

Expanded View Figures

Figure EV1. Validation of F₁F₀ ATP synthase dimerization status.

- A High-resolution image of F₁F₀ ATP synthase dimers and monomers isolated from rat heart mitochondria (HM) by the use of *n*-dodecyl β-D-maltoside (DM) or digitonin (DIG), upon separation by blue-native PAGE and immunostaining for ATP5A.
- B Schematic representation of PLA assay setting in physiological conditions (left) or in the course of MPT (right).
- C Immunoreactivity of the ATP5H-specific antibody used in the PLA assay as compared to an antibody specific for the mitochondrial marker TOMM20.
- D Maximum intensity projection of z-stack from a representative HEK293T cell stained for the (immuno)fluorescence microscopy-based detection of DNA (DAPI, blue), mitochondria (TOMM20, red), and F₁F₀ ATP synthase dimers (PLA, green). Quantification of the PLA signal colocalizing with TOMM20 is reported (whiskers: max and min; box: 10th and 90th percentile; line: median).
- E Assessment of transfection efficiency in HEK293T cells transfected with a control siRNA (siCTR) or a siRNA targeting ATP5H (siATP5H) for 96 h. GAPDH levels were monitored to ensure equal lane loading.
- F, G Representative images (F) and quantification (G) of PLA assays for F₁F₀ ATP synthase dimers in HEK293T cells transfected with siCTR or siATP5H for 96 h. The results are representative of three independent experiments. **P* = 0.0001 (unpaired Student's *t*-test).
- H, I Representative images (H) and quantification (I) of PLA assays for F₁F₀ ATP synthase dimers in ρ⁰ cells and their wt counterpart HPA11. The results are representative of three independent experiments. *****P* = 0.0001 (unpaired Student's *t*-test).

Data information: All results are expressed as mean ± SEM. Scale bar = 10 μm.

