# Fosgonimeton, a Small-Molecule Positive Modulator of the Neurotrophic HGF System, Protects Against Amyloid beta-induced Pathological Alterations in Alzheimer's Disease Models In Vitro and In Vivo

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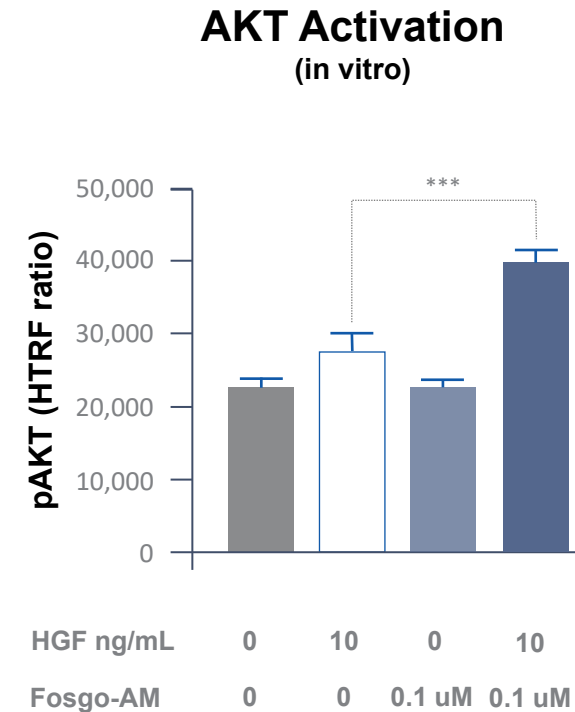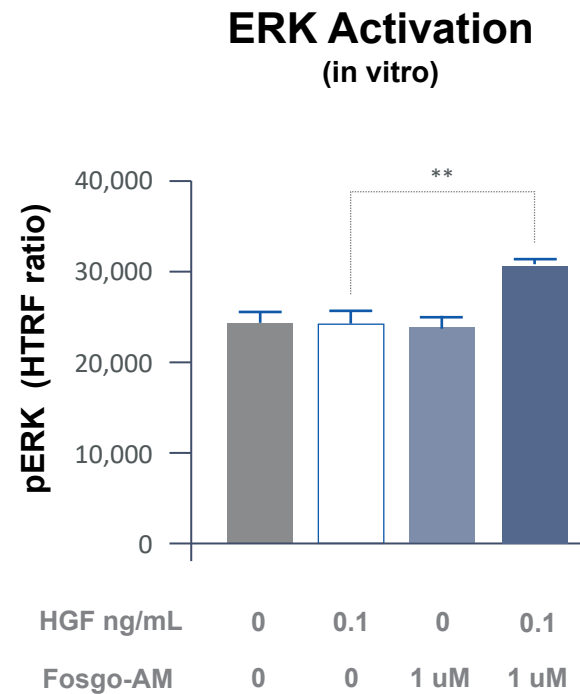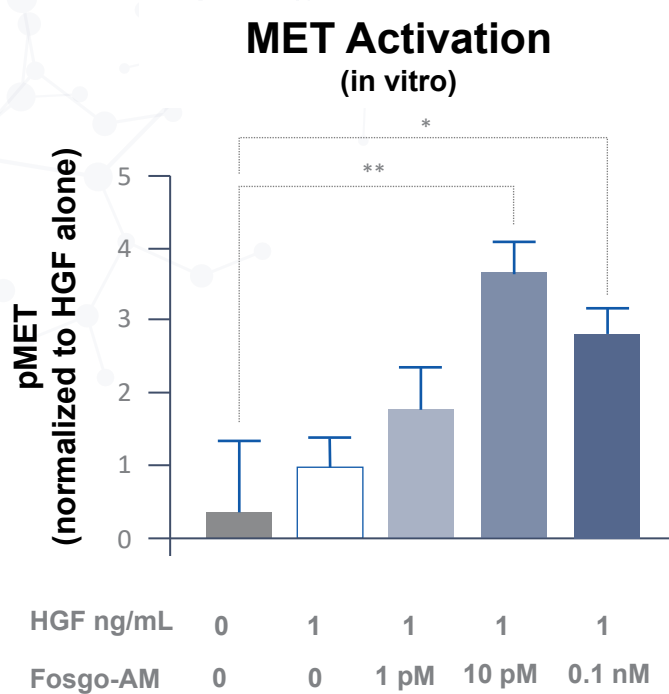
# Positive modulation of the neurotrophic HGF system by fosgonimeton

- Accumulation of amyloid beta (Aβ) and phosphorylated tau proteins are major pathological hallmarks of AD[1]

- Exposure to neurotoxic Aβ peptides results in
  - Mitochondrial & oxidative stress
  - Tau hyperphosphorylation
  - Neuronal loss
  - Synaptic degeneration
  - Cognitive impairment

- Preclinical and clinical data have implicated HGF/MET signaling dysfunction in AD[2, 3]

- Positive modulation of the HGF signaling pathway can protect against neurotoxicity, oxidative stress, and cognitive impairment[4]
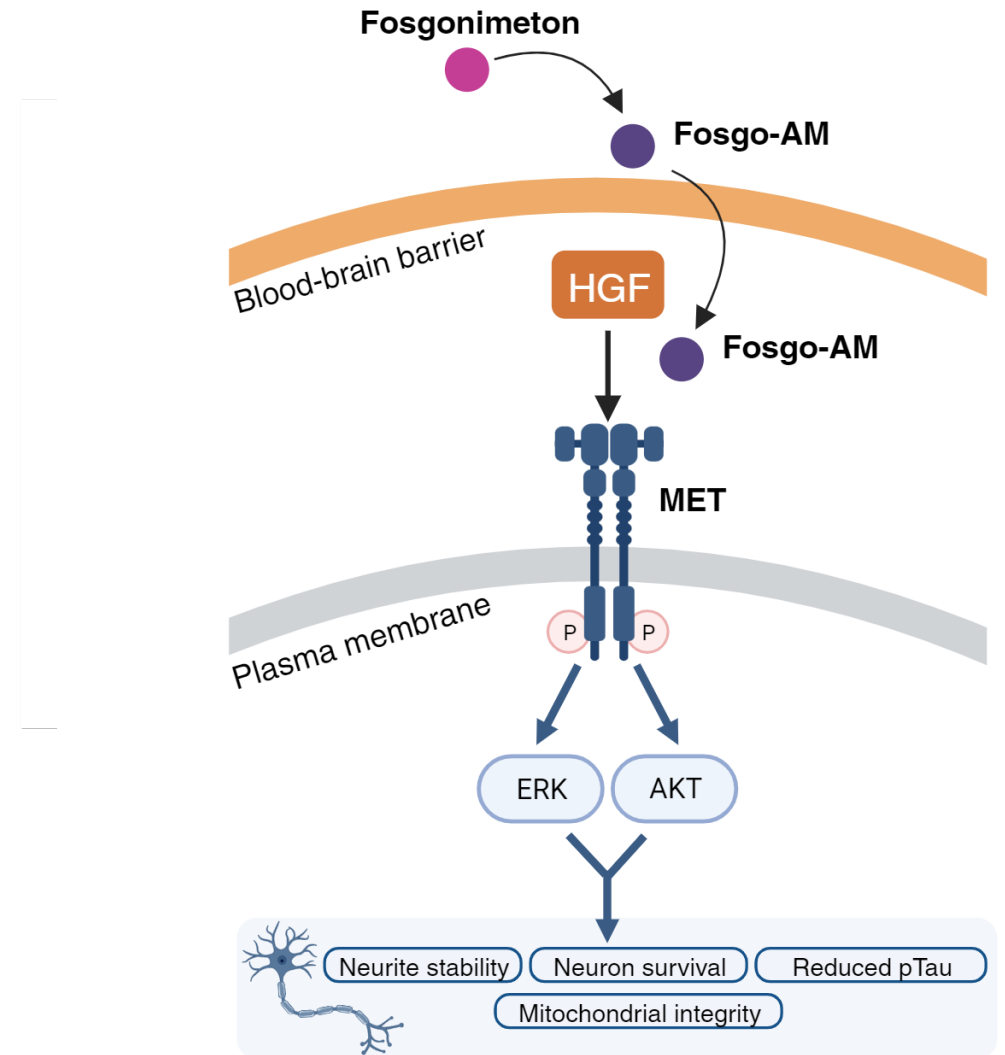
1. Guo et al. *Mol. Neurodegener* 2020; 1, 15
2. Hamasaki et al. *Neuropathology.* 2014; 284-290
3. Wei et al. *Frontiers in Aging Neurosci.* 2022
4. Johnston, et al. *Neurotherapeutics.* 2023;20: 431-451

