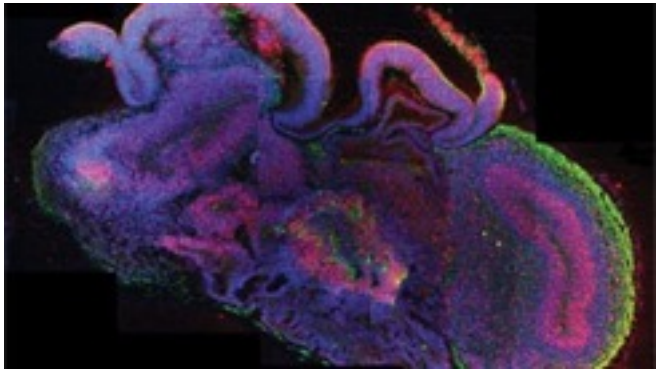
Grow-your-own organs may not be so far off

“If you could use iPSCs to generate a truly functioning organ, then you would have this unlimited suitcase of spare parts that would be genetically matched to individuals,”

Stephen Duncan **(director of the Regenerative Medicine Center at the Medical College of Wisconsin)**



Anahita Rafiei
19.04.2016

Model systems in life sciences

Limitations

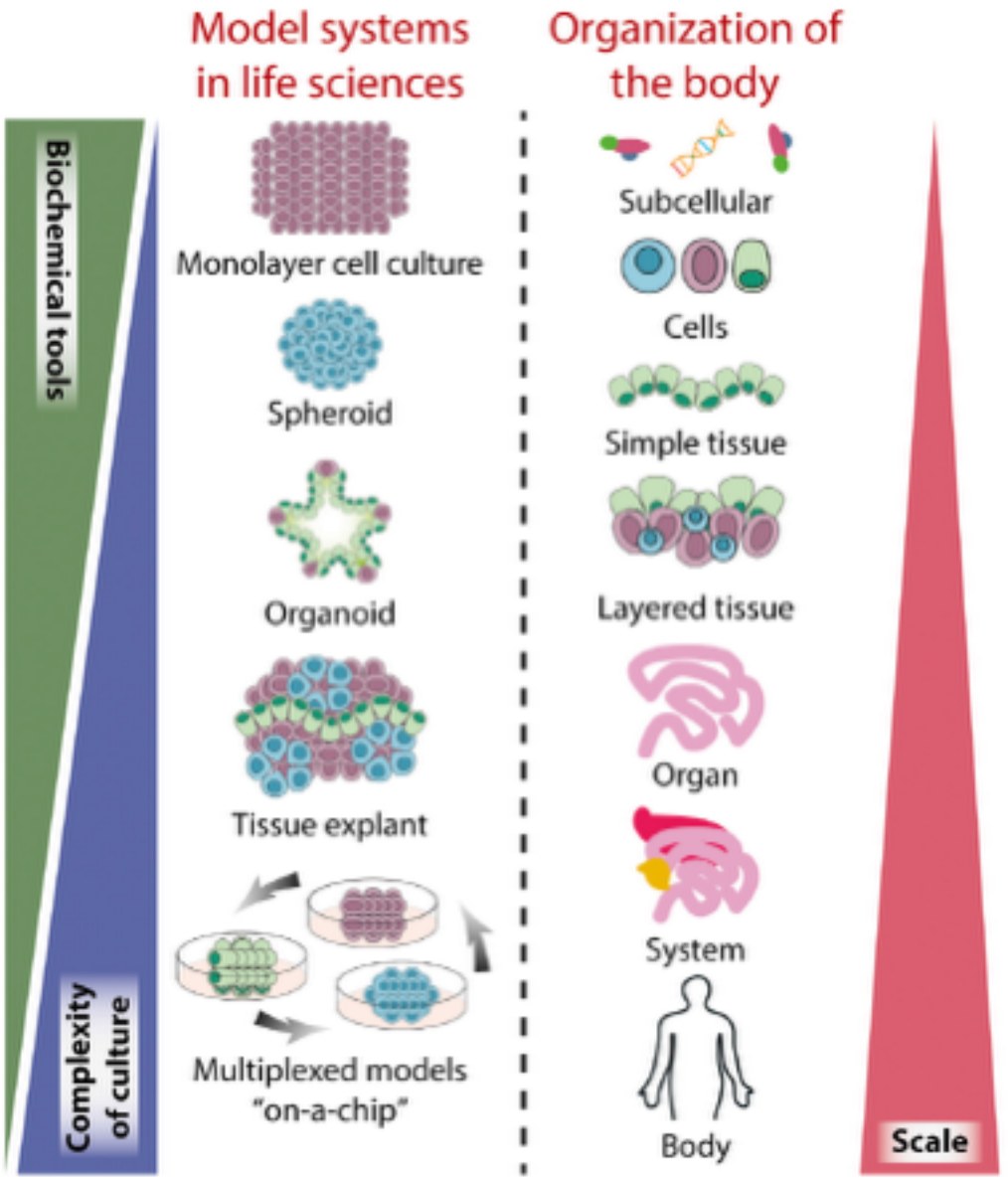
Lack of cell-cell and cell-matrix interactions

Lack stem and progenitor cells to sustain the culture

Moderate complexity, cell-cell interaction, cell-matrix interaction, long term cultures
Representative of in vivo physiology

Lose their phenotype and difficult to maintain for long

Very high complexity



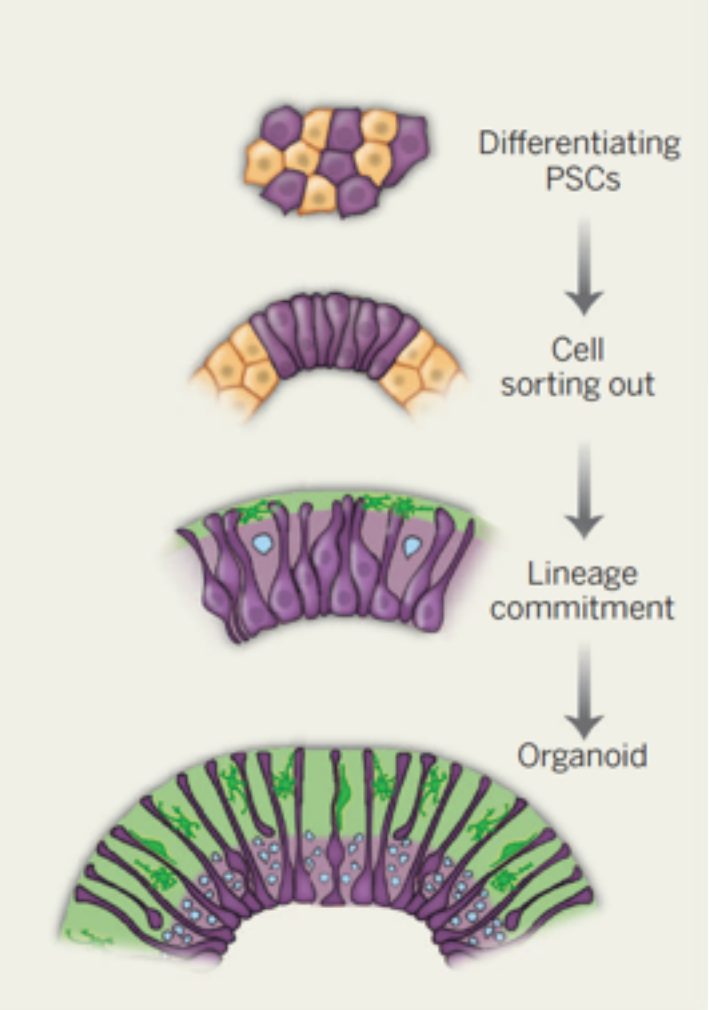
organoids: Resembling an organ

Organoids:

A collection of organ-specific cell types that develops from stem cells or organ progenitors and self-organizes through cell sorting and spatially restricted lineage commitment in a manner similar to in vivo

Organoid formation recapitulates both major processes of self-organization during development:

- **cell sorting out**
- **spatially restricted lineage commitment**

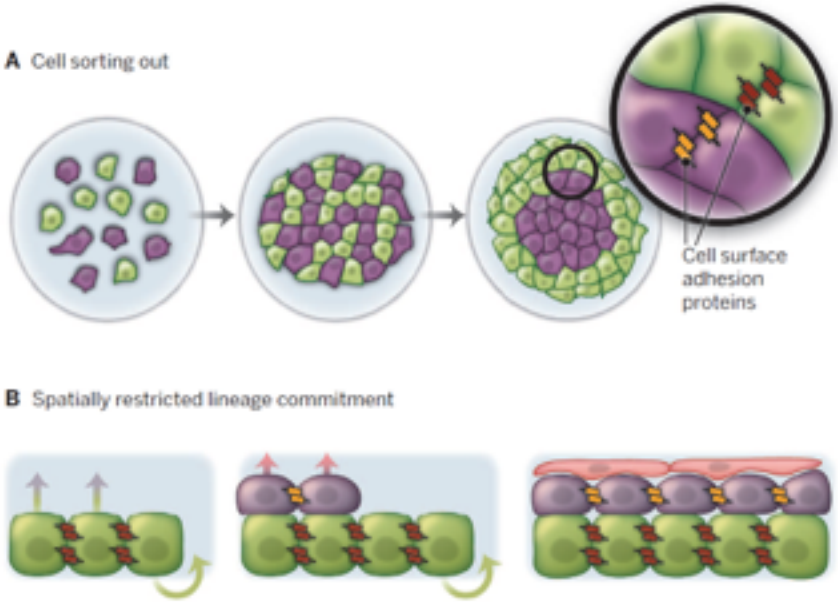


Two approaches to understand the tissue patterning

1- “cell sorting out”
capacity of cells to reorganize and segregate to form structures with much the same histogenic properties as those in vivo

thermodynamically stable pattern

organ self-assembly seems to arise from segregation of cells with similar adhesive properties into domains that achieve the most thermodynamically stable pattern



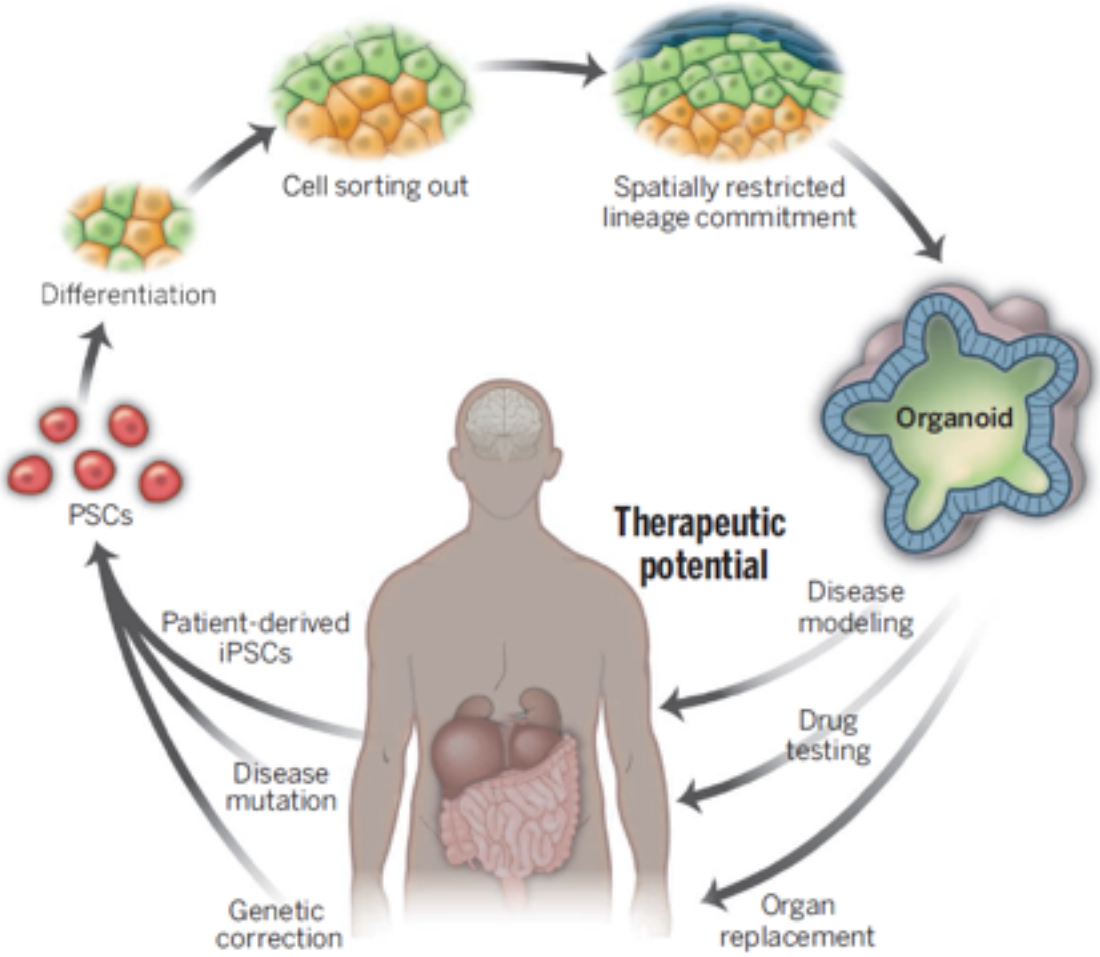
2- proper spatially restricted progenitor fate decisions

What have been done until now?

Table 1. Current state of the art for in vitro self-organizing tissues of various organs. mESCs, mouse embryonic stem cells.

