

Expanded View Figures

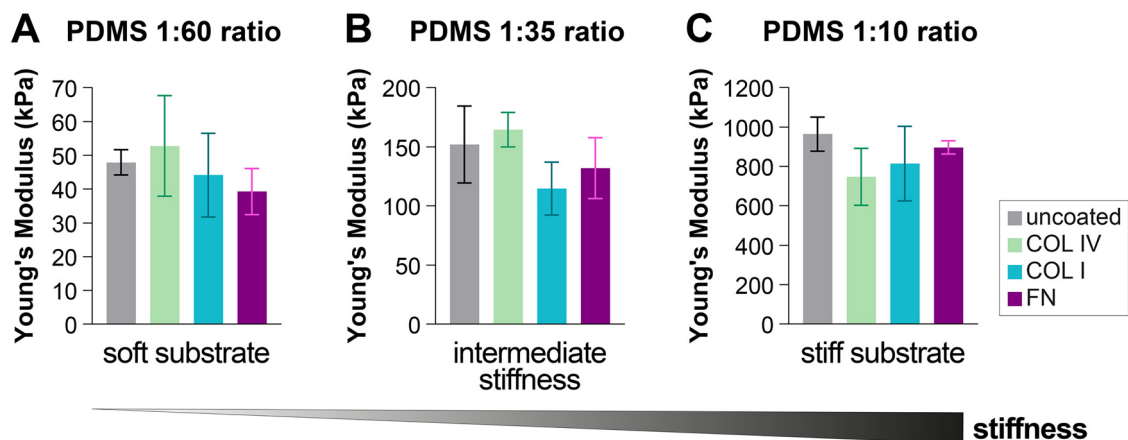


Figure EV1. Validation of PDMS elasticity using atomic force microscopy.

Young's modulus of PDMS substrates with varying crosslinker ratios. (A) PDMS substrate with a crosslinker:silicone base agent ratio of 1:60; means $\pm$ SD, $N = 3$ (biological replicates); Welch ANOVA tests: ns $p = 0.989$ (uncoated vs. COL I), ns $p = 0.4616$ (uncoated vs. FN), ns $p = 0.9842$ (uncoated vs. COL IV), ns $p = 0.982$ (COL I vs. FN), ns $p = 0.9505$ (COL I vs. COL IV), ns $p = 0.6723$ (FN vs. COL IV). (B) PDMS substrate with a 1:35 ratio; means $\pm$ SD, $N \geq 3$ (biological replicates); Welch ANOVA tests: ns $p = 0.2933$ (uncoated vs. COL I), ns $p = 0.8869$ (uncoated vs. FN), ns $p = 0.9686$ (uncoated vs. COL IV), ns $p = 0.9035$ (COL I vs. FN), ns $p = 0.0731$ (COL I vs. COL IV), ns $p = 0.5114$ (FN vs. COL IV). (C) PDMS substrate with a 1:10 ratio; means $\pm$ SD, $N = 3$ (biological replicates); Welch ANOVA tests: ns $p = 0.7579$ (uncoated vs. COL I), ns $p = 0.7492$ (uncoated vs. FN), ns $p = 0.3624$ (uncoated vs. COL IV), ns $p = 0.9483$ (COL I vs. FN), ns $p = 0.9939$ (COL I vs. COL IV), ns $p = 0.5694$ (FN vs. COL IV).

