

YMTHE, Volume 28

Supplemental Information

Comprehensive Molecular Profiles of Functionally Effective MSC-Derived Extracellular Vesicles in Immunomodulation

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Supplemental Table 1. KEGG pathway ranking summary of predicted targets of let-7b-5p and miR-21a-5p.

TERM	TERM_ID	TOTAL_GENES_OF_THE_TERM	UNION_TARGETS_IN_THE_TERM	MIRS_IN_THE_TERM	SCORE
MAPK_SIGNALING_PATHWAY	4010	271	39	2	6.802
PATHWAYS_IN_CANCER	5200	323	35	2	3.962
NEUROTROPHIN_SIGNALING_PATHWAY	4722	131	17	2	3.229
AXON_GUIDANCE	4360	131	18	2	3.137
JAK-STAT_SIGNALING_PATHWAY	4630	153	18	2	2.698
TGF-BETA_SIGNALING_PATHWAY	4350	85	11	2	2.533
FOCAL_ADHESION	4510	197	21	2	2.424
CYTOKINE-CYTOKINE_RECEPTOR_INTERACTION	4060	262	20	2	2.195
MELANOMA	5218	71	11	2	2.173
ENDOCYTOSIS	4144	219	19	2	2.113
COLORECTAL_CANCER	5210	65	9	2	2.07
WNT_SIGNALING_PATHWAY	4310	153	16	2	2.048
INSULIN_SIGNALING_PATHWAY	4910	137	15	2	1.996
REGULATION_OF_ACTIN_CYTOSKELETON	4810	215	17	2	1.906
NOTCH_SIGNALING_PATHWAY	4330	49	7	2	1.835
PROSTATE_CANCER	5215	89	11	2	1.835
CHEMOKINE_SIGNALING_PATHWAY	4062	187	15	2	1.785
ENDOMETRIAL_CANCER	5213	52	8	2	1.756
MTOR_SIGNALING_PATHWAY	4150	53	8	2	1.756
PANCREATIC_CANCER	5212	70	9	2	1.748
GLIOMA	5214	65	9	2	1.71
BASAL_CELL_CARCINOMA	5217	55	8	2	1.683
T_CELL_RECEPTOR_SIGNALING_PATHWAY	4660	109	10	2	1.663
UBIQUITIN_MEDIATED_PROTEOLYSIS	4120	138	11	2	1.653
CHAGAS_DISEASE	5142	102	9	2	1.615
PROTEIN_PROCESSING_IN_ENDOPLASMIC_RETICULUM	4141	167	12	2	1.585
TOXOPLASMOSIS	5145	128	12	2	1.577
CHRONIC_MYELOID_LEUKEMIA	5220	73	9	2	1.548
BLADDER_CANCER	5219	42	6	1	1.427
ADIPOCYTOKINE_SIGNALING_PATHWAY	4920	68	7	2	1.424
GNRH_SIGNALING_PATHWAY	4912	99	9	2	1.395

