Supplementary Figures

Bioactive lipid o-cyclic phytosphingosine-1-phosphate promotes differentiation of human embryonic stem cells into cardiomyocytes via ALK3/BMPR signaling

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Running Title: *cP1P induces ALK3/BMPR signaling during cardiomyocyte differentiation*

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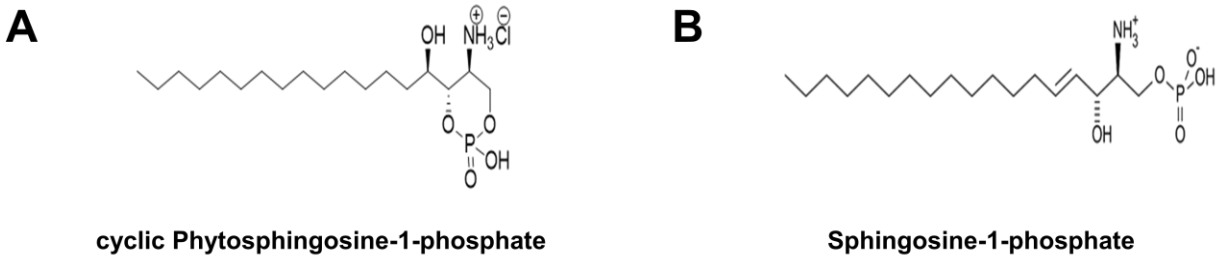
Table of Contents

SUPPLEMENTARY VIDEO S7: Movie representing the beating CM clusters at day 8 in control groups treated with LDN between days 3-5 of differentiation.

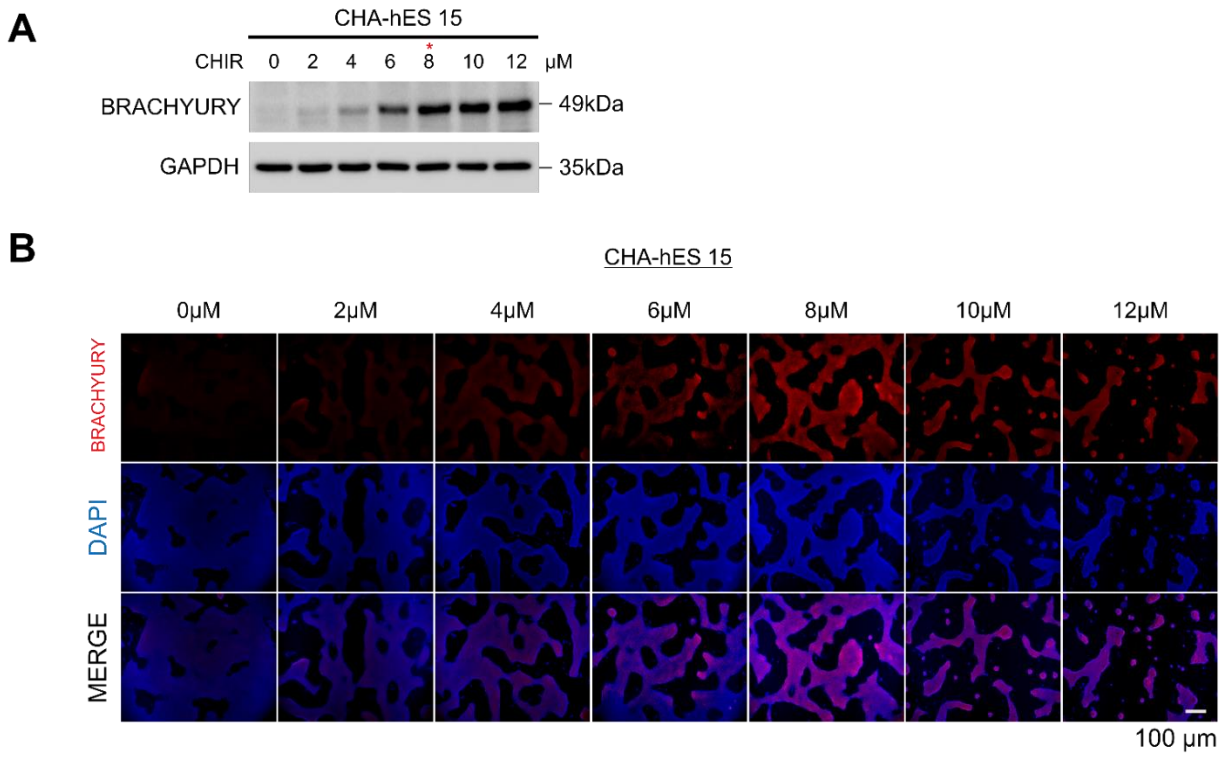
SUPPLEMENTARY VIDEO S8: Movie representing the beating CM clusters at day 8 in cP1P group treated with LDN between days 3-5 of differentiation.

