Human Pluripotent Stem Cells, the Human Embryo, and The Self-Renewing State..

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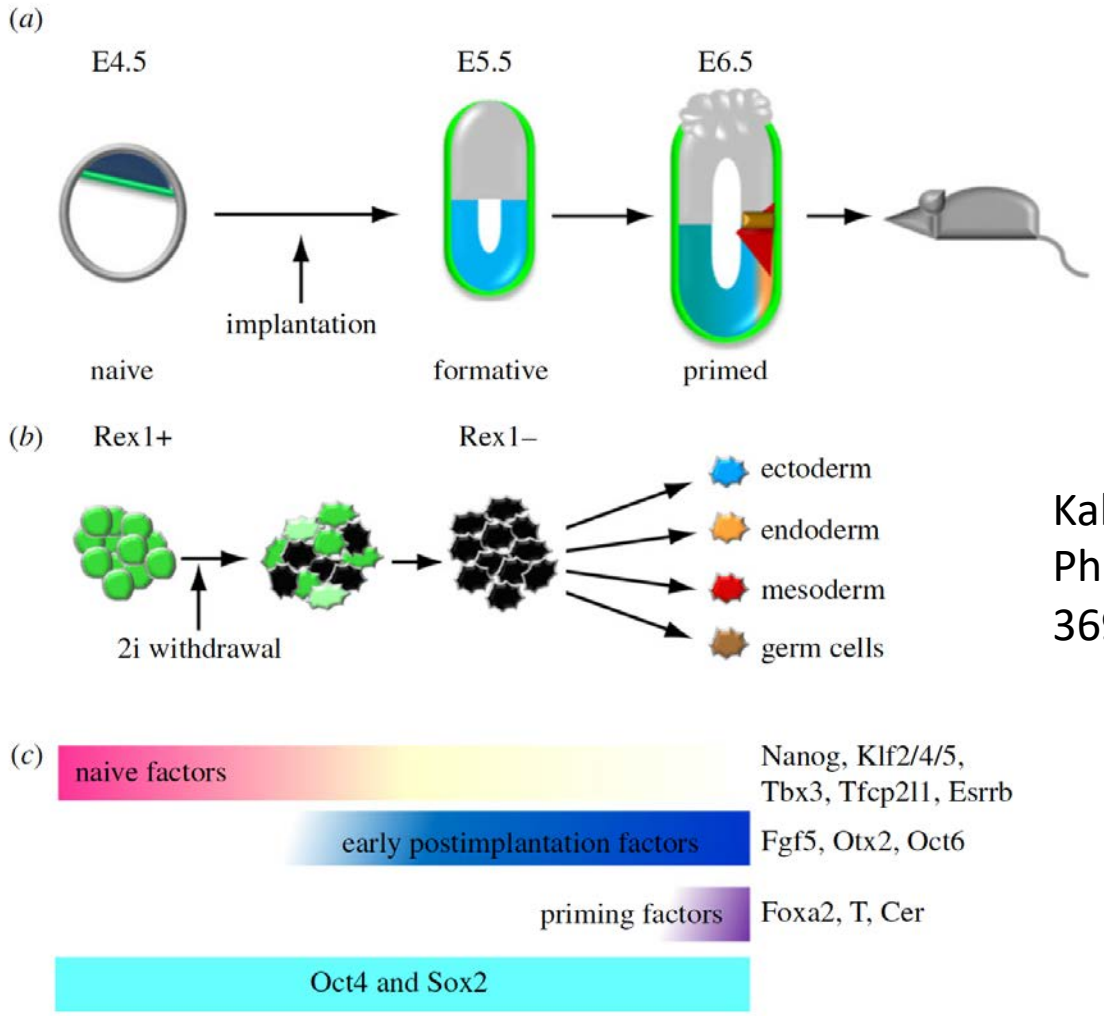
The Jackson Laboratory

What is the developmental status of human PSC?

What stage of mammalian development do hPSC resemble most nearly?

Understanding Pluripotent Cells in a Developmental Context

- Importance for comparative studies of early mammalian embryology
- Important if we are to use human cells as models of human embryonic development
- Important in terms of understanding how to direct lineage specification and differentiation
- Not clear whether we not starting with the optimal pluripotent state, or whether we are working with material that is epigenetically off center



Kalkan and Smith
Phil. Trans. Royal Soc.
369, 20130540 2015

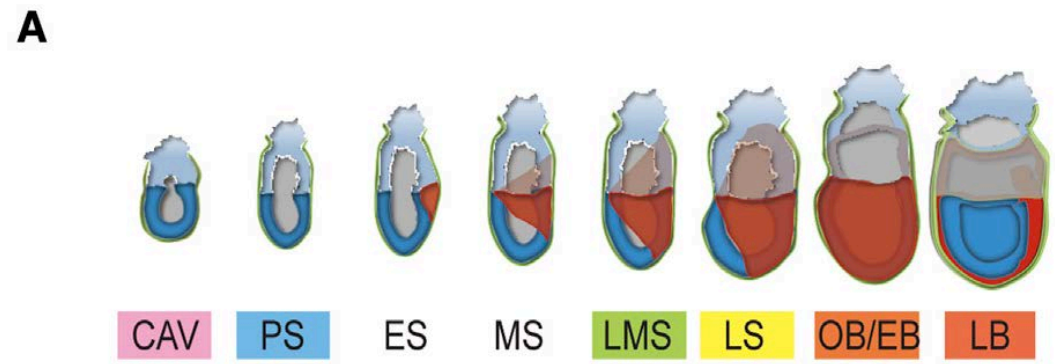
Naïve cells are mouse ES cells maintained under conditions that strongly suppress differentiation (=E4.5 epiblast)

Epiblast stem cells (EpiSC) correspond to late primitive streak (E7.5)

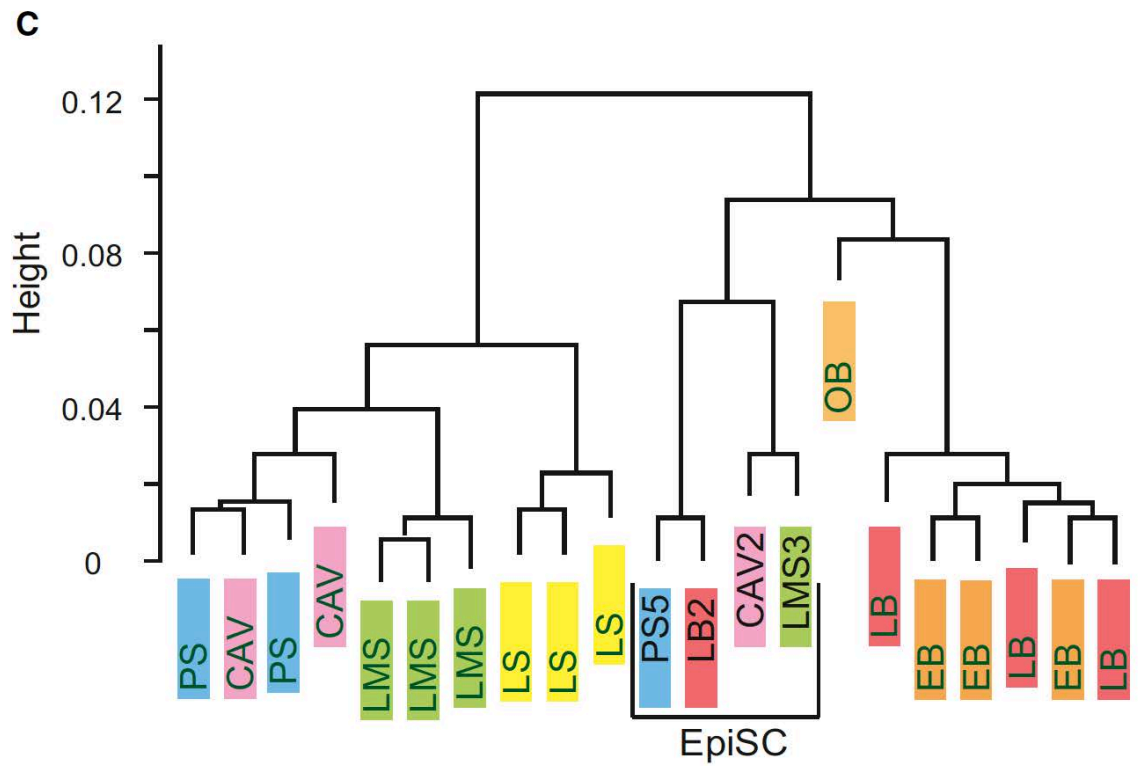
EpiLSC or epiblast like stem cells are an unstable cell type that corresponds to formative pluripotency-the early postimplantation epiblast (E5.5)

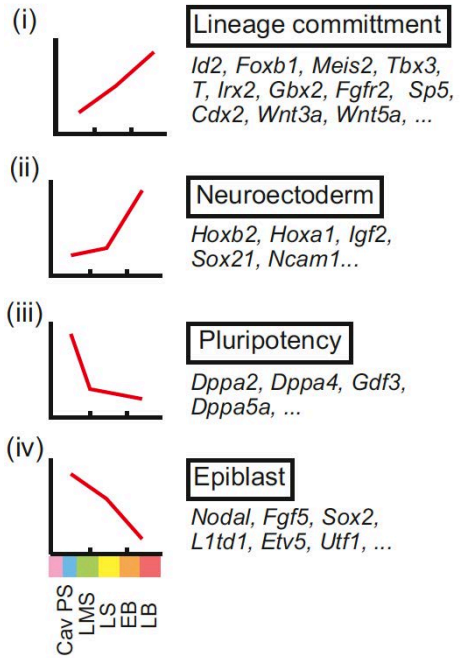
Mouse Embryonic Stem Cells: Naïve or Ground State Pluripotency

- A stage in embryonic development at which the pluripotent cells are capable of forming all somatic tissues and the germ line and are lacking in any bias towards differentiation into any particular fate-the preimplantation epiblast (Ying and Smith)
- Maintained in vitro by strong pharmacologic blockade of signaling pathways that drive differentiation
- In the mouse, only naïve pluripotent cells are capable of generating germ line chimeras



Mouse EpiSC correspond to late gastrula stage embryo and show lineage priming





Kojima et al. Cell Stem Cell 14: 107, 2104

Human versus Mouse PSC

- Human ES and IPS cells resemble in some respects mouse epiblast stem cells, not mouse ES cells, and are widely considered to be equivalent to them
- This interpretation is based on relatively limited interspecies comparative data and does not take into account heterogeneity in stem cell populations

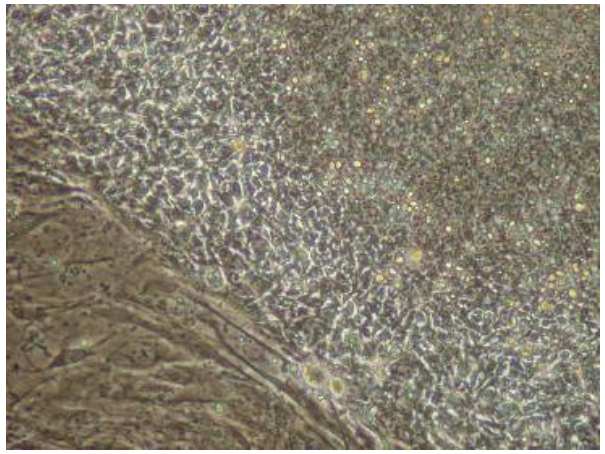
Human Naïve and "Primed" Cells

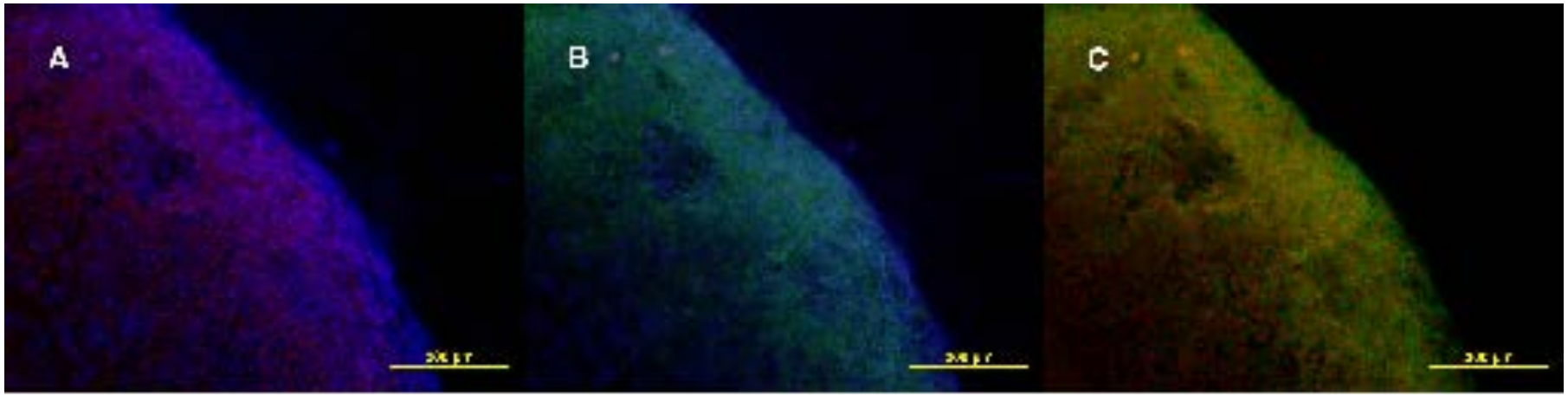
- Human ES and IPS cells resemble in some respects mouse Epiblast stem cells, not mouse ES cells
- Human naïve PSC equivalent to mouse naïve cells have not yet been described, though some cell lines are similar to human pre-implantation epiblast
- Maintenance of genetically stable human naïve PSC has proven problematic; mouse naïve cells may be epigenetically unstable under some conditions
- The embryonic equivalent of the stable attractor state represented by human teratocarcinoma and embryonal carcinoma and and conventional hPSC (ES and iPSC) remains unknown

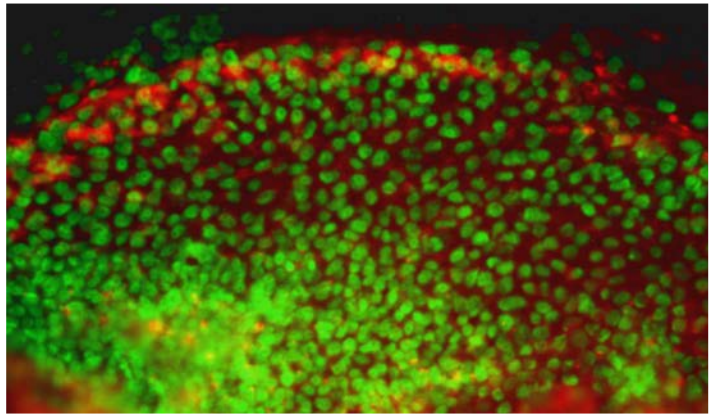
Heterogeneity in ES cultures complicates assessment of developmental status

