

Supporting Information

for

Rapid initiation of cell cycle reentry processes protects neurons from amyloid- β toxicity

Stefania Ippati and Yuanyuan Deng *et al.*

Supporting Text

AAV-Geminin FUCCI green transduction efficiency

FUCCI relies upon cell-cycle-dependent proteolysis of the ubiquitination oscillator Geminin to specifically mark the G1/S transition in living cells (Newman and Zhang 2008). During G1 phase, the nuclei of FUCCI-expressing cells appear blank due to the ubiquitin mediated proteolysis of Geminin. Green fluorescence is maintained throughout S, G2, and M phases until the fluorescent signal is lost between M and G1 (Sakaue-Sawano and Miyawaki 2014). To validate the reliability of our results we tested the transduction efficiency of Geminin AAV virus on primary neurons. We exploited the cell-cycle dependent proteolysis of mAG-hGem (1-110) using a cell permeable proteasome inhibitor (van Eersel et al. 2011) (MG-132, Calbiochem), to test whether viral transduction was extended to the most of neurons in the culture. Four days after AAV-mediated mAG-hGem (1-110) transduction, two-week-old primary hippocampal neurons were treated with MG132 1 μ M and imaged for 10 hours. The proteasome inhibitor blocked the degradation of mAG-hGem (1-110). Image stacks from live videos (**Supplementary Fig. 4**) showed that green fluorescence was widely present in virtually all the neurons imaged confirming good transduction.

