Expanded View Figures

Figure EV1. Assessment of purity of hiMP populations.

A Representative immunofluorescence analysis of MYOD (skeletal myogenic lineage marker, green) and PAX6 (early neuroectodermal lineage marker, red) immunoreactivity in three of the four different hiMP lines used in this study.

B Immunofluorescence analysis of MYOD and MAP2 (late neuroectodermal/neuronal marker, red) in the same hiMPs shown in (A).

C Positive controls for the PAX6 and MAP2 staining shown in (A, B); top panel: spontaneously differentiating hiPSCs; bottom panel: hiPSC-derived neurons.

D Bar graph quantifying the percentages of MYOD-positive nuclei within three populations of hiMPs (experimental replicates = 3; error bars: SD). Data information: Scale bars: (A, B) 75 μm; (C) top 100 μm; bottom 20 μm.

Source data are available online for this figure.

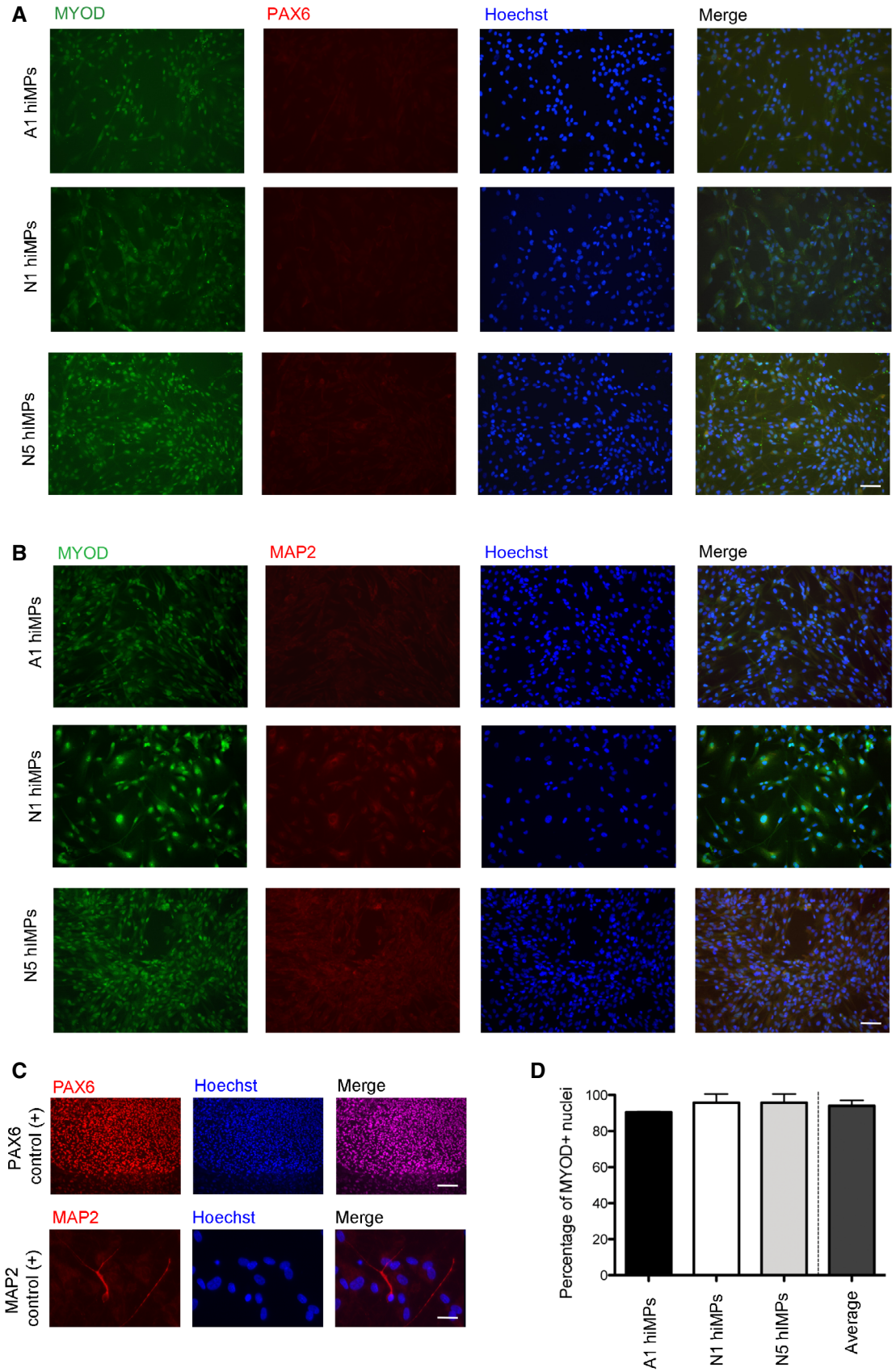
A
MYOD
PAX6
Hoechst
Merge
A1 hiMPs
N1 hiMPs
N5 hiMPs
B
MYOD
MAP2
Hoechst
Merge
A1 hiMPs
N1 hiMPs
N5 hiMPs
C
PAX6
Hoechst
Merge
PAX6 control (+)
MAP2
Hoechst
Merge
MAP2 control (+)
D
Percentage of MYOD+ nuclei
100
80
60
40
20
0
A1 hiMPs
N1 hiMPs
N5 hiMPs
Average

