Additional file 3

Table S1 Exosome proteins enriched within the EV fraction of the whole ADSC secretome.

|  |  |  |
| --- | --- | --- |
| Pathway | Protein | Reference |
| Tetraspanins | CD63 | (Cheng, Zhang, Wu, Cui, & Xu, 2017; Koniusz et al., 2016) |
| Heat shock proteins:  | HSP 70, HSP 90, HSP 105, beta 1, | (Cheng et al., 2017; Koniusz et al., 2016) |
| Lysosomal proteins:  | LAMP2 | (Cheng et al., 2017; Koniusz et al., 2016) |
| Fusion proteins: | Annexin | (Cheng et al., 2017) |
| GTPases | Elongation factor 1-alpha 1: EEF1A1, Eukaryotic Translocation Elongation Factor 2: EEF2 | (Sarvar, Shamsasenjan, & Akbarzadehlaleh, 2016) |
| Metabolic enzymes:  | Glyceraldehyde 3-phosphate dehydrogenase: GAPDH, Lactate Dehydrogenase A: LDHA, Phosphoglycerate Kinase 1: PGK1 | (Sarvar et al., 2016) |
| MSC markers: | CD44 | (Sarvar et al., 2016) |

Table S2 Soluble and EV GO-MF terms enrichment

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Category value | Selection | Category | Enrichment factor | P value | Benj. Hoch. FDR |
| Aminoacyl-tRNA biosynthesis | 13 | 13 | 29,538 | 1,94E-24 | 9,24E-20 |
| Cytoskeleton | 43 | 78 | 4,3506 | 2,99E-25 | 1,28E-19 |
| small molecule metabolic process | 172 | 96 | 2 | 5,23E-26 | 1,50E-19 |
| metabolic process | 172 | 253 | 1,4031 | 2,35E-25 | 3,35E-19 |
| nucleic acid metabolic process | 85 | 84 | 2,958 | 7,27E-24 | 1,73E-18 |
| primary metabolic process | 172 | 227 | 1,4359 | 1,63E-21 | 2,91E-16 |
| regulation of catalytic activity | 33 | 59 | 5,5223 | 3,02E-21 | 5,08E-16 |
| ncRNA metabolic process | 13 | 17 | 22,588 | 4,61E-21 | 5,73E-16 |
| proteolysis | 26 | 30 | 9,8462 | 4,84E-21 | 5,77E-16 |
| ncRNA metabolic process | 13 | 17 | 22,588 | 4,61E-21 | 5,99E-16 |
| tRNA aminoacylation for protein translation | 17 | 13 | 22,588 | 4,61E-21 | 6,28E-16 |
| ribonucleoprotein complex | 64 | 44 | 4,5 | 9,05E-21 | 1,95E-15 |
| proteolysis | 27 | 30 | 9,4815 | 1,82E-20 | 2,08E-15 |
| RNA metabolic process | 85 | 73 | 2,9705 | 4,40E-20 | 4,84E-15 |
| tRNA metabolic process | 17 | 14 | 20,975 | 6,39E-20 | 6,76E-15 |
| cellular metabolic process | 172 | 229 | 1,4136 | 7,21E-20 | 7,37E-15 |
| cellular macromolecule biosynthetic process | 85 | 59 | 3,216 | 3,66E-19 | 3,49E-14 |
| actin cytoskeleton | 25 | 36 | 8,5333 | 2,57E-19 | 3,68E-14 |
| oxoacid metabolic process | 172 | 47 | 2,2326 | 7,35E-19 | 6,18E-14 |
| organic acid metabolic process | 172 | 47 | 2,2326 | 7,35E-19 | 6,37E-14 |
| cellular ketone metabolic process | 172 | 47 | 2,2326 | 7,35E-19 | 6,57E-14 |
| protein folding | 20 | 37 | 9,3405 | 8,79E-19 | 7,18E-14 |
| regulation of gene expression | 85 | 89 | 2,6395 | 1,21E-18 | 9,62E-14 |
| actin cytoskeleton | 43 | 36 | 5,9535 | 6,33E-18 | 5,44E-13 |

Table S3 EV fraction GO-MF terms enrichment

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Category value | Selection | Category | Enrichment factor | P value | Benj. Hoch. FDR |
| Ribosome | 25 | 25 | 12,04 | 4,69E-37 | 6,04E-32 |
| translational termination | 25 | 25 | 12,04 | 4,69E-37 | 1,02E-30 |
| viral transcription | 25 | 26 | 11,577 | 1,22E-35 | 3,81E-30 |
| viral reproductive process | 25 | 26 | 11,577 | 1,22E-35 | 4,44E-30 |
| viral infectious cycle | 25 | 26 | 11,577 | 1,22E-35 | 5,33E-30 |
| translational elongation | 25 | 26 | 11,577 | 1,22E-35 | 6,66E-30 |
| protein complex disassembly | 25 | 26 | 11,577 | 1,22E-35 | 8,88E-30 |
| cellular protein complex disassembly | 25 | 26 | 11,577 | 1,22E-35 | 1,33E-29 |
| SRP-dependent cotranslational protein targeting to membrane | 25 | 27 | 11,148 | 1,65E-34 | 3,27E-29 |
| protein targeting to ER | 25 | 27 | 11,148 | 1,65E-34 | 3,60E-29 |
| establishment of protein localization in endoplasmic reticulum | 25 | 27 | 11,148 | 1,65E-34 | 4,00E-29 |
| cotranslational protein targeting to membrane | 25 | 27 | 11,148 | 1,65E-34 | 4,50E-29 |
| nuclear-transcribed mRNA catabolic process | 25 | 28 | 10,75 | 1,54E-33 | 2,40E-28 |
| macromolecular complex disassembly | 25 | 28 | 10,75 | 1,54E-33 | 2,58E-28 |
| cellular macromolecular complex disassembly | 25 | 28 | 10,75 | 1,54E-33 | 2,80E-28 |

