Supplemental Text and Figures

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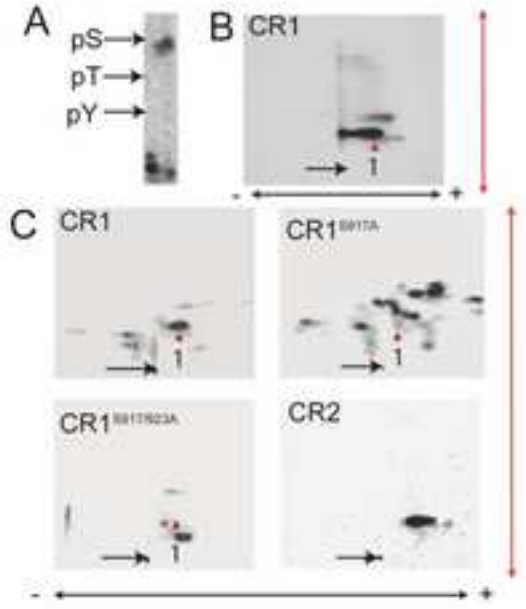
Supplementary Figure 1



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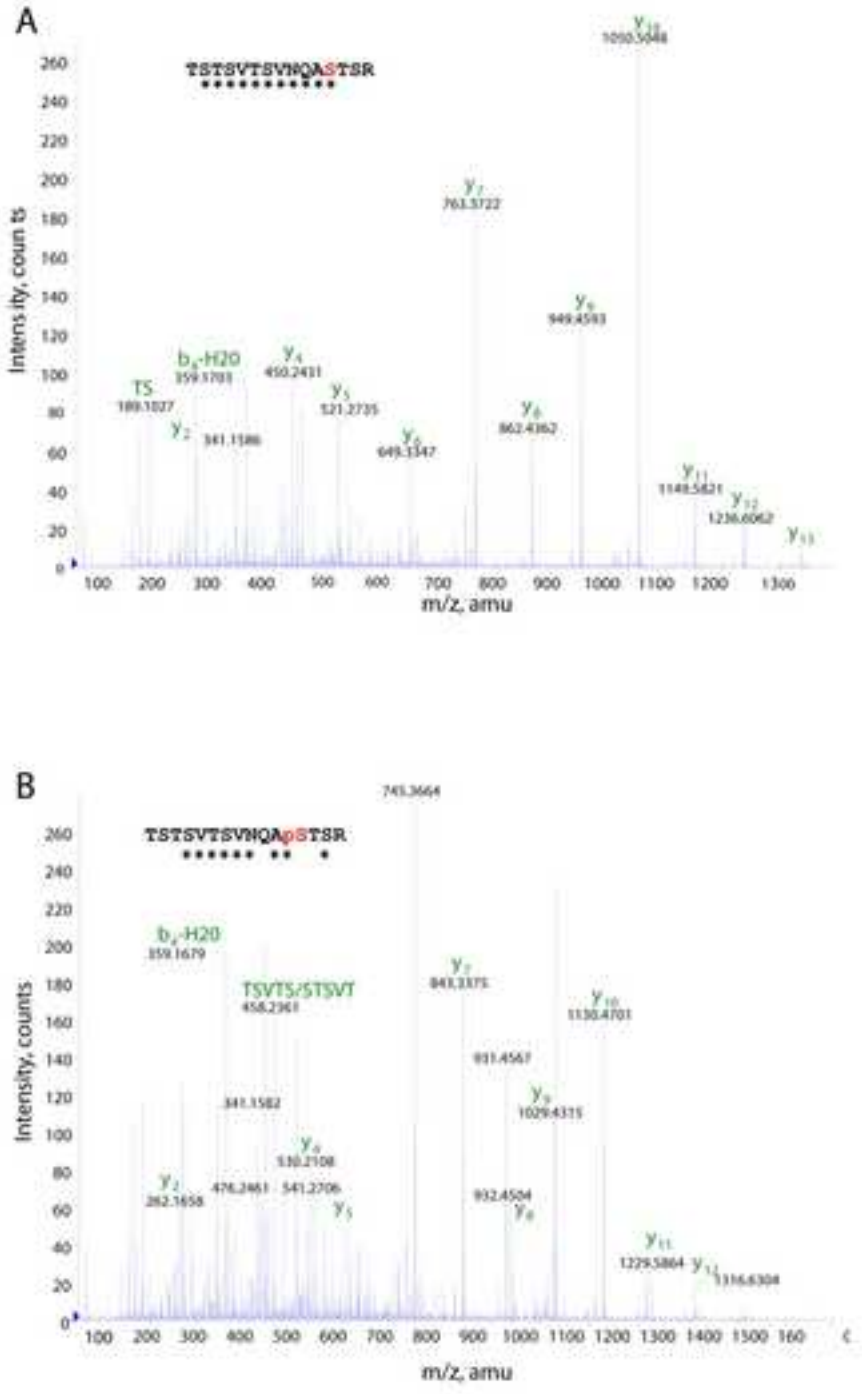
Supplementary Figure 2



