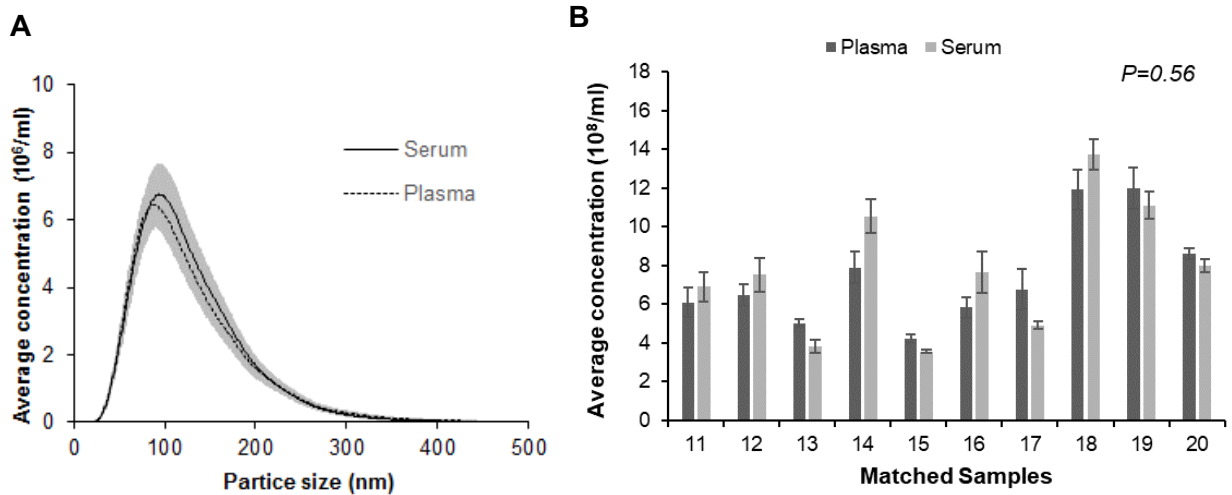
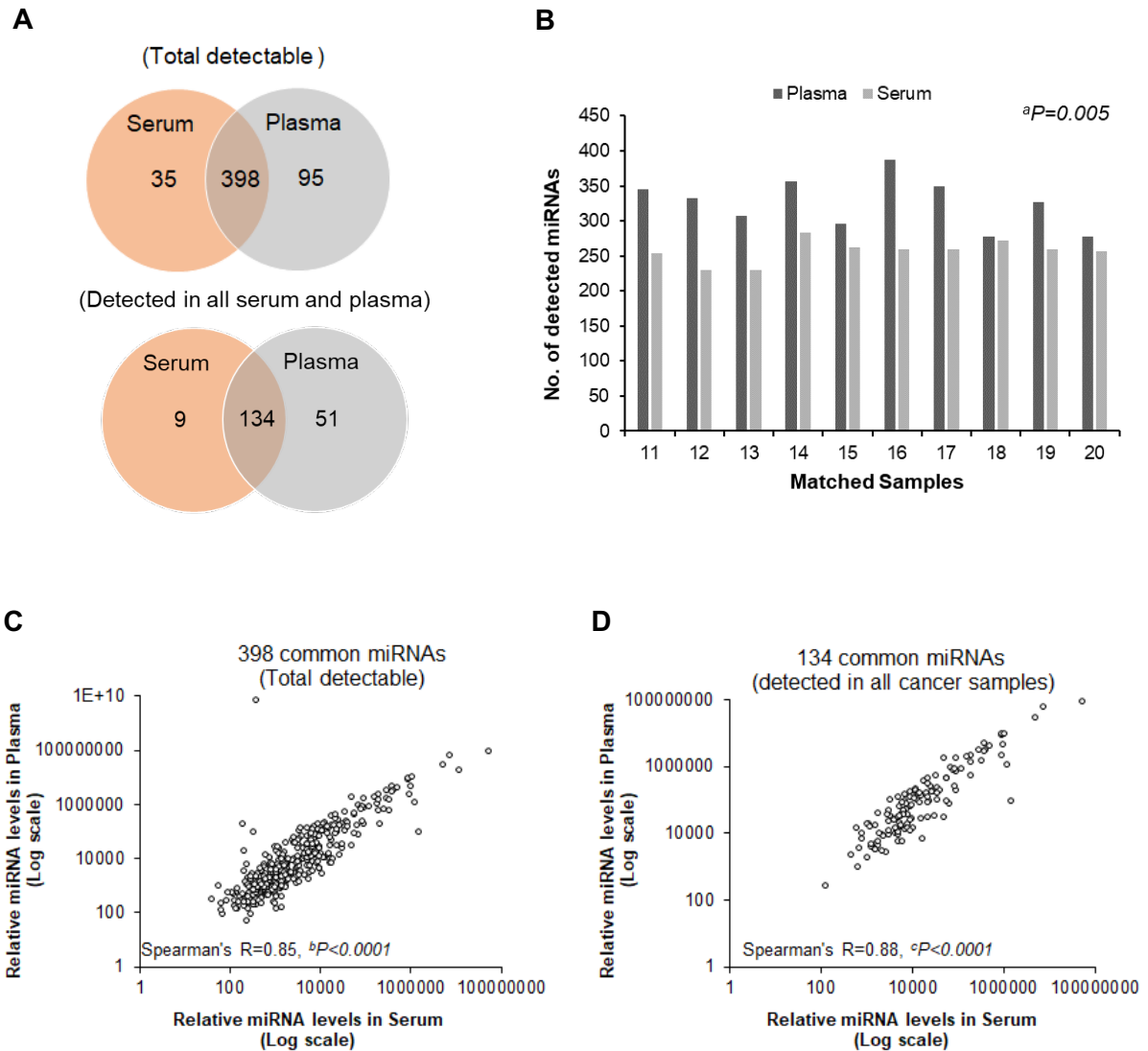


