

Modeling trophoblast differentiation using “pluripotent” stem cells

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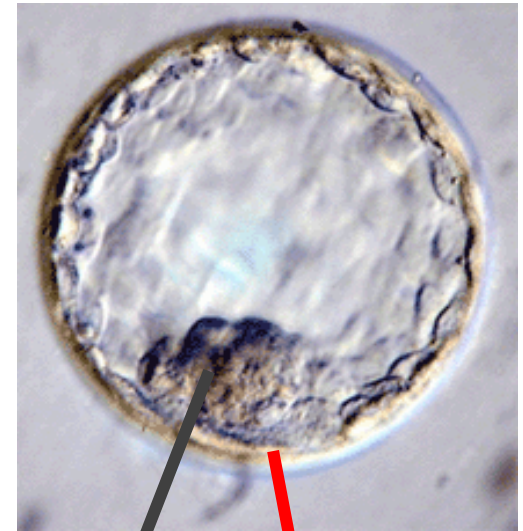
University of California San Diego

Definitions: stem cells

- “Pluripotent”: ability to give rise to embryonic and some extraembryonic cells/tissues
 - Extraembryonic endoderm and mesoderm only
- “Totipotent”: ability to give rise to embryonic and all extraembryonic cells/tissues
 - Including extraembryonic endoderm, mesoderm, and ectoderm (trophoblast)

Conclusions from rodent studies

- ESC from pre-implantation ICM
 - Embryo-proper
 - Extraembryonic endoderm and mesoderm
- TSC from trophectoderm
 - Contribute only to trophoblast (extraembryonic ectoderm)



