Electronic Supplementary Material (ESI) for Lab on a Chip
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Supplemental Information:

a) Preparation of isolated mitochondria

Isolation of mouse skeletal muscle mitochondria

Skeletal muscle mitochondria were isolated from C57BL6/J mice maintained in our animal facility. Briefly, the mice were euthanized by cervical dislocation, and their soleus muscles placed immediately into ice-cold muscle homogenization buffer (100 mM KCl, 50 mM Tris, 5 mM MgCl2, 1.8 mM ATP, and 1 mM EDTA pH 7.2). The fat and connective tissues were removed, and the muscle chopped into small pieces. The chopped muscle was incubated for 2 minutes in 1 mL of protease medium (1 mL homogenization buffer and 5.66 mg protease *from Bacillus licheniformis*, 10.6 U/mg), washed twice with homogenization buffer, and transferred to a homogenizer containing fresh homogenization buffer. The muscle was homogenized using a motor driven homogenizer for 10 minutes at 150 rpm. The homogenate is centrifuged at 720 g for 5 minutes. The pellet was resuspended in homogenization buffer, and centrifuged for a further 5 minutes at 720 g. The supernatants were combined and centrifuged at 10,000 g for 5 minutes. The pellet was resuspended in homogenization buffer, and centrifuged for a further 5 minutes at 9,000 g. The pellet was resuspended in respiration buffer for protein quantification using the BCA Protein Assay (Thermo Scientific, Prod# 23227) and diluted in ice-cold respiration buffer for immediate use.

Isolation of mitochondria Heb7a and 143B TK- osteosarcoma cell lines

Mitochondria were isolated from the HeLa-derived Heb7A and from the 143B TK- osteosarcoma cell lines using a protocol modified from Trounce et al[1]. Approximately 10^7 cells were pelleted, washed in PBS (phosphate buffered saline), and resuspended in ice-cold

H-buffer (210 mM mannitol, 70 mM sucrose, 1 mM EGTA, 5 mM HEPES, 0.5% BSA). The cells were physically sheared with 15–20 passes in a cold dounce and centrifuged at low speed (800×g for 5 min) at 4°C. The cell lysate suspension was incrementally clarified to remove the large cell debris through 4 rounds of low speed spins and the mitochondria were then pelleted with 2 rounds of high speed spins (10 000×g for 20 min). The pellet was resuspended in respiration buffer for protein quantification using the BCA Protein Assay (Thermo Scientific, Prod# 23227) and diluted in ice-cold respiration buffer for immediate use.

b) Composition of respiration buffer

225 mM mannitol, 75 mM sucrose, 10 mM KCl, 10 mM Tris-HCl, 5 mM KH_2PO_4, pH 7.2

c) Discussion on BCA assay

Ultimately, the purpose of our device is to provide a technology which can obtain an accurate and reproducible report of the mitochondrial membrane potential in a minimal amount of isolated mitochondria. For the purposes of this experiment, we were not confined to the tissue culture limitations that can normally affect commercial and research labs. For that reason, we were able to isolate mitochondria in excess of what was needed for our experiments. As such, the BCA protein assay suited our needs well with its broad working range of 20-2000μg/mL. Even with our abundant protein, however, we still routinely dilute our samples 1:50 and 1:100 in water to expand our volume without using up our sample.

The BCA colorimetric assay is by no means the only or best method to use for protein determination. In our lab, we also use the NanoDrop Spectrophotometer system to estimate protein concentration. The advantage of this system is that it reads directly from 1μL of sample- no dilution or indicators needed. We generally re-suspended our final mitochondrial

pellet in 20-30μL H-buffer, but for smaller isolation preparations it may be necessary to use a smaller final volume. For this reason, it will be imperative to find a sensitive and reliable procedure for protein quantification.

