SI Appendix:

Early onset preeclampsia in a model for human placental trophoblast

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Running title: Trophoblast model for early onset preeclampsia

SI Materials and Methods

Primary tissue and iPSC generation. The approach used to provide primary cultures of mesenchymal cells from umbilical cords has been described elsewhere (1). Briefly the tissues were washed to remove blood cells and minced into fragments to deliver adherent cells by the explant method (2). Outgrowths appeared at the periphery of the minced tissues after ~one week of culture. After 10 - 11 days, the fibroblasts were passaged from the 48-well plate into T25 flasks by using TrypLE™ (Invitrogen). The cells reached confluence in the flask by ~14 days and were expanded for reprogramming to iPSC. All cultures used to provide stocks of primary cells and iPSC were performed in a tri-gas incubator set to the following conditions (5 % O_2/ 5 % CO_2/80 % N_2).

Generation of iPSCs from umbilical cord fibroblasts with episomal vectors has also been described elsewhere (3). In brief, a protocol developed by Okita et al (4) with episomal vectors carrying shRNA for p53 suppression and non-transforming L-MYC, in addition to the usual reprogramming genes *POU5F1, SOX2, KLF4* and *LIN-28*, was employed to reprogram the fibroblasts. Three micrograms of the developed Y4 combination (*POU5F1, SOX2, KLF4, LIN28, L-MYC* and *p53 shRNA*) of the episomal plasmids was electroporated with a Nucleofector II device (Lonza, Basel, Switzerland) and Amaxa NHDF Nucleofector kit (Lonza) into the CTL and EOPE fibroblast cells collected from umbilical cords according to the manufacturer's instructions. After 2-4 days of recovery, cells were placed into 100 mm dishes previously coated with Matrigel (BD Bioscience, San Jose, CA). The following day the culture medium was switched to mTeSR1 (StemCell Technologies, Vancouver, Canada). Colonies resembling human ESC were mechanically isolated (around day 20 of culture) and expanded. A total of 10 CTL iPSC and 19 EOPE iPSC lines were established.

RNA extraction and processing. RNA was extracted from each of the primary fibroblast cultures and the respectively generated iPSC lines (cultured under both O_2 concentrations: 4 and 20 %) in STAT60 (Tel-

Test) and further purified according to the manufacturer's instructions. RNA samples were submitted to the University of Texas Southwestern Medical Center Microarray Core Facility (https://microarray.swmed.edu/), and microarray analysis was performed with Illumina HumanHT-12 v4 expression BeadChips. This analysis used the entire 47,159 probes on the BeadChip. Raw intensity data were background-subtracted by using BeadStudio software to generate transcriptomes for each of the cell lines (GeneSpring 12.6 software; Agilent Technologies, Inc.). A raw intensity was then assigned to each gene by taking the average intensity of all probes corresponding to a gene. The raw intensities were normalized by using the quantile normalization method. Principal component analysis was then applied to the top quartile of protein coding genes (4,186 genes) based on the variance across samples in order to provide an unsupervised clustering and visualization approach to the data. Transcriptome data from the gene expression arrays were also used to evaluate pluripotency of the hESC and iPSC lines by using the Pluritest (5) (https://pluritest.org/).

Cell culture and differentiation. Human ESC (H1/WA01 and H9/ WA09) and iPSC were cultured in six-well tissue culture plates (Thermo Scientific) coated with Matrigel (BD Bioscience) under an atmosphere of 5 % CO_2/air at 37°C in mTeSR1 medium (STEMCELL Technologies). Cells were passaged every 5–6 days. Briefly, colonies were exposed to dispase (1 mg/mL) (STEMCELL Technologies) for 5-7 min at 37 °C and then were broken into small clumps with the Stempro EZpassage (ThermoFisher) cutting tool. The method for TB differentiation has been described elsewhere with slight modifications (6). Briefly, cells were passaged onto Matrigel coated dishes into DME/F12 medium (Thermo Scientific) with knock-out serum replacement (KOSR, Invitrogen) that had been conditioned by mouse embryonic fibroblasts for 24 h (MEF-CM) and supplemented with FGF2 (4 ng/ml). After 24 h, the conditioned medium was replaced with daily changes of non-conditioned DME/F12/KOSR medium lacking FGF2 (hESC medium), but containing BMP4 (10 ng/ml), A83-01 (1 μM) and PD173074 (0.1 μM) (BAP treatment) for up to 6 days. These medium changes exposed the culture wells to ambient O_2 conditions for a maximum of 5-6 min to

minimize re-oxygenation of the low O_2 cultures. CTL cultures (undifferentiated) were maintained in MEF-CM containing 4 ng/ml FGF2. After 6 days of BAP treatment, spent medium was collected for immunoassays, total DNA was isolated to determine cell density by using the Wizard® Genomic DNA purification kit (Promega, A2360), and total RNA isolated (STAT60, Tel-Test Inc.) for RNA sequencing.

Measurement of dissolved oxygen in culture media. All measurements were conducted in Columbia, MO which is at an altitude of 274 meters (= 898 ft.). Two 37° C incubators, one a standard CO_2 incubator (NuAire) set to provide 5% CO_2 in air, and a mixed gas O_2/CO_2 incubator (HERAcell150) set to provide 4 % O_2 were used for the dissolved oxygen measurements. Actual CO_2 and O_2 levels of the incubators were monitored with a Bacharach CO_2/O_2 Analyzer (Bacharach, Model 2830, New Kensington, PA), which was calibrated according to the manufacturer's instructions to 20.9% atmospheric oxygen before each measurement. The measured O_2 concentrations were ~19% in the standard incubator and varied between 3.5–4.5% in the low O_2 incubators. The dissolved O_2 concentrations in media were assessed by using an oxygen polarographic electrode, Pinpoint II O_2 Meter (Aquatic Eco-Systems, Inc, Apopka, FL), which provides units in mg of gas per liter of water, i.e. equivalent to parts per million = ppm. The device offsets the effects of temperature from 0 to 50°C (32-122°F) with an accuracy of ± 0.2 ppm. The experiments were carried out by placing 15 ml of medium in either a T25 vented-lid culture flask or by using 15 ml distributed equally among the wells of a 6-well plate (2.5 ml medium/well). At the end of the test equilibration period, the medium (15 ml) was collected into a 50 ml tube and the electrode inserted to a one-inch depth. The medium was gently agitated during the measurements. Values in some experiments were validated by placing the media samples in gas-tight syringes and measuring the partial pressure of O_2 with an IRMA SL Blood Analysis System. Both methods provided comparable values. Also, values obtained from the 6-well and T25 culture dishes were not significantly different.

