

Supplementary Materials

Platelet-derived extracellular vesicles and post-ablation survival in hepatocellular carcinoma: a prospective cohort study

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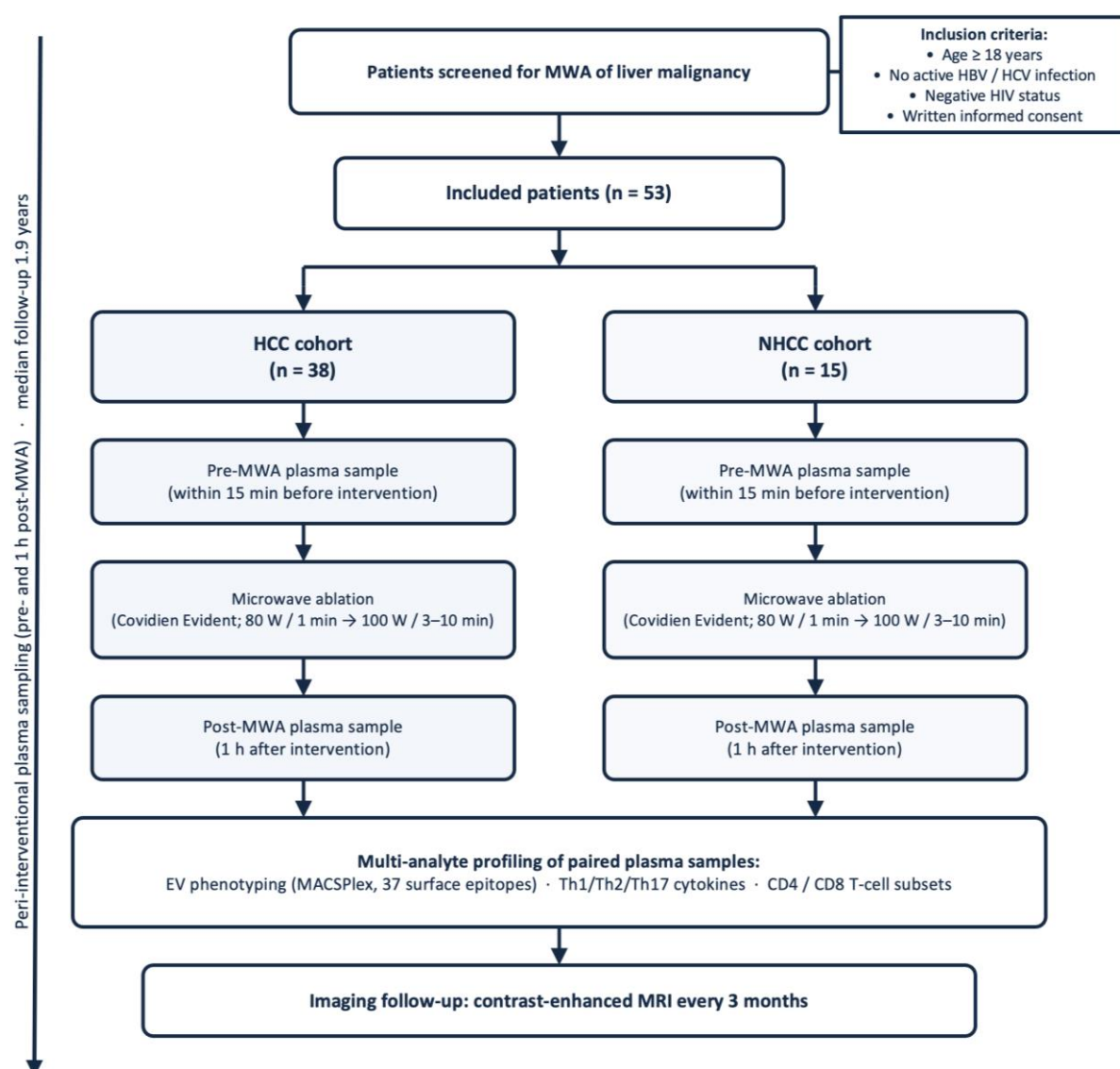
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[#]These authors contributed equally to this work.

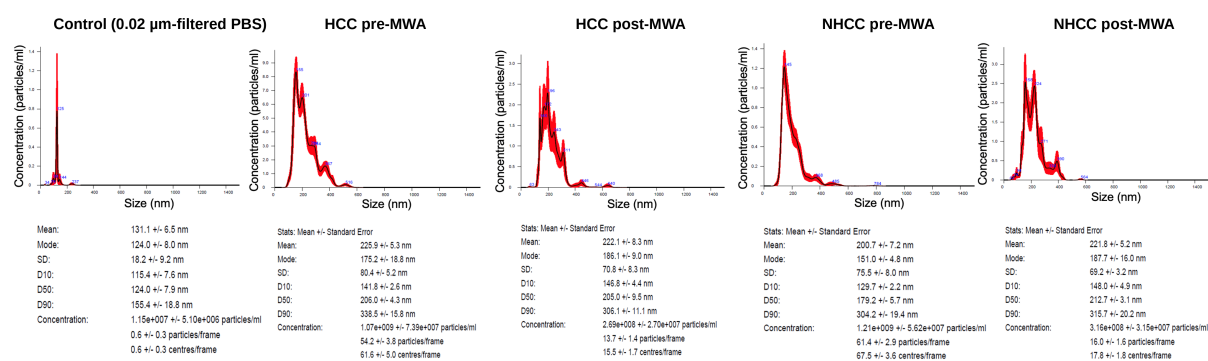
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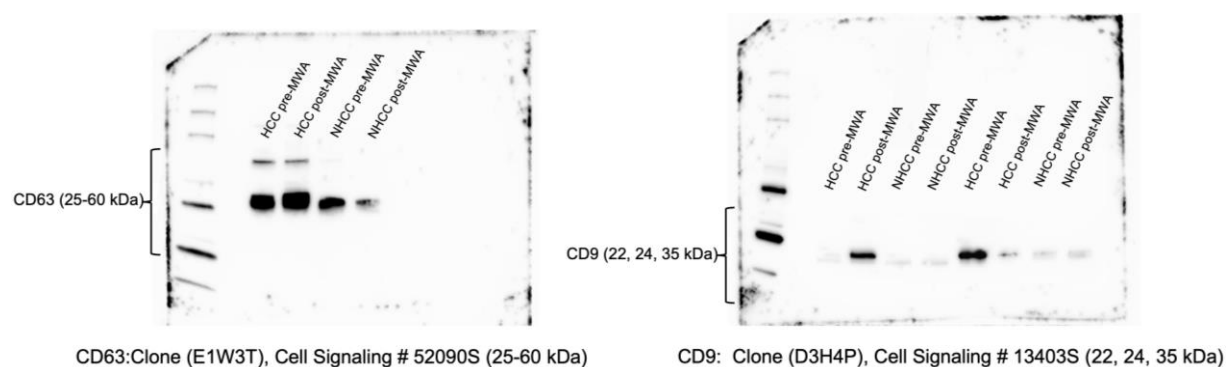
Supplementary Figures and Tables



