Supplemental data table

Table S1: Patient characteristics

HL Patient	Age (Years)	SEX	Diagnosis (histological subgroups)	C-reactive protein (mg/L)	WBC (Gpt/l)	IgG (g/l)	IgA (g/l)	IgM (g/l)	CCL17 (pg/ml)	ESR (mm/hr)	ESR group	Stage	B-Symptoms	Bulky disease	E-Lesions	qPET value	ERAqPET group
HL_G1_11	15.5	female	cHL (Nodular sclerosis)	153.6	18.23	13.65	1.53	1.95	63149.64	72	ESRover30	IV	yes	yes	no	0	Negative
HL_G1_12	17.9	female	cHL (Mixed cellularity))	24	5.03	8.16	1.14	0.8	18143.32	8	ESRlow	III	no	no	no	0	Negative
HL_G1_13	17.9	male	cHL (Nodular sclerosis)	6.8	14.39	12.5	1.82	0.59	265939.92	14	ESRlow	II	no	no	no	0	Negative
HL_G1_5	13.3	male	cHL (Mixed cellularity)	1	5	13.5	2.55	0.25	553.37	15	ESRlow	III	no	no	no	0	Negative
HL_G1_9	9.8	female	cHL (Nodular sclerosis)	4.5	10.3	16	1.76	0.63	23101.64	70	ESRover30	III	no	no	no	0.56	Negative
HL_G1_4	17	male	cHL (Nodular sclerosis)	23	7.36	14.7	2.16	2.1	23088.92	28	ESRlow	II	no	no	no	0.67	Negative
HL_G1_3	3.9	female	cHL (Mixed cellularity)	54.3	5	6.39	0.8	0.34	63050.55	32	ESRover30	II	no	yes	no	0.95	Negative
HL_G1_1	8	male	cHL (Mixed cellularity)	143.5	28.61	14	2.09	1.33	n.d.	90	ESRover30	II	yes	yes	yes	0.95	Negative
HL_G1_10	8.7	male	cHL (Mixed cellularity)	0.3	5.8	10.9	0.67	0.32	14206.62	2	ESRlow	II	no	no	no	0.96	Negative
HL_G1_6	16.9	female	cHL (Nodular sclerosis)	148.68	37.42	14.7	2.55	3.11	n.d.	n.d.	n.d.	II	yes	yes	yes	1.05	Negative
HL_G1_2	9	female	cHL (Mixed cellularity)	23	5.94	12.7	2.16	0.97	27574.43	68	ESRover30	II	no	no	no	1.06	Negative
HL_G1_7	17.9	male	cHL (Nodular sclerosis)	71	11.21	12.55	1.79	1.07	223483.37	25	ESRlow	IV	yes	yes	yes	1.12	Negative
HL_G1_8	15.6	male	cHL (Nodular sclerosis)	22.22	9.98	13.2	2.17	2.44	261702.11	58	ESRover30	II	no	yes	no	1.24	Negative
HL_G2_6	18	male	cHL (Nodular sclerosis)	59.2	11.9	17.6	2.97	1.16	275051.77	6	ESRlow	II	yes	yes	no	1.44	Positive
HL_G2_12	10.5	male	cHL (Mixed cellularity)	2.6	6.1	9.3	2.04	0.34	84054.03	65	ESRover30	II	no	no	no	1.47	Positive
HL_G2_8	11.4	male	cHL (Nodular sclerosis)	42	8.9	15.4	2.02	0.68	165927.62	41	ESRover30	III	no	yes	no	1.55	Positive
HL_G2_1	16	female	cHL (Nodular sclerosis)	111	22.57	10.4	1.1	0.86	281265.14	23	ESRlow	II	no	no	no	1.61	Positive
HL_G2_3	3.4	male	cHL (Mixed cellularity)	25.8	11.3	10.84	1.12	1.35	59857.79	8	ESRlow	II	no	no	no	1.63	Positive
HL_G2_10	17.6	female	cHL (Nodular sclerosis)	49.4	9.02	14.9	2.01	1.85	225287.13	99	ESRover30	II	no	yes	no	1.72	Positive
HL_G2_11	11.1	male	cHL (Mixed cellularity)	20.4	7.69	13.77	2.7	0.52	148023.95	85	ESRover30	IV	yes	no	yes	2.02	Positive
HL_G2_2	5.6	female	cHL (Mixed cellularity)	4	5.6	6.5	0.94	0.59	175332.08	10	ESRlow	III	no	no	no	2.1	Positive
HL_G2_4	16.3	female	cHL (Nodular sclerosis)	24	7.9	17.04	2.81	0.65	273592.16	100	ESRover30	IV	no	no	no	2.15	Positive
HL_G2_7	16.8	female	cHL (Nodular sclerosis)	19	11	n.d.	n.d.	n.d.	89473.85	23	ESRlow	III	no	no	no	2.26	Positive
HL_G2_5	16.2	male	cHL (Nodular sclerosis)	121	15.98	13.4	3.5	0.93	321982.02	119	ESRover30	IV	yes	no	no	2.36	Positive
HL_G2_9	17.3	male	cHL (HL-others)	118	24.4	37.74	5.31	0.97	237536.33	91	ESRover30	II	yes	yes	yes	2.74	Positive
HL_G3_4	13.9	male	cHL (Mixed cellularity)	5	6.4	15.7	1.8	0.9	2527.25	15	ESRlow	IV	no	no	no	3.1	Positive
HL_G3_8	5.5	male	cHL (Mixed cellularity)	116	12.1	10.69	2.23	1.63	54072.44	110	ESRover30	II	yes	yes	yes	3.25	Positive
HL_G3_9	16.9	male	cHL (Nodular sclerosis)	70	24.51	12.9	2.18	0.719	313049.97	83	ESRover30	IV	yes	yes	yes	3.26	positive
HL_G3_1	13.4	male	cHL (Nodular sclerosis)	128	19.15	6.9	0.92	0.9	n.d.	87	ESRover30	IV	yes	No	yes	3.67	Positive
HL_G3_10	14.3	female	cHL (Nodular sclerosis)	87.1	10.1	23.7	2.89	0.56	147170.16	52	ESRover30	III	yes	yes	no	3.83	Positive
HL_G3_3	17.3	female	cHL (Mixed cellularity)	7	16.6	n.d.	n.d.	n.d.	236043.15	33	ESRover30	II	no	yes	no	3.9	Positive
HL_G3_2	16.5	female	cHL (Nodular sclerosis)	91.6	9.7	14	0.86	2.19	280654.76	95	ESRover30	II	no	yes	yes	4.08	Positive
HL_G3_7	9.7	male	cHL (Nodular sclerosis)	34	13.7	23.4	3.17	0.86	255461.41	39	ESRover30	IV	yes	yes	yes	4.71	Positive
HL_G3_5	15.6	male	cHL (Mixed cellularity)	106.2	14.9	32.4	3.72	0.87	296767.86	79	ESRover30	II	yes	yes	yes	6	Positive
HL_G3_6	7.9	female	cHL (Mixed cellularity)	101.5	7.9	n.d.	n.d.	n.d.	269434.83	84	ESRover30	II	yes	yes	yes	8.08	Positive

