Nature of trophoblast generated from ESC and iPSC

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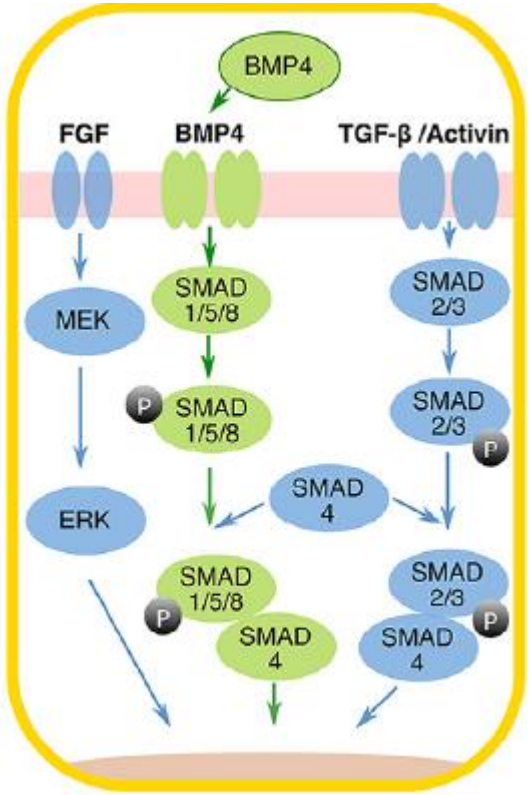
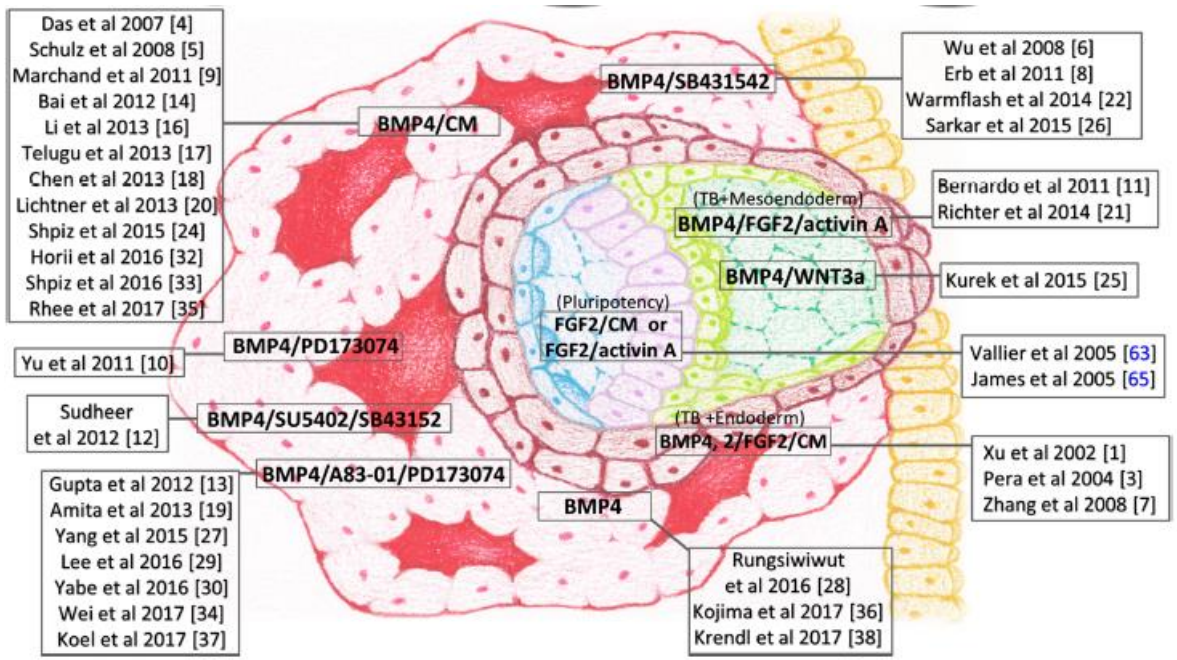
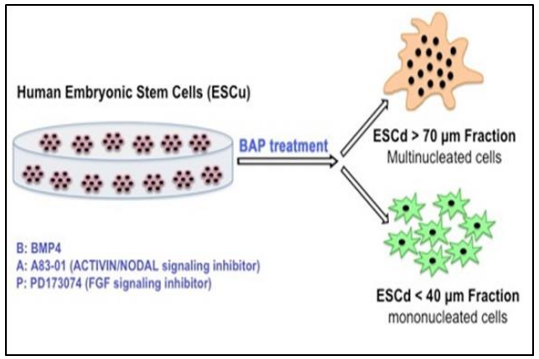
The model utilizes BMP4 plus inhibitors of FGF and TGFB signaling to drive human epiblast-type (primed) ESC and iPSC to trophoblast







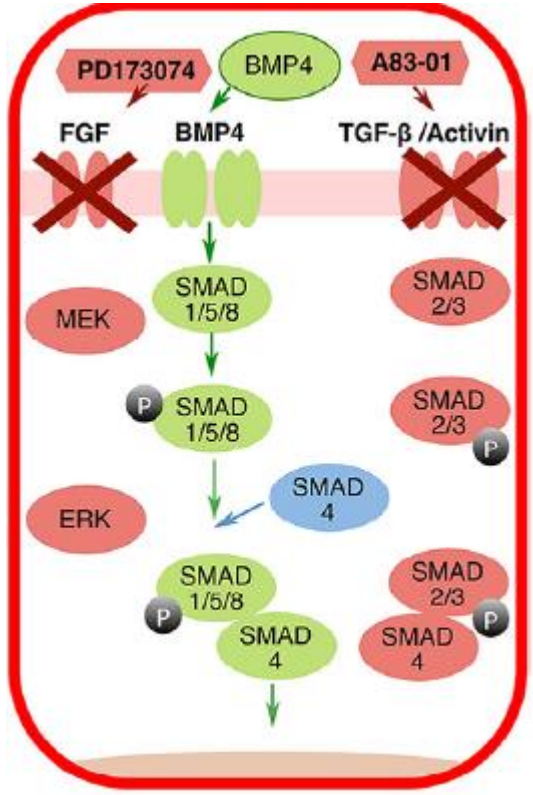
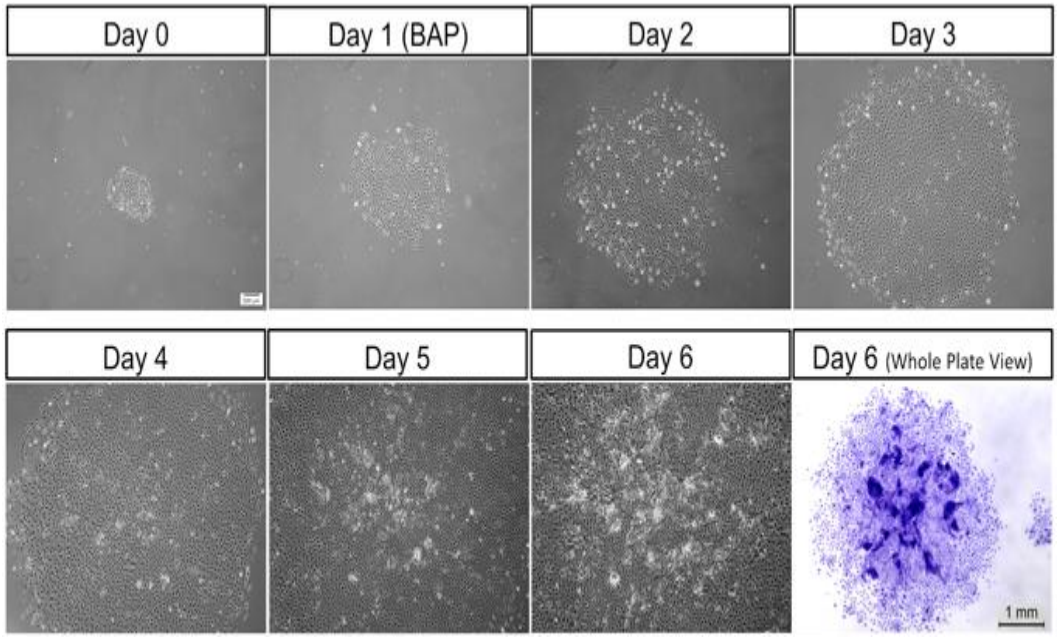


