Molecular Medicine

Supplemental Data

Chronic Lymphocytic Leukemia Cells in a Lymph Node Microenvironment Depict Molecular Signature Associated with an Aggressive Disease

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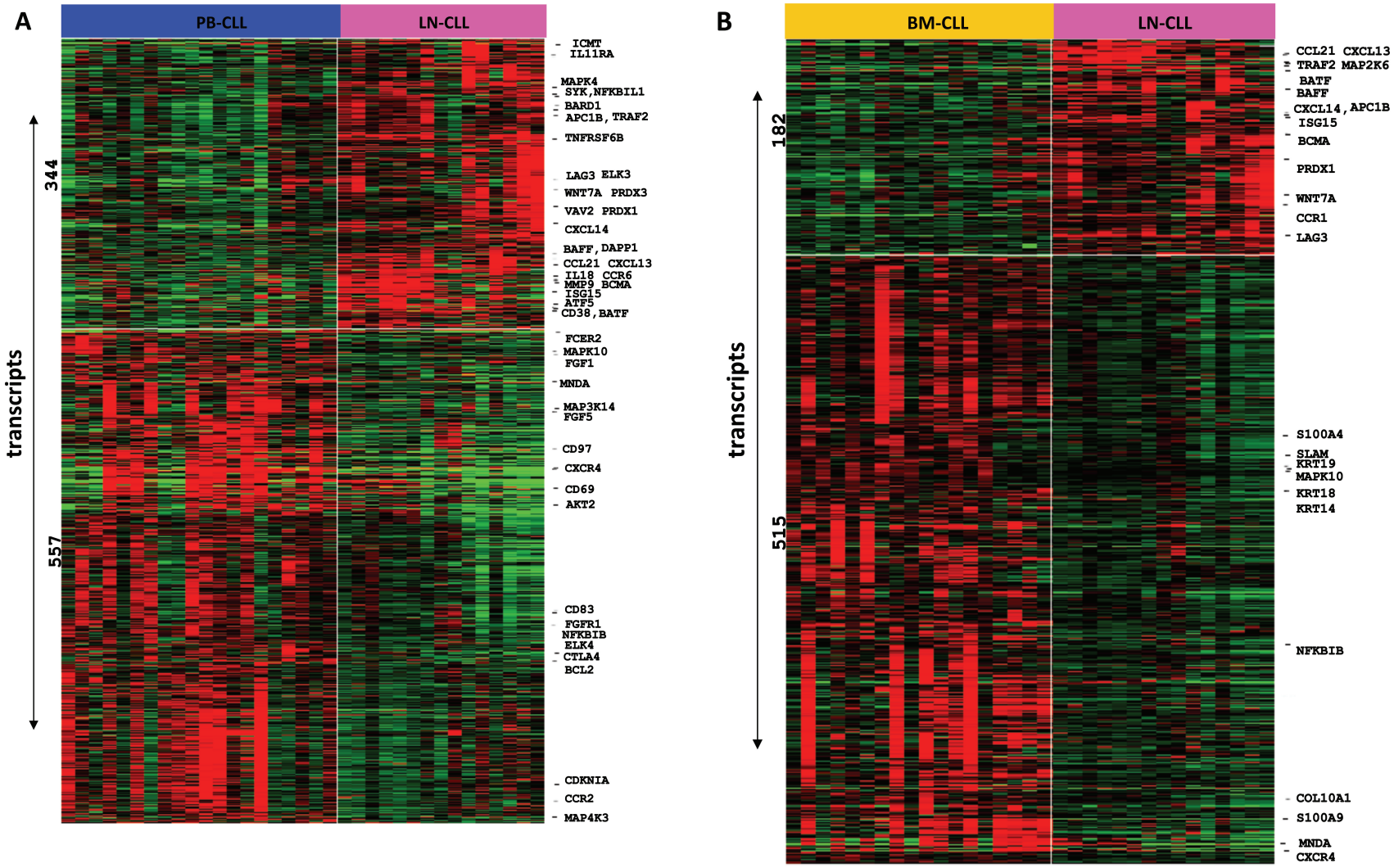
Supplementary Table S1. Clinical characteristics of 37 patients with CLL.

