Electronic Supplementary Information (ESI)

Real-Time Characterization of Uptake Kinetics of Glioblastoma vs. Astrocytes in 2D Cell Culture Using Microelectrode Array

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Control experiments

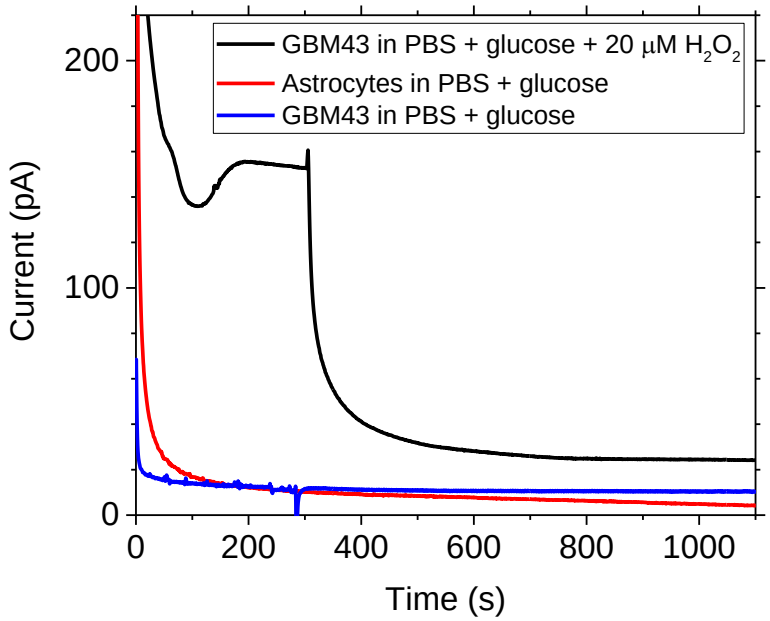

Fig. S-1. Control experiments were performed to detect cellular secretion using the same experimental setup and timeline as in the measurements of H_2O_2 uptake kinetics. The amperometric signals measured for astrocytes (red) and GBM43 cells (blue) exposed to 5.5 mM glucose in PBS (without H_2O_2) were smaller than the smallest measured signal during H_2O_2 exposure, as indicated by the response (black) for GBM43 cells exposed to 20 μM H_2O_2 in the same measurement medium. This observation indicates that the response due to release of analytes by the cells (if any) is below the magnitude of the signals measured during H_2O_2 exposure.

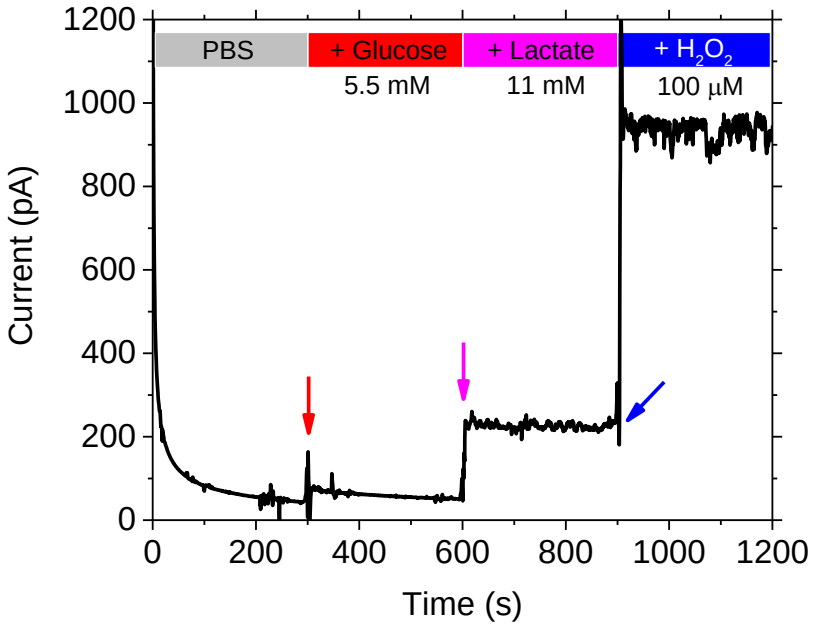

Fig. S-2 Response of representative platinum electrode in the MEA to step changes in concentration of glucose, lactate and H_2O_2. Arrows indicate the time at which each compound is introduced into the solution. The solution is homogenized by stirring using a magnetic bar. The noise level observed in the signal is due to the stirring of the solution. Homogenized concentrations are indicated at the top of the plot.

Sensitivities to glucose, lactate and H_2O_2 are calculated as the ratio of the change in current (ΔI) to the change in concentration (ΔC):
ΔI/ΔC (glucose) = 35.4 pA/5.5 mM = 6 pA/mM
ΔI/ΔC (lactate) = 183.4 pA/11 mM = 16 pA/mM
ΔI/ΔC (H2O2) = 728.8 pA/0.1 mM = 7288 pA/mM

The selectivity to H2O2 is calculated as the sensitivity ratio of H_2O_2 to glucose and lactate:
Selectivity of H2O2 relative to glucose = 1130
Selectivity of H2O2 relative to lactate = 437

From the literature, rat astrocytes exposed to a sustained concentration of 50 µM H_2O_2 for 2 hours exhibited changes in glucose uptake and lactate release, from 1.28 to 1.05 µmol (mg protein)$^{-1}$ h^{-1} for glucose uptake and from 2.40 to 1.50 µmol (mg protein)$^{-1}$ h^{-1} for lactate release [1]. This result indicates that H_2O_2 induced negative changes of 0.23 µmol (mg protein)$^{-1}$ h^{-1} in glucose uptake and 0.9 µmol (mg protein)$^{-1}$ h^{-1} in lactate release, which correspond to 8.3 and 32.5 amol s^{-1} cell^{-1}, respectively. Although these changes in the rates are in the same order as the smallest H_2O_2 uptake rate measured in our experiments, 8 amol s^{-1} cell^{-1} as obtained for astrocytes exposed to 20 µM H_2O_2, the selectivity of H_2O_2 relative to glucose (1130) and lactate (437) indicates that their impact on the H_2O_2 signals is minimal.

Fabrication procedure, cross sections and photograph of microelectrode array (MEA).

