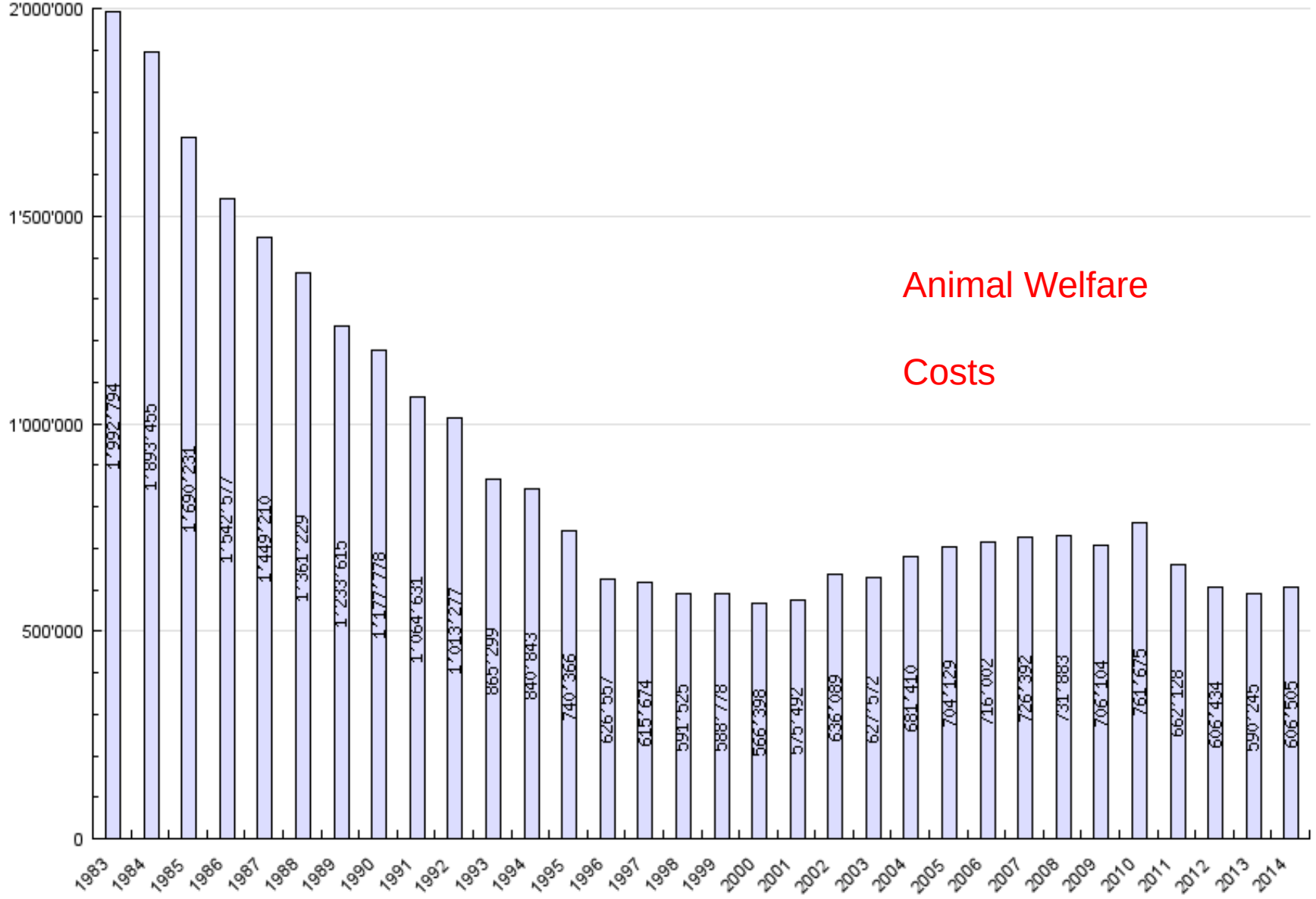
Successful replacement of animal models: cell-based assays

Special series on Laboratory animal science

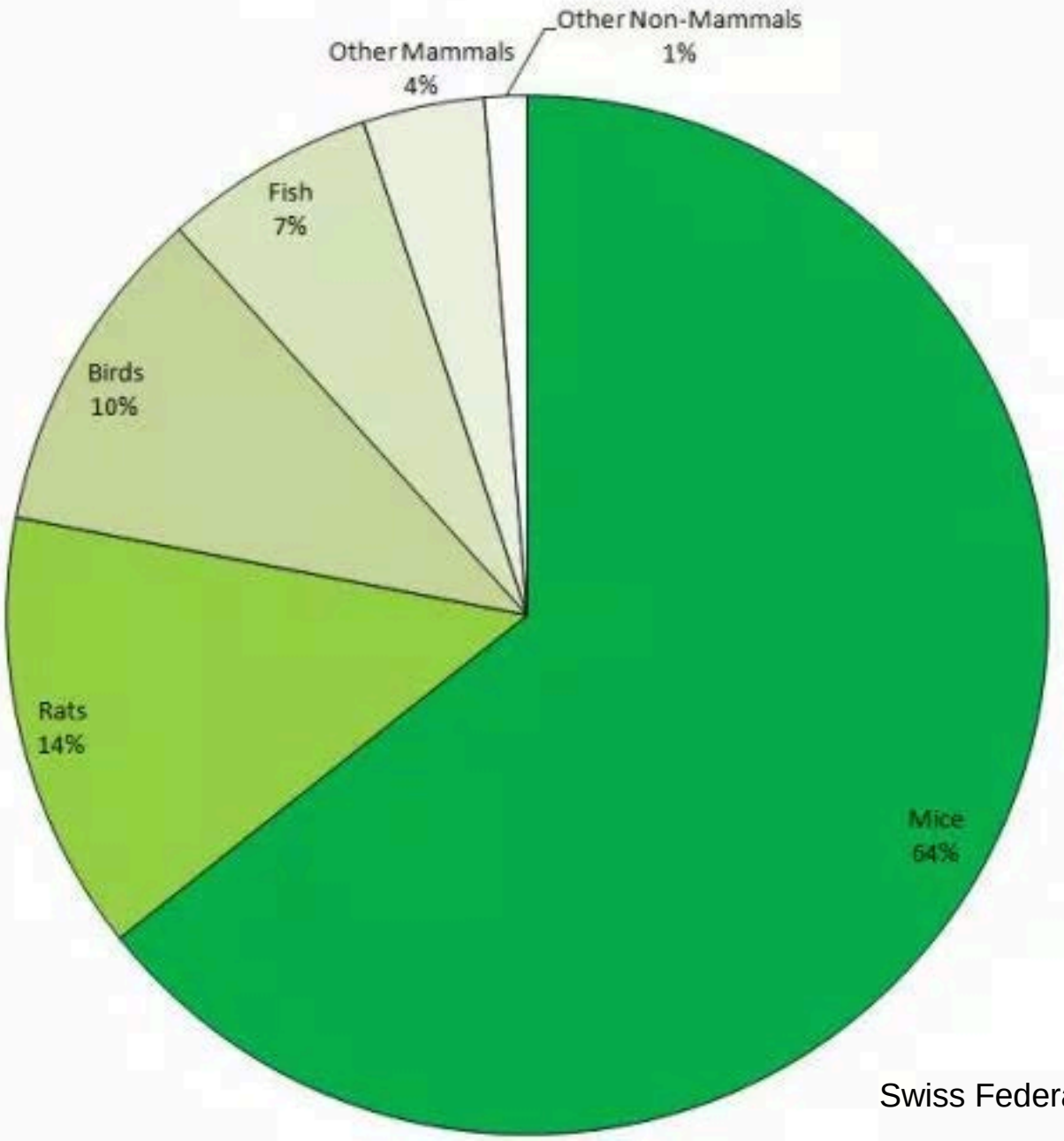
Asvin Lakkaraju

Animal Experimentation in Switzerland



Swiss Federal Statistical Office

Animals used in research in Switzerland in 2014



Swiss Federal Statistical Office

3Rs

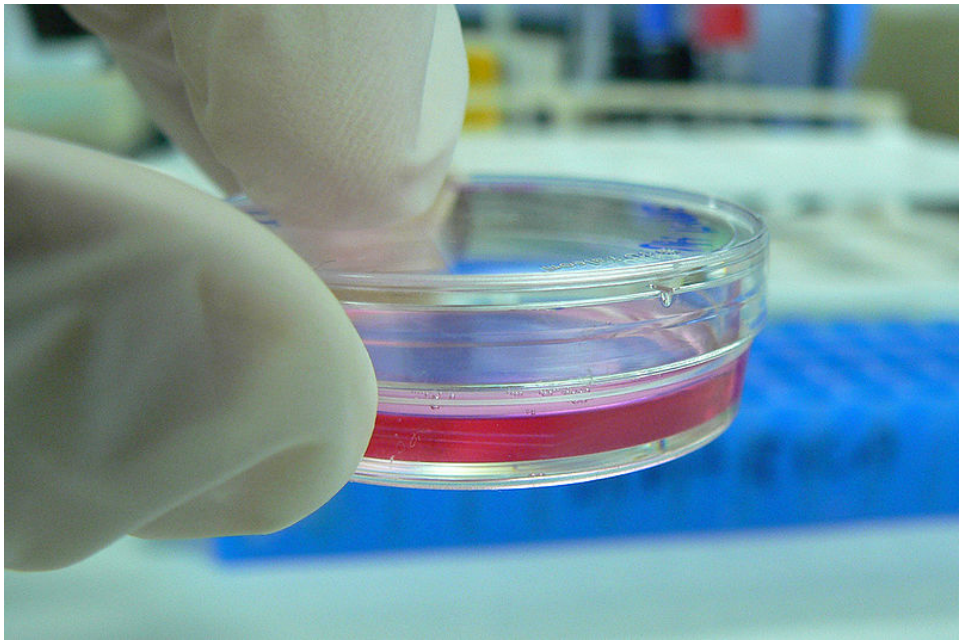
There is a 4 R: Responsibility.

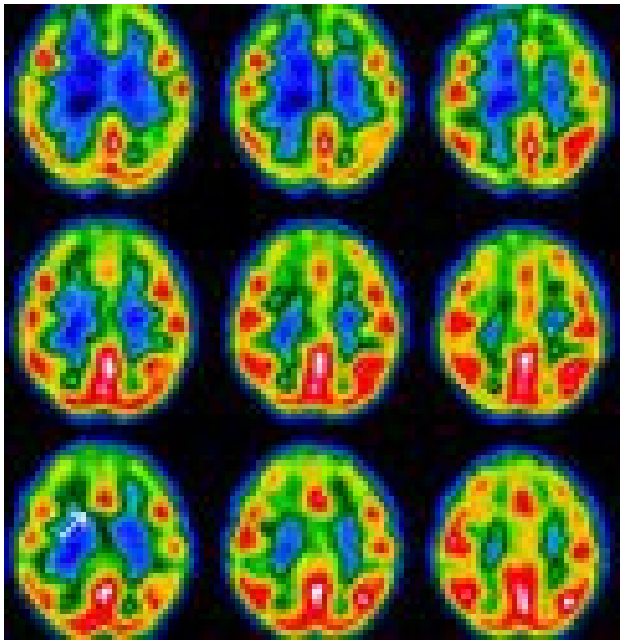
Have a clear hypothesis and plan experiment responsibly

Alternatives

Scientists use many ways to try to replace animals used in research. These include using cell cultures, computer modelling and human studies.

Researchers **must,** by law, use these techniques if they would be as effective as using animals.





Absolutely No Animal Research - Why?

1. **Animals have rights!**
2. Animals surely deserve to live their lives free from suffering and exploitation.
3. Animals are not ours to:
   - eat
   - wear
   - experiment on
   - use for entertainment
   - abuse

People for the Ethical Treatment of Animals

http://www.peta.org/

Benefits as a Result of Animal Research

- *Since the early 1900's life expectancies have increased from 47 to about 77.*
- *Economic Impact – the medical industry alone recognized about a 57 trillion dollar savings in the 70's and 80's*
- *Drug treatments, vaccines and surgical procedures have been developed to improve the quality of life for animals*

But, this is an ongoing debate that we're not here to engage!

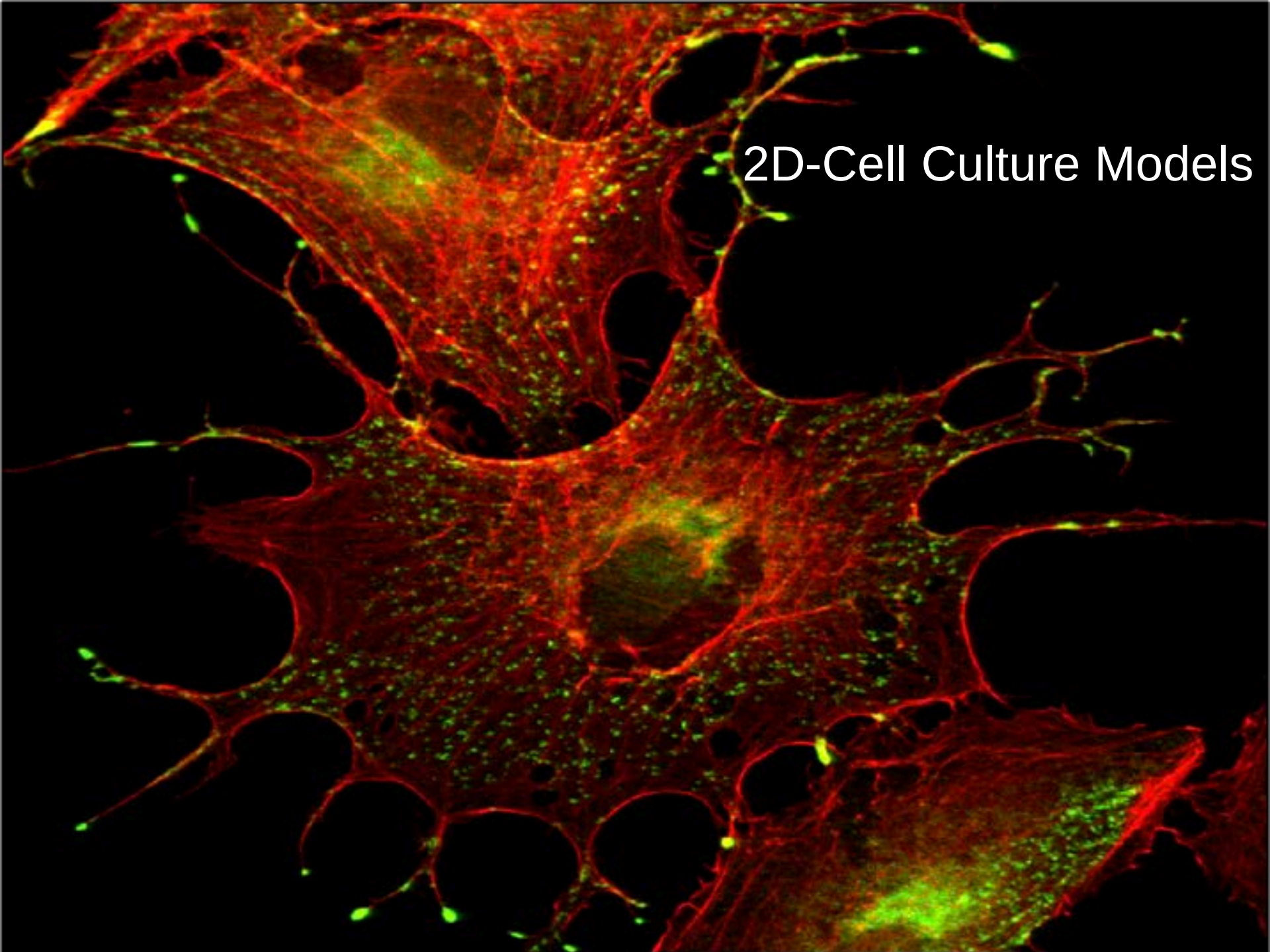
The fact is animal research does occur and we need to know our responsibilities

Complement animal experimentation with other alternatives

Alternatives to Animal Research

Experimental Systems:

1) 2D Cell Culture
2) 3D Cell Culture
3) Organ on Chip
4) Stem Cell Research *X*
5) Computer modeling *X*


2D-Cell Culture Models

Cell Culture Systems do not answer all the questions.

Example: Prion Protein: No toxicity in Cell Lines

But they do offer solutions to understand certain mechanistic events: Simple models.

Examples: signaling cascades, Cell migration…..

Goal is to reduce number of animals by taking advantage of alternate methods!!

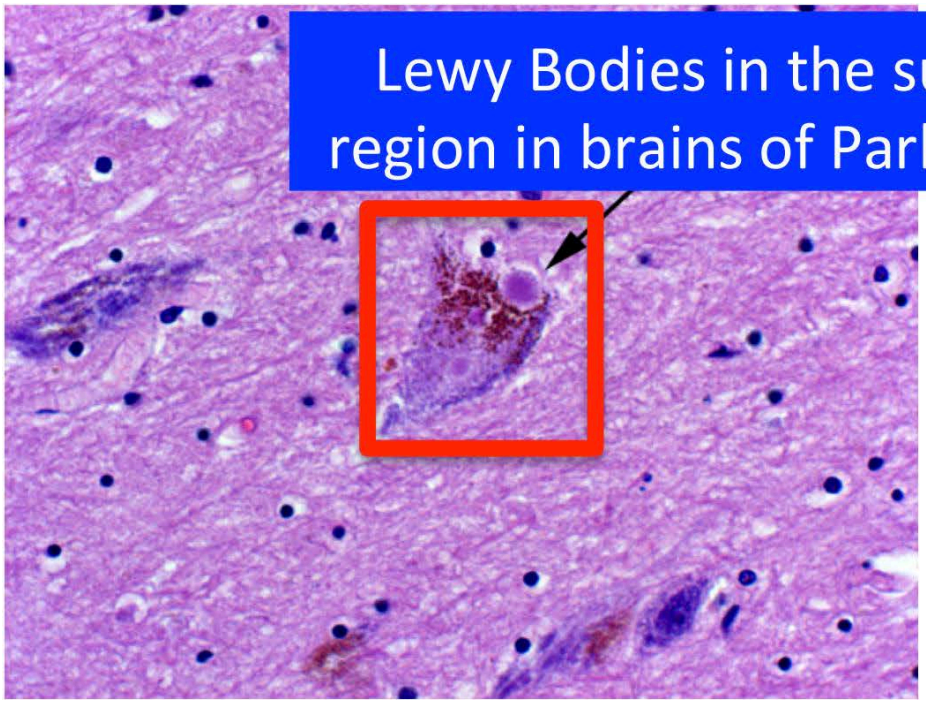
2D Cell Culture

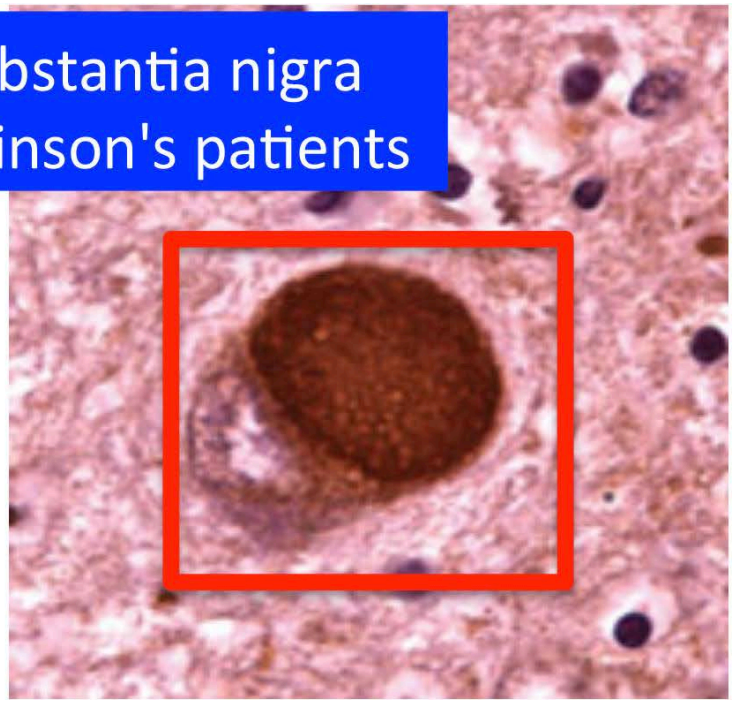
Exogenous α-synuclein fibrils seed the formation of Lewy body-like intracellular inclusions in cultured cells

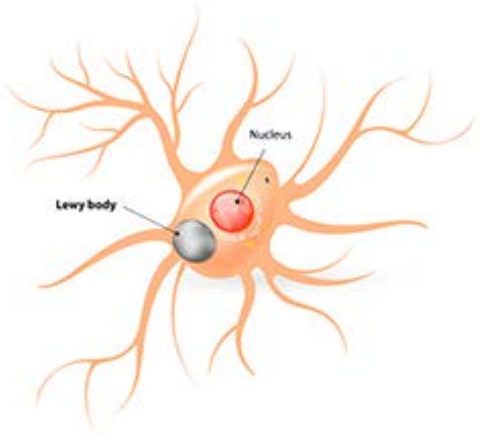
Kelvin C. Luk, Cheng Song, Patrick O'Brien, Anna Stieber, Jonathan R. Branch, Kurt R. Brunden, John Q. Trojanowski, and Virginia M.-Y. Lee[1]

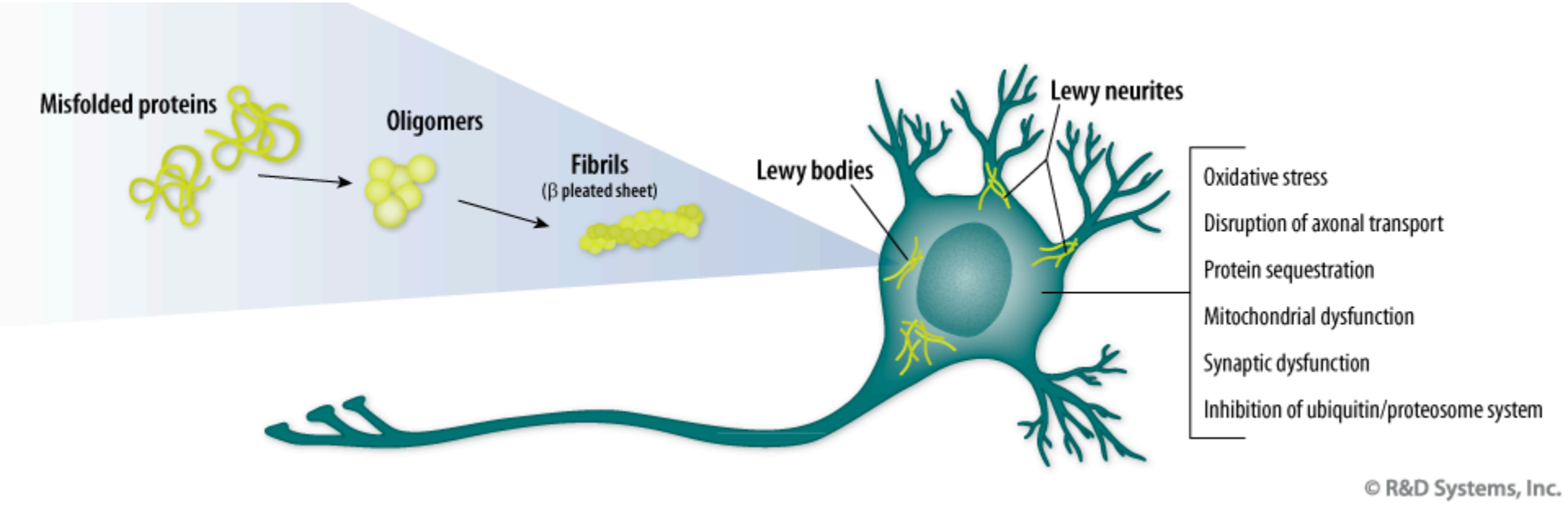
Center for Neurodegenerative Disease Research, Institute on Aging, Department of Pathology and Laboratory Medicine, University of Pennsylvania School of Medicine, Philadelphia, PA 19104-4283

