Expanded View Figures

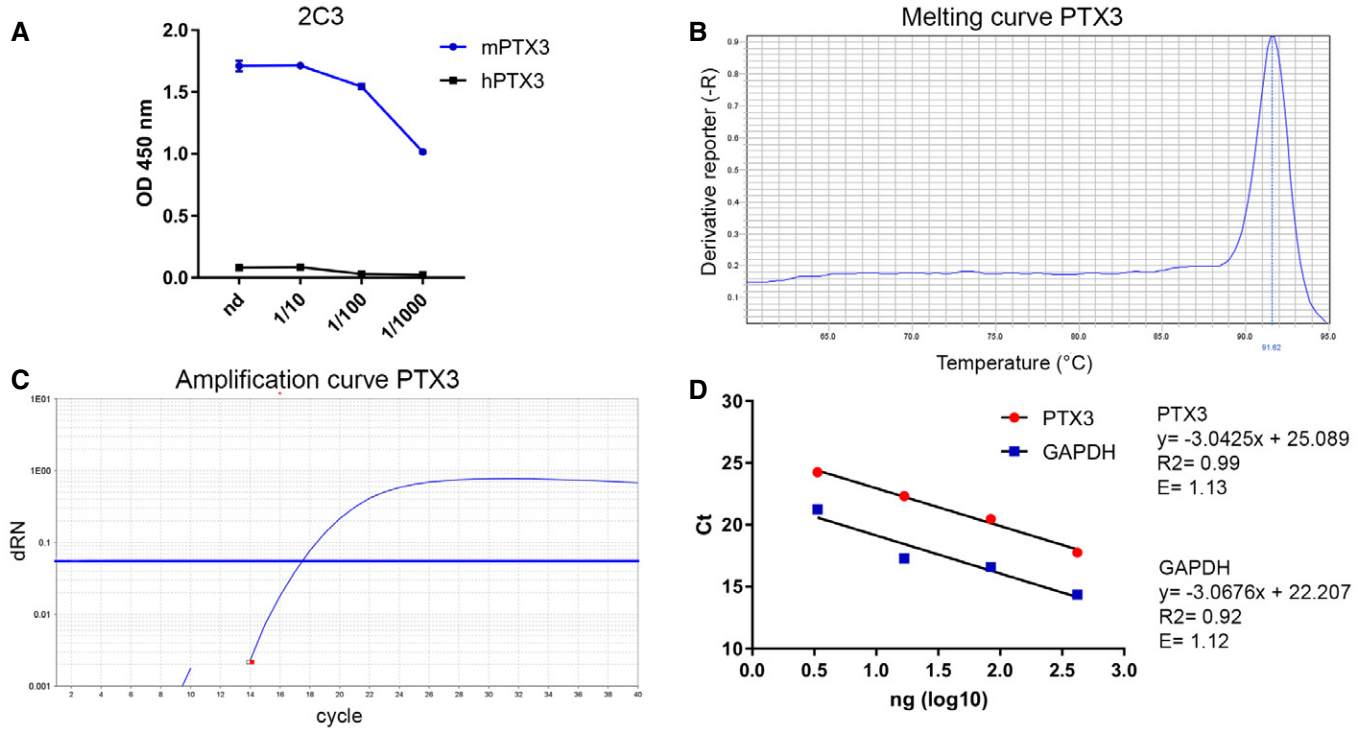

Figure EV1. Specificity of ELISA and RT–PCR assays.

A The specificity of the PTX3 ELISA was tested using different dilutions of 2C3 antibody to measure immobilized murine and human PTX3. Purified recombinant murine and human PTX3 were immobilized in 96-well ELISA plates, and then, different dilutions of 2C3 were added. The graph shows dose–response of 2C3 on immobilized murine or human PTX3. Human PTX3 was not detected by 2C3 antibody.