Figure EV1.

Figure EV2. Additional *in vitro* motility and migration analyses of treated and untreated hiMPs.

A Trajectory plots for visualisation of the migratory paths of untreated and treated cells that were exposed to either 1% BSA or DLL4 and PDGF-BB, respectively, over the course of the motility assay. Each line depicts the path of an individual cell.

B Visualisation of the motility state space of untreated and treated hiMPs using t-SNE (perplexity = 35).

C Hierarchical clustering of the first 30 principal components visualised with a t-SNE plot showing two clusters (Silhouette $S_i = 0.19$).

D Bar charts displaying the normalised motility feature values for comparison between conditions: untreated and DLL4 and PDGF-BB (left), cluster 1 and cluster 2 (right) (experimental replicates = 3; total 412 cells).

E Bar graph showing proportions of untreated and treated hiMPs within the two clusters. Hypothesis testing was performed with a chi-squared (χ^2) test.

F Trajectory plots for visualisation of hiMP migration after 24 h of treatment (top row), or 72 h of treatment (bottom row).

G, H t-SNE plots (perplexity = 35) for visualisation of the motility state space of hiMPs in two-dimensions (left). Cluster assignments after hierarchical clustering ($S_i = 0.13$ (24 h); $S_i = 0.18$ (72 h)). (H) Bar plots showing normalised motility features for both 24 h (top row) and 72 h (bottom row) conditions (experimental replicates = 3; total 876 cells and total 478 cells analysed for 24 and 72 h conditions, respectively.).

I Bar graph displaying proportions of untreated and DLL4 and PDGF-BB-treated hiMPs treated for 24 and 72 h. Hypothesis testing was performed with a chi-squared (χ^2) test.

J Bar graphs depict quantification of parameters obtained from single cell tracking analysed using TrackMate. Motility statistics were calculated for untreated (grey bars) and treated (white bars) hiMPs (experimental replicates = 3; error bars: SD). *P* values within figure: *t*-test.

Source data are available online for this figure.