# Fosgonimeton enhances the HGF signaling pathway in vitro

- Positive modulation of HGF leads to activation of its receptor MET, and stimulation of downstream signaling pathways including ERK (pERK) and AKT (pAKT), which promote neurotrophic and neuroprotective effects[1]



**MET Activation**
(in vitro)

**ERK Activation**
(in vitro)

**AKT Activation**
(in vitro)

| HGF ng/mL | 0 | 1 | 1 | 1 | 1 |
|-----------|---|---|---|---|---|
| Fosgo-AM | 0 | 0 | 1 pM | 10 pM | 0.1 nM |

| HGF ng/mL | 0 | 0.1 | 0 | 0.1 |
|-----------|---|-----|---|-----|
| Fosgo-AM | 0 | 0 | 1 uM | 1 uM |

| HGF ng/mL | 0 | 10 | 0 | 10 |
|-----------|---|----|---|----|
| Fosgo-AM | 0 | 0 | 0.1 uM | 0.1 uM |

**Primary MOA of fosgonimeton** → **Result in downstream neurotrophic and neuroprotective pathway activation**

# Objective: Evaluate the effects of fosgonimeton, a positive modulator of the neurotrophic HGF system, in preclinical models of AD

# Fosgo-AM reduces Aβ-induced neuronal death, neurite degeneration, and tau phosphorylation in primary cortical neurons
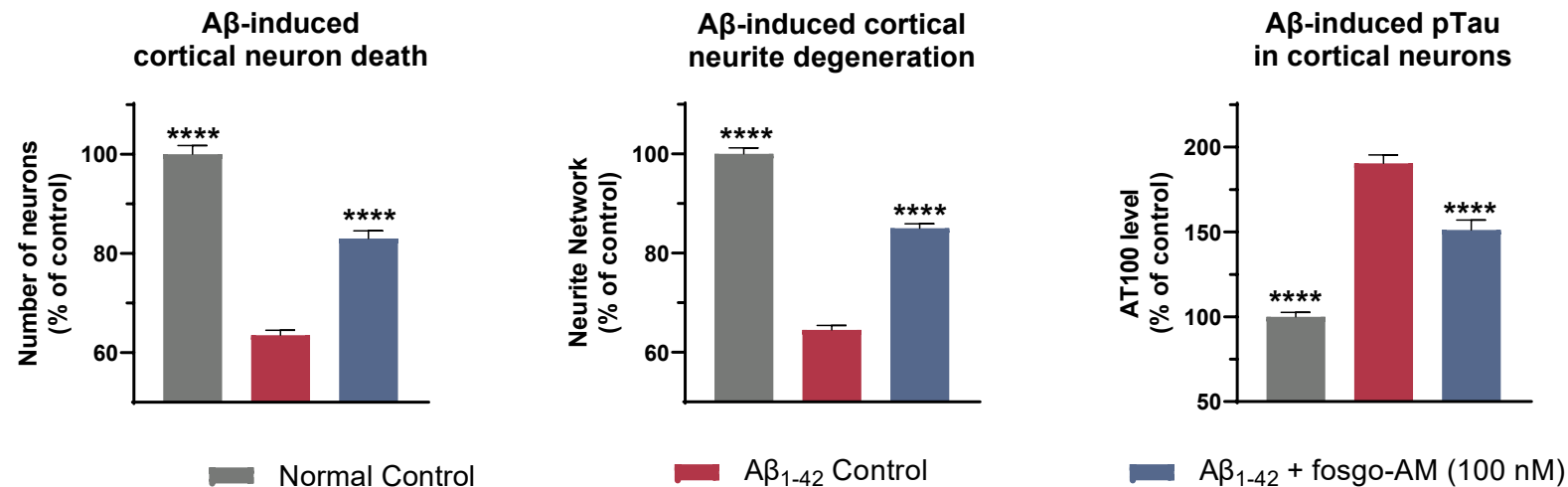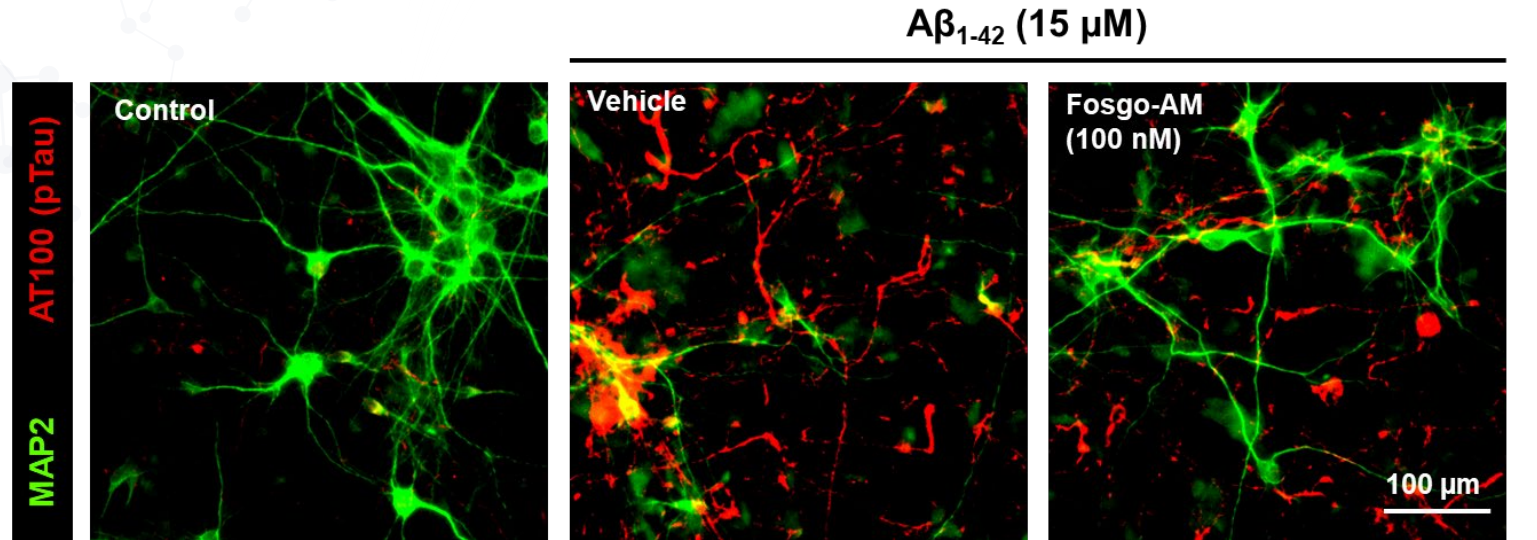