B–D Evaluation of the amplification efficiency of real-time RT–PCR assay designed for PTX3 expression in astrocyte cell cultures. (B, C) Melting curve and amplification plot of PTX3 RT–qPCR assay. (D) Standard curves of PTX3 and GAPDH, used as reference mRNA, obtained using fivefold serial dilutions of the cDNA (420, 84, 16.8, 3.36 ng). The threshold cycle (C_t) values (*y*-axis) are plotted against $\log_{10}$ values of cDNA input amounts (*x*-axis). The graphs are parallel lines and the calculated efficiencies (E) are, respectively, of 1.13 and 1.12 from a y-slope of −3.04 and −3.07 and a correlation coefficient (R^2) > 0.9.

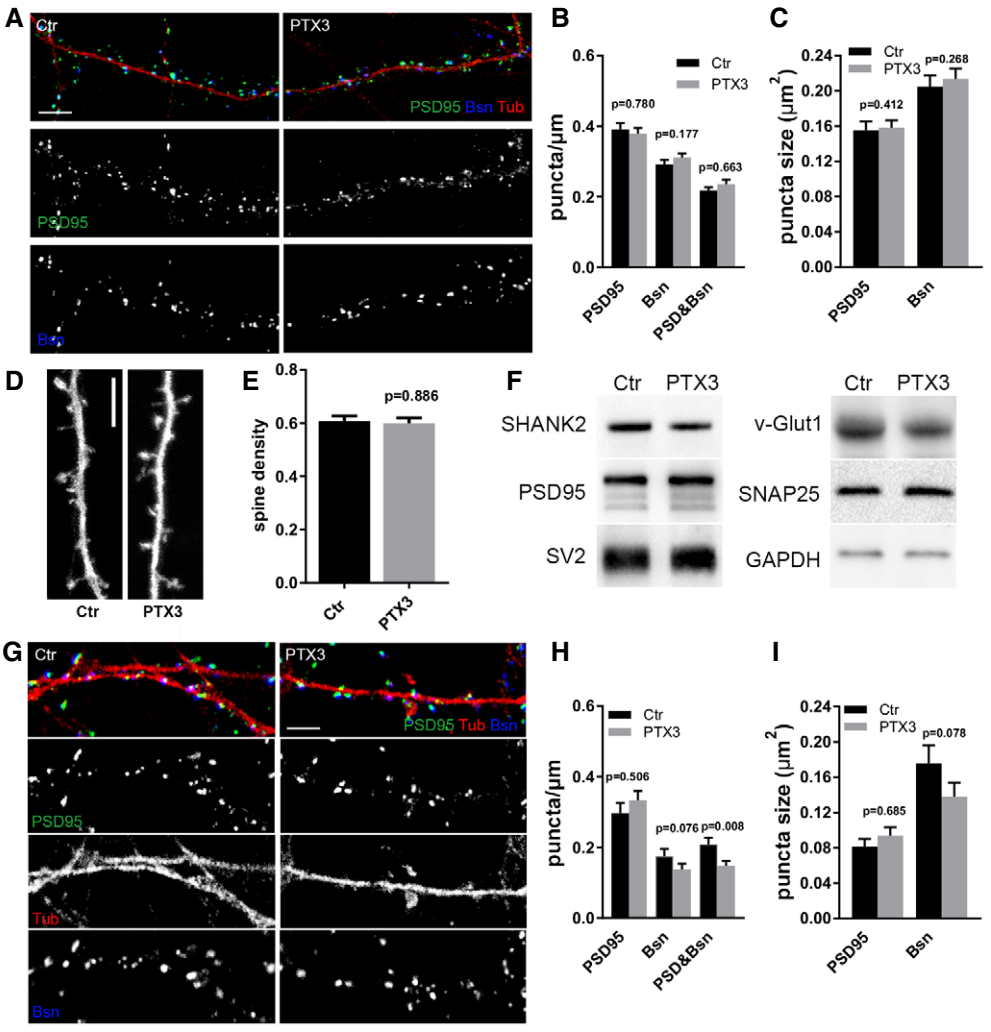

Figure EV2. PTX3 does not affect synapse number and organization.

A Representative images of 14DIV control and PTX3-treated cultures stained for the presynaptic marker Bassoon (blue), the postsynaptic protein PSD95 (green), and the microtubule protein tubulin (red). Scale bar: 5 µm.

