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Supplementary Material

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13 **Liposome-based assay for cancer biomarker detection: Exploring the**
14 **correlation between platelet-derived microvesicles and NSCLC-associated**
15 **miRNAs**

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31 **Chemicals and reagents**

32 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC) ($\geq 99.0\%$), Cholesterol ($\geq 99.0\%$) and 5(6)-
33 carboxyfluorescein ($\geq 95.0\%$) were purchased from Sigma, China. Streptavidin-coated magnetic
34 beads (MBs) were obtained from Thermo Fisher, China. Prostaglandin E1 (PGE1), protease, and
35 phosphatase inhibitors were procured from Aladdin, China. The Anti-CD41a antibody labeled with
36 FITC was sourced from Biolegend, China. Bovine serum albumin (BSA) was obtained from
37 Sigma, China. Additionally, the SanPrep Column MicroRNA Mini-Prep Kit, MicroRNA First
38 Strand cDNA Synthesis Kit, and MicroRNA qPCR Kit (SYBR Green Method) were acquired from
39 Sangon Biotech, China. All other chemicals utilized in the experiment were analytical grade and
40 were used without further purification. Throughout the experimental procedures, ultra-pure water
41 with a resistivity of 18.2 M Ω cm, obtained from the microporous filtration system (Billerica,
42 USA), was employed for the preparation of solutions.

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45 **Table S1** Clinical characteristics of healthy donors and NSCLC patients.

Characteristics		Healthy donors (N= 5)	NSCLC patients (N= 5)
Average age (year)		61.34 ± 1.20	62.85 ± 1.04
Sex (male)		5	5
NSCLC type	Squamous cell carcinoma	/	2
	Adenocarcinoma	/	2
	Large cell carcinoma	/	1
stage		/	(III-IV)

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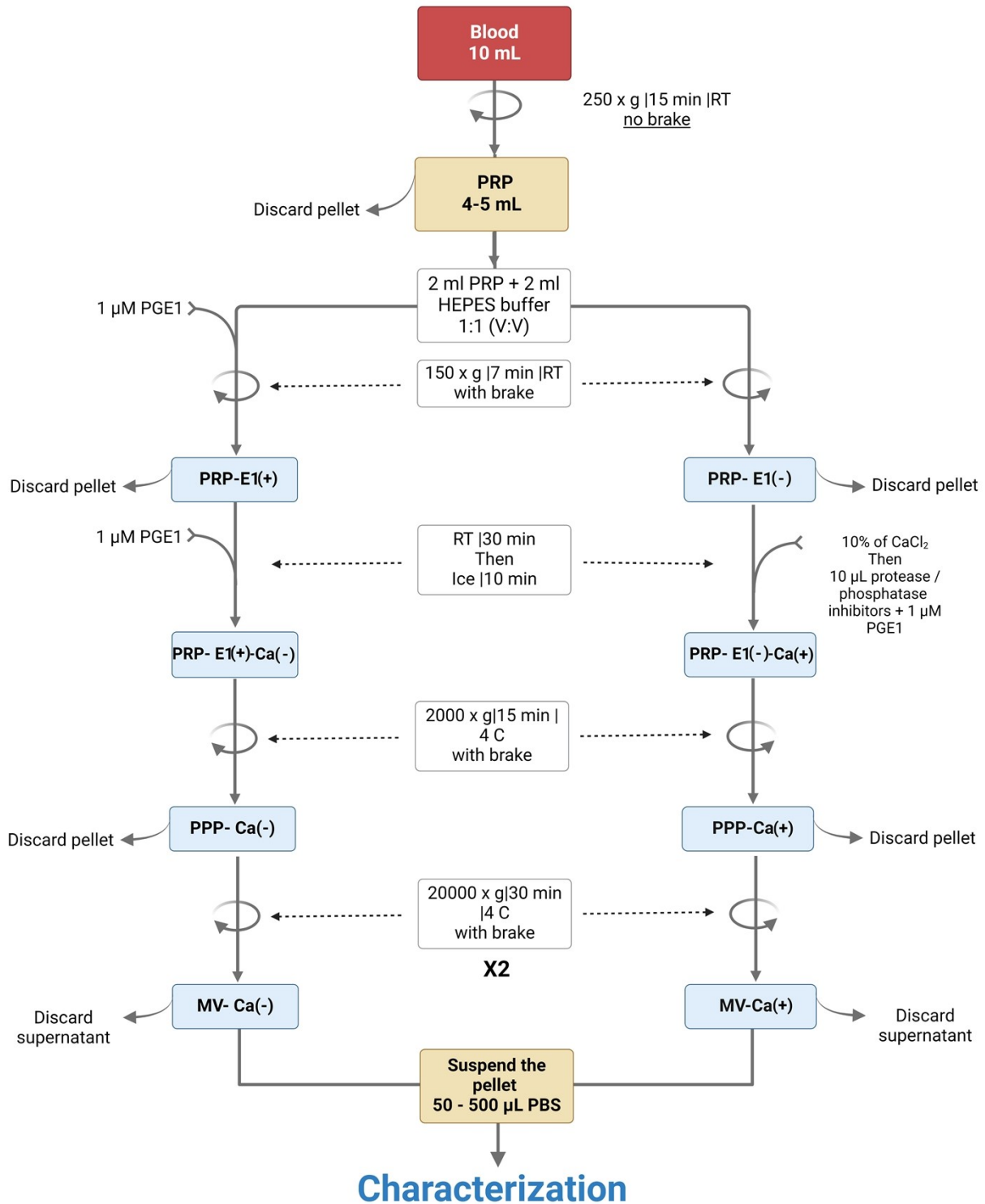
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49 **Table S2** Oligonucleotide sequences.

