High-Throughput Screening of Small Molecules in PLP-EGFP Schwann Cells to Identify Modulators of Myelination

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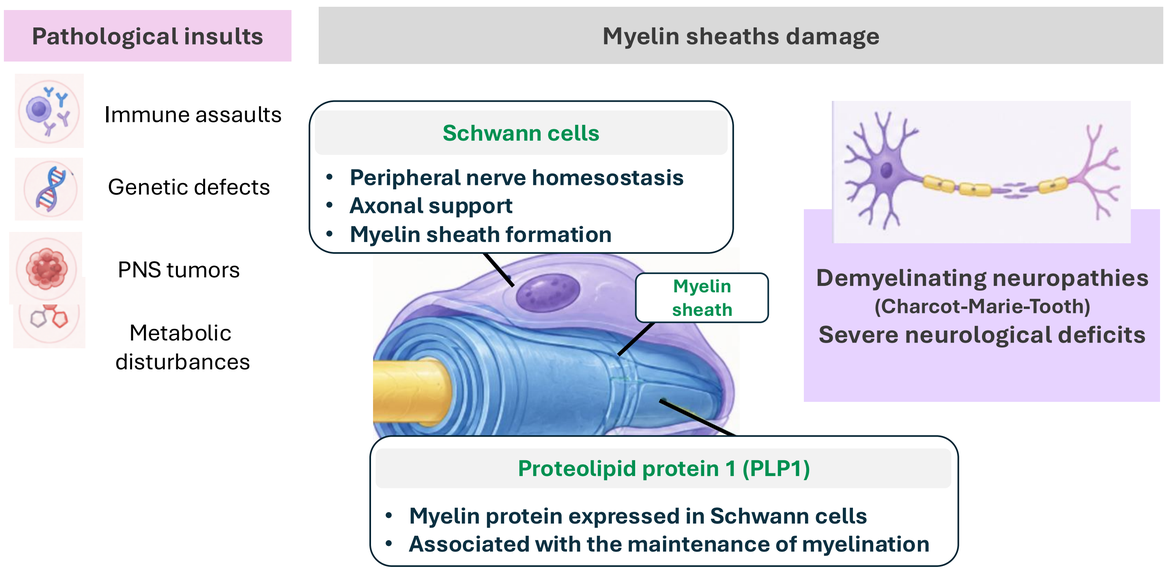
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1. INTRODUCTION

***Myelin sheath damage** caused by diverse pathological insults is an early event that contributes to disease progression and severe neurological deficits.*



The aim is the development of a high-content assay for the identification of ligands able of restoring Schwann cell function and myelination.

2. METHODS

Phenotypic high-throughput screen in primary Schwann cells

1 Schwann cell isolation and seeding



Transgenic mouse model
PLP-EGFP C57BL/6J reporter mice expressing GFP under *PLP1* promoter.



Schwann cell isolation & expansion
Schwann cells purified and expanded from transgenic mice (adapted from Kim & Kim, 2018).



Cell seeding
Spindle-shaped Schwann cells seeded at **5000 cells/well** in 384-well plates and incubated (**24h**, 37°C, 5% CO_2).

2 High-throughput screening



Compound screening
1120 compounds (Prestwick Chemical Library) screened at 10 µM with 0.1% DMSO.



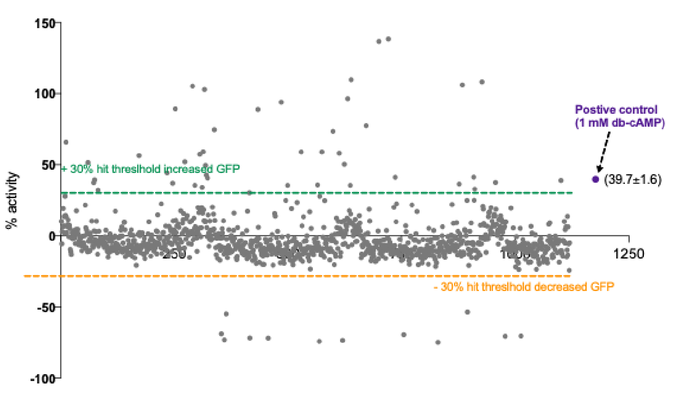
High-content imaging
Fluorescence images acquired on the Operetta CLS High-Content Analysis System.



