

Circulating small extracellular vesicles reflect the severity of myocardial damage in STEMI patients.

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Supplementary Tables

Table S1: Relationship between sEVs profile and relevant clinical and CMR features.

	STEMI site: Anterior	STEMI site: Non-anterior	<i>p value</i>
Number (particles/ml)	$4.7 \times 10^{11} \pm 1.07 \times 10^{11}$	$3.49 \times 10^{11} \pm 9.82 \times 10^{10}$	0.0001
Dimension (nm)	81.49 ± 13.94	80.97 ± 15.50	0.73
	Culprit lesion: LAD	Culprit lesion: Other vessels	
Number (particles/ml)	$4.4 \times 10^{11} \pm 1.29 \times 10^{11}$	$3.71 \times 10^{11} \pm 9.77 \times 10^{11}$	0.045
Dimension (nm)	81.92 ± 13.15	80.47 ± 16.33	0.49
	Presentation time: Late	Presentation time: Early	
Number (particles/ml)	$4.4 \times 10^{11} \pm 1.13 \times 10^{11}$	$3.79 \times 10^{11} \pm 1.18 \times 10^{11}$	0.038
Dimension (nm)	80.68 ± 13.59	81.63 ± 15.66	0.99
	MSI ≤ 0.5	MSI > 0.5	
Number (particles/ml)	$4.02 \times 10^{11} \pm 1.24 \times 10^{11}$	$4.14 \times 10^{11} \pm 1.12 \times 10^{11}$	0.59
Dimension (nm)	77.24 ± 13.44	88.10 ± 14.40	0.014
	Presence of MVO	Absence of MVO	
Number (particles/ml)	$4.1 \times 10^{11} \pm 1.26 \times 10^{11}$	$4.1 \times 10^{11} \pm 1.17 \times 10^{11}$	0.99
Dimension (nm)	74.24 ± 13.74	86.68 ± 13.27	< 0.002

sEVs number and size was evaluated by NTA after isolation from plasma collected 3-5 days post STEMI. Patients were divided according to STEMI site, culprit lesion artery, presentation time (Early < 3 hours, Late \geq 3 hours), MVO presence, MSI (low < 0.5; high > 0.5). Mean \pm SD are reported. In bold the statistically significant values.

Table S2: Correlations between sEV dimension/concentration and clinical/CMR parameters

	sEV concentration	sEV dimension
Peak Troponin	$r = -0.12; p = 0.42$	$r = -0.20; p = 0.20$
LVEDVi	$r = 0.05; p = 0.75$	$r = -0.16; p = 0.33$
LVESVi	$r = 0.13; p = 0.40$	$r = -0.20; p = 0.21$
LVEF	$r = -0.14; p = 0.37$	$r = 0.18; p = 0.25$
LV mass	$r = 0.10; p = 0.54$	$r = 0.01; p = 0.96$
RVEDVi	$r = -0.11; p = 0.50$	$r = 0.01; p = 0.97$
RVESVi	$r = -0.11; p = 0.47$	$r = 0.17; p = 0.30$
RVEF	$r = -0.08; p = 0.60$	$r = -0.19; p = 0.23$
AAR	$r = 0.17; p = 0.27$	$r = -0.02; p = 0.92$
AAR (% LV mass)	$r = 0.20; p = 0.20$	$r = -0.06; p = 0.71$
LGE mass	$r = -0.08; p = 0.63$	$r = -0.37; p = 0.01$
LGE (% LV mass)	$r = -0.07; p = 0.65$	$r = -0.40; p = 0.01$
MVO mass	$r = 0.09; p = 0.55$	$r = -0.32; p = 0.04$
MVO mass (% LV mass)	$r = 0.08; p = 0.62$	$r = -0.33; p = 0.03$
MVO mass (% LGE mass)	$r = 0.07; p = 0.66$	$r = -0.39; p = 0.01$
MSI	$r = 0.09; p = 0.59$	$r = 0.13; p = 0.43$

AAR: area at risk, CMR: cardiac magnetic resonance, LGE: late gadolinium enhancement, LV: left ventricular, LVEDVi: indexed left end-diastolic volume, LVEF: left ventricular ejection fraction, LVESVi: indexed left end-systolic volume, MVO: microvascular obstruction, MSI: myocardial salvage index, RVEDVi: indexed right end-diastolic volume, RVESVi: indexed right end-systolic volume, RVEF: indexed right end.

Table S3: The relation between MACSPlex-antigens and the associated genes.

Antigens	Genes
CD29	ITGB1
CD41b	ITGA2B
CD42a	GP9
CD9	CD9
HLA-ABC	HLA-A
CD81	CD81
CD56	NCAM1
CD62P	SELP
CD24	CD24
HLA-DRDPDQ	HLA-DRB1
CD31	PECAM1
CD45	PTPRC
CD40	CD40
CD3	CD3
CD44	CD44
CD25	IL2RA
CD2	CD2
CD8	CD8A
CD69	CD69
SSEA-4	FUT4
CD49e	ITGA5
CD146	MCAM
CD105	ENG
CD63	CD63
ROR1	ROR1
CD142	F3
CD20	MS4A1
CD11c	ITGAX
CD14	CD14
CD1-c	CD1C
CD4	CD4
CD209	CD209
CD86	CD86
CD133/1	PROM1
CD19	CD19

Gray antigens denote not expressed antigens.