Lewy Bodies in the substantia nigra region in brains of Parkinson's patients



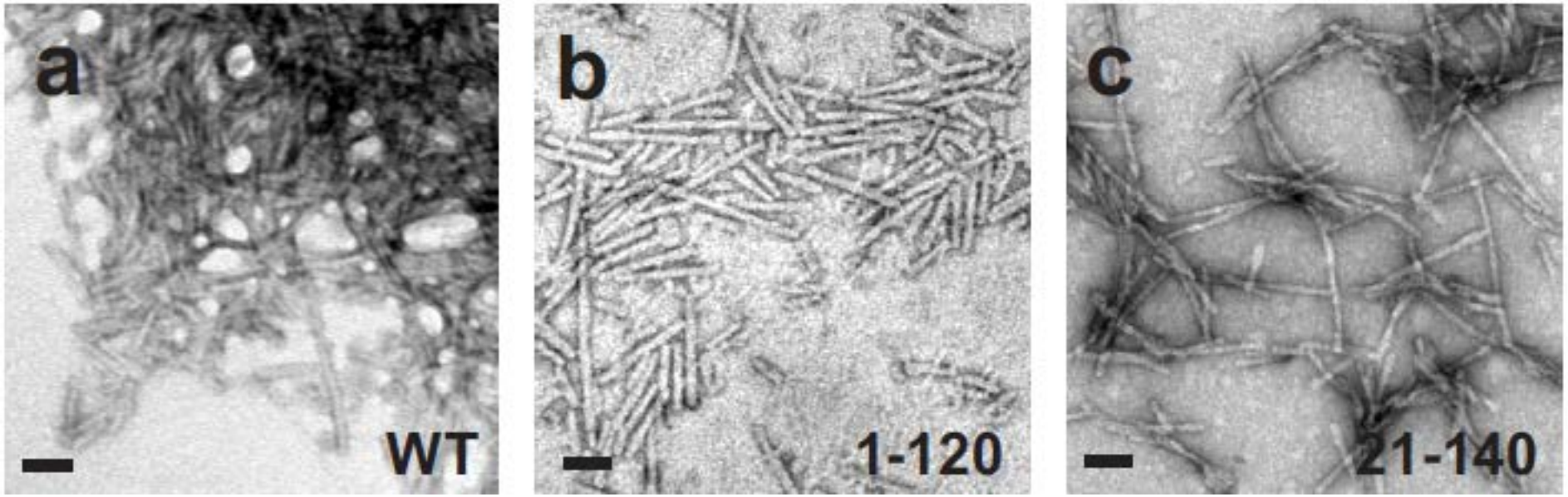






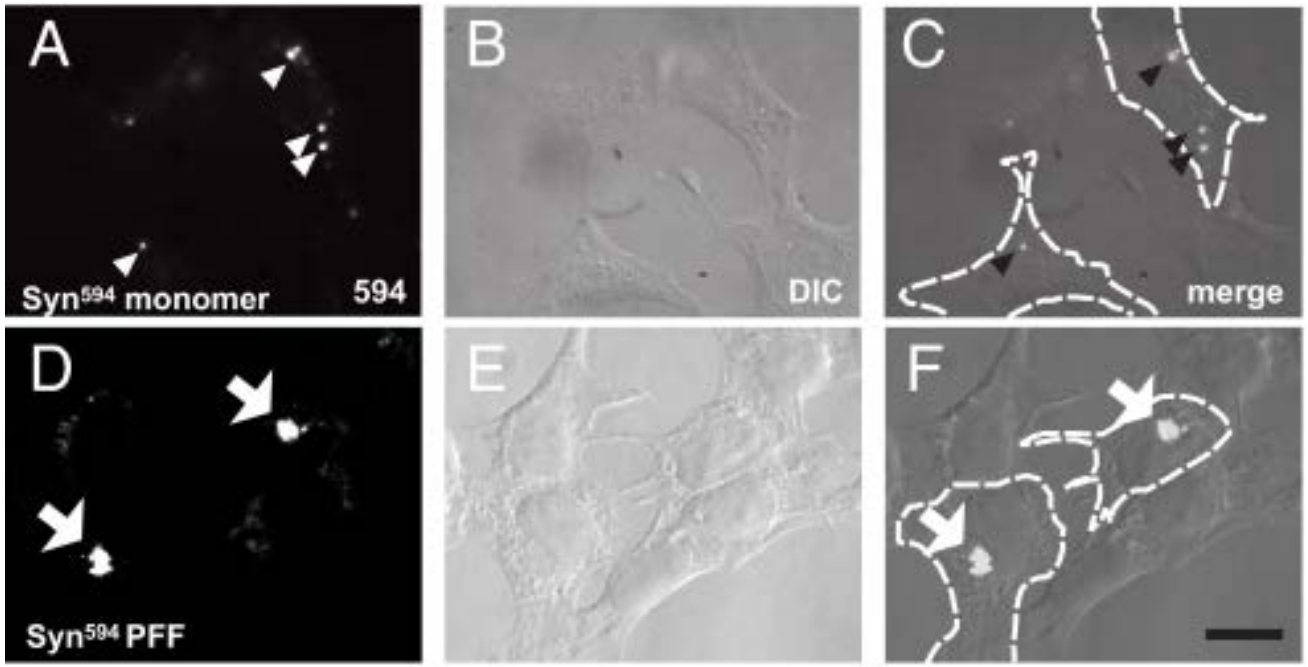
© R&D Systems, Inc.

Synuclein can form Fibrils *In Vitro*



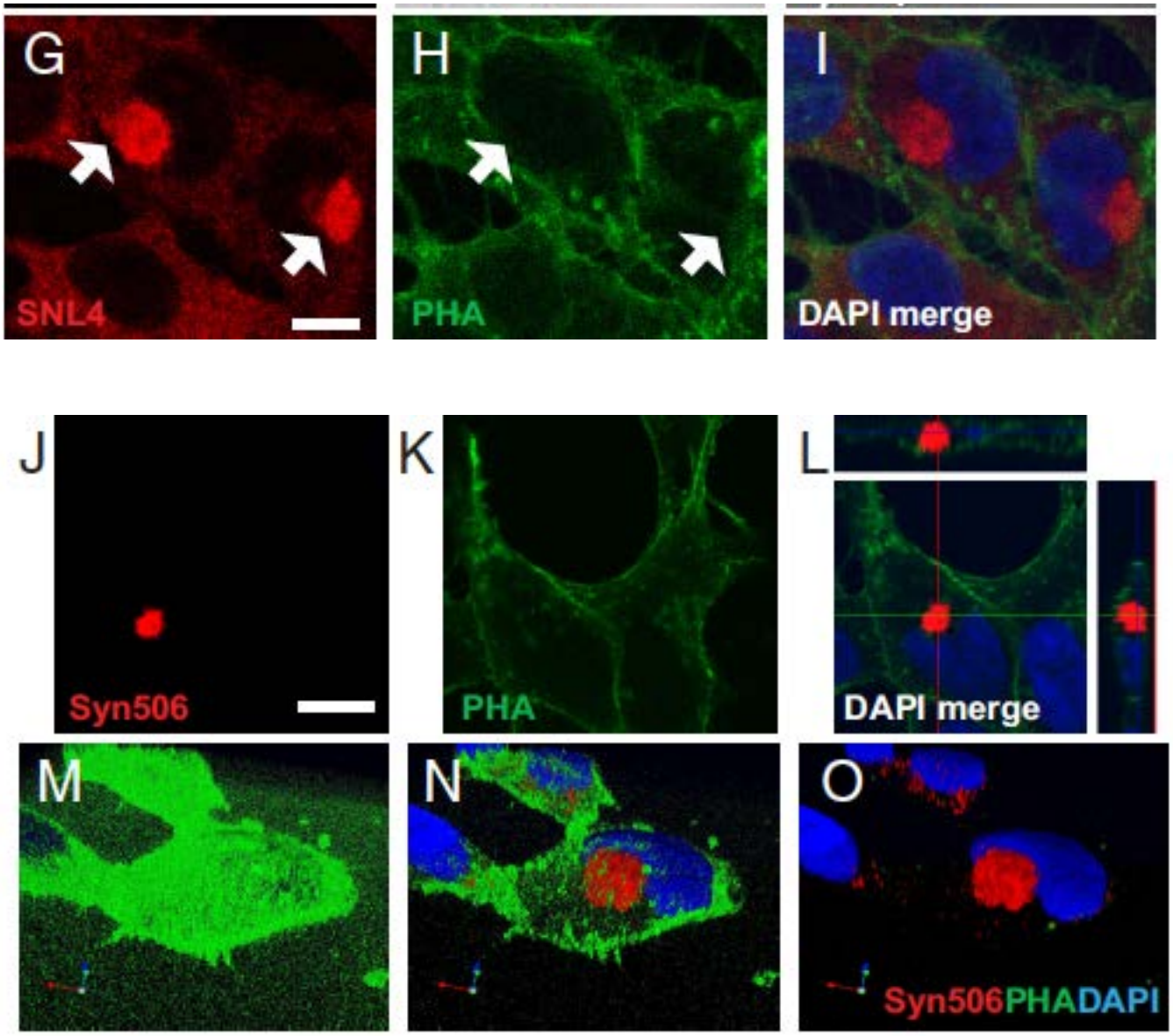
EM Images of recombinant Syn proteins

Synuclein Firbrils induce aggregates in Cells

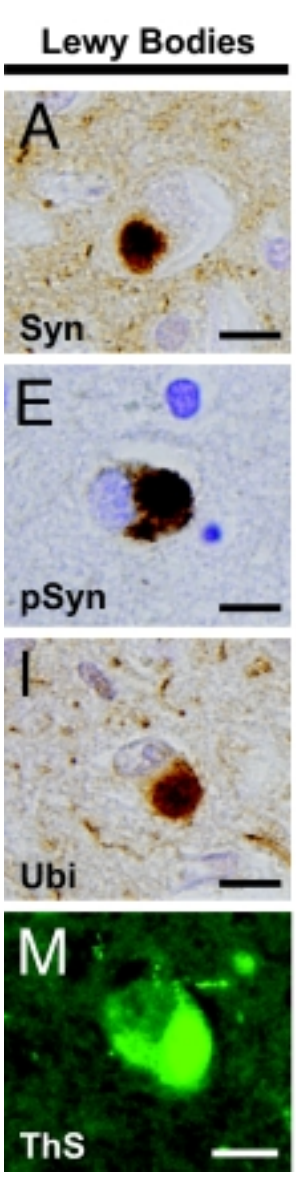


Cells were transduced using special lipophilic compounds

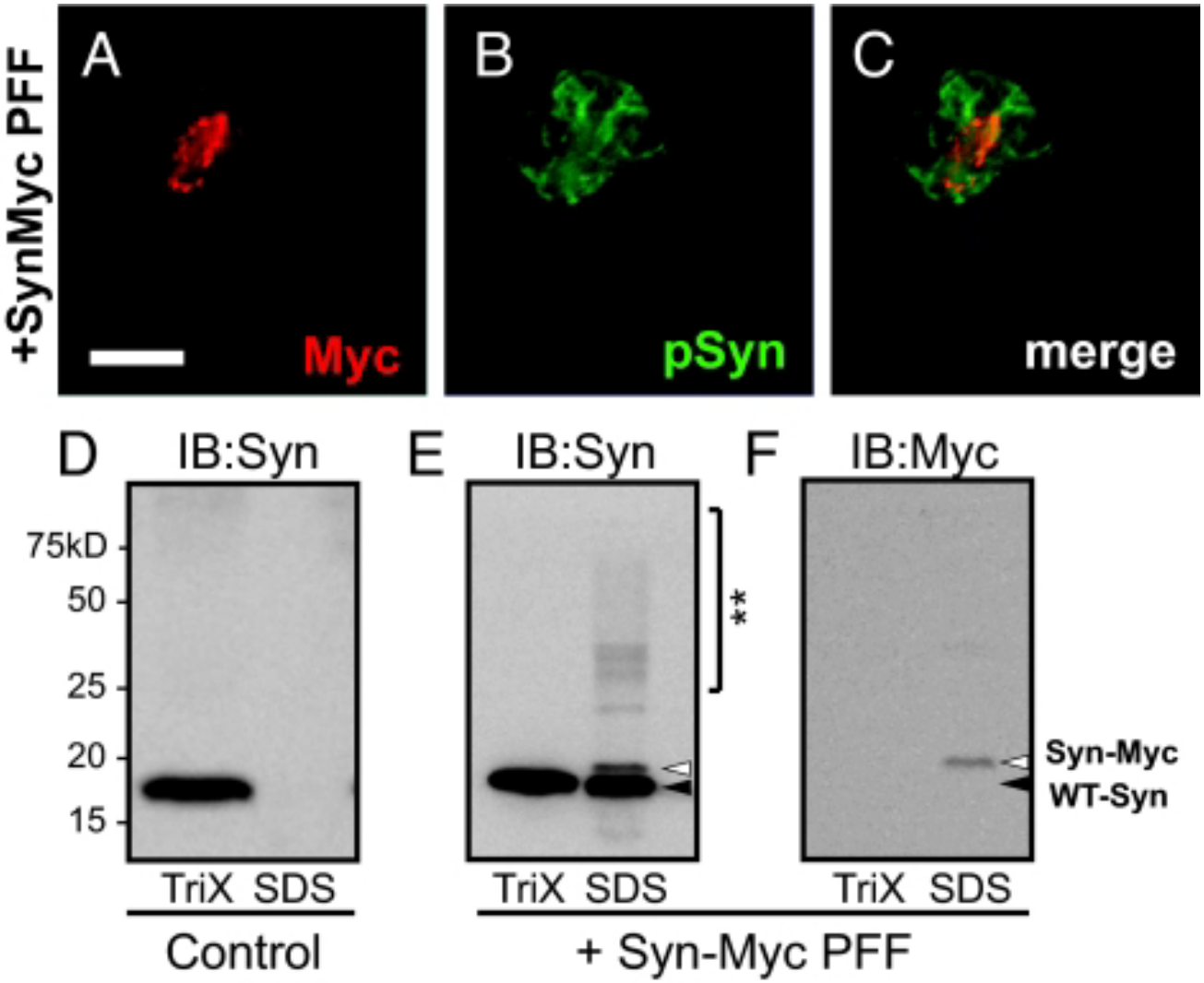
Synuclein Inclusions are inside the Cells



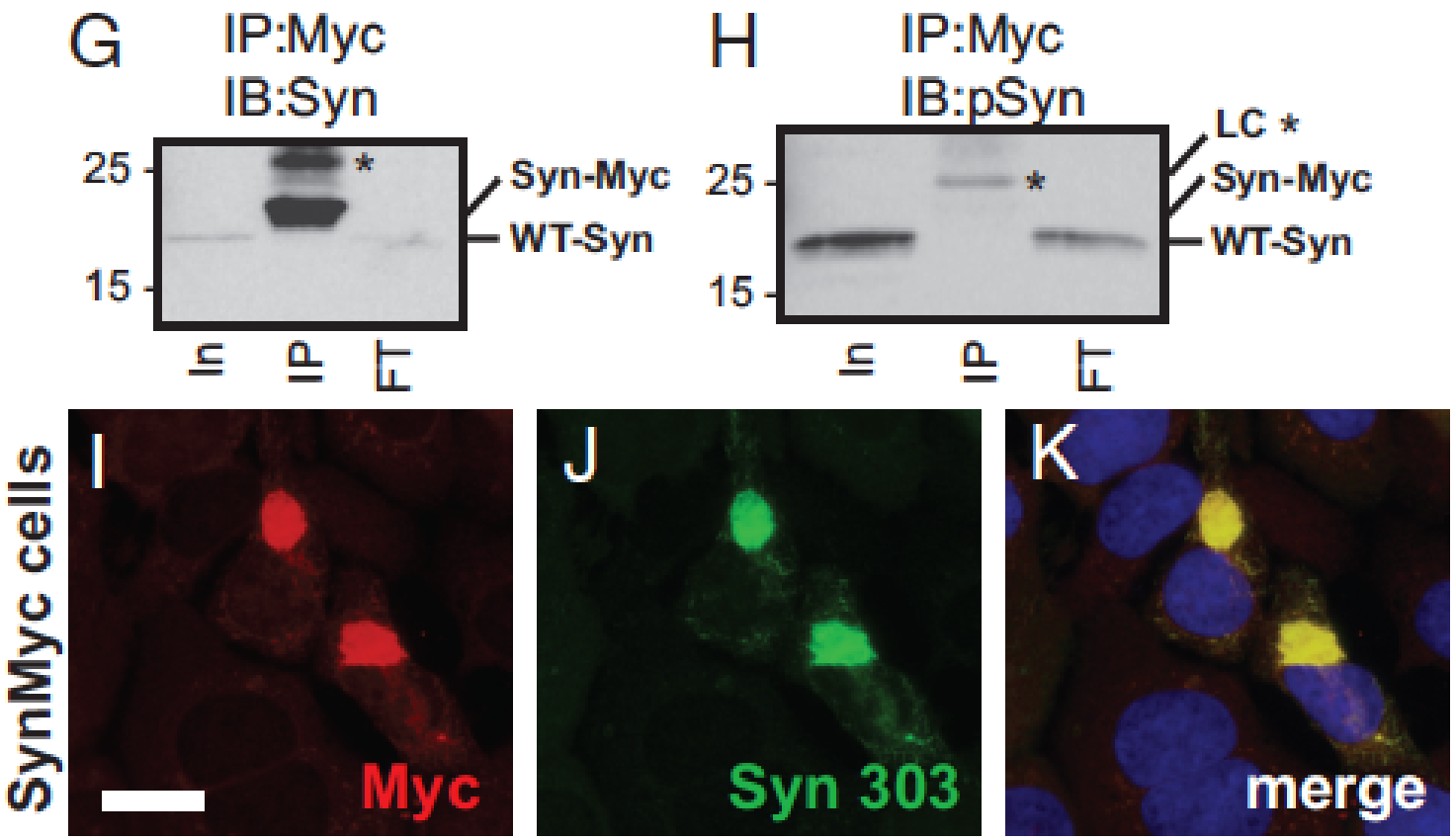
Synuclein inclusions resemble Lewy bodies



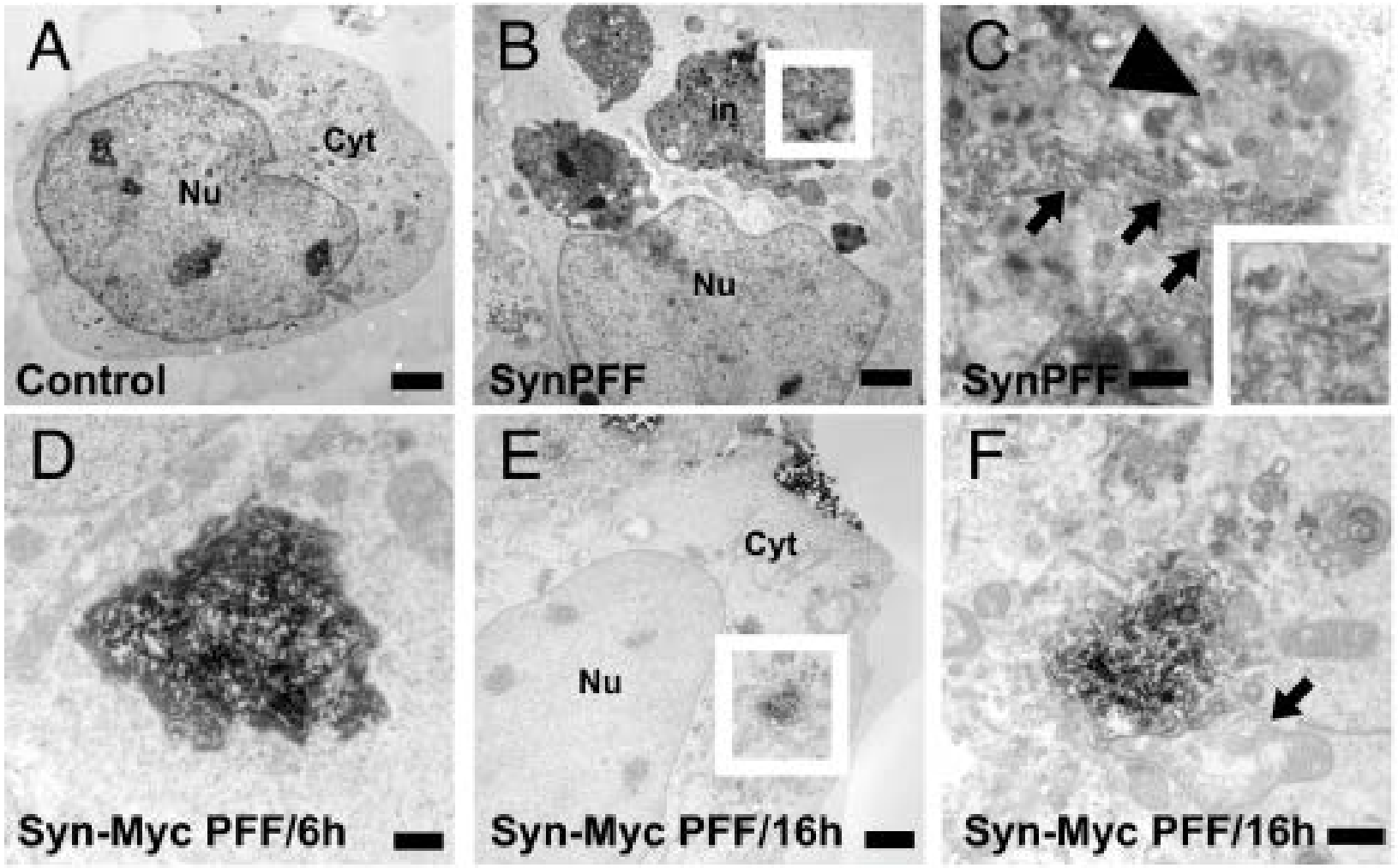
Endogenous Syn is modified



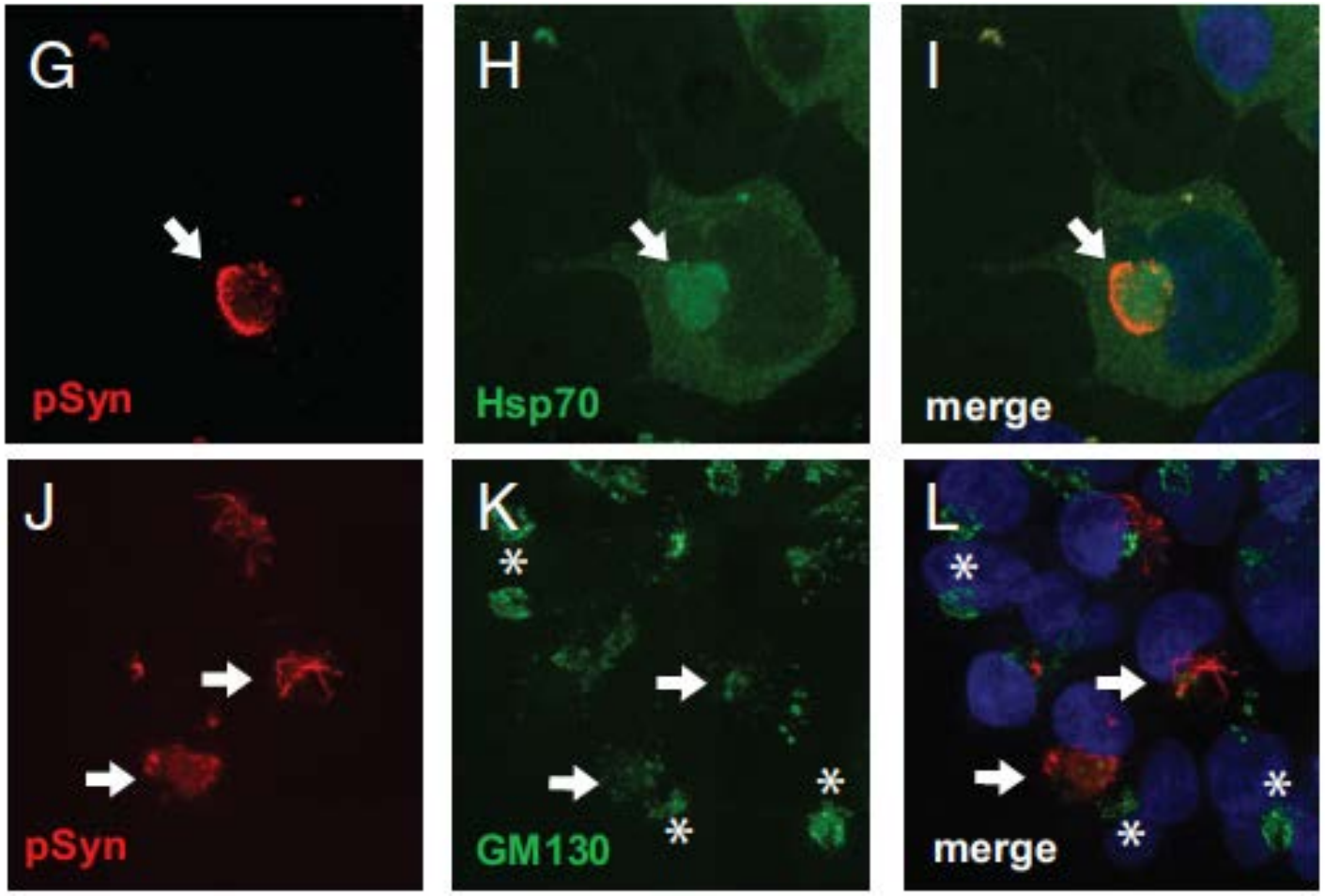
Inclusions contain majority of endogenous Syn



