

Statistically unbiased prediction enables accurate denoising of voltage imaging data

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Here we report SUPPORT (statistically unbiased prediction utilizing spatiotemporal information in imaging data), a self-supervised learning method for removing Poisson–Gaussian noise in voltage imaging data. SUPPORT is based on the insight that a pixel value in voltage imaging data is highly dependent on its spatiotemporal neighboring pixels, even when its temporally adjacent frames alone do not provide useful information for statistical prediction. Such dependency is captured and used by a convolutional neural network with a spatiotemporal blind spot to accurately denoise voltage imaging data in which the existence of the action potential in a time frame cannot be inferred by the information in other frames. Through simulations and experiments, we show that SUPPORT enables precise denoising of voltage imaging data and other types of microscopy image while preserving the underlying dynamics within the scene.

Recent advancements in voltage imaging and calcium imaging have enabled recording of the population activity of neurons at an unprecedented throughput, which opens up the possibility of a system-level understanding of neuronal circuits^{1–3}. To investigate causality within neuronal activities, it is essential to record the activities with high temporal precision. Unfortunately, the inherent limitation in the maximum number of photons that can be collected from a sample in a given time

interval dictates the inherent trade-offs between imaging speed and signal-to-noise ratio (SNR)^{4,5}. In other words, increasing the temporal resolution in functional imaging data inevitably results in a decrease in the SNR. The decrease in SNR not only hinders the accurate detection of the neurons' locations but also compromises the timing precision of the detected temporal events, which nullifies the increase in temporal resolution. Fortunately, all functional imaging data have high inherent

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redundancy in the sense that each frame in a dataset shares a high level of similarity with other frames apart from noise, which offers an opportunity to denoise or distinguish the signal from the noise in the data^{6–9}.

Denosing is a type of signal processing that attempts to extract underlying signals from noisy observations based on previous knowledge of the signal and the noise¹⁰. The fundamental property of noise—randomness—does not allow for exact recovery of the signal, so we can only reduce statistical variance at the cost of increasing statistical bias (that is, an absolute deviation between the mean denoising outcome and the ground truth). In other words, denoising is a statistical estimation of the most probable value based on our previous statistical knowledge of the signal and the noise. Unfortunately, for any given noisy observation, the exact corresponding probability distribution functions (PDFs) of the signal and the noise are almost never known. Therefore, all denoising algorithms start with setting the signal model (that is, PDF of signal) and noise model (that is, PDF of noise), either explicitly or implicitly, and their accuracy determines the denoising performance.

The most common approach starts with applying linear transforms, such as the Fourier transform and the wavelet transform, to noisy observations^{11,12}. Then, a certain set of coefficients that corresponds to a small vector space is preserved, while others are attenuated to reduce statistical variance. This is based on a signal model in which the signal is a random variable drawn from the small vector space, whereas noise is drawn from the entire vector space. An implicit yet important assumption here is that the basis used for the linear transform maps the signal component sharply onto a relatively small and known set of coefficients. When the assumption is not met, denoising leads to a distortion of signals or an increase in statistical bias. Such bias can be reduced by loosening the assumption (for example, the signal is drawn from a larger vector space), but then the variance is increased.

Therefore, building a good signal model that is strong enough to reject noise while being accurate enough to avoid bias is the most critical step in denoising. Previous efforts have focused on finding a handcrafted basis that empirically matches the given data¹³. Some have shown higher general applicability than others¹⁴, but no universal basis that performs well across different types of data has been found, mainly because of the differences in their signal models and noise models¹⁵. This has led to the idea of using a basis learned directly from the dataset for denoising^{6,16,17}. However, these methods still suffer from high bias, as their ability to reduce variance relies on the strong assumption that the data can be represented as a linear summation of a small number of learned vectors.

Recently, the convolutional network has emerged as a strong alternative to existing learning-based image denoising algorithms¹⁸. The high representational power of convolutional networks allows for learning nearly arbitrary signal models in the image domain, resulting in low bias in denoising outcomes without sacrificing variance¹⁹. Owing to its high representational power and the high inherent redundancy in functional imaging data, convolutional networks have shown enormous success in denoising functional imaging data^{7–9}. As a key aspect, these methods learn the signal model from noisy data in a self-supervised manner^{20–23}, so the need for ‘clean’ images as the ground truth for training is alleviated.

Both DeepCAD-RT⁷ and DeepInterpolation⁹ are based on the assumption that the underlying signal in any two consecutive frames in a video can be considered the same, whereas the noise is independent when the imaging speed is sufficiently higher than the dynamics of the fluorescent reporter^{7,9}; the networks are trained to predict the ‘current’ frame using the past and future frames as the input. Unfortunately, this assumption breaks down when the imaging speed is not sufficiently faster than the dynamics, and the bias in the denoising outcome is increased. This is becoming increasingly prevalent due to the development of voltage indicators^{24–28} and calcium indicators with extremely fast dynamics²⁹. In that regard, the question that naturally

follows is how we can implement an accurate statistical model that allows us to accurately predict each pixel value under such conditions.

To this end, we propose SUPPORT (statistically unbiased prediction using spatiotemporal information in imaging data), a self-supervised denoising method for functional imaging data that is robust to fast dynamics in the scene compared to the imaging speed. SUPPORT is based on the insight that a pixel value in functional imaging data is highly dependent on its spatiotemporal neighboring pixels, even when its temporally adjacent frames alone fail to provide useful information for statistical prediction. By learning and using the spatiotemporal dependence among the pixels, SUPPORT can accurately remove Poisson–Gaussian noise in voltage imaging data in which the existence of the action potential in a time frame cannot be inferred from the information in other frames. We demonstrate the capability of SUPPORT using diverse voltage imaging datasets acquired using Voltron1, Voltron2, paQuasAr3-s, QuasAr6a, zArchon1, SomArchon and BeRST1. The analysis of the voltage imaging data with simultaneous electrophysiological recording shows that our method preserves the shape of the spike while reducing the statistical variance in the signal. We also show that SUPPORT can be used for denoising time-lapse fluorescence microscopy images of *Caenorhabditis elegans* (*C. elegans*), in which the imaging speed is not faster than the worm’s locomotion, as well as static volumetric images of *Penicillium* and mouse embryos. SUPPORT is exceptionally compelling for denoising voltage imaging and time-lapse imaging data, and is even effective for denoising calcium imaging data. Finally, we developed software with a graphical user interface (GUI) for running SUPPORT to make it available to the wider community.

Results

Central principle of SUPPORT

The central principle of SUPPORT is to perform denoising based on a statistical prediction model with minimal bias by exploiting all available information in both spatial and temporal domains (Fig. 1a). A functional imaging dataset y is considered a realization of a random variable that is drawn from $p(y) = p(x)p(n|x)$, where x and n are the clean signal and the zero-mean Poisson–Gaussian additive noise, respectively (that is, $y = x + n$). In this setting, the noise in each pixel is independent in both time and space (that is, $\forall(i, k) \neq (j, l)$, $p(n_{i,k}) = p(n_{i,k}|n_{j,l})$, where i, j and k, l are temporal and spatial indices, respectively, where the signal is not (that is, $\forall(i, k, j, l)$, $p(x_{i,k}) \neq p(x_{i,k}|x_{j,l})$). The dependency among $x_{i,k}$ encodes the spatiotemporal structure of the data x (that is, $p(x)$), which can be learned using a statistical prediction model, whereas the spatiotemporal independence of n makes it impossible to predict. The prediction model can be implemented as a neural network that predicts a pixel value $x_{i,k}$ using its spatiotemporal neighboring pixel values by solving the following optimization problem^{20,21}:

$$\theta^* = \arg \min_{\theta} \sum_{i,k} L(f_{\theta}(\Omega_{i,k}), x_{i,k})$$

where $L(\cdot, \cdot)$ is the loss function defined as the L^p distance between the inputs, f_{θ} denotes the neural network parameterized by θ and $\Omega_{i,k}$ denotes the spatiotemporal neighboring pixels of $y_{i,k}$ excluding itself. Evaluating this loss function requires the ground truth x , which is inaccessible, but the zero-mean property of the noise allows us to replace $x_{i,k}$ with $y_{i,k}$ for self-supervised training²¹:

$$\theta^* = \arg \min_{\theta} \sum_{i,k} L(f_{\theta}(\Omega_{i,k}), x_{i,k}) = \arg \min_{\theta} \sum_{i,k} L(f_{\theta}(\Omega_{i,k}), y_{i,k}).$$

For the implementation of the network $f_{\theta}(\Omega_{i,k})$, we devised a network architecture that automatically satisfies the requirements (Fig. 1b,c and Supplementary Figs. 1 and 2). For the prediction of $x_{i,k}$,

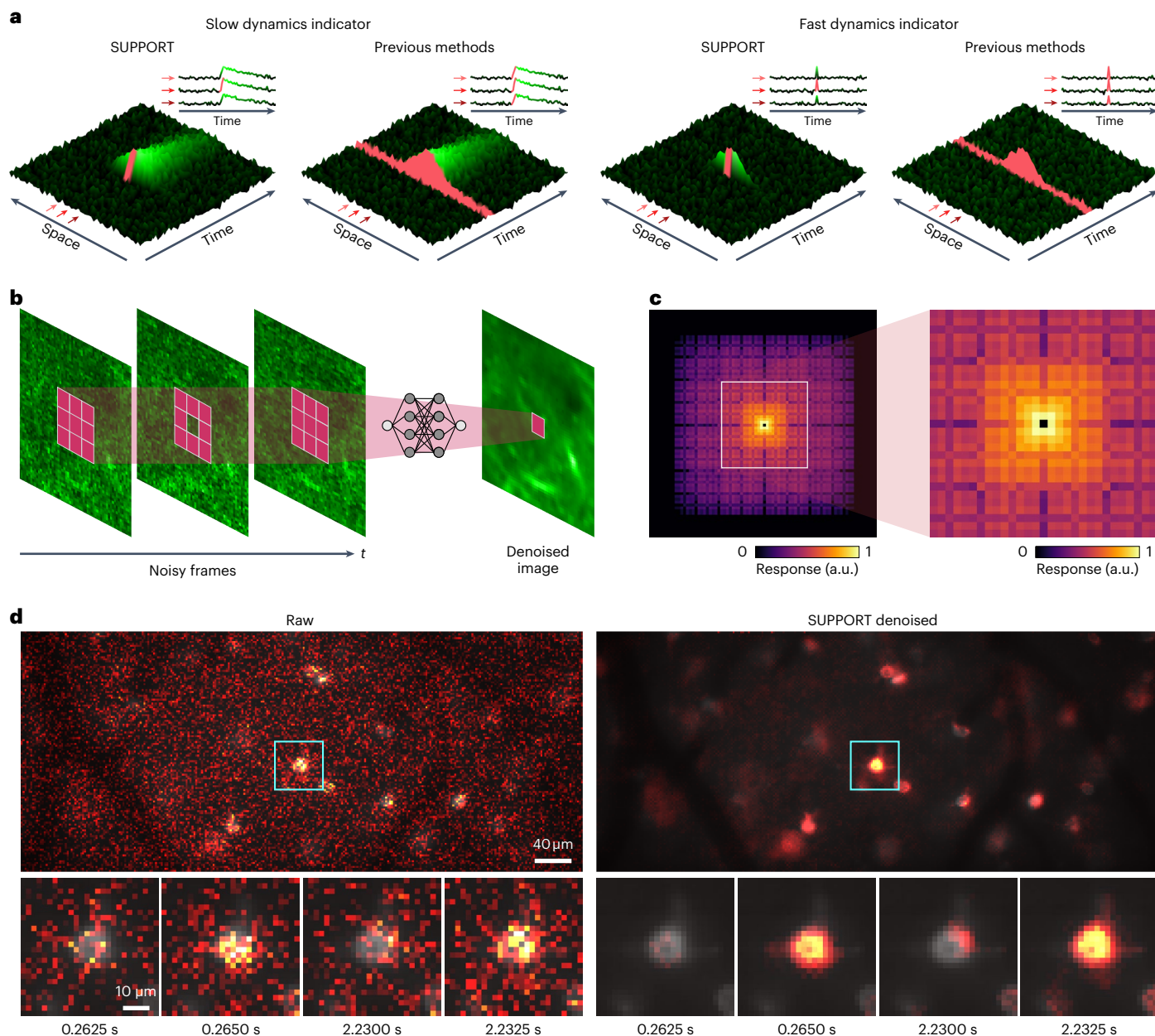


Fig. 1 SUPPORT can be applied to functional imaging data with a fast dynamics indicator. **a**, SUPPORT's self-supervised learning scheme and previous methods that exploit temporally adjacent frames for denoising functional imaging data with slow and fast dynamics indicators. Functional imaging data are represented by green and red surfaces, which indicate the receptive field and prediction target area, respectively. **b**, Noisy frames are fed into the SUPPORT network and output the denoised image. Red tiles indicate the receptive field of the SUPPORT network, which uses spatially adjacent pixels in the same frame. **c**, Impulse response of the SUPPORT network on the current frame. The magnified

view is presented on the right side. Response value of the center pixel is 0, which forces the network to predict the center pixel without using it. **d**, In vivo population voltage imaging data. The left shows the raw data and the right shows the SUPPORT-denoised data. Baseline and activity components are decomposed from raw data and SUPPORT-denoised data. The baseline component with gray colormap and activity component with hot colormap are overlaid. Magnified views of the boxed regions are presented below at the time points near spikes. Consecutive frames of two spikes ($t = 0.2650$ and 2.2325 s).

its spatiotemporal neighbor $\Omega_{i,k}$ excluding $y_{i,k}$ is taken as the input while preserving the spatial invariance. The current frame y_i is fed into a convolutional network that has a zero at the center of the impulse response (Fig. 1b,c); the zero at the center of the impulse response indicates that the pixel value $y_{i,k}$ cannot affect the network's prediction of $x_{i,k}$ (refs. 20,30), which is attained by convolution layers and dilated convolution layers with zeros at the center of the kernels. These layers offer a fractal-shaped receptive field that grows exponentially with depth, enabling the network to integrate information from a large

number of neighboring pixels (Supplementary Fig. 2). In addition, temporally adjacent frames are fed into a U-Net³¹ to extract the available information from the temporally adjacent ones (Supplementary Fig. 1). The outputs from the two convolutional networks are integrated by the following convolutional layers. This architecture 'forces' the network to make a prediction $\hat{x}_{i,k}$ by using its spatiotemporal neighbor $\Omega_{i,k}$ excluding $y_{i,k}$ (that is, $\hat{x}_{i,k} = f_{\theta}(\Omega_{i,k})$).

The major difference between SUPPORT and DeepCAD-RT⁷ or DeepInterpolation⁹, which can also denoise functional imaging data

through self-supervised learning, is that DeepCAD-RT and DeepInterpolation learn to predict a frame given temporally adjacent other frames, whereas SUPPORT learns to predict each pixel value by exploiting the information available from both temporally adjacent frames and spatially adjacent pixels in the same time frame. When the imaging speed is not sufficiently faster than the dynamics in the scene (Fig. 1a), the signal at different time points becomes nearly independent (for example, the existence of the action potential in a time frame cannot be inferred from the information in other frames). In such a case, the major assumptions of the signal models in DeepCAD-RT and DeepInterpolation are violated, which leads to high bias in the denoising outcome. In comparison, SUPPORT relies on the spatiotemporal pixel-level dependence of the signal rather than frame-level dependence, and each pixel value is estimated based on all available information, including its spatially adjacent pixels in the same time frame.

Performance validation on simulated data

For the quantitative evaluation of SUPPORT's performance, we first validated it on synthetic voltage imaging data, which were generated using a NAOmi simulator³². We generated multiple datasets with a frame rate of 500 Hz with different spike widths, ranging from 1 to 9 ms (ref. 33), to verify how the performance of SUPPORT changes as the dependence between the activity in adjacent frames is diminished. The simulation parameters, including spike frequency, dF/F_0 , noise level and level of subthreshold activity, were chosen to match the experimental voltage imaging data acquired using Voltron²⁴ (Methods). Finally, Poisson and Gaussian noise were added to the generated videos. Further details can be found in the Methods section.

We applied SUPPORT, DeepCAD-RT⁷ and penalized matrix decomposition (PMD)⁶ to the synthetic datasets and compared the results. The signals were separated from the backgrounds in the denoised videos (Methods) to compare their accuracy in recovering the time-varying signal (Fig. 2a and Supplementary Video 1). Qualitative comparisons of the results from the dataset with a spike width of 3 ms showed that the denoising outcome from SUPPORT was nearly identical to the ground truth. DeepCAD-RT successfully reduced the variance in the video, but also attenuated the neuronal activity. This was expected because the method was designed for removing noise in calcium imaging data, which has much slower dynamics. PMD showed better performance in preserving neuronal activities, in part because it did not discard the current frame for denoising, but it introduced visible artifacts in the images.

To quantify the performance of each denoising method, we calculated the peak SNR (PSNR) of the denoised videos and calculated the Pearson correlation coefficient between the voltage traces extracted from the clean video and the denoised video. The voltage traces were extracted from 116 cells (Methods). In terms of PSNR, all methods showed substantial enhancements compared to noisy images for every spike width (Fig. 2b and Supplementary Figs. 3–5): noisy (1 ms, 4.57 dB; 9 ms, 15.43 dB), SUPPORT (1 ms, 35.94 dB; 9 ms, 43.08 dB), DeepCAD-RT (1 ms, 30.90 dB; 9 ms, 39.05 dB) and PMD (1 ms, 32.07 dB; 9 ms, 38.61 dB). However, in terms of the Pearson correlation coefficient, only SUPPORT (1 ms, 0.885; 9 ms, 0.991) showed improvement compared to noisy images (1 ms, 0.593; 9 ms, 0.942) for every spike width (Fig. 2c and Supplementary Fig. 6). DeepCAD-RT (1 ms, 0.190; 9 ms, 0.984) and PMD (1 ms, 0.554; 9 ms, 0.983) showed improvement only when the spike width was larger than 5 and 3 ms, respectively, which verifies the importance of exploiting spatially adjacent pixels in the same time frame. We note that this inconsistency between the two metrics stems from the fact that the Pearson correlation coefficient is affected only by the time-varying component of the signals, whereas PSNR is largely determined by the static component.

For further comparison, we analyzed the voltage traces at the single-pixel (Fig. 2d) and single cell levels (Fig. 2e). Only the single-pixel voltage traces from SUPPORT retained the spike waveforms (Fig. 2d),

whereas the spikes were buried under the noise level in the single-pixel voltage traces from the noisy video. DeepCAD-RT and PMD reduced the variance in the single-pixel voltage traces, but the spikes were still not detectable due to the bias introduced by their signal models. The single cell voltage traces showed similar results (Fig. 2e and Supplementary Figs. 7–9), although the difference was less dramatic than the single-pixel traces, as the SNR was improved by averaging multiple pixel values. SUPPORT was able to reduce variance without distorting the waveforms for every spike width. In comparison, the spikes were not detectable in the results from DeepCAD-RT and PMD when the spike width was under 3 ms. It should be noted that the performance of both DeepCAD-RT and PMD was better for larger spike widths, but for different reasons. DeepCAD-RT estimates the current frame given temporally adjacent frames, so the prediction becomes more accurate when the dynamics are slower. PMD attempts to find a low rank approximation of a given matrix that is supposedly closer to the ground truth, so a temporally long event is less likely to be 'ignored' as its contribution to the approximation error is higher.

Denoising single-neuron voltage imaging data

To validate SUPPORT's capability to denoise experimentally obtained voltage imaging data while retaining the spikes, we applied SUPPORT to *in vivo* single-neuron voltage imaging data with simultaneous electrophysiological recordings. The dataset contained light-sheet microscopy images of a single neuron in the dorsal part of the cerebellum of a zebrafish expressing Voltron1 with simultaneous cell-attached extracellular electrophysiological recording. Electrophysiological recordings were taken at a sampling rate of 6 kHz, and light-sheet imaging was performed with a frame rate of 300 Hz (ref. 24).

In the raw data, both the spatial footprint and temporal traces of the neuron were severely corrupted by Poisson–Gaussian noise. We compared temporal traces extracted from the raw video and the denoised video using SUPPORT, DeepCAD-RT and PMD, along with the electrophysiological recording. Spike locations from the electrophysiological recordings were extracted (Methods) and visualized as black dots for a visual aid (Fig. 3a,b). After denoising with SUPPORT, the temporal trace showed a much lower variance compared to the temporal trace of the raw data while preserving the spikes (Supplementary Figs. 9 and 10). In comparison, while the temporal variance in the denoising outcome acquired using DeepCAD-RT was low, the spikes were no longer visible in the traces, which implies that the signal modeling in DeepCAD-RT substantially increased the bias. The temporal trace from PMD was nearly identical to that from the raw video, which indicates that PMD had limited impact on both bias and variance.

After we applied SUPPORT to enhance this data, not only did the neuronal activity become clearly visible in the images, but the spatial footprints of the activity also showed high consistency with the corresponding neuronal shape (Fig. 3c and Supplementary Video 2). Representative frames from the raw and denoised data show that SUPPORT removed the noise very effectively, while the activity was preserved.

For further comparison, we extracted single-pixel fluorescence from the cell membrane pixels and found that the average single-pixel SNR was strongly enhanced with SUPPORT (14.46 dB) compared to DeepCAD-RT (12.21 dB) and PMD (13.46 dB) (Fig. 3d). The spatiotemporal diagram, which visualizes the voltage transients of each 2×2 binned pixel, also verified that SUPPORT successfully reduced the variance while preserving the spikes at the pixel level (Fig. 3e).

Next, we tested the capability of SUPPORT to recover subthreshold activity of neurons using wide-field microscopy images of a single neuron in cortex layer 1 of a mouse brain expressing Voltron1 with simultaneous cell-attached extracellular electrophysiological recording (Fig. 4a). Electrophysiological recordings were taken at a sampling rate of 10 kHz, and imaging was performed at a frame rate of 400 Hz.

After denoising with SUPPORT, we found that even a single-pixel fluorescence trace faithfully reflected the subthreshold signal (Fig. 4b).

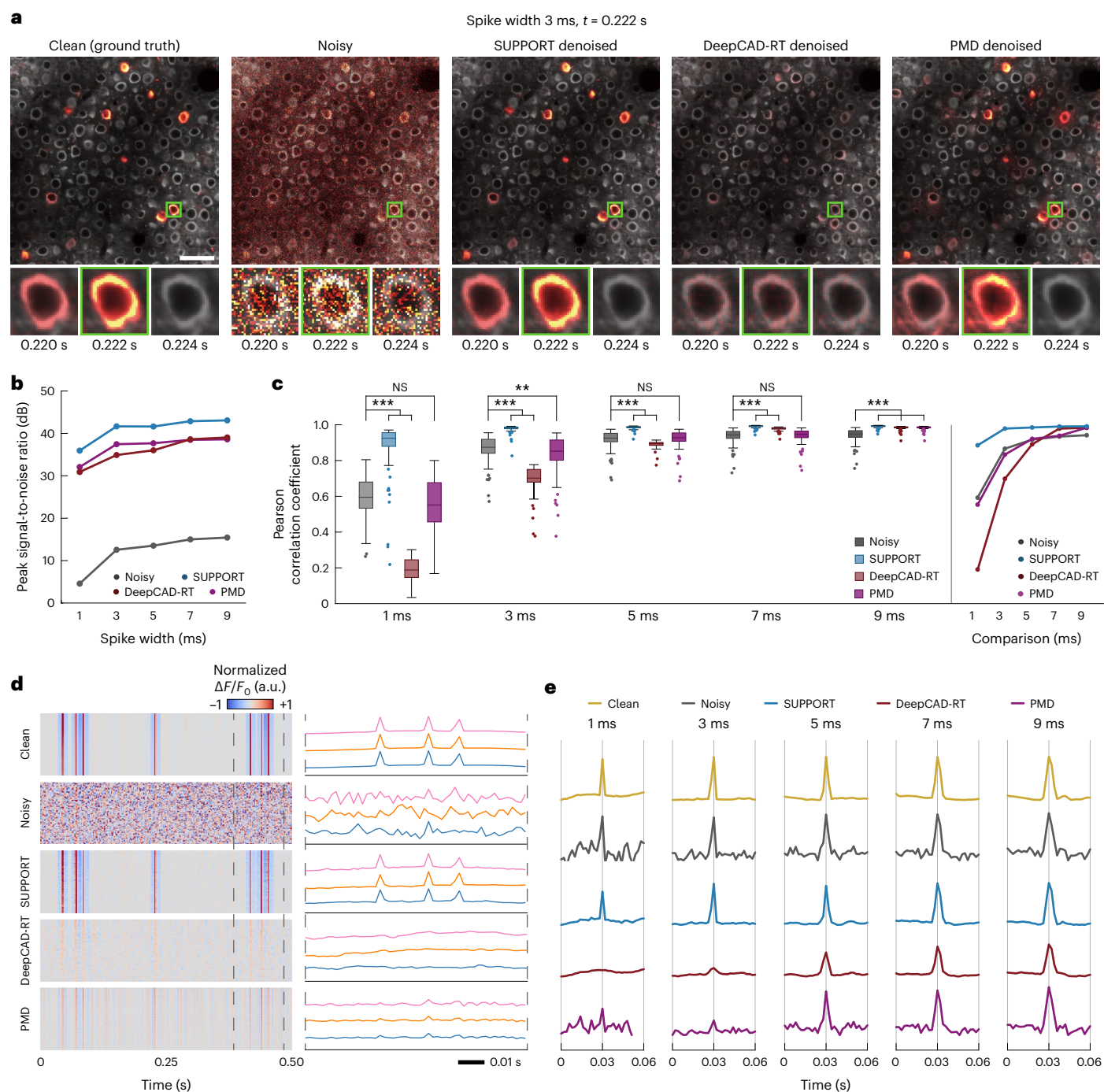


Fig. 2 | Performance validation on simulated data. **a**, Synthetic population voltage imaging data. From left to right are the clean, noisy, SUPPORT, DeepCAD-RT and PMD denoised data. Baseline and activity components are decomposed from the data. The baseline component with a gray colormap and activity component with a hot colormap are overlaid. Magnified views of the boxed regions are presented underneath with the consecutive frames of the spiking event ($t = 0.222$ s). Scale bar, $40 \mu\text{m}$. **b**, PSNR of the baseline-corrected data before and after denoising data with different spike widths. Clean data were used as the ground truth for PSNR calculation. **c**, The left shows a box-and-whisker plot showing Pearson correlation coefficients before and after denoising data with different spike widths. The right shows a line chart showing average Pearson

correlation coefficient before and after denoising data with different spike widths. Two-sided one-way analysis of variance with Tukey–Kramer post hoc test was used. $n = 116$ for each test, which represents the number of neurons (NS, not significant, $*P < 0.1$, $**P < 0.01$, $***P < 0.001$). **d**, Single-pixel fluorescence traces extracted from baseline-corrected data. From top to bottom: clean, noisy, SUPPORT, DeepCAD-RT and PMD denoised data. The left shows each single-pixel trace occupies each row. The right shows three representative single-pixel traces visualized with different colors. **e**, Single cell fluorescence traces near spiking event extracted from baseline-corrected data. From top to bottom: clean, noisy, SUPPORT, DeepCAD-RT and PMD denoised data. From left to right: changing spike widths of 1, 3, 5, 7 and 9 ms.

The average Pearson correlation coefficient, obtained by comparing the fluorescence traces with the electrophysiological recordings, of SUPPORT (0.51 ± 0.18) showed a 0.30 increase compared to the raw

image (0.21 ± 0.12) (Fig. 4c). The power spectral density of the fluorescence traces from the denoised image was also consistent with that of the electrophysiological recordings.

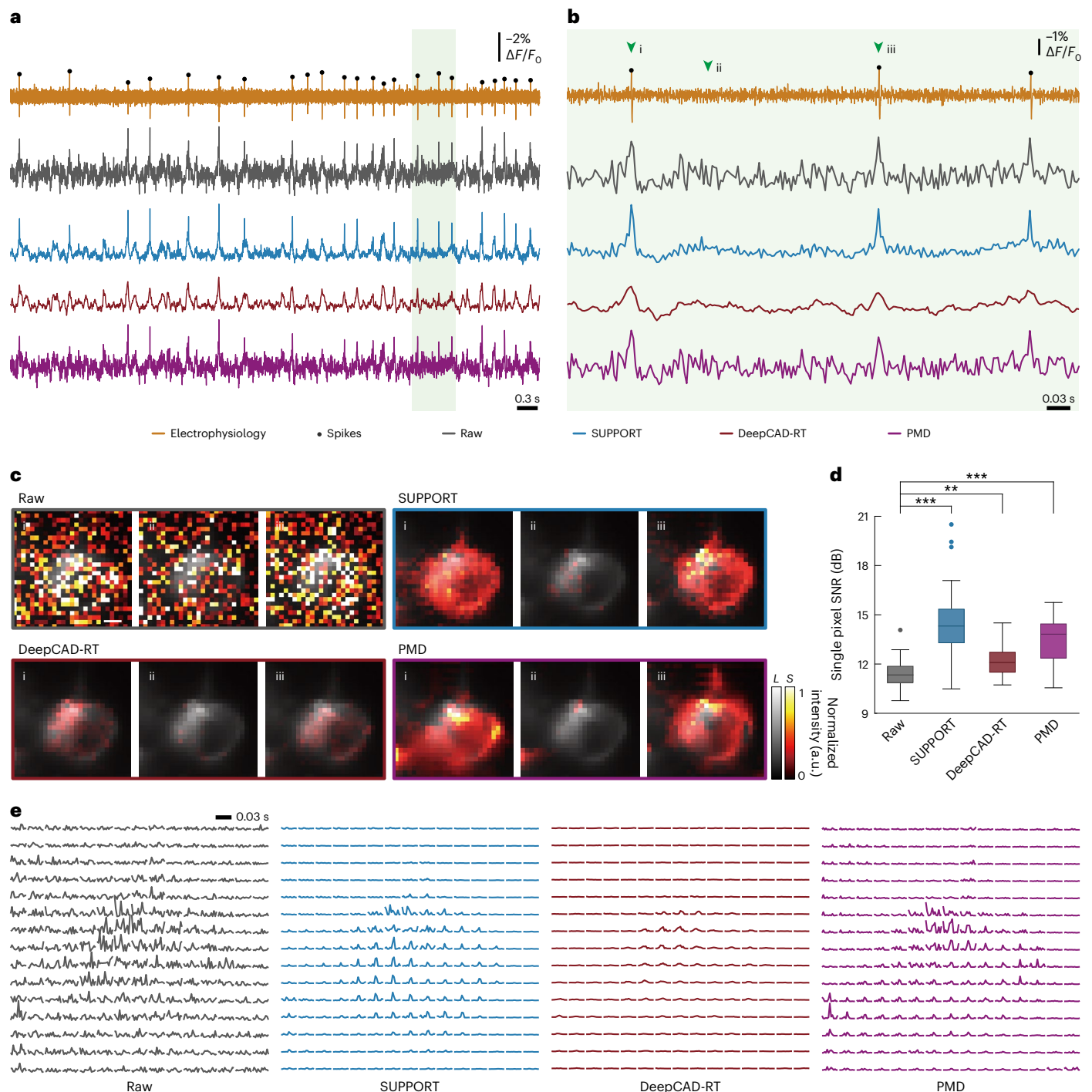


Fig. 3 | Denoising single-neuron voltage imaging data. **a**, Simultaneous electrophysiological recording and voltage imaging data. From top to bottom: electrophysiological recording, raw, SUPPORT, DeepCAD-RT and PMD denoised data. Detected spikes from electrophysiological recordings are marked with black dots. Traces from voltage imaging data were extracted using a manually drawn ROI. **b**, Enlarged view of the green region in **a**. **c**, Three representative frames indicated on **b** with green arrows for raw and denoised data. Baseline and activity components are decomposed from raw data and denoised data. The baseline component with a gray colormap and the activity component with a hot

colormap are overlaid. Scale bar, 1 μ m. **d**, Box-and-whisker plot showing the SNR for the pixels inside the cell region from raw and denoised data. From left to right: raw, SUPPORT, DeepCAD-RT and PMD denoised data. Two-sided one-way analysis of variance with Tukey–Kramer post hoc test was used. $n = 70$ for each test, which represents the number of pixels (* $P < 0.1$, ** $P < 0.01$, *** $P < 0.001$). **e**, Spatiotemporal diagram showing the voltage transients of each 2×2 binned pixel with a small temporal region centered at time point i on **b**. From left to right: raw, SUPPORT, DeepCAD-RT and PMD denoised data.

We confirmed the one-to-one correspondence between the fluorescence trace and the transmembrane potential using wide-field microscopy images of a single neuron in the brain slice from mouse cortex layer 2/3 expressing QuasAr6a (ref. 34), which is known to possess

high linearity (Fig. 4e). The one-to-one correspondence became evident after SUPPORT denoising (Fig. 4f). The average Pearson correlation coefficient between the fluorescence traces and the electrophysiological recordings increased from 0.18 ± 0.11 to 0.65 ± 0.22

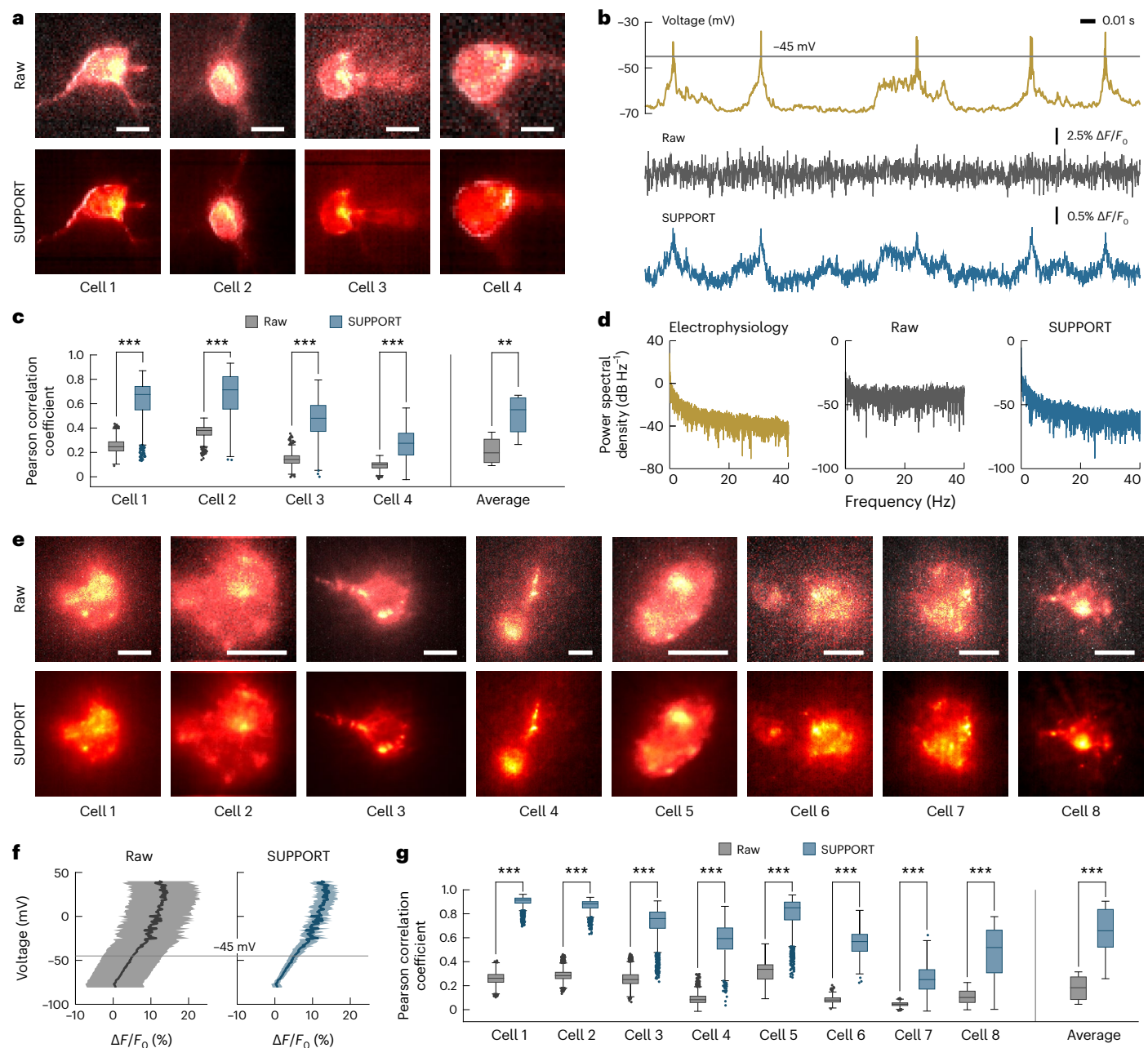


Fig. 4 | Recovering subthreshold activity in voltage imaging data. a, Raw and SUPPORT-denosed images of four neurons in mouse cortex layer 1 expressing Voltron1 are shown after baseline correction. Scale bars, 5 μm . **b**, Electrophysiological recording and single-pixel traces extracted from raw and SUPPORT-denosed data. Spike regions are detected from electrophysiological recording data and excluded in subthreshold analysis. **c**, The left shows box-and-whisker plots showing Pearson correlation coefficient between electrophysiological recording and single-pixel fluorescence traces in subthreshold region. The right shows box-and-whisker plots showing average Pearson correlation coefficients before and after denoising. A two-sided paired-sample *t*-test was used: cell 1, $n = 1,842$; cell 2, $n = 675$; cell 3, $n = 2,610$; cell 4, $n = 506$ and average, $m = 4$, where n represents the number of pixels and m represents the number of cells. **d**, Power spectral density of electrophysiological recording and single-pixel fluorescence traces of raw and denoised data.

e, Raw and SUPPORT-denosed images of eight neurons in the brain slice from mouse cortex L2/3 expressing QuasAr6a are shown after baseline correction. Scale bars, 10 μm . **f**, Relationship between transmembrane potential and dF/F_0 . Average and standard deviation of dF/F_0 values are calculated for corresponding voltage values. Average points are drawn as solid lines and areas between average + standard deviation and average-standard deviation are filled. **g**, The left shows box-and-whisker plots showing Pearson correlation coefficient between electrophysiological recording and single-pixel fluorescence traces in subthreshold region. The right shows box-and-whisker plots showing average Pearson correlation coefficients before and after denoising. A two-sided paired-sample *t*-test was used: cell 1, $n = 3,289$; cell 2, $n = 3,157$; cell 3, $n = 3,458$; cell 4, $n = 3,516$; cell 5, $n = 2,214$; cell 6, $n = 599$; cell 7, $n = 1,240$; cell 8, $n = 427$ and average, $m = 8$, where n represents the number of pixels and m represents the number of cells (** $P < 0.01$, *** $P < 0.001$).

after denoising (Fig. 4g). These results were in line with those from the simulation (Supplementary Fig. 11).

Additionally, we found that SUPPORT precisely revealed the traces from single pixels inside the soma (Supplementary Fig. 12) and along

the dendritic branch (Supplementary Figs. 13–15 and Supplementary Video 3), which indicates SUPPORT's suitability for studies involving voltage dependence along the neuronal processes³⁵. Finally, SUPPORT was able to denoise in vitro cultured neurons labeled with a synthetic

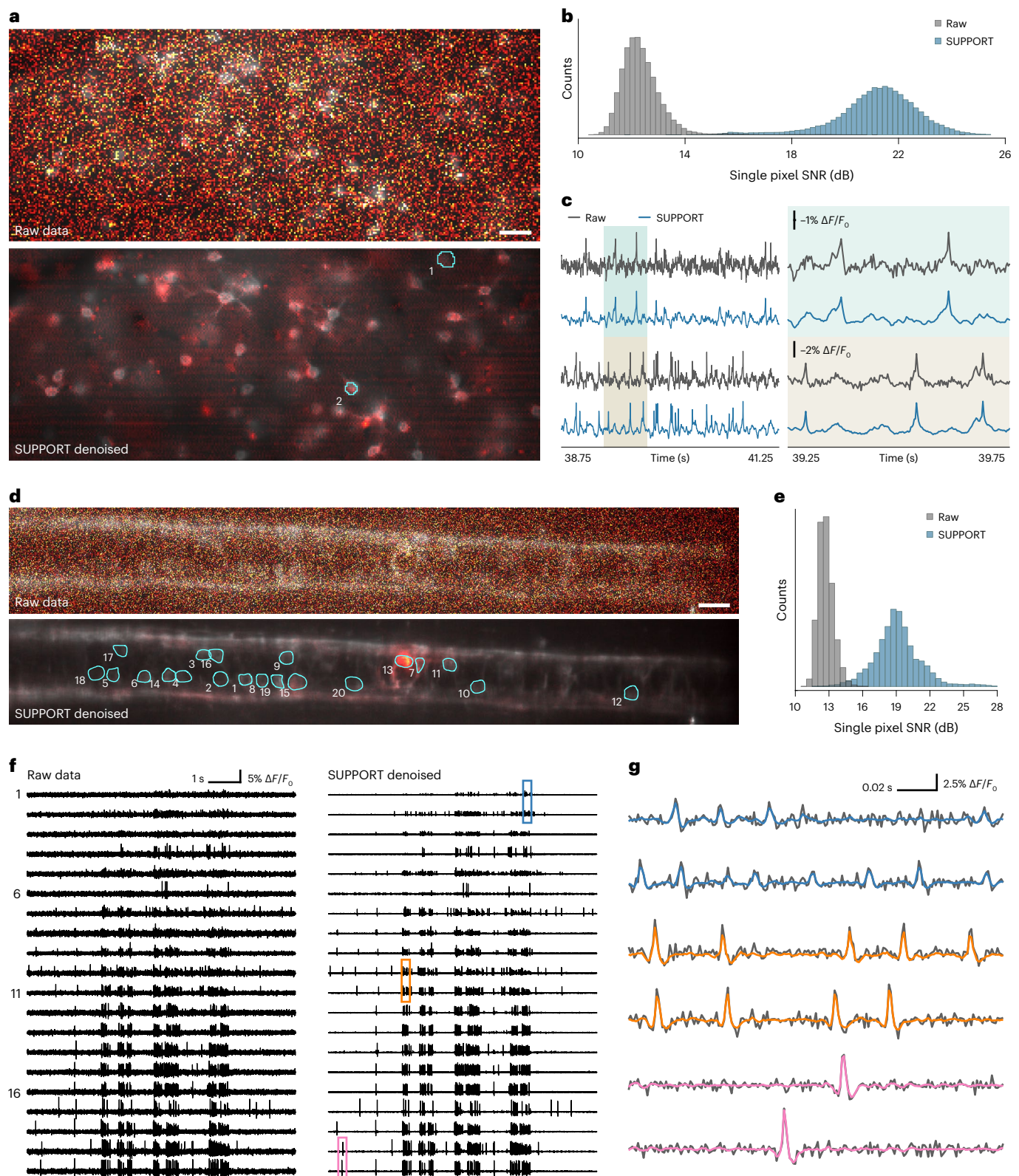


Fig. 5 | Denoising population voltage imaging data. a, Images after baseline correction from mouse dataset. The top shows the baseline-corrected raw data. The bottom shows the baseline-corrected SUPPORT-denoised data. Boundaries of two ROI are drawn with cyan lines. Scale bar, 40 μm . **b**, Distribution of the SNR for all pixels from raw and SUPPORT-denoised data after baseline correction, $n = 65,536$. **c**, Traces from raw and SUPPORT-denoised data extracted from two ROI in **a**. Traces for the smaller temporal region are plotted on the right. The enlarged temporal region is colored blue and brown. **d**, Images from the zebrafish dataset. Baseline and activity components are decomposed from raw

data and SUPPORT-denoised data. The baseline component with a gray colormap and the activity component with a hot colormap are overlaid. Boundaries of 20 ROI are drawn with cyan lines. The top shows raw data. The bottom shows SUPPORT-denoised data. Scale bar, 20 μm . **e**, Distribution of SNR for pixels inside the ROI from raw and SUPPORT-denoised data, $n = 5,722$. **f**, Traces for 20 ROI from raw and SUPPORT-denoised data. The left shows raw data. The right shows SUPPORT-denoised data. **g**, Enlarged view of traces from colored regions in **f** is plotted. Traces from raw data are overlaid with a gray color and denoised data are overlaid with corresponding color in **f**.

voltage dye, which indicates its suitability for designing voltage indicators (Supplementary Fig. 16).

Denoising population voltage imaging data

We applied SUPPORT to voltage imaging data that contained *in vivo* population neuronal activity in awake mouse cortex layer 1 expressing Voltron1 (ref. 24) and zebrafish spinal cord expressing zArchon1 (ref. 27). The mouse dataset was recorded with a wide-field fluorescence microscope with a frame rate of 400 Hz, and the zebrafish dataset was recorded with a light-sheet fluorescence microscope with a frame rate of 1 kHz (ref. 36).

After applying SUPPORT to the voltage imaging data, we applied baseline correction (Methods). Despite the high noise level of the voltage imaging data, the neuronal structures became clearly visible after denoising (Fig. 5a,d, Supplementary Video 4 and Supplementary Fig. 17). The single-pixel SNR was improved by 9.11 dB on average (21.58 ± 1.62 dB for SUPPORT, 12.47 ± 0.89 dB for the raw data) for the mouse dataset (Figs. 5b) and 6.32 dB (19.08 ± 2.07 dB for SUPPORT, 12.72 ± 0.67 dB for the raw data) for the zebrafish dataset (Fig. 5e). For further analysis, we extracted the voltage traces from manually drawn regions of interest (ROI) (Fig. 5c,f,g). In line with the results from the simulation and the single-neuron voltage imaging, the variance was greatly decreased, while the sharp voltage transients induced by spikes were preserved (Supplementary Figs. 18–40).

We also extracted the neurons and corresponding temporal signals using localNMF³⁶, which is an automated cell extraction algorithm, from the mouse and zebrafish datasets (Methods and Supplementary Fig. 41a,b). Owing to the improvement in SNR, we were able to automatically segment 42 neurons from the denoised mouse data compared to 31 neurons from the raw data. For zebrafish data, 27 neurons from the denoised data and nine neurons from the raw data were extracted. We then measured the F_1 score between the ground-truth ROI and the extracted ROI across several intersection-over-union (IoU) threshold values. We quantified the area under F_1 score across the IoU curve, and there was a 1.6-fold improvement for mouse data (0.31 for denoised and 0.19 for raw data) and a 2.0-fold improvement for zebrafish data (0.43 for denoised and 0.21 for raw data) (Supplementary Fig. 41c). The extracted neuronal signal from SUPPORT also clearly shows spikes, while the signal from the raw data shows high variance (Supplementary Fig. 41d), which indicates that SUPPORT facilitates the automated analysis of large-scale population voltage imaging data.

It was shown that SUPPORT could denoise other population voltage imaging data with different regions and voltage indicators, indicating its suitability for the routine use of population voltage recordings (Supplementary Figs. 18–40, 42 and 43). Finally, we observed that SUPPORT trained on single population voltage imaging data accurately denoised another population voltage imaging data without fine-tuning (Supplementary Fig. 44), which demonstrates its generalizability.

Denoising voltage imaging data with motion

The signal model of SUPPORT does not assume that objects in the images remain stationary, which allows for the possibility of denoising image data with motion. To verify this, we applied SUPPORT to synthetic, semisynthetic and experimental voltage imaging datasets with motion.

We first applied random rigid translation to the synthetic datasets generated using a NAOMi simulator as described in the previous section. The translation profile was created by drawing a sequence of random numbers from a zero-mean Gaussian distribution and filtering the sequence with a low-pass filter with a cut-off frequency of 5 Hz to mimic the motion induced by respiration and heartbeat. Subsequently, we applied SUPPORT to the dataset for denoising (Supplementary Figs. 45 and 46). The traces extracted from the SUPPORT-denoised video showed reduced variance while maintaining the spikes (Supplementary Fig. 45d). Quantitatively, the SUPPORT-denoised image

showed an improvement of 6.95 dB in the average SNR (31.23 ± 1.85 dB) compared to the noisy image (24.28 ± 0.02 dB), when motion on a scale larger than the size of the cell body was present (Supplementary Fig. 45e). Additionally, the root-mean-squared error (r.m.s.e.) was lowered by 0.0087 for the SUPPORT-denoised image (0.0074 ± 0.0014) compared to the noisy image ($0.0161 \pm 3.38 \times 10^{-5}$) (Supplementary Fig. 45f). We also found that altering the sequence of preprocessing steps (motion correction, photobleaching correction and SUPPORT) did not significantly affect the results (Supplementary Fig. 47).

Next, we applied random rigid translation, identical to that applied to the synthetic data, to the aforementioned *in vivo* single-neuron voltage imaging data with simultaneous electrophysiological recordings (Fig. 6a–c). We then applied SUPPORT for denoising and aligned the results for motion correction. The outcome was visually indistinguishable from the results obtained by applying SUPPORT to the motionless data (Fig. 6d).