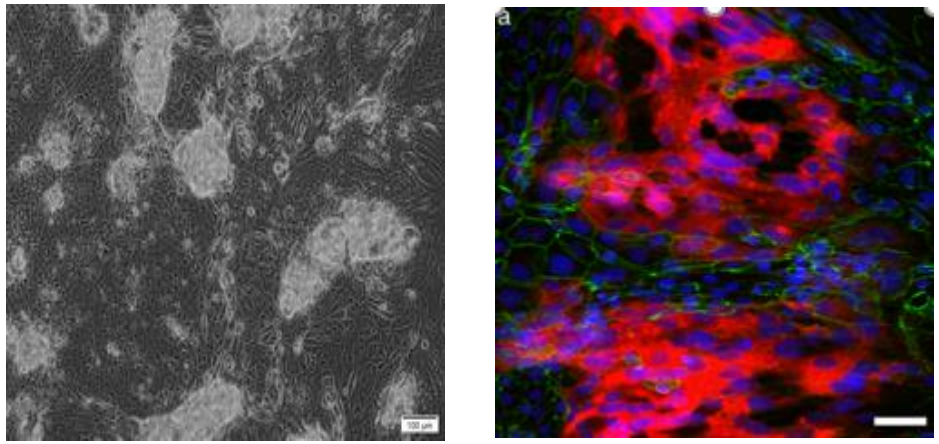
Diagram adapted from Koel M et al. RBM ON LINE (2017)

