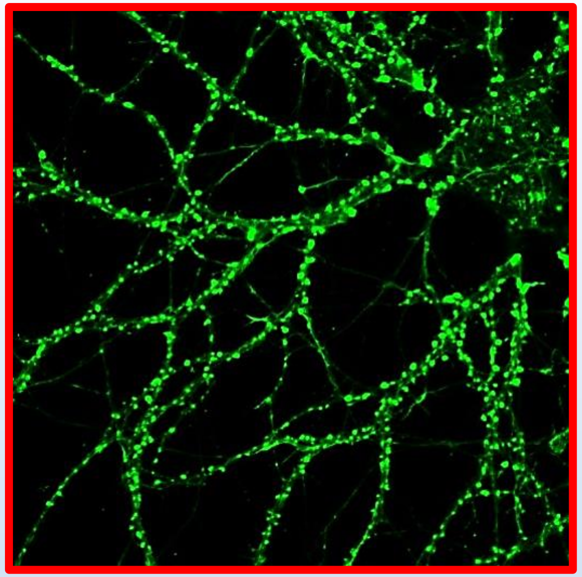
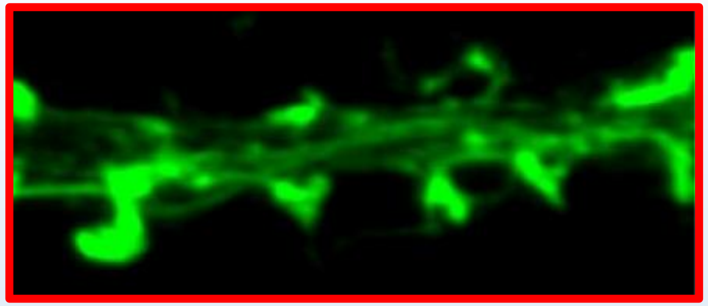
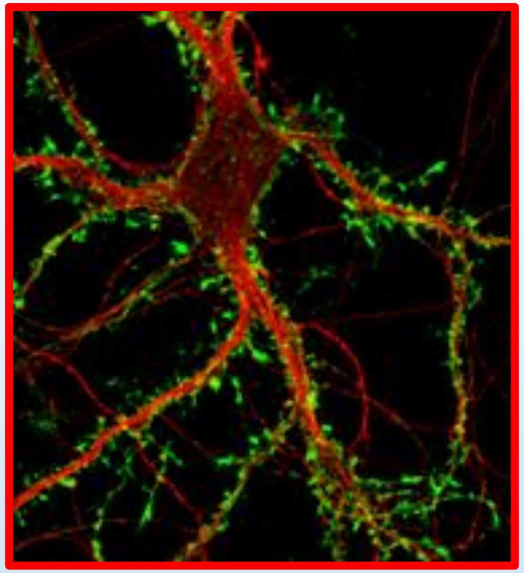


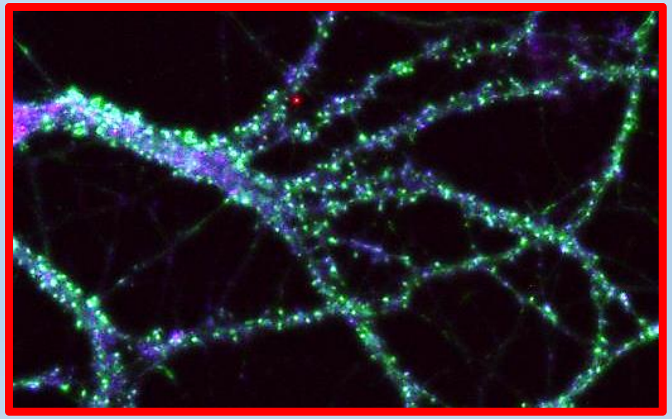


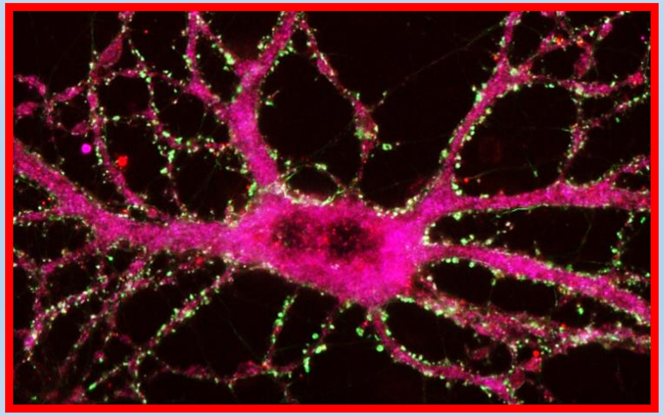


Prion Synaptotoxic Signaling

David A. Harris, M.D., Ph.D.

Department of Biochemistry & Cell Biology
Boston University - School of Medicine

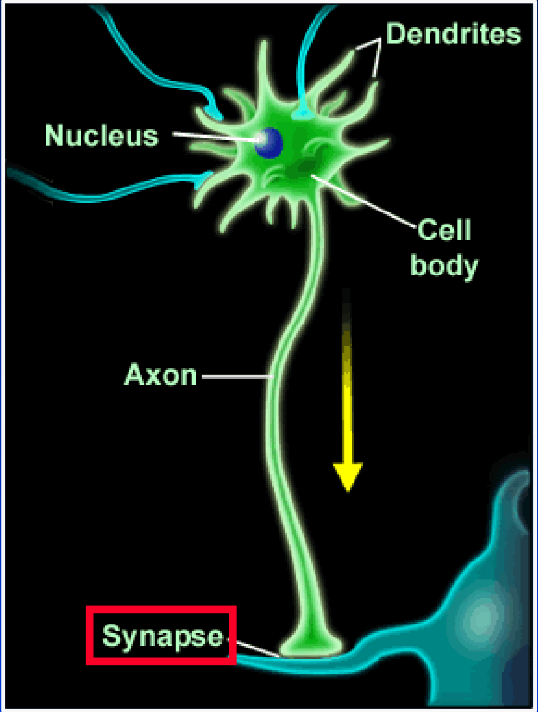


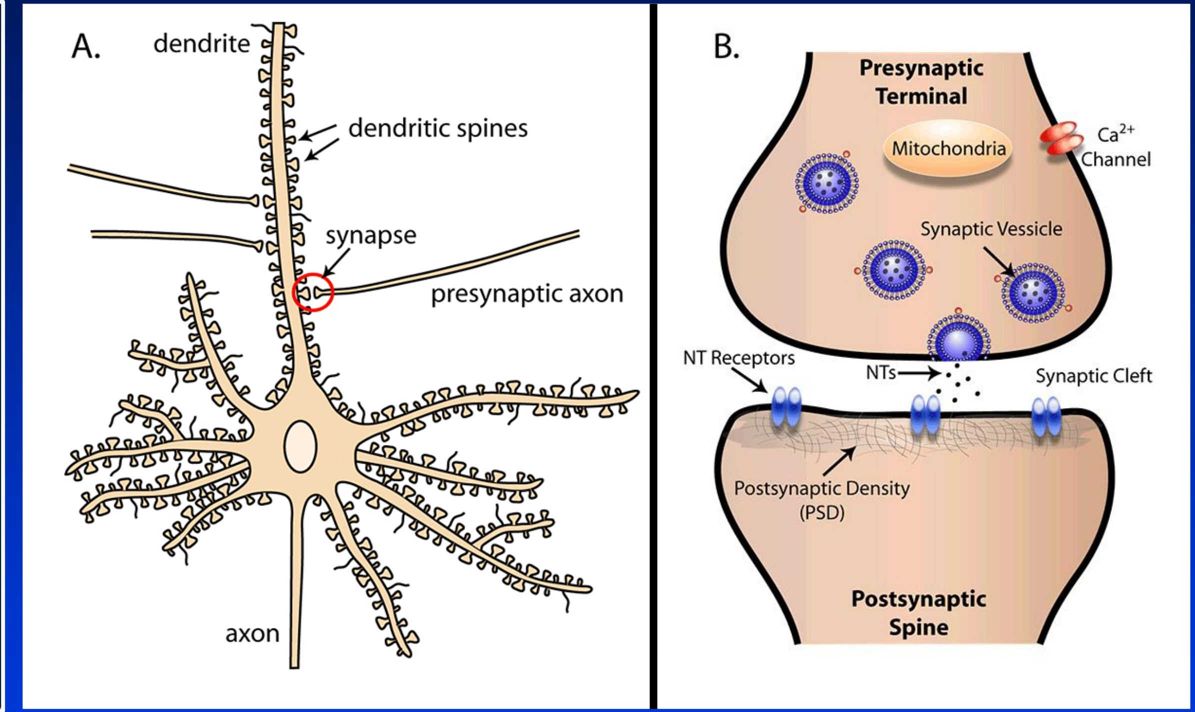


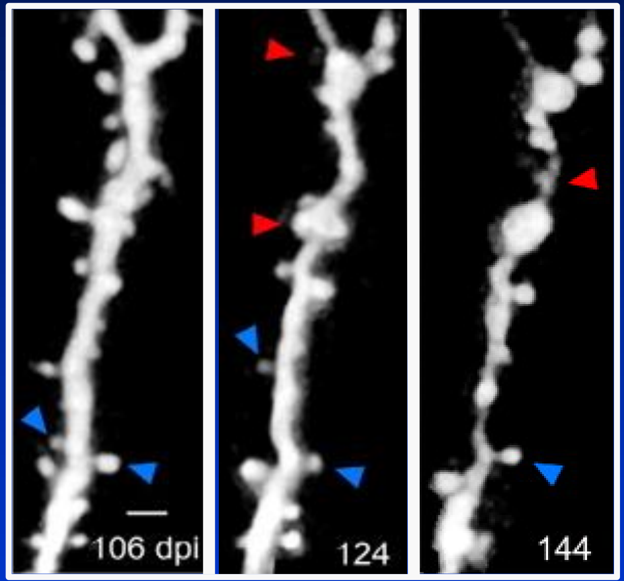
CJD Foundation Conference – Bethesda – November 14, 2024

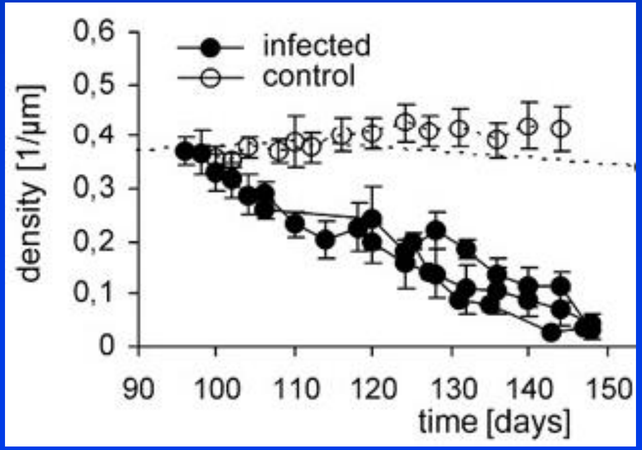
Prion toxicity starts at the synapse

Spine loss begins in presymp. phase







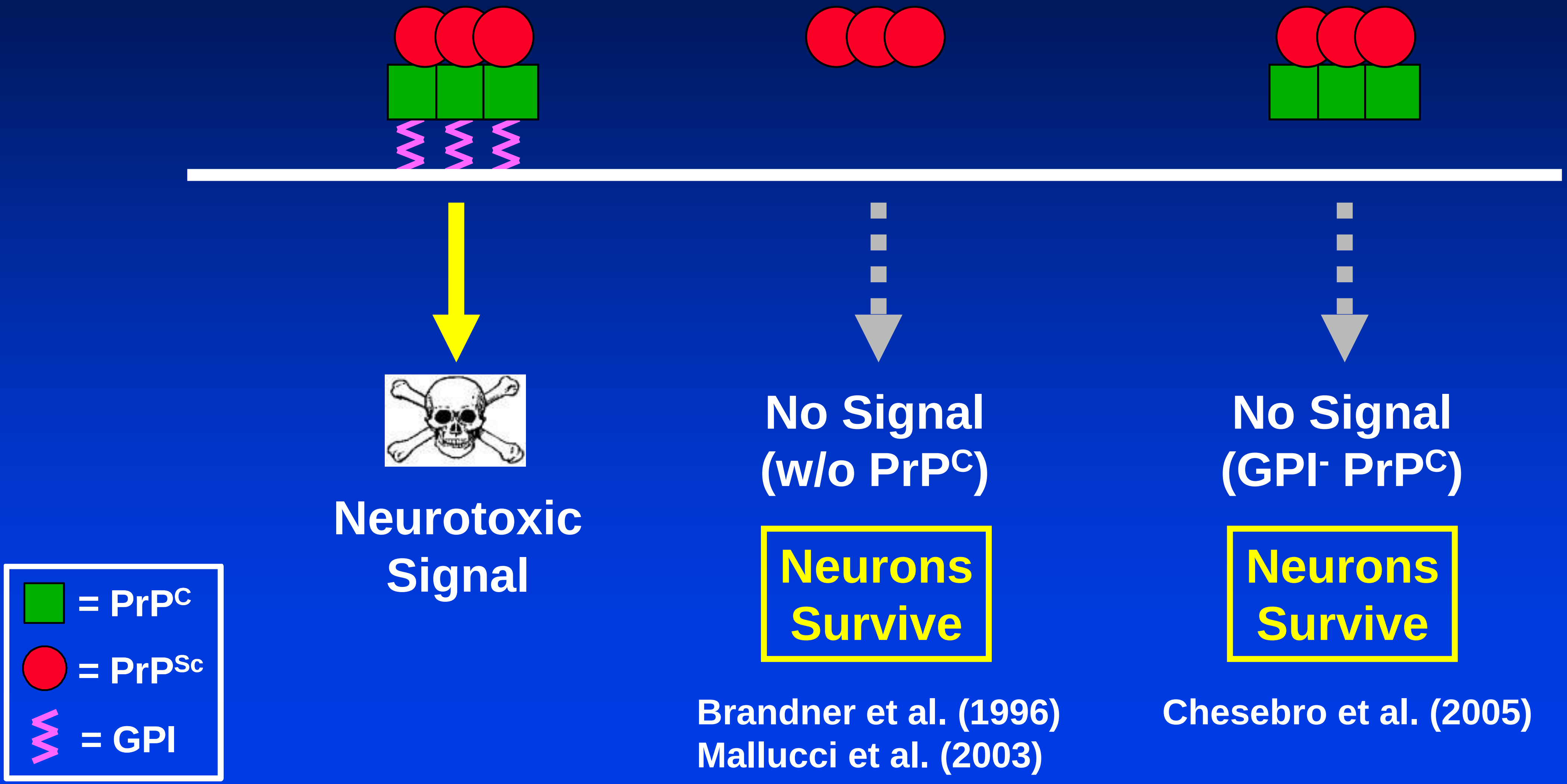


Dendritic spines – the postsynaptic elements of synapses

Role in learning/memory

Fuhrmann et al.
J. Neurosci. (2007)

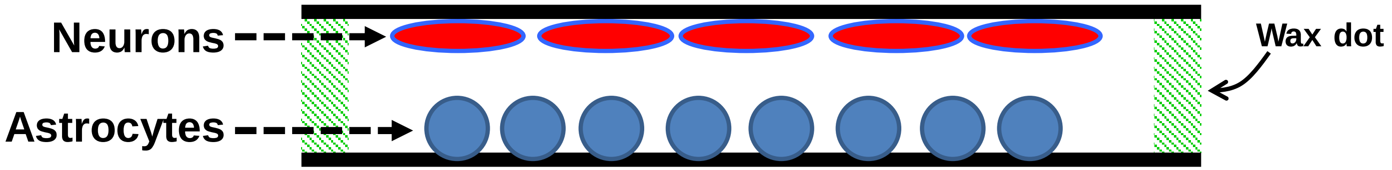
Cell surface PrP^{C} is necessary for PrP^{Sc} neurotoxicity



Brandner et al. (1996)
Mallucci et al. (2003)

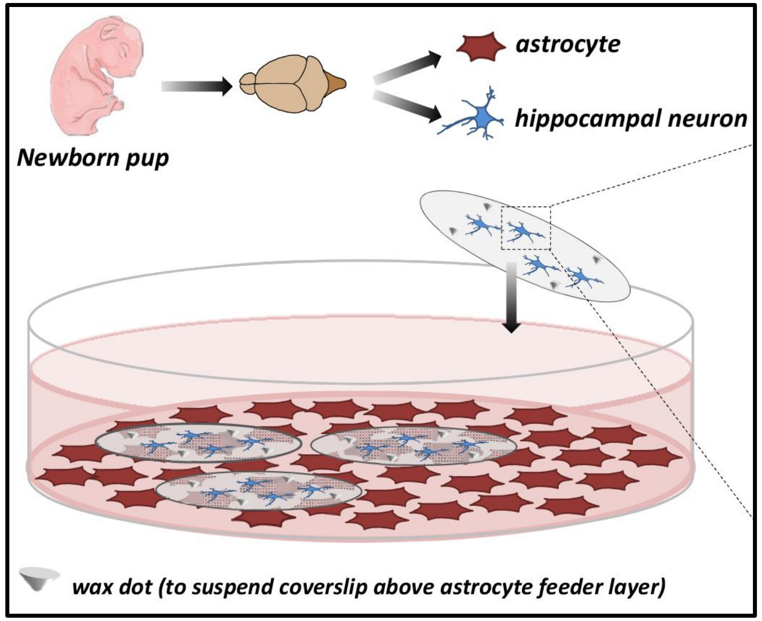
Chesebro et al. (2005)