EM images of Inclusions



Inclusions are associated with chaperones



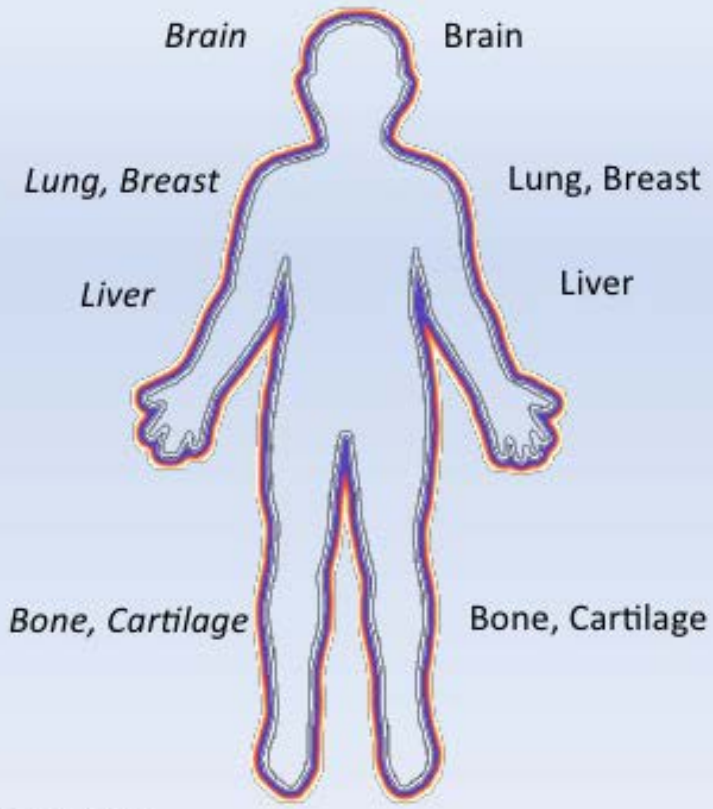
This is also observed with Lewy bodies in patients

Summary

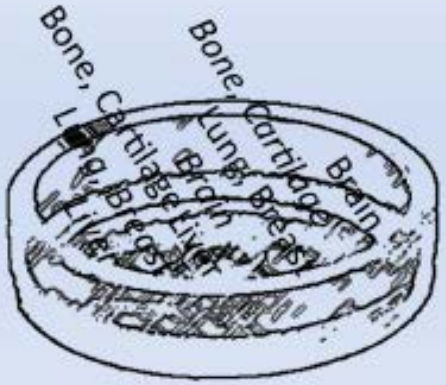
A cell culture model recapitulated Lewy body like structures.

Model system can be used to study mechanistic details as
To how inclusions are formed: CRISPR technology

2D Does not Mimic *in vivo* Geometry



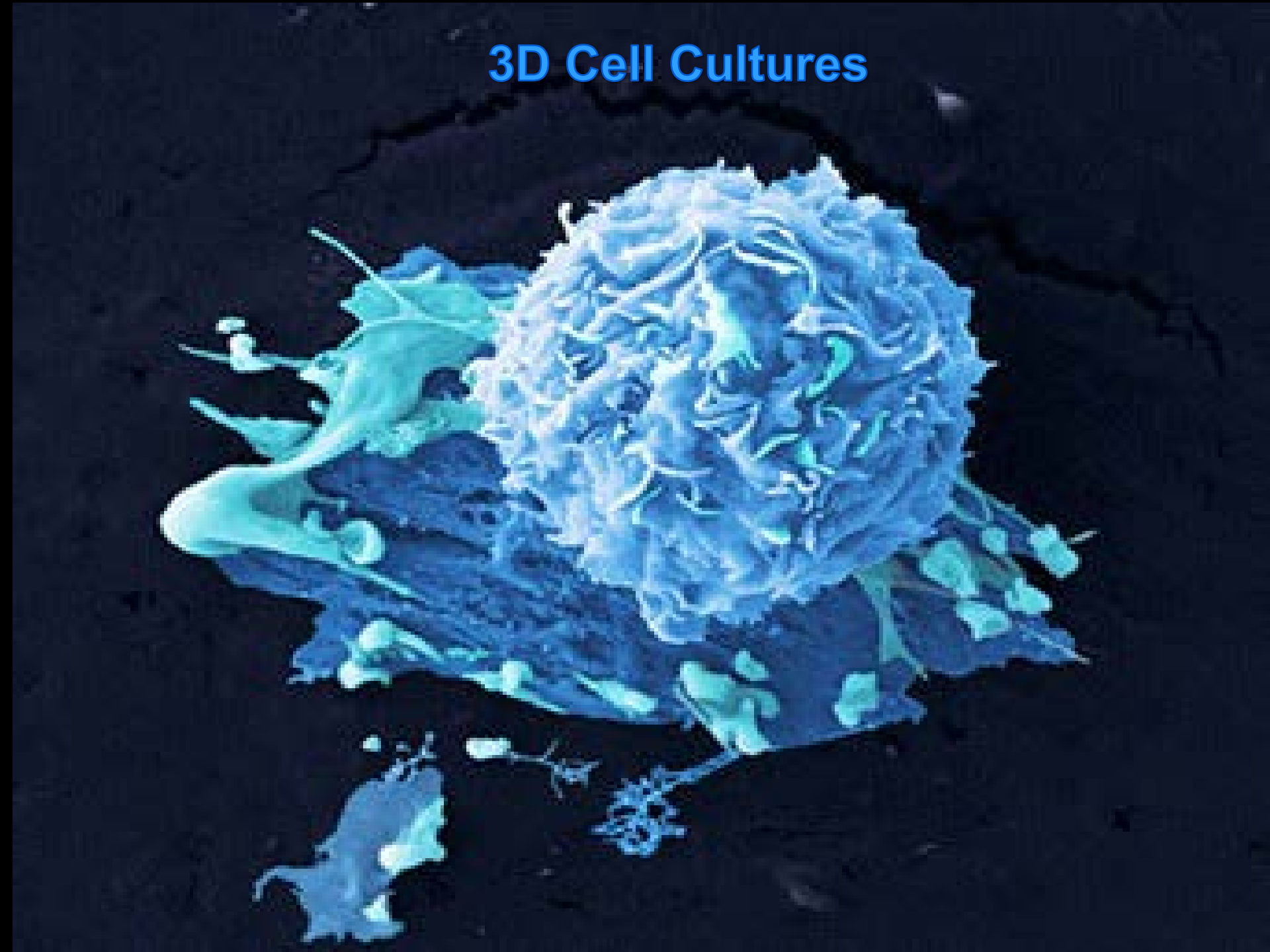
≠

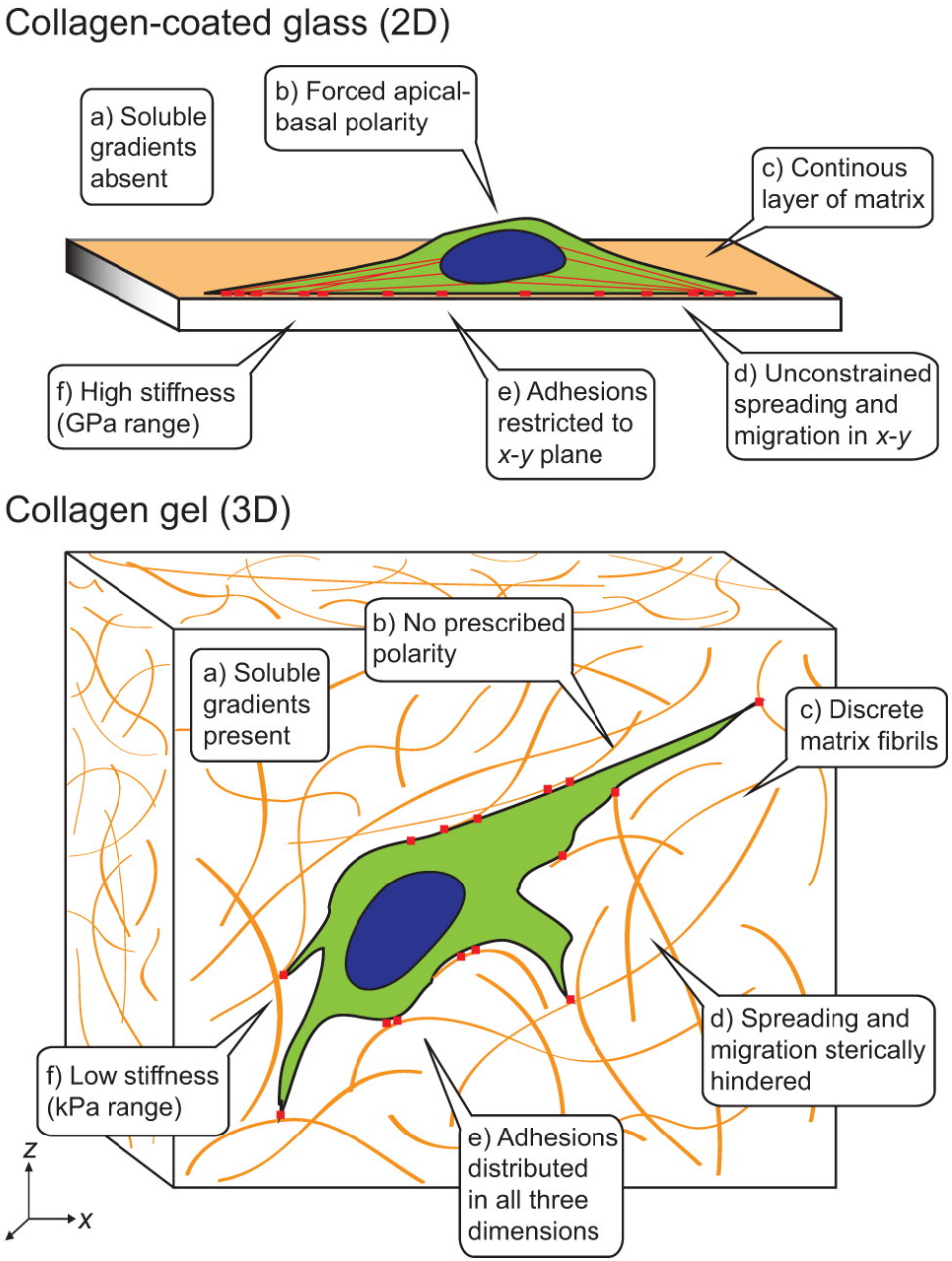


Petri Dish

© 3D BIOTEK, LLC

3D Cell Cultures





Disadvantages of 2D

Altered Cell Shape.

Altered functions/ signaling

Loss of polarity

Advantages of 3D Culture

Growing cells in 3D alters cell proliferation and morphology.

Growing cells in 3D reveals a more realistic drug response.

Growing cells in 3D captures phenotypic heterogeneity.

Growing cells in 3D changes gene expression and cell behavior.

Growing cells in 3D mimics the tumor microenvironment.

3D cell culture movie

PROTOCOL

A 3D human neural cell culture system for modeling Alzheimer's disease

Young Hye Kim[1,2,4], Se Hoon Choi[1,4], Carla D'Avanzo[1,4], Matthias Hebisch[1,3], Christopher Sliwinski[1], Enjana Bylykbashi[1], Kevin J Washicosky[1], Justin B Klee[1], Oliver Brüstle[3], Rudolph E Tanzi[1] & Doo Yeon Kim[1]

LETTER

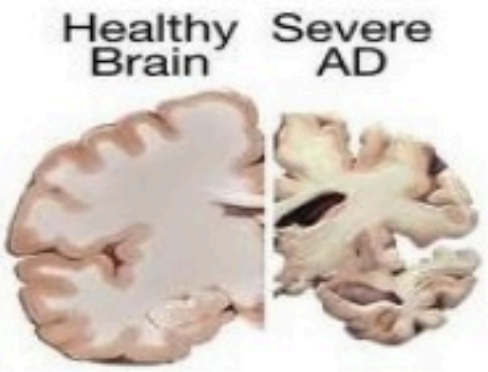
doi:10.1038/nature13800

A three-dimensional human neural cell culture model of Alzheimer's disease

Se Hoon Choi[1]*, Young Hye Kim[1,2]*, Matthias Hebisch[1,3], Christopher Sliwinski[1], Seungkyu Lee[4], Carla D'Avanzo[1], Hechao Chen[1], Basavaraj Hooli[1], Caroline Asselin[1], Julien Muffat[5], Justin B. Klee[1], Can Zhang[1], Brian J. Wainger[4], Michael Peitz[3], Dora M. Kovacs[1], Clifford J. Woolf[4], Steven L. Wagner[6], Rudolph E. Tanzi[1] & Doo Yeon Kim[1]

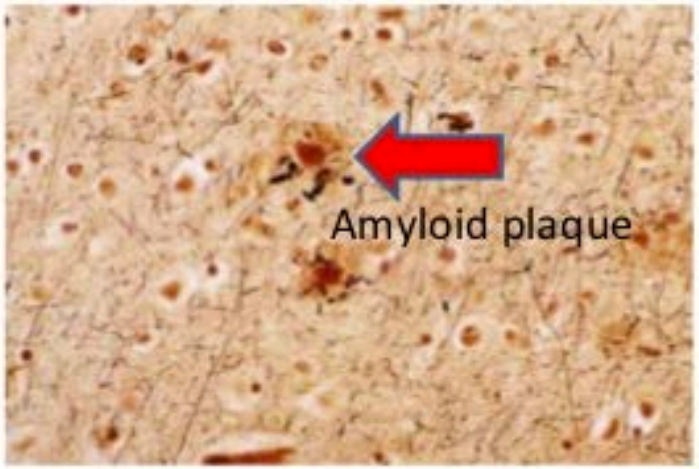
What's Alzheimer's Disease(AD)?

- AD is an illness of the brain. It causes large numbers of nerve cells in the brain to die.
- AD is a progressive, irreversible brain disease that destroys memory and thinking skills.
- Most common cause of dementia in adults



Pathology

- Amyloid plaques,
- Neurofibrillary tangles
- Neuron and Synapse loss
- Neuronal cell death



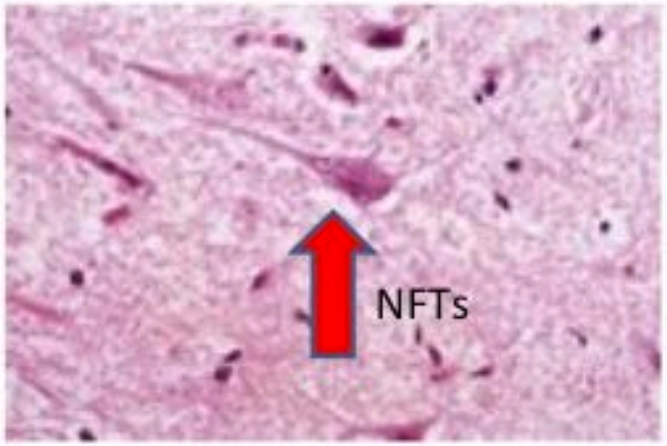
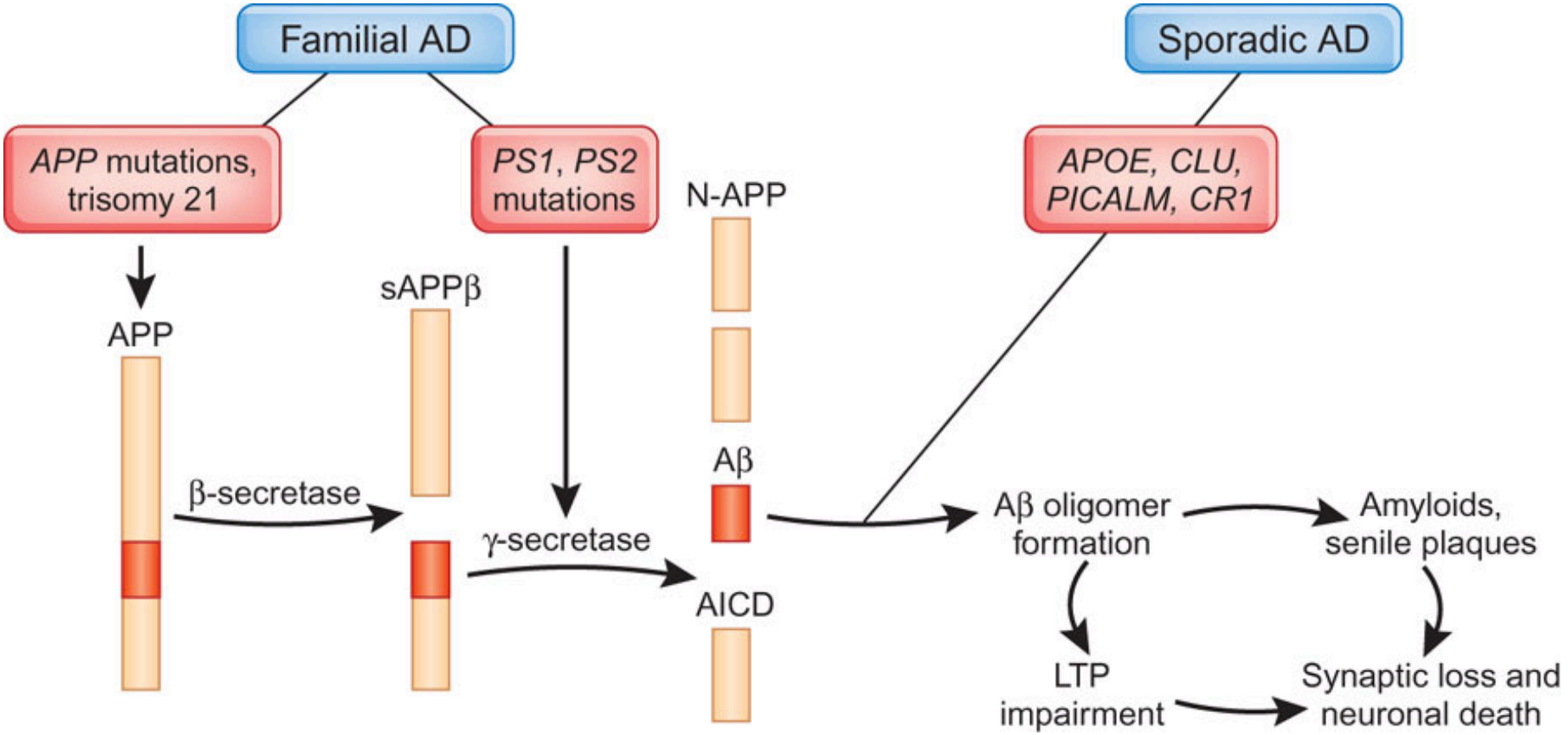


Table 1 - Neuropathological features of the main transgenic mouse models of Alzheimer disease.

