

Appendices

Supplementary Figure 1. KEGG_PATHWAY, hsa04151:PI3K-Akt signaling pathway. Proteins identified by proteomic analysis of MSC-EV are highlighted with red asterisks.

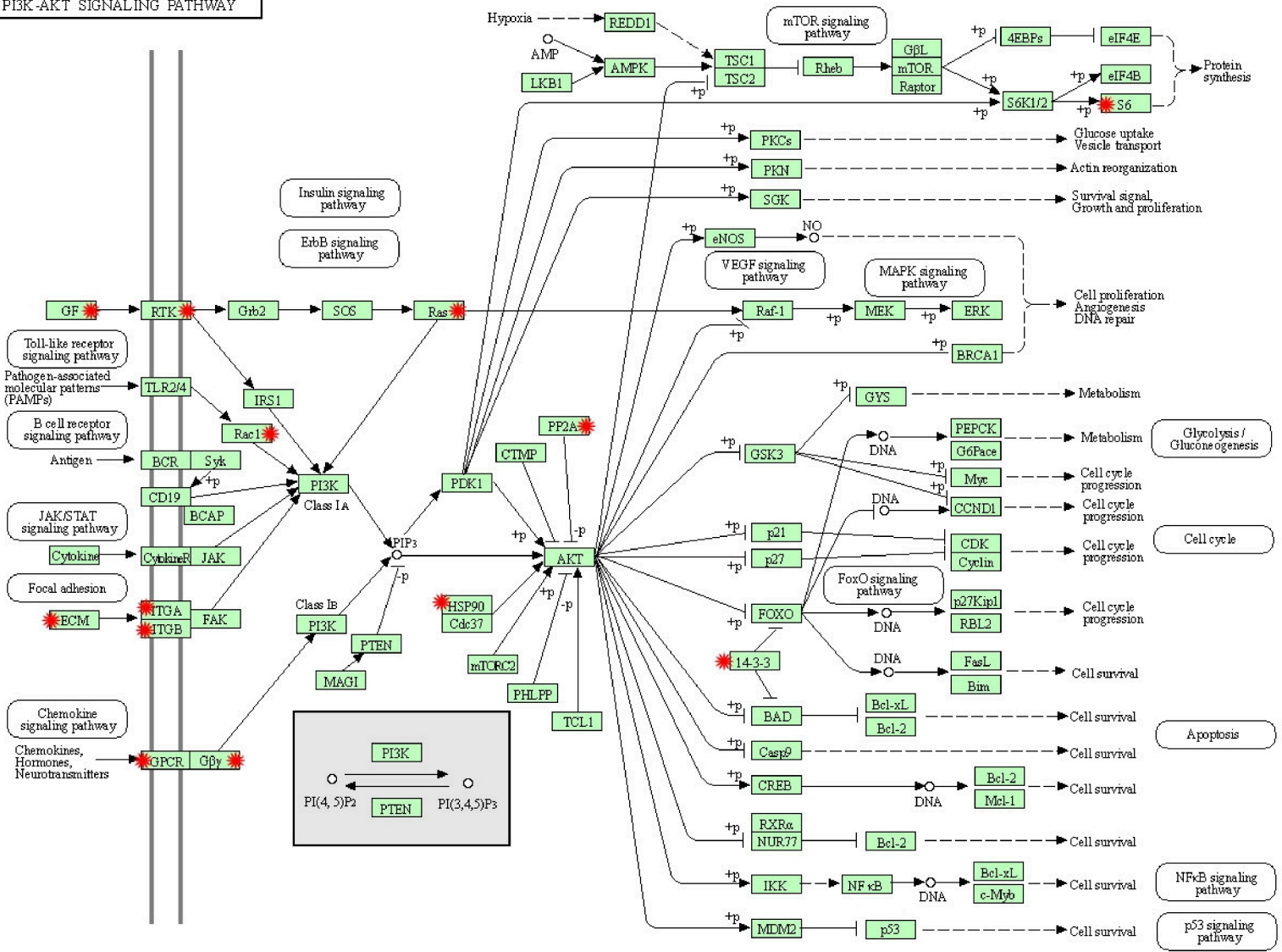
Supplementary Figure 2. The heat map analysis of the cytokine levels in MSC-EV.

Supplementary Table 1. The results of proteomic analysis of MSC-EV and bioinformatic analysis of the MSC-EV proteome.