	Organ	Early reaggregation experiments	Identity derivation from PSCs	3D self-organizing structure or organoid
Endoderm	Thyroid	Embryonic chick thyroid (*90*), adult rat thyroid (*91*)	Thyroid progenitors from mESCs (*92*)	Functional thyroid organoid from mESCs (*70*)
	Lung	Embryonic chick lung (*93*)	Lung progenitors from mESCs and hiPSCs (*92*, *94*)	Bronchioalveolar structures from mouse adult lung stem cells (*71*)
	Pancreas	Mouse embryonic pancreas (*95*)	Pancreatic endocrine cells from mESC (*96*) and hESCs (*97*)	Pancreatic organoids from mouse embryonic pancreatic progenitors (*72*)
	Liver	Chick embryonic liver (*9*)	Hepatocytes from mESCs and hESCs (*98*)	Liver organoids from adult stem cells (*40*); liver buds from human iPSCs (*41*)
	Stomach	Chick embryonic gizzard and proventriculus (*99*)	None	Stomach organoids from adult stem cells (*36*, *37*)
	Intestine	Rat embryonic intestine (*100*)	Intestinal cells from mESCs (*101*) and hPSCs (*33*)	Intestinal organoids from human PSCs (*33*)
Mesoderm	Heart	Chick (*102*) and rat (*103*) cardiac tissue	Spontaneous and directed differentiation of mESCs and hESCs (*104*)	Vascularized cardiac patch from hESCs (*105*)
	Skeletal muscle	Embryonic chick leg skeletal muscle (*76*)	Mesoangioblasts from human iPSCs (*106*)	Anchored contracting skeletal muscle in 3D matrix derived from myoblast progenitors (*107*)
	Bone	Skeletal bone of chick embryonic leg (*77*)	Osteoblasts from mESCs (*108*) and hESCs (*109*)	Bone spheroids from human osteogenic cells (*110*)
	Kidney	Chick embryonic kidney (*9*)	Intermediate mesoderm from mouse (*111*) and human (*112*) PSCs	Ureteric bud (*68*) and metanephric mesenchyme (*29*) renal organoids (*69*) from human and mouse PSCs
Ectoderm	Retina	Embryonic chick retina (*61*)	Retinal progenitors from mouse (*113*) and human PSCs (*114*)	Optic cup organoids from mouse (*64*) and human (*65*) PSCs
	Brain	Embryonic chick brain cells (*45*)	Neural rosettes from mouse and human PSCs (*48*, *49*)	Cerebral organoids from mouse and human PSCs (*28*, *55*)
	Pituitary	Chick anterior pituitary (*115*)	None	Adenohypophysis organoids from mouse PSCs (*73*)
	Mammary gland	Mammary gland from adult virgin mice (*75*)	None	3D breast epithelia embedded in Matrigel (*116*)
	Inner ear	Embryonic chick otocysts (*117*)	Inner ear hair cells from mESCs (*118*)	Inner ear organoids from mESCs (*74*)
	Skin	Embryonic chick skin and feather follicles (*9*)	Keratinocytes from mESCs (*119*)	Stratified epidermis from keratinocytes derived from mESCs (*119*)

What can be done using iPSC-derived organoids



Drug testing

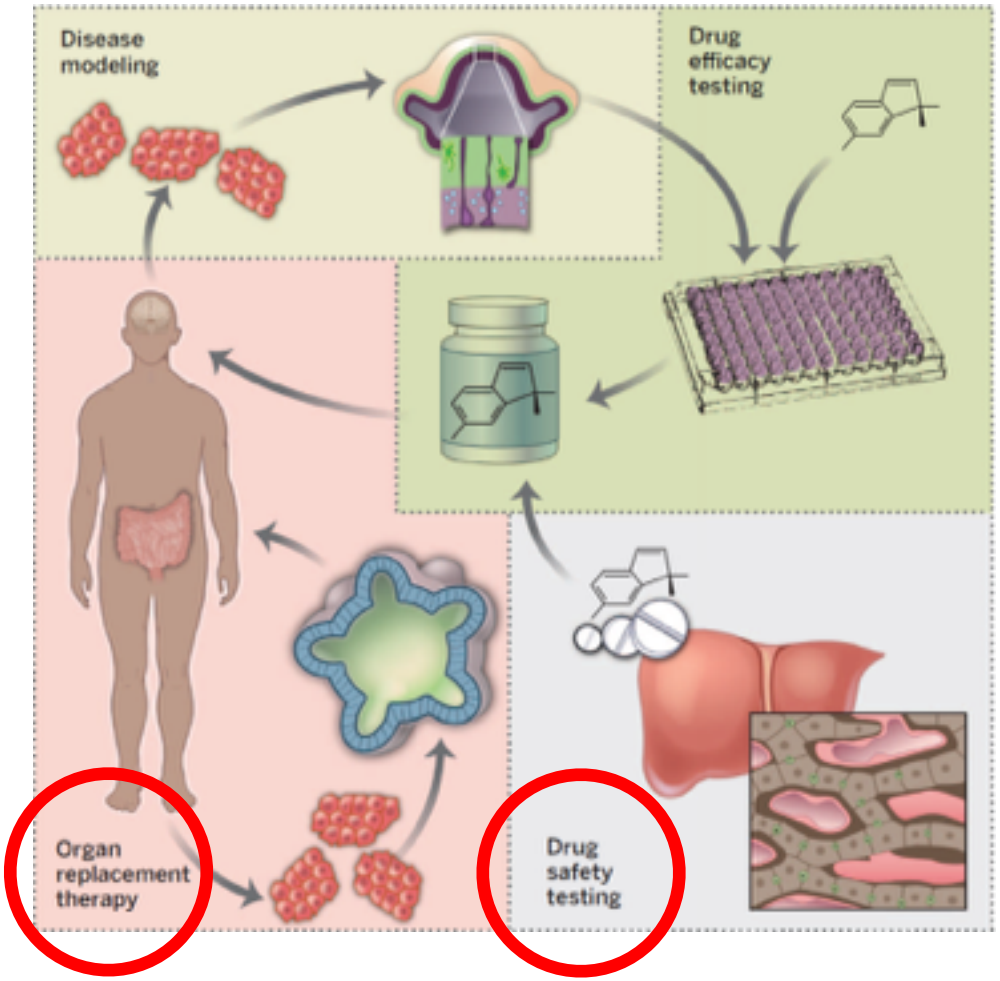
Organoids more appropriate than animal models:

- First tool to test:
  if successful, animal models

- In case of human liver:

metabolizes drugs in a manner distinct from animals
—> Drugs can be removed at early stages of screening when they could otherwise be functional in humans

—> toxic metabolites can be produced specifically in humans but not in tested animals



Earliest process of organogenesis: cellular interactions during organ-bud development

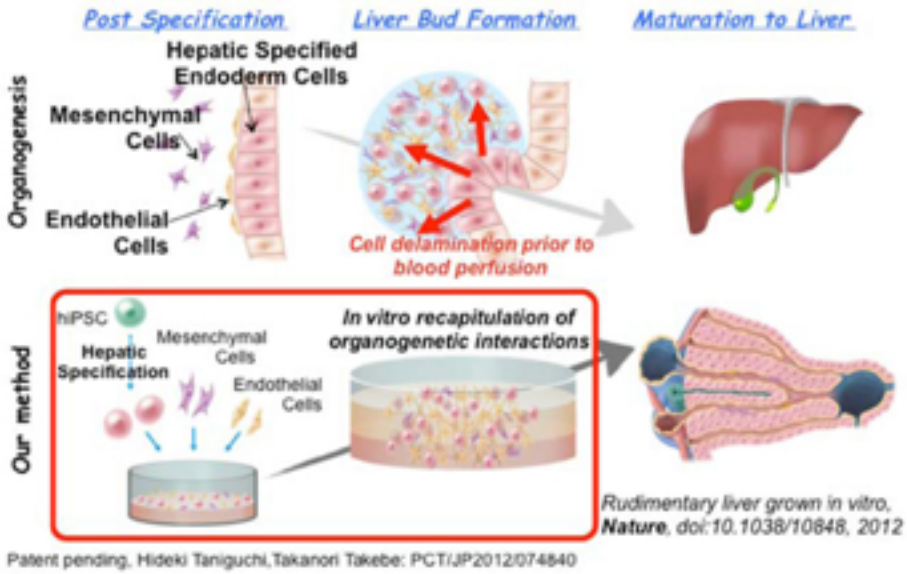
LETTER

doi:10.1038/nature12271

Vascularized and functional human liver from an iPSC-derived organ bud transplant

Takanori Takebe[1,2], Keisuke Sekine[1], Masahiro Enomura[1], Hiroyuki Koike[1], Masaki Kimura[1], Takunori Ogaeri[1], Ran-Ran Zhang[1], Yasuharu Ueno[1], Yun-Wen Zheng[1], Naoto Koike[1,3], Shinsuke Aoyama[4], Yasuhisa Adachi[4] & Hideki Taniguchi[1,2]

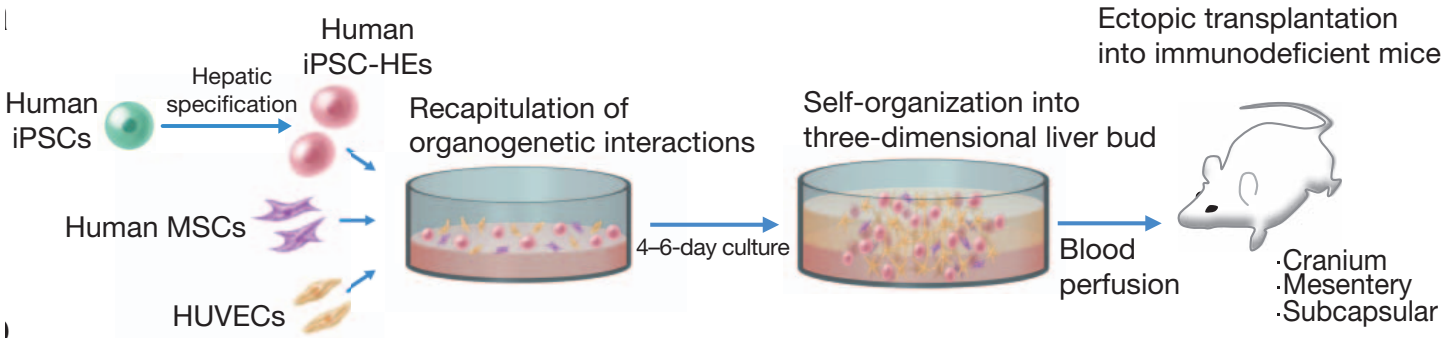
Generation of liver bud from iPSC by mimicking early organogenesis



Hepatic specification:

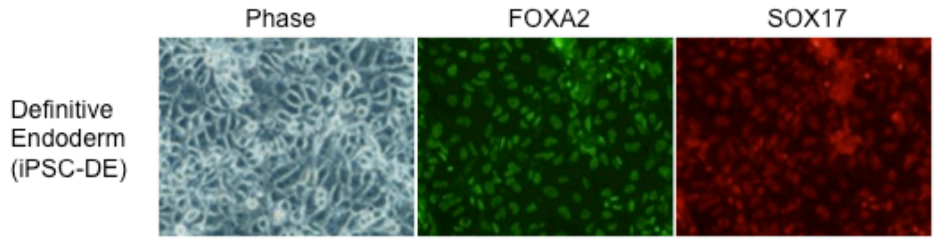
1. human iPSC cells to endoderm: RPMI media + B27 supplement + Activin A

2. Endoderm to hepatocytes: BMP4 + FGF2 (Growth factors important for hepatic specification)



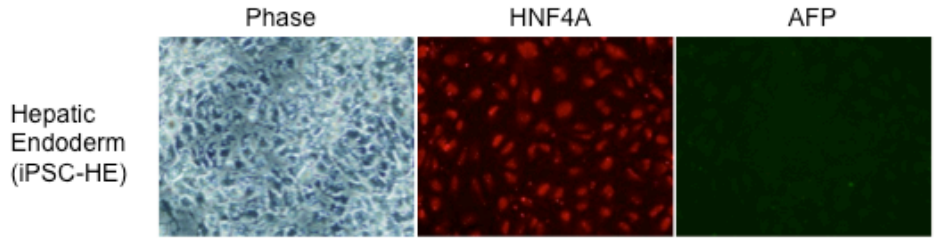
hepatic endoderm cells from human iPSCs (iPSC-HE)

