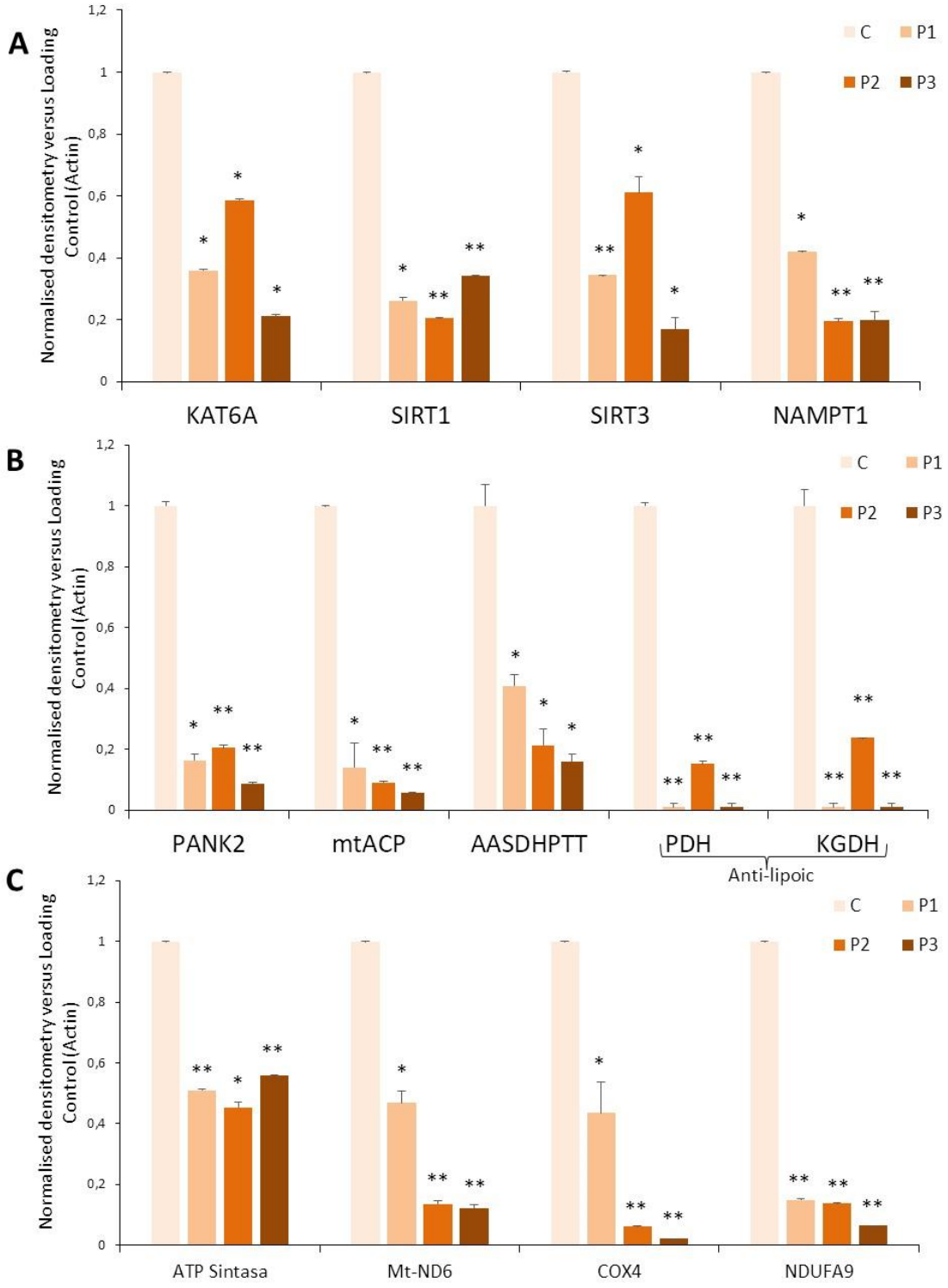
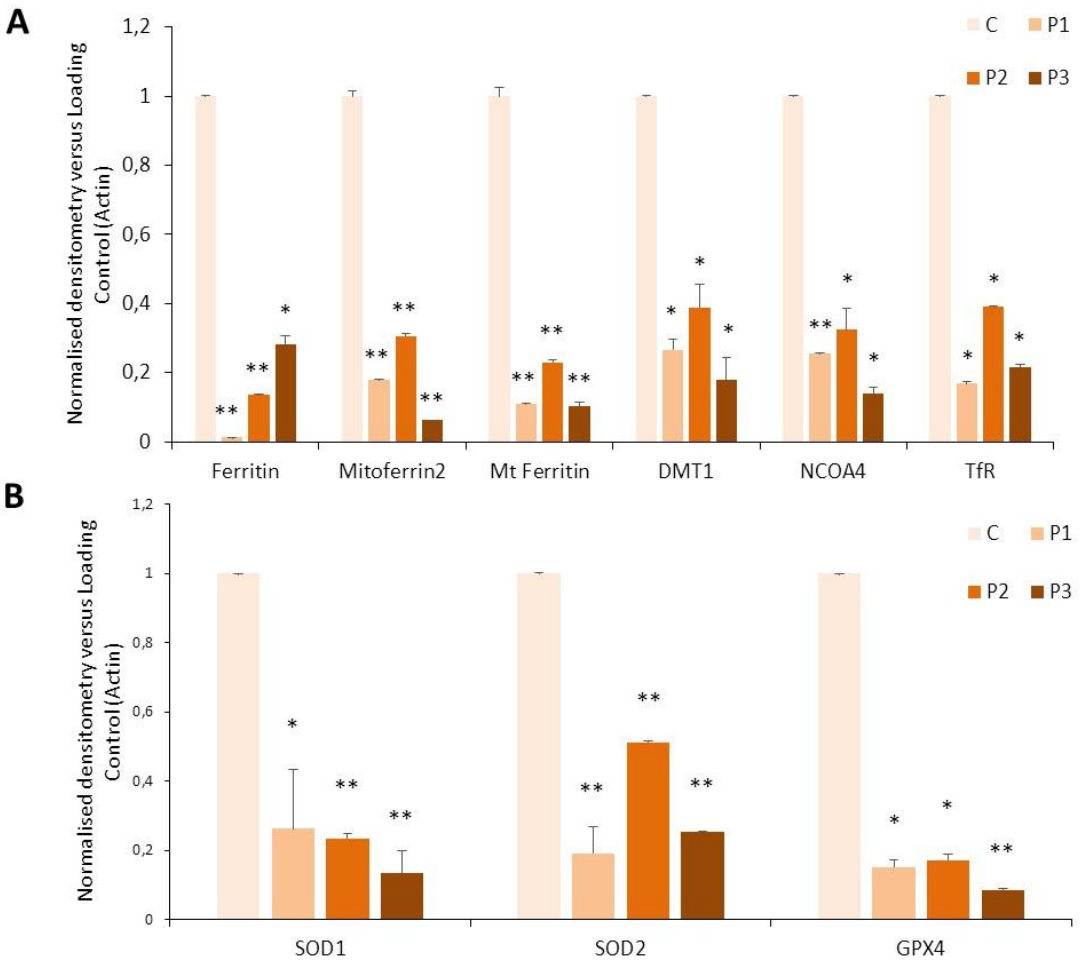
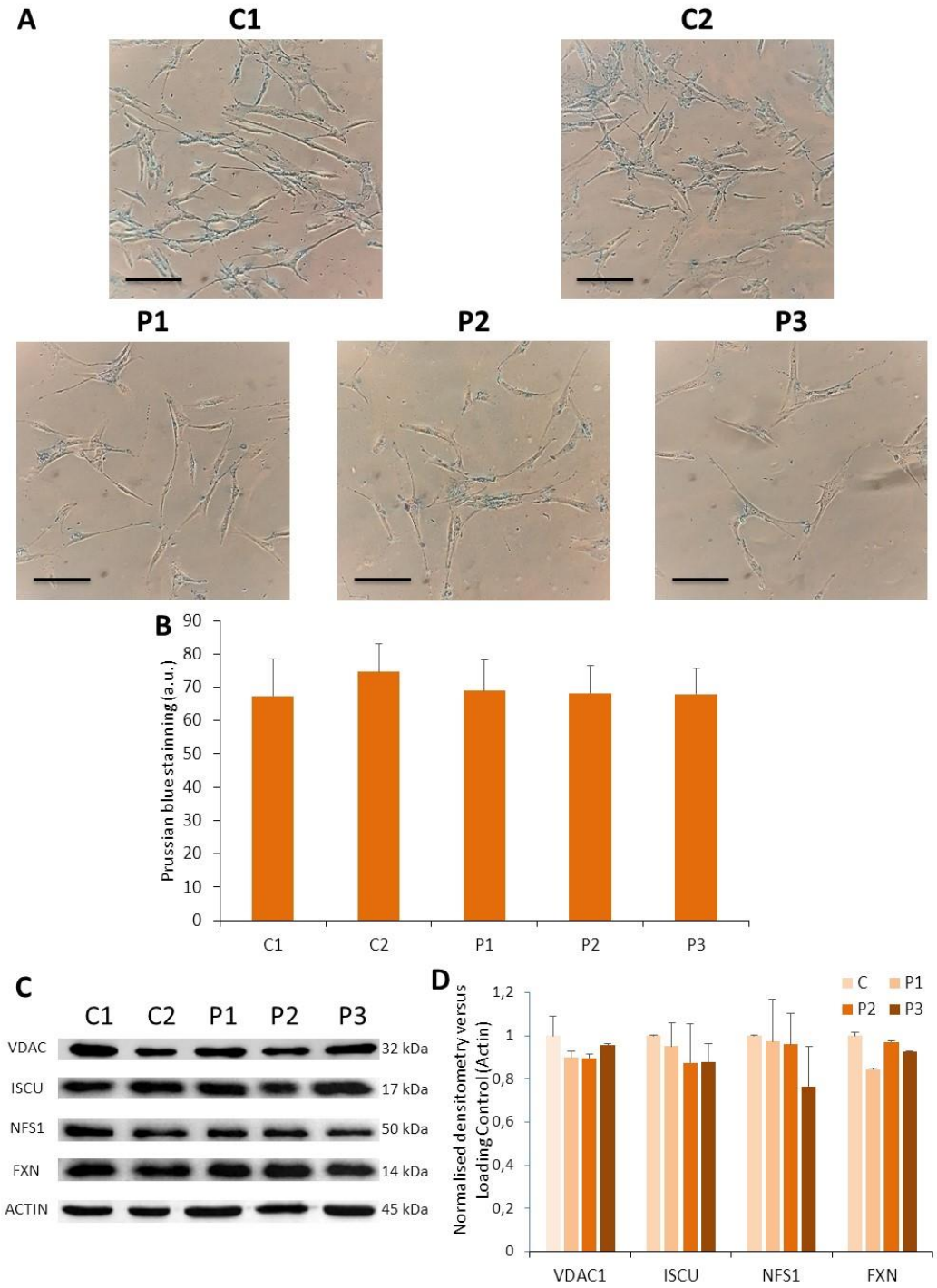


