Science Advances

AAAS

advances.sciencemag.org/cgi/content/full/2/3/e1501462/DC1

Supplementary Materials for

A three-dimensional culture system recapitulates placental syncytiotrophoblast development and microbial resistance

Cameron A. McConkey, Elizabeth Delorme-Axford, Cheryl A. Nickerson, Kwang Sik Kim, Yoel Sadovsky, Jon P. Boyle, Carolyn B. Coyne

Published 4 March 2016, *Sci. Adv.* **2**, e1501462 (2016)
DOI: 10.1126/sciadv.1501462

This PDF file includes:

Other Supplementary Material for this manuscript includes the following:
(available at advances.sciencemag.org/cgi/content/full/2/3/e1501462/DC1)

Data set S1 (Microsoft Excel format). Spreadsheet from RNASeq studies of 2D and 3D cultures of JEG-3 cells, PHT cells, and 3D cultures of HBMECs. Shown

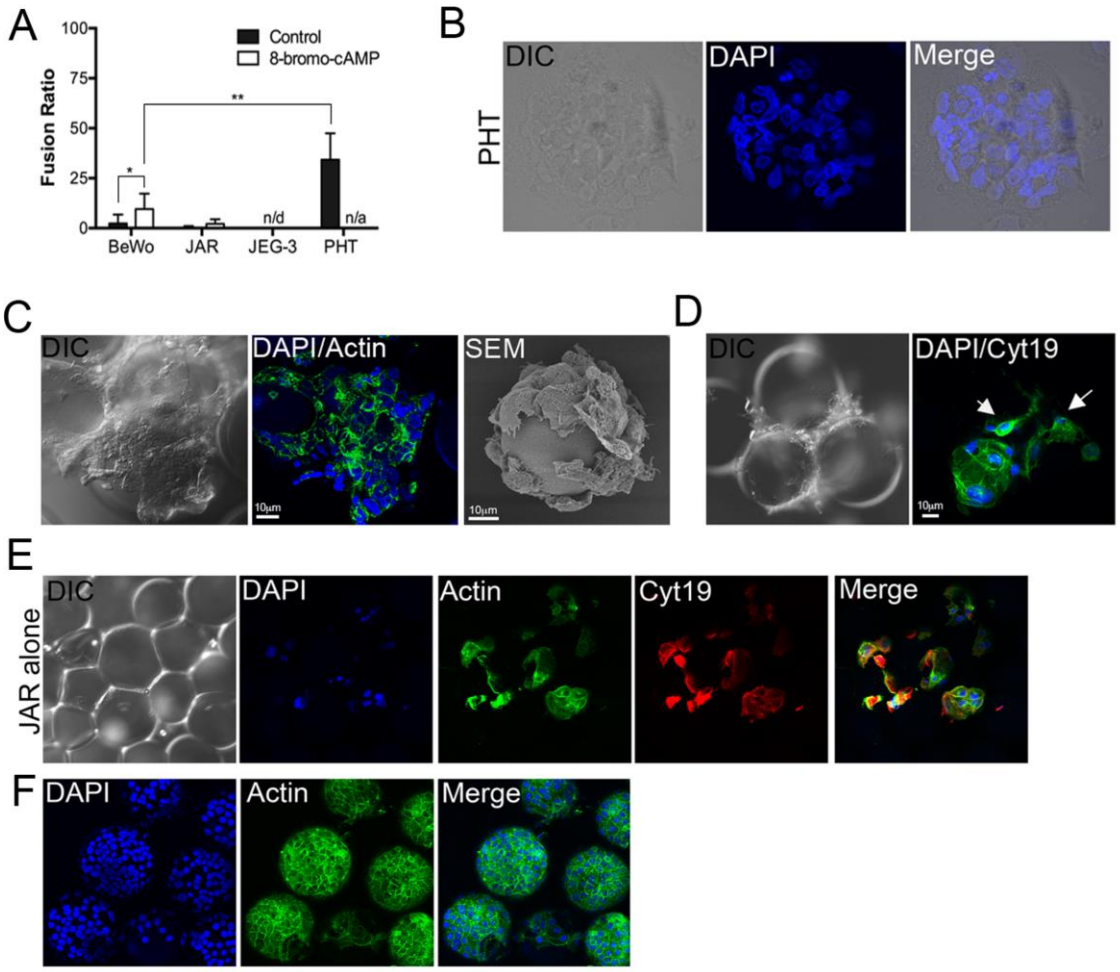
are gene symbols, normalized expression values, and RPKM values from each condition.

Data set S2 (Microsoft Excel format). Spreadsheet from differential expression analyses using DESeq2 of 2D and 3D cultures of JEG-3 cells.

