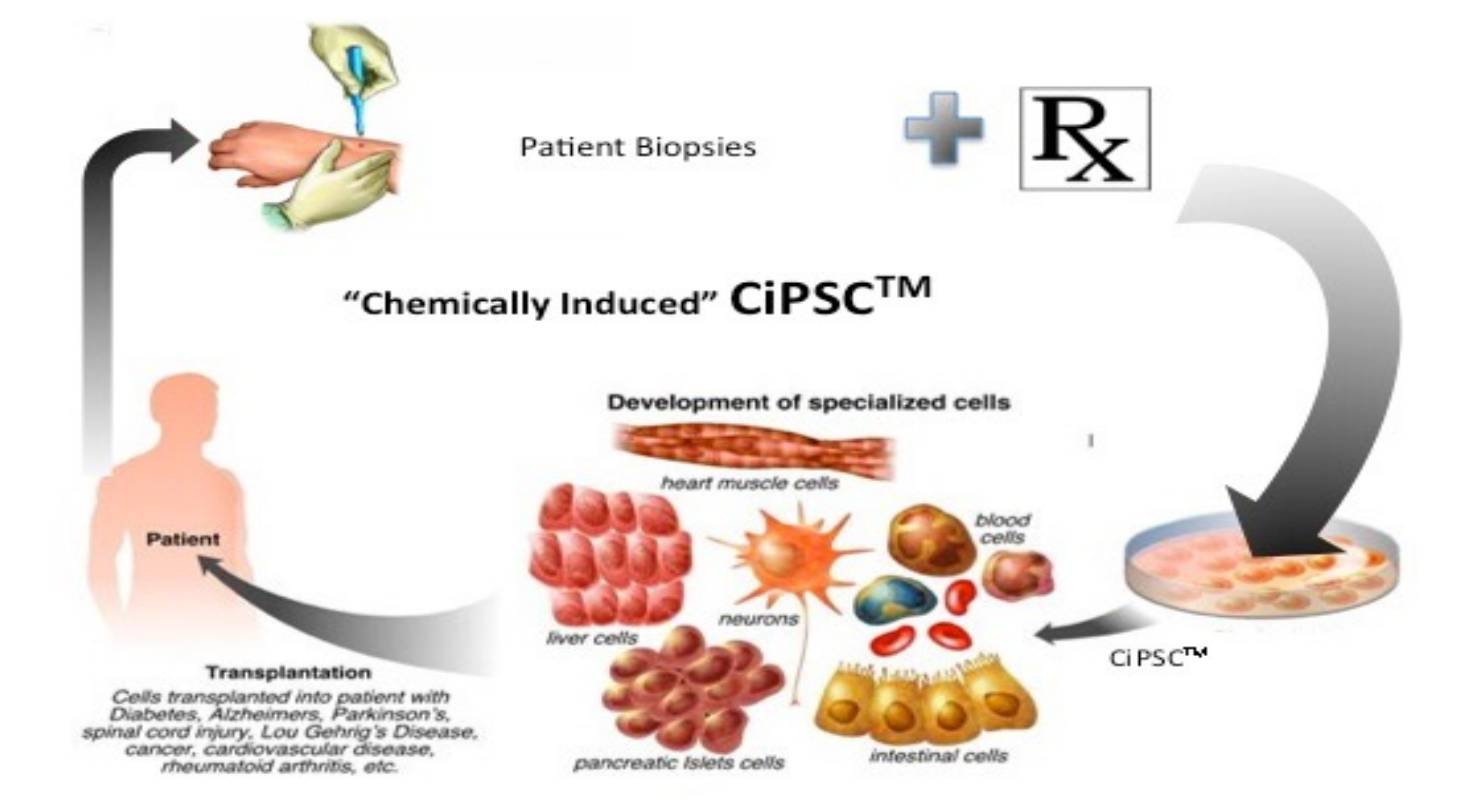


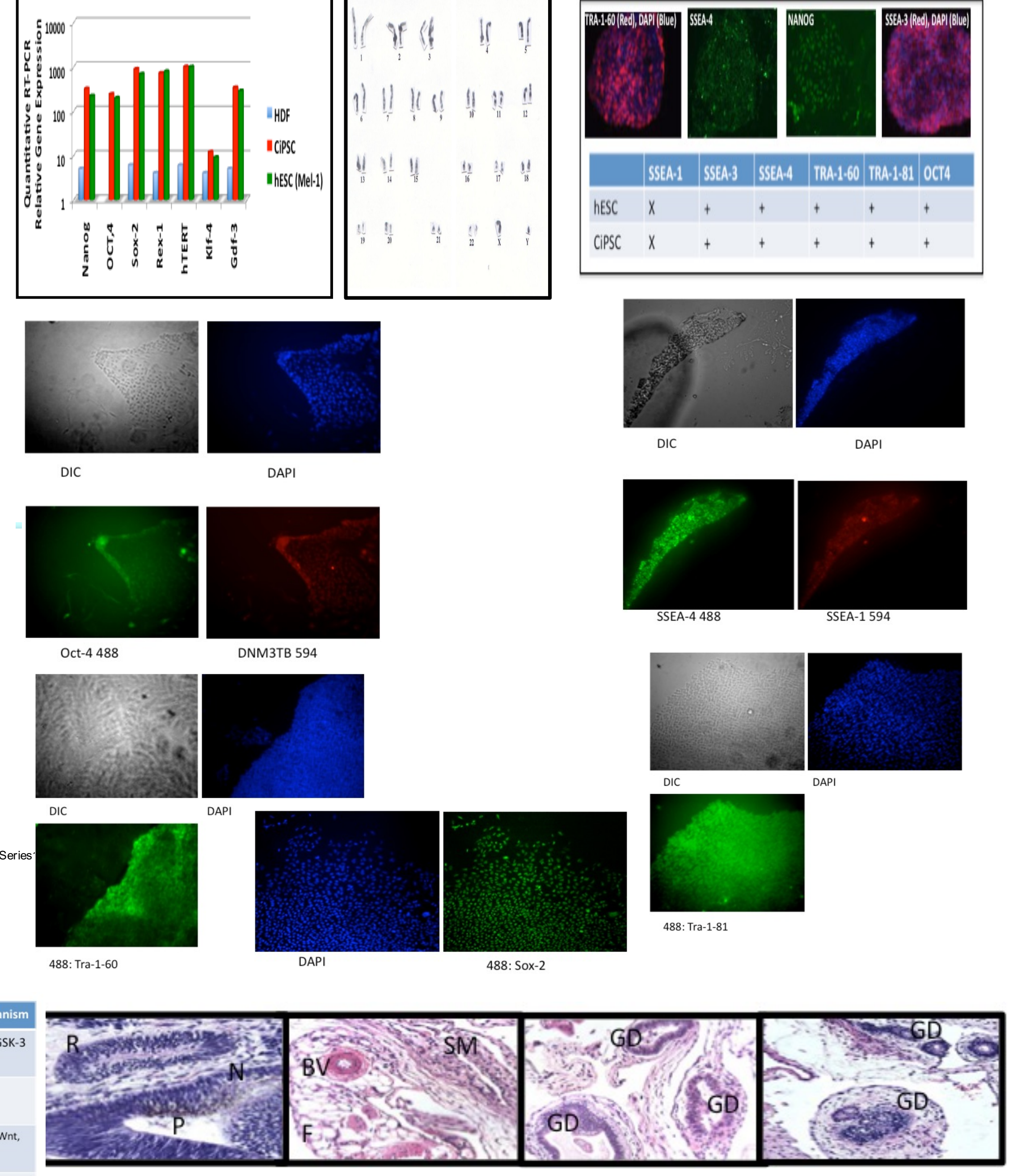