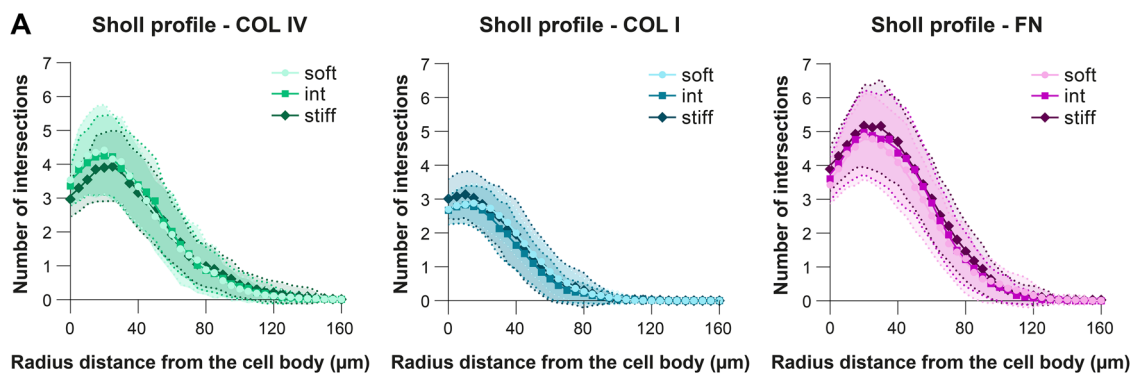


Figure EV2. Sholl profiles for iMCs exposed to different ECM molecules.

(A) Graphs showing the Sholl profile analysis, plotting the number of dendrite intersections against the distance from the cell body. The SEM are represented by the connecting curve (dotted line); $N = 3$ (biological replicates), $n(\text{cells}) \geq 85$. Each curve represents a substrate stiffness condition, and each graph represents an ECM type (same cells as analyzed in Fig. 1C, different plotting of the data); Welch ANOVA test: COL IV: ns $p > 0.9999$ (1:60 vs. 1:35), ns $p = 0.9650$ (1:60 vs. 1:10), ns $p = 0.9489$ (1:35 vs. 1:10); COL I: ns $p = 0.5182$ (1:60 vs. 1:35), ns $p > 0.9999$ (1:60 vs. 1:10), ns $p = 0.5964$ (1:35 vs. 1:10); FN: ns $p = 0.8906$ (1:60 vs. 1:35), ns $p = 0.4342$ (1:60 vs. 1:10), ns $p = 0.7603$ (1:35 vs. 1:10).

