levels of one MCL biomarker (*CCND1*) and five CLL biomarkers (*LEF1*, *CD23*, *CD20*, *FMOD*[1,2] and *KSR2*[2]) in the 19 MCL of the training cohort (7 nnMCL and 12 cMCL) and 54 CLL described in Navarro et al[2].

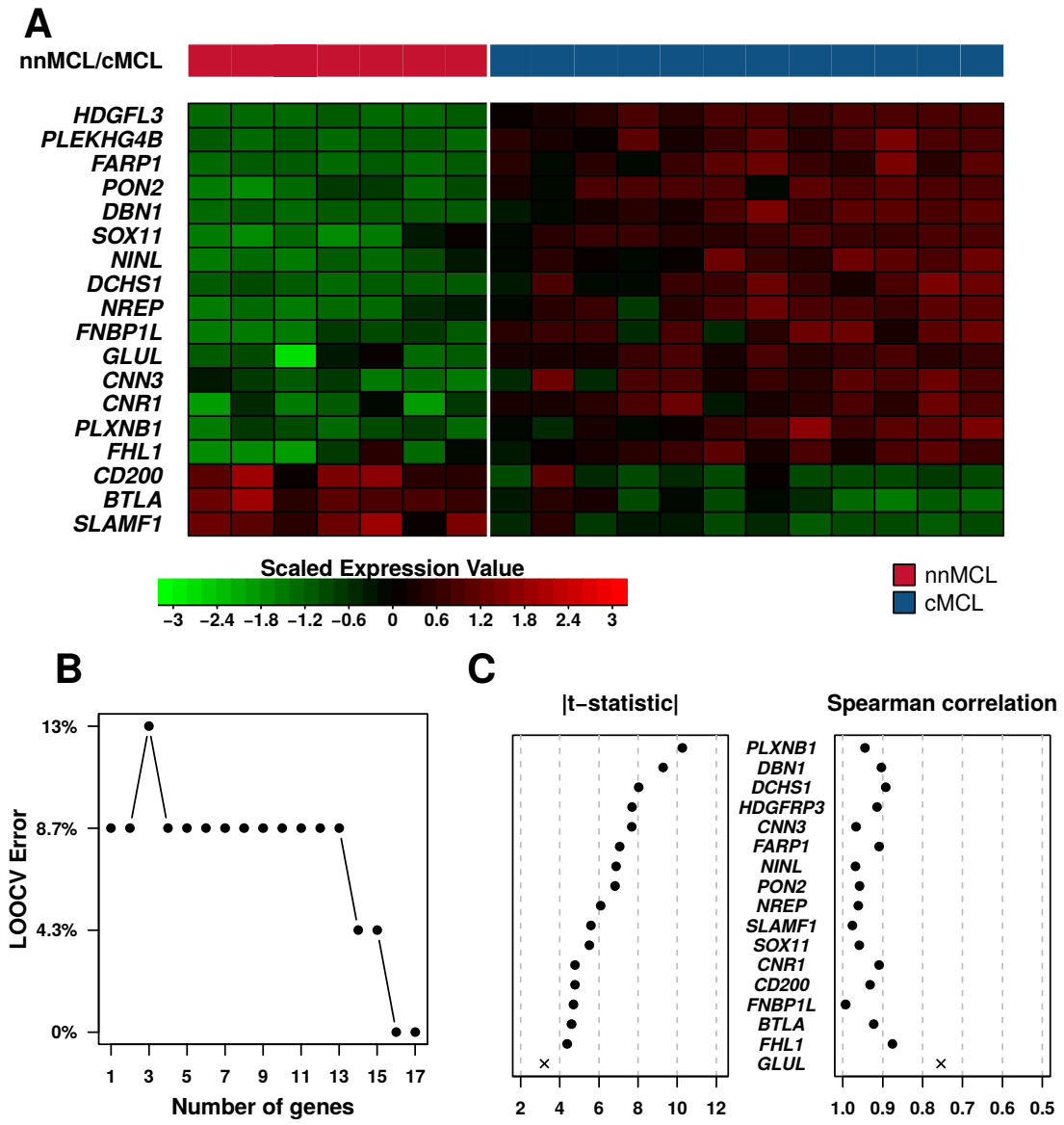

Figure S3. **(A)** Heatmap of the 18-gene signature in the microarray training set. The genes selected to include in a pilot NanoString code set are represented in rows and the 19 samples in columns. Only 3 of the genes were upregulated in the nnMCL cases. **(B)** Leave-one-out cross-validation (LOOCV) error (y-axis) plotted against the number of genes (x-axis) in the pilot NanoString training set. **(C)** The