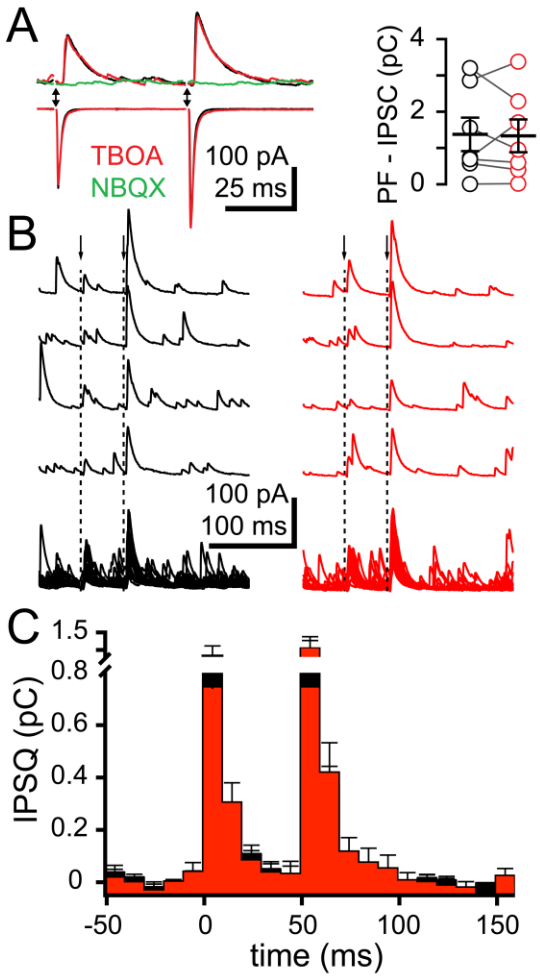

Supplemental Figures

Figure S1. Glutamate spillover does not affect PF feed-forward (FF) IPSCs.

(A) Representative averaged EPSCs (-60 mV) and IPSCs (0 mV) following PF-stimulation show paired-pulse facilitation (black) and insensitivity to TBOA (red). Both EPSCs (not shown) and IPSCs are blocked by NBQX (0 mV, green). PF-mediated IPSCs were quantified by measuring the outward charge (IPSQ for 50 ms) before (black circles) and in the presence of TBOA (red symbols). Horizontal lines are mean values ± SEM for a separate group of six cells. Synaptic stimulation is denoted by arrows.
(B) Individual (top) and superimposed (bottom) MLI-IPSCs following PF-stimulation (at 0 and 50 ms) before (black) and in the presence of TBOA (red).
(C) Average peri-stimulus histogram of the inhibitory charge (IPSQ, 10 ms bins) with paired-PF stimulation (at 0 and 50 ms) in the absence (black) or presence of TBOA (red, n = 3 each).

