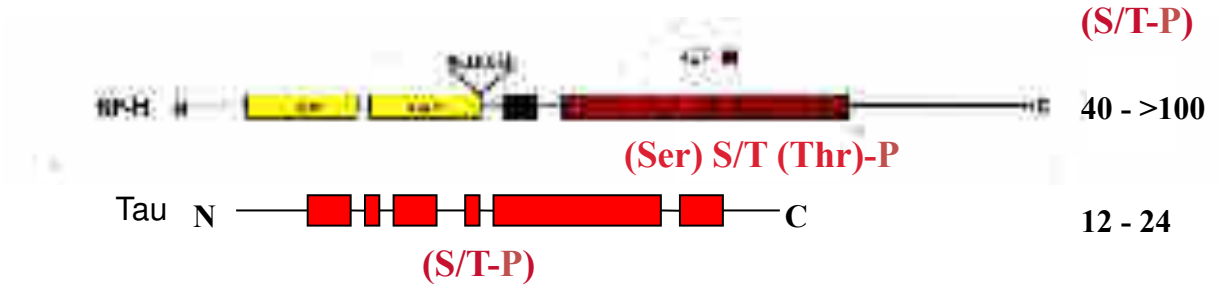
A novel translational approach for neurodegenerative Diseases (e.g. AD, ALS,PD)

Harish c. Pant

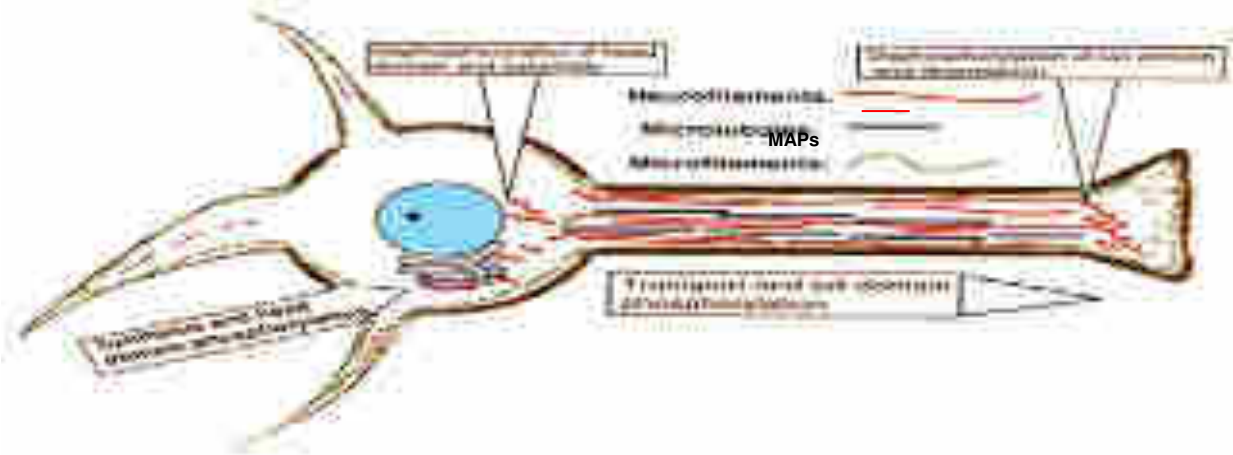
NINDS/NIH, Bethesda, MD

International Conference on Brain Disorders & Therapeutics, August 24-26, 2015, London UK

In mature neurons cytoskeletal protein phosphorylation is topographically & tightly regulated under physiological conditions.



Topographic regulation of cytoskeletal protein phosphorylation



Normal



Human cervical spinal neuron

ALS



Cdk5, Cyclin dependent kinase 5, A neuron specific Cdk;

While studying the compartment specific phosphorylation of neuronal cytoskelelal proteins on proline-directed serine (Ser)/threonine (Thr) residues, we discovered cyclin- dependent kinase 5, (Cdk5), is the major kinase involved in the regulation of phosphorylation in the nervous system. Cdk5 is a multifunctional kinase ,essential for neuronal survival and plays a key role in the nervous system development, maturation of mammalian nervous system and neurodegeneration.

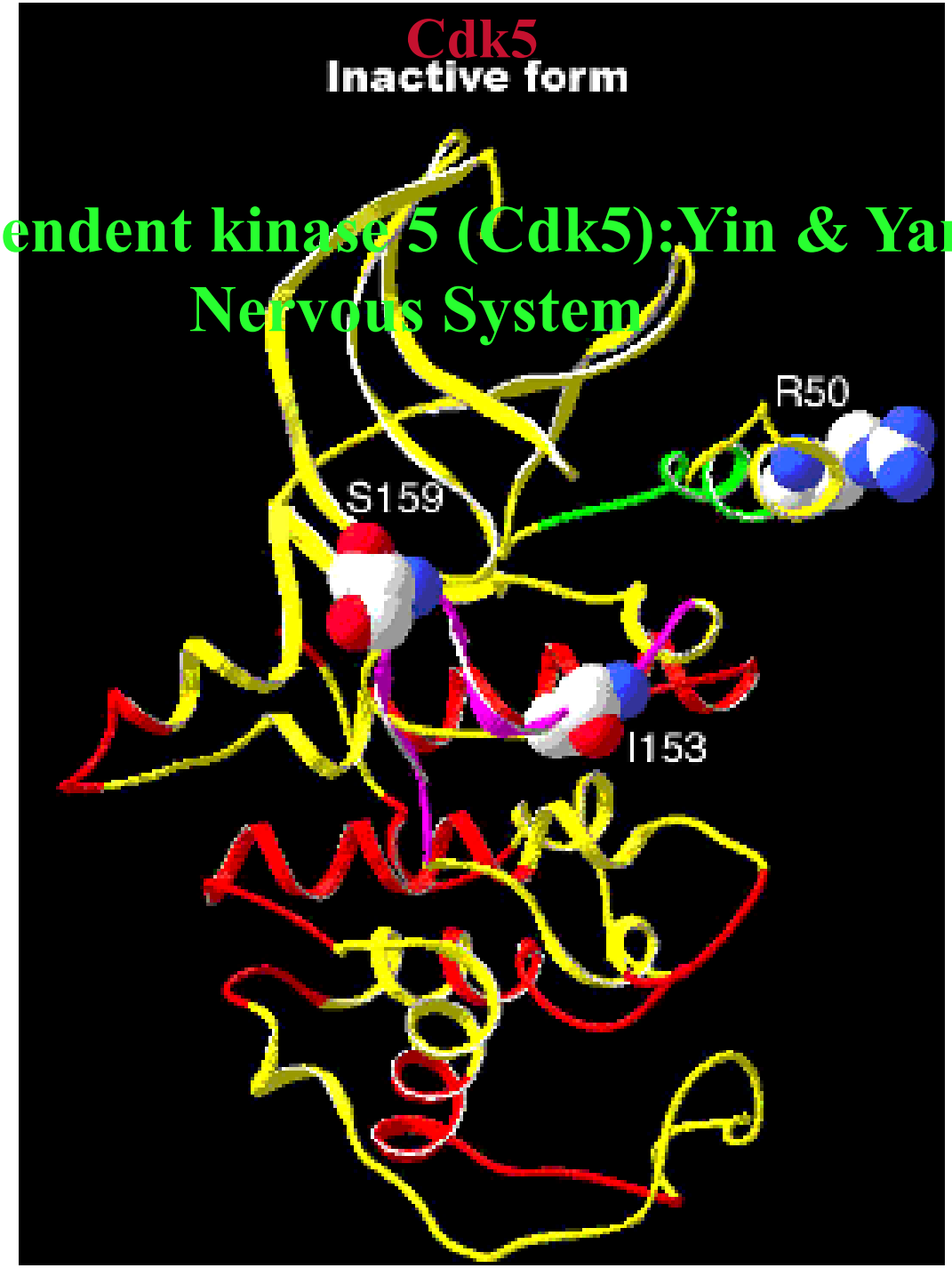
- Cdk5 is unique among the Cdk family of kinases; it is not activated by a cyclin, although it binds with cyclin D/E

- Activity is found primarily in neurons due to its association with neuron specific molecules p35 and p39.

- p35 is the major activator.

Cdk5 is the major kinase phosphorylating S/T-P residues in the nervous tissue

Cyclin dependent kinase 5 (Cdk5):Yin & Yang of the Nervous System



Cdk5; *in vivo* and *in vitro* studies;

Activity is Spatially and Topographically regulated

Cdk5 (-/-) mice,

* Embryonically lethal (E16.5 - P0), wide spread defects in neuronal migration in cortex, hippocampus and cerebellum.

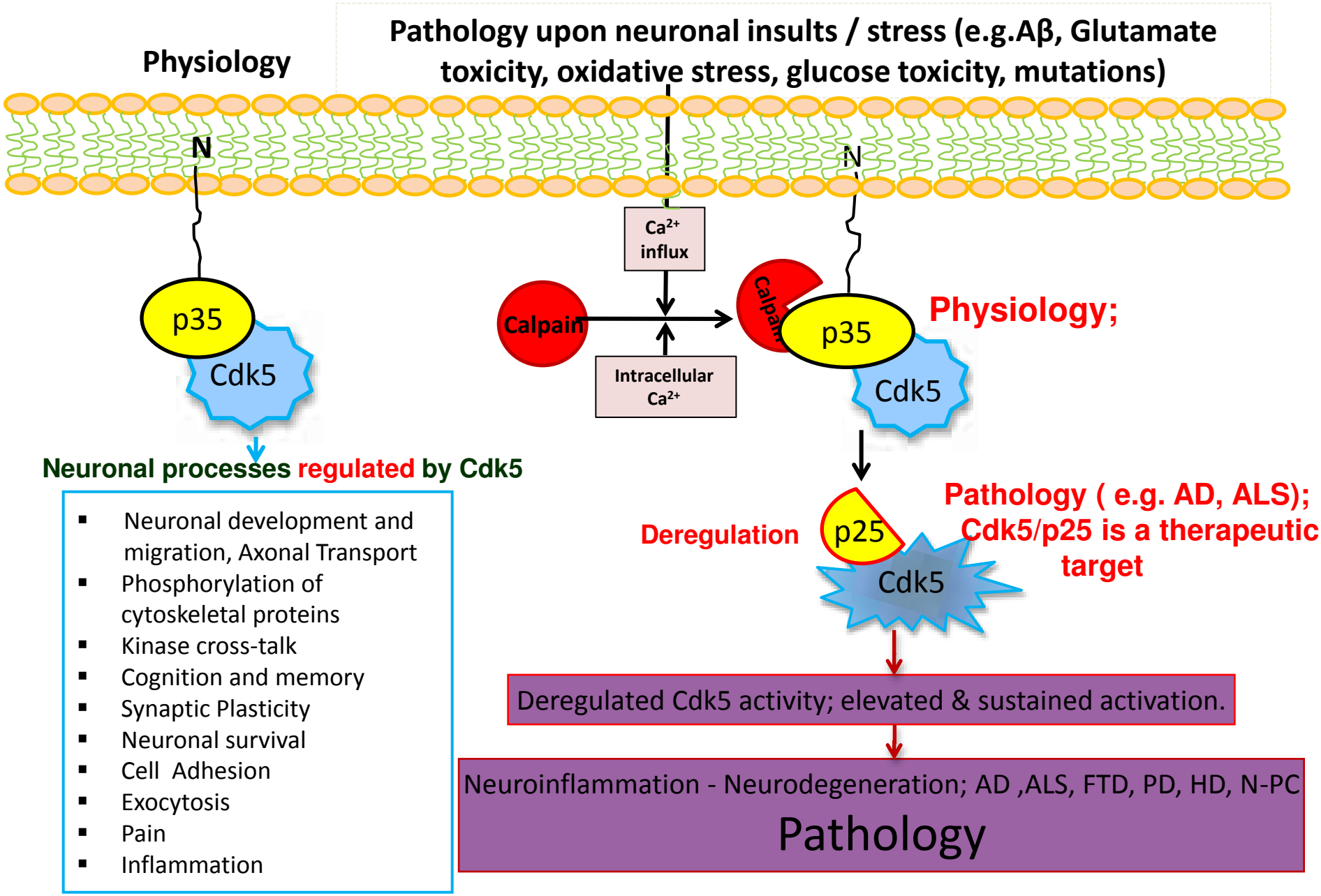
* Ballooned neuronal perikarya of spinal cord and brain stem motor neurons.

* Aberrant phosphorylation of cytoskeletal proteins and apoptosis.

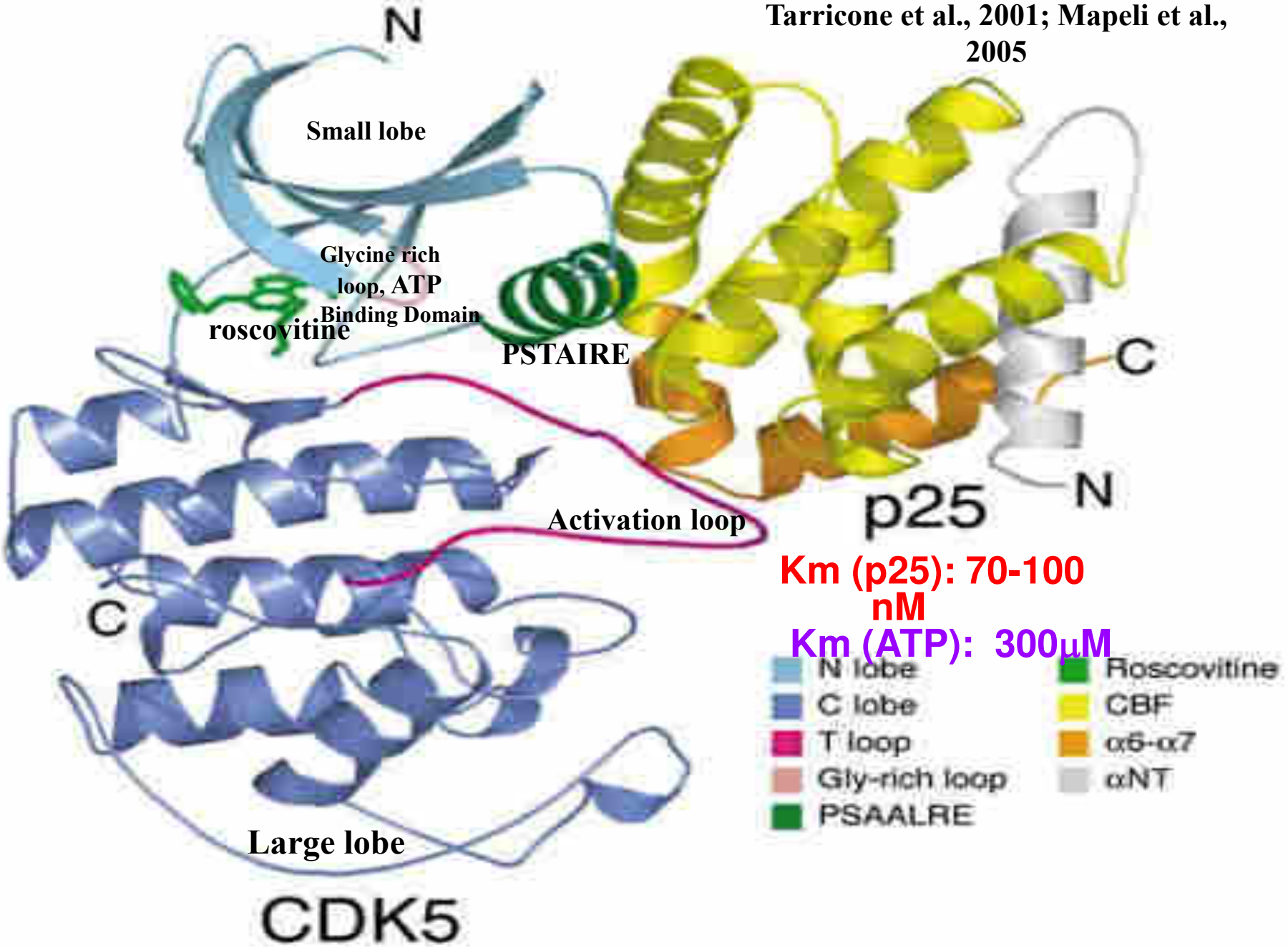
* Higher MAPks (Erk1/2), SAPKs (JNK3) and reduced lipid kinases (PI3K) activity (Cdk5 - cross talk).

These studies suggest that Cdk5 modulates various signal transduction pathways, tightly regulating neuronal function & survival.

Role of Cdk5 in neurophysiology and pathology



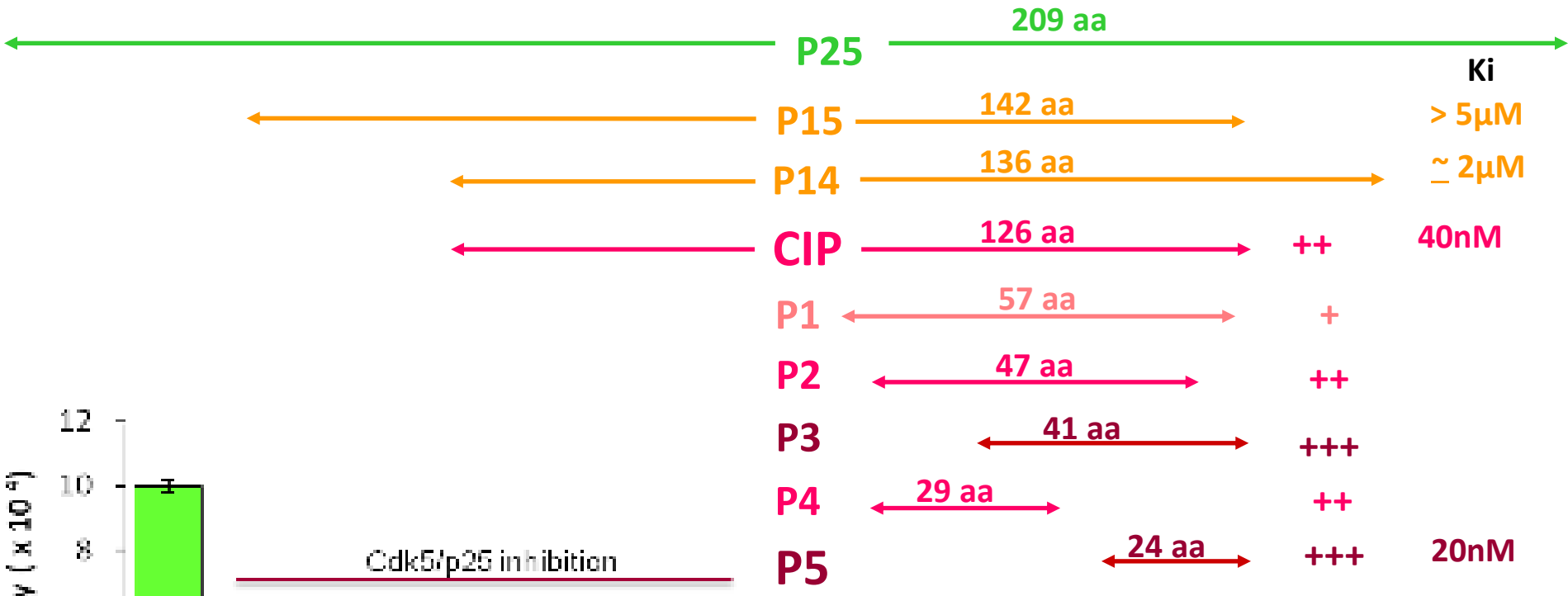
Crystal structure of the Cdk5/p25 complex with roscovitine



What is the effect of smaller peptides derived from P25 fragment Protein?

Can these smaller peptides derived from p25 deregulator, more devastating or rescue the deregulated Cdk5 activity inducing apoptosis?

Cdk5/p25 a therapeutic target; Roscovitine like compounds (ATP analogs) are non specific and toxic; We used truncated peptides derived from p25 within 3.5A interface (using Cdk5/p25 crystal structure) to study the role of these peptides on Cdk5 activity.
To our surprise some of them inhibited Cdk5 activity.



Zheng et al., JBC 2010, EMBO J 2005

Modified p5 – To pass BBB named as TFP5

p5 (24 aa):

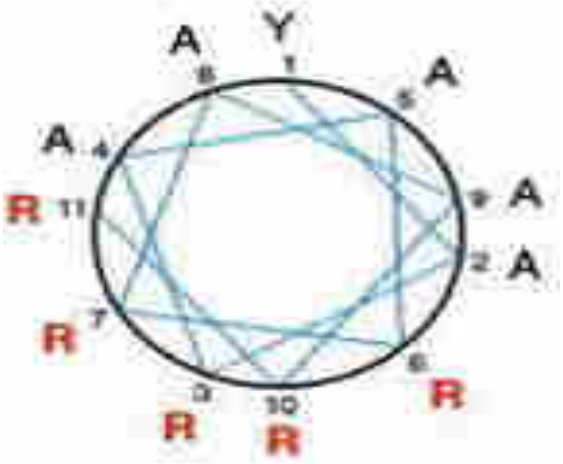
KEAFWDRCLSVINLMSSKMLQINA

TFP5:

FITC-GGGKEAFWDRCLSVINLMSSKMLQINA**YARAARRAARR**

p5

Modified TAT-peptide

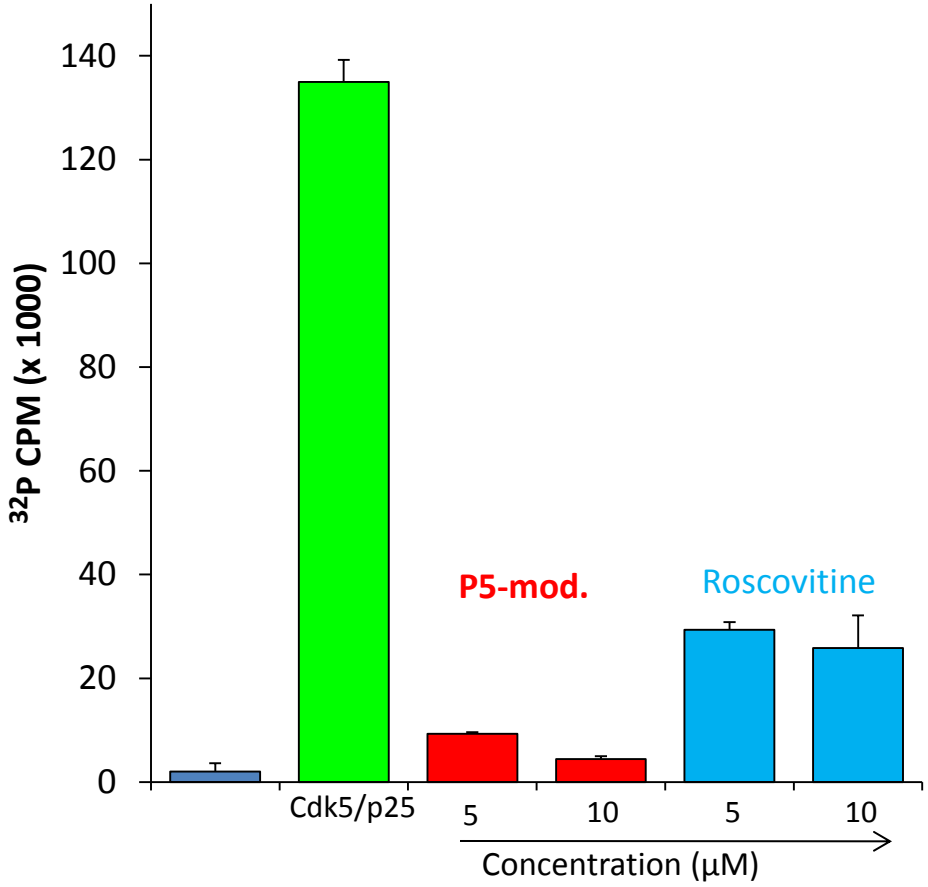


Scrambled peptide:

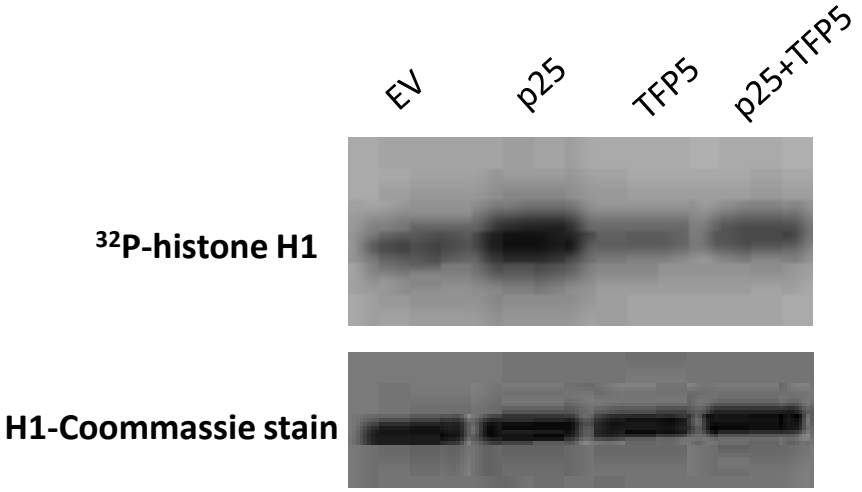
FITC-GGGGGGFWDRCLSGKGKMSSKGGGINA**YARAARRAARR**

TFP5 US Patent # 8597660.

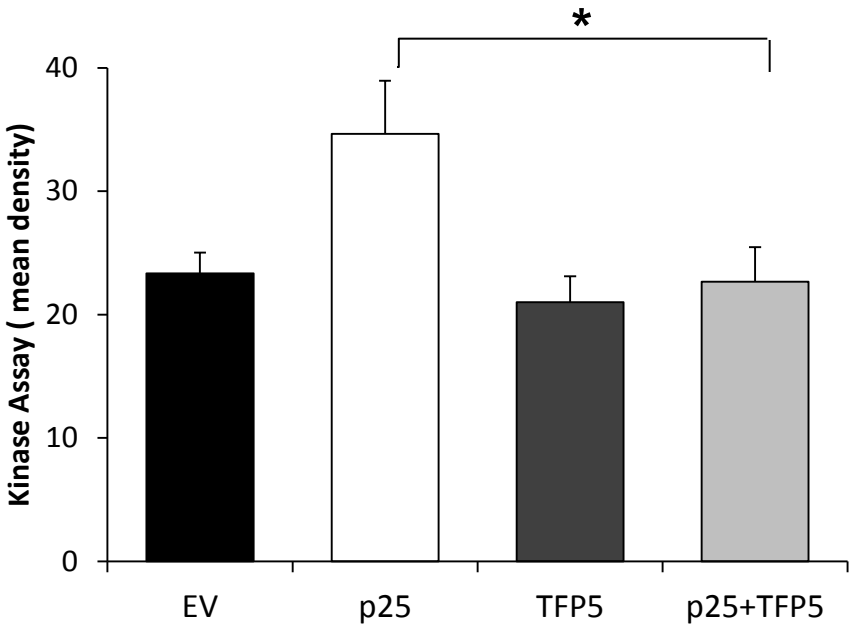
TFPP5-modified peptide is a better inhibitor of Cdk5/p25 activity compared to Roscovitine



A Kinase Assay using cortical neurons with Cdk5 IP

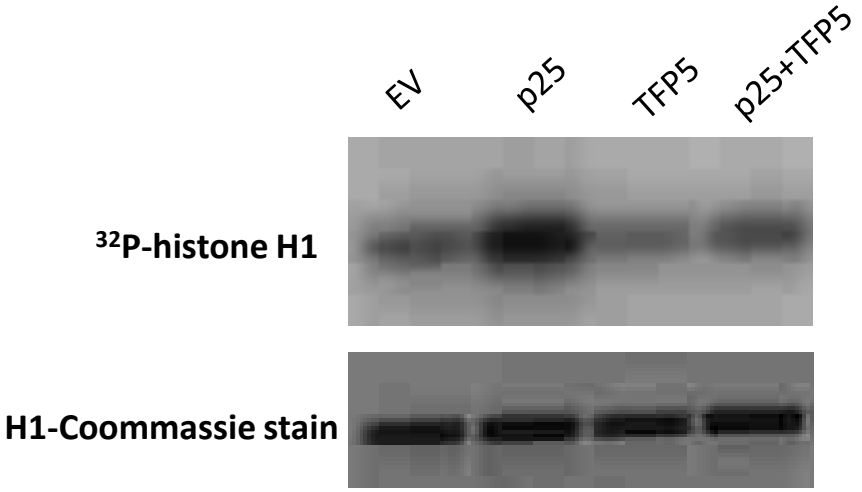


B

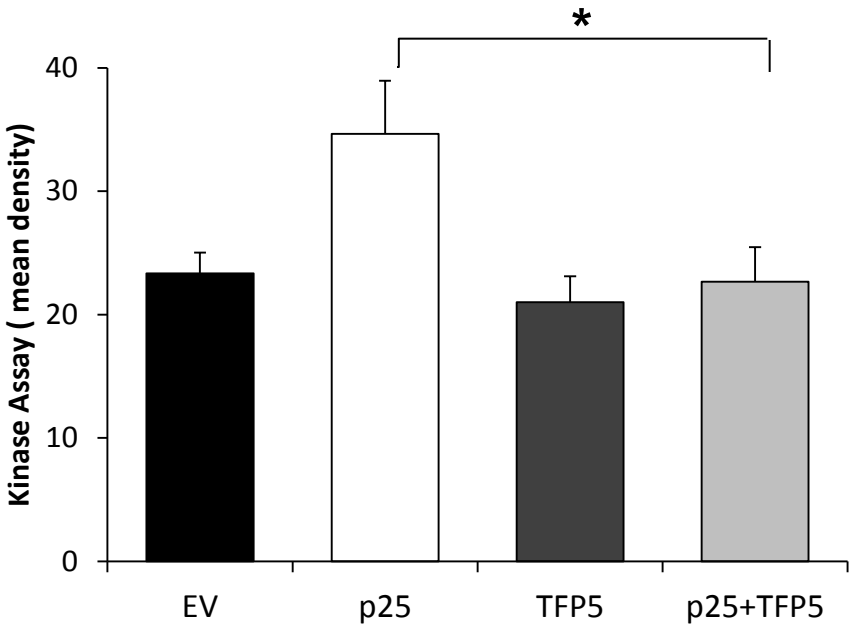


P5 modified peptide inhibits specifically Cdk5/p25 but not endogenous Cdk5/p35 activity *in situ* - neuronal cultures