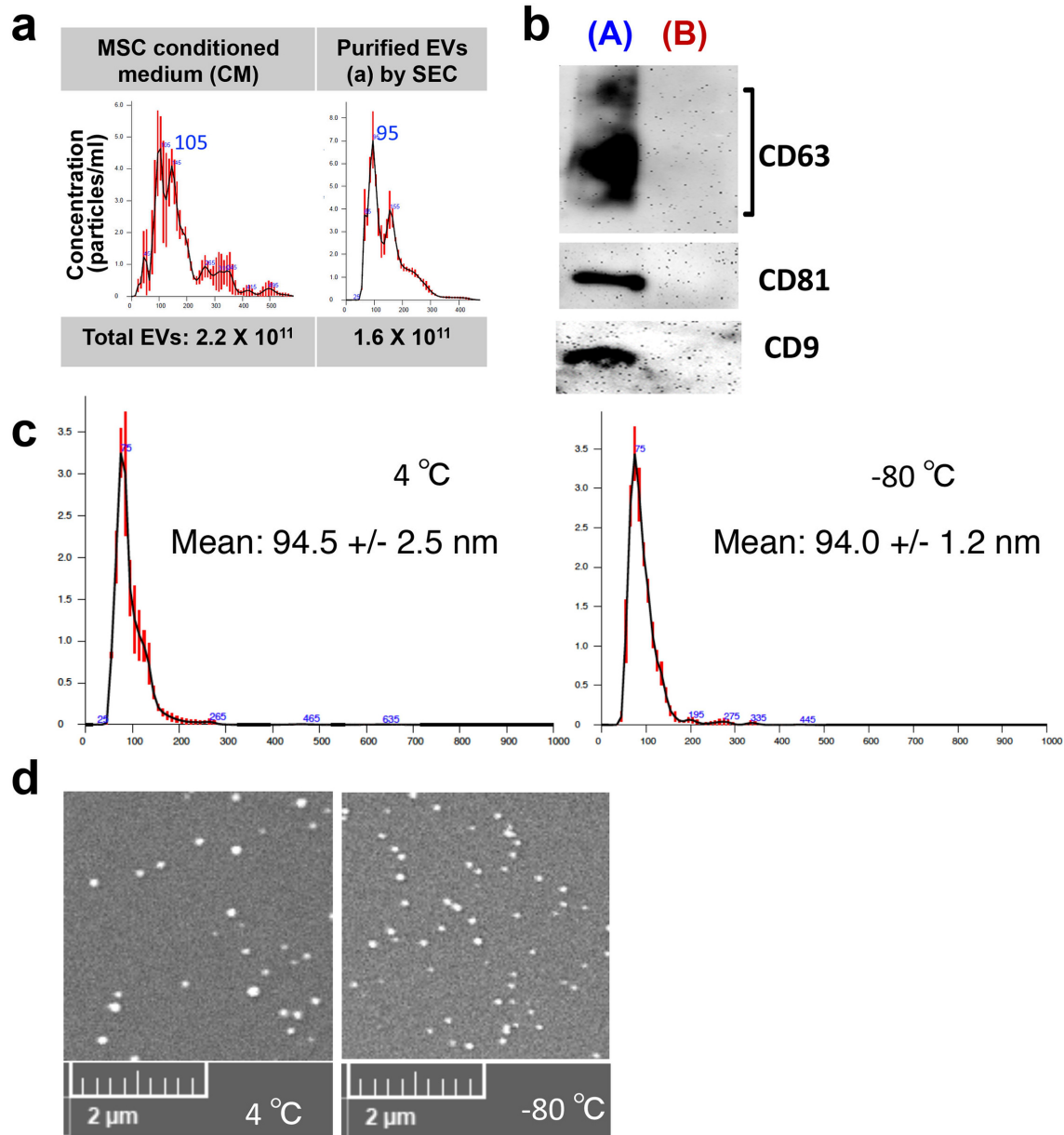
AMOEBIASIS	5146	115	11	2	1.387
MELANOGENESIS	4916	100	10	2	1.382
SPLICEOSOME	3040	126	7	2	1.365
B_CELL_RECEPTOR_SIGNALING_PATHWAY	4662	76	7	2	1.348
ERBB_SIGNALING_PATHWAY	4012	87	9	2	1.345
BACTERIAL_INVASION_OF_EPITHELIAL_CELLS	5100	71	6	2	1.338
PROTEIN_DIGESTION_AND_ABSORPTION	4974	75	8	2	1.328
HEPATITIS_C	5160	137	8	2	1.248
BASAL_TRANSCRIPTION_FACTORS	3022	36	4	2	1.192
TYPE_II_DIABETES_MELLITUS	4930	49	6	2	1.191
SMALL_CELL_LUNG_CANCER	5222	85	8	2	1.185
APOPTOSIS	4210	86	7	2	1.173
PROGESTERONE-MEDIATED_OOCYTE_MATURATION	4914	87	7	2	1.162
P53_SIGNALING_PATHWAY	4115	69	7	2	1.149
VEGF_SIGNALING_PATHWAY	4370	76	7	2	1.129
NATURAL_KILLER_CELL_MEDIATED_CYTOTOXICITY	4650	150	10	2	1.122
NON-SMALL_CELL_LUNG_CANCER	5223	54	6	2	1.102
RENAL_CELL_CARCINOMA	5211	71	7	2	1.088
TYPE_I_DIABETES_MELLITUS	4940	59	4	2	1.076
RIG-I-LIKE_RECEPTOR_SIGNALING_PATHWAY	4622	69	5	2	1.072
FC_GAMMA_R-MEDIATED_PHAGOCYTOSIS	4666	90	8	2	1.071
ACUTE_MYELOID_LEUKEMIA	5221	57	6	2	1.063
ADHERENS_JUNCTION	4520	74	7	2	1.051
GLYCOSPHINGOLIPID_BIOSYNTHESIS_LACTO_AND_NEOLACTO_SERIES	601	26	4	2	1.043
GLYCEROPHOSPHOLIPID_METABOLISM	564	79	7	2	1.03
PPAR_SIGNALING_PATHWAY	3320	80	6	2	1.019
FC_EPSILON_RI_SIGNALING_PATHWAY	4664	80	7	2	0.986
ALLOGRAFT_REJECTION	5330	52	4	2	0.933
CELL_ADHESION_MOLECULES_(CAMS)	4514	149	2	2	0.927
TOLL-LIKE_RECEPTOR_SIGNALING_PATHWAY	4620	101	7	2	0.921
HEDGEHOG_SIGNALING_PATHWAY	4340	54	5	2	0.91

