



Enhanced Autophagy Induction In Niemann-Pick Type C Fibroblasts Compared With Controls Under Starvation And Chloroquine



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INTRODUCTION

Niemann-Pick disease type C (NPC) is a neuro-visceral pathology caused by autosomal recessive mutations in *NPC1* and *NPC2* genes, resulting in an endolysosomal accumulation of sphingomyelin and non-esterified cholesterol mainly in cells of the brain, liver, spleen, and immune system [1]. Autophagy represents the main lysosomal pathway by which cells degrade damaged constituents and protect themselves by eliminating toxic organelles and peptides and recycling their components [2]. Thus, impaired lipid degradation in lysosomal disorders is associated with dysregulated autophagic flux [3].

OBJECTIVE

To assess autophagy induction in Niemann-Pick type C patient-derived fibroblasts versus healthy controls.

METHODS

Autophagy was induced by serum starvation for 6 hours in fibroblast cell lines from three healthy controls and three NPC patients to assess potential differences in the autophagic process. Additionally, cells were treated with 5 μ M Chloroquine for 6 hours. Cell survival (mTOR, p-mTOR), autophagy (LC3-II, p62, HSC70), and heat shock response (HSP70) markers were analyzed by Western blotting.

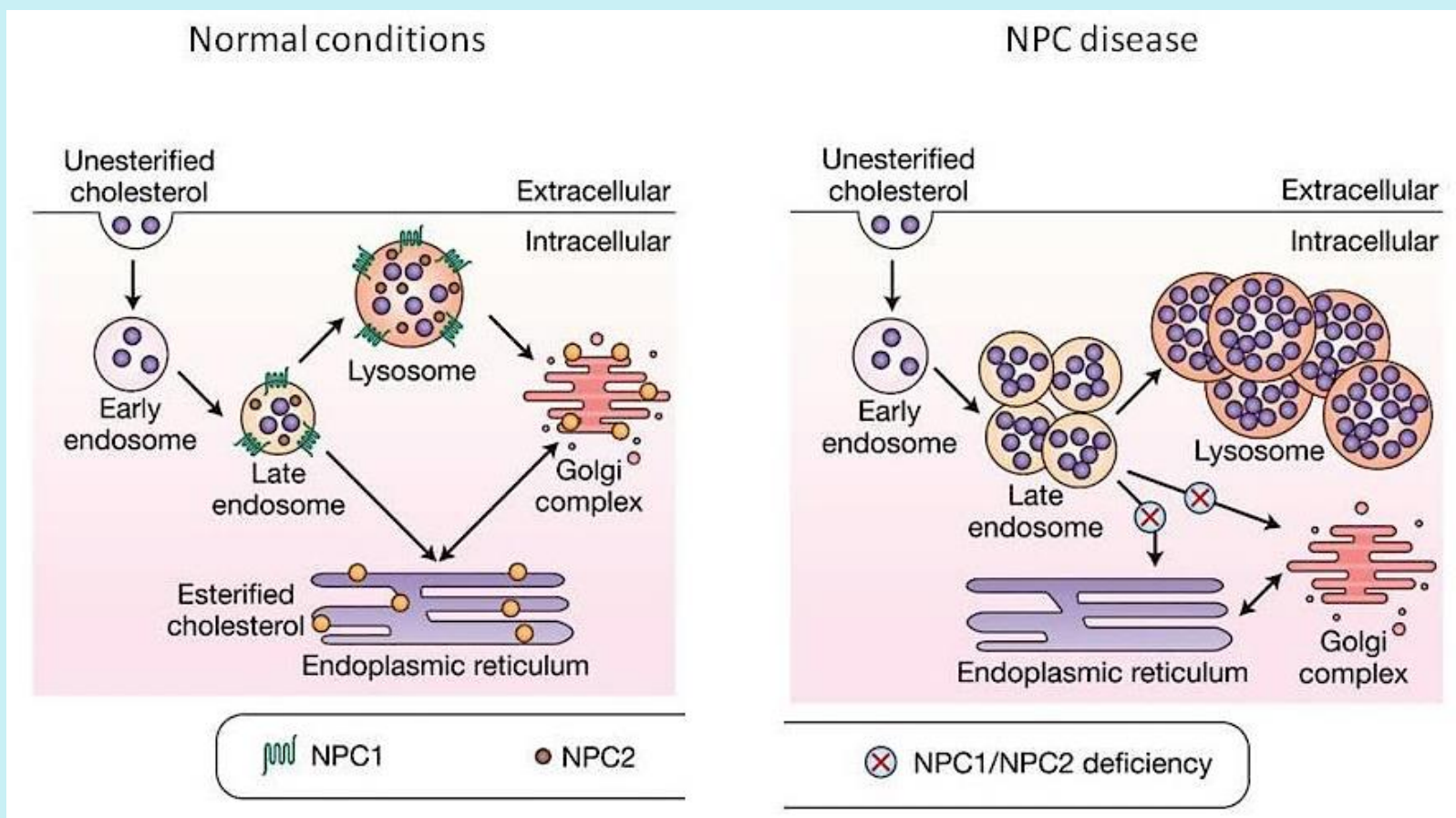
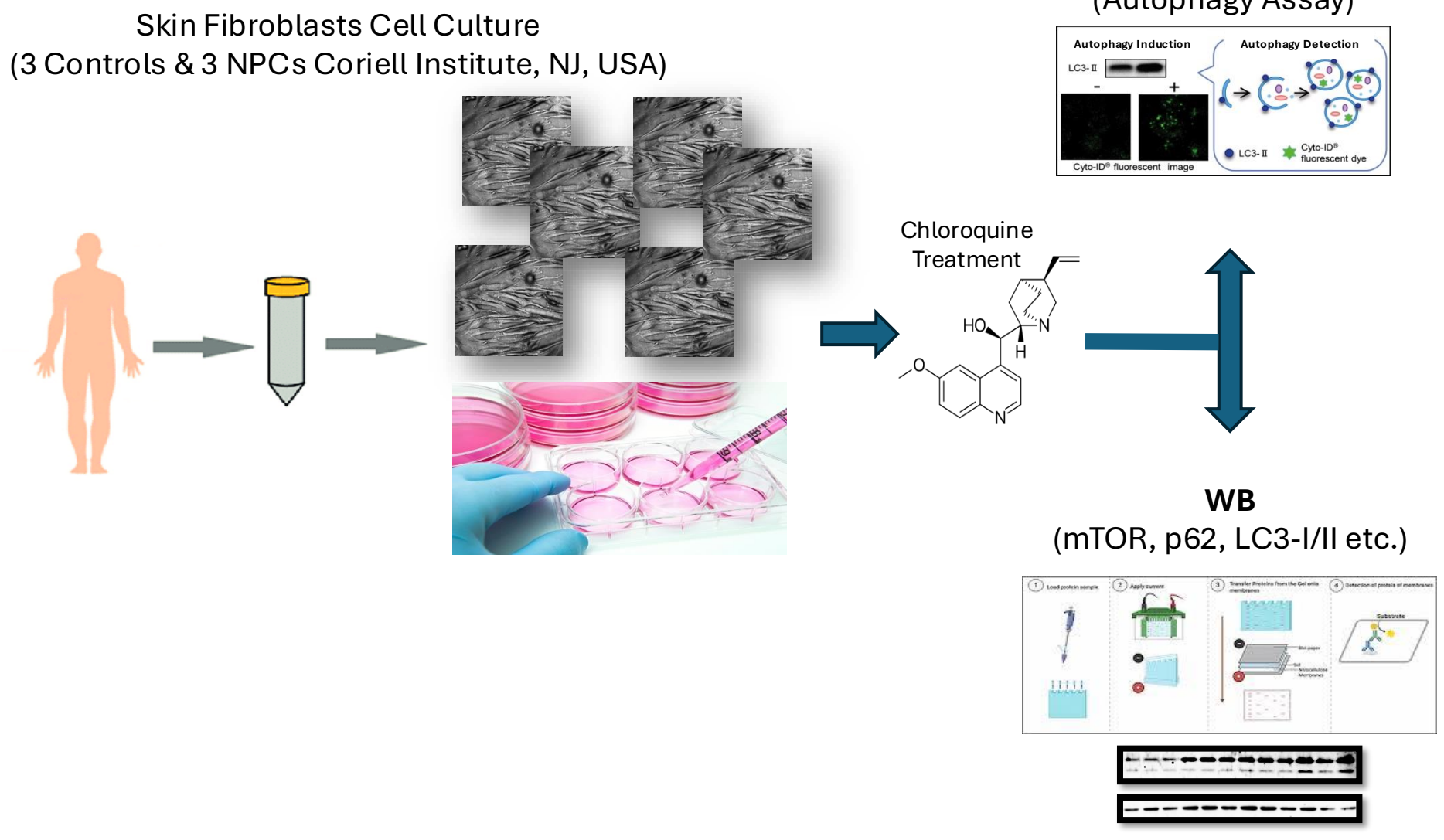