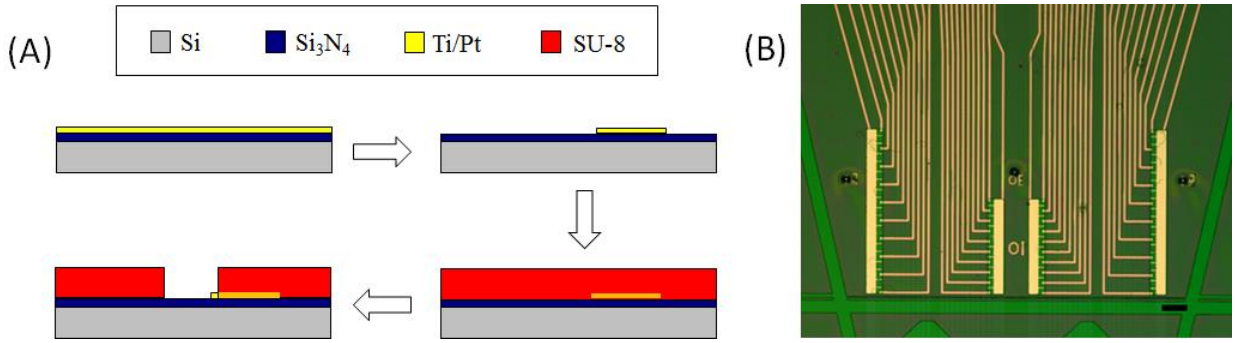

Fig. S-3. Cross sections (A) and photograph of the MEA chip (B). Scale bar is 100 µm. On silicon substrate, 300 nm silicon nitride was deposited by low pressure chemical vapor deposition (LPCVD). Titanium/platinum (10 nm/100 nm) were deposited by electron-beam evaporation, followed by photo-lithographical patterning and lift-off processing to define the electrodes and lead traces. SU-8 photoresist (0.5 µm thick) was spin-coated and then photo-lithographically patterned to expose the electrodes and contact pads. Finally, the wafer was diced, and the dies were wire-bonded to printed circuit boards (PCBs).

Representative pictures of astrocyte and GBM43 cultures before and after exposure to 500 µM H_2O_2.

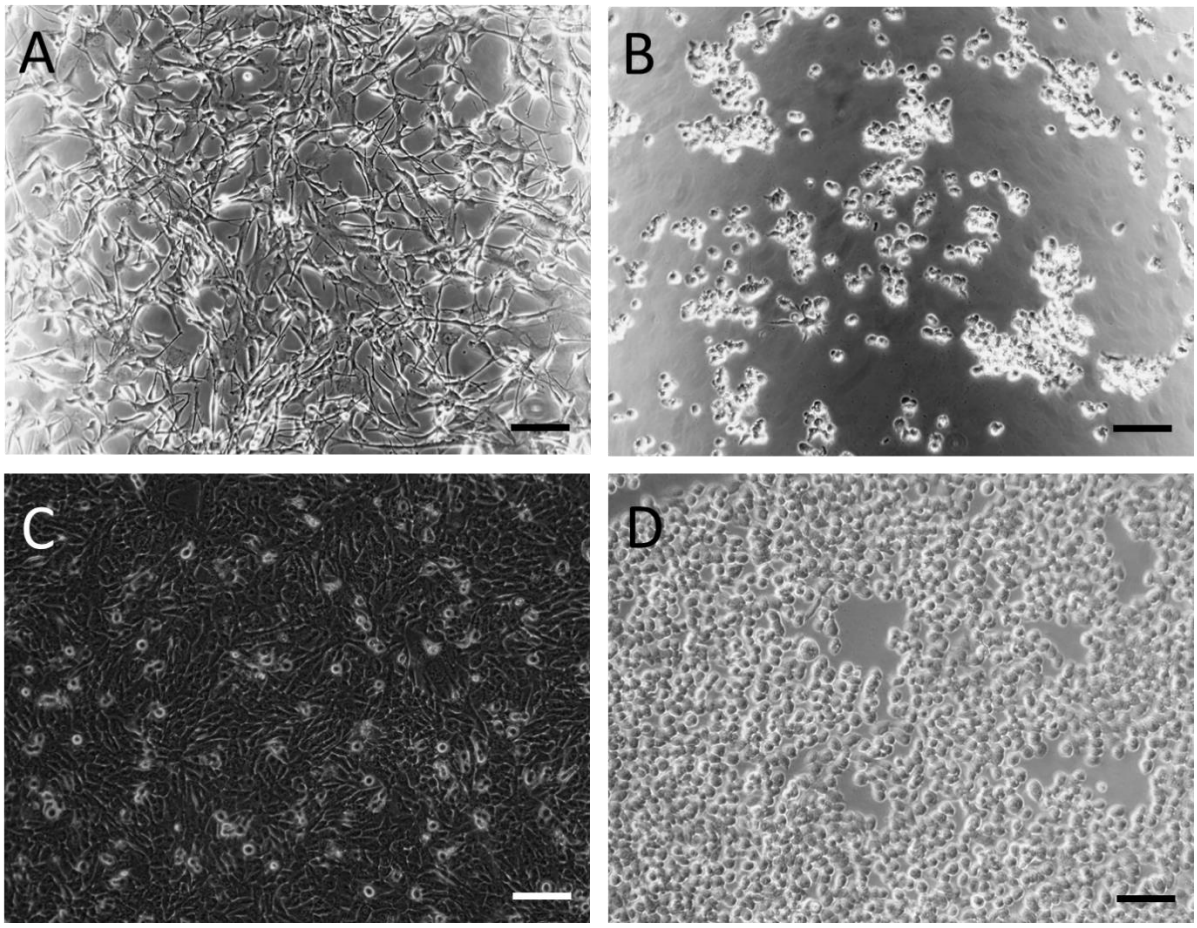

Fig. S-4. Representative pictures of human astrocyte (A-B) and GBM43 (C-D) cultures. (A, C) before treatment with 500 µM H_2O_2 and measurement with MEA for approximately 2 h. (B, D) after the treatment. Scale bars are 100 µm.

Both cell types lose adherence and change morphology after the treatment; however, as shown in Fig. S-5, the cells are highly viable.

Live/dead assay of astrocyte and GBM43 cultures after 2 hours of 500 µM H_2O_2 exposure.

