Supporting Information

Placental Trophoblast-Inspired Lipid Bilayers for Cell-Free Investigation of Molecular Interactions

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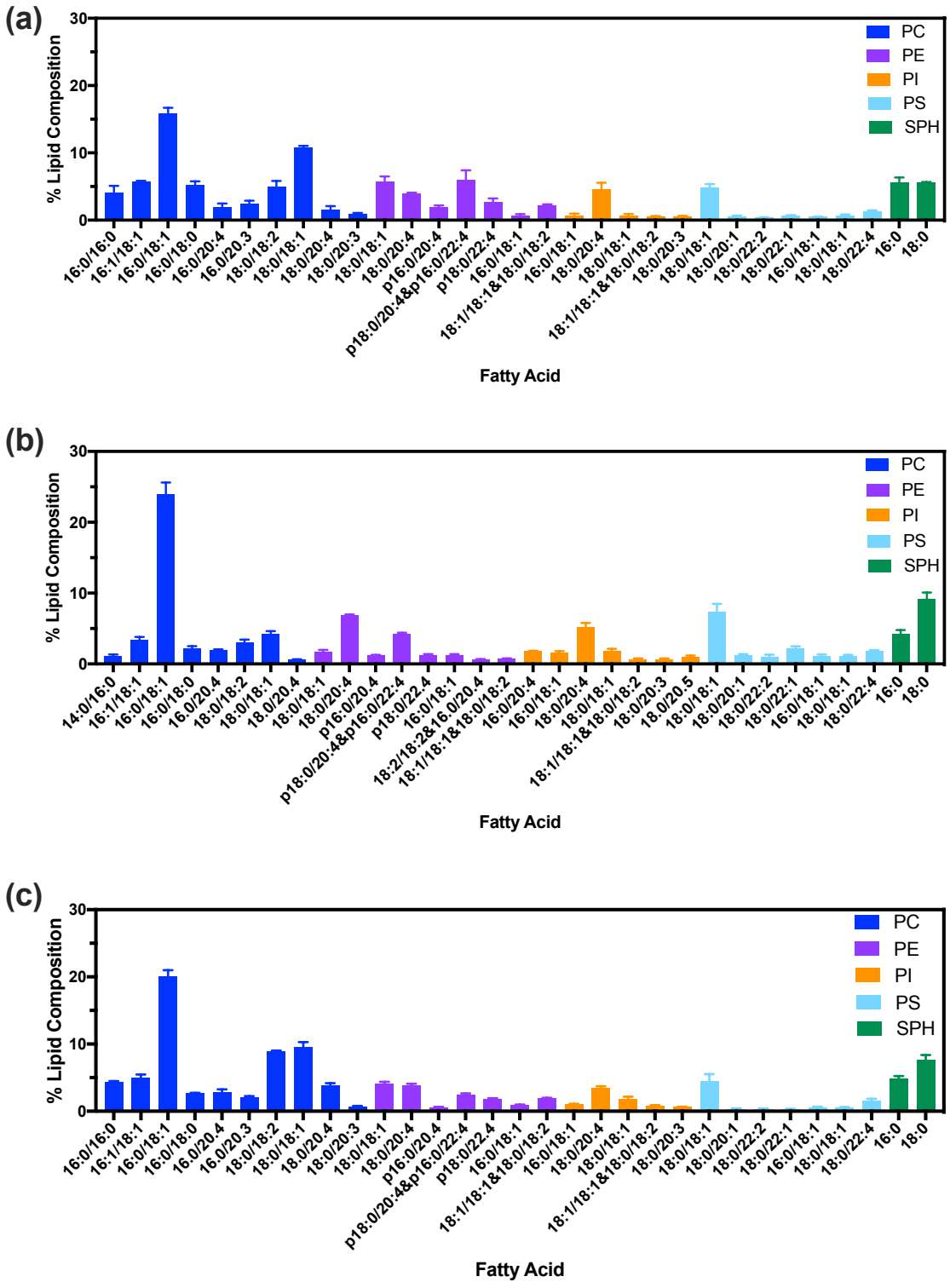

Figure S1. Lipid composition with all fatty acid distributions for (a) HTR-8, (b) TCL-1, and (c) primary trophoblast cells. Results are shown as mean ± standard deviation; n=3.