Supplemental data



Supplemental Figure 1. NanoSight measurements of the isolated sEVs from the matched serum and plasma samples from esophageal adenocarcinoma patients (n=10). A: The overall size distribution of particles (SEM indicated by shaded areas) was similar between the matched serum (n = 10) and plasma samples (n = 10). **B:** Pairwise comparison of the average concentration (\pm SEM) of particles was also similar between serum and plasma (Wilcoxon signed-rank test, $P = 0.56$).



Supplemental Figure 2. Comparison of the miRNA content between serum and plasma sEV preparations from esophageal adenocarcinoma patients (n=10). **A:** The number of total detectable miRNAs (top Venn diagram) and miRNAs detected in all serum or plasma samples (bottom Venn diagram) were higher in plasma. **B:** Pairwise comparison of the number of total detectable miRNAs was significantly higher in the plasma (Wilcoxon signed-rank test, ^a*P* = 0.005). **C, D:** The average relative expression of the common total detectable miRNAs (Spearman's *R* = 0.85, ^b*P* < 0.0001) and the common miRNAs detected in all serum or plasma samples (Spearman's *R* = 0.88, ^c*P* < 0.0001) were significantly correlated.

Supplementary Table 1. Unique miRNAs expressed in at least 5 out of 10 plasma sEV preparations from healthy individuals (n = 10).

Unique	plasma	No. ¹	Relative levels \pm SD
hsa-miR-23a-3p ²		10	16438.7 \pm 7154.9
hsa-miR-141-3p ²		6	2774.9 \pm 933.0
hsa-miR-154-5p		5	1896.1 \pm 858.3
hsa-miR-219a-5p		5	596.1 \pm 155.6
hsa-miR-98-5p ³		10	3654.6 \pm 1614.0
hsa-miR-424-5p ²		8	1422.3 \pm 1052.0
hsa-miR-656-3p		8	944.3 \pm 462.2
hsa-miR-654-5p		9	3883.5 \pm 1881.4
hsa-miR-655-3p		5	864.4 \pm 947.6
hsa-miR-431-5p		7	3770.6 \pm 1521.1
hsa-miR-32-5p		6	351.9 \pm 149.1
hsa-let-7g-3p		5	96.8 \pm 13.6
hsa-miR-342-5p		8	1097.2 \pm 183.8
hsa-miR-337-3p		5	362.7 \pm 280.0
hsa-miR-654-3p		7	195.8 \pm 159.4
hsa-miR-200b-3p		8	1166.5 \pm 253.4
hsa-miR-30d-3p ³		9	1759.1 \pm 986.8
hsa-miR-181c-3p		6	2091.3 \pm 868.4
hsa-miR-489-3p		5	759.7 \pm 403.7
hsa-miR-146b-3p ³		7	364.1 \pm 148.3
hsa-miR-19b-1-5p ³		6	360.9 \pm 303.3
hsa-miR-1271-5p		5	331.8 \pm 150.1
¹ Number of plasma samples with detectable miRNA expression.			
² Protein-associated miRNAs reported by Arroyo 2011.			

Supplementary Table 2. Summary of the number of unique sEV, cell-free and whole blood miRNAs reported by Cheng 2014 assessed in this study.

	Number of miRNAs detected in serum			Number of miRNAs detected in plasma		
	sEV	Cell-free	Whole blood	sEV	Cell-free	Whole blood
Total assessable ¹	22	4	8	12	10	2
In 5 or more samples (healthy individuals)	6	0	1	11	5	0
In 5 or more samples (Cancer)	7	0	1	9	6	0

¹ List of miRNAs reported by Cheng 2014 that were available on the Taqman OpenArray.
Healthy individuals, n=10; esophageal adenocarcinoma patients, n=10

Supplementary Table 3. List of vesicle-associated miRNAs and protein-associated miRNAs in serum (reported Arroyo 2011) that were assessed in our study.

