SUPPLEMENTARY MATERIALS

Disease-specific extracellular matrix composition regulates placental trophoblast fusion efficiency

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Supplementary Methods and Data

S1. Sample preparation of proteolytic digestion of ECM proteins

ECM pellets were resolubilized in 5% sodium dodecyl sulfate (SDS), 100 mM TRIS pH 7.8. Samples were subsequently heated to 99°C for 10 minutes and subjected to probe-based sonication using a Thermo Sonic Dismembrator at 25 % amplitude for 3 cycles x 5 seconds. Remaining debris was pelleted by centrifugation at 20,000g for 5 minutes. An aliquot of the supernatant was diluted to < 1% SDS and used for estimation of protein concentration by bicinchonic acid assay (BCA) (Pierce/Thermo). The remainder of the supernatant was transferred into a new reaction tube and disulfide bonds were reduced by the addition of tris(2-carboxyethyl)phosphine (TCEP) to a final concentration of 20 mM and incubated at 60°C for 30 minutes. Free cysteines were alkylated using iodoacetamide at a final concentration of 25 mM and subsequent incubation at 37°C for 30 minutes in the dark. An equivalent of 10 µg of total protein was used for proteolytic digestion using suspension trapping (STRAP) (1). In brief, proteins were acidified through the addition of phosphoric acid to a final concentration of 1.3% v/v. The sample was subsequently diluted 6-fold in STRAP loading buffer (9:1 methanol water in 100 mM TRIS, pH 7.8) and loaded onto an S-TRAP Micro cartridge (Protifi LLC, Huntington, NY) and spun at 4000g for 2 minutes. Samples were washed three times using 200 µL of STRAP loading buffer. Proteins were then proteolytically digested using sequencing grade trypsin (Promega) at a 1:10 enzyme to substrate ratio for 2 hours at 47°C. Peptides were eluted in 50% acetonitrile, vacuum concentrated, and desalted using homemade R3-STAGE-tip desalting cartridges (2). Desalted peptides were vacuum concentrated and reconstituted in 0.1% formic acid prior to analysis by nLC-MS/MS.

S2. LC-MS/MS and Data Analysis

Samples were analyzed by data dependent acquisition (DDA) using an Easy-nLC 1200 online coupled to a Q Exactive Plus (both Thermo Fisher Scientific). Samples were loaded onto the precolumn (Acclaim PepMap 100 C18, 3 µm particle size, 75 µm inner diameter x 2 cm length) in 0.1% formic acid (buffer A). Peptides were separated using a 100-min binary gradient ranging from 3-40% of buffer B (84% acetonitrile, 0.1% formic acid) on the main column (Acclaim PepMap 100 C18, 2 µm particle size, 75 µm inner diameter x 25 cm length) at a flow rate of 300 nL/min. Full MS scans were acquired from m/z 350-1,500 at a resolution of 70,000, with an automatic gain control (AGC) target of 1 million ions and a

maximum injection time of 50 ms. The 15 most intense ions (charge states +2 to +4) were isolated with a window of m/z 1.2, an AGC target of 20,000 and a maximum injection time of 64 ms and fragmented using a normalized higher-energy collisional dissociation (HCD) energy of 28. MS/MS were acquired with a resolution of 17,500 and the dynamic exclusion was set to 40 s.

MS raw data was processed using Proteome Discoverer 2.4 (ThermoFisher Scientific) and searched using Sequest HT against a human UniProt fasta database (release 23. January 2019; 20,414 target entries). The enzyme specificity was set to trypsin with a maximum of 2 missed cleavages. Carbamidomethylation of cysteine was set as fixed modification and oxidation of methionine as variable modification. The precursor ion mass tolerance was set to 10 ppm, and the product ion mass tolerance was set to 0.02 Da. Percolator was used to assess posterior error probabilities and the data was filtered using a false discovery rate (FDR) <1% on peptide and protein level. The Minora feature detector node of Proteome Discoverer was used for label free quantitation based on precursor areas. Preeclampsia (PE)/healthy ratios were determined for proteins quantified with at least one unique peptide. Normalized spectral abundance factors were calculated as described elsewhere (3).

