Assessing the Current Road Map for Developing Disease Modifying Therapies for Neurodegenerative Diseases .

Todd E. Golde, M.D., Ph.D.
Director, Center for Translational Research in Neurodegenerative Disease
Professor, Department of Neuroscience
University of Florida College of Medicine
tgolde@mbi.ufl.edu

Disclosures:

Previous SABs/Consultancies: Elan, Wyeth, Pfizer, BMS, Novartis, Lundbeck, Sonexa, Genentech, Roche. JANNSEN, IDI, Novartis. Previous Sponsored Research: Lundbeck, Myriad, Pfizer.

Current: Inventor on Patents/Patent Applications relating to GSMs, STLRs, Aβ immunotherapy, and BRI peptides.

Strategic thinking about the next 10 years of AD research (My original Intent)

- How do we maximize our likelihood of developing disease modifying therapies including treatment (as opposed to prevention) for AD and related dementias?
- Strengths, Weaknesses, Opportunities and Threats of our current road map
- An invitation to send me your thoughts and participate as a co-author in a manuscript
- List your top 5 Strengths, Weaknesses, Opportunities and Threats and send them to me at tgolde@ufl.edu
- If a sufficient number of you contribute I'll compile draft a manuscript and engage all contributors to finalize
- I'll likely submit to Alzheimer's Research and Therapy (where I am a co-editor in chief)

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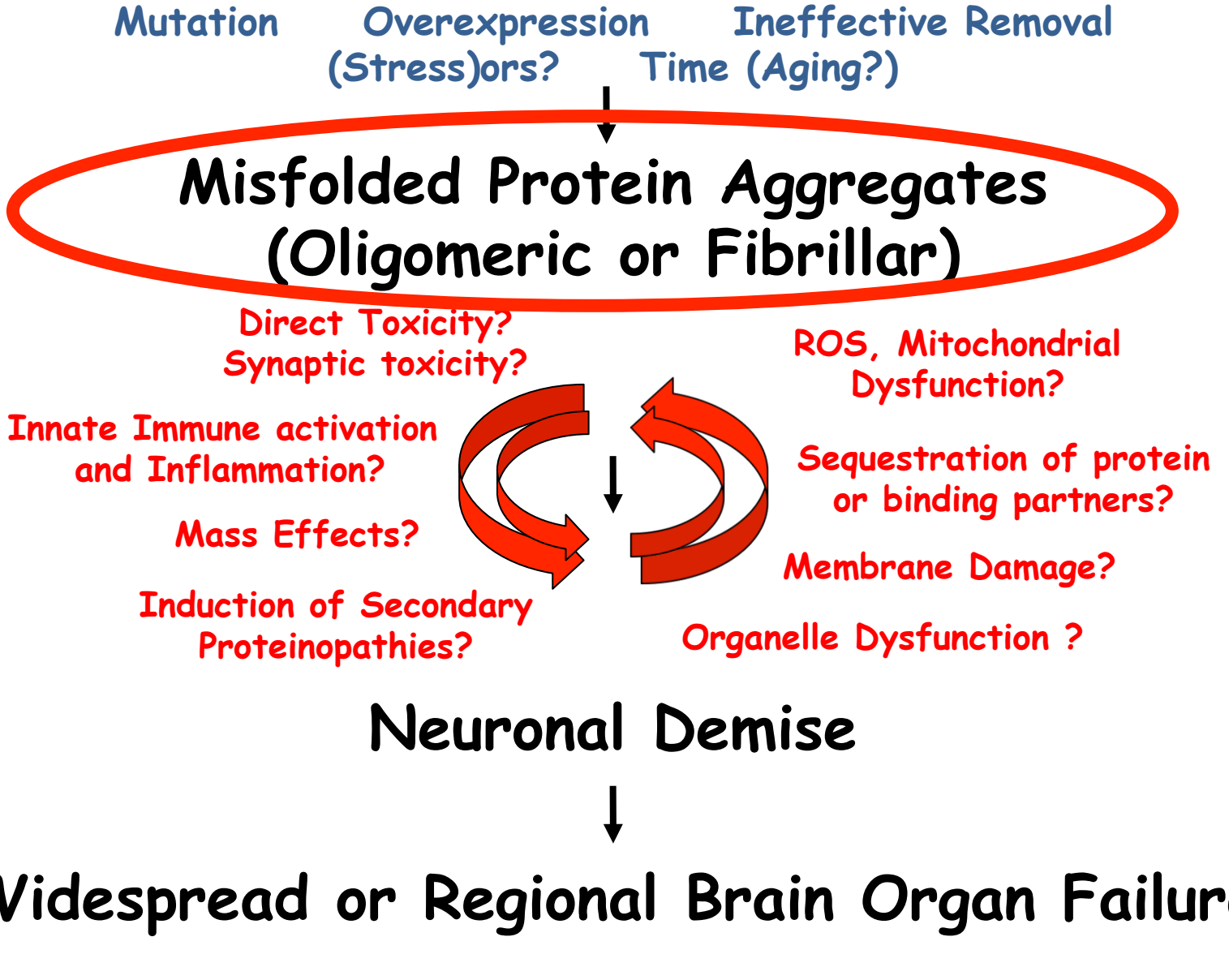
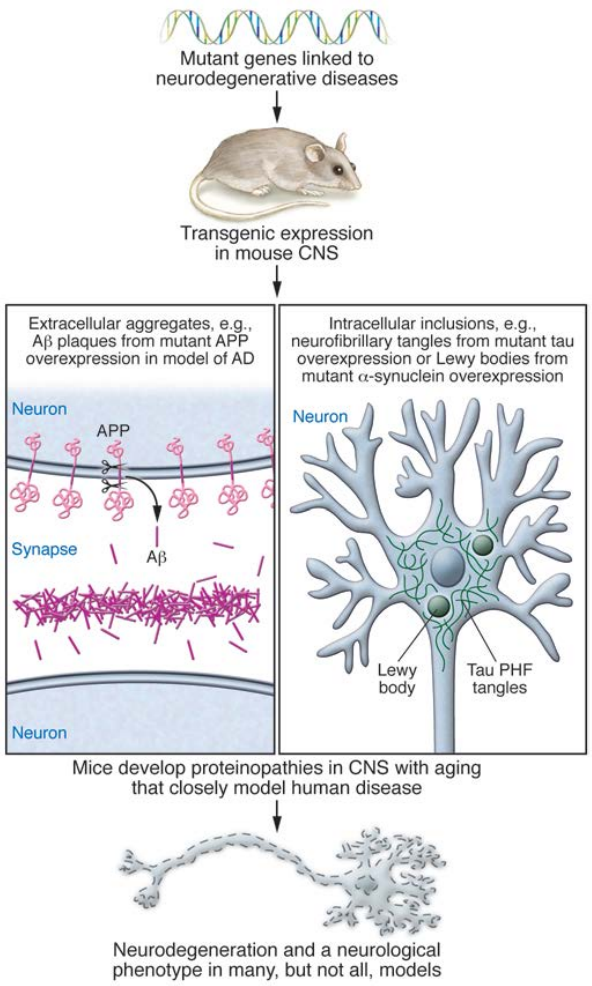
Introduction

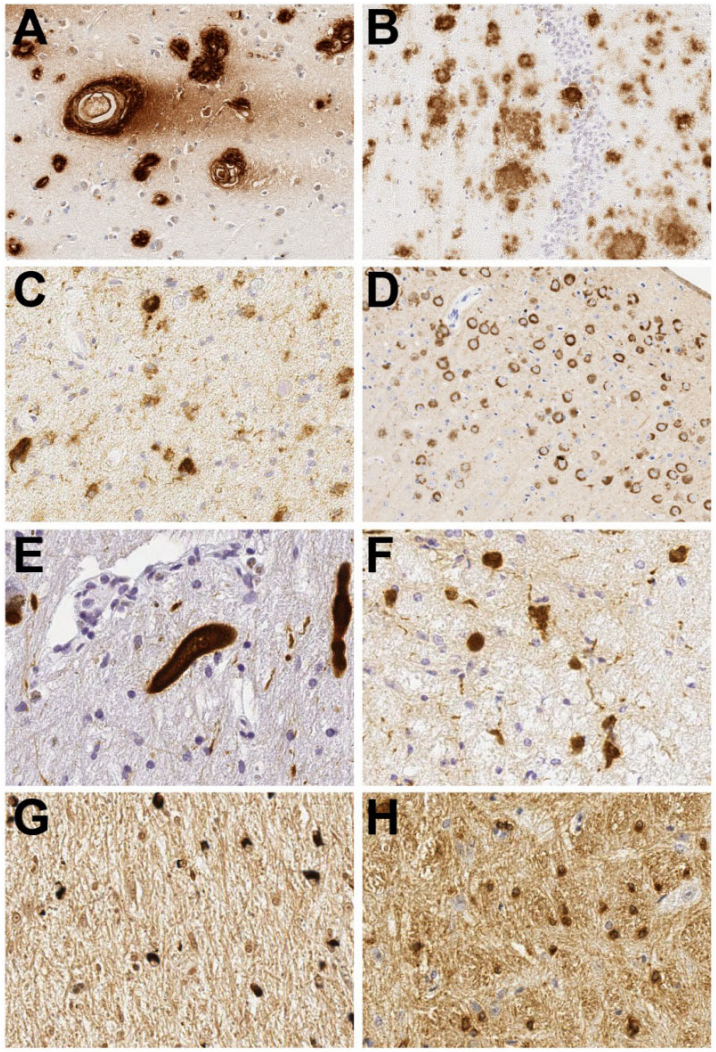
- We need to think laterally about Neurodegenerative Diseases: not in disease-specific silos (see Golde et al JCI 2013)
  - *There has been an amazing convergence in terms of understanding disease triggers but this convergence has not yet been fully leveraged for therapeutic discovery*
- Working Hypothesis: "Aspects of the proteinopathy driven neurodegenerative cascade are shared between various diseases (AD, FTLD, PD, ALS, SCAs, HD)"
- Understanding these shared pathways may lead to therapeutic strategies that can be effective in more than one disease and work as true therapeutics as opposed to prophylactics
- **Evolution of >7 years of activity in trying to identify new therapeutic strategies for neurodegenerative diseases**
- **Trying to answer the question " How do we accelerate preclinical studies that are designed to provide target validation and simultaneously a biologic lead therapeutic"**

The Proteinopathy Hypothesis of Neurodegeneration



The Proteinopathy Hypothesis of Neurodegeneration





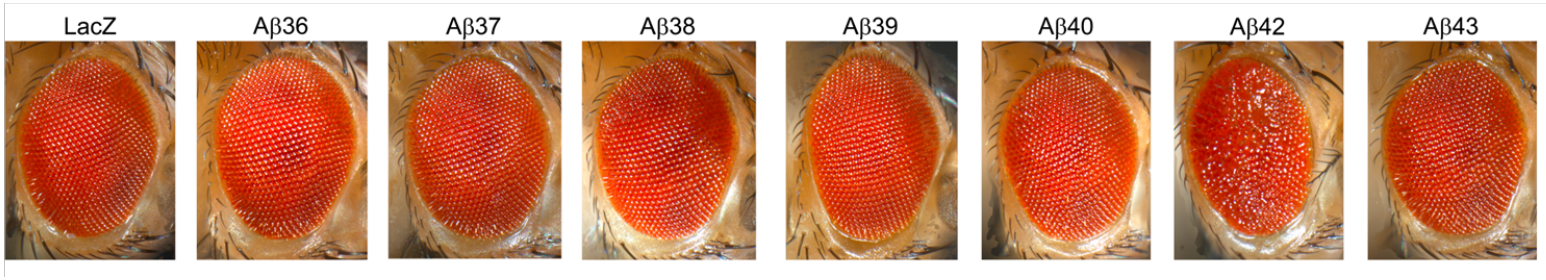
Human Mouse

Toxicity in Most Models Correlates with Aggregation

To examine Aβ1-36,37,38,39,40,42 or 43 by themselves.

GMR-Gal4 ✖ Aβ Peptides/TM6B 1 copy each

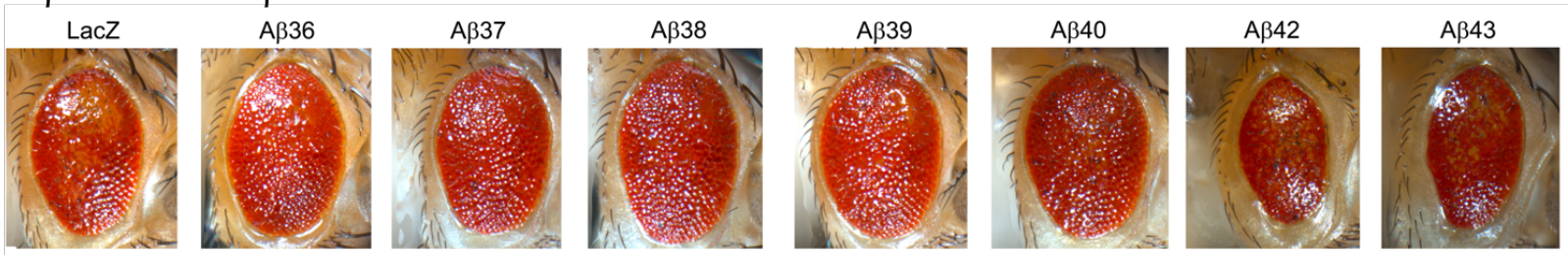
Gal4 X Aβ:



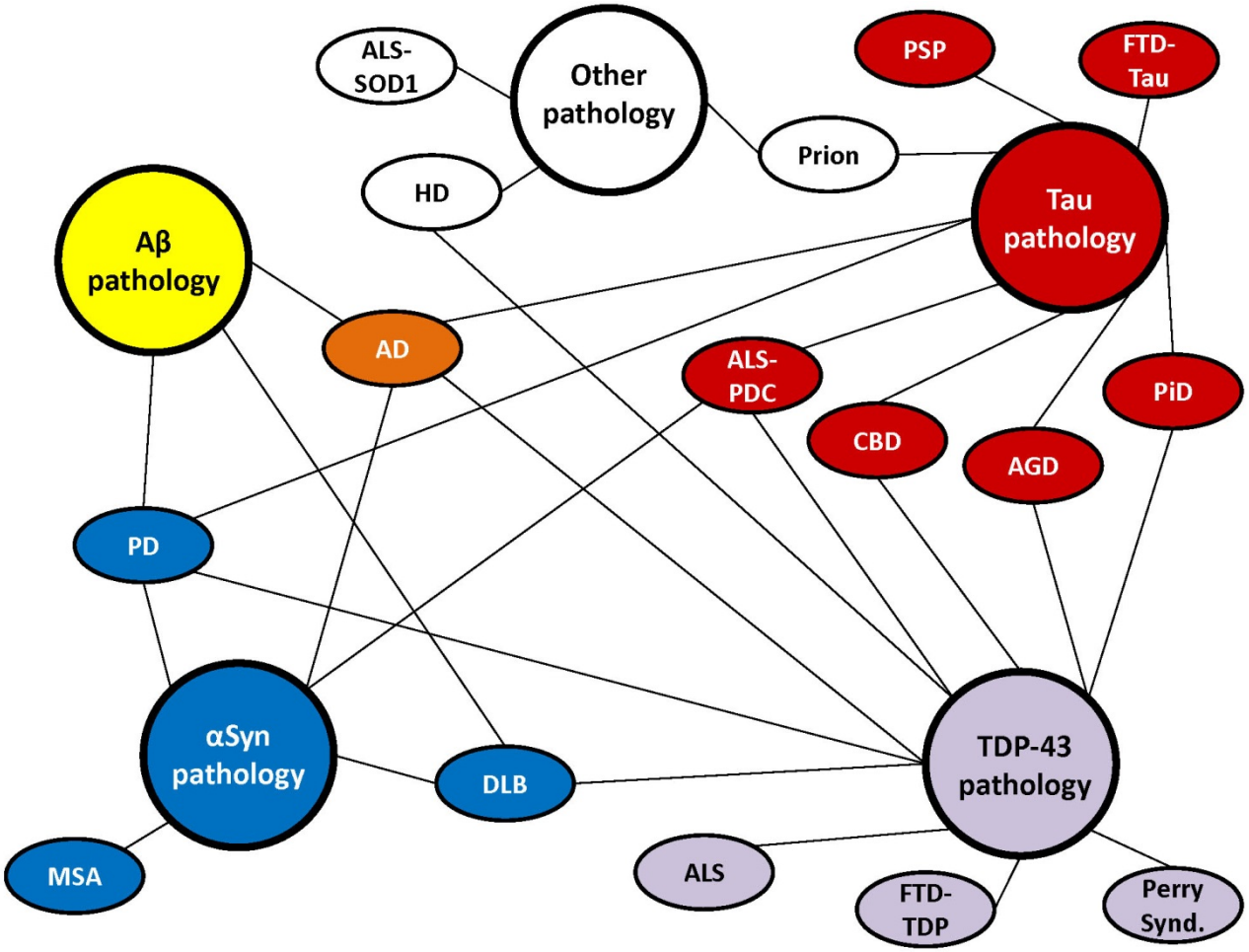
To examine Aβ1-36,37,38,39,40,42 or 43 crossed to Aβ42 fly.

