SUPPLEMENTARY TABLE AND FIGURE LEGENDS

Supplementary Table S1. A) Mass Spectrometric Report of identified Proteins: Human Protein Pilot Search Results for WT and A8/6^{mut} cell lines. B). Mass Spectrometric Report of identified Peptides and Proteins: Human Protein Pilot Search Results for WT and A8/6^{mut} cell lines. C). Mass Spectrometric Identification of Proteins ATP6 and ATP8 Protein Pilot Search Results.

Supplementary Table S2. A). Relative Quantification of ATP6 and ATP8 from WT and A8/6^{mut} strains (Experiment 1). B). Relative Quantification of ATP6 from WT, A8/6^{mut}, and A8/6^{mut} + A8F strains (Experiment 2). C). Relative Quantification of other proteins from WT and A8/6^{mut} strains (Experiment 1). D). Relative Quantification of other proteins from WT, A8/6^{mut}, and A8/6^{mut} + A8F strains (Experiment 2).

Supplementary Table S3. Primer sets to distinguish WT and mutant *ATP8* gene sequences through ARMS (amplification refractory mutation system) sequencing qPCR.

Supplementary Table S4. Selection of Clones by ARMS qPCR. DNA derived from the parental mutant strain and 143B served as controls. ΔC_T is expressed as the average difference in critical threshold cycle number for amplification between the mutant and WT primer sets**.**

Supplementary Table S5. Determination of *ATP5G1-ATP8* and *ATP5G1-ATP6* mRNA. Values are presented as means ± S.D. (n=3), in comparison with parental A8/6^{mut} and using *COX10* as the reference gene. The difference in C_T values ($-\Delta C_{T\ (test-parental)}$) indicates efficiency of priming relative to the parental A8/6^{mut} cell line that does not express any of the exogenous constructs.

Supplementary Table S6. $\Delta\Delta C_T$ values were calculated by first subtracting the C_T values of the housekeeping gene (*MT-CYB*) from each experimental time point, and then subtracting the C_T values for the control (day 0) from the experimental (glucose days 15 or 28 or galactose). Error is SEM.

Supplementry Figure S1. Codon-corrected and codon optimized mitochondrial *ATP8* gene or codon-corrected *ATP6* was cloned into pCMV6 expression vectors (G418 / puromycin resistant; Origene, Rockville, MD). This resulted in the incorporation of a Myc and a FLAG

tag in tandem downstream to the genes. The *ATP5G1* MTS is appended upstream to the *ATP8* and *ATP6* coding sequences.

Supplementary Figure S2. Characterization of proteolytic peptides from ATP8 and ATP6 proteins by tandem mass spectrometry. A). MS/MS spectrum of proteolytic peptide ICSLHSLPPQS from human ATP8 protein (P03928). The precursor ion at *m/z* 619.81 (z=2) was selected for collision-induced dissociation. **B).** MS/MS spectrum of proteolytic peptide LITTQQWLIK from human ATP6 protein (P00846). The precursor ion at *m/z* 622.37 (z=2) was selected for collision-induced dissociation. Skyline spectral libraries were built containing these ATP8 and ATP6 MS/MS spectra.

Supplementary Figure S3. Relative quantification of proteolytic peptides for proteins ATP8 and ATP6 comparing WT and A8/6^{mut} strains. Peak areas under the curve were determined from extracted ion chromatograms (XICs) from data-independent SWATH acquisitions (Experiment 1). Initial assessments: samples from cell lines were acquired in biological duplicates and technical (MS injection) duplicates. **A).** Peak areas for proteolytic peptide LITTQQWLIK from ATP6 with a precursor ion at *m/z* 622.37 (z=2) are displayed. Relative ATP6 levels are significantly reduced to 9% in A8/6^{mut} strains (green) compared to WT strains (blue) with a determined p-value of 9.3 e-09 (***). **B).** Peak areas for proteolytic peptide ICSLHSLPPQS from ATP8 with a precursor ion at *m/z* 619.81 (z=2) are displayed. Relative ATP8 levels of the A8/6^{mut} strain (green) are measured at <3% of ATP8 levels in the WT strains (blue) with a determined p-value of 5.1e-07 (***), which was below background detection levels. ($^{\#}$ see also Supplementary Figure 6A and B).