On day 11 of culture, rat cortical neurons were pre-treated with fosgo-AM or vehicle for 15 minutes and then challenged with AB1-42 solution (15 µM, containing 2 µM Aβ oligomers) for 24 hours and then assessed for the following read-outs: 1) total number of neurons (MAP2+ neurons), 2) neurite network (total length of MAP2+ in µm), and 3) hyperphosphorylated tau (overlap of MAP2+ and AT100 in µm2). Data presented as mean ± SEM; n = 4-6. Statistics applied: One-way ANOVA followed by Fisher's LSD test. **** p < 0.0001 versus Aβ1-42.

# Fosgo-AM attenuates Aβ-induced mitochondrial stress in primary cortical neurons
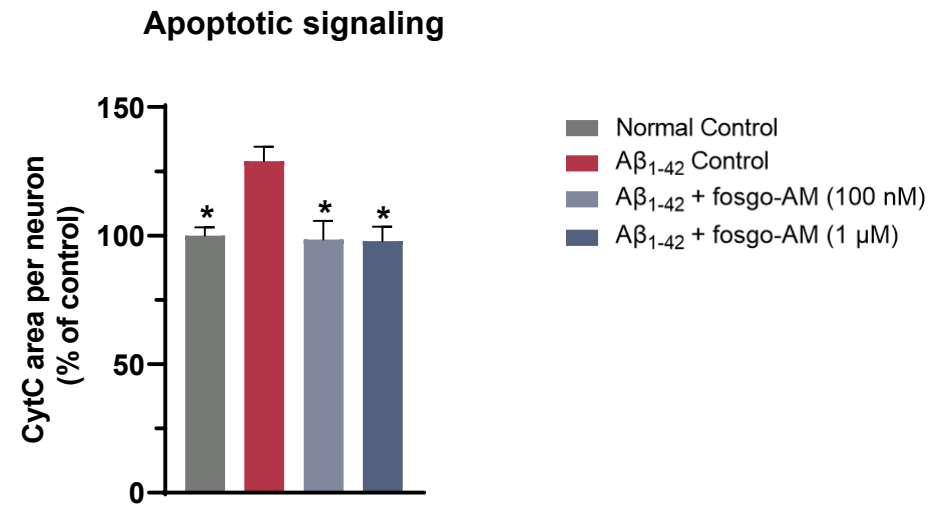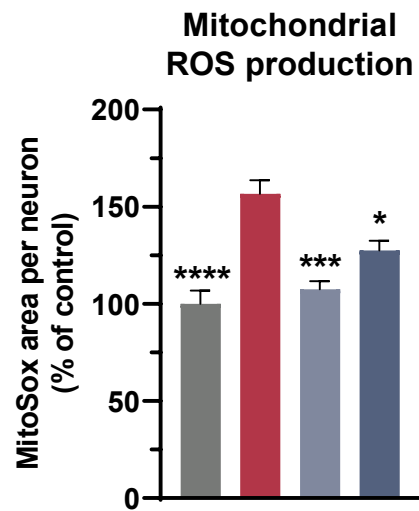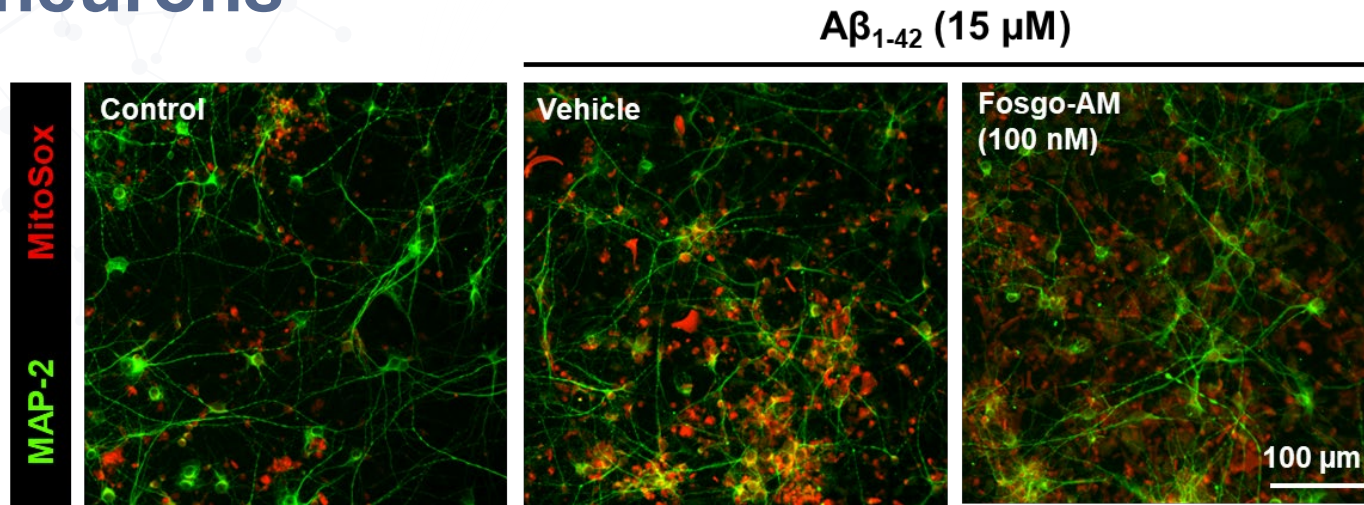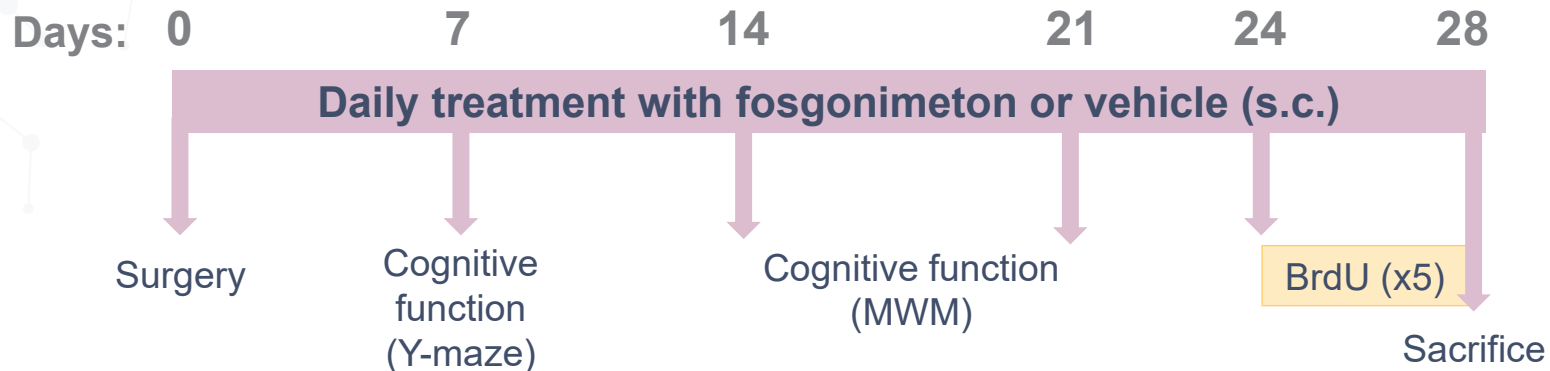


