SUPPLEMENTARY INFORMATION

Methacrylated Pulmonary dECM-Enriched GelMA Bioinks Promote Endothelialization and Angiogenesis in 3D-Printed Tubular Constructs

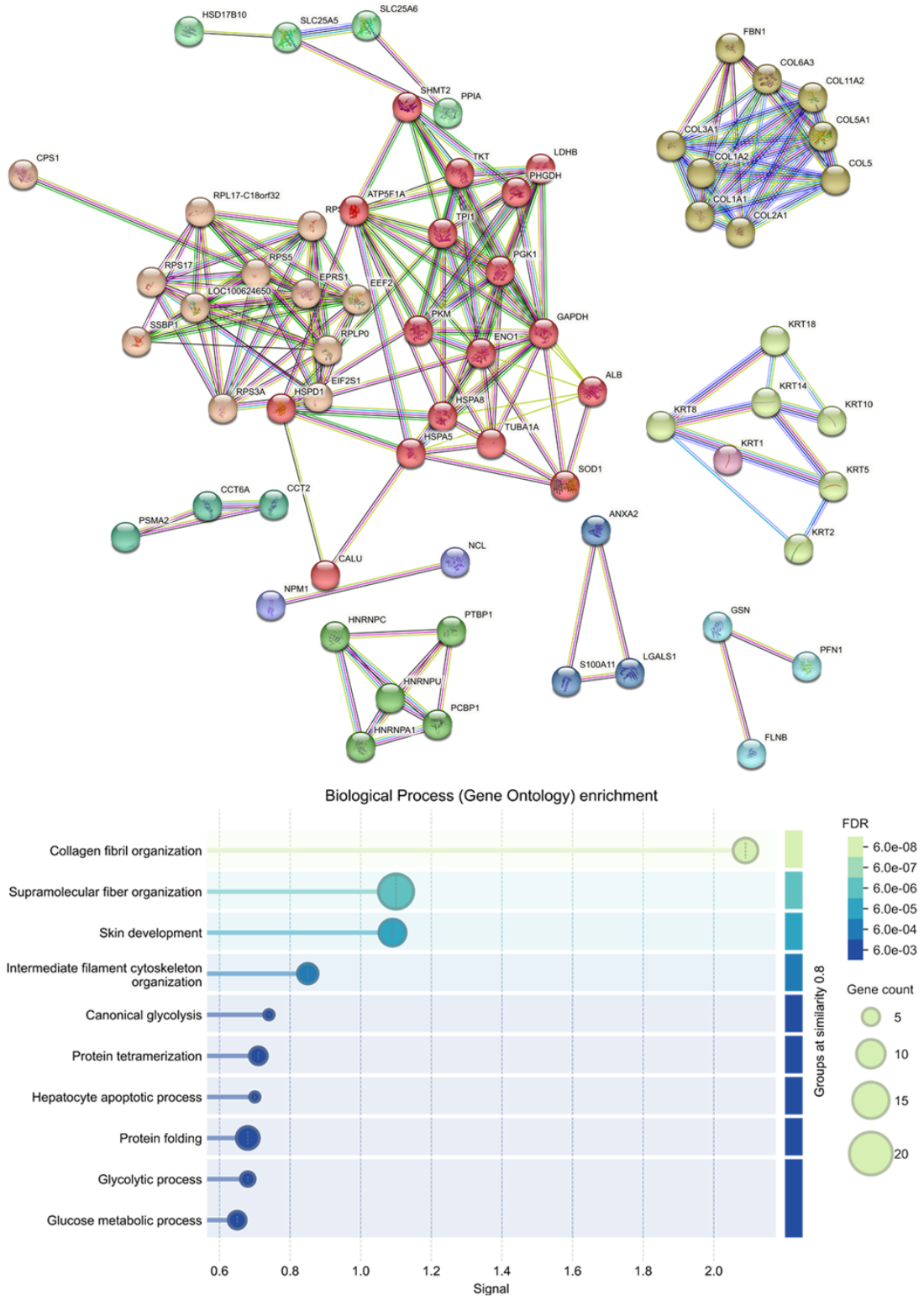

Figure S1. Full proteomic analysis and characterization of porcine-derived pulmonary dECM

