Developing Treatments for Inherited Eye Diseases

Edwin M. Stone, M.D., Ph.D.
Stephen A. Wynn Institute for Vision Research
University of Iowa Carver College of Medicine

Financial Disclosures

NONE

Off Label Use

NONE

What do patients want?

What do patients want?

- Treatment

  Protect what they have.

  Get back what they've lost.

What do patients want?

- Treatment

  Protect what they have.

  Get back what they've lost.

- Diagnosis and Prognosis

  What can they expect?

  Will their kids be affected?

What do patients want?

- Treatment
- Diagnosis and Prognosis
- And, they want it TODAY!

What do patients want?

- REALISTIC HOPE that they will get these things reasonably soon.

What do patients want?

- REALISTIC HOPE that they will get these things reasonably soon.
- Not if, WHEN.

What do patients want?

- REALISTIC HOPE that they will get these things reasonably soon.
- Not if, WHEN.
- Realistic Hope requires a plan (the roadmap) and a dedicated group of people committed to carrying out that plan.

Ed’s Roadmap

Ed's Roadmap

- Nonprofit, philanthropic culture.

Ed's Roadmap

- Nonprofit, philanthropic culture.
- Share ideas freely.

  Publish quickly, share detailed methodology when asked.

Ed's Roadmap

- Nonprofit, philanthropic culture.
- Share ideas freely.

  Publish quickly, share detailed methodology when asked.

- Leave no one behind.

  Work on lots of different diseases (early and late stages) and lots of different genes at the same time.

Ed's Roadmap

- Reduce waste.

  Grant writing, annual reports, institutional overhead, administrative layers.

Ed's Roadmap

- Reduce waste.

  Grant writing, annual reports, institutional overhead, administrative layers.

- Replace animal models with cultured cells whenever possible.

  Use cells for efficacy, animals for safety.

Ed's Roadmap

- Reduce the cost and improve the sensitivity of genetic tests.

  Find patients who might wish to join trials.

  Find the remaining disease-causing genes.

Ed's Roadmap

- Reduce the cost and improve the sensitivity of genetic tests.

  Find patients who might wish to join trials.

  Find the remaining disease-causing genes.

- Develop philanthropically funded GMP facilities to reduce the costs of therapeutic vectors and cells.

Ed's Roadmap

- Develop reusable gene therapy strategies.

  Especially genome editing methods for large and/or expression-sensitive genes.

Ed's Roadmap

- Develop reusable gene therapy strategies.

  Especially genome editing methods for large and/or expression-sensitive genes.

- Develop cell therapies based upon patient-derived stem cells.

  Reduce the risk of immune rejection.

Ed's Roadmap

- Analyze existing clinical data to determine the best timing and anatomic location for therapy.

Ed's Roadmap

- Analyze existing clinical data to determine the best timing and anatomic location for therapy.
- Focus almost entirely on Phase I-II clinical trials with long but fairly conventional follow-up.

Ed's Roadmap

- Analyze existing clinical data to determine the best timing and anatomic location for therapy.
- Focus almost entirely on Phase I-II clinical trials with long but fairly conventional follow-up.
- Do everything with a sense of URGENCY.

Achievable Goal #1 for Rare Genetic Diseases

Have clinical trials of gene therapy for DOZENS of genes underway at the same time.

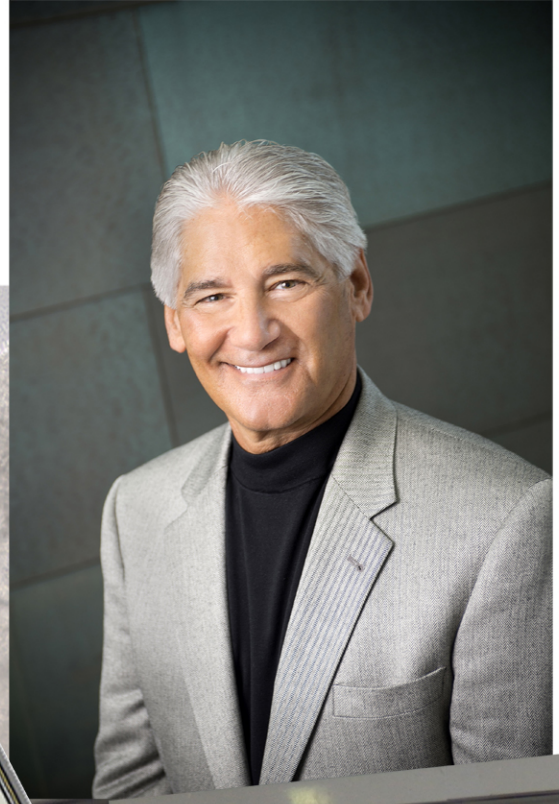
Achievable Goal #2 for Rare Genetic Diseases

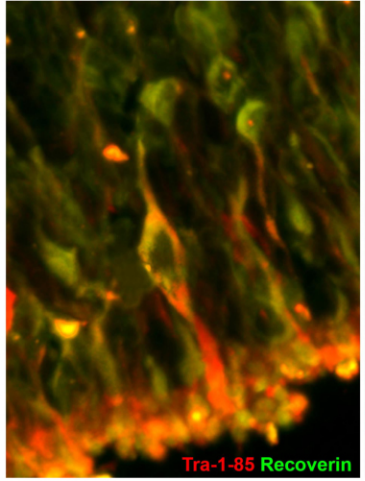
Reduce the time between the initial discovery of a new disease gene and the first gene therapy trial in human subjects to . . .

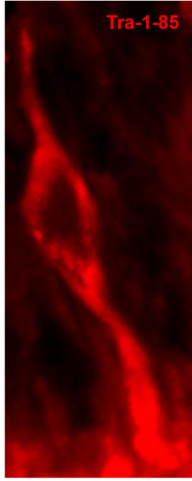
< 24 months

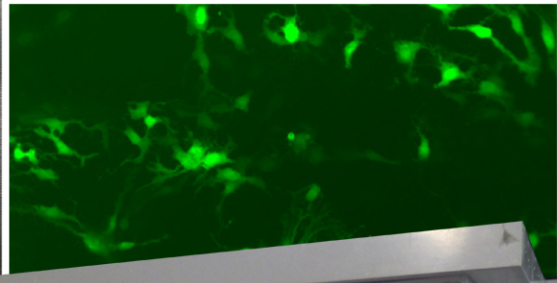
Steven W. Dezii

Translational Vision Research Facility









The Stephen A. Wynn Institute for Vision Research

Vivimus Enim Remedium

Overview of Genetic Testing for Usher Syndrome

Usher Cohort
2204 patients, 1751 families

- William Kimberling
- Sam Jacobson
- Jerry Fishman
- Richard Weleber
- Elias Traboulsi
- Elise Heon
- Byron Lam
- Claes Moller
- Sten Andreasson
- Alex Levin
- Christine Kay
- Raymond Iezzi
- Mina Chung
- Alessandro Iannaccone

Usher Cohort
2204 patients, 1751 families

- 1624 probands screened with an MDPD strategy – 52% positive.
- 35 of the “negative” patients sequenced with whole exome sequencing – 15 positive (42%).
- Suggests that this combination has a 72% sensitivity.

Usher Cohort

2204 patients, 1751 families

- USH2A 575
- MYO7A (1B) 215
- CDH23 (1D) 67
- USH3A 40
- PCDH15 (1F) 28
- USH1C 27
- GPR98 (2C) 9

1000 Consecutive Families

71 with Usher Syndrome

58% positive with MDPD test

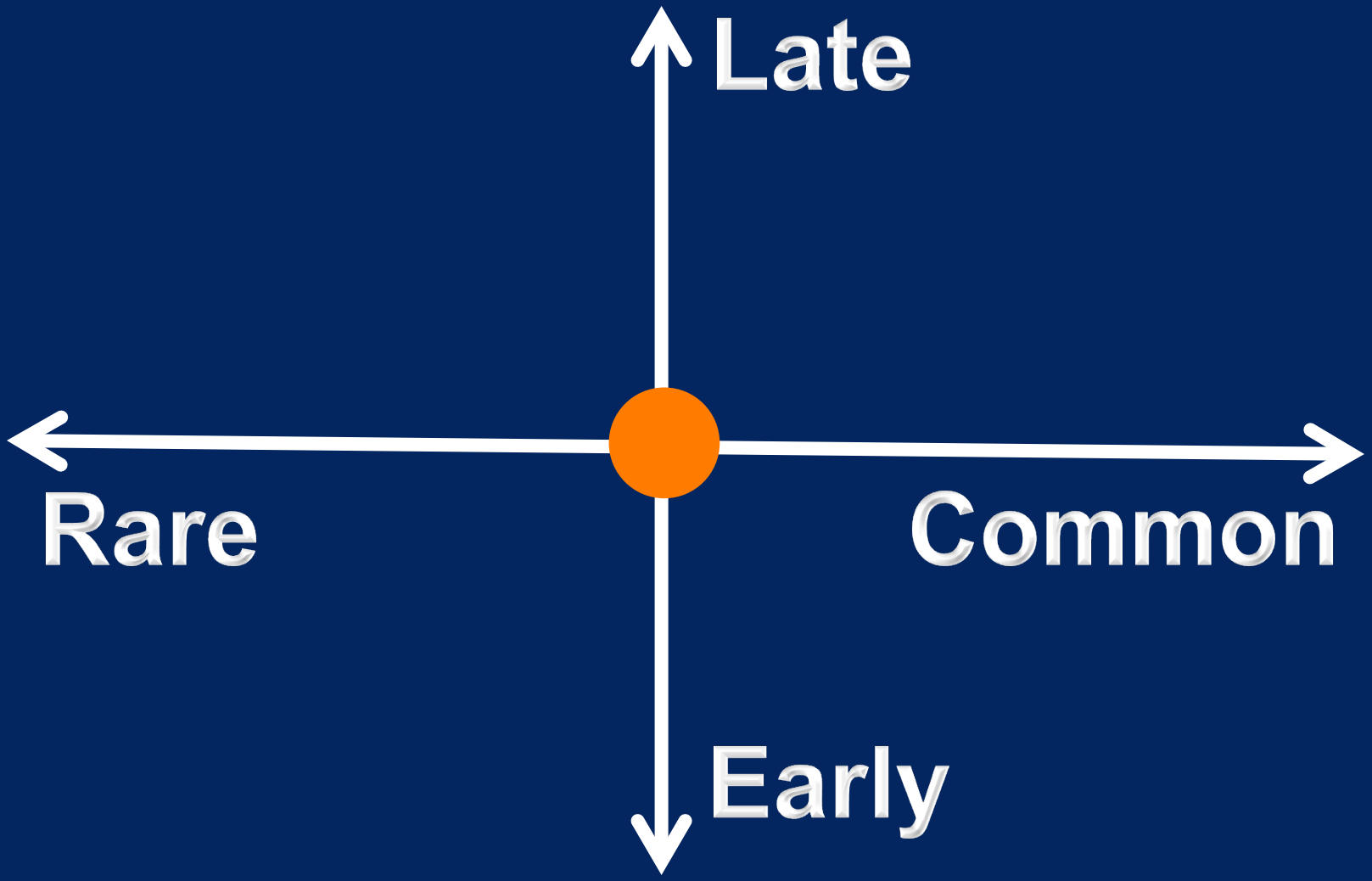
77% after exome

- USH2A 33
- MYO7A (1B) 6
- CDH23 (1D) 8
- USH3A 0
- PCDH15 (1F) 1
- USH1C 3
- GPR98 (2C) 4

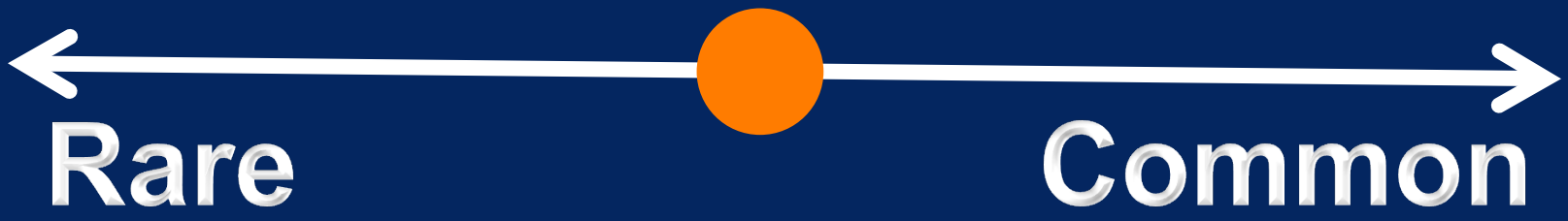
Why the big focus on philanthropy?