DAY 6

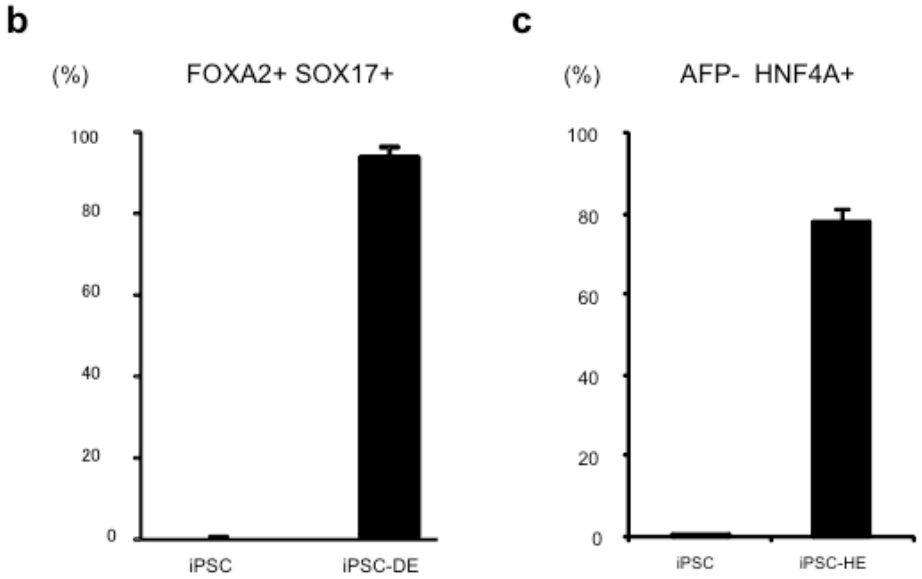


Markers for definitive endoderms

DAY 9



Markers for liver development

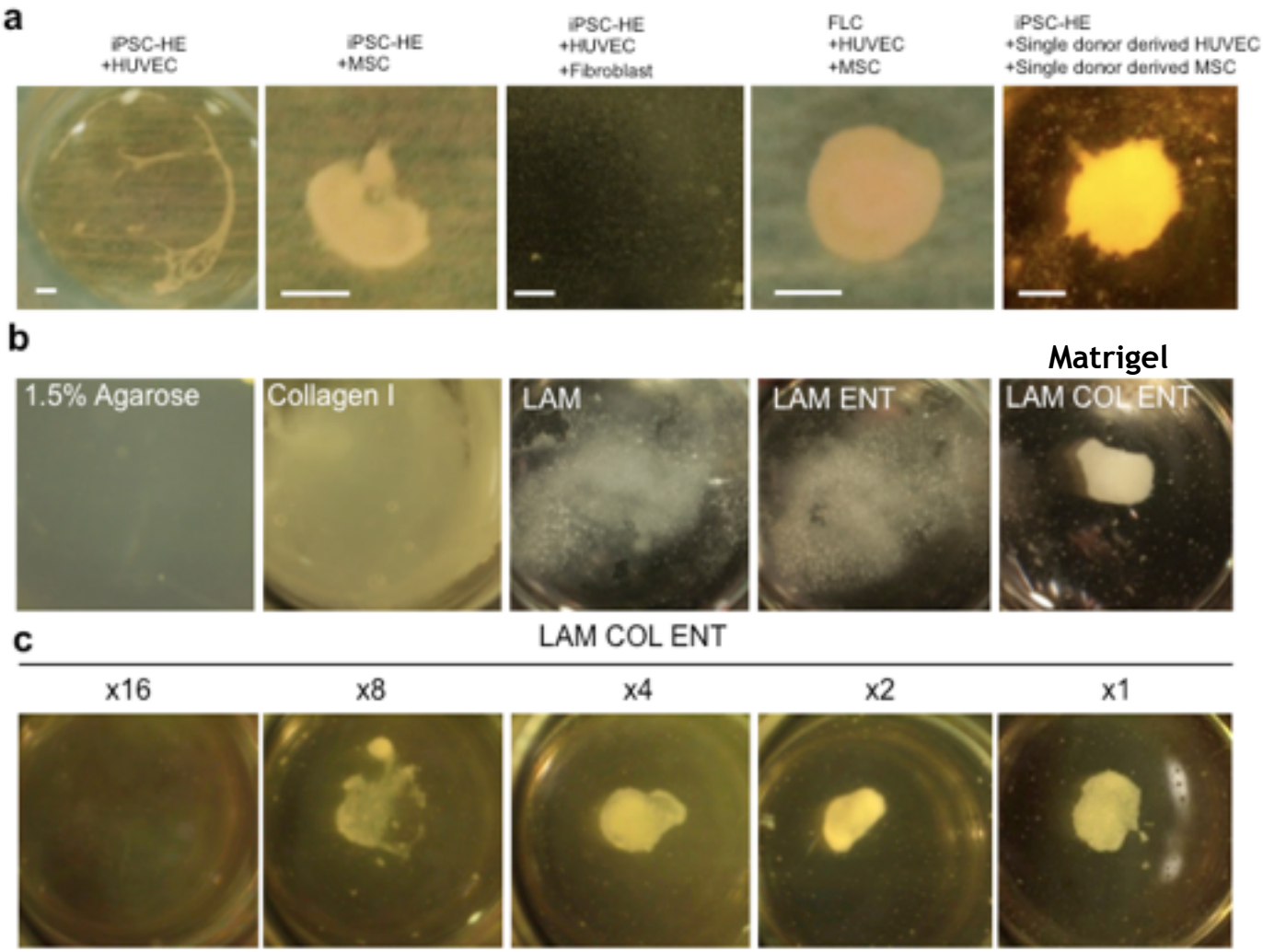


live-bud formation from iPSC + HUVEC + MSC on a Matrigel matrix

various co-cultures

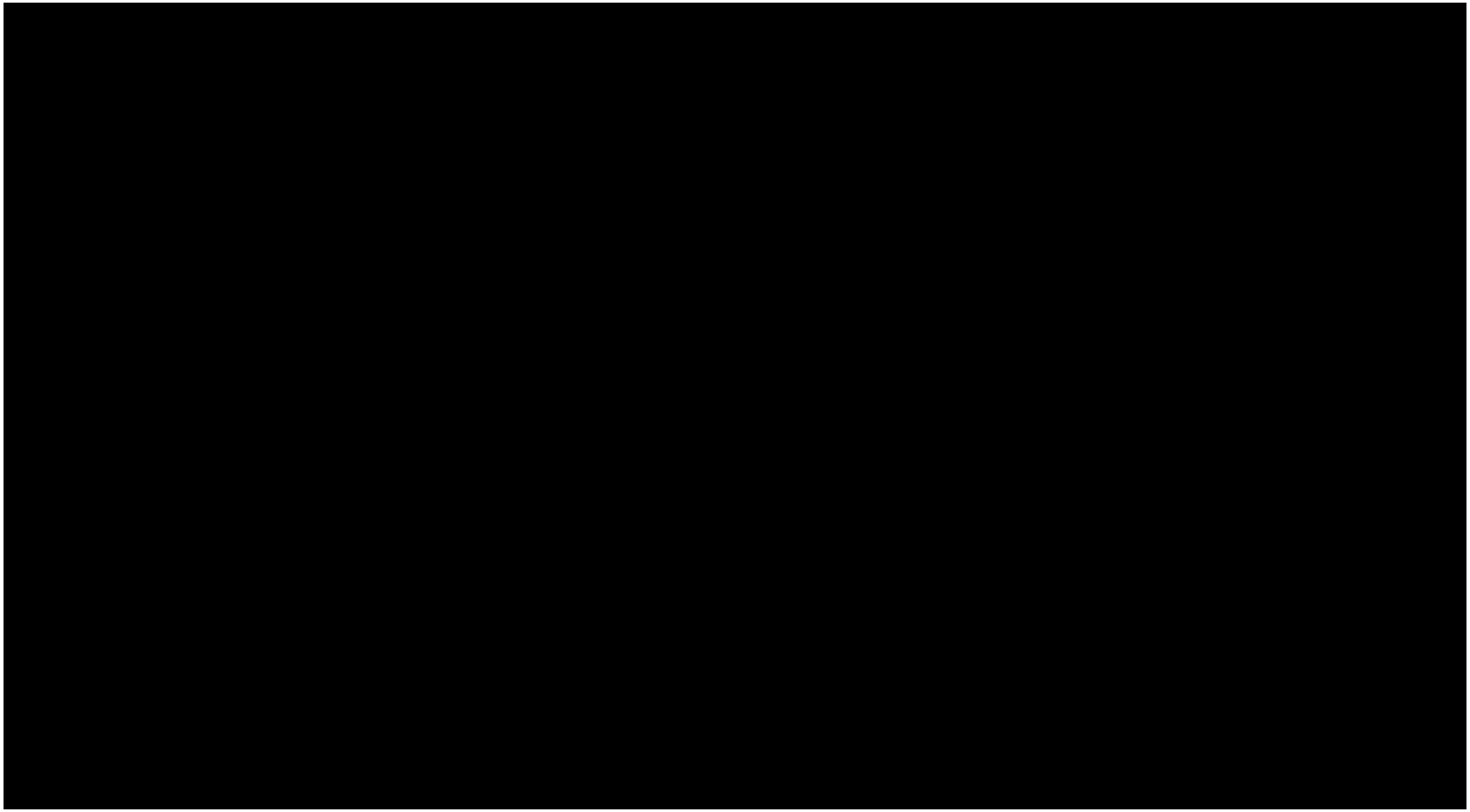
various matrix proteins

matrix protein concentrations

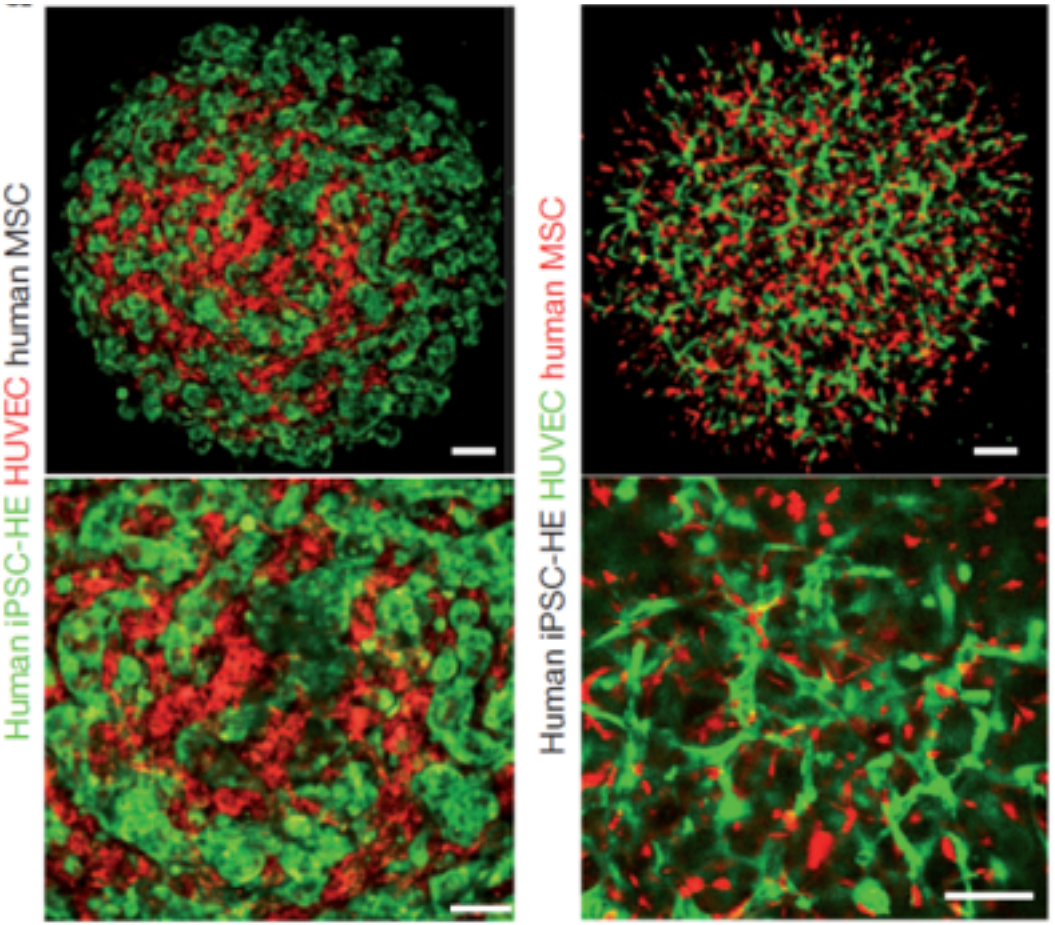


MSC: human mesenchymal stem cell ; HUVEC: human umbilical vein endothelial cell

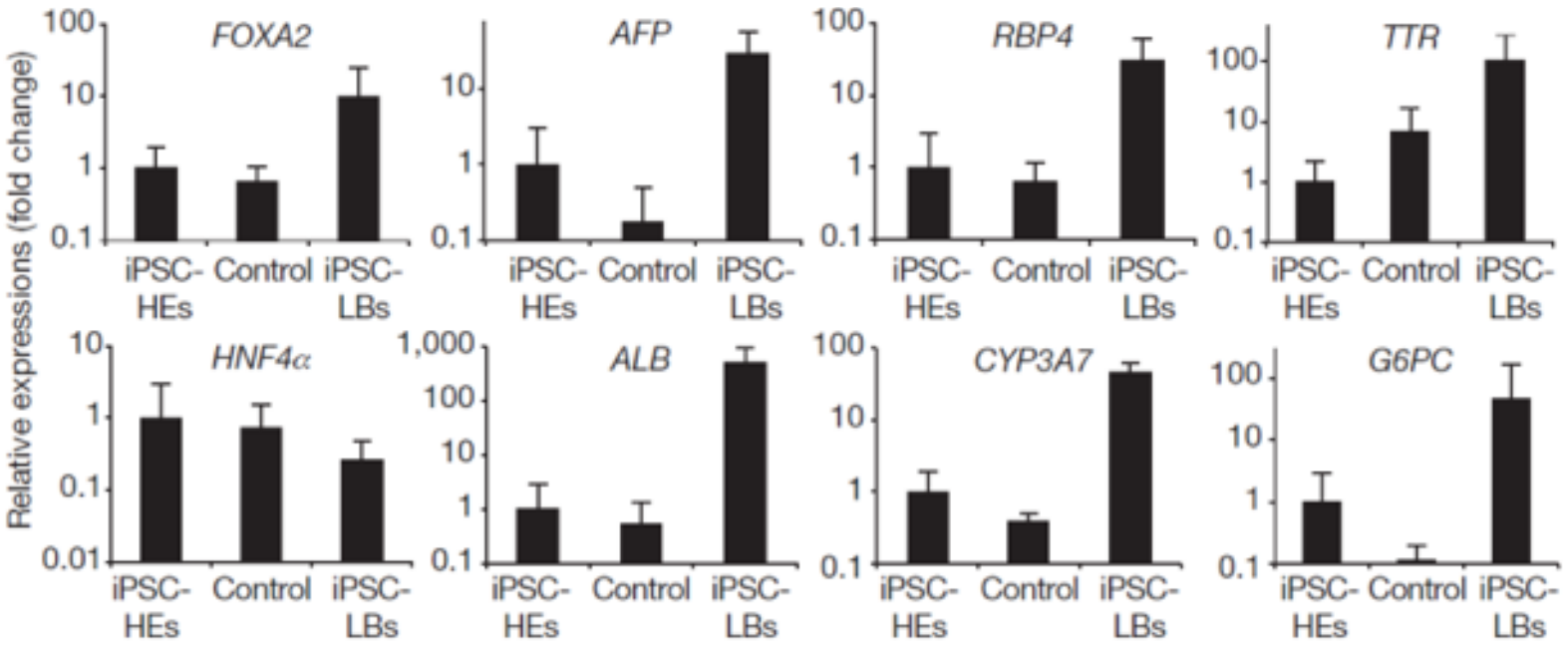
live-bud formation from iPSC + HUVEC + MSC on a Matrigel matrix



Formation of endothelial network and homogeneously distributed iPSC-HE



Expression of hepatic markers in liver bud

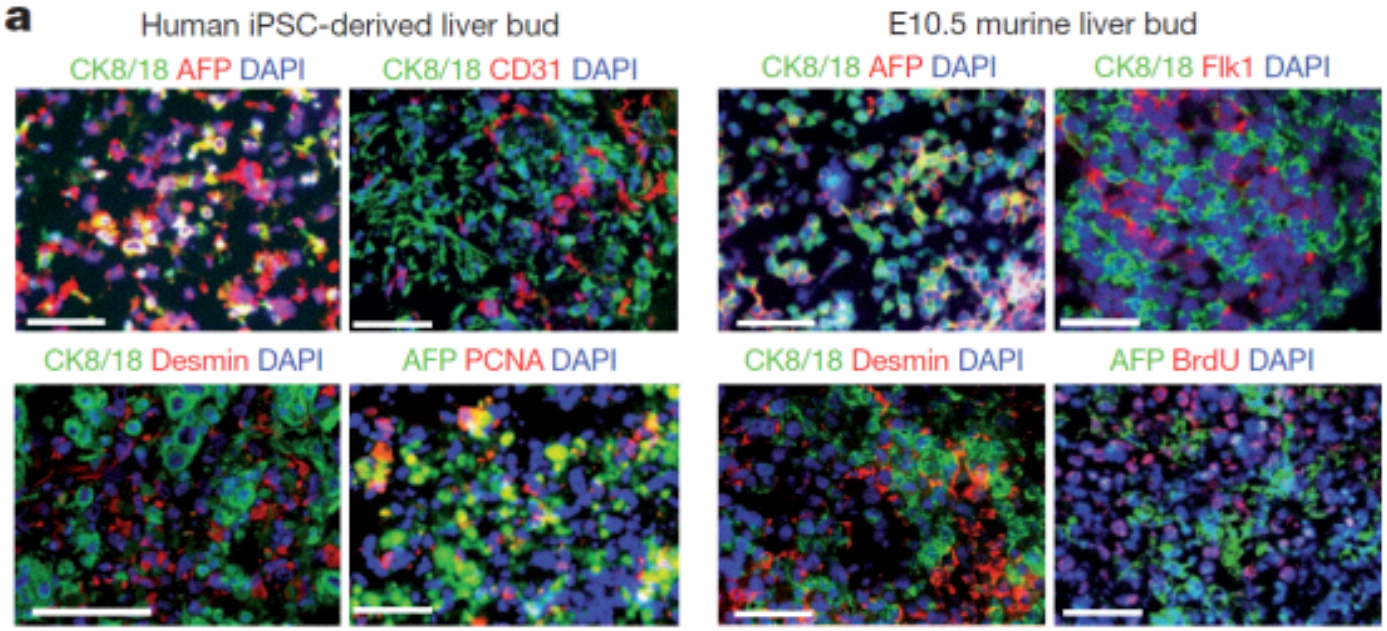


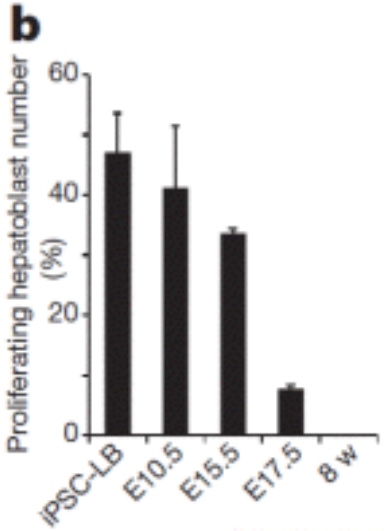
measured at day 6 of culture
control: mixture of separated cultures of iPSC-HE , MSCs, HUVEC

