

Investigating the mechanistic and temporal regulation of inhibitory synapse elimination during cerebral ischemia


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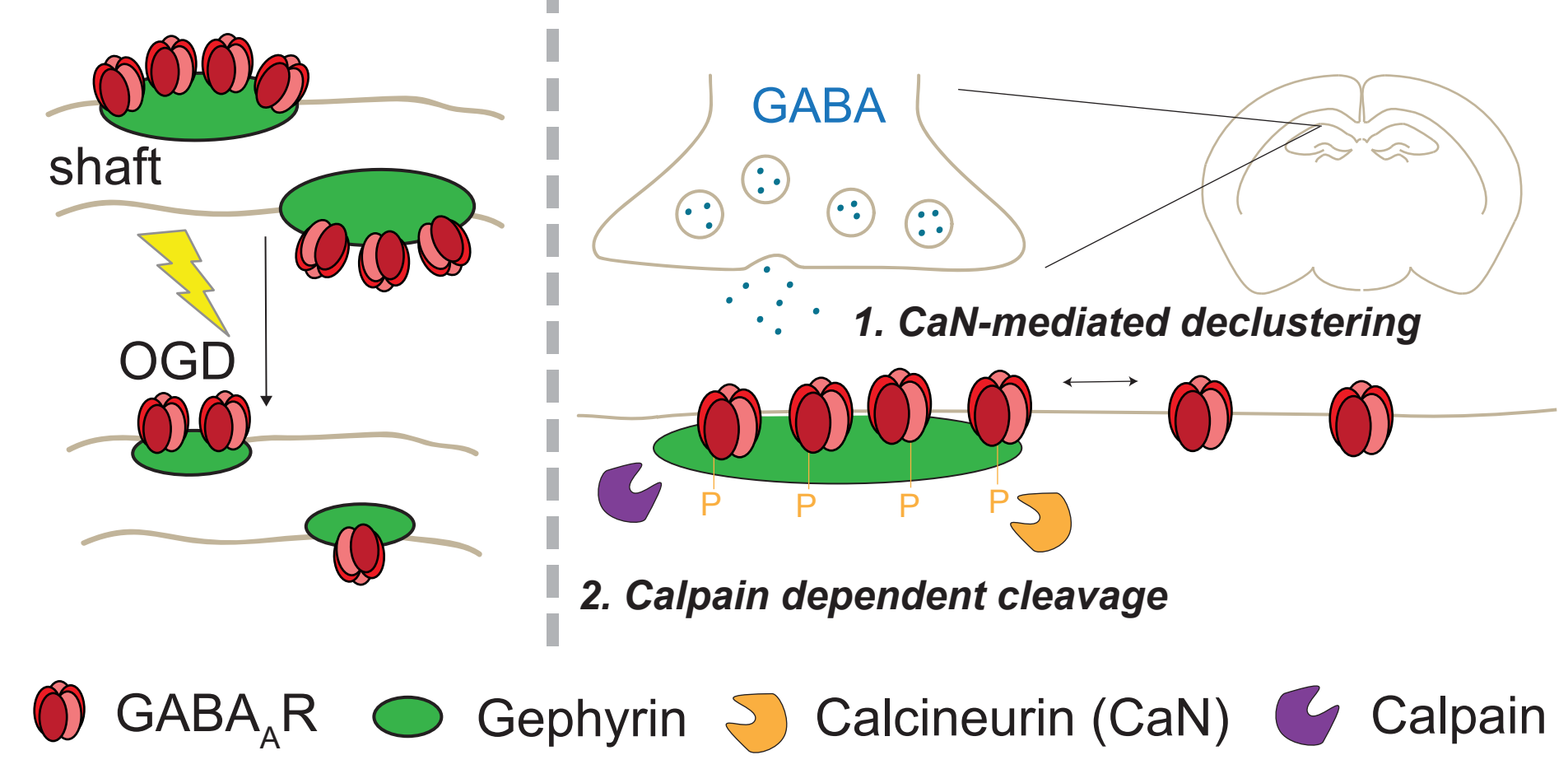
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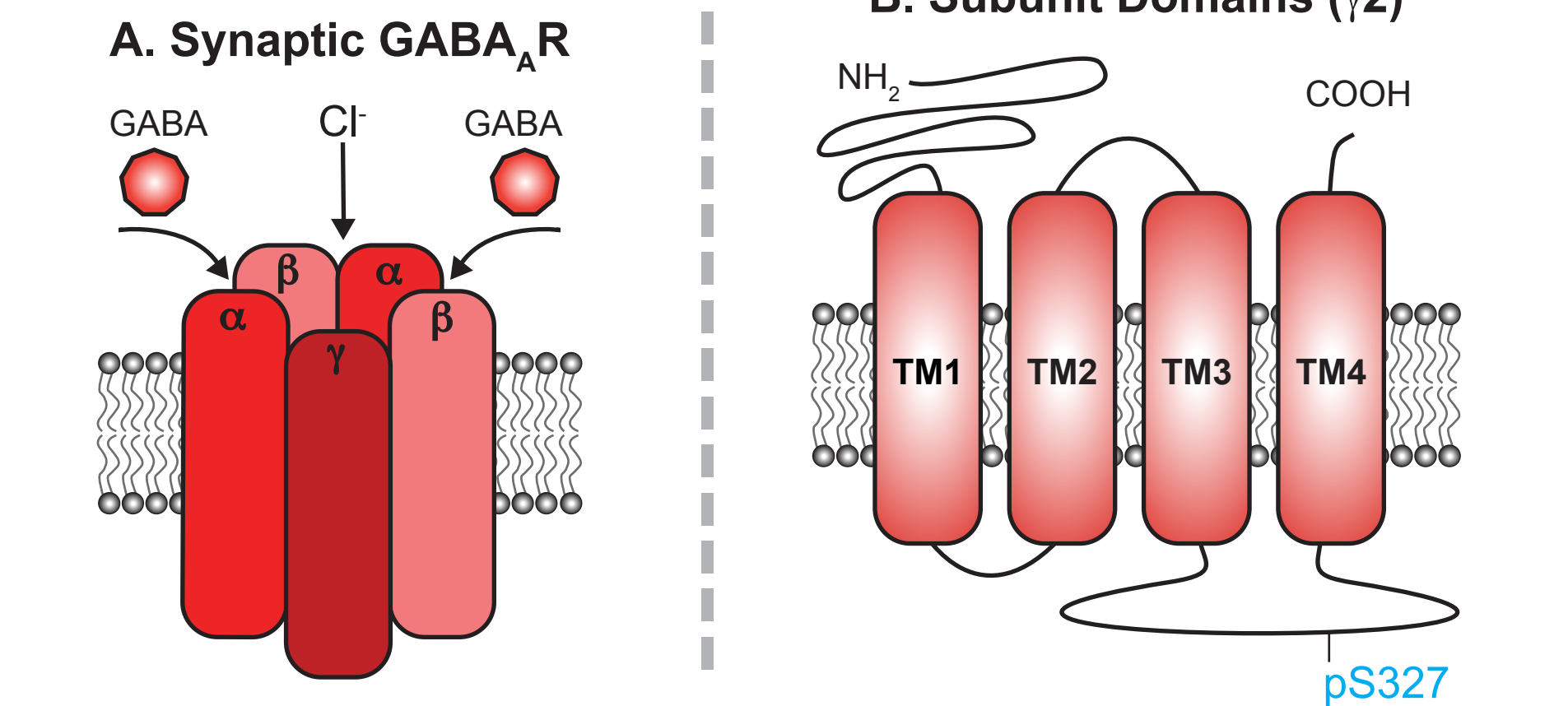
Introduction

