## SUPPLEMENTARY FIGURE LEGENDS

**Suppl. Fig. 1. Experimental design template.** Experimental template designed to assist in designing and executing XF experiments.

**Suppl. Fig. 2. The presence of BSA in MAS buffer preserves mitochondrial coupling. (a)** XF analyzer trace of OCR over time: Smooth muscle cells were pre-exposed to 3 nM rPFO in MAS buffer in the presence or absence of fatty acid-free BSA. After 5 min pre-exposure to saponin, cells were loaded into the XF analyzer and OCR recorded. rPFO; recombinant perfringolysin (XF PMP). **(b)** RCR values in the absence or presence of BSA in MAS buffer. These data show that BSA is required to optimize mitochondrial coupling. Such preservation of mitochondrial coupling is typically observed when assays are performed in MAS buffer containing 2-4 mg/ml BSA.

**Supp. Fig. 3. Comparison of rPFO and saponin for use in the permeabilized cell assay. (a)** XF analyzer traces of OCR in cells exposed to SAP/succ/rot/ADP or to rPFO/succ/rot/ADP. Cells were either pretreated with permeabilizer immediately before inserting plate into the XF24 analyzer (closed circles and triangles) and then given succ/rot/ADP, or media on cells was changed to MAS buffer in the absence of permeabilizing agent and then permeabilizer/succinate/rotenone/ADP were co-injected (opened circles and triangles); **(b)** States 3 and 4 OCR values in rPFO- and saponin-permeabilized cells. Values were calculated from cells to which permeabilizer was co-injected with substrates (i.e., opened circles and triangles); **(c)** RCR values calculated from (b). These results show that, in smooth muscle cells, rPFO (PMP reagent) and saponin work equally well in permeabilized cell assays. Furthermore, these data

suggest that permeabilization before insertion of plate into the XF or during the XF assay works equally well. However, results may be highly dependent on the cell type. The activity of mitochondria in some cell types may be negatively affected by detergent-based permeabilizers such as saponin and the timing of permeabilization could prove critical. Therefore, in untested cell lines, the choice of permeabilizer and the timing of permeabilizer addition would need to be determined empirically.

Date:

Expt. Name:

Expt. Number:

Cell Type:

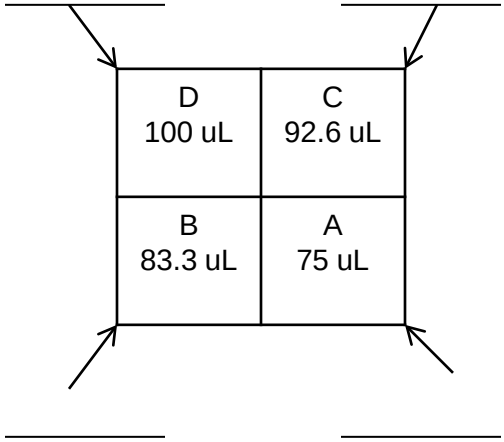
Notes:

Objective:

Plate Layout:

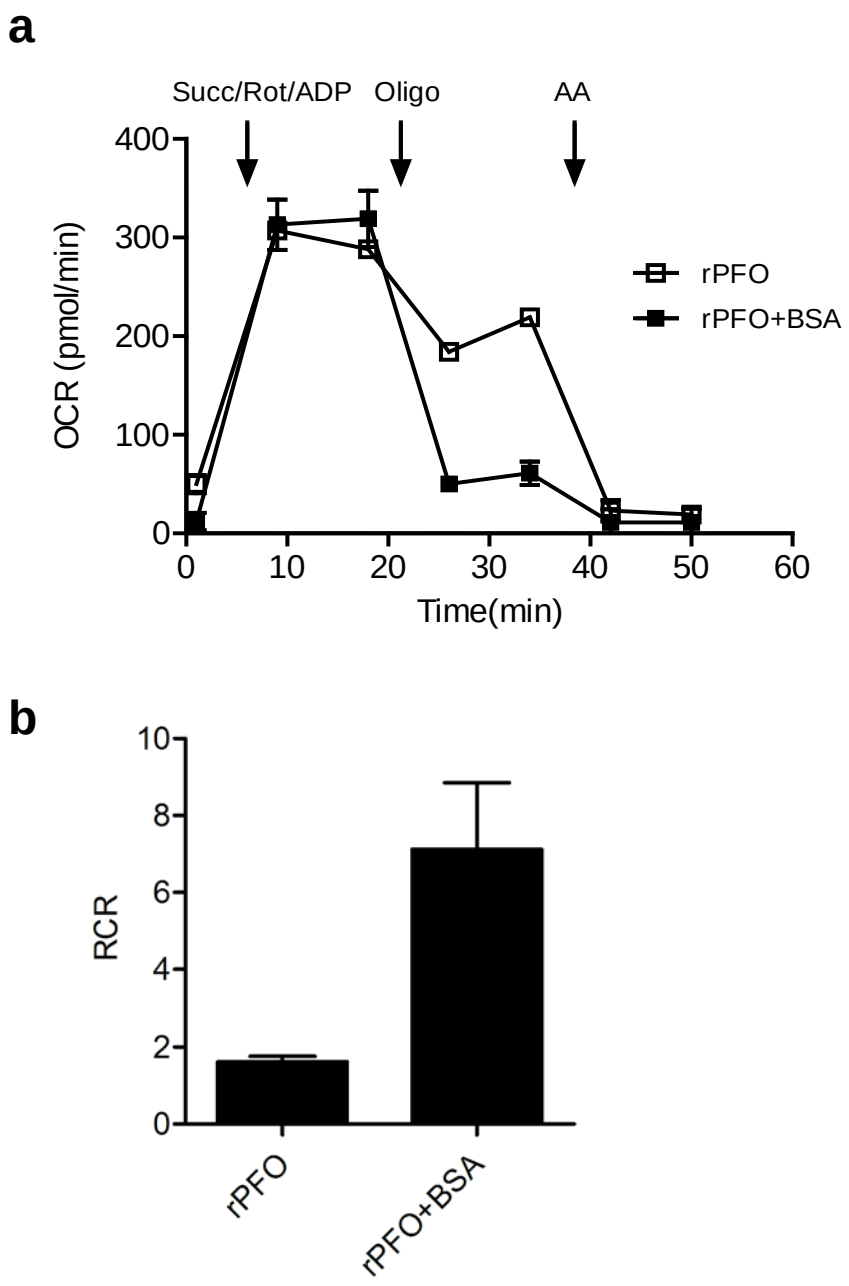
| | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| A | | | | | | |
| B | | | | | | |
| C | | | | | | |
| D | | | | | | |

Ports:

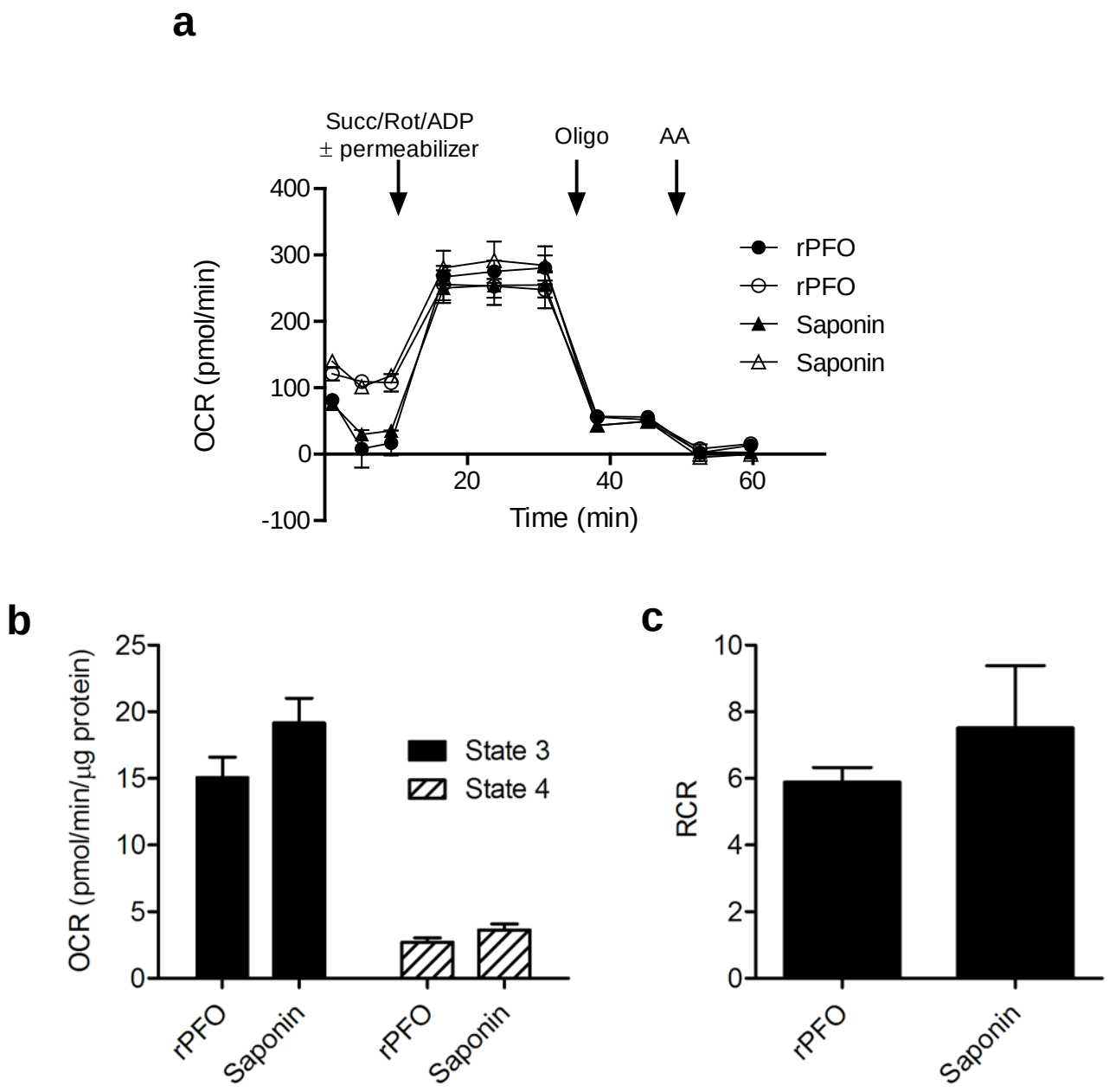



Dilutions :

Other Notes:




**The presence of BSA in MAS buffer preserves mitochondrial coupling. (a)** XF analyzer trace of OCR over time: Smooth muscle cells were pre-exposed to 3 nM rPFO in MAS buffer in the presence or absence of fatty acid-free BSA. After 5 min pre-exposure to rPFO, cells were loaded into the XF analyzer and OCR recorded. rPFO; recombinant perfringolysin (XF PMP). **(b)** RCR values in the absence or presence of BSA in MAS buffer. These data show that BSA is required to optimize mitochondrial coupling. Such preservation of mitochondrial coupling is typically observed when assays are performed in MAS buffer containing 2-4 mg/ml BSA.




**Comparison of rPFO and saponin for use in the permeabilized cell assay. (a)** XF analyzer traces of OCR in cells exposed to SAP/succ/rot/ADP or to rPFO/succ/rot/ADP. Cells were either pretreated with permeabilizer immediately before inserting plate into the XF24 analyzer (closed circles and triangles) and then given succ/rot/ADP, or media on cells was changed to MAS buffer in the absence of permeabilizing agent and then permeabilizer/succinate/rotenone/ADP were co-injected (opened circles and triangles); **(b)** States 3 and 4 OCR values in rPFO- and saponin-permeabilized cells. Values were calculated from cells to which permeabilizer was co-injected with substrates (i.e., opened circles and triangles); **(c)** RCR values calculated from (b). These results show that, in smooth muscle cells, rPFO (PMP reagent) and saponin work equally well in permeabilized cell assays. Furthermore, these data suggest that permeabilization before insertion of plate into the XF or during the XF assay works equally well. However, results may be highly dependent on the cell type. The activity of mitochondria in some cell types may be negatively affected by detergent-based permeabilizers such as saponin and the timing of permeabilization could prove critical. Therefore, in untested cell lines, the choice of permeabilizer and the timing of permeabilizer addition would need to be determined empirically.