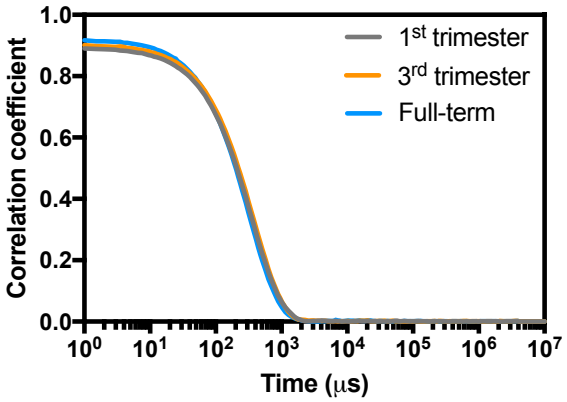

Figure S2. Representative dynamic light scattering correlation curves for 1st trimester, 3rd trimester, and full-term lipid vesicles.

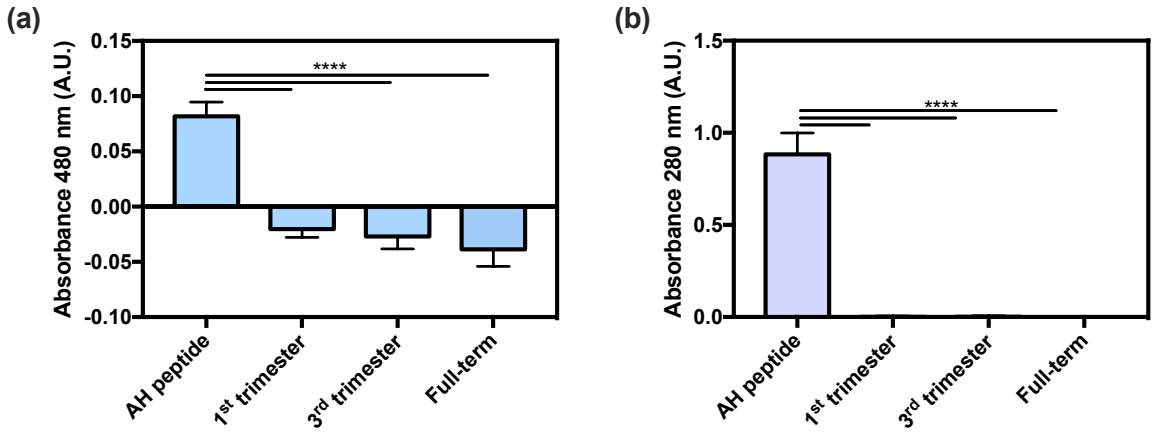

Figure S3. Confirmation of AH peptide removal after rupture of supported placental lipid bilayers. (a) Absorbance measurements for AH peptide using a pierce quantitative colorimetric peptide assay. The control AH peptide concentration was 13 μM, which is the experimental concentration. After bilayer formation, values were below the detection limit (1 μM). (b) Absorbance measurements after 1st trimester, 3rd trimester, and full-term bilayer formation, when QCM-D silica coated quartz crystals were rinsed with 2% (w/v) SDS to remove the adsorbed layer. The control

AH peptide concentration was 13 μM, which is the concentration used in bilayer rupture. After bilayer formation, absorbance values were below the detection limit (0.3 μM). Statistical analysis was performed using one-way ANOVA with Tukey's post hoc analysis; n=4; α=0.05; ****p<0.0001.