B Quantification of synaptic density shows no differences either for postsynaptic marker (PSD95/µm), or for presynaptic marker (Bsn/µm) or as a total number of synapses (PSD95&Bsn/µm) in control and PTX3-treated cultures (PSD95/µm, Ctr = 0.391 ± 0.018; PTX3 = 0.379 ± 0.016; Bsn/µm, Ctr = 0.291 ± 0.012, PTX3 = 0.311 ± 0.012; PSD95&Bsn/µm, Ctr = 0.218 ± 0.009, PTX3 = 0.235 ± 0.013. Number of dendrites: 109 Ctr, 107 PTX3, Mann–Whitney test; five independent experiments, data are presented as mean ± SEM).

C Quantitative analysis of the mean size of PSD95 and Bsn puncta shows no differences in control or PTX3-treated neurons (in μm^2, PSD95: Ctr = 0.155 ± 0.010; PTX3 = 0.158 ± 0.008; Bsn: Ctr = 0.205 ± 0.013, PTX3 = 0.214 ± 0.012; Number of dendrites: 134 ctr, 124 PTX3, Mann–Whitney test; five independent experiments, data are presented as mean ± SEM).

D, E (D) Representative images of GFP-expressing dendritic branches of control and PTX3-treated neurons and (E) quantification of dendritic spine density, i.e., number of spines per µm of parent dendrite (Ctr = 0.607 ± 0.020, PTX3 = 0.598 ± 0.021, number of examined dendrites: 73 and 71 respectively; Mann–Whitney test; three independent experiments, data are presented as mean ± SEM). Scale bar: 5 µm.

F Western blotting analysis of major synaptic proteins on lysates from control or PTX3-treated cultures. GAPDH was used as reference marker.

G–I Chronic PTX3 administration does not increase neither synaptic density nor synaptic puncta size. (G) Representative images of 14DIV control and PTX3-treated cultures stained for the presynaptic marker Bassoon (blue), and the postsynaptic protein PSD95 (green) and tubulin (red). Scale bar: 5 µm. (H) Quantification of synaptic density parameters (PSD95/µm; Bsn/µm; PSD95&Bsn/µm) in control and PTX3-treated neurons (PSD95/µm, Ctr = 0.297 ± 0.029; PTX3 = 0.334 ± 0.026; Bsn/µm, Ctr = 0.261 ± 0.021, PTX3 = 0.221 ± 0.020; PSD95&Bsn/µm, Ctr = 0.209 ± 0.019, PTX3 = 0.148 ± 0.014. Number of dendrites: Ctr = 20, PTX3 = 40, Mann–Whitney test; three independent experiments; data represented as mean ± SEM). (I) Analysis of the mean size of PSD95 and Bsn puncta shows no differences in control or PTX3-treated neurons (in μm^2, PSD95: Ctr = 0.081 ± 0.009; PTX3 = 0.094 ± 0.010; Bsn: Ctr = 0.175 ± 0.021, PTX3 = 0.138 ± 0.016; number of dendrites: Ctr = 22, PTX3 = 48, Mann–Whitney test; three independent experiments, data represented as mean ± SEM).

Source data are available online for this figure.

