Electronic Supplementary Material (ESI) for Biomaterials Science.

Supplementary Materials for

Synthetic Hyaluronic Acid Coating Preserves the Phenotypes of Lymphatic Endothelial Cells

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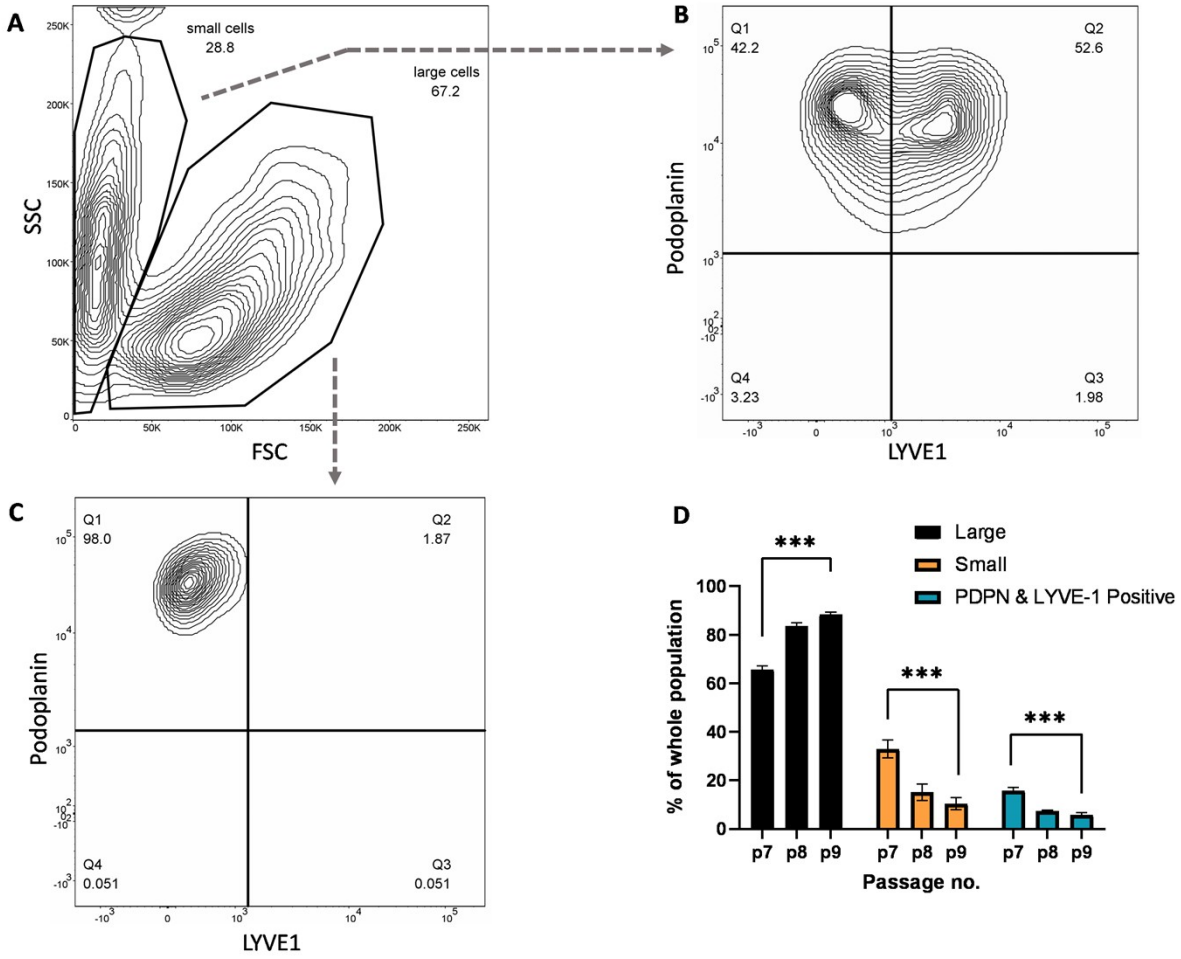
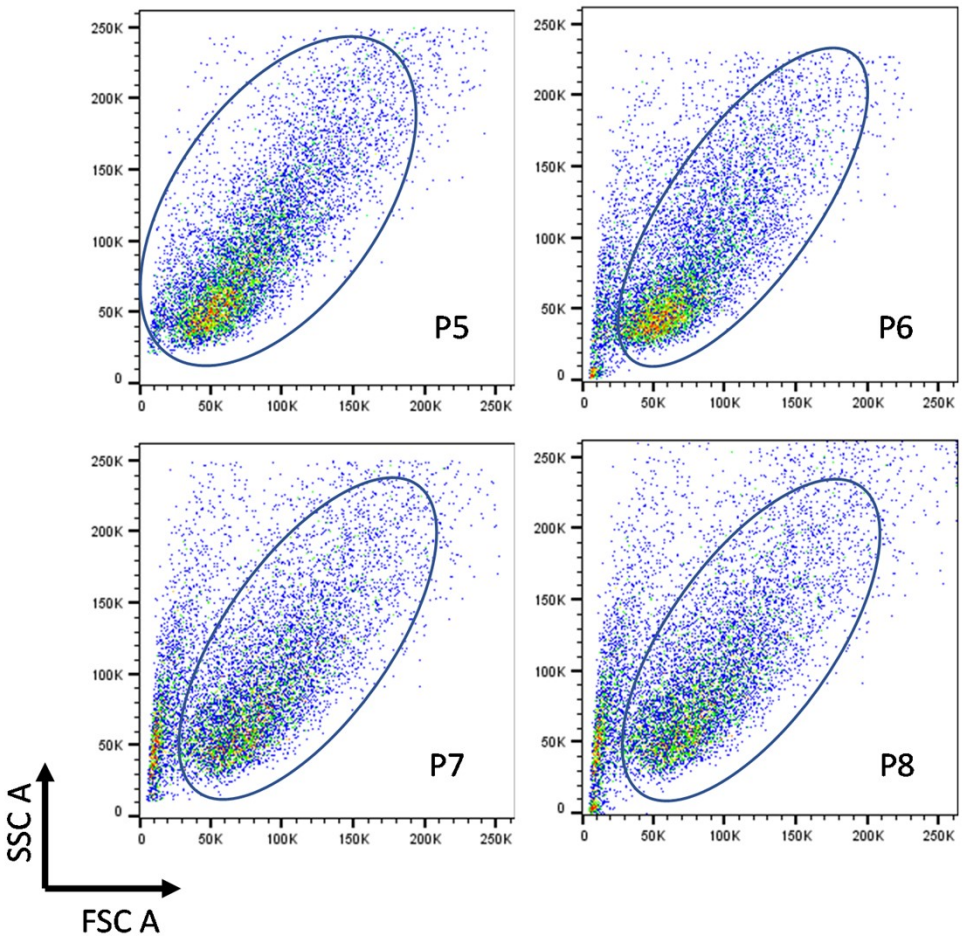
This PDF file includes:

