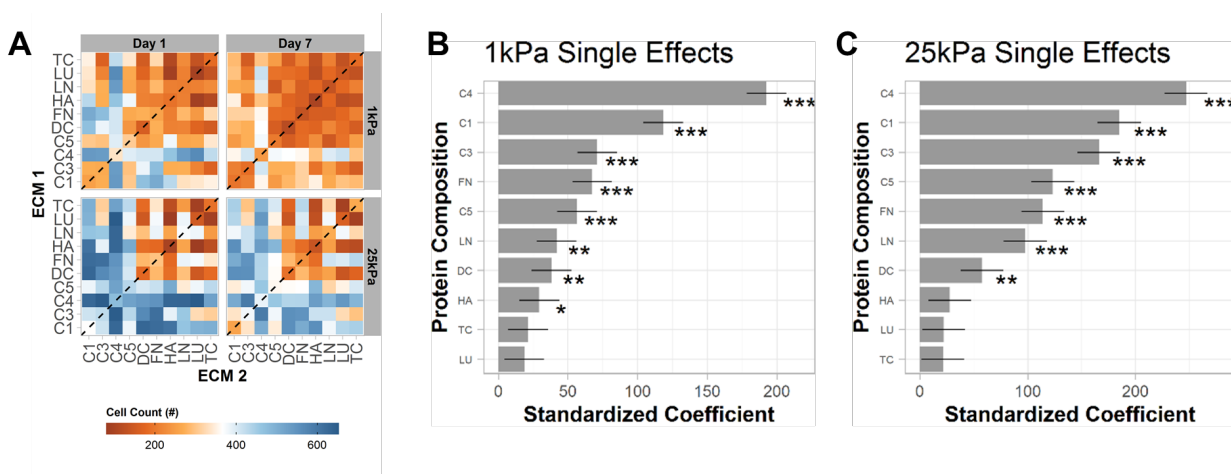


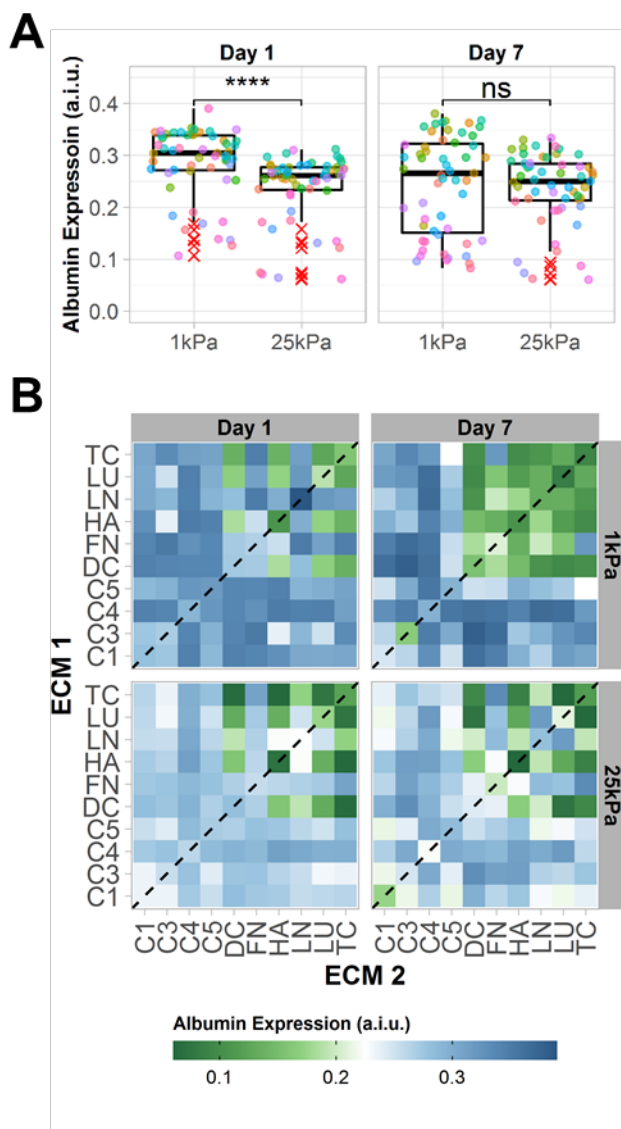
SUPPLEMENTAL DATA

Elucidating Extracellular Matrix and Stiffness Control of Primary Human Hepatocyte Phenotype Via Cell Microarrays