Figure 1. The pathogenesis of Niemann-Pick type C disease. Modified from Pacheco, C. D., & Lieberman, A. P. (2008).

EXPERIMENTAL DESIGN



RESULTS

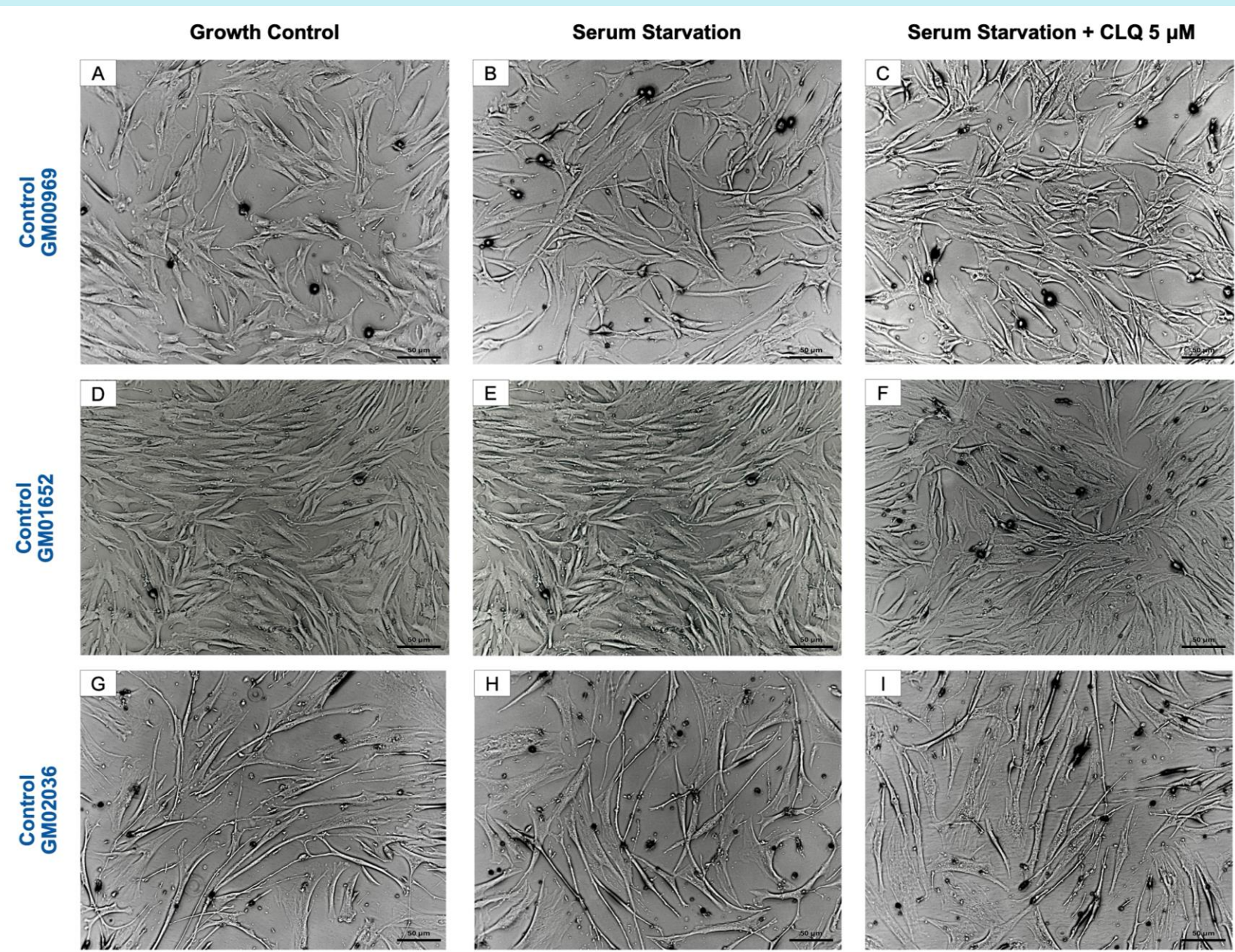


Figure 3. Fibroblast cells from three control subjects in different conditions. In the left column is the growth control condition, the medium column represents the serum starvation condition, and the right column the serum starvation condition with a 5 μ M Chloroquine treatment. Phase contrast images. 10x objective. Scale bar equal to 50 μ m.

FIBROBLAST CULTURES FROM CONTROLS				
ID	Type	Sex	Age	Race
GM00969	Fibroblast	Female	2 years	Caucasian
GM01652	Fibroblast	Female	11 years	Caucasian
GM02036	Fibroblast	Female	11 years	Caucasian

FIBROBLAST CULTURES FROM NPC PATIENTS				
ID	Type	Sex	Age	Race
GM22870	Fibroblast	Female	4 years	Caucasian
GM22871	Fibroblast	Female	4 years	Caucasian
GM03123	Fibroblast	Female	9 years	Caucasian

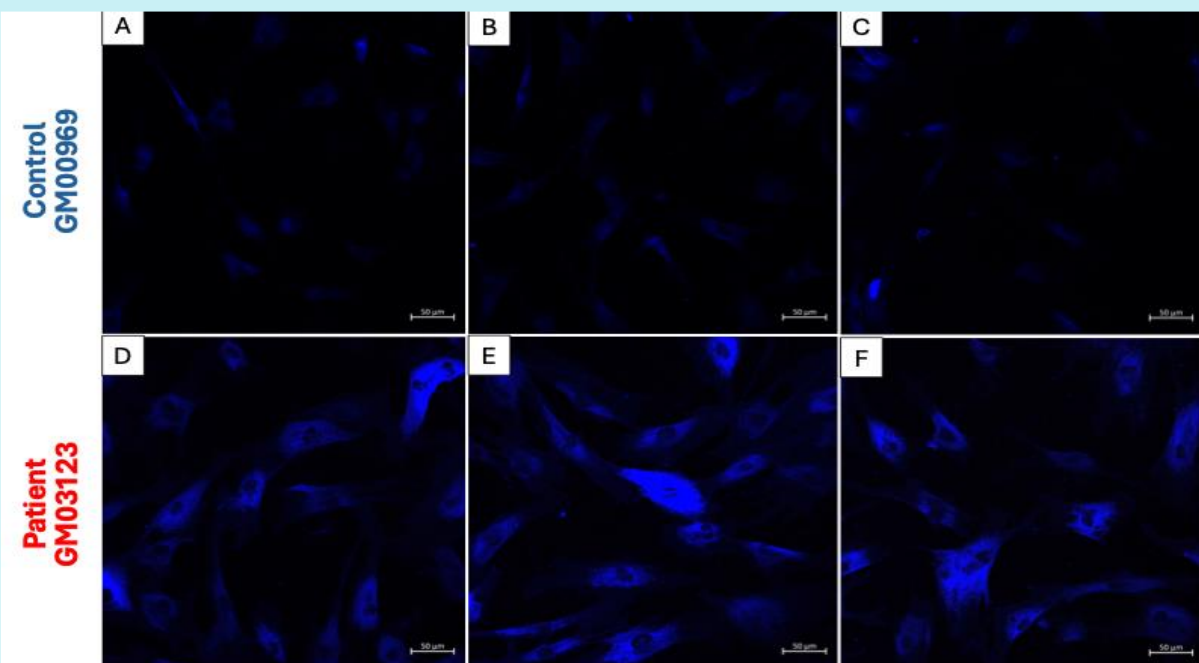


Figure 2. Filipin III staining of fibroblast cultures. The culture from the representative control subject GM00969 is shown in the top row (A-C). The culture from the representative NPC patient GM03123 is shown in the bottom row (D-F). Images obtained with a confocal microscope using the 20x objective. Scale bar equal to 50 μ m.