FOXA2: Fokhead-box-protein A2, **AFP: alpha-fetoprotein**, RBP4: retinol biding protein 4, TTR: transthyretin, **HNF4a: hepatocyte nuclear factor 4 alpha**, **ALB: albumin**, CYP3A7: cytochrome 450 family of proteins, G6PC: glucose-6-phosphatase

iPSC-liver bud vs. murine liver bud

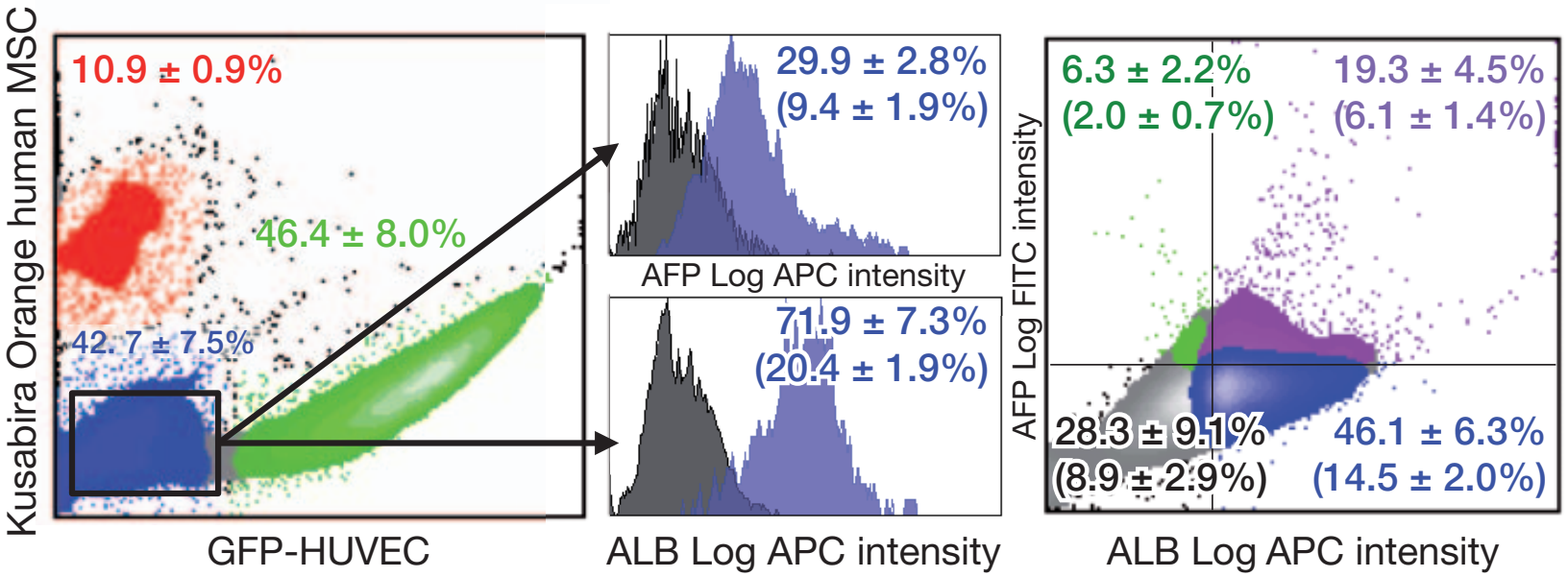
human iPSC-LB is composed of proliferative AFP-positive hepatocytes as well as mesenchymal and endothelial progenitors





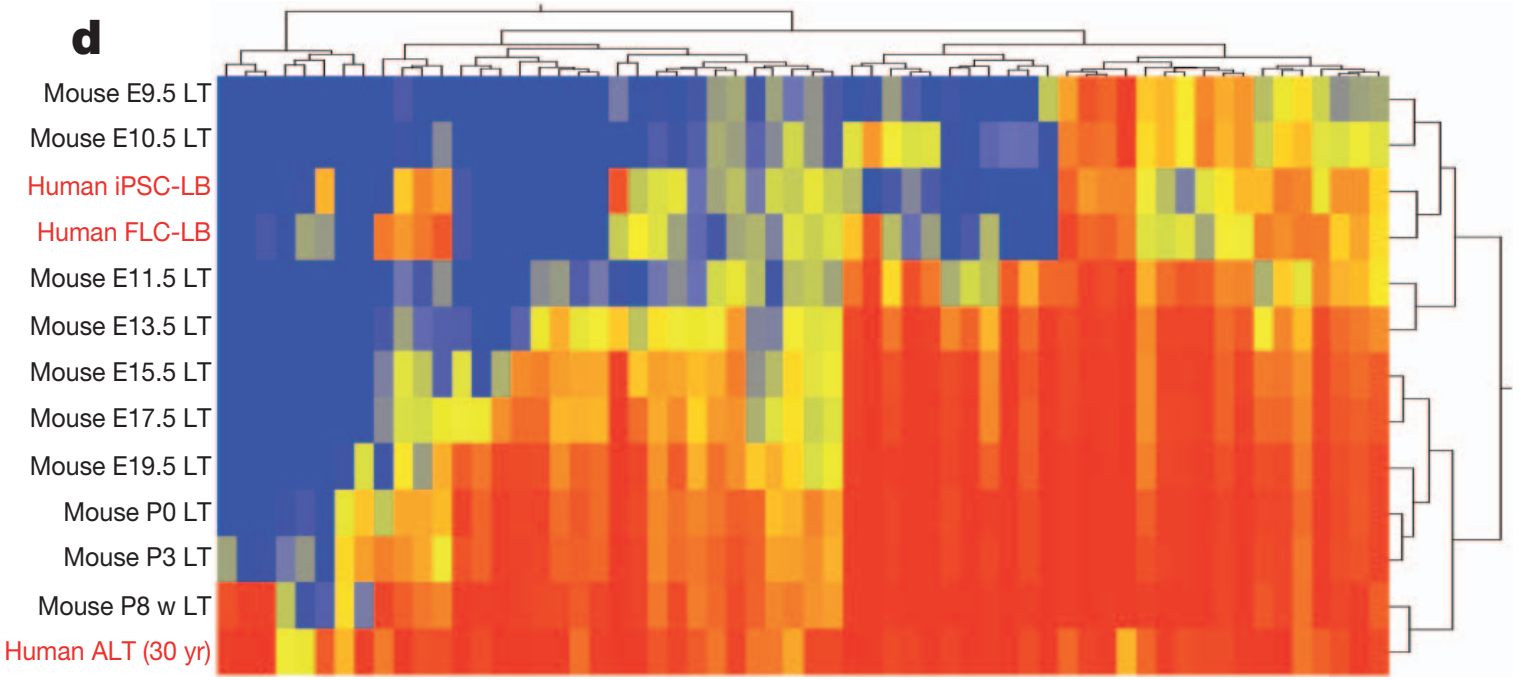
CK8/18 (cytokeratin8):marker for epithelial cells, AFP (alpha-fetoprotein): early hepatic marker, CD31: marker for endothelial cells, Desmin:, PCNA: proliferation marker, Flk1: a VEGF receptor

iPSC-liver bud express ALB and AFP

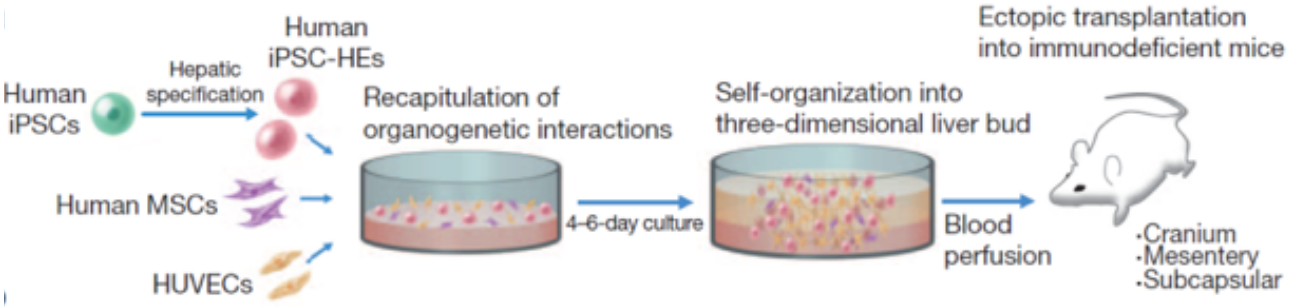


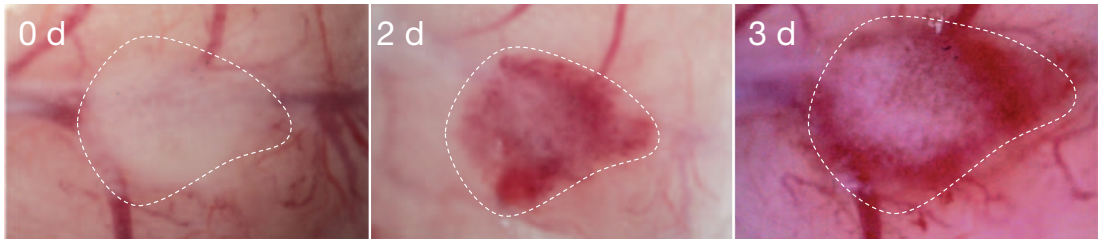
Liver developmental gene signatures in iPSC-LB

- 83 genes upregulated during liver development
- iPSC-LB similar to mouse E11.5 and human FLC-LB

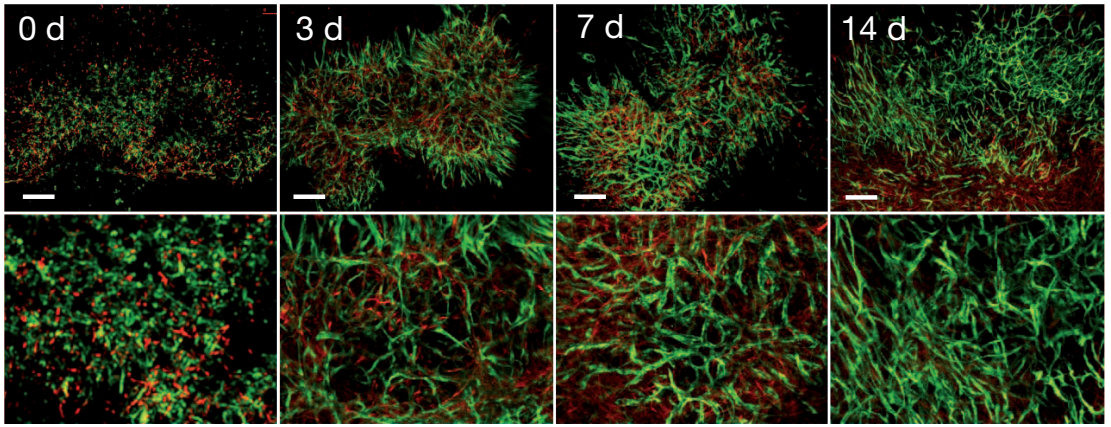


haemodynamic stimulation of iPSC-LB

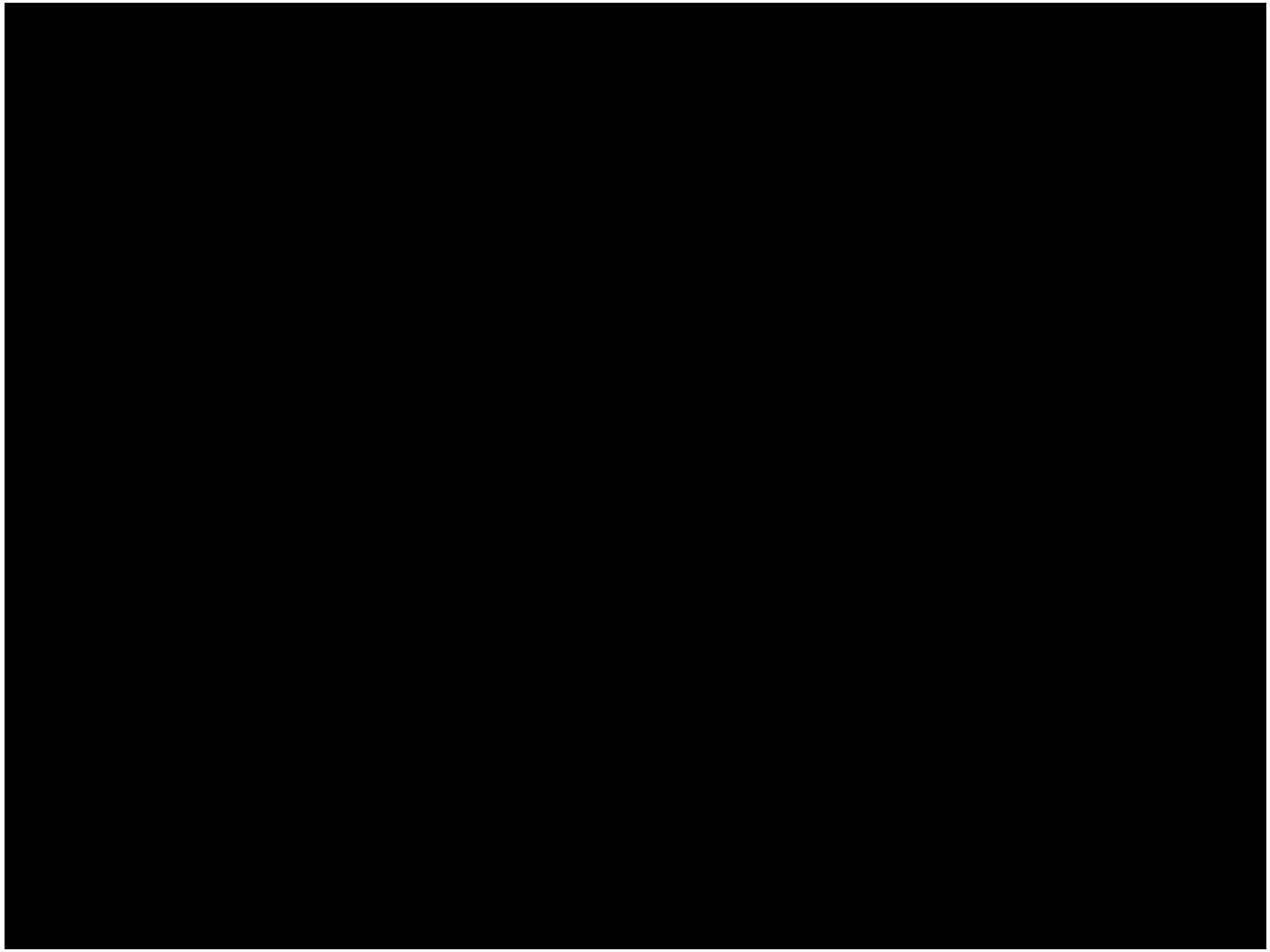




Human iPSC-HE HUVEC human MSC



Vascularisation of the transplant

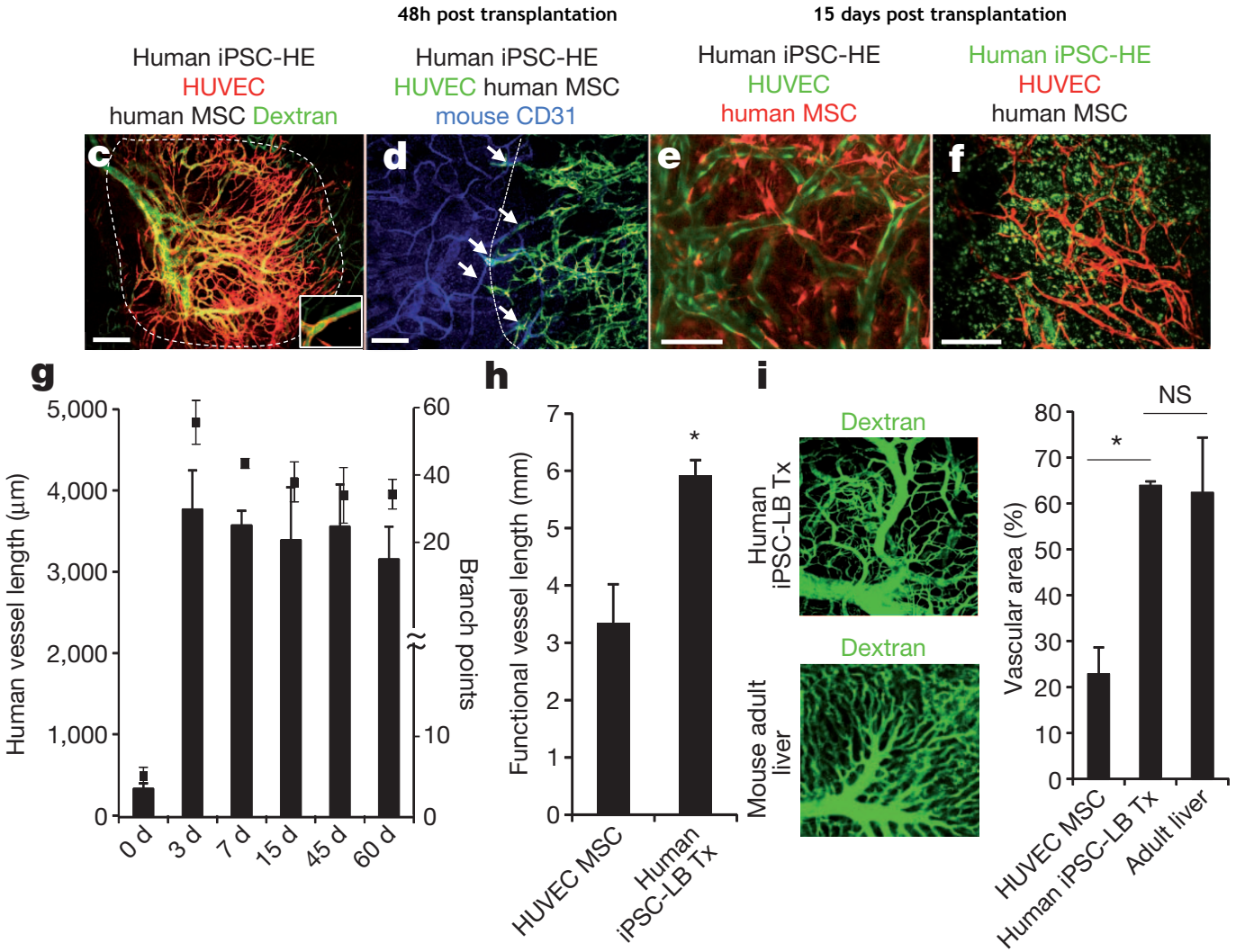


z stack images of patent human vasculatures inside the hiPSC-LB transplants at day 4
FITC-conjugated dextran