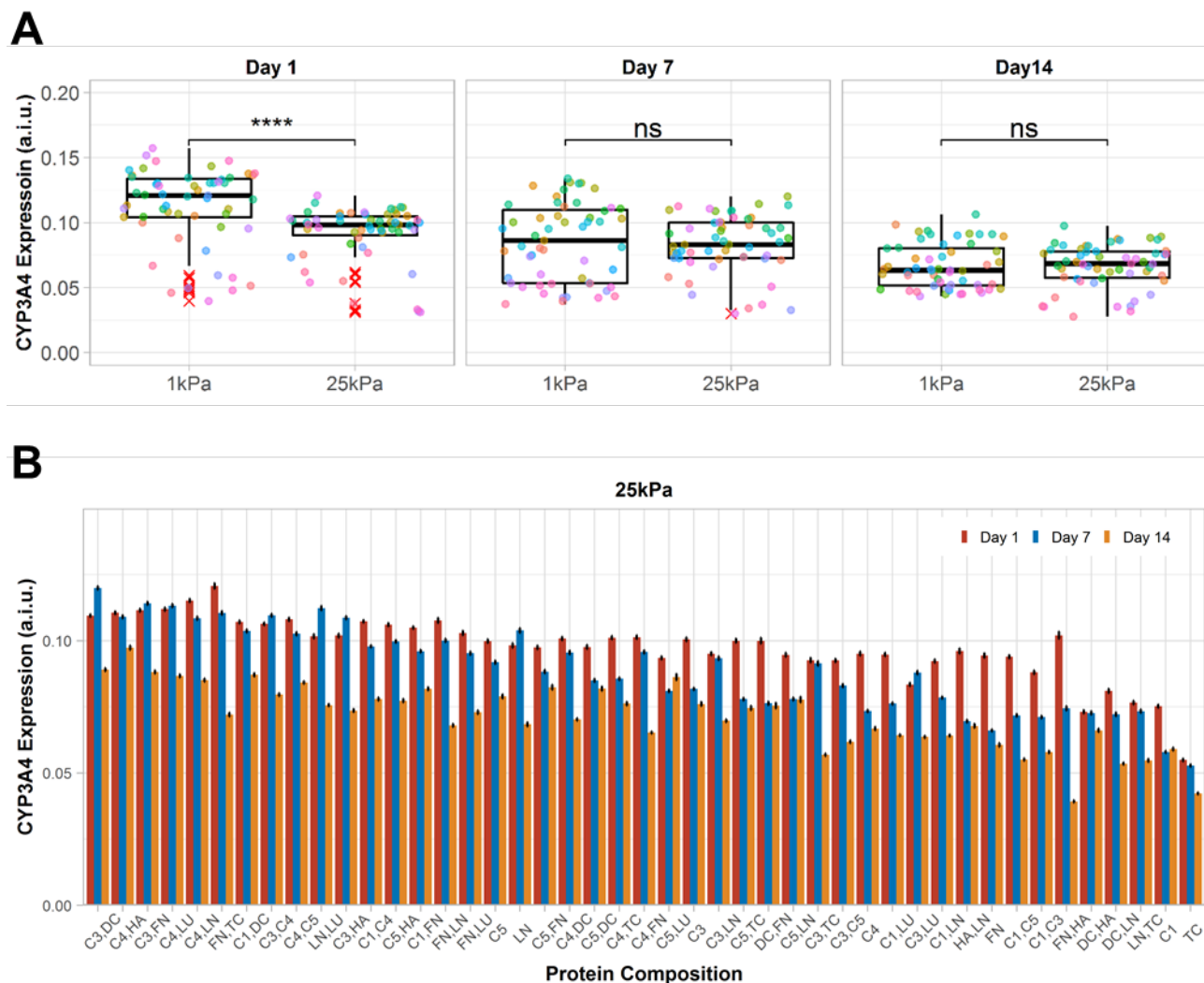
Chase P. Monckton, Aidan Brougham-Cook, Kerim B. Kaylan, Gregory Underhill, and Salman R. Khetani



