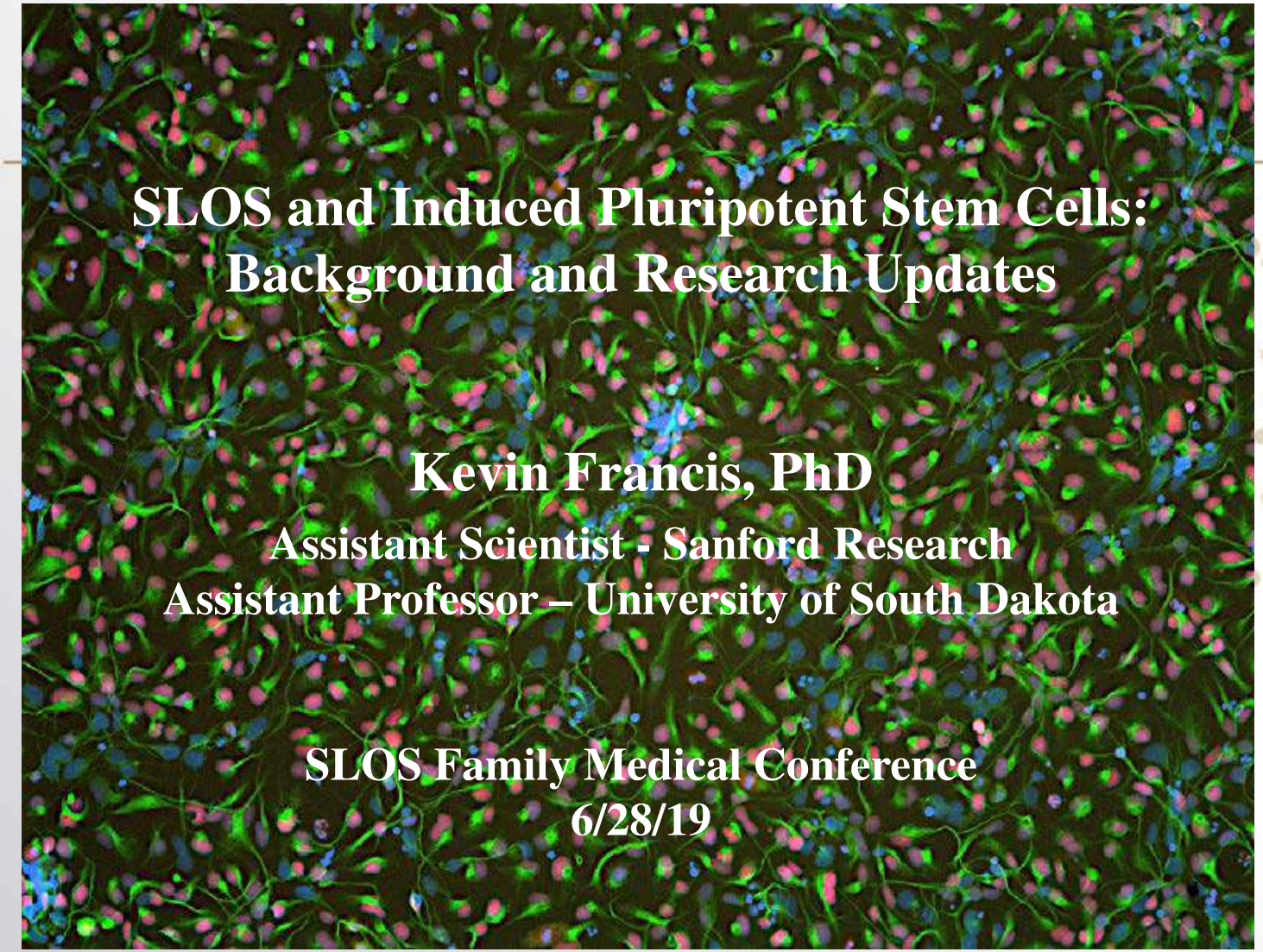

# SLOS and Induced Pluripotent Stem Cells: Background and Research Updates


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**Assistant Scientist - Sanford Research**

**Assistant Professor – University of South Dakota**


**SLOS Family Medical Conference**

**6/28/19**



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# Ways my lab is using stem cells to research DHCR7 effects on development and cell function

1. What are the developmental consequences of *DHCR7* mutation?

2. What are the functional consequences of *DHCR7* mutation?

3. Can we develop therapies to impact patient symptoms to improve quality of life?

** My belief is that understanding how, when, and why DHCR7 discriminately affects specific signaling mechanisms and cell function will help us understand how SLOS manifests and allow us to develop targeted therapies to improve patient function **

# What can stem cells do?

## Self-renewal

- Undergoes rounds of cell division and maintains an undifferentiated state

## Differentiation

- Undifferentiated = no specialization
- Stem cell becomes something different
- More specialized

(Somatic cell)

# Stem cells types:  one size does not fit all

1. Fetal/amniotic/cord blood stem cells
2. **Adult or tissue derived stem cell**
3. Embryonic stem cells
4. **Reprogrammed/induced pluripotent stem cells (iPSCs)**

# Adult or tissue specific stem cells

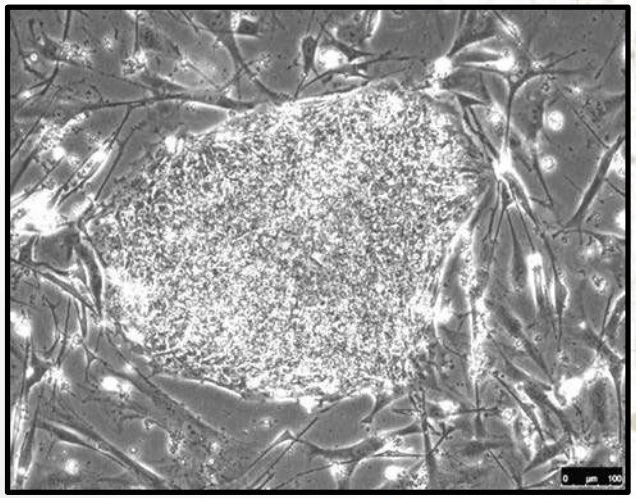
- Found in virtually every organ within the body except the heart
  - Neural, intestinal, bone marrow, fat, etc.
- Adult stem cells reside within areas of organs or tissues which protect them from external stimuli (i.e. bone marrow, lining the ventricles of the brain or crypts within the intestine)
- Adult stem cells are multipotent – can form specific tissues only
  - i.e. neural stem cells can become neurons, not cardiomyocytes
- Usually difficult to obtain and manipulate

  (Bone marrow and fat are the exception)

# Embryonic stem (ES) cells

- Refers to cells isolated from the inner cell mass of the developing blastocyst which exhibit:

- Unlimited self-renewal – (if appropriate conditions are met)
- Pluripotent – can form any cell within the body
- Issues with these include ethical considerations, limited availability, and immune response



# Reversing development with iPSCs

*Developmental potential*

Pluripotent

↓

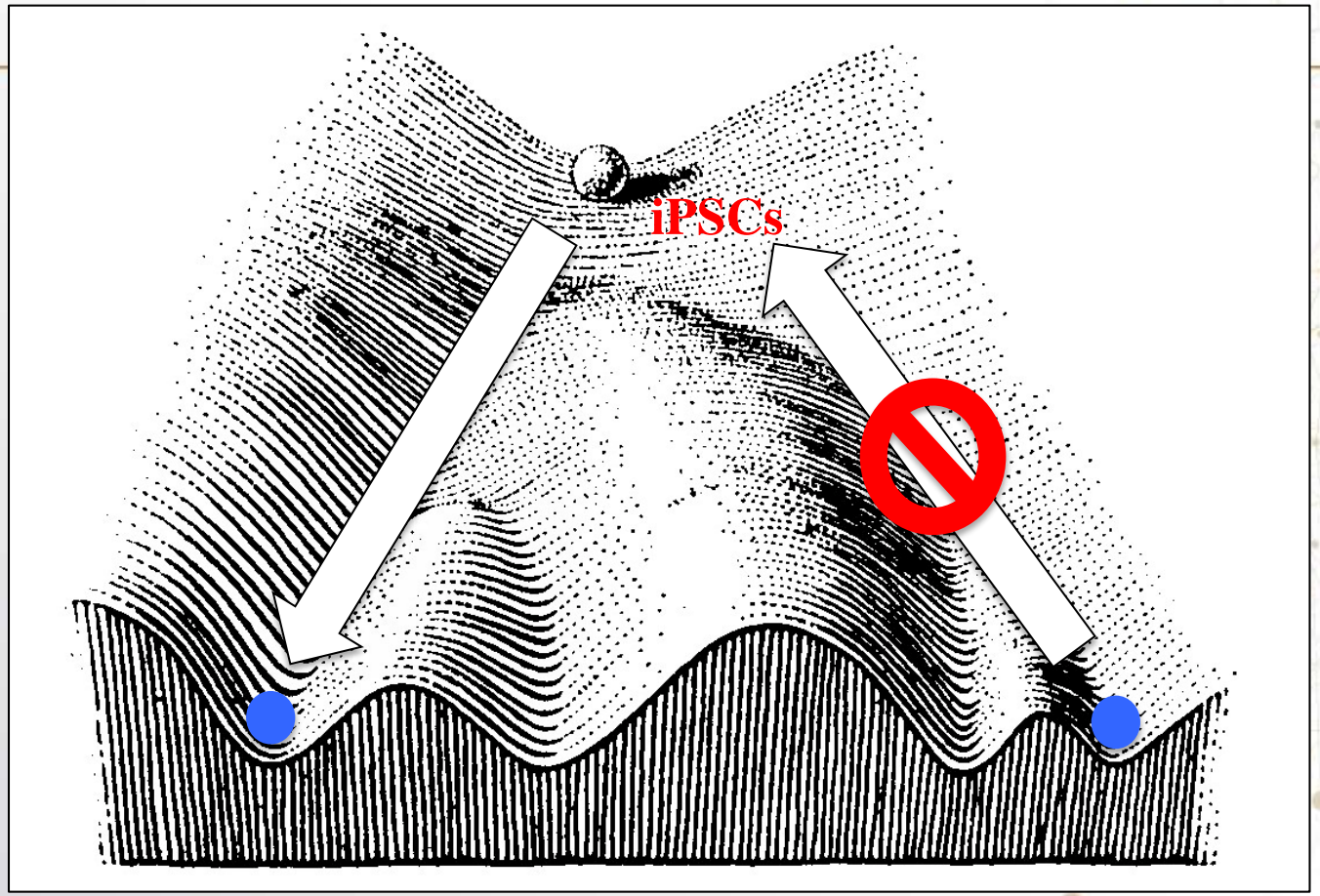
Multipotent

↓

Unipotent

↓

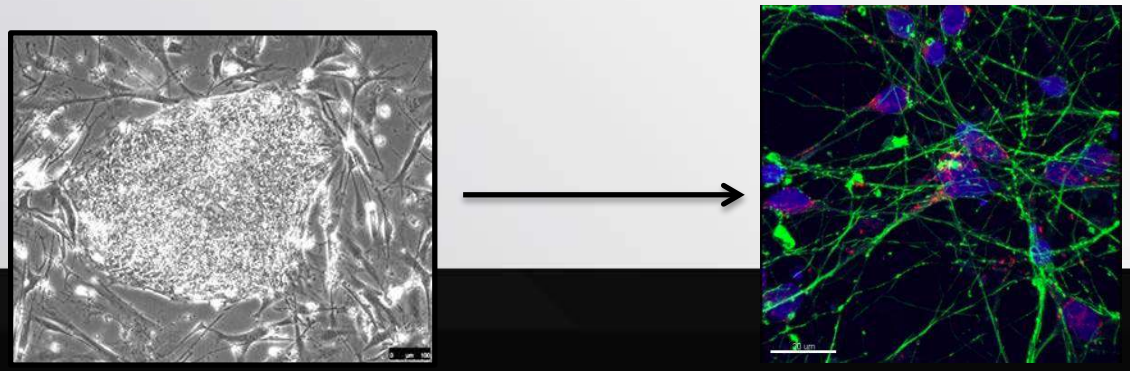
Differentiated




Pluripotent – can become any cell type within the body (outside of the placenta)
Multipotent – can become multiple cell types but within a germ layer

# How are iPS cells different than other stem cells?

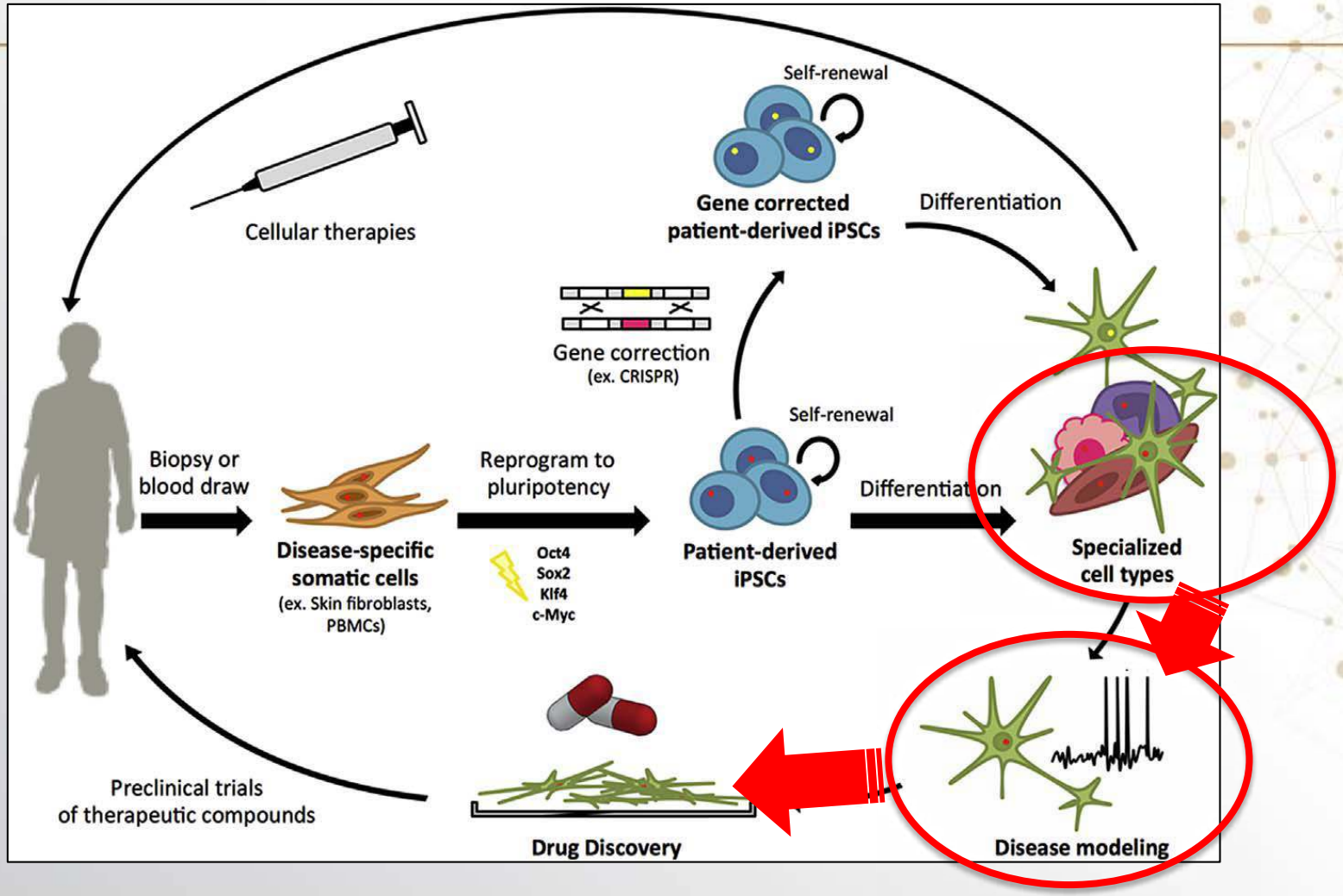
- Exhibit pluripotent capabilities like human ES cells
- Less ethical issues compared to human ES cells
  - No requirement for human embryos or destruction
  - No oocyte donation for cloning
- Can derive from virtually anyone willing to donate a cell sample (blood draw or skin biopsy most common)
- Any cell which can divide can be used (fibroblasts, B or T cells from blood, other types of stem cells
- Can study human diseases outside of the patient
- Should avoid immune rejection



# Human iPSCs as a model – pros and cons

- Exhibit patient's specific genome (pro), but not just your gene of interest (potential con)
- Highly expandable, allowing scale up
- Differentiation efficiency to cell types of interest can vary greatly depending on the target population
- Can identify or test cell type specific effects
- 3D differentiation assays allow analysis in a spatially organized system (pro), but this is not high throughput (con)
- Fairly easy to modulate genomically via nuclease-based genome editing (i.e. CRISPR) for general effects,
- Studies can be technically (and financially) challenging
- Cultures are prone to genomic instability over time