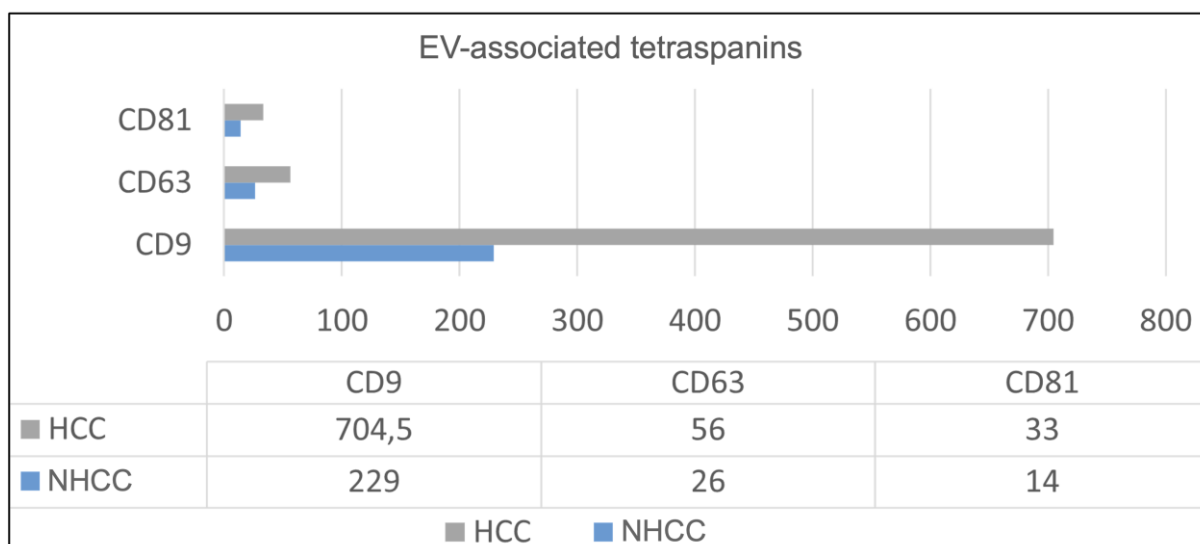
Supplementary Figure 1. Patient flow diagram of the prospective single-center cohort study (n = 53, 38 HCC and 15 NHCC) showing screening, enrolment, paired plasma sampling pre- and 1 h post-MWA, multi-analyte profiling (37-marker MACSPlex EV immunophenotyping, Th1/Th2/Th17 cytokines, CD4/CD8 T-cell subsets), and MRI follow-up every 3 months (median 1.9 years).



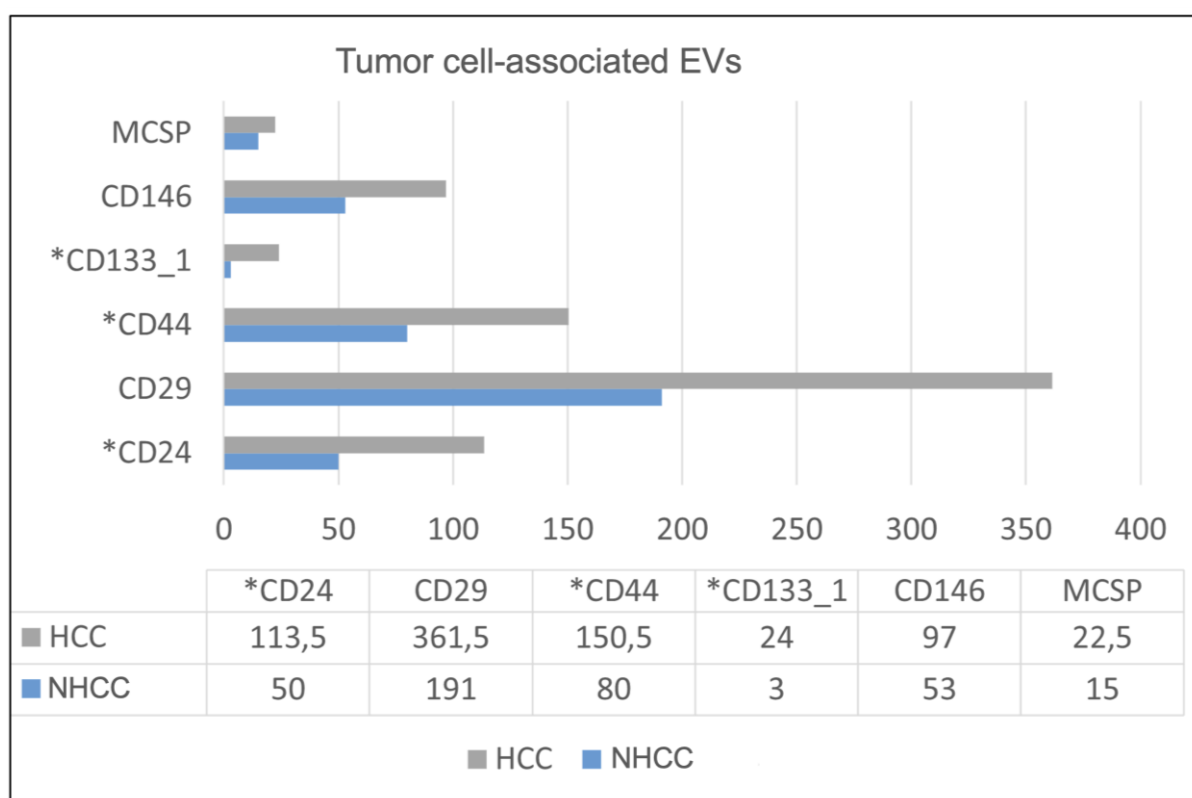
Supplementary Figure 2. NTA of EVs showing particle concentration and size distribution in resuspension buffer (PBS filtered through a 0.02 µm filter) and in pre- and post-MWA plasma samples from the HCC and NHCC groups.