Mouse model	Gene (mutation)	Intraneuronal Aβ	Parenchymal Aβ plaques	Hyperphos-phorylated Tau	Neurofi-brillary tangles	Neuronal loss	Synaptic loss	CAA	Primary reference
PDAPP	APP (V717F)	-	Yes	Yes	No	No	Yes	-	Games et al. 1995
Tg2576	APP (K670N/M671L)	Yes	Yes	-	-	No	No	-	Hsiao et al. 1996
TgCRND8	APP (K670N/M671L, V717F)	-	Yes	-	No	No	-	-	Chishti et al. 2001
APP/PS1	APP (K670N/M671L), PS1 (M146L)	-	Yes	-	-	-	-	-	Holcomb et al. 1998
APP23	APP (K670N/M671L)	-	Yes	Yes	No	Little	Yes	Yes	Sturchler-Pierrat et al. 1997
Tg-SwDI	APP (E693Q, D694N)	-	Yes	-	-	-	-	Yes	Davis et al. 2004
APPDutch	APP (E693Q)	-	Little	-	-	-	-	Yes	Herzig et al. 2004
APPDutch/PS1	APP (E693Q), PS1 (G384A)	-	Yes	-	-	-	-	Little	Herzig et al. 2004
hAPP-Arc	APP (E693G, K670N/M671L, V717F)	-	Yes	-	-	-	-	Little	Cheng et al. 2004
Tg-ArcSwe	APP (E693G, K670N/M671L)	Yes	Yes	-	-	-	-	Yes	Lord et al. 2006 Knobloch et al. 2007
APPArc	APP (E693G)	-	Yes	-	-	-	-	Yes	Rönnbäck et al. 2011
TAPP	APP (K670N/M671L), Tau (P301L)	-	Yes	-	Yes	-	-	-	Lewis et al. 2001
3xTg-AD	APP (K670N/M671L), Tau (P301L), PS1 (M146V)	Yes	Yes	Yes	Yes	-	No	-	Oddo et al. 2003
APP_{SL}/PS1	APP (K670N/M671L, V717I), PS1 (M146L)	Yes	Yes	-	-	Yes	Yes	-	Wirths et al. 2002
APP/PS1KI	APP (K670N/M671L, V717I), PS1 (M233T/L235P)	Yes	Yes	-	-	Yes	Yes	-	Casas et al. 2004
5xFAD	APP (K670N/M671L, I716V, V717I), PS1 (M146L/L286V)	Yes	Yes	-	-	Yes	Yes	-	Oakley et al. 2006

CAA = cerebral amyloid angiopathy; Dash (-) = not reported.


Familial AD
Sporadic AD
APP mutations, trisomy 21
PS1, PS2 mutations
APOE, CLU, PICALM, CR1
APP
sAPPβ
N-APP
Aβ
β-secretase
γ-secretase
AICD
Aβ oligomer formation
Amyloids, senile plaques
LTP impairment
Synaptic loss and neuronal death

Goal

To constitute a cell culture model to observe amyloid plaques and NFT and understand Mechanistic details

PROTOCOL

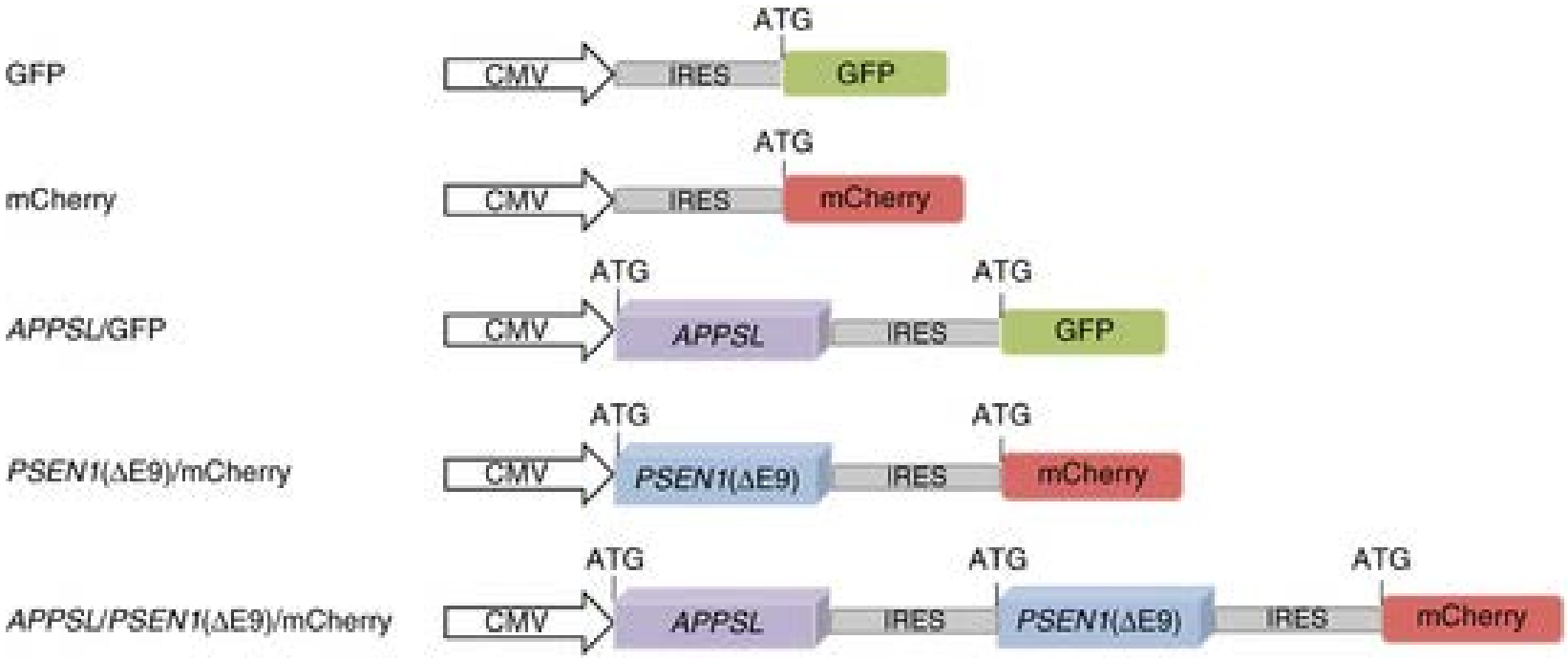
A 3D human neural cell culture system for modeling Alzheimer's disease

Young Hye Kim[1,2,4], Se Hoon Choi[1,4], Carla D'Avanzo[1,4], Matthias Hebisch[1,3], Christopher Sliwinski[1], Enjana Bylykbashi[1], Kevin J Washicosky[1], Justin B Klee[1], Oliver Brüstle[3], Rudolph E Tanzi[1] & Doo Yeon Kim[1]

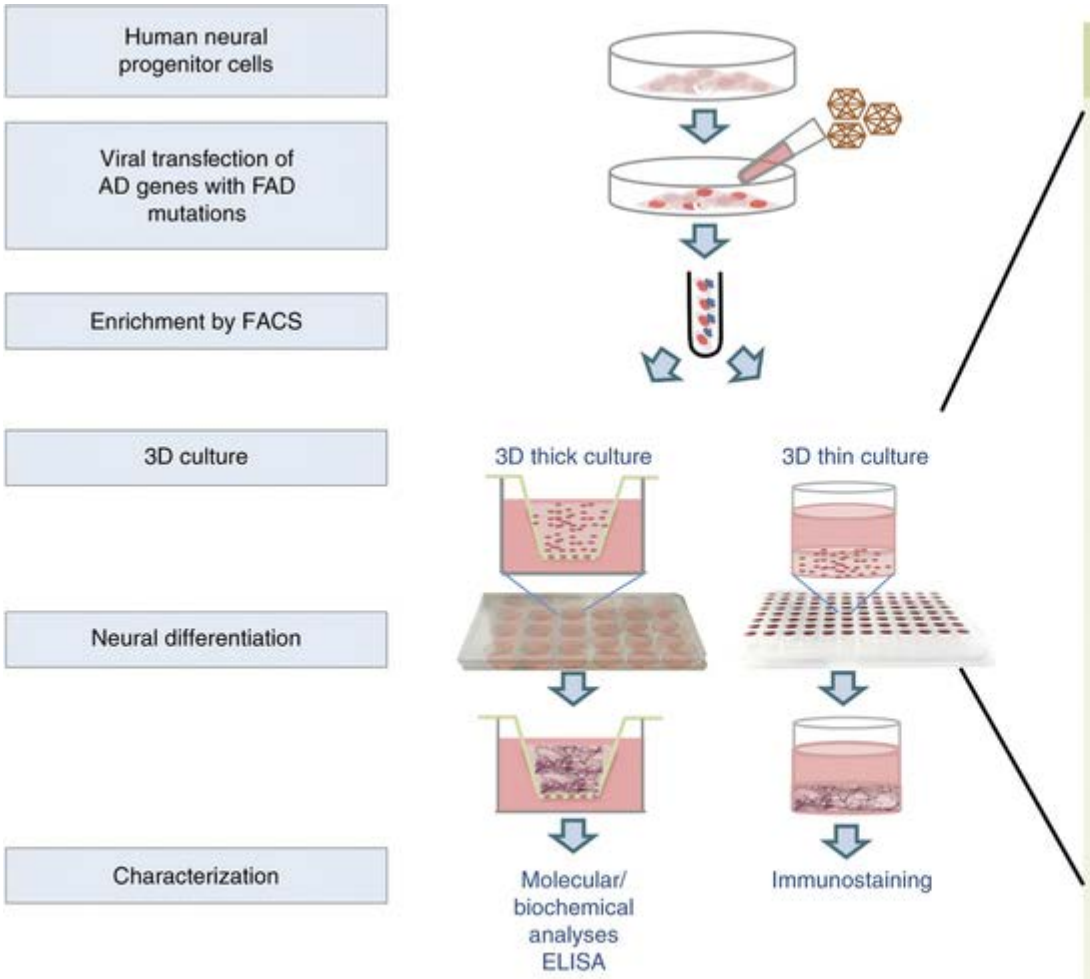
[1]Genetics and Aging Research Unit, MassGeneral Institute for Neurodegenerative Disease, Massachusetts General Hospital, Harvard Medical School, Charlestown, Massachusetts, USA. [2]Biomedical Omics Group, Korea Basic Science Institute, Cheongju-si, Chungbuk, Republic of Korea. [3]Institute of Reconstructive Neurobiology, Life and Brain Center, University of Bonn and Hertie Foundation, Bonn, Germany. [4]These authors contributed equally to this work. Correspondence should be addressed to R.E.T. (tanzi@helix.mgh.harvard.edu) or D.Y.K. (dkim@helix.mgh.harvard.edu).

Published online 11 June 2015; corrected online 1 July 2015 (details online); doi:10.1038/nprot.2015.065

Lenti viral vectors

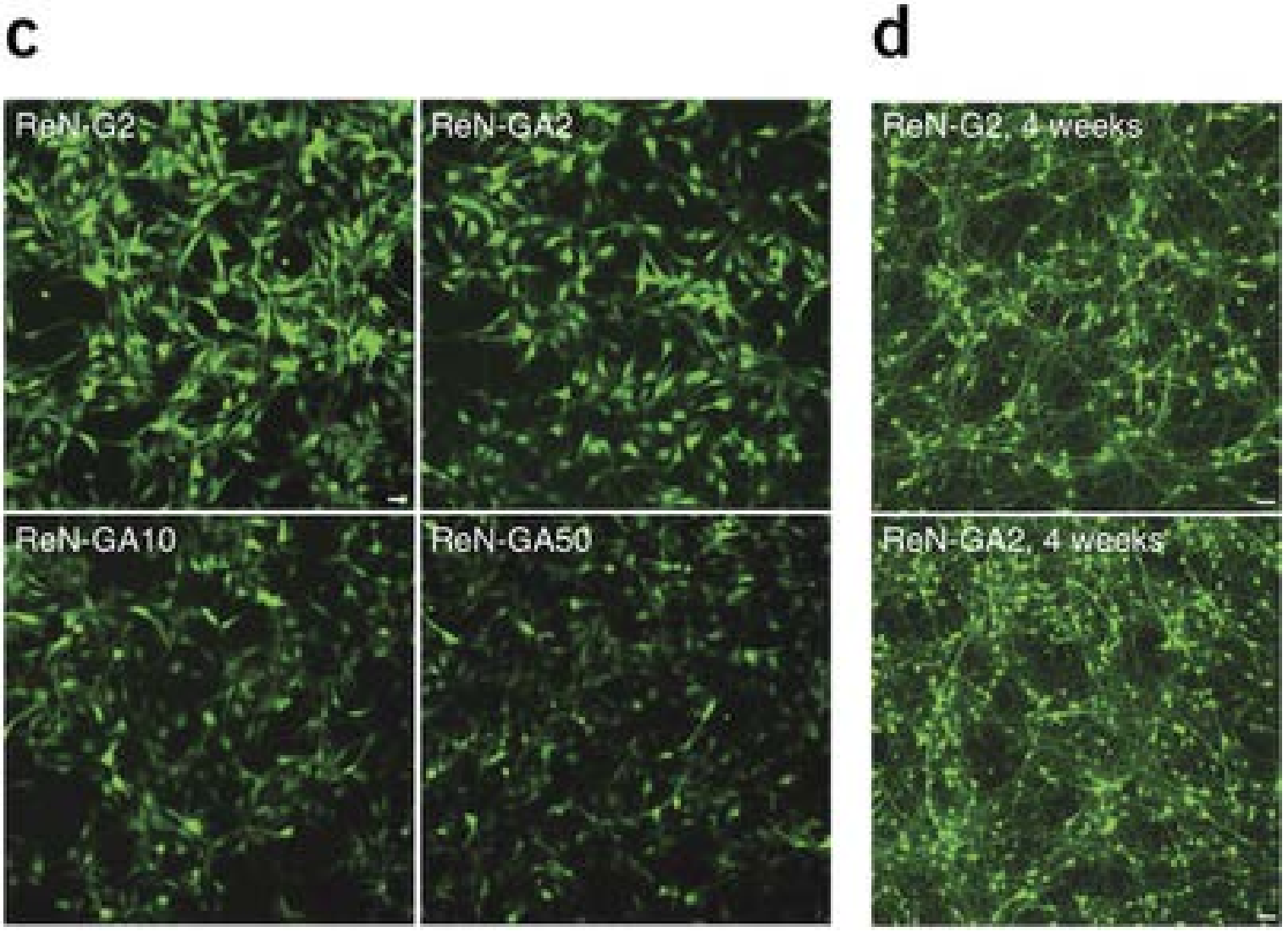


Methodology for 3D Cell Culture

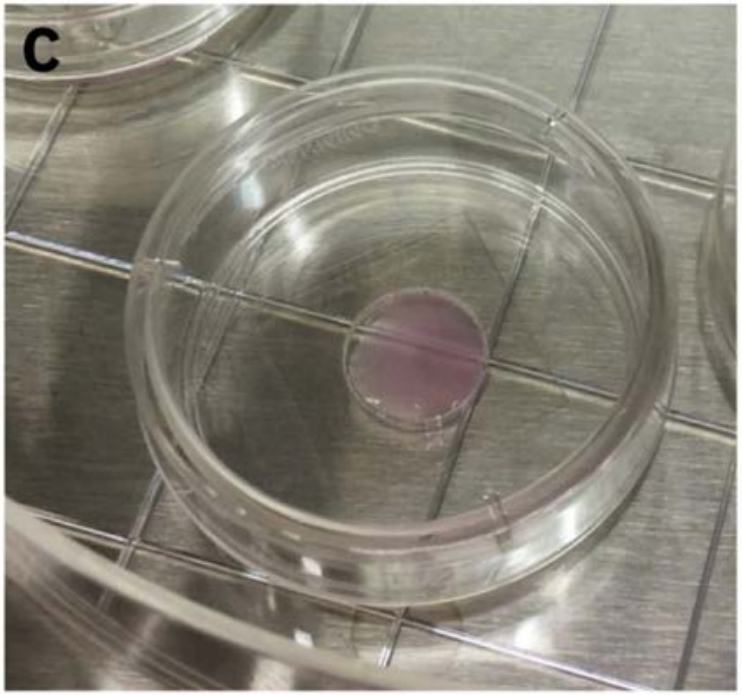


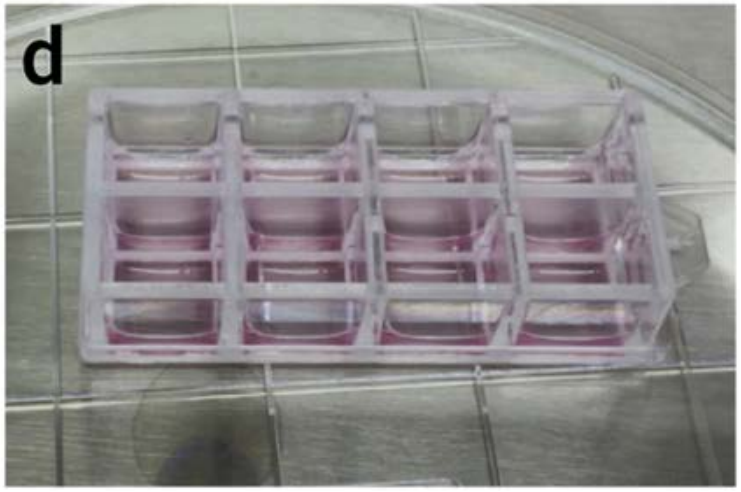
Time		3D culture procedure
−3 d		Seed cells
−1 d		Keep Matrigel at 4 °C
0 d		
	30 min	Keep Matrigel and medium on ice
	<5 min	Detach cells
	<5 min	Resuspend cells
	<5 min	Adjust cell number
	2 min	Mix cells and Matrigel
		Thick culture: 1:1 (vol/vol) dilution; Thin culture: 1:10 ~1:20 (vol/vol) dilution
	2 min	Plate cells/Matrigel
		Thick culture: (~4 mm), 24-Well plates and inserts; Thin culture: (~100–200 µm), 96-Well plates, Glass-bottomed dishes
		Incubate at 37 °C
+1 d		Start differentiation (change medium every 3 d)
+6 weeks		Expression of mature neural markers (i.e., MAP2, 4R tau, vGluT1, NR2B, TH)
		Extracellular aggregation of Aβ40/42
+10–14 weeks		Robust increase in phospho tau in neurites and cell bodies (tau filamentous structures detected in EM)