Data set S3 (Microsoft Excel format). Spreadsheet from differential expression analyses using DESeq2 of 2D and 3D cultures of HBMECs.

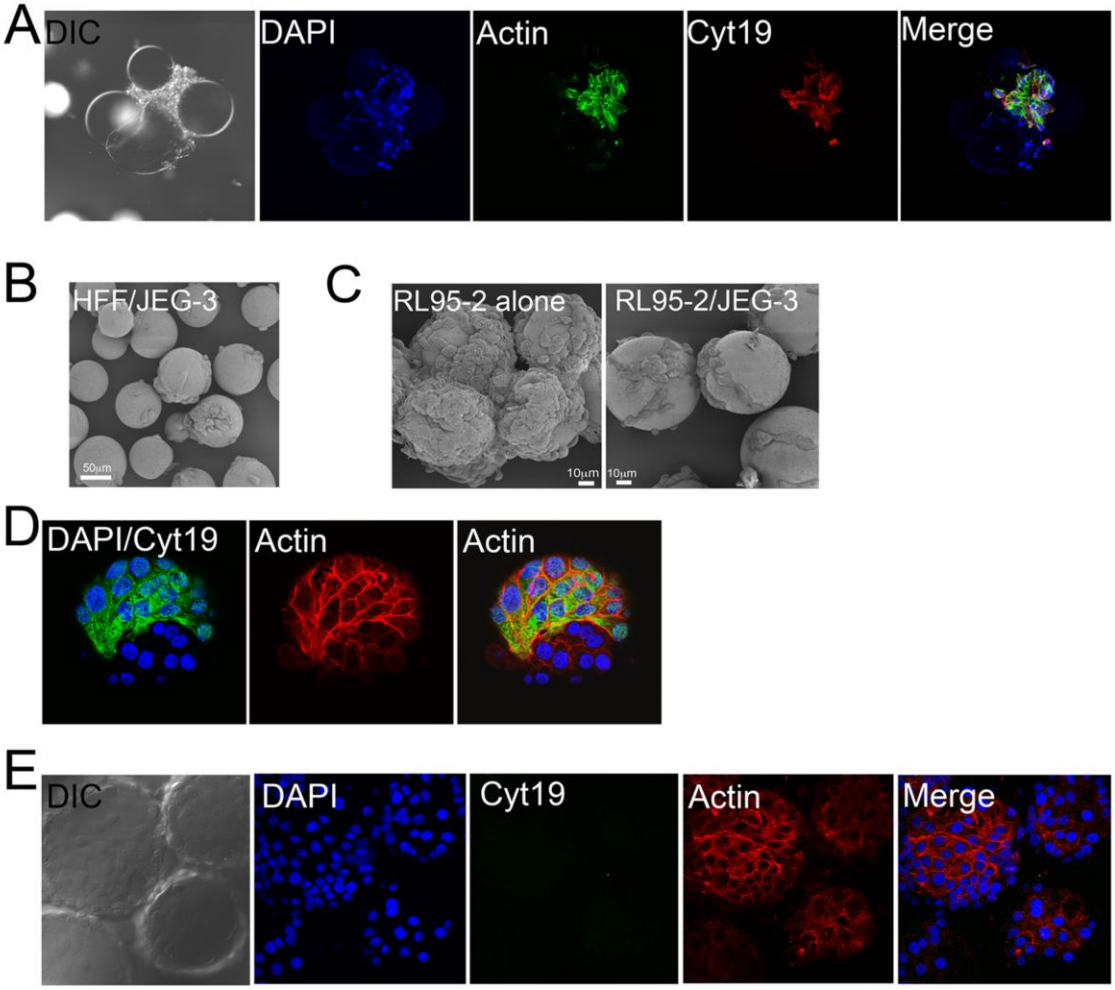
Data set S4 (Microsoft Excel format). Spreadsheet from differential expression analyses using DESeq2 of 2D cultures of JEG-3 cells and PHT cells.