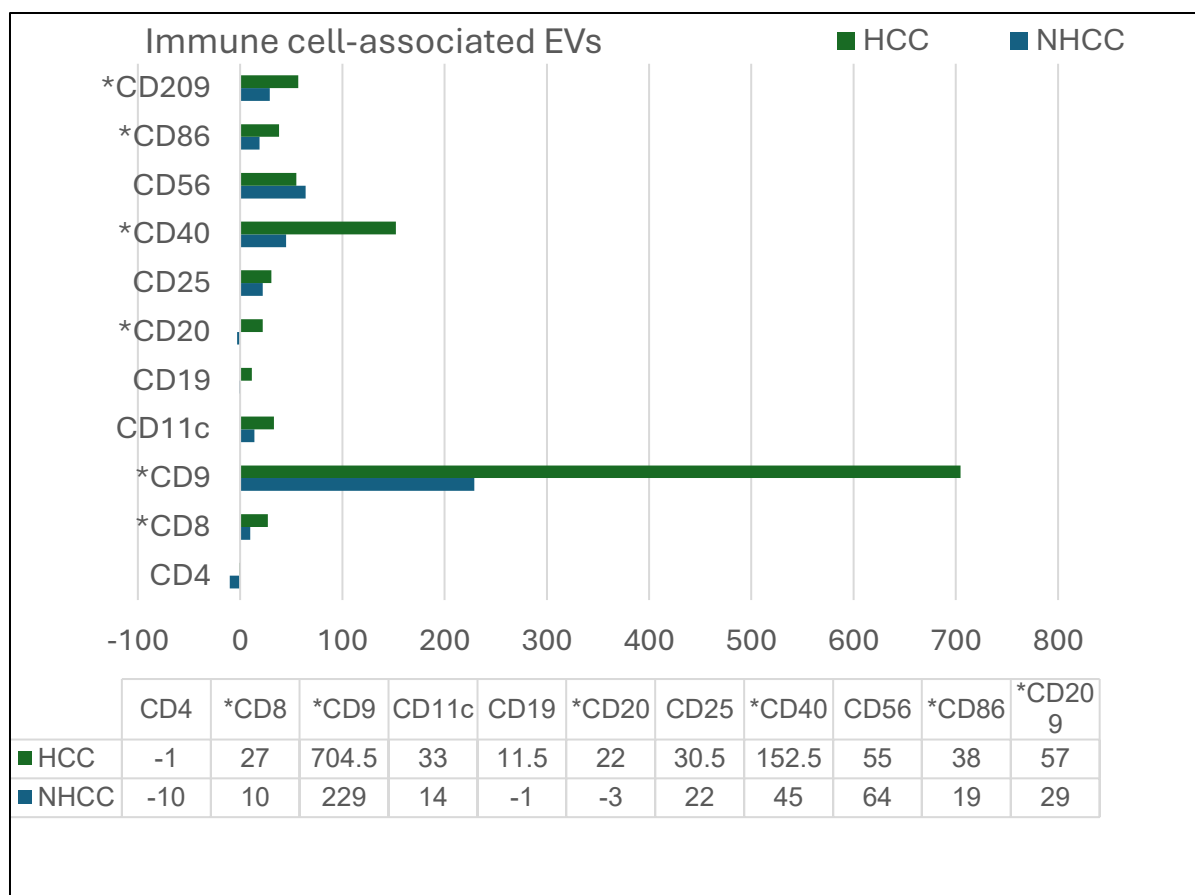
Supplementary Figure 3. Western blot analysis of the exosomal markers CD63 (25–60 kDa) and CD9 (22, 24, 35 kDa) in pre- and post-MWA plasma samples from the HCC and NHCC groups. Primary antibodies: CD63, clone E1W3T (Cell Signaling, #52090S) and CD9, clone D3H4P (Cell Signaling, #13403S).



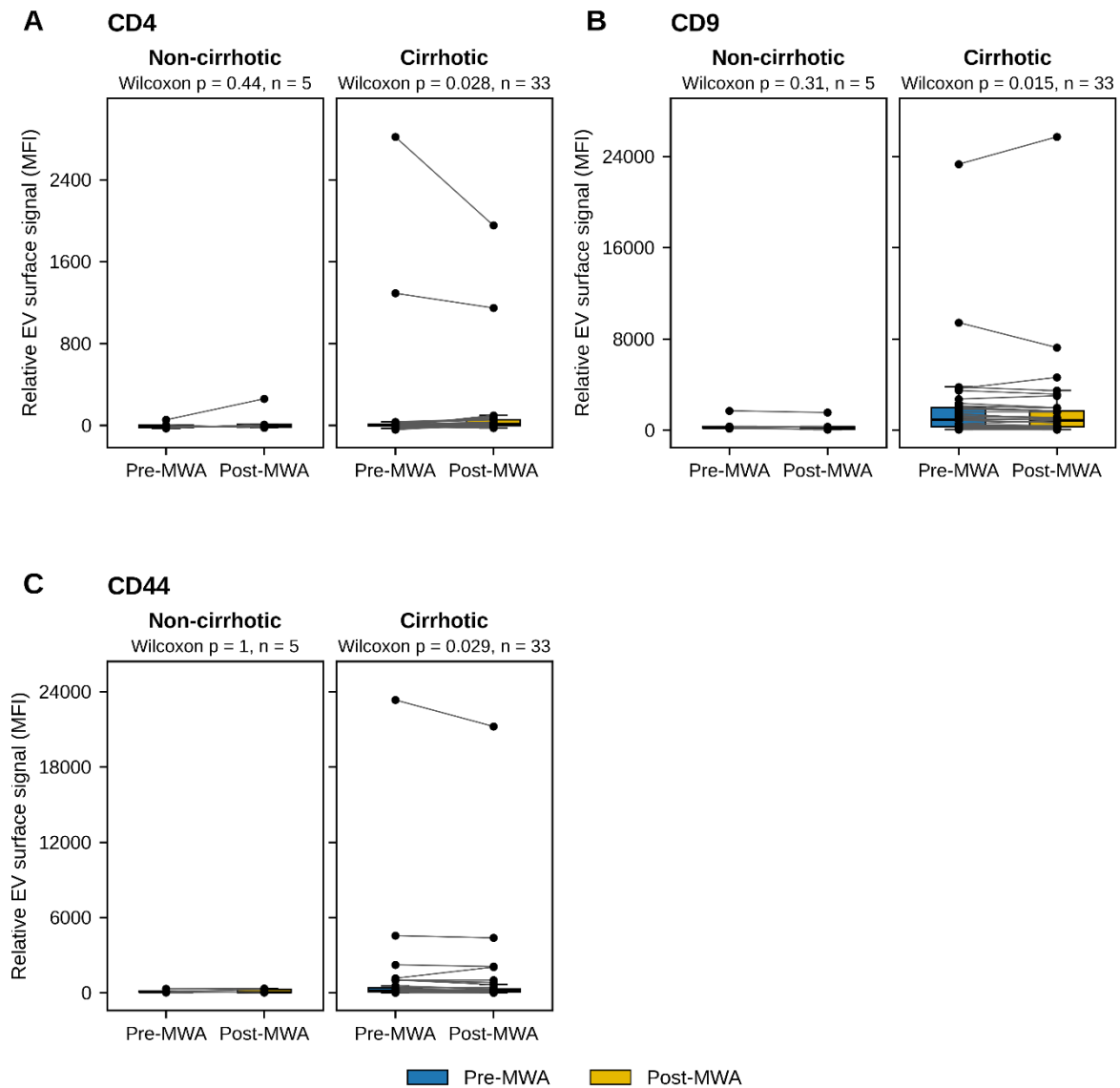
Supplementary Figure 4. Pre-MWA levels of EV-associated tetraspanins CD9, CD63 and CD81 on plasma-derived EVs in HCC and NHCC groups, shown as median fluorescence intensity.