GMR-Aβ42 2 copies ✖ Aβ Peptides/TM6B 1 copy

Aβ42 X Short Aβ:

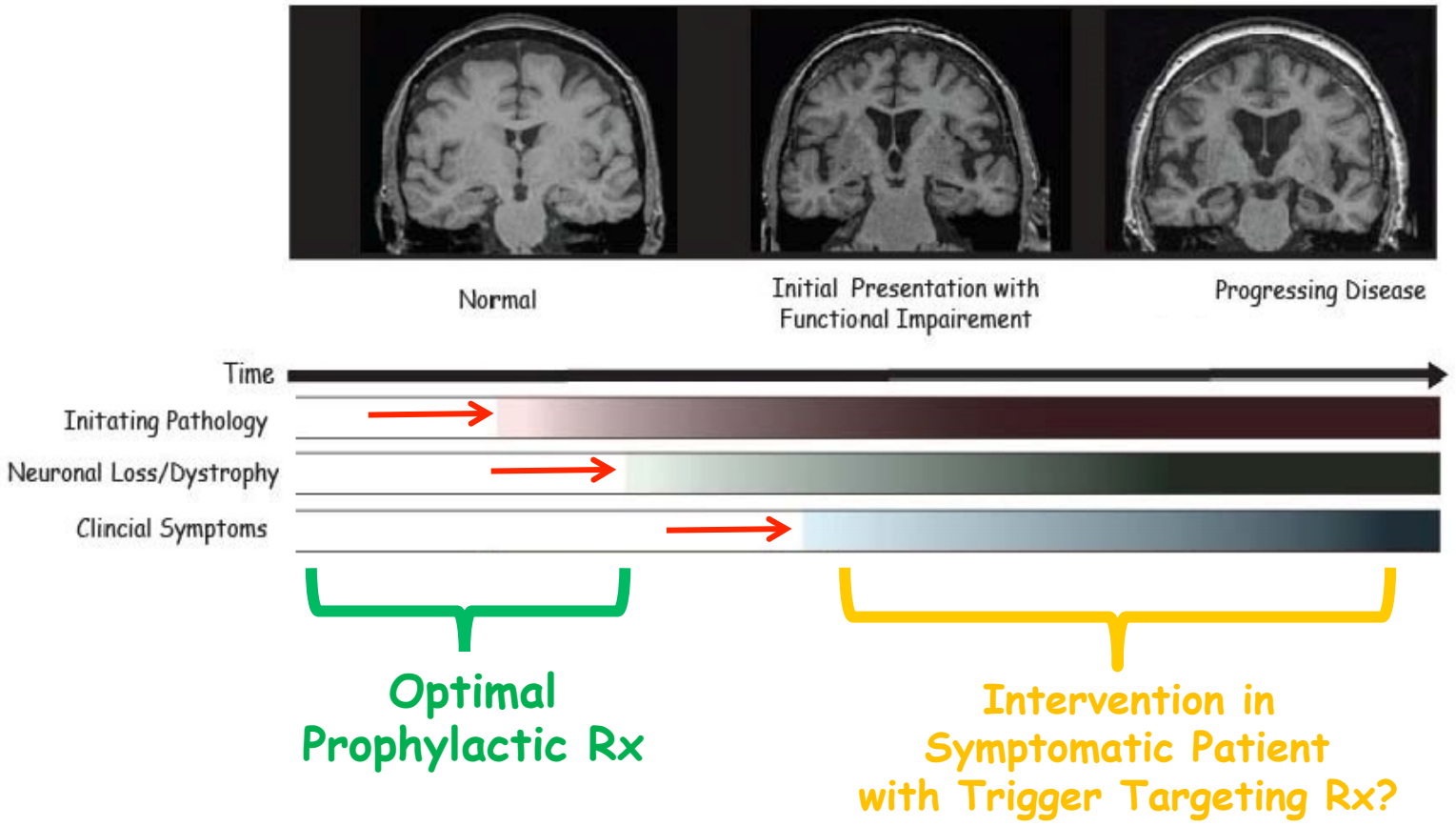


Complex Inclusion Pathology in Most Human CNS Proteinopathies



Reviewed in Golde et al JCI 2013

The Dilemma of Treatment versus Prevention
(see Golde, Schneider and Koo, Neuron 2011)



Efficacy of trigger targeting therapy will decline as pathology progresses! We should avoid Kobayashi Maru Scenarios

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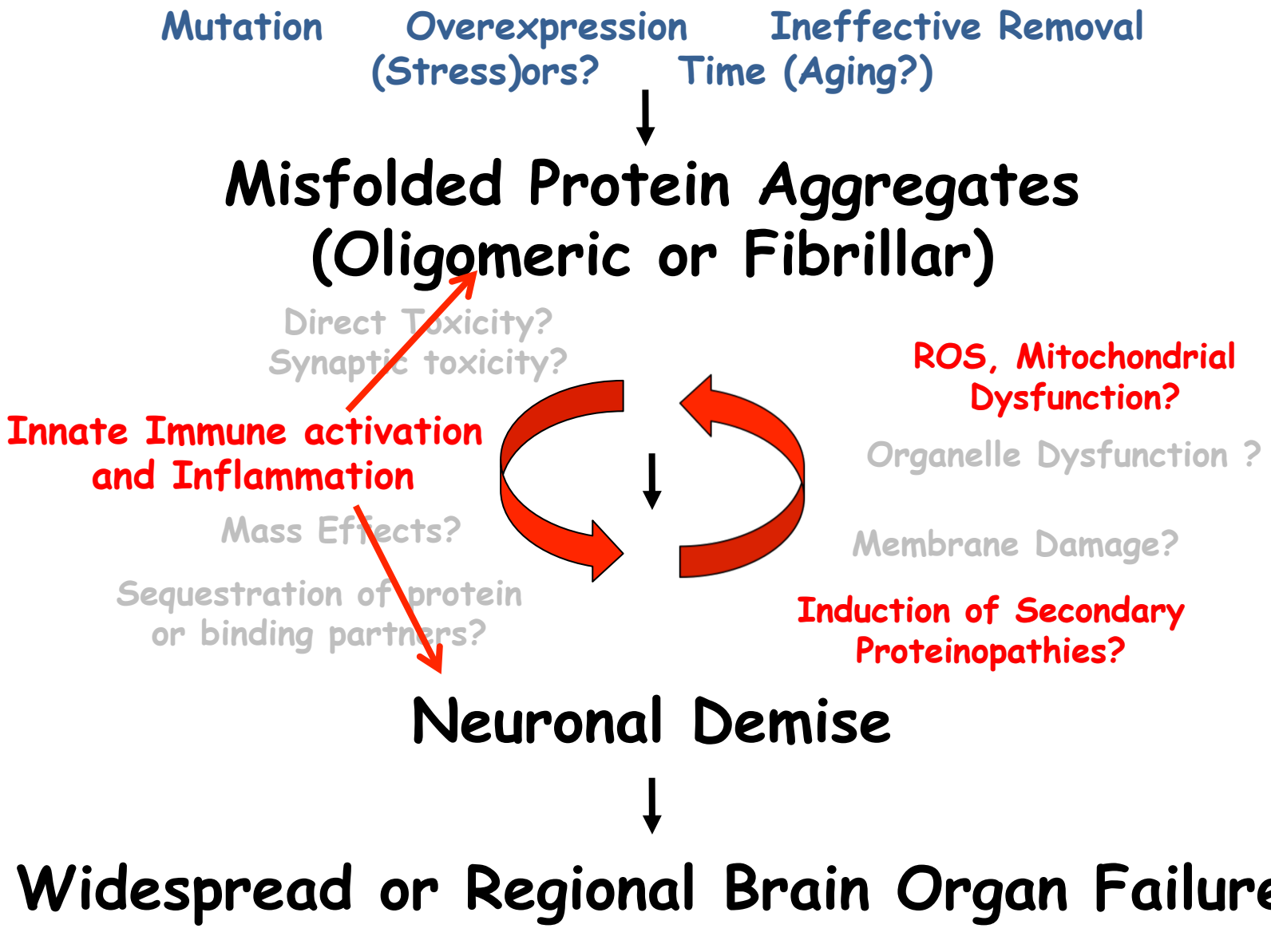
Success in trigger targeting therapy is unlikely to obviate the need for therapies that target downstream pathways

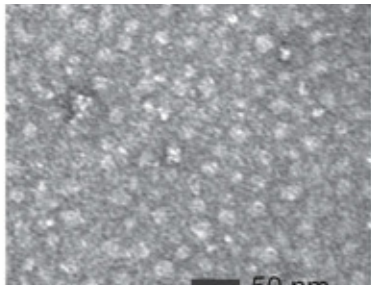
- We need to develop interventions that may modify disease course, even later in the disease. This is challenging as:
  - the biology is much less certain
  - animal models used in preclinical studies are often poor phenocopies of downstream events in the human disease
  - Would a therapy that works in multiple models be more translatable?
  - Can we harness innate immunity to treat multiple neurodegenerative disorders?

Rationale for Targeting Innate Immunity in Neurodegeneration

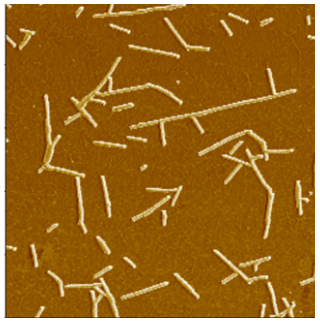


Name that Image

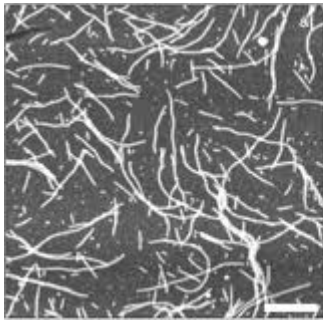
Lysozyme Amyloid and Protofibrils AFM



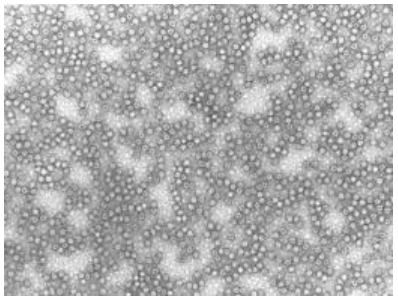
Aβ Oligomers EM



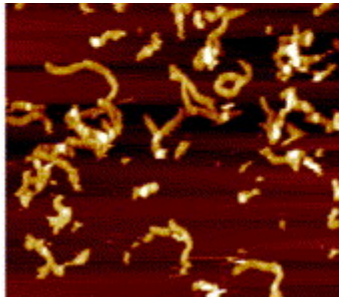
Tobacco Etch Virus AFM



Potato Virus AFM



rAAV EM



Aβ Protofibrils AFM

A normal protein (self) folded into a protein aggregate becomes a danger associated molecular pattern (non-self) that can activate innate immunity like a virus or bacteria.

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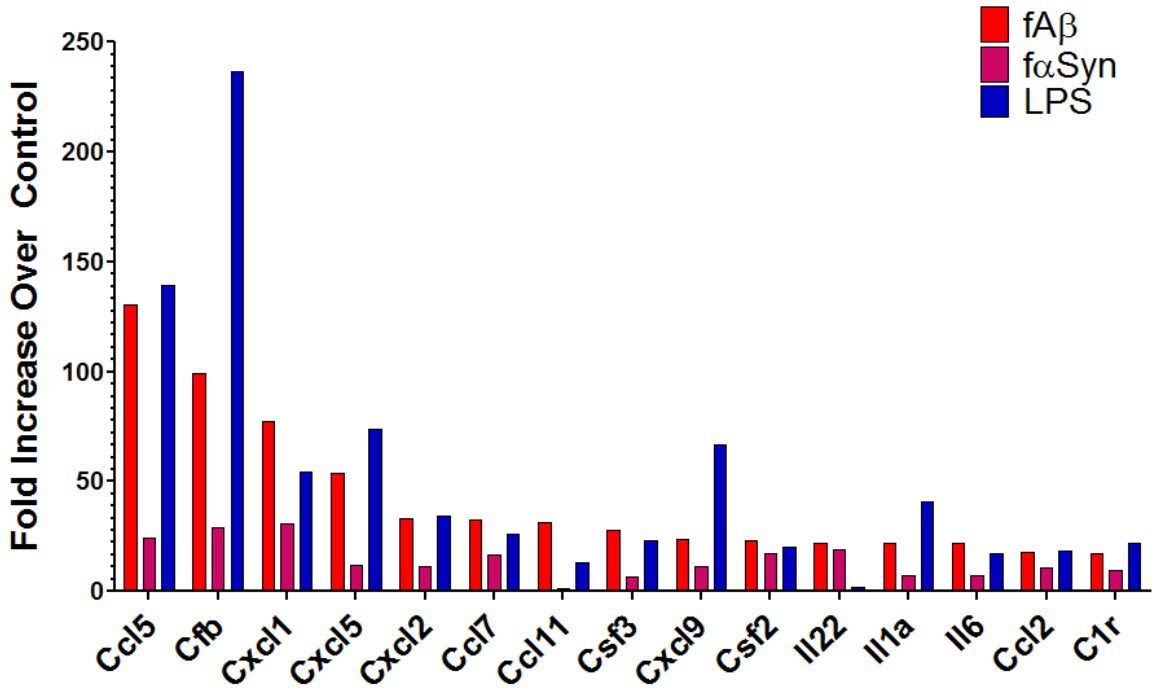
Aβ and other amyloids are DAMPs



Both fibrillar Aβ and α-synuclein activate innate immunity as assessed by Nanostring Gene Counter Arrays

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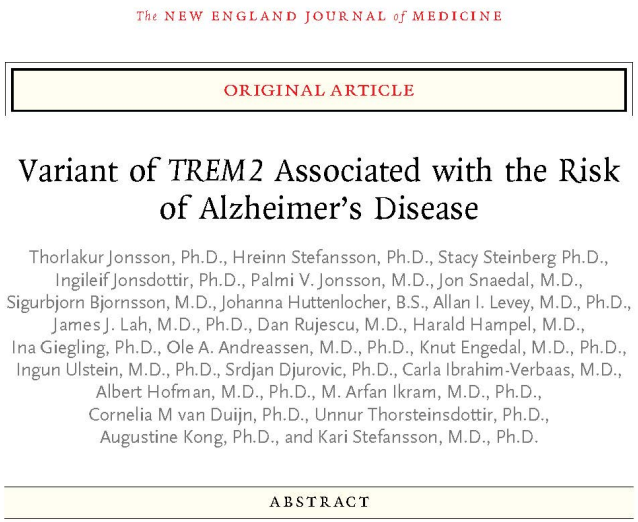
2012-13 Genetics Implicates Innate Immunity in AD



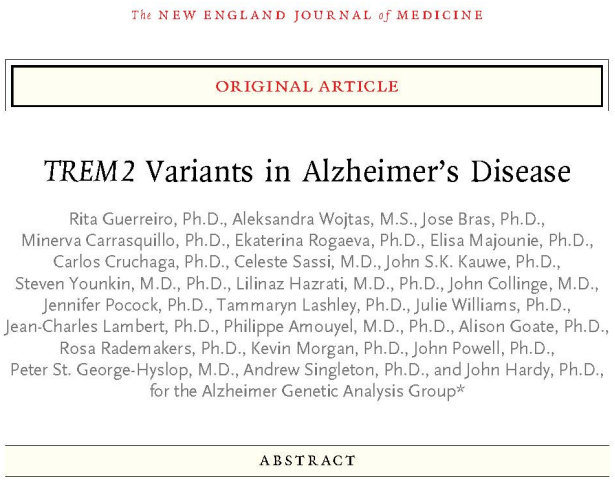
The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Variant of *TREM2* Associated with the Risk of Alzheimer's Disease

Thorlakur Jonsson, Ph.D., Hreinn Stefansson, Ph.D., Stacy Steinberg Ph.D., Ingileif Jonsdottir, Ph.D., Palmi V. Jonsson, M.D., Jon Snaedal, M.D., Sigurbjorn Bjornsson, M.D., Johanna Huttenlocher, B.S., Allan I. Levey, M.D., Ph.D., James J. Lah, M.D., Ph.D., Dan Rujescu, M.D., Harald Hampel, M.D., Ina Giegling, Ph.D., Ole A. Andreassen, M.D., Ph.D., Knut Engedal, M.D., Ph.D., Ingun Ulstein, M.D., Ph.D., Srdjan Djurovic, Ph.D., Carla Ibrahim-Verbaas, M.D., Albert Hofman, M.D., Ph.D., M. Arfan Ikram, M.D., Ph.D., Cornelia M van Duijn, Ph.D., Unnur Thorsteinsdottir, Ph.D., Augustine Kong, Ph.D., and Kari Stefansson, M.D., Ph.D.