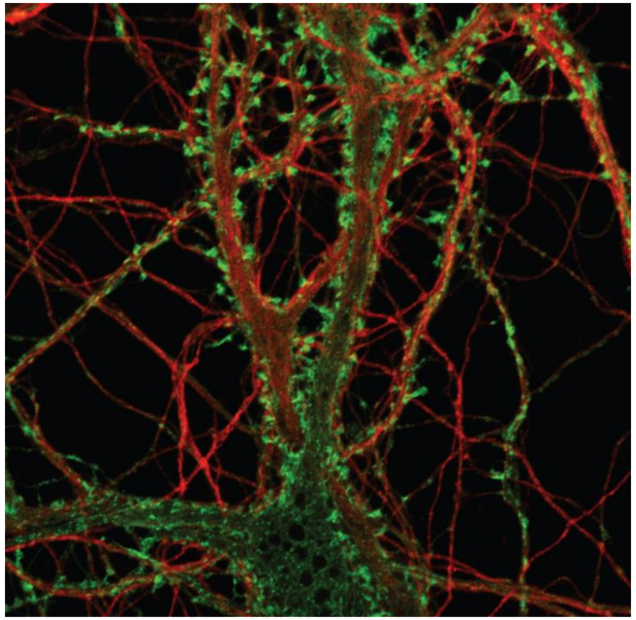
Culture System:
Neonatal mouse hippocampal neurons



Culture at low density for 3 weeks

- Mature axons and dendrites (with spines); active synapses (patch clamping, MEA)
- Separation of neurons/astrocytes: improved visualization; biochemical analysis



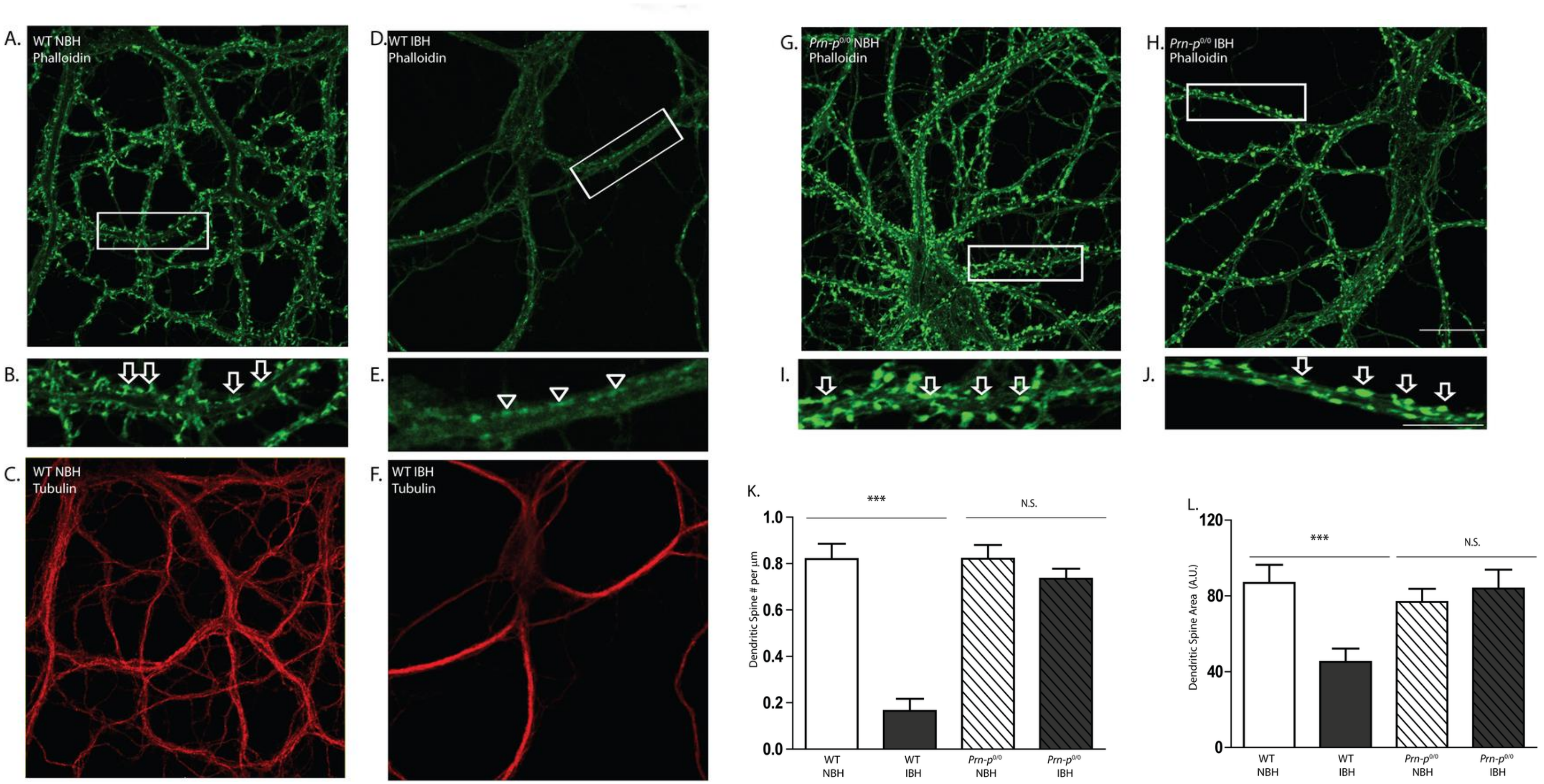


Green: Fl-Phalloidin (F-actin in spines)

Red: anti-tubulin (dendrites, axons)

Cheng Fang
PLoS Pathogens, 2016
(cover photo)

PrP^{Sc} causes PrP^{C}-dependent spine retraction

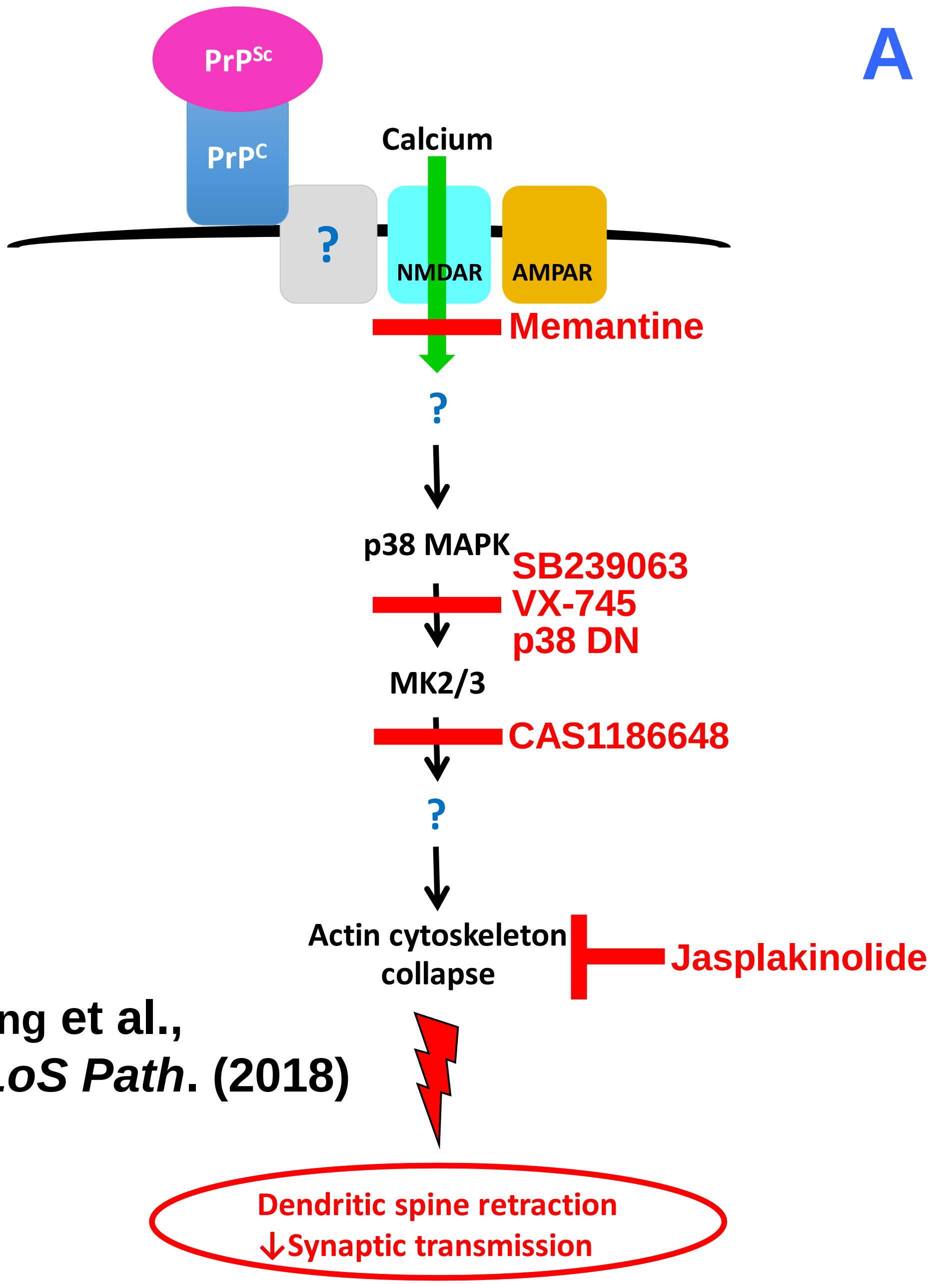


- Flattening/retraction of spines
- No effect on dendritic shafts, axons, viability
- Dependent on PrP^{C} expression

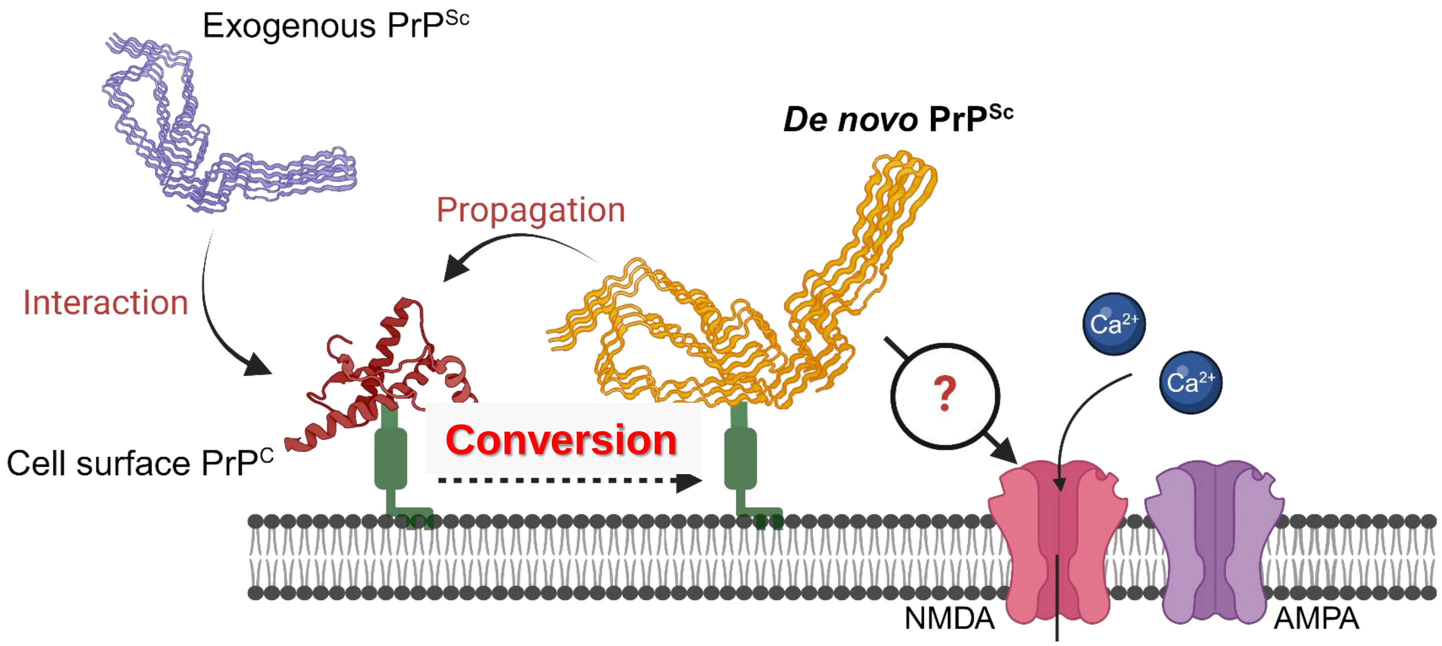
This system reflects earliest events in prion synaptotoxic signaling

Fang et al., *PLoS Path*. (2016)

A prion synaptotoxic signaling pathway

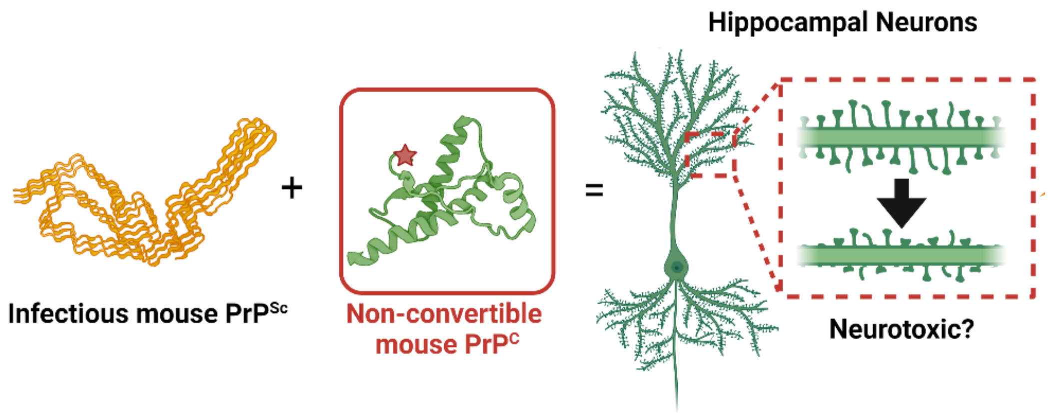


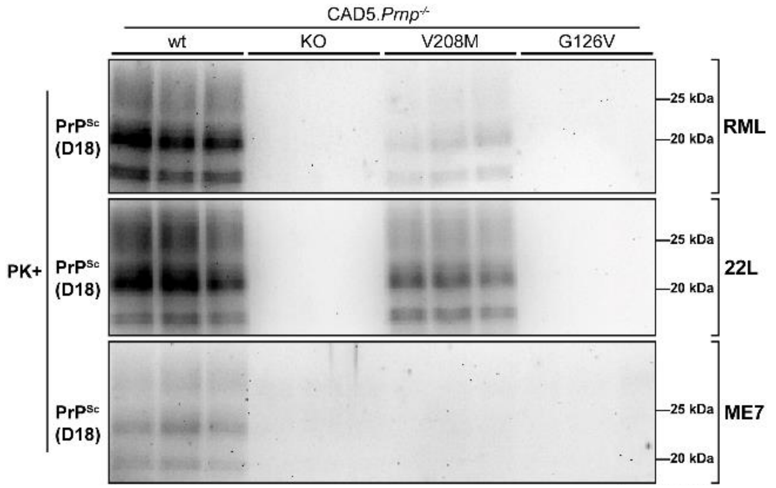
Fang et al., *PLoS Path*. (2018)