The Use of Stem Cell Derived Trophoblasts as a Model for Placentation

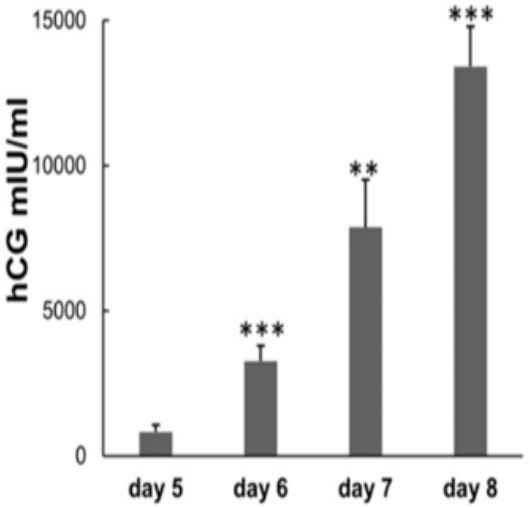
Mechanical passaging by cutting

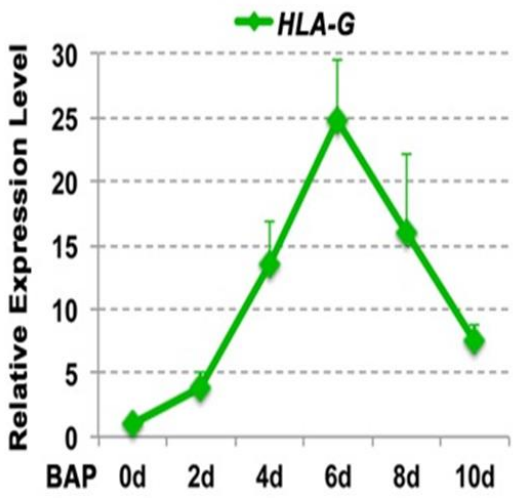


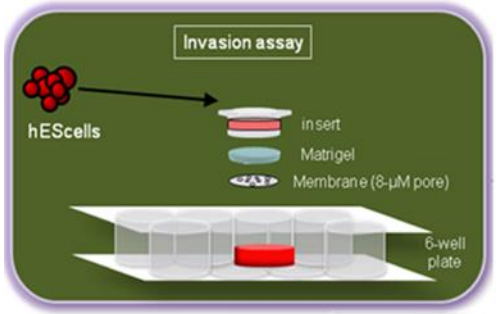
Differentiation

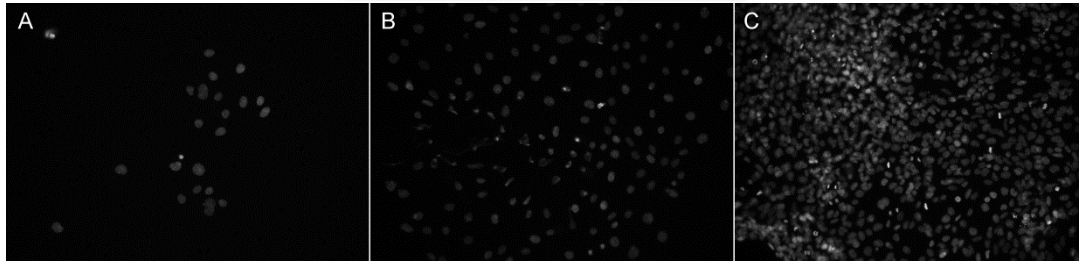


Syncytial trophoblast at d6 and d9



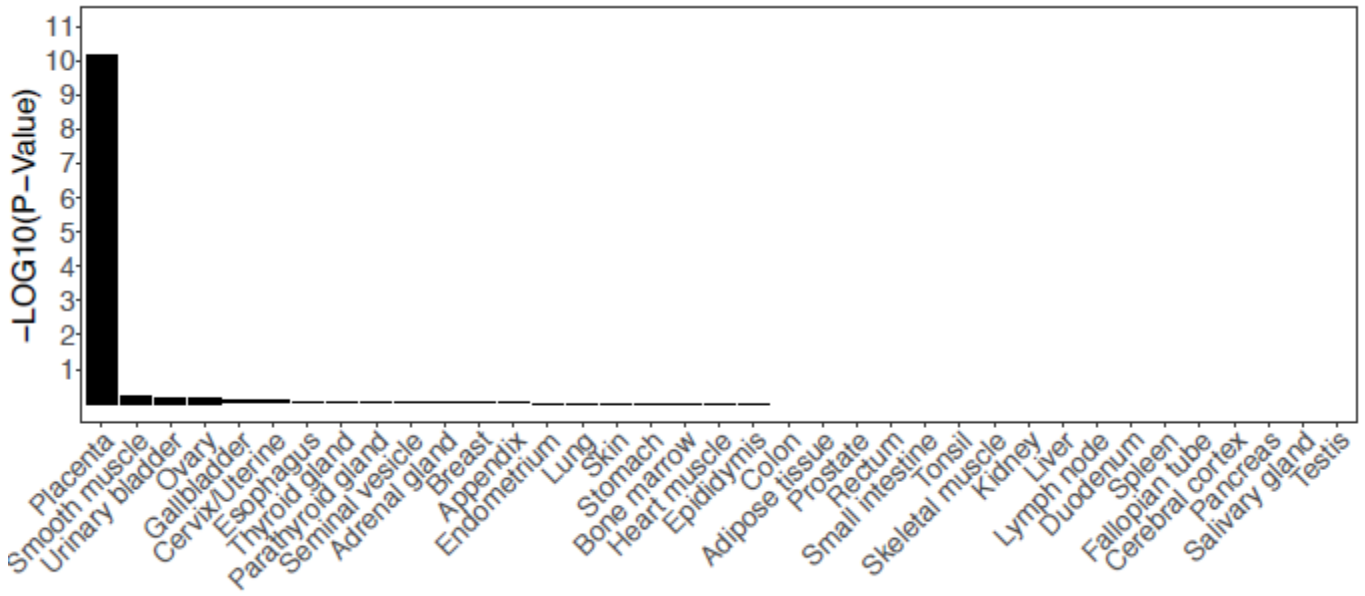




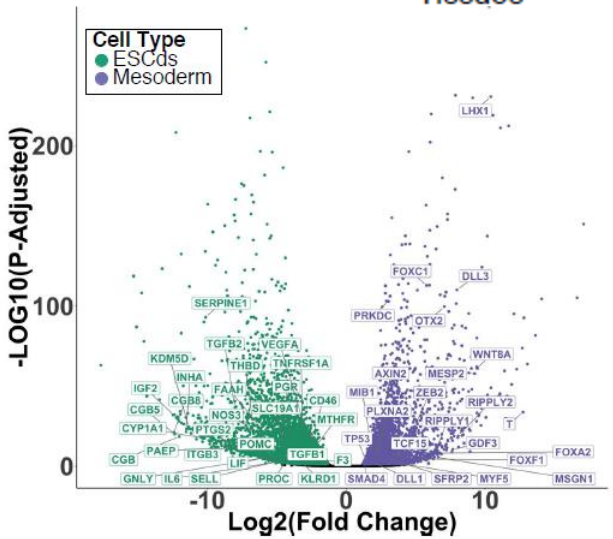


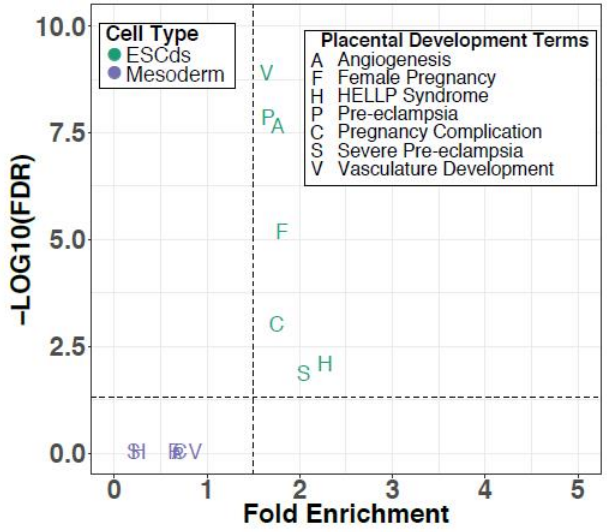
Invasion assays at d3, d4, & d5

The transcriptome of BAP/hESC cells is placental trophoblast-like with invasive features and lacks any signature corresponding to mesoderm



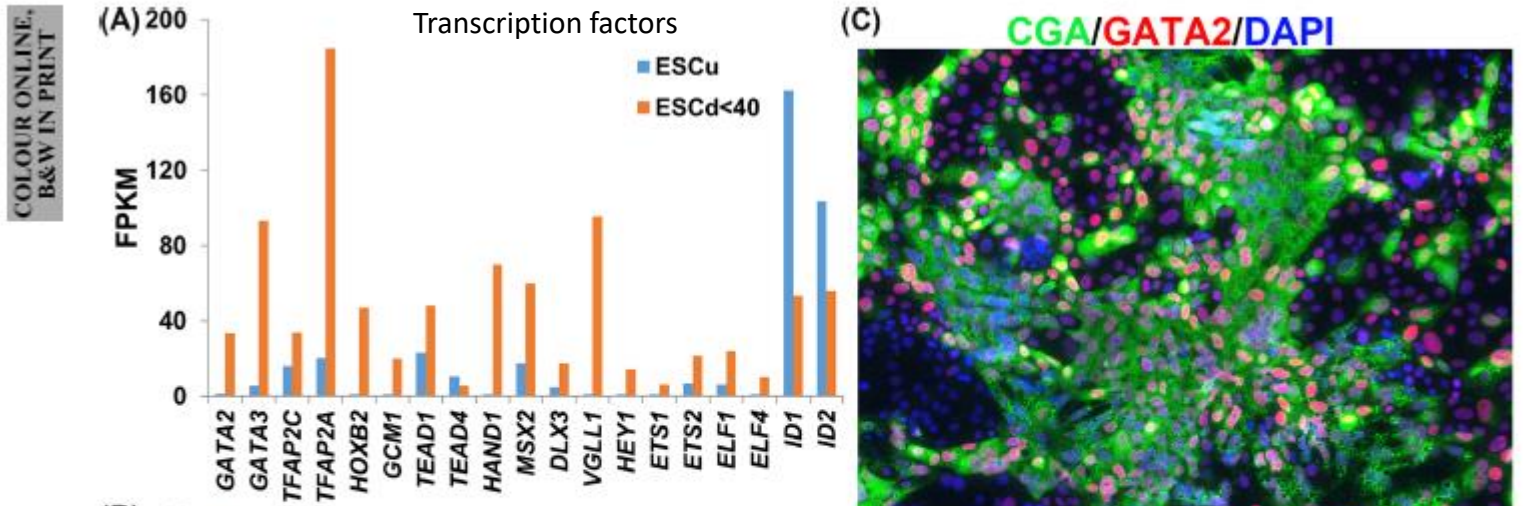
Day 2 mesoderm: Primary data from Szabo, Salzman et al. (2015) GSE64417

