

Supplementary Information

Title:

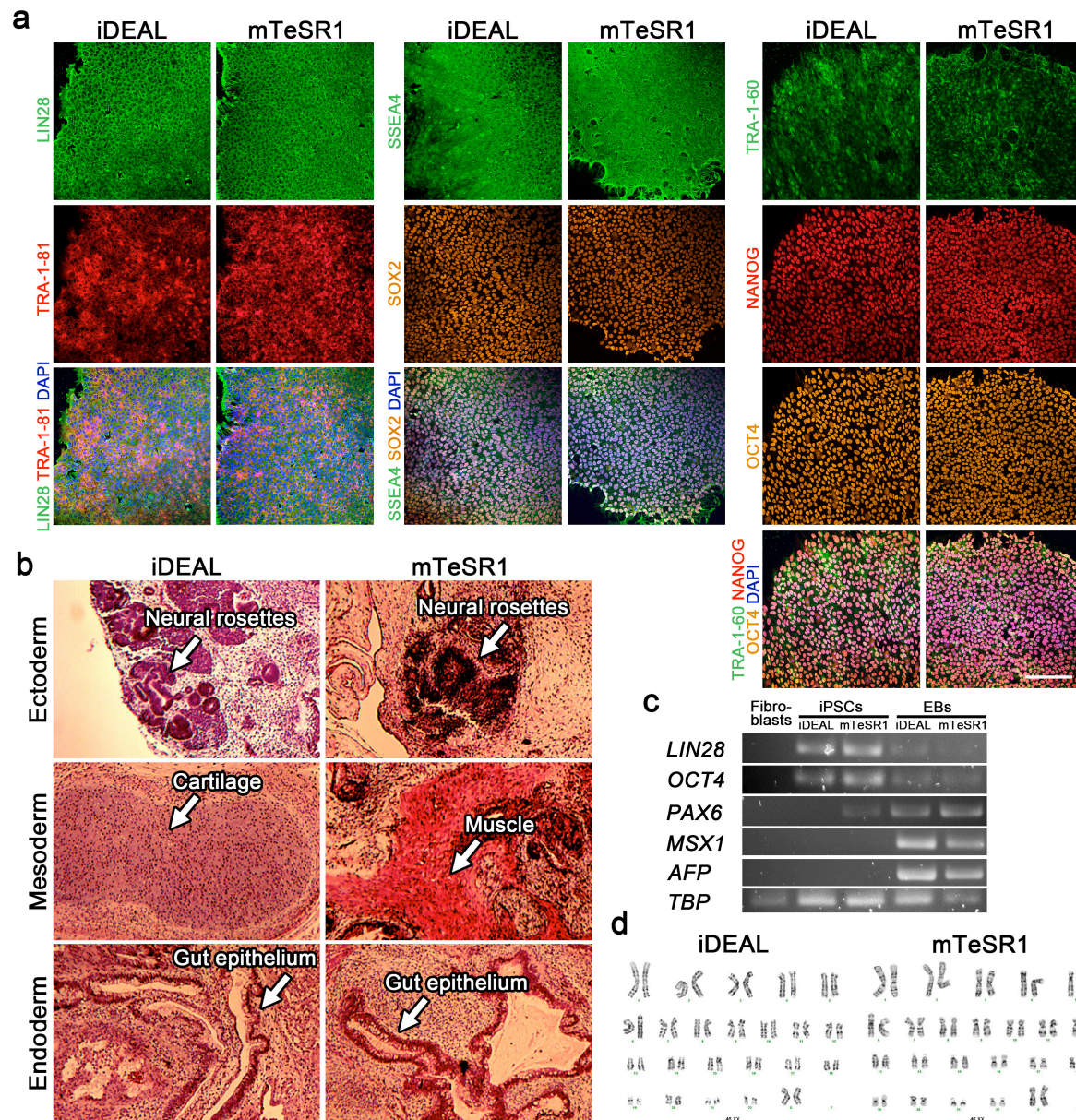
Systematic optimization of human pluripotent stem cells media using Design of Experiments

Authors: Paulo A. Marinho*, Thanathom Chailangkarn* & Alysson R. Muotri

Institution: University of California San Diego, School of Medicine, Department of Pediatrics/Rady Children's Hospital San Diego, Department of Cellular & Molecular Medicine, Stem Cell Program, La Jolla, CA 92093, MC 0695, USA.