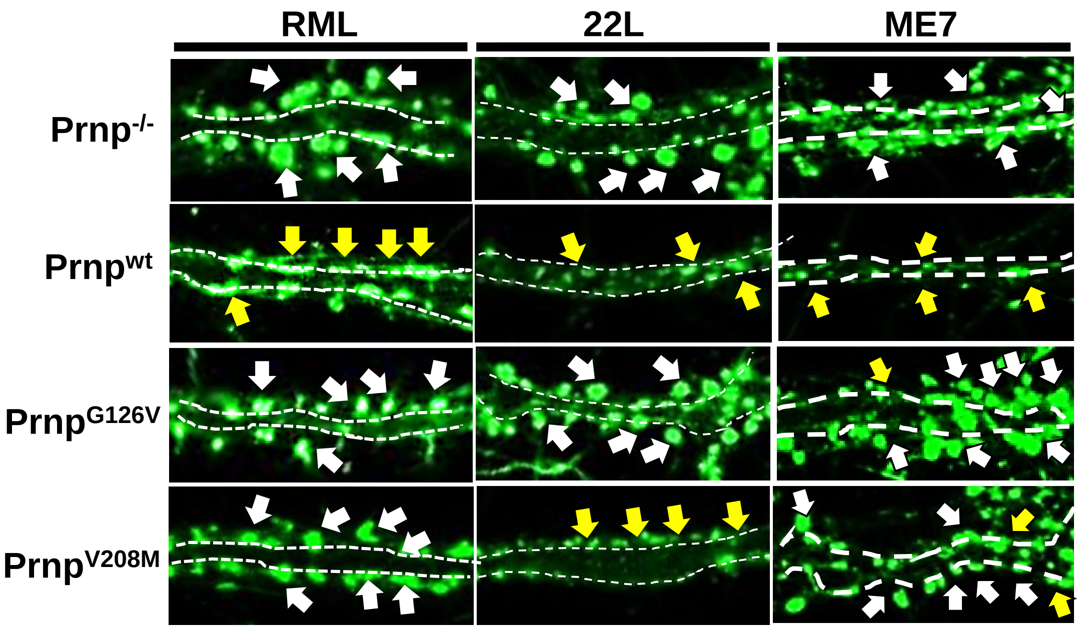


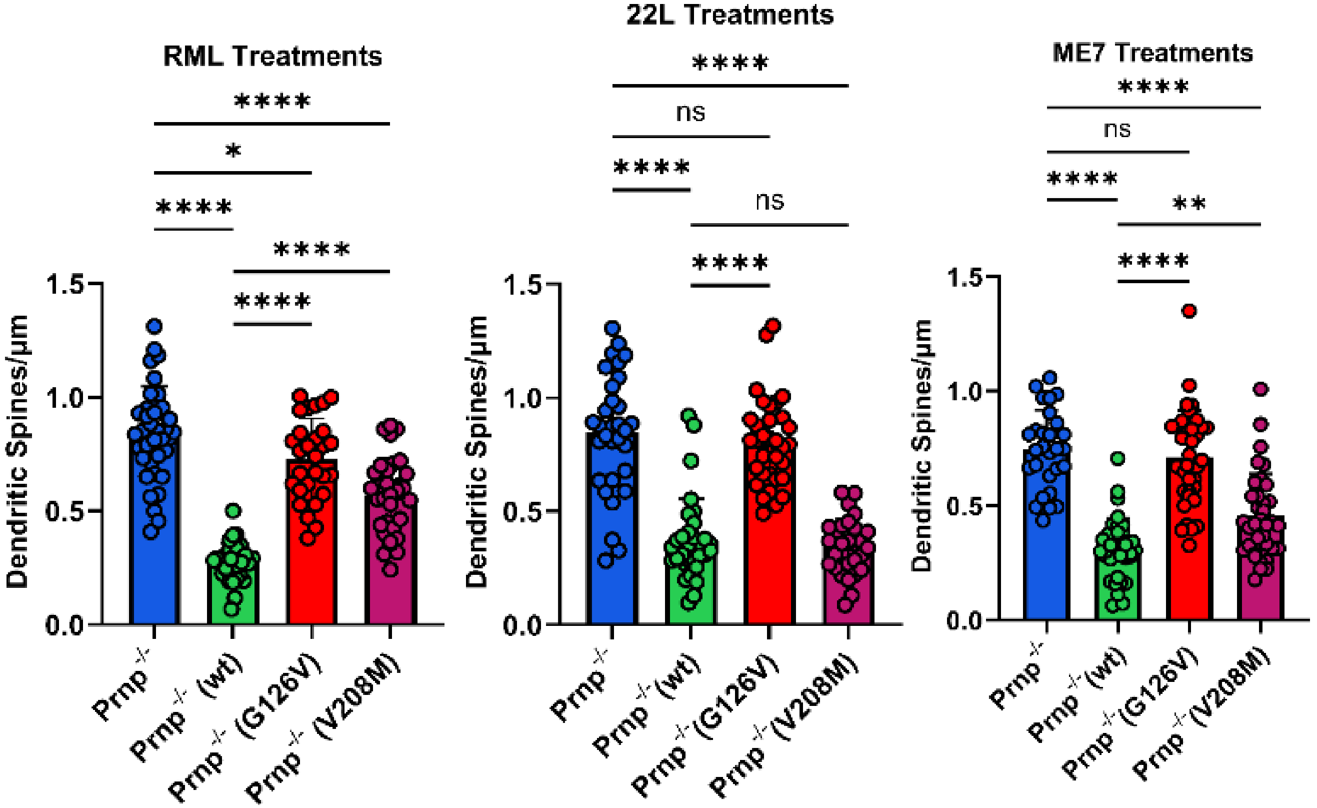
Is conversion of PrP^{C} to PrP^{Sc} essential?
Is PrP^{Sc} itself the synaptotoxic trigger?

Spine retraction is prevented by conversion-resistant PrP^C mutants



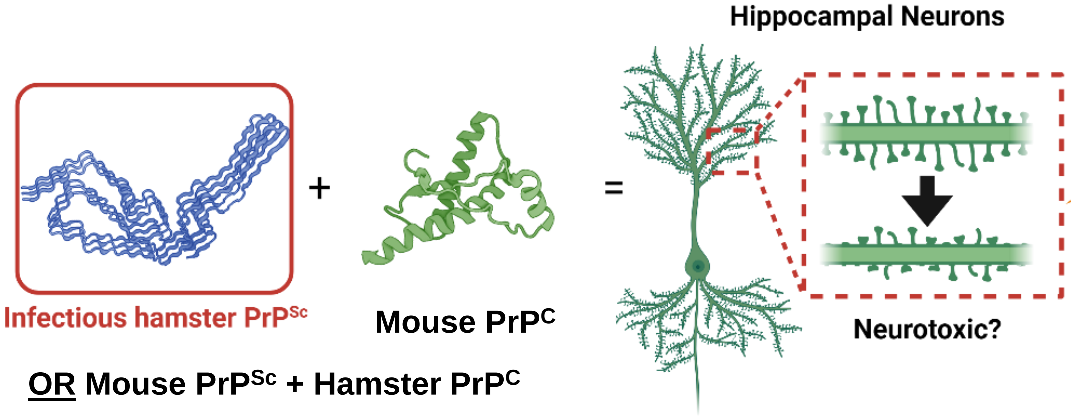


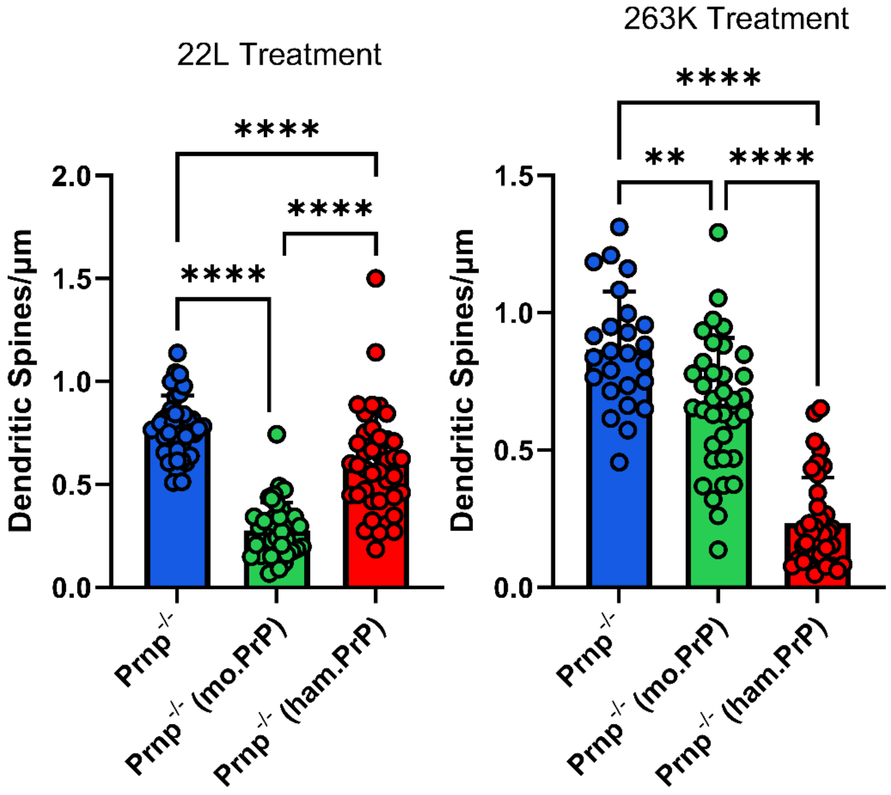


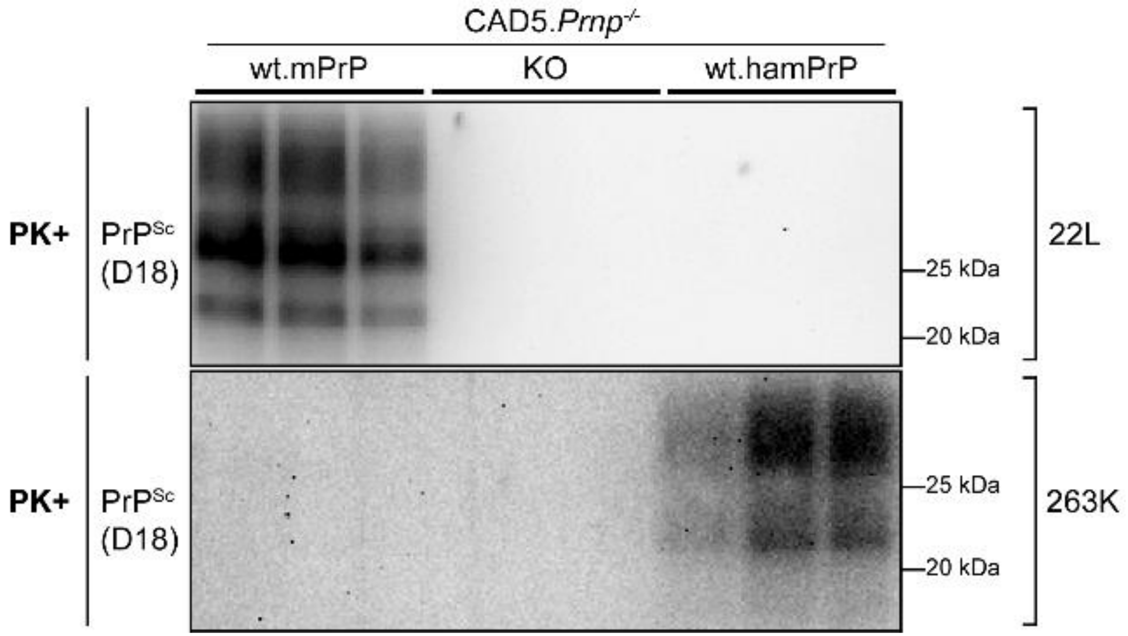


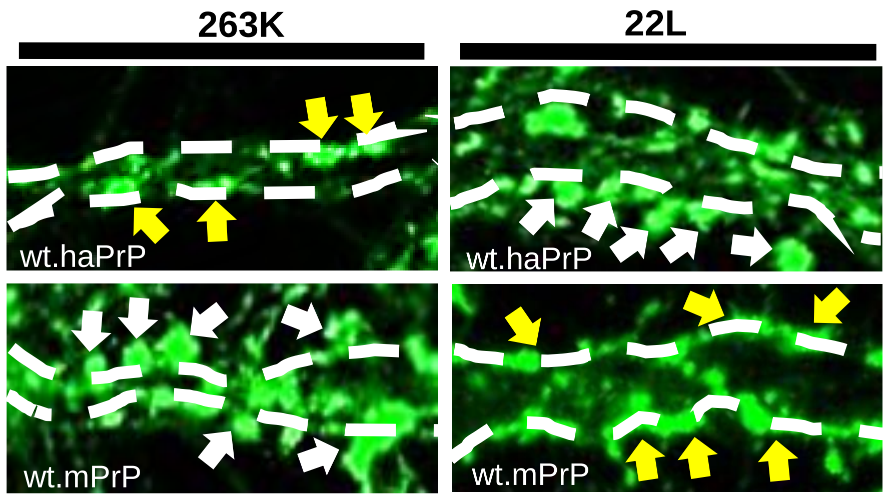
Jean Gatdula (Talk on Tues., Poster)

PrP^C species barrier abrogates spine retraction (mouse/hamster)



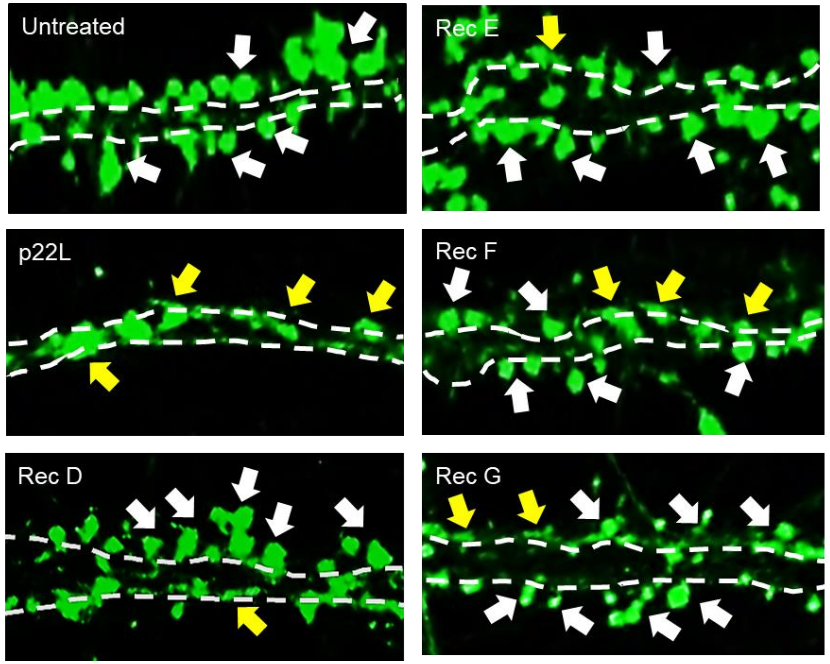


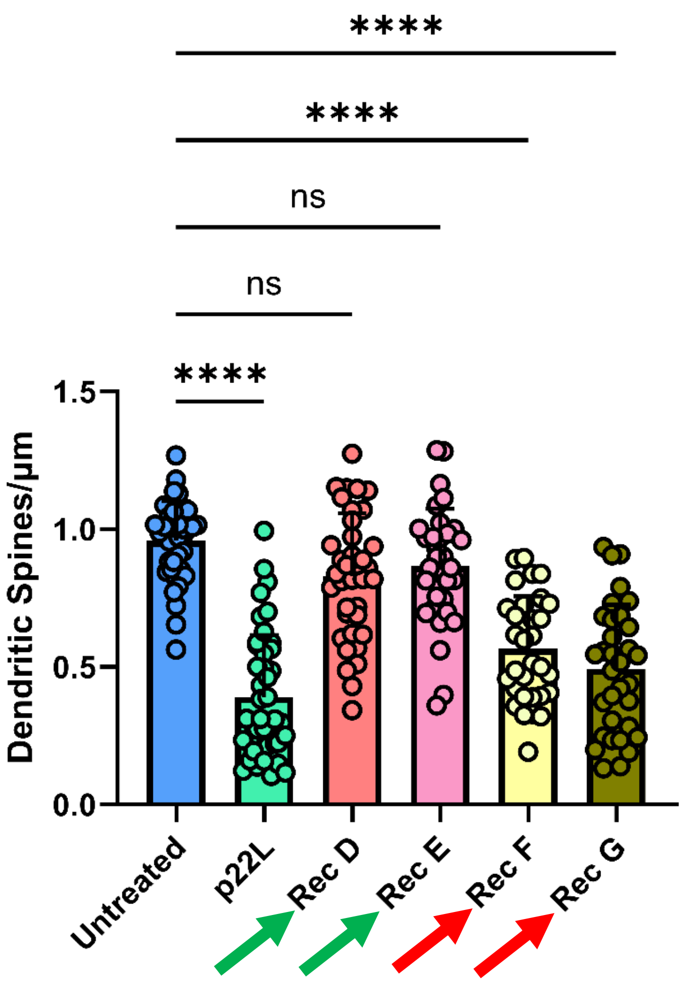




Jean Gatdula (Poster)

recPrP^{Sc} infectivity correlates with spine retraction activity





Recombinant PrP^{Sc}	Incubation Period in C57BL/6 mice (days)
Recombinant D	>600
Recombinant E	412 ± 89
Recombinant F	173 ± 18
Recombinant G	173 ± 13