Treatment of Genetic Eye Disease

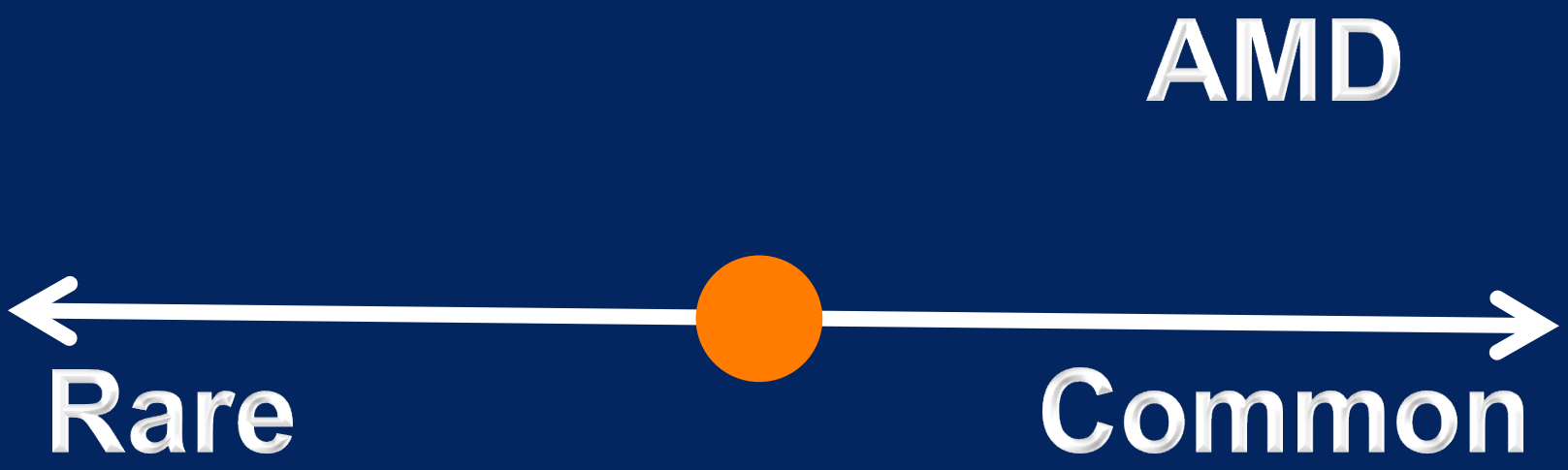
Treatment of Genetic Eye Disease



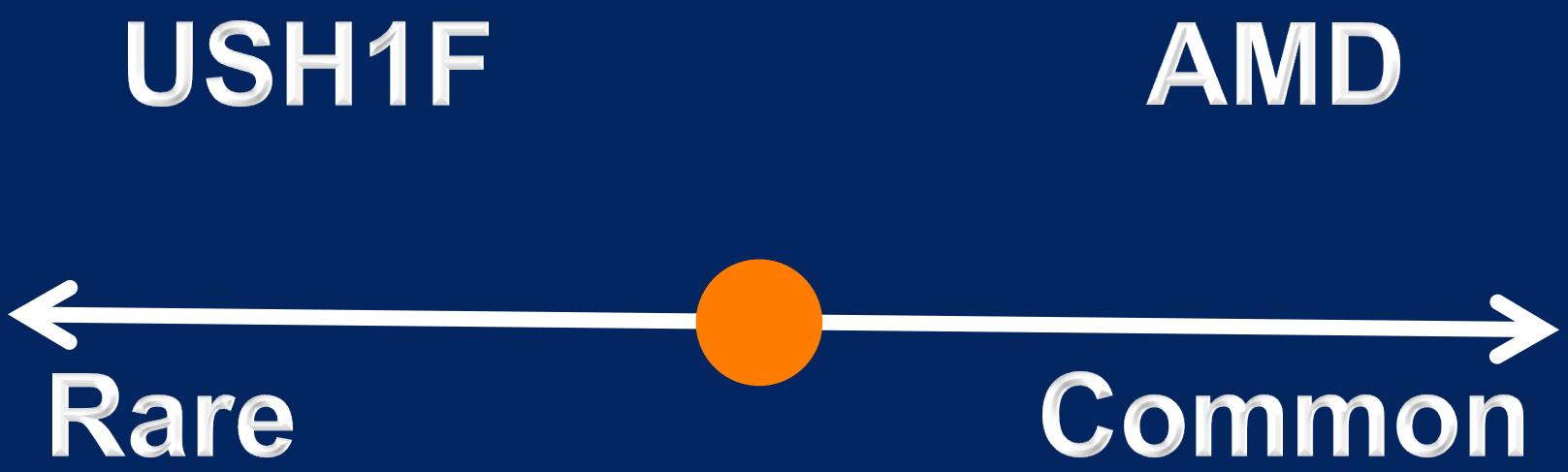
Treatment of Genetic Eye Disease



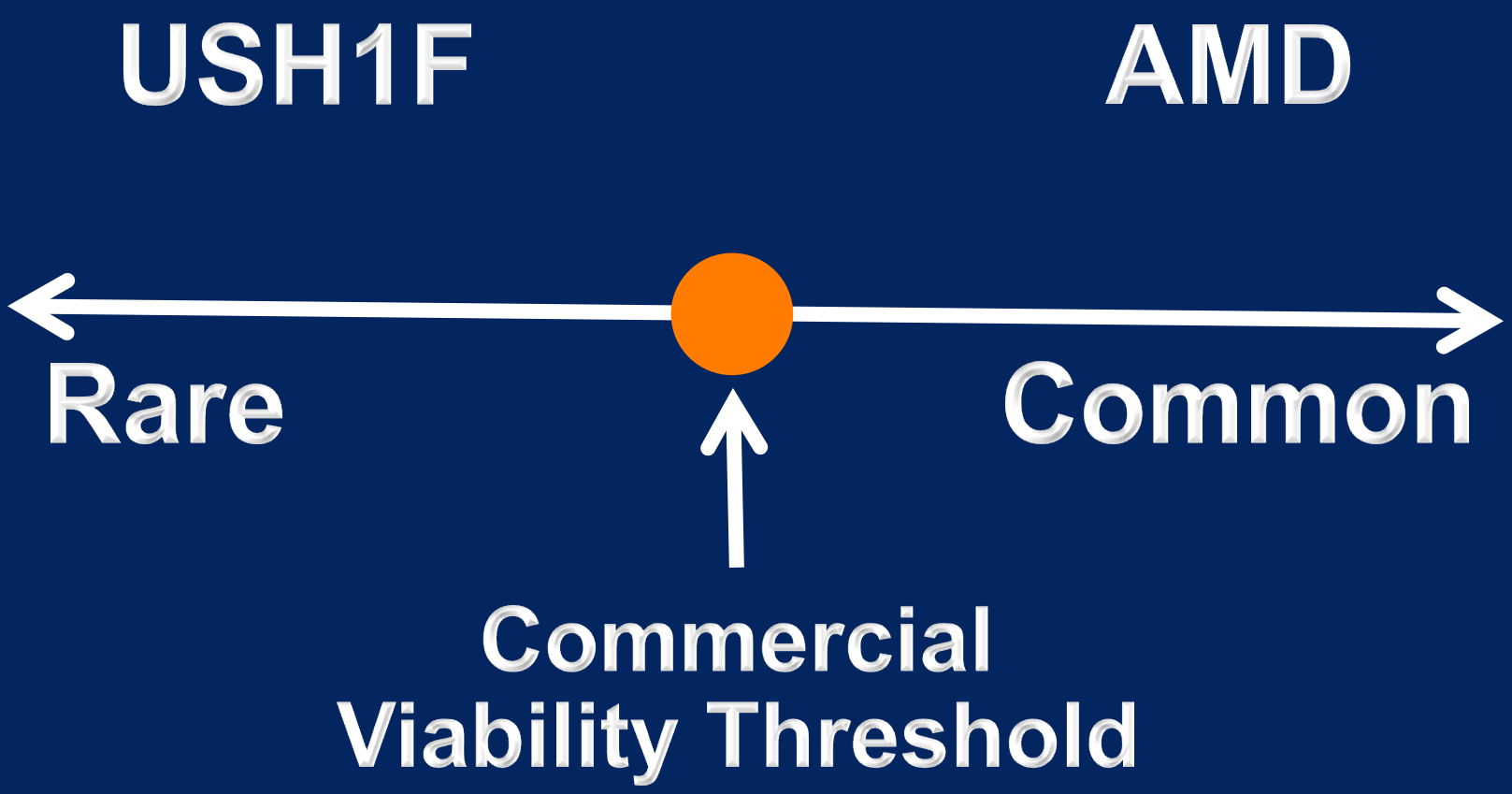
Treatment of Genetic Eye Disease



Treatment of Genetic Eye Disease

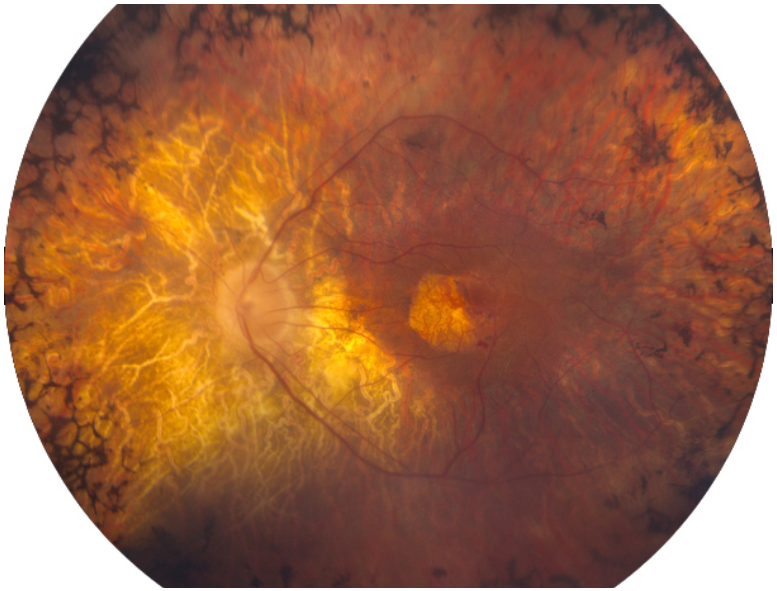


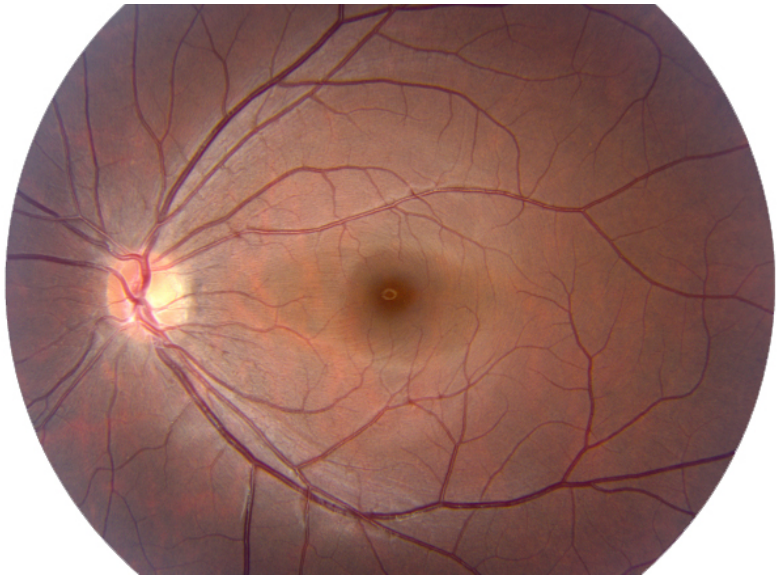
Treatment of Genetic Eye Disease



Why work on lots of diseases (and different types of treatments) at once?

Leave No One Behind

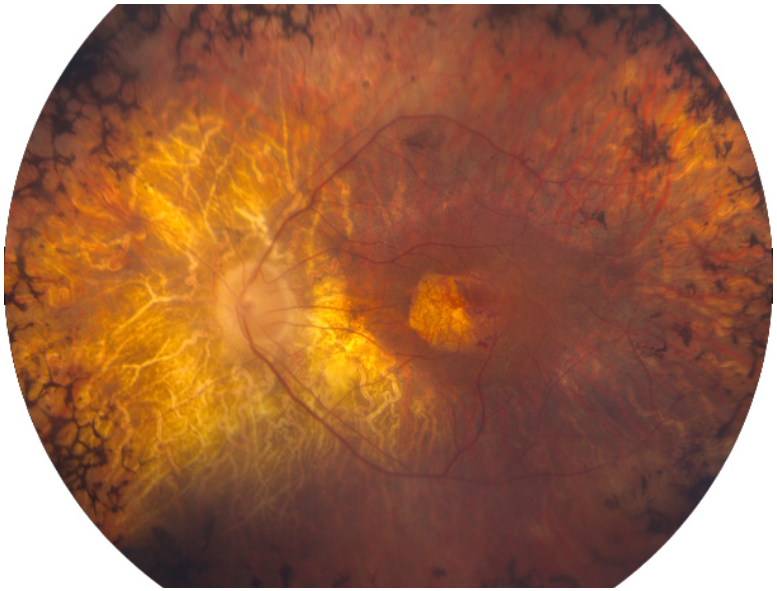


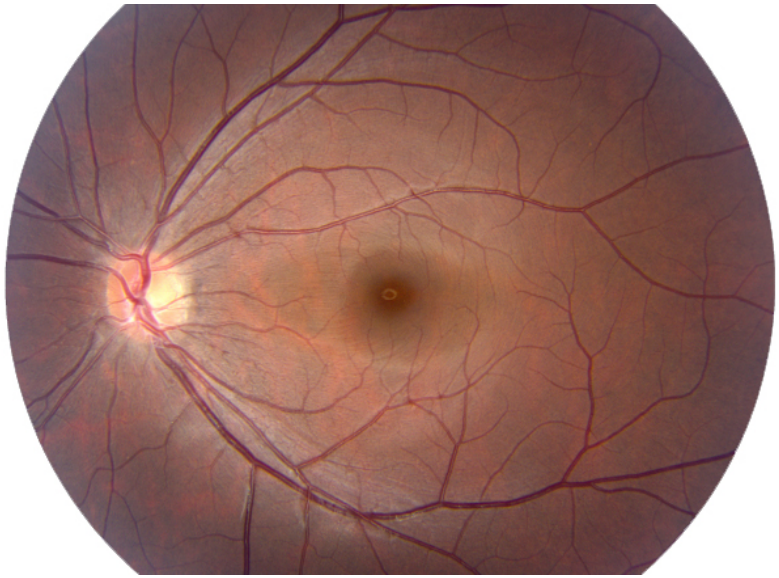


Disease Progression

- Assistive Technology
- Prostheses/Optogenetics
- Cell Therapy
- Gene Therapy
- Drugs/Neuroprotection
- Family Planning

Leave No One Behind





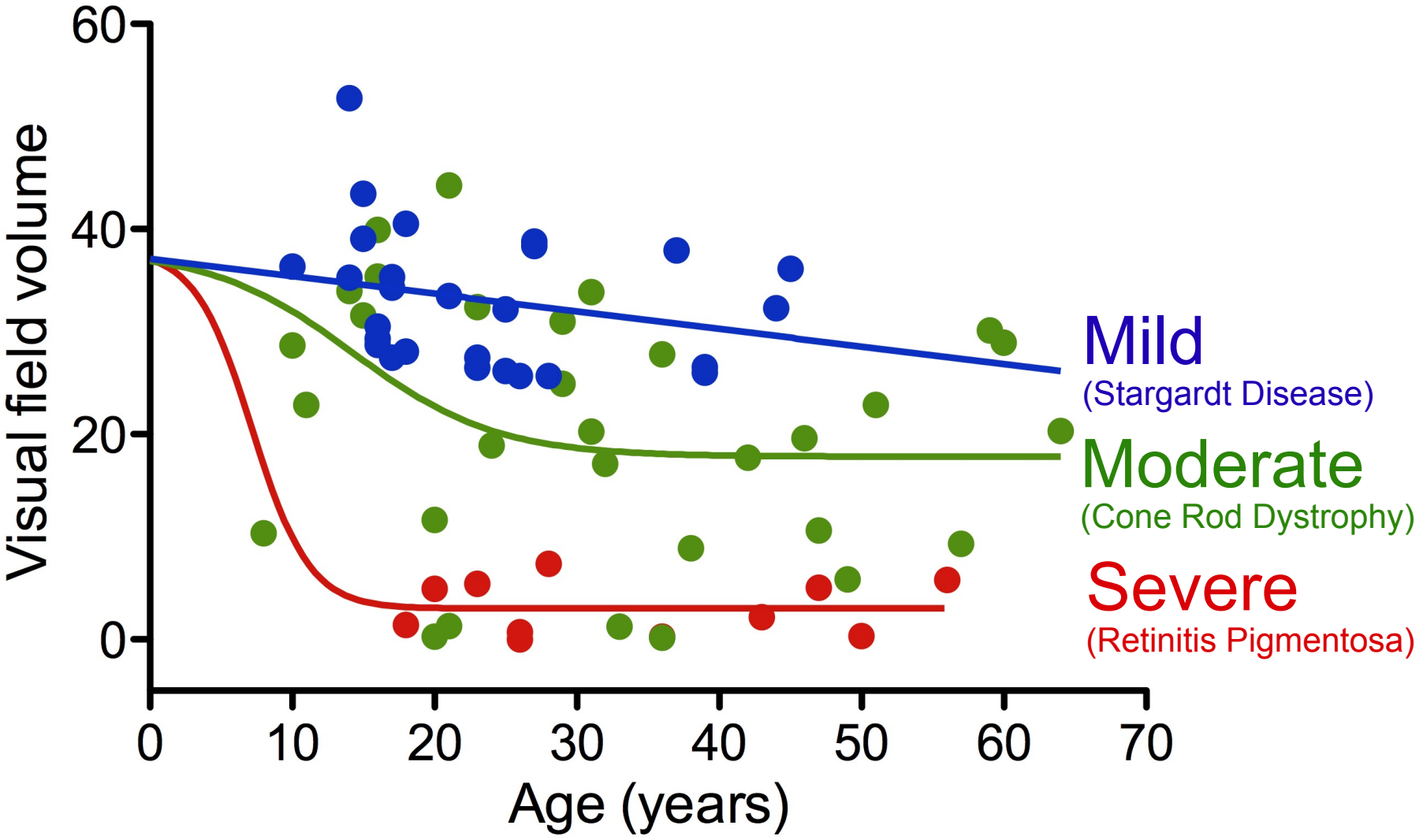
Disease Progression

Gene Therapy


Nurse

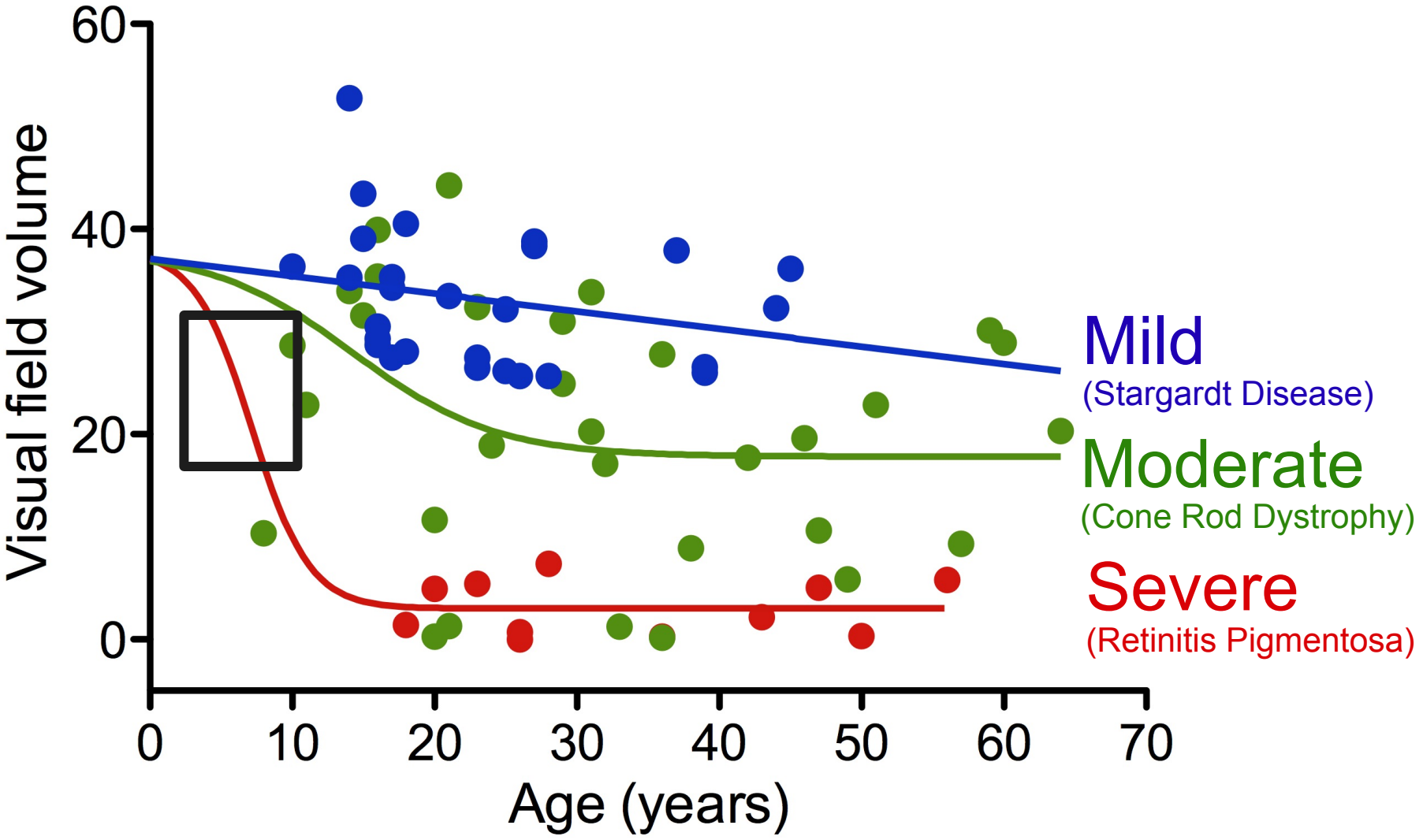
Use existing clinical data to determine the timing and location of therapy.

