

Supplementary information for:

**Circulating microRNAs in cerebrospinal fluid and plasma: sensitive tool for detection
of CNS involvement, monitoring of therapy and prediction of CNS relapse
in aggressive B-NHL**

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Supplementary text:

Multivariate regression analysis (MVA) and correlation with clinical characteristics:

We employed a multivariate analysis to evaluate the effect of oncomiRs, clinical and biochemical characteristics and prognostic indices on lymphoma CNS involvement (Suppl. methods and Suppl. table 5). While oncomiR-indices (as well as individual oncomiRs) were significantly associated with CNS lymphoma involvement (when compared to systemic lymphoma, see Table 1 *P* values), there was no significant effect of either the recorded clinical characteristics or the prognostic indices on CNS lymphoma involvement. In addition, none of them had an effect on the oncomiR-indices, indicating that oncomiRs are an independent prediction factor. However, we observed an enrichment of certain clinical characteristics and prognostic indices in SCNSL compared to the cohorts of systemic patient (see Suppl. table 4 for details).

We observed a partial correlation of oncomiR-indices with FCM positive examinations of CSF in all lymphoma subtypes in CSF ($r = 0.37 - 0.82$, Pearson correlation). In MCL a certain level of correlation of oncomiR-indices with MIPI prognostic index (CSF $r = 0.58$, plasma $r = 0.4$) and CSF LDH levels ($r = 0.82$) was observed.

In addition, a positive but low to moderate correlation of oncomiR-indices levels with circulating lymphoma phase (presence of circulating lymphoma cells at peripheral blood), bone marrow involvement and proliferative index was observed as follows: Circulating lymphoma phase (CSF / plasma): DLBCL ($r=0.16 / 0.05$), MCL ($r=0.23 / 0.05$), BL ($r= 0.48 / 0.46$). Bone marrow involvement (CSF / plasma): DLBCL ($r=0.25 / 0.1$), MCL ($r=0.2 / 0.16$), BL ($r= 0.58 / 0.3$). Proliferative index: DLBCL ($r=0.19 / 0.1$), MCL ($r=0.35 / 0.35$), BL ($r= 0.2 / 0.25$). Categorization of oncomiR-indices (above / below threshold (Tab.1)) increased the correlation coefficient by ~10-20% in CSF and 50-100% in plasma.

Summary of the possible use of cerebrospinal fluid and plasma to detect CNS lymphoma:

i. CSF oncomiRs are suitable for early detection of CNS lymphoma involvement in all studied B-NHL subtypes. Plasma oncomiRs are good markers for detection of CNS involvement in BL (in MCL and DLBCL with lower accuracy). ii. Both CSF and plasma oncomiRs are suitable for monitoring of therapy efficacy (all dg). iii. Increasing oncomiR levels in both CSF and plasma indicate upcoming CNS relapse. iv. OncomiRs in plasma (but not CSF) are markers for prediction of CNS relapse risk at diagnosis of systemic DLBCL (combined CNS-IPI/oncomiR prediction model) and in both CSF and plasma are predictive for overall survival in DLBCL.

Supplementary methods:

MicroRNAs (Assay ID): hsa-miR-21-5p (000397), hsa-miR-19a-3p (000395), hsa-miR-20a-5p (000580), hsa-miR-92a-3p (000431), hsa-miR-155-5p (002623), hsa-let-7a-5p (000377), cel-miR-39-3p (000200) (all Applied Biosystems, Thermo Fisher).

Panel for initial screening included following candidate microRNAs, selected by a compilation of published data of microRNA expression in CNS involving B-NHL: miR-9-5p (000583), miR-15a-5p (000389), miR-16-5p (000391), miR-17-5p (000393), miR-18a-3p (002423), miR-19a-3p (000395), miR-20a-5p (000580), miR-21-5p (000397), miR-24-3p (000402), miR-25-3p (000403), miR-30c-5p (000419), miR-30d-5p (000420), miR-92a-3p (000431), miR-101-3p (002253), miR-106a-5p (002169), miR-106b-5p (000442), miR-142-5p (000465), miR-155-5p (002623), miR-210-3p (000512), miR-223-3p (002295), miR-let7a-5p (000377), RNU44 (001094). miR-let7a-5p, miR-16-5p, miR-24-3p, RNU44 were tested for normalization, best performing let-7a-5p was used [18, 40]. Sequences are available at Applied Biosystems, Thermo Fisher www under the respective assay ID.

OncomiR-index/-ices calculation:

In order to determine the coefficients for oncomiRs-indices, we have first calculated the probability of CNS involvement for each sample according to the following formula:

$$\text{Probability [CNS involvement]} = 1 / [1 + \exp\{\text{X} - \text{sum}(\text{Coefficient}[miRi] * \text{Abundance}[miRi])\}]$$

Initially, each coefficient was set to 1 and the X value to 0.5. The oncomiRs abundances used for the probability calculation were miR-let-7a-normalized, and equalized to average of Ctrl and average of respective systemic lymphoma samples. These scaled values were used in order to assess the relative impact of each oncomiR and not favor primarily those oncomiRs with higher absolute abundance levels and/or disfavor those with lower absolute levels.

Subsequently, the average values of probabilities of CNS positive and systemic samples were calculated. Next, the coefficients which maximize the difference between average probabilities of CNS involvement in CNS positive and systemic lymphoma were then determined using the solver statistical tool (MS Excel). The upper limits of coefficients were initially set to 2 (for X to 10) and if any coefficients reached the upper limit, the solver tool would be rerun only on those coefficients without the maximal value limitation. Finally, determined coefficients were used for calculation of oncomiR-indices by regression the formula:

$$\text{OncomiR-index} = \text{sum}(\text{Coefficient}[miRi] * \text{Abundance}[miRi]).$$

For each lymphoma subtype, the procedure was repeated for multiple oncomiRs combinations. The combination which yielded the best separation of CNS involving and systemic samples, i.e. highest ROC area, specificity and sensitivity, was finally used.

The stratification of patients for Kaplan-Meier and Cox models to estimate relapse risk and overall survival:

1. oncomiRs thresholds for stratification of microRNA (oncomiR) high/low (as in Table 1, determined by ROC as described in method section):

Test:	oncomiR-index	threshold (\log_2)
CNS relapse subsequent (plasma)	miR-21	3.18 (1.67)
CNS relapse current (CSF)	miR-21/20a/155	8.42 (3.07)
CNS relapse current (plasma)	miR-21/19a/20a/155	6.13 (2.62)
Overall survival (CSF)	miR-21/20a/155	8.42 (3.07)
Overall survival (plasma)	miR-21/19a/20a/155	6.13 (2.62)

2. CNS-IPI model stratification (by CNS-IPI score):

- High: (4-6)
Intermediate: (2-3)
Low: (0-1)

3. CNS-IPI/oncomiR model stratification:

- Two risks: (high microRNA and high CNS-IPI)
One risk: (either high microRNA or high CNS-IPI)
No risk: (neither microRNA nor CNS-IPI are high)

For estimation of relapse risk and OS only patients having oncomiR values in both CSF and plasma were included.

Multivariate regression analysis:

In multivariate analysis, the following clinical parameters were tested (in addition to oncomiRs): clinical stage, ECOG, Performance status, IPI, aaIPI, CNS-IPI, MIPI, number of extra nodal lymphoma sites, involvement of the kidneys and adrenal glands, bone marrow and biochemical analysis of CSF and plasma as indicated in Suppl. table 5. Data on cell-of-origin (GCB/non-GCB) and MYC and BCL2 expression and rearrangements were not available in a sufficient number of patients to be employed in MVA.

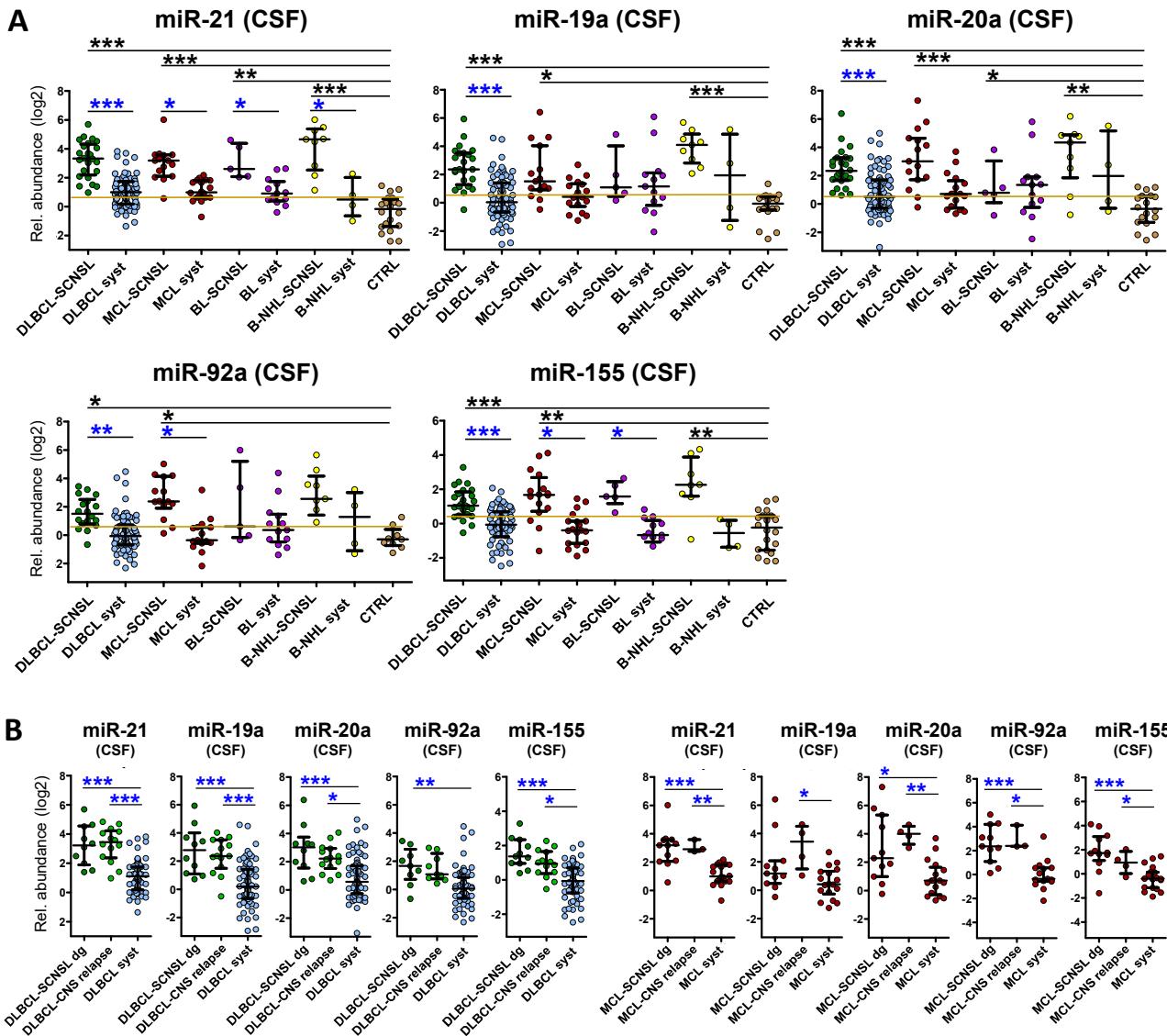


Figure S1. Abundance of oncogenic microRNAs in cerebrospinal fluid of CNS involving lymphomas.

Cerebrospinal fluid (CSF) levels of miR-21/-19a/-20a/-92a/-155 of (A) lymphoma patients of indicated B-NHL diagnoses with secondary CNS involvement (SCNSL), compared to systemic lymphoma (syst) and controls with neurological disorders (CTRL). B-NHL denotes B-NHL-NOS (not otherwise specified, high grade).