Supplemental Figure 1

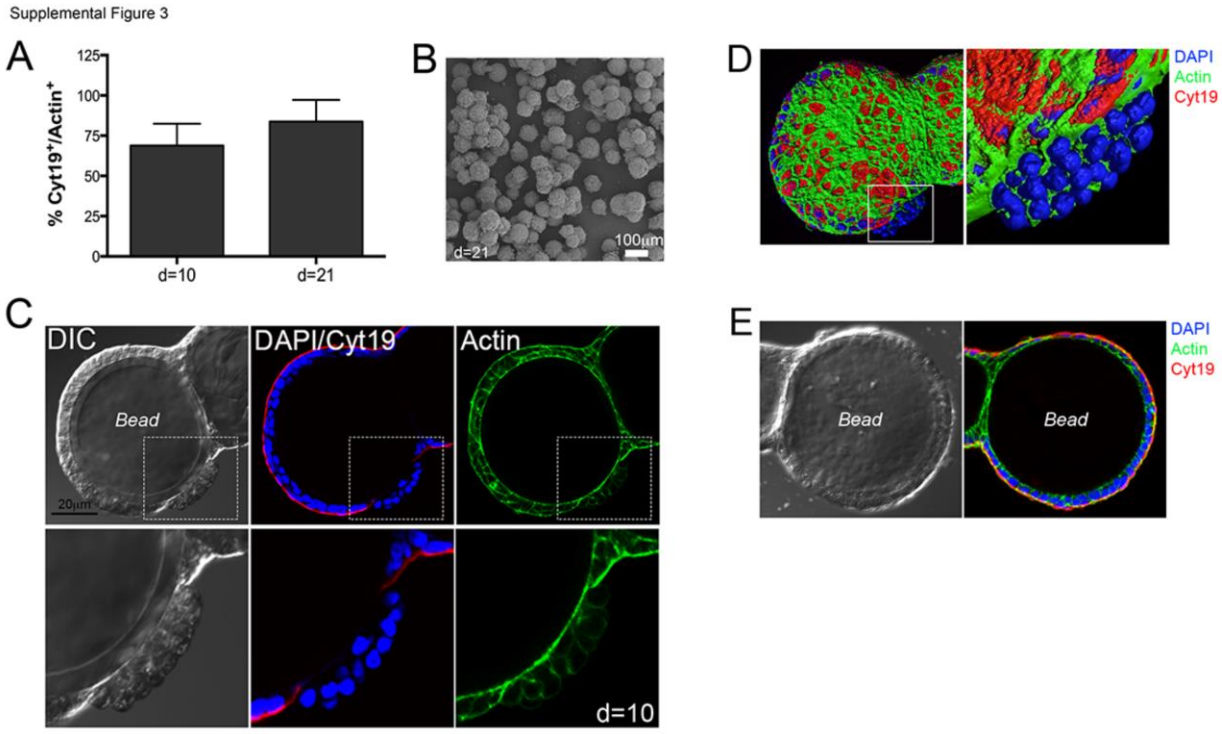


Supplemental Figure 1. (**A**), Fusion ratio of the indicated trophoblast cell lines (BeWo, JAR, or JEG-3) or PHT cells under mock-treated (control) conditions or when treated with 8-bromo-cAMP (for 48hrs). Not applicable (n/a) or not detected (n/d). Data are shown as mean ± standard deviation and are based upon analyses of >250 total nuclei from three independent experiments or PHT preparations. **p<0.01, *p<0.05. (**B**) Confocal microscopy of PHTs isolated from normal term deliveries and grown in culture. Cell nuclei (DAPI; blue) and differential interference contrast (DIC) images demonstrate a differentiated, multinucleated syncytiotrophoblast. (**C**), At left, differential interference contrast (DIC, far left panel) and actin localization (green, second from left) of JEG-3 cells cultured in the RWV bioreactor for 21 days. DAPI-stained nuclei are shown in blue. (**D**). Differential interference contrast (DIC, left panel) and actin localization of (green, right panel) of BeWo cells cultured in the RWV bioreactor for 21 days. DAPI-stained nuclei are shown in blue. (**E**) Confocal microscopy for actin (green) and cytokeratin-19 (red) in JAR cells cultured in 3-D for 21 days. DAPI-stained nuclei are shown in blue. Differential interference contrast (DIC). (**F**), Confocal microscopy for actin (green) in JEG-3 cells cultured on Cytodex beads under static 2-D conditions for 7 days. DAPI-stained nuclei are shown in blue.