S3. Mass Spectrometry Data

The complete list of proteins obtained and quantified with the mass spectrometry analysis, the NSAF for individual normal and preeclamptic ECM samples, and the analysis performed on select structural ECM proteins can be found in the shared data repository:
https://osf.io/ks83z/?view_only=b7a1c5111ba242edba5f6f3cb784f839

S4. Raw datasets - fluorescently-labelled images

Immunofluorescently-labelled image datasets collected for BeWo cells culture on decellularized ECM-coated matrices, and individually-selected matrices; and for vCTBs culture on decellularized ECM-coated matrices. All raw data can found in the shared data repository:
https://osf.io/ks83z/?view_only=b7a1c5111ba242edba5f6f3cb784f839

Supplementary Figures

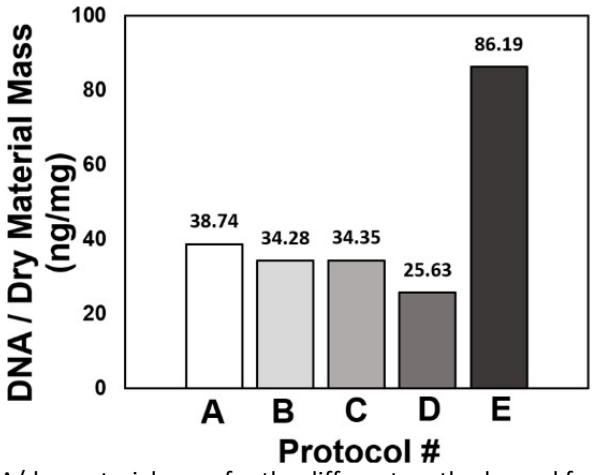

Figure S1. Assessment of DNA/dry material mass for the different methods used for decellularization of placental chunks (n=1 sample per protocol tested).

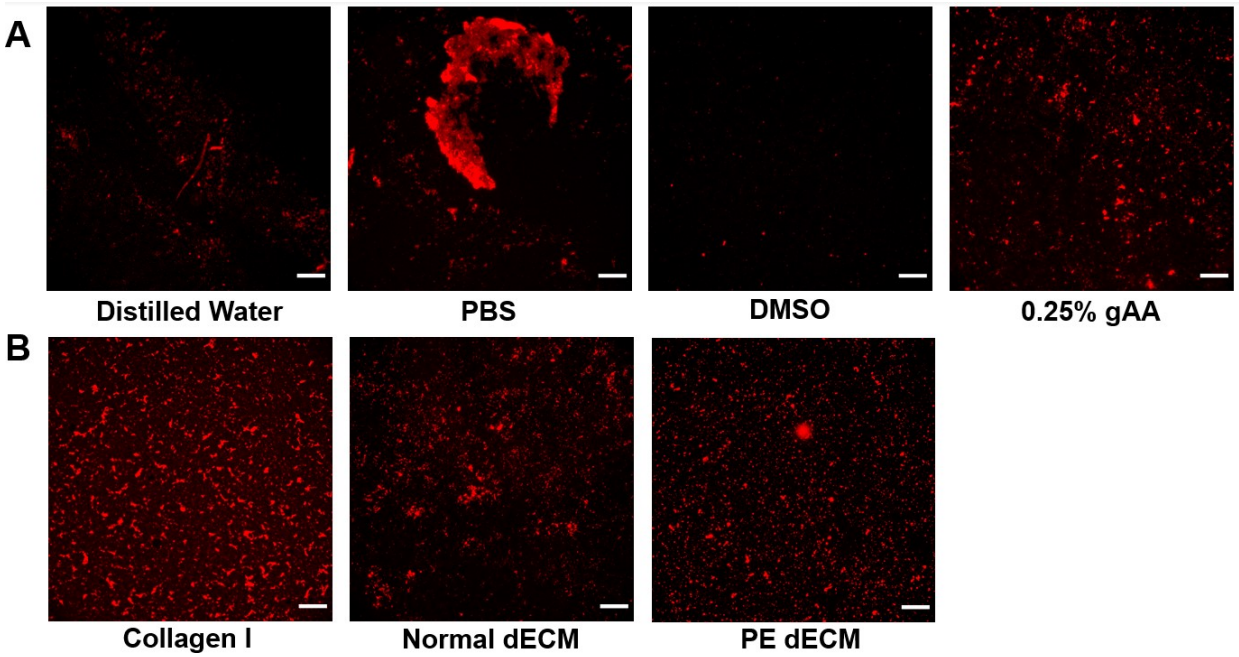

Figure S2: Characterization of dECM: resuspension and attachment to polyacrylamide gels. A) Fluorescent images of normal dECM nanoparticles dissolved in various diluents to assess resuspension. B) Fluorescent images of normal dECM & PE dECM nanoparticles in 0.25% gAA functionalized onto gel surfaces against a gel coated with Collagen I for comparison. Scale bar is 100 µm; red: collagen I. PBS-phosphate buffered saline, DMSO-dimethyl sulfoxide, gAA-glacial acetic acid, PE-preeclamptic; n=1 gel with 3 fields of view.

