Supplement for:

Independent human mesenchymal stromal cell-derived extracellular vesicle preparations differentially affect symptoms in an advanced murine Graft-versus-Host-Disease model

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Supplemental Tables:

Suppl.-Table 1: Applied fluorescence conjugated antibodies

Antigen	Conjugate	Host/isotype	Clone	Supplier
Human CD14	PO	Mouse IgG_1	MEM-15	Exbio
Human CD31	PE	Mouse IgG_1	1F11	Beckman Coulter
Human CD34	APC 750	Mouse IgG_1	581	Beckman Coulter
Human CD44	APC	Mouse IgG2b, kappa	G44-26	BD Biosciences
Human CD45	BV 785	Mouse IgG_1, kappa	HI30	BioLegend
Human CD73	FITC	Mouse IgG_1, kappa	AD2	BD Biosciences
Human CD90	BV 605	Mouse IgG_1, kappa	5E10	BioLegend
Human CD105	BV 421	Mouse IgG_1, kappa	43A3	BioLegend

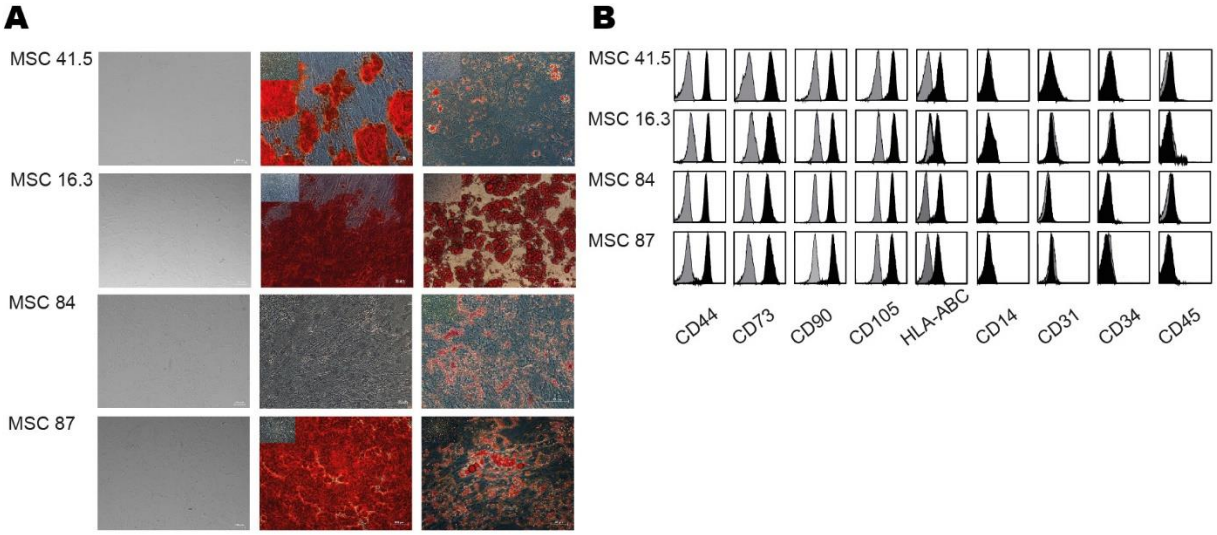
APC: Allophycocyanin, FITC: Fluorescein isothiocyanate, BV: Brilliant Violet, PO: Pacific Orange; PE = Phycoerythrin

Suppl.-Table 2: Band intensities of WB shown in Suppl. Fig. 2 as quantified by Image J.