A Kinase Assay using cortical neurons with Cdk5 IP

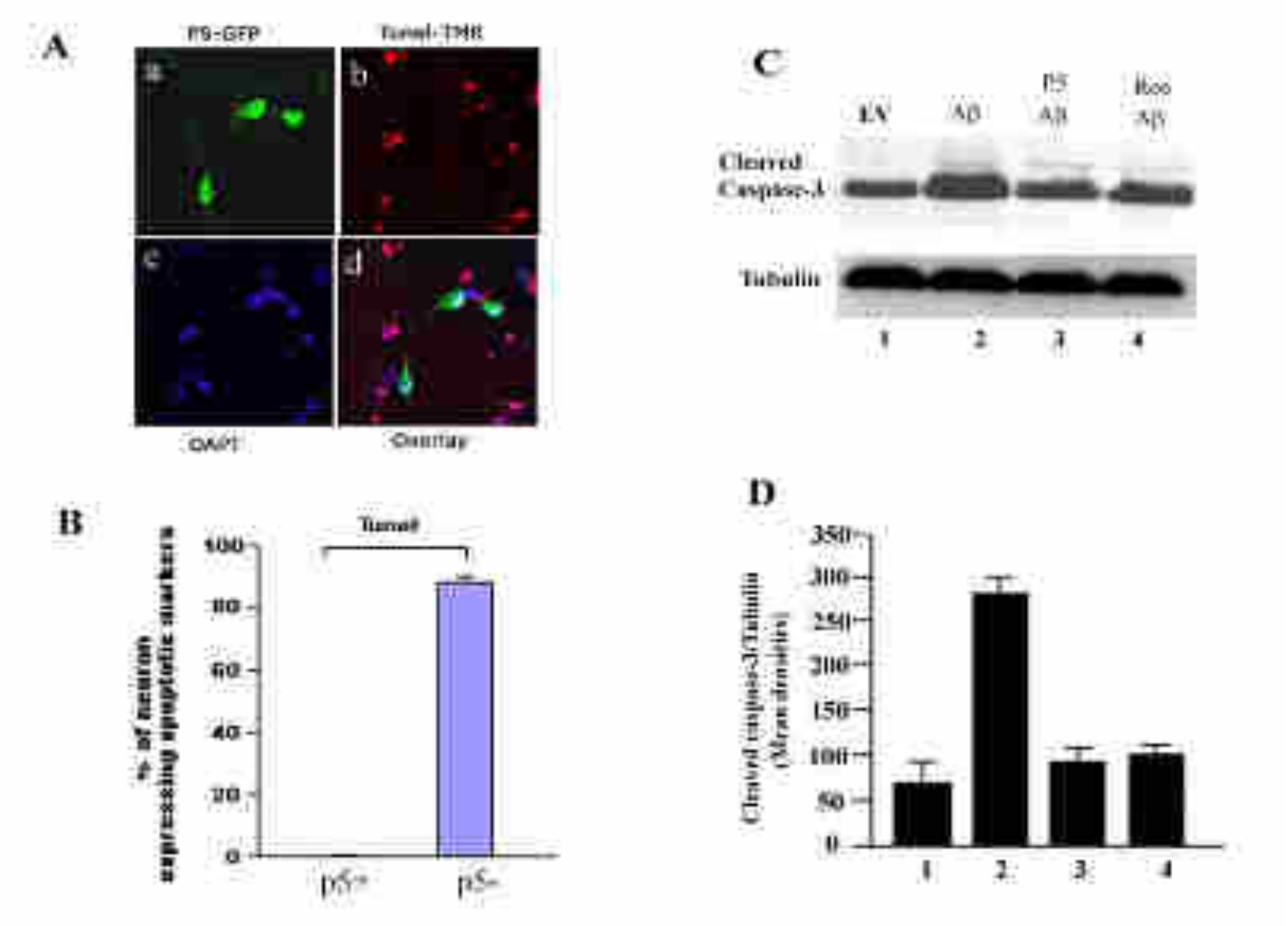


B



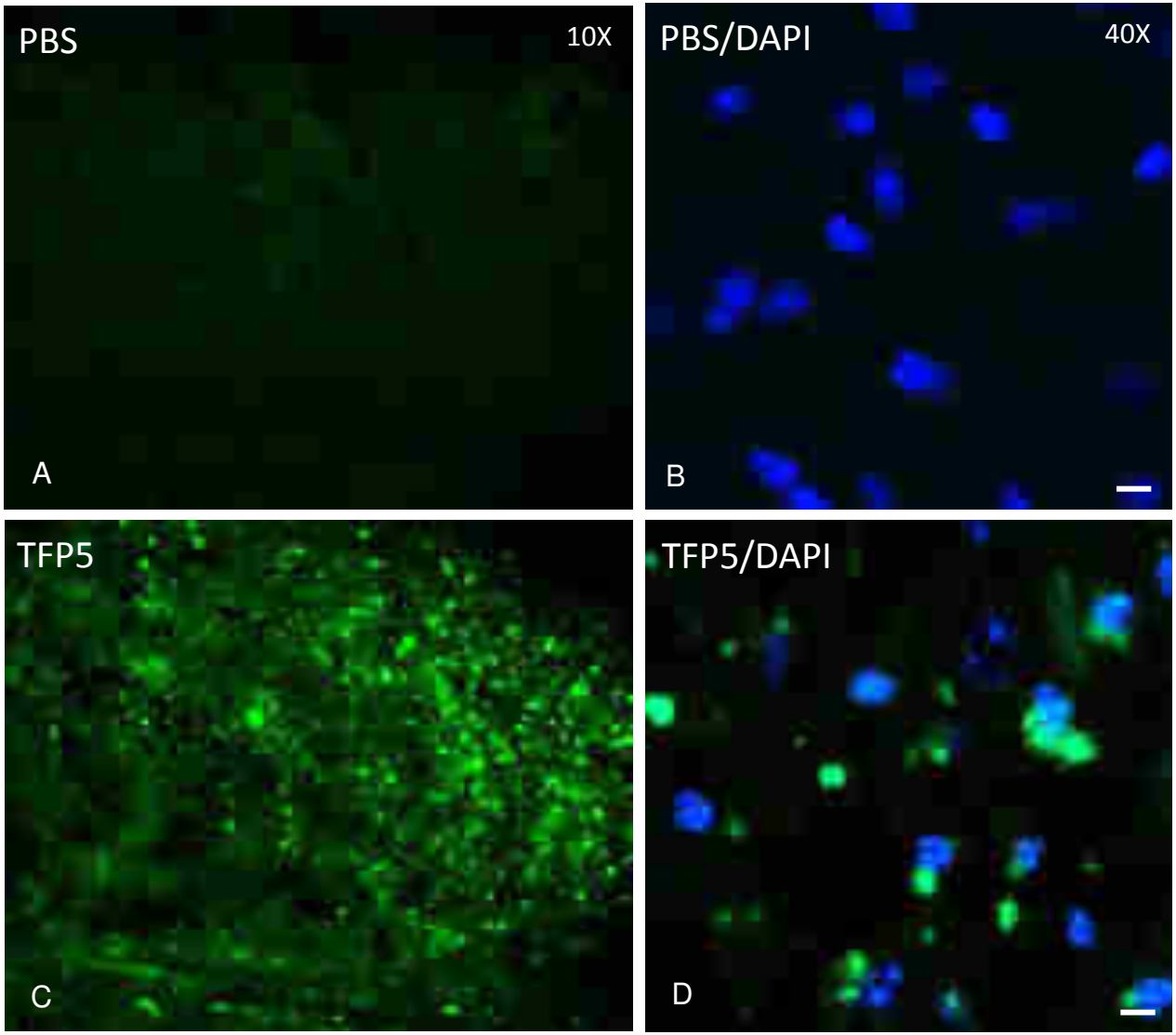
P5 modified peptide inhibits specifically Cdk5/p25 but not endogenous Cdk5/p35 activity *in situ* - neuronal cultures

TFP5 inhibits Aβ induced toxicity in cortical neurons



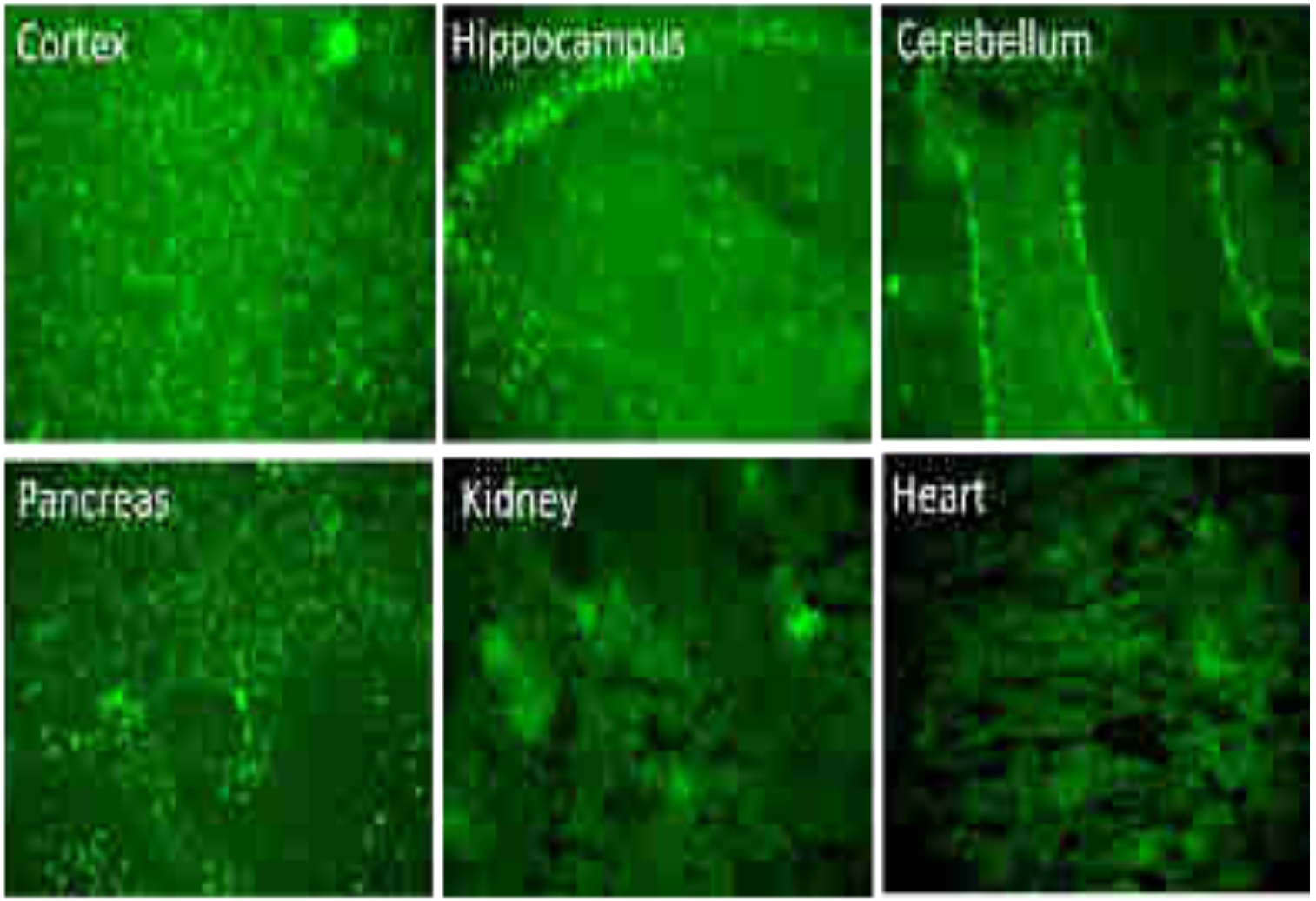
Mouse Cerebral Cortex- Single injection,TFP5 40 mg/Kg

4 days post injection



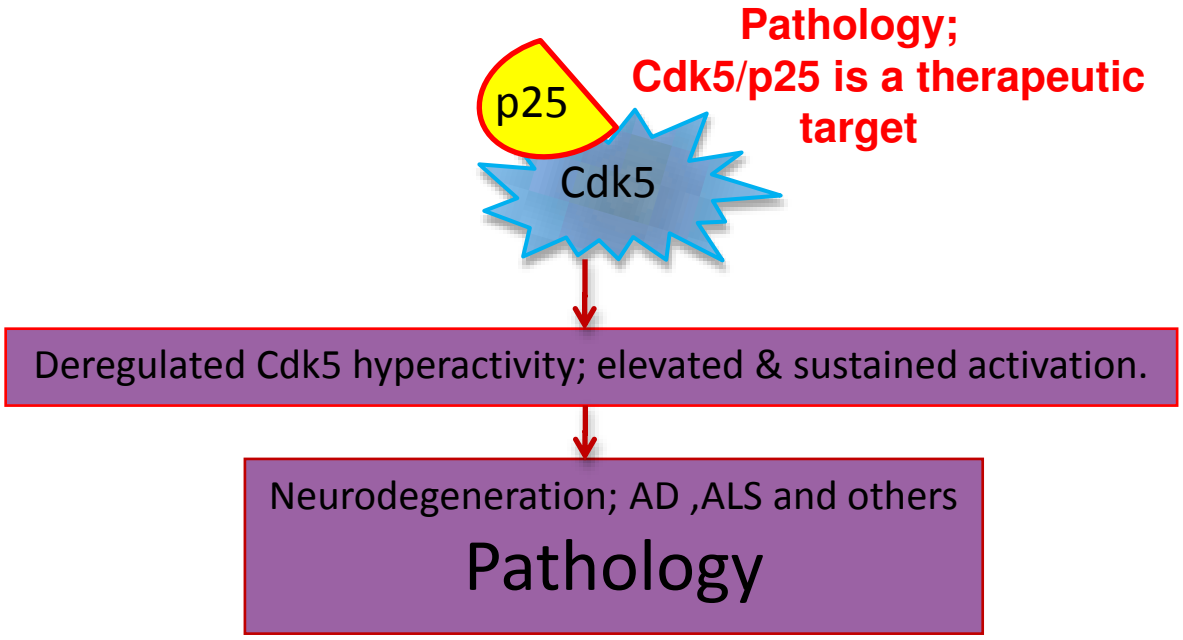
TFP5 Crosses the Blood Brain Barrier

P5- modified peptide distribution in various regions at day 4 after 3 consecutive ip injections



IP injection of TFP5 crosses the blood brain barrier and localized in different parts of brain

Sporadic AD model mouse; p25 Tg



p25 Transgenic mice - AD mouse model in collaboration with Drs Tsai and Kesavapany's laboratories

C57BL/6-Tg(tetO-p25/GFP)



The **p25 transgenic mice** expressing the **p25 protein** bearing a carboxy-terminal **GFP** tag, under the control of the **tetracycline operator**

B6;CBA-Tg(Camk2a-tTA)



The **camk2a transgenic mice** expressing the **tetracycline-controlled transactivator protein (tTA)** under regulatory control of the forebrain-specific **Camk2a promoter**



Bi-Tg(tetO-p25/GFP/Camk2a-tTA)

Induction of p25 expression at the age of **6wk** by the **removal of doxycycline in water**

Phenotypic expression of p25Tg AD model mouse include:

- **p25 expression**
- **Cdk5 Hyperactivity**
- **Aβ plaques, Amyloid plaques**
- **hyperphosphorylation and aggregation of Tau, NFTs and NFPs**
- **Neuroinflammation (activated astrocytes and microglia)**
- **behavioral defects**

Can TFP5 treatment prevent AD phenotypes in sporadic AD model (p25Tg) mice?. A Proof of concept.

Can TFP5 reduce the AD phenotypes in P25 Tg AD model mice?

- Five groups of mice were injected either with saline, TFP5 or scrambled peptide starting after 12 weeks on DOX followed by 6wks off DOX during which the AD phenotypes are expressed.

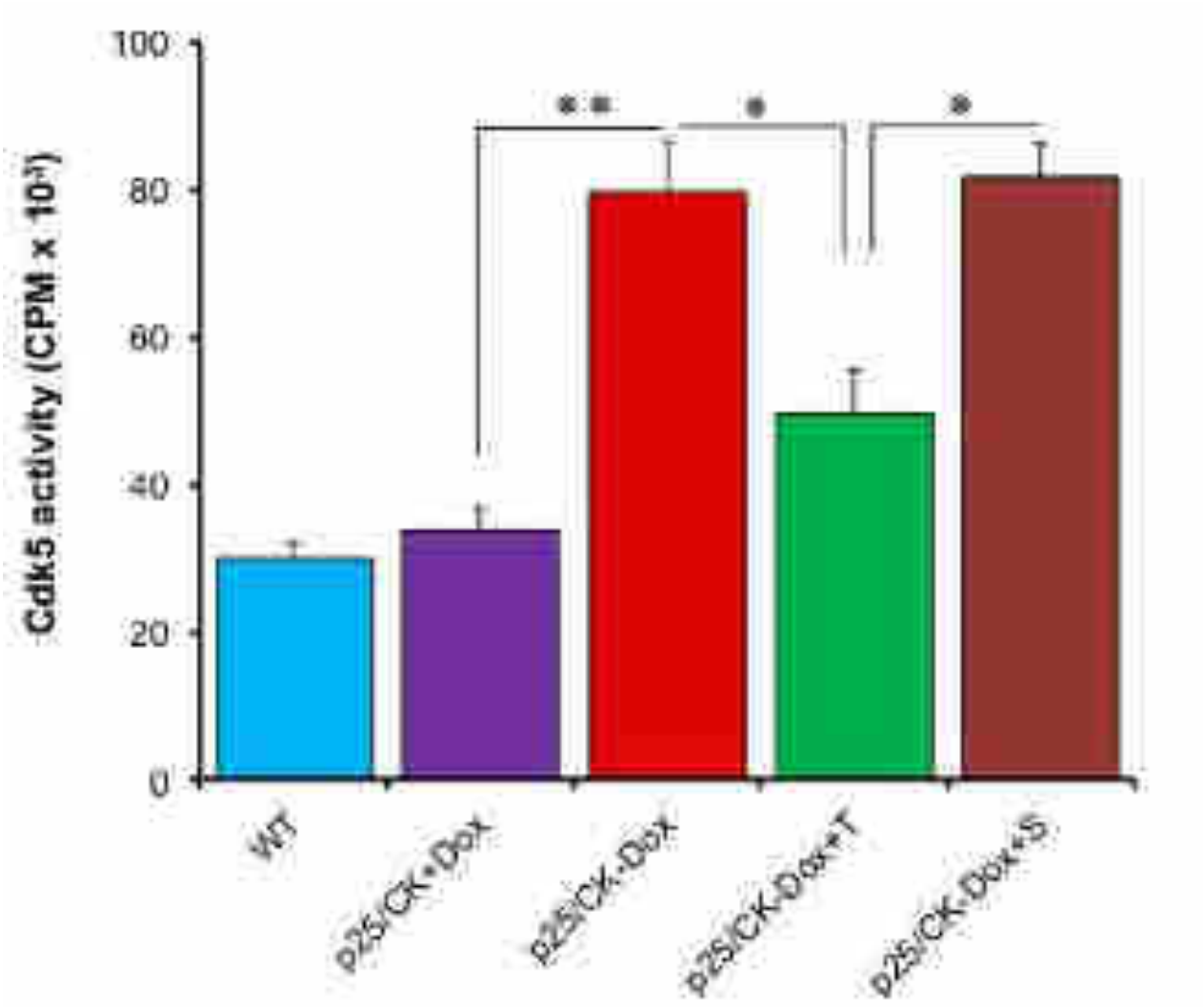
- For six weeks, all injections were given 3 times every week i.e., 18 injections in total.

- After treatment, Y maze and rota-rod and other behavioral studies were carried out before animals were sacrificed for biochemical and cell biological analyses.

- All the behavioral studies are double blinded.

Hyperactivation of Cdk5 is reduced significantly upon TFP5 treatment

Cdk5 Kinase Assay



TFP5 peptide treatment rescues hyperactivation of Cdk5 in P25Tg AD model mice.

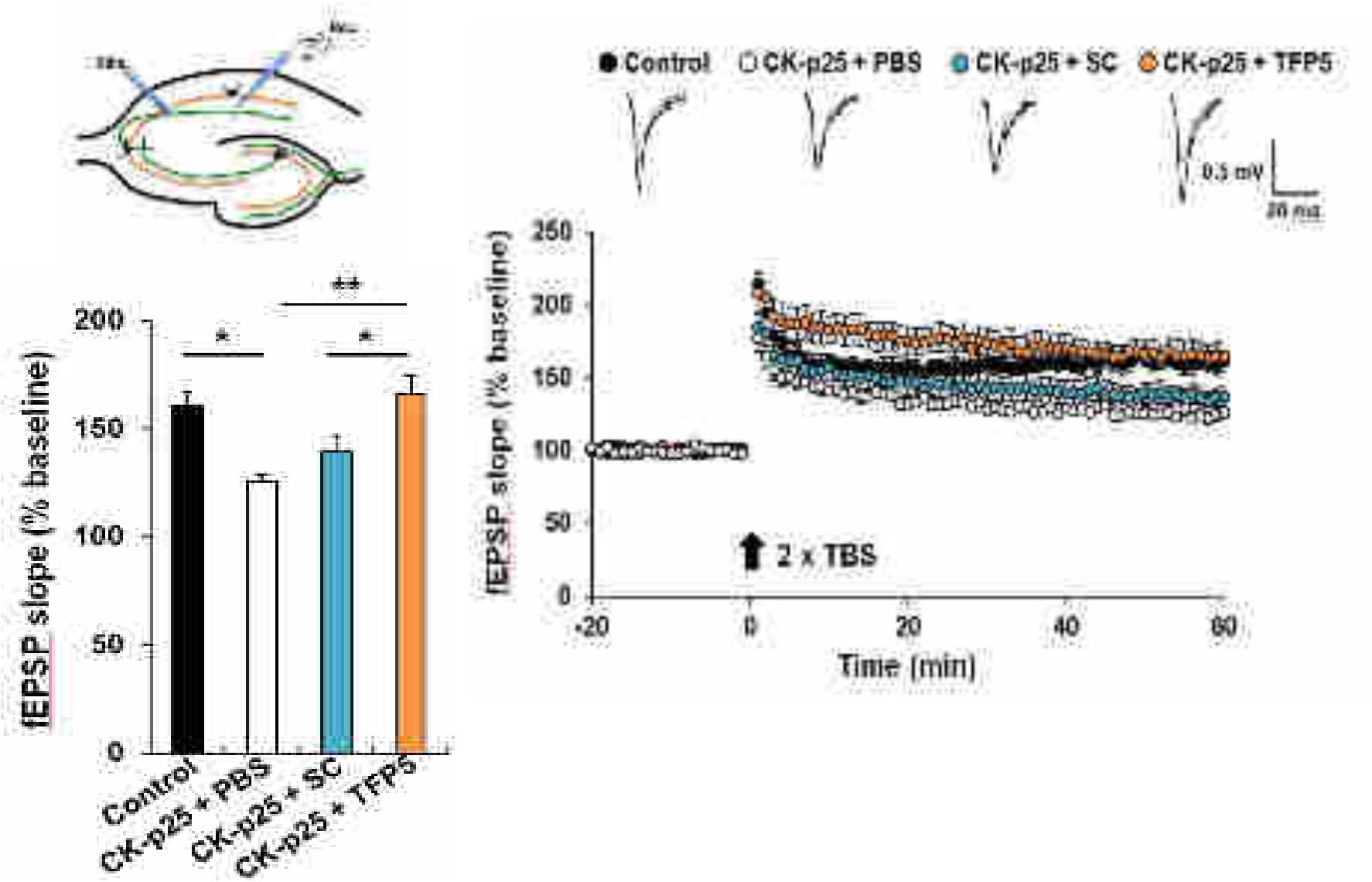
TFP5 treatment rescued synaptic activity in P25Tg AD model mice;

To record field excitatory postsynaptic long term potentials (eLTP), synaptic activity marker of memory and learning measured using transverse hippocampal slices prepared from five different groups of mice (1) WT controls, (2) p25 non- expressing, (3) p25 over expressing (p25Tg, AD model), (4) p25Tg mice treated with TFP5 and (5) p25Tg mice treated with scrambled TFP5.

CA1 field potentials evoked by Schaffer collateral stimulation were measured.

After recording of stable baseline , LTP was induced by two episodes of theta burst stimulation (TBS) with 10 s intervals. TBS consisted of ten bursts (each with four pulses at 100 Hz) of stimuli delivered every 200 ms

p25-mediated synaptic dysfunction rescued in CK-p25 expressing mice post TFP5 administration; loss of synatic activity (eLTP) is prevented in FTP5 treated p25 Tg AD model mice.



Spatial memory (Y- Maze) deficit is rescued in p25Tg TFP5 injections

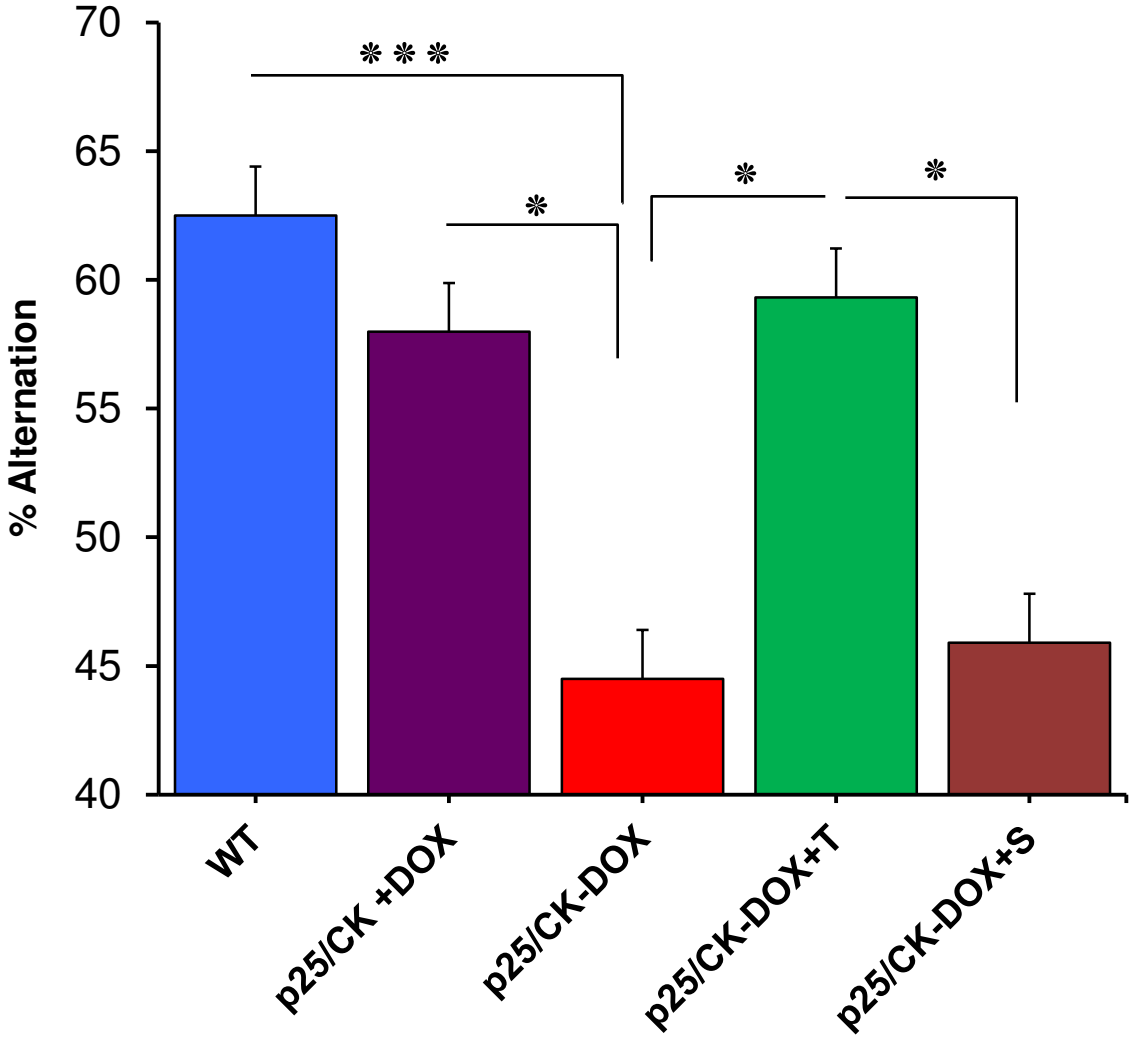
p25Tg

12 weeks on DOX

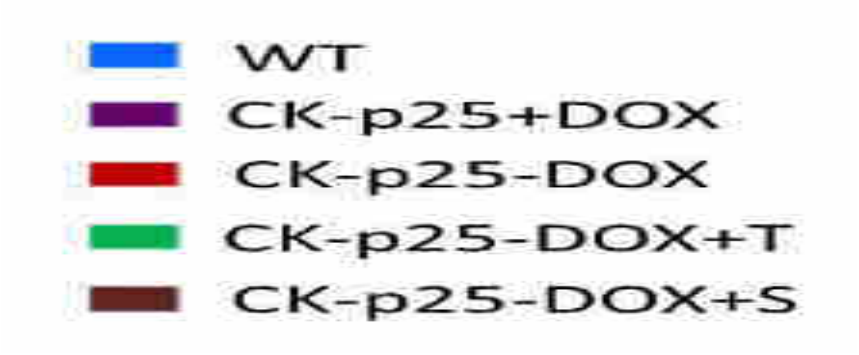
thrice TFP5 0.2 mM / week

6 weeks off DOX

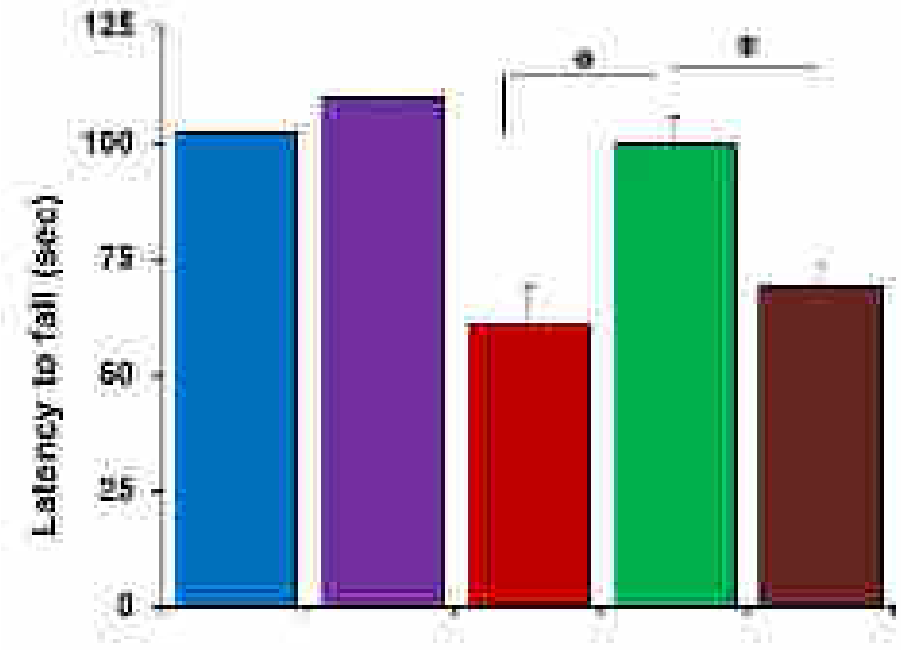
Significant Rescue



TFP5 treatment prevented motor defects in p25 Tg AD model mice

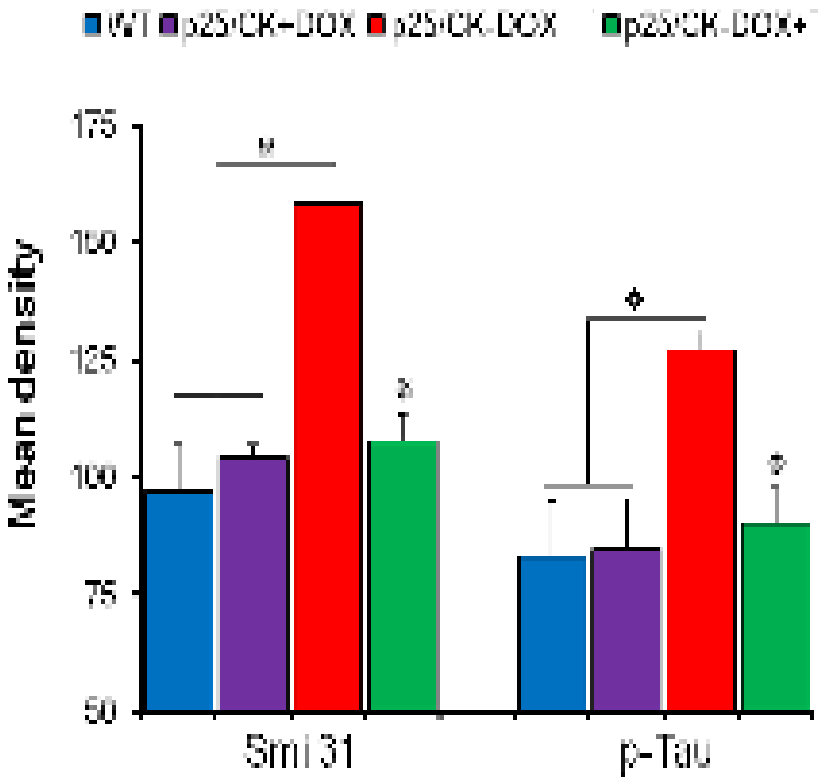


Rota rod: Motor deficit

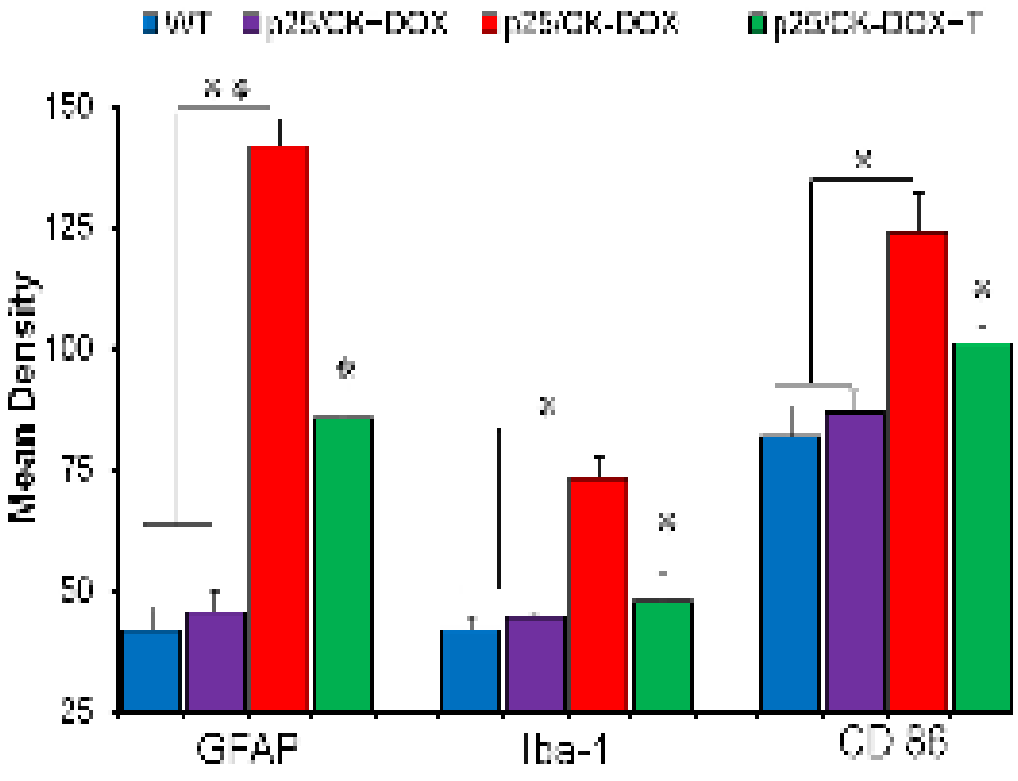


Hyperphosphorylation of cytoskeletal proteins and neuroinflammation is reduced upon TFP5 treatment

TFP5 treatment reduced hyperphosphorylation of p-neurofilament (p-NF-H/M) and p-tau in p25Tg AD model mice



NeuroinflammationTFP5-treatment reduced in p25Tg AD model mice



Does TFP5 prevent hyperactivation of Cdk5 and abnormal phenotypes in double transgenic familial (5XFAD) AD model mice?