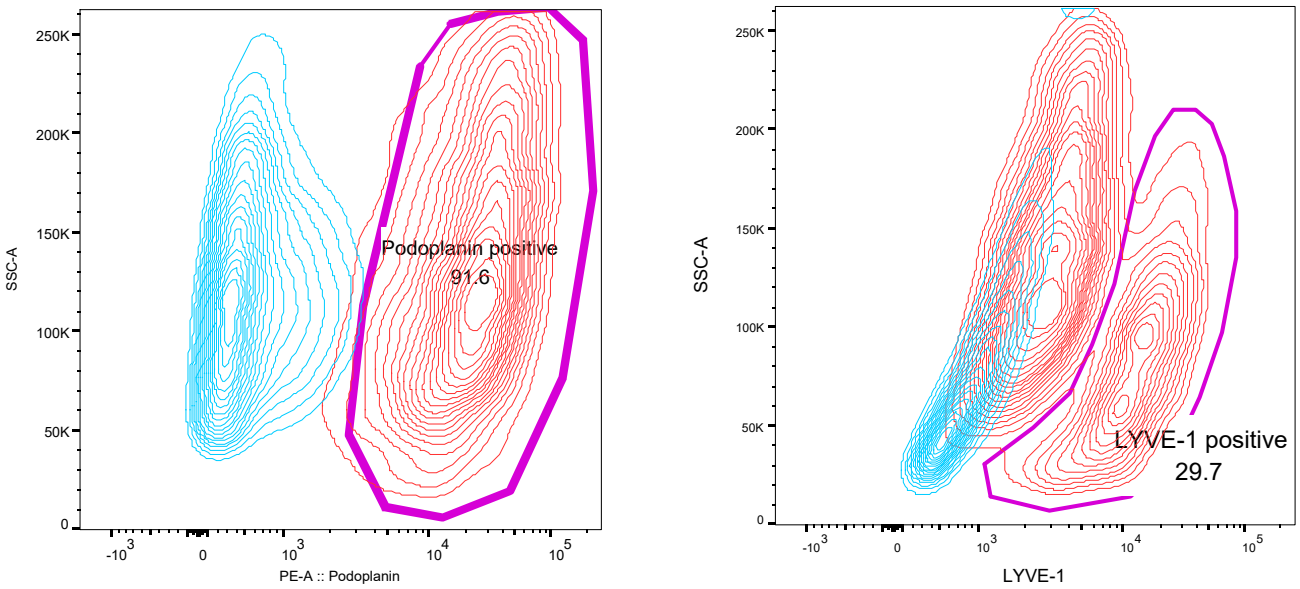
Supplementary Figure S1 to S6
Supplementary Table S1 to S2