Supplementary Figure S4. Relative quantification of proteolytic peptides for protein ATP6 comparing WT, A8/6^{mut}, and A8/6^{mut} + A8F cells. In an independent experimental setup (Experiment 2) tryptic peptide LITTQQWLIK (with a precursor ion at *m/z* 622.37 with z=2) derived from protein ATP6 was quantified during data-independent SWATH acquisitions. Panels **A).** and **B).** show extracted ion chromatograms (XICs) from fragment and precursor ions for WT, A8/6^{mut}, and A8/6^{mut} + A8F cells in the form of bar graphics. WT and A8/6^{mut} cell lines were acquired in biological triplicates, the A8/6^{mut} + A8F strain was analyzed for 4 biological replicates, and normalized peak areas from all biological replicates and strains are displayed. **A).** Normalized peak areas for ATP6 peptide for all individual acquisitions from all different conditions/strains. **B).** Normalized peak areas for ATP6 peptide averaged for each of the three different conditions.

Supplementary Figure S5. Relative quantification of other proteins comparing WT, A8/6^{mut}, and A8/6^{mut} + A8F strain. Additional mitochondrial proteins were quantified from the SWATH acquisitions (Experiment 2) comparing relative abundance levels between the A8/6^{mut} and WT strains (Panel A), and A8/6^{mut} + A8F strain vs. WT strain (Panel B). Protein fold changes are displayed for some mitochondrial housekeeping proteins (CH60: 60 kDa heat shock protein, and ADT2: ADP/ATP translocase 2), as well as several ATP synthase protein subunits of the CV assembly. Displayed relative protein fold changes are mostly ~1, indicating no or small changes, most of them not significant. Relative quantification of other proteins comparing WT, A8/6^{mut}, and A8/6^{mut} + A8F strain. Additional mitochondrial proteins were quantified from the SWATH acquisitions (Experiment 2) comparing relative abundance levels between the A8/6^{mut} and WT strains **(Panel A)**, and A8/6^{mut} + A8F strain vs. WT strain **(Panel B)**. Protein fold changes are displayed for some mitochondrial housekeeping proteins (CH60: 60 kDa heat shock protein, and ADT2: ADP/ATP translocase 2), as well as several ATP synthase protein subunits of the CV assembly. Displayed relative protein fold changes are mostly ~1, indicating no or small changes, most of them not significant.

Supplementary Figure S6. Assessment of XICs and corresponding fragment ion distributions obtained from SWATH acquisitions in comparison to original spectral library MS/MS spectra. A). Peak areas for ATP8 – ICSLHSLPPQS peptide for selected replicates from WT and A8/6^{mut} strain indicating much lower relative abundance in the A8/6^{mut} strain. **B).** Displaying a view "normalized to total (100%)" reveals that while WT SWATH replicates show highly similar fragment ion distribution compared to the spectral library distribution (left bar), the A8/6^{mut} strain shows a non-significant and random fragment ion distribution for the ATP8 peptide ICSLHSLPPQS. The latter is also supported by very low "idotp" values of 0.55 and 0.57 for the A8/6^{mut} strain (the idotp describes a correlation between spectral library MS/MS and SWATH XIC fragment ion distribution, with 1 for highest similarity). These findings conclude that no ATP8 could be confidently measured in the A8/6^{mut} strain with our detection limits (signal is below noise level). **C).** Peak areas for ATP6 – LITTQQWLIK peptide for selected replicates from WT and A8/6^{mut} strain indicating lower relative abundance in the A8/6^{mut} strain. **D).** However, the "normalized to total (100%)" view reveals that both WT and A8/6^{mut} strains show highly similar fragment ion distribution compared to the spectral library distribution (left bar) for LITTQQWLIK, concluding that ATP6 peptide was confidently identified in the different strains.

Supplementary Figure S7. Blue Native Gel Western Blots. A. A8/A6^{mut} cell mitochondrial extracts with or without stably expressed G2A8V5. **B.** Complex I resolution using an NDUFS3 antibody. We believe * to be CI monomers and ** to be CI oligomers. C. Native Western blot of TIM23 containing complexes.