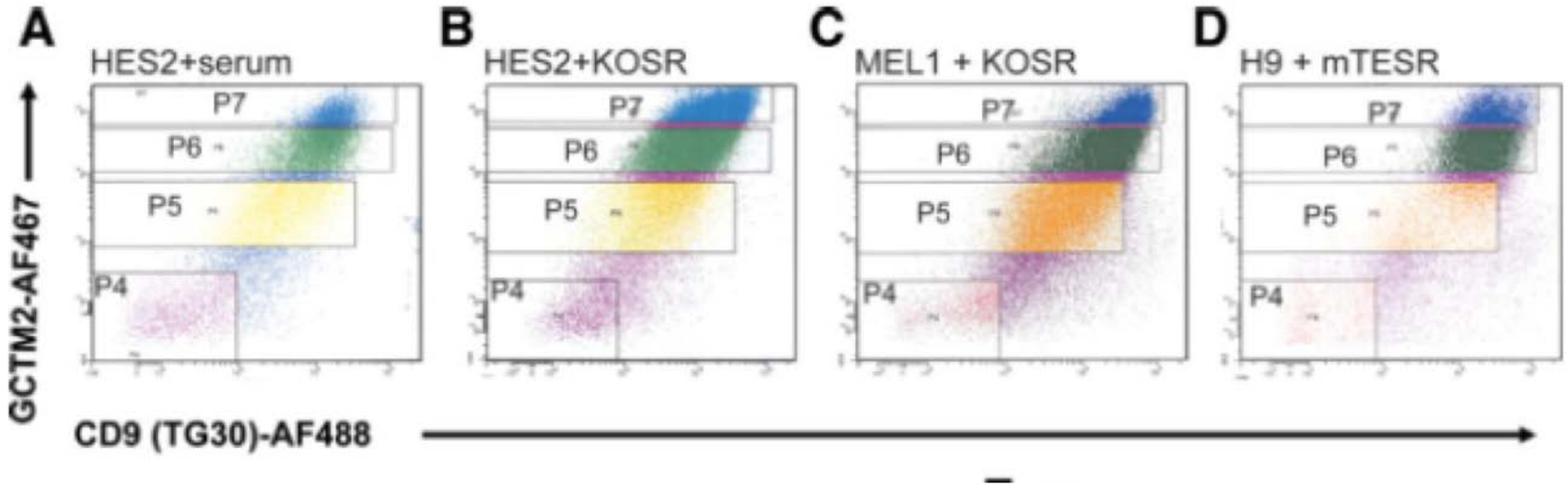
Subpopulations of pluripotent cells with

distinct biological properties and gene expression



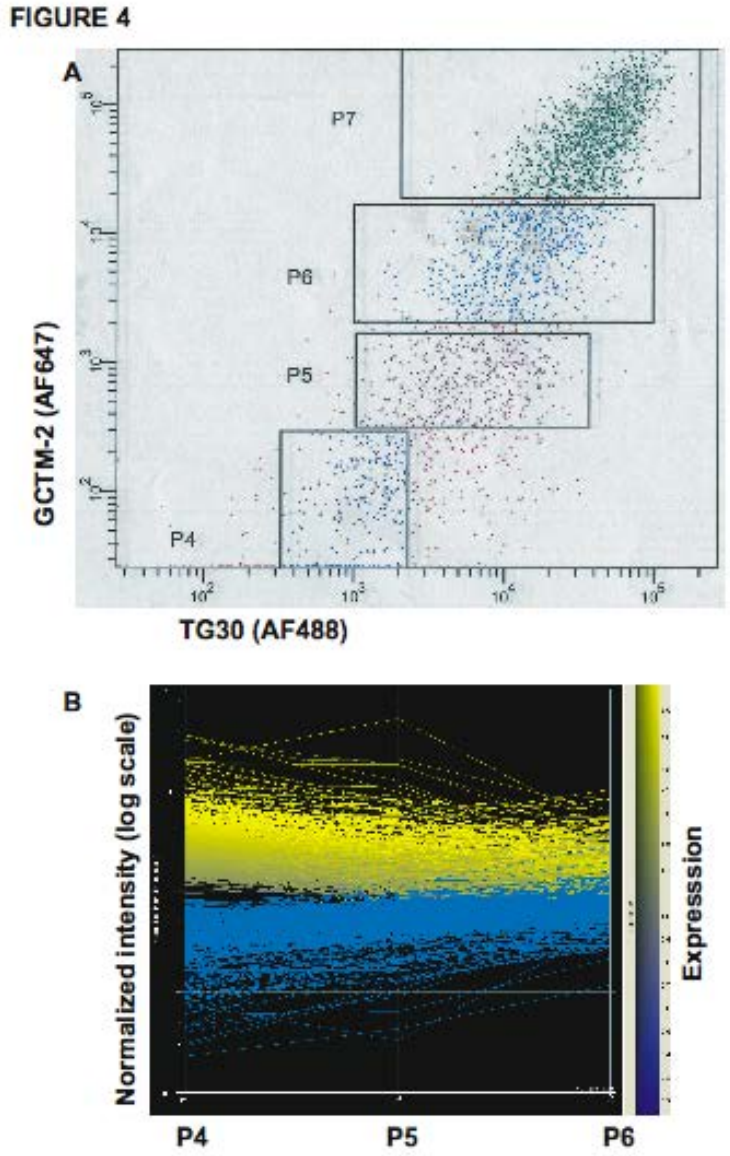






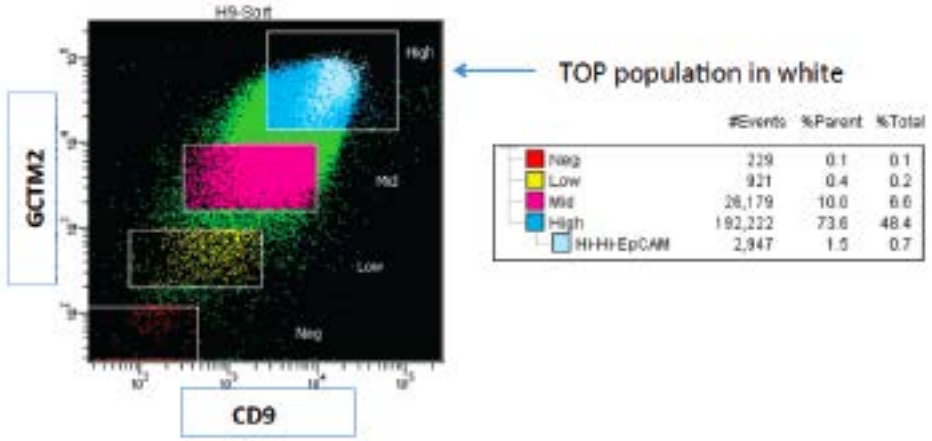
Gradient of antigen expression in hESC grown under different conditions

Laslett BMC Dev Biol. 7: 12, 2007; Kolle et. al Stem Cells 27: 2446, 2009

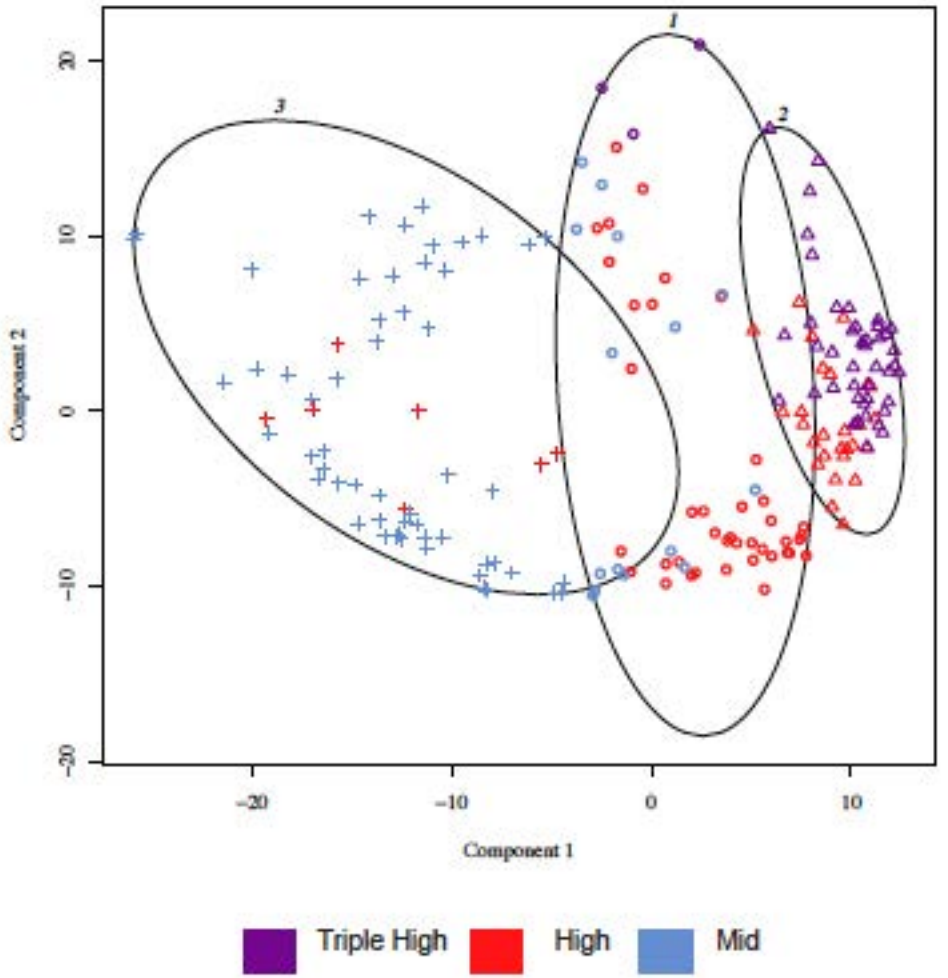


Immunotranscriptional profiling to study earliest phases of stem cell differentiation.

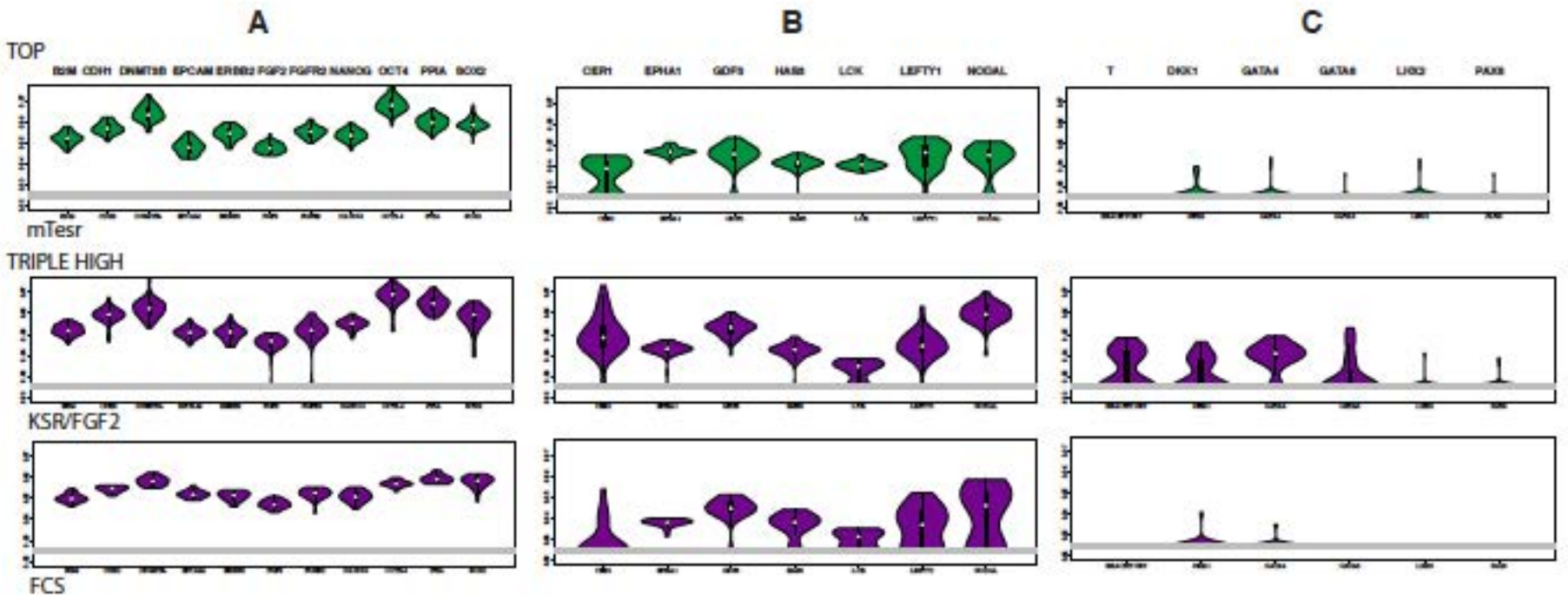
Expression of canonical pluripotency network genes decreases in a continuum across the cell population as surface stem cell markers drop.



Top and high populations show distinct patterns of gene expression at single cell level even in fully defined system supporting high levels of self renewal



Canonical pluripotency genes and signaling genes consistently expressed in all TOP subset at high levels. No lineage priming in TOP subset

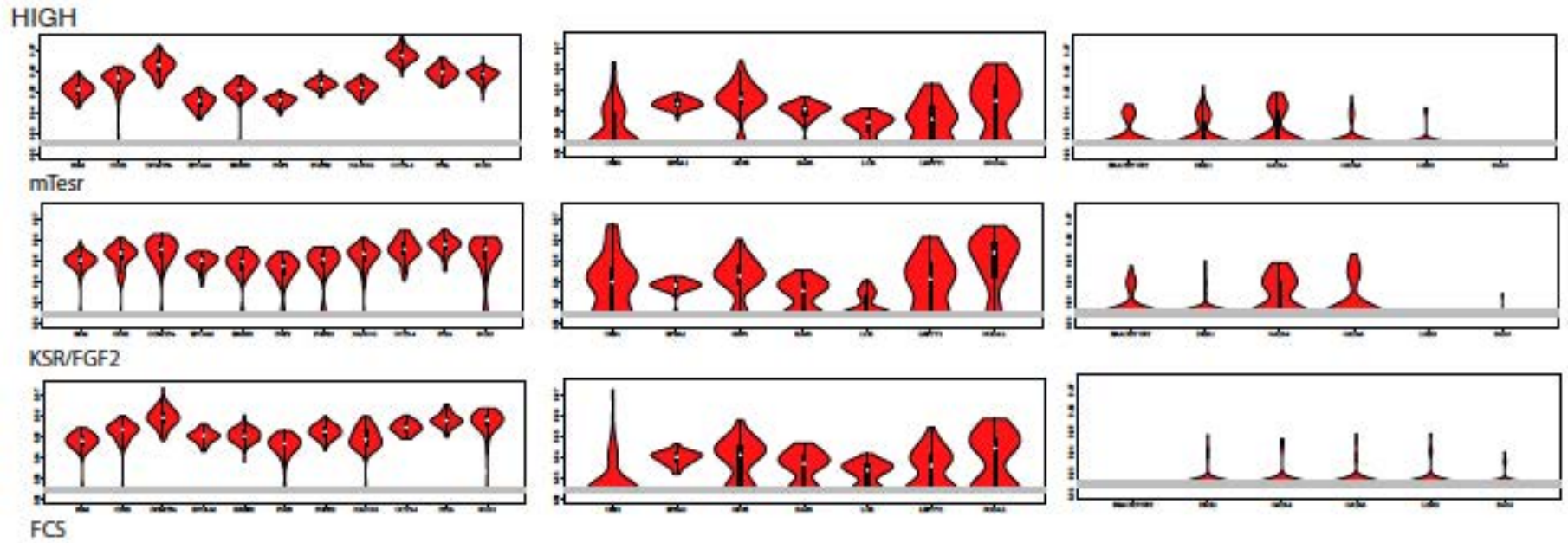


Pluripotency

Intercellular Signaling
Self renewal
Nodal/Activin/TGFbeta

Lineage Specific

High population: pluripotency genes still on signaling molecules begin to switch off