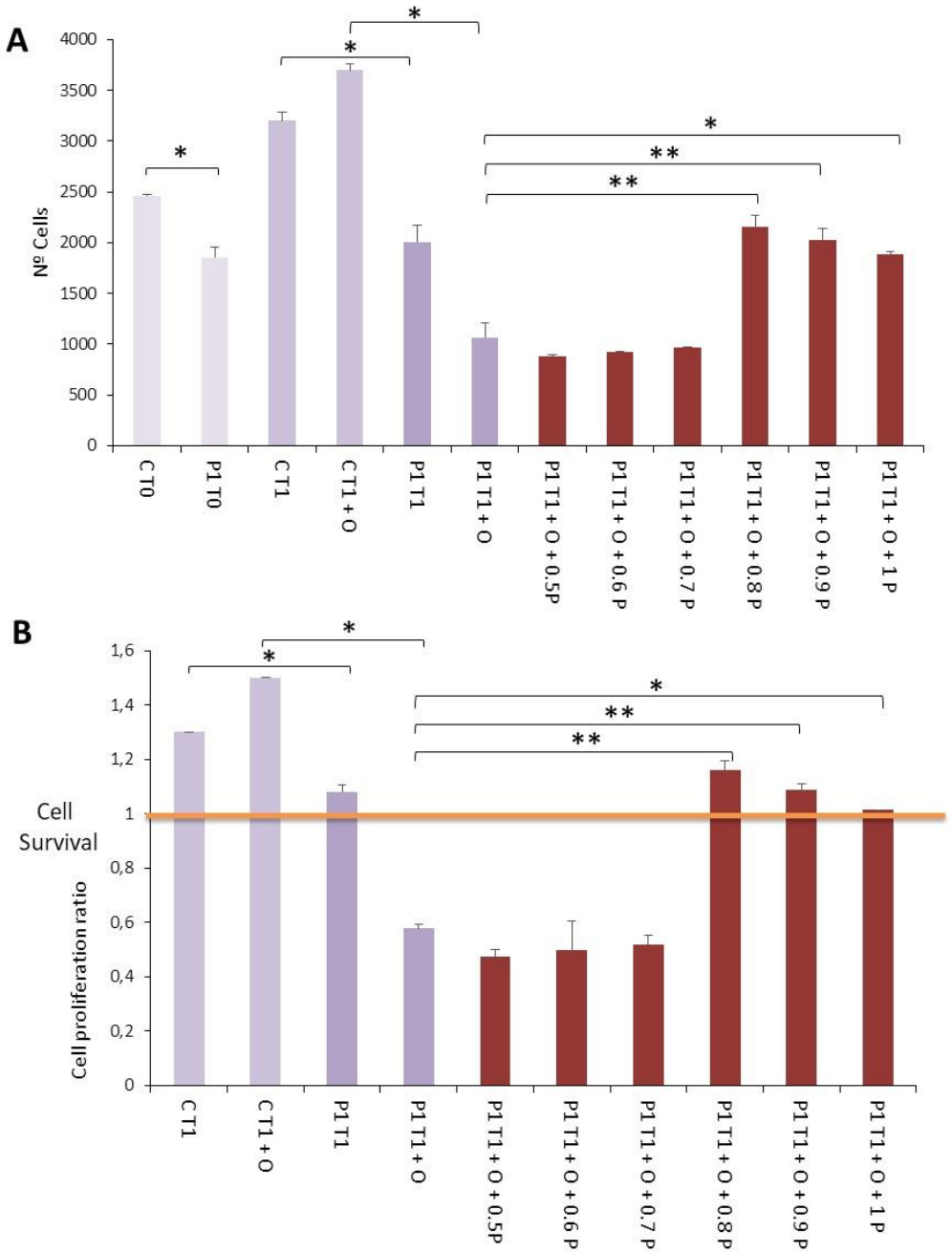
Supplementary Figure 1. Western blotting quantification of figure 1. Band densitometry of western blot shown in figure 1. Pathways in figure panels: acetylation-deacetylation (A), proteins related to CoA metabolism (B) and mitochondrial proteins (C). Data were normalized to actin. Data represent the mean ±SD of three separate experiments. *p-value < 0.05 between control and mutant KAT6A cells; **p-value < 0.01 between control and mutant KAT6A cells.