Quantification of the methacrylation degree of lung-derived dECMMA using ^{1}H NMR

The degree of methacrylation of lung-derived dECMMA was analyzed by ^{1}H NMR spectroscopy, and the spectra were acquired on a Bruker AVANCE 500 MHz equipped with a BBO probe, and analyzed using TopSpin 3.5pl7. Briefly, lyophilized 10 mg dECMMA and dECMMA were transferred into 1.5 mL microcentrifuge tubes. Samples were dissolved in 1 mL DMSO-d_6 (≥99.8% D) by vortexing for 1–2 min. The solutions were then transferred to 5 mm NMR tubes.

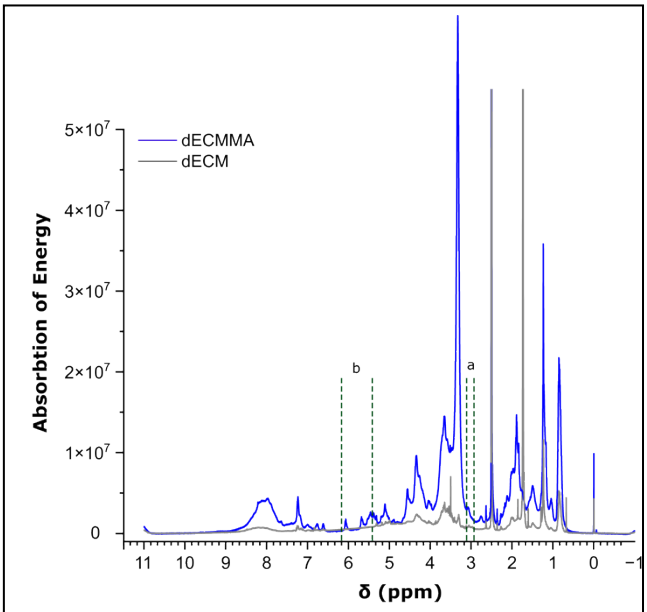

Figure S2. ^{1}HNMR spectra of dECM and dECMMA, a indicating the lysine peak, b indicating the vinyl peaks appearing in the dECMMA spectra

The degree of methacrylation of lung dECMMA was quantified by ^{1}H NMR spectroscopy using the lysine ε-CH_2 proton signal (*I Lys,* 2.95–3.05 ppm) as a molecular indicator of functionalization. The ε-CH_2 signal decreases upon methacrylation due to the substitution of lysine amine groups with methacrylate moieties. To normalize for ECM compositional variability, the lysine region was referenced to an invariant aliphatic region (*I ref,* 0.90–1.40 ppm) that remains unchanged by methacrylation. Integrals were obtained after local baseline correction using absolute-value integration.

The degree of methacrylation (DoM) was calculated using:

$$Degree\ of\ Methacrylation(\%) = [1 - \frac{\left(\frac{I\ Lys}{I\ ref}\right)dECMMA}{\left(\frac{I\ Lys}{I\ ref}\right)dECM}] \times 100.$$

Quantitative comparison of the lysine ε-CH_2 integral, normalized to the invariant aliphatic region (0.90–1.40 ppm), yielded a degree of methacrylation of 34%, consistent across replicate spectra. These results confirm efficient incorporation of methacrylate groups into the lung ECM backbone and validate the chemical modification strategy used to generate dECMMA.

Compressive mechanical properties

To evaluate the mechanical properties of dECM (following thermal crosslinking), dECMMA, GelMA, and G_dECMMA hydrogels, cylindrical discs (10 mm in diameter, 1 mm in thickness, n = 3) were fabricated from their respective prepolymer solutions. For the dECMMA (2% w/v), GelMA (4% w/v), and G_dECMMA (4% + 2% w/v, respectively) groups, 25 µL of prepolymer solution was pipetted into PDMS molds and exposed to UV light (365 nm, 12.5 mW/cm) for 60 seconds to induce permanent crosslinking. For the dECM (2% w/v) group,

