

1 **Supplementary Information**

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4 **Acutely elevated O-GlcNAcylation suppresses hippocampal activity by modulating both intrinsic**
5 **and synaptic excitability factors**

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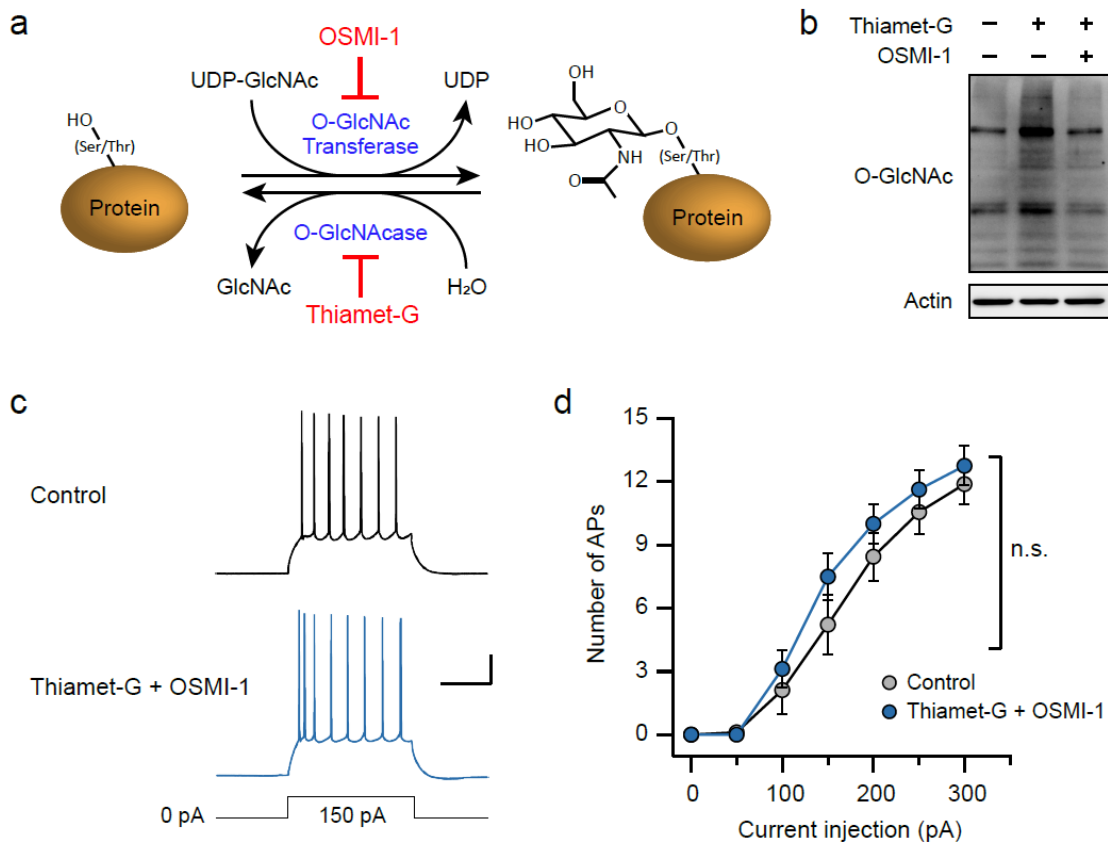
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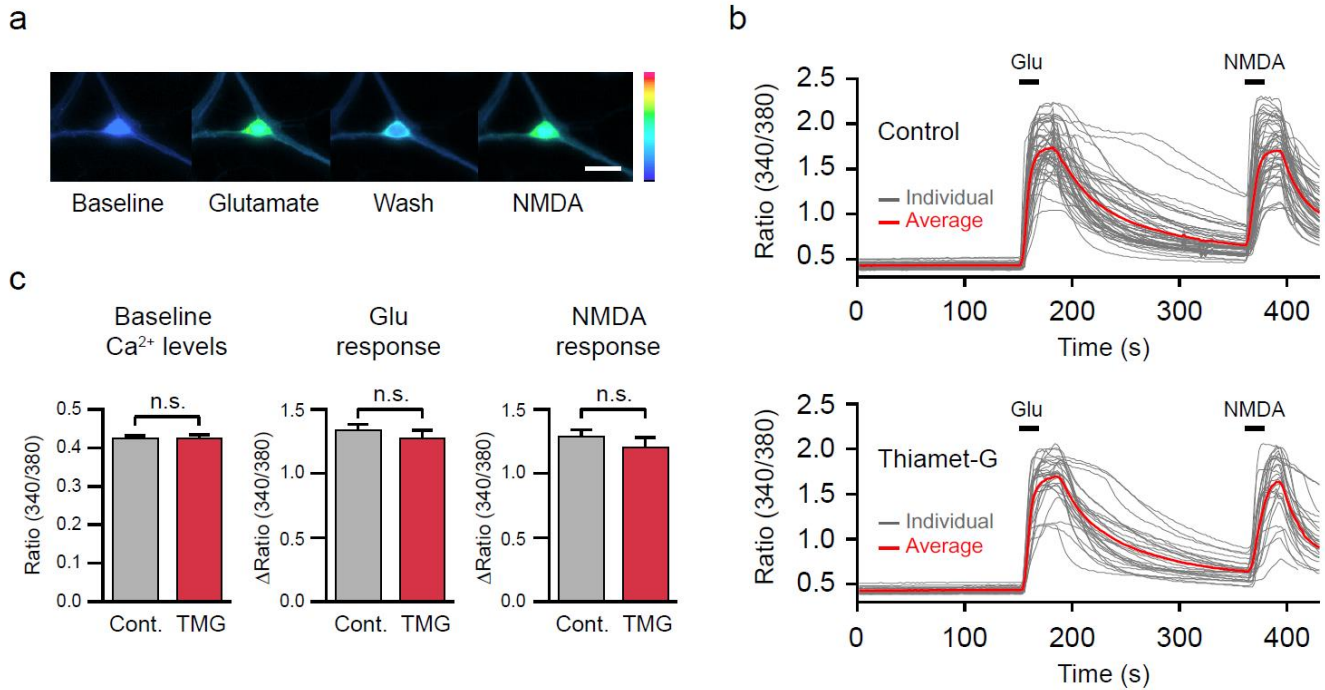
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27 **Supplementary Figure S1. Counteracting the effect of thiamet-G with an OGT inhibitor prevents a**
 28 **decrease in the intrinsic neuronal excitability caused by thiamet-G alone.** (a) Schematic diagram
 29 showing the reversible O-GlcNAc modification. OSMI-1 inhibits the addition of O-GlcNAc to
 30 serine/threonine residues by OGT. (b) OGA inhibition by thiamet-G (1 μ M, 2 hr) increases overall O-
 31 GlcNAcylation levels in acute hippocampal slices, but the co-application of an OGT inhibitor, OSMI-1
 32 (10 μ M, 2 hr), prevents the increase in O-GlcNAcylation levels. (c) Representative recordings of action
 33 potentials from CA1 pyramidal neurons induced by 150-pA current injection for 500 ms (scale bar: 20
 34 mV, 200 ms). (d) Co-treatment of thiamet-G and OSMI-1 does not alter the number of action potentials
 35 triggered by a series of current injection (50-pA increment, 7 steps, Control: n=9, Thiamet-G: n=8). Each
 36 circle represents mean \pm SEM (n.s.; not significant, unpaired t-test). Full-length blots are presented in the
 37 Supplementary Figure S4.