ABSTRACT



The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

TREM2 Variants in Alzheimer's Disease

Rita Guerreiro, Ph.D., Aleksandra Wojtas, M.S., Jose Bras, Ph.D., Minerva Carrasquillo, Ph.D., Ekaterina Rogaeva, Ph.D., Elisa Majounie, Ph.D., Carlos Cruchaga, Ph.D., Celeste Sassi, M.D., John S.K. Kauwe, Ph.D., Steven Younkin, M.D., Ph.D., Lilinaz Hazrati, M.D., Ph.D., John Collinge, M.D., Jennifer Pocock, Ph.D., Tammaryn Lashley, Ph.D., Julie Williams, Ph.D., Jean-Charles Lambert, Ph.D., Philippe Amouyel, M.D., Ph.D., Alison Goate, Ph.D., Rosa Rademakers, Ph.D., Kevin Morgan, Ph.D., John Powell, Ph.D., Peter St. George-Hyslop, M.D., Andrew Singleton, Ph.D., and John Hardy, Ph.D., for the Alzheimer Genetic Analysis Group*

ABSTRACT

TREM2 variants have previously been associated with Nasu Hakola disease (PLOSL) and recently have been associated with ALS, FTD and PD (Paloneva et al Am J Hum Genet. 2002,Rayaprolu et al, Mol Neurodegener. 2013, Cady et al JAMA Neurol. 2014 Apr 1;71(4):449-53.

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Beta Testing a Neologism: Immunoproteostasis

- Aggregated proteins that form the inclusions found in many neurodegenerative diseases can activate the innate immune system
  - i.e., they are Danger Associated Molecular Patterns (DAMPs) that can activate both intra and extracellular pattern recognition receptors (PRRs)
- In turn, innate immune activation can contribute to the degenerative cascade and cognitive dysfunction.
  - Best example is HIV dementia
- Innate immune signaling in the brain can also play a key role in regulating proteostasis of key pathogenic proteins linked to neurodegenerative disorders.
- I'd like to propose that we term this complex interplay between the innate immune system and the proteinopathy, **immunoproteostasis.**

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Chakrabarty et al Neuron 2015 in press

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Can we harness immunoproteostasis to treat AD and other neurodegenerative disorders?

- Challenges:
  - Delicate **balance between positive and negative effects** of innate immune signaling on proteostasis, neurodegeneration and cognitive function
  - This balance may be contextually dependent on the **nature, strength and timing** of the Innate Immune Signals
  - **Immunoproteostasis in mice may be different then in aged humans**
    - Aging skews the human brain towards a "proinflammatory" state in the apparent absence of underlying proteinopathy (Cribbs et al JNI 2012)
  - Potential for "untoward" systemic effects

Can we harness immunoproteostasis to treat AD and other neurodegenerative disorders?

- Opportunities:
  - **Potential for disease modification in later stages of disease –i.e., downstream of trigger**
  - **Efficacy in Multiple Diseases?**
    - Evidence that innate immune activation may be similar in multiple CNS proteinopathies
    - Possibility of cognitive effect in absence of disease modification
    - Possibility of beneficial effect in auto-immune inflammatory conditions
  - **Lots of targets that can be manipulated in non-cell autonomous manner**
  - **Ability to conduct parallel biologic agonist antagonist studies**
  - Strong likelihood of theragnostic biomarker development to assess target engagement

Taking Risks

- When we began this endeavor we had to deal with a lot of uncertainty about what immune targets to manipulate
- We also had no choice but to do this *in vivo*
  - *Skeptical of any "brain in a dish assay"*
- We have used rAAV "somatic transgenesis" as a technology accelerator to cost effectively evaluate potential targets and possibly identify lead biologic therapeutics *in vivo*
  - a modest throughput *in vivo* phenotypic screen in relevant proteinopathy models

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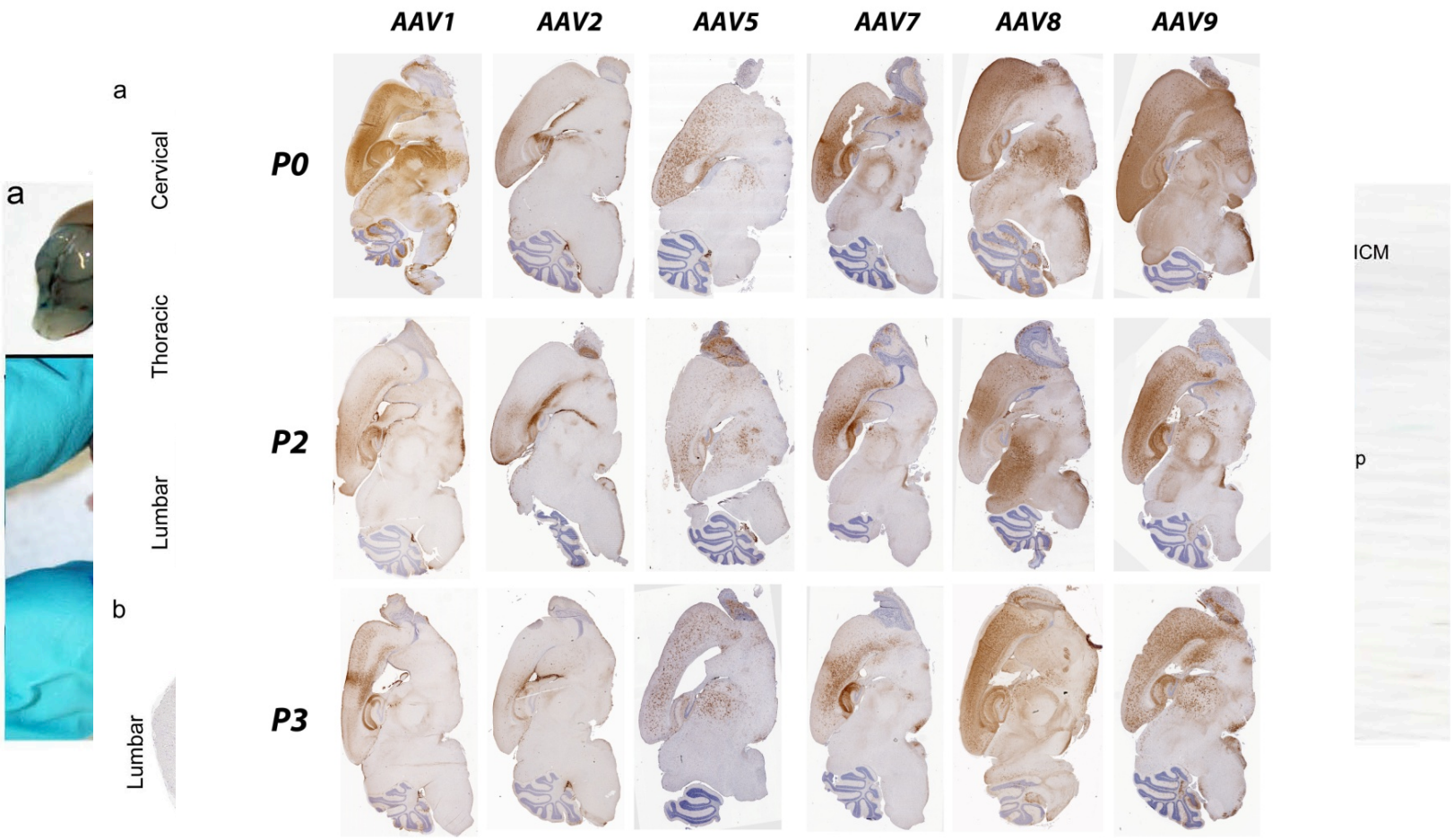
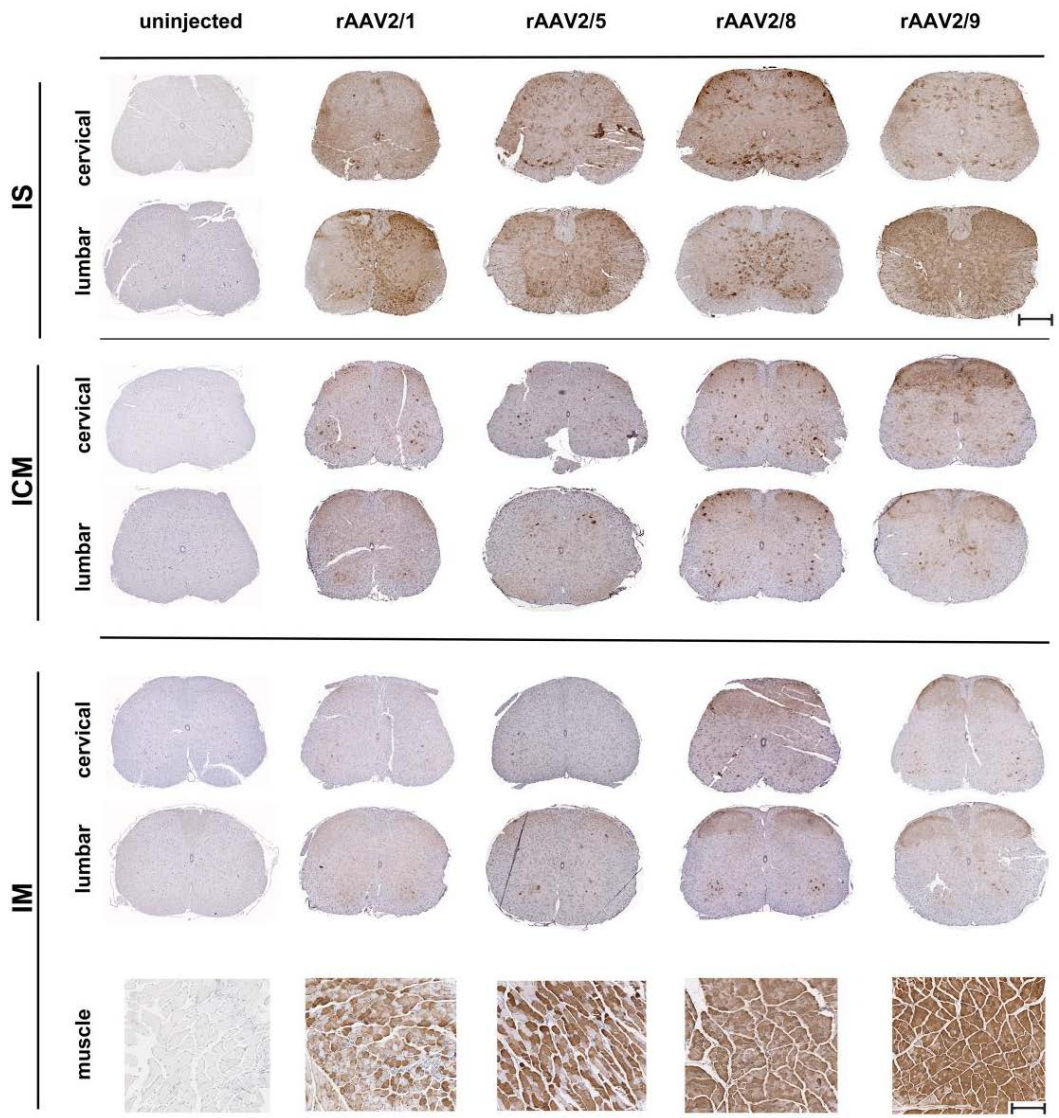
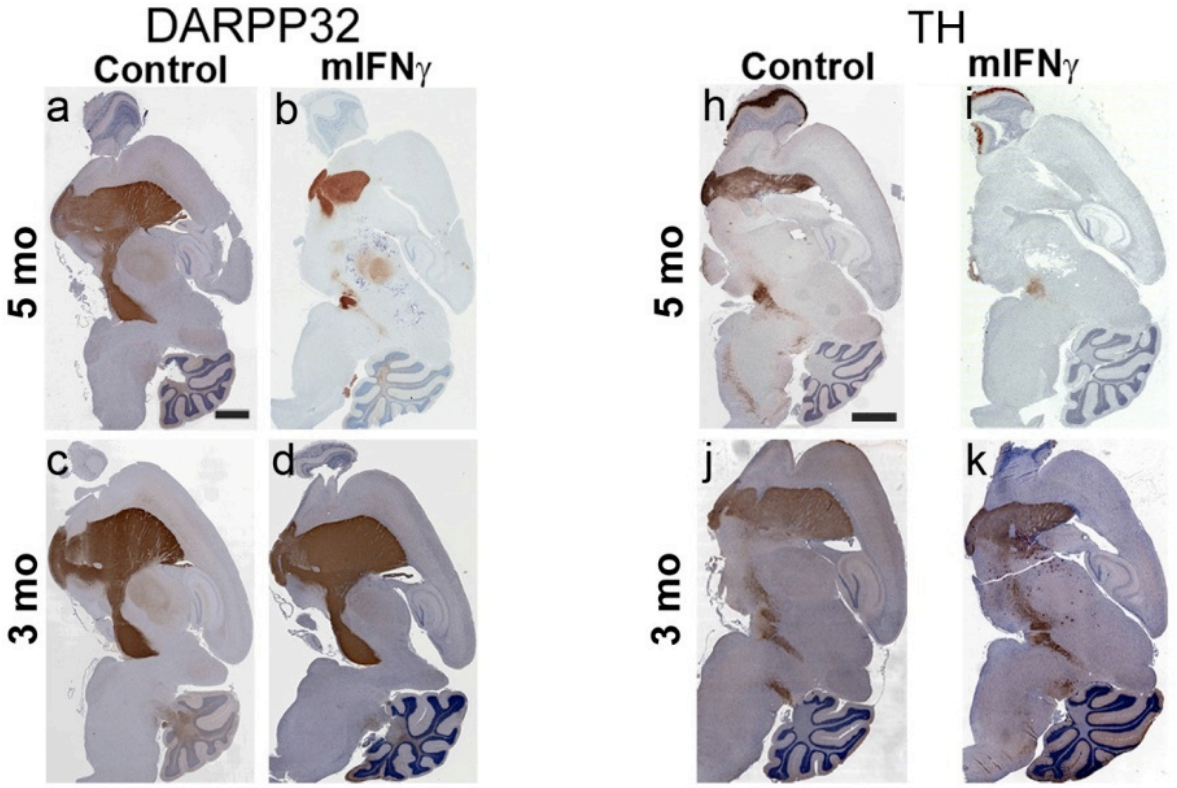
Somatic Brain and Spinal Cord Transgenesis



Somatic Brain and Spinal Cord Transgenesis



Manipulating innate immune activation states in the brain can produce interesting and unexpected phenotypes



Chakrabarty P, et al. Interferon-gamma induces progressive nigrostriatal degeneration and basal ganglia calcification. Nat Neurosci. 2011;14(6): 694-6.