Name	Sequence (5'→3')	F-Primer
miRNA-1246	AAUGGAUUUUUGGAGCAGG	CGCAATGGATTTTTGGAGCAGG
miRNA-21	UAGCUUAUCAGACUGAUGUUGA	CGGTAGCTTATCAGACTGATGTTG
		A
miRNA-223	UGUCAGUUUGUCAAUACCCCA	CGCTGTCAGTTTGTCAAATACCCC
		A
cDNA1	Cholesteryl- TTTTTTTTTTTTCAACATCAGT	////////////////////////////////////
cDNA2	CTGATAAGCTATT- Biotin	////////////////////////////////////

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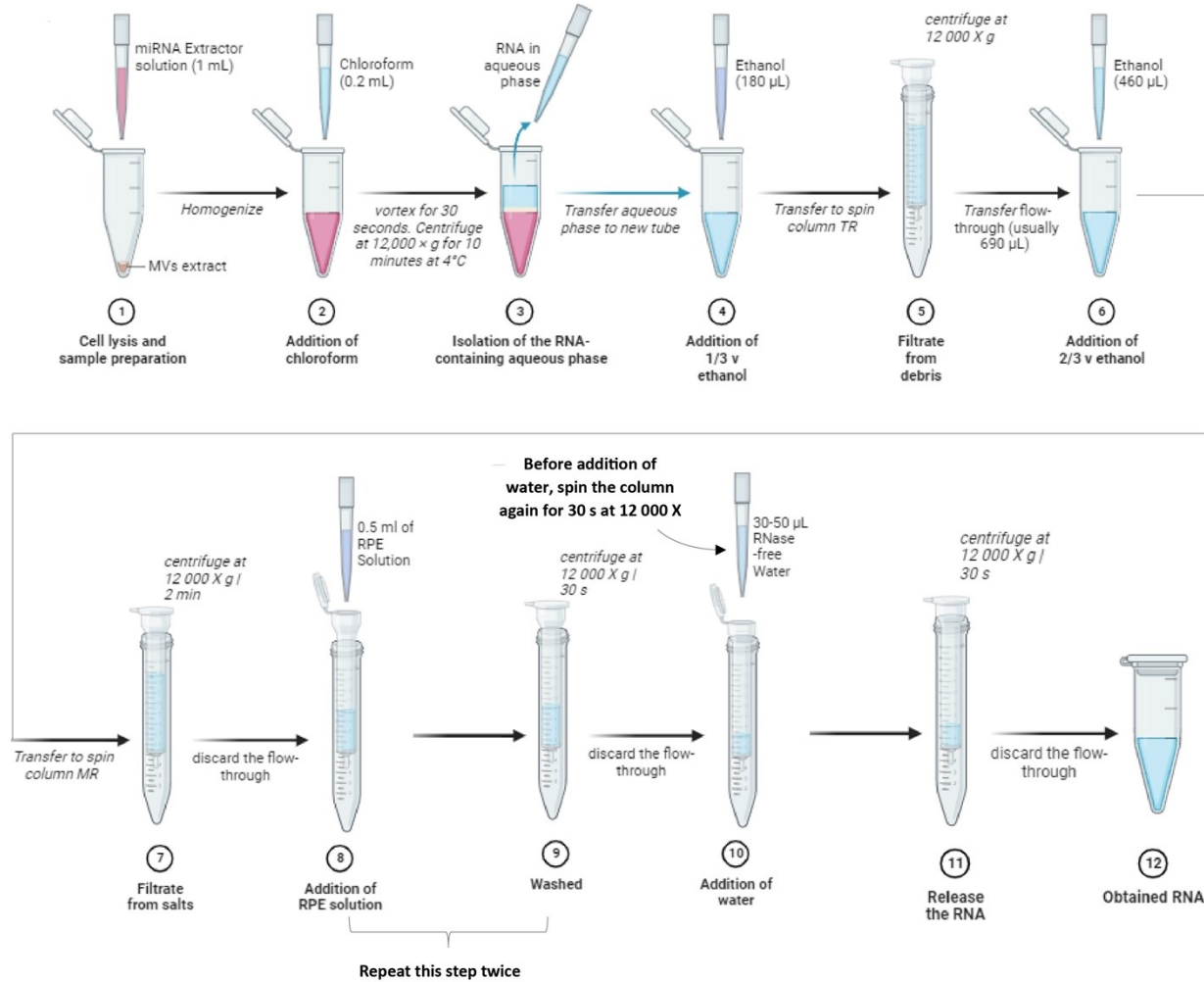
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53 **Scheme S1.** Flowchart illustrating the process of sample preparation from whole human blood