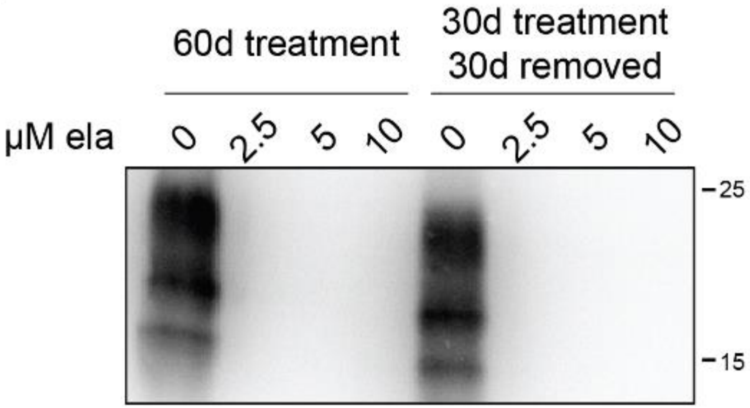
Joaquín Castilla
Hasier Eraña

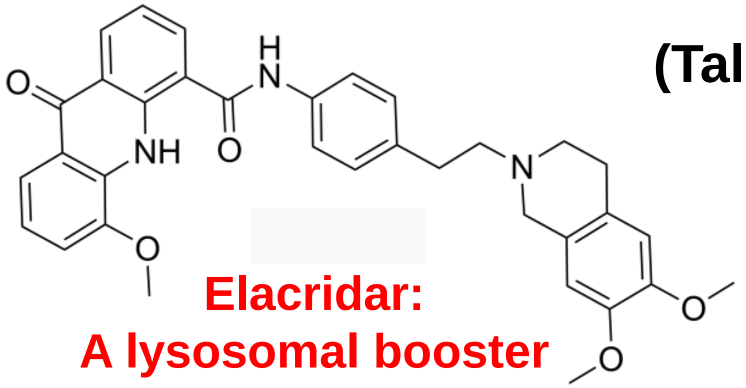
Jean Gatdula
(Poster)

Spine retraction is prevented by a drug that clears PrP^{Sc}, but only within a short time window

Robert Mercer
(Talk on Tues., Poster)

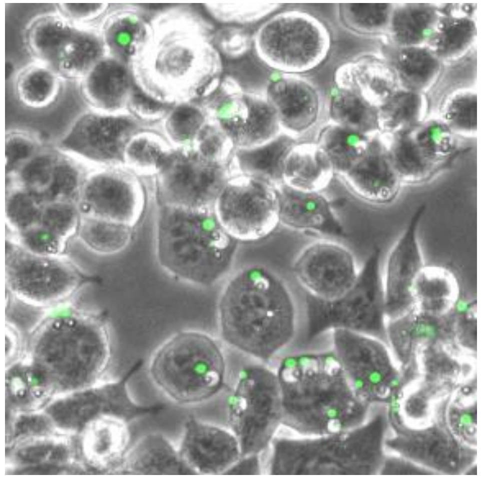
Elacridar clears PrP^{Sc} from infected N2a cells

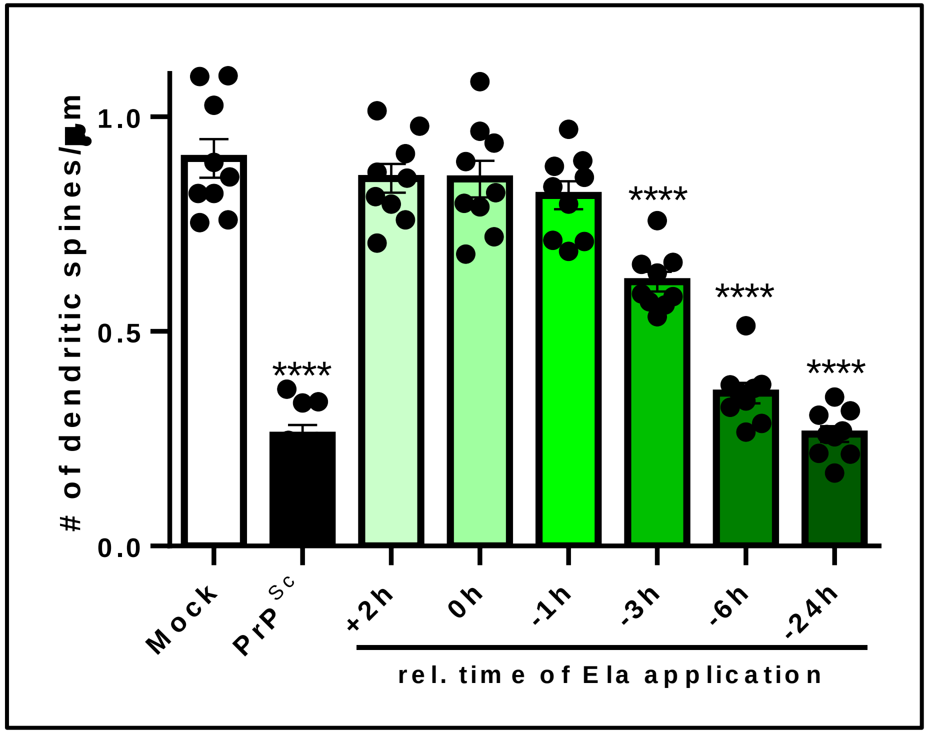




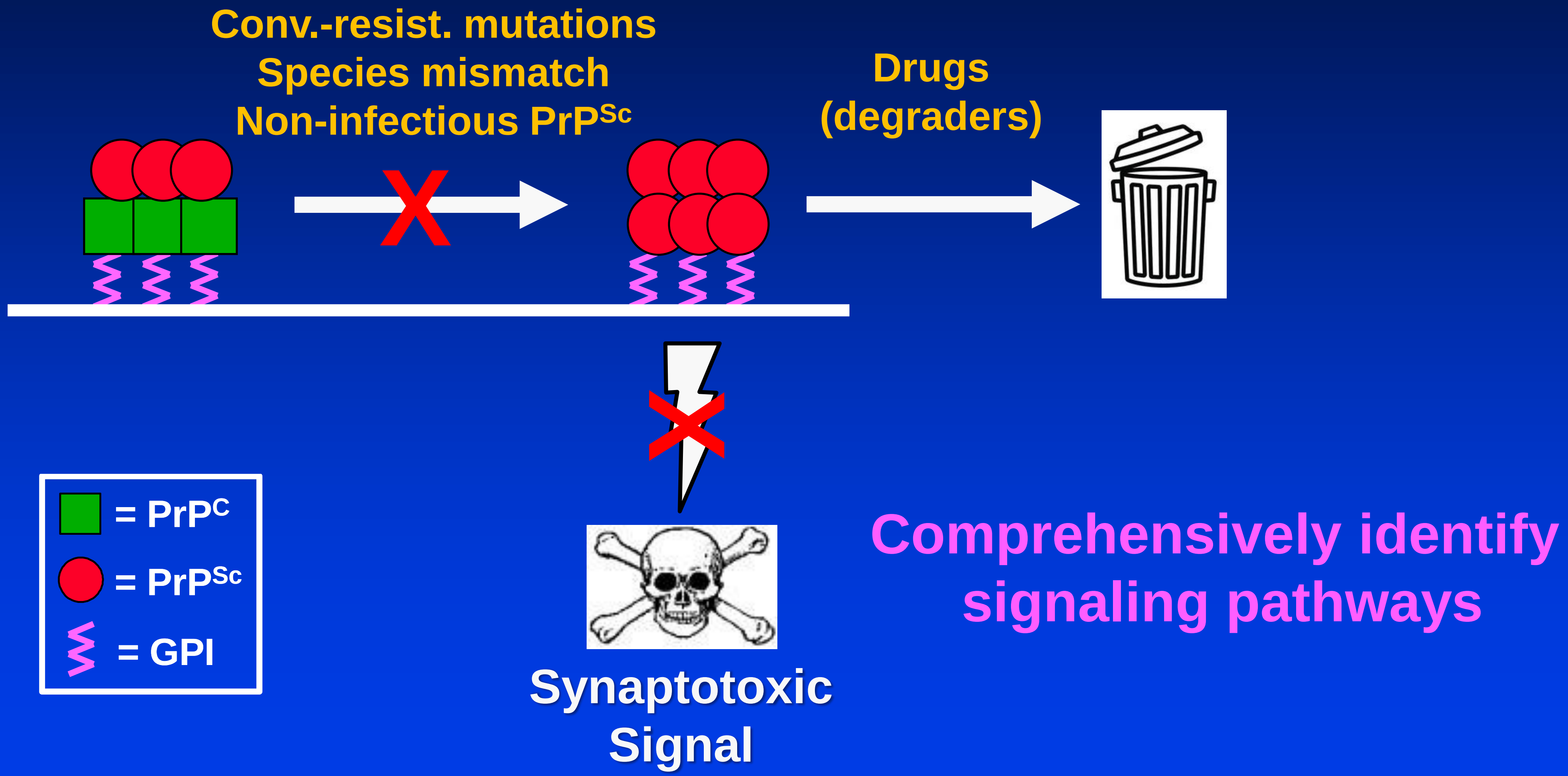
Elacridar:
A lysosomal booster

Elacridar accumulates in enlarged lysosomes





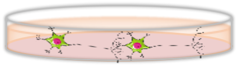
Cell-surface PrP^{Sc} is the synaptotoxic trigger



Chemo-transcriptomic/phosphoproteomic pipeline to identify synaptotoxic signaling pathways and components

Phospho-proteomics (1 hr) → Differentially Phosphorylated Proteins → Phosphoprotein Enrichment Analysis → Synaptotoxic Pathways

Neurons treated with PrP^{Sc}

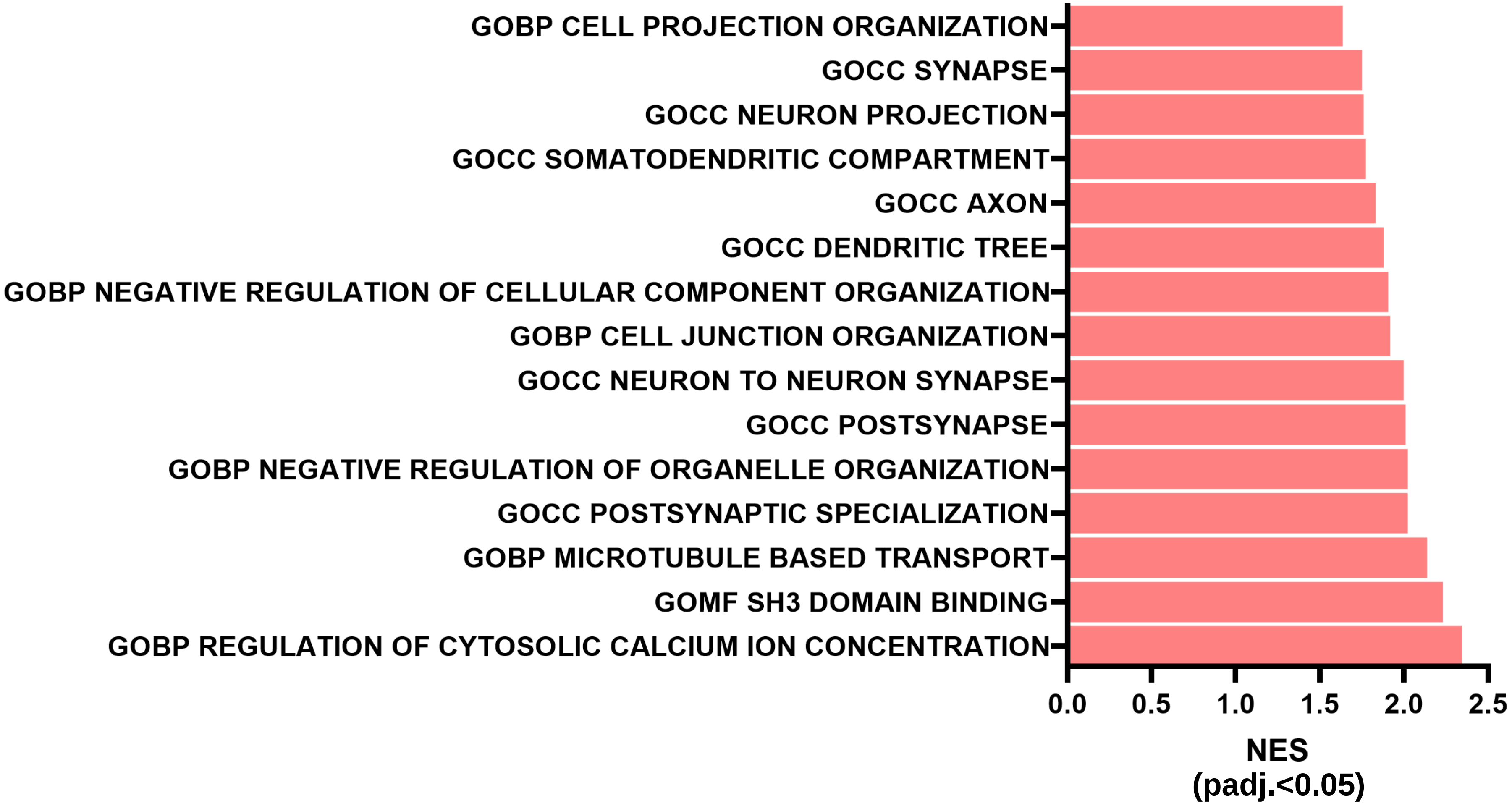


RNA-seq (0.5, 2, 4, 24 hrs) → Differentially Expressed Genes → Gene Set Enrichment Analysis → Synaptotoxic Pathways

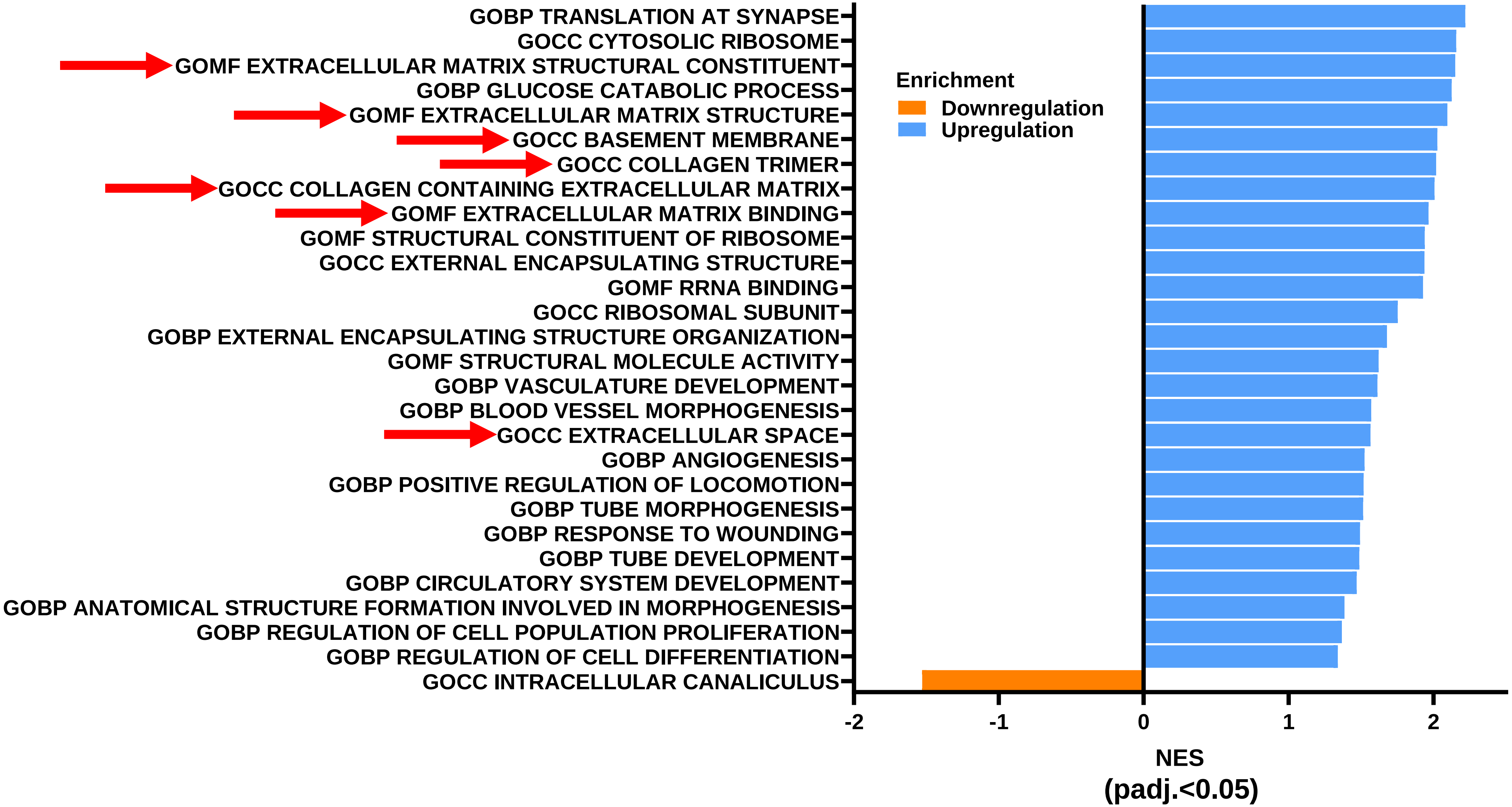
L1000/P100/LINCS databases (Broad Institute): Contain over 1 million transcriptomic signatures derived from 9 different core cell lines that have been subjected to >30,000 small molecule perturbagens. Also, a smaller number of proteomic signatures.