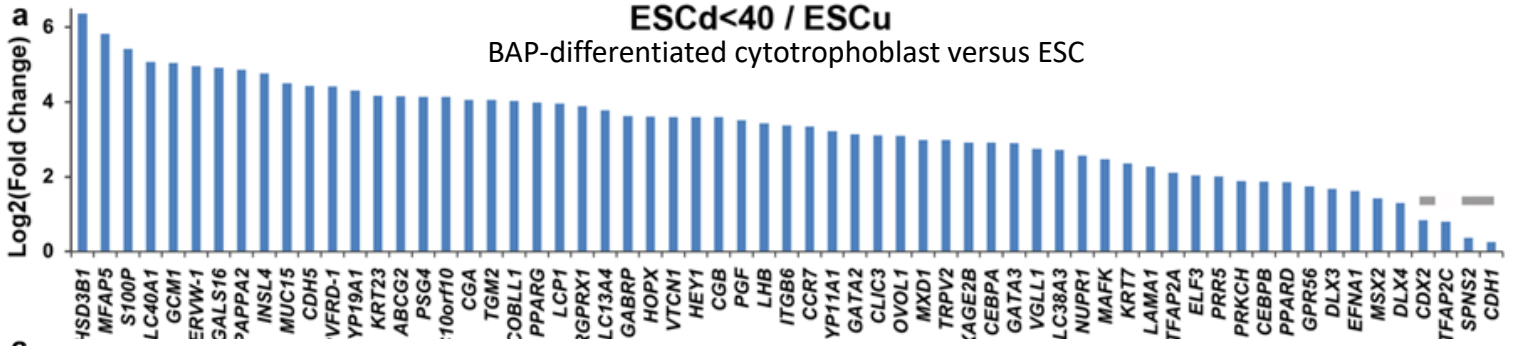


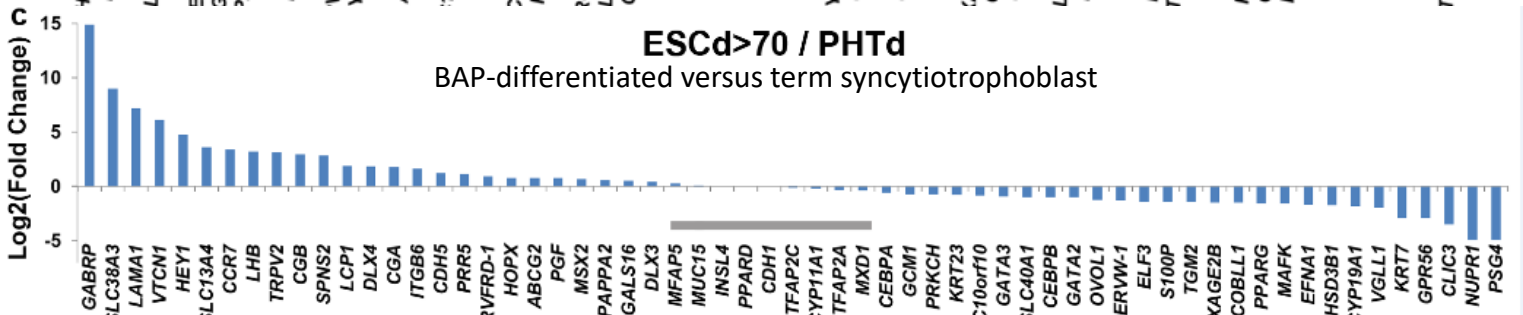


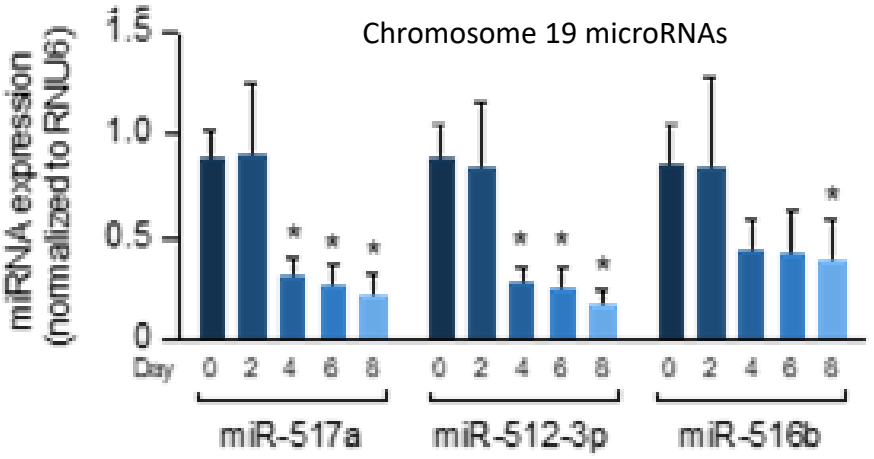
Jain, Ezashi et al. Scientific Reports 2017

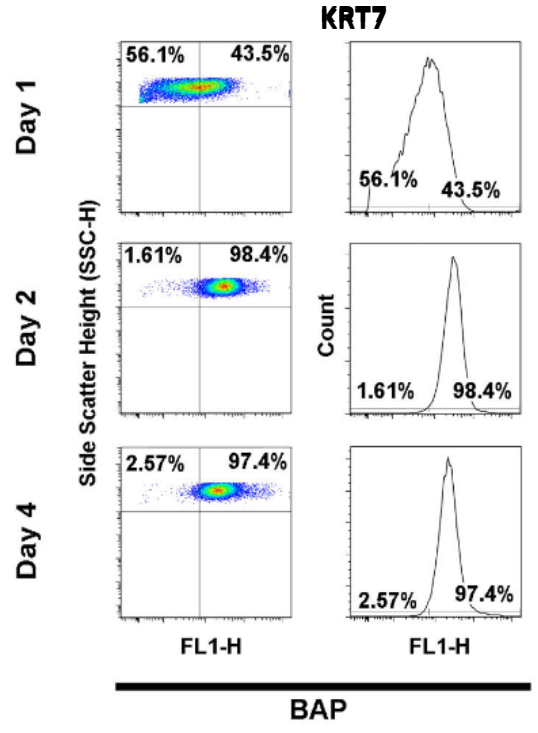
Features of BAP-driven differentiation of ESC/iPSC to trophoblast









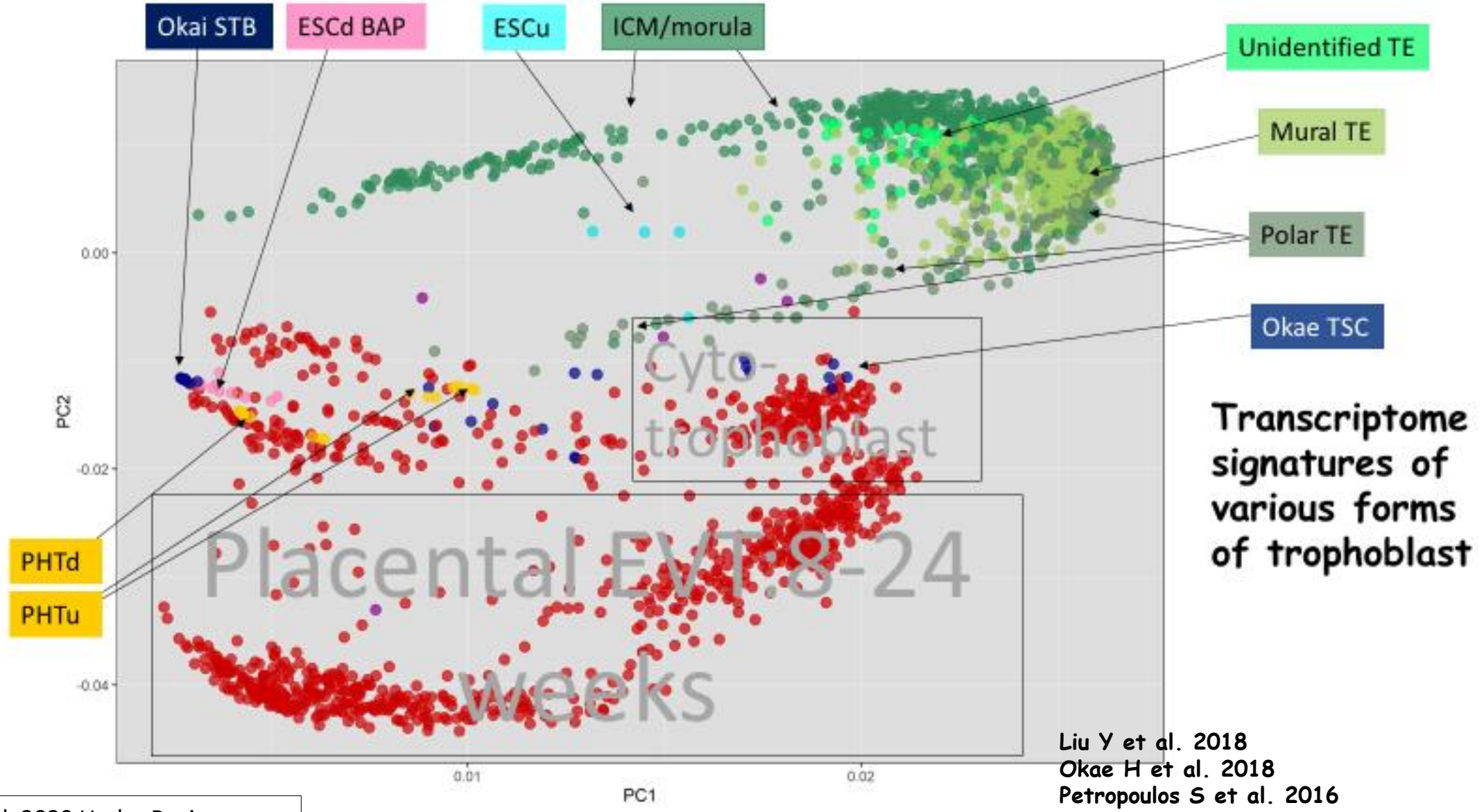


Summary of data collected on the BAP/hESC model

- The BMP4/BAP exposed cells produce a complement of hormones, e.g. P4, hCG, and express a complement of genes consistent with an identity as placental trophoblast.
- At 24 h the cells are transiently CDX2-positive; by 48 h all cells are KRT7 positive but largely CDX2 negative (not fully shown in the presentation)
- HLA-G+ CTB appear at 2-3 days and are maximal at d 6
- Cells exhibit invasive properties
- Syncytium (STB) begins to form after d 5
- The STB and CTB generated have distinct transcriptome profiles from that of villous trophoblast, e.g. DEGs and microRNAs
- The BAP differentiated cells form organoids (not shown)

So, we have trophoblast (and certainly not mesoderm), but what is the nature of this BAP-generated trophoblast?