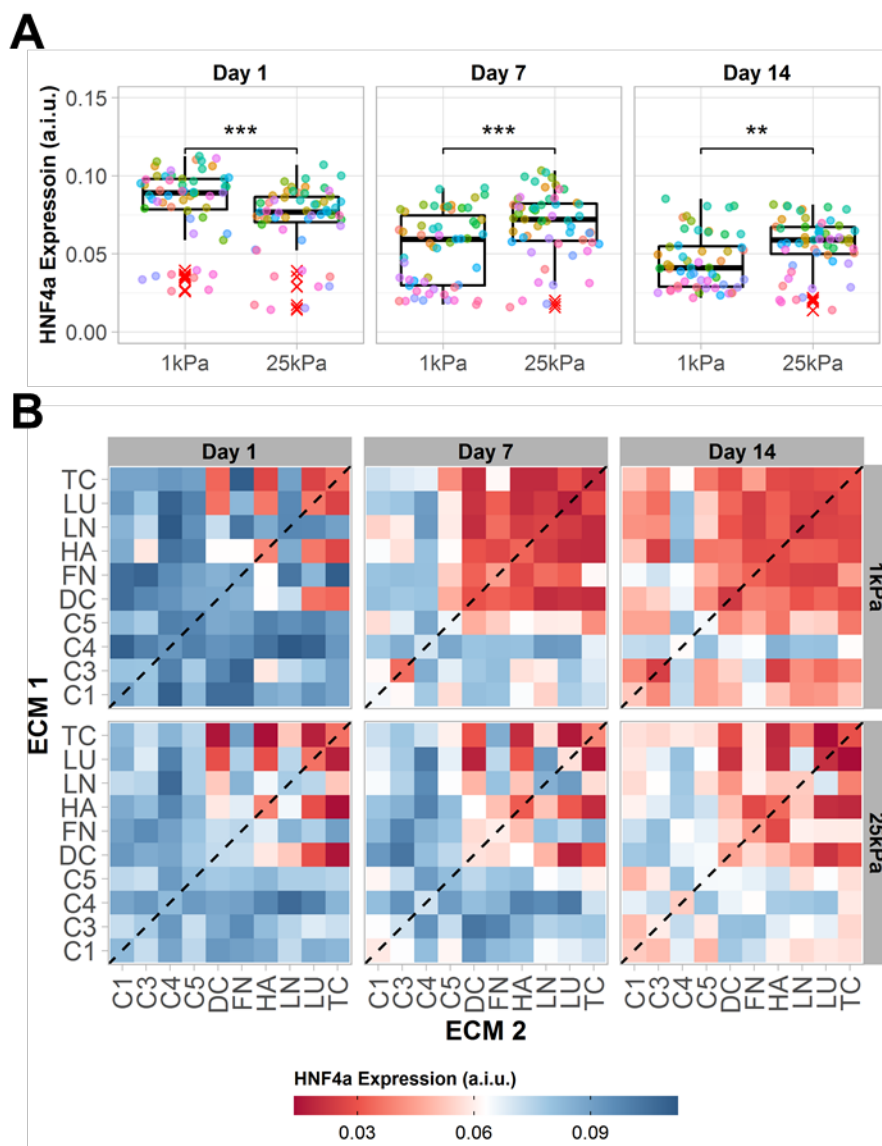
Supplement Figure 1: Attachment of primary human hepatocytes (PHHs) on extracellular matrix (ECM) microarrays. **A)** Heatmaps of average cell counts per island on single- (dashed line) and two-way combinations of the ECM proteins on two different stiffnesses, 1 kPa and 25 kPa, after 1 and 7 days of culture. **B)** Linear regression modeling for the effects of individual ECM proteins on the attachment of PHHs for 1 kPa and **C)** 25 kPa stiffnesses. Error bars represent standard error. * $p \leq 0.01$, ** $p \leq 0.001$, and *** $p \leq 0.0001$. For all panels, data displayed represents summarized single-cell measurements across three PHH donors per condition ($n = 22-24$ ECM islands across representative microarrays).