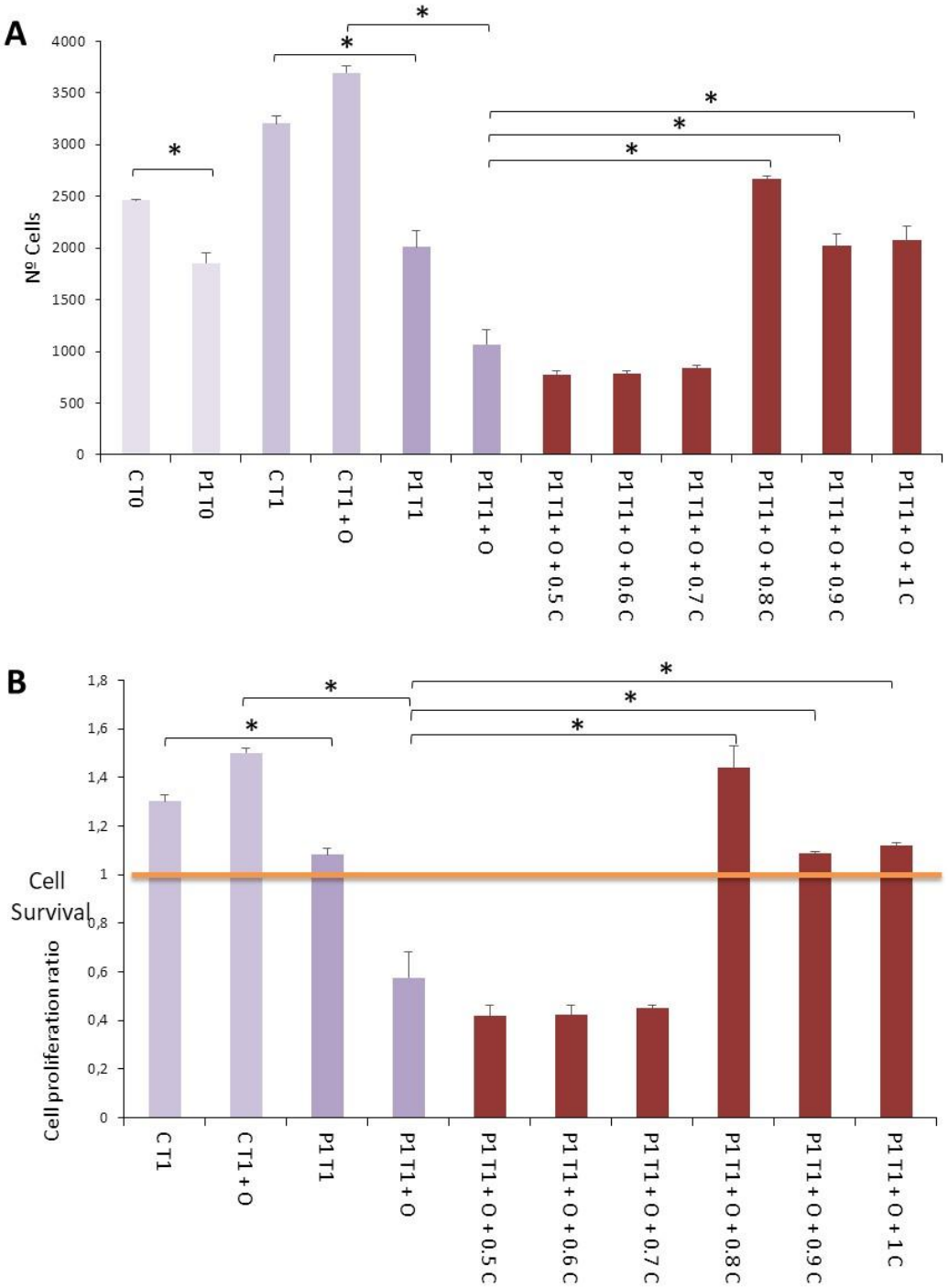
Supplementary Figure 2. Western blotting quantification of figure 1. Pathways in figure panels: proteins related to iron metabolism (A) and antioxidant enzymes (B). Data were normalized to actin. Data represent the mean ±SD of three separate experiments. *p-value < 0.05 between control and mutant KAT6A cells; **p-value < 0.01 between control and mutant KAT6A cells.



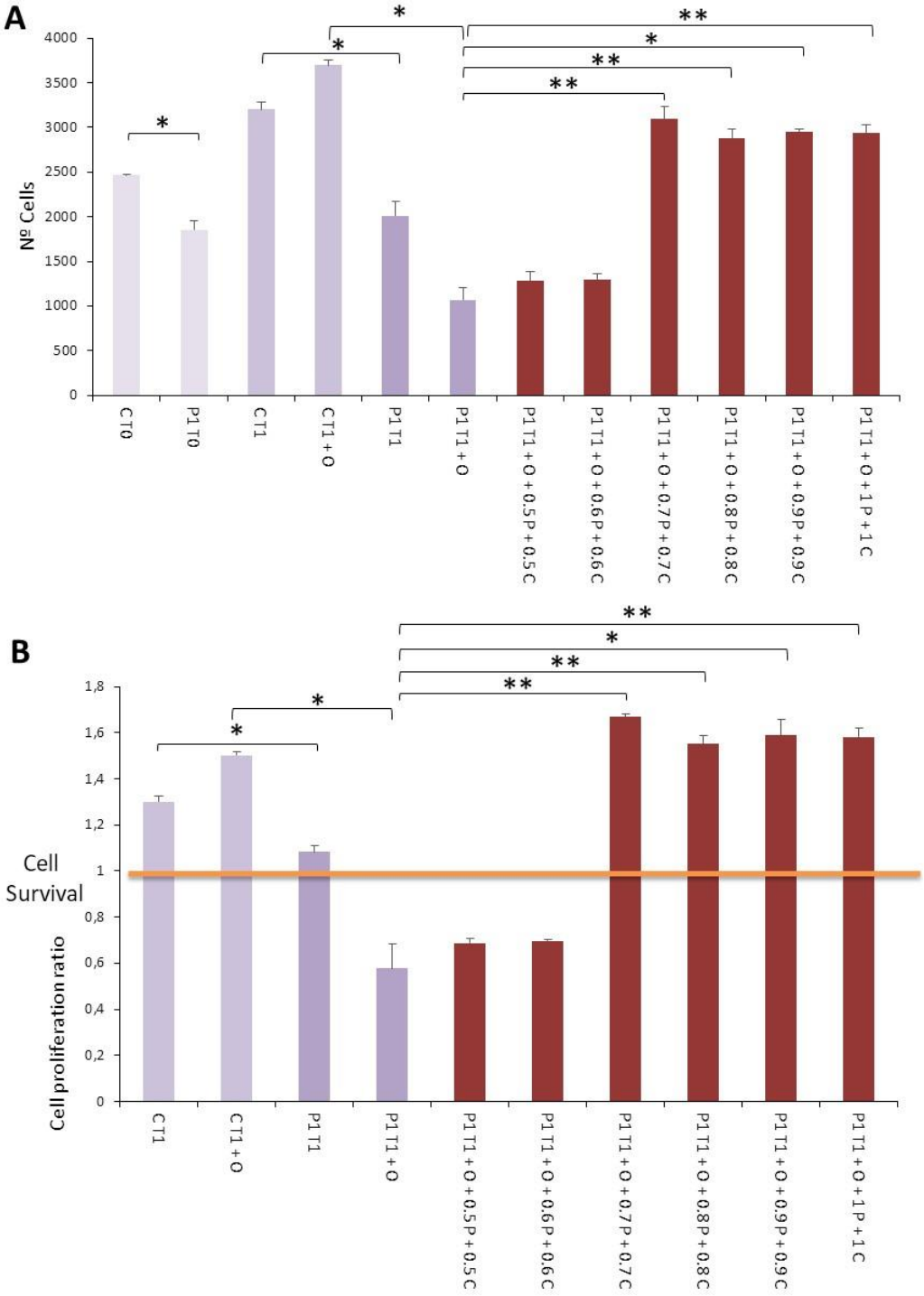
Supplementary Figure 3. Iron accumulation in control (C1 and C2) and mutant KAT6A fibroblasts (P1, P2 and P3) assessed by Prussian Blue staining (A). Quantification of Prussian Blue staining of controls and patient fibroblasts (B). Protein expression patterns in control and KAT6A mutant fibroblasts. Protein extracts of Control (C1 and C2) and patient (P1, P2 and P3) were separated on a SDS polyacrylamide gel and immunostained with primary antibodies. (C) Proteins related to mitochondrial mass and iron-sulfur clusters biosynthesis: VDAC, ISCU, NFS1 and FXN. (D) Western blotting quantification of VDAC, ISCU, NFS1 and FXN. Data represent the mean ±SD of three separate experiments. Scale bar= 100 µm.