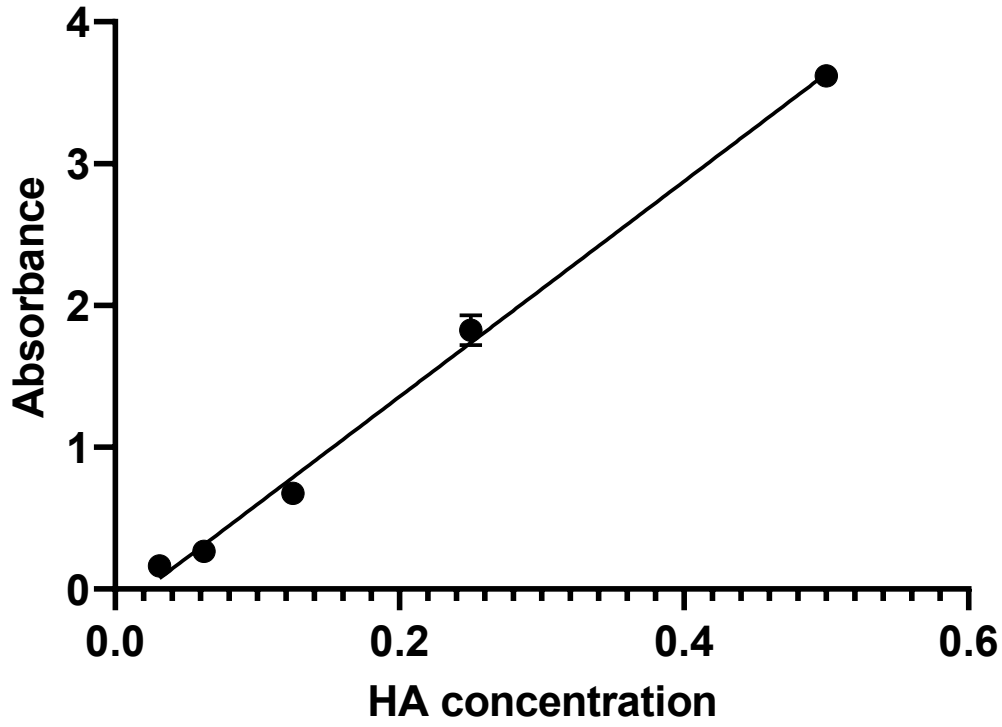
Supplementary Figure 1. (A) Representative gating strategy to analyze human LECs using flow cytometry. Human LECs demonstrate a heterogenous mixture of small (28.8 %) and large cell (67.2 %) populations. **(B)** Human LECs were stained with PE anti-PDPN and APC anti-LYVE1 antibodies. Flow cytometry analysis indicates that 42.2% of the small cell population is PDPN^{+}/LYVE-1^{-}, while 52.6% of the small cell population is PDPN^{+}/LYVE-1^{+}. **(C)** Flow cytometry analysis indicates that 96.0% of the large cell population is PDPN^{+}/LYVE-1^{-}. **(D)** Human LECs were analyzed using flow cytometry after being cultured *in vitro* from passage 7 to 9. Flow cytometry analysis indicates that with an increase in passage number, there is an increase in the amount of large cells and a decrease in the amount of small cell in the population. Flow cytometry analysis using lymphatic markers indicates that as passage number increased, there is a decrease in LYVE-1^{+} cells.



