# Immunoproteastasis: Can we harness it treat AD?

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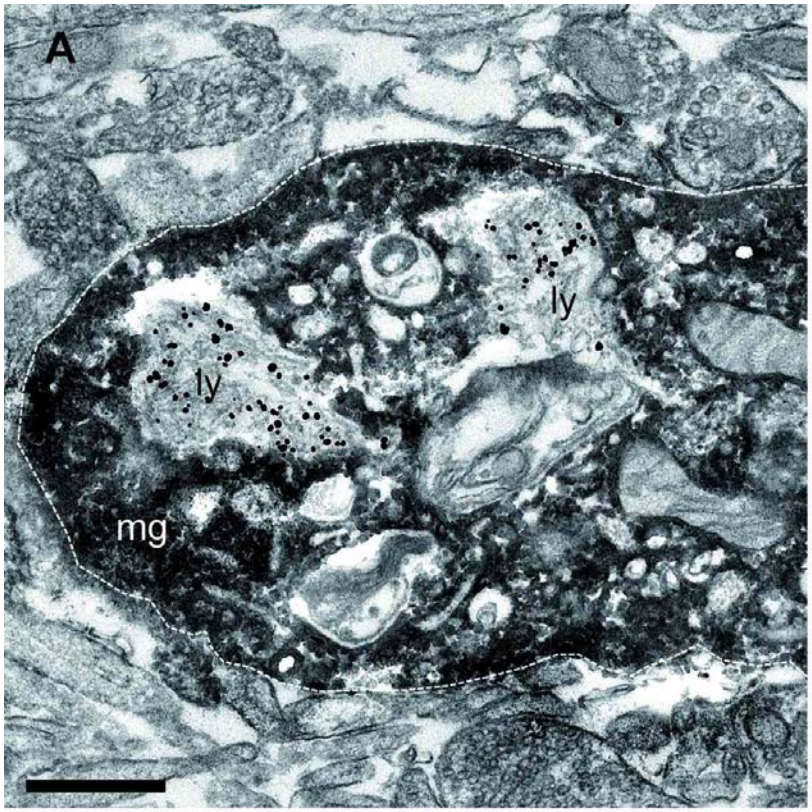
Disclosures:

Current: Inventor on Patents/Patent Applications relating to GSMs, STLRs, Ab immunotherapy, and BRI peptides.
Honorarium Alzheimer's Res and Therapy Co-Editor in Chief

# A little bit of history




Dogma in the field for many years was a pro-inflammatory state would drive the AD cascade (inferred from systemic amyloidosis)

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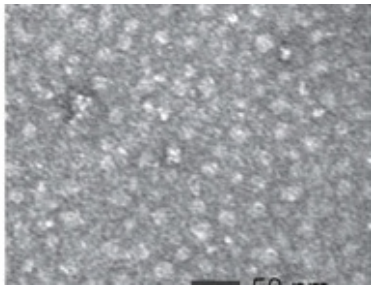
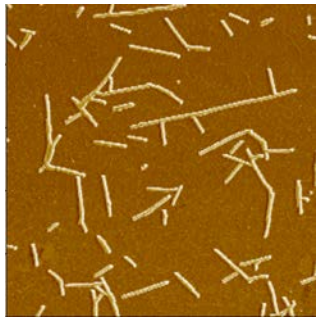
# A Quiz: Amyloid, Oligomer or Virus?

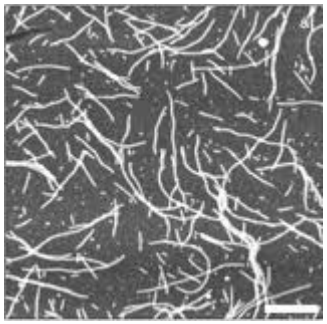
Lysozyme Amyloid and Protofibrils AFM



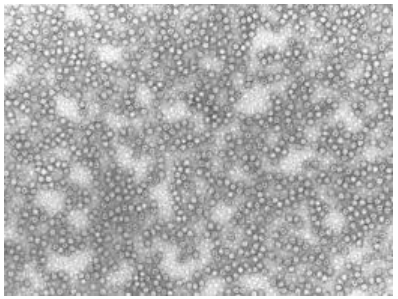
Aβ Oligomers EM



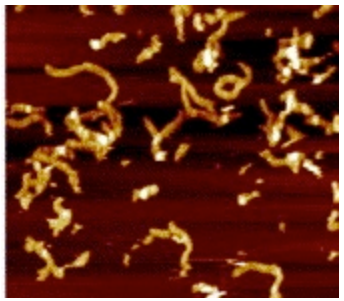
Tobacco Etch Virus AFM



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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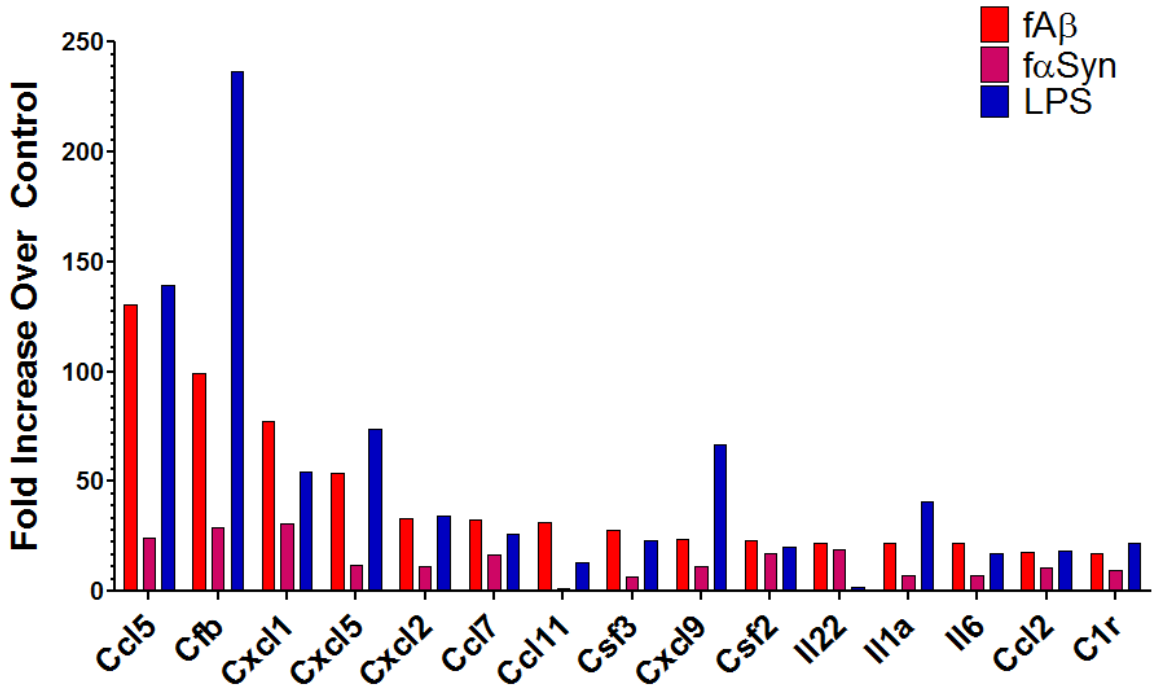
# Aβ and other amyloids (?) are DAMPs