Supplemental Figure 2

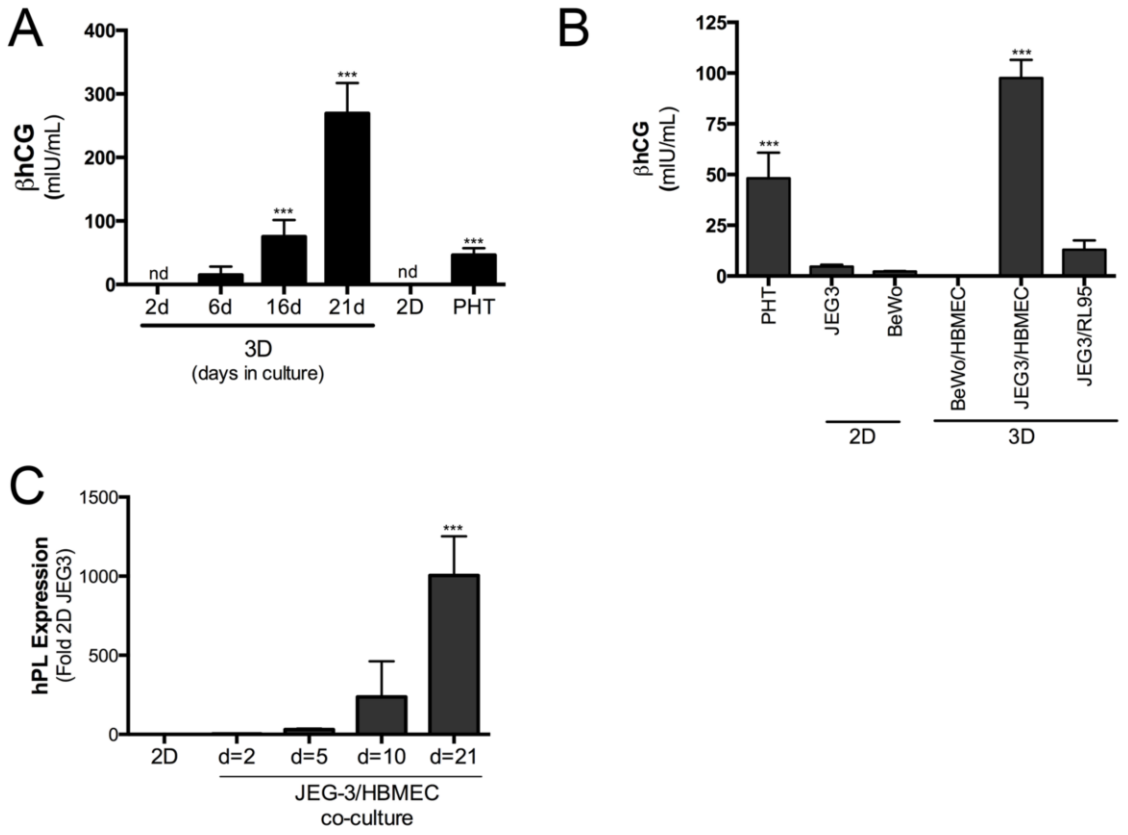


Supplemental Figure 2. (**A**), Confocal microscopy for actin (green) and cytokeratin-19 (red) in BeWo cells cocultured with HBMEC in 3-D for 21 days. DAPI-stained nuclei are shown in blue. Differential interference contrast (DIC). (**B**), Scanning electron micrographs of HFF and JEG-3 cells co-culured in 3-D for 21 days. (**C**), Scanning electron micrographs of RL95-2 cells cultured in 3-D for 21 days alone (left panel) or RL95-2 cells cocultured with JEG-3 cells (right panel). (**D**), Confocal microscopy for cytokeratin-19 (green) and actin (red) in JEG-3 cells cocultured with HBMEC in 3-D for 10 days. DAPI-stained nuclei are shown in blue. (**E**), Confocal microscopy for cytokeratin-19 (green) and actin (red) in Htr8/SVneo cells cocultured with HBMEC in 3-D for 21 days. DAPI-stained nuclei are shown in blue. Differential interference contrast (DIC).



