

## Supplementary Information

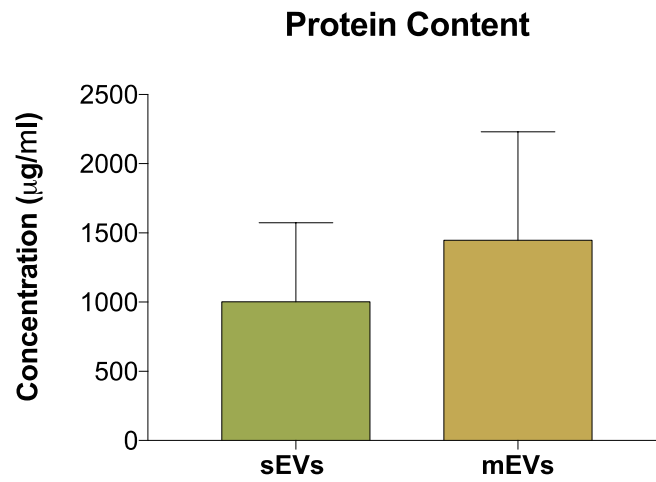
### Controlling the fate of regenerative cells with engineered platelet-derived extracellular vesicles

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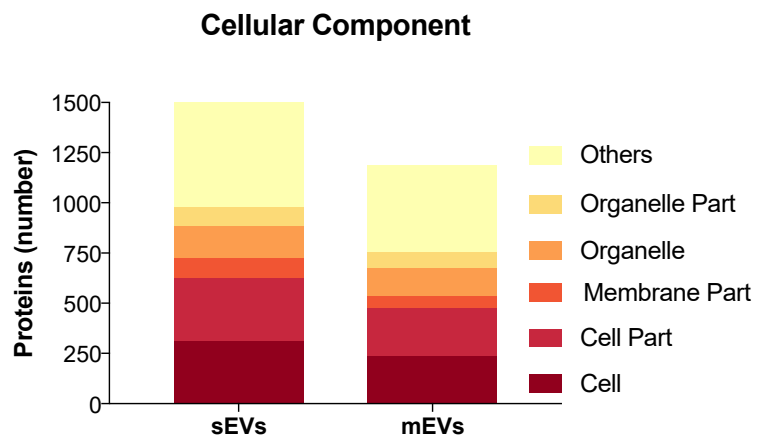
**Table S1** Primers sequences for RT-qPCR analysis.

Gene	Primer Sequence	NCBI Code
<b><i>GADPH</i></b>	F: TGCACCACCAACTGCTTAGC	NM_33197
	R: GGCATGGACTGTGGTCATGAG	
<b><i>ACTIN</i></b>	F: CTGGAACGGTGAAGGTGACA	AK223055
	R: AAGGGACTTCCTGTAACAA	
<b><i>GUSB</i></b>	F: GATGTAGGTGGTGGGTGTCG	NM_000181.4
	R: GCTCCGAATCACTATCGCCA	
<b><i>SOX9</i></b>	F: TTCATGAAGATGACCGACGC	NM_000346.3
	R: GTCCAGTCGTAGCCCTTGAG	
<b><i>COMP</i></b>	F: AGGATGGAGACGGACATCAG	NM_000095.2
	R: TCTGCATCAAAGTCGTCCTG	

