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Supplementary Material

Mathematical Description of the In Vitro Model (Fig.1A)

For this model, O2 concentration in the solution layer is described by planar homogeneous diffusion equation (1):

$$\frac{\partial Cs(X,t)}{\partial t} = Ds \frac{\partial^2 Cs(X,t)}{\partial X^2} \quad X \in [0, Ls] \tag{1}$$

and for the cell layer - as inhomogeneous equation (2) in which specific O_2 consumption rate, *k,* is included in the right part :

$$\frac{\partial Cc(X,t)}{\partial t} = Dc \frac{\partial^2 Cc(X,t)}{\partial X^2} - k \quad X \in (Ls, Ls + Lc] \tag{2}$$

X is the distance from the gas/solution interface towards the bottom; t – time; Cs, Ls, Ds and Cc, Lc, Dc are oxygen concentration, thickness and O_2 diffusion coefficient in the solution (*s*) and the cell (*c*) layer.

Boundary conditions for this system are the following. At the air-solution interface, fast oxygen diffusion in the air provides rapid equilibration and constant O_2 at this boundary point in the solution, with C_0 [μM·ml^{-1}] obeying Henry's Law [1]:

$$Cs(X = 0) = C_0 = S_{O2} pO_2 = S_{O2} (p_b - p_{H2O}) \Phi_{O2} \tag{3}$$

where S_{O2} [μM·ml^{-1}·kPa^{-1}] - oxygen solubility in the solution phase, p_b - total barometric pressure of air, p_{H2O} - partial pressure of water vapor, Φ_{O2} - oxygen volume fraction in dry air (normally 0.20946).

At the cell layer -solution interface ($X=Ls$), boundary conditions are defined by the mass conservation requirement, i.e. equal O_2 fluxes from the solution layer Js and to the cell layer Jc

$$Js\Big|_{X=Ls-0} = -Ds \frac{dCs}{dX}\Big|_{X=Ls-0} = -Dc \frac{dCc}{dX}\Big|_{X=Ls+0} = Jc\Big|_{X=Ls+0} \tag{4}$$

and O_2 partitioning at the boundary:

$$Cc\Big|_{X=Ls+0} = H \cdot Cs\Big|_{X=Ls-0} \tag{5}$$

where H is O_2 partition coefficient at the cells/solution interface.

At the well bottom-cell layer interface ($X=Ls+L_c$), boundary conditions correspond to zero O_2 flux:

$$Jc\Big|_{X=Ls+Lc} = -Dc \frac{dCc}{dX}\Big|_{X=Ls+Lc} = 0 \tag{6}$$

For the samples under steady state conditions (resting cells), oxygen profiles can be obtained in an analytical form:

$$Cs = C_0 - k\frac{Lc}{Ds}X \tag{7}$$

$$Cc = \frac{k}{2Dc}\left(X - Ls\right)^2 - k\frac{Lc}{Dc}\left(X - Ls\right) + H\left(C_0 - k\frac{LcLs}{Ds}\right) \tag{8}$$

for the solution and the cell layers, respectively. Therefore, under steady state dissolved O_2 concentration decreases from the top to the bottom of the vessel: as a linear function in the medium changing from C_0 to $C_0 - k\frac{LcLs}{Ds}$. In the cell layer O_2 concentration decreases as quadratic function: from $H\left(C_0 - k\frac{LcLs}{Ds}\right)$ to the $H\left(C_0 - k\frac{LcLs}{Ds}\right) - k\frac{Lc^2}{2Dc}$.