Supporting Figures

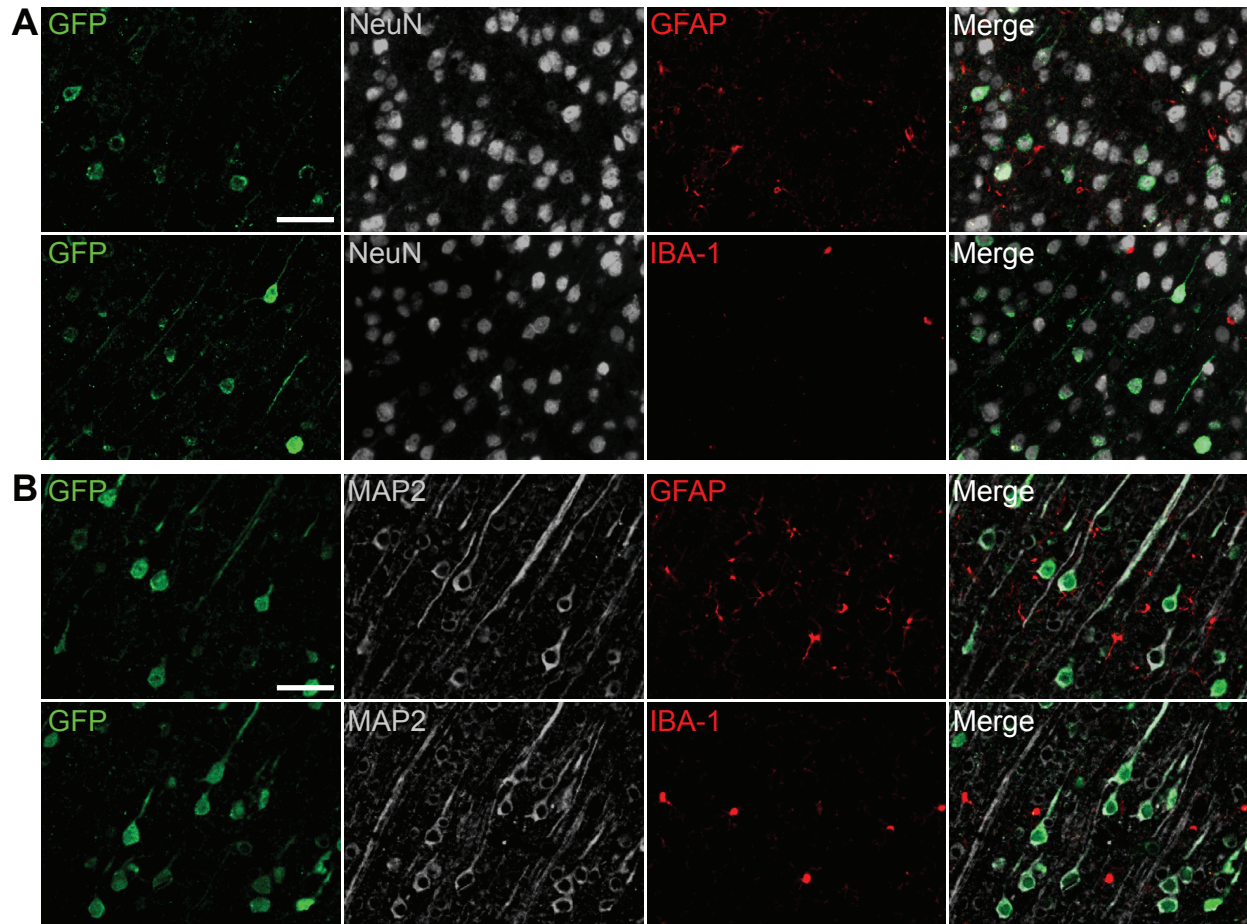


Figure S1. The human synapsin 1 promoter (hSyn1) drives neuron-specific gene expression in the CNS of mice. Wild-type C57Bl/6 mice were injected with AAV-hSyn1-GFP followed by immunofluorescence staining with antibodies to (A) GFP (green), neuronal NeuN (white) and astroglial GFAP or microglial IBA-1 (both red); or (B) GFP (green), neuronal MAP2 (white) and astroglial GFAP or microglial IBA-1 (both red). Overlays of fluorescence channels confirm exclusive neuronal expression. Scale bars, 50 μ m.