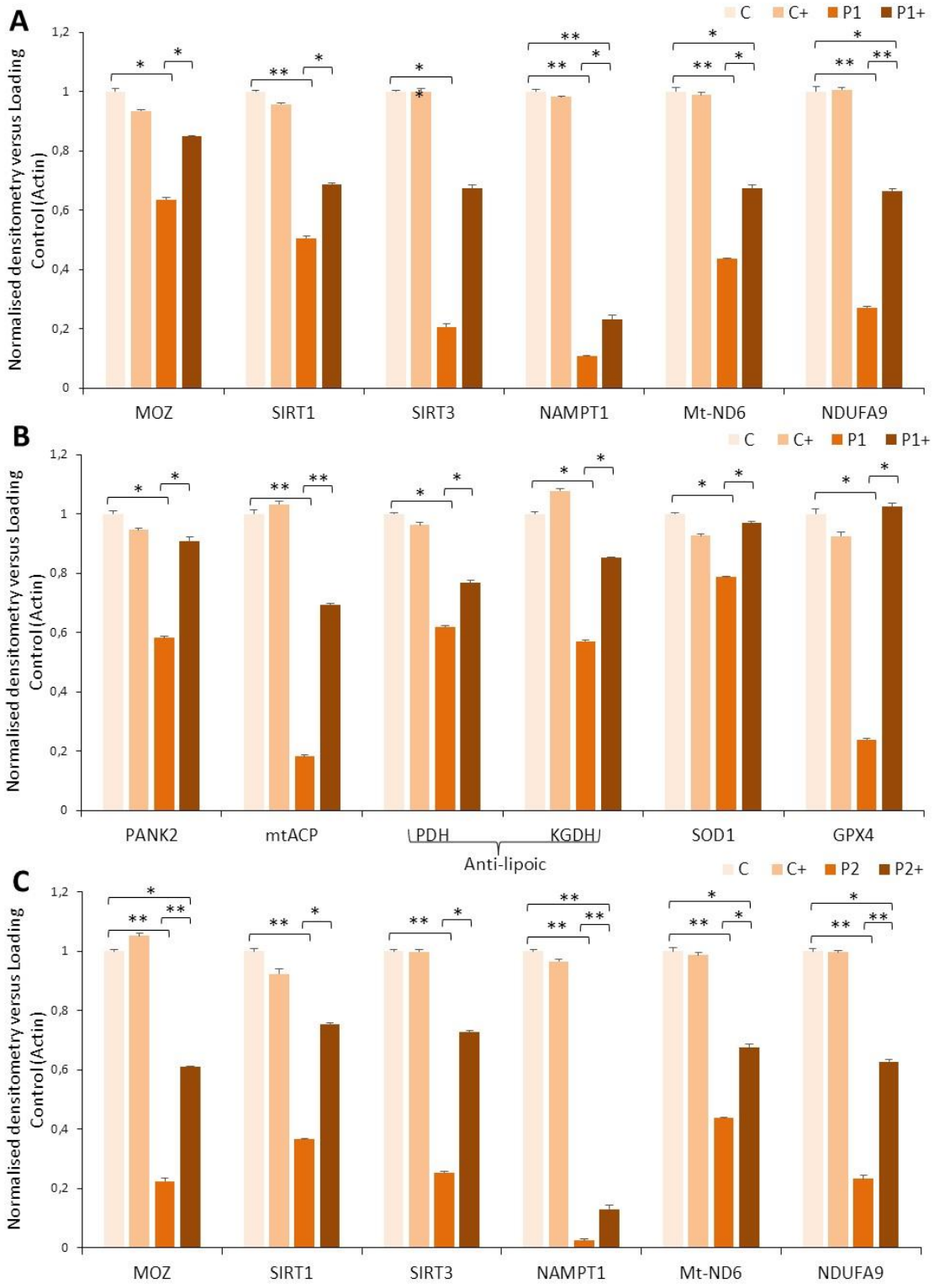
Supplementary Figure 4. (A) Cell quantification control (C1) and mutant KAT6A fibroblasts in galactose medium with oligomycin at 0.5 nM (O). (B) Cell proliferation rate obtained from Nº Cells at T72h (T1)/ Nº cells at T0h (T0). Results ≥ 1 indicate cell survival and cell proliferation, results < 1 indicate cell death. The data represents the mean ±SD of 3 independent experiments. Pantothenate (P) was used at different concentrations (0.5-1 µM). Data represent the mean ±SD of three separate experiments. *p-value < 0.05 and **p-value < 0.01.



