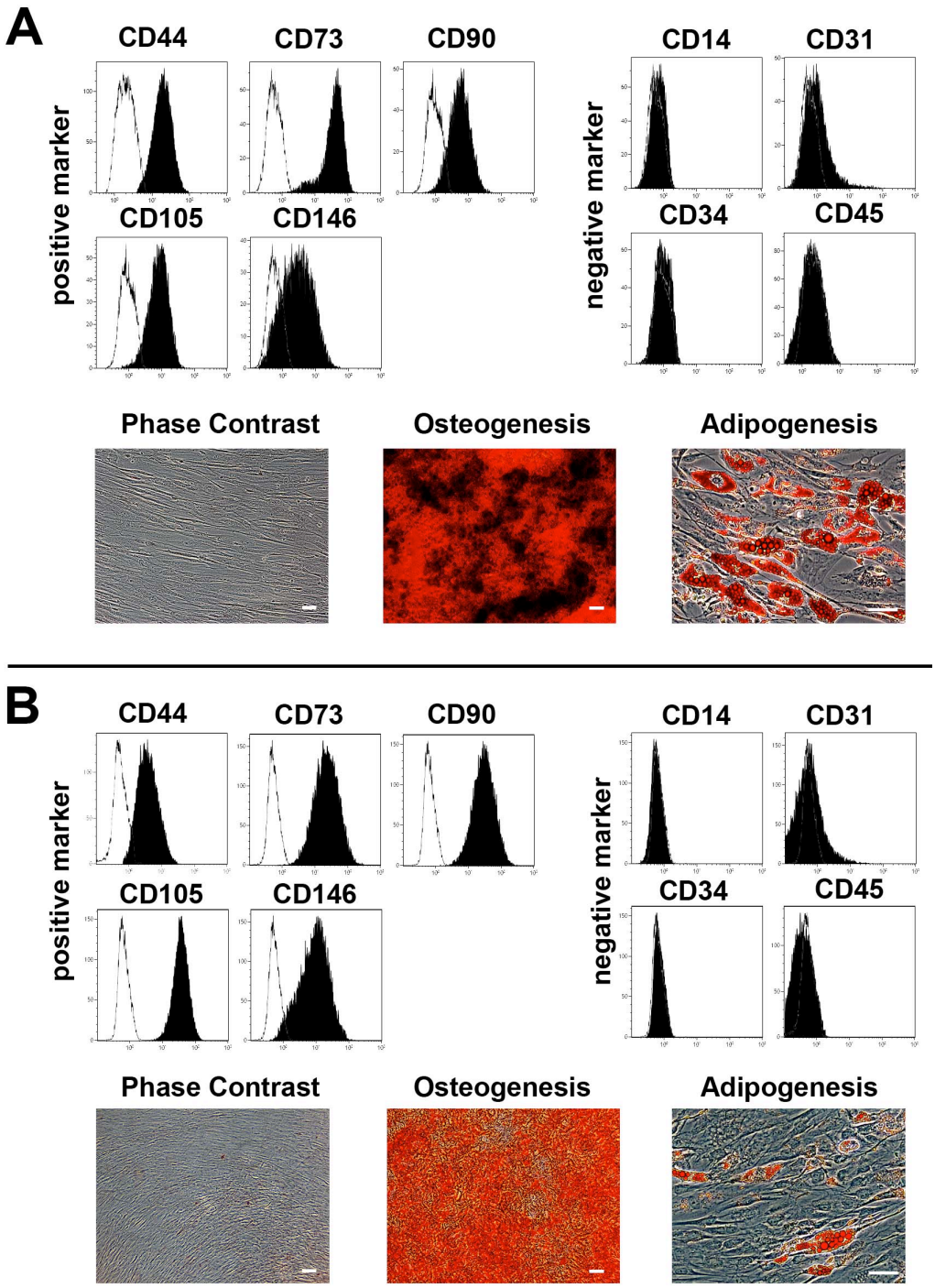
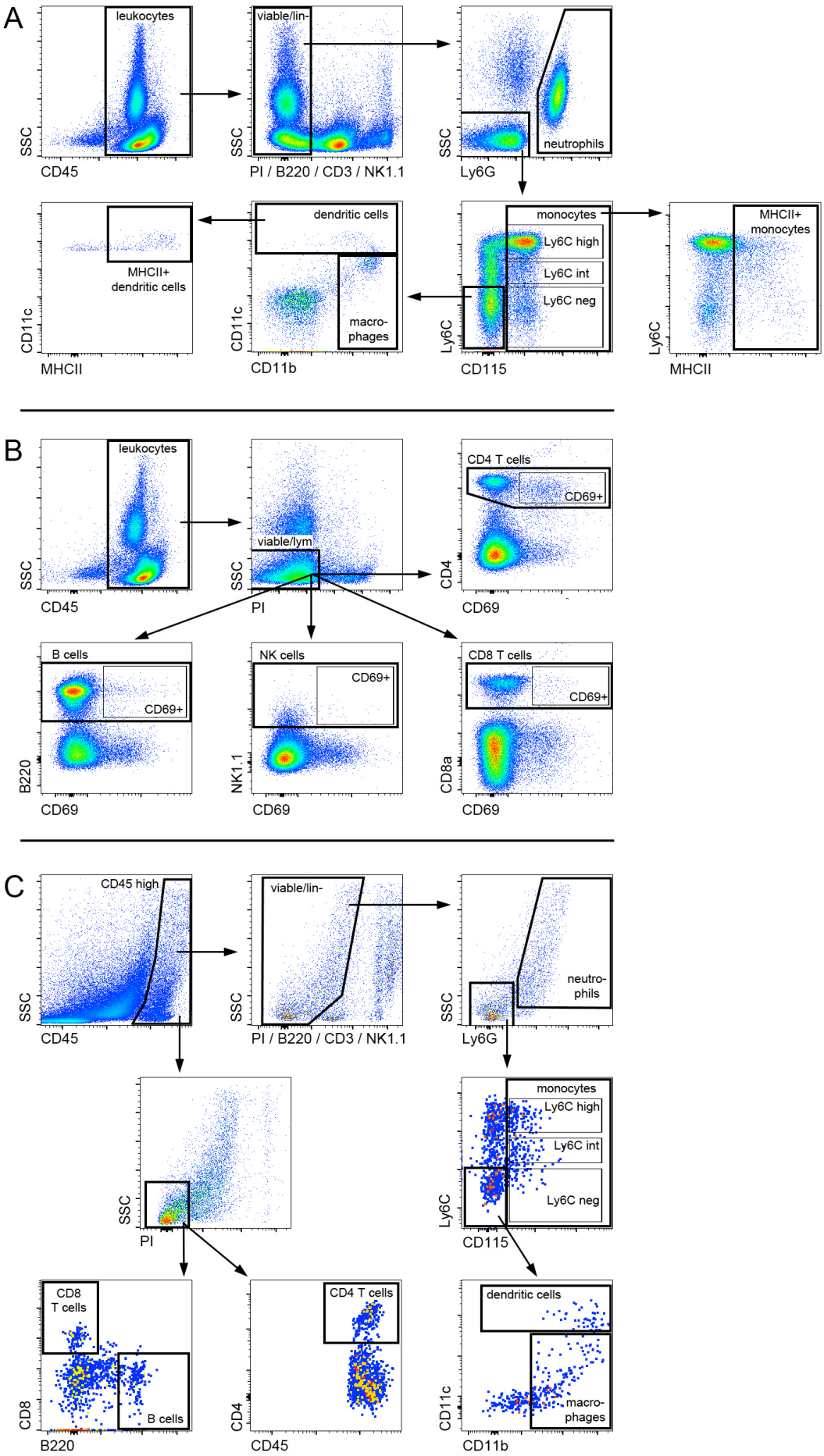
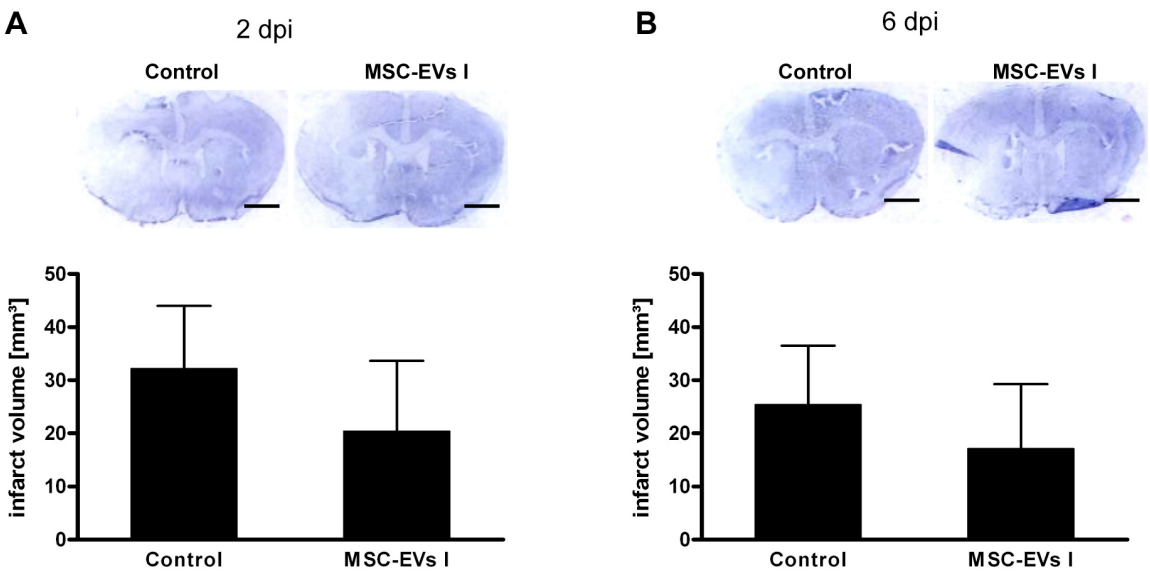


Supplementary Fig. S1. Phenotypical and functional characterization of primary human mesenchymal stem cells (MSCs). Cell surface phenotype and functional differentiation of MSCs derived from bone marrow (BM) of two different donors **(A**,**B)**. Open histograms represent isotype controls, black histograms specific antibodies. Histograms for positive and negative markers are depicted. Mineralization of osteocytes was detected by Alizarin Red S, lipid accumulation in adipocytes by Oil Red O. Scale bar: 50 µm.


A
leukocytes
SSC
CD45
viable/lin-
SSC
PI / B220 / CD3 / NK1.1
SSC
neutrophils
Ly6G
monocytes
Ly6C high
Ly6C int
Ly6C neg
Ly6C
CD115
MHCII+
monocytes
Ly6C
MHCII
dendritic cells
macro-
phages
CD11c
CD11b
MHCII+
dendritic cells
CD11c
MHCII
B
leukocytes
SSC
CD45
viable/lym
SSC
PI
CD4 T cells
CD69+
CD4
CD69
B cells
CD69+
B220
CD69
NK cells
CD69+
NK1.1
CD69
CD8 T cells
CD69+
CD8a
CD69
C
CD45 high
SSC
CD45
viable/lin-
SSC
PI / B220 / CD3 / NK1.1
SSC
neutro-
phils
Ly6G
SSC
PI
monocytes
Ly6C high
