

Nuclear shape, protrusive behaviour and in vivo retention of human bone marrow mesenchymal stromal cells is controlled by Lamin A/C expression.

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Short title: Dorland, *et al.*: Nucleus of MSCs limits migratory behaviour

Key words: Mesenchymal stromal cell, nuclear lamina, Lamin A/C, migration, cellular therapy

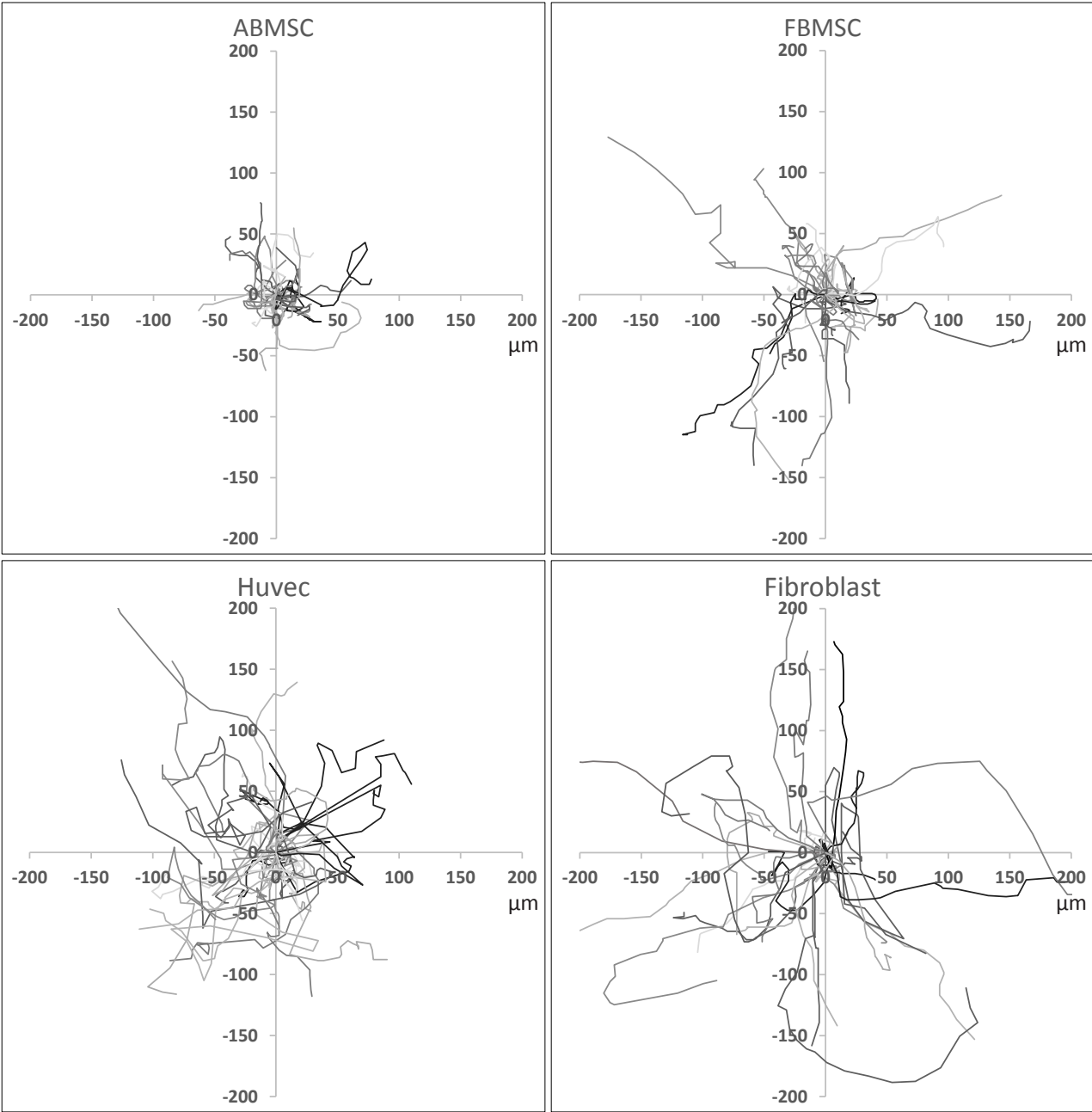
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Figure S1

