

Expanded View Figures

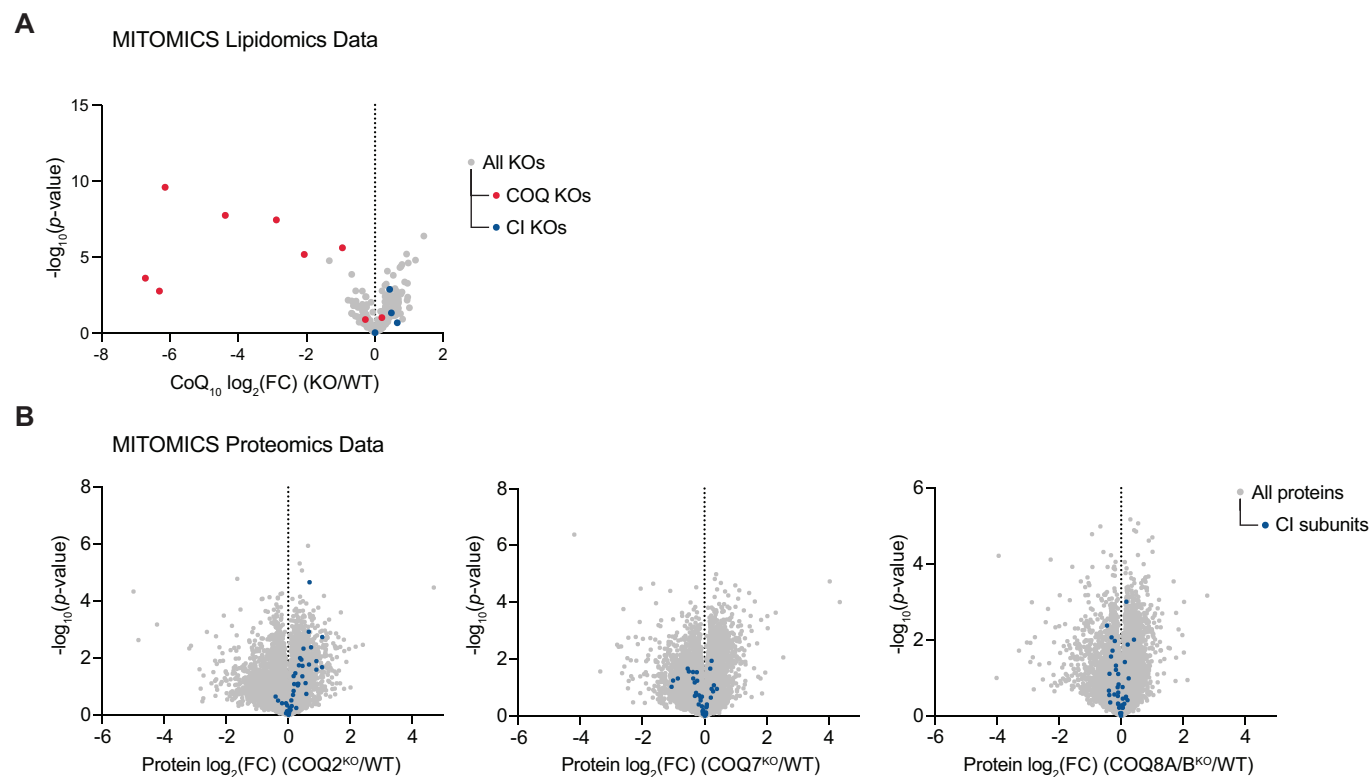


Figure EV1. Perturbations in CI and COQ proteins do not lead to interdependent phenotypes.

(A, B) Targeted lipidomics for CoQ₁₀ abundance (A) and proteomics (B) analyses from the MITOMICS study (Rensvold et al, 2022) investigating the reciprocal interaction between COQ and CI proteins. Volcano plots depict the log₂ fold change of CoQ₁₀ (A) or protein (B) abundance of all HAP1 knock out cell lines relative to WT. In (A), COQ KO cell lines are highlighted in red, CI KO cell lines are highlighted in blue. In (B), CI subunits are highlighted in blue. Data shown as mean ($n = 3$), two-sided Student's t -test.