Nhat Le

Phosphoproteomic enrichment analysis (1 hr): Ca^{2+}, synapses, dendrites, microtubules



Transcriptomic enrichment analysis (24 hrs): Extracellular matrix (up-regulation)



Screening of inhibitors from -omics pipeline for ability to prevent spine retraction

Compound	RNA-seq	Phospho-proteomics	Inhibition of spine retraction	Bioactivity
KN-93		✓	+++	Ca^{2+}/calmodulin-dependent protein kinase II, K+ channel blocker
KN-62			+++	Ca^{2+}/calmodulin-dependent protein kinase II, K+ channel blocker
SB216763	✓	✓	+++	glycogen synthase kinase-3 inhibitor
3F8			++	glycogen synthase kinase-3b inhibitor
TWS119			+++	glycogen synthase kinase-3b inhibitor
GSK3b inhibitor VIII			++	glycogen synthase kinase-3b inhibitor
Tideglusib			+++	glycogen synthase kinase-3b inhibitor
Phorbol ester	✓		Toxic	protein kinase C activator
CID 2858522	✓	✓	+++	Selectively inhibits PKC-induced NF-κB activation
Rottlerin	✓		+++	PKCα, PKCγ, PKCβ, PKCη, CKII and PKA inhibitor
Ro 32-0432			+++	selective cell-permeable protein kinase C inhibitor
GSK 1059615	✓	✓	+++	Phosphoinositide 3-kinase α/β/δ/γ and mTOR inhibitor
AKT inhibitor IV	✓	✓	-	AKT/Phosphoinositide 3-kinase inhibitor
BML-257	✓		-	AKT1 translocation inhibitor
MK2206			+++	AKT inhibitor
GDC0068			++	AKT inhibitor
PI 828			-	Phosphoinositide 3-kinase inhibitor
PF 046			Toxic	Phosphoinositide 3-kinase inhibitor
diphenidol	✓		+	Non-selective muscarinic acetylcholine receptor antagonist
ropinirole	✓		Toxic	D2 and D3 dopamine receptor agonist
Erastin	✓		-	Voltage-dependent anion channel (2/3) inhibitor
Loperamide HCl		✓	Toxic	Ca^{2+} channel blocker
Ouabain	✓		Toxic	Na+/K+ ATPase inhibitor
Cymarin	✓		Toxic	Na+/K+ ATPase inhibitor
Lasalocid	✓		Toxic	Carboxylic acid ionophore
Quinine hemisulfate	✓		+	Mitochondrial ATP-regulated potassium channel inhibitor
Bax channel blocker	✓		+++	Bax-mediated mitochondrial cytochrome C release inhibitor
AT-7519	✓		-	cyclin-dependent kinases1, 2, 4, 6 and 9 inhibitor
BMS-387032	✓		Toxic	cyclin-dependent kinases 2, 7 and 9 inhibitor
HY-11001	✓		-	cyclin-dependent kinases1, 2, 4 and 9 inhibitor
KM-00927	✓		+	histone deacetylase inhibitor
BRD-A57107094	✓		++	histone deacetylase inhibitor
belinostat	✓		toxic	histone deacetylase inhibitor
ISOX	✓		+++	histone deacetylase 6 inhibitor
TPCA-1	✓	✓	+++	nuclear factor-κB kinase 2/NF-κB pathway inhibitor
Ro 106-9920			++	Inhibitor of NF-κB activation
BI 605906			+	IkappaB kinase β inhibitor
PF 184			Toxic	IkappaB kinase β inhibitor
Nafamostat mesylate	✓		-	Serine protease inhibitor
AZD-8055	✓		-	mTOR inhibitor
Anisomycin	✓		+++	Protein synthesis inhibitor
RG108	✓	✓	-	non-nucleoside DNA methyltransferases inhibitor
7-Nitroindazole	✓	✓	+	nitric oxide synthase inhibitor
Nutlin-3	✓		-	P53/ E3 ubiquitin-protein ligase mdm2 inhibitor
Triptolide	✓		-	MDM2 inhibitor through a p53-independent pathway
Celastrol		✓	+++	heat shock protein 90 inhibitor
NVP-AUY922	✓		Toxic	heat shock protein 90 inhibitor
Narciclasine	✓		-	RhoA activator and induces actin polymerization
MLN4924	✓		++	Nedd8 activating enzyme inhibitor
Chloroquine	✓		+++	Lysosomotropic amine; PrP^{Sc} inhibitior
Homoharringtonine	✓		Toxic	Protein translation inhibitor
flubendazole	✓		+++	Inhibitor of microtubule polymerization; autophagy inducer (targeting cysteine protease Atg4B)

CaMKII

GSK3β

PKC

PI3K

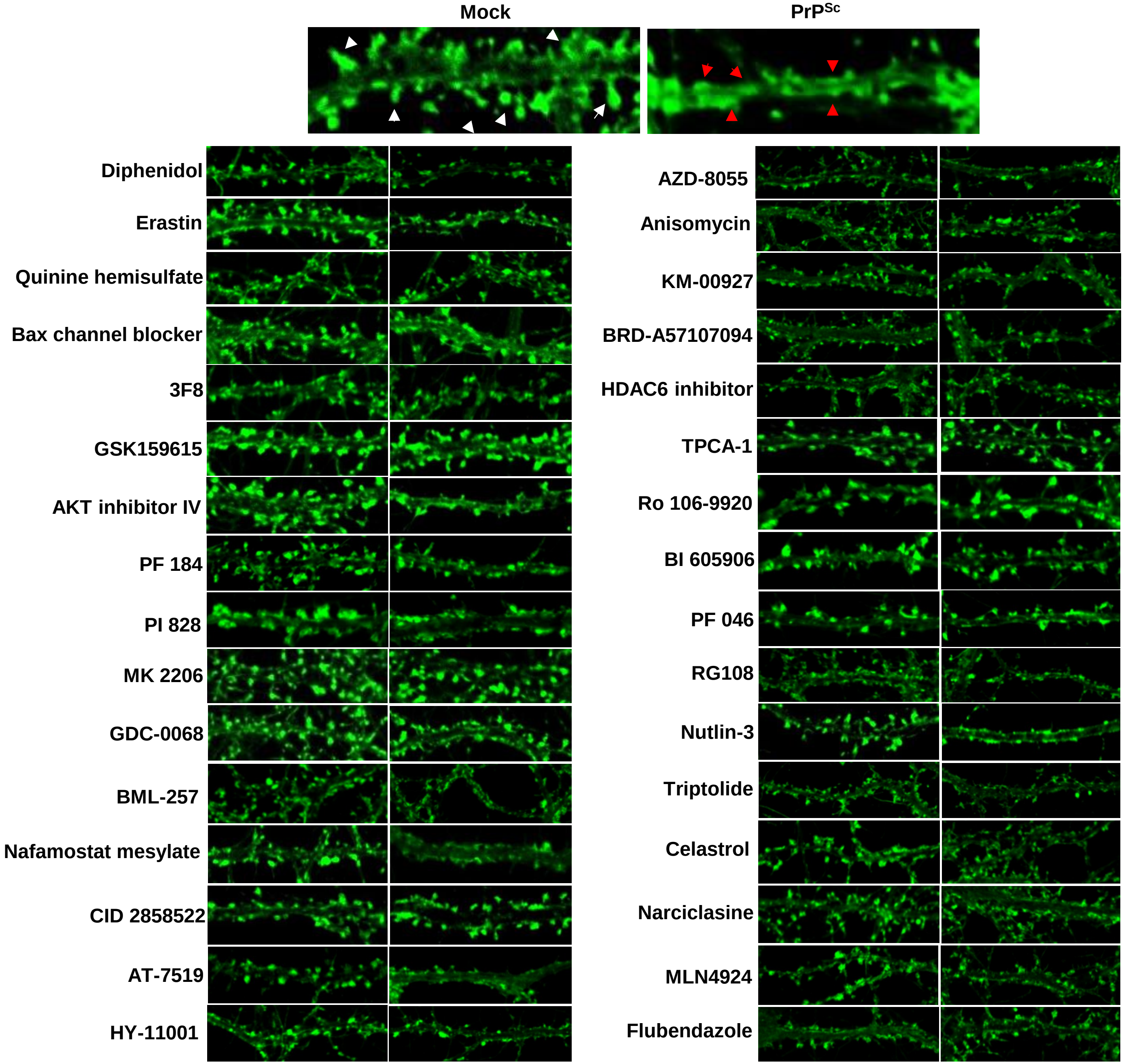
Receptors, Channels

CDKs

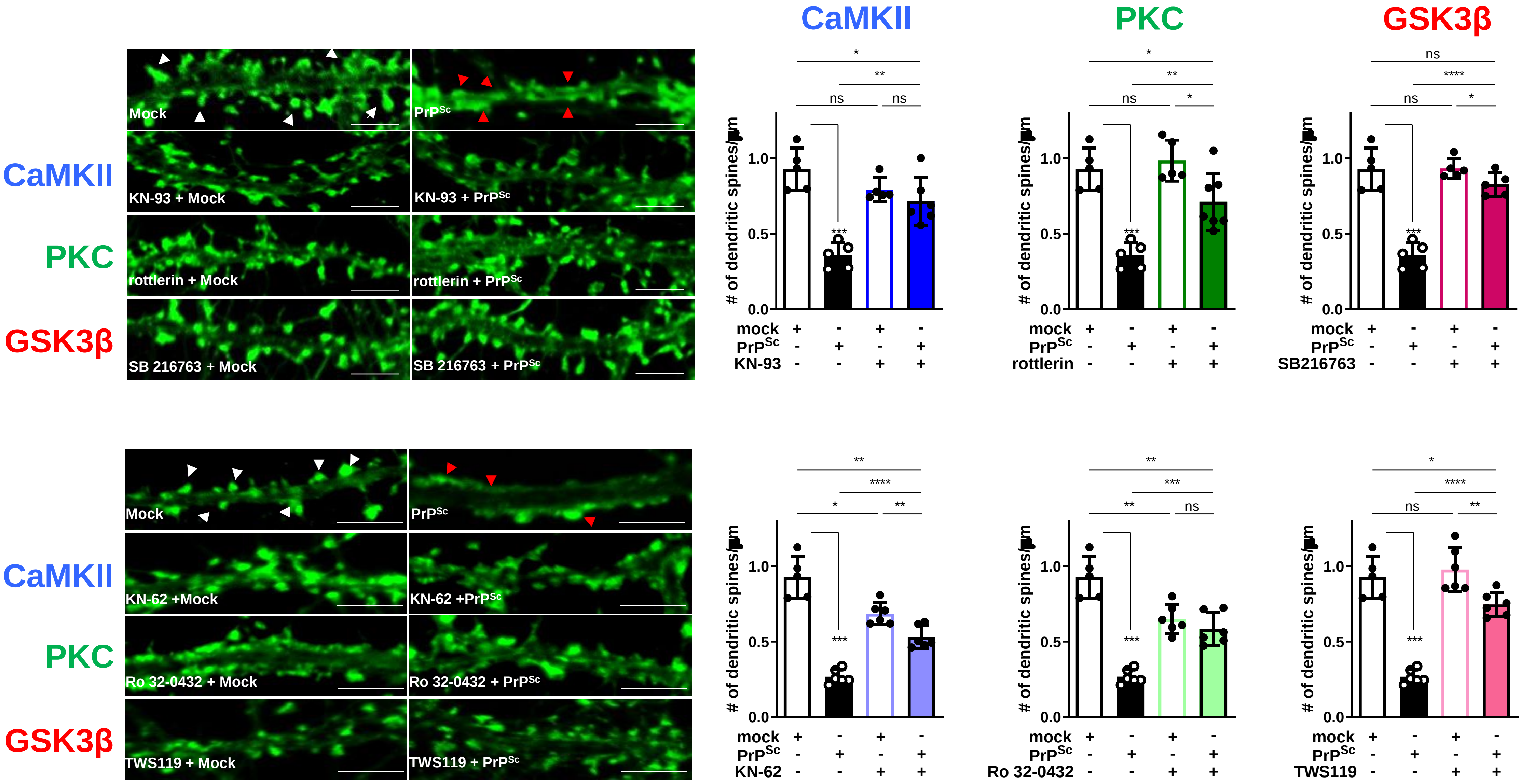
HDACs

NFκB

Misc.

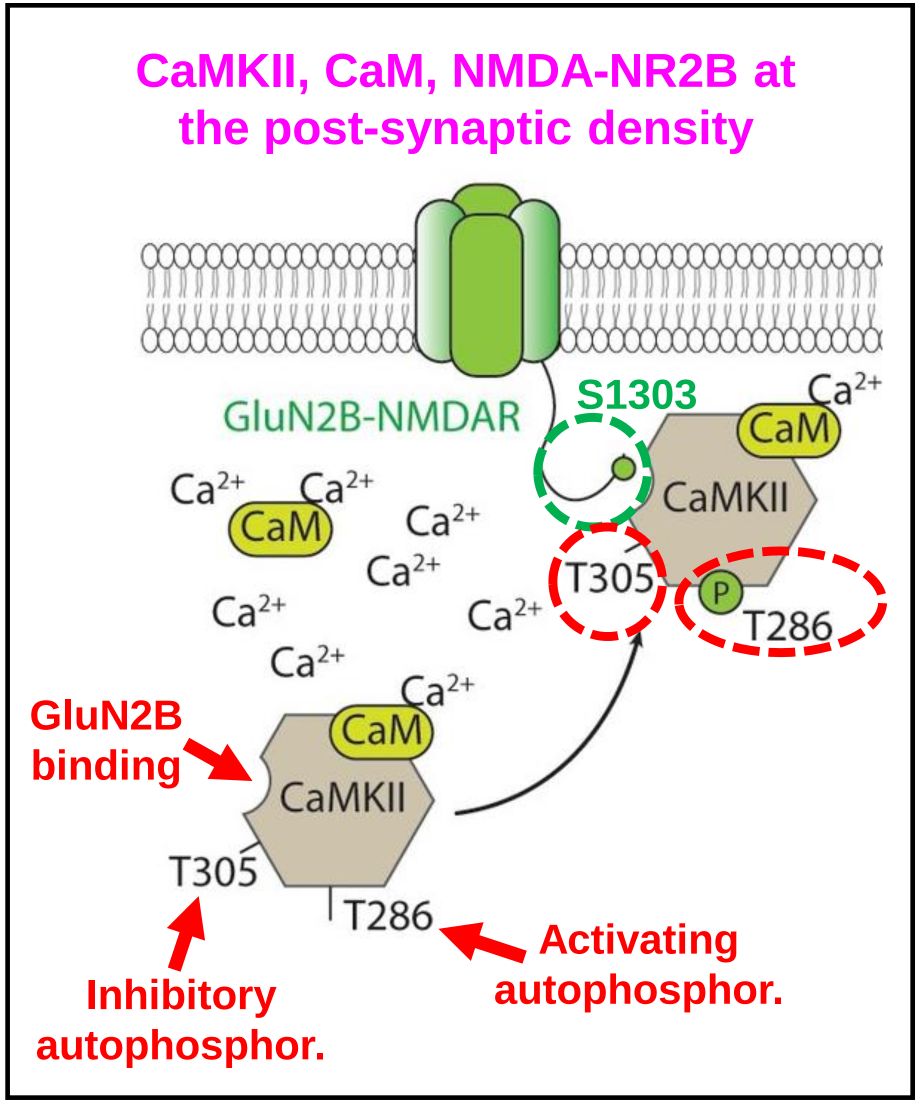


Inhibitors of CaMKII, PKC, and GSK3β prevent spine retraction

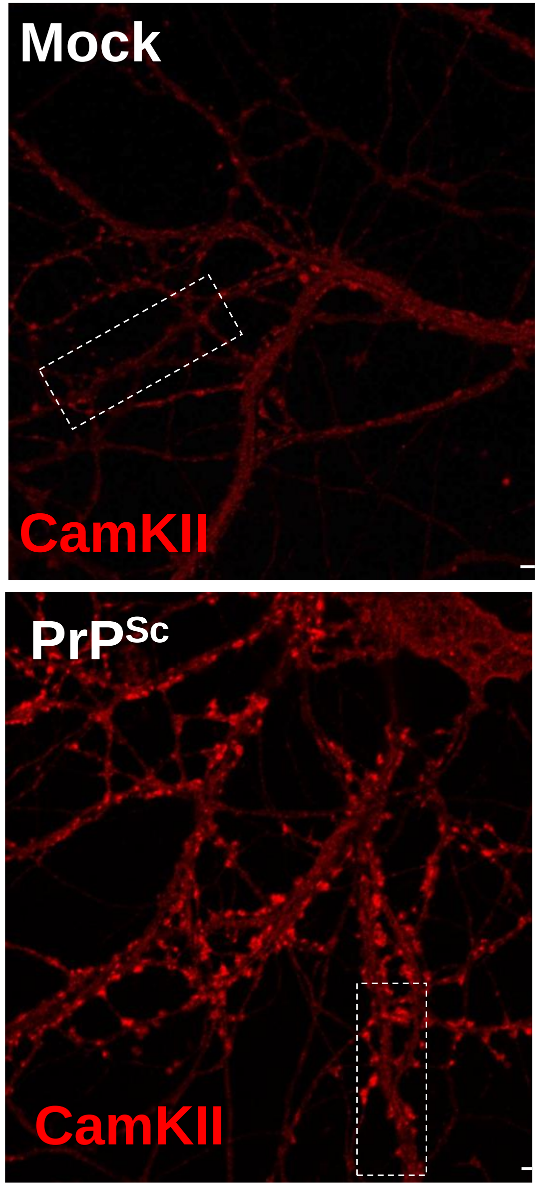


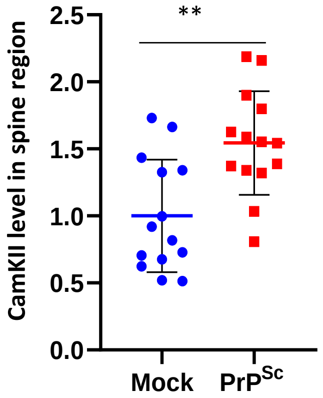
PrP^{Sc} causes dramatic translocation of CaMKII to spines

