Supplementary Information to

CaMKII phosphorylation of neuroligin-1 regulates excitatory synapses

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Supplementary Figure 1: NL-1 and GluA1 c-tails have similar reaction kinetics. (a) GST-NL-1 or GluA1 were incubated with purified CaMKII and $[\gamma-P^{32}]$ ATP and analyzed by autoradiography. Reactions were stopped at their marked time. (b) Protein concentrations are plotted as a ratio of phosphorylated NL-1 (P-32) to total NL-1 (CBB) normalized to the 1 min reaction condition. Saturation of phosphorylated NL-1 and GluA1 occurred at approximately 10 min. Total protein was visualized by CBB protein staining, in **a,b**. Full-length blots are presented in Supplementary Figure 4 when applicable.



Supplementary Figure 2: NL-1 T739A does not affect presynaptic release probability. Paired-pulse ratio (PPR), second EPSC over first EPSC for consecutive stimuli separated by 40 ms. Example traces normalized at first EPSC for (a) NL-1. (b) NL-1 T739A. (c) Second EPSC over first \pm SEM. NL-1 and NL-1 T739A had similar PPRs (P > 0.05, n = 5). Scale bars represent 25 ms.



Supplementary Figure 3: Titration of phosphorylated NL-1 with pT739-Ab (**a**) Protein concentrations from adult WT or NL-1 KO brains were titrated with pT739-Ab. (**b**) Protein concentrations are plotted as ratio of phosphorylated (IP) to total NL-1 (input) normalized to the 0.2 mg protein condition for an individual brain. Saturation of the pT739-Ab occurs between 0.5 and 1 mg of protein.



Supplementary Figure 4: Full-length blots of cropped blots from the manuscript.