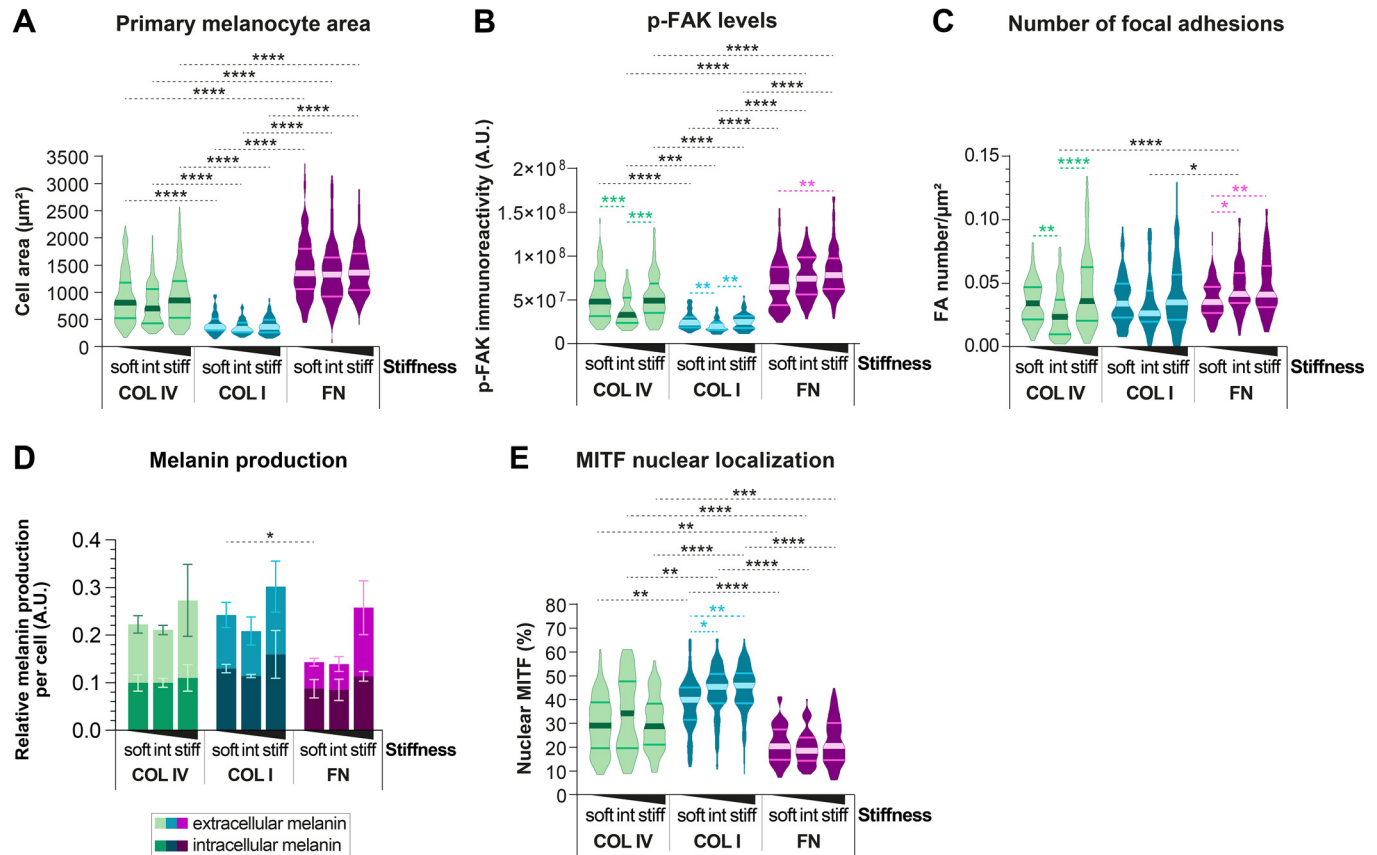


Figure EV3. Response of primary MCs to distinct ECM cues.