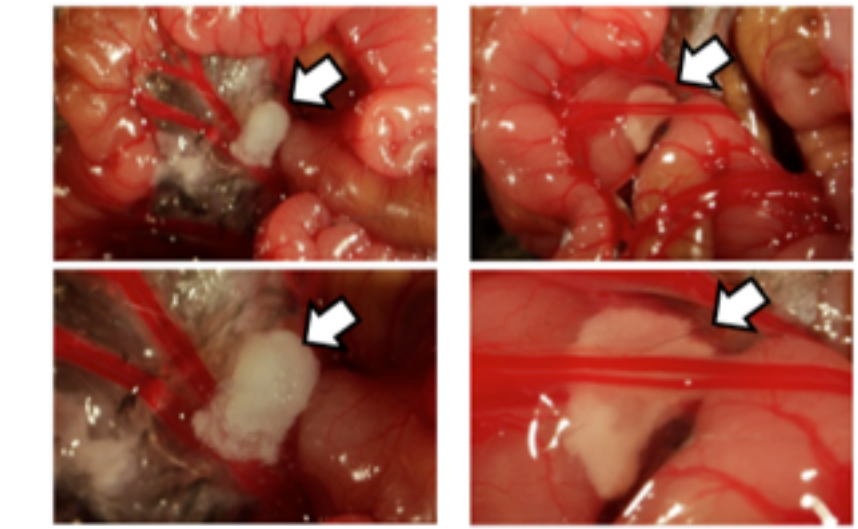
Functional vessel formation in transplanted iPSC-LB

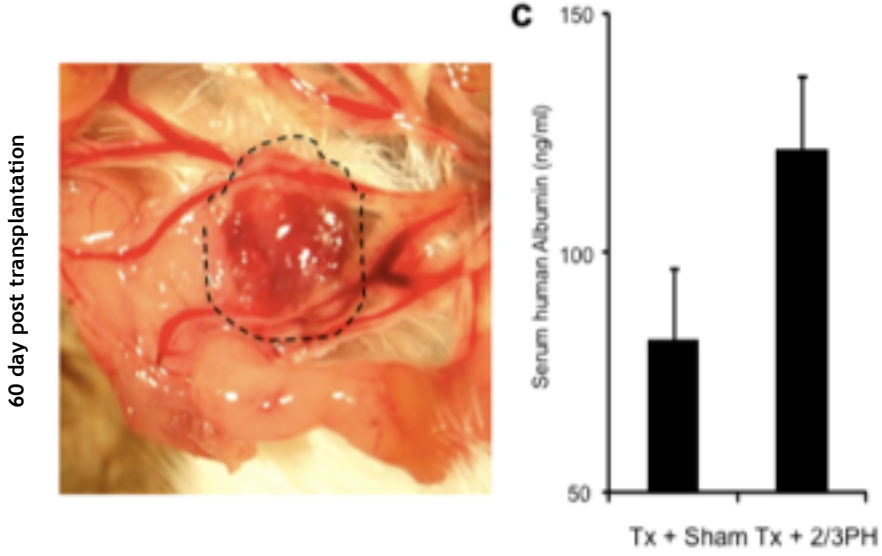
z stack images of patent human vasculatures inside the hiPSC-LB transplants at day 4
Texas red-conjugated dextran

haemodynamics in iPSC-LB



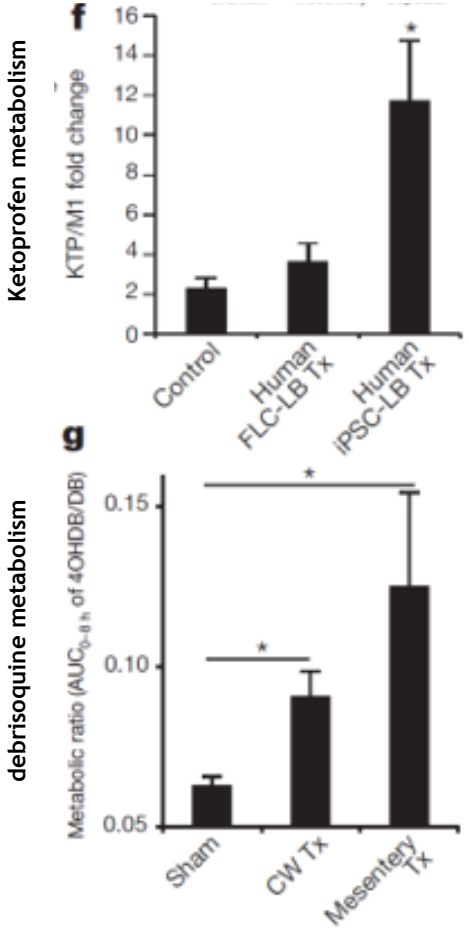
mesenteric transplantation of iPSC-LB in mice



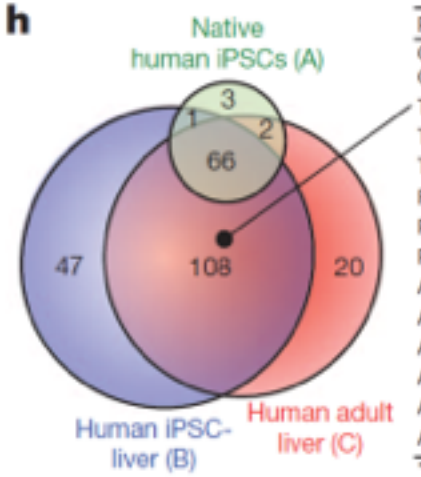


metabolism activity of transplanted iPSC-LB

- transplanted mice were challenged with ketoprofen or debrisoquine (both metabolised differently in mouse and human)
- formation of human-specific metabolites in serum and urine of transplanted mice



- detection of small-molecule metabolites via metabolome
- predicting drug metabolite profiles of humans

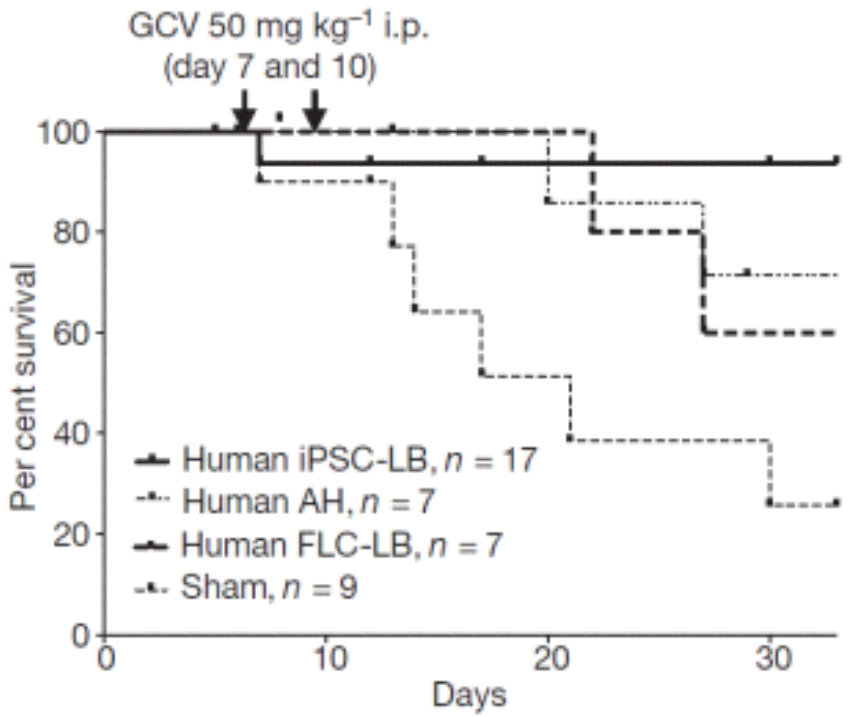


Pathway	Metabolites : A∩(B∩C)
Glycolysis/ Gluconeogenesis	Phosphoenolpyruvic acid
TCA cycle	Succinic acid
TCA cycle	Fumaric acid
TCA cycle	6-Phosphogluconic acid
PPP	Ribose 5-phosphate
PPP	Ribulose 5-phosphate
PPP	Sedoheptulose 7-phosphate
AA Gly, Ser, Cys	2-Hydroxybutyric acid
AA(Gly, Ser, Cys)	CDP-choline
AA(Gly, Ser, Cys)	*N,N*-Dimethylglycine
AA(Gly, Ser, Cys)	Sarcosine
AA(Gly, Ser, Cys)	Taurine
AA(Gly, Ser, Cys)	Taurocholic acid

*PPP, pentose phosphate pathway
*AA, amino acid synthesis/metabolism

Usage of iPSC-LB in liver injury

i



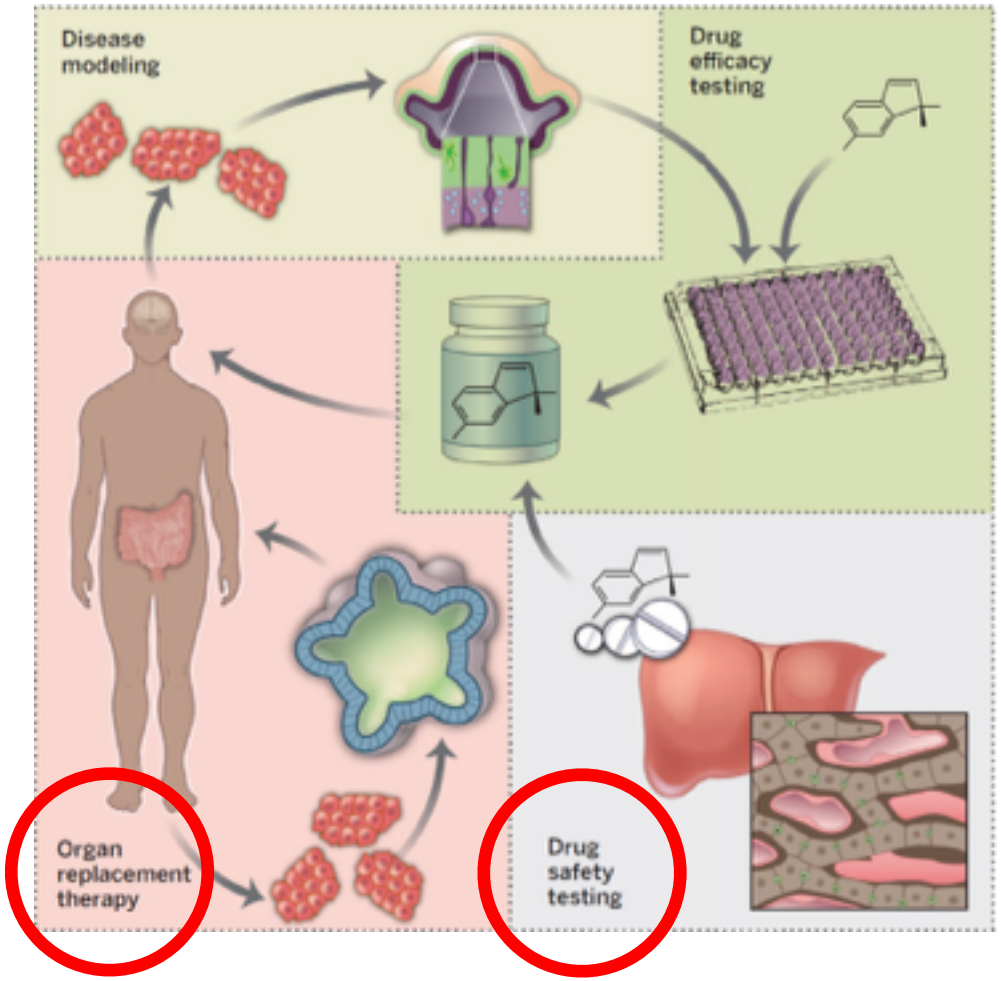
Transplantation:

1- Liver injury:
induction of murine hepatocytes ablation via injection of gancyclovir to TK-NOG mice

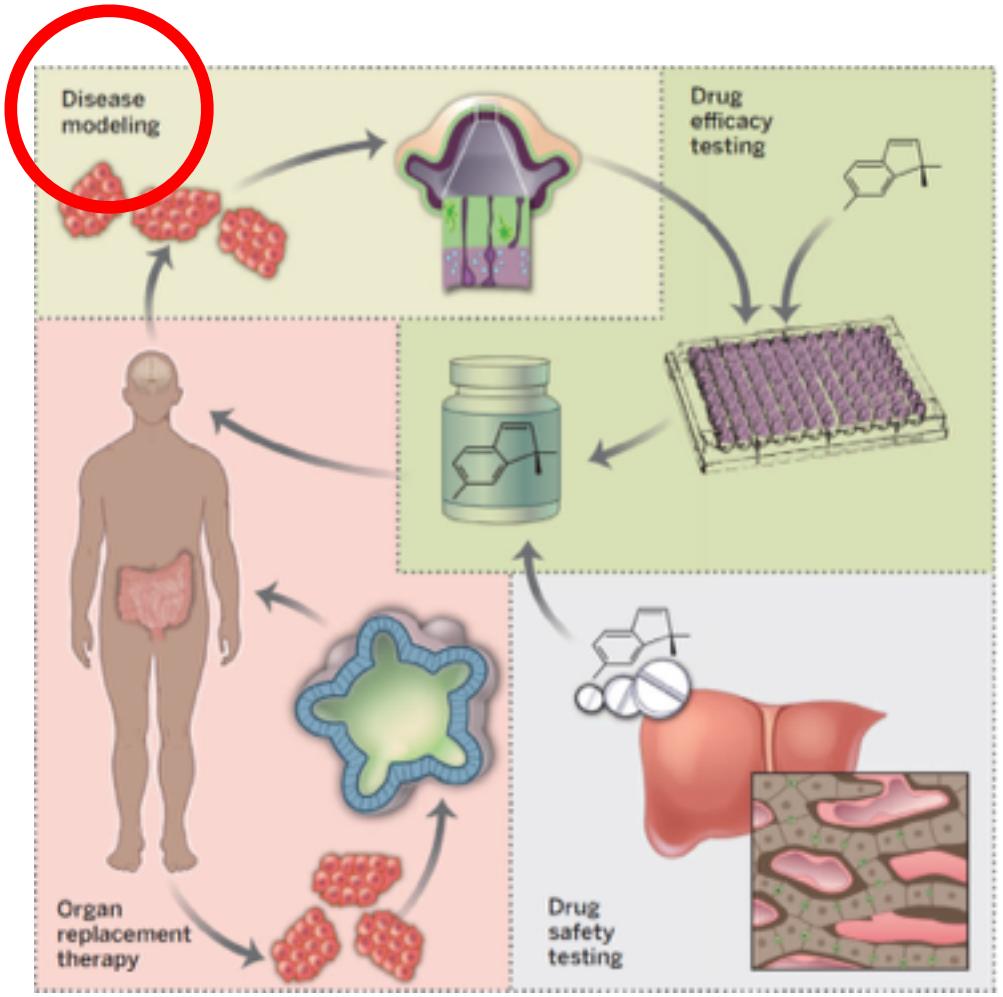
2- comparison between mesenteric transplantation of iPSC-LB, FLC-LB, adult hepatocytes and sham-operated