LONG-TERM DEPRESSION	4730	72	6	1	0.891
CELL CYCLE	4110	125	7	2	0.873
LYSOSOME	4142	121	8	1	0.843
LEUKOCYTE_TRANSENDOTHELIAL_MIGRATION	4670	120	3	2	0.831
NOD-LIKE_RECEPTOR_SIGNALING_PATHWAY	4621	62	5	2	0.831
AMYOTROPHIC_LATERAL_SCLEROSIS (ALS)	5014	56	5	2	0.83
ECM-RECEPTOR INTERACTION	4512	83	6	2	0.796
CALCIUM SIGNALING PATHWAY	4020	178	10	1	0.789
NEUROACTIVE_LIGAND-RECEPTOR INTERACTION	4080	320	12	2	0.781
LYSINE DEGRADATION	310	43	4	2	0.753
SYSTEMIC_LUPUS_ERYTHEMATOSUS	5322	150	3	2	0.746
ALDOSTERONE-REGULATED_SODIUM_REABSORPTION	4960	44	4	2	0.742
PHAGOSOME	4145	174	4	2	0.74
HYPERTROPHIC_CARDIOMYOPATHY (HCM)	5410	87	6	1	0.738
ABC TRANSPORTERS	2010	45	4	2	0.731
GALACTOSE METABOLISM	52	27	3	2	0.729
PHOSPHATIDYLINOSITOL_SIGNALING_SYSTEM	4070	78	5	2	0.727
VASCULAR_SMOOTH_MUSCLE_CONTRACTION	4270	133	7	2	0.72
VIRAL MYOCARDITIS	5416	89	6	1	0.72
GLYCOSAMINOGLYCAN_BIOSYNTHESIS HEPARAN SULFATE	534	26	3	1	0.719
ALZHEIMER'S DISEASE	5010	179	5	2	0.692
LONG-TERM POTENTIATION	4720	69	5	1	0.692
O-GLYCAN BIOSYNTHESIS	512	28	3	1	0.682
HUNTINGTON'S DISEASE	5016	189	2	1	0.655
GRAFT-VERSUS-HOST DISEASE	5332	54	3	2	0.654
MALARIA	5144	54	4	2	0.654
THYROID CANCER	5216	30	3	1	0.649
PARKINSON'S DISEASE	5012	141	1	1	0.63
RNA TRANSPORT	3013	150	5	2	0.616
LEISHMANIASIS	5140	65	4	2	0.592
TIGHT JUNCTION	4530	135	5	2	0.592
AUTOIMMUNE THYROID DISEASE	5320	67	3	2	0.584
HEMATOPOIETIC CELL LINEAGE	4640	83	2	2	0.567