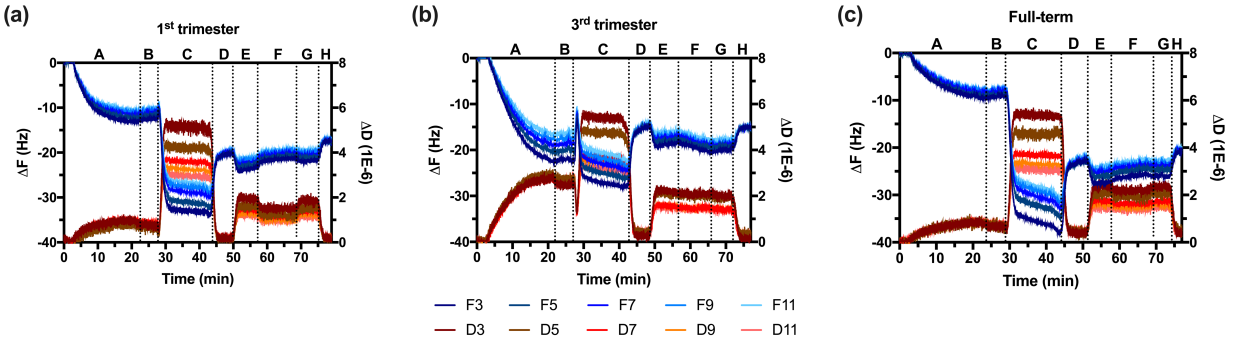

Figure S4. Amphotericin B interactions with (a) 1st trimester, (b) 3rd trimester, and (c) full-term supported placental lipid bilayers investigated via QCM-D. Frequency changes (*ΔF*) and dissipation changes (*ΔD)* are shown in blue and red, respectively for overtones 3, 5, 7, 9, and 11. Solution changes are indicated by letters indicating for A: Vesicles, B: Tris NaCl, C: AH peptide, D: Tris NaCl, E: 1% (v/v) DMSO, F: amphotericin B, G: 1% (v/v) DMSO, H: Tris NaCl. Representative QCM-D traces are shown for n ≥ 3.

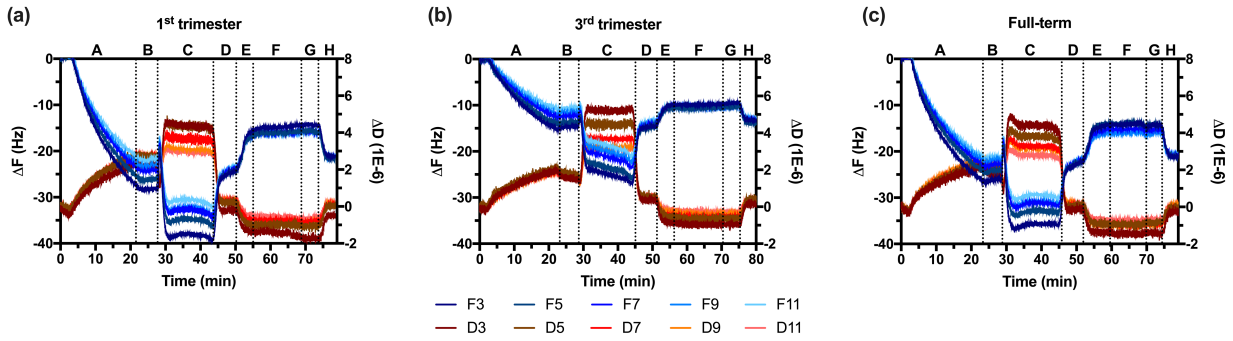

Figure S5. AmBisome interactions with (a) 1st trimester, (b) 3rd trimester, and (c) full-term supported placental lipid bilayers investigated via QCM-D. Frequency changes (ΔF) and dissipation changes (ΔD) are shown in blue and red, respectively for overtones 3, 5, 7, 9, and 11. Solution changes are indicated by letters indicating for A: 1st trimester vesicles, B: Tris NaCl, C: AH peptide, D: Tris NaCl, E: 67.8% (w/w) sucrose and 2% (w/w) succinic acid buffer, which is the clinically utilized suspension buffer, F: AmBisome in the suspension buffer, G: 67.8% (w/w) sucrose and 2% (w/w) succinic acid buffer, H: Tris NaCl. Representative QCM-D traces are shown for $n \geq 3$.