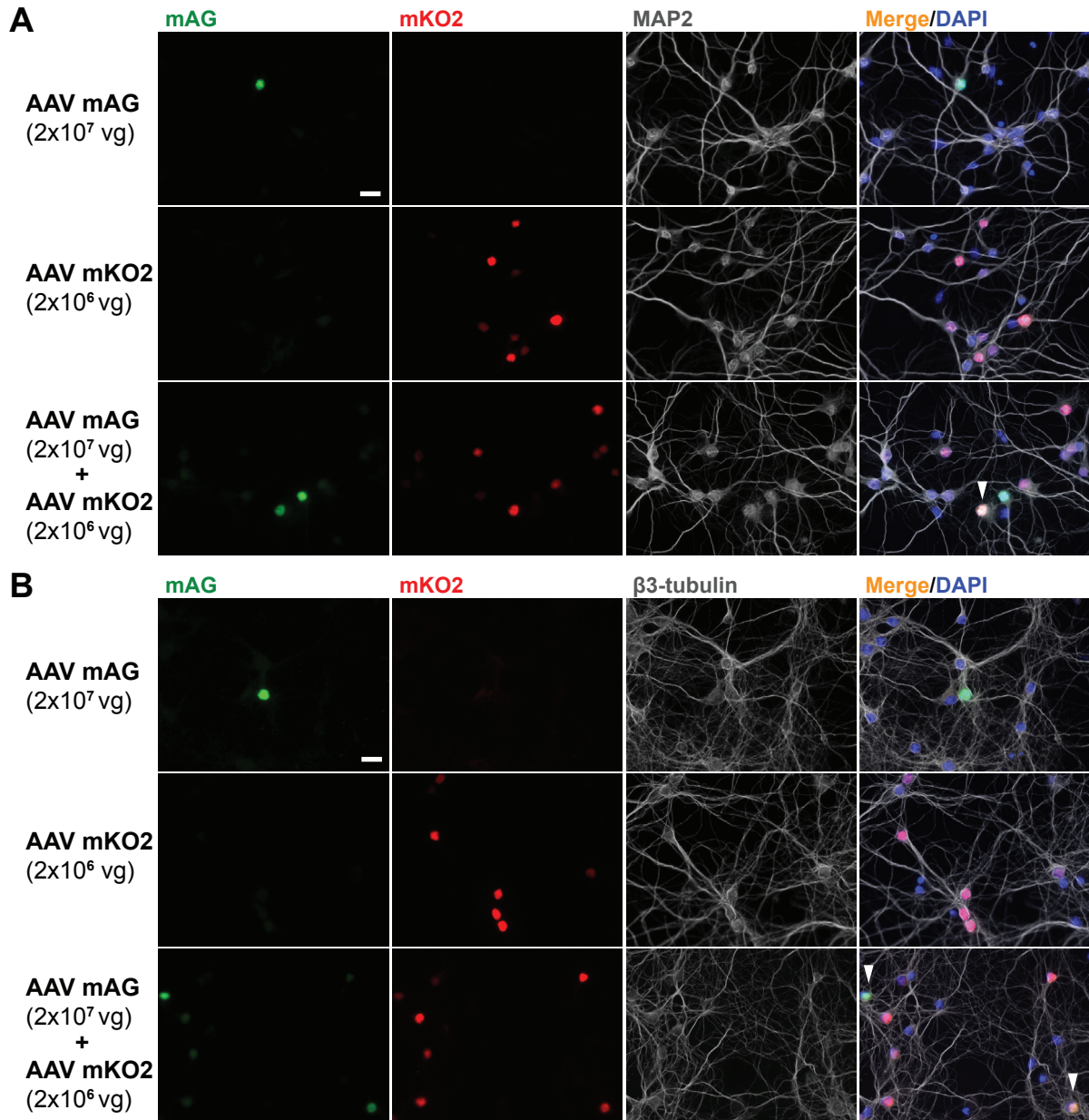


Figure S2. Characterization of FUCCI cell cycle reporters in hippocampal neurons. (A) mKO2 (red) and mAG (green) fluorescence co-immunolabelled for the neuronal marker MAP2 (white) of primary hippocampal neurons transduced with AAVs for mAG-hGem(1-110) (*top row*), mKO2-hCdt1(30-120) (*middle row*) or combined mAG-hGem(1-110) and mKO2-hCdt1(30-120) expression (*bottom row*) at indicated AAV titres in brackets (vg, viral genomes added to each culture). Arrowhead indicates neuron with mKO2/mAG co-activity in merged images including nuclear DAPI staining (blue). (B) mKO2 (red) and mAG (green) fluorescence co-immunolabelled for the neuronal marker β 3-tubulin (white) of primary hippocampal neurons transduced with

AAVs for mAG-hGem(1-110) (*top row*), mKO2-hCdt1(30-120) (*middle row*) or combined mAG-hGem(1-110) and mKO2-hCdt1(30-120) expression (*bottom row*) at indicated AAV titres in brackets (vg, viral genomes added to each culture). Arrowheads indicate neuron with mKO2/mAG co-activity in merged images including nuclear DAPI staining (blue). Scale bars, 20 μ m.