d) Conversion from [TPP^+] to membrane potential

The [TPP^+] measurements were converted to membrane potential and compared according to the following equation[2]:

$$\Delta\Psi_m = \frac{RT}{F}\ln\frac{V_0[TPP^+]_0/[TPP^-]_t - V_t - K_0P}{V_mP + K_iP}, \qquad (1S)$$

where $[TPP^+]_o$ and $[TPP^+]_t$ represent TPP^+ concentration in the test chamber before the addition of mitochondria and at time t respectively. V_o is the initial buffer volume in the chamber and V_t represents the final volume in the chamber which includes the total mass (in mg) of mitochondrial protein (P) added in the assay. For our purposes, the mitochondrial matrix volume (V_m) was assumed to be equal to 1 μL/mg protein. The partition coefficients describe the innate binding and accumulation of the TPP^+ ion to the matrix (K_i) and external (K_o) faces of the inner membrane and are given values of 7.9 μL/mg and 14.3 μL/mg, respectively[3]. For our purposes, we used the values V_0 =70.5 μL; V_t= 75.5 μL and used 750 ng of isolated mitochondrial protein per assay (P).

e) Error Analysis

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The calibration curves for ISE potential (V_{ISE}) vs. [TPP^+] done immediately before and immediately after an example experiment is shown in Fig. 2 (c) of the main text. In principle this defines the uncertainty in the inferred value of [TPP^+] for a given measured value of V_{ISE}. However, in our experiments we also know the value of [TPP^+] just before introduction of mitochondria into the chamber. (It is purposefully kept at 7.2 μM in all of our experiments, including those from different cell lines, to allow more accurate comparison between cell lines.) Therefore, we are only interested in changes in [TPP^+] upon introduction of mitochondria into the chamber. To determine the change in [TPP^+], we need only know the slope of the ISE potential vs. [TPP^+] calibration curve, in order to convert the change in measured ISE potential to a change in [TPP^+].

To determine the slope of the curve, we calculate $dV_{ISE}/d\ ln[TPP^+]$ for the two data points surrounding the central point for the calibration just before and just after the experiment. The choice of the logarithmic slope (as opposed to the linear slope $dV_{ISE}/d[TPP^+]$) is due to the linear response of ISE potential to logarithm of TPP^+ concentration as observed in Fig. 2 (c) of the main text and also mentioned in other references[2] Mathematically, this is equivalent to shifting the calibration curves vertically on the V_{ISE} vs. ln[TPP^+] curve until they both agree at 7.2 μM, and then fitting them to equation 2 of the main text in order to determine the extrapolated TPP^+ for a given V_{ISE} away from the initial point of 7.2 μM.

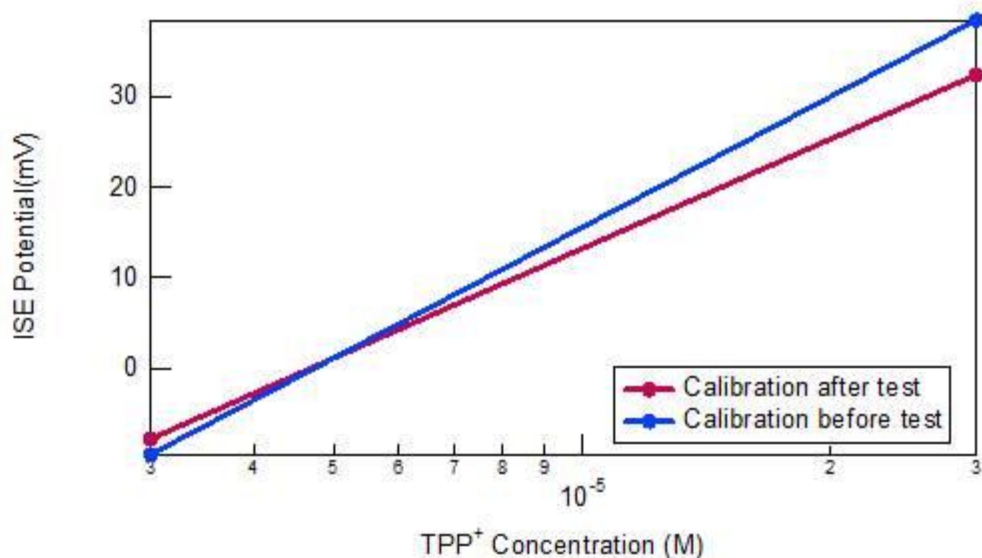

Figure 1S. 2 point calibration curves of the device obtained before and after the mitochondria membrane potential test.

