PROTECTING AND RESTORING SIGHT: HOW NOVEL MOLECULAR PRINCIPLES CAN BE HARNESSED

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USHER SYNDROME COALITION
7th Annual Usher Syndrome FAMILY CONFERENCE
#USH2015

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DISCLOSURE

Academic collaboration with Dr. Dennis S. Rice and colleagues from Lexicon Pharmaceuticals, The Woodlands, Texas.

N. Bazan is the named inventor of technologies and models related to this presentation. IPs are assigned to Louisiana State University Health, New Orleans, Louisiana, USA.

- **A consequence of increased life expectancy is a rise in the occurrence of photoreceptor and neuronal survival failure, as reflected by age-related macular degeneration and other neurodegenerative diseases.**

- **Why does this happen?**

- **What can we learn by teasing out the intimacy of this failure to help develop effective therapies?**

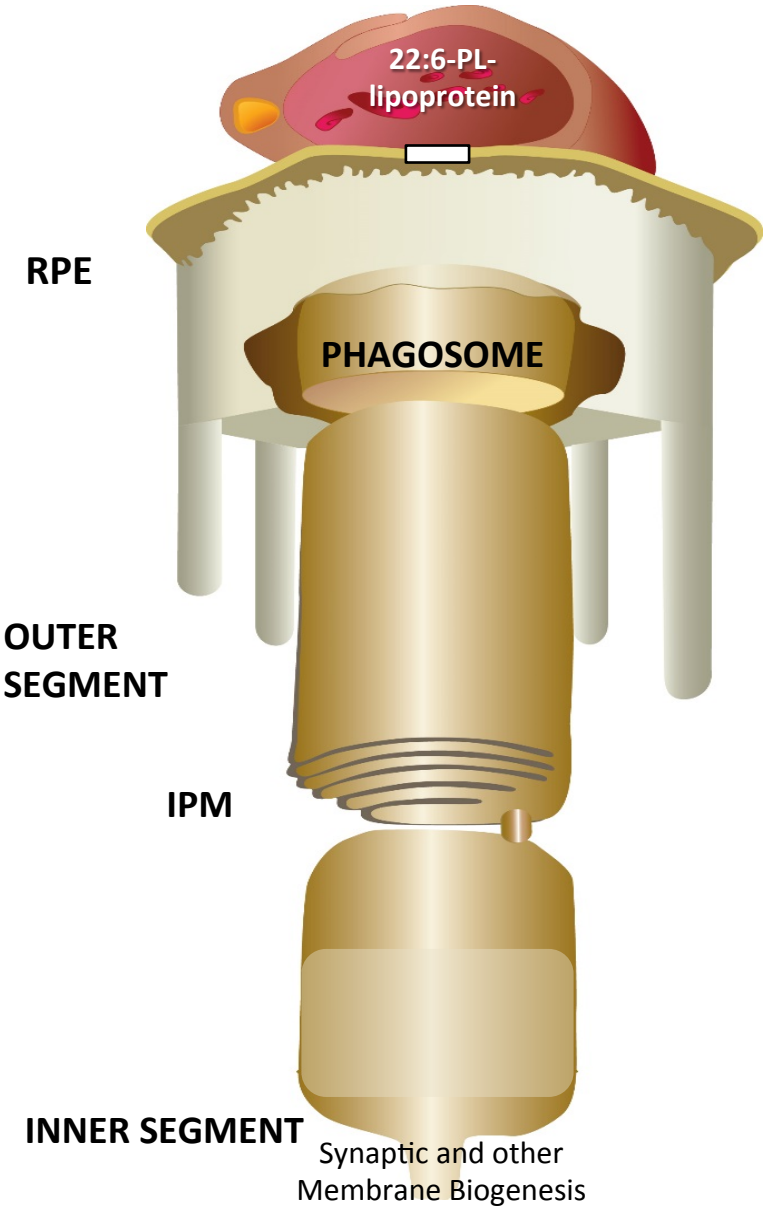
- **This issue is magnified when looking at early onset retinal degenerations, particularly Ushers' syndrome, a dual sensorial (sight and hearing) neurodegenerative condition.**

- **Retinal development, as is the case with the rest of the central nervous system, is driven by neuronal apoptotic cell death, and thereafter neurons, including photoreceptor cells, become post-mitotic cells.**

- **In retinal degenerative diseases, untimely cell death is set in motion, leading to photoreceptor cell loss due to failed biology, not due to developmental failure.**

Beautiful Interdependent Relationship Between Photoreceptor Cells and Retinal Pigment Epithelial Cells

Between these cells, there is a tightly-regulated recycling of key molecules that takes place, driven by the daily shedding of photoreceptor cell tips and RPE phagocytosis.



Bazan, N. *et al*. Annu. Rev. Nutr. 31:321–51, 2011

Is There a Molecular Logic that Sustains Photoreceptor Functional Integrity and Survival?

Why don't diseases manifest during latency in inheritable retinal degenerations (e.g. Retinitis Pigmentosa)**?**

Do pro-homeostatic-response docosanoids, made on demand at the onset of homeostasis disruptions from DHA, sustain latency?
(Bazan N. *Mol. Neurobiol*. 2014 50:1-5.)

We began to decipher molecular principles for:

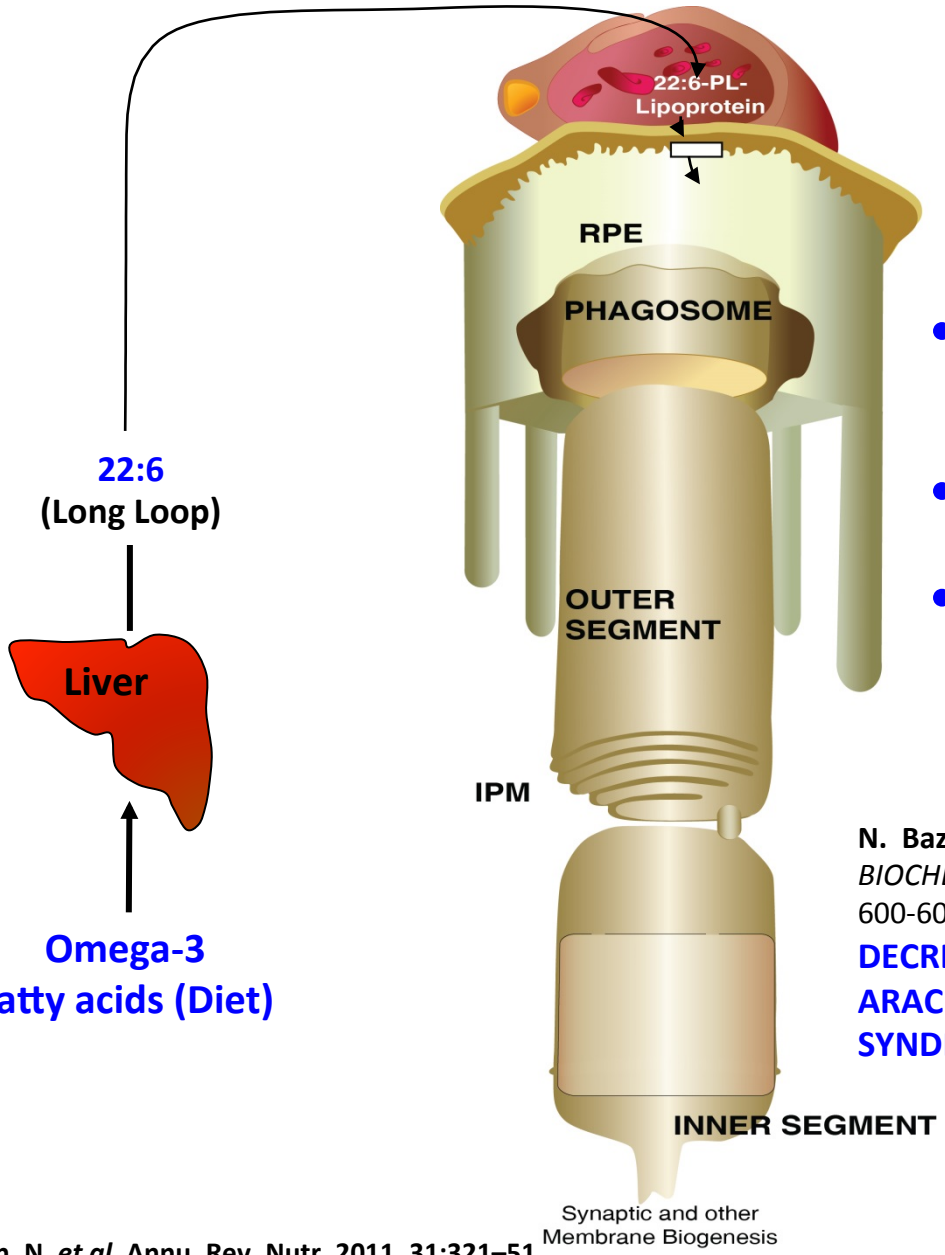
1) Retention specificity of DHA.
Rice, D. *et al*., *Nature Com.* 2015 6:1-14.

2) Intracellular messenger proteins that implement docosanoid bioactivity to counteract homeostasis disruptions and sustain photoreceptor cell survival.
Calandria J.M., *et al*. *Cell Death and Diff.* 2015 1-15.