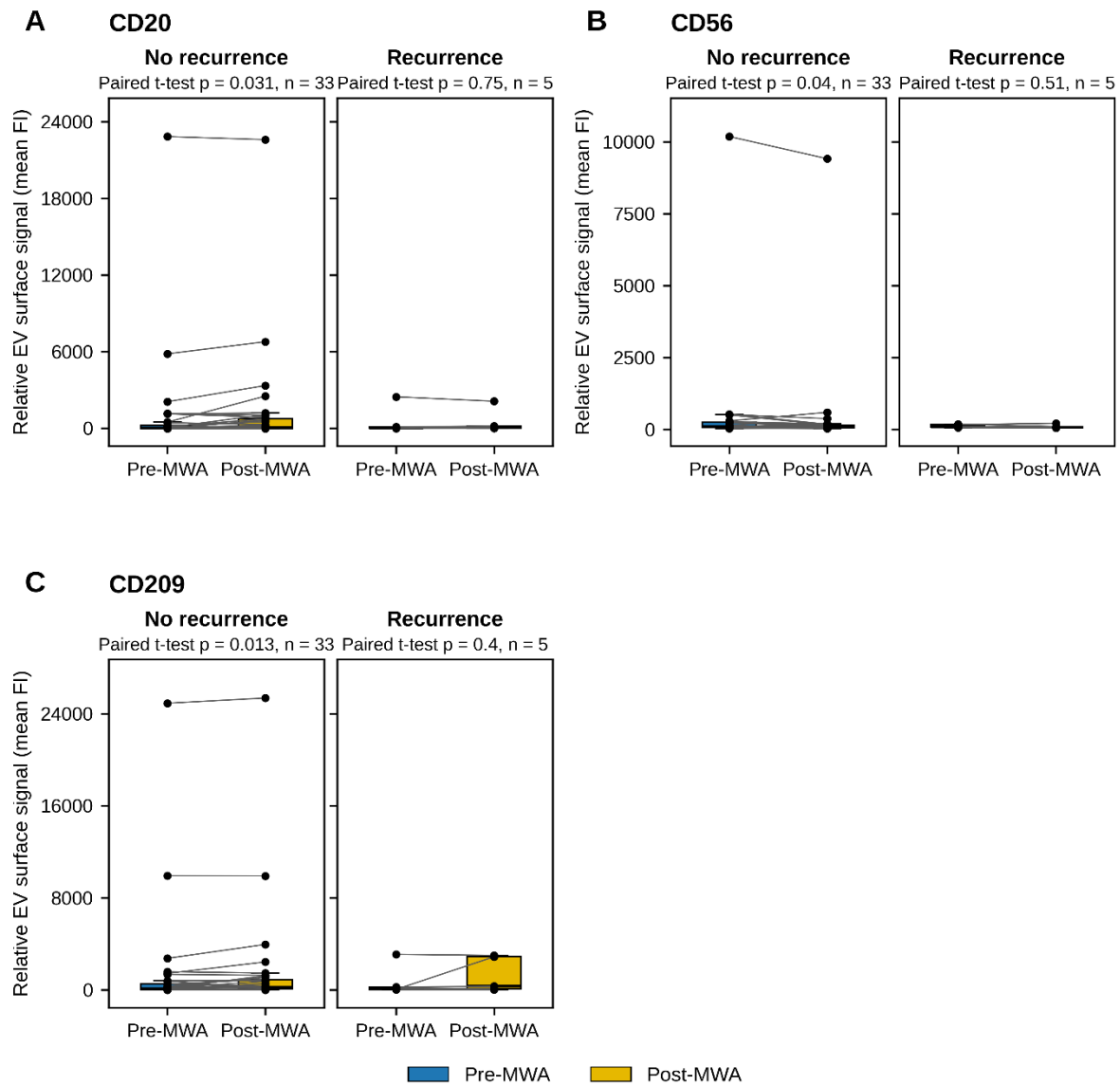
Supplementary Figure 5. Pre-MWA levels of tumor cell-associated markers (CD24, CD29, CD44, CD133, CD146, MCSP) on plasma-derived EVs in HCC and NHCC groups, shown as median fluorescence intensity (* indicates nominal significance, unadjusted $p < 0.05$). The reported p-values are nominal and did not remain significant after Bonferroni correction across the marker panel, and the findings are exploratory.



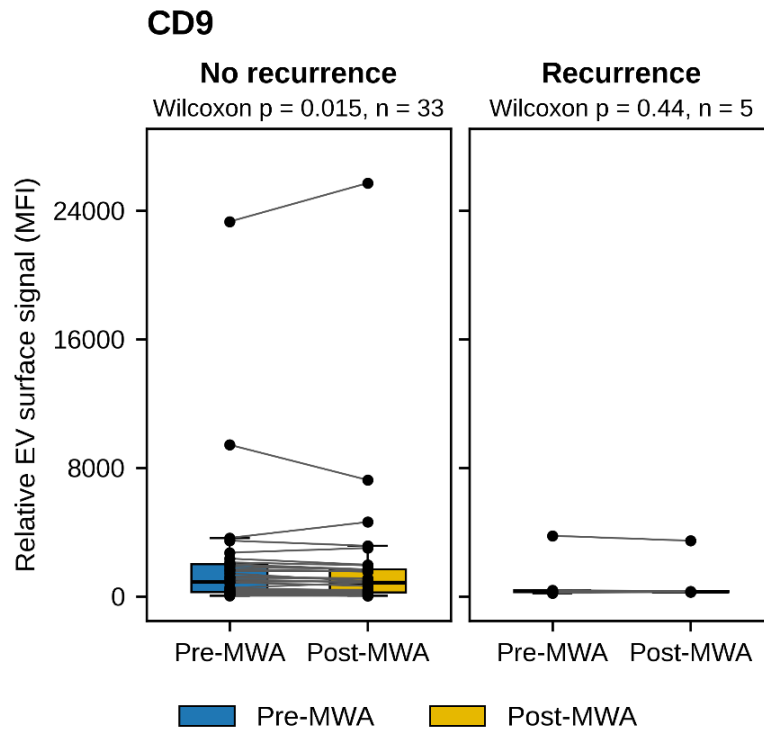
Supplementary Figure 6. Pre-MWA levels of immune cell-associated markers (CD4, CD8, CD9, CD11c, CD19, CD20, CD25, CD40, CD56, CD86, CD209) on plasma-derived EVs in HCC and NHCC groups, shown as median fluorescence intensity (* indicates nominal significance, unadjusted $p < 0.05$). The reported p-values are nominal and did not remain significant after Bonferroni correction across the marker panel, and the findings are exploratory.



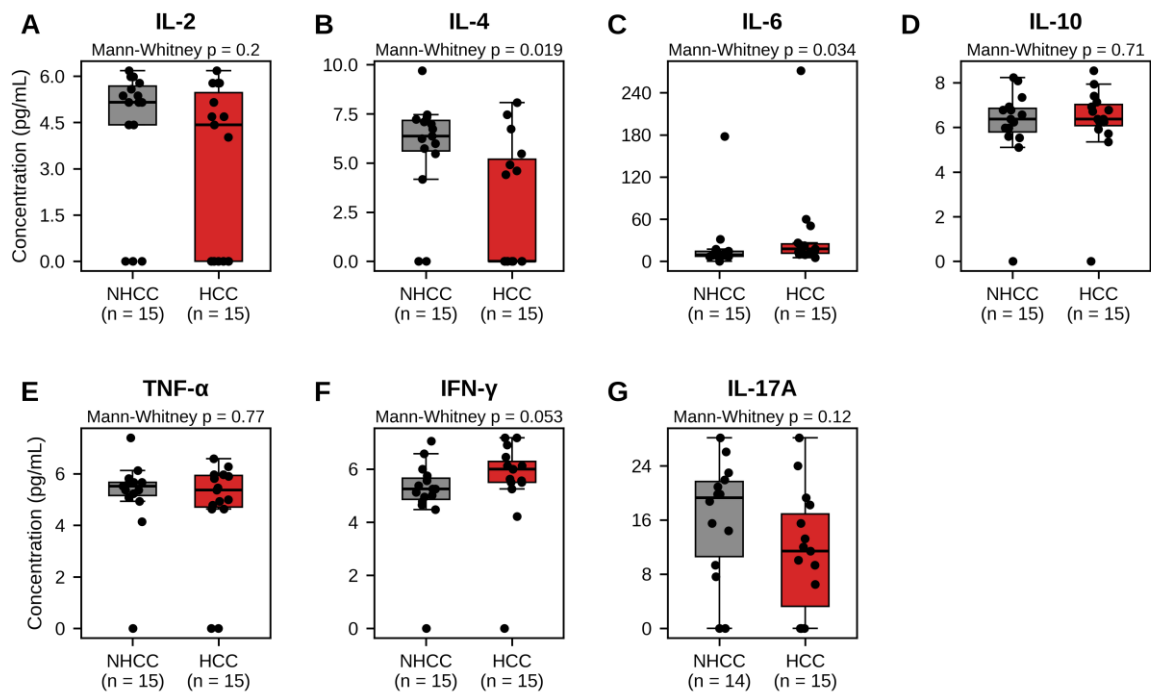
Supplementary Figure 8. (A) CD4, (B) CD9, and (C) CD44 levels on plasma-derived EVs in the HCC group, in non-cirrhotic ($n = 5$) and cirrhotic ($n = 33$) patients, shown pre- and post-MWA as box plots (median and IQR) with paired individual values (paired Wilcoxon signed-rank test on median fluorescence values, nominal p-values).