54 collection until their analysis.

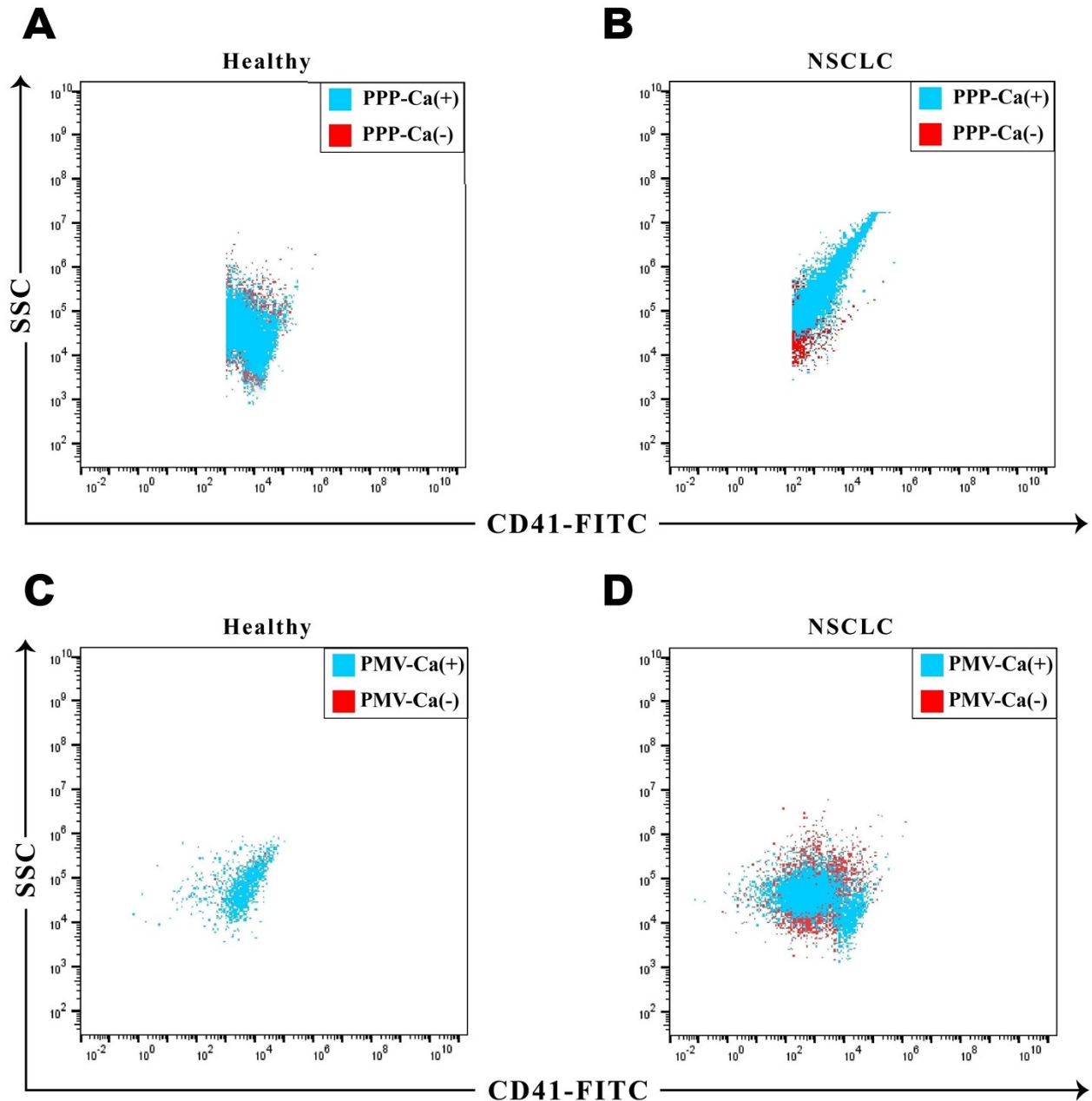


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57 **Scheme S2.** Illustration of the miRNA extraction process following the manufacturer's protocol.