Docosahexaenoic Acid and Sight

- Enriched, avidly retained and attaining the highest content in the body in photoreceptor cells
- Reservoir/precursor of bioactive docosanoid mediators (e.g. NPD1)
- Necessary during pregnancy/early development for vision and synapses
- Decreases in blood in Retinitis Pigmentosa and Usher's Syndrome patients
- NPD1 decreases in CA1 area in early stages of Alzheimer's
- Precursor of very long chain derivatives (38:6,n-3,36:6,n-3) that become part of the molecular species of phosphatidyl-choline (VLC-PUFA-PC):
  - Tightly bound to rhodopsin
  - Decreases in Stargard syndrome
  - Mutated ELOV4 causative of retinal degeneration
  - Decreases also in Age-related Macular Degeneration

DHA (22:6) is Concentrated and Retained in Photoreceptor Cells and in Retinal Pigment Epithelial Cells



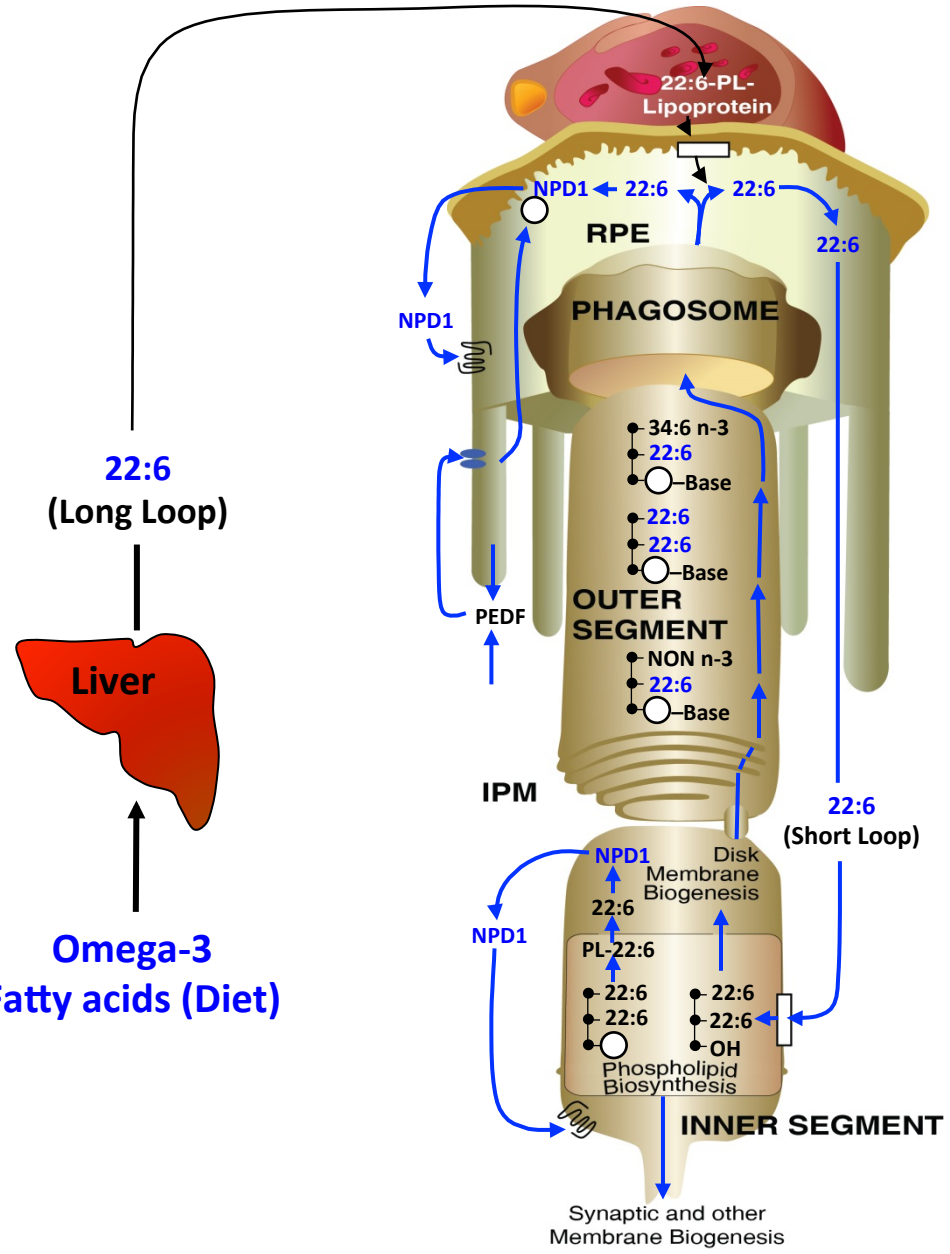
RPE Cells Are the Most Active Phagocytes of the Body

- Necessary for Photoreceptor Vision and Survival
- Post-Mitotic Cell
- The Retina is Part of the Nervous System

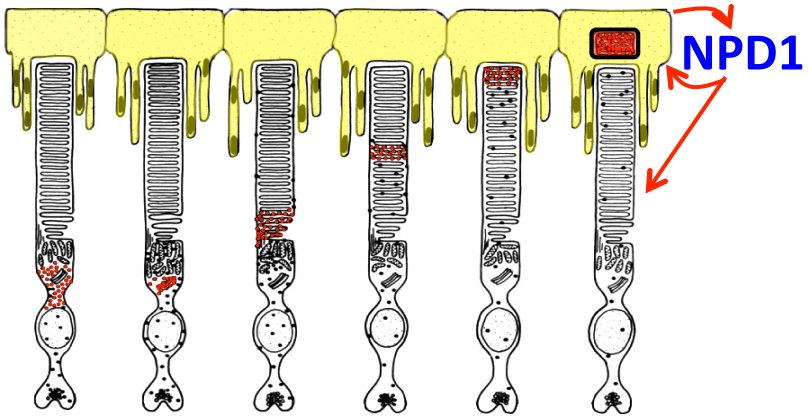
N. Bazan, B. Scott, T.Reddy,and M. Pelias.
BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS. 141, 600-604, 1986
DECREASED CONTENT OF DOCOSAHEXAENOATE AND ARACHIDONATE IN PLASMA PHOSPHOLIPIDS IN USHER'S SYNDROME

Bazan, N. *et al*. Annu. Rev. Nutr. 2011. 31:321–51

DHA is Supplied by the Long Loop and Retained Through Recycling in Photoreceptor and Retinal Pigment Epithelial Cells by Short Loop



Photoreceptor Cell Outer Segment Continuous Renewal: Shedding and Phagocytosis



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Adiponectin receptor 1 conserves docosahexaenoic acid and promotes photoreceptor cell survival

Dennis S. Rice[1,*,§], Jorgelina M. Calandria[2*], William C. Gordon[2,*], Bokkyoo Jun[2], Yongdong Zhou[2], Claire M. Gelfman[1], Songhau Li[2], Minghao Jin[2], Eric J. Knott[2], Bo Change[3], Alex Abuin [1], Tawfik Issa[1], David Potter[1], Kenneth A. Platt[1] & Nicolas G. Bazan[2]

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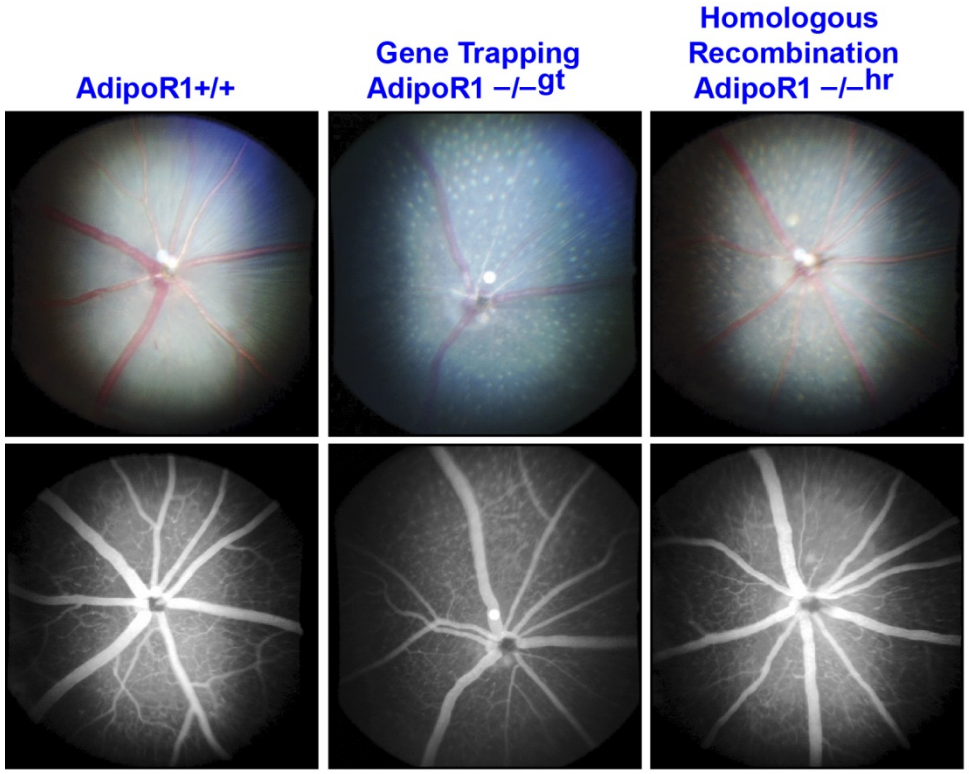
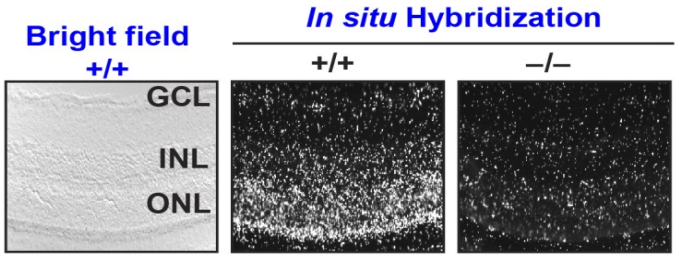
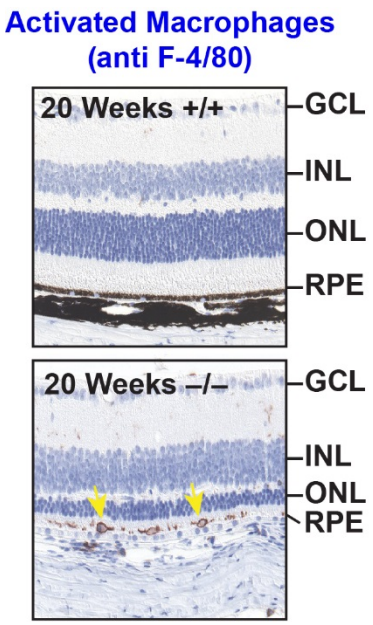
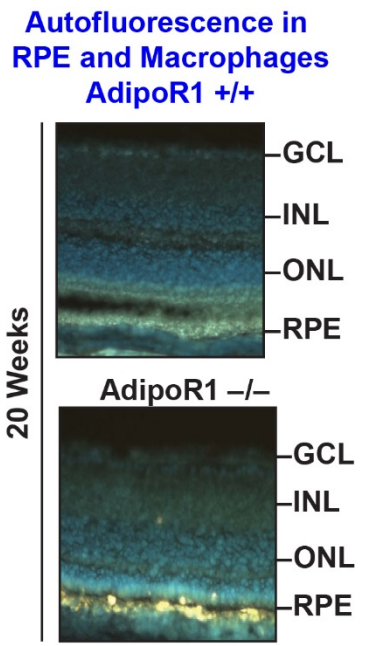
Ablation of AdipoR1 Leads To "Flecked Retina"