left plot shows the t-statistic of the 17 genes included in the pilot NanoString set (without *PLEKHG4B*) in descending order (top to bottom), the right plot shows the corresponding Spearman correlation between the NanoString data and the microarray data of the 19 training samples. The cross (×) indicates a gene (*GLUL*) not included in the final NanoString code set due to lower values of the two statistics.

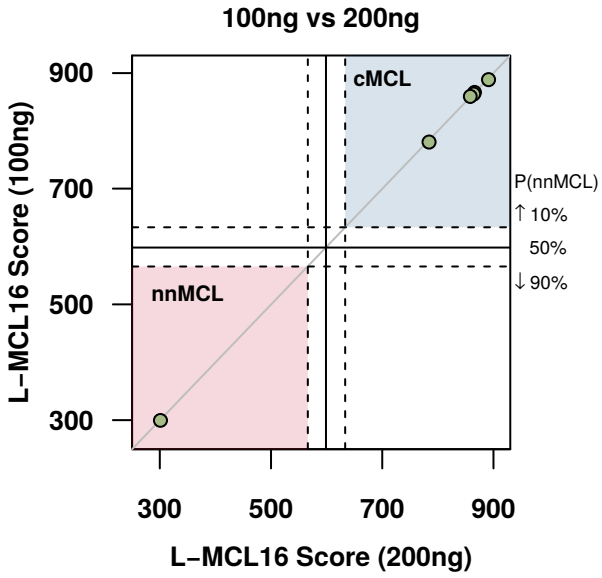

Figure S4. L-MCL16 scores of RNA from six blood samples run with a load of 100 ng and with a load of 200 ng. The x-axis corresponds to the 200-ng score, while the y-axis corresponds to the 100-ng score.