(B) DLBCL-SCNSL (left panel) and MCL-SCNSL (right panel) are subdivided to lymphoma with secondary CNS involvement presented at the time of diagnosis (SCNSL dg) and newly detected CNS relapses. qRT-PCR. Values are equalized to the average of CTRL. Log2 scale. The yellow line marks the 99.9% confidence interval of CTRL. Median \pm interquartile range, *P<0.05, **P<0.01, *** P<0.001, Kruskal-Wallis test.

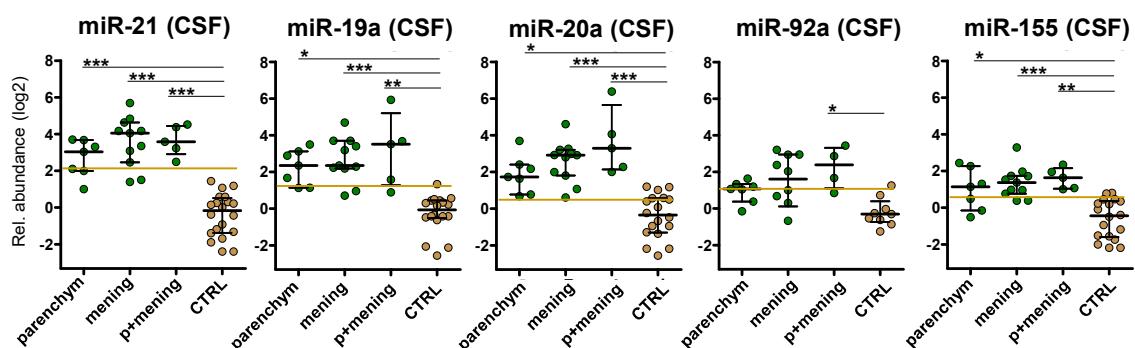


Figure S2. Abundance of oncomiRs in CSF of DLBCL stratified by parenchymal and meningeal involvement.

Levels of indicated microRNAs in the cerebrospinal fluid (CSF) of secondary CNS DLBCL (DLBCL-SCNSL) divided according to parenchymal (parenchym), leptomeningeal (mening) and combined (p+mening) CNS involvement. The yellow line marks the 99.9% confidence interval for CTRL. Values are equalized to average value of CTRL. Log2 scale. Median \pm interquartile range, *P<0.05, **P<0.01, *** P<0.001, Kruskal-Wallis test.

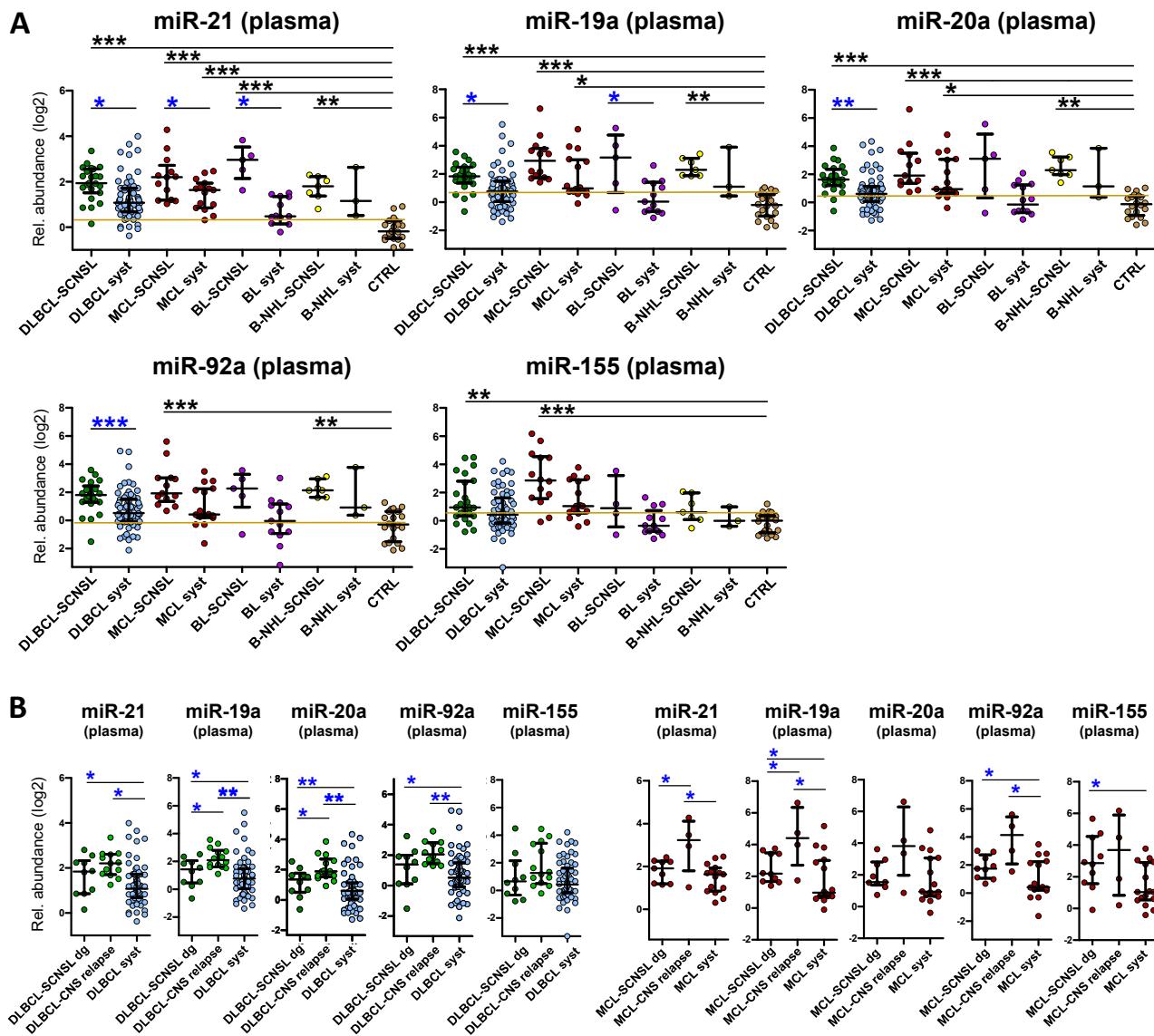


Figure S3. Abundance of oncomiRs is increased in plasma of B-NHL involving CNS.

Plasma levels of miR-21/-19a/-20a/-92a/-155 of (A) lymphoma patients with indicated B-NHL diagnoses, with and without CNS involvement, and controls (CTRL). B-NHL denotes B-NHL-NOS (not otherwise specified, high grade).

(B) DLBCL-SCNSL (left panel) and MCL-SCNSL (right panel) are subdivided to lymphoma with secondary CNS involvement presented at the time of diagnosis (SCNSL dg) and CNS relapses. Values are equalized to average of CTRL. Log2 scale. The yellow line marks 99.9% confidence interval of CTRL. Median \pm interquartile range, * $P<0.05$, ** $P<0.01$, *** $P<0.001$, Kruskal-Wallis test.

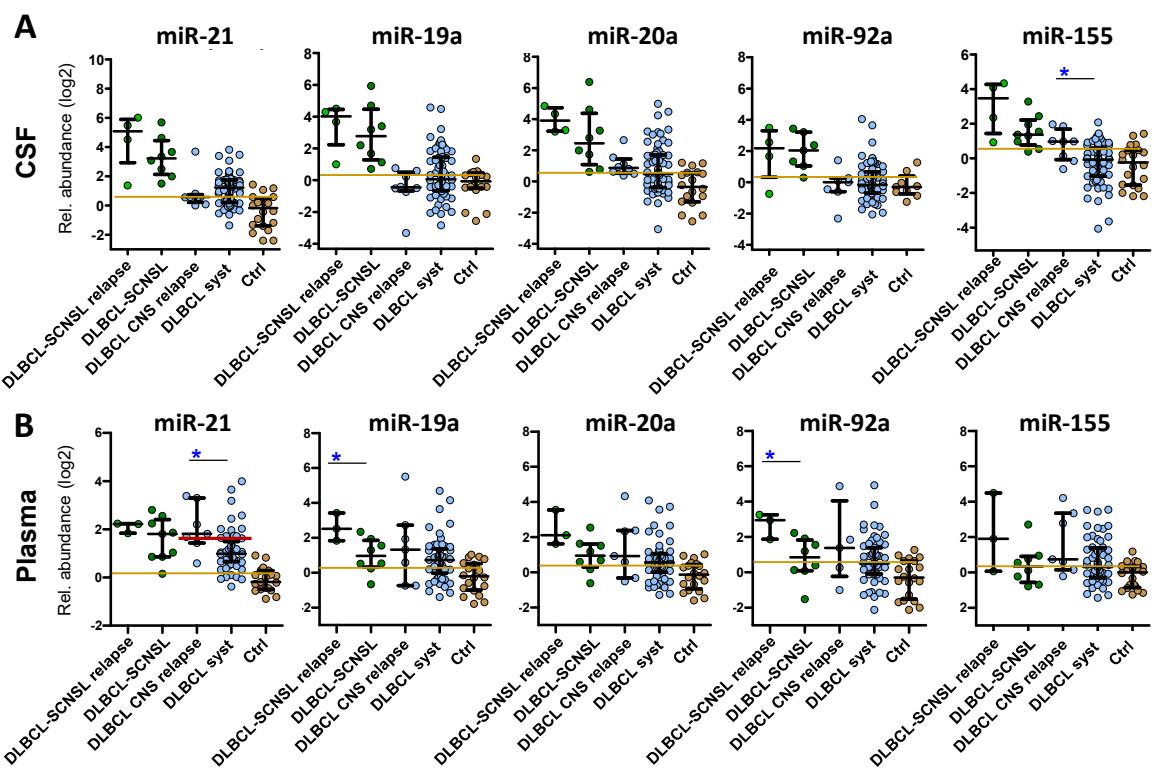


Figure S4. Abundance of oncomiRs in DLBCL patients with subsequent CNS relapse.

Levels of miR-21/-19a/-20a/-92a/-155 in DLBCL patients at the time of initial diagnosis, who subsequently developed CNS relapse. Abbreviation: DLBCL-SCNSL relapse: DLBCL with secondary CNS involvement which developed CNS relapse; DLBCL-SCNSL: non-relapsing group; DLBCL CNS relaps: systemic lymphoma with subsequent relapse/progression to CNS; DLBCL syst: systemic non-relapsing group; CTRL: control patients. CSF (**A**) and plasma (**B**). The yellow line marks the 99.9% confidence interval for controls. The red line (plasma miR-21) indicates the threshold for prediction of CNS relapse. Y-axis values are equalized to average of controls. Log2 scale. Median \pm interquartile range, *P<0.05, **P<0.01, *** P<0.001, Mann-Whitney test.