Table S4. Details of pathway analysis.

ID	Group	Term	Term p-value	% Associated Genes	Nr. Genes	Associated Genes Found
R-HSA:9013408	Cell-Cell interaction	RHOG GTPase cycle	0.008854	2.7	2	[ITGB1, MCAM]
R-HSA:373755	Cell-Cell interaction	Semaphorin interactions	0.00689	3.08	2	[ITGB1, PTPRC]
R-HSA:1566948	Cell-Cell interaction	Elastic fibre formation	0.0033565	4.44	2	[ITGA5, ITGB1]
R-HSA:76009	Platelet activation	Platelet Aggregation (Plug Formation)	0.0025299	5.13	2	[GP9, ITGA2B]
R-HSA:202427	Cell-Cell interaction	Phosphorylation of CD3 and TCR zeta chains	0.0008045	9.09	2	[HLA-DRB1, PTPRC]
R-HSA:416700	Cell-Cell interaction	Other semaphorin interactions	0.0005976	10.53	2	[ITGB1, PTPRC]
R-HSA:913531	Immunity	Interferon Signaling	0.0005135	2.01	4	[CD44, HLA-A, HLA-DRB1, NCAM1]
R-HSA:3000178	Cell-Cell interaction	ECM proteoglycans	0.0004006	3.95	3	[ITGA2B, ITGB1, NCAM1]
R-HSA:75892	Platelet activation	Platelet Adhesion to exposed collagen	0.0003687	13.33	2	[GP9, ITGB1]
R-HSA:1566977	Cell-Cell interaction	Fibronectin matrix formation	0.0000532	33.33	2	[ITGA5, ITGB1]
R-HSA:877300	Immunity	Interferon gamma signaling	0.0000248	4.4	4	[CD44, HLA-A, HLA-DRB1, NCAM1]
R-HSA:76002	Platelet activation	Platelet activation, signaling and aggregation	0.0000076	2.28	6	[CD63, CD9, GP9, ITGA2B, PECAM1, SELP]
R-HSA:76005	Platelet activation	Response to elevated platelet cytosolic Ca ²⁺	0.0000046	3.73	5	[CD63, CD9, ITGA2B, PECAM1, SELP]
R-HSA:198933	Immunity	Immunoregulatory interactions between Lymphoid and	0.0000043	3.79	5	[CD40, CD81, CD8A, HLA-A, ITGB1]

		non-Lymphoid cells				
R-HSA:114608	Platelet activation	Platelet degranulation	0.0000038	3.88	5	[CD63, CD9, ITGA2B, PECAM1, SELP]
R-HSA:373760	Cell-Cell interaction	L1CAM interactions	0.0000026	4.2	5	[CD24, ITGA2B, ITGA5, ITGB1, NCAM1]
R-HSA:216083	Cell-Cell interaction	Integrin cell surface interactions	0.0000005	5.88	5	[CD44, ITGA2B, ITGA5, ITGB1, PECAM1]
R-HSA:202733	Cell-Cell interaction	Cell surface interactions at the vascular wall	0.0000002	4.38	6	[CD2, CD44, ITGA5, ITGB1, PECAM1, SELP]
R-HSA:445144	Cell-Cell interaction	Signal transduction by L1	0.0000001	19.05	4	[ITGA2B, ITGA5, ITGB1, NCAM1]

Table S5: Correlations between CD41-CD61 expression and clinical/CMR parameters

CD41-CD61 expression	
Peak Troponin	$r = -0.09; p = 0.55$
LVEDVi	$r = -0.03; p = 0.83$
LVESVi	$r = -0.15; p = 0.34$
LVEF	$r = 0.31; p = 0.04$
LV mass	$r = 0.05; p = 0.76$
RVEDVi	$r = 0.16; p = 0.32$
RVESVi	$r = 0.01; p = 0.94$
RVEF	$r = 0.19; p = 0.22$
AAR	$r = -0.18; p = 0.25$
AAR (% LV mass)	$r = -0.21; p = 0.19$
LGE mass	$r = -0.20; p = 0.19$
LGE (% LV mass)	$r = -0.20; p = 0.20$
MVO mass	$r = -0.40; p = 0.01$
MVO mass (% LV mass)	$r = -0.39; p = 0.01$
MVO mass (% LGE mass)	$r = -0.35; p = 0.02$
MSI	$r = 0.19; p = 0.24$

AAR: area at risk, CMR: cardiac magnetic resonance, LGE: late gadolinium enhancement, LV: left ventricular, LVEDVi: indexed left end-diastolic volume, LVEF: left ventricular ejection fraction, LVESVi: indexed left end-systolic volume, MVO: microvascular obstruction, MSI: myocardial salvage index, RVEDVi: indexed right end-diastolic volume, RVESVi: indexed right end-systolic volume, RVEF: indexed right end.

