

Supplemental Figures

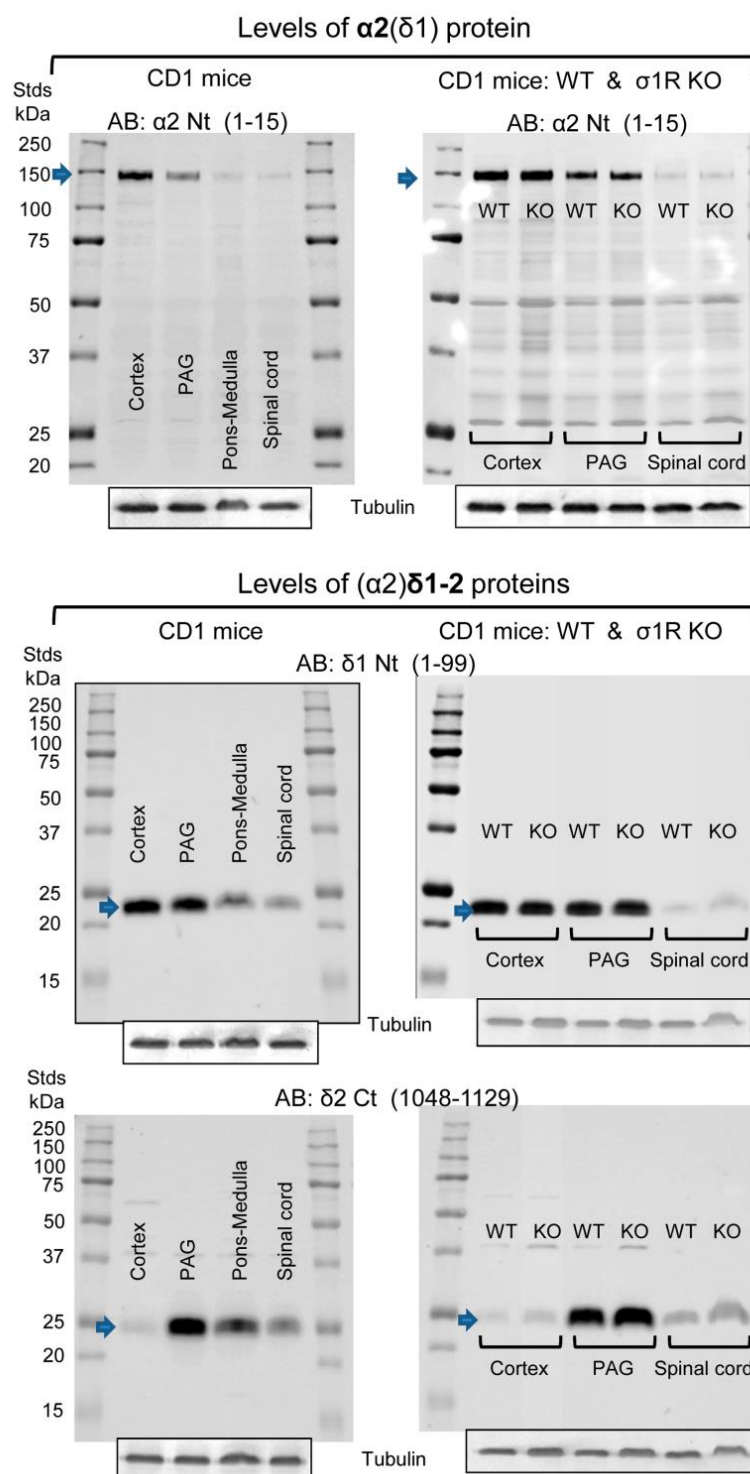


Figure S1. Expression of $\alpha 2(\delta 1)$, $(\alpha 2)\delta 1$ and $(\alpha 2)\delta 2$ in the cerebral cortex, PAG, pons-medulla and spinal cord of CD1 wild type and CD1 $\sigma 1R^{-/-}$ mice. Mouse neural structures were collected and P2 fractions enriched in synaptosomes were obtained, resolving 60 μ g protein/lane by SDS-PAGE and analyzing this extract in Western blots that were probed with antibodies against $\alpha 2\delta 1-2$ proteins (see Methods, representative Western blots are shown).