CLL #	Tissues for GEP	Age (Years)	Gender	Treated	Time to Rx (months)	CD38(PB) %	BLA	Rai Stage	LDT	Cytogenetics
11	LN	59	M	Yes	1	80	Yes	1	<1	Trisomy12, 14q-
12	PB, BM	69	M	Yes	1	22	No	1	>1	NA
16	PB, BM	51	M	Yes	1	89	Yes	3	NA	Trisomy 12, 17p-, 13q14-
22	PB	66	F	No	69	12	No	0	<1	Normal
32	BM	65	F	NA	NA	34	Yes	1	NA	13q14-
34	BM, LN[a]	74	M	Yes	2	63	Yes	2	NA	11q23-
37	PB, BM	53	M	NA	NA	4	No	1	NA	Normal
40	LN[a]	49	M	Yes	1	92	Yes	1	NA	13q14-,13q null
59	LN	62	F	Yes	108	NA	Yes	3	>1	NA
64	LN	72	F	Yes	48	NA	Yes	3	NA	17p13-
66	LN[a]	61	F	Yes	11	84	Yes	4	<1	11q-,13q-, 17p-
67	BM, LN	42	M	Yes	1	17	Yes	3	>1	11q23-, 13q14-
74	BM	55	M	No	38	5	No	0	>1	13q13-
82	PB, LN	79	M	Yes	4	92	Yes	2,3	>1	Trisomy12, 13q-
83	BM, LN[a]	29	M	Yes	22	98	Yes	2	<1	11q-, 14q32-
89	PB, BM	39	M	Yes	10	1	Yes	2,3	>1	Trisomy12
90	PB	66	F	No	35	7	No	0	>1	13q14-
93	LN	50	M	Yes	1	10.55	Yes	4	NA	14q-
96	PB	85	F	Yes	1	9	Yes	2	NA	Normal
97	PB	54	F	Yes	2	51	Yes	2	NA	Normal
106	PB, BM	65	M	No	37	2	No	1	>1	13q null
107	PB	58	M	Yes	2	1.5	Yes	3,4	NA	11q23-, 13q14-
109	PB	44	M	Yes	0.25	44	Yes	2,3	NA	11q-,13q-, 14q-
117	PB,BM	80	M	Yes	48	26.33	Yes	1,2	NA	13q14-, 14q-
120	BM	65	M	Yes	3	0.15	Yes	1,2	NA	14q-
121	PB	47	M	No	41	7	No	1	>1	13q14-
122	BM	50	M	Yes	17	0.14	Yes	3,4	>1	17p-, 20q-, 7p-
124	PB, BM	53	F	No	72	2.11	No	0	>1	14q32-
134	PB	57	M	Yes	82	18.89	No	1	<1	13q null
141	PB	71	M	No	39	20.44	No	0	>1	13q14-
143	PB	69	F	No	25	4.53	No	0,1	<1	13q14-
146	PB	76	F	Yes	17	39.81	No	0,1	<1	11q23-, 13q14-
149	PB, LN	41	M	Yes	6	2.59	Yes	3,4	NA	17p-,13q-, 14q-
152	BM	60	M	No	9	14.41	No	0,1	NA	13q-
163	BM	70	M	No	13	15	No	0,1	NA	13q null, 13q-
166	BM	52	M	No	9	18.66	Yes	0,1	NA	Normal
168	BM	56	F	No	2	NA	Yes	1	NA	13q null, 13q-

Abbreviations: M=male, F= female, NA= not available, Rx= treatment, 11q- = 11q deletion, 17p- = 17p deletion, 13q- = 13q deletion, 14q- = 14q deletion, BLA= Bulky lymphadenopathy, LDT = lymphocyte doubling time, [a] = two specimens were used.

Supplementary Table S2. List of the Primers used in real-time PCR.