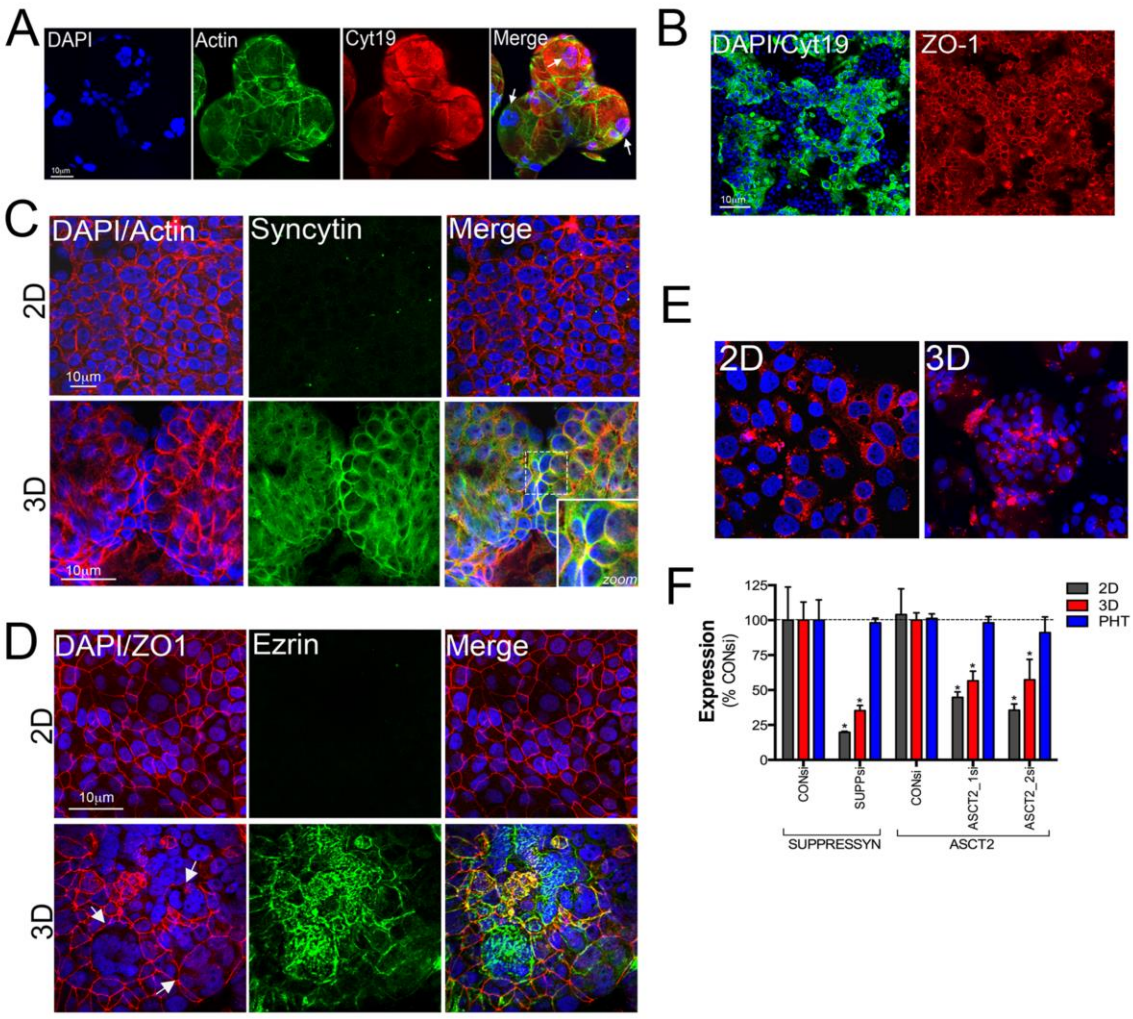
Supplemental Figure 3. (**A**), Image analysis of the percent cytokeratin-19-posiitve cells relative to total actin positive cells at the indicated time of JEG-3/HBMEC co-culture in 3-D. In both cases, >700 total nuclei were used for quantification. Data are shown as mean ± standard deviation. (**B**), Scanning electron micrographs of HBMEC cultured alone 3-D for 21 days. (**C**), Top, confocal microscopy cross-section of cytokeratin-19 (red) and actin (green) in JEG-3/HBMEC co-cultured Cytodex beads cultured for 10 days. At bottom, zoomed region of hatched white box shown in top row. DAPI-stained nuclei are shown in blue. (**D**), Three-dimensional image reconstruction of bead shown in panel (C) demonstrating the extrusion of HBMEC (cytokeratin-19 negative) from the bead surface. At right, zoomed region shown in white box at left. (**E**), Confocal microscopy cross-section of cytokeratin-19 (red) and actin (green) in JEG-3/HBMEC co-cultured Cytodex beads cultured for 21 days. DAPI-stained nuclei are shown in blue. Note the single cell layer coating the bead surface. In (C) and (E), the bead is labeled with white text.