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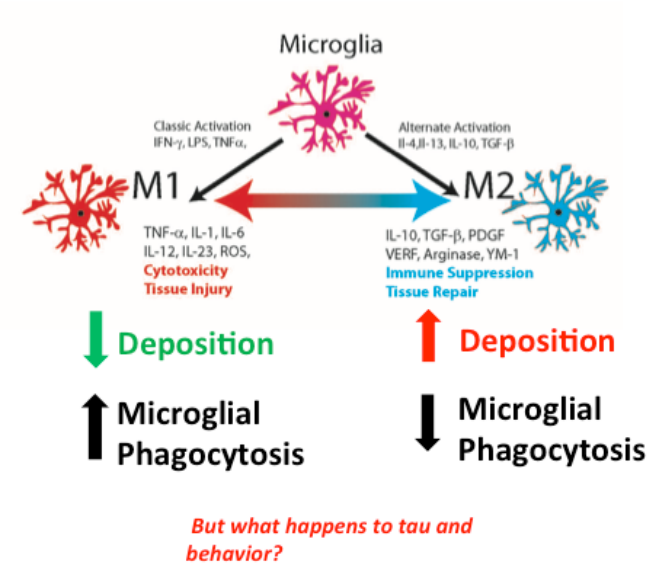
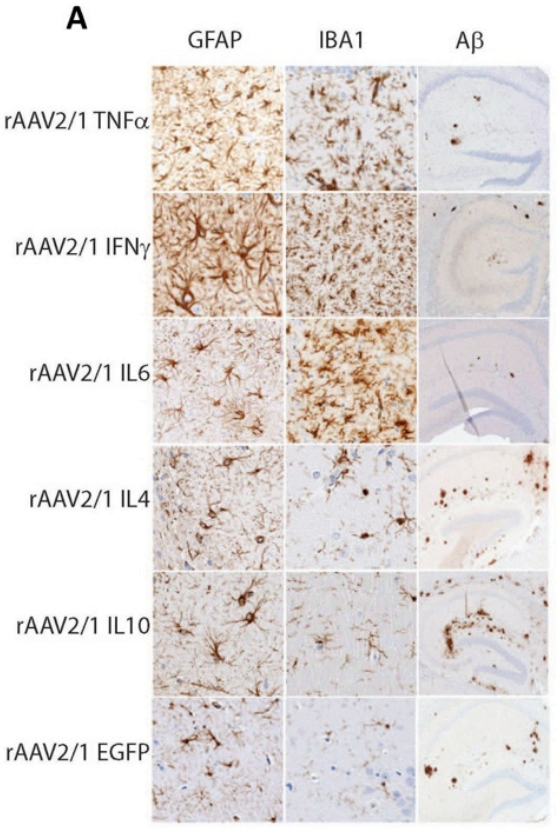
Manipulating Innate Immune Activation States in the APP mouse brain P0 studies in CRND8 mice (rAAV1)





- Il-6 Chakrabarty et al FASEB 2010
- INF-γ Chakrabarty et al J. Immunology 201
- TNF-α Chakrabarty et al Mol. Neurodegen
- Il-4 Chakrabarty et al Mol. Neurodegenera
- Il-10 Chakrabarty et al Neuron in Press 20

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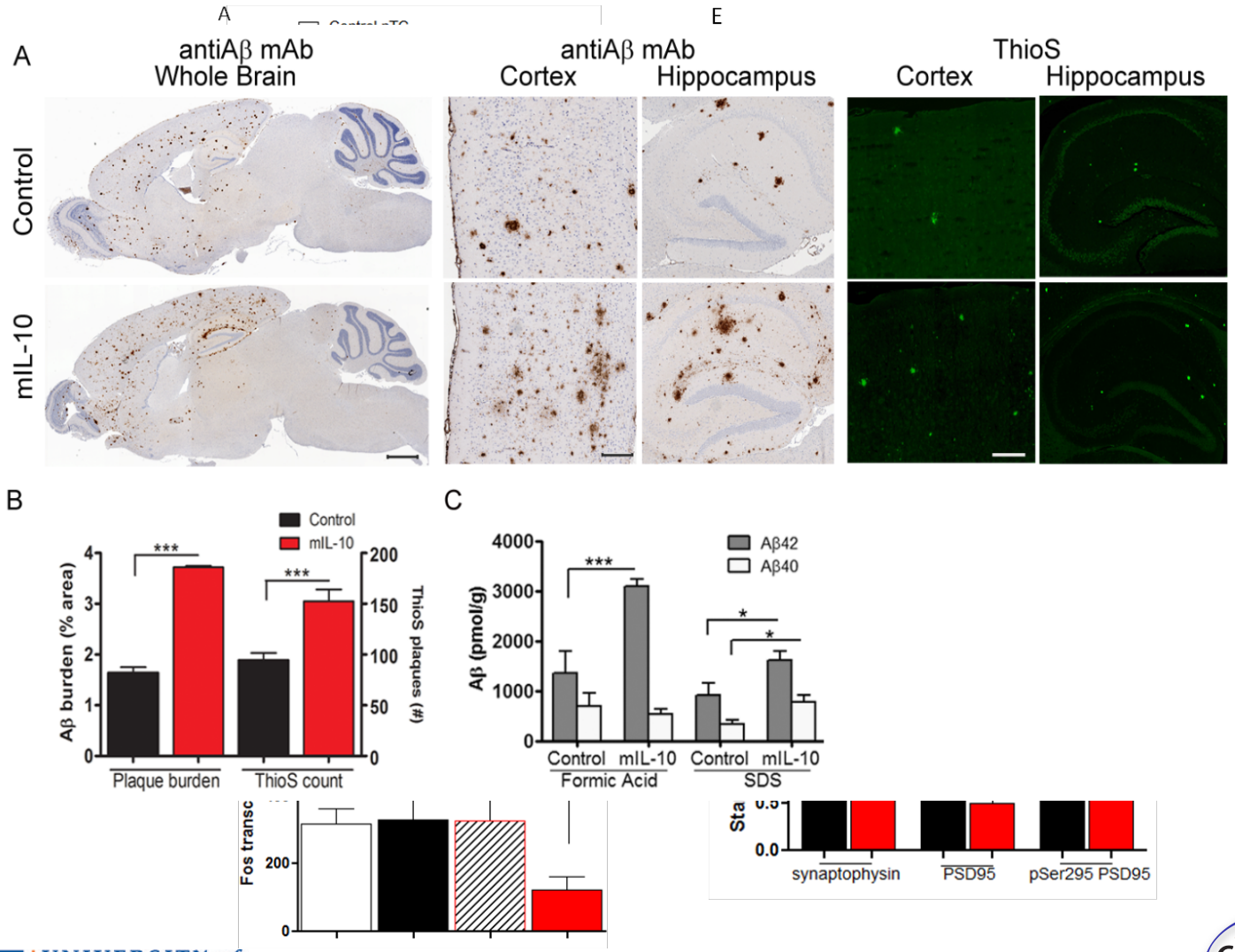
A closer look at the Il-10 studies



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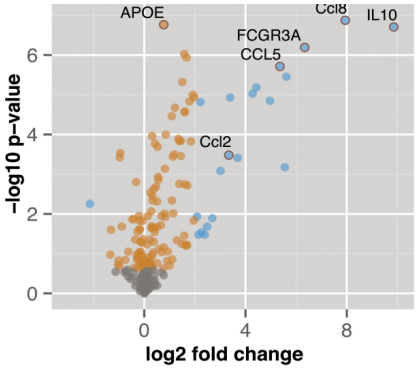
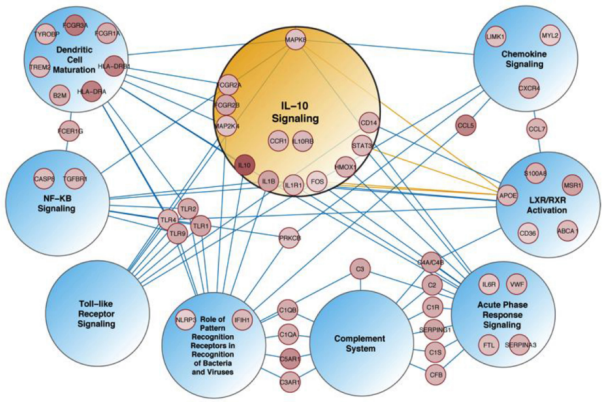
Systems Analysis of Nanostring Based RNA quantification data identify APOE as a possible factor in mediating the IL10 phenotype

H2-Ea	5.21	2E-05
H2-Eb1	3.66	0.0002
C4a	2.37	0.00
C1qb	1.47	0.00
C1qa	1.44	0.00
Cdc42	-0.11	8E-12
Map2k1	-0.18	2E-12
Gnb1	-0.20	3E-18
Rac1	-0.21	2E-09
Atf2	-0.23	9E-08
Rapgef2	-0.23	0.001
Map2k4	-0.24	0.0002
Gnas	-0.26	0.00
Mef2c	-0.29	3E-05
Gnaq	-0.31	0.0006
Mapk8	-0.34	3E-05
Prkca	-0.47	7E-10
Nr3c1	-0.49	1E-06
Prkcb1	-0.54	0.00
Plcb1	-0.65	9E-32



NM_010548.1	IL-10	IL10	9.8607	1.98E-07	1.43E-06
NM_021443.2	Ccl8	Ccl8	7.9403	1.33E-07	1.43E-06
NM_009696.2	ApoE4	APOE	0.775	1.73E-07	1.43E-06
NM_144559.1	FcγRIV	FCGR3A	6.3431	6.43E-07	3.71E-06
NM_009779.2	C3ar1	C3AR1	1.5805	9.37E-07	4.50E-06
NM_009777.2	C1qb	C1QB	1.6722	1.17E-06	4.81E-06
NM_013653.3	CCL5	CCL5	5.3601	1.94E-06	6.99E-06
NM_010382.2	H2-Eb1	HLA-DRB1	5.6153	3.53E-06	1.13E-05
NM_007572.2	C1qa	C1QA	1.5037	4.76E-06	1.37E-05

D



Top Canonical Pathways

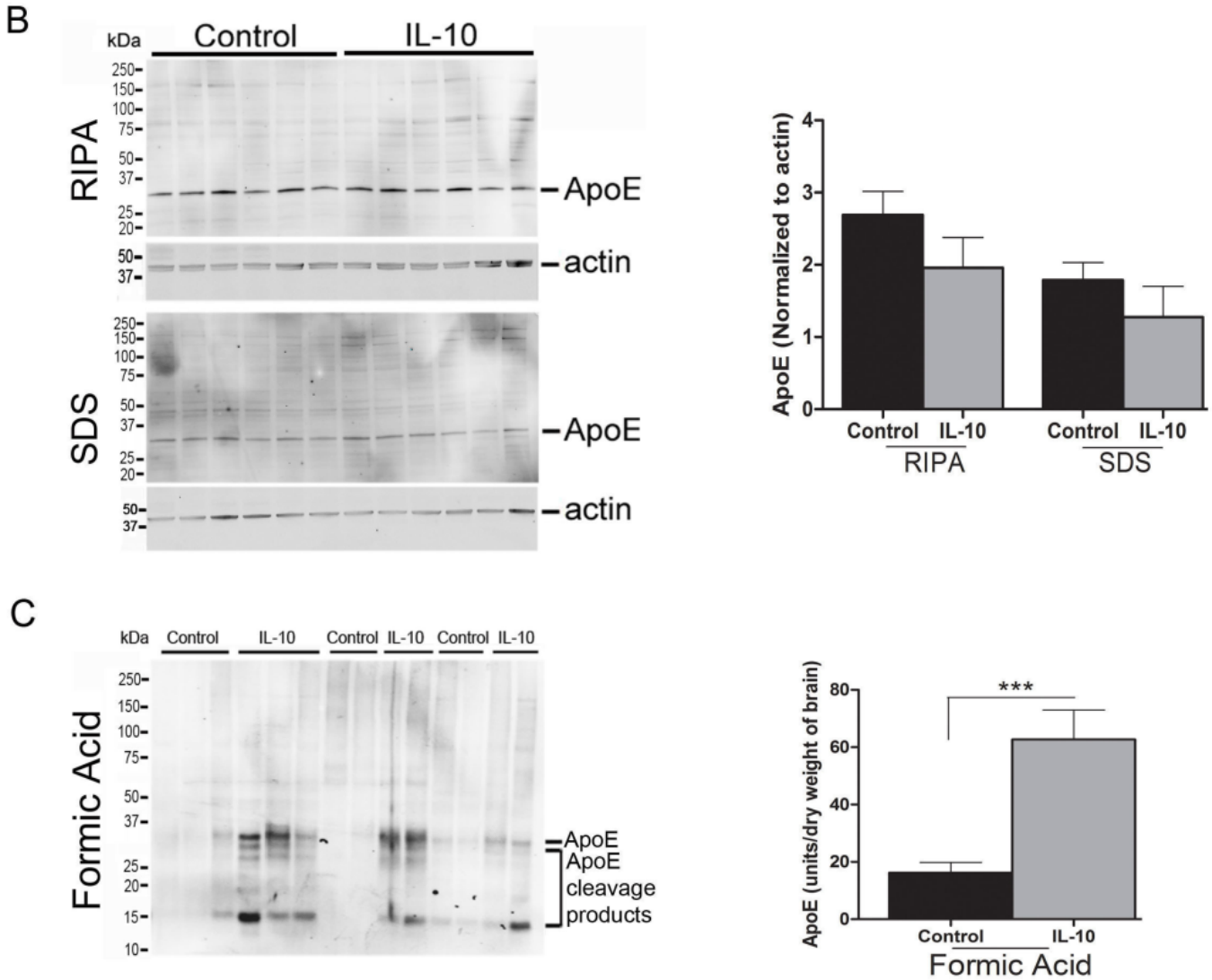
Name	p-value	Ratio
Role of Pattern Recognition Receptors in Recognition of Bacteria and Viruses	9.23E-20	17/109 (0.156)
Acute Phase Response Signaling	9.07E-17	18/181 (0.099)
Complement System	1.55E-16	11/35 (0.314)
IL-10 Signaling	7.99E-16	13/78 (0.167)
Dendritic Cell Maturation	4.32E-15	17/211 (0.081)

