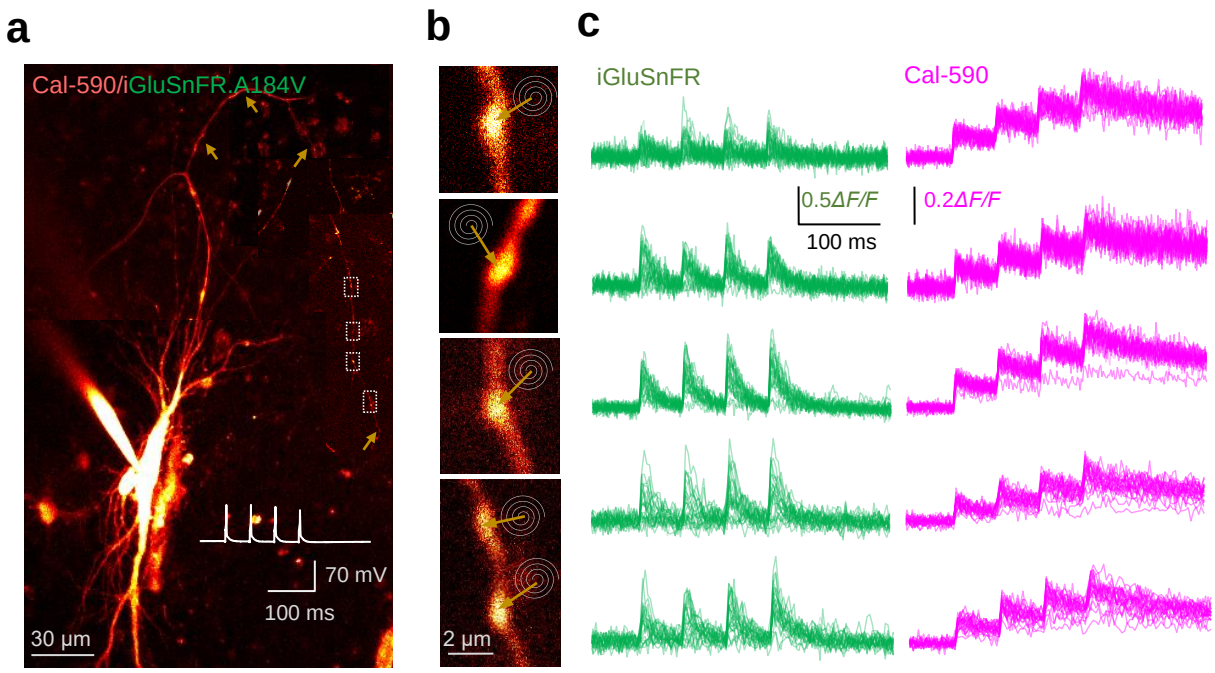