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

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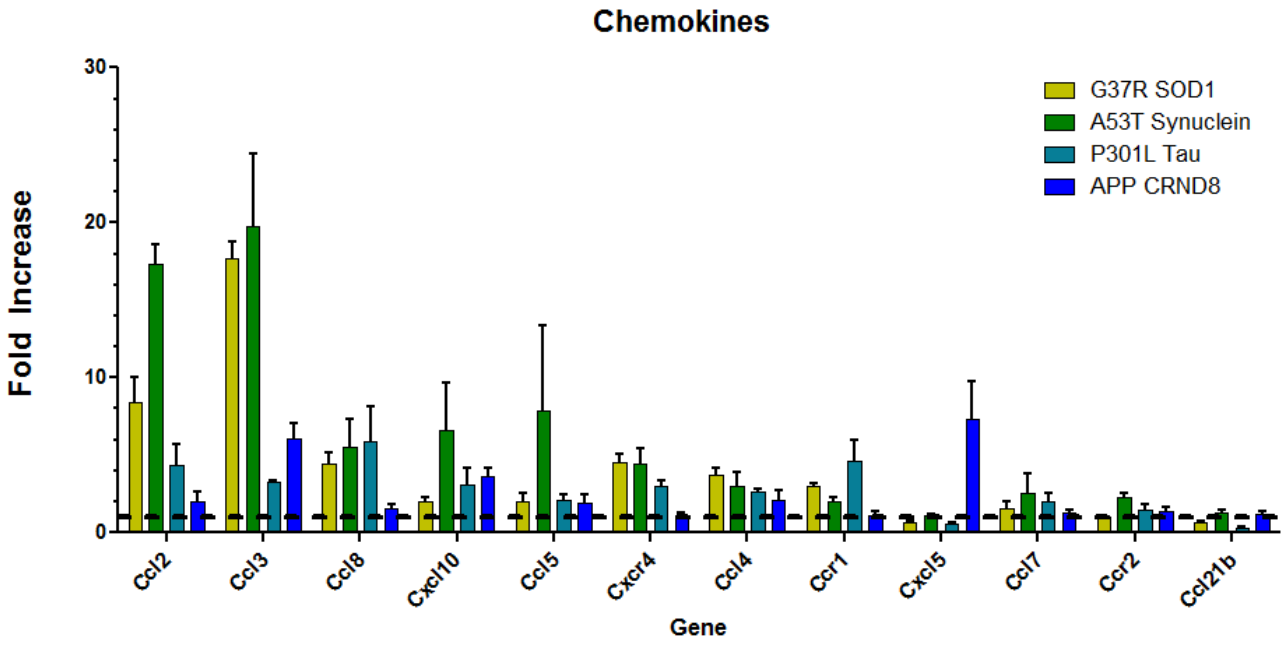
# Widespread Innate Immune Gene Upregulation in Neurodegenerative models of AD, PD, ALS, and FTD

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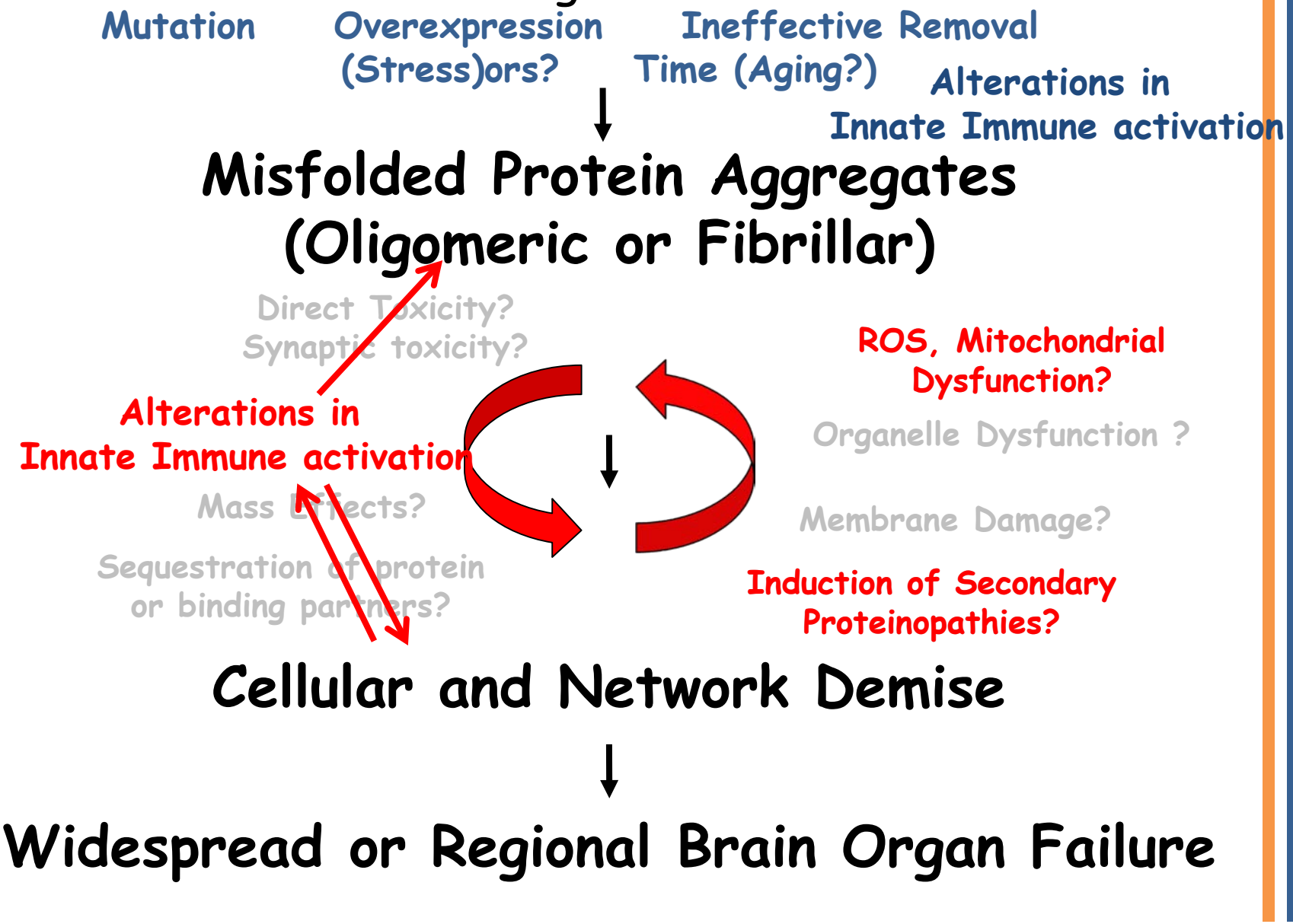
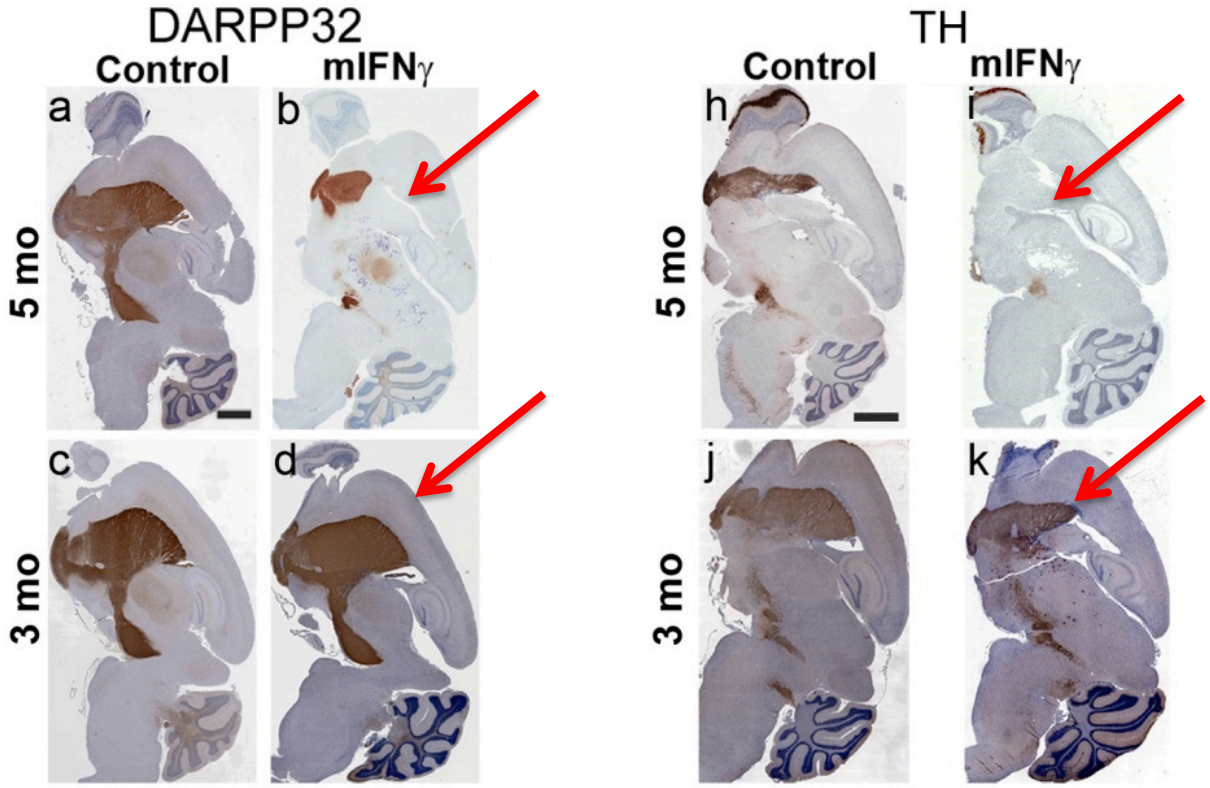
# Immunoproteostasis (Chakrabarty et al Neuron 2015)

- Aggregated proteins forming inclusions found in neurodegenerative proteinopathies 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 examples: HIV dementia, sepsis?*
- **Innate immune** signaling can also **regulate 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*****.**
- Emerging genetic data implicates genes in the immune systems in AD (e.g., TREM2) and PD.
- Outcomes of altered immunoproteostasis may be critically dependent on context: timing, duration and strength of the signals.