E

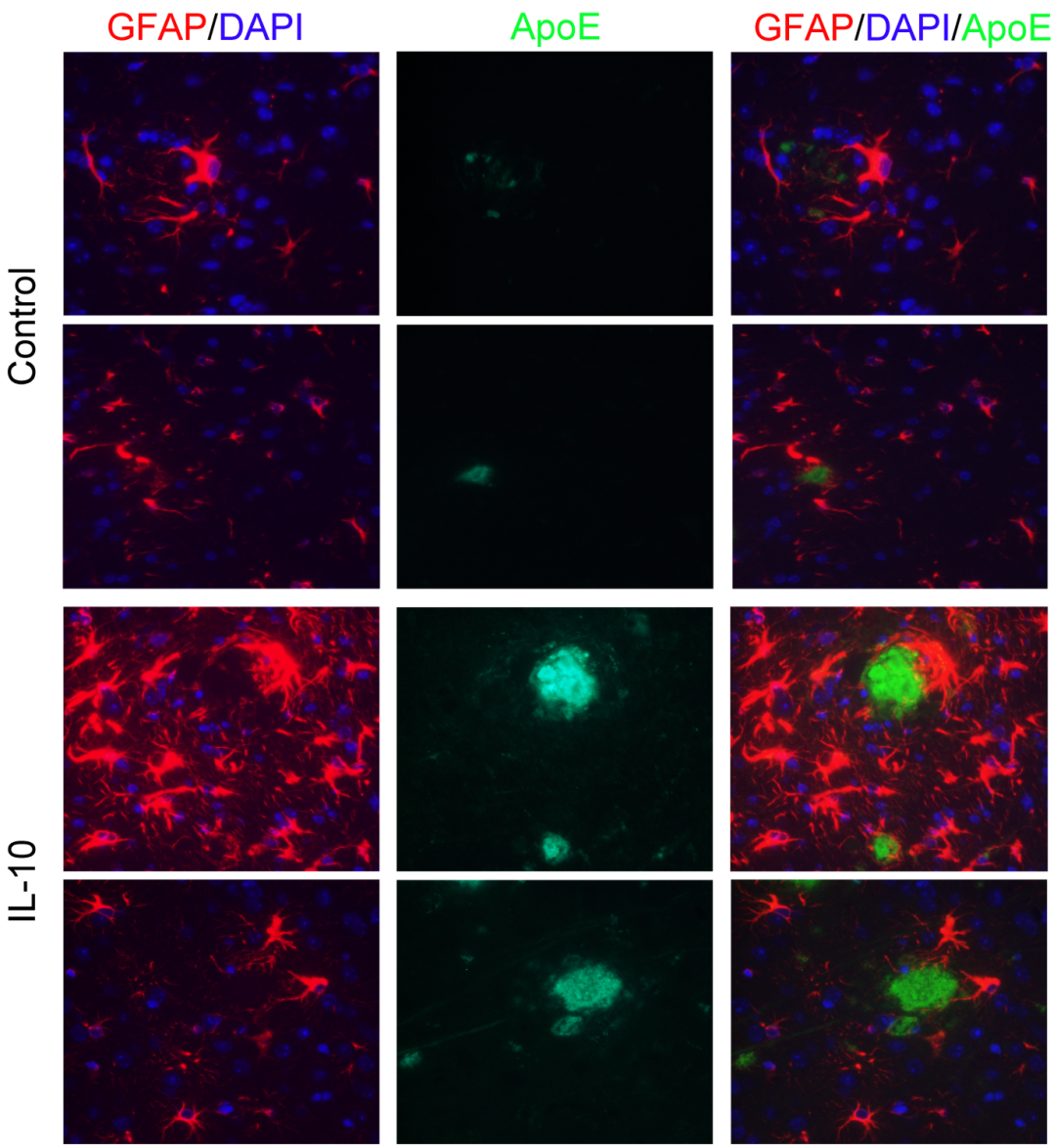
Network name	# of genes	P-value	Q-value
Crosstalk between Dendritic Cells and Natural Killer Cells	14	0.0021645	0.0020297
Fcγ Receptor-mediated Phagocytosis in Macrophages and Monocytes	7	0.0021645	0.0020297
NF-κB Signaling	22	0.0021645	0.0020297
Role of PKR in Interferon Induction and Antiviral Response	7	0.0021645	0.0020297
Protein Ubiquitination Pathway	5	0.0021645	0.0020297
Role of Macrophages, Fibroblasts and Endothelial Cells in Rheumatoid Arthritis	39	0.0021645	0.0020297
Regulation of the Epithelial-Mesenchymal Transition Pathway	12	0.0021645	0.0020297
Role of Osteoblasts, Osteoclasts and Chondrocytes in Rheumatoid Arthritis	25	0.0021645	0.0020297

Figure 7

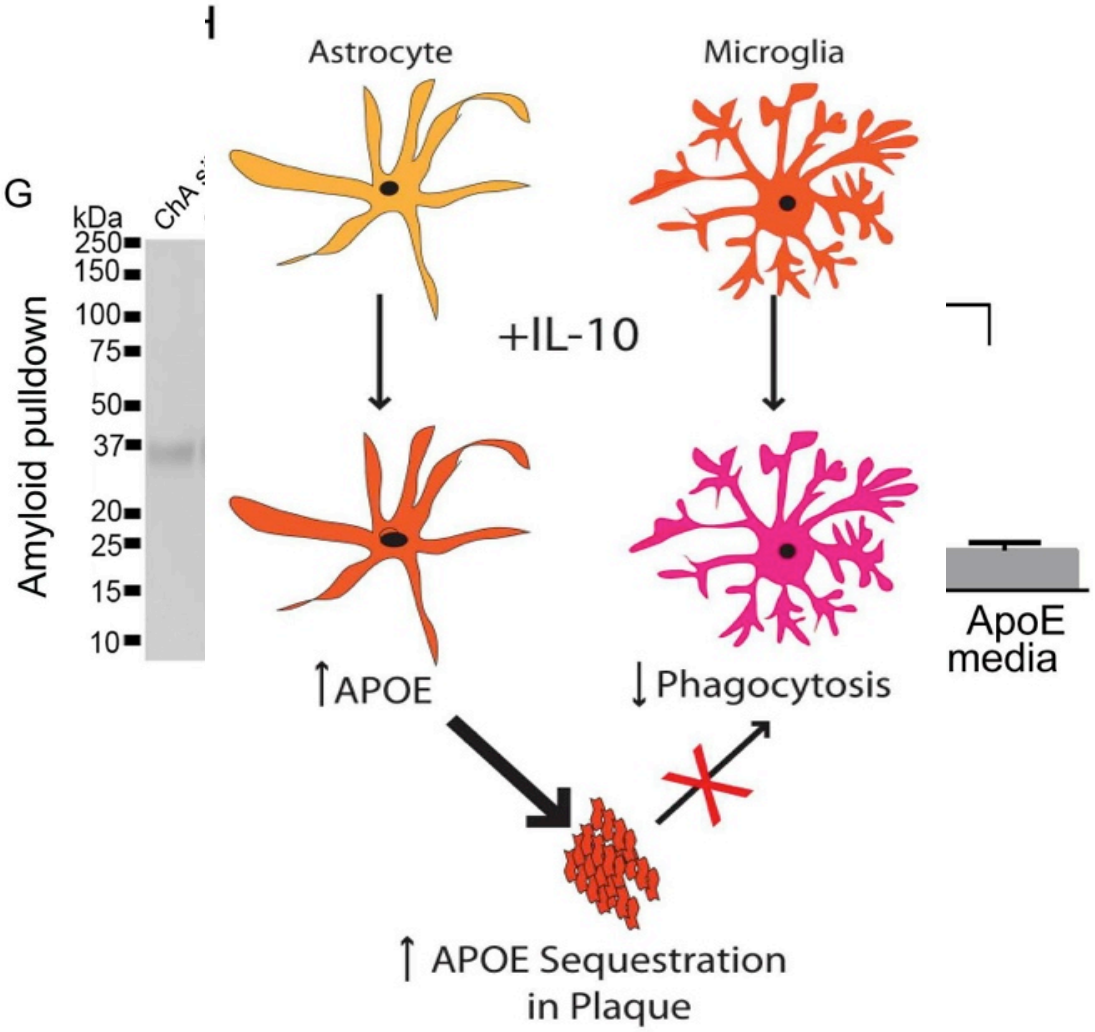
IL-10 increases the levels of plaque associated APOE



IL-10 increases the levels of plaque associated APOE



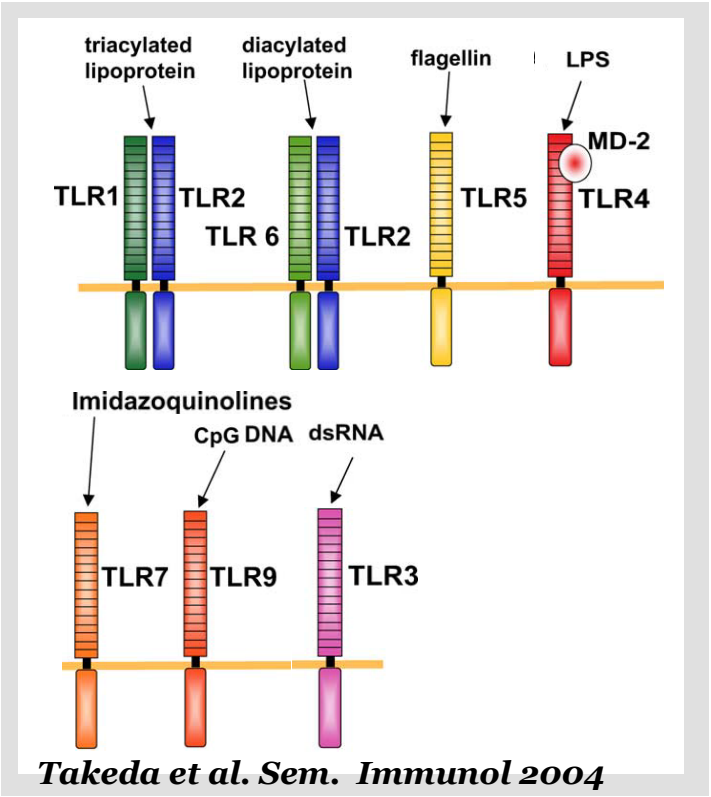
APOE binds aggregated Aβ and impairs microglial phagocytosis

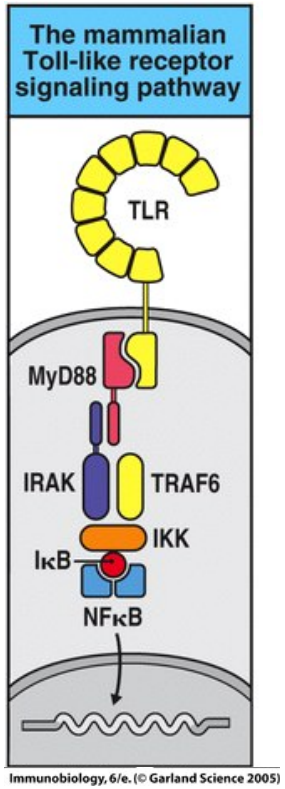


Il-10 study implications

- Without utilizing the less biased transcriptomic approach, we would not have understood IL-10's pro-amyloidogenic effect.
- Innate Immunity may interact with genotype in humans to have divergent effects
  - -IL-10 in APOE4 –harmful
  - -IL-10 in APOE2 –beneficial
  - -Il-10 in APOE3 –hard topredict

Harnessing Toll Like Receptors as AD therapeutics?





TLRs are primary sensors of pathogen and danger associated molecular patterns (PAMPs and DAMPs)

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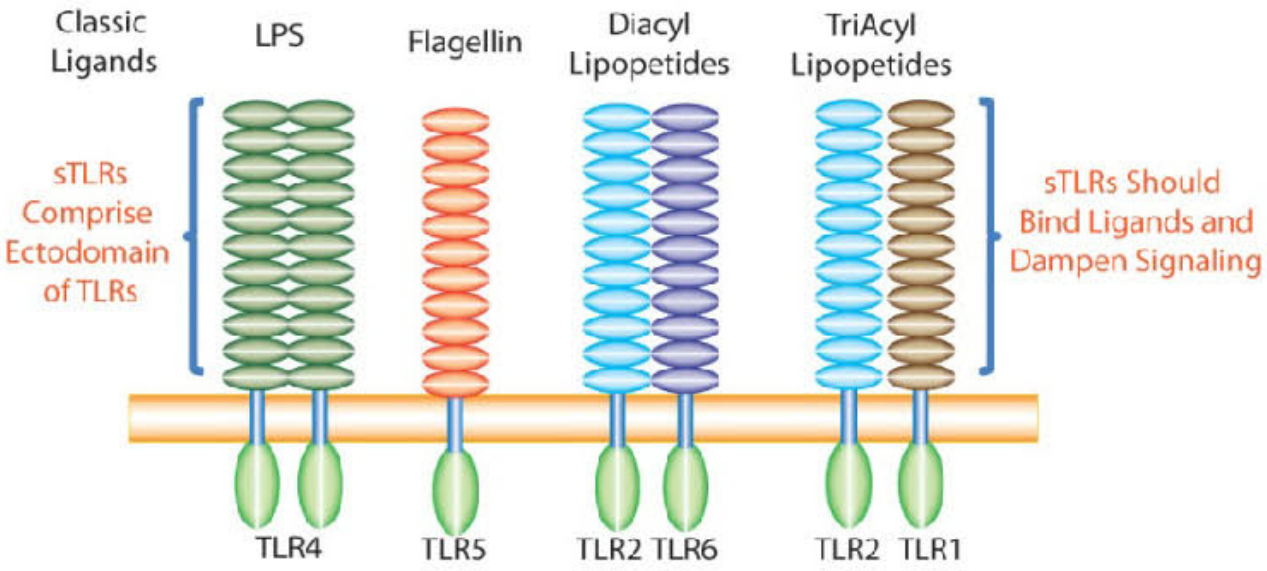
Aβ and other amyloids are DAMPs and Bind TLRs 2,4,6

- ***Aβ binds and activates select TLRs with CD36****. Stewart et al., Nat Immunolgy 2010; Fassbender et al., FASEB J 2004*

TLRs modulate Aβ pathology

- ***TLR/TLR agonists reduce Aβ plaque pathology.*** *Richard et al., J. Neurosci 2008; Chen et al., J Biol Chem 2006; Reed-Geaghan et al., J. Neurosci 2009; Scholtzova et al., J Neurosci 2009.*
- ***TLRs are upregulated in mouse models.*** *Wirths et al., Neurobiol Aging 2010*
- ***TLR4 deficiency increases Aβ plaque pathology but Myd88 deficiency reduces plaque pathology.*** *Tahara et al. Brain 2006; Lim et al., Am J. Path 2011; Hao et al., Brain 2011.*
- ***Myd88 knockdown inhibits Aβ42 induced inflammatory signaling.*** *Jana et al., J. Immunol 2008.*
- ***TLR4 and TLR2 exacerbates Aβ induced neuronal injury.*** *Walter et al., Cel Physiol Biochem 2007;Tang et al., Exp Neurol 2008; Liu et al., J Immunol 2012.*

Harnessing soluble Toll Like Receptors (sTLRs) as AD therapeutics



- Select sTLRs might bind Aβ aggregates but also dampen inflammation.
- What will they do to pathology?
- In addition to sTLR 2,4,6 we tested sTLR5 as it's only known ligand is flagellin
- All of these TLRs are expressed at low levels the mouse and human brain

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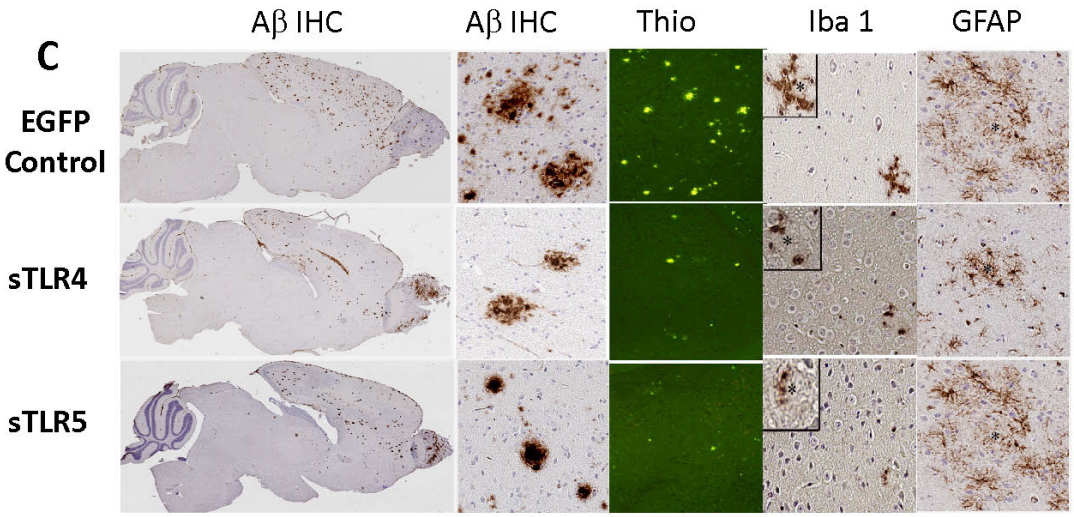
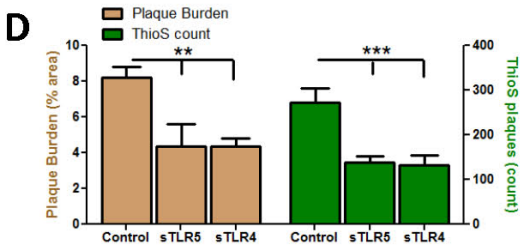
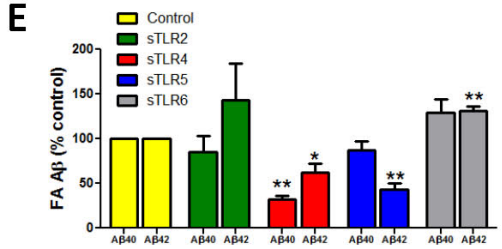
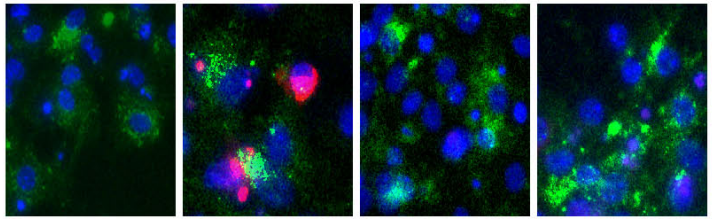
Initial Pilot studies showed that sTLRs 4 and 5 attenuate Aβ deposition and block Aβ toxicity