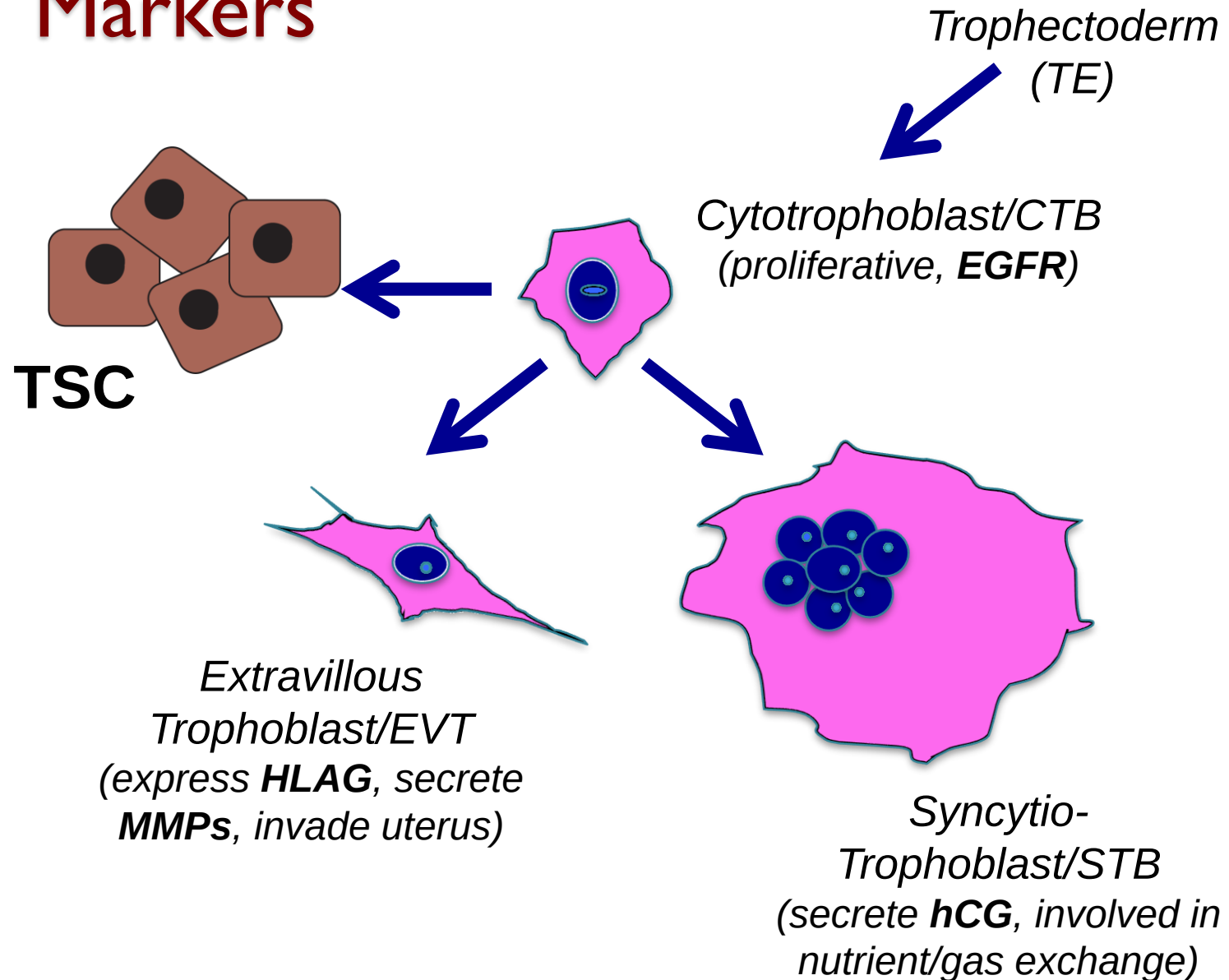
ESC

TSC

Definitions: TSC

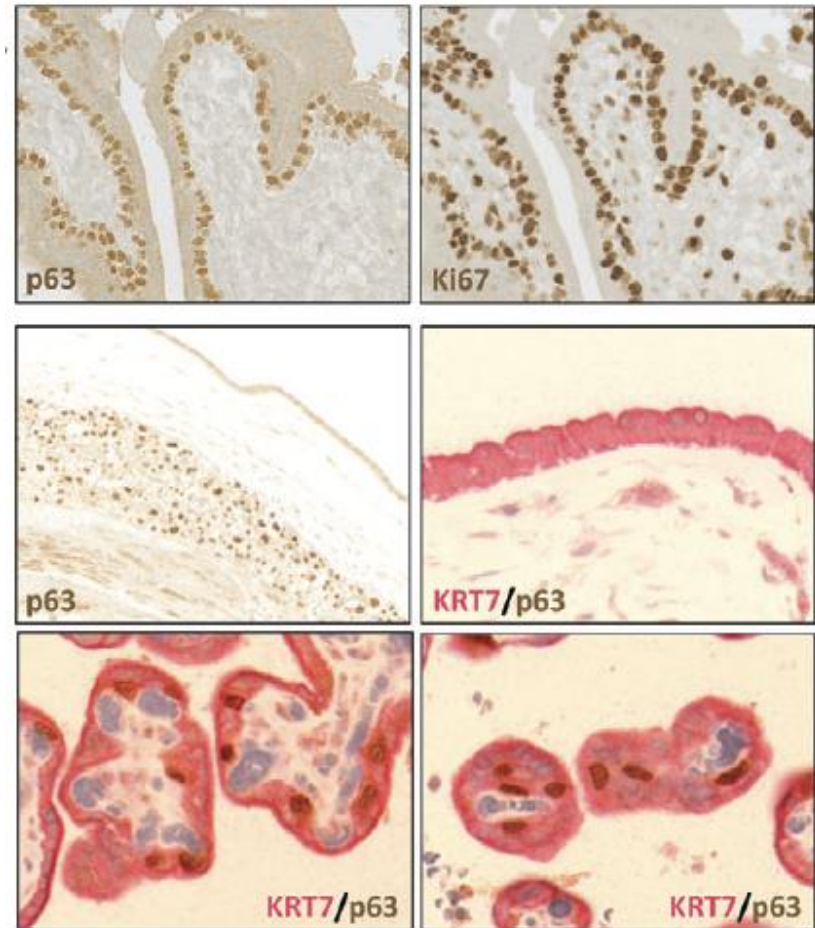
- Mouse: TSC (FGF4/ActA; E3.5-E8.0)
 - labyrinthine trophoblast (SynT-I, SynT-II, sinusoidal giant cell) and junctional zone (spongiotrophoblast and trophoblast giant cells)
- Human: TSC (Wnt, EGF, TGF β inhibitor; blastocyst and first trimester placenta)
 - Part of CTB progenitor layer
 - Gives rise to syncytiotrophoblast (STB) and extravillous trophoblast (EVT)

Human trophoblast differentiation: Markers

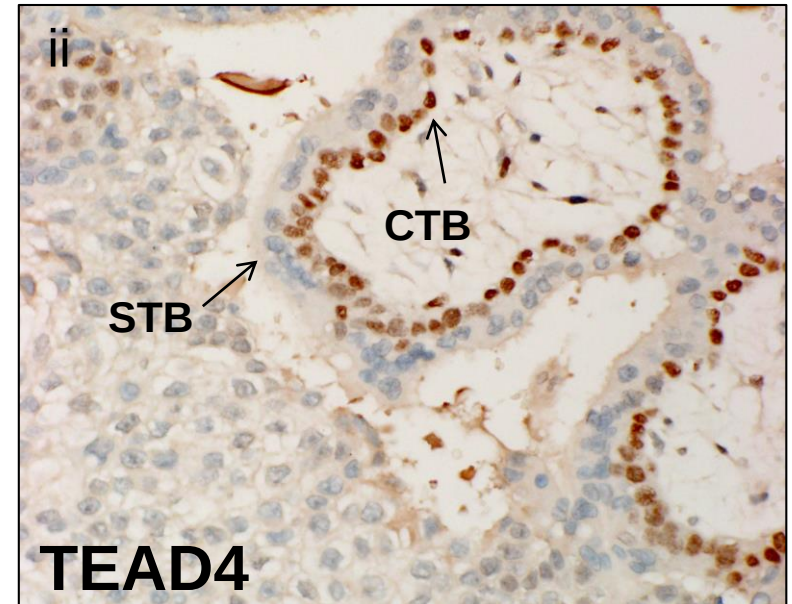
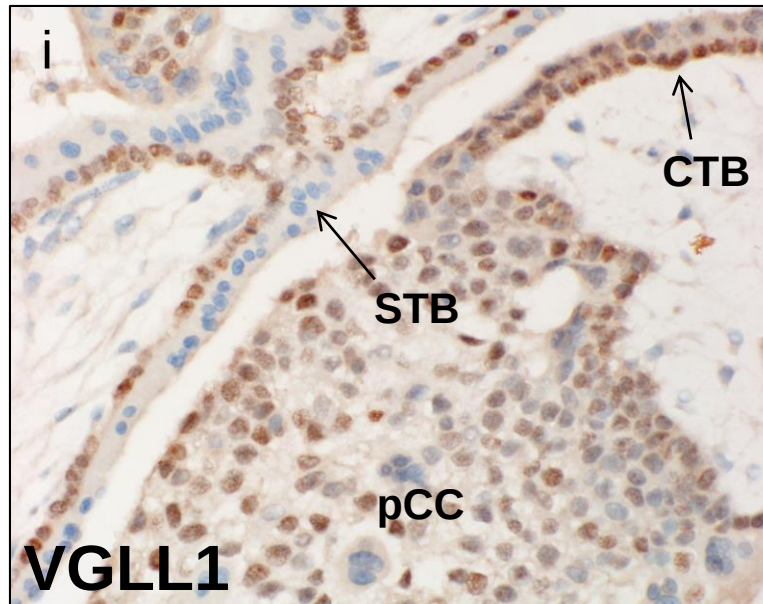


Unique markers of human CTB/TSC

- **p63**
- p53 family of nuclear proteins
- Involved in maintenance and differentiation of stem cells in stratified epithelia
- Expressed in all proliferative CTB (Lee et al., 2007)
- Not expressed in amnion