Perfusion/reperfusion rates for dissolved O_2. When culture dishes or invasion chambers are taken out of the low O_2 incubator for medium changes or other manipulations, there is reperfusion of atmospheric O_2, with resulting fluctuations in culture conditions. The dissolved O_2 concentration of the culture medium held in 91 % N_2/5 % CO_2/ 4 % O_2 was ~3.5 ppm at equilibrium, while it was ~7.8 ppm in air/5 % CO_2 (SI Appendix, Fig. S1A). This translates to partial O_2 pressures of O_2 of 58 mm Hg and 159 mm Hg, respectively. It required ~3h to reach equilibration when the medium was transferred from air/5 % CO_2 to the low O_2 conditions, with an ET50 (exposure time for 50 % equilibration) of ~40 min (SI Appendix, Fig. S1A). Conversely, medium equilibrated under the low (4 %) O_2 condition required ~100 min to reach equilibrium when transferred to 20 % O_2, with an ET50 of 28 min (SI Appendix, Fig. S1B). Thus, partial re-oxygenation of low O_2 cultures cannot be avoided but can be minimized by controlling the exposure time outside incubators and always using medium pre-equilibrated to the designated O_2 condition. For example, "refeeding" colonies can be performed in around 5 min, although passaging can take up to 20 min.

Invasion assay protocol:

A. Preparation of the invasion chambers:

1. BD BioCoat Matrigel Invasion Chambers from BD Biosciences are filled (2 ml bottom chamber, 2 ml top chamber) with pre-equilibrated culture medium (DMEM/F-12) for 2 h in incubators calibrated to provide the desired gas atmosphere (air/5 % CO_2 or 5 % O_2/5 % CO_2/90 % N_2).

2. After 2 h, aspirate the DMEM/F-12 and fill the wells with pre-equilibrated MEF-CM+ FGF2 (4 ng/ml)

B. Cell culture preparation

1. The iPSC cultures were routinely cultured on mTeSR medium as previously described (6).
2. Prior to conducting invasion assays, the selected cell lines were maintained for at least two passages in respective oxygen condition (air/5 % CO_2 and in 5 % O_2/5 % CO_2/90 % N_2) on mTeSR1.

3. Prior to transfer to invasion chambers, the attached 6-day undifferentiated ESC/iPSC colonies cultured in 6-well culture dishes are rinsed twice in DMEM/F-12, Dispase solution (1 mg/ml; I ml) added and incubated with the colonies for ~7 min at 37 ° C. Incubation times vary for each cell line but are typically shorter for colonies cultured in 5% versus 20% O_2. For example, 5-6 min for 5% O_2 cultures is usually long enough before colonies begin to peel off the substratum.
4. Dispase was removed and colonies gently rinsed twice in DMEM/F-12, before addition of 1 ml hESC medium conditioned by irradiated mouse embryonic fibroblasts (iMEF-CM) containing FGF2. Colonies were cut by using the Stempro EZpassage cutting tool.
5. Cells were collected by using a glass pipette, removing only the equally sized squares cut by the tool. A small aliquot (50 µl) was taken from the dissociated squares and placed in another tube before pipetting multiple times with the 200 µl tip on the pipet to break up the squares into single cells. The number of dispersed cells was counted with an automated cell counter (TC20, BioRad).
6. The suspension was shaken thoroughly. An aliquot of 5 x 10^4 cells (approx. 500 squares) was immediately removed and added to the upper compartment of each invasion chamber. There were six cultures (3 CTL and 3 BAP exposed) for each cell line. The suspension was shaken each time before the addition of further aliquots into the top chambers of additional wells. Then the plate was agitated several times from side to side (not by swirling) as it was placed in the incubator, to distribute the cell clumps evenly across the Matrigel-coated membrane. This step is particularly important to obtain reliable outcomes.
7. After 24 h from initial plating, the medium was changed (2 ml in each chamber) to the desired treatments (iMEF-CM+FGF2 for undifferentiated CTL cultures, BAP for differentiation).
8. The medium was then changed daily for an additional 5 days. On the sixth day (7 days after initial plating, see the table below), the cells were gently washed (2 X) by placing PBS in both

Treatment	**Day -1** (day of plating)	**Day 0** (initiate differentiation)	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**
Control	CM+FGF2	CM+FGF2	CM+FGF2	CM+FGF2	CM+FGF2	CM+FGF2	CM+FGF2	Fix cells
BAP	CM+FGF2	BAP	BAP	BAP	BAP	BAP	BAP	Fix cells

chambers. Rough handling was avoided so that colonies would not begin to peel from the membrane. The cells on the lower surface of the membrane were then fixed by adding 1 ml of 4 % (w/v) paraformaldehyde (PFA) to the lower chamber for 12 min. At this stage, the upper surface of the membrane was viewed and an image prepared to record whether colonies were

distributed relatively evenly across the membrane and to ensure that colony peeling had not occurred.

9. The PFA was removed, and both sides of the membrane washed twice with PBS. The cells were then stained with DAPI (diluted in PBS, 1:750). At this stage the upper surface of the membrane was re-imaged to document the overall cell density of each well.
10. The cells on the top of the invasion chamber were then removed with a cotton swab and washed with PBS. This process was continued until all cells are removed. The membrane was then cut from the chamber by using a scalpel and mounted on a glass coverslip.
11. Each membrane was then imaged by taking 12 images per membrane (SI Appendix, Fig. S2) at either 4 x or 10 x magnification and the number of nuclei counted by means of Image J software.

Immunoassays. After 6 days of BAP treatment, spent culture medium was collected to measure hormone and growth factor levels. Total DNA was isolated (Promega, #A2360) from the respective cultures in order to normalize immunoassay results to cell density levels. ELISAs were performed by following the protocols recommended by the manufacturers. The subsequent immunoassays were used: hCG (human chorionic gonadotrophin) (Genway bio, GWB-BQK0F2), PGF (human placental growth factor; ThermoFisher, EHPGF), P4 (progesterone; Genway bio, GWB-BQK0FC), and human VEGF-R1 (sFLT1; ThermoFisher, BMS268-3). Samples were collected in three independent experiments for each cell line under both O_2 conditions.

Statistical procedures. For the invasion assays, data were log transformed due to variance between lines. Then, a Glimmix Procedure was carried out in a 2x2x2 factorial design accounting for sex, disease and treatment as variables. The ELISA data were first normalized to the respective DNA content of the culture. Two-way comparisons were made with the Student's t-test; multiple group comparisons were made by one-way ANOVA followed by Tukey's multiple comparison test (GraphPad PRISM software; GraphPad Software Inc, La Jolla, CA).

Illumina TruSeq RNA library preparation and sequencing. High-throughput sequencing was performed at the University of Missouri DNA Core Facility. Libraries were constructed following the manufacturer's protocol with reagents supplied in Illumina's TruSeq mRNA stranded sample preparation kit. Briefly, the poly-A containing mRNA is purified from total RNA, the RNA was fragmented, double-stranded cDNA generated from fragmented RNA, and the index containing adapters ligated to the ends. The amplified

cDNA constructs were purified by addition of Axyprep Mag PCR Clean-up beads (Fischer Scientific). The final construct of each purified library was evaluated by using the Fragment Analyzer automated electrophoresis system, quantified with the Qubit fluorometer in conjunction with the Qubit HS dsDNA assay kit, and diluted according to Illumina's standard sequencing protocol for sequencing on the NextSeq 500. Raw read counts for each of the 48 DNA samples analyzed are listed in SI Appendix, Table S3. The reads were aligned to the reference human genome (hg19) by using the program HISAT2 (https://ccb.jhu.edu/software/hisat2) (7) an overall alignment of >97 % was achieved in all samples (SI Appendix, Table S3). The reads aligned to genes were further counted by using htseq-count tool from HTseq software package (8). The raw read counts for each biological replicate were normalized by gene length and then by the sequencing depth to calculate the transcripts per million (TPM) (9). The TPM values were then log-transformed for further analysis.