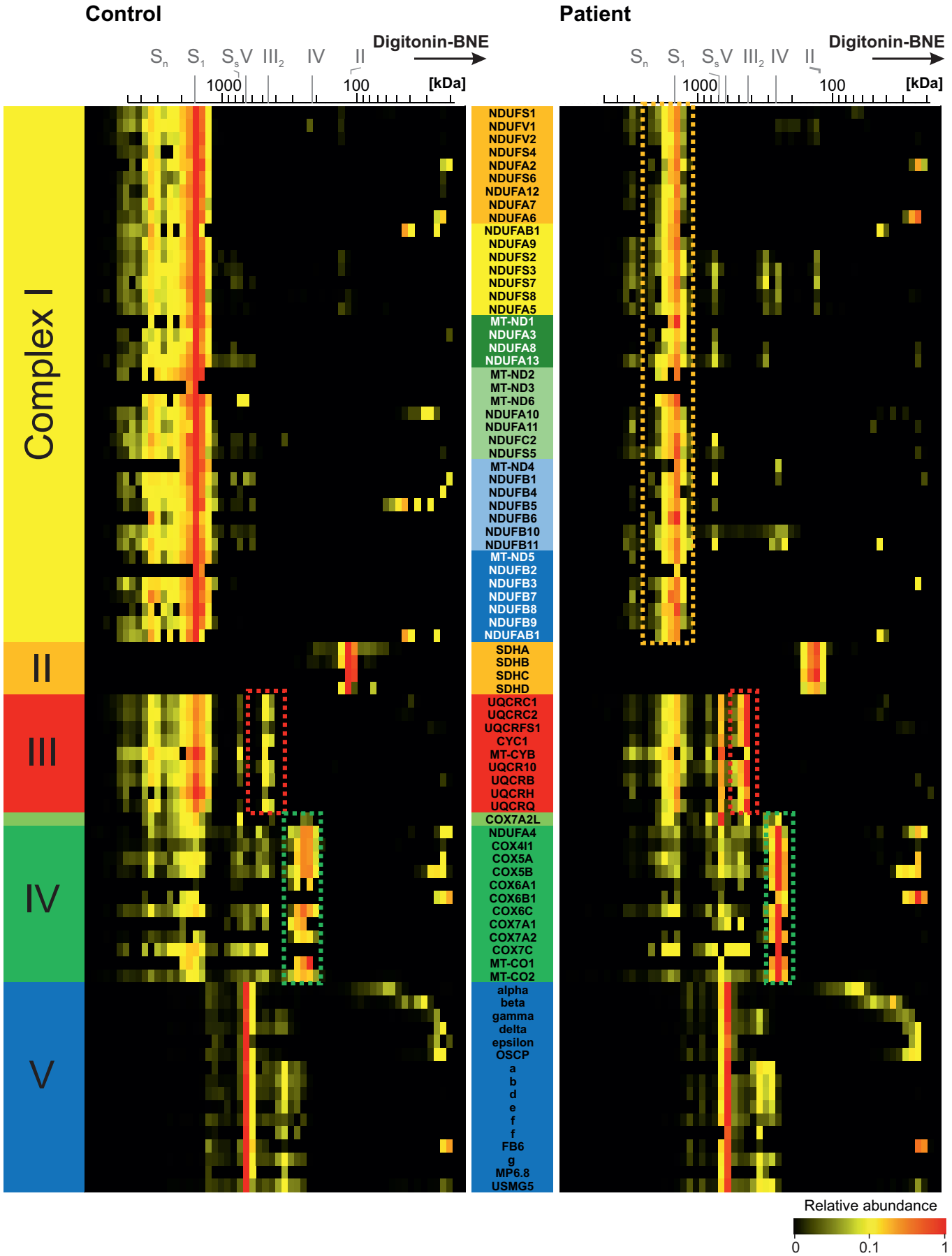


Figure EV2. OxPhos complexes of *RTN4IP1* patient and control fibroblasts analysed by complexome profiling.

Complexome profiling data were presented as a heatmap, corresponding to subunits of individual OxPhos complexes I-V. Mitochondrial complexes were solubilised with digitonin, separated on blue-native gels (BNE), cut into fractions and analysed by quantitative mass spectrometry. Assignment of complexes: complex II (II); complex III dimer (III₂); complex IV (IV); complex V (V); small supercomplex of CIII₂ and CIV (S_s); supercomplex containing CI, III₂ and 1 copy of CIV (S₁) and higher order supercomplexes (S_n). The relative abundance of each protein was represented from low to high according to the colour scale illustrated on the bottom right.