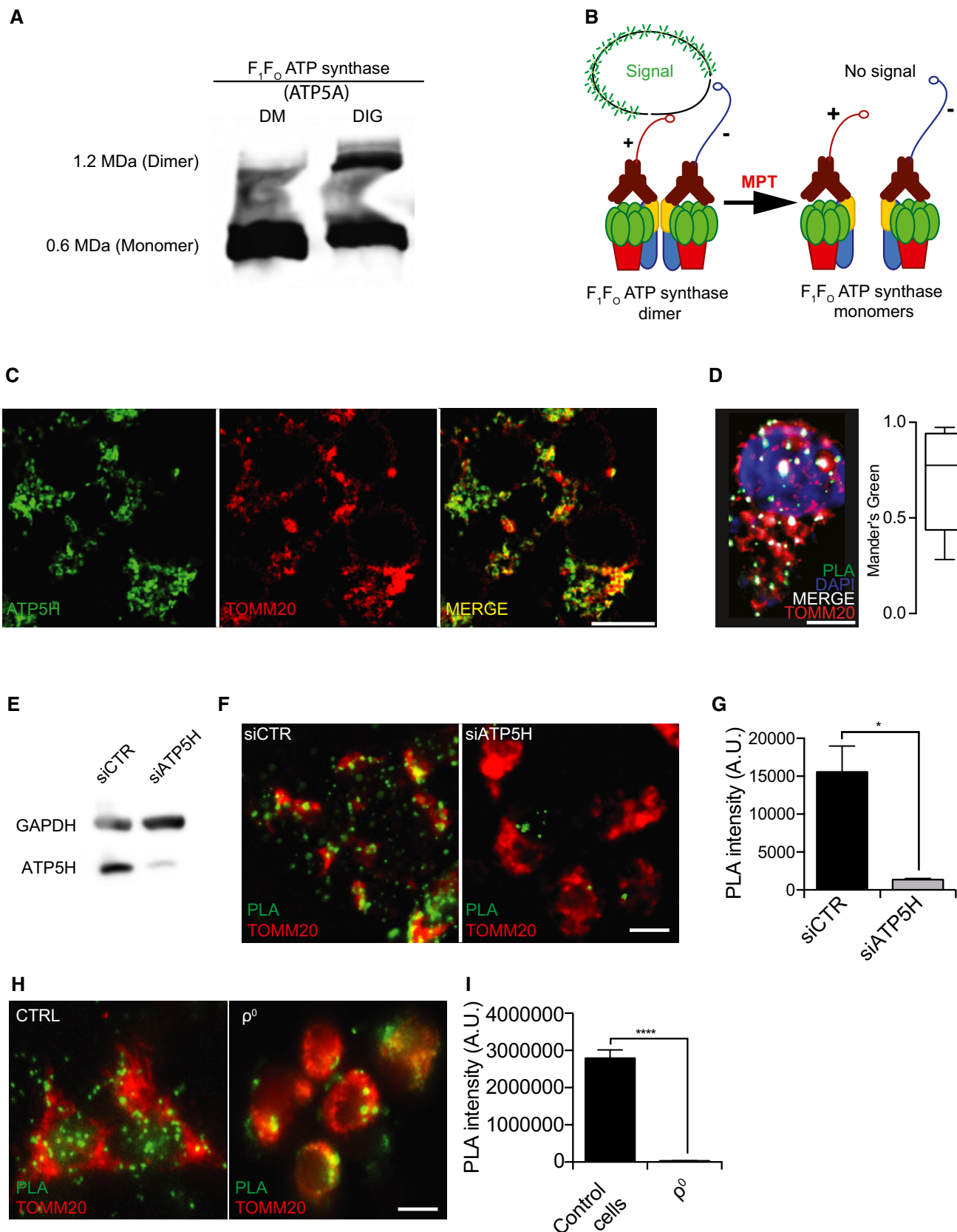


Figure EV1.

Figure EV2. Mitochondrial parameters in ATPIF1-overexpressing HEK293T cells.

- A–D Representative rendering (A) and quantification of morphological parameters (count, B; average volume, C; total volume, D) in HEK293T cells transiently transfected with pcDNA3 or with a plasmid for the overexpression of ATPIF1. The results are representative of three independent experiments. Scale bar = 10 μ m.
- E, F Representative images (E) and quantification (F) of TMRM staining in HEK293T cells transiently transfected with pcDNA3 or with a plasmid for the overexpression of ATPIF1. The results are representative of five independent experiments. * P = 0.0001 (unpaired Student's t -test). Scale bar = 10 μ m.
- G, H Representative traces (G) and quantification (H) of mitochondrial ATP-dependent light emission in HEK293T cells transiently transfected with pcDNA3 or with a plasmid for the overexpression of ATPIF1. 75 μ M *N,N*-dicyclohexylcarbodiimide (DCCD) was employed to completely inhibit mitochondrial ATP synthesis. The results are representative of five independent experiments.
- I, J Representative traces (I) and quantification (J) of mtAlphi/ECFP ratio in HEK293T cells transiently co-transfected with pcDNA3 or with a plasmid for the overexpression of ATPIF1. 30 mM Na acetate or 30 mM NH₄Cl was employed to detect the minimum and maximum ratio, respectively. The results are representative of four independent experiments.
- K, L Representative immunoblotting for the detection of the ATP5A1 subunit after blue-native PAGE (K) and quantification (L) of F₁F₀ ATP synthase dimers (D) and monomers (M) in HEK293T cells transiently transfected with pcDNA3 or with a plasmid for the overexpression of ATPIF1. The results are representative of three independent experiments. * P = 0.0095 (unpaired Student's t -test).

Data information: All results are expressed as mean \pm SEM.