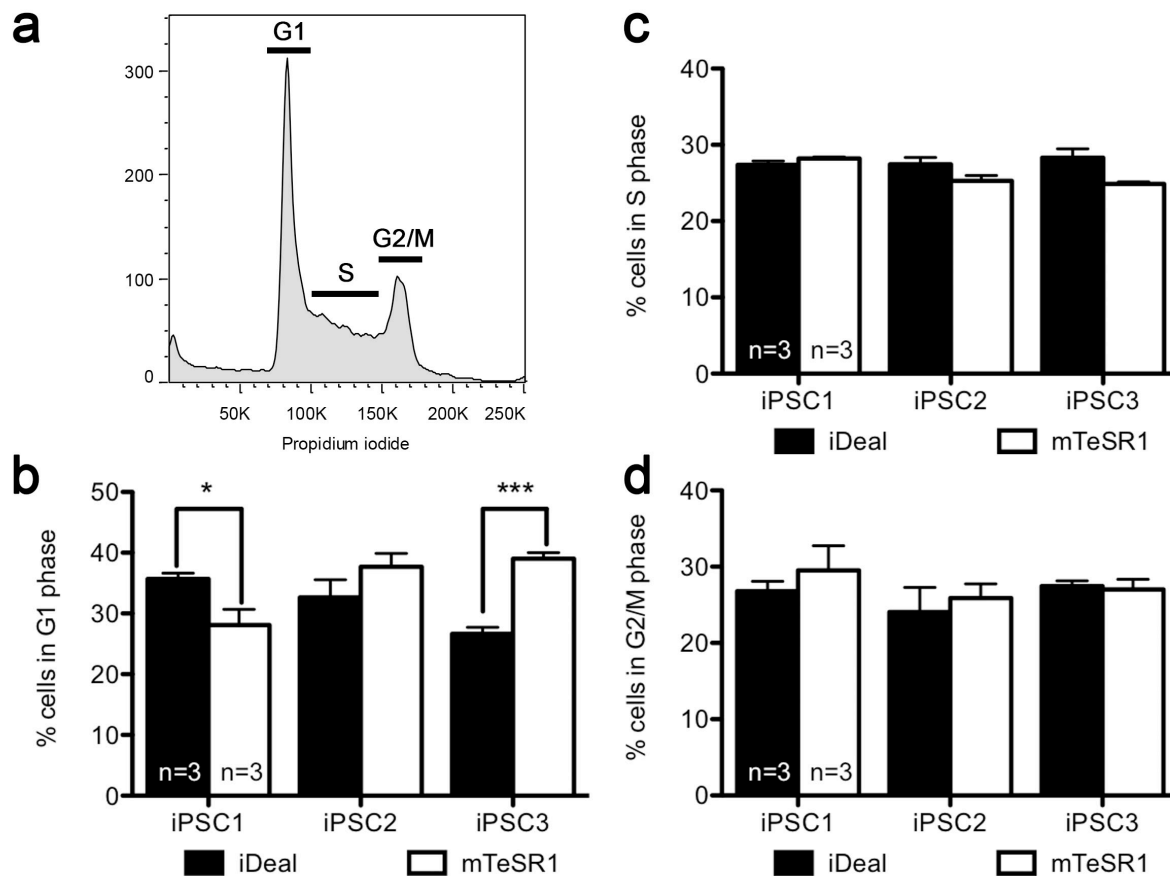
Correspondence: Alysson R. Muotri, Ph.D., University of California San Diego, 2880 Torrey Pines Scenic Drive - Sanford Consortium, Room 3005, La Jolla, CA 92093-0695. Telephone: (858) 534-9320; Fax: (858) 246-1579; email: muotri@ucsd.edu.

*These authors contributed equally to this work.