Using organoids in regenerative medicine

1. Generation of a three-dimensional vascularised liver-bud
2. treating organ failure using organ-bud transplants
3. prediction of drug efficacy



Modeling diseases using iPSC-derived organoids



ARTICLE

doi:10.1038/nature12517

Cerebral organoids model human brain development and microcephaly

Madeline A. Lancaster[1], Magdalena Renner[1], Carol-Anne Martin[2], Daniel Wenzel[1], Louise S. Bicknell[2], Matthew E. Hurles[3], Tessa Homfray[4], Josef M. Penninger[1], Andrew P. Jackson[2] & Juergen A. Knoblich[1]

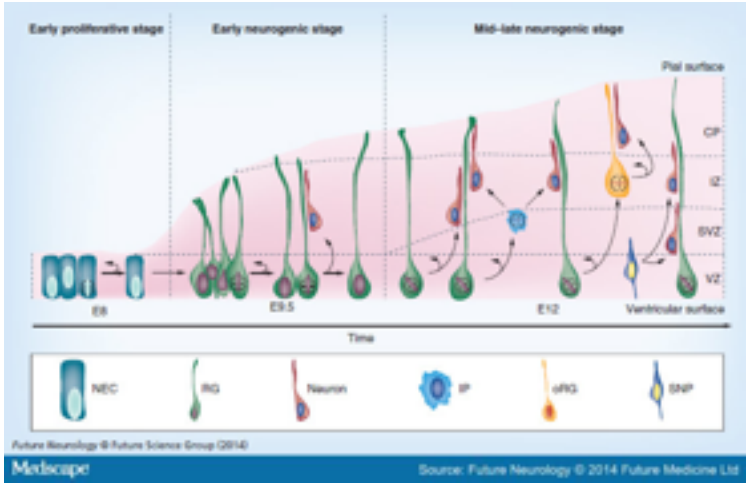
brain development

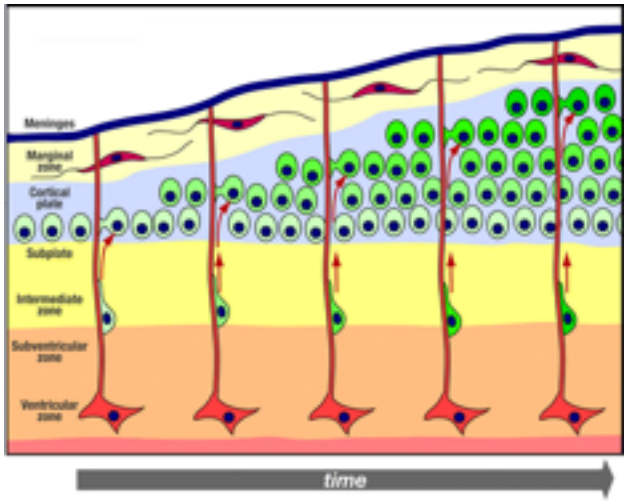
1. radial glial stem cells (RG):

    - derived from neuroepithelium
    - divide and generate neurons and progenitors

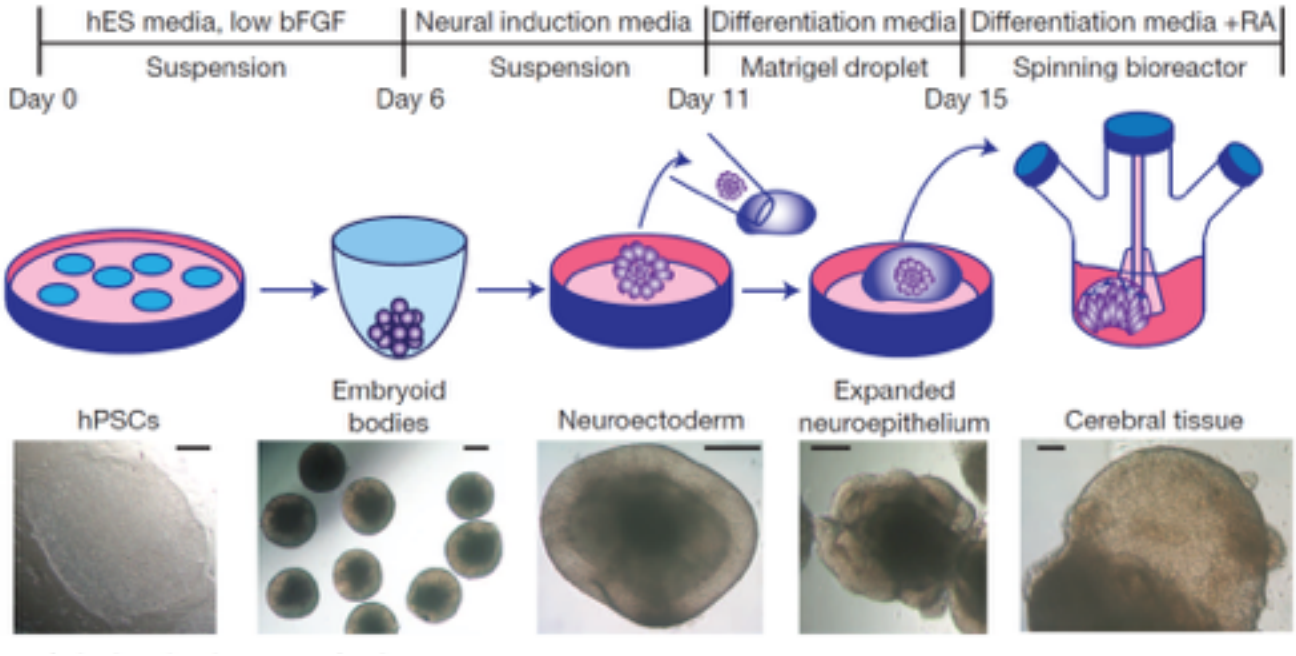
2. Microcephaly:

    - neurodevelopmental disorder where the brain size is reduced
    - several autosomal genes being mutated
    - mouse mutants unable to recapitulate the severely reduced brain size in human disease





neuroepithelial tissue can be generated using hPSCs



Neural induction media:

DMEM + N2 supplement, MEM-NEAA, heparin

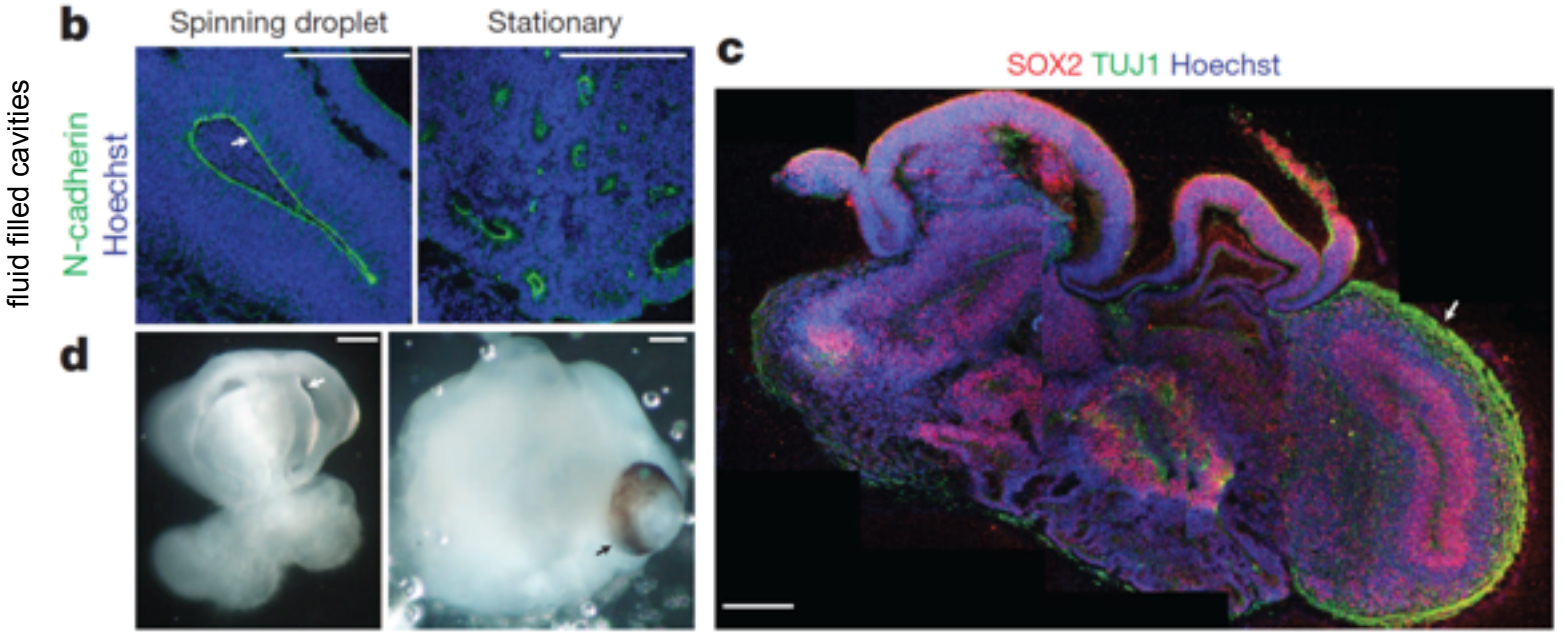
Differentiation media: DMEM + N2 and B27 supplement + insulin + MEM-NEAA

Methods Mol Biol. 2009 ; 549: 51–58. doi:10.1007/978-1-60327-931-4_4.

Differentiation of neuroepithelia from human embryonic stem cells

Xiaofeng Xia and **Su-Chun Zhang***
Departments of Anatomy and Neurology, School of Medicine and Public Health, Waisman Center, University of Wisconsin-Madison, Madison, Wisconsin 53705, USA

Generation of neuroepithelium after 15-20 days



1. N-cadherin:
   - neural-specific cell adhesion molecule
   - play an important role in migration

2. SOX2:
   - marker for neural progenitors

3. TUJ1:
   - expressed in neurons of the PNS and CNS

Immunohistochemistry:

Formation of heterogenous tissue
similar to cerebral cortex, choroid plexus, retina, meninges

human cerebral organoids recapitulate various brain regions

<u>Forebrain/midbrain markers:</u>
FOXG1, SIX3 ,PAX6

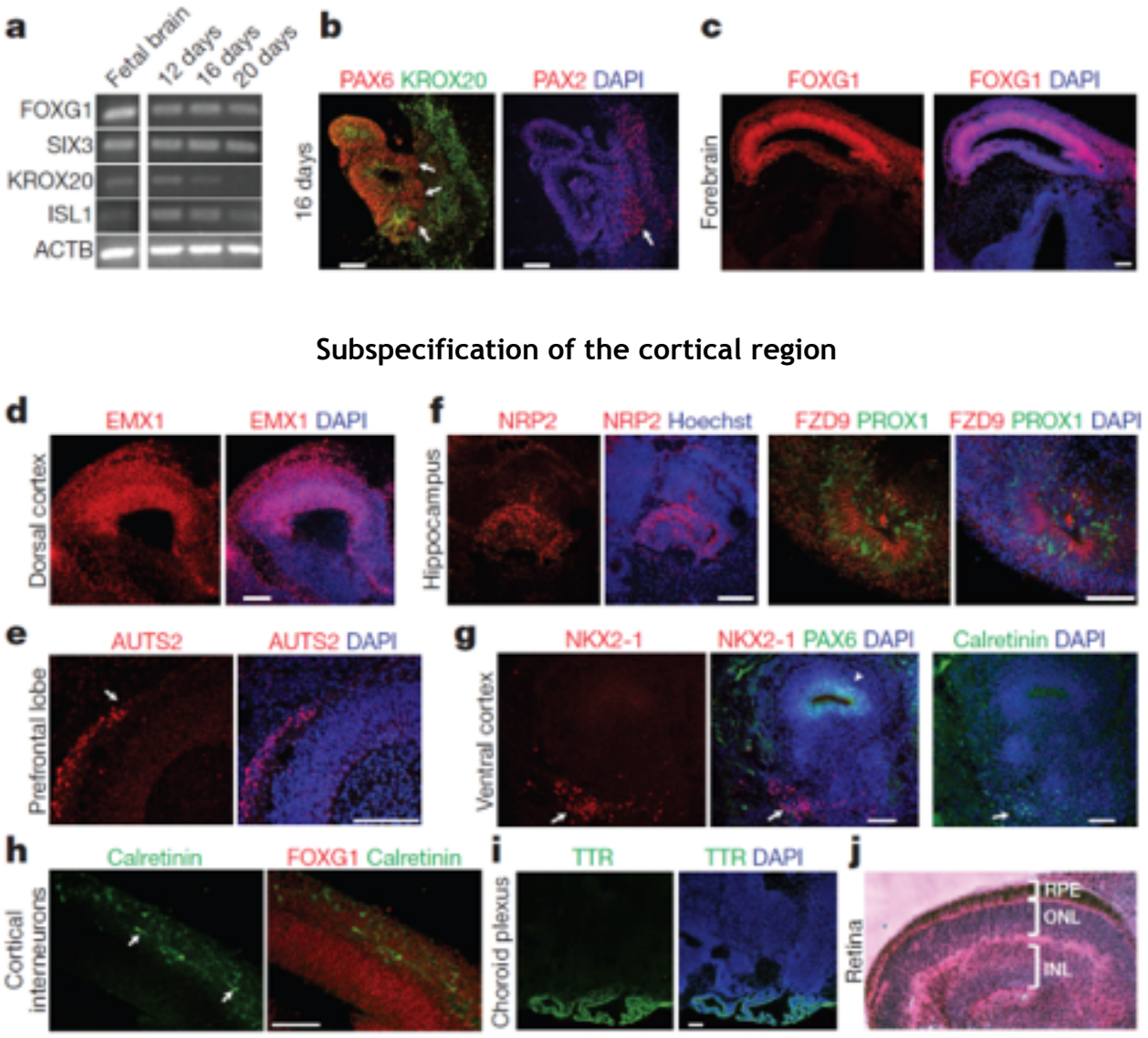
<u>Hindbrain markers:</u>
KROX20, ISL1, PAX2

<u>Regional specification:</u>
FOXG1: forebrain
EMX1: dorsal cortical
AUTS2: prefrontal cortex

<u>Retina- different layers:</u>
retinal pigment epithelium
outer nuclear membrane
inner nuclear membrane

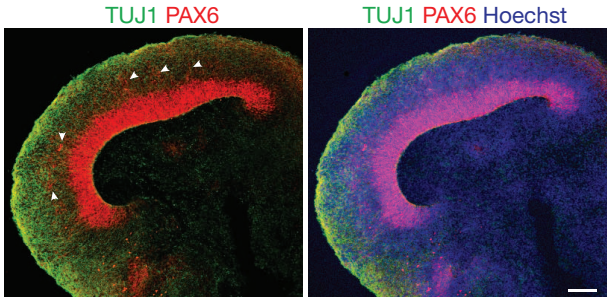
Conclusion:

cerebral organoid developed variety of brain identities organised into discrete, although interdependent domains



