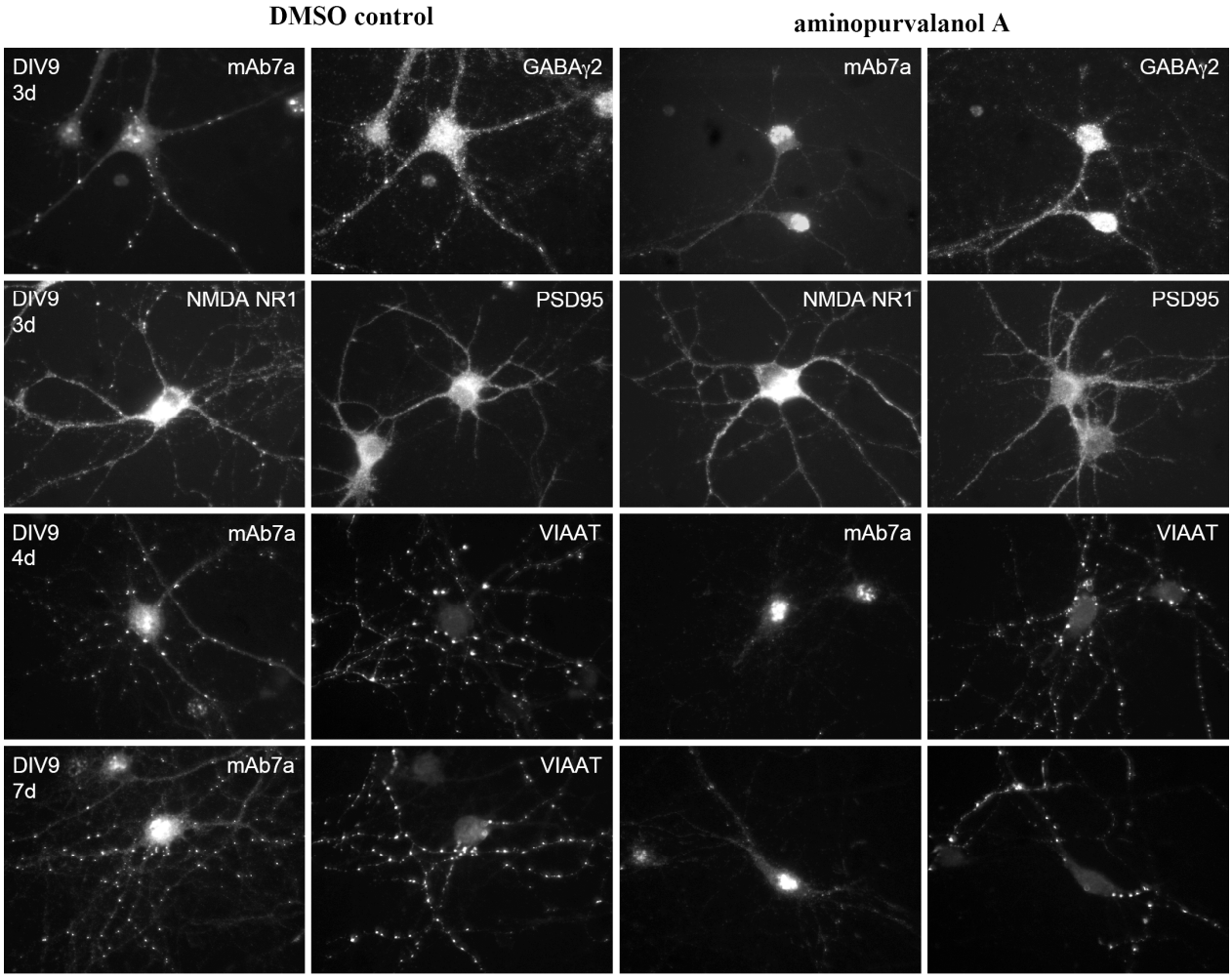
Supplementary figures

Figure 1



Immunofluorescence microscopy of hippocampal neurons upon 3, 4 and 7 days of CDK-inhibition (two right columns) compared to DMSO-treated control cells (left two columns). Note a strong reduction of mAb7a immunoreactivity with all three treatments, whereas the VIAAT staining looks unchanged.

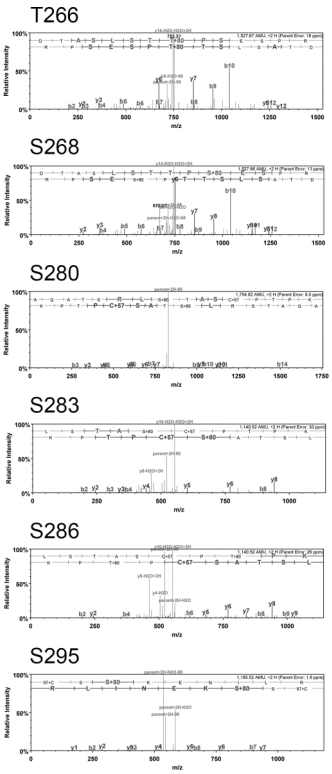


Figure 2

Representation of spectra analysis.

The uninterpreted MS/MS spectra were searched against a small protein database containing the gephyrin sequence using the Mascot software (Matrix Science). The algorithm was set to use semitrypsin as proteolytic specificity, assuming carbamidomethyl as a fixed modification of cysteine, and oxidized methionine and deamidation of asparagines and glutamine as variable modifications. Phosphorylation of serine, threonine and tyrosine was also set to variable modification. Mass tolerance was set to 1.1 Da and 0.1 Da for MS and MS/MS, respectively. Each phosphopeptide found by the software was taken into account but only accepted after manual evaluation of the fragment spectra.

Supplementary methods:

In-gel tryptic digestion and LC-MS/MS analysis-

After SDS-PAGE, coomassie-stained bands were cut out with a scalpel. Gel slices were transferred to a 96-well plate and reduced, alkylated and digested with trypsin (1). Peptides were extracted from the gel pieces with 50% acetonitrile/0.1% TFA, concentrated nearly to dryness in a speedVac vacuum centrifuge and diluted to a total volume of 30 µl with 0.1% TFA. 25 µl of the sample was analysed by a nanoHPLC system (Eksigent, Axel Semrau) coupled to an ESI LTQ Orbitrap mass spectrometer (Thermo Fisher). The sample was loaded on a C18 trapping column (Inertsil, LC Packings) with a flow rate of 10 µl/min 0.1% TFA. Peptides were eluted and separated on an analytical column (75µm x 150mm) packed with Inertsil 3 µm C18 material (LC Packings) with a flow rate of 200 nl/min in a gradient of buffer A (0.1% formic acid) and buffer B (0.1% formic acid, acetonitrile): 0-6 min: 3% B; 6-60 min: 3-40% B; 60-65 min: 60-90% B. A parent mass list with the calculated m/z values of possible single or doubly phosphorylated peptides was used for preferred selection of phosphopeptide candidates. One survey scan (res: 60000) was followed by 3 information- dependent product ion scans in the Orbitrap (res: 7500). 2+, 3+ and 4+ charged ions were selected for fragmentation.

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1. Catrein, I., Herrmann, R., Bosserhoff, A., and Ruppert T. (2005) Experimental proof for a signal peptidase I like activity in Mycoplasma pneumoniae, but absence of a gene encoding a conserved bacterial type I SPase. *FEBS J*. **272**, 2892-2900