SUPPLEMENTARY FIGURE S1. Chemical structures of cP1P and S1P.



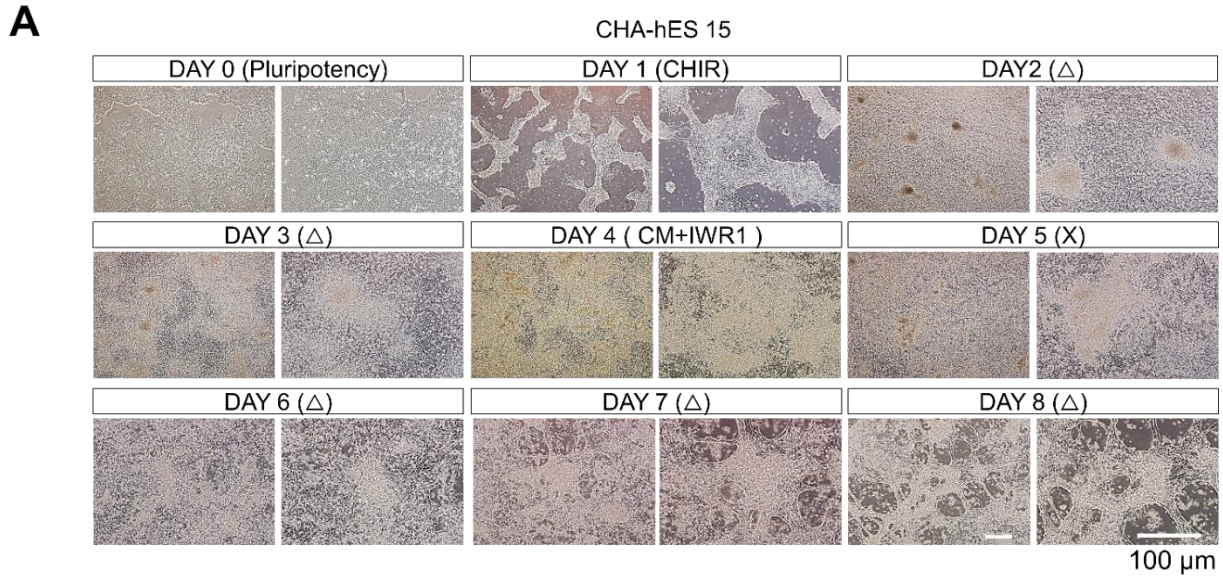
SUPPLEMENTARY FIGURE S1: Chemical structures of **(A)** cP1P and **(B)** S1P.

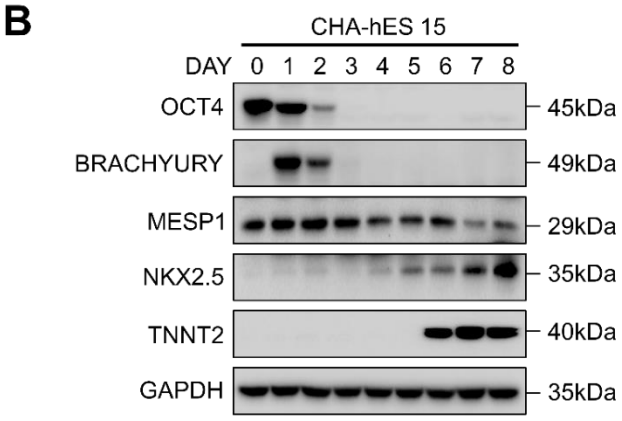
SUPPLEMENTARY FIGURE S2. Optimization of CHIR concentration for efficient mesoderm induction.



SUPPLEMENTARY FIGURE S2: Optimization of CHIR concentration for efficient mesoderm induction. **(A)** Immunoblotting and **(B)** immunofluorescence analysis for the mesodermal marker BRACHYURY were conducted at a range of CHIR concentrations (0-12 μM) in hESCs at day 1 of mesodermal induction, Scale bar, 100 μm.

SUPPLEMENTARY FIGURE S3. Characterization of hESC-derived cardiac differentiation.