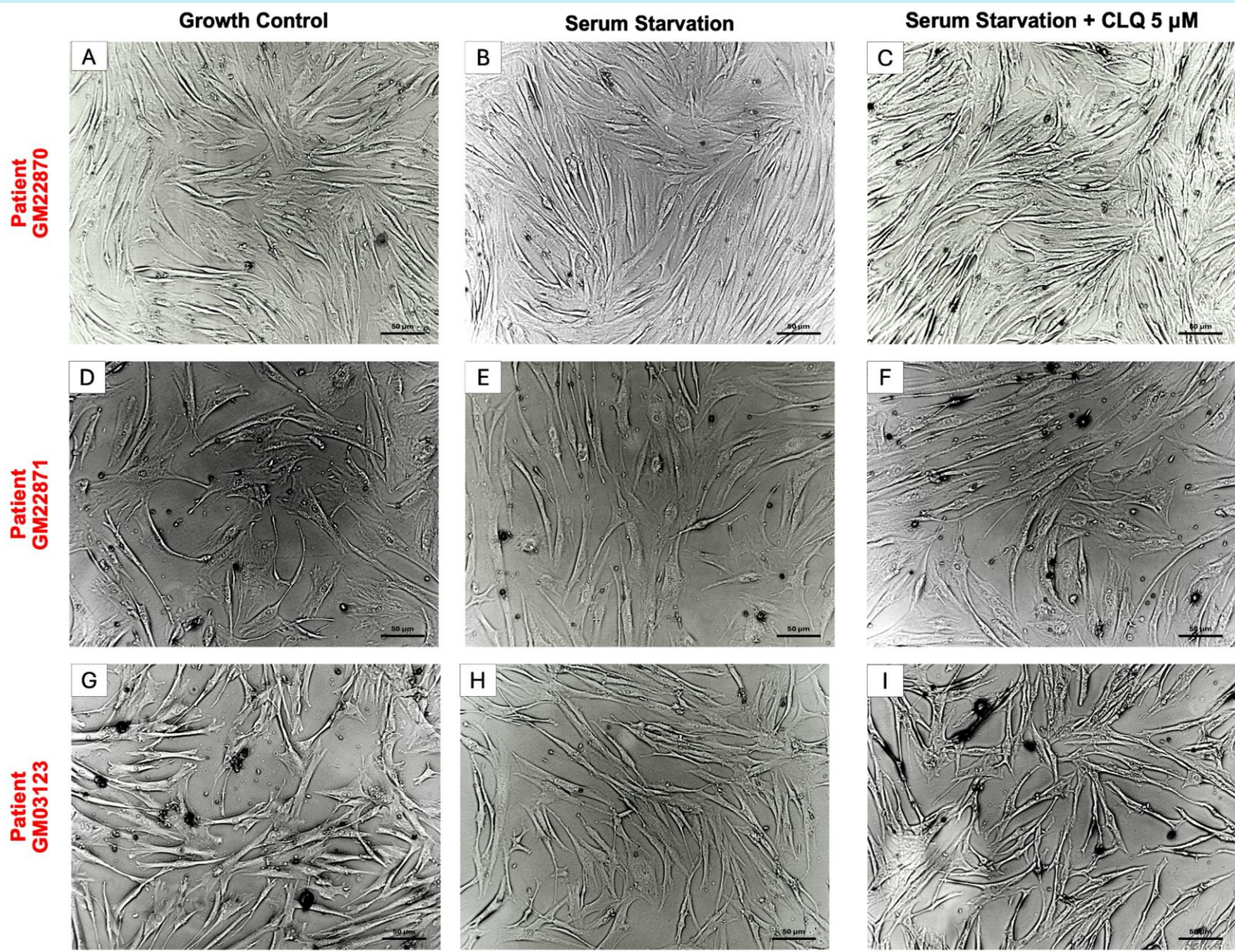
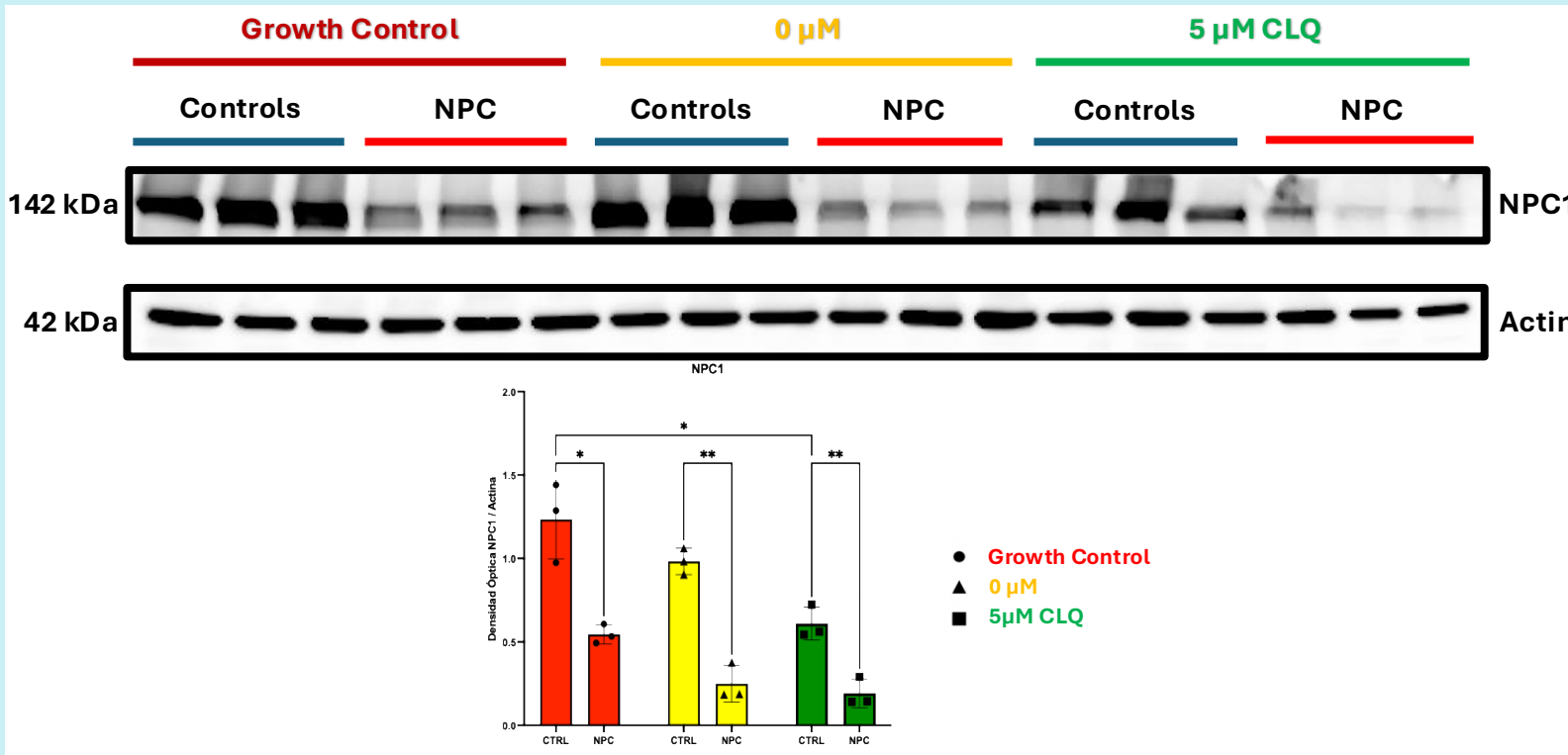


Figure 4. Fibroblast cells from three NPC patients in different conditions. In the left column is the growth control condition, the medium column represents the serum starvation condition, and the right column the serum starvation condition with a 5 μ M Chloroquine treatment. Phase contrast images. 10x objective. Scale bar equal to 50 μ m.