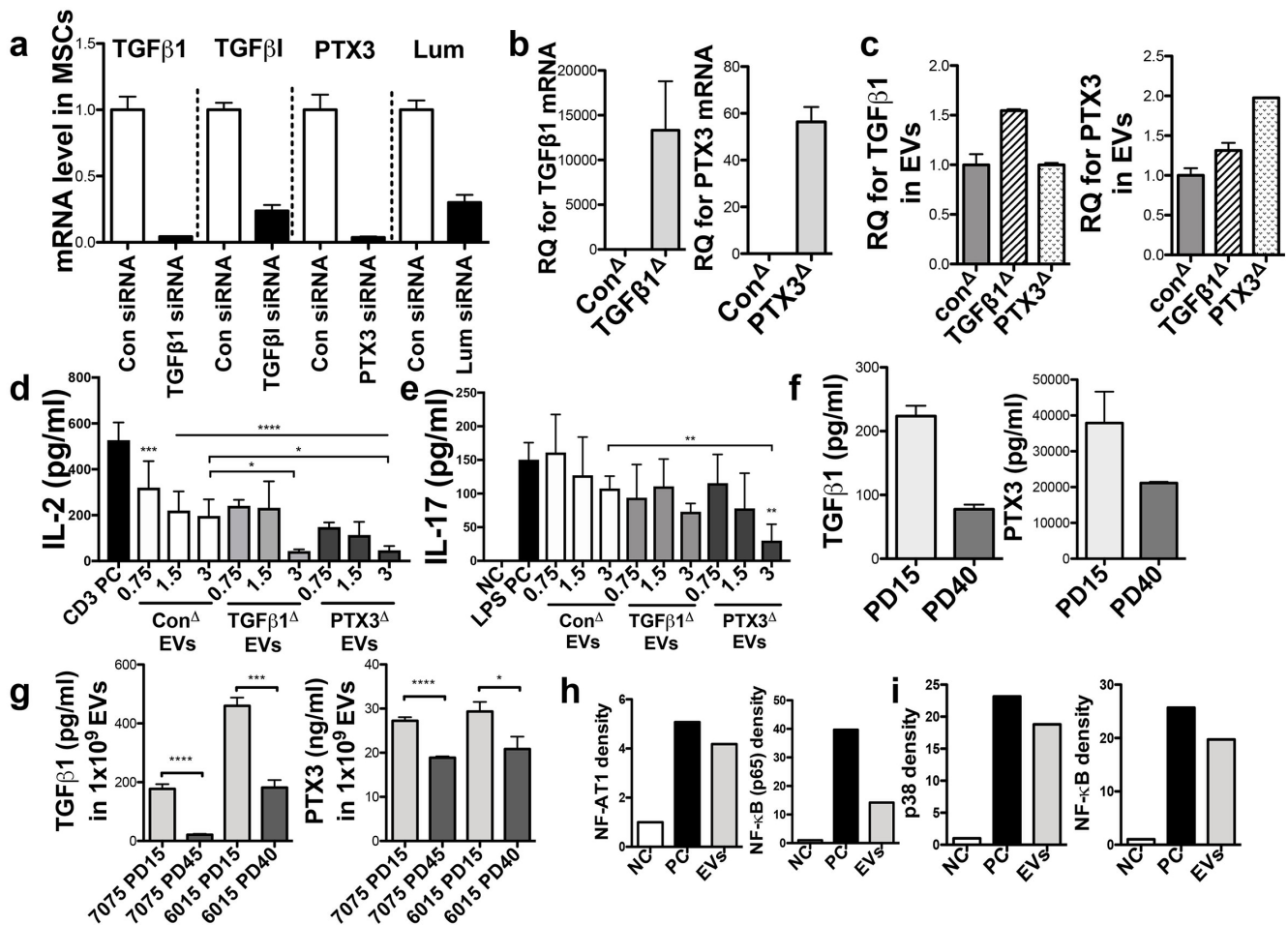
PANCREATIC SECRETION	4972	105	4	2	0.543
COMPLEMENT_AND_COAGULATION_CASCADES	4610	76	2	2	0.541
INOSITOL_PHOSPHATE_METABOLISM	562	57	3	2	0.515
PEROXISOME	4146	80	3	2	0.507
RNA_DEGRADATION	3018	58	2	2	0.491
CARBOHYDRATE_DIGESTION_AND_ABSORPTION	4973	39	2	2	0.487
INTESTINAL_IMMUNE_NETWORK_FOR_IGA_PRODUCTION	4672	44	3	1	0.486
VASOPRESSIN-REGULATED_WATER_REABSORPTION	4962	43	2	2	0.482
AMINO_SUGAR_AND_NUCLEOTIDE_SUGAR_METABOLISM	520	47	2	2	0.48
OOCYTE_MEIOSIS	4114	112	5	1	0.443
N-GLYCAN_BIOSYNTHESIS	510	50	3	1	0.442
PURINE_METABOLISM	230	165	3	1	0.432
BIOSYNTHESIS_OF_UNSATURATED_FATTY_ACIDS	1040	25	2	1	0.43
CYTOSOLIC_DNA-SENSING_PATHWAY	4623	55	3	1	0.412
DILATED_CARDIOMYOPATHY	5414	89	4	1	0.408
GAP_JUNCTION	4540	88	1	1	0.373
ETHER_LIPID_METABOLISM	565	35	2	1	0.349
PRION_DISEASES	5020	35	2	1	0.349
SNARE_INTERACTIONS_IN_VESICULAR_TRANSPORT	4130	35	2	1	0.349
ARRHYTHMOGENIC_RIGHT_VENTRICULAR_CARDIOMYOPATHY_(ARVC)	5412	74	3	1	0.344
SALIVARY_SECRETION	4970	77	3	1	0.338
PYRIMIDINE_METABOLISM	240	98	3	1	0.321
SPHINGOLIPID_METABOLISM	600	41	2	1	0.32
PENTOSE_PHOSPHATE_PATHWAY	30	28	1	1	0.314
GASTRIC_ACID_SECRETION	4971	73	1	1	0.312
LINOLEIC_ACID_METABOLISM	591	45	2	1	0.306
BUTANOATE_METABOLISM	650	30	1	1	0.304
ARACHIDONIC_ACID_METABOLISM	590	86	2	1	0.3
ALANINE_ASPARTATE_AND_GLUTAMATE_METABOLISM	250	32	1	1	0.295
STAPHYLOCOCCUS_AUREUS_INFECTIION	5150	51	2	1	0.292
CARDIAC_MUSCLE_CONTRACTION	4260	79	2	1	0.289

