

Article

Patients with cholangiocarcinoma present specific RNA profiles in serum and urine extracellular vesicles mirroring the tumor expression: novel liquid biopsy biomarkers for disease diagnosis

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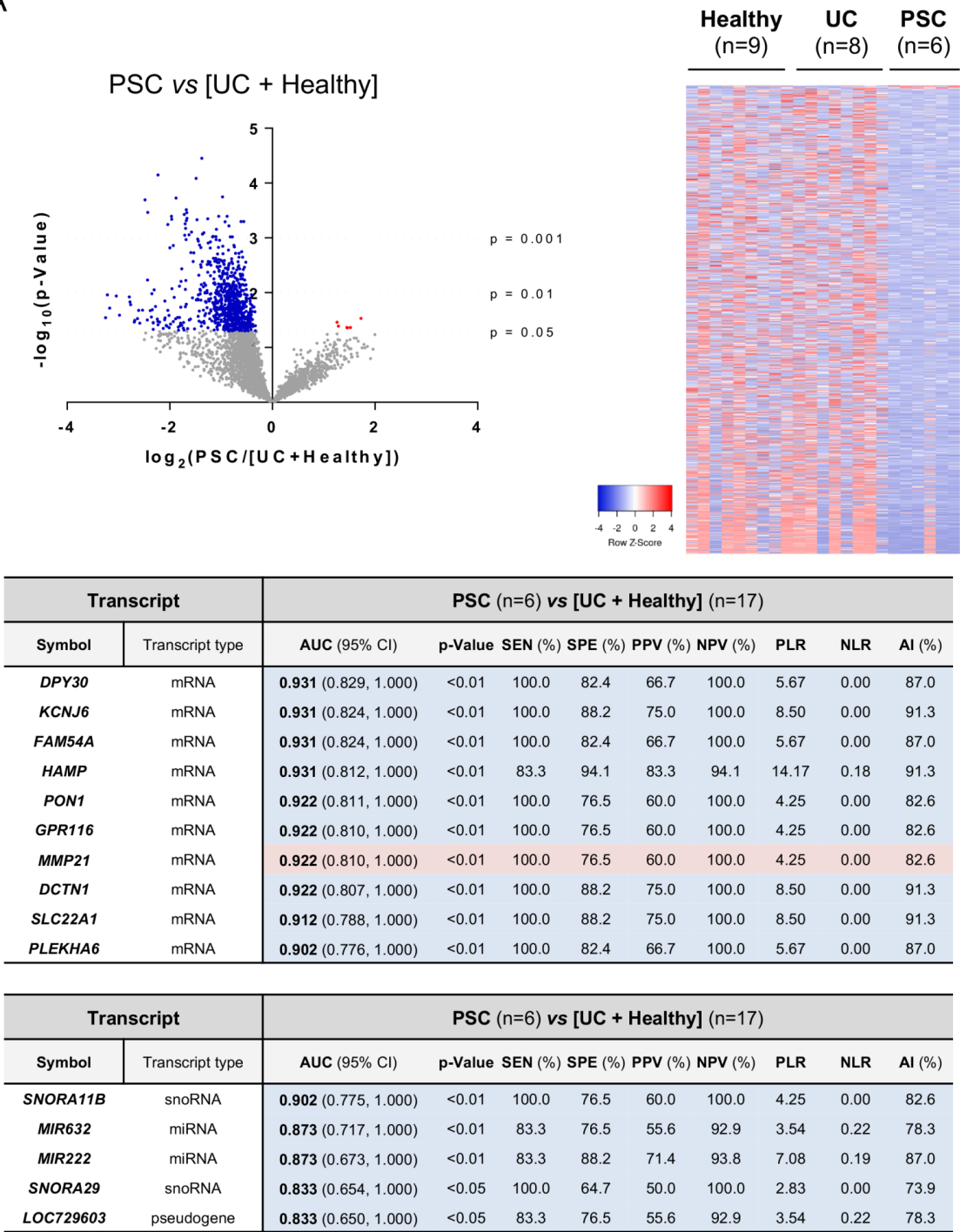
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Supplementary information