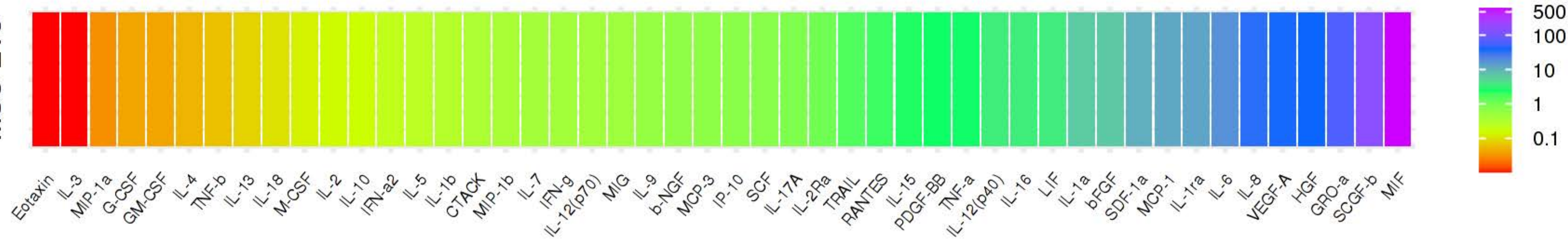
Supplementary Table 2. EV markers identified by proteomic analysis.

Supplementary Table 3. Analysis of cytokines in MSC-EV.

PI3K-AKT SIGNALING PATHWAY



MSC-EVs



Supplementary Table 2. EV markers identified by proteomic analysis.

MISEV2018	Results of proteomic analysis
1- Transmembrane or GPI-anchored proteins associated to plasma membrane and/or endosomes	
1a: non-tissue specific	
Tetraspanins (CD63, CD81, CD82)	CD63, CD81, CD82
other multi-pass membrane proteins (CD47; heterotrimeric G proteins GNA*)	GNA11, GNA13, GNAQ
MHC class I (HLA-A/B/C, H2-K/D/Q)	HLLA, HLAB, HLAC, HLAH, HLAH
Integrins (ITGA*/ITGB*)	ITGB1, ITGB3, ITGAV, ITGA2B, ITGA5, ITGA4, ITGA2, ITGB5, ITGA6, ITGA3, ITGA1, ITGA11
transferrin receptor (TFR2)	-
LAMP1/2	LAMP2
heparan sulfate proteoglycans including syndecans (SDC*)	SDC1
EMMPRIN (BSG)	BSG
ADAM10	ADAM10
GPI-anchored 5'nucleotidase CD73 (NT5E)	NT5E
complement-binding proteins CD55 and CD59	CD55, CD59
sonic hedgehog (SHH)	-
2 - Cytosolic proteins recovered in EVs	
2a: with lipid or membrane protein-binding ability	
ESCRT-I/II/III (TSG101, CHMP*)	TSG101, CHMP4B
accessory proteins: ALIX (PDCD6IP), VPS4A/B	PDCD6IP
ARRDC1	-
Flotillins-1 and 2 (FLOT1/2)	FLOT1, FLOT2
caveolins (CAV*)	CAV1
EHD*	EHD4, EHD1, EHD3, EHD2
RHOA	-
annexins (ANXA*)	ANXA1, ANXA2, ANXA6, ANXA5, ANXA4, ANXA7, ANXA11
Heat shock proteins HSC70 (HSPA8), and HSP84 (HSP90AB1)	HSPA8, HSP90AB1
ARF6	ARF6
syntenin (SDCBP)	SDCBP
2 - Cytosolic proteins recovered in EVs	
2b: promiscuous incorporation in EVs (and possibly exomeres)	
Heat shock protein HSP70 (HSPA1A)	HSPA1A
cytoskeleton: actin (ACT*), tubulin (TUB*)	ACTB, ACTA, TUBA1B, TUBA4A, TUBB4B, TUBB3, TUBB8, TUBA1C, TUBB6, TUBB4A, TUBB
enzymes (GAPDH)	GAPDH
Subtypes of EVs (e.g. large oncosomes, large EVs) and/or pathologic/atypical state	
4- Transmembrane, lipid-bound and soluble proteins associated to other intracellular compartments than PM/endosomes	
4a: nucleus	
Histones (HIST1H**)	HIST1H2AJ, HIST1H2BL
Lamin A/C (LMNA)	-
4b: mitochondria	
IMMT	-
cytochrome C (CYC1)	-
TOMM20	-
4c: secretory pathway	
Calnexin (CANX)	CANX