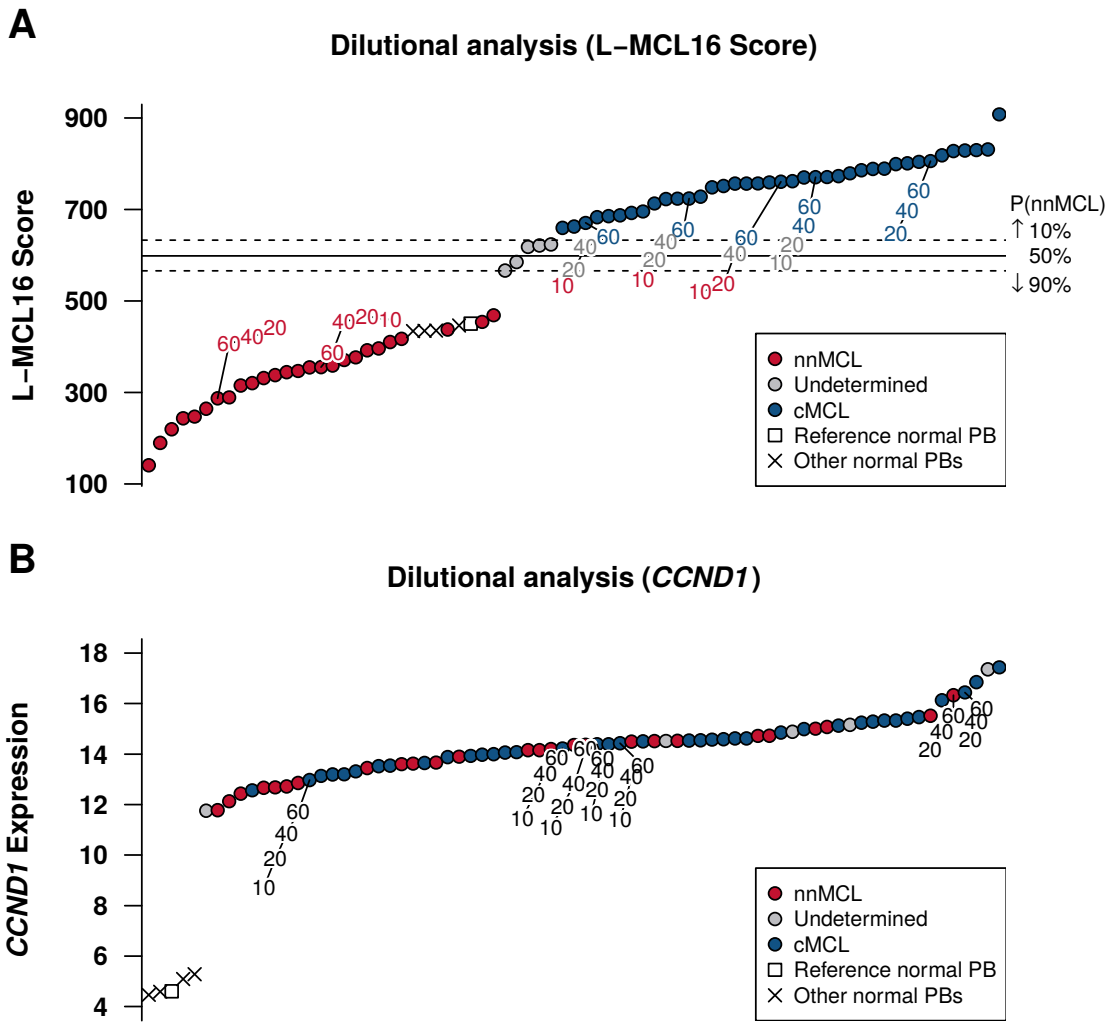

Figure S5. L-MCL16 scores **(A)** and *CCND1* expression **(B)** of the dilution experiments. The values (L-MCL16 score or *CCND1* expression) of the validation samples are shown in ascending order (from left to right). The values of the dilution experiments of the same sample are linked with a line, and the percentage of the dilution is indicated with numbers in the plot. The squares (□) represent the values of the normal peripheral blood (PB) sample used to perform the dilutions, while the crosses (×) represent the values of other normal PB samples.