Figure

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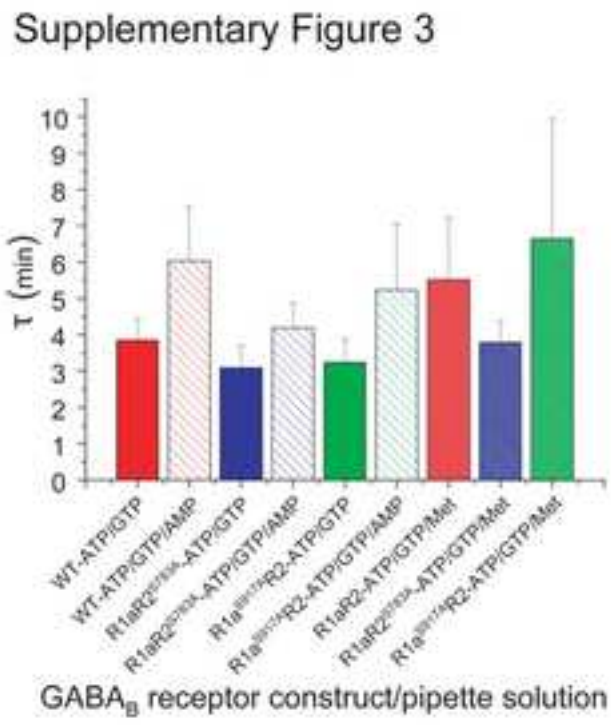

Figure 4

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Supplementary Figure 4

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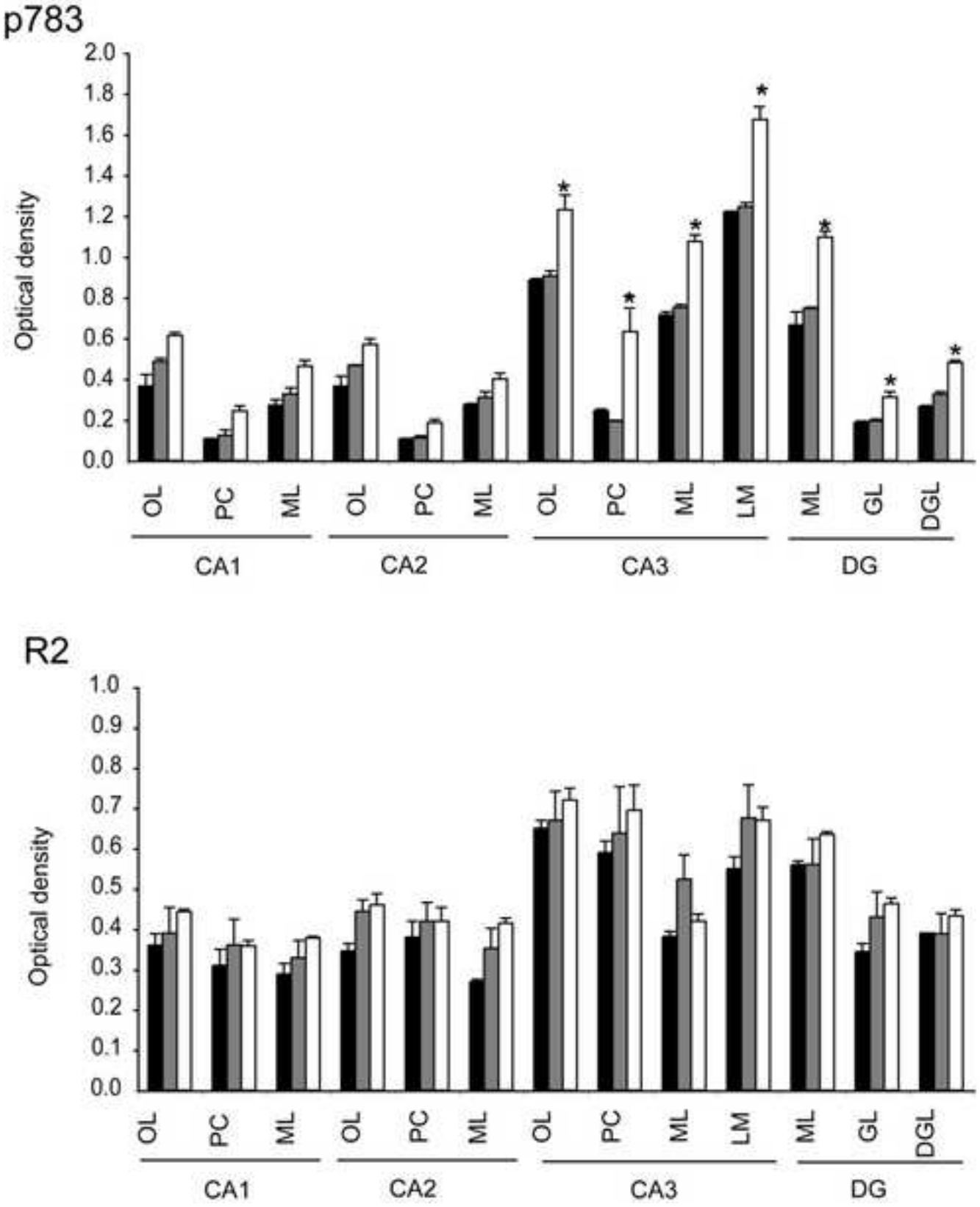
Supplementary Figure 5