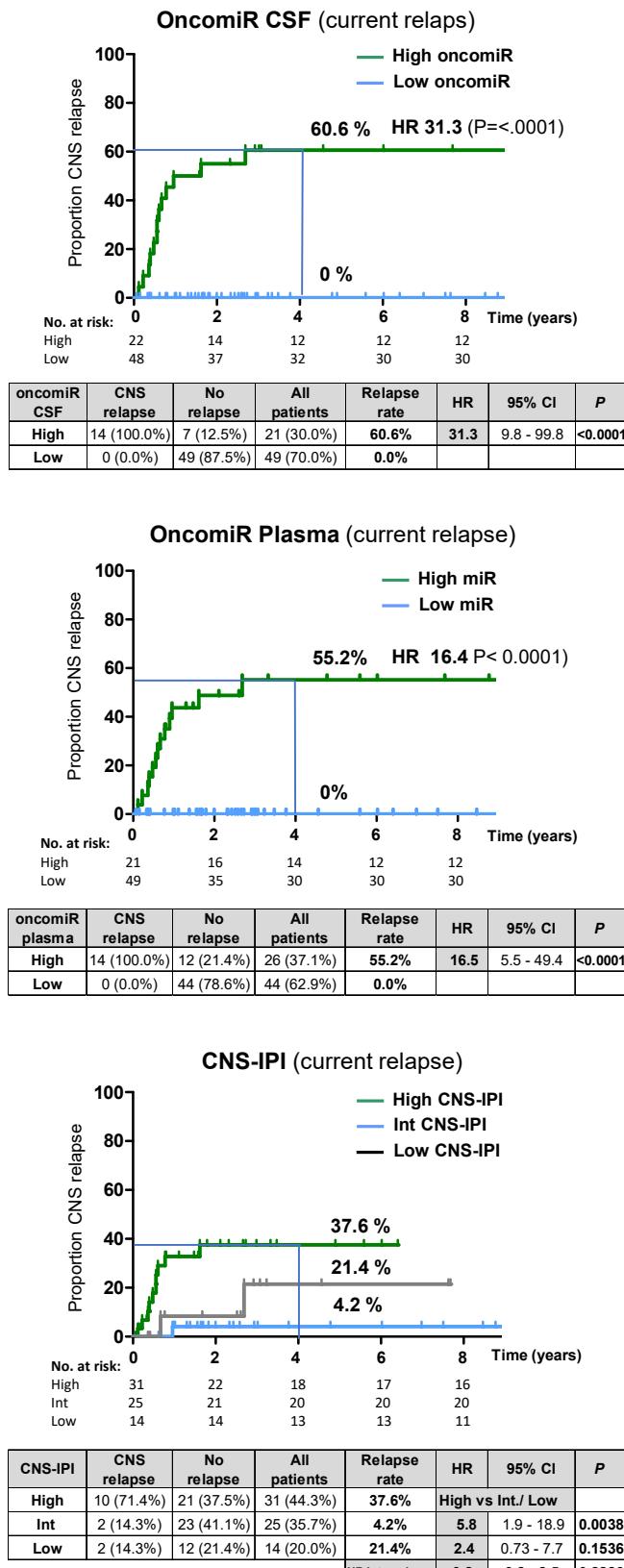


Figure S5. Prognostic value of circulating oncomiRs in DLBCL at the time of CNS relapse.

Risk for CNS relapse stratified by oncomiR levels in cerebrospinal fluid (CSF) or plasma at the time of CNS relapse (current relapse) and by CNS-IPI score (Kaplan-Meier estimates). DLBCL systemic patients were stratified by high / low microRNA levels acquired at the time of CNS relapse (current relapse, upper panels) or by CNS-IPI (lower panel). Note: HR, 95% CI and P in the tables below charts were obtained from univariate models of indicated categories. Abbreviations: CNS, central nervous system; IPI, International Prognostic Index; Int., intermediate; HR, hazard ratio. For details on risk stratification see Suppl. methods.

Figures S6, S7, S8 follow behind Supplementary tables starting at page 15.

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Figure S6. Receiver operating analysis of oncomiRs in CSF of CNS lymphoma compared to controls or systemic lymphoma.

Receiver operating characteristic (ROC) curves for the possibility of indicated oncomiRs in CSF to discriminate between CNS lymphoma and controls (left panels) and between CNS involving and systemic lymphoma (right panels) in: (A) DLBCL, (B) MCL, (C) BL and (D) B-NHL-NOS. The X-axes show the percent of specificity and the Y-axes denote percent of sensitivity (100-sensitivity).

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Figure S7. Receiver operating analysis of oncomiRs in plasma of CNS lymphoma compared to controls or systemic lymphoma.

Receiver operating characteristic (ROC) curves for the possibility of indicated oncomiRs in plasma to discriminate between CNS lymphoma and controls (left panels) and between CNS involving and systemic lymphoma (right panels) in: (A) DLBCL, (B) MCL, (C) BL and (D) B-NHL-NOS. The X-axes show the percent of specificity and the Y-axes denote percent of sensitivity (100-sensitivity).

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Figure S8. Receiver operating analysis of oncomiR-indices in CSF (A) and plasma (B) of CNS lymphoma compared to control samples.

A

CSF	CNS vs.	miR-21		miR-19a		miR-20a		miR-92a		miR-155		oncomiR-index		
		AUC	p-value	miR combination	AUC	p-value								
DLBCL-SCNSL	CTRL	0.99	< 0.0001	0.96	< 0.0001	0.97	< 0.0001	0.91	0.0011	0.91	< 0.0001	miR-21/-20a/-155	1.00	< 0.0001
	Syst	0.91	< 0.0001	0.86	< 0.0001	0.81	< 0.0001	0.85	< 0.0001	0.85	< 0.0001	miR-21/-20a/-155	0.96	< 0.0001
MCL-SCNSL	CTRL	0.98	< 0.0001	0.89	0.0001	0.93	< 0.0001	0.97	0.0003	0.89	0.0001	miR-21/-20a/-92a/-155	0.99	0.0003
	Syst	0.95	< 0.0001	0.82	0.0009	0.84	0.0013	0.90	0.0002	0.92	< 0.0001	miR-21/-20a/-92a/-155	0.93	< 0.0001
BL-SCNSL	CTRL	1.00	0.0007	0.88	0.0102	0.79	0.0527	0.84	0.0482	0.98	0.0015	miR-21/-155	1.00	0.0007
	Syst	0.92	0.0067	0.54	0.8054	0.52	0.8825	0.62	0.4598	0.98	0.0022	miR-21/-155	1.00	0.0014
B-NHL-NOS-SCNSL	CTRL	0.99	< 0.0001	1.00	< 0.0001	0.90	0.0008	0.88	0.0071	0.93	0.0007	miR-21/-155	0.94	0.0002
	Syst	0.94	0.0175	0.69	0.3082	0.63	0.4969	0.69	0.3082	0.93	0.0234	miR-21/-155	0.94	0.0136

B

Plasma	CNS vs.	miR-21		miR-19a		miR-20a		miR-92a		miR-155		oncomiR-index		
		AUC	p-value	miR combination	AUC	p-value								
DLBCL-SCNSL	CTRL	0.98	< 0.0001	0.94	< 0.0001	0.94	< 0.0001	0.91	< 0.0001	0.83	0.0002	miR-21/-19a/-20a/-155	0.94	< 0.0001
	Syst	0.79	< 0.0001	0.79	< 0.0001	0.81	< 0.0001	0.76	0.0004	0.66	0.0187	miR-21/-19a/-20a/-155	0.79	< 0.0001
MCL-SCNSL	CTRL	1.00	< 0.0001	1.00	< 0.0001	0.98	< 0.0001	0.98	< 0.0001	0.93	< 0.0001	miR-21/-19a/-155	1.00	< 0.0001
	Syst	0.72	0.0503	0.75	0.0200	0.67	0.1145	0.77	0.0150	0.74	0.0276	miR-21/-19a/-155	0.84	0.0016
BL-SCNSL	CTRL	1.00	0.0007	0.88	0.0109	0.83	0.0250	0.86	0.0145	0.74	0.1412	miR-21/-19a/-155	1.00	0.0020
	Syst	1.00	0.0016	0.82	0.0385	0.75	0.1039	0.80	0.0546	0.71	0.2130	miR-21/-19a/-155	1.00	0.0033
B-NHL-NOS-SCNSL	CTRL	0.98	0.0005	1.00	0.0003	1.00	0.0001	1.00	0.0001	0.77	0.0356	miR-19a/-155	1.00	0.0001
	Syst	0.61	0.6056	0.67	0.4386	0.67	0.4251	0.67	0.4251	0.71	0.3051	miR-19a/-155	0.67	0.4251

Table S1. Summary of Receiver operating characteristics (ROC) of oncomiRs in CSF and plasma.

Comparison of area under curve (AUC) of indicated CNS involving lymphoma vs. control (grey rows) or CNS vs. systemic lymphoma (white rows). **Left panel:** the single oncomiRs (from Suppl. fig. 5 and 6), **right panel:** oncomiR-indices (from Fig. 4). **(A)** CSF, **(B)** plasma. The area under curve (AUC) is a measure of how well the respective oncomiR distinguishes between CNS lymphoma from CTRL or systemic lymphoma. Higher value = better separation, ROC=1 denotes absolute separation. OncomiR-index combines expression of individual oncomiRs (best combination) to one variable, yielding higher specificity/sensitivity and separation than individual oncomiR.

A CSF

CSF	CNS lymphoma	CNS vs Ctrl - single oncomiR					oncomiR-index		
		miR-21	miR-19a	miR-20a	miR-92a	miR-155	Index	Percent	
	DLBCL	95.8	100.0	100.0	94.4	88.0	-21/20a/155	100.0	
	MCL	93.3	80.0	80.0	100.0	81.3	-21/20a/92a/155	93.3	
	BL	100.0	80.0	80.0	80.0	100.0	-21/155	100.0	
	B-NHL-NOS	100.0	100.0	77.8	88.9	87.5	-21/155	88.9	
CSF	Specificity	miR-21	miR-19a	miR-20a	miR-92a	miR-155	Index	Percent	
		DLBCL	95.0	94.4	83.3	87.5	77.8	-21/20a/155	100.0
		MCL	100.0	94.4	100.0	87.5	88.2	-21/20a/92a/155	100.0
		BL	100.0	94.4	81.3	75.0	88.2	-21/155	100.0
		B-NHL-NOS	90.0	100.0	100.0	88.9	100.0	-21/155	100.0
CSF	Youden index	miR-21	miR-19a	miR-20a	miR-92a	miR-155	Index	Percent	
		DLBCL	90.8	94.4	83.3	81.9	65.8	-21/20a/155	100.0
		MCL	93.3	74.4	80.0	87.5	69.5	-21/20a/92a/155	93.3
		BL	100.0	74.4	61.3	55.0	88.2	-21/155	100.0
		B-NHL-NOS	90.0	100.0	77.8	77.8	87.5	-21/155	88.9

B Plasma

Plasma	CNS lymphoma	miR-21	miR-19a	miR-20a	miR-92a	miR-155	Index	Percent	
		DLBCL	95.7	83.3	91.7	81.8	79.2	-21/19a/20a/155	91.3
		MCL	100.0	100.0	92.3	92.3	85.7	-21/19a/155	100.0
		BL	100.0	80.0	80.0	80.0	75.0	-21/19a/155	100.0
		B-NHL-NOS	100.0	100.0	100.0	100.0	85.7	-19a/155	100.0
Plasma	Specificity	miR-21	miR-19a	miR-20a	miR-92a	miR-155	Index	Percent	
		DLBCL	94.7	100.0	85.0	95.0	75.0	-21/19a/20a/155	85.0
		MCL	100.0	100.0	90.0	95.0	100.0	-21/19a/155	95.0
		BL	100.0	100.0	90.0	100.0	80.0	-21/19a/155	100.0
		B-NHL-NOS	89.5	100.0	100.0	100.0	55.0	-19a/155	100.0
Plasma	Youden index	miR-21	miR-19a	miR-20a	miR-92a	miR-155	Index	Percent	
		DLBCL	90.4	83.3	76.7	76.8	54.2	-21/19a/20a/155	76.3
		MCL	100.0	100.0	82.3	87.3	85.7	-21/19a/155	95.0
		BL	100.0	80.0	70.0	80.0	55.0	-21/19a/155	100.0
		B-NHL-NOS	89.5	100.0	100.0	100.0	40.7	-19a/155	100.0

Table S2. Sensitivity and specificity of oncomiRs in CSF and plasma of secondary CNS lymphoma patients compared to controls.