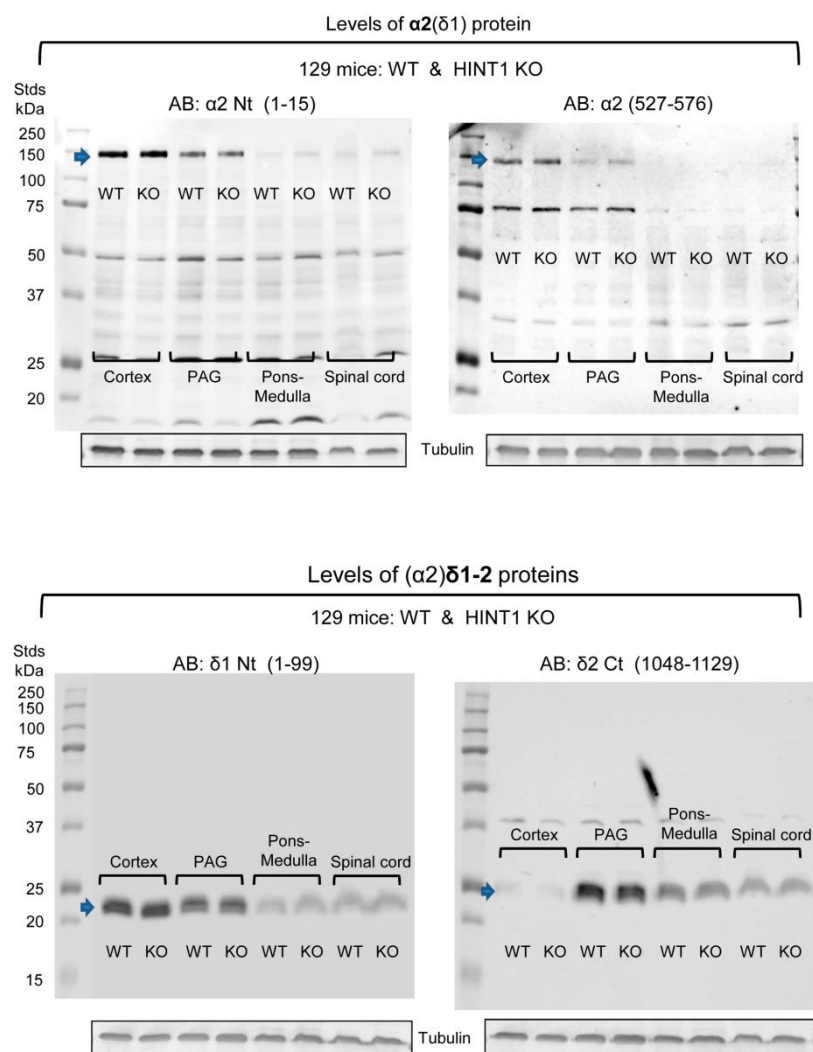


Figure S2. Expression of $\alpha 2(\delta 1)$, $(\alpha 2)\delta 1$ and $(\alpha 2)\delta 2$ in the cerebral cortex, PAG, pons-medulla and spinal cord of 129 HINT1^{+/+} and 129 HINT1^{-/-} mice (for details see Figure S1, representative Western blots are shown).