LTP/LTD/Memory

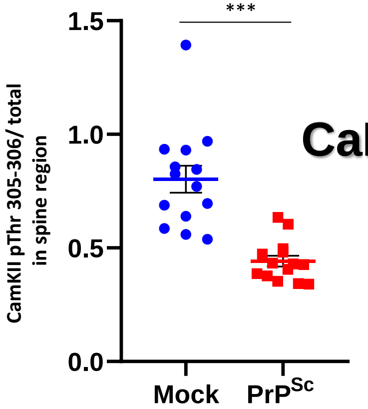


Ge et al., (2023)

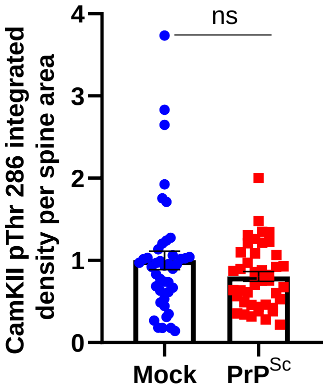




Total CaMKII in spines

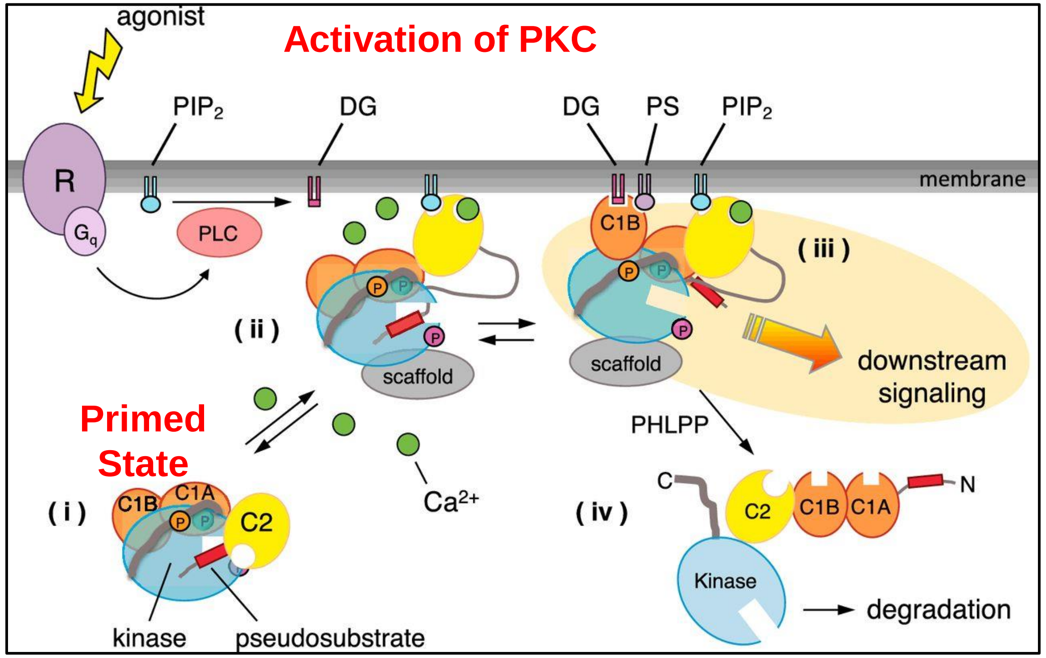


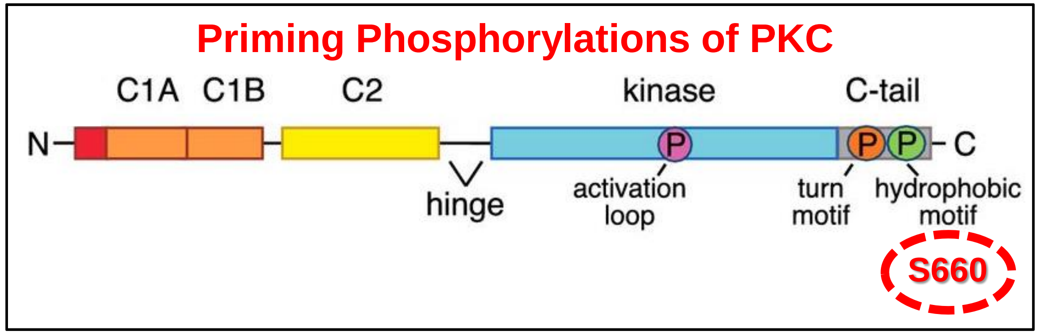
CaMKII-pT305/306 in spines



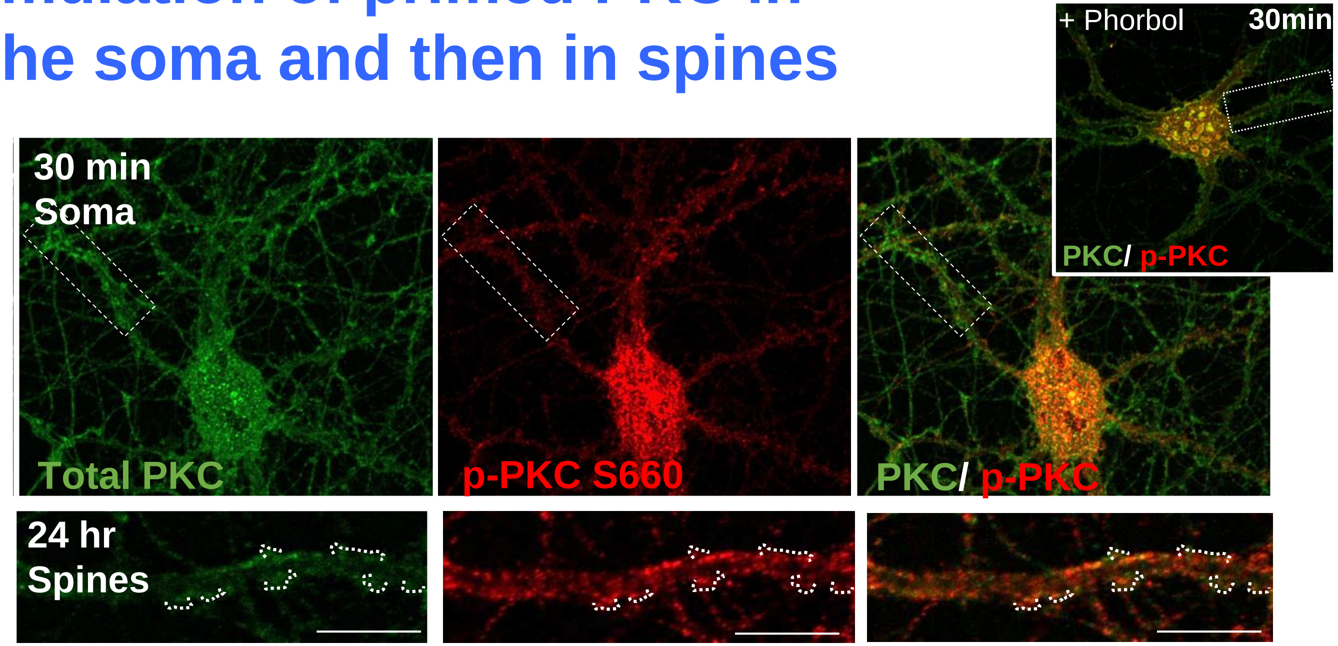
CaMKII-pT286 in spines

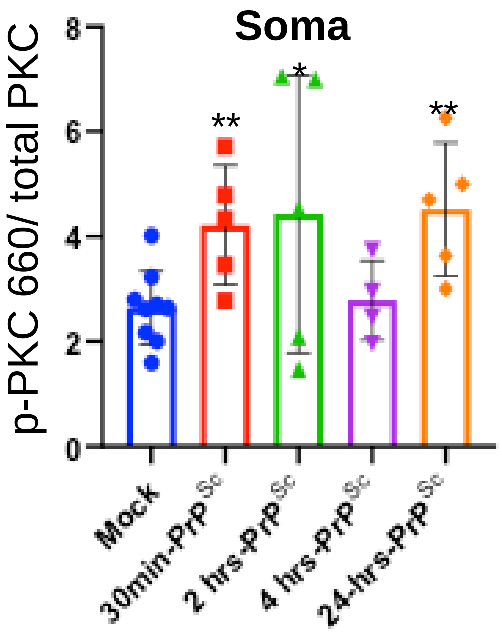
PrP^{Sc} causes accumulation of primed PKC in endosomes within the soma and then in spines

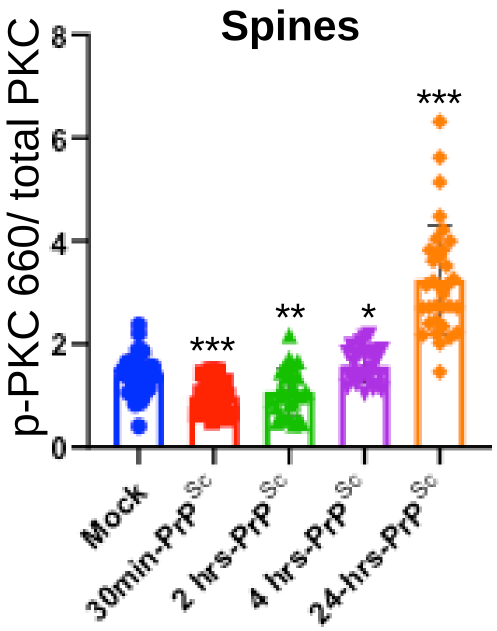




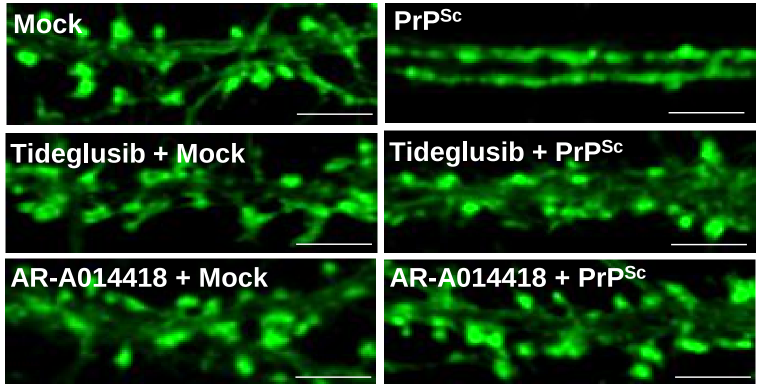
Calender & Newton (2017)



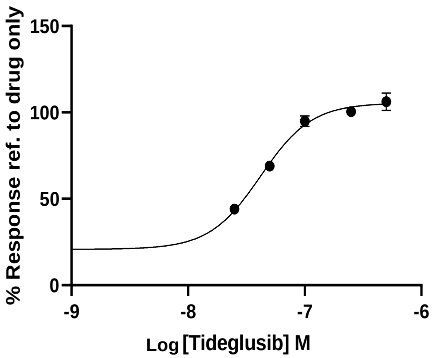


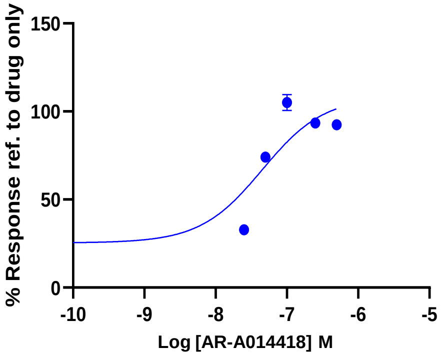


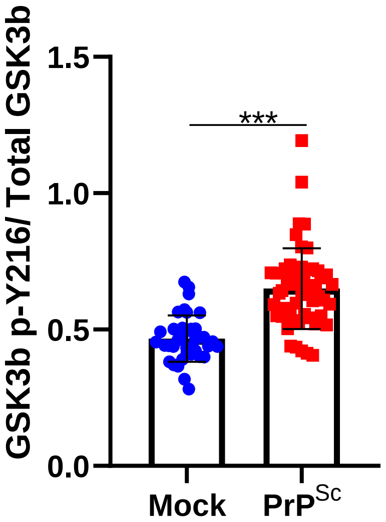
GSK3β inhibitors prevent spine retraction