# Induced pluripotent stem cells (iPSC) to model rare neurological diseases



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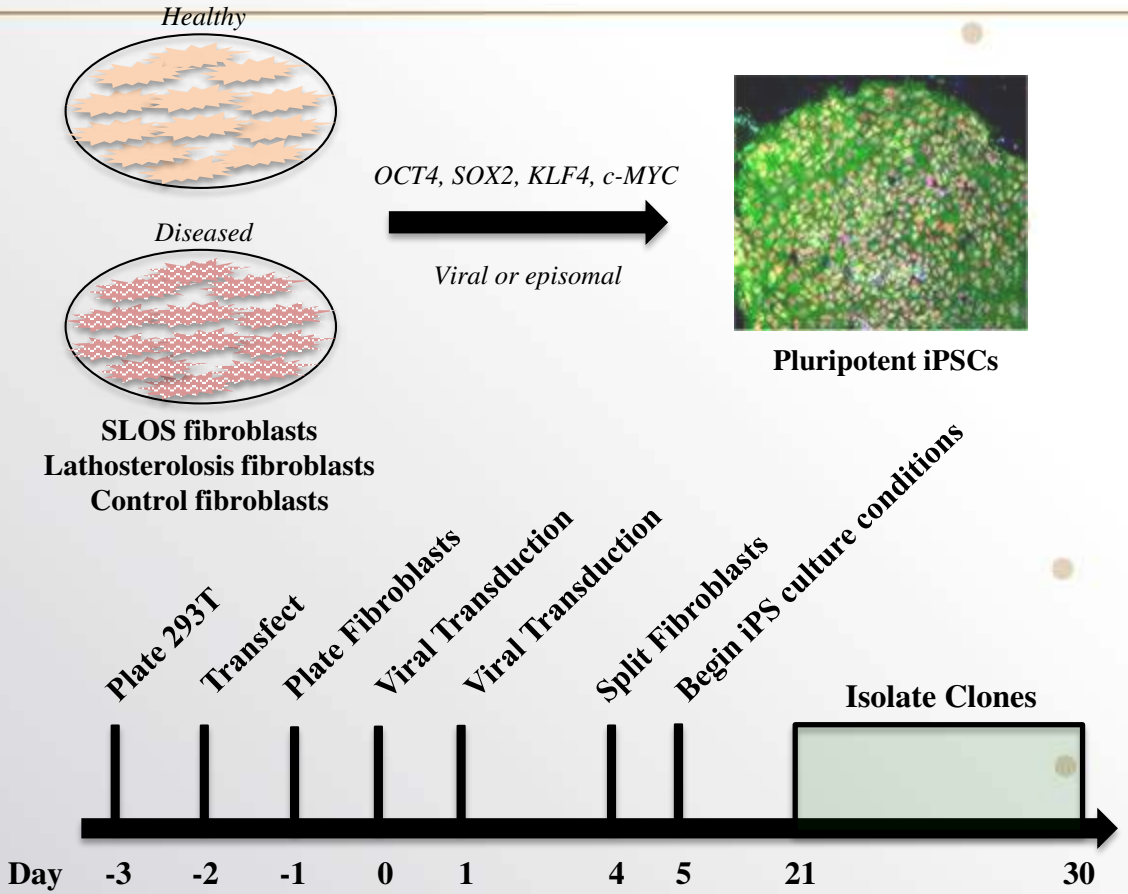
# SLOS iPSC models within my lab (via NIH/Porter lab)

| Subject | Cell Lines | Starting Tissue | Phenotype | Mutations | Biopsy age | Gender |
|---|---|---|---|---|---|---|
| SLOS-066 | MN 4F-1<br>MN 4F-2 | Skin biopsy; fibroblast | Mild SLOS | p.M1V/<br>p.Q98X | 2 years | M |
| SLOS-003 | MW 4F-1<br>MW 4F-2 | Skin biopsy; fibroblast | Mild SLOS | p.T154M/<br>c.964-1G>C | 4 years | M |
| SLOS-029 | CWI 4F-1<br>CWI 4F-2 | Skin biopsy; fibroblast | Classical SLOS | p.T93M/<br>c.964-1G>C | 6 months | F |
| SLOS-001 | CW 3F-1<br>CW 3F-2 | Skin biopsy; fibroblast | Classical SLOS | p.T93M/<br>c.964-1G>C | 8 months | M |
| SLOS-098 | A2 3F-1<br>A2 3F-2<br>A2 4F-5 | Skin biopsy; fibroblast | Severe SLOS | c.964-1G>C<br>homozygous | 1 day | M |

# Cellular reprogramming to pluripotency – methodology



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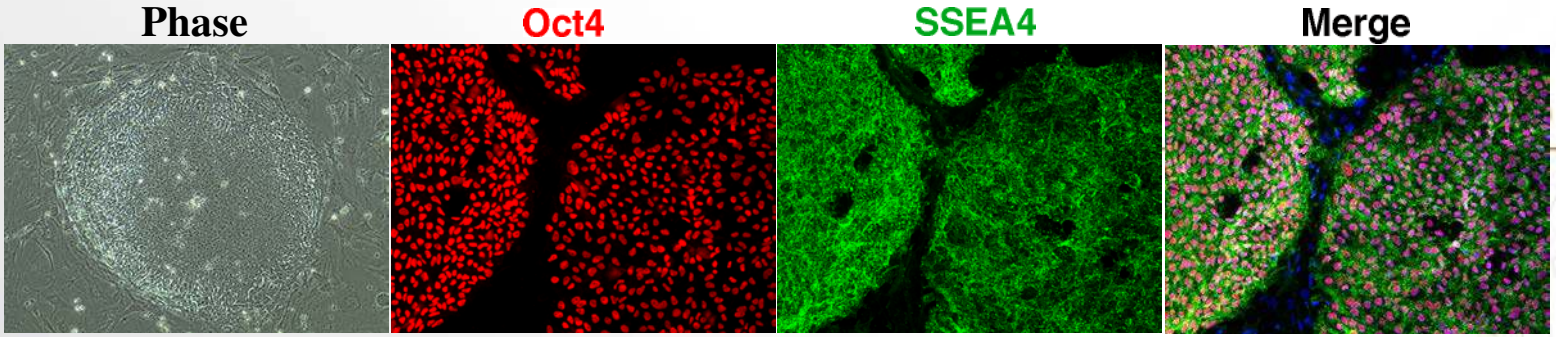
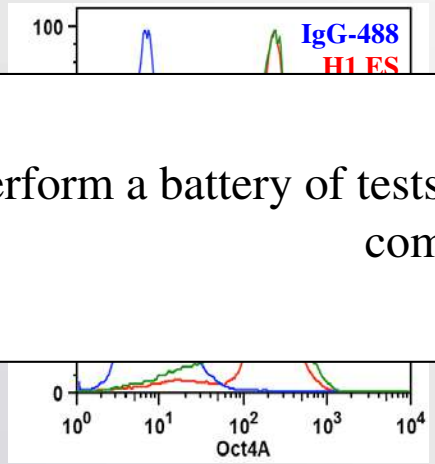
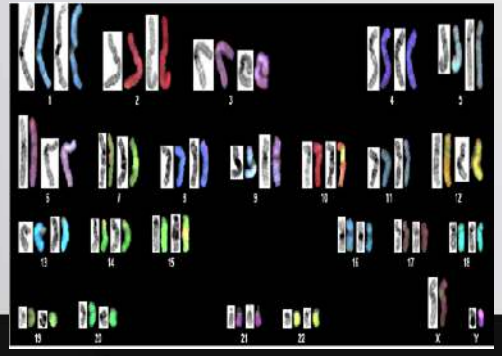
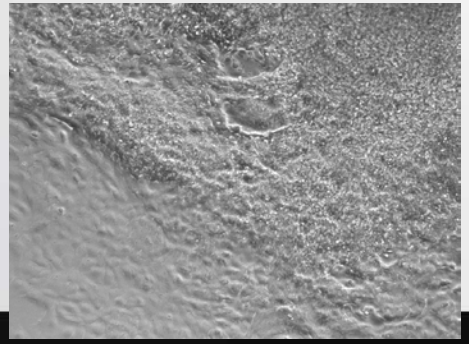
# Characterization of SLOS iPSCs

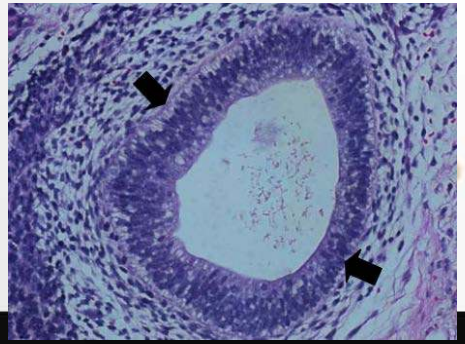



- We perform a battery of tests to make sure the SLOS iPSC lines we generated can be compared to pre-validated controls









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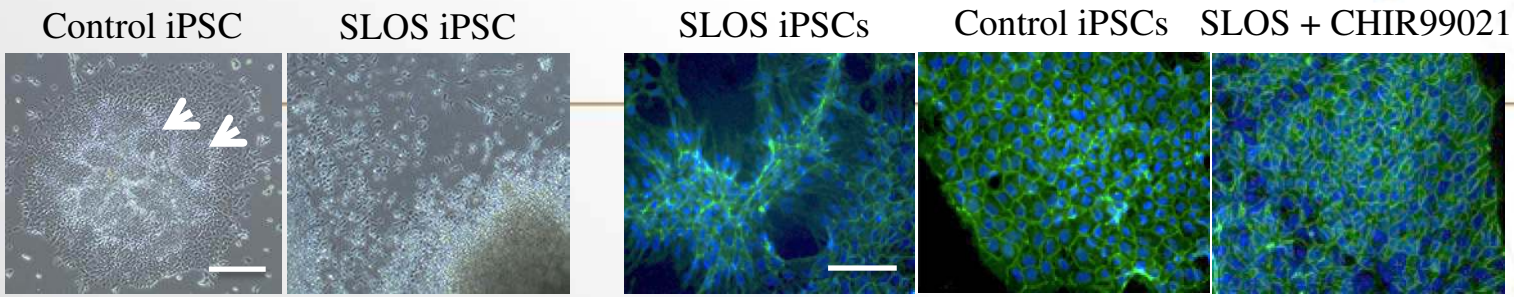
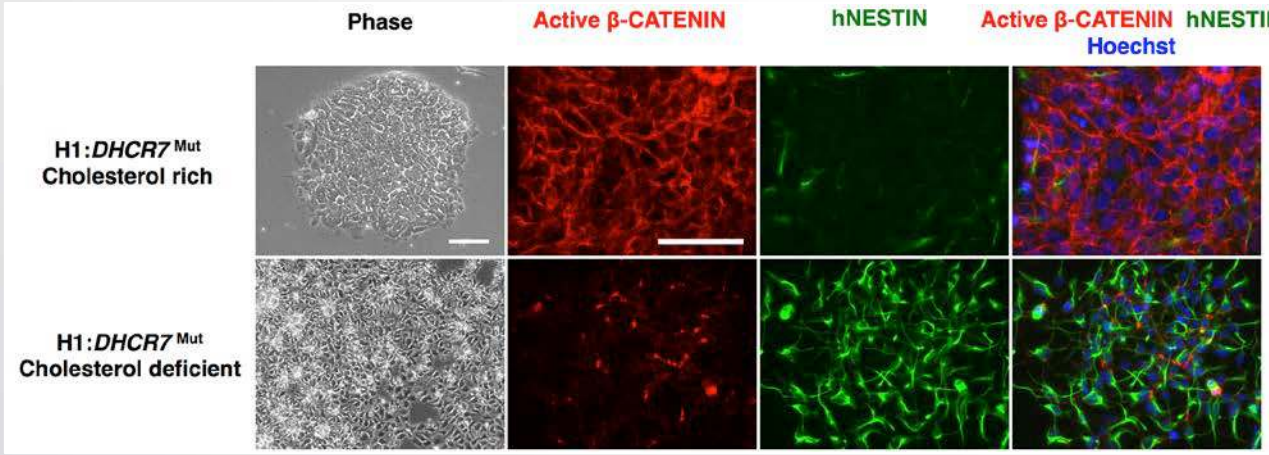
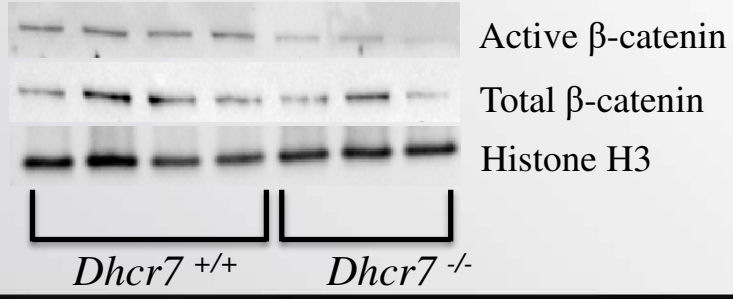
# Loss of β-catenin activity in iPSCs carrying *DHCR7* mutations




E18.5 Cortex



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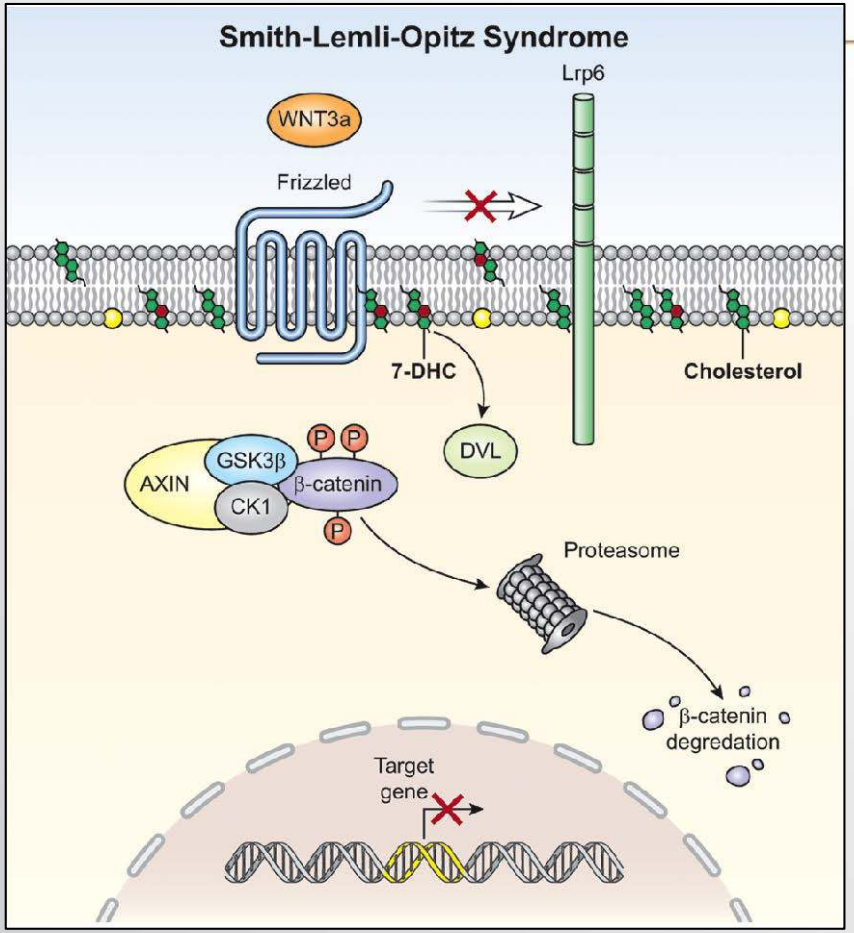
# Wnt signaling deficits in Smith-Lemli-Opitz syndrome




Francis et al. 2016. *Nat Med* 22(4):388-96.