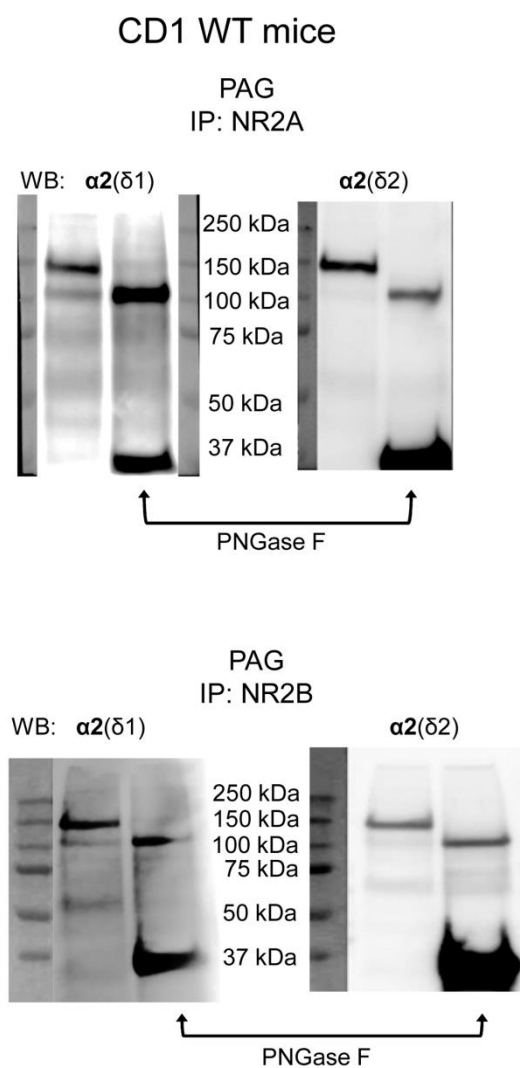


Figure S3. Representative Western blots of the assay presented in the Figure 2 main text.

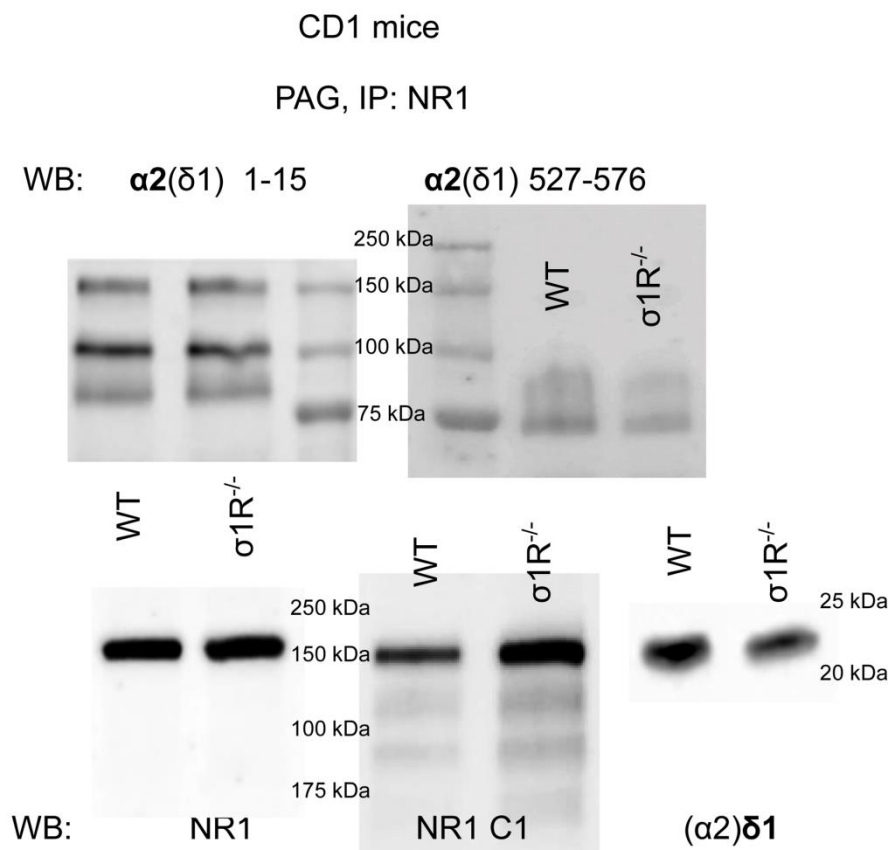


Figure S4. Representative Western blots of the assay presented in the Figure 4 main text.