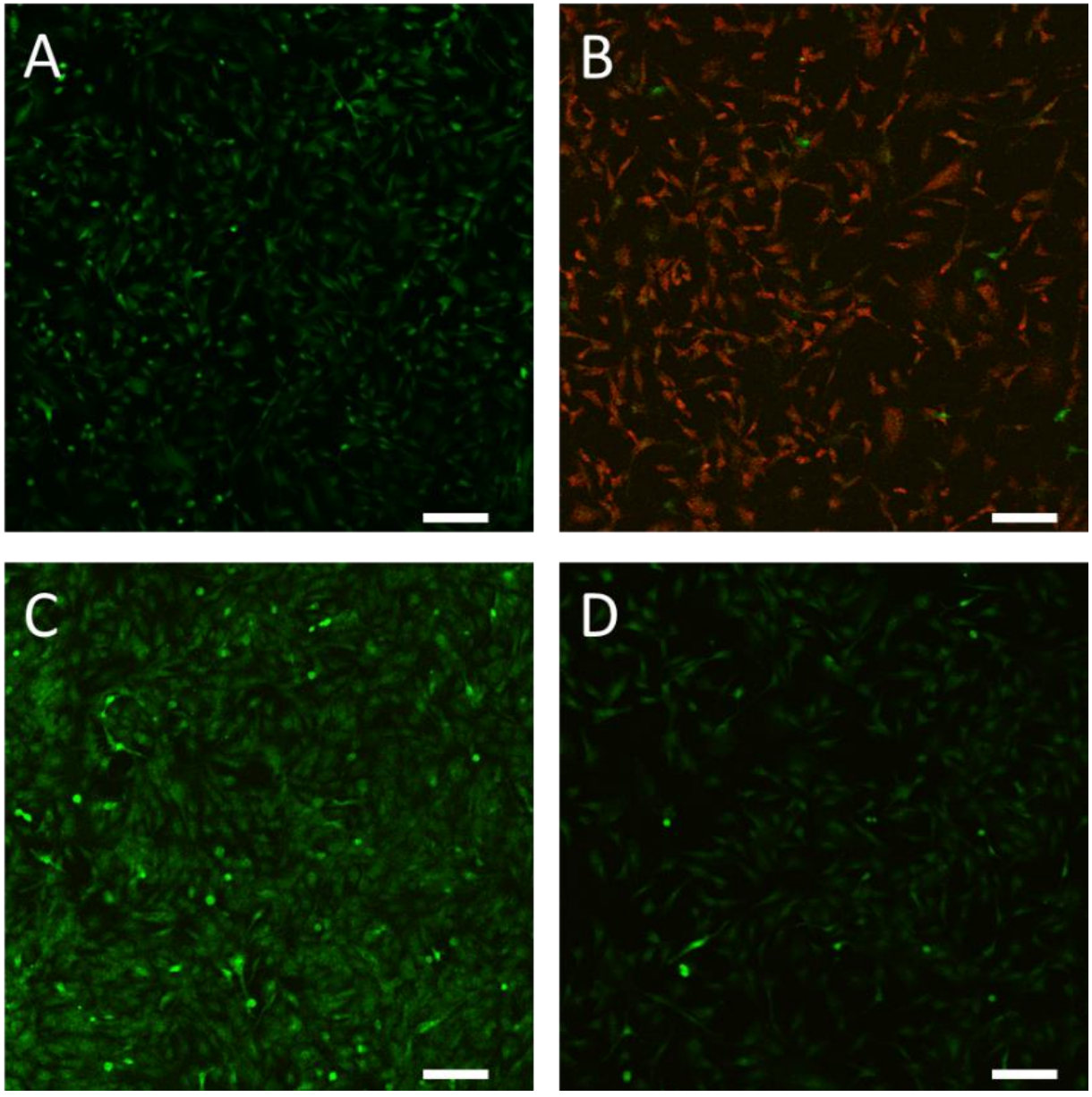

Fig. S-5. Live/dead assays for astrocytes (A-D) and GBM43 (E-H in next page) cultures. (A) positive control (no treatment). (B) negative control (fixed with formalin for 20 minutes). (C) incubated for 2 h in PBS (pH 7.4) + 5.5 mM glucose. (D) incubated for 2 h in PBS + 5.5 mM glucose + 500 µM H_2O_2. Cell viability determined by CellTracker Green (live) and propidium iodide (red, dead) labeling. Scale bars are 100 µm. (Continue on next page).

Two hours in H_2O_2 caused a fraction of astrocytes to lose adherence and thus being washed away during the live/dead assay, which would explain the apparent reduction in cell confluence in (D) compared to (C). However, the astrocytes that remained adhered were viable.