Gene	Forward Oligo/Reverse Oligo (5′ to 3′)	Product Size	Tm
ATF2	ATGGTGCCTAGTGTTCCAGG/ATCCGCTACCATGACCTTTG	145bp	60°C
BAFF	ACCGCGGGACTGAAAATCT/CACGCTTATTTCTGCTGTTCTGA	98bp	60°C
BCMA	GCAGTTTTCGTGCTAATGTTTT/TCATCACCAGTCCTGCTCTTTT	131bp	60°C
BCL2	TTGTTGTTGTTCAAACGGG/GGCTGGGCACATTTACTGTT	140bp	60°C
BTK	AAAGCAGTTCCTTCACCGAGACCT/ACCGGACTGGAAATTTGGAGCCTA	146 bp	60°C
CAMLG	TGCTCATGAACTCGGAACAG/AATCACCCAGCACTACTCGC	162bp	60°C
CAV1	TGAGATTCAGTGCATCAGCC/AACTTGAAATTGGCACCAGG	191bp	60°C
CCND2	CTTCGCTTCTGGTATCTGGC/TGAGGAATGTTGTGATGGG	104 bp	60°C
CCL21	CAAGACACCATCCCCACA/TGTGACCGACTCAGTCCTC	124 bp	60°C
CCR7	GTCCAGAAACTGTTCCCACCTGC/TTCCTCACCAAGCCAAGAAGTCT	198 bp	60°C
CXCR4	TGCTTGCTGAATTGGAAGTG/ATGGAGTAGATGGTGGGCAG	173bp	60°C
FCER2	TTCGAGCTGAACAGCAGAGA/TCTCGTTCAATTCCTGGGAC	108bp	60°C
IDH	TTGTCCAGATGGCAAGACAG/GCTTTGCTCTGTGGGCTAAC	149bp	60°C
IL-6	AGATGCAATAACCACCCCTG/AACAACAATCTGAGGTGCCC	180bp	60°C
MAP2K6	TCAATGCTCTCGGTCAAGTG/ATGCCCAGACTCCAAATGTC	175bp	60°C
MCM3	TCAAGCCTGTCCTGACACAG/CAGGTCCACAGTCTTGCTCA	185bp	60°C
NFκBIB	TAATTAGGCACCGACCTTGG/CACTTCTGCCCAAGCTCTTC	117bp	60°C
STAT1	TCATCTTCTCTGGCGACAGTTTTC/GCGAATTTGCTGGCCTTTCT	109 bp	60°C
SYK	GGAAGTGAAGTCACCGCTATG/GGGAGCGGTTAGTTCACCAC	185bp	60°C
TRAF2	TGAGAACATTGTCTGCGTCC/CCAAGACCTTCTGCTCCAAG	185bp	60°C
TRAF1	ACTTCATGCAGGTGAAACCC/TACGGGATTCTGGAAAGCAC	131bp	60°C
ZAP-70	GTTGACTCATCCTCAGAGACGAATC/AGGTTATCGCGCTTCAGGAA	183bp	60°C



Supplementary Figure S1. Differential gene expression of LN-CLL compared to PB-CLL and BM-CLL cells. CLL cells from PB (n=20), BM (n=18), and LN (n=15) samples were used for microarray analyses. Significant Analyses of Microarray (SAM) identified differentially expressed genes (numbers are shown on left side of figure) between microenvironments. The red color demonstrates overexpression and green color represents underexpression of target genes. These overexpressed and underexpressed genes are separated by arrows. (A) Comparison of LN-CLL versus PB-CLL; (B) Comparison of LN-CLL versus BM-CLL; (C) Comparison of PB-CLL versus BM-CLL. SAM identified differentially expressed genes (numbers are shown on left side of figure) between microenvironments.