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61 **Fig. S1** Platelet-poor plasma (PPP) from healthy donors (A) and patients with non-small cell lung

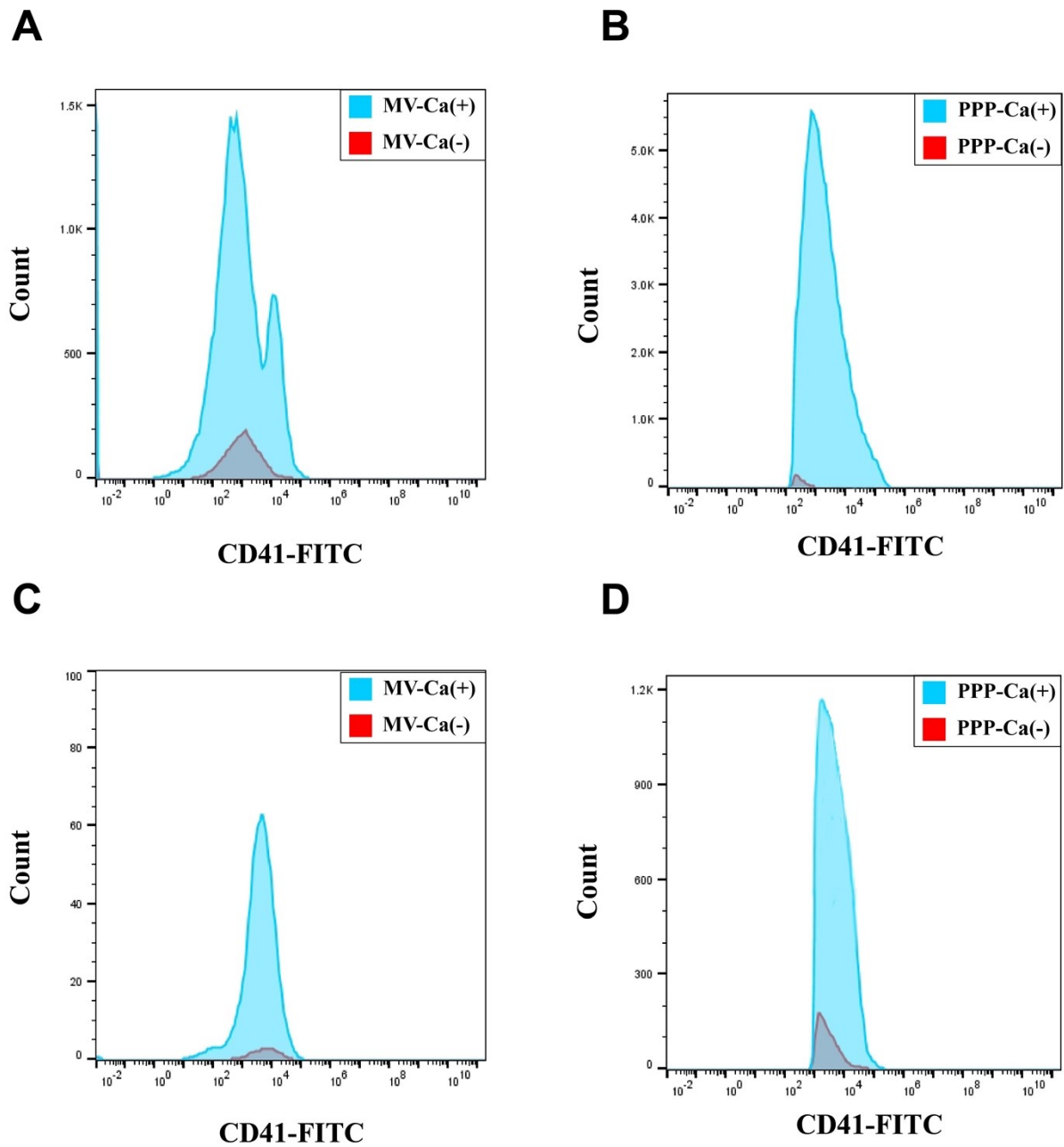
62 cancer (NSCLC) (B) before calcium activation (PPP-Ca(-)) overlaid with PPP after calcium