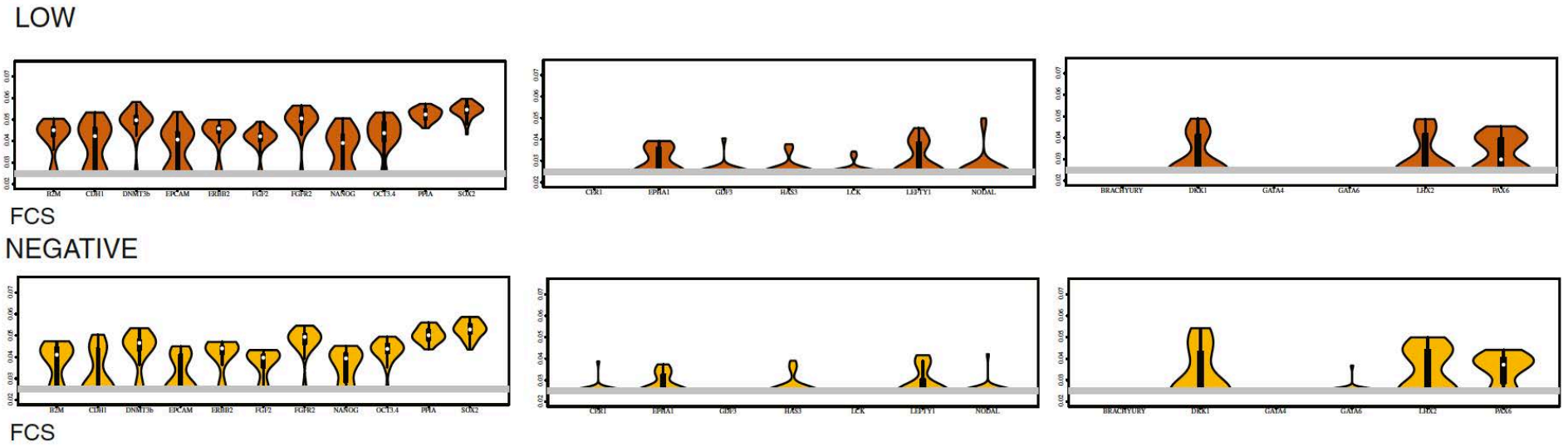


Pluripotency

Intercellular Signaling
Self renewal
Nodal/Activin/TGFbeta

Lineage Specific

Marker low population: Pluripotency genes still on Signaling molecules off, lineage specific genes on

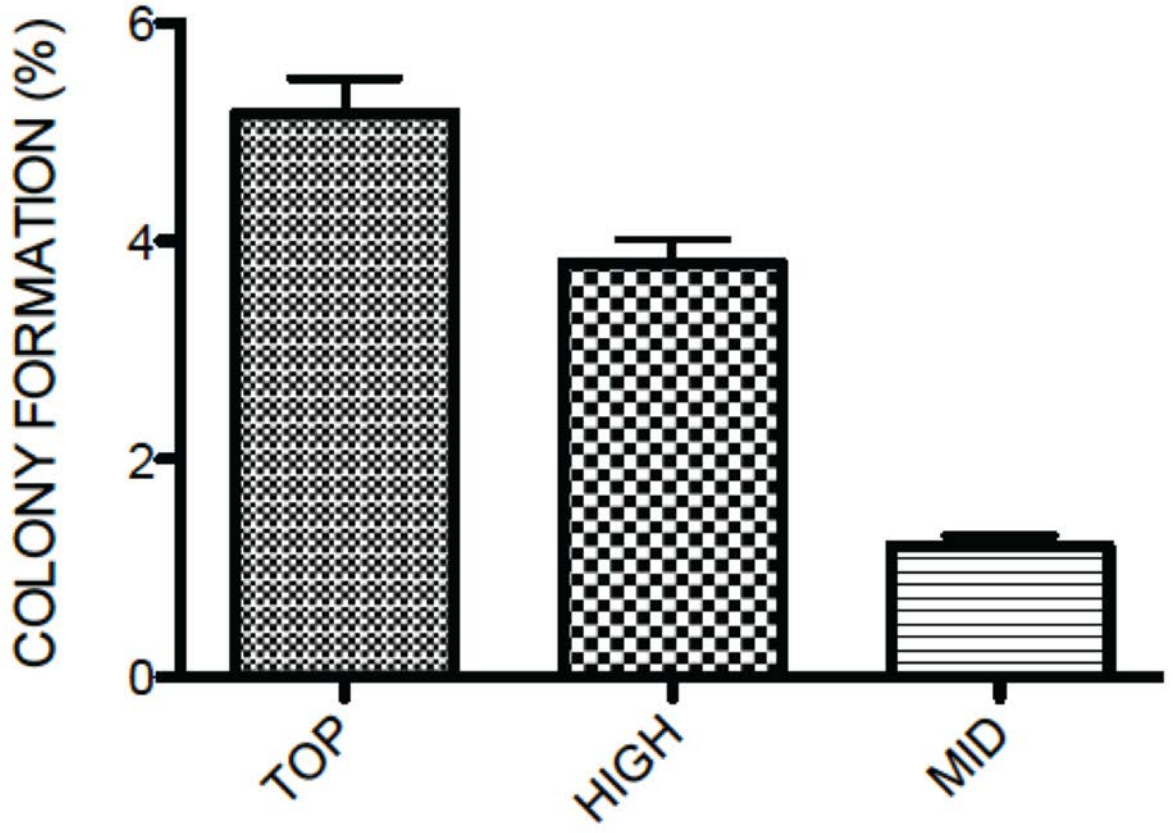


Pluripotency

Intercellular Signaling
Self renewal
Nodal/Activin/TGFbeta

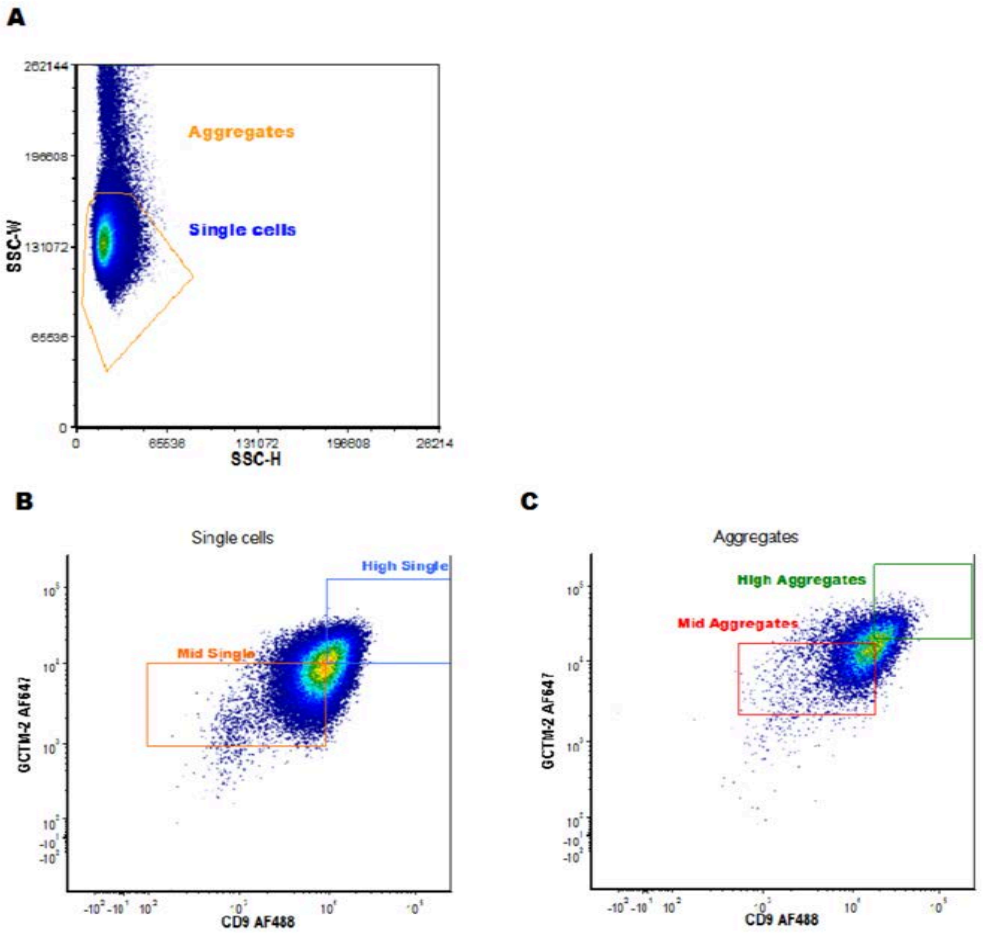
Lineage Specific

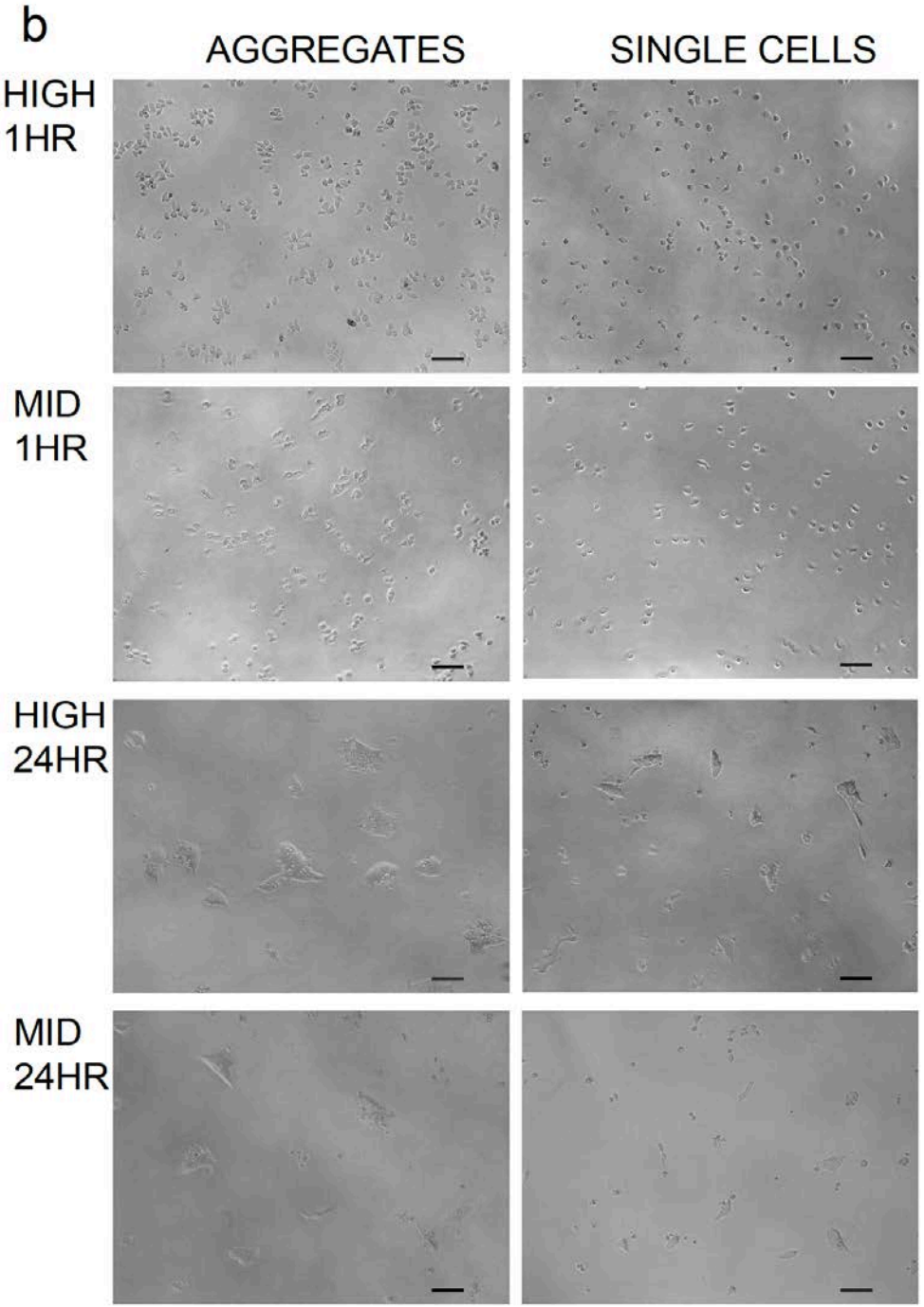
In defined culture system, self renewal mainly restricted to TOP and HIGH populations

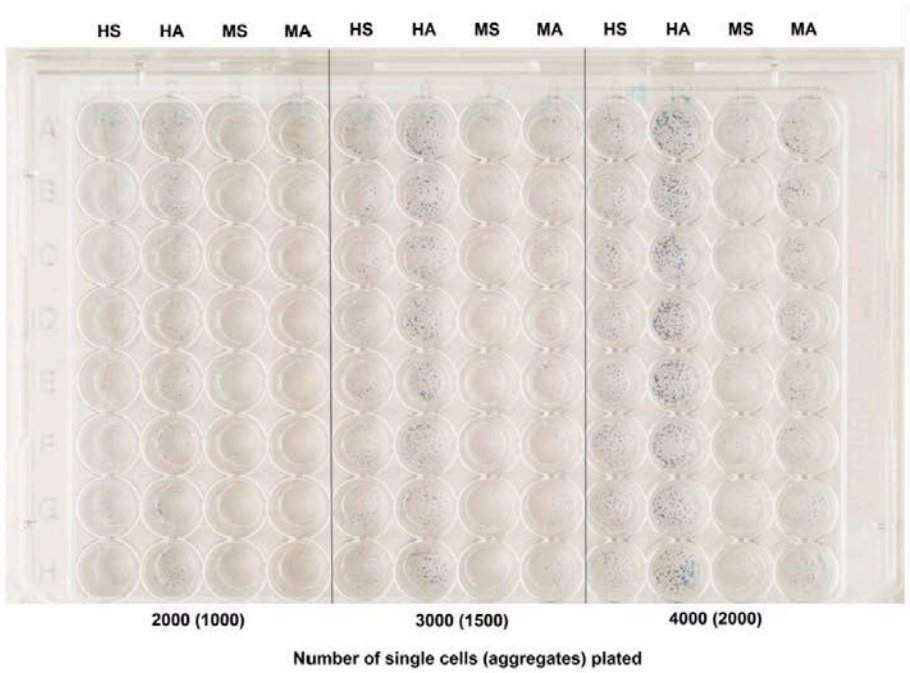


But all subpopulations can differentiate into derivatives of all three germ layers i.e. are pluripotent

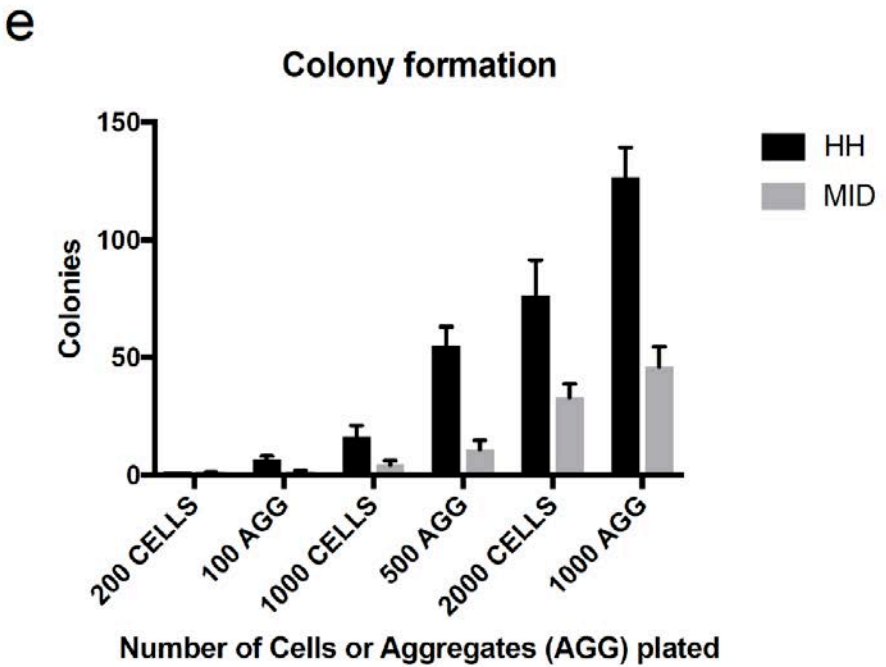
Sorting cells as aggregates preserves cell-cell contacts, enables high cell survival and measurement of self-renewal independently of initial cell survival



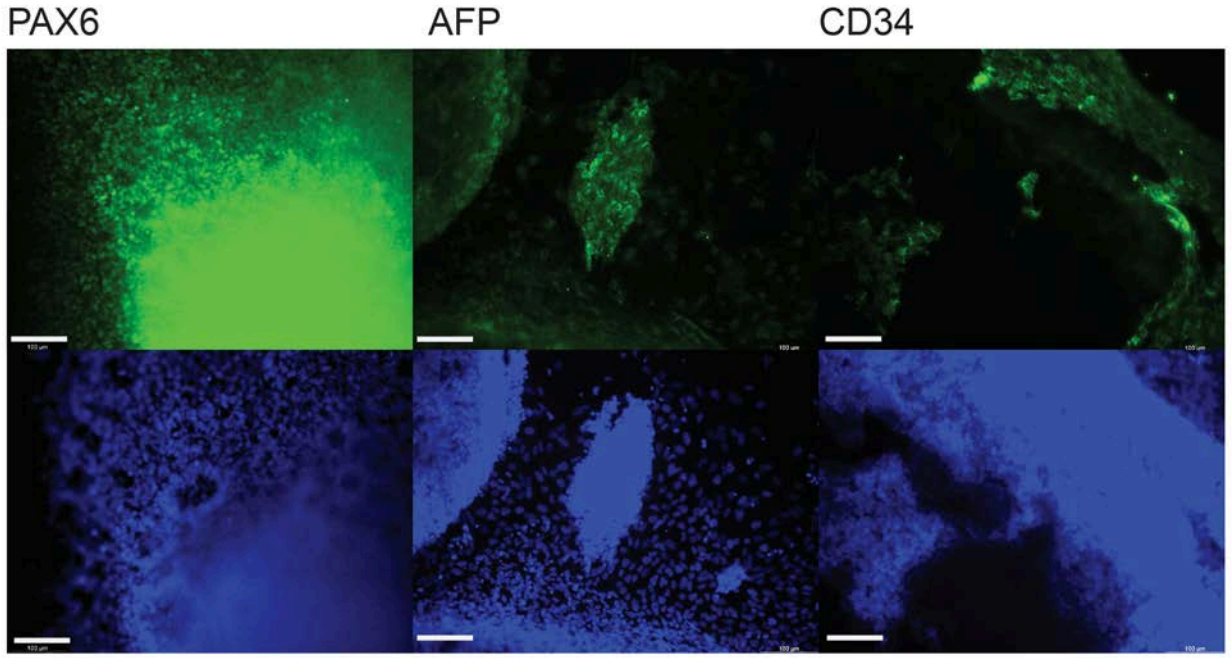




Cells at top of hierarchy show high levels of self-renewal as aggregates or as single cells.
Initial survival was similar in High and Mid populations
Self-renewal capacity is separate from ability to survive as single cells.