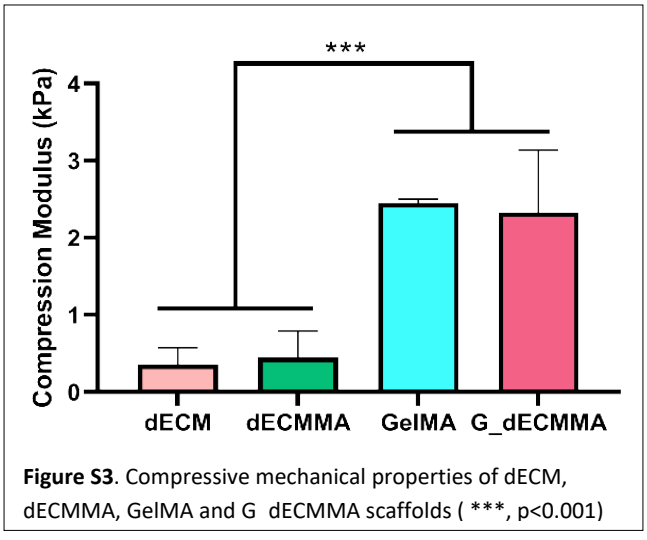

Figure S3. Compressive mechanical properties of dECM, dECMMA, GelMA and G dECMMA scaffolds (***, p<0.001)

25 µL of the prepolymer solution was similarly cast into PDMS molds and incubated at 37 °C for 15 minutes to allow thermal crosslinking.

Compression testing was performed at 37 °C after a 5-minute thermal equilibrium period. A preload force of 0.001 N was applied prior to testing. Samples were compressed in strain-controlled mode at a rate of 2.5% strain per minute until reaching −30% strain, followed by an unloading phase at 5% strain per minute. Stress–strain curves were generated from the data, and the elastic modulus was calculated as the slope of the curve within the 0–5% strain range ($R^2 > 0.9$). The compressive modulus dECM (thermally crosslinked), dECMMA (2% w/v), GelMA (4% w/v), and G-dECMMA (4%-2% w/v) were 0.35 ±0.2 kPa, 0.44±0.2 kPa, 2.45± 0.05 kPa, and 2.33 ±0.8 kPa, respectively. The results clearly indicate that the mechanical stability of the scaffolds was significantly maintained by the GelMA component. Moreover, the methacrylation and UV crosslinking of dECMMA biomaterial inks did not show a significant increase in the mechanical properties compared to its unmodified thermally crosslinked derivative.

Endothelial Cell Tube Formation Assay

For the endothelial cell tube formation Assay, 300 µL of chilled Corning Matrigel Matrix (positive control, 10 mg/mL) was pipetted into each well of an ibidi µ-Slide 8 Well chamber. To allow gelation, the slide was incubated at 37 °C for 20 minutes. Separately, 300 µL of GelMA (4% w/v) and G_dECMMA (4% GelMA + 2% dECMMA, w/v) solutions were pipetted into individual wells and crosslinked by exposure to UV light (365 nm, 12.5 mW/cm²) for 60 seconds. HUVEC suspensions were prepared by trypsinizing confluent monolayers and resuspending the cells in Endothelial Cell Growth Medium (PeloBiotech, Germany) at a concentration of 1×10^6 cells/mL. A total of 200 µL of the suspension (2×10^5 cells per sample) was added onto crosslinked Matrigel, GelMA, and G_dECMMA hydrogels, each in duplicate.