Inhibitory synapses are crucial for maintaining correct neuronal excitability, which is important for efficient circuitry and proper brain function. Inhibitory GABA_A receptors (GABA_ARs) mediate the majority of fast synaptic inhibition in the brain. Thus, the number of postsynaptic GABA_ARs influences inhibitory strength. Shifts in neuronal excitability have been implicated in a variety of neurological disorders, including ischemia. The oxygen and glucose deprivation (OGD) observed during ischemic insult in hippocampal regions leads to synaptic depression through GABA_AR and gephyrin loss from synaptic sites. *However, mechanisms that regulate GABA_AR declustering and gephyrin elimination following an ischemic insult remain undefined.* In this project, I propose that GABA_AR declustering is mediated by calcineurin activity and this is the first step in facilitating synapse elimination. Furthermore, I speculate a role of the cysteine protease, **Calpain**, in mediating gephyrin loss during OGD. Based on this, I plan to investigate (i) mechanisms of synaptic GABA_AR declustering and gephyrin elimination in hippocampal pyramidal neurons following OGD (ii) probe the temporal regulation to determine the sequential flow of events promoting GABA_AR and gephyrin loss and (iii) use an *in vivo* model of cerebral ischemia to compare cell-type specific mechanisms in the CA1 hippocampus.



Synaptic GABA_ARs mediate inhibitory transmission

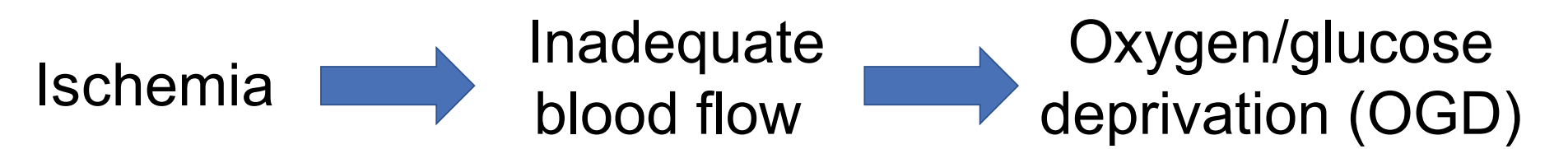
Subunit composition and PTMs determines both GABA_AR localization and function.



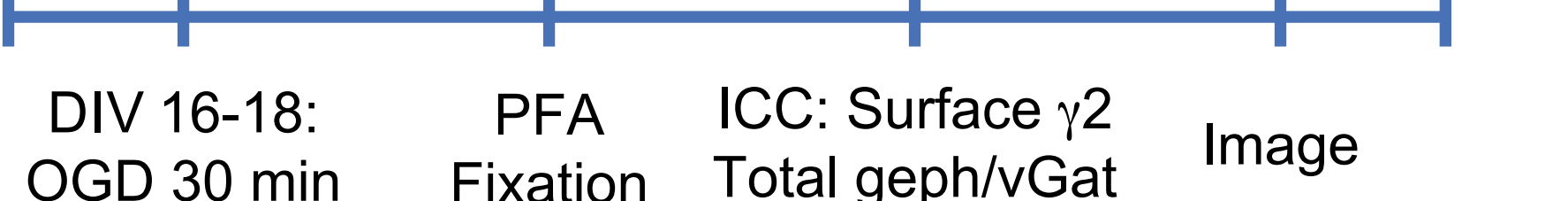
Synaptic GABA_ARs mediate phasic inhibition in the adult CNS. GABA_ARs are heteropentameric structures, with each subunit containing 4 transmembrane domains and an intracellular loop. The intracellular loop is where majority of post translational modifications of the receptor occurs.