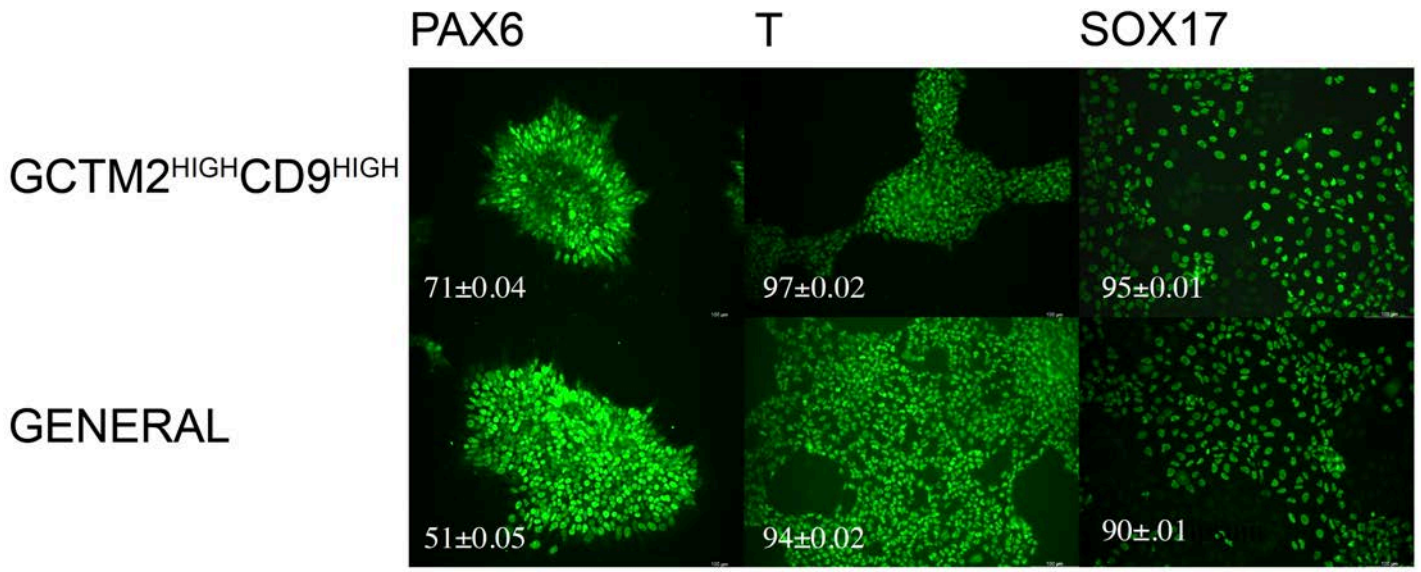


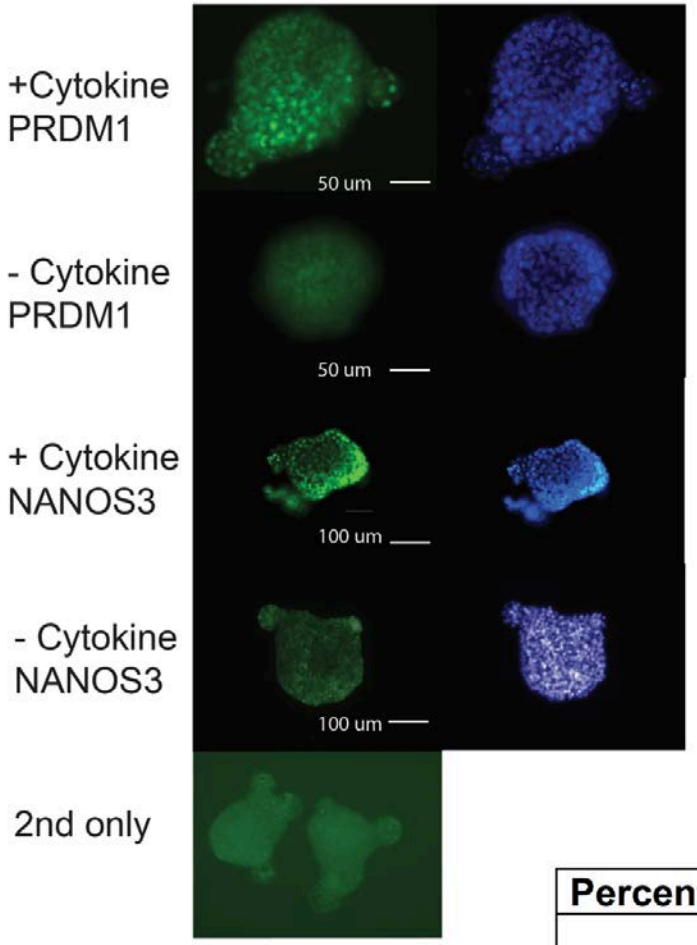
Most of the self-renewal activity resides in cells at the top of the hierarchy

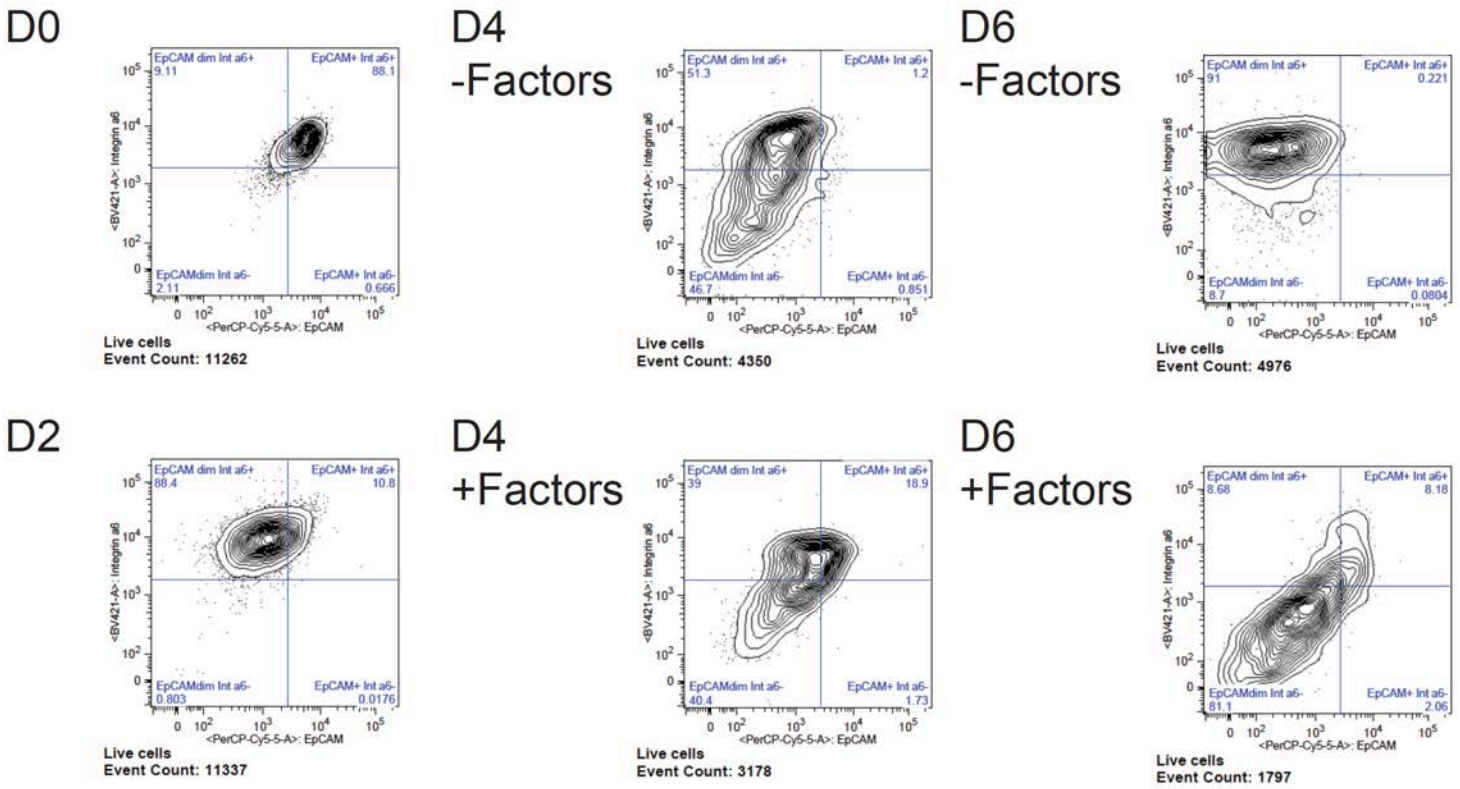


Self renewing population is pluripotent.
So is the remainder of the population.
Capacity for self renewal is distinct to pluripotency.

Spontaneous differentiation in EB
Directed differentiation in adherent culture

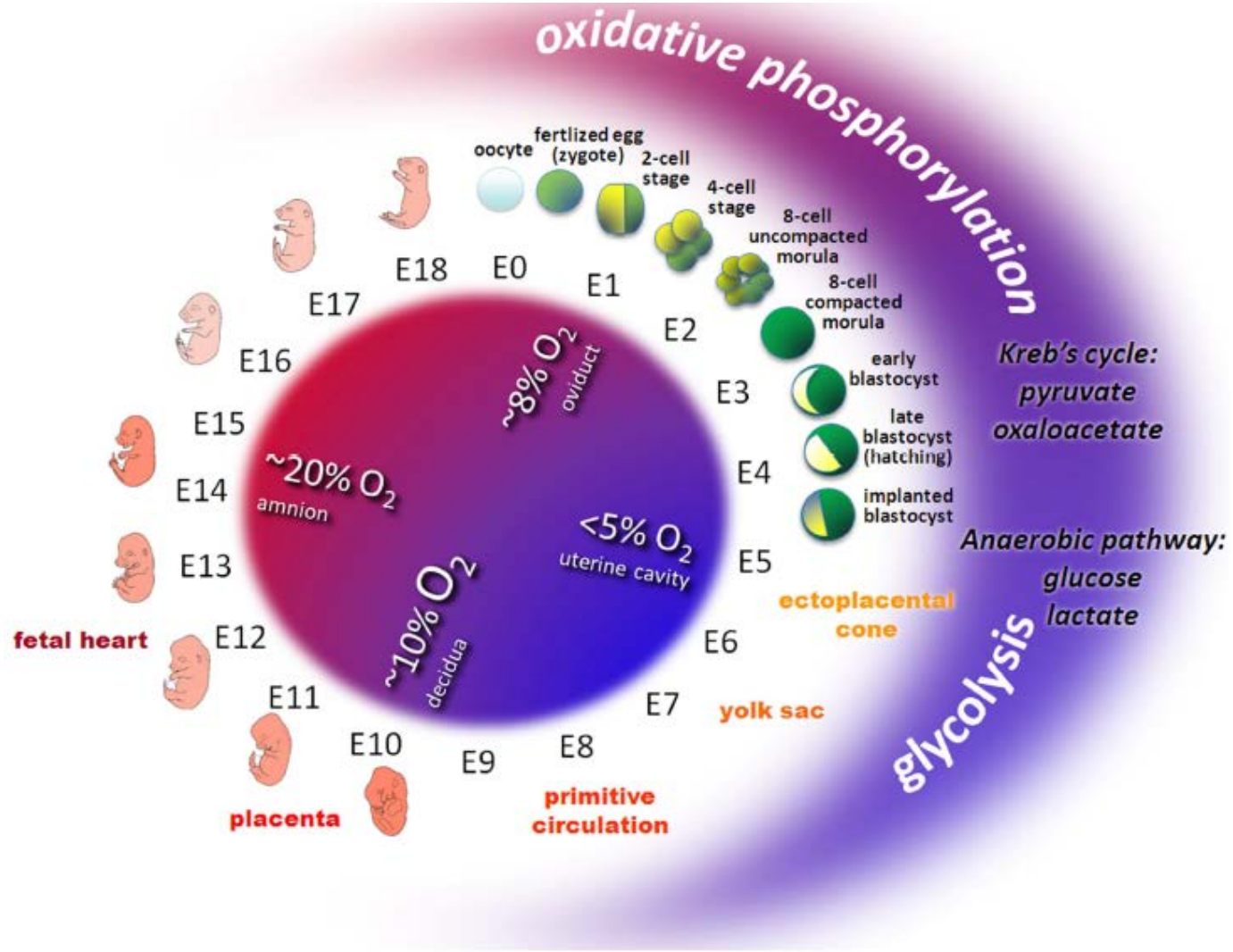






Percentage of PGC-Like Cells on Day 4			
	EXP 1	EXP2	EXP3
WA01			
GCTM-2^{high}CD9^{high}EPCAM^{high}	7.1	36.0	50.0
GCTM-2^{mid}CD9^{mid}	2.9	20.2	29.9
WA09			
GCTM-2^{high}CD9^{high}EPCAM^{high}	18.9	58.3	ND
GCTM-2^{mid}CD9^{mid}	8.1	44.9	ND

Self renewing population can give rise to primordial germ cells, unlike primed cells. These cells have the differentiation potential of early post-implantation epiblast.