n.d. – No data

Table S2: List of miRNAs (TMM >50) dysregulated in pre-induction ERAqPET–positive versus –negative samples, previously reported in adult classical HL studies

miRNA detected in pediatric cHL study	Reported in adult cHL studies	Expression in cHL tissue compared to non-malignent tissue	Reported expression
let-7a-5p	Reference [1,2,3]	Plasma, Whole Blood, cHL cell lines	Up/down
let-7b-5p	Reference [2,3]	cHL cell lines, Whole Blood	Up/down
let-7d-3p	Reference [1,3]	Plasma, Serum	Up/down
let-7e-5p	Reference [4]	cHL cell lines	Up/down
let-7f-5p	Reference [1,2,4,5]	Plasma, cHL cell lines	Up/down
let-7g-5p	Reference [4,6,5]	HRS cells, cHL cell lines	Up
let-7i-5p	Reference [1]	Plasma	Down
miR-1	Reference [1,3]	Plasma, Whole Blood	Up/Down
miR-100-5p	Reference [1,4]	Plasma, cHL cell lines	Up/down
miR-101-3p	Reference [1,2,3]	Plasma, Serum, cHL cell lines	Up/down
miR-103a-3p	Reference [4,5]	cHL cell lines	Up
miR-10b-5p	Reference [5]	cHL cell lines	Up
miR-1180-3p	Reference [5]	cHL cell lines	Up
miR-122-5p	Reference [1,3]	Plasma, Whole Blood	Down
miR-125a-5p	Reference [1,7,6]	Plasma, HRS cells, cHL LN-RLN,	Up/down
miR-125b-5p	Reference [1,2]	Plasma, cHL cell lines	Up/down
miR-126-3p	Reference [1,3,5]	Plasma, Serum, cHL cell lines	Up/down
miR-126-5p	Reference [1,5]	Plasma, cHL cell lines	Up/down
miR-151a-3p	Reference [3]	Whole Blood, Serum	Up/down
miR-1301-3p	Reference [3,2,5]	Whole Blood, cHL cell lines	Up
miR-190b	Reference [3]	Whole Blood	Down
miR-130b-3p	Reference [8,4,1,2,5]	cHL LN-HLcl, cHL cell lines	Up
miR-140-3p	Reference [6,7,1]	Plasma, HRS cells, cHL cell lines, cHL LN-RLN	Up/down
miR-142-3p	Reference [4,7]	cHL LN-RLN, cHL cell lines	Up/Down
miR-142-5p	Reference [1,4,2]	Plasma, cHL cell lines	Up/Down
miR-143-3p	Reference [1,3,6,2]	Plasma, Whole Blood, HRS cells, cHL cell lines	Down/Up
miR-144-3p	Reference [1]	Plasma	Down
miR-145-5p	Reference [1,9,7,6]	Plasma, cHL LN-RLN, HRS cells	Up/down
miR-193a-5p	Reference [3]	Serum, Whole Blood	Up/down
miR-148a-3p	Reference [1,2,5]	Plasma, cHL cell lines	Down
miR-150-5p	Reference [3,4,6,2,5]	Serum, HRS cells, cHL cell lines	Up/down
miR-199b-5p	Reference [3]	Whole Blood	Up
miR-155-5p	Reference [9,10,4,6,2,5]	Plasma, HRS cells, cHL LN-RLN, cHL cell lines	Up
miR-15a-5p	Reference [1]	Plasma	Down
miR-15b-3p	Reference [4,7]	cHL LN-RLN, cHL cell lines	Up/down
miR-16-5p	Reference [4,6]	HRS cells, cHL cell lines	Up
miR-17-5p	Reference [4]	cHL cell lines	Up
miR-181a-2-3p	Reference [2,5]	cHL cell lines	Down
miR-181a-3p	Reference [7,2]	cHL LN-RLN, cHL cell lines	Up
miR-320b	Reference [3]	Serum	Up
miR-182-5p	Reference [1,7,6]	Plasma, cHL LN-RLN, cHL cell lines	Up/down
miR-139-3p	Reference [4,10]	cHL cell lines, cHL-RLN	Down
miR-186-5p	Reference [1,6]	Plasma, HRS cells, cHL cell lines	Up
miR-320c	Reference [3]	Serum	Up
miR-320d	Reference [3]	Serum	Up
miR-191-3p	Reference [1]	Plasma	Up
miR-191-5p	Reference [4,2]	cHL cell lines, cHL cell lines	Up
miR-421	Reference [3]	Whole Blood	Up

