SUPPLEMENTARY APPENDIX

The involvement of microRNA in the pathogenesis of Richter syndrome

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SUPPLEMENTARY MATERIAL (SM)

miRNAs involvement in the pathogenesis of Richter's syndrome

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Supplementary Methods

Xenogeneic mouse transplantation

We analyzed the miRNA expression in PBMCs from 5 CLL patients before transfer into NSG mouse (**pre-transfer**) and the paired human $CD5^+CD19^+$ cells from xenografted NSG mice (**post-transfer**). At euthanasia, spleens were collected and human $CD5^+CD19^+$ cells isolated by fluorescence activated cell sorting. In all 5 cases the cells proliferated in the spleen.

Firefly miRNA profiling assay

Starting material was RNA extracted from FFPE tissue (BM, if involved, or LN) with the RNeasy FFPE kit (Qiagen, Germantown, MD, USA) according to the manufacturer's instruction. Samples were run at the Abcam Service Lab and raw data were background-subtracted and normalized with a geNorm-like algorithm using the three most stable miRNAs (hsa-miR-15a-5p, hsa-miR-191-5p and hsa-miR-26a-5p) with the Firefly Analysis Workbench software.

Quantitative reverse transcription (qRT)-PCR

RNA was extracted from FFPE tissue with the RNeasy FFPE kit and deparaffinization solution (Qiagen, Germantown, MD, USA) according to the manufacturer's protocol. RT reaction and qRT-PCR was performed with TaqMan miRNA assays as previously described [1] for hsa-let7g, hsa-miR-15a, hsa-miR-16, hsa-miR-17, hsa-miR-21, hsa-miR-23b, hsa-miR-24, hsa-miR-24-1*, hsa-miR-26a,

hsa-miR-29a, hsa-miR-29b, hsa-miR-29c, hsa-miR-34a, hsa-miR-146a, hsa-miR-146b, hsa-miR-150, hsa-miR-155, hsa-miR-181a, hsa-miR-181b, hsa-miR-191, hsa-miR-195, hsa-miR-221, hsa-miR-223, ebv-miR-BART4 and ebv-miR-BART16 (Life Technologies, Carlsbad, CA, USA). The geometric mean of U6 and RNU48 was used to normalize expression, and was found to not be significantly different between analyzed groups. Relative expression levels were determined with the comparative Ct method (delta delta CT).

miRNA gene expression profiling

Gene expression profiling was performed on the Agilent miRNA microarray platform (Sanger miRBase Release 14.0) for n = 58 samples (Ulm University cohort). 100 ng of total RNA per sample was labeled and subsequently hybridized at 55°C for 20h using the Agilent miRNA Complete Labeling and Hyb Kit, subsequently washed according to the Agilent Gene Expression Wash Buffer Kit and scanned with the Agilent microarray Scanner. Raw data were extracted using the Agilent Feature Extraction Software. Arrays used to generate miRNA expression profiles were adjusted for potential batch effects and normalized by using the least-variant set (LVS) of genes method [2]. Expression data was clustered and depicted as heatmap using the "Genesis platform" [3]. Hierarchical clustering was used with Pearson correlation as agglomeration rule and average linkage as distance metric.

miRNA target and Ingenuity pathway analysis

We performed enrichment analysis using QIAGEN's Ingenuity Pathway Analysis (IPA, QIAGEN, Redwood City, https://www.qiagenbioinformatics.com/products/ingenuity-pathway-analysis/) to explore the biological functions, potential molecular interactions, and canonical pathways for identified miRNAs in our study. First, validated targets of miR-17, miR-29c, miR-191, miR-21, miR-146b, miR-150, miR-181b and miR-26a were identified using the publicly available algorithm miRTarBase (**Supplementary Table S5A and S5B**) [4]. We restricted the validated targets to those that were supported by strong experimental evidence, such as luciferase reporter assay, western blot or qRT-PCR, as defined by miRTarBase. We uploaded the validated target genes of the selected miRNAs into the IPA software and identified the most significant pathways from the IPA library of canonical

pathways. The statistical significance of the association between the validated targets and the canonical pathway was calculated using Fisher's exact text with a cut-off for P value set at 0.05.

Network analyses

Briefly, the correlation coefficient method was used to build networks based on the expression levels of the miRNAs measured [5-7]. We generated networks for each of the patient groups using the expression levels of miRNAs from qRT-PCR and, independently, the expression levels obtained by the Firefly assay. We also included the miRNAs that did not show any difference in expression between the patient groups in the networks, as this does not affect the connectivity of miRNAs inside a network.

For each patient group, we searched for a linear correlation between each miRNA in a matrix of 25 elements (all miRNAs measured by qRT-PCR) or 40 elements (all miRNA measured by Firefly). In order to check if there is any statistical difference between the number of edges of the networks built by qRT-PCR or Firefly, we used the chi-square test. Additionally, we calculated the connectivity of each node and evaluated if there is a statistical difference between networks (1a vs 1b and 2a vs 2b, respectively) by employing a paired t-test.

For the qRT-PCR method, we used Spearman r values of +/- 0.8 as a correlation threshold, like in our previous study [5] which corresponds to a P value of 0.01 in the smallest patient cohort (Richter cohort). The value of this threshold determines if an edge can exist between two miRNA nodes. For the Firefly expression data, we choose +/- 0.66 as a threshold, because this is the smallest Spearman r value for which we obtained a significant statistic correlation ($P < 0.05$) between two miRNAs in the smallest patient cohort (Richter cohort, $n = 9$). We selected the miRNA pairs from the generated matrices that show a correlation higher/lower than the mentioned thresholds and drew an edge between the corresponding nodes of the network.

We used as a measure of connectivity of a node the fraction between the numbers of edges and the maximum number of edges a node can form inside a given network ($l = 24$ in the qRT-PCR networks and $l = 39$ in the Firefly networks). We considered a hub the nodes with high level of connectivity (≥ 0.25 for the 1a qRT-PCR network and ≥ 0.3 for the 1b, 2a and 2b qRT-PCR networks).

Cell lines, cell culture and transfections

B-chronic lymphocytic leukemia MEC1 cell line was obtained from DSMZ and HG3 cell line was kindly provided by Dr. Anders Rosen. [8, 9] The DLBCL cell line HB was gifted by Dr. Ken H. Young (UTMDACC, Department of Hematopathology). Prior to the start of the *in vitro* experiments cells were authenticated by DNA fingerprinting and were tested for mycoplasma contamination. Cells were cultured in RPMI 1640 (Invitrogen) supplemented with 10% fetal bovine serum at 37°C in 5% CO_2 incubator. miRNA mimic transfections were done using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instruction.

Cell proliferation analysis

Cells transfected with miR-21 mimic or scramble mimic were seeded into 96-well plates at a density of 5×10^3 cells per well in 100 µl medium. The CellTiter 96 AQueous One Solution (Promega) (20 µl) was added into each well at the time-point 24, 48 and 72 h and cell viability was measured according to the manufacturer's instructions.