Differential expression analysis. EdgeR was used for differential expression analysis (10, 11). The reads were normalized by using the trimmed mean of M-values (TMM) normalization method (12). The differentially expressed genes (DEG) were defined as the genes with an absolute fold-change ≥ 1.5 and adjusted p-value ≤ 0.05.

Functional enrichment analysis. Functional enrichment analysis of gene sets was carried out by using the Genomic Regions Enrichment of Annotations Tool (GREAT) (12). The GO Biological Process and Disease Ontology were used for enrichment analysis using the hypergeometric statistic. The ontology terms with an FDR ≤ 0.05 and a fold-change ≥ 2 were considered significant.

Weighted co-expression network analysis. Weighted correlation network analysis (WGCNA) (13) was used to construct a co-expression network from the gene expression data from CTL and EOPE samples. The data from the invasion assays were used to calculate the correlation between co-expressed gene modules and invasion cell counts. The oxygen treatment, disease group information, and sex were also incorporated into the evaluation. Analysis was carried out as described previously (14) with minor modifications. Genes that had an expression level lower than 1 across all samples were removed from the analyses. Then, the top quartile of genes based on variance were selected. After that, the adjacency matrix for the selected genes was calculated by means of the signed hybrid Pearson correlation method with a soft thresholding power. The power was used to make a scale-free network ($R^2 = 0.8$) that retains a good number of connections. The adjacency matrix was then used to calculate the interconnectedness score (topological overlap) that was used to bundle the genes by hierarchical clustering. The hierarchical tree was further cut into gene modules by using the dynamic tree cut tool. Closely related gene clusters

(Correlation > 0.75) were merged together to obtain the final gene modules (15). The identified gene modules were decomposed such that each module was represented by its weighted expression (module eigengene) in the form of its first principal component. The correlation between the gene expression and the module eigengene defined as module membership (K_{ME}) was also calculated for each gene. Based on the K_{ME}, the genes were assigned to modules with the correlation value greater than 0.75, thereby allowing a gene to be a part of multiple modules or multiple regulatory pathways. The significance of the association between the module and different features, including invasion cell count and oxygen treatment, was also estimated by calculating the correlation between the module eigengene and the corresponding feature.

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SI Appendix, Figures

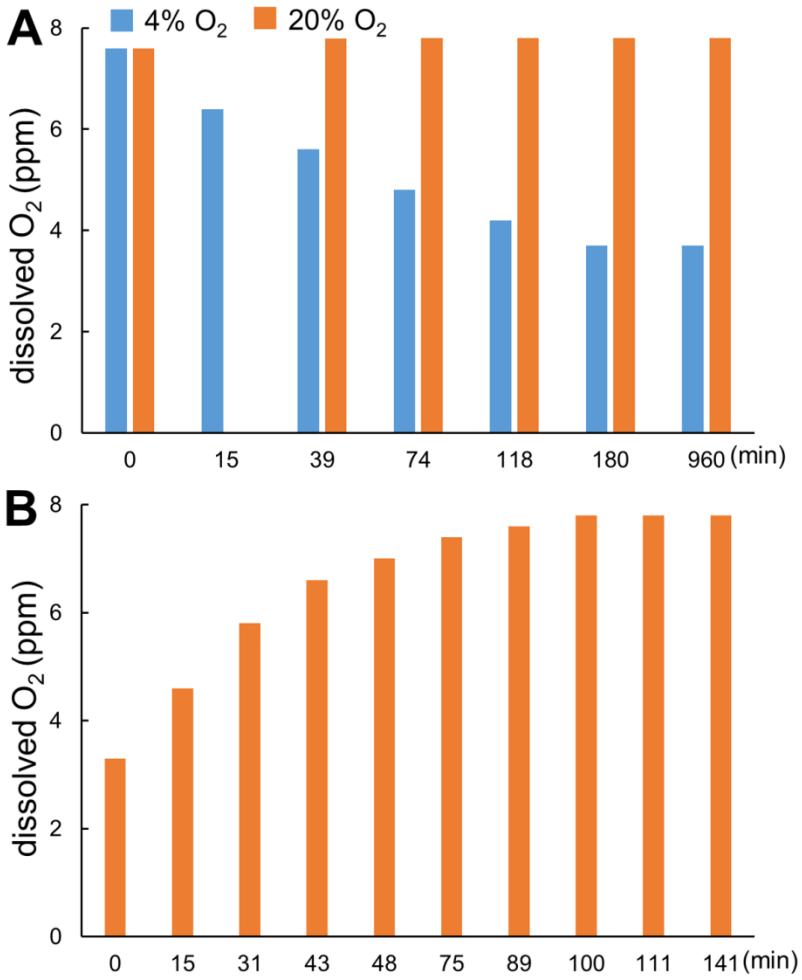

Fig. S1: Equilibration of O_2 levels in culture medium in 6-well culture plates. (A) Initial O_2 concentration in the medium held under standard O_2 conditions measured 7.8 ppm. Upon transfer to 4 % O_2, levels decreased to 5.6 ppm after 39 min and reached equilibrium (3.7 ppm) after 3 h. (B) The O_2 concentration under 4 % O_2 conditions was measured at 3.3 ppm. Upon transfer to air/5 % CO_2, O_2 levels in the medium increased to 5.8 ppm in the first 31 min and to 7.8 ppm after 100 min.