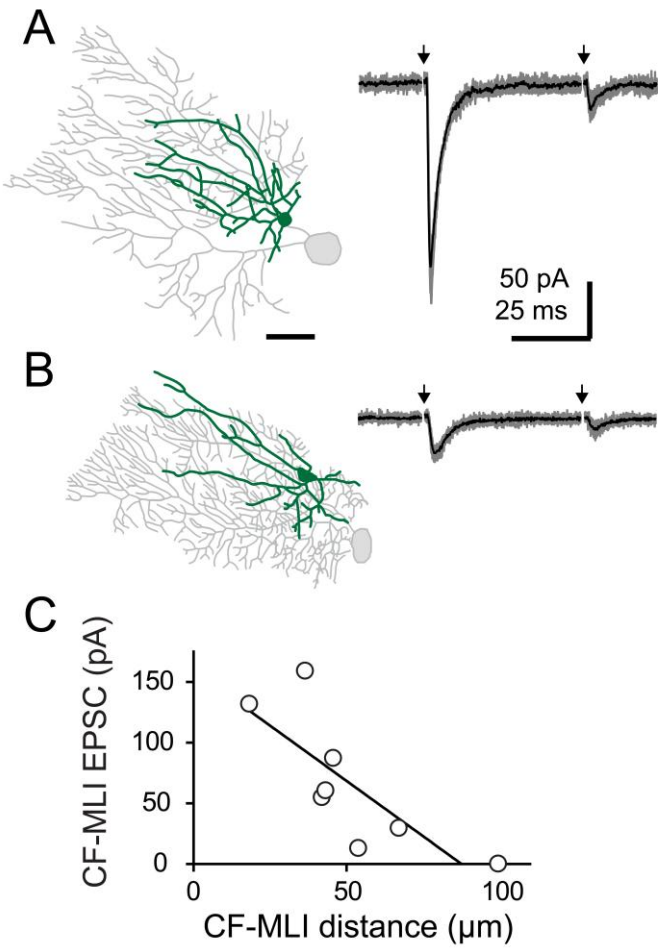

Figure S2. Correlation of PC-MLI intersomatic distance with CF-MLI EPSC.

(A and B) Left: Examples of PC and MLI reconstructions. CF location was inferred by the PC that receives a single CF input (CF-PC EPSCs not shown). Scale = 30 µm. Right: Superimposed (grey) and averaged (black) CF-MLI EPSCs in response to paired-pulse stimulation (50 ms). Synaptic stimulation is denoted by arrows.
(C) Correlation of intersomatic distance between PC and MLI CF-MLI versus EPSC amplitudes suggests that larger CF-MLI EPSCs are closer to CF release sites. Solid line is the linear regression fit. Spearman correlation coefficient r = -0.86 (P = 0.005).

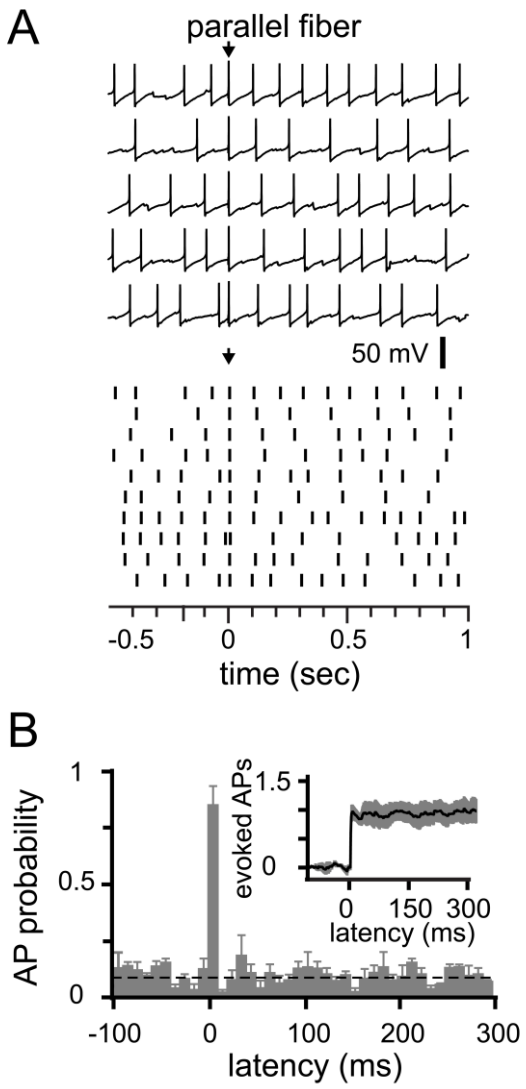

Figure S3. Timecourse of PF-MLI excitability.

(A) Representative examples of spontaneous APs (top) and raster plots (bottom) following PF stimulation at 0 ms (arrow).
(B) Peri-stimulus probability histogram (10 ms bins) of APs and cumulative spike probability plot (inset) following PF stimulation. Dotted line represents the average baseline firing probability. Note the brief duration of PF-mediated excitation compared to CF-mediated excitation (Figures 3 and 4)

