

Application of the 3R principles: Vertebrae as an additional source of murine bone marrow cells

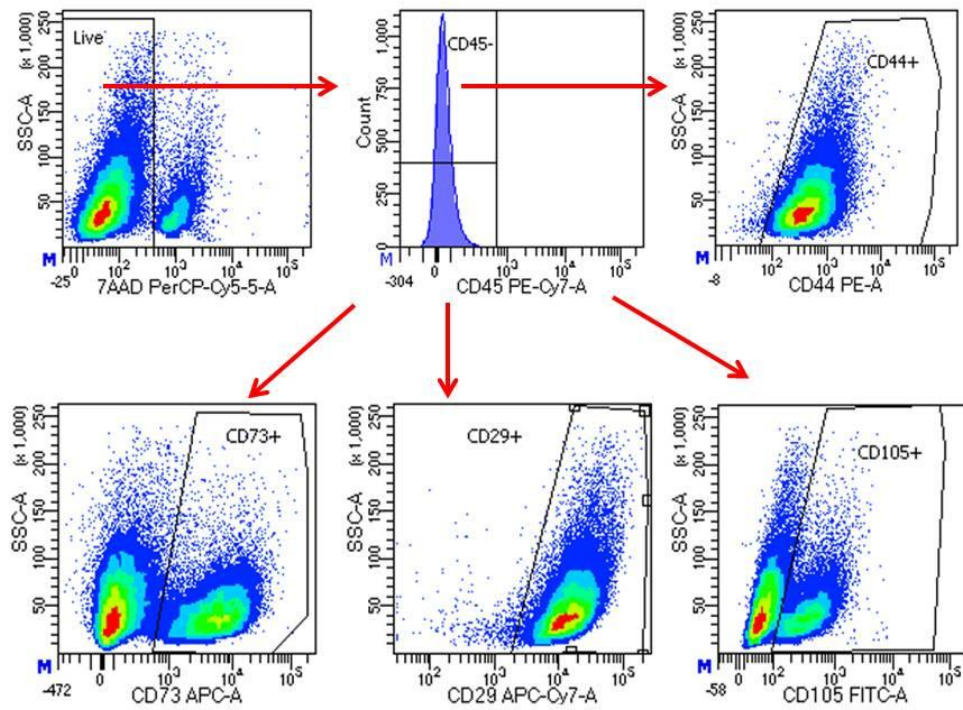
Supplementary material

Supplementary Table 1. Antibodies used for flow cytometry analysis of the expression of MSC surface markers.

Cells analyzed	Marker	Fluorochrome/ channel	Isotype	Clone	Catalog no.	Supplier
Cultured mesenchymal stromal/stem cells	CD45	PE-Vio770	rat IgG2bk	30F11	130-117-529	Miltenyi Biotec
	CD44	PE	rat IgG2bk	IM7.8.1	130-102-606	Miltenyi Biotec
	CD29	APC-Vio770	hamster IgG λ	HM β 1-1	130-105-126	Miltenyi Biotec
	CD73	APC	rat IgG1 κ	TY/11.8	130-102-586	Miltenyi Biotec
	CD105	VioBright 515	rat IgG2ak	MJ7/18	130-110-304	Miltenyi Biotec
	7-AAD	PerCP-Cy5.5	/	/	130-111-568	Miltenyi Biotec

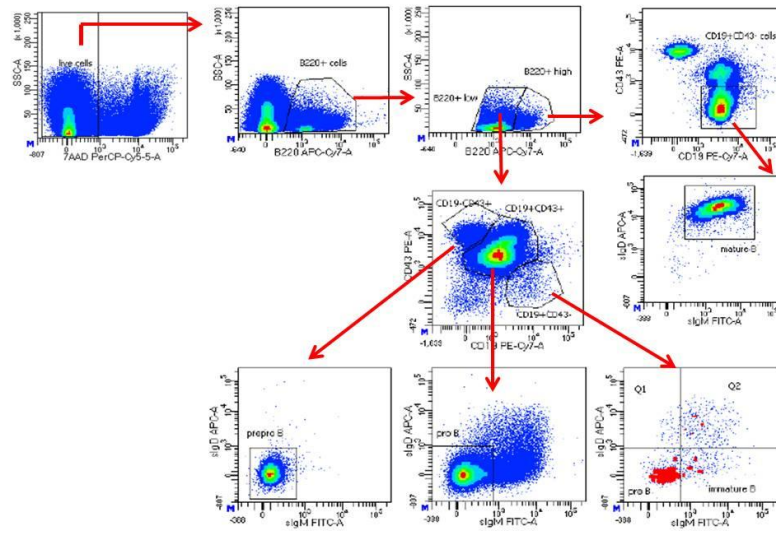
Supplementary Table 2. Antibodies used for flow cytometry analysis of immune cell populations.