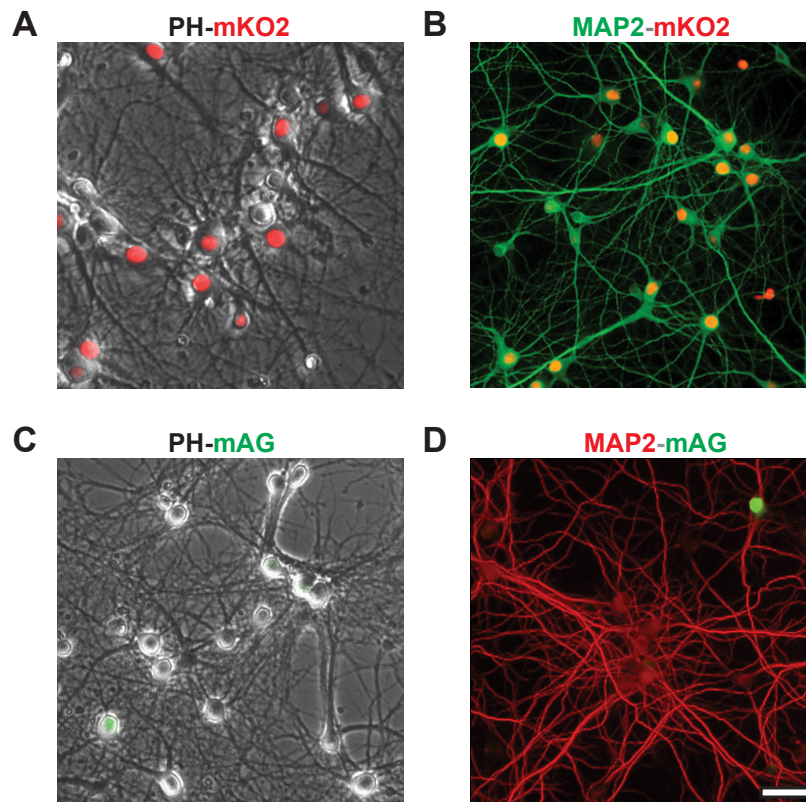


Figure S3. Fucci reporter activity in primary neurons transduced with same AAV titres (2×10^7 vg) for quantification. (A) Combined live phase contrast (PH) and fluorescence image of neurons expressing mKO2 (red). (B) mKO2 fluorescence (red) co-immunolabelled for the neuronal marker MAP2 (green). Note that the few mKO2⁺/MAP2⁻ cells are likely due to the toxicity with MAP2 loss of mKO2-hCdt1(30-120) expression by high AAV titers in this experimental setting. (C) Combined live phase contrast and fluorescence image of neurons expressing mAG (green). (D) mAG fluorescence (green) co-immunolabelled for the neuronal marker MAP2 (red). Scale bars, 25 μ m. Quantification of cell numbers are presented in Fig. 1C and 1D.

MG-132 1 μ M - mAG

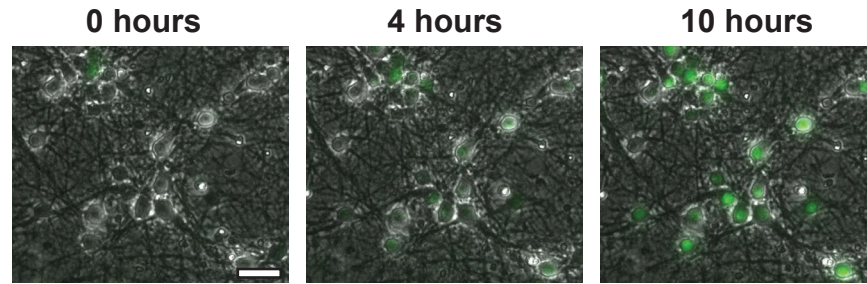


Figure S4. High AAV-pFUCCI-S/G2/M Green mAG transduction efficacy. High AAV-pFUCCI-S/G2/M Green mAG transduction efficacy is demonstrated by mAG activity (green) in virtually all neurons upon inhibition of mAG degradation upon treatment with the proteasome inhibitor MG-132 after 4 and 10 hours (n = 6). Scale bar, 25 μ m.

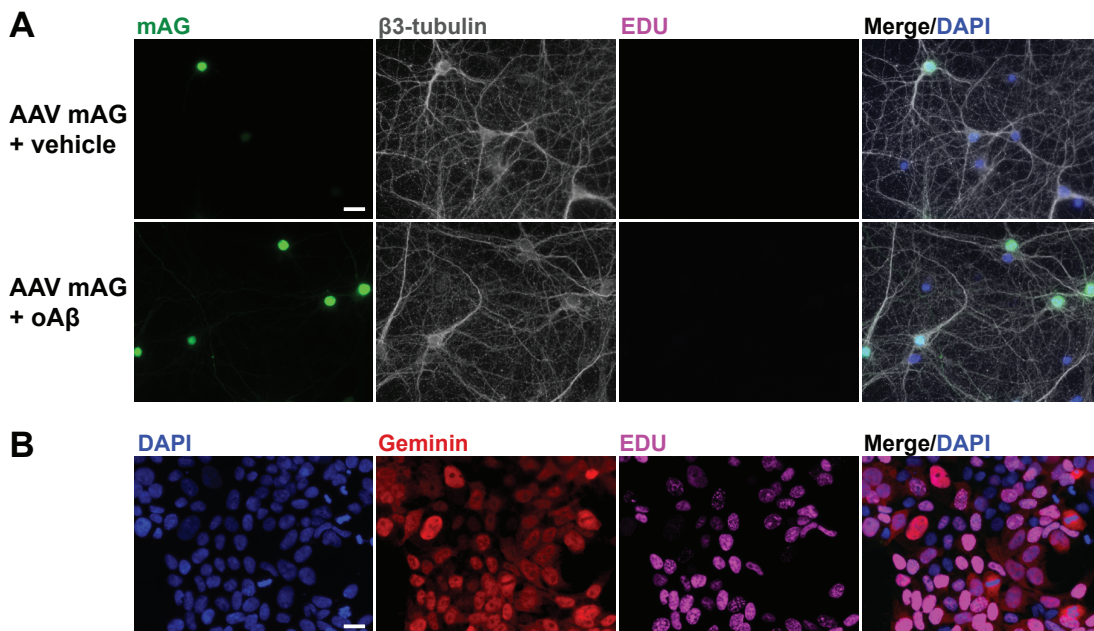


Figure S5. Absence of DNA synthesis and replication in mAG-positive neurons. (A) Absence of EdU staining (purple) in vehicle- and oA β -treated primary hippocampal neurons that were transduced with AAV for mAG-hGem (1-110) expression (green) and co-stained for neuronal β 3-tubulin (white). Merged images of all channels include nuclear DAPI staining (blue). Scale bar, 20 μ m. (B) Abundant EdU staining (purple) in dividing HEK293T cells co-stained for endogenous geminin (red) and nuclear DAPI (blue) as an experimental positive control.