Fibroblasts Cells Treated with 5 μ M CLQ for 6 h

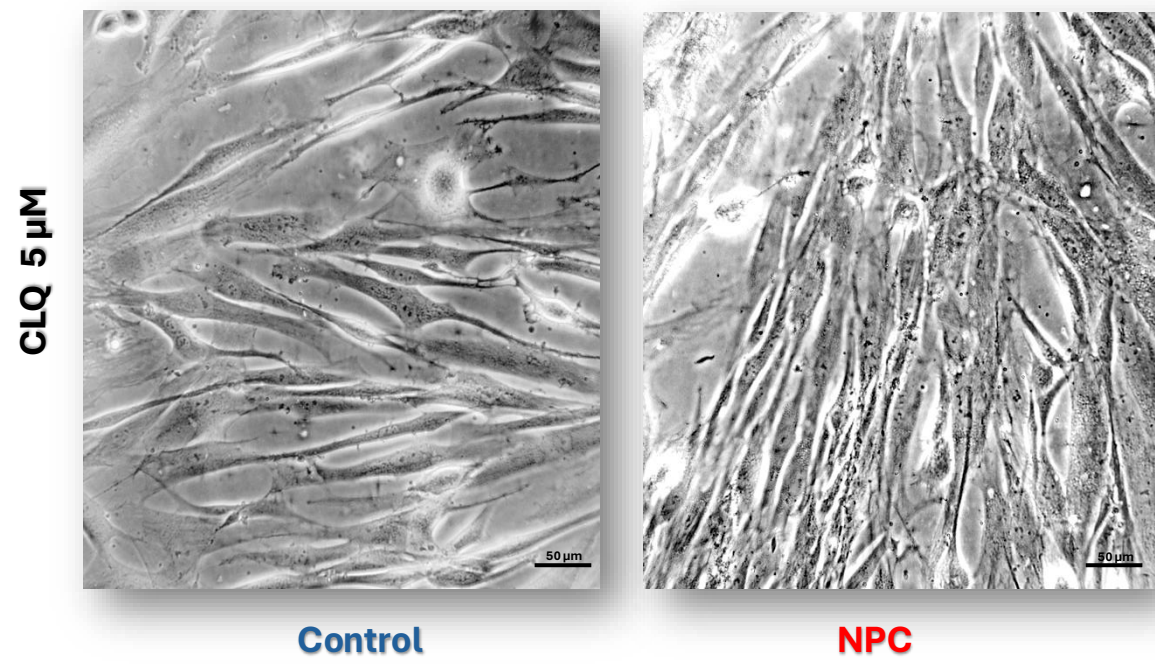


Figure 5. Fibroblast cells treated with Chloroquine (CLQ) for 6 hrs. On the left are the cells from the control subject, on the right are the cells of the NPC patient. Phase contrast images. 20x objective. Scale bar equal to 50 μ m.