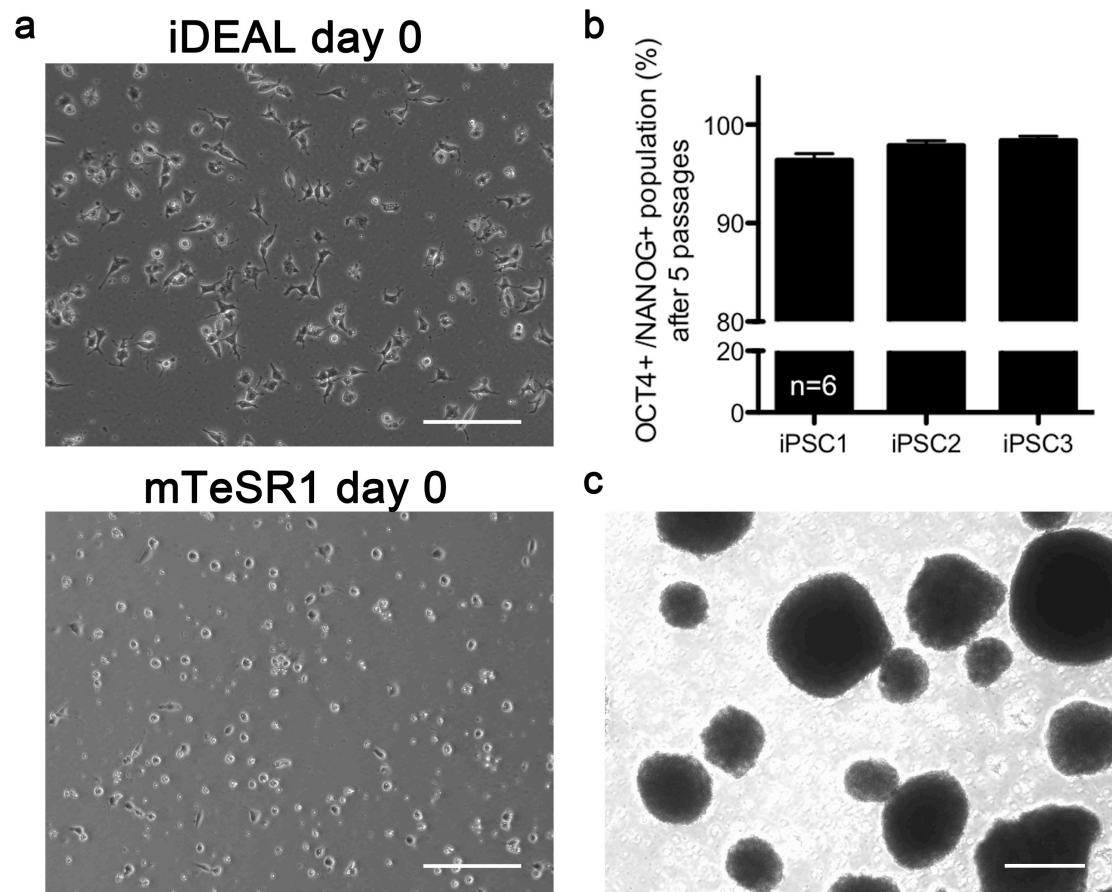


Supplementary Fig. 1: Characterization of iPSCs derived in iDEAL and mTeSR1. (a) Representative images of iDEAL- and mTeSR1-derived iPSCs expressing pluripotent markers; LIN28 and TRA-1-81 (left), SSEA4 and SOX2 (middle), and NANOG, OCT4 and Tra-1-60 (right) assessed by immunofluorescence staining. Scale bar, 200 μ m. (b) Representative images of teratoma generated from iPSCs derived in iDEAL and mTeSR1 showing tissues of 3 germ layers; neural rosettes (ectoderm), cartilage (mesoderm), muscle (mesoderm) and gut

epithelium (endoderm). **(c)** Embryoid bodies (EBs) generated from iPSCs expressed 3 germ layer markers; *PAX6* (ectoderm), *MSX1* (mesoderm) and *AFP* (endoderm) assessed by semi quantitative RT-PCR. *TBP*, housekeeping control. **(d)** iPSCs-derived from both media were genetically stable assessed by G-banding karyotype analysis.