Supplemental Figure 4

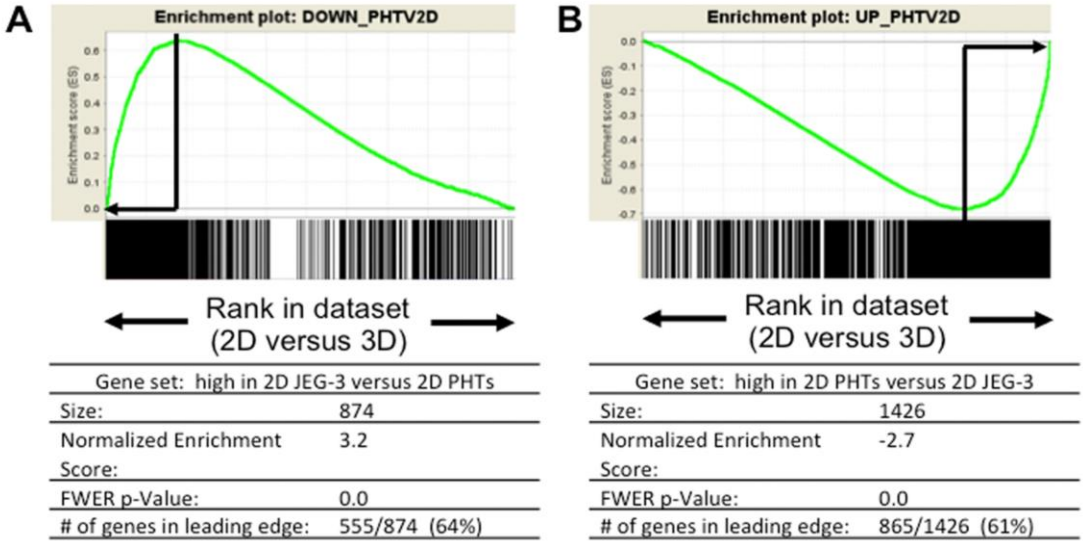


Supplemental Figure 4. (**A**), ELISAs for βhCG from supernatants of JEG-3 cells cultured in two-dimensions (2-D for 72-96hrs) or three-dimensions (3-D, for 21 days) at the indicated days post co-cultured with HBMEC, or from PHT cells (blue, cultured 48-72hrs). In all cases, media was replaced ~24hrs prior to ELISA. (**B**), ELISAs for βhCG from supernatants of JEG-3 or BeWo cells cultured in 2-D or 3-D cocultured with wither HBMEC or RL95-2 cells, or from PHT cells. In all cases, media was replaced ~24hrs prior to ELISA. (**C**), Expression of human placental lactogen (hPL) as assessed by RT-qPCR in JEG-3 cells cultured in 2-D, or in JEG-3 cells cultured in 3-D for the indicated days post co-culture. In all, data are shown as mean ± standard deviation, ***p<0.001.

Supplemental Figure 5



Supplemental Figure 5. (**A**), Confocal microscopy for actin (green) and cytokeratin-19 (red) in 3-D cultures of JEG-3 cells after 21 days. DAPI-stained nuclei are shown in blue. White arrows denote large syncytia. (**B**)**,** Confocal microscopy for ZO-1 (red) and cytokeratin-19 (green) in JEG-3 and HBMEC co-cultured in 2-D for 10 days. DAPI-stained nuclei are shown in blue. (**C**), Confocal microscopy for actin (red) and syncytin (green) in JEG-3 cells cultured in 2-D (top row) or 3-D (bottom row). DAPI-stained nuclei are shown in blue. (**D**), Confocal microscopy for ZO-1 (red) and ezrin (green) in JEG-3 cells cultured in 2-D (top row) or 3-D (bottom row). DAPI-stained nuclei are shown in blue. White arrows denote syncytia. (**E**), Cy3-conjugated scrambled siRNA (red) ~48hrs following transfection in 2-D (left) or 3-D (right) cultures of JEG-3 cells. DAPI-stained nuclei are shown in blue. (**F**), RT-qPCR for suppressyn or ASCT2 in 2-D or 3-D JEG-3 cultures or PHT cells transfected with scrambled control siRNAs (CONsi) or with siRNAs against suppressyn (SUPPsi) or ASCT2 (ASCT2si). Data are shown as mean ± standard deviation, *$p<0.05$.



Gene set: high in 2D JEG-3 versus 2D PHTs	
Size:	874
Normalized Enrichment Score:	3.2
FWER p-Value:	0.0
# of genes in leading edge:	555/874 (64%)

Gene set: high in 2D PHTs versus 2D JEG-3	
Size:	1426
Normalized Enrichment Score:	-2.7
FWER p-Value:	0.0
# of genes in leading edge:	865/1426 (61%)

Supplemental Figure 6. GSEA enrichment plots and corresponding statistical output for (**A**) a gene set containing all transcripts that were of higher abundance in 2D JEG-3 compared to PHTs ("2D JEG3 UP") and (**B**) a gene set containing all transcripts that were of higher abundance in PHTs compared to 2D JEG-3 ("PHT enriched"). Both gene sets were significantly (FWER=0.0) enriched in transcripts that differed between 2-D and 3-D JEG-3, providing further evidence for the high similarity between 3-D JEG-3 cultures and PHT cells.