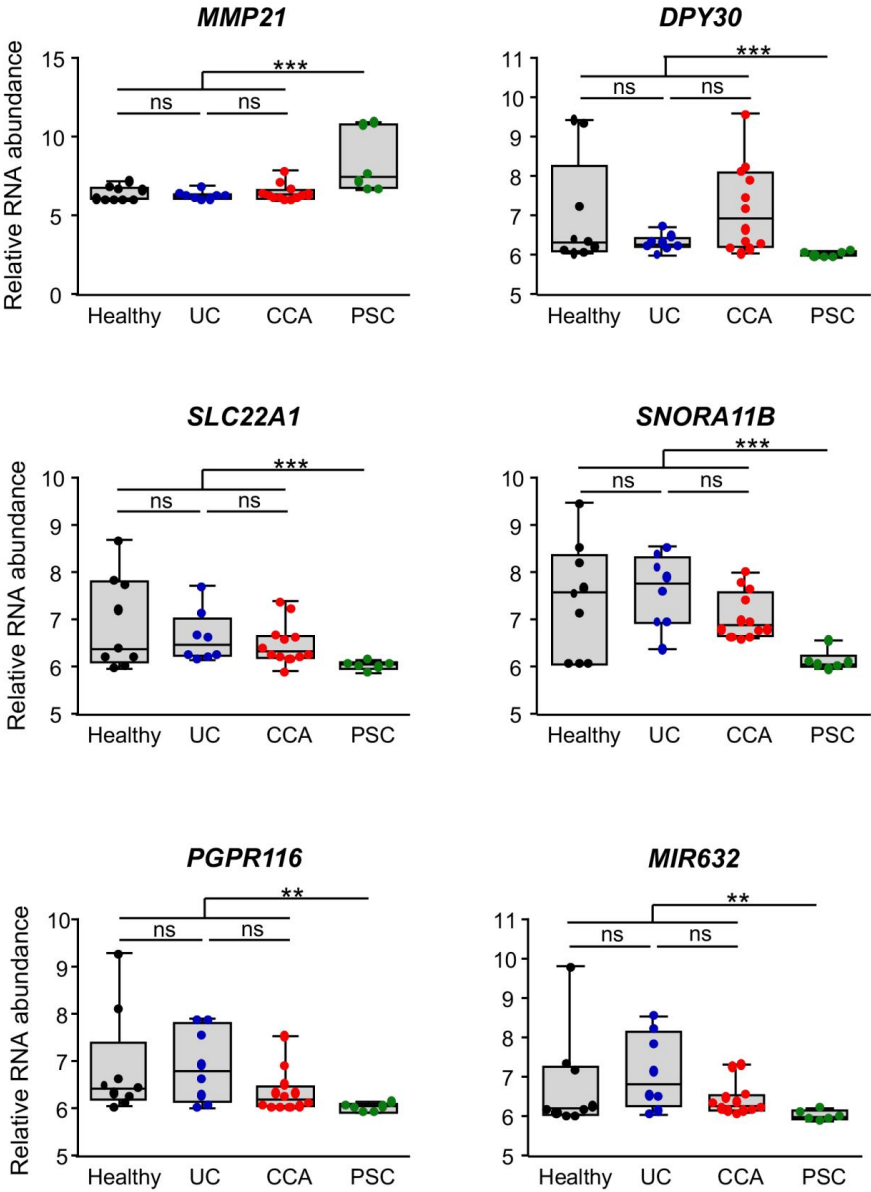
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Supplementary Figure 1 – Differential transcriptomic profile and diagnostic efficacy of serum EV transcripts. A) Volcano plot [$-\log_{10}(\text{p-value})$ and $\log_2(\text{fold-change})$; up left], heatmap of the differentially expressed transcripts (up right) and diagrams with the diagnostic capacity with the highest AUC values of the 10 selected mRNAs and 5 selected non-coding RNAs in serum EVs from PSC vs [UC + Healthy individuals]. Abbreviations: AI, accuracy index; AUC, area under the receiver operating characteristic curve; CI, confidence interval; lncRNA, long non-coding RNA; miRNA, microRNA; NLR, negative likelihood ratio; NPV, negative predictive value; PLR, positive likelihood ratio; PPV, positive predictive value; SEN, sensitivity; snoRNA, small nucleolar RNA; SPE, specificity;