Quantitatively, using simultaneously recorded electrophysiological recordings as ground truth, the SUPPORT-denoised image with motion on a scale comparable to the cell body size showed a substantial improvement of 0.46 in the average Pearson correlation coefficient (0.75 ± 0.12) compared to the raw image (0.29 ± 0.12) (Fig. 6e). Similarly, when using SUPPORT-denoised data without motion as ground truth, the average Pearson correlation coefficient showed an improvement of 0.57 for the SUPPORT-denoised image (0.95 ± 0.05) compared to the raw image (0.38 ± 0.19) (Fig. 6f). Additionally, the SNR was enhanced by 17.04 dB for the SUPPORT-denoised image (40.05 ± 0.44 dB) compared to the raw image (23.01 ± 0.51 dB) (Fig. 6g).

Finally, we evaluated SUPPORT using a voltage imaging dataset obtained from an awake mouse hippocampus expressing SomArchon³⁷ (Fig. 6h). This dataset contained natural motion with a scale comparable to the size of the cell body (Fig. 6i,j). Consistent with the findings from the synthetic and semisynthetic datasets, the variance was substantially reduced, while maintaining the distinct voltage transients associated with spikes (Fig. 6k). Furthermore, the single-pixel SNR showed an average improvement of 3.40 dB (17.30 ± 1.38 dB for SUPPORT, 13.90 ± 0.86 dB for the raw data) (Fig. 6l).

SUPPORT denoises imaging data of freely moving *C. elegans*

To assess the broad applicability of SUPPORT, we tested its capability to denoise three-dimensional time-lapse fluorescence microscopy images of *C. elegans*³⁸, in which the differences among the frames came from the motion of the worm, which was not sampled with a sufficiently high imaging speed. The nuclei of all neurons in the worm were labeled using red fluorescent protein mCherry³⁹ under the H20 promoter. The volume images with 20 axial slices were recorded with spinning disk confocal microscopy at a volume rate of 4.75 Hz.

We denoised the video using SUPPORT, DeepCAD-RT and PMD in a plane-by-plane manner. We first compared the noisy data and the denoised results for a single axial slice. SUPPORT successfully denoised the images without any visible artifacts, whereas the denoising outcomes acquired using DeepCAD-RT and PMD suffered from motion-induced artifacts (Extended Data Fig. 1a and Supplementary Fig. 48), which again proves the importance of using an appropriate signal model for denoising. The difference between the SUPPORT output and the noisy input, which was expected to be white noise, did appear purely white. However, the difference between the outputs from DeepCAD-RT and PMD and the noisy input contains low frequency components that are highly correlated with the structure of the input image (Extended Data Fig. 1b).

In the consecutive frames shown in Extended Data Fig. 1c, the worm's locomotion is considerably faster than the imaging speed, which precludes the accurate prediction of the current frame based on adjacent frames. Nevertheless, SUPPORT successfully denoised the image without suffering from motion artifacts by incorporating information from neighboring pixels in the current frame.

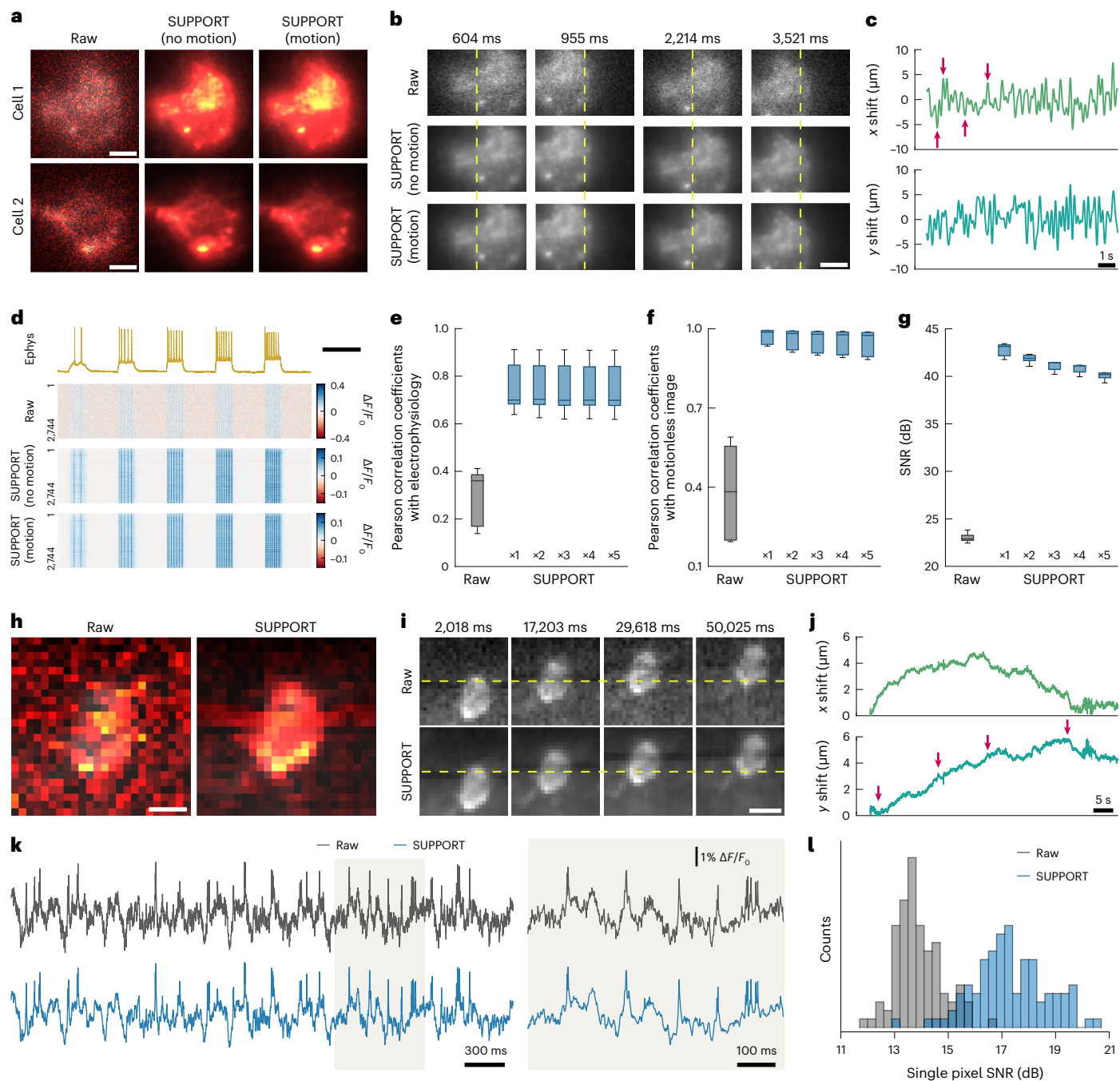


Fig. 6 | Denoising voltage imaging data with motion. **a**, Representative frames of raw video and SUPPORT-denoised videos without and with motion after baseline correction. Motion was synthetically applied to the images of neurons in mouse cortex L2/3 expressing QuasAr6a, simultaneously recorded with electrophysiology. Scale bars, 5 μm . **b**, Representative frames of a spatially expanded view of cell 1 in **a** at the timings indicated by red arrows in **c**. From left to right: frames at 604, 955, 2,214 and 3,521 ms. From top to bottom: raw video, SUPPORT-denoised video without motion and SUPPORT-denoised video with motion. Scale bar, 5 μm . **c**, Line plot showing the x and y direction motions in the micrometer scale. **d**, Electrophysiology trace and single-pixel fluorescence traces extracted from the videos. From top to bottom: electrophysiology, raw video, SUPPORT-denoised video without motion and SUPPORT-denoised video with motion. Scale bar, 500 ms. **e**, Box-and-whisker plot showing Pearson correlation coefficients between fluorescence traces and electrophysiology, before and after denoising. $\times 5$ indicates a five times higher motion compared to $\times 1$. $n = 5$,

which represents the number of cells. **f**, Box-and-whisker plot showing Pearson correlation coefficients between ground-truth image (SUPPORT-denoised image without motion) and images with motion before and after denoising. $n = 5$, which represents the number of cells. **g**, Box-and-whisker plot showing SNR acquired by comparing ground-truth image and images with motion before and after denoising. $n = 5$, which represents the number of cells. **h**, Representative frames of raw video and SUPPORT-denoised videos after baseline correction. The images show a neuron expressing SomArchon in the hippocampus of an awake mouse. Scale bar, 3 μm . **i**, Representative frames in **h** at the timings indicated by red arrows in **j**. From left to right: frames at 2,018, 17,203, 29,618 and 50,025 ms. Scale bar, 5 μm . **j**, Line plot showing x and y directional motions in the micrometer scale. **k**, Traces extracted from a single cell in raw video and SUPPORT-denoised video. Temporally expanded traces from the brown area on the left are shown on the right. **l**, Histogram of SNR from the raw video and SUPPORT-denoised video.

By contrast, DeepCAD-RT and PMD failed to predict the location of each cell, which was manifested as motion-induced artifacts in the images. The denoising outcome (Extended Data Fig. 1d, Supplementary Fig. 49 and Supplementary Video 5) demonstrates that SUPPORT can be used for denoising not only functional imaging data but also volumetric time-lapse images in which the speed of dynamics is faster than the imaging speed.

SUPPORT denoises volumetric structural imaging data

To demonstrate the generality of SUPPORT, we evaluated it on denoising volumetric structural imaging data in which no temporal redundancy could be exploited for denoising. SUPPORT was tested on two volumetric datasets that contained *Penicillium* imaged with confocal microscopy and mouse embryos imaged with expansion microscopy⁴⁰. *Penicillium* was imaged with two different recording settings to generate a pair of low-SNR and high-SNR volumes (Methods).

The volumetric images were denoised with SUPPORT regarding each z-stack as a time series. The qualitative analysis showed that SUPPORT was able to enhance the signal of volumetric structural imaging data, revealing the structures that were hidden by the noise (Extended Data Fig. 2a,b,e,f, Supplementary Fig. 50 and Supplementary Video 6). The fine structure of *Penicillium* was recovered with SUPPORT (Extended Data Fig. 2d), demonstrating the signal model's capability to learn statistics from a wide range of data. For the quantitative evaluation of SUPPORT with the *Penicillium* dataset, the Pearson correlation coefficients and SNR were measured by regarding the high-SNR image as a ground truth for each plane along the z axis (Extended Data Fig. 2c). The average Pearson correlation coefficient of SUPPORT (0.76 ± 0.07) showed 0.29 increments compared to the low-SNR image (0.47 ± 0.09) and the average SNR of SUPPORT (8.65 ± 0.62 dB) showed 5.98 dB increments compared to the low-SNR image (2.67 ± 0.51 dB). The qualitative and quantitative studies showed that SUPPORT is capable of enhancing not only time-lapse images but also static volumetric images. Thus, SUPPORT can be used in a wide range of biological research involving microscopic imaging.

Discussion

SUPPORT, a self-supervised denoising method, has demonstrated its ability to denoise diverse voltage imaging datasets acquired using Voltron1, Voltron2, paQuasAr3-s, QuasAr6a, zArchon1, SomArchon and BeRST1 (Supplementary Table 1). Thanks to its statistical prediction model that predicts a pixel value $x_{i,k}$ by integrating the information from its spatiotemporal neighboring pixels $\Omega_{i,k}$ (that is, $\hat{x}_{i,k} = f_{\theta}(\Omega_{i,k})$), it showed high robustness when faced with the fast dynamics in the scene. While this design allows SUPPORT to simultaneously achieve low bias and low variance, it still leaves room for fundamental improvement, as it does not exploit the information contained in $y_{i,k}$. The reason $y_{i,k}$ was not exploited as an input is because it is used as the target in place of the ground truth for self-supervised learning; we cannot use $y_{i,k}$ as both the input and the target of the network, as the network will simply become an identity function. This means that the cost of truly exploiting all available information is to give up the self-supervised learning scheme that does not require ground truth.

It should be noted that SUPPORT is specifically designed to remove zero-mean 'stochastic' noise, which includes Poisson noise and Gaussian noise originating from photons, dark current and sensor readout. However, it is not capable of addressing 'deterministic' artifacts such as motion-induced artifacts, photobleaching or fixed-pattern noise. As a result, a specifically designed data processing pipeline is needed to process data containing such artifacts (Supplementary Fig. 47).

Denoising time-lapse imaging data in which a *C. elegans* exhibited rapid movement and a single volumetric image demonstrated that SUPPORT is not limited to denoising voltage imaging data; it can be used for denoising any form of time-lapse imaging data (Supplementary Figs. 51–58 and Supplementary Videos 7–9) including calcium

imaging in which the imaging speed is slow compared to the underlying dynamics or volumetric structural imaging data. This is an important finding, as it indicates that the data do not need to be low rank to be denoised using SUPPORT, which is often required by many denoising algorithms^{6,41}. Also, SUPPORT could be trained with only 3,000 frames (Supplementary Figs. 59 and 60), which would facilitate its general usage in many laboratories with common desktop settings, especially with our GUI-based SUPPORT (Supplementary Fig. 61). We also note that the performance of SUPPORT comes at the typical computational cost of 2 days of training time with an NVIDIA RTX 3090 GPU. Overall, its self-supervised learning scheme, robustness to fast dynamics, low variance in denoising outcomes and compatibility with motion make it a versatile tool for processing a wide range of image data. We expect that SUPPORT's core strategy, learning the statistical relationships between neighboring entities in an n -dimensional array, will extend beyond image denoising and be adapted to process a broader range of biological data.

Online content

Any methods, additional references, Nature Portfolio reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at <https://doi.org/10.1038/s41592-023-02005-8>.

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Methods

SUPPORT network architecture

The architecture of the SUPPORT network consists of two subnetworks: two-dimensional (2D) U-Net and the blind spot network. 2D U-Net exploits the information of the temporally adjacent frames. The input data are first separated into two blocks: (1) temporal neighboring frames and (2) the center frame. The temporal neighboring frames are concatenated in the channel dimension and passed through 2D U-Net. Then, the center frame and the output of 2D U-Net are concatenated in the channel dimension and passed through the blind spot network, which has a zero at the center of the impulse response. Finally, the outputs of 2D U-Net and the blind spot network are concatenated in the channel dimension and passed through 1×1 convolution layers. The overall architecture is illustrated in Supplementary Fig. 1a.

The 2D U-Net³¹ consists of a 2D encoder, a 2D decoder and skip connections from the encoder to the decoder (Supplementary Fig. 1b). In the 2D encoder, there are four encoder blocks. Each block consists of a $3(x) \times 3(y)$ convolutional layer, followed by a BatchNorm, a LeakyReLU and a $2(x) \times 2(y)$ maximum pooling layer. In the decoder, there are four decoder blocks, each of which contains a bilinear interpolation followed by a $3(x) \times 3(y)$ convolutional layer, a BatchNorm and a LeakyReLU. The skip connections link low- and high-level features by concatenating feature maps in the channel dimension. We designed 2D U-Net to take the previous 30 frames and next 30 frames as the input. For denoising structural imaging data, the previous ten frames and next ten frames were used as the input.

The blind spot network was designed to efficiently increase the receptive field of the network over computation (that is, memory and the number of multiply-add operations). A comparison to previous blind spot network designs^{20,30} is shown in Supplementary Fig. 2. The blind spot network consists of (1) two sequential parts and (2) an aggregating part (Supplementary Fig. 1c). There are two sequential paths that use convolutional layers with kernel sizes of 3×3 and 5×5 . Each sequential path consists of sequential blind spot convolutional layers with ‘shortcut connections’ (Supplementary Fig. 1c). The center value of the weight of the blind spot convolutional layer is masked as 0 to make the blind spot property. For the kernel size of 3×3 , the dilation and padding are both set as 2^i for the i th layer to preserve blind spot properties for each feature after the layer. Similarly, for the kernel size of 5×5 , the padding and dilation are set as 2×3^i . The shortcut connection links the input to the features by adding the input, passed by the 1×1 convolutional layer, to the intermediate features. In the aggregating path, all features after each layer in the sequential paths are concatenated in the channel dimension and then passed through three 1×1 convolutional layers to finally predict the signal. The receptive field of the blind spot network is illustrated in Fig. 1b, which shows the fractal-like pattern.

For the data in which structured noise can be predicted from the neighboring pixels, options to change the size of the blind spot were also implemented (Supplementary Fig. 51). To increase the size of the blind spot to p , we added additional dilation and padding of $\lfloor p/2 \rfloor$ for the last blind spot convolutional layers of two sequential paths. Also, only the final features of two sequential paths, rather than all intermediate features, were passed through the aggregating path. Overall, we adhered to the default network architecture (Supplementary Fig. 1) except for the following instances (Supplementary Table 2):

- (1) For structural imaging dataset, we reduced the size of temporal (or ‘axial’) receptive field to 21 due to the limited availability of the axial slices.
- (2) For dataset with motion, we increased the network capacity by multiplying the number of channels in the U-Net by a factor of four.
- (3) For dataset with correlated noise on neighboring pixels, we increased the size of the blind spot.

Training SUPPORT network

The network was trained on Pytorch 1.12.1 and CUDA 11.3 with an NVIDIA RTX 3090 GPU and an Intel Xeon Silver 4212R CPU. For the loss function, the arithmetic average of L1-loss and L2-loss was used. As a preprocessing step, each input video was normalized by subtracting the average value and dividing by the standard deviation. Patches with a size of $128(x) \times 128(y) \times 61(t)$ were extracted from the input video with an overlap of $61(x) \times 61(y) \times 1(t)$. If the spatial dimension of the data was smaller than 128, we reduced the patch size to match the spatial dimension of the data. Then, random flipping and rotation by integer multiples of 90° were used for data augmentation. A batch size of 16 was used by default. An Adam optimizer⁴² with a learning rate of 5×10^{-4} without weight decay was used for gradient-based optimization. To ensure reproducibility, random seeds for all relevant libraries, NumPy and PyTorch, were fixed at 0. The network was trained for 500 epochs, with each epoch containing a loop through all patches by default. The loss values were tracked for every gradient update to monitor the training procedure. Training SUPPORT for processing the zebrafish dataset that had a size of $1,024(x) \times 148(y) \times 24,000(t)$ took 47 h for 14 million gradient updates. The inference for the same dataset took 30 min. We note that overfitting was avoided by using 1,500 or more frames and training the network over an extended period did not lead to overfitting (Supplementary Figs. 60 and 62). For both training and inference, we used zero padding to match the input and output sizes, which had minimal impact on the results (Supplementary Fig. 63).

The dependency of the denoising performance and loss function was investigated through denoising simulation and experimental data. The weighted average of L1 and L2 loss, $\mathcal{L} = \alpha \mathcal{L}_1 + (1 - \alpha) \mathcal{L}_2$, with $\alpha \in \{0, 0.3, 0.5, 0.7, 1\}$ for simulated data and $\alpha \in \{0, 0.5, 1\}$ for experimental data were used as a loss function (Supplementary Figs. 64 and 65).

Synthetic voltage imaging data generation

Simulating synthetic voltage imaging data includes the pipeline of first generating clean video (ground truth) and then adding Poisson and Gaussian noise. To generate a realistic spatial profile that resembles neurons in a mouse brain, we used a NAOMI³² simulator that was originally developed for simulating a two-photon calcium imaging dataset. The code was modified to generate voltage transients instead of calcium transients as temporal components. We generated five different videos with 15,000 frames and a frame rate of 500 Hz with different spike widths, ranging from 1 to 9 ms. The constructed voltage signals were matched to the parameters of Voltron. Every other parameter was set as default apart from increasing the simulated field of view twofold. The noisy video was generated by adding Poisson and Gaussian noise. To add Poisson noise to the images, we first normalized the input images and multiplied them by 1,000, and then used each pixel value as the parameter (that is, mean value) of the Poisson distribution. Thereafter, Gaussian noise with a mean of 0 and a standard deviation of 5 was added to the images. Finally, negative values were truncated to 0.

In vivo simultaneous voltage imaging and electrophysiology

The data from simultaneous structured illumination fluorescence imaging and patch-clamp electrophysiological recordings of single-neuron activity were recorded with mouse cortex L2/3 pyramidal neurons using a digital micromirror device or spatial light modulator with a frame rate of 1,000 Hz. Voltron2 and QuasAr6a were expressed using in utero electroporation. NDNF-Cre± mice (JAX catalog no. 028536) of 6 weeks to 8 months were used for in vivo QuasAr6 voltage imaging. All procedures involving animals were in accordance with the National Institutes of Health guide for the care and use of laboratory animals and were approved by the Institutional Animal Care and Use Committee (IACUC) at Harvard University.

In vitro single-neuron voltage recording

We prepared primary rat hippocampal neurons cultured on a 35 mm glass bottom dish (P35G-1.5-14-C, MatTek). At 9 days in vitro, neurons were stained with a voltage-sensitive dye (BeRST1, 2 μM) dissolved in an imaging solution containing 140 mM NaCl, 3 mM KCl, 3 mM CaCl_2 , 1 mM MgCl_2 , 10 mM HEPES and 30 mM glucose (pH 7.3) for 15 min, and then rinsed with a fresh imaging solution before optical imaging²⁸. Time-lapse imaging of spontaneous neural activity was acquired using an inverted microscope (Eclipse Ti2, Nikon) equipped with a $\times 40$ water-immersion objective lens (numerical aperture (NA) 1.15; MRD7710, Nikon), while maintaining the sample temperature at 30 °C. For excitation, an LED (SOLIS-623C, Thorlabs) with a band-pass filter (ET630/20x, Chroma Technology) was used at an irradiance of 20 mW mm^{-2} at the sample. Emission was passed through a dichroic mirror (T660lpxr, Chroma Technology) and an emission filter (ET665lp, Chroma Technology), and was collected by an sCMOS camera (Orca Flash v.4.0, Hamamatsu) at a 1-kHz frame rate with 4×4 binning and subarray readout (361×28 pixels) for a duration of 25 s. All the animal experiments were performed according to the Institute of Animal Care and Use Committee guidelines of Seoul National University (Seoul, Korea) (SNU-220616-1-2).

In vivo simultaneous calcium imaging and electrophysiology

A craniotomy over V1 was performed, and neurons were infected with adeno-associated virus (AAV2/1-hSynapsin-1) encoding jGCaMP8f. At 18–80 days after the virus injection, the mouse was anesthetized, the cranial window was surgically removed and a durotomy was performed. The craniotomy was filled with 10–15 μl of 1.5% agarose, and a D-shaped coverslip was secured on top to suppress brain motion and leave access to the brain on the lateral side of the craniotomy. The mice were then lightly anesthetized and mounted under a custom two-photon microscope. Two-photon imaging (122 Hz) was performed of L2/3 somata and neuropil combined with a loose-seal, cell-attached electrophysiological recording of a single neuron in the field of view. Temporally fourfold downsampling was held to the data to reduce the sampling rate before the analysis. After excluding some outlier recordings with a low correlation between calcium signal and action potentials, an ROI was manually drawn around the neuron, and fluorescence traces were extracted from the mean signal of the ROI in the temporal stack. All surgical and experimental procedures were conducted in accordance with protocols approved by the IACUC and Institutional Biosafety Committee of Janelia Research Campus.

Volumetric structural imaging of *Penicillium*

For the volumetric structural imaging of *Penicillium*, the specimen was imaged using a point-scanning confocal microscopy system (NIS-Elements AR v5.11.01, C2 Plus, Nikon) equipped with a $\times 16$ 0.8 NA water dipping objective lens (CFI75 LWD 16X W, Nikon). The imaging was performed using a 488 nm excitation laser with a laser power of 0.075 mW for the low-SNR image and a laser power of 1.5 mW for the high-SNR image. The frame rate was 0.5 Hz for $1,024 \times 1,024$ pixels with a pixel size of 0.34 μm and each volume consisted of 1,000 z-slices with a z-step size of 0.1 μm .

Expansion microscopy of mouse embryos

Mouse embryos were isolated on day 15.5 of pregnancy in C57BL/6J mice and fixed with ice-cold fixative (4% paraformaldehyde in 1 \times phosphate buffered saline) for a day at 4 °C. Fixed mouse embryos were embedded in 6% (w/w) low-gelling-temperature agarose and then sliced to a thickness of 500 μm with a vibratome. Embryo slices were then processed for anchoring, gelation, Alexa Flour 488 NHS-ester staining, digestion, decalcification and expansion according to the previously described whole-body ExM protocol⁴⁰. Following a 4.1-fold expansion of the embryo slices in the hydrogel, the sample was attached to cover glass and imaged using a confocal microscope (Nikon Eclipse Ti2-E) with a

spinning disk confocal microscope (Fusion v.2.1.0.34, Dragonfly 200; Andor, Oxford Instruments) equipped with a Zyla 4.2 sCMOS camera (Andor, Oxford Instruments) and a $\times 10$ 0.45 NA air lens (Plan Apo Lambda, Nikon). The z-stack images were obtained with a z-step size of 1 μm for intestine and bone, and 0.5 μm for tail. All animal experiments involving mouse embryos conducted for this study were approved by the IACUC of KAIST (KA-2021-040).

In vivo calcium imaging of zebrafish brain

For zebrafish experiments, transgenic larval zebrafish (*Danio rerio*) expressing GCaMP7a calcium indicator under control of GAL4-UAS system and *huc* promoter (*Tg(huc:GAL4);Tg(UAS:GCaMP7a)*)^{43–45} with a *Casper* (*mitfa(w2/w2);mpv17(a9/a9)*)⁴⁶ mutant were imaged at 3–4 days postfertilization.

The larvae were paralyzed by bath incubation with 0.25 mg ml^{-1} of pancuronium bromide (Sigma-Aldrich) solution for 2 min (ref. 47). After paralysis, the larvae were embedded in agar using a 2% low melting point agarose (TopVision) in a Petri dish. The dish was filled with standard fish water after solidifying the agarose gel. Specimens were imaged using a point-scanning confocal microscopy system (NIS-Elements AR v.5.11.01, C2 Plus, Nikon) equipped with a $\times 16$ 0.8 NA water dipping objective lens (CFI75 LWD 16X W, Nikon). The imaging was performed using a 488 nm excitation laser (0.15–0.75 mW). All animal experiments involving zebrafish conducted for this study were approved by the IACUC of KAIST (KA-2021-125).

Imaging spontaneous neurotransmission

Primary cultures of rat hippocampal neurons were obtained from embryonic day 18 Sprague-Dawley fetal rats and plated onto glass coverslips that were precoated with poly-D-lysine. Neurons were transfected with SF.iGluSnFR A184V (Addgene catalog no. 106199) or iGABASnFR F102G (Addgene catalog no. 112160) using calcium-phosphate method, along with Synapsin1-mCherry to serve as a presynaptic bouton marker. Transfected hippocampal neurons at day 16 in vitro were placed in a perfusion chamber (Chamlide, LCI) and mounted onto the 35 °C heating stage of an inverted microscope (IX71, Olympus) equipped with a $\times 40$ oil-immersion objective lens (UPlanApo, $\times 40/1.00$). The imaging was conducted in Tyrode's solution (136 mM NaCl, 2.5 mM KCl, 2 mM CaCl_2 , 2 mM MgCl_2 , 10 mM glucose, 10 mM HEPES; pH 7.4; 285–290 mOsm) containing 1 μM tetrodotoxin to block action potential firing. A total of 12 trials were obtained, each consisting of 500 frames captured at a frame rate of 100 Hz (iGluSnFR) or 50 Hz (iGABASnFR), using an Andor Sona-2BV11 sCMOS camera (Andor) driven by MetaMorph Imaging Software (Molecular Devices) with a binning of 2 and a cropped mode of 110×110 pixels. All the animal experiments were performed according to the Institute of Animal Care and Use Committee guidelines of Seoul National University (SNU-220525-4).

Baseline and activity decomposition of voltage imaging data

For visualization, the data were decomposed into the underlying baseline and neuronal activity. The baseline estimation was performed using the temporal moving average. Window length was chosen in accordance with the recording rate for the data. For the data that only required photobleaching correction, b-spline fit was used to estimate baseline without using the moving average (Fig. 5a). For the positive-going voltage indicators (zArchon1, QuasAr6a, paQuasAr3-s, SomArchon), the activity component was acquired by subtracting the estimated baseline from the data. For the negative-going voltage indicators (Voltron1, Voltron2), the activity component was acquired by subtracting the data from the estimated baseline.

Spike detection for F_1 score calculation

To calculate spike detection accuracy, we measured the F_1 score for a given dF/F_0 threshold. The spikes were detected through the following steps: (1) calculating dF/F_0 from the fluorescence trace,

(2) hard-thresholding the dF/F_0 trace and (3) finding local maximum locations. The dF/F_0 threshold refers to the threshold value used in step (2). In simulated data, clean voltage traces were used to obtain ground-truth spike locations. In experimental data, electrophysiological recordings were used as the ground truth. For subthreshold analysis, we calculated Pearson correlation coefficients between electrophysiological recording and single-pixel voltage traces in the subthreshold regime.

Cell detection in neuronal populations imaging data

For voltage imaging data, the SGPMD-NMF pipeline was applied to detect ROI and corresponding temporal signals, which is available on GitHub (<https://github.com/adamcohenlab/invivo-imaging>). In the pipeline, detrending based on b-spline fitting and demixing based on localNMF were used without additional denoising. For the mouse cortex data, detrended data was flipped before the demixing step by subtracting the data from the maximum value of the data, since the data were recorded with Voltron1, which is a negative-going voltage indicator. After extraction, we removed nonneuronal spatial components with the following simple heuristics: (1) reject if the number of pixels in the component is smaller than α , (2) reject if the width or height of the component is larger than β and (3) reject if the width/height is not in (γ, δ) .

For mouse cortex data, only the first heuristic was used, with α set as 10, where the size of the neurons was small in the data. For zebrafish data, all heuristics were used, with $\alpha = 100$, $\beta = 50$, $\gamma = 0.5$ and $\delta = 1.5$ (Supplementary Fig. 41).

Cellpose⁴⁸ was applied to a single frame image of SUPPORT-denoised video to detect cells (Supplementary Fig. 55). All parameters were set to default except 'flow' and 'cellprob', which were set by empirical values that best fit the data.

Real-time intravital imaging in anesthetized mouse

H2B-GFP (Jackson Laboratory, Stock No. 006069) and mTmG (Jackson Laboratory, stock no. 007676) mice were purchased from the Jackson Laboratory. Flowing red blood cells in various tissues of the offspring of H2B-GFP crossbred with mTmG were imaged using a confocal and two-photon microscope (IVM-CMS, IVIM Technology Inc.). For real-time intravital imaging, mice were anesthetized using an intramuscular injection of a mixture of Zoletil (20 mg kg⁻¹) and Xylazine (11 mg kg⁻¹). Red blood cells fluorescently labeled by far-red fluorophore DiD (Thermo Fisher) were intravenously injected through the tail vein. To image ear skin, the right ear of the anesthetized mouse was gently attached to transparent coverslip with saline water^{49–51}. To image kidney, a 15 mm incision was made on both the skin and the retroperitoneum and then the kidney was gently exteriorized with round forceps. The exposed kidney surface was covered by transparent coverslip^{52,53}. A wet gauze soaked in warm saline was placed between the kidney and the underlying tissue to reduce motion artifacts^{54–56}. To image muscle, a 10 mm incision was made on thigh skin and then muscle was exposed and covered by transparent coverslip⁵⁷. A high NA water-immersion objective lens (CFI75 Apochromat 25XC W, NA 1.1, Nikon) was used, and 488, 561 and 640 nm lasers were used to excite green fluorescent protein (GFP), mT and DiD, respectively. All animal experiments involving live anesthetized mice conducted for this study were approved by the IACUC of KAIST (KA-2021-058, KA-2022-010).

Performance metrics

SNR, PSNR and r.m.s.e. were used as metrics to evaluate the pixel-level consistency between SUPPORT-denoised images and ground-truth images. The r.m.s.e. between the signal x and the reference signal y is defined as $\text{r.m.s.e.}(x, y) = \sqrt{\mathbb{E}[(x - y)^2]}$, where \mathbb{E} denotes the arithmetic mean. The SNR between the signal x and the reference signal y is defined as $\text{SNR}(x, y) = 10 \log_{10} \frac{\mathbb{E}[x^2]}{\text{r.m.s.e.}(x, y)^2}$. The PSNR between the signal x and the

reference signal y is defined as $\text{PSNR}(x, y) = 10 \log_{10} \frac{\max(x)^2}{\text{r.m.s.e.}(x, y)^2}$. The Pearson correlation between the signal x and the reference signal y is defined as $R = \frac{\mathbb{E}[(x - \mu_x)(y - \mu_y)]}{\sigma_x \sigma_y}$ where μ_x and μ_y are the mean values of signal x and y , respectively, and σ_x and σ_y are the standard deviations of signal x and y , respectively. As a measure of the performance of denoising experimental voltage imaging data, SNR was used with modification, as the ground truth was not available. The SNR of the signal x is defined as $\text{SNR}(x) = 10 \log_{10} \frac{\max(x)}{\sigma_x}$. Metrics were calculated after baseline correction.

For the population voltage imaging data, we measured the performance of cell extraction. After cell detection using localNMF, we first calculated IoU between all pairs of extracted components and manually segmented cells. The cell extraction was given as correct if the IoU between the extracted component and the manual segmentation was higher than the threshold. Then, the F₁ score (that is, harmonic mean of precision and recall) was calculated.

Comparison with other denoising algorithms

We used the publicly available implementations of PMD (<https://github.com/ikinsella/trefide>), DeepCAD-RT (<https://github.com/cabooster/DeepCAD-RT>), NOSA⁵⁸ (<https://github.com/DavideR2020/NOSA>) and Volpy⁵⁹ (<https://github.com/flatironinstitute/CalMan>).

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

The dataset of one-photon epifluorescence imaging with targeted illumination of QuasAr6a expressing mouse cortex L2/3 neurons simultaneously recorded with patch clamp can be downloaded from <https://zenodo.org/record/8176722>. The dataset of one-photon epifluorescence imaging with targeted illumination of Voltron2 expressing mouse cortex L2/3 neurons simultaneously recorded with patch clamp can be downloaded from <https://zenodo.org/record/8176722>. The dataset of wide-field fluorescence imaging of SomArchon expressing mouse hippocampus neurons can be downloaded from <https://zenodo.org/record/8176722>. The dataset of confocal imaging of volumetric structural imaging of *Penicillium* can be downloaded from <https://zenodo.org/record/8176722>. The dataset of confocal imaging of volumetric structural imaging of Alexa fluor 488 NHS-ester stained mouse embryos can be downloaded from <https://zenodo.org/record/8176722>. The dataset of in vivo single-neuron simultaneous calcium recording of jGCaMP8f and electrophysiology can be downloaded from the DANDI (<https://dandiarchive.org/dandiset/000168?search=jgcam8f&pos=1>). The dataset of confocal imaging of GCaMP7a expressing zebrafish neurons can be downloaded from (<https://zenodo.org/record/8176722>). The datasets from previous publications are publicly available, and the corresponding links can be found in each respective publication. Source data are provided with this paper.

Code availability

Code for Pytorch implementation of SUPPORT is available online at GitHub repository (<https://github.com/NICALab/SUPPORT>).

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Author contributions

M.E. and S.H. designed denoising algorithm. M.E. and S.H. designed and performed experiments and analyzed data. P.P. performed simultaneous electrophysiology and voltage imaging experiments with H.T. under supervision of A.E.C. G.K. performed calcium imaging data analysis. E.-S.C. performed confocal imaging of zebrafish and *Penicillium*. J.S. performed expansion microscopy imaging of mouse embryos under supervision of J.-B.C. K.-H.L. performed zebrafish experiments under supervision of C.-H.K. S.K. performed in vitro single-neuron voltage recording under supervision of M.C. U.L.B. performed population voltage imaging in zebrafish under supervision of A.E.C. E.L. and H.-a.T. performed voltage imaging of cardiac cells and population voltage imaging in mice under supervision of X.H. J.C. and S.E.L. performed intravital imaging in mice under supervision of P.K. S.H.R. performed spontaneous neurotransmission imaging under supervision of S.C. K.D.P. performed voltage imaging in mice. M.R. performed in vivo single-neuron simultaneous calcium recording and electrophysiology. M.E., S.H. and Y.-G.Y. wrote the manuscript with input from all authors. Y.-G.Y. conceived and led this work.

Competing interests

Y.-G.Y., M.E. and S.H. declare the following competing interests. Y.-G.Y., M.E. and S.H. are co-inventors on patent applications owned by KAIST covering SUPPORT (KR10-2023-0091724).

Additional information

Extended data is available for this paper at <https://doi.org/10.1038/s41592-023-02005-8>.

Supplementary information The online version contains supplementary material available at <https://doi.org/10.1038/s41592-023-02005-8>.

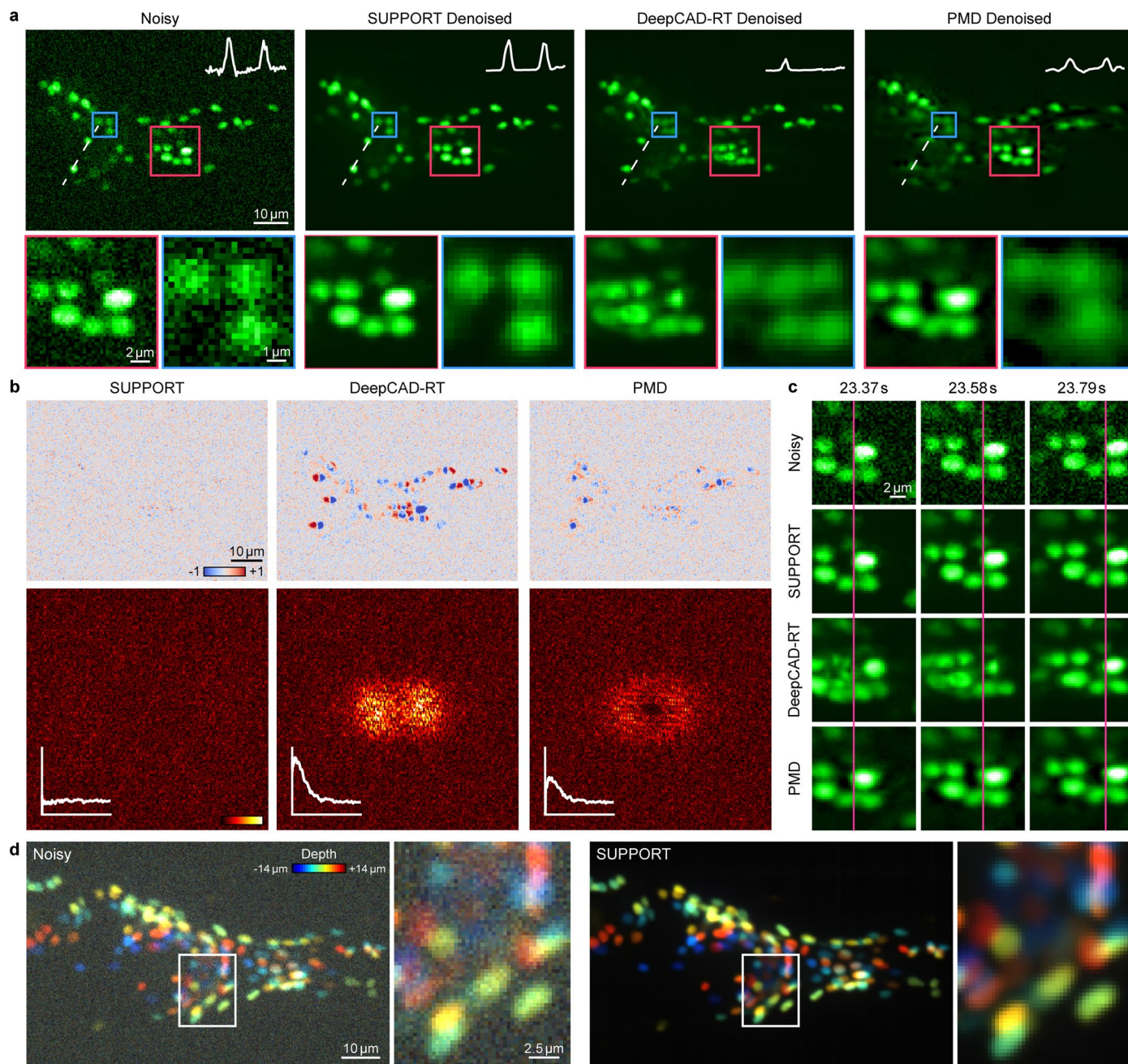
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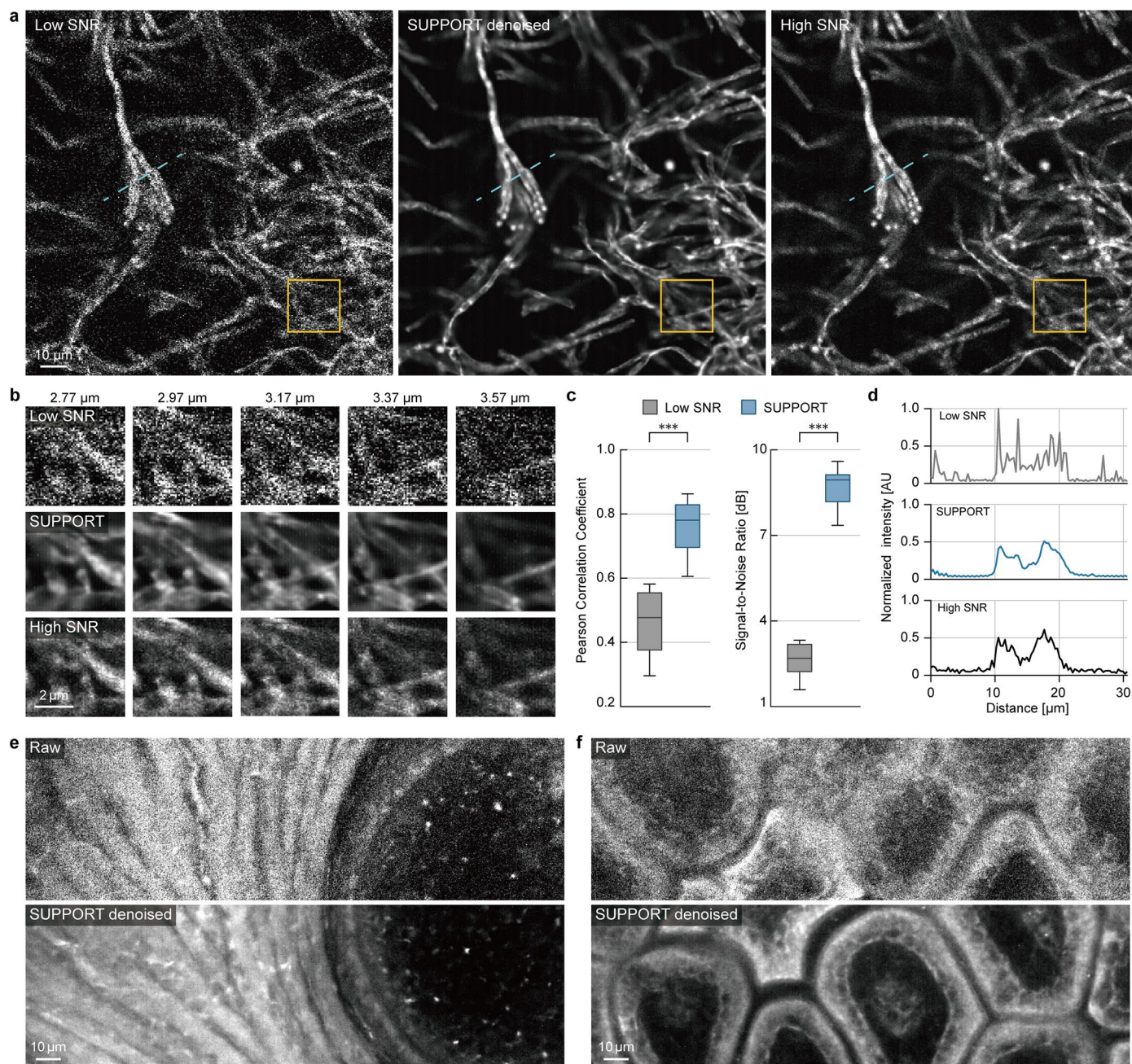
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Extended Data Fig. 1 | SUPPORT denoises freely moving *Caenorhabditis elegans* imaging data. **a**, Images of freely moving *C. elegans*. From left to right: Noisy, SUPPORT, DeepCAD-RT, and PMD denoised data. Inset shows the intensity profile along the dashed line. Magnified views of the boxed regions are presented underneath. **b**, Pixel-wise difference between denoised data and noisy data. Squared norm of Fourier transform of each difference are shown in the lower images. Inset shows the logarithm of the squared norm of Fourier transform

against the distance to the origin. **c**, Magnified views of the red boxed region in **a** at consecutive neighboring time points. Magenta lines were set on the left side of the brightest neuron in the noisy data. From top to bottom: Noisy, SUPPORT, DeepCAD-RT, and PMD denoised data. **d**, Noisy volume and denoised volume are depth coded and presented. Magnified views of the boxed regions are presented on the right.



Extended Data Fig. 2 | SUPPORT denoises volumetric structural imaging data. **a**, Representative axial slice from low-SNR, SUPPORT-denoised, high-SNR volumes of *Penicillium*. **b**, Magnified views of the yellow boxed region in **a** at multiple axial locations. Axial location of **a** corresponds to 3.37 μm . **c**, Box-and-whisker plot showing Pearson correlation coefficient and signal-to-noise ratio for axial slices. A two-sided paired-sample t-test is used, $N = 381$, which represents

the number of planes along the z-axis (***: p-value < 0.001). **d**, Intensity profiles of the cyan dashed line in **a**. **e**, Example frame of bone of a mouse embryo after expansion for the raw data (top) and denoised image using SUPPORT (bottom). **f**, Raw (top) and denoised image (bottom) of intestine of a mouse embryo. **e-f**, Length scales are presented in pre-expansion dimensions.

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|-------------------------------------|-----------------------------------------------------------------|
| n/a | <input type="checkbox"/> Involved in the study |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Antibodies |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Eukaryotic cell lines |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Palaeontology and archaeology |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> Animals and other organisms |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Clinical data |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Dual use research of concern |

Methods

- | | |
|-------------------------------------|-------------------------------------------------|
| n/a | <input type="checkbox"/> Involved in the study |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> ChIP-seq |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Flow cytometry |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> MRI-based neuroimaging |

Animals and other research organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research, and [Sex and Gender in Research](#)

Laboratory animals

CD-1 mice of 3-4 weeks were used for simultaneous patch clamp and voltage imaging in brain slices. NDNF-Cre+/- (JAX #028536) or PV-Cre+/- (JAX #017320) of 6 weeks - 8 months were used for in vivo QuasAr6 voltage imaging. Mice were housed in standard conditions with a reverse 12-h light/dark cycle at 23°C and 40-60% humidity. Up to five mice were housed per cage after weaning, with water and food provided ad libitum.

Mouse embryos isolated on day 15.5 of pregnancy in C57BL/6J mice were used for volumetric structural imaging using expansion microscopy.

Rat embryos isolated on day 18 of pregnancy were used for imaging spontaneous neurotransmission.

Transgenic zebrafish larvae (Tg(huc:GAL4);Tg(UAS:GCaMP7a)) with Casper background at 3-4dpf were used for calcium imaging experiments. Zebrafish were maintained under standard conditions at 28°C and a 14:10 hour light:dark cycle.

H2B-GFP (Jackson Laboratory, Stock No. 006069) and mTmG (Jackson Laboratory, Stock No. 007676) mice were purchased from the Jackson Laboratory (Bar Harbor, USA). The mice were housed in cages with independent ventilation, controlled temperature (22.5 °C), and humidity (52.5%). They were provided ad libitum access to a standard diet and water under a 12/12 hours light/dark cycle.

Mice for calcium imaging were cared for in compliance with the Guide for the Care and Use of Laboratory Animals. All experiments were approved by the Janelia Research Campus IACUC and IBC committees. Mice were housed on a free-standing, individually ventilated (approximately 60 air changes hourly) rack (Allentown). The holding room was ventilated with 100% outside filtered air with 15–20 air changes hourly. Each ventilated cage (Allentown) was provided with corncob bedding (Shepard Specialty Papers), at least 8g of nesting material (Bed-r’Nest, The Andersons) and red Mouse Tunnel (Bio-Serv). Mice were maintained on a 12:12-h light:dark cycle. The holding room temperature was maintained at 68–72 °F with a relative humidity of 30–70%. Irradiated rodent laboratory chow (LabDiet 5053) was provided ad libitum.

Wild animals

Not involved in this study.

Reporting on sex

(mice) Mice of both sexes were used without regard to sex in this study. (zebrafish larvae, mouse embryos, rat embryos) Sex is not specified at this developmental stage and was therefore not determined.