# Global Rationale for Targeting Immunoproteostasis in Neurodegeneration

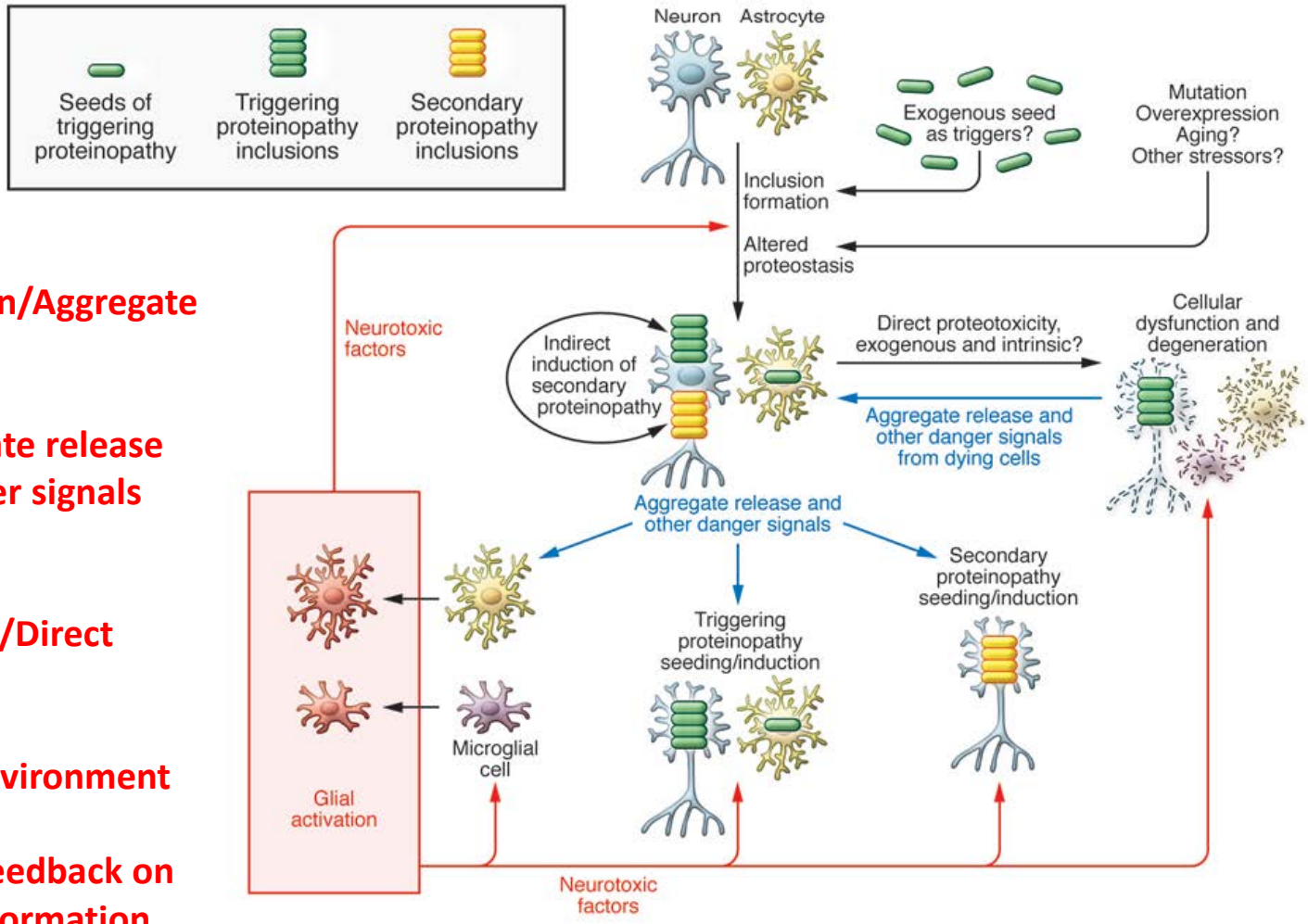


# Selective Dopaminergic Cell Death by rAAV-IFN-γ




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

# Immunoproteostasis may contribute to pathology spread
## Golde et al JCI 2013




**1. Inclusion/Aggregate Formation**

**2. Aggregate release plus danger signals**

**3. Glia Activation/Direct Toxicity**

**4. Toxic Environment**

**Positive Feedback on inclusion formation**

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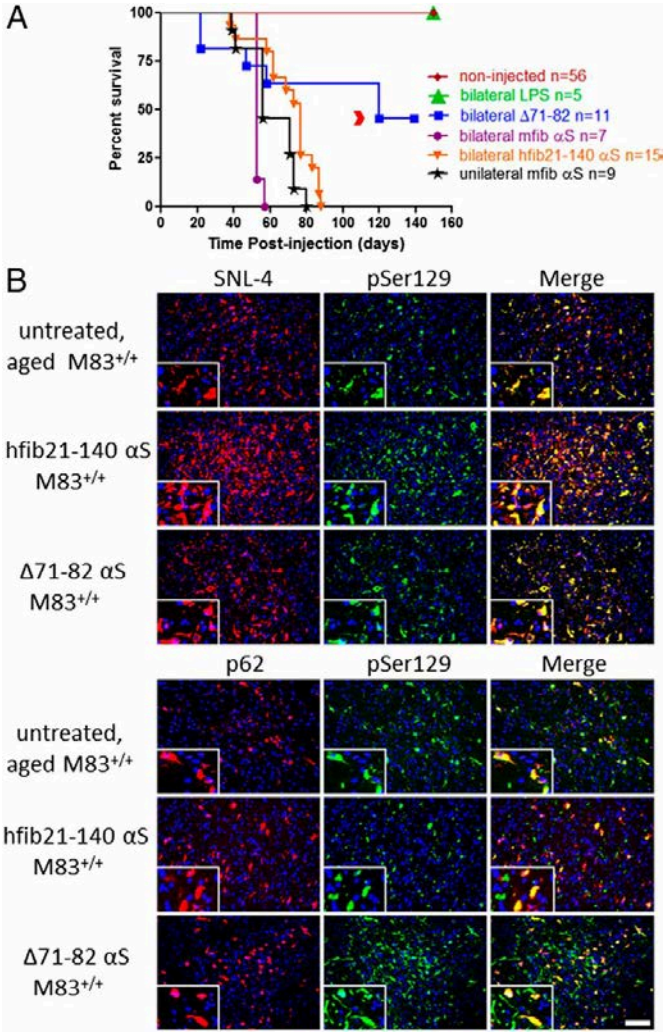
# αS IM injection reduces survival in M83+/+ Tg mice.