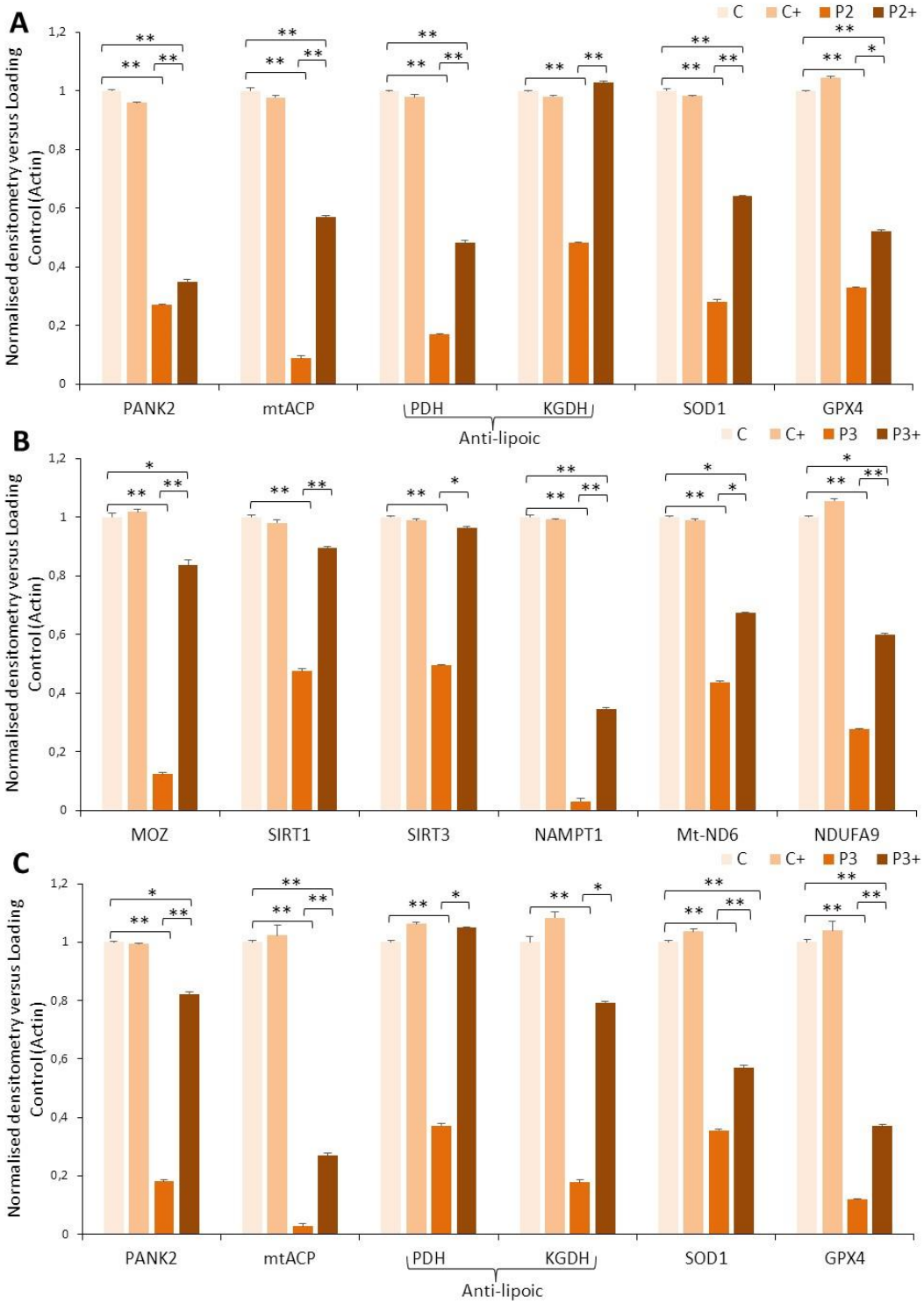
Supplementary Figure 5. (A) Cell quantification of control (C1) and mutant KAT6A fibroblasts in galactose medium with oligomycin at 0,5 nM (O). (A) Cell proliferation rate was obtained by the equation: Nº Cells at T72h (T1)/ Nº cells at T0h (T0). (B) Results ≥ 1 indicate cell survival and proliferation. Results < 1 indicate cell death. The data represents the mean ±SD of 3 independent experiments. L-carnitine (C) was used at different concentrations (0.5-1 µM). Data represent the mean ±SD of three separate experiments. *p-value < 0.05 and **p-value < 0.01.



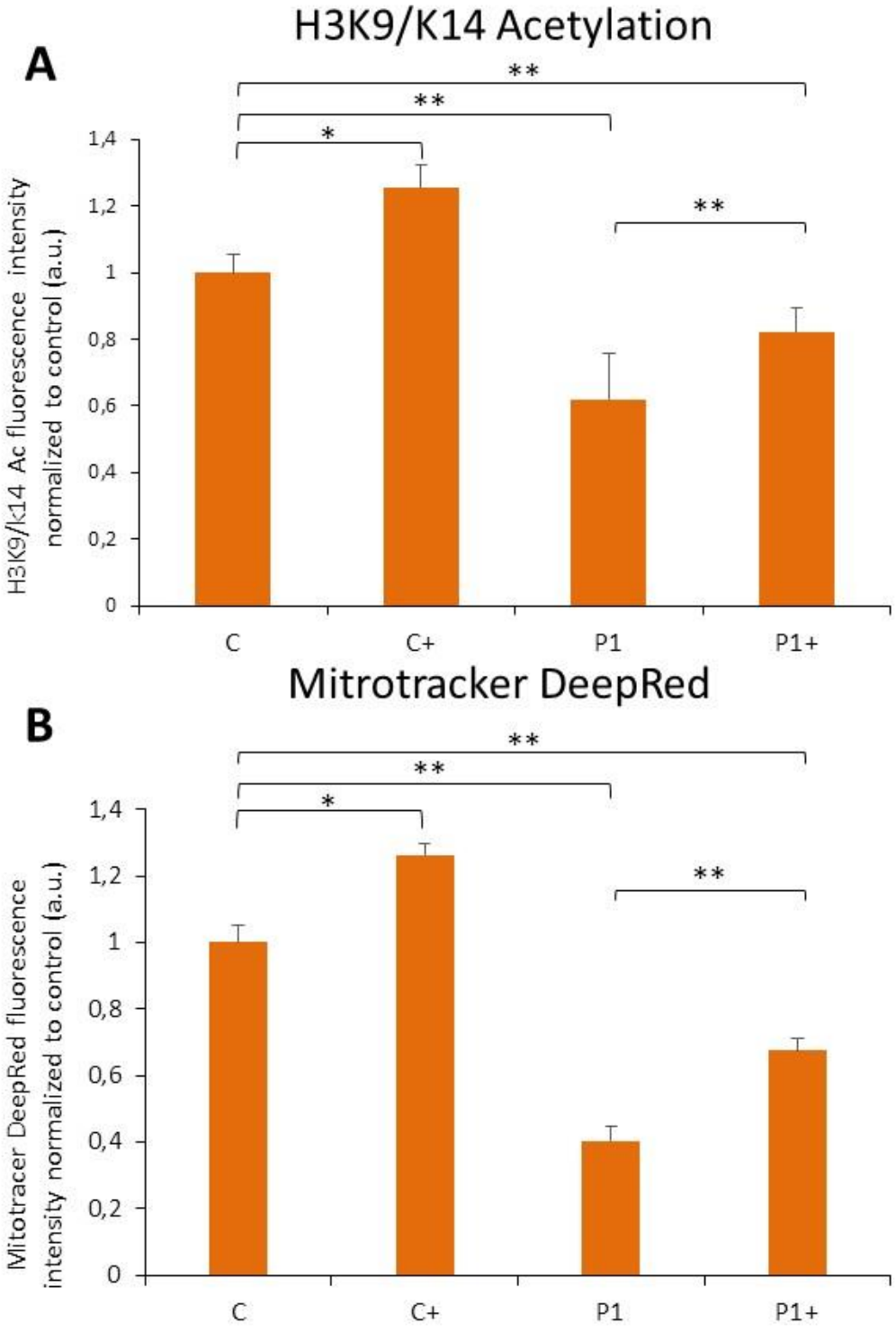
Supplementary Figure 6. (A) Cell quantification control (C1) and mutant KAT6A fibroblasts in galactose medium with oligomycin at 0.5 nM (O). (B) Cell proliferation rate was obtained by the equation: Nº Cells at T72h (T1)/ Nº cells at T0h (T0). Results ≥ 1 indicate cell survival and proliferation. Results < 1 indicate cell death. The data represents the mean± SD of 3 independent experiments. The combination of pantothenate (P) and L-carnitine (C) was used at different concentrations (0.5-1 µM). Data represent the mean ±SD of three separate experiments. *p-value < 0.05 and **p-value < 0.01.