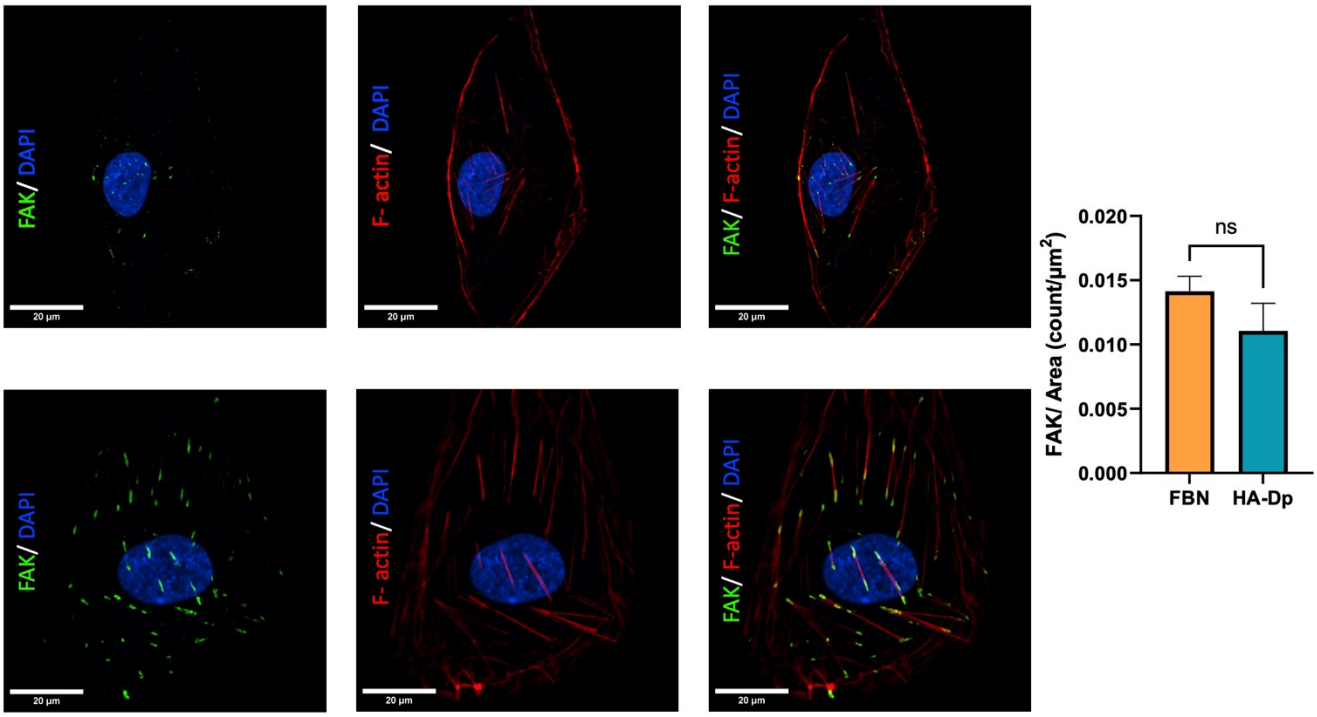
Supplementary Figure 2. Representative scatter plots SSC-A vs. FSC-A. The oval area indicating the gating strategy for LEC population from P5-8.