ABCA4-associated Retinal Disease

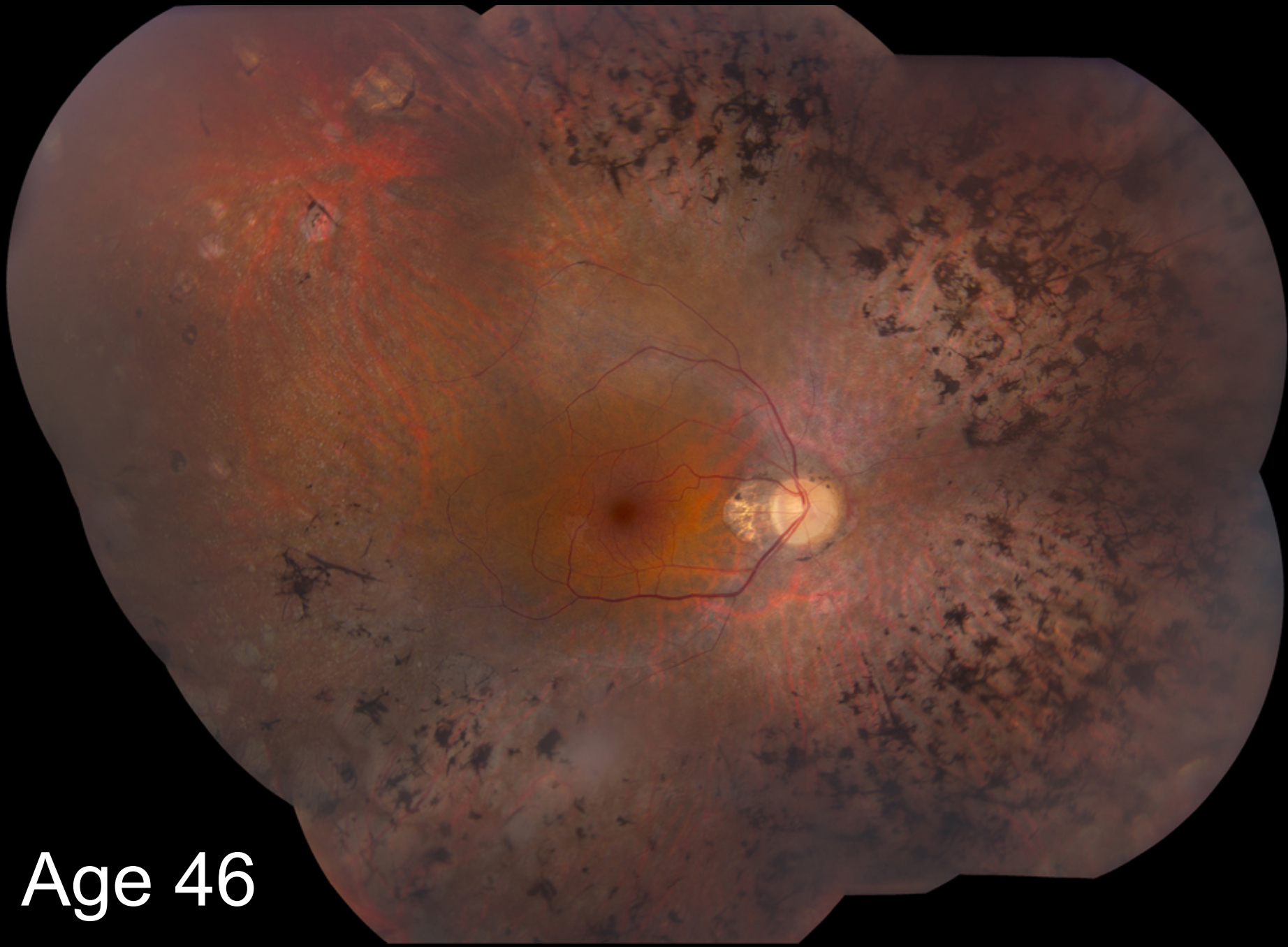


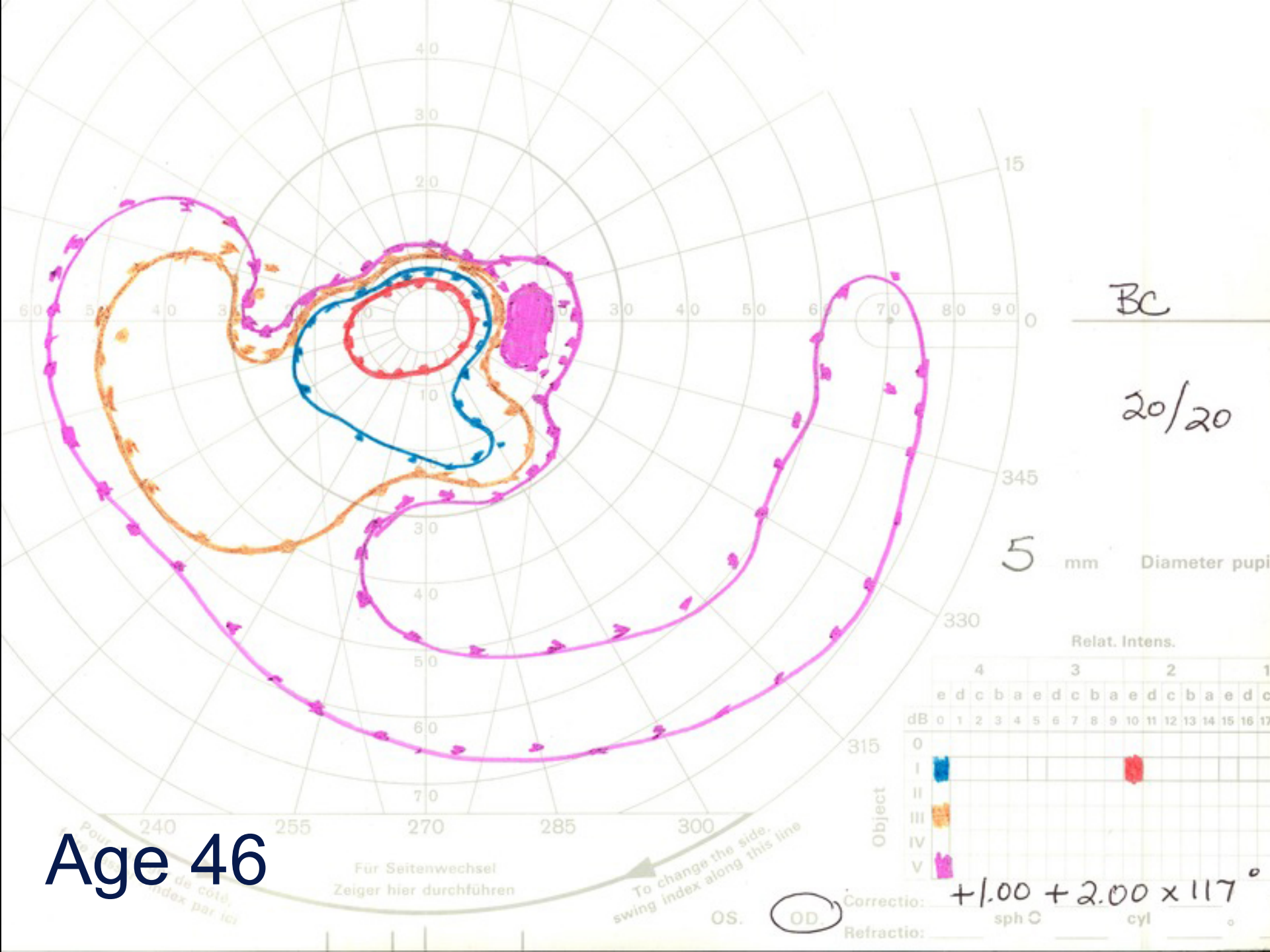
Redrawn from Schindler, et al., *Human Molecular Genetics*, 2010

ABCA4-associated Retinal Disease

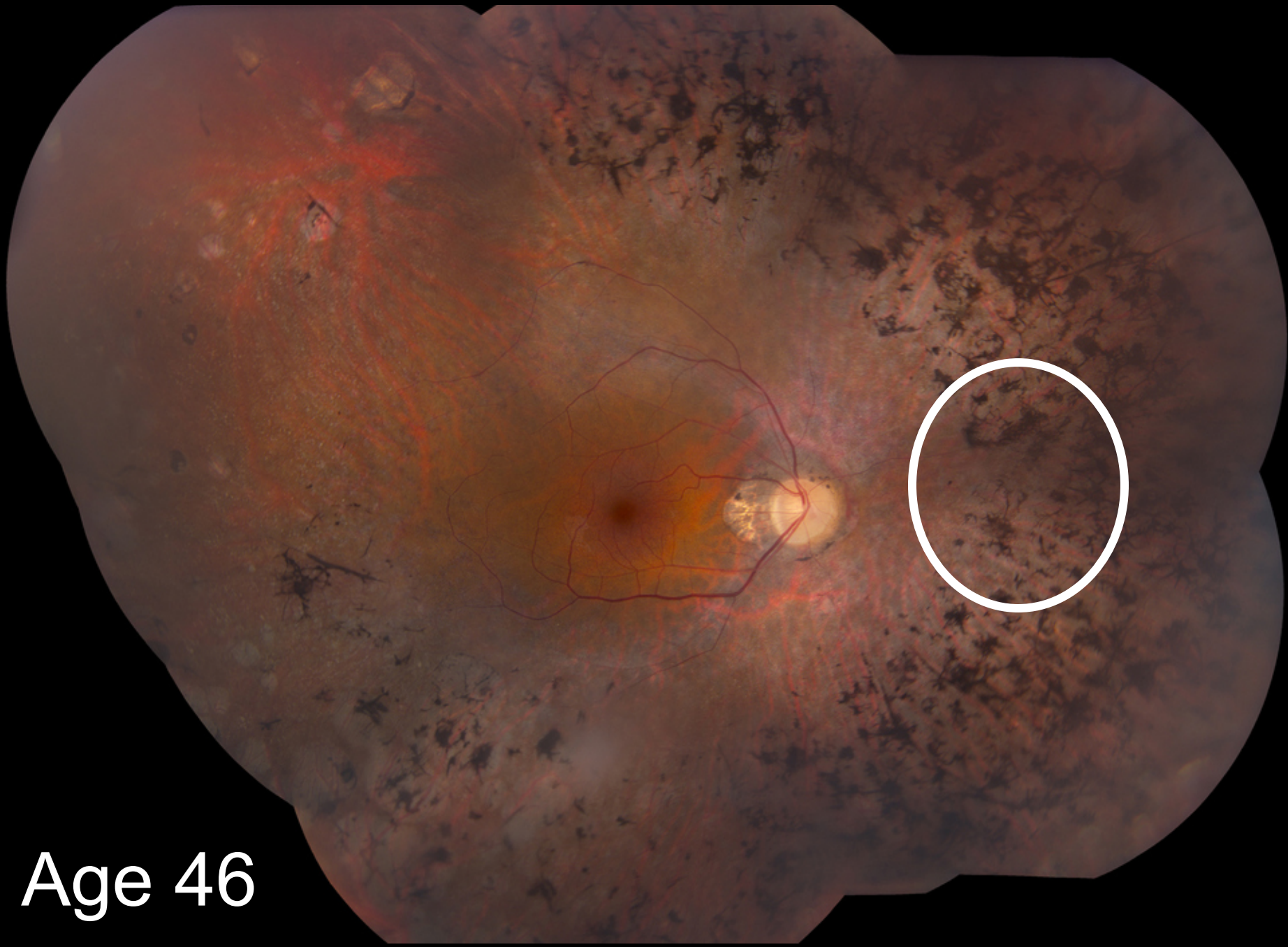


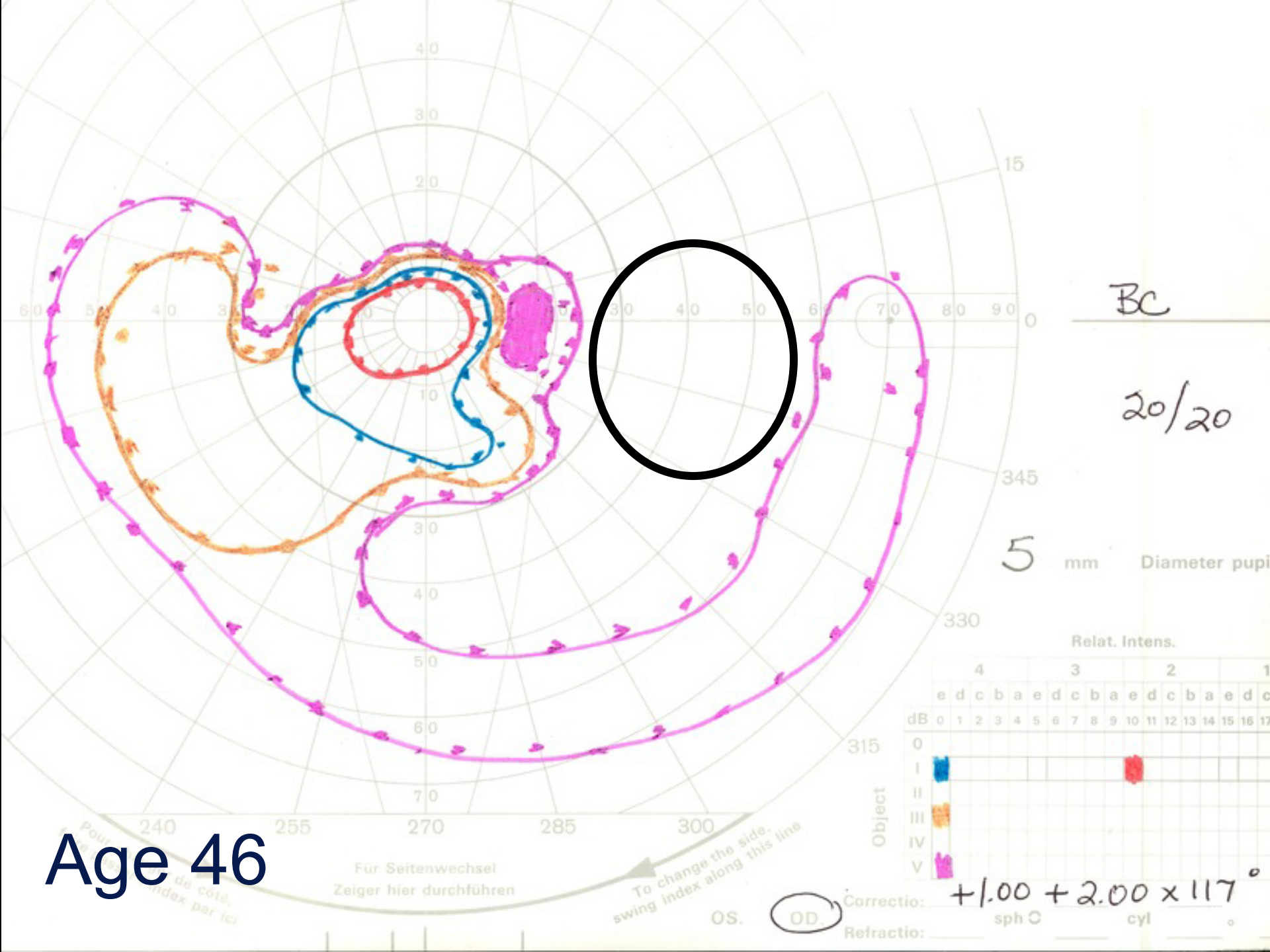
Redrawn from Schindler, et al., *Human Molecular Genetics*, 2010


Age 46


BC
20/20
5
mm
Diameter pupi
Relat. Intens.
Object
OS.
OD.
+1.00 +2.00 x 117 °
sph
cyl
Correctio:
Refractio:
Für Seitenwechsel
Zeiger hier durchführen
To change the side,
swing index along this line

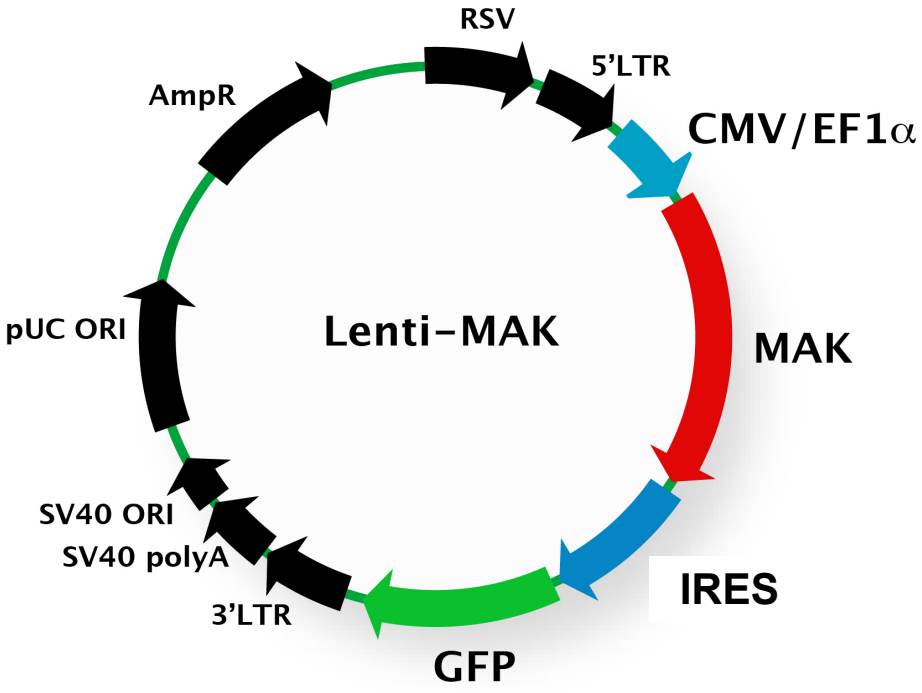
Age 46

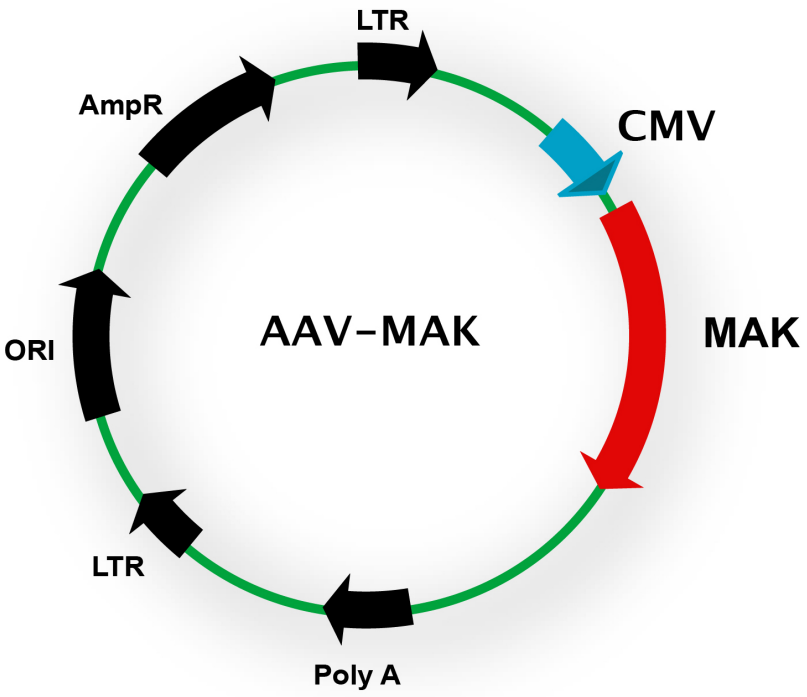

Age 46


BC
20/20
5
mm
Diameter pupi
Relat. Intens.
Object
+1.00 +2.00 x 117 °
sph
cyl
OS.
OD.
Für Seitenwechsel
Zeiger hier durchführen
To change the side,
swing index along this line

Age 46

MAK gene transfer vector designs



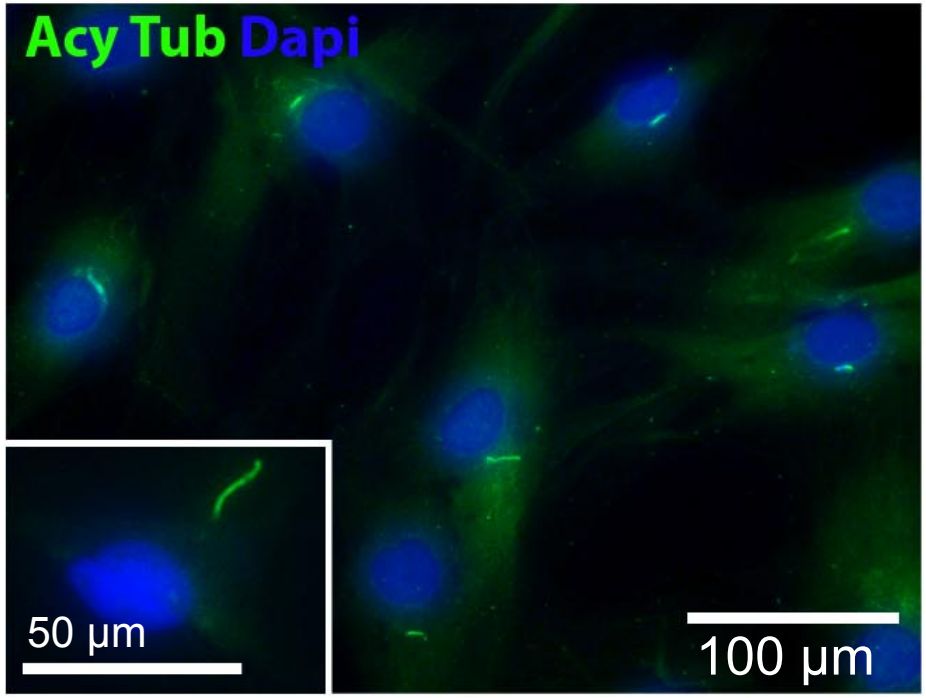


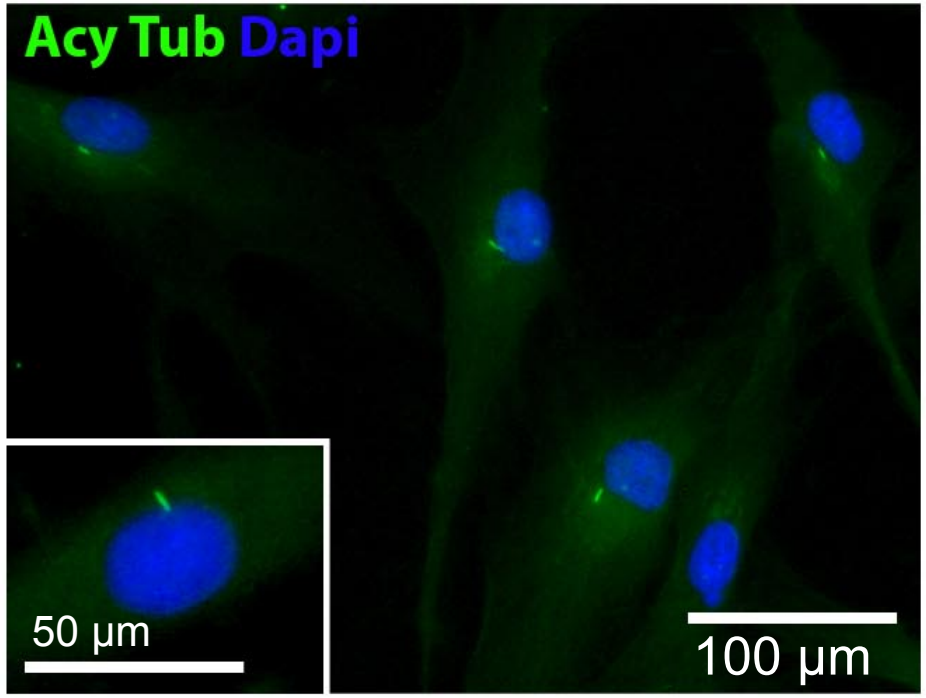
MAK cDNA = 1800 bp

Replace animal models with cultured cells whenever possible.