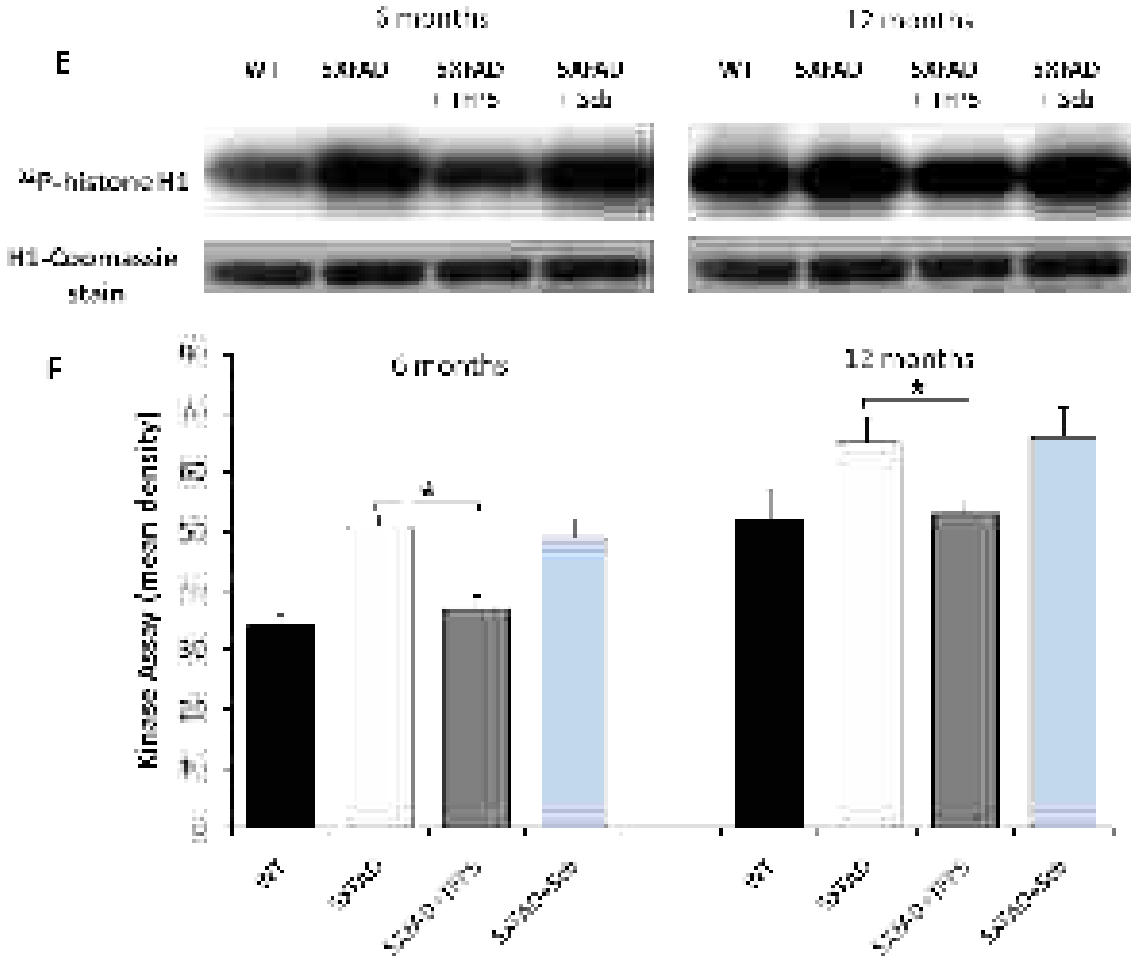
5XFAD mouse has the following five familial Alzheimer Disease (FAD) mutations:

- Three APP (695) mutations:
  - Swedish (K670N/ M671L)
  - Florida (I716V)
  - London (V717I)
- Two Presenilin I (PS1) mutations:
  - M146L
  - L286V

Phenotypic expression of 5XFAD mouse include:

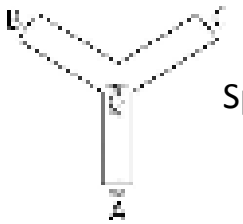
- **p25 expression**
- **Cdk5 Hyperactivity**
- **Aβ plaques, Amyloid plaques**
- **hyperphosphorylation and aggregation of Tau, NFTs and NFPs**
- **Neuroinflammation (activated astrocytes and microglia)**
- **behavioral defects**

TFP5 peptide prevents hyperactivation of Cdk5 in 5XFAD mouse brain



Rescue of spatial memory deficit by TFP5 treatment in AD model mice:

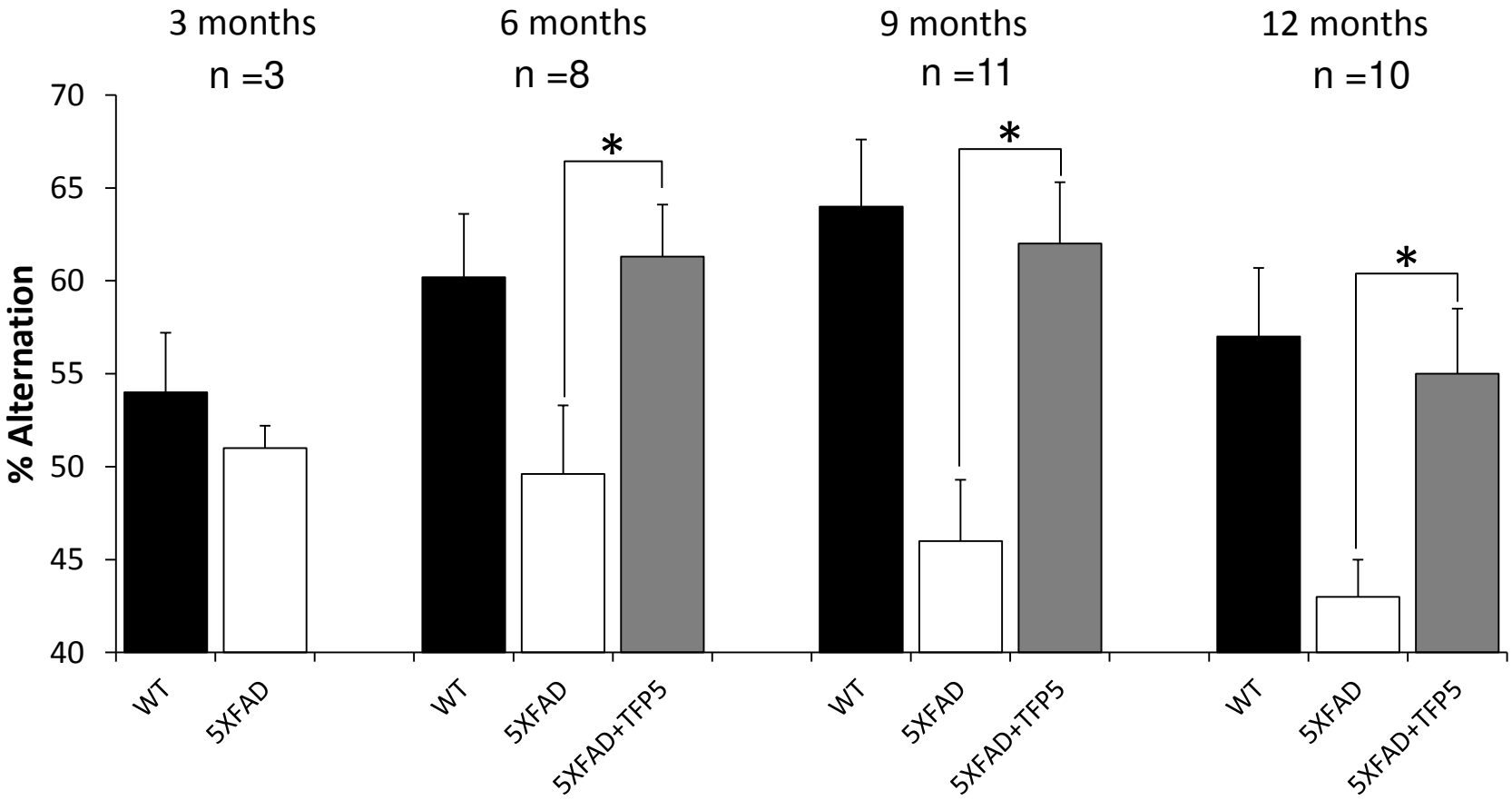
Y-maze Spontaneous Alternation Test (memory).



Spontaneous alternation behavior (SAB) % = (working memory)

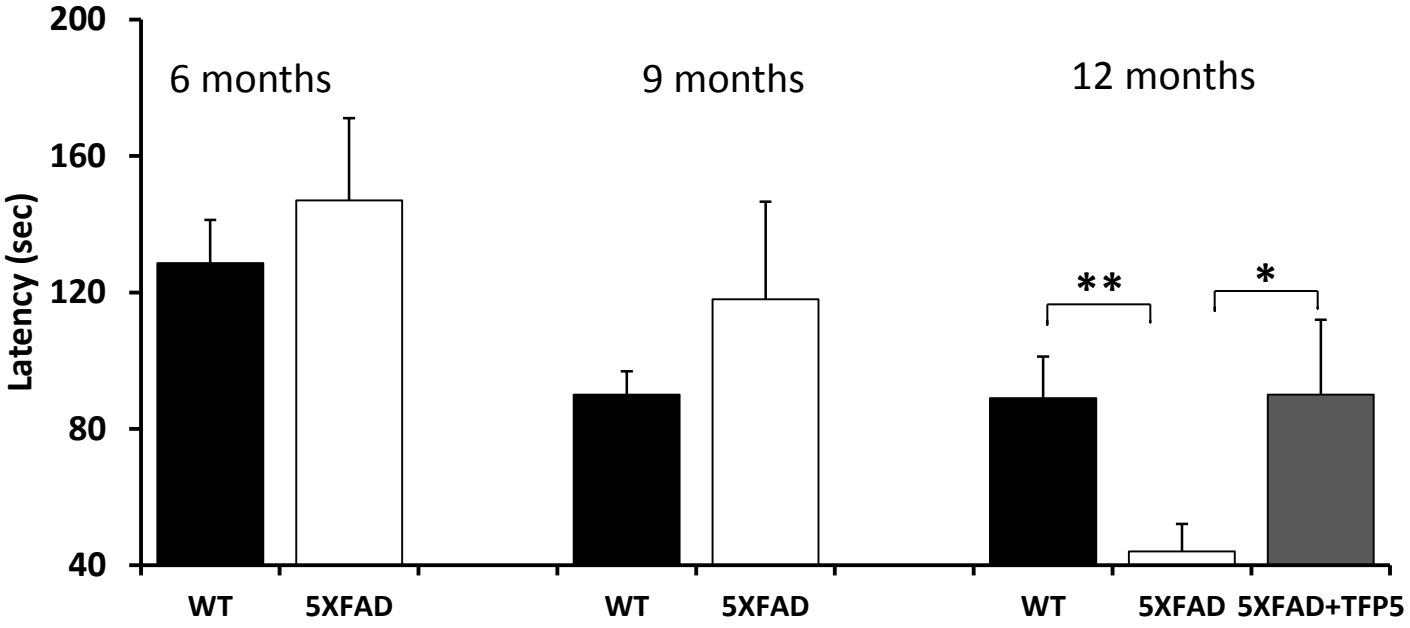
$$\frac{\text{Total number of triad entry}}{\text{Total number of arm entry - 2}} \times 100$$

Hsiao et al., Science (1996)
Holocomb et al., Nature (1998)



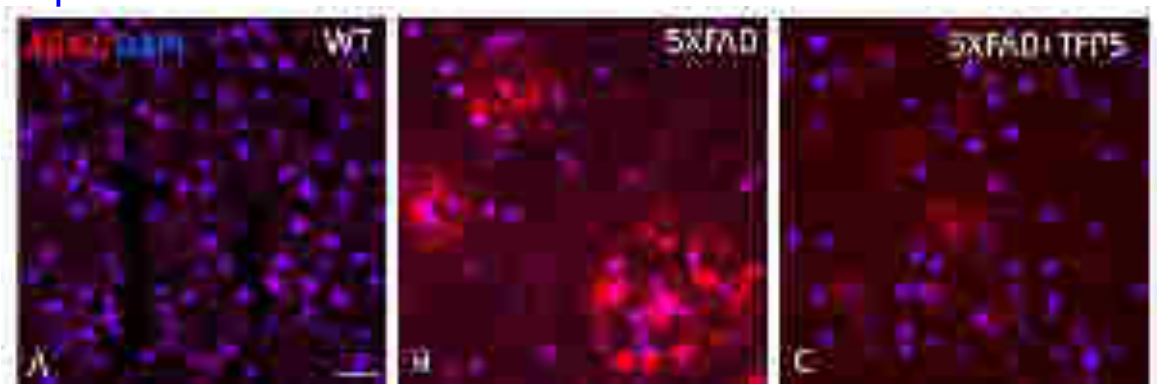
All the behavioral studies were double blinded.

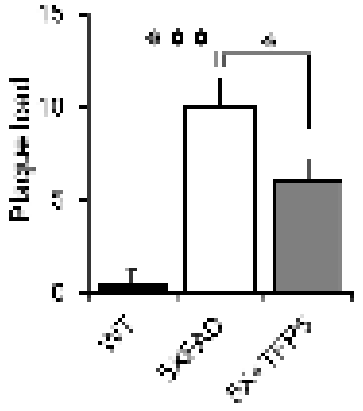
TFP5 injection rescued motor deficit in 12 month old 5XFAD model mice (Rota-rod test, motor behavior)



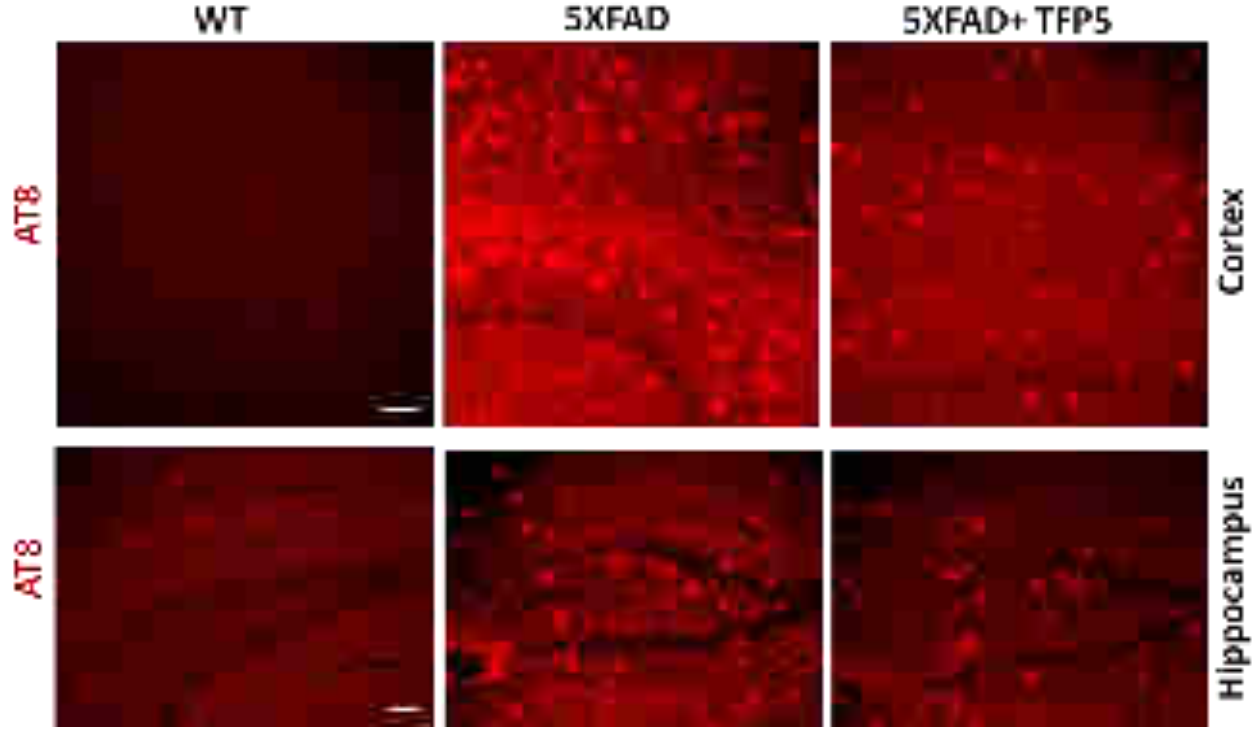
Aβ and phospho-tau were reduced post TFP5 treatment

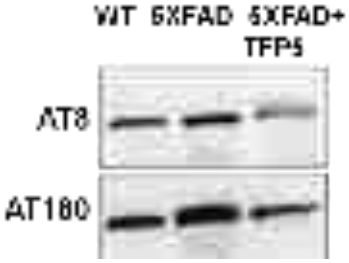
Aβ plaques

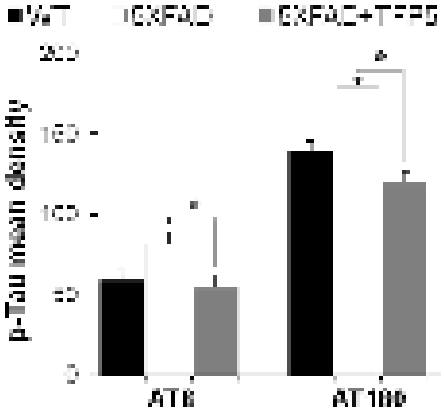




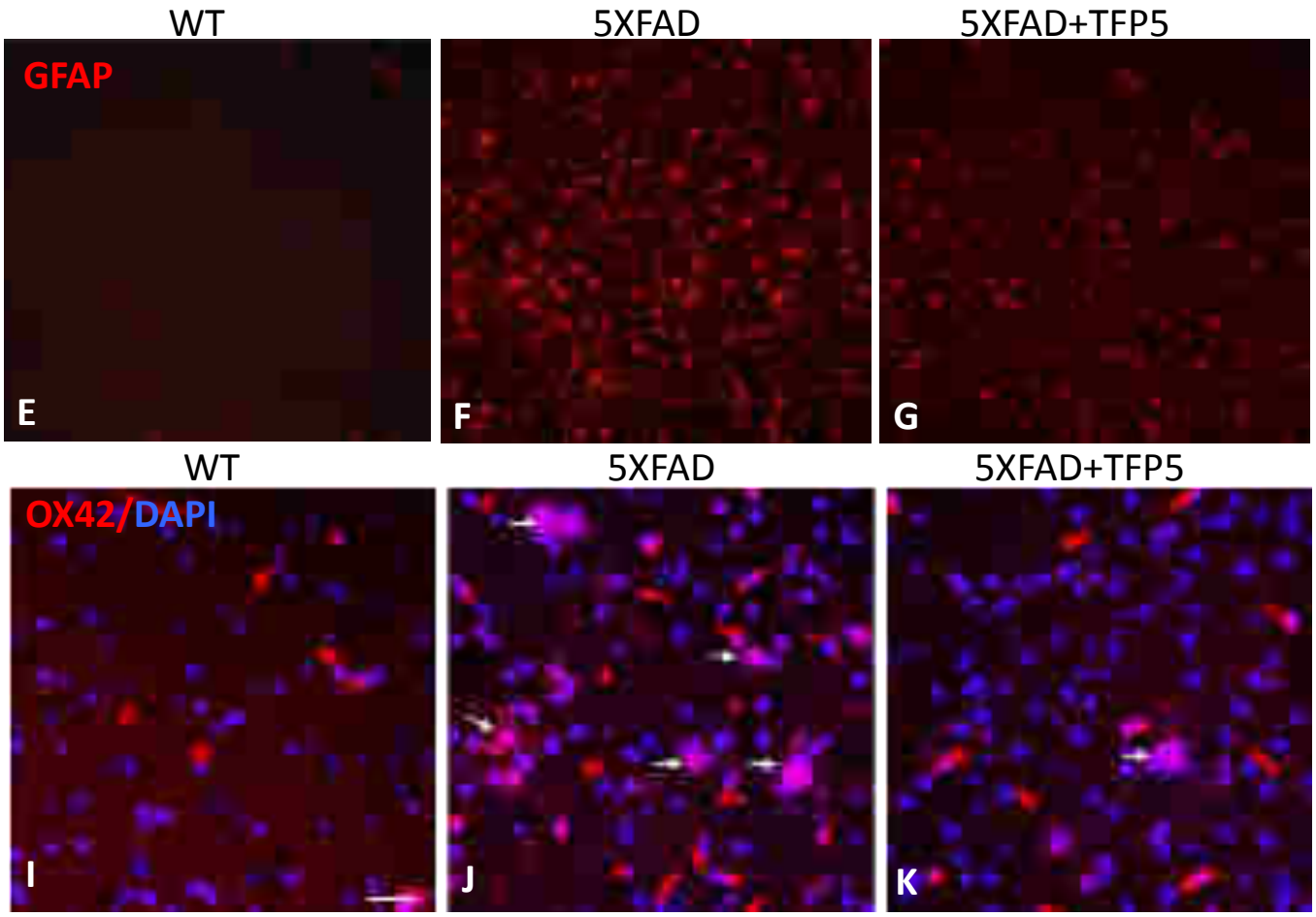
Phospho-tau

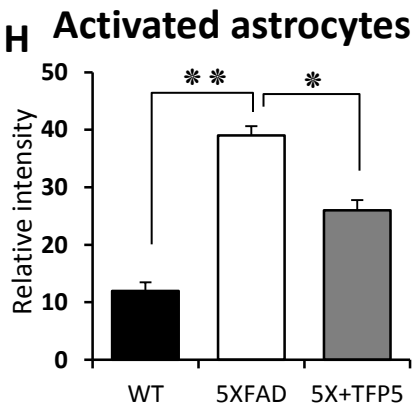


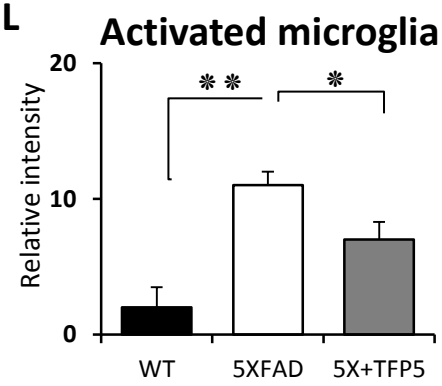




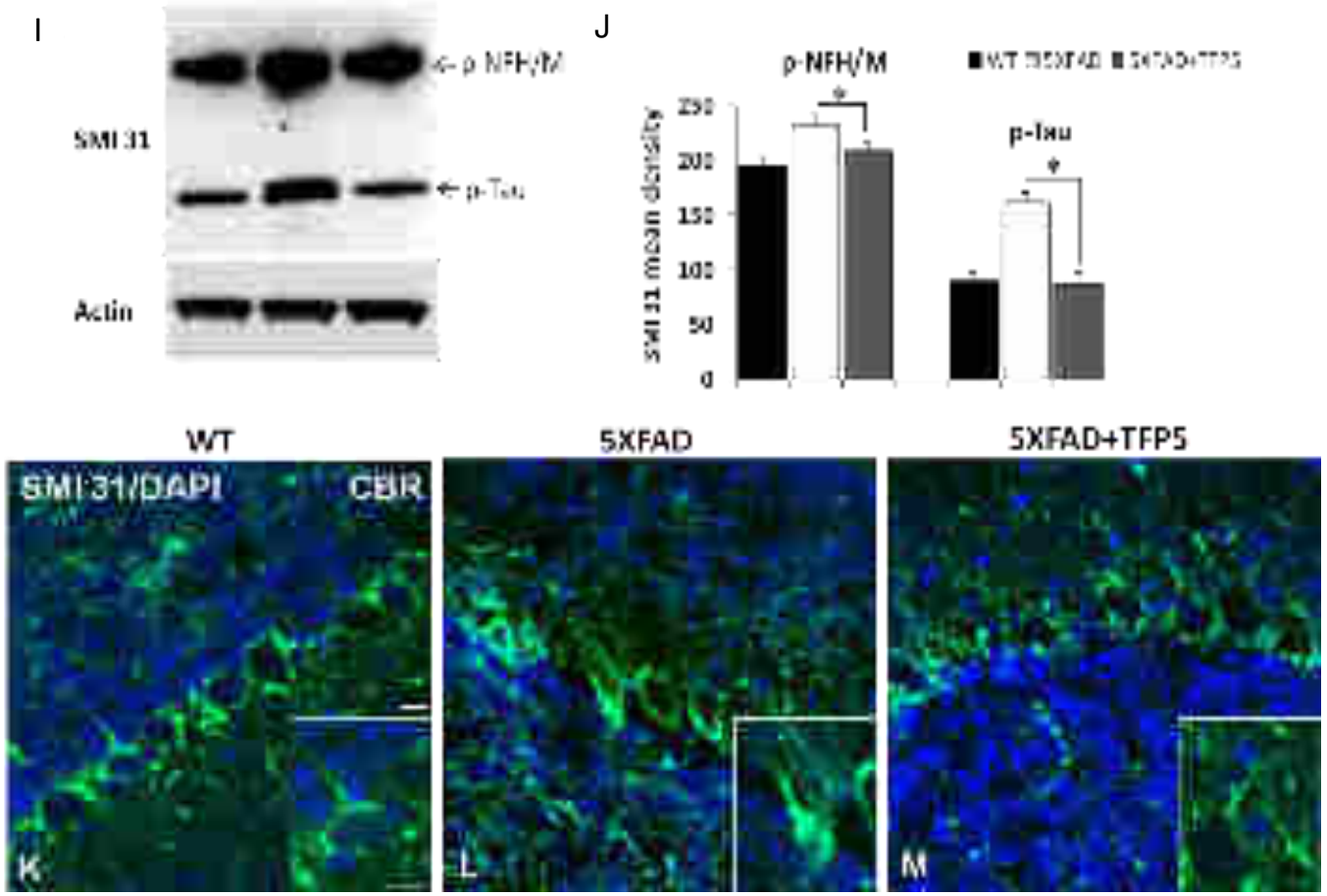
Neuroinflammation is reduced upon TFP5 treatment





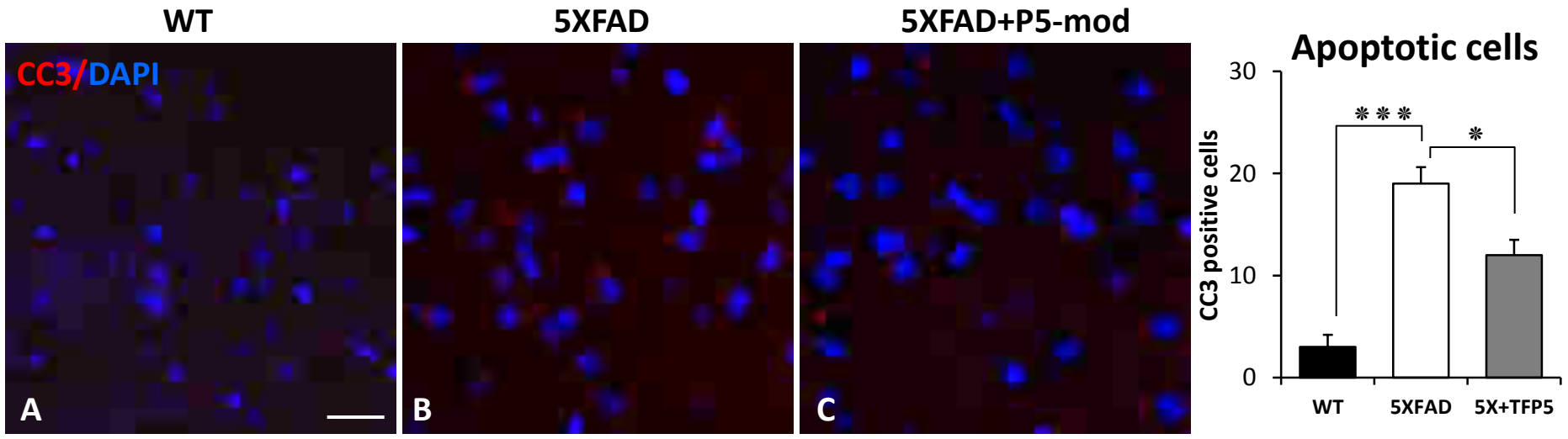


P5-mod treatment inhibits hyperphosphorylation of NF-M/H and Tau of 5XFAD mice brain ; Western blots and ICC using p-NF-M/H and p-tau specific antibodies



Neuronal cell loss is reduced upon P5-modified peptide treatment of AD mice

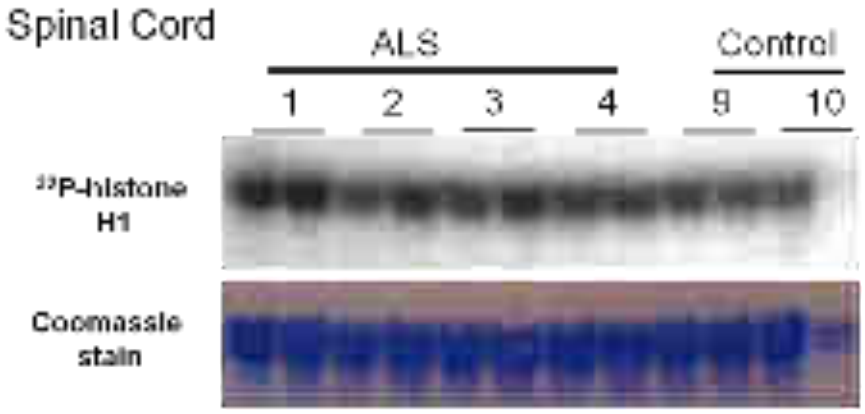
Cleaved caspase 3 (apoptotic cell marker)

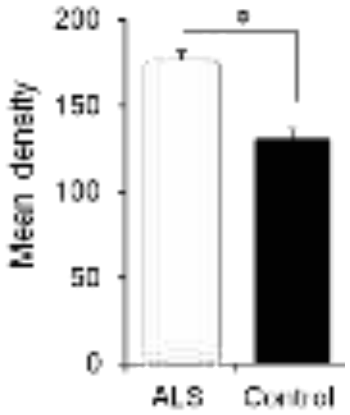


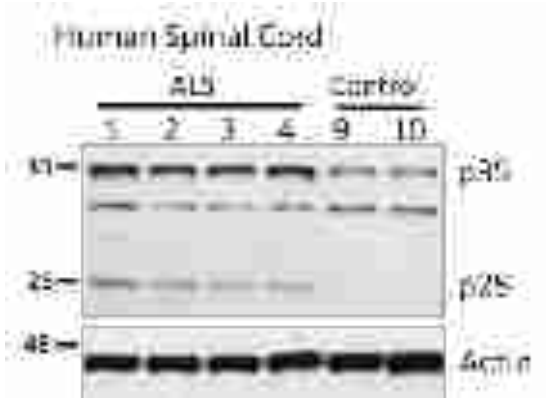
Since it is well documented that deregulation of Cdk5 activity is associated with ALS pathology, and expression of p25 and Cdk5 hyperactivity have been demonstrated in ALS brain and spinal cords (Nguyen et al.,2001; Cruz et al., 2004), we asked the following question:

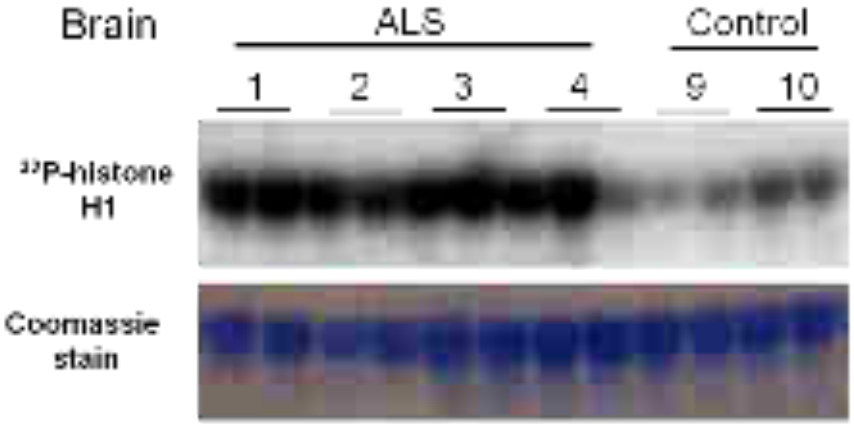
Can TFP5 ameliorate ALS pathology?