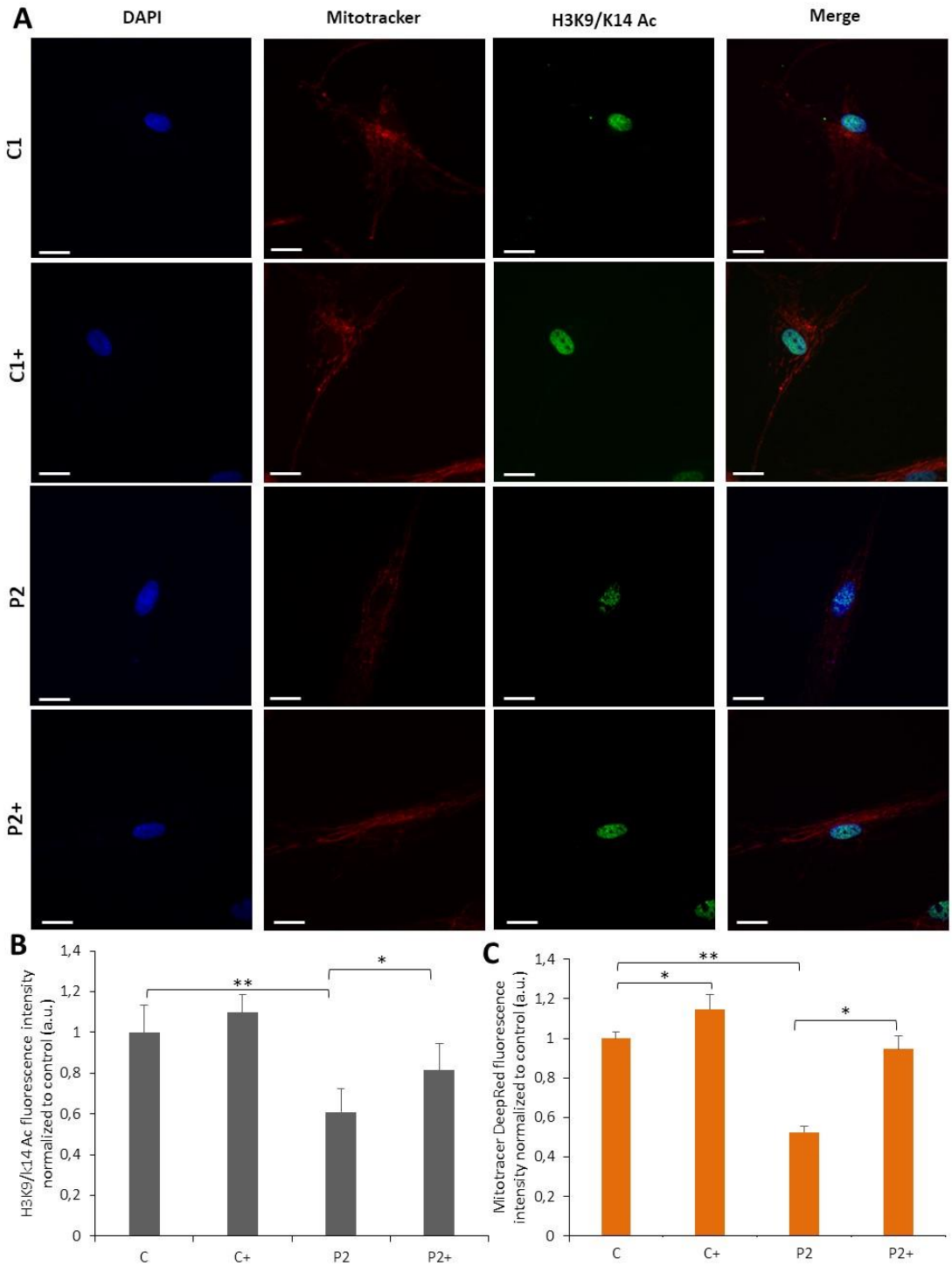
Supplementary Figure 7. Western blotting quantification of figure 3. Patients in figure panels: patient 1 (P1) (A-B) and patient 2 (P2) (C). Data were normalized to actin. Data represent the mean ±SD of three separate experiments. *p-value < 0.05 and **p-value < 0.01.



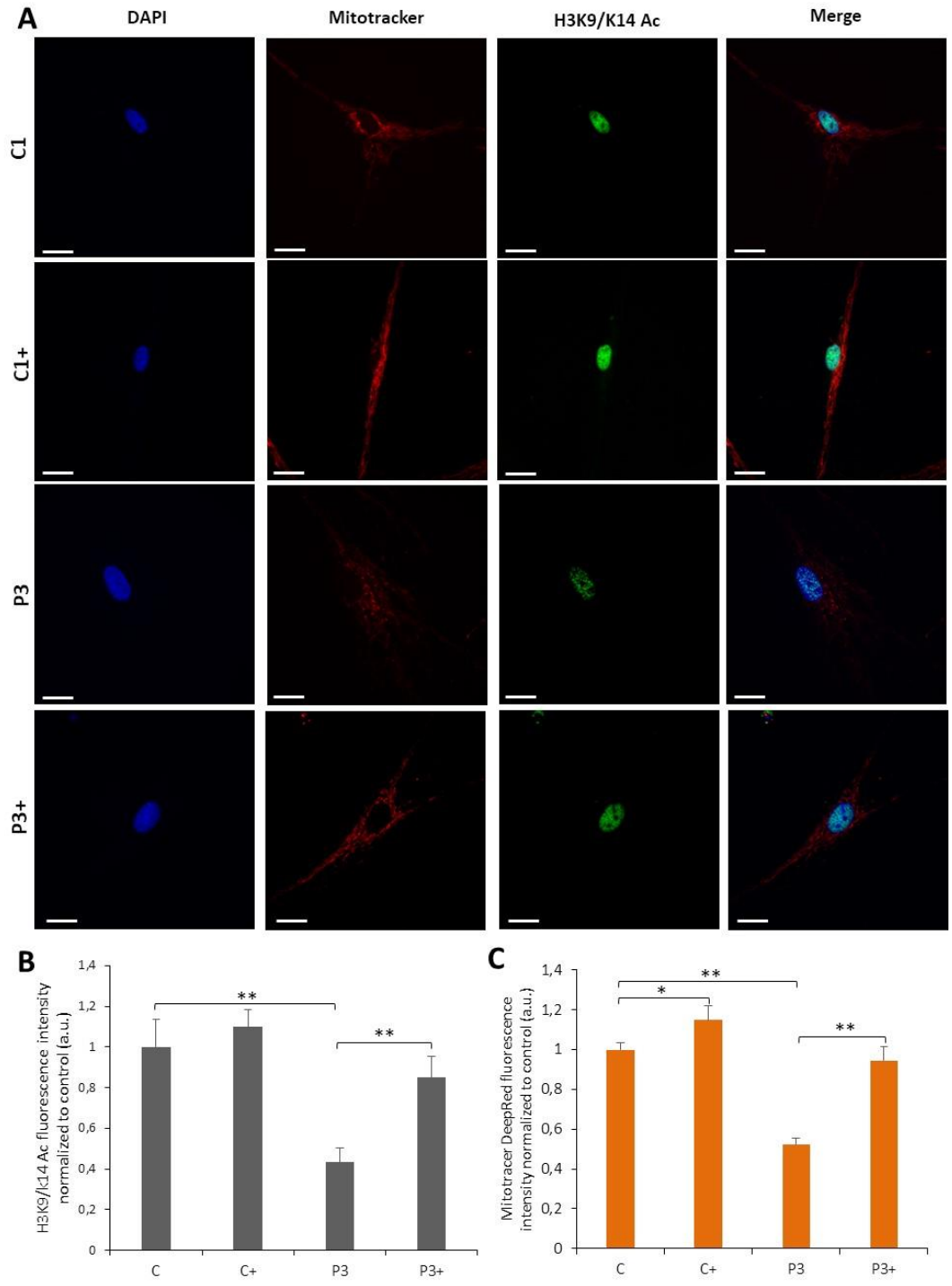
Supplementary Figure 8. Western blotting quantification of figure 3. Patients in figure panels: patient 2 (P2) (A) and patient 3 (P3) (B-C). Data were normalized to actin. Data represent the mean ±SD of three separate experiments. *p-value < 0.05 and **p-value < 0.01.



