Unravelling the mechanisms regulating embryonic epicardial cell proliferation

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INTRODUCTION

Epicardial development is a highly complex process that relies on the precise coordination of cell proliferation and differentiation. The epicardium originates from an extracardiac cluster of cells, the proepicardium, which initially migrates to the myocardium and then massively expand to form the epicardial epithelial layer. Finally, some epicardial epithelial cells transform into mesenchymal cells via Epithelial-to-Mesenchymal Transition (EMT), and progressively invade the myocardial walls.

The regulatory signals that govern epicardial cell proliferation remain largely unknown. To unveil the molecular signals involved in controlling epicardial cell proliferation, we examined the proliferation status of epicardial cells at different embryonic stages, and performed an RNA-seq analysis to identify candidate signalling pathways operating within the proepicardium. Then, we conducted *in vivo* and *in vitro* research to carefully dissect epicardial proliferation. Our results show that both canonical and non-canonical Wnt signals are involved in the regulation of epicardial proliferation during embryonic development.

RESULTS

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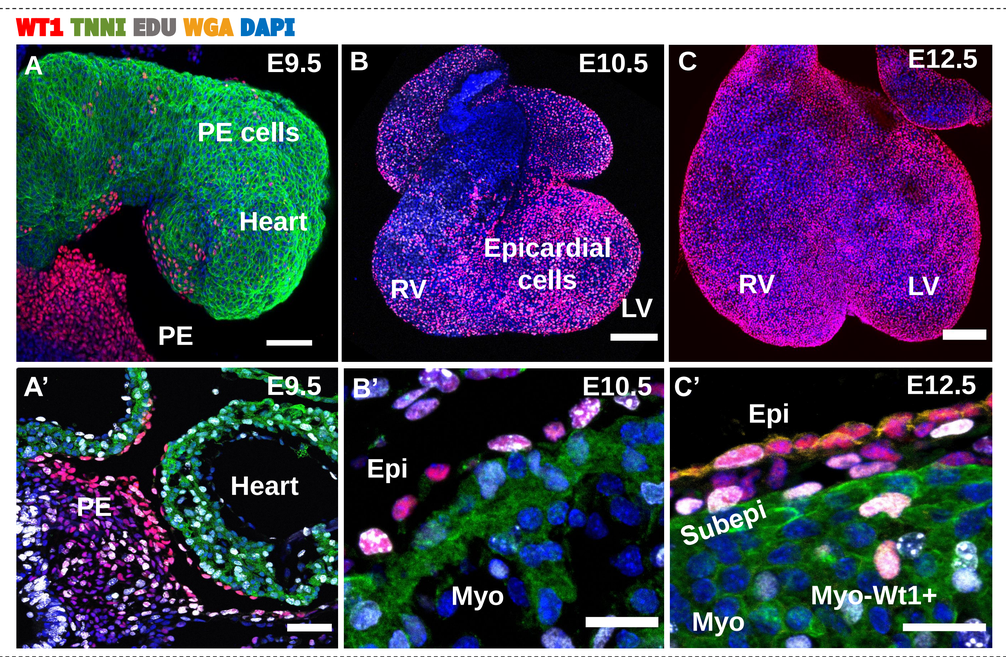


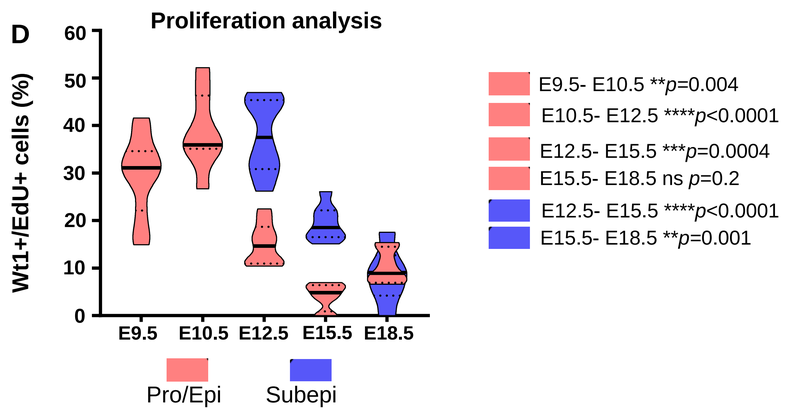
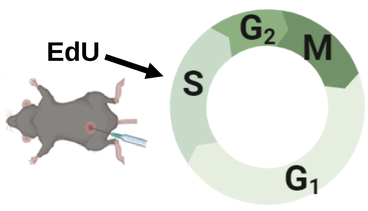