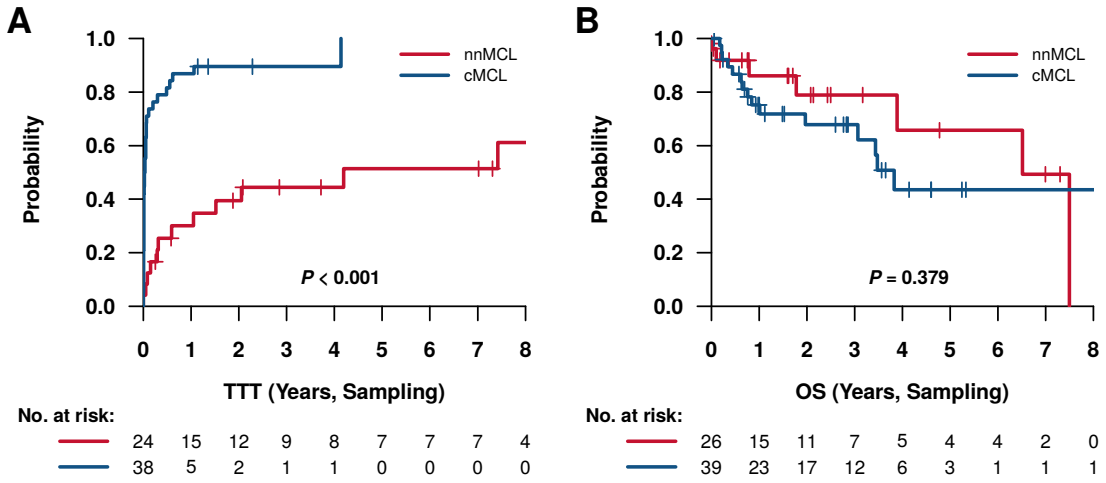

Figure S6. **(A)** Kaplan-Meier (KM) curves of the time to first treatment (TTT) from sampling time of the nnMCL and cMCL subgroups identified by the L-MCL16 assay (validation cohort). **(B)** KM curves of the overall survival (OS) from sampling time of the nnMCL and cMCL subgroups identified by the L-MCL16 assay (validation cohort).

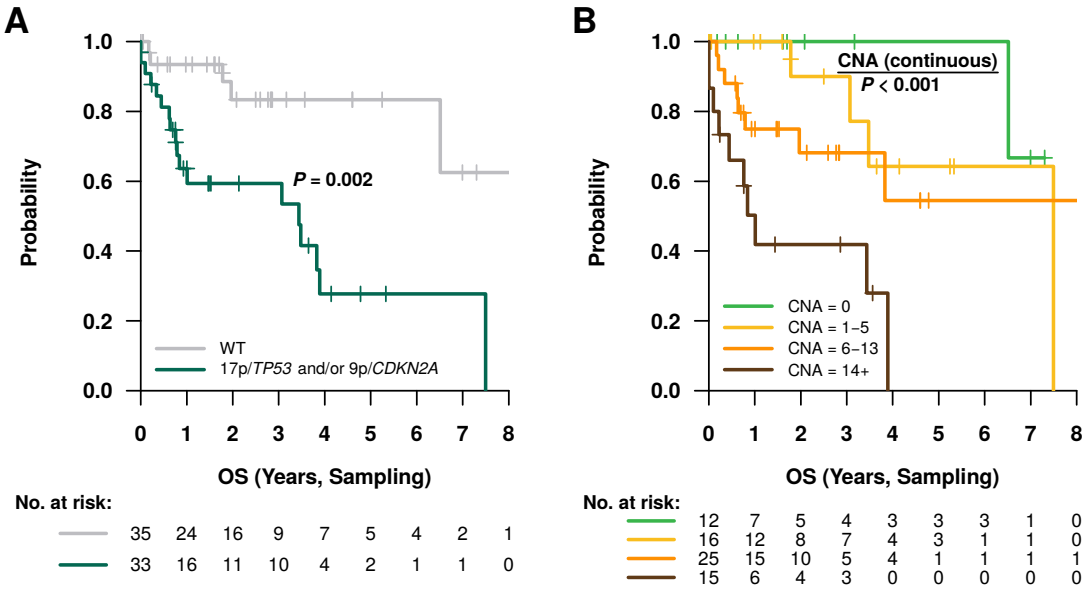

Figure S7. Prognostic impact of molecular features in the validation cohort. **(A)** Kaplan-Meier (KM) curves of the overall survival (OS) from sampling time of the patients with 17p/*TP53* and/or 9p/*CDKN2A* alterations versus patients with wild-type 17p/*TP53* and 9p/*CDKN2A*. **(B)** KM curves of the OS from sampling time according to the number of copy number alterations (CNAs), grouped into four categories. The number of CNAs was associated with OS as a continuous variable, the KM curves represented in this figure are a visual approximation of the relationship.