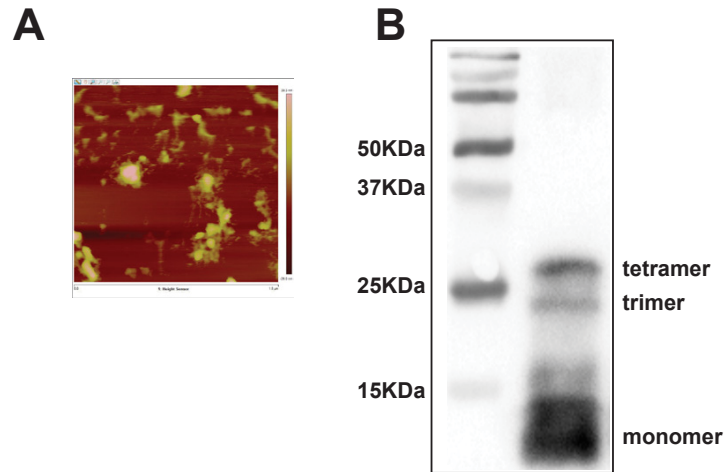


Figure S6. Characterization of Amyloid- β_{1-42} oligomers (oA β). (A) Atomic force microscopy topography imaging of oA β . After a 24-h incubation under oligomer-forming conditions, A β_{1-42} assembled into predominantly oligomeric structures (B) Representative Western blots of oA β , separated by SDS-PAGE on a 12% agarose gel, and probed with the specific monoclonal antibody, revealing A β_{1-42} monomers, trimers, and tetramers, and larger oligomeric assemblies ranging from 30 to 60 kDa.