Figure 1: **Epicardial cells exhibit a transmural (epicardium-subepicardium-myocardium) proliferation gradient, as shown by EdU incorporation (S-phase of the cell cycle).**

PE, proepicardium; Epi, epicardium; LV, left ventricle; RV, right ventricle; Myo, myocardium

Scale bars: 200 µm (B, C), 100 µm (A, A'), 20 µm (B', C')



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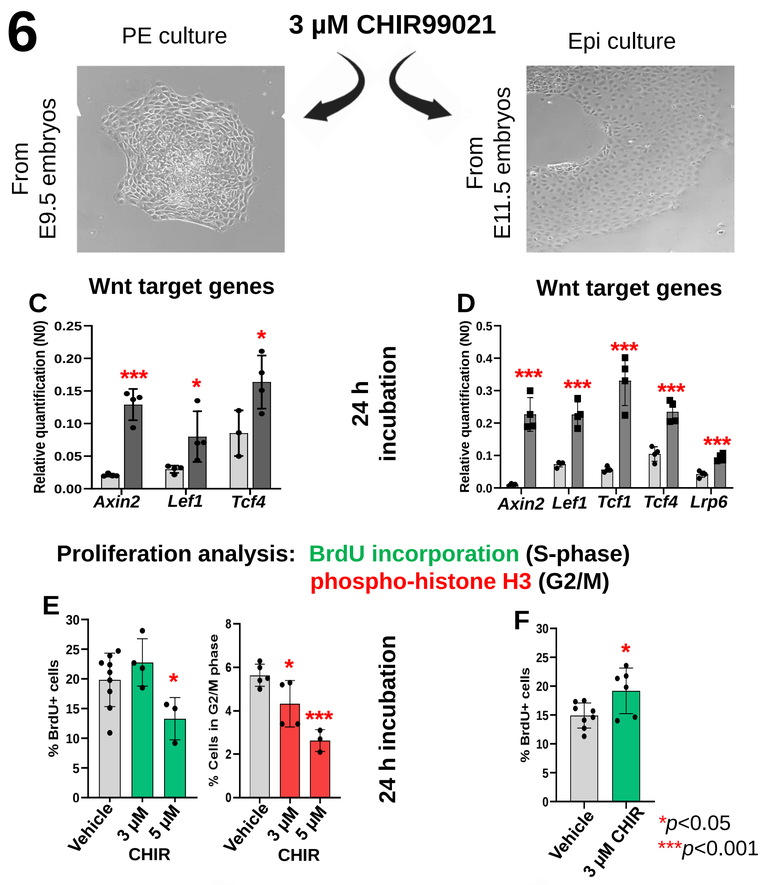
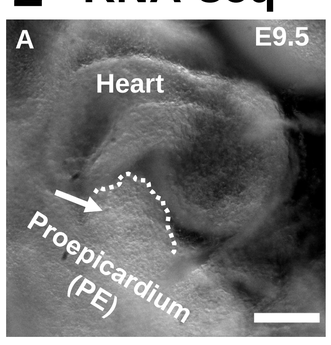


2 RNA-seq



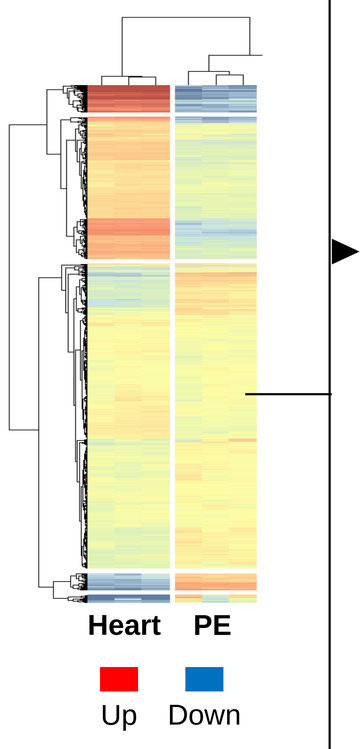


Figure 2: **Comparative transcriptional profiling of the proepicardium and heart tube tissues reveals a prominent proepicardial Wnt signalling gene signature, including an upregulation of *Tcfs*, *Lef1* and *Jun* genes, along with a downregulation of the *Wnt5a* ligand in the proepicardium**. Scale bars: 200 µm (A).