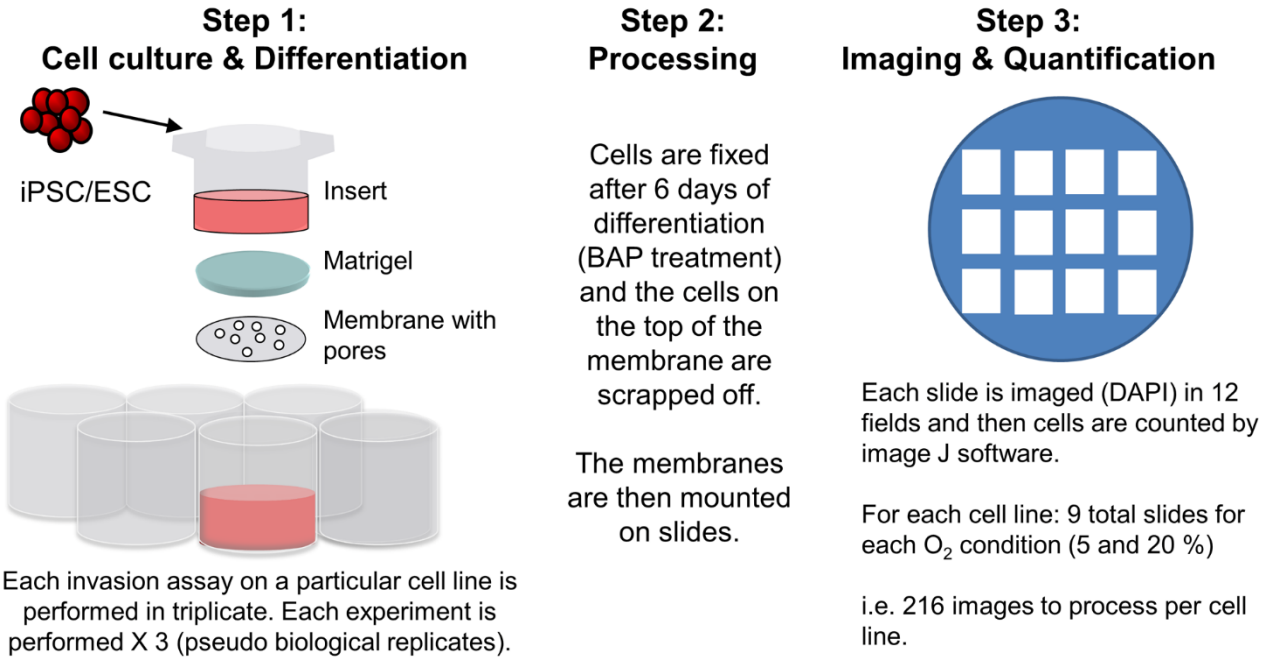

Fig. S2. Cartoon summarizing the methods used in the invasion experiments

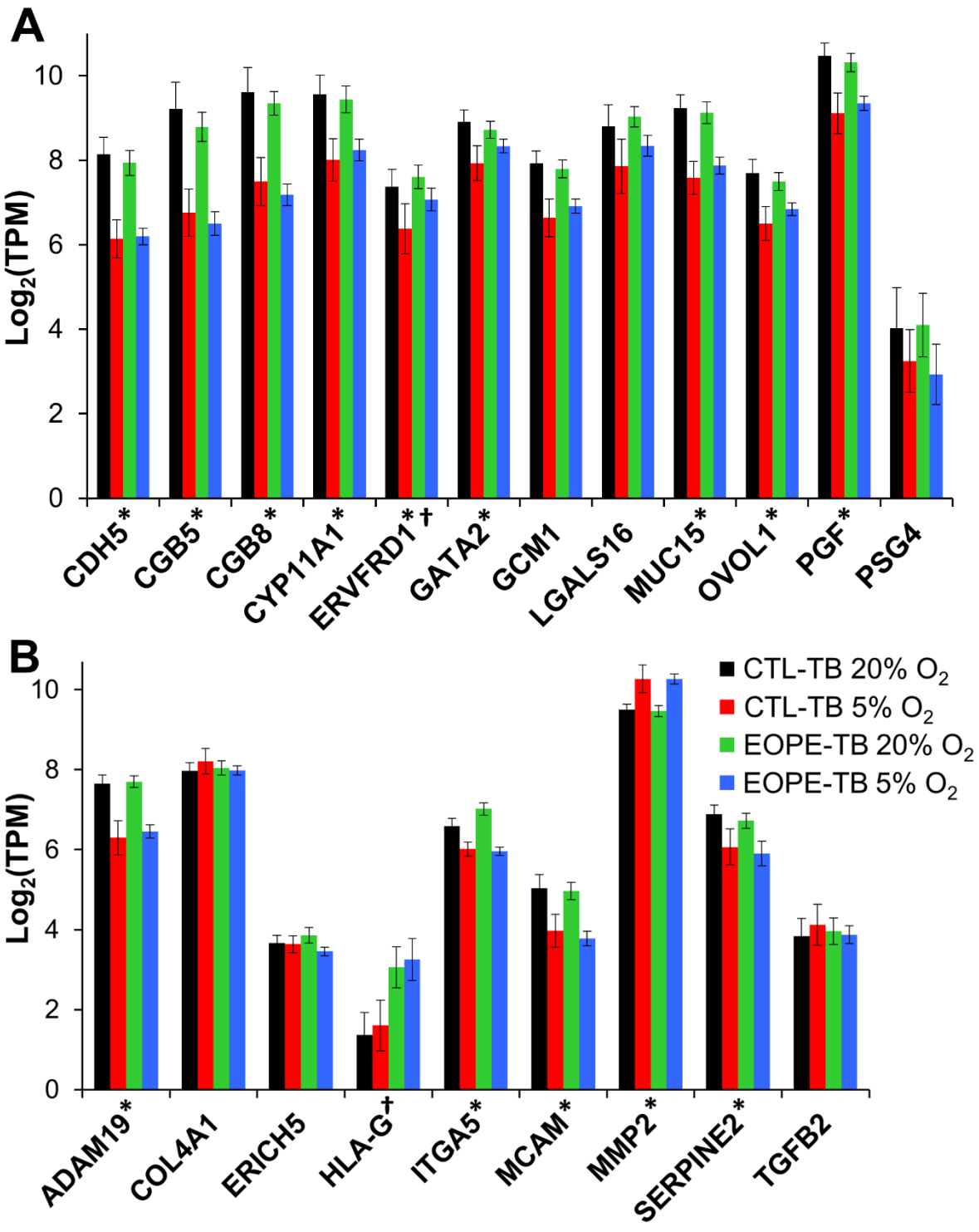

Fig. S3. Transcript levels for gene expression markers with typically enriched expression in syncytiotrophoblast (A) and extravillous trophoblast (B) were compared by two-way ANOVA (GraphPad Prism), with disease and oxygen level as factors. Genes marked with * differed significantly ($p<0.05$) between 5 and 20 % oxygen. Genes marked with † differed significantly between CTL and EOPE.