Feature ID	2D-1 Normalized expression values	2D-1Unique gene reads	2D-1-Total gene reads	2D-1 RPKM	2D-2 Normalized expression values	2D-2 Unique gene reads	2D-2 Total gene reads	2D-2- RPKM	PHT Normalized expression values	PHT Total gene reads	PHT - Unique gene reads	PHT - RPKM
FBN1	0.1	60	60	0.08906576	0.1	69	69	0.09320361	32.4	16751	16751	29.58801111
KIAA0040	0	9	9	0.046941608	0.1	14	14	0.075389298	47.3	7307	7307	43.25741741
CYP27A1	0	24	24	0.047002587	0.1	55	55	0.12077957	48.4	4668	4668	44.21892307
CD14	0.1	6	6	0.089210212	0.1	8	8	0.117557862	24.1	1443	1443	22.03852404
RARRES3	0.2	11	11	0.151110241	0.1	4	4	0.064005233	26.7	1136	1136	24.38110599
ERVMER34-1	0.2	29	29	0.158194774	0	24	24	0.040786263	25.6	3497	3497	23.43202543
SEMA3B	0.1	12	12	0.058349388	0	11	11	0.048056643	41.4	7719	7719	37.8222129
ITGB6	0	38	38	0.035339311	0	66	66	0.04802409	25.9	5827	5827	23.70522774
EMP1	0	6	6	0.036004806	0	6	6	0.035584335	19.4	2882	2882	17.68434825
SPP1	0.1	6	6	0.07353604	0	1	1	0.012112878	133.1	9660	9660	121.6554324
HTRA1	0	4	4	0.013227237	0.1	15	15	0.078436602	209.7	14554	14554	191.6514178
CDKN1C	0.1	10	10	0.108436749	0.1	14	14	0.150038563	1273.1	104444	104444	1163.443098
S100A9	0.1	3	3	0.145797625	0.1	3	3	0.14409497	343	6400	6400	313.4382118

Supplemental Table 1. Thirteen “core” genes identified using GSEA gene clustering as being up-regulated in both 3D JEG-3 and PHT cells, while being of low abundance in both 2D JEG-3 cells and 3D HBMECs. These data were used as a template for Pavlidis template matching as described in the text. Shown are gene symbols, normalized expression values, and RPKM values from each condition.

Feature ID	2D1	2D2	3D1	3D2	PHT	3DHBMEC
CDKN1C	-4.6153584	-4.1468787	1.5144567	0.2626656	8.773901	-1.7887869
PSG1	-3.921119	-3.9380667	-0.22617082	0.7427797	9.460972	-2.1183946
S100A9	-3.287176	-3.3041232	1.1652021	2.2976515	7.7828217	-4.6543765
PSG5	-1.4406893	-3.0425992	1.4121387	-0.6632169	8.027683	-4.293317
COL3A1	-3.8660176	-3.298002	-0.45486197	2.908913	4.709969	NaN
CST6	-3.3526158	-3.1996381	0.92729485	1.7335161	3.8914433	NaN
PGLYRP4	-3.6192062	-2.051191	0.07574192	2.4452305	3.149425	NaN
TLR3	-3.4982266	-1.7782086	0.5725885	0.5515633	4.847786	-0.69550234
INHA	-3.215372	-2.8697493	0.7837297	1.2734401	5.584989	-1.5570376
MX2	-3.1090999	-3.5410843	0.50641423	1.3866765	6.0634685	-1.306375
DPEP2	-2.7727022	NaN	0.2848162	-0.7526594	5.7954855	-2.5549402
MEST	-2.004034	-2.4360187	0.29859698	0.73482716	6.4559345	-3.0493062
GLI3	-1.6825411	-1.6994882	0.10452571	1.0758541	5.04494	-2.8432906
PDE10A	-1.883922	-1.2227973	-0.121859215	1.3585134	4.8581533	-2.9880881
C1orf132	-1.943281	-2.6606684	0.4137977	0.5260693	4.6306095	-0.96652734
PDCD1LG2	-2.528641	-2.8086226	0.79635787	0.15436682	4.386539	NaN
SLC15A3	-2.6784124	-1.7161185	0.8078183	0.46973506	5.1219487	-2.004971
COL1A2	-2.2726433	-1.8745531	0.029987501	1.1624368	5.0096536	-2.0548813
TRIM22	-2.2016459	-2.2185931	0.40841398	1.9394126	4.056296	-1.9838837
ZNF703	-1.7419285	-2.5280955	-0.12697016	1.1411628	4.4938407	-1.2380093
SCNN1A	-1.91741	-1.9343573	-0.029816518	0.8681675	4.034992	-1.021576
SRGAP3	-2.2366235	-1.4055741	-0.085995875	0.9850531	3.4989674	-0.7558271
BST2	-2.1571171	-1.3260676	-0.1719128	1.0392485	3.806743	-1.1908938
IL2RG	-2.2775428	-1.4464931	-0.011437804	1.3863561	3.4828992	-1.1337814
KCNH7	-1.8846995	NaN	-0.4121433	0.13534348	3.8284366	-1.6669375
IRF8	-2.0342352	-1.3593049	-0.3235194	-0.07077594	3.7878354	NaN
VWA5A	-1.9947506	-1.3137263	0.14918274	0.11086799	4.2087436	-1.1603171
PCDH1	-2.3226817	-1.2267348	0.087014385	0.16994983	4.3587503	-1.0662982
GPR115	-1.2335644	-1.3451484	1.3702362	1.0235177	4.1780415	-3.9930823
NUPR1	-0.8869548	-1.6899252	0.78675747	1.0436988	3.9485207	-3.202097
PDZD2	-1.2410015	-0.9718706	1.7307273	-0.13682331	3.4032292	-2.784261
TMEM40	-1.2311027	-1.4392341	1.0187767	0.23622842	2.5625994	-1.1472675