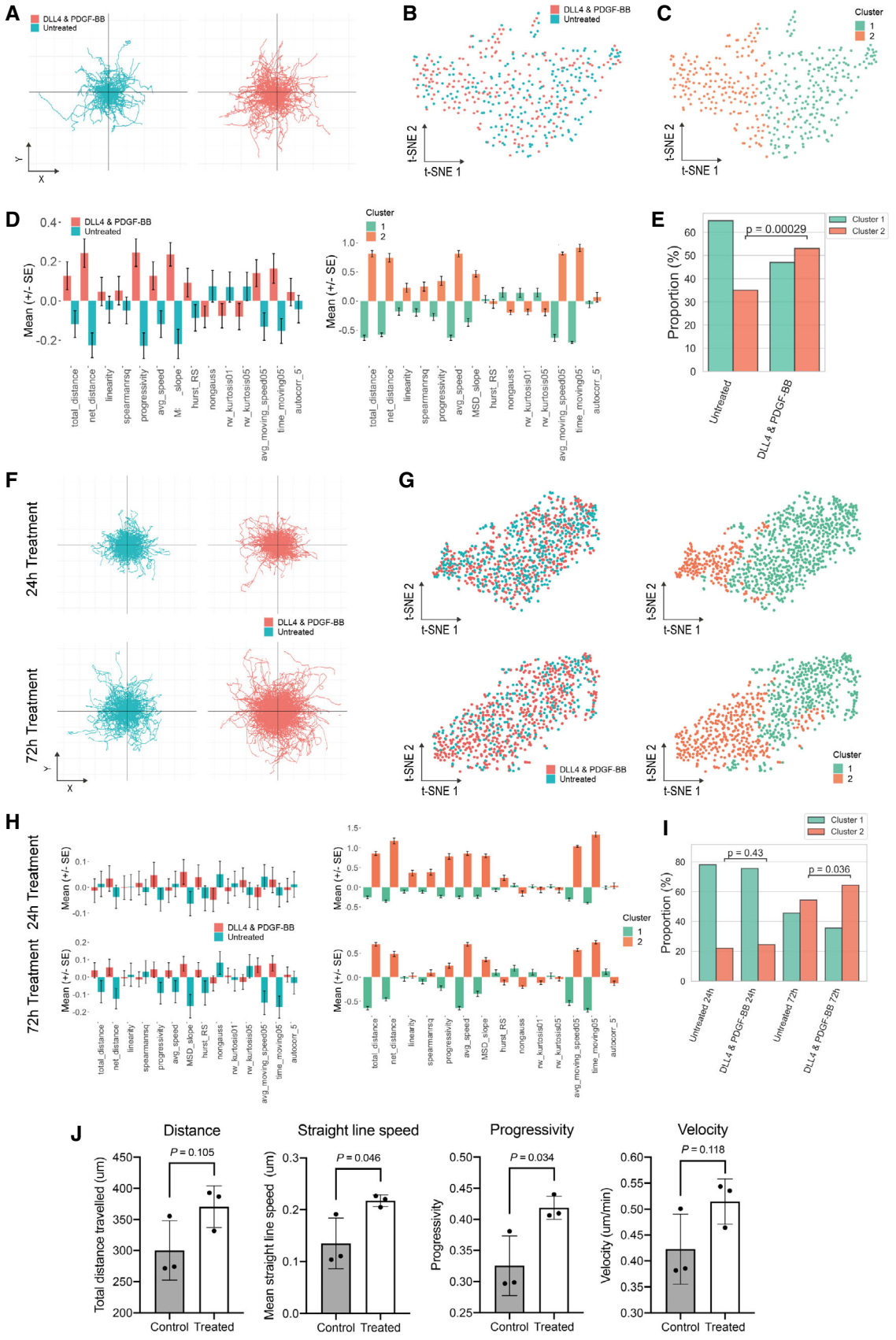
A
DLL4 & PDGF-BB
Untreated
B
C
Cluster
1
2
t-SNE 1
t-SNE 2
D
Mean (+/- SE)
total_distance
net_distance
linearity
spearmanrsq
progressivity
avg_speed
MSD_slope
hurst_RS
nongauss
rw_kurtosis01
rw_kurtosis05
avg_moving_speed05
time_moving05
autocorr_5
E
p = 0.00029
Proportion (%)
Untreated
DLL4 & PDGF-BB
F
24h Treatment
72h Treatment
G
H
I
p = 0.43
p = 0.036
Untreated 24h
DLL4 & PDGF-BB 24h
Untreated 72h
DLL4 & PDGF-BB 72h
J
Distance
P = 0.105
Total distance travelled (um)
Straight line speed
P = 0.046
Mean straight line speed (um)
Progressivity
P = 0.034
Velocity
P = 0.118
Velocity (um/min)
Control
Treated

Figure EV2.

Figure EV3. Additional *in silico* analyses and assessment of permeability of endothelialised 3D blood vessel-like microfluidic channels.

A Heatmaps displaying genes that are involved in negative regulation of cellular extravasation (left; GO: 002692) and positive regulation of cellular extravasation (right; GO: 002693). *$P < 0.05$.

B *P* value-adjusted hierarchical clustering heatmap showing a manually curated list of genes involved in enhanced trans-endothelial migration of cancer cells (*P* set al 0.05).

C Maximum intensity projection of a microfluidic channel immunostained for CD31 showing cobblestone-like morphology of HUVECs lining the top perfusion channel. Scale bar = 30 μm.

D Representative fluorescence images of 20 kDa FITC-conjugated dextran (top two rows) and 150 kDa TRITC-conjugated dextran (bottom two rows) added to the top perfusion channel of OrganoPlate® chips with and without 3D endothelial monolayers generated by HUVECs. Chips were imaged every 3 min for 15 min. Scale bar = 100 μm.