63 activation (PPP-Ca(+)). Platelet-derived microvesicles (PMVs) isolated from healthy donors (C)

64 and patients with non-small cell lung cancer (NSCLC) (D) before calcium activation (PMV-Ca(-)

65)) overlaid with PMVs after calcium activation (PMV-Ca(+)). The data shown represent one of at

66 least three independent experiments.



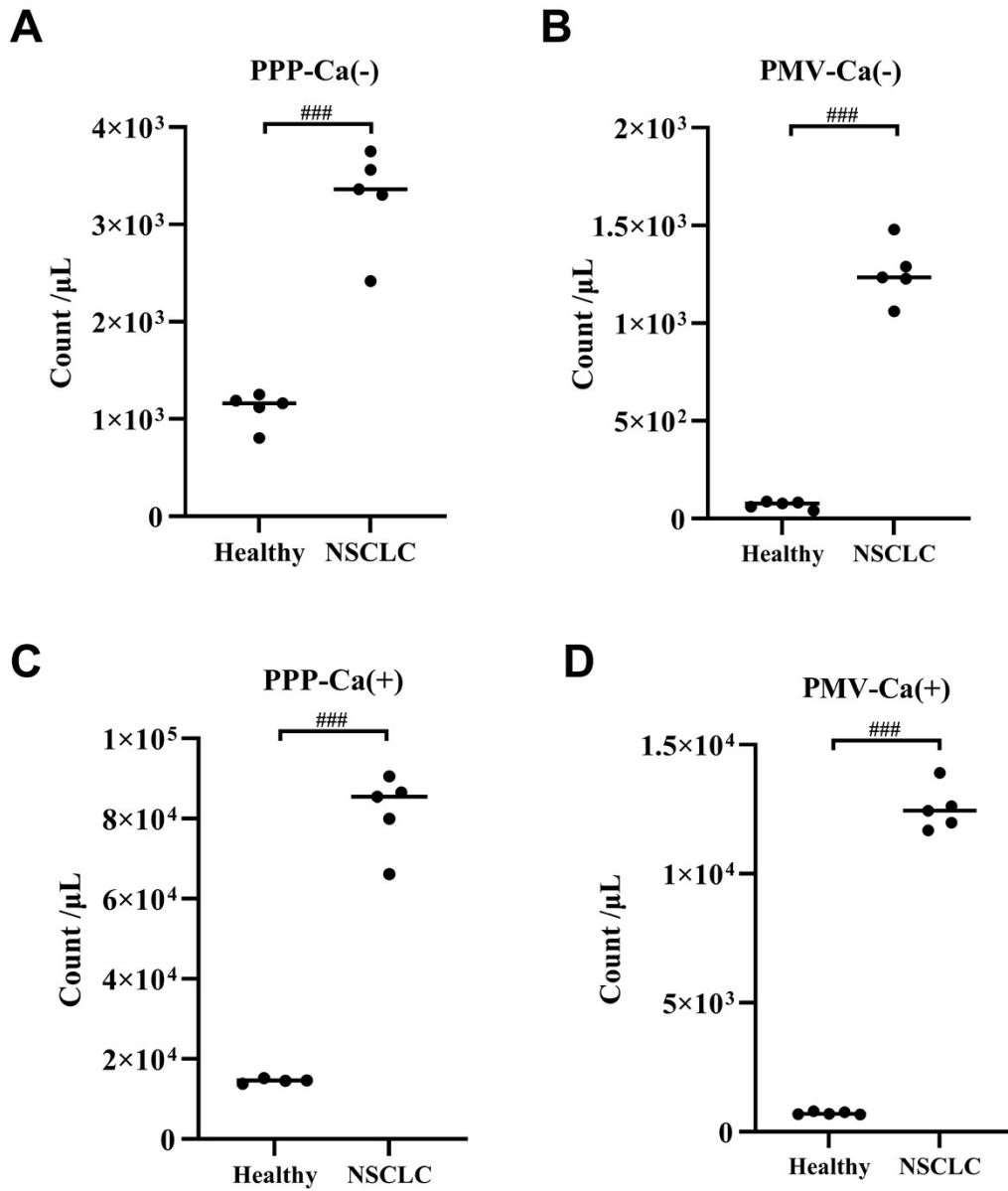
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69 **Fig. S2** CD41a levels in platelet-poor plasma (PPP) and isolated microvesicles (MVs) before and
 70 after calcium activation in patients with non-small cell lung cancer (NSCLC) (A, B) and healthy
 71 donors (C, D). The data shown represent one of at least three independent experiments.