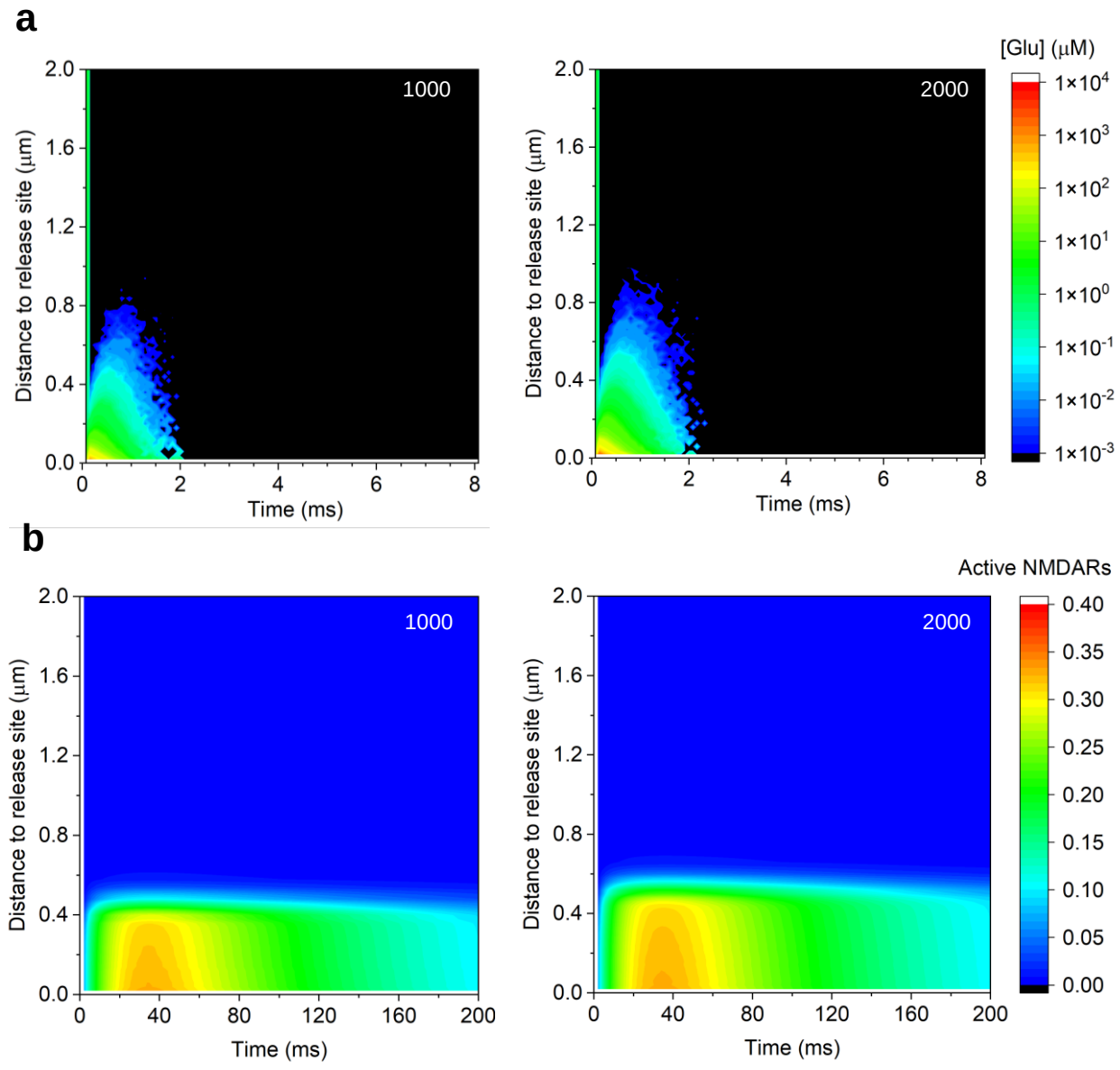