miR-195-5p	Reference [2,5]	HRS, cHL cell lines	Up
miR-4732-3p	Reference [3]	Whole Blood	down
miR-197-3p	Reference [1]	Plasma	Down
miR-532-3p	Reference [3]	Serum	Up
miR-19a-3p	Reference [4]	cHL cell lines	Up
miR-19b-3p	Reference [4]	cHL cell lines	Up
miR-629-5p	Reference [3]	Serum	Up
miR-205-5p	Reference [3,7]	Serum, cHL cell lines	Up/down
miR-20a-5p	Reference [9,7,4,6]	cHL LN-RLN, cHL cell lines, HRS cells	Up
miR-20b-5p	Reference [4,2]	cHL cell lines	Down/Up
miR-210-3p	Reference [1]	Plasma	Down
miR-21-5p	Reference [8,7,10,4,6,2,5]	HRS, cHL LN-RLN, cHL cell lines	Up
miR-221-3p	Reference [1]	Plasma	Down
miR-222-3p	Reference [1]	Plasma	Down
miR-223-3p	Reference [2,6]	HRS cells, cHL cell lines	Up
miR-874-3p	Reference [3]	Whole Blood	Down
miR-23a-3p	Reference [8,4,1,2,5]	cHL cell lines, cHL LN RLN, cHL cell lines	Up/down
miR-23b-3p	Reference [7,5]	cHL cell lines	Up/down
miR-24-3p	Reference [1,6,2]	Plasma, HRS cells	Up/down
miR-25	Reference [8,1,4,6]	Plasma, cHL LN-RLN, cHL cell lines	Up
miR-26a-5p	Reference [8,7,1]	Plasma, cHL LN-RLN, cHL cell lines	Up/Down
miR-26b-3p	Reference [1,6]	Plasma, cHL cell lines	Up
miR-146b-5p	Reference [5]	cHL cell lines	Up
miR-27a-3p	Reference [3,7,2,5]	cHL cell lines, Serum	Up
miR-27b-3p	Reference [2,5]	cHL cell lines	Up
miR-28-3p	Reference [2,3]	cHL cell lines, Whole Blood	Up
miR-29a-3p	Reference [4,10,2]	cHL-RLN, cHL LN-RLN, cHL cell lines	Up/down
miR-29b-3p	Reference [4]	cHL cell lines	Up
miR-29c-3p	Reference [1,4]	Plasma, cHL cell lines	Up/down
miR-30a-5p	Reference [6,2,5]	HRS, cHL cell lines	Down/Up
miR-30c-5p	Reference [1]	Plasma	Down
miR-30d-5p	Reference [1]	Plasma	Up
miR-30e-5p	Reference [1,2]	Plasma, cHL cell lines	Up
miR-320a	Reference [9,8,2]	cHL LN-RLN, cHL cell lines	Up
miR-196b-5p	Reference [5]	cHL cell lines	Up
miR-339-3p	Reference [5]	cHL cell lines	Down
miR-98-5p	Reference [5]	cHL cell lines	Up
miR-328-3p	Reference [9,10,3]	Whole Blood, cHL LN-RLN	Up/down
miR-335-5p	Reference [3,10,7]	Whole Blood, cHL cell lines	Up/down
miR-130a-3p	Reference [2]	cHL cell lines cells	Down
miR-181a-5p	Reference [2]	cHL cell lines	Up
miR-345-5p	Reference [3,2,5]	cHL cell lines, Serum	Up
miR-34a-5p	Reference [7,5,3]	Whole Blood, cHL LN-RLN, cHL cell lines	Up
miR-363-3p	Reference [6,2]	cHL cell lines	Down
miR-370-3p	Reference [9,7]	cHL LN-RLN	Up
miR-190a-5p	Reference [2]	cHL cell lines	Up
miR-223-5p	Reference [2]	cHL cell lines	Down
miR-340-5p	Reference [2]	cHL cell lines	Down
miR-378a-3p	Reference [2]	cHL cell lines	Up
miR-423-3p	Reference [1]	Plasma	Up
miR-424-5p	Reference [2,1]	cHL cell lines, Plasma	Up/down
miR-760	Reference [2]	cHL cell lines	Up
miR-200c-3p	Reference [6]	HRS cells	Up
miR-486-3p	Reference [1]	Plasma	Up
miR-501-3p	Reference [1,3]	Plasma, Serum	Up/down
miR-503-5p	Reference [3,5]	Whole Blood, cHL cell lines	Up
miR-26b-5p	Reference [6]	cHL cell lines	Up
miR-532-5p	Reference [1,3]	Plasma, Whole Blood	Up/down