Percent of oncomiR sensitivity, specificity and Youdan index in CSF (**A**) and plasma (**B**) of indicated secondary CNS lymphoma compared to controls. The sensitivity and specificity is determined according to the minimal distance of the ROC curve to the top left corner of the plot square, evaluated by Receiver operating analysis. The Youden index is the sum of sensitivity and specificity subtracted by 100. **Left panel** represents individual oncomiR, **right panel**: oncomiR-index combining expression of individual oncomiRs into one classifier.

A CSF

CSF	Sensitivity	CNS lymphoma	CNS vs Systemic lymphoma - single miR					oncomiR-index	
		miR-21	miR-19a	miR-20a	miR-92a	miR-155	Index	Percent	
		DLBCL	83.3	75.0	75.0	83.3	72.0	-21/20a/155	91.3
		MCL	93.3	80.0	80.0	85.7	87.5	-21/20a/92a/155	87.5
	BL	100.0	60.0	80.0	60.0	100.0	-21/155	100.0	
	B-NHL-NOS	77.8	77.8	66.7	66.7	87.5	-21/155	88.9	
	Specificity	miR-21	miR-19a	miR-20a	miR-92a	miR-155	Index	Percent	
		DLBCL	87.5	77.0	77.1	79.7	82.2	-21/20a/155	90.4
		MCL	91.7	75.0	76.5	86.7	87.5	-21/20a/92a/155	93.3
		BL	84.6	46.2	53.9	53.9	91.7	-21/155	100.0
	B-NHL-NOS	66.7	66.7	66.7	66.7	100.0	-21/155	100.0	
	Youden index	miR-21	miR-19a	miR-20a	miR-92a	miR-155	Index	Percent	
		DLBCL	70.8	52.0	52.1	63.0	54.2	-21/20a/155	81.7
		MCL	85.0	55.0	56.5	72.4	75.0	-21/20a/92a/155	80.8
		BL	84.6	6.2	33.9	13.9	91.7	-21/155	100.0
	B-NHL-NOS	44.5	44.5	33.3	33.3	87.5	-21/155	88.9	

B Plasma

Plasma	Sensitivity	miR-21	miR-19a	miR-20a	miR-92a	miR-155	Index	Percent	
		DLBCL	78.3	79.2	79.2	77.3	62.5	-21/19a/20a/155	83.3
		MCL	61.5	71.4	69.2	69.2	71.4	-21/19a/155	78.6
		BL	100.0	80.0	80.0	80.0	75.0	-21/19a/155	100.0
	B-NHL-NOS	66.7	66.7	71.4	71.4	57.1	-19a/155	100.0	
	Specificity	miR-21	miR-19a	miR-20a	miR-92a	miR-155	Index	Percent	
		DLBCL	74.6	79.6	78.3	77.6	65.0	-21/19a/20a/155	78.3
		MCL	73.3	62.5	62.5	62.5	62.5	-21/19a/155	100.0
		BL	100.0	76.9	61.5	92.3	69.2	-21/19a/155	100.0
	B-NHL-NOS	66.7	66.7	66.7	66.7	66.7	-19a/155	66.7	
	Youden index	miR-21	miR-19a	miR-20a	miR-92a	miR-155	Index	Percent	
		DLBCL	52.8	58.8	57.5	54.9	27.5	-21/19a/20a/155	61.7
		MCL	34.9	33.9	31.7	31.7	33.9	-21/19a/155	78.6
		BL	100.0	56.9	41.5	72.3	44.2	-21/19a/155	100.0
	B-NHL-NOS	33.3	33.3	38.1	38.1	23.8	-19a/155	66.7	

Table S3. Sensitivity and specificity of oncomiRs in CSF and plasma of secondary CNS lymphoma compared to systemic lymphoma.

Percent of oncomiR sensitivity, specificity and Youden index in CSF (**A**) and plasma (**B**) of indicated secondary CNS lymphoma compared to systemic lymphoma. The sensitivity and specificity is determined according to the minimal distance of the ROC curve to the top left corner of the plot square, evaluated by Receiver operating analysis. The Youden index is the sum of sensitivity and specificity subtracted by 100. **Left panel** represents individual oncomiR, **right panel**: oncomiR-index combining expression of individual oncomiRs into one classifier.

Characteristics	DLBCL		MCL		BL		B-NHL NOS		All diagnoses							
	SCNSL (n=25)	Systemic (n=72)	All patients (N=97)	SCNSL (n=15)	Systemic (n=19)	All patients (N=34)	SCNSL (n=5)	Systemic (n=13)	All patients (N=18)	SCNSL (n=9)	Systemic (n=4)	All patients (N=13)	SCNSL (n=13)	Systemic (n=54)	All patients (N=108)	All patients (N=162)
Age, median (range), years	59 (27-79)	61 (23-81)	65 (47-83)	66 (44-82)	65 (44-83)	62 (39-70)	47 (19-85)	54 (19-85)	63 (53-73)	48 (33-72)	61 (33-73)	62.5 (27-83)	62 (19-85)	62 (19-85)	62 (19-85)	
<60	13 (52.0)	32 (44.4)	5 (33.3)	11 (32.4)	1 (20.0)	9 (69.2)	10 (55.6)	2 (50.0)	5 (41.7)	2 (41.5)	2 (41.5)	22 (41.5)	49 (45.4)	49 (45.4)	71 (43.8)	
>=60	12 (48.0)	40 (55.6)	52 (53.6)	10 (66.7)	4 (20.0)	23 (67.6)	4 (30.8)	8 (44.4)	5 (62.5)	2 (50.0)	7 (58.3)	31 (58.5)	59 (54.6)	59 (54.6)	91 (56.2)	
Sex																
Male	15 (60.0)	44 (61.1)	59 (60.8)	15 (100.0)	14 (73.7)	29 (85.3)	3 (60)	10 (76.9)	13 (72.2)	7 (77.8)	3 (75.0)	10 (76.9)	40 (74.1)	71 (65.7)	111 (68.5)	
Female	10 (40.0)	28 (38.9)	38 (39.2)	0 (0.0)	5 (26.3)	5 (14.7)	2 (40)	3 (23.1)	5 (27.8)	2 (22.2)	1 (25.0)	3 (23.1)	14 (25.9)	37 (34.3)	51 (31.5)	
ECOG/PC																
0-1	9 (36.0)	38 (52.8)	47 (48.4)	7 (46.7)	15 (78.9)	22 (64.7)	3 (60.0)	6 (46.1)	9 (50.0)	2 (25.0)	3 (75.0)	5 (41.7)	21 (39.6)	62 (57.4)	83 (51.6)	
2-4	16 (64.0)	34 (47.2)	50 (51.6)	4 (21.1)	8 (53.3)	12 (35.3)	2 (40.0)	7 (53.8)	9 (50.0)	6 (75.0)	1 (25.0)	7 (58.3)	32 (60.4)	46 (42.6)	78 (48.4)	
Ann Arbor stage																
I or II	4 (16.0)	17 (23.6)	21 (21.6)	0 (0.0)	3 (15.8)	3 (8.8)	0 (0.0)	0 (0.0)	0 (0.0)	1 (14.3)	2 (50.0)	3 (27.3)	5 (9.6)	22 (20.4)	27 (16.9)	
III or IV	21 (84.0)	55 (76.4)	76 (78.4)	15 (100.0)	16 (84.2)	31 (91.2)	5 (100.0)	18 (100.0)	6 (85.7)	2 (50.0)	8 (72.7)	47 (90.4)	86 (79.6)	133 (83.1)		
Extranodal sites, n																
0-1	9 (36.0)	35 (48.6)	44 (45.4)	2 (13.3)	11 (57.9)	13 (38.2)	2 (40.0)	3 (23.1)	5 (27.8)	3 (37.5)	3 (75.0)	6 (50.0)	16 (30.2)	52 (48.1)	68 (42.2)	
>1	16 (64.0)	37 (51.4)	53 (54.6)	13 (82.7)	8 (42.1)	21 (61.8)	3 (60.0)	10 (76.9)	13 (72.2)	5 (62.5)	1 (25.0)	6 (50.0)	37 (69.8)	56 (51.9)	93 (57.8)	
Involvement of kidney and/or adrenal glands																
IP																
Low	3 (12.0)	14 (19.4)	17 (17.5)	0 (0.0)	3 (15.8)	3 (9.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (50.0)	2 (18.2)	3 (5.9)	19 (17.6)	22 (13.8)	
Low-intermediate	2 (8.0)	13 (18.1)	15 (15.5)	2 (14.3)	3 (15.8)	5 (15.2)	1 (20.0)	2 (15.4)	3 (16.7)	2 (29.0)	1 (25.0)	3 (27.2)	7 (13.7)	19 (17.6)	26 (16.4)	
High-intermediate	4 (16.0)	17 (23.6)	21 (22.7)	3 (21.4)	9 (47.4)	12 (36.4)	2 (40.0)	4 (30.8)	6 (33.3)	2 (29.0)	0 (0.0)	2 (18.2)	11 (21.6)	30 (27.8)	41 (25.8)	
High	16 (64.0)	28 (38.9)	44 (45.3)	9 (64.3)	4 (21.0)	13 (39.4)	2 (40.0)	7 (53.8)	9 (50.0)	3 (42.0)	1 (25.0)	4 (36.4)	40 (37.0)	40 (37.0)	70 (44.0)	
CNS-IP1																
Low (0-1)	2 (8.7)	13 (18.1)	15 (15.8)	31 (32.6)	1 (2.9)	0 (0)	1 (2.9)	0 (0)	4 (30.8)	4 (22.2)	0 (0.0)	0 (0.0)	9 (17.0)	15 (13.9)	24 (14.9)	
Intermediate (2-3)	4 (17.4)	27 (37.5)	32 (44.4)	49 (51.6)												
High (4-6)	17 (73.9)															
MPI																
Low (<5.7)																
Intermediate (5.7-6.2)																
High (>6.2)																
Non-GCB cell-of-origin	10 (62.5)	33 (55.9)	43 (57.3)													
Bone marrow involvement	12 (48.0)	17 (23.3)	29 (29.6)	14 (87.5)	14 (70.0)	28 (77.8)	5 (100.0)	6 (46.2)	11 (61.1)	4 (50.0)	0 (0.0)	4 (36.4)	35 (64.8)	37 (33.9)	72 (44.2)	
Peripheral blood involvement	6 (26.1)	6 (9.4)	12 (13.8)	12 (80.0)	12 (60.0)	24 (68.6)	2 (40.0)	0 (0.0)	2 (11.8)	3 (42.9)	0 (0.0)	3 (30.0)	23 (46.0)	18 (18.2)	41 (27.5)	
LDH (plasma) > Norm	14 (66.7)	42 (60.0)	56 (60.8)	11 (84.6)	10 (52.6)	21 (65.6)	3 (60.0)	12 (92.3)	15 (83.3)	6 (75.0)	2 (50.0)	8 (66.7)	34 (72.3)	66 (61.1)	100 (64.7)	
Lactate (CSF) > Norm	10 (55.6)	10 (20.4)	20 (29.9)	4 (44.4)	5 (33.3)	9 (37.5)	3 (50.0)	3 (25.0)	5 (31.3)	7 (87.5)	0 (0.0)	7 (70.0)	23 (59.0)	18 (23.1)	41 (35.0)	
Total protein level (CSF) > Norm	8 (44.4)	12 (24.0)	20 (29.4)	4 (44.4)	3 (20.0)	7 (29.2)	3 (75.0)	2 (16.7)	5 (31.3)	5 (62.5)	0 (0.0)	5 (50.0)	20 (51.3)	17 (21.5)	37 (31.4)	
B2 microglobulin (Plasma) > Norm	11 (68.8)	43 (61.8)	54 (63.5)	11 (100.0)	13 (72.2)	24 (82.8)	4 (80.0)	11 (91.7)	15 (88.2)	2 (28.6)	1 (33.3)	3 (30.0)	28 (71.8)	68 (66.2)	96 (69.1)	
Albumin (Plasma) < Norm	7 (50.0)	14 (41.2)	21 (43.8)	3 (37.5)	9 (69.2)	12 (57.1)	1 (33.3)	7 (63.6)	8 (51.1)	3 (60.0)	0 (0.0)	3 (37.5)	14 (46.7)	36 (59.0)	50 (54.9)	

Table S4: Summary of baseline clinical characteristics of CNS involving and systemic lymphoma patients. Data are presented as n (%). SCNSL, secondary CNS lymphoma. DLBCL, Diffuse large B-cell lymphoma. MCL, Mantle-cell lymphoma. BL, Burkitt's lymphoma. B-NHL-NOS (not otherwise specified, high grade).