Unique markers of human CTB/TSC: VGLL1



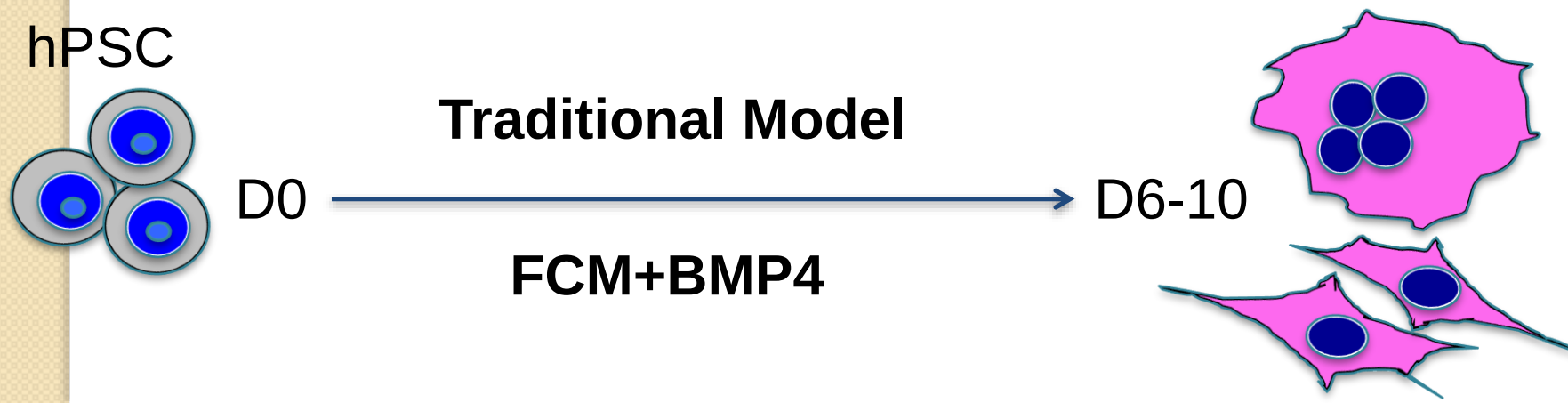
Binding partner for TEAD proteins: possible role in induction of p63/trophoblast lineage

Previously-established models of human trophoblast differentiation

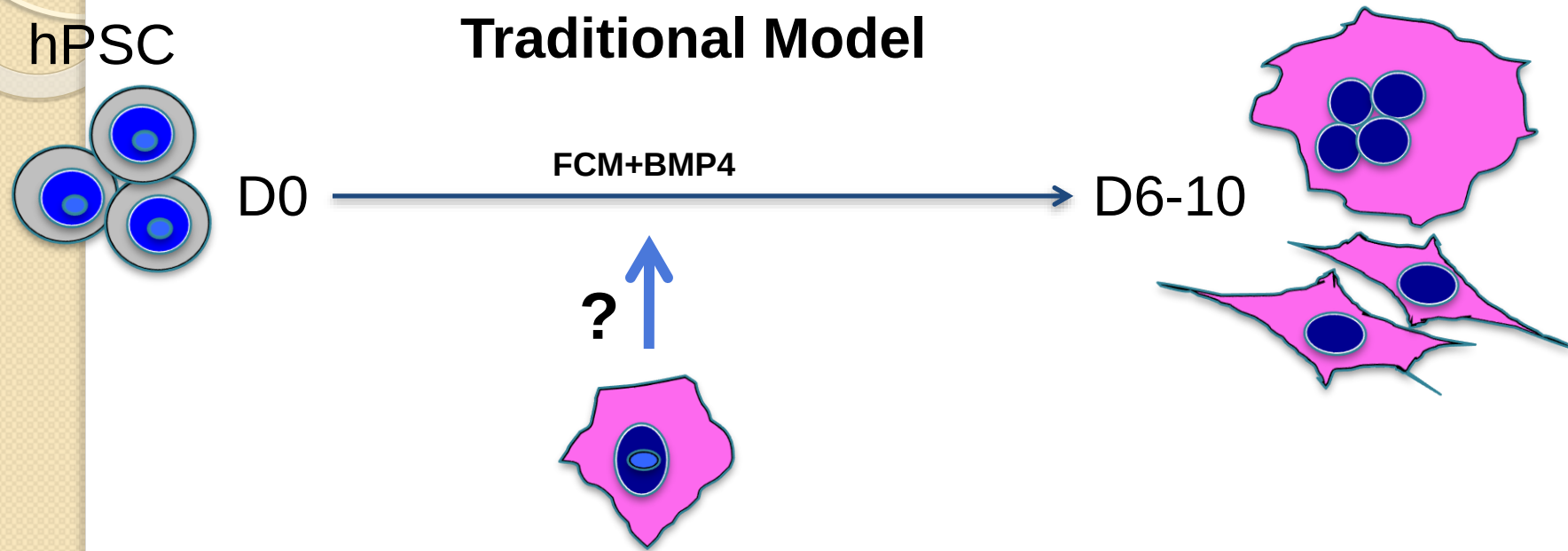
- Immortalized troph/chorioCA cell lines
 - JEG3, HTR8, JAr, BeWo
 - Not fully representative of the in vivo cell type
- Primary cells
 - Tissue availability: mostly term placentas
 - Term CTB can only differentiate into STB
 - First trimester CTB can differentiate into both STB and EVT, but
 - ***Ethical concerns***

Human pluripotent stem cells as a model of trophoblast differentiation

- Embryoid body-derived trophoblast on Matrigel (3D)
 - Giakoumopoulos and Golos, J Endocrinol, 2013
- BMP4 treatment of hPSC (2D)
 - Xu et al. Science, 2002
 - ~12 other groups
 - Roberts et al. Biol Reprod, 2018