Soft agar colony formation assay

Pre-warmed RPMI-1640 medium containing 20% FBS was mixed with melted 1.2% agar by microwave in 6 well plates. miR-21 mimic or scramble mimic transfected cells were resuspended in medium containing 0.7% agar and overlaid on 1.2% agar in 6 well plates (5000 cells/well). 1 ml medium was added to the top layer and cells were incubated for two to three weeks at 37°C. The formed colonies were stained with crystal violet and counted. Images of the plates were captured and the number of colonies was calculated under a microscope. Experiments were performed in triplicate.

Statistical analysis

Statistical analyses were carried out with the GraphPad Prism 6 software. To determine whether the data followed a normal Gaussian distribution, the Shapiro–Wilk normality test was performed. For the paired RS/CLL cohort from UTMDACC, P values were determined with a paired t-test (normal distribution) or the Wilcoxon matched-pairs signed rank test (non-normal distribution). For the extended RS/CLL cohort from UTMDACC and the independent cohort from Ulm University, P values were determined with an unpaired t test if the data were normally distributed, while the non-parametric

Supplementary Methods

Mann–Whitney–Wilcoxon test was applied on data with a non-normal distribution. All tests were two-sided, and P values <0.05 were considered statistically significant.

Supplementary Figures and Tables.

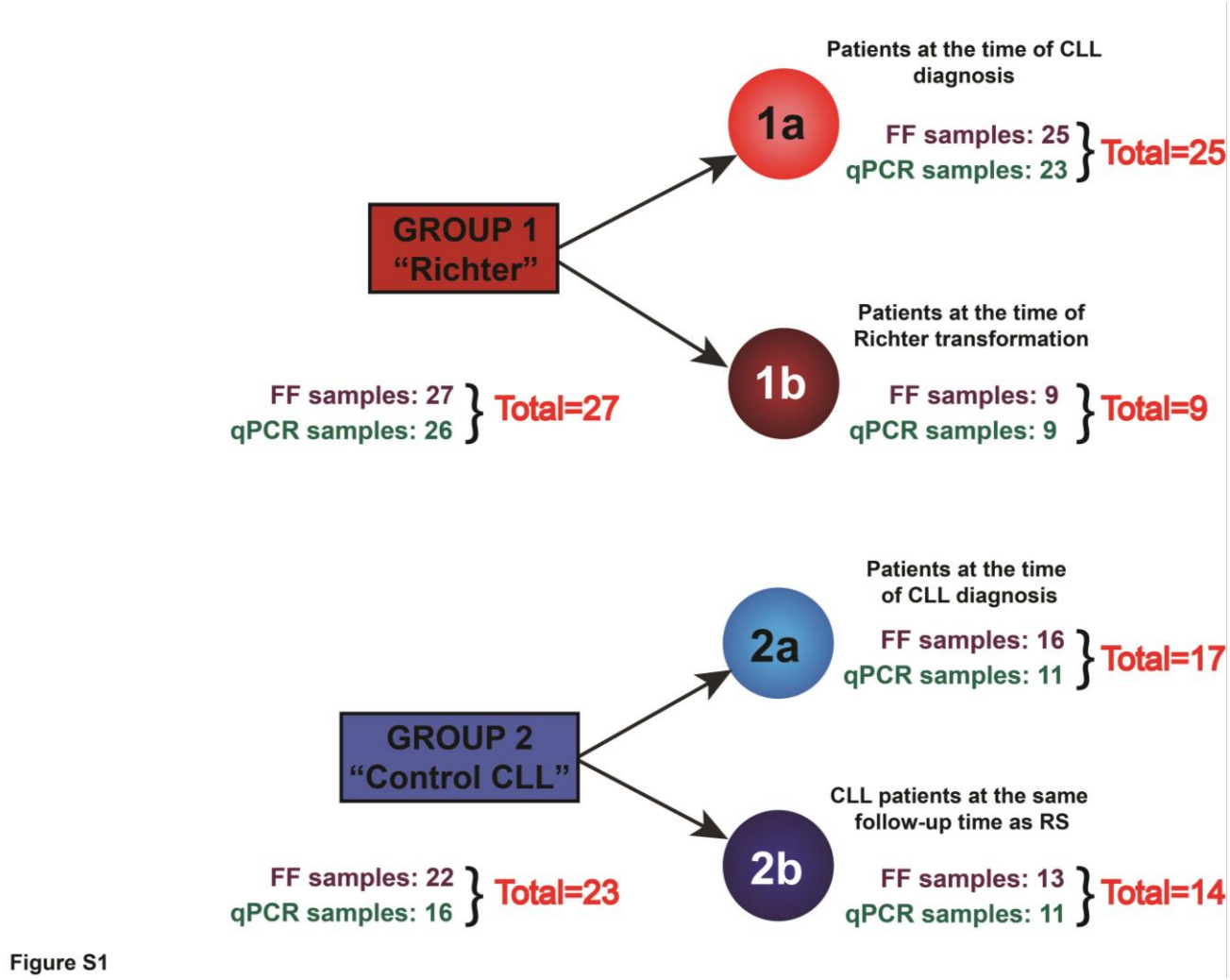

Figure S1

Supplementary Figure S1. A schematic representation of the extended cohort samples used for the Firefly and qRT-PCR analysis. In red are the total number of patients used, in purple are the number of samples used for the Firefly analysis (FF) and in green the number of samples used for the qRT-PCR analysis (qPCR). Some of the patients of group 1a and 1b overlap (only the time of sampling differs – paired samples) and also samples from the group 2a and 2b overlap (for details see second and third column of Supplementary Table S2).