Sacino A N et al. PNAS 2014;111:10732-10737

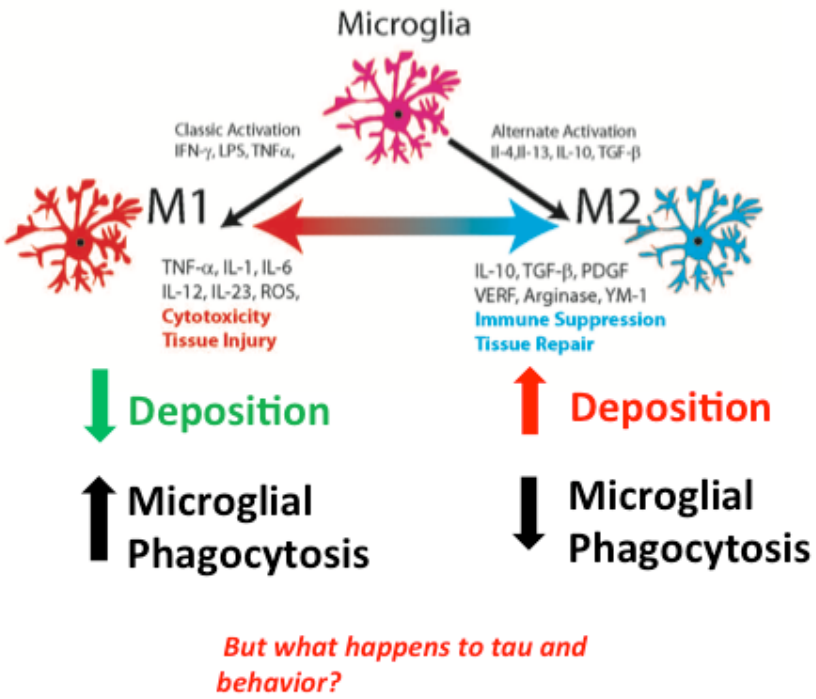
# Can we harness immunoproteostasis to treat AD and other neurodegenerative Disorders

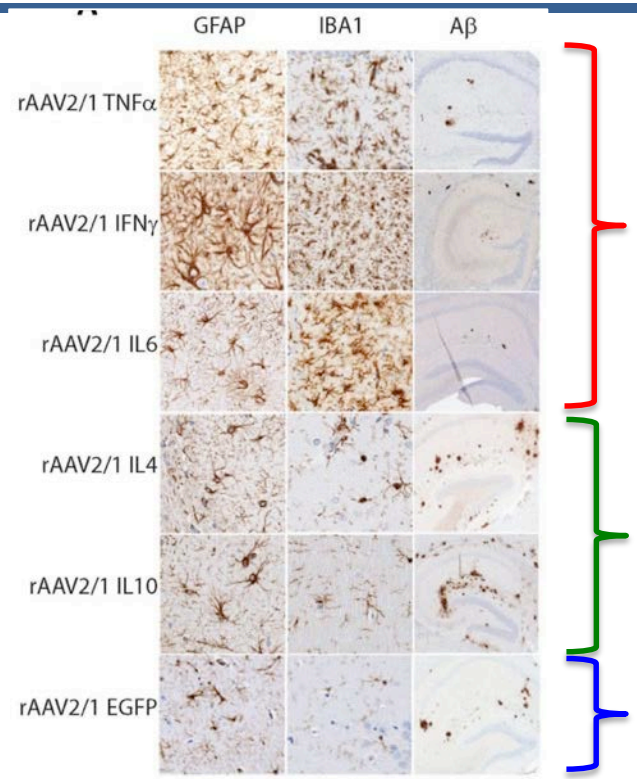
- Opportunities:
  - Potential for disease modification in later stages of disease –downstream of trigger
  - Efficacy in multiple diseases?
  - Possibility of cognitive effect in absence of disease modification
  - Lots of targets
  - Potential  to genetically validate target in humans

# 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 human brain towards a "proinflammatory" state in the apparent absence of underlying proteinopathy (Cribbs et al JNI 2012)
  - Potential for "untoward" systemic effects

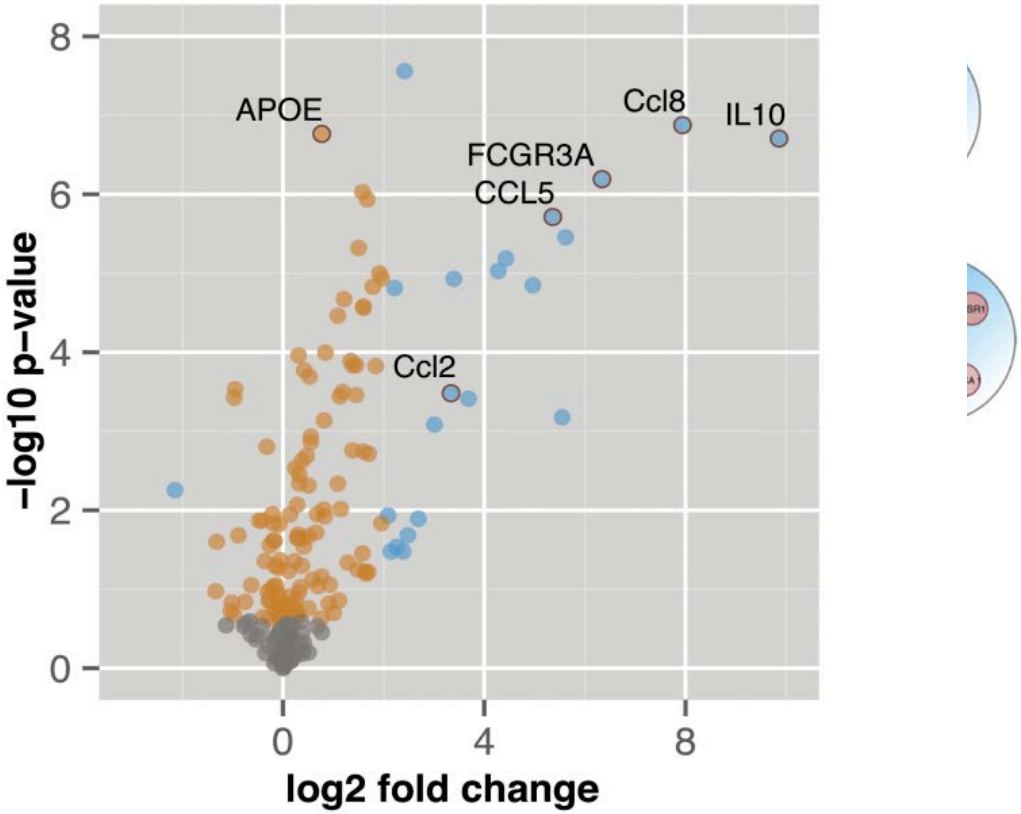
# Manipulating Innate Immune Activation States in the APP mouse brain studies in CRND8 mice (rAAV1)



