Supplementary tables and figures

Assay	VEC donor	VEC passage	Conditions evaluated	Biological replicates	Technical replicates
Proliferation	VEC2	P1	Control, Vtn, CM 3T3-FC, CFC, VFC, DFC	1 VEC; 3 for CFC/VFC /DFC	3 per condition and time point
CFE	VEC3	P1–P2	Control, Vtn, CM 3T3-FC, CFC, VFC, DFC	1 VEC; 3 for CFC/VFC /DFC	1 flask for culturing and 3 flasks for CFE per condition or biological replicate
Migration	VEC1	P2	Control, Vtn, CM 3T3-FC, CFC, VFC, DFC	1 VEC; 3 for CFC/VFC /DFC	6 wells initially; ≥3 retained after exclusion
2D IF	VEC2	P1	Control, Vtn, CM 3T3-FC, CFC, VFC, DFC	1 VEC; 3 for CFC/VFC /DFC	One coverslip per donor/cell line/control
3D collagen hydrogels	VEC1	P2	Control; feeder and native fibroblast conditions	1 VEC; 3 for CFC/VFC /DFC	One gel per donor/cell line/control
Growth-factor array	N/A	N/A	Control, 3T3-FC, CFC, VFC, DFC	1 VEC; 3 for CFC/VFC /DFC	One conditioned-media sample per donor/cell line/control; two technical replicates per GF analyzed

Supplementary Table S1. Overview of the biological and technical replicate structure used for each assay. The table summarizes the VEC donor, VEC passage, culture conditions evaluated, and the numbers of biological and technical replicates. One biological VEC donor was used for each assay, while three independent biological donor-derived fibroblast populations were used for each primary fibroblast tissue source where applicable. Technical replicates are indicated for each assay. P = passage, CFE = colony formation assay, IF = immunofluorescence, VEC = Vaginal epithelial cells, GF = Growth factor.

Concentration µg/cm^2	Well 1	Well 2	Well 3	Average cell number pr well
1	3.125	1.250	1.875	2083
0.75	625	625	1.250	833
0.5	2.500	1.250	3.125	2292
0.25	625	625	1.250	833
0.1	1.875	0	0	625

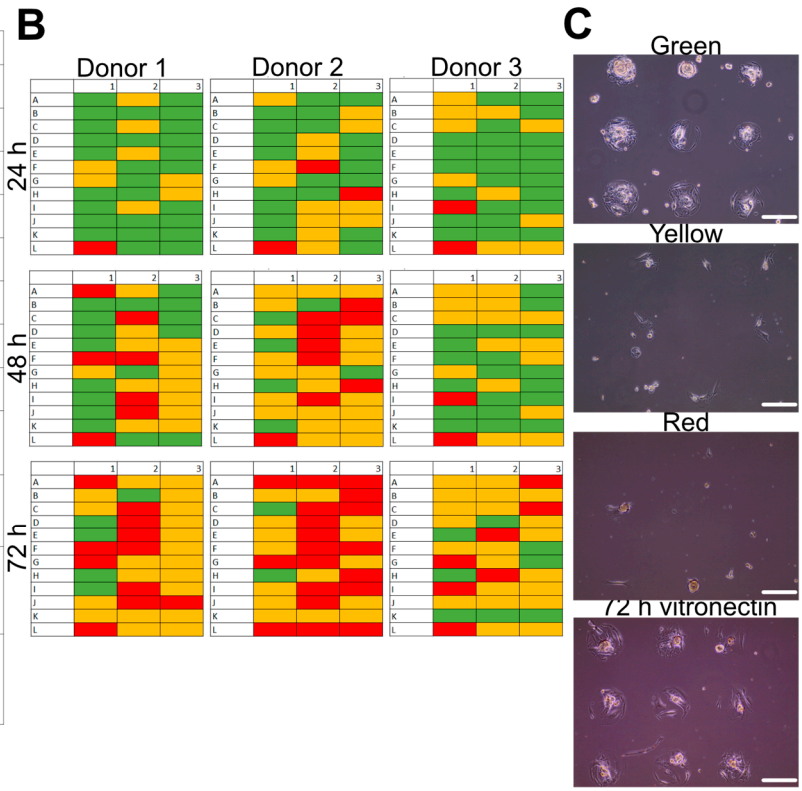
Supplementary Table S2. VEC numbers for varying concentrations of Vitronectin coatings on a 12-well.

Condition	Cell count pass 1	Cell count pass 2
Control	280 000	-
Vtn	300 000	50 000
CM	-	-
3T3-FC	1 980 000	1 375 000
C1	740 000	140 000
C2	200 000	1 080 000
C3	420 000	360 000
V1	940 000	1 175 000
V2	1 140 000	700 000
V3	800 000	350 000
D1	2 060 000	400 000
D2	2 220 000	1 700 000
D3	1 220 000	325 000

Supplementary Table S3. Cell counts from passage in the CFE assay. The counts have not been deducted feeder cell numbers. Vtn = Vitronectin coating, CM = commercial medium, 3T3-FC = J2 3T3 feeder cells, C1-C3 = Cervical feeder cell donors, V1-V3 = Vaginal feeder cell donors, D1-D3 = Dermal feeder cell donors.

