Supplemental Figures

Dissecting the Role of Myeloid and Mesenchymal Fibroblasts in Age-dependent Cardiac Fibrosis

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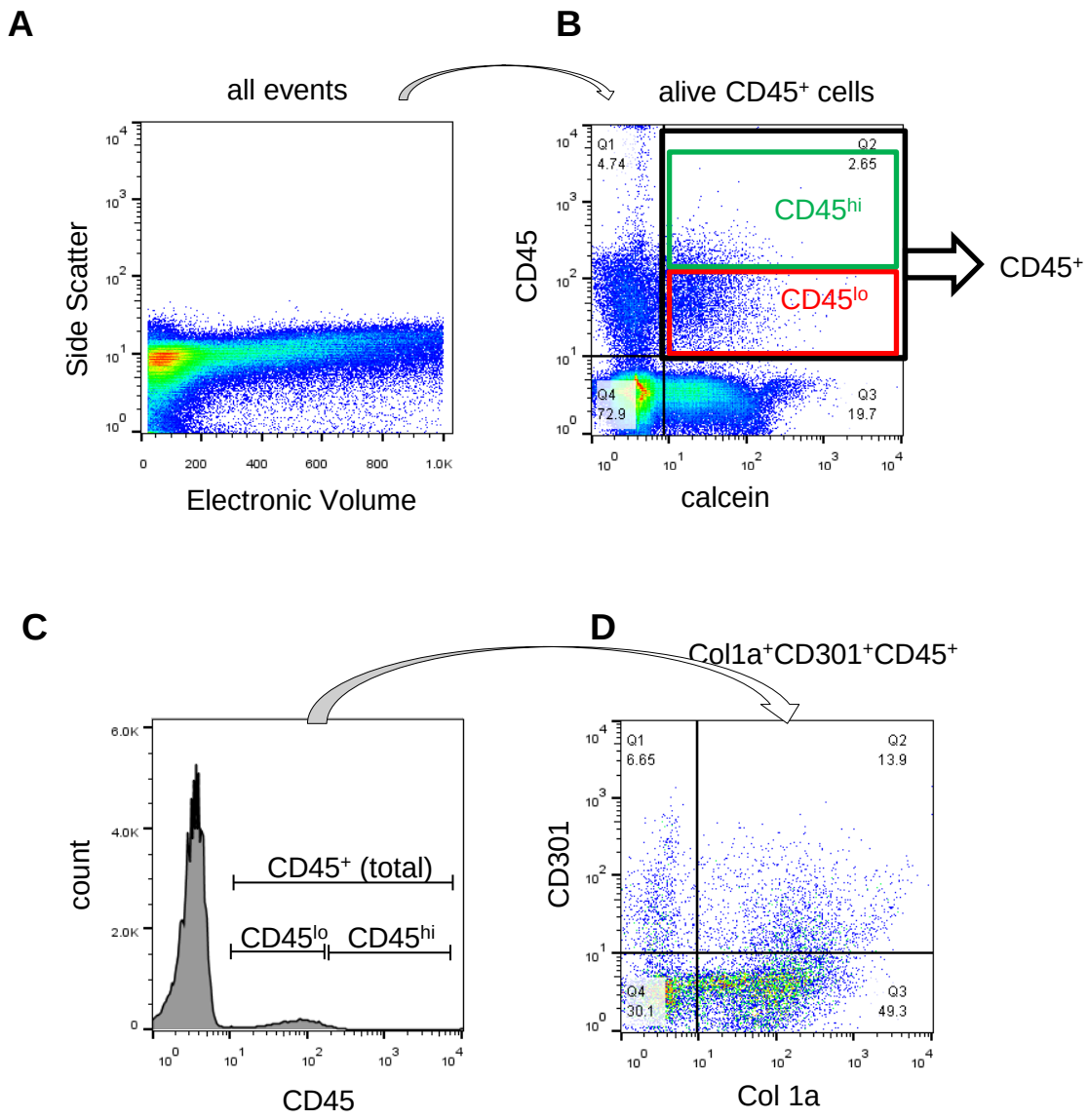
Supplemental Table 1. Analysis of the heart function in mice of various genetic backgrounds. Aged denotes 21-124 month-old mice. N = 10 mice per aged B6J, aged MCP-1KO, aged B6N group respectively. Data presented as a mean value ± SEM. * denotes $p<0.05$ when compared with B6J group.