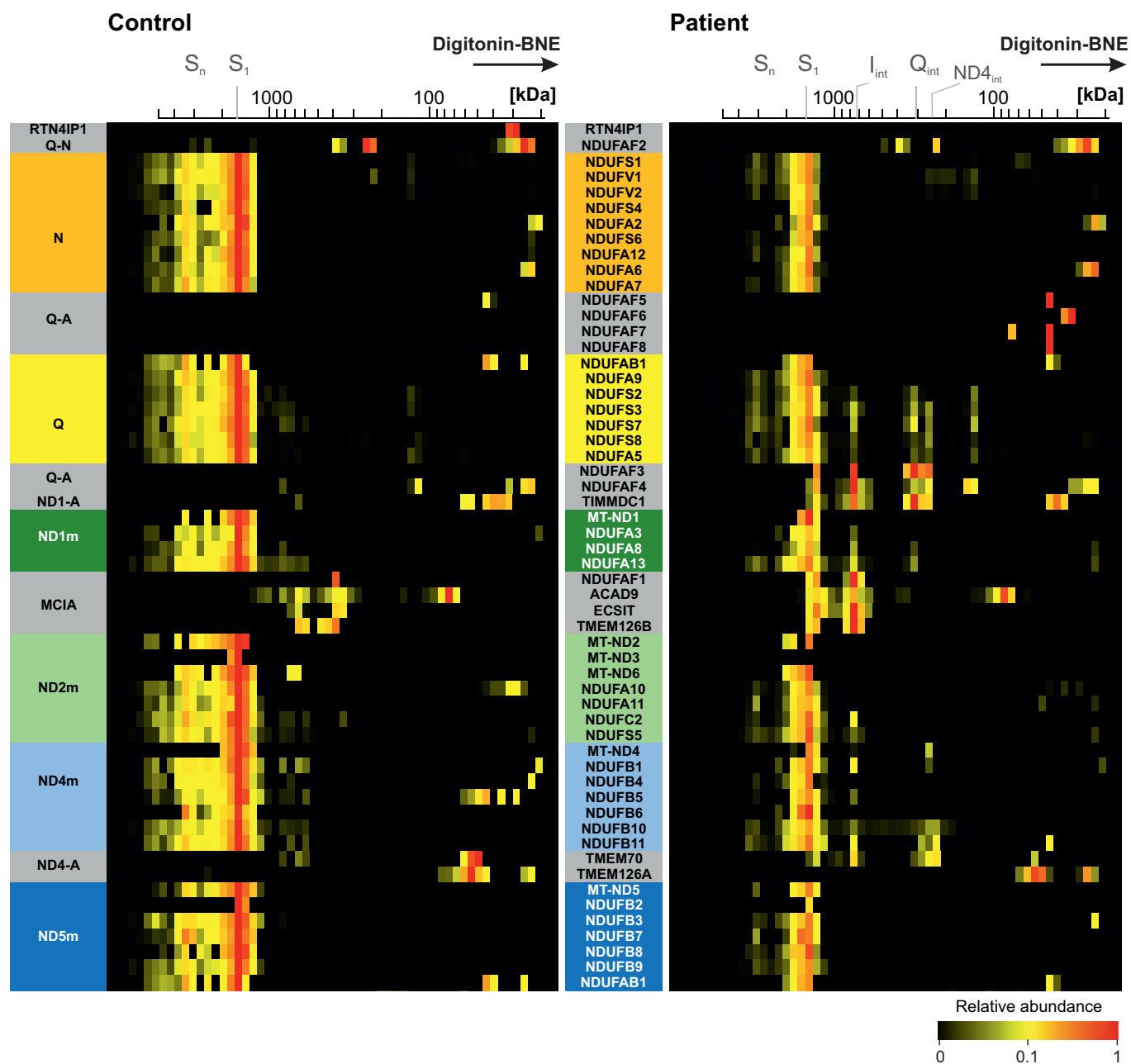
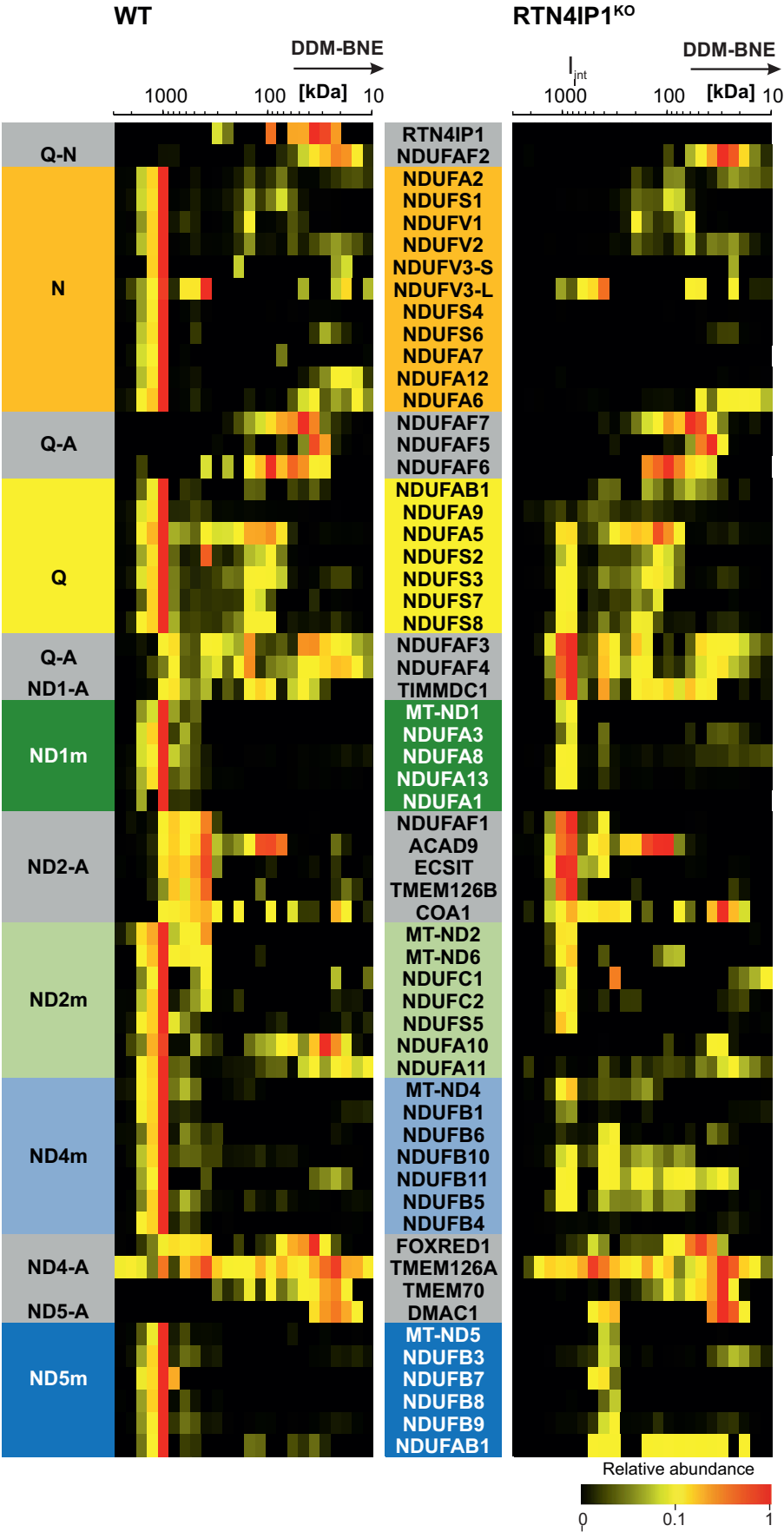