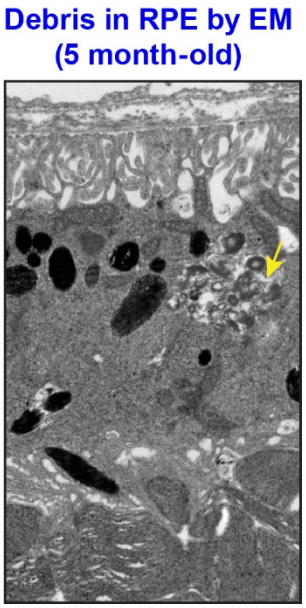


(14 - 16 weeks of age)





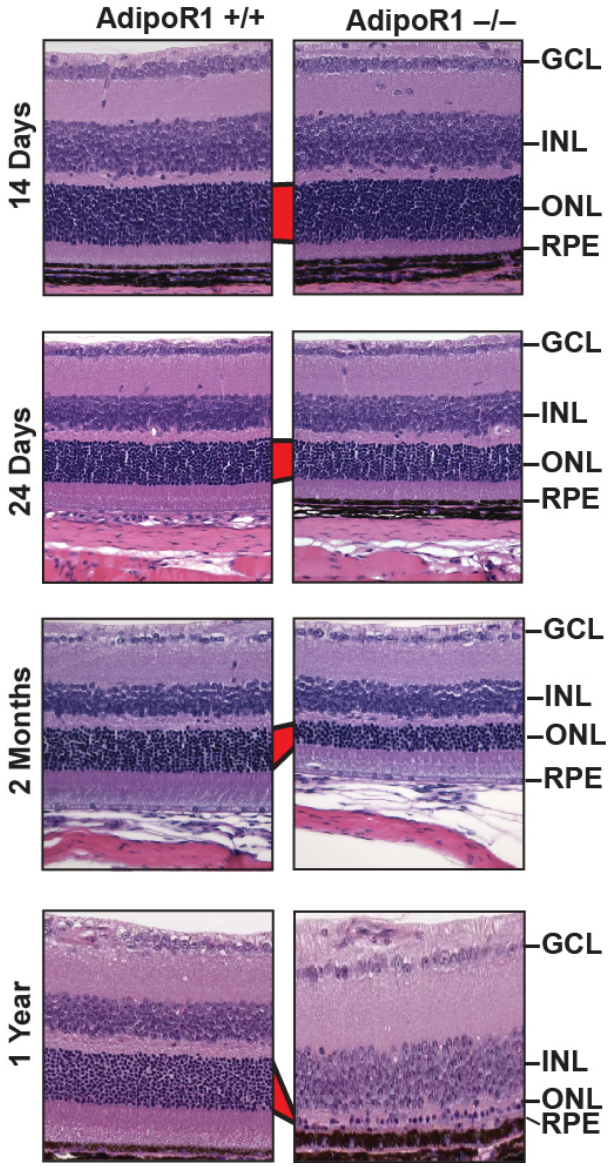




D. Rice, *et.al.*, *Nature Comm.* 2015

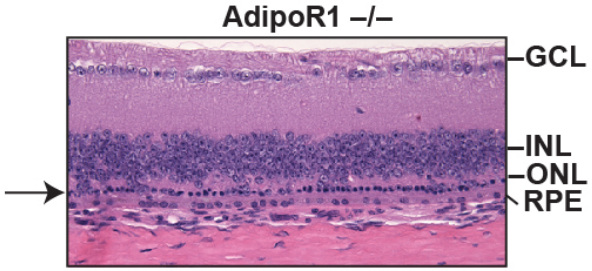
Ablation of AdipoR1 Leads To Photoreceptor Cell Degeneration

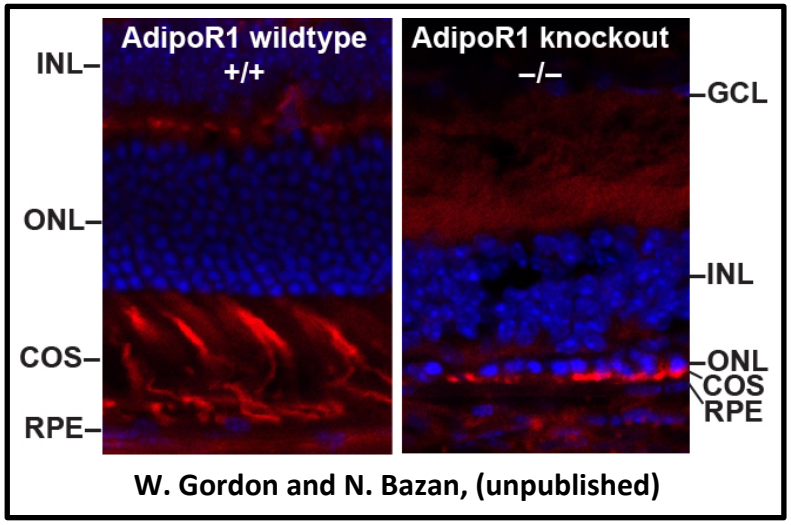
Progressive Loss of Photoreceptors



Rice, *et al.*, *Nature Comm.* 2015

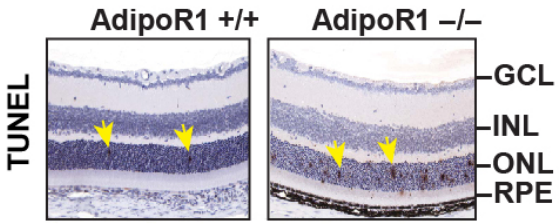
Single Row of Cone Photoreceptors (15 months old)

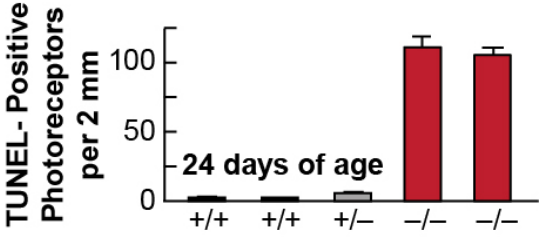




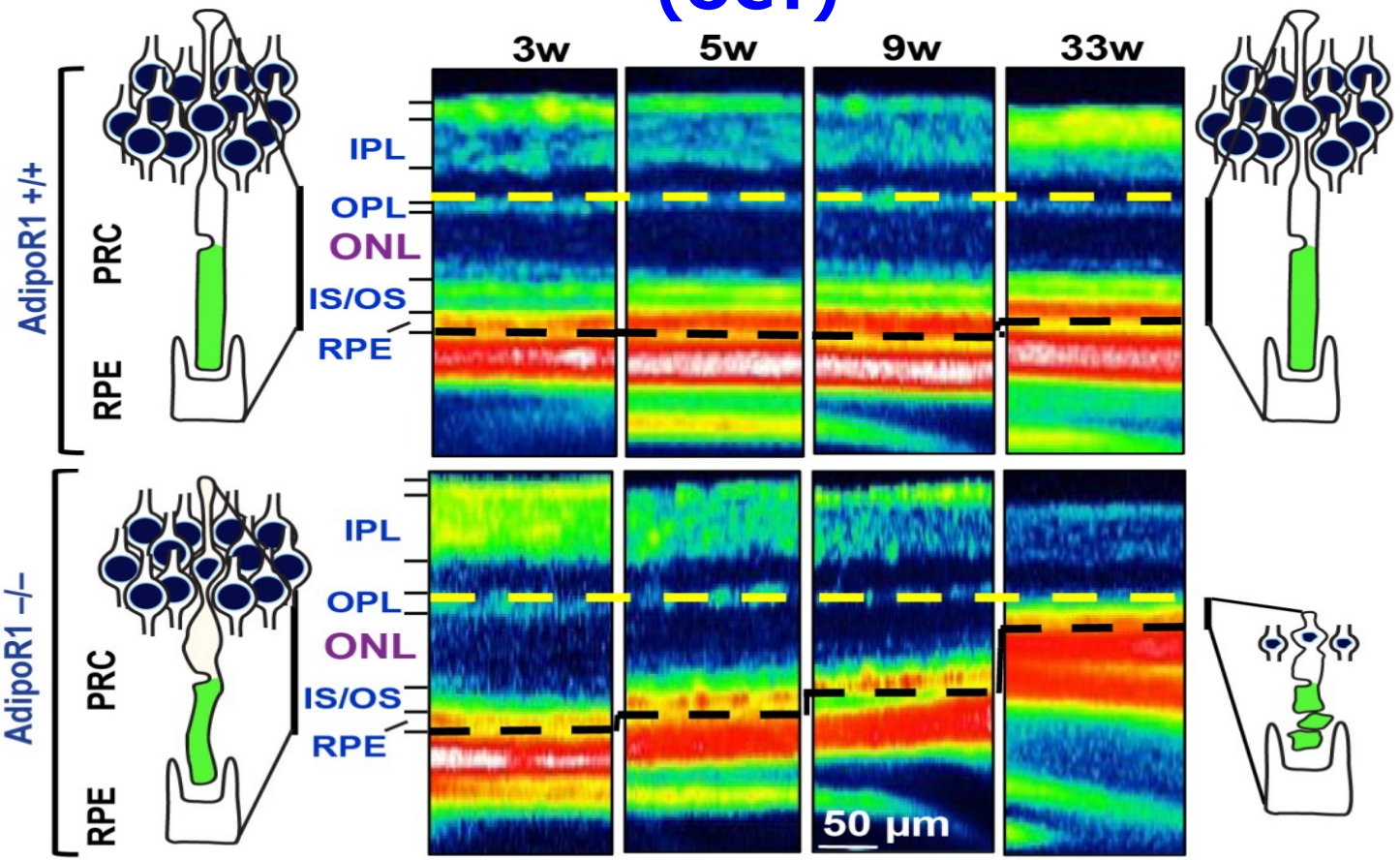
W. Gordon and N. Bazan, (unpublished)

