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Supplemental information

High-fidelity estimates of spikes and subthreshold waveforms from 1-photon voltage imaging *in vivo*

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Brain region	Reporter	# of Cells	Relative Variance (mean ± s.e.m.)		
			Signal	Background	Residual
Mouse hippocampus	SomArchon1	6	0.76 ± 0.05	0.09 ± 0.02	0.01 ± 0.00
Mouse cortex L1	Voltron	59	0.66 ± 0.02	0.15 ± 0.01	0.00 ± 0.00
Mouse cortex L1	SomArchon1	6	0.41 ± 0.09	0.35 ± 0.07	0.01 ± 0.00
Zebrafish spinal cord	zArchon1	23	0.16 ± 0.02	0.52 ± 0.06	0.00 ± 0.00

Table S1. **Performance of SGPMD-NMF across different *in vivo* datasets.** Relative variance was defined as the variance of the indicated SGPMD-NMF waveform divided by the variance of a flat average across the corresponding ROI. The relative variances do not sum to 1 because the signal, background, and residual were not necessarily mutually orthogonal. Related to Figs. 5 and 6 and Table 1.

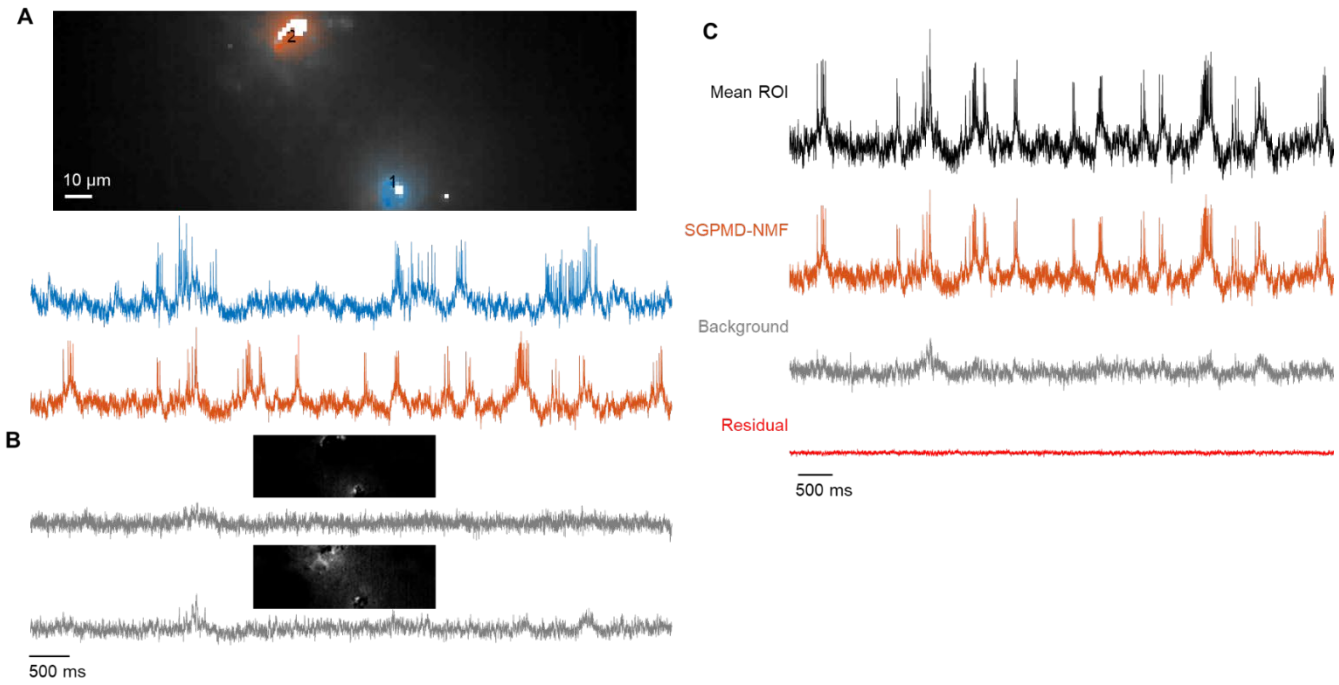

Figure S1. Voltage imaging in mouse cortical L1 using SomArchon1. The cells expressed SomArchon1 and were imaged via holographic membrane-targeted illumination. A) Top: Image of the field of view, showing well-separated neurons as occurs in L1. The cell footprints are overlaid in blue and orange. Regions contaminated by blood flow are masked in white. Bottom: extracted single-cell traces. Elevated spike rates clearly reside atop subthreshold depolarizations, giving confidence that the sub-threshold voltages are at least approximately correct. B) Background components from SGPMD-NMF. The two components that explained the most variance in the movie are included, with each component's spatial profile above the corresponding temporal trace. C) Average across pixels in cell 2 in the denoised movie (Mean ROI), SGPMD-NMF signal (SGPMD-NMF), background (Background) and residual (Residual). Related to Table 1.