miR-542-3p	Reference [3,2,5]	cHL cell lines, Whole Blood	Up
miR-374a-5p	Reference [6]	HRS cells, cHL cell lines	Up
miR-375	Reference [6]	HRS cells	Up
miR-652-3p	Reference [1,6]	Plasma, cHL cell lines	Up/Down
miR-744-5p	Reference [1,2]	cHL cell lines, Plasma	Up
miR-485-3p	Reference [6]	HRS cells	Up
miR-574-3p	Reference [6]	HRS cells	Down
miR-92a-3p	Reference [8,3,4,2,5]	cHL LN-RLN, Whole Blood, cHL cell lines	Up/down
miR-92b-3p	Reference [2,5]	cHL cell lines	Up
miR-93-5p	Reference [4,8,1]	Plasma, cHL LN-RLN, cHL cell lines	Up/down
miR-1294	Reference [3]	Whole Blood	Up/down
miR-99a-5p	Reference [2,1,3]	Plasma, Whole Blood, cHL cell lines	Up/down
miR-99b-5p	Reference [1,5]	Plasma, cHL cell lines	Up/down

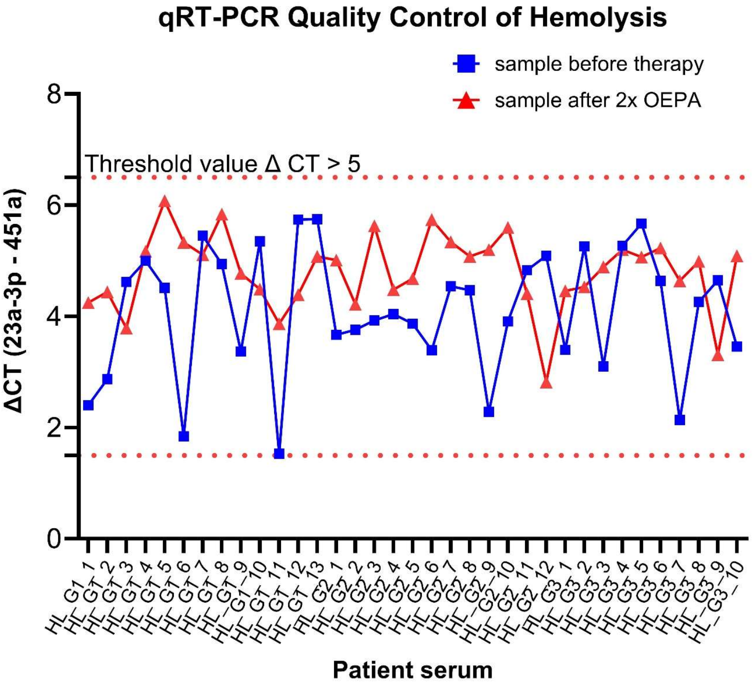
Note: Reference list in the end of supplementary data

Supplemental data figure:

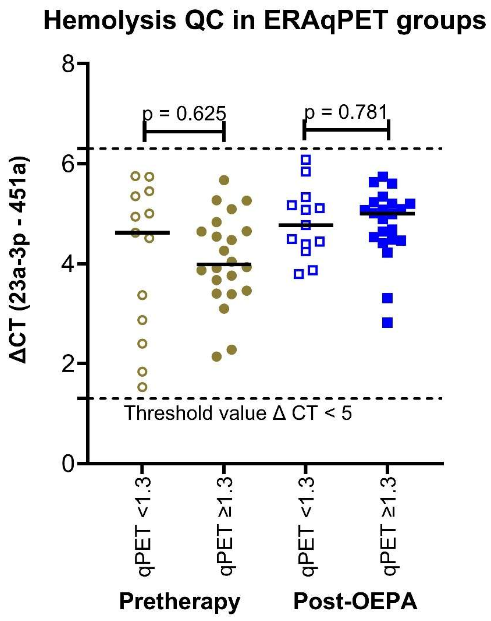
Figure S1: Serum sample quality assessment