On day 11 of culture, rat cortical neurons were pre-treated with fosgo-AM or vehicle for 15 minutes then challenged with AB1-42 solution (15 μM, containing 2 μM Aβ oligomers) for 4 hours. Analyses were directly and automatically performed by MetaXpress® (Molecular Devices) to quantify the following read-outs: 1) total number of neurons (MAP2+ neurons), 2) mitochondrial ROS in neurons (overlap MitoSox and MAP2 in μm²) in plate 1and 3) cytochrome c release in neuron cytoplasm (overlap CytC and MAP2 in μm²) in plate 2. Data presented as mean ± SEM; n = 4-6. Statistics applied: One--way ANOVA followed by Fisher's LSD test. * p < 0.05, ** p < 0.01, *** p < 0.001, **** p < 0.0001 versus Aβ1-42.

# Intrahippocampal injection of $A\beta_{1-42}$ in aged mice: Study design

**The hippocampus is the main brain region involved in learning and memory, which is affected in AD**

**Experimental Design**

Days: 0    7    14    21    24    28

Daily treatment with fosgonimeton or vehicle (s.c.)

Surgery | Cognitive function (Y-maze) | Cognitive function (MWM) | BrdU (x5)

Sacrifice

**Groups:** 12 mice per group

1. **Sham Control**
   Aged mice treated with vehicle daily

2. **$A\beta_{1-42}$ Control**
   Aged mice that received intrahippocampal $A\beta_{1-42}$, treated with vehicle daily

3. **$A\beta_{1-42}$ + Fosgonimeton 0.5 mg/kg**
   Aged mice that received intrahippocampal $A\beta_{1-42}$, treated with fosgonimeton 0.5 mg/kg daily

4. **$A\beta_{1-42}$ + Fosgonimeton 1 mg/kg**
   Aged mice that received intrahippocampal $A\beta_{1-42}$, treated with fosgonimeton 1 mg/kg daily
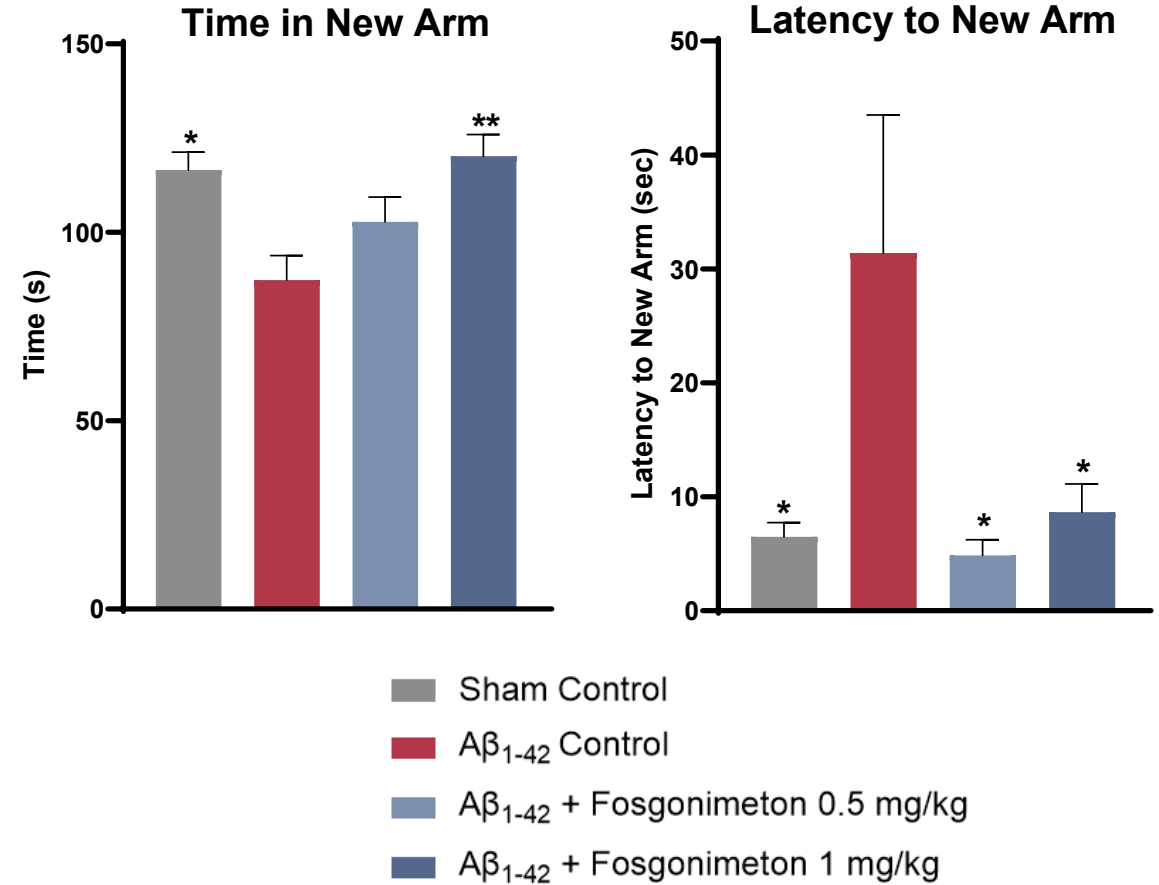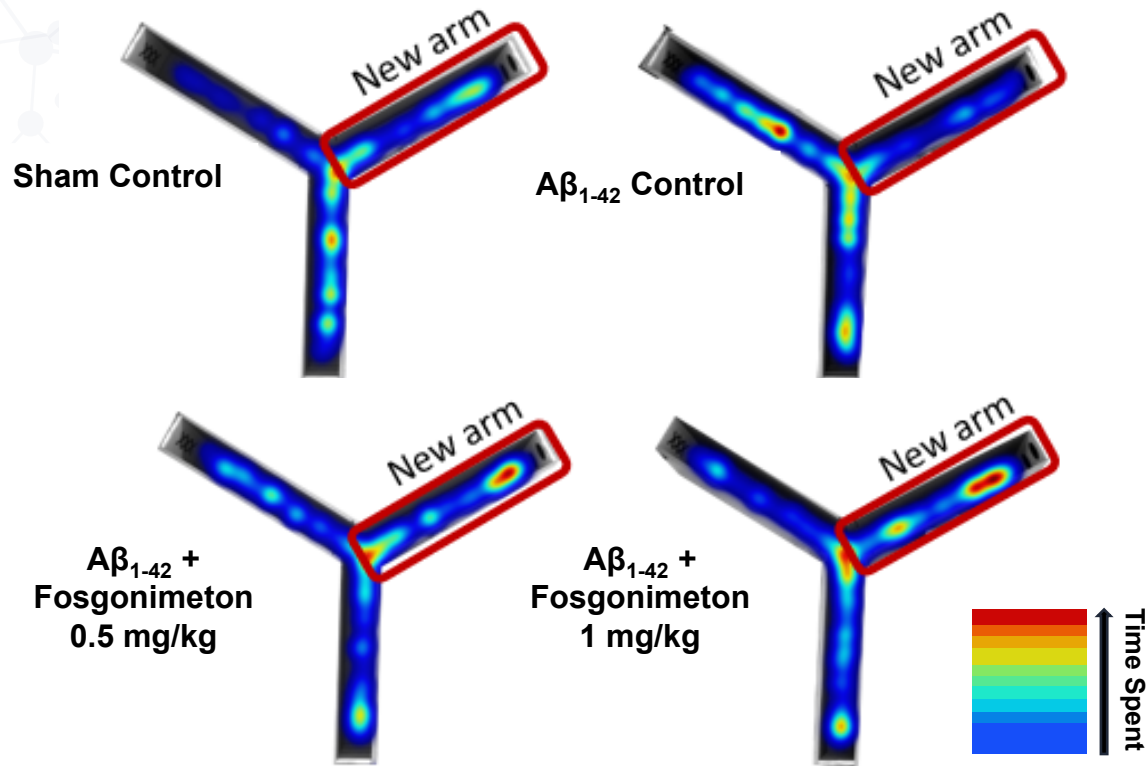
**$A\beta_{1-42}$**
100 uM, 15 uM oligomers
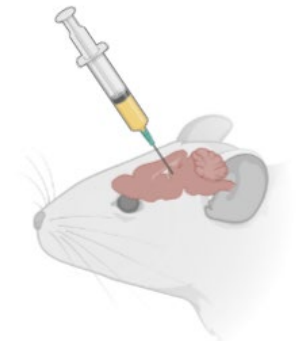
**Aged mouse**
18 months old