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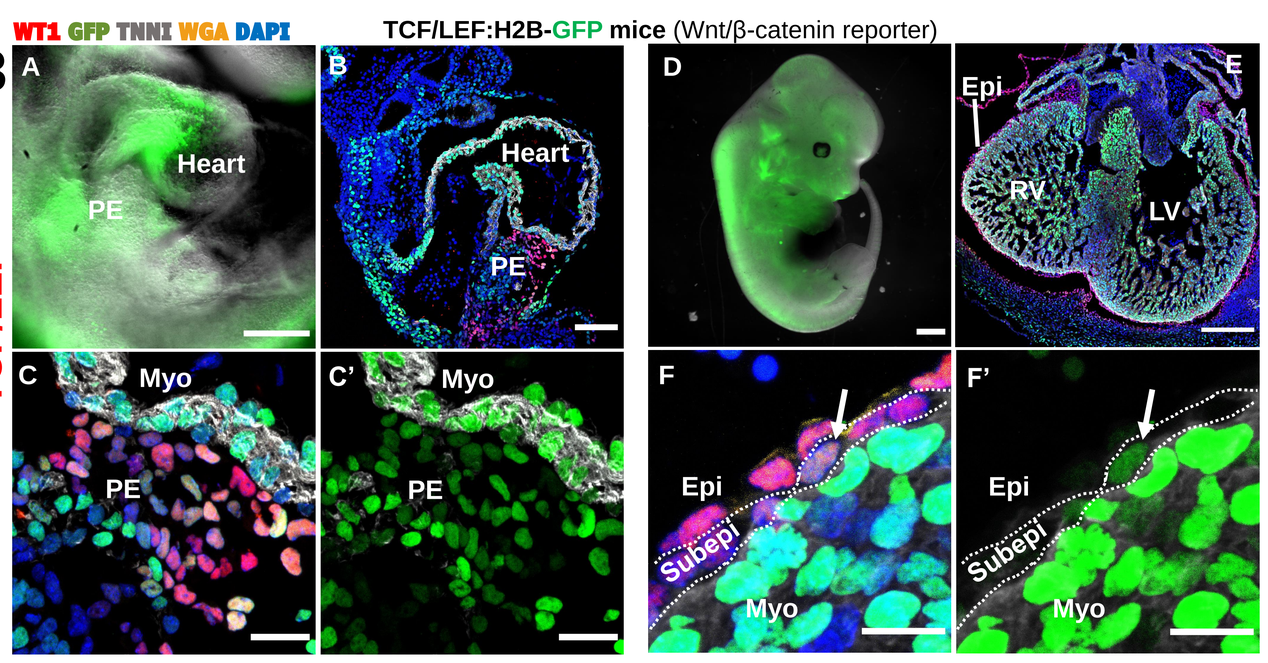

Figure 3: **TCF/LEF:H2B-GFP mice line demonstrates Wnt/β-catenin activity (GFP) in both proepicardial cells (E9.5 stage) and subepicardial cells (E12.5 stage).** Scale bars: 1000 µm (D), 250 µm (A, E), 100 µm (B), 50 µm (C, C'), 15 µm (F, F').

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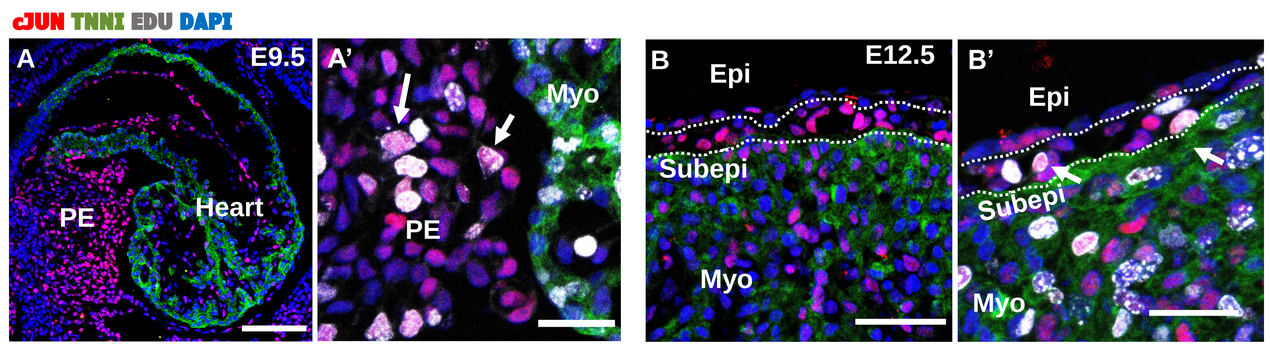