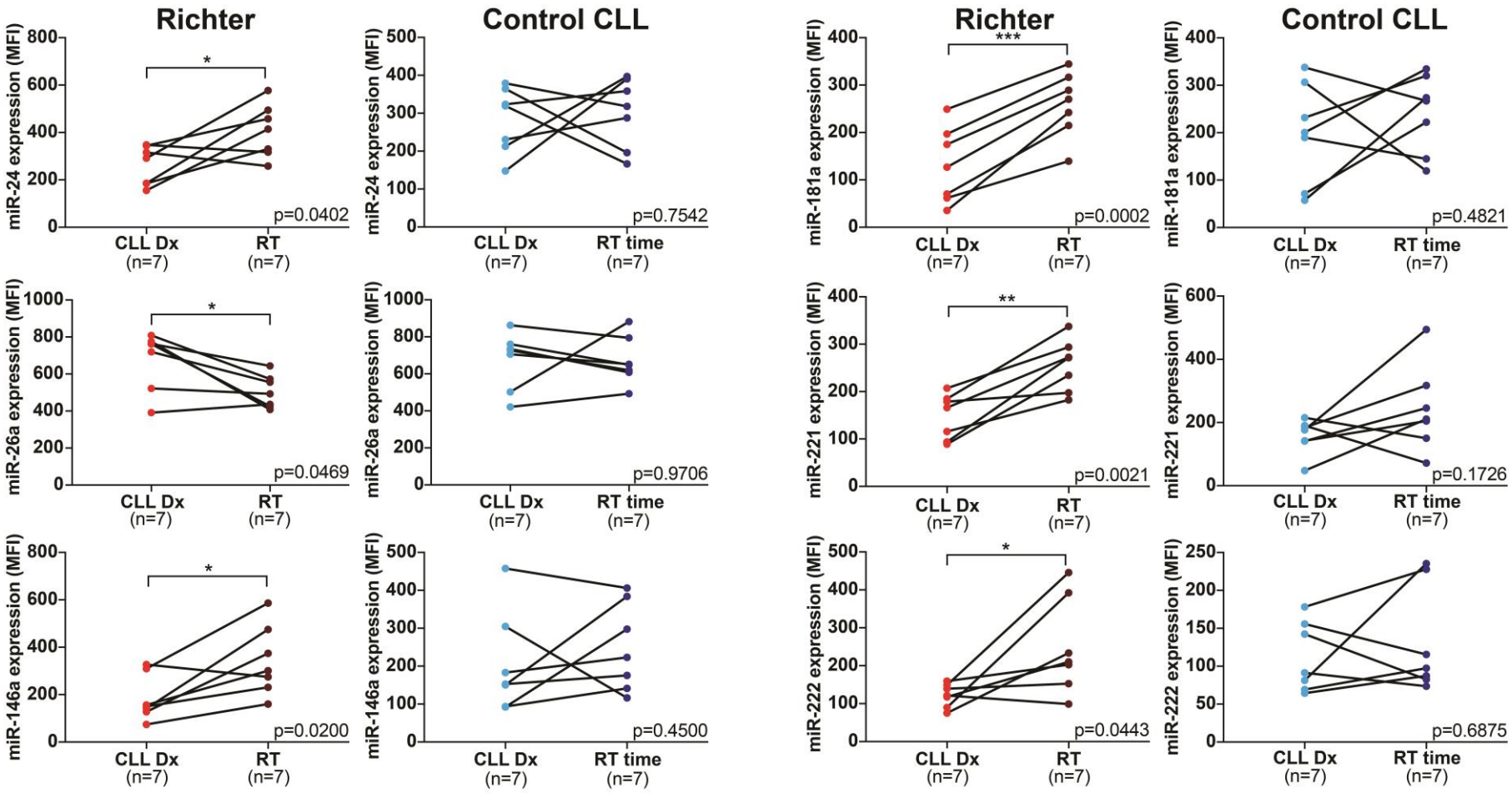

Figure S2

Supplementary Figure S2. Firefly analysis of miR-24, miR-26a, miR-146a, miR-181a, miR-221 and miR-222 in paired samples at CLL diagnosis (Dx) and at Richter transformation (RT) time. All miRNAs are differently expressed at RT time when compared to CLL diagnosis time for the Richter syndrome samples, but not for the control CLL samples. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

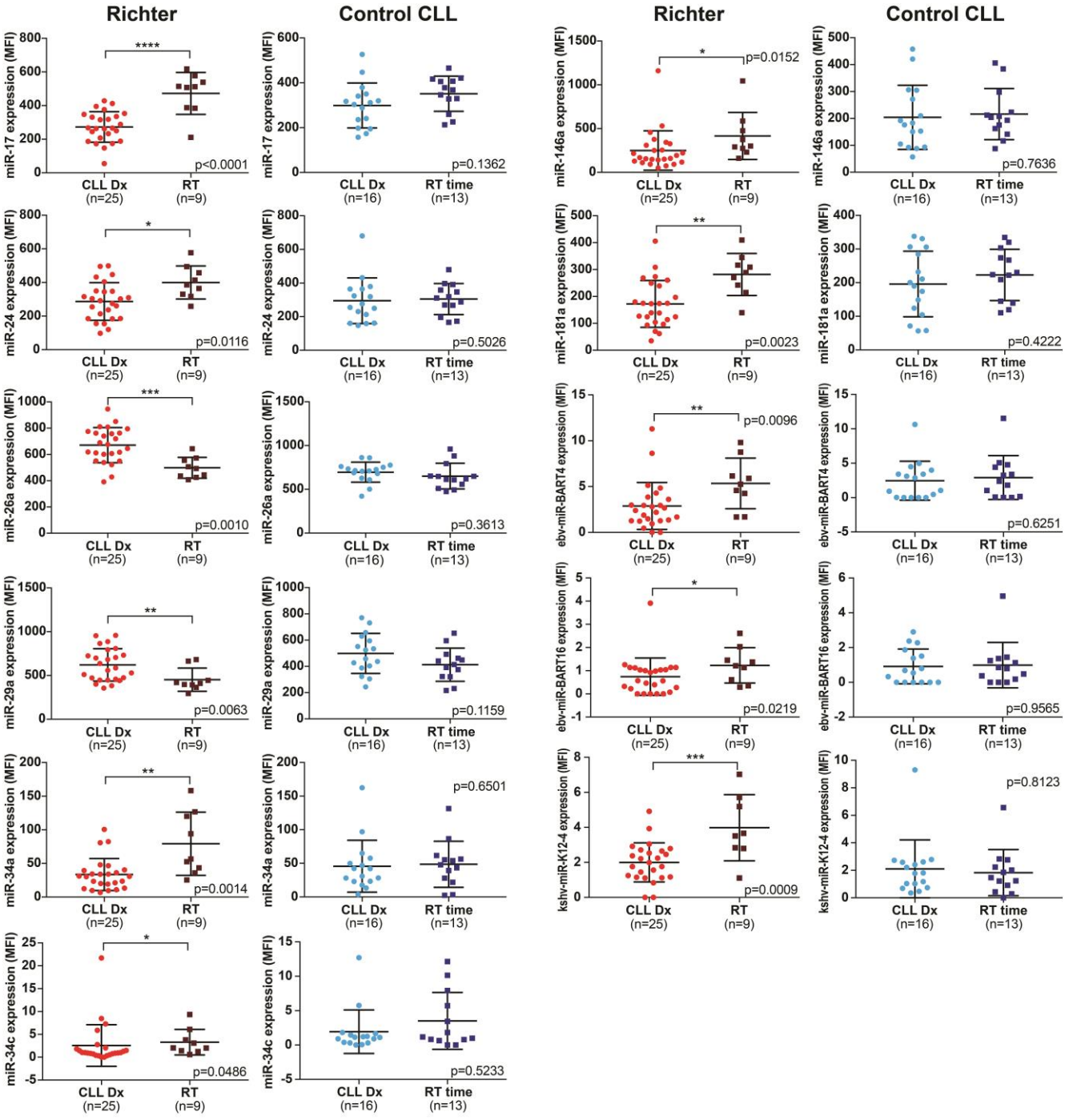

Figure S3

Supplementary Figure S3. Firefly analysis of miR-17, miR-24, miR-26a, miR-29a, miR-34c, miR-146a, miR-181a, ebv-miR-BART4, ebv-miR-BART16 and kshv-miR-K12-4 in an extended set of samples at CLL diagnosis (Dx) and at Richter transformation (RT) time. All miRNAs are differently expressed at RT time when compared to CLL diagnosis time for the Richter syndrome samples, but not for the control CLL samples. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$.

