Summary of supplementary data

In supplementary Figures 1 and 2 we corroborate findings of the main text stating that complex I is the most decisive regulator of mitochondrial bioenergetics in intact mitochondria while complex III is the major regulator in conditions of cyt-c depletion after its release from the inner membrane space. In addition to the mitochondrial membrane potential $\Delta\Psi_m$, as shown in the main text, we investigated the influence of model parameter changes on the respiratory flux and the protomotive force. The respiratory flux is considered to be the electron transfer between respiratory complexes and is determined by calculating the flux J_{C1} in Table II of the main text (J_{C1}, J_{C3} and J_{C4} are equal during equilibrium). The protomotive force is given by pmf = $\Delta\Psi_m$ + 2.3 Log (H_x / H_i), with the mitochondrial membrane potential $\Delta\Psi_m$ and the matrix and inter membrane space hydrogen concentration H_x and H_i as defined in Table I of the main text. In cells with intact mitochondria, similar to the data shown in Fig 3 of the main text, the respiratory flux and the protomotive force are (Supplementary Figure 1AB and 2AB, respectively) more decisively regulated by metabolites associated with complex I (NADH and ubiquinone) and by complex I activity than by complex III and IV and their associated metabolite (cyt-c). In cells depleted of cyt-c (to 5% of original levels), this decisive regulation role shifted to complex III (Supplementary Figure 1AB for respiratory flux and 2AB~~,~~ for protomotive force).

In supplementary Figure 3 we determined the influence of model parameters on the ATP synthase flux, which is defined in Table II of the main text (J_{F1}). In each scenario - (A) intact mitochondria in state 4, (B) intact mitochondria in state 3, (C) cyt-c depletion to 5%, (D) cyt-c depletion to 0.5% parameters related to ANT (#19, #20) and Mg^{2+} concentration as a co-factor for ATP production more decisively influencing ATP synthase flux than its own enzymatic activity (#19, parameter X_{F1} in Table III).

An illustration of the reasoning that ATP synthase is a passive component driven by ANT is depicted in Supplementary Fig. 4. The mathematically exact relationship between ATP synthase activity (#15) and ANT activity (#19) on the fluxes of ATP synthase and ATP transferase is indicated by solid red arrows. The dark yellow shaded box indicates that varying ANT activity (#19) had a strong influence on $\Delta\Psi_m$ while varying ATP synthase enzymatic activity (#15) only mildly influenced $\Delta\Psi_m$ (indicated by light yellow shading). Since ANT does not directly contribute to $\Delta\Psi_m$ we concluded that the severe impact of ANT activity on $\Delta\Psi_m$ must be put into effect through regulation of the ATP synthase flux (black dashed arrow).

Supplementary Figure 1: Shift from complex I to complex III as driver for respiratory flux after cyt-c depletion

Single parameter variation of model parameters was performed as described. The influence of model parameter changes on the respiratory flux (electron transfer through respiration complexes) was assessed. (A-B) Complex I activity was more important than complex II and IV activity in intact mitochondria. (A) Resting (state-4, ATP:ADP = 100:1) mitochondria had a significantly smaller respiratory flux (dotted line) than state-3 mitochondria (B) and were more significantly influenced by proton leaks and by cytosolic ATP consumption (B) ATP-producing (state-3, ATP:ADP = 3:1) mitochondria were highly influenced by ANT activity. (C-D) Complex III activity was more important than complex I and complex IV in mitochondria depleted of cyt-c to levels of (C) 0.5 % and (D) 5% of initial levels. Dark blue, light blue, green, orange and brown bars refer to a tenfold decrease, fivefold decrease, reference value, fivefold increase and tenfold increase of model parameters, respectively.

Parameters are ranked according to their influence on the respiratory flux with lowest rank indicating highest influence.