Ly6C int
Ly6C neg
Ly6C
CD115
CD8
T cells
B cells
CD8
B220
CD4 T cells
CD4
CD45
dendritic cells
macro-
phages
CD11c
CD11b

Supplementary Fig. S2. Gating strategy to identify peripheral immune responses and brain infiltrated myeloid and lymphoid leukocyte subsets by flow cytometry. (A) For analysis of myeloid cells white blood cells were gated for side scatter (SSC) characteristics and CD45 expression. CD45 positive cells were further gated by propidium iodide (PI) and lymphocyte lineage markers (B220, CD3, NK1.1) to exclude contamination with dead cells and lymphoid subsets, which can partially express myeloid markers such as CD11b. Viable and lymphoid-depleted cells were subdivided by scatter characteristics and Ly6G expression to identify neutrophils. Monocytes were defined by CD115 expression in the SSC^{low} $Ly6G^{-}$ cell population. Monocyte subsets were identified according to their Ly6C expression. $CD115^{-}$ cells were subdivided by CD11b and CD11c expression into dendritic cells and macrophages. Activation of monocytes and dendritic cells was determined by quantification of MHCII expression. **(B)** For analysis of lymphoid cells in the blood, CD45 positive cells were gated for viable lymphoid cells according to scatter characteristics and propidium iodide (PI). B lymphocyte, natural killer cells and T lymphocyte subsets were defined by B220, NK1.1, CD4 and CD8 antigen characteristics. Activation states of lymphocyte subsets were determined by CD69 expression. **(C)** Brain infiltrated immune cells were gated for side scatter (SSC) characteristics and $CD45^{high}$ expression. $CD45^{high}$ cells were further gated according to analysis of white blood cells **(A,B)** except for natural killer cells since NK1.1 positive events were too low for quantitative analysis.



Supplementary Fig. S3. Acute brain injury is not modulated by MSC-EVs. Infarct volume as determined by cresyl violet staining **(A)** 2 days after middle cerebral artery occlusion (MCAO) and **(B)** 6 days after MCAO in mice (n=8 per condition) that had been intravenously treated with normal saline (control) or MSC-EVs at 1 day post-ischemia (dpi; in **(A)**) or 1, 3 and 5 dpi (in **(B)**). Representative photographs of cresyl violet stainings are also shown. Scale bar: 1 mm. Data are mean ± S.D. values.

