

Supporting Information for:

Estradiol and dihydrotestosterone exert sex-specific effects on human fibroblast and endothelial proliferation, bioenergetics, and vasculogenesis

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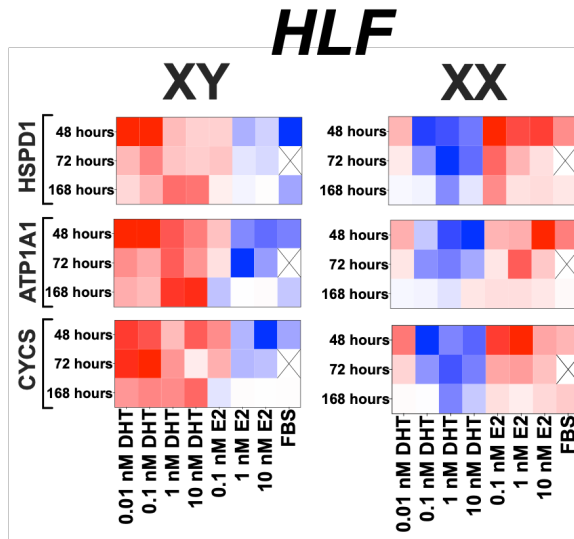
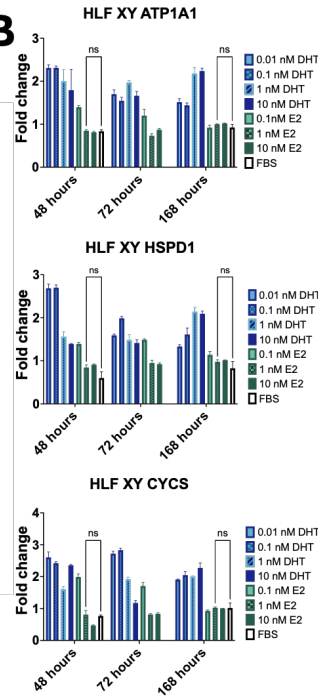
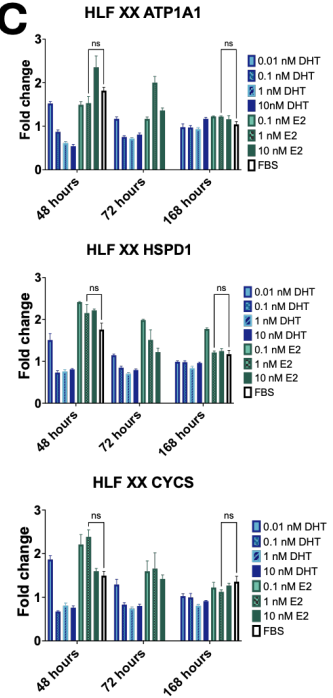
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Figure S7 – PEG incorporation in bulk PDMS minimizes Nile Red absorption.