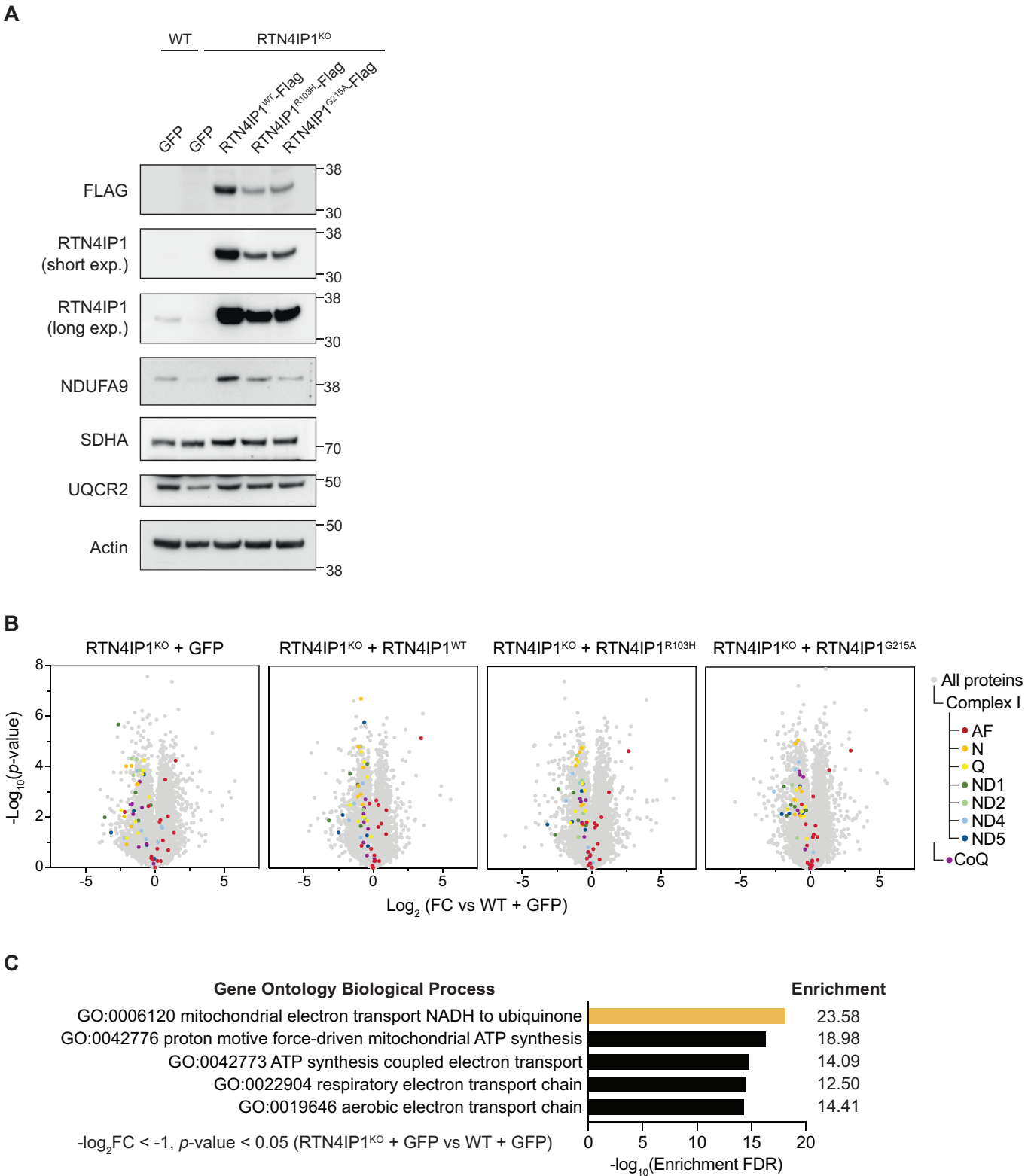
Figure EV3. CI assembly in *RTN4IP1*-derived patient fibroblasts corresponding to control cells.