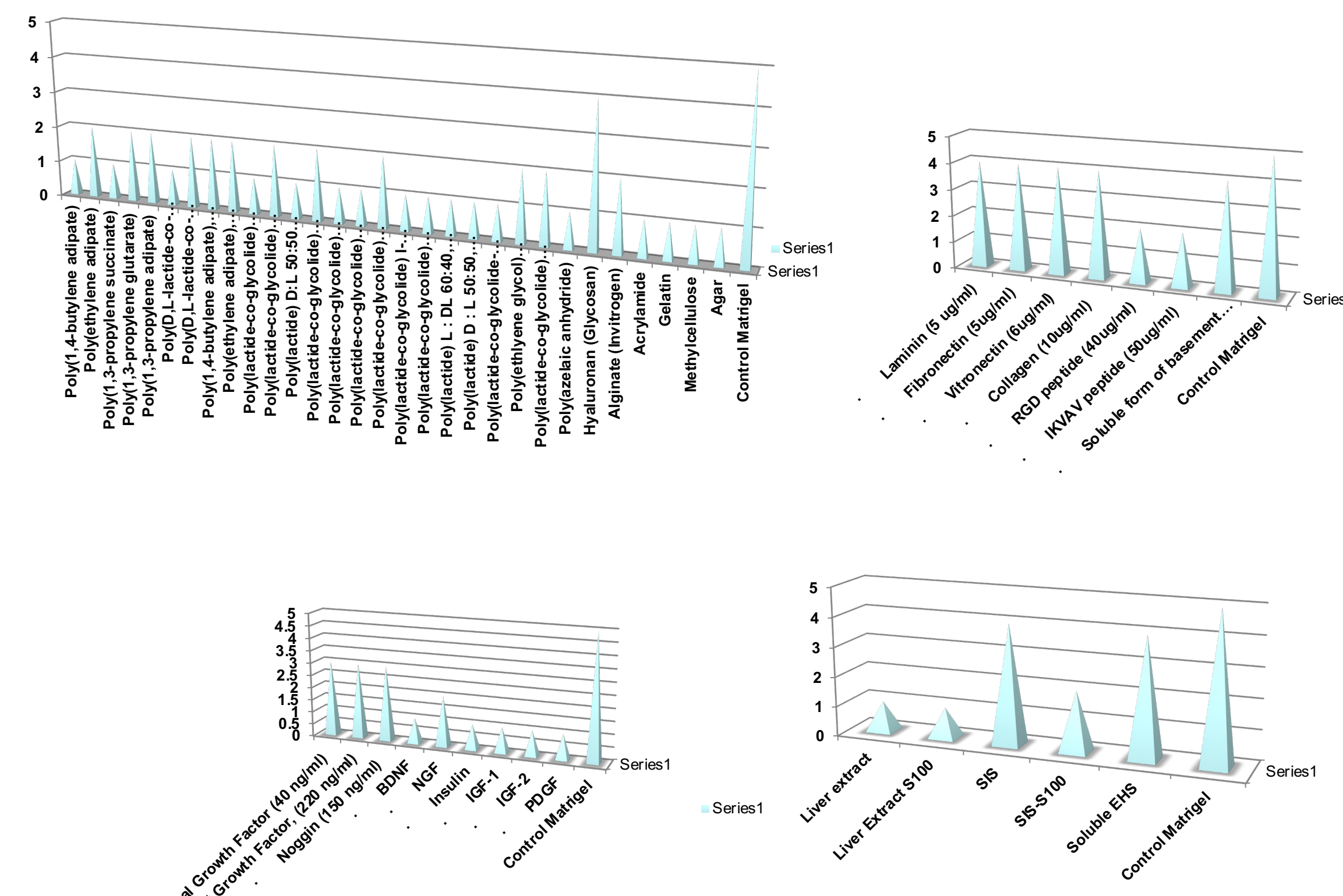
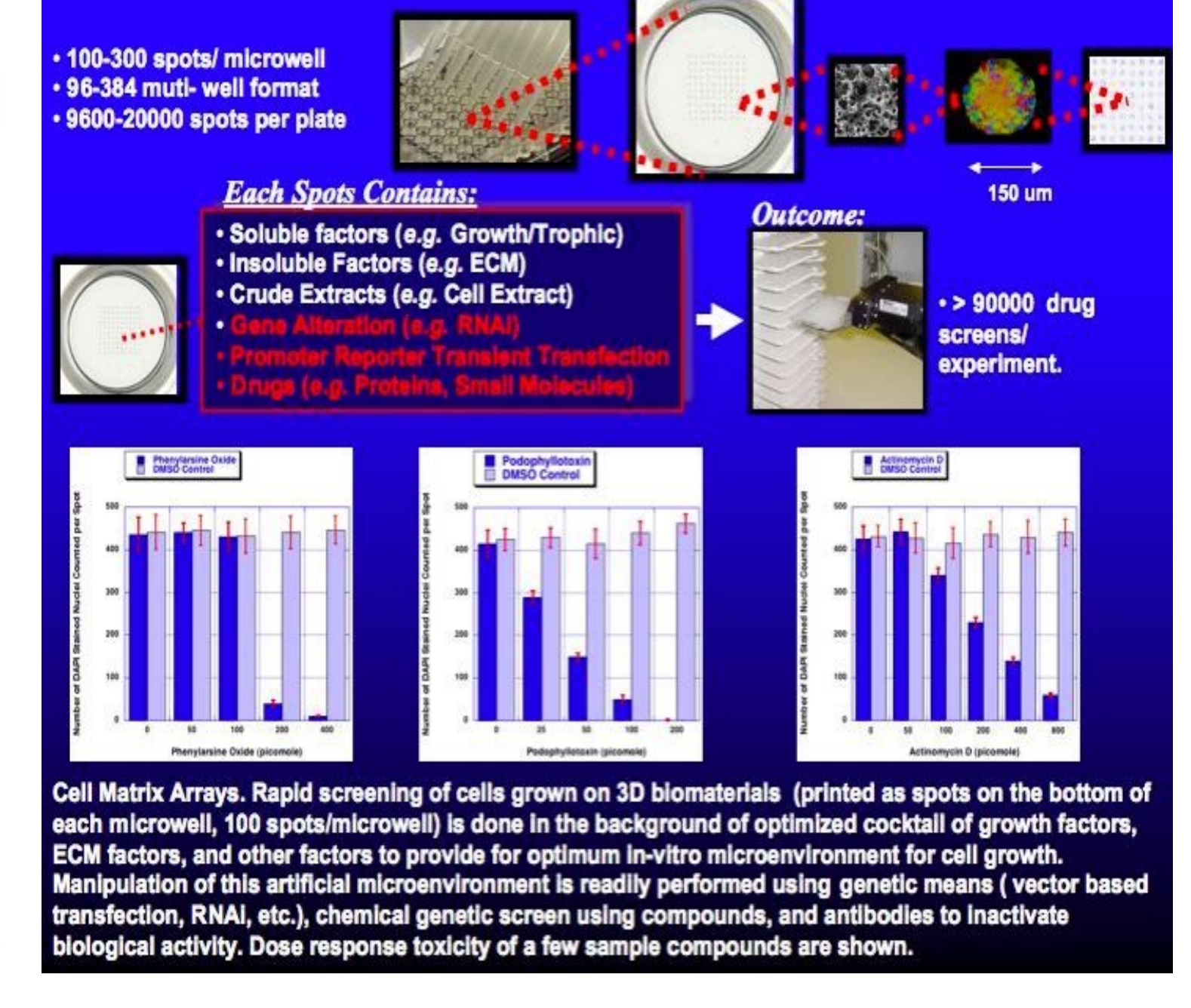