Characteristics	DLBCL						MCL			B-NHL NOS		
	Systemic to CNS relapse subsequent (n=7)	Systemic to CNS relapse all (n=13)	Systemic no CNS relapse (n=65)	SCNSL relapse subsequent (n=4)	SCNSL relapse all (n=8)	SCNSL no CNS relapse (n=10)	CNS relapse (n=4)	SCNSL no CNS relapse (n=11)	Systemic no CNS relapse (n=19)	CNS relapse (n=1)	SCNSL no CNS relapse (n=8)	Systemic no CNS relapse (n=4)
Age, median(range), years	46 (27-74)	55 (27-78)	61 (23-81)	71 (59-79)	66.5 (46-79)	61.5 (37-79)	77 (62-83)	63 (47-75)	66 (44-82)	58	60 (57-73)	48 (33-72)
<60	4 (57.1)	8 (61.5)	28 (43.1)	1 (25.0)	2 (25.0)	5 (50.0)	0 (0.0)	5 (45.5)	6 (31.6)	2 (28.5)	2 (50.0)	2 (50.0)
≥60	3 (42.9)	5 (38.5)	37 (56.9)	3 (75.0)	6 (75.0)	5 (50.0)	4 (100.0)	6 (54.5)	13 (68.4)	0 (0.0)	5 (71.5)	2 (50.0)
Sex												
Male	7 (100.0)	10 (76.9)	37 (56.7)	3 (75.0)	5 (62.5)	6 (60.0)	4 (100.0)	11 (100.0)	14 (73.7)	0 (0.0)	7 (87.5)	3 (75.0)
Female	0 (0.0)	3 (23.1)	28 (43.1)	1 (25.0)	3 (37.5)	4 (40.0)	0 (0.0)	0 (0.0)	5 (26.3)	1 (100.0)	1 (12.5)	1 (25.0)
ECOG/PC												
0-1	2 (28.6)	5 (38.5)	36 (55.4)	1 (25.0)	2 (25.0)	3 (30.0)	4 (100.0)	3 (27.3)	15 (78.9)	0 (0.0)	2 (28.5)	3 (75.0)
2-4	5 (71.4)	8 (61.5)	29 (43.6)	3 (75.0)	6 (75.0)	7 (70.0)	0 (0)	8 (72.7)	4 (21.1)	1 (100.0)	5 (71.5)	1 (25.0)
Ann Arbor stage												
I or II	0 (0.0)	3 (23.1)	17 (26.2)	0 (0.0)	0 (0.0)	1 (10.0)	0 (0.0)	0 (0.0)	3 (15.8)	0 (0.0)	1 (16.7)	2 (50.0)
III or IV	7 (100.0)	10 (76.9)	48 (73.8)	3 (200.0)	7 (100.0)	9 (90.0)	4 (100.0)	11 (100.0)	16 (84.2)	1 (100.0)	5 (83.3)	2 (50.0)
Extranodal sites, n												
0-1	2 (28.6)	7 (53.8)	33 (50.8)	2 (50.0)	2 (25.0)	2 (20.0)	1 (25.0)	1 (9.1)	11 (57.9)	0 (0.0)	3 (42.9)	3 (75.0)
>1	5 (71.4)	6 (46.2)	32 (49.2)	2 (50.0)	6 (75.0)	8 (80.0)	3 (75.0)	10 (90.9)	8 (42.1)	1 (100.0)	4 (57.1)	1 (25.0)
Involvement of kidney and/or adrenal glands												
IPI	1 (16.7)	1 (7.7)	10 (15.4)	0 (0.0)	1 (14.3)	5 (50.0)	0 (0.0)	1 (9.1)	0 (0)	0 (0.0)	0 (0.0)	0 (0.0)
Low	1 (14.3)	4 (30.8)	13 (20.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (15.8)	0 (0.0)	2 (50.0)
Low-intermediate	0 (0.0)	1 (7.7)	13 (20.0)	0 (0.0)	1 (10.0)	1 (10.0)	0 (0.0)	2 (18.2)	3 (15.8)	0 (0.0)	2 (33.3)	1 (25.0)
High-intermediate	1 (14.3)	0 (0)	16 (24.6)	2 (66.7)	2 (28.6)	3 (30.0)	2 (66.7)	1 (9.1)	9 (47.4)	1 (100.0)	1 (16.7)	0 (0.0)
High	5 (71.4)	8 (61.5)	23 (35.4)	1 (33.3)	5 (71.4)	6 (60.0)	1 (33.3)	8 (72.7)	4 (21.0)	0 (0.0)	3 (50.0)	1 (25.0)
CNS-IPI												
Low(0-1)	0 (0.0)	2 (15.4)	13 (20.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (15.8)	0 (0.0)	0 (0.0)	2 (50.0)
Intermediate (2-3)	1 (14.3)	3 (23.1)	26 (40.0)	1 (25.0)	1 (12.5)	2 (20.0)	1 (20.0)	1 (10.0)	3 (15.8)	0 (0.0)	2 (33.3)	1 (25.0)
High (4-6)	6 (85.7)	8 (61.5)	26 (40.0)	3 (75.0)	7 (87.5)	8 (80.0)	6 (60.0)	8 (72.7)	4 (21.0)	0 (0.0)	1 (16.7)	0 (0.0)
MPI												
Low (<5.7)												
Intermediate (5.7-6.2)												
High (>6.2)												
Non-GCB cell-of-origin												
3 (60.0)	8 (72.7)	30 (56.6)	0 (0.0)	1 (33.3)	3 (50.0)			0 (0)	1 (9.1)	4 (15.0)		
Bone marrow involvement	3 (42.9)	3 (23.1)	14 (21.5)	3 (75.0)	7 (87.5)	4 (40.0)	3 (75.0)	11 (100.0)	14 (70.0)	1 (100.0)	3 (37.5)	0 (0.0)
Peripheral blood involvement	0 (0.0)	0 (0.0)	12 (18.5)	3 (75.0)	5 (62.5)	3 (30.0)	2 (50.0)	10 (90.9)	12 (60.0)	1 (100.0)	2 (25.0)	0 (0.0)
LDH (plasma) > Norm	4 (80.0)	5 (50.0)	38 (58.5)	2 (50.0)	4 (57.1)	9 (90.0)	1 (50.0)	11 (100.0)	10 (52.6)	1 (100.0)	5 (83.3)	2 (50.0)
Lactate (CSF) > Norm	2 (50.0)	5 (50.0)	8 (18.2)	2 (100.0)	6 (100.0)	3 (37.5)	2 (100.0)	2 (28.6)	5 (33.3)	1 (100.0)	6 (85.7)	0 (0.0)
Total protein level (CSF) > Norm	2 (50.0)	4 (40.0)	10 (22.2)	2 (100.0)	5 (83.3)	3 (37.5)	1 (50.0)	2 (28.6)	3 (20.0)	1 (100.0)	4 (57.1)	0 (0.0)
β2 microglobuline (Plasma) > Norm	3 (75.0)	2 (28.6)	40 (62.5)	1 (33.3)	3 (60.0)	9 (90.0)	1 (100.0)	10 (100.0)	13 (72.2)	0 (0.0)	2 (50.0)	1 (33.3)
Albumin (Plasma) < Norm	1 (33.3)	4 (44.4)	13 (41.9)	1 (100.0)	2 (50.0)	3 (75.0)	2 (100.0)	3 (50.0)	9 (69.2)	0 (0.0)	3 (75.0)	0 (0.0)

Table S5. Baseline clinical characteristics of patients who developed CNS relapse compared with patients with no CNS relapse.

Data are presented as n (%). In DLBCL, the category of **Systemic to CNS relapse subsequent** denotes systemic DLBCL with samples collected at time of initial diagnosis (n=7), who subsequently relapsed/progressed to CNS. The category of **Systemic to CNS relapse all** contains in addition the “current” relapses (n=6), which were collected at the time of CNS relapse. The category **SCNSL relapse subsequent** denotes secondary CNS patients, with samples collected at time of diagnosis, who after achieving complete remission developed subsequently CNS relapse (n=4). The category **SCNSL relapse all** in addition contains SCNSL relapses collected at time of SCNSL relapse (n=4). Note: several DLBCL CNS relapses have samples from both, systemic diagnose and from CNS relapse, these patients are not duplicated in “relapse all” category. All relapses in MCL and B-NHL-NOS are relapses of systemic disease to CNS.

Risk Factor	HR	95% CI	P
OncomiR high vs. low	9.4	1.8 - 49.9	0.0088
CNS-IPI			
high (vs. low)	4.9	0.88 - 26.7	0.0695
int. (vs. low)	4.8	0.08 - 283	0.4497
high (vs. int.)	4.8	1.7 - 21.5	0.0402
CNS-IPI / Oncomir			
both risk (vs. no risk)	31.9	4.7 - 219	0.0004
one risk (vs. no risk)	1.4	0.08 - 22.6	0.8278
both risks (vs. one risk)	12.1	2.1 - 69.5	0.0056

Table S6. Predictive value of circulating oncomiRs in plasma for CNS relapse in DLBCL.

Risk for CNS relapse stratified by oncomiR levels in plasma (at time of diagnosis), CNS-IPI and CNS-IPI/oncomiR combined risk categories (univariate analysis). Abbreviations: CNS, central nervous system; IPI, International Prognostic Index; Int., intermediate; HR, hazard ratio. For details on risk stratification, see Suppl. methods.

A	CSF	miR-21	miR-19a	miR-20a	miR-92a	miR-155
DLBCL	1.83			1.31		1.78
MCL	1.36			0.83	1.30	1.84
BL	1.57					1.75
B-NHL-NOS	1.10					1.64

Plasma	miR-21	miR-19a	miR-20a	miR-92a	miR-155
DLBCL	0.07	0.03	6.93		0.43
MCL	2.42	0.15			1.25
BL	2.12	0.08			0.10
B-NHL-NOS		5.85			0.45

B	CSF	miR-21	miR-19a	miR-20a	miR-92a	miR-155
DLBCL	0.09			0.16		1.15
MCL	0.05			0.12	0.13	1.28
BL	0.08					1.59
B-NHL-NOS	0.07					1.54

Plasma	miR-21	miR-19a	miR-20a	miR-92a	miR-155
DLBCL	0.003	0.003	0.37		1.76
MCL	0.12	0.01			3.22
BL	0.18	0.02			1.04
B-NHL-NOS		0.40			4.00

Table S7. Coefficients for oncomiR-indices calculations.

The values stand for the coefficients calculated as described in the methods and supplementary methods sections. A) Equalized coefficients (let-7a/Ctrl/systemic) B) unequalized coefficients.

p 15-18 :

Figure S6. Receiver operating analysis of oncomiRs in CSF of CNS lymphoma compared to controls or systemic lymphoma.

Receiver operating characteristic (ROC) curves for the possibility of indicated oncomiRs in CSF to discriminate between CNS lymphoma and controls (left panels) and between CNS involving and systemic lymphoma (right panels) in: (A) DLBCL, (B) MCL, (C) BL and (D) B-NHL-NOS. The X-axes show the percent of specificity and the Y-axes denote percent of sensitivity (100-sensitivity).

p 19-22:

Figure S7. Receiver operating analysis of oncomiRs in plasma of CNS lymphoma compared to controls or systemic lymphoma.