ARGININE_AND_PROLINE_METABOLISM	330	53	2	1	0.288
RETINOL_METABOLISM	830	77	2	1	0.287
AMINOACYL-TRNA_BIOSYNTHESIS	970	64	1	1	0.28
FRUCTOSE_AND_MANNOSE_METABOLISM	51	36	1	1	0.279
CYSTEINE_AND_METHIONINE_METABOLISM	270	38	1	1	0.272
GLUTATHIONE_METABOLISM	480	54	1	1	0.249
GLYCEROLIPID_METABOLISM	561	50	1	1	0.238
FATTY_ACID_METABOLISM	71	47	1	1	0.232
GLYCOLYSIS_GLUONEOGENESIS	10	60	1	1	0.228
STARCH_AND_SUCROSE_METABOLISM	500	44	1	1	0.226
NUCLEOTIDE_EXCISION_REPAIR	3420	43	1	1	0.224
GLYCOSYLPHOSPHATIDYLINOSITOL(GPI)-ANCHOR_BIOSYNTHESIS	563	25	1	1	0.22
ANTIGEN_PROCESSING_AND_PRESENTATION	4612	78	1	1	0.217
BASE_EXCISION_REPAIR	3410	38	1	1	0.217
RNA_POLYMERASE	3020	28	1	1	0.216
PHOTOTRANSDUCTION	4744	29	1	1	0.215
CITRATE_CYCLE_(TCA_CYCLE)	20	32	1	1	0.214
GLYCINE_SERINE_AND_THREONINE_METABOLISM	260	34	1	1	0.214
REGULATION_OF_AUTOPHAGY	4140	35	1	1	0.214

