Mechanotransduction-on-Chip: Vessel-Chip Model of Endothelial YAP Mechanobiology Reveals Matrix Stiffness Impedes Shear Mechanoresponse

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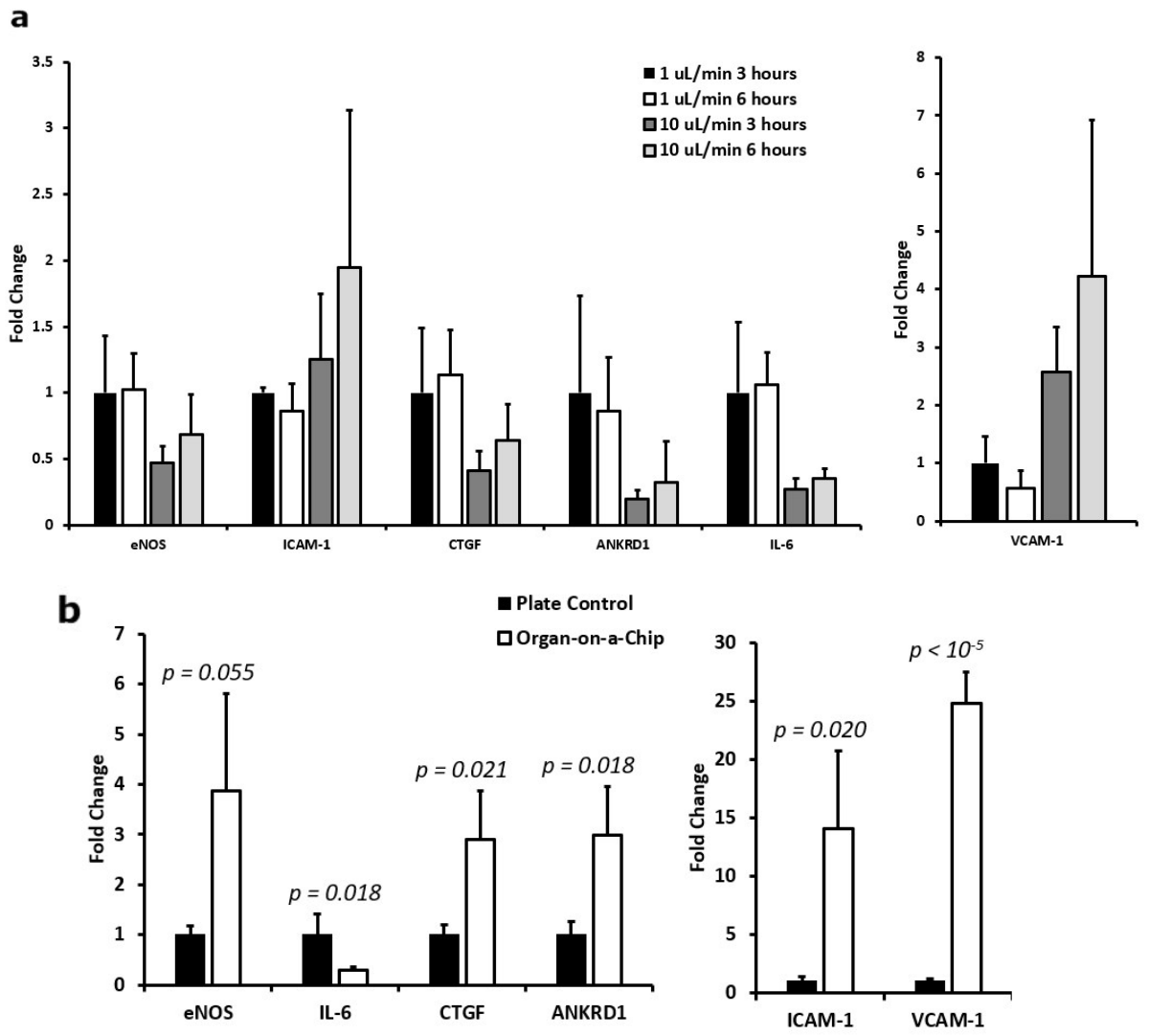
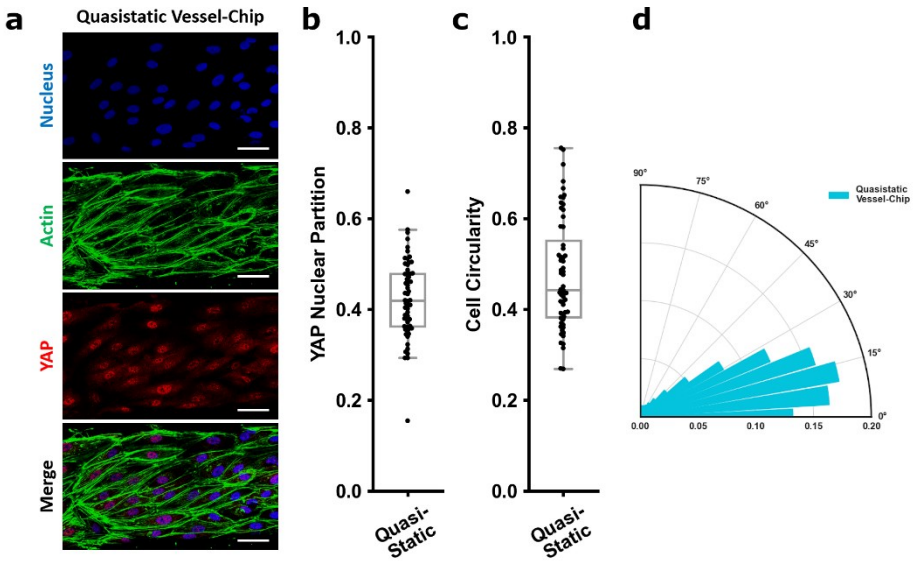
Supplementary Methods/Figures

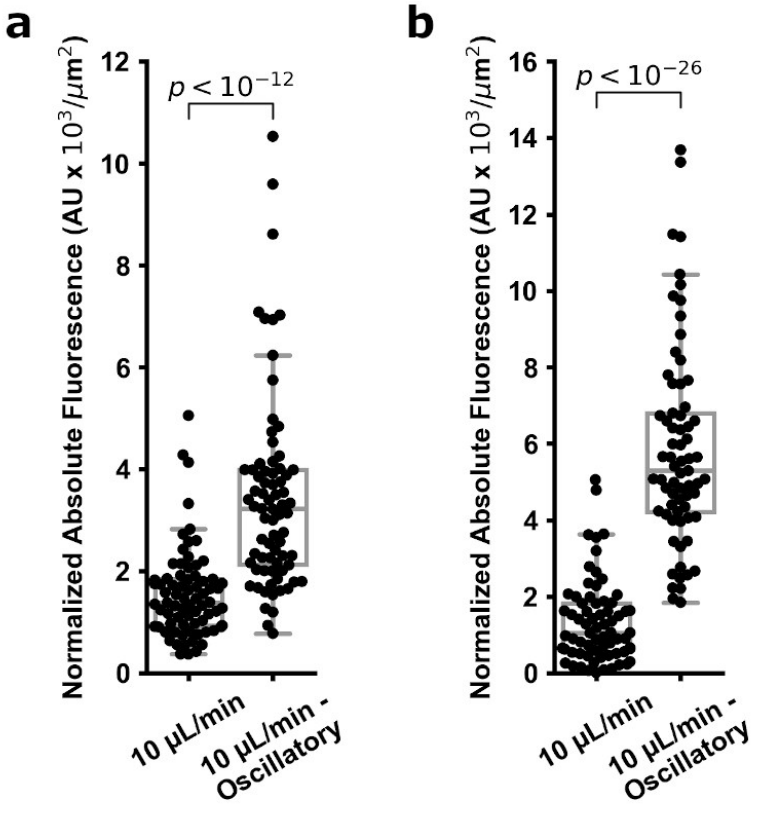
Vessel-on-a-Chip Cell Extraction: Trypsinizing cells from the vessel-on-a-chip system for processing and later use in experimentation required a modified protocol for consistent results, which is laid out here for reproducibility. After removal of the chip from the experimental conditions (connections, syringe pump, etc.), place an empty pipette tip (200 µL is the lower limit since this protocol relies on gravity flow, but higher volume tips are acceptable) on one of the two ports of the microfluidic chamber. In the other, still open port, fill a pipette tip to full volume with PBS and place it on the other port. Remove the tip from the pipettor *without* pushing any of the PBS through the