A robust vasculogenic microfluidic model using human immortalized endothelial cells and Thy1 positive fibroblasts

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Supplemental Figures

Figure S1:

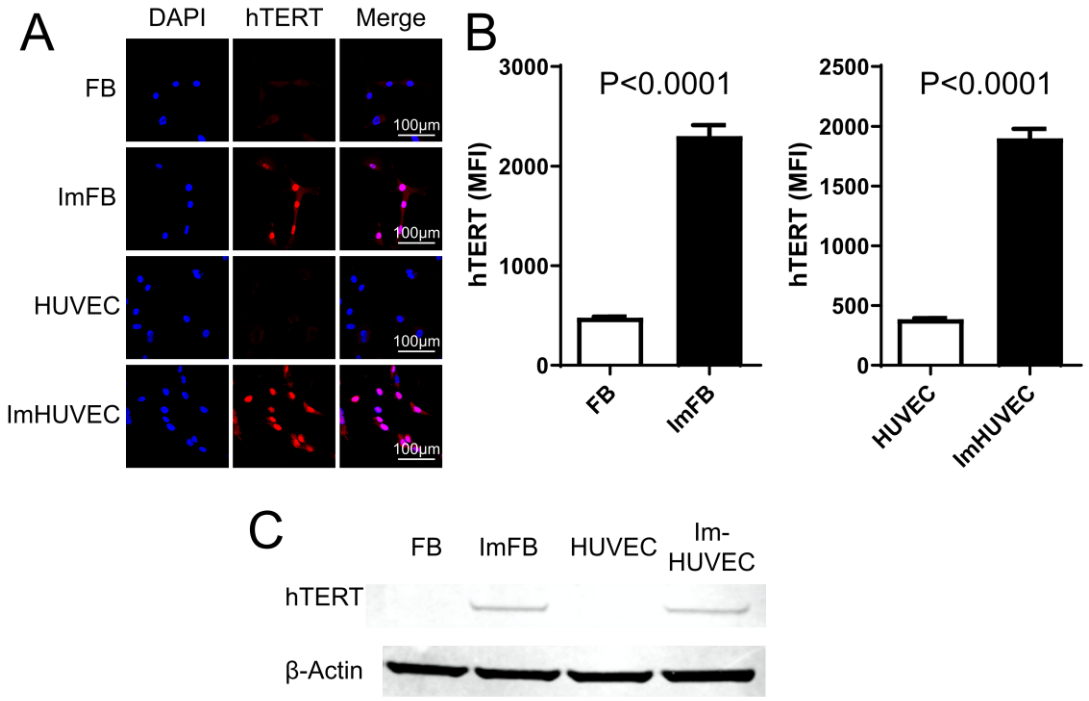

Figure S1: Expression of hTERT in FBs, ImFBs, HUVECs and ImHUVECs.

(A) Representative confocal images of nucleus (DAPI, in blue) and hTERT (red) staining in FBs, ImFBs, HUVECs and ImHUVECs. Scale bar is 100 μm.

(B) Statistical quantification for the mean fluorescent intensity (MFI) of hTERT in FBs, ImFBs, HUVECs and ImHUVECs. Bars represent mean ± SD. Two-tailed *t* tests were performed for the statistical comparisons.

(C) hTERT detection in FBs, ImFBs, HUVECs and ImHUVECs by western blot.

Figure S2:

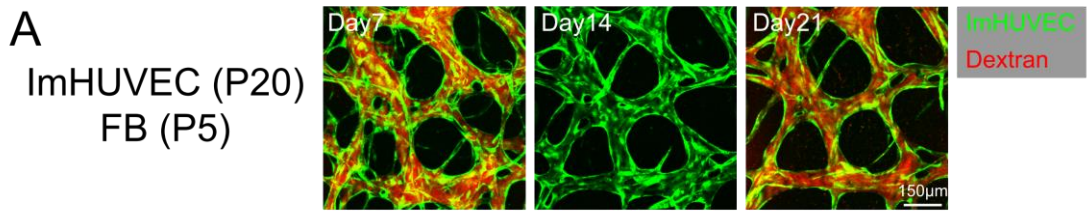

Figure S2: μVNs made of ImHUVECs from P20 with ImFBs from P5 remain perfusable over 3 weeks.