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43 **Supplementary Figure S2. Acutely elevated O-GlcNAc modification does not affect glutamate- and**
 44 **NMDA-induced calcium influx.** (a) Sample images of Fura-2-based intracellular calcium levels in
 45 dissociated hippocampal neuron culture treated with a pulse of glutamate (100 μ M, 30 s) or NMDA (100
 46 μ M, 30 s). Images are shown in pseudocolor reflecting the ratio of 340-nm images to 380-nm images
 47 (scale bar: 20 μ m, 340/380 ratio range in pseudocolor: 0 - 3). (b) Glutamate and NMDA pulses triggered
 48 robust calcium influx in hippocampal neurons. Calcium responses from each individual neuron are
 49 displayed as thin gray lines, and averaged calcium responses are shown in red. (c) Increasing O-
 50 GlcNAcylation levels by thiamet-G did not alter resting calcium levels and the size of glutamate- and
 51 NMDA-mediated calcium influx (Control: n=51, Thiamet-G: n=27). Error bars represent \pm SEM (n.s.; not
 52 significant, unpaired t-test).

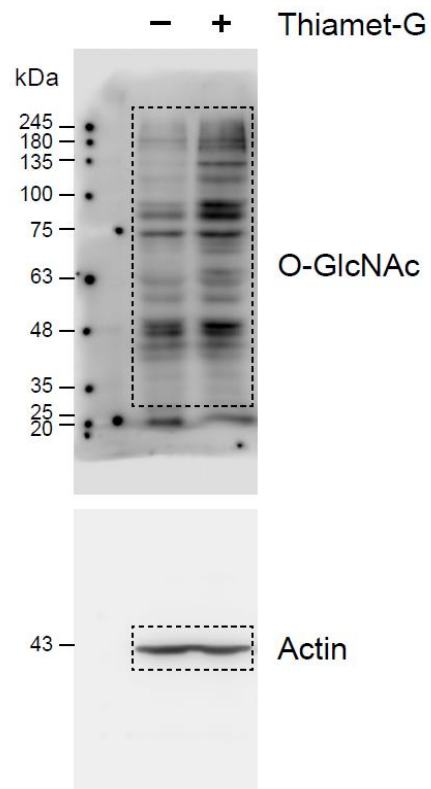
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60 **Supplementary Figure S3. Full-length blots.** The original images for the blots in Figure 1b are shown.

61 Dashed boxes indicate the cropped areas presented in the main figure.

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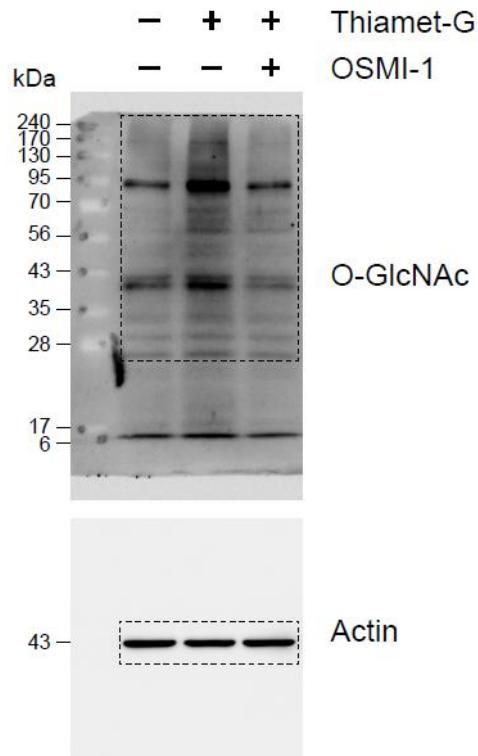
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73 **Supplementary Figure S4. Full-length blots.** The original images for the blots in Supplementary Figure
74 S1b are shown. Dashed boxes indicate the cropped areas presented in the supplementary figure.

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