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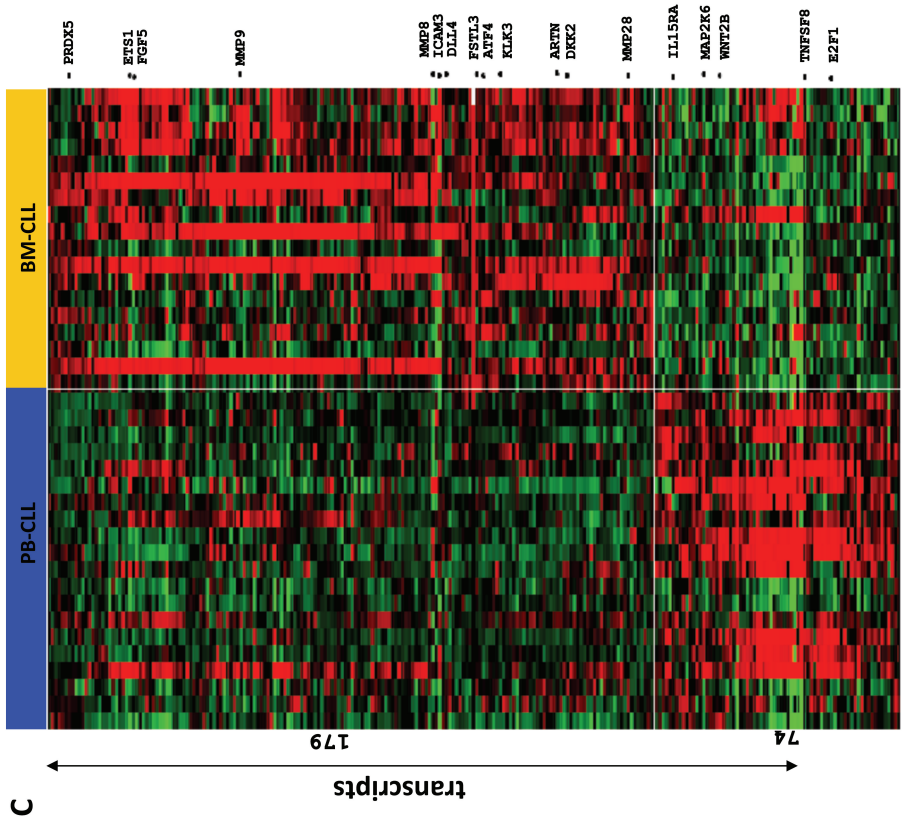
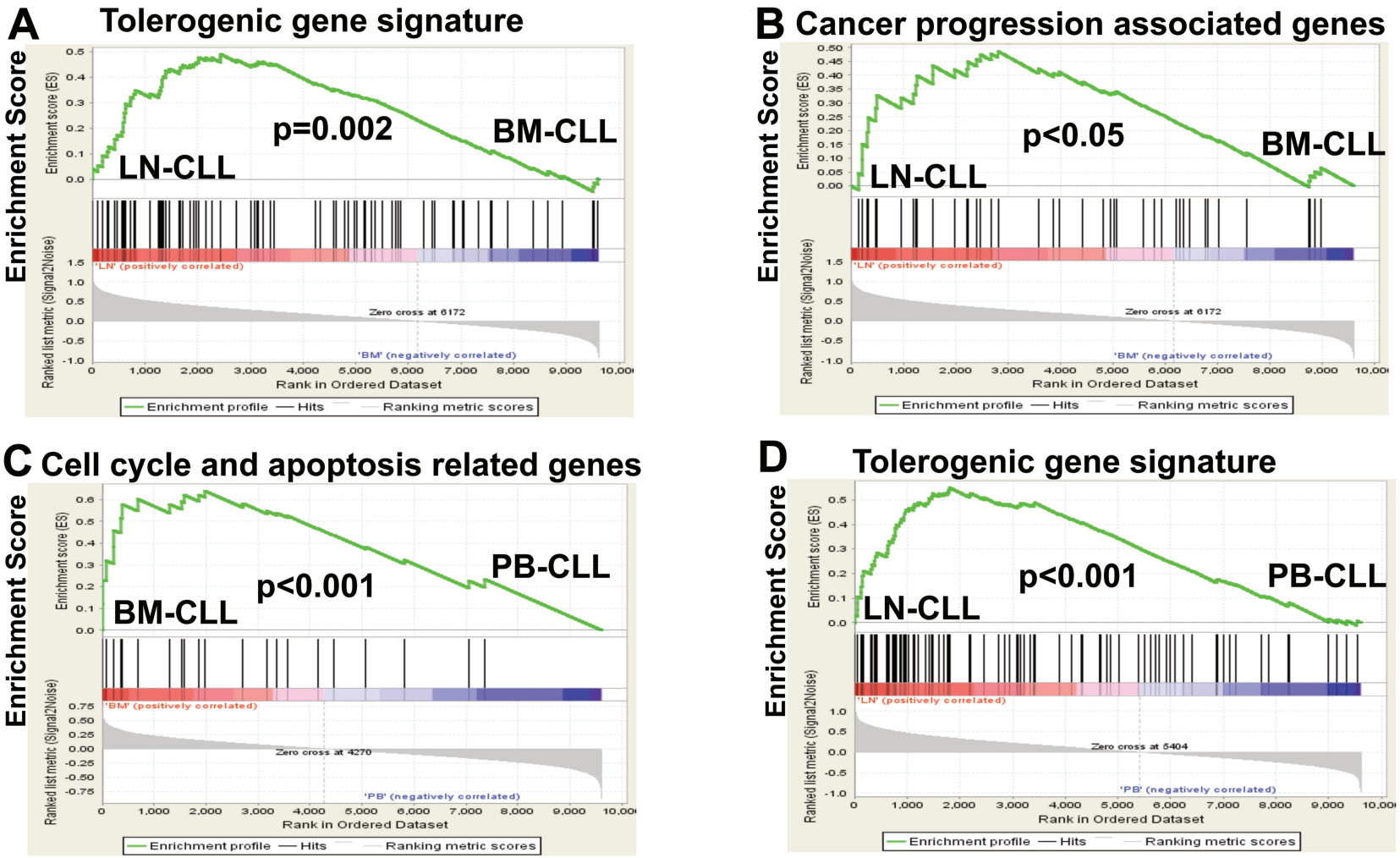