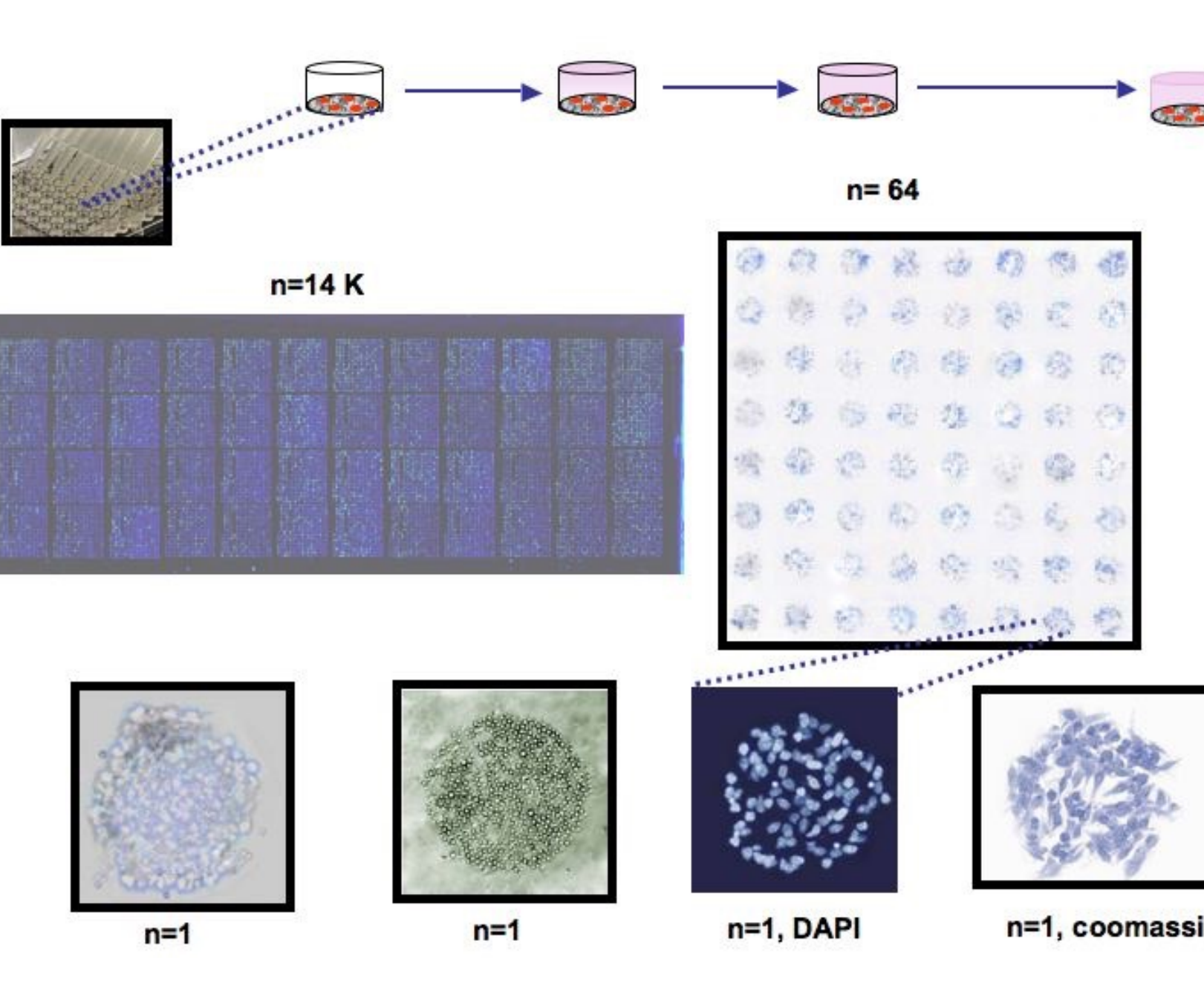
# Highly Efficient Complete Reprogramming and Lineage Differentiation Propensity of Chemically induced Pluripotent Stem Cells (CiPSC™)