NAV2-AS2	-1.5380079	-1.9699925	0.2564761	0.2893898	2.9621346	NaN
TCHH	-1.3179518	-1.7291778	0.80096734	0.048893902	3.3615885	-1.1643201
C1orf95	-1.9121823	-1.514092	0.7827664	0.107860625	3.230067	-0.6944198
RND3	-1.6834618	-1.4828176	0.25966632	0.6883935	2.5327551	-0.31453562
DDX60L	-1.6531256	-1.2550355	0.24833995	0.721917	2.489326	-0.5514218
BTBD19	-1.5392642	-1.1036992	0.77282697	-0.1821865	2.5342898	-0.48196685
LINC00456	-1.5052557	-0.9372404	0.082777515	-0.31528786	2.6750064	NaN
C1QTNF6	-1.0499498	-1.3331771	0.08300948	0.033518434	3.1315765	-0.8649776
OPHN1	-0.7527505	-1.5399107	0.43901783	-0.003025845	2.431114	-0.5744447
KIAA0513	-1.2466872	-1.7703719	0.65982014	0.4432071	2.113411	-0.19937916
CORO6	-0.8971628	-1.6186543	0.861916	0.36388823	1.7009248	-0.41091195
SLC52A3	-0.92498904	-1.2003394	1.2112641	0.40112284	1.3924148	-0.8794734
AHR	-1.2073641	-0.6651328	0.3812052	0.8126788	1.3901583	-0.7115454
PTPN3	-0.9925945	-0.8897841	0.34897923	0.39887762	1.2850306	-0.15050888
MMAA	-0.77313805	-0.87581515	0.344753	-0.07849553	1.8571519	-0.4744561
LGALS8	-0.6396121	-0.8789517	0.035941597	0.06769704	1.7769657	-0.36204058
ZNF91	-0.8050284	-0.74200726	0.02732651	0.044622228	1.7782933	-0.30320638
TAB3	-0.4902263	-0.6079214	-0.06731907	0.05275734	1.5286711	-0.41596165
CALCOCO1	-0.59683055	-0.5576529	-0.1868159	0.29094562	1.6267303	-0.5763766
TMBIM1	-1.0127681	-0.8527115	-0.21817477	0.563013	1.9514407	-0.43079934
INSR	-0.43935192	-0.8641373	-0.08222854	0.5734552	2.1957803	-1.3835176
CTA-384D8.31	0.3444299	NaN	-1.2530932	-2.1206439	3.0293074	NaN
CARD16	1.9207737	-3.0077684	-0.10322784	0.3511496	2.0271697	-1.1880968

Supplemental Table 2. Spreadsheet of gene expression profiles from RNASeq in 2D and 3D cultures of JEG-3 cells, PHT cells, and 3D cultures of HBMECs. These genes were identified using GSEA followed by Pavlidis Template matching (PTM), with the genes from Table S5 as a template (PTM *P*-value <0.01). Data are shown as log2 transformed RPKM values.

Supplemental Dataset 1. Spreadsheet from RNASeq studies of 2D and 3D cultures of JEG-3 cells, PHT cells, and 3D cultures of HBMECs. Shown are gene symbols, normalized expression values, and RPKM values from each condition.

Supplemental Dataset 2. Spreadsheet from differential expression analyses using DESeq2 of 2D and 3D cultures of JEG-3 cells. Shown are gene symbols from each condition as well as log2 fold changes, lfcSE, and p-values. These expression differences were prior to the removal of HBMEC-enriched genes by GSEA.

Supplemental Dataset 3. Spreadsheet from differential expression analyses using DESeq2 of 2D and 3D cultures of HBMECs. Shown are gene symbols from each condition as well as log2 fold changes, lfcSE, and p-values

Supplemental Dataset 4. Spreadsheet from differential expression analyses using DESeq2 of 2D cultures of JEG-3 cells and PHT cells. Shown are gene symbols, log2 fold changes, lfcSE, and p-values. These genes represent the 'PHT enriched library' used in subsequent GSEA studies.