chamber and place it in the incubator for 3-5 minutes. This serves as the PBS wash step. After the short incubation, check to see PBS flow-through on the other side, indicating that PBS is now the fluid volume in the channel. Remove both pipette tips from all ports. Repeat the steps for a wash with 0.25% trypsin. However, for this step, incubate within the cell incubator for 2 minutes. Remove the chips after and observed under a microscope to detect cell detachment. If cells are not fully detached, the chamber may be gently percussed to facilitate detachment. Afterwards, in the chamber, if cells are not present, they are now detached, and the fluid may be removed from the chamber. To do so, prime a pipette for suction by pressing the top dispensation plunger all the way down and connect it to the pipette tip with the lower volume. Let go of the plunger and suction the cells in the trypsin through. Once a majority of the volume is withdrawn, remove the tip and add the trypsin to an equivalent or greater volume of cell media in a centrifuge tube. Media may be perfused through the chamber after to pick up any cells not obtained through the first suction. The tube may be centrifuged for a pellet, and any subsequent protocol may be performed. It is important to note that trypsinization rapidly deactivates YAP, though gene expression is dependent on the mRNA half-life. Thus, all trypsinization steps to RNA extraction should be kept to under 10 minutes to ensure preservation of the phenotype and not introduce artifacts.



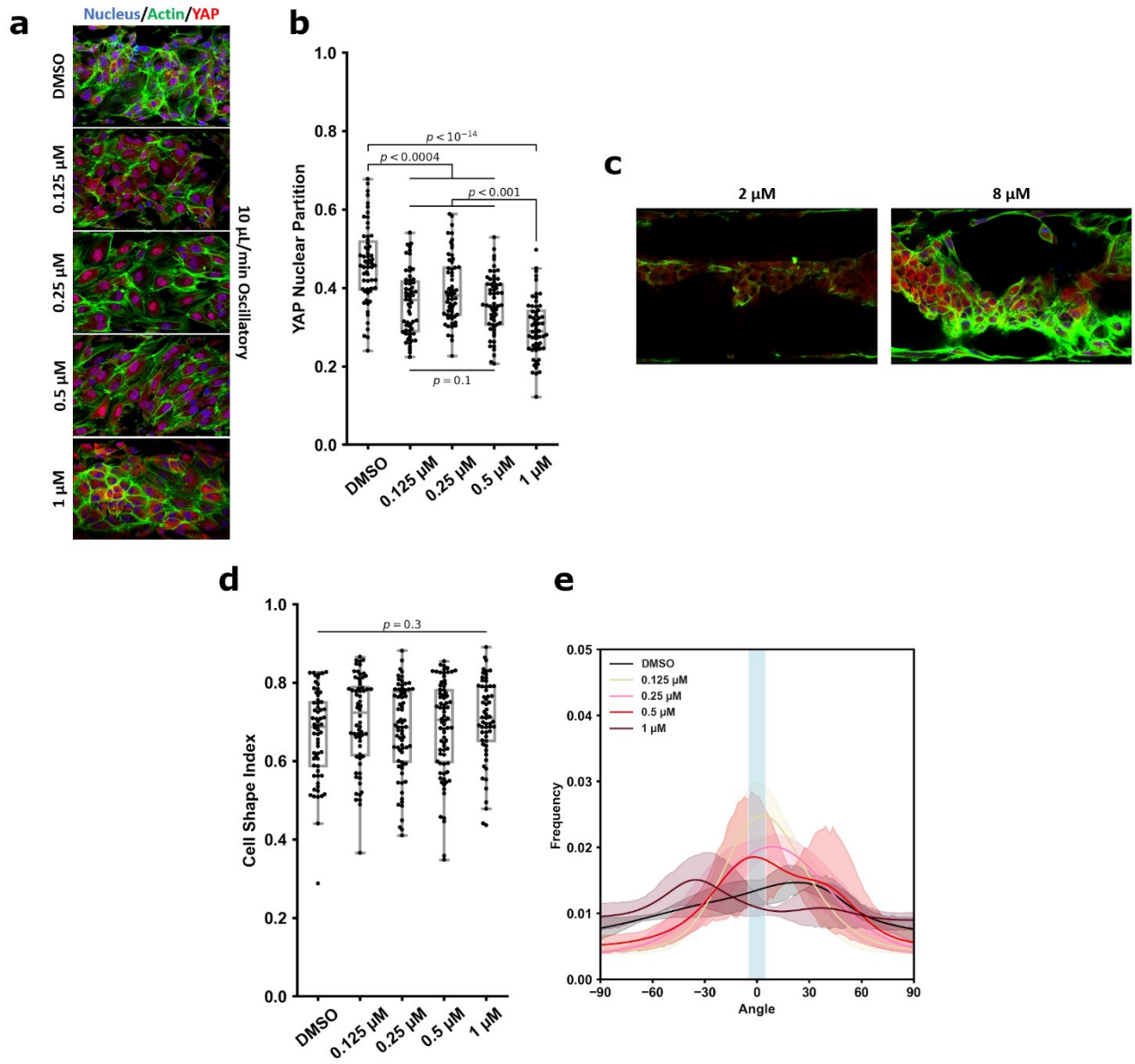
Supplementary Figure 1: RTqPCR gene expression analysis of various endothelial genes at 3- and 6-hour timepoints for venous and arterial shear (**a**). Each group for each gene was run n=3. Note that in the manuscript proper, the 1 vs 10 μL/min regimes were repeated an addition 4 times, and the n=3 from this graph were analyzed together with the repeat for the n=7 in the main manuscript. The data here is intended only to illustrate the timepoint trends. A fundamental limitation but repeatable