Supplementary Table 1. Antibodies used for flow cytometry of blood and brain tissues.

antigen / conjugate		host / istoype	clone	supplier
CD16/CD32	-	Rat IgG2a, kappa	2.4G2	BD Pharmingen
Panel 1: myeloid				
CD45	Pacific Orange	Rat IgG2b	30-F11	invitrogen
Ly6G	FITC	Rat IgG2a, kappa	1A8	BD Pharmingen
CD115	APC	Rat IgG2a, kappa	AFS98	eBioscience
Ly6C	BD Horizon V450	Rat IgM, kappa	AL-21	BD Horizon
CD11b	PE-Cy7	Rat IgG2b, kappa	M1/70	eBioscience
CD11c	PE	Hamster IgG	N418	Miltenyi Biotec
CD3	PerCP-Cy5.5	American Hamster IgG1, kappa	145-2C11	BD Pharmingen
CD45R (B220)	PerCP-Cy5.5	Rat IgG2a, kappa	RA3-6B2	eBioscience
NK1.1	PerCP-Cy5.5	Mouse IgG2a, kappa	PK136	eBioscience
MHCII	APC efour 780	Rat IgG2b, kappa	M5/114.15.2	eBioscience
Panel 2: lymphoid				
CD45	Pacific Orange	Rat IgG2b	30-F11	invitrogen
CD4	PE-Cy7	Rat IgG2a, kappa	RM4-5	BD Pharmingen
CD8a	FITC	Rat IgG2a, kappa	53-6.7	BD Pharmingen
CD45R (B220)	APC efour 780	Rat IgG2a, kappa	RA3-6B2	eBioscience
NK1.1	BD Horizon V450	Mouse IgG2a, kappa	PK136	BD Horizon
CD69	PE	American Hamster IgG1, lambda3	H1.2F3	BD Pharmingen