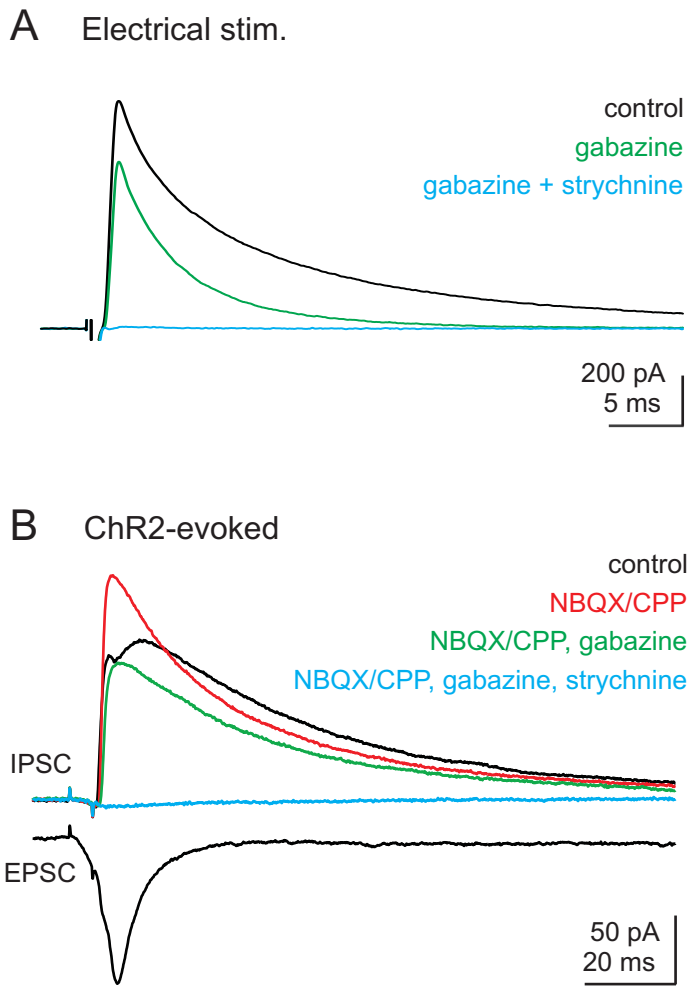

Figure S1. Atypical glycinergic IPSCs recorded at two Golgi cells in response to electrical stimulation or ChR2 evoked MF activation. A. In 1 of 20 experiments where IPSCs were evoked in Golgi cells (Vm = +15 mV) with electrical stimulation in the presence of NBQX and CPP (5 and 2.5 M), a large fraction of the current was insensitive to gabazine (5 M, green). This current was blocked by the application of strychnine (1 M, blue), indicating that it was glycinergic. This was the only experiment where a large glycinergic input was recruited with electrical stimulation. **B.** In this experiment, synaptic currents were evoked by light activation of mossy fibers in Thy1 ChR2-YFP mice. Initially the EPSC and IPSC were measured in control conditions (V-clamp, at EPSC and IPSC reversal potentials respectively). The delay between excitation and inhibition still suggests that this experiment reflects polysynaptic inhibition rather than direct activation of an interneuron. Blocking glutamatergic transmission did not block the IPSC (NBQX/CPP = red). Furthermore, application of gabazine produced only a moderate reduction in the evoked IPSC (green), which was completely blocked by the glycine receptor antagonist strychnine (1 M, blue). While some MFs contain neurotransmitters other than glutamate , it is unclear what transmitter is responsible for this IPSC. It is possible that the IPSC results from a Lugaro cell, which inhibit Golgi cells via synapses with a prominent glycinergic component (Dieudonné and Dumoulin, 2000). NBQX/CPP blocked evoked IPSCs in all other experiments. We therefore conclude that these two examples represent synapses that are not readily activated under our experimental conditions.