pMCs were cultured overnight on substrates of varying stiffness (soft, intermediate or stiff) coated with COL IV, COL I, or FN. (A) Violin plots showing the medians and distributions of the cell area of pMCs; $N \geq 5$ (biological replicates), $n(\text{cells}) \geq 96$; Kruskal-Wallis test: COL IV: ns $p = 0.5372$ (1:60 vs. 1:35), ns $p > 0.9999$ (1:60 vs. 1:10), ns $p = 0.1807$ (1:35 vs. 1:10); COL I: ns $p = 0.1552$ (1:60 vs. 1:35), ns $p = 0.7629$ (1:60 vs. 1:10), ns $p > 0.9999$ (1:35 vs. 1:10); FN: ns $p = 0.4268$ (1:60 vs. 1:35), ns $p > 0.9999$ (1:60 vs. 1:10), ns $p = 0.0972$ (1:35 vs. 1:10); 1:60: **** $p < 0.0001$ (COL I vs. FN), **** $p < 0.0001$ (COL I vs. COL IV), **** $p < 0.0001$ (FN vs. COL IV); 1:35: **** $p < 0.0001$ (COL I vs. FN), **** $p < 0.0001$ (COL I vs. COL IV), **** $p < 0.0001$ (FN vs. COL IV); 1:10: **** $p < 0.0001$ (COL I vs. FN), **** $p < 0.0001$ (COL I vs. COL IV), **** $p < 0.0001$ (FN vs. COL IV). (B) Quantification of p-FAK levels; violin plots showing the medians and distributions of the integrated density of total p-FAK per cell; $N = 3$ (biological replicates), $n(\text{cells}) \geq 52$; Kruskal-Wallis test: COL IV: *** $p = 0.0004$ (1:60 vs. 1:35), ns $p > 0.9999$ (1:60 vs. 1:10), *** $p = 0.0005$ (1:35 vs. 1:10); COL I: ** $p = 0.002$ (1:60 vs. 1:35), ns $p > 0.9999$ (1:60 vs. 1:10), ** $p = 0.0024$ (1:35 vs. 1:10); FN: ns $p = 0.3084$ (1:60 vs. 1:35), ** $p = 0.0035$ (1:60 vs. 1:10), ns $p = 0.7979$ (1:35 vs. 1:10); 1:60: **** $p < 0.0001$ (COL I vs. FN), **** $p < 0.0001$ (COL I vs. COL IV), ns $p = 0.1$ (FN vs. COL IV); 1:35: **** $p < 0.0001$ (COL I vs. FN), *** $p = 0.0003$ (COL I vs. COL IV), **** $p < 0.0001$ (FN vs. COL IV); 1:10: **** $p < 0.0001$ (COL I vs. FN), **** $p < 0.0001$ (COL I vs. COL IV), **** $p < 0.0001$ (FN vs. COL IV). (C) Quantification of FAs; violin plots showing the medians and distributions of the number of focal adhesions per μm^2 per cell; $N = 3$ (biological replicates), $n(\text{cells}) \geq 65$; Kruskal-Wallis test: COL IV: ** $p = 0.0081$ (1:60 vs. 1:35), ns $p = 0.507$ (1:60 vs. 1:10), **** $p < 0.0001$ (1:35 vs. 1:10); COL I: ns $p = 0.7166$ (1:60 vs. 1:35), ns $p > 0.9999$ (1:60 vs. 1:10), ns $p = 0.1991$ (1:35 vs. 1:10); FN: * $p = 0.0117$ (1:60 vs. 1:35), ** $p = 0.0071$ (1:60 vs. 1:10), ns $p > 0.9999$ (1:35 vs. 1:10); 1:60: ns $p > 0.9999$ (COL I vs. FN), ns $p > 0.9999$ (COL I vs. COL IV), ns $p > 0.9999$ (FN vs. COL IV); 1:35: * $p = 0.0167$ (COL I vs. FN), ns $p = 0.5377$ (COL I vs. COL IV), **** $p < 0.0001$ (FN vs. COL IV); 1:10: ns $p > 0.9999$ (COL I vs. FN), ns $p > 0.9999$ (COL I vs. COL IV), ns $p > 0.9999$ (FN vs. COL IV). (D) Quantification of intra- and extracellular melanin content by spectrophotometry at 405 nm from pMCs cultured 72 h on substrates of varying stiffness (soft, intermediate or stiff) coated with COL IV, COL I or FN; means $\pm$ SD, $N = 3$ (biological replicates); multiple t- tests: Soft vs. Intermediate: ns $p = 0.4947$ (COL I), ns $p = 0.8479$ (FN), ns $p = 0.8479$ (COL IV); Soft vs. Stiff: ns $p = 0.619892$ (COL I), ns $p = 0.10379$ (FN), ns $p = 0.619892$ (COL IV); Intermediate vs. Stiff: ns $p = 0.361807$ (COL I), ns $p = 0.118759$ (FN), ns $p = 0.361807$ (COL IV); COL I vs. COL IV: ns $p = 0.868243$ (1:60), ns $p = 0.935696$ (1:35), ns $p = 0.935696$ (1:10), COL IV vs. FN: ns $p = 0.053003$ (1:60), ns $p = 0.053003$ (1:35), ns $p = 0.836335$ (1:10); COL I vs. FN: * $p = 0.0139$ (1:60), ns $p = 0.0797$ (1:35), ns $p = 0.5564$ (1:10). (E) Quantification of nuclear MITF; violin plots showing the medians and distributions of the percentage of nuclear MITF per cell; $N = 3$ (biological replicates), $n(\text{cells}) \geq 67$; Kruskal-Wallis test: COL IV: ns $p = 0.2081$ (1:60 vs. 1:35), ns $p > 0.9999$ (1:60 vs. 1:10), ns $p = 0.3965$ (1:35 vs. 1:10); COL I: * $p = 0.0186$ (1:60 vs. 1:35), ** $p = 0.0034$ (1:60 vs. 1:10), ns $p > 0.9999$ (1:35 vs. 1:10); FN: ns $p = 0.7181$ (1:60 vs. 1:35), ns $p > 0.9999$ (1:60 vs. 1:10), ns $p = 0.2589$ (1:35 vs. 1:10); 1:60: **** $p < 0.0001$ (COL I vs. FN), ** $p = 0.0056$ (COL I vs. COL IV), ** $p = 0.0013$ (FN vs. COL IV); 1:35: **** $p < 0.0001$ (COL I vs. FN), ** $p = 0.0046$ (COL I vs. COL IV), **** $p < 0.0001$ (FN vs. COL IV); 1:10: **** $p < 0.0001$ (COL I vs. FN), **** $p < 0.0001$ (COL I vs. COL IV), *** $p = 0.0009$ (FN vs. COL IV). AU, arbitrary units.