SI Tables

Sample Name	AMEL	CSF1PO	D13S317	D16S539	D18S51	D19S433	D21S11	D2S1338	D3S1358	D5S818	D7S820	D8S1179	FGA	TH01	TPOX	vWA
H1ESC	X, Y	12, 13	8,11	9, 13	17, 18	13, 15	18, 32.2	18, 19	15, 15	9, 11	8, 12	12, 13	20, 24	9.3, 9.3	8, 11	15, 17
H9ESC	X, X	11, 11	9, 9	12, 13	12.5, 13	12, 15	30, 30	18, 24	13, 16	11, 12	9, 11	8, 14	26, 28	9.3, 9.3	10, 11	17, 17
uc1p4	X, Y	10, 12	8, 12	10, 11	13, 15	15, 15.2	29, 30	18, 20	15, 17	11, 13	8, 9	12, 16	25, 25	9, 9	8, 8	16, 17
MRuc1i-1p9	X, Y	10, 12	8, 12	10, 11	13, 15	15, 15.2	29, 30	18, 20	15, 17	11, 13	8, 9	12, 16	25, 25	9, 9	8, 8	16, 17
uc3p2	X, Y	11, 11	8, 10	11, 11	13, 17	12, 13	28, 29	17, 22	16, 16	11, 12	10, 11	13, 16	22, 22	7, 9.3	8, 8	16, 17
MRuc3i-7p5	X, Y	11, 11	8, 10	11, 11	13, 17	12, 13	28, 29	17, 22	16, 16	11, 12	10, 11	13, 16	22, 22	7, 9.3	8, 8	16, 17
uc4p2	X, Y	10, 12	11, 12	11, 11	13.2, 15	12, 15.2	28, 28	17, 23	15, 17	11, 11	10, 11	14, 15	21, 24	7, 9	8, 11	16, 17
MRuc4i-13p6	X, Y	10, 12	11, 12	11, 11	13.2, 15	12, 15.2	28, 28	17, 23	15, 17	11, 11	10, 11	14, 15	21, 24	7, 9	8, 11	16, 17
uc5p2	X, X	9, 10	13, 13	9, 10	15, 15	13, 14	28, 30.2	25, 26	16, 17	10, 12	9, 11	11, 13	20, 23	9, 9.3	8, 8	17, 18
MRuc5i-3p4	X, X	9, 10	13, 13	9, 10	15, 15	13, 14	28, 30.2	25, 26	16, 17	10, 12	9, 11	11, 13	20, 23	9, 9.3	8, 8	17, 18
uc6p2	X, X	12, 12	11, 13	10, 12	19, 22	13, 15	29, 31.2	19, 19	15, 16	11, 13	11, 13	13, 14	25, 26	6, 7	8, 12	16, 17
MRuc6i-4p4	X, X	12, 12	11, 13	10, 12	19, 22	13, 15	29, 31.2	19, 19	15, 16	11, 13	11, 13	13, 14	25, 26	6, 7	8, 12	16, 17
uc7p4	X, Y	11, 12	12, 14	11, 13	15, 17	15.2, 17.2	29, 29	16, 17	16, 19	9, 12	8, 11	13, 13	21, 23	9, 9.3	8, 8	17, 19
MRuc7i-1p8	X, Y	11, 12	12, 14	11, 13	15, 17	15.2, 17.2	29, 29	16, 17	16, 19	9, 12	8, 11	13, 13	21, 23	9, 9.3	8, 8	17, 19
uc8p4	X, X	10, 11	10, 11	10, 13	12, 17	13, 15.2	30, 31	17, 24	14, 18	9, 11	10, 12	10, 11	23, 23	6, 9.3	8, 11	16, 17
MRuc8i-1p7	X, X	10, 11	10, 11	10, 13	12, 17	13, 15.2	30, 31	17, 24	14, 18	9, 11	10, 12	10, 11	23, 23	6, 9.3	8, 11	16, 17
uc9p4	X, X	11, 12	10, 11	11, 12	12, 19	14, 16	32.2, 32.2	19, 20	16, 18	10, 13	9, 11	11, 15	18, 24	6, 8	8, 8	17, 18
MRuc9i-2p6	X, X	11, 12	10, 11	11, 12	12, 19	14, 16	32.2, 32.2	19, 20	16, 18	10, 13	9, 11	11, 15	18, 24	6, 8	8, 8	17, 18
uc10p5	X, Y	11, 12	8, 12	12, 13	13, 20	12, 15	30, 30	17, 17	14, 15	11, 12	10, 10	11, 11	23, 25	6, 7	8, 11	15, 17
MRuc10i-5p6	X, Y	11, 12	8, 12	12, 13	13, 20	12, 15	30, 30	17, 17	14, 15	11, 12	10, 10	11, 11	23, 25	6, 7	8, 11	15, 17
ucAp3	X, X	9, 12	8, 13	9, 12	13, 17	14.2, 15	29, 32.2	17, 25	16, 17	12, 12	9, 11	11, 12	20, 22	9.3	8, 11	18, 19
MRucAi-3p3	X, X	9, 12	8, 13	9, 12	13, 17	14.2, 15	29, 32.2	17, 25	16, 17	12, 12	9, 11	11, 12	20, 22	9.3	8, 11	18, 19
ucBp4	X, Y	10, 12	8, 8	9, 12	13, 14	13, 14	28, 32.2	20, 23	16, 17	10, 12	8, 10	12, 14	21, 25	7, 9	8, 9	16, 19
MRucBi-1p4	X, Y	10, 12	8, 8	9, 12	13, 14	13, 14	28, 32.2	20, 23	16, 17	10, 12	8, 10	12, 14	21, 25	7, 9	8, 9	16, 19
ucCp1	X, Y	11, 12	9, 10	10, 12	19, 21	14, 14	31.2,	19, 23	15, 16	11, 13	12, 13	13, 13	27, 28	6, 9.3	9, 12	16, 18
MRucCi-2p7	X, Y	11, 12	9, 10	10, 12	19, 21	14, 14	31.2,	19, 23	15, 16	11, 13	12, 13	13, 13	27, 28	6, 9.3	9, 12	16, 18
ucDp4	X, Y	10, 11	11, 12	12, 12	14, 15	14, 16	28, 30	16, 17	17, 18	12, 12	9, 10	12, 13	23, 23	9, 9.3	8, 12	14, 16
MRucDi-1p7	X, Y	10, 11	11, 12	12, 12	14, 15	14, 16	28, 30	16, 17	17, 18	12, 12	9, 10	12, 13	23, 23	9, 9.3	8, 12	14, 16
ucEp1	X, Y	12, 13	10, 14	11, 13	17, 17	13, 15	27, 31	17, 18	15, 15	13, 13	8, 9	14, 14	18, 21	8, 9.3	12, 12	15, 18
MRucEi-6p6	X, Y	12, 13	10, 14	11, 13	17, 17	13, 15	27, 31	17, 18	15, 15	13, 13	8, 9	14, 14	18, 21	8, 9.3	12, 12	15, 18
ucGp4	X, Y	11, 13	10, 11	12, 14	14, 15	14, 14	30, 30.2	16, 19	14, 15	12, 13	8, 11	9, 13	22, 23	8, 9	10, 11	16, 19
MRucGi-3p4	X, Y	11, 13	10, 11	12, 14	14, 15	14, 14	30, 30.2	16, 19	14, 15	12, 13	8, 11	9, 13	22, 23	8, 9	10, 11	16, 19
ucIp4*	X, Y	11, 11	8, 11	11, 12	14, 15	13, 14	29, 29	17, 22	17, 17	11, 13	8, 12	13, 14	19, 24	6, 8	11, 11	16, 18
MRucIi-4p4*	X, Y	11, 11	8, 11	11, 12	14, 15	13, 14	29, 29	17, 22	17, 17	11, 13	8, 12	13, 14	19, 24	6, 8	11, 11	16, 18
ucJp4*	X, Y	11, 11	8, 11	11, 12	14, 15	13, 14	29, 29	17, 22	17, 17	11, 13	8, 12	13, 14	19, 24	6, 8	11, 11	16, 18
MRucJi-1p4*	X, Y	11, 11	8, 11	11, 12	14, 15	13, 14	29, 29	17, 22	17, 17	11, 13	8, 12	13, 14	19, 24	6, 8	11, 11	16, 18
ucKp2	X, Y	10, 11	8, 12	10, 11	13, 16	14, 15.2	28, 28	17, 24	15, 15	11, 12	11, 12	12, 14	20, 22	9.3, 9.3	8, 8	17, 17
MRucKi-2p3	X, Y	10, 11	8, 12	10, 11	13, 16	14, 15.2	28, 28	17, 24	15, 15	11, 12	11, 12	12, 14	20, 22	9.3, 9.3	8, 8	17, 17
ucLp2	X, Y	10, 15	8, 11	9, 11	16, 16	13, 14	32.2, 32.2	17, 18	14, 15	12, 12	10, 12	13, 14	22, 22	6, 7	8, 11	15, 18
MRucLi-4p13	X, Y	10, 15	8, 11	9, 11	16, 16	13, 14	32.2, 32.2	17, 18	14, 15	12, 12	10, 12	13, 14	22, 22	6, 7	8, 11	15, 18
ucMp4	X, Y	10, 12	8, 10	11, 12	16, 18	14, 14	28, 32.2	20, 21	16, 18	11, 12	9, 12	8, 15	22, 24	6, 9	8, 9	17, 18
MRucMi-4p4	X, Y	10, 12	8, 10	11, 12	16, 18	14, 14	28, 32.2	20, 21	16, 18	11, 12	9, 12	8, 15	22, 24	6, 9	8, 9	17, 18
ucPp2	X, X	10, 12	11, 12	9, 11	13, 14	14, 14	29, 31.2	16, 20	15, 17	13, 13	10, 13	13, 14	19, 22	6, 9	8, 8	16, 17
MRucPi-1p7	X, X	10, 12	11, 12	9, 11	13, 14	14, 14	29, 31.2	16, 20	15, 17	13, 13	10, 13	13, 14	19, 22	6, 9	8, 8	16, 17
ucQp4	X, X	11, 12	8, 12	13, 14	13, 17	13, 15	30, 30	18, 23	16, 18	9, 11	9, 10	8, 11	20, 25	7, 10	8, 11	17, 20
MRucQi-15p4	X, X	11, 12	8, 12	13, 14	13, 17	13, 15	30, 30	18, 23	16, 18	9, 11	9, 10	8, 11	20, 25	7, 10	8, 11	17, 20
ucRp2	X, X	11, 11	9, 9	11, 13	13, 17	13, 14	29, 32.2	17, 23	14, 17	11, 12	11, 12	13, 14	19, 24	6, 6	8, 11	18, 18
MRucRi-7p4	X, X	11, 11	9, 9	11, 13	13, 17	13, 14	29, 32.2	17, 23	14, 17	11, 12	11, 12	13, 14	19, 24	6, 6	8, 11	18, 18
ucS**	X, X	10, 12	11, 12	9, 9	16, 18	14.2, 16.2	28, 31.2	23, 25	14, 15	11, 12	14, 14	12, 13	22, 26	7, 9	11, 11	14, 15
MRucSi-4p3	X, X	10, 12	11, 12	9, 9	16, 18	14.2, 16.2	28, 31.2	23, 25	14, 15	11, 12	14, 14	12, 13	22, 26	7, 9	11, 11	14, 15

