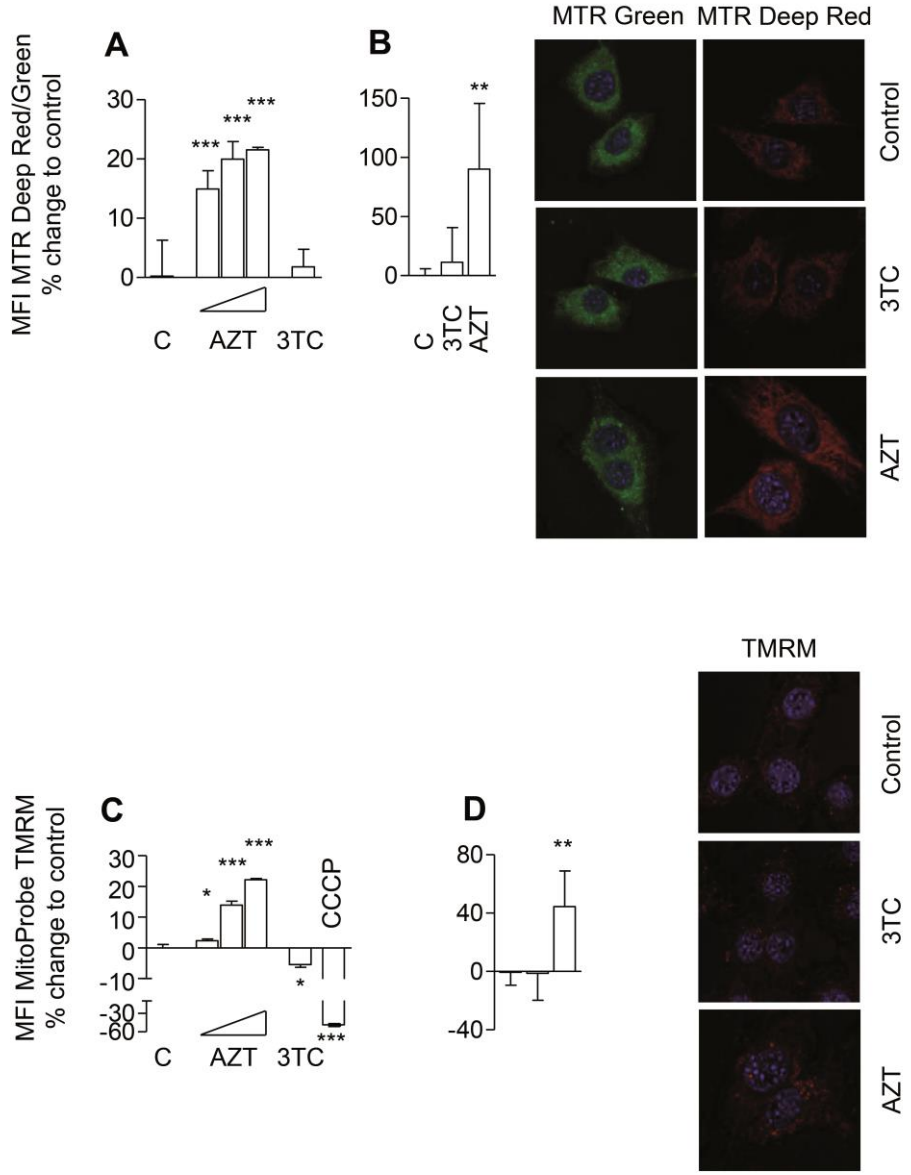
Supporting Information

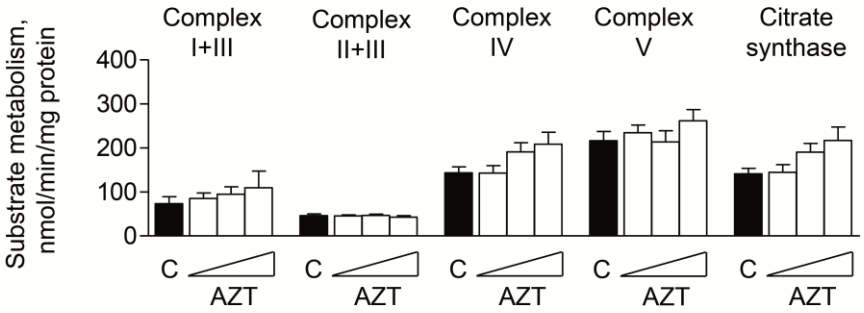
Supplementary Figure S1



Supplementary Fig S1. AZT incubation leads to mitochondrial membrane hyperpolarization. C2C12 cells were incubated in the absence or presence of AZT (6, 30 and 150µM) or 3TC (200µM) for up to 8 days. Flow cytometry **(A)** and confocal microscopy **(B)** analysis of potential dependent MitoTracker Deep Red (MTR Deep Red) and potential independent MitoTracker Green (MTR Green) staining. Data is presented as percent change to untreated control of the MFI MTR Deep Red/MTR Green ratio **(A)** with **(B)** quantification of confocal microscopy (*left panel*) and

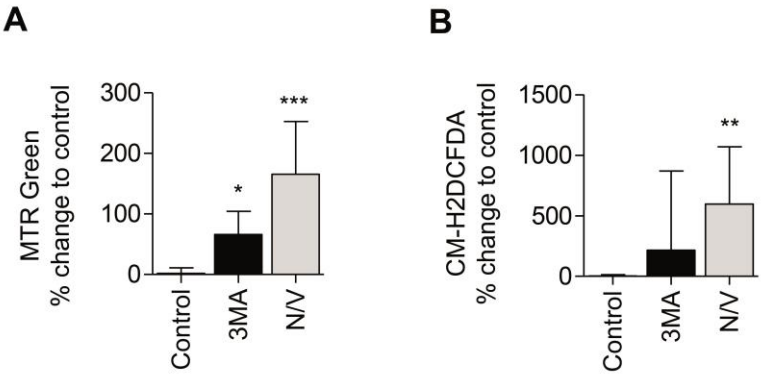
representative pictures (*right panel*) Flow cytometry **(C)** and confocal microscopy **(D)** analysis of mitochondrial potential specific MitoProbe TMRM (TMRM) staining. Specific detection of mitochondrial potential confirmed using carbonyl cyanide 3-chlorophenylhydrazone (CCCP) induced mitochondrial membrane depolarization. **(C** and **D)** data is presented as percent change to untreated control with **(D)** quantification of confocal microscopy (*left panel*) and representative pictures (*right panel*). All experiments are presented as mean±SD and are representative for at least three independent experiments with four replicates. *P<0.05; **P<0.01;***P<0.001.

Supplementary Figure S2



Supplementary Fig S2. Effects of AZT on the respiratory chain complex activity in C2C12 cells. C2C12 cells were incubated in the absence or presence of AZT (6, 30 and 150μM) for up to 8 days. Cells were sonicated, and individual substrates were added to the cell lysates, after which levels of substrate conversion by the respiratory chain complexes I and III, II and III, IV, and V and citrate synthase were measured photometrically. All experiments are presented as mean±SD and are representative for at least three independent experiments. *P<0.05

Supplementary Figure S3



Supplementary Fig S3. Pharmacological autophagy inhibition reiterates the effect of AZT on dysfunctional mitochondria accumulation. C2C12 cells were treated with 3MA (5mM) or nocodasole plus vinblastin N/V (50µM) for 24h. **(A)** Flow cytometry analysis of total mitochondrial mass using MitoTracker Green (MTR Green). Data is presented as percent change to untreated control. **(C)** Flow cytometry analysis of ROS production using CM-H2DCFDA. Data is presented as percent change to untreated control. All experiments are presented as mean ±SD and are representative for at least three independent experiments with four replicates. *P<0.05; **P<0.01.