A



B

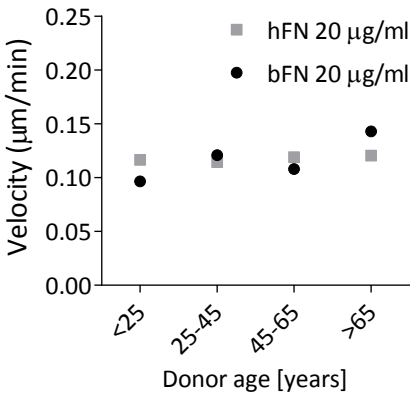
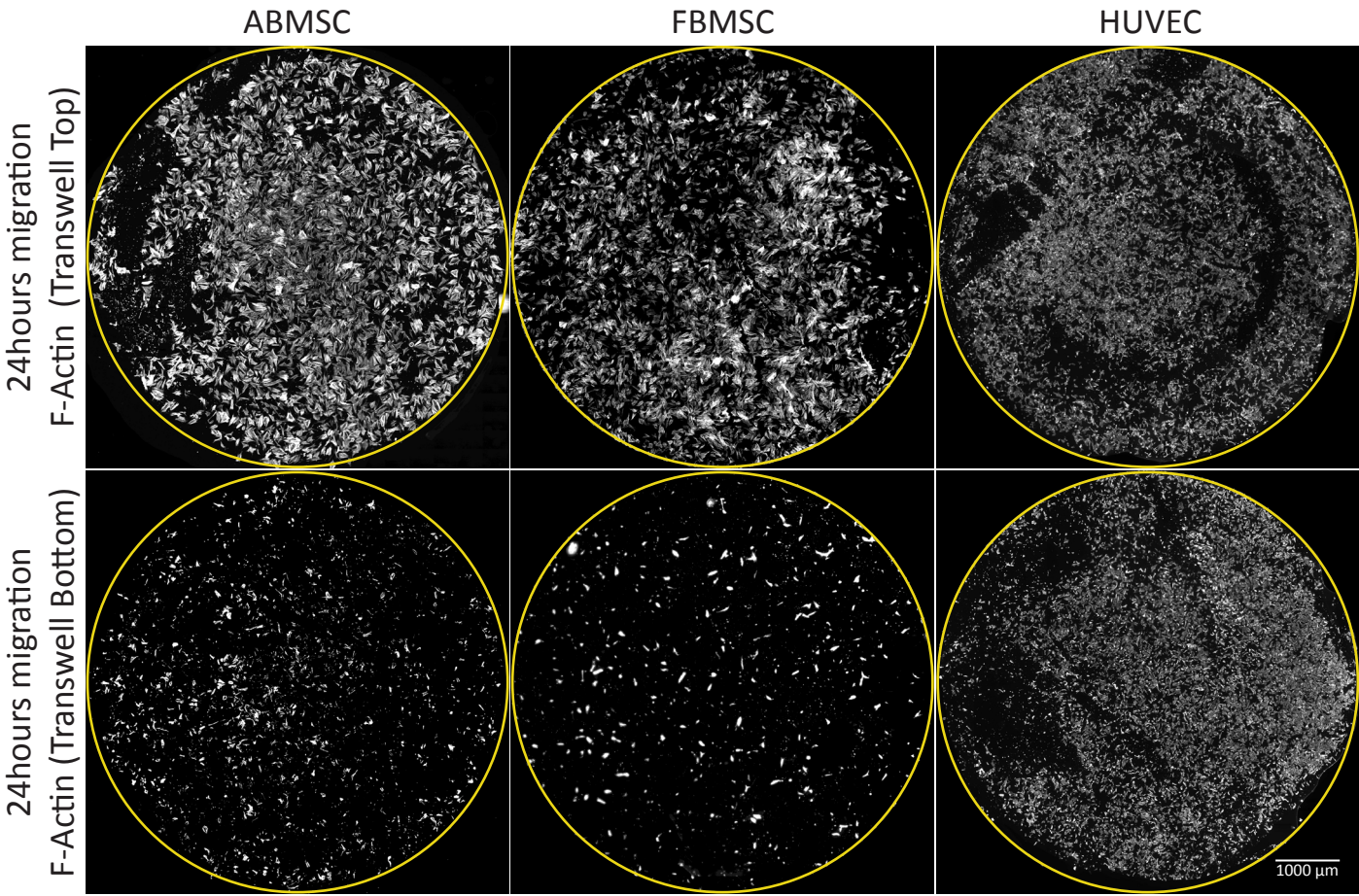
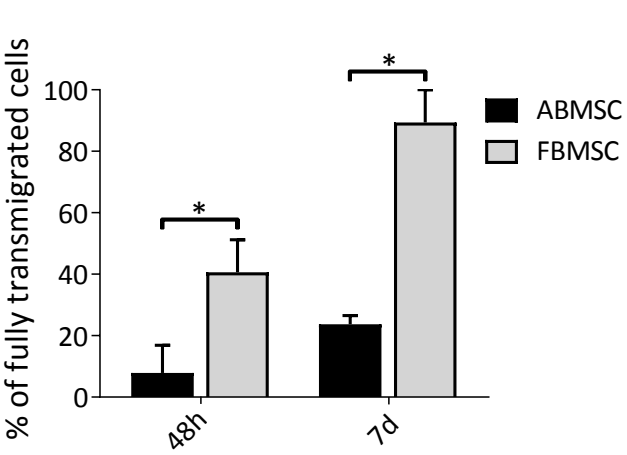