Field-collected samples

Not involved in this study.

Ethics oversight

All experimental methods involving mice and zebrafish were approved by the Korea Advanced Institute of Science and Technology Institutional Animal Care and Use Committee (KAIST-IACUC).

All of the animal experiments were performed according to the Institute of Animal Care and Use Committee guidelines of Seoul National University (Seoul, Korea).

All procedures involving animals were in accordance with the National Institutes of Health guide for the care and use of laboratory animals and were approved by the Institutional Animal Care and Use Committee at Harvard University (Harvard-IACUC).

All surgical and experimental procedures were conducted in accordance with protocols approved by the Institutional Animal Care and Use Committee (IACUC) and Institutional Biosafety Committee (IBC) of Janelia Research Campus.

Note that full information on the approval of the study protocol must also be provided in the manuscript.



Statistically unbiased prediction enables accurate denoising of voltage imaging data

In the format provided by the authors and unedited

1 **SUPPLEMENTARY INFORMATION**

2

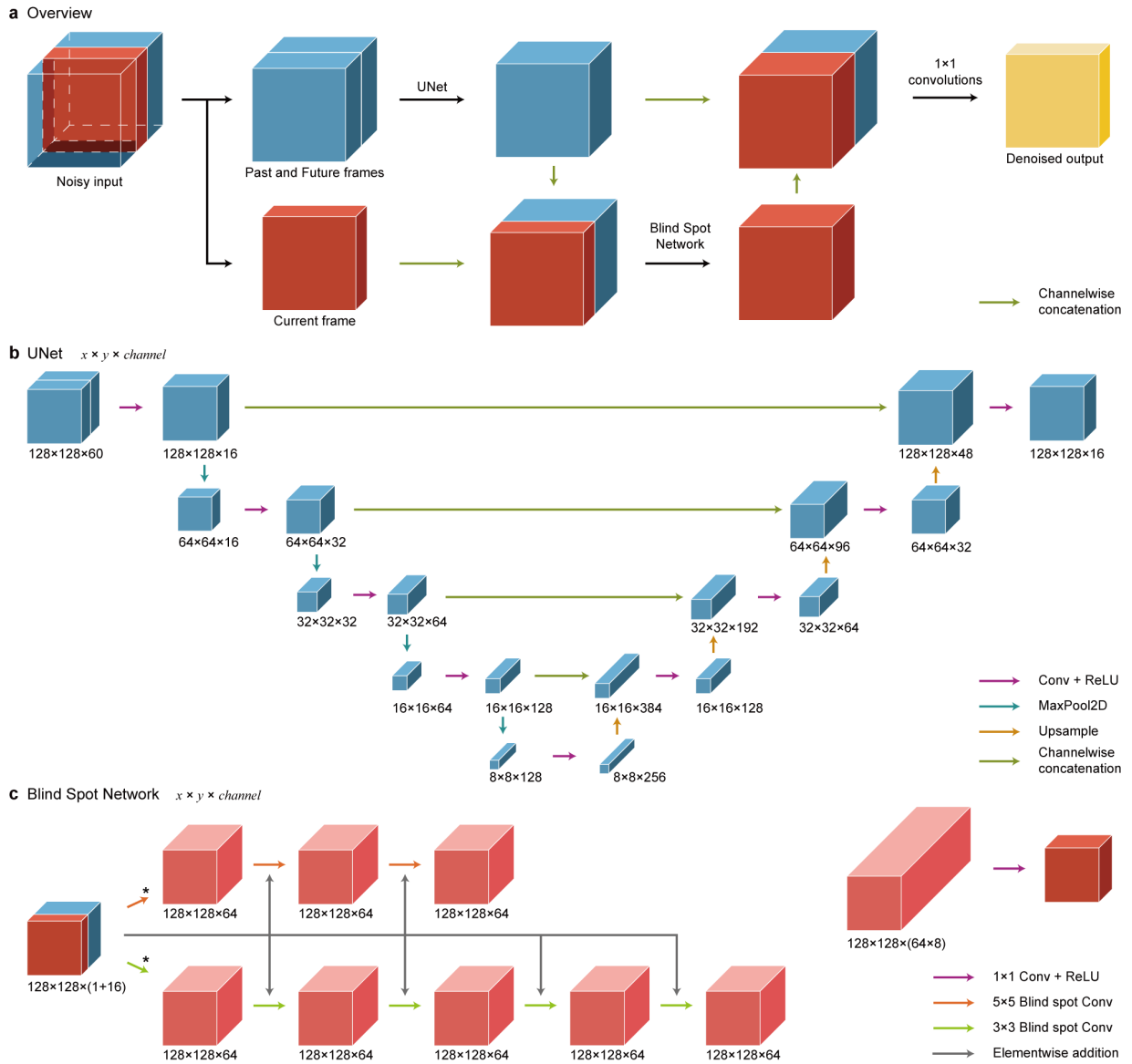
3 **Supplementary Figures 1–65**

4 **Supplementary Videos 1–9**

5 **Supplementary Tables 1-2**

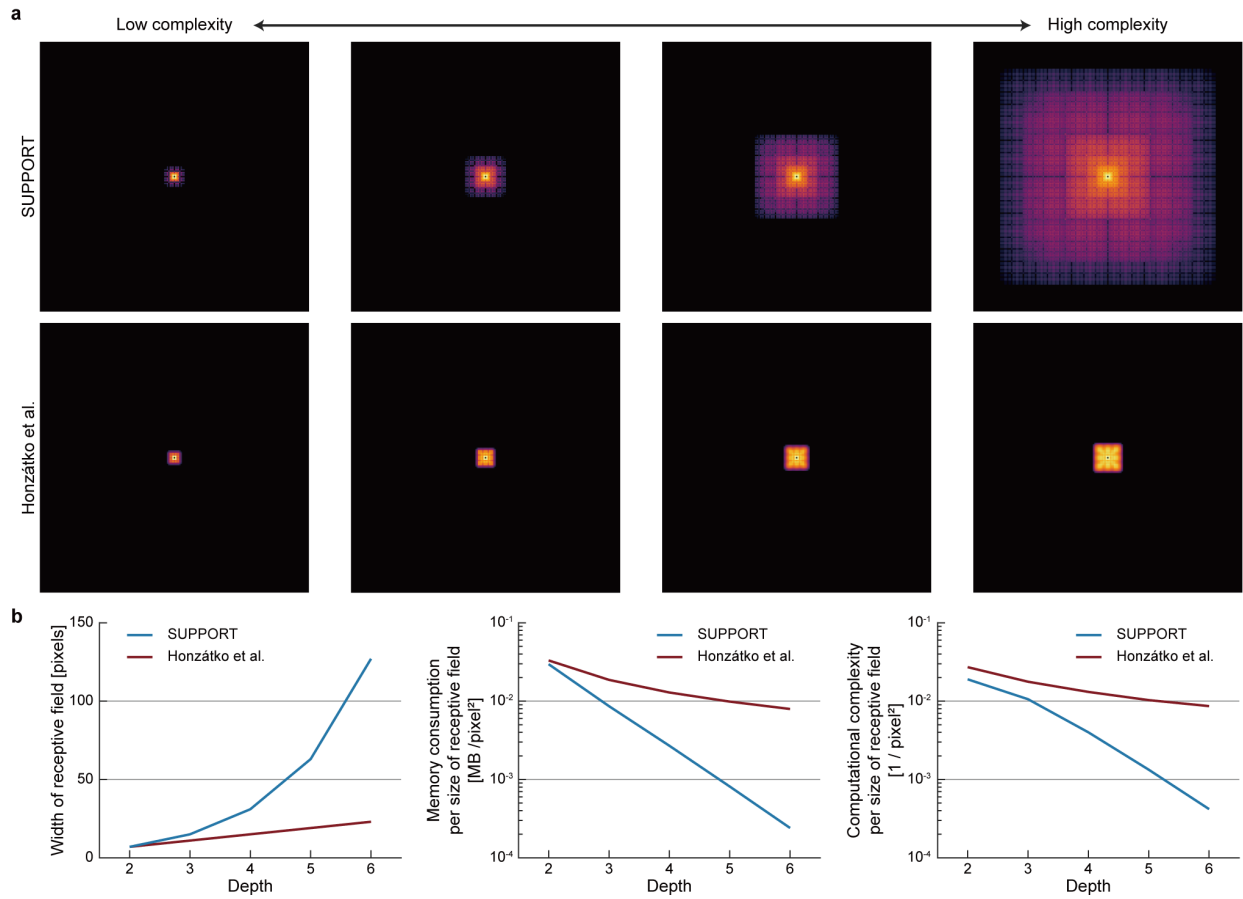
6 **References**

7 **SUPPLEMENTARY FIGURES**

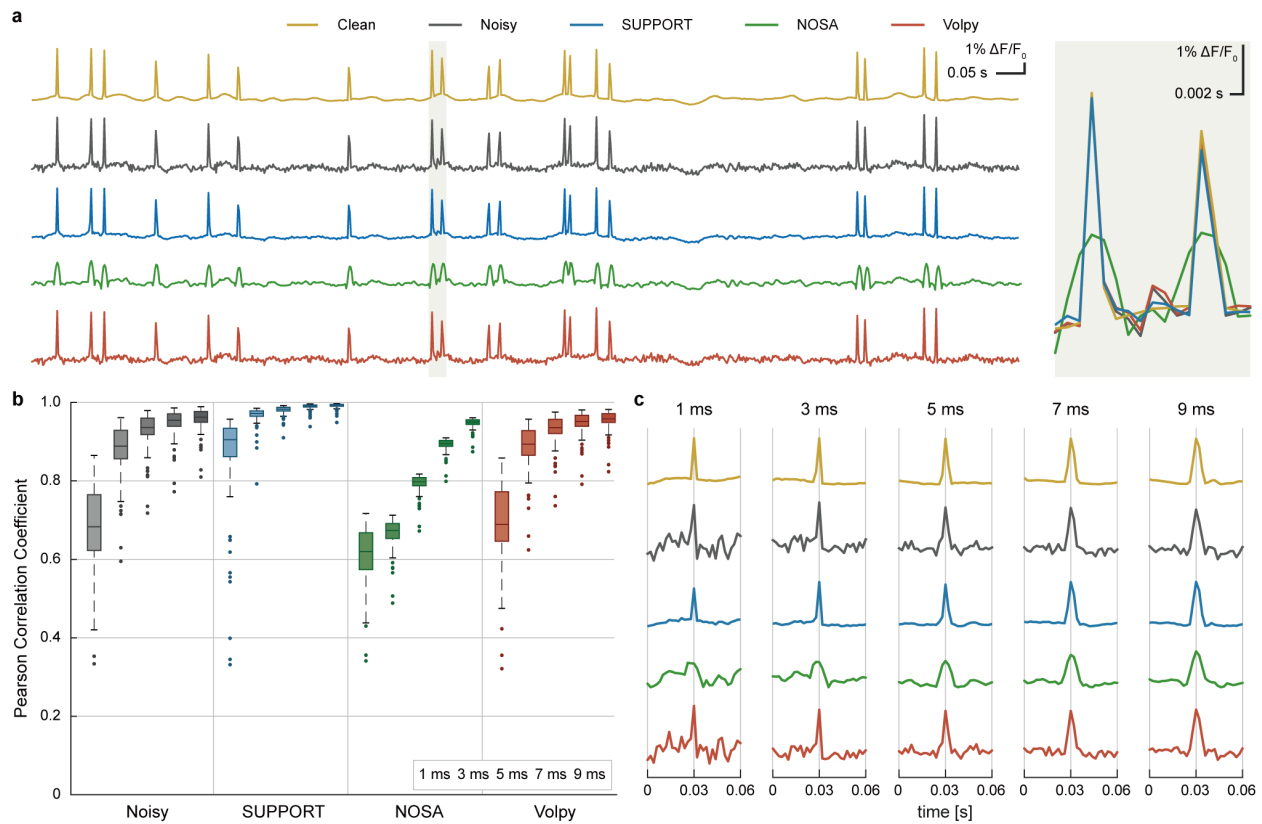


8

9 **Supplementary Fig. 1: Network architecture.** **a**, Overall architecture of the network. The network
 10 predicts the center value based on its spatiotemporally neighboring pixels. Pixels in past and future frames
 11 are processed by 2D U-Net, and spatially neighboring pixels in the current frame are processed by a blind
 12 spot network. **b**, 2D U-Net, which consists of a 2D encoder, a 2D decoder, and skip connections, processes
 13 the past and future frames. The past and future frames are channelwise concatenated and given to the 2D
 14 U-Net as input. **c**, The blind spot network consists of blind spot convolution layers, which are convolution
 15 layers with zero at the center of the kernels. In addition to the current frame, feature maps from the 2D U-
 16 Net, which encode the information in temporally neighboring frames, are channelwise concatenated and
 17 given as input to the blind spot network. (*) The first blind spot convolution layer has a blind spot property
 18 only in the first channel, which corresponds to the current frame. For the other layers, there are blind spots
 19 in all channels.

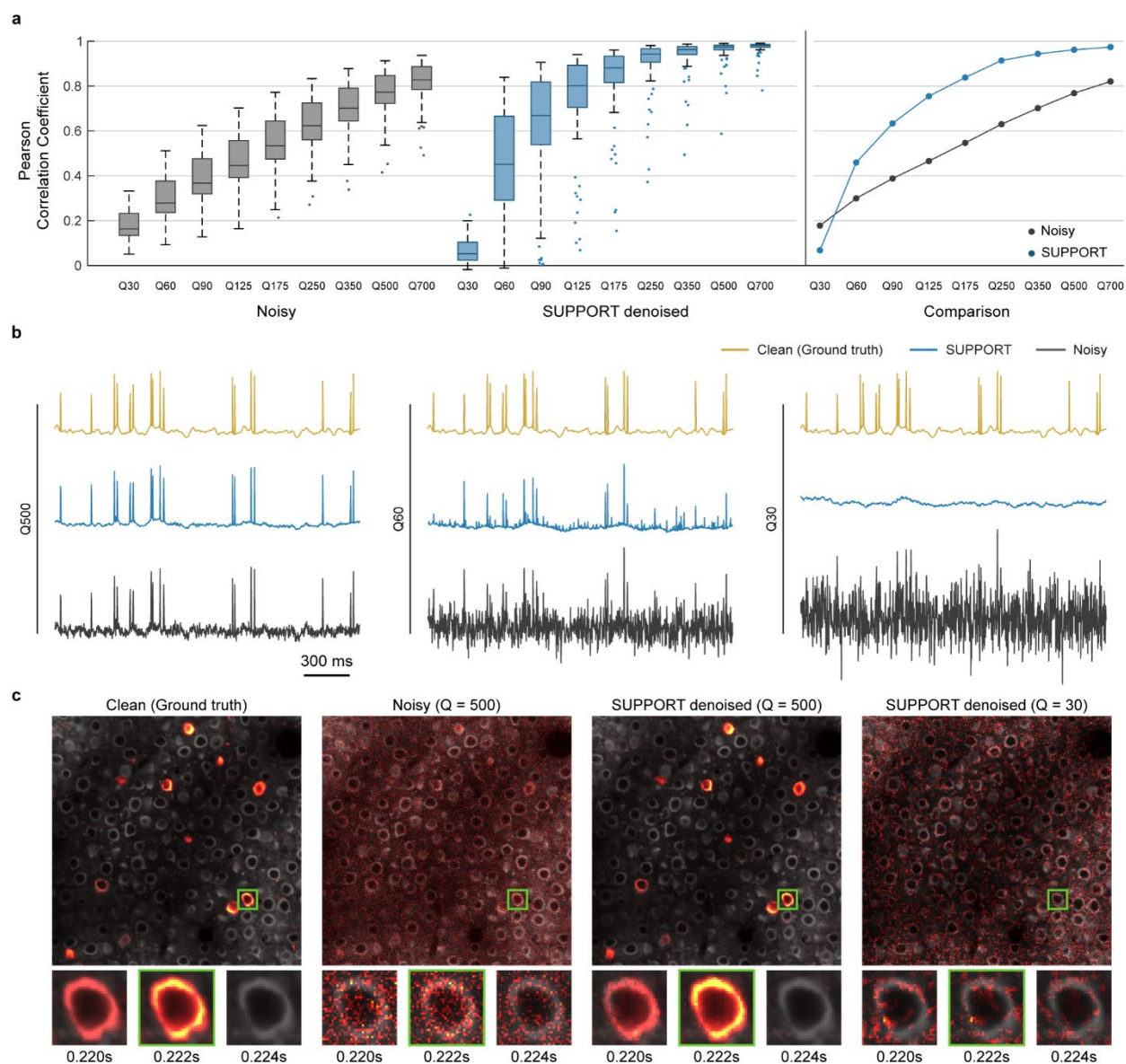


20
 21 **Supplementary Fig. 2: Comparison of blind spot networks with different complexities.** **a**, Impulse
 22 response of two blind spot network architectures (SUPPORT, Honzátko et al.¹) with different network
 23 complexities. While the width of SUPPORT’s receptive field increases exponentially, Honzátko et al.
 24 increases linearly. **b**, The width of the receptive field, memory consumption, and the number of multiply-
 25 add operations over the size of the receptive field of SUPPORT and Honzátko et al. for different depths.



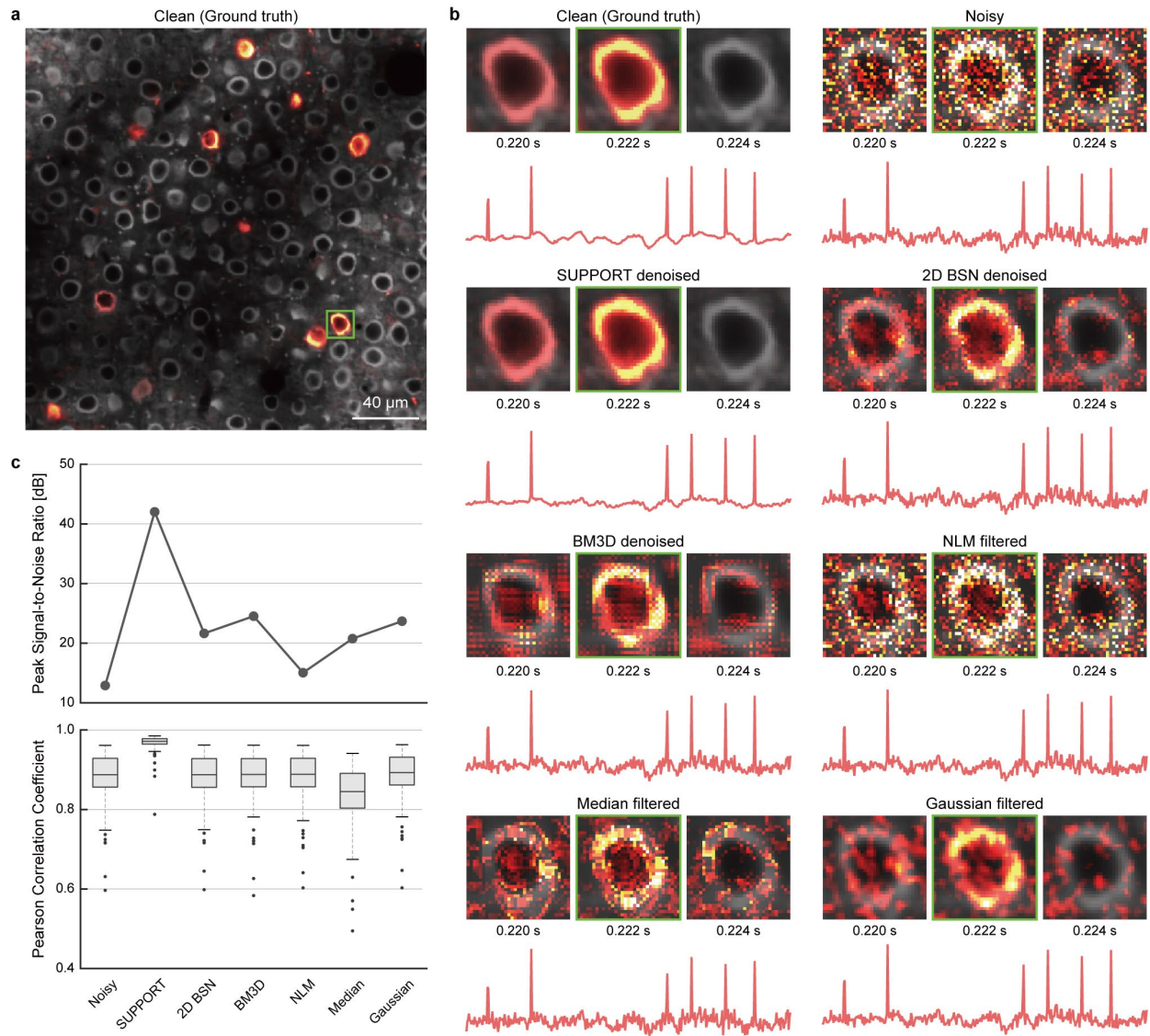
26

27 **Supplementary Fig. 3: SUPPORT performance comparison with other voltage imaging data analysis**
 28 **algorithms on simulated data.** **a**, Traces from clean, noisy, SUPPORT-denoised, NOSA-denoised, and
 29 Volpy-denoised data. Enlarged view of the gray region in **a** is displayed on the right. **b**, Box-and-whisker
 30 plot showing Pearson correlation coefficients before and after denoising for different spike widths. N=116
 31 for each test, which represents the number of neurons. **c**, Single cell fluorescence traces near spiking events.
 32 From top to bottom: Clean, noisy, SUPPORT-denoised, NOSA-denoised, and Volpy-denoised data. From
 33 left to right: spike widths of 1 ms, 3 ms, 5 ms, 7 ms, and 9 ms.



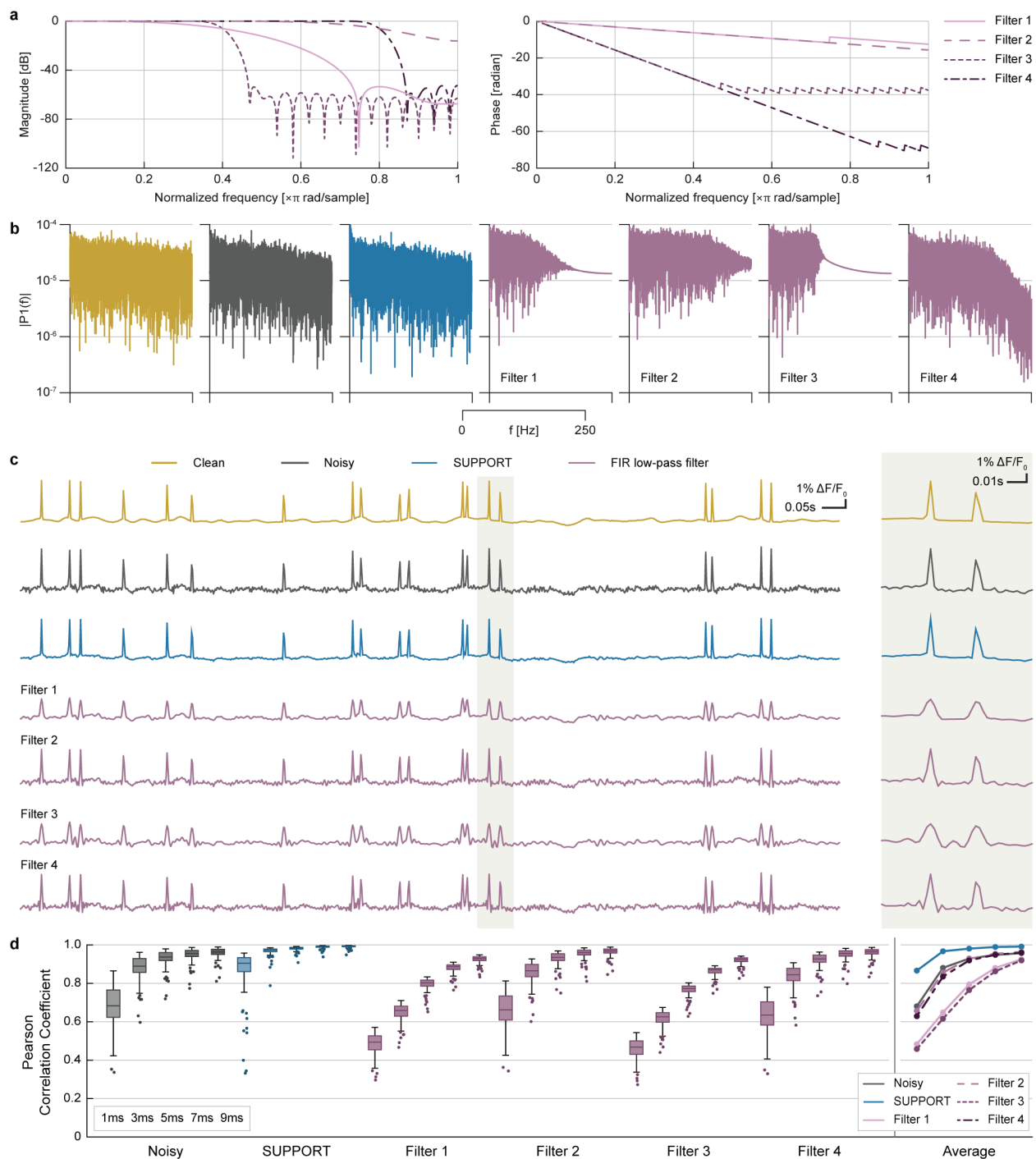
34

35 **Supplementary Fig. 4: SUPPORT denoising of simulated images with various noise levels.** **a**, Left:
 36 Box-and-whisker plot showing Pearson correlation coefficients before and after denoising data with the
 37 different noise levels. Right: Line chart showing average Pearson correlation coefficients before and after
 38 denoising data with the different noise levels. Q30 indicates each pixel captured 30 photons in maximum
 39 and contained corresponding level of Poisson noise. $N=116$ for each test, which represents the number of
 40 neurons. **b**, Fluorescence traces extracted by averaging the ROI of the image. From left to right: noise level
 41 of Q500, Q60, and Q30. From top to bottom: Ground truth, SUPPORT denoised data, and noisy data. **c**,
 42 Representative frames of clean, noisy (Q500) and SUPPORT denoised data (Q500 and Q30). Baseline and
 43 activity components are decomposed from the data. The baseline component with a gray colormap and
 44 activity component with a hot colormap are overlaid. Magnified views of the boxed regions are presented
 45 underneath with the consecutive frames of the spiking event ($t=0.222s$).



46

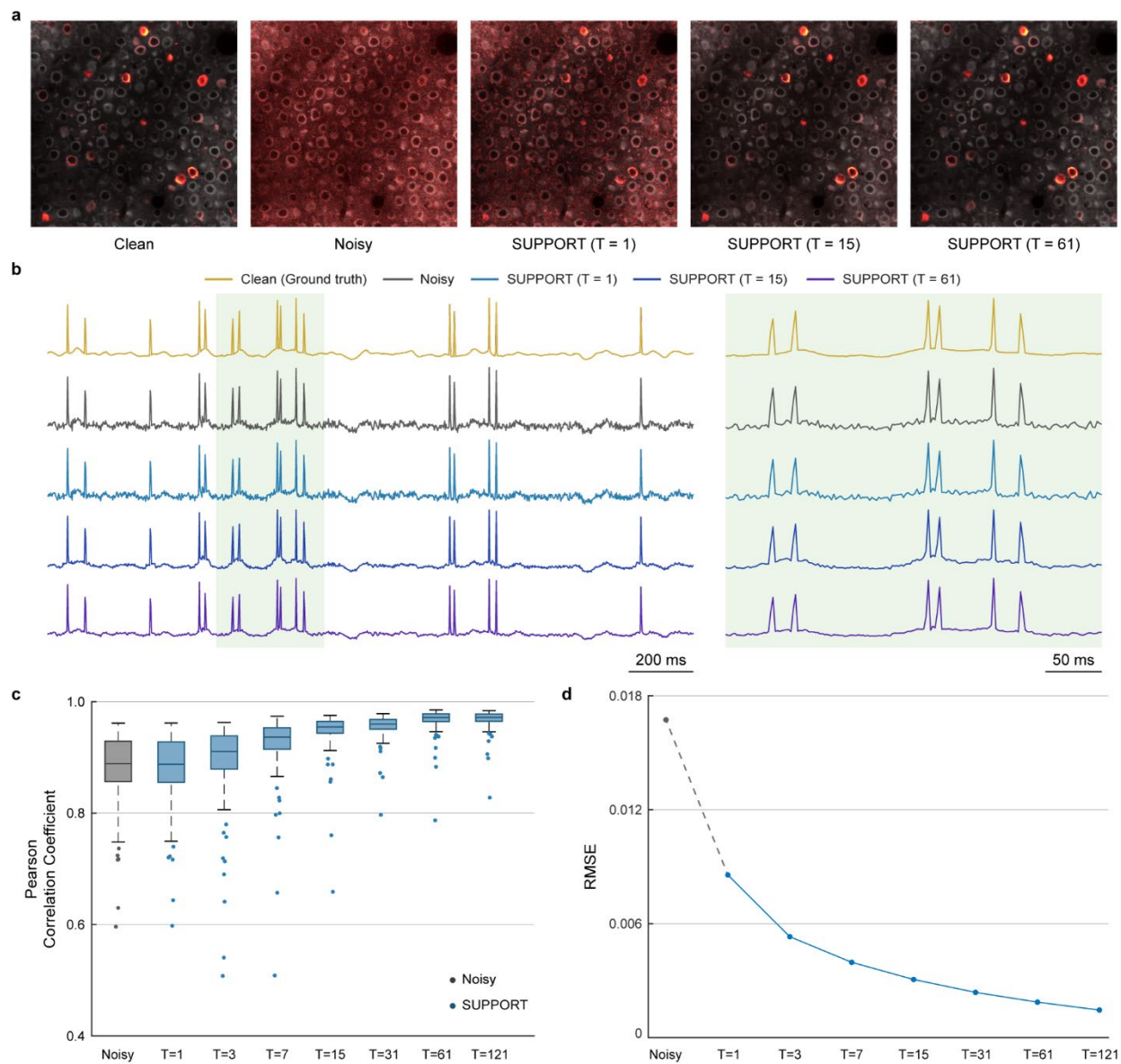
47 **Supplementary Fig. 5: Denoising performance compared with other 2D denoising methods on**
 48 **simulated data.** **a**, Clean synthetic population voltage imaging data ($t=0.222$ s). Baseline and activity
 49 components are decomposed from the data. The baseline component with a gray colormap and activity
 50 component with a hot colormap are overlaid. **b**, Consecutive frames of a green box in **a** from clean, noisy,
 51 SUPPORT, 2D BSN, BM3D denoised, NLM, median and gaussian filtered data. Traces are plotted below.
 52 **c**, PSNR and Pearson correlation coefficients of the baseline-corrected data before and after denoising.
 53 $N=116$ for each test, which represents the number of neurons.



54

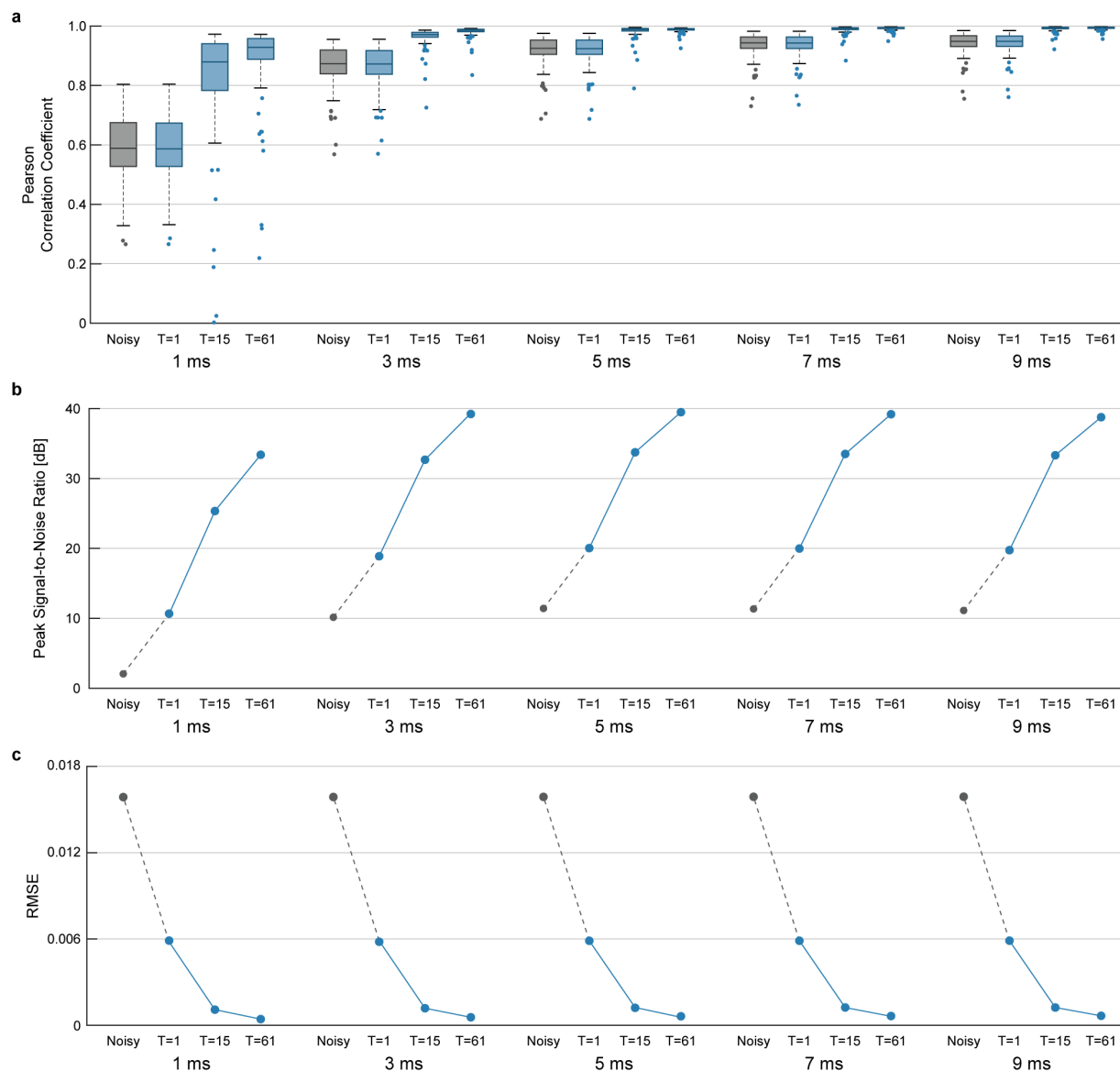
55 **Supplementary Fig. 6: Comparison between SUPPORT and low-pass FIR filtering on simulated**
 56 **voltage imaging data.** **a**, Frequency responses of FIR filters with different filter order and cutoff
 57 frequencies. Filter specifications are (filter order, cutoff frequency): Filter 1 = (10, 100Hz), Filter 2 = (10,
 58 200Hz), Filter 3 = (50, 100Hz), Filter 4 = (50, 200Hz). **b**, Single-sided amplitude spectrum of traces. Voltage
 59 traces with 3ms spike width are shown. **c**, Traces used in **b**. Magnified view is presented on the right side.
 60 **d**, Left: Box-and-whisker plot showing Pearson correlation coefficients before and after denoising data with
 61 different spike widths. Right: Line chart showing average Pearson correlation coefficients before and after

62 denoising data with different spike widths, $N = 116$ for each test, which represents the number of neurons.



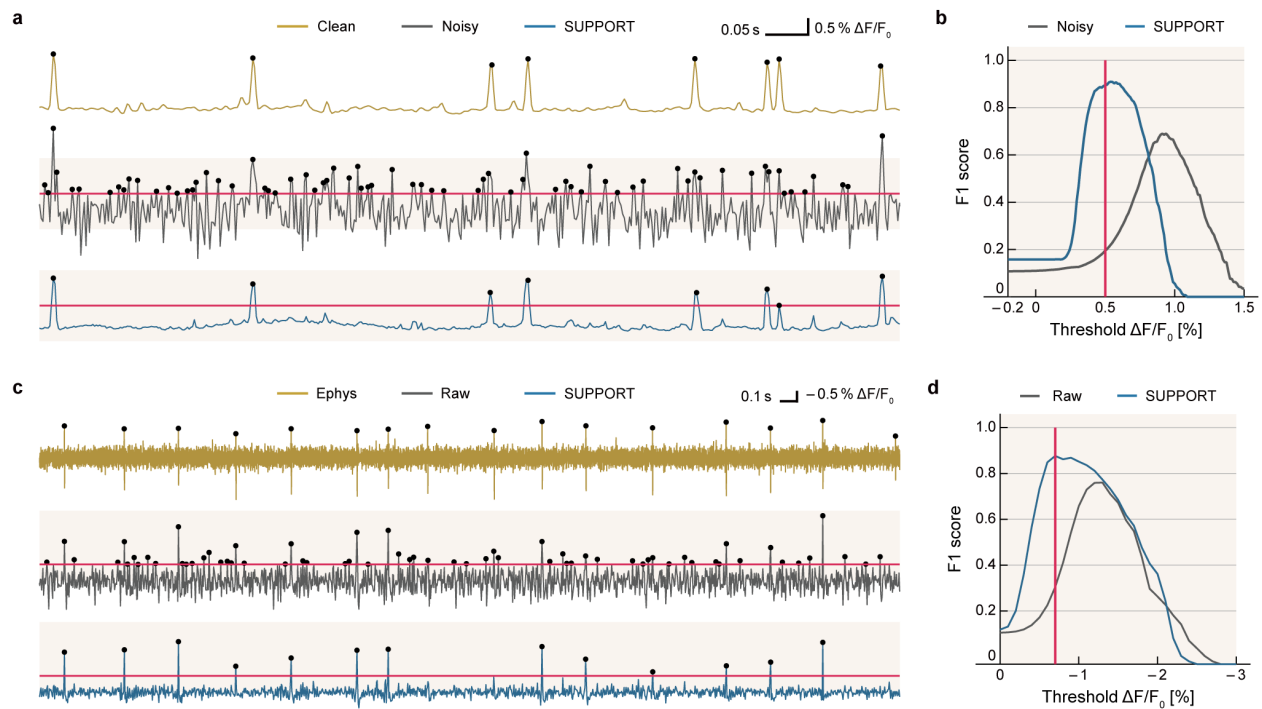
63

64 **Supplementary Fig. 7: Comparison of denoising performance of SUPPORT with varying temporal**
 65 **receptive field sizes. a**, Representative frames of a clean video, a noisy video, and SUPPORT-denoised
 66 videos using different receptive fields after baseline correction. Simulated data with spike width of 3ms
 67 was used. Receptive fields of 1, 15, and 61 in the temporal direction were used. **b**, Traces extracted from a
 68 single cell in the clean video, the noisy video, and the SUPPORT-denoised videos. Temporally expanded
 69 traces from the green area on the left are shown on the right. **c**, Box-and-whisker plot showing Pearson
 70 correlation coefficients from the extracted traces before and after denoising data with different receptive
 71 fields. N=116 for each test, which represents the number of neurons. **d**, Line plot showing RMSE before
 72 and after denoising data with different receptive fields.



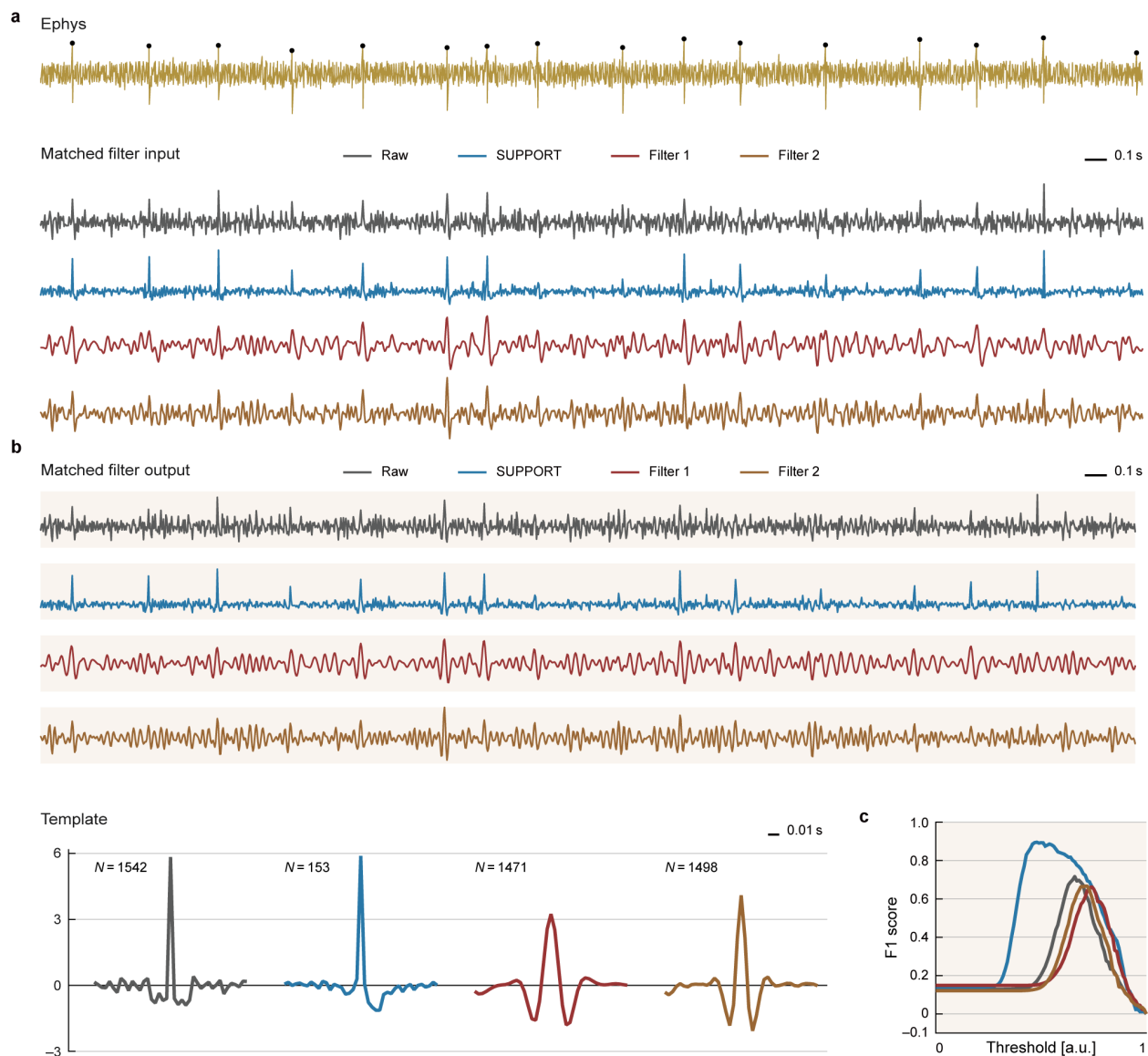
73

74 **Supplementary Fig. 8: Denoising performance of SUPPORT on simulation data with various spike**
 75 **widths and receptive fields. a, Box-and-whisker plot showing Pearson correlation coefficients before and**
 76 **after denoising data with different receptive fields in the temporal direction. Simulated data with changing**
 77 **spike widths of 1ms, 3ms, 5ms, 7ms, and 9ms were used. N=116 for each test, which represents the number**
 78 **of neurons. b, Line plot showing peak signal-to-noise ratio before and after denoising data with different**
 79 **receptive fields in the temporal direction. c, Line plot showing RMSE before and after denoising data with**
 80 **different receptive fields in the temporal direction.**



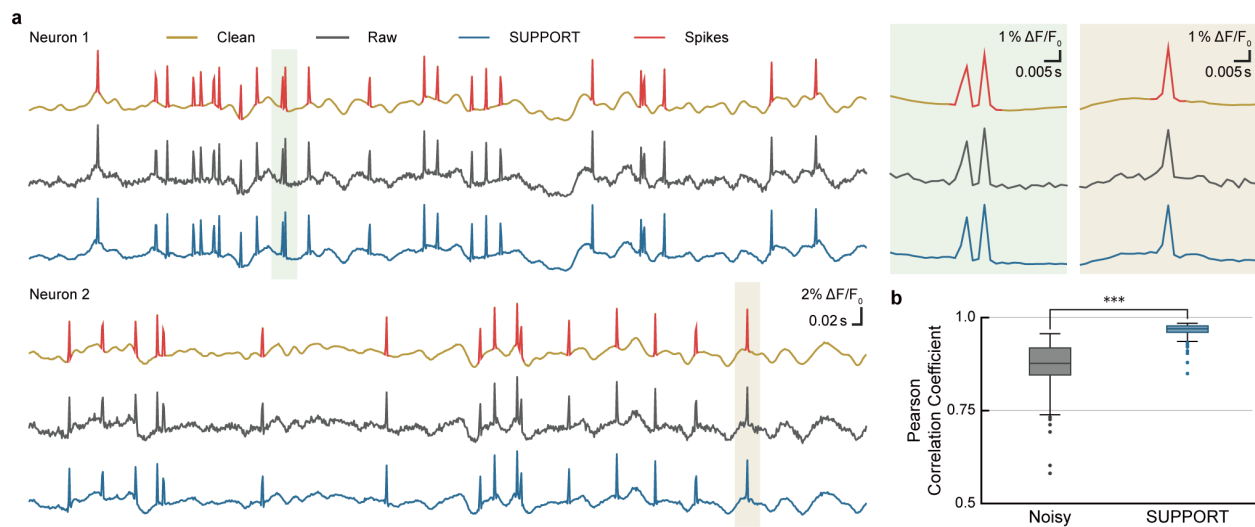
81

82 **Supplementary Fig. 9: SUPPORT enhances spike detection accuracy.** **a**, Traces from clean, noisy, and
 83 SUPPORT-denoised data tested with synthetic population voltage imaging data. Spikes are detected by
 84 thresholding the clean trace and finding local maximum locations. Red horizontal lines indicate the example
 85 threshold within the threshold region colored in pink. **b**, F1 scores across dF/F_0 threshold values used for
 86 spike detection. Red vertical line and pink region correspond to those in **a**. **c**, Electrophysiological recording
 87 and traces from voltage imaging data before and after SUPPORT denoising. Spikes are detected by
 88 thresholding the electrophysiological recording and finding local maximum locations. **d**, F1 scores across
 89 dF/F_0 threshold values used for spike detection.



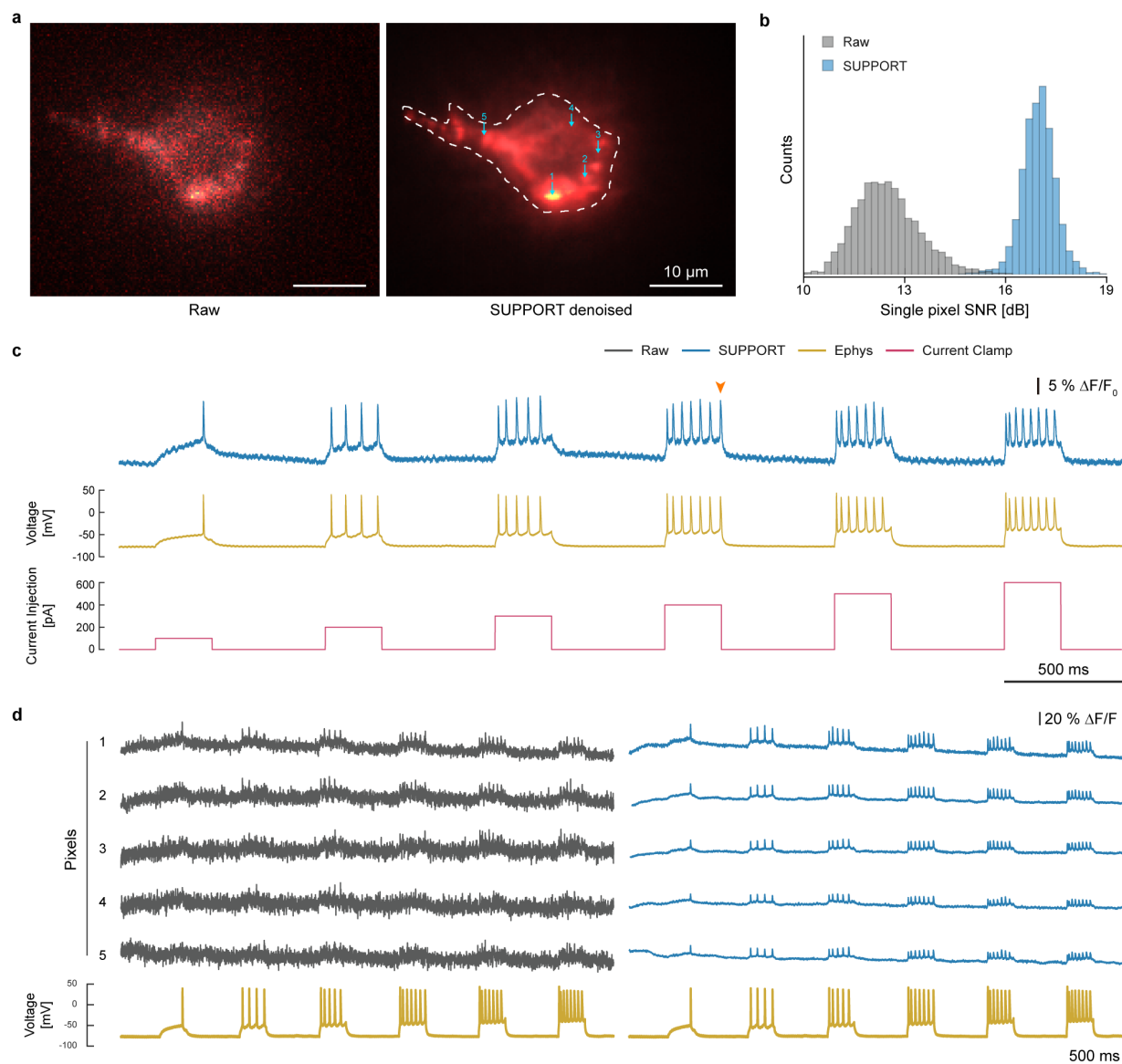
90

91 **Supplementary Fig. 10: Comparison between SUPPORT and low-pass FIR filtering on single-neuron**
 92 **voltage imaging data.** **a**, Signal traces from simultaneous electrophysiological recording and voltage
 93 imaging. From top to bottom: Electrophysiological recording, traces extracted from raw data, SUPPORT-
 94 denoised data, and FIR low-pass filtered data. Filter orders are 10 for both FIR filters. Cutoff frequencies
 95 of Filter 1 and Filter 2 are 25Hz and 75Hz, respectively. Traces from voltage imaging are used in
 96 matched filtering. **b**, Matched filtering outputs and extracted templates. Templates are obtained from the
 97 corresponding voltage traces. The salmon-colored areas behind the traces indicate the region of the
 98 threshold value used for F1 score calculation in **c**. **c**, F1 scores across threshold values used for spike
 99 detection.