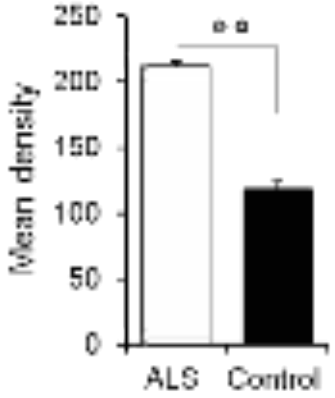
Cdk5 activity is elevated brain and spinal cord of ALS patients

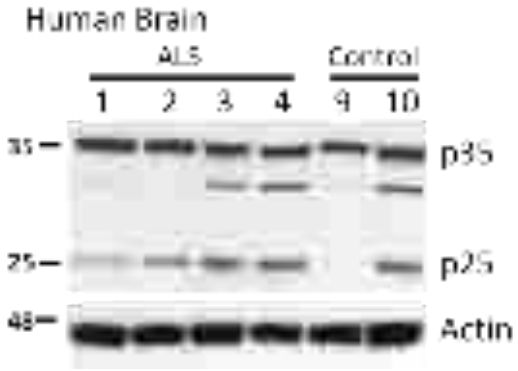












TFP5 Injection paradigms for ALS mice

Slow ALS (SOD1 G37R)

- Onset 140-150 days
- End stage 170 days
- G37R (line 42) mice treated with **TFP5 (0.4mM)/ once a week** starting from 80 days carried out till the end stage of mice (criteria set by Vet.)

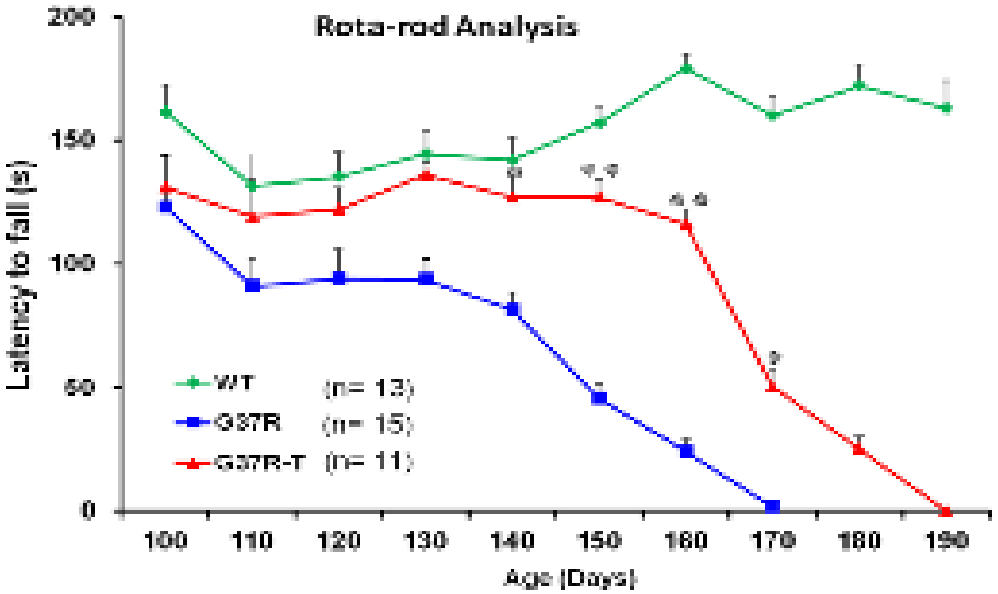
80 days were chosen to make sure that injections are given well before the onset of the disease.
We also did 0.2mM one injection per week which did not show any rescue.

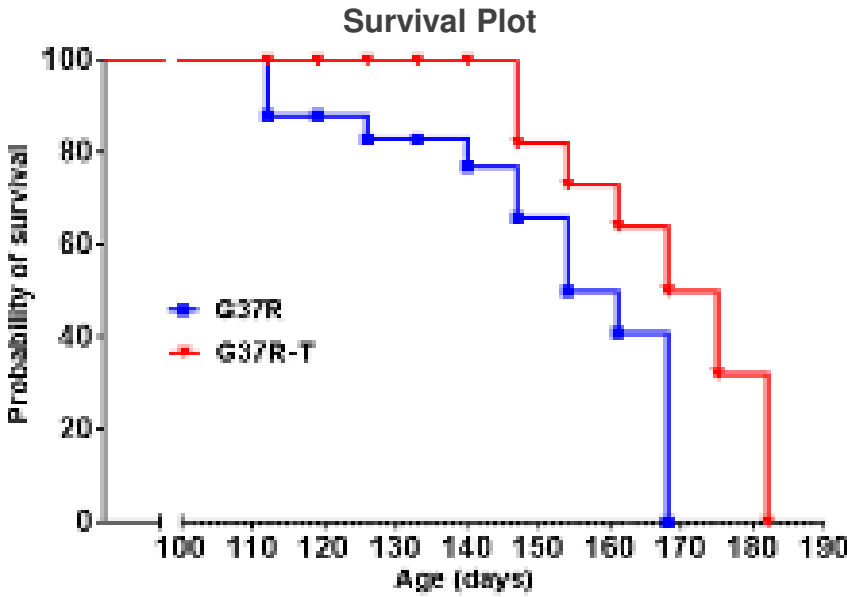
Fast ALS (SOD1 G93A)

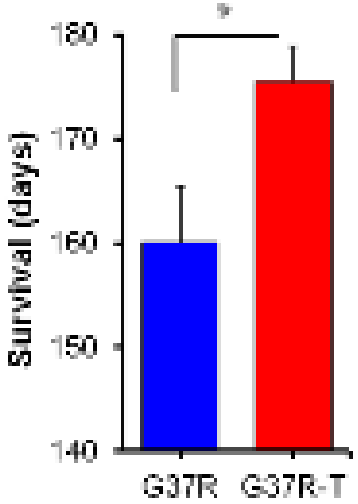
- Onset 100-110 days
- End stage 130 days
- G93A mice treated with **TFP5 (0.4mM)/twice a week** starting from 35 days carried out till the end stage of mice (Criteria by Vet.)

35 days were chosen to make sure that injections are given well before the onset of the disease.
We also did one injection per week which was not effective. Therefore changed to two injections per week.

Survival plot of G37R (line 42) mice treated with TFP5 (0.4mM ; 100mg/kg)/ once a week starting from 80 days

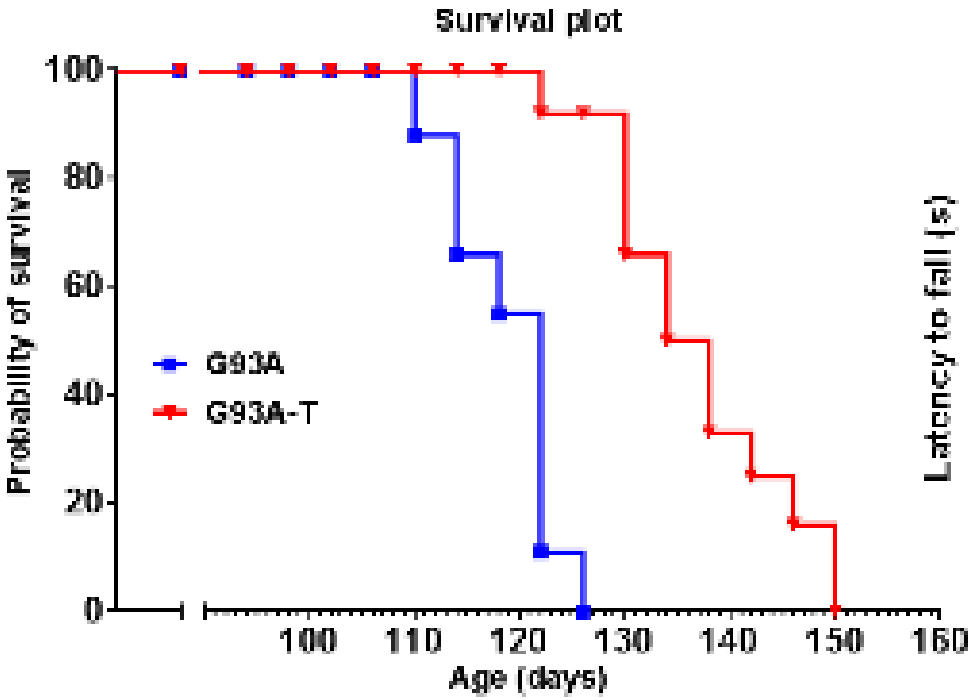


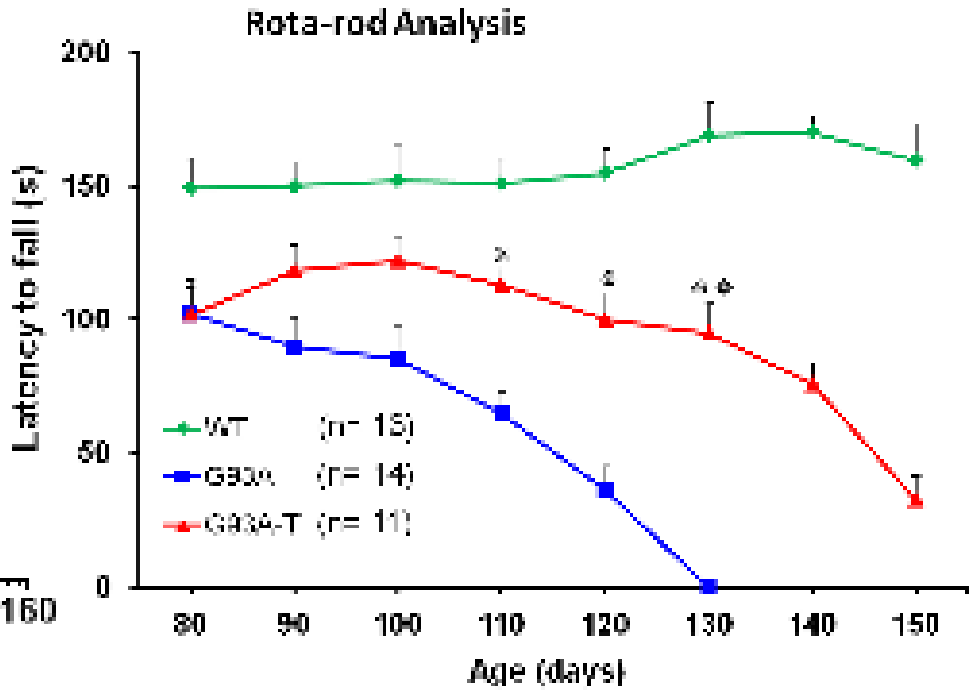


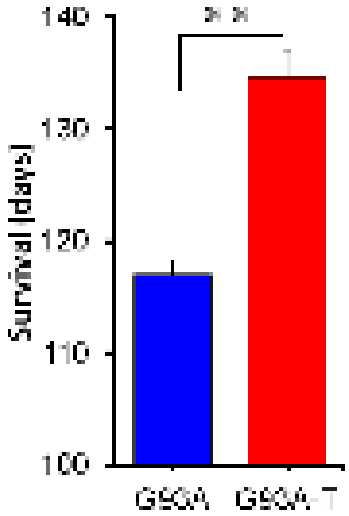


G37R survival = 160 days ± 5
G37R-T survival = 175 days ± 3.5
Survival increased by 15 days

Survival plot of G93A mice treated with TFP5 (0.4mM)/twice a week starting from 35 days





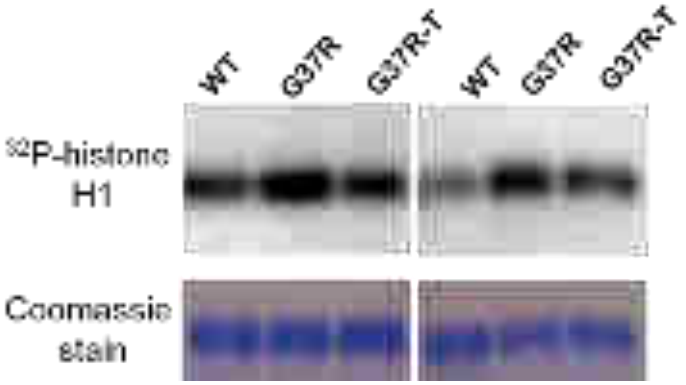


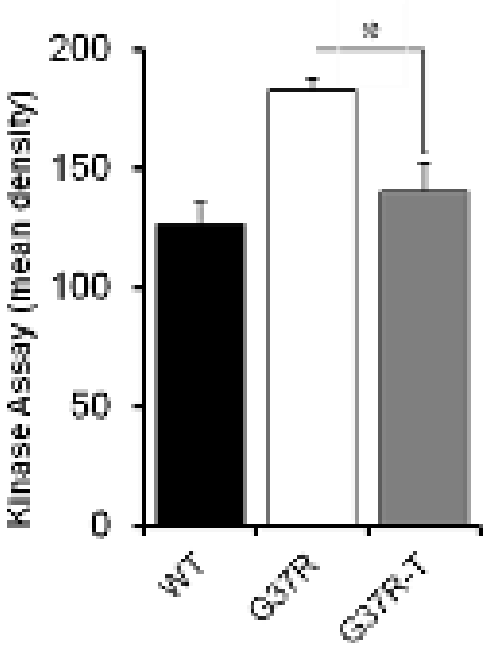
G93A survival = 117 days ± 1

G93A-T survival = 134.6 days ± 2.5

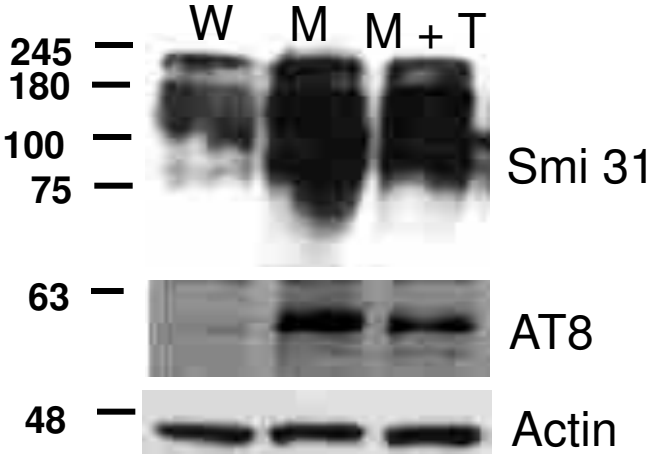
Survival increased by 17.5 days

Elevated Cdk5 activity of G37R ALS model mice brain reduced to wild type activity upon TFP5 treatment

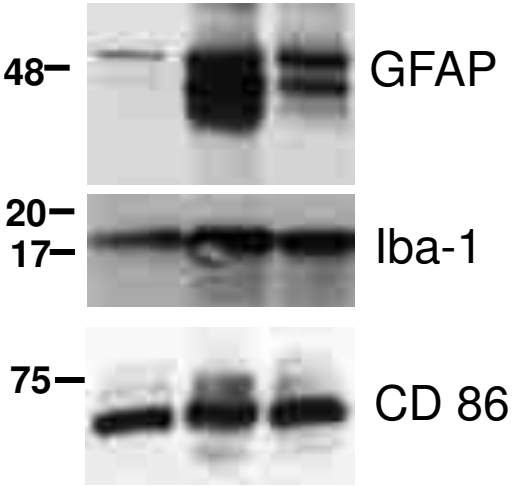




Reduction in phospho - NF and pTau after TFP5 treatment in G37R mice

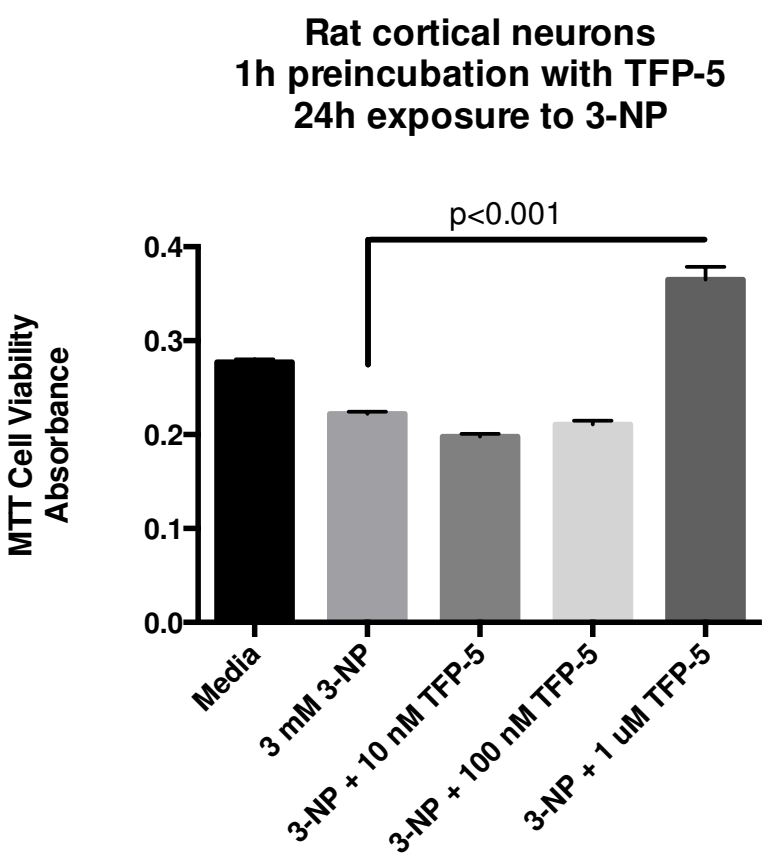


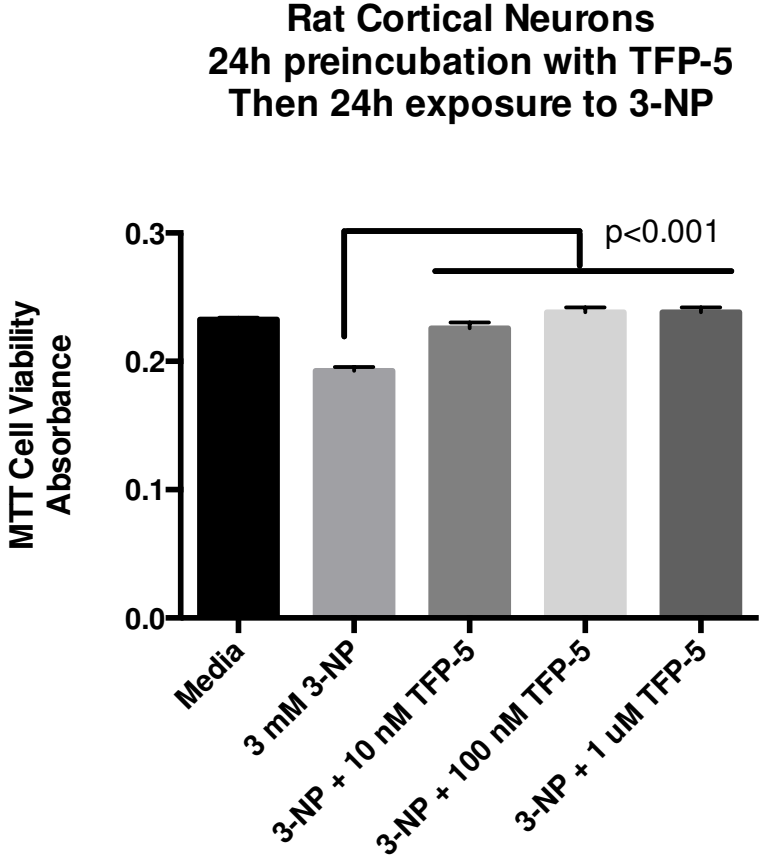
Neuroinflammation is reduced post TFP5 treatment in G37R mice



TFP5 can ameliorate ALS phenotypes in SOD1 mutant mice

Longer preincubation facilitates TFP-5 mediated neuroprotection in cells treated with 3NP (mitochondrial electron transport complex1)

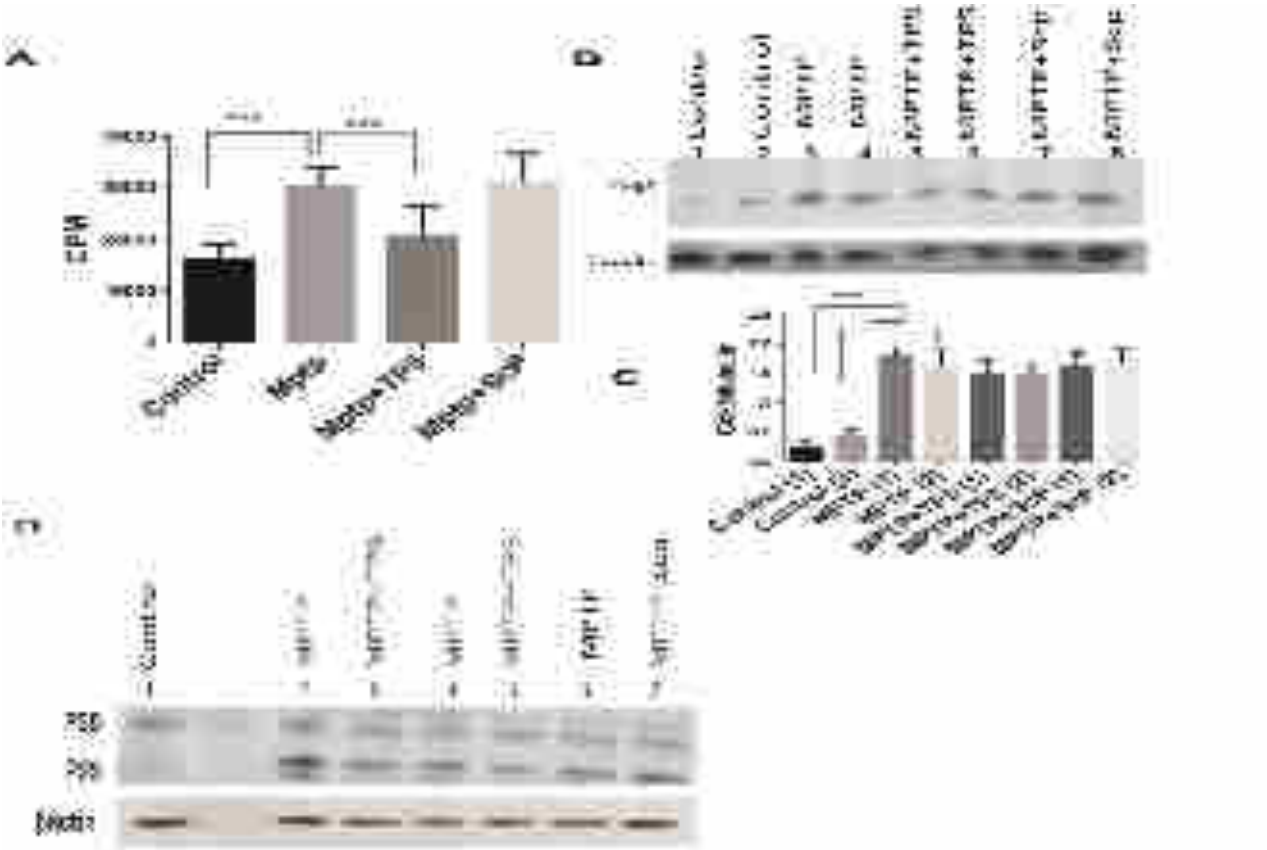




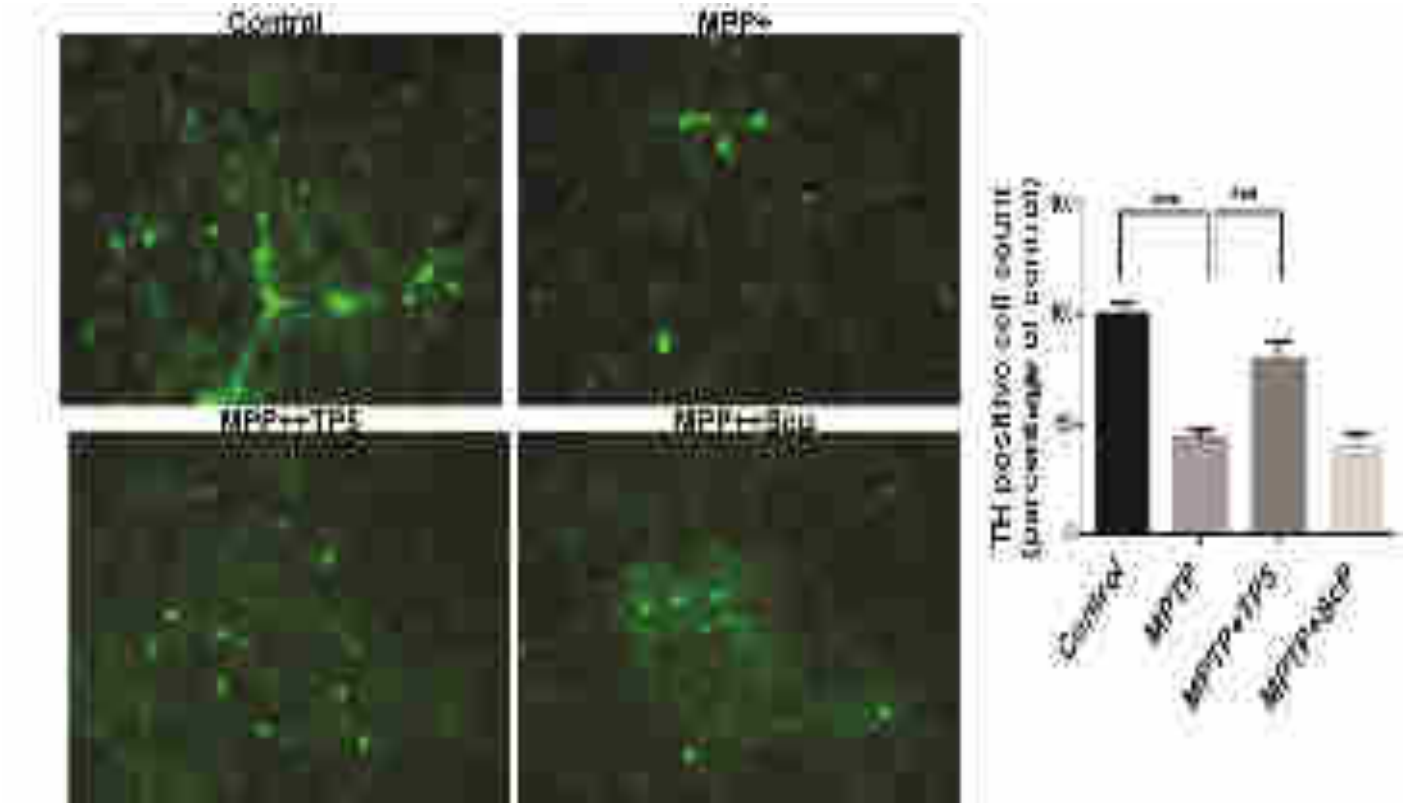
Cdk5 deregulation in Parkinsion's Disease (PD)

Recent evidence indicates that Cdk5 is also hyperactivated in PD. Following few experiments show that TFP5 or TP5 also inhibit Cdk5 hyperactivation *in vivo* and *in vitro*, rescues nigrostriatal dopaminergic (NS) neurodegeneration induced by 1-methyl- 4-phenyl-1,2,3,6-tetrahydropyridine (MPTP/MPP+), in a mouse model of PD. TFP5 peptide treatment also blocked dopamine depletion in the striatum and improved gait dysfunction due to MPTP administration. The neuroprotective effect of TFP5 peptide is also associated with marked reduction in neuroinflammation and apoptosis.