- Wnt signaling is a critical protein signaling pathway for controlling tissue development and stem cell activity
- Helps explain some of the developmental malformations observed in these patients
- However, the precise consequences of Wnt disruption on tissue development and function, as well as other signaling that are also disrupted, in these children are unknown



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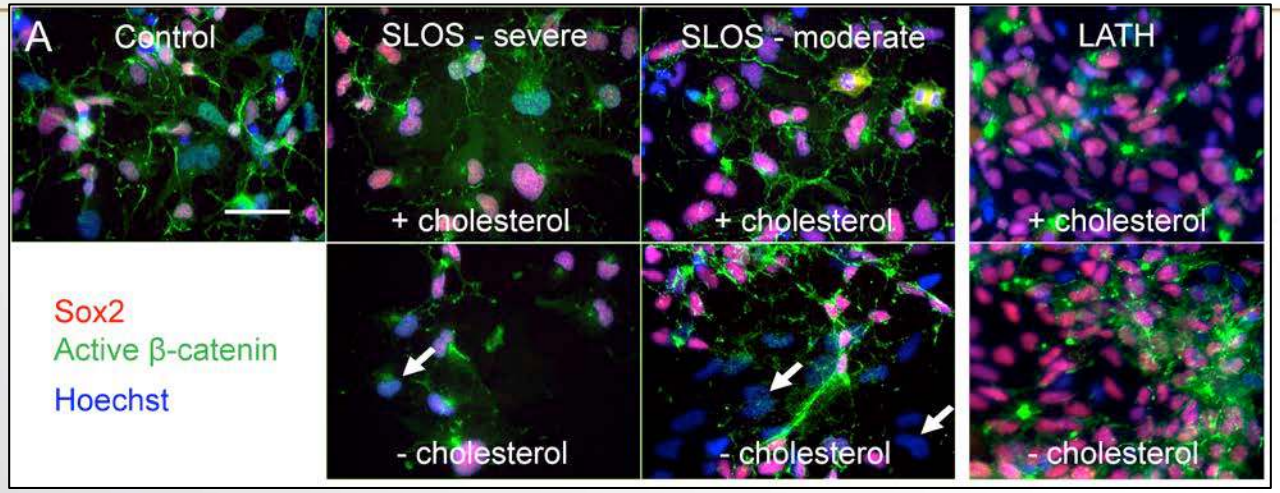
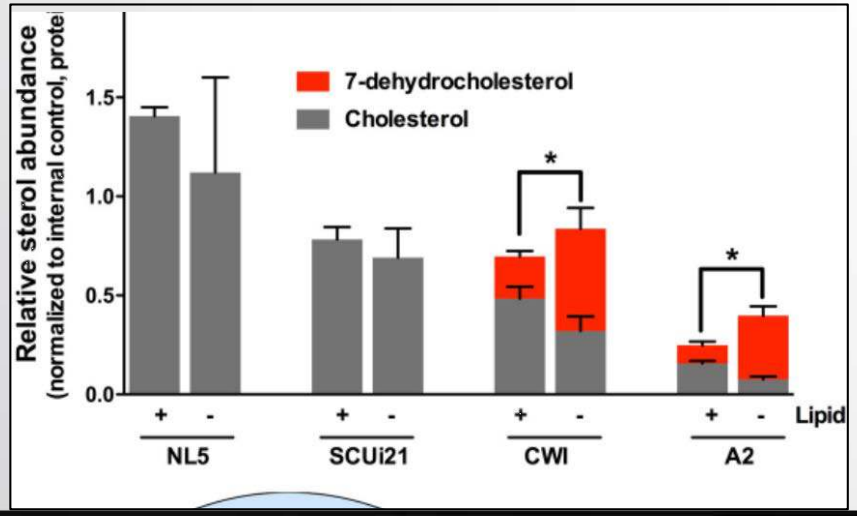
# Cholesterol synthesis mutations induces β-catenin deficits and loss of Sox2/Nestin$^{+}$ progenitors



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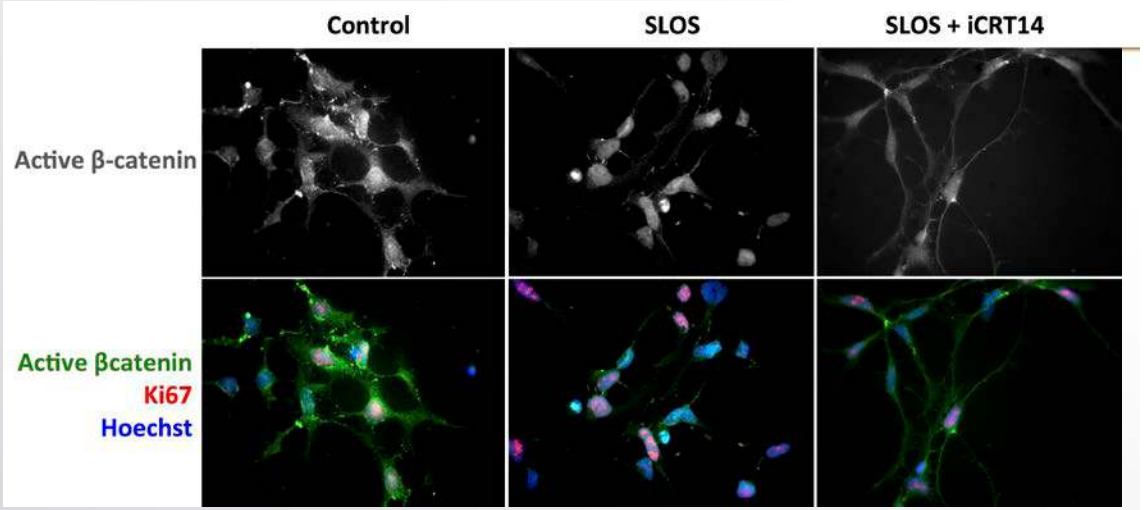
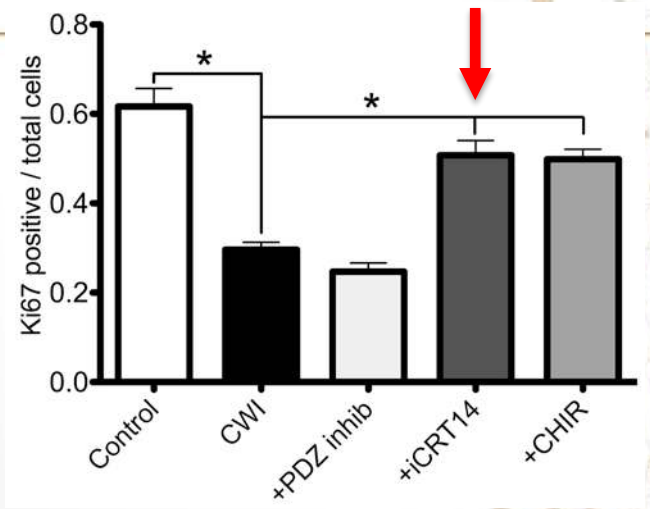
# Wnt signaling is both lost and localizes differently in SLOS

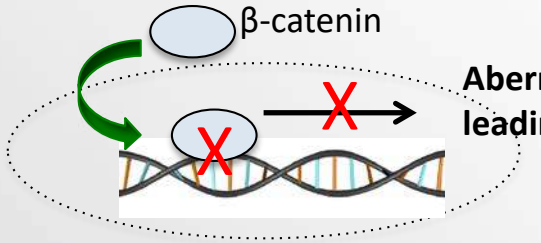



iCRT14

- Inhibits TCF/LEF DNA binding by β-catenin

- iCRT14 treated SLOS NSCs maintain normal proliferation




**Aberrant activation of target genes, leading to developmental deficits?**

Ongoing – if we shift β-catenin localization, does this affect cellular phenotypes in SLOS?



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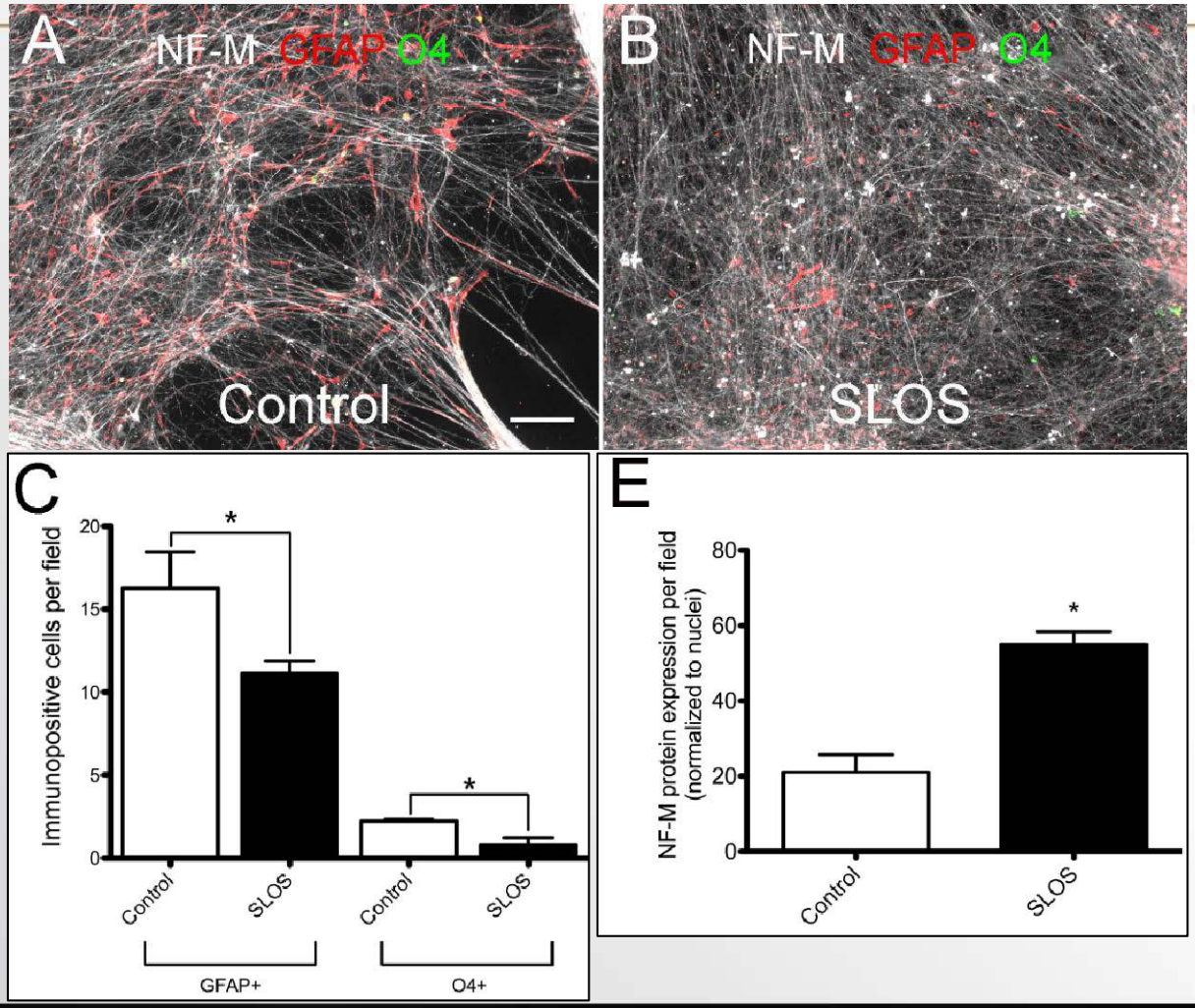
# Stem cell differentiation reveals a shift away from astrocytes in SLOS iPSCs



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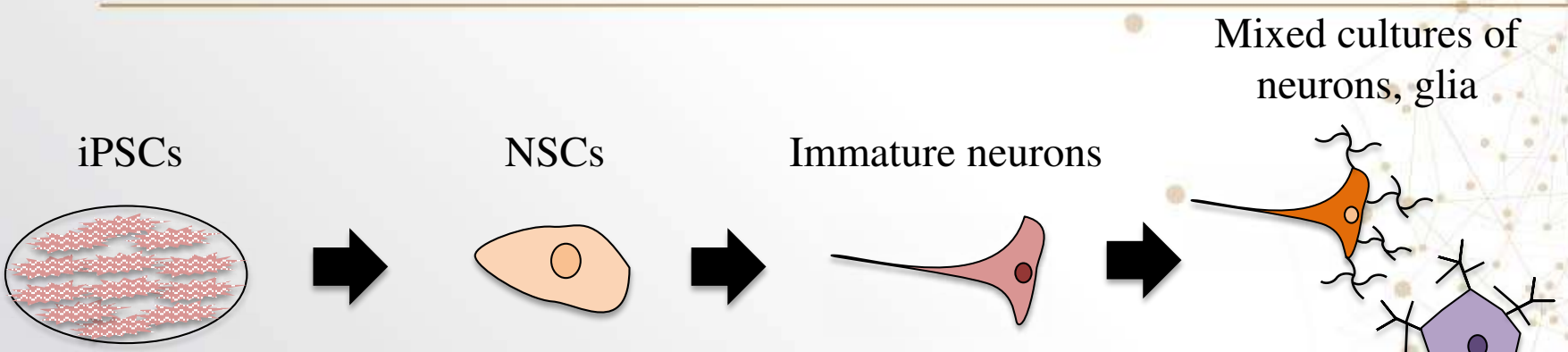
# Developmental phenotypes are only partially Wnt dependent



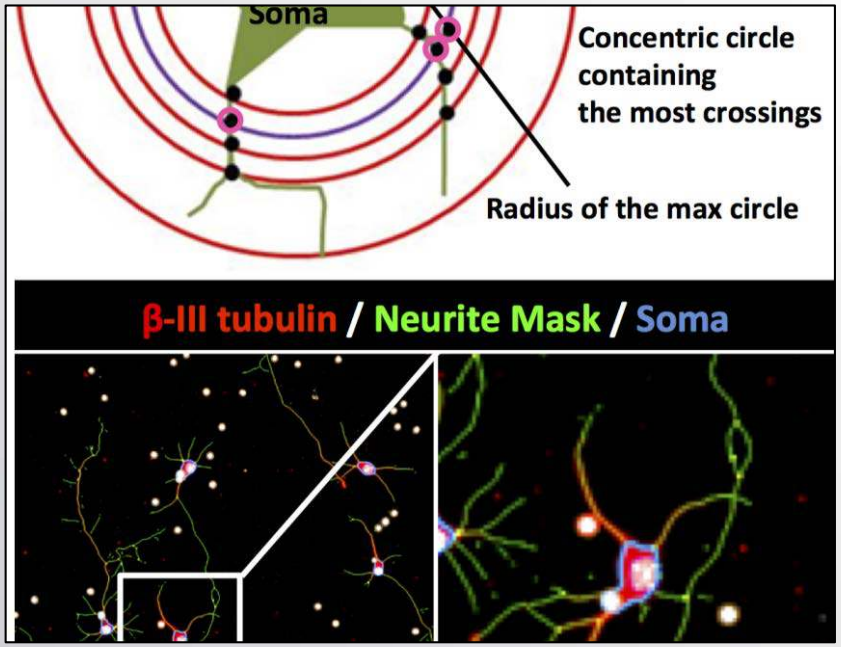

Wnt/β-catenin
FGF-2
Ventral/dorsal regulators
Cell-cell junctions
Axonal guidance
Cytoskeletal

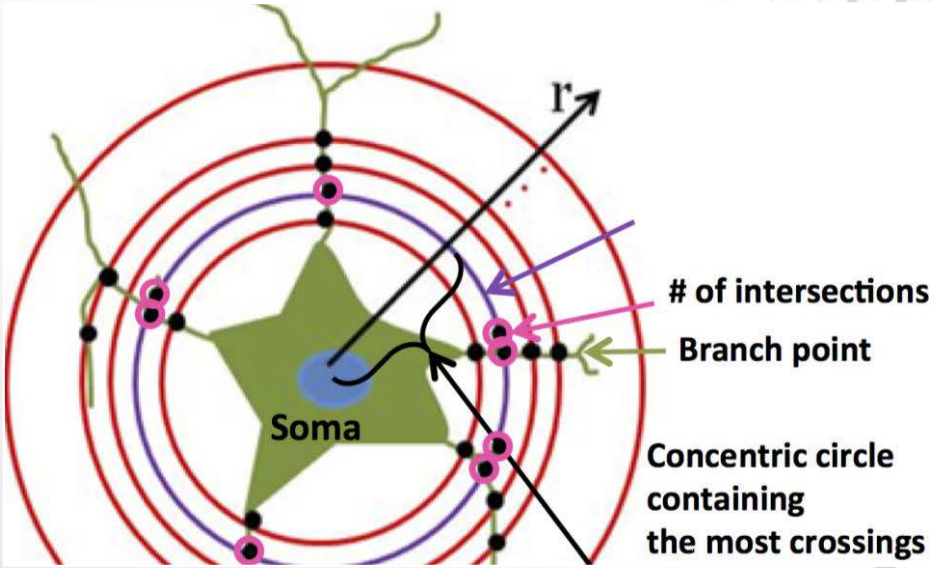
Wnt/β-catenin
SHH
TGFβ
Astrogenesis
Cerebellar formation
Notch signaling
Axonal guidance
Skeletal differentiation
Estradiol signaling

Wnt/β-catenin
SHH
TGFβ
Astrogenesis
Synaptic dysfunction
Cerebellar dysfunction
Axonal guidance
Skeletal differentiation
Estradiol signaling