Metabolic changes during early development
Peri-implantation epiblast displays bivalent metabolism (OXPHOS and Glycolysis)

Ufer and Wang Front. Mol. Neurosci. 4: 1, 2011

Self-renewing Population has More Mitochondria with Higher Membrane Potential- Bivalent metabolism similar to early epiblast

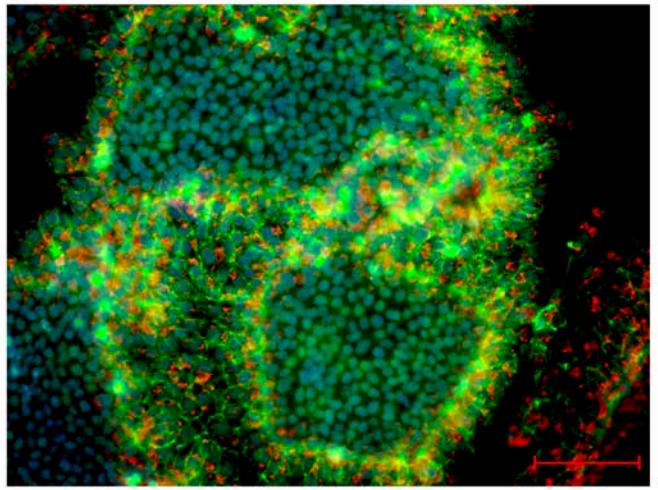
Uptake of TMRM dye
Red/Green ration of JC-1 fluorescence

LOW

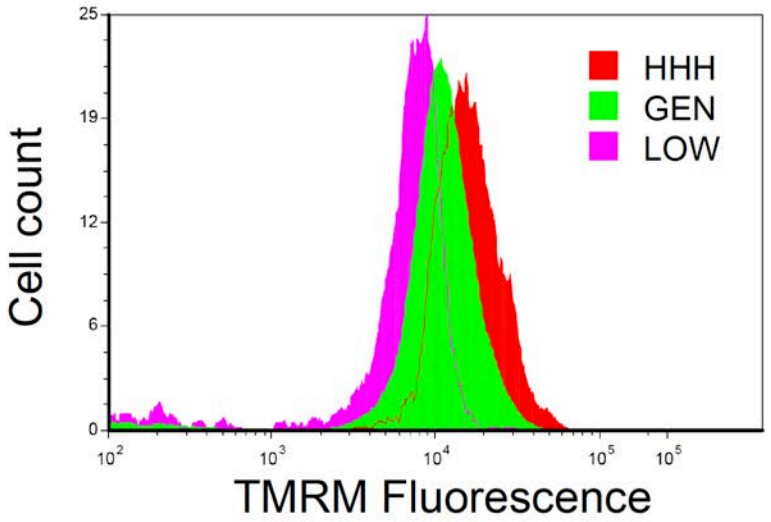
TOTAL

HIGH

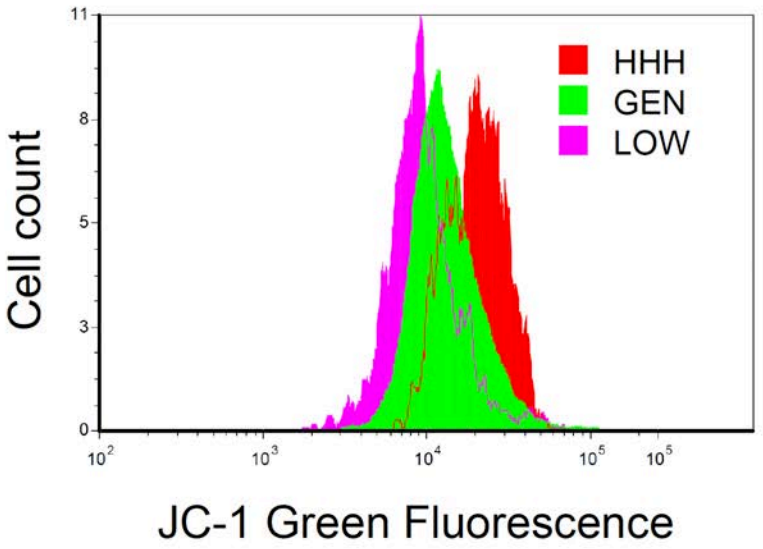
a

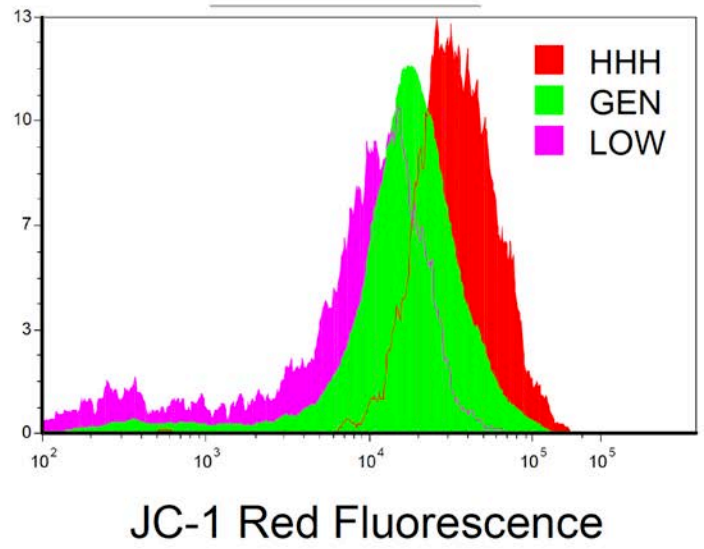


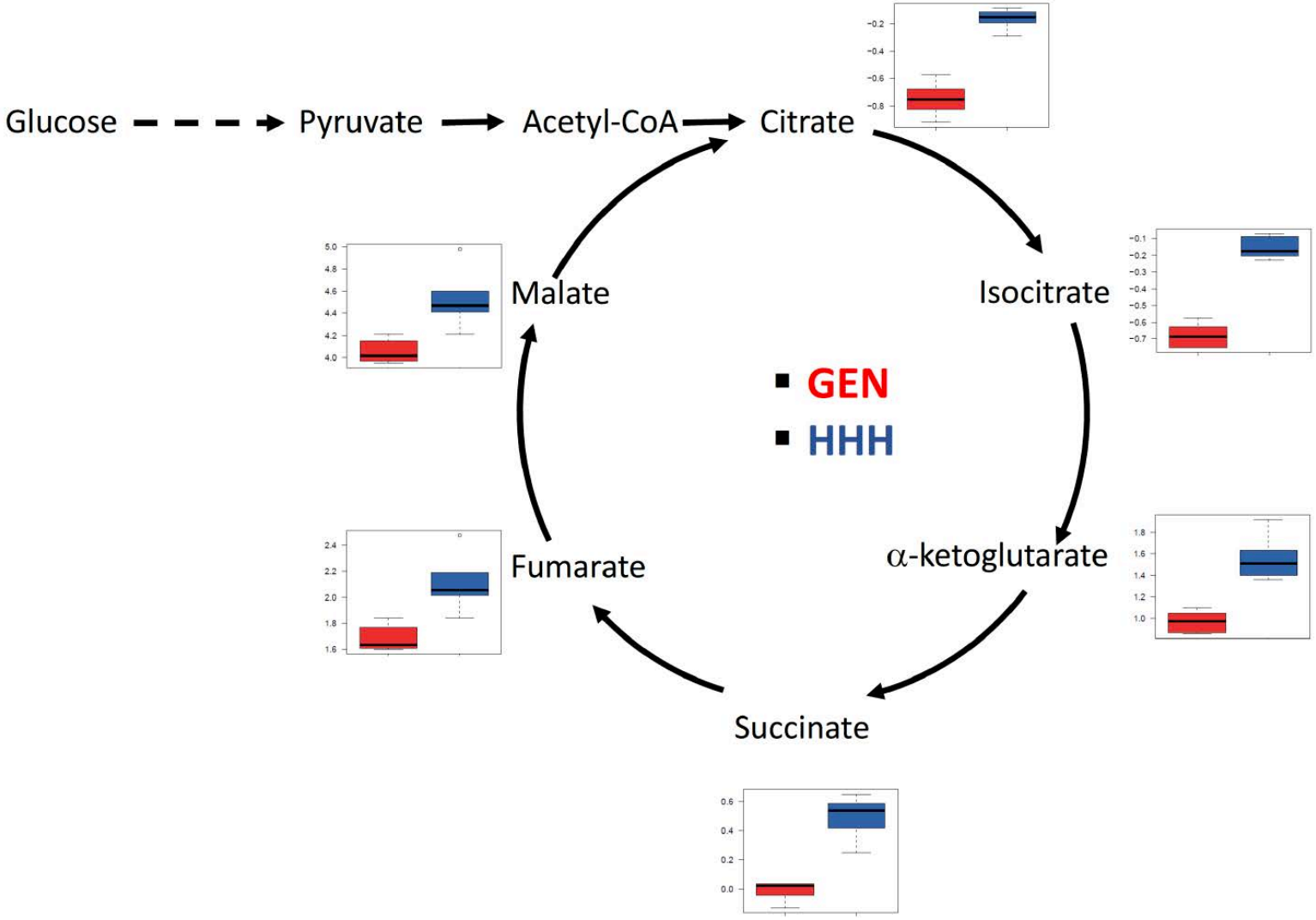
b



c

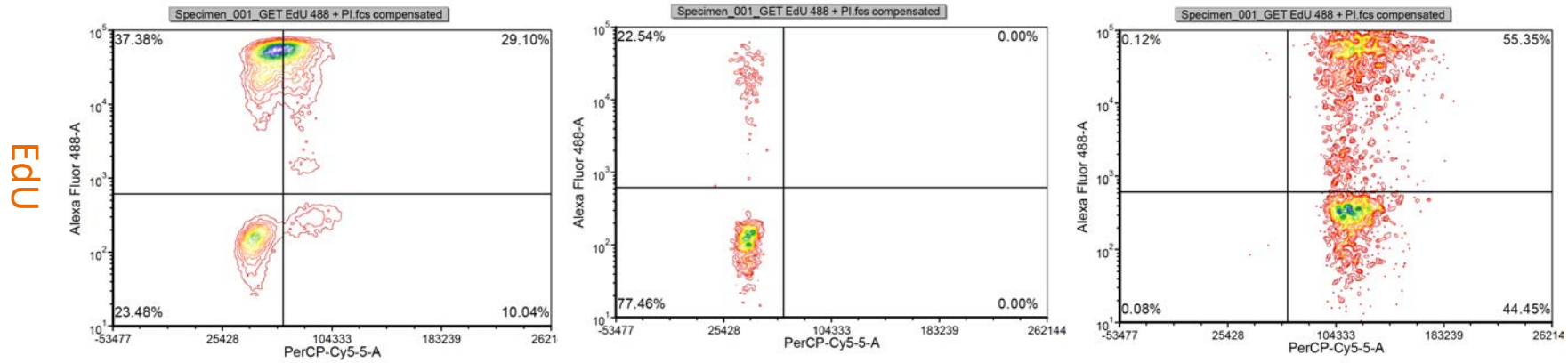






LC-MS and GC-MS analysis shows higher levels of TCA intermediates in HHH (blue) versus GEN (red) population

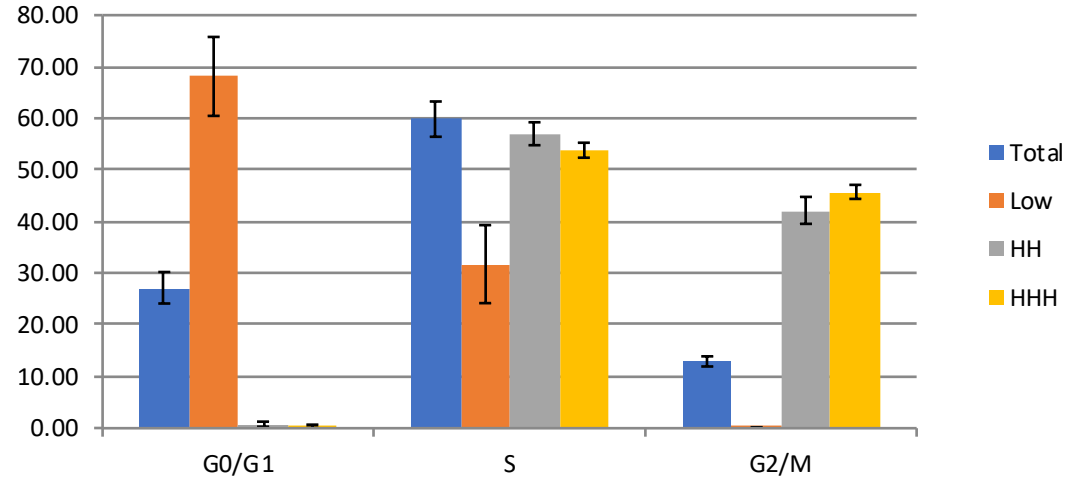
Self-Renewing Population has Unique Cell Cycle