1. Crank J. 1975. The Mathematics of Diffusion, 2ed., Oxford university press, 1975, 414p.

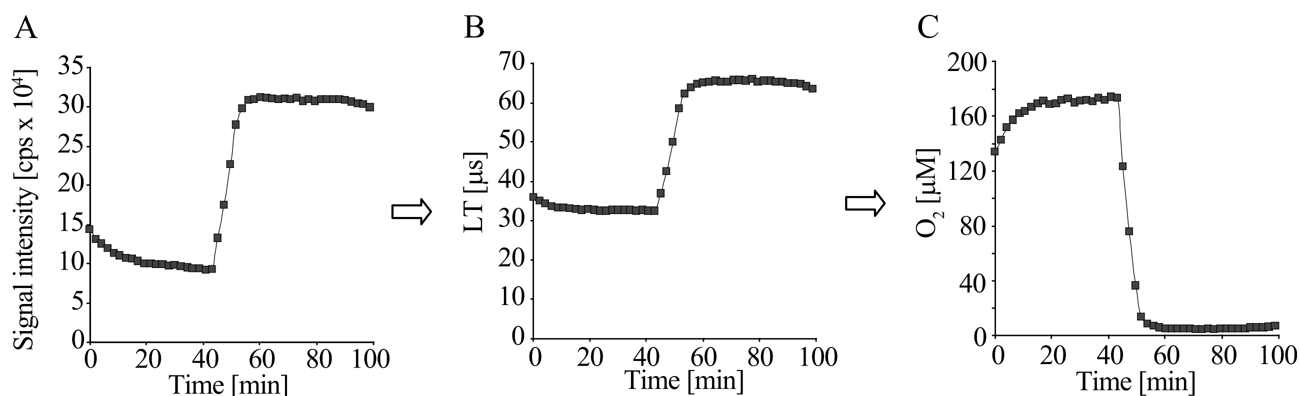

Figure S1. Representative example of raw fluorescence and processed data for a typical measurement experiment with dPC12 cells loaded intracellularly with MitoXpress probe, i.e. one well of a 96-well plate: A – raw TR-F intensity profile (delay time 30 µs). Blank TR-F signals (cells without probe) are normally under 1000 cps, thus giving a signal-to-blank ratio of >100. B – phosphorescence lifetime profile which corresponds to A, with lifetime values calculated with the formula: $\tau = (t_2-t_1)/\ln(F_1/F_2)$ (see Methods). C – Intracellular oxygen concentration profile which corresponds to A, generated using the lifetime calibration shown in Fig. S2 below: $Cc = -0.0016\tau^3 + 0.3231\tau^2 - 22.244\tau + 522.96$ (see Methods). After the initial signal drift due to plate equilibration (0-20 min), steady state condition is achieved (20-40min). Subsequent activation of resting cells by the addition of 5 mM EGTA (at ~40min) induces sustained anoxia within the cell layer (60-100 min).

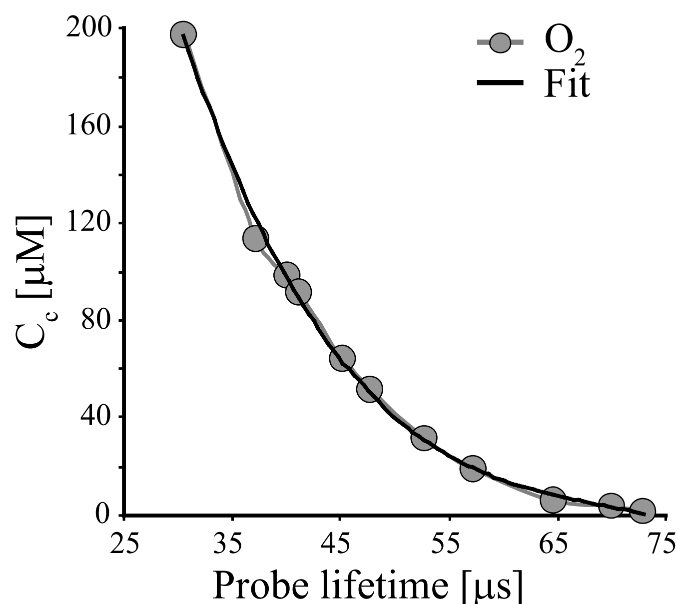

Figure S2. Calibration plot for calculating O_2 concentration in dPC12 cells by means of the intracellular probe MitoXpress and phosphorescence lifetime measurements on Victor^3 TR-F reader.