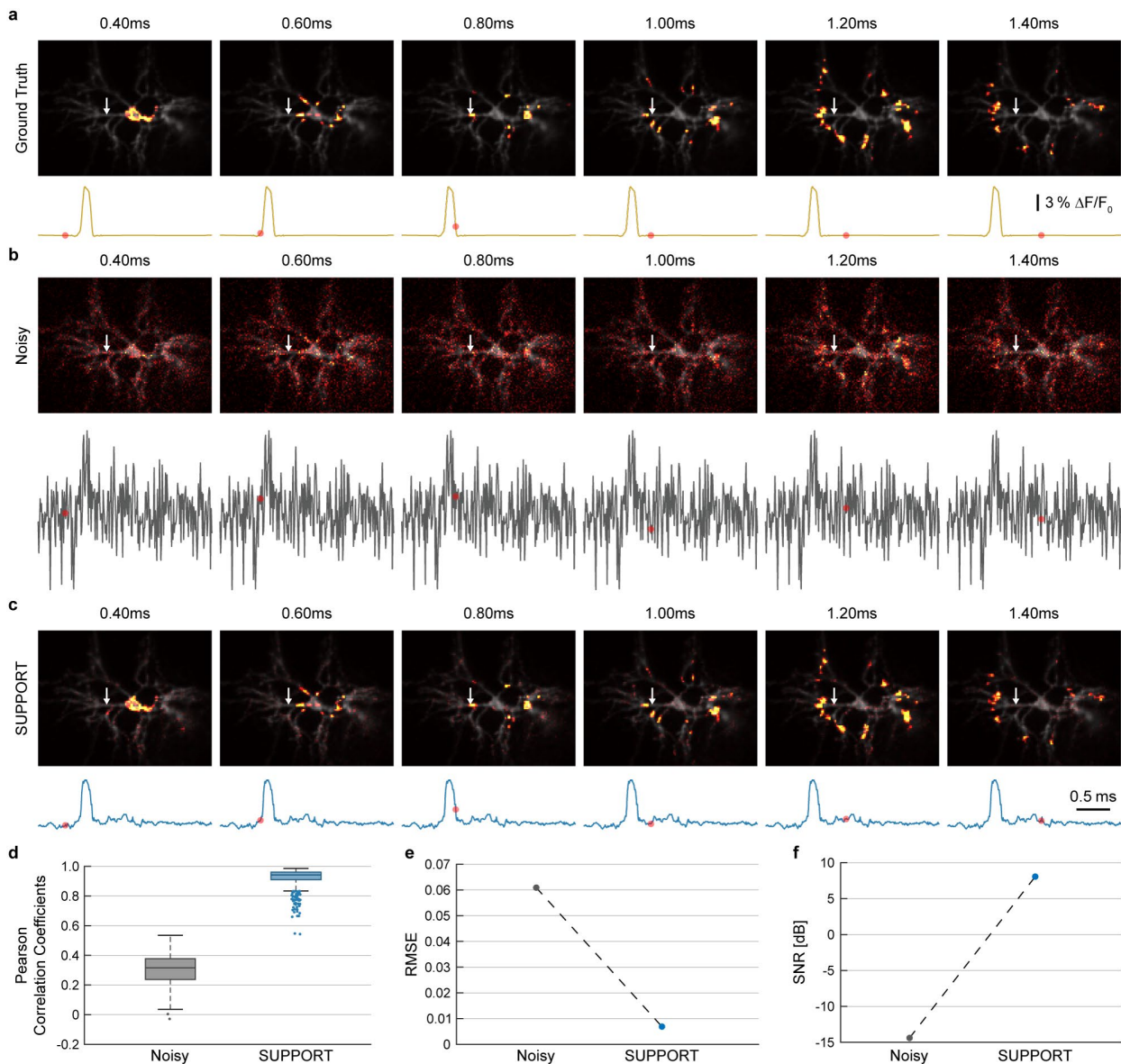


100

101 **Supplementary Fig. 11: SUPPORT can be applied for subthreshold signal recovery from simulated**
 102 **voltage imaging data. a.** Traces from raw and SUPPORT-denoised data extracted from two ROIs. Detected
 103 spike regions are indicated in red color. Spike regions are excluded in subthreshold analysis. **b.** Box-and-
 104 whisker plot showing Pearson correlation coefficient of subthreshold regions. A two-sided paired-sample t-
 105 test is used (***, p-value<0.001). N=116, which represents the number of pixels.

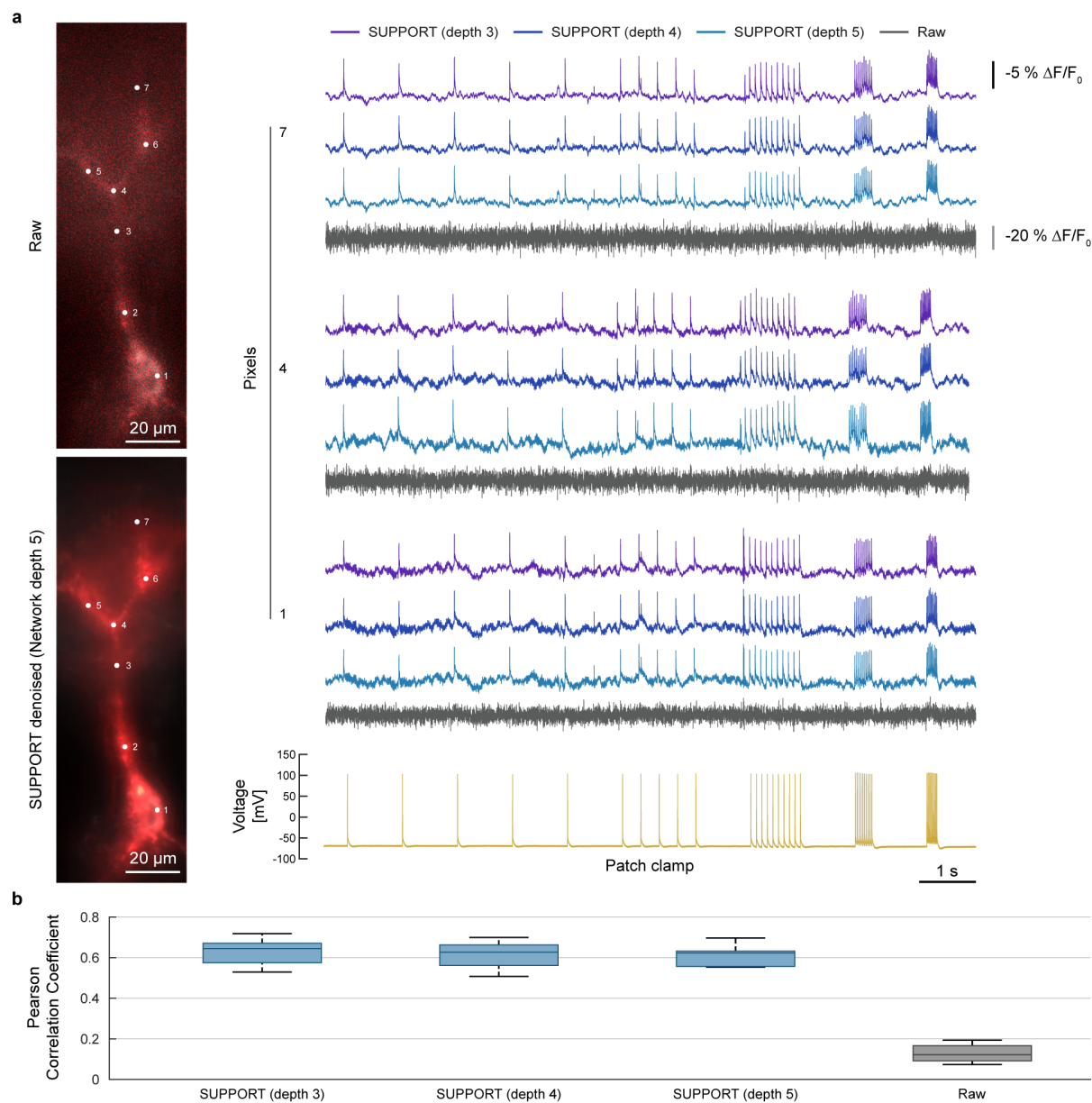


106
 107 **Supplementary Fig. 12: SUPPORT reveals single pixel traces from voltage imaging data with**
 108 **simultaneous electrophysiological recording.** **a**, Representative frames of raw and SUPPORT-denoised
 109 videos are shown after baseline correction. The frames used are indicated on **c** with an orange arrow.
 110 QuasAr6a-expressing mouse cortex L2/3 was used as a dataset (Supplementary Table 1). The baseline
 111 component with gray colormap and the activity component with hot colormap are overlaid. The boundary
 112 of the region of interest (ROI) is drawn with a white dotted line. Five single pixels to be analyzed are
 113 marked with cyan arrows. **b**, Histogram of single pixel signal-to-noise ratio (SNR) from raw data and
 114 SUPPORT-denoised data. **c**, Traces extracted from SUPPORT-denoised data for the ROI in **a**,
 115 electrophysiological recording, injected currents. **d**, Traces extracted from raw and SUPPORT-denoised
 116 videos from single pixels in **a** and electrophysiological recording. Left: From raw video. Right: From
 117 SUPPORT-denoised video.



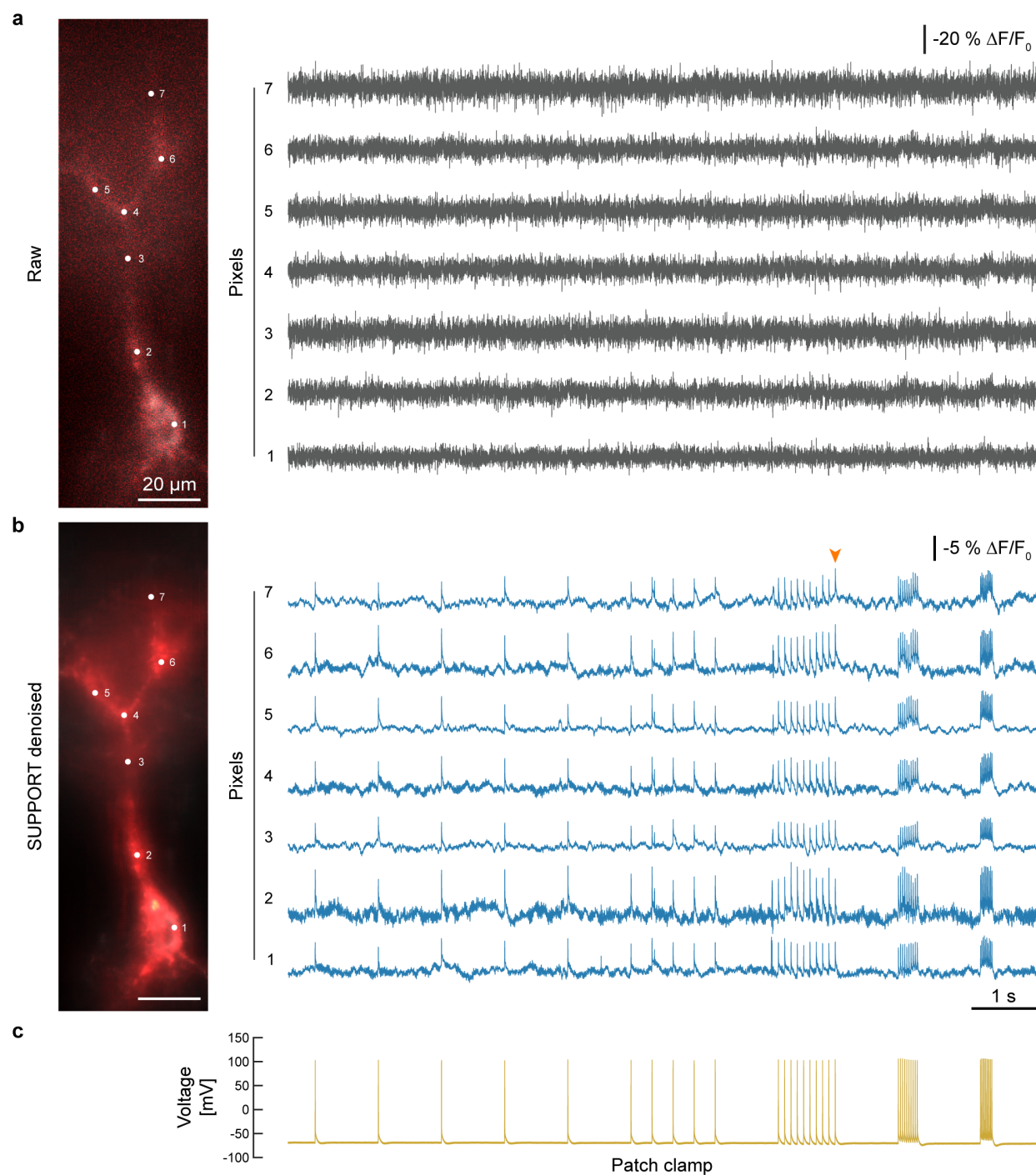
118

119 **Supplementary Fig. 13: SUPPORT improves voltage imaging data during propagating action**
 120 **potential.** **a**, Top: Synthetic voltage imaging data recorded at 100kHz, shown at six different time points.
 121 Baseline and activity components are decomposed from the data. The baseline component with a gray
 122 colormap and the activity component with a hot colormap are overlaid. Bottom: A trace extracted from the
 123 single pixel indicated by a white arrow was plotted. The red dot indicates the current frame. **b**, Gaussian
 124 noise added to the ground truth video is shown. **c**, Denoised videos using SUPPORT are shown. **d**, Box-
 125 and-whisker plot of single pixel Pearson correlation coefficients before and after denoising. $N=12800$ which
 126 represents the number of pixels. **e**, Line plot of single pixel RMSE before and after denoising. **f**, Line plot
 127 of single pixel SNR before and after denoising.



128

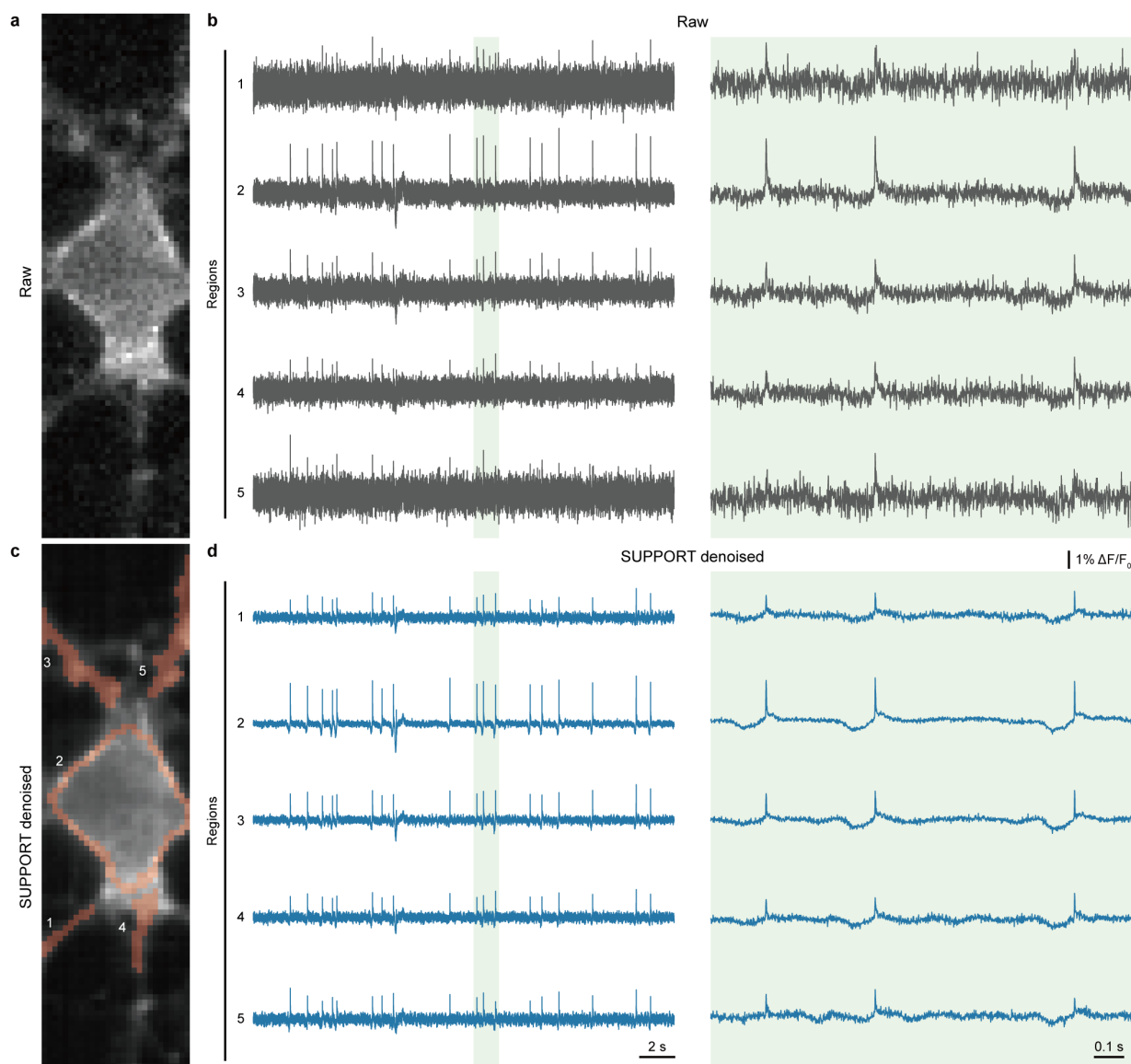
129 **Supplementary Fig. 14: Denoising performance comparison of SUPPORT with various receptive**
 130 **field sizes in the XY-direction on single neuron voltage imaging data with dendritic branch. a, Left: A**
 131 **representative frame of raw and SUPPORT-denoised video after baseline correction. Voltron2-expressing**
 132 **mouse cortex L2/3 was used as a dataset (Supplementary Table 1). The baseline component with gray**
 133 **colormap and the activity component with hot colormap are overlaid. Single pixels to be analyzed along**
 134 **the dendritic branch are marked with white dots and numbers. Right: Traces extracted from the single pixels**
 135 **of denoised and raw video. Three different models of SUPPORT were used which exhibit different sizes of**
 136 **the receptive fields in the XY-direction. Bottom: Corresponding voltage traces from the**
 137 **electrophysiological recording. b, Box plot showing Pearson correlation coefficients between the traces**
 138 **extracted from the single pixels represented as a white dot in a and electrophysiological recording. N=7,**
 139 **which represents the number of pixels.**



140

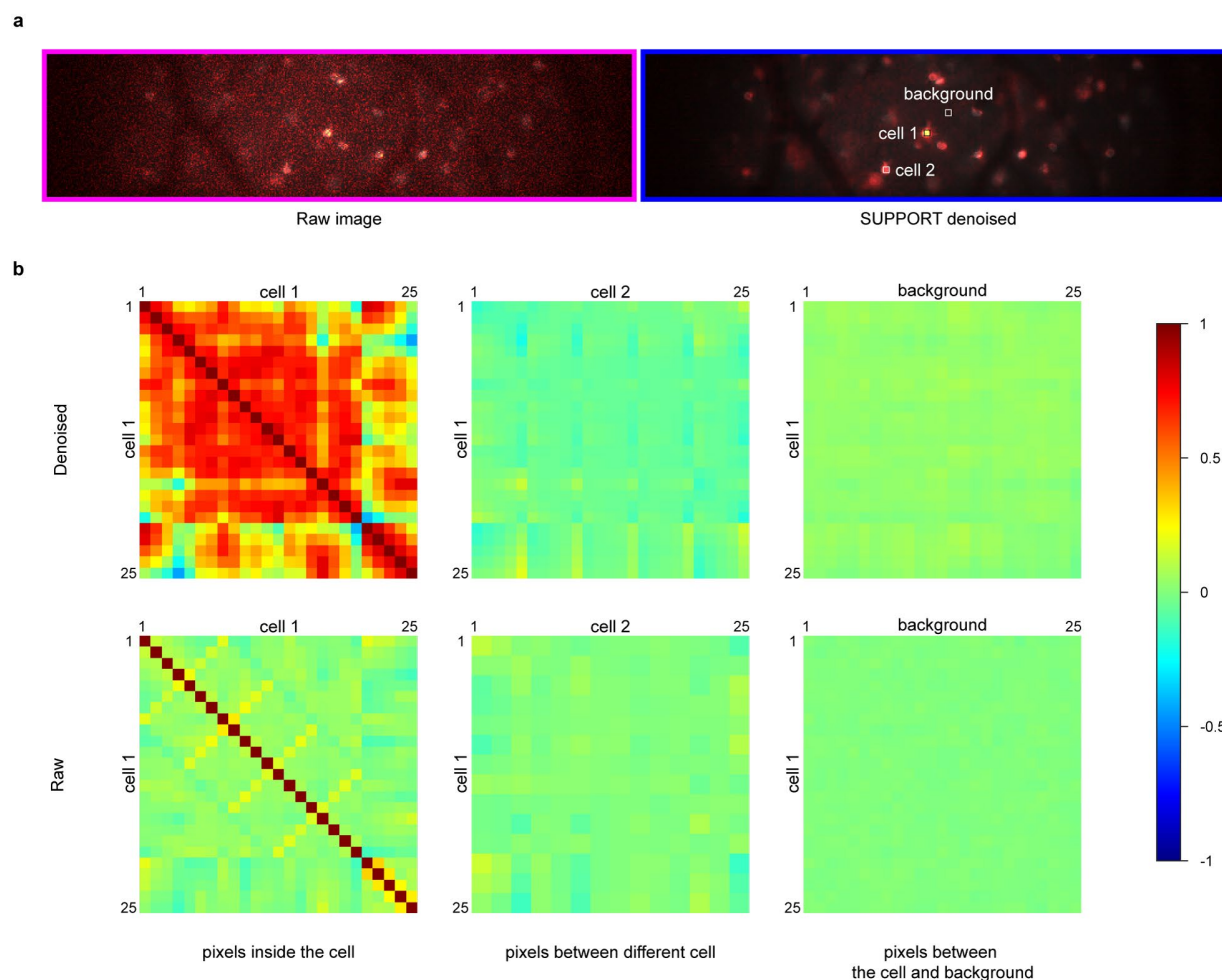
141 **Supplementary Fig. 15: SUPPORT reveals the voltage signal along the dendritic branch.** **a**, Left: A
 142 representative frame of raw video after baseline correction. The frame used is marked on **b** with an orange
 143 arrow. Voltron2-expressing mouse cortex L2/3 was used as a dataset (Supplementary Table 1). The baseline
 144 component with gray colormap and the activity component with hot colormap are overlaid. Single pixels
 145 to be analyzed along the dendritic branch are marked with white dots and numbers. Right: Traces extracted
 146 from the single pixels of raw video. **b**, Left: A representative frame of SUPPORT-denoised video after
 147 baseline correction. Right: Traces extracted from the single pixels of SUPPORT-denoised video. **c**,

148 Corresponding voltage traces from the electrophysiological recording.



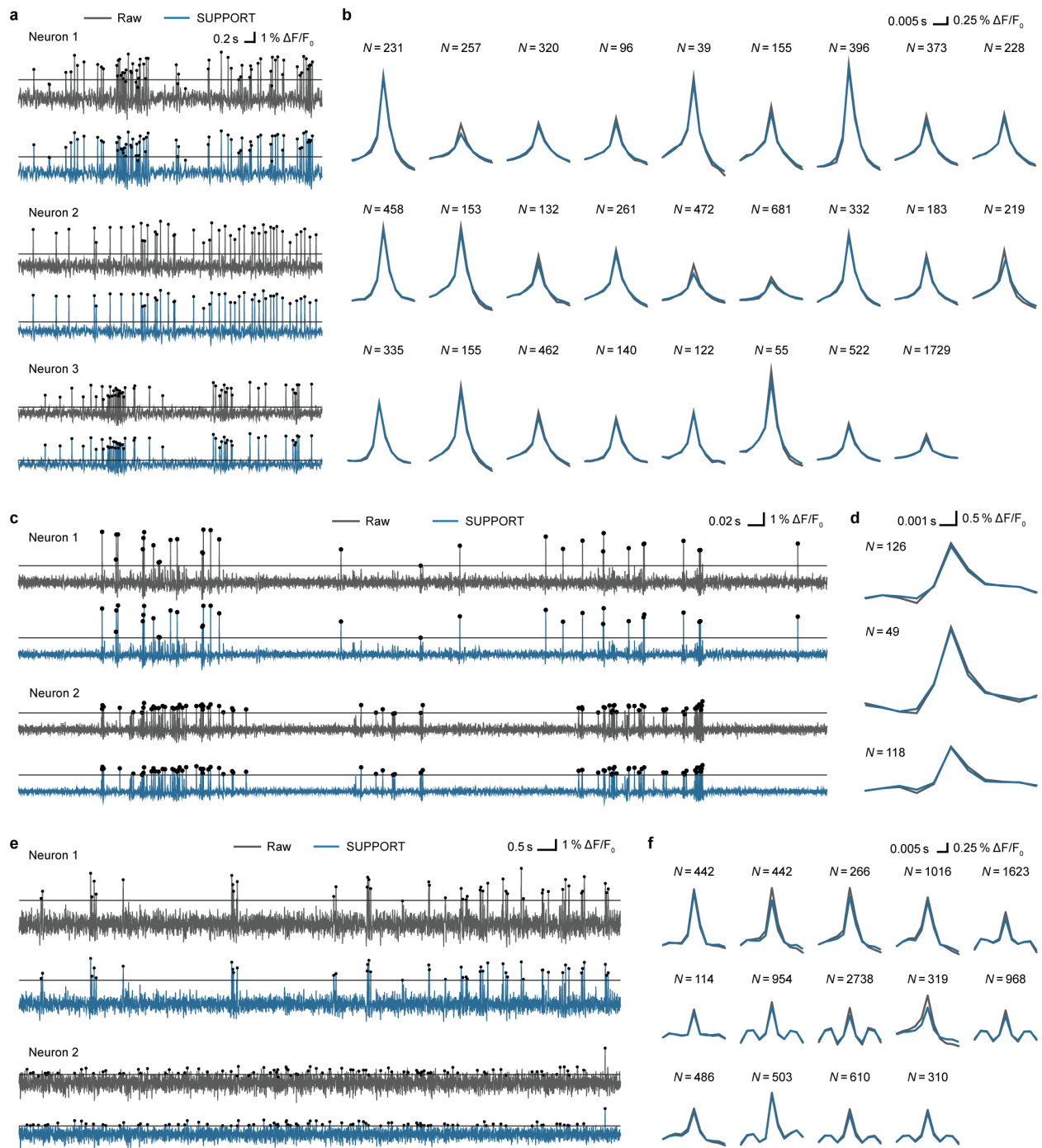
149

150 **Supplementary Fig. 16: SUPPORT reveals the voltage signal in several regions.** **a**, Representative
 151 frame of raw video. BeRST1-labeled mouse hippocampus cultured cells were used as a dataset
 152 (Supplementary Table 1). **b**, Traces extracted from the five regions of interest (ROIs) shown in **c** from the
 153 raw video. Temporally expanded traces from the green area on the left are shown on the right. **c**,
 154 Representative frame of SUPPORT-denoised video. ROIs to be analyzed are marked in orange. **d**, Left:
 155 Traces extracted from the five ROIs shown in **c** from the SUPPORT-denoised video. Right: Temporally
 156 expanded traces from the green area on the left.



157

158 **Supplementary Fig. 17: Pixelwise correlation coefficient map between different regions in voltage**
 159 **imaging data.** **a**, Representative frames of raw video and SUPPORT-denoised video, for which the raw
 160 data was never shown to SUPPORT on training. Dataset of in vivo mouse cortex layer 1 expressing Voltron1
 161 was used. Three regions which are cell1, cell2, and background were indicated with boxes. **b**, Pixel-level
 162 Pearson correlation coefficients. From left to right: Pearson correlation coefficients calculated between the
 163 same cell, between another cell, and between the background. Top: Pearson correlation coefficients
 164 calculated on SUPPORT-denoised video. Bottom: Pearson correlation coefficients calculated on the raw
 165 video.

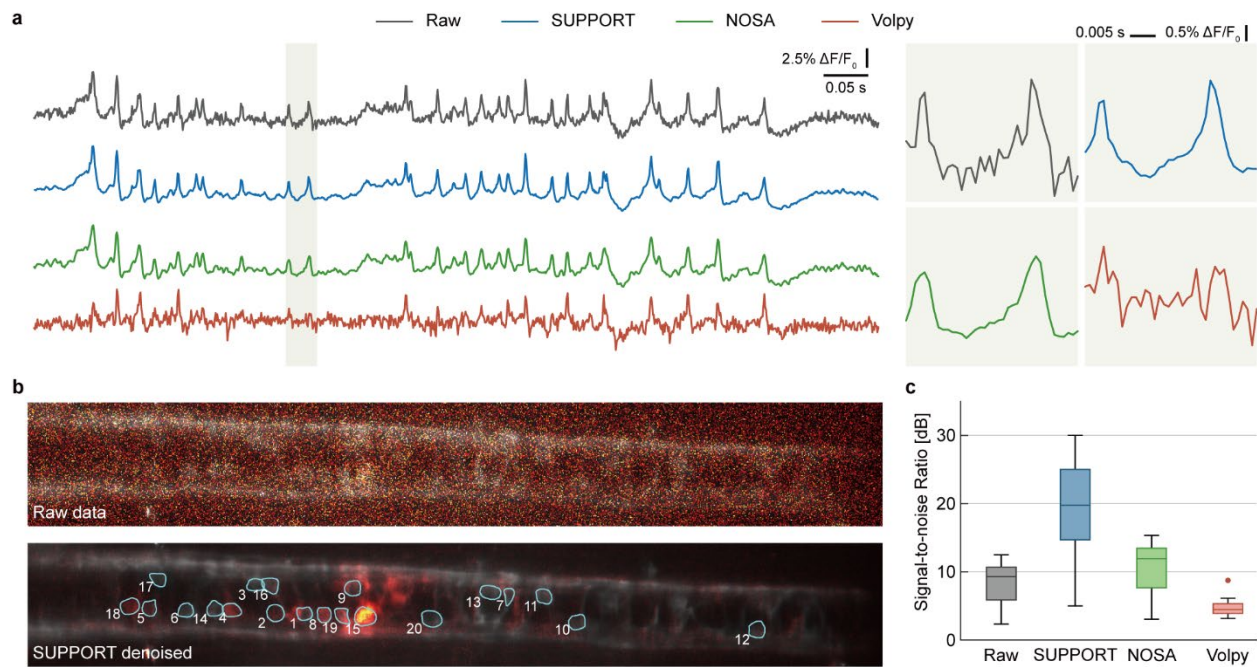


166

167 **Supplementary Fig. 18: SUPPORT preserves temporal spike shapes in voltage imaging data.** **a**, Traces
 168 extracted from representative 3 neurons from mouse dataset. Spikes are detected by thresholding the clean
 169 trace and finding local maximum locations. **b**, Average of spikes of all 26 neurons from raw and SUPPORT-
 170 denoised data. **c**, Traces extracted from representative 2 neurons from population voltage imaging data with
 171 paQuasAr3s. **d**, Average of spikes of all 3 neurons from raw and SUPPORT-denoised data. **e**, Traces
 172 extracted from representative 2 neurons from population voltage imaging data with Voltron1. **f**, Average of

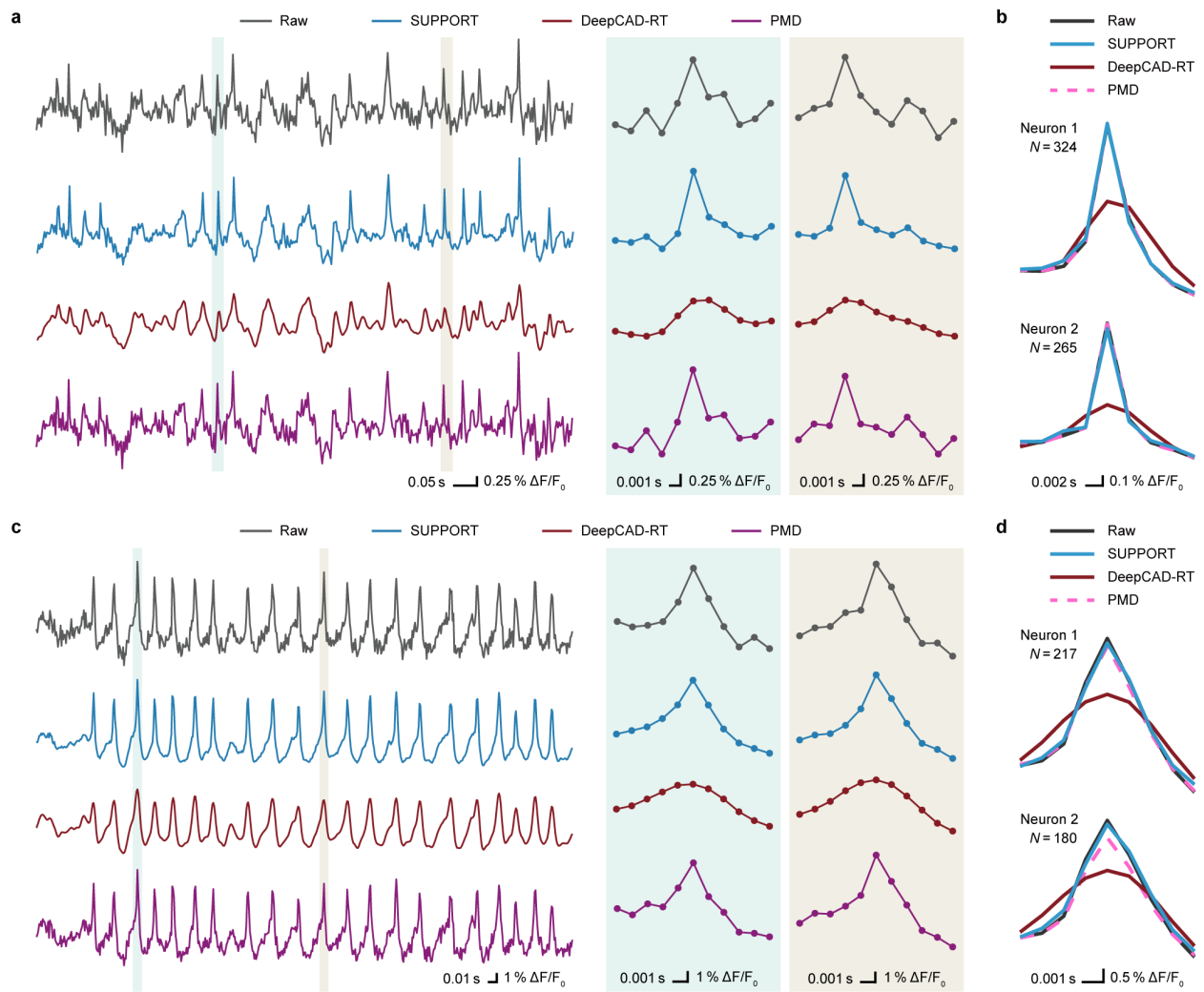
173 spikes of 14 neurons from raw and SUPPORT-denoised data. Neurons with less than 100 spikes or dF/F_0
174 lower than 2% were excluded.

175



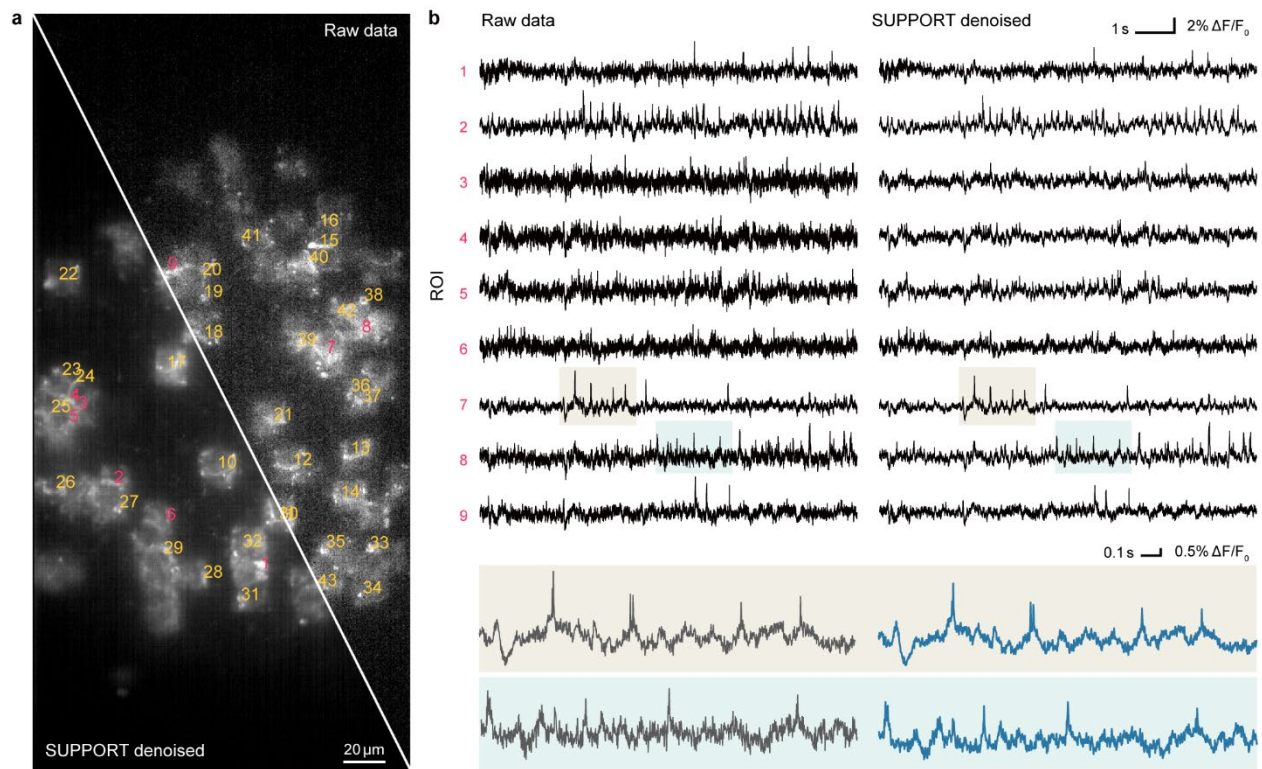
176

177 **Supplementary Fig. 19: SUPPORT performance comparison with other voltage imaging data**
 178 **analysis algorithms on zebrafish dataset. a**, Traces from raw, SUPPORT, NOSA, and Volpy
 179 data. Enlarged view of the gray region in a is displayed on the right side. **b**, Images from the zebrafish
 180 dataset. Baseline and activity components are decomposed from raw data and SUPPORT-denoised data.
 181 The baseline component with a gray colormap and the activity component with a hot colormap are overlaid.
 182 Boundaries of 20 ROIs are drawn with cyan lines. **c**, Box-and-whisker plot showing signal-to-noise ratio
 183 of neuronal traces from raw, SUPPORT, NOSA, and Volpy denoised data. $N=20$, which represents the
 184 number of neurons.



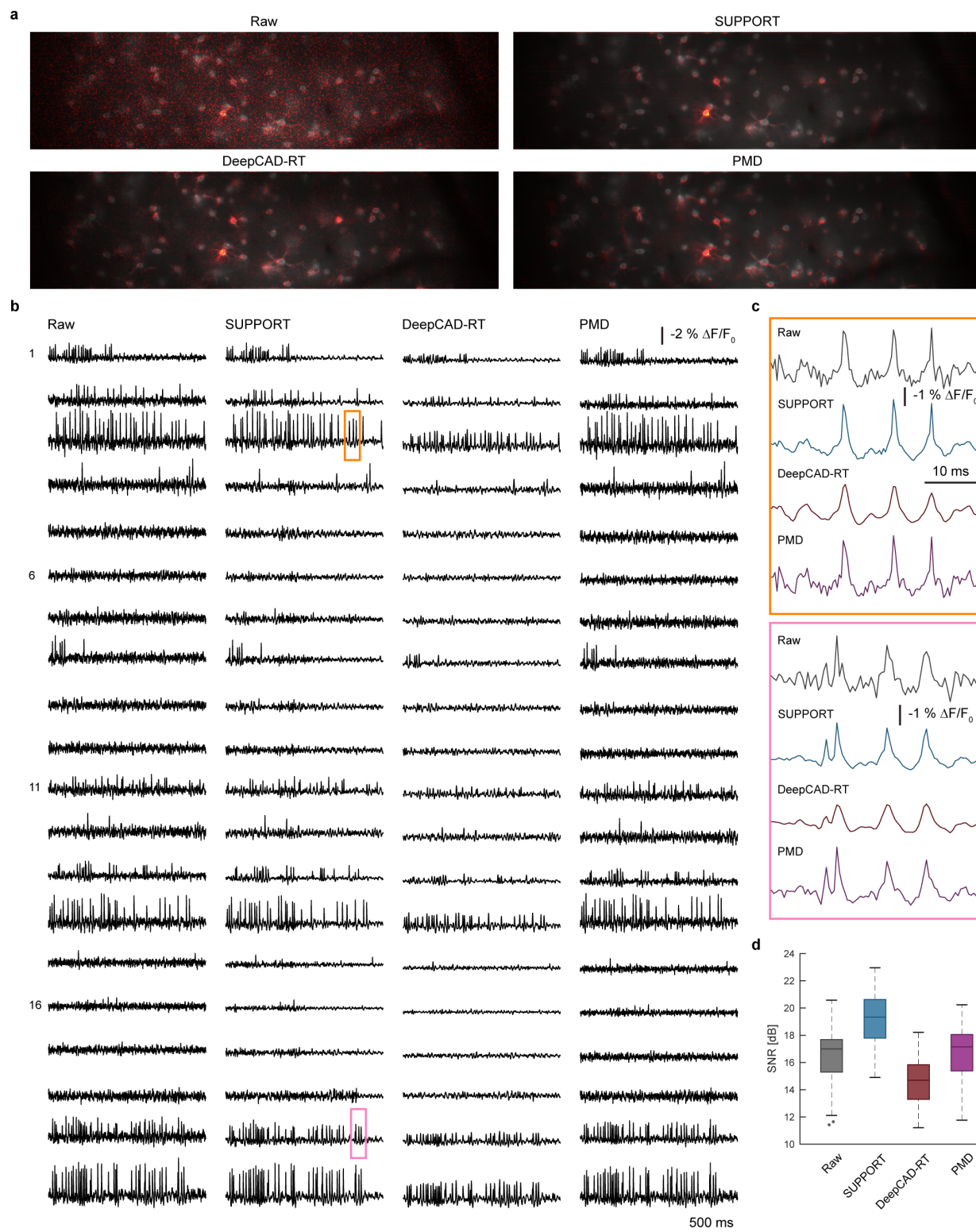
185

186 **Supplementary Fig. 20: Spike shape analysis on experimental population voltage imaging data. a,**
 187 **Traces from raw and denoised data using SUPPORT, DeepCAD-RT, and PMD from mouse dataset. Traces**
 188 **for the smaller temporal region are plotted on the right. b, Average spike shapes of representative two**
 189 **neurons extracted from raw and denoised data. c, Traces from raw and denoised data from zebrafish dataset.**
 190 **d, Average spike shapes of representative two neurons extracted from raw and denoised data.**



191

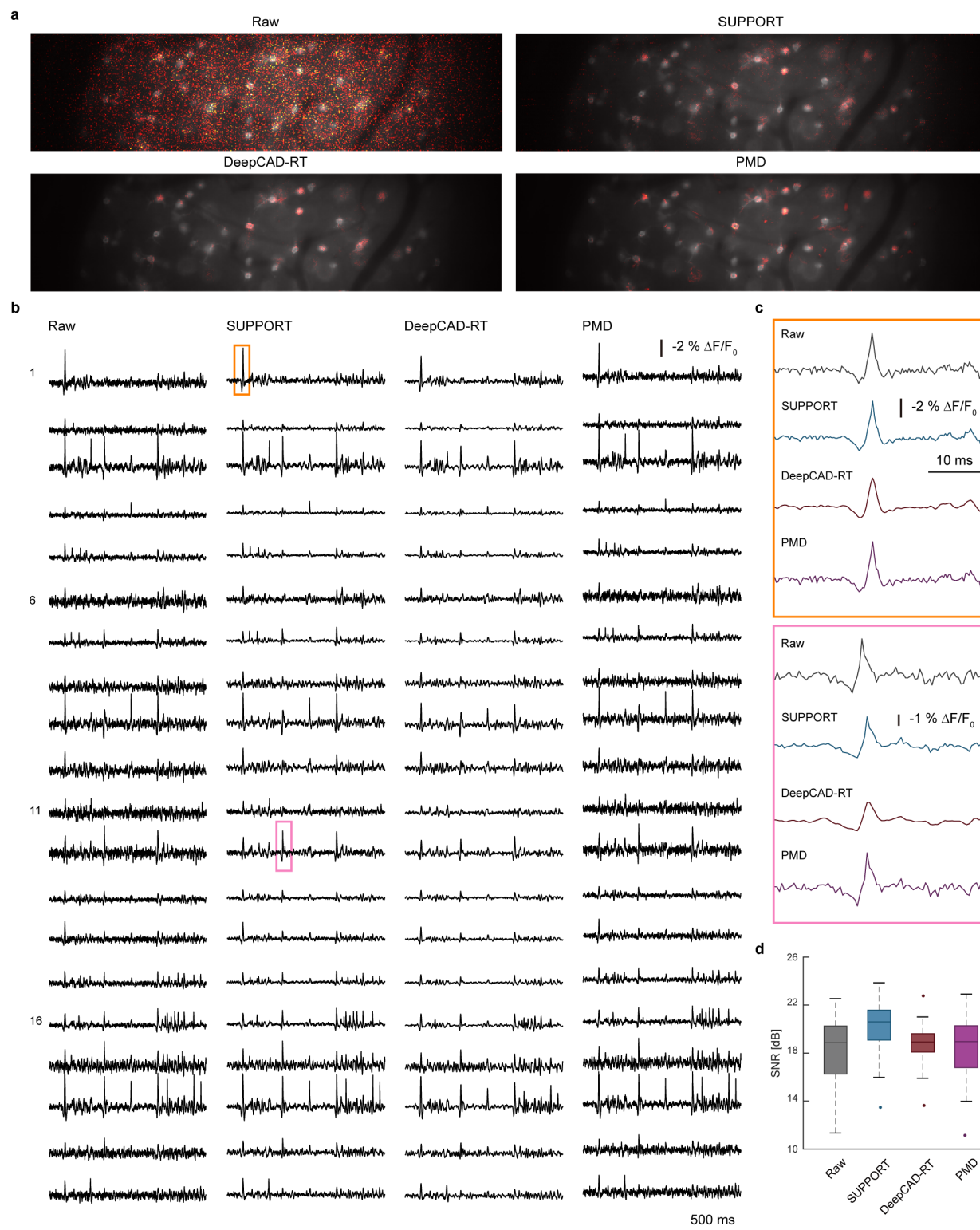
192 **Supplementary Fig. 21: Applying SUPPORT to population voltage imaging data with SomArchon**
 193 **indicator. a,** Cultured neurons expressing SomArchon indicator. **b,** Traces from 9 neurons before and after
 194 SUPPORT denoising. Traces for the smaller temporal regions are plotted below.