Supplementary Figure 2: Protomotive Force for state-4, state-3 and cyt-c depleted mitochondria

Single parameter variation of model parameters was performed as described and the influence on the protomotive force is given for (A) resting (state-4, ATP:ADP = 100:1) and (B) ATP-producing (state-3, ATP:ADP = 3:1) intact mitochondria and mitochondria depleted of cyt-c to levels of (C) 0.5 % and (D) 5% of initial levels. Importance was shifted from complex I to complex III as the driver of the protomotive force after cyt-c depletion. Dark blue, light blue, green, orange and brown bars refer to a tenfold decrease, fivefold decrease, reference value, fivefold increase and tenfold increase of model parameters, respectively. Parameters are ranked according to their influence on the protomotive force with lowest rank indicating highest influence.

Supplementary Figure 3: ATP synthase flux in state-3, state-4 and cyt-c depleted mitochondria

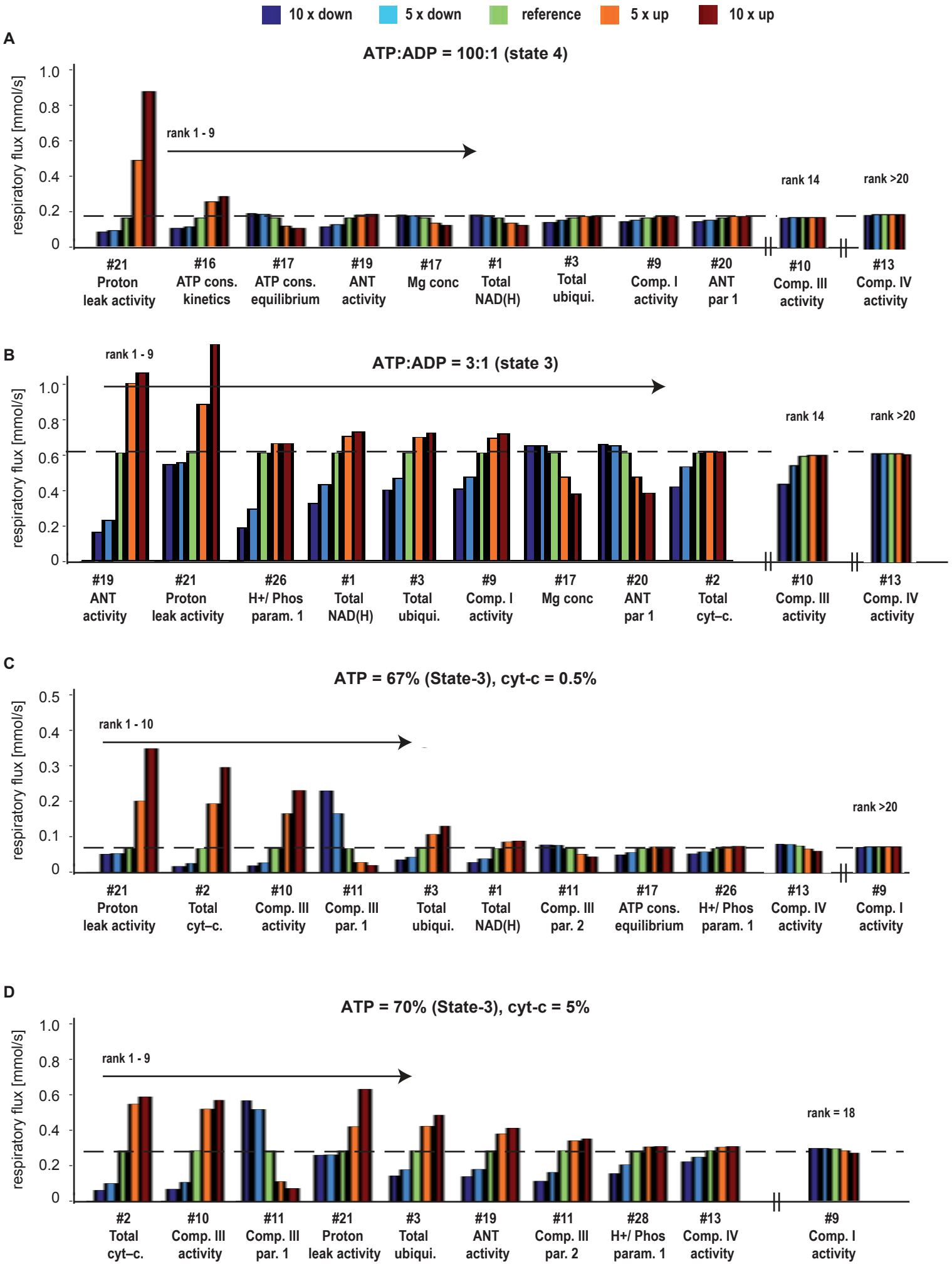
Single parameter variation of model parameters was performed as described and the influence on the ATP synthase flux is given for (A) resting (state-4, ATP:ADP = 100:1) and (B) ATP-producing (state-3, ATP:ADP = 3:1) intact mitochondria and mitochondria depleted of cyt-c to levels of (C) 0.5 % and (D) 5% of initial levels. In all scenarios, ATP synthase flux was not driven by the enzymatic activity of ATP synthase, but rather by ANT activity, or by respiratory components. Importance shifted from complex I to complex III as driver for ATP synthase flux after cyt-c depletion. Dark blue, light blue, green, orange and brown bars refer to a tenfold decrease, fivefold decrease, reference value, fivefold increase and tenfold