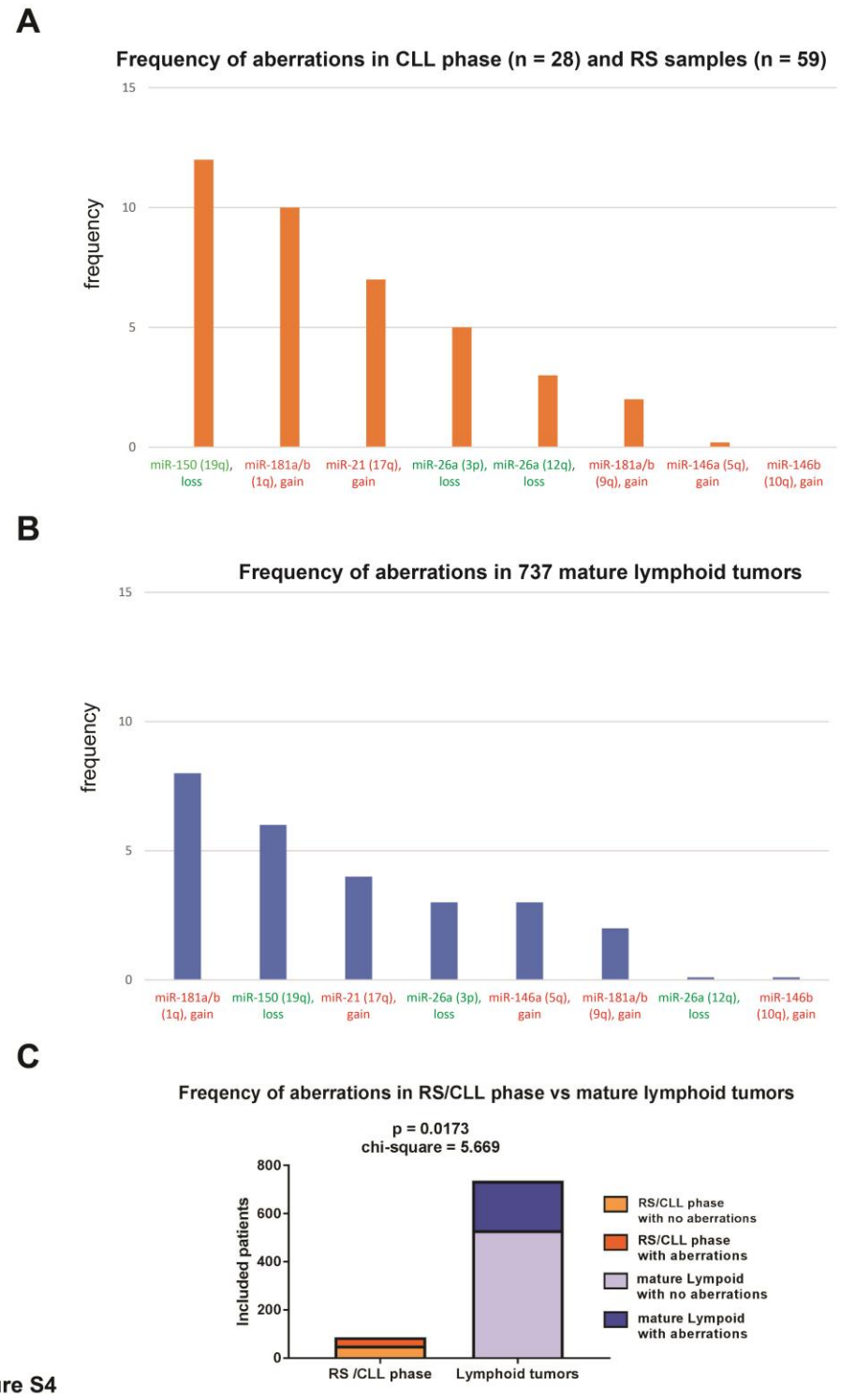

Figure S4

Supplementary Figure S4. (A) Frequency of miRNA specific gains and losses associated with Richter syndrome and CLL phase (B) Frequency of miRNA specific gains and losses associated with B-lymphoid tumors. (C) Comparison of the frequency of the DNA aberrations between RS and B-lymphoid tumors.

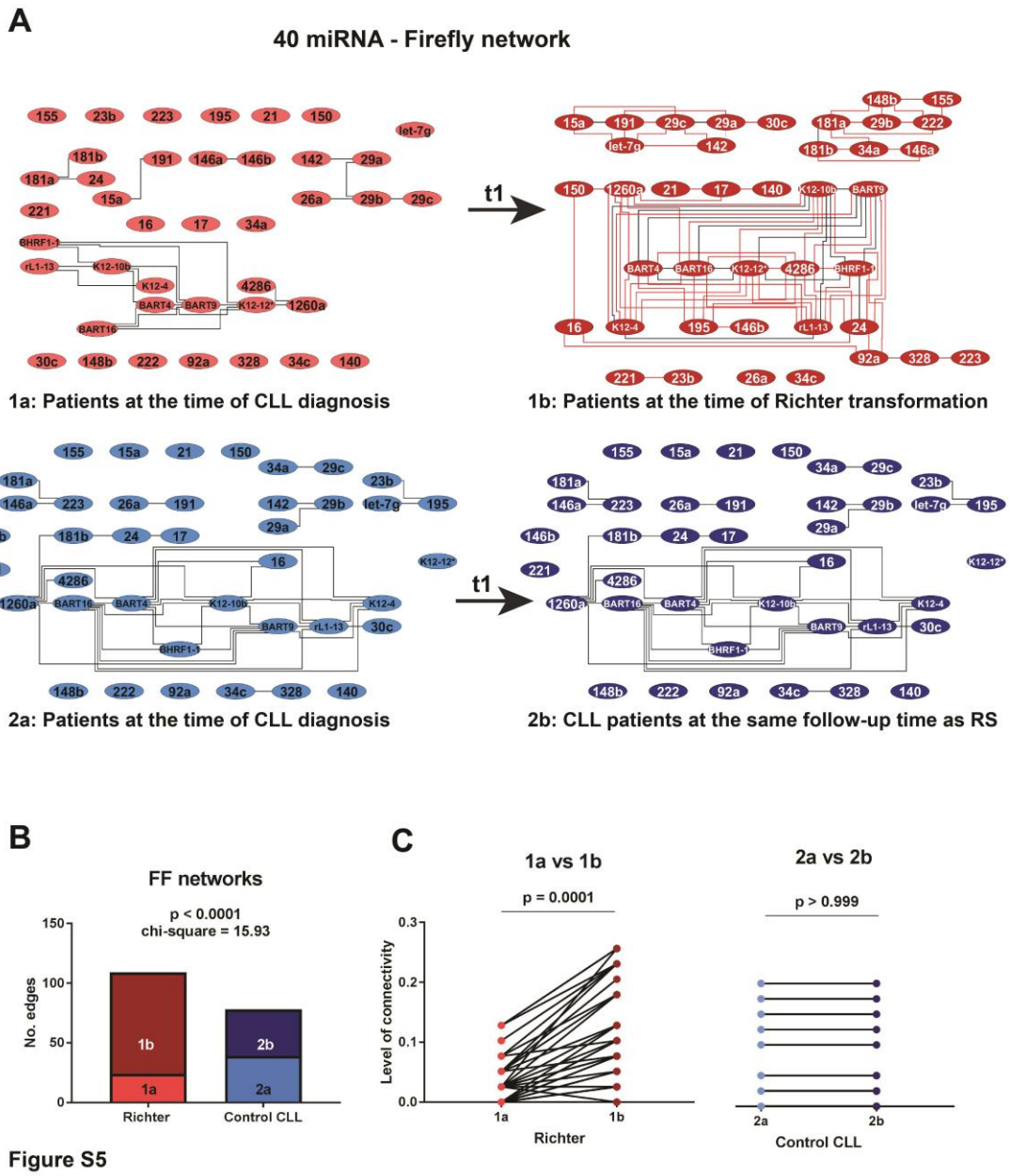

Figure S5

Supplementary Figure S5. The 40-miRNA expression network based on Firefly assay in the four analyzed groups. (A) We observed an increase in edges between 1a and 1b networks (24 to 85) and no difference between 2a and 2b FF networks (P = 0.0001, chi-square = 15.93). The miRNA nodes showed an increase in connectivity between graphs 1a and 1b (P = 0.0001) and no difference between 2a and 2b (P = 0.999). The FF networks confirmed that Richter transformation leads to a complete rearrangement of the miRNA graph, with an increase in the number of edges and addition of new miRNA hubs. (B-C) The analysis of the difference between the number of edges of the networks (B) and of the connectivity (C).

