Expanded View Figures

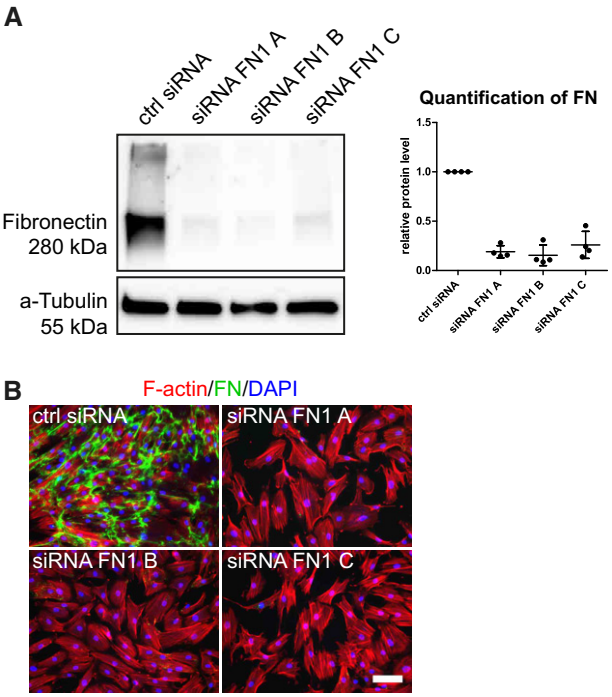

Figure EV1. Verification of fibronectin knockdown in BM-MSCs by siRNA gene silencing.

A Efficiency of siRNA-mediated fibronectin knockdown in BM-MSCs analyzed by Western blot analysis 48 h after knockdown initiation. Quantification shows the Western blot signal intensity of fibronectin normalized to a-tubulin, $n = 4$, individual data points and mean (line) ± SD.

B Absence of cellular fibronectin networks analyzed by immunofluorescence of cell-derived fibronectin 3 days after successful knockdown. Scale bar: 100 μm.

Source data are available online for this figure.