Cells analyzed	Marker	Fluorochrome/ channel	Isotype	Clone	Catalog no.	Supplier
B cells	B220/CD45R	APC-Vio770	rat IgG2ak	RA3-6B2	130-102-267	Miltenyi Biotec
	CD19	PE-Vio770	rat IgG2ak	6D5	130-102-361	Miltenyi Biotec
	CD43	PE	rcomb. human IgG1	REA364	130-105-338	Miltenyi Biotec
	Membrane IgM	FITC	rat IgG2a	X-54	130-095-906	Miltenyi Biotec
	Membrane IgD	APC	rat IgG2ak	11-26c.2a	130-102-445	Miltenyi Biotec
	7-AAD	PerCP-Cy5.5	/	/	130-111-568	Miltenyi Biotec
Naïve, effector, central memory CD4 T cells	CD44	PE	rat anti-mouse	IM7	51-9007324	BD Biosciences
	CD4	APC-Vio770	rat IgG2bk	GK1.5	130-102-786	Miltenyi Biotec
	CD62L	APC	rat anti-mouse	MEL-14	51-9007326	BD Biosciences
	CD3	FITC	rcomb. human IgG1	REA641	130-109-836	Miltenyi Biotec
	7-AAD	PerCP-Cy5.5	/	/	130-111-568	Miltenyi Biotec
Naïve, effector, central memory CD8 T cells	CD44	PE	rat IgG2bk	IM7.8.1	130-102-606	Miltenyi Biotec
	CD8	FITC	rat, IgG $_{2a}$, κ	53-6.7	553031	BD Biosciences
	CD62L	APC		MEL-14	51-9007326	BD Biosciences
	CD3	APC-Cy7	rat, IgG 2b, κ	17A2	560590	BD Biosciences
	7-AAD	PerCP-Cy5.5	/	/	130-111-568	Miltenyi Biotec
Monocytes (inflammatory, non-inflammatory), neutrophils	Ly6G	PE	rat IgG2ak	1A8	130-102-392	Miltenyi Biotec
	Ly6C	APC	rat IgG2a	1G7.G10	130-102-341	Miltenyi Biotec
	CD11b	PE-Vio770	rat IgG2bk	M1/70.15.1 1.5	130-099-704	Miltenyi Biotec
	7-AAD	PerCP-Cy5.5	/	/	130-111-568	Miltenyi Biotec

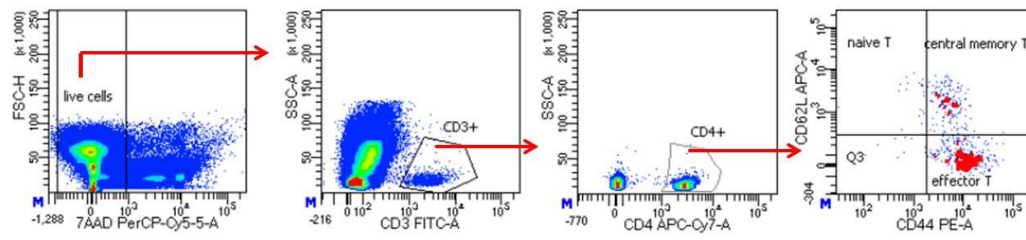


Supplementary figure 1. Flow cytometry analyses of MSCs. Gating strategy for immunophenotyping MSC cells. FSC/SSC graphs and exclusion of doublets are not shown.

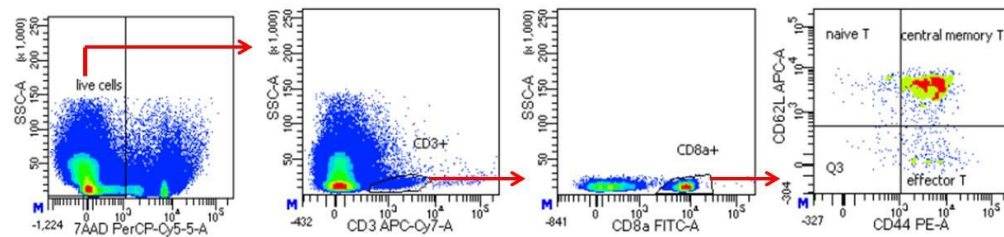
A



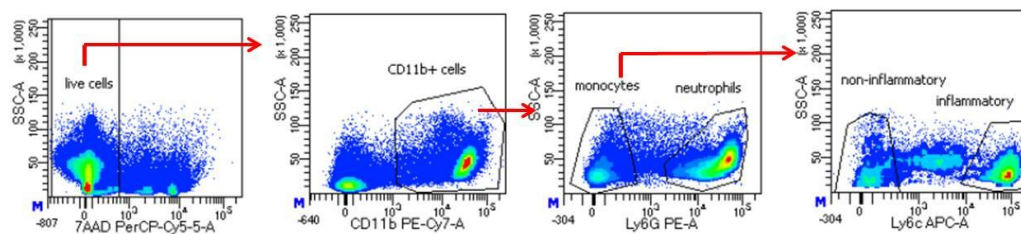
B



C



D



Supplementary figure 2. Flow cytometry analyses of immune cell populations: representative density plots and gating strategy for prepro B, pre B, pro B, immature and mature B cells. The percentages are calculated per B cells (B220+) (**A**). Representative density plots and gating strategy for central memory and effector CD4 T cells. The percentages are calculated per CD3+CD4+ T cells (**B**). Representative density plots and gating strategy for central memory and effector CD8 T cells. The percentages are calculated per CD3+CD8+T cells (**C**). Representative density plots and gating strategy for monocytes, neutrophils, inflammatory and non-inflammatory monocytes. Monocytes and neutrophils were calculated per live single cells and inflammatory and non-inflammatory monocytes were calculated per monocytes (CC11b+Ly6G-) (**D**). FSC/SSC graphs and exclusion of doublets are not shown.