Establishment of 3D Cell Culture

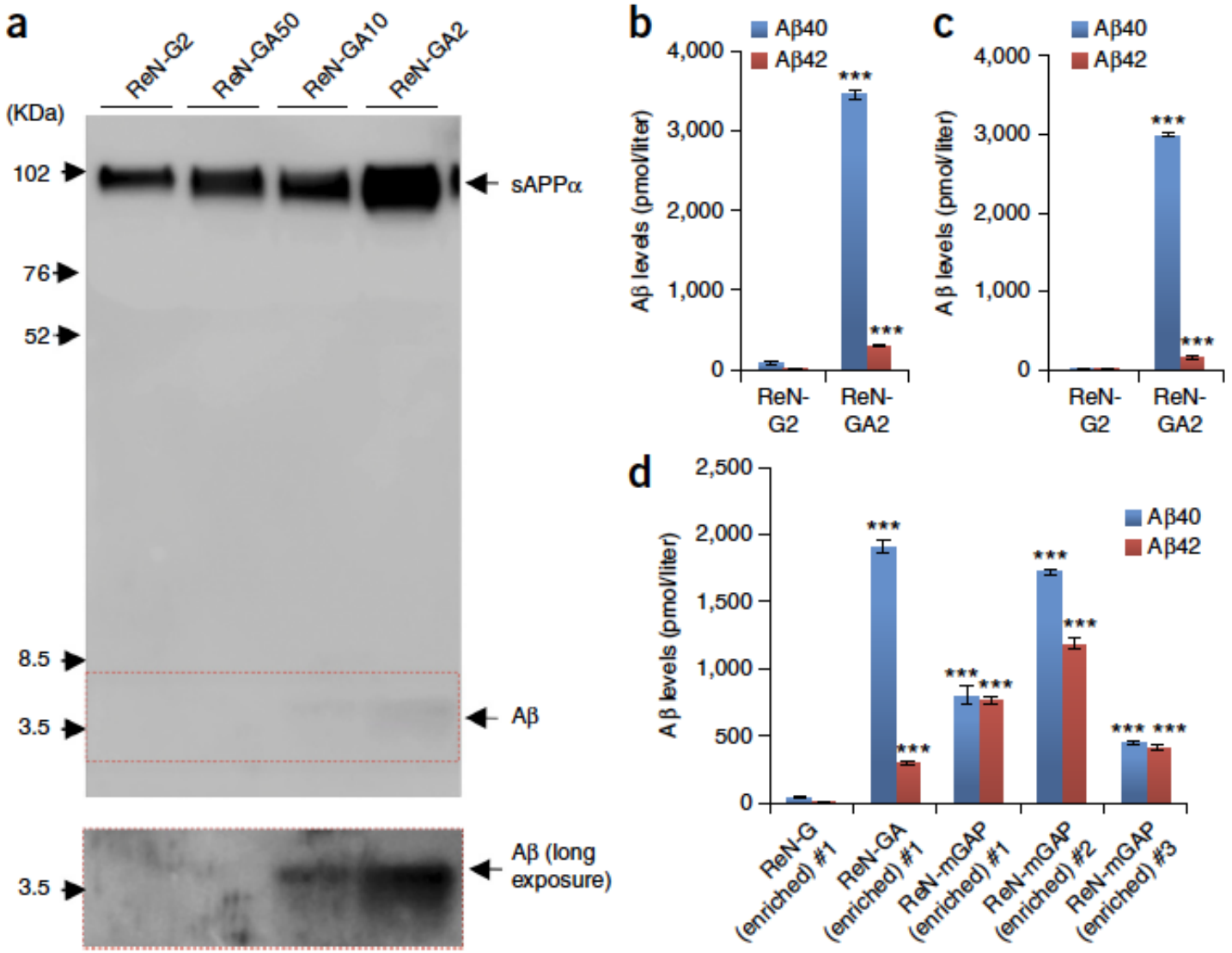


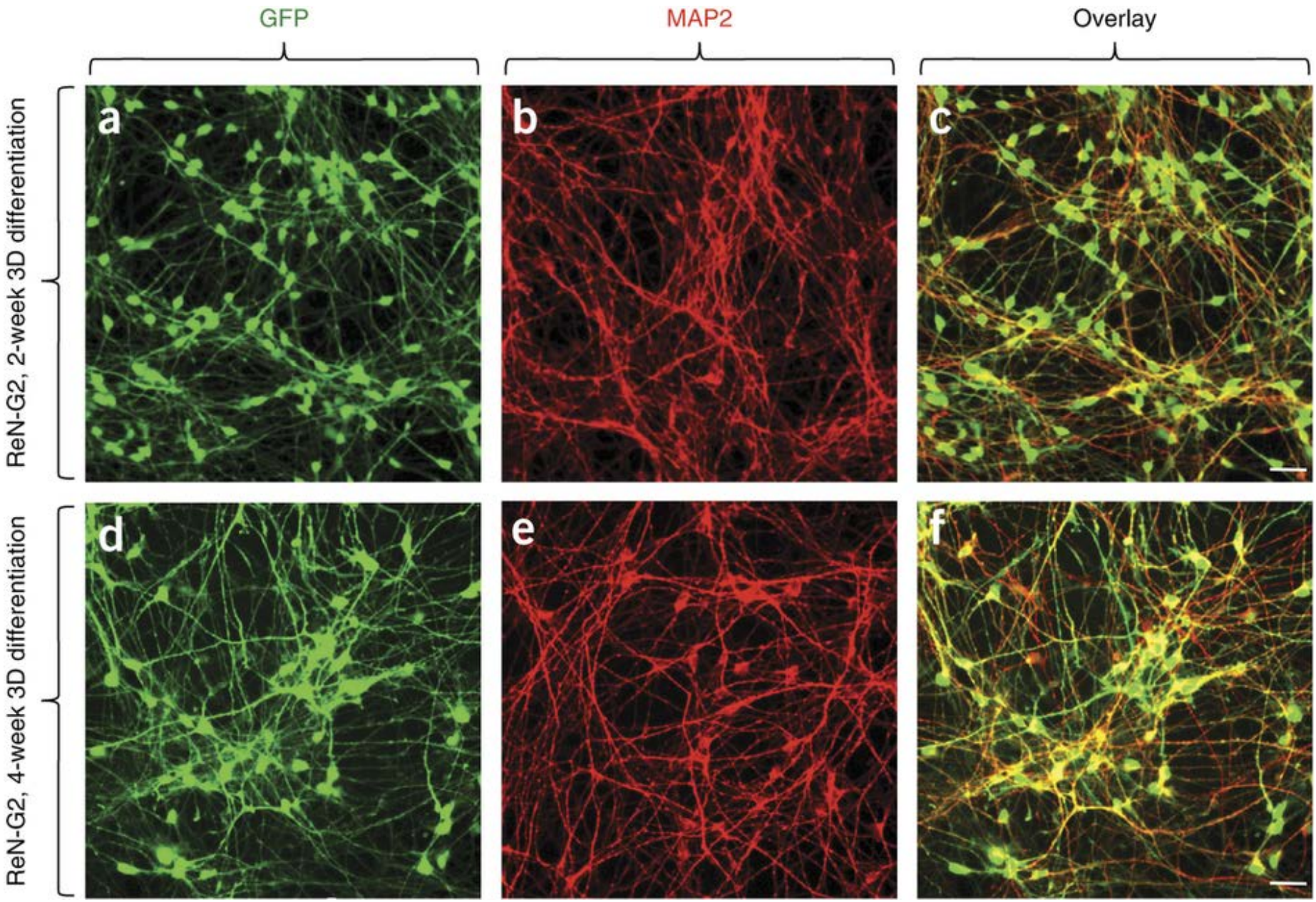
Thick and Thin Cultures



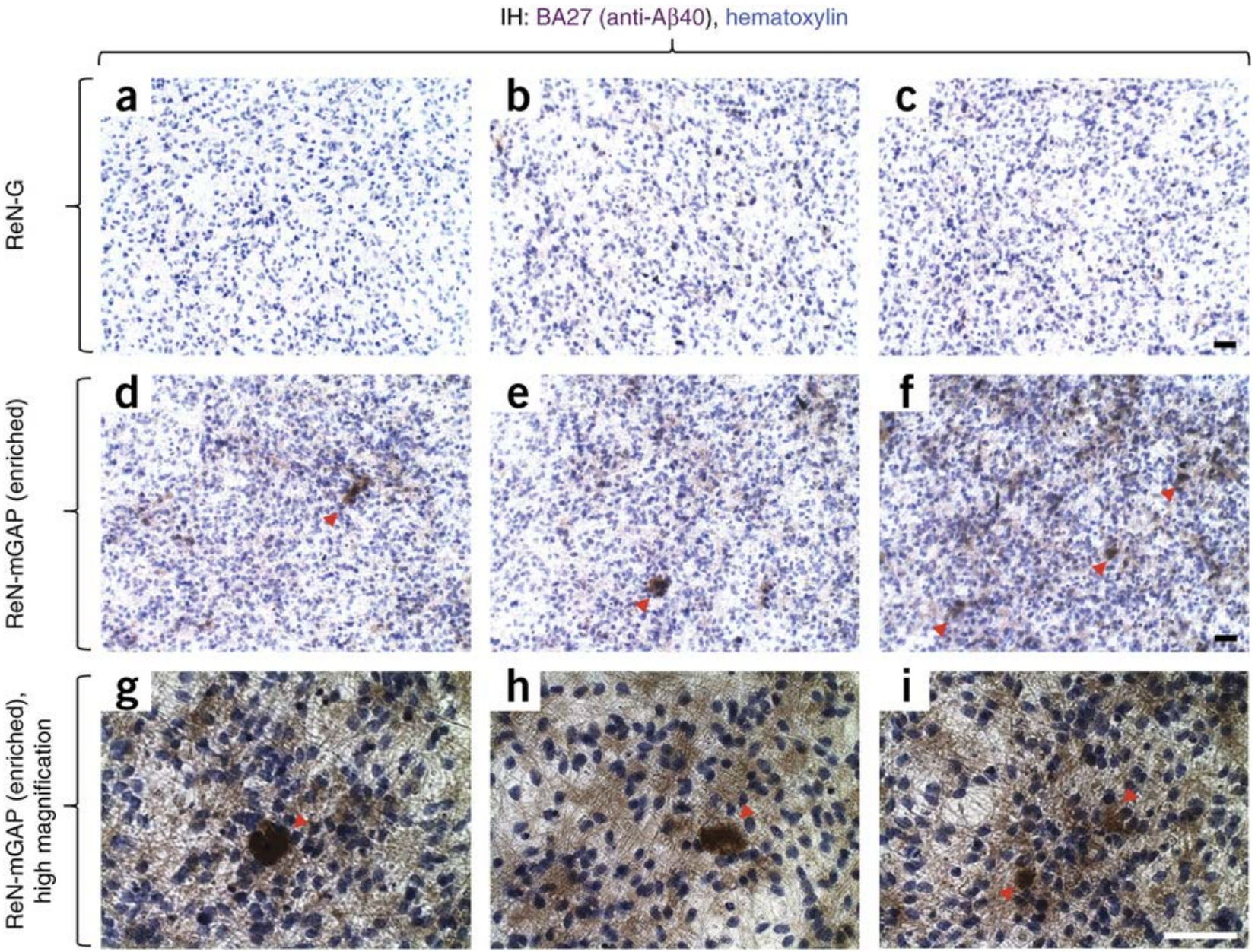


Cells release Aβ




GFP
MAP2
Overlay
ReN-G2, 2-week 3D differentiation
ReN-G2, 4-week 3D differentiation
a
b
c
d
e
f

Accumulation of Aβ aggregates



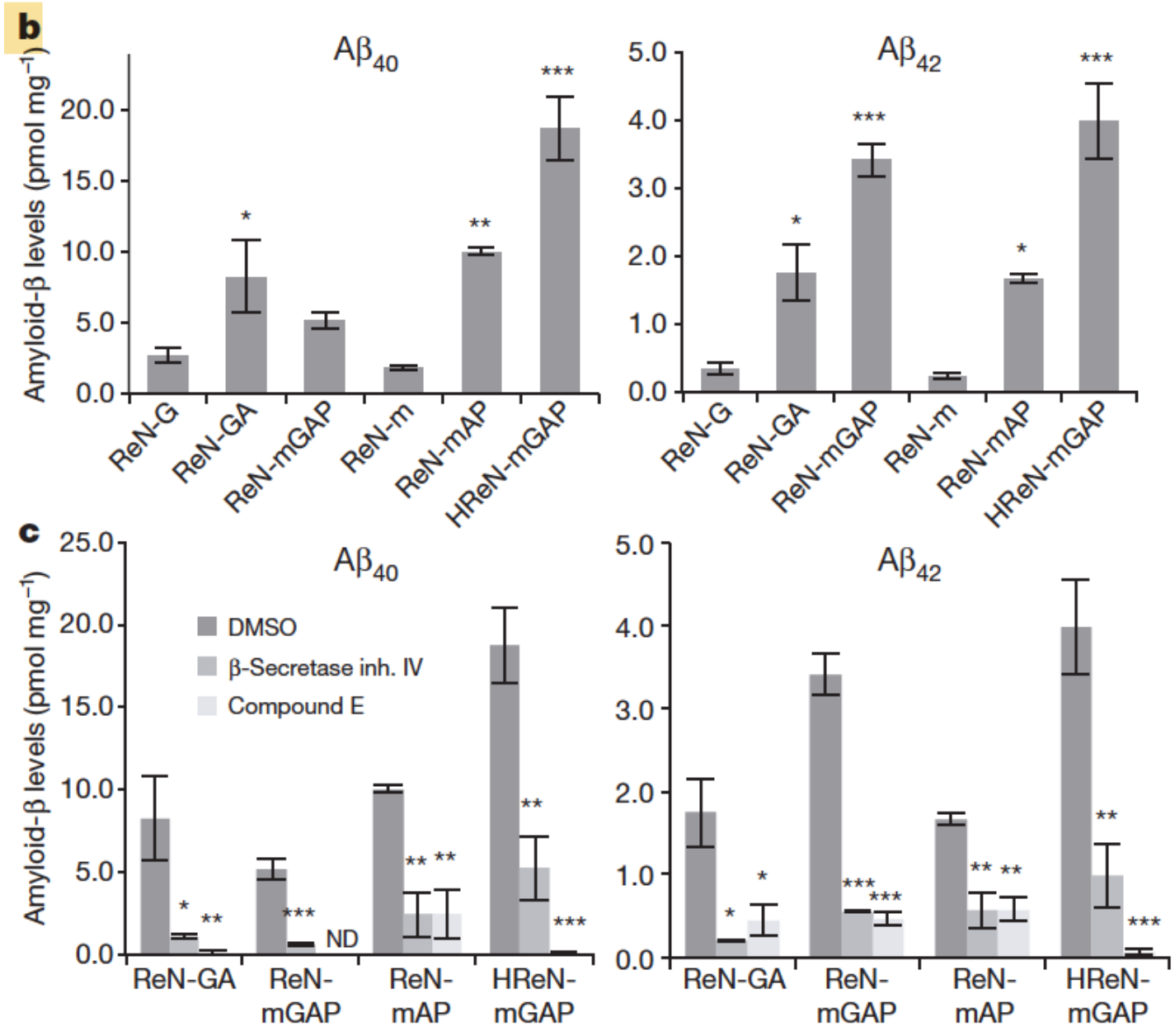
LETTER

doi:10.1038/nature13800

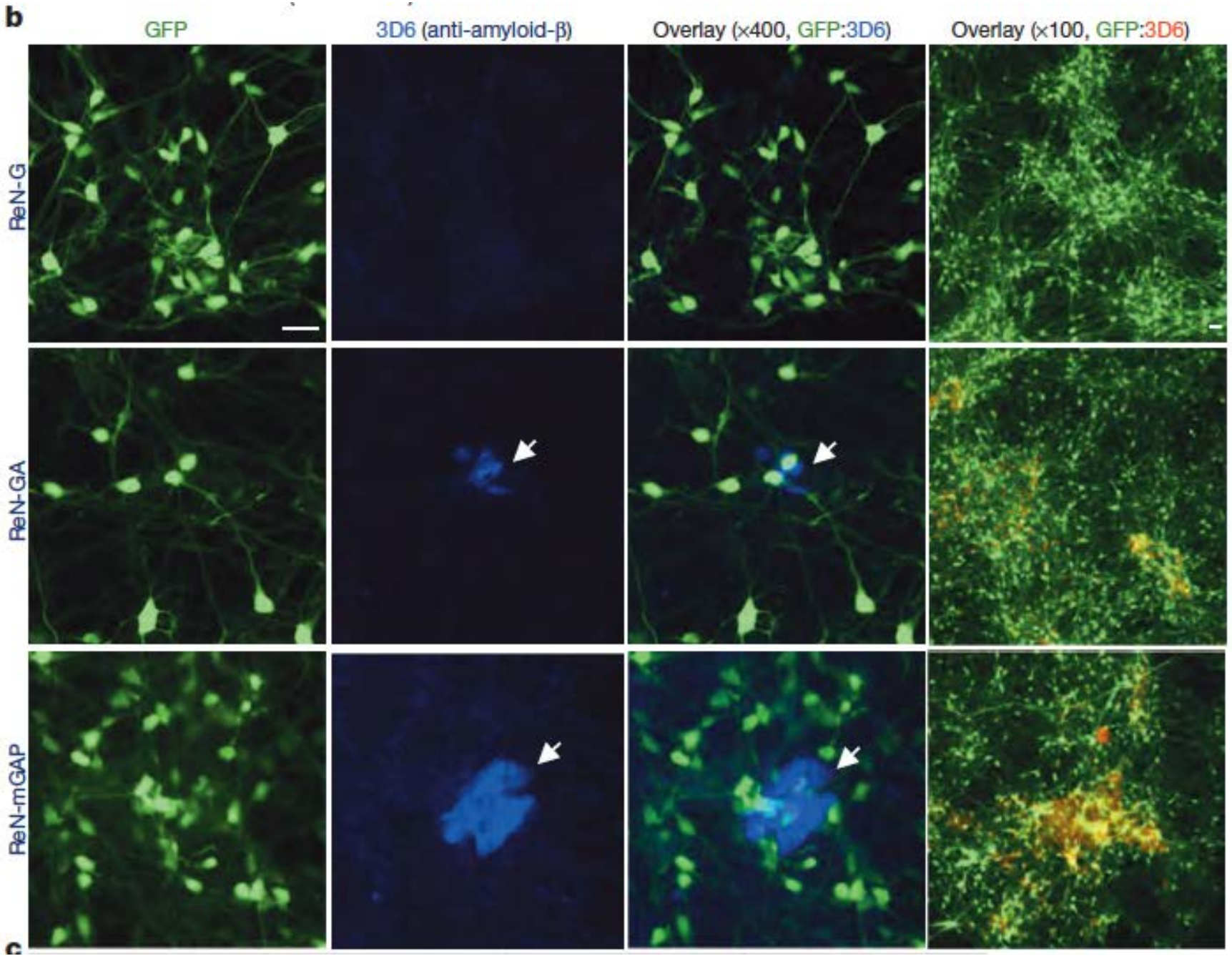
A three-dimensional human neural cell culture model of Alzheimer's disease

Se Hoon Choi[1]*, Young Hye Kim[1,2]*, Matthias Hebisch[1,3], Christopher Sliwinski[1], Seungkyu Lee[4], Carla D'Avanzo[1], Hechao Chen[1], Basavaraj Hooli[1], Caroline Asselin[1], Julien Muffat[5], Justin B. Klee[1], Can Zhang[1], Brian J. Wainger[4], Michael Peitz[3], Dora M. Kovacs[1], Clifford J. Woolf[4], Steven L. Wagner[6], Rudolph E. Tanzi[1] & Doo Yeon Kim[1]