Untreated

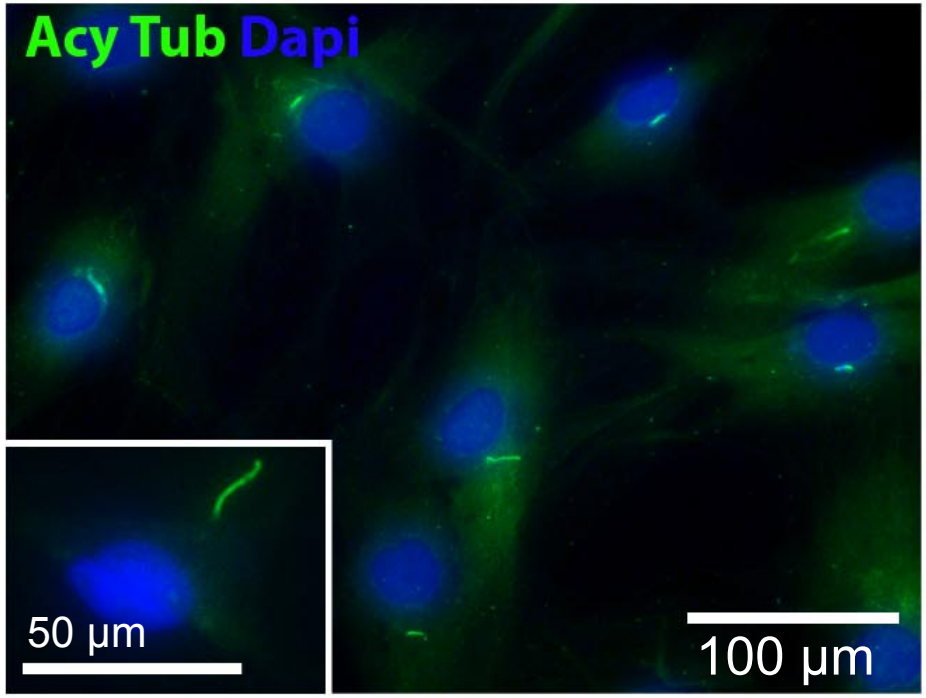
Treated

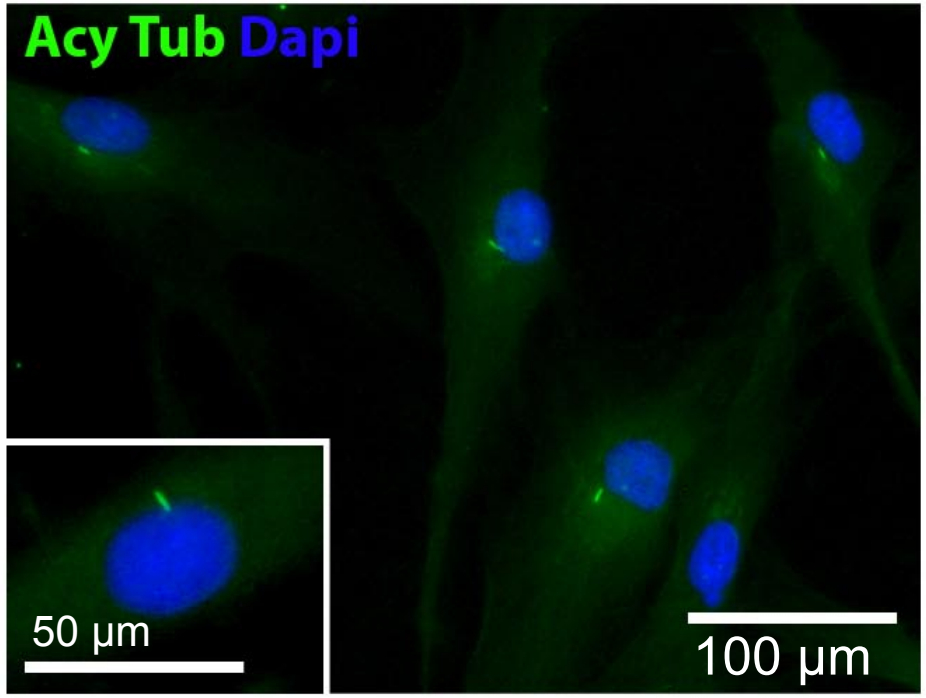


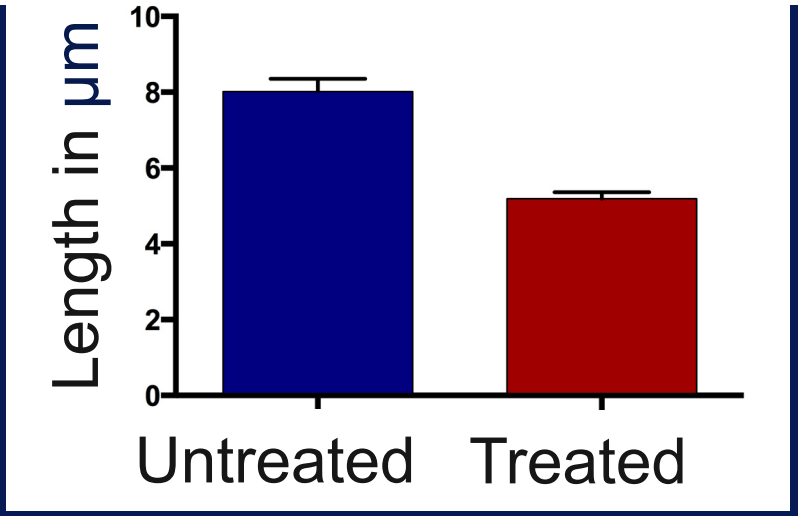


Untreated

Treated







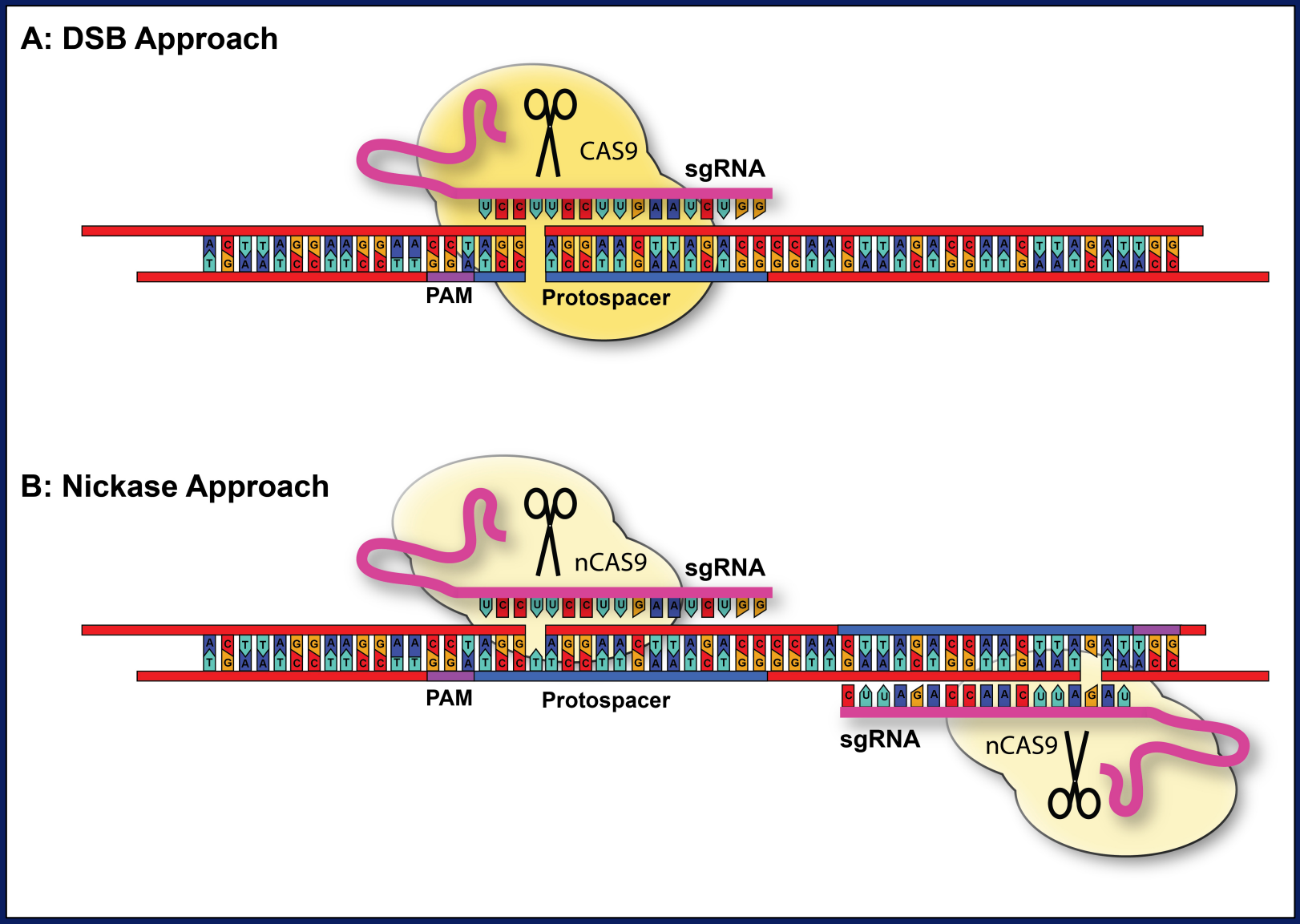
Genome editing for very large or expression sensitive genes.

CRISPR/Cas9

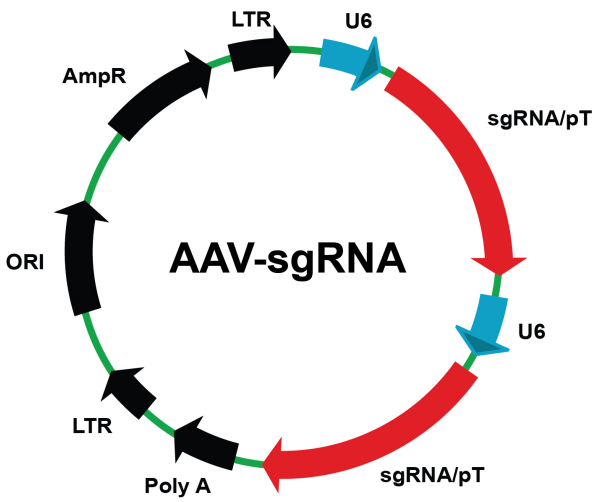
(clustered regularly interspaced short palindromic repeats)

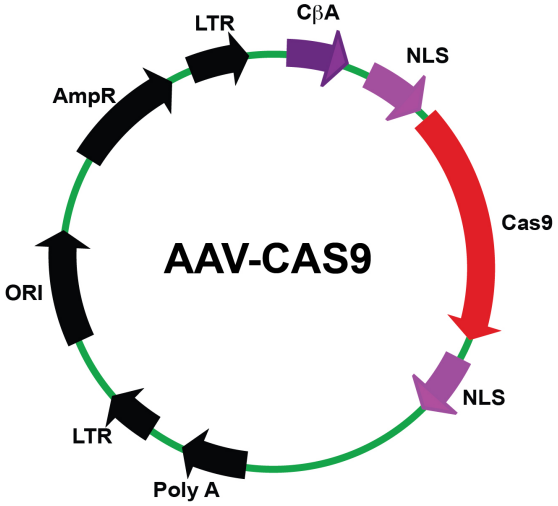
Feng Zhang
Keith Joung
George Church

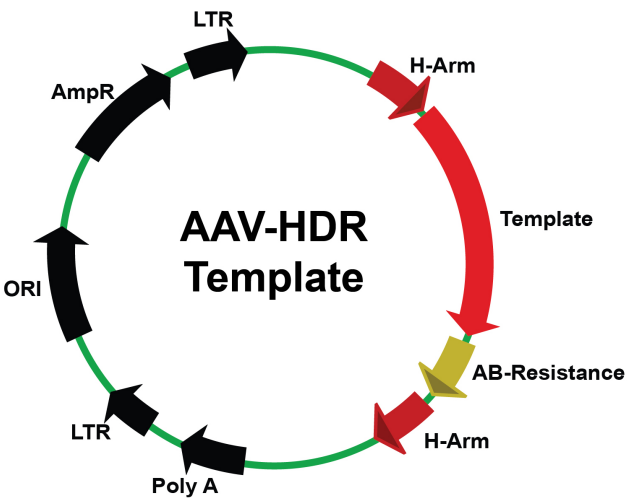
CRISPR/Cas9


A: DSB Approach
CAS9
sgRNA
PAM
Protospacer
B: Nickase Approach
nCAS9
sgRNA
PAM
Protospacer
sgRNA
nCAS9

CRISPR-CAS9/HDR for genome editing



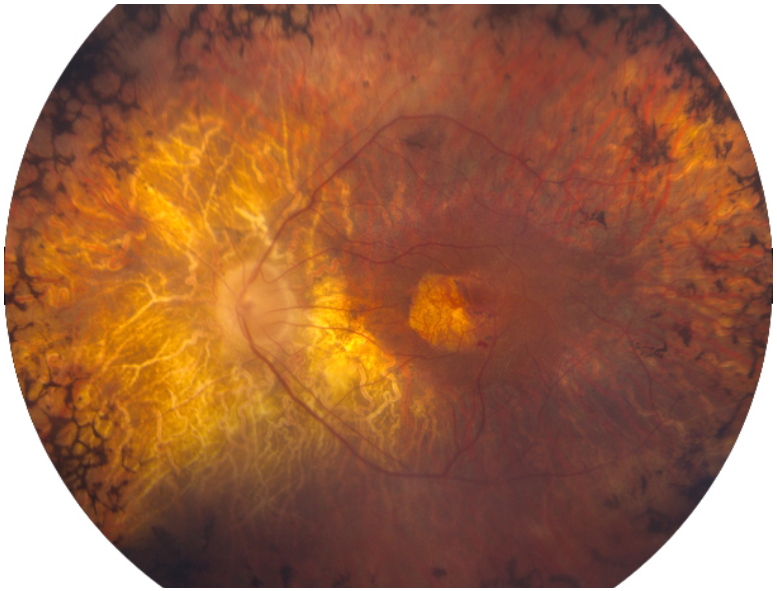


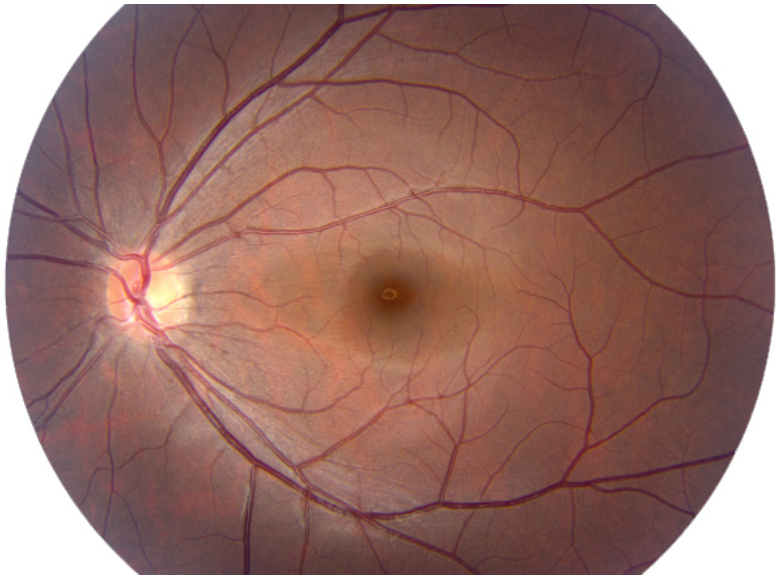


Simultaneous delivery of 3 separate AAVs to induce "homologous directed repair" (HDR)

1) Tandem guide RNAs for efficient targeting
2) Mutant Nicking CAS9 for specific cutting
3) Repair template with selection cassette to efficiently select corrected iPSCs