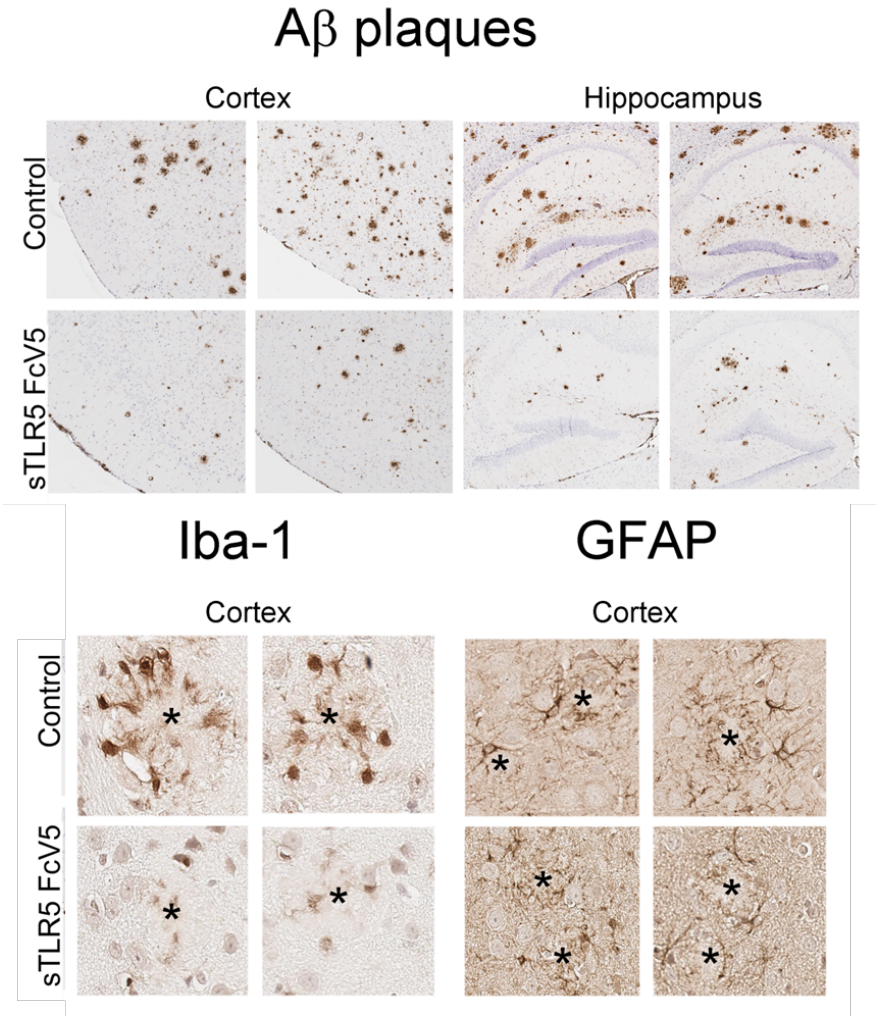




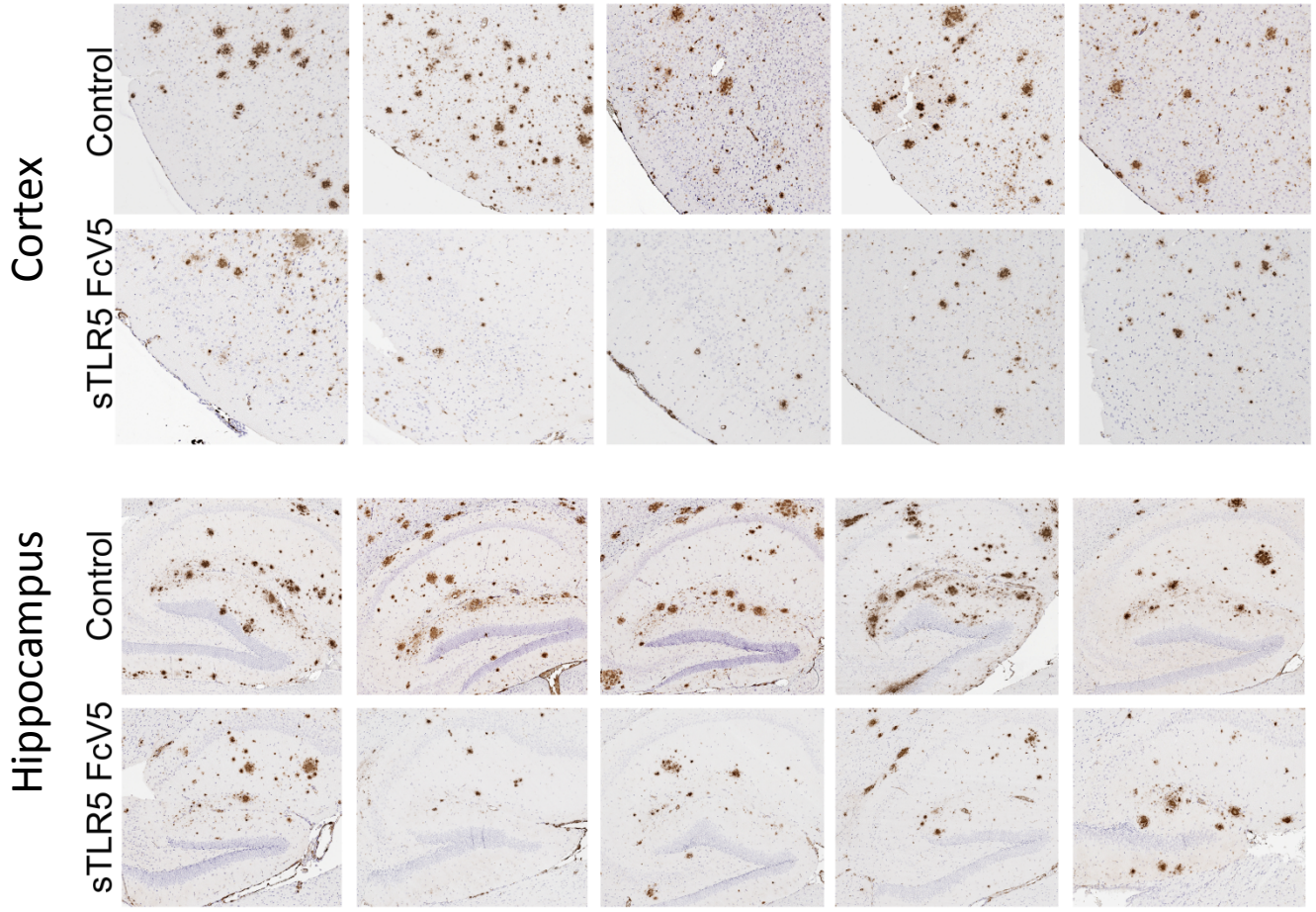
DAPI
PI
Calcein

Control Aβ Aβ+sTLR4 Aβ+sTLR5

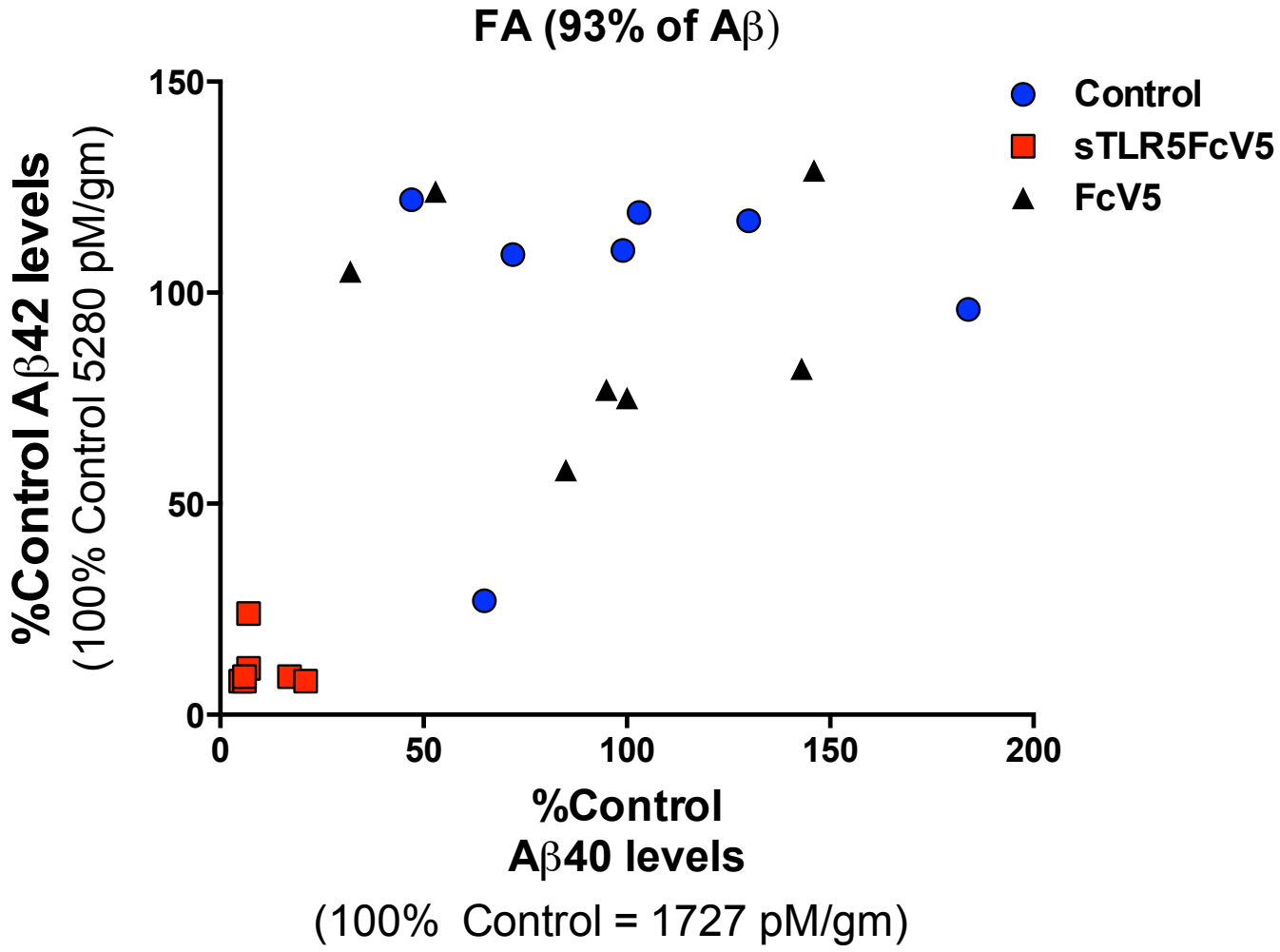
rAAV-sTLR-FcV5 tranduciton dramatically reduces Aβ plaque pathology and associated micogliosis



sTLR5FcV5 expression leads to reduction in Aβ plaque load compared to control mice

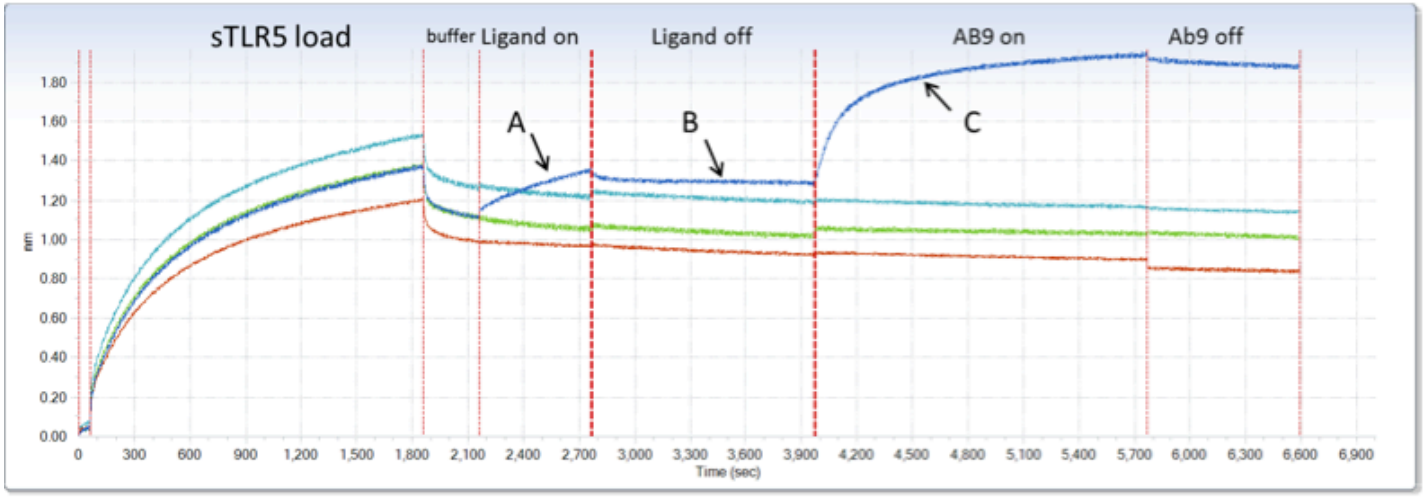


sTLR-FcV5 fusion expression dramatically alters Aβ plaque pathology



How is sTLR5 working?

Biolayer Interferometry (BLI) using anti-hu FC biosensors loaded with sTLR5 FC V5.
Associated with oligomeric Aβ42 and confirmed with antibody AB9.



Dark blue: 10uM Aβ42oligomer

Red: 10ug/mL flagellin (*B. subtilis*)

Light Blue: 10ug/mL flagellin (*S. typhimurium*)

Green: Control (oligomer vehicle)

Direct Binding of Aβ

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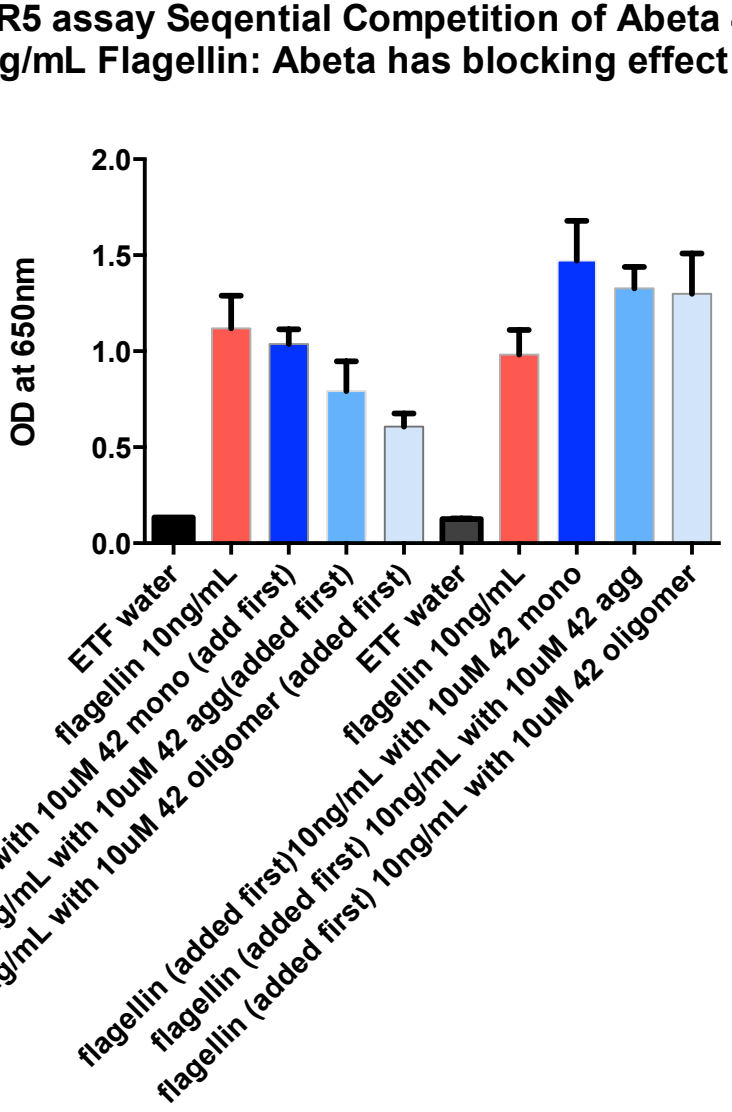
Aβ modulates TLR5 activation by Flagellin, but does not activate TLR5 by itself



sTLR5Fc as novel AD immunotherapies?