Transcriptomic landscapes of trophoblasts of different origins



Transcriptome signatures of various forms of trophoblast

Liu Y et al. 2018
Okae H et al. 2018
Petropoulos S et al. 2016
Yabe S et al. 2016

Karvas et al. 2020 Under Review

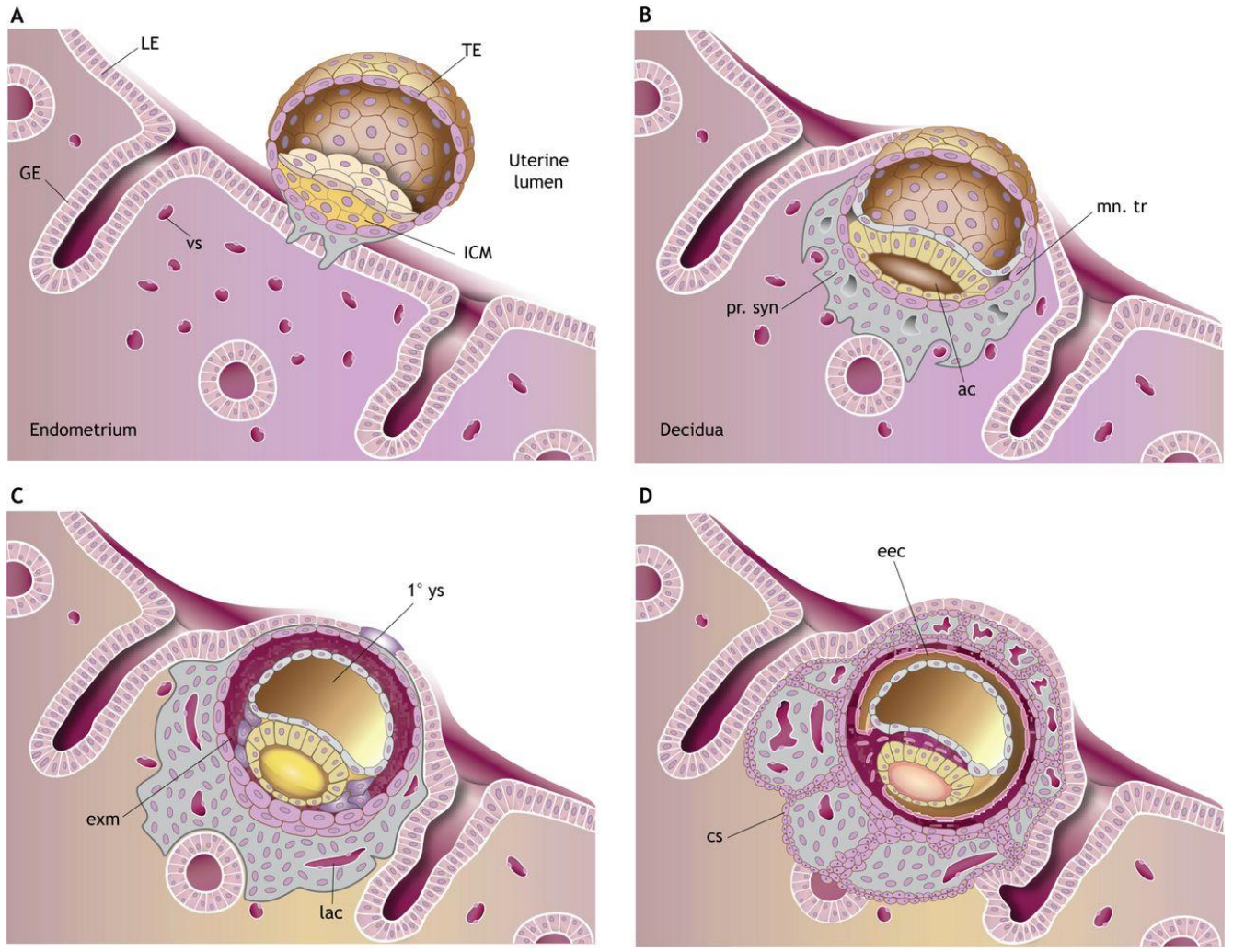
The early stages of human placental development

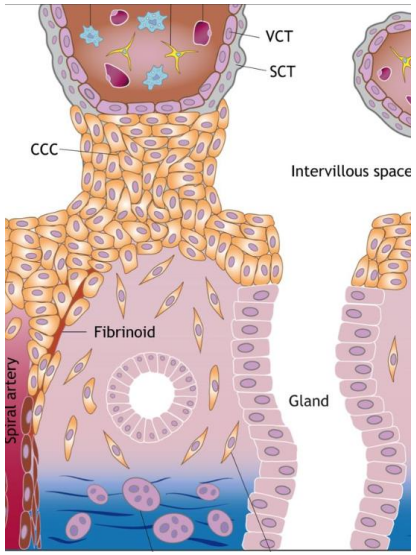
There are three kinds of syncytial trophoblast

a) Invasive syncytiotrophoblast during implantation during weeks 2-3

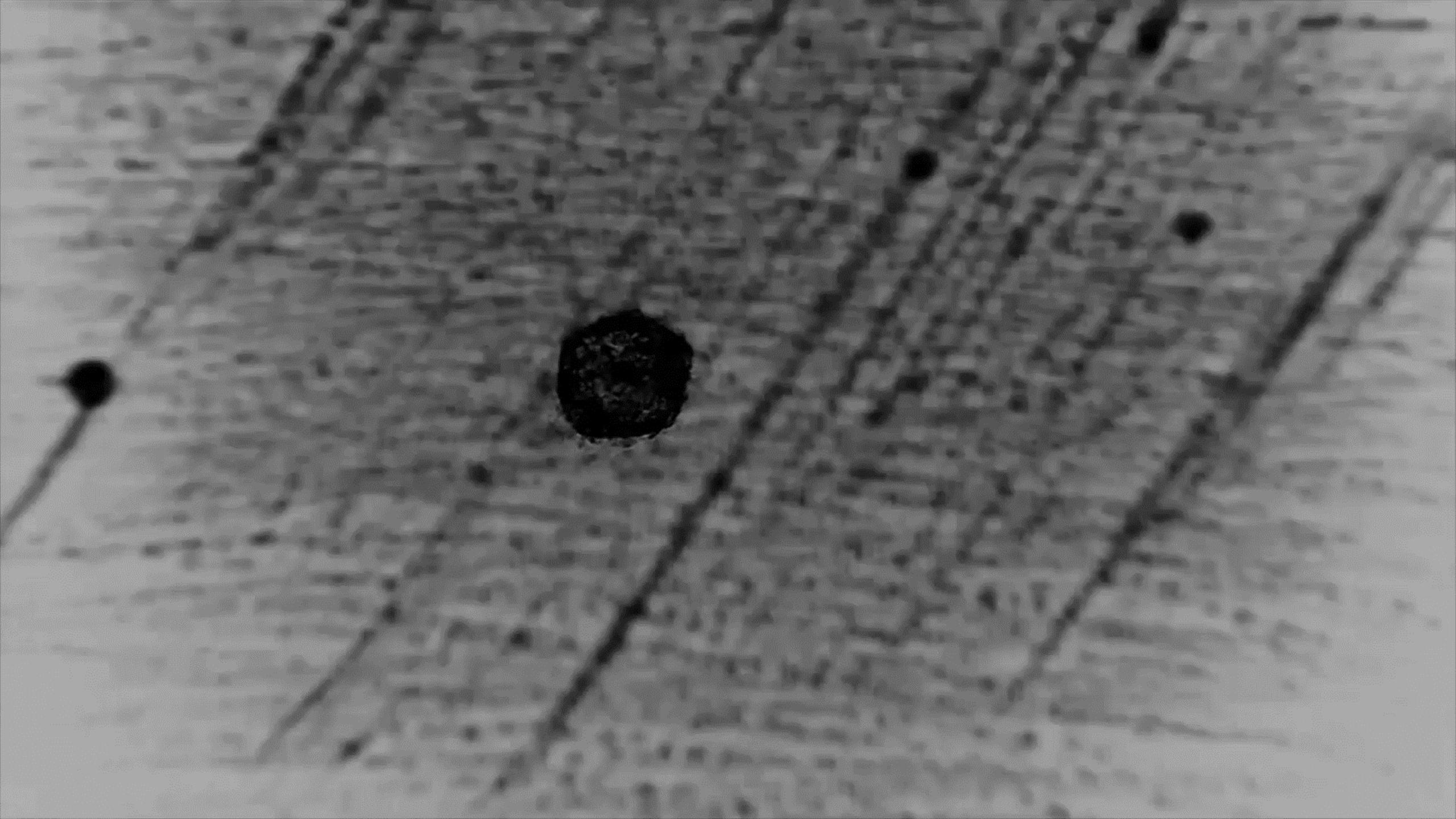
b) Villous (non-invasive) syncytiotrophoblast from week 4 until term

c) Trophoblast “giant cells”, whose origins are unclear but would appear to be invasive