Table S4 Top 20 significantly enriched GO BP terms secretome-wide

|  |  |  |  |
| --- | --- | --- | --- |
| p-value | GO term ID | GO term | Semantic class |
| 3.1E-24 | GO:0006457 | protein folding | protein metabolism - folding |
| 2.46E-20 | GO:0045055 | regulated exocytosis | transport - exocytosis |
| 2.8E-20 | GO:0016192 | vesicle-mediated transport | transport - intracellular - vesicle  |
| 2.89E-20 | GO:0006887 | exocytosis | transport - exocytosis |
| 1.98E-19 | GO:0036230 | granulocyte activation | immune system - leukocytes |
| 4.77E-19 | GO:0002283 | neutrophil activation involved in immune response | immune system - leukocytes |
| 4.77E-19 | GO:0043312 | neutrophil degranulation | immune system - leukocytes |
| 6.53E-19 | GO:0002275 | myeloid cell activation involved in immune response | immune system - gene expression |
| 9.53E-19 | GO:0042119 | neutrophil activation | immune system - leukocytes |
| 1.13E-18 | GO:0002446 | neutrophil mediated immunity | immune system - leukocytes |
| 5.06E-18 | GO:0043299 | leukocyte degranulation | immune system - leukocytes |
| 8.22E-18 | GO:0002444 | myeloid leukocyte mediated immunity | immune system - leukocytes |
| 2.16E-17 | GO:0051179 | localization | protein metabolism - localisation |
| 2.35E-17 | GO:0051234 | establishment of localization | protein metabolism - localisation |
| 4.26E-17 | GO:0002274 | myeloid leukocyte activation | immune system - differentiation |
| 6.3E-17 | GO:0032940 | secretion by cell | transport - exocytosis |
| 2.23E-16 | GO:0002366 | leukocyte activation involved in immune response | immune system - leukocytes |
| 2.94E-16 | GO:0002263 | cell activation involved in immune response | immune system |
| 9.19E-16 | GO:0046903 | secretion | transport - exocytosis |
| 5.85E-15 | GO:0001775 | cell activation | general |

Table S5 Top 20 significantly enriched GO BP terms in ADSC and AFS EV fractions

|  |  |  |  |
| --- | --- | --- | --- |
| p-value | GO term ID | GO term | Semantic class |
| 2.56E-32 | GO:0006614 | SRP-dependent cotranslational protein targeting to membrane | protein metabolism - localisation - membrane |
| 2.31E-31 | GO:0006613 | cotranslational protein targeting to membrane | protein metabolism - localisation - membrane |
| 4.2E-31 | GO:0045047 | protein targeting to ER | protein metabolism - localisation - ER |
| 1.32E-30 | GO:0072599 | establishment of protein localization to endoplasmic reticulum | protein metabolism - localisation - ER |
| 2.59E-30 | GO:0070972 | protein localization to endoplasmic reticulum | protein metabolism - localisation - ER |
| 9.58E-30 | GO:0016071 | mRNA metabolic process | RNA metabolism |
| 2.43E-29 | GO:0000184 | nuclear-transcribed mRNA catabolic process, nonsense-mediated decay | RNA metabolism - stability |
| 8.57E-29 | GO:0006413 | translational initiation | RNA metabolism - translation |
| 2.57E-27 | GO:0006401 | RNA catabolic process | RNA metabolism |
| 3.77E-27 | GO:0006402 | mRNA catabolic process | RNA metabolism |
| 7.47E-26 | GO:0006612 | protein targeting to membrane | protein metabolism - localisation - membrane |
| 1.84E-24 | GO:0019083 | viral transcription | physiology - virus |
| 1.53E-23 | GO:0019080 | viral gene expression | physiology - virus |
| 1.56E-23 | GO:0034655 | nucleobase-containing compound catabolic process | metabolism |
| 2.36E-22 | GO:0000956 | nuclear-transcribed mRNA catabolic process | RNA metabolism |
| 2.41E-22 | GO:0022613 | ribonucleoprotein complex biogenesis | RNA metabolism |
| 2.44E-22 | GO:0046700 | heterocycle catabolic process | metabolism |
| 2.79E-22 | GO:0044270 | cellular nitrogen compound catabolic process | metabolism |
| 5.42E-22 | GO:0019439 | aromatic compound catabolic process | metabolism |
| 6.39E-22 | GO:0006412 | translation | RNA metabolism - translation |