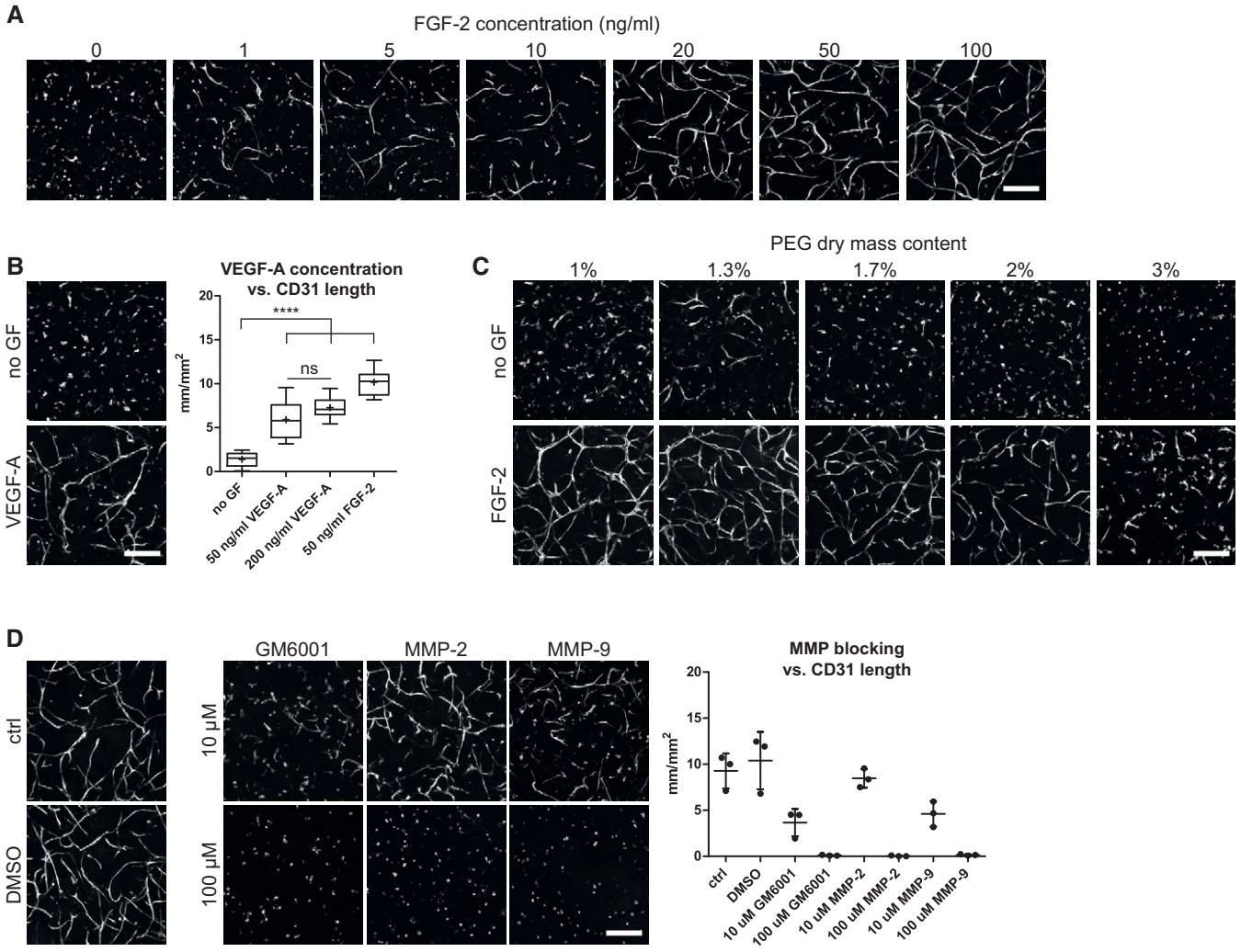

Figure EV2. 3D micro-capillary network formation in PEG hydrogels depends on growth factor concentration, PEG matrix stiffness, and MMP activity.

Representative images of micro-capillary networks by CD31-immunostains at day 7.

A Impact of FGF-2 concentration in the medium.

B Impact of VEGF-A concentration in the medium. Quantitative analysis of the absolute length of CD31-positive networks depending on VEGF-A concentration (n = 8). Box plot shows 25th and 75th percentiles with whiskers at 5th and 95th percentiles, median (line), and mean (+). ANOVA with Bonferroni's *post hoc* test (****$P < 0.0001$) shows significant differences from no growth factor (no GF) control. Both VEGF-A conditions are not significantly different from each other, but each from the FGF-2 group.

C Impact of physical matrix properties by PEG dry mass content.

D Impact of MMP activity by MMP inhibitors. Quantitative analysis of the MMP inhibition effect on the absolute length of CD31-positive micro-capillary networks. Individual data points and mean (line) ± SD, n = 3, ANOVA with Bonferroni's *post hoc* test: All MMP-blocking conditions except 10 µM MMP-2 are significantly reduced compared to control and DMSO control groups, with $P < 0.05$.

Data information: Scale bars: 200 µm.

Source data are available online for this figure.

Figure EV3. Genome-wide transcriptional comparison of BM-MSCs isolated from micro-capillary networks with control cultured BM-MSCs.

A Schematic of experimental workflow. BM-MSCs (n = 3 donors) were cultured alone or in micro-capillary co-cultures with endothelial cells (EC). After 7 days, cells were retrieved from PEG hydrogels and BM-MSCs were isolated by FACS-sorting for non-endothelial (CD31-negative) cells. BM-MSC-isolated RNAs were analyzed by next-generation sequencing and differential gene expression.

B FACS separation of BM-MSCs and ECs by means of CD31-staining. Black represents the full cell population of beforehand as single and live determined cells. Red represents the staining pattern of the isotype control antibody.

C *CD31/PECAM1* mRNA expression in FACS separated cell populations after 7 days of culture in PEG hydrogels measured by qRT–PCR. n = 3, individual data points and mean (line) ± SD.

D Volcano plot of differentially expressed genes between mono- and co-cultured BM-MSCs. The horizontal line corresponds to a FDR = 0.01 and genes below this line are considered as not significant (not), while genes above are considered as significant (signif). The two vertical lines correspond to a $\log_2$-fold change of 1 in expression and genes outside this range are considered as strongly affected (strong; signif & strong).

E Heatmap of gene expression by BM-MSCs monocultured or cultured in the perivascular microenvironment. Depicted are the 100 most significantly changed genes due to culture in the perivascular microenvironment. Hierarchical clustering separates genes with positive $\log_2$ fold change (increased expression in perivascular BM-MSCs, red) from negative change (reduced expression in perivascular BM-MSCs, blue) (A full list of differentially expressed genes is displayed in Dataset EV1).

Source data are available online for this figure.