(A) Representative images of μVNs made of ImHUVECs (green) from passage 20 (P20) and ImFBs from passage 5 (P5) at the same location on day 7, 14 (did not perform dextran perfusion experiment), and 21. μVNs are perfused with 70 kDa MW Texas Red dextran (red).

Figure S3:

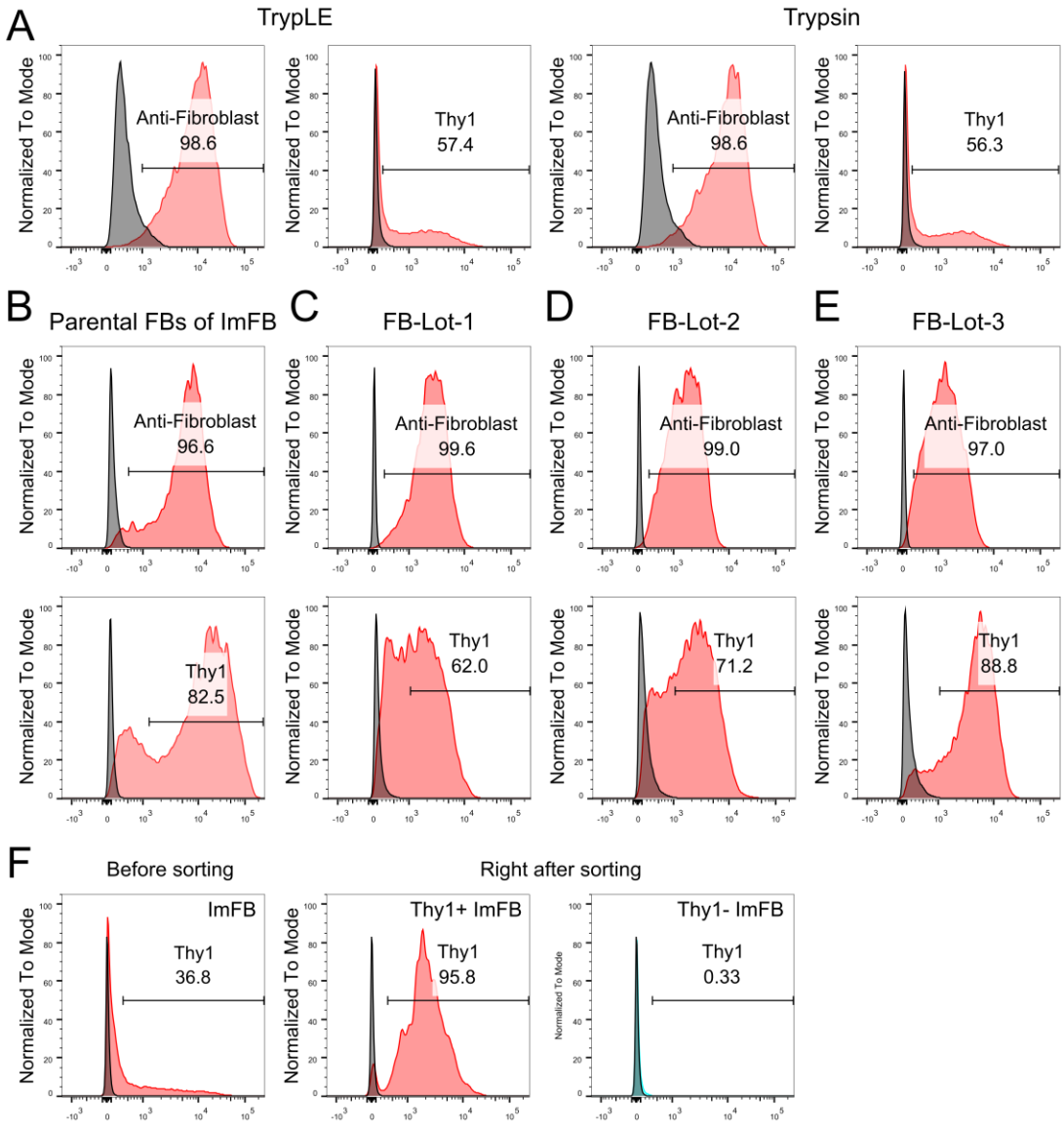

Figure S3: Evaluation of anti-Fibroblast and Thy1 expression in FBs from different experimental conditions.

(A) FACS measurement of anti-Fibroblast and Thy1 in FBs detached by TrypLE (left) or Trypsin (right).

(B-E) Representative histograms showing the anti-Fibroblast (top) and Thy1 (bottom) expression in parental FBs of ImFBs **(B)**, and FBs from different lot numbers. **(C-E)**.

(F) Representative histograms of anti-Fibroblast and Thy1 expression in ImFBs before and right after Thy1 based cell sorting.

Figure S4:

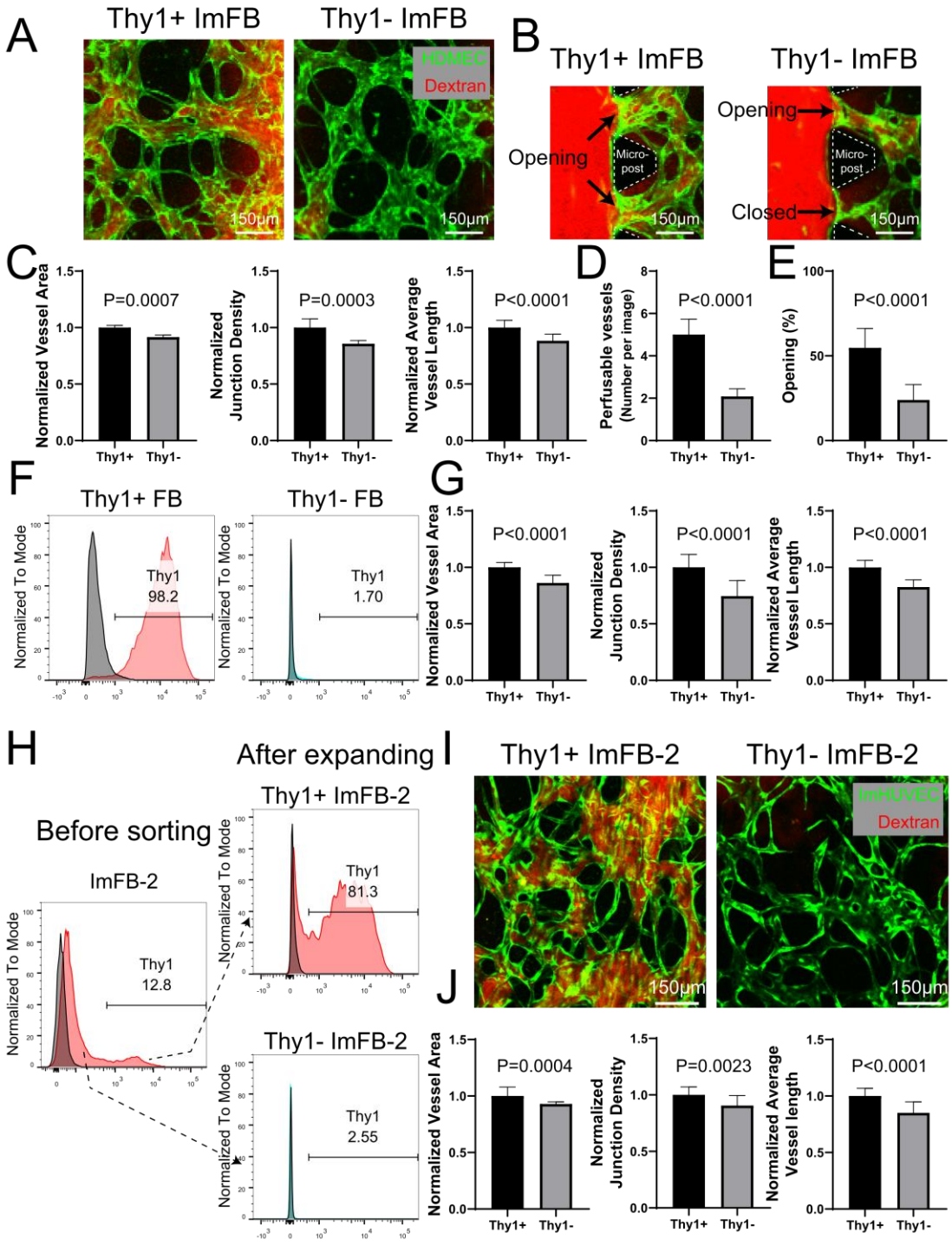

Figure S4: Thy1+ FBs promote better μVN formation than Thy1- FBs.

(A) Representative images of μVNs made of human dermal microvascular endothelial cells (HDMEC) with Thy1+ ImFBs or Thy1- ImFBs. Green,

HDMEC. Red, dextran. Scale bar is 150 μm.

(B) Representative images of dermal μVNs formed with Thy1+ or Thy1- ImFBs at the gap regions between microposts. Arrows point out the microvessel openings and closed structure. Green, HDMEC. Red, dextran. Scale bar is 150 μm.

(C) Statistical analysis of normalized vessel area (left), junction density (middle), and average vessel length (right) of dermal μVNs formed with Thy1+ ImFBs or Thy1- ImFBs.

(D-E) Quantification of the number of perfusable microvessels in each image **(D)** and the percentage of the openings at the gap regions between microposts in each device **(E)**.

(F) Representative histograms showing Thy1 expression in Thy1+ primary FBs and Thy1- primary FBs after sorting.

(G) Statistical analysis of normalized vessel area (left), junction density (middle), and average vessel length (right) of μVNs made of ImHUVECs with Thy1+ or Thy1- primary FBs.

(H) FACS measurements of Thy1 in ImFBs made from another batch of FBs (ImFB-2) before sorting (left) and after expanding the sorted Thy1+ (top right) and Thy1- (bottom right) cells.

(I) Representative images of μVNs made of ImHUVECs with Thy1+ ImFB-2 or Thy1- ImFB-2. Green, HDMEC. Red, dextran. Scale bar is 150 μm.

(J) Statistical analysis of normalized vessel area (left), junction density (middle), and average vessel length (right) of μVNs formed with Thy1+ ImFB-2 or Thy1- ImFB-2.

Bars represent mean ± SD. Two-tailed *t* tests were performed for the statistical comparisons.

Figure S5:

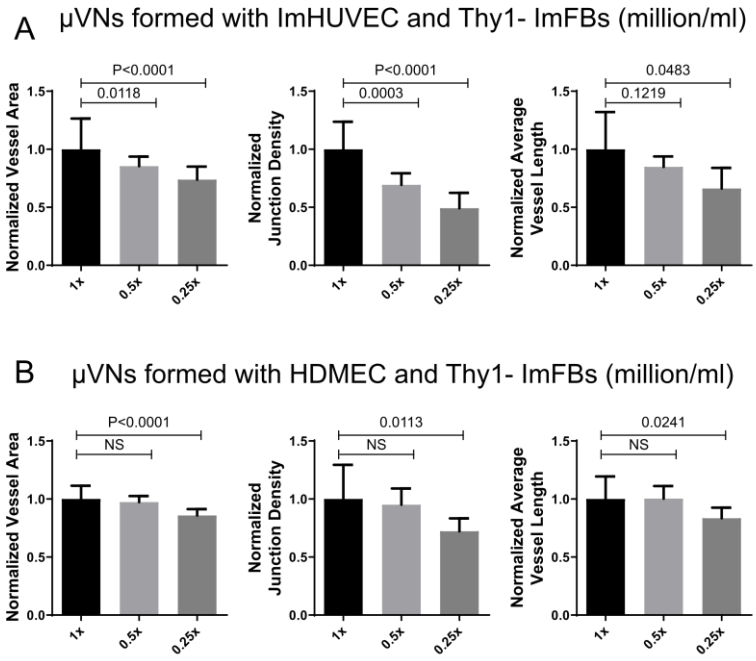

Figure S5: Reducing seeding density of Thy1- ImFBs cannot rescue the defective μVNs.

(A-B) Statistical analysis of normalized vessel area (left), junction density (middle), and average vessel length (right) of μVNs made of ImHUVEC **(A)** or HDMEC **(B)** with Thy1- ImFBs at different seeding density.

Bars represent mean ± SD. Two-tailed *t* tests were performed for the statistical comparisons.

Figure S6:

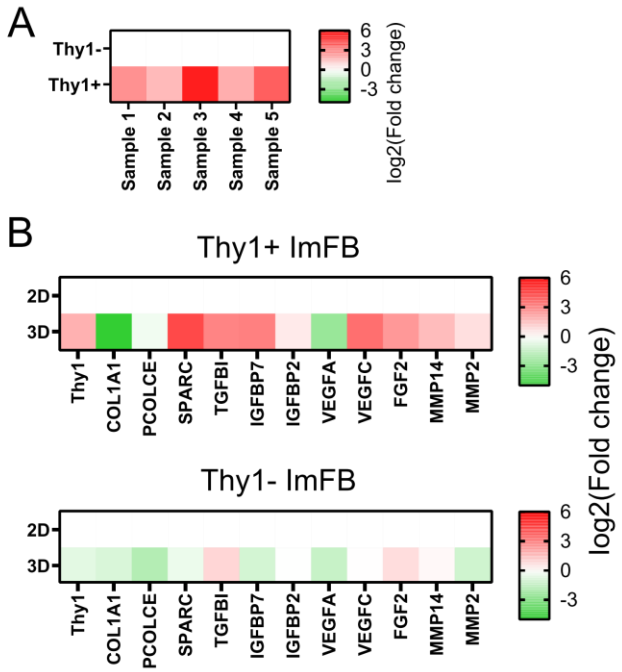

Figure S6: Expression of angiogenic and lumen formation factors in FBs.

(A) Heatmap of RT-PCR results of IGFBP2 expression in Thy1+ and Thy1- ImFBs or primary FBs from different samples cultured in 2D. Fold change was relative to Thy1- ImFBs or primary FBs of each sample.

(B) Heatmap of RT-PCR results of genes involved in lumen formation and angiogenesis in Thy1+ ImFBs (top) and Thy1- ImFBs (bottom) cultured in 2D or 3D. Fold change was relative to 2D data of each sample.

Figure S7:

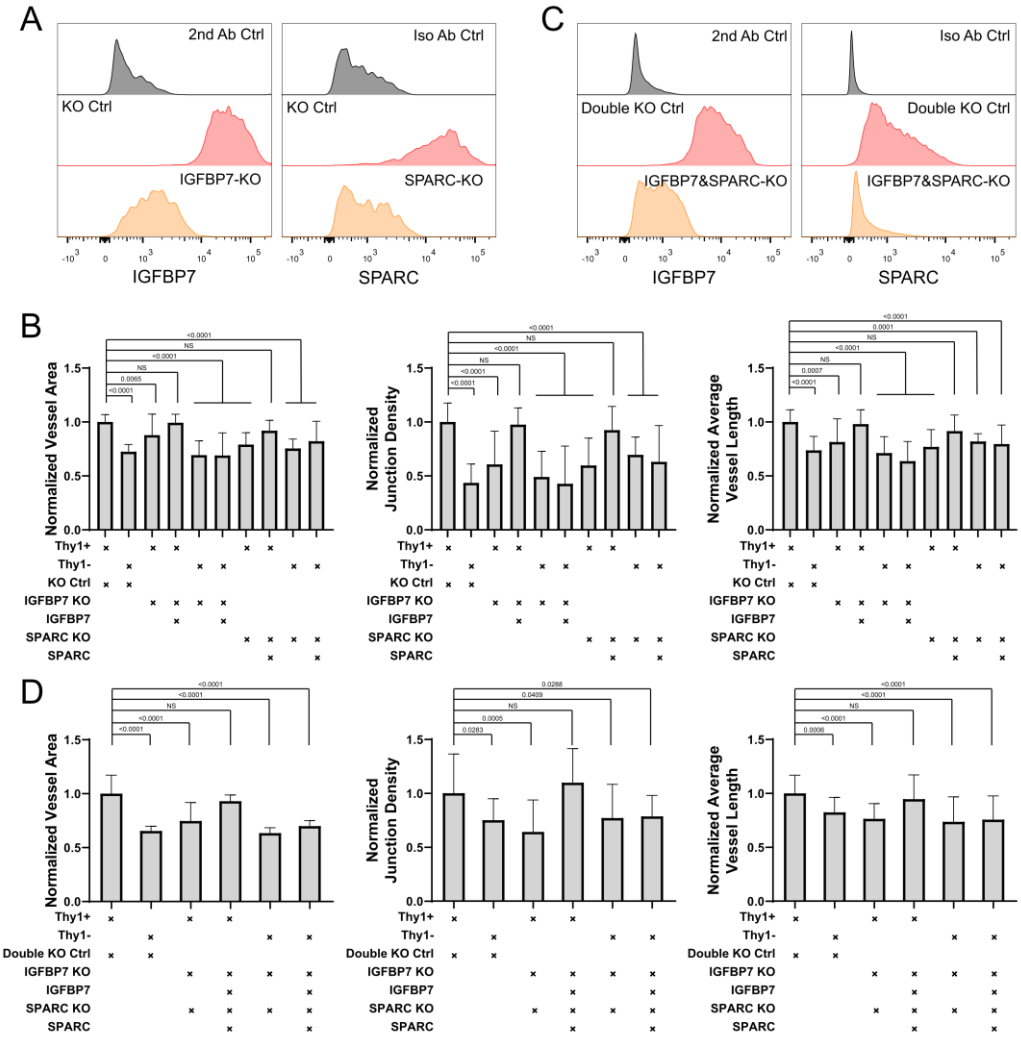

Figure S7: IGFBP7 and SPARC are required for µVN formation.

(A) Representative histograms showing IGFBP7 (left) or SPARC (right) expression in KO control ImFBs and IGFBP7 KO ImFBs, or SPARC KO ImFBs, respectively.

(B) Statistical analysis of normalized vessel area (left), junction density (middle), and average vessel length (right) of µVNs made of ImHUVECs with Thy1+ or Thy1- KO control ImFBs, IGFBP7 KO ImFBs, or SPARC KO ImFBs.

(C) Representative histograms showing IGFBP7 (left) or SPARC (right) expression in double KO control ImFBs and IGFBP7 and SPARC double KO ImFBs.

(D) Statistical analysis of normalized vessel area (left), junction density (middle),

and average vessel length (right) of μVNs made of ImHUVECs with Thy1+ or Thy1- double KO control ImFBs, IGFBP7 and SPARC double KO ImFBs. Bars represent mean ± SD. Two-tailed *t* tests were performed for the statistical comparisons.