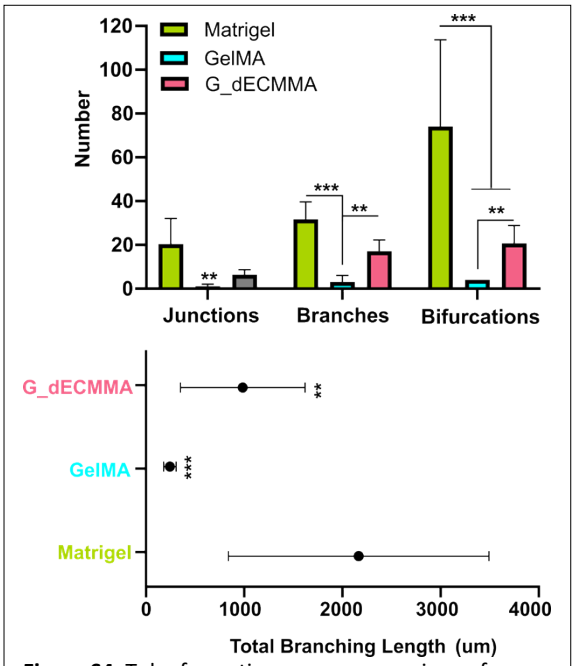

Figure S4. Tube formation assay, comparison of braching and branch length Matrigel, GelMA and G_dECMMA

The HUVEC seeded wells were visualized with a confocal microscope over 24 hours in an incubation chamber. The time-lapse images were quantified using ImageJ Angiogenesis Analyser. Quantitative analysis of endothelial network formation revealed significant differences regarding the number of junctions, branches and bifurcations as well as total branching length. Matrigel, the positive control, supported the most complex vascular-like structures, exhibiting a significantly high number of junctions, branches, and bifurcations, indicative of dense and well-organized endothelial networks. G_dECMMA, on the other hand, demonstrated an enhanced performance compared to GelMA, with significantly more network features and an increased branching length. Notably, addition of the dECMMA to GelMA was not sufficient to improve angiogenic potential to replace Matrigel; nevertheless, the significant improvement compared to pristine GelMA hydrogels implies the formation of more extended and densely interconnected structures. Additionally, GelMA, showing minimal endothelial organization, with low values across all measured parameters, highlights its limited ability to promote vascular morphogenesis without bioactive supplementation.

KEGG analysis of porcine-derived pulmonary dECM angiogenesis-related proteins

The proteomic analysis indicated the presence of LDHA, PGK1, ENO1, GAPDH, and PFK2, which are glycolytic enzymes that are upregulated under hypoxia via the HIF-1 signaling pathway. Through KEGG analysis, we may suggest that these enzymes may support the metabolic shift toward anaerobic glycolysis, providing rapid ATP production, which is essential for endothelial cell proliferation and migration during angiogenesis. Notably, LDHA-mediated lactate production may act as a signaling molecule that stabilizes HIF-1α and induces VEGF expression[1], further promoting vascular growth. Additionally, the presence of enzymes like GAPDH and PGK1 exhibits secondary roles in gene regulation and extracellular matrix remodeling, which can also support endothelialization. When we analysed the ECM–receptor interaction pathway—particularly collagen and its associated integrins (e.g., α1β1, α2β1)—we can conclude these enzymes play a central role in regulating angiogenesis and endothelialization[2]. These extracellular matrix proteins provide structural and biochemical cues that bind integrins on endothelial cells, activating downstream signaling cascades such as focal adhesion kinase (FAK) and PI3K/Akt, which enhance cell adhesion, migration, and survival. Likewise, collagen-integrin binding could support endothelial attachment and directional migration along matrix fibers during neovascularization. Altogether, the presence of these ECM components could reinforce the formation of a supportive microenvironment for endothelial cells to organize into stable vascular structures. Additionally, the focal adhesion proteins, such as ECM, Filamin, and Actin, highlight key regulators that are directly linked to angiogenesis and endothelialization. Furthermore, these ECM–integrin interactions activate focal adhesion kinase (FAK) signaling, which coordinates cytoskeletal remodeling and cell motility, enabling endothelial cells to migrate and form neovascularization. Specifically, filamin crosslinks actin filaments and stabilizes the actin cytoskeleton, promoting lamellipodia and filopodia formation that are essential for sprouting angiogenesis[3]. Meanwhile, Actin polymerization drives the dynamic structural changes required for endothelial cell migration, adhesion turnover, and vessel lumen formation. Together, these components are promising building blocks to enhance endothelialization by fostering cell–ECM interactions and cytoskeletal reorganization critical for vascular network formation.

References

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3 R. S. Fischer, P.-Y. Lam, A. Huttenlocher and C. M. Waterman, *Dev Biol*, 2019, 451, 86–95.