The recorded ISE potential at the beginning of the experiment, before addition of the mitochondria into the chamber, was 1.79 mV. The shifted calibration curves are fit to equation 2 to obtain a set of J and K pairs for each calibration curve (assuming V_{ISE} is in units of mV, and [TPP^+] in units of μM):

Table 1S. Calibration coefficients obtained by curve fitting after vertically shifting the two calibration curves.

	K (mV)	J (mV)
Calibration before test	0.2484	0.0208
Calibration after test	0.2085	0.0174

In Fig. 2S below, we plot the inferred change in [TPP^+] vs. the measured change in ISE potential for both calibration slopes using equation 2 and coefficients in table 1S. In Fig. 3S we plot the difference between these two curves, which tells us the error in the inferred change in [TPP^+] upon introduction of mitochondria into the chamber as a function of

measured changed in ISE potential. Typically in our experiments we measure a change of V_{ISE} of less than 2-3 mV so that our error in the inferred change in [TPP^+] is less than about 150 nM. In Fig. 4S, we plot the *error* in the inferred change in [TPP^+] upon introduction of mitochondria into the chamber vs. the *actual* inferred change in [TPP^+] upon introduction of mitochondria into the chamber. (This plot was generated by converting the horizontal axis of Fig. 3S into [TPP^+] change using the first calibration slope.) What can be seen is that for inferred changes of less than 1 μM, the error is less than about 120 nM. For changes of [TPP^+] less than this, the error is less.

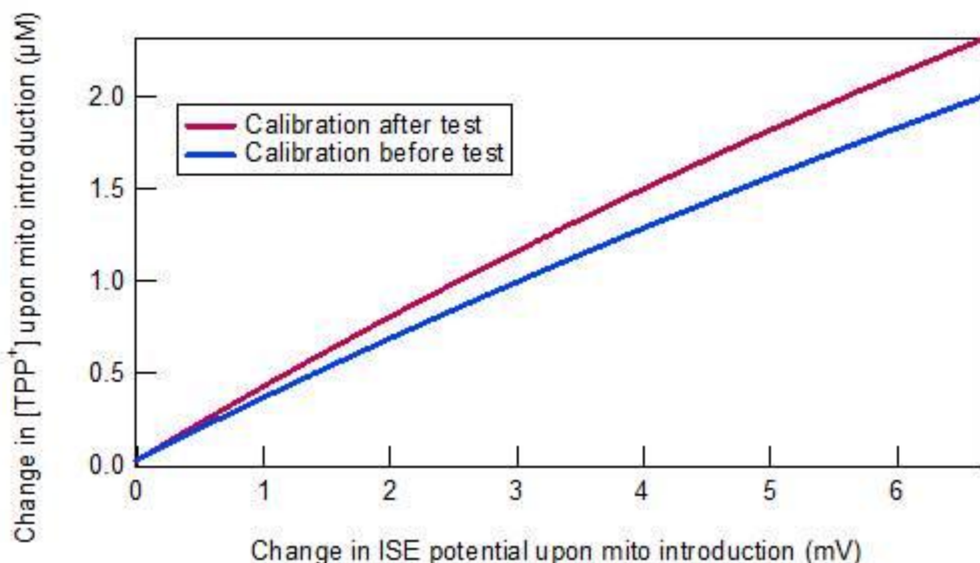

Figure 2S. Change in TPP^+ concentration vs. change in ISE potential obtained by inserting the calbration coefficients in Table 1S into equation 2 of the main text. Initial TPP^+ concentration and ISE potential are 7.2 μM and 1.79 mV respectively.