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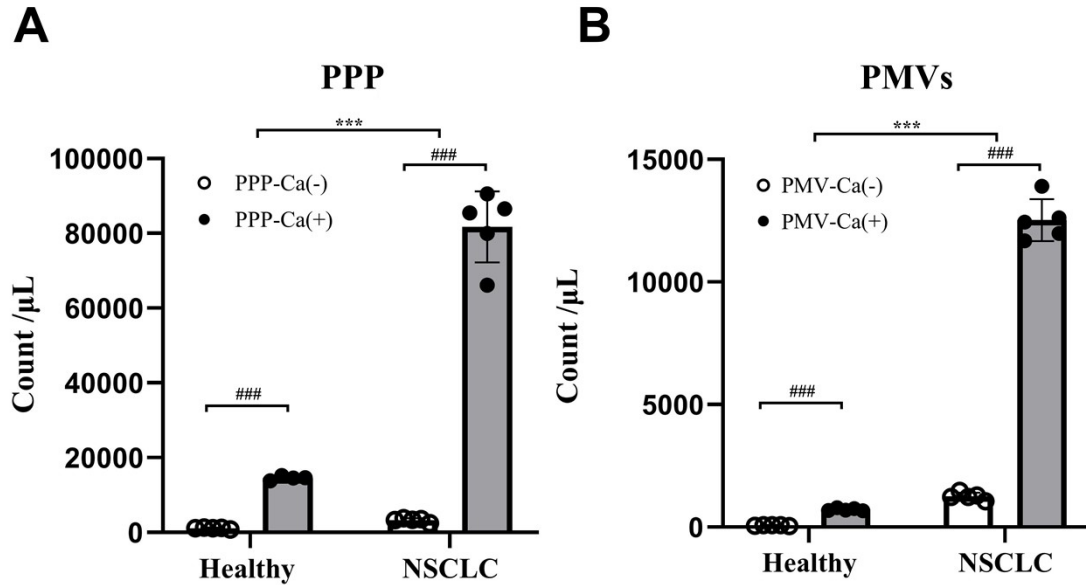
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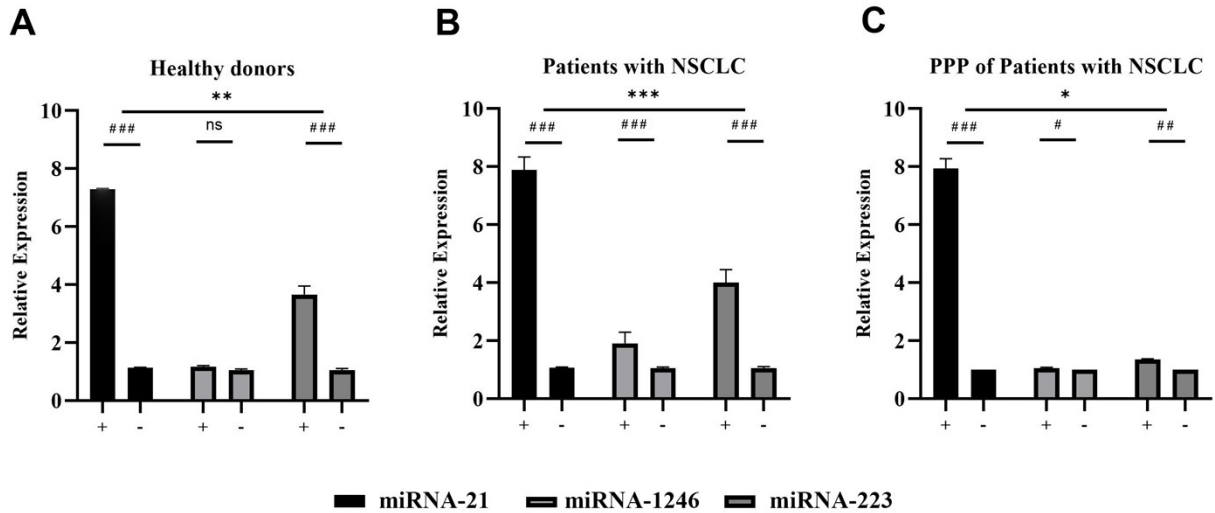
77 **Fig. S3** Comparison of CD41a levels in platelet-poor plasma (PPP) and isolated platelet-derived
 78 microvesicles (PMVs) before (A, B) and after (C, D) calcium activation in healthy donors and
 79 patients with non-small cell lung cancer (NSCLC). Data are expressed as mean \pm SD of five
 80 replicate measurements for each donor in both healthy and unhealthy groups. ### $p < 0.001$ (paired
 81 t-test).