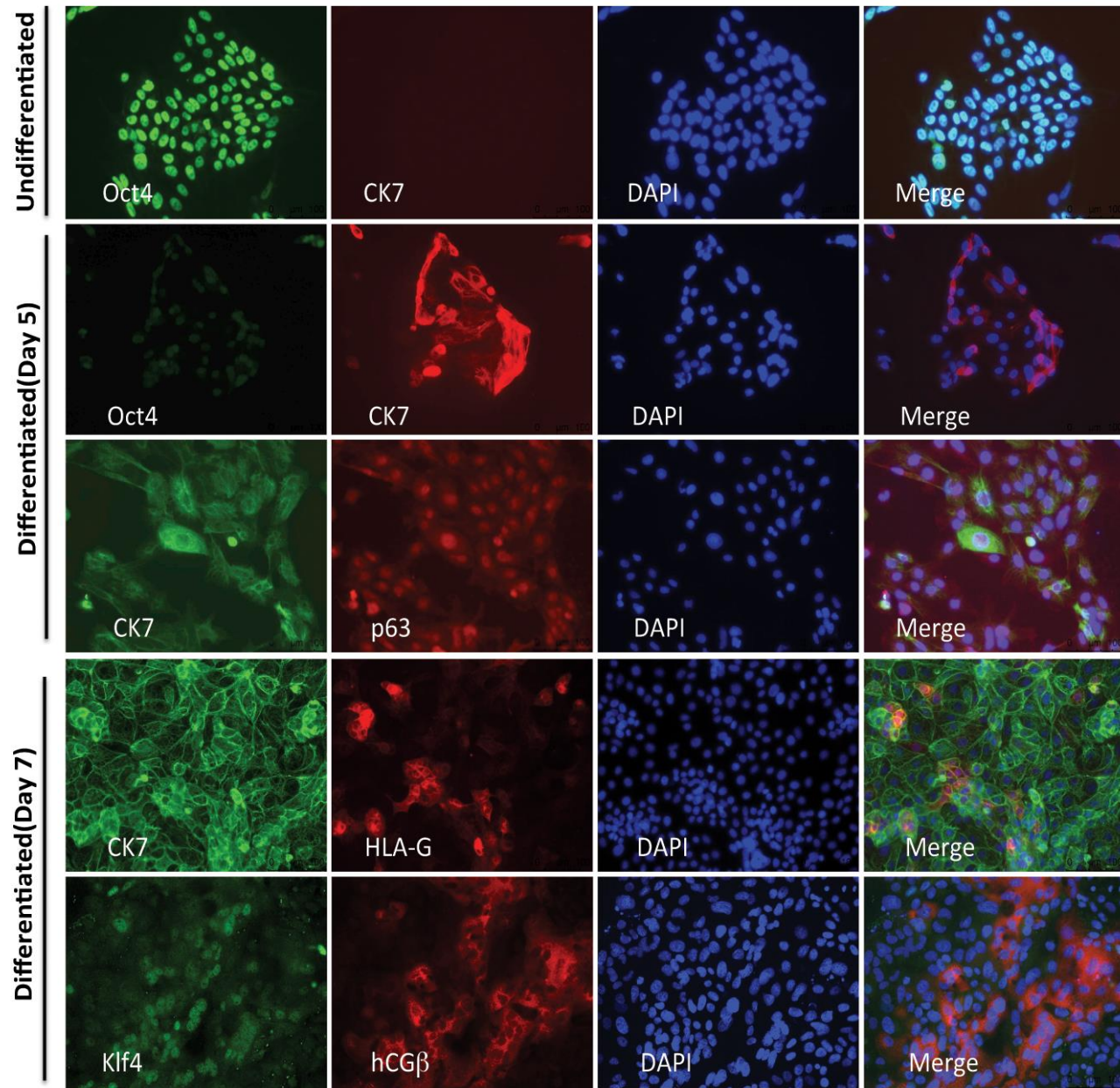


Human pluripotent stem cells as a model of trophoblast differentiation

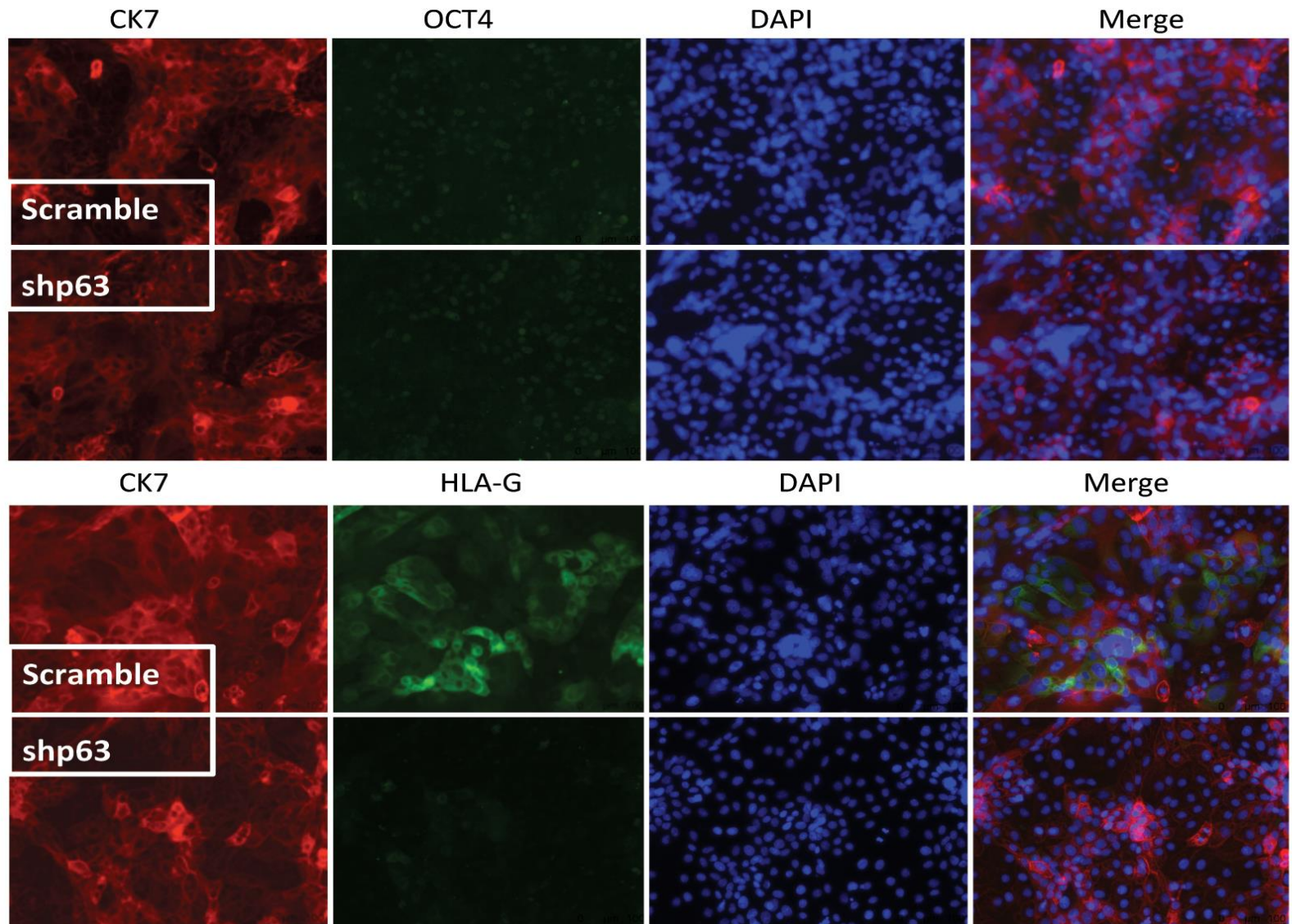


- Developmentally accurate?
 - **hESC** → **CTB** → terminally differentiated trophoblast (**STB/EVT**)