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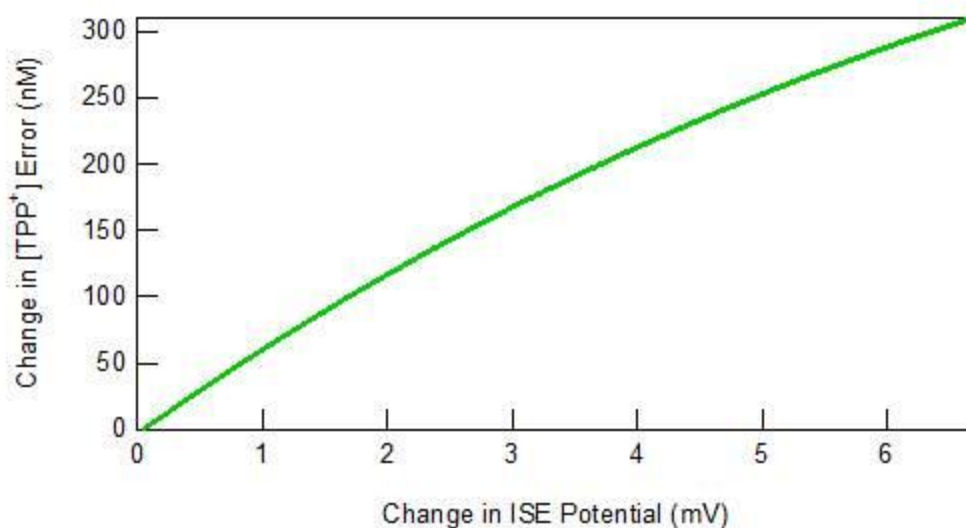

Figure 3S. Worst case error in calculation of change in [TPP$^+$] vs. measured change in ISE potential upon introduction of mitochondria in the sensing chamber.

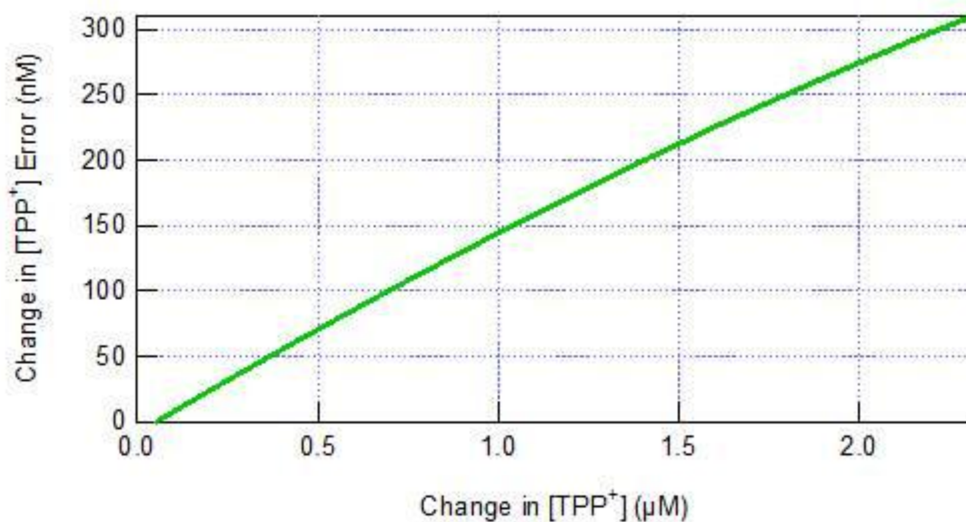

Figure 4S. Worst case error in calculation of change in [TPP+] vs. change in [TPP$^+$] upon introduction of mitochondria into the sensing chamber.

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References

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