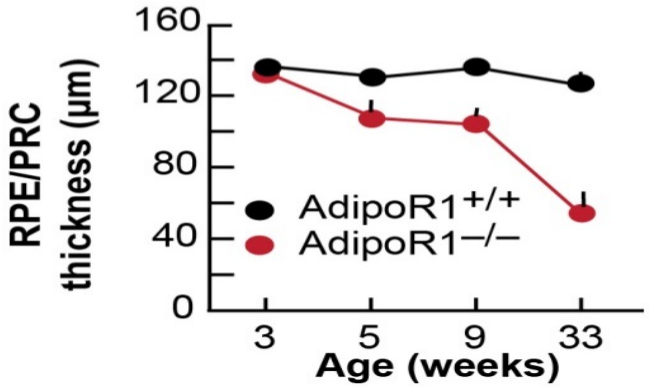
TUNEL Positive Photoreceptor Nuclei (24 day-old)





Progressive Loss of Photoreceptors in AdipoR1$^{-/-}$ Mice (OCT)

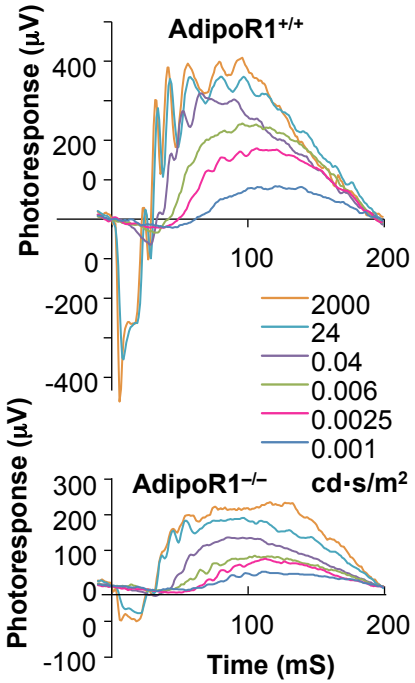


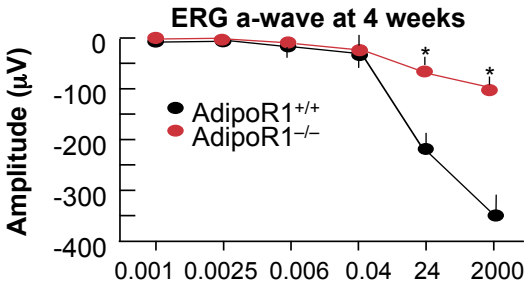


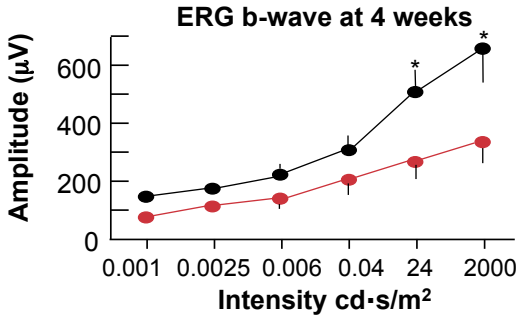
Rice *et al.*, *Nature Comm*. 2015

Attenuated ERGs, Prolonged Dark Recovery and Impaired Retinol Visual Cycle in AdipoR1 $^{-/-}$-Deficient Mice

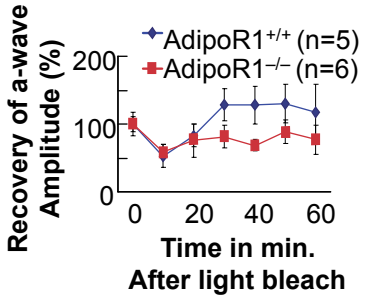
Before Photoreceptor Loss (3-4 Weeks Old)



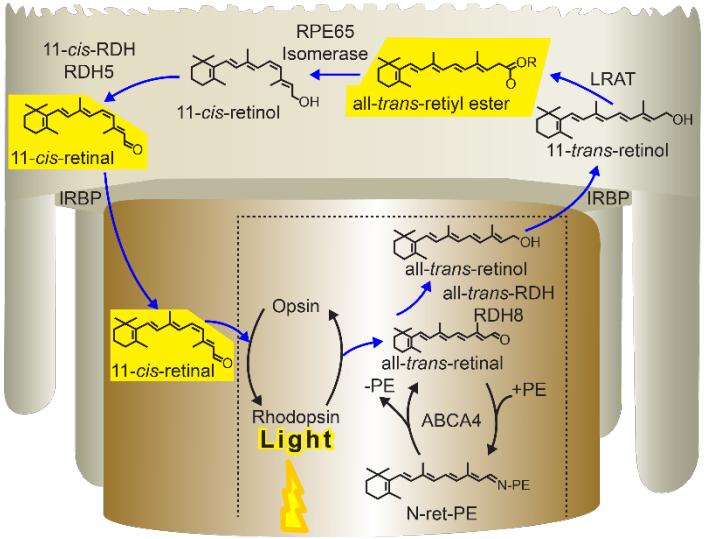


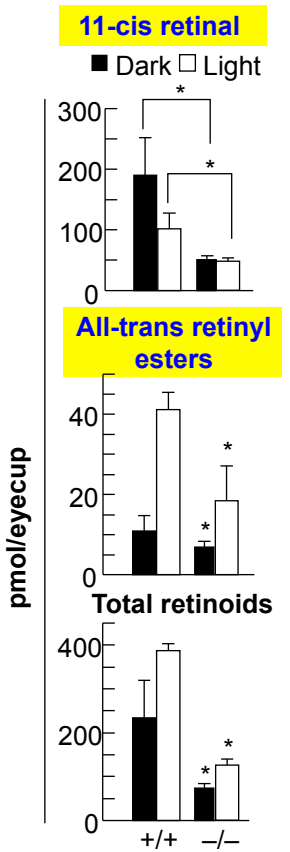


Failure in Dark Adaptation



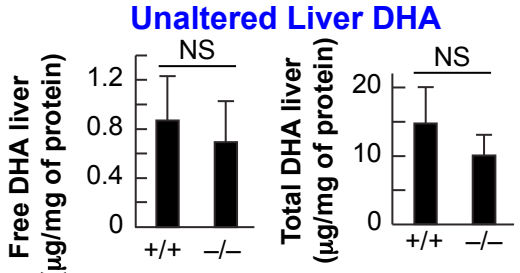
Impaired Visual Cycle

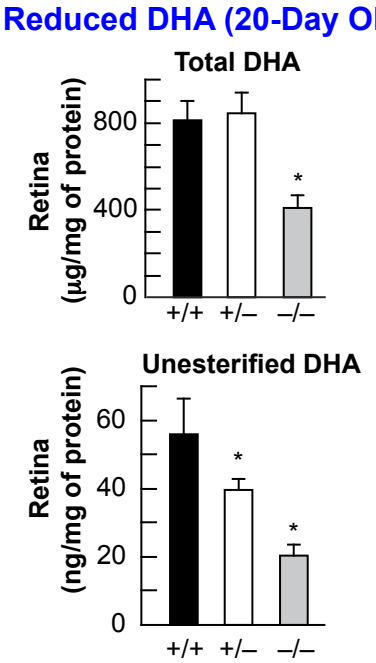


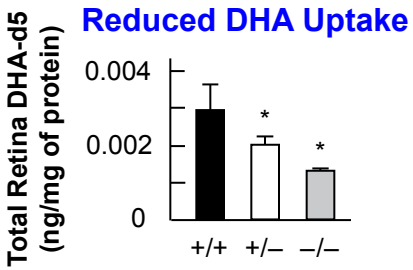


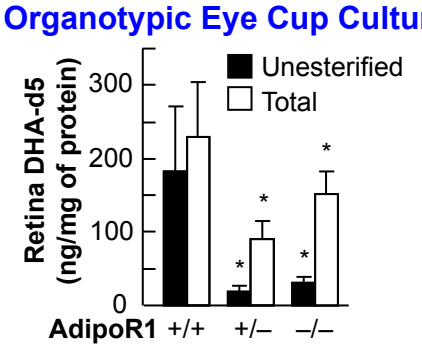
Rice *et al.*, *Nature Comm.* 2015

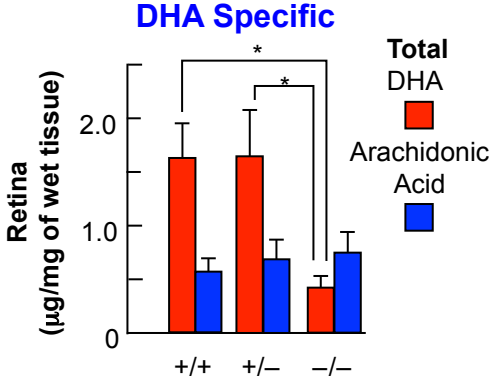
Cell-Selective DHA Lipidome-Specific Impairments in AdipoR1$^{-/-}$ Mice