observation for the vessel-on-a-chip model was that the arterial shear regime displayed upregulated surface adhesion molecules (ICAM-1 and VCAM-1). RTqPCR gene expression analysis of the vessel-on-a-chip system at 72 hours incubation total (**b**). Each group performed at n=3. This figure highlights than, in comparison to a plate control, longer incubation times seem to aberrantly regulate gene expression contrary to the expected patterns. Notably, surface adhesion molecules and targets of YAP/TAZ are all upregulated in the vessel-on-a-chip system vs. the polystyrene plate controls. This is remedied by the lumenization protocol established, and was the main driving force for developing a rigorous protocol.



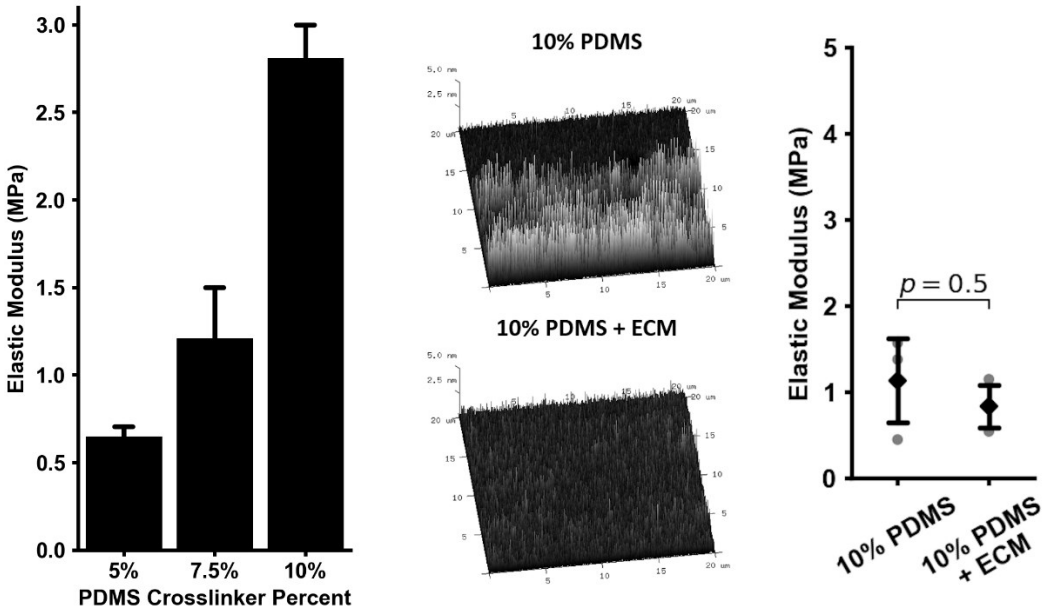
Supplementary Figure 2: Quasistatic control IF is shown in **a** (scale bar: 50 µm). YAP partially partitioned to the cytoplasm (**b**). Cells elongated and aligned under flow (**c-d**). This control set required 48-hour culture under <2 µL/hour to achieve confluence and lumen formation.