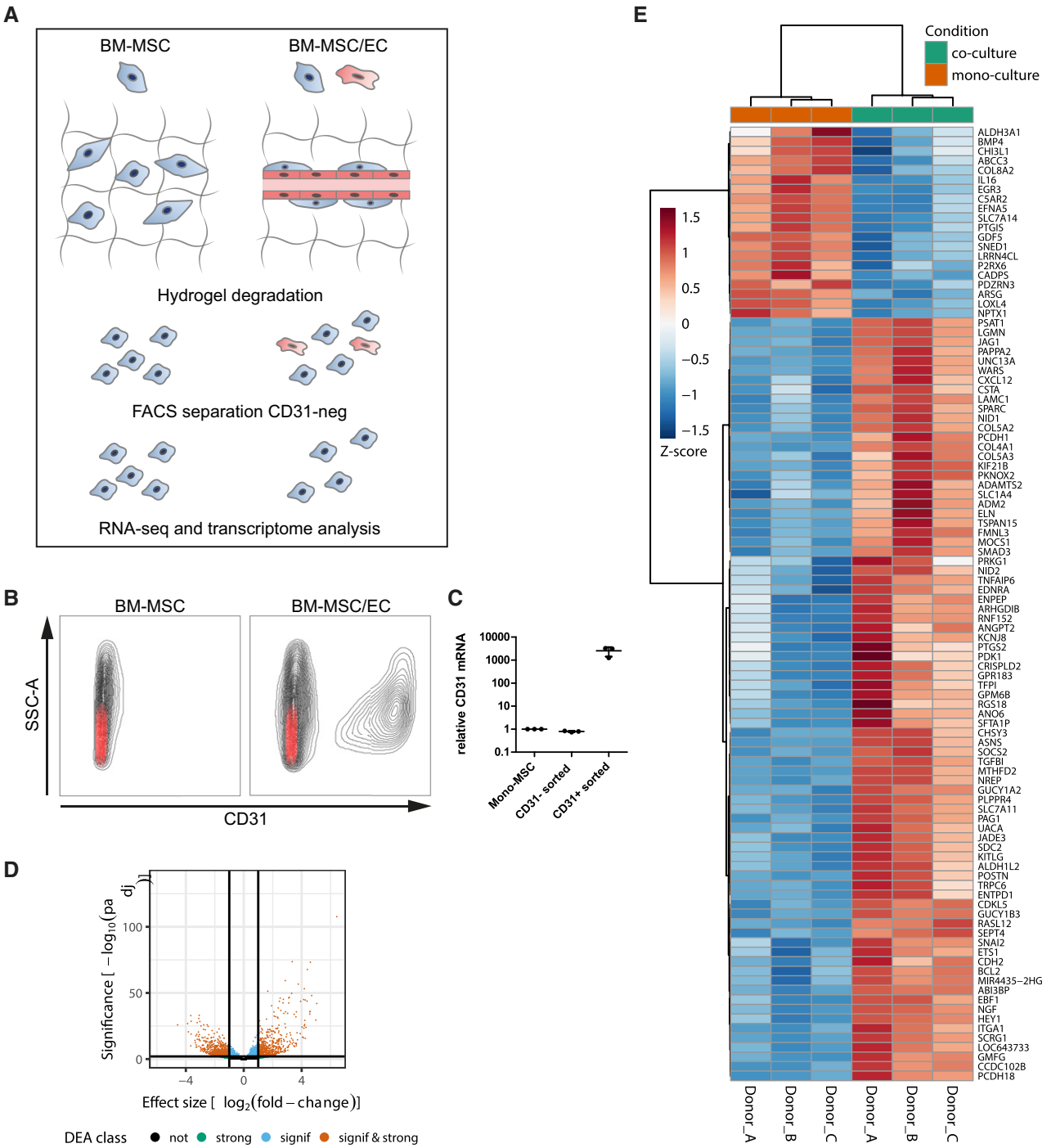

Figure EV3.

Figure EV4. Functional enrichment map of differentially expressed genes (DEG) and induction of Notch pathway target genes and perivascular markers in BM-MSCs isolated from micro-capillary networks.

A Genes identified as DEG following transcriptome analysis are enriched and clustered by the gene ontology domain GO Biological Process. Up- and downregulated genes in perivascular BM-MSCs are displayed in red and blue, respectively. GO Biological Process clusters show clusters of blood vessel regulation, cell–matrix interactions, and metal and ion homeostasis (a list of differentially expressed genes with their corresponding Biological Process terms is displayed in Dataset EV3).

B Induction of Notch pathway target genes and perivascular markers in BM-MSCs isolated from micro-capillary networks. Log_2-fold change of identified differentially expressed genes following transcriptome analysis in BM-MSCs isolated from micro-capillary networks compared to monocultured BM-MSCs.