Models of cerebral ischemia

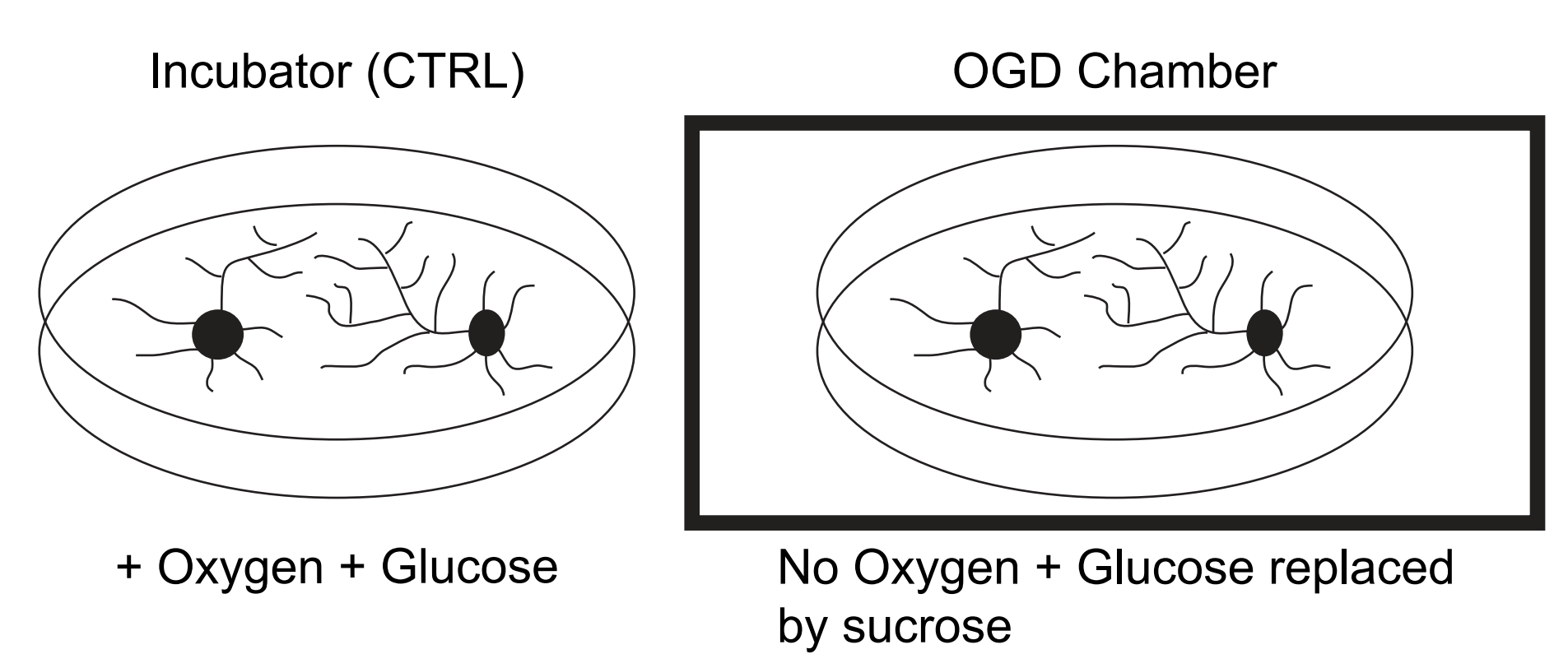
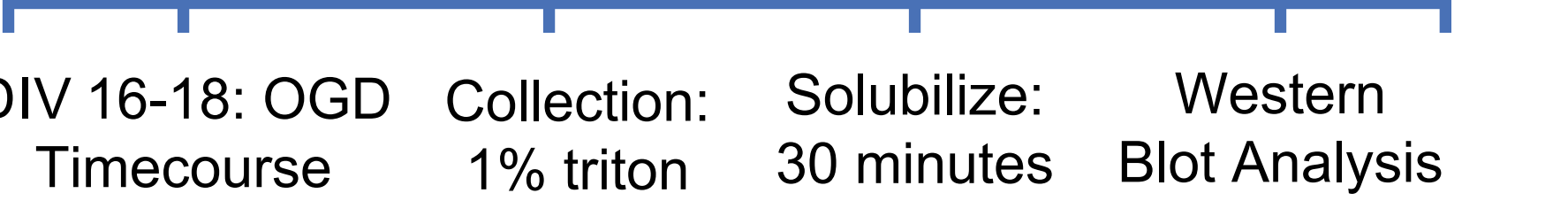
Anoxic chamber and sucrose mimics ischemia *in vitro*. CA/CPR mouse model induces cerebral ischemia *in vivo*.



Fixed imaging (in vitro)



Biochemistry (in vitro)

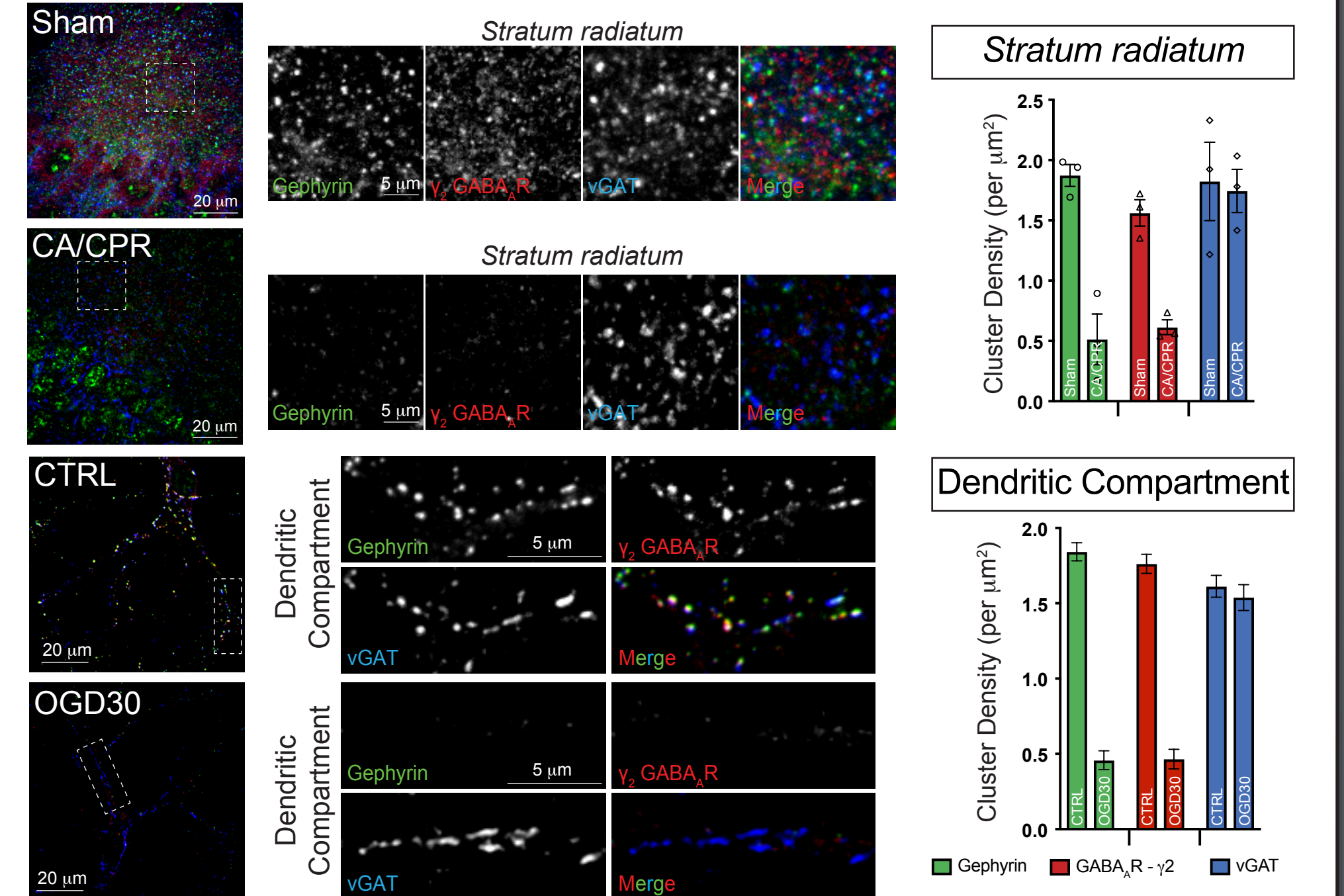


CA/CPR mouse model (in vivo)