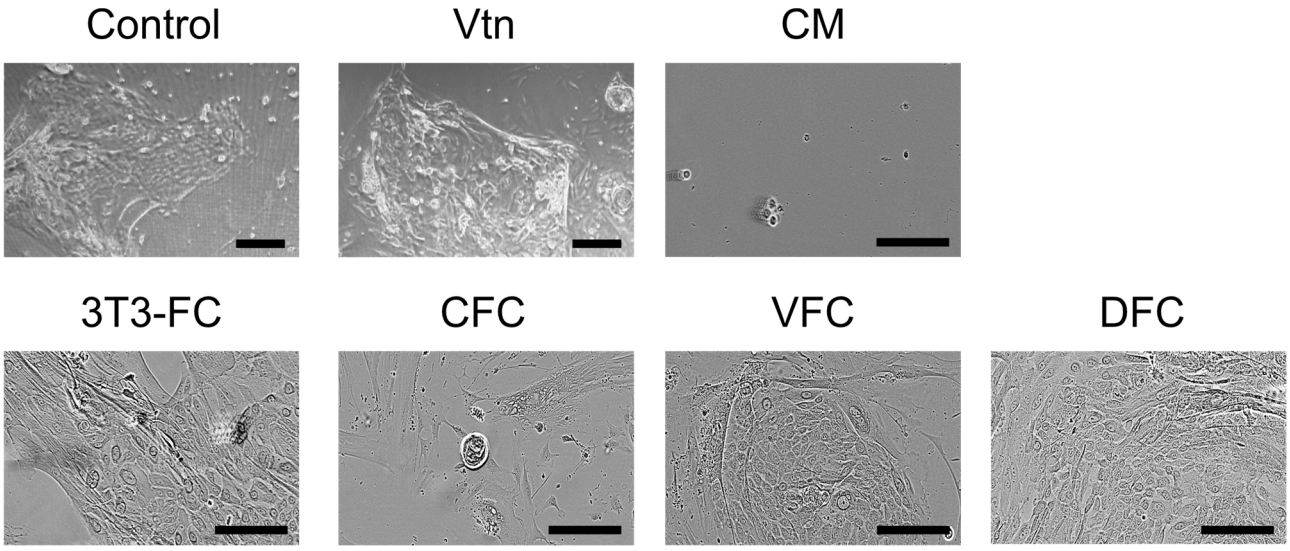
A

	1	2	3
A	Collagen I	Collagen I Collagen III	Fibronectin Collagen IV
B	Collagen III	Collagen I Collagen IV	Fibronectin Collagen VI
C	Collagen IV	Collagen I Collagen V	Fibronectin Laminin
D	Collagen V	Collagen I Fibronectin	Fibronectin Vimentin
E	Collagen VI	Collagen I Laminin	Laminin Collagen IV
F	Fibronectin	Collagen I Vitronectin	Laminin Collagen VI
G	Laminin	Collagen I Vitronectin	Vitronectin Collagen IV
H	Vitronectin	Collagen I Tropoelastin	Vitronectin Collagen VI
I	Tropoelastin	Fibronectin Laminin Collagen I	Vitronectin Laminin
J	Collagen IV Collagen VI	Fibronectin Laminin Collagen IV	Vitronectin Tropoelastin
K	Collagen III Collagen V	Vitronectin Laminin Collagen I Collagen IV	Tropoelastin Collagen IV
L	Negative control	Fibronectin Laminin Collagen I Collagen IV	Tropoelastin Collagen VI



Supplementary Figure S1. ECM array. **A.** Coatings tested in the ECM array (adapted from the array manual). **B.** The amount of cells adherent to the nine technical replicates of the array coating. Green colors signify at least one cell adherent to all the nine technical repeats, yellow colors signify adherent cells in 1-8 of the technical replicates, and red colors signify no adherent cells. Results displayed from each of the three biological replicates (donors) with evaluation of adherence every 24 hours for a total

of 72 hours. **C.** 40x images of green, yellow, and red adherence and of vitronectin after 72 hours of incubation (Scale bar: 400 µm).



Supplementary Figure S2. Morphological examples of VEC colonies after 8 days of incubation on the different culturing conditions using a phase-contrast microscopy, 20x magnification (Scale bar: 100 µm). Vtn = Vitronectin coating, CM = commercial medium, 3T3-FC = J2 3T3 feeder cells, CFC = Cervical feeder cells, VFC = Vaginal feeder cells, DFC = Dermal feeder cells.