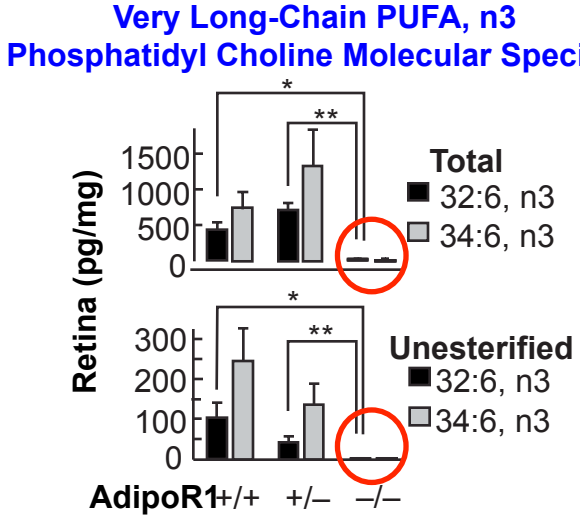


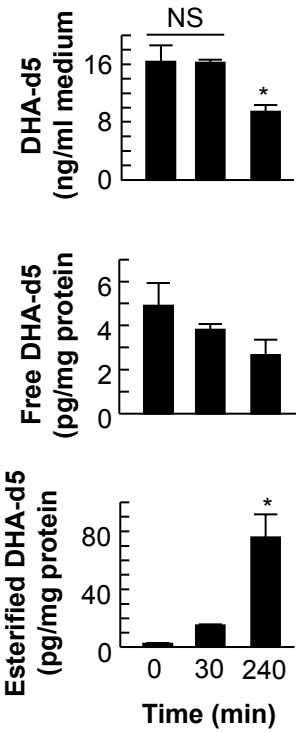


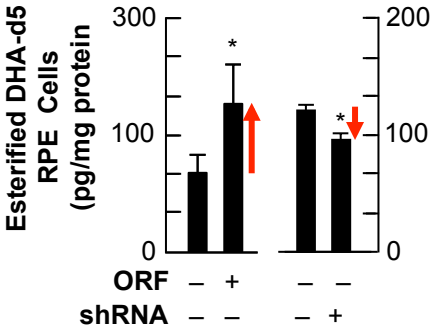










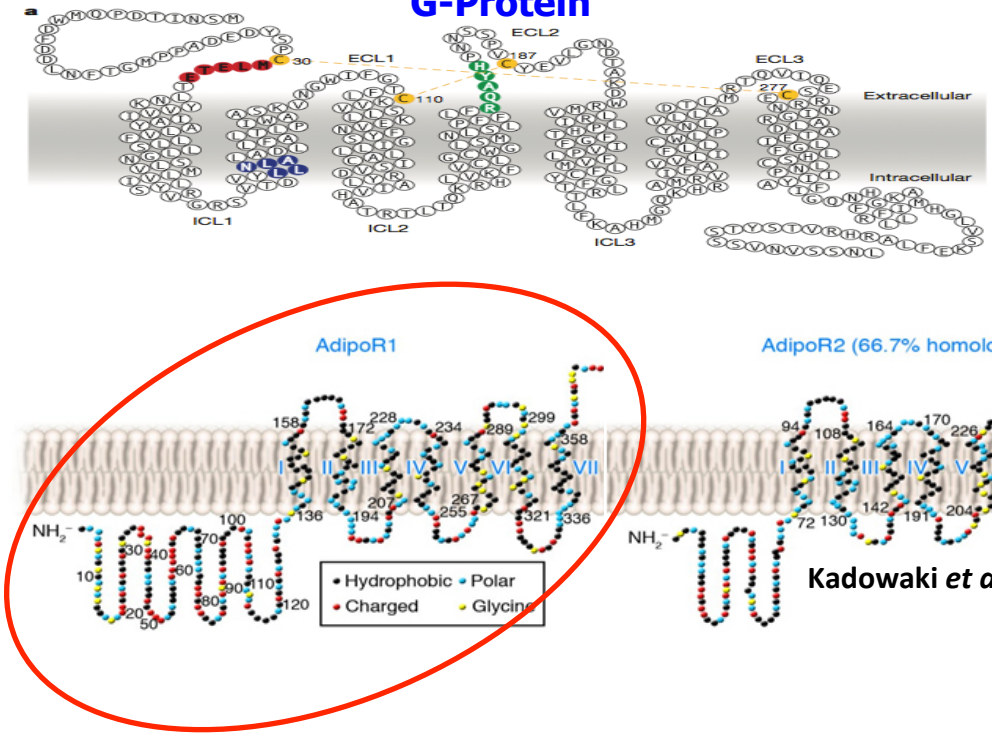


Rice *et al., Nature Comm.* 2015

AdipoR1 is a Seven Transmembrane-Spanning Domain Receptor

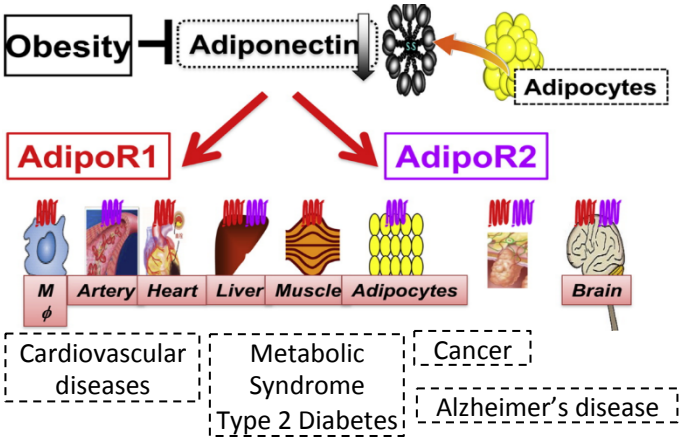
Cloning of adiponectin receptors that mediate antidiabetic metabolic effects *Nature* 423:762-769 (2003)

Toshimasa Yamauchi, Junji Kamon, Yusuke Ito, Atsushi Tsuchida, Takehiko Yokomizo, Shunbun Kita, Takuya Sugiyama, Makoto Miyagishi, Kazuo Hara, Masaki Tsunoda, Koji Murakami, Toshiaki Ohteki, Shoko Uchida, Sato Takekawa, Hironori Waki, Nelson H. Tsuno, Yoichi Shibata, Yasuo Terauchi, Philippe Froguel, Kazuyuki Tobe, Shigeo Koyasu, Kazunari Taira, Toshio Kitamura, Takao Shimizu, Ryozo Nagai & Takashi Kadowaki



