**Supplementary Figure 3.** Functional GABAARs, evident on some cholinergic MG neurons, do not contribute to spontaneous or evoked synaptic events. **A.** Time course of GCaMP6f mediated Ca2+ fluorescence changes in 10 neuron somas (Black traces) in response to focally applied GABA (Black bar, 100 µM). Red triangles above the traces depict the timing of peak responses (AA,Peak). The response average from these neurons and of those after incubation with bicuculline (10 μM) are depicted by the blue and purple traces, respectively. **B.** GABA response parameters. Overall 18 ± 2% of cholinergic MG neurons (n = 338, N = 22) responded to GABA application. *Left:* Average AA,Peak responses to 100 µM GABA (0.121 ± 0.033, n = 30; N = 2) or 1 µM GABA (0.095 ± 0.012, n = 308, N = 20) were indistinguishable (*p > 0.05*). *Right:* Incubation with the GABAAR selective antagonist Bicuculline (20 µM; 10-15 min) reduced the AE,Peak response of cholinergic MG neurons (n = 132; N =9) to 1 µM GABA by 47 ± 7% relative to the same neurons tested before antagonist application (*p < 0.05*; Black bar, Control). **C.** GABAARs do not contribute to the synapse-dependent spontaneous Ca2+ transients. Graphs depict spontaneous Ca2+ transient frequency (FS, Left panel) and amplitude (AS,Peak, Right panel) measurements from n = 30 cholinergic neuron pairs in N = 2 ChAT+/ GCaMP6f+ MG explants tested before (Control, Gray bars) and after treatment with 10 µM Bicuculline (Purple bars). Bars represent group FS and AS,Peak averages while the superimposed dot plots and connecting lines depict their values from individual neurons before and after treatment. Neither FS nor AS,Peak were detectably changed by inhibiting GABAARs (*p > 0.05* for both). **D.** GABAARs do not contribute to the stimulus-evoked synaptic Ca2+ responses. F/F0 responses (AE.Peak) evoked by 20X MG connective stimulation cholinergic MG neurons (n = 30, N = 2) assayed before (Control, Black bar) and after treatment with 10 µM Bicuculline (Purple bar) were not detectably different (*p > 0.05*).