Leave No One Behind

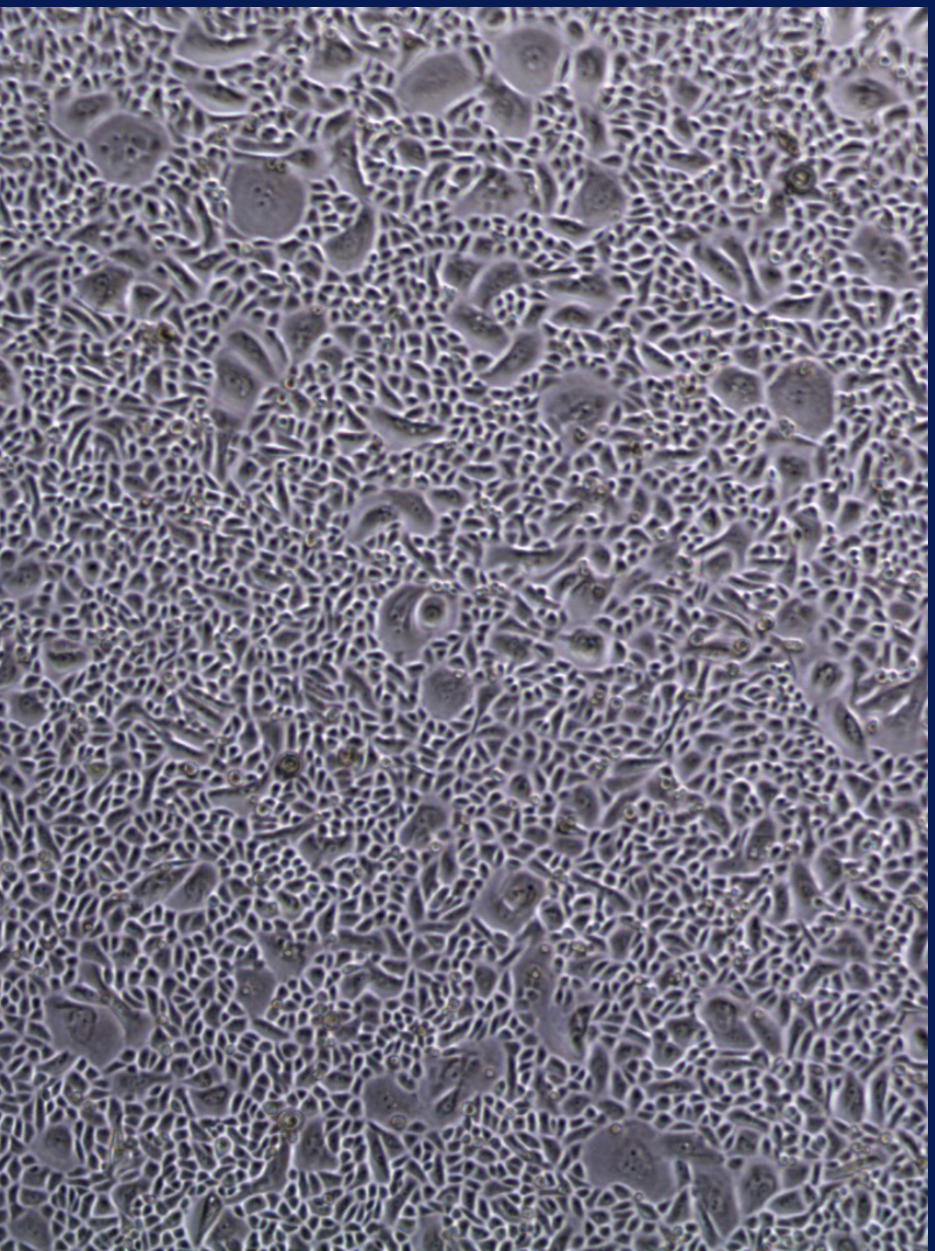




Disease Progression

Cell Therapy

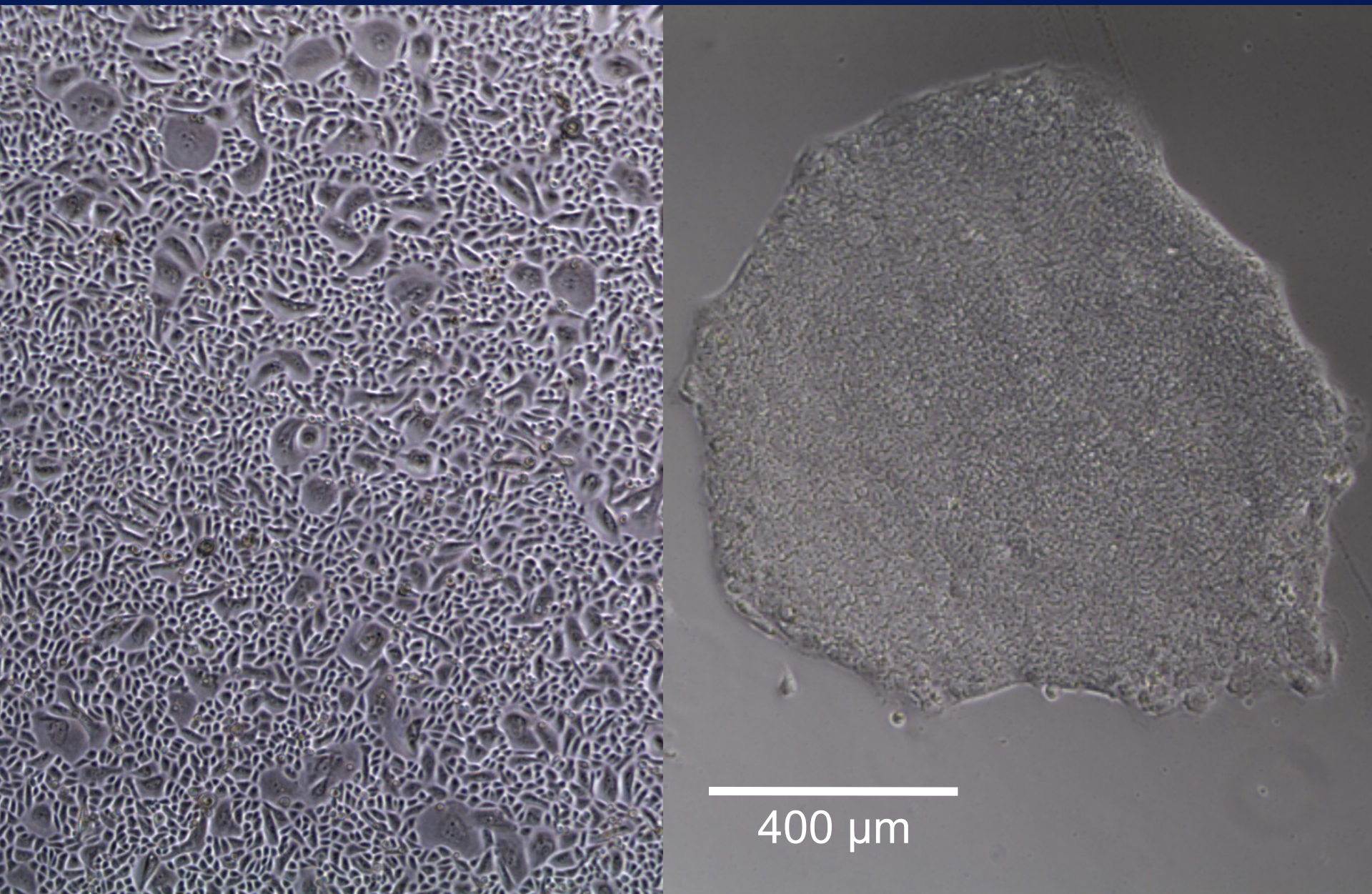
Keratinocytes

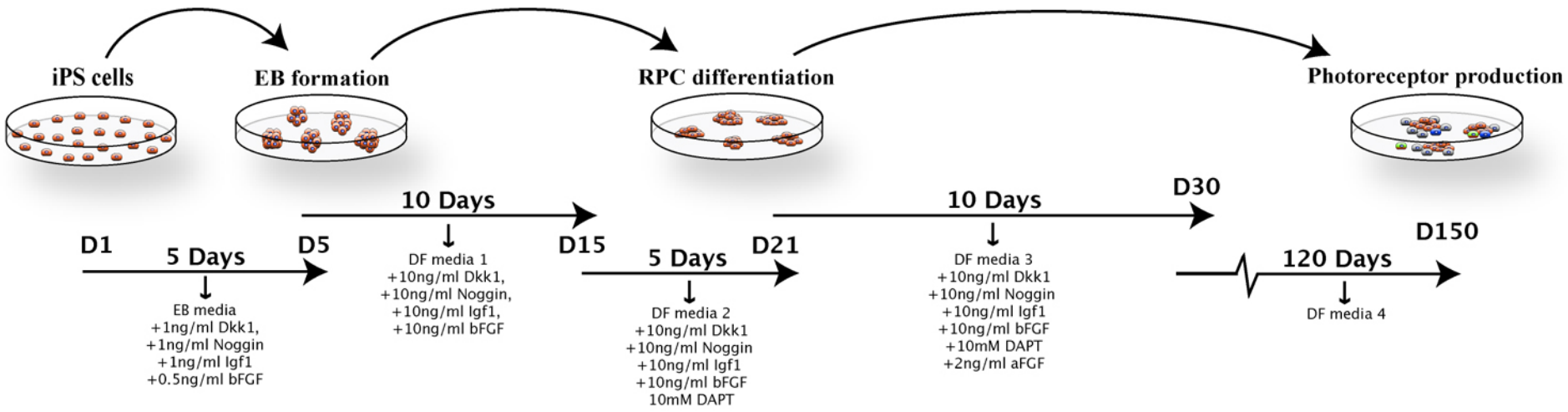


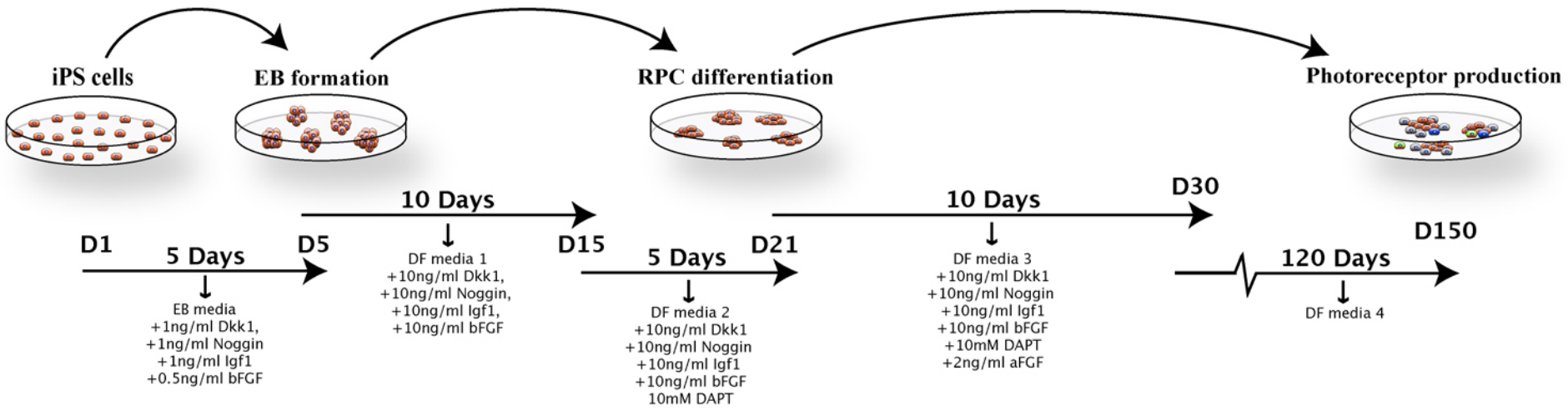
400 µm

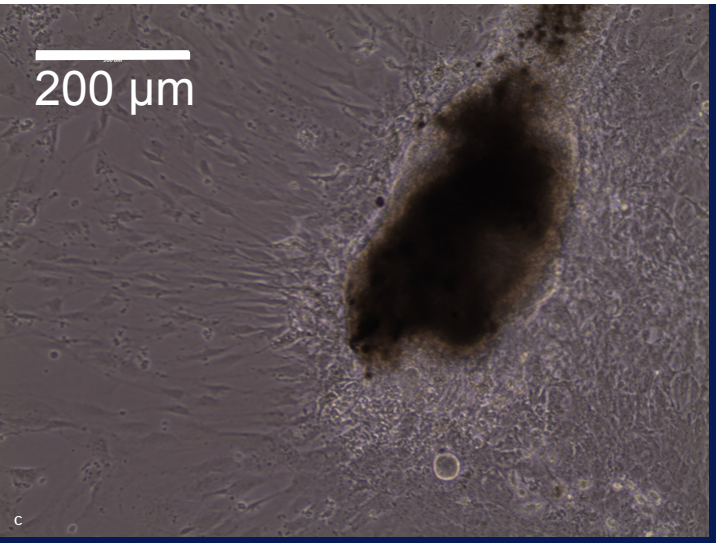
Keratinocytes

Isolated iPSCs

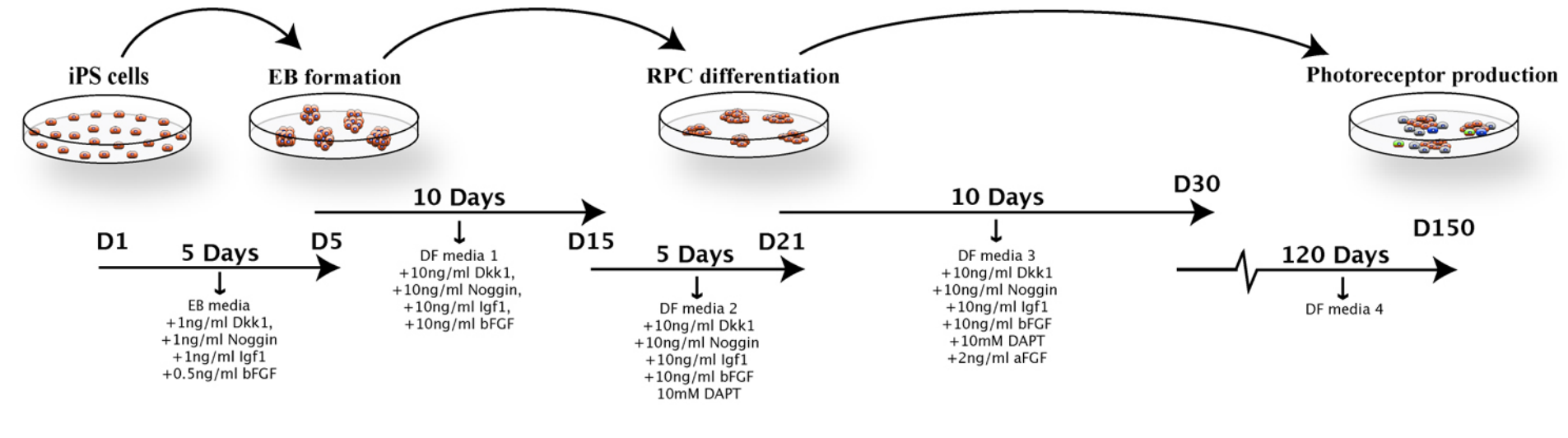


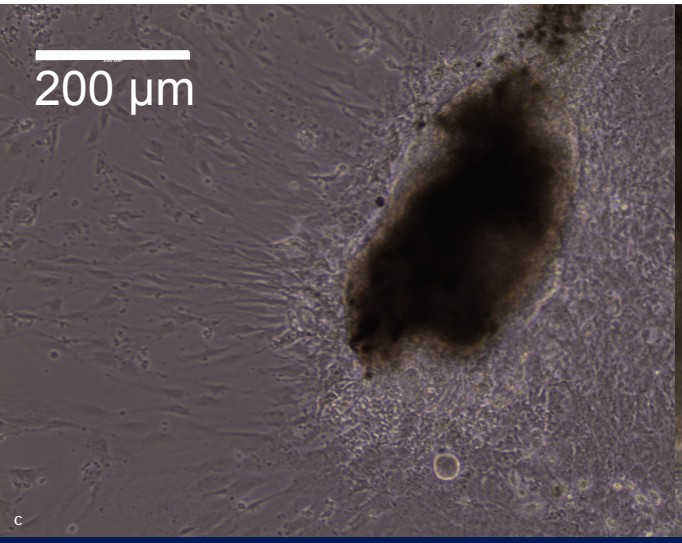

iPS cells
EB formation
RPC differentiation
Photoreceptor production
D1
5 Days
D5
EB media
+1ng/ml Dkk1,
+1ng/ml Noggin
+1ng/ml Igf1
+0.5ng/ml bFGF
10 Days
DF media 1
+10ng/ml Dkk1,
+10ng/ml Noggin,
+10ng/ml Igf1,
+10ng/ml bFGF
D15
5 Days
D21
DF media 2
+10ng/ml Dkk1
+10ng/ml Noggin
+10ng/ml Igf1
+10ng/ml bFGF
10mM DAPT
10 Days
DF media 3
+10ng/ml Dkk1
+10ng/ml Noggin
+10ng/ml Igf1
+10ng/ml bFGF
+10mM DAPT
+2ng/ml aFGF
D30
120 Days
D150
DF media 4

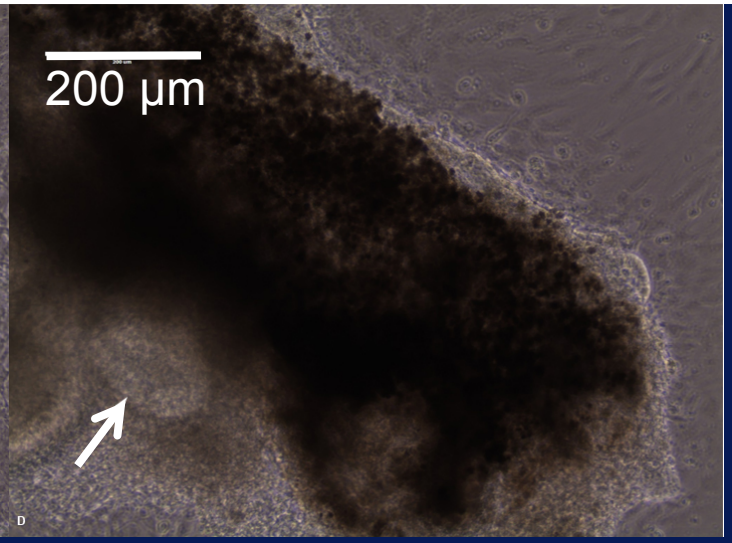

iPS cells
EB formation
RPC differentiation
Photoreceptor production
D1
5 Days
D5
EB media
+1ng/ml Dkk1,
+1ng/ml Noggin
+1ng/ml Igf1
+0.5ng/ml bFGF
10 Days
D15
DF media 1
+10ng/ml Dkk1,
+10ng/ml Noggin,
+10ng/ml Igf1,
+10ng/ml bFGF
5 Days
D21
DF media 2
+10ng/ml Dkk1
+10ng/ml Noggin
+10ng/ml Igf1
+10ng/ml bFGF
10mM DAPT
10 Days
D30
DF media 3
+10ng/ml Dkk1
+10ng/ml Noggin
+10ng/ml Igf1
+10ng/ml bFGF
+10mM DAPT
+2ng/ml aFGF
120 Days
D150
DF media 4