E Bar chart quantifying images shown in (D) using the normalised intensity calculated as the ratio of fluorescence between the ECM channel and top perfusion channel at each time point for cell-free and HUVEC chips containing 20 and 150 kDa fluorescent dextrans (technical replicates = 3; error bars; SD).

Source data are available online for this figure.

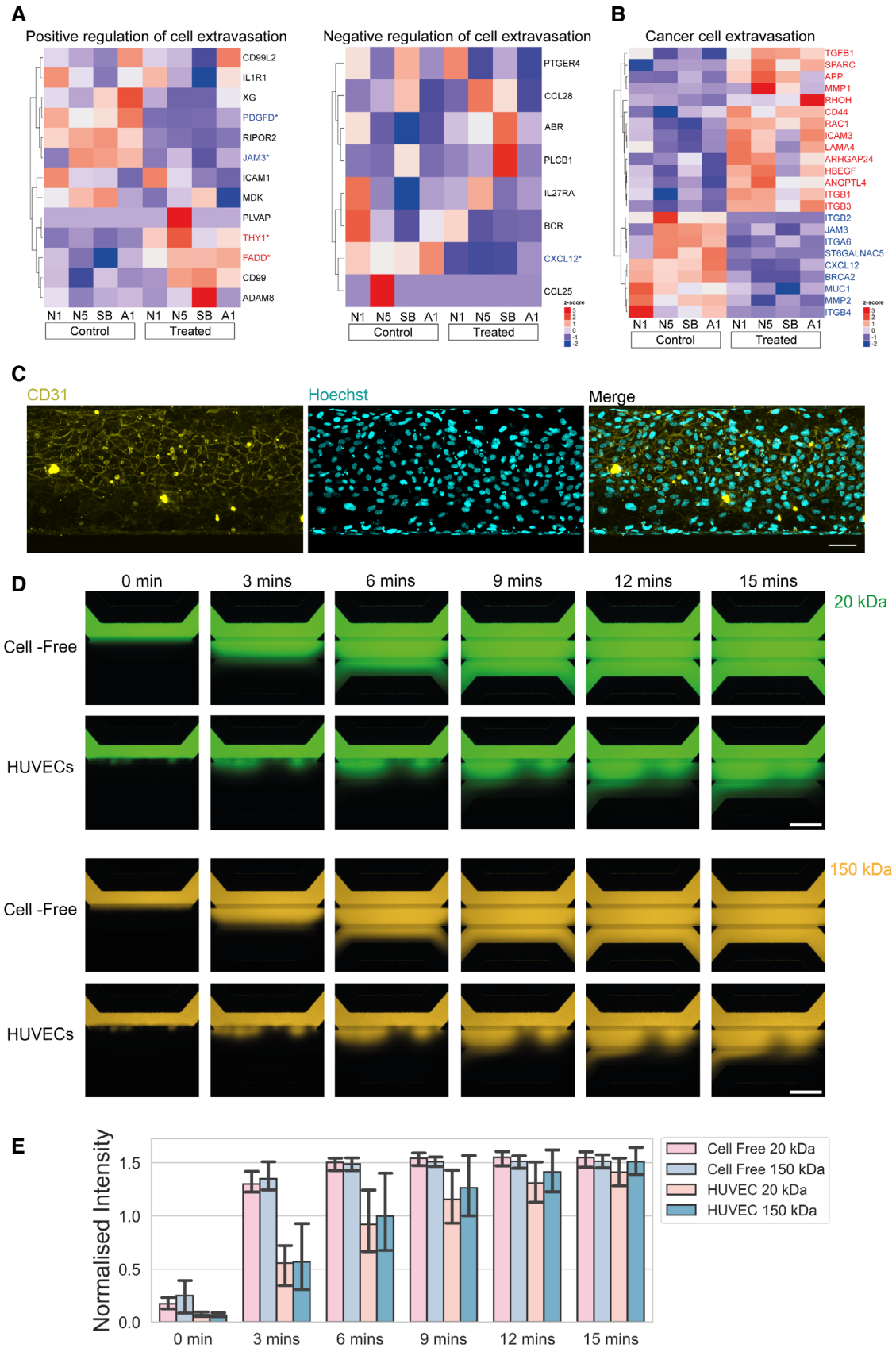

Figure EV3.