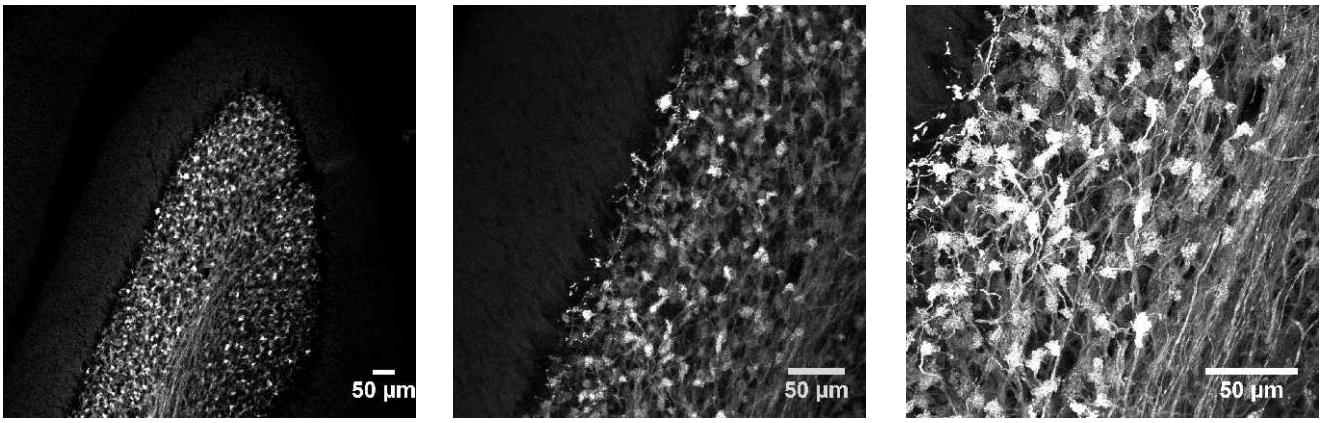

Figure S2. ChR2/EYFP expression is restricted to cerebellar mossy fibers in Thy1-ChR2/EYFP line 18 mice. Three different magnifications showing EYFP labeling in mossy fibers within the cerebellar granule cell layer. No labeling was ever observed in the molecular layer, and no cell bodies were ever observed in the granule cell layer. Mossy fiber labeling was dense in all lobules of the cerebellar cortex. Imaging was performed with a Zeiss LSM510 using 515 nm illumination and detecting 535 – 590 nm fluorescence.

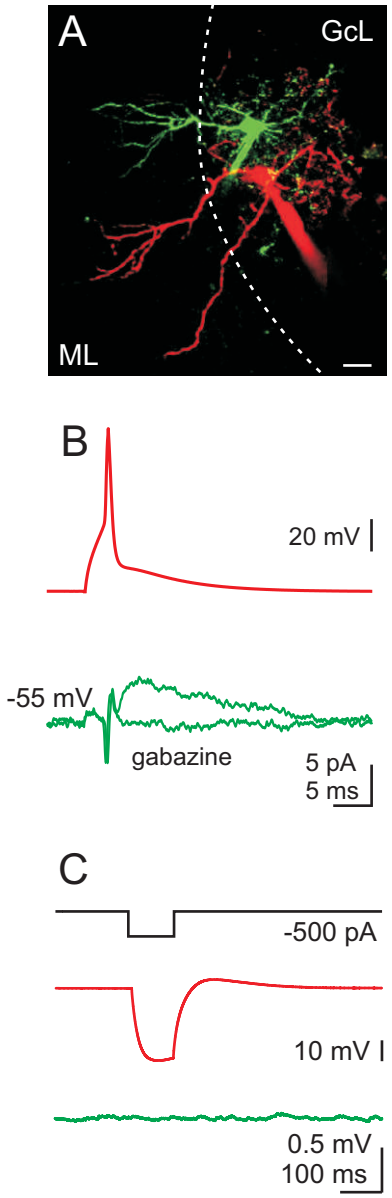

Figure S3. Lack of dendritic overlap explains the lack of electrical connections between a pair of Golgi cells. Paired recording between two Golgi cells. **A.** Two recorded Golgi cells were filled with Alexa 594 (red) and Alexa 488 (green) respectively and imaged with 2-photon microscopy. Note that their dendrites do not overlap in the molecular layer. ML = molecular layer, GcL = granule cell layer, dotted line is the boundary of the molecular layer, scale bar = 20 m **B.** An action potential (current-clamp) in the red cell produced a GABAergic IPSC in the green cell (V-clamp, held at -55 mV) that was blocked by gabazine. **C.** A 500 pA hyperpolarizing step to the red Golgi cell in current-clamp does not produce a detectable hyperpolarization in the green Golgi cell (also in current-clamp), indicating a lack of electrical coupling between these cells. These cells were also uncoupled in the opposite direction (not shown).