The degree of hemolysis was determined using qRT-PCR by quantifying the level of miR-451a relative to the level of miR-23a (hemolysis ratio (ΔCT) = CTmiR-23a – CTmiR-451a). **(A)** Assessment of haemolysis; none of the serum samples showed a ratio above the threshold of Delta (Δ)CT <5. **(B)** Hemolysis quality control in ERAqPET groups; comparison of ΔCT values between ERAqPET subgroups (<1.3 vs. ≥1.3) at Pretherapy (day 0) and Post-OEPA (day 57) showing no significant differences in hemolysis levels between response groups (p = 0.625 and p = 0.781, respectively). **(C)** Endogenous microRNA expression; Radar plots showing homogenous relative expression of endogenous miRNAs between the samples measured before sequencing

A.



B.


Hemolysis QC in ERAqPET groups
ΔCT (23a-3p - 451a)
8
6
4
2
0
p = 0.625
p = 0.781
Threshold value Δ CT < 5
qPET <1.3
qPET ≥1.3
qPET <1.3
qPET ≥1.3
Pretherapy
Post-OEPA

C.

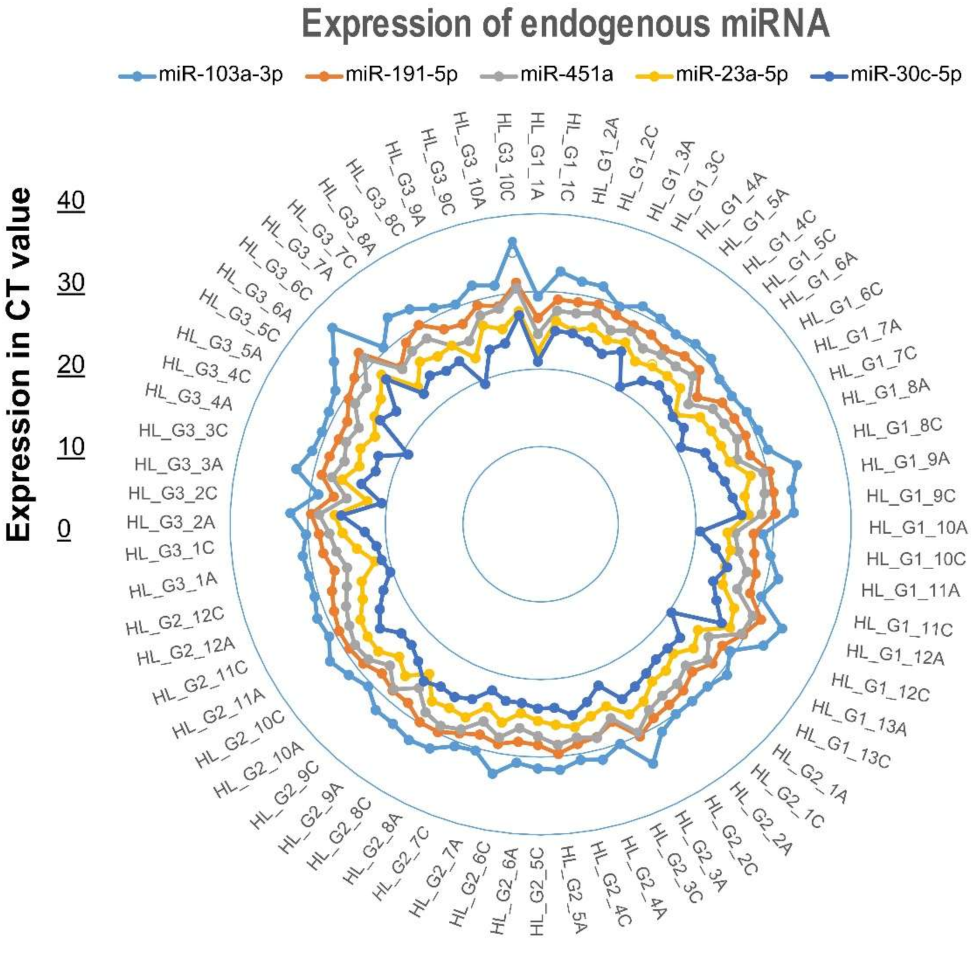
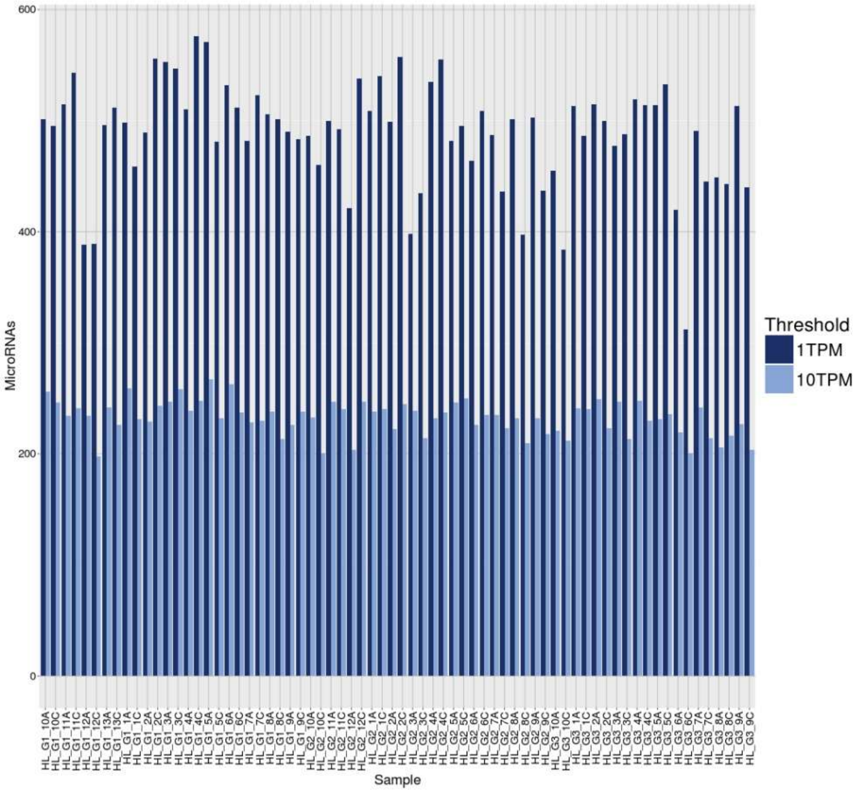