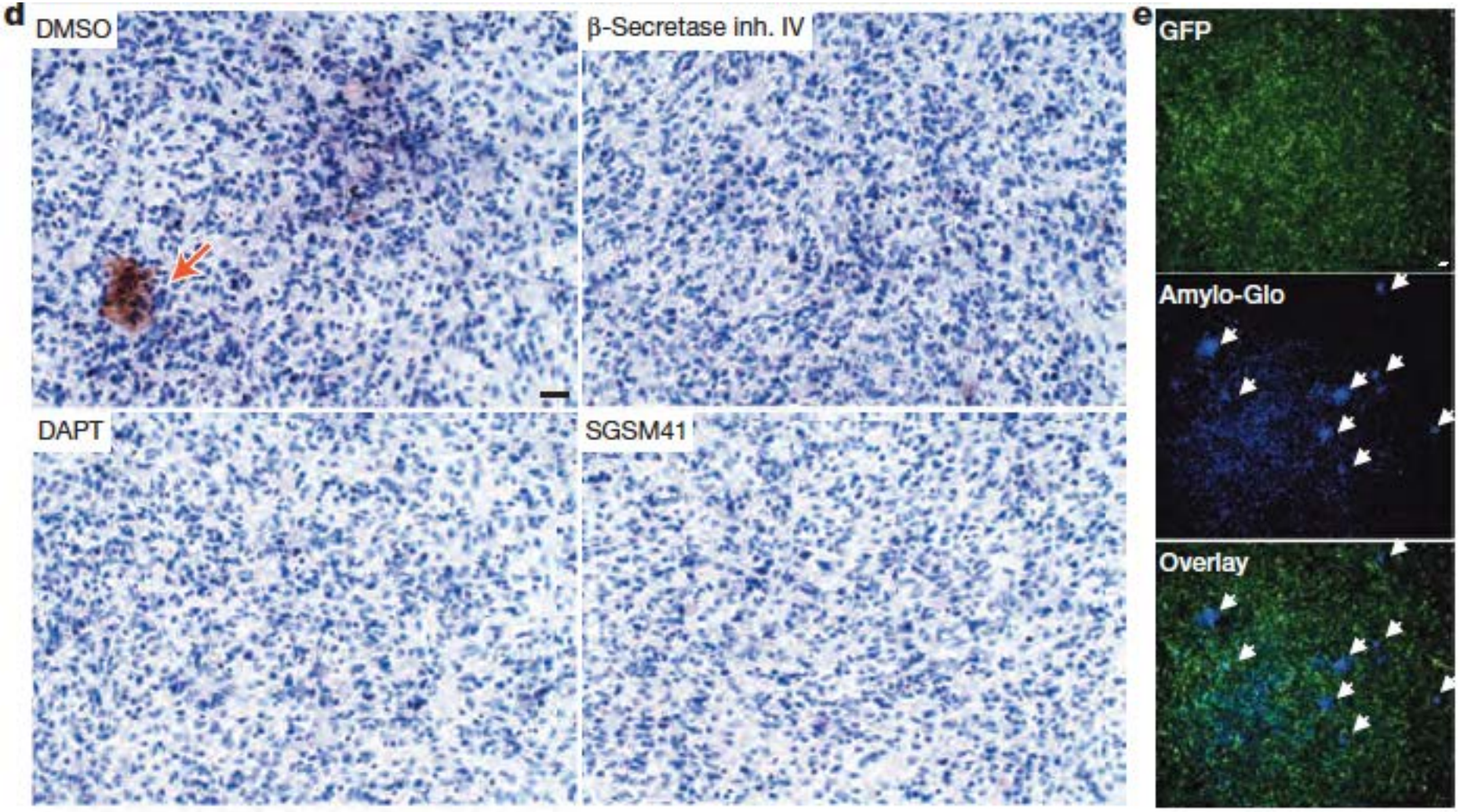
Accumulation of Aβ peptides

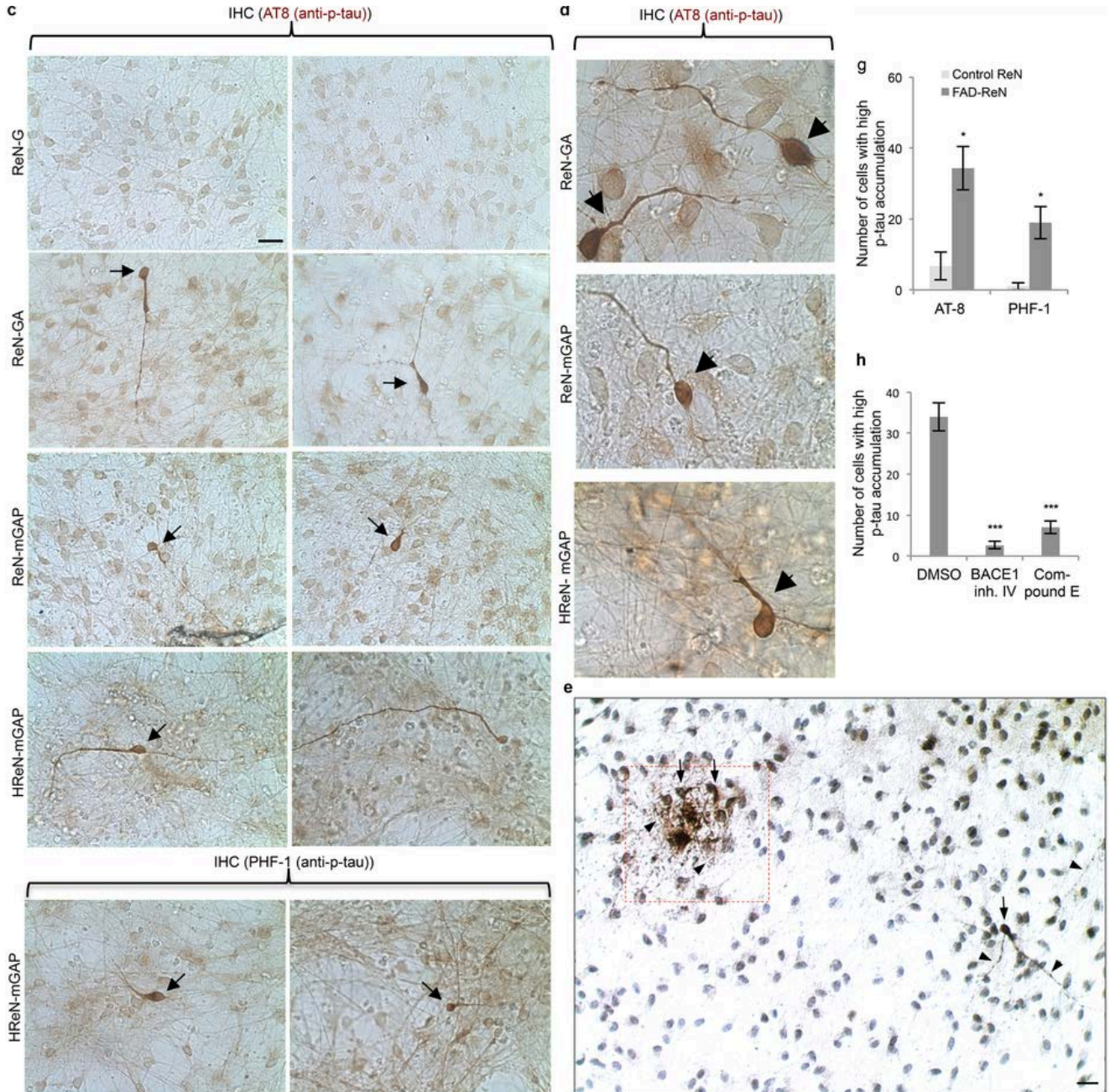


Accumulation of amyloid

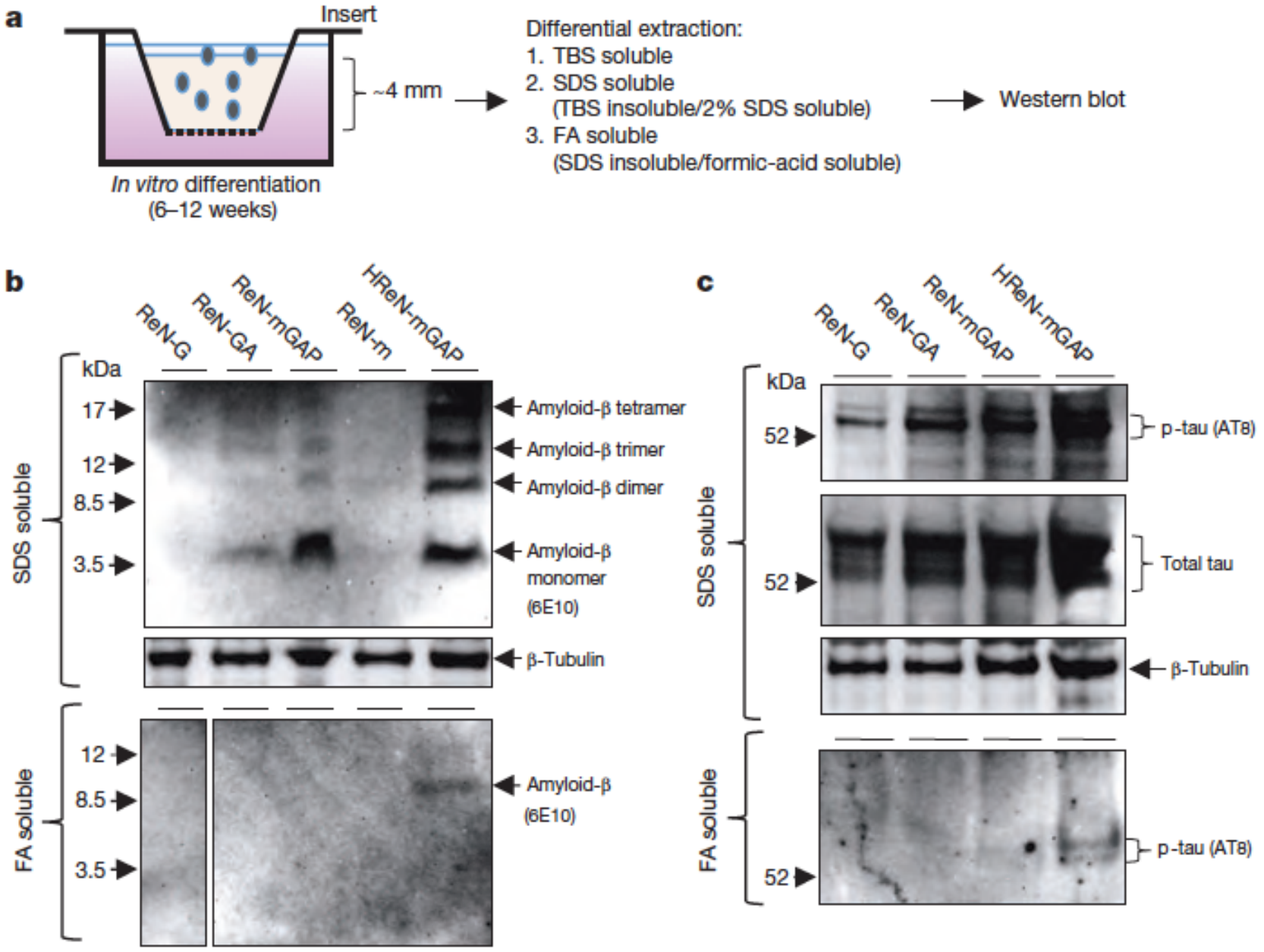


Pharmacological Modulation

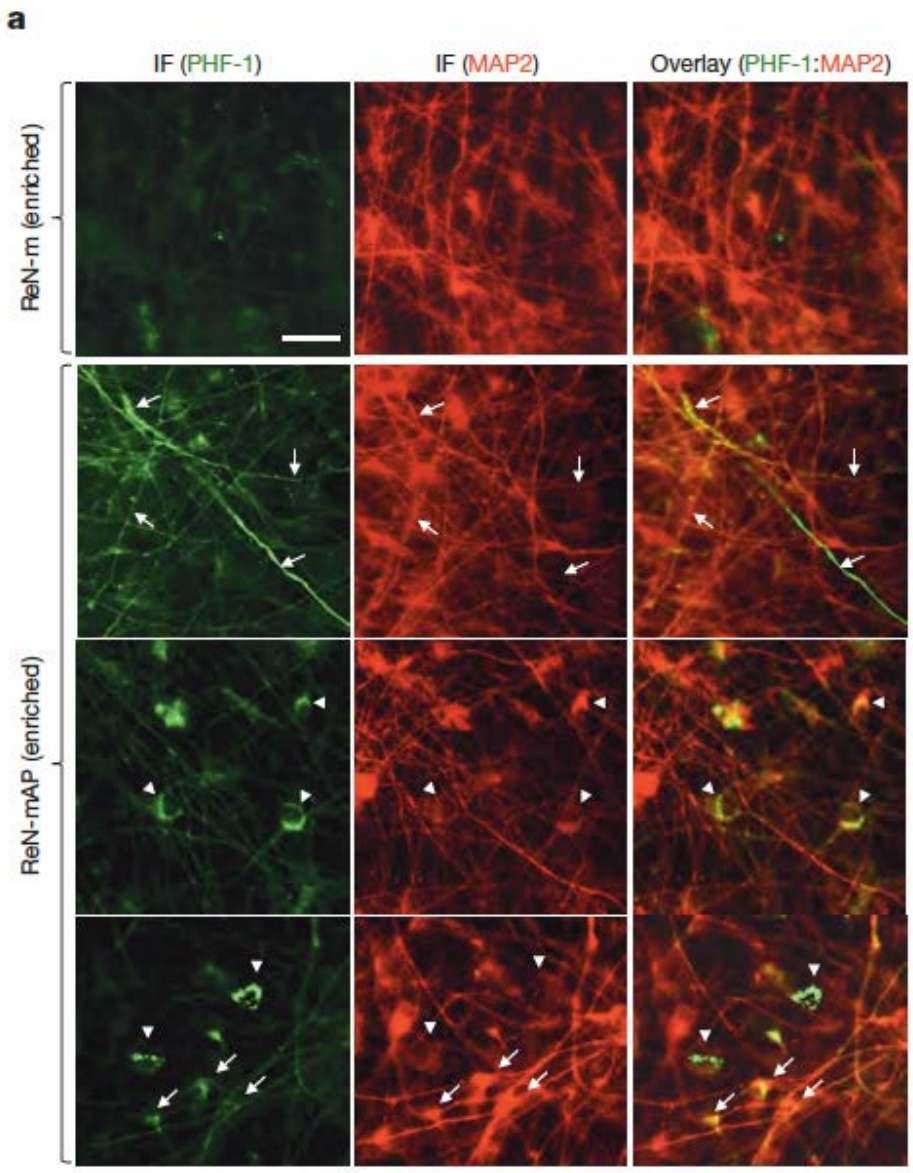


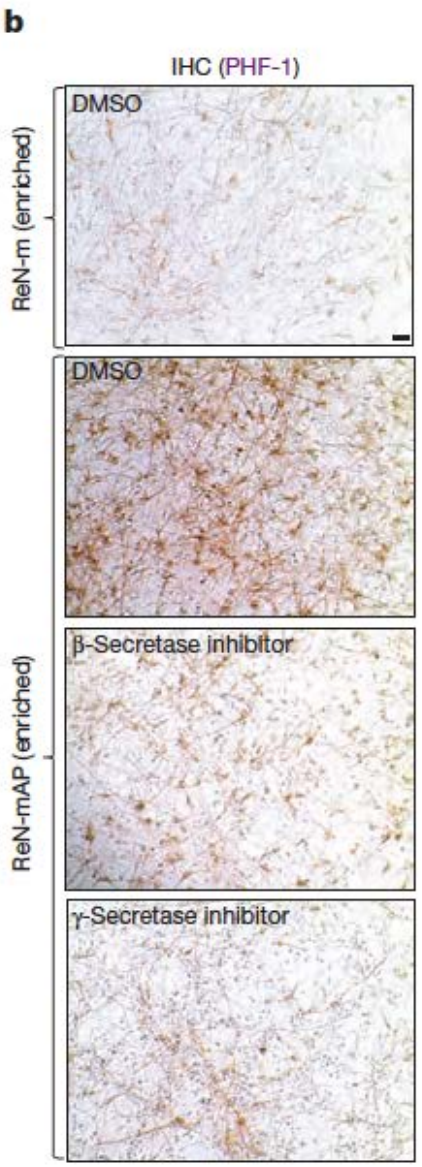

c
IHC (AT8 (anti-p-tau))
ReN-G
ReN-GA
ReN-mGAP
HReN-mGAP
IHC (PHF-1 (anti-p-tau))
HReN-mGAP
a
IHC (AT8 (anti-p-tau))
ReN-GA
ReN-mGAP
HReN- mGAP
g
Control ReN
FAD-ReN
Number of cells with high p-tau accumulation
60
40
20
0
*
*
AT-8
PHF-1
h
Number of cells with high p-tau accumulation
40
30
20
10
0
***
***
DMSO
BACE1 inh. IV
Com-pound E
e

Aβ and Tau are in insoluble fractions

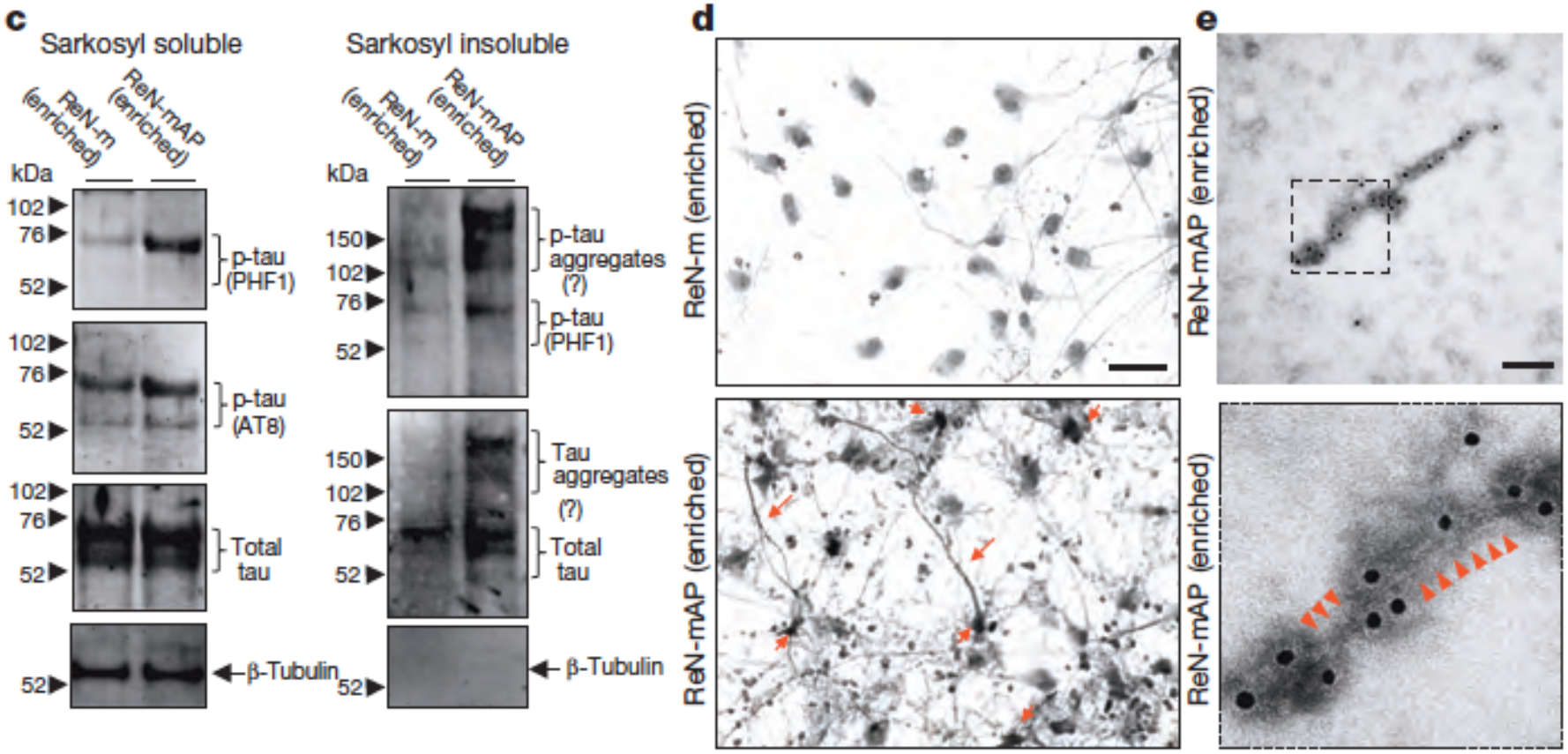


Aggregated p-Tau

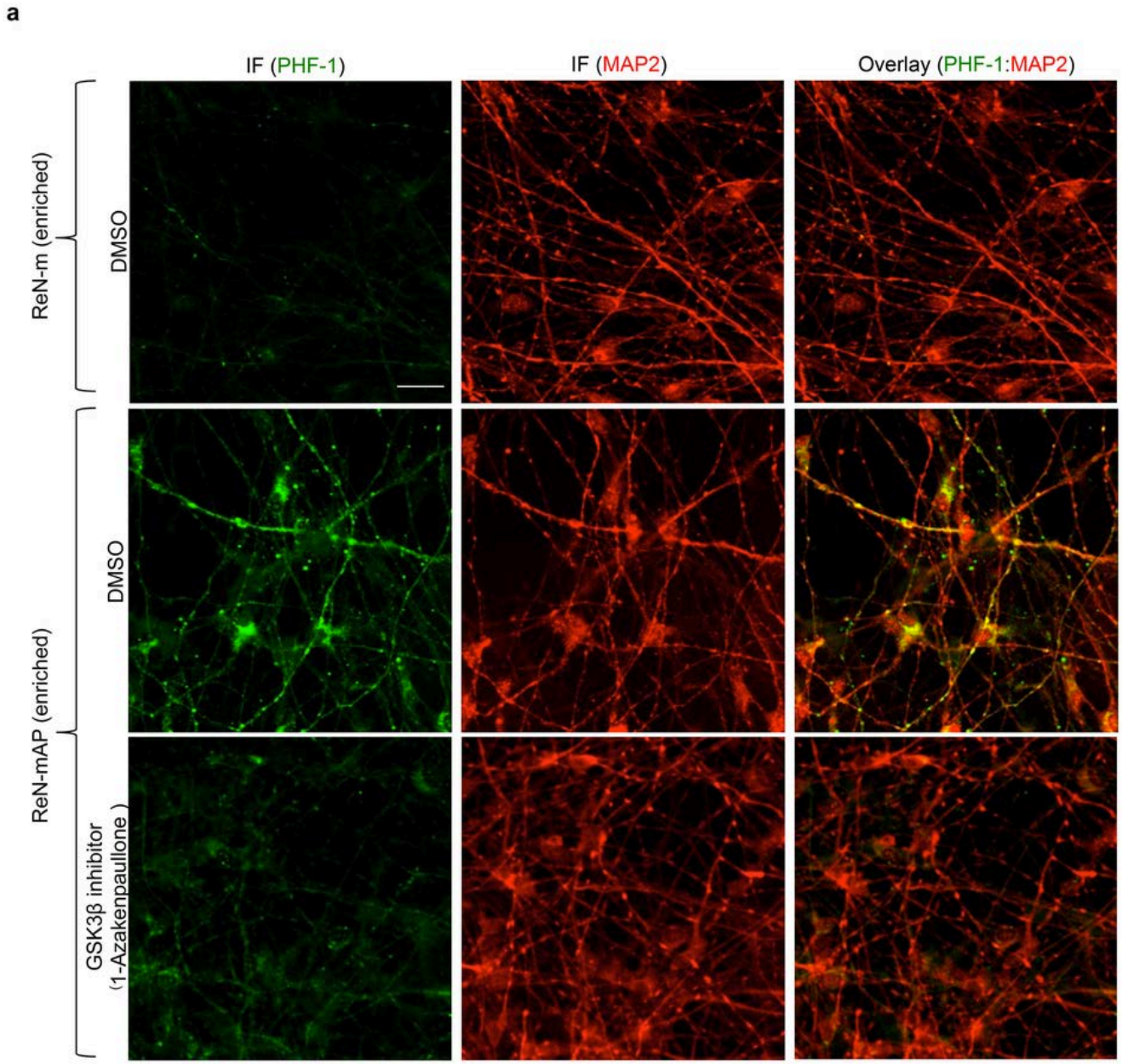




Aggregated p-Tau



Aggregated p-Tau upon GSK3β inhibition



Disadvantages

Organoids are highly variable in size and shape, and it is difficult to maintain cells in consistent positions in these structures for extended analysis.

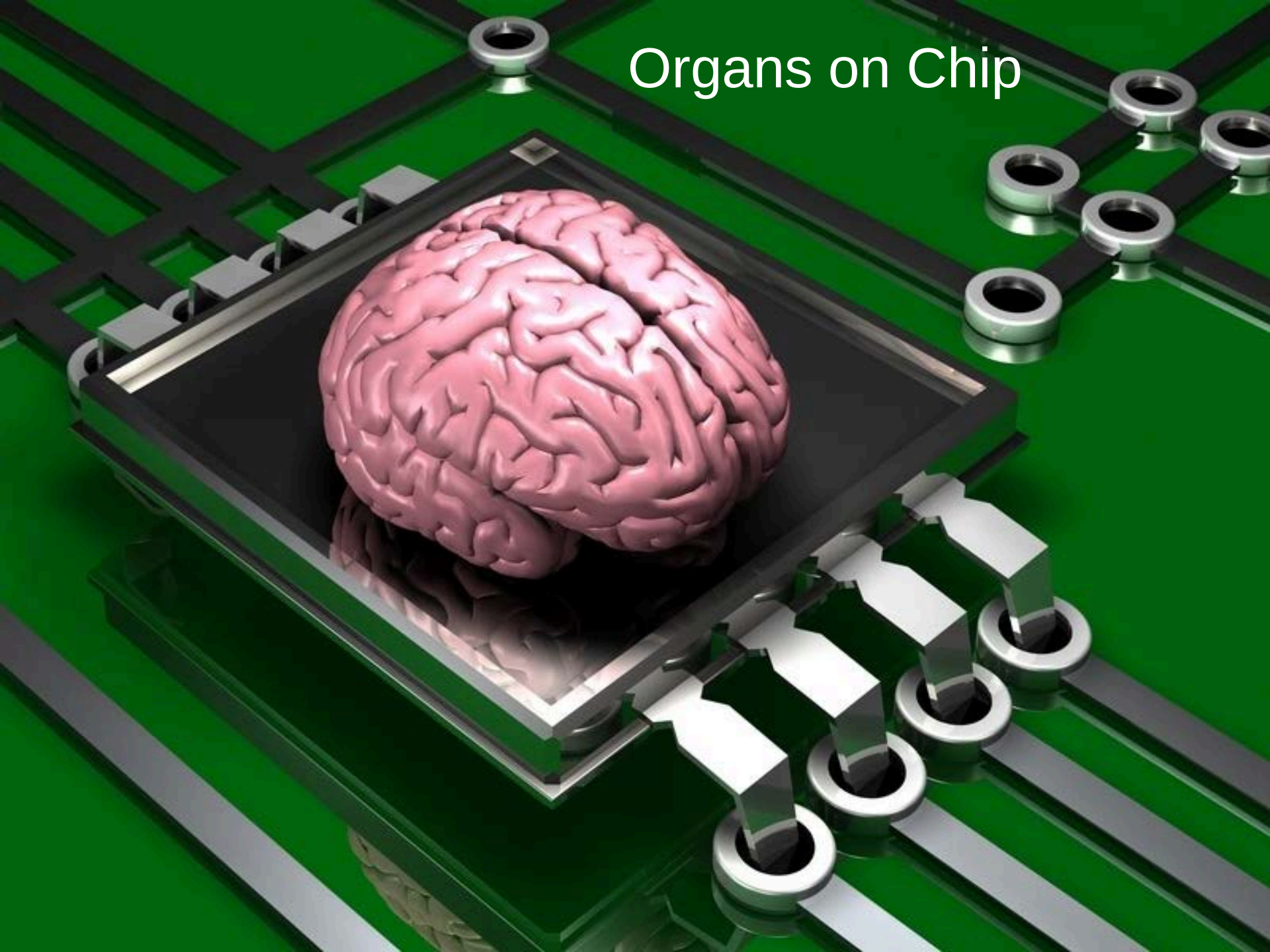
Another drawback of 3D models is that functional analysis of entrapped cells—for example, to quantify transcellular transport, absorption or secretion—is often hampered by the difficulty of sampling luminal contents,

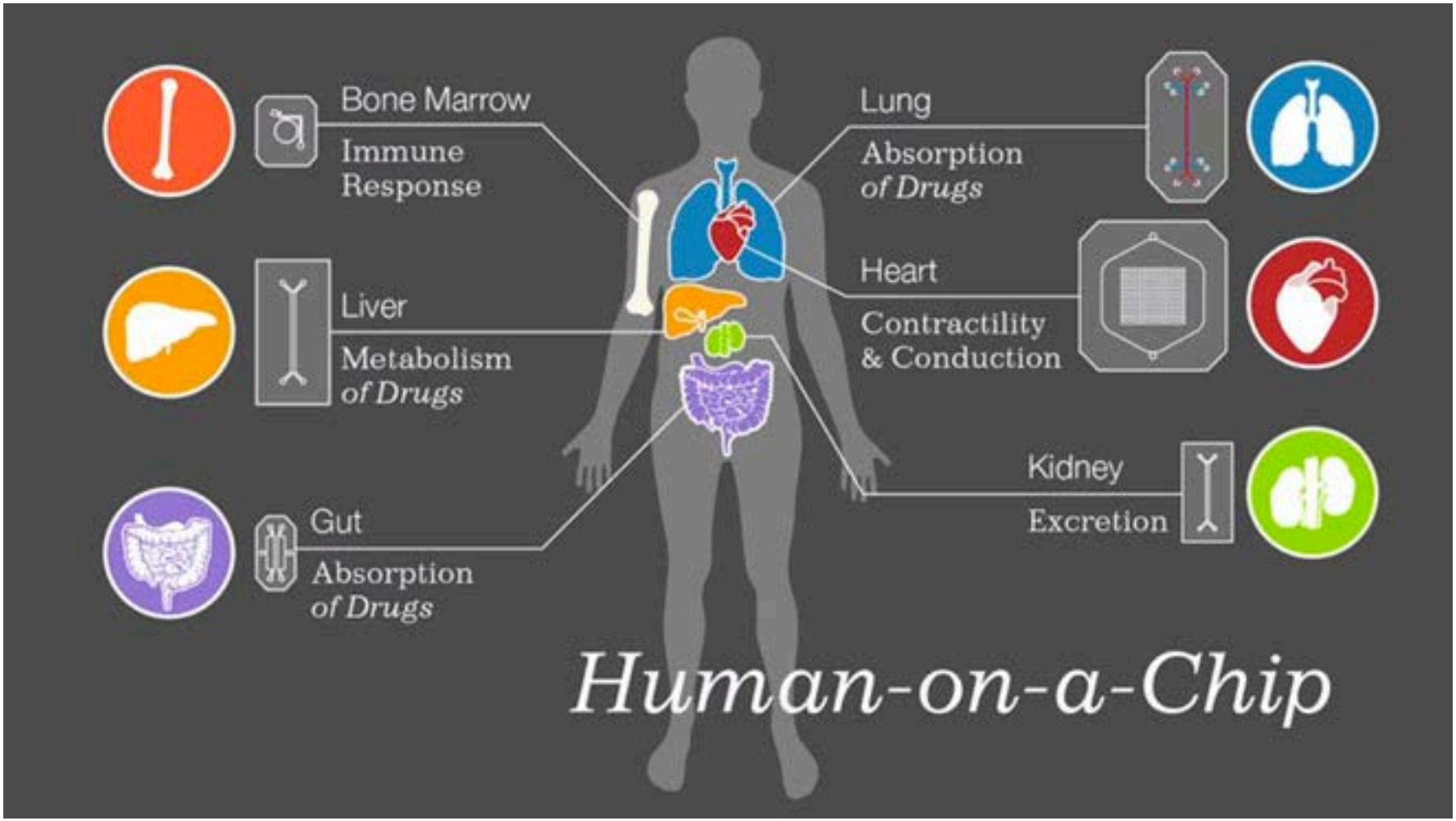
It is difficult to harvest cellular components for biochemical and genetic analysis.

Reproducibility of results.