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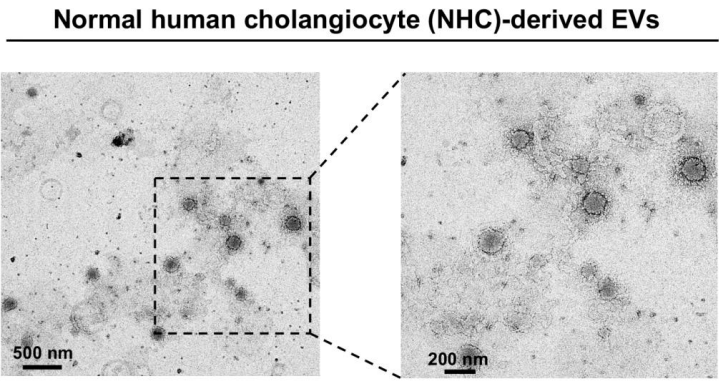
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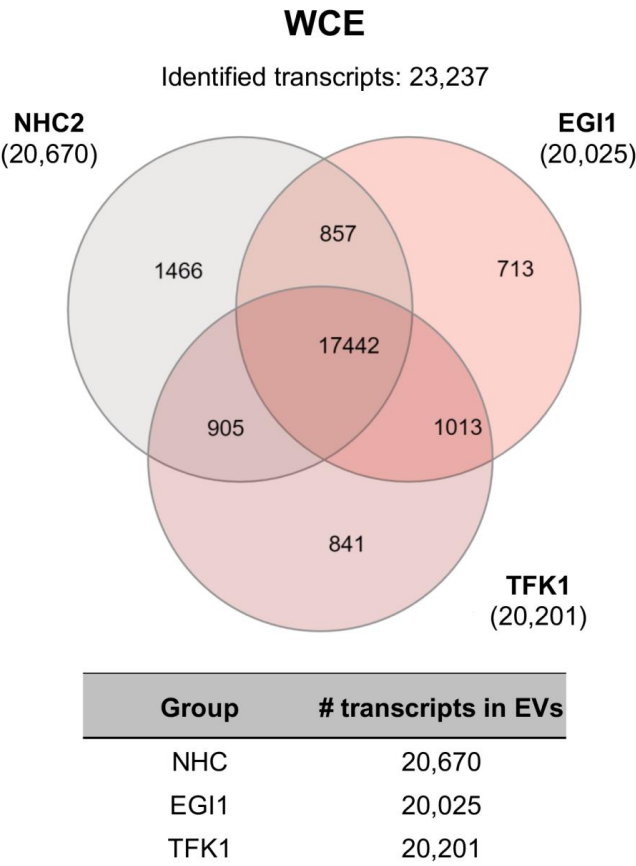
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Supplementary Figure 1 (continued) – Differential transcriptomic profile and diagnostic efficacy of serum EV transcripts. B) Potential serum EV biomarkers for PSC.