Vesicle-	No.	Average Ct ±	Protein-	No.	Average Ct ±
hsa-miR-30c-5p	9	21.8 ± 0.9	hsa-miR-26b-5p	10	24.8 ± 1.3
hsa-miR-30b-5p ²	10	22.7 ± 1.4	hsa-miR-222-3p	10	25.8 ± 1.2
hsa-miR-197-3p	10	24.1 ± 0.8	hsa-miR-215-5p	10	27.6 ± 1.1
hsa-miR-142-3p	10	24.5 ± 1.9	hsa-miR-29a-3p	10	27.8 ± 1.8
hsa-let-7a-5p	6	27.4 ± 0.4	hsa-miR-194-5p	10	28.9 ± 1.5
hsa-miR-574-3p	10	28.1 ± 0.9	hsa-let-7g-5p	10	29.0 ± 0.8
hsa-miR-590-5p	10	28.9 ± 1.7	hsa-miR-101-3p	9	29.5 ± 1.6
hsa-miR-542-5p	0	NA ³	hsa-miR-140-3p	10	30.3 ± 1.3
			hsa-miR-29b-3p	5	30.7 ± 1.2
			hsa-let-7d-3p	1	31.1 ± NA ³
			hsa-miR-338-3p	2	33.1 ± 0.3
			hsa-miR-596	0	NA ³
			hsa-let-7g-5p	10	29.0 ± 0.8

¹ Number of serum samples with detectable miRNA expression.

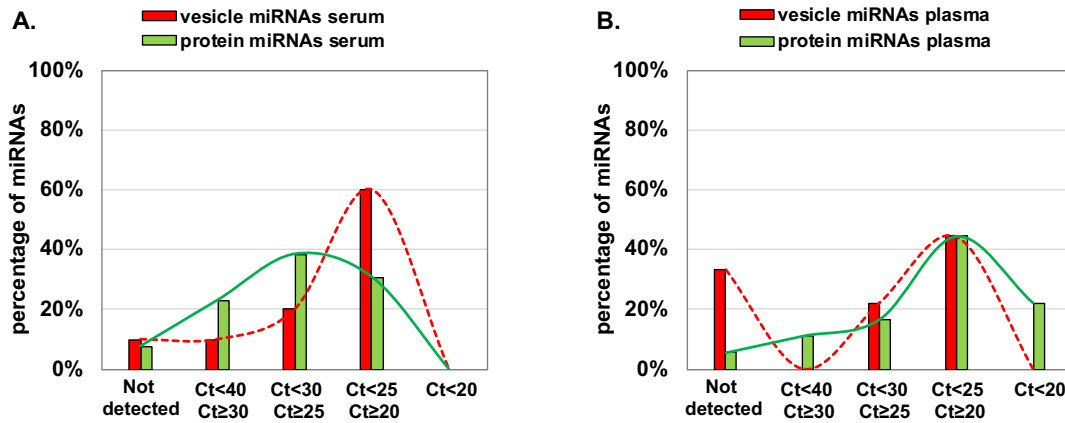
² Top 20 abundant miRNAs in the serum samples.

Supplementary Table 4. List of vesicle-associated miRNAs and protein-associated miRNAs in plasma (reported by Arroyo 2011) that were assessed in our study.

Vesicle-	No.	Average Ct ±	Protein-	No.	Average Ct ±
hsa-miR-142-3p	9	20.7 ± 1.0	hsa-miR-30a-5p ²	10	17.9 ± 1.0
hsa-miR-193a-5p	10	24.3 ± 1.4	hsa-miR-328-3p ²	10	19.2 ± 0.4
hsa-miR-146b-5p	10	24.5 ± 0.7	hsa-miR-146a-5p	10	19.9 ± 1.7
hsa-miR-744-5p	10	27.7 ± 1.2	hsa-miR-210-3p	10	23.2 ± 1.2
hsa-miR-668-3p	3	28.2 ± 0.3	hsa-miR-376a-3p	10	24.0 ± 1.2
hsa-miR-886-3p	10	28.9 ± 0.8	hsa-let-7d-5p	10	24.7 ± 0.8
hsa-miR-629-5p	10	29.7 ± 1.1	hsa-miR-425-5p	10	24.9 ± 0.5
hsa-miR-491-3p	1	30.0 ± NA ³	hsa-miR-122-5p	10	25.7 ± 1.7
hsa-miR-501-5p	0	NA ³	hsa-miR-20b-5p	10	25.8 ± 1.3
			hsa-miR-100-5p	10	26.8 ± 1.6
			hsa-miR-140-3p	10	27.9 ± 1.0
			hsa-miR-141-3p	6	28.7 ± 0.6
			hsa-miR-598-3p	10	29.3 ± 1.2
			hsa-miR-378a-5p	7	29.5 ± 1.0
			hsa-miR-622	0	NA ³

¹ Number of plasma samples with detectable miRNA expression.

² Top 20 abundant miRNAs in the plasma samples.



Supplemental Figure 3. Percentage of vesicle-associated (red) and protein-associated (green) miRNAs expressed at levels within the indicated Ct range in sEV preparations from esophageal adenocarcinoma patients. A. serum, B. plasma. The list of vesicle-associated and protein-associated miRNAs assessed in the serum and plasma were derived from Arroyo 2011. The bar graphs represent the percentage of miRNAs within each Ct range out of the total vesicle-associated miRNAs or protein-associated miRNAs assessed respectively in each sample type. Smoothed lines were added to aid visualisation of the trends.