* Summary of microarray, RNAseq, and qPCR analyses

# Neurite outgrowth as a suggestion of neural function



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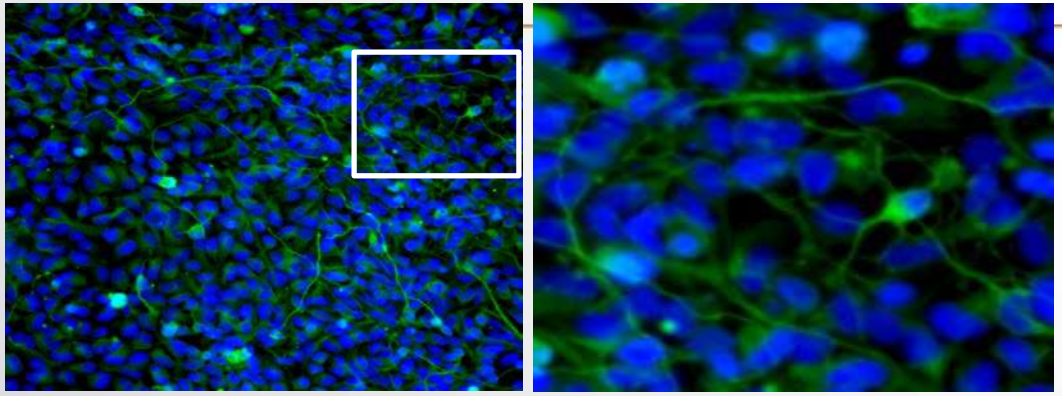
# SLOS human NSCs exhibit aberrant neurite outgrowth

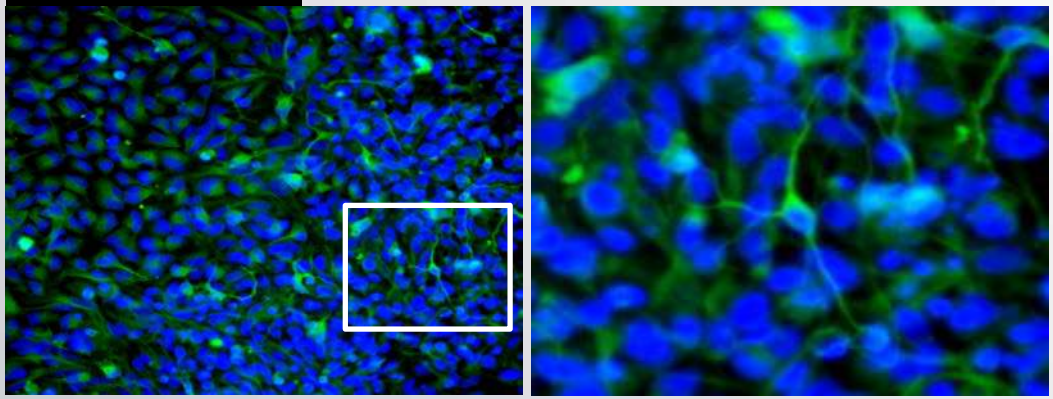
**SLOS - CWI**

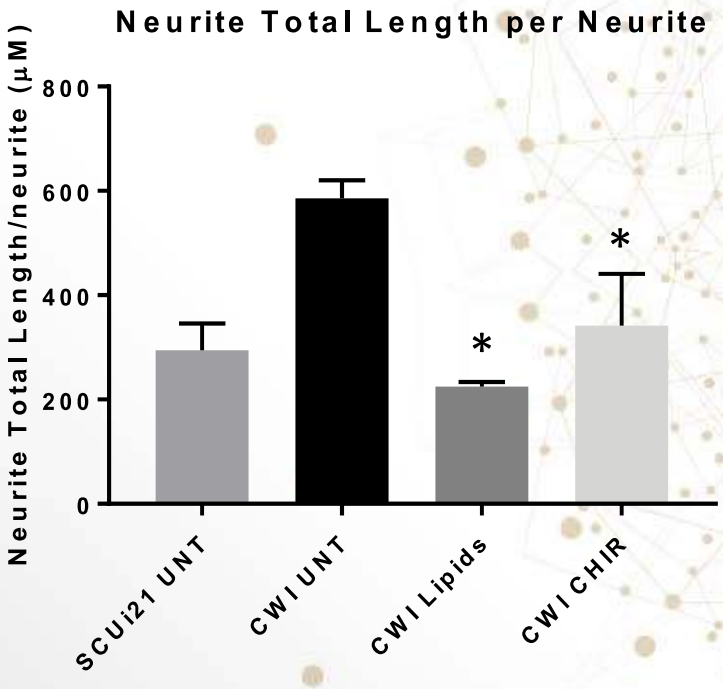
Untreated



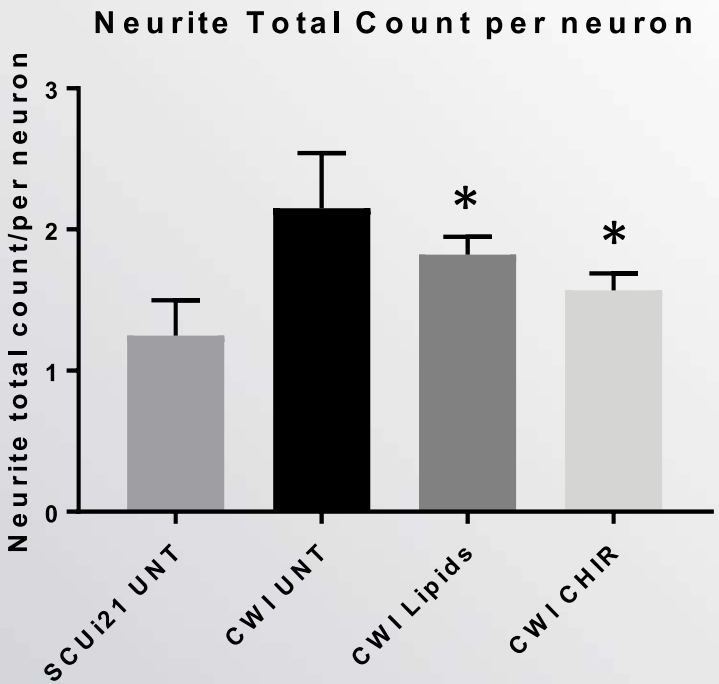
**Control – Scui21**

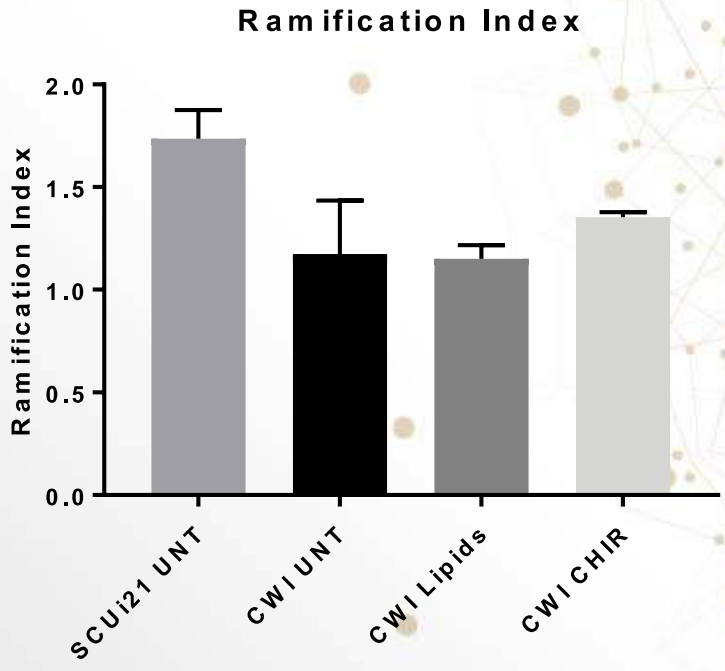
Untreated





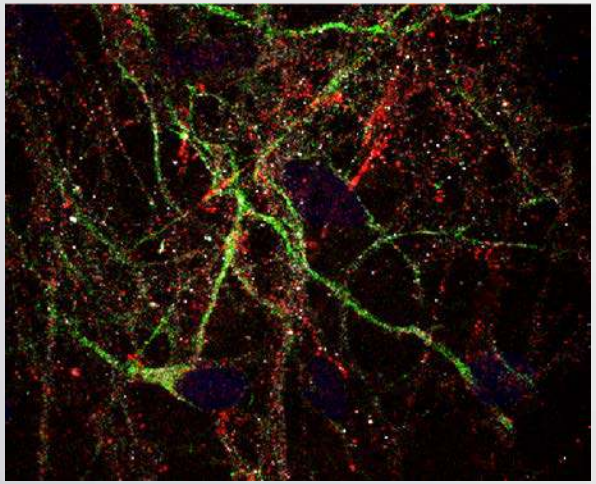
# SLOS human NSCs exhibit aberrant neurite outgrowth



# Additional functional read-outs ongoing in SLOS iPSCs

Formation of synapses




Localization of defined proteins

Network level function

# Summary

- *DHCR7* mutations have significant effects on development and neural differentiation
- Wnt deficits in SLOS are likely a significant contributor to developmental issues within, though there are other pathways at play also
- Reduced numbers of astrocytes would likely significantly impair things like synapse formation, synapse function, nutrient transport to neurons, neurotransmitter recycling
- Data collected using non-biased, automated quantitation reveals neurite deficits of interest
- Need more data from multiple cell lines spanning the spectrum, as well as quantitation of other functional analyses

# Acknowledgements


Lab members at Sanford Research

- ***Nick Coungeris***
- *Ruthellen Anderson*
- *Jordan Sheets*
- *Bethany Freel*
- Dallas Soyland
- Eric Sandhurst

Collaborators

- ***Forbes Porter – NIH/NICHD***
- Amina Qutub – Rice University
- Karen Litwa - ECU
- Wonhwa Cho – UIC
- Stephanie Cologna – UIC
- Vijay Gokhale – Univ of Arizona
- Jon Cooper – Wash U
- Bill Pavan – NIH/NHGRI
- Jerry Vockley – UPMC
- Dan Cohn – UCLA

Smith-Lemli-Opitz | RSH FOUNDATION

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CBBRe Center for Brain and Behavior Research

Special thank you to the patients and families



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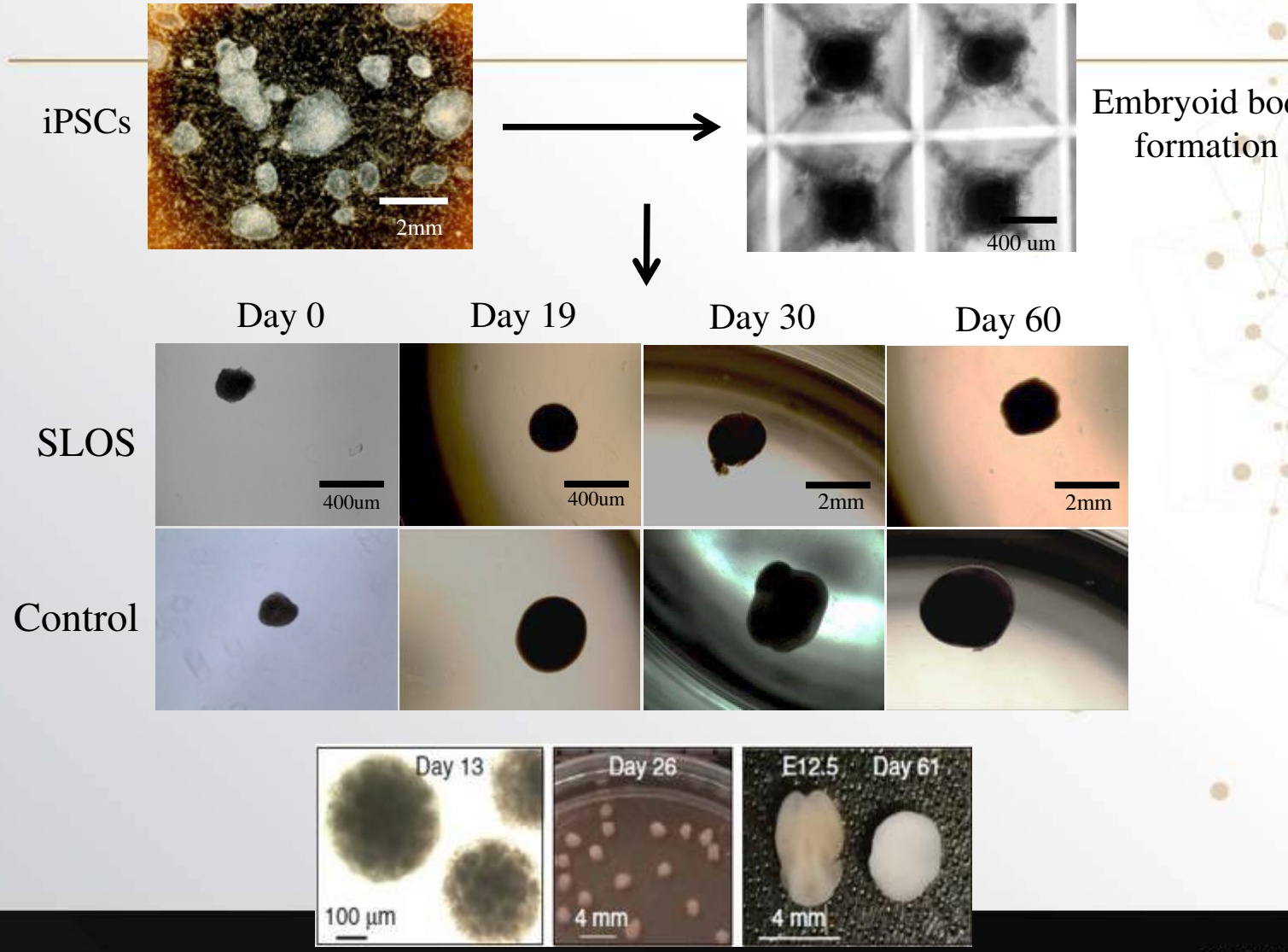
# Three-dimensional modeling of human neurodevelopment




Pasca, A.M. *et al. Nat. Methods*, 671–678 (2015)



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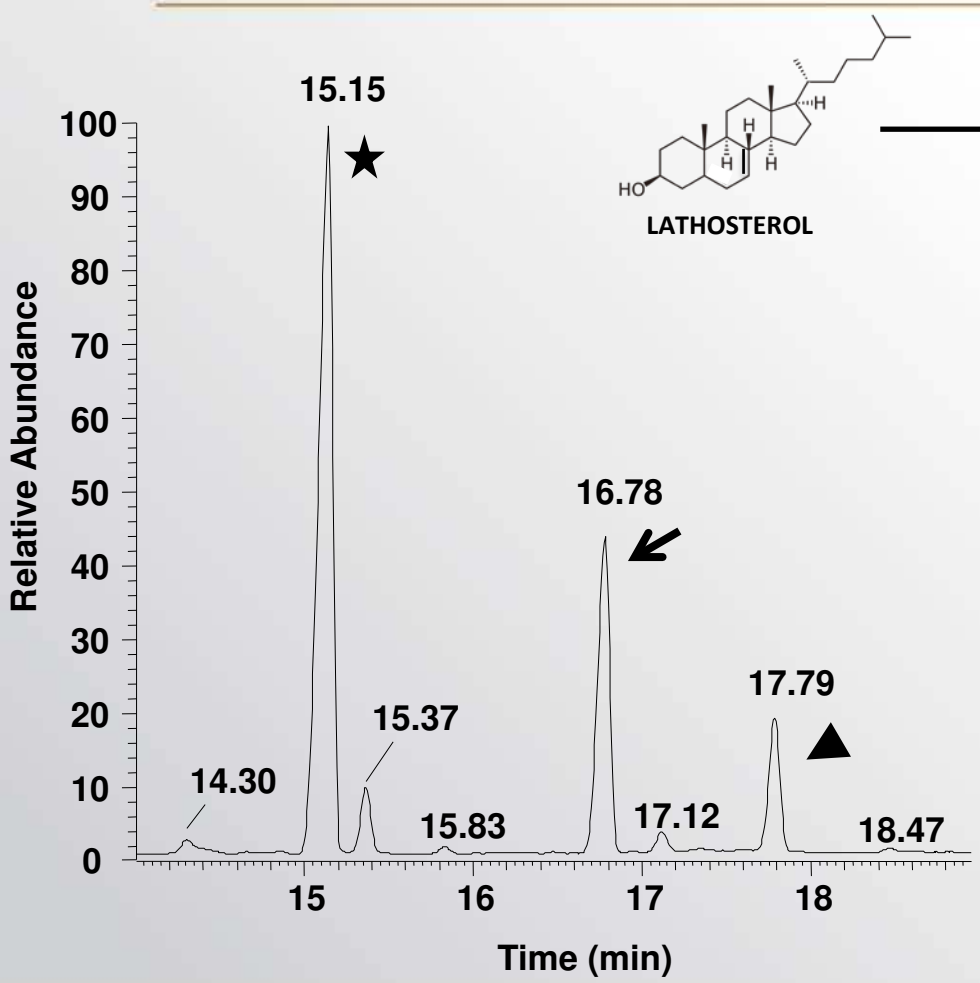
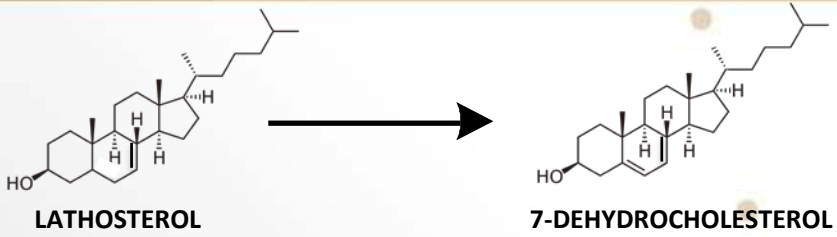
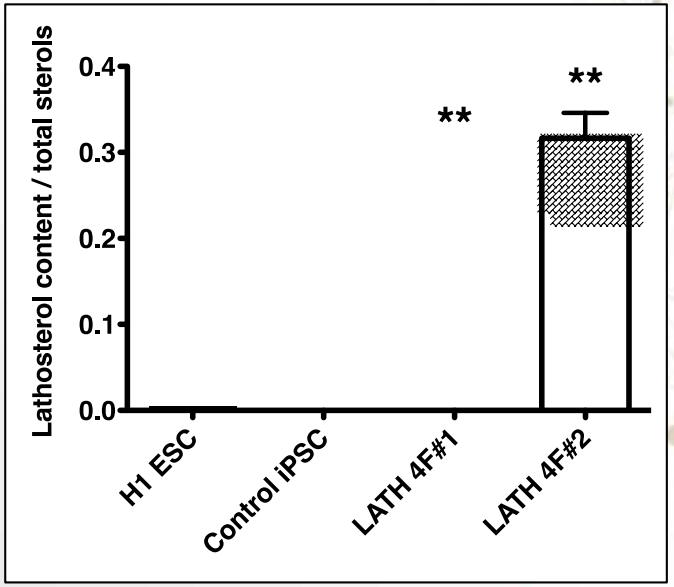
# Lathosterolosis iPS cells exhibit proper biochemistry