Figure S2

A



B



C

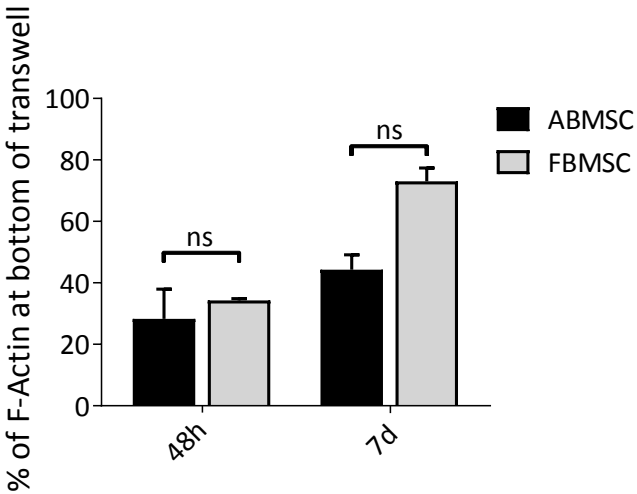
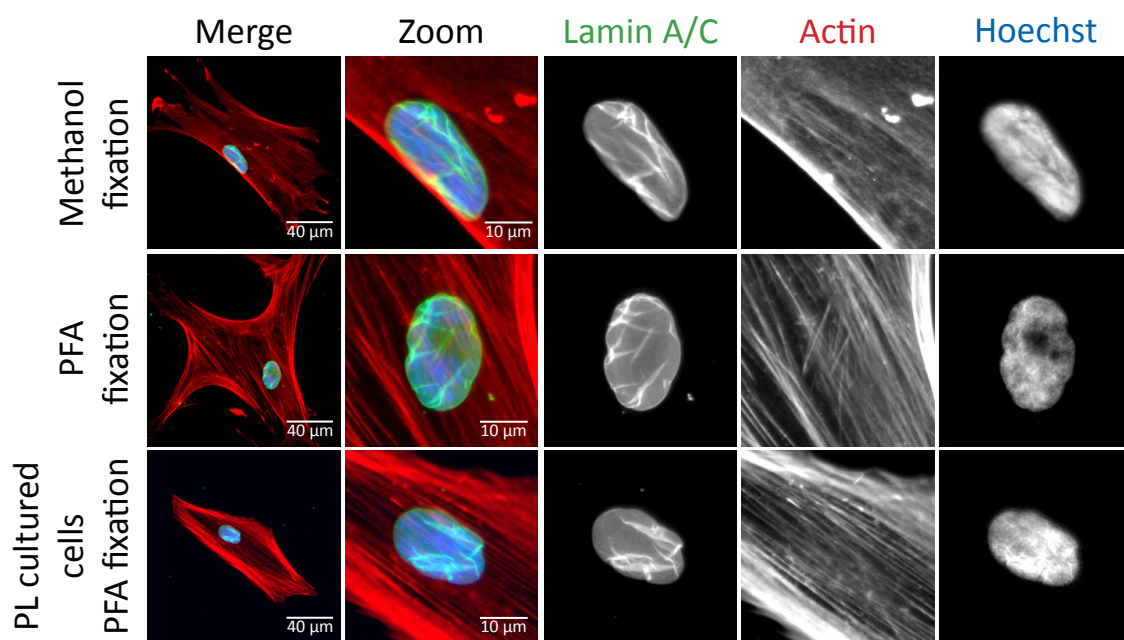
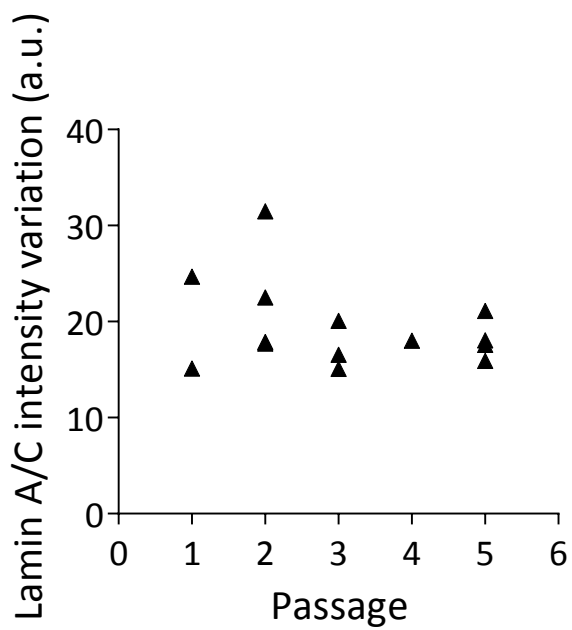