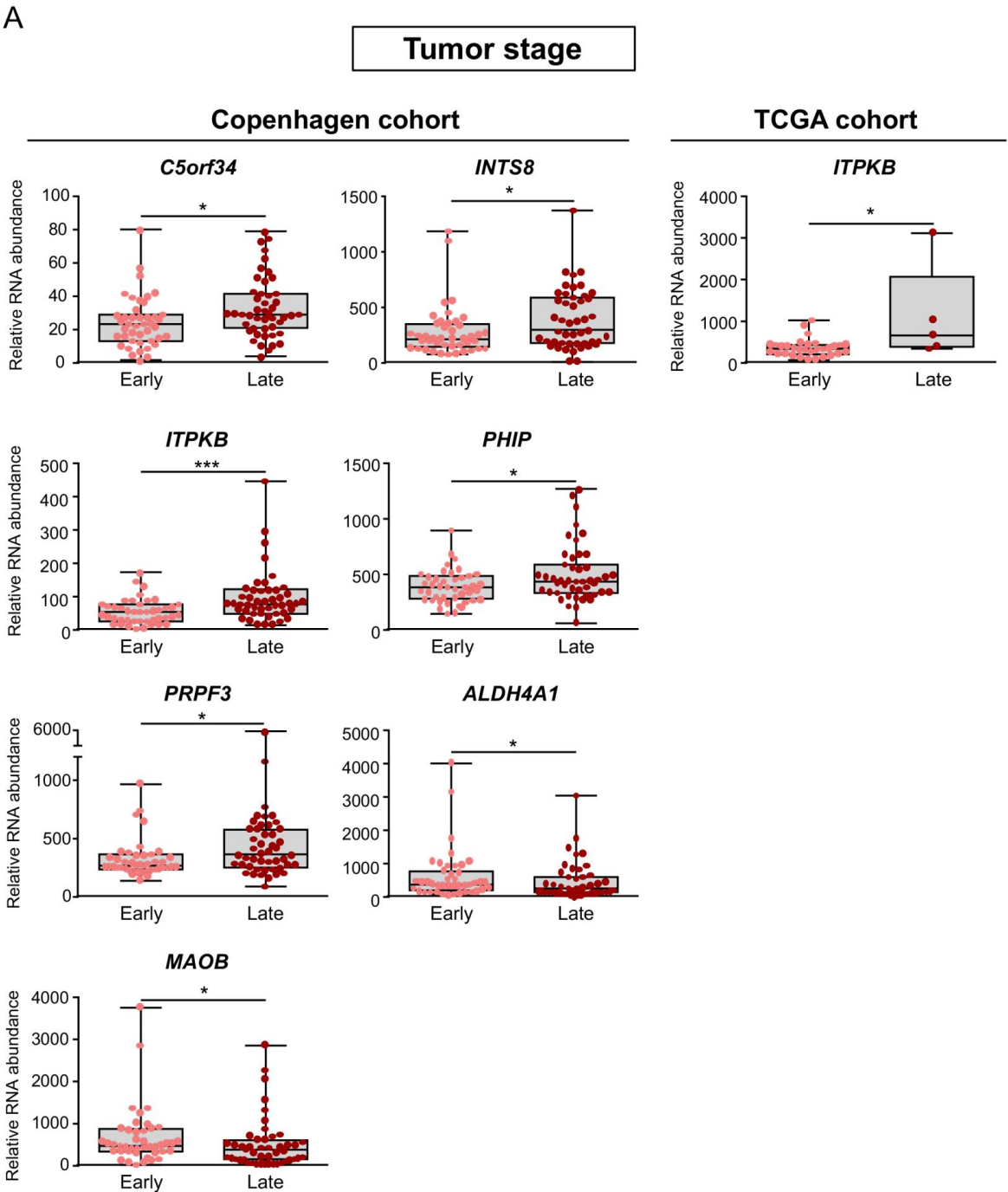
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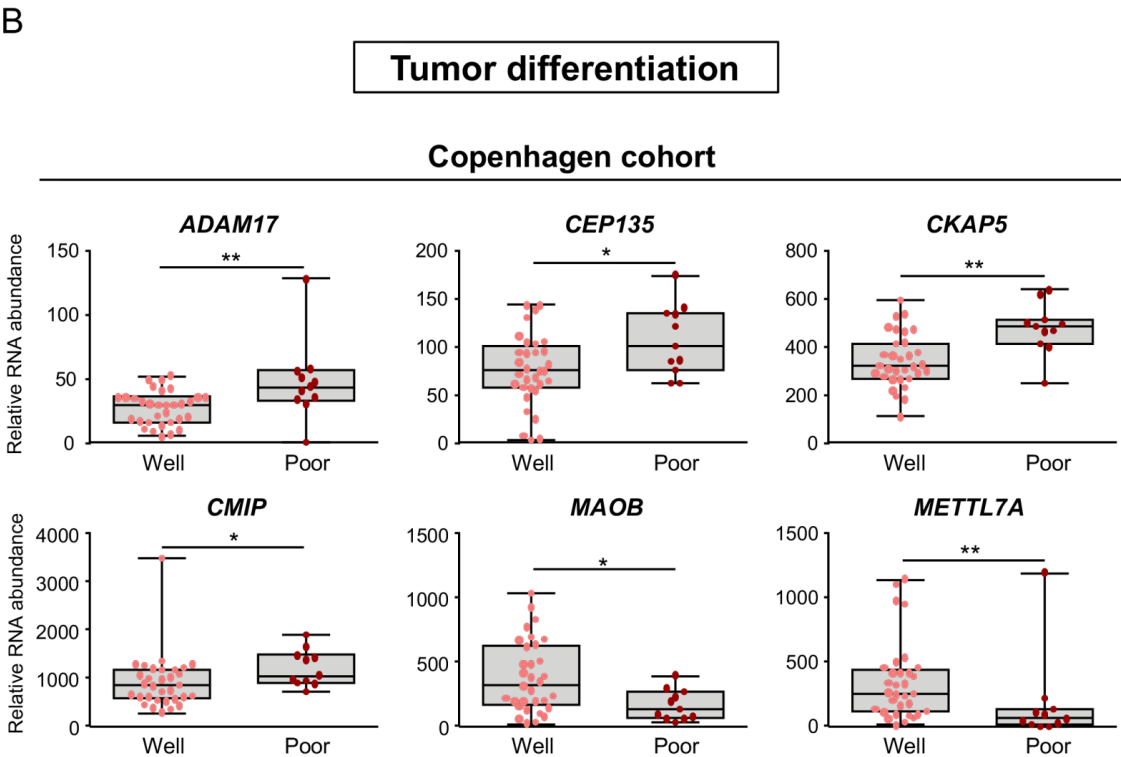
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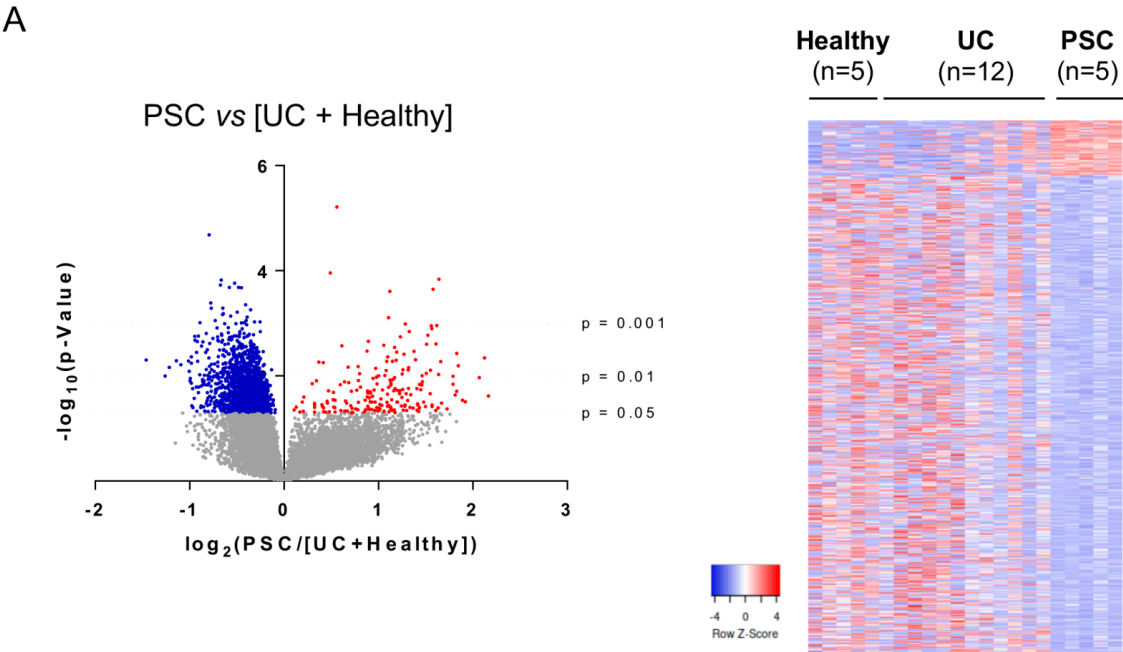
Supplementary Figure 2 – Characterization of cholangiocyte-derived EVs transcriptome. In order to validate the protocol for EVs isolation from cholangiocytes, we used normal human cholangiocytes (NHCs). **A)** TEM images of NHC-derived EVs showcasing the typical round shape (~150 nm) and morphology. **B)** Venn diagrams showing the number of transcripts altered for each comparison and the total transcripts identified in each group.