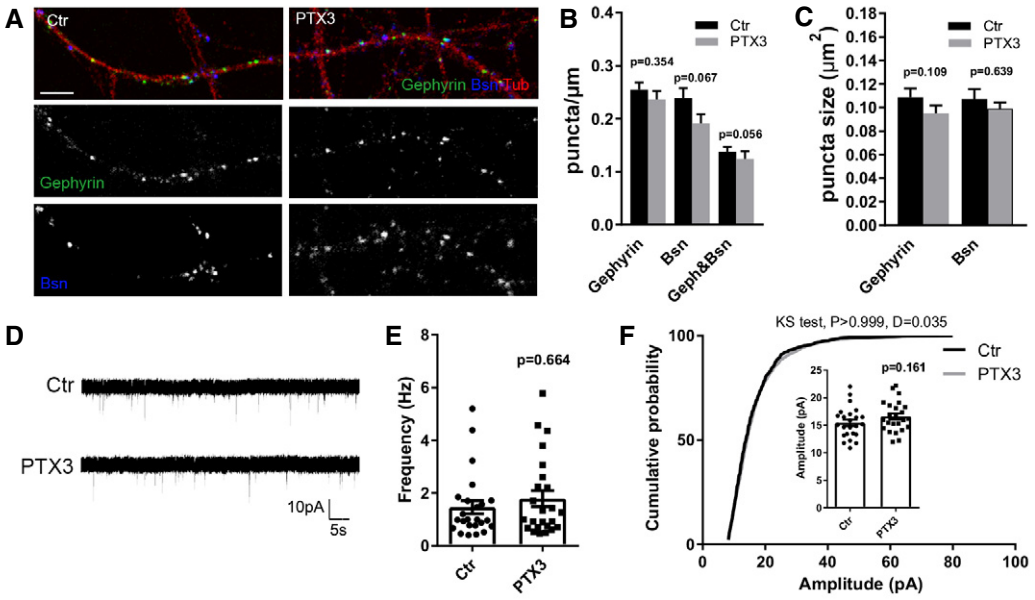

Figure EV3. PTX3 does not modify structure and function of inhibitory synapses.

A Representative images of 14DIV control and PTX3-treated cultures stained for the presynaptic marker bassoon (blue), and the postsynaptic inhibitory protein gephyrin (green) and the microtubule protein tubulin (red). Scale bar: 5 µm.

B Quantification of synaptic density showing no differences either for postsynaptic marker (gephyrin) or for presynaptic marker (Bsn) or as a total number of synapses (geph&Bsn) in control or PTX3-treated neurons (gephyrin/µm, Ctr = 0.255 ± 0.014, PTX3 = 0.237 ± 0.016; Bsn/µm, Ctr = 0.239 ± 0.018, PTX3 = 0.191 ± 0.018; gephyrin&Bsn/µm, ctr = 0.138 ± 0.010, PTX3 = 0.125 ± 0.014. Number of dendrites: Ctr = 71, PTX3 = 56; Mann–Whitney test. Three independent experiments, data are presented as mean ± SEM).

C Quantitative analysis of the mean size of gephyrin and bassoon puncta showing no differences in control and PTX3-treated neurons (in μm^2, gephyrin: Ctr = 0.109 ± 0.008; PTX3 = 0.095 ± 0.006; Bsn: Ctr = 0.107 ± 0.008, PTX3 = 0.099 ± 0.005, number of dendrites: Ctr = 53, PTX3 = 41; Mann–Whitney test; three independent experiments, data are presented as mean ± SEM).

D Representative traces of mIPSCs recorded from control and PTX3-treated neurons.

E mIPSC frequency quantitation (Hz, Ctr = 1.464 ± 0.250; PTX3 = 1.792 ± 0.306. Number of neurons: Ctr = 24, PTX3 = 24; five independent experiments. Mann–Whitney test, data are presented as a distribution plus mean ± SEM).

F Cumulative probability plot and (inset) average of mIPSC amplitude (pA, Ctr = 15.43 ± 0.572; PTX3 = 16.58 ± 0.565. Number of neurons: Ctr = 24, PTX3 = 24; five independent experiments. Unpaired *t*-test, data are presented as a distribution, mean ± SEM and cumulative probability distribution of amplitudes analyzed with Kolmogorov–Smirnov test).