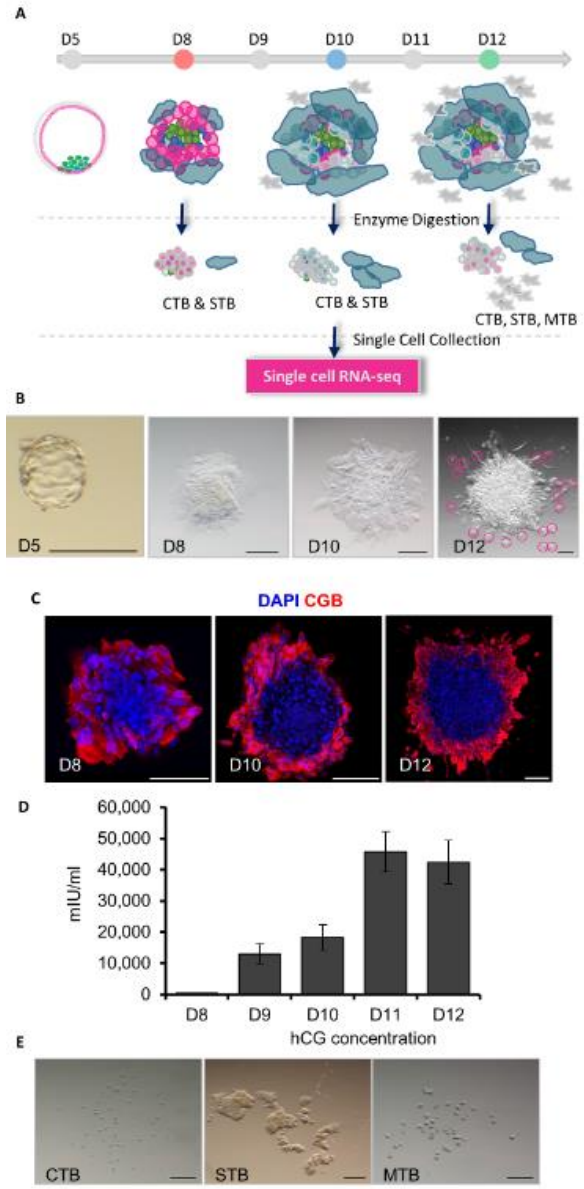


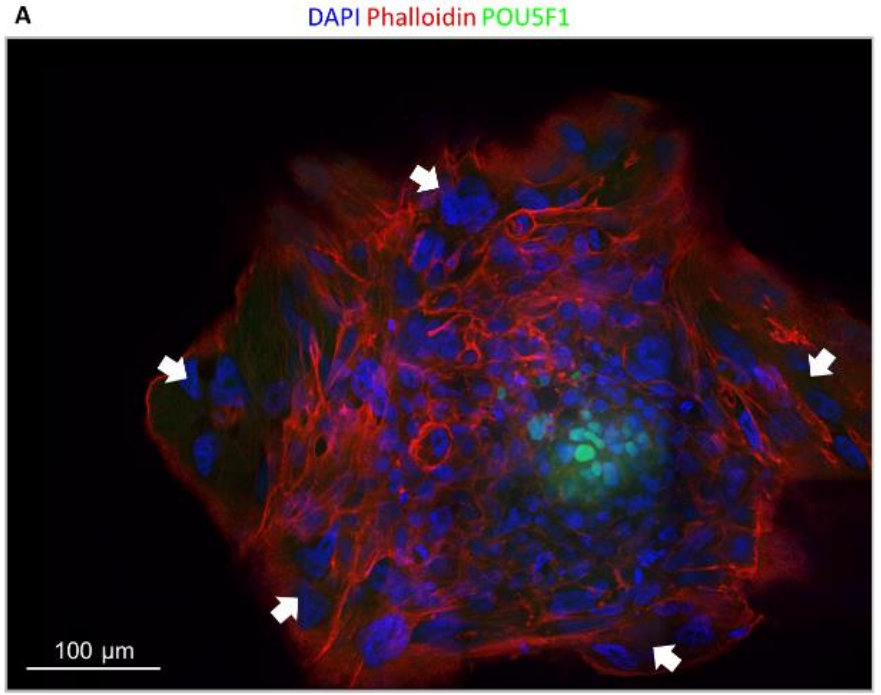


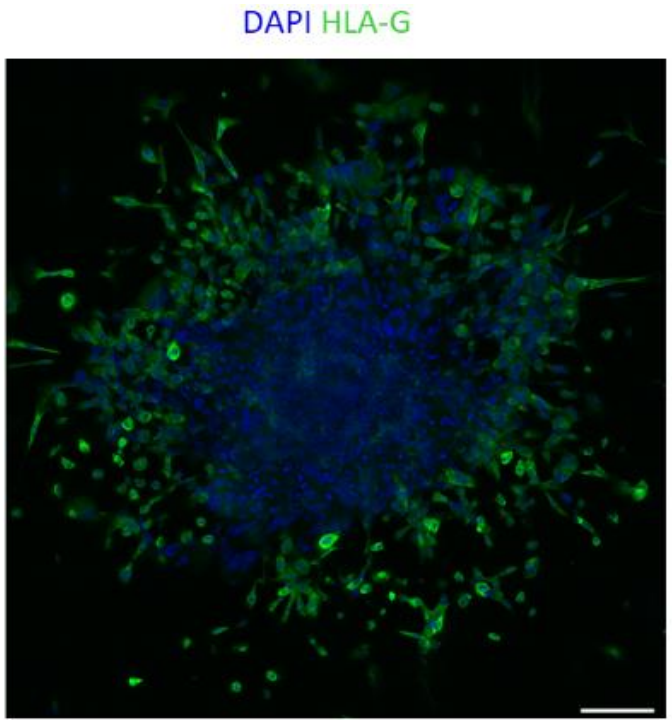
Margherita Y. Turco, and Ashley Moffett Development 2019;146:dev163428

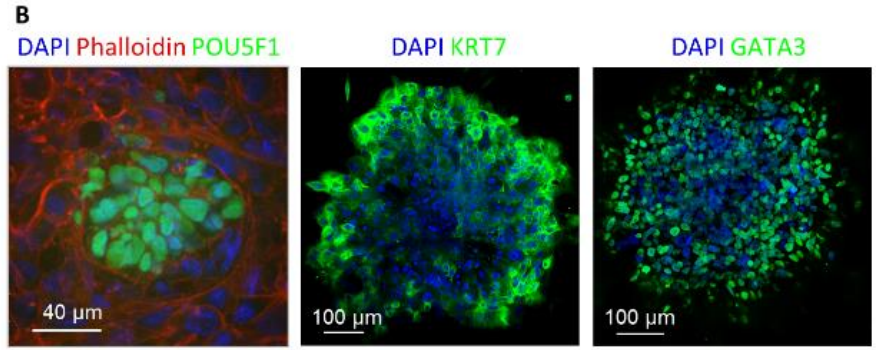


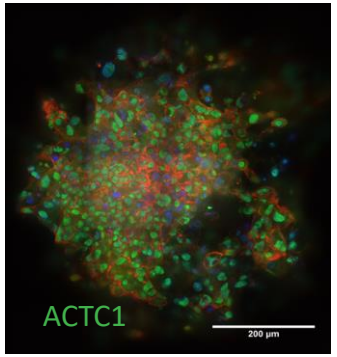
Dynamics of early trophoblast development in cultured human embryos: West et al. PNAS 116 (2019)