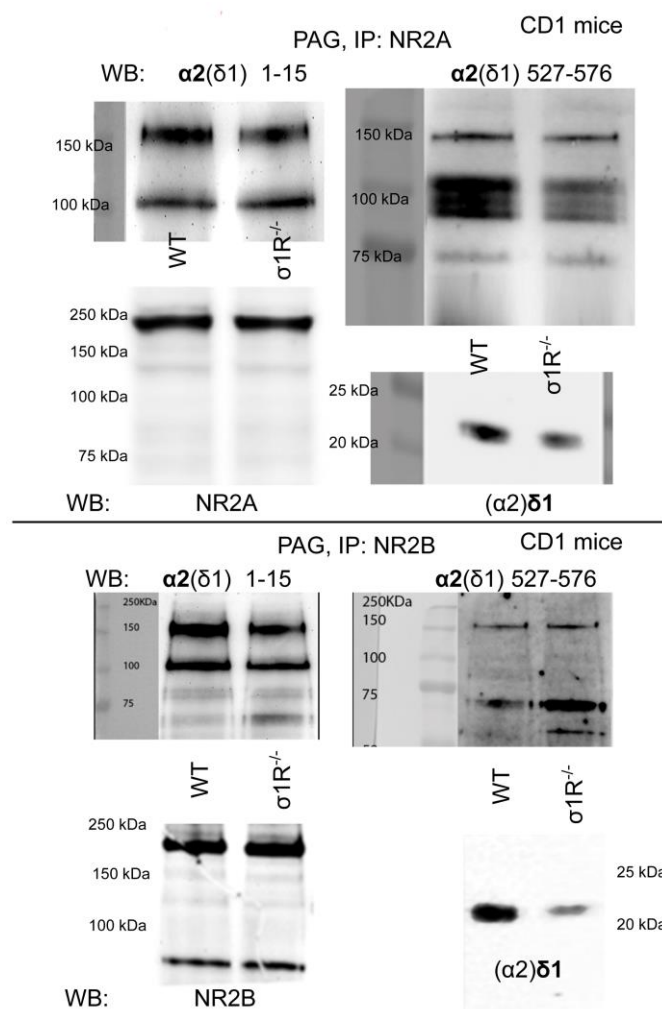


Figure S5. Representative Western blots of the assay presented in the Figure 4 main text.

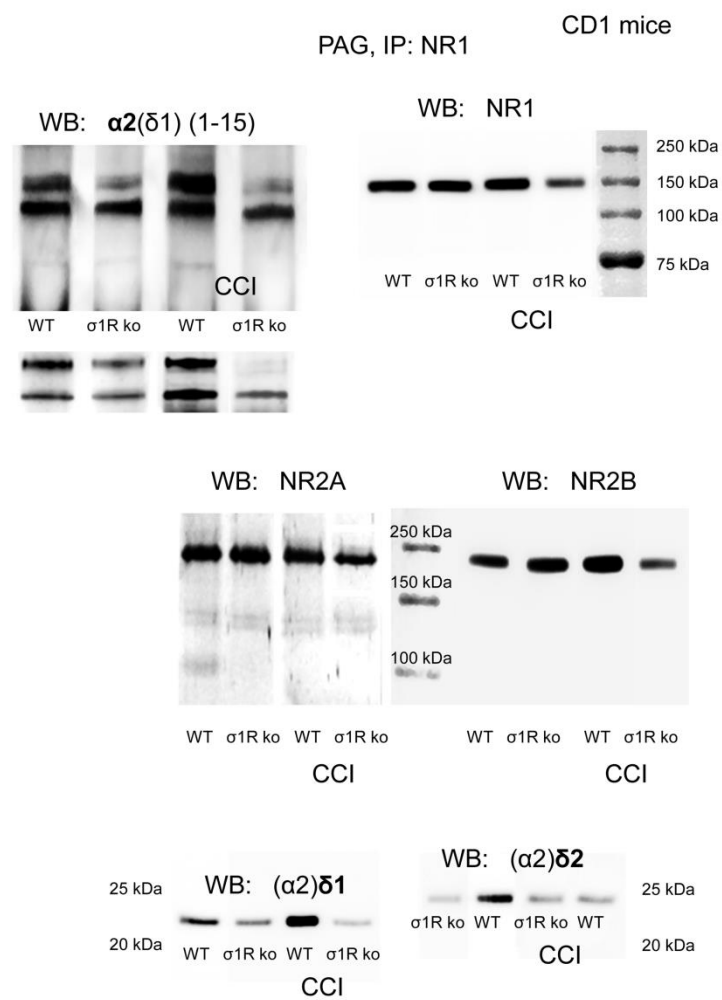


Figure S6. Representative Western blots of the assay presented in the Figure 5 main text.

