Supplementary information

New engineered-botulinum toxins inhibit the release of pain-related mediators

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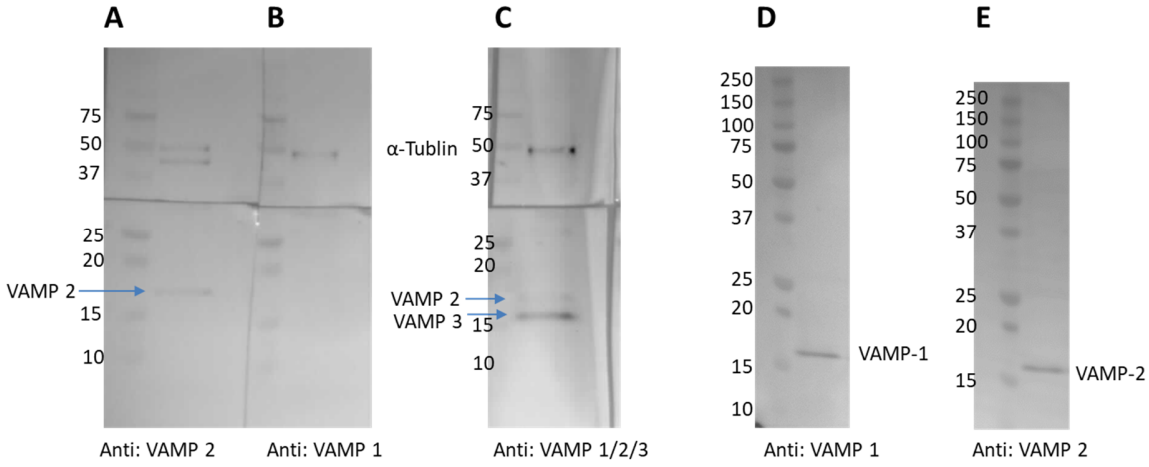

Figure S1. RAW 264.7 cells express predominately VAMP3 isoform. (**A-C**) Cell lysates from cultured RAW cells were subjected to SDS-PAGE followed by Western blotting using indicated antibodies against VAMP1, VAMP2, VAMP1/2/3 or α-Tublin. Note that, samples were incubated with VAMP antibodies for 20 h at 4°C whereas anti α-Tublin antibody was incubated with samples for 1 h at 22°C. Western blots show RAW cells express predominately VAMP3 isoform. Anti-VAMP2 antibody detected a faint band in WB, corresponding to predicted size of VAMP 2 (~17 kDa). VAMP1 was not detected under the same condition. In contrast, VAMP 1 (**D**) and VAMP 2. (**E**) in cell lysates from cultured mDRGs were easily detected in WB.