195

196 **Supplementary Fig. 22: Denoising population mouse voltage imaging data.** **a**, Images after baseline
 197 correction from mouse dataset expressing Voltron1 in cortex layer 1. **b**, Traces from 20 ROIs from raw,

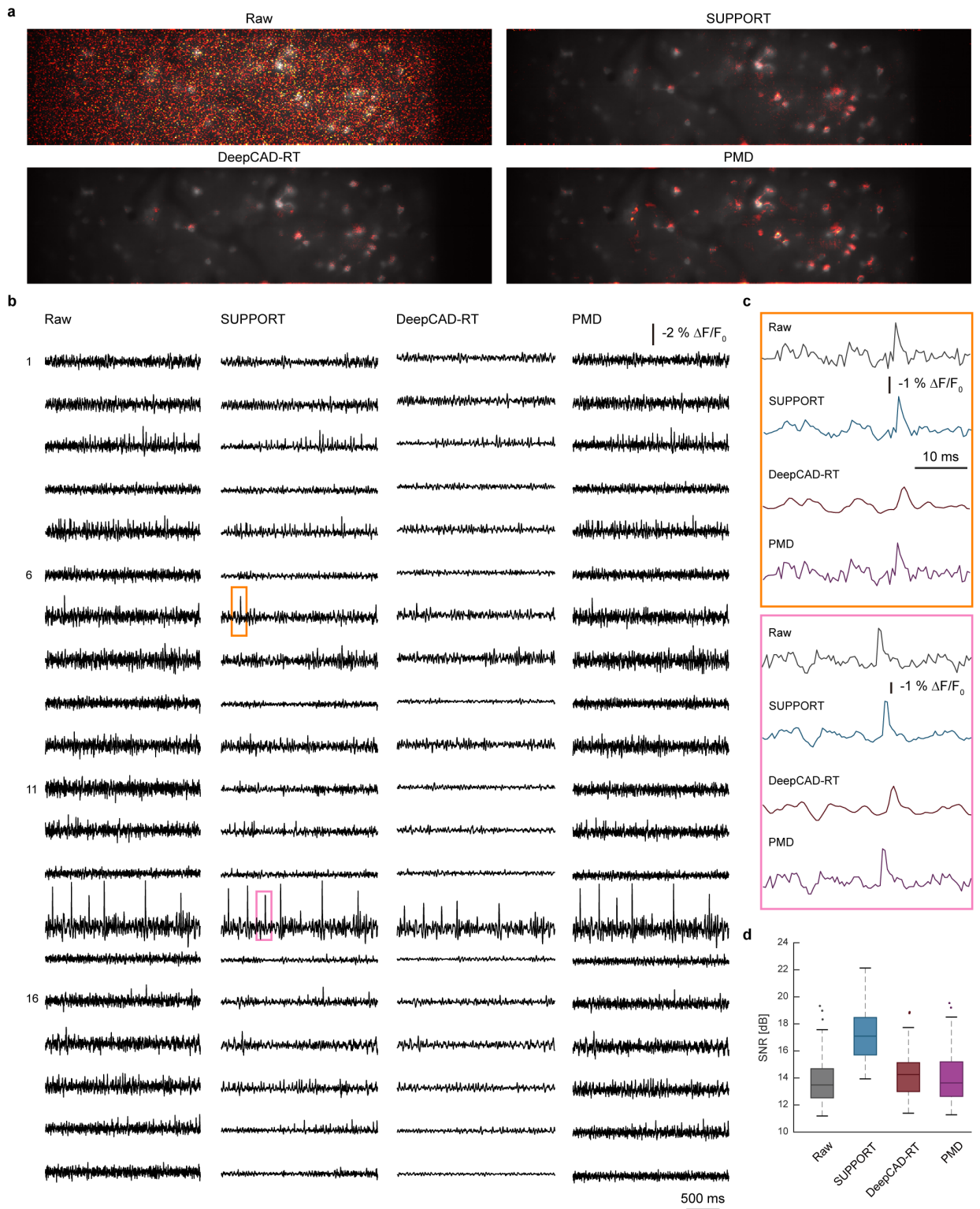
198 SUPPORT, DeepCAD-RT, and PMD. **c**, Enlarged view of traces from colored box in **b** are plotted. **d**, Box-
199 and-whisker plot showing the signal-to-noise ratio for the extracted traces. $N=79$, which represents the
200 number of neurons.



201

202 **Supplementary Fig. 23: Denoising population mouse voltage imaging data.** **a**, Images after baseline
 203 correction from mouse dataset expressing Voltron1 in cortex layer 1. **b**, Traces from 20 ROIs from raw,

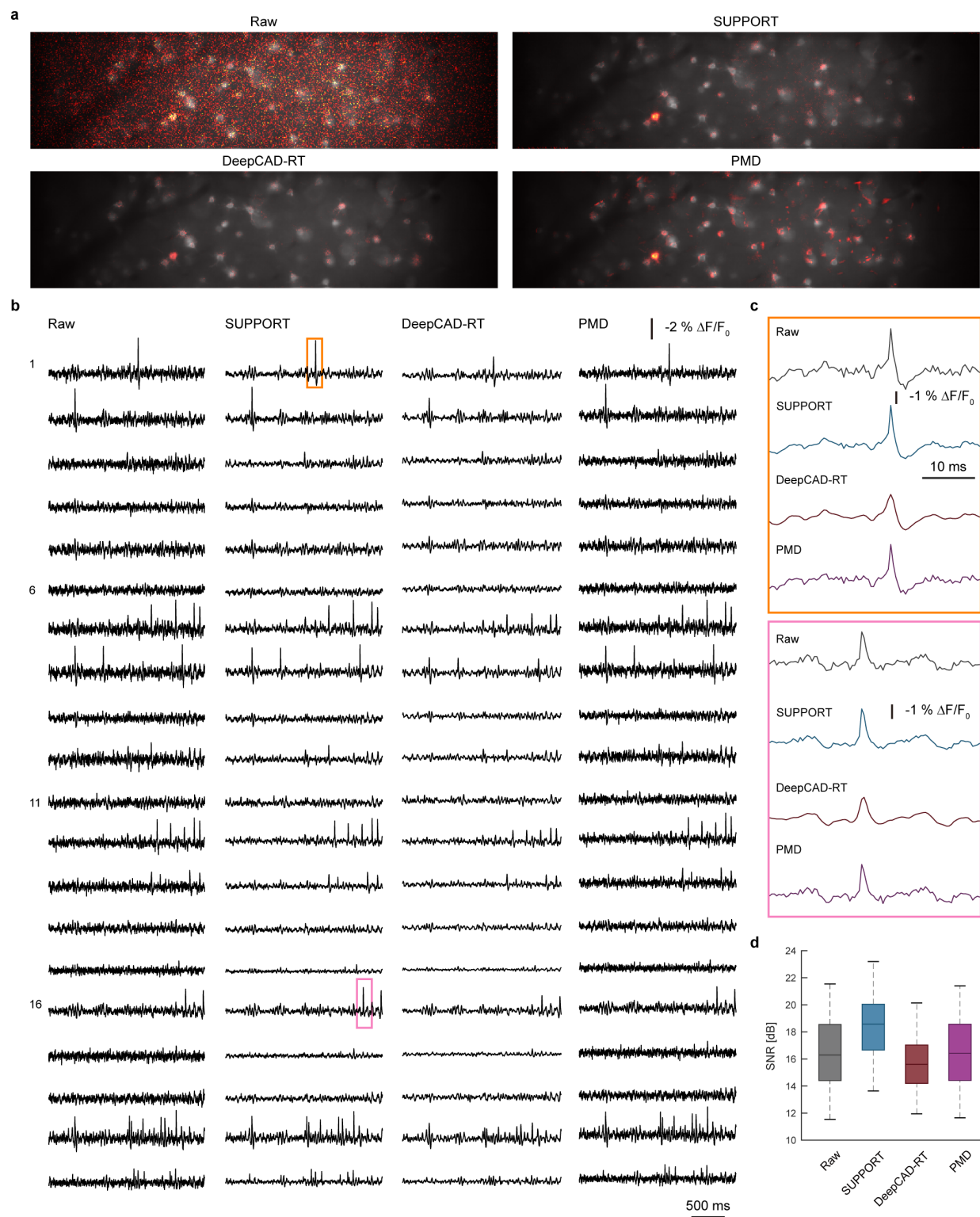
204 SUPPORT, DeepCAD-RT, and PMD. **c**, Enlarged view of traces from colored box in **b** are plotted. **d**, Box-
205 and-whisker plot showing the signal-to-noise ratio for the extracted traces. N=50, which represents the
206 number of neurons.



207

208 **Supplementary Fig. 24: Denoising population mouse voltage imaging data.** **a**, Images after baseline
 209 correction from mouse dataset expressing Voltron1 in cortex layer 1. **b**, Traces from 20 ROIs from raw,

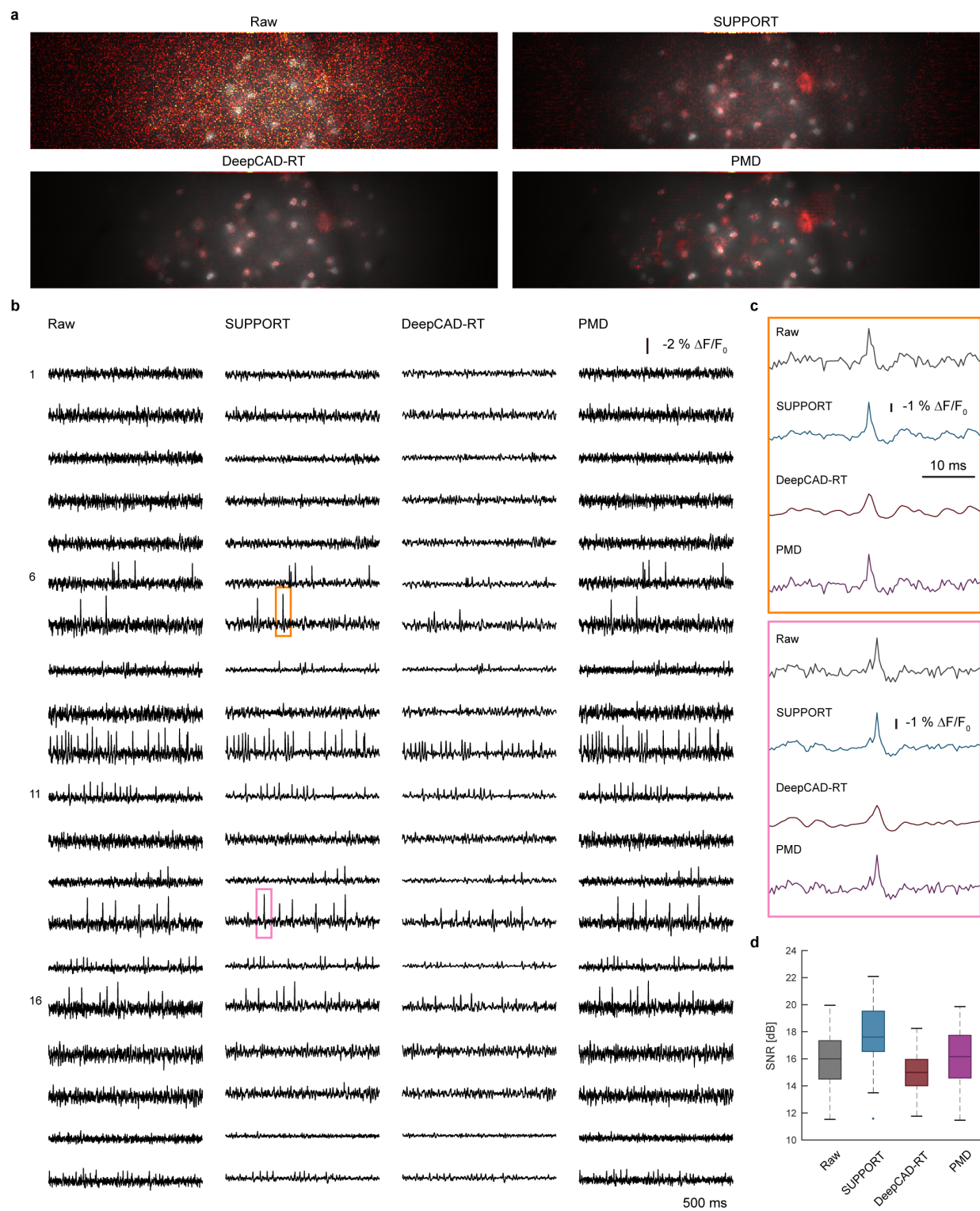
210 SUPPORT, DeepCAD-RT, and PMD. **c**, Enlarged view of traces from colored box in **b** are plotted. **d**, Box-
211 and-whisker plot showing the signal-to-noise ratio for the extracted traces. N=65, which represents the
212 number of neurons.



213

214 **Supplementary Fig. 25: Denoising population mouse voltage imaging data.** **a**, Images after baseline
 215 correction from mouse dataset expressing Voltron1 in cortex layer 1. **b**, Traces from 20 ROIs from raw,

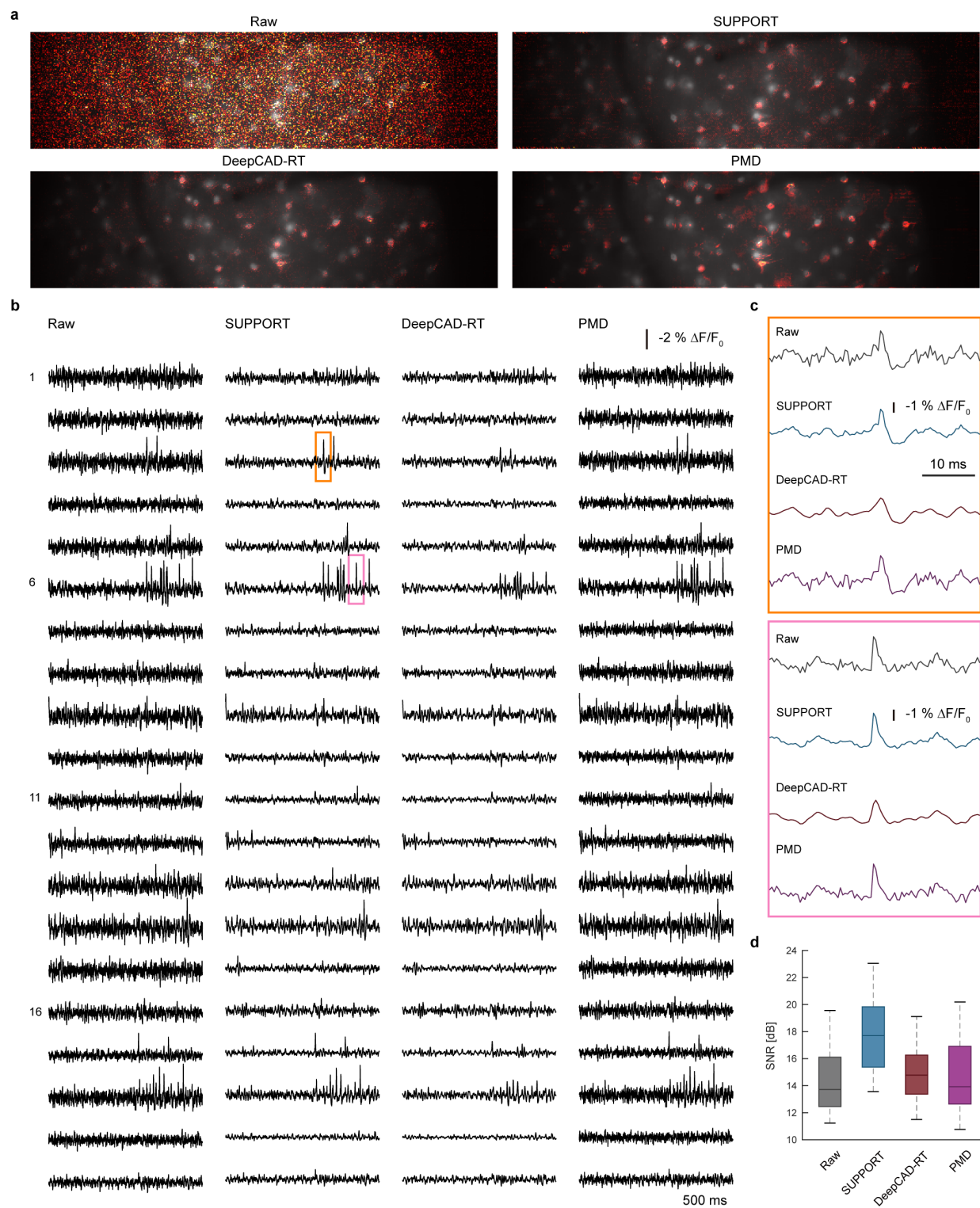
216 SUPPORT, DeepCAD-RT, and PMD. **c**, Enlarged view of traces from colored box in **b** are plotted. **d**, Box-
217 and-whisker plot showing the signal-to-noise ratio for the extracted traces. $N=63$, which represents the
218 number of neurons.



219

220 **Supplementary Fig. 26: Denoising population mouse voltage imaging data.** **a**, Images after baseline
 221 correction from mouse dataset expressing Voltron1 in cortex layer 1. **b**, Traces from 20 ROIs from raw,

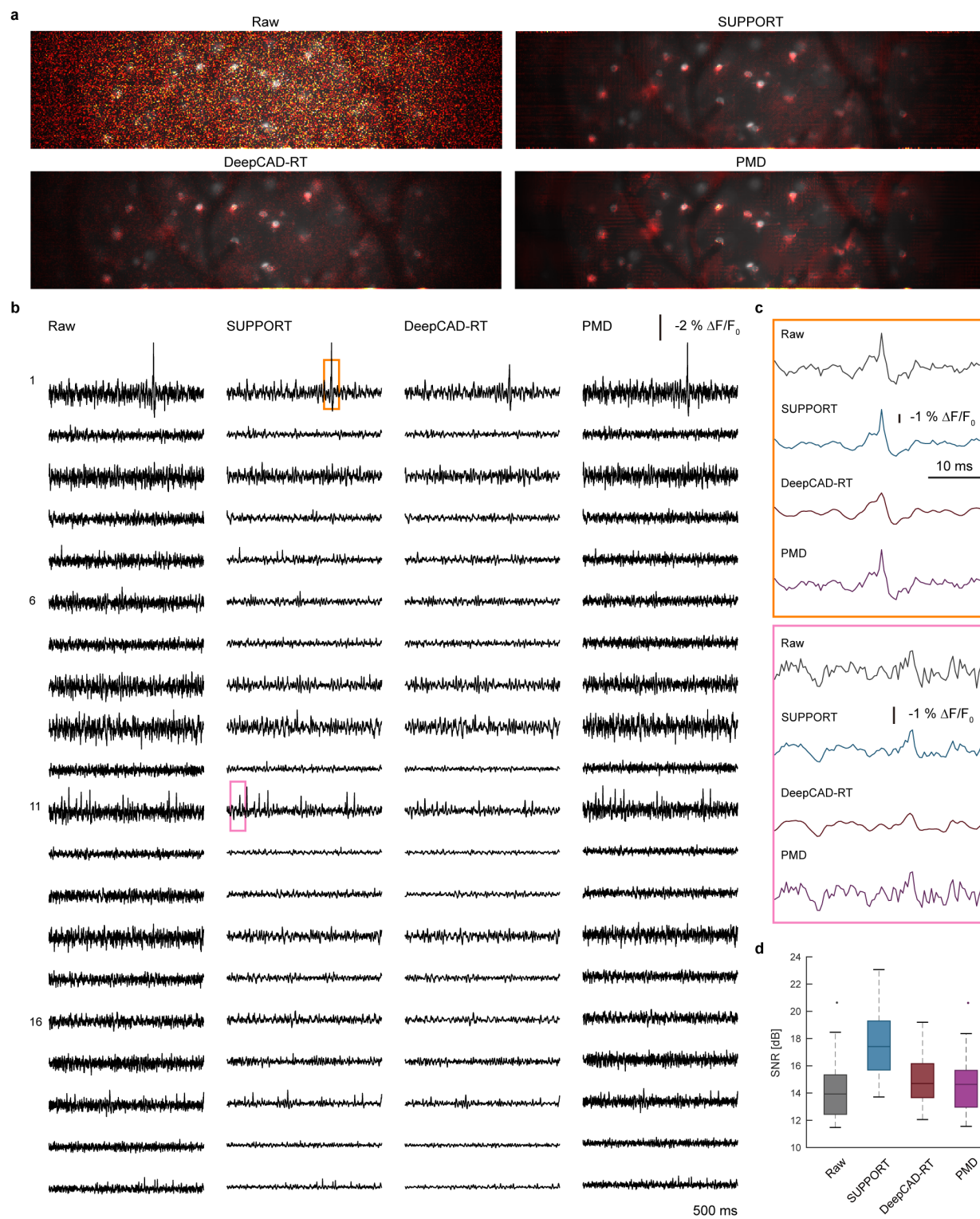
222 SUPPORT, DeepCAD-RT, and PMD. **c**, Enlarged view of traces from colored box in **b** are plotted. **d**, Box-
223 and-whisker plot showing the signal-to-noise ratio for the extracted traces. N=39, which represents the
224 number of neurons.



225

226 **Supplementary Fig. 27: Denoising population mouse voltage imaging data.** **a**, Images after baseline
 227 correction from mouse dataset expressing Voltron1 in cortex layer 1. **b**, Traces from 20 ROIs from raw,

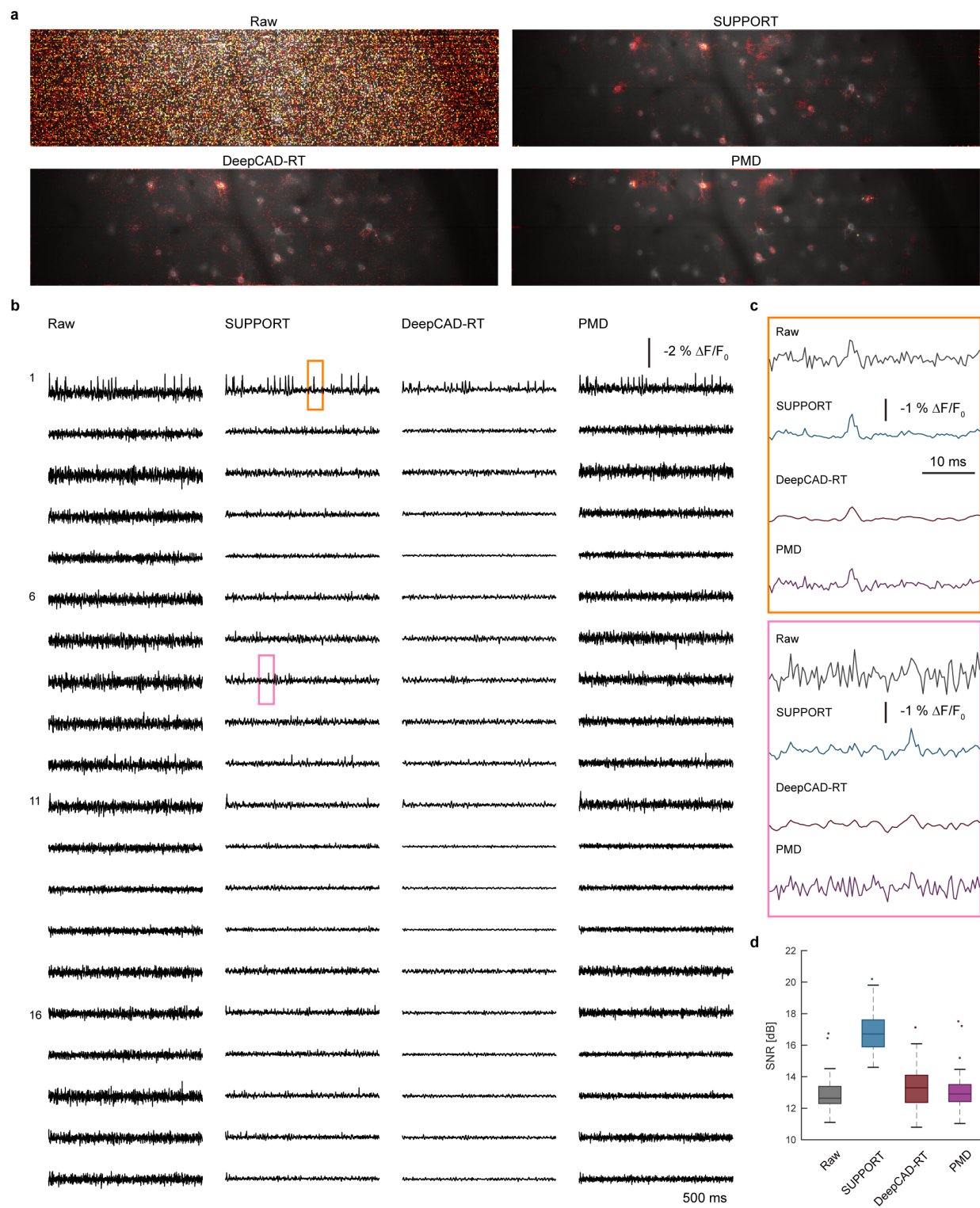
228 SUPPORT, DeepCAD-RT, and PMD. **c**, Enlarged view of traces from colored box in **b** are plotted. **d**, Box-
229 and-whisker plot showing the signal-to-noise ratio for the extracted traces. $N=77$, which represents the
230 number of neurons.



231

232 **Supplementary Fig. 28: Denoising population mouse voltage imaging data.** **a**, Images after baseline
 233 correction from mouse dataset expressing Voltron1 in cortex layer 1. **b**, Traces from 20 ROIs from raw,

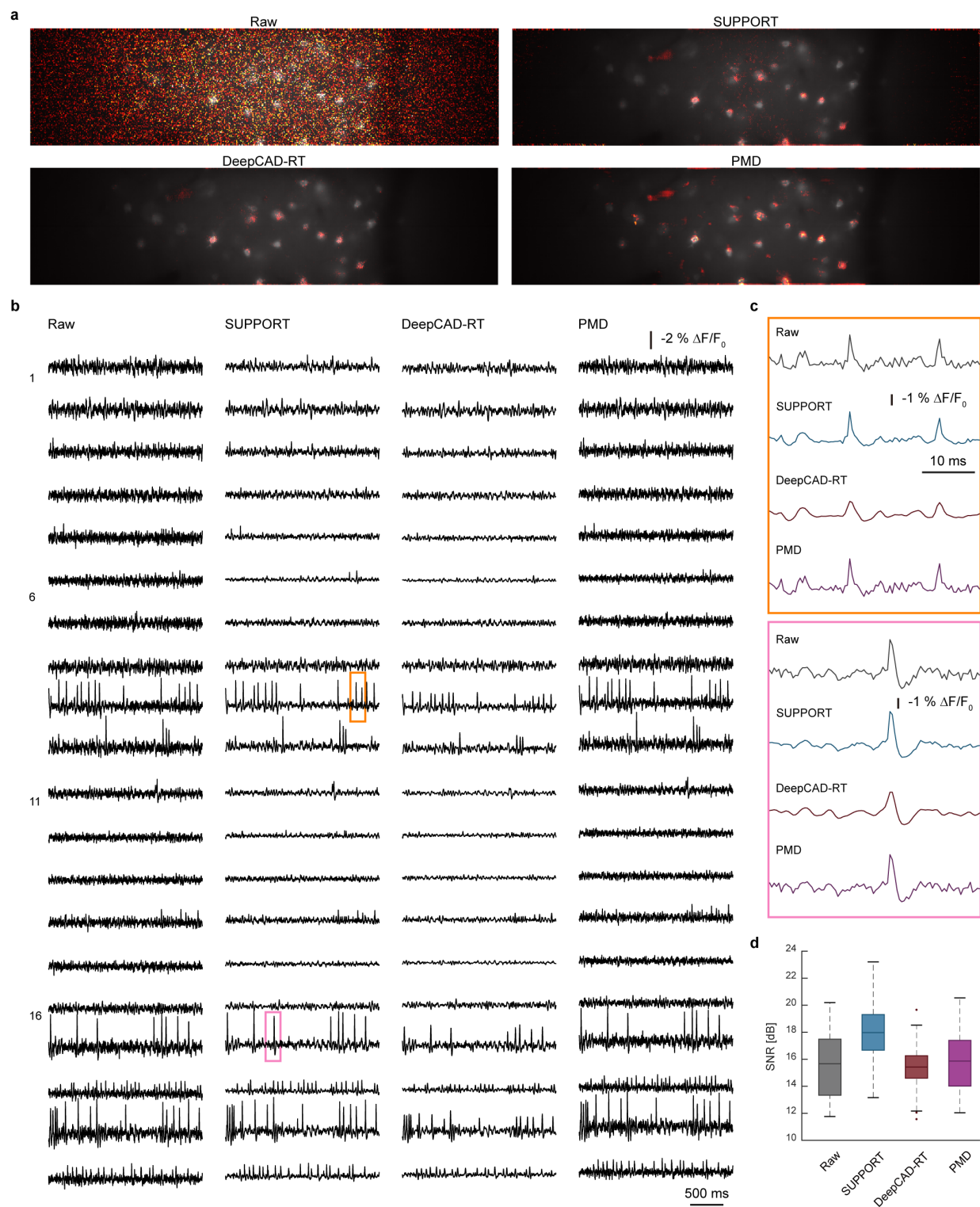
234 SUPPORT, DeepCAD-RT, and PMD. **c**, Enlarged view of traces from colored box in **b** are plotted. **d**, Box-
235 and-whisker plot showing the signal-to-noise ratio for the extracted traces. N=49, which represents the
236 number of neurons.



237

238 **Supplementary Fig. 29: Denoising population mouse voltage imaging data.** **a**, Images after baseline
 239 correction from mouse dataset expressing Voltron1 in cortex layer 1. **b**, Traces from 20 ROIs from raw,

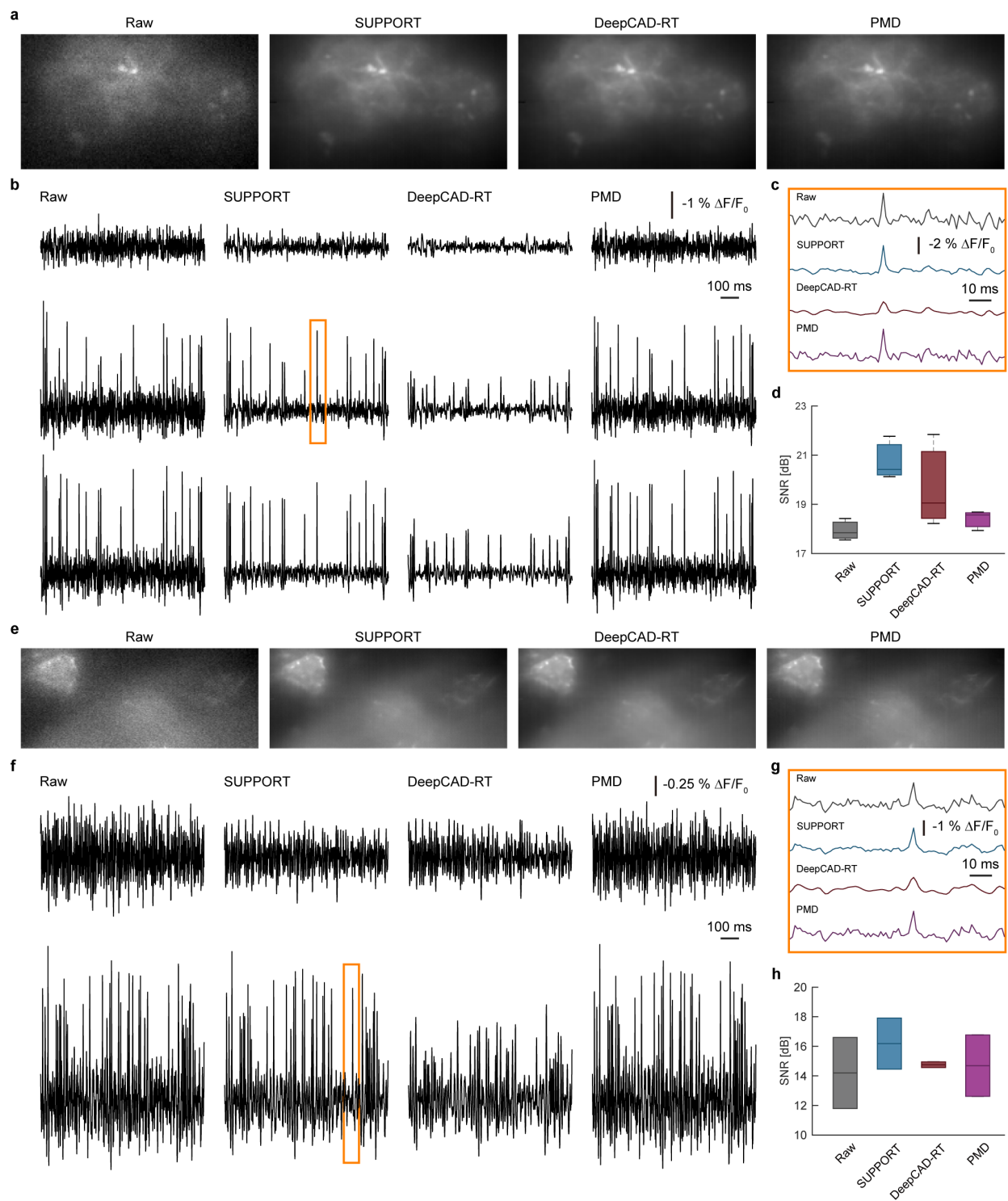
240 SUPPORT, DeepCAD-RT, and PMD. **c**, Enlarged view of traces from colored box in **b** are plotted. **d**, Box-
241 and-whisker plot showing the signal-to-noise ratio for the extracted traces. N=39, which represents the
242 number of neurons.



243

244 **Supplementary Fig. 30: Denoising population mouse voltage imaging data.** **a**, Images after baseline
 245 correction from mouse dataset expressing Voltron1 in cortex layer 1. **b**, Traces from 20 ROIs from raw,

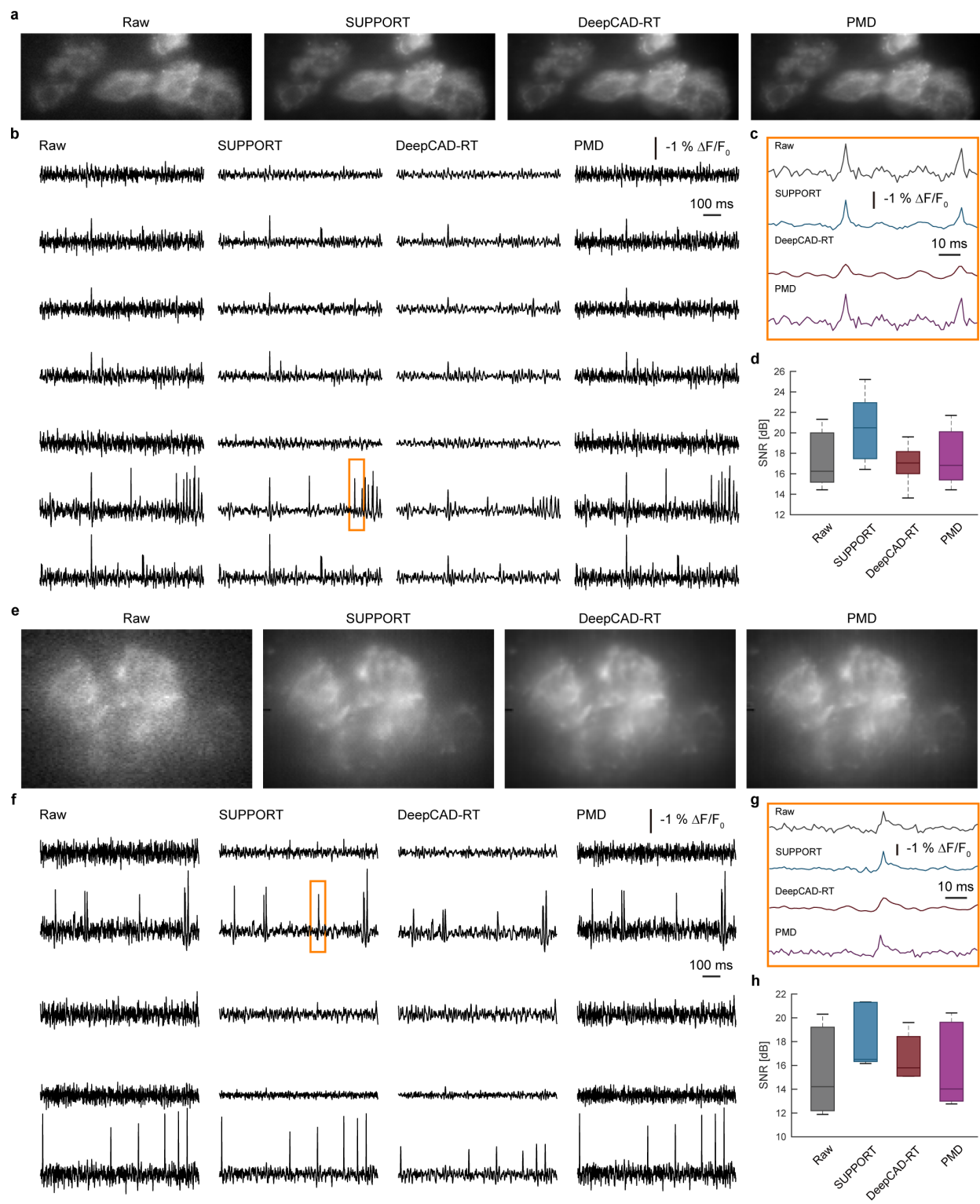
246 SUPPORT, DeepCAD-RT, and PMD. **c**, Enlarged view of traces from colored box in **b** are plotted. **d**, Box-
247 and-whisker plot showing the signal-to-noise ratio for the extracted traces. N=33, which represents the
248 number of neurons.



249

250 **Supplementary Fig. 31: Denoising population mouse voltage imaging data.** **a**, Images from mouse
 251 dataset expressing paQuasAr3s in hippocampus. **b**, Traces from 3 ROIs from raw, SUPPORT, DeepCAD-
 252 RT, and PMD. **c**, Enlarged view of traces from colored box in **b** are plotted. **d**, Box-and-whisker plot
 253 showing the signal-to-noise ratio for the extracted traces. $N=3$, which represents the number of neurons. **e**,

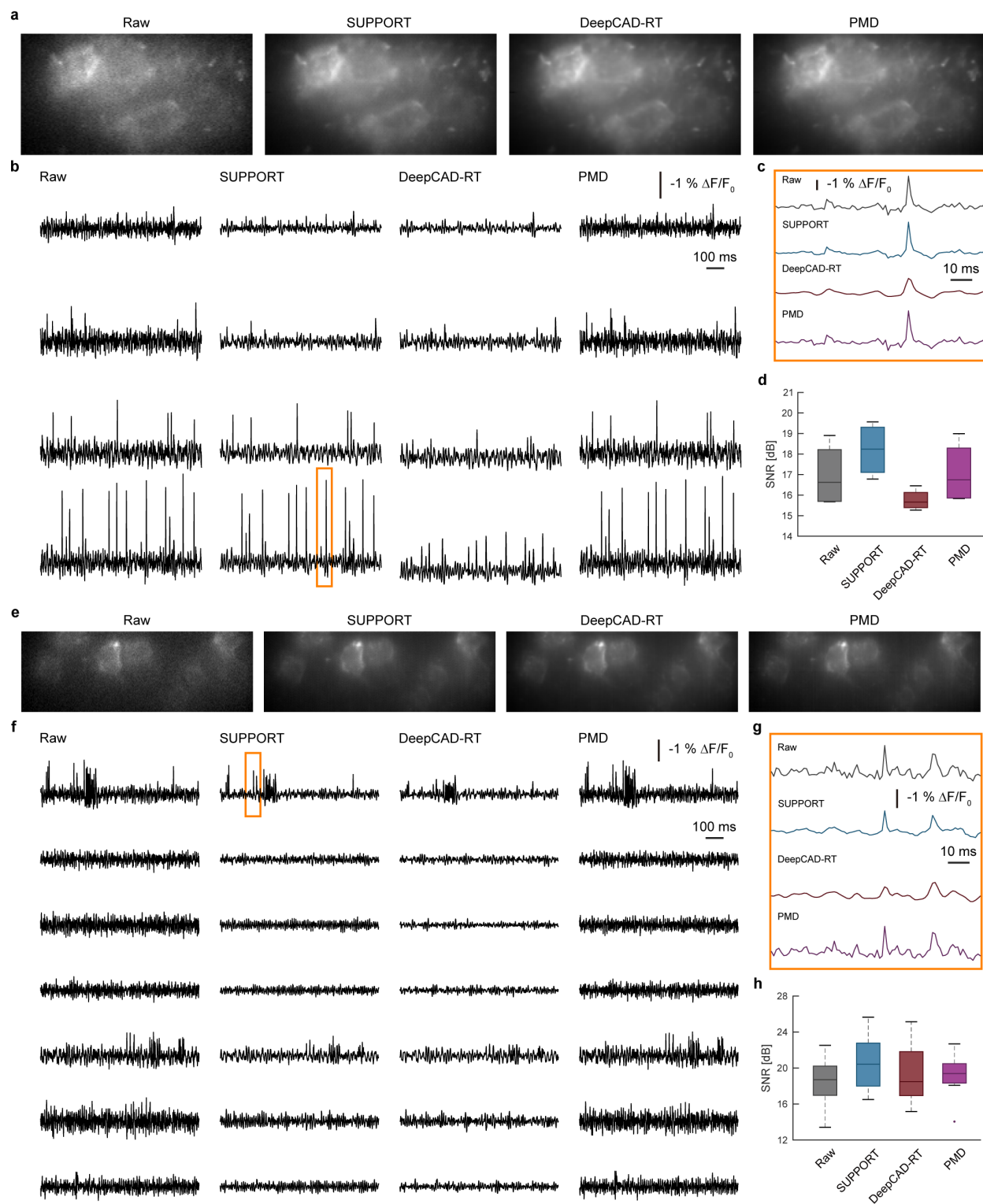
254 Images from mouse dataset expressing paQuasAr3s in hippocampus. **f**, Traces from 2 ROIs from raw,
255 SUPPORT, DeepCAD-RT, and PMD. **g**, Enlarged view of traces from colored box in **f** are plotted. **h**, Box-
256 and-whisker plot showing the signal-to-noise ratio for the extracted traces. $N=2$, which represents the
257 number of neurons.



258

259 **Supplementary Fig. 32: Denoising population mouse voltage imaging data.** **a**, Images from mouse
 260 dataset expressing paQuasAr3s in hippocampus. **b**, Traces from 7 ROIs from raw, SUPPORT, DeepCAD-
 261 RT, and PMD. **c**, Enlarged view of traces from colored box in **b** are plotted. **d**, Box-and-whisker plot
 262 showing the signal-to-noise ratio for the extracted traces. $N=7$, which represents the number of neurons. **e**,

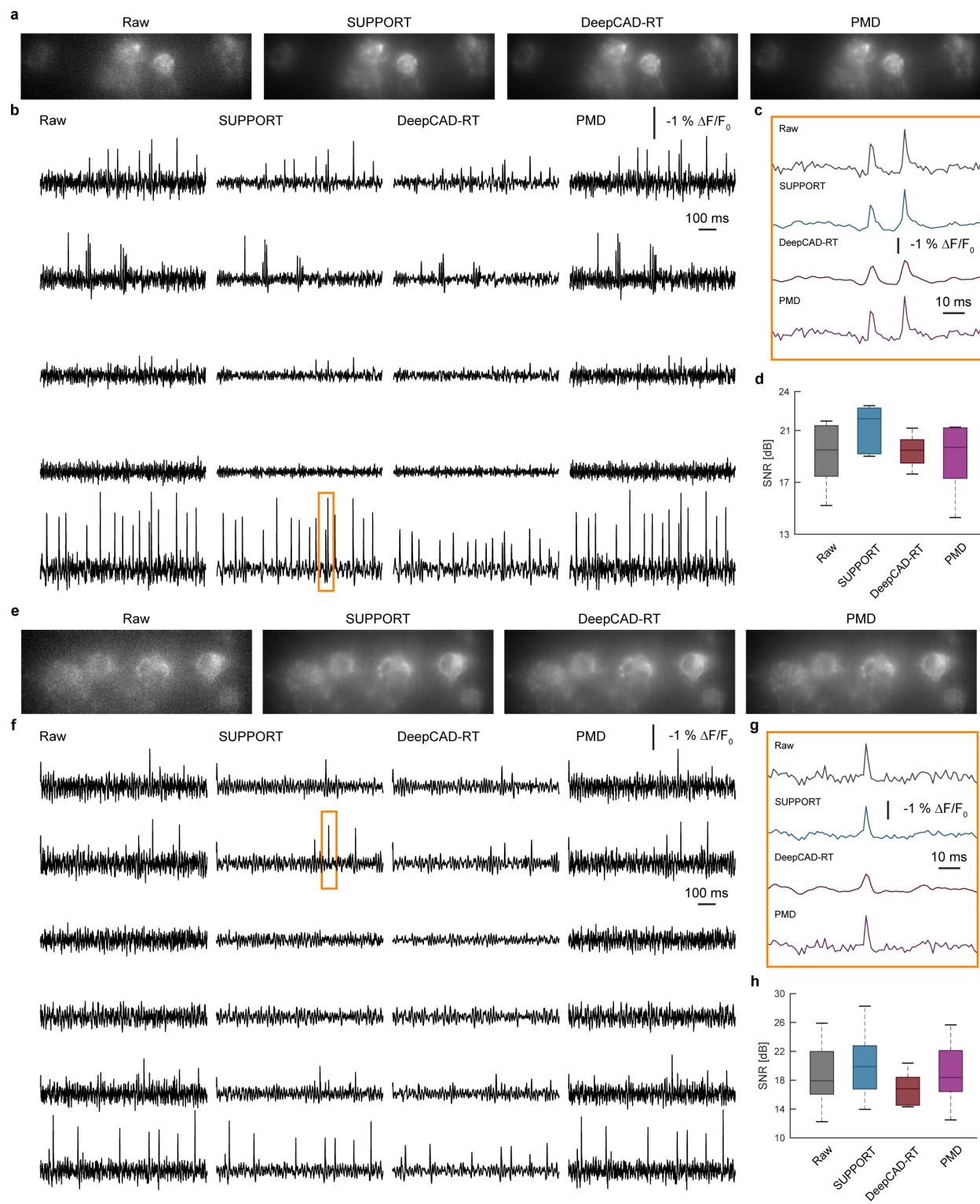
263 Images from mouse dataset expressing paQuasAr3s in hippocampus. **f**, Traces from 5 ROIs from raw,
264 SUPPORT, DeepCAD-RT, and PMD. **g**, Enlarged view of traces from colored box in **f** are plotted. **h**, Box-
265 and-whisker plot showing the signal-to-noise ratio for the extracted traces. $N=5$, which represents the
266 number of neurons.