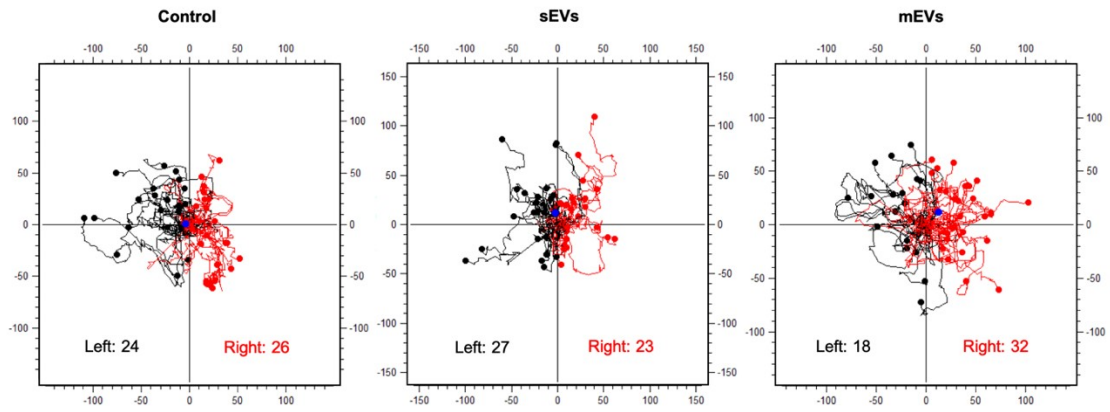
	F: TTCCAGACCAGCAGCACTC	
<b><i>RUNX2</i></b>	R: CAGCGTCAACACCATCATTC	NM_001024630
	F: CCAAGGACGCTGGGAAATCT	
<b><i>ALP</i></b>	R: TATGCATGAGCTGGTAGGCG	NM_000478.3
	F: TCGCACAGACACTCTGGAAAA	
<b><i>MKX</i></b>	R: TGTTAAGGCCATAGCTGCGT	NM_173576.2
	F: CCGCGTCTGTGAACCTTTAC	
<b><i>TNDM</i></b>	R: CACCCACCAGTTACAAGGCA	NM_022144.2
	F: GGACATCGCGTGGGTGAA	
<b><i>NOS2</i></b>	R: TTTATCGCTCGGAGCCTGC	NM_000625.4
	F: GGAAAACCAAGTGGGAGCAT	
<b><i>ARG-1</i></b>	R: TGTGGTTGTCAGTGGAGTGT	NM_000045.4
	F: TGCTCTACAAGGGATCGGGT	
<b><i>MRC1</i></b>	R: ACACGCCAAACAAGAACATGA	NM_002438.4
	F: GAAGAGTGTGGTTGGGCGTA	
<b><i>CCR7</i></b>	R: GCATTTGTAGTCCTGCTGCG	NM_001301716.2
	F: TTTTGGGCTGTTTCAGTGCAG	
<b><i>IL-15</i></b>	R: GCTGTTACTTTGCAACTGGGG	NM_000585.5



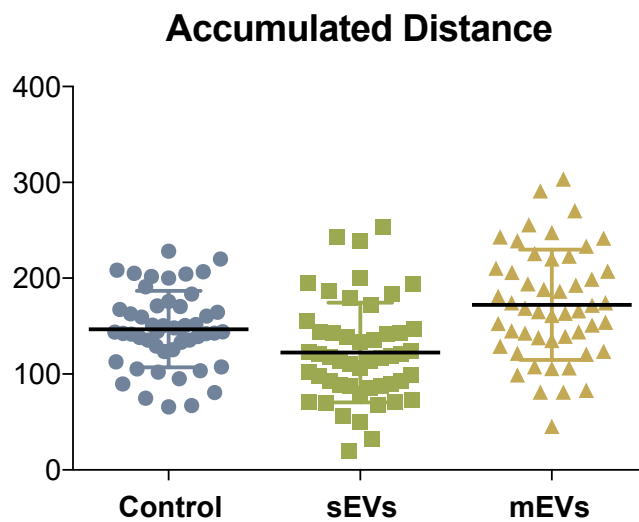
**Fig. S1** Protein content of sEVs and mEVs assessed by BCA analysis. Quantification from three independent isolations (n = 3).