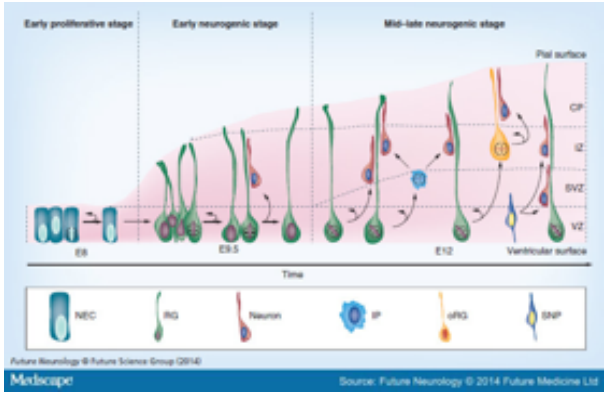
Recapitulation of dorsal cortical organization

The region for most dramatic changes in brain evolution from rodent to human



Organization of dorsal cortical region into a layer with neurons located at the basal surface

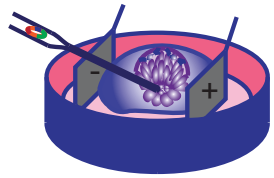
TUJ1: neurons
PAX6: RGs

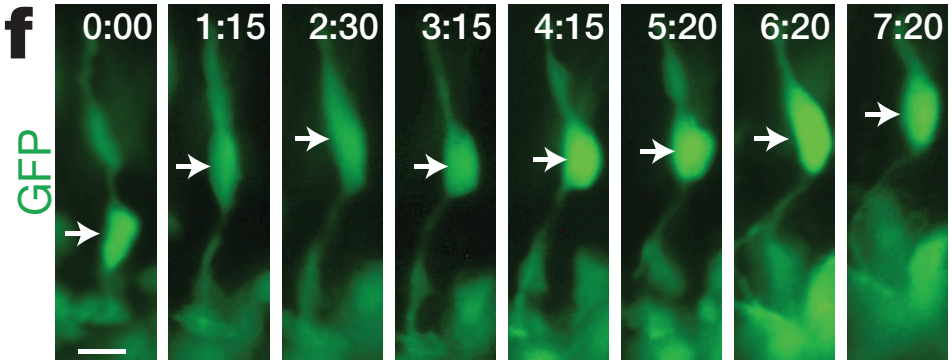


Test for inter kinetic nuclear migration (IKNM):

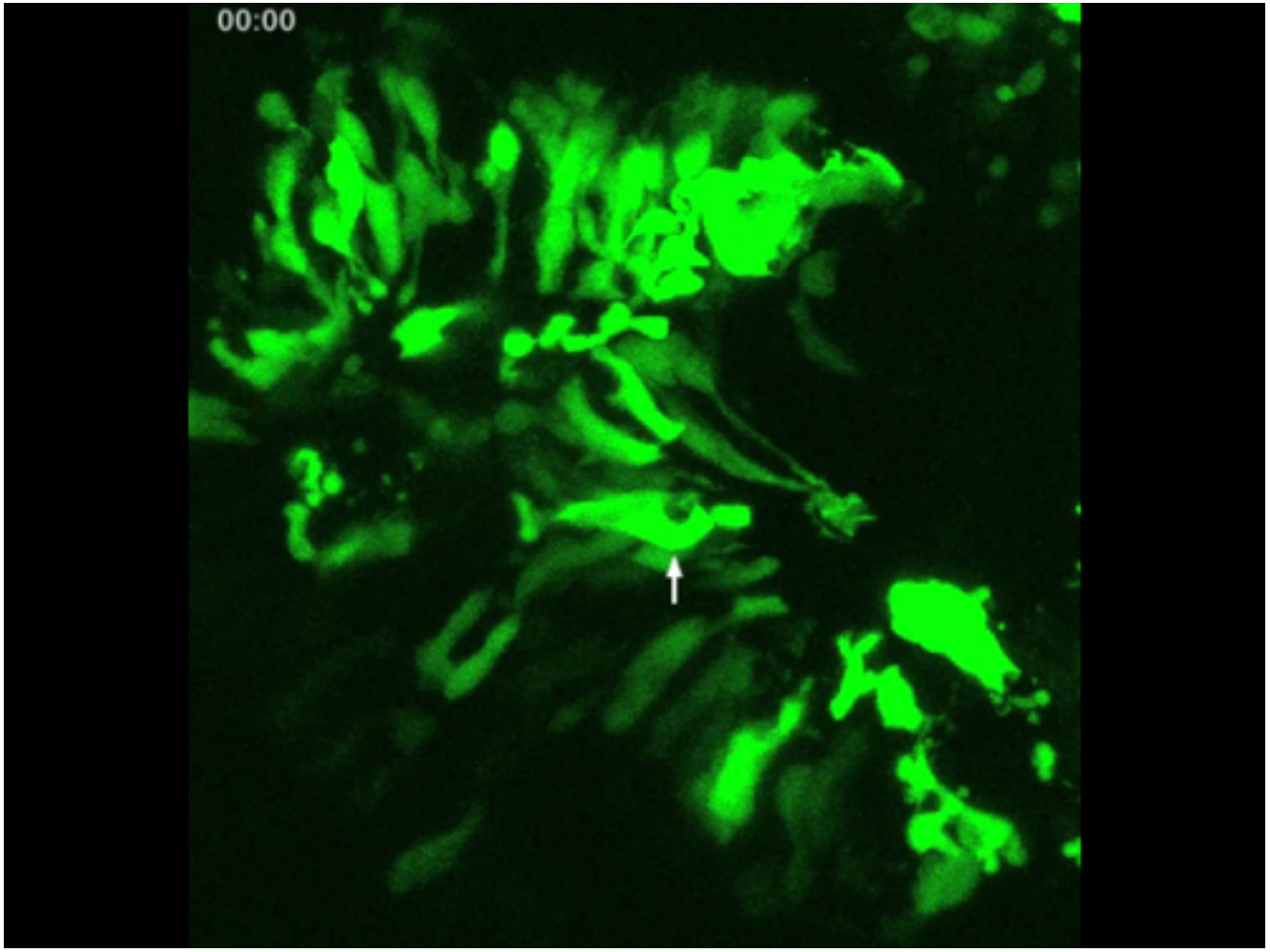
- Mitotic divisions occur at ventricular zone (VZ)
- Nuclei of cells in S phase located at the basal side of VZ

- Injection of GFP plasmid in to fluid-filled cavities of cerebral organoid
- electroporated the RG adjacent to the cavities
- Live imaging of GFP electroporated RGs

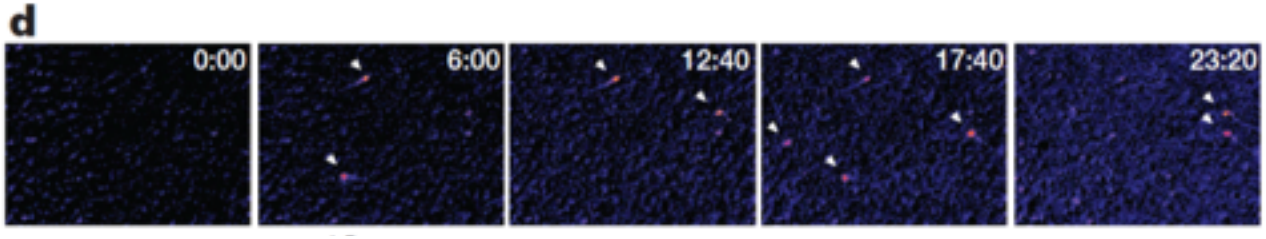




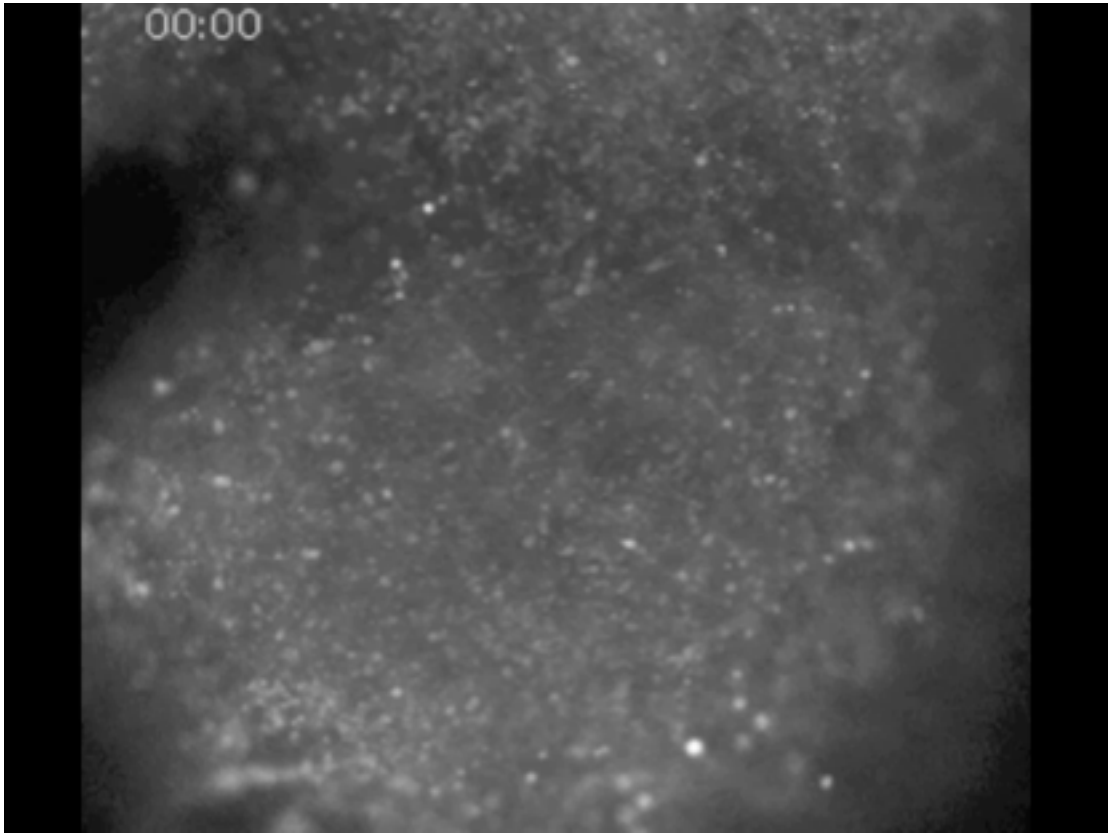
movement of nuclei along apical and basal processes of RG



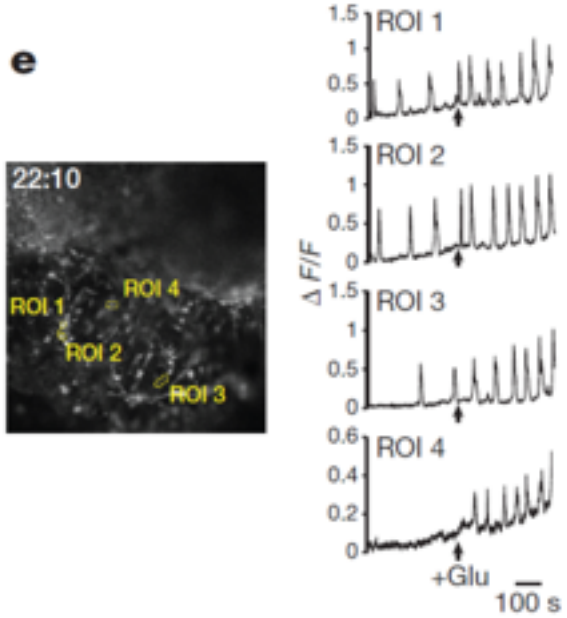
Neural activity within cerebral organoid



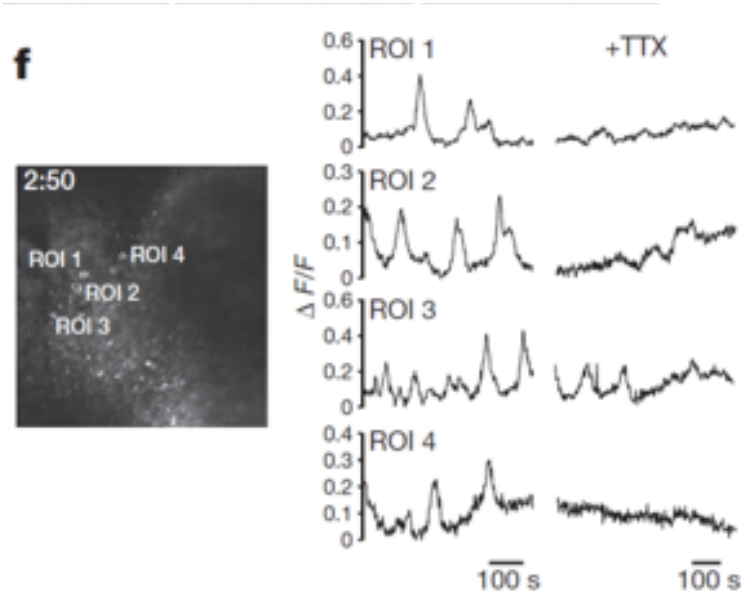
Fluo-4-Calcium live imaging shows calcium surges



Calcium spikes depend on neural activity

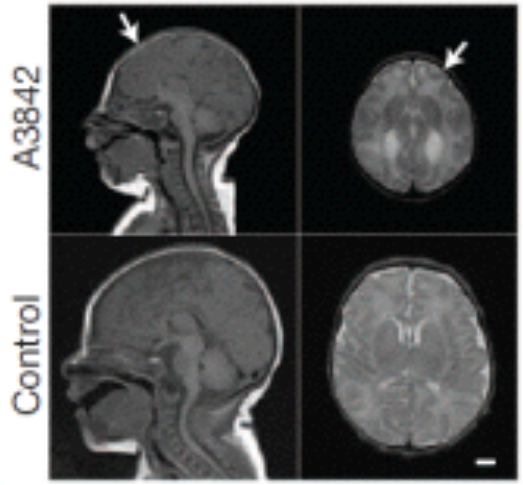


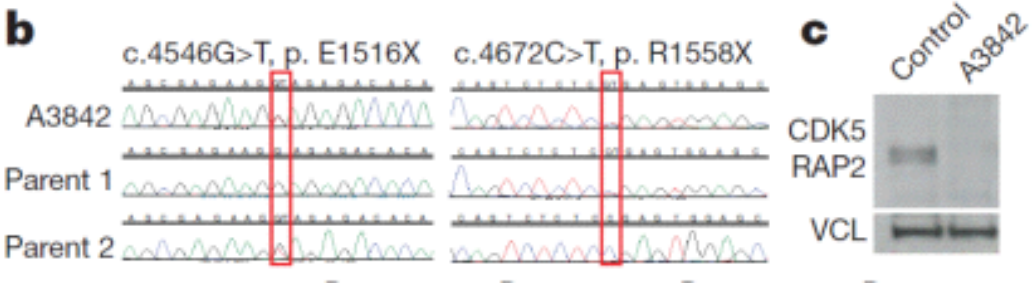
exogenous glutamate application: more frequent calcium spikes
—> glutamatergic receptor activity