200 µm

45 days

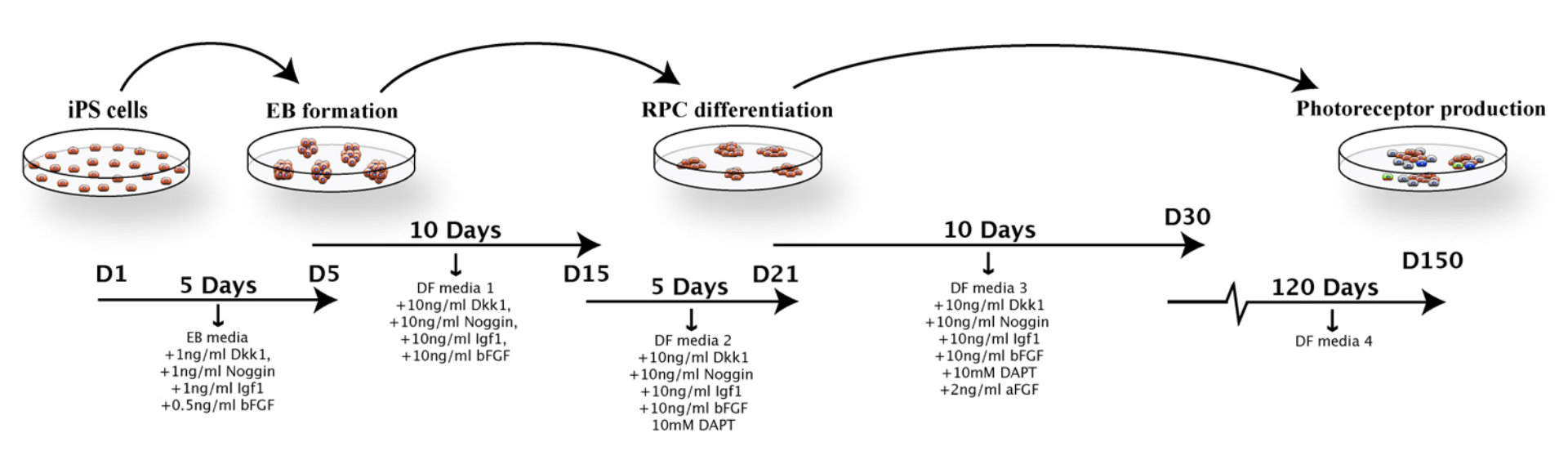


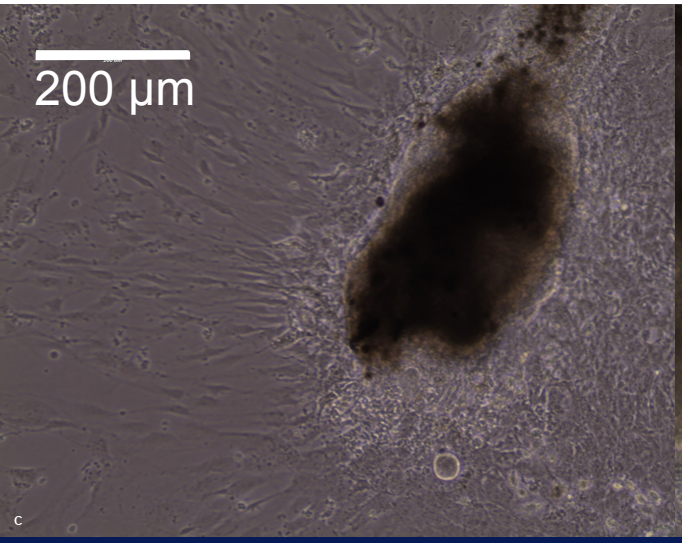


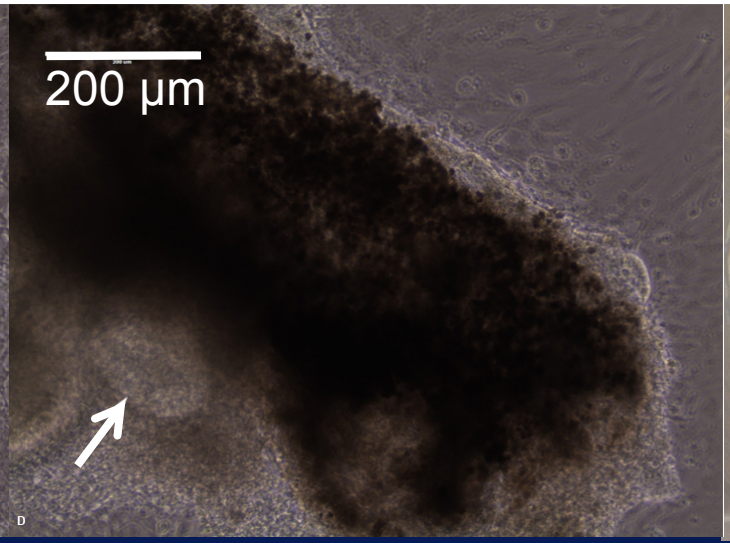


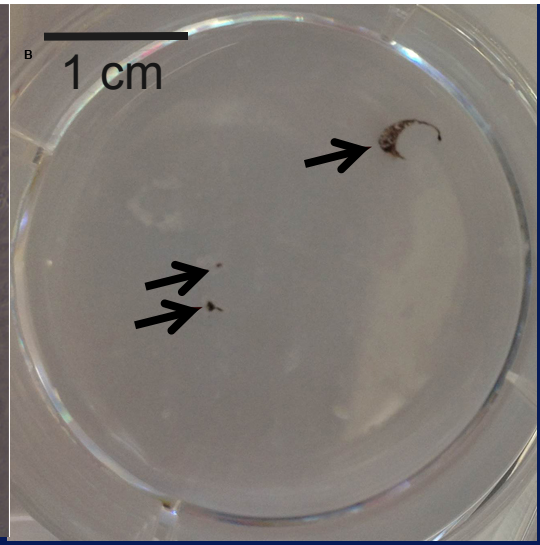
45 days

70 days





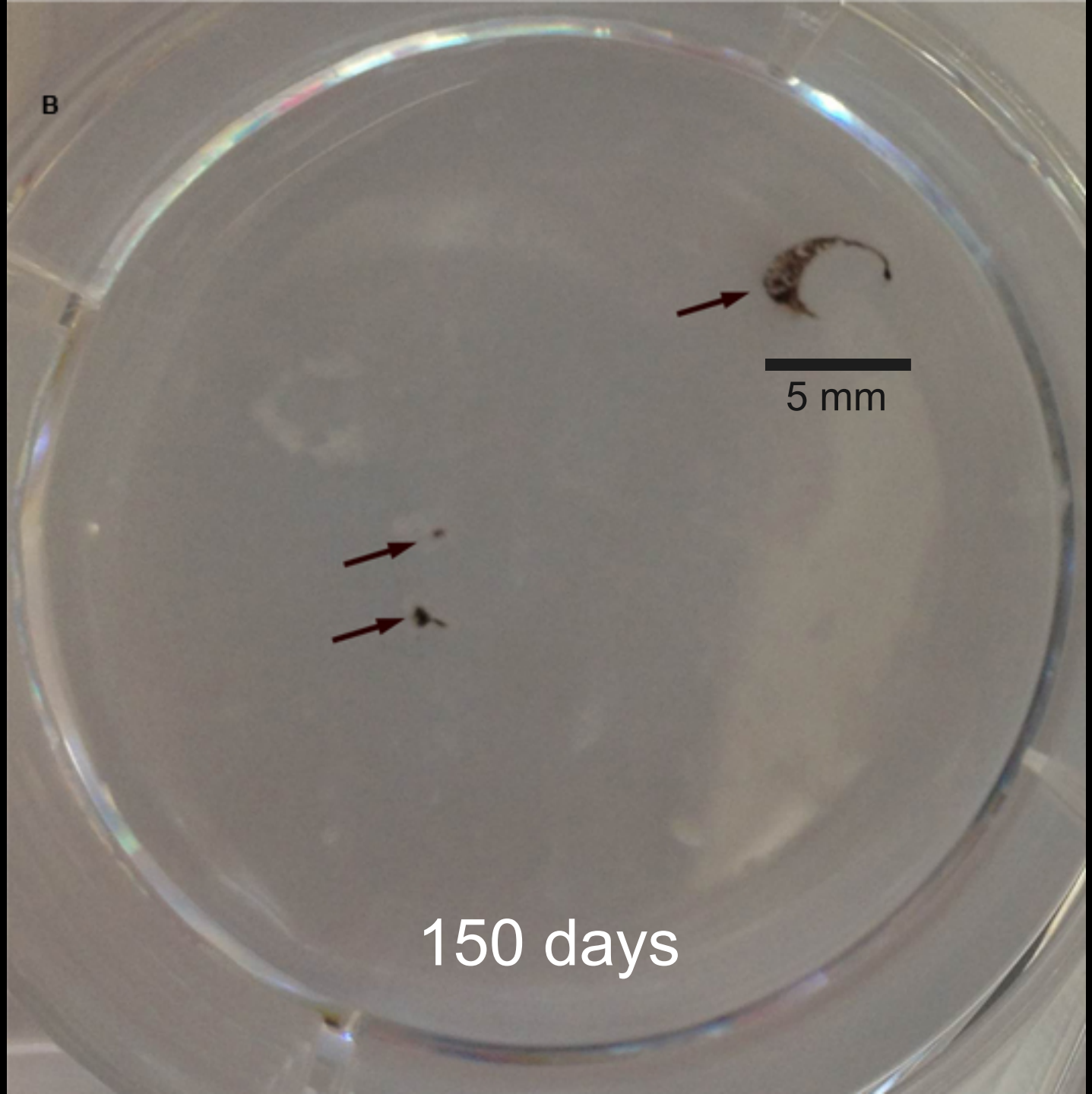




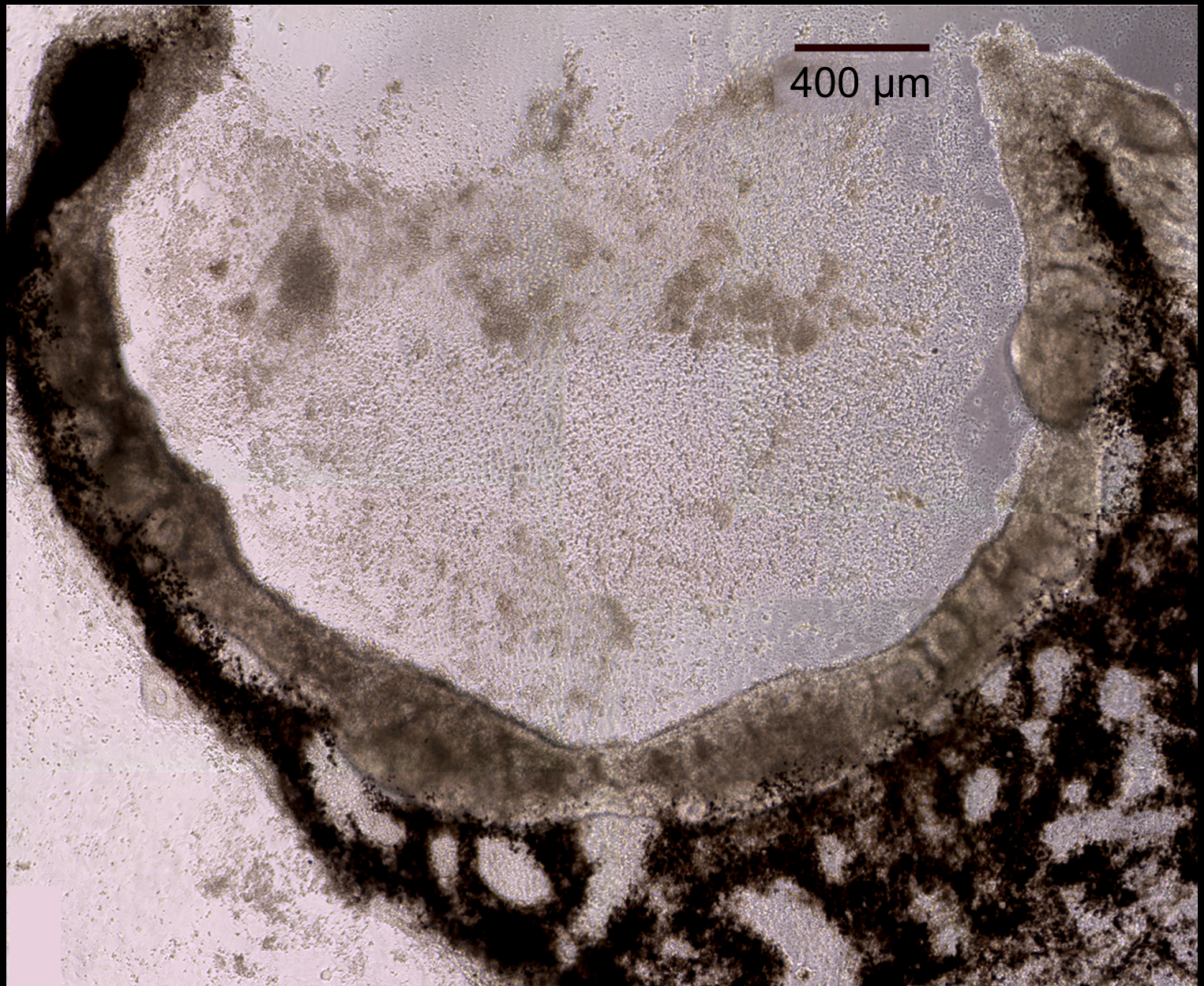
45 days

70 days

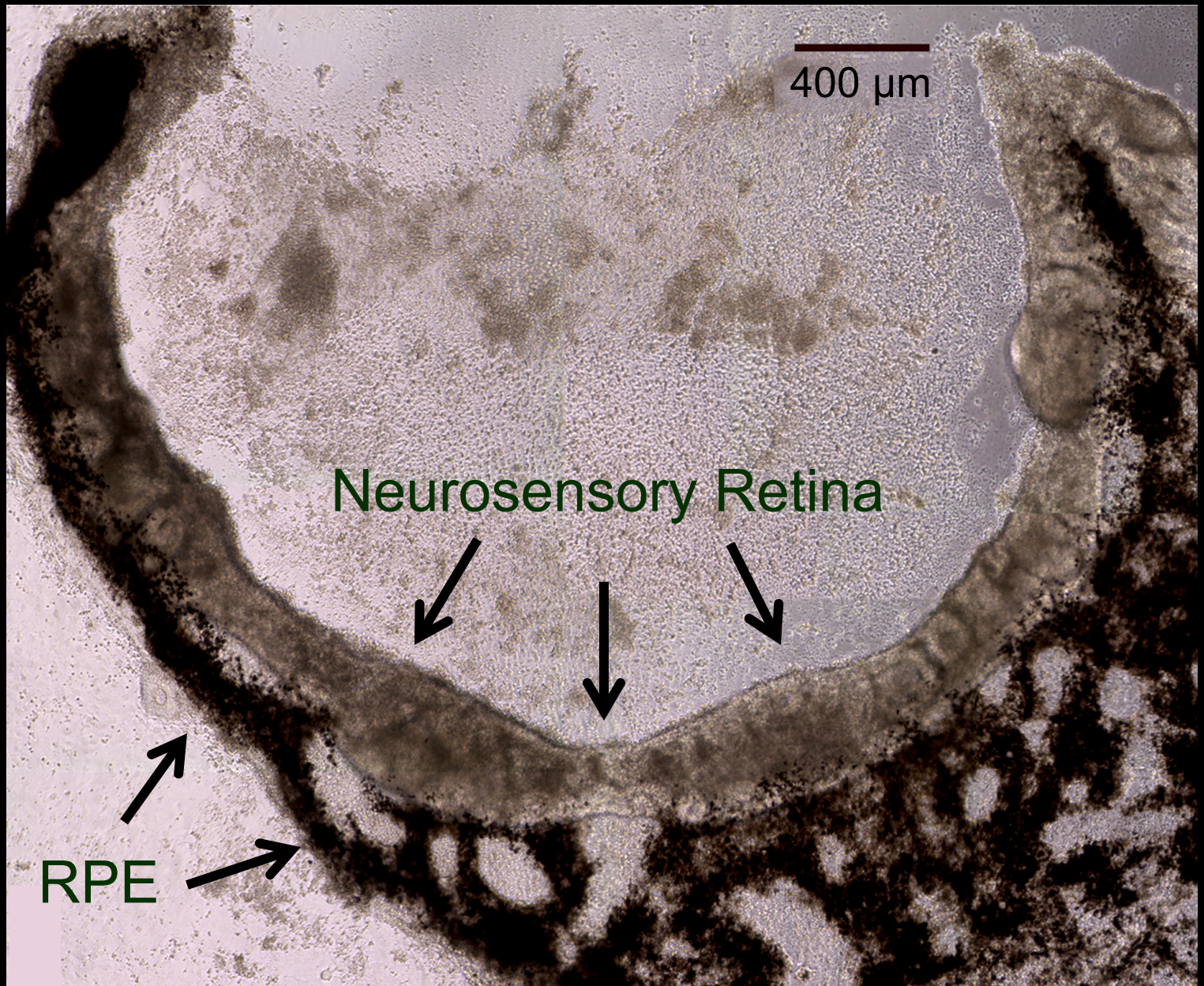
150 days


B
5 mm
150 days

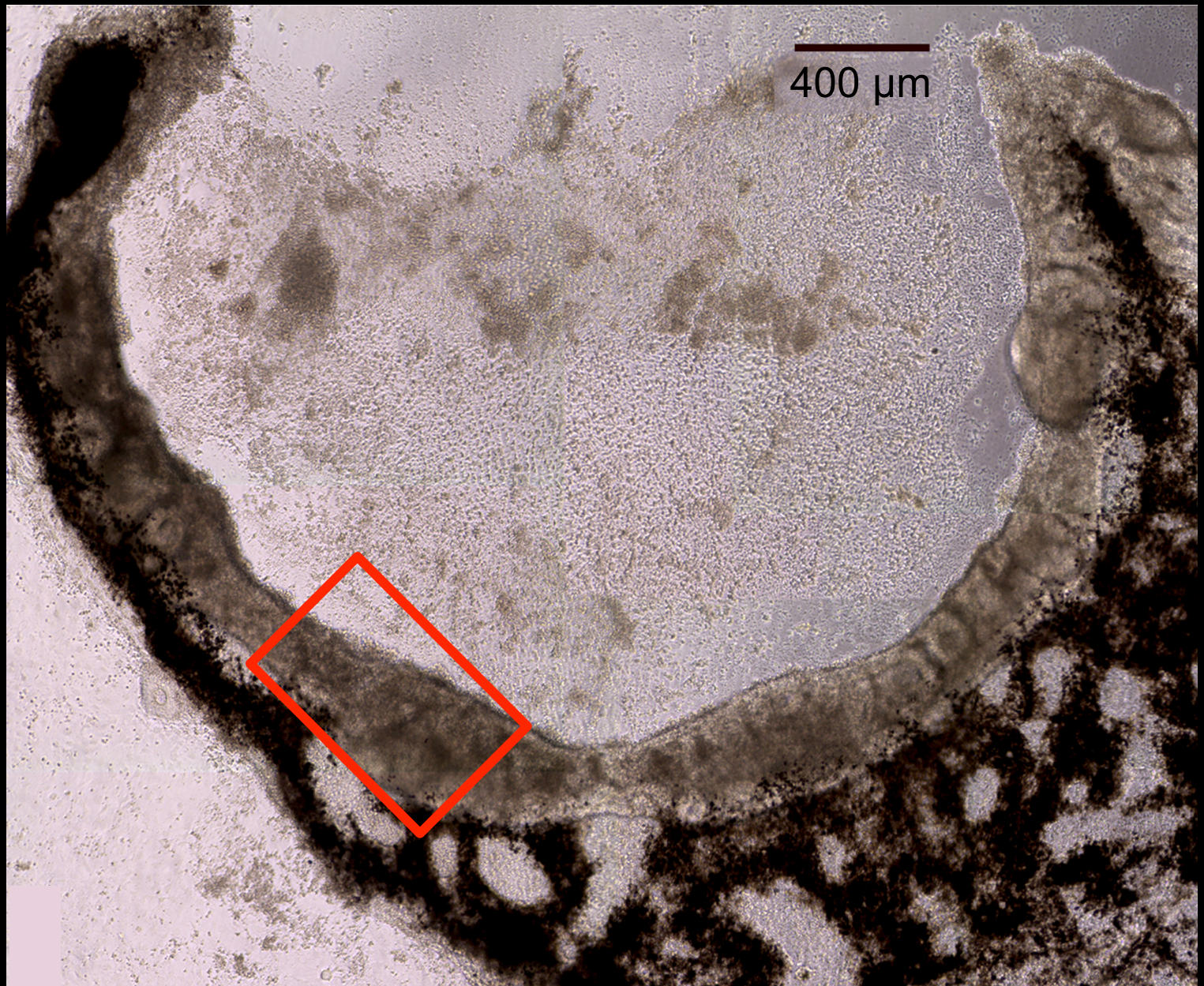
Multi-layer Eyecup-like Structure

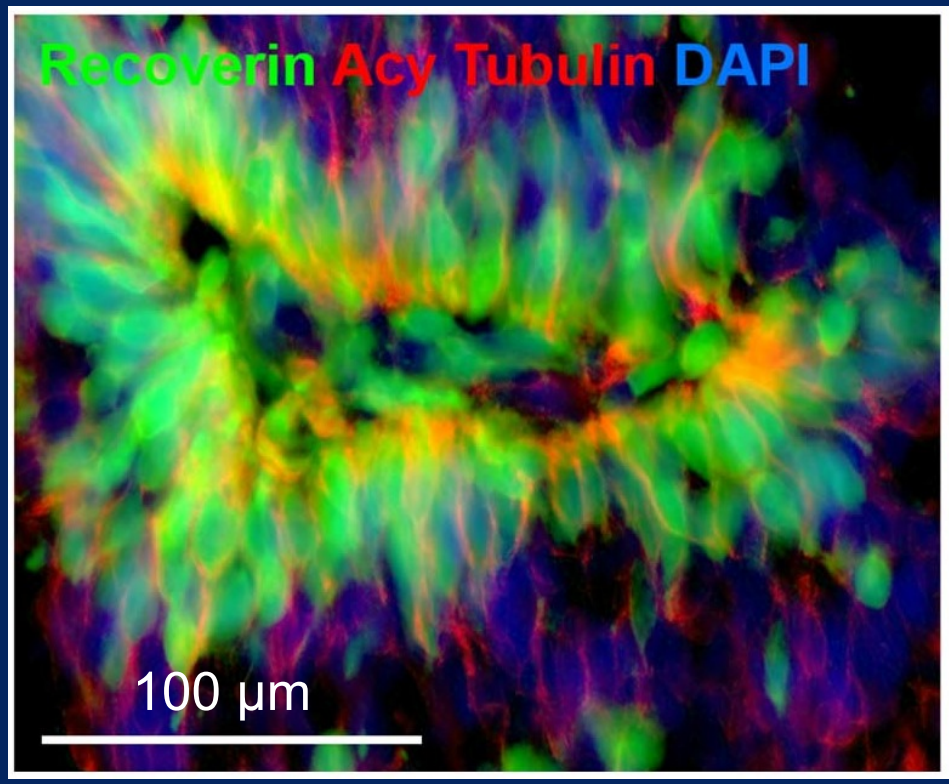


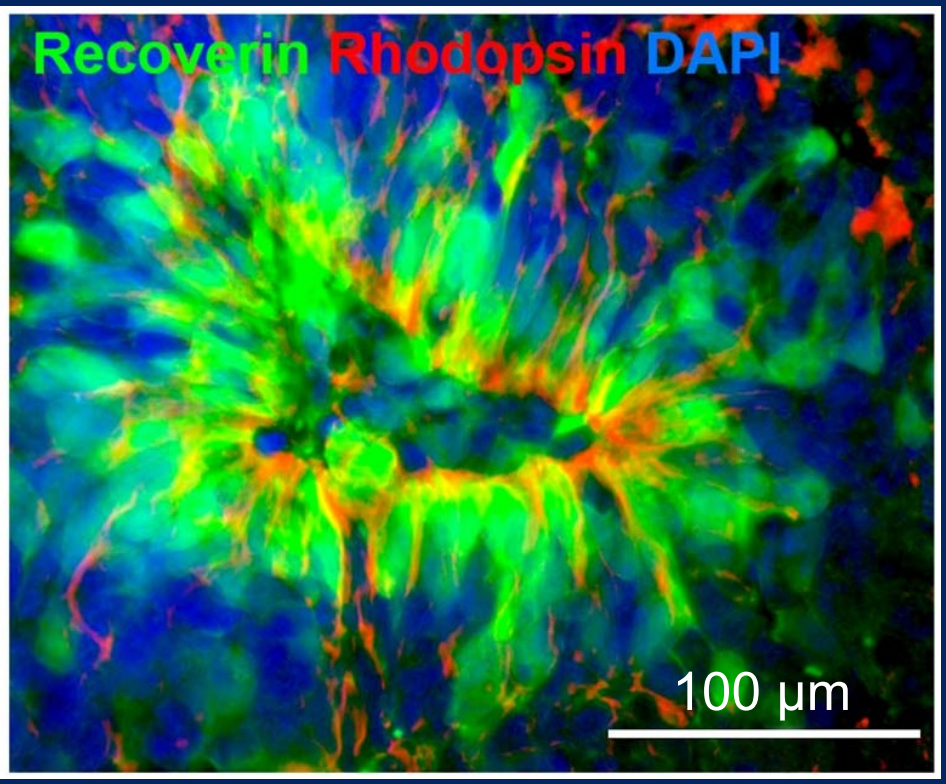
Multi-layer Eyecup-like Structure

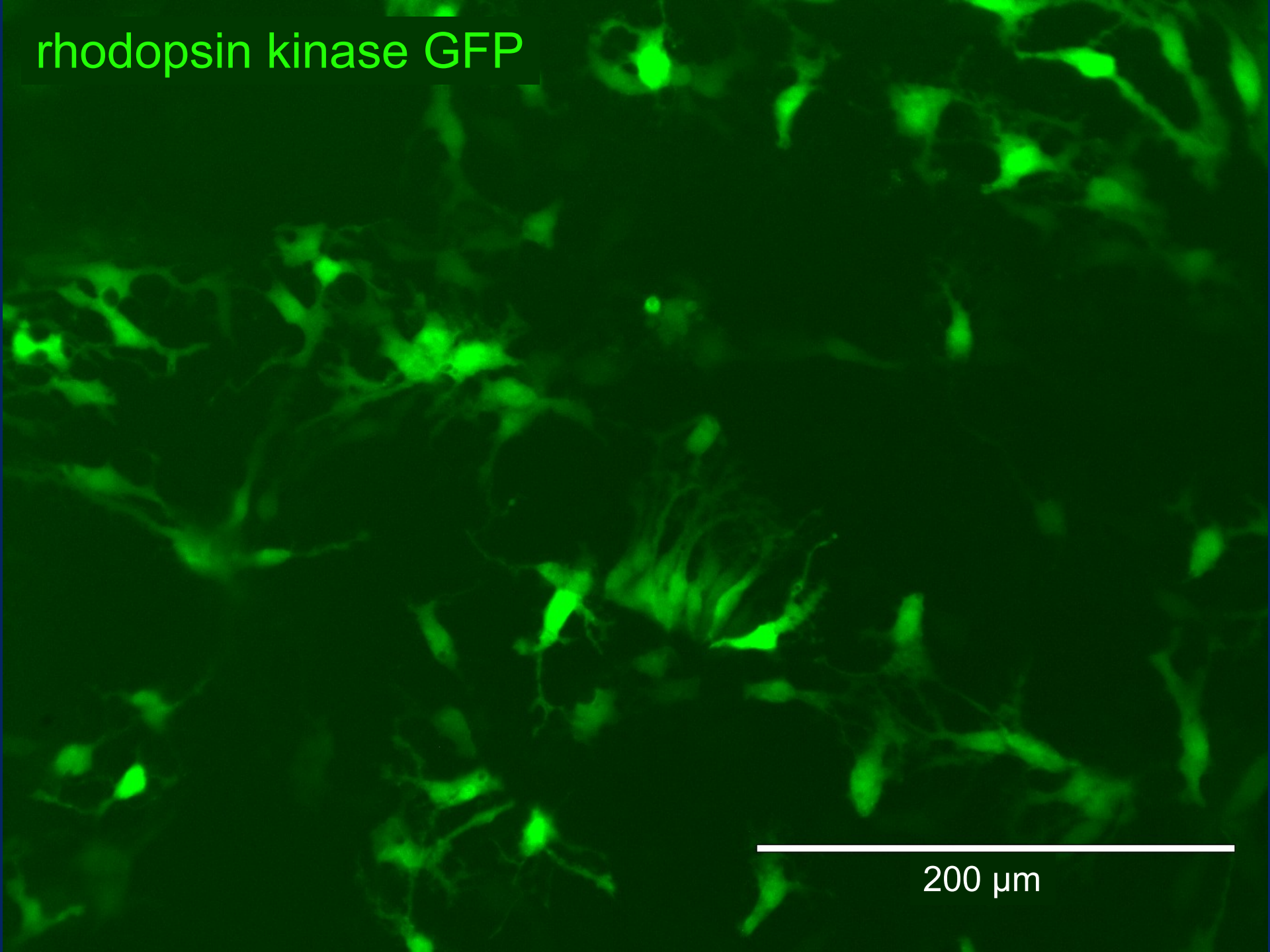


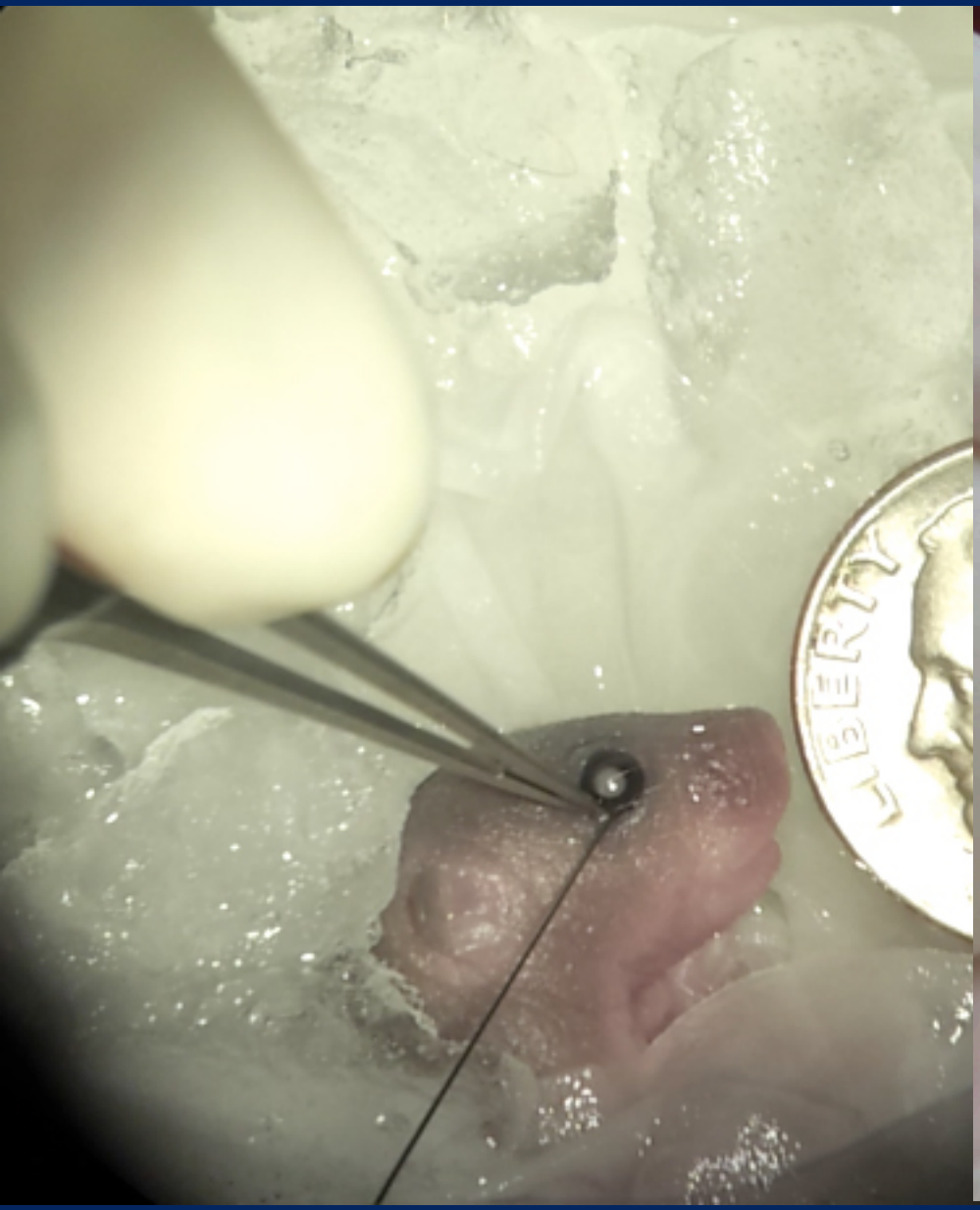
Multi-layer Eyecup-like Structure

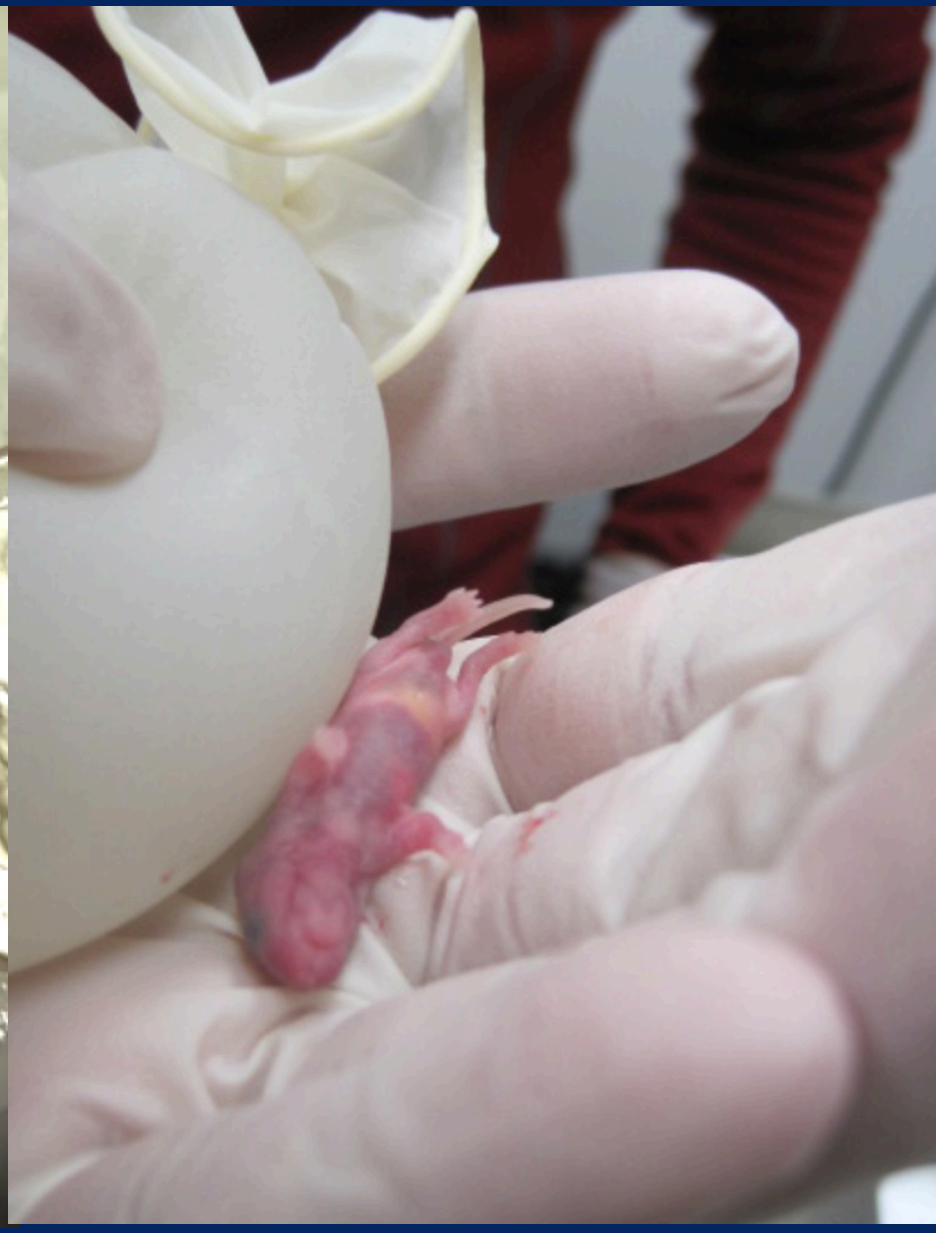


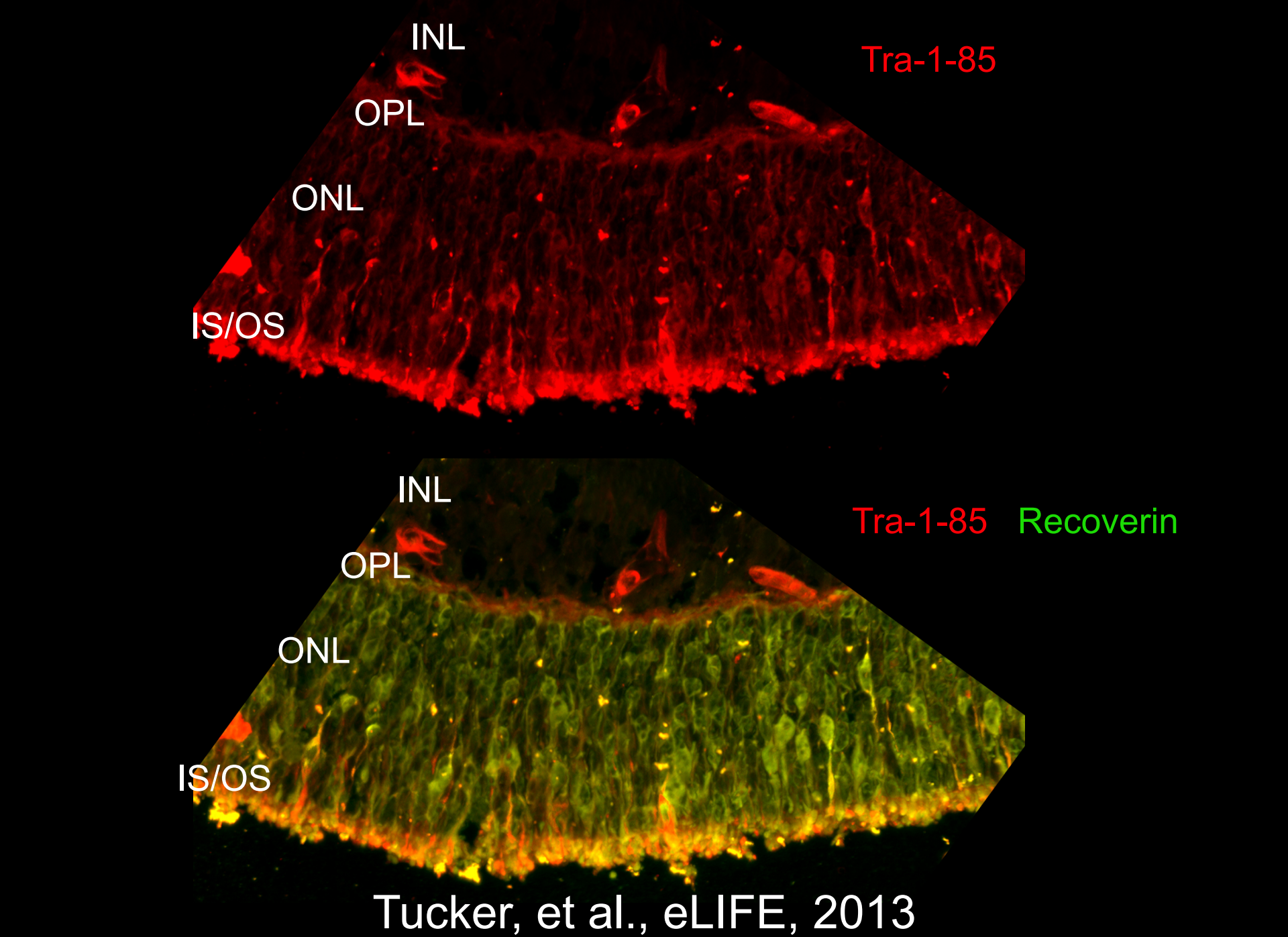

Recoverin Acy Tubulin DAPI
100 µm


Recoverin Rhodopsin DAPI
