Supplemental data

Mesenchymal Stromal Cell Exosomes Prevent and Revert Experimental Pulmonary Fibrosis Through Modulation of Monocyte Phenotypes

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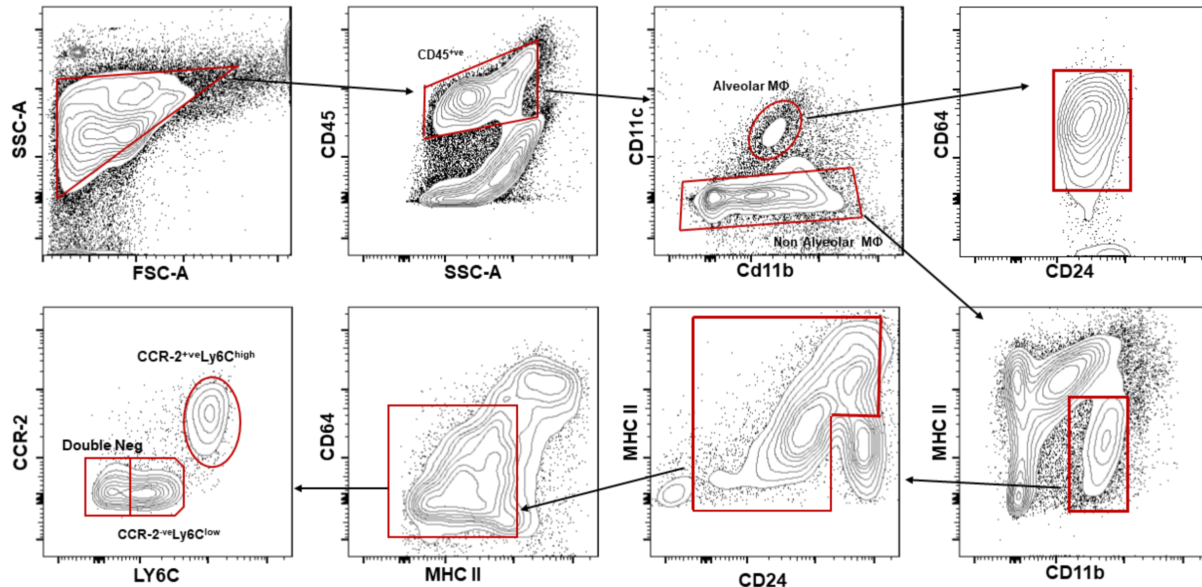

Figure S1: Representative *in vivo* gating strategy of lung macrophage, monocyte and bone marrow derived monocytes. Cells were isolated from whole lung after enzymatic digestion. Lung aggregates and cell debris were excluded based on forward and side scatter parameters. Immune cells were identified by CD45 staining. Alveolar macrophages (Alveolar MΦ) were identified using a sequential gating strategy to identify $CD45^{+}CD11b^{-}CD11c^{+}$ $CD64^{+}$ population. In order to identify monocyte subpopulation, sequential gating strategy was performed on non-alveolar MΦ subset of $CD45^{+}$cells ($CD11b^{+}$ $CD11C^{-}$). Myeloid cells were separated from lymphoid cells (B, T cells and natural killer cells) by gating on $CD11b^{hi}$ population. Granulocytes (neutrophils and eosinophils) were gated out as $CD24^{+}$ $CD11C^{-}$. Gating on $MHCII^{-}$ population was used to separate the interstitial macrophages and $CD11b^{+}$ dendritic cells from monocytes. Further gating was performed on $CD11b^{+}$ MHC II^{-} $CD64^{low/int}$ CCR-2^{+} $Ly6C^{high}$ and $CD11b^{+}$ MHC II^{-} $CD64^{low/int}$ CCR-2^{+} $Ly6C^{high}$ CCR-2^{-} $Ly6C^{low}$ population to reflect classical or non-classical monocyte phenotype respectively. BMDMo gating strategy was similar to above, with the exclusion of CD11c (a marker of Alveolar MΦ) staining. Gating strategy performed according to Fluorescence-minus-one controls. FSC, forward scatter; SSC, side scatter; MHC II, major histocompatibility complex class II.

A

qs.Fex

B

qs.Mex

Figure S2: QuSAGE gene set analysis. Differential mean and 95% confidence interval of the mean of individual genes belonging to the FEx (A) and MEx (B) signatures in publicly available transcriptome data of LyC6^{pos} vs. Ly6C^{neg} bone marrow-derived monocytes.