Receiver operating characteristic (ROC) curves for the possibility of indicated oncomiRs in plasma to discriminate between CNS lymphoma and controls (left panels) and between CNS involving and systemic lymphoma (right panels) in: (A) DLBCL, (B) MCL, (C) BL and (D) B-NHL-NOS. The X-axes show the percent of specificity and the Y-axes denote percent of sensitivity (100-sensitivity).

Figure S6A. Receiver operating analysis of oncomiRs in CSF of CNS lymphoma compared to controls or systemic lymphoma.

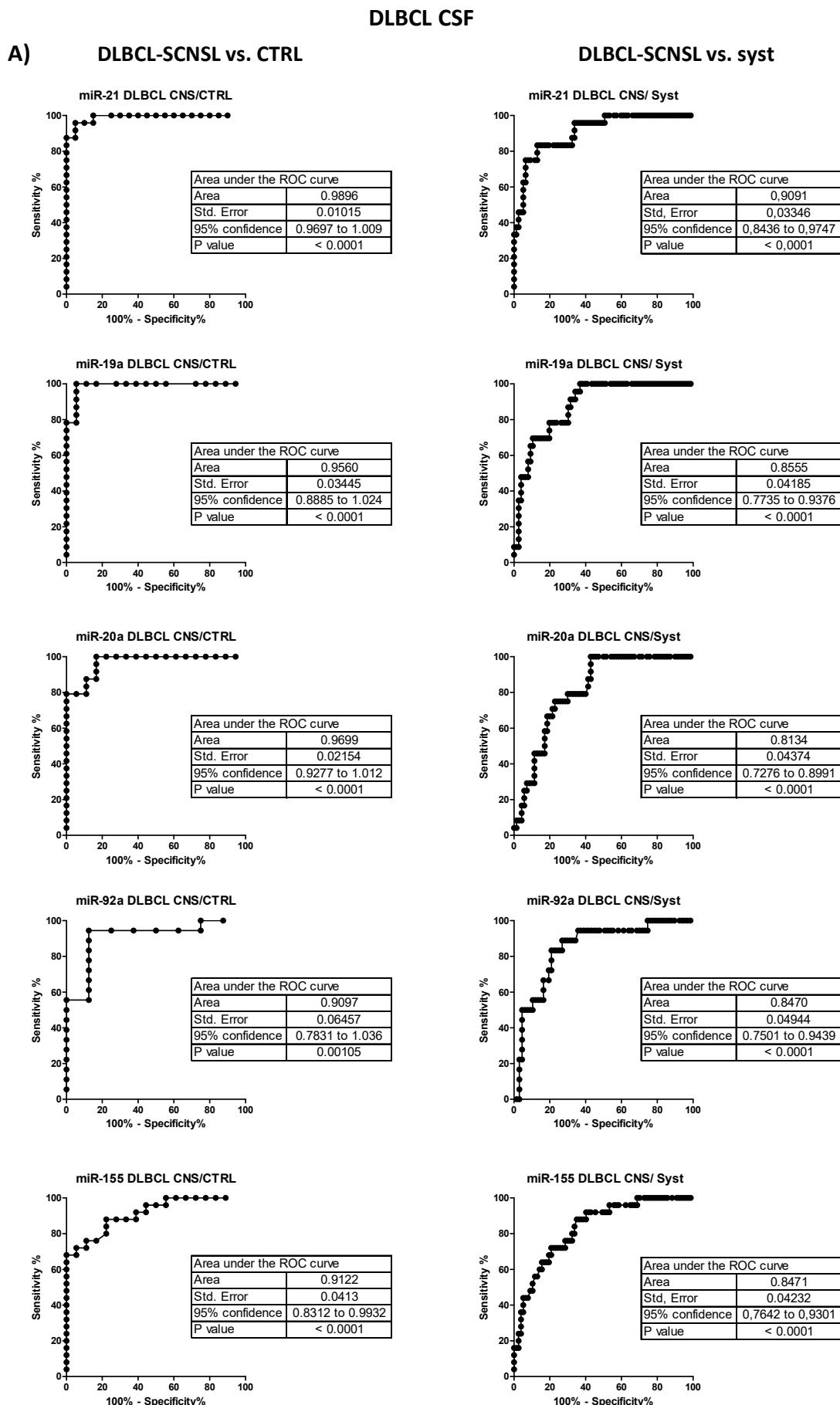


Figure S6B. Receiver operating analysis of oncomiRs in CSF of CNS lymphoma compared to controls or systemic lymphoma.

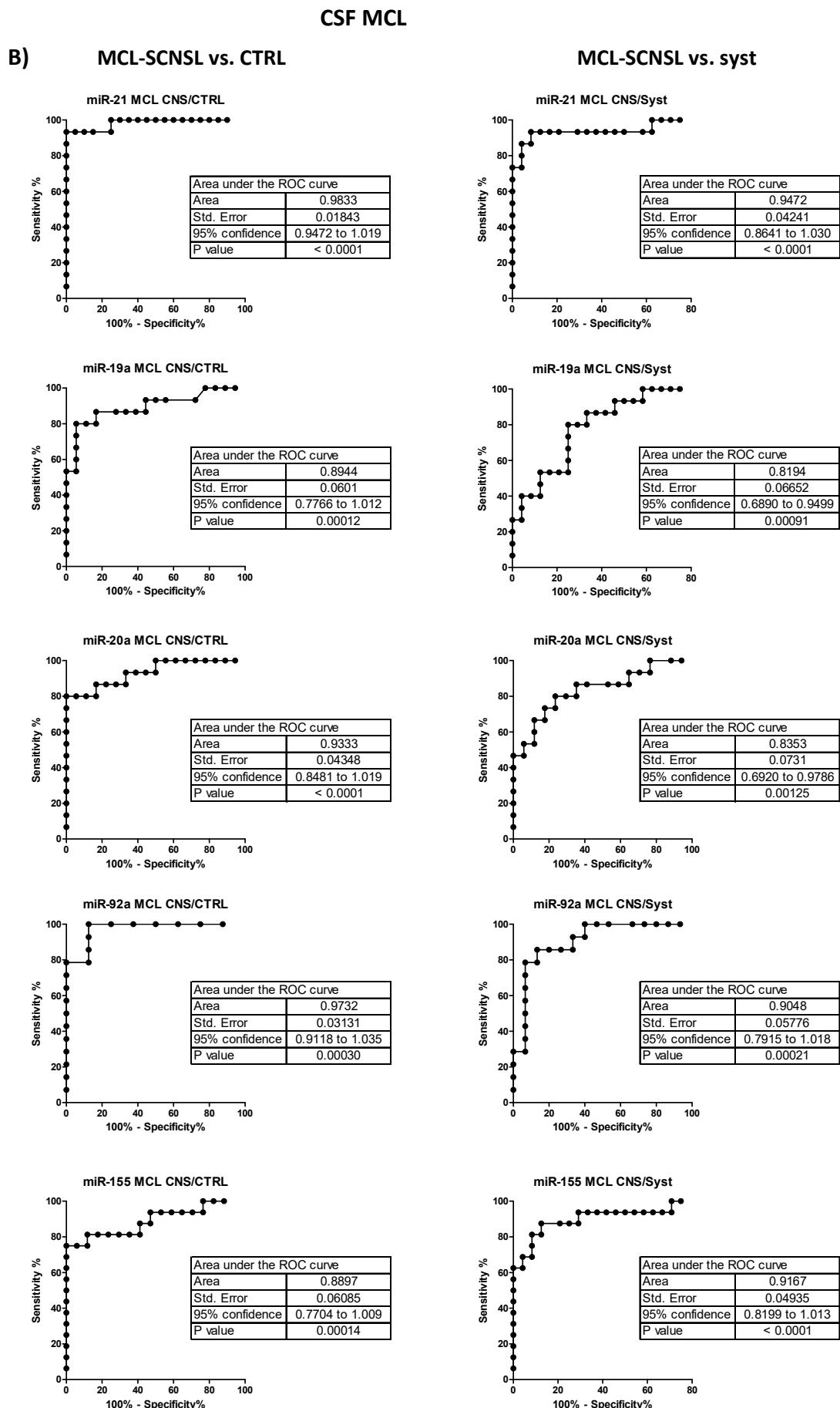


Figure S6C. Receiver operating analysis of oncomiRs in CSF of CNS lymphoma compared to controls or systemic lymphoma.

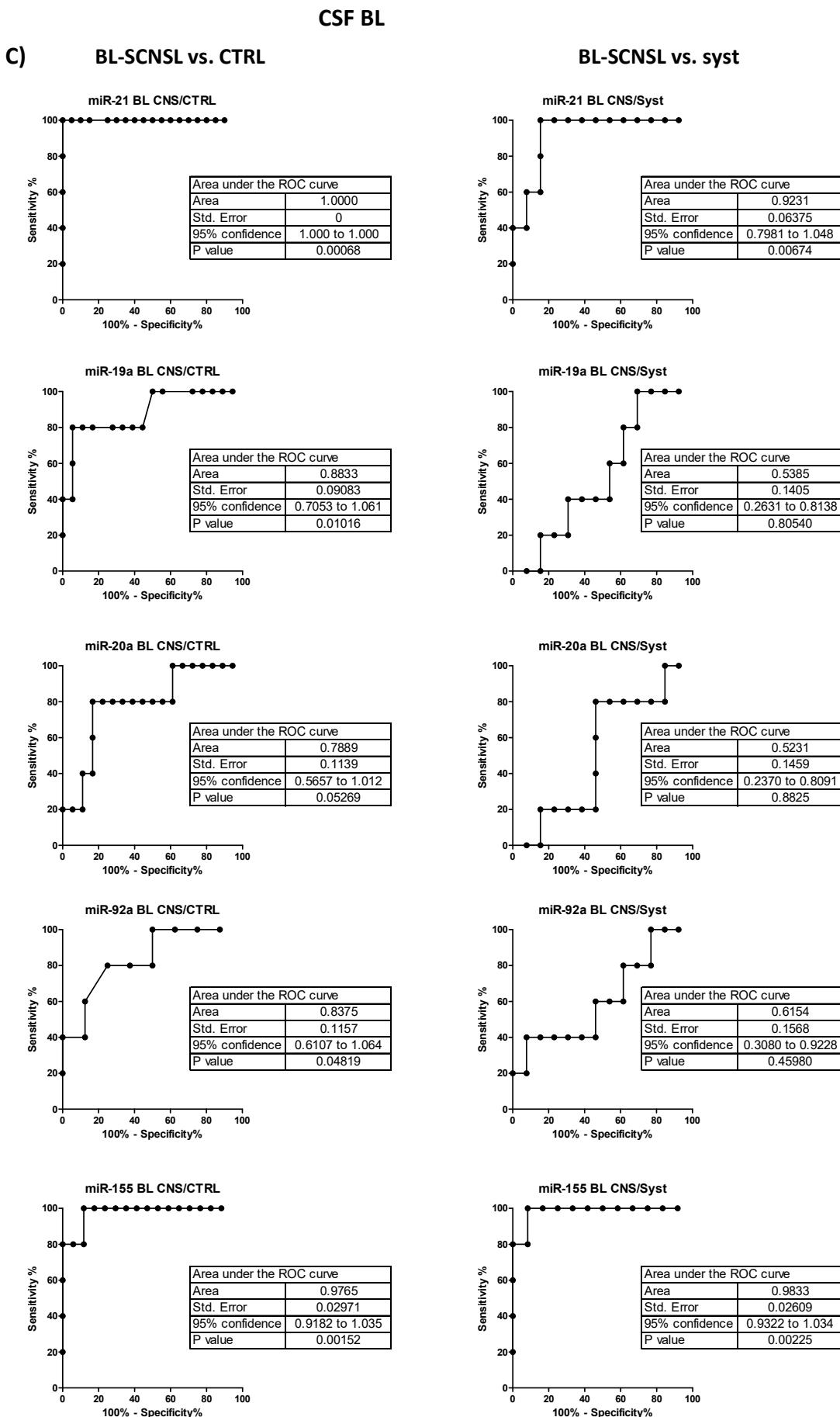


Figure S6D. Receiver operating analysis of oncomiRs in CSF of CNS lymphoma compared to controls or systemic lymphoma.