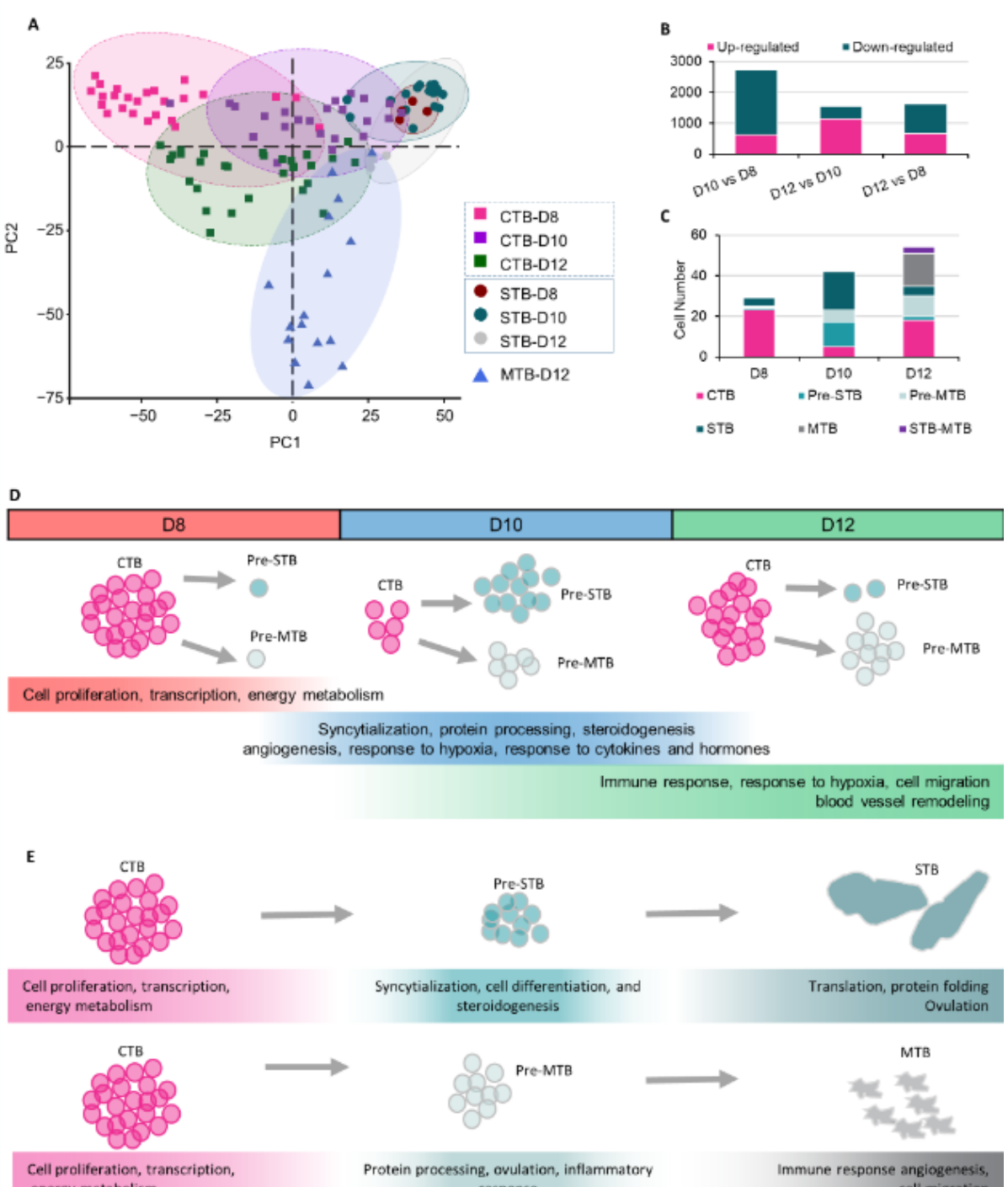




West RC, Ming H et al. 2019 PNAS

Funding and embryos from Colorado Center for Reproductive Medicine, Lone Tree, CO 80124

Dynamics of CTB differentiation



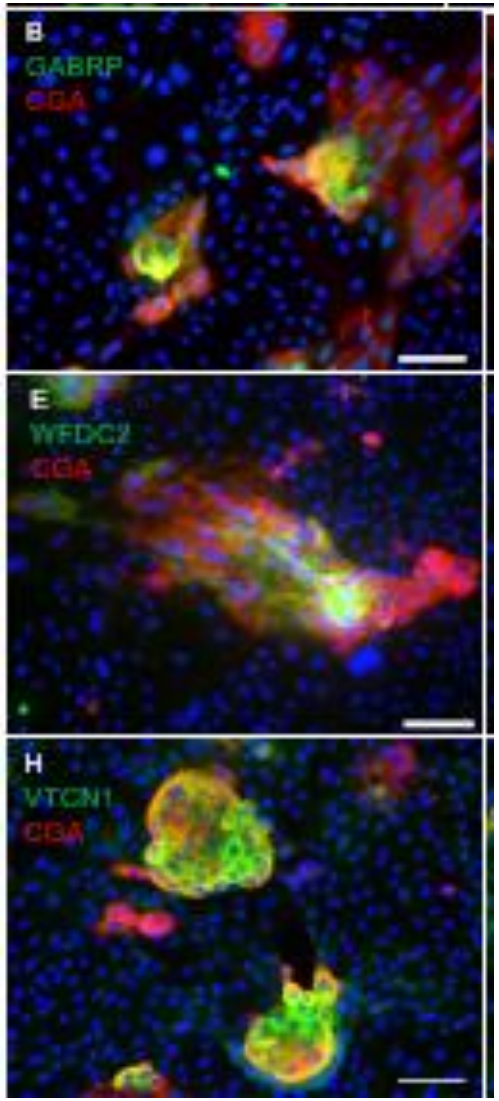
(**A**) PCA of trophoblast cells clustered by cell type and developmental stages. Data suggest than CTB cells are considerably more dynamic than STB in different developmental stages. (**B**) Numbers of differentially expressed genes in CTB from different developmental stages. (**C**) The number of cells in each cell type collected at different developmental stages. (**D**) Diagram to illustrate the switch of cell functions of CTB at different developmental stages as revealed by GO terms and pathway analysis. (**E**) Diagram to illustrate the switch of cell functions of CTB as they undergo differentiation to STB (upper panel) and MTB (bottom panel) as revealed by GO terms and pathway analysis.

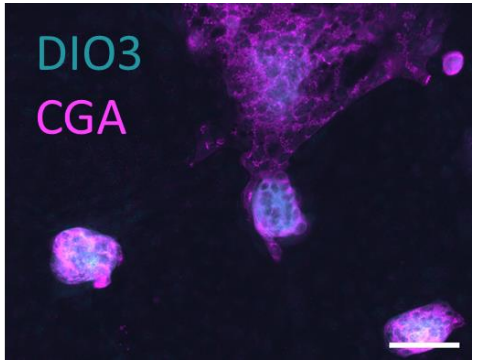
Colorado Center for Reproductive Medicine, Lone Tree, CO 80124

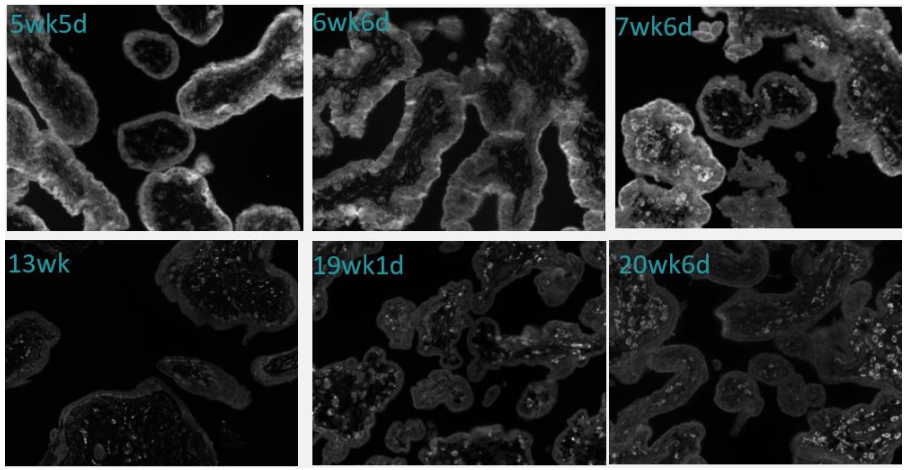
West RC, Ming H et al. 2019 PNAS

Genes highly upregulated in response to BAP that are not expressed in late pregnancy trophoblast