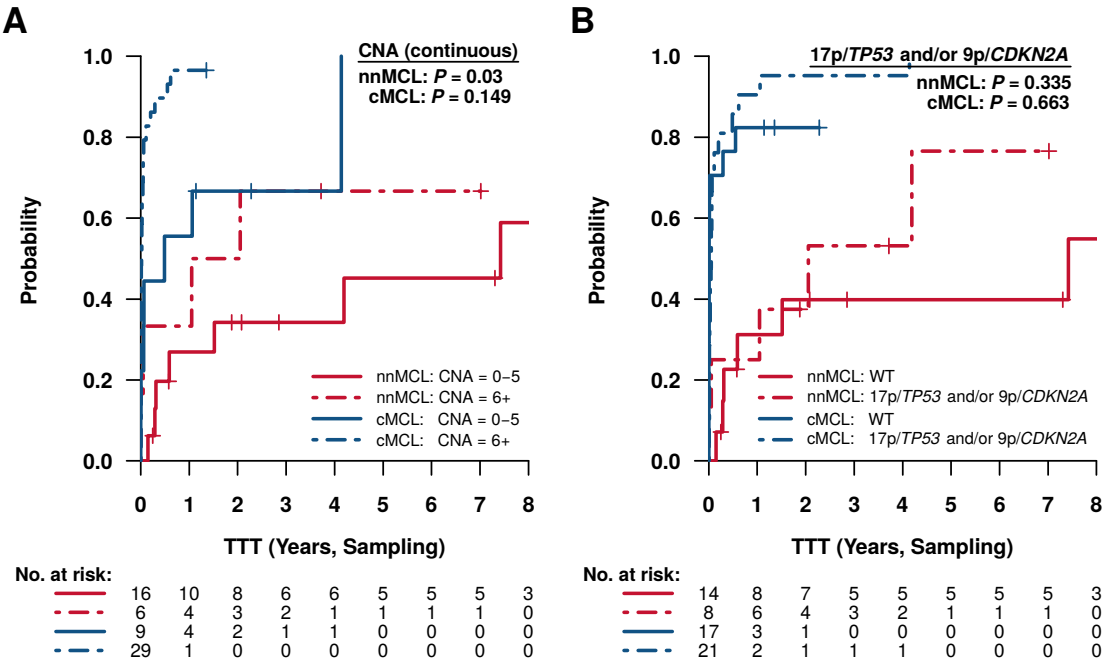

Figure S8. **(A)** Kaplan-Meier (KM) curves of the time to first treatment (TTT) from sampling time according to the number of copy number alterations (CNAs) for the nnMCL and cMCL subgroups (validation cohort). The number of CNAs was associated with TTT as a continuous variable. **(B)** KM curves of the TTT from sampling time according to the presence of 17p/*TP53* and/or 9p/*CDKN2A* alterations for the nnMCL and cMCL subgroups (validation cohort).