Supplementary Figure 3 – Comparative analysis of the levels of candidate serum liquid biopsy transcripts with CCA disease severity in the Copenhagen and TCGA cohorts. A) Tumour stage.
Abbreviations: Early, T1/2 tumor stage; Late, T3/4 tumor stage.



Supplementary Figure 3 (continued) – Comparative analysis of the levels of candidate serum liquid biopsy transcripts with CCA disease severity in the Copenhagen and TCGA cohorts. B) Tumour differentiation. Abbreviations: Well, well + well to moderate + moderate tumor differentiation; Poor, moderate to poor + poor differentiation.

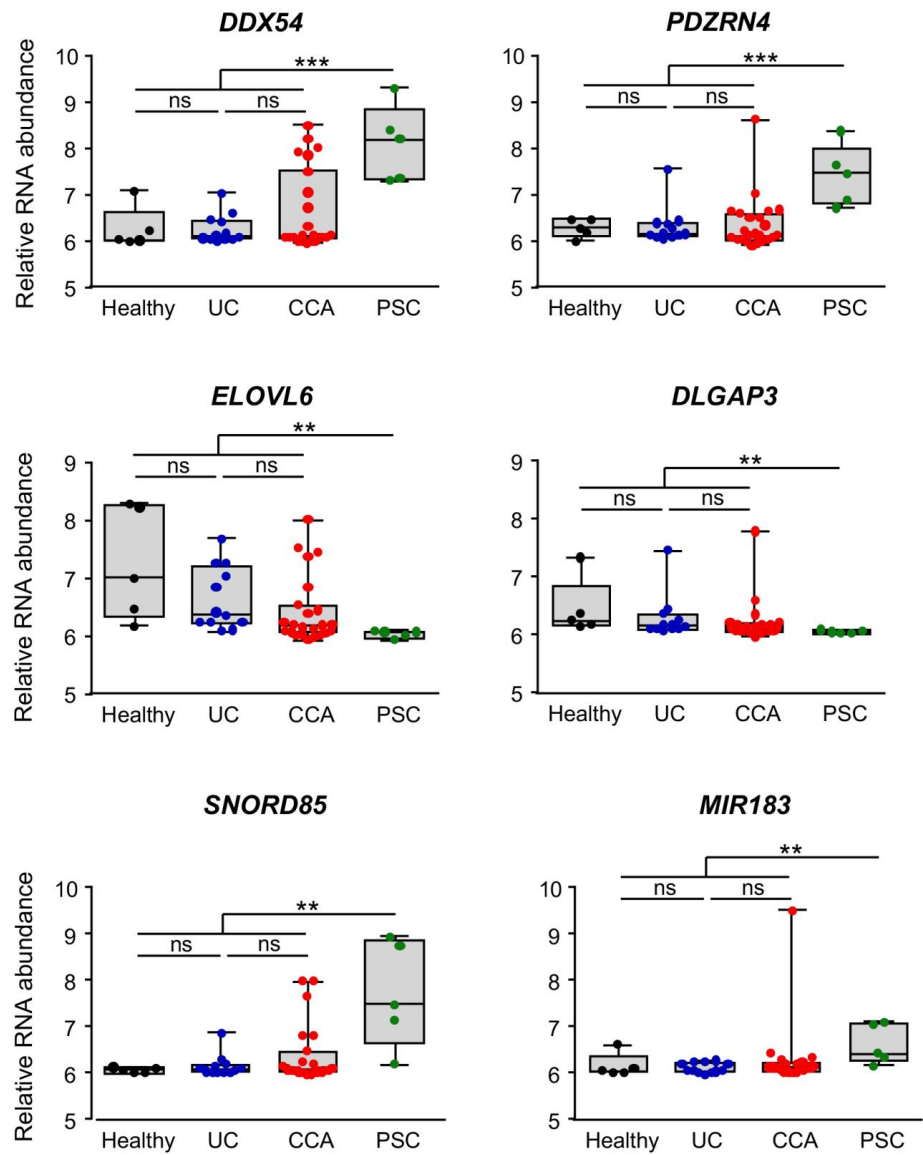


Transcript		PSC (n=5) vs [UC + Healthy] (n=17)								
Symbol	Transcript type	AUC (95% CI)	p-Value	SEN (%)	SPE (%)	PPV (%)	NPV (%)	PLR	NLR	AI (%)
ZSCAN2	mRNA	1.000 (1.000, 1.000)	<0.001	100.0	100.0	100.0	100.0	>100	0.00	100.0
LRRC8D	mRNA	1.000 (1.000, 1.000)	<0.001	100.0	100.0	100.0	100.0	>100	0.00	100.0
DDX54	mRNA	1.000 (1.000, 1.000)	<0.001	100.0	100.0	100.0	100.0	>100	0.00	100.0
DLGAP3	mRNA	0.988 (0.952, 1.000)	<0.001	100.0	94.1	83.3	100.0	17.00	0.00	95.5
ELOVL6	mRNA	0.988 (0.952, 1.000)	<0.001	100.0	94.1	83.3	100.0	17.00	0.00	95.5
KIAA0195	mRNA	0.988 (0.952, 1.000)	<0.001	100.0	94.1	83.3	100.0	17.00	0.00	95.5
SEMA3F	mRNA	0.976 (0.920, 1.000)	<0.001	100.0	94.1	83.3	100.0	17.00	0.00	95.5
BCL2L1	mRNA	0.976 (0.918, 1.000)	<0.001	100.0	88.2	71.4	100.0	8.50	0.00	90.9
PDZRN4	mRNA	0.965 (0.889, 1.000)	<0.01	100.0	94.1	83.3	100.0	17.00	0.00	95.5
FOXP1	mRNA	0.965 (0.889, 1.000)	<0.01	100.0	94.1	83.3	100.0	17.00	0.00	95.5