Supplementary Figure 1. Documenting the profile of glutamate escape at individual presynaptic boutons of CA3 pyramidal cells.

(a) CA3 pyramidal cell (organotypic hippocampal slice) expressing SF-iGluSnFR.A184V and dialysed whole-cell with Cal-590 and 4 µM Alexa-594, with 4 ROIs along the axon (arrowheads, dotted rectangles; collage of 8-17 µm deep image stack projections); inset trace, four action potentials 50 ms apart elicited at the soma in current-clamp.

(b) Presynaptic axonal boutons (ROIs shown in **a**); spirals and arrows, Tornado scan positions.

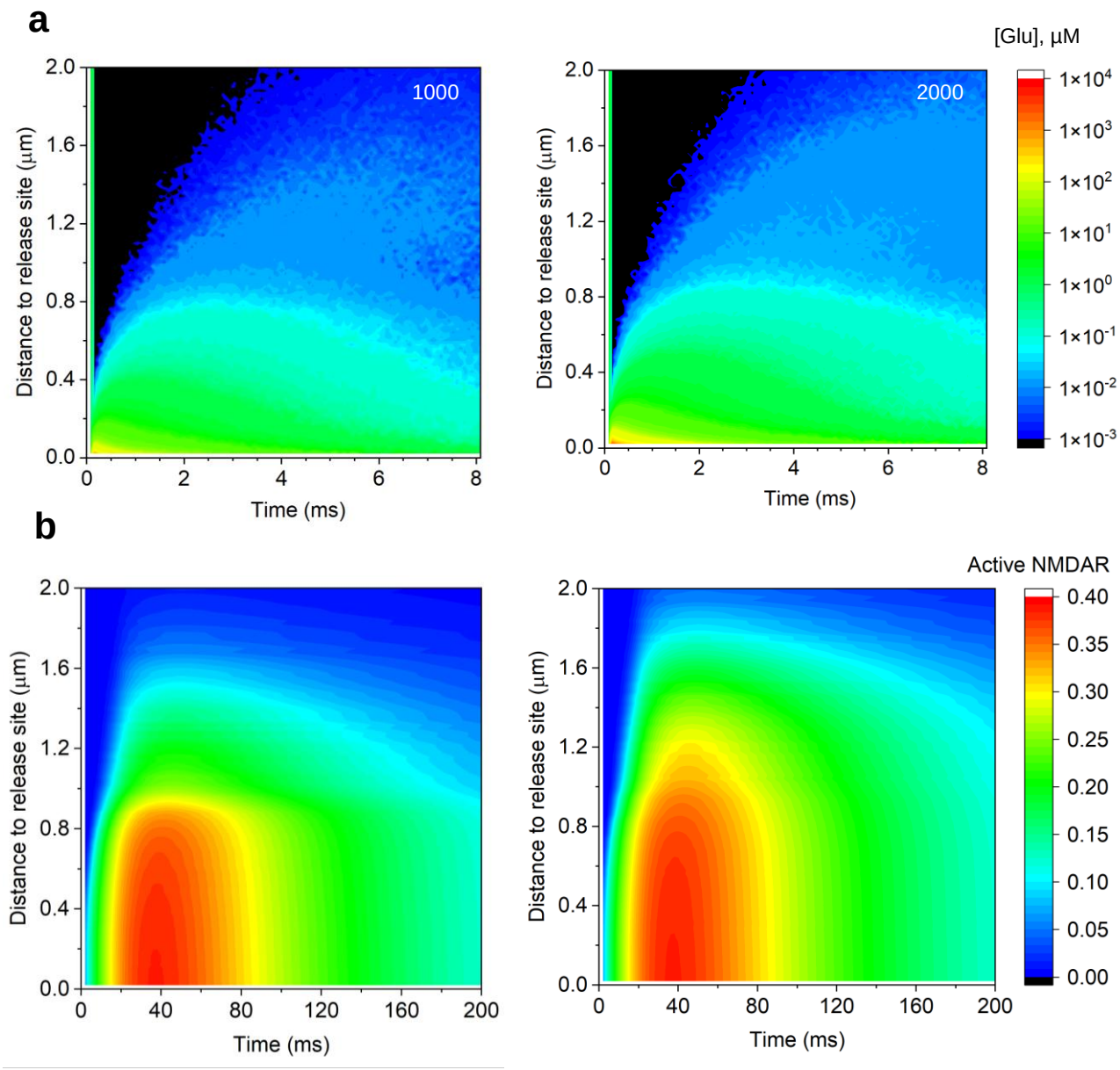
(c) Fluorescence time course; integrated Tornado scan signal, 500hz sampling rate in iGluSnFR (green) and Cal-590 (magenta) channels (λ_x^{2P}=910 nm), in response to four action potentials 50 ms apart, as indicated, for the boutons in **b**; 20-24 trials shown.



Supplementary Figure 2. Concentration profile of free glutamate and NMDA receptor activation following synaptic release, with transporters distributed across neuropil.

(a) The spatiotemporal dynamics of the free glutamate concentration in simulation experiments illustrated in Fig. 2a, with 1000 (*left*) or 2000 (*right*) molecules released, as indicated.

(b) The spatiotemporal dynamics of NMDA receptor activation, assuming no Mg^{2+} block, computed based on the free glutamate dynamics as shown in **a**, with the corresponding numbers of molecules released, as indicated; see Methods for further detail.



Supplementary Figure 3. Concentration profile of free glutamate and NMDA receptor activation following synaptic release, with transporters concentrated on astrocyte surfaces.

(a) The spatiotemporal dynamics of the free glutamate concentration in simulation experiments illustrated in Fig. 2d, with 1000 (*left*) or 2000 (*right*) molecules released, as indicated.

(b) The spatiotemporal dynamics of NMDA receptor activation, assuming no Mg^{2+} block, computed based on the free glutamate dynamics as shown in **a**, with the corresponding numbers of molecules released, as indicated; see Methods for further detail.