



**Figure S1.** NKCC1 diffusion in the axon is increased upon GABA<sub>A</sub>R blockade: **A**, Reconstructed trajectories of NKCC1 in the axon depicting the increased surface exploration upon gabazine (yellow) and muscimol (orange) treatment as compared to control (blue). **B**, Time-averaged MSD functions of axonal QDs in control (blue) vs. gabazine (yellow) or muscimol (orange) exposure. The MSD versus time relationship for axonal trajectories shows a steeper initial slope upon drug exposure, suggesting that trajectories were less confined.  $N_{\text{Control}} = 390$  QDs,  $N_{\text{gabazine}} = 145$  QDs,  $N_{\text{muscimol}} = 274$  QDs, 37 cells from 5 cultures. **C-D**, Boxplots of  $\log(D)$  of NKCC1 in control condition (blue) or upon application of gabazine (yellow) or muscimol (orange) showing increased diffusion upon gabazine application.  $N = 390$  QDs (control, 48 cells),  $n = 145$  QDs (gabazine, 64 cells), Welch t-test,  $p = 3.5 \times 10^{-2}$ ,  $n = 274$  QDs (muscimol, 28 cells), Welch t-test,  $p = 2.1 \times 10^{-12}$ , 5 cultures. **D**, Median explored area EA in control vs. gabazine or muscimol conditions show reduced confinement upon gabazine (Welch t-test,  $p = 8.46 \times 10^{-6}$ ) or muscimol (Welch t-test,  $p = 4.2 \times 10^{-11}$ ) application. **C**:  $D$  in  $\mu\text{m}^2 \cdot \text{s}^{-1}$ ; **D**: EA in  $\mu\text{m}^2$ . In all graphs, \*,  $p < 5.0 \times 10^{-2}$ ; \*\*\*,  $p < 1.0 \times 10^{-3}$ .