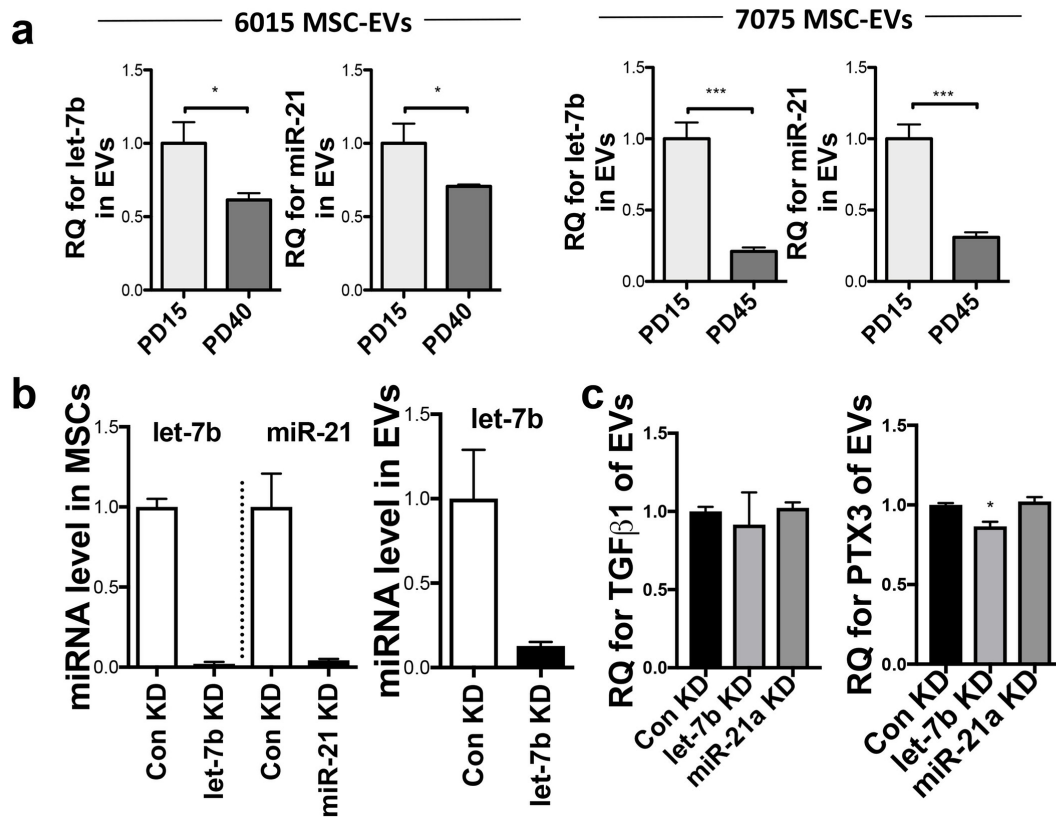
Supplemental Figure 1. Characterization of MSC-EVs isolated by a size exclusion chromatography method. **a** Particle sizes of MSC conditioned medium and purified MSC-EVs were analyzed by nanoparticle tracking system. **b** Western blot assays with EVs and protein fractions using CD63, CD81, and CD9 antibodies. **c-d** Particle sizes and electron microscopy images of MSC-EVs before and after storage at -80°C .