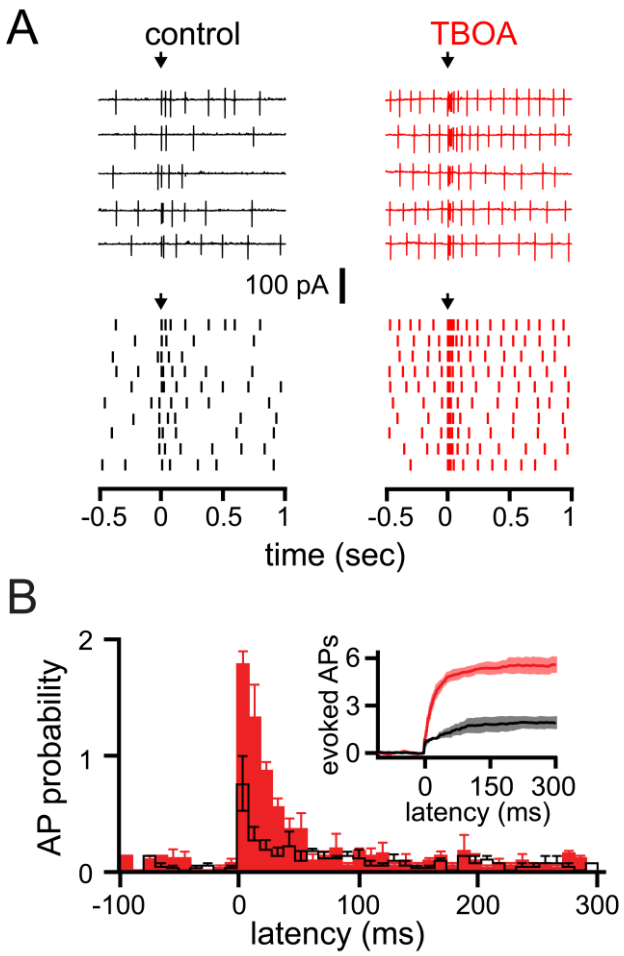

Figure S4. Cell-attached CF-stimulation.

(A) Representative examples of spontaneous APs recorded in cell-attached configuration (top) and raster plots (bottom) either in control (black) or TBOA (red). Arrows at 0 ms indicates CF stimulation.
(B) Peri-stimulus probability histogram (10 ms bins) of APs and cumulative spike probability plot (inset) in control (black) and TBOA (red). These results demonstrate that CF-mediated excitation persists during non-invasive cell-attached recordings.

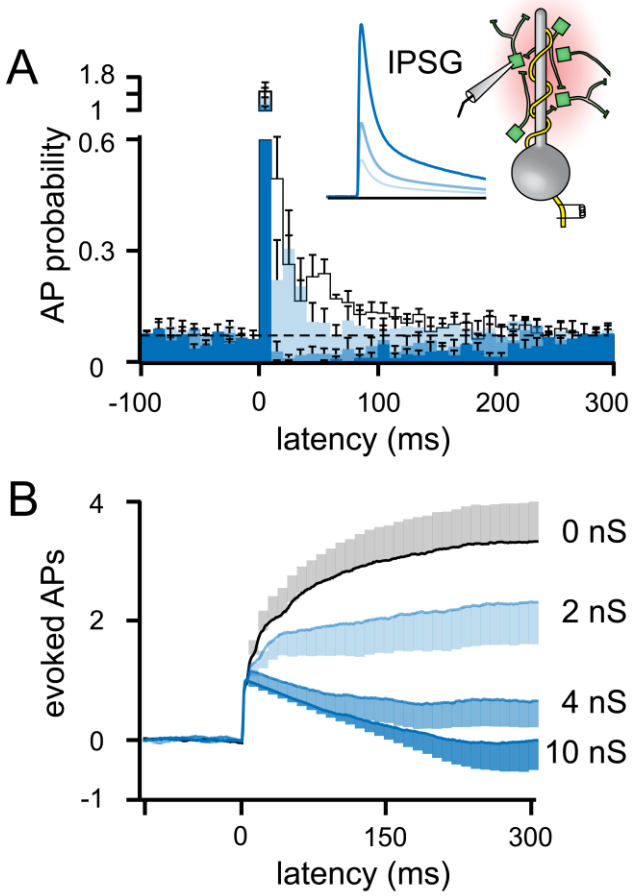

Figure S5. The influence of somatic inhibition on CF-evoked APs.