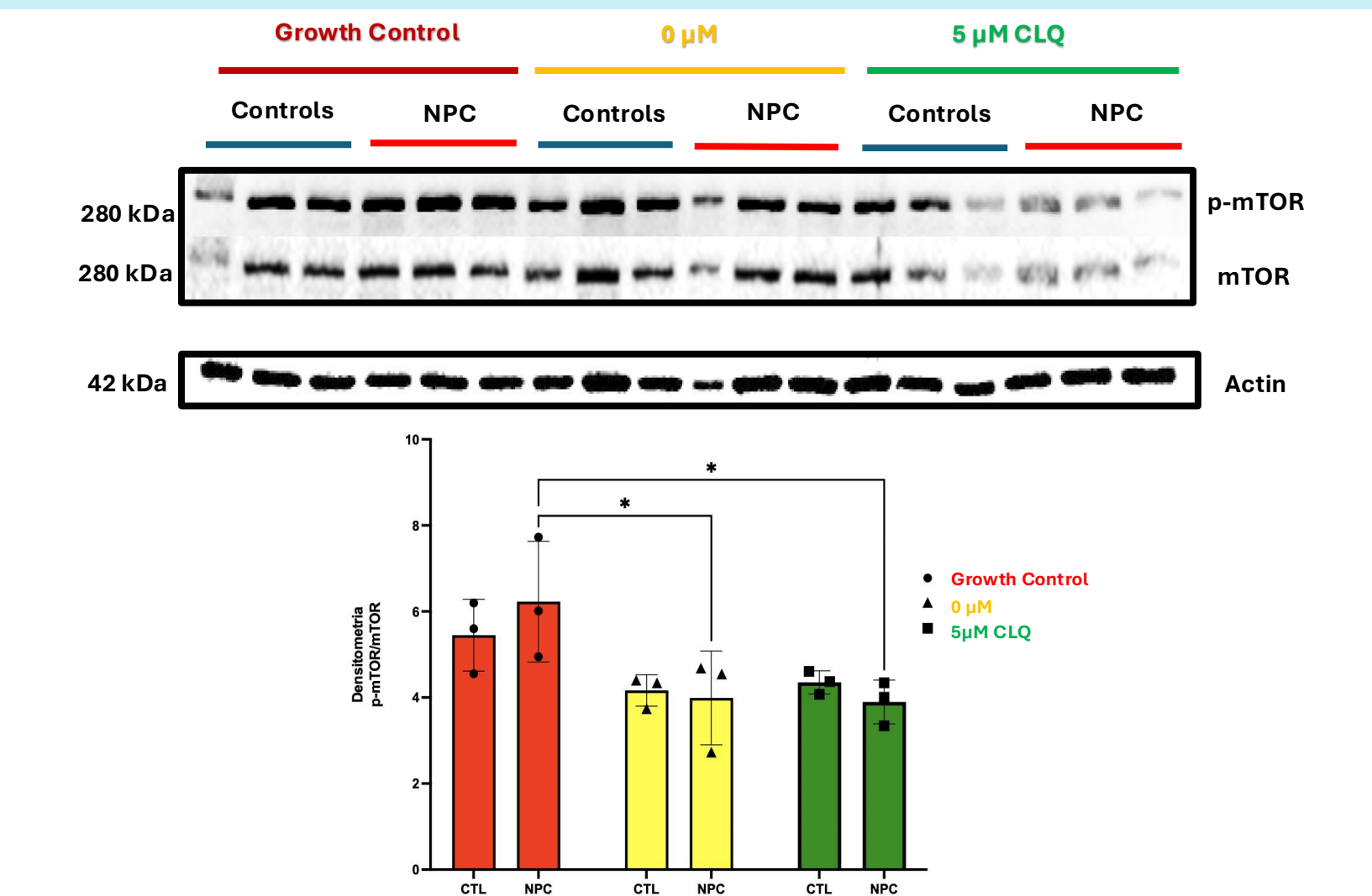


Figure 7. Immunoblot of p-mTOR in fibroblasts samples from healthy controls and NPC. Cell were cultured either on Growth medium (Growth control) or in medium without serum with or without Chloroquine (CLQ) from 0 or 5 μ M for 6 h. Actin detection was used as loading control. At the bottom of the figure is the densitometry of the Western blots.

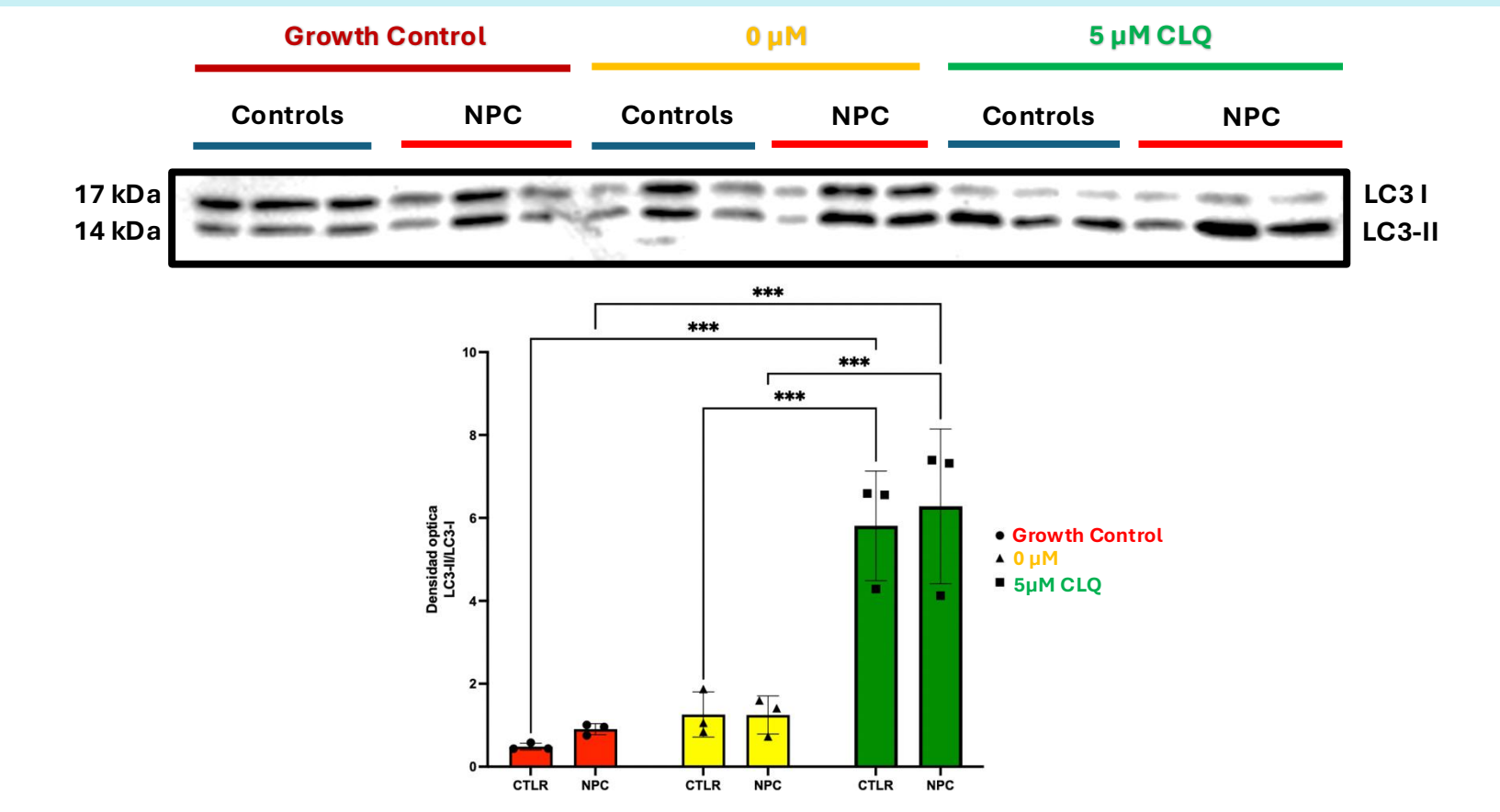


Figure 9. Immunoblot of LC3-II in fibroblasts samples from healthy controls and NPC. Cell were cultured either on Growth medium (Growth control) or in medium without serum with or without Chloroquine (CLQ) from 0 or 5 μ M for 6 h. Actin detection was used as loading control. At the bottom of the figure is the densitometry of the Western blots.

DISCUSSION

Fibroblast cultures from NPC patients exhibited a more pronounced accumulation of autophagic vesicles than controls, both under serum starvation and upon treatment with 5 μ M Chloroquine.

CONCLUSIONS

- NPC fibroblasts demonstrated increased sensitivity to autophagy induction during serum starvation, particularly when treated with Chloroquine, compared to healthy control fibroblasts; which resulted in a greater accumulation of potential autophagic vesicles.
- A lower expression of p-mTOR and mTOR was observed in fibroblasts from healthy subjects and NPC patients under starvation and starvation combined with a Chloroquine treatment.
- Skin fibroblasts from Niemann Pick patients are a good cellular model to study pathological processes associated with this neurodegenerative disease.