267

268 **Supplementary Fig. 33: Denoising population mouse voltage imaging data.** **a**, Images from mouse
 269 dataset expressing paQuasAr3s in hippocampus. **b**, Traces from 4 ROIs from raw, SUPPORT, DeepCAD-
 270 RT, and PMD. **c**, Enlarged view of traces from colored box in **b** are plotted. **d**, Box-and-whisker plot
 271 showing the signal-to-noise ratio for the extracted traces. $N=4$, which represents the number of neurons. **e**,

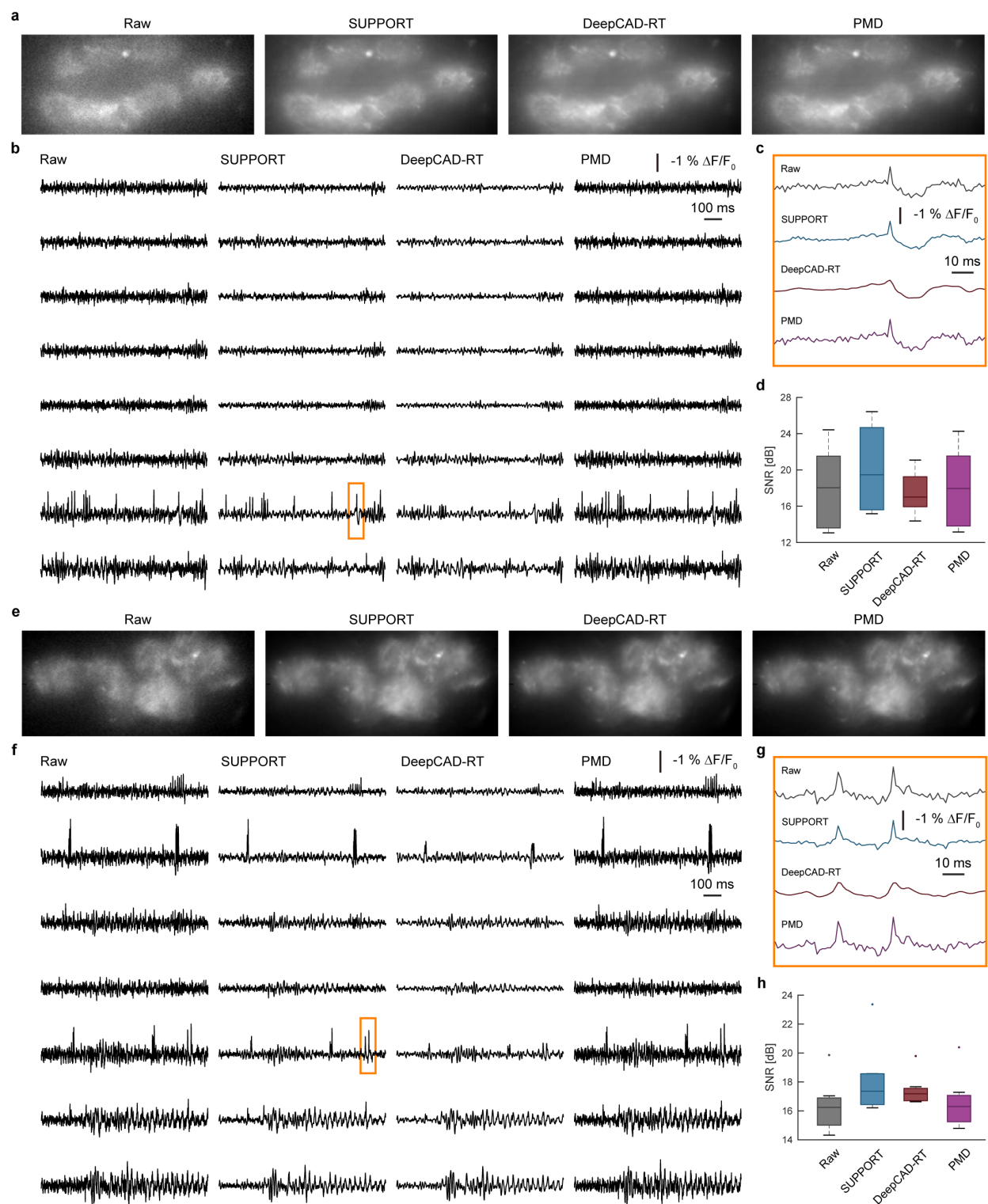
272 Images from mouse dataset expressing paQuasAr3s in hippocampus. **f**, Traces from 7 ROIs from raw,
273 SUPPORT, DeepCAD-RT, and PMD. **g**, Enlarged view of traces from colored box in **f** are plotted. **h**, Box-
274 and-whisker plot showing the signal-to-noise ratio for the extracted traces. $N=7$, which represents the
275 number of neurons.



276

277 **Supplementary Fig. 34: Denoising population mouse voltage imaging data.** **a**, Images from mouse
 278 dataset expressing paQuasAr3s in hippocampus. **b**, Traces from 5 ROIs from raw, SUPPORT, DeepCAD-
 279 RT, and PMD. **c**, Enlarged view of traces from colored box in **b** are plotted. **d**, Box-and-whisker plot
 280 showing the signal-to-noise ratio for the extracted traces. $N=5$, which represents the number of neurons. **e**,

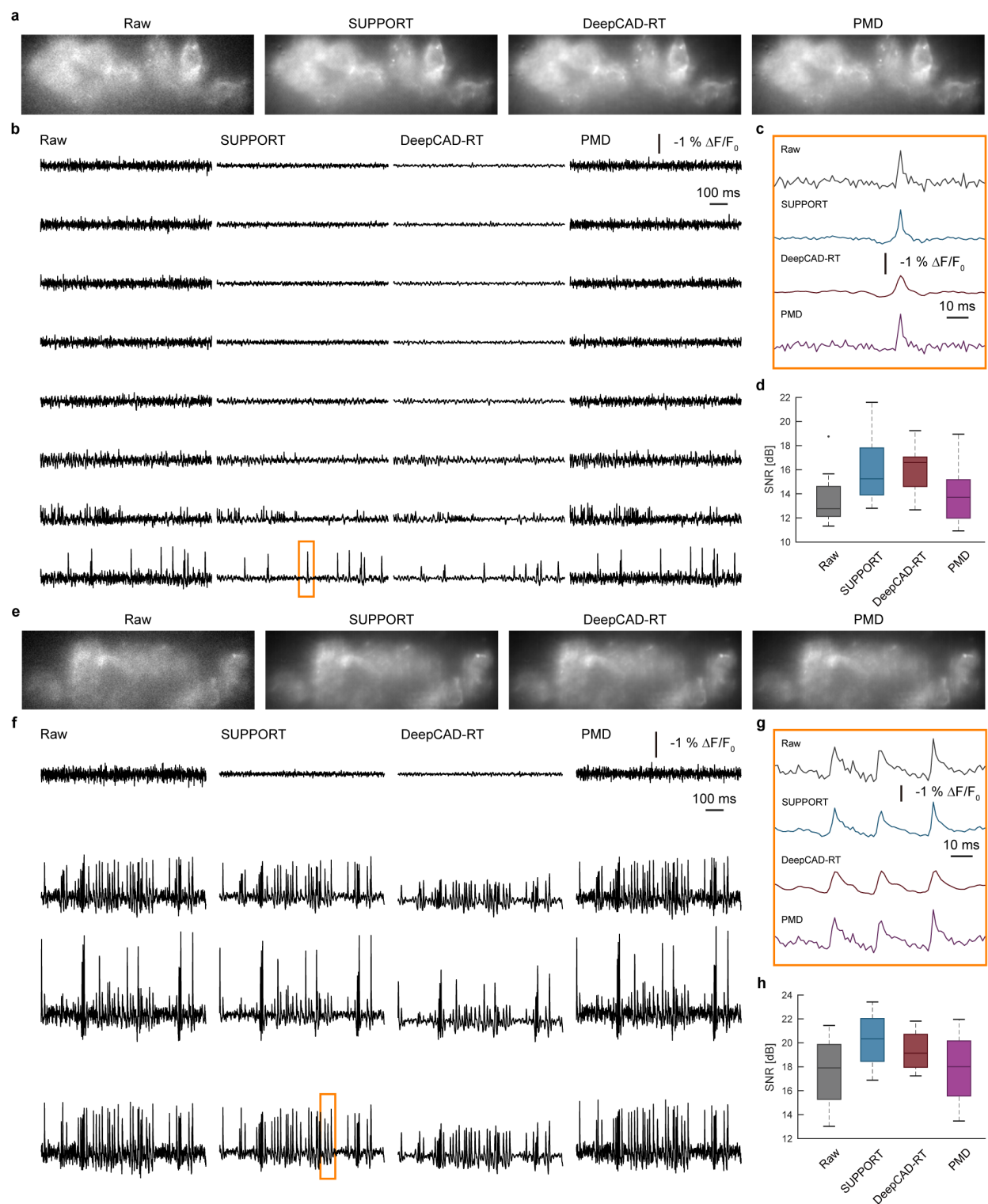
281 Images from mouse dataset expressing paQuasAr3s in hippocampus. **f**, Traces from 6 ROIs from raw,
282 SUPPORT, DeepCAD-RT, and PMD. **g**, Enlarged view of traces from colored box in **f** are plotted. **h**, Box-
283 and-whisker plot showing the signal-to-noise ratio for the extracted traces. $N=5$, which represents the
284 number of neurons.



285

286 **Supplementary Fig. 35: Denoising population mouse voltage imaging data.** **a**, Images from mouse
 287 dataset expressing paQuasAr3s in hippocampus. **b**, Traces from 8 ROIs from raw, SUPPORT, DeepCAD-
 288 RT, and PMD. **c**, Enlarged view of traces from colored box in **b** are plotted. **d**, Box-and-whisker plot
 289 showing the signal-to-noise ratio for the extracted traces. $N=8$, which represents the number of neurons. **e**,

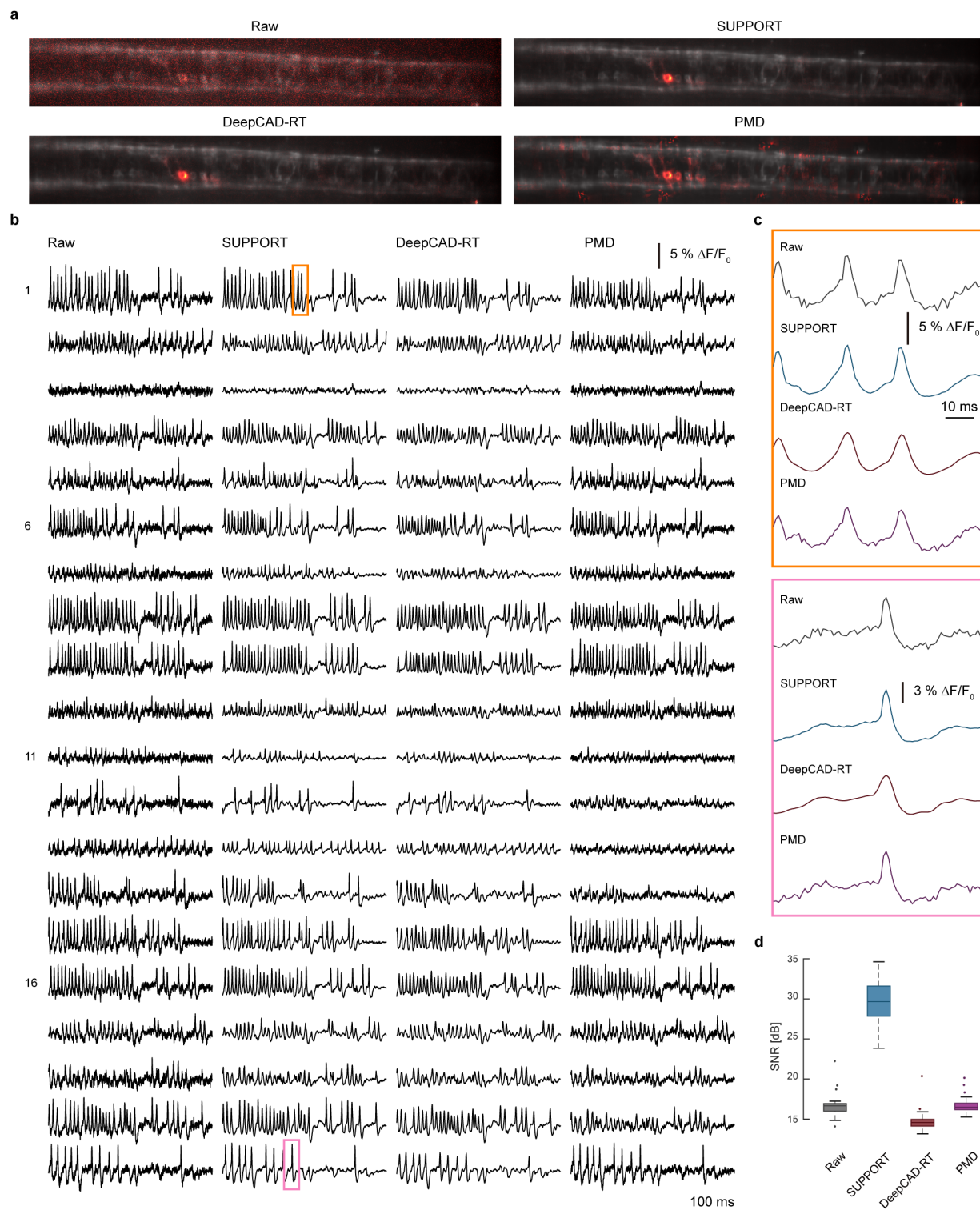
290 Images from mouse dataset expressing paQuasAr3s in hippocampus. **f**, Traces from 7 ROIs from raw,
291 SUPPORT, DeepCAD-RT, and PMD. **g**, Enlarged view of traces from colored box in **f** are plotted. **h**, Box-
292 and-whisker plot showing the signal-to-noise ratio for the extracted traces. $N=7$, which represents the
293 number of neurons.



294

295 **Supplementary Fig. 36: Denoising population mouse voltage imaging data.** **a**, Images from mouse
 296 dataset expressing paQuasAr3s in hippocampus. **b**, Traces from 8 ROIs from raw, SUPPORT, DeepCAD-
 297 RT, and PMD. **c**, Enlarged view of traces from colored box in **b** are plotted. **d**, Box-and-whisker plot
 298 showing the signal-to-noise ratio for the extracted traces. N=8, which represents the number of neurons. **e**,

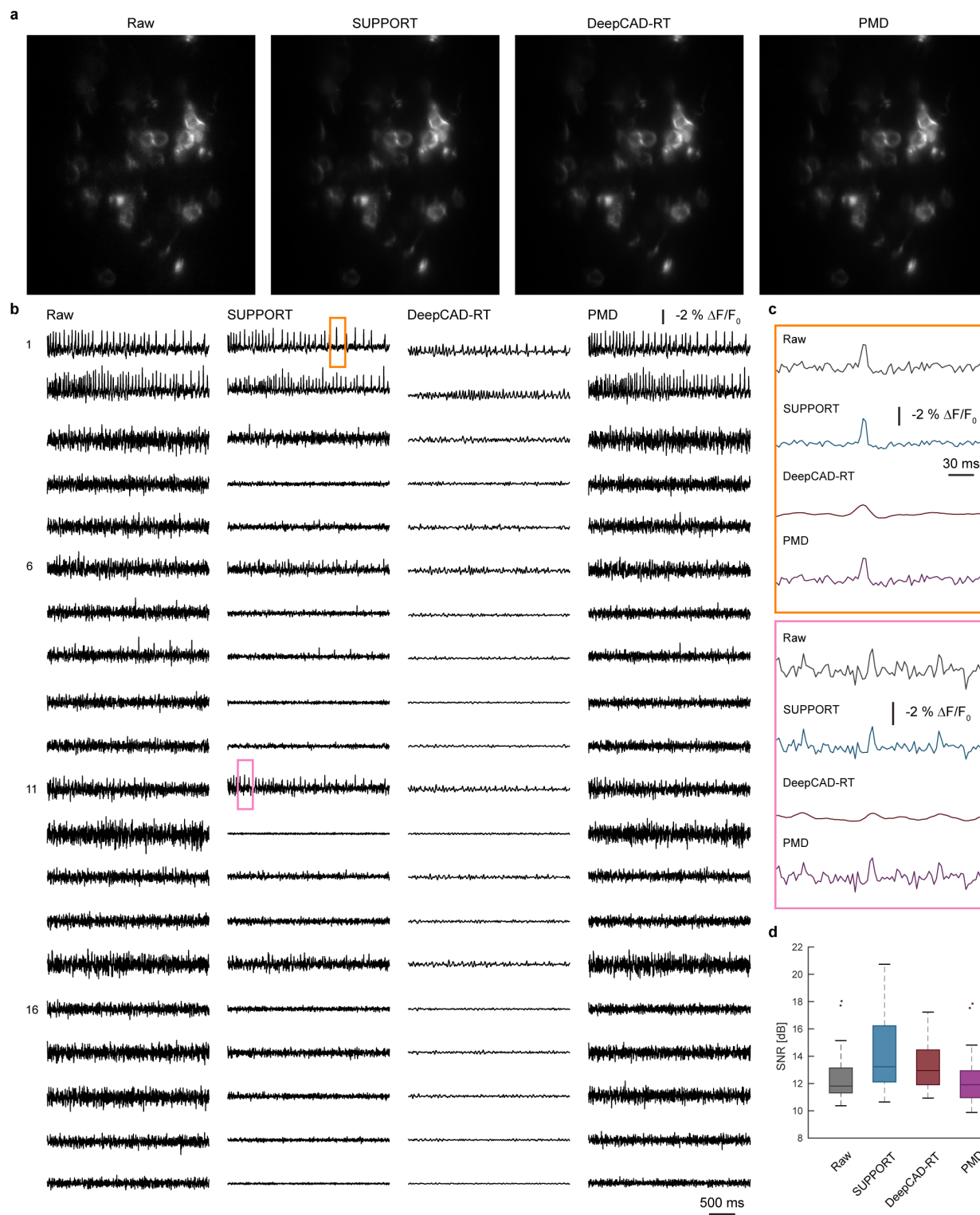
299 Images from mouse dataset expressing paQuasAr3s in hippocampus. **f**, Traces from 4 ROIs from raw,
300 SUPPORT, DeepCAD-RT, and PMD. **g**, Enlarged view of traces from colored box in **f** are plotted. **h**, Box-
301 and-whisker plot showing the signal-to-noise ratio for the extracted traces. $N=4$, which represents the
302 number of neurons.



303

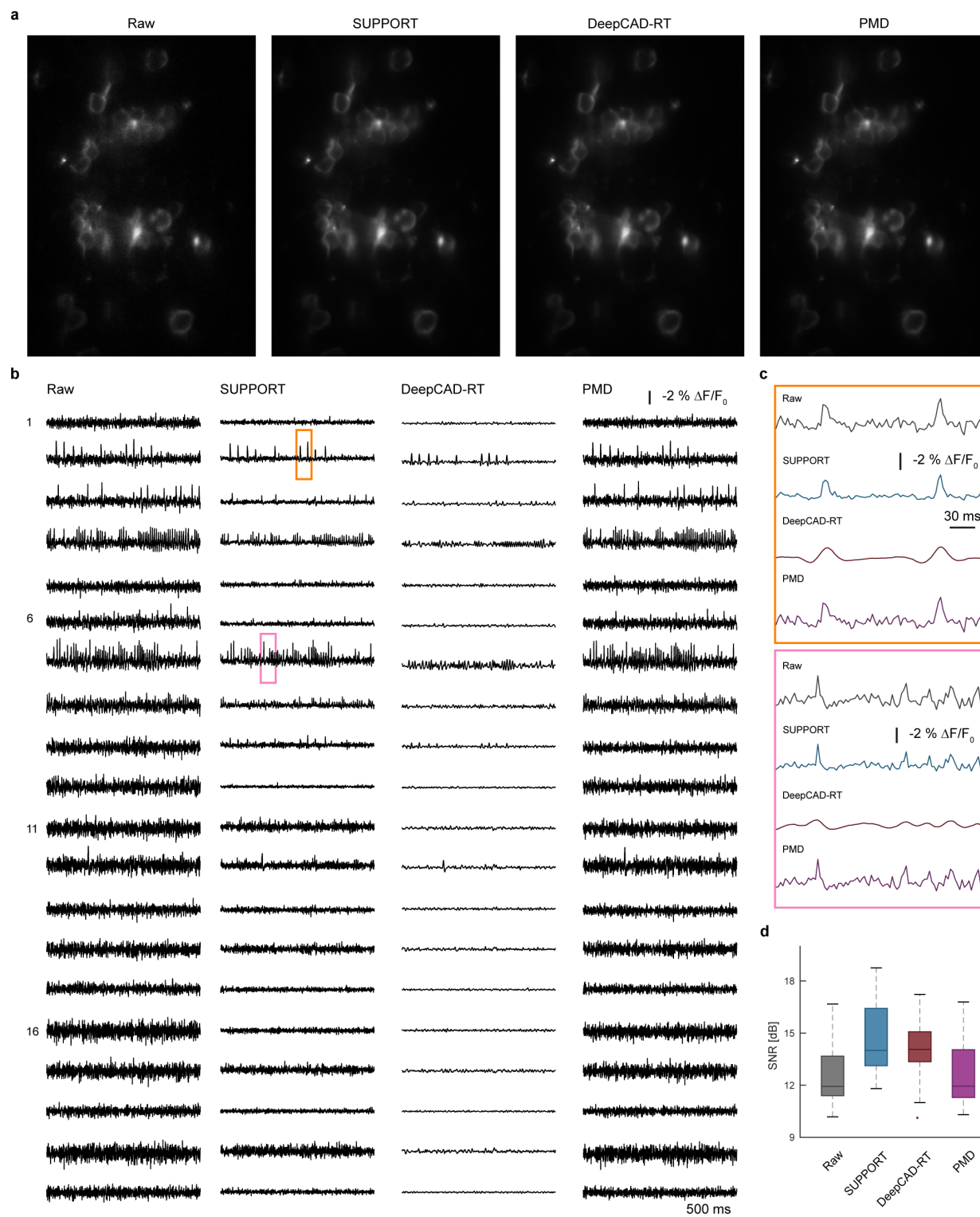
304 **Supplementary Fig. 37: Denoising population zebrafish voltage imaging data.** **a**, Images after baseline
 305 correction from zebrafish dataset expressing zArchon in spinal cord. **b**, Traces from 20 ROIs from raw,
 306 SUPPORT, DeepCAD-RT, and PMD. **c**, Enlarged view of traces from colored box in **b** are plotted. **d**, Box-
 307 and-whisker plot showing the signal-to-noise ratio for the extracted traces. N=20, which represents the

308 number of neurons.



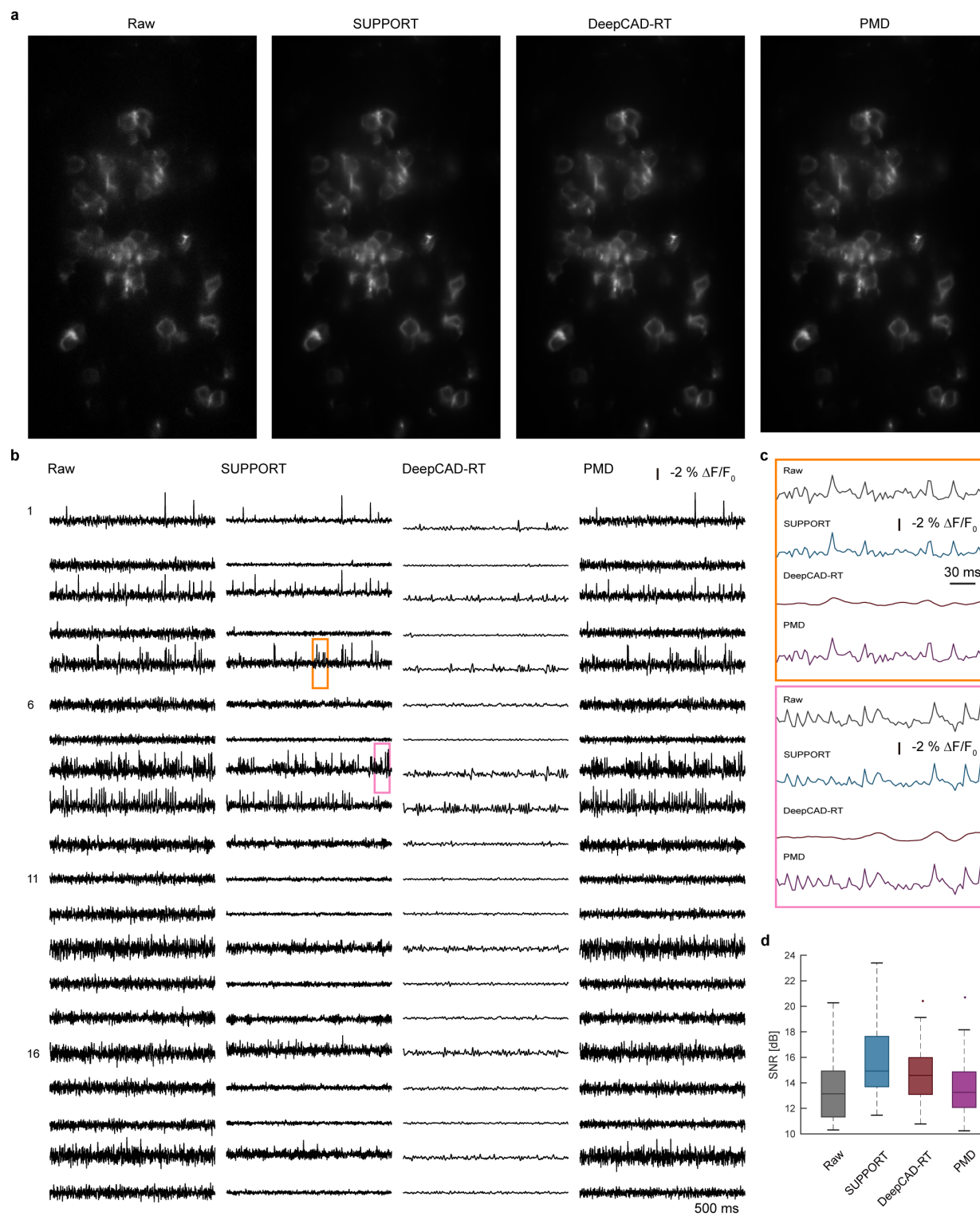
309

310 **Supplementary Fig. 38: Denoising population zebrafish voltage imaging data.** **a**, Images from zebrafish
 311 dataset expressing Voltron1 in tegmental region. **b**, Traces from 20 ROIs from raw, SUPPORT, DeepCAD-
 312 RT, and PMD. **c**, Enlarged view of traces from colored box in **b** are plotted. **d**, Box-and-whisker plot
 313 showing the signal-to-noise ratio for the extracted traces. N=31, which represents the number of neurons.



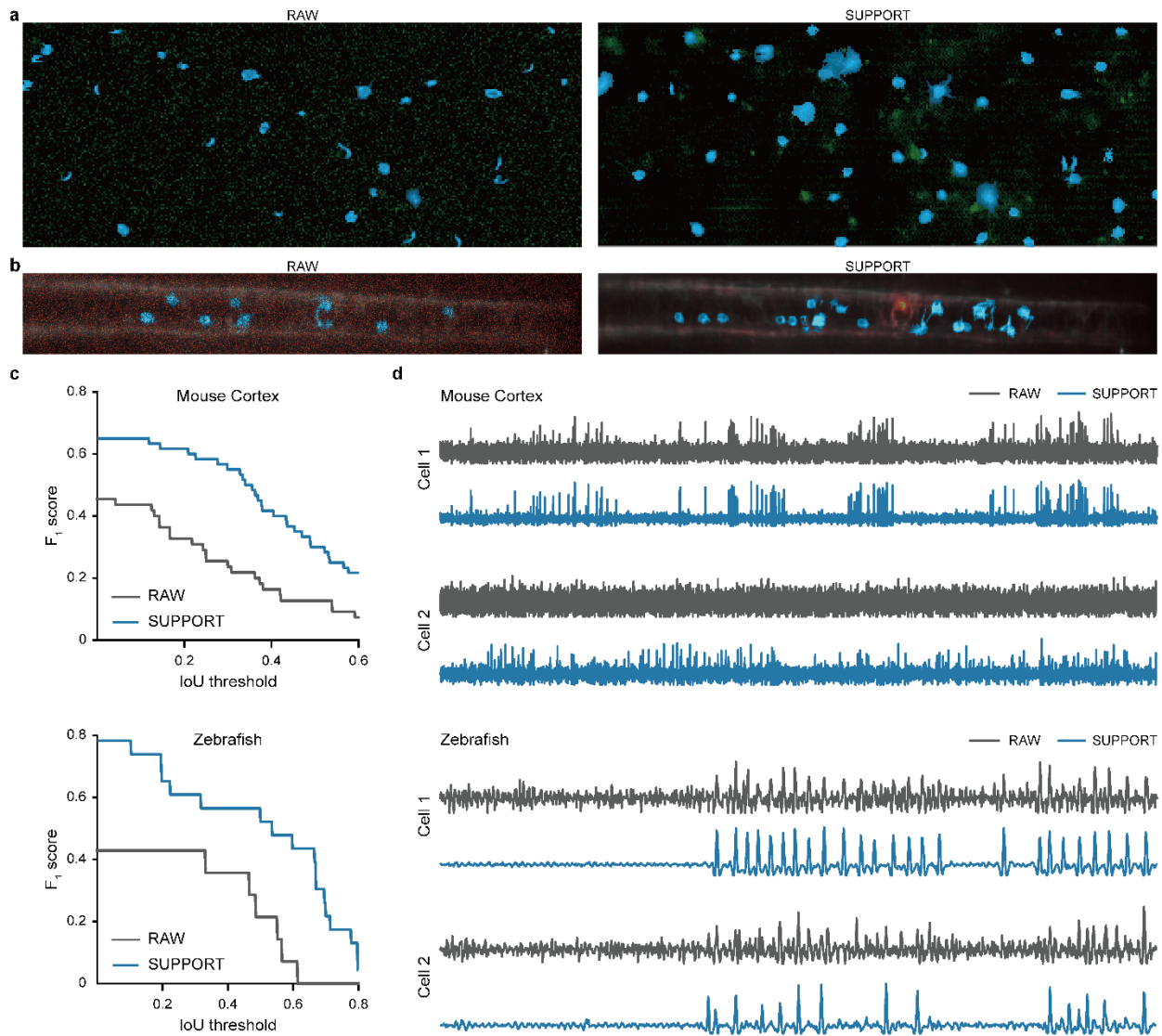
314

315 **Supplementary Fig. 39: Denoising population zebrafish voltage imaging data.** **a**, Images from zebrafish
 316 dataset expressing Voltron1 in tegmental region. **b**, Traces from 20 ROIs from raw, SUPPORT, DeepCAD-
 317 RT, and PMD. **c**, Enlarged view of traces from colored box in **b** are plotted. **d**, Box-and-whisker plot
 318 showing the signal-to-noise ratio for the extracted traces. N=28, which represents the number of neurons.



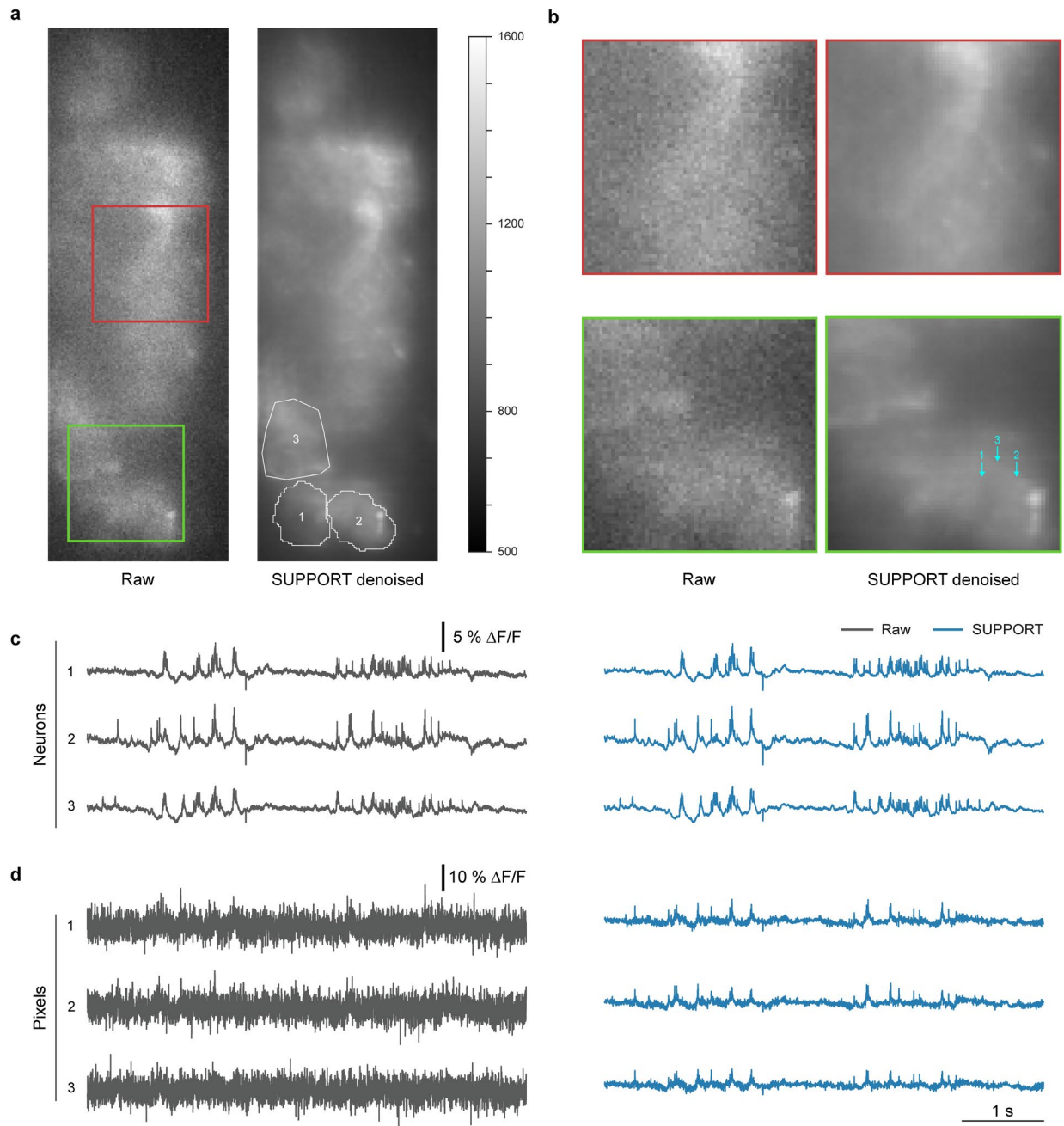
319

320 **Supplementary Fig. 40: Denoising population zebrafish voltage imaging data.** **a**, Images from zebrafish
 321 dataset expressing Voltron1 in tegmental region. **b**, Traces from 20 ROIs from raw, SUPPORT, DeepCAD-
 322 RT, and PMD. **c**, Enlarged view of traces from colored box in **b** are plotted. **d**, Box-and-whisker plot
 323 showing the signal-to-noise ratio for the extracted traces. N=41, which represents the number of neurons.



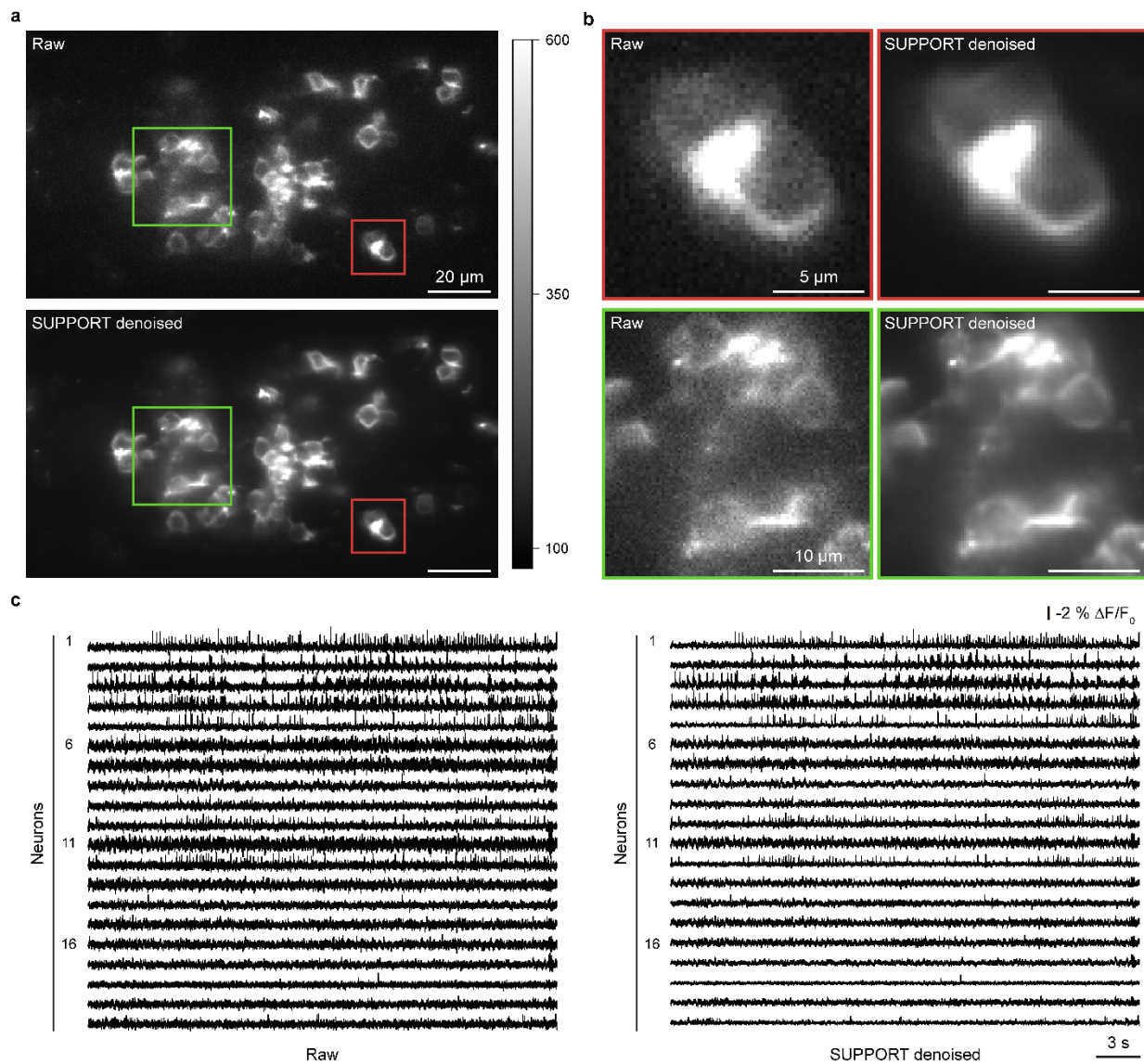
324

325 **Supplementary Fig. 41: Neuronal source extraction using localNMF from population voltage imaging.**
 326 **a**, Extracted neurons from raw and SUPPORT-denoised mouse videos are colored in blue and overlaid on
 327 the images of Fig. 4a. **b**, Extracted neurons from raw and SUPPORT-denoised zebrafish videos are colored
 328 in blue and overlaid on the images of Fig. 4d. **c**, F_1 scores across intersection-over-union thresholds. **d**,
 329 Extracted temporal signals from raw and SUPPORT-denoised video for two representative cells.



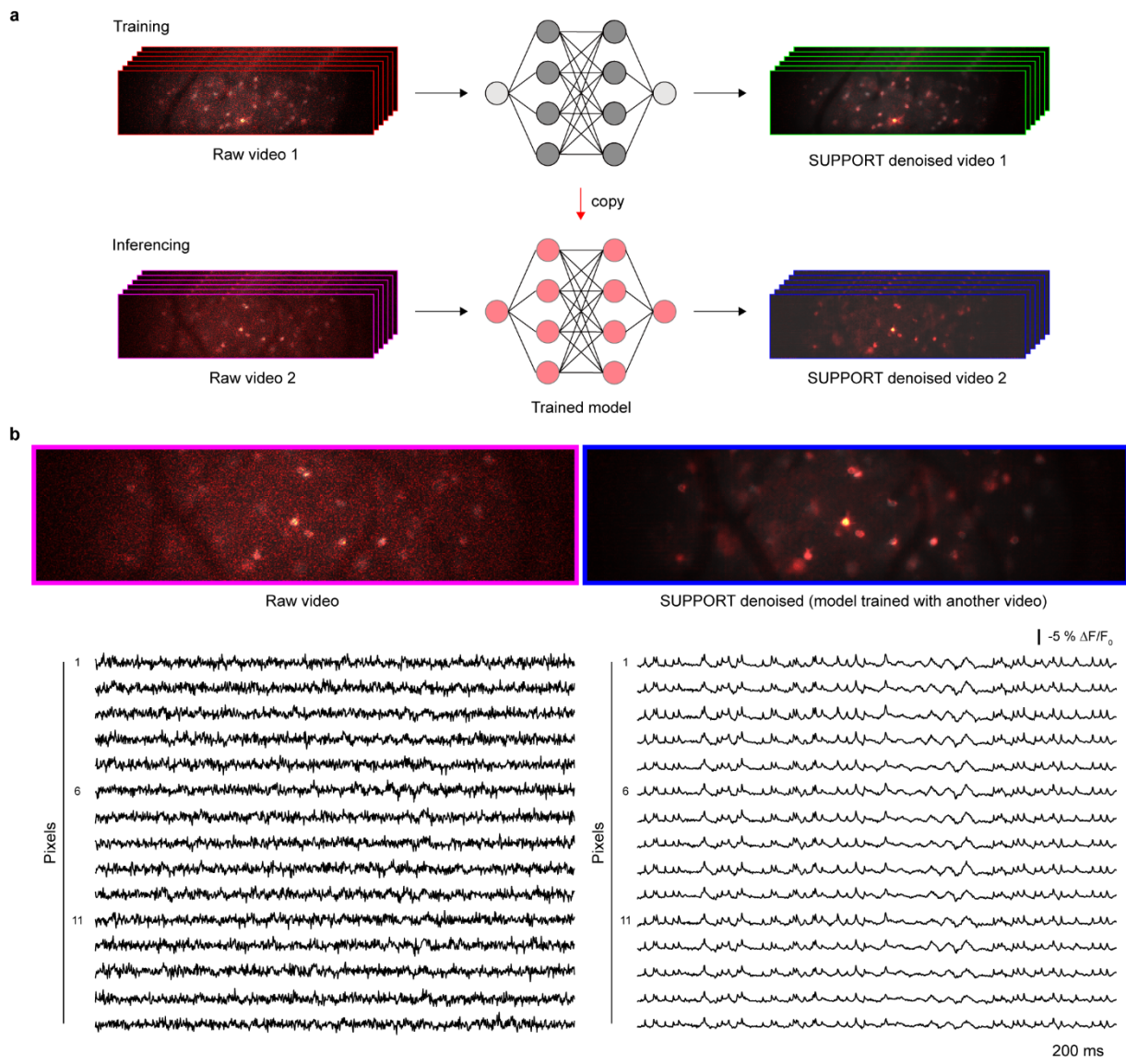
330

331 **Supplementary Fig. 42: Applying SUPPORT to population voltage imaging data with paQuasAr3s**
 332 **indicator.** **a**, Representative frames from raw video and SUPPORT-denosed video. paQuasAr3s-
 333 expressing mouse hippocampus CA1 was used as a dataset (Supplementary Table 1). Boundaries of three
 334 regions of interest (ROIs) are drawn with white lines. **b**, Magnified views of the boxed regions in **b**. **c**,
 335 Traces extracted from raw and SUPPORT-denosed videos for three ROIs in **a**. **d**, Single pixel traces from
 336 raw and SUPPORT-denosed video for three pixels indicated in **b**.



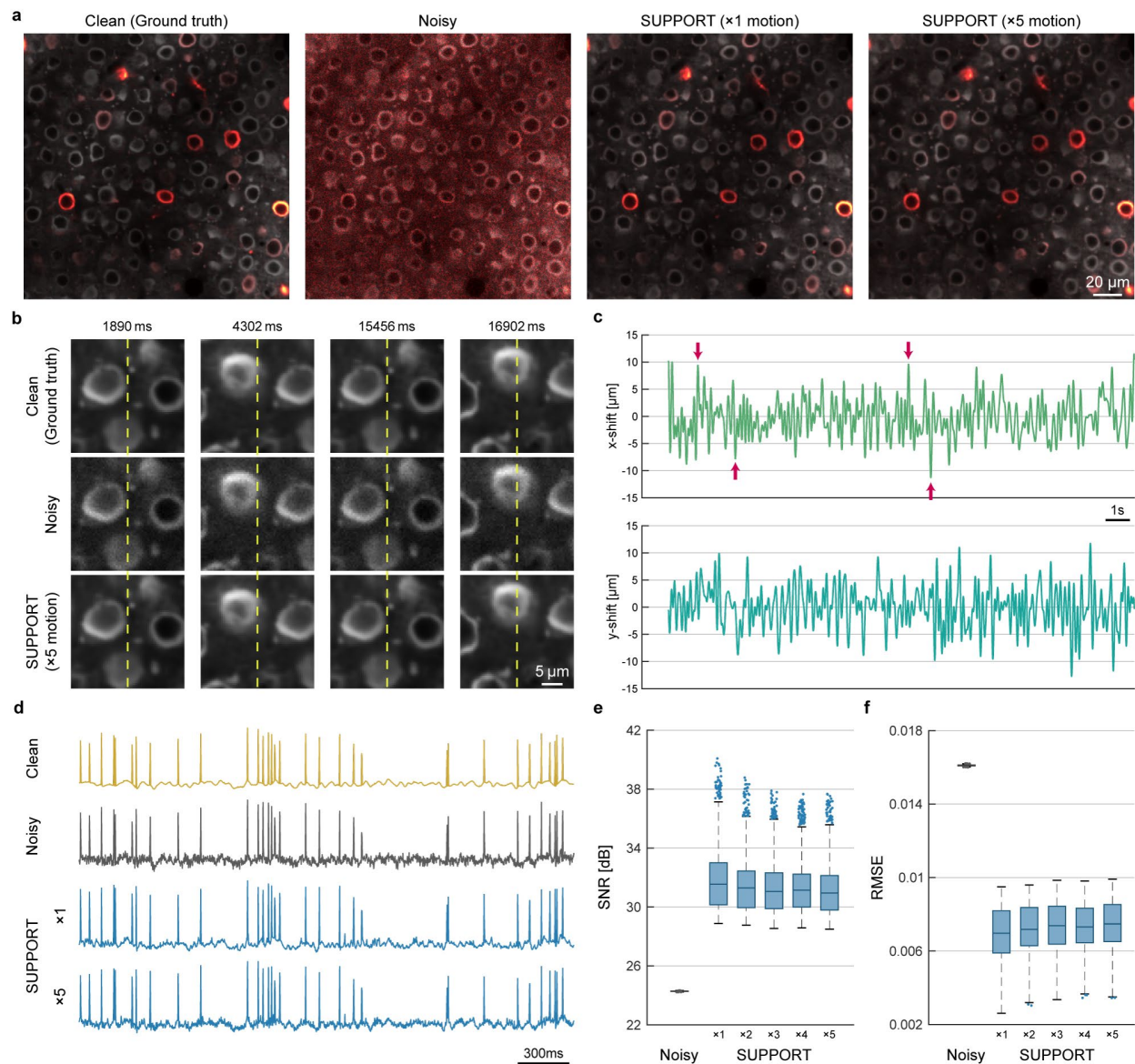
337

338 **Supplementary Fig. 43: Applying SUPPORT to population voltage imaging data with Voltron1**
 339 **indicator.** **a**, Representative frames from raw video and SUPPORT-denoised video. Voltron1-expressing
 340 zebrafish tegmental area was used as a dataset (Supplementary Table 1). **b**, Magnified views of the boxed
 341 regions in **a**. **c**, Traces extracted from the raw and SUPPORT-denoised video for 20 neurons. Left: From
 342 the raw video. Right: From SUPPORT-denoised video.