In vivo and in vitro OGD models alter inhibitory synapse morphology

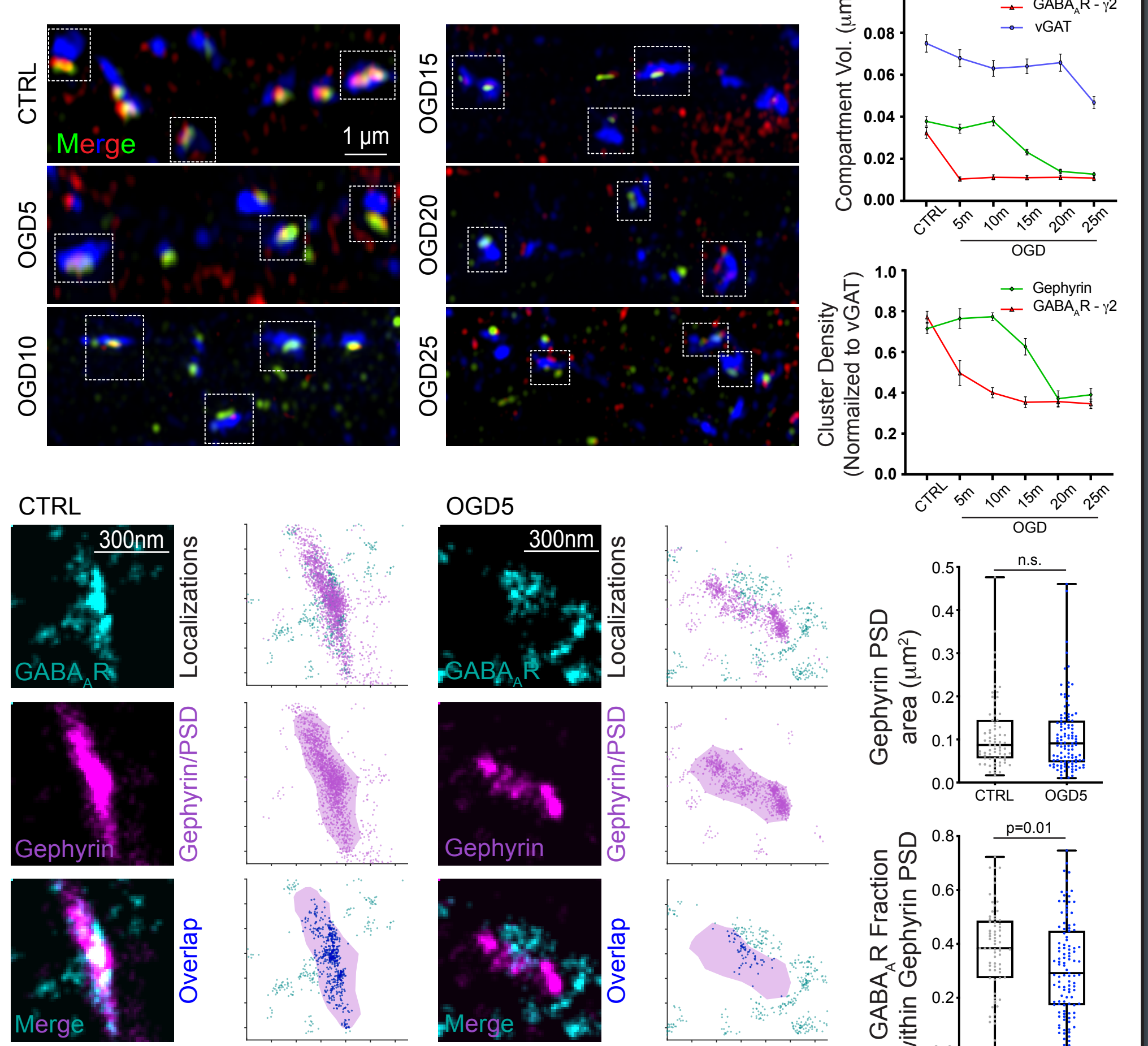
Postsynaptic components of the inhibitory synapse are removed before the presynapse during *in vivo* and *in vitro* models of OGD



Following ischemic insult, GABA_AR and gephyrin clusters decreases in hippocampal pyramidal neurons. This decrease is not observed for vGAT suggesting that an OGD insult initially targets postsynaptic components of the inhibitory synapse.

Super resolution imaging reveals nanoscale alterations to postsynapse

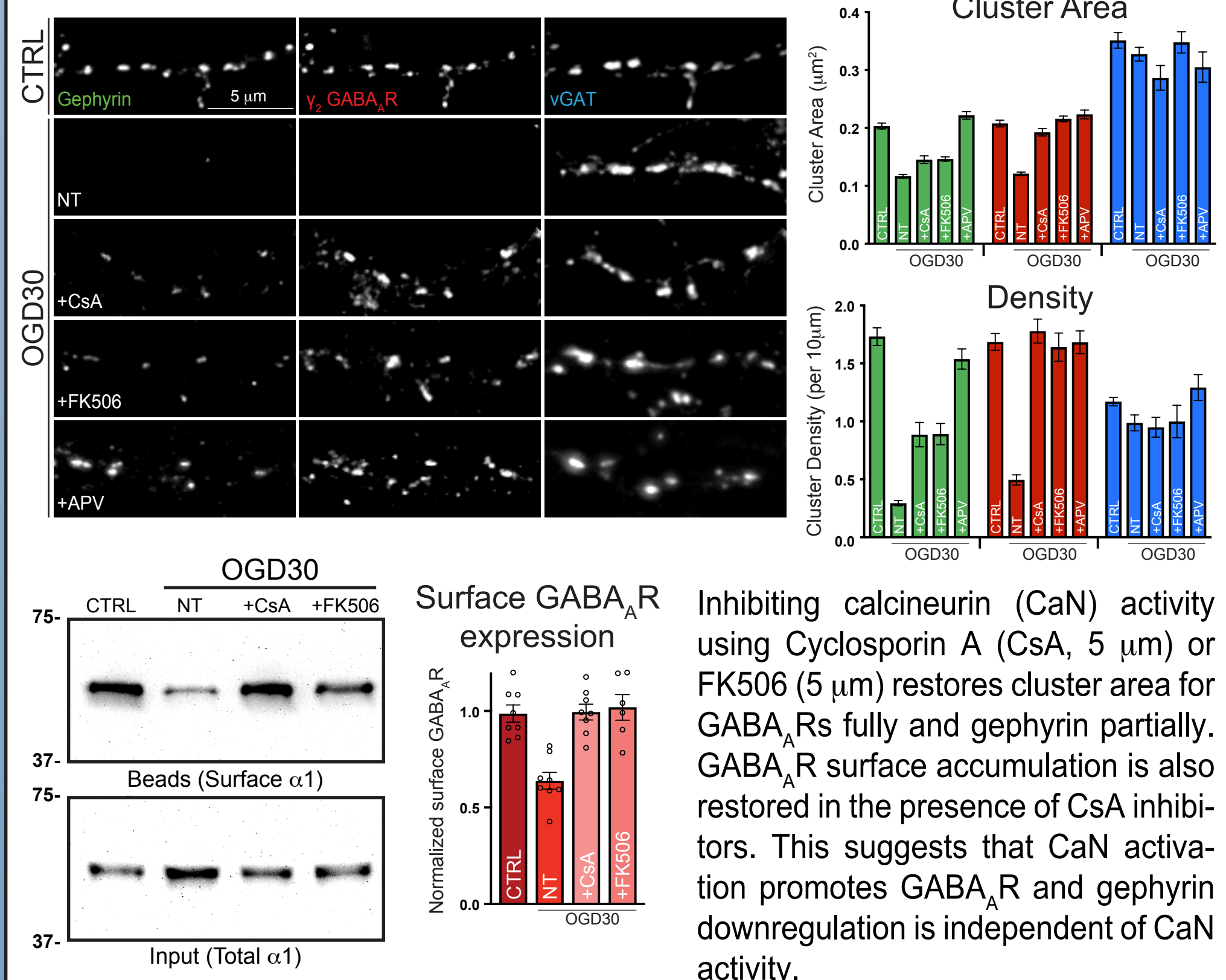
SIM and STORM imaging show initial loss (5min) of GABA_ARs, which is followed by gephyrin loss at later time points (10-15min).