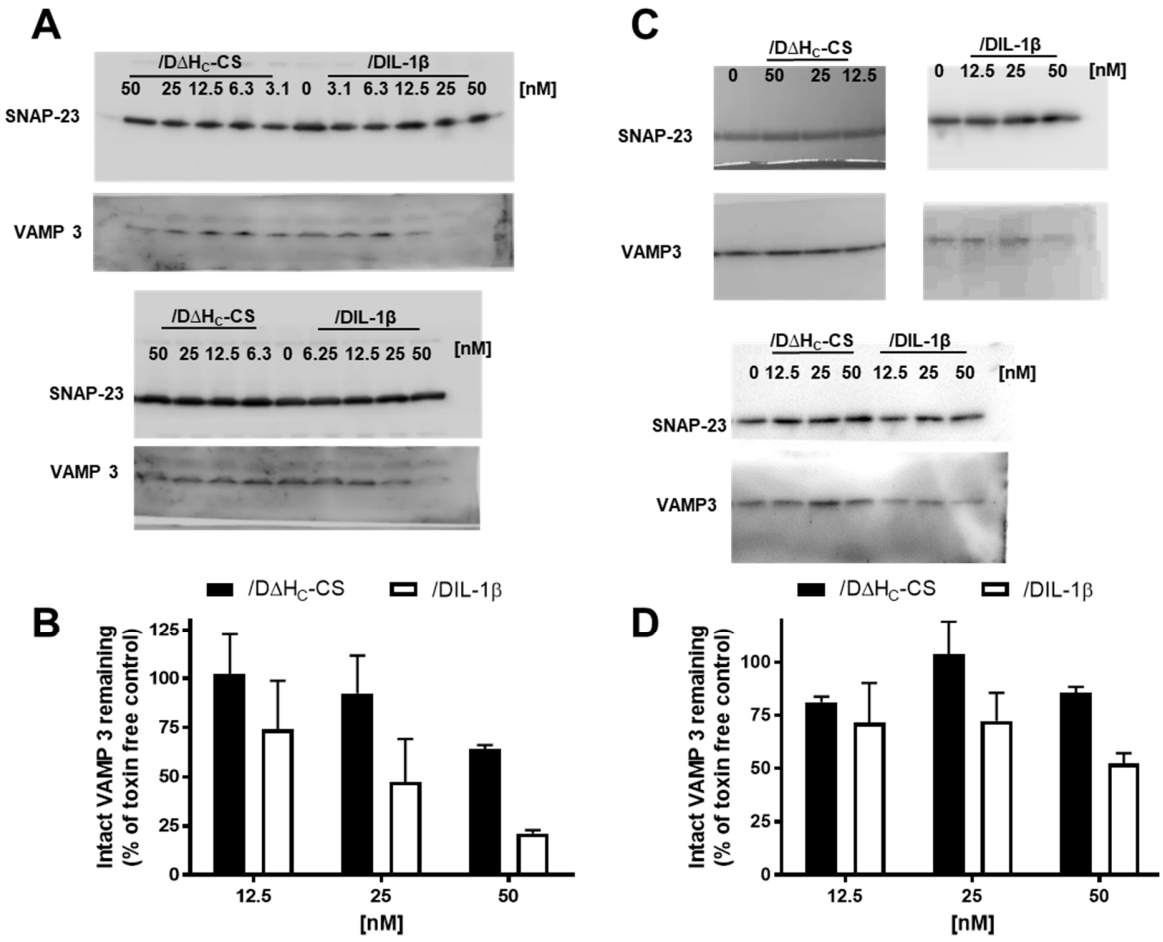

Figure S2. Effect of /DIL-1β and /DΔH_C-CS on cleavage of VAMP 3 in cultured macrophages. Cultured RAW264.7 cells (A, B) and primary mouse macrophage cells (C, D) were treated with /DIL-1β or /DΔH_C-CS for 6 h before stimulation with LPS and IFNγ for 42 h. Cells were dissolved in LDS sample and cell lysates were subjected to SDS-PAGE followed by Western blotting (A, C) using an antibody against VAMP1/2/3. Detection of VAMP3 proved to be more difficult than SNAP-23. (B, D) Plots showing the differential cleavage of VAMP 3 by /DIL-1β and /DΔH_C-CS. VAMP 3 cleavage was normalized according to a loading control (SNAP-23) before analysis relative to the toxin free control cells. Data plotted are mean ± S.E.M.