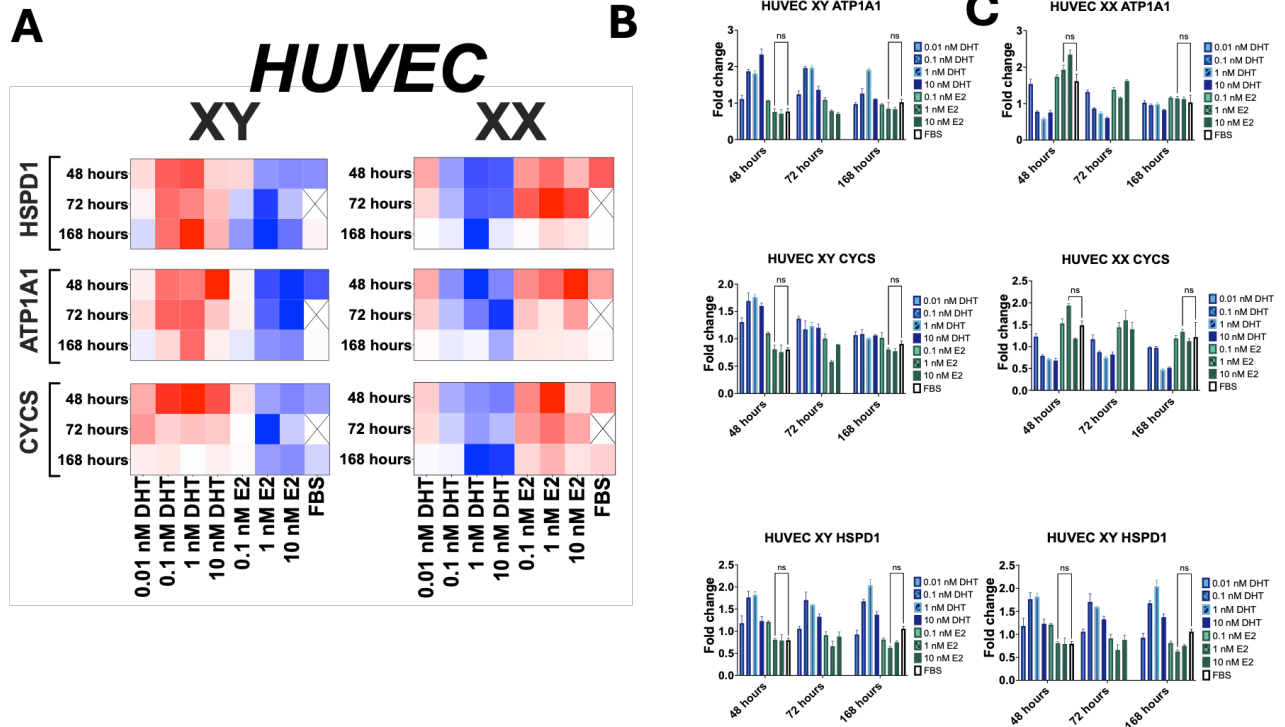
Supplemental Figures

Cell donor information											
Human Umbilical Vein Endothelial Cells (HUVEC)											
Supplier	Catalog #	Donor #	Donor Sex	Donor Race	Donor Age	Average SRY Ct	Average ER α Ct	Average ER β Ct	Average GPER Ct	Average AR Ct	Average GAPDH Ct
Lonza	CC-2517	0000315132	Female	Caucasian	N/A	Undetected	20.929	20.812	24.787	25.117	17.216
Lonza	CC-2517	0000234484	Female	Caucasian	N/A	37.990	21.249	24.482	23.158	23.284	17.438
Stemcell	200-0630	W243422007450P	Female	N/A	N/A	Undetected	19.663	17.445	26.245	26.245	16.130
ATCC	PCS-100-010	70013502	Female	N/A	N/A	Undetected	20.224	22.235	24.465	27.457	16.606
Lonza	CC-2517	0000318930	Male	Caucasian	N/A	24.503	20.621	22.433	24.346	24.396	16.663
Lonza	CC-2517	0000273864	Male	Caucasian	N/A	22.978	19.630	19.945	21.690	24.564	15.844
Stemcell	200-0630	W243422007461J	Male	N/A	N/A	22.643	18.796	19.077	27.242	25.242	17.127
Stemcell	200-0630	W243422007038J	Male	N/A	N/A	21.242	18.521	18.799	24.500	24.339	16.411
Human Lung Fibroblasts (HLF)											
Supplier	Catalog #	Donor #	Donor Sex	Donor Race	Donor Age	Average SRY Ct	Average ER α Ct	Average ER β Ct	Average GPER Ct	Average AR Ct	Average GAPDH Ct
ATCC	PCS-201-013	70014625	Female	Caucasian	29	Undetected	19.359	23.884	21.842	25.241	16.437
ATCC	PCS-201-013	700451445	Female	Caucasian	20	Undetected	18.746	21.217	21.064	21.714	16.519
Lonza	CC-2512	22TL151174	Female	Caucasian	45	Undetected	21.831	23.163	21.256	20.425	16.819
Lonza	CC-2512	000343490	Female	Caucasian	24	Undetected	18.570	21.572	21.922	23.687	16.998
ATCC	PCS-201-013	70002722	Male	Hispanic	14	20.364	19.937	21.943	20.689	24.289	16.789
Lonza	CC-2512	23TL268440	Male	Hispanic	45	21.308	22.815	18.977	21.120	21.470	17.129
Lonza	CC-2512	23TL275471	Male	Black	32	20.923	22.109	21.507	20.453	21.253	16.826
Lonza	CC-2512	0000615568	Male	Hispanic	22	22.274	24.558	24.699	20.388	20.374	16.217
Human Retinal Microvascular Endothelial Cells (HRMVEC)											