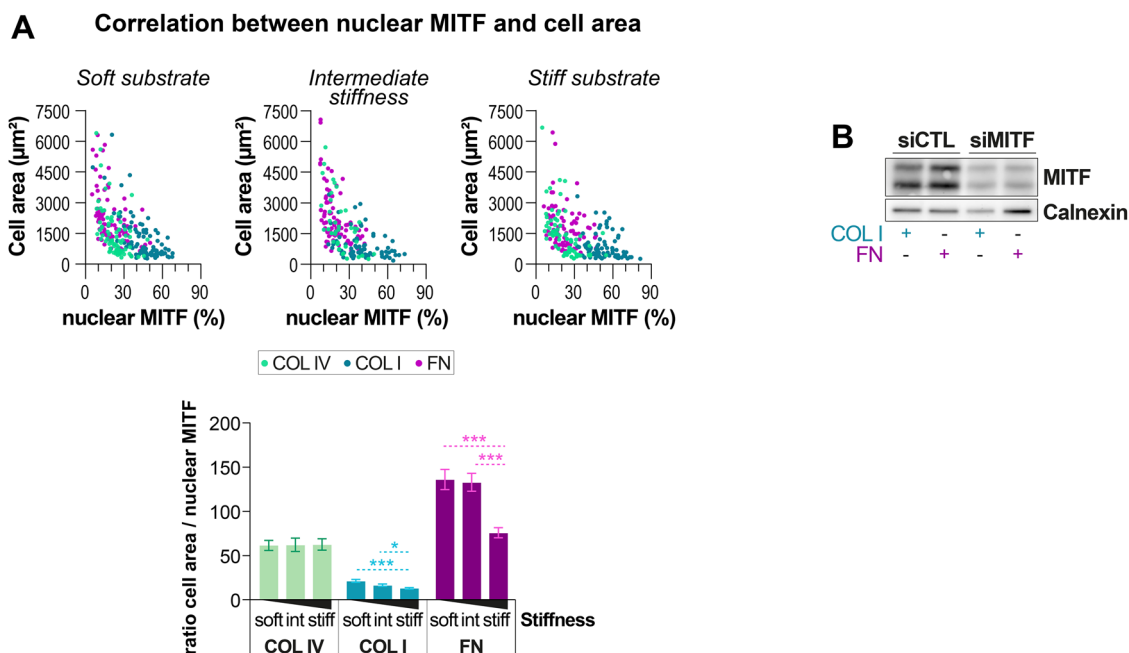


Figure EV4. Negative correlation between nuclear MITF levels and MC area and siMITF validation.

(A) The percentage of nuclear MITF was plotted against the cell area of the corresponding MC for all stiffnesses and ECM types tested. The bar diagram depicts the means $\pm$ SEM of the ratio of cell area to percentage of nuclear MITF per cell; $N = 3$ (biological replicates), $n(\text{cells}) \geq 47$; Kruskal-Wallis test: COL IV: ns $p > 0.9999$ (1:60 vs. 1:35), ns $p = 0.5839$ (1:60 vs. 1:10), ns $p > 0.9999$ (1:35 vs. 1:10); COL I: ns $p = 0.5497$ (1:60 vs. 1:35), *** $p = 0.0001$ (1:60 vs. 1:10), * $p = 0.0329$ (1:35 vs. 1:10); FN: ns $p > 0.9999$ (1:60 vs. 1:35), *** $p = 0.0001$ (1:60 vs. 1:10), *** $p = 0.0001$ (1:35 vs. 1:10); 1:60: **** $p < 0.0001$ (COL I vs. FN), **** $p < 0.0001$ (COL I vs. COL IV), ** $p = 0.0014$ (FN vs. COL IV); 1:35: **** $p < 0.0001$ (COL I vs. FN), **** $p < 0.0001$ (COL I vs. COL IV), *** $p = 0.0006$ (FN vs. COL IV); 1:10: **** $p < 0.0001$ (COL I vs. FN), **** $p < 0.0001$ (COL I vs. COL IV), ns $p = 0.0603$ (FN vs. COL IV). (B) Western blot analysis of MITF expression in iMCs plated on COL I or FN and transfected for 72 h with siCtrl or siMITF, performed in parallel with melanin quantification shown in Fig. 4D.