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 2011, ISSCR Conference Meeting, Gladstone Institute, San Francisco, California.

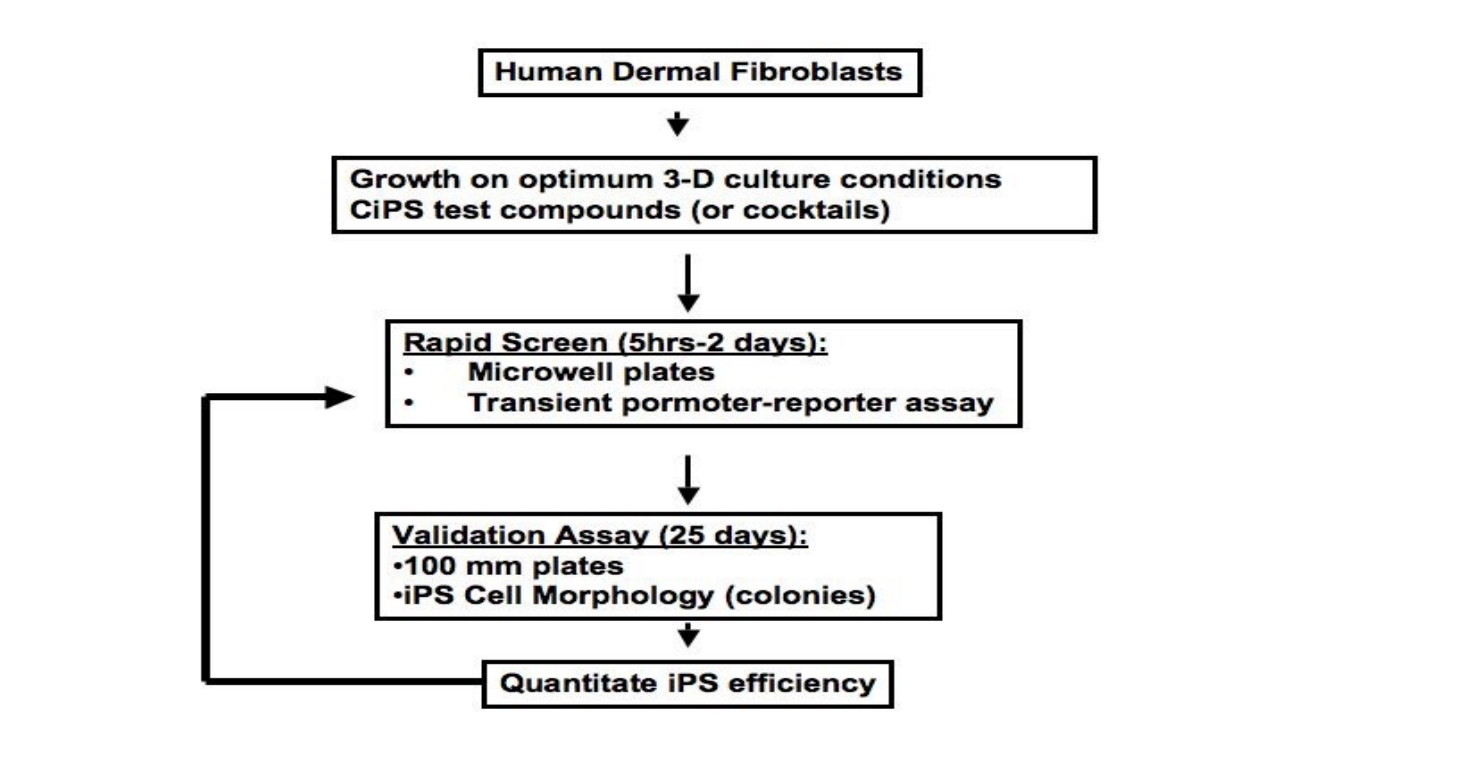
## CiPSC Pluripotency *in-vitro* & *in-vivo* (cont.)



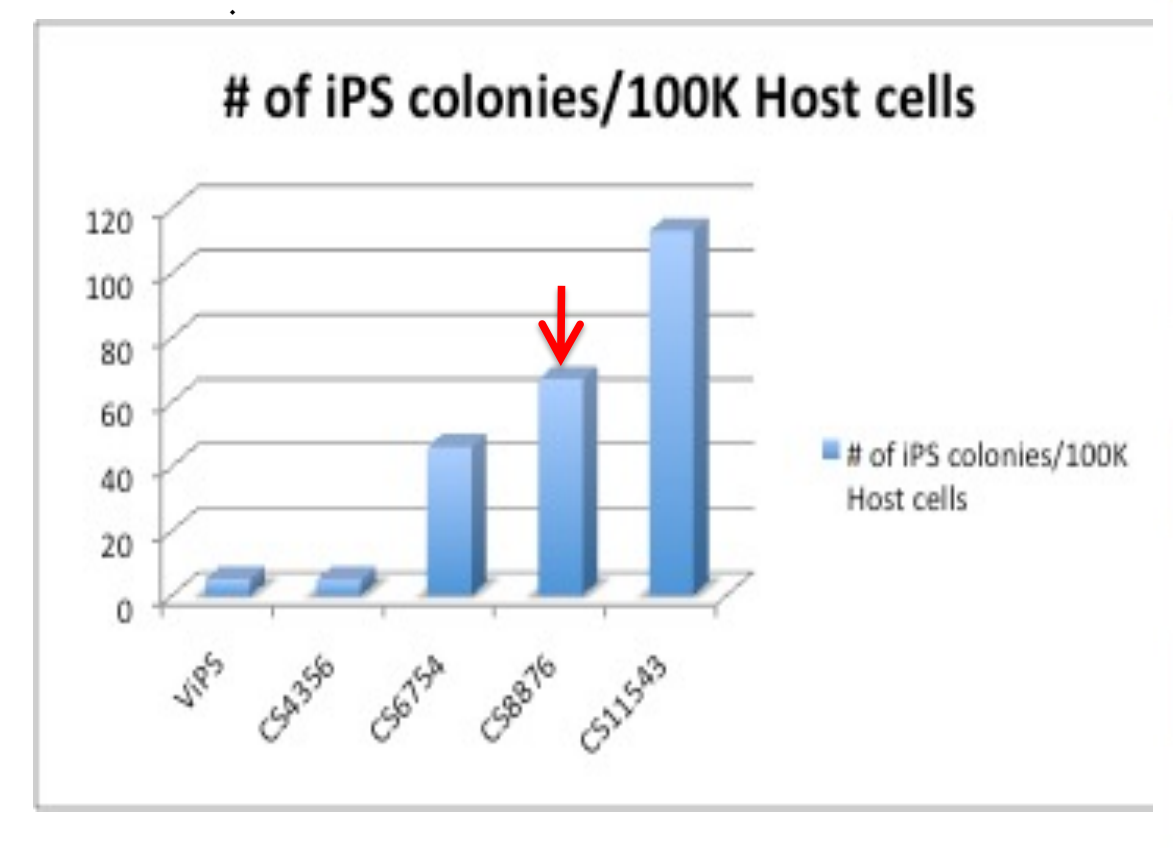
## High Throughput Screen of Optimal CiPSC 3-D Scaffold