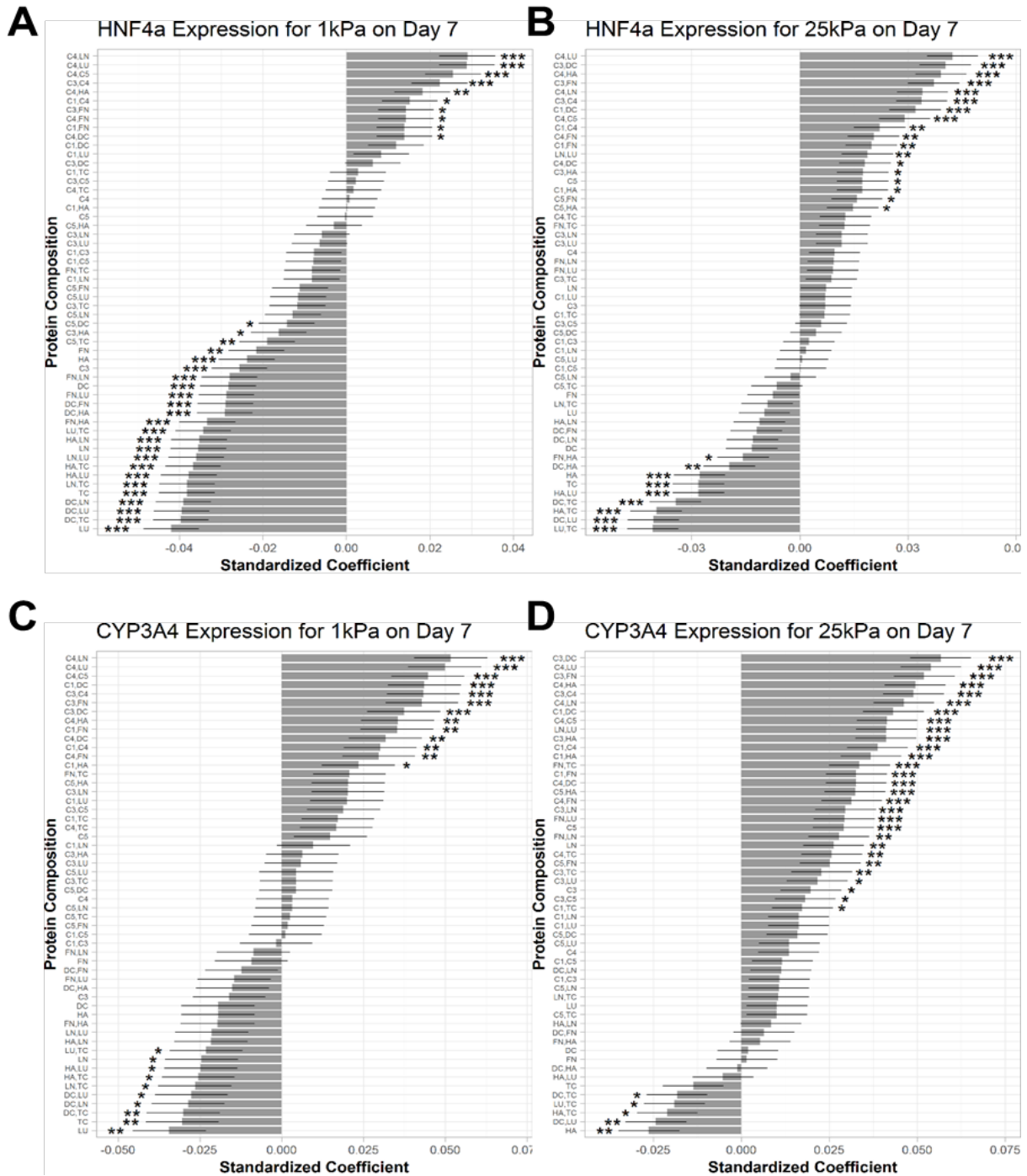
Supplemental Figure 2: Albumin expression in primary human hepatocytes (PHHs) on extracellular matrix (ECM) microarrays. A) Box plots show the median albumin expression (center bar) and interquartile range (IQR) for 55 ECM conditions separated for each microarray stiffness at 1 and 7 days of culture (each dot represents the mean albumin expression of an individual ECM composition; red asterisks represent outliers beyond $1.5 \times \text{IQR}$). A.i.u. = arbitrary intensity units. Comparison of median values was performed using the Wilcoxon Test. **** $p < 0.0001$. **B)** Heatmaps of mean albumin expression on single- (dashed line) and two-way combinations of the ECM proteins on two stiffnesses after 1 and 7 days of culture. For all panels, data displayed represents summarized single-cell measurements across three PHH donors per condition ($n = 22$ -24 ECM islands across representative microarrays).