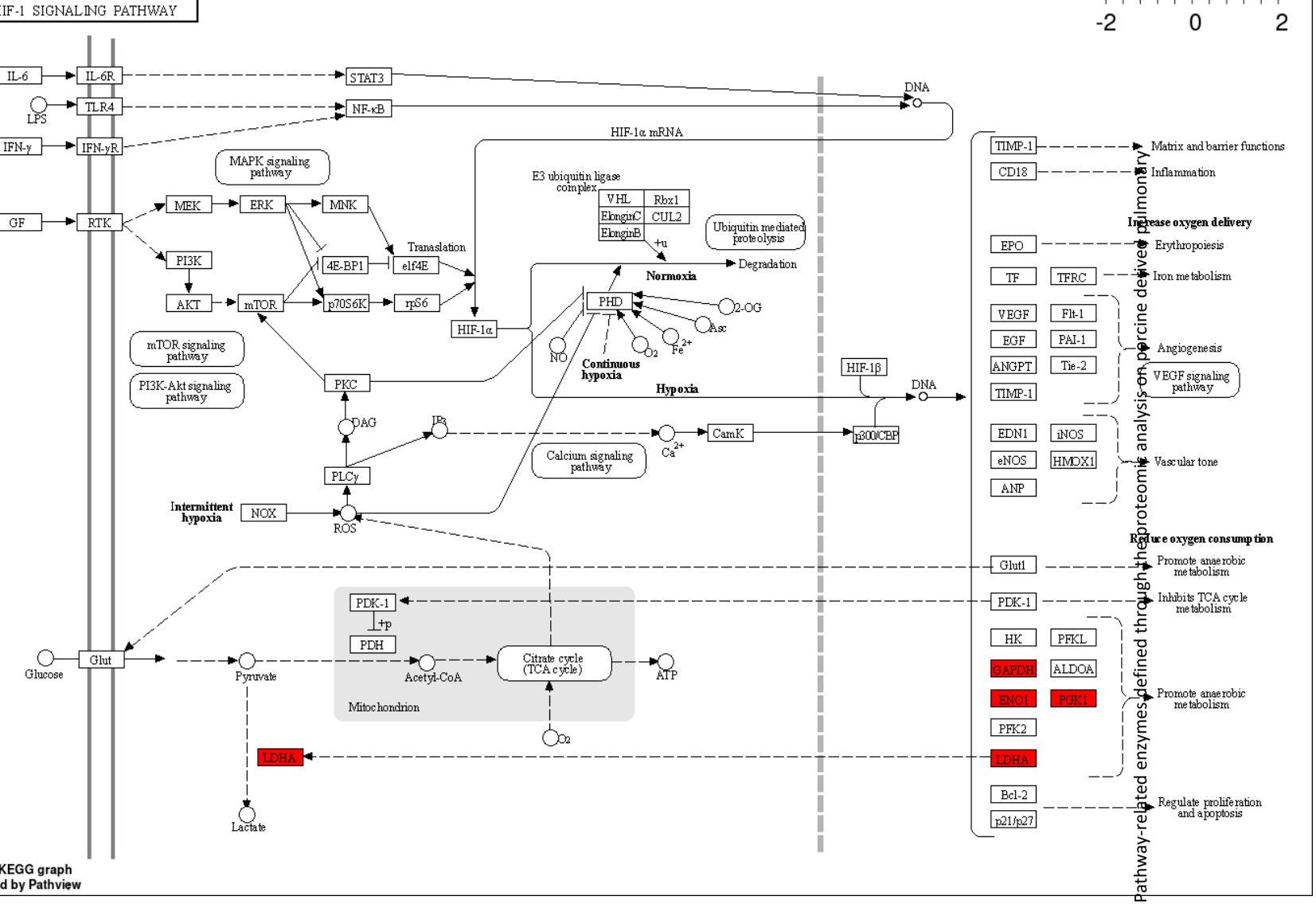

Figure SSi. HIF-1 Signalling Pathway-related enzymes defined through the proteomic analysis on porcine derived pulmonary dECM