Supplier	Catalog #	Donor #	Donor Sex	Donor Race	Donor Age	Average SRY Ct	Average ER α Ct	Average ER β Ct	Average GPER Ct	Average AR Ct	Average GAPDH Ct
Celprogen	36052-03	230501	Female	Caucasian	35	Undetected	26.606	21.294	24.157	23.631	16.514
Celprogen	36052-03	230371	Female	Asian	25	Undetected	25.414	22.542	21.682	24.520	16.815
Celprogen	36052-03	230625	Female	Caucasian	30	37.666	21.977	22.681	20.170	24.333	17.636
Celprogen	36052-03	230502	Male	Caucasian	37	21.992	25.205	24.889	22.021	20.547	16.634
Celprogen	36052-03	230002	Male	Black	41	21.566	25.782	21.720	19.745	24.907	17.706
Celprogen	36052-03	230666	Male	Caucasian	28	24.053	18.112	19.335	21.048	23.885	17.413
Human Ocular Fibroblasts (HOF)											
Supplier	Catalog #	Donor #	Donor Sex	Donor Race	Donor Age	Average SRY Ct	Average ER α Ct	Average ER β Ct	Average GPER Ct	Average AR Ct	Average GAPDH Ct
CellBiologics	H-6204	208536	Female	Caucasian	36	Undetected	27.118	21.413	28.041	22.1697	16.448
ScienCell	6620	30574	Female	Caucasian	26	Undetected	20.912	23.157	24.451	26.2843	16.483
ScienCell	6620	30448	Female	Black	39	37.920	25.729	22.718	19.725	23.7130	16.889
CellBiologics	H-6204	208206	Male	Caucasian	40	21.592	23.983	25.542	26.560	24.7739	16.785
ScienCell	6620	30733	Male	Hispanic	32	20.409	21.449	27.666	23.510	27.6758	17.583
ScienCell	6620	30222	Male	Caucasian	17	23.339	26.691	26.535	22.427	19.4397	16.063

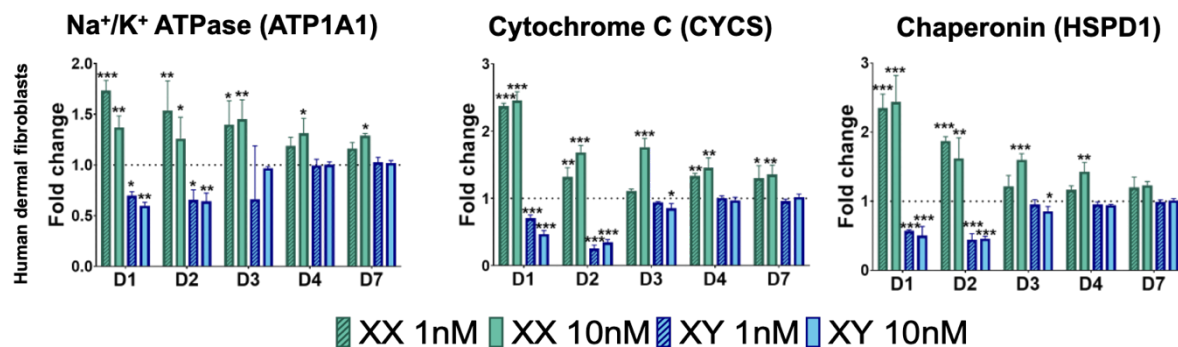
Supplemental Figure 1: Cell donor information. HLF, HOF, HUVEC, and HRMVEC were tested for SRY gene expression (i.e. XY chromosomal makeup) and the expression of steroidal hormone receptors. Data for SRY is represented in the table as cycle threshold (C_T); C_T over 36 is assumed to be background signal as per PCR setup parameters. Expression of estrogen receptor alpha (ER α), estrogen receptor beta (ER β), G-protein coupled estrogen receptor (GPER), and androgen receptor (AR) show that cells utilized in experimentation test positive for expression of estrogen and androgen receptors. PCR preformed in triplicate per gene.