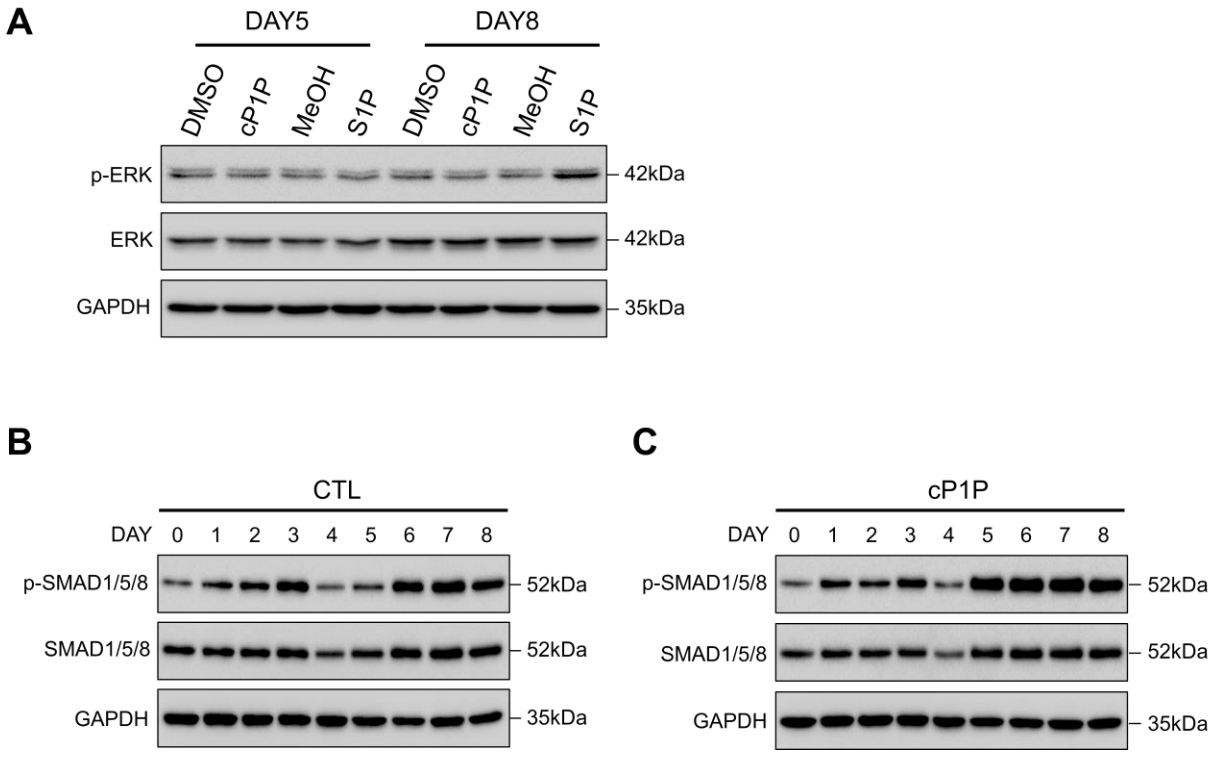




SUPPLEMENTARY FIGURE S3: Characterization of hESC-derived cardiac differentiation. **(A)** Phenotypic overview of hESC-derived CMs during cardiac differentiation from days 0 to 8. Differentiation started when the confluence of hESCs reached 80–90% (Day 0). The morphology of the hESCs were analyzed visually to identify homogeneous colonies with clear borders that showed no obvious differentiating zones. hESCs were treated with 8 μM CHIR for 24 h on day 1 followed by treatment with IWR1

on day 3 for 48 hours. Beating islands of cardiomyocytes were observed to cover maximum spaces by the 8^{th} day of differentiation. Pictures were taken using an Olympus IX71 inverted microscope equipped with an Olympus DP70-IFAD camera. Scale bars=100 µm. **(B)** Immunoblots for the expressions of specific proteins during different stages of cardiac differentiation.

SUPPLEMENTARY FIGURE S4. Immunoblots to analyze the expression profiles of key signaling pathway intermediates during cardiac differentiation.



SUPPLEMENTARY FIGURE S4: Immunoblots to analyze the expression profiles of key signaling pathway intermediates during cardiac differentiation. **(A)** Immunoblot to analyze the ERK signaling pathway upon the treatment with cP1P or S1P at days 5 and 8 of cardiac differentiation. Immunoblot to analyze the SMAD signaling pathway upon the treatment with **(B)** DMSO and **(C)** cP1P during different stages of cardiac differentiation.