Figure S3

A



B



C

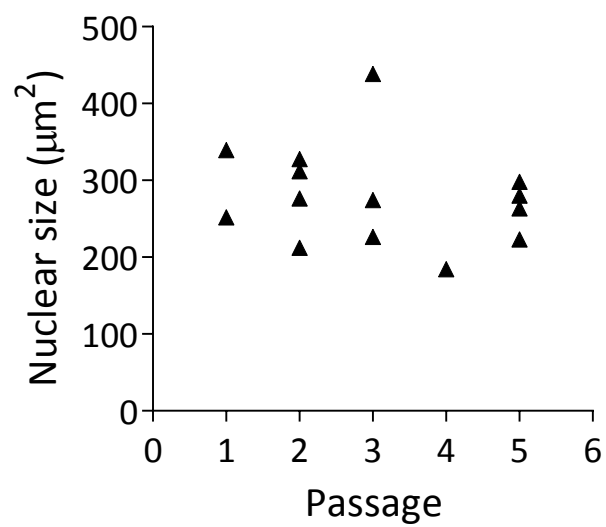


Figure S4

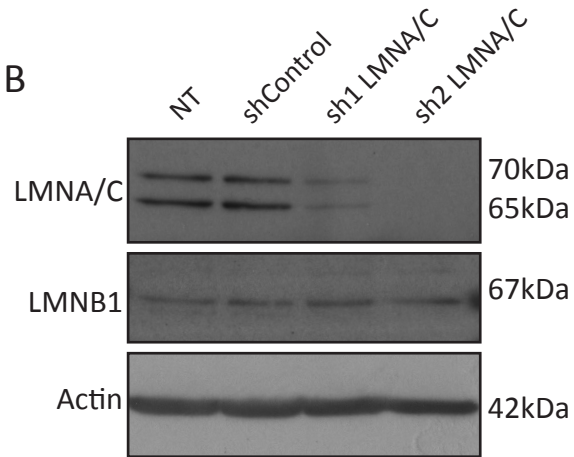
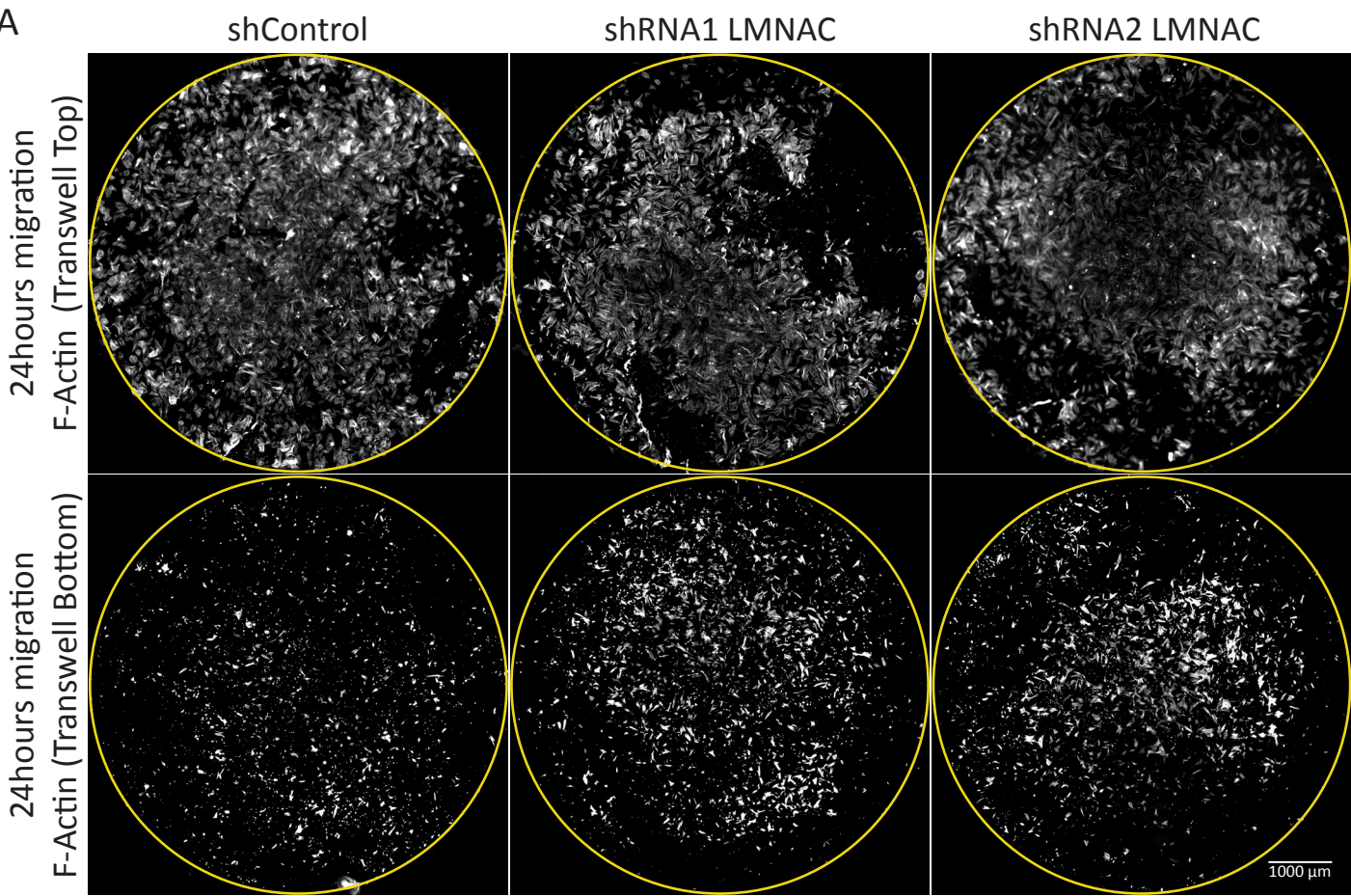