(A) Peri-stimulus probability histogram (10 ms bins) of CF-evoked APs in the absence (empty bars) or presence of increasing inhibitory conductances (E_{rev} = -65 mV; shown in increasing shades of blue). The peak AP probability decreased from 1.5 ± 0.2 (0 nS) to 1.3 ± 0.2, 1.2 ± 0.2, 1.0 ± 0.1 with a 2, 4, and 10 nS inhibitory conductance triggered 5 ms following CF stimulation, respectively (n = 5, P < 0.001; ANOVA with Dunnett's post test). The CF-stimulated inhibitory conductance (4.5 ± 0.8 nS, n = 33) was measured in a separate group of cells. Inset: The dual component inhibitory conductance (IPSG) mimicked the time course of the CF-mediated IPSQ (see Figure 2; 80% fast: rise 1 ms; decay 8 ms and 20% slow: rise 10 ms; decay 80 ms). Recordings are in the presence of SR95531.
(B) Cumulative spike probability plot in the absence (0 nS) or presence of the inhibitory conductances (2, 4, and 10 nS) from (A). The number of CF-evoked APs (at 300 ms) decreased from 3.3 ± 0.6 to 2.2 ± 0.7, 0.7 ± 0.4, -0.02 ± 0.4 with a 2, 4, and 10 nS inhibitory, respectively (n = 5, P < 0.001; ANOVA with Dunnett's post test). Inhibition was blocked with SR95531. These results demonstrate that somatic injection of an inhibitory conductance similar to CF-mediated IPSG effectively blocks CF-mediated excitation, suggesting that the small effect of blocking synaptic inhibition on MLI spiking could result from dendritic-localized CF-mediated inhibition.