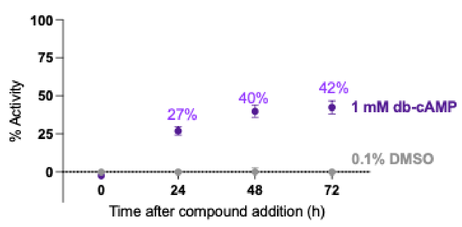
Fluorescence quantification
GFP intensity measured at **0, 24, 48 and 72h** after compound addition with Harmony software.

3. RESULTS

A. **56 of 1120 compounds showed ± 30% activity threshold at 48 h**



B. **1 mM db-cAMP increases GFP fluorescence from 24 h, reaching a plateau by 48 h**



C. **56 of 1120 compounds changed GFP fluorescence at 48 h**

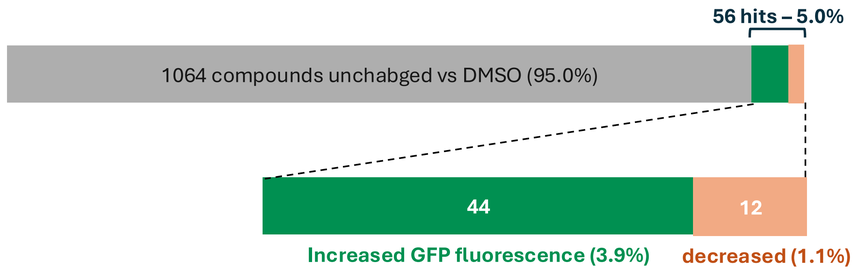


***Figure 1.* Hit selection from the primary screen.** A. Primary Plp1-EGFP Schwann cells were treated with 1120 compounds (Prestwick Chemical Library, 10 µM, 0.1% DMSO) and GFP fluorescence intensity was quantified 48 h after addition. B. Positive controls (1mM db-cAMP) for increased GFP fluorescence intensity were included in each plate. C. Compounds were classified relative to the DMSO vehicle control as increased (n=44, 3.9%), decreased (n=12, 1.1%) or unchanged (n=1064; 95.0%).

Compound 1 increases GFP fluorescence in primary Schwann cells

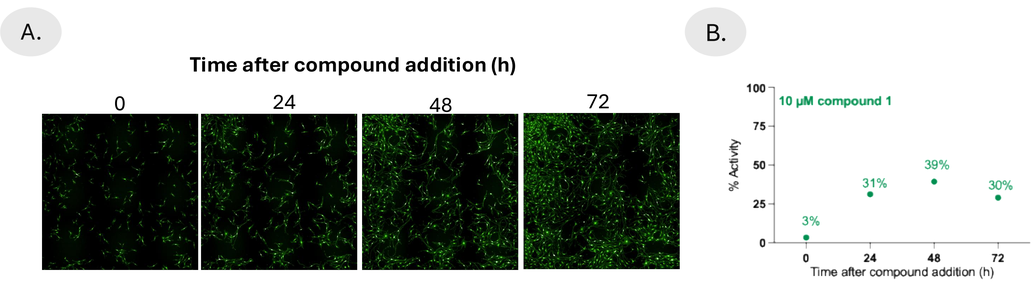


Figure 2. Representative fluorescence images (A) and quantification of GFP fluorescence intensity in primary Schwann cells following treatment with 10 µM compound 1.

Confirmed hits induce a concentration-dependent increase in GFP fluorescence

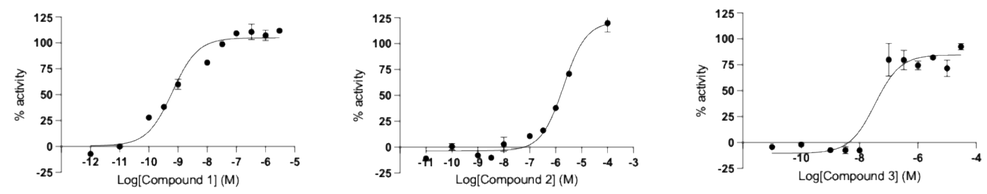


Figure 3. Concentration-response confirmation of three primary-screen hits. Plp1-EGFP Schwann cells were treated with serial dilution of compounds 1, 2 and 3 and GFP fluorescence intensity was quantified at 48 h and expressed as % activity relative to DMSO control.

4. CONCLUSIONS

- **A primary Schwann cell culture was developed as a physiologically relevant model for performing high-throughput screening.**
- **This platform provides a valuable tool for identifying compounds that enhance myelination and may be relevant to the treatment of peripheral neuropathies.**

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