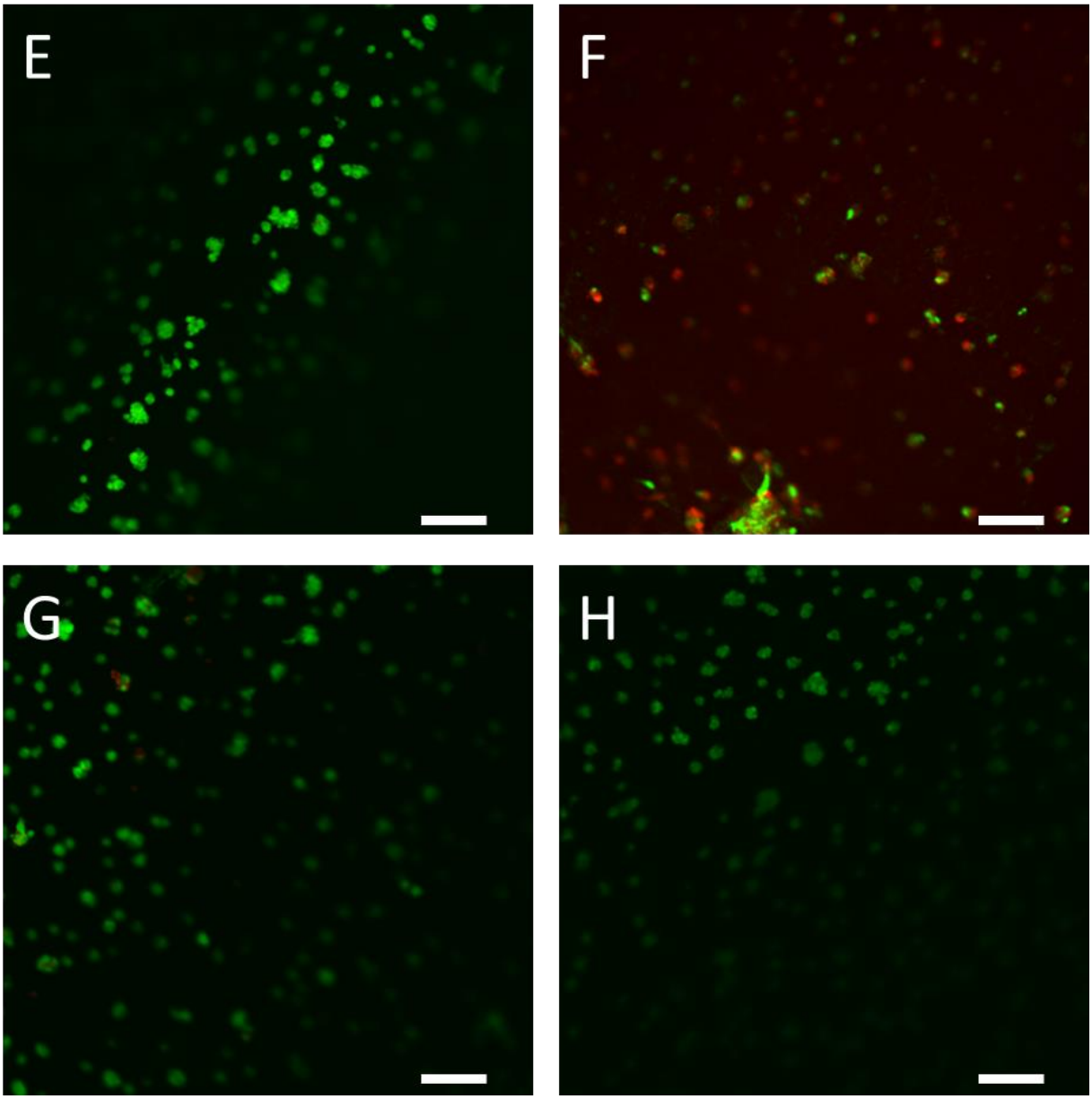

Fig. S-5. (Continued from previous page) Live/dead assays for astrocytes (A-D in previous page) and GBM43 (E-H) cultures. (E) positive control (no treatment). (F) negative control (fixed with formalin for 20 minutes). (G) incubated for 2 h in PBS (pH 7.4) + 5.5 mM glucose. (H) incubated for 2 h in PBS + 5.5 mM glucose + 500 µM H_2O_2. Cell viability determined by CellTracker Green (live) and propidium iodide (red, dead) labeling. Scale bars are 100 µm.

Despite losing adherence, two hours in 500 µM H_2O_2 had no apparent harmful effect on GBM43 cells viability (H).

Schematic of the simulation geometry and boundary conditions.

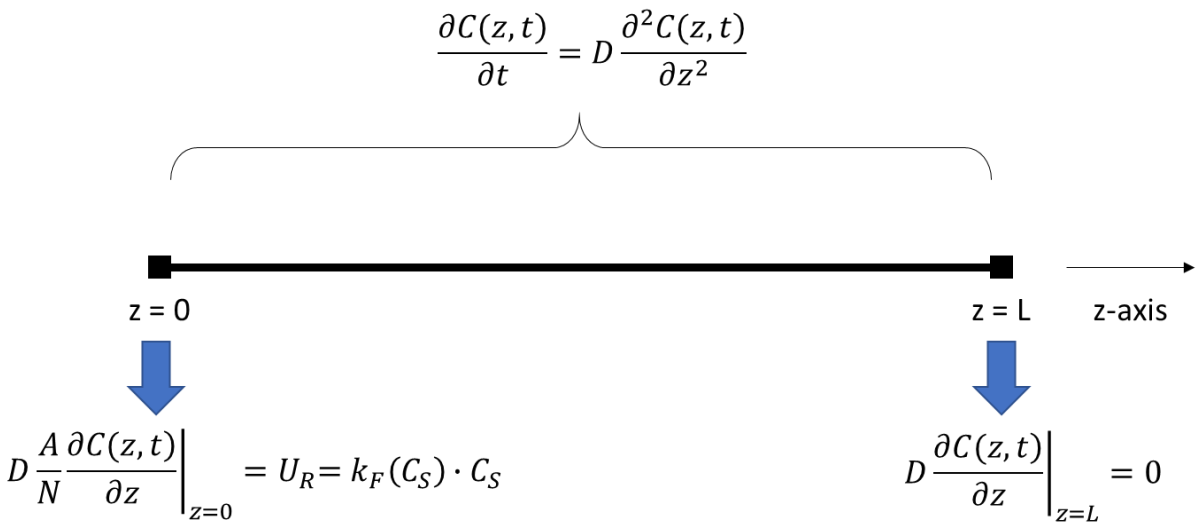

Fig. S-6. Simulation geometry and boundary conditions. The surface of the cells is defined as the plane z = 0, wherein the boundary condition is set to U_R. The interface air/solution is defined as the plane z = L, wherein the boundary condition is set to zero flux. The initial condition, C(z,0) for $0 \leq z \leq L$, is set to C_0 where C_0 = 20, 60, 100, 200, 300 or 500 µM.

Representative concentration transients measured in real time at the electrode positions.