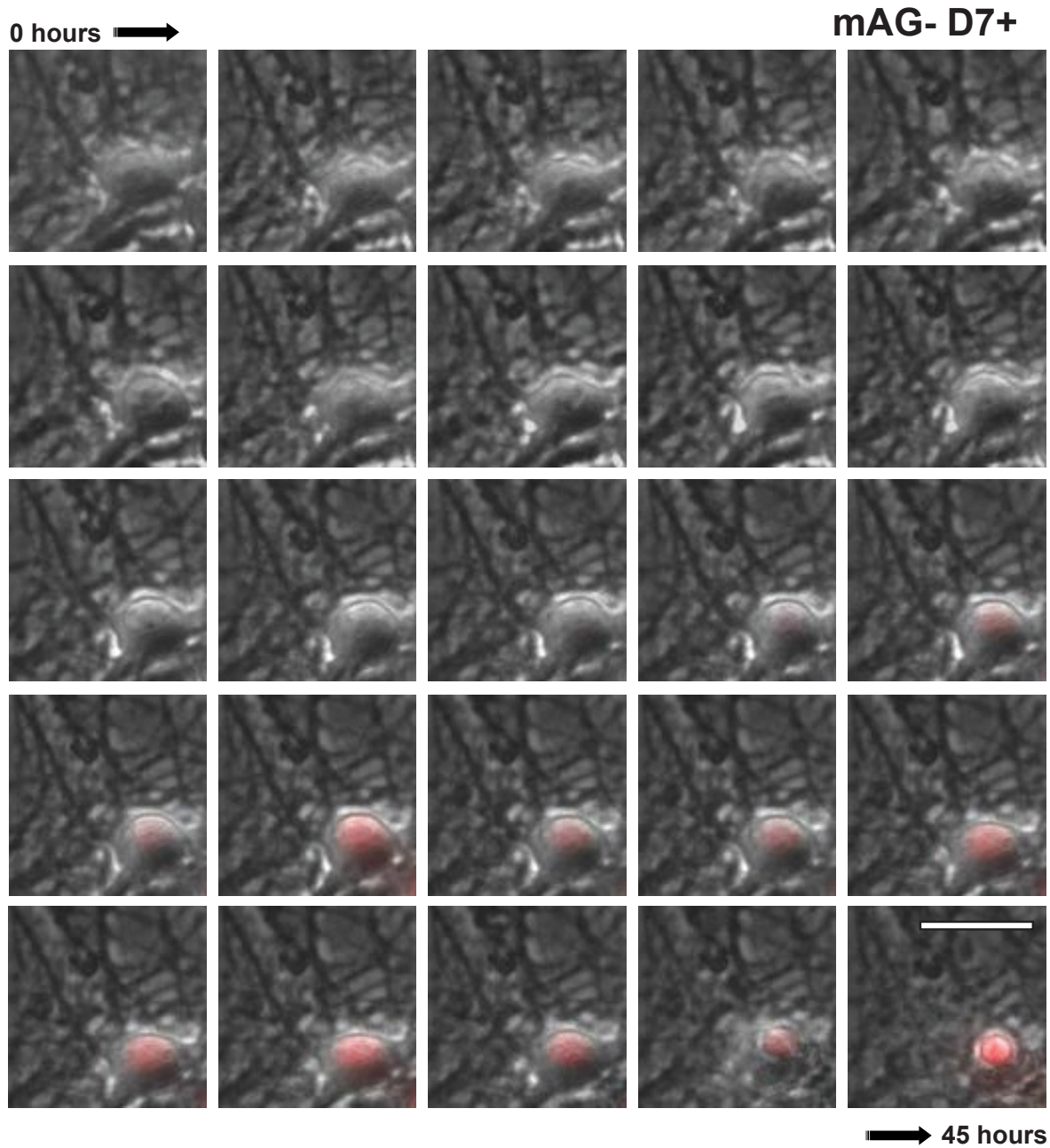


Figure S7. α A β -induced cell death in the absence of cell cycle activity. Image stack from live cell recordings upon α A β exposure at 0 hours of mAG-hGem (1-110)-transduced neurons that show no mAG fluorescence activity (mAG-) but undergo cell death as indicated by DRAQ7 uptake (D7+; red) during 45 hours recording. Scale bar, 25 μ m.