A**B****C**

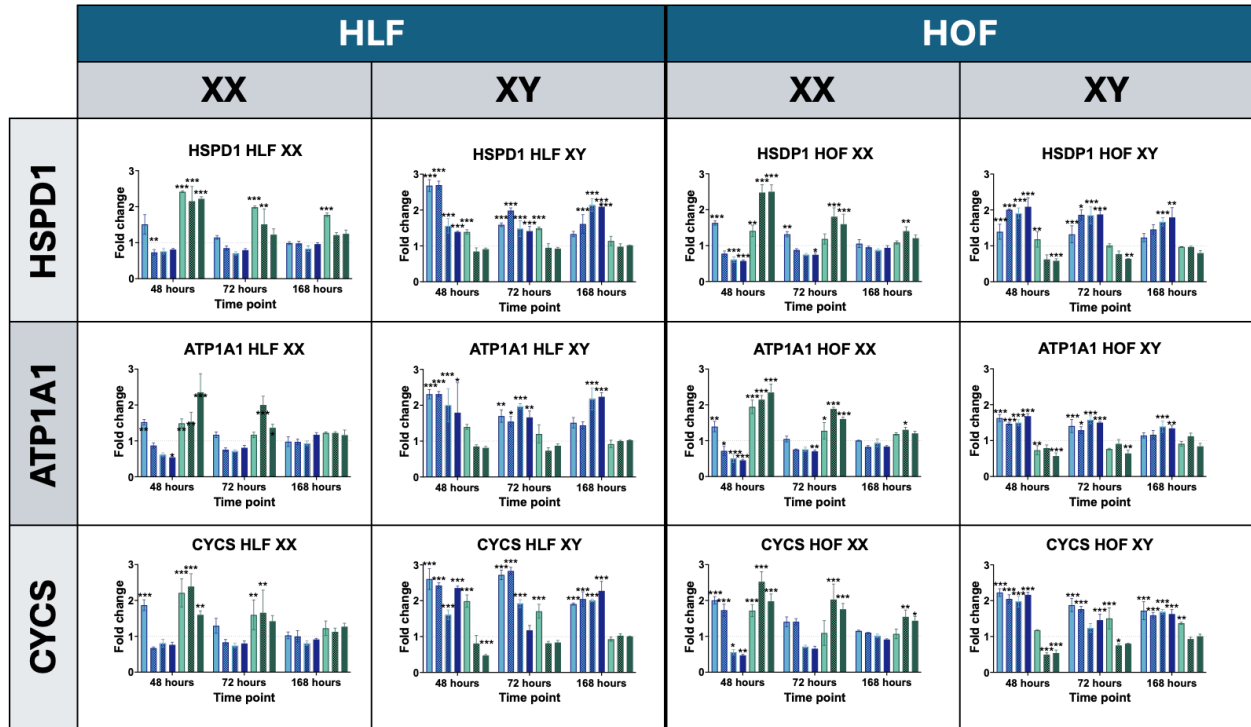
Supplemental Figure 2: 2% FBS-containing medium and CSS-containing medium with 1 nM E2 induce similar gene expression responses in HLF. **A:** RT-qPCR results for XY (left) and XX (right) show that FBS induces changes that mirror culture with 1nM E2. **B:** XY HLF showed no statistical differences between 1nM E2 and FBS in ATP1A1, HSPD1, or CYCS at 48 or 168 hours. **C:** Similarly, XX HLF also showed no significant differences between FBS and 1nM E2. Analyzed using the $2^{-\Delta\Delta C_t}$ method with GAPDH as a housekeeping gene and the same cells grown in media containing charcoal stripped serum as a control. n=4 donors per sex for HLF, 3 technical replicas per donor, PCR preformed in triplicate.