Table S6. sEV characteristics analyzed according to gender.

	Male	Female	<i>p</i>
Number (particles/ml)	$4.19 \times 10^{11} \pm 1.20 \times 10^{11}$	$3.76 \times 10^{11} \pm 1.19 \times 10^{11}$	0.27
Dimension (nm)	81.82 ± 15.58	76.86 ± 10.99	0.51
CD41-CD61 expression (ng/sEV)	$9.2\text{e-}009 \pm 8.2\text{e-}009$	$8.7\text{e-}009 \pm 2.9\text{e-}009$	0.41

Table S7. Correlation between sEV parameters and age of patients

	r	p
Number (particles/ml)	0.06	0.68
Dimension (nm)	-0.18	0.25
CD41-CD61 expression	-0.17	0.27

Table S8. ROC analysis

	AUC	95% CI
Concentration	0.53	0.34-0.70
Size	0.75	0.59-0.90
CD41-CD61	0.71	0.55-0.86
Peak Troponin	0.84	0.74-0.97
Model	0.95	0.89-1.00

AUC: area under the curve, CI: confidence interval

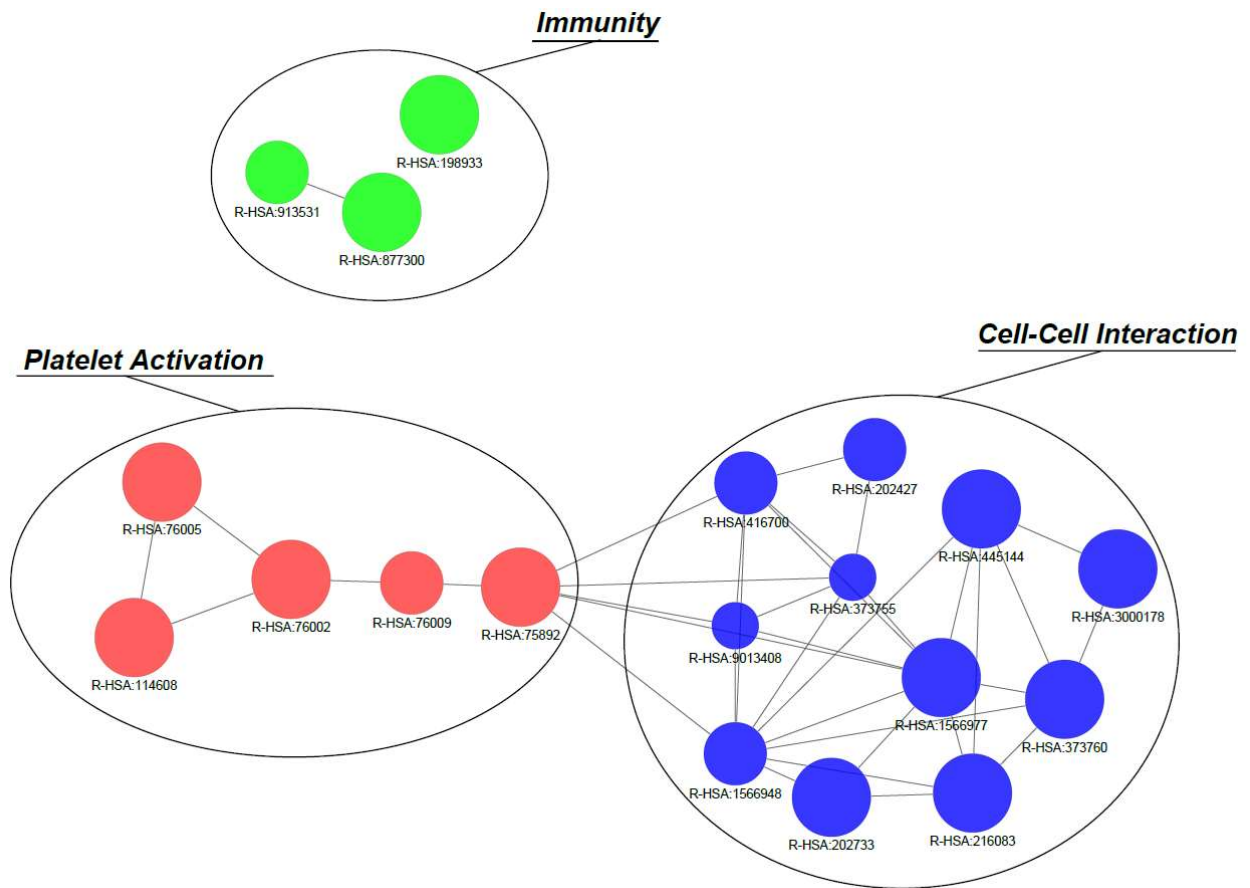


Figure S1: Network graph summarizing pathways analysis results, performed using the expressed antigens. Each node represents a specific Reactome pathway while the links thickness denotes the overlapping degree (k-score) between nodes. The node size reflects the significance: the higher the node, the more significant the pathway.