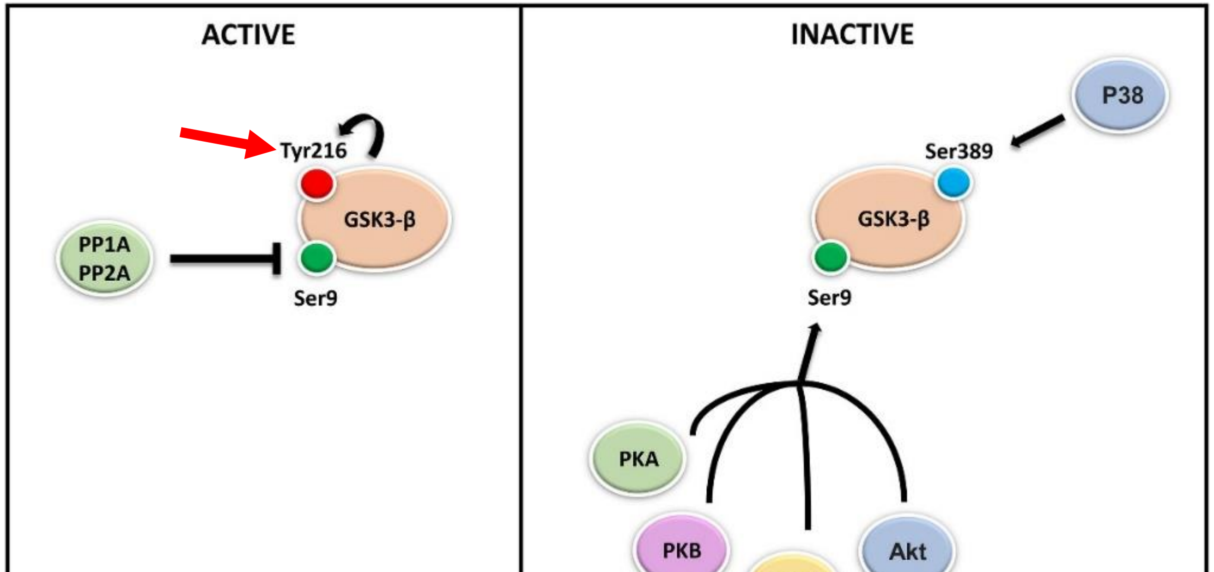


24 hrs treatment



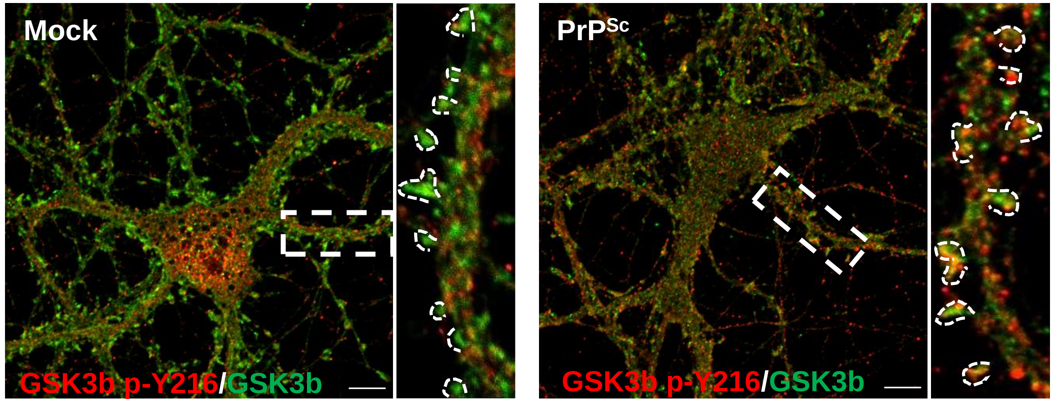






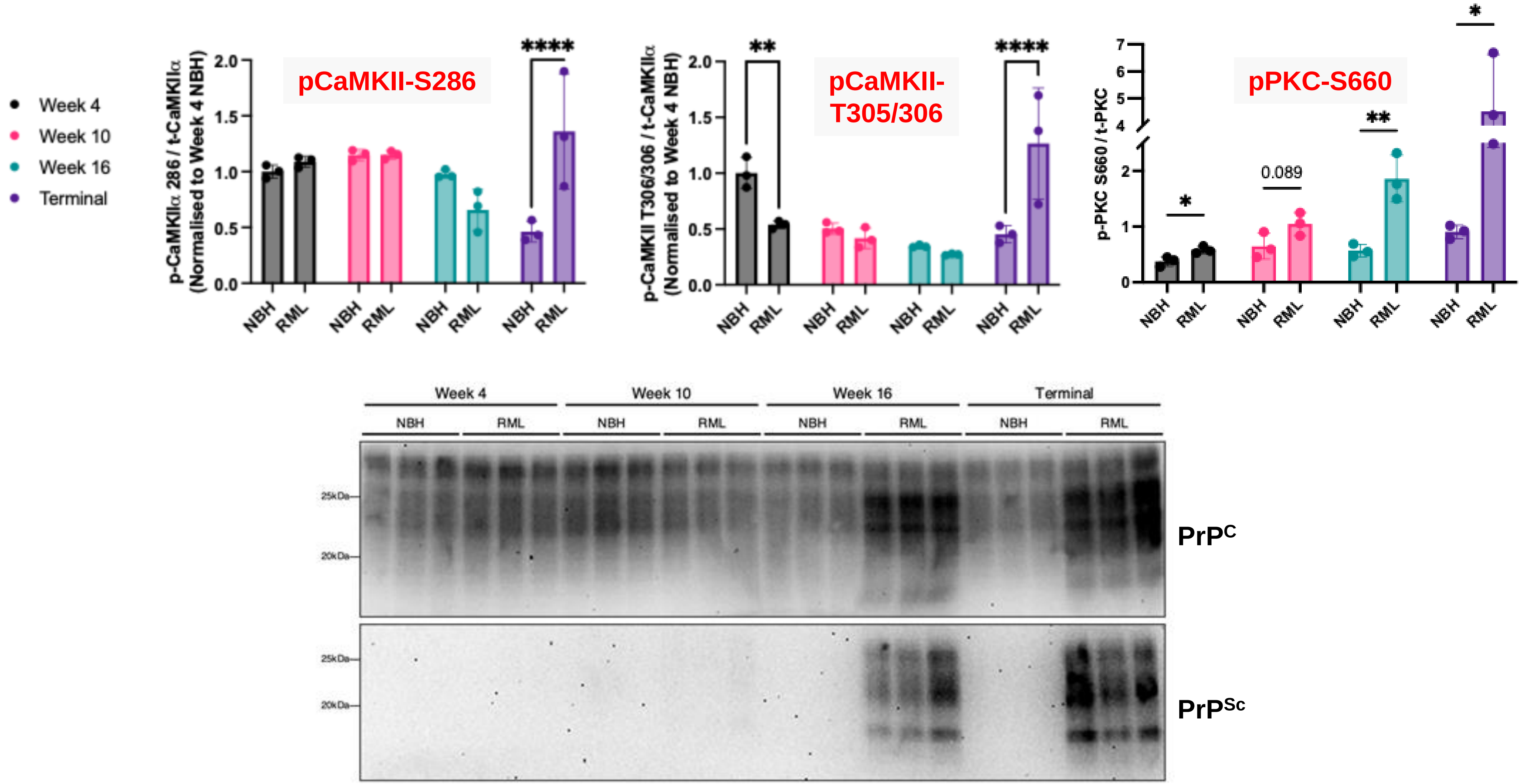
Lauretti et al. *BBA* (2020)

PrP^Sc increases active GSK3β p-Y216 in spines

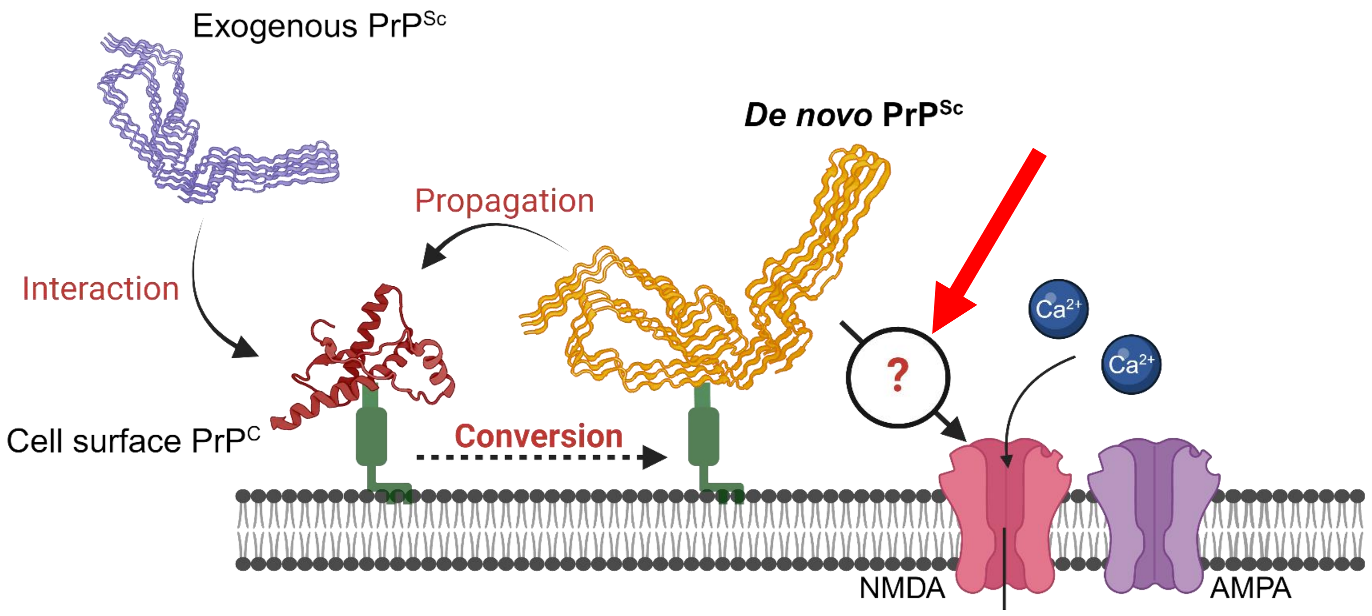


1 hr treatment

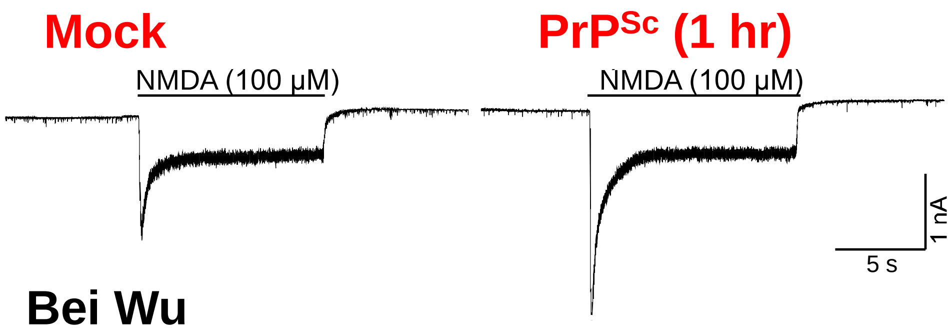
Changes in phosphorylation of CamKII and PKC in prion-infected brain, coincident with appearance of PrP^{Sc}



How does PrP^{Sc} activate NMDARs?



PrP^{Sc} rapidly increases sensitivity of cultured neurons to NMDA



Bei Wu

Is the interaction between PrP^{Sc} and NMDARs direct or indirect?

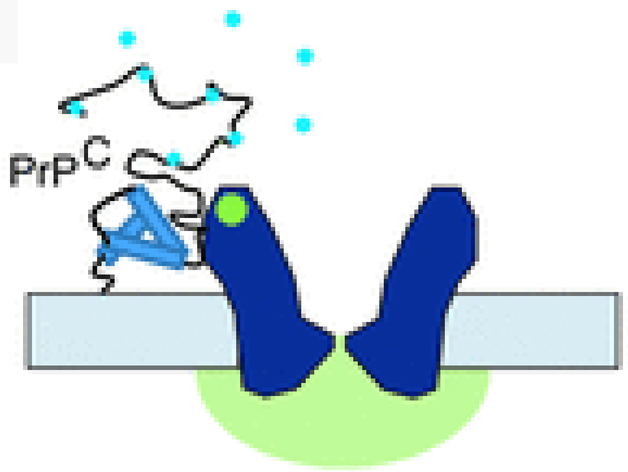
- **Direct: Physical interaction of PrP^{Sc} and NMDARs**
- **Indirect: PrP^{Sc} perturbs the lipid bilayer**

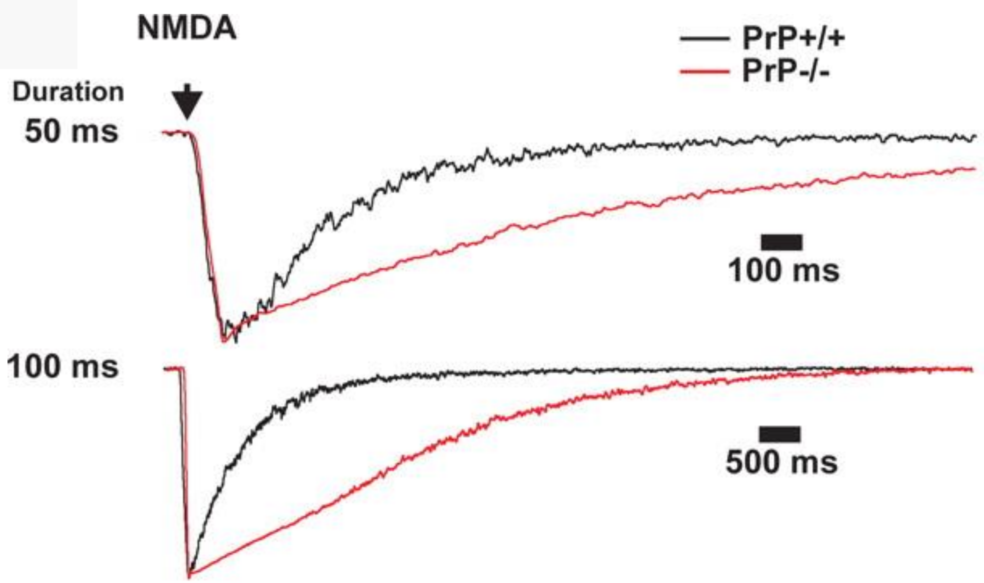
PrP^{C} binds to NMDARs and dampens their activity -- PrP^{Sc} may do the opposite

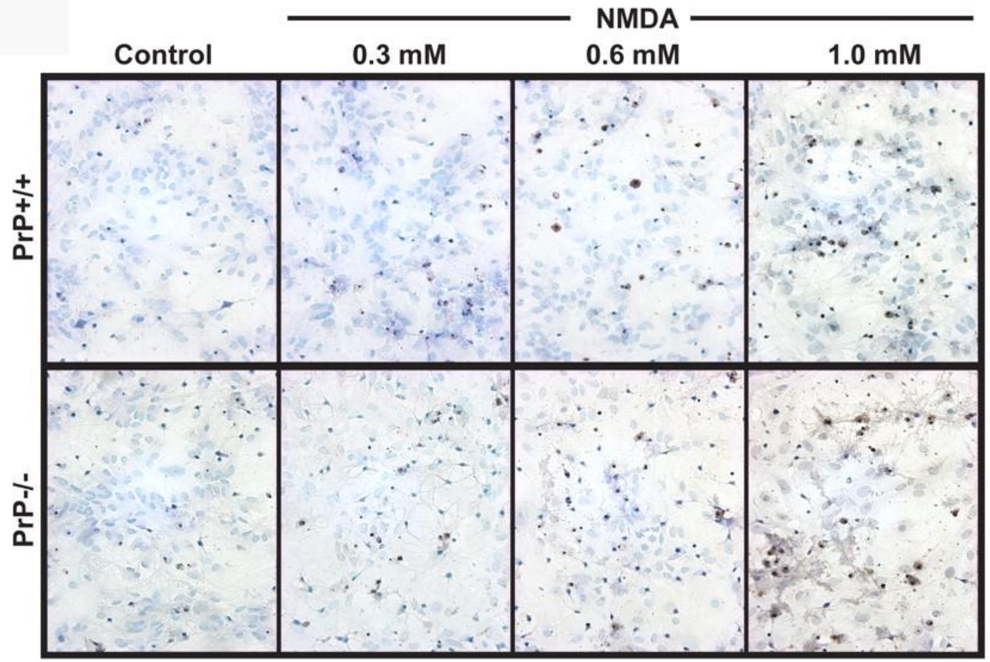
Prion protein attenuates excitotoxicity by inhibiting NMDA receptors

Houman Khosravani,[1] Yunfeng Zhang,[1] Shigeki Tsutsui,[2] Shahid Hameed,[1] Christophe Altier,[1] Jawed Hamid,[1] Lina Chen,[1] Michelle Villemaire,[2] Zenobia Ali,[2] Frank R. Jirik,[2] and Gerald W. Zamponi[1]

J. *Cell Biol.* (2008)



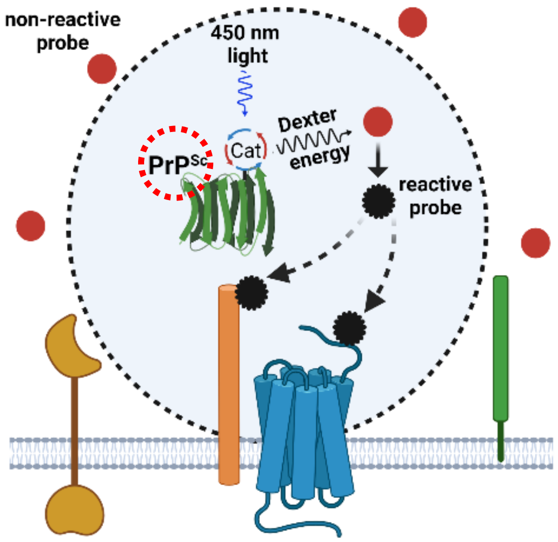




Identify PrP^{Sc} interactors

Micro-mapping:

Photo-activated proximity labeling technique

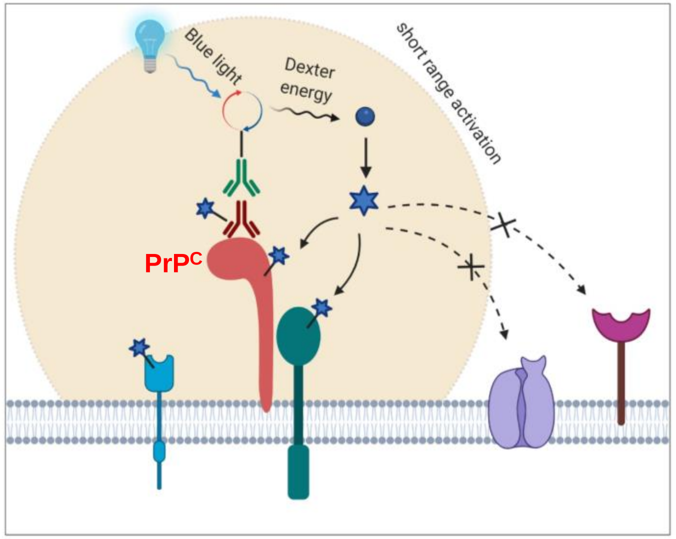


Microenvironment mapping via Dexter energy transfer on immune cells

Jacob B. Geri[1]*, James V. Oakley[1]*, Tamara Reyes-Robles[2]*, Tao Wang[1]*, Stefan J. McCarver[1], Cory H. White[2], Frances P. Rodriguez-Rivera[3], Dann L. Parker Jr.[3], Erik C. Hett[2], Olugbeminiyi O. Fadeyi[2]†, Rob C. Oslund[2]†, David W. C. MacMillan[1]†

Science 367, 1091–1097 (2020)