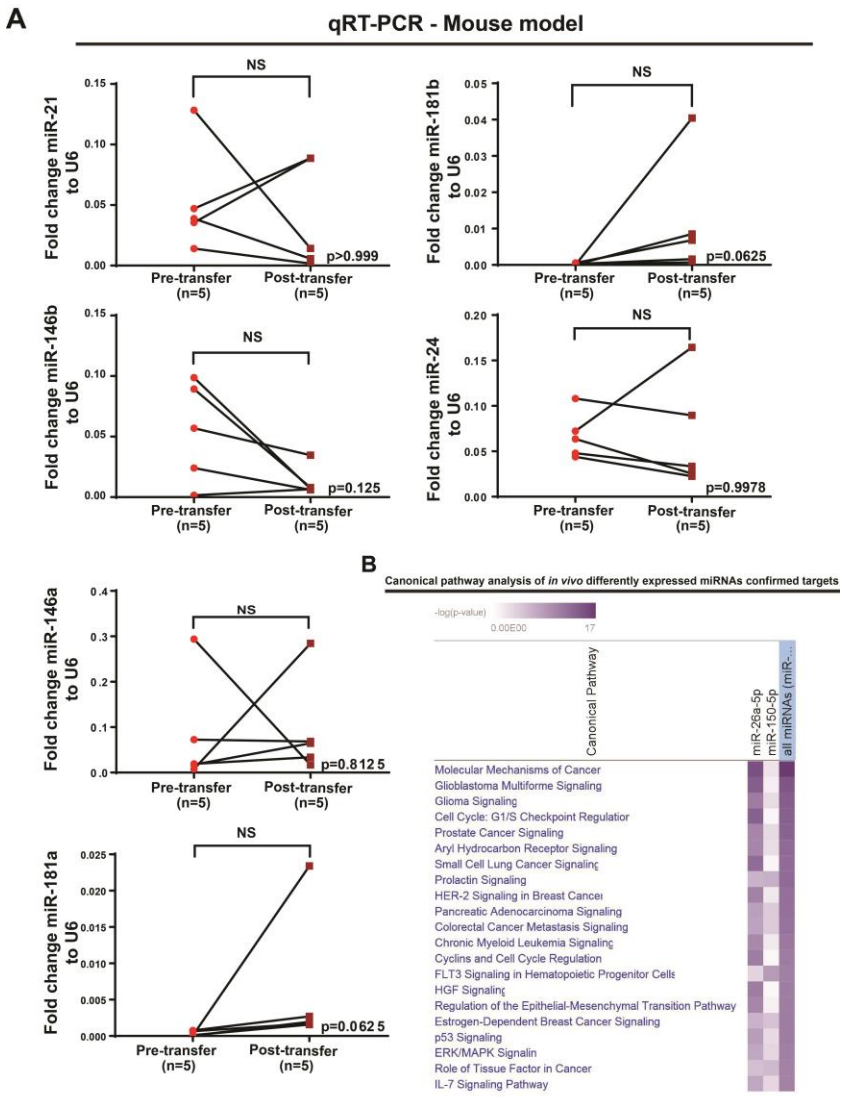

Figure S6

Supplementary Figure S6. Enlarged signature miRNA expression by qRT-PCR after transfer of CLL B cells into NGS mice and canonical pathway analysis of the in vivo differently expressed miRNAs. (A) By qRT-PCR we observed that miR-181a and miR-181b are upregulated (n.s.) after the transfer of the human CLL cells in the NSG mouse model, similar to the trend we detected in the patient samples. The other 4 miRNAs of the enlarged RS signature (miR-21, miR-146b, miR-146a and miR-24) did not show a clear change between pre-transfer and post-transfer (B) IPA canonical pathway analysis of common confirmed targets of the two significantly downregulated miRNAs, miR-26a and miR-150 that we confirmed both in patients and *in vivo* model showed that most of the targets are involved in cancer related mechanism, including p53 signaling pathway.

Supplementary Table S1. Selection criteria for the RS and matched CLL control cohort				
		Richter (n=7)	**Matched CLL Control (n=7)**	**p-value***
Age at diagnosis, years				0.1977
	Median	50	55	
	Average	50.1	56.0	
	Range	35-68	49-66	
Gender				1.000
	Male	2	2	
	Female	5	5	
Time to transformation for RS patients/matching follow-up time for CLL control patients				0.5096
	Median	93	104	
	Average	83	105.9	
	Range	8-204	30-190	

*p-value for gender calculated by two-tailed Fisher's exact test; p-value for age and time to transformation calculated by two-tailed unpaired t-test

Supplementary Table S2 – detailed clinical data of Richter (group 1a and 1b) and CLL (group 2a and 2b) patients included in the study.