3D-SIM and 2D-STORM increases imaging resolution for synapses, which are at the resolution limit of conventional imaging methods. Changes in GABA_AR compartment volume occurs within 5 minutes of OGD, while gephyrin compartment volume decreases by 15 minutes. Additionally, GABA_AR localizations overlapping with the gephyrin PSD decrease by 5 minutes of OGD.

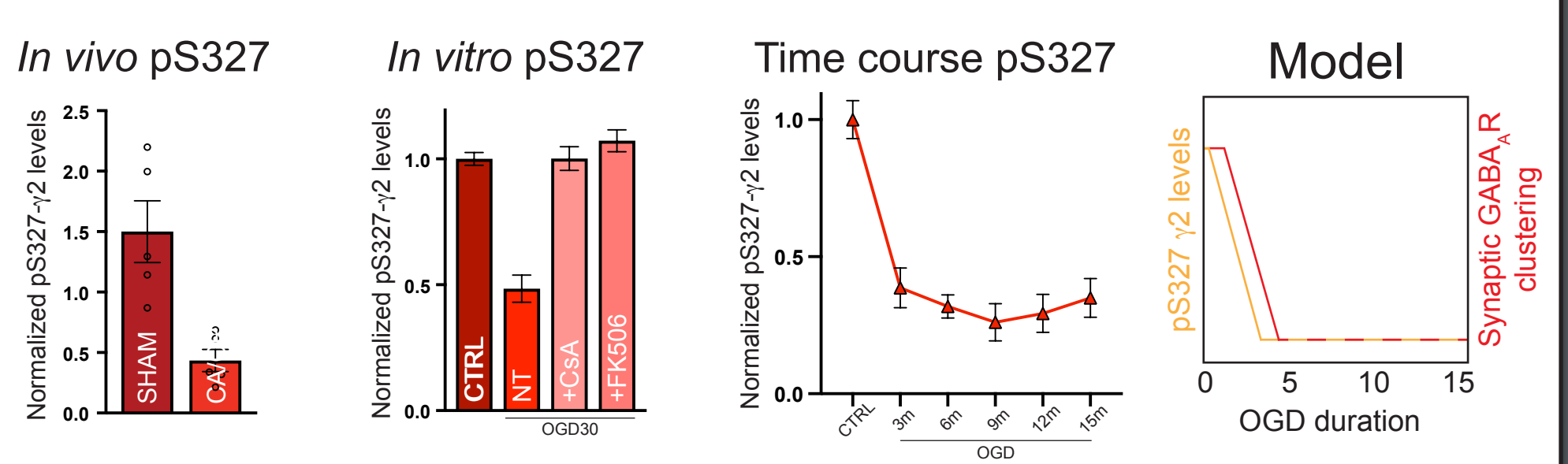
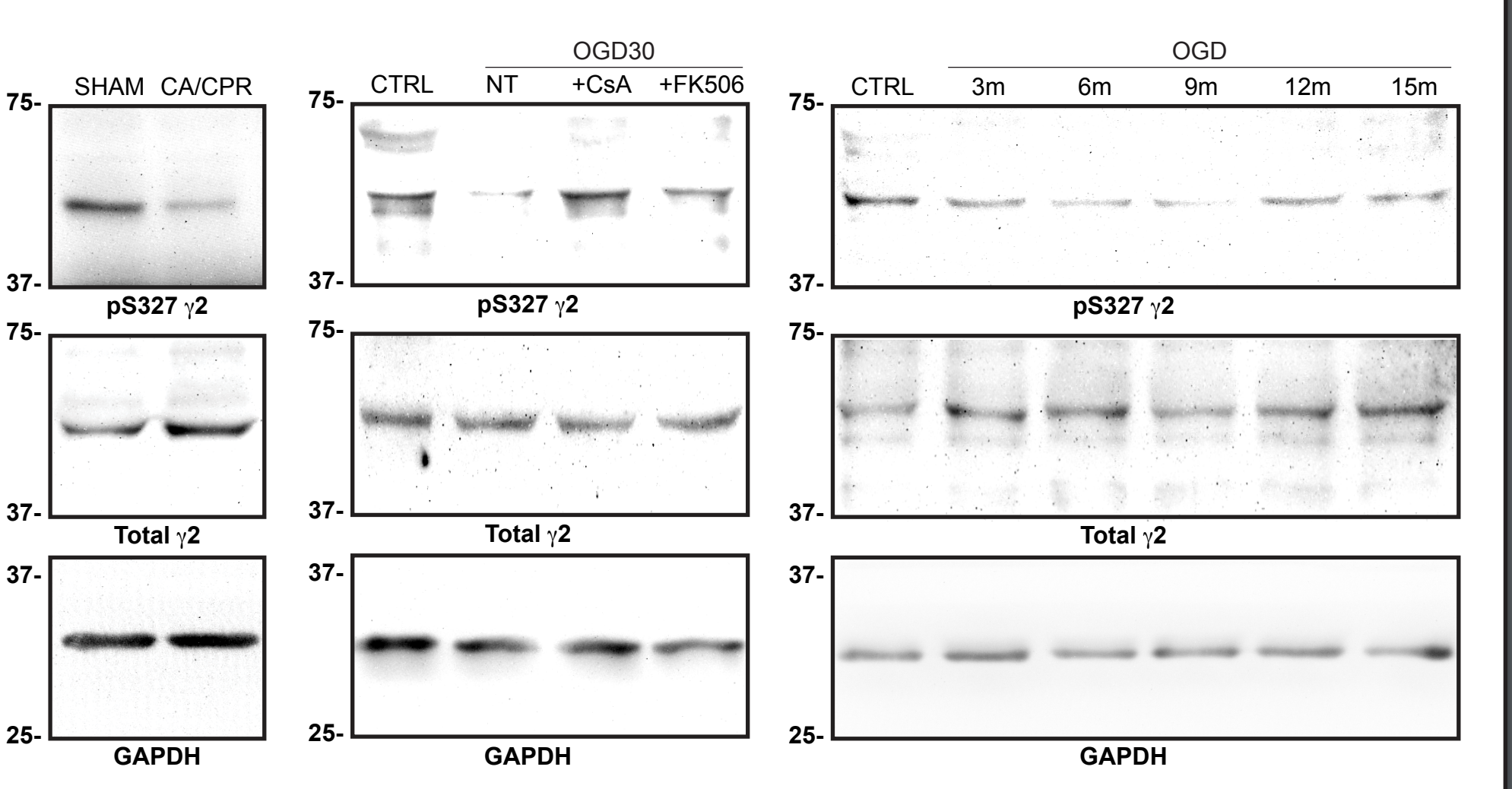
Calcineurin activation promotes GABA_AR loss during OGD