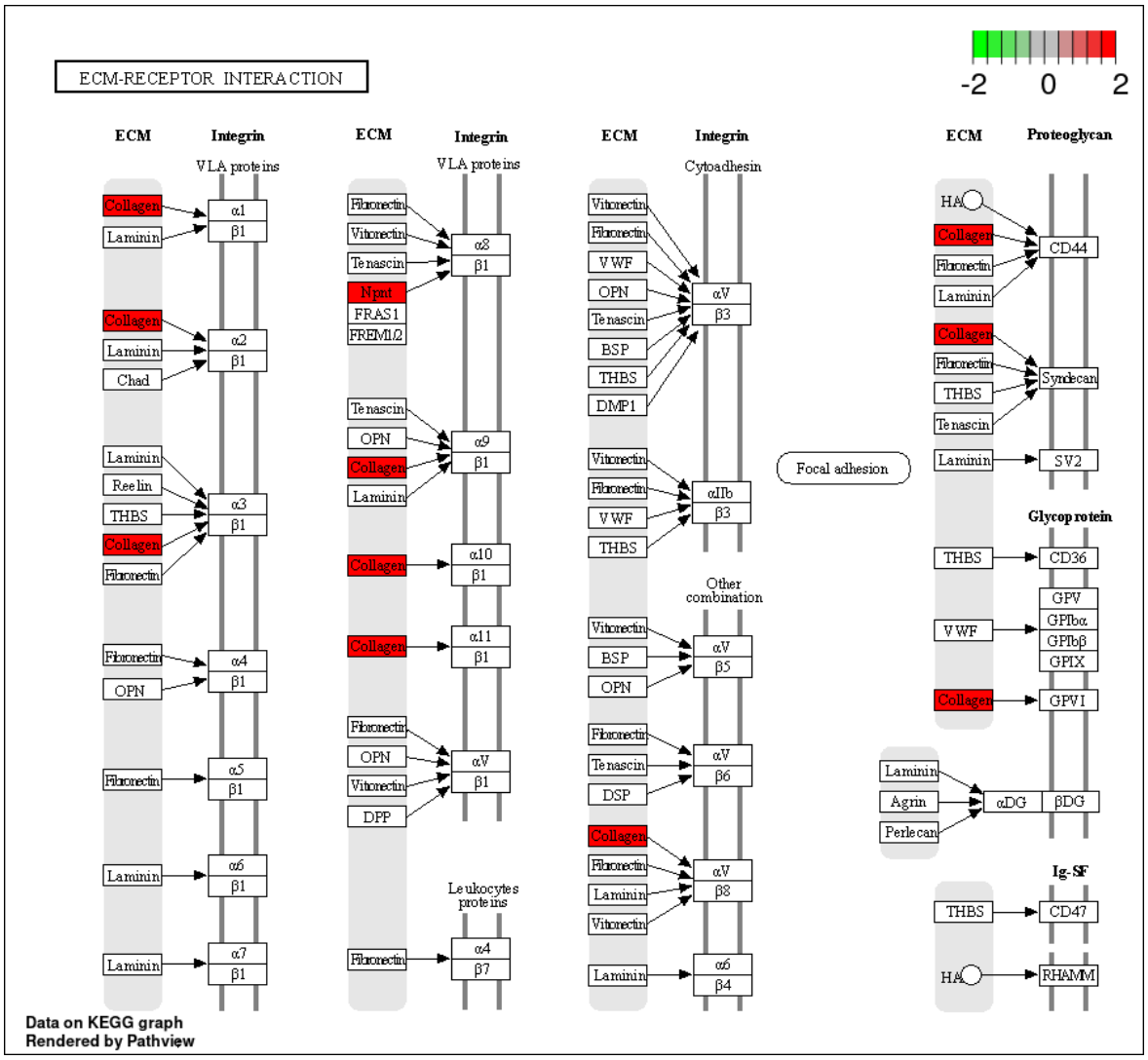

Figure S5ii. ECM-Receptor Interaction-related proteins defined through the proteomic analysis on porcine-derived pulmonary dECM