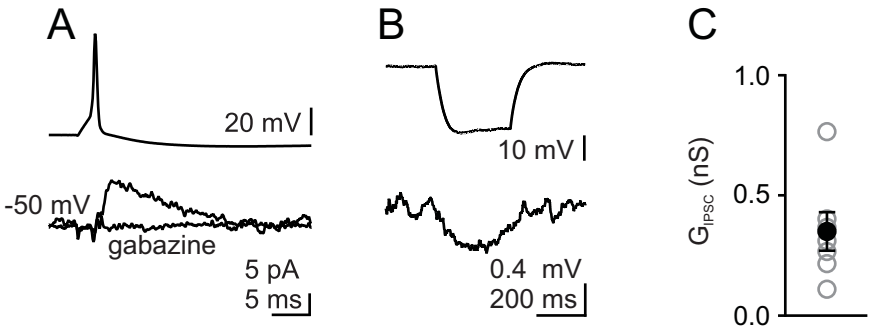

Figure S4. Paired recordings between Golgi cells reveal GABAergic inhibition under 2 mM external Ca^{2+} with no CGP. Experiments were performed in 2 mM external calcium, no CGP, and a K-based internal solution that had an IPSC reversal potential of -85 mV. To check the most connections, neurons were not filled with dye and imaged in some experiments, and we did not repeat pharmacological block with gabazine in most experiments. Recordings were obtained from pairs of nearby Golgi cells, and action potentials were evoked in one cell while responses were measured in the other cell (V-clamp at -50 mV). As shown in a representative experiment, IPSCs were observed that were blocked by the $GABA_A$ receptor antagonist gabazine (A) and hyperpolarization of one Golgi cell produced a smaller hyperpolarization in another Golgi cell, indicating that the cells were electrically coupled (B). In these experiments synaptic connections were observed in 7 of 56 (13%) directions tested. It is likely that small connections were missed in some cases, because gabazine, which is invaluable in identifying small currents by separating them from gap junction transmitted action potential conductances, was only used in a small number of experiments where IPSCs were obvious and large. Hence, the average from this small number of connections is likely biased toward large conductances. C. The amplitudes of the conductances are summarized, with open circles representing individual experiments and the closed circle representing the average. Gabazine reduced the IPSC amplitude to 1 % of initial values, n=2.

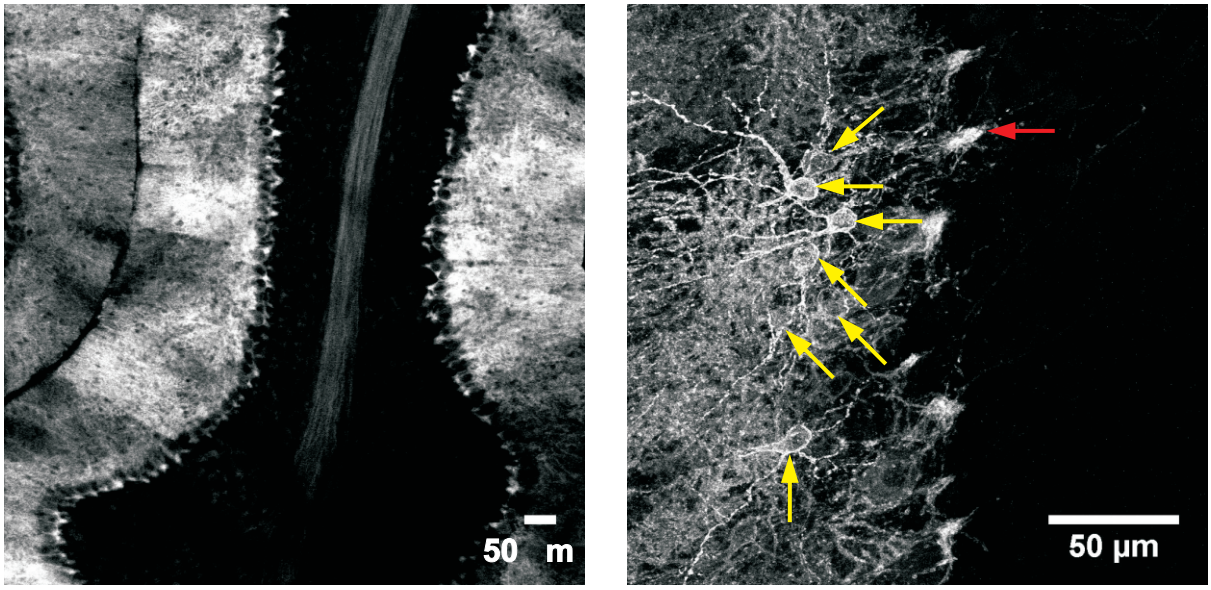

Figure S5. ChR2/EYFP expression in the molecular layer of Prv-mhChR2-EYFP mice. Two different magnifications showing EYFP labeling in the molecular layer. No cell bodies were ever observed in the granule cell layer. MLIs are brightly labeled (yellow arrows), and the axon of basket cells (red arrow) can be seen clearly at the bottom of the Purkinje cell layer. We also observed weaker labeling in Purkinje cells, though this does affect our conclusion that MLIs make no synapses onto Golgi cells. At higher gain settings, Purkinje cell axons could be seen exiting the cerebellar cortex below the basket cell axons, and this likely accounts for the weak labeling of the white matter below the granule cell layer in the *left* image. Imaging was performed with a Zeiss LSM510 using 515 nm illumination and detecting 535 – 590 nm fluorescence.

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