Calcineurin activity facilitates surface GABA_AR cluster removal during OGD.



OGD onset promotes CaN-mediated dephosphorylation of GABA_ARs (S327)

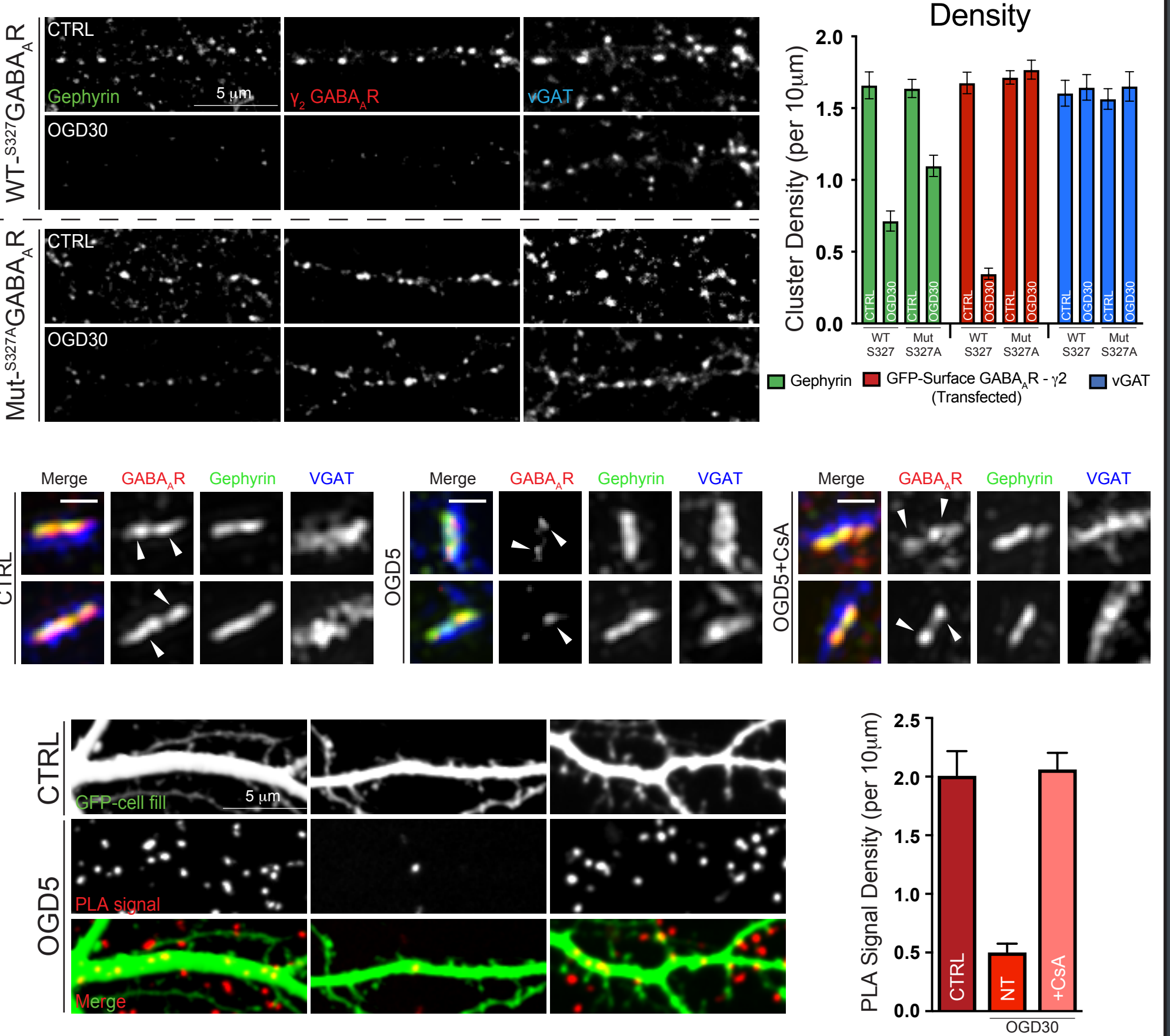
Serine 327 (S327) dephosphorylation on the gamma2 subunit of GABA_ARs occurs immediately following OGD via CaN activation.



Synaptic declustering of GABA_ARs from postsynaptic sites is mediated through CaN-dependent dephosphorylation of serine 327 (S327) on the gamma2 subunit during inhibitory synaptic depression. This site is dephosphorylated both *in vivo* and *in vitro* following OGD. S327 dephosphorylation is facilitated by CaN during OGD and occurs immediately following an insult.