Figure S5

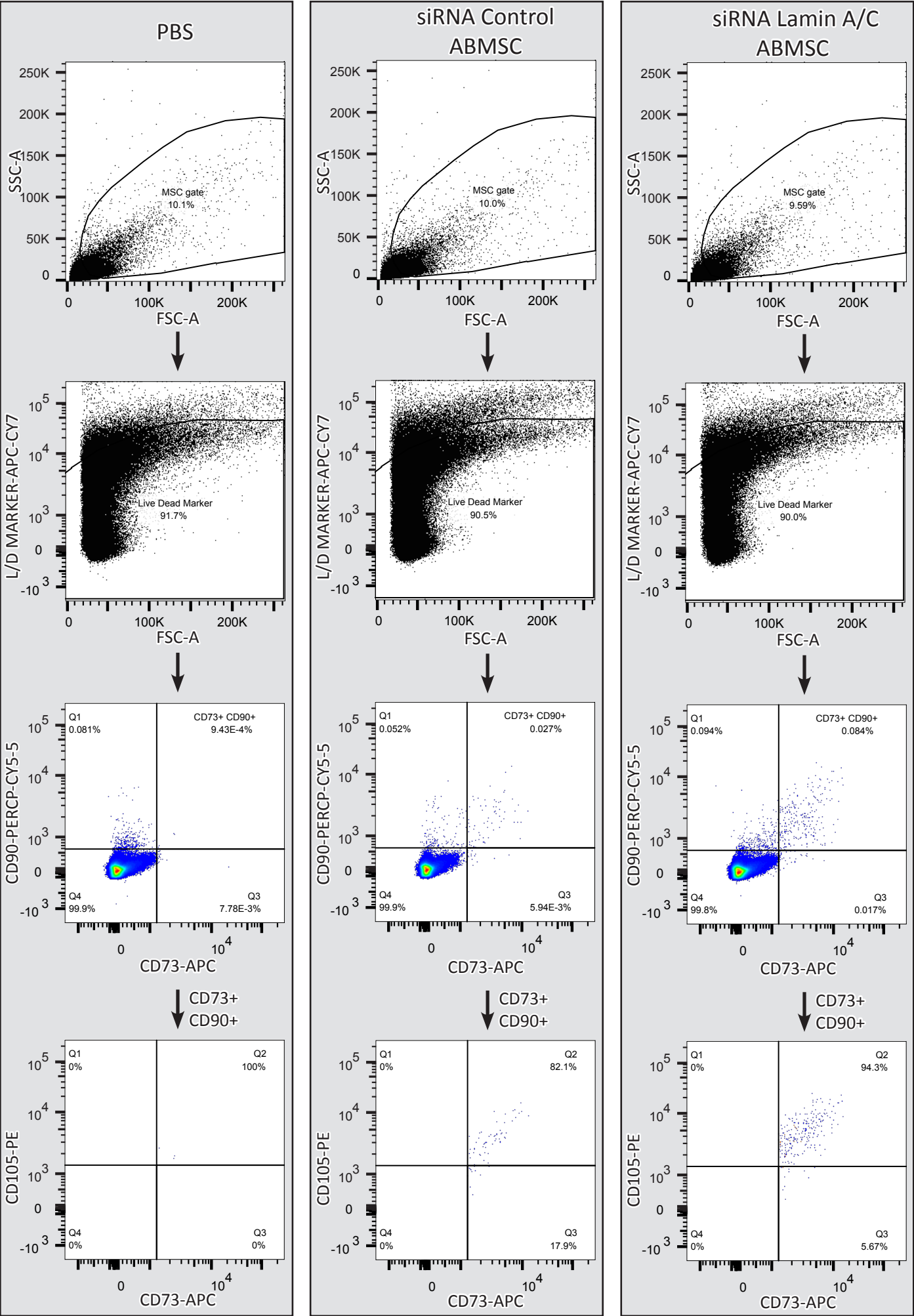