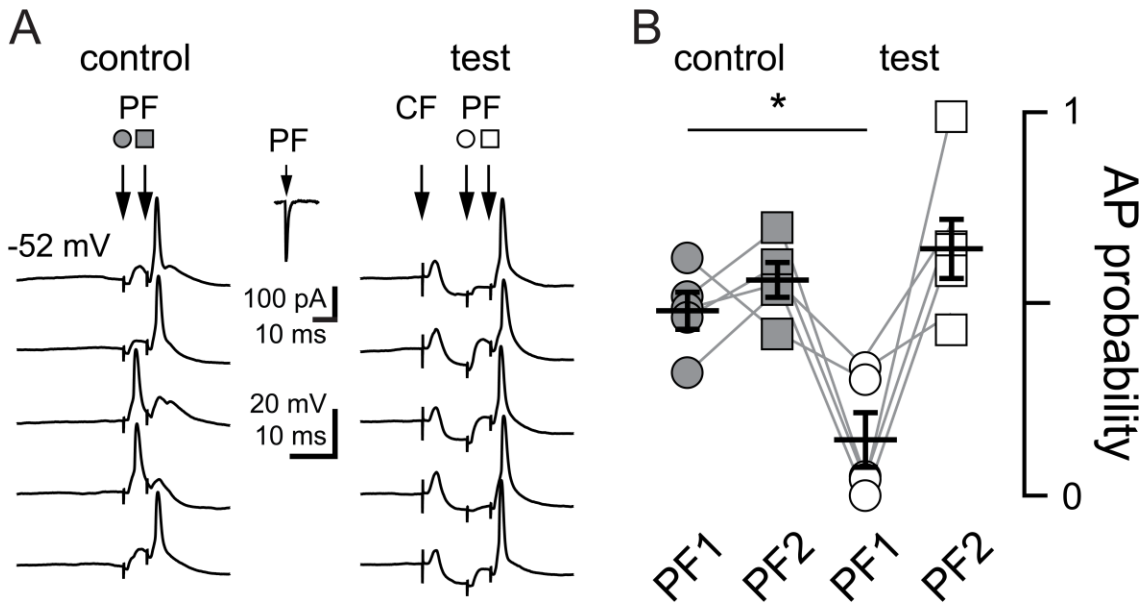

Figure S6. CF-FFI reduces PF-evoked spiking in MLIs.
(A) Five representative traces showing paired PF-evoked spiking in MLIs in the absence (control) and presence of CF stimulation (test). PF-stimulation was set to evoke spiking in 50% of trials (PF1, filled circle). CF-MLI stimulation (10 ms before PF stimulation) evokes an EPSP followed by an IPSP that reduced the PF-evoked AP probability at the first stimulus (PF1; empty circle). Paired-pulse PF stimulation (5 ms interval) generated robust PPF of EPSCs (not shown), but no significant difference in PF-evoked spiking (PF2, compare filled circles and squares), presumably due to robust PF-evoked FFI at this short interval (see Figure S1). Inset: the rapid PF-EPSC in voltage clamp (-60 mV) in the same cell.
(B) Summary of AP probability for control and test PF stimulation, as in (A) (probability = 0.48 ± 0.05, 0.56 ± 0.05, 0.17 ± 0.07, 0.64 ± 0.08, for control PF1, PF2 and test PF1, PF2 conditions, respectively, n = 5 each. Control PF1 and test PF1 are significantly different, ANOVA with Dunnett's post test, $p < 0.05$).

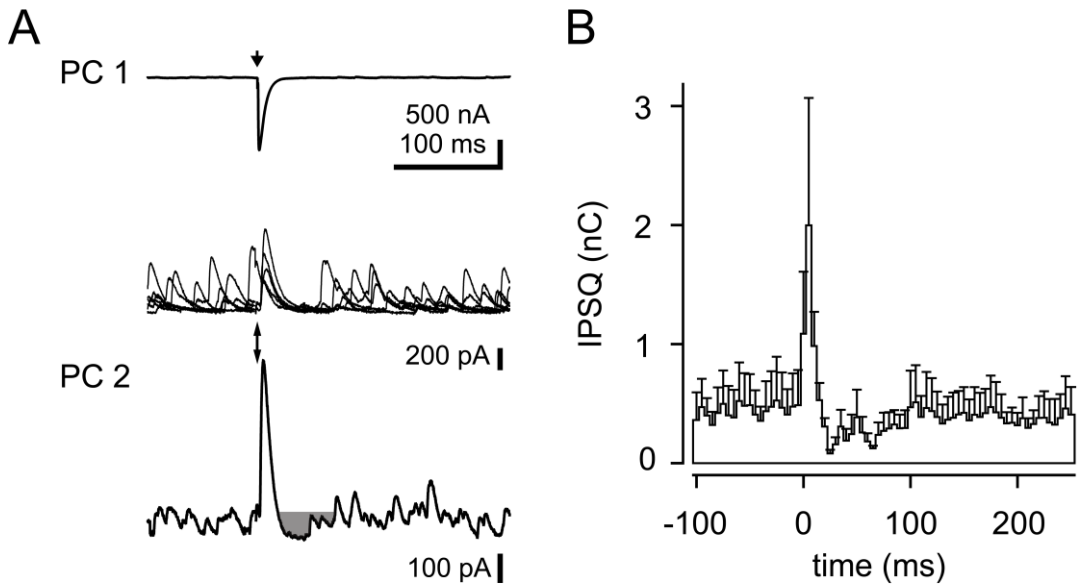

Figure S7. Spillover FFI to neighboring PCs generates biphasic change in IPSQ at elevated temperature and physiological extracellular [Ca^{2+}].
(A) Top: averaged whole cell currents for PC1 (-20 mV) with supra-threshold CF stimulation (arrow). Middle and bottom: simultaneous individual (five traces overlaid) and averaged IPSCs recorded in PC2 at 0 mV following CF EPSCs in PC1. Shaded region denotes disinhibition. Recording configuration as in Figure 7A, except using 1.5 mM extracellular Ca^{2+} and 1 mM Mg^{2+} between 36 - 37ºC..
(B) Average peri-stimulus histogram of the inhibitory charge (IPSQ, 5 ms bins) with supra-threshold CF stimulation (at 0 ms). Supra-threshold CF-stimulation evoked coincident all-or-none IPSCs with an onset latency of 3.9 ± 0.6 ms (n = 4). CF-evoked all-or-none inhibition was brief (5.2 ± 0.9 ms half-width, n = 4) and resulted in an increase of charge above the spontaneous inhibition (709.4 ± 336.2%, n = 4, $P < 0.05$). CF stimulation reduced the charge of spontaneous IPSCs by 67.3 ± 10.8 % (n = 4, $P < 0.01$) for a duration of 31.5 ± 5.8 ms (half-width, n = 4).