Blocking dephosphorylation maintains GABA_AR clustering with gephyrin

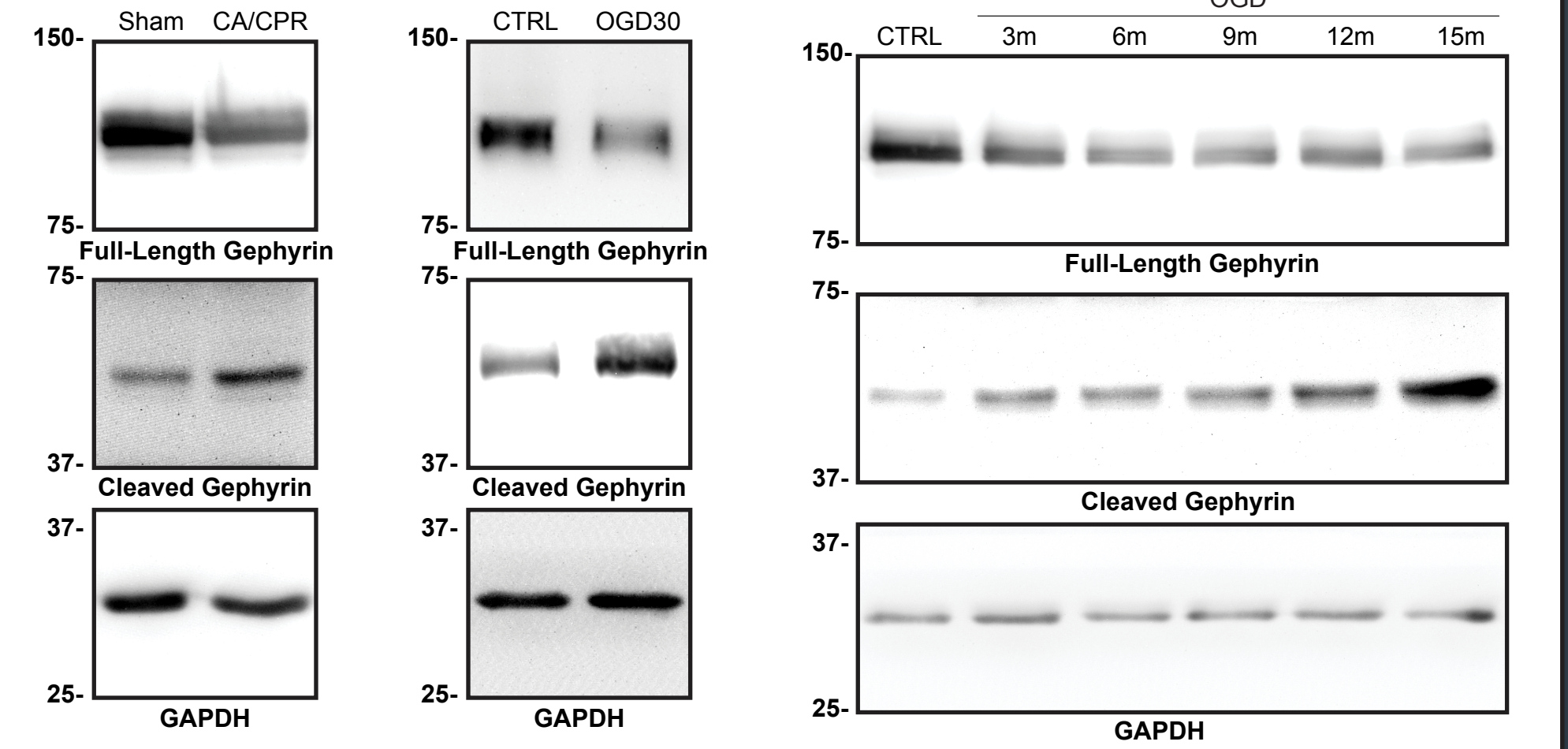
CaN dependent dephosphorylation of S327 reduces synaptic GABA_AR clustering by uncoupling from the scaffold gephyrin at the synapse.



Expression of a Super-Ecliptic pHluorin (SEP) tagged gamma2-GABA_AR shows that mutating serine 327 to alanine prevents the loss of surface GABA_AR clustering during OGD and partially restores gephyrin clustering. Using proximity ligation assays (PLA), which detects interactions under 40nm, the physical interaction between GABA_ARs and the scaffold gephyrin is lost by 5 minutes following OGD. This interaction is maintained during OGD when CaN activity is blocked. Together, this data shows an important role for S327 dephosphorylation in synaptic declustering of GABA_ARs from gephyrin during OGD.

Gephyrin is cleaved during OGD

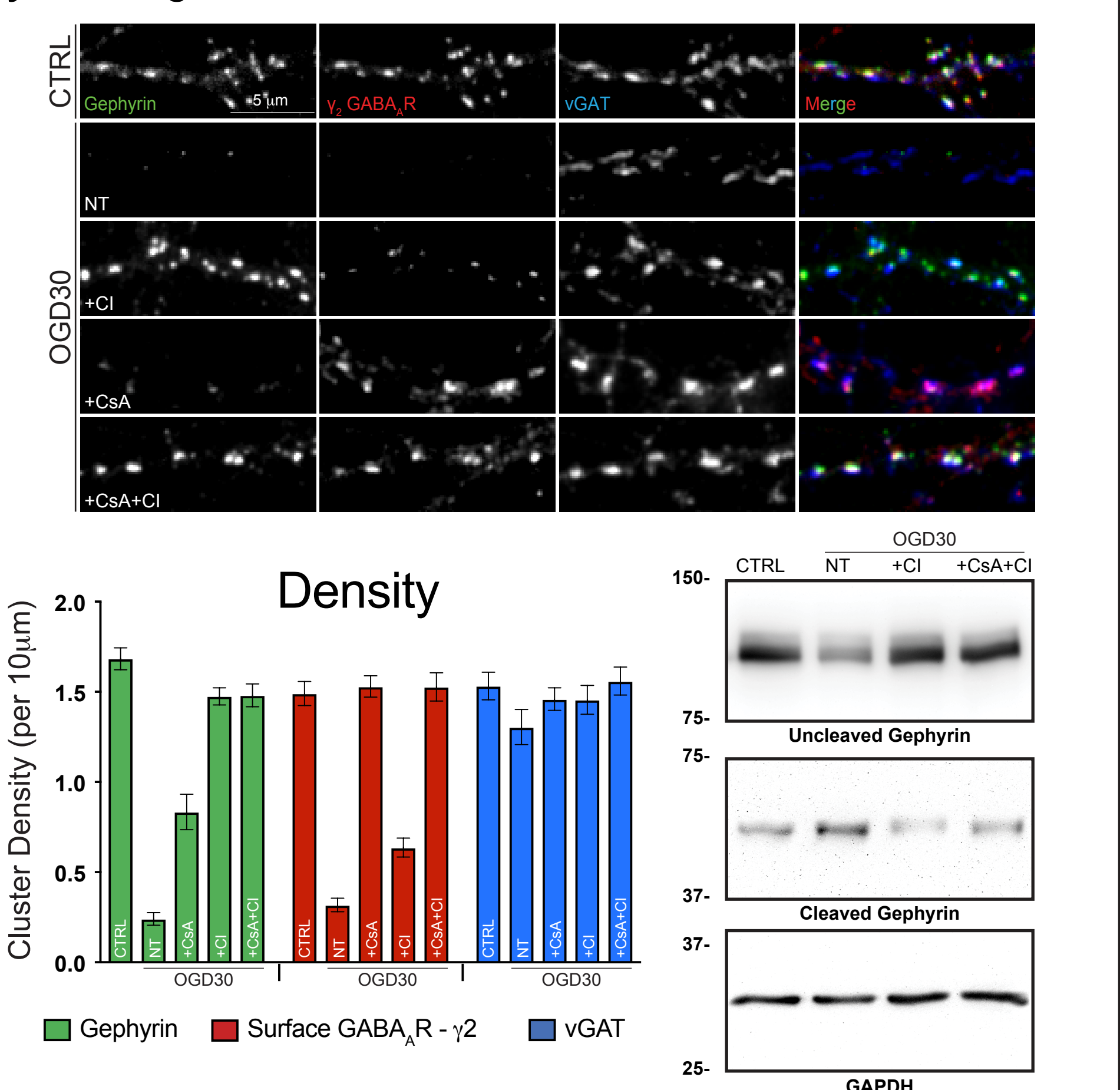
The scaffolding molecule gephyrin, which is found primarily at inhibitory synapses to anchor GABA_ARs, is cleaved following OGD.