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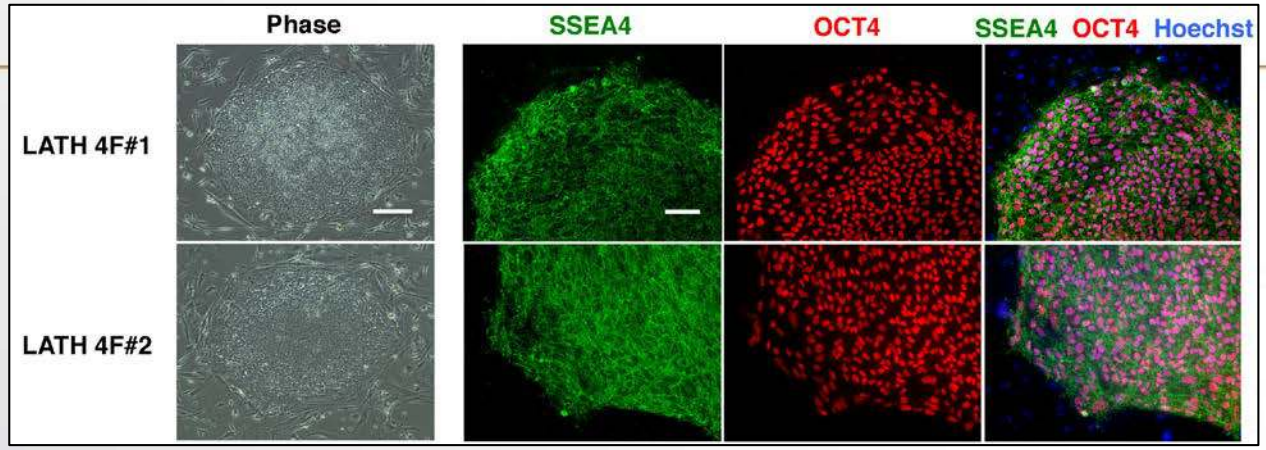
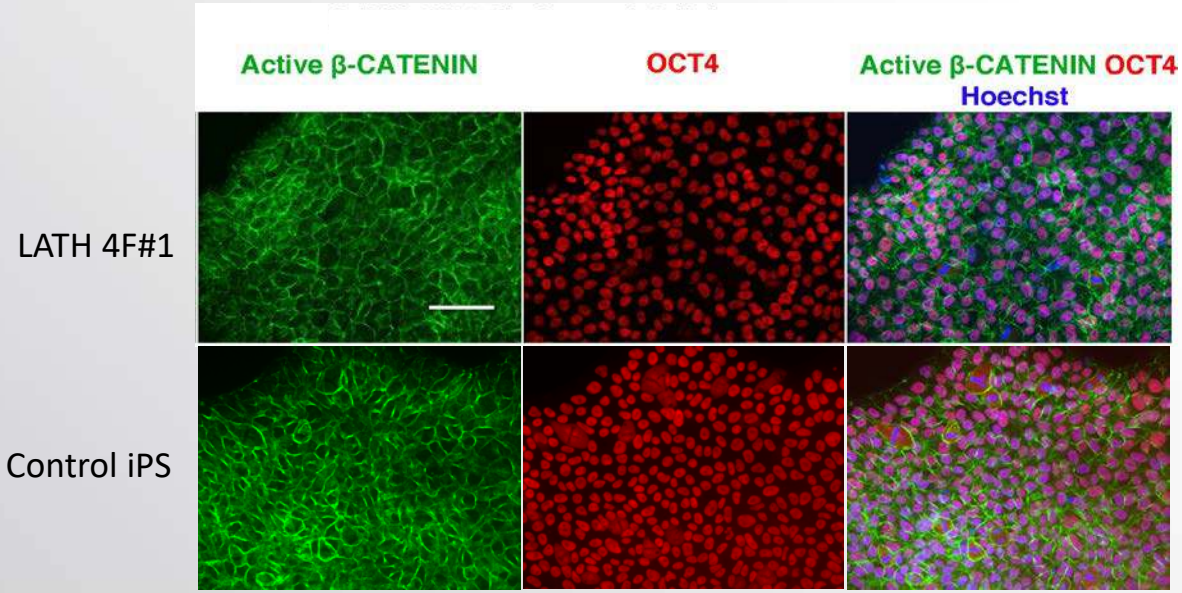
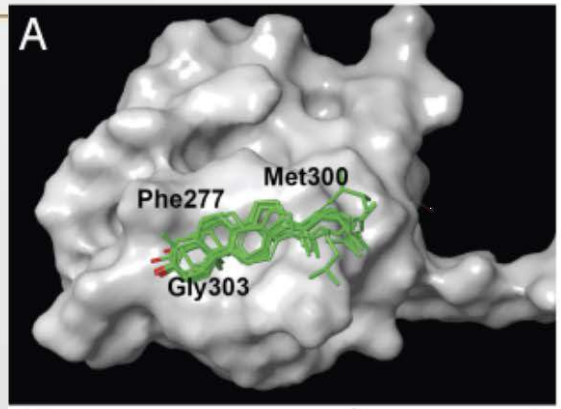
# LATH iPS cells exhibit no observable phenotype in cholesterol deficient conditions

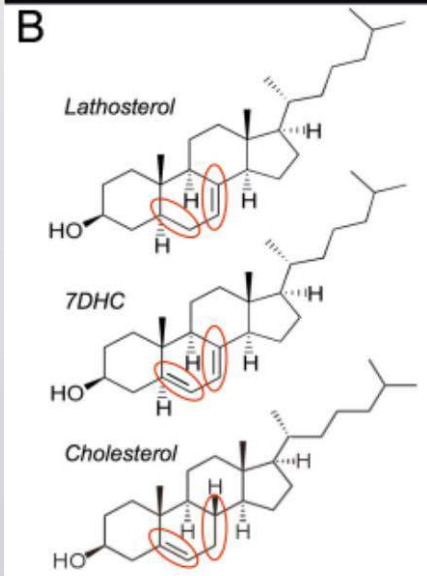


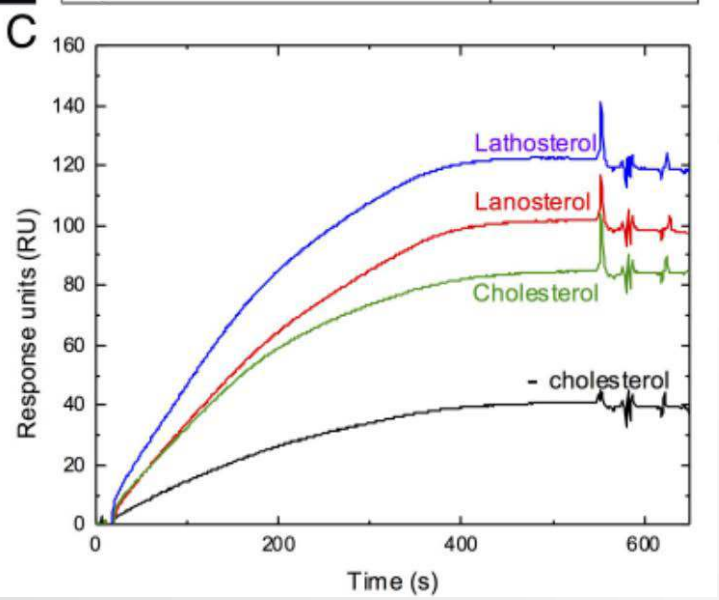



# Sterols display varying degrees of specificity for protein-lipid interactions




| Molecule | Glide emodel |
|---|---|
| cholesterol | -23.161 |
| lathosterol | -22.706 |
| zymostenol | -22.519 |
| dimethylcholesta-8,24-dien-ol | -21.98 |
| dehydrocholesterol | -21.764 |
| zymosterol | -20.227 |
| desmosterol | -19.694 |
| ergosterol | -19.024 |



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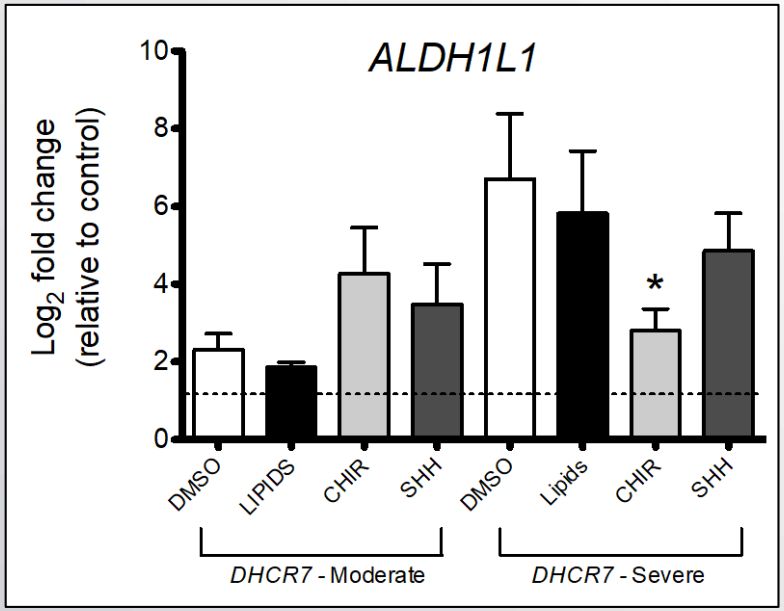
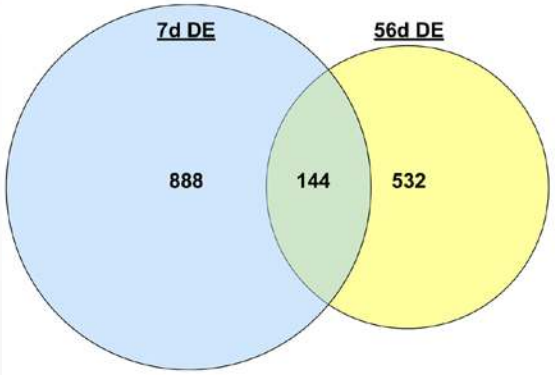
# Prolonged stabilization of Wnt signaling versus other differentially expressed pathways on glial formation



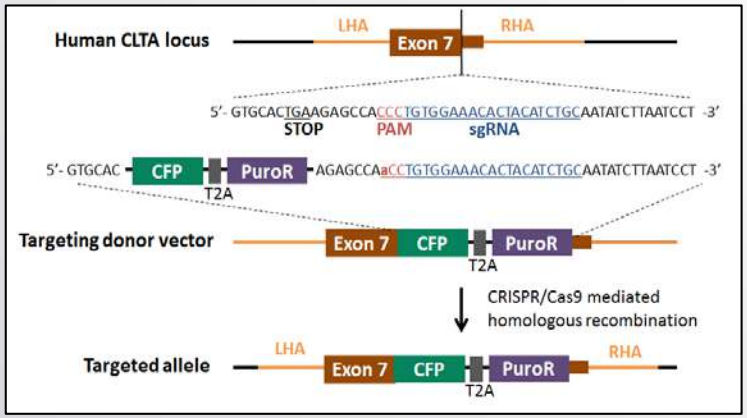

| Genes | Predicted changed | Expr Log Ratio | Findings |
|---|---|---|---|
| KRT17 | Inhibited | -7.555 | Upregulates |
| FLT1 | Inhibited | -5.508 | Upregulates |
| PTGER2 | Inhibited | -5.476 | Upregulates |
| IL33 | Inhibited | -5.448 | Upregulates |
| ELN | Inhibited | -5.38 | Upregulates |
| CFI | Inhibited | -4.924 | Upregulates |
| MYL3 | Inhibited | -4.506 | Upregulates |
| CEMIP | Affected | -4.48 | Regulates |
| WNT11 | Inhibited | -4.431 | Upregulates |
| TNNT2 | Inhibited | -4.398 | Upregulates |
| NGF | Inhibited | -4.383 | Upregulates |
| ABLIM3 | Inhibited | -4.225 | Upregulates |
| ASPN | Inhibited | -4.17 | Upregulates |

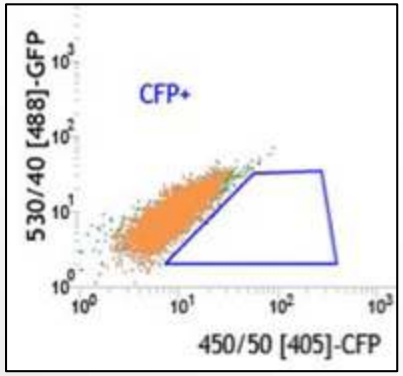
TGFβ signaling is disrupted in 7d and 56d cultures

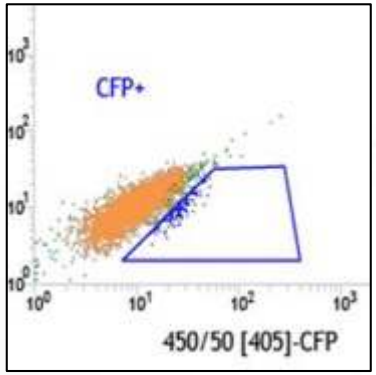


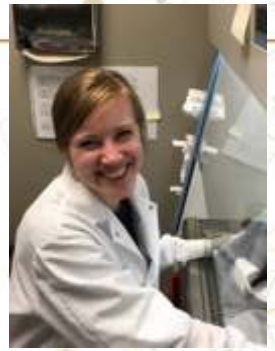
SANFORD HEALTH

# Measuring the effects of sterol substitution on endocytic activity

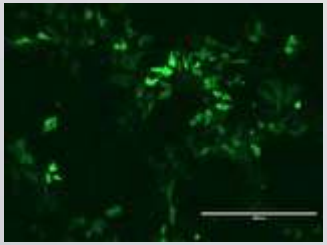


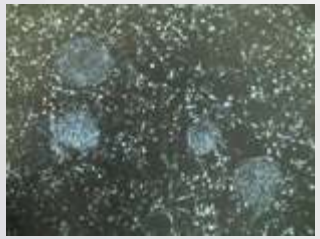



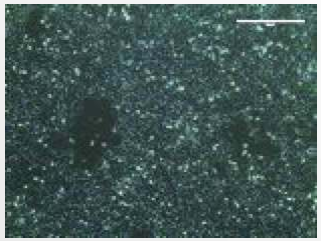



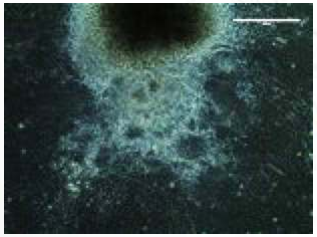



Elle Anderson, BS
MD/PhD student









Day 1: Nucleofection
Donor: Cas9/sgRNA
4:2.5 ug ratio

→

Day 4-7: Cell sorting
Plated onto feeder colonies

→

Feeder-free transition
After several passages

→

- Differentiation assays
- Cellular trafficking quants
- Assessing sterol specific effects