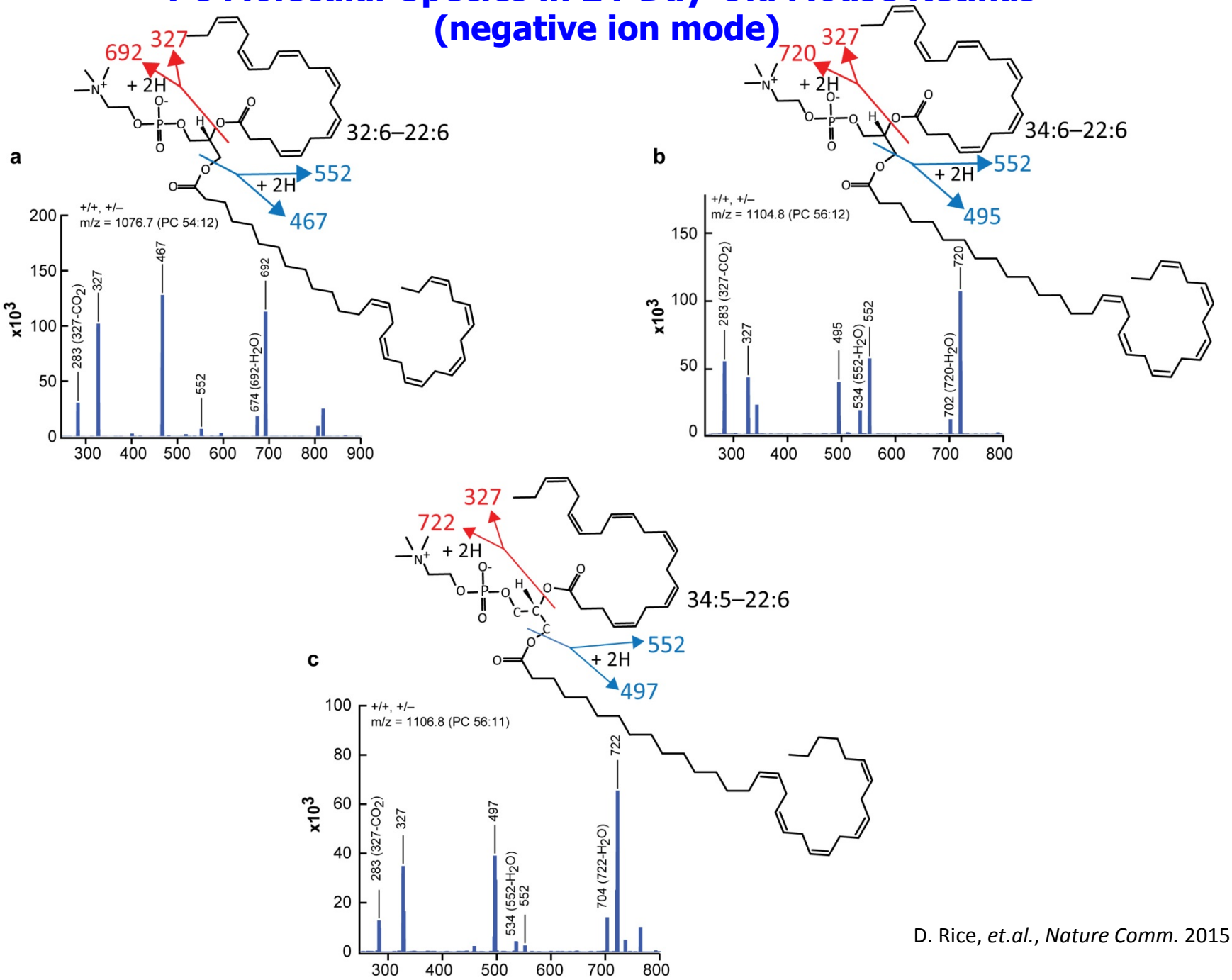
Kadowaki *et al.*, JCI, 2014

Impaired Adiponectin Action Takes Place in Obesity and of Other Diseases



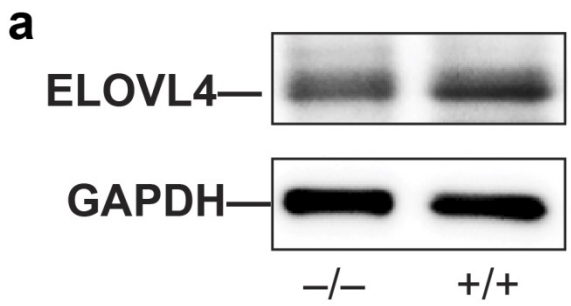
Toshimasa Yamauchi *et al.*, *Best Pract Res Clin Endocrin Metab* 2014

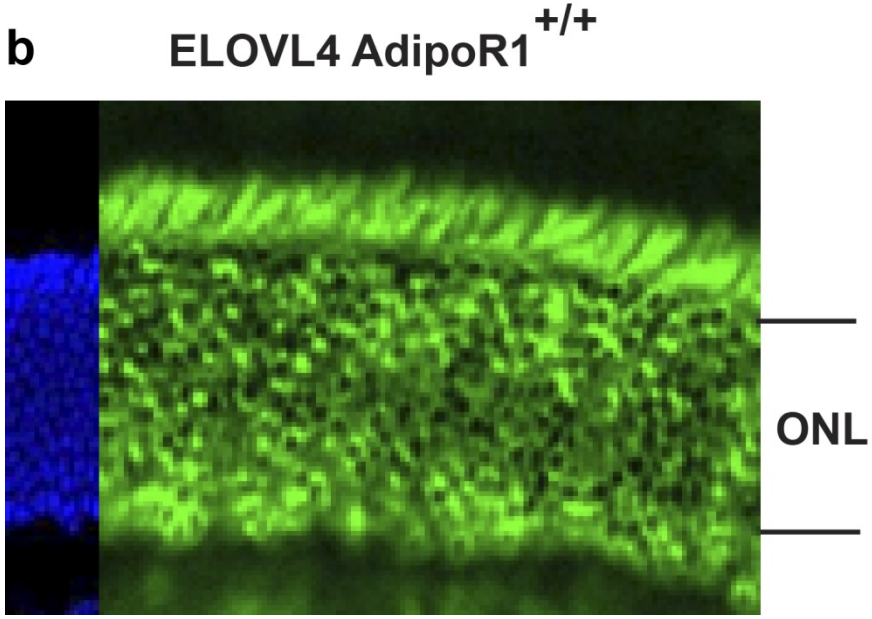
PC Molecular Species in 24-Day-Old Mouse Retinas (negative ion mode)