RS	Group FF analysis	Group PCR analysis	Age at Dx	Sex	RAI stage	FISH (% of total cells)							ZAP70	CD38	B2M	IGHV status		LDH	Time to transformation in months	Survival time in months	Time to 1st Tx in months	Number of Tx	Tumor Burden	
						del13q	tri12	del11q	del17p	NA	Normal	PCH				mutated	unmutated	313-618 UI/L					% of DLBC	% of CLL
1	1a + 1b	1a + 1b	68	F	II		+					+	NA	NA	3.6		+	679	108	108	0	6	80	20
2	1a + 1b	1a + 1b	35	F	I	26		25					NA	+	1.6		+	630	114	117	10	4	60	40
3	1a + 1b	1a + 1b	50	M	IV			94					+	+	3.2	+		431	204	217	5	5	14	86
4	1a + 1b	1a + 1b	52	F	I		10						NA	NA	2.6	NA		303	14	20	1	1	30	70
5	1a + 1b	1a + 1b	49	M	II			97					NA	+	7.5		+	1477	8	26	2	1	90	10
6	1a + 1b	1a + 1b	52	F	III						+		NA	NA	5.2	NA		480	93	94	36	4	30	70
7	1a + 1b	1b	45	F	III					+			NA	+	2	NA		1171	40	42	0	5	60	40
8	1b	1b	46	M	III			75					NA	+	7.1		+	739	64	114	2	2	Left calf mass Bx - DLBCL	
9	1b	1b	54	M	I						+		NA	NA	3.7		+	497	119	198	36	1	BM Bx: 30% of total celularity represents Hodgkin lymphoma (considered a variant of RS)	
10	1a	1a	44	M	I				77				NA	+	2.6		+	857	47	81	9	6	BM normal (not involved with RS or CLL)	
11	1a	1a	56	M	II						+		NA	+	3.6	+		506	132	134	14	2	BM normal (not involved with RS or CLL)	
12	1a	-	48	F	NA					+			NA	NA	NA		+	438	155	158	0	4	BM sample could not be cut (close to exhausting)	
13	1a	1a	60	M	I					+			NA	NA	2.3	NA		490	55	180	12	1	BM not involved with RS but minimal CLL(<5%)	
14	1a	1a	49	M	I		50						NA	–	2.9	+		512	68	73	6	3	BM Bx not done	
15	1a	1a	61	M	NA	90							+	+	5.7		+	535	39	44	16	2	BM not involved with RS but CLL present	
16	1a	1a	56	F	0	19			11				NA	–	2		+	1201	29	88	24	4	BM not involved with RS but CLL present	
17	1a	1a	69	M	0	+	+	+				+	+	+	10		+	851	51	75	0	4	BM Bx not done	
18	1a	1a	59	M	I		48		41				+	+	2.7		+	1242	29	30	1	5	BM not involved with RS but CLL present	
19	1a	1a	48	M	II	52							+	+	4.8	+		413	213	216	5	5	BM not involved with RS but CLL present	
20	1a	1a	31	M	I	+						+	NA	–	2.8	+		427	118	245	12	3	BM not involved with RS but CLL present	
21	1a	1a	57	M	II		+					+	NA	+	4.3		+	780	137	156	60	2	BM not involved with RS but CLL present	
22	1a	1a	78	F	III						+		NA	+	7.7		+	914	43	43	30	1	BM not involved with RS (but CLL present 10%, plus BM highly suggestive of MDS)	
23	1a	1a	70	M	0					+			NA	NA	2.1		+	582	163	198	156	0	BM not involved with RS but CLL present	
24	1a	1a	64	F	I		+					+	NA	+	4.3		+	701	61	75	2	2	BM not involved with RS but CLL present	
25	1a	1a	78	F	0		60		68				NA	+	3		+	1587	75	78	12	6	BM not involved with RS but CLL present	
26	1a	1a	59	M	I	+		+				+	+	+	4.5		+	647	69	147	60	1	BM Bx normal (not involved with RS or CLL)	
27	1a	1a	48	M	I	21			37				NA	+	3.7		+	828	143	151	48	4	BM not involved with RS but CLL present	

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CLL	Group FF analysis	Group PCR analysis	Age at Dx	Sex	RAI stage	FISH (% of total cells)							ZAP70	CD38	B2M	IGHV status		LDH	Follow-up time	Survival time in months	Time to 1st Tx in months	Number of Tx	Tumor Burden
						del13q	tri12	del11q	del17p	NA	Normal	PCH				mutated	unmutated						
1	2a + 2b	2a	66	F	0	74		78					+	NA	NA	NA		516	104	131	32	4	Not applicable
2	2a + 2b	2a + 2b	55	F	0	77							+	–	2.2	NA		516	127	120	72	2	Not applicable
3	2a + 2b	2a + 2b	53	M	I					+			NA	NA	2.1	NA		577	190	234	126	1	Not applicable
4	2a + 2b	2a + 2b	49	F	I					+			NA	NA	1.8	NA		522	156	192	156	0	Not applicable
5	2a + 2b	2a + 2b	60	M	IV	+		+				+	+	+	2.4	NA		401	59	96	56	1	Not applicable
6	2a + 2b	2b	57	F	0		+					+	+	–	2.4		+	647	30	60	26	1	Not applicable
7	2a + 2b	2a + 2b	52	F	0	82							–	–	2	NA		408	75	109	75	1	Not applicable
8	-	2b	65	M	I	+	+	+				+	+	+	4.1		+	1175	42	88	30	1	Not applicable
9	2b	2b	59	M	IV	32							+	–	2.2		+	430	44	86	39	1	Not applicable
10	2b	2a + 2b	45	M	I	+						+	+	–	2.9	+		455	65	113	60	1	Not applicable
11	2b	2b	49	M	I	+						+	–	–	NA	NA		426	10	53	0	0	Not applicable
12	2b	-	67	M	I	75							NA	–	2.5	+		519	32	60	27	1	Not applicable
13	2a	-	60	M	I		35						+	+	3.7		+	513	24	58	12	1	Not applicable
14	2b	2b	58	M	I				+			+	–	–	2.2	NA		517	54	96	8	3	Not applicable
15	2b	-	64	F	0	46							NA	NA	NA	+		399	5	72	0	0	Not applicable
16	2a	-	70	F	I	74							NA	–	3.8	+		477	77	110	63	1	Not applicable
17	2a	2a	67	F	I	10							–	–	2.9	+		551	NA	113	57	NA	Not applicable
18	2a	2a	65	M	I		35						–	–	2.7		+	445	NA	156	102	NA	Not applicable
19	2a	-	62	M	0	51							–	–	1	+		357	NA	56	0	0	Not applicable
20	2a	-	43	M	II	+						+	–	–	2.3		+	608	NA	15	1	1	Not applicable
21	2a	2a	70	M	0		58						–	–	2.5	NA		479	NA	108	60	1	Not applicable
22	2a	2a	55	F	I	59							–	+	2.3	+	+	465	NA	96	65	1	Not applicable
23	2a	-	59	M	I	32							–	–	3.1	+		542	NA	94	31	NA	Not applicable

(+)	**means data was obtained from patient clinical history only**
PCH	**Patient's clinical history**
	means paired samples
Bold inside Box	**means patients from groub 1b**

Note: FF – firefly; PCR – qRT-PCR; NA – not available.
The same patient could be analyzed in the two groups or in only one group if: a) the samples were unavailable because of lack of the "paired" samples, and b) the detection with the specific method failed. More details are presented in **Supplementary Figure 1**.

Supplementary Table S3. Rationale for selection of miRNAs for the Firefly Custom Multiplex miRNA assay.					
#	**Origin**	**miRNA**	**miRBase (v21) mature sequence**	**Source**	**Rationale**
1	Human	miR-15a	hsa-miR-15a-5p	*10*	Part of 13 miRNA signature associated with prognosis and progression in CLL
				11	miRNA/TP53 feedback associated with CLL pathogenesis and outcome
				12	Differentially expressed between CLL del17p and CLL NL cyto/FISH
				13	Downregulated in CLL
				14	Among top 30 highest expressed miRs in RNA seq
2	Human	miR-16-1/miR-16-2	hsa-miR-16-5p	*10*	Part of 13 miRNA signature associated with prognosis and progression in CLL
				11	miRNA/TP53 feedback associated with CLL pathogenesis and outcome
				15	High expression associated with increased risk of disease progression in CLL
				14	Among top 30 highest expressed miRs in RNA seq
3	Human	miR-23b	hsa-miR-23b-3p	*10*	Part of 13 miRNA signature associated with prognosis and progression in CLL
4	Human	miR-24-1	hsa-miR-24-3p	*10*	Part of 13 miRNA signature associated with prognosis and progression in CLL
5	Human	miR-29a	hsa-miR-29a-3p	*10*	Part of 13 miRNA signature associated with prognosis and progression in CLL
				16	Differentially expressed between CLL patients sensitive and resistant to fludarabine
				17	Upregulated in indolent human B-CLL compared to aggressive B-CLL and NL CD19+ B-cells
6	Human	miR-29b	hsa-miR-29b-3p	*10*	Part of 13 miRNA signature associated with prognosis and progression in CLL
				18	Low expression associated with disease progression in del17p CLL
				19	Expression correlated with IGVH mutation status in CLL
7	Human	miR-29c	hsa-miR-29c-3p	*10*	Part of 13 miRNA signature associated with prognosis and progression in CLL
				20	Downregulation associated with prognostic factors of aggressive disease
				15	High expression associated with reduced risk of disease progression in CLL