	Syntenin	*CD9*	*CD81*
MSC-EVs 16.3	14805,874	18058,551	13391,258
MSC-EVs 41.5a	6380,196	38671,563	19032,108
MSC-EVs 41.5b	9656,752	40201,078	25338,078
MSC-EVs 41.5c	19968,844	55239,735	36101,584
MSC-EVs 70.2	9799,702	37841,158	33019,049
MSC-EVs 87	3598,589	33405,823	11888,217

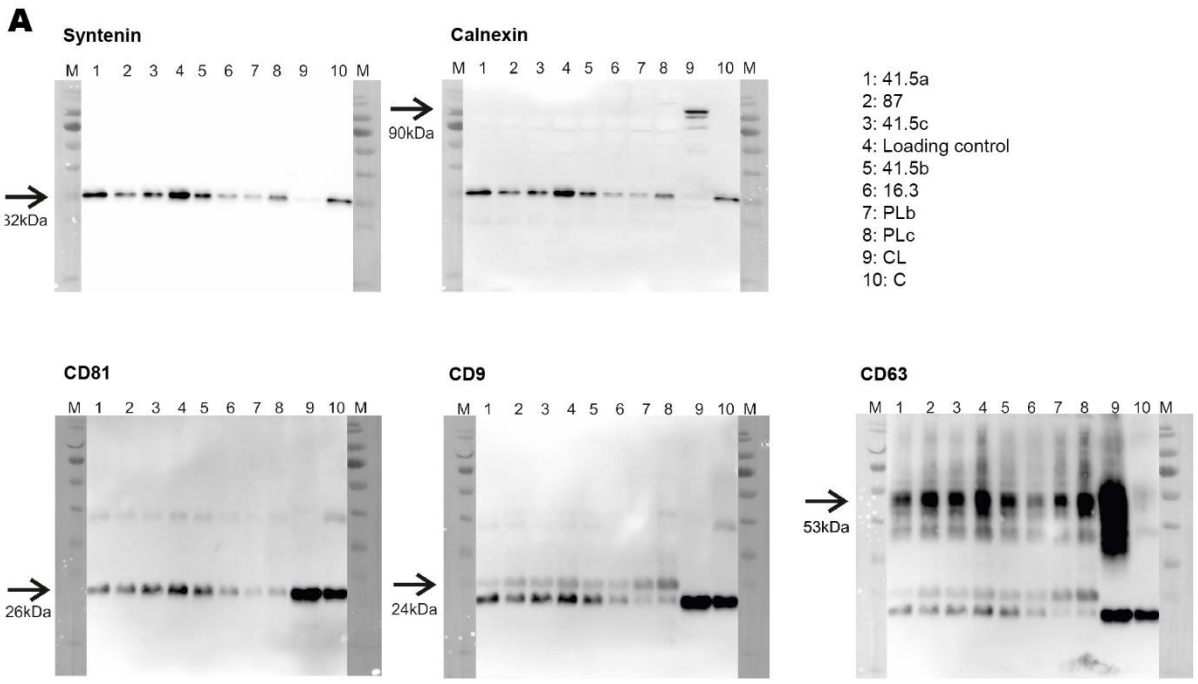
Supplement figures

Suppl.-Fig. 1



Suppl.-Figure 1: All MSCs provide MSC *bona fide* criteria. (A) Morphological appearance of expanded MSCs (phase contrast image, first column) and their osteogenic (2nd column) and adipogenic differentiation potential (3rd column) following alizarin red or oil red staining, respectively. Inserts in the 2nd and 3rd column show negative controls. (B) cell surface phenotype of expanded MSCs analysed by flow cytometry. Cells were labelled with fluorochrome conjugated antibodies against the MSC marker proteins CD44, CD73, CD90, CD105 and HLA-ABC and the negative markers CD14, CD31, CD34 and CD45.

Suppl.-Fig. 2



Suppl.-Figure 2: All MSC-EV preparations contain exosomal marker proteins and lack the impurity marker Calnexin. (A**)** Western blots of all MSC-EV preparations used in the study. The plot in the upper row was initially stained with anti-Synthenin antibodies. Following documentation the blot was additionally labelled with anti-Calnexin antibodies (no stripping). The plot in the lower row was sequentially stained with anti-CD81, anti-CD9 and anti-CD63 antibodies. Results after each detection round are depicted. Lane 9 contains MSC lysates (CL) and lane 10 a non-MSC derived EV preparation we internally use as a CD81 positive control.

<u>Supplement figures</u>

Suppl.-Figure 1: All MSCs provide MSC *bona fide* criteria.

Suppl.-Figure 2: All MSC-EV preparations contain exosomal marker proteins and lack the impurity marker Calnexin.