CD271 antigen defines a subset of multipotent stromal cells with immunosuppressive and lymphohematopoietic engraftment-promoting properties

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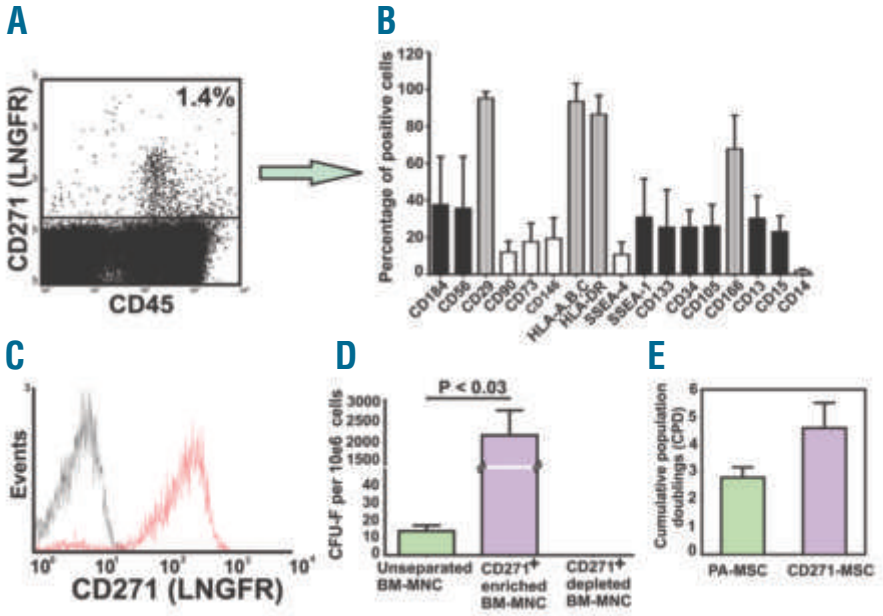
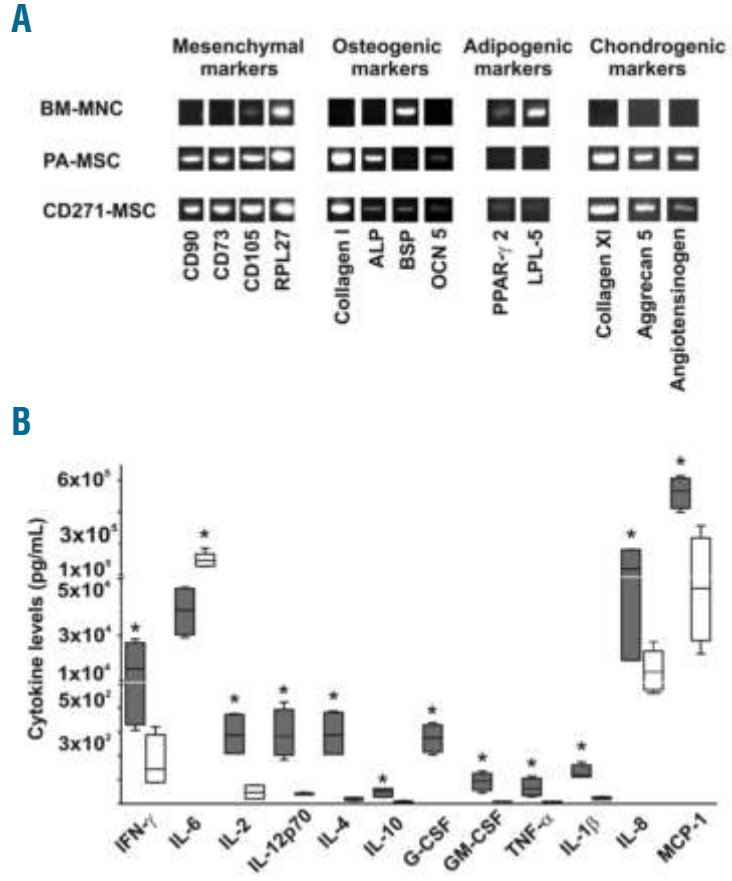
Online Supplementary Table S1. Percentage of human DNA in the organs of mice transplanted with CD271-MSC and PA-MSC separately at different doses.