Table S1. *Related to Figures 1-5 and Table 1.* Short tandem repeat (STR) profiling for hESC lines (H1 and H9) and cell lines derived from explants of umbilical cords (uc) before and after conversion to iPSC. The parental primary cells are numbered 1-10 for CTL lines and A-S for EOPE lines (see Table 1). The number following the lower case "p" is the passage number at which the analysis was performed. The iPSC lines were named according to convention (laboratory, MR; tissue source, uc; cell line designation, 1-10 CTL and A-S EOPE; iPSC clone number, e.g, i-4; and p, passage number). The STR multiplex assay amplifies the Amelogenin (AMEL) sex-determining marker and 15 tetranucleotide repeat loci. Note that the original primary explant cells and the iPSC lines derived from them invariably show identical STR patterns. * Two PE samples I and J were from monozygotic twin boys (Table 1). As expected, both their

explant cells (ucI and ucJ) and the two iPSC lines (MRucIi and MRucJi) showed identical STR patterns. ** The genomic DNA of ucS was collected from umbilical cord tissue.

CTL ESC & iPSC				**EOPE** iPSC			
Sample ID	Pluri-raw	Novelty	Result	Sample ID	Pluri-raw	Novelty	Result
H1 ESC	24.534	1.599	Pass	MRucAi-3p7	29.713	1.341	Pass
MRuc1i-1p10	28.507	1.532	Pass	MRucBi-1p14	20.232	1.488	Pass
MRuc2i-4p4	25.03	1.188	Pass	MRucCi-2p7	22.641	1.383	Pass
MRuc3i-7p5	29.419	1.505	Pass	MRucDi-5p8	25.081	1.489	Pass
MRuc4i-2p7	20.256	1.523	Pass	MRucEi-6p6	24.347	1.499	Pass
MRuc5i-3p8	29.55	1.35	Pass	MRucFi-4p9	25.419	1.377	Pass
MRuc6i-4p4	28.943	1.382	Pass	MRucGi-3p4	20.139	1.429	Pass
MRuc7i-1p9	20.688	1.263	Pass	MRucHi-5p5	20.498	1.63	Pass
MRuc8i-1p7	20.302	1.172	Pass	MRucIi-4p4	20.911	1.338	Pass
MRuc9i-2p6	29.92	1.492	Pass	MRucJi-1p4	24.897	1.397	Pass
MRuc10i-5p7	25.13	1.23	Pass	MRucKi-2p5	22.107	1.378	Pass
				MRucLi-4p17	21.096	1.621	Pass
				MRucMi-4p11	26.203	1.687	Further evaluate
				MRucNi-3p7	29.503	1.628	Pass
				MRucOi-2p5	23.775	1.573	Pass
				MRucPi-1p7	27.218	1.253	Pass
				MRucQi-6p7	25.637	1.458	Pass
				MRucRi-7p3	34.4	1.45	Pass
				MRucSi-4p16	18.566	1.361	Further evaluate

Table S2. *Related to Figures 1-5 and Table 1.* Pluritest scores and Pluritest assessments for CTL iPSC (left) and EOPE iPSC (right). The cell line name (explanation in legend for Table S1) is accompanied with the clone number and with the passage (p) number when the RNA was prepared. Slightly deviant scores but within the range considered to represent pluripotency are in red font. High quality pluripotent lines have a Pluri-raw Score ≥ 20 and a Novelty Score of ≤ 1.67 (https://pluritest.org/).