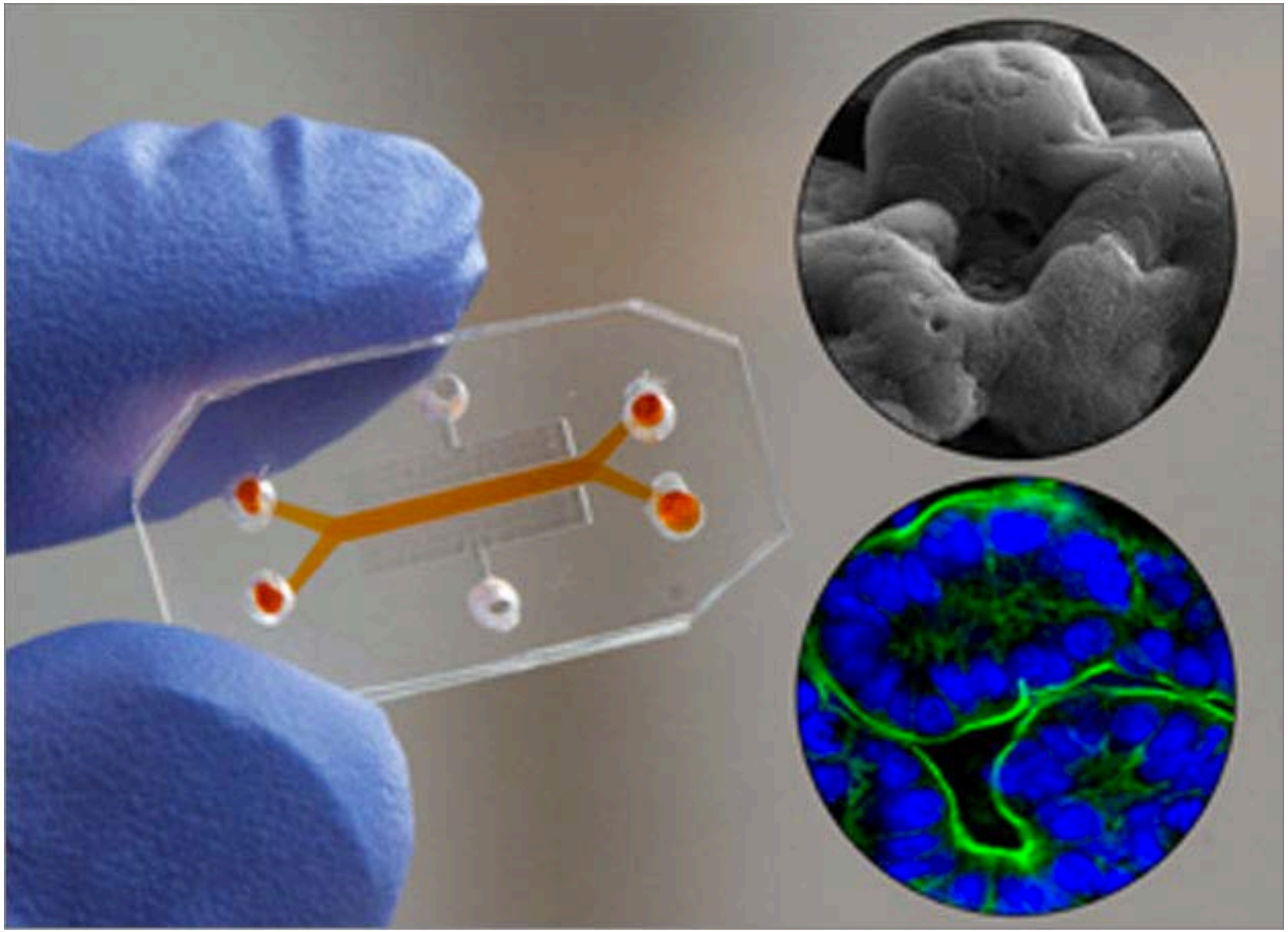
The absence of fluid flow also precludes the study of how cultured cells interact with circulating blood and immune cells

Organs on Chip

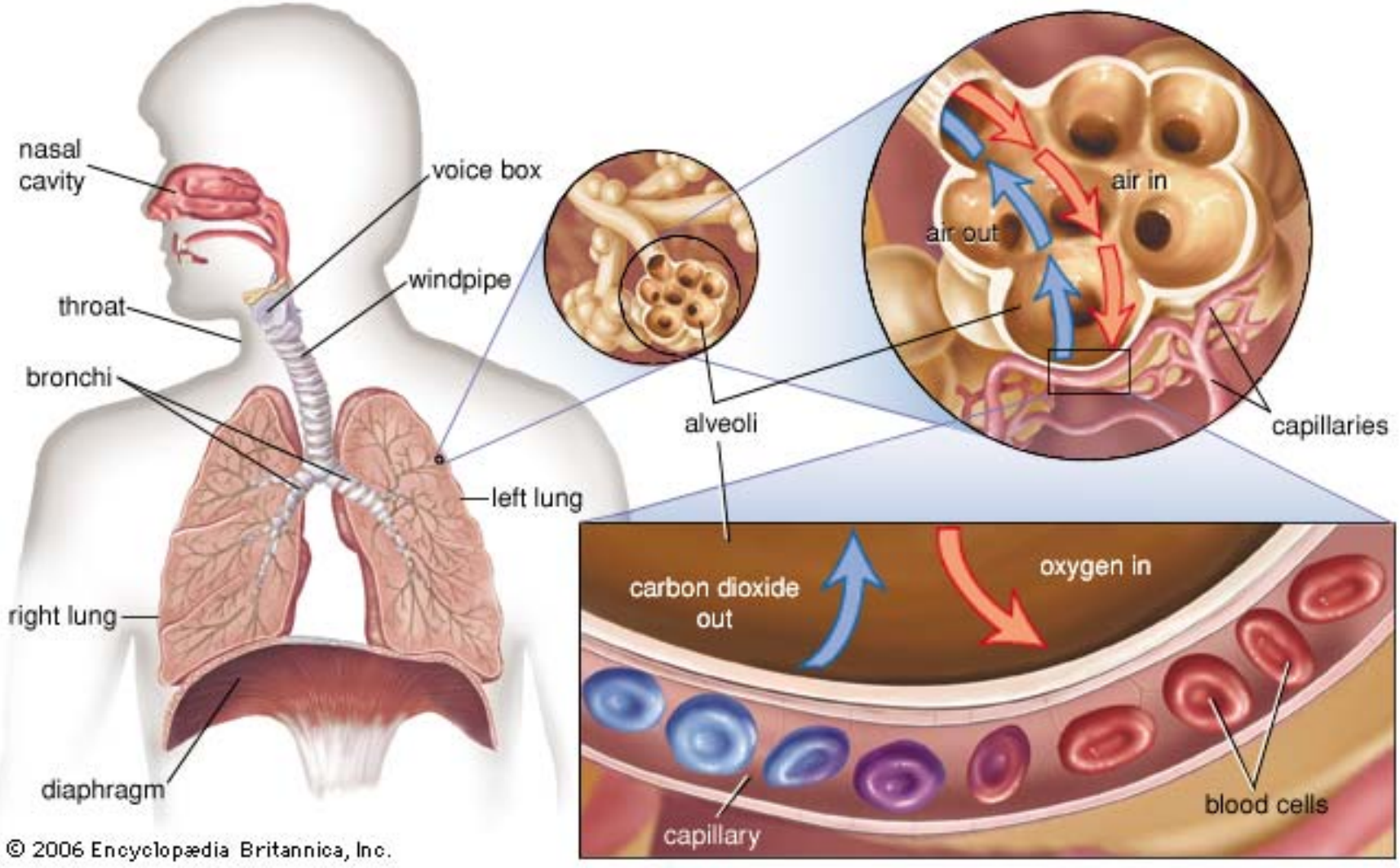




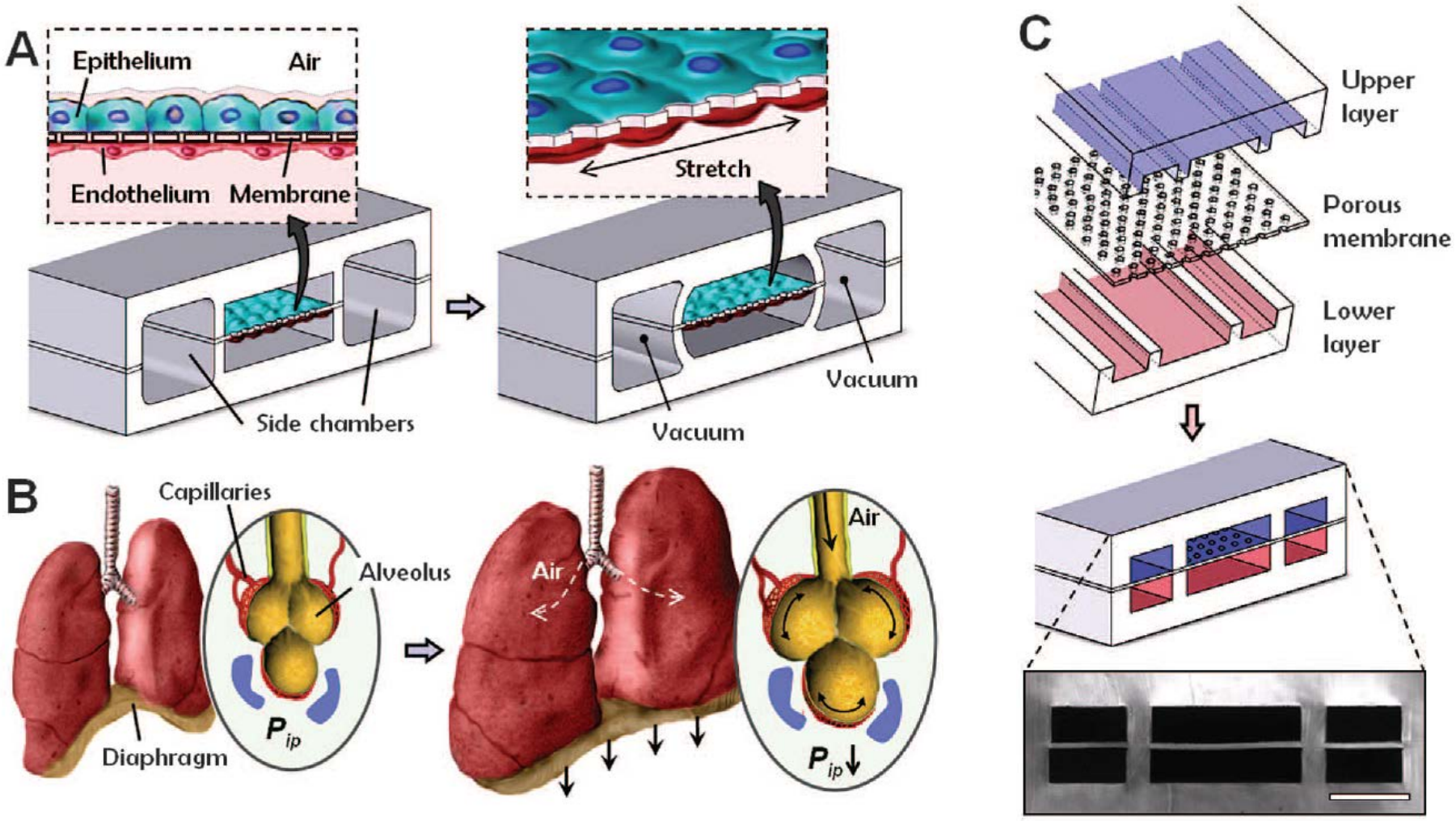
An organ-on-a-chip is a microfluidic cell culture device that contains continuously perfused chambers inhabited by living cells arranged to simulate tissue- and organ-level physiology.



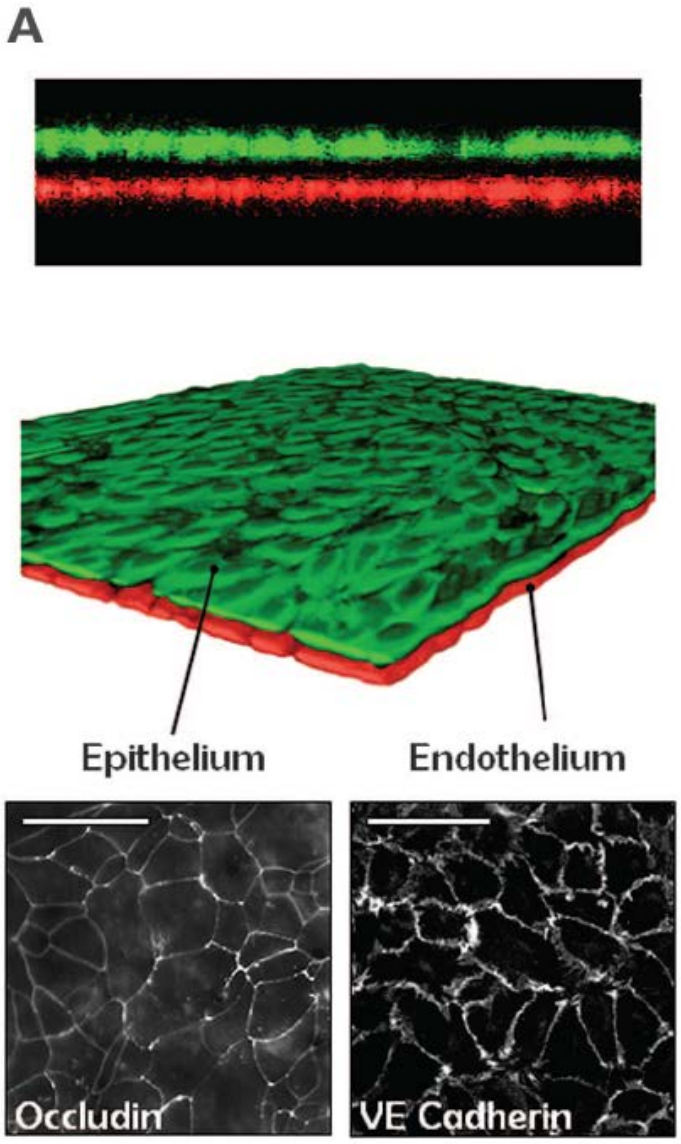
Lung on Chip

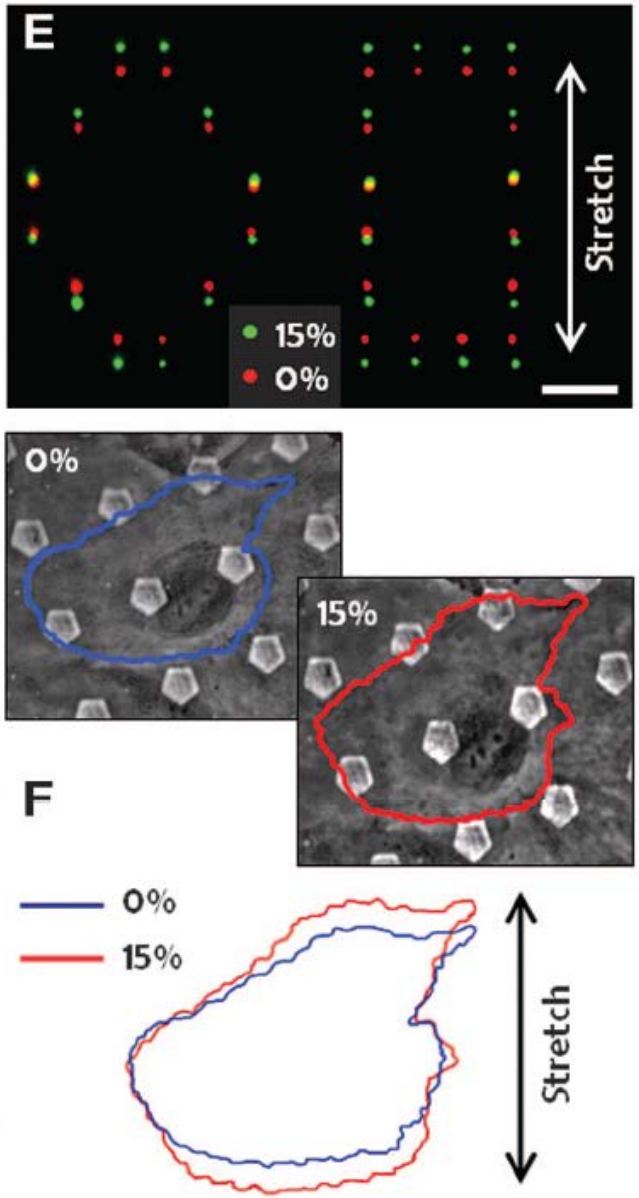

nasal cavity
voice box
windpipe
throat
bronchi
left lung
right lung
diaphragm
alveoli
air in
air out
capillaries
carbon dioxide out
oxygen in
capillary
blood cells
© 2006 Encyclopædia Britannica, Inc.