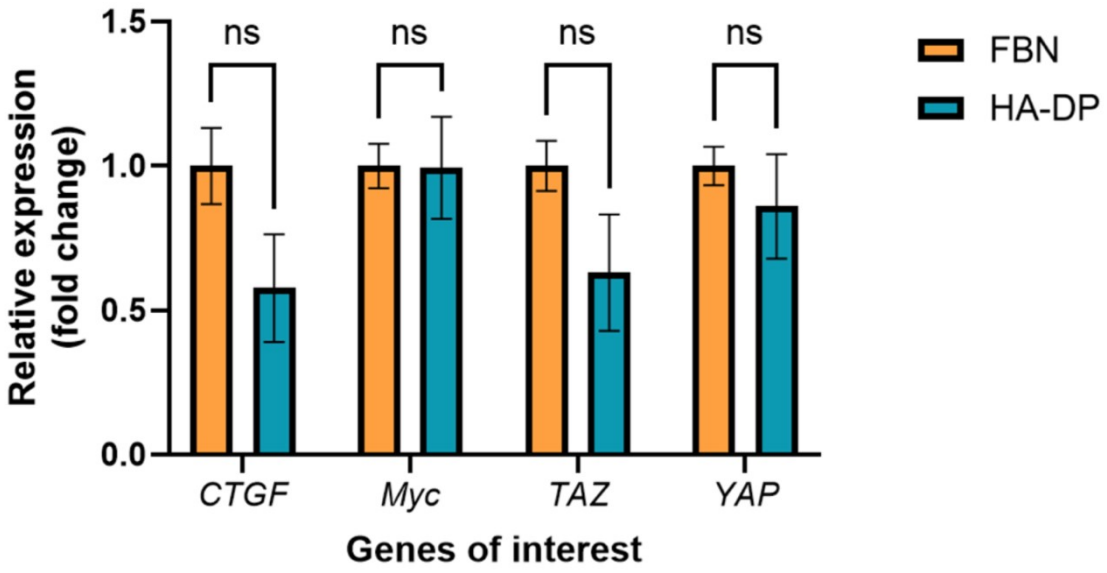
Supplementary Figure 3. Representative contour plots for SSC-A vs. Podoplanin (left) and SSC-A vs. LYVE-1 (right). The areas highlighted in purple indicating podoplanin$^+$ cells (91.6%) and LYVE-1$^+$ cells (29.7%).



Supplementary Figure 4. Standard curve to correlate absorbance (634 nm) to HA-concentration (mg/ml).



Supplementary Figure 5. Focal adhesion kinase (FAK) and F-actin staining for LEC (P.4) on HA-DP (top panel) and FBN (bottom panel). Quantification of FAK is shown in the adjacent graph.



Supplementary Figure 6. Early passage LEC (P.4) cultured on FBN and HA-DP were analyzed using qRT-PCR for *CTGF, MYC, YAP, and TAZ*. Statistical significance was set at * $p<0.05$ and ^{ns} $p>0.05$

Supplementary Table 1. Antibodies used for immunostaining and FASC in this study.

Name of Reagent	Company	Host Species	Catalog Number	Dilution Factor
LYVE-1	Abcam	Mouse	ab-219556	1:1,000
Podoplanin	Abcam	Rabbit	ab-10288	1:400
Phalloidin	Thermo Fisher	594 conjugated	ab-176753	1:1,000
FAK	Sigma-Aldrich	FITC conjugated	16-233	2 µg/ml
Prox1	R&D systems	Goat	AF2727	1:200
VECAD	BD Bioscience	Mouse	555661	1:100
DAPI	Thermo Fisher	n/a	D-1306	300 nM
LYVE-1	R&D systems	Mouse	FAB20892A	1 µg/ml
Podoplanin	BioLegend	Rat	337004	1 µg/ml
Prox1	Novus Biologicals	Mouse	NBP1-30045AF488	1 µg/ml

Supplementary Table 2. Primers used for this study

Genes	Catalog Number
LYVE-1	Thermo Hs00272659_m1
PDPN	Thermo Hs00366766_m1
Prox-1	Thermo Hs00896294_m1
FLT4	Thermo Hs01047677_m1
GAPDH	Thermo Hs02786624_g1
YAP	Thermo Hs00902712_g1
TAZ	Thermo Hs00902887_g1
MYC	Thermo Hs00153408_m1
CTGF	Thermo Hs02786624_g1