Source data are available online for this figure.

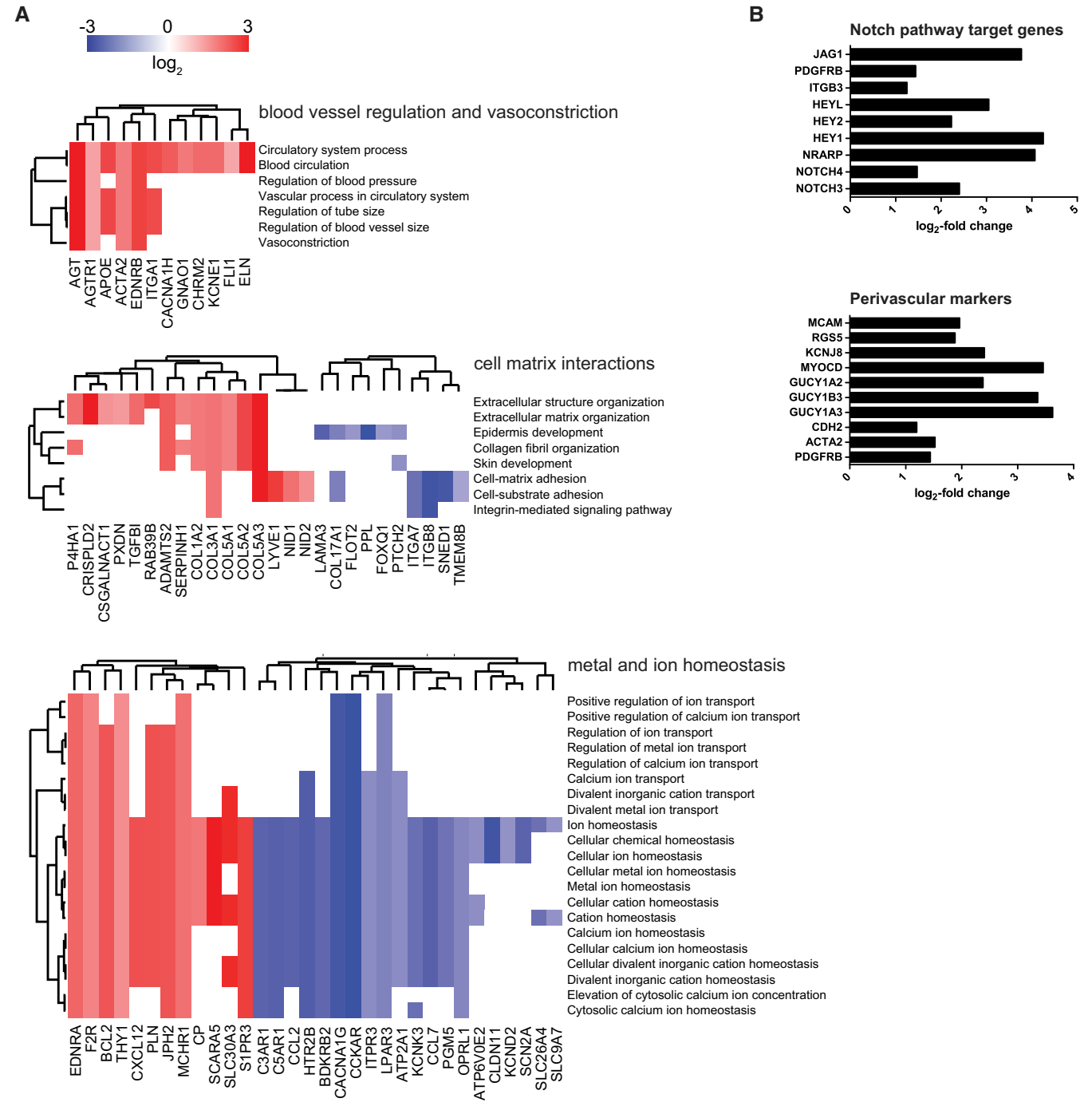

Figure EV4.