Total Population

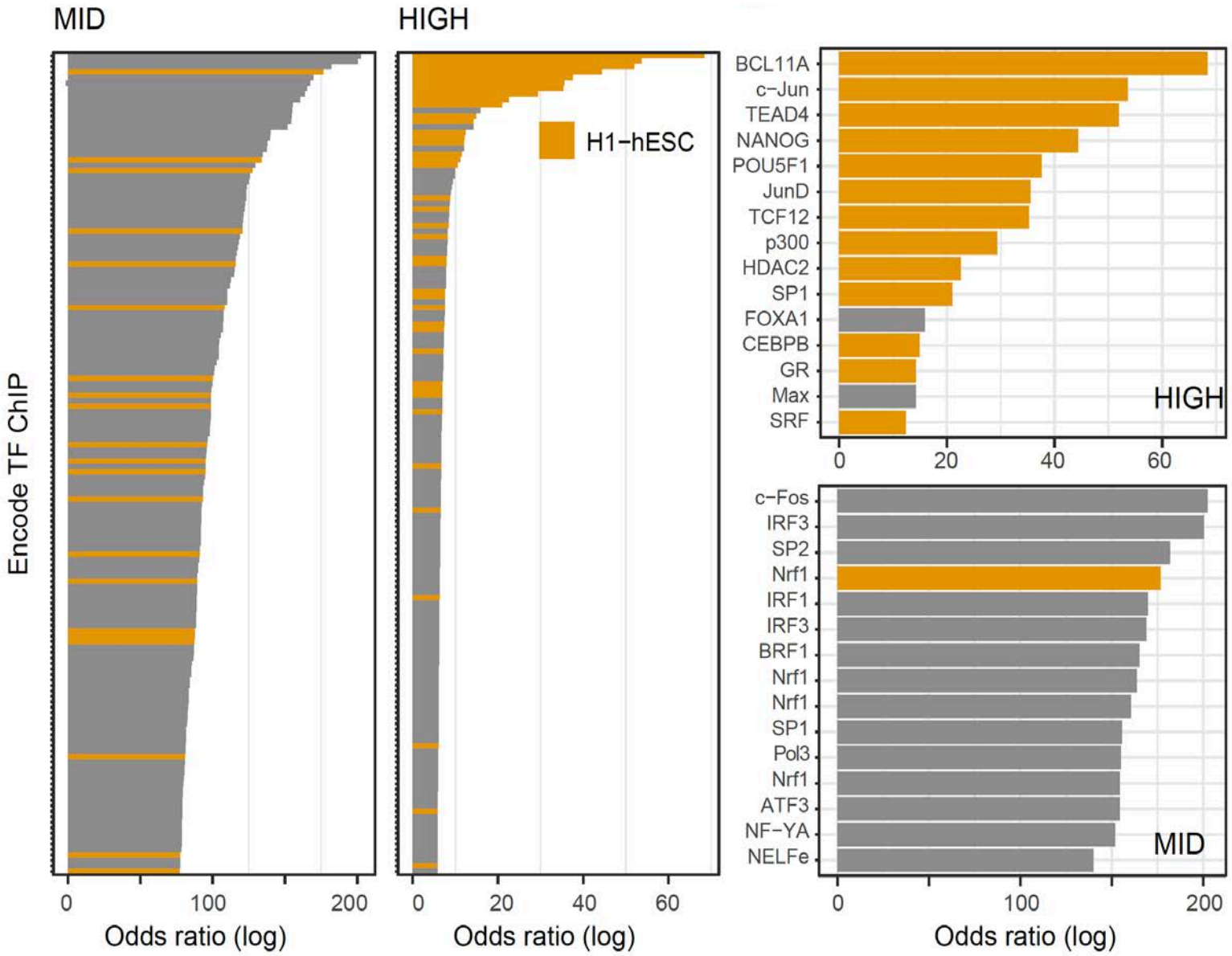
Low Population

HHH Population



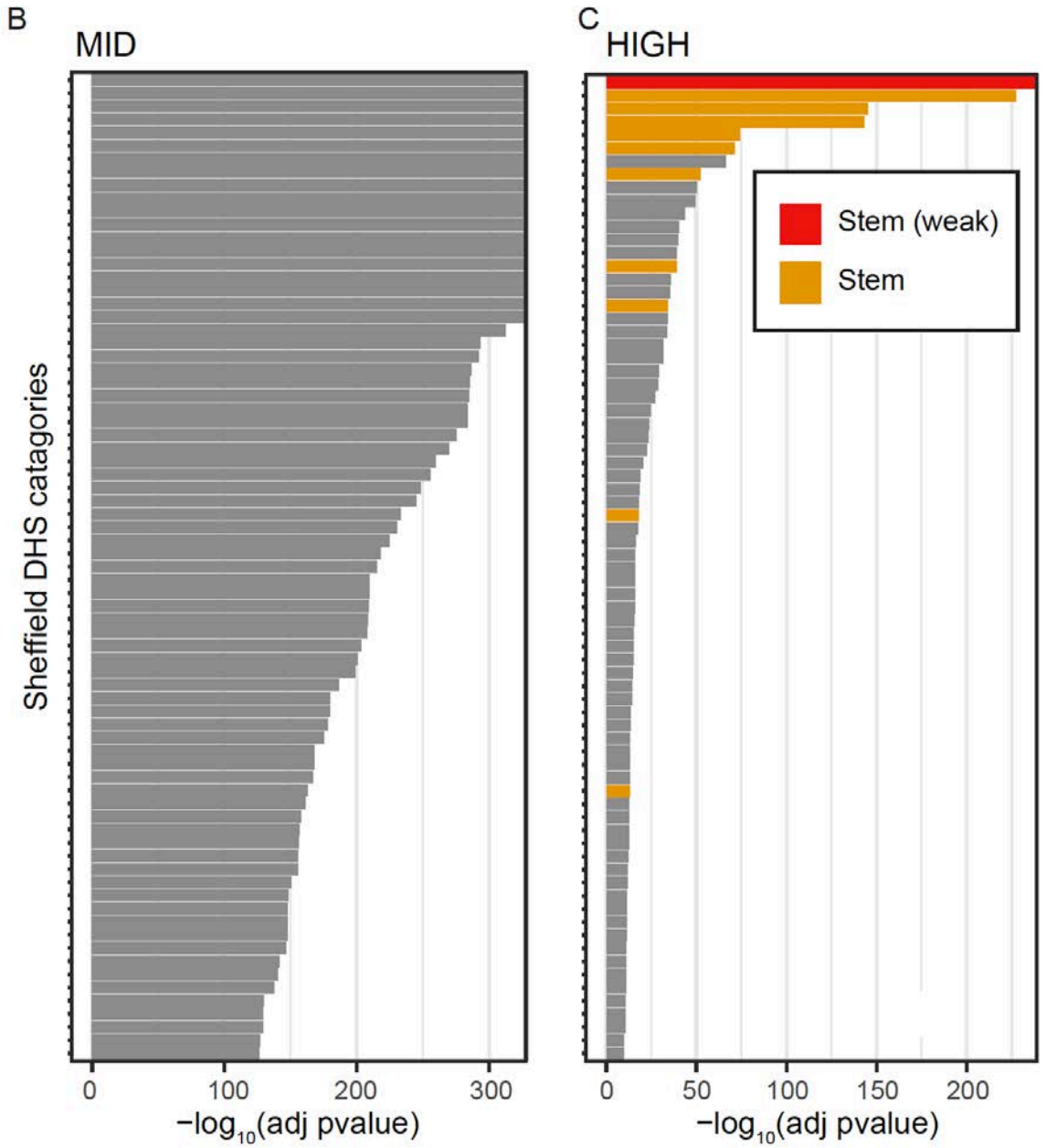
Very low G1 content, similar to mouse peri-implantation epiblast.
G1 is the gateway to lineage specification

Differential ATAC-seq analysis reveals unique open chromatin configuration in self-renewing hPSC



Open chromatin sites in hPSC subpopulation enriched for self-renewal map to ENCODE TF binding sites that are:

- Specific to hPSC
- Occupied by pluripotency associated TFs



Differential ATAC-seq shows that open chromatin peaks specific to self-renewing population map to hPSC specific DNAase Hypersensitive Sites

- Open chromatin specific to self-renewing cells
- Open chromatin common to pluripotent population
- Open chromatin specific to primed cells

The High Self-Renewing Compartment of Conventional hPSC

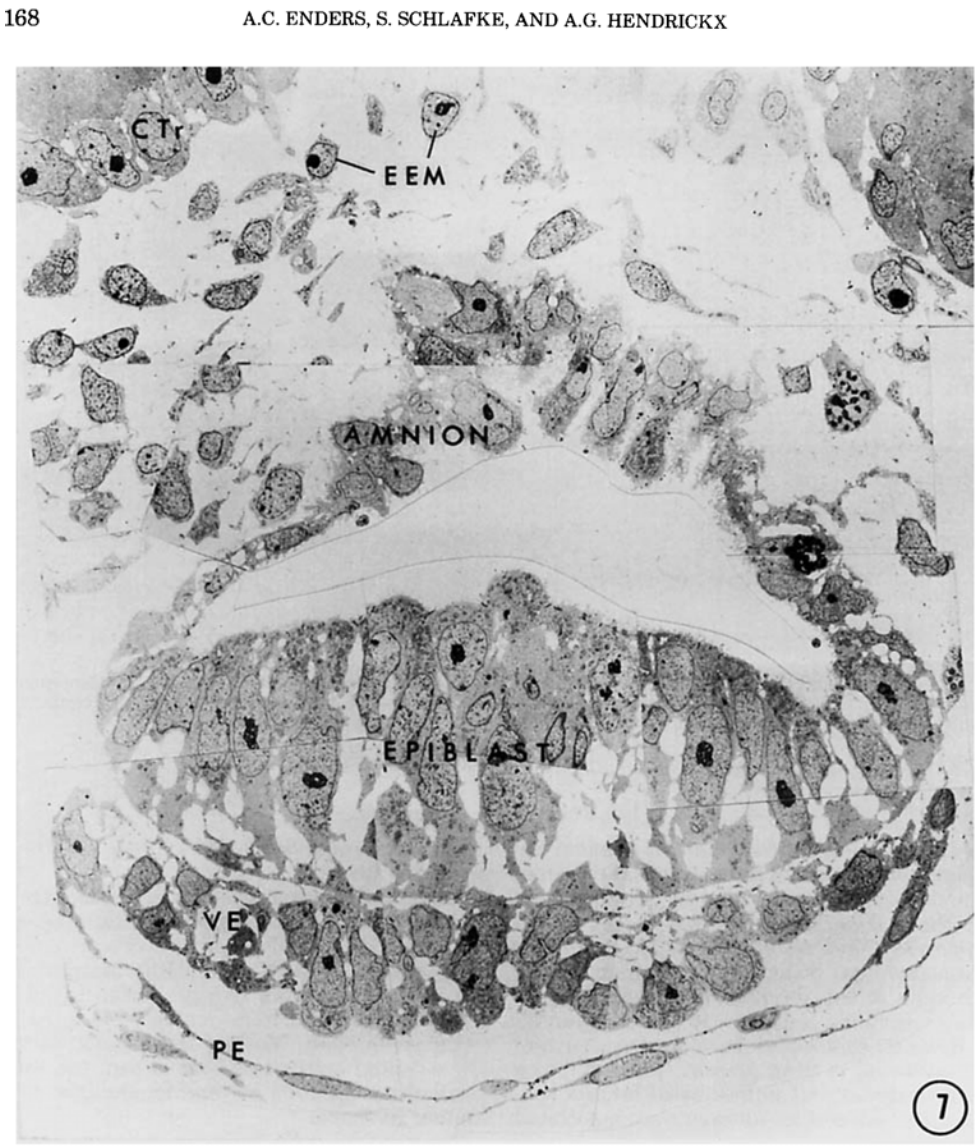
- Shows uniform and high levels of pluripotency genes
- Shows no lineage priming
- Has abundant active mitochondria, bivalent metabolism, and a cell cycle with little G1 component, similar to mouse peri-implantation cells, not mouse epiblast stem cells
- Has a specific set of open chromatin sites that are unique to pluripotent stem cells and are occupied in hPSC by pluripotency related transcription factors

Molecular baseline for understanding development status of human pluripotent cells has not been available

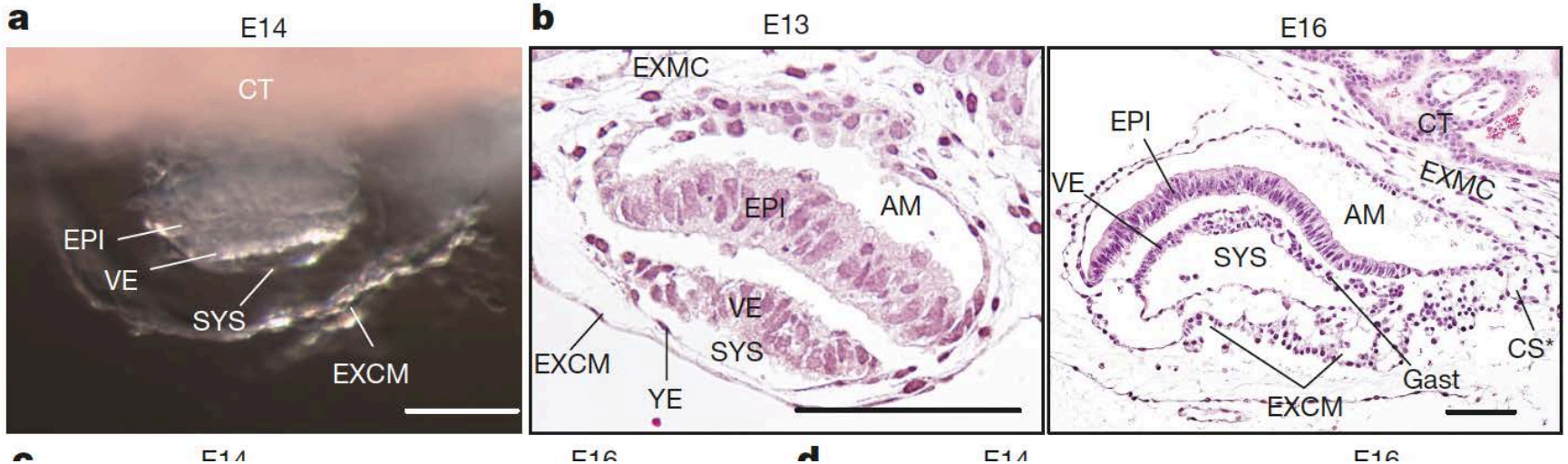
Until recently our knowledge of post-implantation primate development has been limited to a handful of histological and ultrastructural studies

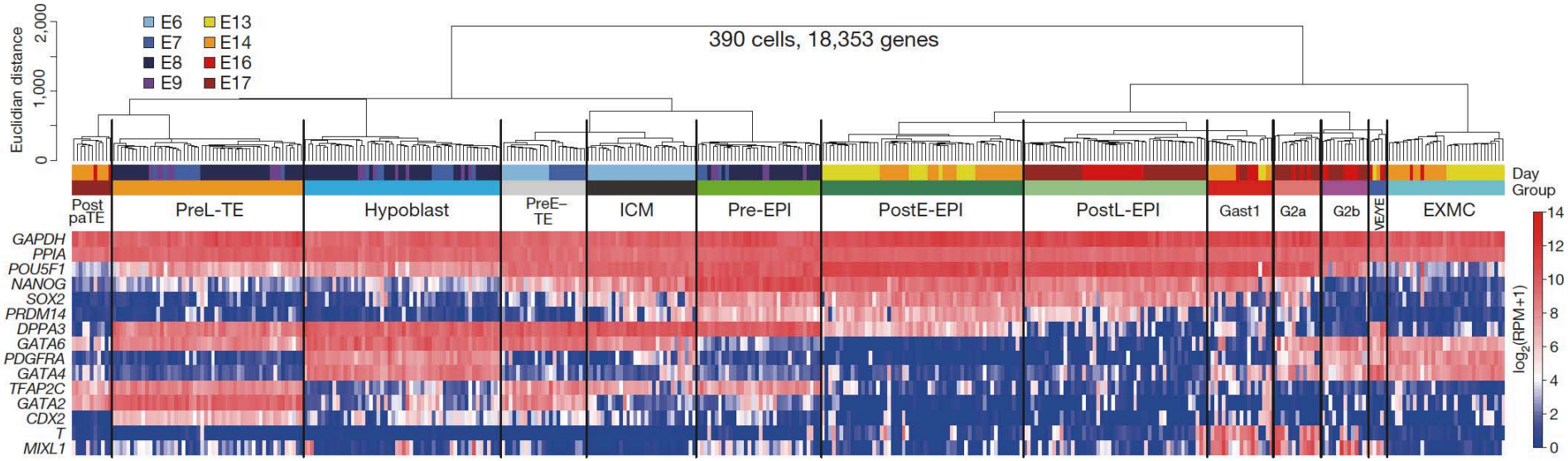
Rhesus Monkey Embryo E14

Enders Am. J. Anat. 177: 161, 1986

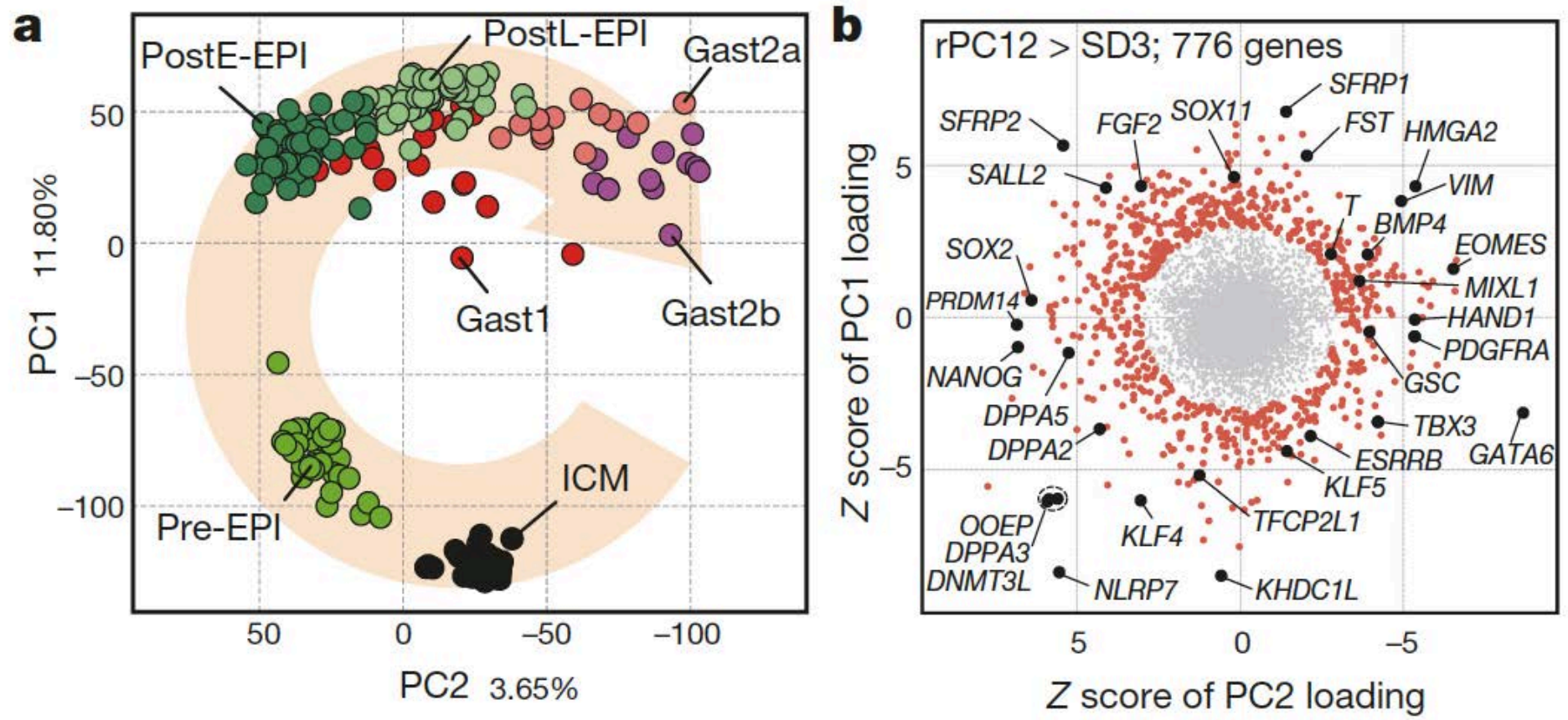


Single cell RNA-seq analysis of pre- and post-implantation development in cynomolgus monkey