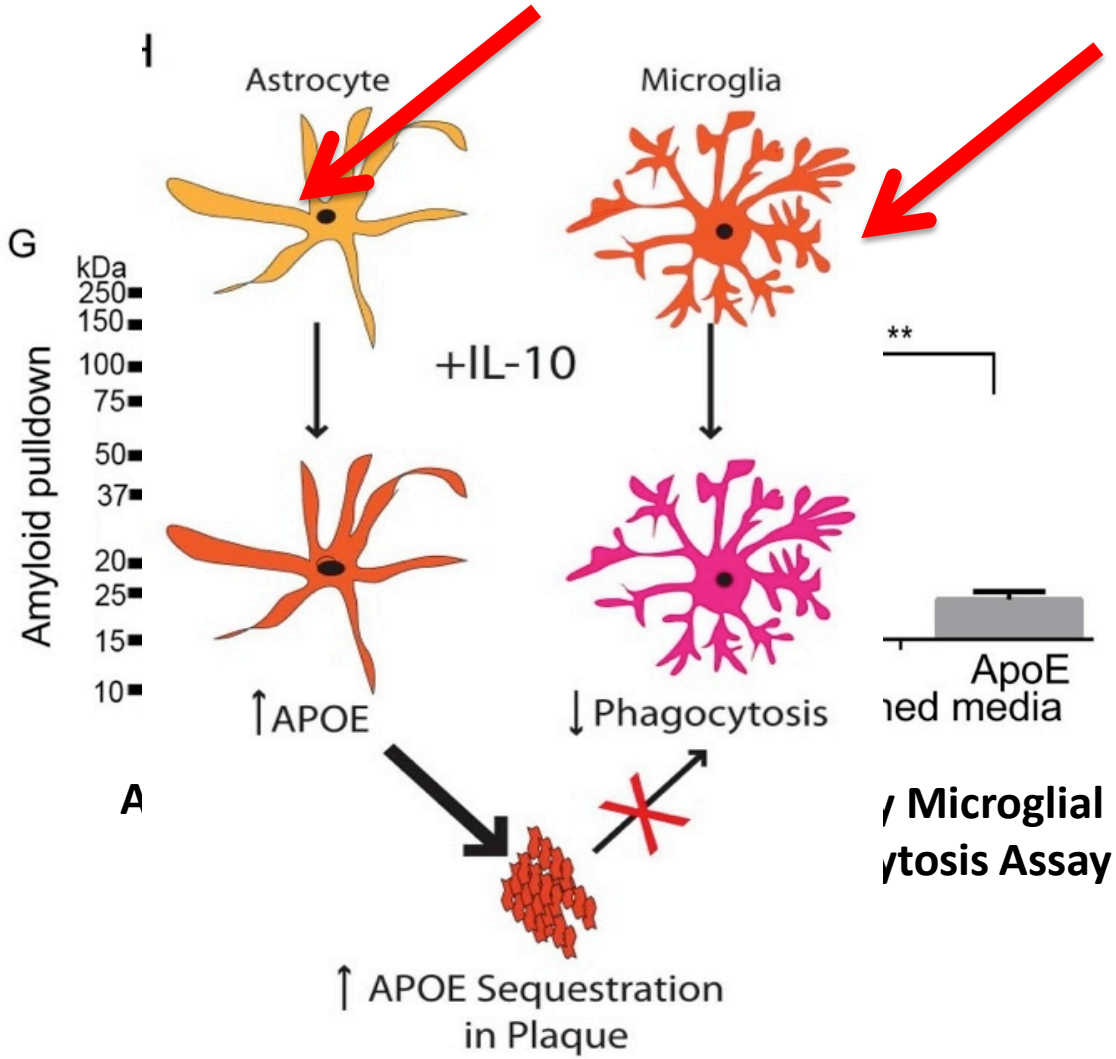

- Same effects observed whether cytokine was expressed from chronic infections (P2/3 vs P0)
- Il-6 Chakrabarty et al FASEB 2010
- INF-γ Chakrabarty et al J. Immunology 2010
- TNF-α Chakrabarty et al Mol. Neurodegeneration 2011
- Il-4 Chakrabarty et al Mol. Neurodegeneration 2012
- Il-10 Chakrabarty et al Neuron 2015

# Systems Analysis of Nanostring Based RNA quantification data identify APOE as a possible factor in mediating the IL-10 phenotype