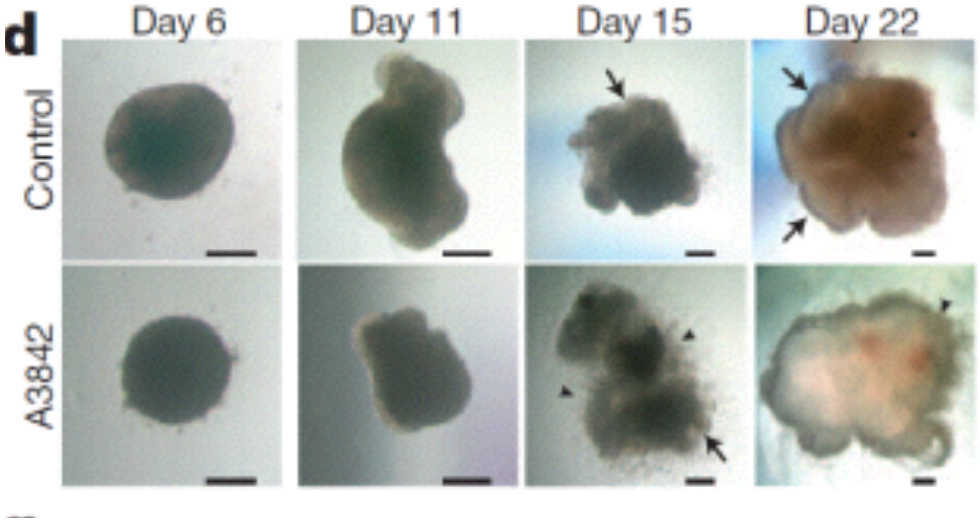


tetrodotoxin: blockade of signal

cerebral organoid modelling of microcephaly







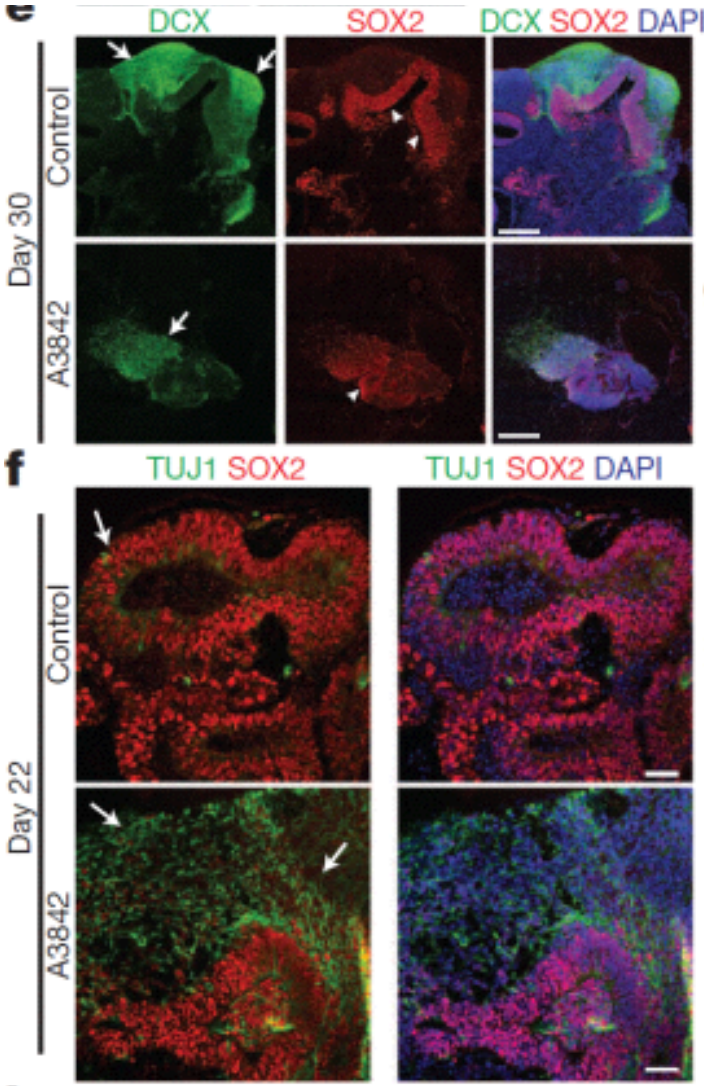
Patient iPSC:

skin fibroblast + Yamanaka factors

Pluripotent cells + cerebral organoid culture

smaller neuroepithelial tissue

cerebral organoid modelling of microcephaly



Patient-derived cerebral organoid:

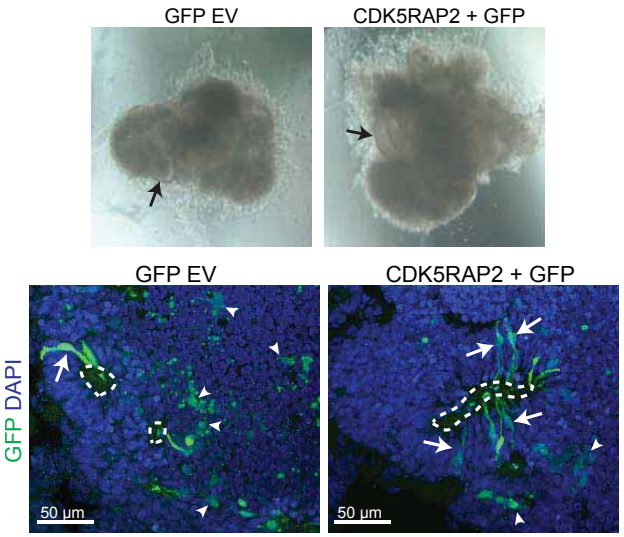
- **smaller neural tissues with few progenitors**

DCX: marker for neurons
SOX2: marker for progenitors

- **reduced RGs**
- **increased neurons**

——-> premature neural differentiation

Loss of CDK5RAP2 leads to premature neural differentiation at the expense of progenitors



<u>Over-expression of CDK5RAP2 in patient cerebral organoid:</u>

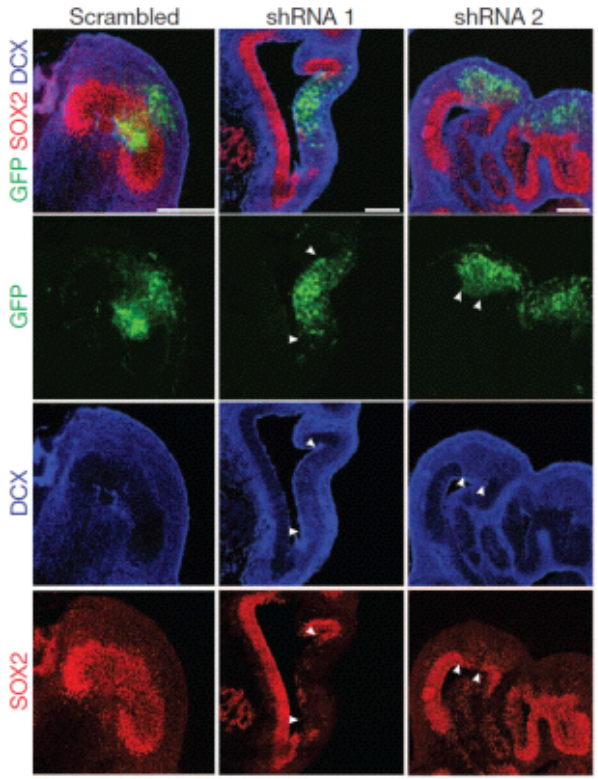
Larger neuroepithelium compared to control

increased number in cells with RG morphology and decreased in neurons

<u>Down-regulation of CDK5RAP2 in cerebral organoid:</u>

increased in neurons (DCX marker)

decreased of progenitors (SOX2 marker)



What is shown in this paper

1. A three dimensional culture system for driving brain tissue in vitro

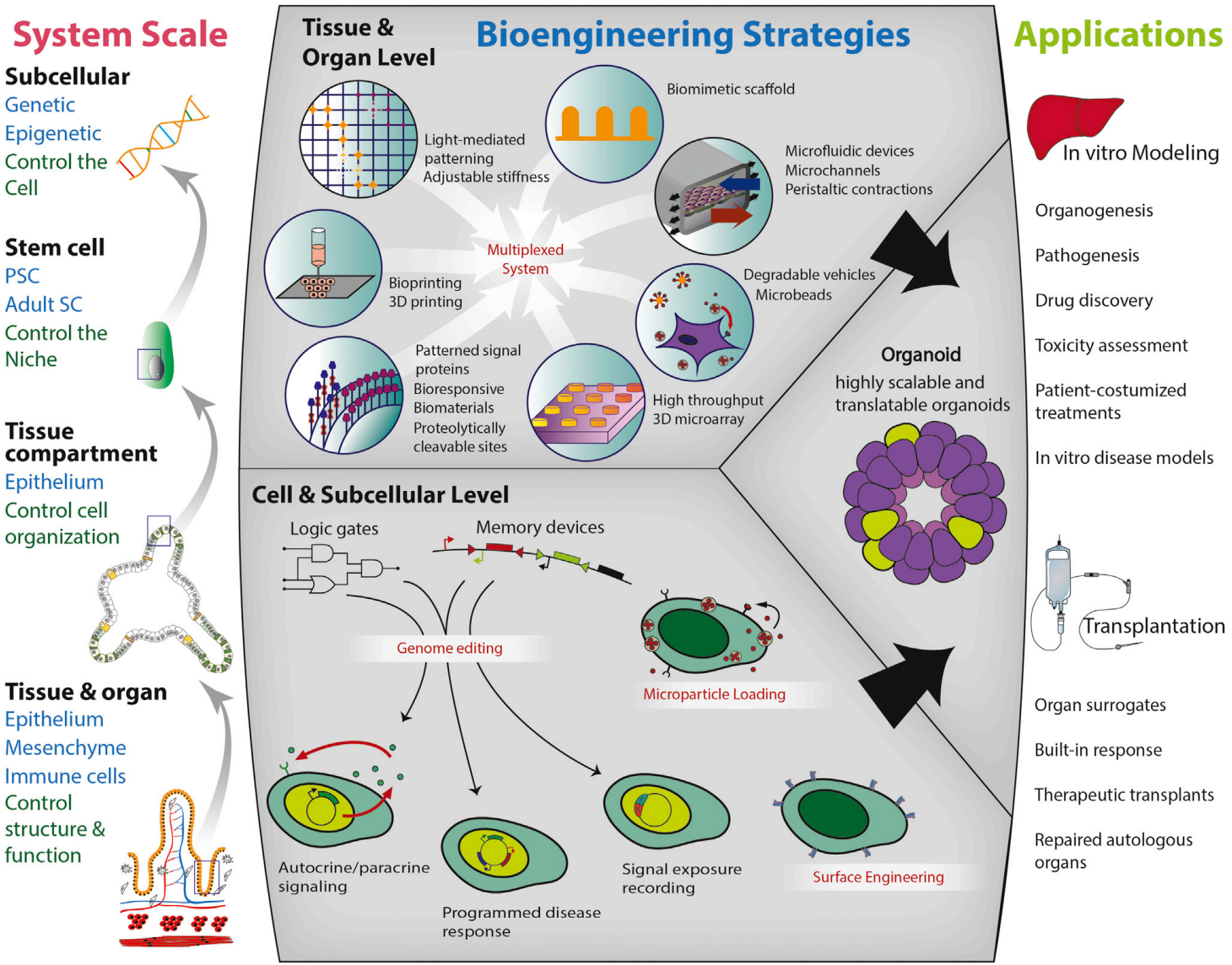
2. These organoids develop a variety of regional identities capable of influencing one other

3. Similar to human brain at early stages

4. Cortical neurons mature and form pyramidal identities

5. Patient derived iPS models the CDK5RAP2-dependent pathogenesis of microcephaly

Achievements using organoids as model systems



Limitations of organoid systems

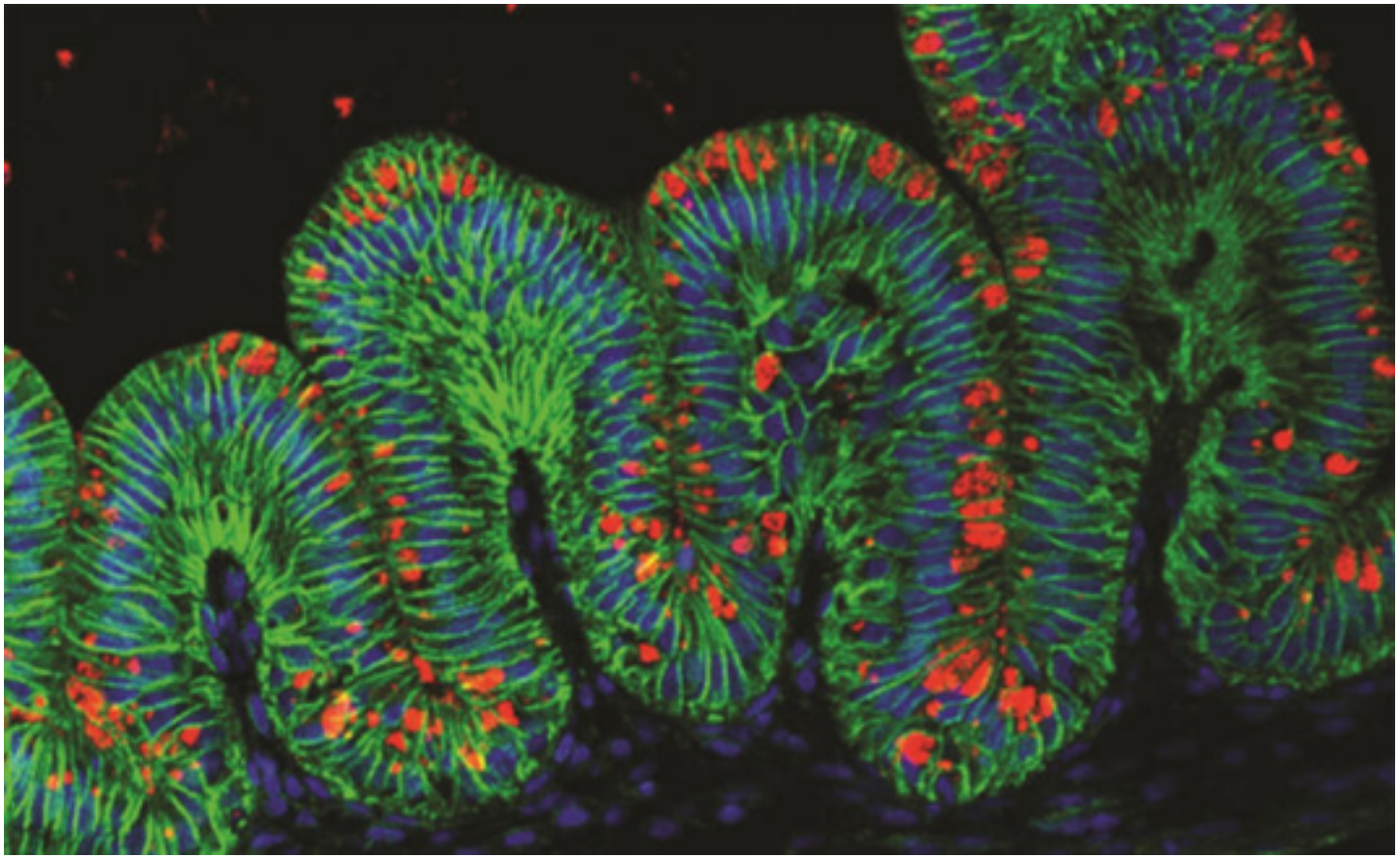
1. Unclear in what extent they recapitulate in vivo development
    - retinal organoids nicely display typical laminar organization
    - outer segments fail to form
    - photoreceptors fail to fully mature to become light-sensitive

2. Maturation level of organoids
    - Cerebral organoids recapitulate fairly early events in brain development
    - later features such as cortical plate layers, fail to fully form

3. lack of vascularization
    - co-culture with endothelial cells
    - transplantation of these tissues stimulates invasion from host vasculature

4. limited growth potential, which can also affect their maturation
    - spinning bioreactors can provide better nutrient exchange

Thanks for your attention



Stomach organoid- McCracken *et al*, Nature, 2014