				18	Low expression associated with disease progression in del17p CLL
				19	Downregulation associated with disease aggressiveness and poor survival in CLL
				21	Downregulated in CLL patients with TP53 abnormalities
8	Human	miR-146	hsa-miR-146a-5p	10	Part of 13 miRNA signature associated with prognosis and progression in CLL
9	Human	miR-155	hsa-miR-155-5p	10	Part of 13 miRNA signature associated with prognosis and progression in CLL
				15	High expression associated with increased risk of disease progression in CLL
				12	Differentially expressed between CLL del17p and CLL NL cyto/FISH
				13	Overexpression in CLL
				22	Overexpressed in proliferation centers from bone marrow and lymphoid tissue
				14	Among top 30 highest expressed miRs in RNA seq
10	Human	miR-195	hsa-miR-195-5p	10	Part of 13 miRNA signature associated with prognosis and progression in CLL
11	Human	miR-221	hsa-miR-221-3p	10	Part of 13 miRNA signature associated with prognosis and progression in CLL
				16	Differentially expressed between CLL patients sensitive and resistant to fludarabine
12	Human	miR-223	hsa-miR-223-3p	10	Part of 13 miRNA signature associated with prognosis and progression in CLL
				20	Downregulation associated with prognostic factors of aggressive disease
				18	Low expression associated with disease progression in del17p CLL
13	Human	miR-34a	hsa-miR-34a-5p	12	Differentially expressed between CLL del17p and CLL NL cyto/FISH
				23	Low expression associated with DNA damage response, fludarabine-refractory CLL
				21	Downregulated in CLL patients with TP53 abnormalities
14	Human	miR-34c	hsa-miR-34c-5p	11	miRNA/TP53 feedback associated with CLL pathogenesis and outcome
				24	Epigenetically silenced in CLL
15	Human	miR-21	hsa-miR-21-5p	12	Differentially expressed between CLL del17p and CLL NL cyto/FISH
				13	Overexpression in CLL
				25	Higher expression in non-responders to fludarabine therapy in CLL

				14	Among top 30 highest expressed miRs in RNA seq
16	Human	miR-181b	hsa-miR-181b-5p	12	Differentially expressed between CLL del17p and CLL NL cyto/FISH Downregulated in therapy-refractory CLL
17	Human	miR-181a/b	hsa-miR-181a-5p / hsa-miR-181b-5p	26	Enhance drug sensitivity in CLL
				19	Expression correlated with IGVH mutation status in CLL
				27	miR-181b biomarker for progression of CLL from indolent to aggressive
				18	Low expression associated with disease progression in del17p CLL
				16	miR-181a differentially expressed between CLL patients sensitive and resistant to fludarabine
				14	Among top 30 highest expressed miRs in RNA seq
18	Human	miR-150	hsa-miR-150-5p	13	Overexpression in CLL
				28	Lower in CLL with UM IGHV and ZAP70 expression
				22	Lower in CLL proliferation centers from bone marrow and lymphoid tissue
				14	Highest expressed miR in RNA seq
19	Human	miR-92	hsa-miR-92a-3p	13	Downregulated in CLL
20	Human	miR-146b-5p	hsa-miR-146b-5p	15	High expression associated with reduced risk of disease progression in CLL
21	Human	miR-222	hsa-miR-222-3p	15	High expression associated with reduced risk of disease progression in CLL
				25	Higher expression in non-responders to fludarabine therapy in CLL
22	Human	miR-148a	hsa-miR-148a-3p	25	Higher expression in non-responders to fludarabine therapy in CLL
23	Human	miR-17	hsa-miR-17-5p	29	13q13.3-qter region containing miR17-92 cluster acquired at time of Richter transformation
				30	miR-17 expression higher in UM and ZAP70 high cases
				31	AntagomiR17 reduced proliferation of MEC1 cells, reduced tumor growth and increased survival in SCID mice with MEC1-generated tumors
				21	Downregulated in CLL patients with TP53 abnormalities
				14	Among top 30 highest expressed miRs in RNA seq
24	Human	let-7g	hsa-let-7g-5p	14	Among top 15 highest expressed miRs in RNA-seq

25	Human	miR-191	hsa-miR-191-5p	*14*	Among top 15 highest expressed miRs in RNA-seq
26	Human	miR-142	hsa-miR-142-5p	*14*	Among top 15 highest expressed miRs in RNA-seq
27	Human	miR-1260a	hsa-miR-1260a	*14*	Among top 15 highest expressed miRs in RNA-seq
28	Human	miR-4286	hsa-miR-4286	*14*	Among top 15 highest expressed miRs in RNA-seq
29	Human	miR-26a	hsa-miR-26a-5p	*14*	Among top 15 highest expressed miRs in RNA-seq
30	Epstein Barr virus	BART4	ebv-miR-BART4-5p	*14*	Overexpressed in plasma of CLL patients
31	Epstein Barr virus	BART16	ebv-miR-BART16	N/A	N/A
32	Epstein Barr virus	BHRF1-1	ebv-miR-BHRF1-1	*14*	Overexpressed in plasma of CLL patients
33	Kaposi sarcoma-associated herpesvirus	KSHV-miR-K12-4-3p	kshv-miR-K12-4-3p	N/A	N/A
34	Kaposi sarcoma-associated herpesvirus	KSHV-miR-K12-10b	kshv-miR-K12-10b	N/A	N/A
35	Kaposi sarcoma-associated herpesvirus	KSHV-miR-K12-12*	kshv-miR-K12-12-5p	N/A	N/A
36	Epstein Barr virus	BART9	ebv-miR-BART9-3p	*14*	The two highest expressed xeno-miRs in RNA-seq
37	Rhesus lymphocryptovirus	miR-rL1-13	rlcv-miR-rL1-13-3p	*14*	The two highest expressed xeno-miRs in RNA-seq
38	Human	hsa-miR-140	hsa-miR-140-5p	Normalizer	
39	Human	hsa-miR-30c	hsa-miR-30c-5p	Normalizer	
40	Human	hsa-miR-328	hsa-miR-328-3p	Normalizer	

Supplementary Table S4. Expression data for the miRNAs used from the array data generated in the Ulm University cohort.

Uploaded as separate file.

Supplementary Table S5A. List of validated targets for miR-21-5p, miR-146b-5p, miR-150-5p and miR-181b-5p (restricted signature), and Table S4B for miR-17-5p, miR-29c-3p, miR-191-5p (Richter miRNA hubs), and miR-26a (in vivo downregulated miRNA).