Designing Lung on Chip

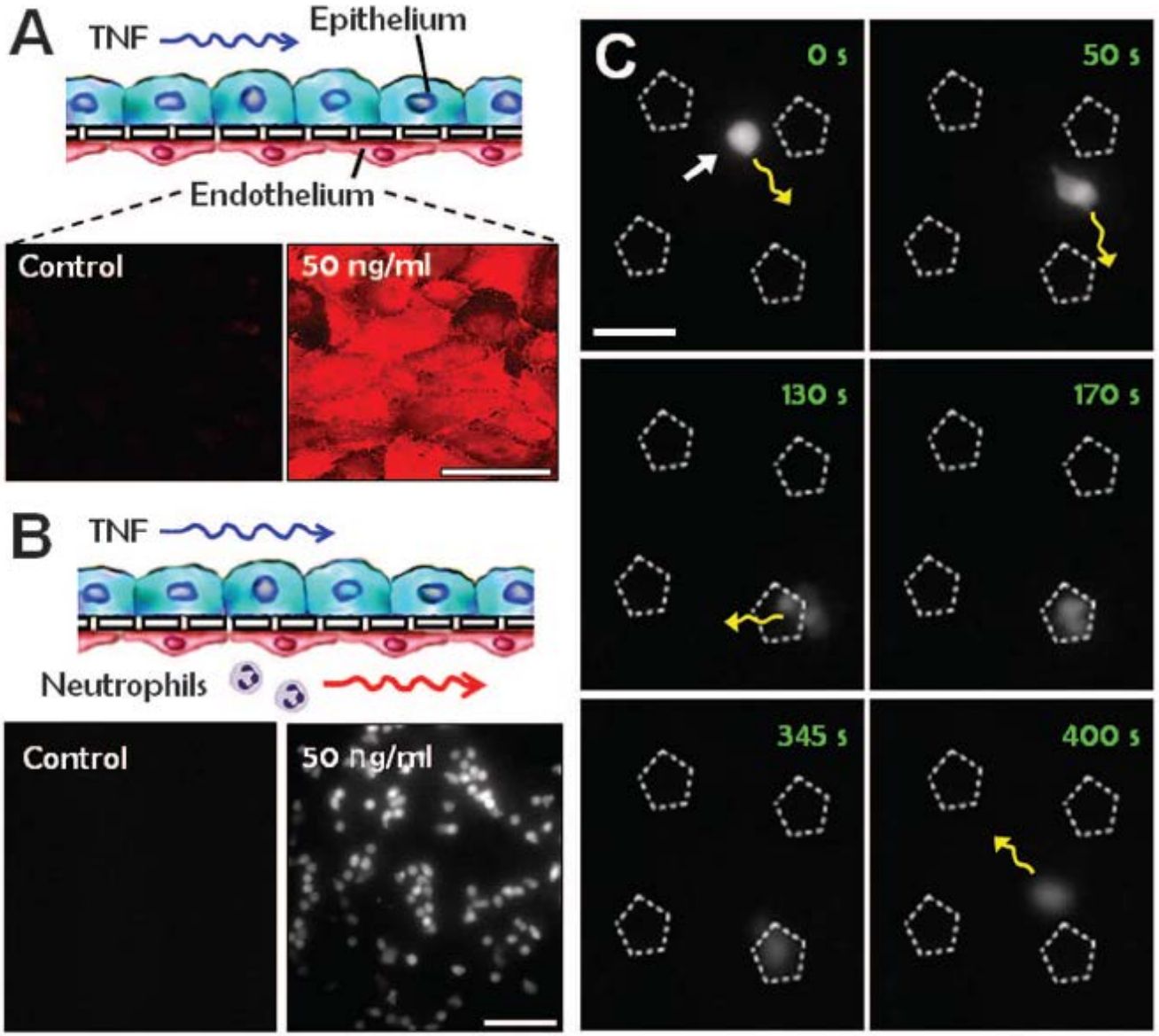


Mechanical Stretching

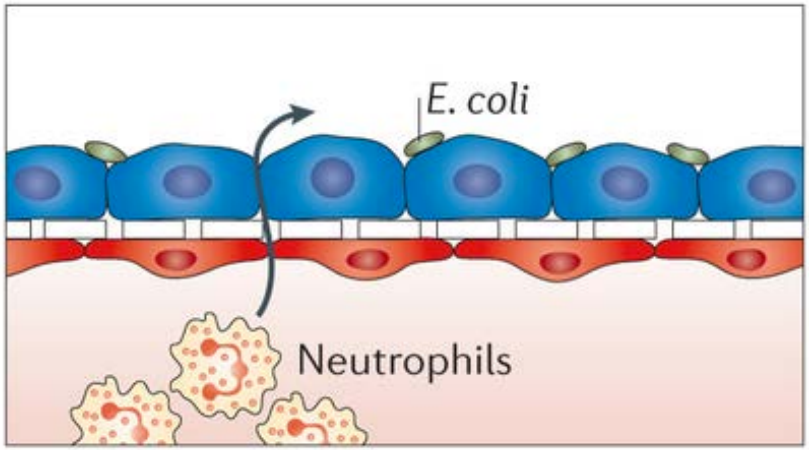


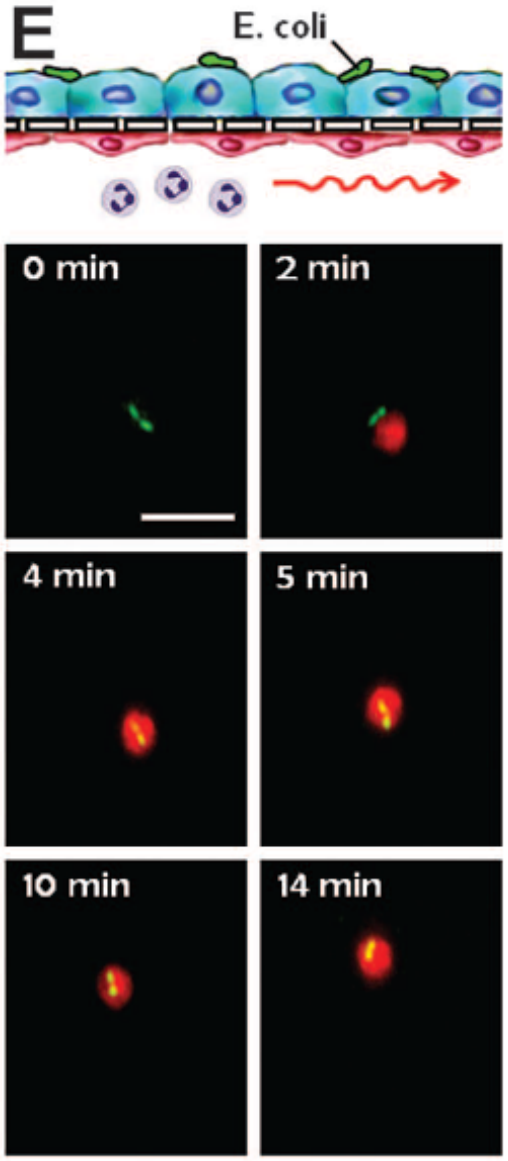


Pulmonary Inflammation Model



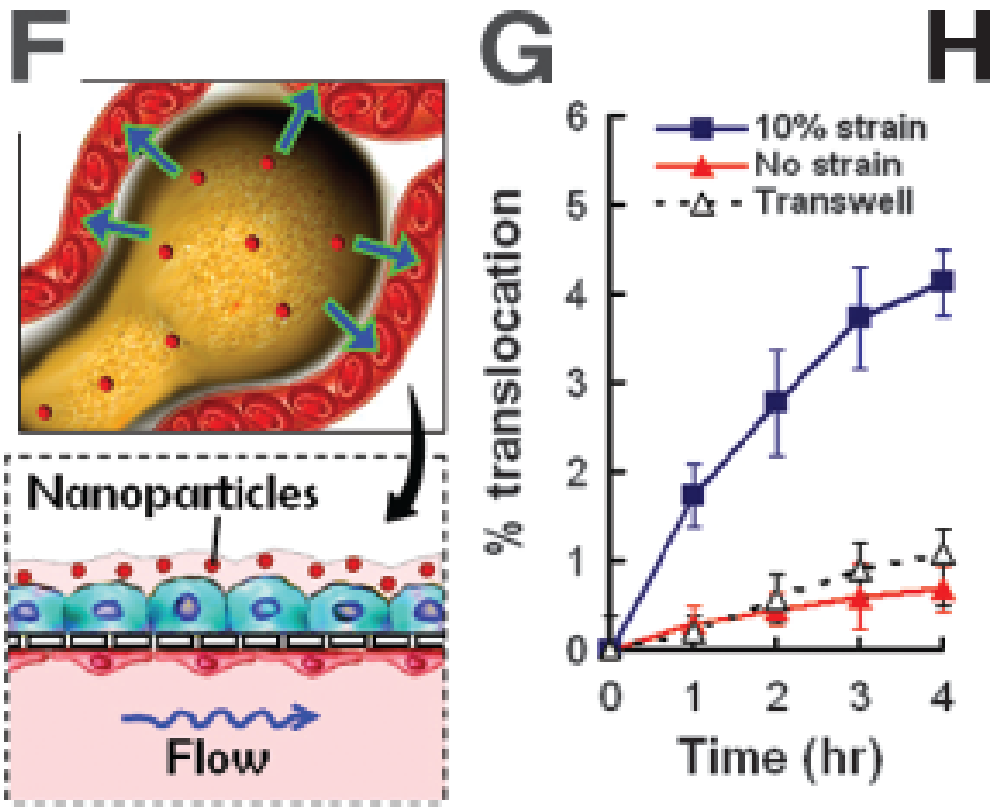
Bacterial Infection Model



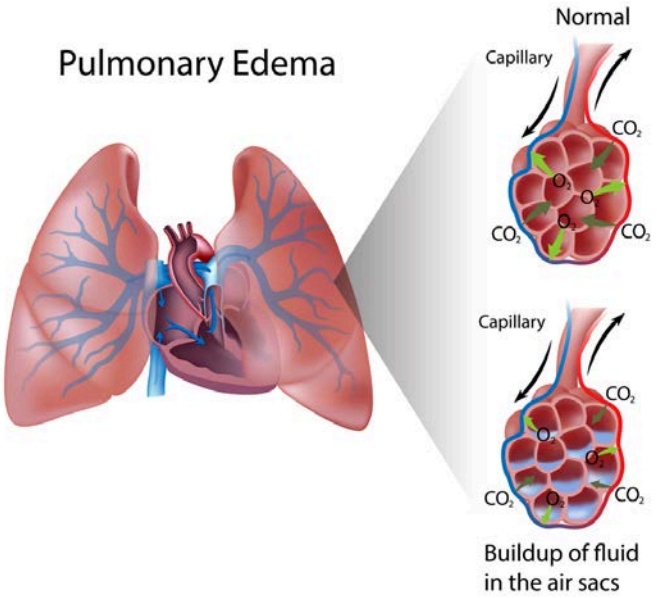


Movie

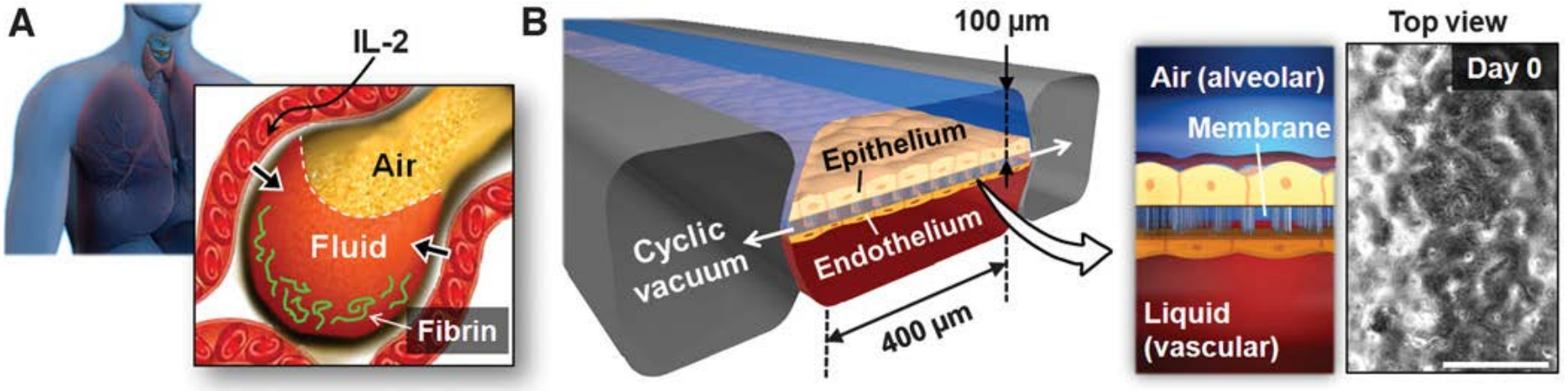
Nanoparticles can enter blood stream



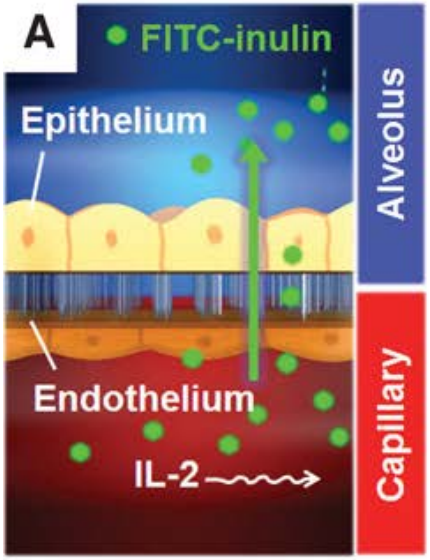
Pulmonary Edema Model

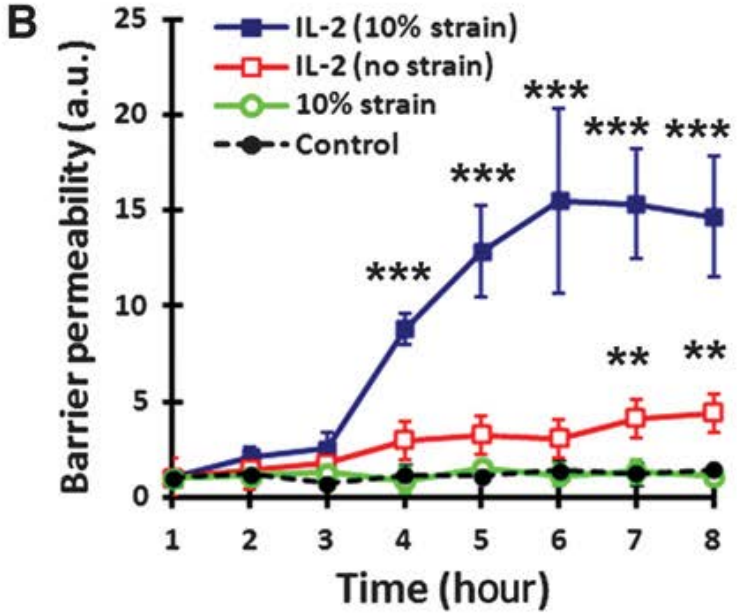


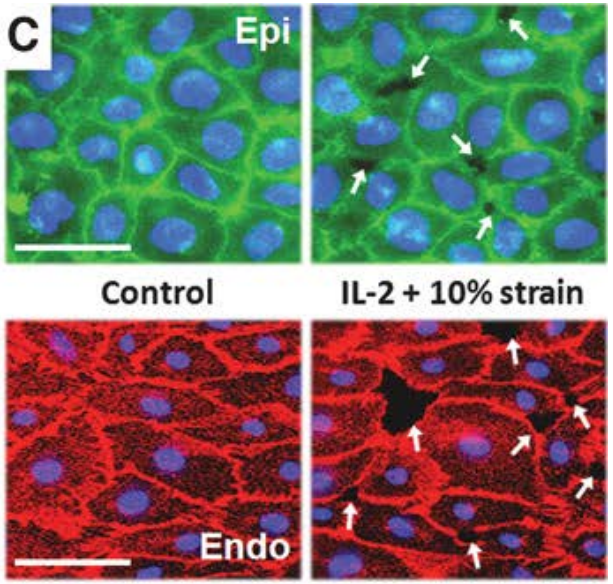
Observed when cancer patients
Were treated with IL-2

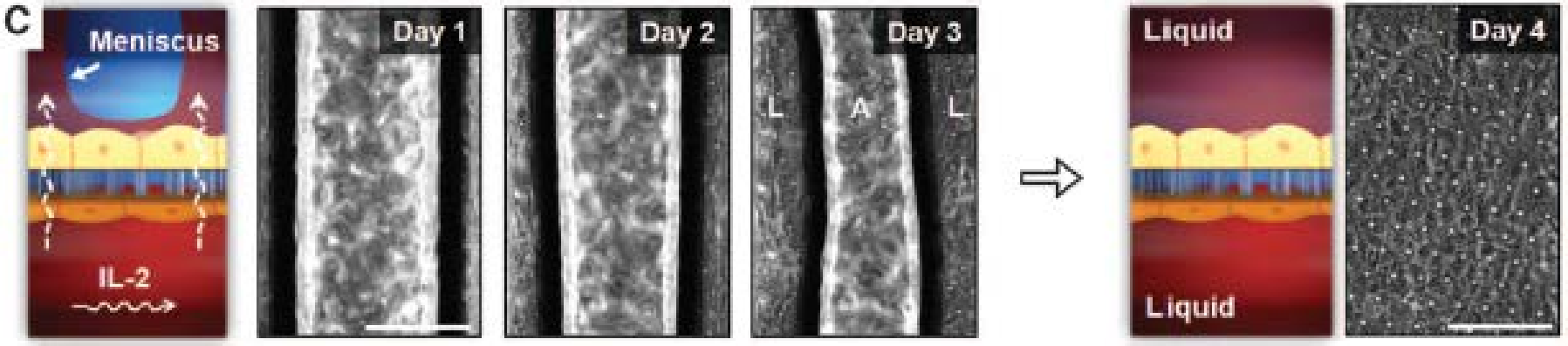


IL-2 crosses the membrane barrier

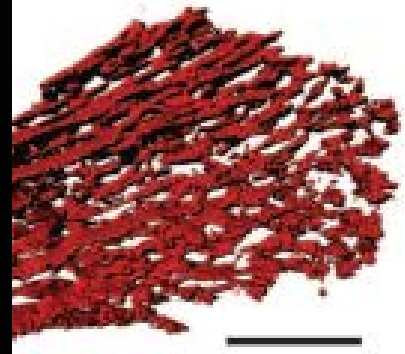




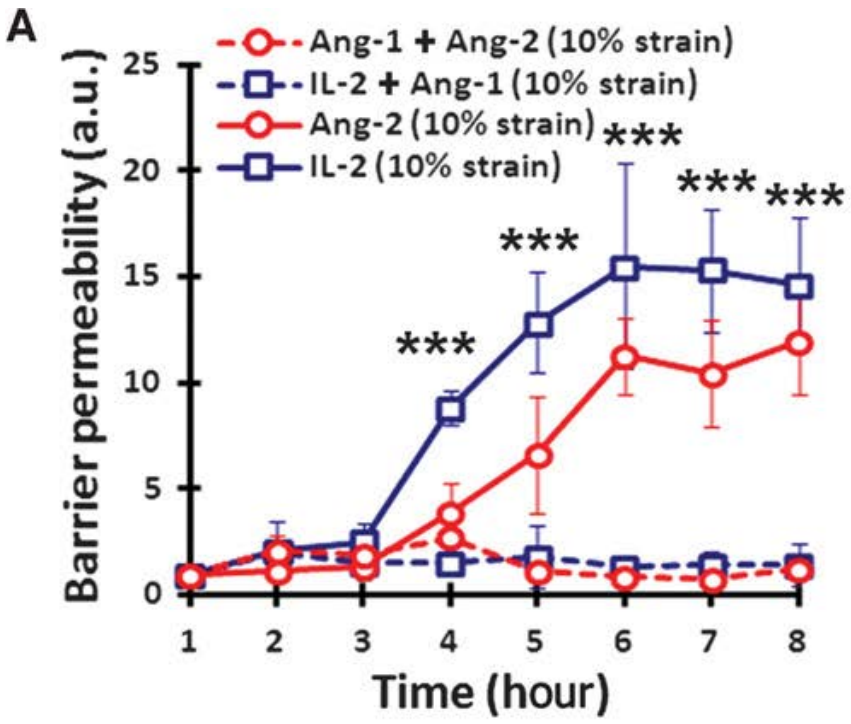


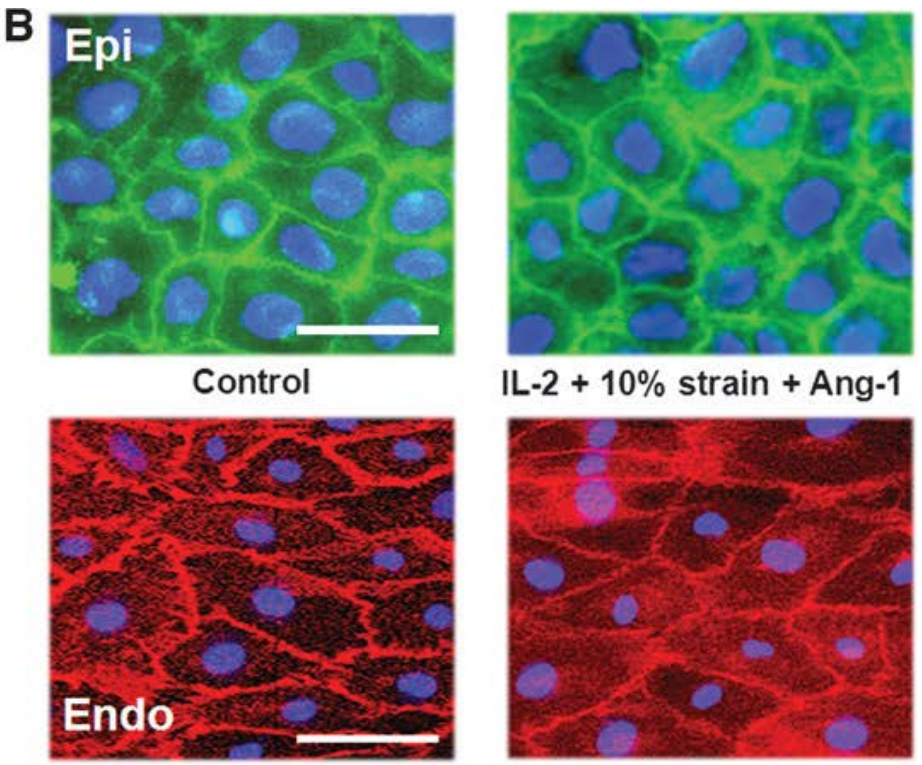


Blood Clots

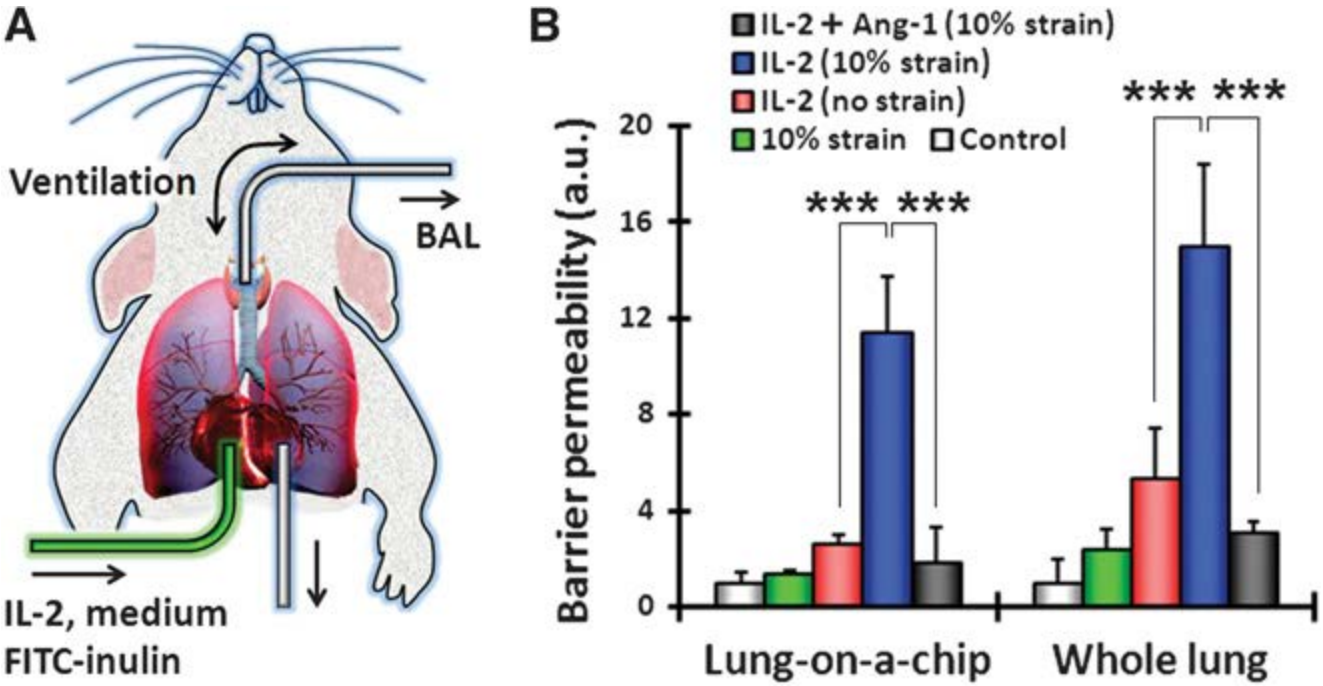


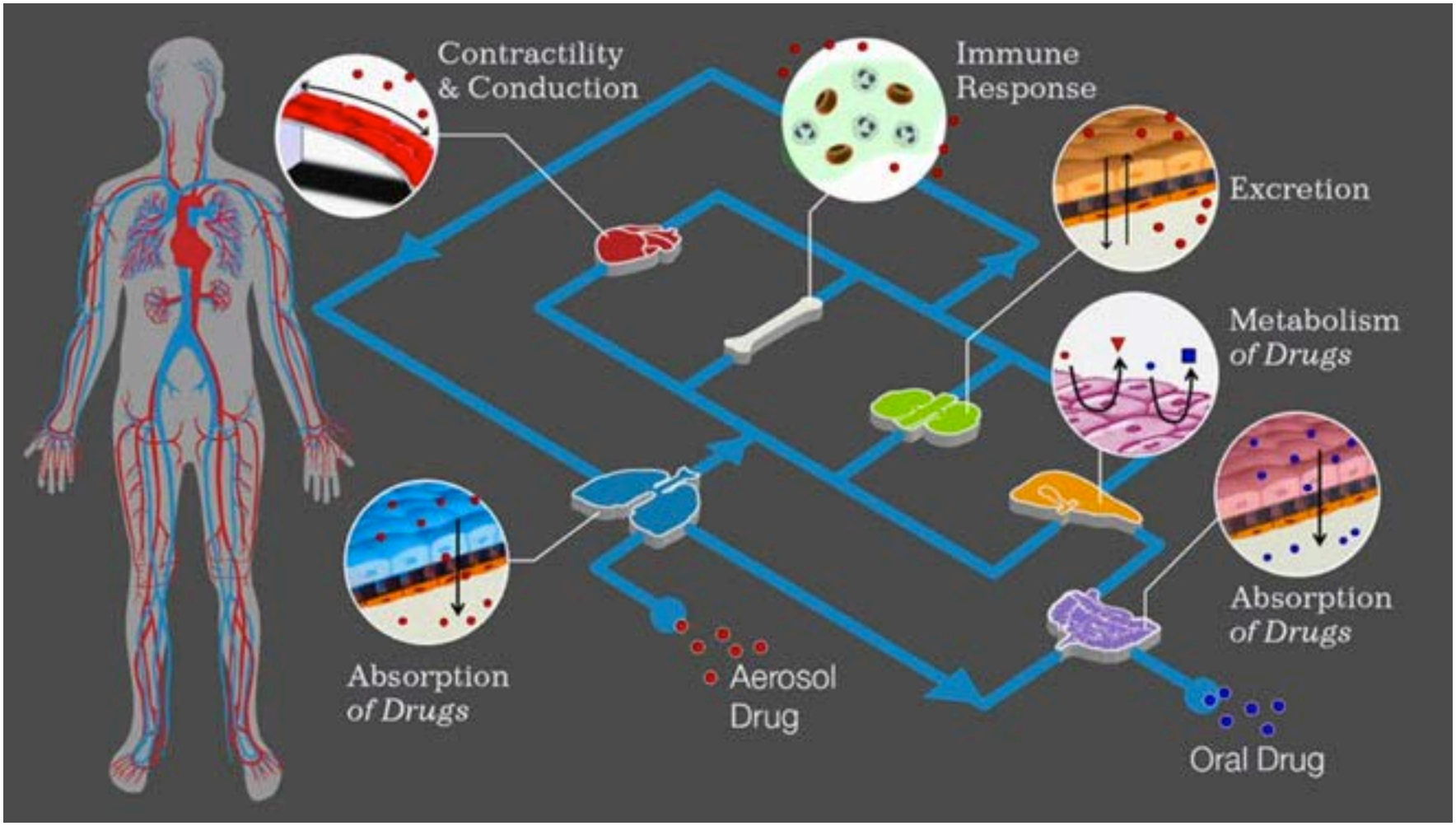
Pharmacological modulation





Comparison with animal model




Contractility
& Conduction
Immune
Response
Excretion
Metabolism
of Drugs
Absorption
of Drugs
Absorption
of Drugs
Aerosol
Drug
Oral Drug

Thank You