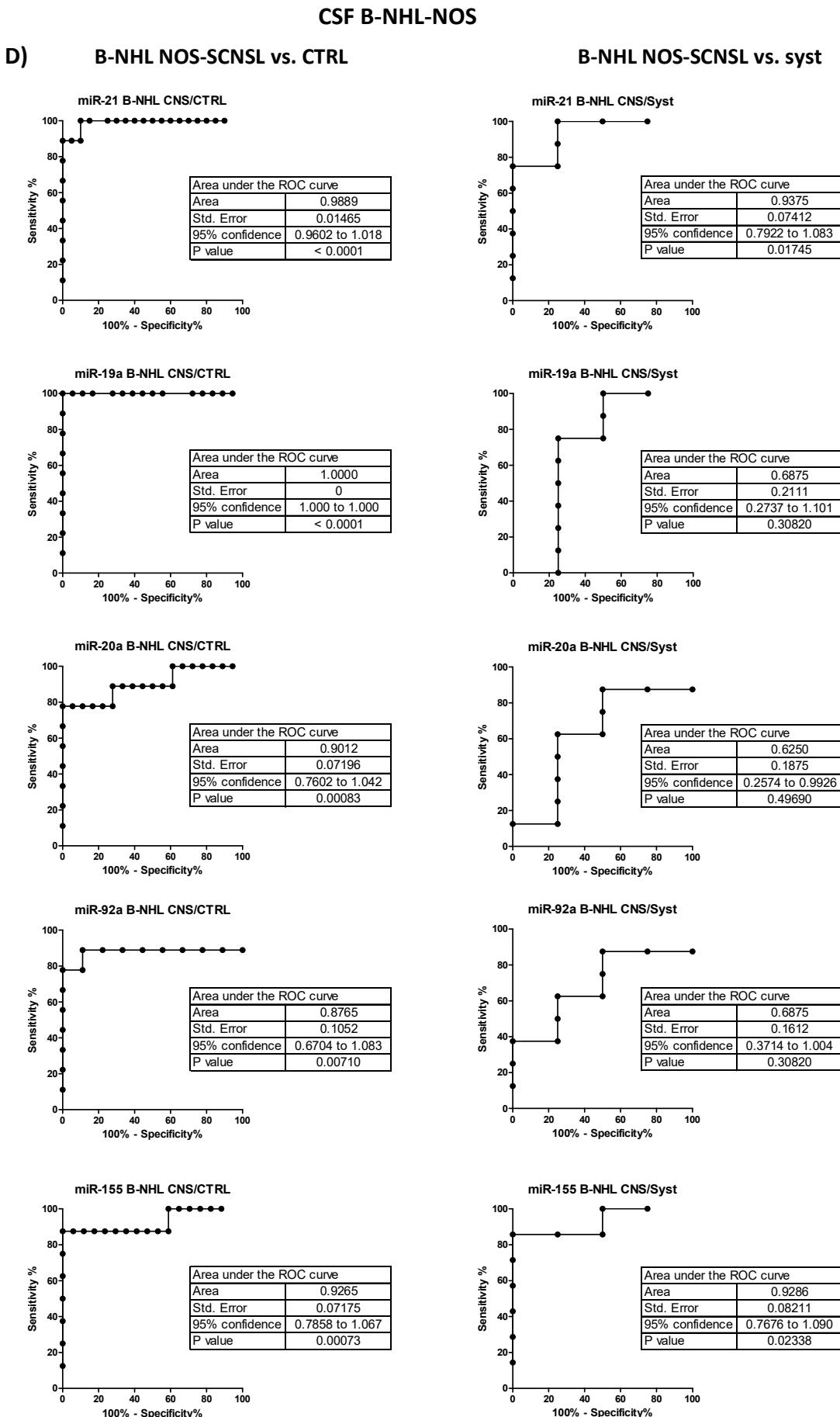


Figure S7A. Receiver operating analysis of oncomiRs in plasma of CNS lymphoma compared to controls or systemic lymphoma.

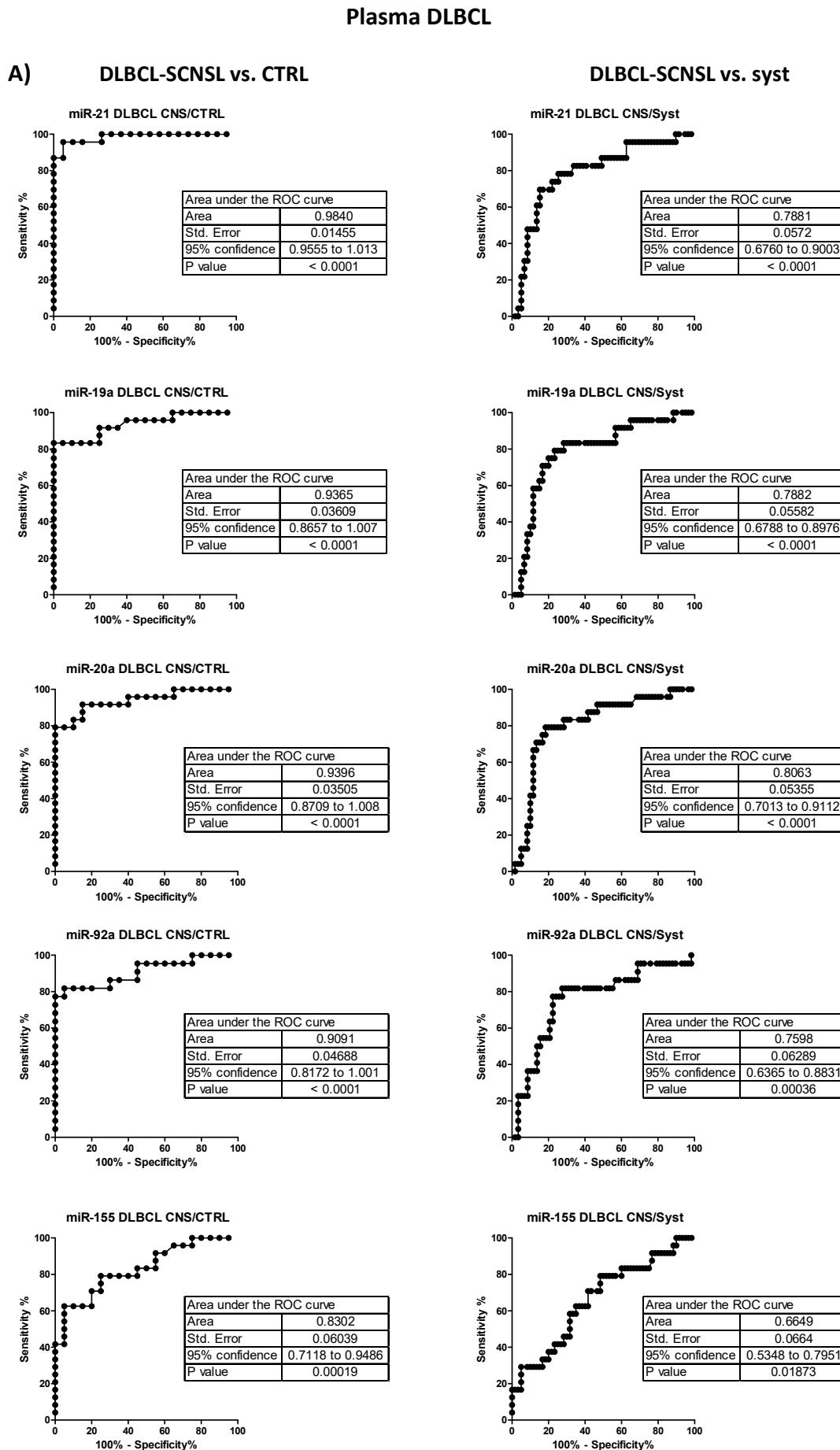


Figure S7B. Receiver operating analysis of oncomiRs in plasma of CNS lymphoma compared to controls or systemic lymphoma.

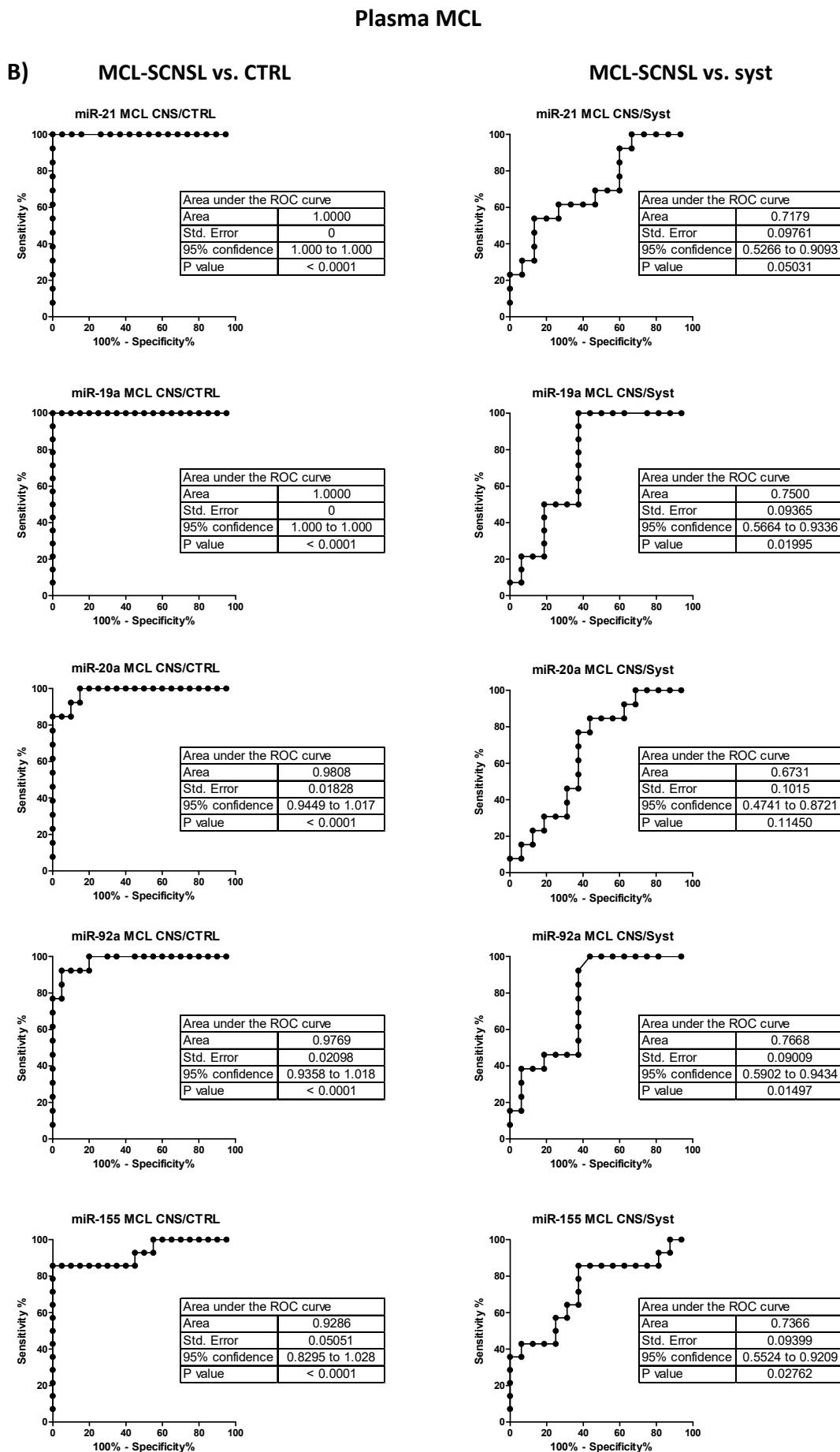


Figure S7C. Receiver operating analysis of oncomiRs in plasma of CNS lymphoma compared to controls or systemic lymphoma.