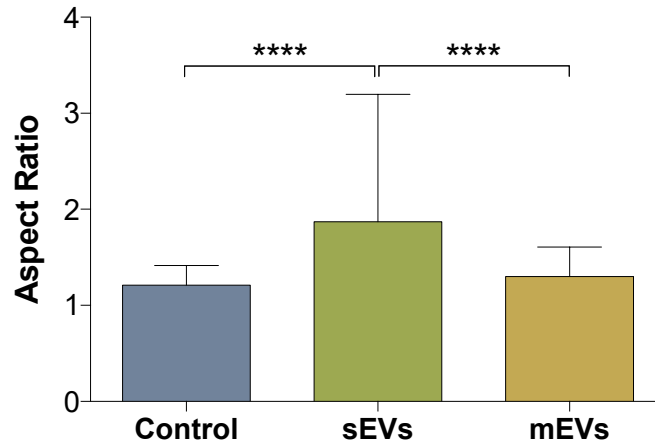
**Fig. S2** Number of proteins identified in each EVs populations grouped according to their cellular component.



**Fig. S3** Cell trajectory plots of HUVECs cultured on chemotaxis  $\mu$ -slides, consisting of two chambers separated by a central channel. HUVECs were seeded on the left chamber while the EVs were added in the right chamber. Movement to the right (red trajectory) denotes chemoattraction by sEVs or mEVs, while movement to the left (black trajectory) denotes random movement. Blue spot represents the calculated center of mass.



**Fig. S4** Accumulated distance representing cell trajectories and mobility during the chemotaxis migration assay (n = 50 cells).



**Fig. S5** Aspect ratio of macrophages incubated with sEVs and mEVs or unstimulated (control). The aspect ratio was calculated dividing the long by the short axis of 80 - 100 cells from 8 - 10 different images taken from three independent samples (\*\*\*\* $p < 0.0001$ ).

**Table S2** Most abundant proteins found in platelet-derived sEVs and mEVs through mass spectrometry analysis, listed according to their relative abundance.

Protein ID	Gene Symbol	Description
P08514	<i>ITGA2B</i>	Integrin alpha-IIb
P05106	<i>ITGB3</i>	Integrin beta-3
P02751	<i>FNI</i>	Fibronectin
P07996	<i>THBS1</i>	Thrombospondin-1
P04003	<i>C4BPA</i>	C4b-binding protein alpha chain
P01024	<i>C3</i>	Complement C3
P60709	<i>ACTB</i>	Actin, cytoplasmic 1

<b>P18206</b>	<i>VCL</i>	Vinculin
<b>P24844</b>	<i>MYL9</i>	Myosin regulatory light polypeptide 9
<b>P06396</b>	<i>GSN</i>	Gelsolin
<b>H7BYY1</b>	<i>TPM1</i>	Tropomyosin 1 (Alpha), isoform CRA_m
<b>P12814</b>	<i>ACTN1</i>	Alpha-actinin-1
<b>P35579</b>	<i>MYH9</i>	Myosin-9
<b>P21333</b>	<i>FLNA</i>	Filamin-A
<b>Q9Y490</b>	<i>TLN1</i>	Talin-1
<b>P04264</b>	<i>KRT1</i>	Keratin, type II cytoskeletal 1
<b>P01871</b>	<i>IGHM</i>	Immunoglobulin heavy constant mu
<b>P01834</b>	<i>IGKC</i>	Immunoglobulin kappa constant
<b>A0A4W9A917</b>	<i>IGHG3</i>	Immunoglobulin heavy constant gamma 3 (Fragment)
<b>A0A0B4J231</b>	<i>IGLL5</i>	Immunoglobulin lambda-like polypeptide 5
<b>P01857</b>	<i>IGHG1</i>	Immunoglobulin heavy constant gamma 1
<b>P01876</b>	<i>IGHA1</i>	Immunoglobulin heavy constant alpha 1
<b>A0A286YHEY4</b>	<i>IGHG2</i>	Immunoglobulin heavy constant gamma 2 (Fragment)
<b>P00738</b>	<i>HP</i>	Haptoglobin
<b>P02790</b>	<i>HPX</i>	Hemopexin
<b>P02768-1</b>	<i>ALB</i>	Serum albumin isoform 1
<b>P02787</b>	<i>TF</i>	Serotransferrin
<b>D6RF35</b>	<i>GC</i>	Vitamin D-binding protein
<b>P01023</b>	<i>A2M</i>	Alpha-2-macroglobulin
<b>C9JEU5</b>	<i>FGG</i>	Fibrinogen gamma chain