Indices	Aged B6J	Aged MCP-1KO	Aged B6N
Weight [g]	36.327 ± 1.566	44.353 ± 2.755	45.86 ± 1.937
Heart Rate [bpm]	424.672 ± 15.024	417.946 ± 14.222	424.172 ± 20.205
Systolic Parameters			
LVID;d [mm]	4.658 ± 0.089	4.559 ± 0.118	5.146 ± 0.064*
LVPW;d [mm]	0.731 ± 0.028	0.851 ± 0.033*	0.811 ± 0.034
Volume;d [μl]	104.16 ± 4.043	97.282 ± 5.329	130.875 ± 4.555*
Fractional Shortening [%]	24.421 ± 0.787	25.665 ± 0.725	22.251 ± 0.707
Cardiac Output [ml/min]	21.804 ± 0.923	17.644 ± 0.925*	22.127 ± 1.567
Peak Velocity [cm/s]	89.746 ± 4.929	100.933 ± 10.779	105.012 ± 7.749
Diastolic Parameters			
E-Peak Velocity [cm/s]	72.582 ± 2.711	70.824 ± 3.603	71.096 ± 3.720
E/A Peak Velocity Ratio	1.328 ± 0.057	1.416 ± 0.058	1.623 ± 0.124*
Isovolumic Relaxation Time [ms]	20.811 ± 1.173	21.422 ± 1.139	20.275 ± 0.958
IVRT/RR	0.145 ± 0.006	0.148 ± 0.007	0.140 ± 0.006
LAV [μl]	33.386 ± 2.309	37.668 ± 2.221	38.349 ± 2.416
LA/Weight	0.947 ± 0.089	0.889 ± 0.095	0.842 ± 0.048

Supplemental Table 2. Kovacs analysis of Doppler mitral early flow predicts left ventricular diastolic stiffness. Young denotes 3 month-old and aged denotes 21- 24 month-old mice. N = 6, 7, 4, 5 mice per young B6J, aged B6J, aged MCP-1KO, aged B6N group respectively. C denotes damping constant, k - spring constant and x_o- initial displacement. Data presented as a mean value ± SEM.