Gephyrin cleavage is one mechanism that facilitates turnover of the scaffold found mainly at inhibitory synapses. The scaffold is cleaved both *in vivo* and *in vitro* following OGD. Gephyrin cleavage follows S327 dephosphorylation and GABA_AR declustering.

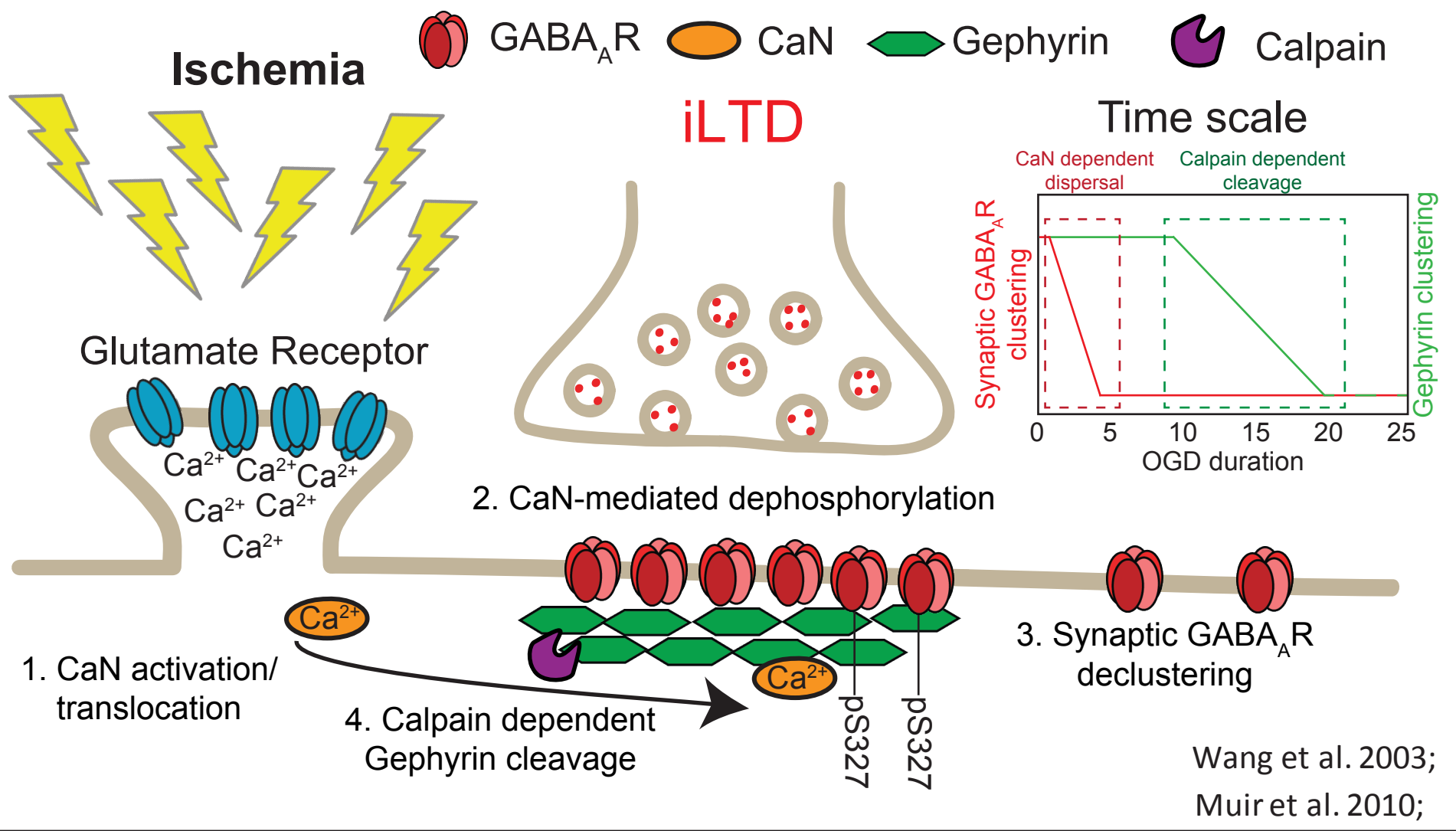
Calpain activity leads to gephyrin cleavage during OGD

Activation of the cysteine protease calpain cleaves full-length gephyrin during OGD.



Blocking calpain activity (Calpain Inhibitor III, CI, 1uM) prevents loss of synaptic gephyrin and partial loss of GABA_ARs. Use of both CsA and CI together restores clustering of both GABA_ARs and gephyrin.

Model of GABA_AR and gephyrin loss during OGD



Conclusions

- Post synaptic components of the inhibitory synapse are lost initially during an ischemic insult both *in vitro* and *in vivo*.
- SIM imaging reveals GABA_AR clustering is lost first (5min) followed by gephyrin loss (15min).
- STORM imaging shows GABA_AR outside of gephyrin PSD following OGD.
- CaN mediates synaptic declustering of GABA_ARs through S327 dephosphorylation.
- Blocking S327 dephosphorylation prevents GABA_AR cluster loss and restores gephyrin interaction.
- Gephyrin is cleaved by calpain during OGD.
- Blocking both CaN and calpain activity restores both the GABA_AR and gephyrin

Future Directions

- Investigate the role of GABA_AR endocytosis following declustering.
- Examine if inhibitory synapse loss is persistent following reperfusion.
- Investigate GABAAR sorting to late endosomes or lysosomes for receptor degradation.
- Explore neuroprotection to cell-death when GABA_AR declustering or gephyrin cleavage is blocked.