Table S6 Top significantly enriched GO BP terms in ADSC and AFS soluble fractions

|  |  |  |  |
| --- | --- | --- | --- |
| p-value | GO term ID | GO term | Semantic class |
| 0.00346 | GO:0008635 | activation of cysteine-type endopeptidase activity involved in apoptotic process by cytochrome c | cell death - enzymes |
| 0.0211 | GO:0006749 | glutathione metabolic process | metabolism |

Table S7 Comparison of miRNA cargo between different ADSC and AFS cell types and secretome pre-conditioning methods.

|  |  |  |  |
| --- | --- | --- | --- |
| **ADSC** | **AFS** | **(Wen et al., 2014)** | **(Lo Sicco et al., 2017)** |
| **Top Expressed** | **Top Expressed** | **Top Expressed** | **Under Expressed** | **Over Expressed** |
| miR-4454 | miR-1273g-3p | miR-652 | miR-376c | miR-855-5p |
| miR-3960 | miR-4454 | miR-29c | miR-708 | miR-872 |
| miR-1273g-3p | miR-3613-3p | miR-222 | miR-539 | miR-636 |
| miR-4497 | miR-3960 | miR-146a | miR-494 | miR-628-5p |
| miR-3613-3p | miR-3665 | miR-377 | miR-484 | miR-518f |
| miR-3665 | miR-6087 | miR-195 | miR-411 | miR-509-5p |
| miR-6089 | miR-6089 | miR-146b | miR-382 | miR-454 |
| Let-7b-5p | miR-6090 | miR-92a | miR-376a | miR-451 |
| miR-6869-5p | miR-7704 | miR-323 | miR-370 | miR-374-5p |
| miR-24-3p | miR-24-3p | miR-877 | miR-345 | miR-302c |
| miR-6087 | miR-4787-5p | miR-466c | miR-155 | miR-202a |
| miR-4787-5p | miR-4668-5p | miR-466b | miR-324-3p | miR-223 |
| miR-145-5p | miR-6869-5p | miR-196c | miR-301 | miR-214 |
| miR-23a-3p | miR-4497 | miR-664 | miR-210 | miR-199a-3p |
| miR-6090 | miR-23a-3p | miR-466d | miR-196b | miR-184 |
| miR-125b-5p | miR-8069 | let-7b | miR-194 | miR-150 |
| miR-7704 | miR-4508 | miR-685 | miR-181a | miR-146b |
| miR-221-3p | miR-6088 | miR-351 | miR-149 | miR-142-3p |
| miR-222-3p | miR-6729-5p | miR-3541 | miR-146a | miR-139-5p |
| miR-4668-5p | miR-6125 | miR-181c | miR-138 | miR-133b |
| miR-3196 | miR-3196 | miR-142-5p | miR-130b | miR-126 |
| miR-8069 | miR-4466 | miR-92b | miR-127 | miR-122 |
| miR-31-5p | miR-5787 | miR-323 | miR-100 |  |
| miR-22-3p | miR-221-3p | miR-182 | miR-99b |  |
| miR-100-5p | miR-4516 | miR-1 | miR-99a |  |
| miR-6125 | miR-1915-3p | miR-3591 | miR-34a |  |
| miR-4466 | miR-149-3p | miR-140 | miR-31 |  |
| miR-6729-5p | miR-320a | miR-708 | miR-30c |  |
| miR-5100 | miR-6727-5p | miR-34c | miR-29a |  |
| miR-7977 | miR-222-3p | miR-872 | miR-28 |  |
| miR-1915-3p | miR-4488 | miR-450a | miR-27a |  |
| miR-6088 | miR-103a-3p | miR-34b | miR-210 |  |
| Let-7a-5p | miR-320b | miR-214 | miR-20a |  |
| miR-3178 | miR-762 | miR-3593-3p | miR-20b |  |
| Let-7c-5p | miR-638 | miR-210 | miR-19a |  |
| miR-103a-3p | let-7b-5p | miR-34a | miR-19b |  |
| miR-4516 | miR-145-5p | miR-31 | miR-15b |  |
| miR-3656 | miR-1237-5p | miR-542-3p | miR-10b |  |
| miR-27a-3p | miR-31-5p | miR-290 | let-7e |  |
| miR-16-5p | miR-100-5p | miR-532-5p | let-7d |  |
| miR-23b-3p | miR-3656 | miR-25 | let-7a |  |
| miR-4488 | miR-3178 | miR-130a |  |  |
| Let7e-5p | miR-1469 | miR-31 |  |  |
| miR-638 | miR-4530 | miR-23b |  |  |
| miR-5787 | miR-125b-5p | miR-181a |  |  |
| miR-6727-5p | miR-23b-3p |  |  |  |
| miR-149-3p | miR-320c |  |  |  |
| miR-4508 | miR-107 |  |  |  |
| miR-1260b | miR-92a-3p |  |  |  |
| miR-214-3p | miR-8072 |  |  |  |
| **NA** | **80.0%** | **11.1%** | **14.6%** | **4.6%** |

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