On day 0, animals received bilateral hippocampal injections of either vehicle (Sham Control) or $A\beta_{1-42}$ solution. The animals that received $A\beta_{1-42}$ were split into 3 groups, receiving either daily subcutaneous injections of saline ($A\beta_{1-42}$ Control) or fosgonimeton at a dose of 0.5 mg/kg or 1 mg/kg. At day 7 following surgery, animals were assessed for cognitive performance in Y-maze. On days 14-21, animals were assessed for cognitive performance in Morris water maze (MWM). On days 24-28, animals received daily injections of bromodeoxyuridine (BrdU) to assess new cell proliferation before sacrifice in day 28.

# Fosgonimeton improves cognitive performance in Y-maze

- During the Y-maze retention trial, longer latency to enter or less time spent in new arm is associated with greater cognitive impairment



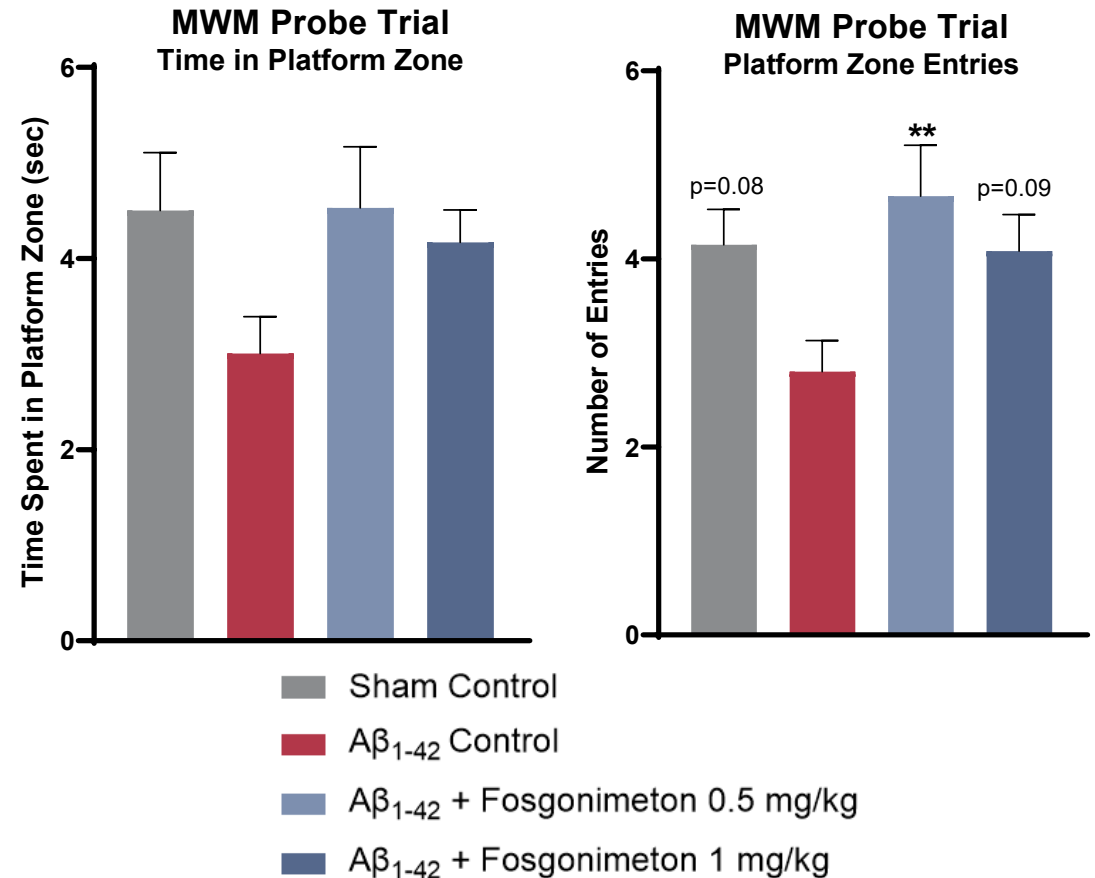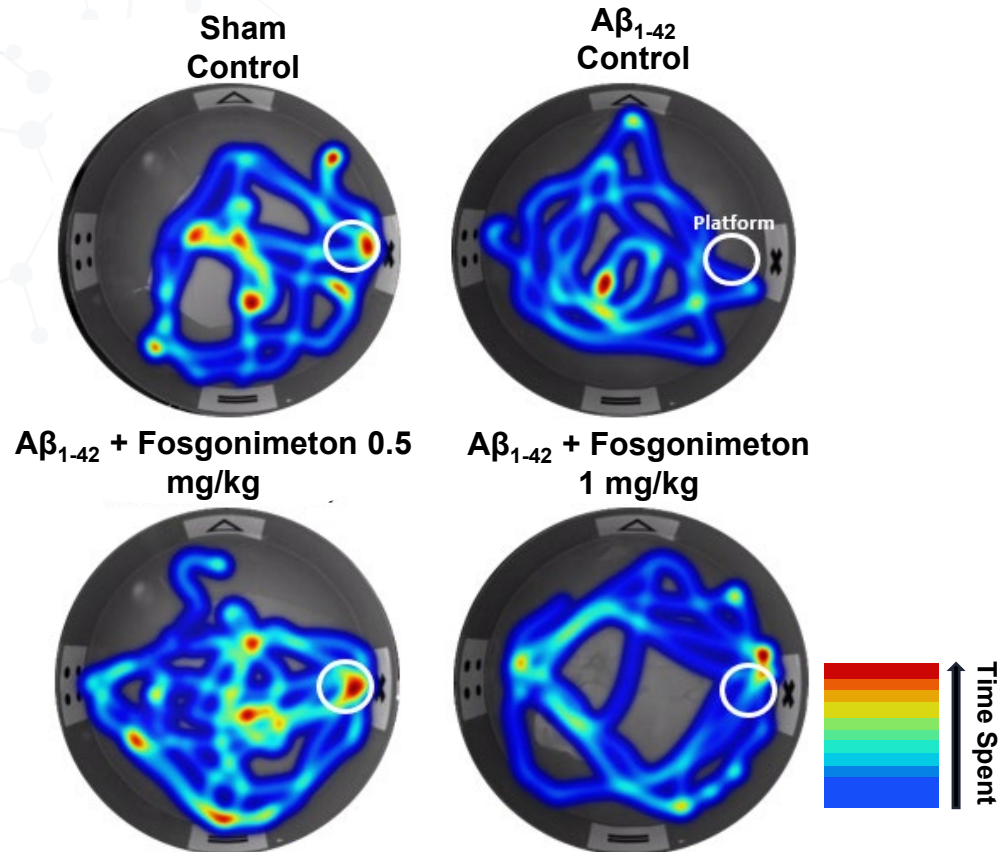**Representative Heat Maps of Time Spent**