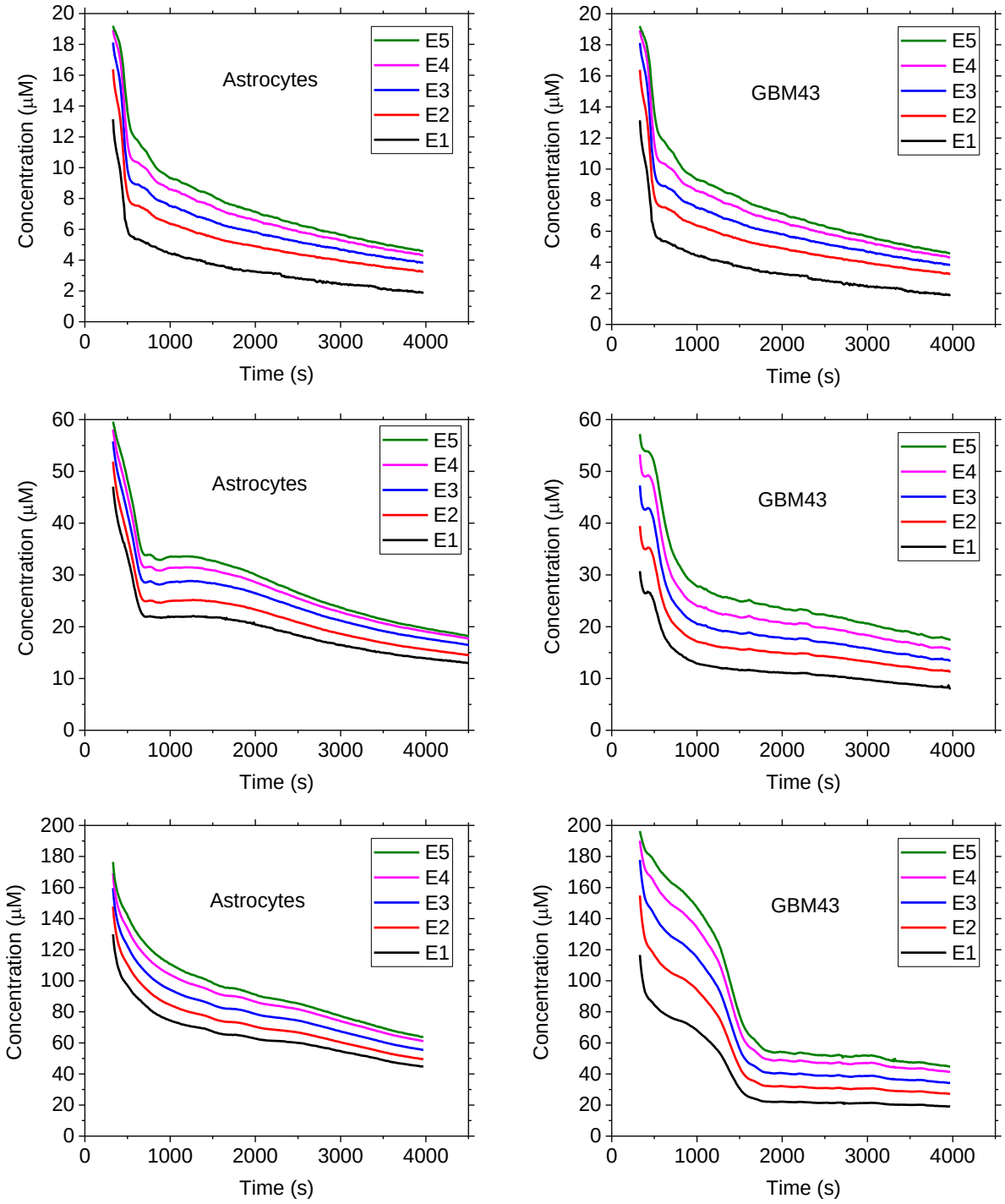

Fig. S-7. Representative concentration transients measured in real time at the electrode positions during experiments wherein the cell cultures of astrocytes and GBM43 are exposed to C_0 of 20, 60, 200, 300 and 500 μM H_2O_2. (Continue in next page).

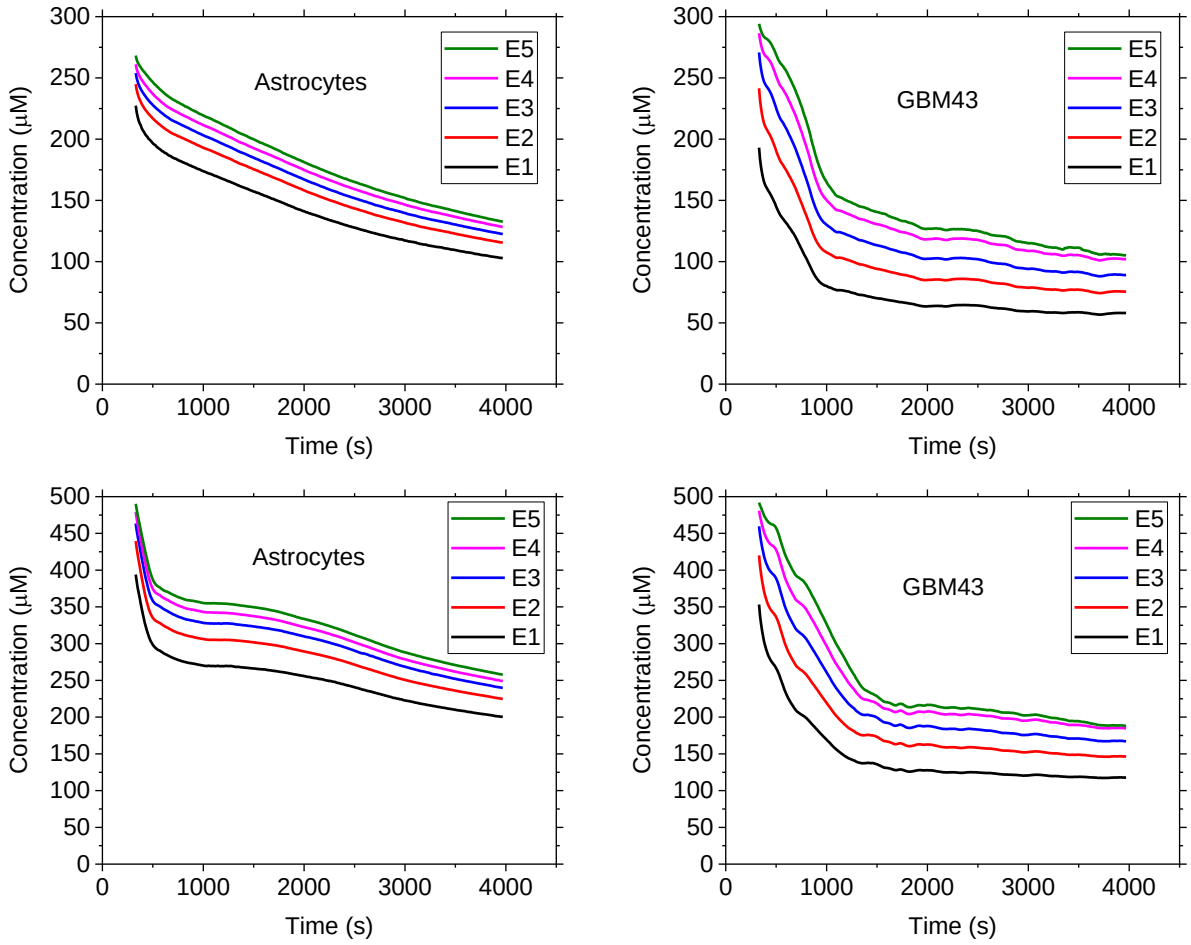

Fig. S-7. (Continued from previous page) Representative concentration transients measured in real time at the electrode positions during experiments wherein the cell cultures of astrocytes and GBM43 are exposed to C_0 of 20, 60, 200, 300 and 500 μM H_2O_2.