Supplementary Figure 9. (A) CD20, (B) CD56, and (C) CD209 levels on plasma-derived EVs in the HCC group, in patients without ($n = 33$) and with ($n = 5$) tumor recurrence, shown pre- and post-MWA as box plots (median and IQR) with paired individual values (paired t-test on mean fluorescence values, nominal p-values).



Supplementary Figure 10. CD9 levels on plasma-derived EVs in the HCC group, in patients without ($n = 33$) and with ($n = 5$) tumor recurrence, shown pre- and post-MWA as box plots (median and IQR) with paired individual values (paired Wilcoxon signed-rank test on median fluorescence values, nominal p -values).



Supplementary Figure 11. Pre-MWA cytokine levels (IL-2, IL-4, IL-6, IL-10, TNF- α , IFN- γ , IL-17A) in plasma of the HCC and NHCC groups, shown as box plots (median and IQR) with individual values (Mann-Whitney U test, nominal p -values). Concentrations are given in pg/mL. Cytokine measurements were available for 15 HCC

A IL-2
Wilcoxon $p = 0.35$, $n = 14$
Concentration (pg/mL)
Pre-MWA Post-MWA

B IL-4
Wilcoxon $p = 0.037$, $n = 14$
Concentration (pg/mL)
Pre-MWA Post-MWA

C IL-6
Wilcoxon $p = 0.36$, $n = 14$
Concentration (pg/mL)
Pre-MWA Post-MWA

D IL-10
Wilcoxon $p = 0.028$, $n = 14$
Concentration (pg/mL)
Pre-MWA Post-MWA

E TNF- α
Wilcoxon $p = 0.55$, $n = 14$
Concentration (pg/mL)
Pre-MWA Post-MWA

F IFN- γ
Wilcoxon $p = 1$, $n = 14$
Concentration (pg/mL)
Pre-MWA Post-MWA

G IL-17A
Wilcoxon $p = 0.22$, $n = 14$
Concentration (pg/mL)
Pre-MWA Post-MWA

Legend: Pre-MWA (blue), Post-MWA (yellow)

A IL-2
Wilcoxon $p = 0.81$, $n = 14$

Concentration (pg/mL)

Pre-MWA Post-MWA

B IL-4
Wilcoxon $p = 0.51$, $n = 15$

Concentration (pg/mL)

Pre-MWA Post-MWA

C IL-6
Wilcoxon $p = 0.43$, $n = 15$

Concentration (pg/mL)

Pre-MWA Post-MWA

D IL-10
Wilcoxon $p = 0.12$, $n = 15$

Concentration (pg/mL)

Pre-MWA Post-MWA

E TNF- α
Wilcoxon $p = 0.31$, $n = 15$

Concentration (pg/mL)

Pre-MWA Post-MWA

F IFN- γ
Wilcoxon $p = 0.73$, $n = 15$

Concentration (pg/mL)

