

Upon completing my BSc (Hons) in Biomedical Science at the University of Southampton, I had the opportunity to undertake a ten-week research project under the supervision of Dr John Parrington at the University of Oxford.

With a profound passion for drug discovery and development, in February 2024 I reached out to Dr John Parrington to explore opportunities for an internship in his lab. I was thrilled to discover a summer project that aligned perfectly with my interests and provided the chance to enhance my skills in cutting-edge laboratory techniques at such a prestigious institution. In May 2024, I was delighted to receive the news from the British Pharmacological Society of being granted a Vacation Studentship, an esteemed award that financially supported me throughout my stay in the lab, enabling me to fully immerse myself in the invaluable experience.

The original objective of my project was to culture melanoma cell lines B16 and CHL1 WT and two-pore channel 2 (TPC2) knockout for testing a new TPC2 agonist (SG-094) safety, toxicity and off-target effects through various assays. However, I encountered significant challenges in maintaining sterile technique, precise pipetting, and proper thawing procedures. I initially struggled with making mistakes and worked at a slower pace to prevent them from recurring. Nonetheless, through consistent practice, I was able to enhance my skills and build my confidence over time. Despite my best efforts and assistance from a fellow lab member, I faced difficulty in initiating cell growth as the cells appeared non-viable and unsuitable for the planned experiments. This setback prompted me to modify my experimental approach slightly. I shifted focus to utilising previously obtained protein samples from WT and TPC1 and TPC2 knockout B16 and CHL1 melanoma cell lines to investigate mTOR and MiTF expression. Initially, I familiarised myself with conducting a BCA protein assay to standardise protein concentration for western blot, ensuring uniform protein levels across all samples. Subsequently, I performed electrophoresis, protein transfer to a membrane, blocking, as well as application of primary and secondary antibodies. Regrettably, no significant results were obtained from this analysis (Figure 1).

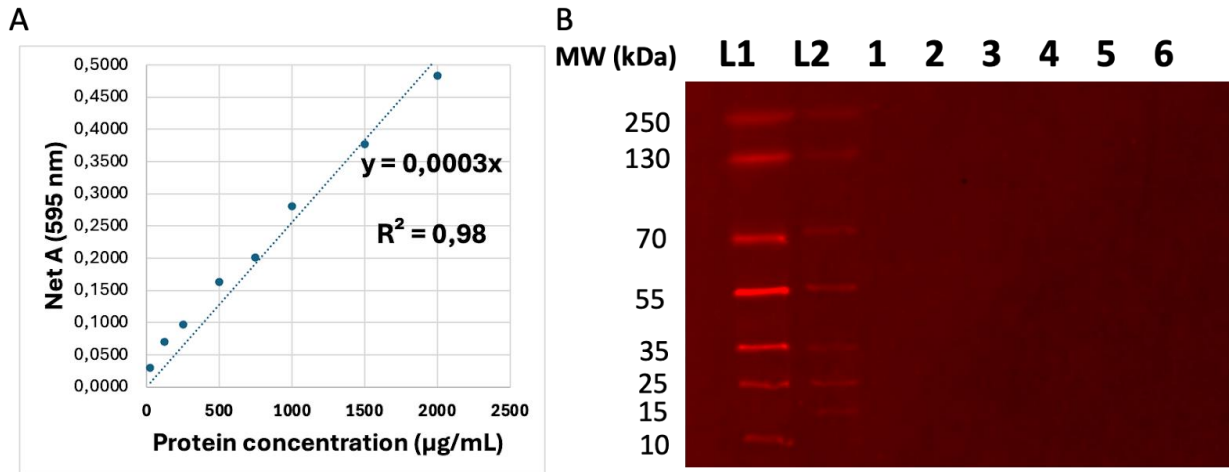


Figure 1. Sample results from BCA protein assay and western blot. (A) BCA protein assay to calculate the concentration of samples from B16 and CHL1 TPC1 and TPC2 knockout cell lines. (B) Western Blot analysis of protein samples from B16 TPC1 and TPC2 Knockout cell lines against MitF antibody. The PVDF membrane was scanned by an Odyssey® M Imaging System (LI-COR). L1 and L2 = PageRuler™ Plus Protein Ladder (Thermo Scientific™); 1 = Sample from B16 wild-type; 2 = Sample from B16 TPC1 knockout; 3 = Sample from B16 TPC2 knockout; 4 = Sample from CHL1 wild-type; 5 = Sample from CHL1 TPC1 knockout; 6 = Sample from CHL1 TPC2 knockout.