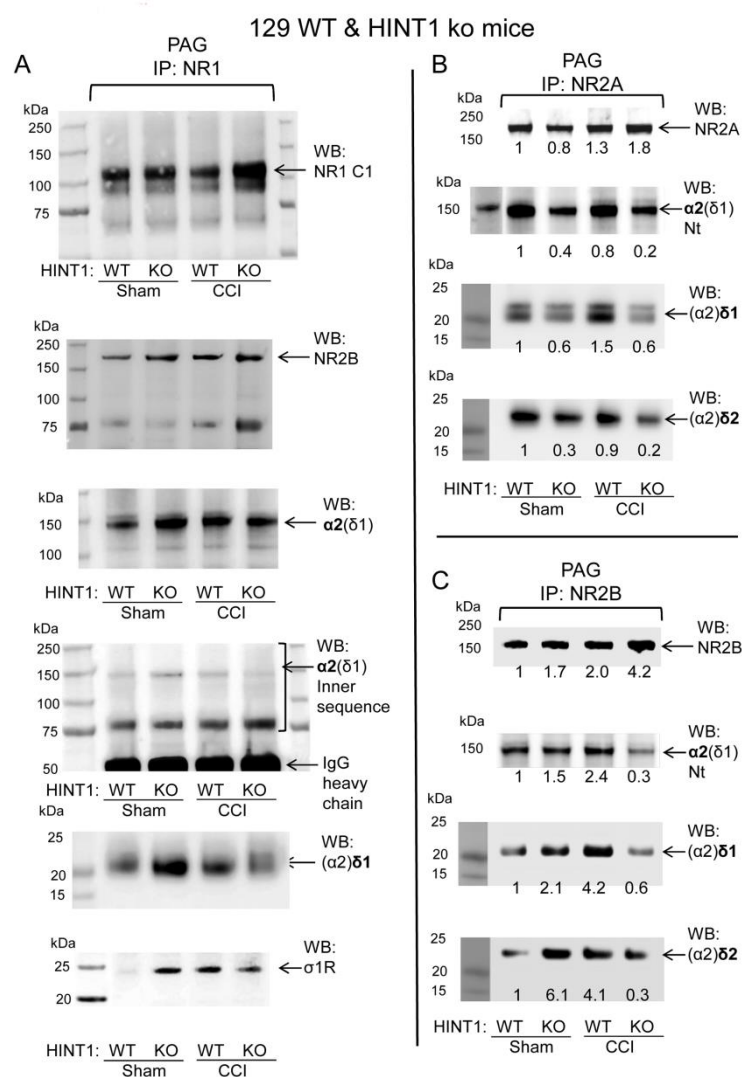


Figure S7. Effect of HINT1 on the molecular associations between the $\alpha 2\delta 1$ -2 and NR1, NR2A or NR2B subunits in the CCI model of neuropathic pain. 129 WT and 129 HINT1^{-/-} (KO) mice were sacrificed, and PAG synaptosomal fractions were obtained 7 days after surgery. Following membrane solubilization, (A) NR1, (B) NR2A and (C) NR2B subunits were immunoprecipitated (IP), and the co-precipitated proteins were resolved by SDS-PAGE and then analyzed in Western blots (WB). Representative Western blots are shown (for details see Figure 6).