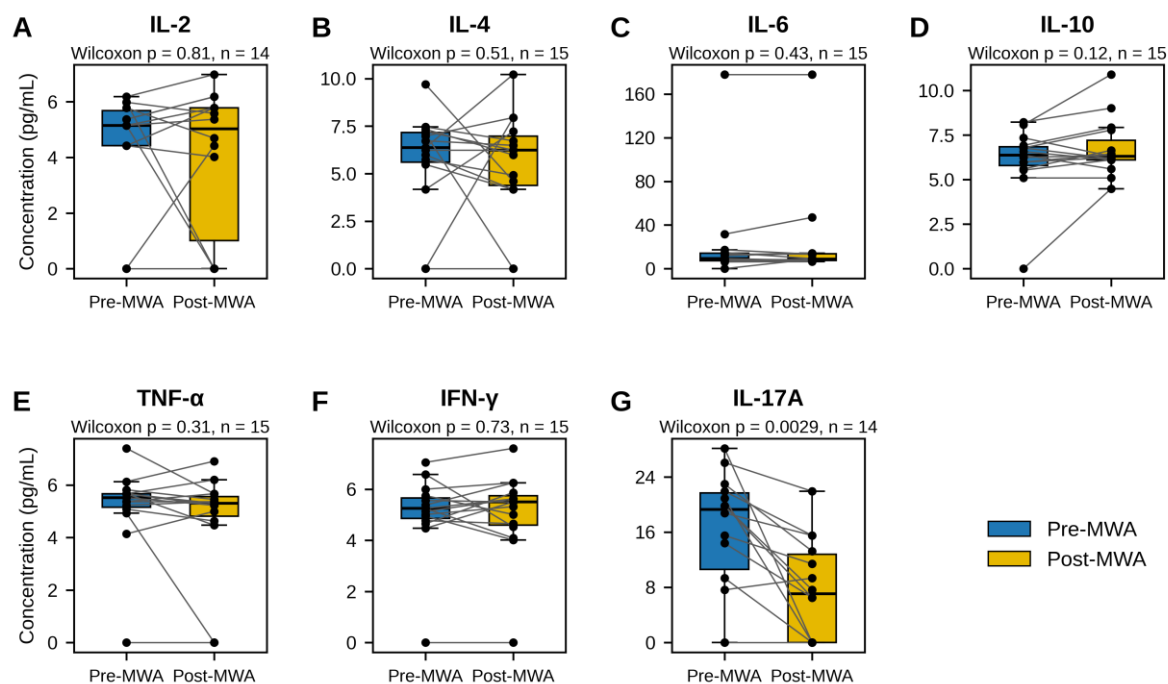
Pre-MWA Post-MWA

G IL-17A
Wilcoxon $p = 0.0029$, $n = 14$

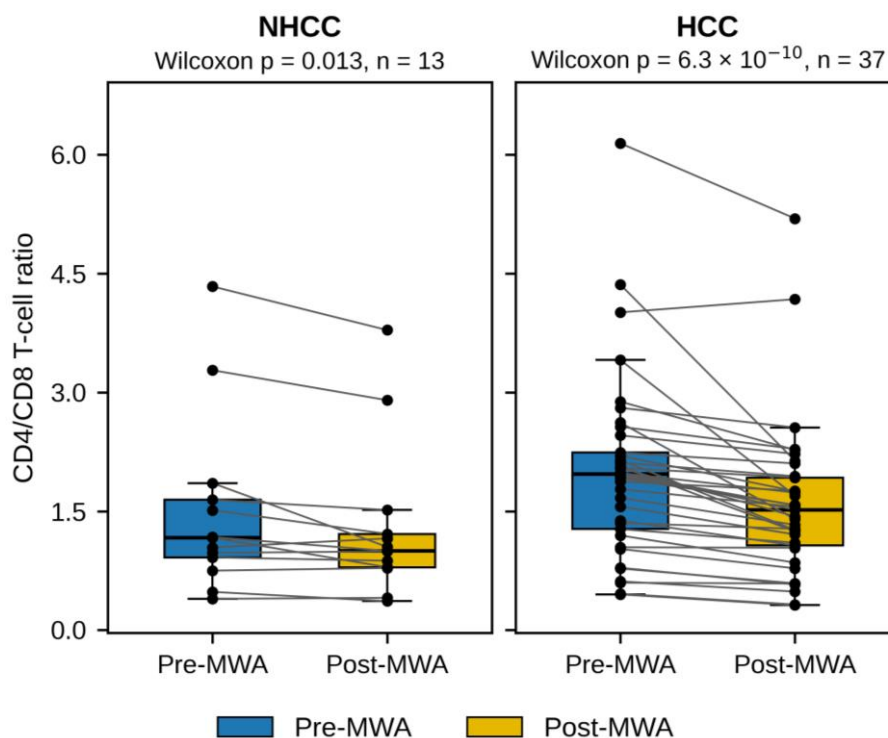
Concentration (pg/mL)

Pre-MWA Post-MWA

Legend:
■ Pre-MWA
■ Post-MWA



Supplementary Figure 13. Pre- and post-MWA cytokine levels (IL-2, IL-4, IL-6, IL-10, TNF- α , IFN- γ , IL-17A) in plasma of the NHCC group (n = 14 to 15 patients with paired samples, as given in each panel), shown pre- and post-MWA as box plots (median and IQR) with paired individual values (paired Wilcoxon signed-rank test, nominal p-values). Concentrations are given in pg/mL. Reported p-values are nominal (unadjusted), and the differences did not remain significant after Bonferroni correction across the cytokine panel.



Supplementary Figure 14. CD4/CD8 T-cell ratio determined by flow cytometry of whole blood pre- and post-MWA in the NHCC (n = 13) and HCC (n = 37) groups, shown as box plots (median and IQR) with paired individual values and analyzed using the Wilcoxon signed-rank test. p-values for the pre- versus post-MWA changes are given above the panels, with $p < 0.05$ considered nominally significant. As the CD4/CD8 ratio was the single analyte compared, no multiplicity correction was applied, and the findings are interpreted as exploratory.

Supplementary Table 1. Pre- and post-MWA levels of EV surface antigens on plasma-derived EVs in NHCC and HCC groups, shown as median values with IQR

Pre-MWA	NHCC	HCC	<i>p</i> -Value
	Median (IQR), MFI	Median (IQR), MFI	
CD8	10.0 (-4.5 – 33.5)	27.0 (15.0 – 42.0)	0.024*
CD105	54.0 (30.0 – 98.0)	111.5 (45.0 – 197.0)	0.036*
CD209	29.0 (5.5 – 49.5)	57.0 (20.0 – 246.0)	0.02*
CD9	229.0 (160.5 – 666.5)	704.5 (262.0 – 1999.0)	0.033*
CD40	45.0 (19.0 – 89.5)	152.5 (37.0 – 336.0)	0.022*
CD81	14.0 (1.5 – 26.5)	33.0 (16.0 – 56.0)	0.005*
CD24	50.0 (42.5 – 93.0)	113.5 (72.0 – 290.0)	0.004*
CD86	19.0 (7.5 – 30.0)	38.0 (22.0 – 144.0)	0.003*
CD44	80.0 (39.5 – 132.0)	150.5 (68.0 – 331.0)	0.029*
CD133	3.0 (-9.5 – 20.5)	24.0 (6.0 – 133.0)	0.009*
CD142	32.0 (17.0 – 54.5)	59.5 (40.0 – 110.0)	0.007*
CD20	-3.0 (-9.5 – 5.5)	22.0 (0.0 – 166.0)	0.005*
Post-MWA	NHCC	HCC	<i>p</i> -Value
	Median (IQR), MFI	Median (IQR), MFI	
CD19	-4.0 (-8.0 – 12.0)	8.5 (-7.0 – 209.0)	0.047*
CD2	-8.0 (-20.0 – 9.5)	7.0 (-7.0 – 17.0)	0.021*
CD1c	-26.0 (-34.0 – -19.5)	-15.5 (-23.0 – 3.0)	0.021*
CD25	9.0 (-7.5 – 20.5)	36.5 (10.0 – 252.0)	0.008*
CD49e	1.0 (-14.5 – 13.0)	18.0 (6.0 – 35.0)	0.017*
CD209	26.0 (8.0 – 35.0)	47.5 (21.0 – 250.0)	0.025*
CD9	278.0 (161.5 – 557.5)	754.5 (267.0 – 1683.0)	0.041*
CD81	22.0 (9.5 – 35.5)	34.0 (23.0 – 55.0)	0.027*

CD146	36.0 (18.5 – 65.0)	93.0 (35.0 – 188.0)	0.021*
CD86	27.0 (15.0 – 50.0)	57.0 (25.0 – 296.0)	0.035*
CD326	34.0 (23.0 – 45.5)	49.5 (31.0 – 325.0)	0.023*
CD142	25.0 (20.5 – 55.0)	54.5 (36.0 – 181.0)	0.014*
CD20	1.0 (-6.0 – 17.0)	31.0 (2.0 – 294.0)	0.014*

Asterisks indicate nominal significance (unadjusted $p < 0.05$). Note on multiplicity: the tabulated p -values are nominal (unadjusted) two-sided values (Mann-Whitney U test), and asterisks denote nominal $p < 0.05$ without correction for multiple testing. Because 37 EV surface markers were compared, the Bonferroni-adjusted significance threshold is $p < 0.0014$ ($0.05/37$). Under this conservative correction, the tabulated differences are not statistically significant and are presented as exploratory, hypothesis-generating findings. **Abbreviations:** HCC, hepatocellular carcinoma; NHCC, non-HCC group with hepatic metastasis other than HCC; IQR, interquartile range; MFI, median fluorescence intensity; CD, cluster of differentiation.

Supplementary Table 2. Pre- and post-MWA cytokine levels (IL-2, IL-4, IL-6, IL-10, TNF- α , IFN- γ , IL-17A) in plasma of the NHCC and HCC groups, shown as median concentrations (pg/mL) with IQR and compared using the Mann-Whitney U test

	NHCC	HCC	p -Value
	Median (IQR), pg/mL	Median (IQR), pg/mL	
IL-2 pre-MWA	5.2 (4.4 – 5.7), n = 15	4.4 (0.0 – 5.5), n = 15	0.198
IL-4 pre-MWA	6.4 (5.6 – 7.2), n = 15	0.0 (0.0 – 5.2), n = 15	0.019*
IL-6 pre-MWA	9.2 (7.3 – 14.1), n = 15	17.6 (11.4 – 24.8), n = 15	0.034*
IL-10 pre-MWA	6.4 (5.8 – 6.8), n = 15	6.4 (6.1 – 7.0), n = 15	0.708
TNF-α pre-MWA	5.5 (5.2 – 5.7), n = 15	5.4 (4.7 – 5.9), n = 15	0.771
IFN-γ pre-MWA	5.2 (4.9 – 5.7), n = 15	6.0 (5.5 – 6.3), n = 15	0.053
IL-17A pre-MWA	19.3 (10.6 – 21.7), n = 14	11.4 (3.3 – 16.9), n = 15	0.125
IL-2 post-MWA	5.0 (1.0 – 5.8), n = 14	4.9 (1.1 – 5.2), n = 14	0.561
IL-4 post-MWA	6.2 (4.4 – 7.0), n = 15	4.8 (4.2 – 6.2), n = 14	0.430
IL-6 post-MWA	8.8 (7.6 – 13.7), n = 15	15.7 (12.4 – 21.1), n = 14	0.021*
IL-10 post-MWA	6.3 (6.1 – 7.2), n = 15	7.1 (6.4 – 7.6), n = 14	0.230