Figure S6

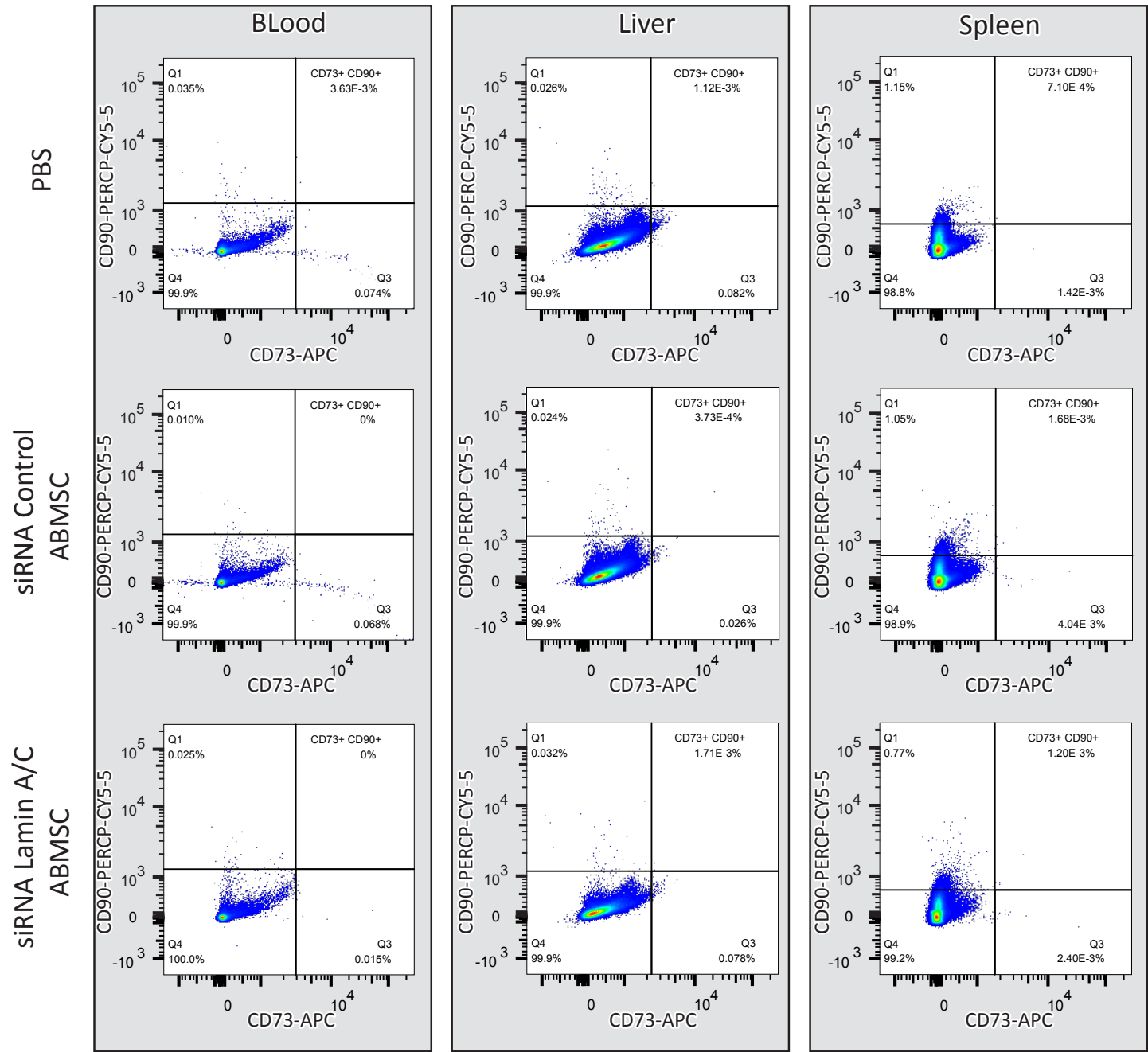