The measured traces indicate the presence of gradients in H_2O_2 over the entire experiment for all the tested C_0 values.

Representative concentration as a function of distance from the cell surface at selected time points.

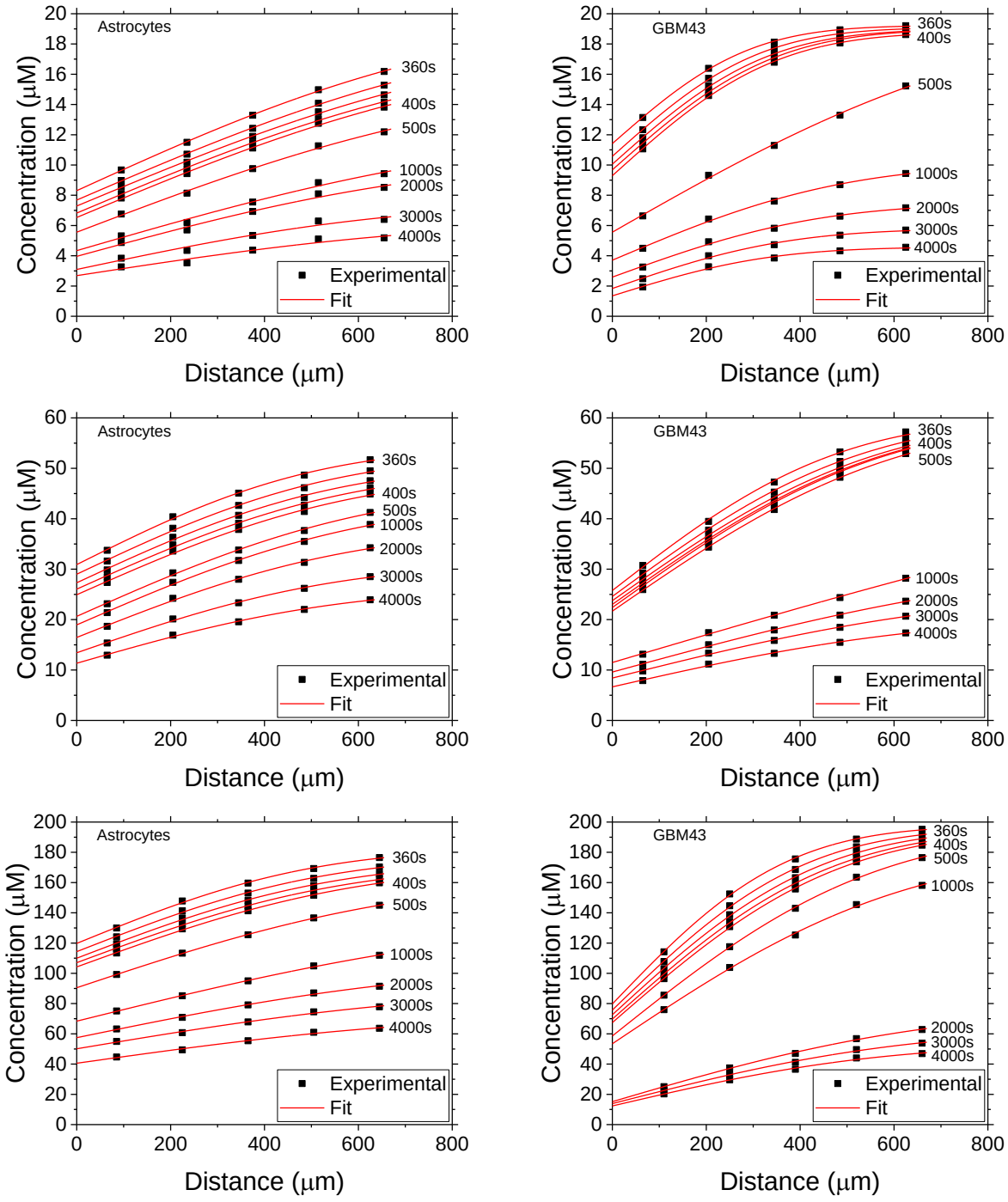

Fig. S-8. Representative concentration as a function of distance from the cell surface at the indicated time points for both astrocytes and GBM43 cells exposed to C_0 of 20, 60, 200, 300 and 500 μM H_2O_2. (Continue in next page).