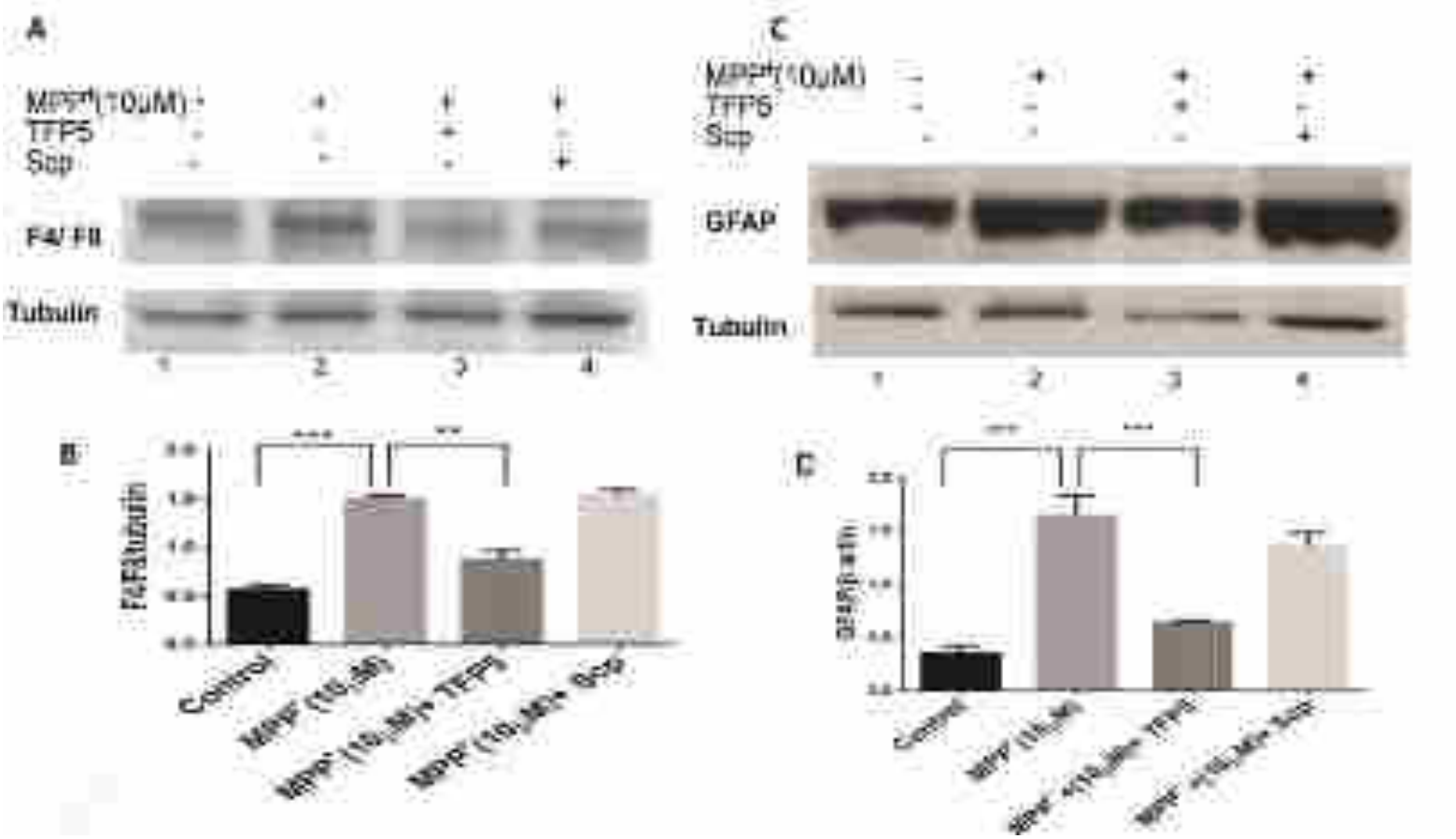
TFP5 inhibits MPTP-induced Cdk5/p25 hyperactivity in PD model mice



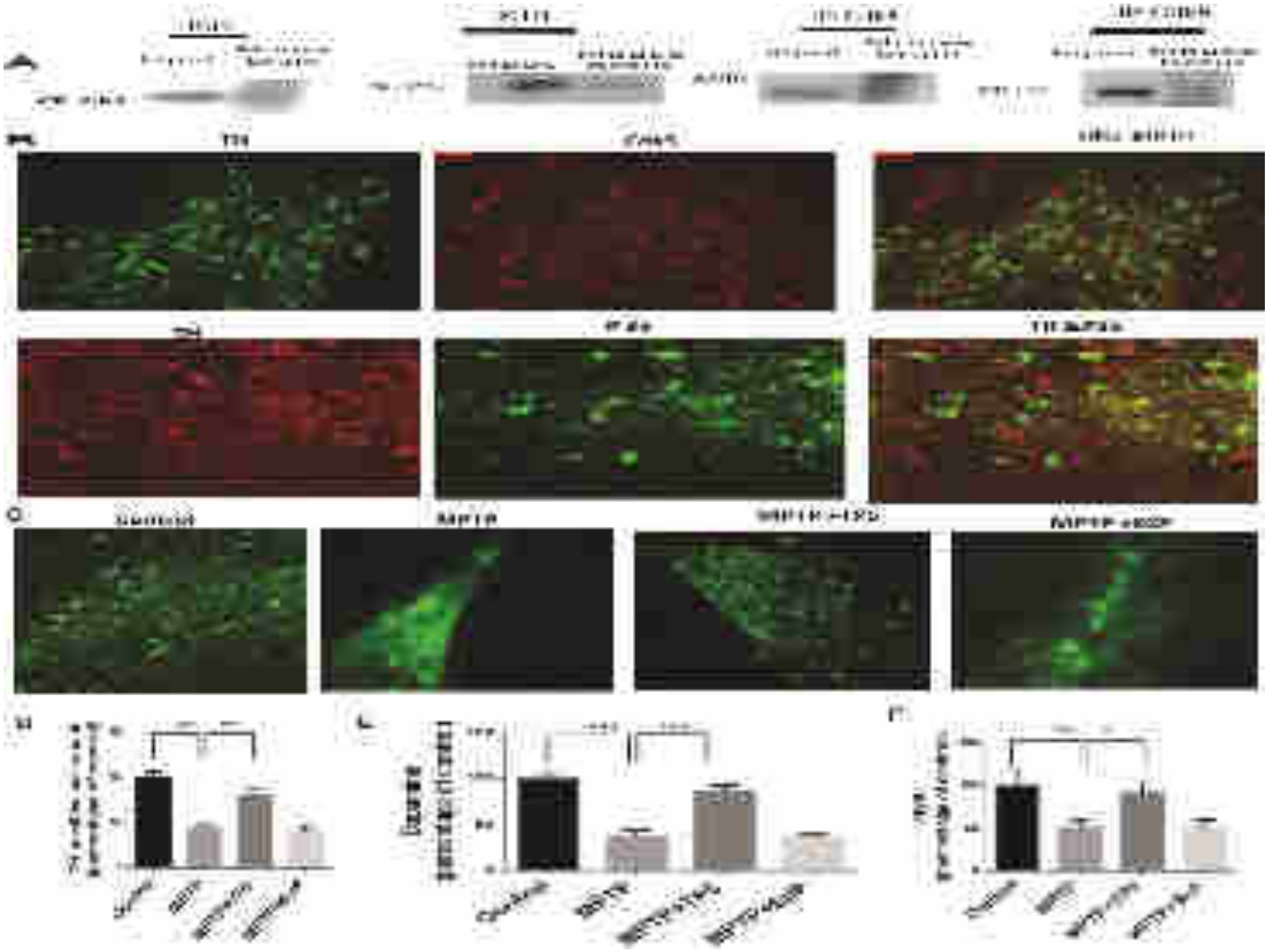
Dopaminergic neuronal loss in SN due to MPTP is reduced significantly by TP5 treatment



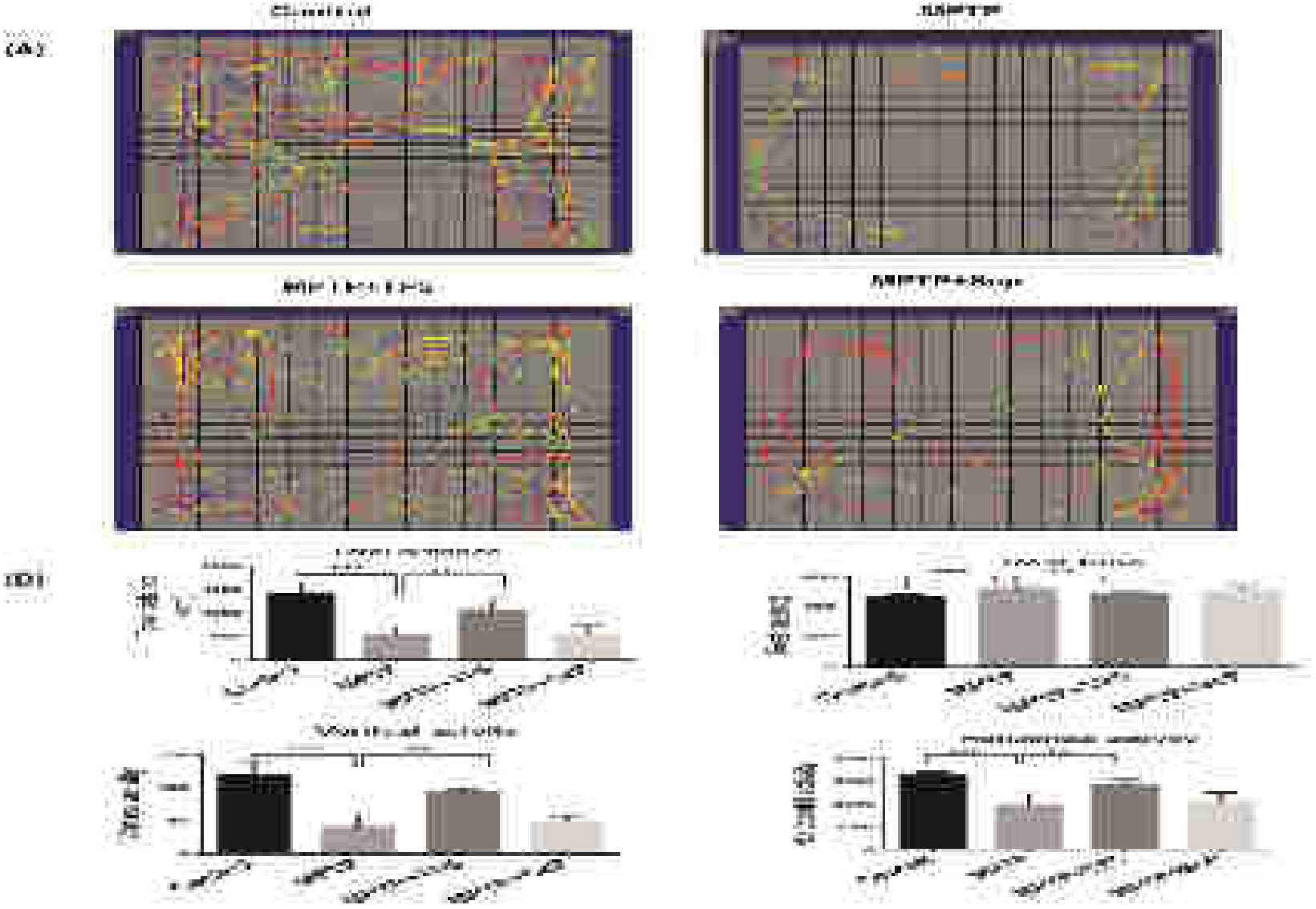
TFP5 treatment inhibits MPP+ -induced inflammation and apoptosis in mesencephalic primary cultures



Dopaminergic neurons of the SN express Cdk5/p35 and are protected from cell death by TP5 after MPTP induction



TP5 treatment improves significantly the Locomotor Function in MPTP exposed Mice



Conclusion

TFP5 provides neuroprotection in the 1-methyl- 4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) model of Parkinson's disease mice.

TFP5 – A therapeutic candidate

- Passes through BBB due to modified TAT attached.
- Can be visualized due to FITC.
- Does not cause toxicity in the mouse even at the concentration of >2000mg/Kg.
- No neurological stress observed post TFP5 injections.
- No change in the body weight before and after injections.
- Rescues following AD phenotypes:
  - Specifically inhibits Cdk5/p25 hyperactivity
  - Rescues spatial memory deficit
  - Reduces p-tau, p-NF aggregates and beta-amyloid plaques
  - Reduces neuroinflammation and apoptosis

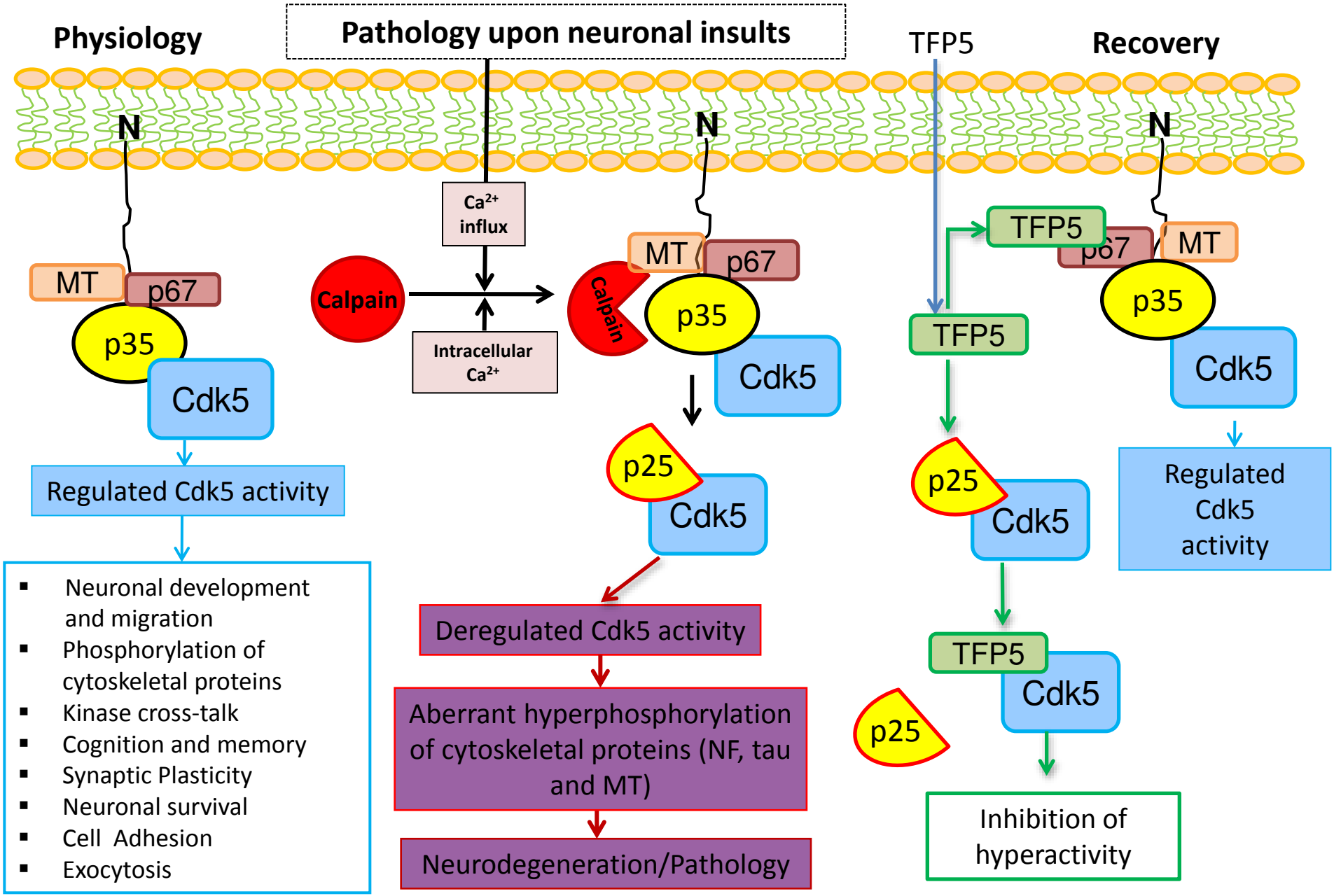
Summary

- A 24 amino acid, modified peptide (TFP5), reduced / prevented AD phenotypes in familial 5XFAD model mice upon ip injection of TFP5.

- TFP5 treatment also reduced AD phenotypes in p25 Tg AD model mice, **proof of concept**.

- **On going studies are also providing the evevience that TFP5 can ameliorate PD and ALS pathologies in Model mice.**

Summary: TFP5 selectively inhibits the deregulated Cdk5/p25 but not Cdk5/p35, the regulated, normal activity *in situ* & *in vivo* , ameliorate pathology.



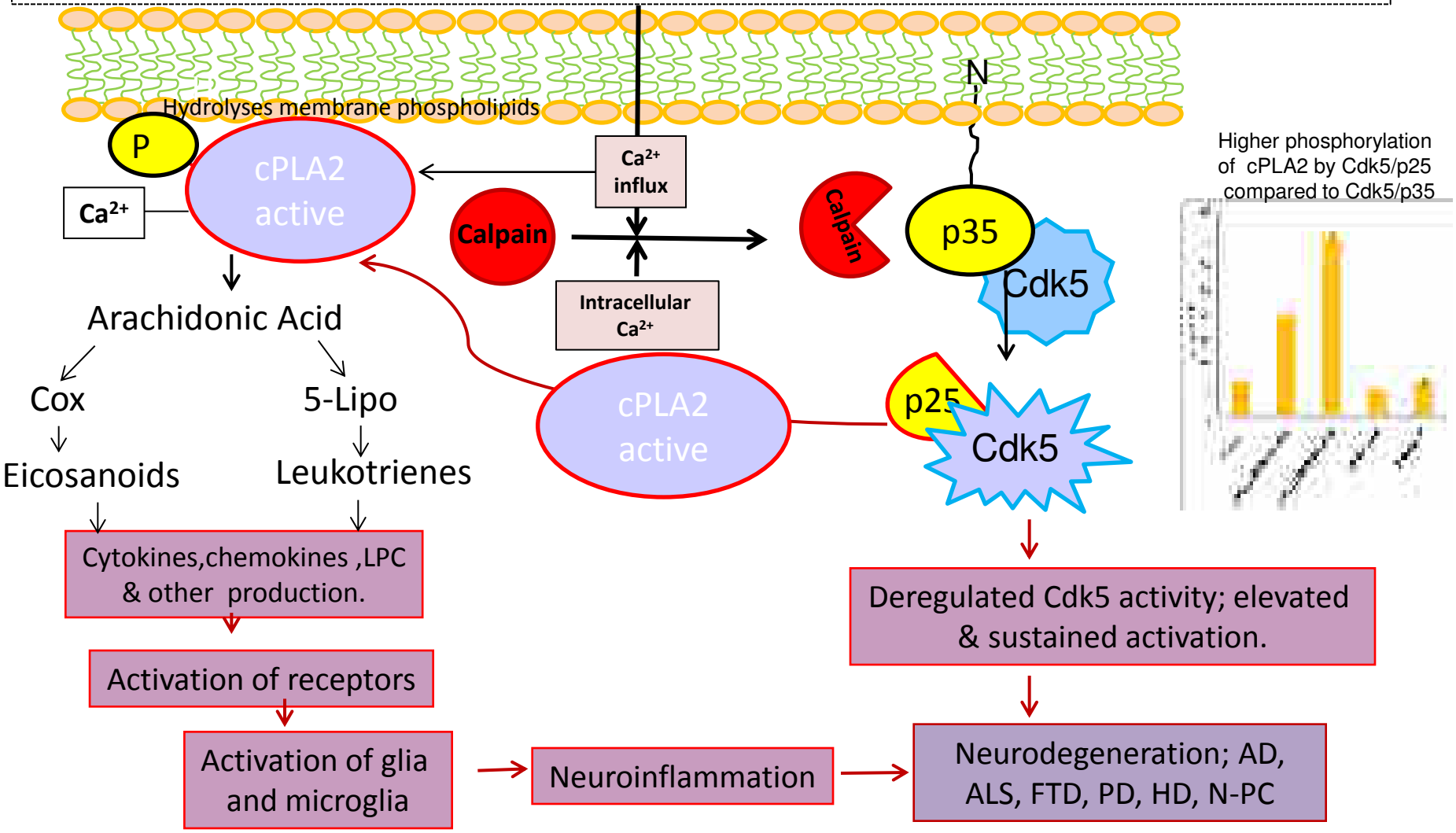
In p25Tg mice neuroinflammation occurs before Aβ and pTau expression ; (Sundaram JR et al.; J. Neurosci 32, 1020-34, 2012).

On the basis of these studies we propose Cdk5/p25 acts like a double edged sword in neuropathology.

Hypothesis

Deregulated Cdk5 activity acts like a double edged sword in neuropathology

Neuronal insults (e.g. Aβ, Glutamate toxicity, oxidative stress, Mutations (e.g.SOD1), neuroinflammation)



CPR-NINDS laboratory, NINDS, NIH

Colleagues

N. Amin
P. Grant
V. Shukla
Binu Kumar
Susan Kuntz
Joe Steiner
M. Bhaskar

Collaborators

Y. Zheng, Department of Nephrology,
Ningxia People's Hospital, Province, China
S. Kesavapany GSK, Singapore
L.H. Tsai, , J. Soe MIT, Boston

Thank You

WT mouse : single i.p 40mg/kg wt (0.2mM)

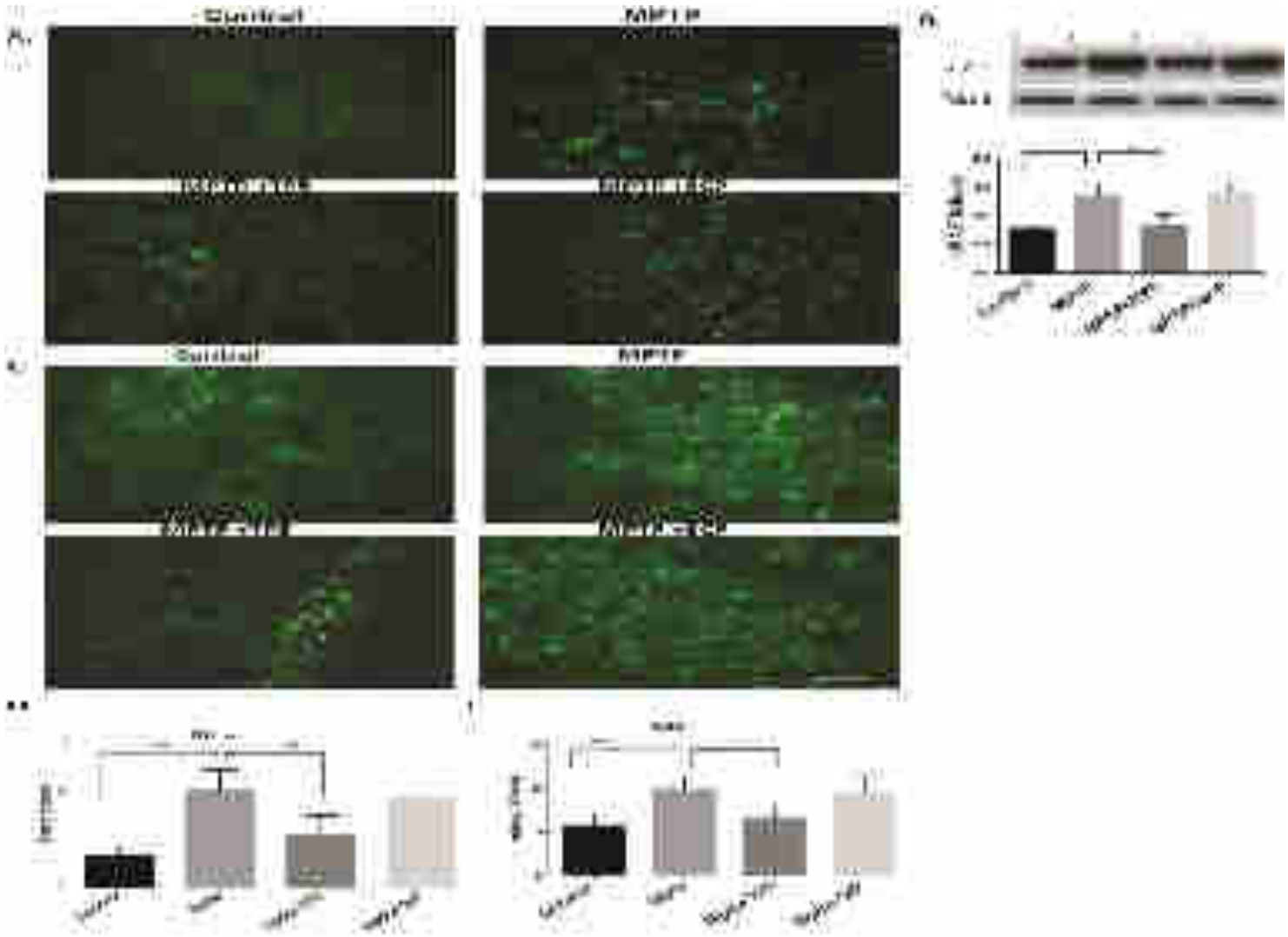
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After 4^{th} and 7^{th} day, TFP5 was observed in the following regions:
(12µm thick frozen sections)

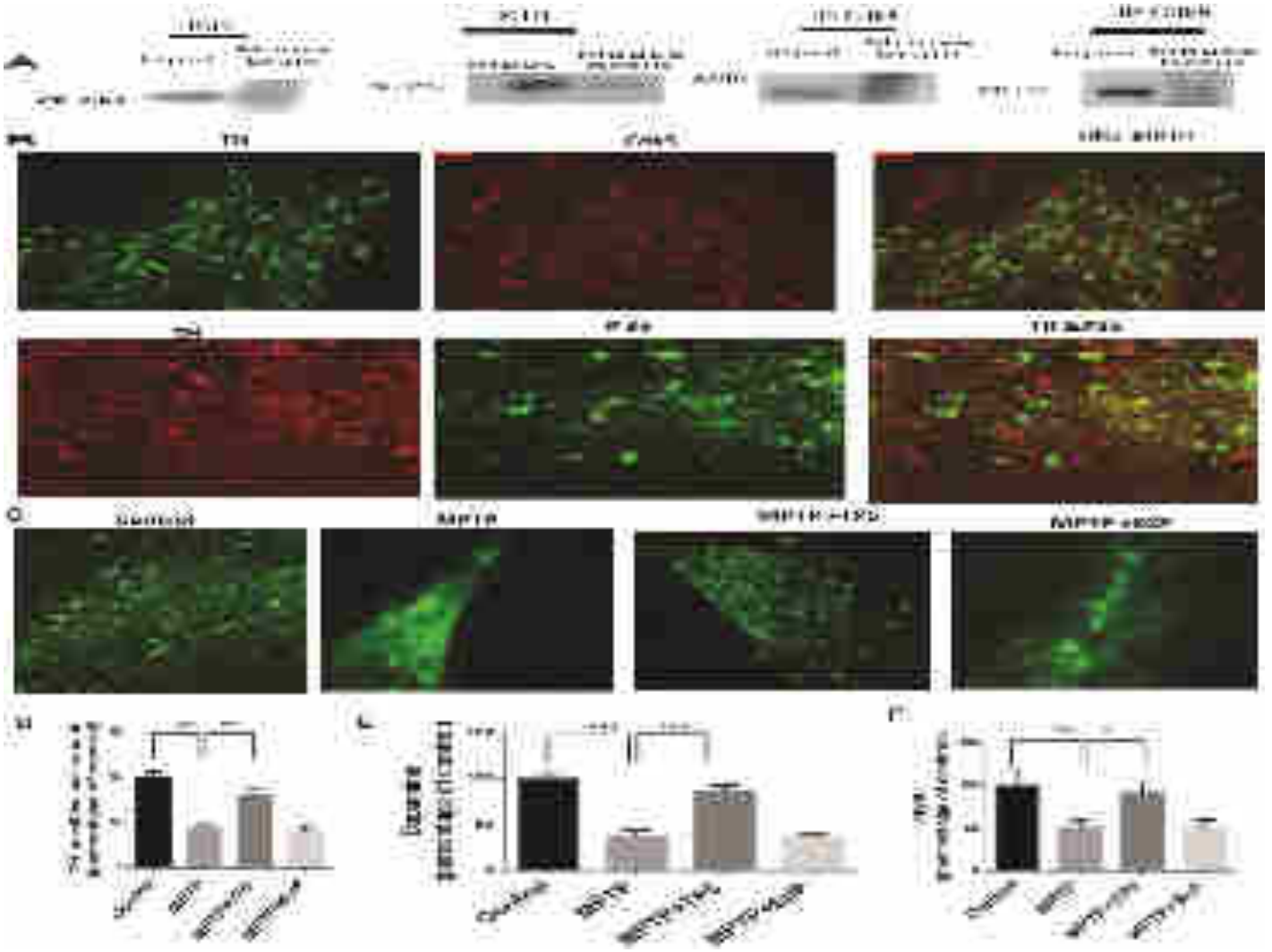
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Regions	Relative expression	
	Day 4	Day 7
Brain:		
Cortex	++++	+++
Hippocampus	+++	++
Cerebellum	+++	+
Pancreas	++++	+++
Kidney	+++	++
Heart	++	+
Liver	+	-

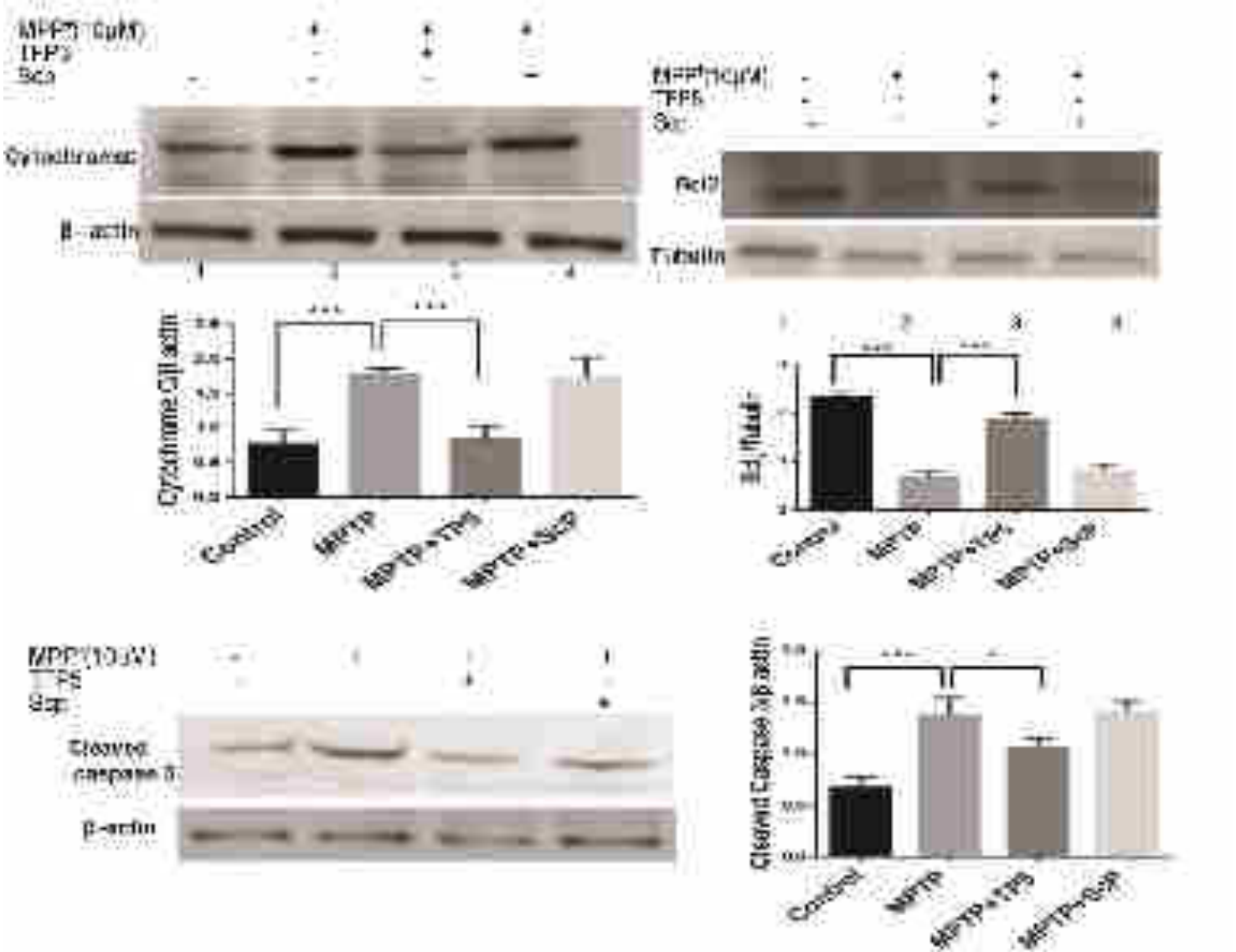
TP5 suppresses MPTP-induced astroglial and microglial activation in the SN in vivo



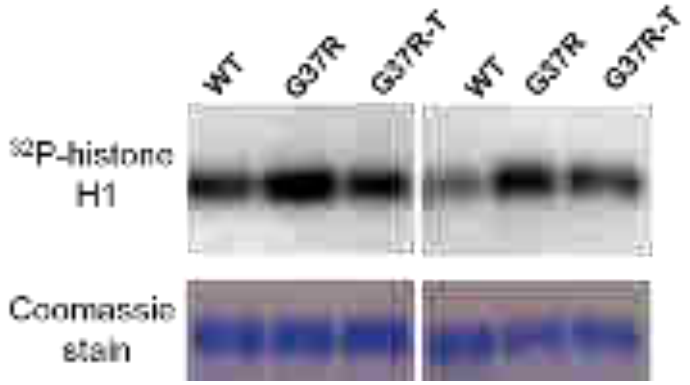
Dopaminergic neurons of the SN express Cdk5/p35 and are protected from cell death by TP5 after MPTP induction

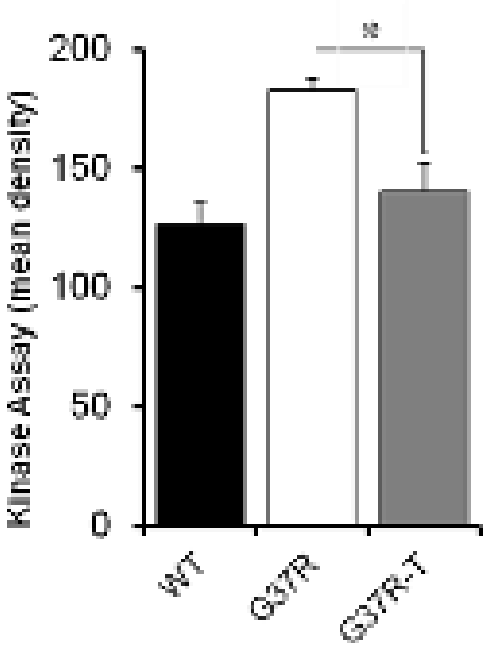


Apoptosis is prevented in SN neurons of PD model mice(in vivo studies) upon TFP5 treatment