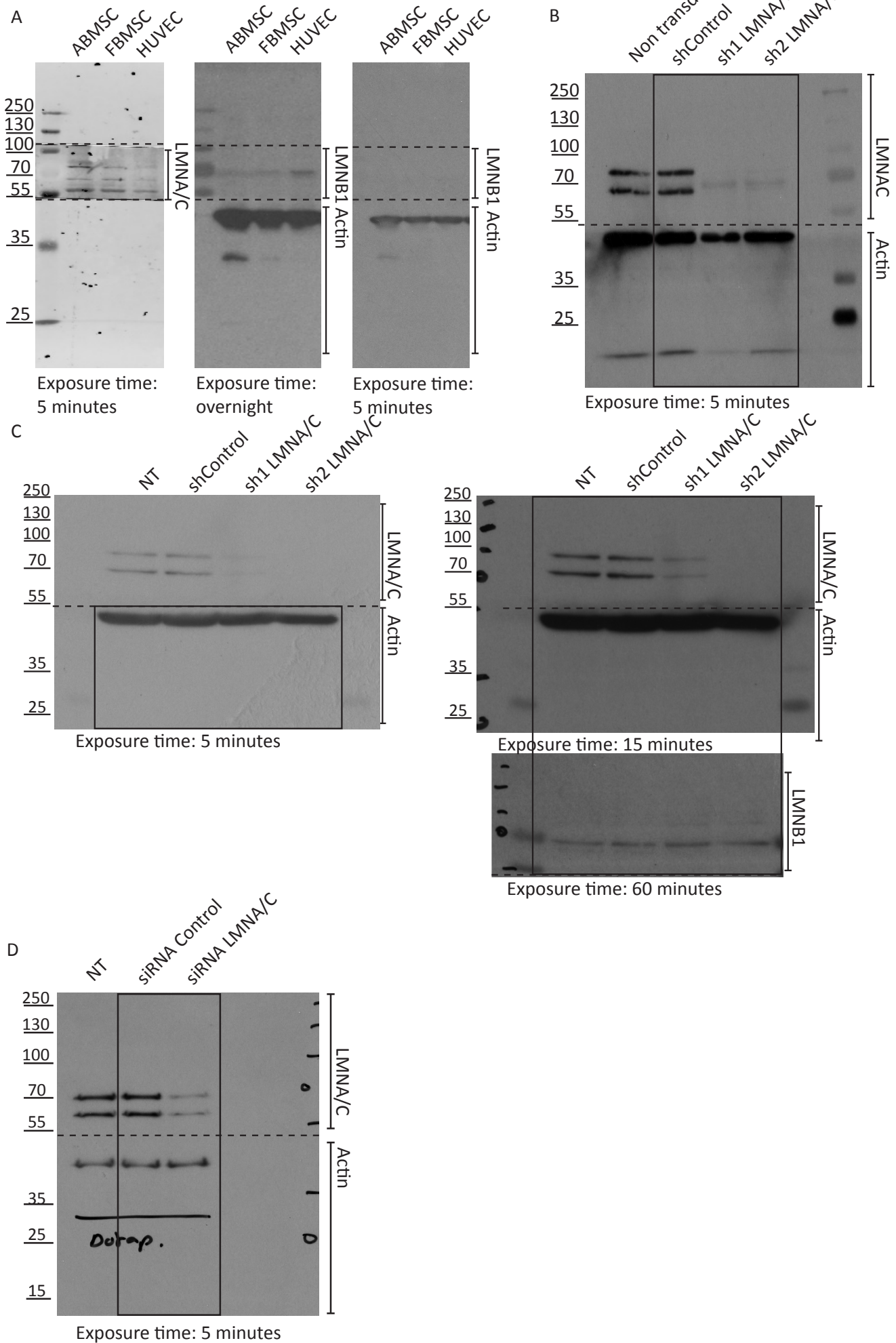