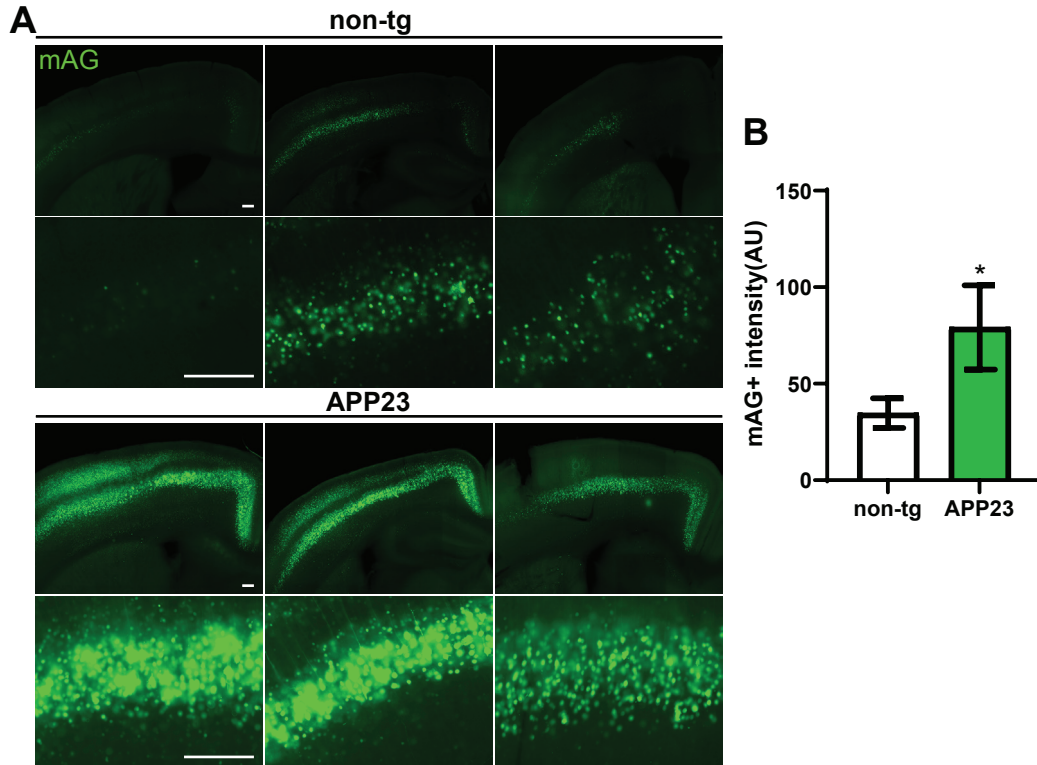


Figure S8. High intensity mAG activity in APP23 brains. (A) Representative fluorescence images of 50 μm -thick brain sections from 3 non-tg mice and 3 APP23 littermates at 3 months of age to show reproducibility of data. Higher resolution images show layer IV/V in the cortex. Scale bars, 200 μm . (B) mAG fluorescence intensity per mAG+ cell in non-tg and APP23 mice ($n=5-10$; $*P=0.0319$; Student's t -test). Error bars indicate means \pm SEM.

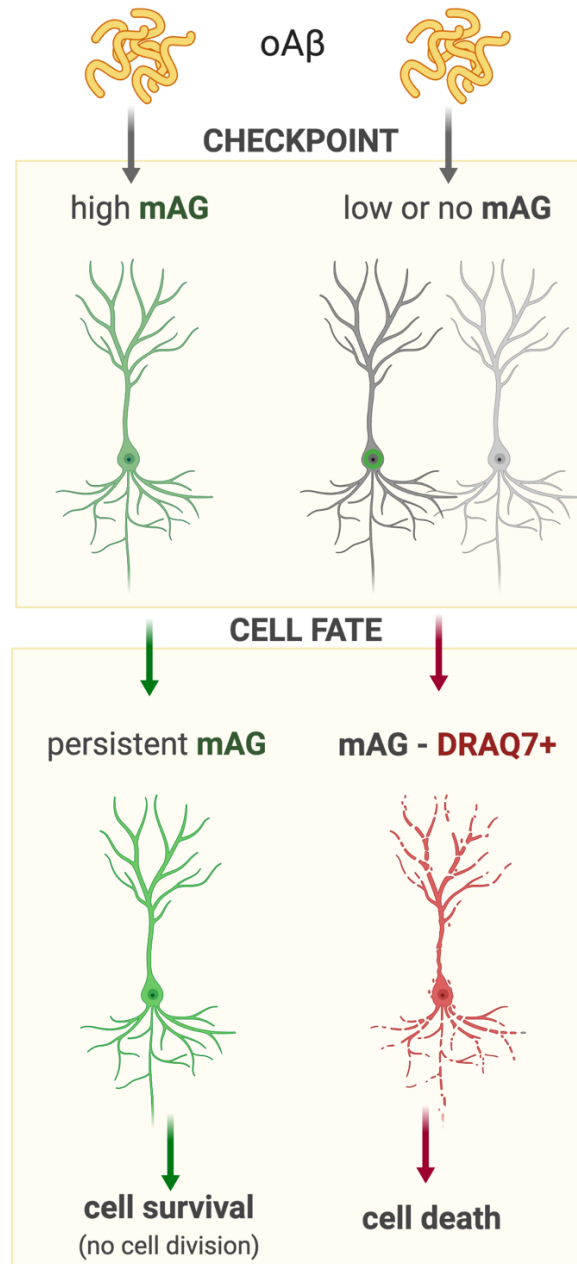


Figure S9. Proposed cell fate pathways. Neurons respond to oAβ depending on their cell cycle state at the time of the challenge. Depending on unknown check-point processes, neurons either commit to a progressive and persisting cell cycle-like state (mAG^{high}) without cell division that is associated with cell survival, or only transient (mAG^{low}) or, most commonly, absence of cell cycle pathway activity, which both result in cell death with 48 hours of oAβ exposure. Absence of mitosis in mAG^{low} cells suggest that cell death is different from mitotic apoptosis observed in dividing cell types.

Supporting video legend

Video S1. Time-lapse video of 48 hours live imaging of representative vehicle treated mature mKO2/mAG AAV-transduced neuron showing maintained mKO2 and transient mAG expression. Live imaging of individual channels for mKO2 (red), mAG (green) and phase contrast is followed by sequences of merged channels, demonstrating continuous mKO2 signals while mAG activity is transient in the same neurons. AAV titres were 6.6×10^6 viral genomes (vg) for mAG-hGem(1-110) and 2×10^6 viral genomes (vg) for mKO2-hCdt1(30-120) expression. Stacks of this video are presented in Fig. 1E.