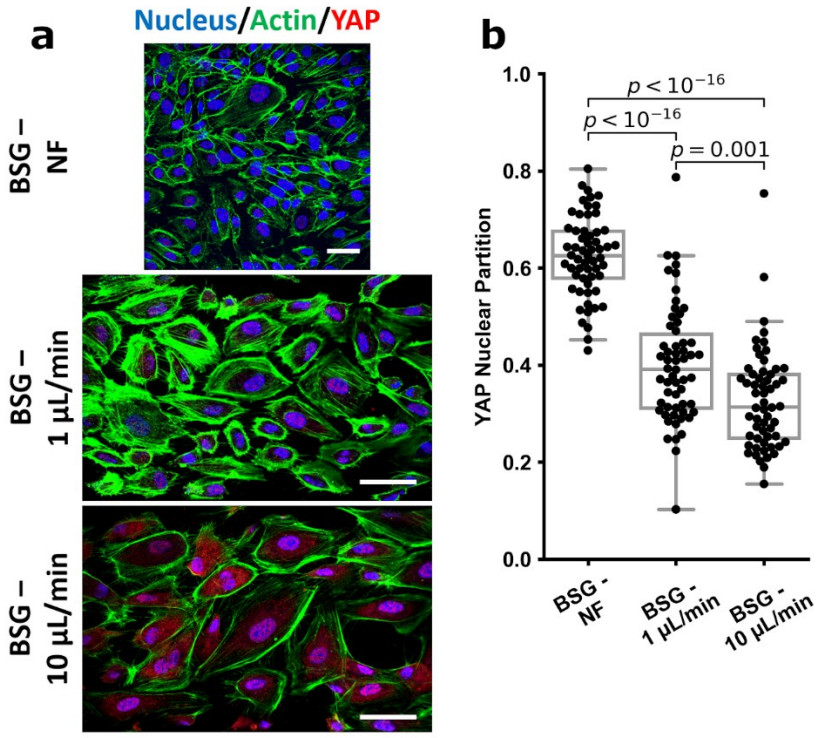
Supplementary Figure 3: Immunofluorescence data identical to **Figure 3a-d**, but normalized to DAPI fluorescence instead of cell area. Relationships, observations, and conclusions made in the area normalized data remain preserved when normalized to DAPI. Statistical analysis was performed on each group using an unpaired Student's t-test. All p-values are reported natively. Values reported as normalized intensity, mean ± standard deviation.



Supplementary Figure 4: Full verteporfin dose response curve with ECs subject to 10 μL/min oscillatory shear, showing the inhibition of YAP nuclear localization with increasing concentrations (**a-b**). In **c**, representative high concentration regimes are shown to highlight how excessive verteporfin induces excessive cellular detachment. Both the cell circularity (cell shape index) and alignment are not affected by verteporfin (**d-e**). The manuscript displays the data seen here only from DMSO and 1 μM.



Supplementary Figure 5: Elastic moduli of the various PDMS formulations and confirmation via AFM that chemical modification of a 10% PDMS thin film does not alter the mechanical properties. Conical fit using 0-10% of the AFM curve was used to fit data of PDMS thin films, >15 technical replicates taken per sample with iterative outlier analysis to remove technical replicates with large deviations.



Supplementary Figure 6: Borosilicate glass (BSG) studies demonstrating that high shear can downregulate YAP activity when HUVECs are culture on glass. The NF (no flow) control is conventional glass coverslips used commonly in cell culture, HUVECs within the device require delivery of media, and static controls within the flow chamber is suboptimal. Flow experiments are performed using non-coated BSG glass as the vessel-chip bottom. Note that YAP partitioning on BSG is statistically identical to the 10% PDMS group (**Figure 4b** in manuscript, 10% PDMS=0.32±0.1; BSG=0.32±0.1; p=0.7). Note the coverslip control is a conventional glass coverslip as perfusion is required on the chip to maintain EC media delivery – however the morphology of HUVECs grown in conventional culture is different from those grown under shear fundamentally, and this control only illustrates changes in YAP partition.