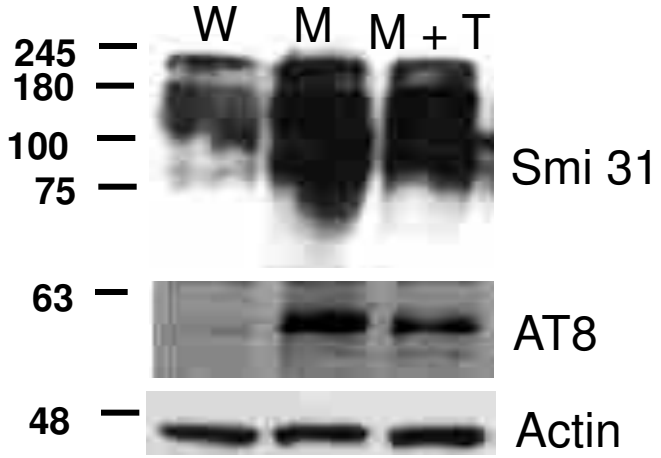


Elevated Cdk5 activity of G37R ALS model mice brain reduced to wild type activity upon TFP5 treatment

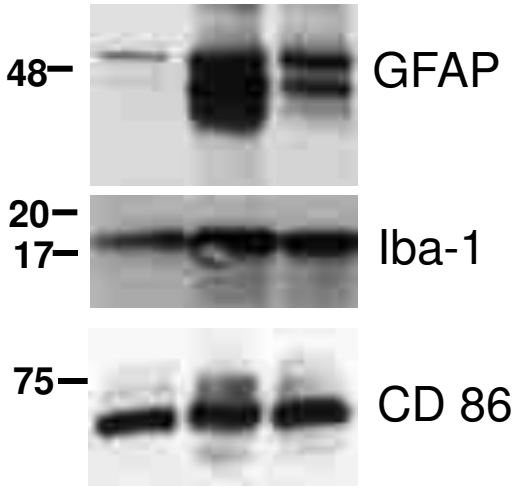




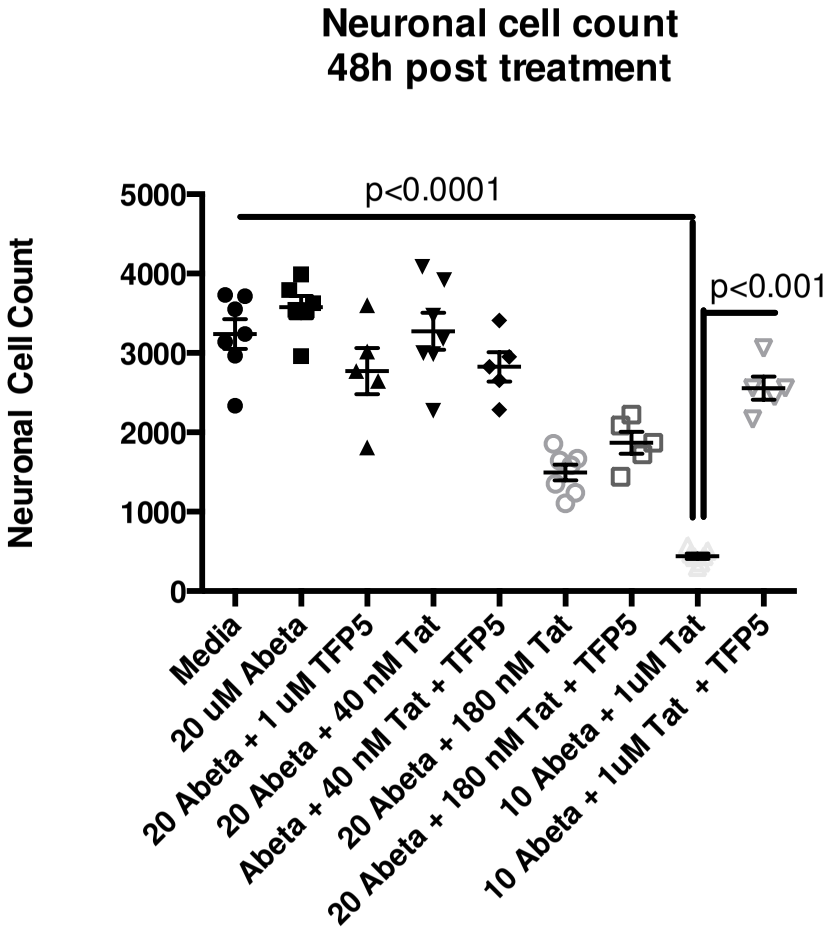
Reduction in phospho - NF and pTau after TFP5 treatment in G37R mice



Neuroinflammation is reduced post TFP5 treatment in G37R mice



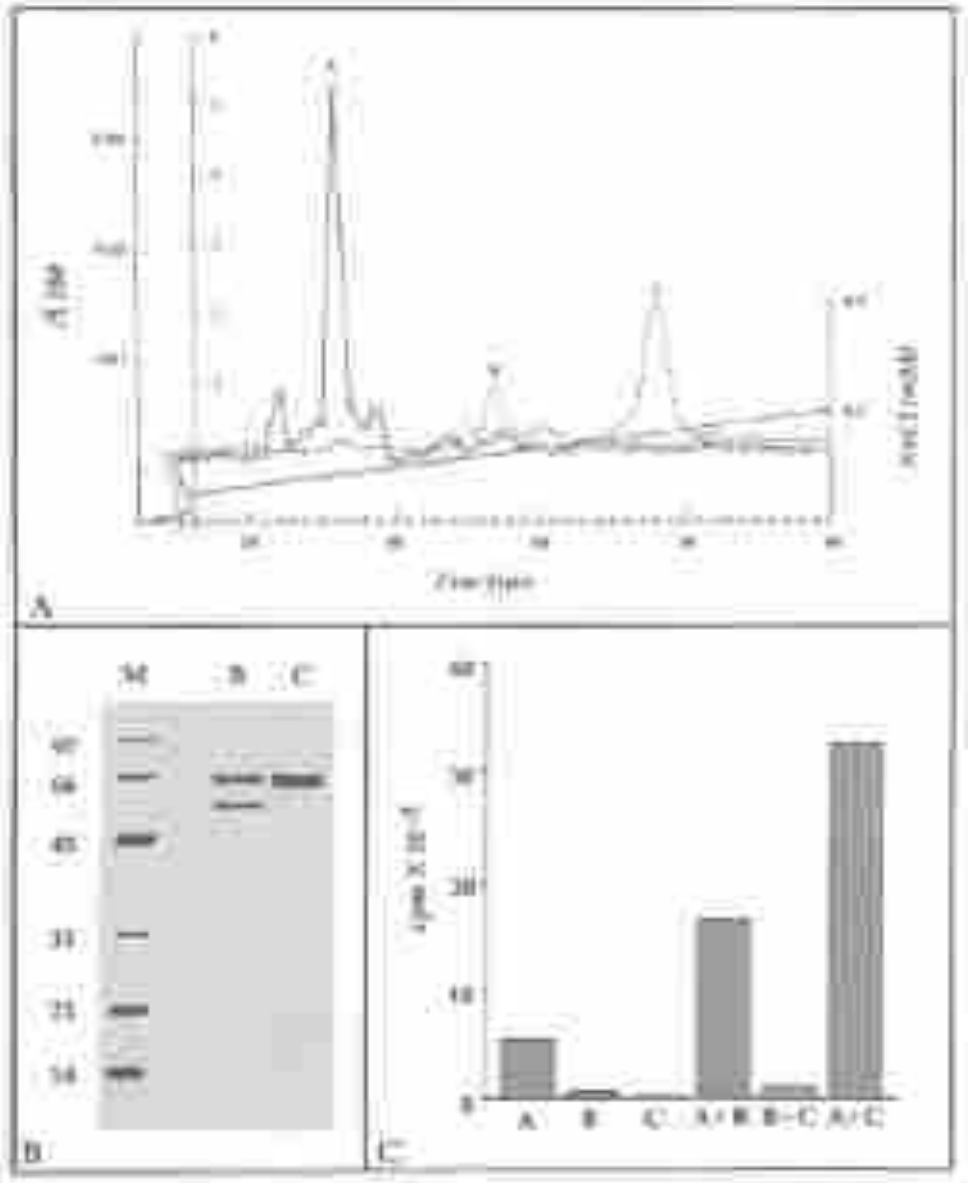
TFP-5 prevents neuronal cell loss in Abeta/Tat treated neurons

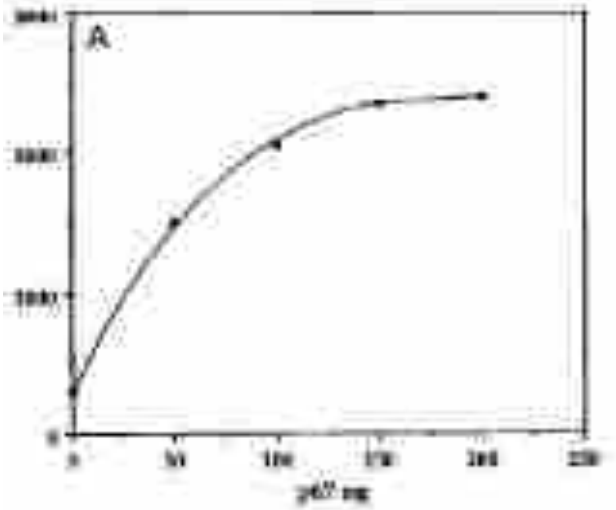


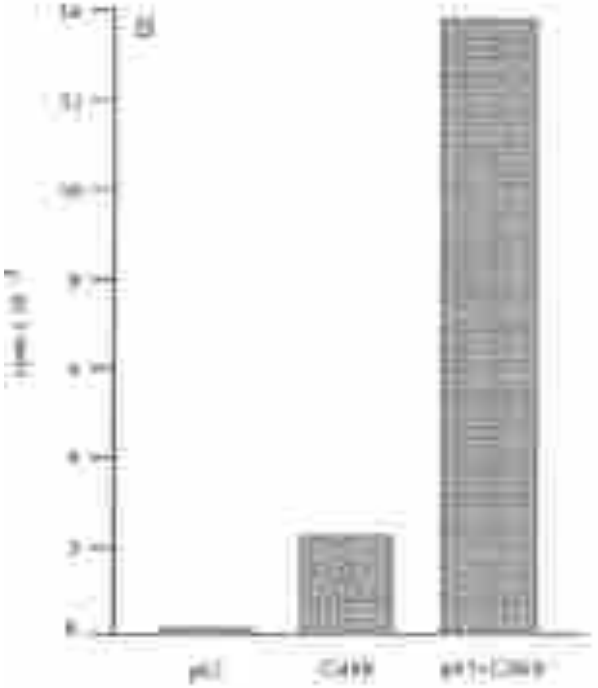
Selectivity of TFP5

***In situ and in vivo* Cdk5 exists as a multimeric complex , with cytoskeletal and synaptic proteins.**

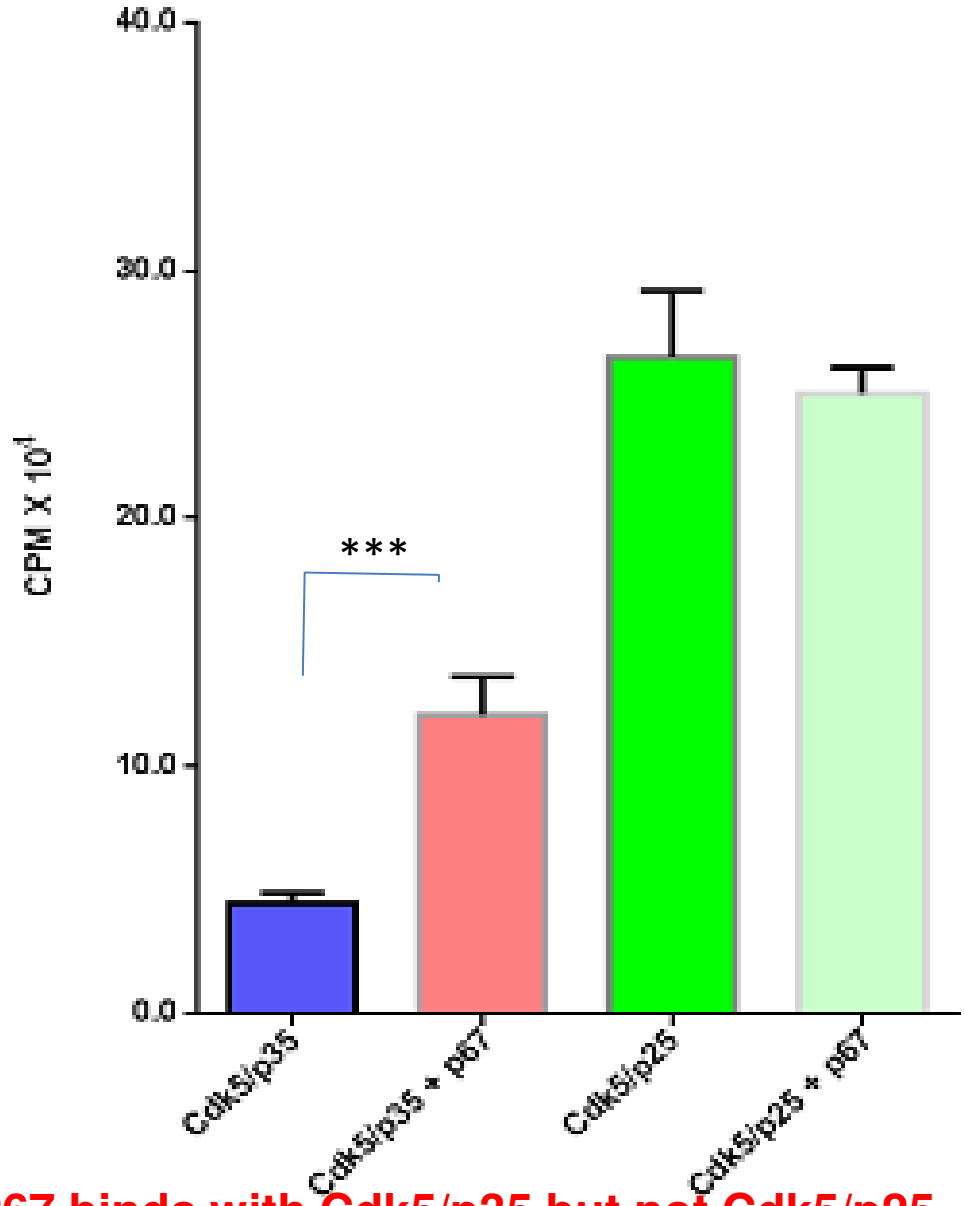
Cdk5 associates with p67 (Munc/18) and elevates its activity





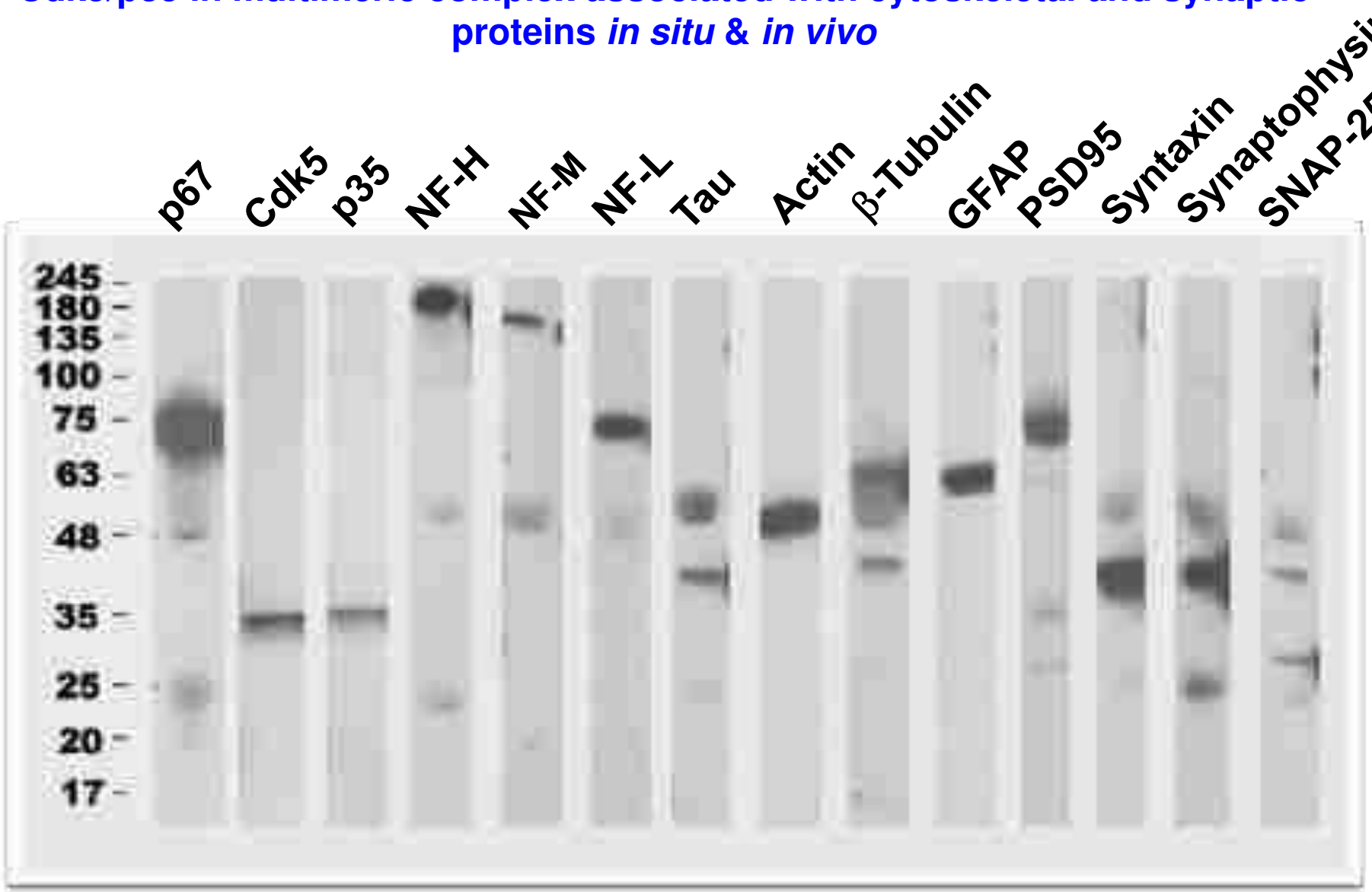


p67 (Munc 18/ n-sec1); P67 affects Cdk5/p35 but not Cdk5/p25 activity



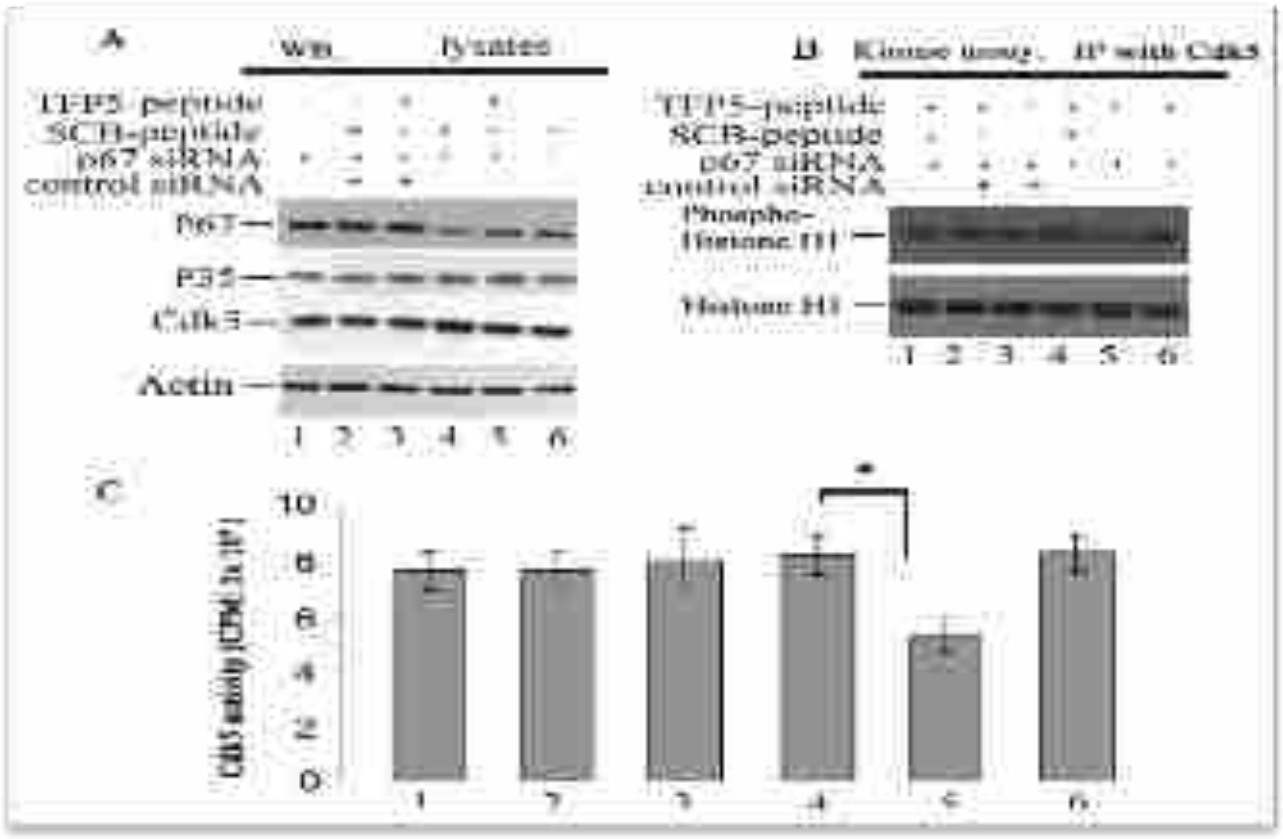
P67 binds with Cdk5/p35 but not Cdk5/p25

Cdk5/p35 in multimeric complex associated with cytoskeletal and synaptic proteins *in situ* & *in vivo*



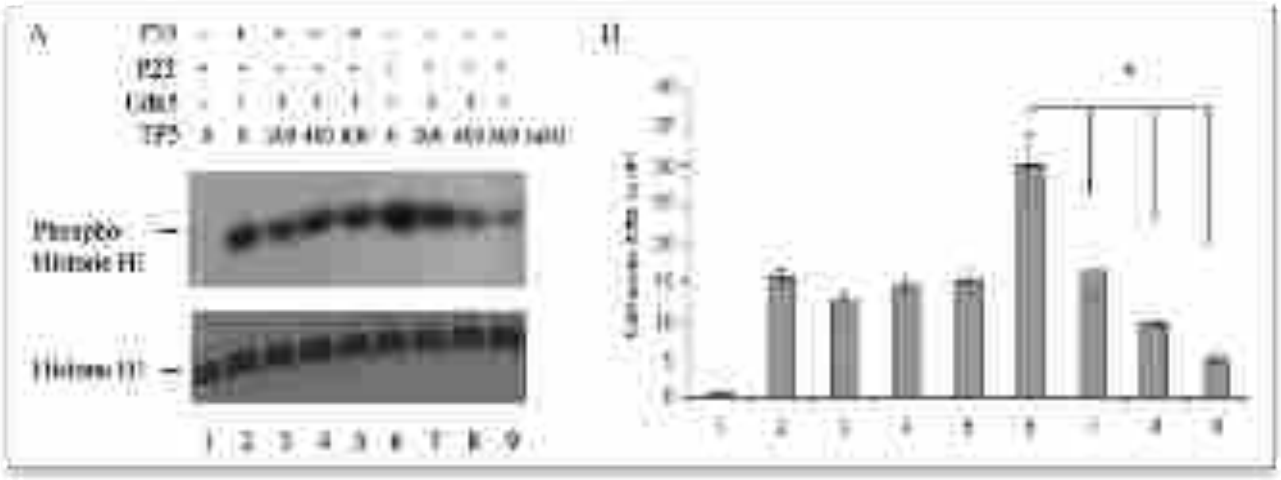
Rat brain lysate was immunoprecipitated (IP) with p67 (Munc 18) antibody and then immunodetected with Western blot (WB) analysis using respective antibodies as shown

Cdk5/p35 activity in cortical neurons transfected with p67siRNA is inhibited by TFP5



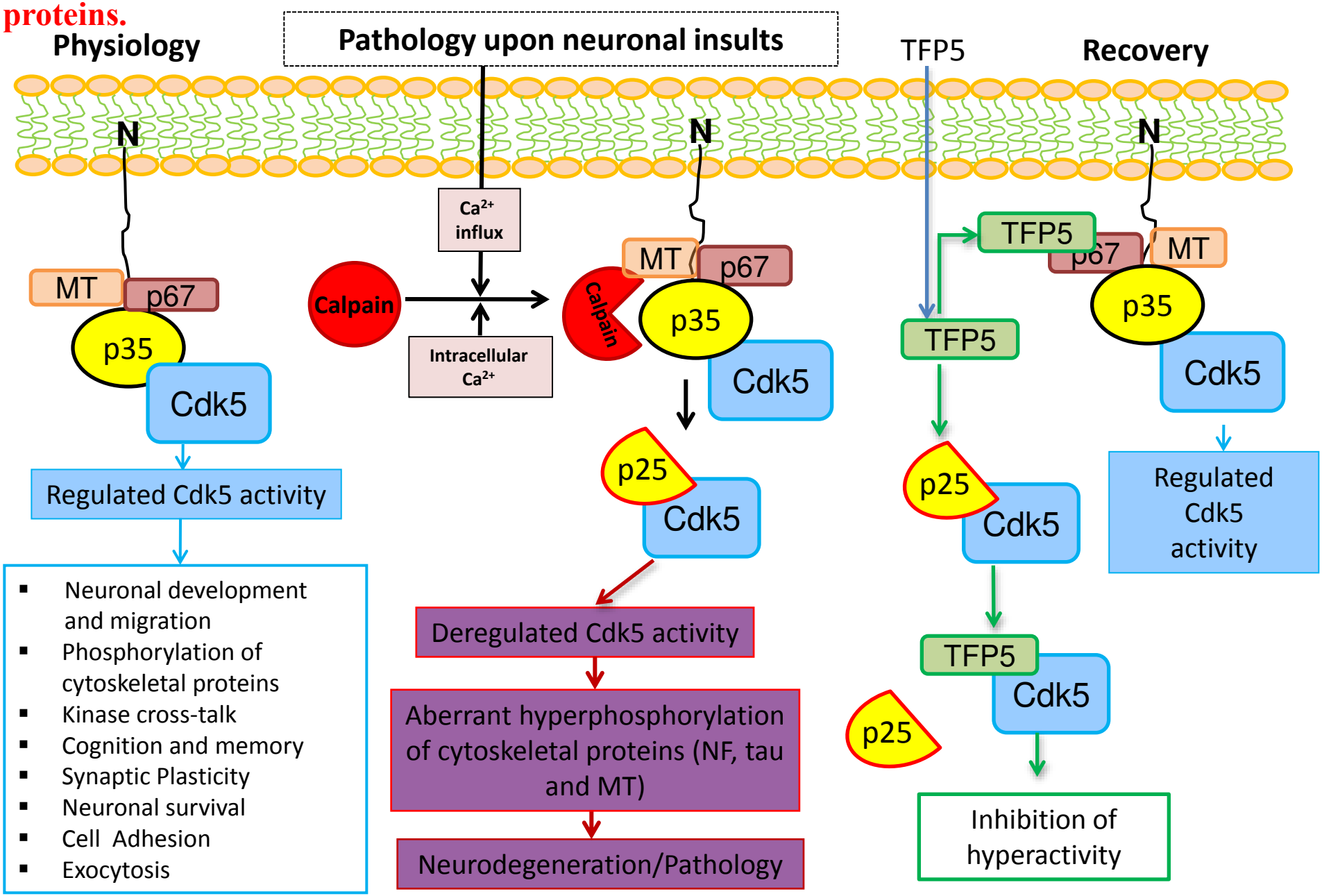
The expression of p67 was "knocked down" in cortical neurons transfected with p67 siRNA (upper panel, lane 4-6); however, p35 and Cdk5 expressions were unaffected (two middle panels). B: The Cdk5 kinase activity in the cells transfected with p67 siRNA and treated with TFP5 was inhibited (lane 5), C: The bar graph shows the mean density of autoradiographs of the Cdk5 kinase activity. The results represent the values of three experiments. * $p<0.01$.

Only Cdk5/p25 activity is inhibited by TP5 in HEK 293 cells overexpressing Cdk5/p35 or Cdk5/p25. p35 interaction with macromolecules (e.g,microtubules) spares Cdk5/p35 activity.



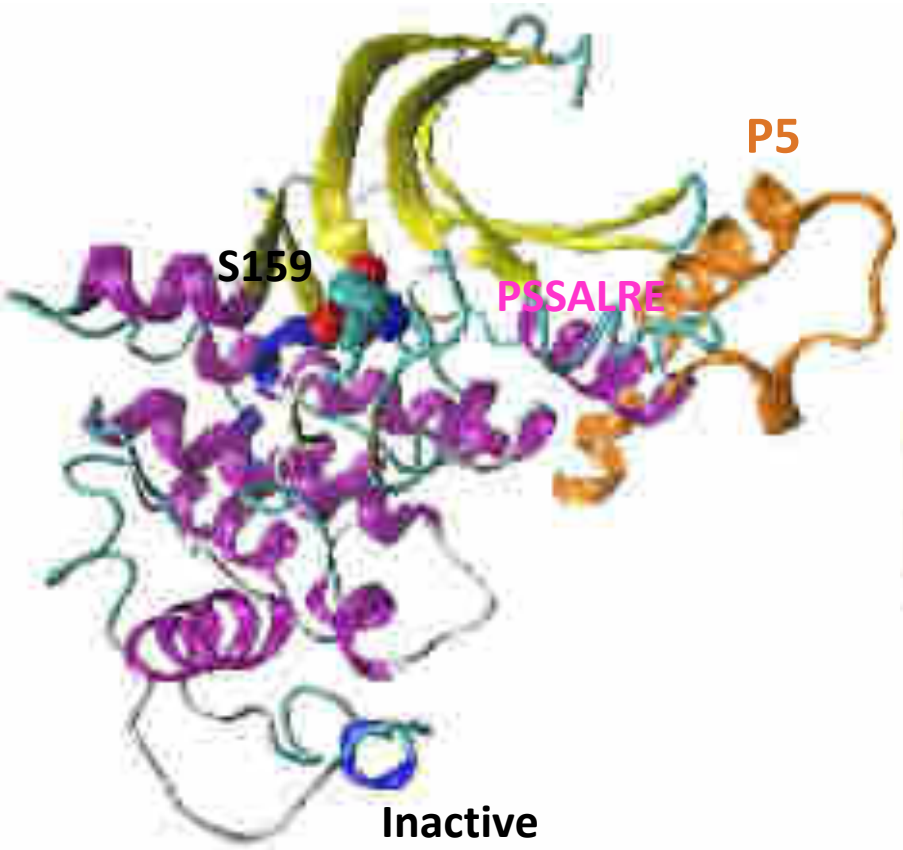
HEK293 cells over expressing p35/Cdk5 or p25/Cdk5 were treated with different concentrations of the TFP5. p35/Cdk5 kinase activities (lane 2-5) were unaffected; p25/Cdk5 kinase activities , however, were inhibited (lane 6-9). B. The bar graph quantitates the results. Standard errors are shown for three experiments* p<0.01

Summary: CIP and or TFP5 selectively inhibits the deregulated Cdk5/p25 but not Cdk5/p35, the regulated, normal activity in the presence of cytoskeletal and or synaptic proteins.