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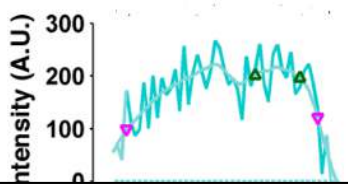
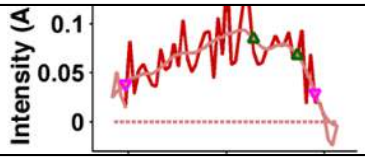
# Disruption of clathrin-mediated endocytosis, suggestive of inhibition of membrane scission

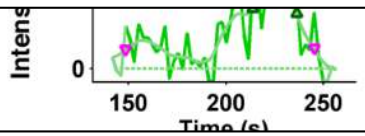
**Control**



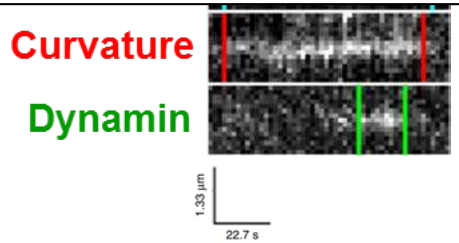

Curvature but no dynamin recruitment



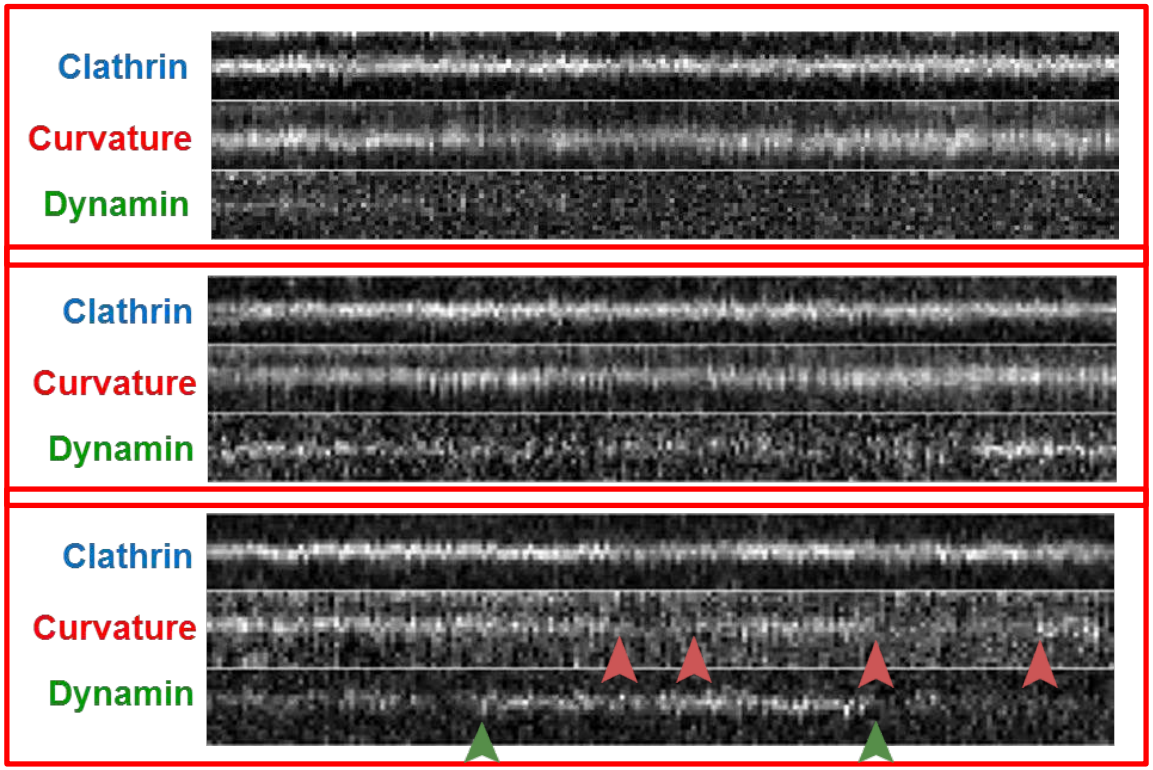

Curvature and consistent dynamin recruitment




Fluctuating curvature and fluctuating dynamin recruitment

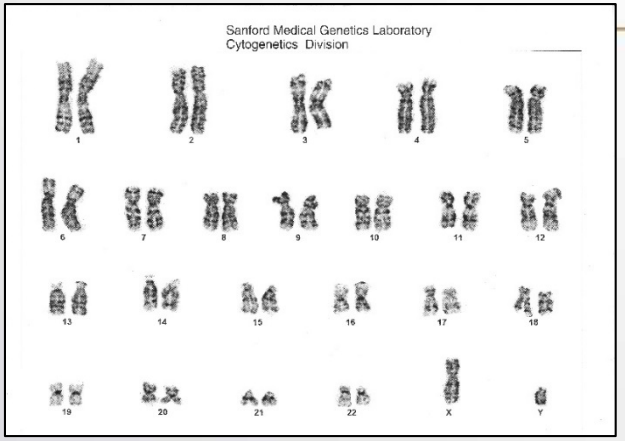


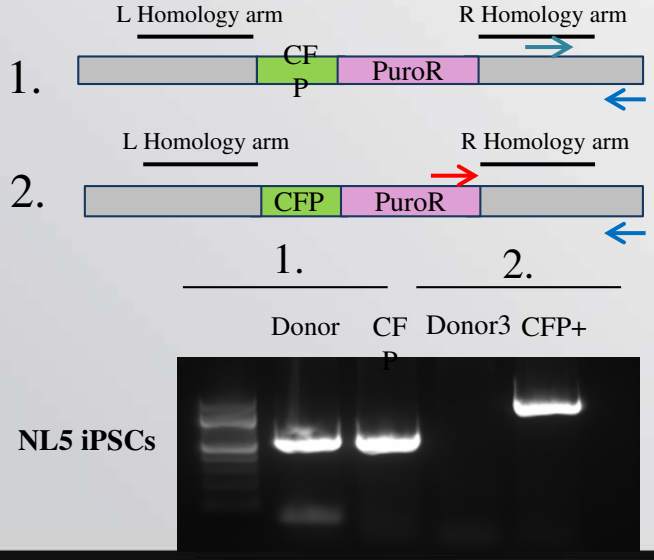

**2.5 µM AY9944** (SLOS) - **Persistent tracks**

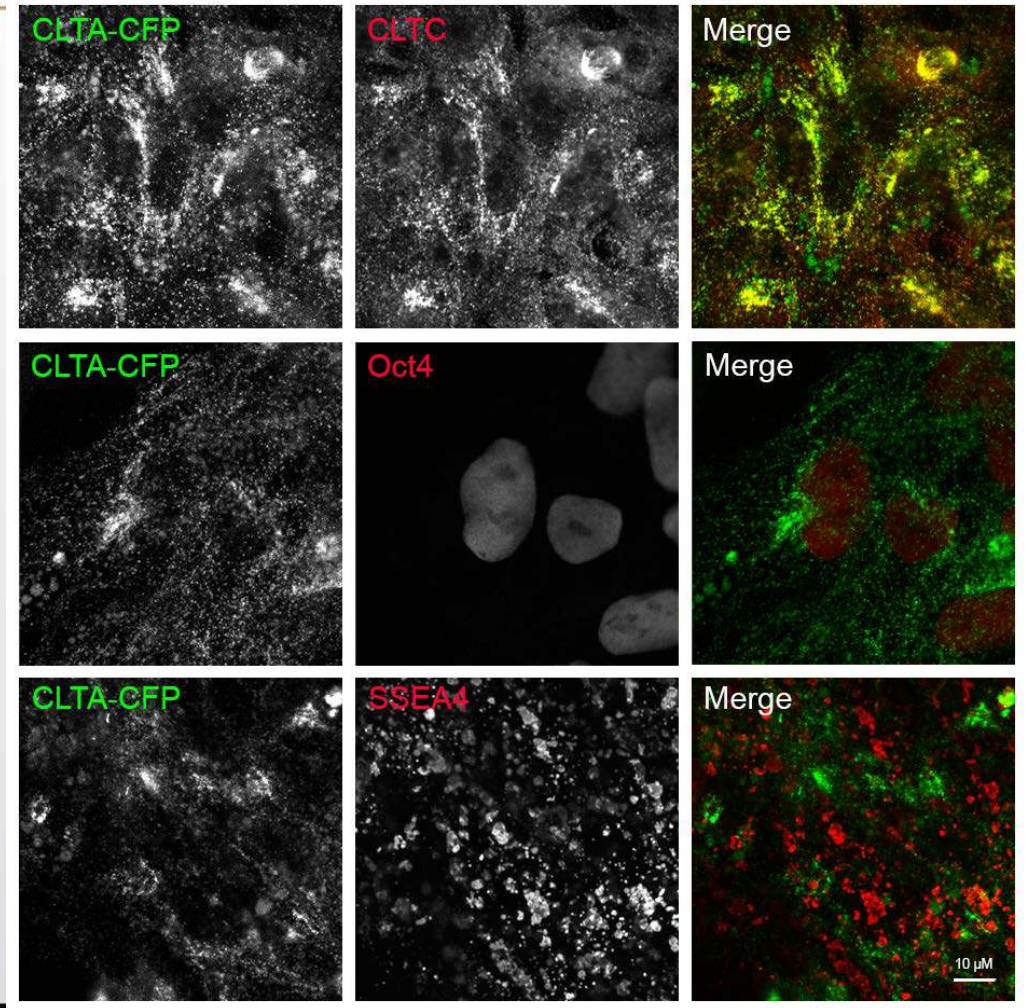


# Human iPSC CLTA-CFP+ for cell type specific analysis of CME

**CLTA-CFP+**



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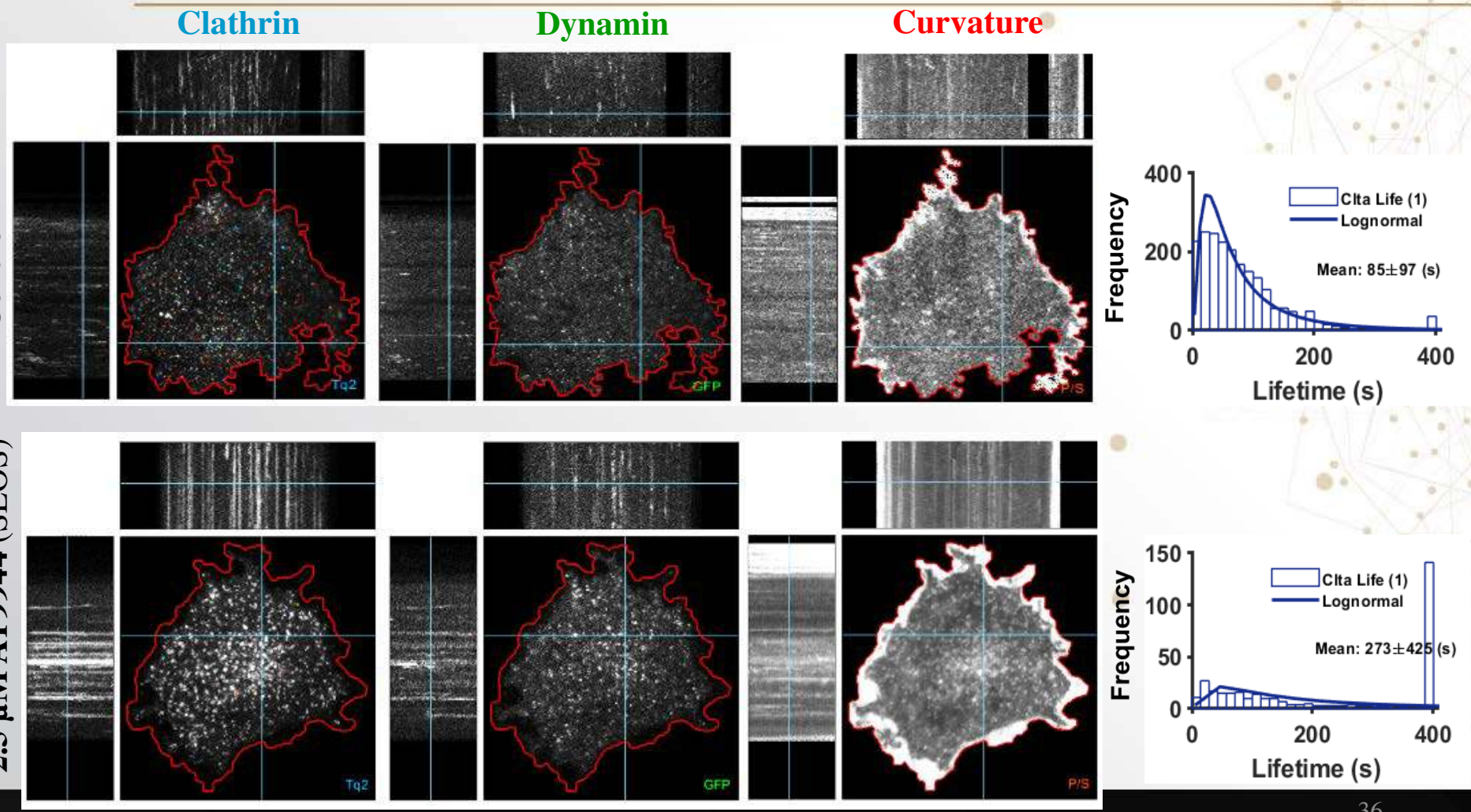
# Disruption of clathrin-mediated endocytosis by altered sterol homeostasis



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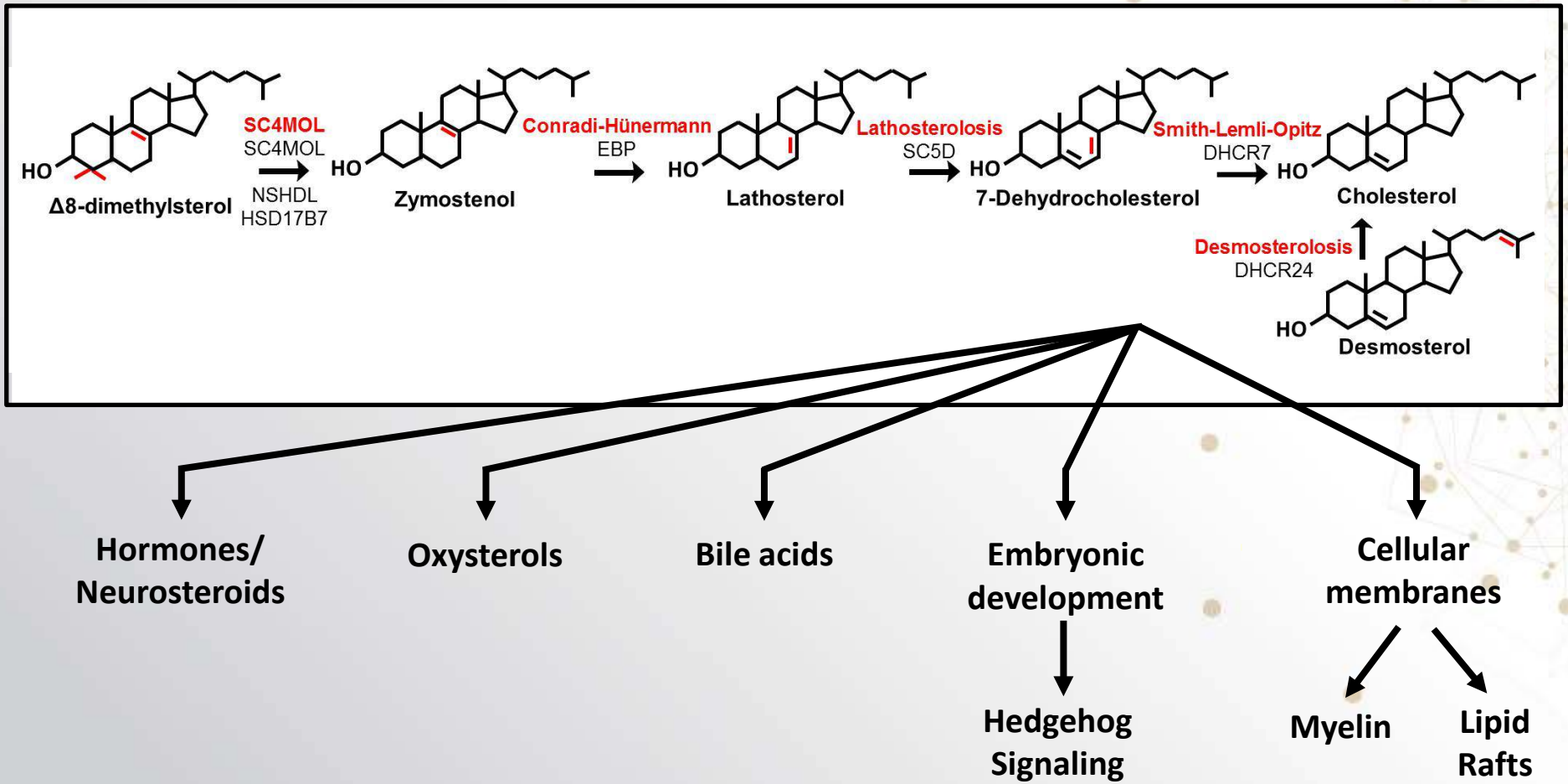
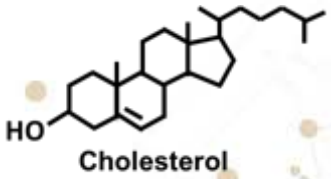
- Signaling pathways – cell type dependent
- Wnt – yes in NSCs, early differentiation, but aberrant differentiation in more mature cultures