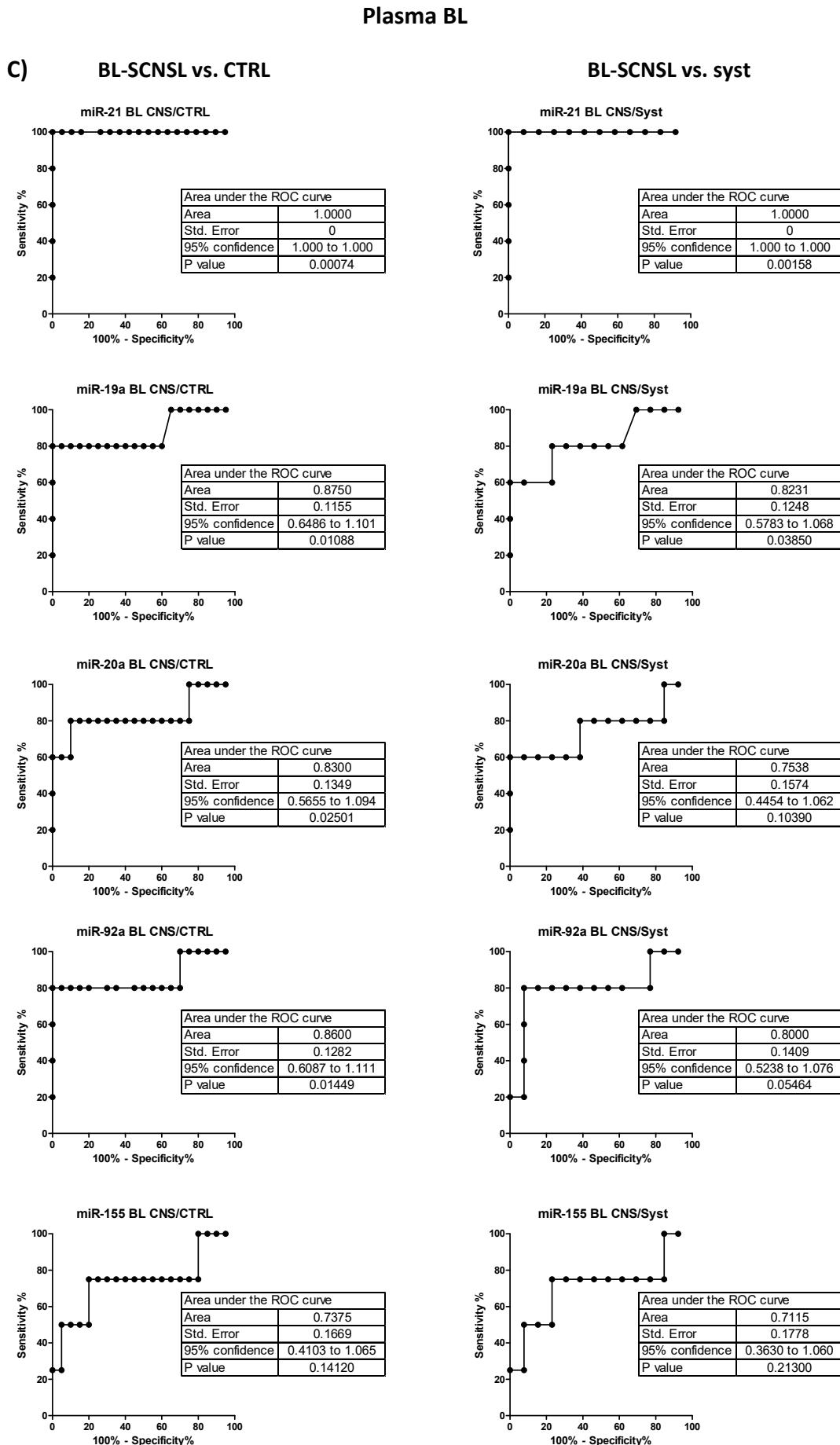
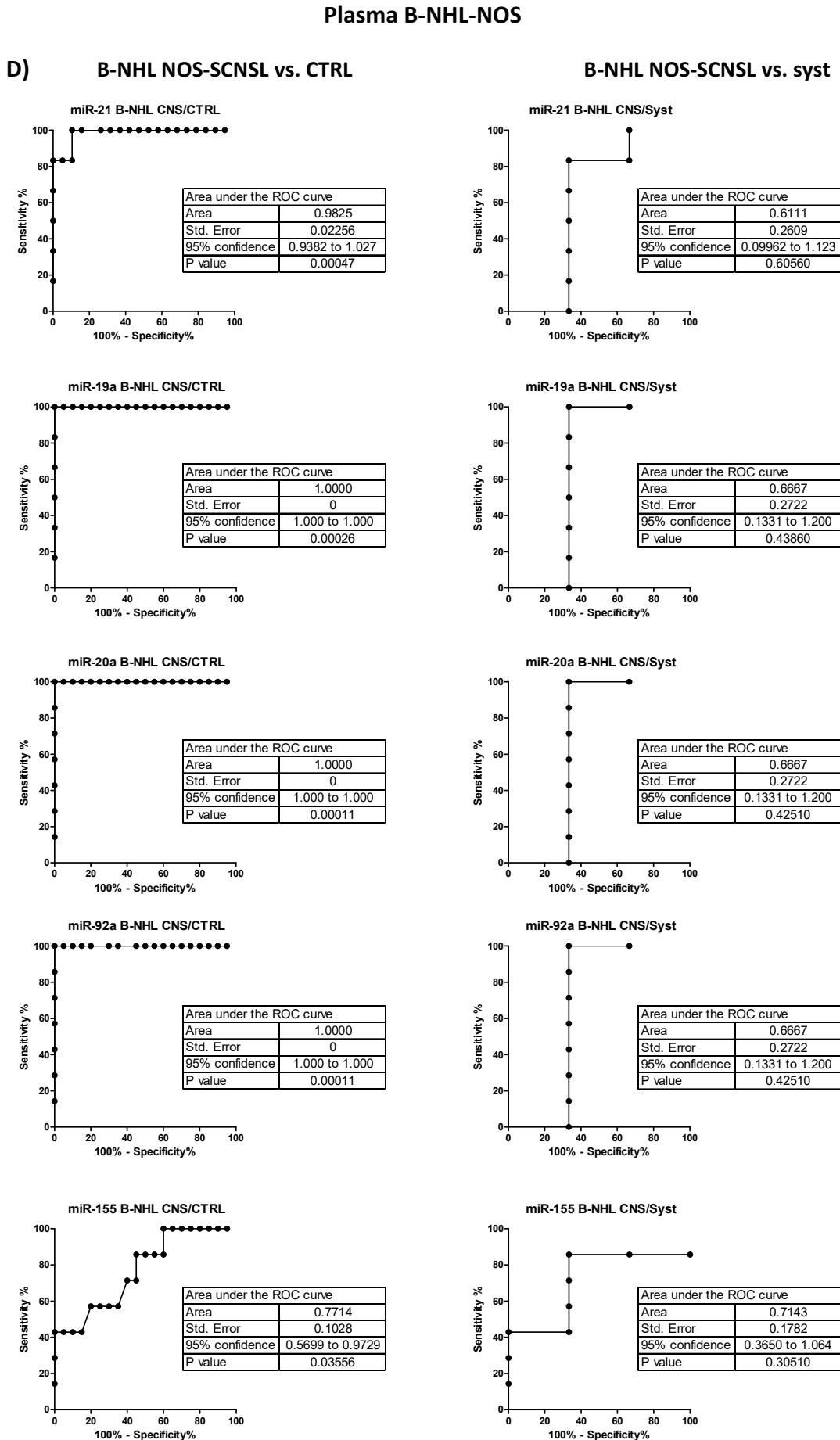


Figure S7D. Receiver operating analysis of oncomiRs in plasma of CNS lymphoma compared to controls or systemic lymphoma.



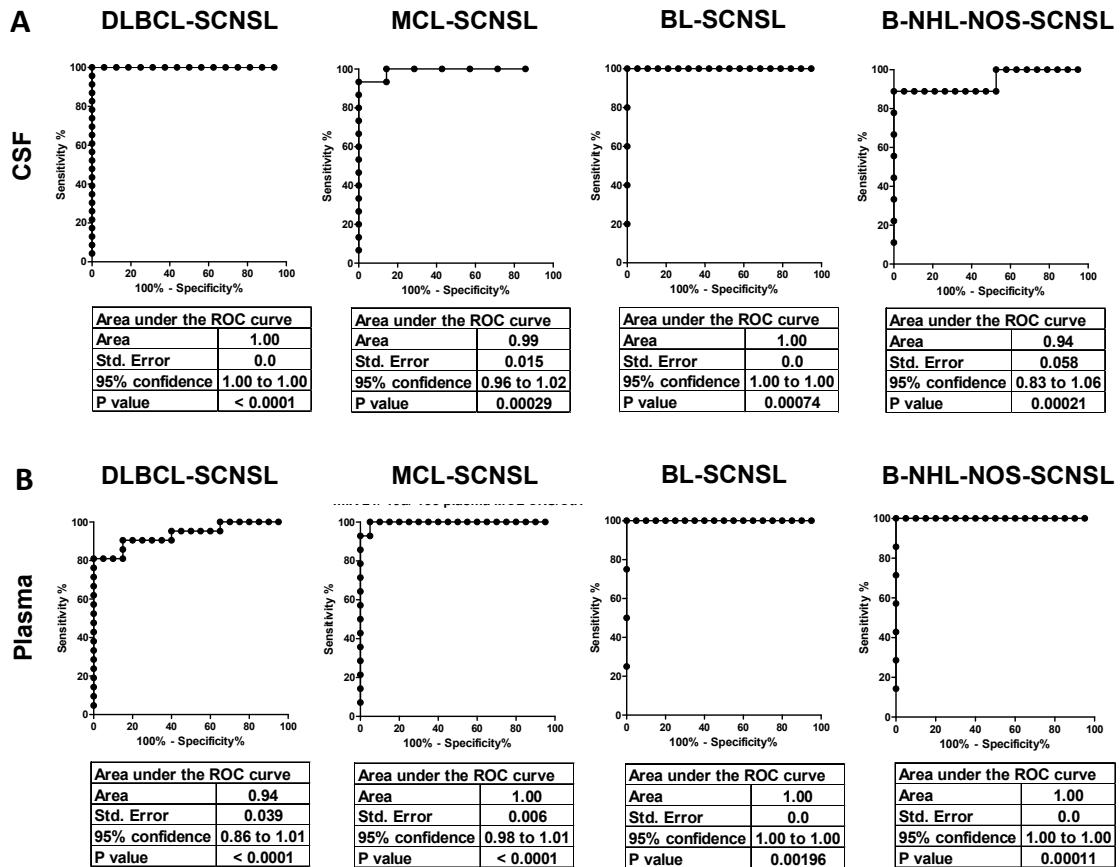


Figure S8. Receiver operating analysis of oncomiR-indices in CSF (A) and plasma (B) of CNS lymphoma compared to control samples.

Receiver operating characteristic (ROC) curves for the possibility of indicated oncomiR-indices to discriminate between CNS lymphoma of indicated lymphoma subtypes and control samples. The X-axes show the percent of specificity and the Y-axes percent of sensitivity (100-sensitivity).