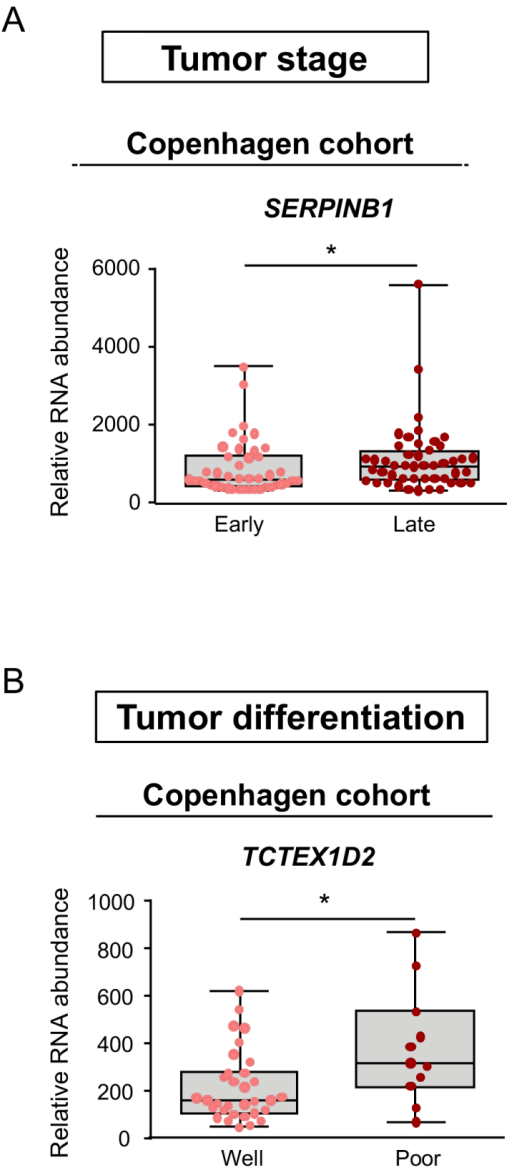
Transcript		PSC (n=5) vs [UC + Healthy] (n=17)								
Symbol	Transcript type	AUC (95% CI)	p-Value	SEN (%)	SPE (%)	PPV (%)	NPV (%)	PLR	NLR	AI (%)
SNORD85	snoRNA	0.965 (0.886, 1.000)	<0.01	100.0	82.4	62.5	100.0	5.67	0.00	86.4
LOC286135	lincRNA	0.941 (0.843, 1.000)	<0.01	100.0	82.4	62.5	100.0	5.67	0.00	86.4
MIR34B	miRNA	0.906 (0.780, 1.000)	<0.01	100.0	82.4	62.5	100.0	5.67	0.00	86.4
LOH12CR2	lincRNA	0.906 (0.779, 1.000)	<0.01	100.0	82.4	62.5	100.0	5.67	0.00	86.4
MIR183	miRNA	0.906 (0.762, 1.000)	<0.01	80.0	94.1	80.0	94.1	13.60	0.21	90.9

Supplementary Figure 4 – Differential transcriptomic profile and diagnostic efficacy of urine EV transcripts. A) Volcano plot [$-\log_{10}(\text{p-value})$ and $\log_2(\text{fold-change})$; up left], heatmap of the differentially expressed transcripts (up right) and diagrams with the diagnostic capacity with the highest AUC values of the 10 selected mRNAs and 5 selected non-coding RNAs in serum EVs from PSC vs [UC + Healthy individuals]. Abbreviations: AI, accuracy index; AUC, area under the receiver operating characteristic curve; CI, confidence interval; lincRNA, long non-coding RNA; miRNA, microRNA; NLR, negative likelihood ratio; NPV, negative predictive value; PLR, positive likelihood ratio; PPV, positive predictive value; SEN, sensitivity; snoRNA, small nucleolar RNA; SPE, specificity;

B



Supplementary Figure 4 (continued) – Differential transcriptomic profile and diagnostic efficacy of urine EV transcripts. B) Potential serum EV biomarkers for PSC.