# Cholesterol synthesis, tissue development, and cellular function



# Cholesterol synthesis disorders – common pathway, drastically different clinical phenotypes

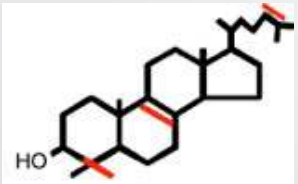


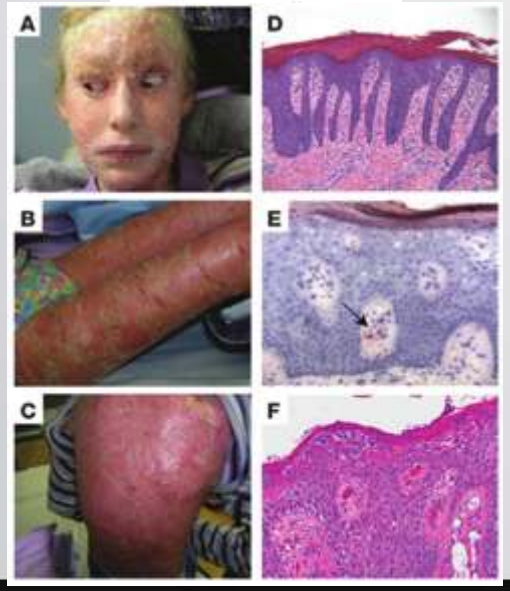

## SC4MOL/CHILD

***SC4MOL, NSHDL, HSD17B7***

**Incidence: ?**

**Carrier: 0.05%**



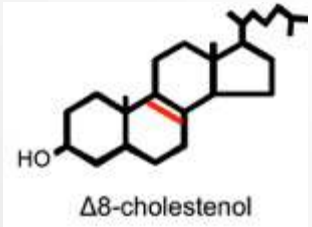



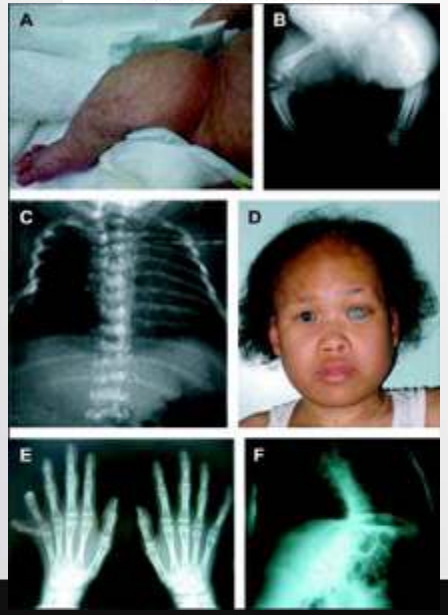

## Conradi-Hünermann syndrome

***EBP***

**Incidence: 1:100,000**

**Carrier: 0.3%**



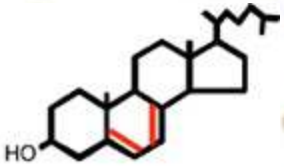



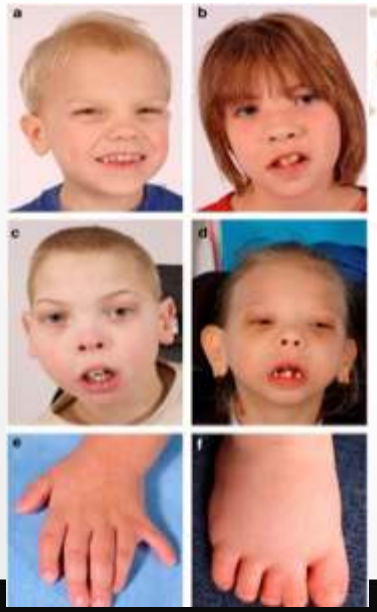

## Smith-Lemli-Opitz syndrome

***DHCR7***

**Incidence: 1:50,000**

**Carrier: 3%**

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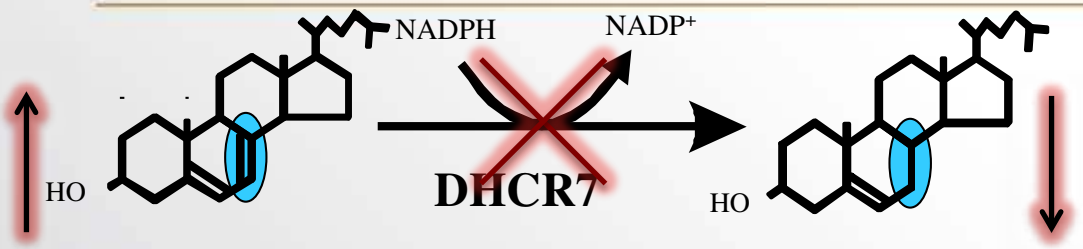
# Cholesterol biosynthesis, *DHCR7*, and tissue dysfunction




7-dehydrocholesterol

- Microcephaly
- Agenesis of corpus callosum, cerebellum
- Ambiguous genitalia
- Motor dysfunction

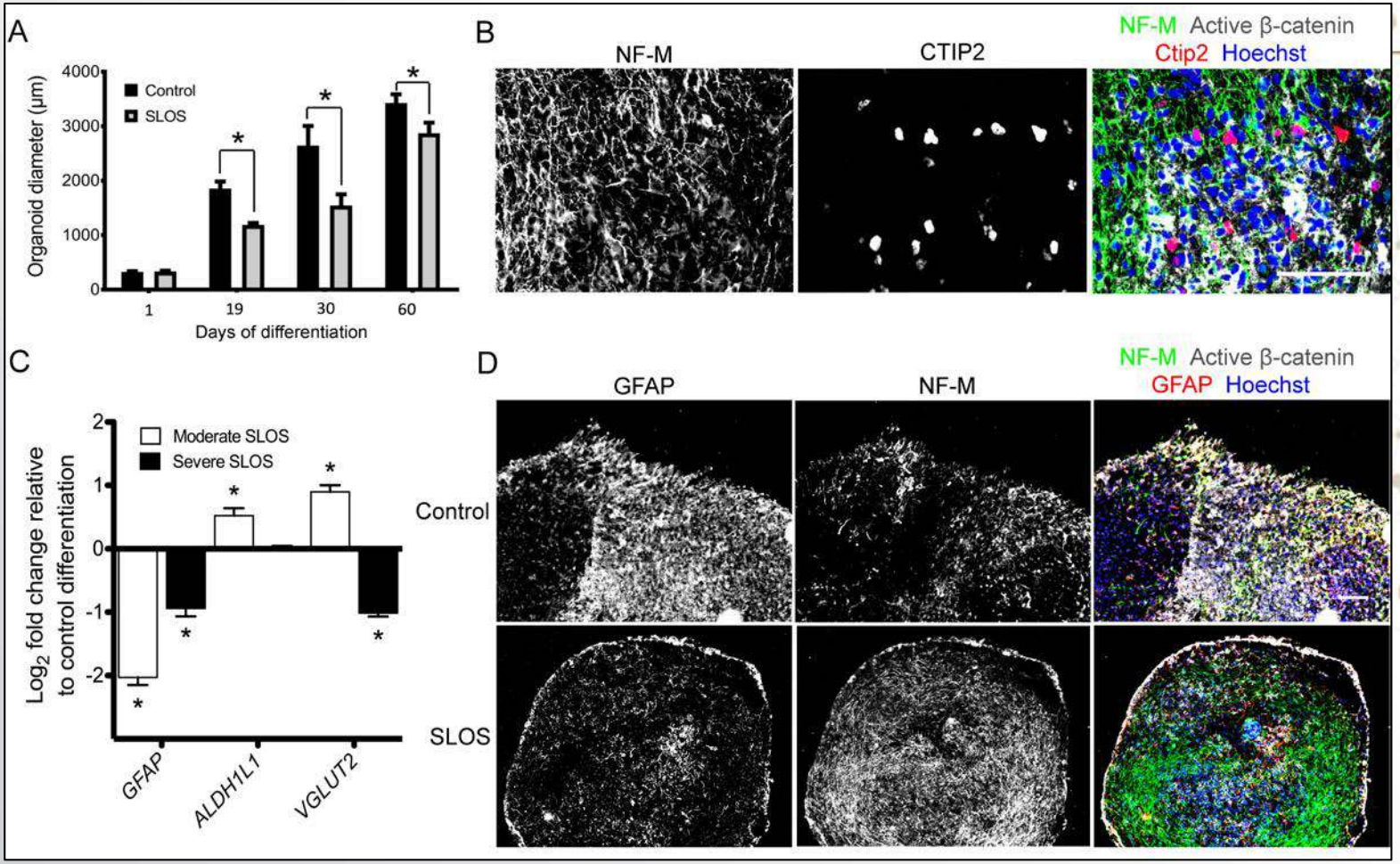
Cholesterol

- Autistic behaviors
- Depression
- Cognitive impairment
- Dandy-Walker malformation
- Perinatal to adult lethality

Incidence → 1:50,000

- Likely causes biophysical changes within cellular membranes, affecting protein localization, protein-protein binding, cytoskeletal remodeling
- Unclear if effects are direct or indirect through changing lipid composition of membranes
- Our animal models are limited due to lethality and poor metabolic correlation between mouse, human

# Stem cell differentiations reveal a shift away from astrocytes in SLOS iPSCs



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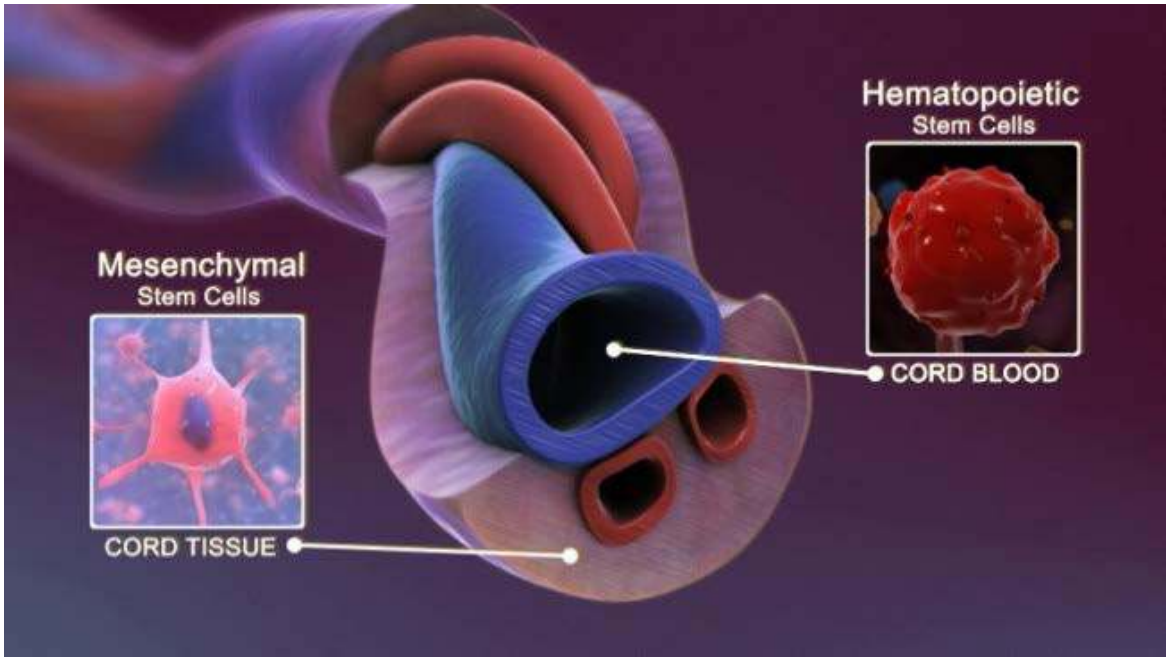
# Fetal/amniotic/cord blood stem cells

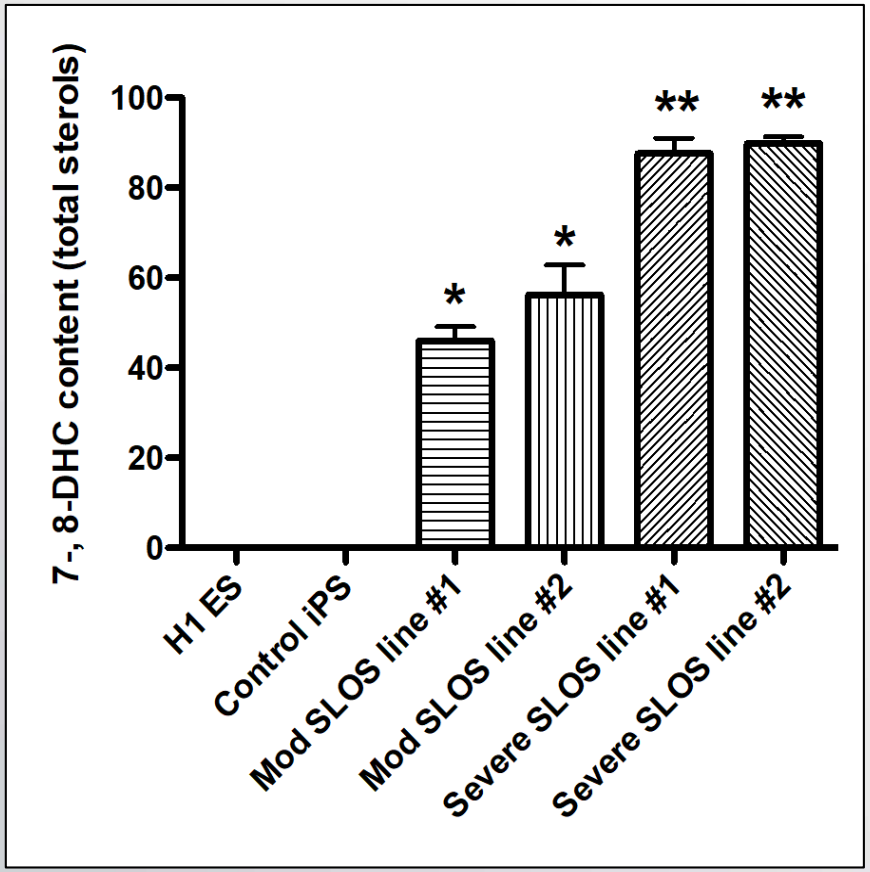


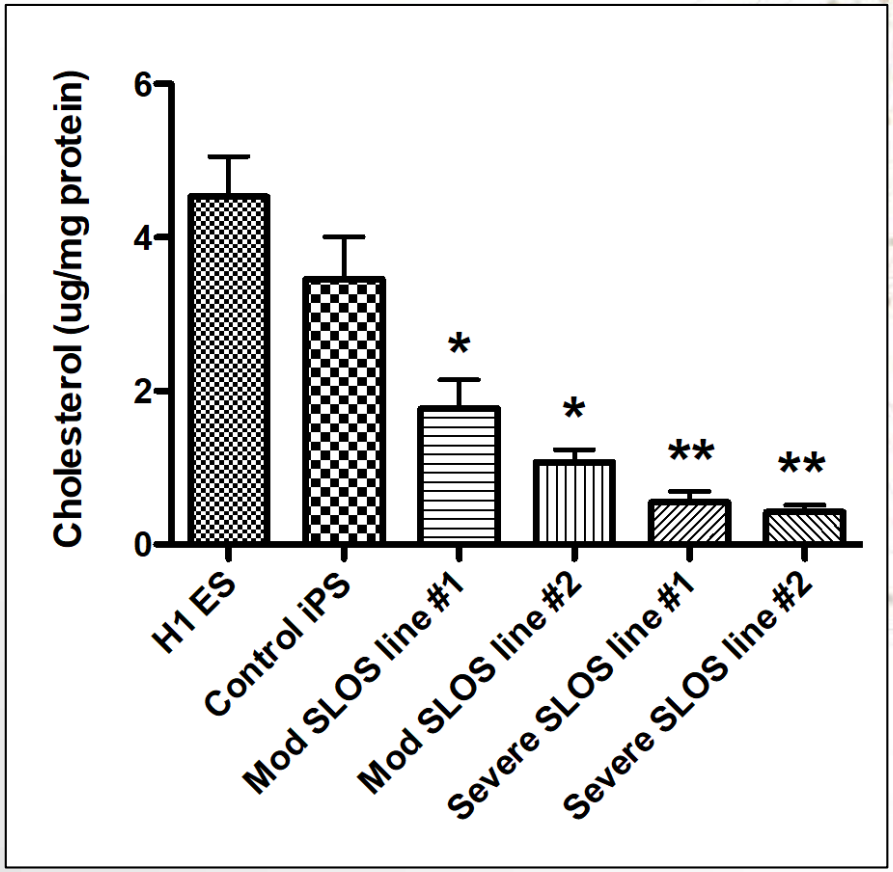

- Unipotent or multipotent cells with a limited capacity for differentiation (bone, muscle, cartilage; mesodermal restricted)
- Need to be isolated early in life and used immediately or cryogenically stored
- ***Fetal stem cells are one of the best resources for scientists to study early human development (individual specific and require little to no manipulation in the lab to study)***
- Clinically, the applicability of these cells broadly across diseases is probably limited