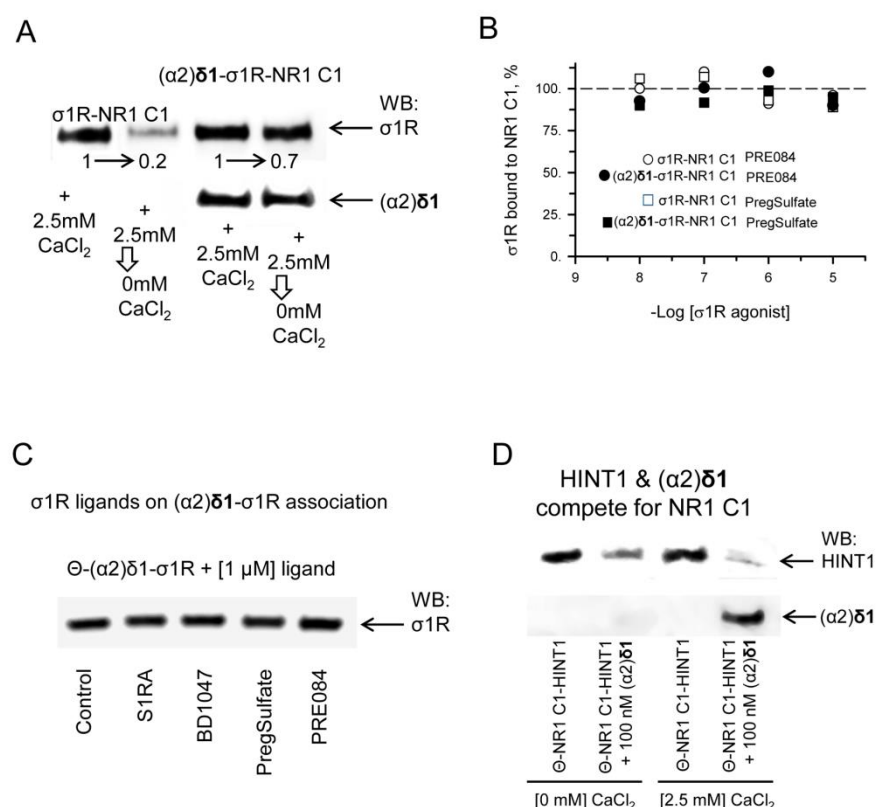


Figure S8. (A) The NR1 C1- $\sigma 1R$ -($\alpha 2$) $\delta 1$ trimer exhibits more stability than the NR1 C1- $\sigma 1R$ dimer at low calcium concentrations. Dimers and trimers were formed in the presence of $CaCl_2$ (2.5 mM) and after removing the calcium by three washes the presence of NR1 C1-bound $\sigma 1R$ s and ($\alpha 2$) $\delta 1$ proteins was determined in Western blots. (B) The influence of increasing concentrations of $\sigma 1R$ agonists, PRE084 and Pregnenolone sulfate, on the NR1 C1- $\sigma 1R$ dimers and NR1 C1- $\sigma 1R$ -($\alpha 2$) $\delta 1$ trimers was assessed. (C) Agonists and antagonists of $\sigma 1R$ s do not alter the association of $\sigma 1R$ s with ($\alpha 2$) $\delta 1$ peptides. (D) When bound to NR1 C1 subunits, HINT1 proteins can interchange with ($\alpha 2$) $\delta 1$ peptides. In the presence or absence of calcium, ($\alpha 2$) $\delta 1$ reduces HINT1 binding to NR1 C1 subunits, but in the presence of $CaCl_2$ (2.5 mM), ($\alpha 2$) $\delta 1$ remains bound to the NR1 C1 subunit (for details see Figures 7A and 9A).