100 µm

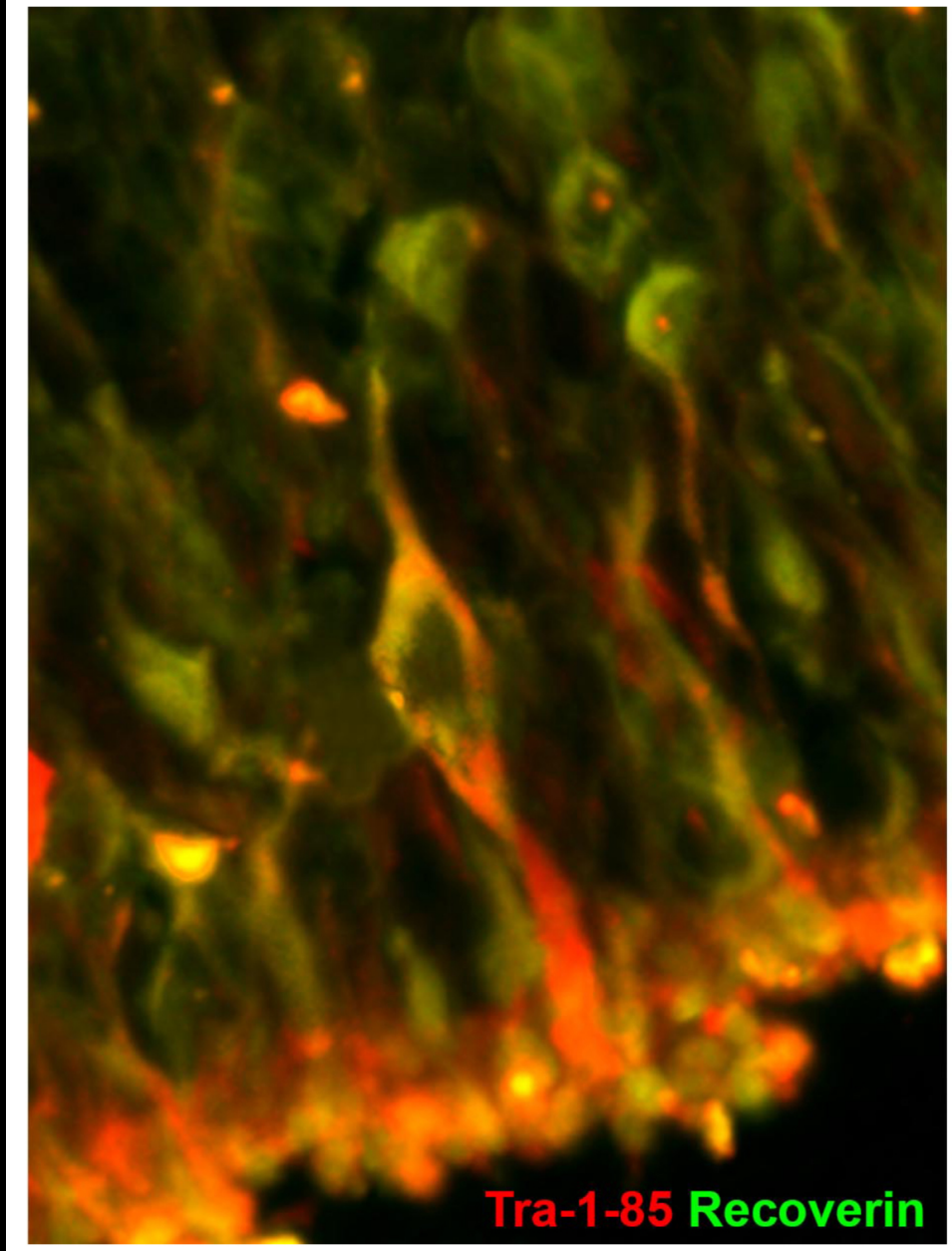

rhodopsin kinase GFP
200 µm

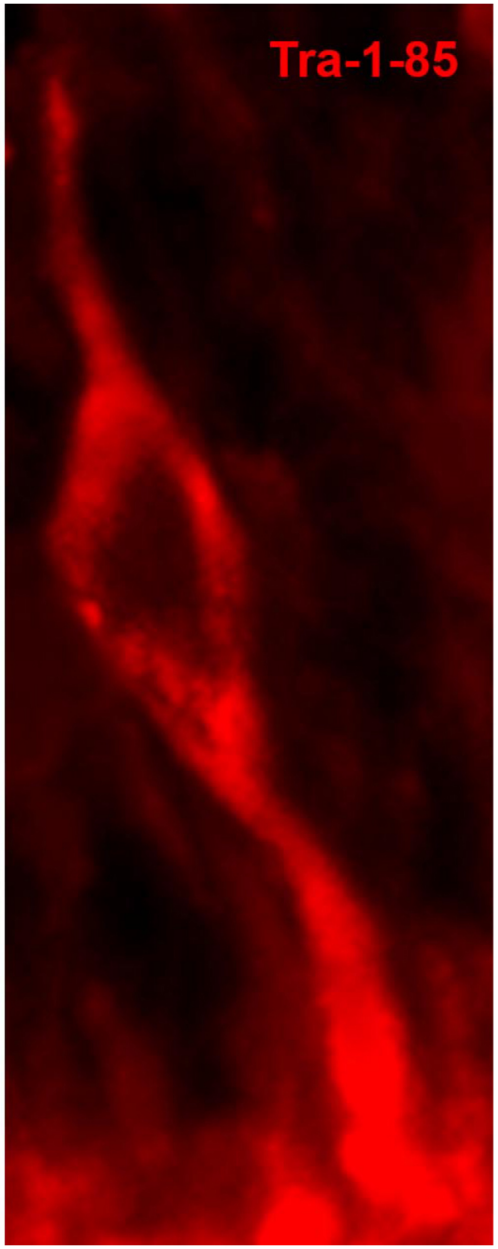

LIBERTY





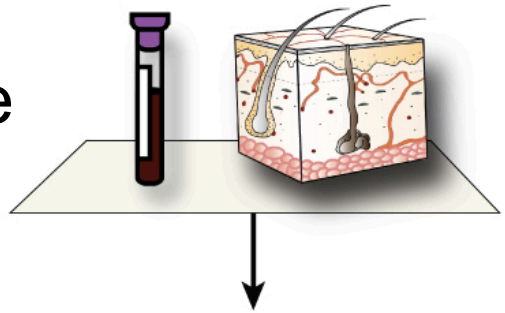
Tucker, et al., eLIFE, 2013





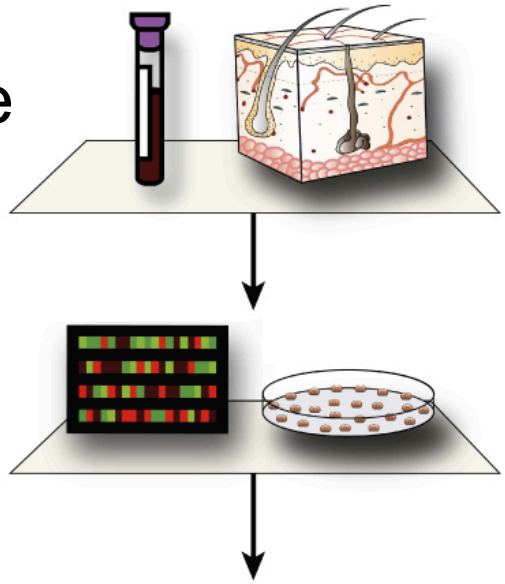
Tucker, et al., eLIFE, 2013

Blood Sample



Skin Biopsy

Blood Sample

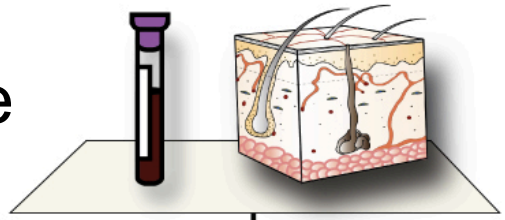


Skin Biopsy

Genetic Testing

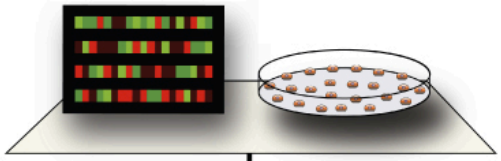
Establish Cell Lines

Blood Sample



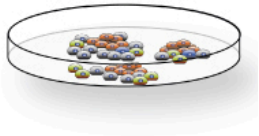
Skin Biopsy

Genetic Testing

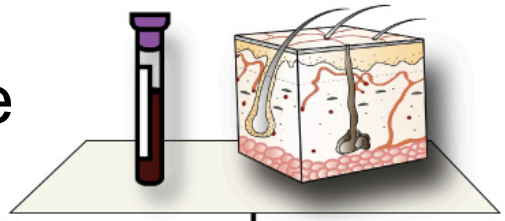


Establish Cell Lines

Evaluate Mutations

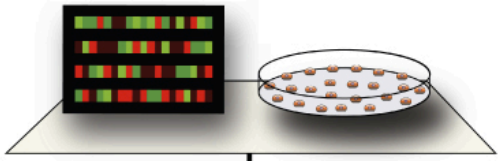


Blood Sample



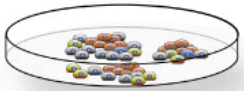
Skin Biopsy

Genetic Testing

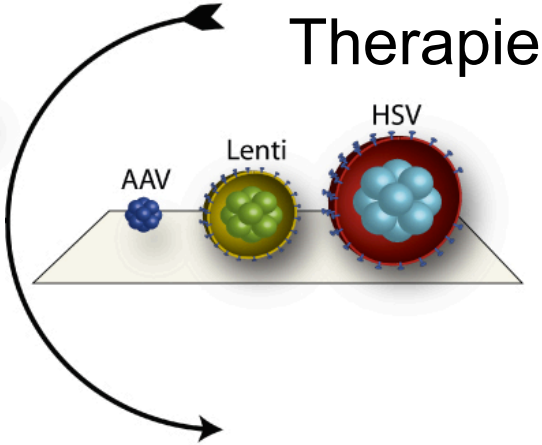


Establish Cell Lines