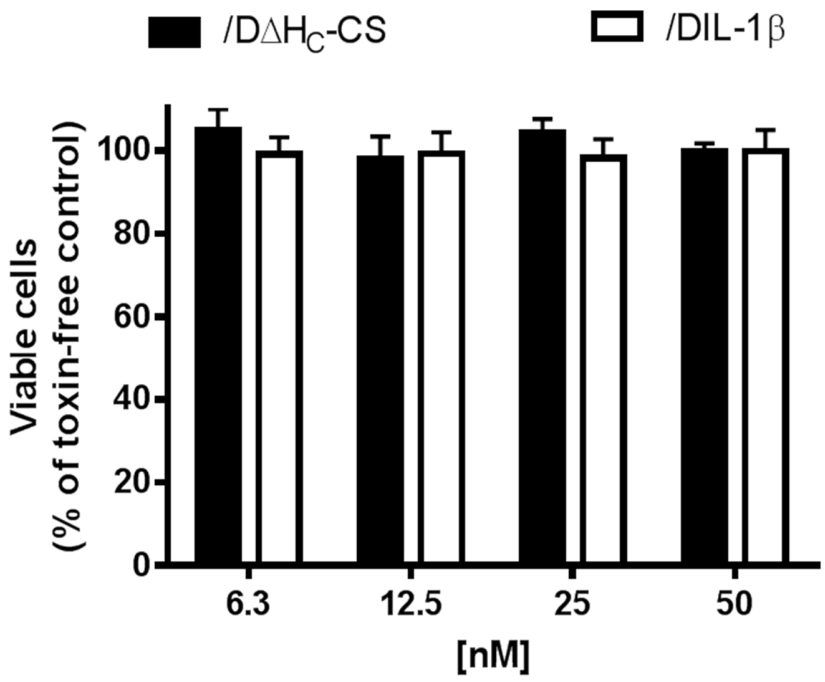

Figure S3. Effect of /DIL-1β and /DΔHc-CS on RAW 264.7 cell viability. RAW cells were plated into a 96 well plate with ~ 0.4 × 10^5 cells/well and cultured for 24 h at 37°C, 5% CO_2. On the following day, the cells were incubated with various doses of /DIL-1β or /DΔH_C-CS for 44 h, followed by 4 h incubation with alamar blue contained culture medium before reading at absorbance 570 nm. Viable cells after treatment was quantified as a % of the toxin free control sample (vehicle) treated cells. Data graphed are mean ± S.E.M. from 2 independent experiments.

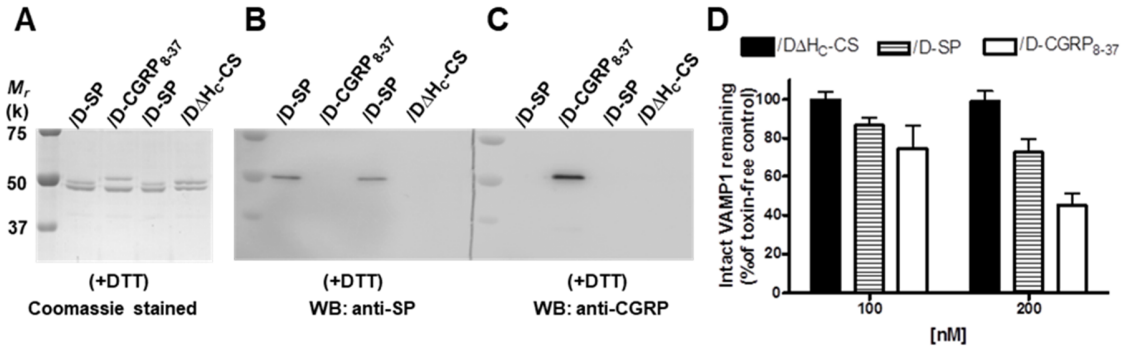

Figure S4. Effect of /D-SP conjugate on VAMP 1 cleavage in cultured DRGs. /D-SP conjugate was produced as for /D-$CGRP_{8-37}$. DTT reduced conjugate samples were subjected to SDS-PAGE followed by Coomassie staining (**A**) or Western blotting using an antibody against substance P (**B**) or an antibody against CGRP (**C**). (**D**) Rat DRGs were incubated with /D-SP conjugate for 24 h at 37°C. The cells were then

harvested in LDS-sample buffer for Western blotting. Intact VAMP1 remaining after overnight treatment was plotted as % of toxin-free control. SP: substance P. Data are mean ± SEM from two independent experiments. Data for /D-CGRP$_{8-37}$ conjugate and control protein from Fig. 5 were replotted here for comparison.