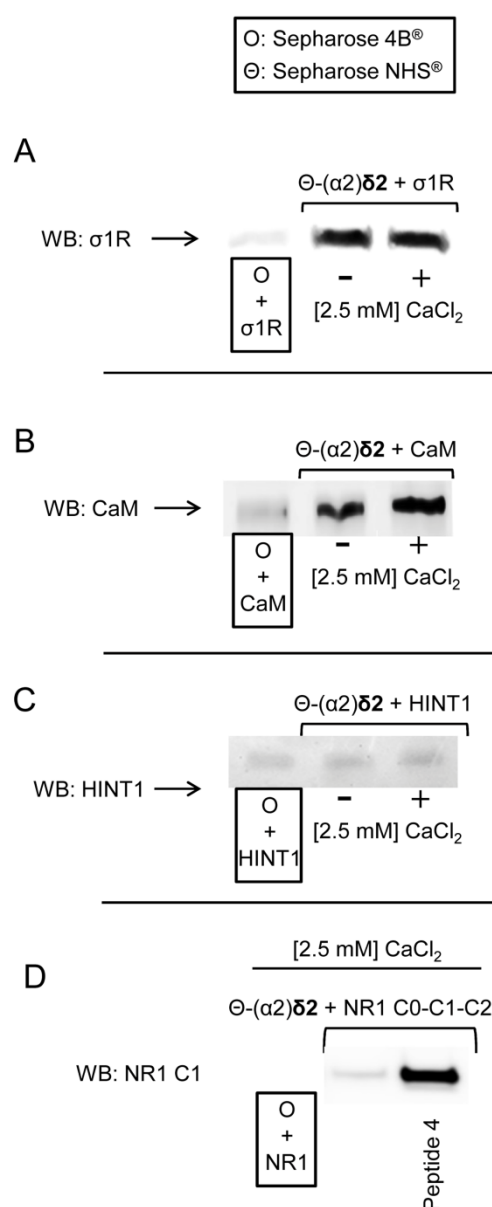


Figure S9. The effect of calcium on the physical interactions of (α 2) δ 2 peptides with σ 1Rs, HINT1 proteins and calmodulin CaM. **A**, The (α 2) δ 2 peptide binds to σ 1Rs in the absence of calcium. **B**, The (α 2) δ 2 peptide binds to CaM in the absence of calcium. **C**, Calcium does not promote HINT1 protein binding to the (α 2) δ 2 peptide. **D**, The (α 2) δ 2 peptide binds to NR1 C1 NMDAR subunits in presence of peptide 4. O and Θ represents plain agarose and NHS-Sepharose®, respectively. For details see Figure 7A.