Supplementary Table S5A. miR-21-5p, miR-146b-5p, miR-150-5p and miR-181b-5p validated targets.

miR-21-5p			miR-146b-5p	miR-150-5p	miR-181b-5p
ABCB1	HNRNPK	RHO	CDKN1A	ARRB2	ADCY9
AKT2	HPGD	RHOB	EGFR	BIRC5	ATM
ANKRD46	ICAM1	RPS7	ERBB4	CBL	BCL2
ANP32A	ICOSLG	RTN4	HNRNPD	CCR6	BCL2L11
APAF1	IGF1R	SATB1	IL6	CISH	CARD10
BASP1	IL12A	SERPINB5	IRAK1	COL4A4	CBX7
BCL10	IL1B	SERPINI1	KIT	CREB1	CDX2
BCL2	IRAK1	SETD2	MALAT1	CXCR4	CREB1
BCL6	ISCU	SIRT2	MMP16	EGR2	CYLD
BMI1	JAG1	SMAD7	NFKB1	EP300	E2F1
BMPR2	JMY	SMARCA4	PAX8	FLT3	ELN
BTG2	LRP6	SMN1	PDGFRA	IGF2	FOS
CADM1	LRRFIP1	SOCS1	RARB	MMP14	GATA6
CASC2	MAP2K3	SOCS6	SLC5A5	MUC4	GRIA2
CASP8	MARCKS	SOD3	TLR4	MYB	HK2
CBX4	MEF2C	SOX2	TRAF6	NANOG	HMGB1
CCL20	MSH2	SOX5	UHRF1	NOTCH3	IGF1R
CCR1	MSH6	SP1	ZNRF3	P2RX7	KAT2B
CDC25A	MSLN	SPRY2		POLD3	KPNA4
CDK2AP1	MTAP	STAT3		PRKCA	LATS2
CEBPB	MYD88	STUB1		SLC2A1	MAP2K1
CLU	NAV3	TAP1		SP1	MAP3K10
COL4A1	NCAPG	TCF21		SRCIN1	MCL1
COX2	NCOA3	TGFB2		SSSCA1	MEG3
DAXX	NFIA	TGFBI		STAT1	NFIA
DDAH1	NFIB	TGFBR2		STAT5B	NLK
DERL1	NTF3	TGFBR3		TP53	PBX3
DNM1L	OXTR	TGIF1		VEGFA	PDCD10
DOCK4	PCBP1	TIAM1		ZEB1	PDCD4
DOCK5	PCGF2	TICAM2		ZNF350	PLAG1
DOCK7	PDCD4	TIMP3			PTEN
DUSP10	PIAS3	TLR3			RAP1B
E2F1	PIK3R1	TM9SF3			RASSF1
EGFR	PLAT	TNFAIP3			RNF2
EGLN1	PLOD3	TNFRSF10B			SIRT1
EIF4A2	PPARA	TOPORS			SIX2
ERBB2	PPIF	TP53BP2			SPP1
FASLG	PSMD9	TP63			TCL1A
FBXO11	PTEN	TPM1			TIMP3
FMOD	PTPN14	TRAF7			TMED7
FOXO1	PTX3	UBE2N			VSNL1
FZD6	RASA1	VEGFA			XIAP
GAS5	RASGRP1	VHL			
GDF5	RECK	WWP1			
HMGB1	REST	YOD1			

Supplementary Table S5B. Richter specific miRNA hubs and miR-26a (*in vivo model* down-regulated miRNA) validated targets

miR-17-5p		miR-29c-3p		miR-191-5p	miR-26a	
ABCA1	PKNOX1	ADAM12	PDGFRB	BASP1	HMGA2	WEE1
ADAR	PPP2R2A	AKT2	PER1	CCND2	HMGA1	TET2
APP	PTEN	AKT3	PHLDB2	CDK6	CCNE2	PTPN13
BCL2	PTPRO	BACE1	PPP1R13B	CDK9	CCND2	ADAM17
BCL2L11	RAD21	BCL2	PTEN	CEBPB	CDK8	PIK3C2A
BMP2	RB1	CCND2	RCC2	CTDSP2	CDC6	CHEK1
BMPR2	RBL1	CD274	RFX7	EGR1	PTEN	TDG
BRCA2	RBL2	CDC42	SERPINH1	IL1A	EZH2	PHB
CCL1	RND3	CDK6	SIRT1	LRRC8A	PLAG1	LOXL2
CCND1	RUNX1	CNOT6	SP1	MDM4	SERBP1	ITGA5
CCND2	SIRPA	COL10A1	SPARC	NDST1	SMAD1	DUSP4
CDKN1A	SMAD4	COL15A1	SRSF10	NOTCH2	MAP3K2	NRAS
CLOCK	SMURF1	COL1A1	TARBP1	RPS6KA3	RB1	E2F2
CLU	SOCS6	COL1A2	TDG	SATB1	SMAD4	PTGS2
CYP7B1	STAT3	COL21A1	TET2	SLC16A2	MYC	JAG1
DAPK3	TBC1D2	COL3A1	TFAP2C	SOX4	CTGF	DUSP5
DNAJC27	TCEAL1	COL4A1	TGIF2	TMC7	STRADB	ST8SIA4
DNMT1	TCF3	COL4A2	TIAM1	YBX3	IFNB1	MALT1
E2F1	TGFBR2	COL5A2	VEGFA		GSK3B	PRDX3
EGR2	TIMP3	COL7A1	WNT4		CPEB2	PIK3CG
EPAS1	TLR7	CREB5			CPEB3	IGF1
ETV1	TNF	CTNND1			CPEB4	PLOD2
FBXO31	TNFSF12	DNMT3A			GDAP1	FUT8
GPR137B	TP53COR1	DNMT3B			MTDH	ST3GAL6
HBP1	TP53INP1	FBN1			CDK6	NRP1
HIF1A	TRIM8	FGA			CCNE1	TRPC6
HSPB2	UBE2C	FGB			ESR1	HOXA5
IGFBP3	VEGFA	FRAT2			ABCA1	LARP1
ITGB8	VLDLR	FZD4			ARL4C	NAMPT
JAK1	WEE1	FZD5			NOS2	
KAT2B	YES1	HMGCR			IL6	
LDLR	ZBTB4	IGFBP1			MCL1	
MAP3K12	ZFYVE9	ITGA6			CHD1	
MAPK9		ITGB1			CKS2	
MEF2D		KLF4			PRKCD	
MFN2		LAMC1			BAG4	
MMP2		LAMC2			FGF9	
MYC		LOX			ACVR1	
NABP1		LRP6			AMACR	
NCOA3		MCL1			ATM	
NPAS3		MMP15			RCBTB1	
NPAT		MMP2			HGF	
PDLIM7		MMP24			LIN28B	
PHLPP1		MYCN			ZCCHC11	
PKD2		NASP			DNMT3B	

Supplementary Table S6. miRNAs of the restricted signature and their expression in Richter syndrome and sepsis.

iR	RS	up/down in sepsis vs control	Examples
miR-21	UP	up	*32*
		up	*33*
miR-146b	UP	up (in vitro)	*34*
		down (in vivo LPS stimulation)	*35*
miR-181b	UP	up	*36*
miR-150	DOWN	down	*37*

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