Supplementary Figure 1. Analyzing $GABA_B$ receptor phosphorylation by phospho-peptide mapping and phospho-amino analysis. A. GST-CR1 phosphorylated by brain extracts and purified by SDS-PAGE was subject to trypsin digestion and the resulting phospho-peptides were hydrolyzed in 6N HCl and subject to TLC followed by autoradiography. The migration of phospho-serine (PS), threonine (PT) and tyrosine (PY) are indicated. **B**. GST-CR1 phosphorylated by brain extracts was digested with trypsin and then subject to thin layer electrophoresis (black double-headed arrow) followed by ascending chromatography (red double-headed arrow) and then by autoradiography. The single black arrow represents the origin. The red arrow signifies peptide 1. **C.** Wild-type and mutant fusion proteins were phosphorylated *in vitro* by AMPK, purified SDS-PAGE and subject to digestion with trypsin. The respective peptides were then separated as outlined in **B**.

Supplementary Figure 2. Identification of S783 as an AMPK phosphorylation site within $GABA_BR2$. A. Collisional induced dissociation spectrum of a doubly-charged peptide of m/z = 763.4, retention time 20.2 min. Y-ion series is labeled where present. Spectrum matches unphosphorylated peptide TSTSVTSVNQASTSR, with matched y-ions indicated by asterisks. **B**. Collisional induced dissociation spectrum of a doubly-charged peptide of m/z = 803.3, retention time 20.8 min. Y-ions are labeled where present. Spectrum matches phosphopeptide TSTSVTSVNQApSTSR, with matched y-ions indicated

by asterisks. Note 80 Da increases in mass for ions y4 to y12 (but not y2), unambiguously showing the addition of phosphate at S783.

Supplementary Figure 3. Analysis of the GABA-current run-down. $GABA_BR1$ and R2 subunits, either wild-type or mutants, were co-expressed in GIRK cells and subjected to whole–cell patch clamp electrophysiology to record GABA-activated currents. The patch pipette electrolyte was always supplemented with 2mM ATP and 1mM GTP either in the absence or presence of either 1mM AMP or 1mM metformin (Met), as indicated. From the time-stability relationships shown in text figure 3, the decays were fitted with a monoexponential function to determine the decay time constants (τ) shown here. Each bar represents the mean ± s.e.m. from n = 5-9 determinations.

Supplementary Figure 4. Analysis of S783 phosphorylation in hippocampal neurons using immunofluorescence. 12-14 DIV hippocampal neurons were fixed and processed for immunohistochemistry with anti-pS783 antibody and a FITC-conjugated secondary. Anti-pS783 staining was also performed in a 500-fold molar excess of the phospho (P-Pep) or dephospho (Pep) antigens. Scale bars represent 10 μm in each panel.

Supplementary Figure 5. Comparing levels of S783 phosphorylation and $GABA_BR2$ subunit expression in the hippocampus of MCAO and control

rats. Digital images of brain sections stained with p783 or R2 as indicated were exported to *MetaMorph* and the optical density measurements were made for varying domains of the hippocampus. OL = oriens layer, PC = pyramidal cell layer, LM = stratum lacunosum moleculare, GL = granule cell layer of the dentate gyrus, DGL = polymorph layer of the dentate gyrus for sham (black bars), Stroke R (control; grey) and Stroke L (injury; open). * = significantly different from control ($p<0.01$; students t-test; n=4).