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84 **Fig. S4** Comparison of CD41a concentration levels in platelet-poor plasma (PPP) (A) and isolated
85 microvesicles (MV)s (B) before and after calcium activation in healthy donors and patients with
86 non-small cell lung cancer (NSCLC). Data are expressed as mean \pm SD of five replicate
87 measurements for each donor in both healthy and unhealthy groups. *** p < 0.001 (One-Way
88 ANOVA with Duncan's multiple comparisons test), ### p < 0.001 (paired t-test).

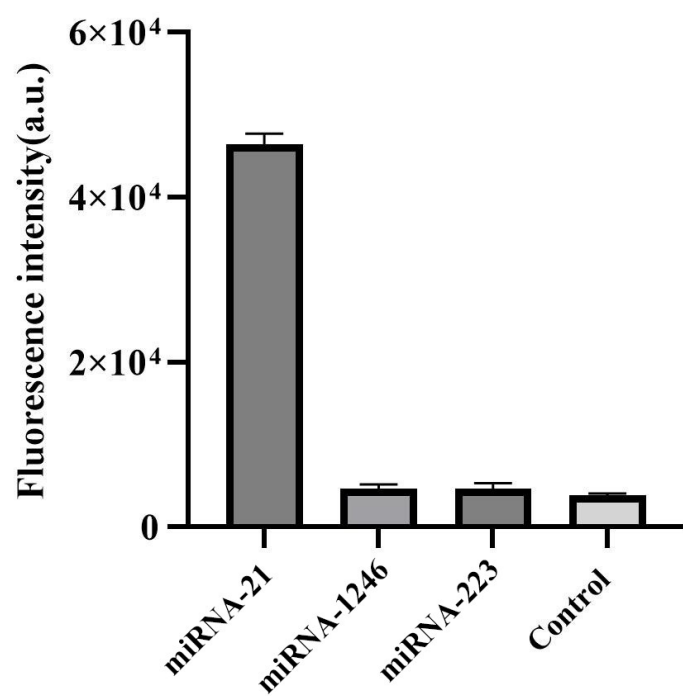
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93 **Fig. S5** Effect of calcium treatment on miRNA expression in platelet-derived microvesicles
 94 (PMVs) of healthy donors and NSCLC patients (A, B), and PPP of NSCLC patients (C). Data are
 95 expressed as mean \pm SD of five independent experiments. * $p < 0.05$, ** $p < 0.01$, *** $p <$
 96 0.001 (One-Way ANOVA with Duncan's multiple comparisons tests), ns: no significance,
 97 # $p < 0.05$, ## $p < 0.01$ ### $p < 0.001$ (paired t-test).

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100 **Fig. S6** Test of specificity of the liposome-based assay for mi-RNA21 detection (10 ng/mL) in the
101 presence of different miRNA sequences. The data are shown as the mean \pm SD (n = 3).