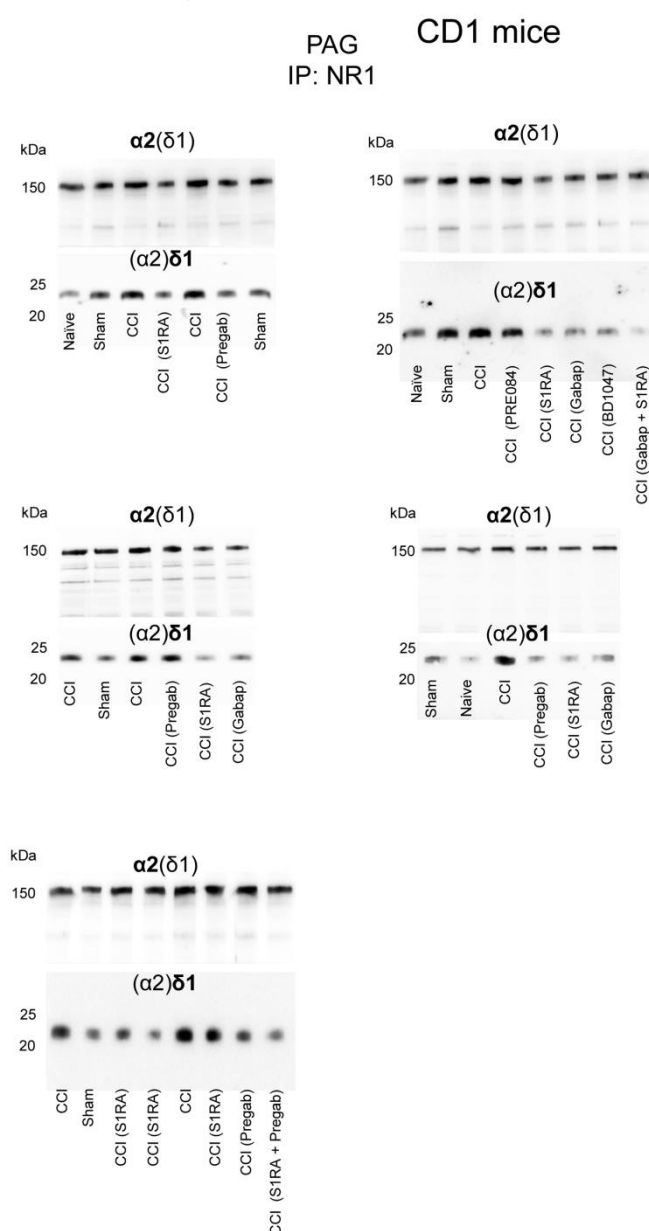


Figure S10. CCI model of neuropathic pain: the effect of S1RA, BD1047 and gabapentinoids on the molecular associations between the $\alpha 2\delta 1$ proteins and NR1 C1 subunits in PAG synaptosomes. S1RA, Pregabalin, Gabapentin or BD1047 (all 3 nmol) were injected icv into CCI CD1 WT mice 7 days after surgery and after 30 min, the animals were sacrificed and PAG synaptosomal fractions were obtained. The NR1 subunits were immunoprecipitated from the solubilized membrane preparations and the co-precipitated $\alpha 2\delta 1$ proteins were analyzed in Western blots (for further details see Methods and Figure 2). Gabap (Gabapentin); Pregab (Pregabalin).

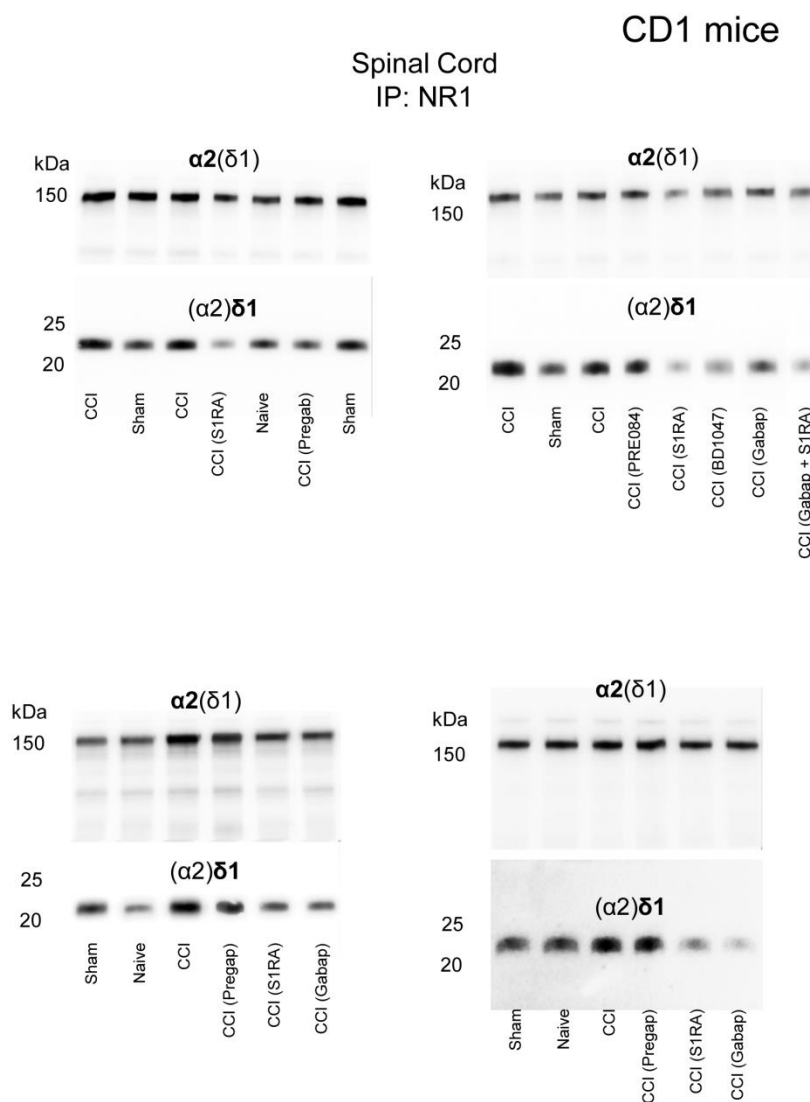


Figure S11. CCI model of neuropathic pain: the effect of S1RA, BD1047 and gabapentinoids on the $\alpha 2\delta 1$ -NR1 association in spinal cord synaptosomal fraction (for details see Figure S10).