Gene	ESCu	ESCd<40	ESCd>70	PHTu	PHTd
***GABRP**	**1.3**	**1994.8**	**1074.2**	**<0.1**	**<0.1**
DIO3	5.8	248.5	1076.7	0.0	0.0
NTRK2	0.1	142.0	99.1	<0.1	<0.1
NDNF	0.8	151.4	99.7	<0.1	0
CPE	7.8	327.25	227.1	.25	0.1
***VTCN1**	**0.4**	**443.5**	**259.0**	**0.6**	**3.8**
MATN2	3.4	288.0	208.9	0.4	0.2
***WFDC2**	**37.7**	**224.5**	**181.3**	**0.3**	**0.2**
IGFBPL1	21.3	123.1	111.5	0.25	0
***ACTC1**	**56.2**	**388.2**	**273.9**	**0.8**	**0.2**
IGFBP5	1.4	275.1	195.6	0.7	0.2
COL4A5	26.1	242.0	85.2	0.7	1.3
NID2	8.2	237.6	136.3	0.7	0.6
CTHRC1	2.7	286.5	180.7	0.8	0.6
TNC	9.0	299.5	180.3	0.9	1.2
AADAT	8.1	158.8	88.8	0.85	7.6
PXDN	57.8	100.2	99.6	0.6	0.3
SESN3	28.3	134.1	77.1	0.9	1.2
CGB8	0.2	102.1	1008.3	0.8	41.4



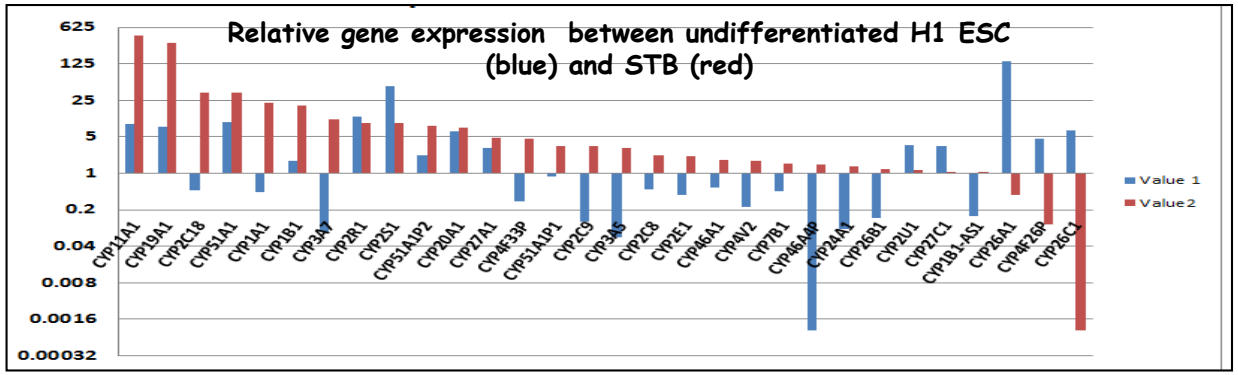




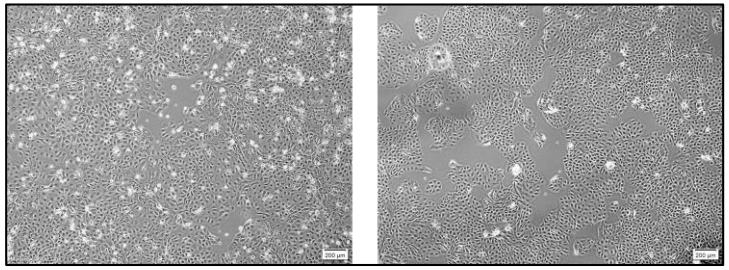
Karvas R, Schulz L et al. Under Review 2020

Other potential uses of trophoblast generated from ESC and iPSC

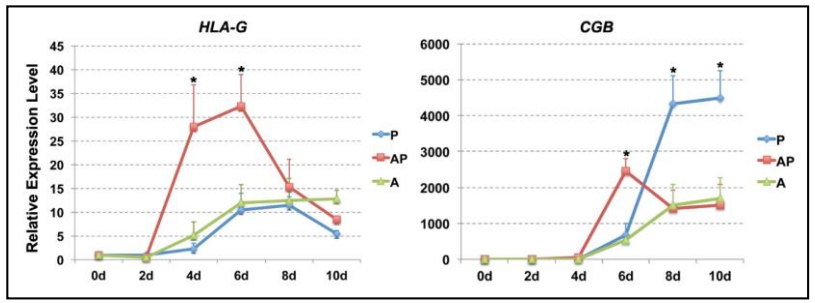
1. Toxicology and responses to foreign agents



2. Generation of trophoblast stem cells

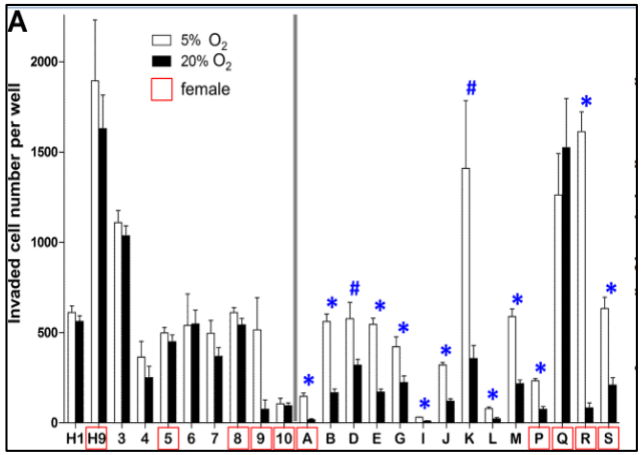


3. Following trophoblast lineage divergence



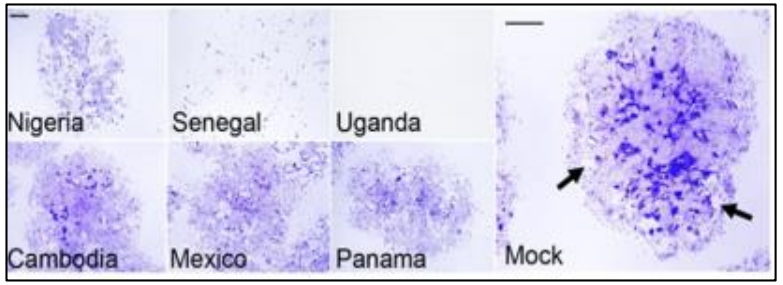
Yang Y, Adachi K et al. PNAS: 2015

4. Studying origins of placental disease, e.g. preeclampsia



Sheridan MA, Yang Y et al. PNAS: 2019

5. Susceptibility of early trophoblast to pathogens, e.g. ZIKV



Sheridan MA, Balaraman V PloS one, 2018

"Failed" Criteria for ESC-Derived Trophoblast: Lee et al. (2016) Stem Cell Reports 6: 257-272

- Hypermethylated *ELF5* promoter
- Lack of expression of C19-encoded miRNA
- Lack of expression of HLA-G and uncharacteristic HLA profile
- Lack of certain "positive trophoblast markers"

Conclusion: BMP-treated human ESC have not fully differentiated to trophoblast

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Colorado Center for Reproductive Medicine, Lone Tree, CO 80124

HLA expression during human embryo culture from d 8 until d 12