Expression of endogenous miRNA
miR-103a-3p
miR-191-5p
miR-451a
miR-23a-5p
miR-30c-5p
Expression in CT value
40
30
20
10
0
HL_G1_1A
HL_G1_1C
HL_G1_2A
HL_G1_2C
HL_G1_3A
HL_G1_3C
HL_G1_4A
HL_G1_5A
HL_G1_4C
HL_G1_5C
HL_G1_6A
HL_G1_6C
HL_G1_7A
HL_G1_7C
HL_G1_8A
HL_G1_8C
HL_G1_9A
HL_G1_9C
HL_G1_10A
HL_G1_10C
HL_G1_11A
HL_G1_11C
HL_G1_12A
HL_G1_12C
HL_G1_13A
HL_G1_13C
HL_G2_1A
HL_G2_1C
HL_G2_2A
HL_G2_2C
HL_G2_3A
HL_G2_3C
HL_G2_4A
HL_G2_4C
HL_G2_5A
HL_G2_5C
HL_G2_6A
HL_G2_6C
HL_G2_7A
HL_G2_7C
HL_G2_8A
HL_G2_8C
HL_G2_9A
HL_G2_9C
HL_G2_10A
HL_G2_10C
HL_G2_11A
HL_G2_11C
HL_G2_12A
HL_G2_12C
HL_G3_1A
HL_G3_1C
HL_G3_2A
HL_G3_2C
HL_G3_3A
HL_G3_3C
HL_G3_4A
HL_G3_4C
HL_G3_5A
HL_G3_5C
HL_G3_6A
HL_G3_6C
HL_G3_7A
HL_G3_7C
HL_G3_8A
HL_G3_8C
HL_G3_9A
HL_G3_9C
HL_G3_10A
HL_G3_10C

Figure S2:

Small RNA sequencing of serum miRNA expression profiles in HL patient and principal component analysis of sample variability.

(A) Number of identified known miRNAs; The mapped reads sequenced for each sample were annotated using Genome databases. Bar plot shows almost consistent number of known miRNAs detected at >1 TPM; average 488 miRNAs (dark blue) and >10 TPM; 232 miRNAs (light blue) thresholds across all samples in the discovery cohort (n=35). **(B)** Principal Component Analsis of serum-miRNA expression profiles; with a projection of the first two principal components (PC) of all serum samples within this cohort. The Quadrat represents the centre and the 95% confidence interval of the samples in all each group. Three samples out of quadrat were considered outliers (based on PC scores) and were excluded from further analysis.

A.



B.