Evaluate Mutations

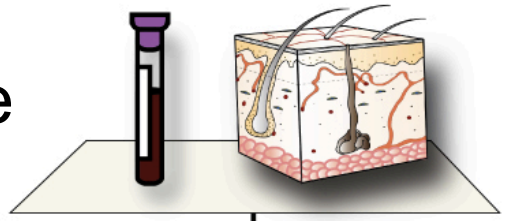


Test Efficacy of Gene and Drug Therapies



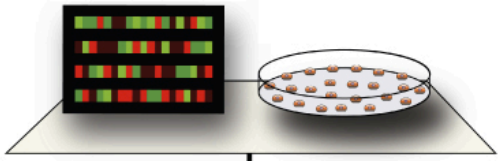
Blood Sample

Skin Biopsy



Genetic Testing

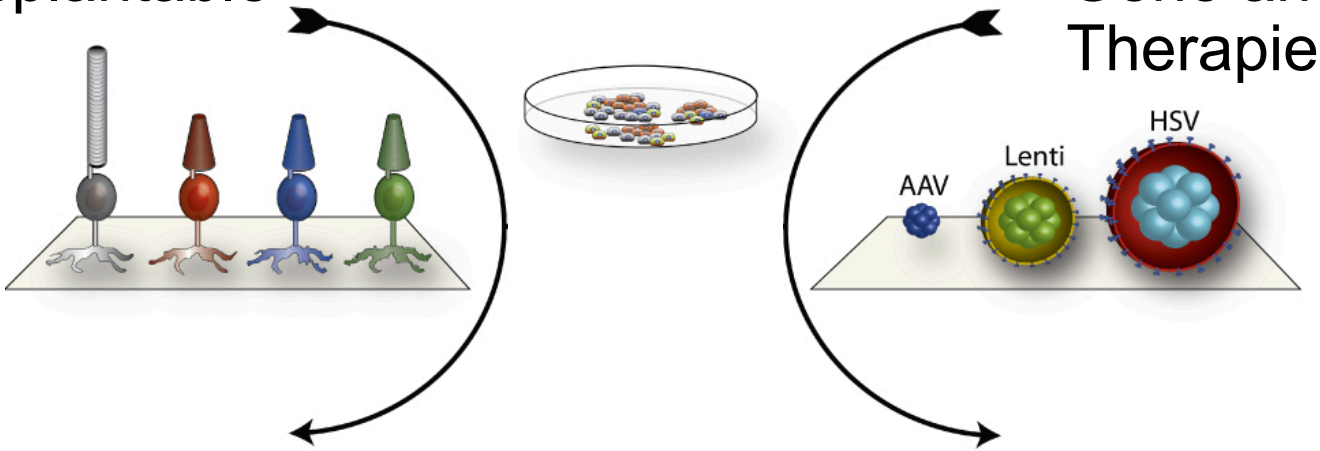
Establish Cell Lines

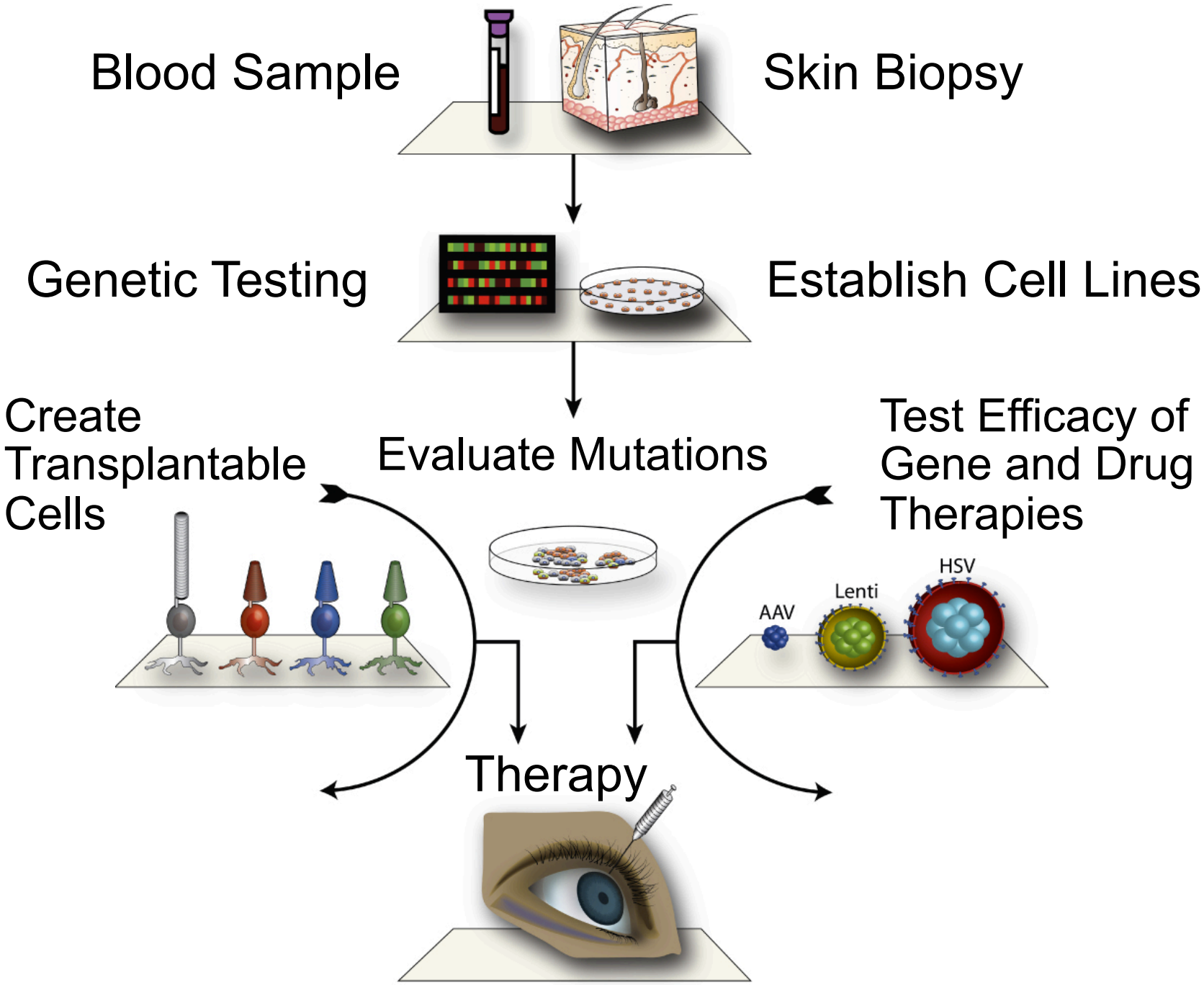


Create Transplantable Cells

Evaluate Mutations

Test Efficacy of Gene and Drug Therapies




Blood Sample
Skin Biopsy
Genetic Testing
Establish Cell Lines
Create Transplantable Cells
Evaluate Mutations
Test Efficacy of Gene and Drug Therapies
AAV
Lenti
HSV
Therapy

Acknowledgements

Budd Tucker

Rob Mullins

Bill Kimberling

Adam DeLuca

Jean Andorf

Heather Daggett

Steve Wynn

Steve Dezii

Wyc Grousbeck

Vivimus Enim

WIVR

Remedium

HHMI

FOUNDATION FIGHTING BLINDNESS

National Eye Institute

NATIONAL INSTITUTES OF HEALTH