- It appears to work but how?
  - **Acting like antibodies** ?
  - Blocking aggregation?
  - Blocking Inflammation?
  - Neutralizing toxicity?
- Can it work when administered peripherally?
- Can it work as therapeutics as opposed to prophylactics?
  - Some preliminary data says yes
- Can it work in other neurodegenerative proteinopathies?
  - Tau studies are underway
- What about other sTLRs?
  - sTLR4 did not reproduce
  - sTLR4Fc also showed no clear effect

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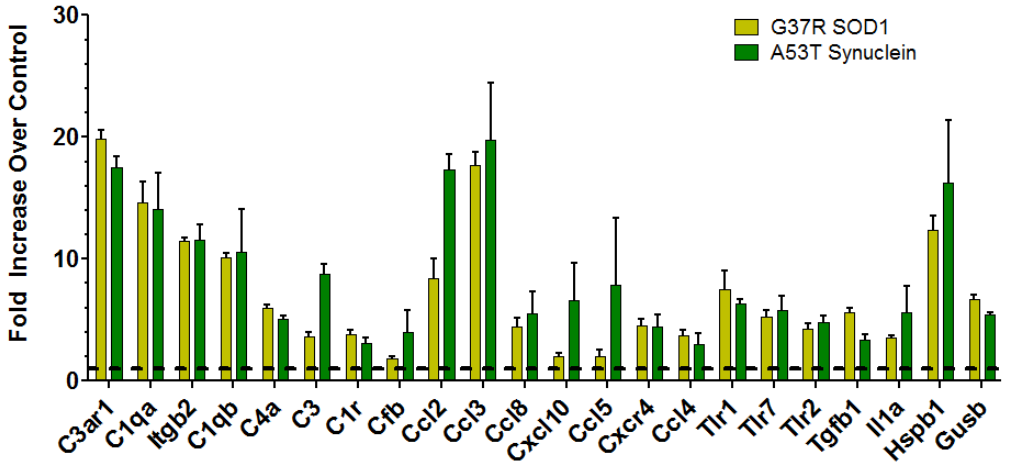
Targeting Innate Immunity in fALS mutant SOD1 models

- There is a massive alteration in innate immune gene expression accompanying a massive gliosis in SOD1 fALS models (Buovsky et al JCI 2012) that may be similar to both human fALS and sALS
- Multiple innate immune pathways are upregulated dramatically (complement, chemokines, acute phase proteins, cytokines, toll-like receptors)
- Mutant SOD1 mice are an excellent phenocopy of human ALS mediated by SOD1



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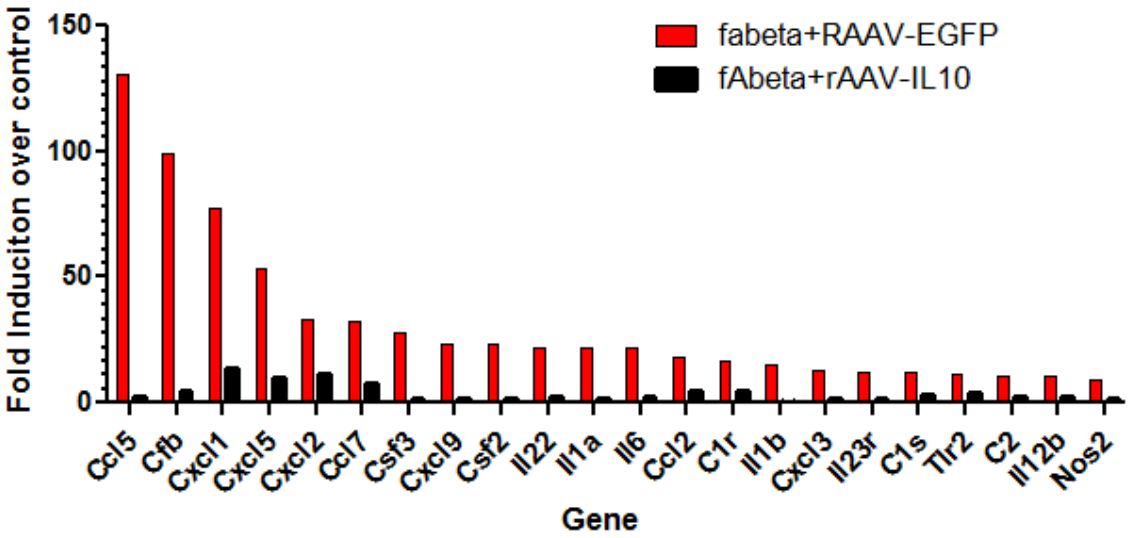
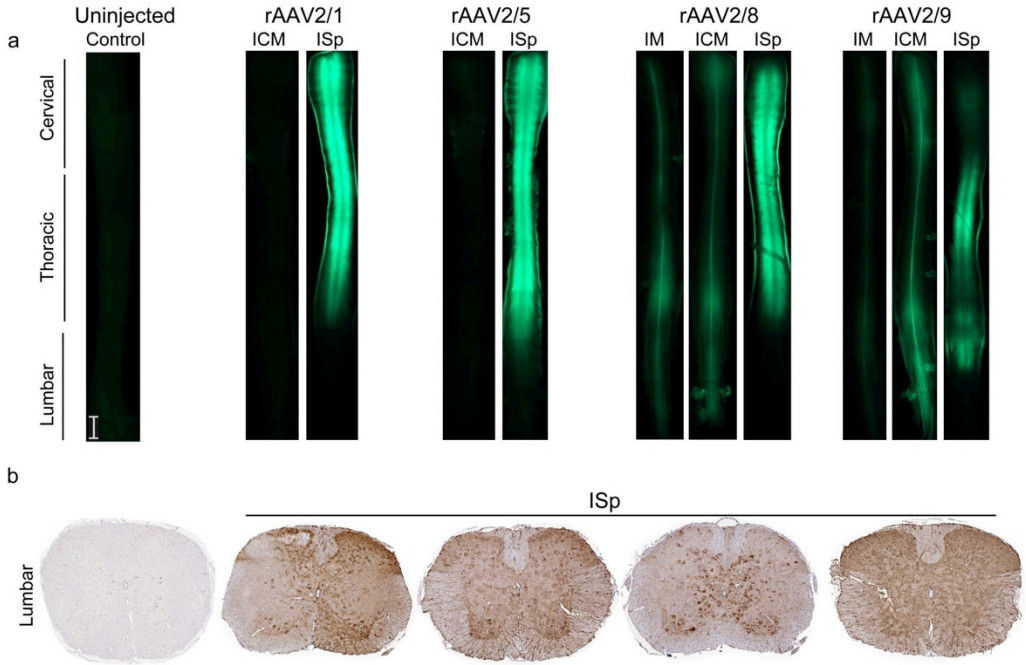
So many targets, but which one?

- We chose IL-10 as a "master" anti-inflammatory cytokine that acts in a non-cell autonomous fashion
  - Also because recombinant Il-10 was well tolerated but lacked efficacy in human HCV/hepatitis trials and we had shown in vivo effects of rAAV-IL-10 in APP mouse models
  - In primary mixed neuroglial culture rAAV2/1-IL-10 can dramatically suppress innate immune gene activation induced by Aβ, synuclein or LPS



Study design

- Intraspinal rAAV2/1-murine IL-10 injected into SOD1 G93A model at P0
- Survival Study (mice aged till they are moribund due to paralysis)
- Initial Goal >25% increase in lifespan



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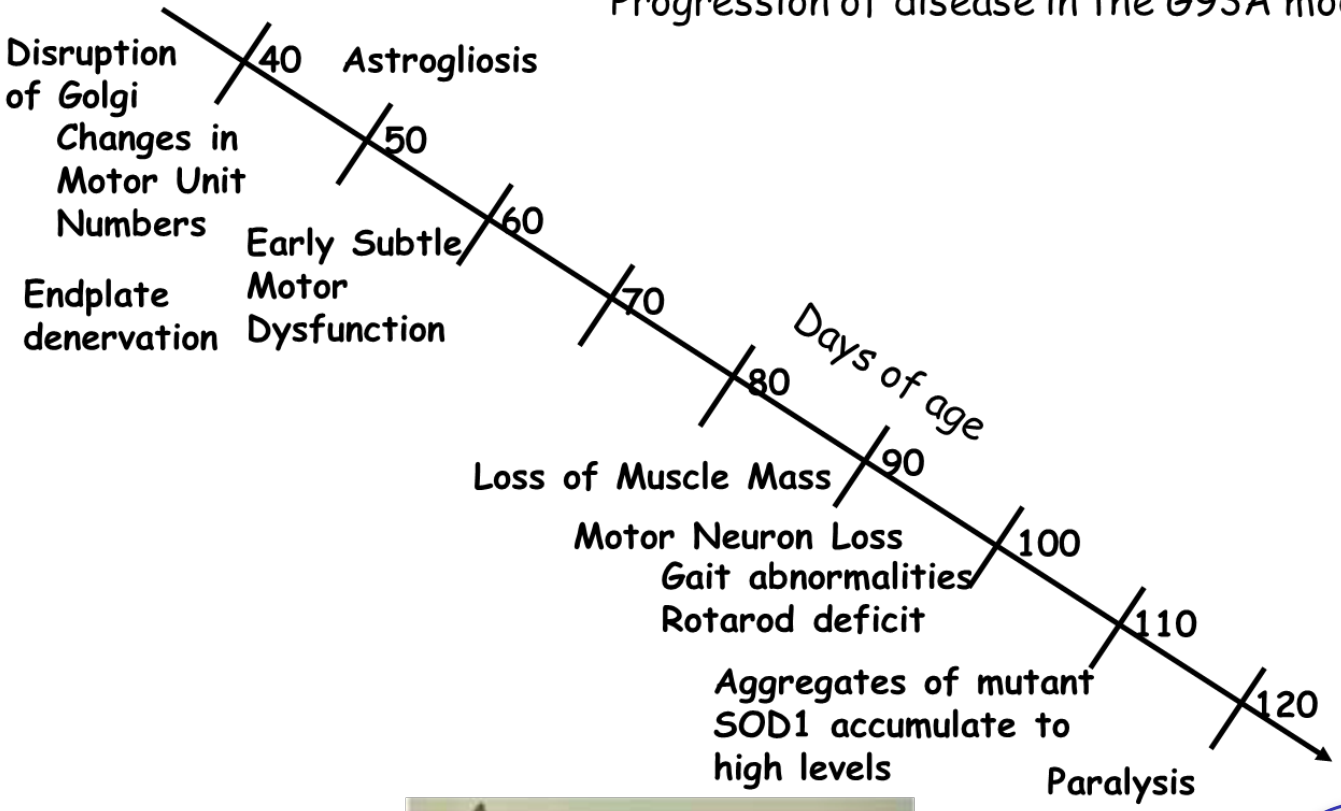
G93A SOD1 mice develop a motor phenotype

Motor neuron degeneration in mice that express a human Cu,Zn superoxide dismutase mutation. *Science*. 1994. Gurney ME, Pu H, Chiu AY, Dal Canto MC, Polchow CY, Alexander DD, Caliendo J, Hentati A, Kwon YW, Deng HX, et al.

Progression of disease in the G93A model



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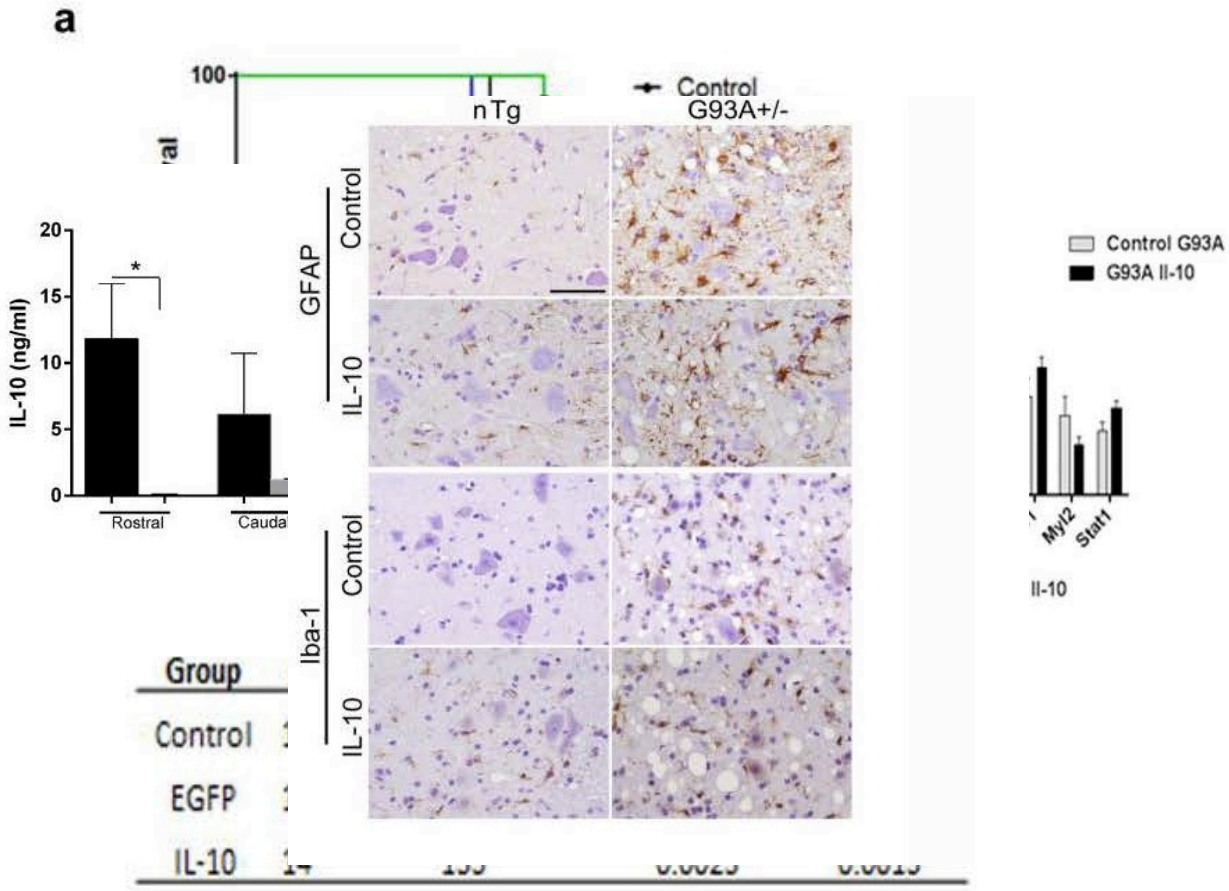
rAAV2-IL-10 Prolongs survival (Ayers et al 2014 Molecular Therapy)