SUPPLEMENTARY FIGURE S5. The original uncropped images for immunoblots in the main figures.

Figure 1B.

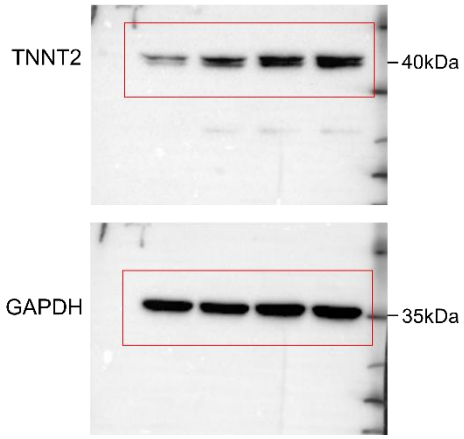


Figure 2E.

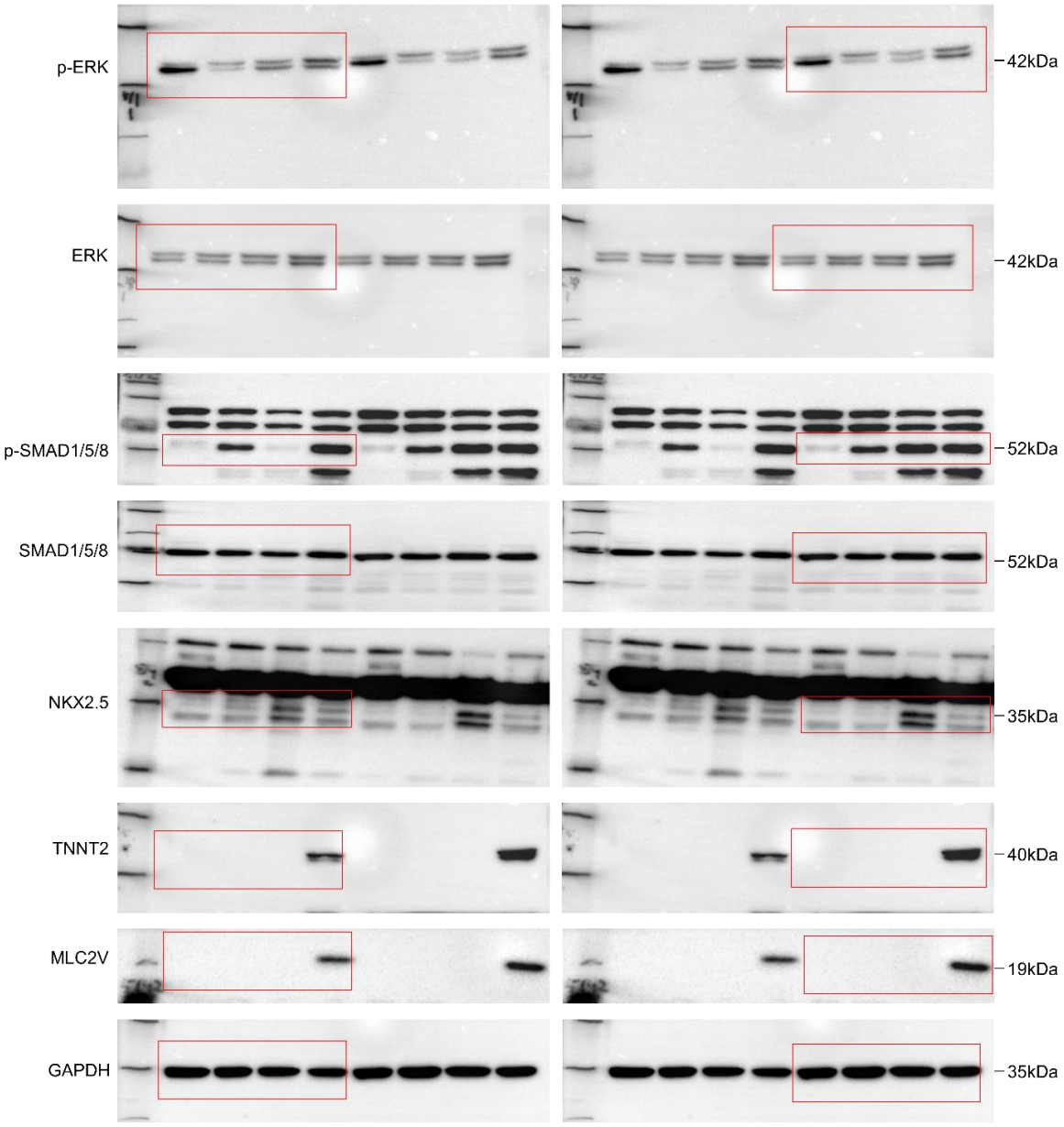

p-ERK
42kDa
ERK
42kDa
p-SMAD1/5/8
52kDa
SMAD1/5/8
52kDa
NKX2.5
35kDa
TNNT2
40kDa
MLC2V