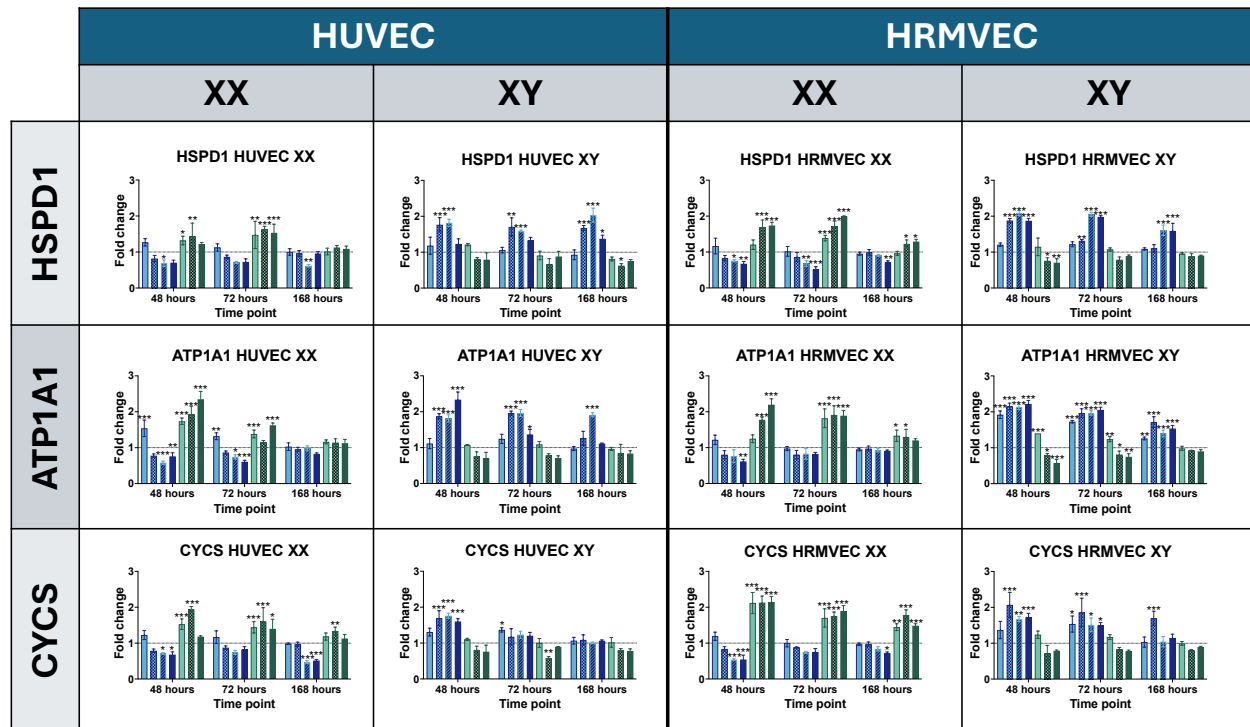
Supplemental Figure 3: 2% FBS-containing medium and CSS-containing medium with 1 nM E2 induce similar gene expression responses in HUVEC. **A:** RT-qPCR results for XY (left) and XX (right) HUVEC show that FBS again induces changes that mirror culture with 1nM E2. **B:** XY HUVEC showed no statistical differences between 1nM E2 and FBS in ATP1A1, HSPD1, or CYCS at 48 or 168 hours. **C:** Similarly, XX HUVEC also showed no significant differences between FBS and 1nM E2. Analyzed using the $2^{-\Delta\Delta C_t}$ method with GAPDH as a housekeeping gene and the same cells grown in media containing charcoal stripped serum as a control. n=4 donors per sex for HLF, 3 technical replicas per donor, PCR preformed in triplicate.