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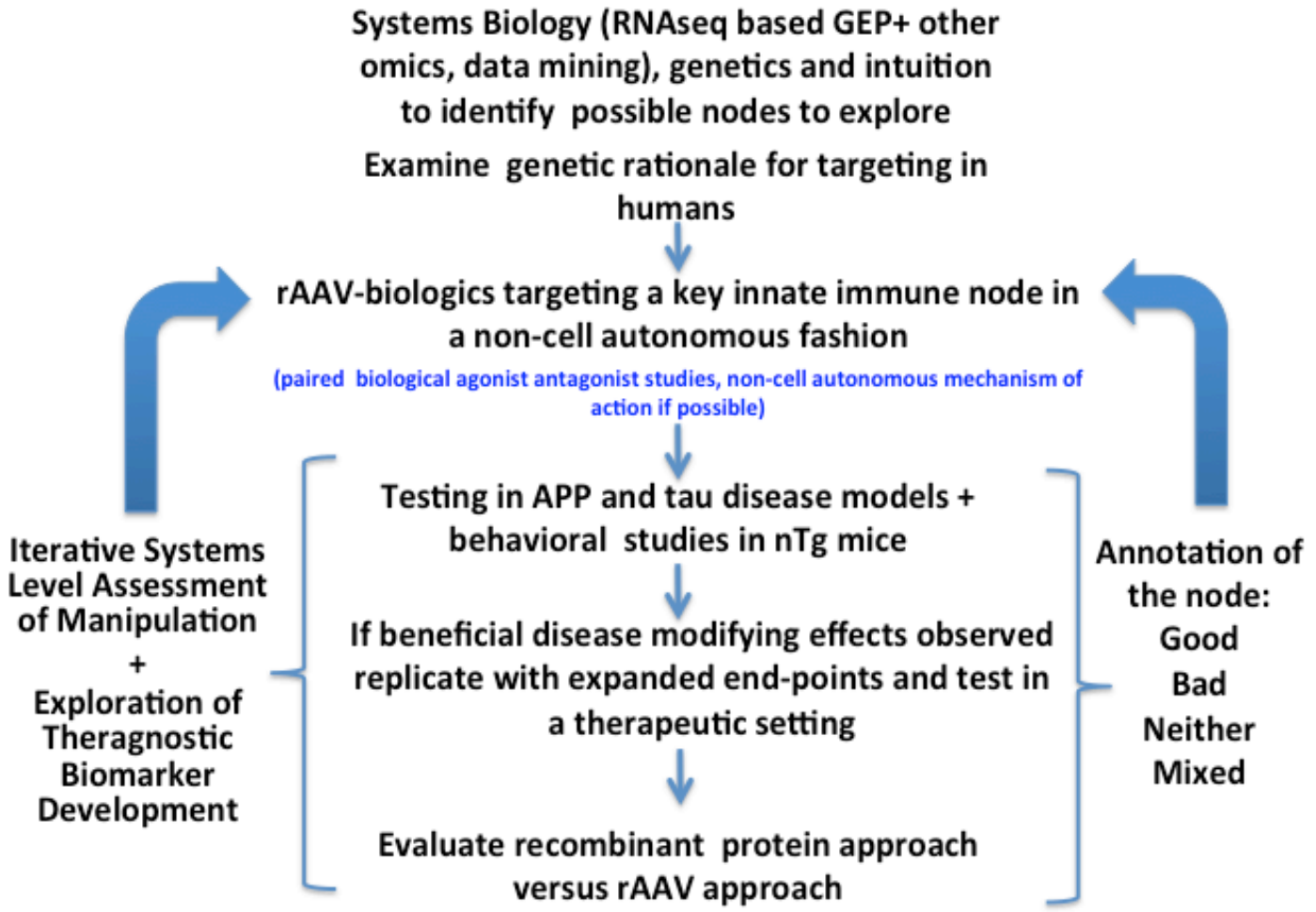
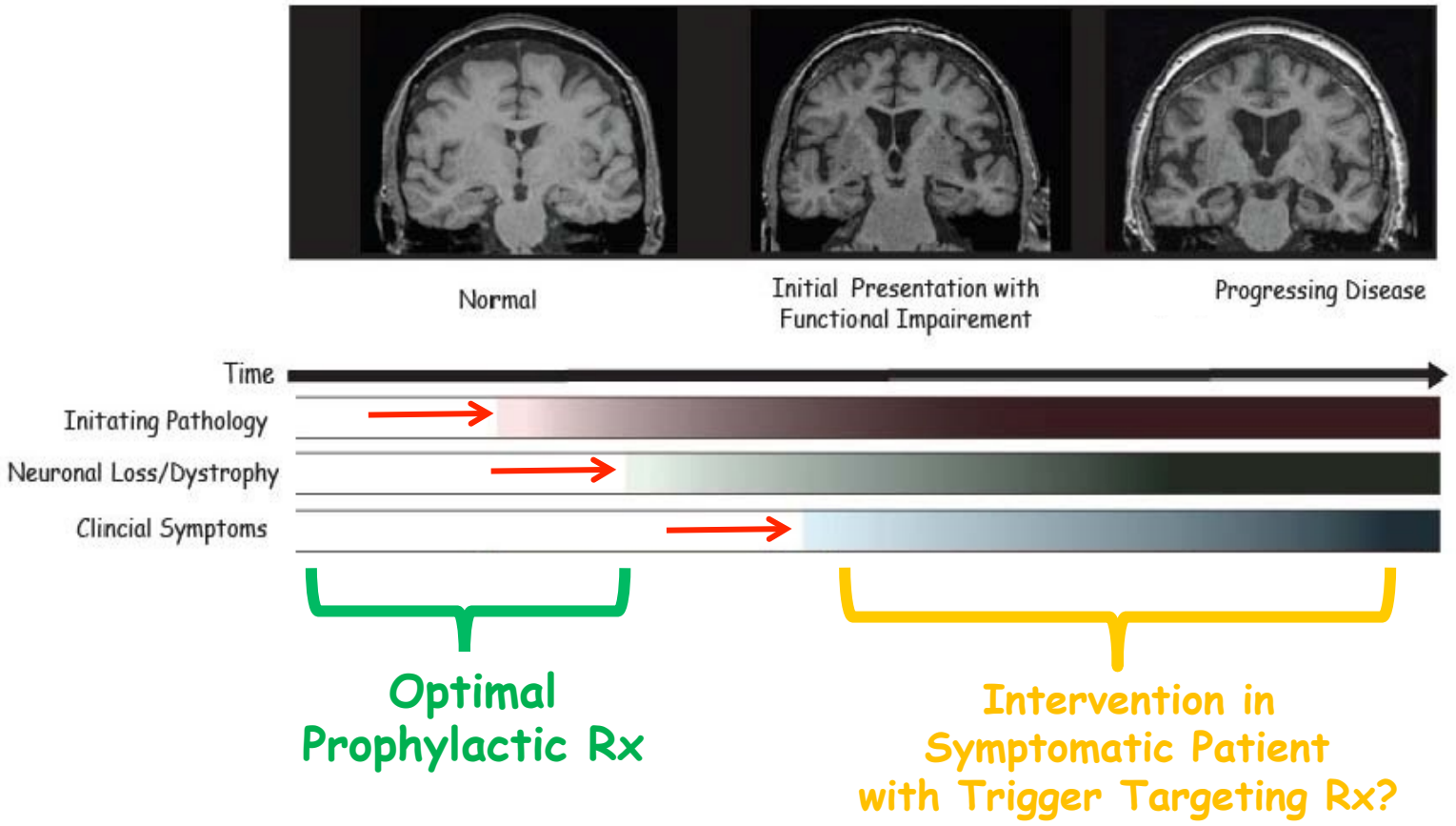
Targeting Innate Immunity in fALS

- Il-10 is not yet a breakthrough as we did not even reach the 25% life span extension target: Can we optimize
  - Dose finding studies
  - Peripheral delivery (recombinant or viral)
  - Therapeutic as opposed to Prophylactic delivery
- Nevertheless, first POC that innate immunity could be harnessed to provide disease modification in fALS
- Illustrates complexity of manipulating innate immunity
  - Effects may strongly influenced by context
- Establishment of a cost-effective technology platform to evaluate multiple individual targets or even combination therapies
- rAAV based therapies can be directly on the clinical development path
  - Delivery and scale up issues still need to be completely solved

A Systems Approach to Harnessing Innate immunity for Neurodegenerative Proteinopthies



The Dilemma of Treatment versus Prevention
(see Golde, Schneider and Koo, Neuron 2011)



Efficacy of trigger targeting therapy will decline as pathology progresses! We should avoid Kobayashi Maru Scenarios

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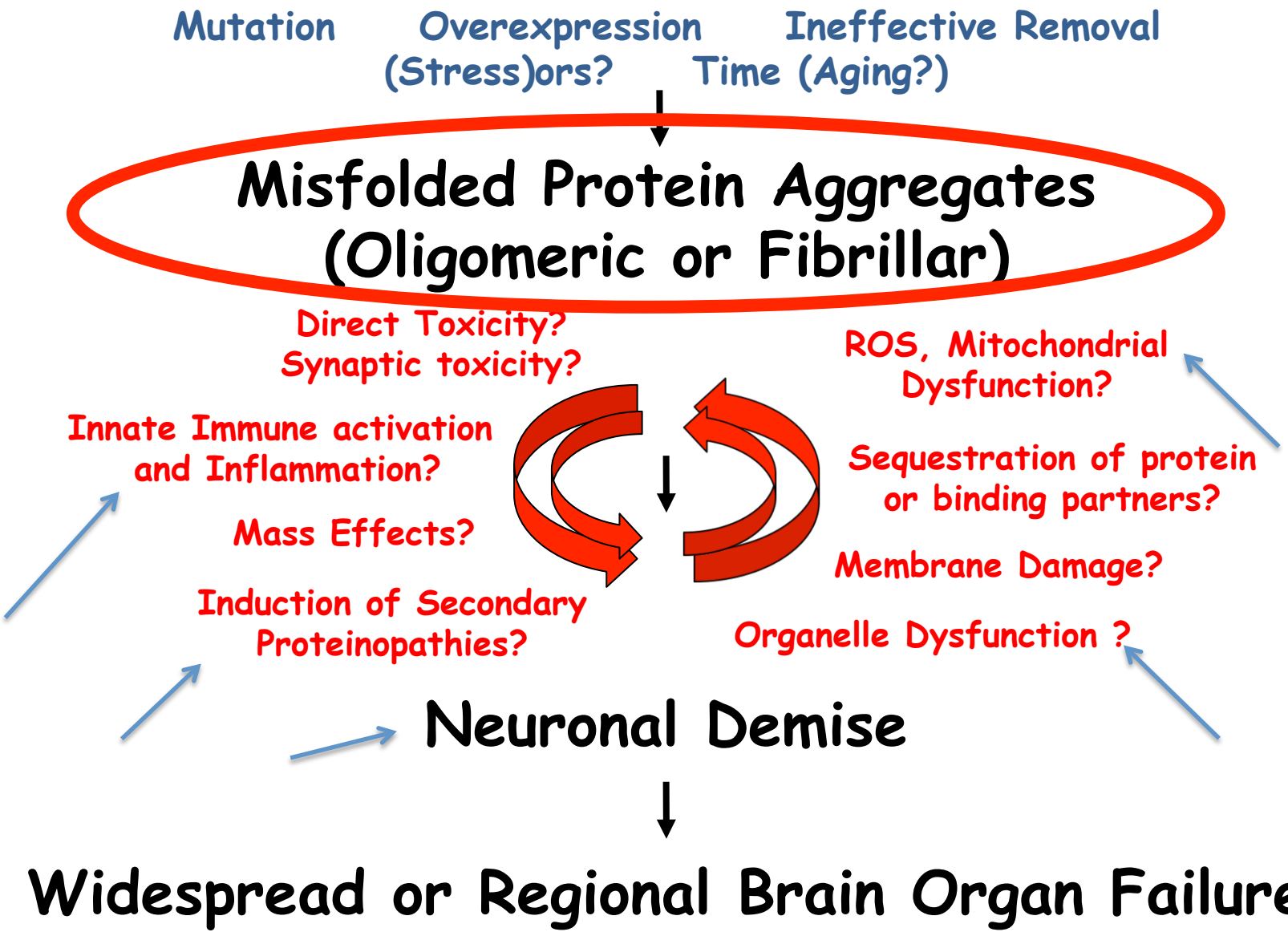
The Proteinopathy Hypothesis of Neurodegeneration



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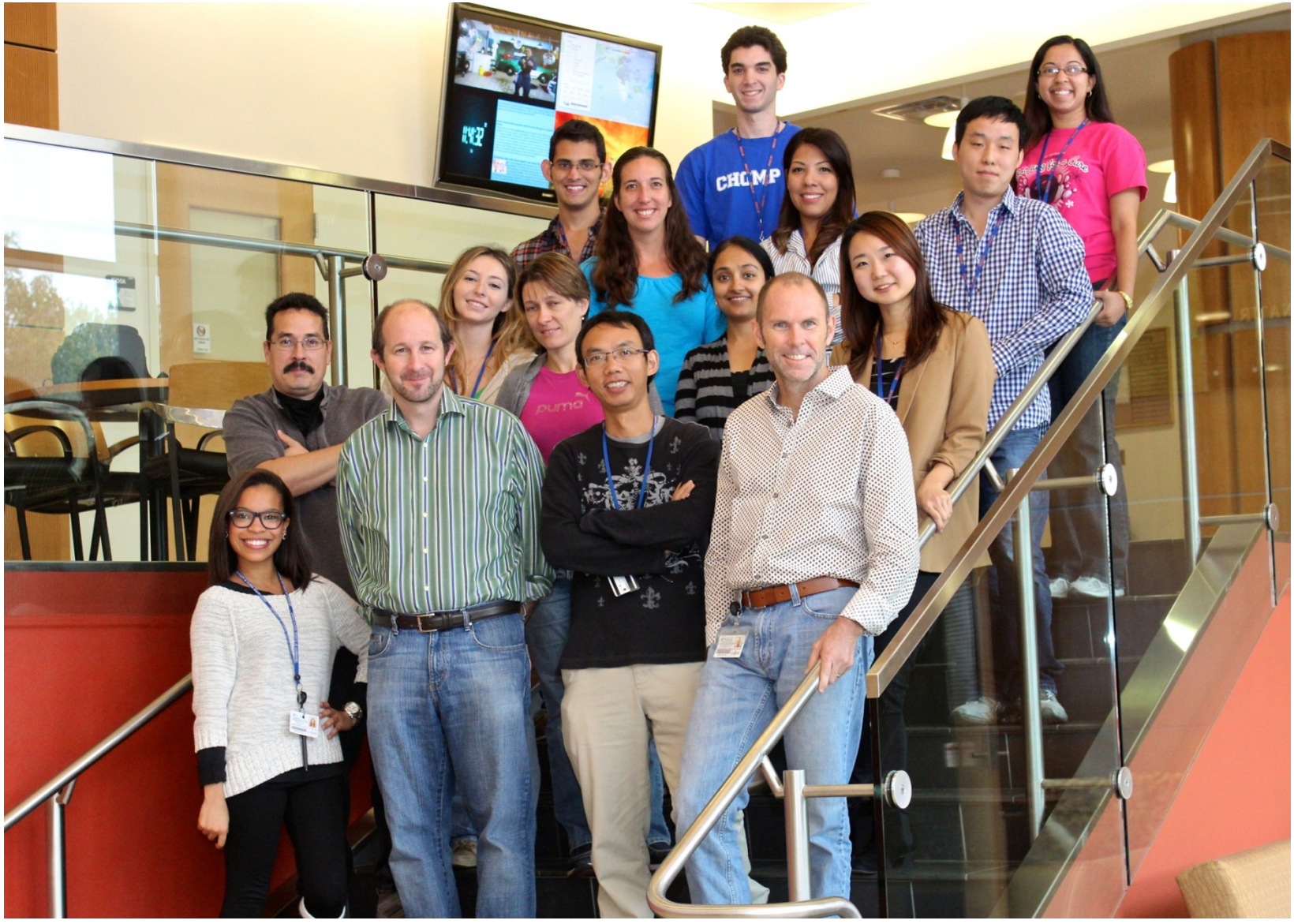
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