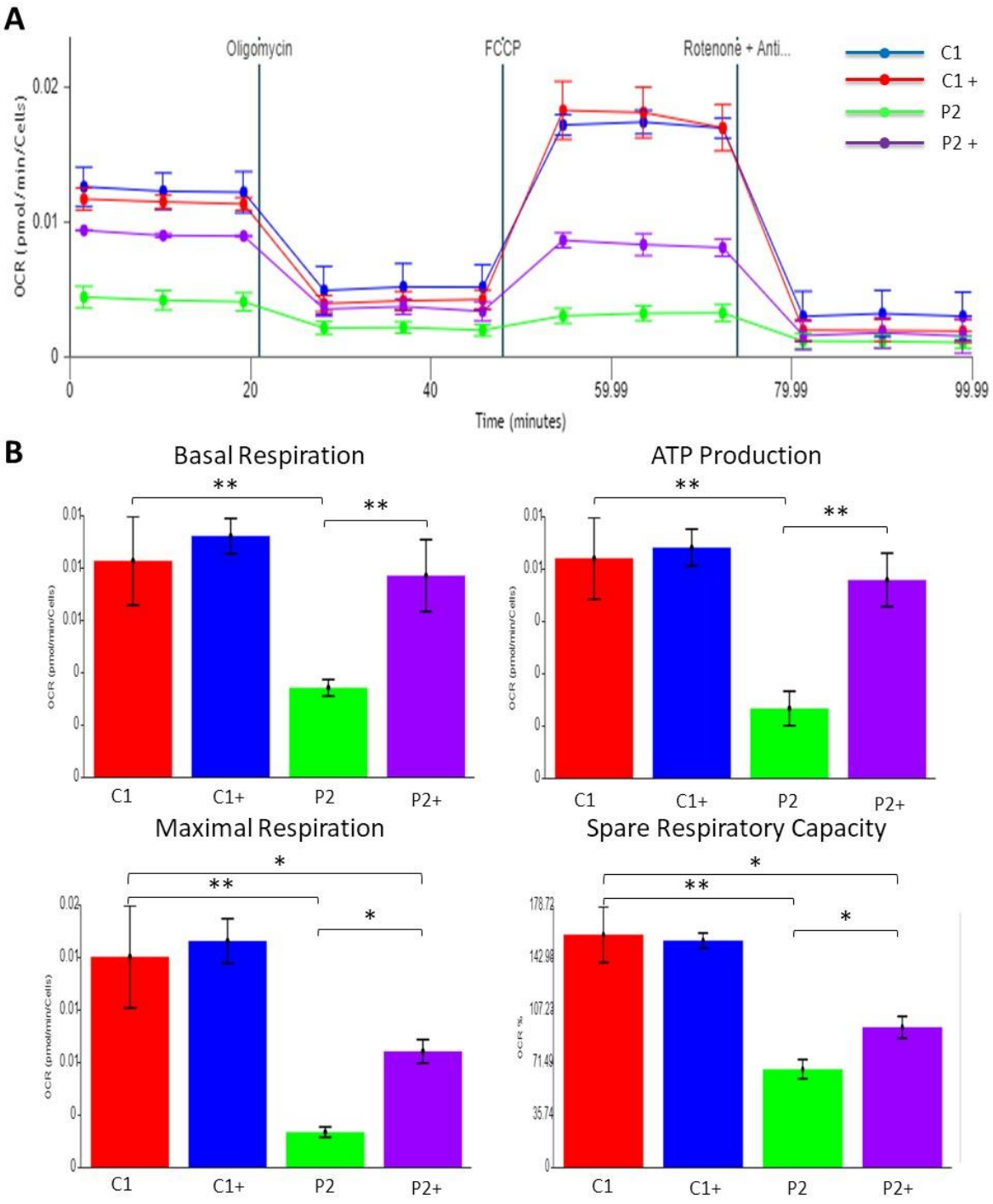
Supplementary Figure 9. Quantification of H3K9/K14 Acetylation and MitoTracker staining of figure 4 using softWoRx and ImageJ software. Data were normalized to control. Fifty cells per condition were analyzed. Data represent the mean ±SD of three separate experiments. *p-value < 0.05 and **p-value < 0.01.



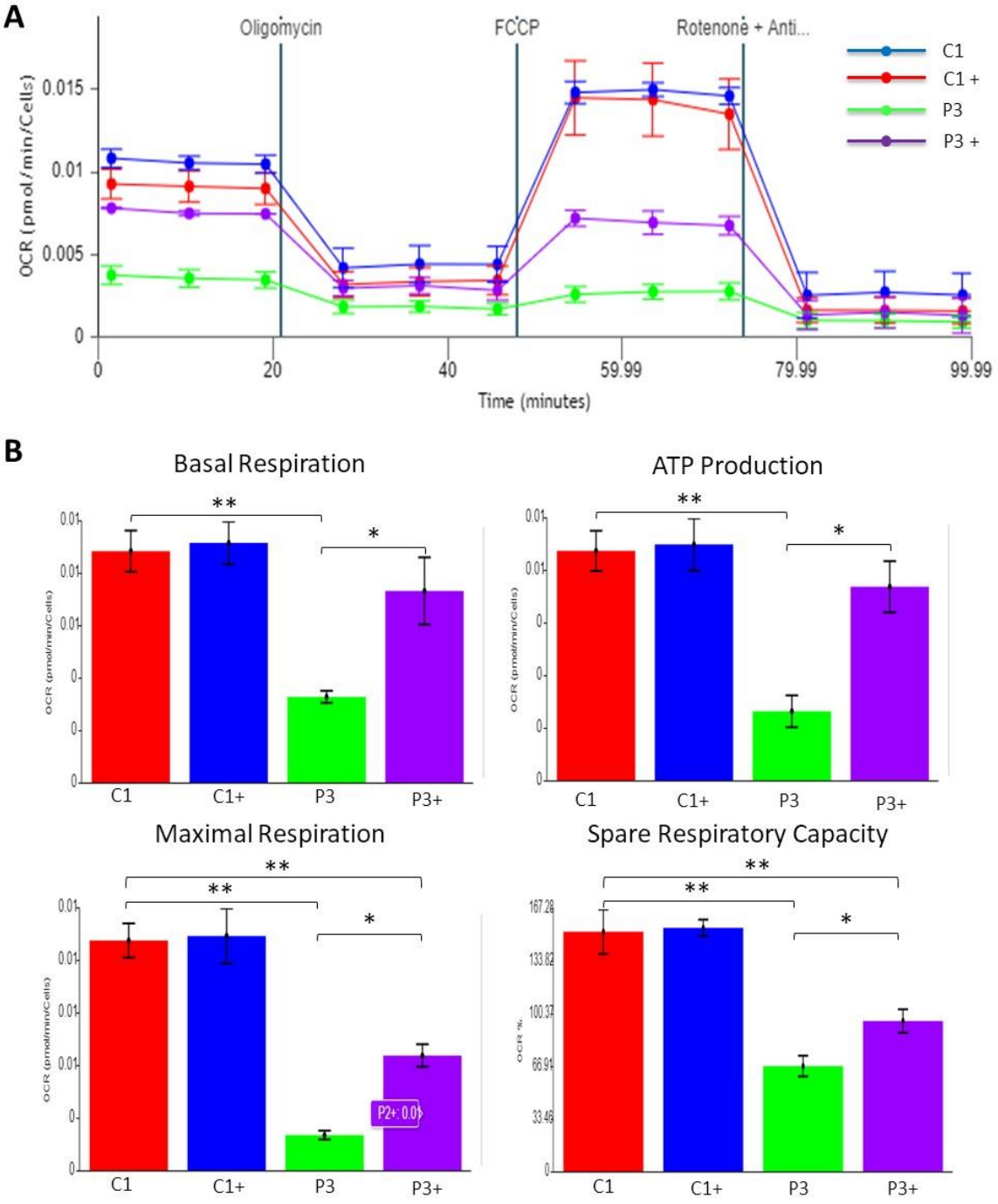
Supplementary Figure 10. Effect of 0.4 µM pantothenate and 0.4 µM L-carnitine on histone acetylation levels in control (C) and KAT6A fibroblasts (Patient 2 – P2). (A) Control and KAT6A fibroblasts were incubated with Mitotracker DeepRed FM 100 nM for 45 min, then they were fixed and immunostained with anti-H3K9/K14 and examined by fluorescence microscopy. (B) Quantification of H3K9/K14 Acetylation immunostaining. (C) Quantification of MitoTracker staining. Fifty cells per condition were analyzed. Fluorescence was quantified using softWoRx and ImageJ software. Data represent the mean ±SD of three separate experiments. *p-value < 0.05 and **p-value < 0.01. Scale bar= 15 µm. C+ and P2+, treated control and P2 cell lines.