Figure S7



Supplemental Figure and Video legends

Supplemental Figure S1. (a) Individual trajectories of ABMSC, FBMSC, HUVEC and fibroblasts live imaged for 12 hours using brightfield imaging and tracked in ImageJ. $n=3$ independent experiments, shown are 10 cells per condition. **(b)** Average migration velocity of ABMSCs obtained from donors with varying ages, cultured on 20 μ g/ml hFN or bFN. 1 donor per condition, analysis includes 15 cells per condition. Spearman correlation test non-significant.

Supplemental Figure S2. (a) Representative widefield IF tilescan images from top and bottom of transwell filters. ABMSC, FBMSC and HUVEC migrated for 24 hours and were subsequently fixed and stained for F-actin. **(b)** Quantification of fully transmigrated ABMSC and FBMSC. Cells were incubated for 48 hours and 7 days and were subsequently fixed and stained for Lamin A/C and F-actin. Widefield images from the bottom of the filter were analysed manually and the number of fully transmigrated cells (stage 3) was calculated as percentage of total protruding cells (stage 2+3). Mean $\pm$ s.e.m. $n\geq 3$ independent experiments including 30 cells per condition comprising cells derived from at least 3 different donors. Kruskal-Wallis-test followed by an uncorrected Dunn's test, $*=p<0.05$ **(c)** Quantification of total F-actin at the bottom of the Transwell for ABMSC and FBMSC. Cells were allowed to transmigrate for 48 hours and 7 days and were subsequently fixed and stained for F-actin. F-actin surface area coverage at top and bottom of the Transwell filters was determined from tilescan widefield images covering the entire transwell filter area Cell-body migration (F-actin at Transwell bottom (stage 2+3)) was calculated as a percentage of total F-actin (all stages combined). Mean $\pm$ s.e.m. $n\geq 3$ independent experiments comprising cells from at least 3 different donors. Kruskal-Wallis-test followed by an uncorrected Dunn's test.

Supplemental Figure S3.

(a) Representative widefield immunofluorescence (IF) images of ABMSC stained for Lamin A/C (green), F-actin (red) and Hoechst (blue). Magnified images from regions of interest (Zoom) show detailed structures of the nuclear envelope containing Lamin A/C. Cells were fixed with either methanol (top row) or paraformaldehyde (PFA) (middle and bottom row). Cells were cultured in DMEM with 10% FCS (Top and middle row) or DMEM with 5% platelet lysate (bottom row). Scalebars: 20 μ m and 10 μ m. **(b,c)** Quantification of the intensity variation **(b)** in a.u. and nuclear size **(c)** based on Lamin A/C IF widefield imaging. Experiments are displayed in order of cell passage number, each data point comprises the analysis of 30 cells, 11 ABMSC donors are included. Spearman correlation test non-significant.

Supplemental Figure S4. (a) Representative widefield IF tilescan images from top and bottom of transwell filters. ABMSC transduced with shRNA Control or shRNA1,2 Lamin A/C migrated for 24 hours and were subsequently fixed and stained for F-actin. **(b)** Western blot analysis of Lamin

A/C, Lamin B1 and actin (loading control) in lysates of ABMSC transduced with shRNA Control or shRNA1,2 targeting Lamin A/C. NT indicates non transduced cells. Images are cropped scans of blots, corresponding whole Western blot scans are shown in Supplemental Figure S7C.

Supplemental Figure S5.

Representative graphs from FACS analysis of siRNA control and Lamin A/C treated ABMSC in murine lung tissue, 14 hours after intravenous administration. From top to bottom the applied gating strategy to quantify the number of ABMSC is displayed. Antibodies were validated to be specific for ABMSC only and did not bind murine cells. Tissues of PBS-injected mice served as a negative control. ABMSC were defined as CD73+, CD90+ and CD105+.

Supplemental Figure S6.

Representative graphs from FACS analysis of siRNA control and Lamin A/C treated ABMSC in murine blood (left), liver (middle) and spleen (right), 14 hours after intravenous administration. Applied gating strategies were the same as for the FACS analysis in lung tissue. Markers against CD73 and CD90 are shown.

Supplemental Figure S7.

Whole scans of Western blots corresponding to data in Figure 4B, 4D, 5A and Supplemental Figure S4. **(a)** Western blot showing Lamin A/C, Lamin B1 and actin (loading control) in lysates of ABMSC, FBMSC and HUVEC. **(b)** Western blot showing Lamin A/C and actin (loading control) in lysates of ABMSC transduced with shRNA Control or shRNA1,2 targeting Lamin A/C. **(c)** Western blot showing Lamin A/C and actin (loading control) in lysates of ABMSC transduced with GFP-control or GFP-Lamin B1. **(d)** Western blot showing Lamin A/C and actin (loading control) in lysates of ABMSC transfected with control or siRNA's against Lamin A/C. NT indicates non transduced cells. Black rectangles indicate the areas used in the figures, dotted lines indicate sliced membranes.

Supplemental movie M1. Migration of ABMSC, FBMSC, HUVEC and fibroblasts. Live widefield microscopy (Zeiss Observer Z1) imaging using EC Plan-Neofluar 10x/0.30 Ph 1 air objective. Frames were taken every 30 minutes for 12 hours at 37°C, 5% CO₂. ABMSC: passage 4, FBMSC: passage 6, HUVEC: passage 5, Fibroblasts: passage 5.

Supplemental movie M2. Z-stack of ABMSC migrating in Transwell assay. ABMSC in 8 µm pore size transwells migrated for 24 hours and were subsequently fixed and stained for F-actin and Lamin A/C. IF Z-stacks of ABMSC were acquired using confocal microscopy (Leica) using a 40x/1.30 oil objective. Cell passage: 4 Total image z-stack comprises 40 µm.