Supplemental Figure 3: CYP3A4 expression in primary human hepatocytes (PHHs) on extracellular (ECM) microarrays. A) Box plots show the median CYP3A4 expression (center bar) and interquartile range (IQR) for 55 ECM two microarray stiffnesses over time (each dot represents the mean CYP3A4 expression of an individual ECM composition; red asterisks represent outliers beyond $1.5 \times \text{IQR}$). A.i.u. = arbitrary intensity units. Comparison of median values was performed using the Wilcoxon Test **** $p < 0.0001$. **B)** Bar plot showing mean CYP3A4 expression on ECM combinations over time on the 25 kPa stiffness. Error bars represent standard error of mean. For all panels, data displayed represents summarized single-cell measurements across three PHH donors per condition ($n = 22-24$ ECM islands across representative microarrays).



Supplemental Figure 4: HNF4 α expression in primary human hepatocytes (PHHs) on extracellular matrix (ECM) microarrays. A) Box plots show the median HNF4 α expression (center bar) and interquartile range (IQR) for 55 ECM two microarray stiffnesses over time (each dot represents the mean CYP3A4 expression of an individual ECM composition; red asterisks represent outliers beyond 1.5*IQR). A.i.u. = arbitrary intensity units. Comparison of the median values was performed using the Wilcoxon Test. ** $p < 0.01$ and *** $p < 0.001$. **B)** Heatmaps of mean HNF4 α expression on single- (dashed line) and two-way combinations of the ECM proteins on two stiffnesses over time. For all panels, data displayed represents summarized single-cell measurements across three PHH donors per condition (n = 22-24 ECM islands across representative microarrays).



Supplemental Figure 5: Rank ordering the effects of extracellular matrix (ECM) protein composition and stiffness on primary human hepatocyte (PHH) HNF4 α and CYP3A4 expression. **A)** Multiple linear regression model for the effects of ECM protein combinations on mean HNF4 α expression after 7 days of culture on cellular microarrays of 1 kPa and **B)** 25 kPa stiffnesses. **C)** Effects of ECM protein combinations on mean CYP3A4 expression after 7 days of culture on cellular microarrays of 1 kPa and **D)** 25 kPa stiffnesses. Conditions are ranked in descending order based on the corresponding

standardized coefficient. Error bars represent standard error. * $p \leq 0.01$, ** $p \leq 0.001$, and *** $p \leq 0.0001$. For all panels, data displayed represents summarized single-cell measurements across three PHH donors per condition (n = 22-24 ECM islands across representative microarrays).