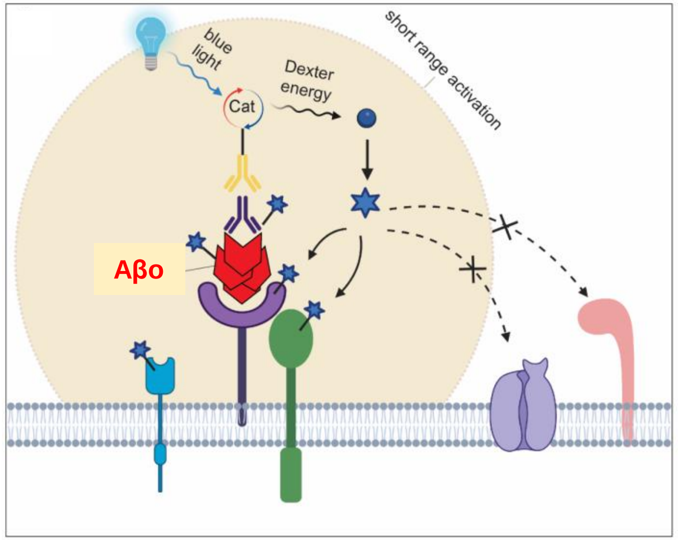
Identify PrP^{C} interactors



Gene	Protein
Prnp*	**PrP**
Gpc1*	**Glypican 1**
Pcdh1	Protocadherin 1
Cntfr	Ciliary neurotrophic factor receptor
Cadm1	Cell adhesion molecule 1
Marcksl1	MARCKS-like protein 1
Pcdhgb1	Protocadherin Gamma Subfamily B, 1
Slc39a10*	**Solute Carrier Family 39 Member 10**
Igf1r	Insulinlike growth factor1 receptor
L1cam	L1 Cell Adhesion Molecule
Ncam1*	**Neural Cell Adhesion Molecule 1**
Nes	Nestin
Gprin1	G protein regulated inducer of neurite outgrowth 1
Dbn1	Drebrin 1
Epn1	Epsin 2
Ldlr	low-density lipoprotein receptor
Dgl3	Discs Large MAGUK Scaffold Protein 3
Lrch2	Leu Rich Repeats & Calponin Homo Dom Cont 2

* previously reported PrP interacting protein

Identify Aβ interactors



Gene Symbol	logFC	adj.P.Val
Icam5	**2.025802**	**2.63E-06**
Negr1	1.866689	0.000752
Cntn1	1.818421	1.03E-06
Prnp	**1.69149**	**4.95E-05**
Thy1	1.509525	0.001211
Dcc	1.461998	2.63E-06
Nrcam	1.454929	1.97E-05
Adamtsl4	1.425197	0.019307
Alcam	1.415064	0.000181
Cdh13	1.413514	7.32E-05
Opcml	1.384585	0.000317
Atp1b1	1.381895	0.021432
Olfm1	1.361352	1.29E-05
Nfasc	1.294088	4.29E-05
Map6	1.220426	1.12E-05
Ntm	1.214847	0.003033
Slc39a10	1.092461	3.87E-05
Sorbs2	1.049221	0.00028
Gpr158	1.003294	0.000221

Ladan Amin

The "Shmerling effect": spontaneous toxicity of internally deleted PrP

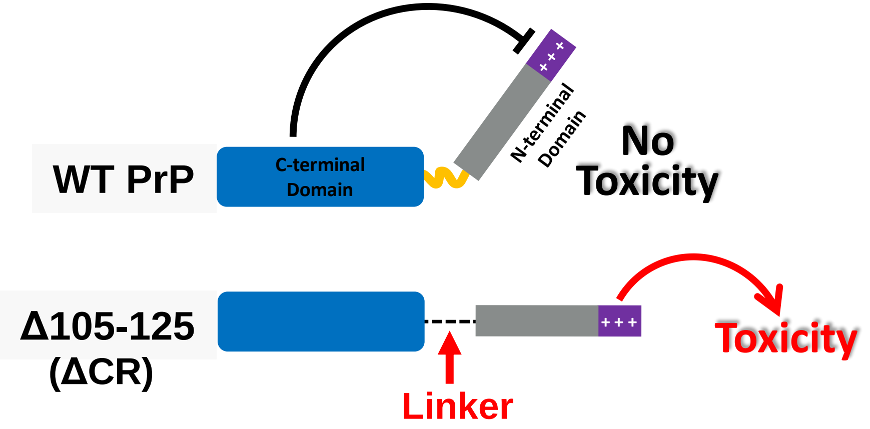
Cell (1998)

Expression of Amino-Terminally Truncated PrP in the Mouse Leading to Ataxia and Specific Cerebellar Lesions

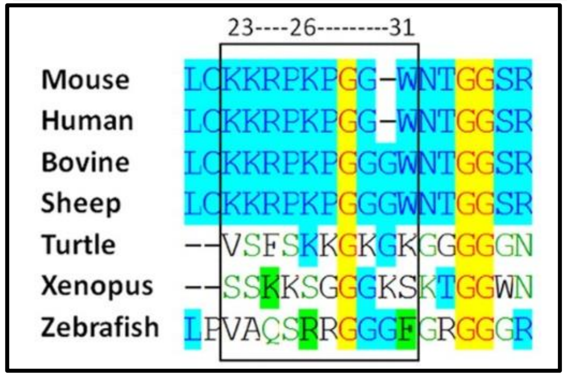
Doron Shmerling,‖ Ivan Hegyi,† Marek Fischer,# Thomas Blättler,† Sebastian Brandner,† Jürgen Götz,* Thomas Rülicke,‡ Eckhard Flechsig,* Antonio Cozzio,* Christian von Mering,* Christoph Hangartner,* Adriano Aguzzi,† and Charles Weissmann*§

Internal deletions of PrP (Δ32-121 or 32-134) cause spontaneous neurodegeneration in Tg mice

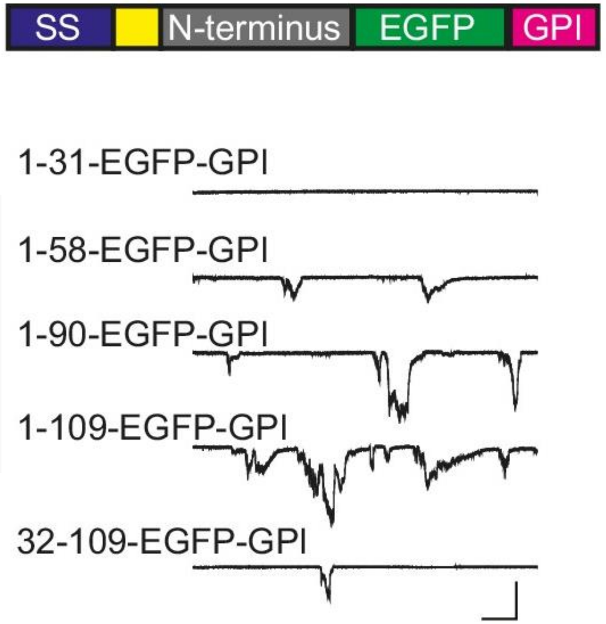
Deletions of the central linker region (e.g. 105-125) free the N-terminal domain to induce toxicity

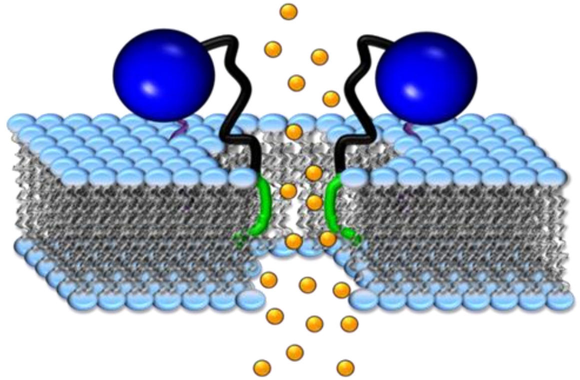


The conserved, polybasic N-terminus of PrP



The N-terminal domain (23-31) is necessary & sufficient to form pores



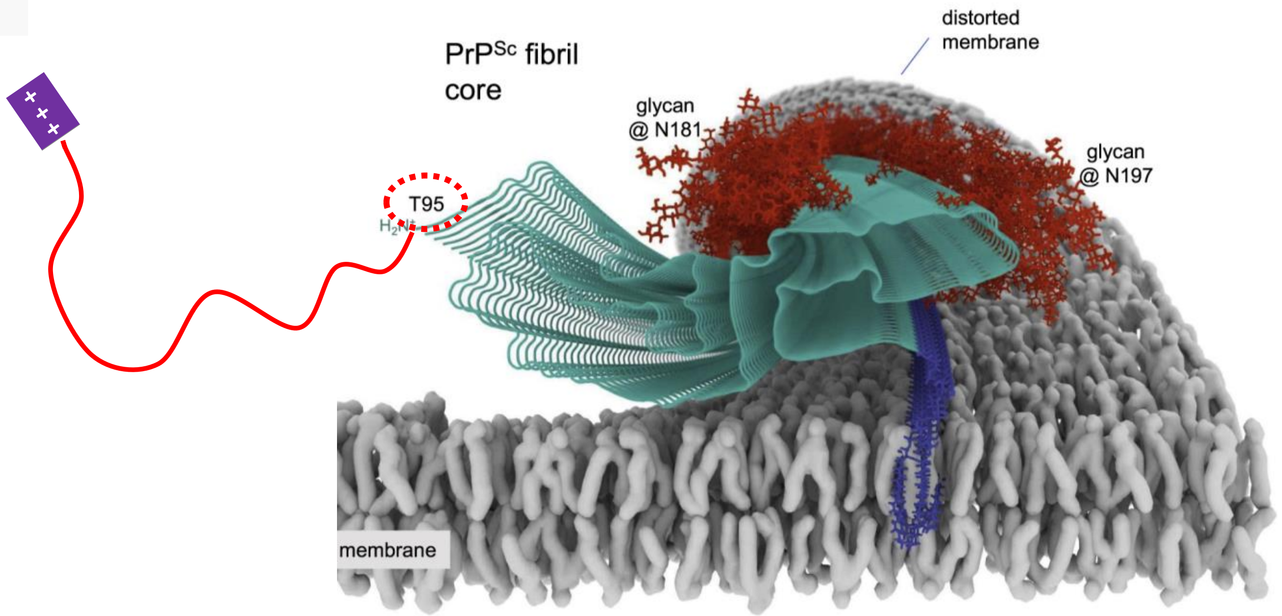


Residues 23-31 interact w/ the lipid bilayer to form pores

Li *et al*. *EMBO J*. (2007)
Solomon *et al*. *JBC* (2012)
Wu *et al*. *eLife* (2017)
McDonald *et al*. *Structure* (2019)

The N-terminus of PrP^{Sc} may destabilize the lipid bilayer, altering the activity of NMDARs and other membrane proteins

The N-terminal domain of PrP^{Sc} is untethered (not part of the amyloid core)

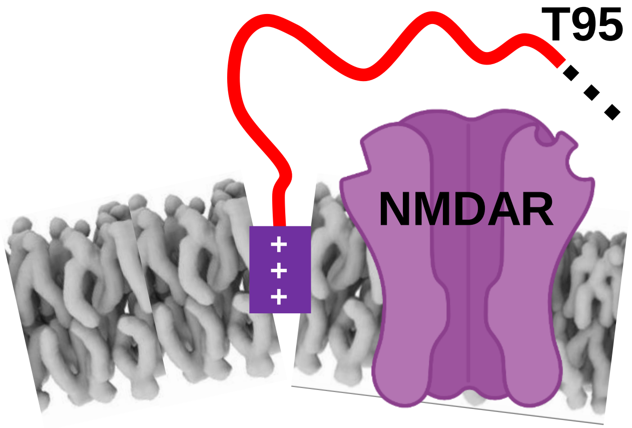


High-resolution structure and strain comparison of infectious mammalian prions

Allison Kraus,[1,*] Forrest Hoyt,[2] Cindi L. Schwartz,[2] Bryan Hansen,[2] Efrosini Artikis,[3] Andrew G. Hughson,[3] Gregory J. Raymond,[3] Brent Race,[3] Gerald S. Baron,[3] and Byron Caughey[3,4,*]

Molecular Cell (2021)

Perturbation of the lipid bilayer by the untethered N-terminal domain

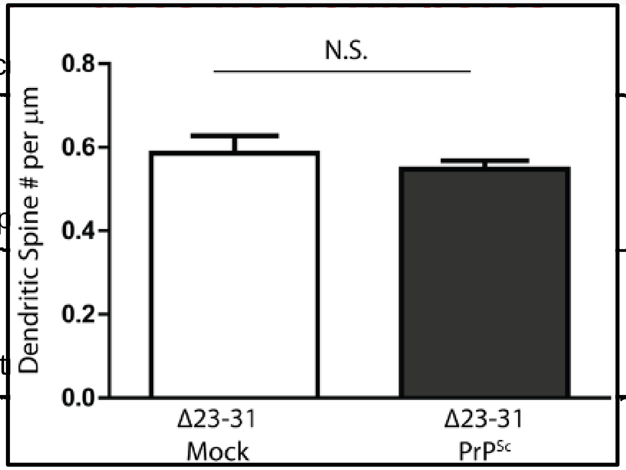


Residues 23-31 are essential for PrP^{Sc} synaptotoxicity

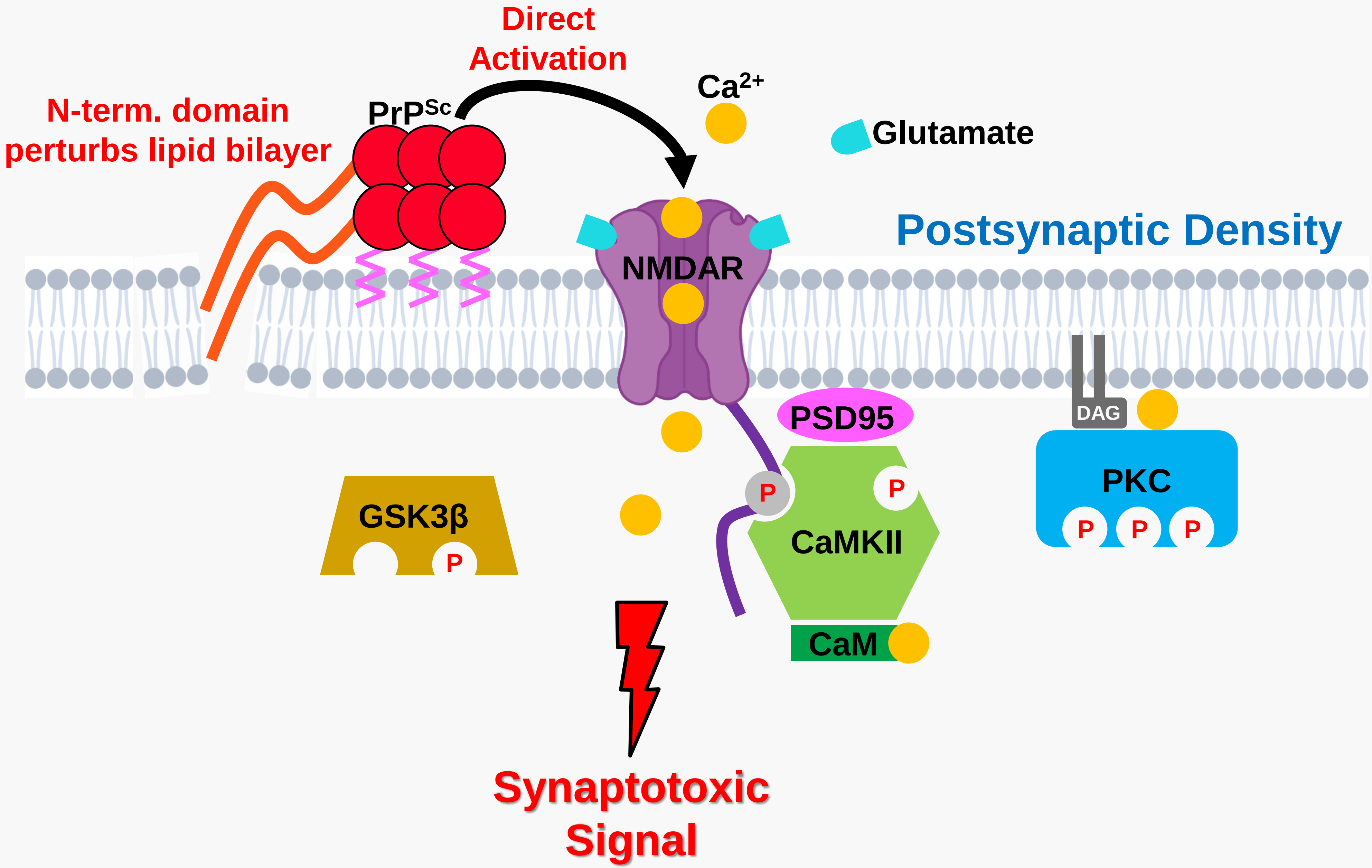
Infected N2a.3 c

N2a cell overexp

Tga20 neurons t



PrP^{Sc} synaptotoxic signaling



Harris Laboratory

Nhat Le (now at Ohio State)
Jean Gatdula (Grad. Student)
Robert Mercer (Res. Asst. Prof.)
Jose Alepuz Guillen (Postdoc)
Nadia Mirza (Grad. Student)
Isabel Orbe (Grad. Student)
Samantha Tolton (Grad. Student)
Janelle Vultaggio (Lab Mgr.)
Beulah Ackah (BU Undergrad.)
Haixu Wang (BU Undergrad.)
Timothy Liu (BU Undergrad.)
Aravind Sundaravadivelu (Bioinformatician)
Ladan Amin (now at Novartis)

CIC BioGUNE, Spain

Joaquín Castilla
Hasier Eraña
Glenn Millhauser (UCSC)
Glenn Telling (CSU)
Jason Bartz (Creighton)

Joel Watts (Toronto)
Gerold Schmitt-Ulms (Toronto)
Gustavo Mostoslavsky (Boston U.)

Merck (Cambridge)

Olugbeminiyi Fadeyi
Rob Oslund
Tamara Reyes-Robles