increase of model parameters, respectively. Parameters are ranked according to their influence with lowest rank indicating highest influence.

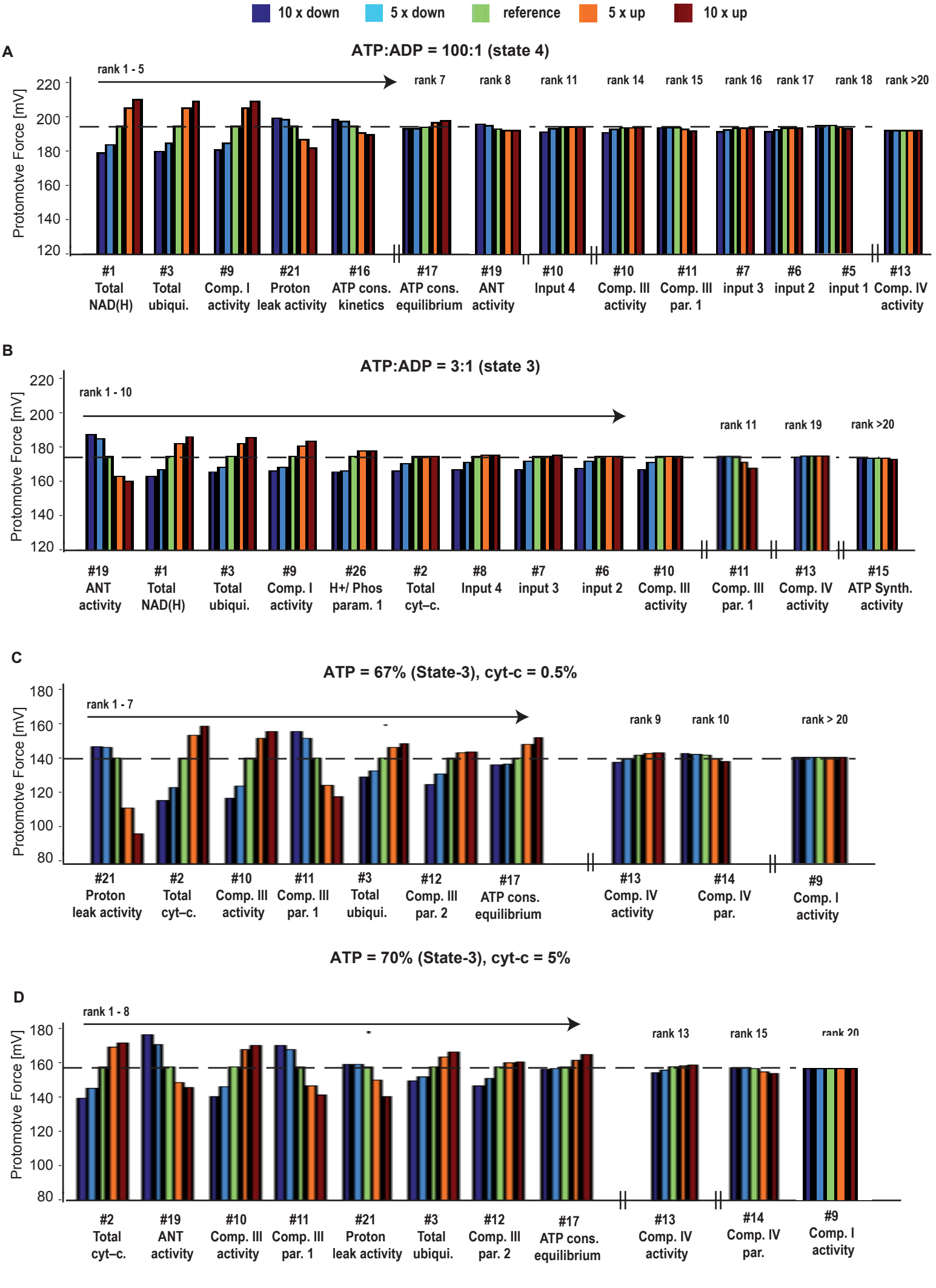
Supplementary Figure 4: ATP synthase is a passive component of $\Delta\Psi_m$ regulation