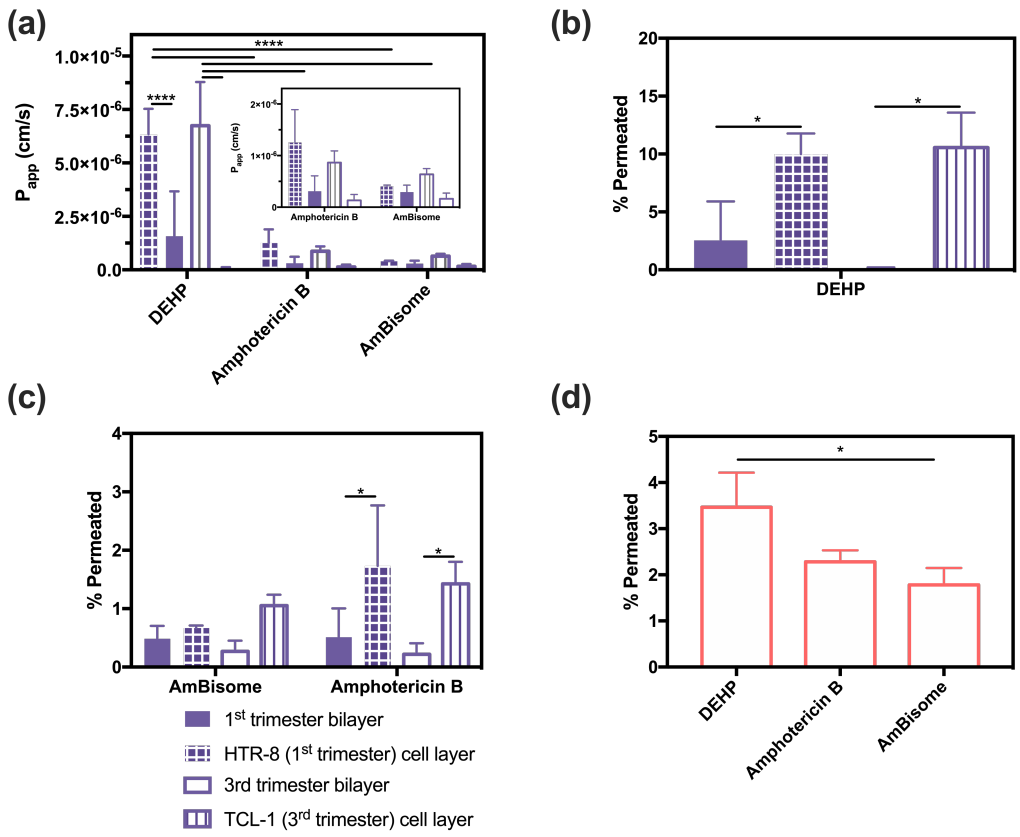

Figure S6. Examining transport across 1st and 3rd trimester suspended placental lipid bilayers and corresponding cell monolayers (HTR-8 and TCL-1, respectively). (a) Apparent permeability (P_{app}) of DEHP, amphotericin B, and AmBisome for placental lipid bilayer and cell monolayers. (b) Percent of 200 μM DEHP that transports across the placental lipid bilayer after 2 h at 25°C or cell monolayer after 2 h at 37 °C. (c) Percent of AmBisome and amphotericin B that transports across the placental lipid bilayer after 2 h at 25°C or cell monolayer after 2 h at 37 °C. (d) Percent of DEHP, amphotericin B, and AmBisome that transports across a placental collagen coated transwell plate. Statistical analysis was performed using one- or two-way ANOVA with Tukey's post hoc analysis; $n \geq 3$; $\alpha = 0.05$; *$p < 0.05$; ****$p < 0.0001$.