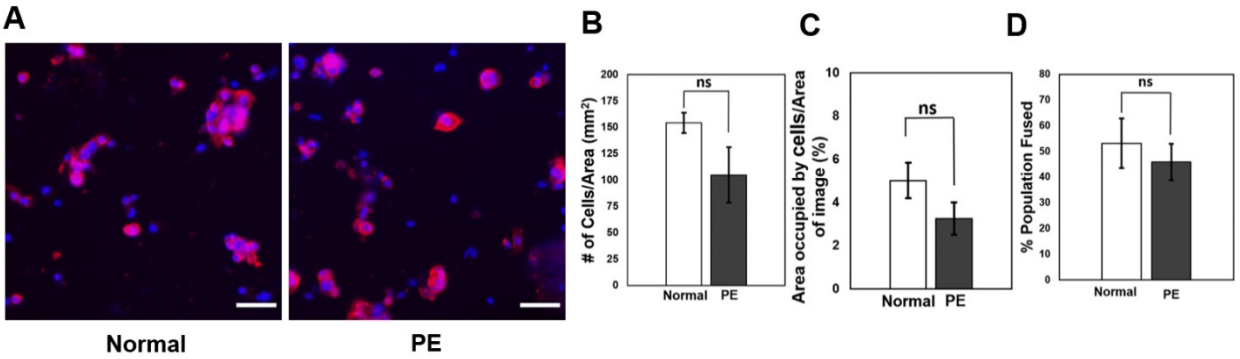

Figure S3: Effect of disease-specific ECM composition on fusion in primary vCTBs (villous cytotrophoblasts). A) Fluorescent images of fused vCTBs on substrates containing normal dECM and preeclamptic (PE) dECM. Scale bar is 50 µm; red: E-cadherin, blue: nuclei. B) Quantification of cell adhesion, C) cell spreading and C) fusion efficiency. (data presented as mean ± standard deviation; n = 3 independent gels; ns-not significant; by one-way ANOVA with Tukey post-hoc analysis). Similarly-sized experiments were repeated thrice using different placentas and showed similar results.