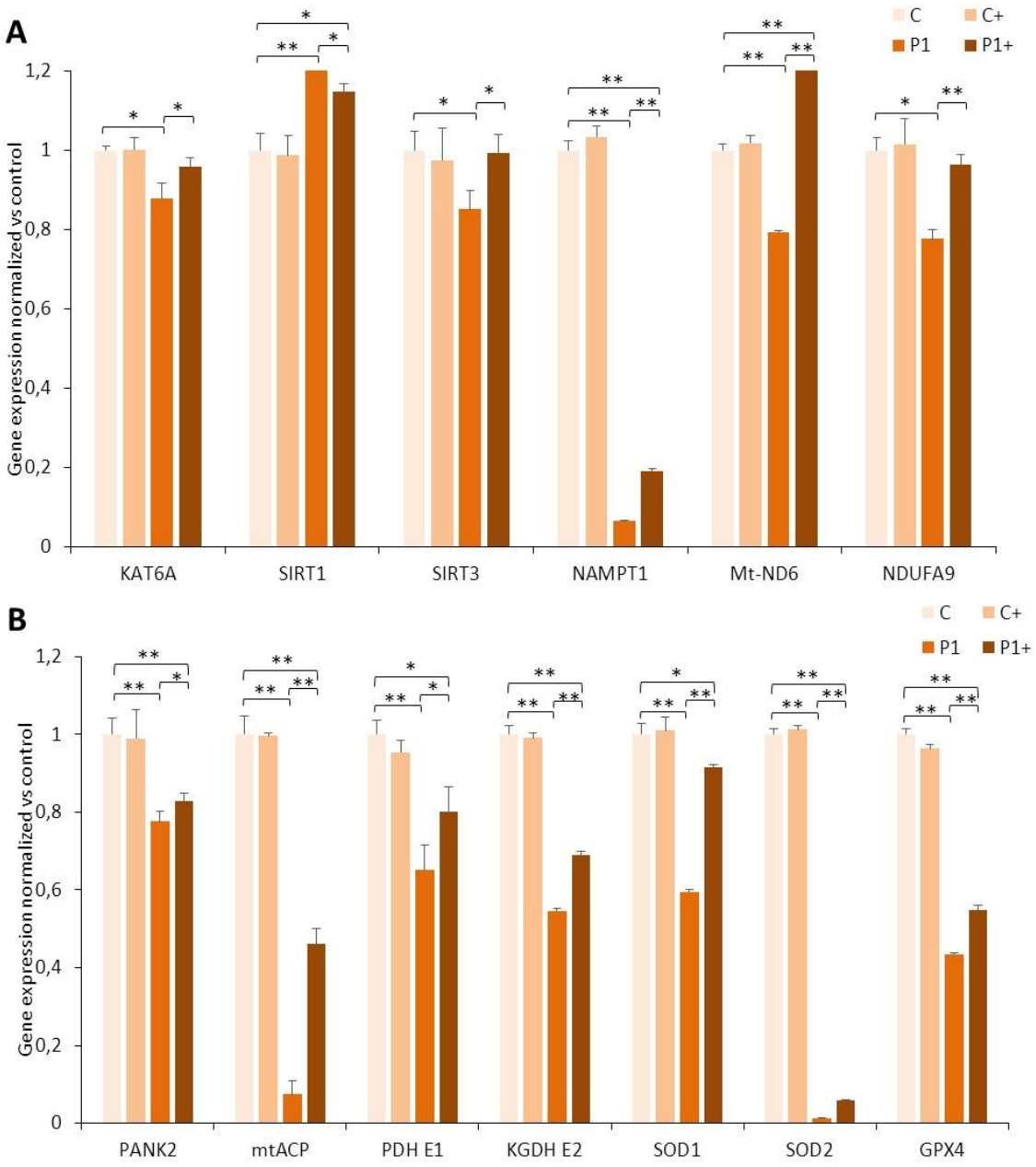
Supplementary Figure 11. Effect of 0.7 µM pantothenate and 0.7 µM L-carnitine on histone acetylation levels in control (C) and KAT6A fibroblasts (Patient 3 – P3). (A) Control and KAT6A fibroblasts were incubated with Mitotracker DeepRed FM 100 nM for 45 min, then they were fixed and immunostained with anti-H3K9/K14 and examined by fluorescence microscopy. (B) Quantification of H3K9/K14 Acetylation immunostaining. (C) Quantification of MitoTracker staining. Fifty cells per condition were analyzed. Fluorescence was quantified using softWoRx and ImageJ software. Data represent the mean ±SD of three separate experiments. *p-value < 0.05 and **p-value < 0.01. Scale bar= 15 µm. C+ and P3+, treated control and P3 cell lines.



Supplementary Figure 12. Effect of pantothenate and L-carnitine supplementation on mitostress bioenergetic assay in control (control – C1) and mutant KAT6A fibroblasts (Patient 2- P2). (A) Mitochondrial respiration profile was measured with a Seahorse XFe24 analyzer. Fibroblasts were treated for 15 days with 0.4 µM pantothenate and 0.4 µM L-carnitine. (B) Basal respiration, ATP production, maximal respiration and spare respiratory capacity were assessed by Seahorse analytics website. *p-value < 0.05 and **p-value < 0.01.



Supplementary Figure 13. Effect of pantothenate and L-carnitine supplementation on mitostress bioenergetic assay in control (control – C1) and mutant KAT6A fibroblasts (Patient 3- P3). (A) Mitochondrial respiration profile was measured with a Seahorse XFe24 analyzer. Fibroblasts were treated for 15 days with 0.7 µM pantothenate and 0.7 µM L-carnitine. (B) Basal respiration, ATP production, maximal respiration and spare respiratory capacity were assessed by Seahorse analytics website. *p-value < 0.05 and **p-value < 0.01.



Supplementary Figure 14. Transcript expression levels of key altered genes in control and mutant KAT6A fibroblasts with and without pantothenate and L-carnitine treatment. Gene expression were retrieved from the DESeq2 RNAseq differential expression analysis. DESeq2 provides methods to test for differential expression by using negative binomial generalized linear models. Data was normalized versus control. Data represent the mean ±SD of three separate experiments. *p-value < 0.05 and **p-value < 0.01.

Supplementary Figure 15. Quality control in control and mutant KAT6A fibroblasts treated and non treated with a Phred-Score (Q) greater than 30.