Supplemental Figure 4: Human dermal fibroblasts (HDF) mirror HOF and HLF gene expression responses to E2. RT-qPCR results for energetic and stress-related genes in human dermal fibroblasts (HDF) exposed to varying levels of E2 over 7 days with baseline expression (Fold change = 1) represented by the dotted line. In XX cells, administration of both 1nM and 10nM E2 induced significant upregulation of ATP1A1, CYCS, and HSPD1 in a transient manner with some levels returning to baseline by day 7. Conversely, in XY cells, E2 exposure caused a downregulation of these genes that peaked at 24 and 48 hours before also returning to baseline by day 7. Analyzed using the $2^{-\Delta\Delta C_t}$ method with GAPDH as a housekeeping gene and the same cells grown in media containing charcoal-stripped serum as a control. n= 3 donors per sex for each cell type, 3 technical replicas per donor, 3 technical replicas per gene.

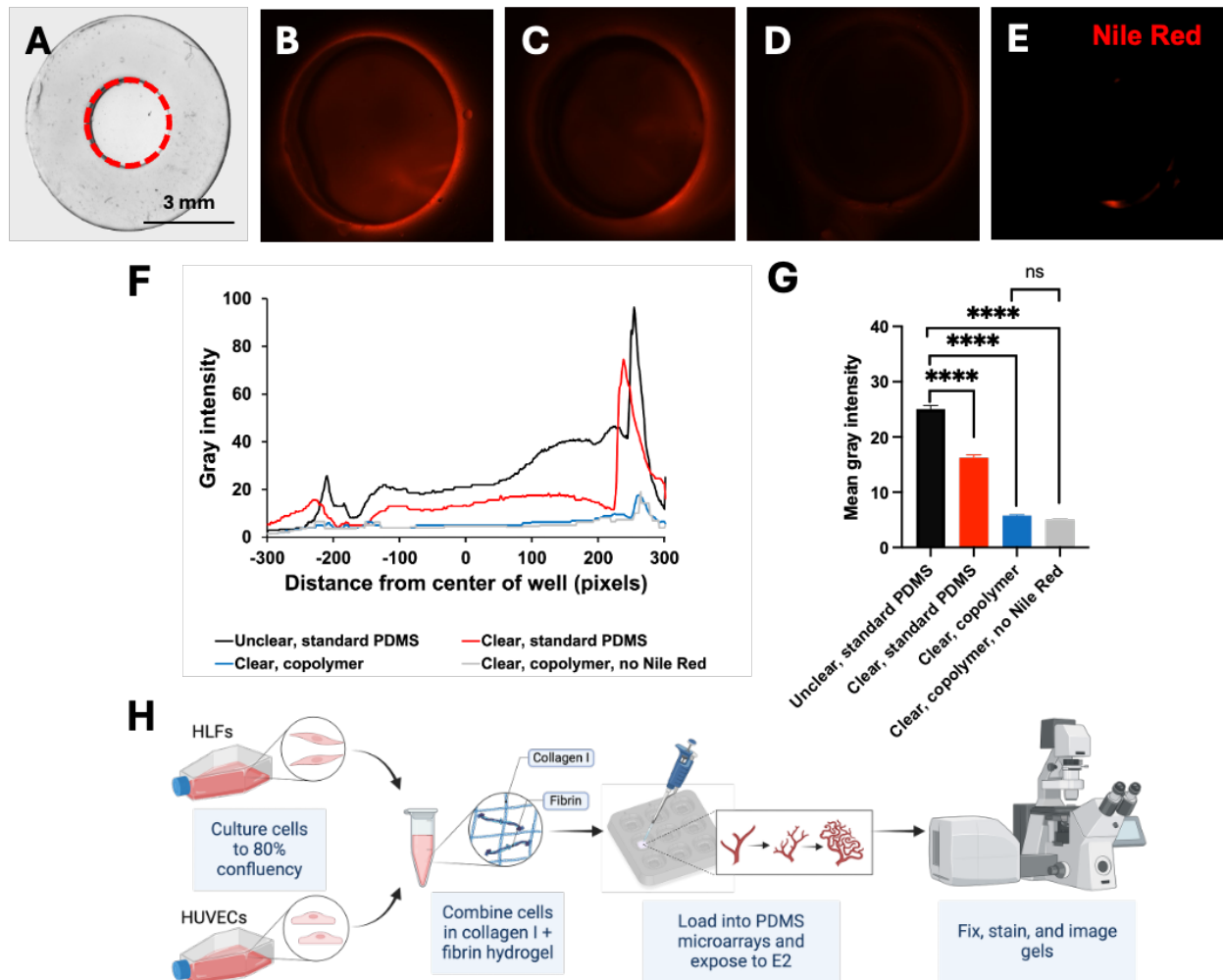


Supplemental Figure 5: HLF and HOF display significant fold changes in gene expression upon exposure to E2 and DHT. HLF and HOF showed sex-specific fold changes in result to exposure to E2 and DHT when compared to the same cells grown in CSS as a baseline (Fold change = 1). Analyzed using the $2^{-\Delta\Delta C_t}$ method with GAPDH as a housekeeping gene and the same cells grown in media containing charcoal-stripped serum as a control. n = 4 donors per sex for HLF and 3 donors per sex for HOF, 3 technical replicas per donor, PCR preformed in triplicate. Compared to control via 2-way ANOVA for chromosomal sex and hormones. * = p < 0.05, ** = p < 0.01, *** = p < 0.001, **** = p < 0.0001.



0.01 nM DHT
 0.1 nM DHT
 1 nM DHT
 10 nM DHT
 0.1 nM E2
 1 nM E2
 10 nM E2