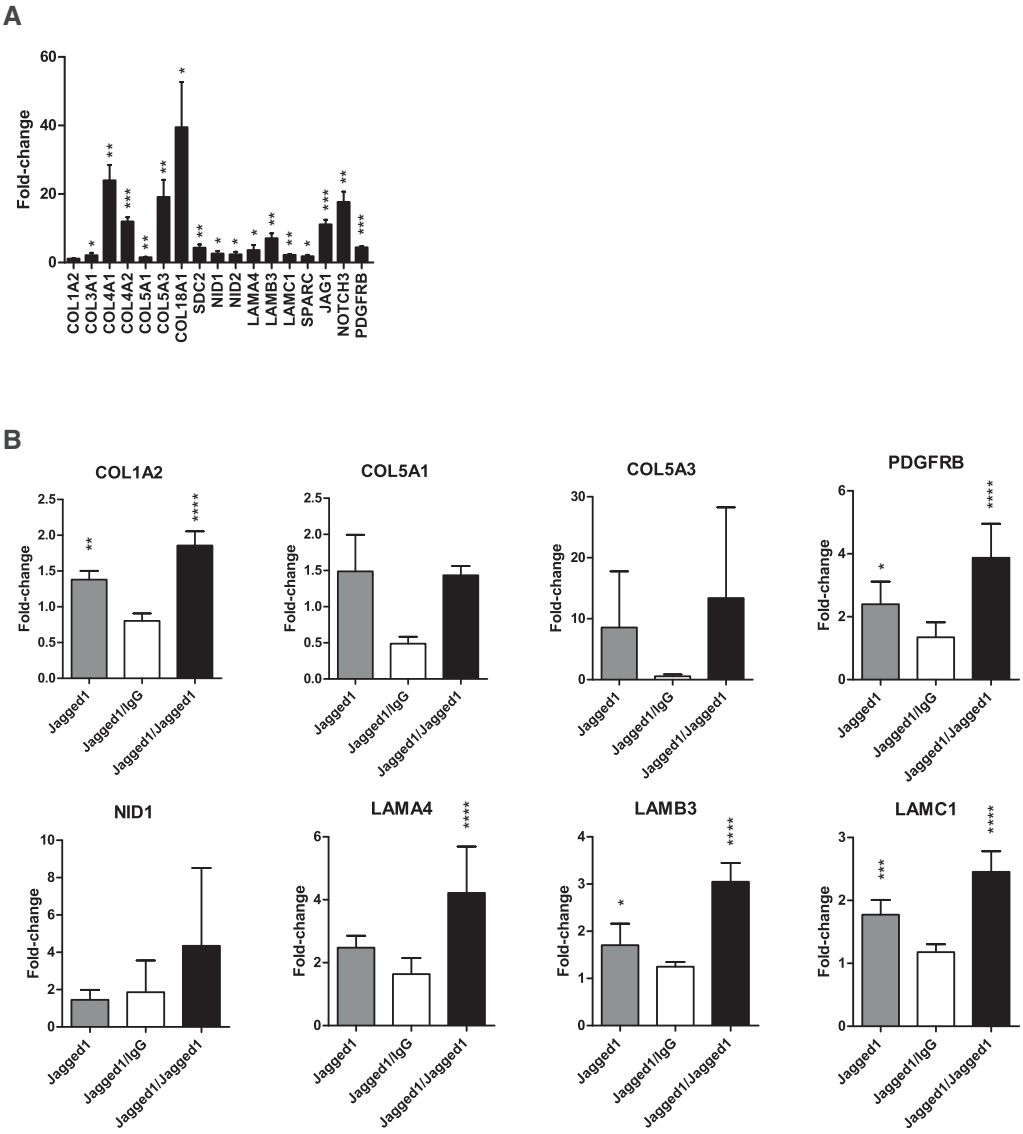

Figure EV5. ECM reprogramming in BM-MSCs isolated from micro-capillary co-cultures induced by VEGF-A (A) and reversible microenvironment-controlled ECM switch in MSCs (B).

A Induction of gene expression in BM-MSCs after 7 days of participation in 3D micro-capillary networks versus monocultured BM-MSCs in the presence of 200 ng/ml VEGF-A (n = 4). Data are displayed as relative fold changes compared to MSCs monocultured. Gene expression was analyzed by qRT–PCR and normalized on three reference genes (*GAPDH*, *YWHAZ*, *EEF1A1*). Bars represent mean values ± SD, and one-sample t-tests address the significance of the induction: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

B Gene expression of BM-MSCs after (7 days) culture in Jagged1-functionalized 3D microenvironments and subsequent (7 days) exposure to new Jagged1-functionalized or IgG control microenvironments. Gene expression was analyzed by qRT–PCR and normalized on three reference genes (*GAPDH*, *YWHAZ*, *EEF1A1*). Bars represent mean values ± SD, n = 5; ANOVA with Bonferroni's *post hoc* test shows significant differences from IgG control: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$,****$P < 0.0001$.

Source data are available online for this figure.