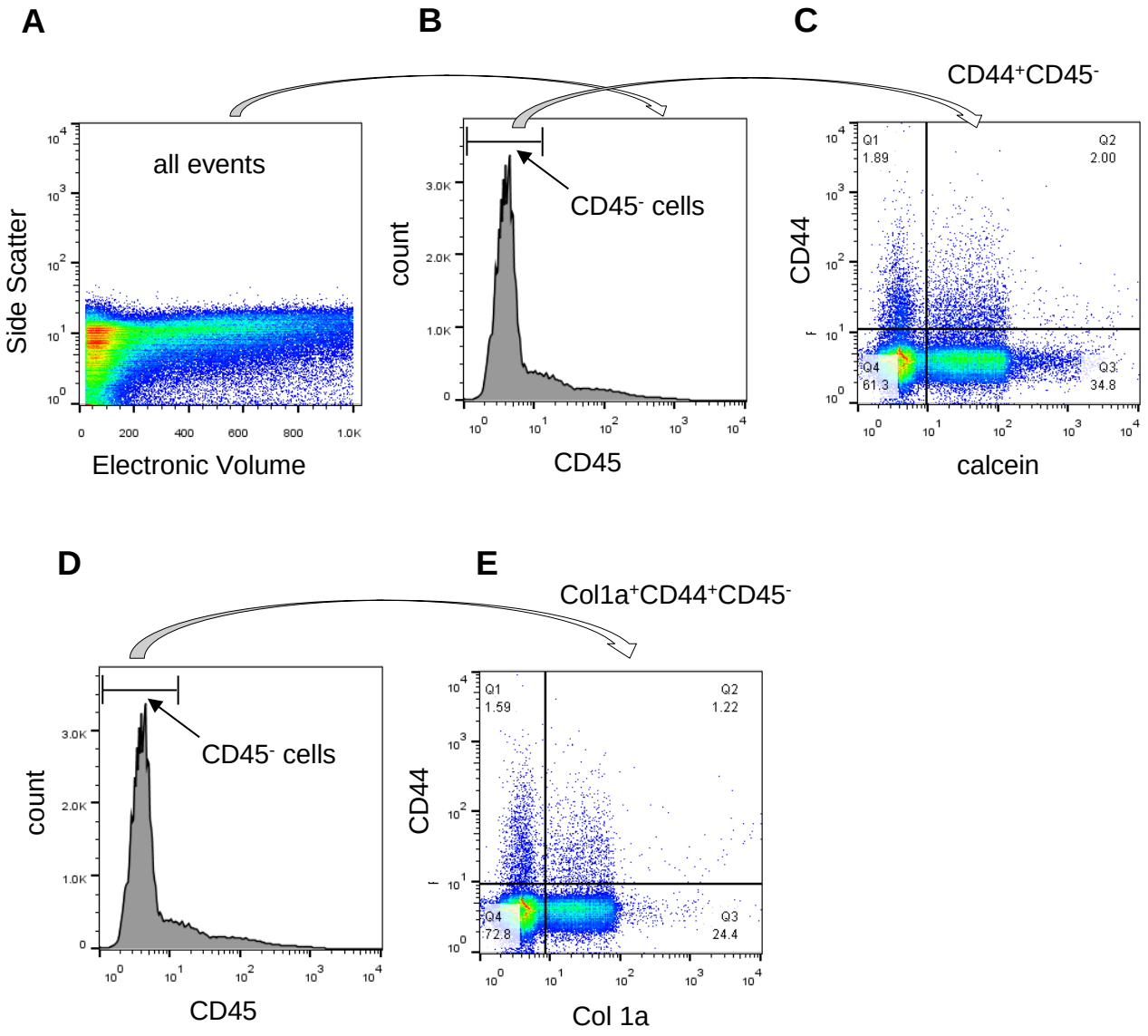
Mice	c [g/s]	k [g/s^2]	x_o [cm]
Aged B6J	195.15 ± 14.271	10417 ± 534.38	-0.016 ± 0.0012
Aged MCP-1KO	154.64 ± 6.592	8992.7 ± 411.43	-0.017 ± 0.0012
Aged B6N	204.14 ± 14.973	7935.1 ± 800.13	-0.023 ± 0.0036

Supplemental Figure 1



Supplemental Figure 1. Flow cytometry gating strategy for a myeloid lineage. A, Ungated events. **B,** Cells positive for calcein (alive) and CD45 were selected. Color coded frames point to $CD45^{lo}$, $CD45^{hi}$ and $CD45^{+}$ (total) populations. **C,** Fixed and permeabilized cells were gated based on their expression of $CD45^{+}$. **D,** Double positive cells ($Col1a^{+}CD301^{+}$) on $CD45^{+}$ gated population. To calculate the number of M2a macrophages/myeloid fibroblasts in all non-myocytes we used the following formula: $Col1a^{+}CD301^{+}CD45^{+}$ (from D) x $CD45^{+}calcein^{+}$ (from B) / 100 = % of $Col1a^{+}CD301^{+}CD45^{+}$ in all non-myocytes. Same strategy was used to calculate number of $Col1a^{+}CD301^{+}CD45^{lo}$ and $Col1a^{+}CD301^{+}CD45^{hi}$. A and B shows alive cells; C and D depict fixed and permeabilized cells.

Supplemental Figure 2



Supplemental Figure 2. Flow cytometry gating strategy for the mesenchymal lineage. A, Ungated events. **B,** Cells of non-myeloid origin selected (CD45$^-$). **C,** Gated on CD45$^-$ population, CD44$^+$calcein$^+$ cells are selected. **D,** Fixed and permeabilized cells gated based on CD45$^-$ population. **E,** Double positive cells (Col1a$^+$CD44$^+$) analyzed on CD45$^-$ gated population. To calculate the number of mesenchymal fibroblasts in all non-myocytes we used the following formula: Col1a$^+$CD44$^+$CD45$^-$ (from E) x CD44$^+$calcein$^+$CD45$^-$ (from B) / 100 = % of Col1a$^+$CD44$^+$CD45$^-$ in all non-myocytes. A, B and C shows alive cells; D and E depict fixed and permeabilized cells.