Supplemental Figure 6: HUVEC and HRMVEC display significant fold changes in gene expression upon exposure to E2 and DHT. HUVEC and HRMVEC showed sex-specific fold changes in result to exposure to E2 and DHT when compared to the same cells grown in CSS as a baseline (Fold change = 1). Analyzed using the $2^{-\Delta\Delta C_t}$ method with GAPDH as a housekeeping gene and the same cells grown in media containing charcoal-stripped serum as a control. n = 4 donors per sex for HUVEC and 3 donors per sex for HRMVEC, 3 technical replicas per donor, PCR preformed in duplicate. Compared to control via 2-way ANOVA for chromosomal sex and hormones. * = p < 0.05, ** = p < 0.01, *** = p < 0.001, **** = p < 0.0001.



Supplemental Figure 7: PEG incorporation in bulk PDMS minimizes Nile Red absorption. **A:** Brightfield image of sample PDMS disc. Area of interest is the well in the center outlined with red dashed line. **B-D:** Fluorescence microscopy images of area of interest of PDMS discs after exposure to 1 ug/ml Nile Red for 1 hour. Standard PDMS molded on an unclear coated mold (**B**), standard PDMS molded on a clear coated mold (**C**), and PDMS+PEG Copolymer molded on a clear coated mold (**D**) were tested. **E:** Copolymer molded on clear coated mold not exposed to Nile Red and instead exposed to control (PBS) showed some background positivity around the edge of the well, but not significant fluorescence. **F:** Linear fluorescent intensity across PDMS discs. Lines represent the average value of 3 discs. **G:** Comparison of mean gray intensity shows a significantly higher fluorescent signal corresponding to significantly increased absorption in standard, unclear PDMS than standard, clear PDMS. PDMS+PEG copolymer showed significantly decreased absorption that was not significantly greater than naturally occurring background fluorescence seen in control discs not exposed to Nile Red. Bars represent SEM. $n = 3$ devices per condition. Compared via one-way ANOVA. * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$, **** = $p < 0.0001$. **G:** Workflow of utilizing treated copolymer PDMS arrays to create vascular gels. Created in BioRender. Martier, A. (2025) <https://BioRender.com/gzsd4>