<b>P02675</b>	<i>FGB</i>	Fibrinogen beta chain
<b>P02671</b>	<i>FGA</i>	Fibrinogen alpha chain
<b>O43866</b>	<i>CD5L</i>	CD5 antigen-like
<b>P02647</b>	<i>APOAI</i>	Apolipoprotein A-I
<b>P02749</b>	<i>APOH</i>	Beta-2-glycoprotein 1
<b>P27105</b>	<i>STOM</i>	Erythrocyte band 7 integral membrane protein

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**Table S3** Selected proteins of sEVs and mEVs according to their structural function based on total proteome of the vesicles by mass spectrometry analysis, listed according to their relative abundance.

<b>Protein ID</b>	<b>Gene Symbol</b>	<b>Description</b>
<b>P13501</b>	<i>CCL5</i>	C-C motif chemokine 5
<b>P02775</b>	<i>PPBP</i>	Platelet basic protein
<b>P01137</b>	<i>TGFB1</i>	Transforming growth factor beta-1 proprotein
<b>Q14766</b>	<i>LTBP1</i>	Latent-transforming growth factor beta-binding protein 1
<b>P62993</b>	<i>GRB2</i>	Growth factor receptor-bound protein 2
<b>Q04756</b>	<i>HGFAC</i>	Hepatocyte growth factor activator
<b>P26927</b>	<i>MST1</i>	Hepatocyte growth factor-like protein
<b>O00602</b>	<i>FCN1</i>	Ficolin-1
<b>Q15485</b>	<i>FCN2</i>	Ficolin-2
<b>O75636</b>	<i>FCN3</i>	Ficolin-3

<b>O75882</b>	<i>ATRN</i>	Attractin
<b>P11226</b>	<i>MBL2</i>	Mannose-binding protein C
<b>P05556</b>	<i>ITGB1</i>	Integrin beta-1
<b>P08514</b>	<i>ITGA2</i>	Integrin alpha-2
<b>D3DSM0</b>	<i>ITGB2</i>	Integrin beta
<b>P08648</b>	<i>ITGA5</i>	Integrin alpha-5
<b>A0A0A0MTH3</b>	<i>ILK</i>	Integrin-linked protein kinase
<b>Q15363</b>	<i>TMED2</i>	Transmembrane emp24 domain-containing protein 2
<b>Q8NBN3</b>	<i>TMEM87A</i>	Transmembrane protein 87A
<b>A0A494BZZ8</b>	<i>TMEM87B</i>	Transmembrane protein 87B (Fragment)
<b>Q9BVK6</b>	<i>TMED9</i>	Transmembrane emp24 domain-containing protein 9
<b>Q99805</b>	<i>TM9SF2</i>	Transmembrane 9 superfamily member 2
<b>P49755</b>	<i>TMED10</i>	Transmembrane emp24 domain-containing protein 10
<b>Q8WWA1</b>	<i>TMEM40</i>	Transmembrane protein 40
<b>V9GY93</b>	<i>TMEM165</i>	GDT1 family protein (Fragment)
<b>Q15836</b>	<i>VAMP1</i>	Vesicle-associated membrane protein 1
<b>P51809</b>	<i>VAMP7</i>	Vesicle-associated membrane protein 7
<b>Q9BV40</b>	<i>VAMP8</i>	Vesicle-associated membrane protein 8
<b>P04275</b>	<i>vWF</i>	von Willebrand factor
<b>Q16610</b>	<i>ECM1</i>	Extracellular matrix protein 1
<b>P05452</b>	<i>CLEC3B</i>	Tetranectin
<b>P07359</b>	<i>GP1BA</i>	Glycoprotein Ib (Platelet), alpha polypeptide



**Table S4** Comparison of the PANTHER pathways between the *Homo sapiens* proteome and all the proteins identified in sEVs and mEVs. Bolt common PANTHER pathways detected in both EVs subpopulations.