TNF-α post-MWA	5.3 (4.8 – 5.6), n = 15	5.8 (4.9 – 6.4), n = 14	0.274
IFN-γ post-MWA	5.5 (4.6 – 5.7), n = 15	5.7 (5.4 – 6.2), n = 14	0.148
IL-17A post-MWA	7.6 (0.0 – 14.4), n = 15	18.2 (9.5 – 18.8), n = 14	0.085

Cytokine measurements were available for 15 HCC and 15 NHCC patients before MWA and for 14 HCC and 15 NHCC patients after MWA, and the number of measured samples (n) is given for each group. Asterisks indicate nominal significance (unadjusted $p < 0.05$). Note on multiplicity: the tabulated p-values are nominal (unadjusted) two-sided values, and asterisks denote nominal $p < 0.05$. Because seven cytokines were compared, the Bonferroni-adjusted significance threshold is $p < 0.0071$ ($0.05/7$). Under this correction, none of the tabulated differences remain significant, and they are interpreted as exploratory. **Abbreviations:** HCC, hepatocellular carcinoma; NHCC, non-HCC group with hepatic metastasis other than HCC; IQR, interquartile range; IL, interleukin; TNF, tumor necrosis factor; IFN, interferon.

Supplementary Table 3. Pre- and post-MWA CD4/CD8 ratios in NHCC (n = 13) and HCC (n = 37) groups, shown as median ratios (dimensionless) with IQR

	NHCC (n=13)	HCC (n=37)	p-Value
	Median (IQR)	Median (IQR)	
CD4/CD8 pre-MWA	1.2 (0.9 – 1.6)	2.0 (1.3 – 2.2)	0.043*
CD4/CD8 post-MWA	1.0 (0.8 – 1.2)	1.5 (1.1 – 1.9)	0.085
Δ CD4/CD8	-0.1 (-0.4 – 0.0)	-0.2 (-0.4 – -0.1)	0.081

Asterisk indicates nominal significance (unadjusted $p < 0.05$). Note: the tabulated p-values are nominal (unadjusted) two-sided values. As the CD4/CD8 ratio was the single analyte compared, no multiplicity correction was applied, and the findings are interpreted as exploratory. **Abbreviations:** HCC, hepatocellular carcinoma; NHCC, non-HCC group with hepatic metastasis other than HCC; IQR, interquartile range; CD, cluster of differentiation.

STROBE Statement

Item #	Section / Topic	STROBE recommendation	Where reported in manuscript	Reported?
Title and abstract				
1 (a)	Title and abstract	Indicate the study's design with a commonly used term in the title or the abstract.	<i>Title; Abstract → Background & Aims</i>	Yes
1 (b)	Title and abstract	Provide in the abstract an informative and balanced summary of what was done and what was found.	<i>Abstract → Background & Aims; Approach & Results; Conclusions</i>	Yes
Introduction				
2	Background / rationale	Explain the scientific background and rationale for the investigation being reported.	<i>Introduction (paragraphs 1–4): epidemiology of liver malignancies, BCLC framework, role of MWA, biology of EVs and tumor-immune interactions.</i>	Yes
3	Objectives	State specific objectives, including any pre-specified hypotheses.	<i>Introduction (final paragraph): multi-analyte EV / cytokine / lymphocyte profiling to characterize immunomodulatory effects of MWA and to identify candidate biomarkers of therapeutic response.</i>	Yes
Methods				
4	Study design	Present key elements of study design early in the paper.	<i>Methods → "Patients and study design": prospective single-center observational cohort with peri-interventional blood sampling and longitudinal MRI follow-up.</i>	Yes
5	Setting	Describe the setting, locations and relevant dates, including periods of recruitment, exposure, follow-up, and data collection.	<i>Methods → "Patients and study design": Frankfurt University Hospital; ethics approval 19-443 (10 Sep 2020); enrolment 2021–2022; median follow-up 1.9 years (MRI every 3 months).</i>	Yes
6 (a)	Participants — eligibility	Cohort study — Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up.	<i>Methods → "Patients and study design" / "Study characteristics" / "Follow Up Investigation": age ≥ 18 years, absence of active HBV / HCV / HIV; consecutive patients undergoing MWA for HCC (n = 38) or hepatic metastasis (n = 15).</i>	Yes
6 (b)	Participants — matching	Cohort study — For matched studies, give matching criteria and number of exposed and unexposed.	<i>Not applicable — non-matched design comparing HCC versus NHCC and pre- versus post-MWA samples.</i>	N/A

7	Variables	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable.	<i>Methods → Tables 1 and 2: clinical variables (BCLC, Child-Pugh, MELD, fibrosis / cirrhosis, hepatitis serology, MASH, AFP, tumor volume, histologic grading, diabetes, recurrence, mortality). EV variables: 37 surface epitopes by MACSPlex. Cytokines: Th1/Th2/Th17 panel. T-cell subsets: CD4/CD8 by flow cytometry. Outcome: overall mortality during the observation period.</i>	Yes
8	Data sources / measurement	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group.	<i>Methods → "Isolation of EVs" / NTA / Western blot / MACSPlex / Cytokine determination / CD4–CD8 ratio determination. Standardized commercial assays (Miltenyi Pan EV Isolation Kit, MACSPlex Exosome Kit, BD CBA Th1/Th2/Th17, BD CD3/CD8/CD45/CD4 Multitest). EV-TRACK reporting (EV-TRACK ID: EV260032) and MISEV2023 nomenclature compliance disclosed in "Data and methodology reporting" subsection.</i>	Yes
9	Bias	Describe any efforts to address potential sources of bias.	<i>Methods → "Statistical analysis"; Limitations: single-center design, residual confounding from underlying liver disease and prior treatments acknowledged. Pre-specified Bonferroni correction across the EV / cytokine panel to limit type I inflation; sensitivity analysis excluding an extreme platelet-EV outlier disclosed.</i>	Yes
10	Study size	Explain how the study size was arrived at.	<i>Methods → "Patients and study design": pragmatic prospective enrolment of all eligible consecutive MWA patients during the inclusion window (no formal a-priori power calculation). Post-hoc power analysis explicitly addressed in Limitations ($\leq 50\%$ for Cohen's $d = 0.74$; sample sizes of ~75–210 patients with 20–55 events required for 80 % power).</i>	Yes
11	Quantitative variables	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen, and why.	<i>Methods → "Statistical analysis": continuous variables tested for normality (Shapiro–Wilk); non-normal data reported as median (IQR), and additionally as mean $\pm$ SD where conventional. EV-surface markers normalized as median fluorescence intensity (MFI). Pre-versus post-MWA evaluated as paired measures. Survival comparison stratified by overall mortality status at end of follow-up.</i>	Yes
12 (a)	Statistical methods — main	Describe all statistical methods, including those used to control for confounding.	<i>Methods → "Statistical analysis": Mann–Whitney U for between-group comparisons; Wilcoxon signed-rank for paired pre- vs post-MWA comparisons; χ^2 / Fisher's exact for categorical data; Pearson's r for normally distributed correlation pairs and Spearman's ρ</i>	Yes