Supplementary Fig. 2: Cell cycle analysis. (a) Representative image of cell cycle histogram of iPSCs stained with propidium iodide followed by FACS analysis showing population in each cycle phase (G1, S and G2/M). (b-d) Percentage of iPSCs in G1(b), S(c) and G2/M (d) phase at day 7 after passaging when media were changed daily. Black bars represent iPSC derived in iDEAL, White bars represent iPSC derived in mTeSR1. Data were shown as mean $\pm$ s.e.m for each of 3 iPSC lines (one clone each). n (technical replicates) = 3. * $P < 0.05$, *** $P < 0.001$, two-sided unpaired Student's *t* test.



Supplementary Fig. 3: Single-cell plating. **(a)** Representative images of single-cell iPSCs at hour 3 after plated (day 0) in iDEAL (top) or mTeSR1 (bottom) confirm singularity of plated cells. Scale bar, 200 μm . **(b)** iDEAL-derived iPSC population (%) with OCT4 and NANOG expression after 5 passages of single-cell passaging. Data were shown as mean $\pm$ s.e.m for each of 3 iPSC lines (one clone each). n (technical replicates) = 6. **(c)** Representative image of EBs generated from iDEAL-derived iPSCs that had been passaged as single cells for 5 passages. Scale bar, 200 μm .

Supplementary Table 1. Components of human pluripotent stem cell medium previously optimized (per liter)

Component	Concentration
Dulbecco's Modification of Eagle's Medium/Ham's F-12 + 80uL of concentrated hydrochloric acid	-
citric acid	12.9 mg
sodium selenite	15.4 µg
non essential amino acids	1.95 %
glutamine or glutamax	2 mM
basic fibroblast growth factor	60 ug
Recombunin® Alpha	0.4 g
optiferrin	12 mg
transforming growth factor beta-1	2 µg
pipecolic acid	1 µM
GABA gamma aminobutyric acid	1 mM
CDL chemically defined lipid	0.50 %
long Arg3 insulin-like growth factor-1	240 µg

Supplementary Table 2. Components of iDEAL (per liter)

Component	Concentration
Dulbecco's Modification of Eagle's Medium/Ham's F-12 + 80 μ L of concentrated hydrochloric acid	-
citric acid	12.9 mg
sodium selenite	15.4 μ g
non essential amino acids	1.95 %
glutamine or glutamax	2 mM
basic fibroblast growth factor	51 μ g
Recombinant [®] Alpha	0.4 g
optiferrin	12 mg
transforming growth factor beta-1	2 μ g
pipecolic acid	1 μ M
GABA gamma aminobutyric acid	1 mM
CDL chemically defined lipid	0.10 %
neuregulin-1 beta 1	21 μ g
long Arg3 insulin-like growth factor-1	240 μ g

Supplementary Table 3. Cells used in each experiment.

Experiments	H9	HUE9	Subject1				Subject2				Subject3						
			Fibro- blast1	iDEAL- derived		mTeSR1- derived		Fibro- blast2	iDEAL- derived		mTeSR1- derived		Fibro- blast3	iDEAL- derived		mTeSR1- derived	
				iPSC1	EB1	iPSC1	EB1		iPSC2	EB2	iPSC2	EB2		iPSC3	EB3	iPSC3	EB3
iDEAL optimization	1	1*															
iPSC characterization																	
Immunostaining				1		1			1		1			1		1	
Semi-qPCR			1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Teratoma assay				1		1			1		1			1		1	
Karyotyping				1		1			1		1			1		1	
iDEAL testing																	
iPSC culture maintenance																	
Pluripotency analysis				1(3)		1(3)			1(3)		1(3)			1(3)		1(3)	
Apoptosis analysis				1(3)		1(3)			1(3)		1(3)			1(3)		1(3)	
Single-cell passaging																	
Pluripotency analysis				1(4)		1(4)			1(4)		1(4)			1(4)		1(4)	
Survival analysis				1(4)		1(4)			1(4)		1(4)			1(4)		1(4)	
X chromosome inactivation			1	6		6											

* described in patent (reference 16)

One clone per cell line was used in all experiments except X chromosome inactivation in which six clones per line were used. Number in each parenthesis represents technical replicates.