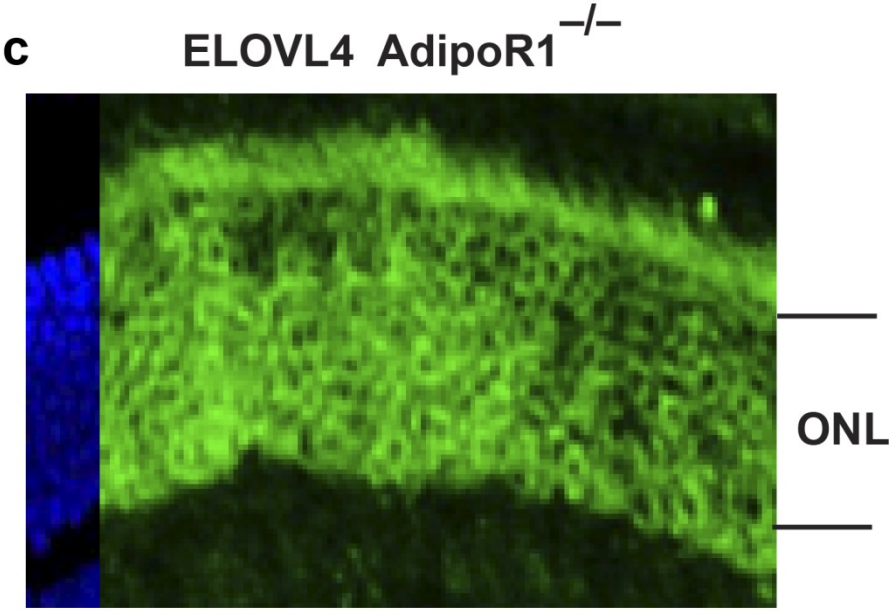


D. Rice, *et.al.*, *Nature Comm.* 2015

ELOVL4 is not Down-Regulated in AdipoR1$^{-/-}$ Retinas

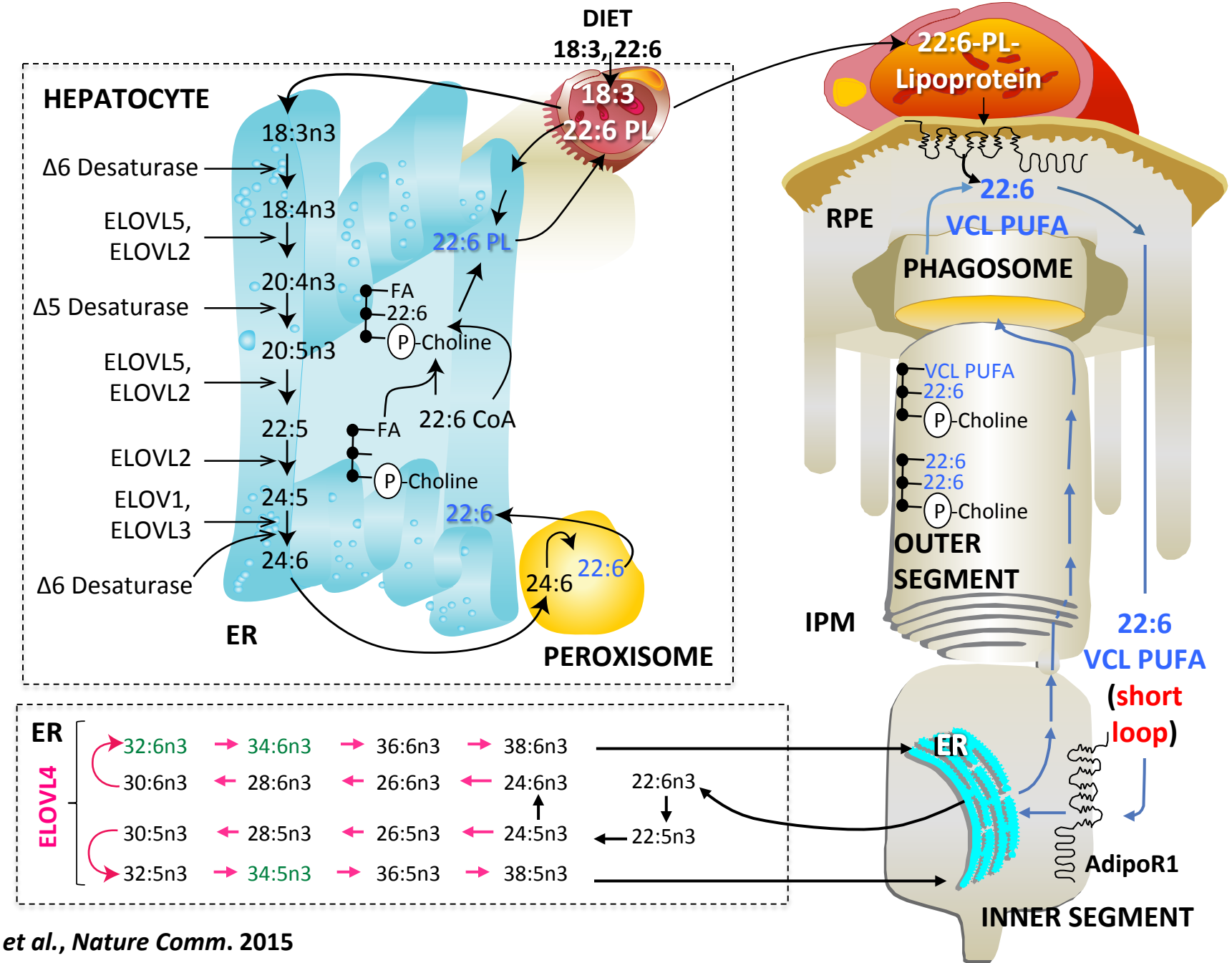






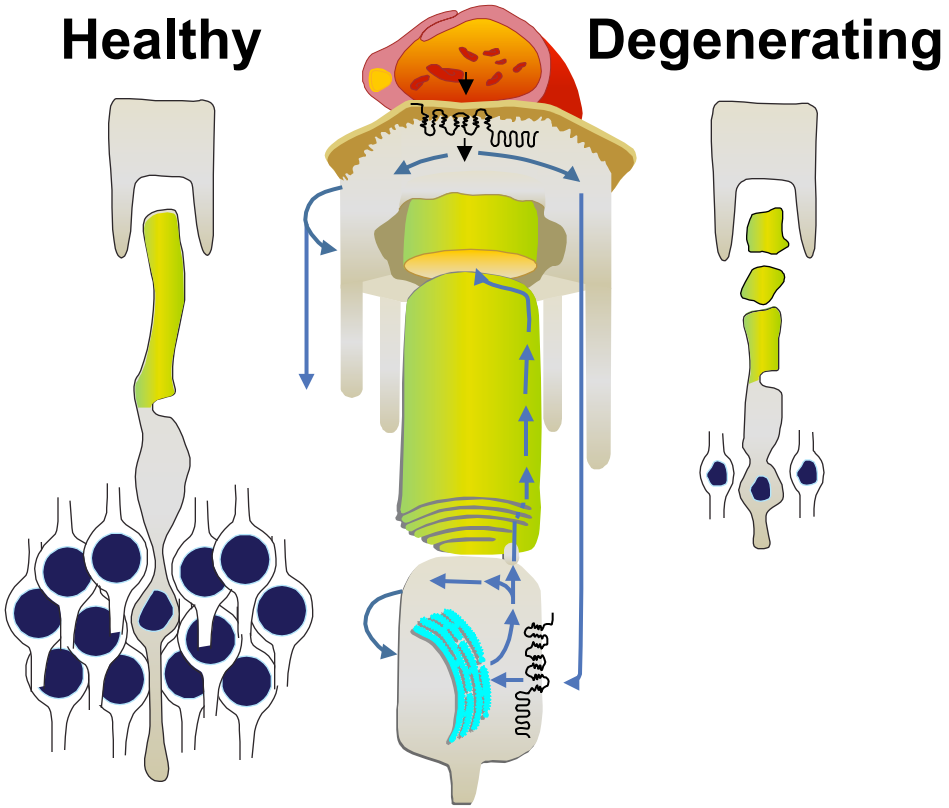
Rice *et al.*, *Nature Comm*. 2015

Docosanomics: A Novel Molecular Switch



Rice *et al.*, *Nature Comm*. 2015

AdipoR1: Organizes the DHA Lipidome in Photoreceptor Cell Membranes and is Necessary for Cell Integrity and Survival, Hence Essential for Vision



Novel Molecular Switch that Selectively and Specifically Controls the DHA Lipidome

Adiponectin Receptor 1 is an Integral Membrane Protein Necessary for Maintenance of Photoreceptor Cell Structure and Function Because It Sustains Specific Molecular Species of Phosphatidylcholine

Ablation of AdipoR1:

1. **Flecked Retinahotoreceptor Cell Degeneration**
2. **Attenuated ERG and Prolonged Dark Recovery**
3. **Impaired Retinal Visual Cycle**
4. **Adiponectin KO (cognate ligand) does not display Retinal Degeneration Phenotype.**

SNP in human AdipoR1 gene associated with AMD

Kaarniranta K. *et al.*, Adiponectin receptor 1 gene variant is associated with advanced age-related macular degeneration in Finnish population. *Neurosci Lett.* 2012 ,513:233-7.

The Adiponectin Receptor 1 is a Regulatory Switch of the DHA Lipidome in the Retinal Pigment Epithelium and Photoreceptor Cells

Ablation of AdipoR1 leads to:

1. **Reduction in retinal DHA levels.**
2. **Decreased DHA uptake into the RPE when eyecups are incubated with d5-DHA. DHA is also decreased in human RPE cells by silencing or overexpressing AdipoR1.**
3. **Absence of phosphatidyl choline molecular species with VLC-PUFAs in retinas by 24 days of life.**
4. **Specific for DHA metabolism:**
   - **uptake**
   - **retention**
   - **conservation**
   - **elongation**
   - **availability for NPD1 synthesis and other docosanoids.**

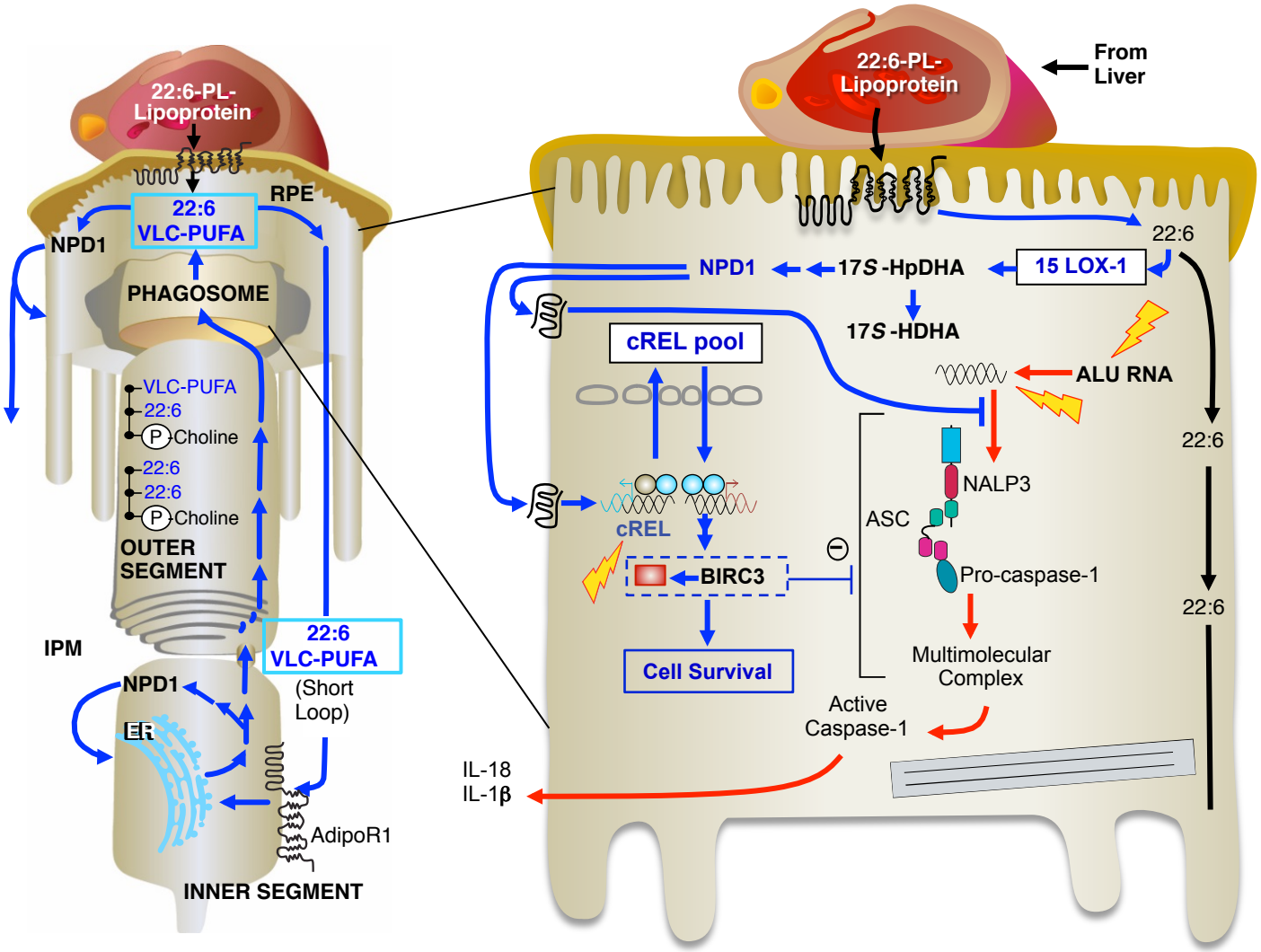
OPEN

Cell Death and Differentiation (2015), 1–15

www.nature.com/cdd

NPD1-mediated stereoselective regulation of BIRC3 expression through cREL is decisive for neural cell survival