During my time in the lab, I was also involved in a separate project from a fellow lab member “PKC- α expression and immune regulation in U937 WT cell lines”. This collaboration enabled me to observe and gain expertise in suspension cell culture and extraction of cytosolic and membrane-bound proteins. I then independently conducted BCA assays and western blot analysis targeting PKC- α expression in the samples. Furthermore, I performed ELISA to measure IL-10 levels in U937 WT samples treated with novel TPC2 agonists from a German lab. Unfortunately, the western blot results were inconclusive, but I obtained intriguing results from the ELISA that necessitate additional analysis (Figure 2).

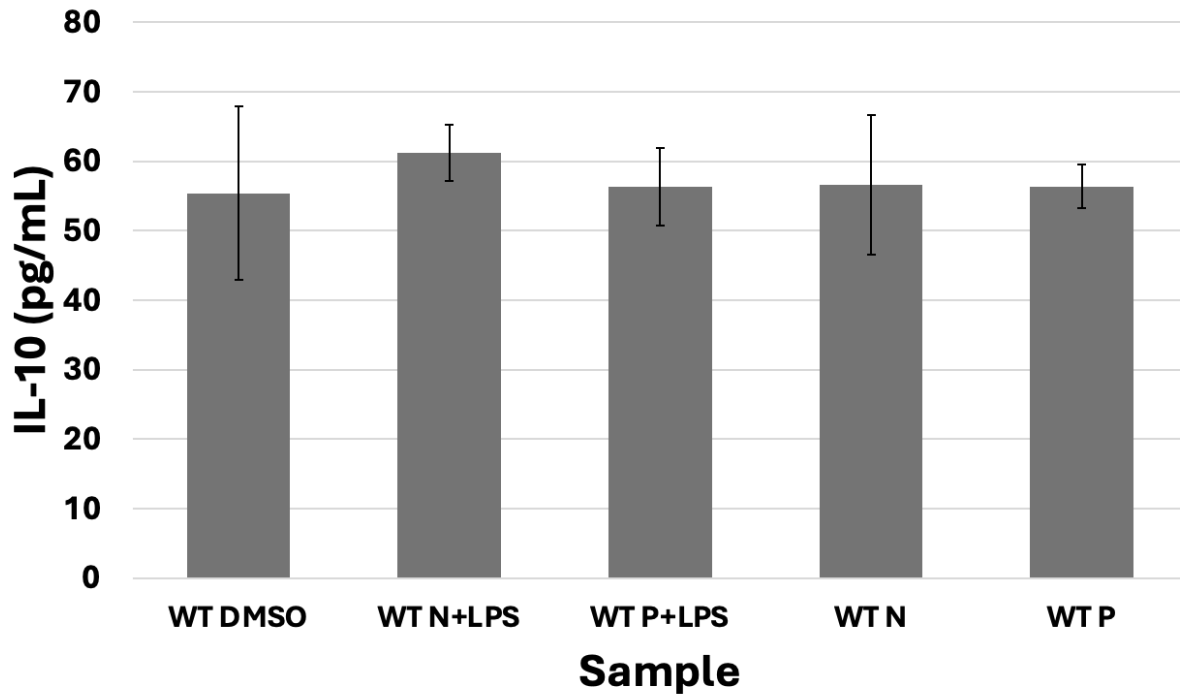


Figure 2. IL-10 levels of TPC2 agonist-treated WT U937 cell lines. WT DMSO = WT U937 treated with DMSO; WT N+LPS = WT U937 + LPS + TPC2-A1-N agonist; WT P+LPS = WT U937 + LPS + TPC2-A1-P agonist; WT N = WT U937 + TPC2-A1-N agonist; WT P = WT U937 + TPC2-A1-P agonist.

Overall, the experience I engaged in proved to be a multifaceted success. It afforded me the valuable opportunity to integrate into the vibrant and international research community at Oxford. This opportunity also enhanced my capacity to work independently, design and reshape experimental plans upon unexpected outcomes, as well as refine my technical expertise. Moreover, the limited yield of significant results allowed me to improve my skills in troubleshooting laboratory challenges at various stages. Consequently, I now stand as a more versatile and well-prepared researcher, equipped to tackle the array of research challenges throughout my future career.

I am currently pursuing a MSc in Biomedical Sciences at the University of Amsterdam, embarking on my first year of studies. While the nature of my upcoming programme will differ greatly from the research I carried out alongside Dr Parrington during the summer, the acquired soft skills and the technical proficiencies (e.g., cell culture, western blotting and ELISA) will undoubtedly drive forward my forthcoming research experiences.

ACKNOWLEDGMENTS

I would like to express my gratitude to the British Pharmacological Society for their financial support, which enabled me to dedicate myself fully to this project as well as join the unique research community at Oxford. I also extend my heartfelt thanks to Dr John Parrington for his invaluable assistance and support throughout the ten weeks.