Proposed molecular model; p5/TFP5 competes with p25 and inhibits Cdk5 deregulated activity

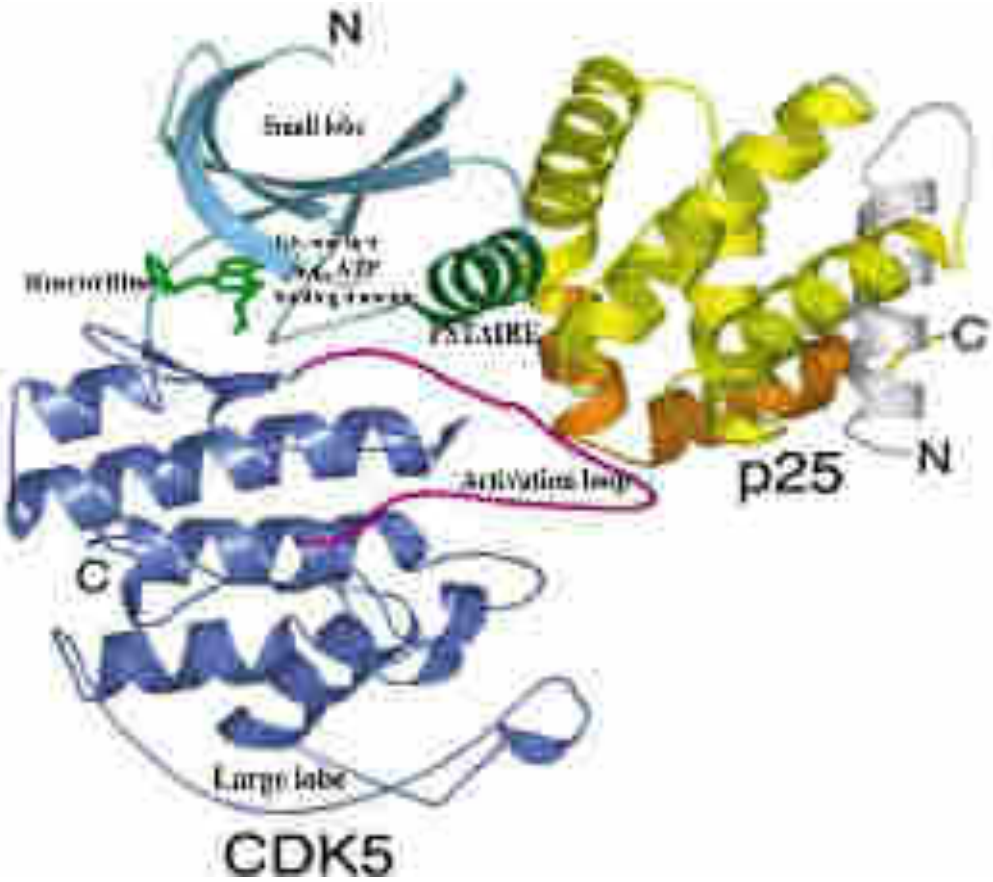
Approximate position of p5 peptide in the inactive Cdk5 (Cdk2 homology structure)



Computational Molecular Modeling

Position of p5 peptide in Cdk5/p25 crystal (1H4L)

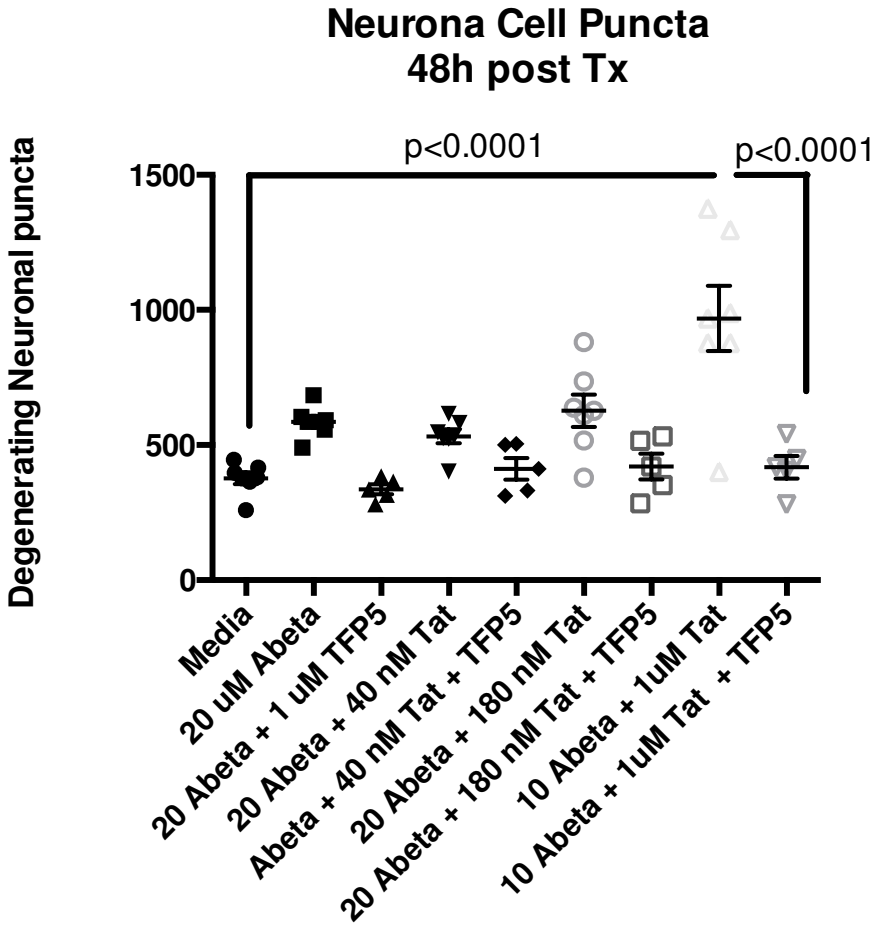
Tarricone et al., 2001; Mapeli et al., 2005



Active

Km (p25): 70-100 nM
Ki (p5): 20nM

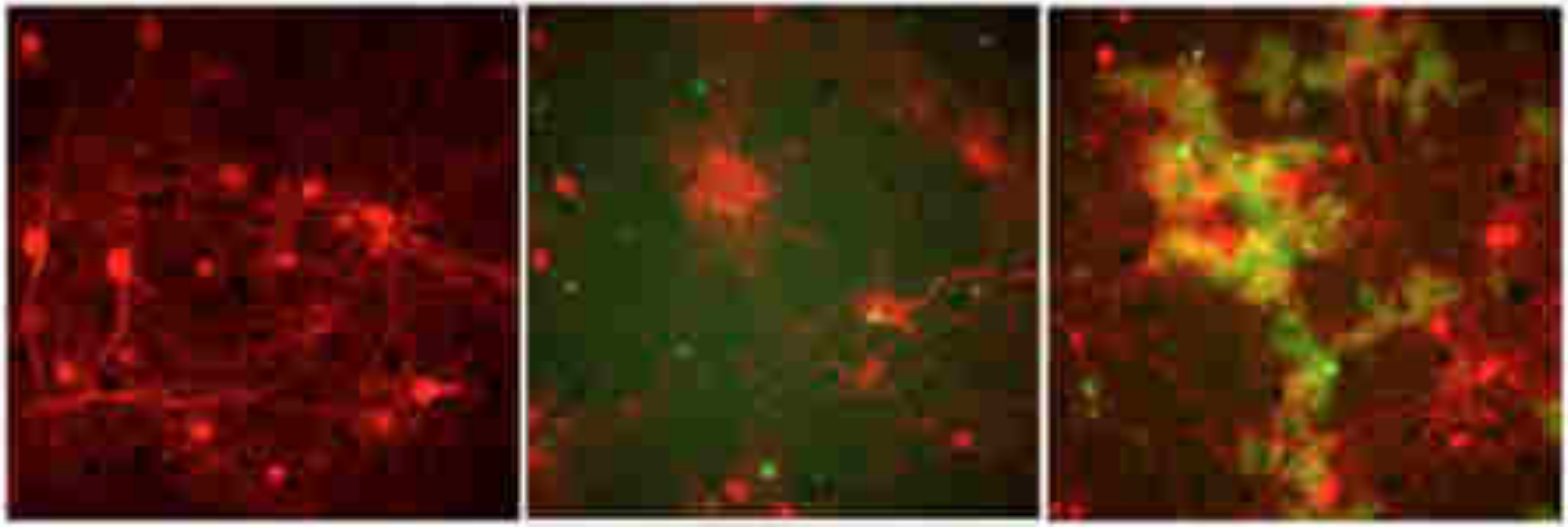
TFP-5 attenuates neuronal fragmentation following Ab/Tat treatment for 48h



Media/Saline

Media/1 μM TFP-5

20 μM Aβ + 1 μM TFP-5



βIII Tubulin-tdTomato expressing neurons
TFP-5

TFP5 Reduces significantly ALS phenotypes in SOD1 mutant mice; A potential therapeutic candidate for ALS

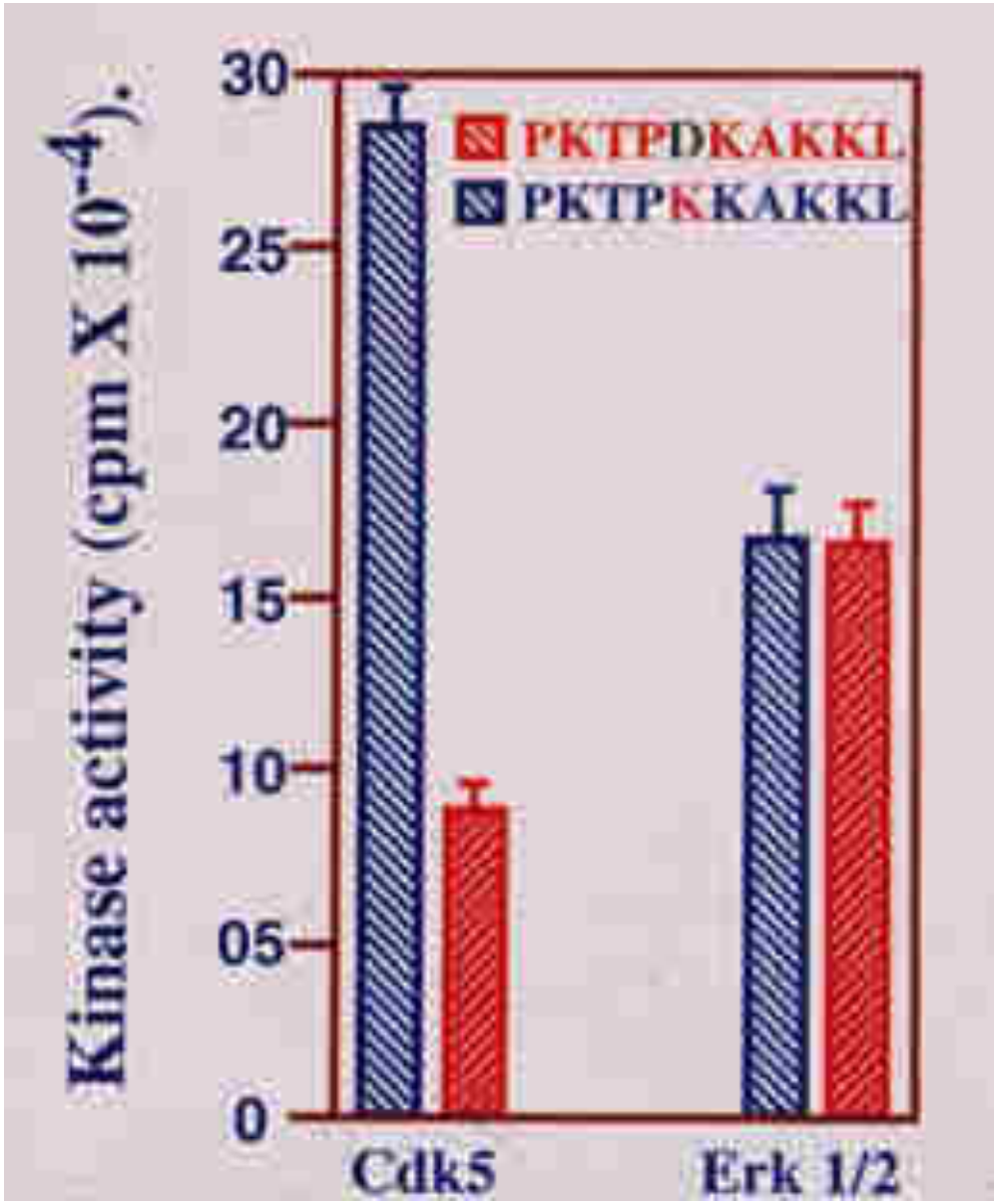
Conclusion:

TFP5 treatment prevents / reduces AD phenotypes in P25 & 5XFAD Tg, familial and p25 Tg sparodic AD model mice and can be a therapeutic candidate for AD pathology.

The higher levels of p25 expression (Cdk5 deregulation & hyperactivation) in this sporadic AD model mice induces robust expression of inflammation/neurodegeneration.

To understand the progression of neuroinflammation , it is important to study the early time course of expression of inflammatory factors in p25Tg mice which will provide a better understanding of the onset of pathology.

Unlike MAKPs (e.g. Erk1/2), Cdk5 activity depends upon the nature of the amino acid residue following the proline



Kinetic Mechanisms (unique to Cdk5)

- 1. CDK5/p35 has higher affinity for substrate peptides/proteins compared to ATP
- Km (ATP) =300μM
- Km (peptide) =100μM

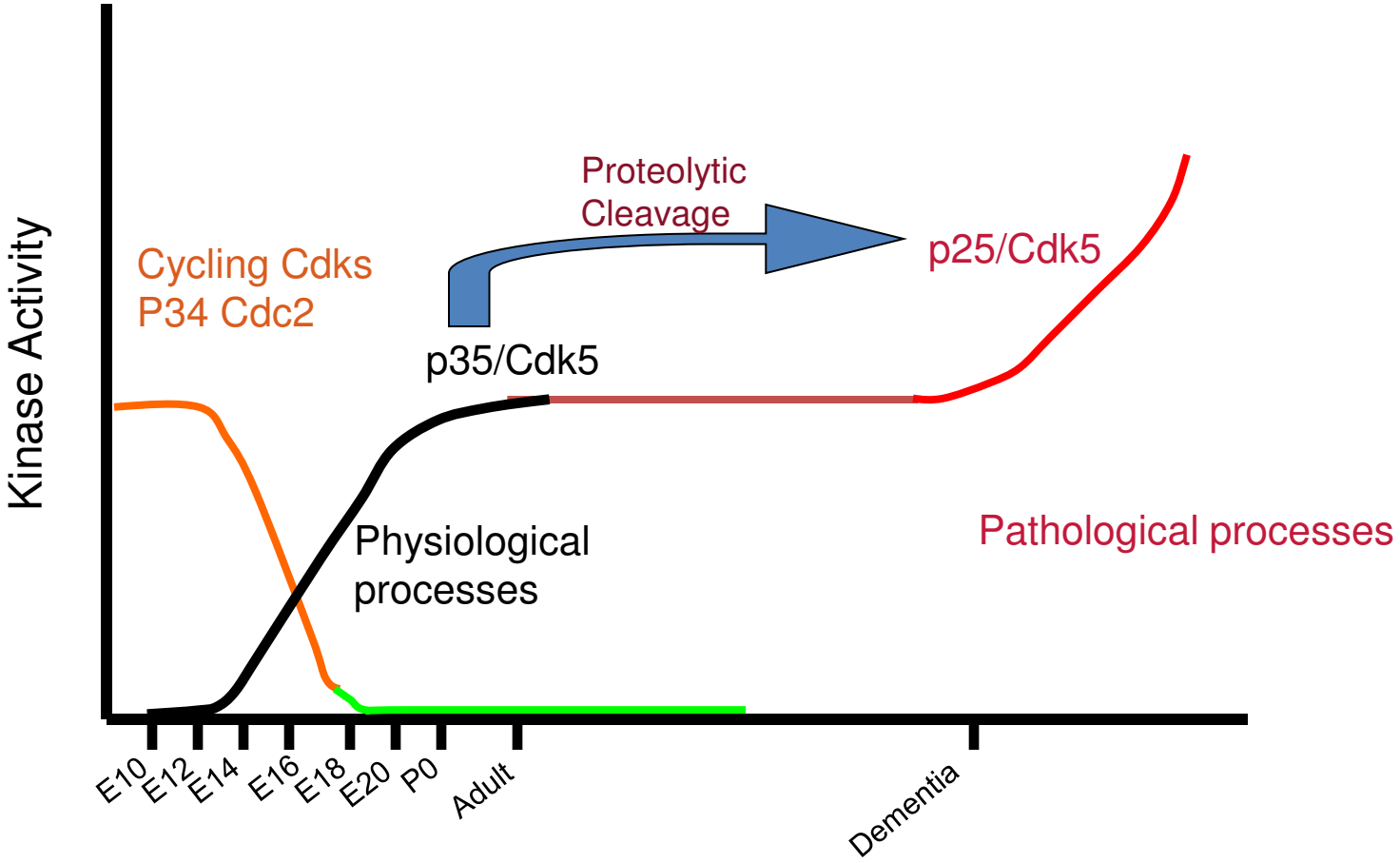
2. Therefore, the enzyme kinetics can be explained with a two substrate binding model for CDK5/p35 activation. First, with peptides/proteins, then second ATP.

- Cdk5 has also very high affinity for its activators
- Km (activator): 70-100 nM

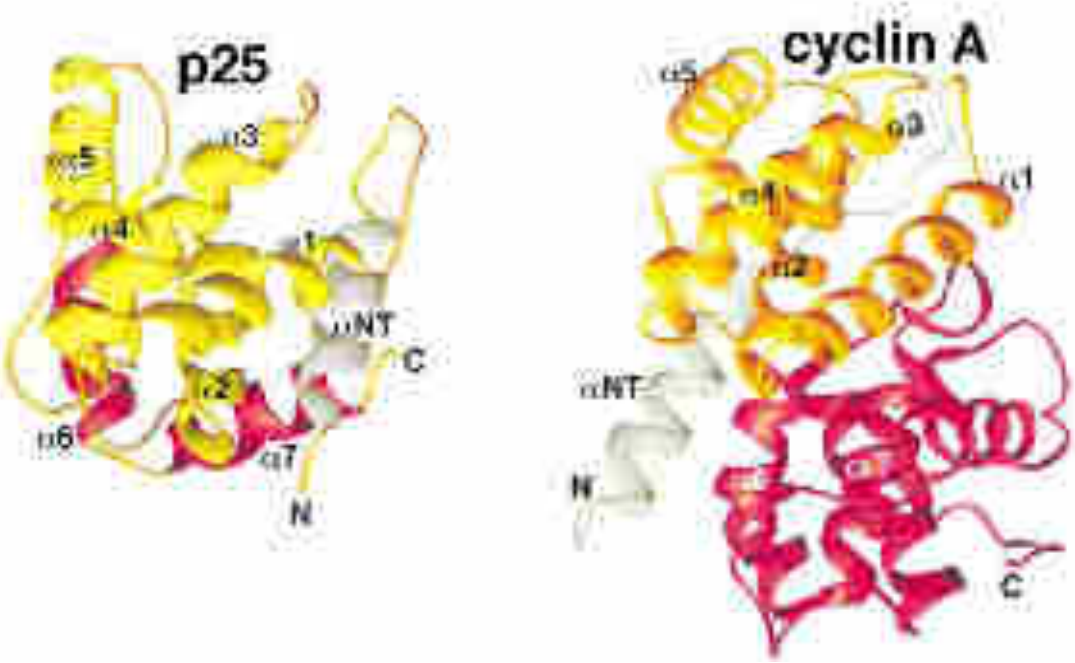
- 3. CDK5 has an order of magnitude lower affinity for ATP compared to other kinases
- CDK5, Km (ATP): 300- Other kinases Km (ATP): 10-20μM

- 4. Consensus sequence X-S/TP-K/R-X; X, neutral or basic amino acid

Regulation and Deregulation of Cdk's activity



Structural basis of Cdk5 activation by p35/p25
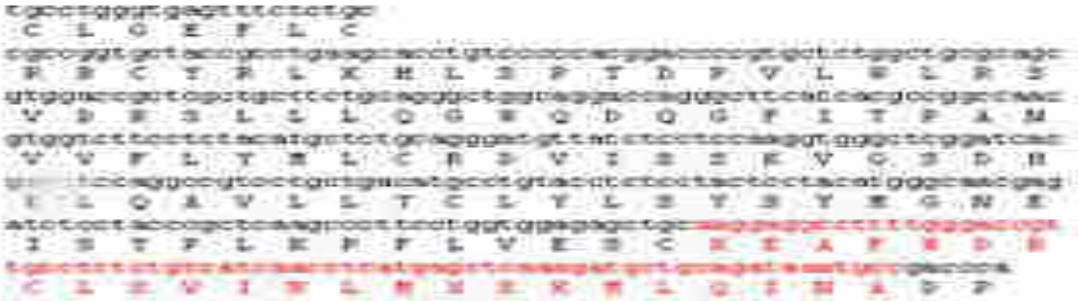


Tarricone et al., Molecular cell, 2001

P5 is a better inhibitor than CIP

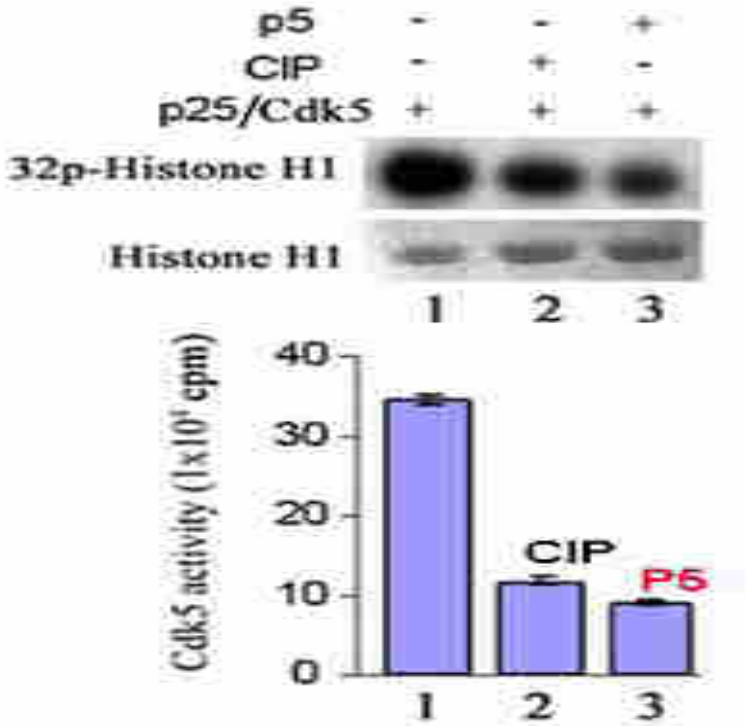
A

CIP

P5



B



Kinetic Mechanisms (unique to Cdk5)

- 1. CDK5/p35 has higher affinity for substrate peptides/proteins compared to ATP
- Km (ATP) =300μM
- Km (peptide) =100μM

2. Therefore, the enzyme kinetics can be explained with a two substrate binding model for CDK5/p35 activation. First, with peptides/proteins then ATP.

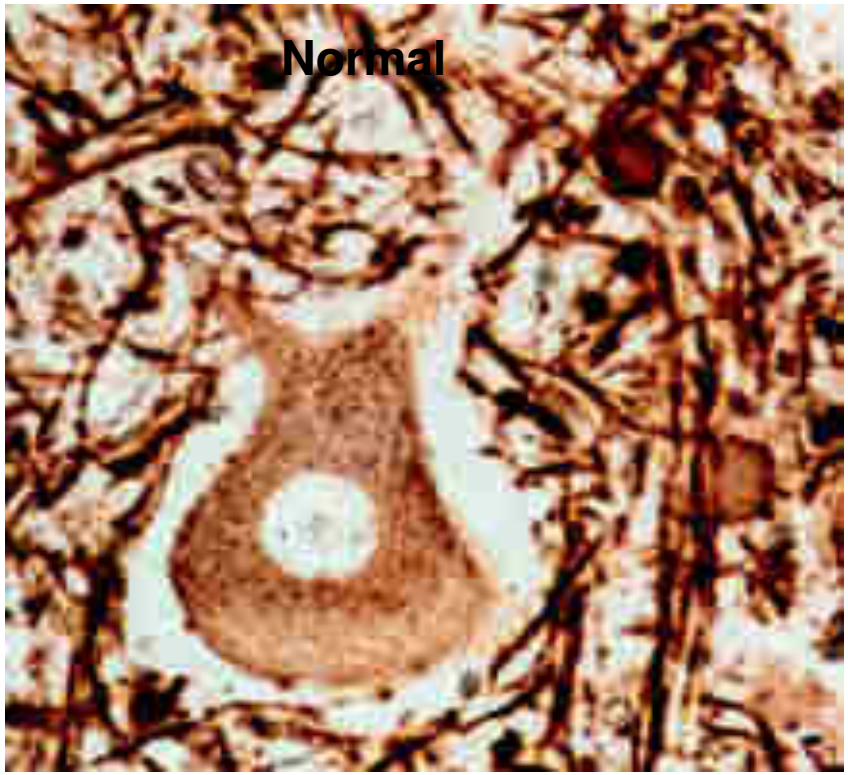
- CDK5 has also very high affinity for its activators
- Km (activator): 70-100 nM (p35)

- 3. CDK5 has an order of magnitude lower affinity for ATP compared to other kinases
- CDK5, Km (ATP): 300μM
- Other kinases Km (ATP): 10-20μM

In mature neurons cytoskeletal protein phosphorylation is topographically & tightly regulated under physiological conditions.

Human cervical spinal neuron

Control



ALS



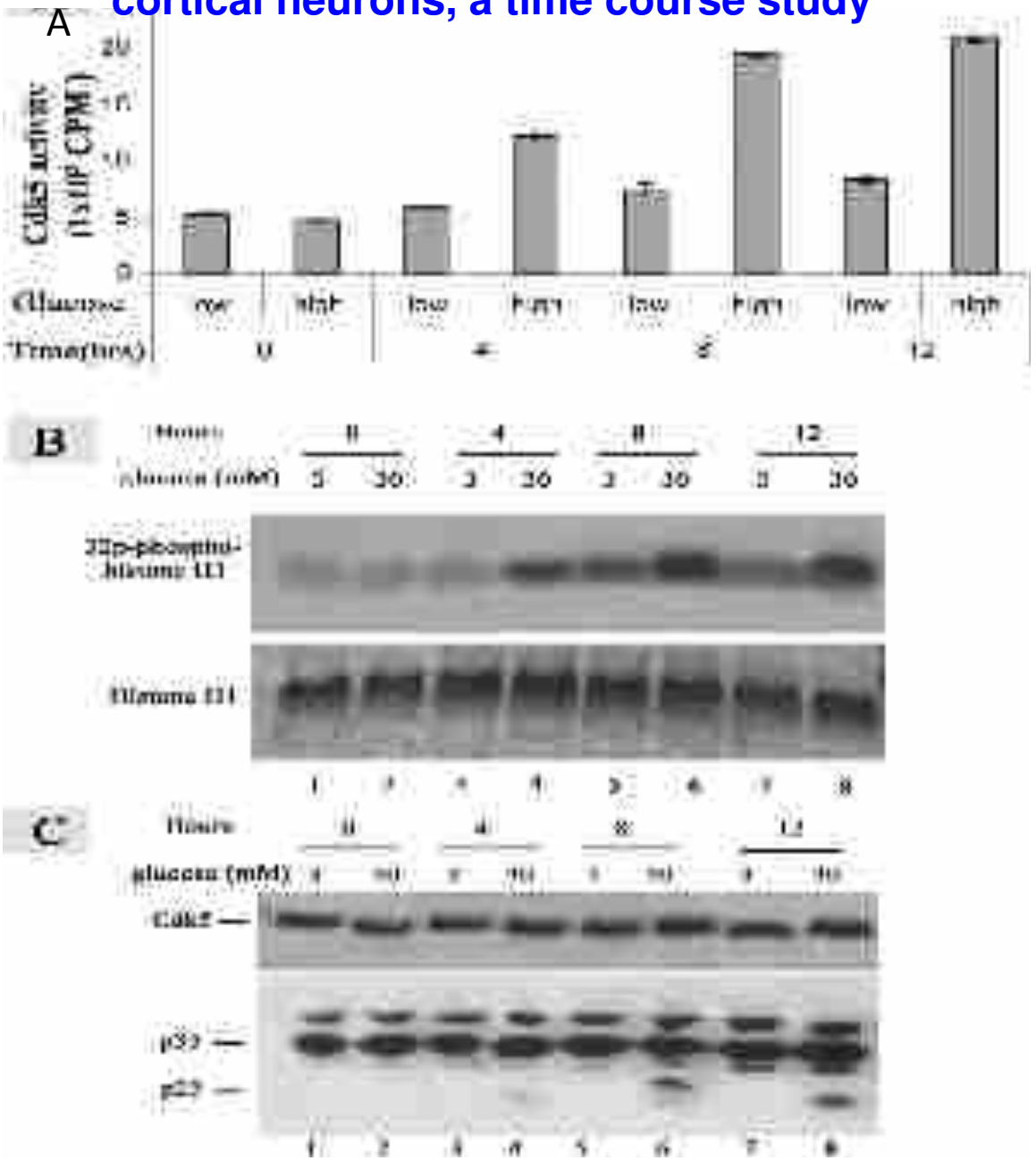
To record field excitatory postsynaptic potentials, transverse hippocampal slices were prepared from mice. In brief, the brain was rapidly removed and transferred to ice-cold, oxygenated (95 % O_2 and 5 % CO_2) cutting solution containing (mM) 211 sucrose, 3.3 KCl, 1.3 NaH_2PO_4, 0.5 $CaCl_2$, 10 $MgCl_2$, 26 $NaHCO_3$ and 11 glucose. Hippocampal slices were cut with a Leica VT1000S vibratome (Leica) and transferred for recovery to a holding chamber containing oxygenated artificial cerebrospinal fluid (ACSF) consisted of (mM) 124 NaCl, 3.3 KCl, 1.3 NaH_2PO_4, 2.5 $CaCl_2$, 1.5 $MgCl_2$, 26 $NaHCO_3$ and 11 glucose at 28-30 ° C for at least 1 h before recording. CA1 field potentials evoked by Schaffer collateral stimulation were measured. After recording of stable baseline (at least 20 min of stable responses), LTP was induced by two episodes of theta burst stimulation (TBS) with 10 s intervals. TBS consisted of ten bursts (each with four pulses at 100 Hz) of stimuli delivered every 200 ms. Recordings were performed using an AM-1800 Microelectrode amplifier (A-M systems) and a Digidata 1440A A-D converter (Axon Instruments). All data were digitized and analyzed by the use of pClamp 10 software (Axon Instruments).

Sample traces represent fEPSPs at 1 min before (gray trace) and 1 hr after (black trace) TBS.