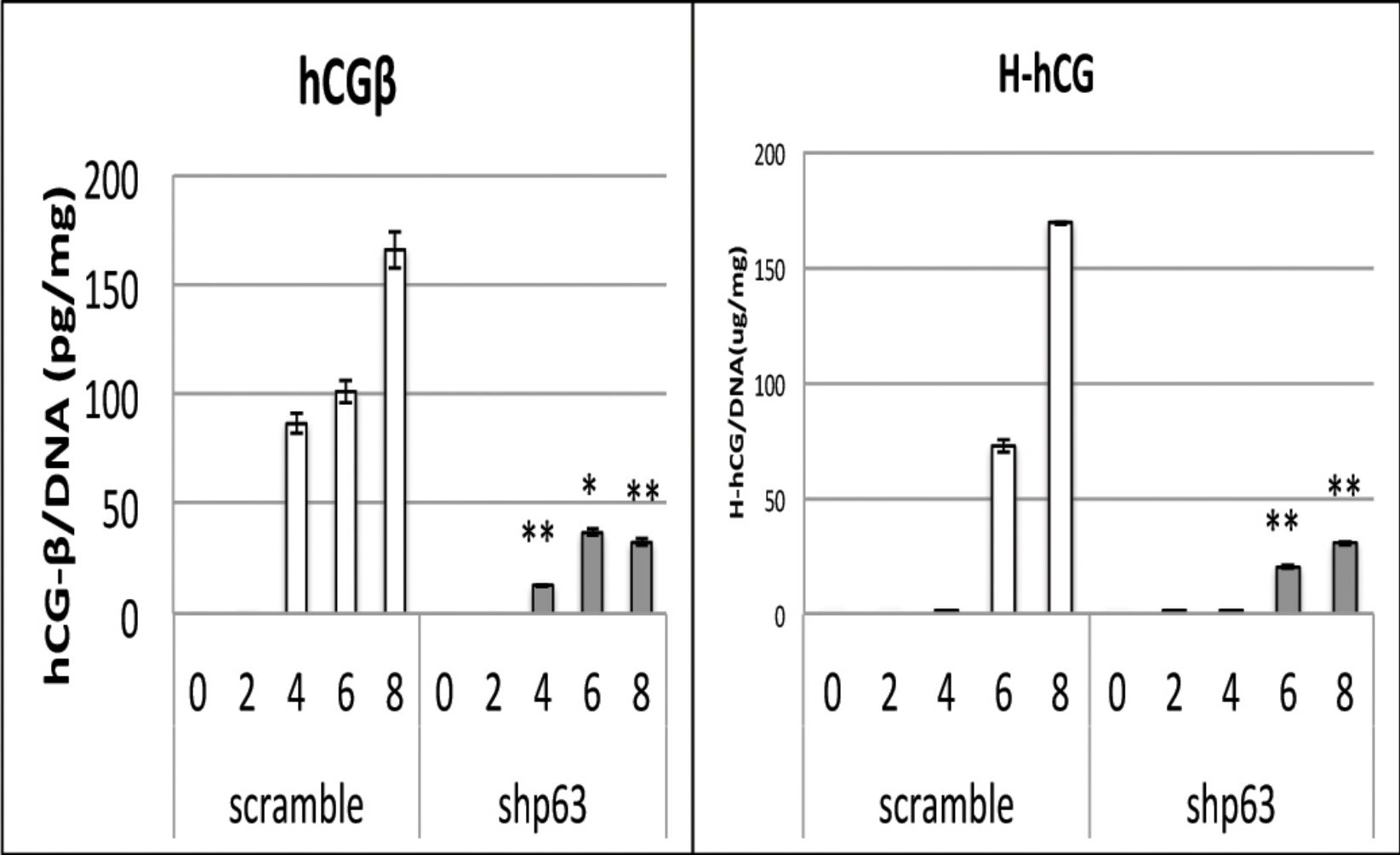
hESCs following BMP4 treatment



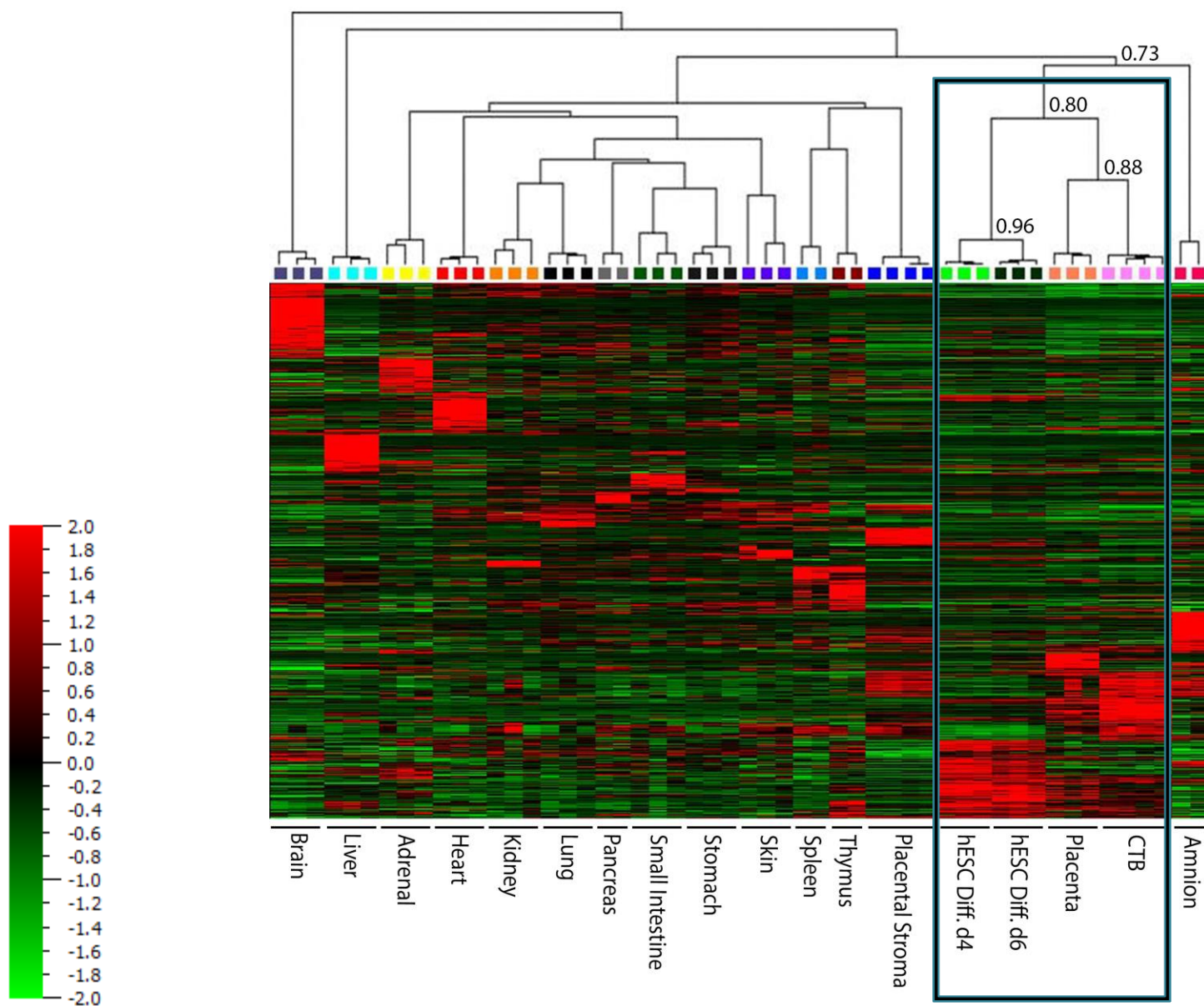
Downregulation of p63 inhibits trophoblast differentiation of hESCs