Grp94 (HSP90B1)	HSP90B1
BIP (HSPA5)	HSPA5
GM130 (GOLGA2)	-
4d: others (autophagosomes, cytoskeleton...)	
ATG9A	-
Actinin1/4 (ACTN1/4)	ACTN1, ACTN4
cytokeratin 18 (KRT18)	-
5- Secreted proteins recovered with EVs secreted or luminal proteins that can associate with EVs by binding to specific (e.g. growth factor receptors) or to promiscuous (e.g. proteoglycan, lipid) receptors on the EV surface	
5a: Cytokines and growth factors	
TGFB1/2	-
IFNG	-
VEGFA	-
FGF1/2	-
PDGF*	PDGFD
EGF	-
interleukins (IL*)	-
5b: adhesion and extracellular matrix proteins	
Fibronectin (FN1)	FN1
Collagen (COL**)	COL1A2, COL6A1, COL6A2, COL6A3, COL5A1, COL12A1, COL1A1
MFGE8	MFGE8
galectin3-binding protein (LGALS3BP)	LGALS3BP
CD5L	-
fetuin-A (AHSG)	-
3- Major components of non-EV co-isolated structures All EVs as purity control	
3a: lipoproteins (produced by liver, abundant in plasma, serum)	
Apolipoproteins A1/2 and B APOA1/2, APOB; APOB100; albumin (ALB)	APOB
3b: protein and protein/nucleic acid aggregates	
Ribosomal proteins	RPS20, RPS3A, RPL26, RPL15, RPL27, RPS8, RPS15A, RPS16, RPS14, RPS23, RPS18, RPS13, RPS11, RPL7A, RPS4X, RPS6, RPL23, RPS15, RPS25, RPL30, RPL10A, RPL32, RPL11, RPL8, RPS27A, RPL24, RPL19, RPL18A, RPL6, RPL18, RPL36, RPLP2, RPLPOP6, RPSA, RPS2, RPL7, RPL17, RPS3, RPS12, RPL13, RPL10, RPL12, RPL9, RPL4, RPL3, RPL13A, RPS27, RPL27A, RPL5, RPL21, RPL28, RPS9, RPS5, RPL14

Notice. Left column – groups of EVs protein markers, recommended by MISEV2018 [PMID: 30637094]; right column – proteins of MSC-EVs identified by proteomic analysis; ‘-’ – the protein was not identified by current proteomic analysis.

Supplementary Table 3. Analysis of cytokines in MSC-EV.

Cytokine	MSC-EV (pg/mL)
bFGF	6,68 ± 4,83
CTACK	0,35 ± 0,01
Eotaxin	0,01 ± 0,003
G-CSF	0,04 ± 0,03
GM-CSF	0,04 ± 0,03
GRO-a	61,6 ± 50,57
HGF	40,79 ± 32,74
IFN-a2	0,21 ± 0,01
IFN-g	0,44 ± 0,18
IL-1a	6,39 ± 4,82
IL-1b	0,29 ± 0,23
IL-1ra	12,78 ± 1,13
IL-2	0,15 ± 0,09
IL-2Ra	1,08 ± 0,38
IL-3	0,01 ± 0
IL-4	0,05 ± 0,03
IL-5	0,24 ± 0,07
IL-6	17,97 ± 8,32
IL-7	0,43 ± 0,38
IL-8	34,43 ± 21,76
IL-9	0,56 ± 0,45
IL-10	0,16 ± 0,06
IL-12(p40)	3,4 ± 0,14
IL-12(p70)	0,44 ± 0,13
IL-13	0,08 ± 0,03
IL-15	2,34 ± 0,42
IL-16	3,41 ± 1,4
IL-17A	0,86 ± 0,18
IL-18	0,1 ± 0,06
IP-10	0,71 ± 0,45
LIF	3,52 ± 1,86
MCP-1	12,06 ± 6,07
MCP-3	0,66 ± 0,39
M-CSF	0,13 ± 0,04
MIF	633 ± 132,43
MIG	0,48 ± 0,34
MIP-1a	0,03 ± 0,02
MIP-1b	0,37 ± 0,04
b-NGF	0,64 ± 0,54
PDGF-BB	2,46 ± 0,63
RANTES	1,99 ± 0,39
SCF	0,75 ± 0,32
SCGF-b	150,9 ± 80,78
SDF-1a	10,91 ± 10,74
TNF-a	2,6 ± 0,69
TNF-b	0,06 ± 0,01

TRAIL	$1,65 \pm 1,21$
VEGF-A	$37,82 \pm 21,05$