			<i>otherwise; Bonferroni correction for multiple testing; Cohen's d for standardized between-group effect sizes (small ≥ 0.2, medium ≥ 0.5, large ≥ 0.8).</i>	
12 (b)	Statistical methods — subgroups	Describe any methods used to examine subgroups and interactions.	<i>Methods + Results → "Subgroup Analyses Based on Clinical Parameters": pre-specified stratification by cirrhosis status and recurrence; separate analyses within the HCC subpopulation.</i>	Yes
12 (c)	Statistical methods — missing data	Explain how missing data were addressed.	<i>Methods + Results: differential blood-cell counts not available for all patients and therefore excluded from analyses; histologic grading missing in a small number of patients (n disclosed in Table 1). No imputation performed; analyses were complete-case.</i>	Yes
12 (d)	Statistical methods — follow-up	Cohort study — If applicable, explain how loss to follow-up was addressed.	<i>Methods → "Follow Up Investigation": MRI every three months; median follow-up 1.9 years; all 53 patients had documented vital status at the end of the observation period (no loss to follow-up).</i>	Yes
12 (e)	Statistical methods — sensitivity	Describe any sensitivity analyses.	<i>Results / Limitations: sensitivity analysis of the platelet-EV survival comparison excluding the single patient with extreme CD29 expression disclosed; attenuation of effect estimates reported.</i>	Yes

Results

13 (a)	Participants — numbers	Report numbers of individuals at each stage of study — e.g. numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analyzed.	<i>Results → "Patient characteristics"; Table 1; Supplementary Figure S1 (study flow diagram): 53 patients enrolled (38 HCC, 15 NHCC); all analyzed; no exclusions after enrolment.</i>	Yes
13 (b)	Participants — non-participation	Give reasons for non-participation at each stage.	<i>Methods → "Patients and study design"; Supplementary Figure S1: all consecutive eligible MWA patients during the enrolment window who provided written informed consent were included; no documented refusals or exclusions.</i>	Yes
13 (c)	Participants — flow diagram	Consider use of a flow diagram.	<i>Provided as Supplementary Figure S1: patient flow from screening through inclusion, peri-interventional plasma sampling (pre- and 1 h post-MWA), multi-analyte profiling and MRI follow-up for both HCC and NHCC cohorts.</i>	Yes

14 (a)	Descriptive data — characteristics	Give characteristics of study participants (e.g. demographic, clinical, social) and information on exposures and potential confounders.	<i>Results → "Patient characteristics"; Table 1: age, sex, diagnosis, BCLC stage, Child-Pugh, MELD, fibrosis / cirrhosis, alcohol use, hepatitis status, MASH, diabetes, AFP, tumor volume, histologic grading, recurrence, mortality.</i>	Yes
14 (b)	Descriptive data — missing data	Indicate number of participants with missing data for each variable of interest.	<i>Table 1: denominators reported per variable (e.g., n = 37 for diabetes, n = 31 for histologic grading) reflecting partially missing data.</i>	Yes
14 (c)	Descriptive data — follow-up time	Cohort study — Summarize follow-up time (e.g., average and total amount).	<i>Methods → "Follow Up Investigation": median follow-up 1.9 years; MRI every 3 months. Supplementary Figure S1 displays the follow-up timeline schematically.</i>	Yes
15	Outcome data	Cohort study — Report numbers of outcome events or summary measures over time.	<i>Results / Table 1: mortality 10 / 38 (26.3 %) HCC and 6 / 15 (40.0 %) NHCC; recurrence 5 / 38 (13.2 %) HCC and 2 / 14 (14.3 %) NHCC.</i>	Yes
16 (a)	Main results	Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (e.g., 95 % CI). Make clear which confounders were adjusted for and why they were included.	<i>Results → "Pre- and post-MWA markers"; Table 1 (between-group differences with χ^2 p-values); Table 2 (platelet-EV median (IQR), Mann–Whitney U-test p-values and Cohen's d). Univariable estimates only; no multivariable adjustment owing to limited event count, as explicitly discussed in Limitations.</i>	Yes
16 (b)	Main results — categories	Report category boundaries when continuous variables were categorized.	<i>Results / Tables: no novel categorization of continuous variables introduced (standard clinical categories: BCLC stages 0/A/B/C/D; Child-Pugh A/B/C; histologic grading G1–G3 according to WHO).</i>	Yes
16 (c)	Main results — absolute risk	If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period.	<i>Not applicable — primary outcomes reported as group-level frequencies and standardized effect sizes; relative-risk metrics were not computed.</i>	N/A
17	Other analyses	Report other analyses done — e.g. analyses of subgroups and interactions, and sensitivity analyses.	<i>Results: subgroup analyses by cirrhosis and recurrence status; cytokine–EV correlations (Pearson's r / Spearman's ρ based on normality); CD4/CD8 ratio dynamics; sensitivity analysis of platelet-EV survival comparison without the extreme outlier (Limitations).</i>	Yes
Discussion				
18	Key results	Summarize key results with reference to study objectives.	<i>Discussion → opening paragraphs and "Conclusions": EV signatures discriminating HCC from NHCC, MWA-induced peri-interventional remodeling, platelet-EV</i>	Yes

			<i>directional association with shorter overall survival, candidate biomarkers warranting validation.</i>	
19	Limitations	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias.	<i>Discussion → "Several limitations warrant acknowledgment": modest sample size, limited follow-up, multiple-testing inflation, single-center design, clinical heterogeneity, semi-quantitative nature of bead-based EV phenotyping, absence of TEM / AFM ultrastructural confirmation, lack of co-isolated lipoprotein / albumin markers, no functional in-vitro or in-vivo follow-up; explicit underpower discussion with post-hoc Cohen's d power calculation and required validation cohort sizes (~75–210 patients with 20–55 events).</i>	Yes
20	Interpretation	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence.	<i>Discussion → effect-size-based interpretation paragraph and biological context: directional concordance across four functionally related platelet-membrane epitopes interpreted in the context of established platelet-EV biology in cirrhosis-associated HCC; hypothesis-generating framing emphasized throughout.</i>	Yes
21	Generalizability	Discuss the generalizability (external validity) of the study results.	<i>Discussion → "Validation in larger, independent, ethnically diverse cohorts with longer follow-up is required"; need for standardized preanalytical workflows and inter-laboratory harmonization before clinical implementation discussed.</i>	Yes
Other information				
22	Funding	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based.	<i>"Funding acknowledgement" section: Rudolf Geißendoerfer-Stiftung, Frankfurter Forschungs Foerderung, B. Braun-Stiftung, Helio Health Inc., DFG SFB 1382 (project ID 403224013), BMBF DEEP-HCC, HMWK ENABLE and ACLF-I clusters, EU Horizon 2020 (MICROB-PREDICT, DECISION, GALAXY, LIVERHOPE, IHMCSA). "The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript."</i>	Yes