Single parameter variation can be exploited to study the influence of individual model components on model output. This illustration shows the influence of ATP synthase (Table III, #15) and adenosine nucleotide transferase (ANT) (#19) on the mitochondrial membrane potential, $\Delta\Psi_m$, in intact, ATP-producing mitochondria (refer also to Fig 3A). Red arrows indicate the mathematically defined relationships between parameters and components, given in Table II. While an increase in ANT activity greatly increases $\Delta\Psi_m$ (indicated by dark yellow shading), a similar increase in ATP synthase activity only induces a negligible $\Delta\Psi_m$ increase (indicated by light yellow shading). Since ANT does not directly influence $\Delta\Psi_m$, we concluded that ANT influences $\Delta\Psi_m$ by driving ATP synthase flux (black dashed arrow), implicating ATP synthase as a passive parameter in the regulation of $\Delta\Psi_m$.

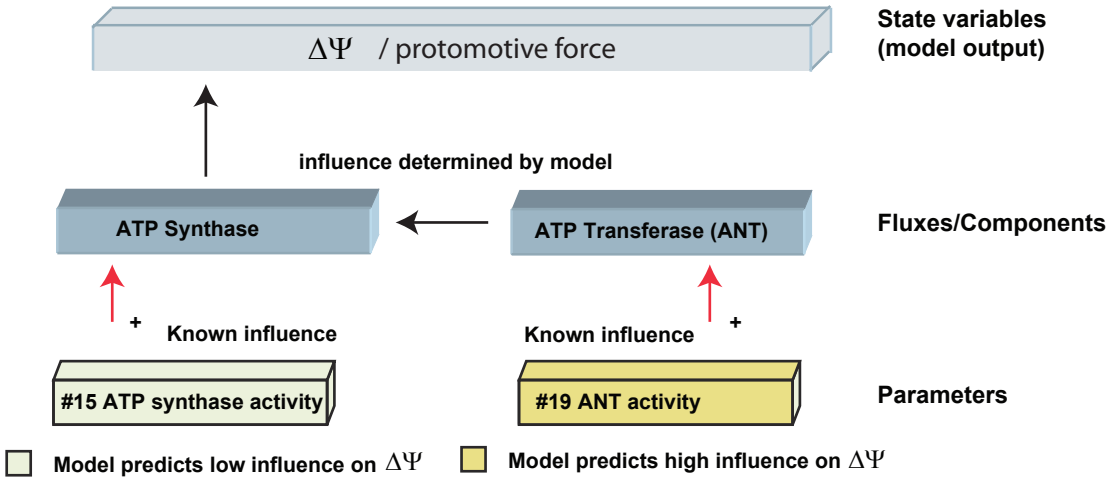
Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 4



Supplementary Figure 3