Figure S8:

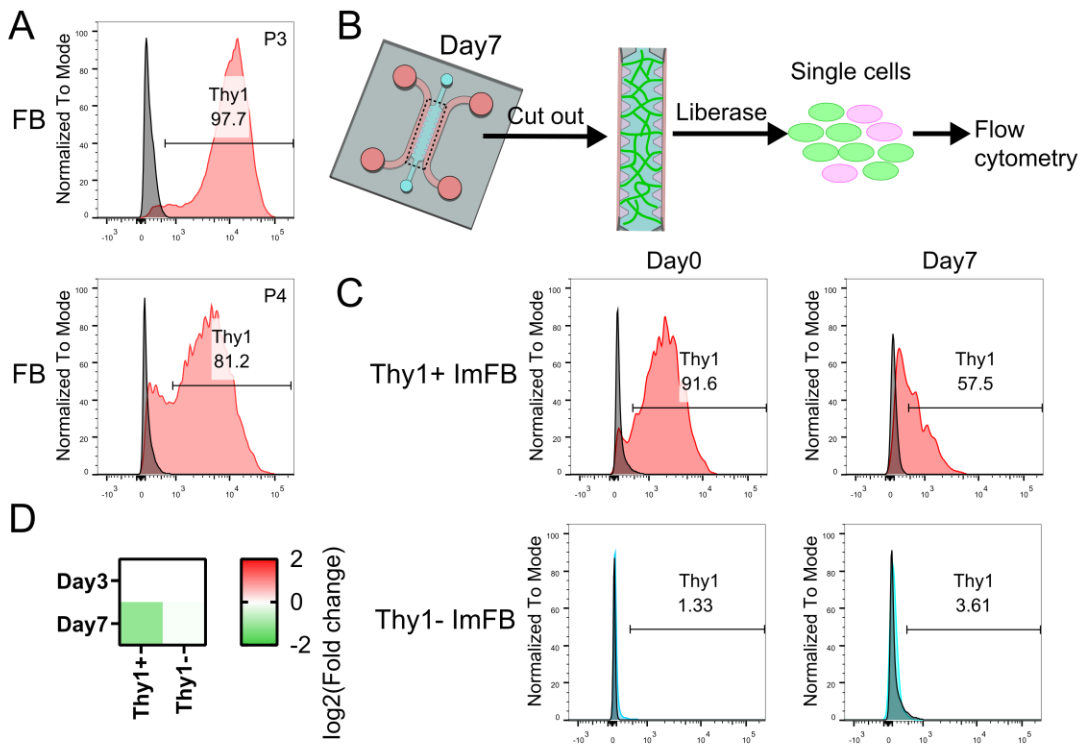

Figure S8: Thy1 expression in FBs decreased when expanding in both 2D and 3D.

(A) Representative histograms showing Thy1 expression in FBs from P3 (top) and P4 (bottom).

(B) Schematic representation of the flow cytometry procedure to run from μVNs. Gel channels containing cells were cut out from the device, and further treated with Liberase to dissociate single cells from matrix.

(C) Representative histograms showing Thy1 expression in Thy1+ ImFBs and Thy1- ImFBs on day 0 and day 7.

(D) Heatmap of RT-PCR results of Thy1 expression in Thy1+ and Thy1- ImFBs cultured in 3D fibrin gel for 3 days and 7 days. Fold change was relative to day 3 data of each sample.

Figure S9:

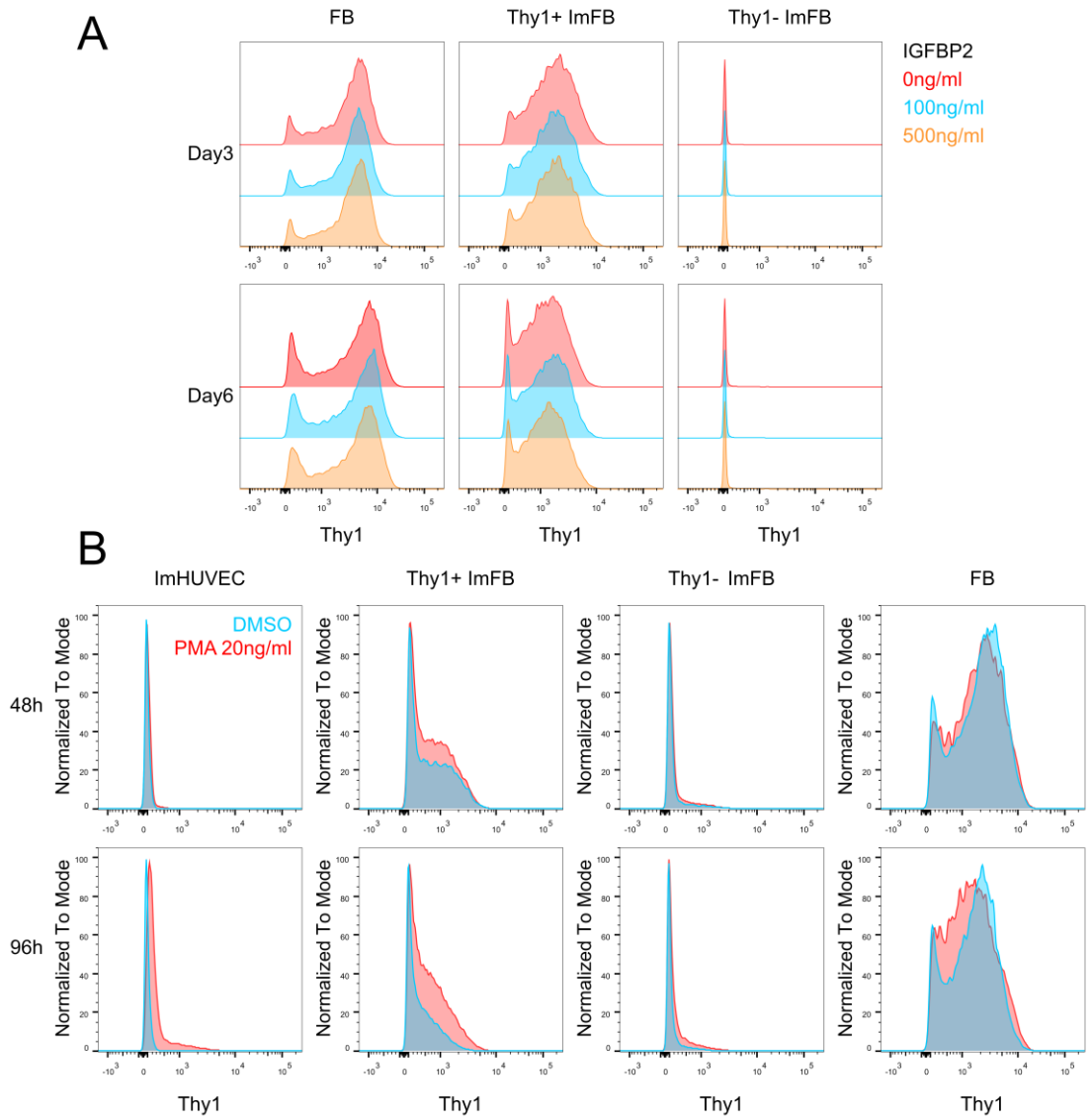

Figure S9: Thy1 expression in FBs treated with IGFBP2 or PMA.

(A) Representative histograms showing Thy1 expression in FBs (left), Thy1+ ImFBs (middle), and Thy1- ImFBs (right) treated with IGFBP2 at 0, 100, or 500 ng/ml for 3 days (top) or 6 days (bottom).

(B) Representative histograms showing Thy1 expression in ImHUVECs (far left), Thy1+ ImFBs (left), Thy1- ImFBs (right), and FBs (far right) treated with DMSO or PMA for 48h (top) or 96h (bottom).