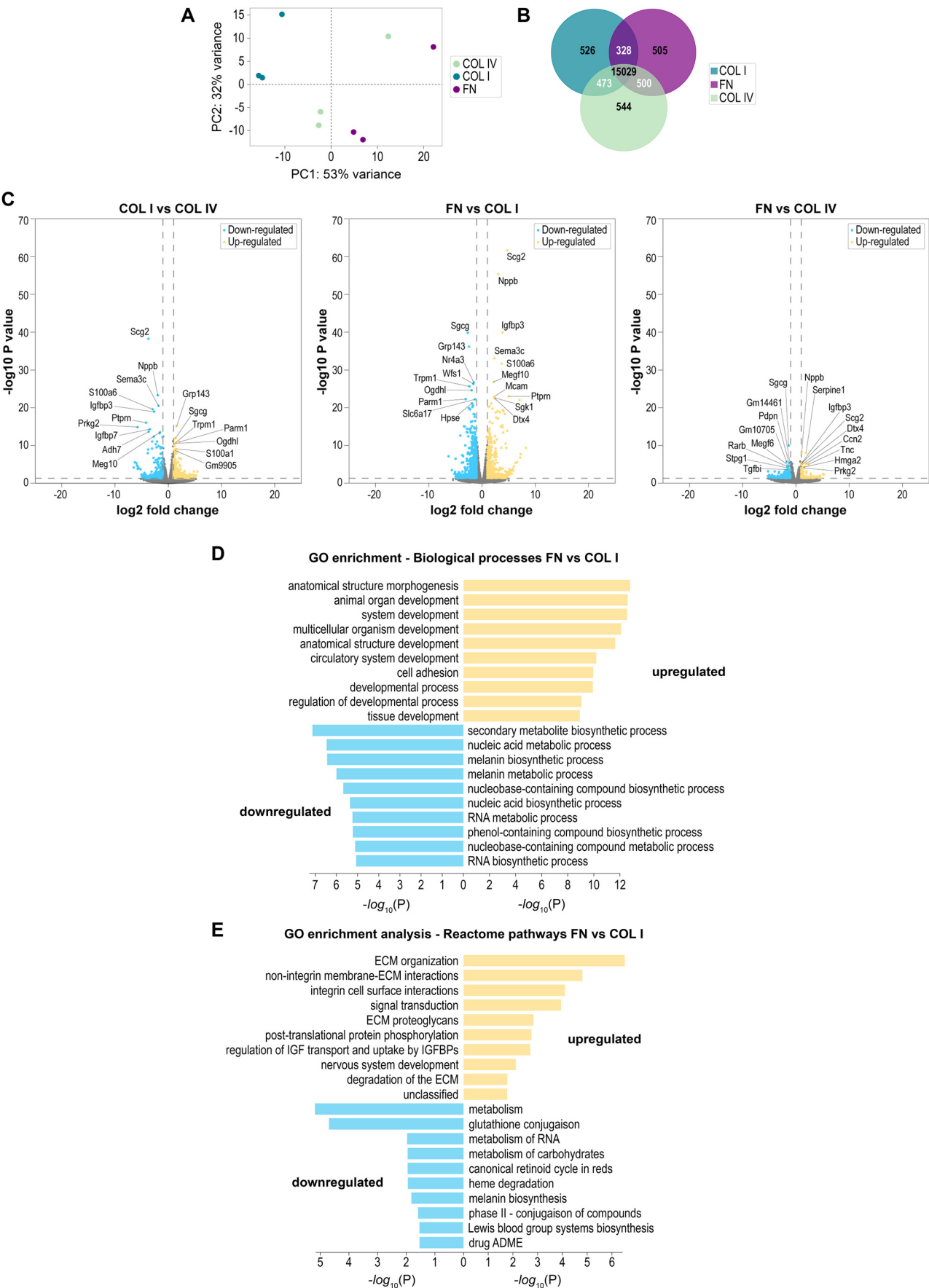


Figure EV5. Transcriptomic profiling across ECM conditions.