19kDa
GAPDH
35kDa

Figure 3A.

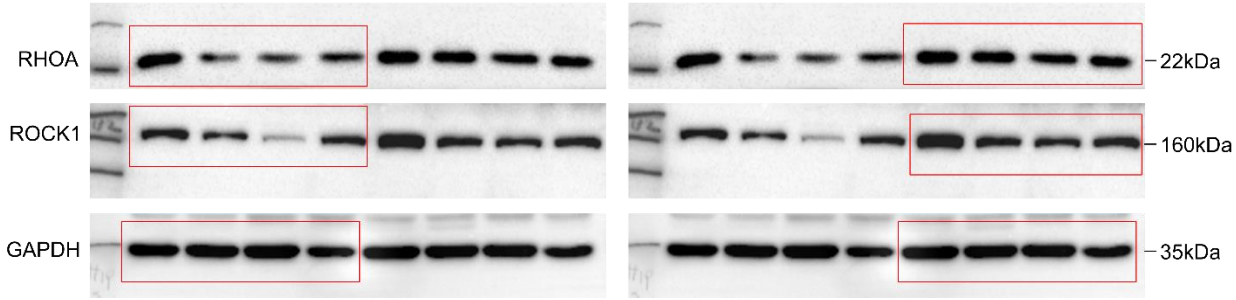


Figure 3C

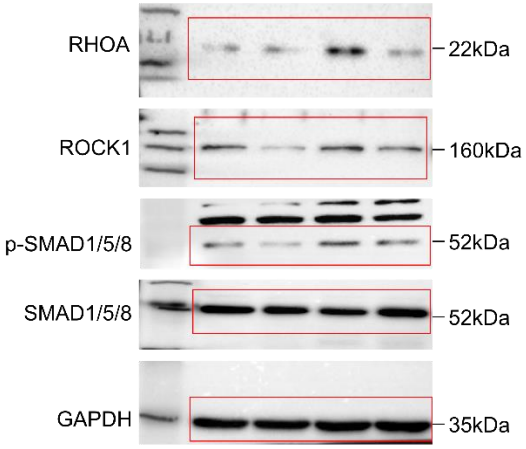
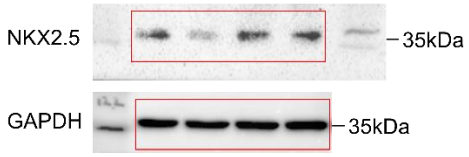


Figure 3D.



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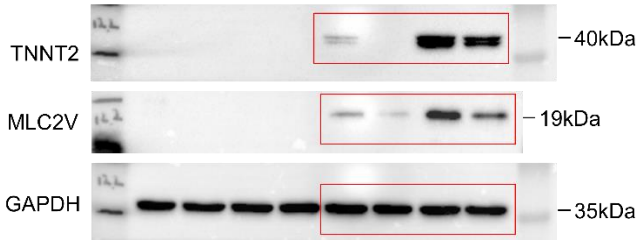


Figure 3G.