Sham Control

$A\beta_{1-42}$ Control

$A\beta_{1-42}$ + Fosgonimeton 0.5 mg/kg

$A\beta_{1-42}$ + Fosgonimeton 1 mg/kg

Time Spent

**Time in New Arm**

**Latency to New Arm**

Legend:
- Sham Control
- $A\beta_{1-42}$ Control
- $A\beta_{1-42}$ + Fosgonimeton 0.5 mg/kg
- $A\beta_{1-42}$ + Fosgonimeton 1 mg/kg

# Fosgonimeton improves cognitive performance in MWM

- During the Morris water maze (MWM) probe trial, reduced frequency of entries in the platform zone indicative of cognitive impairment



**Representative Heat Maps of Time Spent**

Sham Control

$A\beta_{1-42}$ Control

$A\beta_{1-42}$ + Fosgonimeton 0.5 mg/kg

$A\beta_{1-42}$ + Fosgonimeton 1 mg/kg

Platform

Time Spent

**MWM Probe Trial**
Time in Platform Zone

Time Spent in Platform Zone (sec)

**MWM Probe Trial**
Platform Zone Entries

Number of Entries

p=0.08

**

p=0.09

Sham Control

$A\beta_{1-42}$ Control

$A\beta_{1-42}$ + Fosgonimeton 0.5 mg/kg

$A\beta_{1-42}$ + Fosgonimeton 1 mg/kg

Data presented as mean ± SEM; n = 10-12 for MWM. Statistics applied: One--way ANOVA followed by Dunnett's multiple comparisons test.
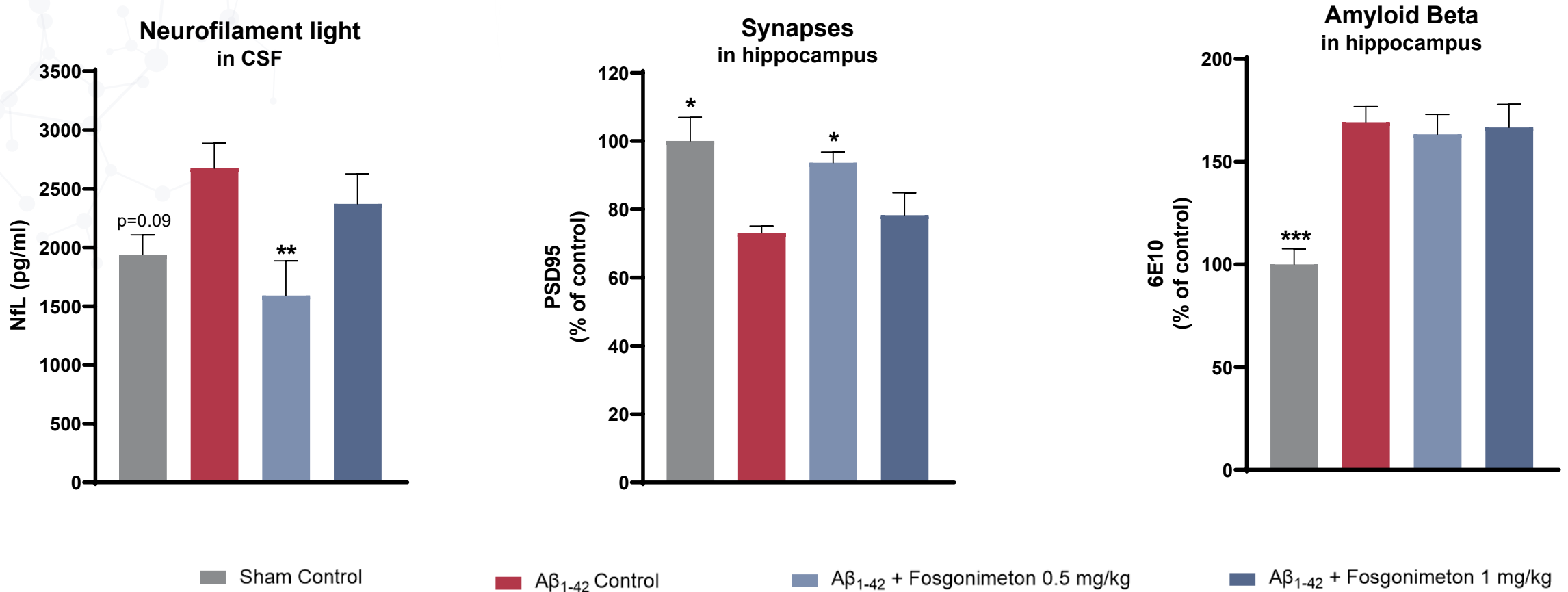** $p < 0.01$ versus $A\beta_{1-42}$ Control.