iMCs were cultured overnight on stiff substrates coated with COL IV, COL I, or FN; $N = 3$ (biological replicates). (A) Principal component analysis (PCA) of gene expression across substrates. Each dot represents one sample: COL IV (green), COL I (blue), and FN (purple). Axes indicate principal components capturing the highest variance. (B) Venn diagram showing the number of genes expressed in the samples grown on COL IV, COL I, and FN substrates. (C) Volcano plots depicting differentially expressed genes between substrates: (i) COL I vs. COL IV, (ii) FN vs. COL I, and (iii) FN vs. COL IV (p values derived from Wald tests in DESeq2 with Benjamini-Hochberg adjustment). Significantly downregulated genes are shown in blue; upregulated genes in yellow. (D) Gene Ontology (GO) Biological Process enrichment analysis of significantly regulated genes across substrates. The x-axis indicates the adjusted p value of Fisher's Exact test; the y-axis lists the most significantly enriched biological processes. (E) Reactome pathway enrichment analysis performed on differentially expressed genes across ECM conditions. The x-axis represents the adjusted p value of Fisher's exact test; the y-axis lists the top significantly enriched Reactome pathways.

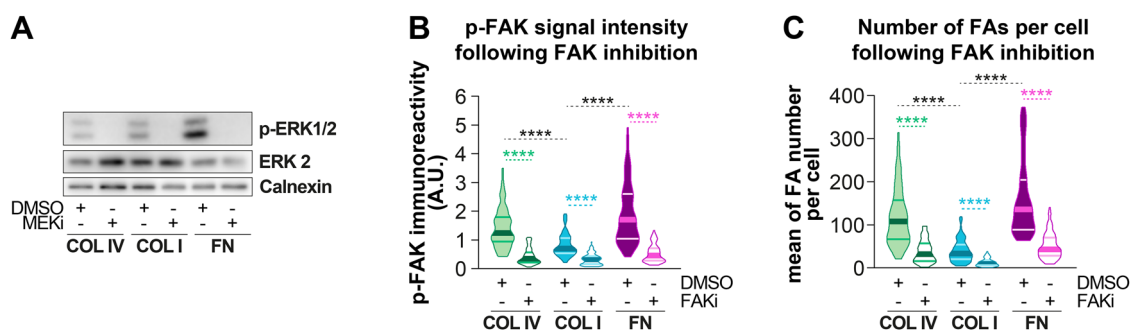


Figure EV6. Functional validation of MEK and FAK inhibitors in iMCs cultured on ECM-coated substrates.

iMCs were cultured on stiff substrates coated with COL IV, COL I, or FN. Four hours post-plating, a 16-h treatment with DMSO, 100 nM of MEK inhibitor (MEKi = Trametinib) or FAK inhibitor (FAKi = Ifebemtinib) was commenced. **(A)** Representative Western blot showing the inhibition of ERK1/2 phosphorylation with Trametinib treatment. **(B)** Quantification of p-FAK (Y397) intensity per cell following treatment with DMSO or FAK inhibitor (FAKi); violin plots display medians and distributions; $N = 3$ (biological replicates), $n(\text{cells}) \geq 88$; Kruskal-Wallis test: DMSO: **** $p < 0.0001$ (COL IV vs. COL I), ns $p > 0.9999$ (COL IV vs. FN), **** $p < 0.0001$ (COL I vs. FN); DMSO vs. FAKi: **** $p < 0.0001$ (COL IV DMSO vs. COL IV FAKi 24 h), **** $p < 0.0001$ (COL I DMSO vs. COL I FAKi 24 h), **** $p < 0.0001$ (FN DMSO vs. FN FAKi 24 h). **(C)** Quantification of focal adhesion (FA) number per cell following FAKi treatment; violin plots show medians and distributions; $N = 3$ (biological replicates), $n(\text{cells}) \geq 70$; Kruskal-Wallis test: DMSO: **** $p < 0.0001$ (COL IV vs. COL I), ns $p = 0.6525$ (COL IV vs. FN), **** $p < 0.0001$ (COL I vs. FN); DMSO vs. FAKi: **** $p < 0.0001$ (COL IV DMSO vs. COL IV FAKi), **** $p < 0.0001$ (COL I DMSO vs. COL I FAKi), **** $p < 0.0001$ (FN DMSO vs. FN FAKi).