PANTHER Pathways	<i>Homo sapiens</i>				Fold	
	- REFLIST (20851)	Sample	Expected	+/-	Enrichment	p-value
<b>sEVs</b>						
5HT1 type receptor mediated signaling pathway (P04373)	46	8	1.13	+	7.08	6.27E-03
5HT2 type receptor mediated signaling pathway (P04374)	67	9	1.65	+	5.47	1.28E-02
5HT4 type receptor mediated signaling pathway (P04376)	33	7	0.81	+	8.64	6.30E-03
Angiotensin II-stimulated signaling through	39	7	0.96	+	7.31	1.60E-02

G proteins and beta-arrestin (P05911)

B cell activation (P00010)	70	10	1.72	+	5.82	3.23E-03
Beta1 adrenergic receptor signaling pathway (P04377)	46	8	1.13	+	7.08	6.27E-03
Beta2 adrenergic receptor signaling pathway (P04378)	46	8	1.13	+	7.08	6.27E-03
Beta3 adrenergic receptor signaling pathway (P04379)	29	7	0.71	+	9.83	3.07E-03
<b>Blood coagulation (P00011)</b>	<b>46</b>	<b>20</b>	<b>1.13</b>	<b>+</b>	<b>17.71</b>	<b>6.11E-15</b>
CCKR signaling map (P06959)	172	15	4.22	+	3.55	7.32E-03
Corticotropin releasing factor receptor signaling pathway (P04380)	32	8	0.79	+	10.18	6.22E-04
Cytoskeletal regulation by Rho GTPase (P00016)	83	20	2.04	+	9.81	6.82E-11
Dopamine receptor mediated signaling	57	13	1.40	+	9.29	1.55E-06

pathway (P05912)						
EGF receptor signaling pathway (P00018)	136	16	3.34	+	4.79	1.24E-04
Enkephalin release (P05913)	35	7	0.86	+	8.14	8.75E-03
<b>FGF signaling pathway (P00021)</b>	<b>121</b>	<b>15</b>	<b>2.97</b>	<b>+</b>	<b>5.05</b>	<b>1.50E-04</b>
GABA-B receptor II signaling (P05731)	36	7	0.88	+	7.92	1.02E-02
Glycolysis (P00024)	19	8	0.47	+	17.15	2.36E-05
Gonadotropin-releasing hormone receptor pathway (P06664)	232	20	5.70	+	3.51	4.94E-04
Huntington disease (P00029)	143	22	3.51	+	6.27	1.16E-08
Inflammation mediated by chemokine and cytokine signaling pathway (P00031)	255	32	6.26	+	5.11	8.27E-11
<b>Integrin signalling pathway (P00034)</b>	<b>191</b>	<b>31</b>	<b>4.69</b>	<b>+</b>	<b>6.61</b>	<b>3.93E-13</b>
Metabotropic glutamate receptor group II pathway (P00040)	48	10	1.18	+	8.48	1.64E-04
Metabotropic glutamate receptor group III	69	10	1.69	+	5.90	2.89E-03

pathway (P00039)							
Muscarinic acetylcholine receptor 1 and 3 signaling pathway (P00042)	60	9	1.47	+	6.11	5.92E-03	
Muscarinic acetylcholine receptor 2 and 4 signaling pathway (P00043)	61	11	1.50	+	7.34	1.67E-04	
Nicotinic acetylcholine receptor signaling pathway (P00044)	105	12	2.58	+	4.65	3.81E-03	
Opioid prodynorphin pathway (P05916)	33	8	0.81	+	9.87	7.56E-04	
Opioid proenkephalin pathway (P05915)	34	8	0.83	+	9.58	9.15E-04	
Opioid proopiomelanocortin pathway (P05917)	34	8	0.83	+	9.58	9.15E-04	
Oxytocin receptor mediated signaling pathway (P04391)	58	9	1.42	+	6.32	4.66E-03	
Parkinson disease (P00049)	98	17	2.41	+	7.06	3.36E-07	
Pentose phosphate pathway (P02762)	9	4	0.22	+	18.10	3.02E-02	