# Significant issues related to SLOS research and therapy

- No good therapeutic option
  - Clinical studies on dietary cholesterol inconclusive
  - Maternal-embryonic cholesterol transport during first trimester only
- No treatment for CNS effects
- Virtually no understanding of pathophysiology and signaling defects in SLOS CNS
- Poor correlation between predicted mutational severity and clinical symptoms
- Animal models are limited
  - *Dhcr7* -/- mouse - Lethal at P0
  - Hypomorphic model - Biochemical phenotype has only short term effect

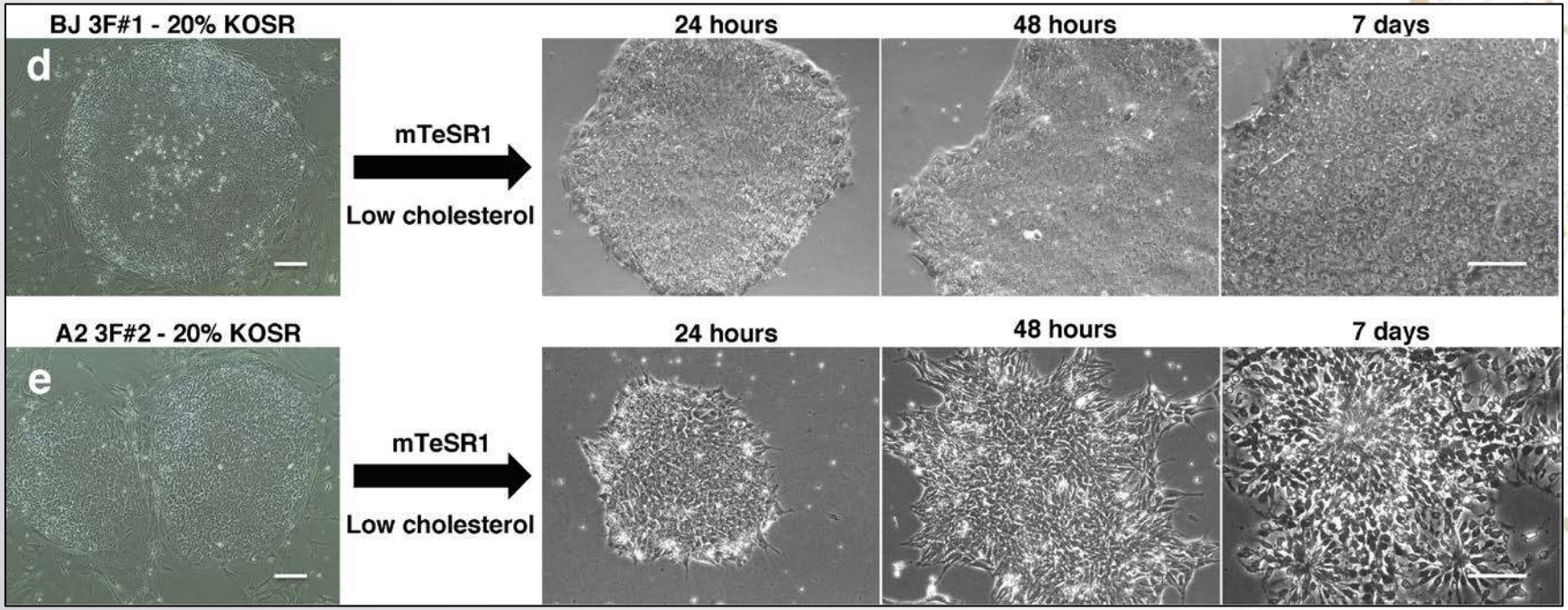
# Biochemical phenotype within SLOS iPSCs



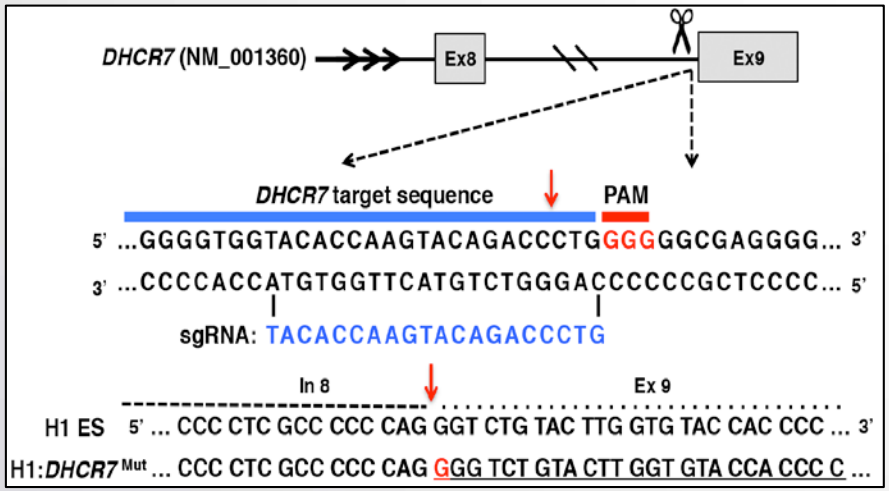

Ionize and analyze by mass spectrometry

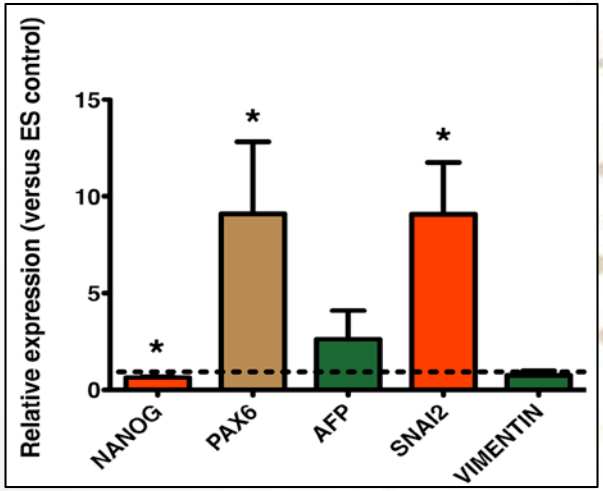
# Dramatic shift in SLOS iPS morphology in cholesterol free/pluripotent conditions

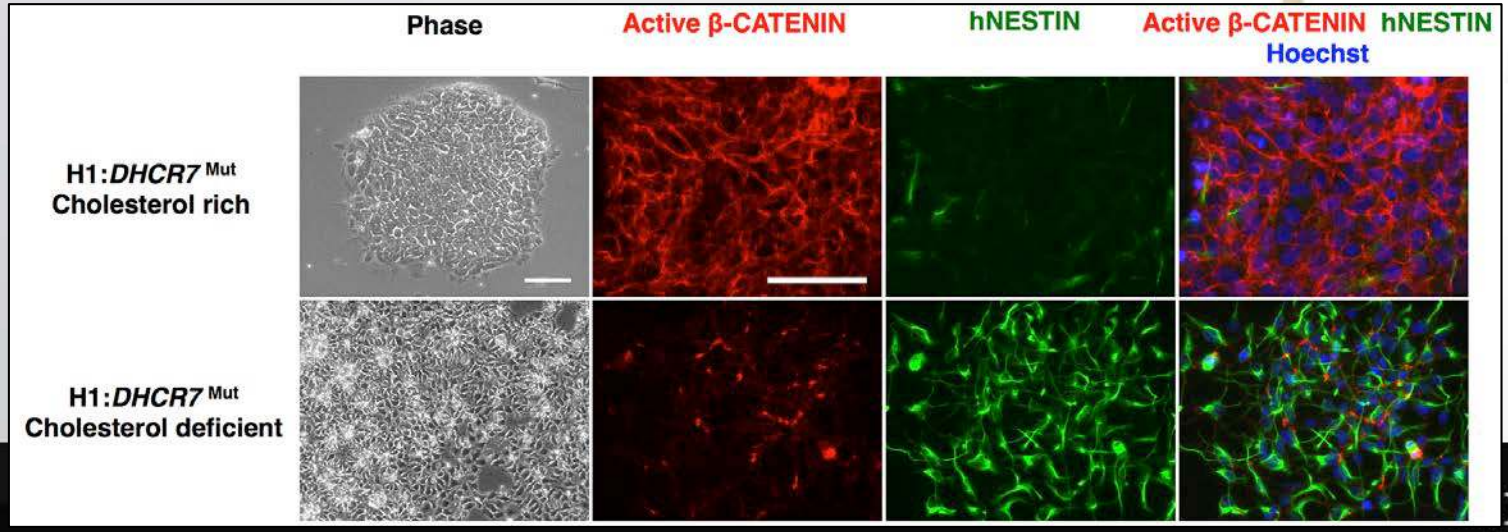



Ionize and analyze by mass spectrometry

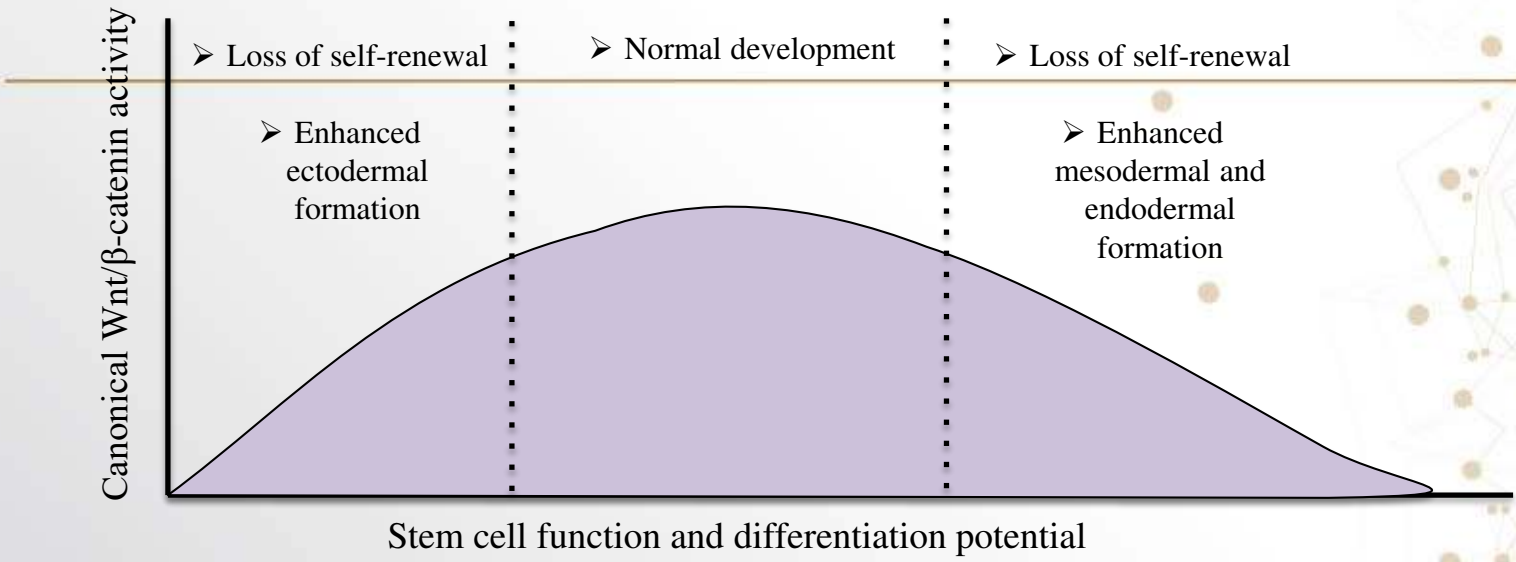
# CRISPR/Cas9 mutated *DHCR7* mimics Wnt defects

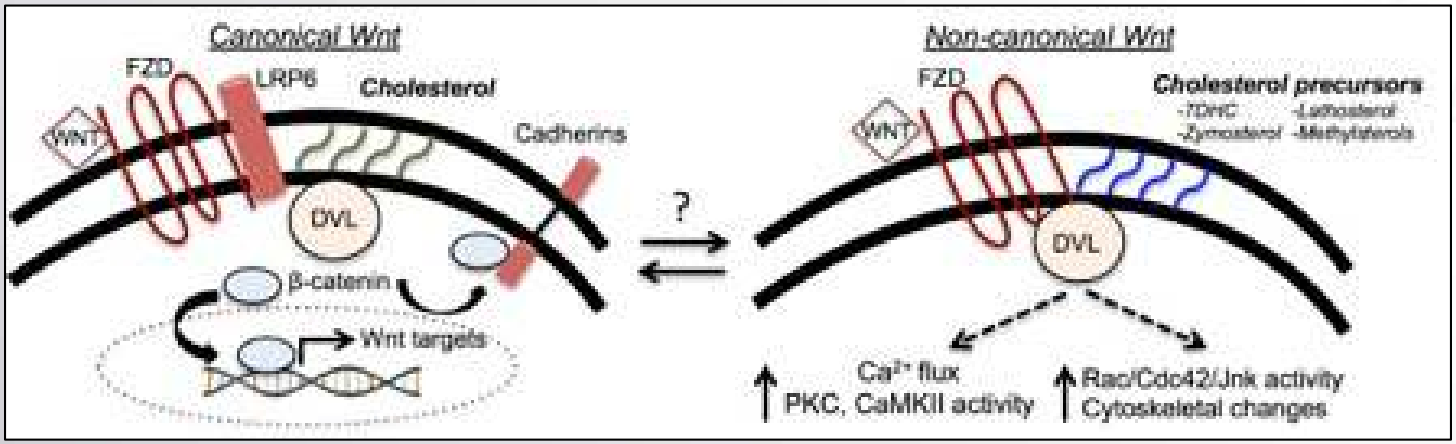


# Wnt signaling, stem cell function, and tissue development



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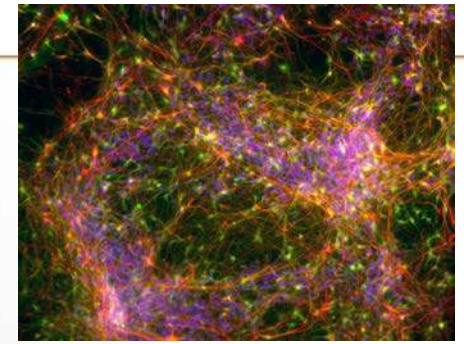
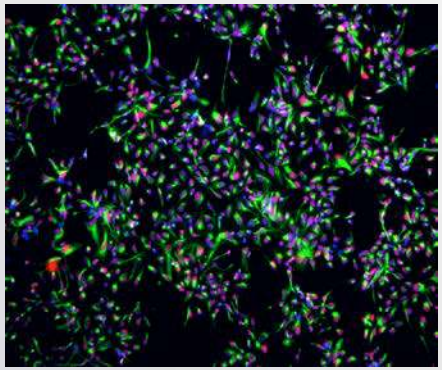
# Using iPSCs to identify altered signaling pathways and cellular deficits suggestive of disease pathology



**Expandable neural progenitors (NSCs)**



**Induce neural differentiation**

**7d (immature differentiated)**

**28d (mature, differentiated)**

**60d (mature, fully specified)**

**Genomic analyses (RNAseq, ChIPseq, etc.)**

**Proteomics**

**Functional cellular assays**

- **Identify disrupted pathways**
- **Identify cell types susceptible to mutations**
- **Determine if mechanisms are common across models or correlate to clinical severity**