## High Throughput Screen of CiPS Inducer Cocktails



Compound	Category	Target	Pathway	Outcome (promotes)
1	GSK-3	Wnt	Self Renewal	
2	GSK-3	Wnt	Differentiation	
3	TBD	Wnt	Self Renewal	
4	TBD	Wnt	Differentiation	
5	Smoothened	Hedgehog	Apoptosis, Anti-Proliferation	
6	TBD	Hedgehog	Apoptosis, Anti-proliferation	
7	TBD	Hedgehog	Differentiation	
8	TBD	NF-κB	Self Renewal	
9	TBD	NF-κB	Differentiation	
10	Cox-1	PGE-2	Anti-Proliferation	
11	Cox-2	PGE-2	Anti-Proliferation	
12	PKC	PKC	Apoptosis, Necrosis	
13	TBD	TBD	Self Renewal	
14	TBD	TBD	Differentiation	



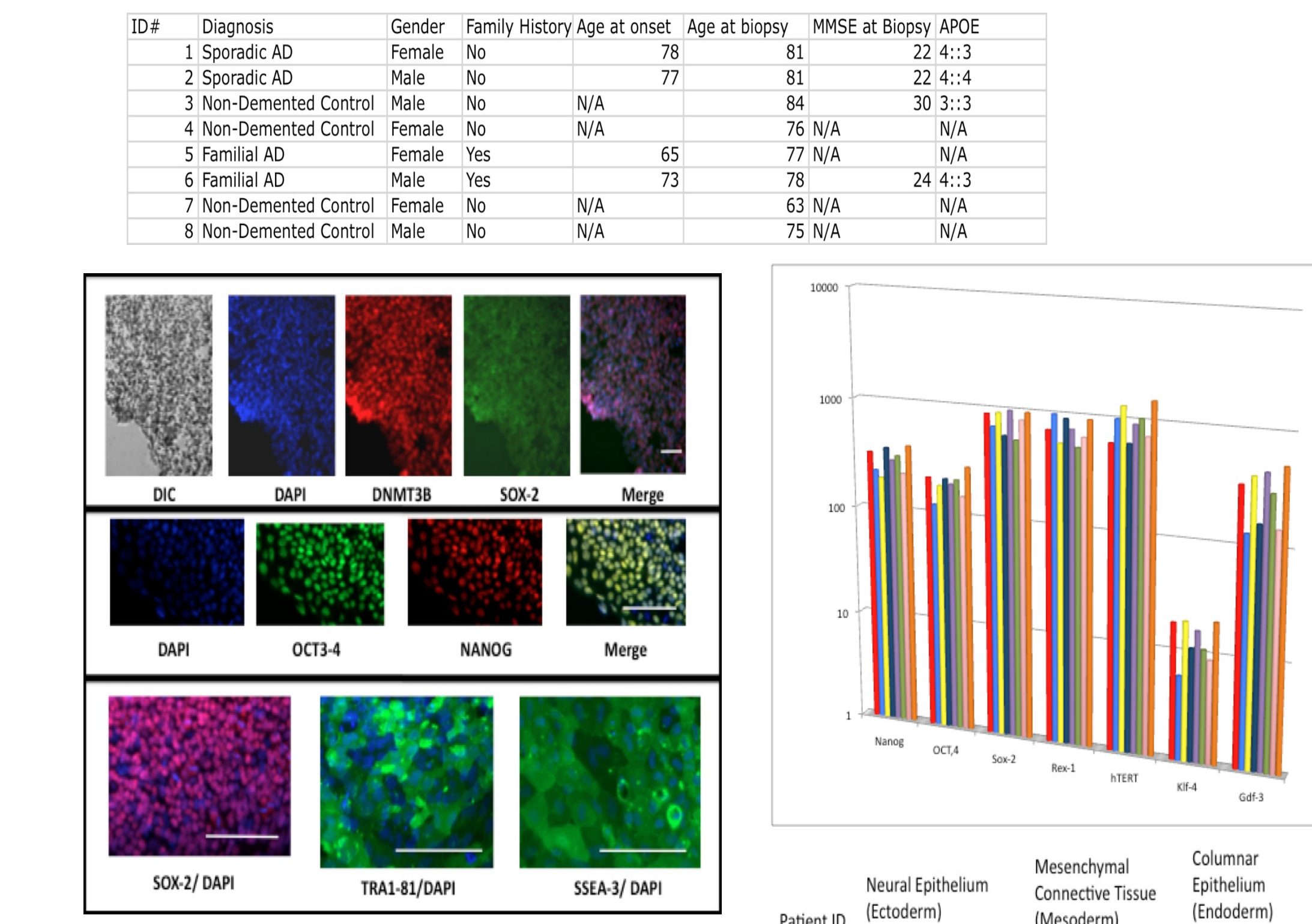
Compound	Structure	Name	Mechanism
D9-6452		6-bromoindirubin-3-oxime (BIO)	Wnt, GSK-3
D10-8976		Indirubin-5-nitro-3-oxime (INO)	Wnt
D3-8976		Valproic Acid	HDAC, Wnt, KLF-4
D21-8765		2-(3-(6-Methylpyridin-2-yl)-1H-pyrazol-4-yl)-1,5-naphthyridine	TGF-β
D12-2234		1-(4-Methylphenyl)-2-(4,5,6,7-tetrahydro-2-imino-3(2H)-benzothiazolyl)ethanone hydrobromide	PS3
D9-9811		Prostaglandin I2	KLF-4
D2-6542		Prostaglandin E2	Wnt-wt/akt
C-569812		Colcemid	Cell Cycle

CiPSC spontaneously differentiated into all three germ layers (Ectoderm, Mesoderm, and Endoderm) as evident in teratomas in SCID mice. Ectoderm: Neuron (N), Neuronal Rosette (R), Pigment Cells (P). Mesoderm: Blood Vessel (BV), Fat Cell (F), Smooth Muscle (SM). Endoderm: Glands and Ducts (GD).