Downregulation of p63 inhibits trophoblast differentiation of hESCs

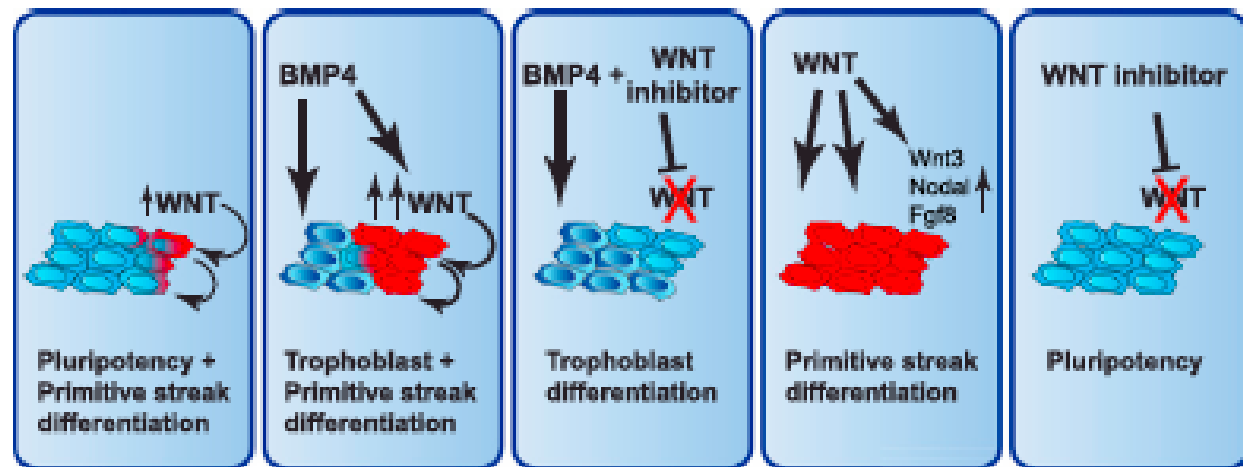


Comparison to other fetal and placental cells/tissues

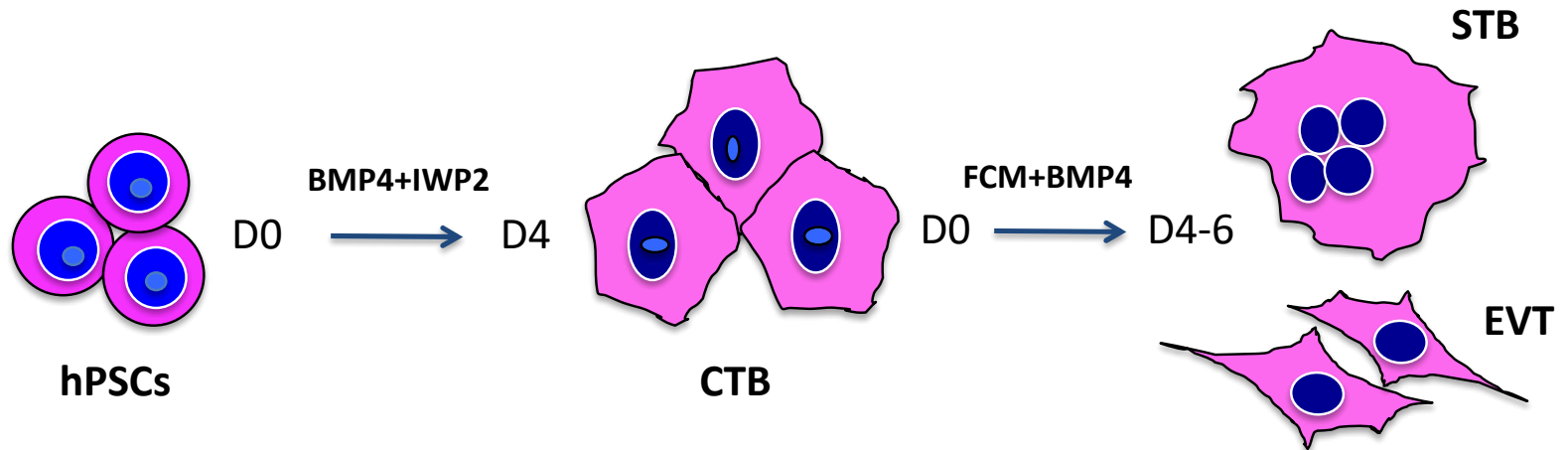


“BMP4 also induces mesoderm”

- BMP4 induction of mesoderm is Wnt-dependent, while induction of trophoblast is Wnt-independent