Complexome profiling data of CI and identified assembly factors were sorted to their assembly modules and presented as a heatmap. Assignment of complexes: higher order supercomplexes (S_n); supercomplex S_1 of CI, CIII₂ and CIV (S_1); intermediate of CI including modules Q, ND1m, ND2m, ND4m and assembly factors (I_{int}); intermediate of Q-module containing assembly factors (Q_{int}) and intermediate of ND4-module containing assembly factors ($ND4_{int}$).



**Figure EV4. CI assembly in U2OS WT and U2OS RTN4IP1^{KO} cells.**

DDM-solubilised mitochondria complexome profiling data of CI and identified assembly factors were sorted to their assembly modules and presented as heatmap. Assignment of complexes: intermediate of CI including modules Q, ND1m, ND2m, ND4m and assembly factors (I_{int}).



◀ **Figure EV5. Validation of the U2OS RTN4IP1 rescue cell lines.**

(A) Western blotting analysis validating the stable expression of RTN4IP1-FLAG constructs in the U2OS RTN4IP1^{KO} cell lines. WT and RTN4IP1^{KO} cells were infected with a GFP or FLAG-tagged RTN4IP1-encoding lentivirus, resulting in the generation of WT and RTN4IP1^{KO} cells expressing GFP, and RTN4IP1^{KO} cells expressing RTN4IP1-FLAG mutants (RTN4IP1^{WT}, RTN4IP1^{R103H} or RTN4IP1^{G215A}). Actin and SDHA were used as loading controls. (B) Volcano plot of proteomics experiments depicted in main Fig. 5A,B, showing protein abundances in U2OS RTN4IP1^{KO}(+GFP) or U2OS RTN4IP1^{KO}(+RTN4IP1^{WT}, RTN4IP1^{R103H} or RTN4IP1^{G215A}) cells relative to WT(+GFP). CI proteins are highlighted, and colour coordinated to match individual CI modules. Data shown as mean ($n = 3$), two-sided Student's t -test. (C) Gene Ontology of Biological Process showing enrichment of mitochondrial electron transport from NADH to ubiquinone proteins based on proteomics analysis of RTN4IP1^{KO}(+GFP) versus WT(+GFP), for proteins whose $\log_2$ FC < -1 and $*p$ value < 0.05 . FDR for the relevant gene sets (top to bottom): 7.58e-19, 4.81e-17, 1.64e-15, 3.08e-15, 4.82e-15; hypergeometric test. Source data are available online for this figure.