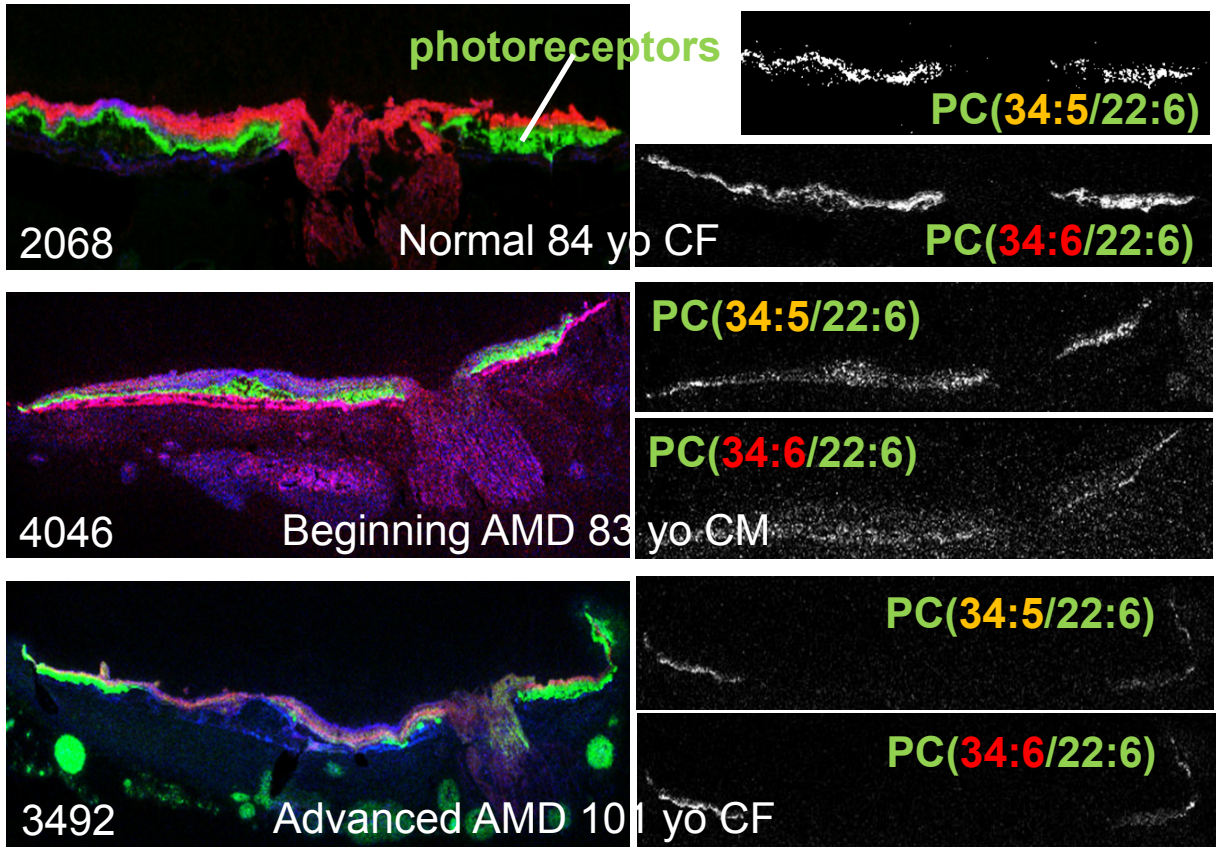
JM Calandria[1], A Asatryan[1], V Balaszczuk[1], EJ Knott[1], BK Jun[1], PK Mukherjee[1], L Belayev[1] and NG Bazan*[1]



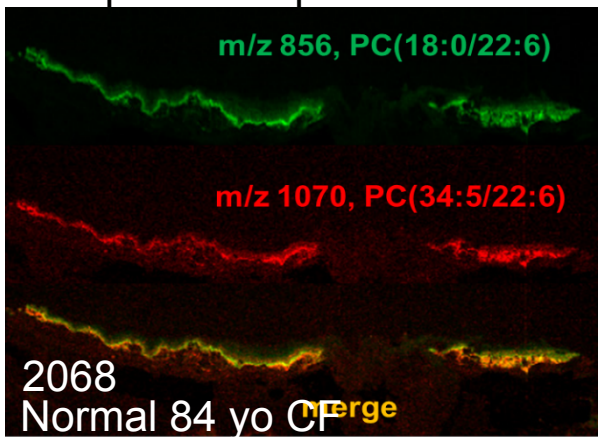
Spatial Organization of DHA-Containing Phospholipid Molecular Species in the Human Retina by Imaging Mass Spectrometry

Red: m/z 810.5
PE(16:0/22:6)+K$^+$
Green: m/z 856.5
PC(18:0/22:6)
Blue: m/z 818.6
PC(p18:0/22:6)

VLC-PUFAs are diminished in degenerating retinas

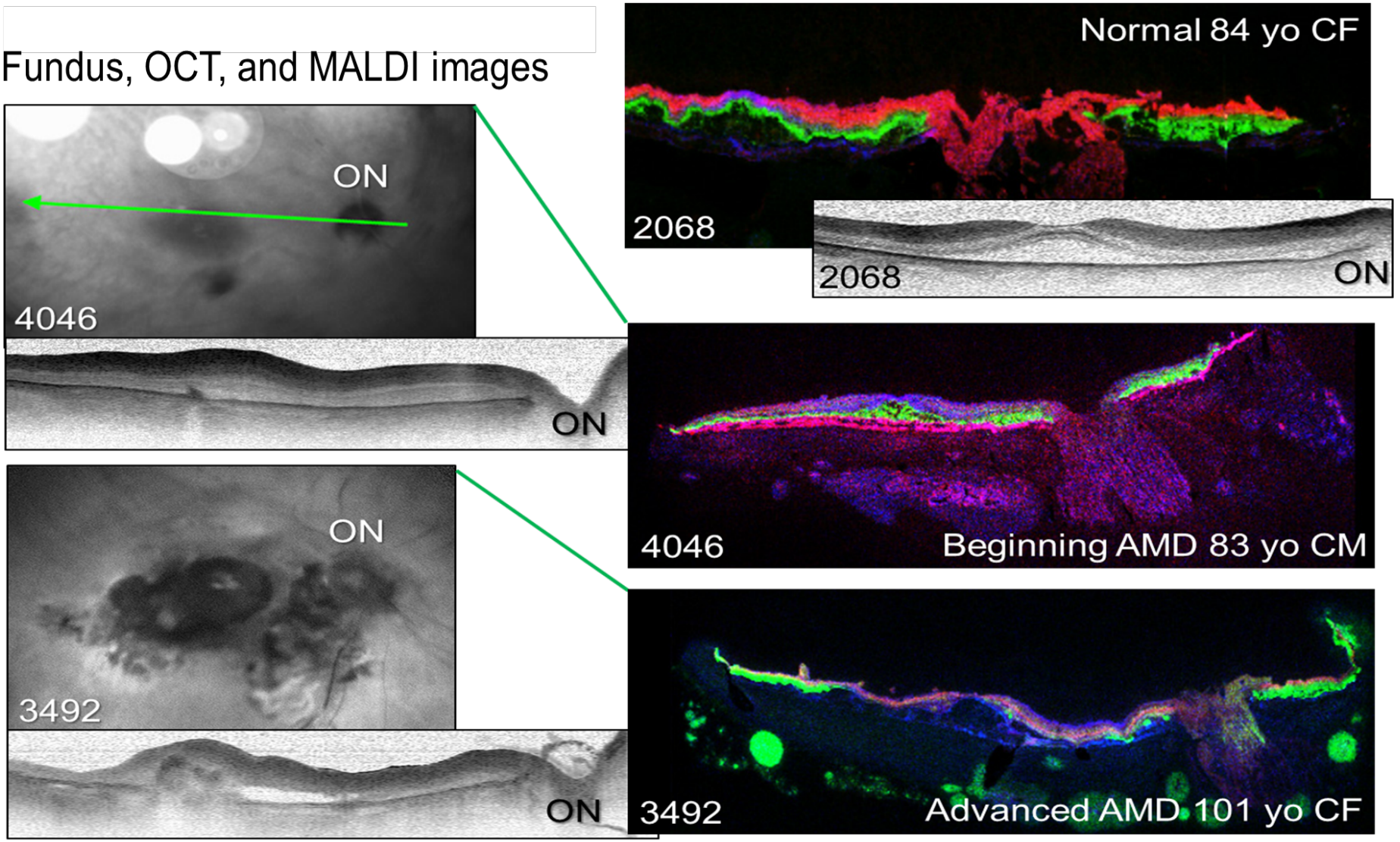


VLC-PUFAs co-localize with photoreceptors



W. Gordon, B. Jun and N. Bazan, to be submitted for publication

Retinal Spatial Organization of DHA-Containing Phospholipid Molecular Species in AMD by Imaging Mass Spectrometry



W. Gordon, B. Jun and N. Bazan, to be submitted for publication

HOW NOVEL MOLECULAR PRINCIPLES CAN BE HARNESSED

1. Mimicking nature responses to onset and progression of photoreceptor and hearing cell dysfunction and loss

2. Develop analogs of the naturally occurring survival mediators to make them bioavailable and to attain longer lasting actions

3. Still need to define disease modifying proteins/lipids in Usher's

4. For deafness, neurological insights are evolving at the molecular level based on new understandings of neuronal plasticity

5. Combination of therapy with genetic/antisense or other approaches

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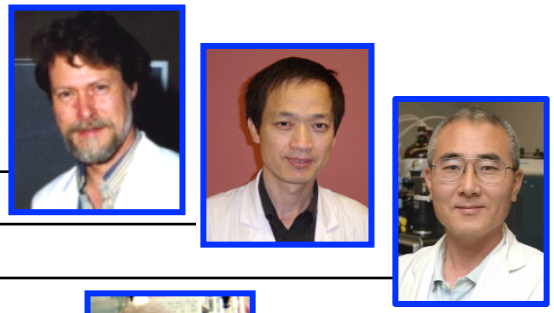

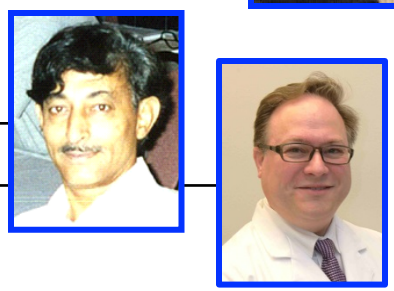

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