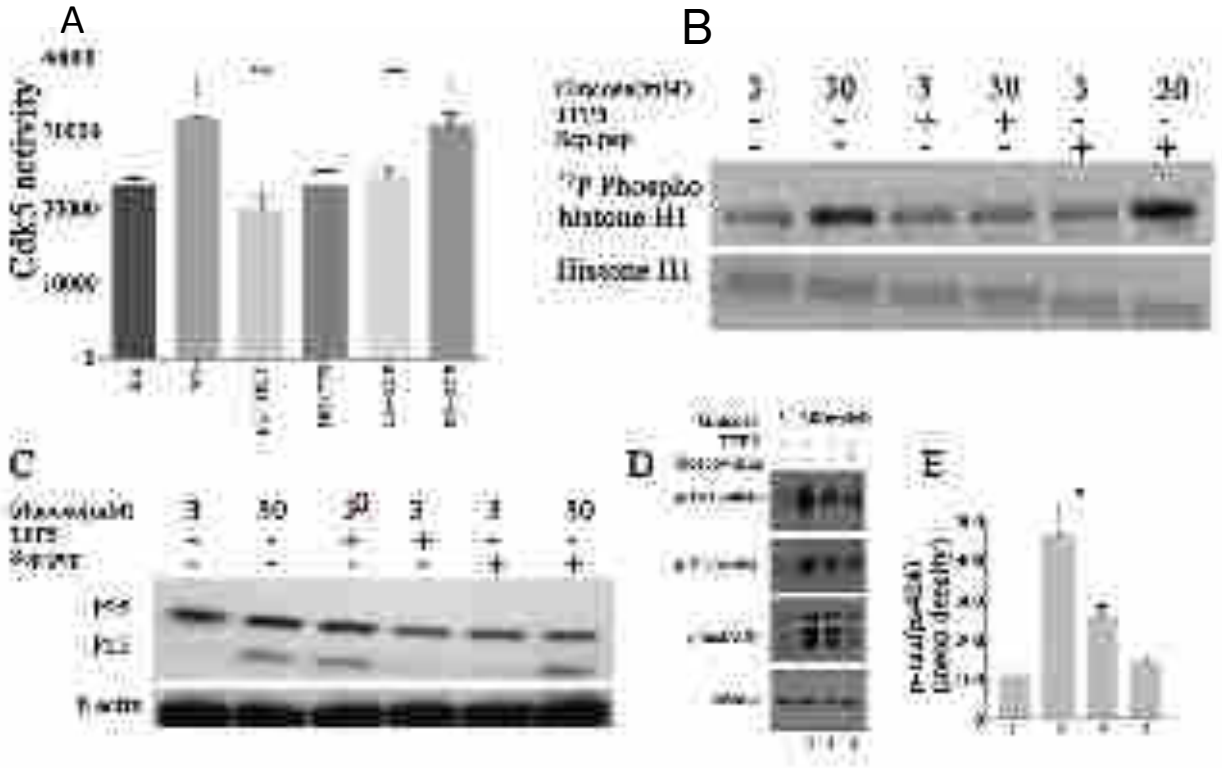
Bar graph : average slopes of fEPSP during the last 10 min of recording (percentage of baseline response)

Control (p25 only - Dox off) : 160.6 ± 6.1 %, 6 slices from 4 mice
CK-p25 + PBS : 125.2 ± 3.7 %, 5 slices from 3 mice
CK-p25 + SC : 137.5 ± 6.2 %, 7 slices from 4 mice
CK-p25 + TFP5 : 165.7 ± 9.0 %, 6 slices from 4 mice

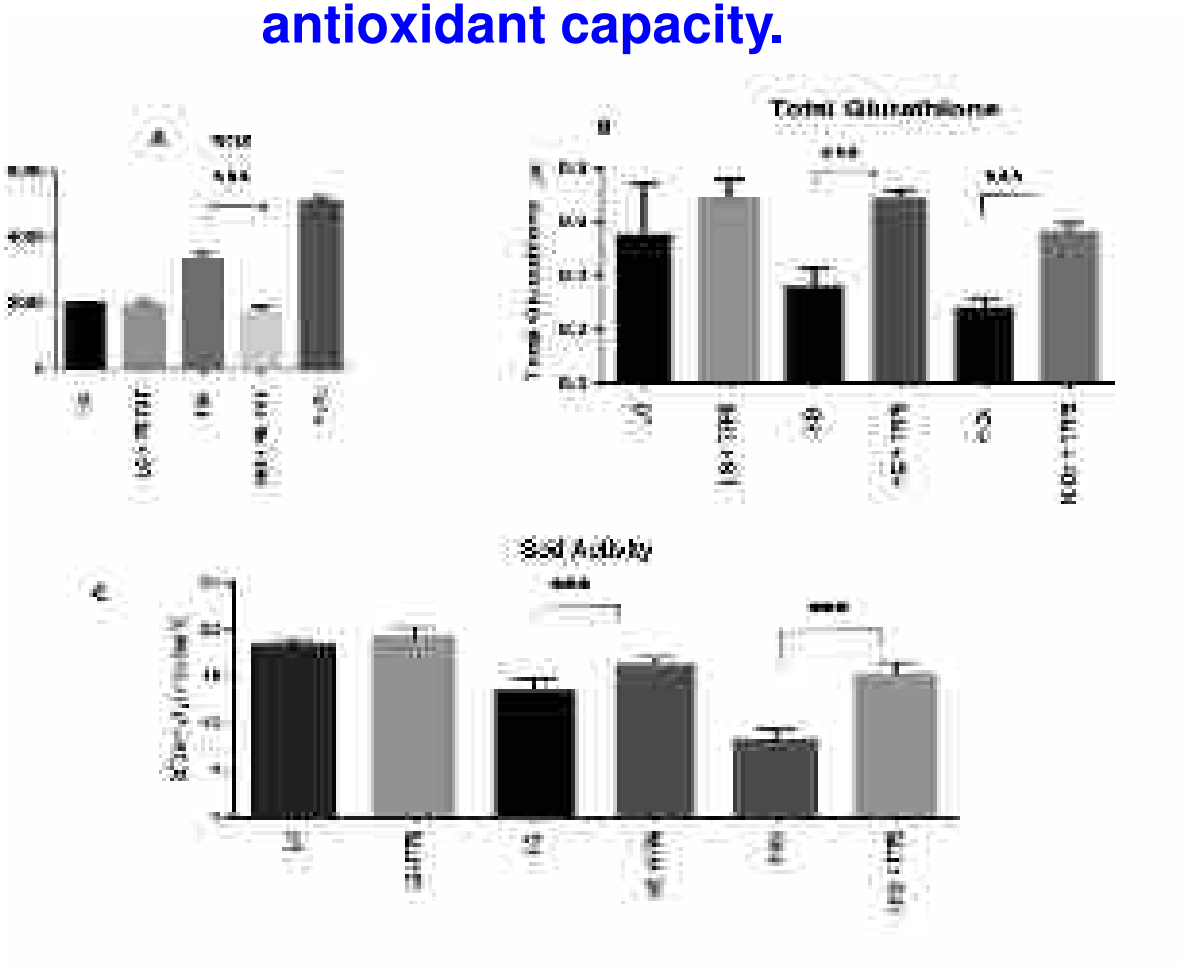
High glucose induces p25 expression and hyper activates Cdk5 activity in cortical neurons, a time course study



Cdk5 hyper activity and tau hyper phosphorylation induced by high glucose is inhibited by TFP5 pretreatment in cortical neurons.

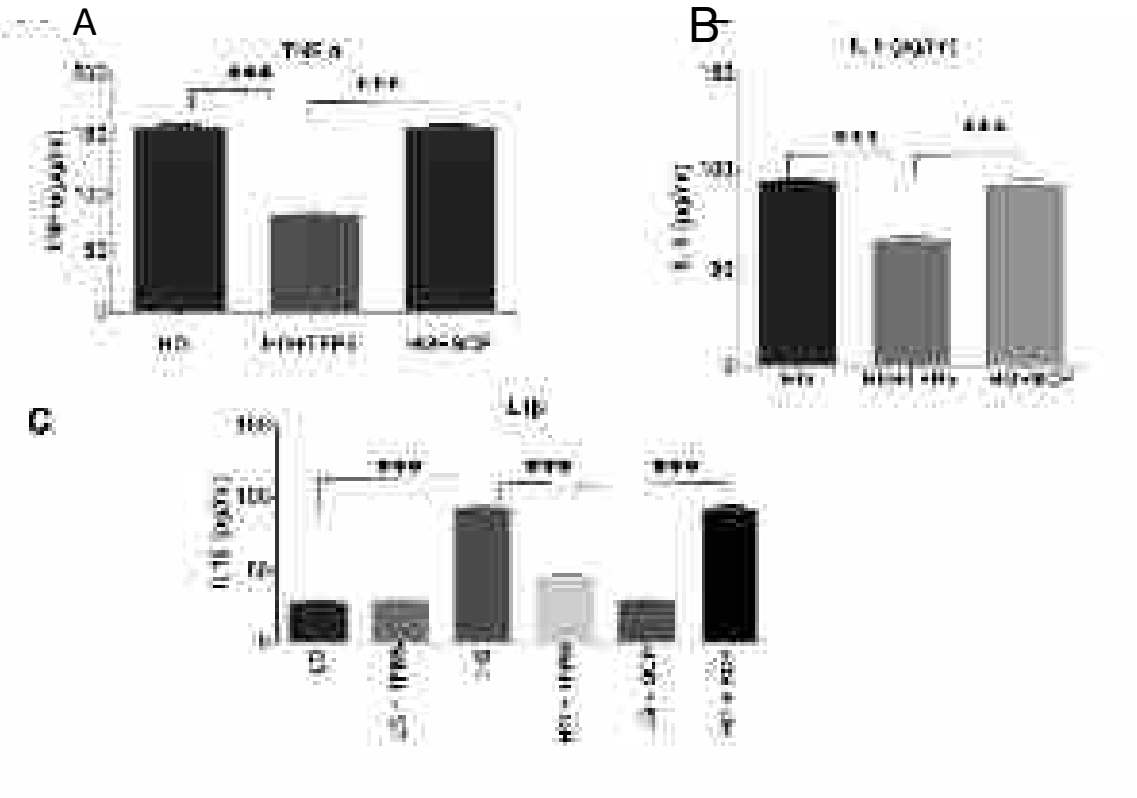


Pre-treatment with TFP5 rescues glucotoxic cortical neurons from oxidative stress due to increased ROS production and decreased antioxidant capacity.



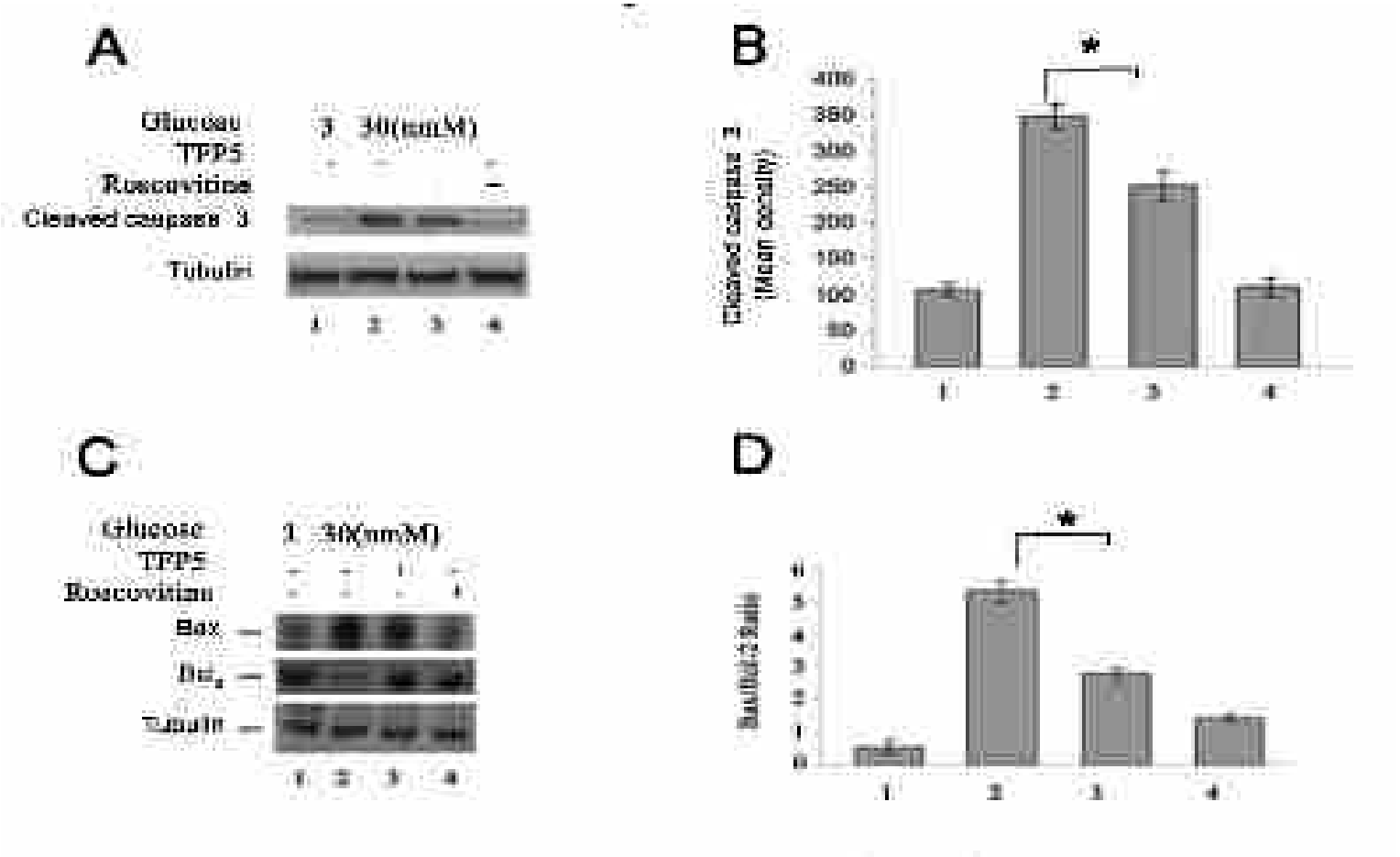
TFP5 behaves like antioxidant

TFP5 reduces increased production of inflammatory cytokines induced by high glucose in cultures of primary cortical neurons; scrambled peptide has no effect



TFP5 as a anti inflammatory agent

TFP5 protects neuronal loss due to HG toxicity.



TFP5 is anti apoptotic

Modified p5 – To pass BBB as TFP5

p5 (24 aa):

KEAFWDRCLSVINLMSSKMLQINA

TFP5:

Modified HIV-TAT-peptide

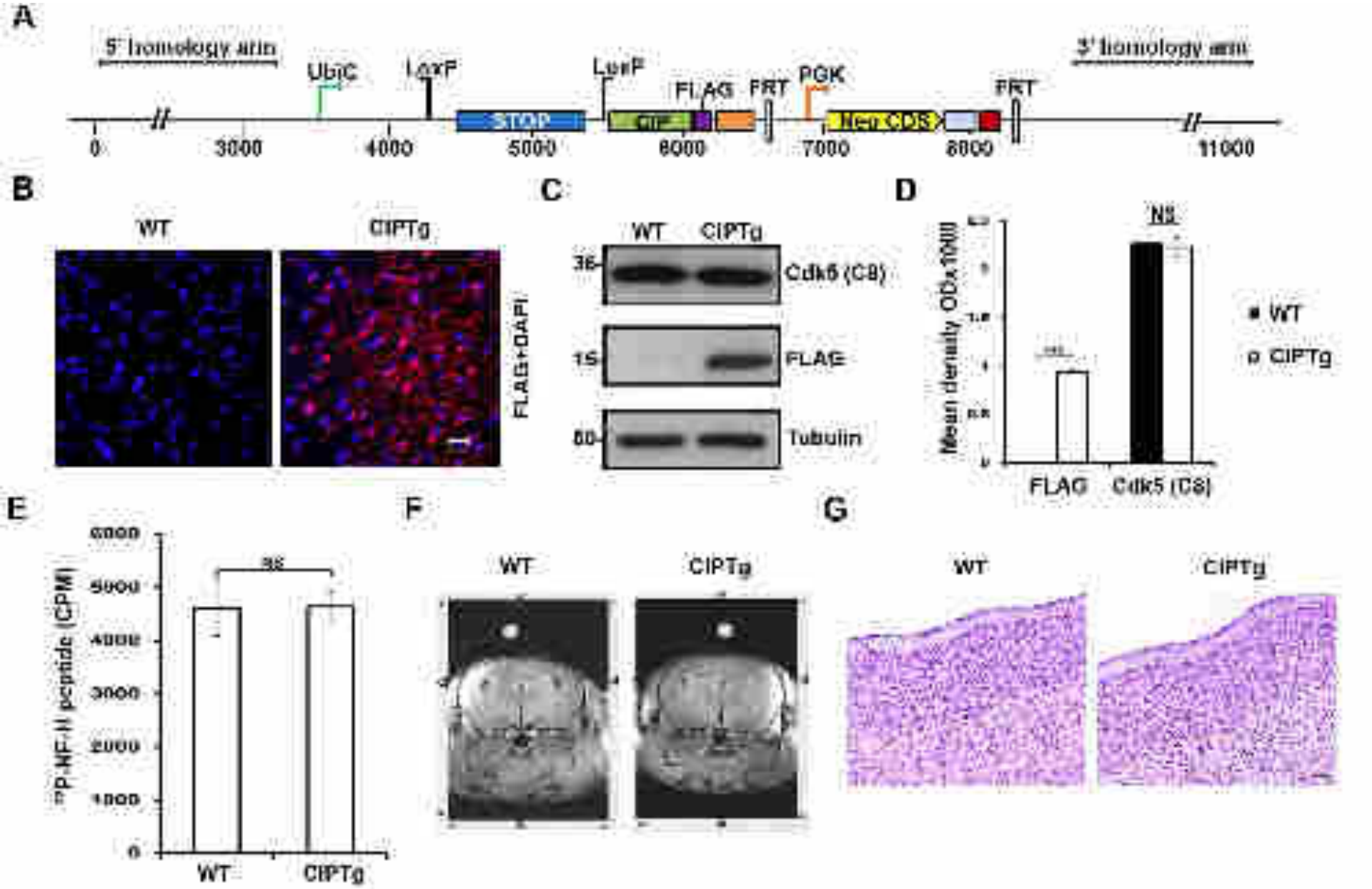
FITC-GGG KEAFWDRCLSVINLMSSKMLQINA YARAARRAARR

p5

Scrambled peptide:

FITC-GGGGGGFWDRCLSGKGKMSSKGGGINAYARAARRAARR

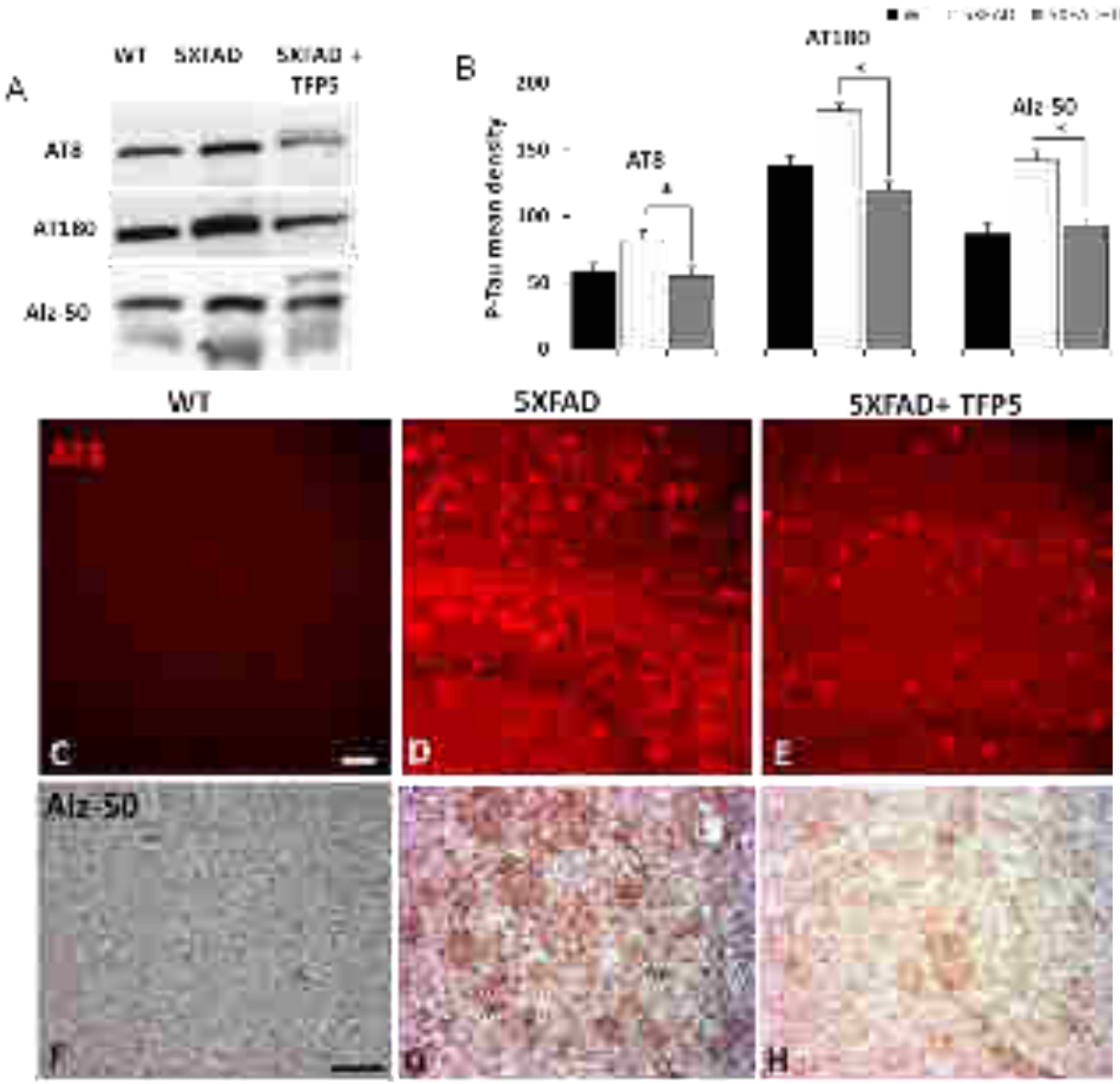
TFP5 US Patent # 8597660.


A
5' homology arm
UbiC
LoxP
LoxP
FLAG
FRT
PGK
Neo CDS
FRT
3' homology arm
0
3000
4000
5000
6000
7000
8000
11000
B
WT
CIPTg
FLAG+DAPI
C
WT
CIPTg
36
Cdk5 (C8)
15
FLAG
60
Tubulin
D
NS
Mean density ODx10(II)
WT
CIPTg
FLAG
Cdk5 (C8)
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5000
4000
3000
2000
1000
0
WT
CIPTg
F
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CIPTg

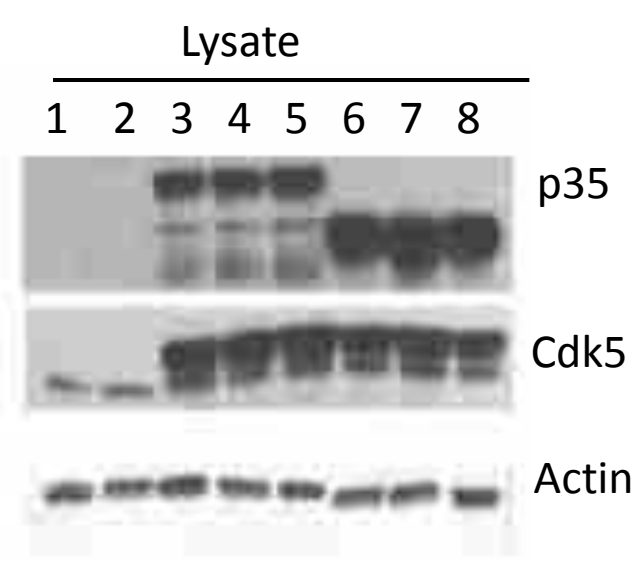
Summary

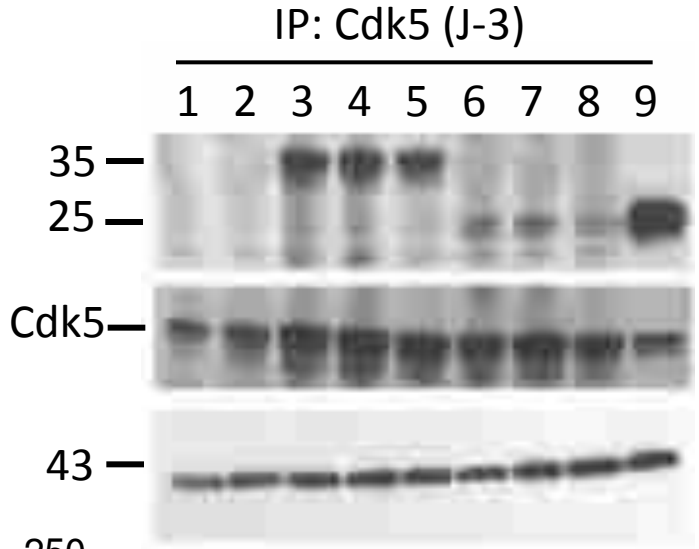
- Cdk5 activity is tightly regulated in the nervous system, its regulation and deregulation correlates with cytoskeleton phosphorylation and neurophysiology and pathology.
- Cdk5 is involved regulation of signal transduction pathways implicated in neuronal survival.
- Deregulation of Cdk5 activity causes neuronal cell death.
- CIP, a truncated peptide from p25, specifically inhibits Cdk5 deregulation, aberrant tau hyperphosphorylation and induces neuronal survival without affecting normal activity of cdk5 (Cdk5/p35).
- P5, (24 aa) a truncated peptide from CIP inhibits cdk5/p25 activity.

P5-mod reduced pathological hallmarks of AD in cortex: Hyperphosphorylation of aggregated & tangled Tau (AT8)& Alz50

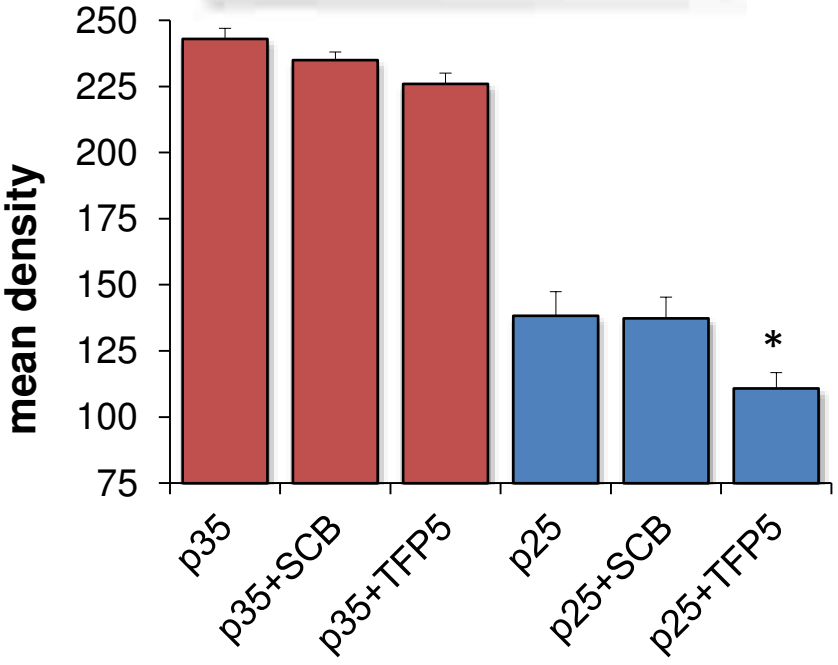


HEK 293 cells transfected with Cdk5/p35 or Cdk5/p25 in the presence or absence of TFP5 and binding with Cdk5 of p35 and p25 is evaluated using Cdk5 antibody





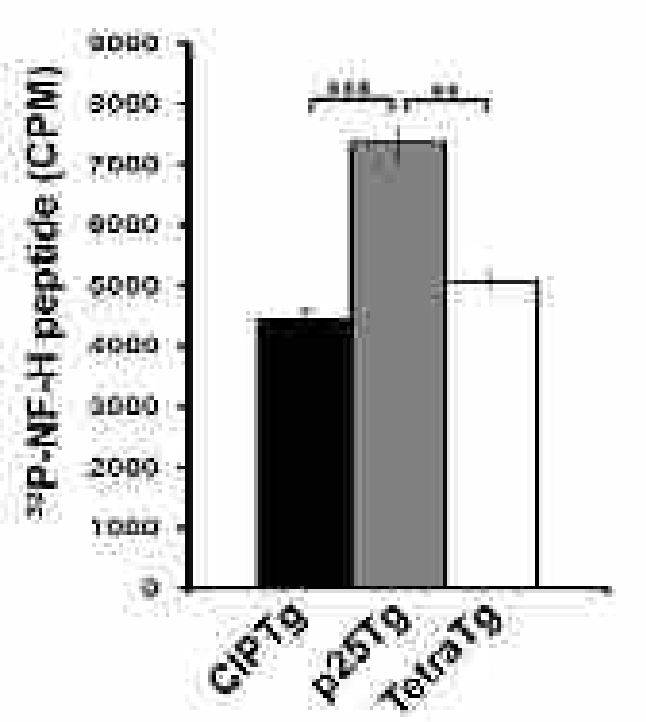
1. EV
2. EV+TFP5
3. P35
4. P35+ SCB
5. P35+ TFP5
6. P25
7. P25+SCB
8. P25+TFP5
9. P25 (lysate, positive control)

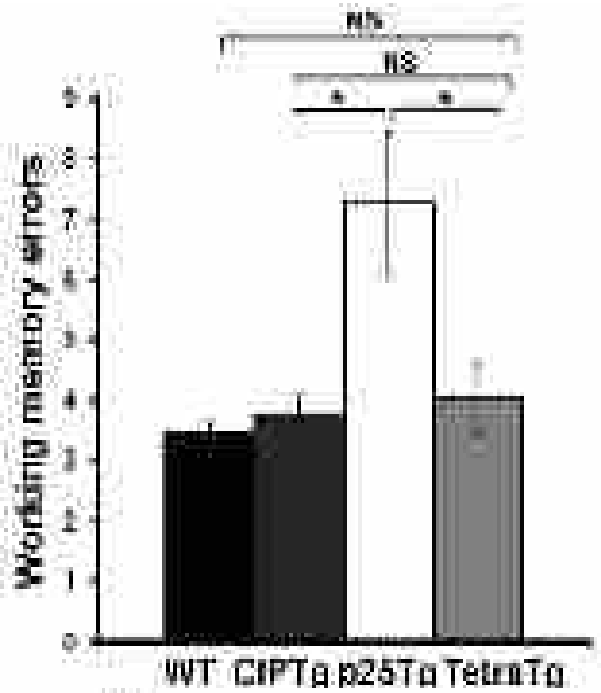


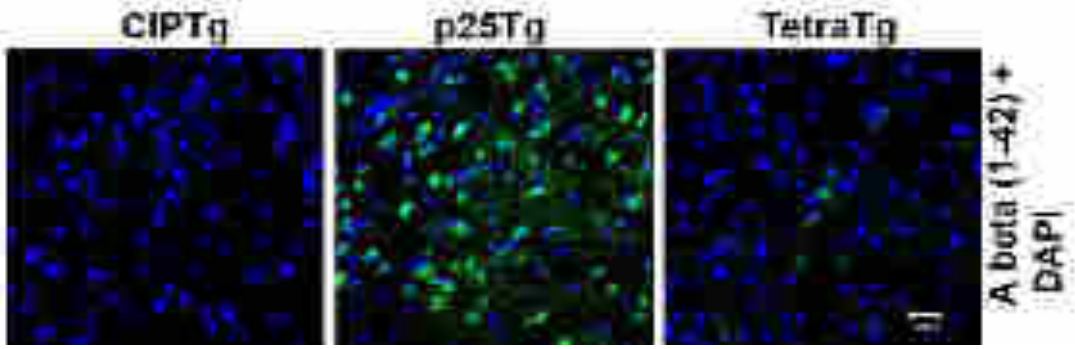
TFP5 competes with p25/cdk5 but not with p35/Cdk5

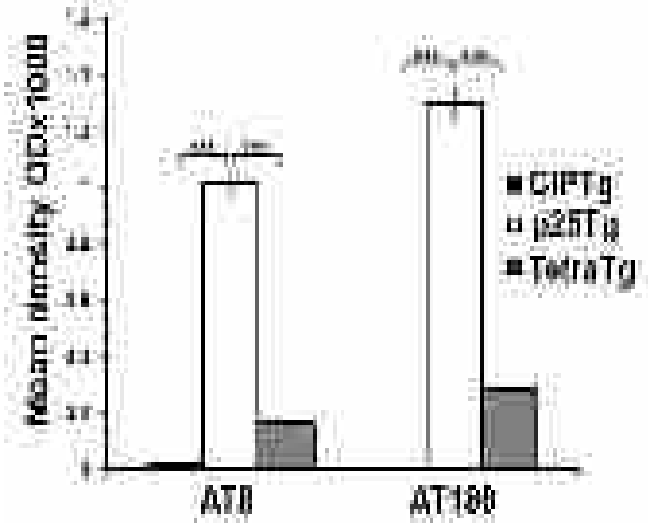
CIP Tg reduced AD like pathology from 12 week induced p25 Tg, in tetra Transgenic (CIP Tg X p25Tg) mice.

All the behavioral studies were double blinded









(Sundaram, JR et al., J. Neurosci. 2013)

In the presence of p67/ Munc-18, TFP5 selectively inhibits Cdk5/p25 but not Cdk5/p35 activity, (+/- 0.18mg p67)