Sample #	Cell Line	Clinical Condition	Sex	O_2 %	# Raw Reads	% Alignment
1	H1 WA01	CTL	M	5	9544478	97.78%
2	H1 WA01	CTL	M	20	8487826	98%
3	H9 WA09	CTL	F	5	9923104	97.83%
4	H9 WA09	CTL	F	20	8754450	97.75%
5	MRuc3i	CTL	M	5	8354043	97.81%
6	MRuc3i	CTL	M	20	9930415	97.94%
7	MRuc4i	CTL	M	5	8457322	97.96%
8	MRuc4i	CTL	M	20	9306064	97.78%
9	MRuc5i	CTL	F	5	10178388	97.77%
10	MRuc5i	CTL	F	20	9089732	97.94%
11	MRuc6i	CTL	M	5	10142239	97.82%
12	MRuc6i	CTL	M	20	8804329	97.69%
13	MRuc7i	CTL	M	5	10036158	97.78%
14	MRuc7i	CTL	M	20	9837780	97.80%
15	MRuc8i	CTL	F	5	10859080	97.87%
16	MRuc8i	CTL	F	20	10138845	97.73%
17	MRuc9i	CTL	F	5	9852303	97.73%
18	MRuc9i	CTL	F	20	8432784	97.64%
19	MRuc10i	CTL	F	5	10146448	97.71%
20	MRuc10i	CTL	F	20	9170474	97.86%
21	MRucAi	EOPE	F	5	8943860	98.03%
22	MRucAi	EOPE	F	20	9514962	97.90%
23	MRucBi	EOPE	M	5	9940119	97.95%
24	MRucBi	EOPE	M	20	9066300	97.94%
25	MRucDi	EOPE	M	5	8782427	97.52%
26	MRucDi	EOPE	M	20	8615882	98.08%
27	MRucEi	EOPE	M	5	6593496	97.76%
28	MRucEi	EOPE	M	20	8842144	97.87%
29	MRucGi	EOPE	M	5	8628925	97.96%
30	MRucGi	EOPE	M	20	9657760	98.01%
31	MRucIi	EOPE	M	5	9436505	97.81%
32	MRucIi	EOPE	M	20	9570690	97.87%
33	MRucJi	EOPE	M	5	6614438	97.90%
34	MRucJi	EOPE	M	20	7461183	97.96%
35	MRucKi	EOPE	M	5	8020706	97.84%
36	MRucKi	EOPE	M	20	7002708	97.74%
37	MRucLi	EOPE	M	5	7288412	97.84%
38	MRucLi	EOPE	M	20	7690860	97.86%
39	MRucMi	EOPE	M	5	8016411	97.8%
40	MRucMi	EOPE	M	20	7151914	97.85%
41	MRucPi	EOPE	F	5	8102036	97.66%
42	MRucPi	EOPE	F	20	8245633	97.77%
43	MRucQi	EOPE	F	5	8751919	97.84%
44	MRucQi	EOPE	F	20	9053055	97.82%
45	MRucRi	EOPE	F	5	8855108	97.73%
46	MRucRi	EOPE	F	20	10245320	97.68%
47	MRucSi	EOPE	F	5	9581929	97.67%
48	MRucSi	EOPE	F	20	6043823	97.67%

Table S3. *Related to Figures 4 and 5.* Raw read counts and overall alignment for each sample of RNA analyzed. A total of 48 RNAseq samples were analyzed by NextSeq 500 (Illumina).

Replicate #	1	2	3	4	ave	std	1	2	3	4	ave	std	P value
Cell line	5% O_2						20% O_2						
H1	543	654	638		612	60	561	616	508		562	54	0.344
H9	1435	1696	2551		1894	584	1987	1536	1365		1629	322	0.529
*MRuc**3**i-7*	1121	990	1217		1109	114	928	1103	1077		1036	94	0.438
*MRuc**4**i-2*	205	389	500		365	149	122	290	181	262	214	77	0.136
*MRuc**5**i-3*	474	464	556		498	51	397	429	522		449	65	0.365
*MRuc**6**i-4*	257	596	1009	295	539	348	399	659	583		547	134	0.971
*MRuc**7**i-1*	294	505	592	595	496	141	465	313	329		369	84	0.229
*MRuc**8**i-1*	649	559	625		611	47	609	485	532		542	62	0.200
*MRuc**9**i-2*	360	871	313		515	309	18	28	179		75	90	0.078
*MRuc**10**i-5*	153	49	115		79	68	122	95	69		71	52	0.772
*MRuc**A**i-3*	181	138	122		147	30	26	10	17		18	8	0.002
*MRuc**B**i-1*	561	633	489		561	72	134	204	163		167	36	0.001
*MRuc**D**i-5*	740	561	431		577	155	271	311	378		320	54	0.054
*MRuc**E**i-6*	510	647	518	505	545	69	135	153	192	202	170	32	0.000
*MRuc**G**i-3*	328	423	515		316	224	177	197	296		167	123	0.038
*MRuc**I**i-4*	28	33	30		23	15	8	10	13		8	5	0.000
*MRuc**J**i-1*	314	348	302		321	24	95	140	124		120	23	0.000
*MRuc**K**i-2*	2130	868	1230		1409	650	217	458	395		357	125	0.051
*MRuc**L**i-4*	78	95	62		78	17	19	36	12		22	12	0.009
*MRuc**M**i-4*	566	531	670		589	72	214	254	186		218	34	0.001
*MRuc**P**i-1*	250	212	240		234	20	102	50	75		76	26	0.001
*MRuc**Q**i-6*	1707	1142	937		1262	399	1454	2026	1094		1525	470	0.501
*MRuc**R**i-7*	1789	1411	1636		1612	190	33	84	130		83	49	0.000
*MRuc**S**i-4*	585	758	556		633	109	234	265	129		209	71	0.005

Non-Significant (P > 0.05)
Almost Significant (P = 0.051 -0.055)
Significant (P < 0.05)

Table S4. *Related to Figure 2.* Invaded cell numbers for 10 CTL (upper, gray) and 14 EOPE lines (lower, beige) after differentiation under BAP conditions for 6 days. Data are summarized in Fig. 3. The cell numbers are derived from either three or four independent experiments, each performed in triplicate invasion chamber wells. These values were averaged (ave), the standard deviation (std) calculated according to whether the cells had been cultured under 5 % O_2 (left) or 20 % O_2 (right). Significance of pairwise comparisons (5 % vs 20 % O_2) is highlighted (non-significant P > 0.05: green, almost significant P = 0.051 -0.055: yellow, significant P < 0.05: brown).

EOPE vs. CTL	**Upregulated in EOPE**	**Upregulated in CTL**
EOPE 20 % O_2 vs. CTL 20% O_2	0	2 (*RPS17*, FDR = 0.0005; *MTRNR2L2*, FDR = 0.005)
EOPE 5 % O_2 vs. CTL 5 % O_2	0	0

Table S5. *Related to Figures 4 and 5.* Summary of the differential expression analysis between the CTL and EOPE samples across the two different O_2 conditions. Genes with a p-adjusted ≤ 0.05 and fold-change ≥ 1.5 were considered differentially expressed.