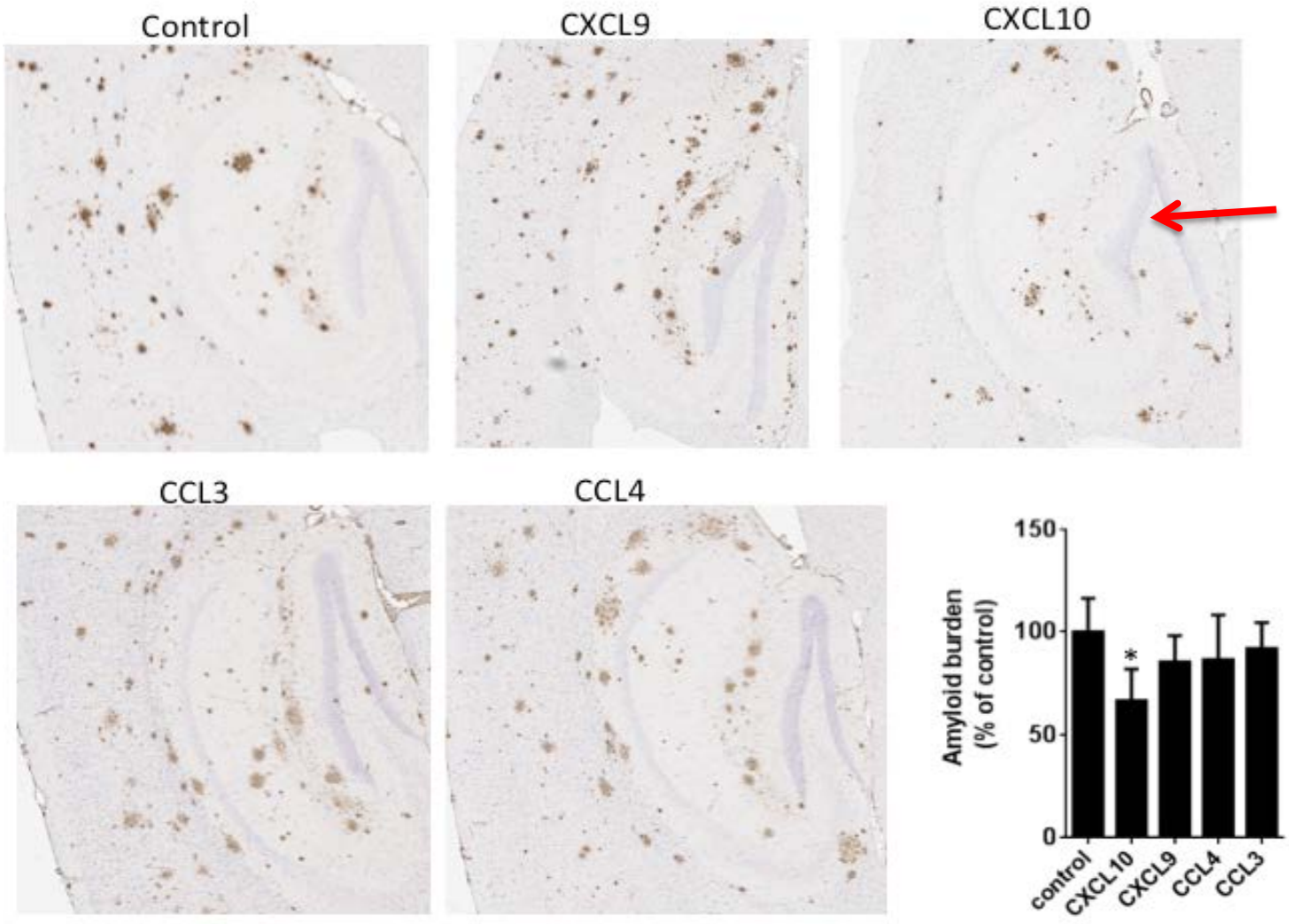

Chakrabarty et al Neuron 2015

# APOE binds aggregated Aβ and impairs microglial phagocytosis

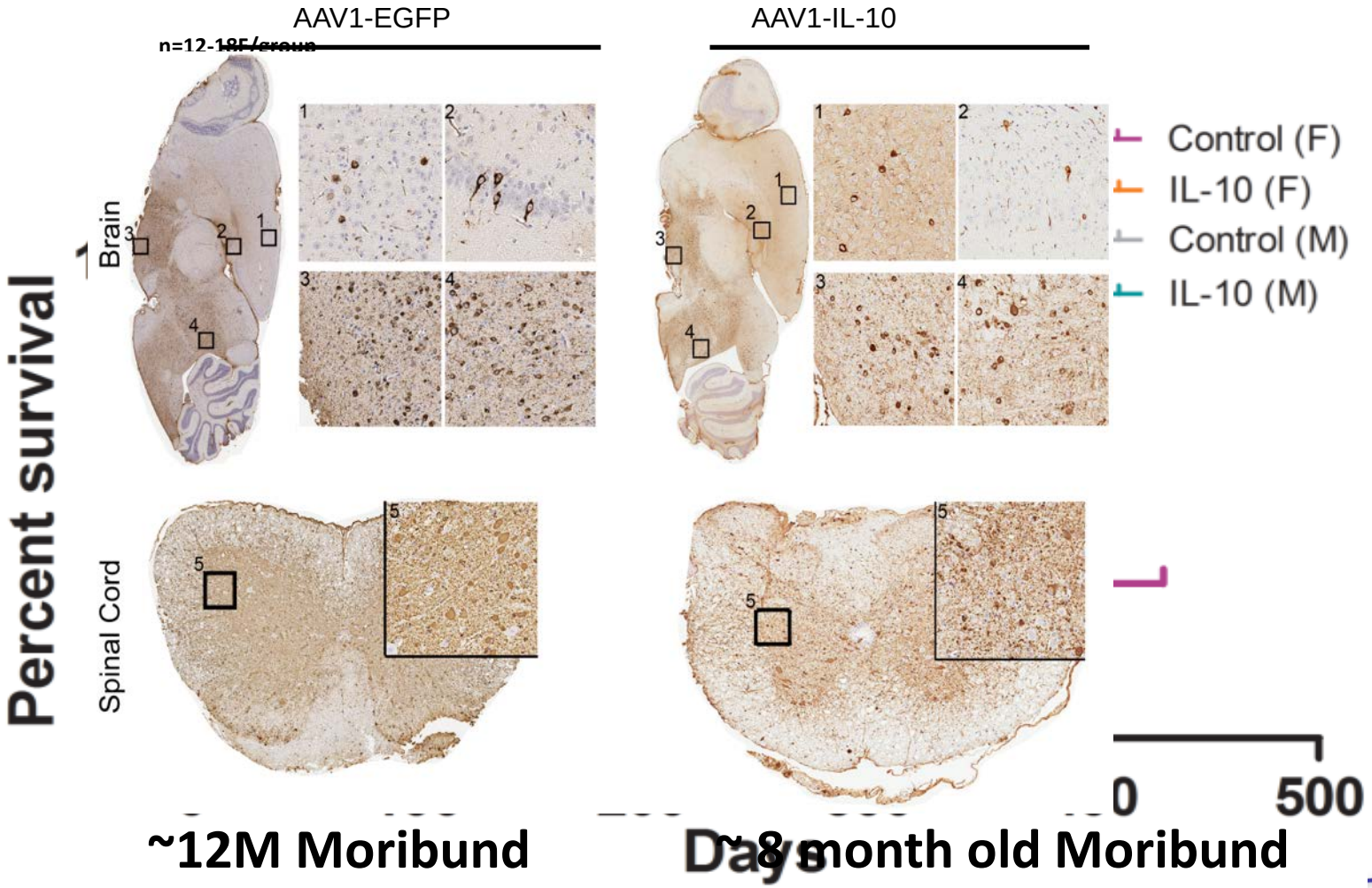



Chakrabarty et al Neuron 2015

# CXCL10 reduces Aβ loads



# rAAV-IL-10 accelerates tauopathy in homozygous JNPL3 mice (in progress)




~12M Moribund

~8 month old Moribund

Chakrabarty & Golde, unpublished

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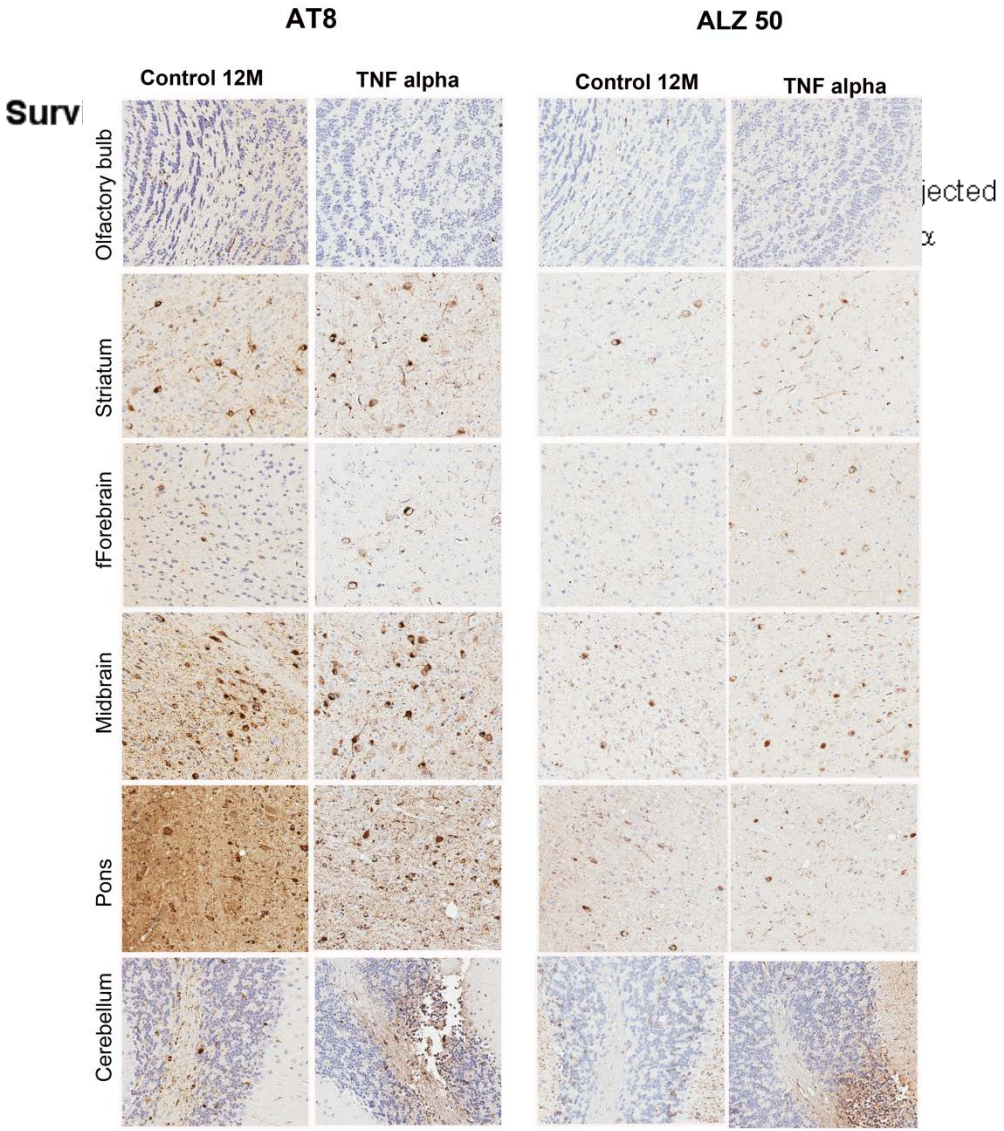
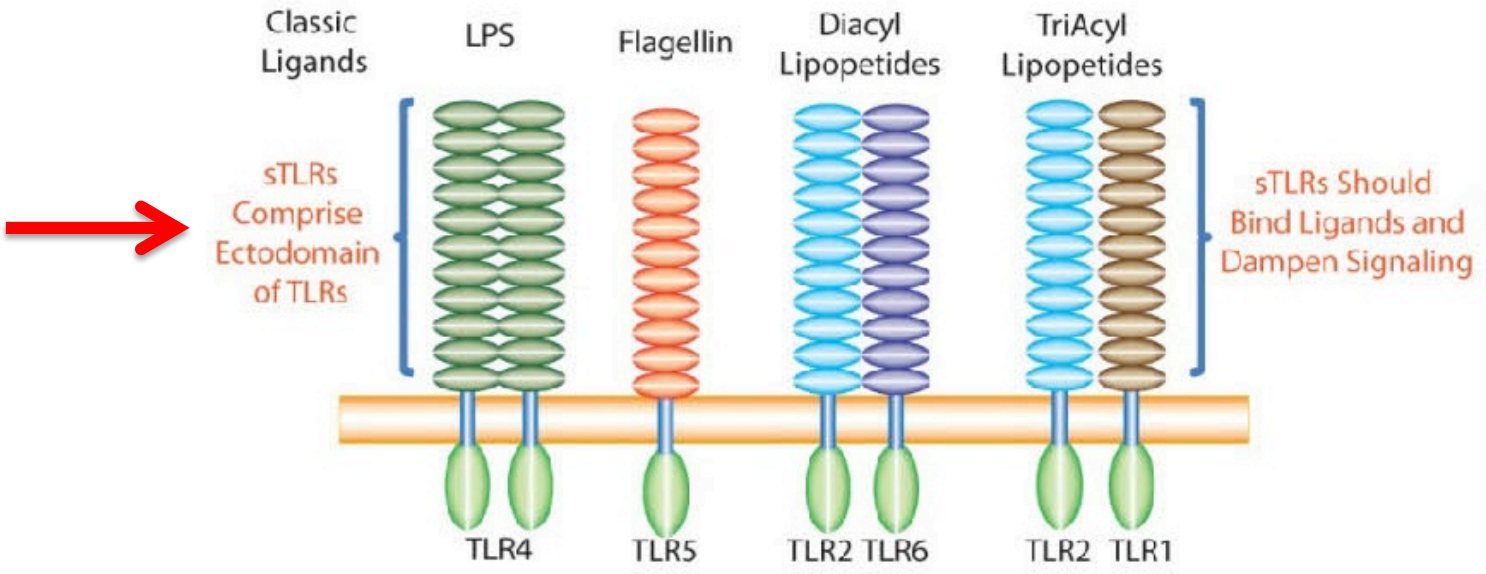
# rAAV-TNFα Makes tau mice better??