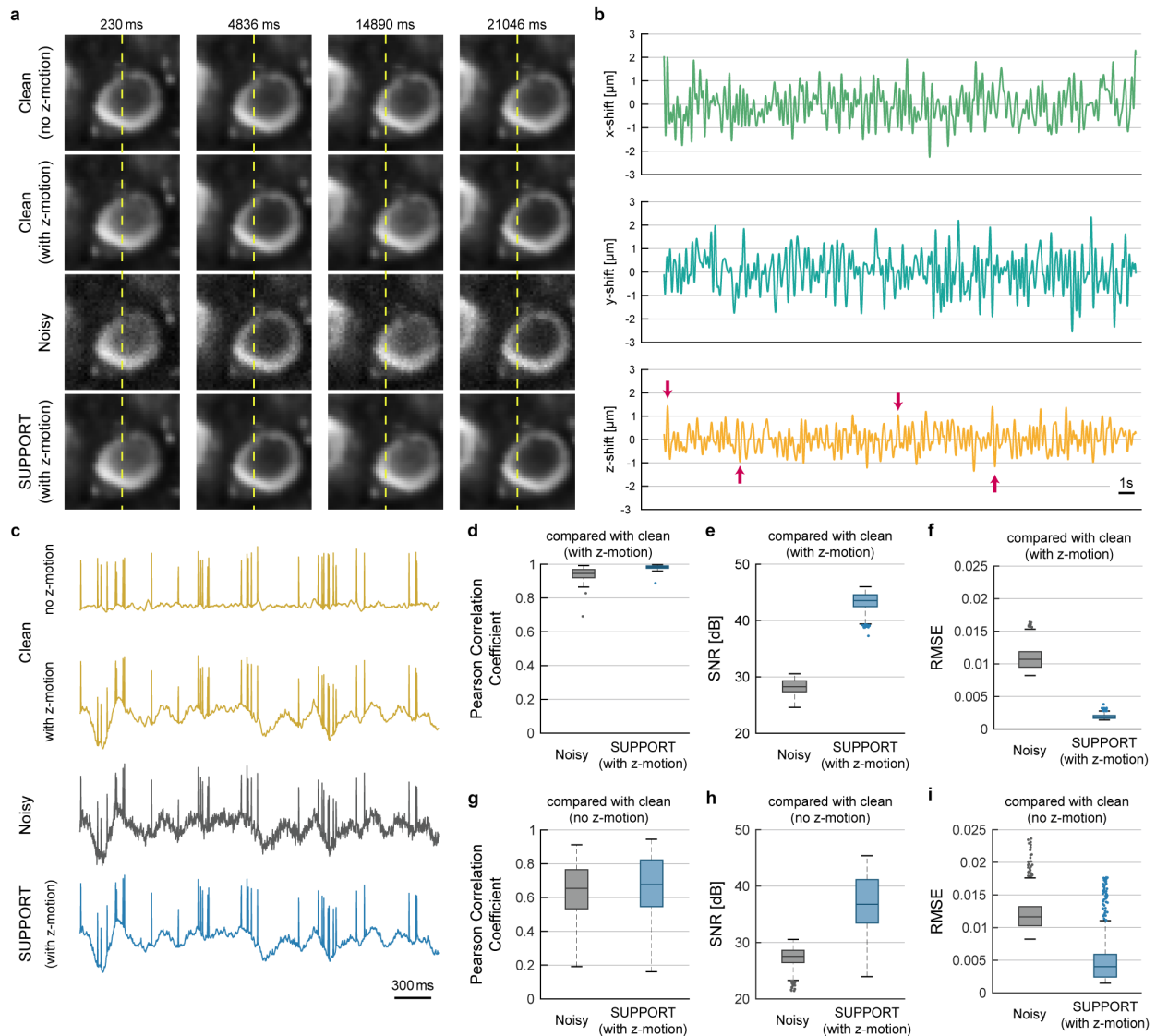
343

344 **Supplementary Fig. 44: Network generalization for population voltage imaging data.** **a**, Ability to
 345 generalize SUPPORT for denoising unseen dataset. Top: SUPPORT is first trained with raw video 1. Bottom:
 346 Trained SUPPORT network is used for denoising raw video 2, which is recorded from different fields of
 347 view and time. **b**, SUPPORT was able to denoise the unseen dataset without additional fine-tuning. Top: A
 348 representative frame of raw video and SUPPORT-denoised video, for which the raw data was never shown
 349 to SUPPORT on training. Bottom left: Single pixel traces inside one neuron from the raw video. Bottom
 350 right: Traces from SUPPORT-denoised video. This shows that the traces from SUPPORT-denoised video
 351 are highly correlated.



352

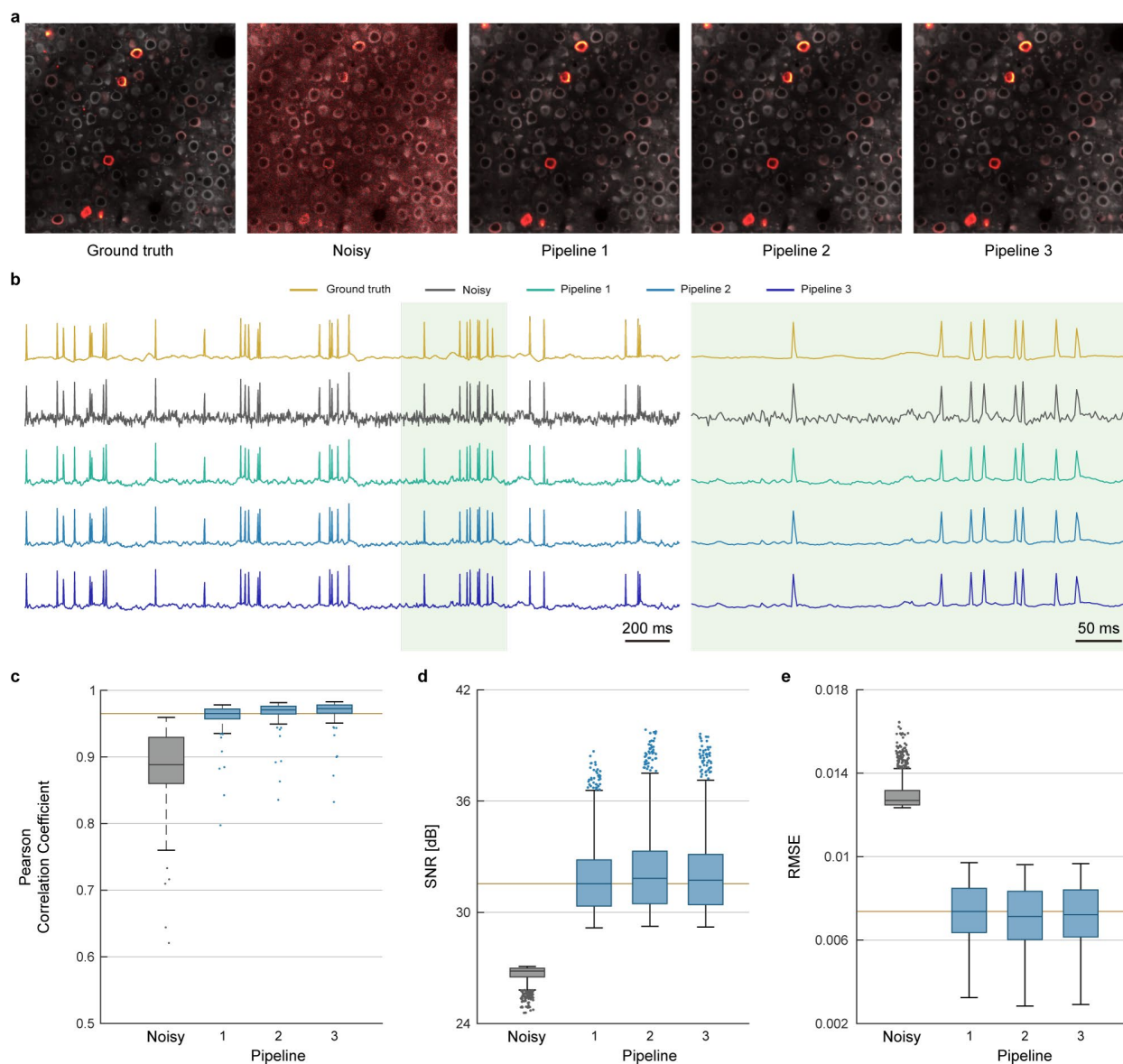
353 **Supplementary Fig. 45: Denoising simulated voltage imaging data with motion in the x and y**
 354 **directions.** **a**, Representative frames of clean video, noisy video, and SUPPORT-denoised videos with
 355 motion after baseline correction. $\times 5$ indicates a five times higher motion compared to $\times 1$. **b**, Spatially
 356 expanded view of representative frames at the timings indicated by red arrows in **c**. From left to right:
 357 frames at 1890ms, 4302ms, 15456ms, and 16902ms. From top to bottom: clean video, noisy video, and
 358 SUPPORT-denoised video with motion. **c**, Line plot showing x and y-direction motions in the micrometer
 359 scale. **d**, Traces extracted from a single cell of the clean video, noisy video, and SUPPORT-denoised video
 360 with motion. **e**, Box-and-whisker plot showing SNR before and after denoising data with motion. $N=1440$,
 361 which represents the number of planes along the t-axis. **f**, Box-and-whisker plot showing RMSE before and
 362 after denoising data with motion. $N=1440$, which represents the number of planes along the t-axis.



363

364 **Supplementary Fig. 46: Denoising simulated voltage imaging data with motion in the x, y, and z**
 365 **directions. a**, Representative frames indicated with red arrows in **b**. From left to right: frames at 230ms,
 366 4836ms, 14890ms, and 21046ms. From top to bottom: clean video without and with z-motion, noisy video,
 367 and SUPPORT-denoised video with z-motion. **b**, line plot showing x, y, and z-direction motions in the
 368 micrometer scale. **c**, Traces extracted from a single cell of the clean video without and with z-motion, noisy
 369 video, and SUPPORT-denoised video with z-motion. **d**, Box-and-whisker plot showing Pearson correlation
 370 coefficient before and after denoising data with z-motion compared with clean data with z-motion. N=102,
 371 which represents the number of neurons. **e**, Box-and-whisker plot showing SNR before and after denoising
 372 data with z-motion compared with clean data with z-motion. N=1470, which represents the number of
 373 planes along the t-axis. **f**, Box-and-whisker plot showing RMSE before and after denoising data with z-
 374 motion compared with clean data with z-motion. N=1470, which represents the number of planes along the
 375 t-axis. **g**, Box-and-whisker plot showing Pearson correlation coefficient before and after denoising data
 376 with z-motion compared with clean data without z-motion. N=102, which represents the number of neurons.
 377 **h**, Box-and-whisker plot showing SNR before and after denoising data with z-motion compared with clean

378 data without z-motion. N=1470, which represents the number of planes along the t-axis. **i**, Box-and-whisker
379 plot showing RMSE before and after denoising data with z-motion compared with clean data without z-
380 motion. N=1470, which represents the number of planes along the t-axis.



381

382 **Supplementary Fig. 47: Performance comparison between SUPPORT pipelines using simulated data.**

383 **a**, Representative frames of clean video, noisy video, and SUPPORT-denoised videos acquired with

384 different pipelines after baseline correction. Simulated data was used. Three different pipelines were used

385 in which the difference was the order of operations between motion correction, denoising, and

386 photobleaching correction. Pipeline 1: SUPPORT denoising followed by motion correction followed by a

387 photobleaching correction. Pipeline 2: motion correction followed by SUPPORT denoising followed by a

388 photobleaching correction. Pipeline 3: motion correction followed by a photobleaching correction followed

389 by SUPPORT denoising. **b**, Traces extracted from a single cell of the clean video, noisy video, and

390 SUPPORT-denoised videos. Temporally expanded traces from the green area on the left are shown on the

391 right. **c**, Box-and-whisker plot showing the Pearson correlation coefficients of extracted traces from the

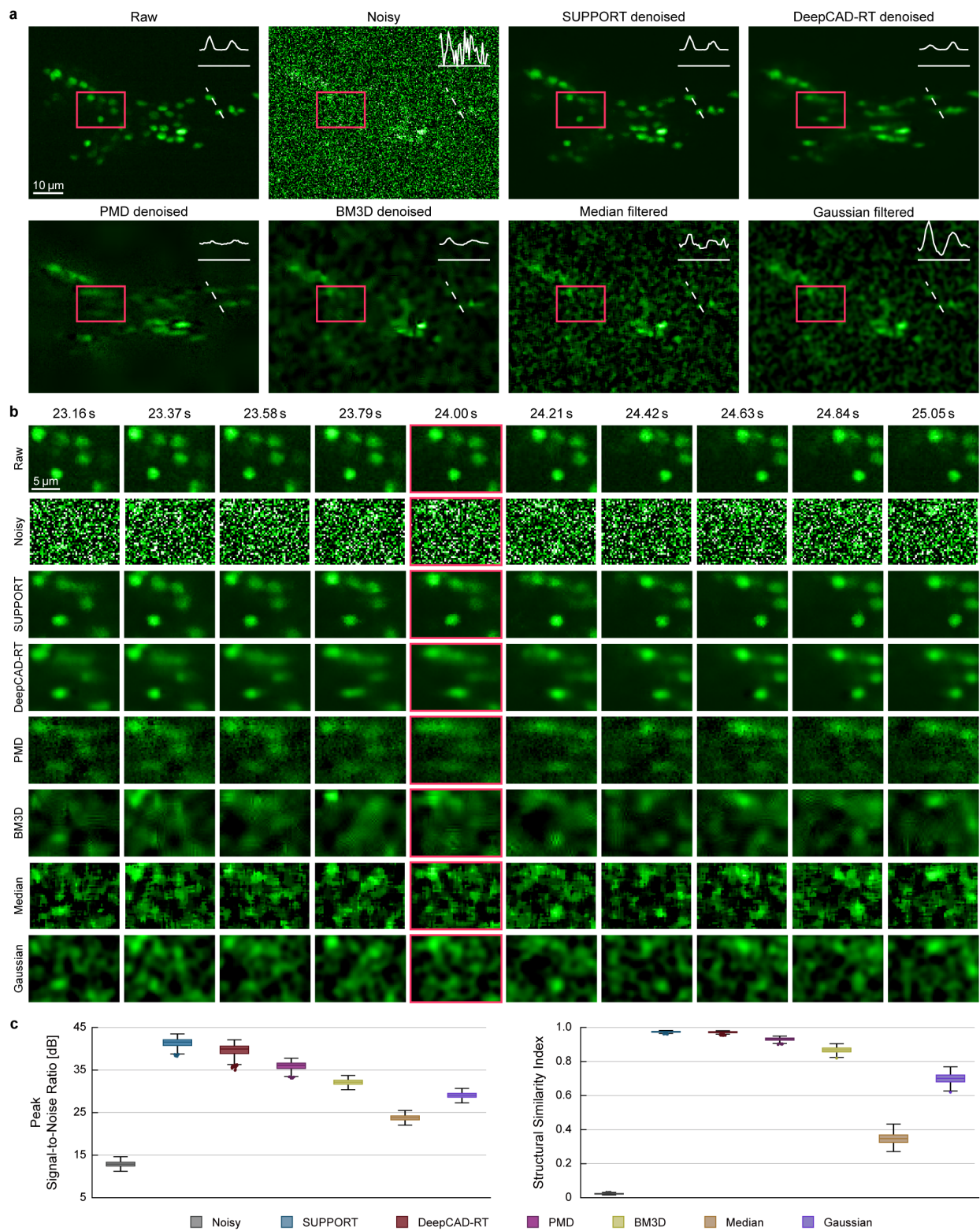
392 ROIs before and after denoising data with different pipelines. N=102, which represents the number of

393 neurons. **d**, Box-and-whisker plot showing the frame-wise SNR before and after denoising data with

394 different pipelines. N=1500, which represents the number of planes along the t-axis. **e**, Box-and-whisker

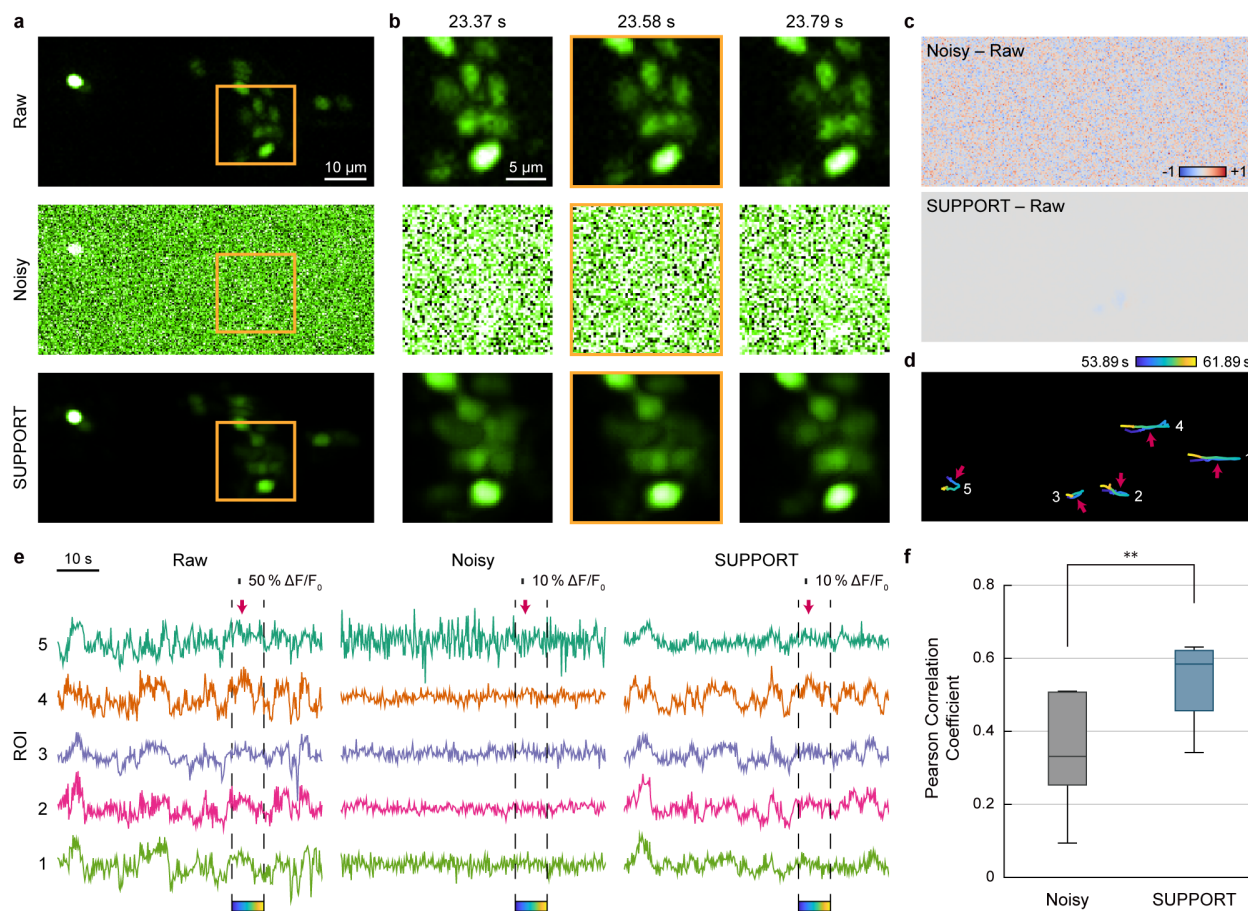
395 plot showing the frame-wise RMSE before and after denoising data with different pipelines. N=1470, which

396 represents the number of planes along the t-axis.



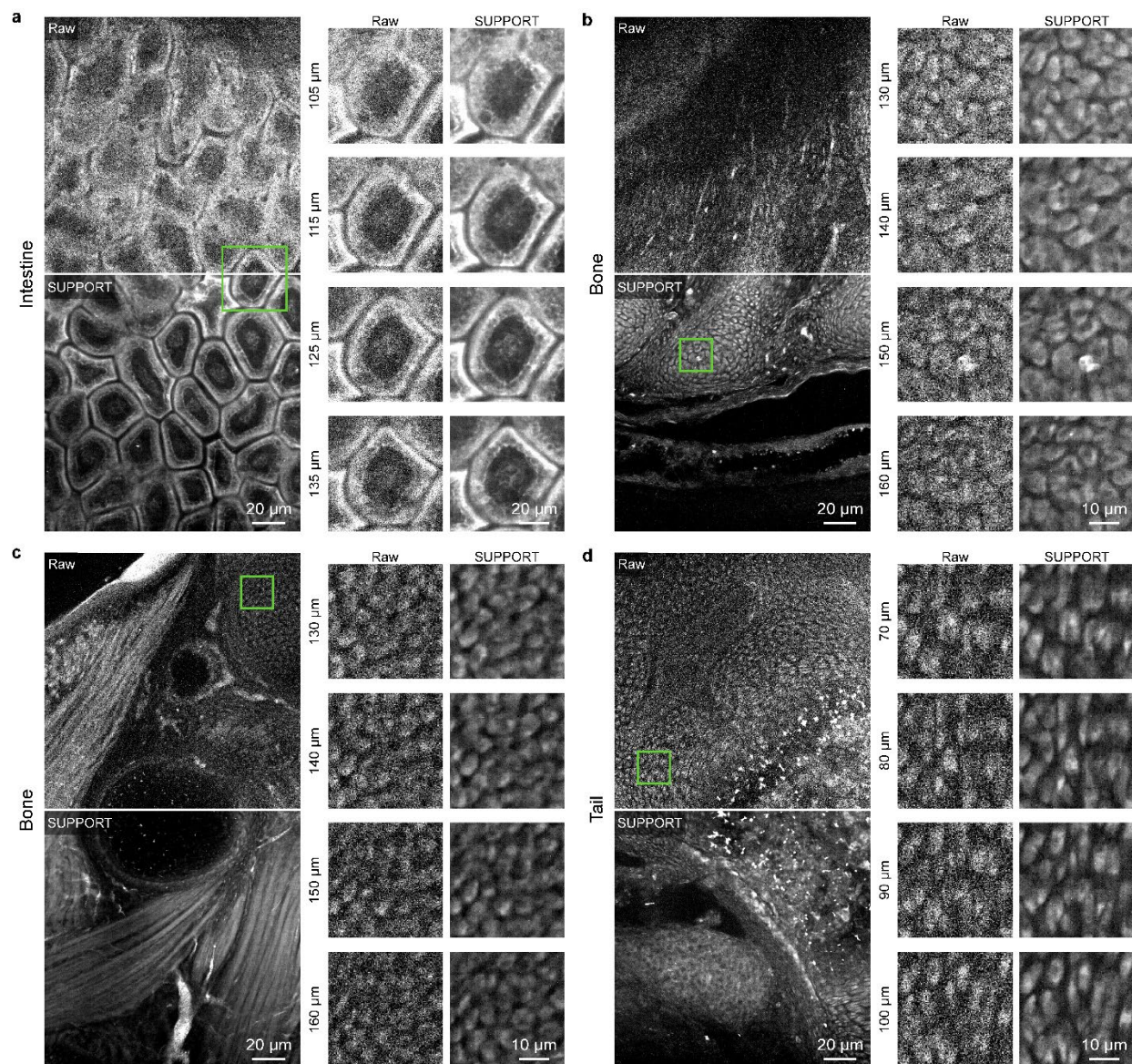
397
398 **Supplementary Fig. 48: Denoising performance compared with other 2D denoising methods on freely**

399 **moving *C.elegans* imaging data. a**, Raw, noisy, and denoised images of freely moving *C. elegans*
400 expressing NLS-mCherry. Inset shows the intensity profile along the dashed line. **b**, Magnified views of
401 the red boxed region in **a** at consecutive neighboring time points. From top to bottom: raw, noisy,
402 SUPPORT-denoised, DeepCAD-RT-denoised, PMD-denoised, BM3D-denoised, median filtered, and
403 gaussian filtered. **c**, Peak signal-to-noise ratio (PSNR) and Structural similarity index between raw and
404 denoised data. N=531, which represents the number of planes along the t-axis.



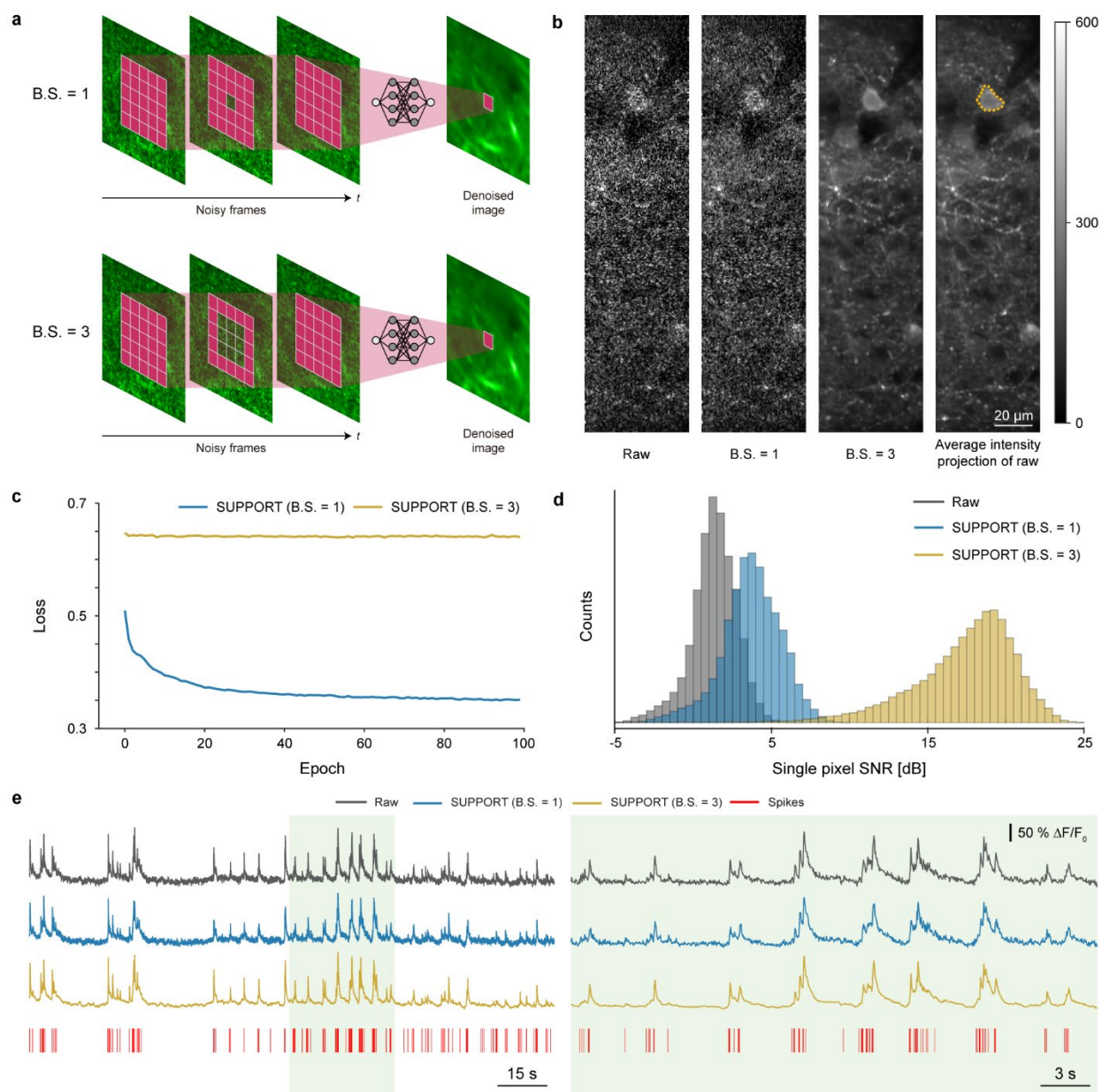
405

406 **Supplementary Fig. 49: Denoising freely moving *C.elegans* calcium imaging data.** **a**, Calcium imaging
 407 data of freely moving *C. elegans* expressing NLS-YC2. From top to bottom: Raw, Noisy, and SUPPORT-
 408 denoised data. **b**, Magnified views of the boxed regions in **a** at consecutive neighboring time points. **c**,
 409 Pixel-wise difference acquired by subtracting raw data from noisy and denoised data. **d**, Location of
 410 centroid over time is color-coded for five neuronal ROIs. **e**, Traces for 5 ROIs from raw, Noisy, and
 411 SUPPORT-denoised data. Area between two dashed lines corresponds to the temporal area visualized in **d**.
 412 Location indicated by red arrows corresponds to the red arrows in **d**. **f**, Box-and-whisker plot showing
 413 Pearson correlation coefficients between traces from Noisy and SUPPORT-denoised data and traces from
 414 raw data. Two-sided paired-sample t-test is used. N=5, which represents the number of neurons (**: p-
 415 value<0.01).



416

417 **Supplementary Fig. 50: Applying SUPPORT to volumetric structural images of mouse embryos**
 418 **acquired with expansion microscopy. a–d, Left: A frame at depth 125 μm from raw data (top) and**
 419 **SUPPORT-denoised data (bottom). Right: Expanded view of green box on the left at multiple depths. a,**
 420 **Intestine. b–c, Bone. d, Tail. Biological data shown are representative from $n=5$ stacks.**

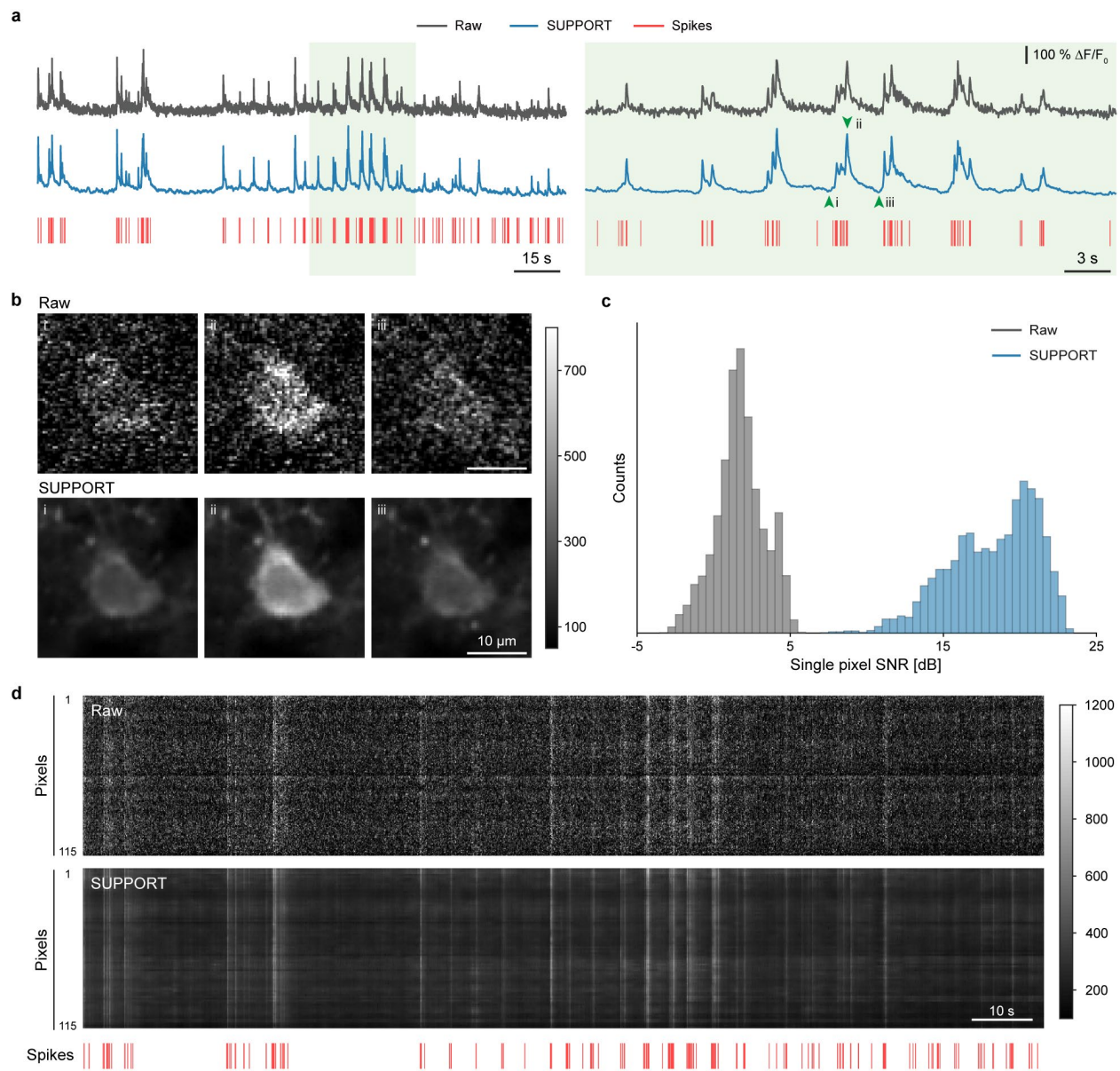


421

422 **Supplementary Fig. 51: Increasing the size of the blind spot to denoise the data with structured noise.**

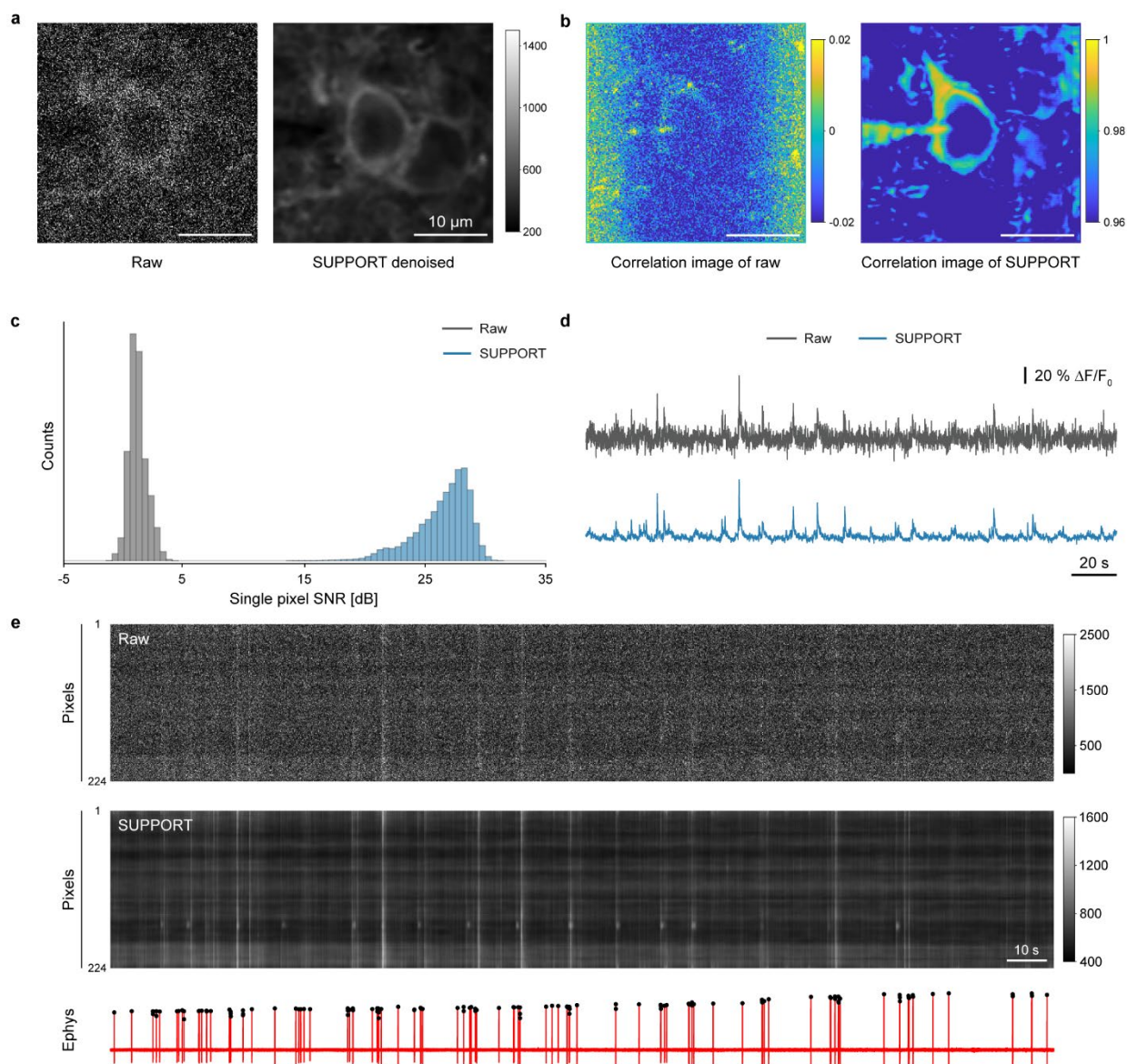
423 **a**, Receptive fields with different sizes of the blind spot. Top: Blind spot size of 1. Bottom: Blind spot size
 424 of 3. The center frame of noisy frames is the current frame to be denoised. **b**, Representative frames of raw
 425 and SUPPORT-denoised data with structural noise. Raw data were recorded from a jRCaMP8f-expressing
 426 mouse cortex L2/3 (Supplementary Table 1). From left to right: Raw data, SUPPORT-denoised data with
 427 blind spot sizes of 1 and 3, and the average intensity projection of the raw data. The boundary of the region
 428 of interest (ROI) is drawn with a yellow dotted line. **c**, Training loss curve of SUPPORT with blind spot
 429 sizes of 1 and 3. With a blind spot size of 1, SUPPORT learned to predict the structured noise, and training
 430 loss was significantly lower than for a blind spot size of 3. **d**, Histogram of single pixel signal-to-noise ratio
 431 (SNR) from raw data, SUPPORT-denoised data with blind spot sizes of 1 and 3. While a blind spot size of
 432 3 significantly increased the SNR, a size of 1 only slightly increased the SNR. **e**, Traces extracted from the
 433 raw data, SUPPORT-denoised data with blind spot sizes of 1 and 3 for the ROI in **b**, and spikes (action

434 potentials) detected from simultaneous electrophysiological recording. Temporally expanded traces from
435 the green area of the left are shown on the right.



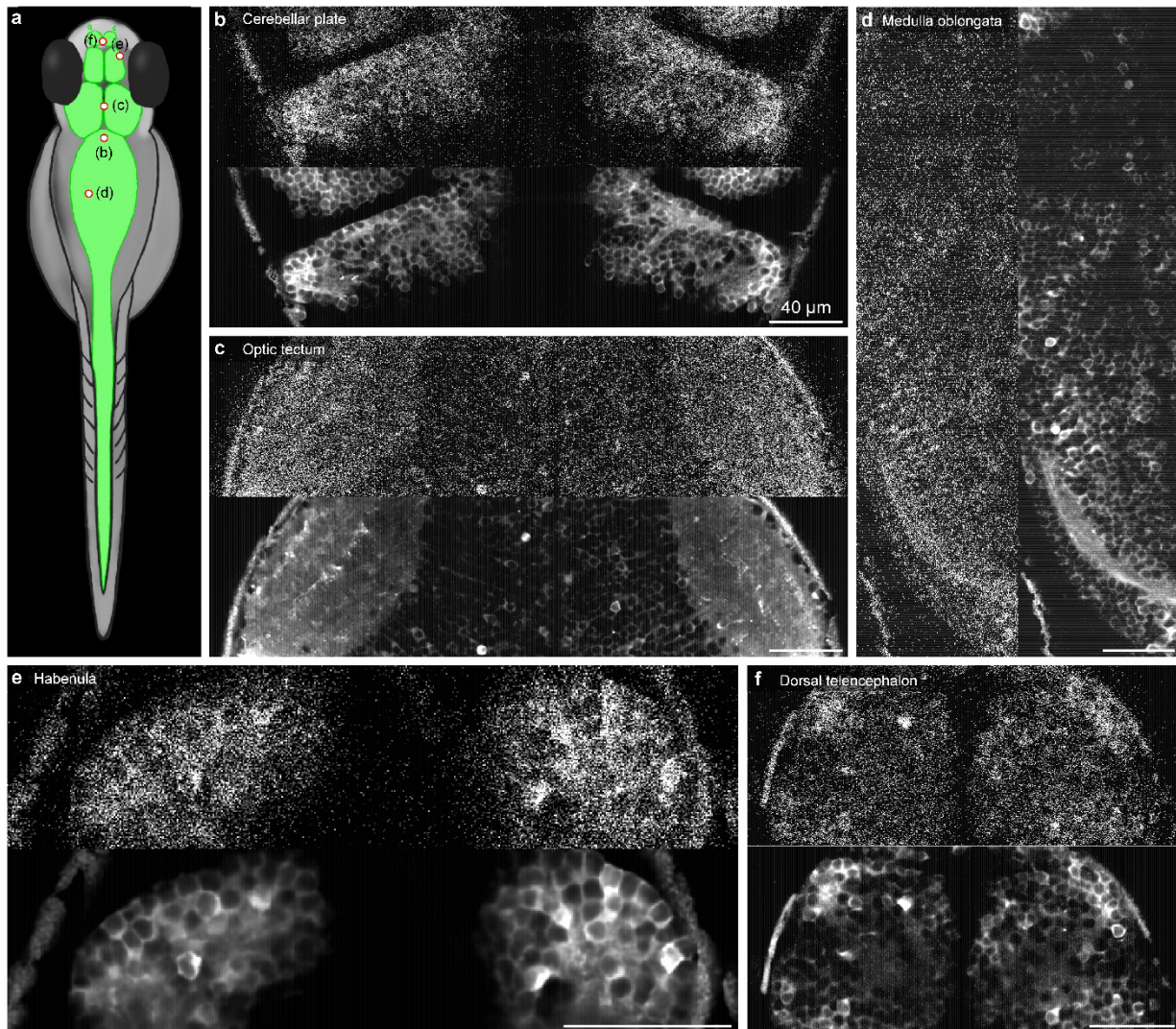
436

437 **Supplementary Fig. 52: Applying SUPPORT to simultaneous calcium imaging and**
 438 **electrophysiological recording data with jRCaMP8f indicator. a,** Traces extracted from the cytoplasmic
 439 area of raw and SUPPORT-denoised video. Raw data were recorded from a jRCaMP8f-expressing mouse
 440 cortex L2/3 (Supplementary Table 1). Spikes (action potentials) detected from simultaneous
 441 electrophysiological recordings are drawn at the bottom. Temporally expanded traces from the green area
 442 of the left are shown on the right. **b,** Representative frames of the raw and SUPPORT-denoised video
 443 marked with green arrows on **a**. Top: From the raw video. Bottom: From the SUPPORT-denoised video. **c,**
 444 Histogram of single pixel signal-to-noise ratio (SNR) from the raw video and SUPPORT-denoised video.
 445 **d,** Traces for pixels in the cell from raw and denoised data. Each row corresponds to a signal of each pixel.
 446 Spikes detected from simultaneous electrophysiological recordings are plotted underneath.



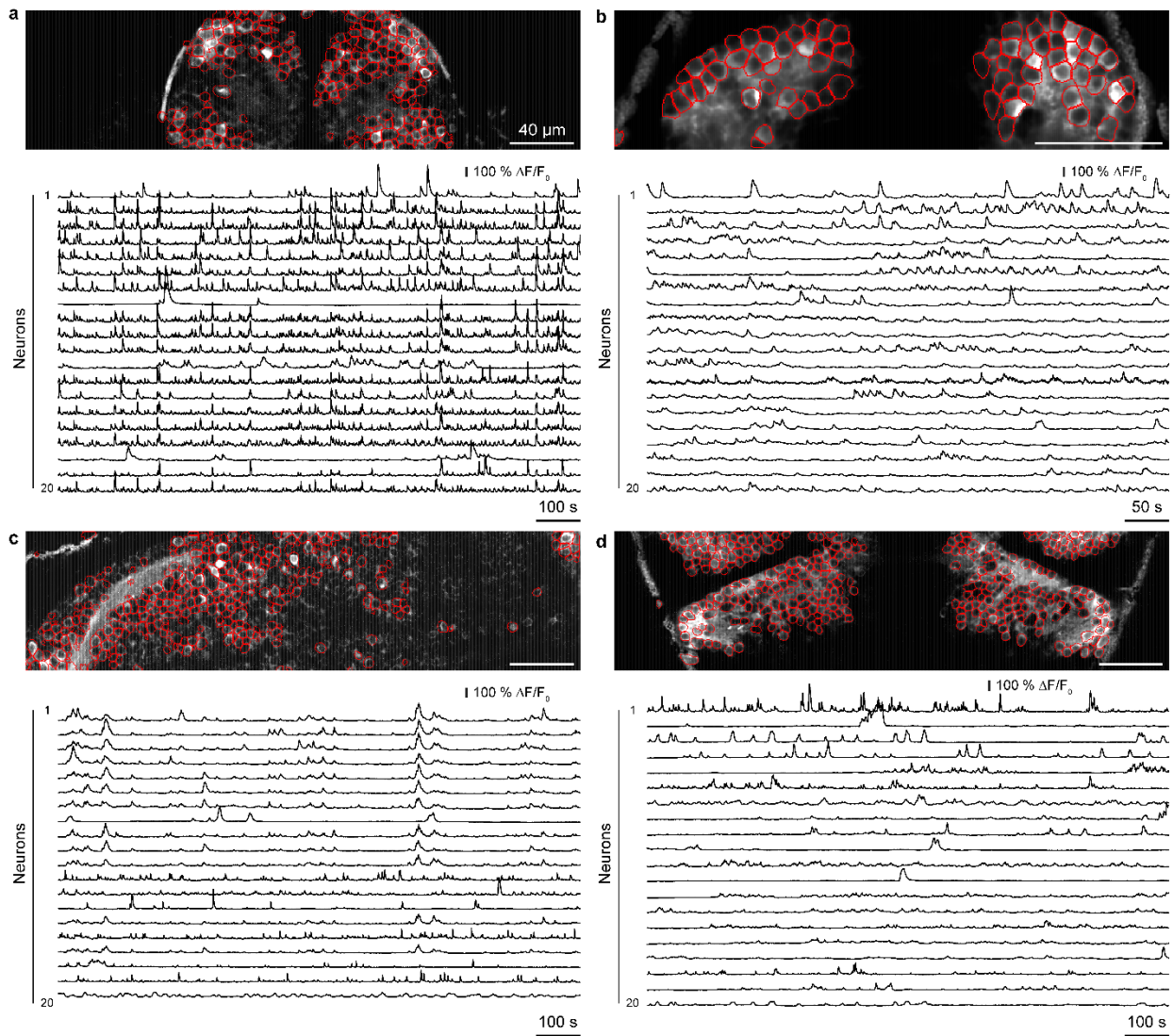
447

448 **Supplementary Fig. 53: Applying SUPPORT to simultaneous calcium imaging and**
 449 **electrophysiological recording data with GCaMP6f indicator. a,** Representative frames of raw video
 450 and SUPPORT-denoised video. Raw data were recorded from a GCaMP6f-expressing mouse cortex V1
 451 (Supplementary Table 1). **b,** Correlation images of raw video and SUPPORT-denoised video. The
 452 correlation image for each pixel is the average of the correlation coefficients between the signal of that pixel
 453 and the signals of neighboring pixels. **c,** Histogram of single pixel signal-to-noise ratio (SNR) from raw
 454 and SUPPORT-denoised videos. **d,** Traces extracted from the ROI of raw and SUPPORT-denoised videos.
 455 The ROI contains 100 pixels inside the cytoplasm. **e,** Traces for pixels in the cell from raw and SUPPORT-
 456 denoised video. Each row corresponds to a signal of each pixel. Simultaneous electrophysiological
 457 recording is plotted underneath, with black dots for detected spikes.



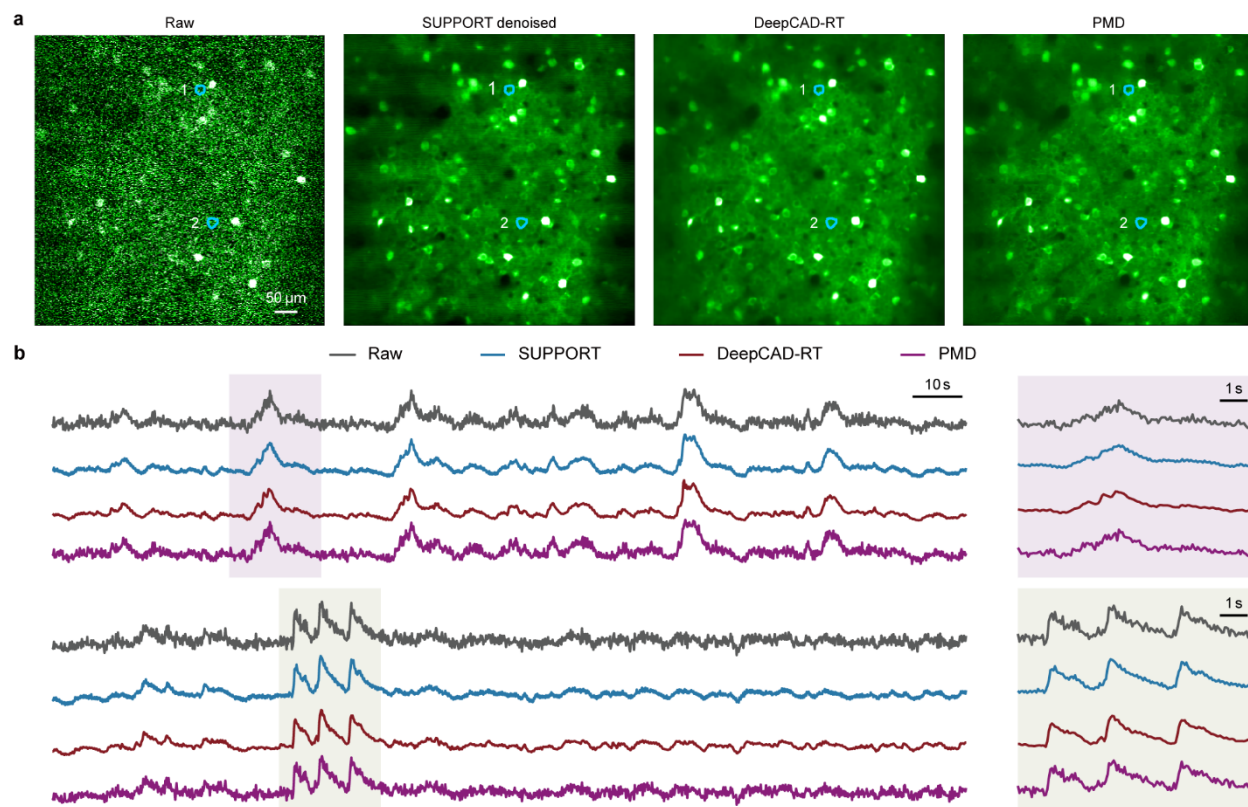
458

459 **Supplementary Fig. 54: SUPPORT denoises calcium imaging in larval zebrafish.** a, Larval zebrafish
 460 expressing GCaMP7a calcium indicator under control of huc promoter. **b–f**, Top left: Representative frames
 461 of raw video. Bottom right: Representative frame of SUPPORT-denoised video. **b**, Cerebellar plate. **c**, Optic
 462 tectum. **d**, Medulla oblongata. **e**, Habenula. **f**, Dorsal telencephalon.