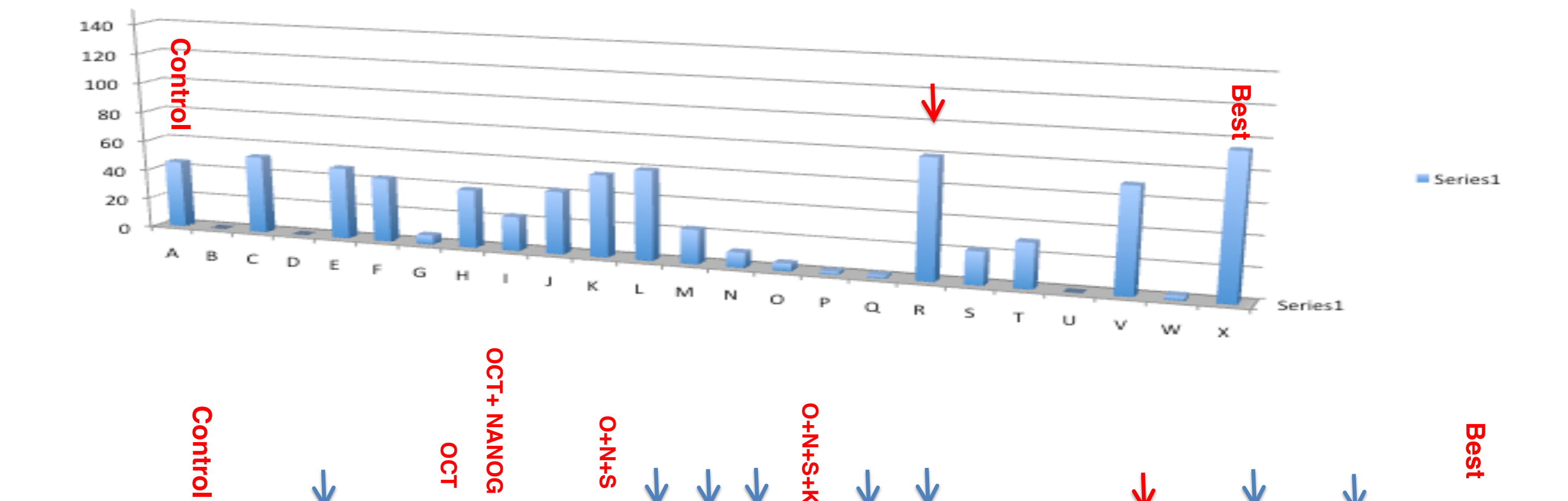
## Protocol for CiPSC Derivation

- 1) CiPSC are derived from Human Dermal Fibroblasts (HDF) grown in 3D cultures of optimal Hydrogel.
- 2) The hydrogel is enriched with optimum concentrations of growth factors and ECM components refined for CiPSC derivation.
- 3) HDF are embedded in this hydrogel and grown for 32 days of the CiPSC derivation at 37 degrees Celsius and 5% CO2 with media changes every 2 days.
- 4) During days 1-32 cells are grown in mTeSR-1 supplemented with Inducer Drug Cocktail of drugs.
- 5) At day 32, CiPSC are recovered from the HA hydrogel via treatment with a collagenase/hyaluronidase solution, counted and plated on matrigel coated plates and grown in mTeSR-1 medium.