Acknowledgments

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Cyto-ID Assay in NPC and control Fibroblasts

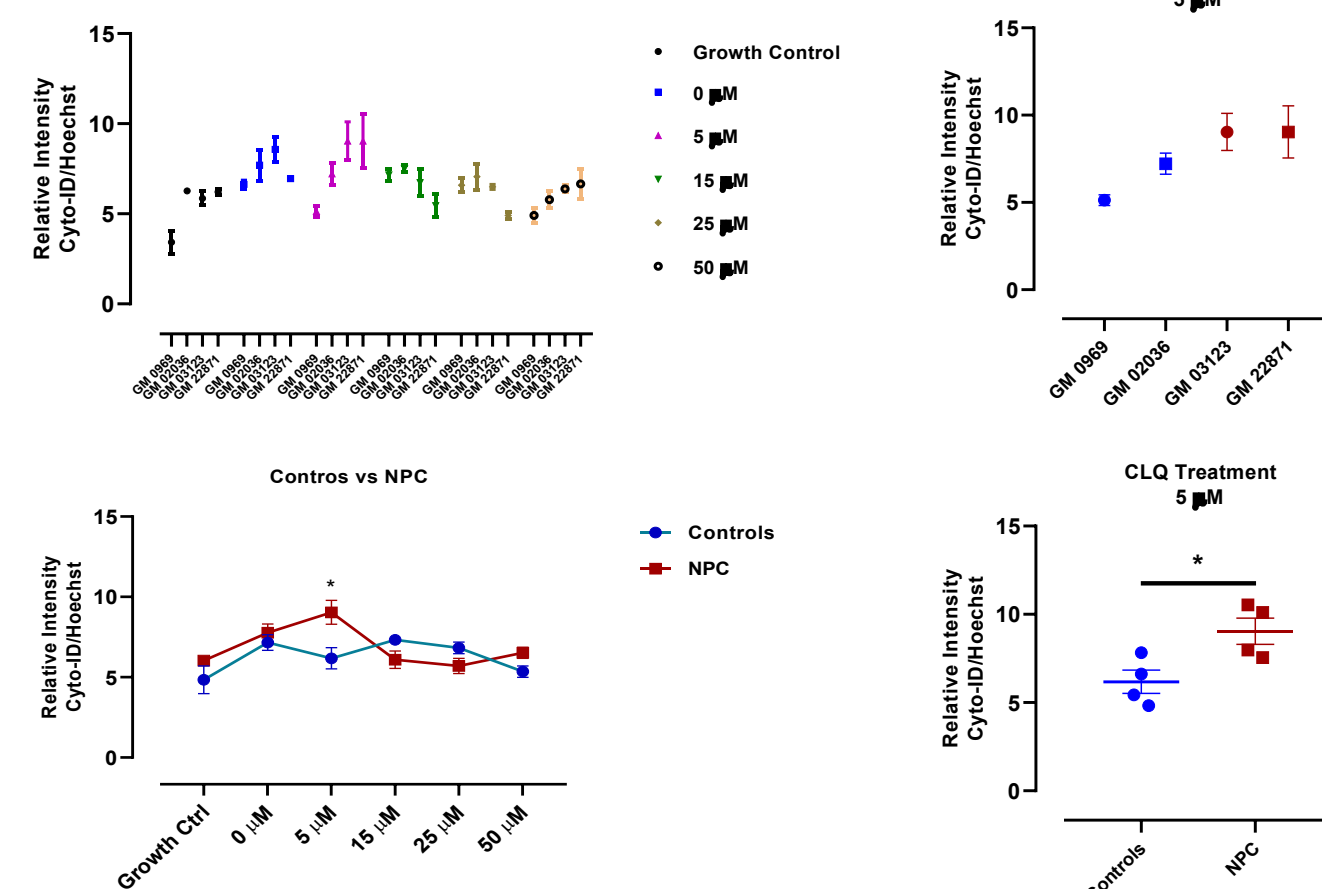


Figure 6. Autophagy detection with Cyto-ID in fibroblast cells from healthy controls and NPC patients. Cells were cultured either on Growth medium (Growth control) or in medium without serum with or without Chloroquine (CLQ) from 0 to 50 μ M for 6 h. Asterisks denote statistical significance between Healthy controls and NPC at 5 μ M of CLQ.