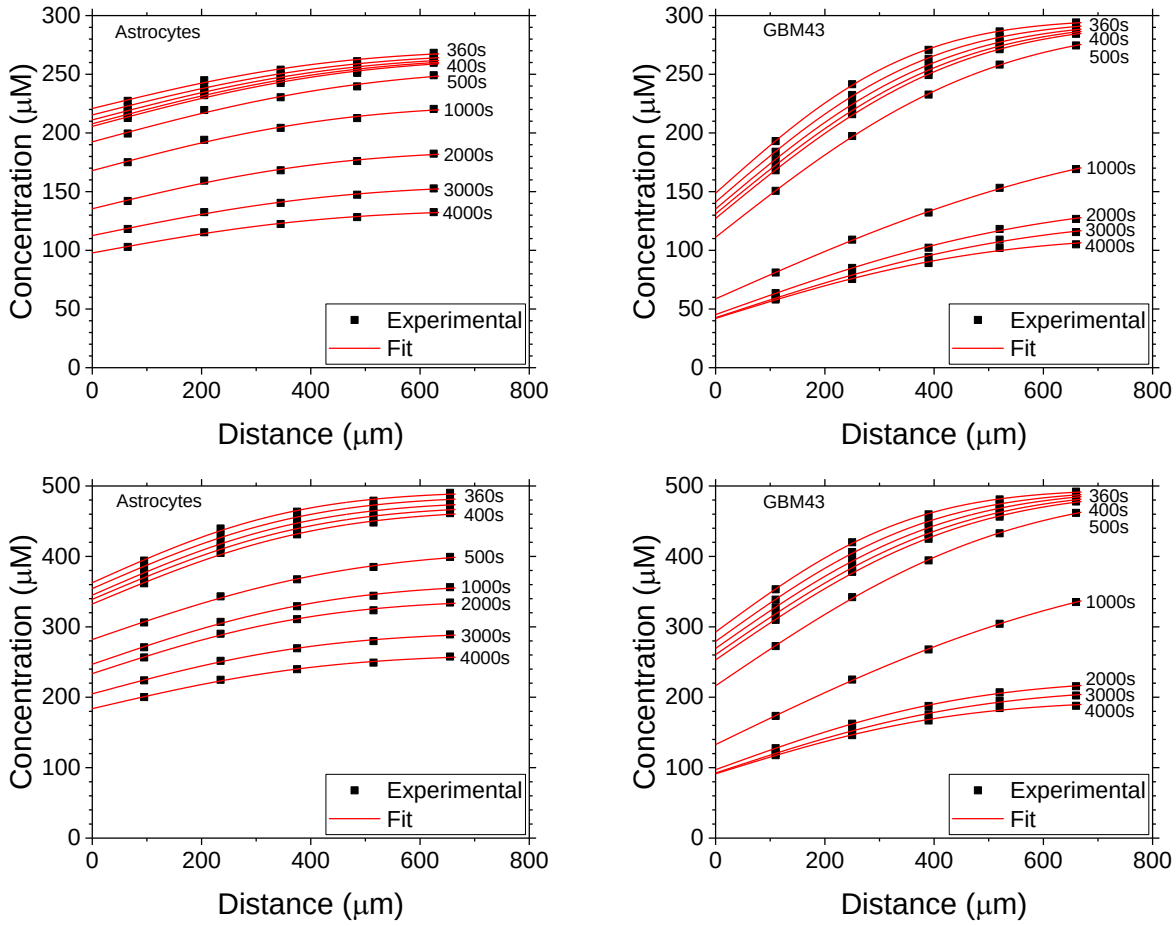

Fig. S-8. (Continued from previous page) Representative concentration as a function of distance from the cell surface at the indicated time points for both astrocytes and GBM43 cells exposed to C_0 of 20, 60, 200, 300 and 500 μM H_2O_2.

For both cell types, C(z,t) was measured for all the C_0 values, including triplicates of each. All the fitted curves (red lines) were obtained using a single fitting function whose parameters were adjusted at each time point without carrying information over from previous time points.

Discussion on the fitting expression $C(z) = A_1[1 + A_2 erfc(A_3 z)]$.

The established expression that describes C(z,t) for a first-order irreversible reaction at a planar electrode in contact with a semi-infinite volume of solution is given by Eq. (S1) [2],

$$\bar{C}(z,s) = C_0\left[\frac{1}{s} - \frac{k_+ e^{-(s/D)^{1/2}z}}{D^{1/2}s\left(\frac{k_+}{D^{1/2}} + s^{1/2}\right)}\right] \qquad \text{(S1)}$$

where
$\bar{C}(z,s)$ is the Laplace transform of $C(z,t)$,
z and s are the spatial variable and Laplace variable, respectively,
C_0 is the initial uniform concentration,
k_+ is the heterogeneous reaction rate constant, and
D is the diffusion coefficient.

The inverse transform of Eq. (S1) is given by Eq. (S2)

$$C(z,t) = C_0\left\{1 - \left[1 - \frac{e^{\left(\frac{k_+ z}{D} + \frac{k_+^2 t}{D}\right)} erfc\left(\frac{z}{2\sqrt{Dt}} + \frac{k_+\sqrt{t}}{\sqrt{D}}\right)}{erfc\left(\frac{z}{2\sqrt{Dt}}\right)}\right] erfc\left(\frac{z}{2\sqrt{Dt}}\right)\right\} \qquad \text{(S2)}$$

The expression $C(z) = A_1[1 + A_2 erfc(A_3 z)]$ is a generalized expression derived from Eq. (S2) by lumping the variables, parameters and associated relationships into the coefficients A_1, A_2 and A_3, for a given time point. The coefficients A_1, A_2 and A_3 were used as parameters to fit the data of experimental concentration vs. distance at time points spaced by 10 s. As shown in Fig. 3 (in manuscript) and Fig. S-6, the expression $C(z) = A_1[1 + A_2 erfc(A_3 z)]$ fitted all the experimental data sets satisfactorily.

Since in the current study the depth of the solution is finite and the 2D monolayer of cells is expected to act as H2O2 sink exhibiting kinetics beyond first-order, the fitting parameters A_1, A_2 and A_3 will have somewhat different but related physical interpretations from the original equation (S2). However, further investigation on the specifics of the fitting parameters is out of the scope of this work.

Simulation results using constant k_F extracted from linear regressions within 0–20 µM H_2O_2.