# Fosgonimeton reduces Aβ-induced cytochrome c release and neuron loss in the hippocampus
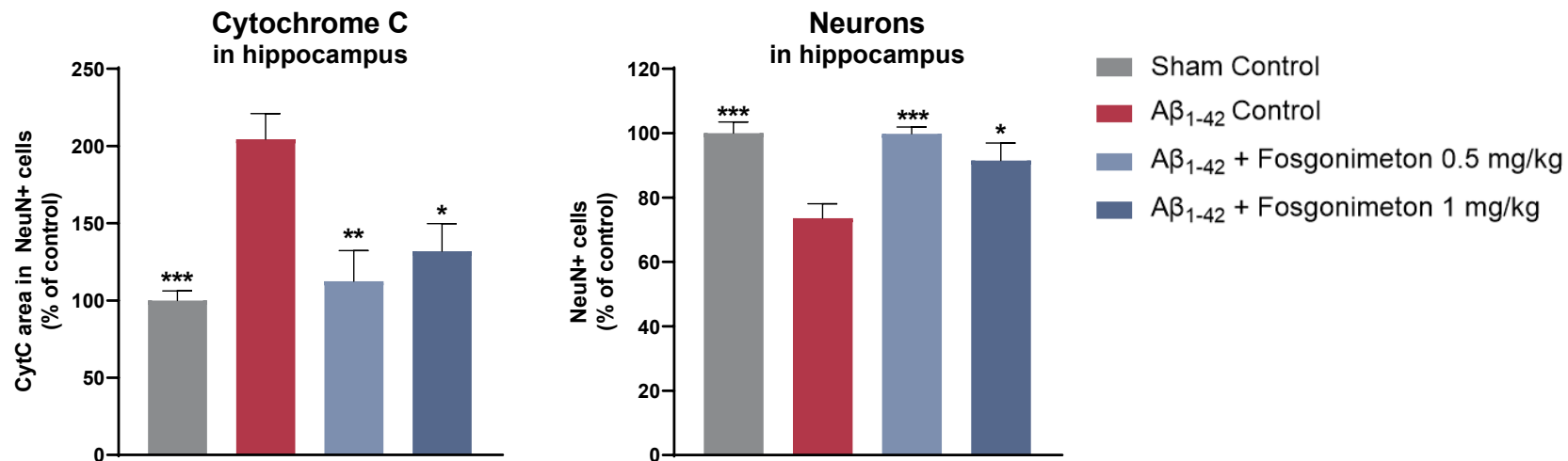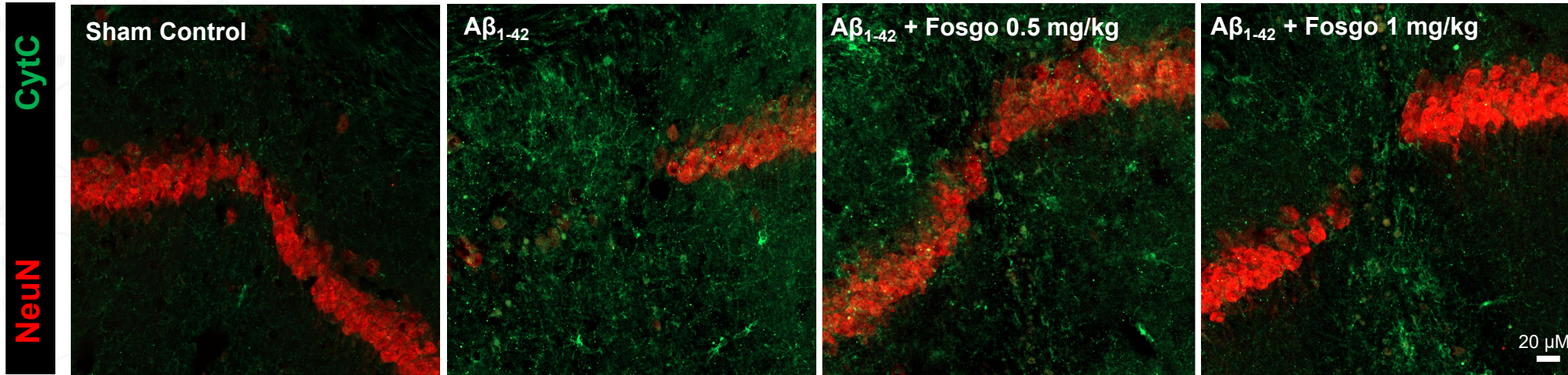


Data presented as mean ± SEM; n = 5-7. Statistics applied: One-way ANOVA followed by Dunnett's multiple comparisons test.
* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ versus Aβ1-42 Control. Scale bar = 20 μM.

# Fosgonimeton attenuates Aβ-induced neurodegeneration and synapse loss without affecting Aβ load



**Neurofilament light**
in CSF

**Synapses**
in hippocampus

**Amyloid Beta**
in hippocampus

■ Sham Control    ■ Aβ$_{1-42}$ Control    ■ Aβ$_{1-42}$ + Fosgonimeton 0.5 mg/kg    ■ Aβ$_{1-42}$ + Fosgonimeton 1 mg/kg

# Fosgonimeton increases cell proliferation in an area of neurogenesis

**The subgranular zone (SGZ) of the hippocampus is one of the few brain regions that contains neuronal progenitor cells in the adult brain, with the potential to support neurogenesis[1]**

1. Abbott & Nigussie. *Anat Histol Embryol.* 2020; 3-16.

# Summary and conclusions

**In primary cortical neurons, fosgo-AM treatment:**

1. Protected against $A\beta_{1-42}$-induced neurodegeneration, neurite degeneration and tau hyperphosphorylation
2. Attenuated the impact of $A\beta_{1-42}$ on mitochondrial stress

**In an aged $A\beta_{1-42}$ mouse model of AD, fosgonimeton treatment:**

1. Improved cognitive performance in Y-maze and MWM
2. Protected against $A\beta_{1-42}$-induced mitochondrial stress, neurodegeneration, and synapse loss
3. Promoted cell proliferation in the subgranular zone, an area of adult neurogenesis