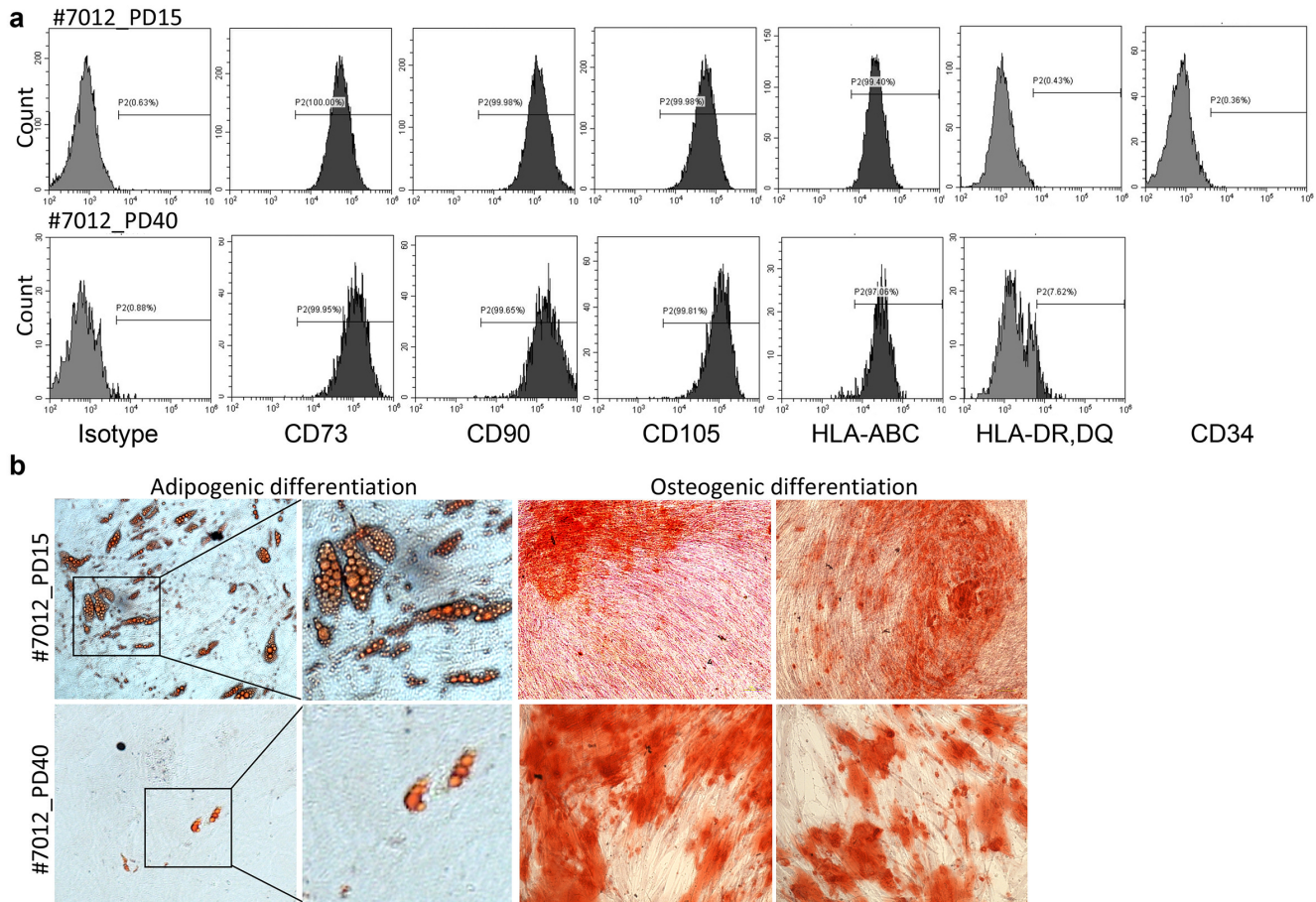
Supplemental Figure 2. Supporting data for the results in Figure 6. **a** Relative quantification (RQ) of TGFβ1, TGFβ1, PTX3 and Lum mRNAs in PD15 MSCs after target siRNAs transfection by RT-PCR assays. **b** RQ of TGFβ1 and PTX3 mRNAs in PD15 MSCs after target DNA plasmid transfection by RT-PCR assays. **c** RQ of TGFβ1 and PTX3 protein levels in EVs by ELISAs. EVs were isolated from PD15 MSCs after target DNA plasmid transfection. **d** IL-2 ELISA with conditioned medium of splenocytes activated by plate-bound anti-CD3 and **e** IL-17 ELISA with conditioned medium of LPS stimulated splenocytes for 24 h with EVs derived from PD15 MSCs transfected with target DNA plasmids (n=4). All data are presented as means ± SD.; *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 by one-way ANOVA with Tukey's test. **f** TGFβ1 and PTX3 ELISAs in conditioned medium of early-passage and late-passage MSCs (donor #7012). **g** TGFβ1 and PTX3 ELISAs in early-passage and late-passage MSC-EVs from donors # 7075 and # 6015 (n=3). Data are presented as means ± SD. *p < 0.05, ***p < 0.001, ****p < 0.0001 by student's t-test. **h-i** Quantitative band-intensity of western blots in Figure 6i-k.



Supplemental Figure 3. Supporting data for the results in Figure 7. a Relative expressions of let-7b and miR-21 in early- and late-passage MSC-EVs from two additional donors #6015 and #7075. **b** Relative expressions of let-7b and miR-21 in PD15 MSCs and let-7b in PD15 MSC-EVs after miRNA inhibitor transfection. **c** TGFβ1 and PTX3 ELISAs with EVs isolated from PD15 MSCs after miRNA inhibitor transfection. All data are presented as means ± SD; *p < 0.05 by Student's t-test or one-way ANOVA with Dunnett's test.



Supplemental Figure 4. Characterization of early-passage and late-passage MSCs (donor #7012). **a** Flow cytometry analysis with antibodies to the MSC surface markers (CD73, CD90 and CD105), HLAs (human leukocyte antigens) and hematopoietic marker CD34 in early-passage (PD15) and late-passage (PD40) MSCs. **b** Representative images of Oil red O and Alizarin Red staining after adipogenic and osteogenic differentiation induction with PD15 and PD40 MSCs. Both early- and late-passage MSCs expressed the MSC markers, but adipogenic differentiation potential of late passage MSCs was dramatically decreased.



Supplemental Figure 5. Karyotyping of early-passage (PD15) and late-passage (PD35) MSCs (donor #7012). Both early- and late-passage MSCs showed normal diploid genotypes, but some early signs of instability (possible fusions and polyploidization) were also observed in late passage MSCs.