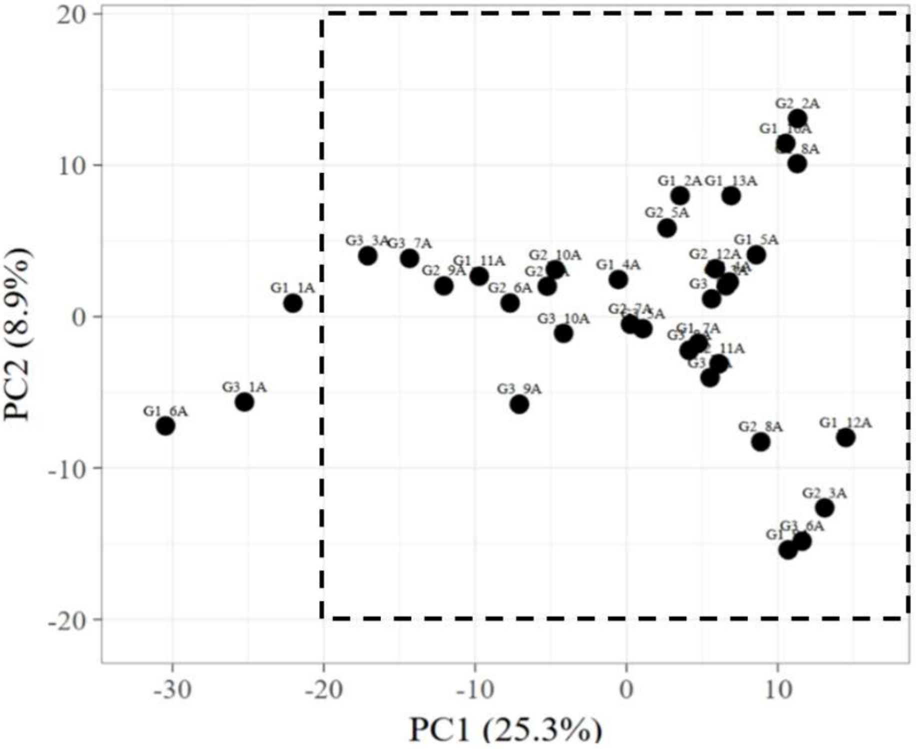

PC2 (8.9%)
PC1 (25.3%)
20
10
0
-10
-20
-30
-20
-10
0
10
G2 2A
G1 10A
8A
G1 2A
G1 13A
G2 5A
G3 3A
G3 7A
G1 5A
G2 12A
G2 10A
G1 11A
G2 9A
G2
G1 4A
4A
3A
G3
G1 1A
G2 6A
G3 10A
G2 7A
5A
G1 7A
G3
11A
G3
G3 1A
G3 9A
G1 6A
G2 8A
G1 12A
G2 3A
G3 6A
G1

Figure S3:

Extended PCA of differentially expressed miRNAs in Induction therapy responder patients. To further explore the variance within the dataset and investigate the central overlap observed in the primary PCA (PC1 vs. PC2), additional principal components were analyzed. PCA score plots illustrate the relationship between PC3 vs. PC4 and PC5 vs. PC6. Patient samples are categorized into ERAqPET negative <1.3 (red dots) and ERAqPET positive ≥1.3 (blue dots) groups.

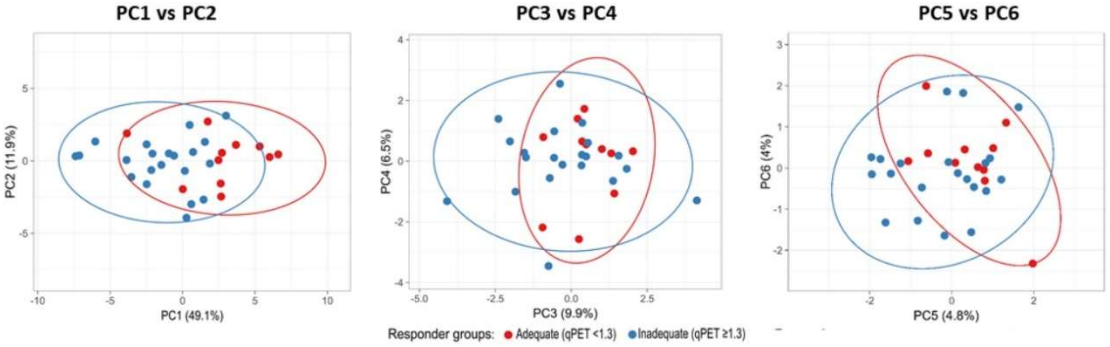


Figure S4.

Expression of miR-148a-3p post-induction-therapy and comparison of change in miR-148a-3p expression (Δ) after induction-therapy

(A) Post-induction miRNA expression at diagnosis (Day 57). Boxplots display normalized read counts ($\log_2$-TMM values) for miR-148a-3p. **(B)** Comparison of changes in miRNA expression (Δd0–d57). Boxplots display changes in expression levels between Day 0 and Day 57 for candidate miRNAs on the y-axis. Boxes represent interquartile ranges (Tukey method); horizontal lines indicate medians, and whiskers show data ranges. Significant differential expression between groups is indicated ($p < 0.05$).