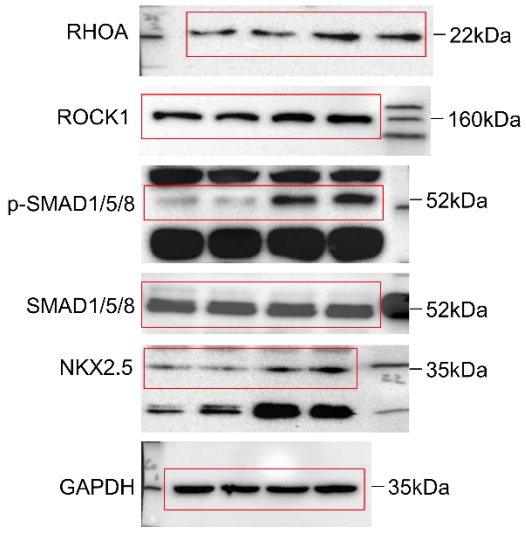


Figure 3H

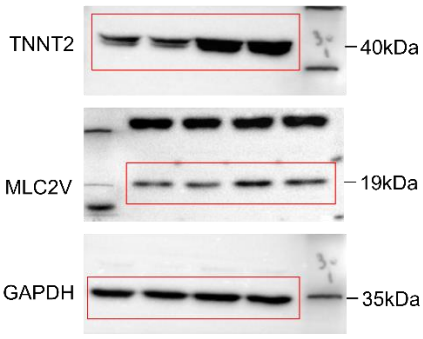


Figure 4A.

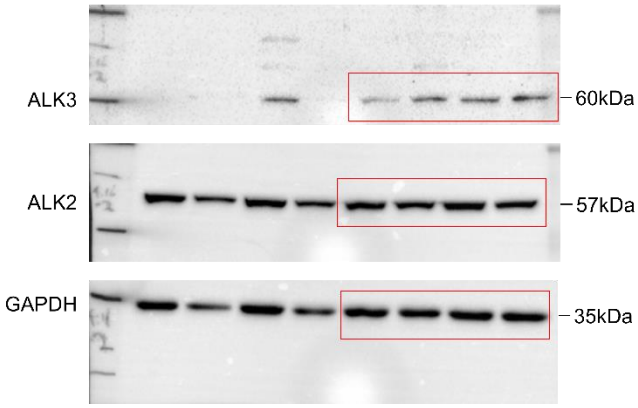


Figure 4C

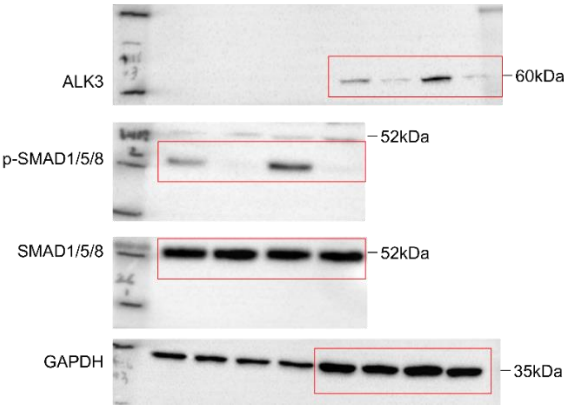
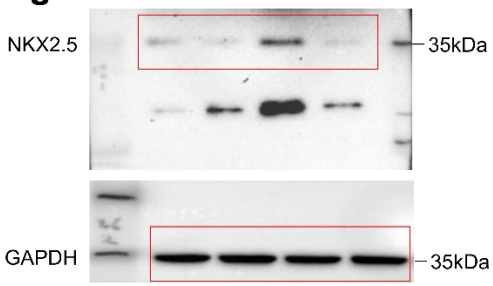


Figure 4D



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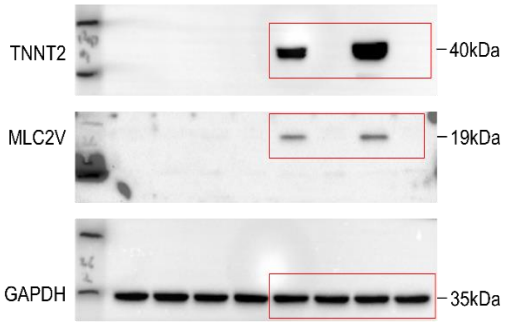

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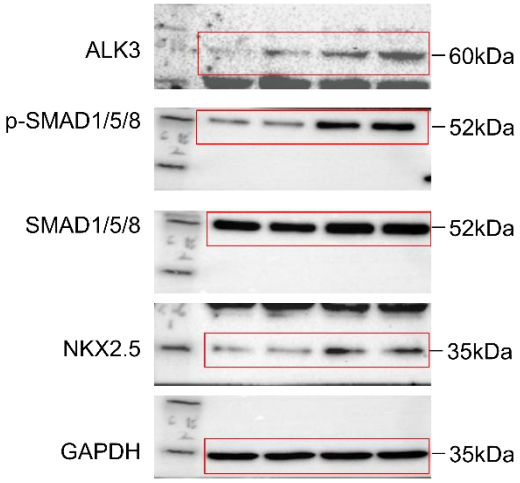

Figure 4H.