Supplementary Figure S1. *Continued.*



Supplementary Figure S2. Cellular pathways among PB-CLL, BM-CLL, and LN-CLL cells. CLL cells from PB (n=20), BM (n=18), and LN (n=15) samples were used for Gene Set Enrichment Analyses (GSEA) analyses. GSEA identified active pathways associated with CLL microenvironments. Representative enrichment plots of the active pathways among PB-CLL, BM-CLL, and LN-CLL are shown. Upregulation of these pathways was significant ($p<0.05$, false discovery rate (FDR)<0.25). (A,B) Active pathways associated with LN-CLL compared to BM-CLL; (C) Active pathways associated with BM-CLL compared to PB-CLL; (D-F) Active pathways associated with LN-CLL compared to PB-CLL.

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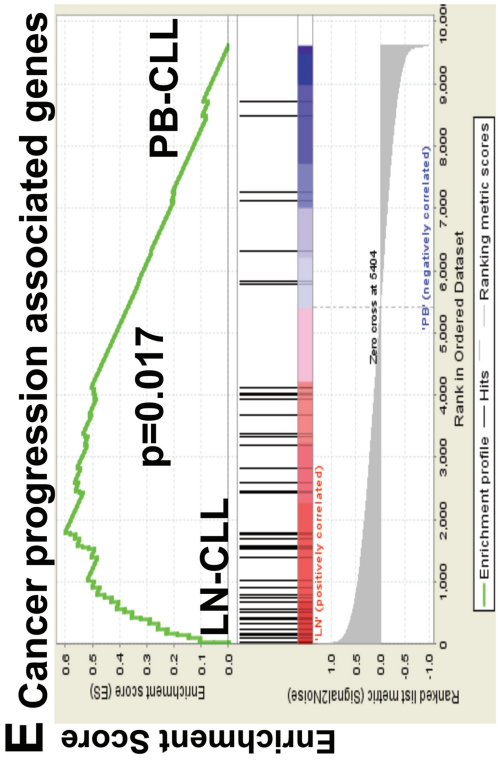
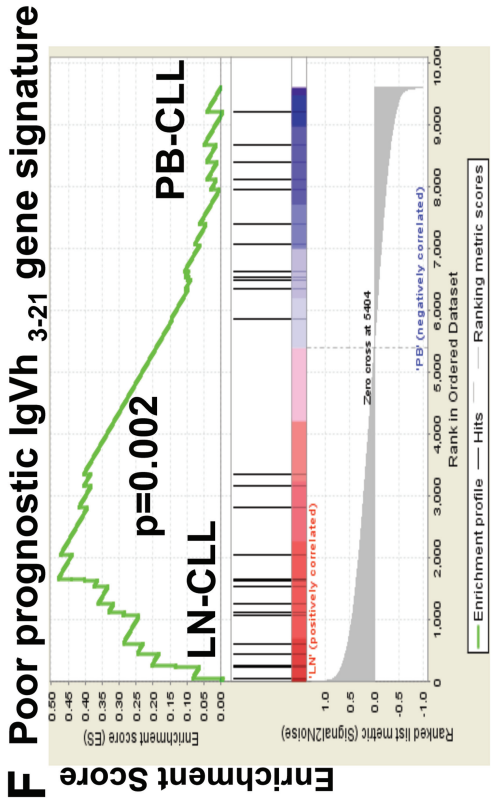
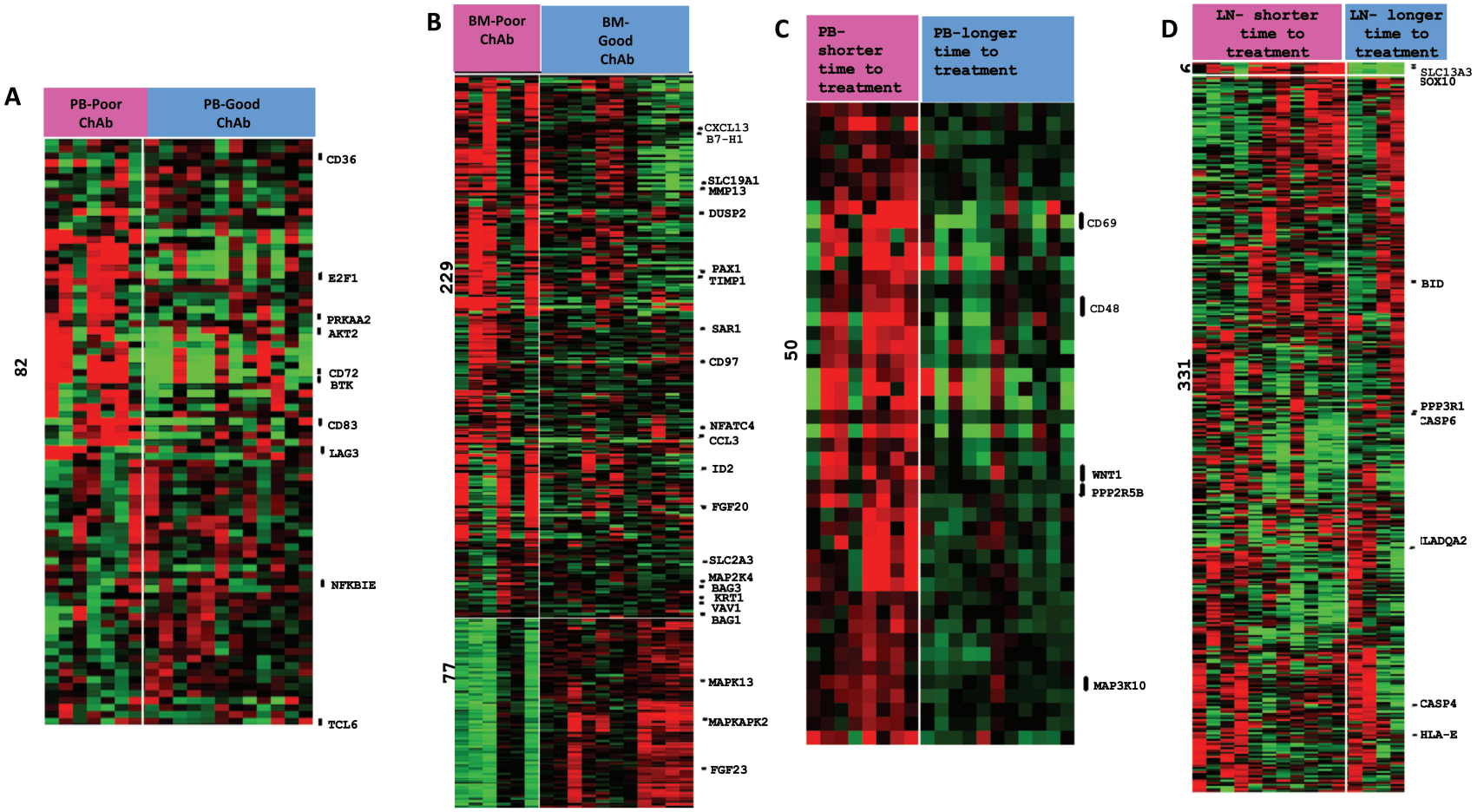




Supplementary Figure S2. *Continued.*



Supplementary Figure S3. Correlation of differential gene expression in PB-CLL, BM-CLL, and LN-CLL with known prognostic markers. Gene expression profiles of PB-CLL, BM-CLL, or LN-CLL were correlated with good versus poor clinical outcome using SAM analyses. The CLL samples were grouped based on known prognostic indicators such as short (<12 months) versus long (>12 months) time to treatment, and poor prognosis chromosomal abnormalities (poor ChAb; 11q deletion, 17p deletion, and trisomy 12) versus good prognosis chromosomal abnormalities (good ChAb; 13q deletion and normal karyotype). (A) Gene expression profiles in PB-CLL with poor ChAb, n=7 versus good ChAb, n=12; (B) BM-CLL with poor ChAb, n=6 versus good ChAb, n=11; (C) Comparison of gene expression profiles in PB-CLL with shorter time to treatment, n=9 versus longer time to treatment, n=10; and, (D) LN-CLL with shorter time to treatment, n=11 versus longer time to treatment, n=4.