**These data demonstrate that fosgonimeton can promote neuroprotective and neurotrophic effects in preclinical models of AD, supporting its further investigation as a potential therapeutic for AD**
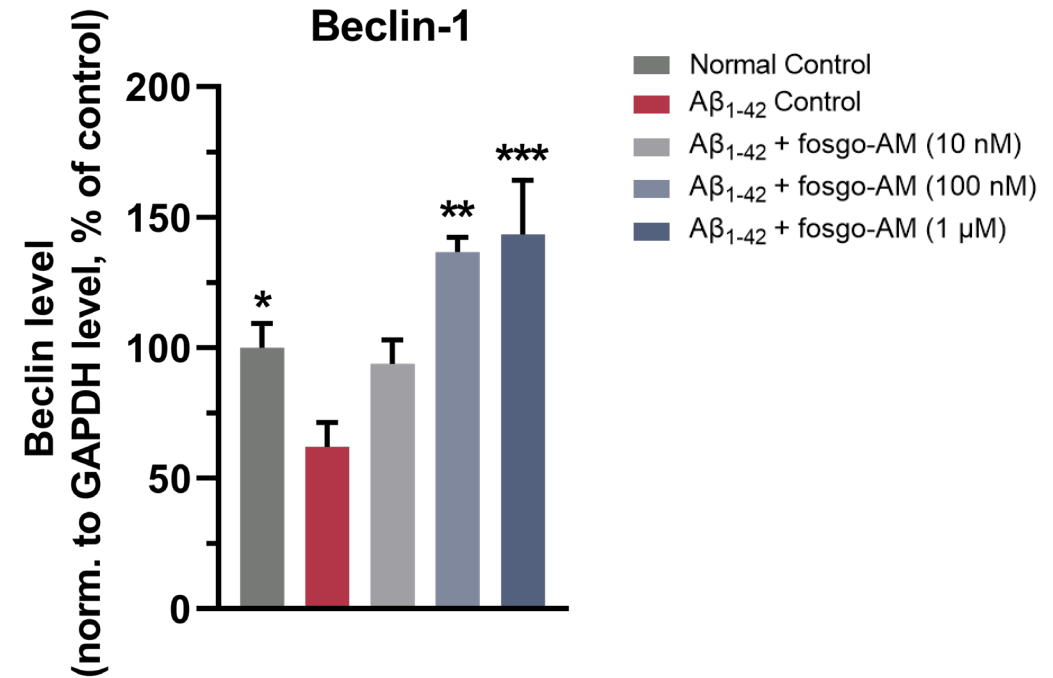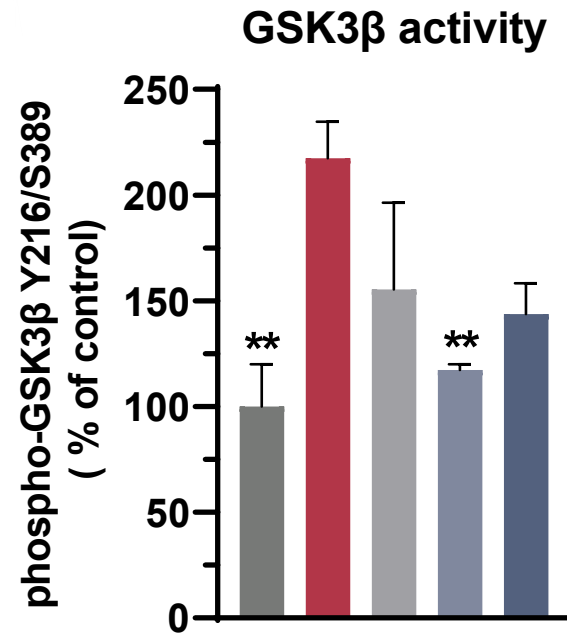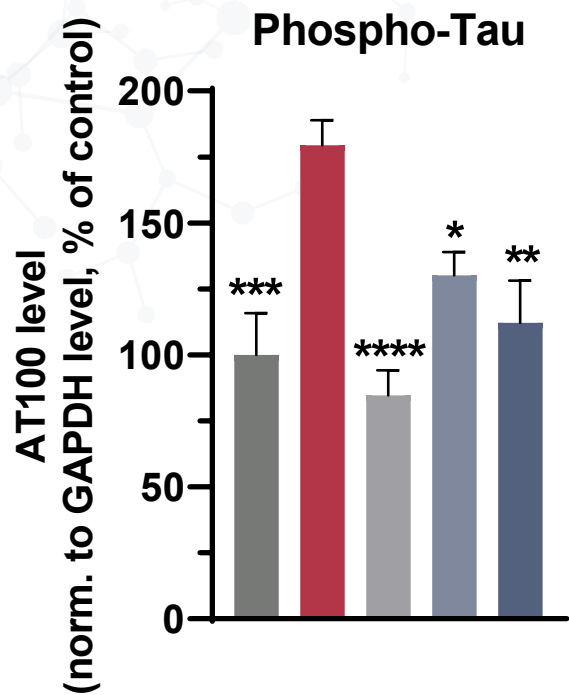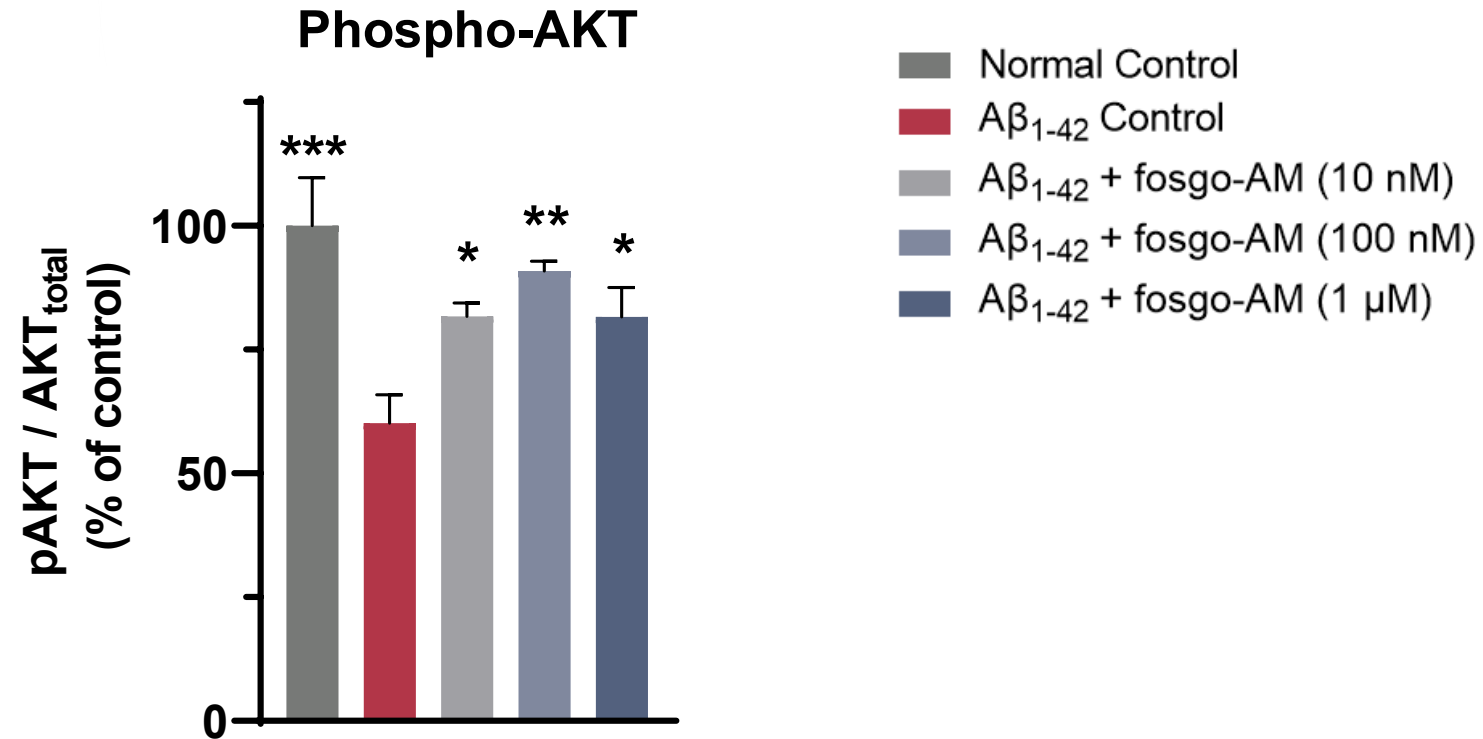
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# Thank you for your attention!

# Appendix

# Fosgonimeton reduces phospho-tau levels, GSK3β activation, and increases levels of Beclin-1

# Fosgonimeton treatment increases AKT activation following Aβ injury



Phospho-AKT

pAKT / AKT$_{total}$ (% of control)

**Legend:**
- Normal Control
- Aβ$_{1-42}$ Control
- Aβ$_{1-42}$ + fosgo-AM (10 nM)
- Aβ$_{1-42}$ + fosgo-AM (100 nM)
- Aβ$_{1-42}$ + fosgo-AM (1 μM)

# No differences in groups in MWM acquisition



**MWM Acquisition**

Legend:
- Sham Control
- Aβ1-42 Control
- Aβ1-42 + Fosgonimeton 0.5 mg/kg
- Aβ1-42 + Fosgonimeton 1 mg/kg

X-axis: Day
Y-axis: Latency to Platform (sec)