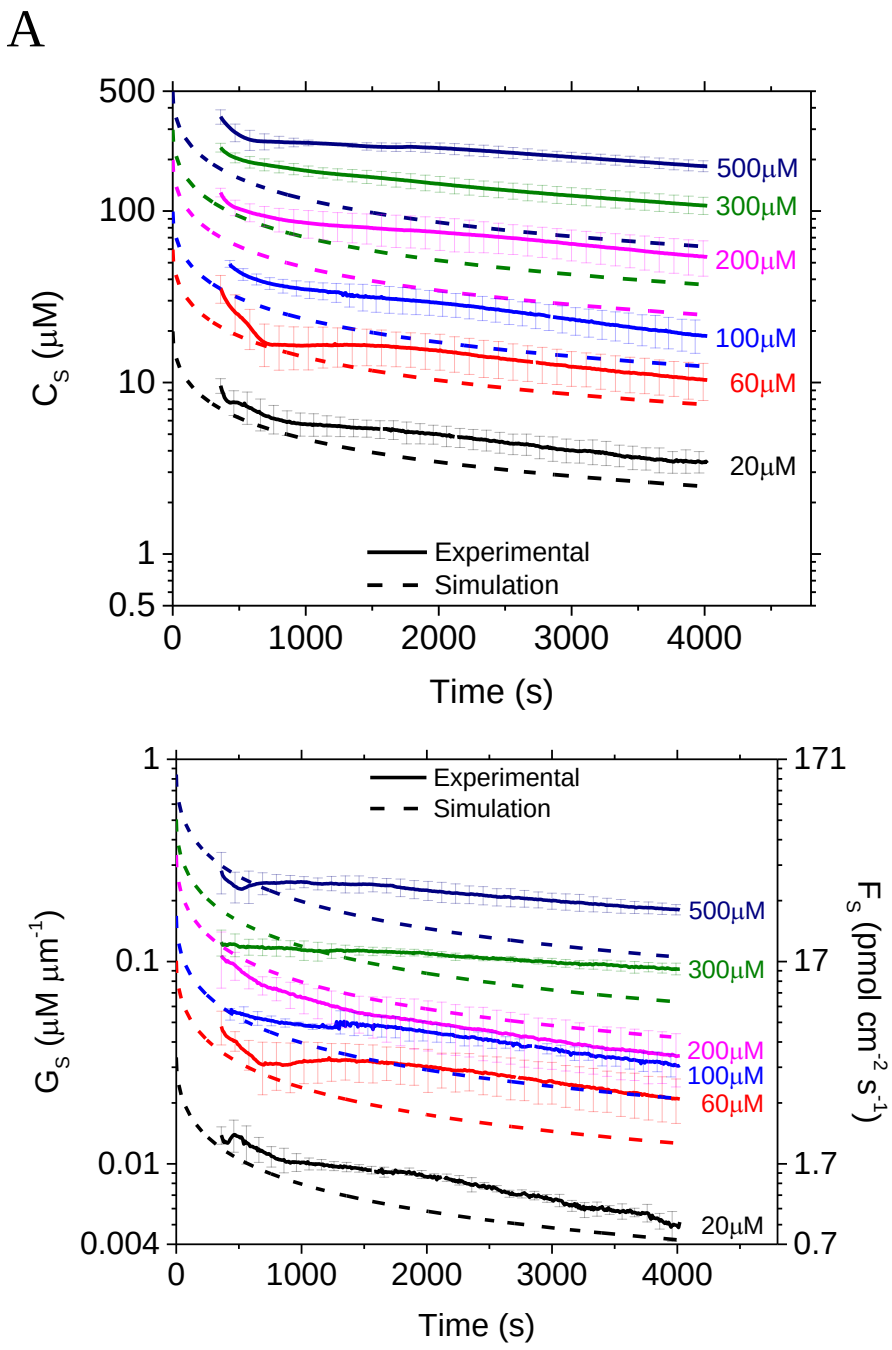

Fig. S-9. Simulation results using constant k_F extracted from linear regressions of the experimental data within 0–20 µM H_2O_2. (A) Astrocytes, using k_F of 2.63×10^{-12} L s^{-1} cell^{-1}. (B) GBM43, using k_F of 4.2×10^{-12} L s^{-1} cell^{-1}. (Continue in next page).

B

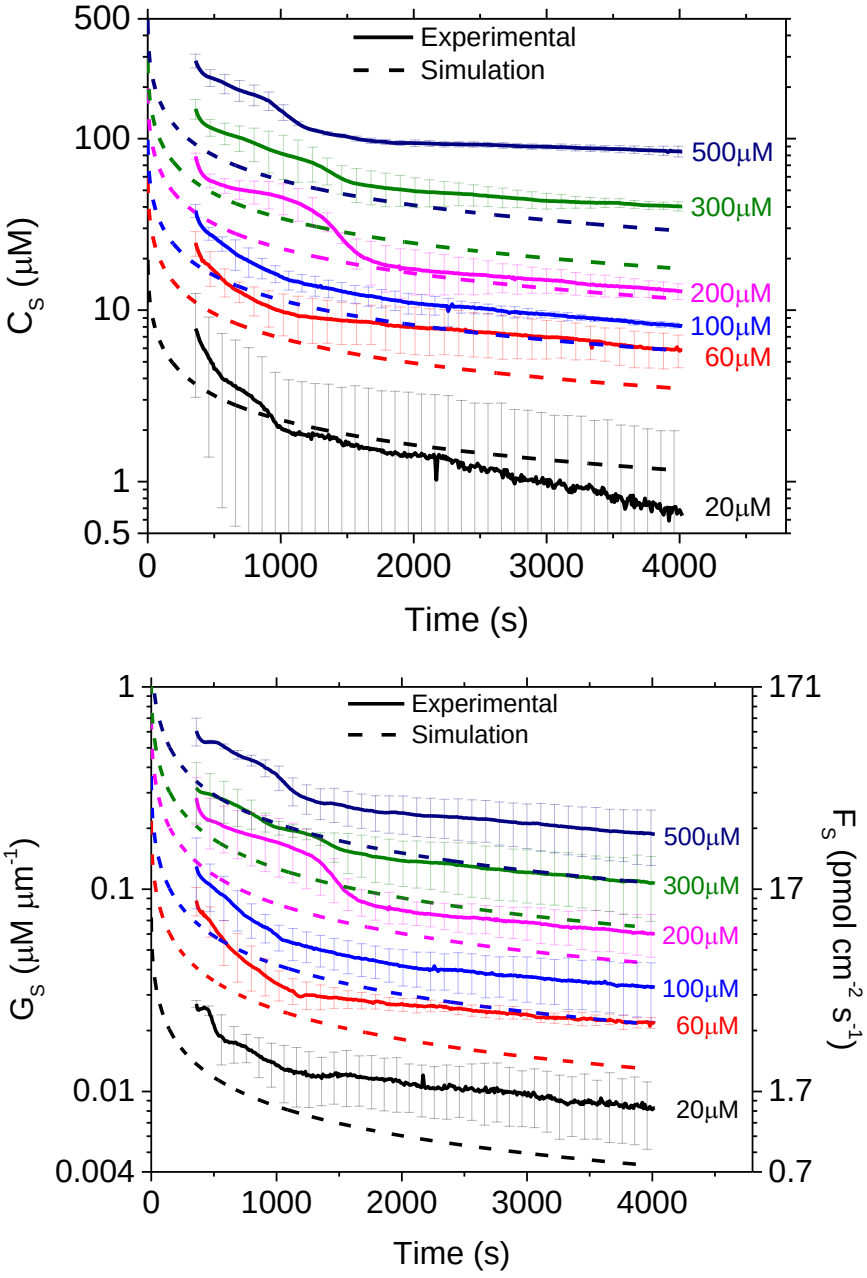

Fig. S-9. (Continued from previous page) Simulation results using constant k_F extracted from linear regressions of the experimental data within 0–20 μM H_2O_2. (A) Astrocytes, using k_F of 2.63×10^{-12} L s^{-1} cell^{-1}. (B) GBM43, using k_F of 4.2×10^{-12} L s^{-1} cell^{-1}.

Compared to Figures 4 and 5 (in manuscript), the simulation results using constant k_F are in well agreement at low C_S but deviate progressively from the experimental data as C_S increases.

References

[1] J. R. Liddell, C. Zwingmann, M. M. Schmidt, A. Thiessen, D. Leibfritz, S. R. Robinson and R. Dringen, *Journal of neuroscience research*, 2009, **87**, 2696–2708.

[2] Bard, A. and Faulkner, L., Electrochemical Methods: Fundamentals and Applications, Wiley, 2000, p. 192.