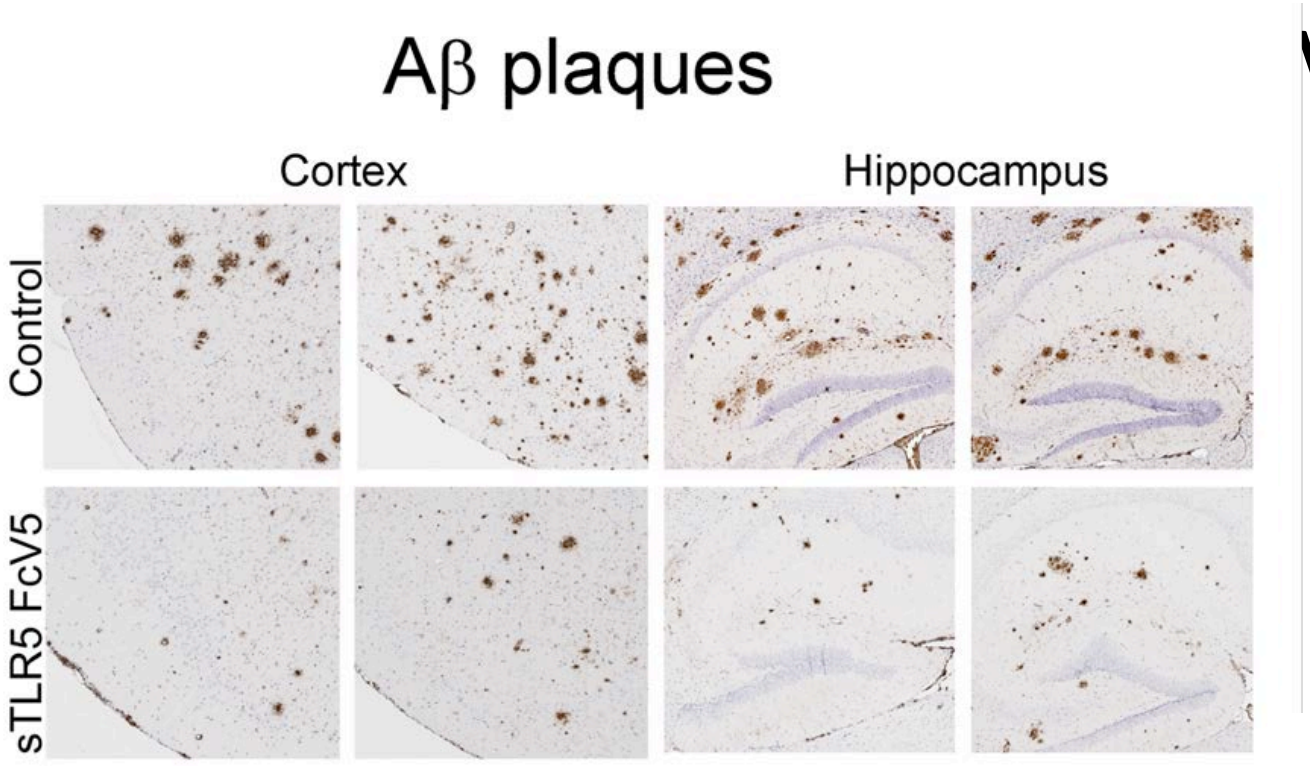


# Harnessing soluble Toll Like Receptors (sTLRs) as AD therapeutics?



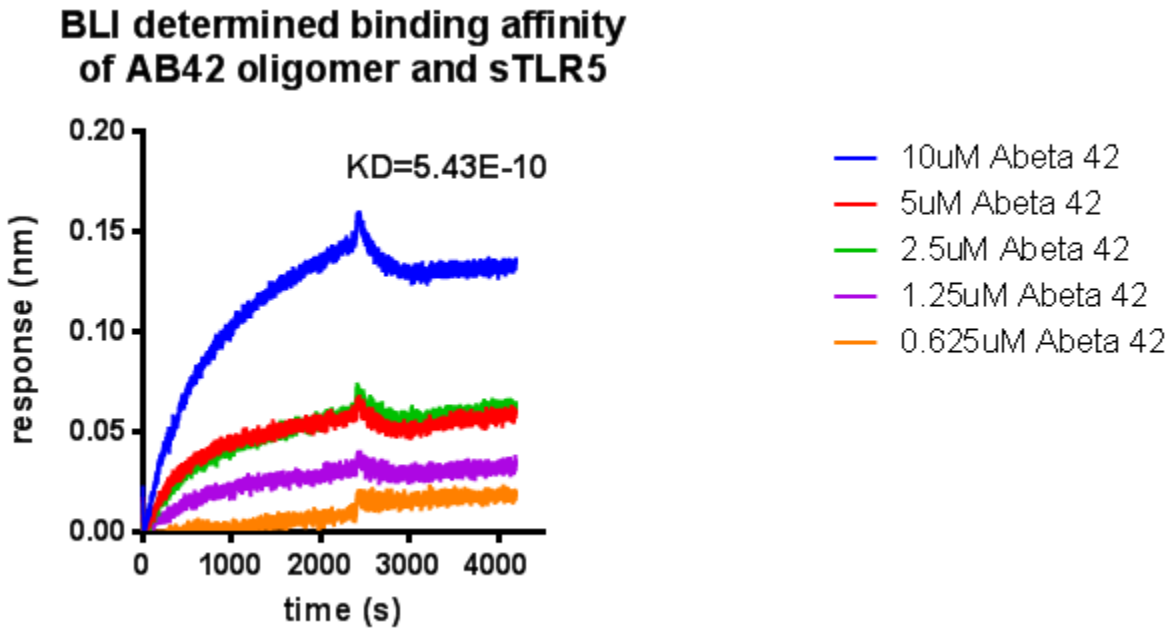

- TLRs are cell surface or endosomal PRR that paly major roles in innate immune activation
- Select sTLRs might bind Aβ aggregates but also dampen inflammation.
- What will they do to pathology?
- In addition to sTLR 2,4,6 we tested sTLR5 as it's only known ligand is flagellin a fibrillar protein
- All of these TLRs are expressed at low levels the mouse and human brain but can be induced in AD/aging