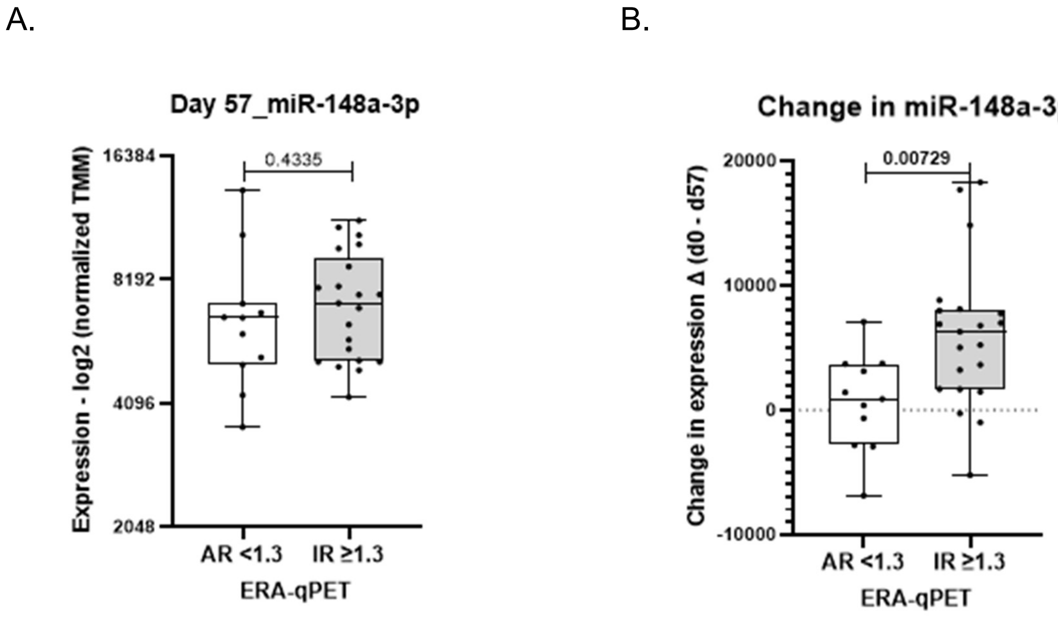
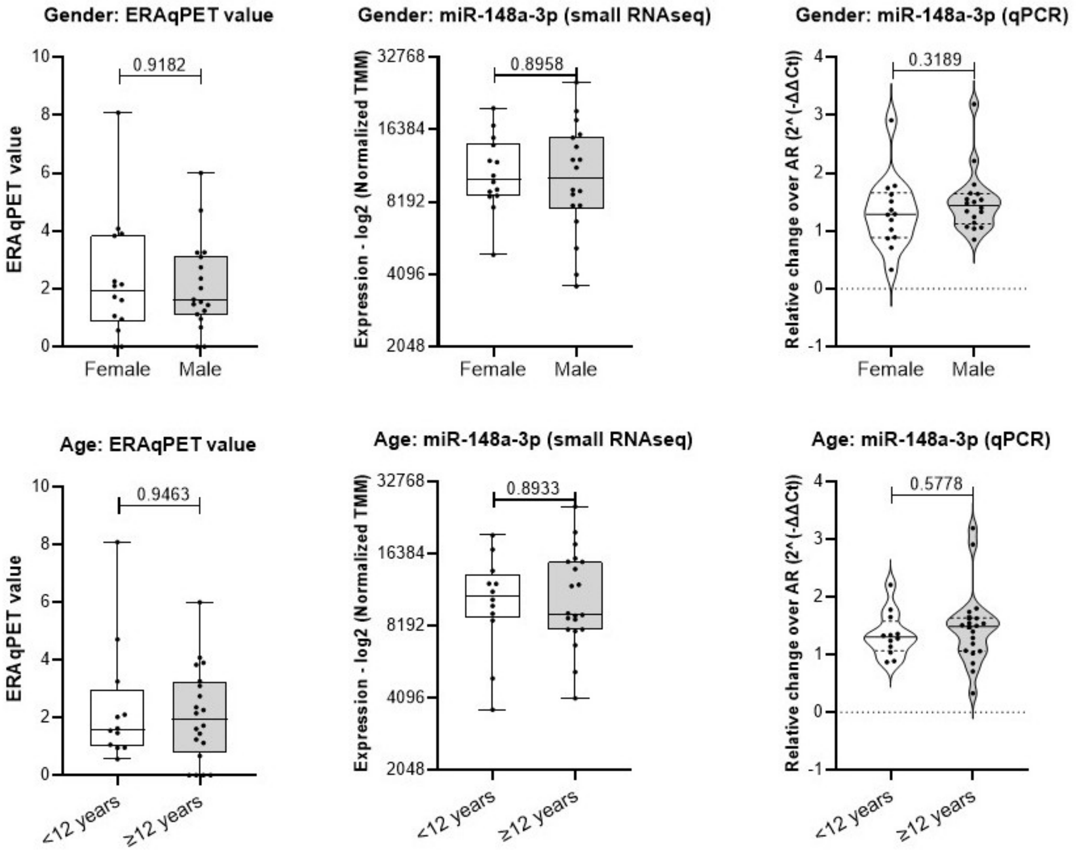


Figure S5.

Comparative analysis of early response qPET and baseline miR-148a-3p expression across Sex and Age subgroups. Early response ERAqPET values (first column) and baseline miR-148a-3p expression measured by small RNA-seq (second column) and qRT-PCR (third column) are shown for sex (top row: Female vs Male) and age (bottom row: <12 years vs ≥12 years) subgroups. miR-148a-3p qRT-PCR values are expressed as relative change over the ERAqPET-negative (AR) group mean using 2^(-ΔΔCt) values. Small RNA-seq values are shown as log2 expression normalized by TMM. Box and Violin plots display the distribution of values; boxes and violins represent the interquartile range (Tukey method), solid horizontal lines indicate medians, and whiskers show the full data range (whiskers are dashed when drawn on violins). Significant differences between groups ($p < 0.05$) are indicated.



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