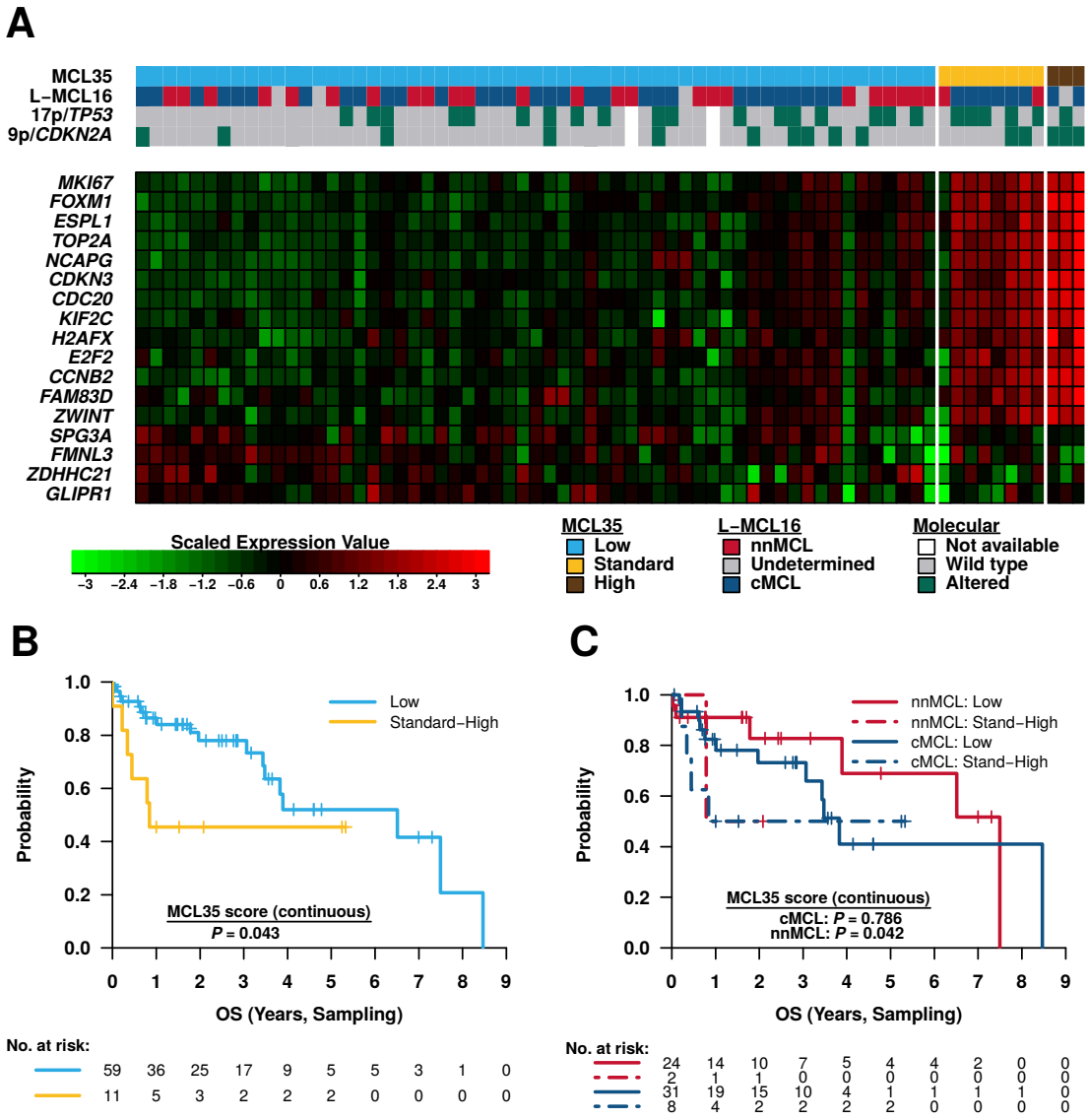

Figure S9. Prognostic impact of the proliferation MCL35 assay in the validation cohort. **(A)** Heatmap of the 17 informative genes included in the proliferation MCL35 assay, genes are shown as rows and samples as columns. Samples are ordered in ascending order according to the MCL35 score (from left to right). Shown above are several molecular features (MCL35 risk group, L-MCL16 subgroup, 17p/*TP53* and 9p/*CDKN2A* alterations). **(B)** Kaplan-Meier curves (KM) of the overall survival (OS) from sampling time of the groups identified by the proliferation MCL35 assay. The *P* value corresponds to the MCL35 score analyzed as a continuous variable. **(C)** KM curves of the OS from sampling time according to the groups identified with the proliferation MCL35 assay for the nnMCL and cMCL subgroups. The *P* values correspond to the MCL35 score analyzed as a continuous variable.

REFERENCES

1. McCarthy BA, Yancopoulos S, Tipping M et al. A seven-gene expression panel distinguishing clonal expansions of pre-leukemic and chronic lymphocytic leukemia B cells from normal B lymphocytes. Immunol.Res. 2015;63:90-100.

2. Navarro A, Clot G, Martinez-Trillos A et al. Improved classification of leukemic B-cell lymphoproliferative disorders using a transcriptional and genetic classifier. Haematologica. 2017;102(9):e360-e363