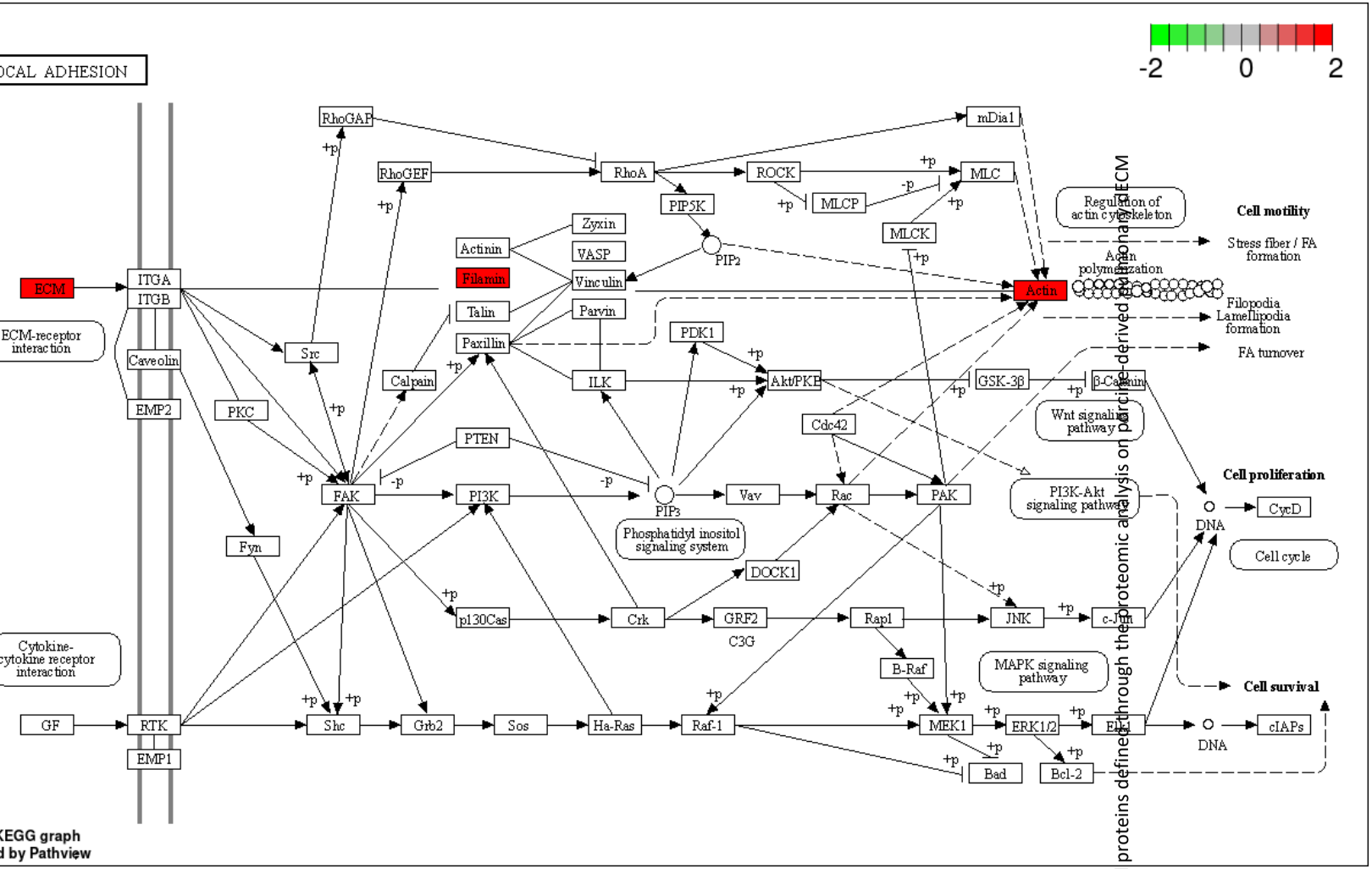

Figure S5iii. Focal Adhesion-related proteins defined through the proteomic analysis on pericite-derived cultured ECM