Supplementary Figure 5 – Comparative analysis of the levels of candidate urine liquid biopsy transcripts with CCA disease severity in the Copenhagen and TCGA cohorts. A) Tumour stage; B) Tumour differentiation. Abbreviations: Early, T1/2 tumor stage; Late, T3/4 tumor stage; Well, well + well to moderate + moderate tumor differentiation; Poor, moderate to poor + poor differentiation.

117 **Supplementary Table 1: Clinical information from study individuals**

		Serum				Urine			
		Healthy	UC	PSC	CCA	Healthy	UC	PSC	CCA
Patients (n)		9	8	6	12	5	12	5	23
Age, mean [range] (years)		41.4 [26-72]	54.3 [33-82]	45.5 [16-75]	67.4 [59-79]	66.2 [53-72]	58.8 [42-82]	49.6 [26-75]	67.8 [50-82]
Gender, M / F (n/n)		4 / 5	5 / 3	6	9 / 3	3 / 2	6 / 6	4 / 1	16 / 7
ALT, mean [range] (IU/L)		NA	16.50 [9-29]	44.33 [12-128]	56.67 [11-200]	NA	15.83 [9-30]	36.60 [18-62]	115.96 [11-672]
AST, mean [range] (IU/L)		NA	16.25 [10-20]	36.83 [16-83]	46.09 [19-104]	NA	17.67 [10-28]	35.80 [20-56]	85.00 [19-379]
GGT, mean [range] (IU/L)		NA	24.88 [13-38]	122.33 [17-361]	750.33 [87-3011]	NA	22.25 [14-38]	119.40 [35-241]	781.87 [87-3011]
Bilirubin, mean [range] (mg/dL)		0.85 [0.4-1.3]	0.68 [0.3-1.1]	1.10 [0.5-2.4]	2.65 [0.3-16.7]	0.68 [0.4-1.3]	0.63 [0.3-1.1]	1.00 [0.3-2.4]	6.00 [0.3-31.3]
ALP, mean [range] (mg/dL)		57.50 [52-63]	60.38 [42-95]	237.50 [54-634]	320.33 [68-699]	70.00 [52-95]	67.67 [42-95]	301.8 [54-634]	406.91 [68-1956]
CA19-9, mean [range] (U/mL)		12.95 [5.6-38.5]	NA	16.33 [5-35]	5346.10 [51.5-22039]	13.18 [4.9-38.5]	NA	13.33 [0-35]	5967.60 [51.5-23244]
CCA type	iCCA	-	-	-	4	-	-	-	5
	pCCA	-	-	-	6	-	-	-	9
	dCCA	-	-	-	2	-	-	-	9
Tumor stage	I	-	-	-	1	-	-	-	1
	II	-	-	-	3	-	-	-	7
	III	-	-	-	4	-	-	-	4
	IV	-	-	-	3	-	-	-	8
	Unknown	-	-	-	1	-	-	-	3
Tumor different iation grade	Well	-	-	-	2	-	-	-	6
	Moderate	-	-	-	1	-	-	-	4
	Poor	-	-	-	0	-	-	-	0
	Unknown	-	-	-	9	-	-	-	13
IBD	No IBD	0	0	1	12	0	0	1	23
	UC	0	8	3	0	0	12	2	0
	Crohn	0	0	1	0	0	0	2	0
	Unspecified	0	0	1	0	0	0	0	0

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Supplementary Table 2: Number of transcripts identified in each experimental group

	Array transcripts	Serum				Urine			
		Healthy	UC	PSC	CCA	Healthy	UC	PSC	CCA
mRNA	27,580	9,516	10,526	3,949	23,029	10,949	22,801	21,145	24,817
non-coding RNA	726	310	326	131	635	202	581	542	637
microRNA	518	105	123	49	309	57	273	224	319
small nucleolar RNA	263	119	95	43	199	70	175	162	201
misc RNA	48	16	16	6	43	14	39	39	42
guide RNA	23	11	13	5	19	5	20	16	20
small nuclear RNA	16	15	14	12	15	14	15	15	15
antisense RNA	10	4	4	2	7	4	8	8	7
small cytoplasmic RNA	5	5	5	5	5	5	5	5	5
RNase MRP RNA	1	1	1	1	1	1	1	1	1
ribosomal RNA	1	1	1	1	1	1	1	1	1
telomerase RNA	1	1	0	0	1	1	1	1	1
		Total: 25,804				Total: 27,319			