Development of a “step-wise” trophoblast differentiation model

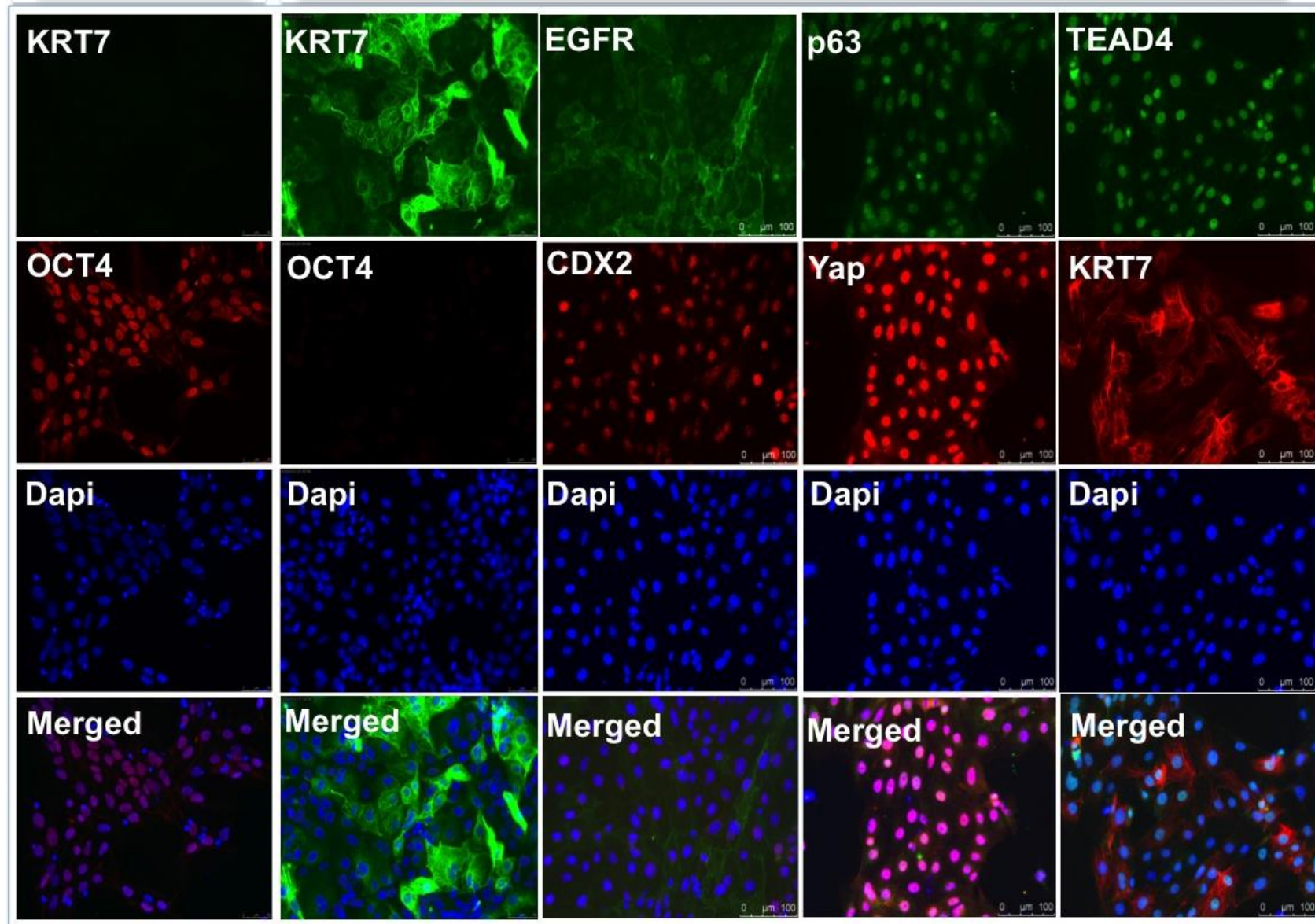


- Consistent and uniform differentiation of hPSCs into CTB
- Separation of trophoblast lineage specification from terminal trophoblast differentiation

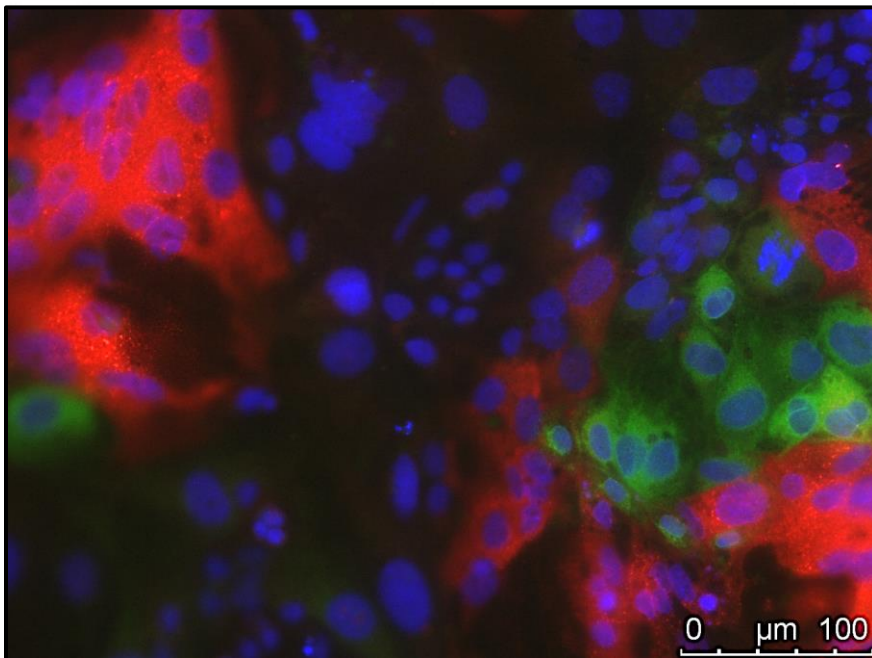
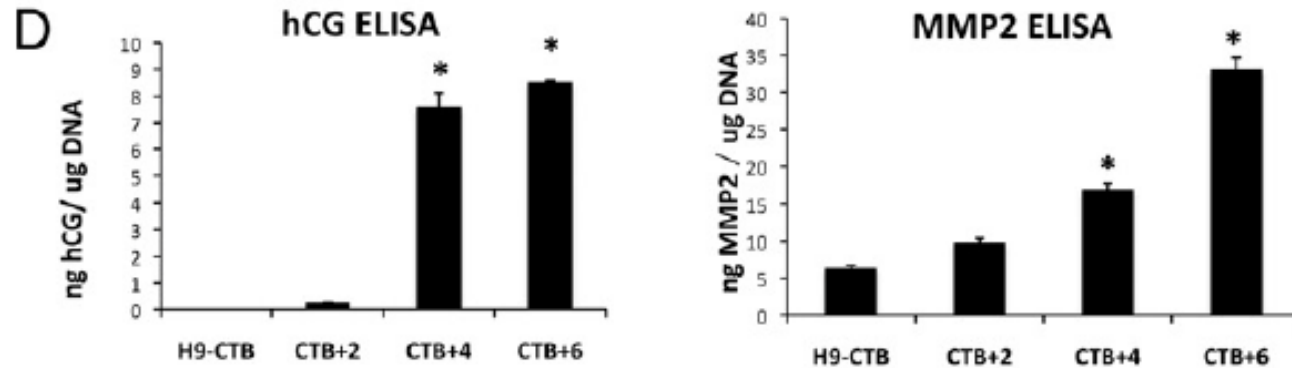
Step I: CTB induction