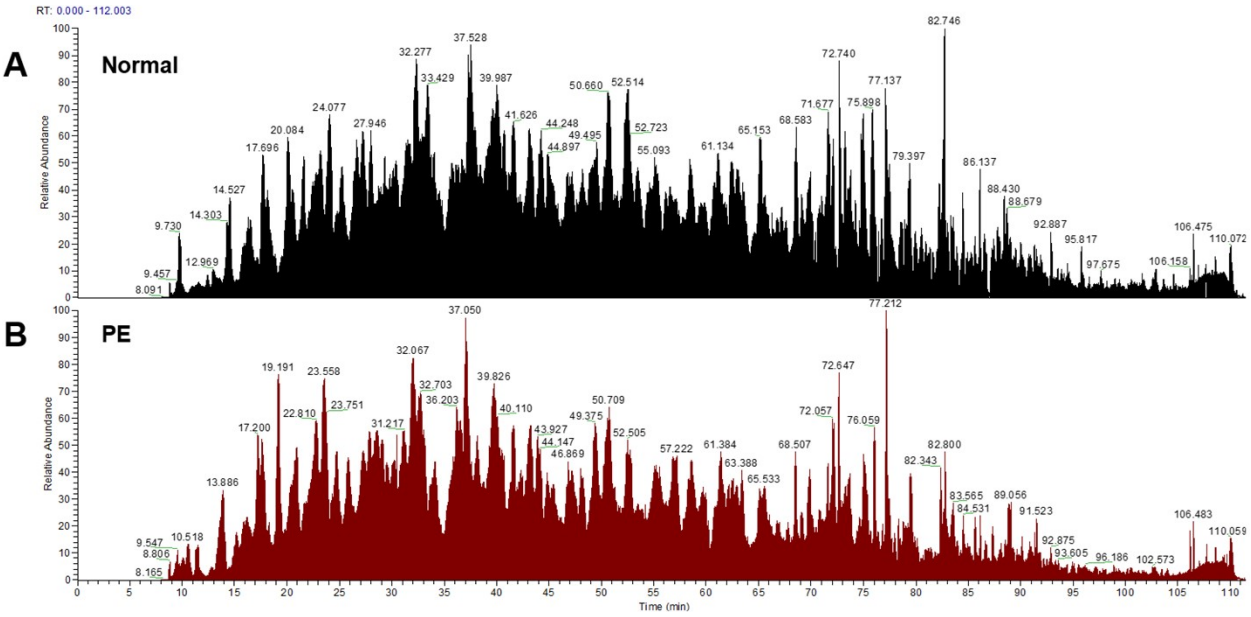

Figure S4: Total ion chromatographs of normal (A) and preeclamptic (B) placental dECM nanoparticles.

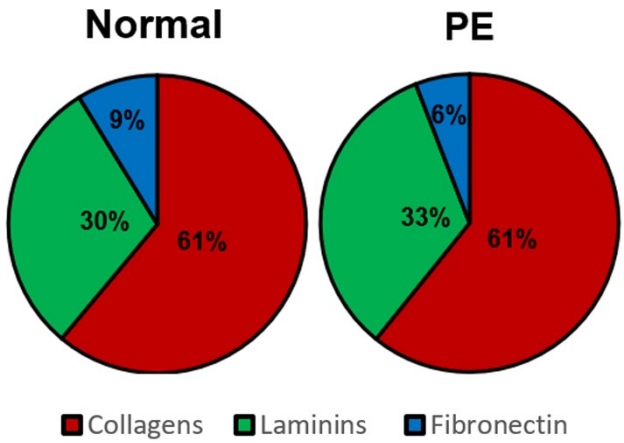

Figure S5: Comparison of NSAF distributions for selected protein groups in normal and preeclamptic (PE) samples.

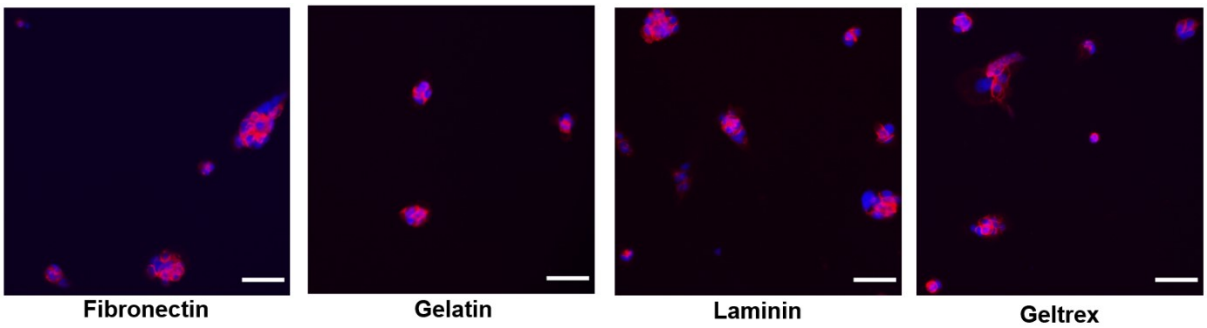

Figure S6: Effect of specific ECM components and mixtures on fusion in BeWo cells. Fluorescent images of fused BeWo cells on substrates coated with different ECM materials such as Fibronectin, Gelatin, Laminin and an ECM mixture of Geltrex. Scale bar is 100 µm; red: E-cadherin, blue: nuclei; n=3 independent gels.

Supplementary References

1. Zougman A, Selby PJ, Banks RE. Suspension trapping (STrap) sample preparation method for bottom-up proteomics analysis. Proteomics. 2014 Jun;14(9):1006–1000.

2. Dickhut C, Radau S, Zahedi RP. Fast, efficient, and quality-controlled phosphopeptide enrichment from minute sample amounts using titanium dioxide. Methods Mol Biol. 2014;1156:417–30.

3. Paoletti AC, Parmely TJ, Tomomori-Sato C, Sato S, Zhu D, Conaway RC, et al. Quantitative proteomic analysis of distinct mammalian Mediator complexes using normalized spectral abundance factors. Proc Natl Acad Sci U S A. 2006 Dec 12;103(50):18928–33.