PI3 kinase pathway (P00048)	52	9	1.28	+	7.05	2.15E-03
Plasminogen activating cascade (P00050)	18	5	0.44	+	11.31	2.81E-02
Ras Pathway (P04393)	74	9	1.82	+	4.95	2.55E-02
Thyrotropin-releasing hormone receptor signaling pathway (P04394)	60	9	1.47	+	6.11	5.92E-03

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**mEVs**

<b>Blood coagulation (P00011)</b>	<b>46</b>	<b>23</b>	<b>0.92</b>	+	<b>25.06</b>	<b>2.23E-20</b>
Corticotropin releasing factor receptor signaling pathway (P04380)	32	6	0.64	+	9.40	1.35E-02
Cytoskeletal regulation by Rho GTPase (P00016)	83	16	1.66	+	9.66	1.36E-08
Dopamine receptor mediated signaling pathway (P05912)	57	11	1.14	+	9.67	1.22E-05
<b>FGF signaling pathway (P00021)</b>	<b>121</b>	<b>10</b>	<b>2.41</b>	+	<b>4.14</b>	<b>3.99E-02</b>

Glycolysis (P00024)	19	9	0.38	+	23.74	2.94E-07
Huntington disease (P00029)	143	15	2.85	+	5.26	8.12E-05
Inflammation mediated by chemokine and cytokine signaling pathway (P00031)	255	18	5.09	+	3.54	1.23E-03
<b>Integrin signalling pathway (P00034)</b>	<b>191</b>	<b>25</b>	<b>3.81</b>	<b>+</b>	<b>6.56</b>	<b>1.65E-10</b>
Metabotropic glutamate receptor group II pathway (P00040)	48	8	0.96	+	8.35	1.93E-03
Metabotropic glutamate receptor group III pathway (P00039)	69	8	1.38	+	5.81	2.00E-02
Muscarinic acetylcholine receptor 2 and 4 signaling pathway (P00043)	61	9	1.22	+	7.40	1.34E-03
Opioid prodynorphin pathway (P05916)	33	6	0.66	+	9.11	1.57E-02
Opioid proenkephalin pathway (P05915)	34	6	0.68	+	8.85	1.82E-02
Opioid proopiomelanocortin pathway (P05917)	34	6	0.68	+	8.85	1.82E-02

Oxytocin receptor mediated signaling pathway (P04391)	58	7	1.16	+	6.05	4.20E-02
Parkinson disease (P00049)	98	18	1.96	+	9.21	1.83E-09
Pentose phosphate pathway (P02762)	9	4	0.18	+	22.28	1.38E-02
Plasminogen activating cascade (P00050)	18	6	0.36	+	16.71	8.31E-04
Unclassified	18243	280	363.97	-	0.77	0.00E00

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**Table S5** Quantification of the cytokines released by macrophages after the sEVs and mEVs stimulus and in control group (no stimulation).

	<b>GM-CSF</b> (pg/mL)	<b>IL-1<math>\beta</math></b> (pg/mL)	<b>IL-6</b> (pg/mL)	<b>IL-8</b> (pg/mL)	<b>IL-10</b> (pg/mL)
<b>Control</b>	2.30 $\pm$ 0.33	16.33 $\pm$ 7.64	2.91 $\pm$ 0.21	5684.10 $\pm$ 340.32	3.51 $\pm$ 0.60
<b>sEVs</b>	9.24 $\pm$ 5.92	255.67 $\pm$ 249.34	15.61 $\pm$ 4.87	18014.65 $\pm$ 934.07	6.96 $\pm$ 3.45
<b>mEVs</b>	2.10 $\pm$ 0.17	13.33 $\pm$ 4.04	2.88 $\pm$ 0.18	4837.09 $\pm$ 561.55	3.58 $\pm$ 0.48

**Video S1 and S2** The co-localization of small and medium extracellular vesicles and hASCs was assessed by confocal laser scanning microscopy (CLSM, TCS SP8, Leica, Wetzlar, Germany). 3D images were generated from z-stacked images previously acquired (1.0  $\mu$ m pixel size) and converted to a film sequence using the Leica application suite LAS X 3D vs 3.5.0 software.