Day -2

Day 4



Step II: Terminal differentiation of hPSC-derived CTB



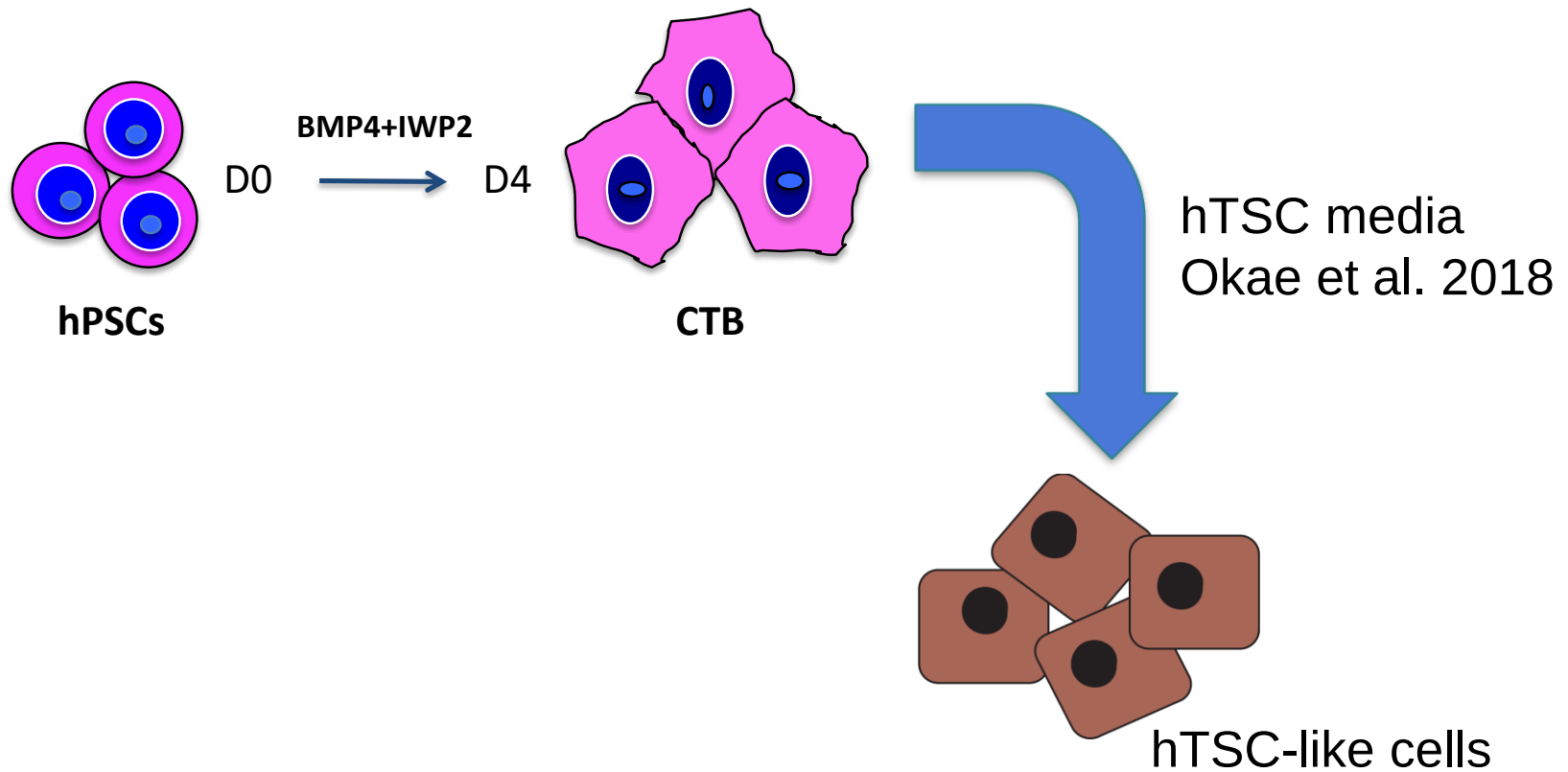
hCG (majority)
HLAG (5-20%)

Horii et al., *PNAS*, 2016;
Horii et al. *Curr Prot Stem Cell Biol*, 2019

An improved method to evaluate hPSC-derived trophoblast?

- hPSC-derived CTB can recapitulate primary CTB in:
 - Response to low oxygen (inhibition of STB and HIF-dependent induction of EVT)
 - Trisomy 21 (blunted STB differentiation, rescued by treatment with Activin A)

hTSC-like cells can be derived from hPSCs



hPSC-derived trophoblast: real or not real?

- Human-specific?
 - BMP4 induces some trophoblast markers in mESC and mEpiSC
- Only an in vitro phenomenon?
 - “Transdifferentiation”
 - In vivo “stress” conditions where ESC-EpiSC-derived cells can contribute to trophoblast?
- hPSC (or subpopulation) are ***totipotent***
 - mESC cycle between totipotent (2C) and pluripotent ESC state (Macfarlan et al. 2012)

Thank you!

- Parast lab

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- Collaborators

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