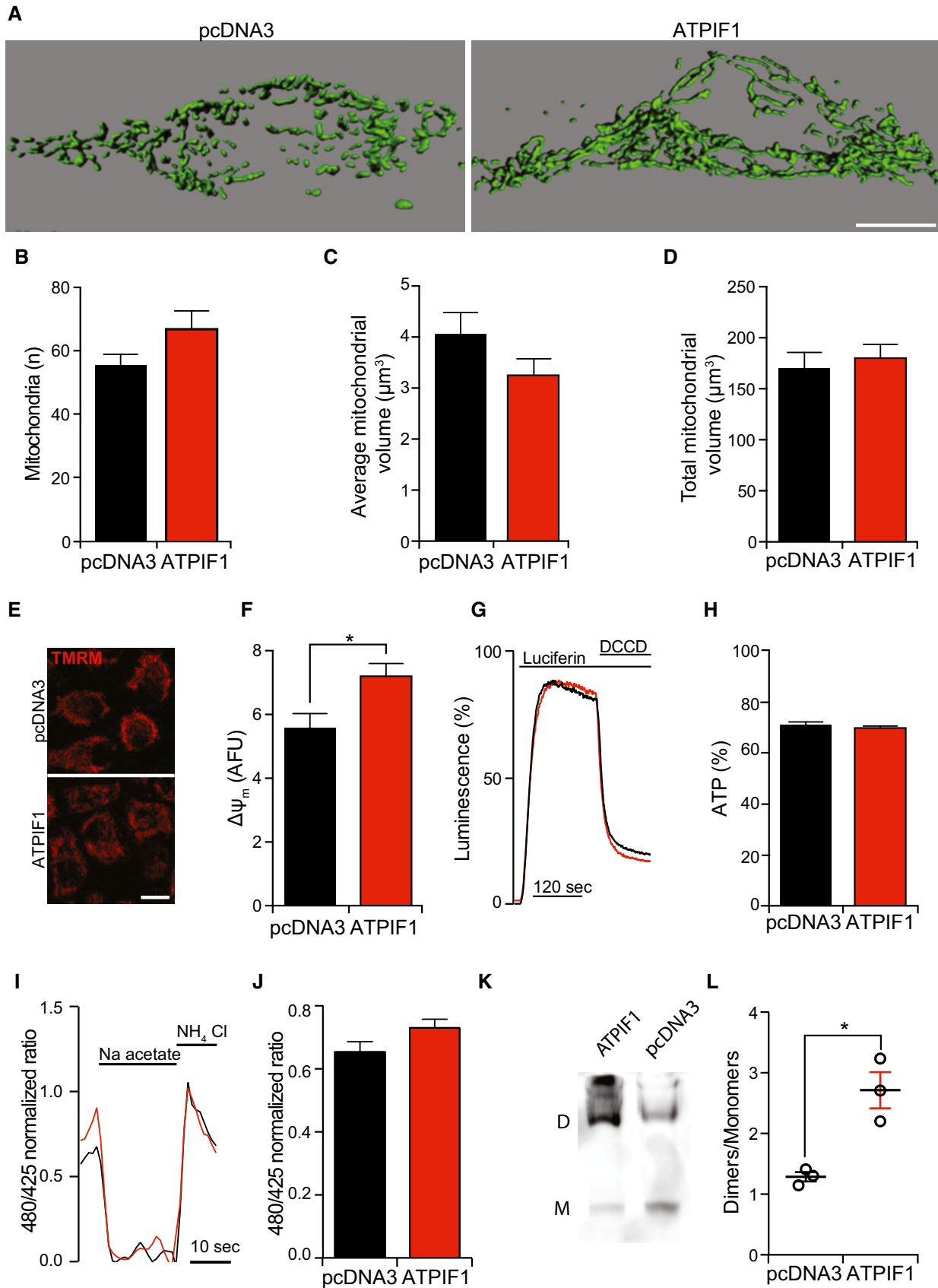


Figure EV2.

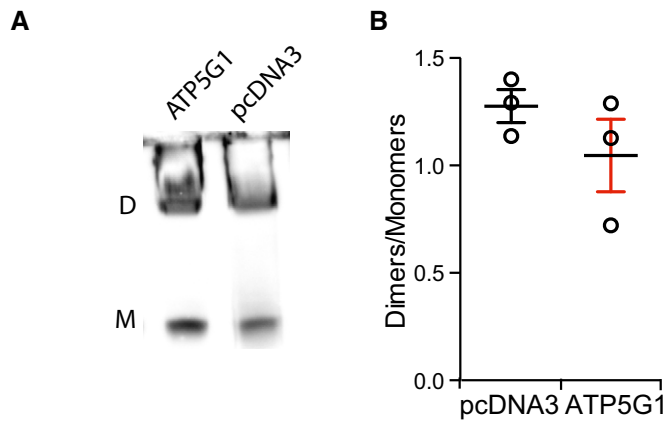


Figure EV3. Dimerization status during ATP5I overexpression.

A, B Representative immunoblotting for the detection of the ATP5A1 subunit after blue-native PAGE (A) and quantification (B) of F₁F₀ ATP synthase dimers (D) and monomers (M) in HEK293T cells transiently transfected with pcDNA3 or with a plasmid for the overexpression of ATP5G1. The results are representative of three independent experiments and expressed as mean ± SEM.