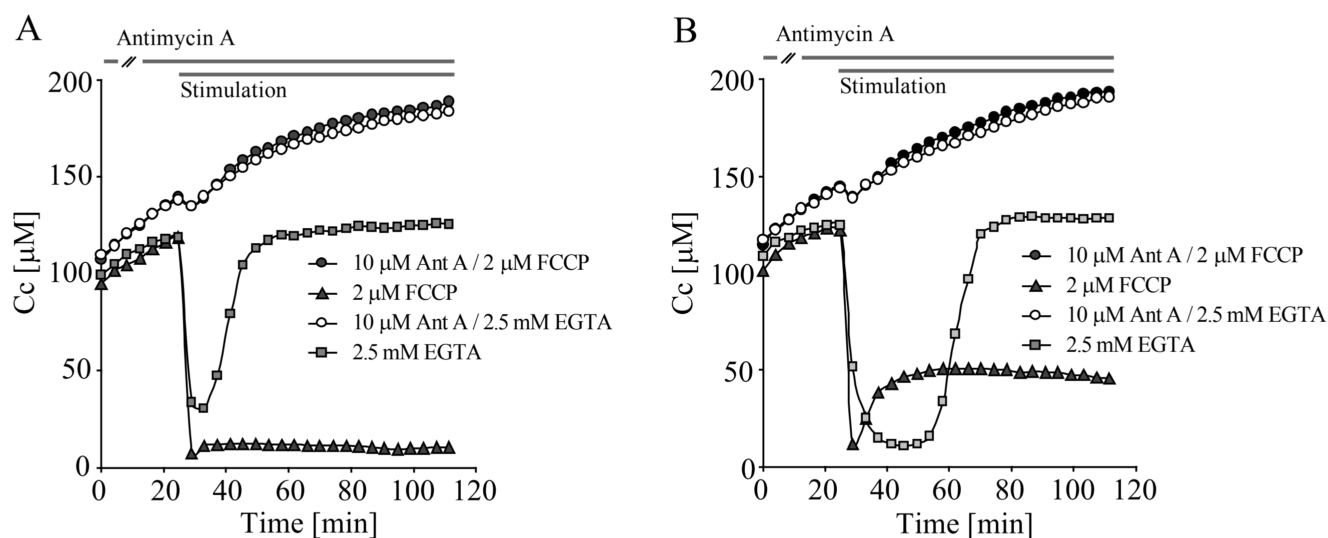

Figure S3. Profiles of cellular oxygenation upon treatment with activators and inhibitors of respiration in 100 µl (A) and 200 µl (B) of galactose (+) medium. Cells treated with FCCP (mitochondrial uncoupler) or EGTA show sustained and transient activation of respiration, respectively. Pre-treatment of cells with antimycin A abolishes these responses.

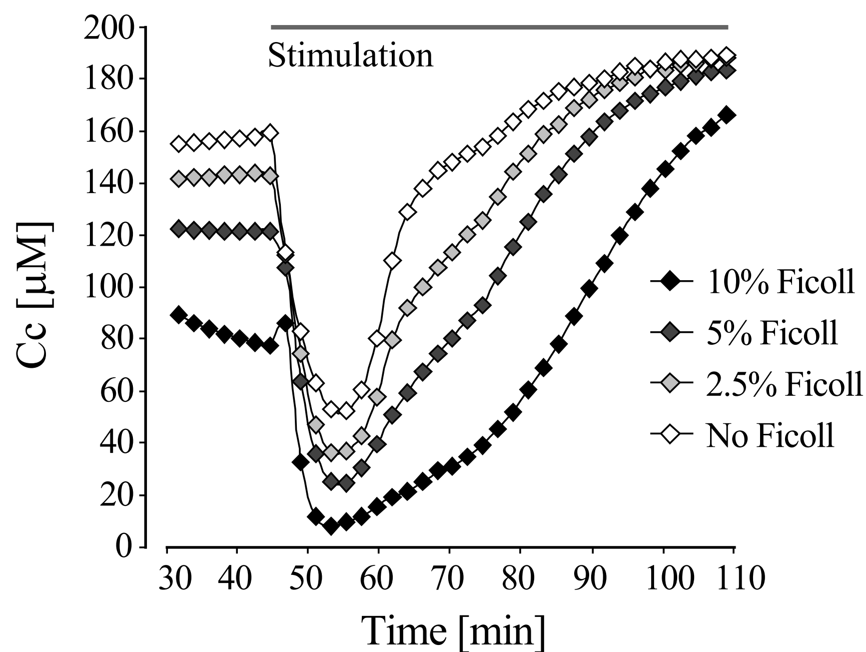

Figure S4. Effect of viscosity on the respiratory response to stimulation. Increase in the concentration of Ficoll makes the deoxygenation phase faster and reoxygenation on the cells slower. Experiment was performed in 100 µl of galactose (+) medium. Cells were stimulated by 5 mM EGTA.