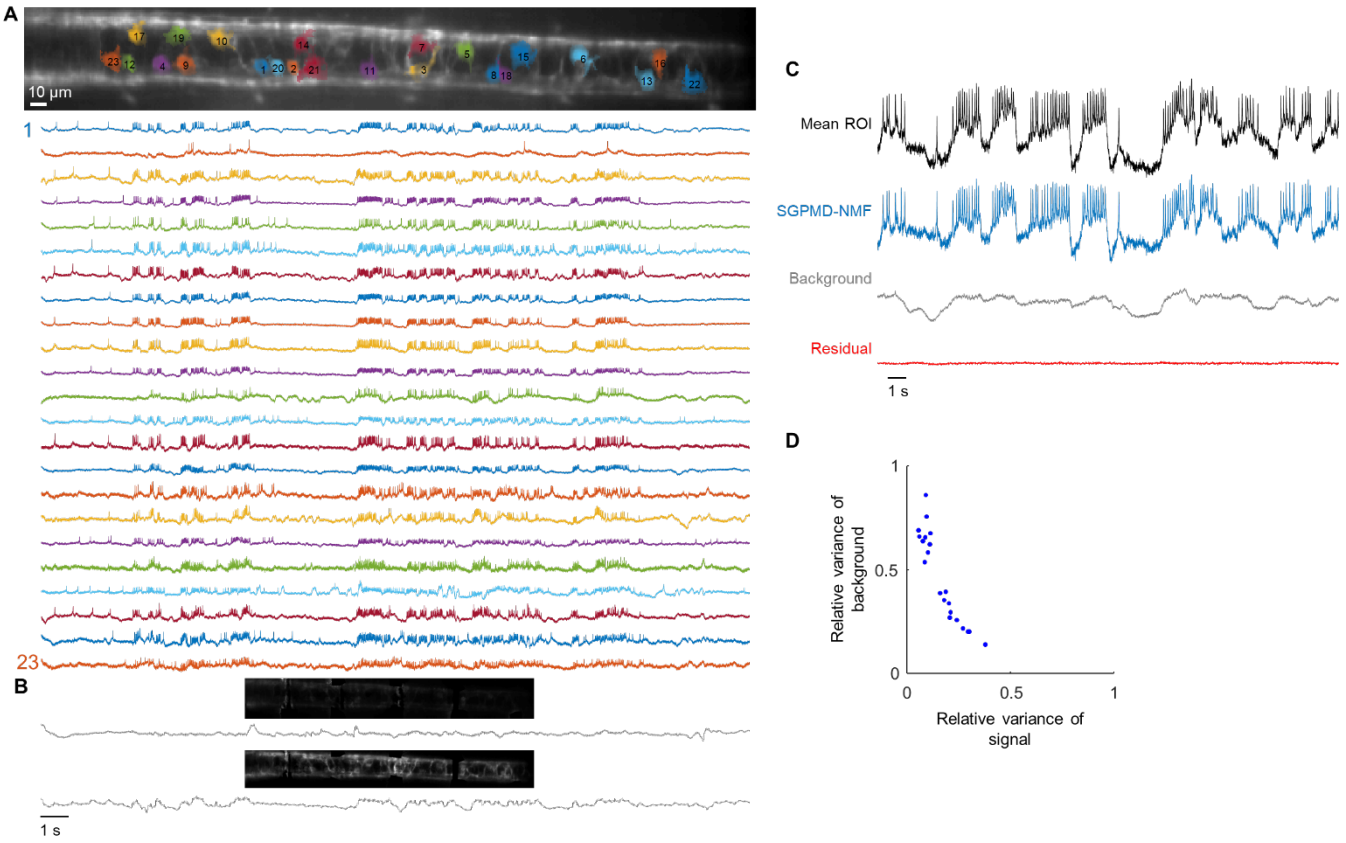

Figure S2. Voltage imaging in zebrafish spinal cord using zArchon1. The cells expressed zArchon1 and were imaged via light sheet fluorescence microscopy. A) Top: Image of the field of view. The cell footprints are overlaid. Bottom: extracted single-cell traces. B) Background components from SGPMD-NMF. The two components that explained the most variance in the movie are included, with each component's spatial profile above the corresponding temporal trace. C) Average over pixels in cell 8 in the denoised movie (Mean ROI), SGPMD-NMF signal (SGPMD-NMF), background (Background) and residual (Residual). D) Scatter plot of the relative variance of each cell background vs. signal. As in Fig. 6E, relative variance of background vs signal is anticorrelated. Related to Table 1.