Mitinori Saitou/ Nakamura et al. Nature 537: 57, 2016

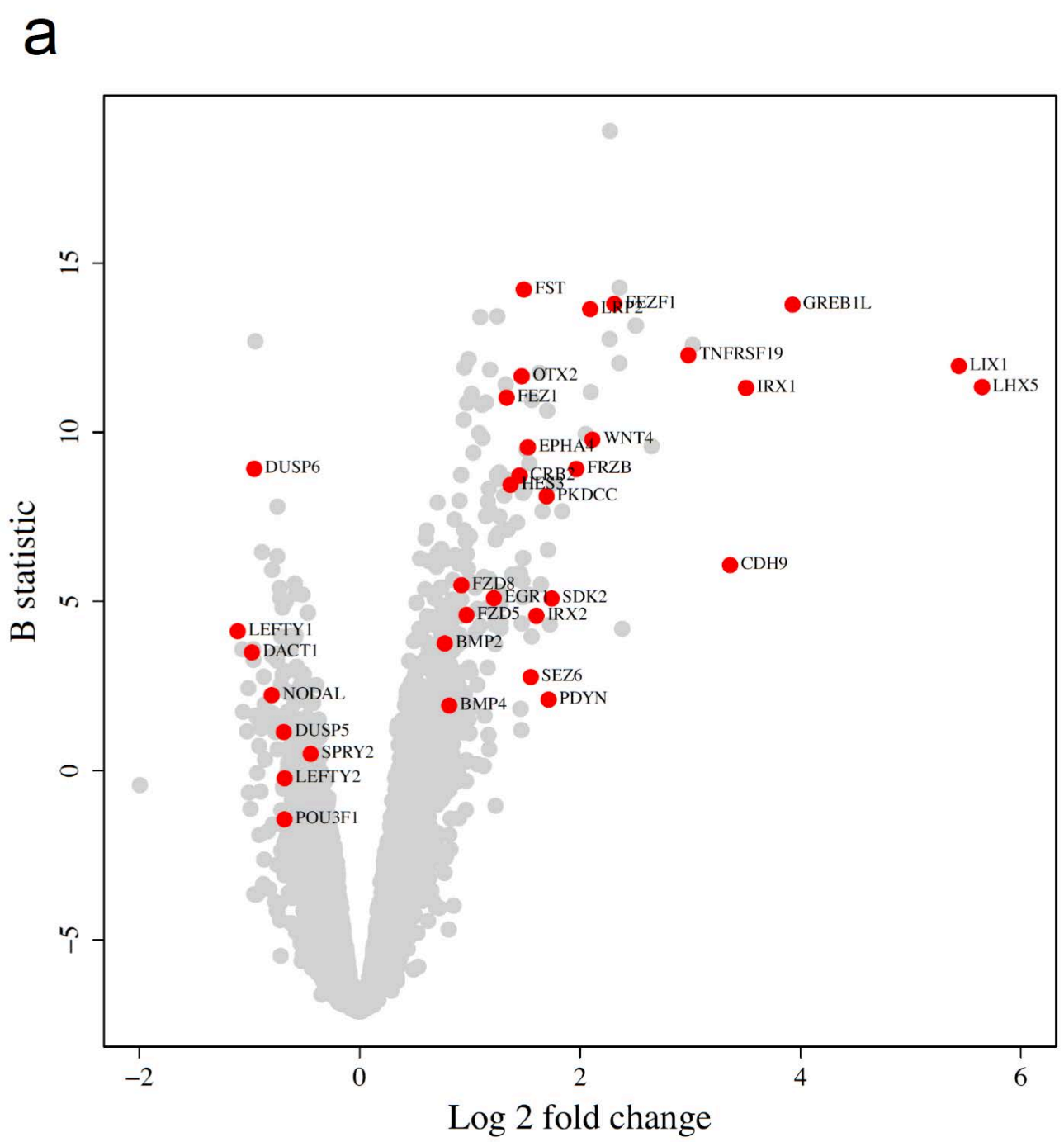


Major transitions in primate pluripotency during preimplantation development

Nakamura et al. Nature 537: 57, 2016

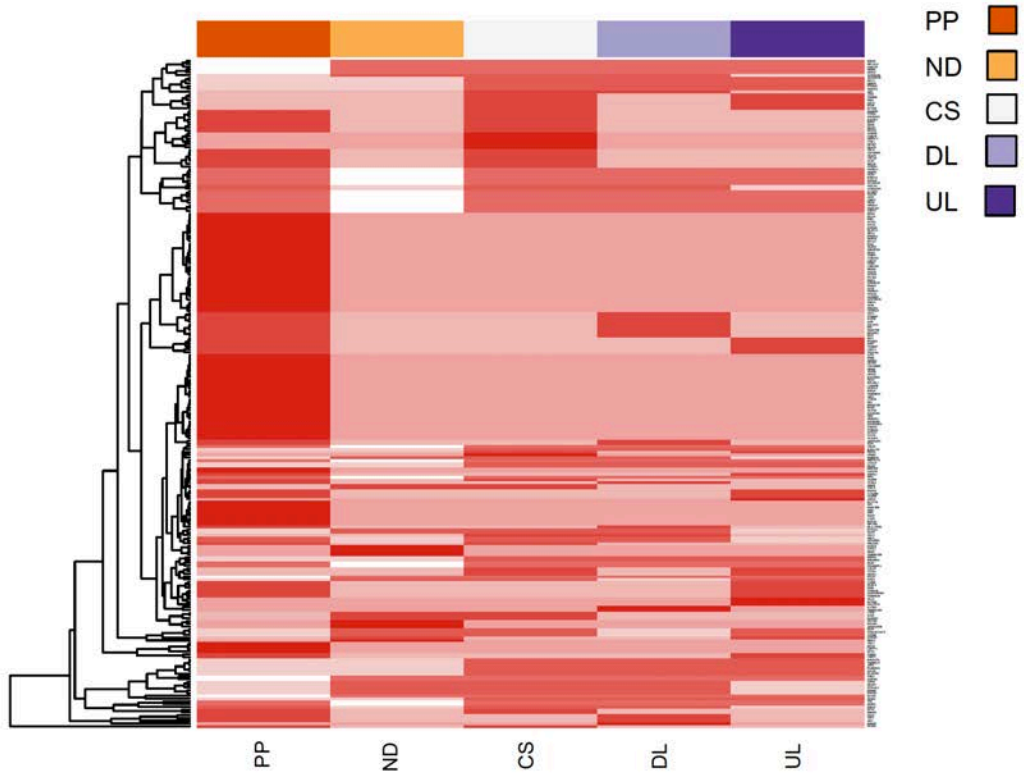
RNA-seq on sorted HHH versus GEN hPSC subpopulations

Many genes turned on in GEN population are involved in neural differentiation

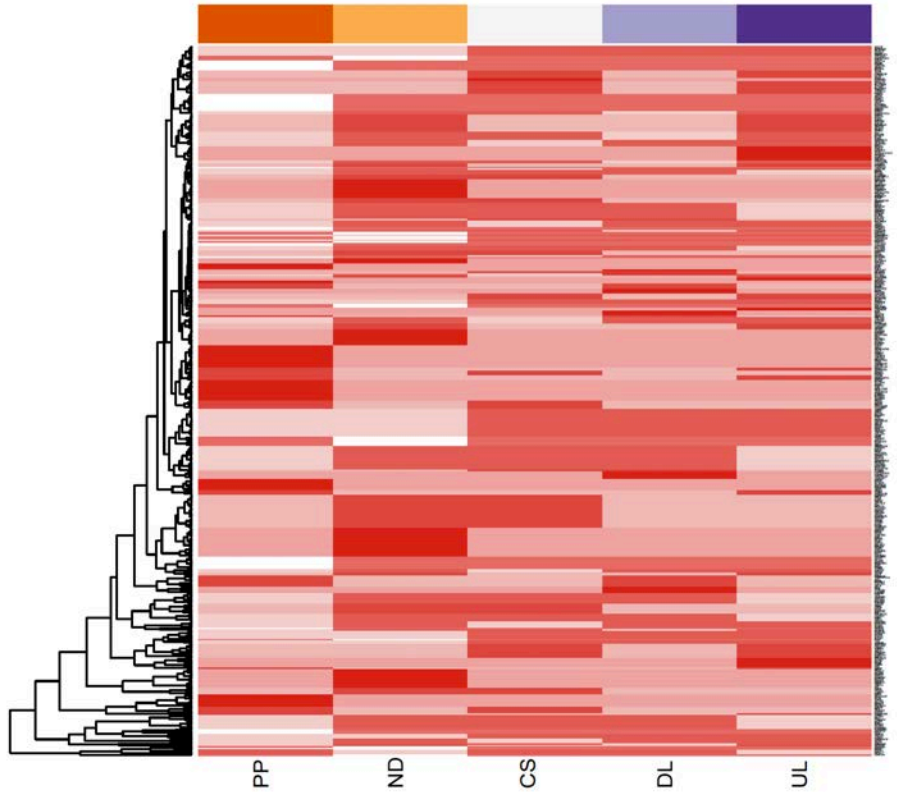


Comparison of our RNA-seq data with CORTECON analysis of neural specification shows that the HHH Fraction is enriched for pluripotency and the GEN population is enriched for neural specification

HHH

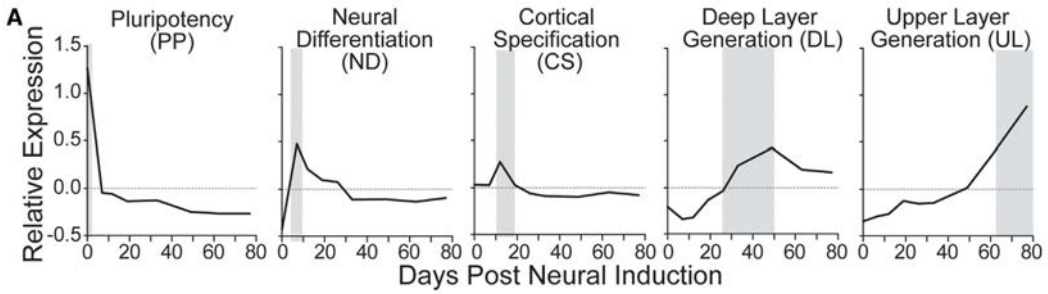


GEN

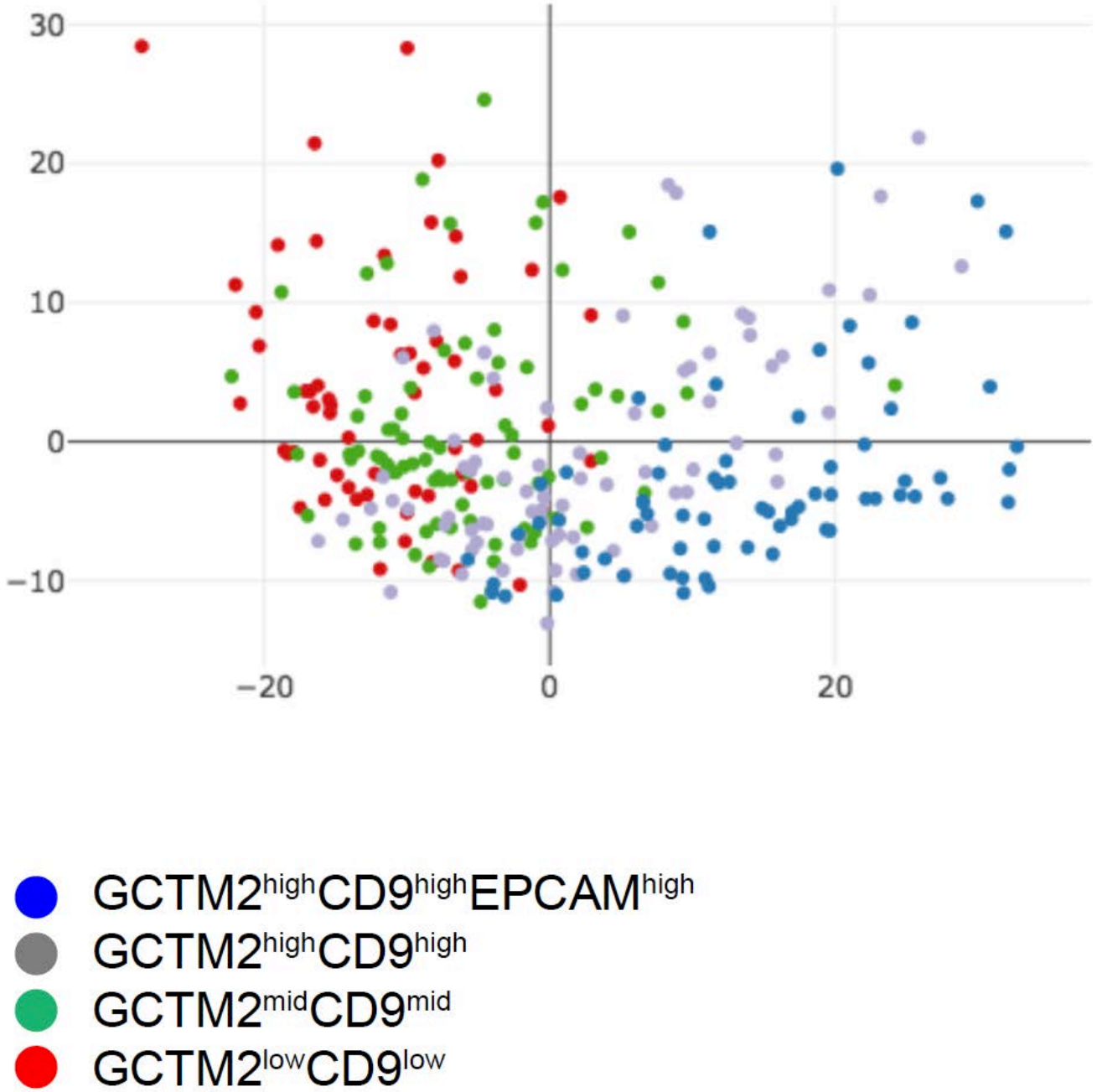


CORTECON: A Temporal Transcriptome Analysis of In Vitro Human Cerebral Cortex Development from Human Embryonic Stem Cells

http://dx.doi.org/10.1016/j.neuron.2014.05.013



PCA of single cell RNA-seq shows separation of hPSC subpopulations along a continuum of cell states



Interspecies PCA shows that hPSC map between pre-and post-implantation states of monkey epiblast hPSC are clearly distinguished from gastrulating stages that correspond to mouse epiblast stem cells