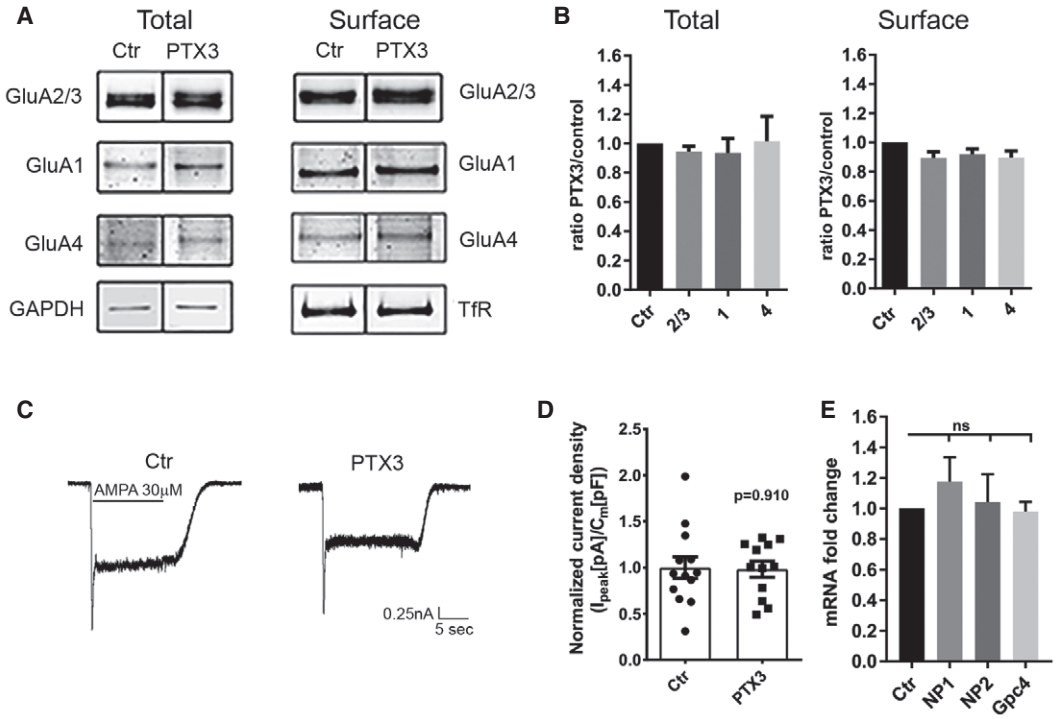

Figure EV4. PTX3 does not affect the total amount of surface AMPARs.

A Western blotting analysis of total and surface (biotinylated) GluA subunits of control and PTX3-treated neurons as indicated showing no differences either for the total levels of expression or for the global surface levels of the different GluA subunits.

B Quantification of total receptors levels was normalized to GAPDH in each sample, whereas surface GluA subunits were normalized to surface transferrin receptor, TfR, in each sample. Data are shown as ratio PTX3 over control for each GluA subunit (total: Ctr = 1 ± 0; GluA2/3 = 0.943 ± 0.036, GluA1 = 0.93 ± 0.098, GluA4 = 1.014 ± 0.17. Number of samples: 7, 6, 7, 7 respectively; Kruskal–Wallis test, $P = 0.472$. Surface: Ctr = 1 ± 0; GluA2/3 = 0.89 ± 0.041, GluA1 = 0.92 ± 0.038, GluA4 = 0.89 ± 0.047; number of samples: 6, 6, 6, 6, respectively. Kruskal–Wallis test, $P = 0.112$; 3 independent experiments, normalized values, mean ± SEM).

C, D (C) Representative traces and (D) quantitative analysis of total current density evoked by application of 30 μM AMPA in control and PTX3-treated cultures (Ctr = 1 ± 0.117; PTX3 = 0.983 ± 0.87; Student's *t*-test; number of neurons: Ctr = 13, PTX3 = 12; 3 independent experiments, data are presented as distribution plus normalized mean ± SEM).

E PTX3 does not affect neuronal pentraxins and glypican 4 expression. qRT–PCR analysis of mRNA levels for NP1, NP2, and glypican 4 (Gpc4) performed in pure neuronal cultures treated with PTX3 (1 μg/ml for 48 h). qPCR fold change normalized on control: Ctr = 1.000 ± 0.000; NP1 = 1.174 ± 0.16; NP2 = 1.041 ± 0.182; Gpc4 = 0.980 ± 0.062. Wilcoxon test: Ctr vs. NP1: $P = 0.312$, Ctr vs. NP2: $P = 0.688$, Ctr vs. Gpc4: $P > 0.999$. Graphs show mean ± SEM. At least 5 independent experiments.