	Brain	Lungs	Liver	Heart	Skeletal muscle
CD271- MSC ($1x10^5$ cells/mouse)	ND	0.02 ± 0.01	ND	ND	ND
PA-MSC ($1x10^5$ cells/mouse)	ND	0.005 ± 0.005	ND	0.007 ± 0.007	0.0031 ± 0.003
CD271- MSC ($7x10^5$ cells/mouse)	ND	0.09 ± 0.02**	0.006 ±0.003	0.002 ± 0.002	0.003 ± 0.002
PA-MSC ($7x10^5$ cells/mouse)	ND	0.035 ± 0.01**	ND	0.006 ± 0.0007	0.003 ± 0.003

*The values represent the mean value ± standard error for each group (n=3 mice); ND- not detected; **P< 0.003, the amount of human DNA detected in the lungs of the group transplanted with $7x10^5$ CD271-MSC compared to the group transplanted with $1x10^5$ CD271-MSC; **P< 0.001, the amount of human DNA detected in the lungs of the group transplanted with $7x10^5$ PA-MSC compared to the group transplanted with $1x10^5$ PA-MSC.*



Online Supplementary Figure S1. Phenotypic characterization of CD271$^+$ BM-MNC, clonogenic potential of enriched CD271$^+$ cells and proliferative potential of MSCs derived from these cells. (A) A representative dot plot of CD271$^+$ bone marrow mononuclear cells. (B) Cell surface profile of CD271$^+$ bone marrow mononuclear cells after density gradient separation. The positivity for analyzed antigens was determined by gating on all CD271$^+$ BM-MNC. The bars represent mean values of the analyzed bone marrow samples ± standard error of mean (n= 20). (C) A representative histogram of positively selected CD271$^+$ bone marrow mononuclear cells. The black line denotes the isotype control, whereas the red line denotes positively selected CD271$^+$ bone marrow mononuclear cells (n=10). (D) Colony- forming unit- fibroblast assay (CFU-F). Highly enriched CD271$^+$ BM-MNC were cultured for 14 days in the culture medium. On day 14, the number of fibroblast-like colonies was scored and the frequency of CFU-F per 1x10^6 BM-MNC was calculated. (E) Determination of population doublings in MSC derived from CD271$^+$ BM-MNC and MSC derived from unseparated BM-MNC (n=3).



Online Supplementary Figure S3. Genetic profile of mesenchymal and differentiation markers in bone marrow, and cytokine profile of CD271-MSC and PA-MSC. (A) Total RNA was isolated from bone marrow mononuclear cells and CD271-MSC and PA-MSC at passage 4 using RNeasy Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. A representative gel shows expression of mesenchymal stromal cell markers CD90, CD73, CD105 and ribosomal protein L27, osteogenic markers OCN5, ALP, BSP and Col I, adipogenic markers PPAR-γ2 and LPL-5 and chondrogenesis Col XI, angiotensinogen and aggrecan 5. (B) Cytokine production by CD271-MSC (gray bars) compared to PA-MSC (white bars) is presented. The y-axis is divided into 0-500, 501-5,000 and 5,001-40,000 pg/mL/10^6 cells cytokine secretion. Different concentration scales are used for these three parts of the y-axis. Significant increases of cytokine production of CD271-BMSC compared to PA-BMSC could be shown for the cytokines IFN-γ, IL-2, IL-12p70, IL-4, IL-10, GM-CSF, TNF-α, IL-1β, IL-8 and MCP-1 (*P<0.05). The PA-BMSC showed low cytokine secretion of IL-12p70, IL-4, IL-10, GM-CSF, TNF-α, IL-1 β and no cytokine secretion was observed for G-CSF. The major cytokine produced by these cells was IL-6. Values represent mean ± SEM of four independent experiments.