# rAAV2/1-sTLR-FcV5 transduction dramatically reduces Aβ plaque pathology and microgliosis



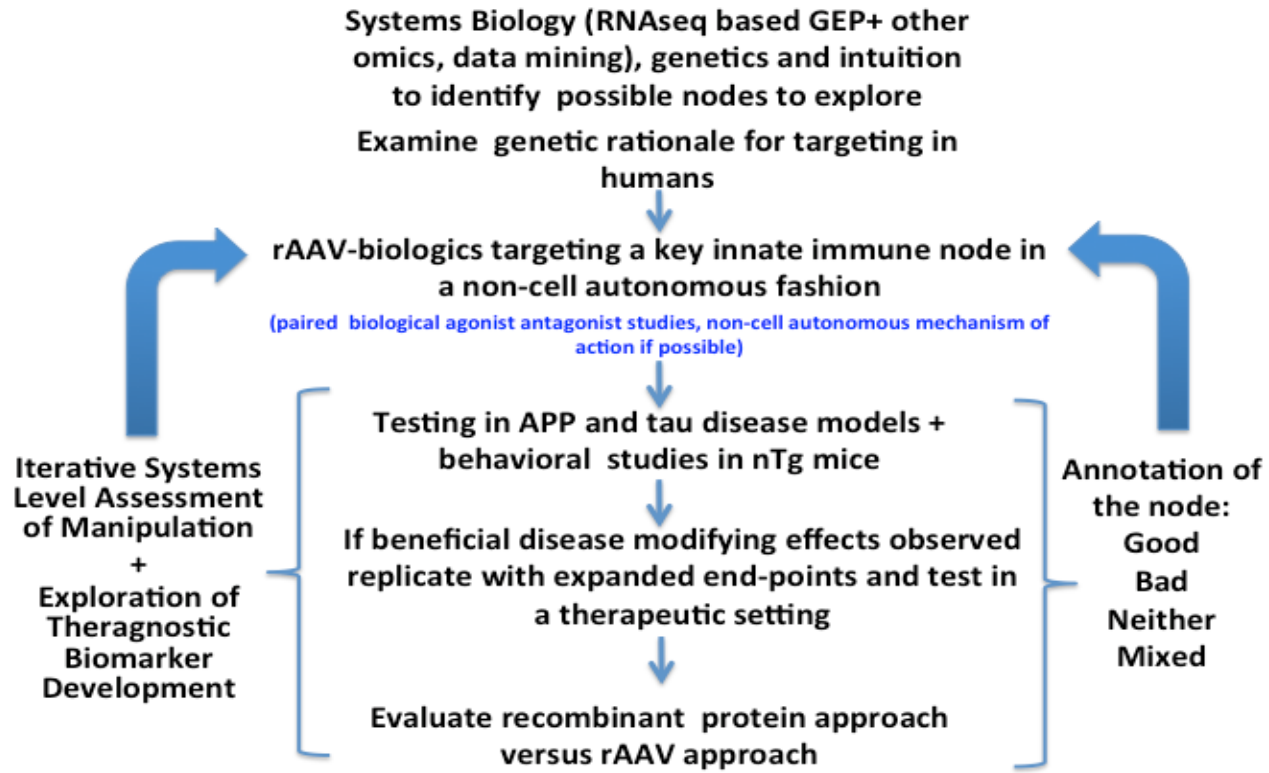

**CRND P0 to 6 months (n >12 in all groups)**
**+ Preliminary Data (n= 4-5) an ~50 reduction of Aβ loads following Hippocampal rAAV delivery from 6 to 9 months**

# How is sTLR5 working? Direct Binding of Aβ




| | Kon | Kdis | KD |
|---|---|---|---|
| sTLR5Fc | 5.43E-10 | 1.61E+02 | 8.75E-08 |
| AB5 | 5.06E-10 | 2.68E+04 | 1.36E-05 |

# A systems level approach may help (RNA seq data on SAGE SYNAPSE)




**Funding Provided by the NIA/NIH Accelerating Medicine Partnership (U01 AG-01493)**

# Discordant DEG between AD and 20 month old CRND8 APP mice

| | log FC AD v C | FDR | Log Fc Tg v Non Tg | FDR |
|---|---|---|---|---|
| DUSP1 | 0.56 | 0.000279964 | -0.81 | 0.00000223 |
| JUNB | 0.29 | 0.073092398 | -0.51 | 0.013088013 |
| HAVCR2 | -0.25 | 0.07683679 | 1.24 | 0.000338239 |
| GUSB | -0.28 | 0.001435684 | 0.74 | 0.00000555 |
| MLXIPL | -0.30 | 0.004527746 | 0.67 | 0.33775106 |
| SELPLG | -0.31 | 0.056524677 | 0.44 | 0.025928842 |
| MPEG1 | -0.33 | 0.089103971 | 1.77 | 4.7E-39 |
| PNPLA7 | -0.36 | 0.000289267 | 0.46 | 0.028309628 |
| NLRP3 | -0.38 | 0.009241254 | 1.15 | 0.189043447 |
| MMP19 | -0.41 | 0.00102898 | 0.69 | 0.522921551 |
| AIF1 | -0.41 | 0.018854884 | 0.58 | 0.195938201 |
| CAPN3 | -0.41 | 0.012136044 | 0.64 | 0.060402145 |
| LILRB4 | -0.44 | 0.017139569 | 2.10 | 0.002516225 |
| P2RY13 | -0.50 | 0.004898898 | 0.82 | 0.000168063 |
| CD22 | -0.54 | 0.005084601 | 1.75 | 0.033218481 |
| PLD4 | -0.55 | 0.000312735 | 0.96 | 0.0000401 |
| P2RY12 | -0.72 | 0.004559564 | 0.58 | 0.002706418 |
| CX3CR1 | -0.89 | 0.000394162 | 0.98 | 4.27E-22 |

# A systems level approach may help (RNA seq data on SAGE SYNAPSE) example 20 month old CRND8 APP mice

| Gene | Name | p value | % Control |
|---|---|---|---|
| Prmt3 | protein arginine N-methyltransferase 3 | 2.7917E-10 | 59% |
| Laptm5 | lysosomal-associated protein transmembrane 5 | 2.42551E-09 | 251% |
| Hspb6 | heat shock protein, alpha-crystallin-related, B6 | 2.79078E-09 | 204% |
| C4b | complement component 4B (Chido blood group) | 7.42587E-09 | 384% |
| Gfap | glial fibrillary acidic protein | 1.96049E-08 | 520% |
| 463242 | RIKEN cDNA 4632428N05 gene | 2.58914E-08 | 193% |
| St14 | suppression of tumorigenicity 14 (colon carcinom | 2.59738E-08 | 370% |
| Csf3r | colony stimulating factor 3 receptor (granulocyte) | 3.38337E-08 | 227% |
| Gusb | glucuronidase, beta | 3.48591E-08 | 162% |
| Cybrd1 | cytochrome b reductase 1 | 5.57443E-08 | 298% |
| Lag3 | lymphocyte-activation gene 3 | 5.58617E-08 | 340% |
| Trem2 | triggering receptor expressed on myeloid cells 2 | 5.80413E-08 | 389% |
| Dap | death-associated protein | 9.05299E-08 | 154% |
| Inpp5d | inositol polyphosphate-5-phosphatase D | 9.42382E-08 | 172% |
| Cd68 | CD68 antigen | 2.07126E-07 | 330% |
| Ctsz | cathepsin Z | 2.20427E-07 | 210% |
| Man2b1 | mannosidase 2, alpha B1 | 2.35119E-07 | 187% |

**N = 8 per group, Illumina High Seq 101 bp reads**
**~100 million reads per sample**

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# Discordant gene expression changes between primary microglia treated with Aβ and old APP mice.

| | Primary Microglia | | 20 M CRND8 APP Mice | | |
|---|---|---|---|---|---|
| Gene | P value | % Change | P value | % Change | |
| ALOX5AP | 2.8E-05 | 19% | NS | 85% | |
| PTGS1 | 3.02E-05 | 22% | NS | 114% | |
| CTSD | 6.12E-06 | 34% | 0.002975 | 203% | |
| IGF1 | 7.57E-06 | 35% | NS | 157% | |
| CTSB | 2.59E-05 | 36% | NS | 96% | |
| SCARB1 | 7.38E-05 | 41% | NS | 87% | |
| Ubiquilin2 | 0.000149 | 41% | 0.000921 | 79% | neuron enriched |
| CX3CR1 | 8.73E-06 | 44% | 0.00108 | 184% | |
| CD68 | 2.16E-05 | 47% | 3.14E-05 | 348% | |
| APOE | 1.65E-05 | 47% | NS | 114% | |
| LAMP1 | 6.8E-06 | 51% | NS | 103% | |
| C5AR1 | 5.42E-07 | 52% | NS | 180% | |
| ADAM10 | 1.49E-05 | 53% | NS | 86% | |
| GUSB | 2.02E-05 | 55% | 0.005787 | 180% | |
| STAT3 | 4.41E-05 | 66% | NS | 131% | |
| JAK2 | 0.00011 | 343% | NS | 78% | |
| TNFSF10 | 1.26E-05 | 345% | NS | 92% | |
| IFIH1 | 0.000106 | 353% | NS | 96% | |
| DDX58 | 3.87E-05 | 512% | NS | 144% | |
| ZBP1 | 7.87E-05 | 1060% | NS | 159% | |

# Concluding Thoughts

- Converging Pathological, Biologic and Genetic Evidence implicates alterations in innate immunity in Alzheimer's disease and other Neurodegenerative Proteinopathies
- Preconceived concepts of how innate immunity alters phenotypes need to be tested empirically
- Clear potential  to identify novel disease modifying targets/therapies
- But we may need systems level analysis to understand how a given manipulation alters the phenotype
  - Preconceived notions may not be correct

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# Concluding Thoughts (following my marching orders)

- Age: Yes
- Selective vulnerability: Yes
- Pathogenic interactions: Yes
- Sequence of events: More work to done
- Progression: More work to done
- Mixed pathology: Not AD specific
- Multiple clinical phenotypes: Certainly could play a role?
- Biomarkers: Needs more study?
- Risk factors: Yes Evidence For Genetic and Environmental
  - Why do the elderly often tank cognitively when the get an infection
- Translational potential: Yes, but a cautious approach is warranted

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