Source data are available online for this figure.

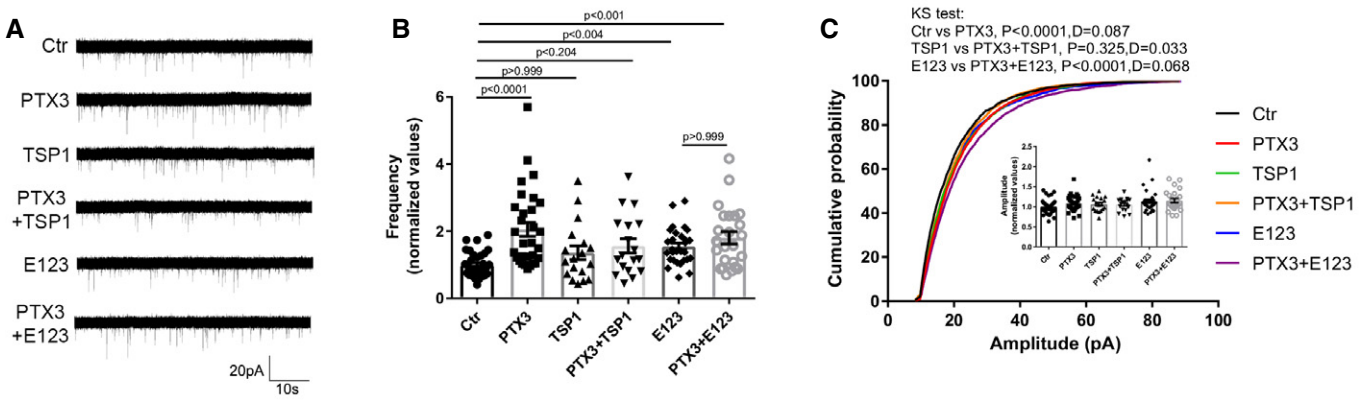

Figure EV5. Full-length TSP1 exerts a negative regulation of PTX3 activity.

A Examples of mEPSC recordings in the indicated conditions.

B mEPSC frequency quantification showing that co-incubation of PTX3 and full-length TSP1 did not result in increased miniature frequency. Of note application of E123 alone induced *per se* a statistically significant increase of mEPSC frequency, whereas no difference was detectable upon co-incubation of E123 fragment +PTX3 with respect to E123 alone (normalized average frequency: Ctr = 1.000 ± 0.065; PTX3 = 2.055 ± 0.209; TSP1 = 1.358 ± 0.202; PTX3+TSP1 = 1.562 ± 0.212; E123 = 1.548 ± 0.100; PTX3+E123 = 1.803 ± 0.183. Number of recorded cells: 33, 30, 18, 18, 29, 24, respectively, from at least 3 different culture preparations. Kruskal–Wallis test $P < 0.0001$ followed by post hoc Dunn's test. Data are presented normalized mean ± SEM along with distribution of values).

C Cumulative probability plot of mEPSCs amplitudes showing that there is a significant shift in the distribution by the Kolmogorov–Smirnov test in Ctr vs. PTX3 and E123 vs. E123 + PTX3 but not in TSP1 vs. TSP1 + PTX3 further supporting the evidence that full-length TSP1, but not E123 fragment, inhibits PTX3 activity. Inset: No statistically significant difference was instead detectable in the average amplitude (normalized average amplitude: Ctr = 1.000 ± 0.033; PTX3 = 1.088 ± 0.036; TSP1 = 1.06 ± 0.043; PTX3+TSP1 = 1.053 ± 0.038; E123 = 1.137 ± 0.051; PTX3 + E123 = 1.152 ± 0.052. Number of recorded cells: 33, 30, 18, 18, 29, 24, respectively, from at least three different culture preparations. Kruskal–Wallis test $P = 0.172$ followed by post hoc Dunn's test. Data are presented normalized mean ± SEM along with the distribution of values.).