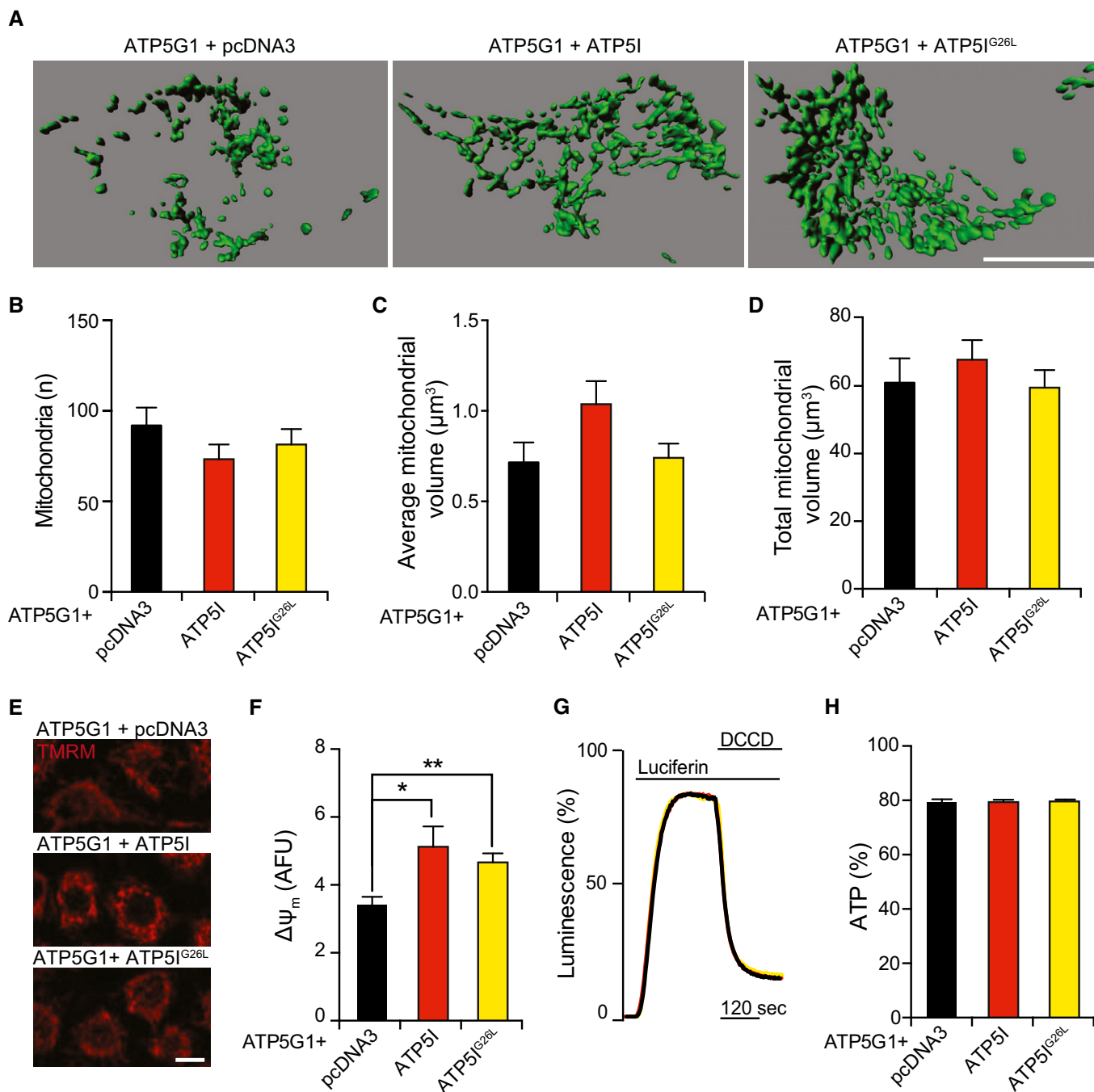


Figure EV4. Mitochondrial parameters in ATP5G1- and ATP5I-co-overexpressing HEK293T cells.

A–D Representative rendering (A) and quantification of morphological parameters (count, B; average volume, C; total volume, D) in HEK293T cells transiently transfected with a construct for the overexpression of ATP5G1 plus pcDNA3 or a plasmid for the overexpression of ATP5I or ATP5I^{G26L}. The results are representative of three independent experiments. Scale bar = 10 μm.

E, F Representative images (E) and quantification (F) of TMRM staining in HEK293T cells transiently transfected with a construct for the overexpression of ATP5G1 plus pcDNA3 or a plasmid for the overexpression of ATP5I or ATP5I^{G26L}. The results are representative of three independent experiments. **P* = 0.0060, ***P* = 0.0345 (Kruskal–Wallis test with Dunn's correction for multiple comparison). Scale bar = 10 μm.

G, H Representative traces (G) and quantification (H) of mitochondrial ATP-dependent light emission in HEK293T cells transiently transfected with a construct for the overexpression of ATP5G1 plus pcDNA3 or a plasmid for the overexpression of ATP5I or ATP5I^{G26L}. 75 μM DCCD was employed to completely inhibit mitochondrial ATP synthesis at the end of the assay. The results are representative of three independent experiments.

Data information: All results are expressed as mean ± SEM.