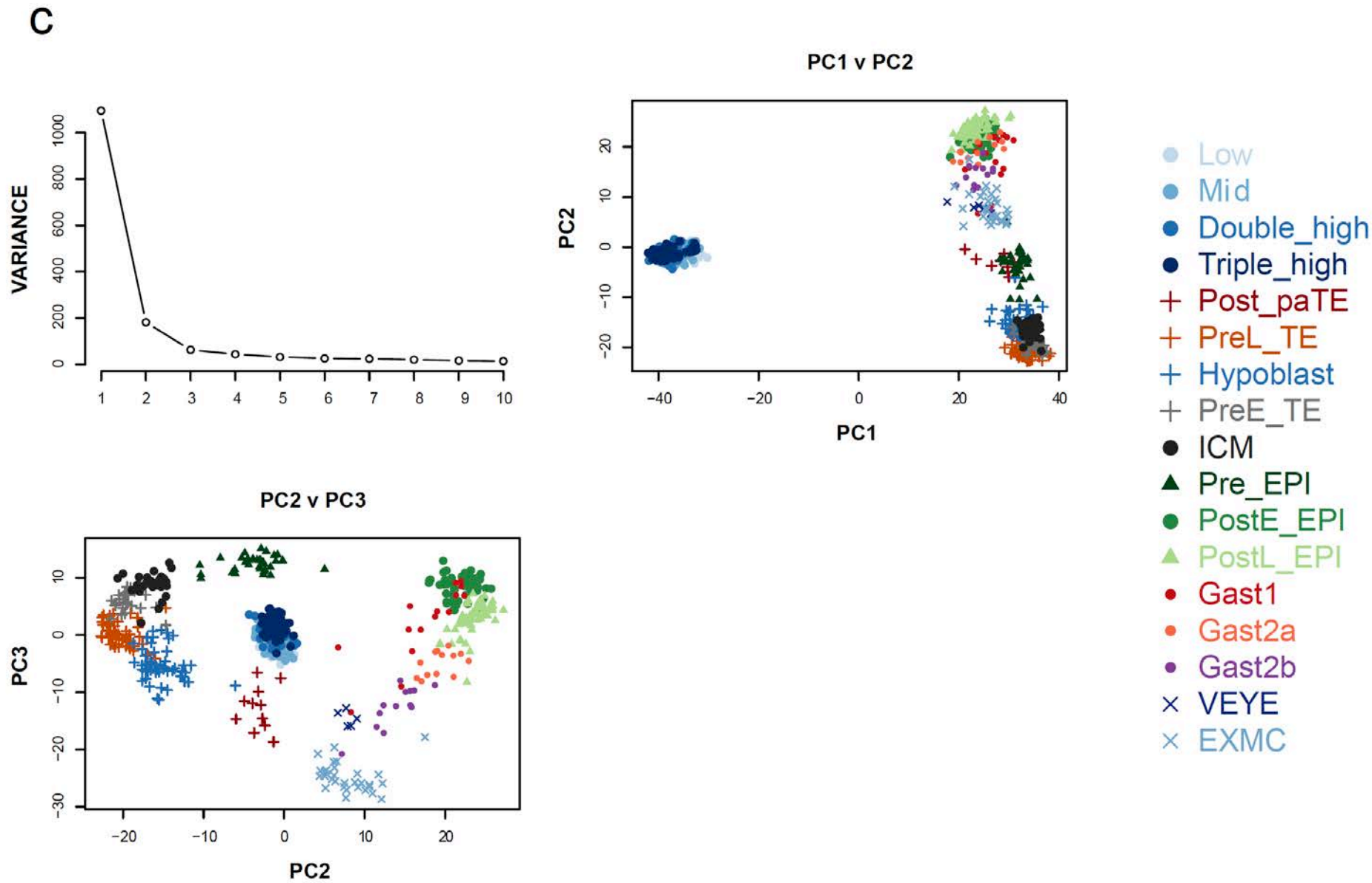
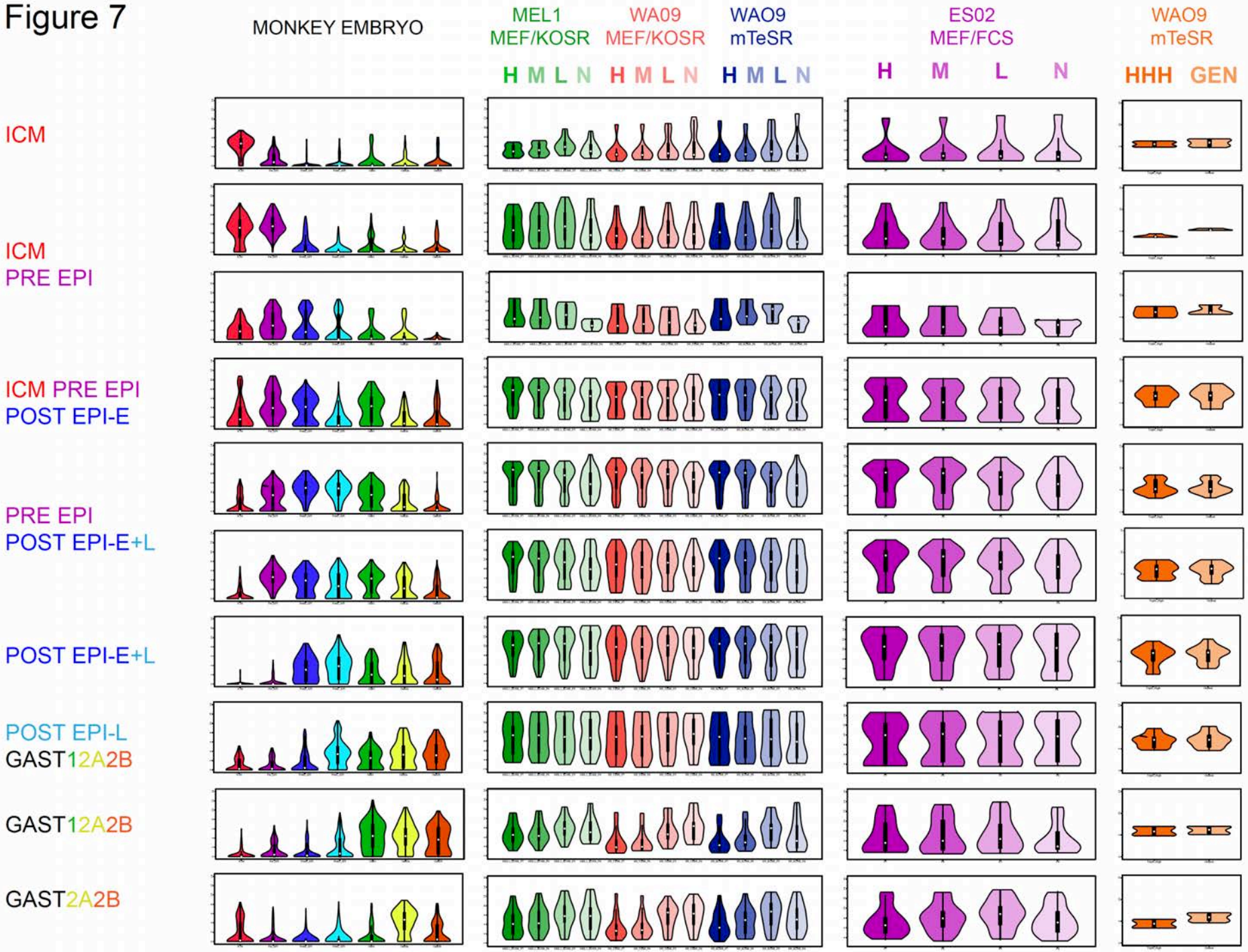


Figure 7


MONKEY EMBRYO
MEL1 MEF/KOSR
WA09 MEF/KOSR
WAO9 mTeSR
ES02 MEF/FCS
WAO9 mTeSR
H M L N H M L N H M L N
H M L N
HHH GEN
ICM
ICM PRE EPI
ICM PRE EPI POST EPI-E
PRE EPI POST EPI-E+L
POST EPI-E+L
POST EPI-L GAST12A2B
GAST12A2B
GAST2A2B

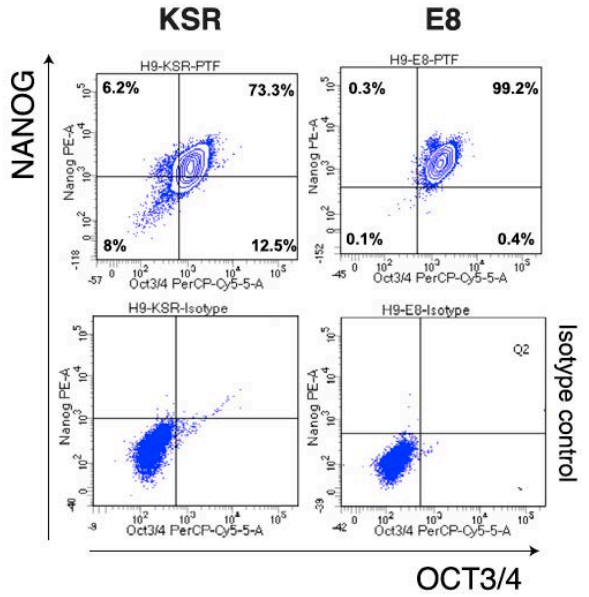
Human Pluripotent Stem Cells

- Self renewing conventional hPSC have metabolic and cell cycle status similar to mouse epiblast
- Transcriptionally they equate most nearly to peri-implantation primate epiblast E13-E16
- Conventional hPSC are competent to give rise to germline cells similar to early mouse epiblast
- Self-renewing hPSC do not resemble mouse epiblast stem cells and are not primed for any particular fate

Lipid Deprivation Induces a Stable, Naive-to-Primed Intermediate State of Pluripotency in Human PSCs

Daniela Cornacchia,[1,15] Chao Zhang,[2,3,15] Bastian Zimmer,[1,11] Sun Young Chung,[1] Yujie Fan,[1,4] Mohamed A. Soliman,[1,5] Jason Tchieu,[1] Stuart M. Chambers,[1,12] Hardik Shah,[6,13] Daniel Paull,[7] Csaba Konrad,[8] Michelle Vincendeau,[1,14] Scott A. Noggle,[7] Giovanni Manfredi,[8] Lydia W.S. Finley,[9,10] Justin R. Cross,[6] Doron Betel,[2,3] and Lorenz Studer[1,16,*]

https://doi.org/10.1016/j.stem.2019.05.001

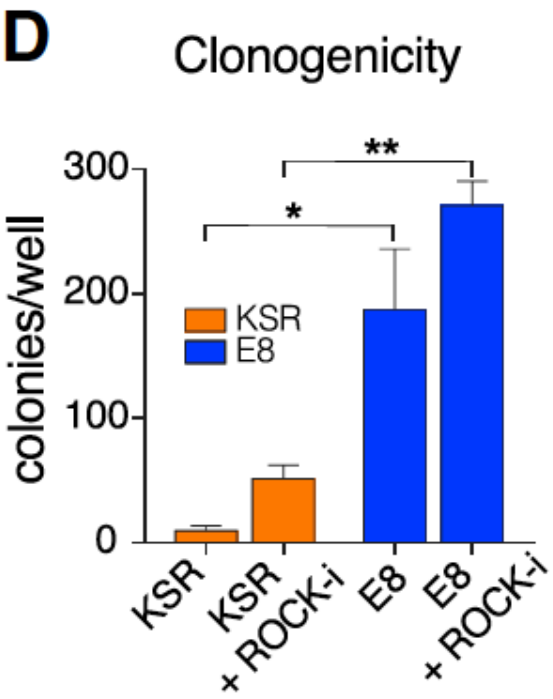


Enhanced Clonogenicity

Increased Expression of Pluripotency Markers

Bivalent Metabolism

Pre-Implantation Epiblast Marker



Human Pluripotency Is Initiated and Preserved by a Unique Subset of Founder Cells

Mio Nakanishi,[1] Ryan R. Mitchell,[1] Yannick D. Benoit,[1] Luca Orlando,[1] Jennifer C. Reid,[1,2] Kenichi Shimada,[3] Kathryn C. Davidson,[4] Zoya Shapovalova,[1] Tony J. Collins,[1] Andras Nagy,[4,5] and Mickie Bhatia[1,2,6,*]

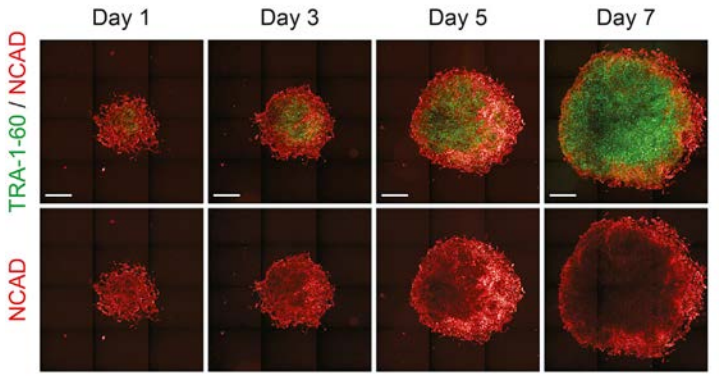
https://doi.org/10.1016/j.cell.2019.03.013

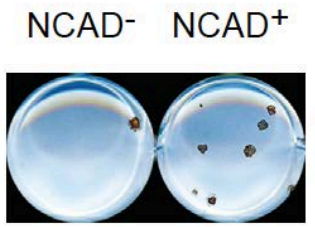
Reside at Colony Periphery

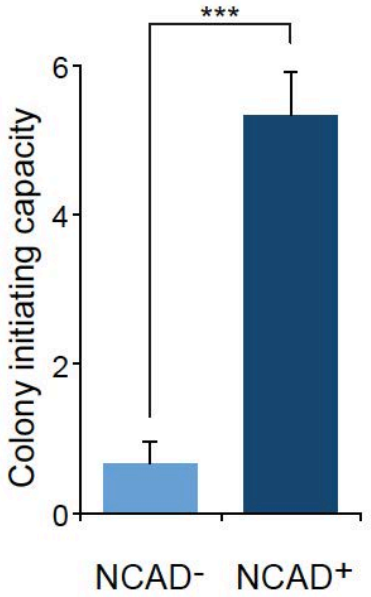
NCAM+

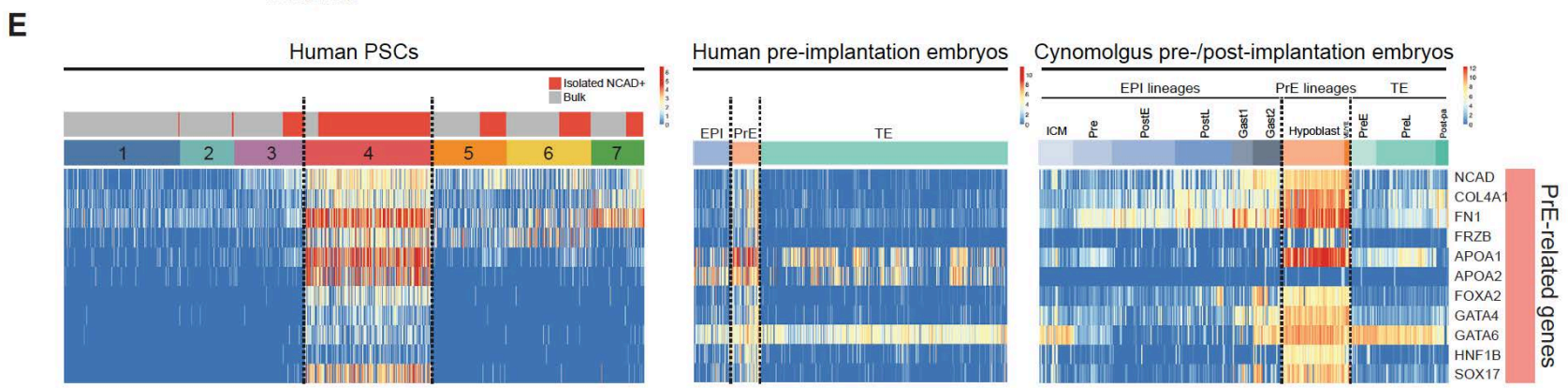
High Clonogenicity

Co-expression of PrEndo Genes









Questions

- Can the self-renewing subpopulation of hPSC be maintained in a pure form in culture?
- If we can understand how to maintain pure self-renewing subpopulation of hPSC in vitro, will this improve clonal growth of cells and their genetic stability?
- Does variation in differentiation capacity of hPSC cell lines arise from variation in the stability of substates within the cultures?

Studying the human embryo in a dish

- Infertility and contraception, early embryo loss
- Birth defects
- Embryonic origins of adult disease-epigenetic reprogramming during this phase of development (cardiometabolic, neurological)
- Improving the fidelity of stem cell differentiation

Kevin Lau Lizzie Mason,
Christine wells, Josh kie
Shelley Hough,
Bioplatforms australia,
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