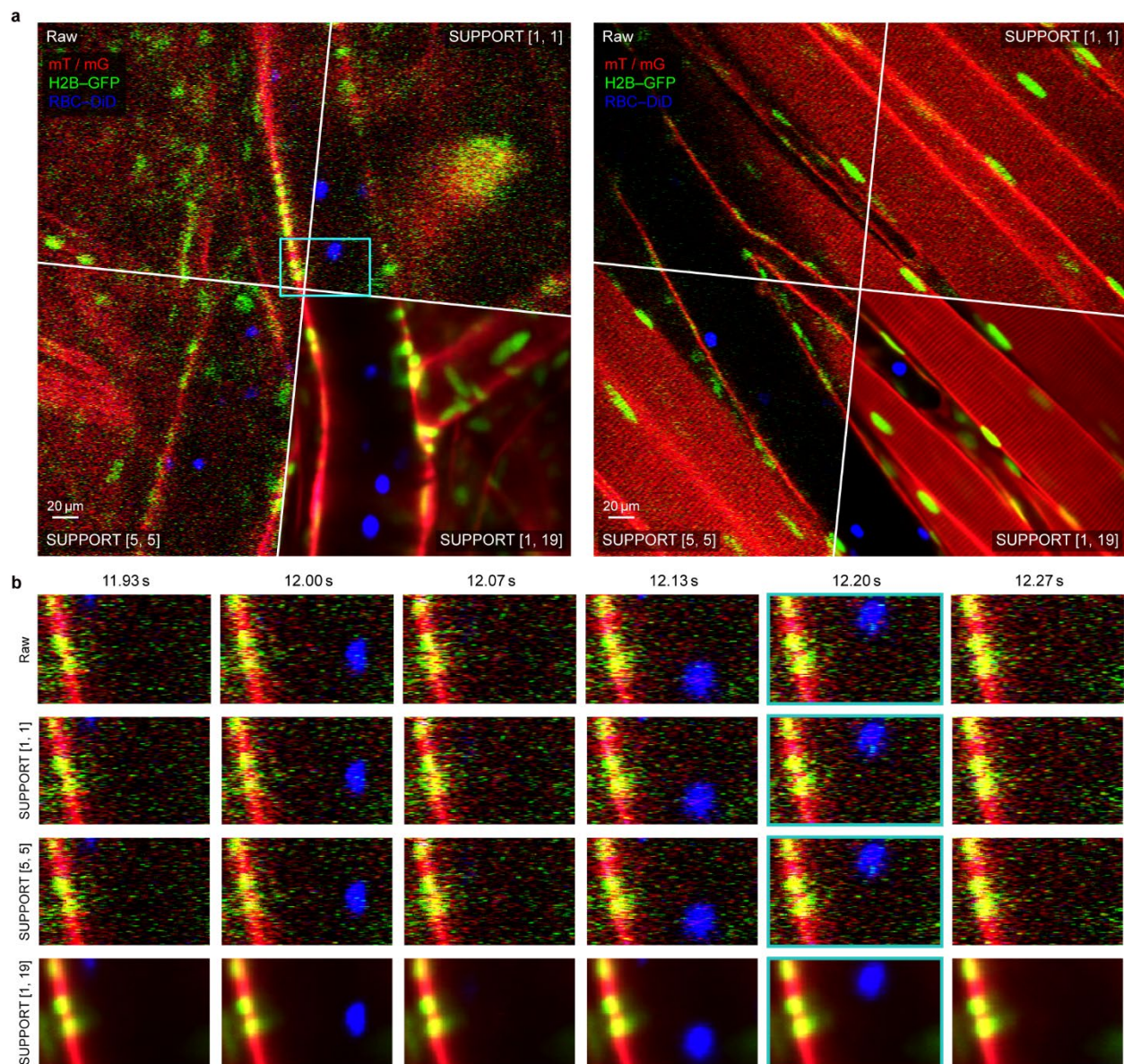
463

464 **Supplementary Fig. 55: SUPPORT denoising enables single frame cell detection.** a–d, Top: Neurons
 465 extracted from a single frame of SUPPORT-denoised video. Bottom: Representative calcium traces of 20
 466 neurons. **a**, Dorsal telencephalon. **b**, Habenula. **c**, Medulla oblongata. **d**, Cerebellar plate.



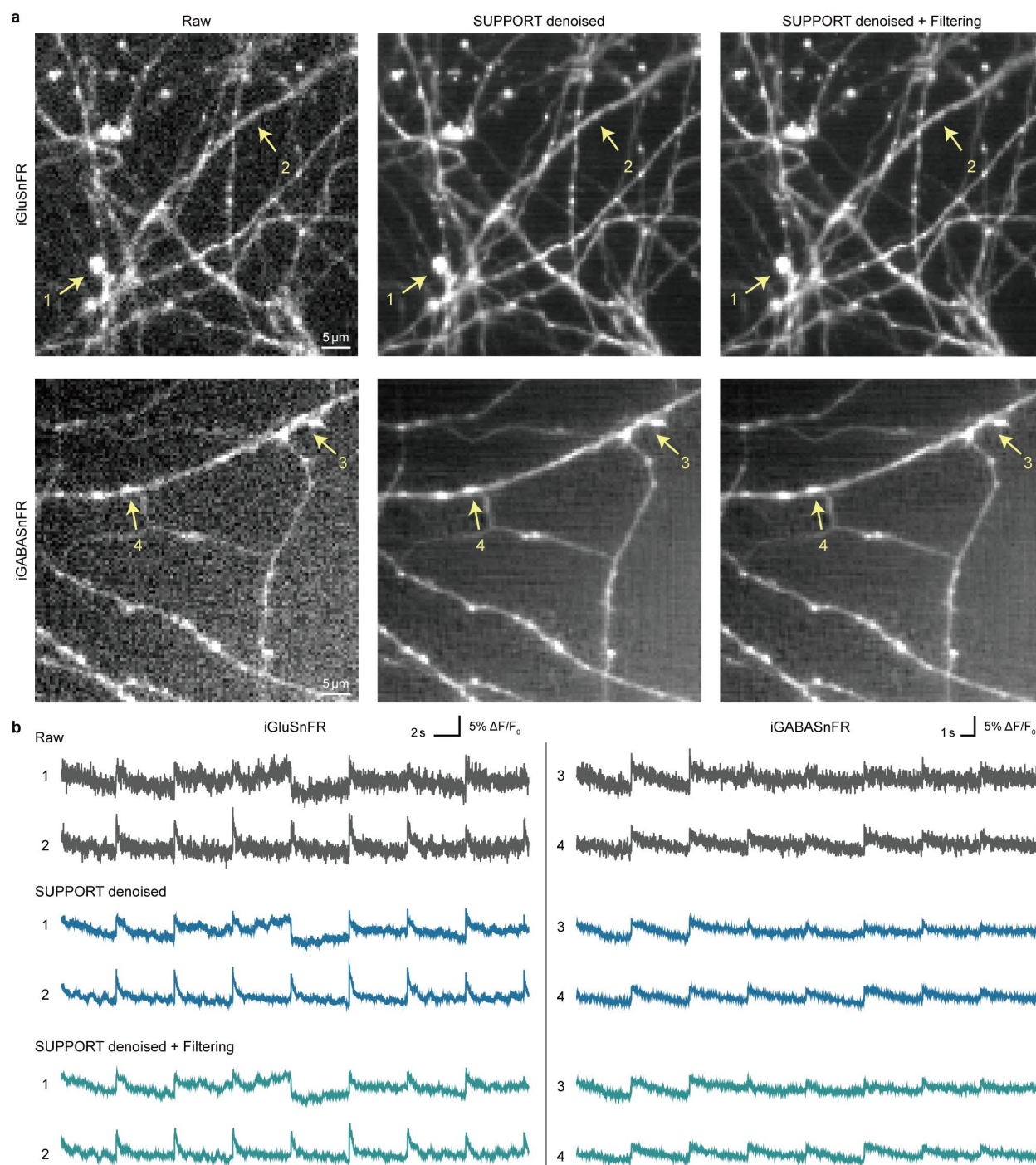
467

468 **Supplementary Fig. 56: Applying SUPPORT to Neurofinder calcium imaging dataset. a,**
 469 **Raw, SUPPORT, DeepCAD-RT, and PMD denoised. b, Two neuronal traces from**
 470 **raw and denoised data. Traces for the smaller temporal region are plotted on the right.**



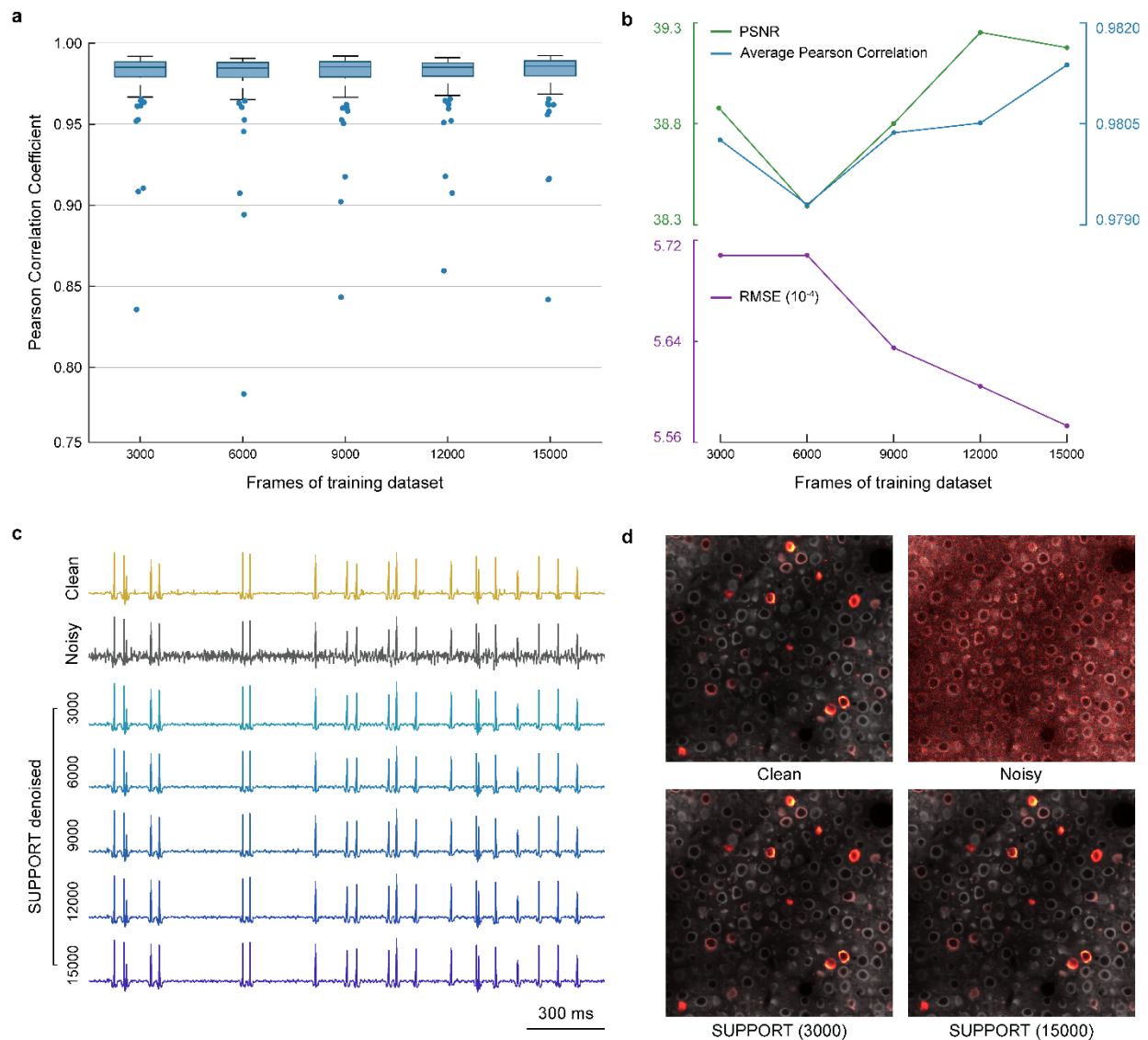
471

472 **Supplementary Fig. 57: Applying SUPPORT to multi-color in vivo imaging of mouse organs. a,**
 473 **Confocal image of ear skin and muscle of H2B-GFP (green) and mTmG (red) mice with red blood cells**
 474 **fluorescently labelled by far-red fluorophore DiD (blue). Left: Ear skin, Right: muscle. Comparisons of raw**
 475 **and SUPPORT-denoised data with blind spot sizes of 1×1 , 5×5 , and 1×19 are displayed. b, Expanded view**
 476 **of cyan box in a with consecutive frames are displayed. Biological data shown are representative from $n=5$**
 477 **experiments.**



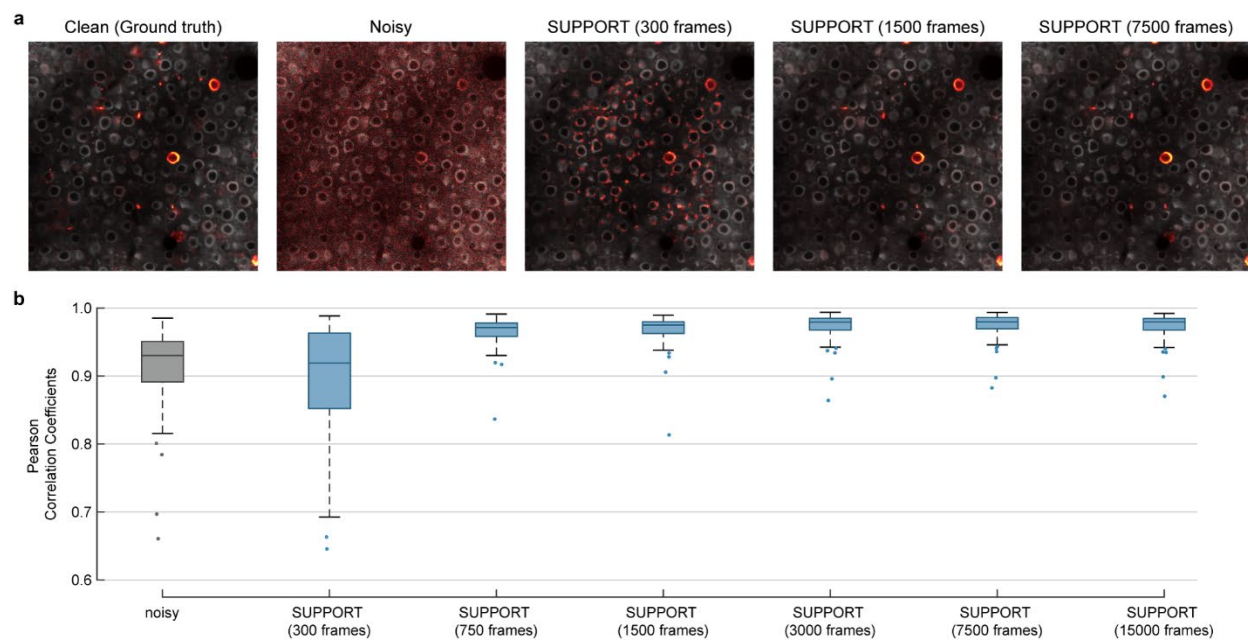
478

479 **Supplementary Fig. 58: Applying SUPPORT to functional imaging data of cultured cells of rat**
 480 **hippocampal neurons expressing iGluSnFR and iGABASnFR.** **a**, Primary cultures of rat hippocampal
 481 neurons expressing SF.iGluSnFR A184V and iGABASnFR F102G along with SynapsinI-mCherry marker.
 482 Representative frames of raw, SUPPORT-denoised, and SUPPORT-denoised followed by temporally
 483 filtered data. Four regions of interest (ROIs) are indicated by yellow arrows. **b**, Traces from raw, SUPPORT-
 484 denoised, and SUPPORT-denoised followed by filtered data extracted from four ROIs in **a**.



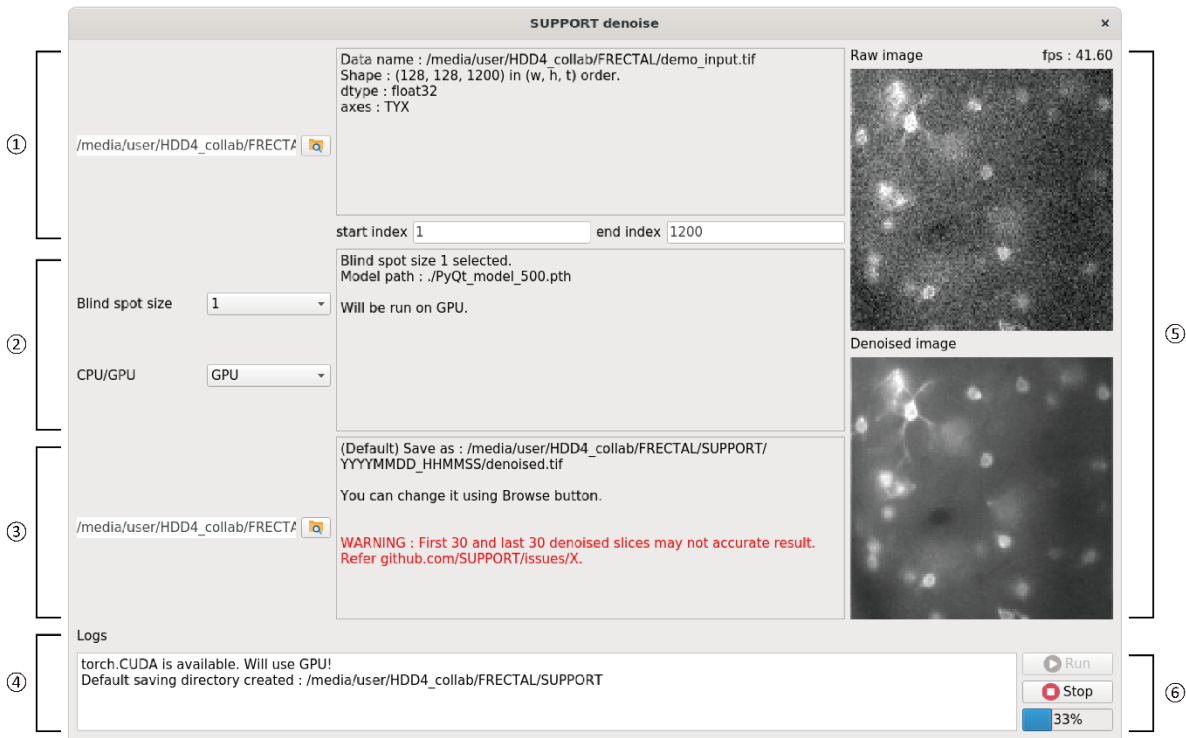
485

486 **Supplementary Fig. 59: Denoising performance as a function of the size of training data.** **a**, Pearson
 487 correlation coefficients between trace extracted from clean (ground truth) video and SUPPORT-denoised
 488 videos. Synthetic data with spike width of 3 ms were used for comparison. SUPPORT was trained with five
 489 different sizes of training datasets to evaluate the correlation between denoising performance and dataset
 490 size. $N=116$, which represents the number of neurons. **b**, PSNR, average Pearson correlation coefficients,
 491 and RMSE between clean video and SUPPORT-denoised videos. All three metrics show a trend of
 492 performance increases with larger training datasets. **c**, Traces extracted from a single cell of the clean video,
 493 SUPPORT-denoised videos, and the noisy video. While performance improves with larger datasets, traces
 494 show that SUPPORT was able to denoise with 3,000 frames of training data. **d**, Representative frames of
 495 clean video, noisy video, and SUPPORT-denoised videos after baseline correction. SUPPORT trained with
 496 3,000 frames and 15,000 frames were used. Representative frames of clean and SUPPORT-denoised videos.



497

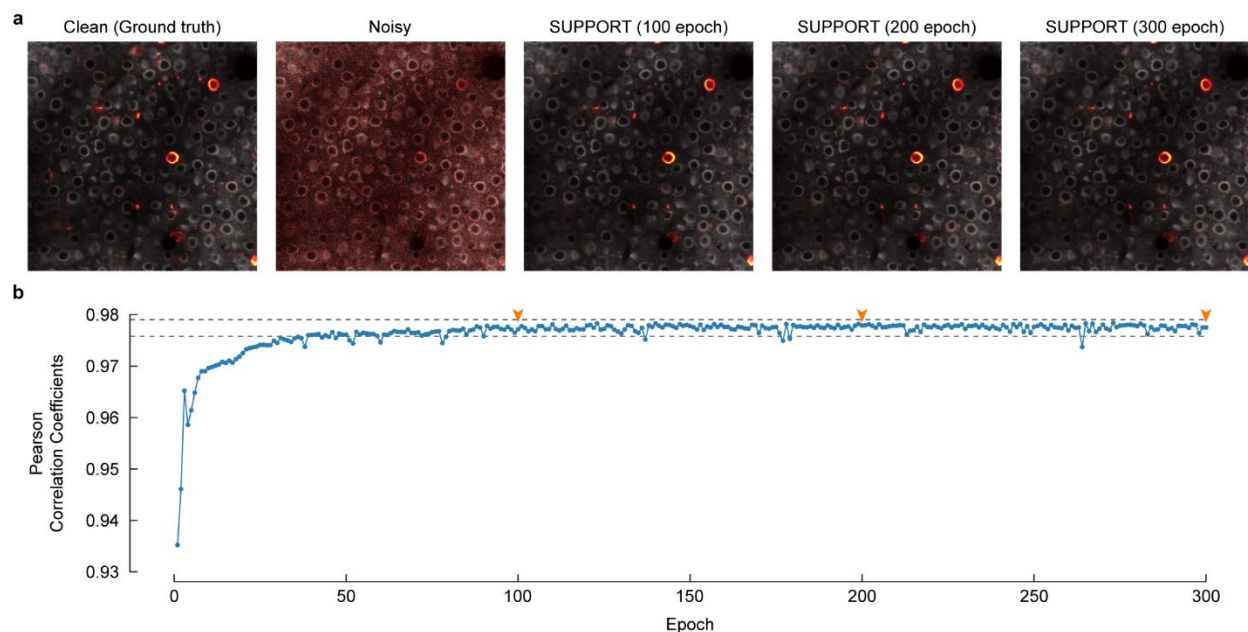
498 **Supplementary Fig. 60: Extended denoising performance analysis as a function of the size of training**
 499 **data.** **a**, Representative frames of clean video, noisy video, and SUPPORT-denoised videos after baseline
 500 correction. SUPPORT trained with 300, 1500, and 7500 frames were used. **b**, Box-and-whisker plot
 501 showing Pearson correlation coefficients before and after denoising data trained with different sizes of
 502 training data. N=116, which represents the number of neurons.



- ① (Input) Image path and information
- ② (Model) Blind spot size and CPU/GPU setting
- ③ (Save) Saving directory for denoised image and log files
- ④ Logs
- ⑤ Raw and denoised image in real time
- ⑥ Run / Stop button and progress bar

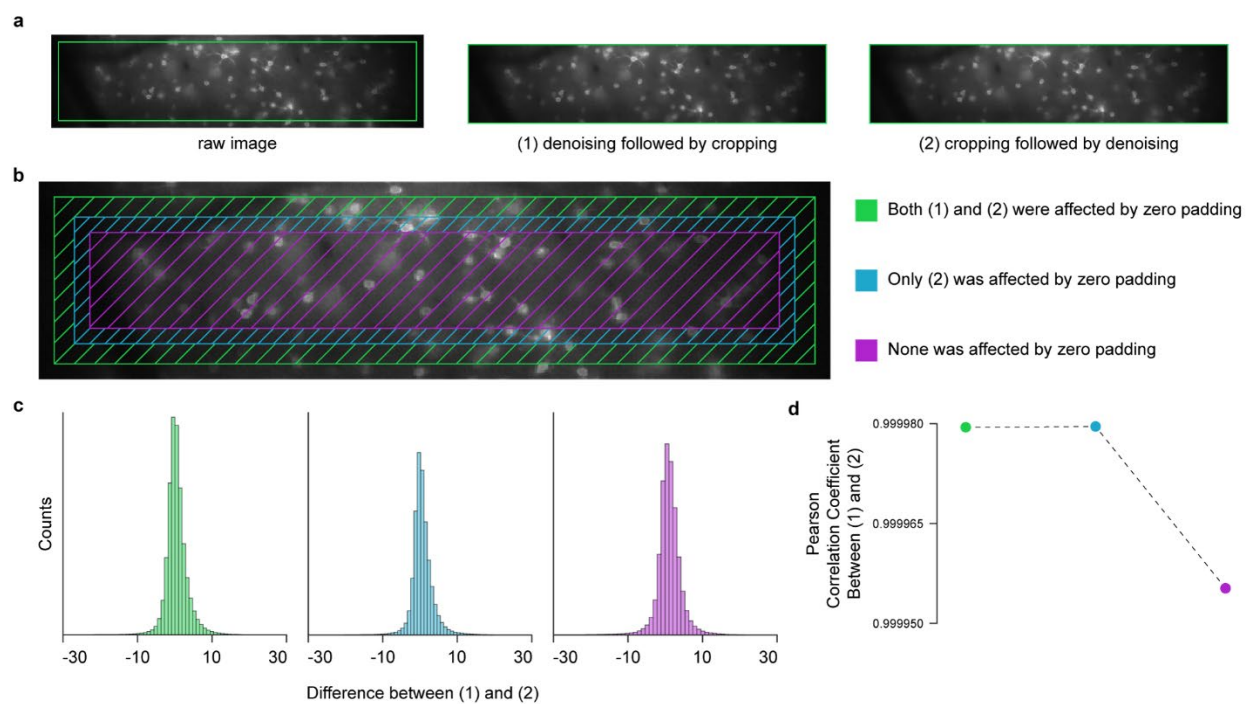
503

504 **Supplementary Fig. 61: Python GUI: SUPPORT software with a graphical user interface.** The user
 505 can choose the size of the blind spot and CPU/GPU option. Once the Run button is pressed, the software
 506 loads and denoises using a selected model, shows the denoised frame in real time, and saves denoised
 507 frames.



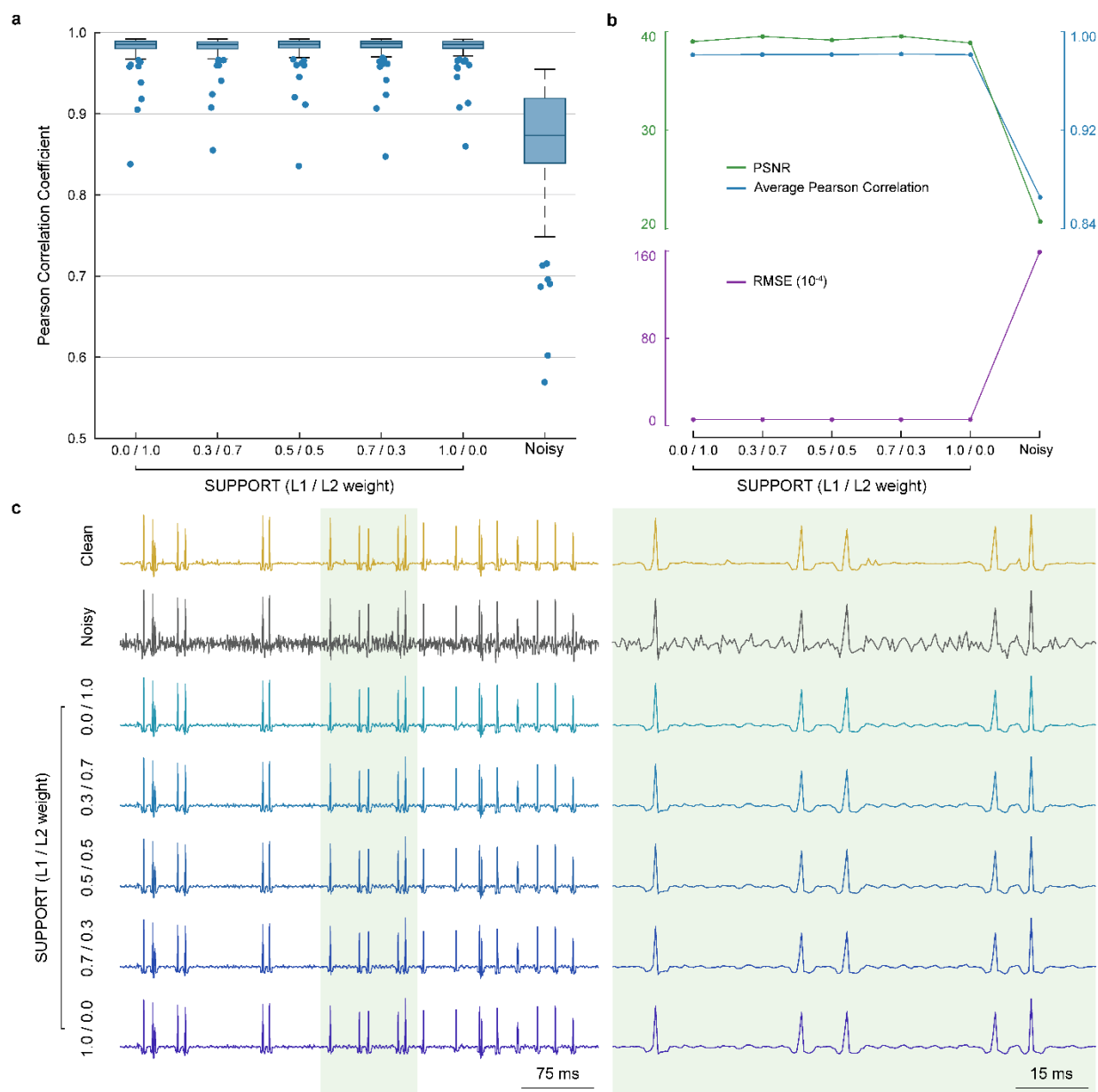
508

509 **Supplementary Fig. 62: Denoising performance as a function of the iteration of training.** **a**,
 510 Representative frames of clean video, noisy video, and SUPPORT-denoised videos after baseline correction.
 511 SUPPORT trained for 100, 200, and 300 epochs were used. **b**, Line plot showing Pearson correlation
 512 coefficients as a function of training iterations.



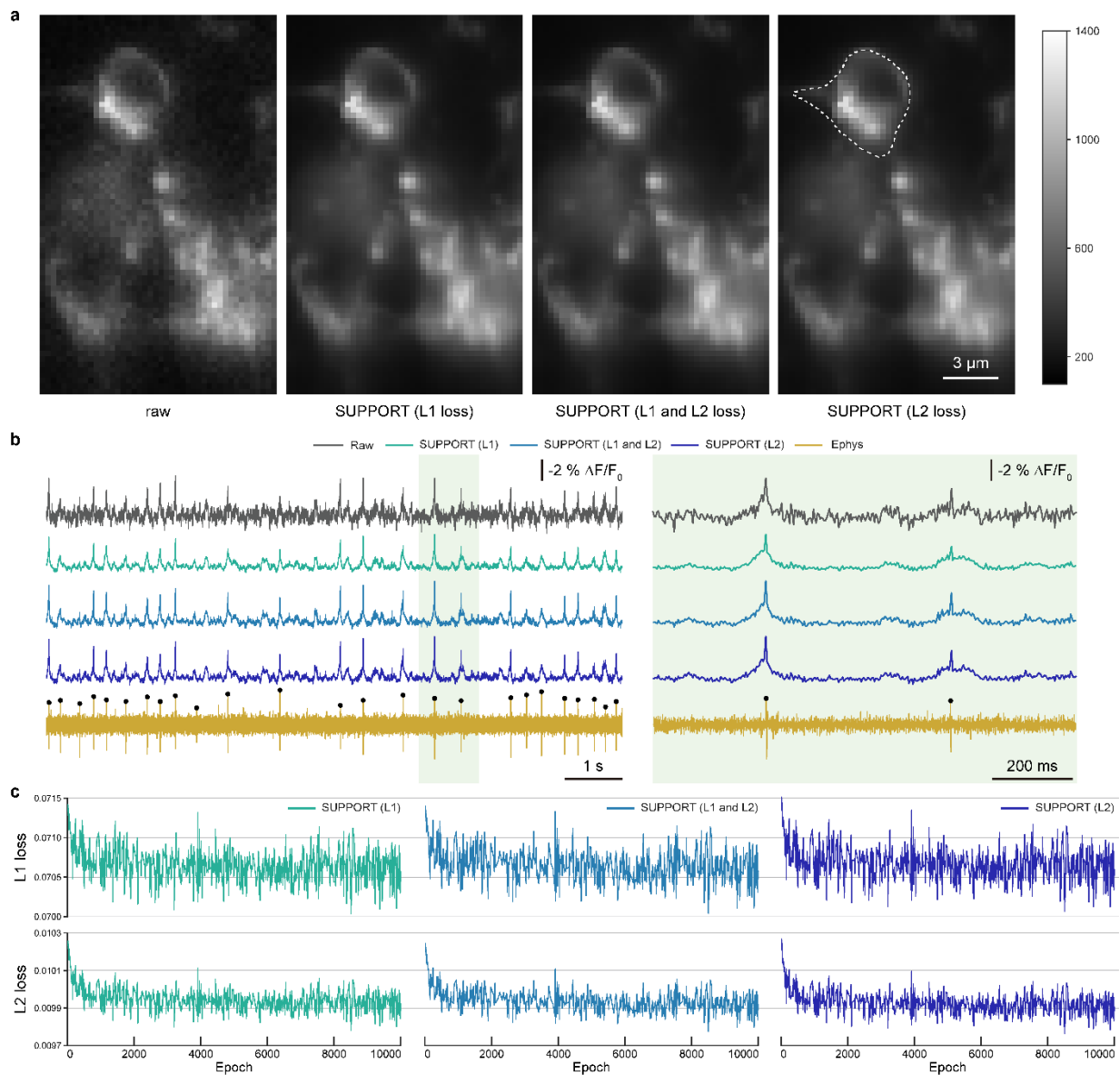
513

514 **Supplementary Fig. 63: Analysis of the effect of padding at the edge of the images on SUPPORT**
 515 **denoising.** **a**, Representative frames of raw video, (1) SUPPORT-denoised video followed by cropping,
 516 and (2) cropped video followed by SUPPORT-denoising. Dataset of in vivo mouse cortex layer 1 expressing
 517 Voltron1 was used. **b**, Regions, where both denoised videos are affected by zero padding, were colored
 518 green, and only one affected was colored blue, none were affected were colored purple. **c**, Histogram of
 519 pixel value differences between the two videos in each region described in **b**. **d**, Line plot showing Pearson
 520 correlation coefficient between the two videos for each region.



521

522 **Supplementary Fig. 64: L1 and L2 loss comparison on simulation data.** **a**, Pearson correlation
 523 coefficients between traces from clean (ground truth) video and SUPPORT-denoised video or noisy video.
 524 SUPPORT-denoised videos were acquired with five different training loss settings. $N=116$, which
 525 represents the number of neurons. **b**, PSNR, average Pearson correlation coefficients, and RMSE between
 526 clean video and SUPPORT-denoised videos or noisy video. All three metrics indicate that the weight of L1
 527 and L2 loss for training SUPPORT did not significantly change denoising performance. **c**, Traces extracted
 528 from a single cell of the clean video, SUPPORT-denoised videos, and noisy video. Temporally expanded
 529 traces from the green area of the left are shown on the right. This again shows that the weight of L1 and L2
 530 loss does not significantly change SUPPORT's output.



531

532 **Supplementary Fig. 65: L1 and L2 loss comparison on voltage imaging data with simultaneous**
 533 **electrophysiological recording. a**, From left to right: Representative frames from the raw data, SUPPORT
 534 trained using L1 loss function, both L1 and L2 loss functions, and L2 loss function. For the training setting
 535 that used both L1 and L2 loss, equal weights of L1 and L2 were used. The boundary of the region of interest
 536 (ROI) is drawn with a white dotted line. **b**, Traces extracted from the raw data, SUPPORT trained using L1
 537 loss function, both L1 and L2 loss function, and L2 loss function for the ROI in **a**, and electrophysiological
 538 recording. Detected spikes are marked with black dots. Temporally expanded traces from the green area of
 539 the left are shown on the right. Using additional L2 loss showed better reconstruction of the spikes after
 540 SUPPORT denoising. **c**, L1 loss and L2 loss were tracked during the training procedure for SUPPORT
 541 using L1 loss function, both L1 and L2 loss functions, and L2 loss function.

542 **SUPPLEMENTARY VIDEOS**

543 **Supplementary Video 1. Synthetic voltage imaging**

544 **Supplementary Video 2. Single-neuron simultaneous electrophysiology and voltage imaging of larval**
545 **zebrafish**

546 **Supplementary Video 3. Synthetic voltage imaging data with propagating action potential**

547 **Supplementary Video 4. Population voltage imaging of larval zebrafish spinal cord**

548 **Supplementary Video 5. Volumetric time-lapse imaging of *C. elegans***

549 **Supplementary Video 6. Volumetric structural imaging of mouse embryos recorded with expansion**
550 **microscopy**

551 **Supplementary Video 7. Calcium imaging of larval zebrafish**

552 **Supplementary Video 8. Voltage imaging of cardiac cells**

553 **Supplementary Video 9. Multi-color in vivo imaging of various mouse organs**

SUPPLEMENTARY TABLES

| Region | Cell type | Recording rate (Hz) | Reporter | Imaging modality | Reference |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------|---------------------|--------------------------------------------------|-------------------------|-----------------------------------------------------------------------------------------|
| Voltage Imaging | | | | | |
| Mouse cortex L1 (Fig. 1, 5, Supplementary Fig. 17, Supplementary Fig. 18, Supplementary Fig. 20, Supplementary Fig. 22, Supplementary Fig. 23, Supplementary Fig. 24, Supplementary Fig. 25, Supplementary Fig. 26, Supplementary Fig. 27, Supplementary Fig. 28, Supplementary Fig. 29, Supplementary Fig. 30, Supplementary Fig. 41, Supplementary Fig. 44, Supplementary Fig. 63) | interneurons (<i>Ndnf</i> +) | 400 | Voltron1 | wide-field fluorescence | reporter and data: Abdelfattah, A. S. et al. ² |
| Zebrafish dorsal part of the cerebellum (Fig. 3, Supplementary Fig. 9, Supplementary Fig. 10, Supplementary Fig. 65, Supplementary Video 2) | excitatory (<i>vGlut2a</i>) | 300 | Voltron1 + cell-attached extracellular recording | light sheet | reporter and data: Abdelfattah, A. S. et al. ² |
| Zebrafish spinal cord (Fig. 5, Supplementary Fig. 19, Supplementary Fig. 20, Supplementary Fig. 37, Supplementary Fig. 41, Supplementary Video 3) | excitatory (<i>vGlut2a</i>) | 1000 | zArchon1 | light sheet | reporter: Piatkevich, K. D. et al. ³ data: Xie, M. E. et al. ⁴ |

| | | | | | |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------|------|------------------------|------------------------------------------------------------------|---------------------------------------------------------------------------------------------|
| Mouse cortex L2/3 (Fig. 4, 6, Supplementary Fig. 12) | pyramidal cells | 1000 | QuasAr6a + patch clamp | one-photon epifluorescence microscopy with targeted illumination | reporter: Tian, H. et al. ⁵ data: this work |
| Mouse cortex L2/3 (Supplementary Fig. 14, Supplementary Fig. 15) | pyramidal cells | 1000 | Voltron2 + patch clamp | one-photon epifluorescence microscopy with targeted illumination | reporter: Abdelfattah, A. S. et al. ⁶ data: this work |
| Cultured rat hippocampal neuron (Supplementary Fig. 16) | primary neurons | 1000 | BeRST1 | wide-field fluorescence | reporter: Huang, Y. L., Walker, A. S. & Miller, E. W. ⁷ data: this work |
| Mouse hippocampus CA1 (Supplementary Fig. 18, Supplementary Fig. 31, Supplementary Fig. 32, Supplementary Fig. 33, Supplementary Fig. 34, Supplementary Fig. 35, Supplementary Fig. 36, Supplementary Fig. 42) | interneurons | 1000 | paQuasAr3-s | micromirror-based, somatargeted, structured illumination | reporter and data: Adam, Y. et al. ⁸ |
| Zebrafish tegmental area (Supplementary Fig. 18, Supplementary Fig. 38, Supplementary Fig. 39, Supplementary Fig. 40, Supplementary Fig. 43) | excitatory (<i>vGlut2a</i>) | 300 | Voltron1 | light sheet | reporter and data: Abdelfattah, A. S. et al. ² |
| Mouse cortex L1 (Fig. 4) | Interneurons (Ndnf+) | 400 | Voltron1 + patch clamp | wide-field fluorescence | reporter: Abdelfattah, A. S. et al. ² data: Rozsa, Marton et al. ⁹ |
| Mouse culture (Supplementary Fig. 21) | primary neurons | 500 | SomArchon | DMD-targeted illumination | reporter: Piatkevich, K.D. et al. ¹⁰ data: Xiao, S. et al. ¹¹ |

| | | | | | |
|----------------------------------------------------------------------------------------|-----------------------------|---------|--------------------------------------------------------------------|-------------------------|----------------------------------------------------------------------------------------------------|
| Mouse hippocampus (Fig. 6) | N/A | 800 | SomArchon | wide-field fluorescence | reporter and data: Piatkevich, K.D. et al. ¹⁰ |
| Structural Imaging | | | | | |
| <i>C. elegans</i> (Extended data Fig. 1, Supplementary Fig. 48, Supplementary Video 4) | pan-neuronal (<i>H20</i>) | 4.75 | mCherry | confocal | reporter: Shaner, N. C. et al. ¹⁰ data: Toyoshima, Y. et al. ¹² |
| <i>Penicillium</i> (Fig. 6) | entire | Static | N/A | confocal | data: this work |
| Mouse embryos; intestine, bone, tail (Extended data Fig. 2, Supplementary Fig. 50) | entire | Static | Alexa Fluor 488 NHS-ester | confocal | data: Sim, J. et al. ¹³ |
| Calcium Imaging | | | | | |
| Mouse cortex L2/3 (Supplementary Fig. 51, Supplementary Fig. 52) | pyramidal cells | 122 | jGCaMP8f + loose-seal cell attached electrophysiological recording | two-photon | reporter: Zhang, Y. et al. ¹⁴ data: Rozsa, M. et al. ¹⁵ |
| Mouse cortex V1 (Supplementary Fig. 53) | excitatory (<i>syn</i>) | 60 | GCaMP6f + loose-seal cell attached electrophysiological recording | two-photon | reporter: Chen, T. W. et al. ¹⁶ data: GENIE project ¹⁷ |
| Zebrafish (Supplementary Fig. 54, Supplementary Fig. 55, Supplementary Video 5) | pan-neuronal (<i>huc</i>) | 1, 2, 4 | GCaMP7a | confocal | reporter: Muto, A., Ohkura, M., Abe, G., Nakai, J. & Kawakami, K. ¹⁸ data: this work |
| Mouse cortex V1 (Supplementary Fig. 56) | N/A | 7.5 | GCaMP6s | two-photon | reporter: Chen, T. W. et al. ¹⁶ data: Neurofinder ¹⁹ |
| <i>C. elegans</i> (Supplementary Fig. 49) | pan-neuronal (<i>H20</i>) | 4.75 | YC2.6 | confocal | reporter: Shaner, N. C. et al. ²⁰ data: Toyoshima, Y. et al. ¹² |

| Others | | | | | | |
|-----------------------------------------------------------|----------------|-----|---------------------|-------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--|
| Mouse ear skin, kidney, muscle (Supplementary Fig. 57) | N/A | 15 | H2B-GFP, mTmG | confocal | reporter: Kanda, T., Sullivan, K.F., & Wahl, G.M. ²¹ , Muzumdar, M.D., Tasic, B., Miyamichi, K., Li, L., & Luo, L. ²² data: Hwang, Y. et al. ^{49,50,51,52,53,54,55,56,31} | |
| Rat hippocampus culture (Supplementary Fig. 58) | primary neuron | 100 | iGluSnFR, iGABASnFR | wide-field fluorescence | reporter: Marvin, J.S. et al. ³² data: this work | |

Supplementary Table 1. List of datasets analyzed in this study.

| Dataset | Network configuration |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Voltage imaging | |
| Mouse cortex L1 (Fig. 1, 5, Supplementary Fig. 17, Supplementary Fig. 18, Supplementary Fig. 20, Supplementary Fig. 22, Supplementary Fig. 23, Supplementary Fig. 24, Supplementary Fig. 25, Supplementary Fig. 26, Supplementary Fig. 27, Supplementary Fig. 28, Supplementary Fig. 29, Supplementary Fig. 30, Supplementary Fig. 41, Supplementary Fig. 44, Supplementary Fig. 63) | Default (input frame = 61, blind conv channels = 64, one by one channels = [32, 16], last layer channels = [64, 32, 16], Unet channels = [16, 32, 64, 128, 256], blind spot size = [1, 1]) |
| Zebrafish dorsal part of the cerebellum (Fig. 3, Supplementary Fig. 9, Supplementary Fig. 10, Supplementary Fig. 65, Supplementary Video 2) | Changed blind spot size to [3, 3] from default |
| Zebrafish spinal cord (Fig. 5, Supplementary Fig. 19, Supplementary Fig. 20, Supplementary Fig. 37, Supplementary Fig. 41, Supplementary Video 3) | Default |
| Mouse cortex L2/3 (Fig. 4, 6, Supplementary Fig. 12) | Default |
| Mouse cortex L2/3 (Supplementary Fig. 14, Supplementary Fig. 15) | Default |
| Cultured rat hippocampal neuron (Supplementary Fig. 16) | Default |
| Mouse hippocampus CA1 (Supplementary Fig. 18, Supplementary Fig. 31, Supplementary Fig. 32, Supplementary Fig. 33, Supplementary Fig. 34, Supplementary Fig. 35, Supplementary Fig. 36, Supplementary Fig. 42) | Changed blind spot size to [3, 3] from default |
| Zebrafish tegmental area (Supplementary Fig. 18, Supplementary Fig. 38, Supplementary Fig. 39, Supplementary Fig. 40, Supplementary Fig. 43) | Changed blind spot size to [3, 3] from default |
| Mouse cortex L1 (Fig. 4) | Changed blind spot size to [3, 3] from default |
| Mouse culture (Supplementary Fig. 21) | Changed blind spot size to [3, 3] from default |
| Mouse hippocampus (Fig. 6) | Changed Unet channels to [64, 128, 256, 512, 1024] from default |
| Structural Imaging | |
| <i>C. elegans</i> (Extended data Fig. 1, Supplementary Fig. 48, Supplementary Video 4) | Changed input frame to 21 from default, Changed blind spot size to [3, 3] from default |
| <i>Penicillium</i> (Fig. 6) | Changed input frame to 21 from default |
| Mouse embryos; intestine, bone, tail (Extended data Fig. 2, Supplementary Fig. 50) | Changed input frame to 21 from default |
| Calcium Imaging | |
| Mouse cortex L2/3 (Supplementary Fig. 51, Supplementary Fig. 52) | Changed blind spot size to [3, 3] from default |
| Mouse cortex V1 (Supplementary Fig. 53) | Default |
| Zebrafish (Supplementary Fig. 54, Supplementary Fig. 55, Supplementary Video 5) | Default |
| Mouse cortex V1 (Supplementary Fig. 56) | Changed blind spot size to [3, 3] from default |

| | |
|--------------------------------------------------------|-------------------------------------------------|
| <i>C. elegans</i> (Supplementary Fig. 49) | Changed blind spot size to [3, 3] from default |
| Others | |
| Mouse ear skin, kidney, muscle (Supplementary Fig. 57) | Changed blind spot size to [1, 19] from default |
| Rat hippocampus culture (Supplementary Fig. 58) | Default |

Supplementary Table 2. List of network configurations used for each dataset.

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