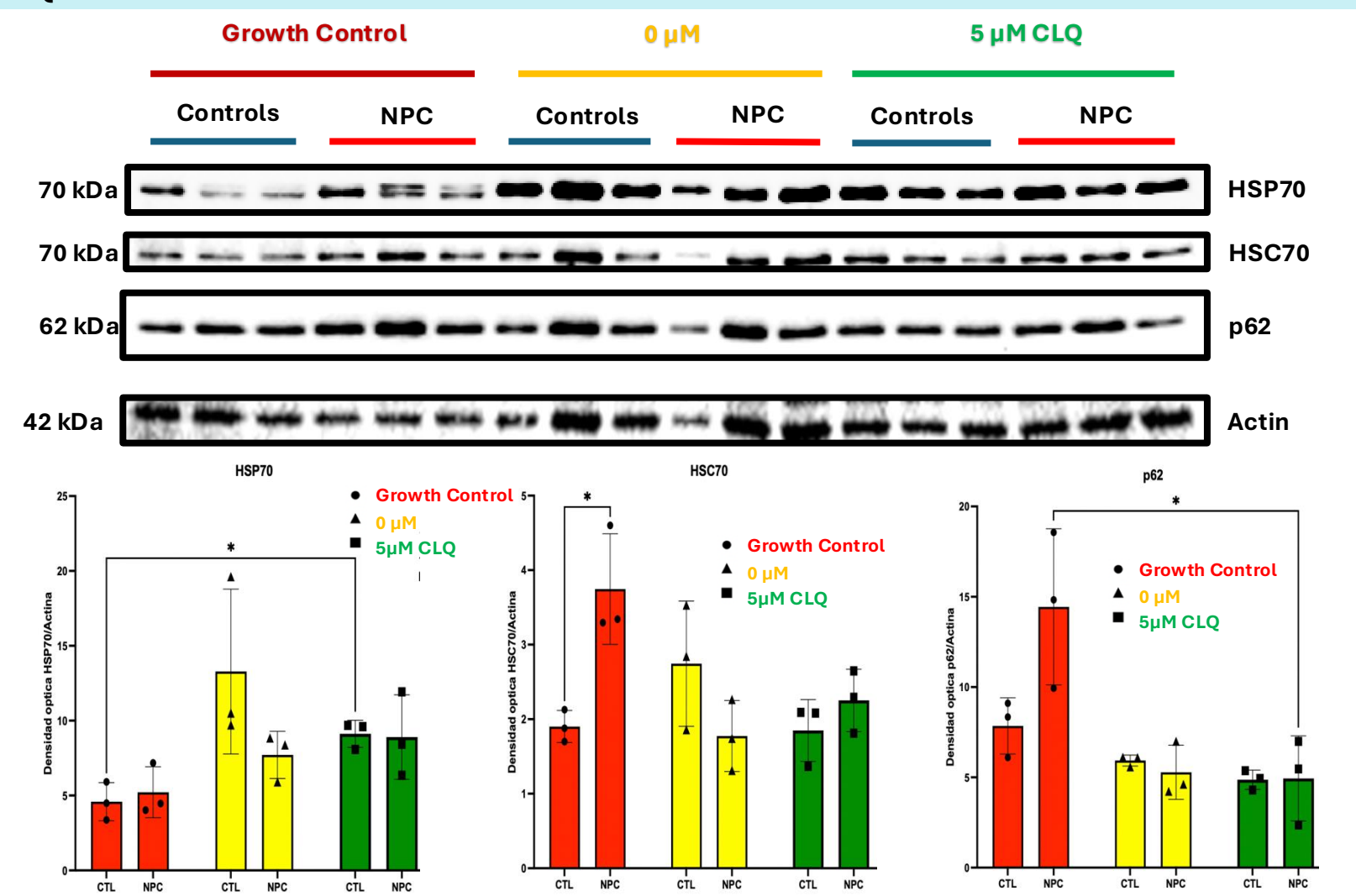
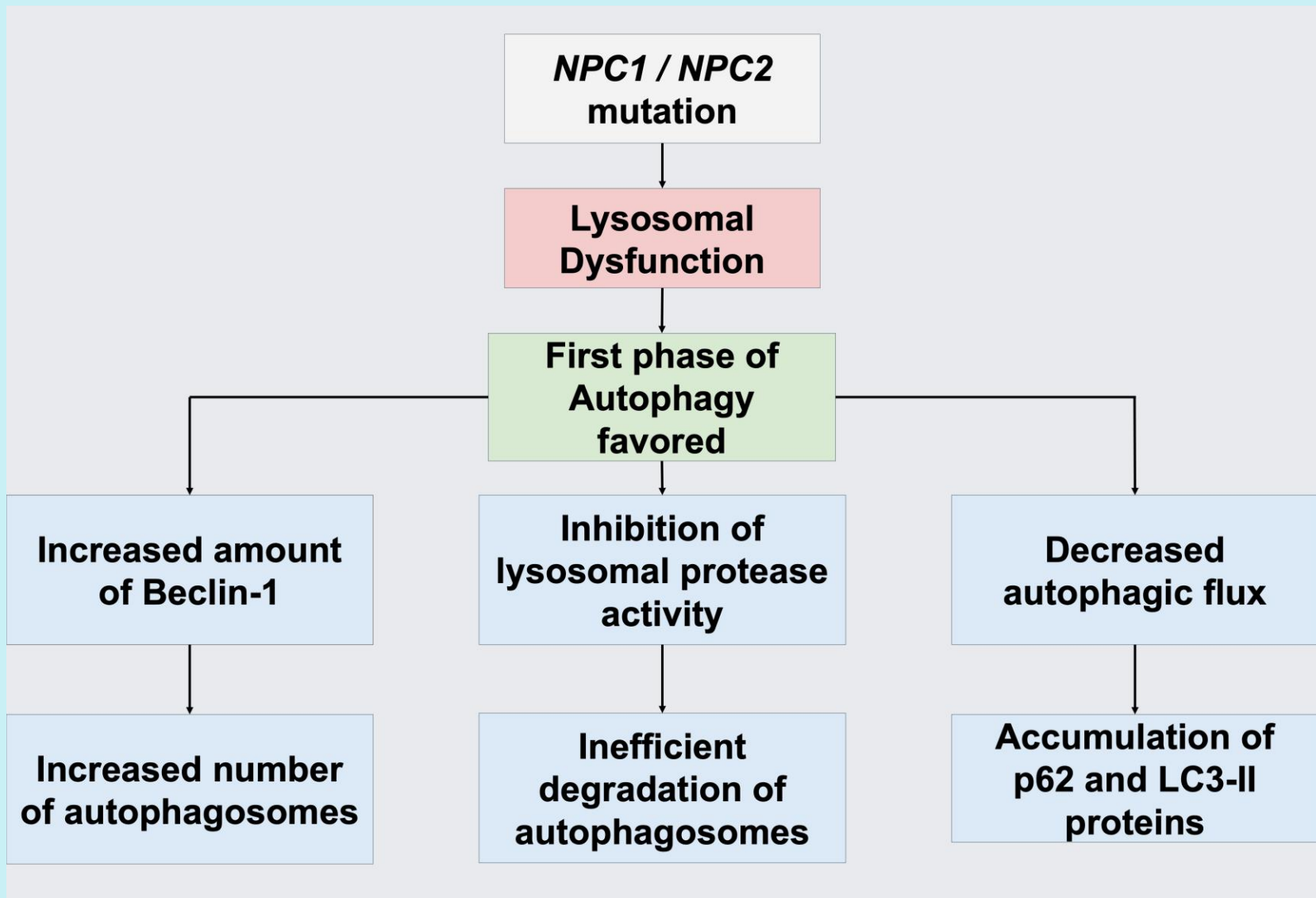


Figure 8. Immunoblots of HSP70, HSC70 and p62 (autophagy substrate) in fibroblasts samples from healthy controls and NPC. Cell were cultured either on Growth medium (Growth control) or in medium without serum with or without Chloroquine (CLQ) from 0 or 5 μ M for 6 h. Actin detection was used as loading control. At the bottom of the figure is the densitometry of the Western blots.



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