Gene	K_{ME}-score	Correlation	P value	adjusted p value
NEURL1	0.99166957	-0.6411007	0.00744369	0.02560337
FDX1	0.99091683	-0.6083999	0.01239258	0.02947238
PRKCZ	0.99086532	-0.6447414	0.00700842	0.02558895
SPIRE2	0.98841279	-0.603806	0.01325637	0.03046182
FURIN	0.98831897	-0.6497263	0.00644554	0.02558895
DIRC2	0.9863337	-0.6096594	0.01216364	0.02923006
CARS	0.98631635	-0.6653172	0.00491341	0.02390458
CD274	0.98279084	-0.6312065	0.00873579	0.02621377
KLB	0.98272879	-0.620513	0.01032544	0.02719032
TOP1	0.98155635	-0.6738276	0.00420953	0.02390458
HSD17B1	0.9811725	-0.6489189	0.00653418	0.02558895
FHDC1	0.97970679	-0.60061	0.01388462	0.03148952
GLTP	0.97951539	-0.6153631	0.01116816	0.02835914
SDC1	0.9793054	-0.615697	0.01111192	0.02835914
RASA1	0.97922142	-0.6120512	0.01173806	0.02877848
DACT2	0.97892185	-0.5898711	0.01616774	0.03341645
HSPB1	0.97828964	-0.65382	0.00601074	0.0254383
EIF2B2	0.97814308	-0.6515325	0.00625071	0.02558895
FBRS	0.97793016	-0.7116555	0.00198867	0.02390458
LOC113230	0.97781417	-0.6107899	0.011961	0.02915451
SIN3B	0.97725403	-0.6377227	0.00786649	0.02564547
FLVCR2	0.97718879	-0.6707064	0.00445759	0.02390458
PVRL3	0.97691328	-0.662831	0.00513589	0.02436061
ATP2B4	0.97677145	-0.5853696	0.01720742	0.03460592
KCNN4	0.9767095	-0.6300196	0.008902	0.02621377
CYP11A1	0.97621394	-0.6763923	0.0040141	0.02390458
NR2F6	0.97596883	-0.703291	0.00236981	0.02390458
STK40	0.97590351	-0.6305614	0.00882582	0.02621377
MAST4	0.97371456	-0.6364498	0.00803066	0.02564547
HSPB8	0.9735902	-0.6796668	0.00377525	0.02390458
MAN1A2	0.97339963	-0.5947613	0.0150944	0.03263461
ABHD5	0.97338435	-0.5999523	0.01401674	0.03148952

HOPX	0.97314074	-0.5672509	0.02192745	0.03840005
HDAC5	0.97303063	-0.7028993	0.00238901	0.02390458
WNK2	0.97297518	-0.6512691	0.00627882	0.02558895
RPS6KA5	0.97205721	-0.6078925	0.01248576	0.02947238
FAR2	0.97178342	-0.579826	0.01855837	0.03585598
RAP1B	0.97158959	-0.6125698	0.01164734	0.02865488
HES2	0.97148598	-0.5997961	0.01404826	0.03148952
EPHB4	0.97130053	-0.6488553	0.0065412	0.02558895
C10orf10	0.97129963	-0.6227831	0.00997028	0.02716041
SLC38A3	0.97125717	-0.6155016	0.0111448	0.02835914
FADS3	0.97102879	-0.5384711	0.03140453	0.04781289
PPARD	0.97005064	-0.6909845	0.00303616	0.02390458
TGFBR3	0.9700302	-0.6266532	0.00938708	0.02669685
SLC9A1	0.96925952	-0.6567301	0.00571613	0.02540107
DNAJB9	0.96924202	-0.6314663	0.00869974	0.02621377
CYP2C18	0.96921708	-0.6135383	0.01147941	0.02849609
DYSF	0.96885228	-0.6298209	0.00893008	0.02621377
ABTB2	0.96884381	-0.6234473	0.00986822	0.02708997
RHOV	0.96847483	-0.6880178	0.00321749	0.02390458
FANCE	0.96756867	-0.6678152	0.00469771	0.02390458
KIAA0040	0.96729316	-0.6487824	0.00654926	0.02558895
PDIA3	0.96725717	-0.5356062	0.03249534	0.04905347
DUSP8	0.96698369	-0.6358387	0.00811045	0.02564547
TFCP2L1	0.96690281	-0.6235222	0.00985676	0.02708997
SMAD7	0.96621996	-0.6906892	0.00305383	0.02390458
ZFHX3	0.96539458	-0.6825858	0.00357209	0.02390458
TNFRSF1B	0.96534737	-0.6206727	0.01030014	0.02719032
PREX1	0.96526445	-0.5000356	0.04856169	0.06261643
CD200	0.96498495	-0.5472816	0.02822382	0.04459364
HSPBAP1	0.96410273	-0.6831032	0.00353702	0.02390458
TMEM180	0.96380099	-0.7130433	0.00193055	0.02390458
AKR1B1	0.96354796	-0.5410392	0.03045058	0.0469639
DAB2	0.96326738	-0.6597813	0.00541974	0.0250223

FAM129B	0.96290069	-0.6137791	0.01143795	0.02849609
NUS1	0.96282667	-0.7104303	0.00204115	0.02390458
ETV4	0.96271108	-0.6181707	0.01070228	0.02787297
QSOX1	0.96261213	-0.5982472	0.01436387	0.03161829
PWWP2B	0.96252792	-0.7145294	0.00186984	0.02390458
IGSF5	0.96213923	-0.5147032	0.04134703	0.05620982
OPN3	0.9618784	-0.5769319	0.01929558	0.03629407
ADCY5	0.9614719	-0.5055396	0.04575245	0.06057727
ATG9B	0.96076855	-0.590457	0.01603608	0.03327293
SNX27	0.96050103	-0.565159	0.02253082	0.0390061
RYBP	0.9603951	-0.686237	0.00333046	0.02390458
CGA	0.96031027	-0.5140306	0.04165912	0.0563616
TBX3	0.95991503	-0.6750475	0.00411564	0.02390458
TIPARP	0.95973179	-0.6439204	0.00710476	0.02558895
HPCAL1	0.95954664	-0.6799469	0.00375536	0.02390458
PPM1D	0.95899303	-0.7106327	0.00203241	0.02390458
SEMA7A	0.95895385	-0.646545	0.00680043	0.02558895
KMT2C	0.95869919	-0.6941591	0.00285135	0.02390458
KHK	0.95844973	-0.5632021	0.0231068	0.03917643
AFF1	0.95839151	-0.6547746	0.00591279	0.0254383
ZNF845	0.9582506	-0.6816408	0.00363688	0.02390458
EGFL7	0.95814564	-0.5765623	0.01939133	0.03637793
RRBP1	0.95814532	-0.5420523	0.03008041	0.04649385
GMPPB	0.95784947	-0.6050624	0.01301558	0.03014357
CPEB4	0.95783445	-0.6753486	0.00409273	0.02390458
FAM83G	0.95755978	-0.613666	0.01145739	0.02849609
RHOBTB1	0.95709274	-0.669073	0.004592	0.02390458
GNE	0.95684241	-0.6882198	0.00320488	0.02390458
OTUB2	0.95651532	-0.5979943	0.01441591	0.03162062
HEXIM1	0.95644364	-0.7719927	0.00045862	0.02390458
PHLPP1	0.95617519	-0.6775982	0.00392477	0.02390458
EPPK1	0.9558326	-0.6806944	0.0037027	0.02390458
SIAH1	0.95559814	-0.6211877	0.01021886	0.02719032

BAIAP2	0.9555462	-0.642037	0.00732975	0.02558895
ADCK2	0.95542233	-0.5287028	0.03524191	0.05082556

Table S6. *Related to Figure 2.* The top 100 genes sorted by their module membership values (K_{ME}) in the CTL9 module. The correlation values associated with the corresponding invasion potential are listed as well as the p value and adjusted p value.