## CiPSC Complete Reprogramming and High Lineage Differentiation Propensity using Alzheimer's Disease Patient Cells

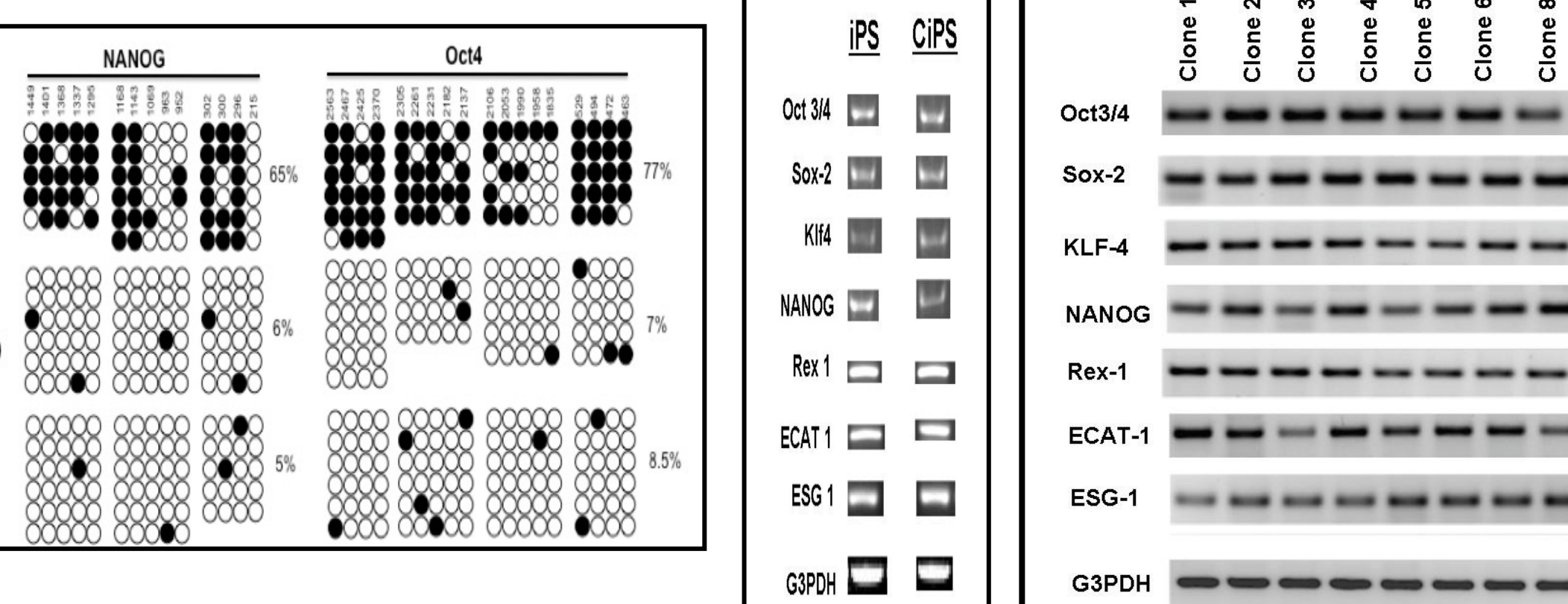


## Rescue of ViPSC Phenotype using a Cocktail of Small Molecule CiPSC Inducers



	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X
OCT4	+	+	++	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sox2	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Nanog	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
KLF-4	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
D9-6452	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
D10-8976	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
D3-8976	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
D21-8765	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
D12-2234	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
D9-9811	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
D2-6542	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
D23-1276	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
D6-982	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
D-2217	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
D23-1276	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C-569812	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

## CiPSC Pluripotency *in-vitro* & *in-vivo*



Patient ID	B. Number of iPSC colonies per 100K starting skin fibroblasts (visual ID)	C. Randomly selected subset of cell lines from column B isolated for further study	D. Subset of cell lines from column C with normal growth rate	E. Subset of cell lines from column C with low growth rate, challenging propagation	F. Completely reprogrammed cell lines (subset of those in column C)	G. Partial reprogrammed cell lines (subset of those in column C)	H. Teratoma producing cell lines (4 random subset of complete reprogrammed cell lines (from column C))	I. Cell lines with High differentiation propensity (in-vitro) number of cell lines tested (same cell lines as column H)								
1	CI-PSC:150	VPSC:10	CI-PSC:8	VPSC:8	CI-PSC:7	VPSC:2	CI-PSC:1	VPSC:6	CI-PSC:6	VPSC:1	CI-PSC:2	VPSC:7	CI-PSC:44	VPSC:14	CI-PSC:44	VPSC:14
2	CI-PSC:142	VPSC:5	CI-PSC:8	VPSC:5	CI-PSC:0	VPSC:4	CI-PSC:0	VPSC:4	CI-PSC:1	VPSC:4	CI-PSC:3	VPSC:4	CI-PSC:34	VPSC:14	CI-PSC:34	VPSC:14
3	CI-PSC:102	VPSC:12	CI-PSC:8	VPSC:8	CI-PSC:7	VPSC:2	CI-PSC:1	VPSC:6	CI-PSC:6	VPSC:2	CI-PSC:2	VPSC:6	CI-PSC:44	VPSC:14	CI-PSC:44	VPSC:14
4	CI-PSC:136	VPSC:10	CI-PSC:8	VPSC:8	CI-PSC:5	VPSC:2	CI-PSC:3	VPSC:6	CI-PSC:6	VPSC:3	CI-PSC:2	VPSC:5	CI-PSC:44	VPSC:14	CI-PSC:44	VPSC:14
5	CI-PSC:122	VPSC:10	CI-PSC:8	VPSC:8	CI-PSC:8	VPSC:3	CI-PSC:0	VPSC:5	CI-PSC:8	VPSC:2	CI-PSC:0	VPSC:6	CI-PSC:34	VPSC:14	CI-PSC:34	VPSC:14
6	CI-PSC:105	VPSC:10	CI-PSC:8	VPSC:8	CI-PSC:7	VPSC:1	CI-PSC:1	VPSC:6	CI-PSC:8	VPSC:1	CI-PSC:0	VPSC:6	CI-PSC:44	VPSC:14	CI-PSC:44	VPSC:14
7	CI-PSC:108	VPSC:4	CI-PSC:8	VPSC:4	CI-PSC:8	VPSC:1	CI-PSC:0	VPSC:3	CI-PSC:7	VPSC:2	CI-PSC:1	VPSC:2	CI-PSC:44	VPSC:14	CI-PSC:44	VPSC:14
8	CI-PSC:115	VPSC:9	CI-PSC:8	VPSC:8	CI-PSC:8	VPSC:2	CI-PSC:2	VPSC:6	CI-PSC:7	VPSC:0	CI-PSC:1	VPSC:8	CI-PSC:44	VPSC:14	CI-PSC:34	VPSC:14