Figure 4: **cJun expression is conspicuous in proepicardial cells and cells within the subepicardium, showing a strong correlation with proliferative cells**. Scale bars: 100 µm (A), 50 µm (B), 25 µm (A', B')

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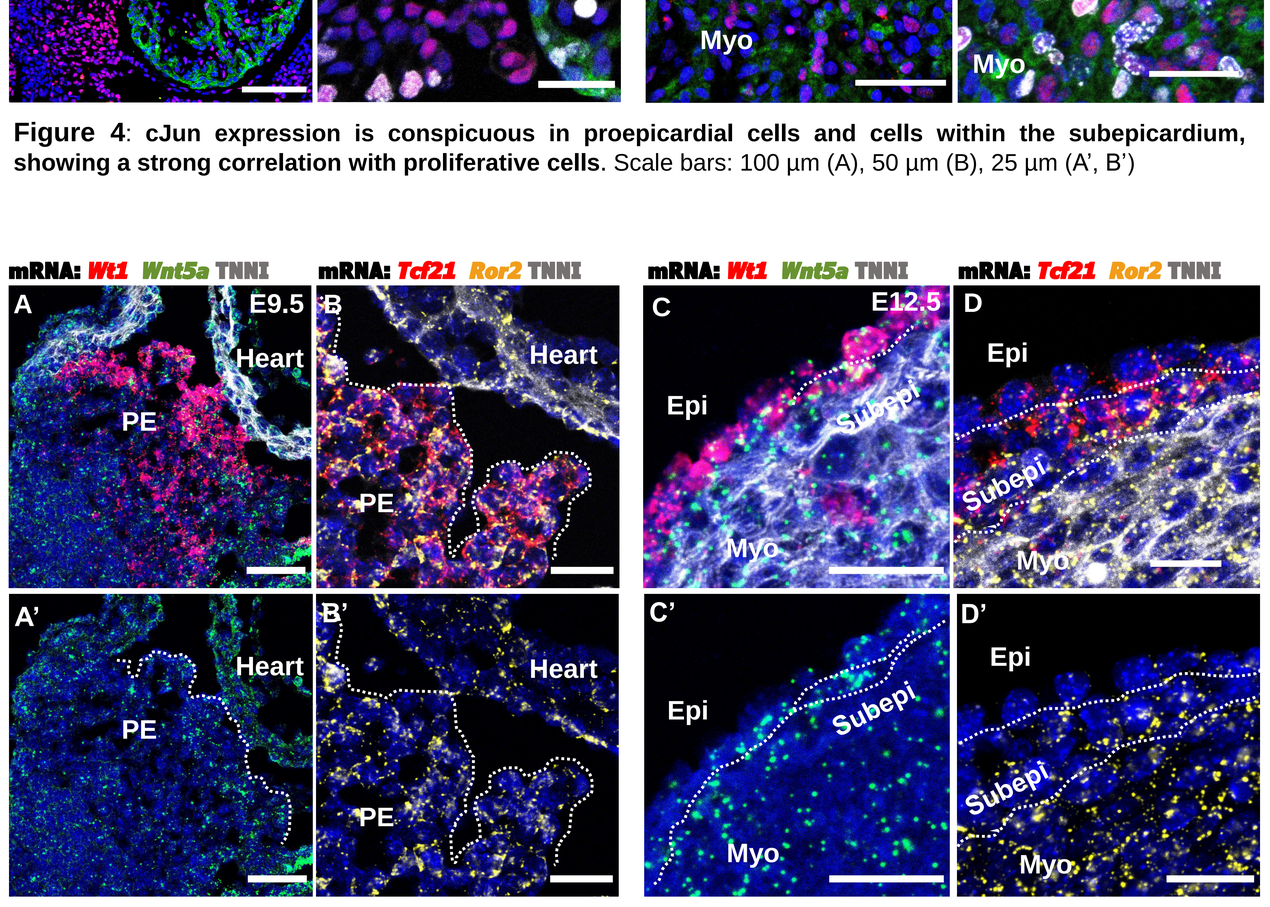
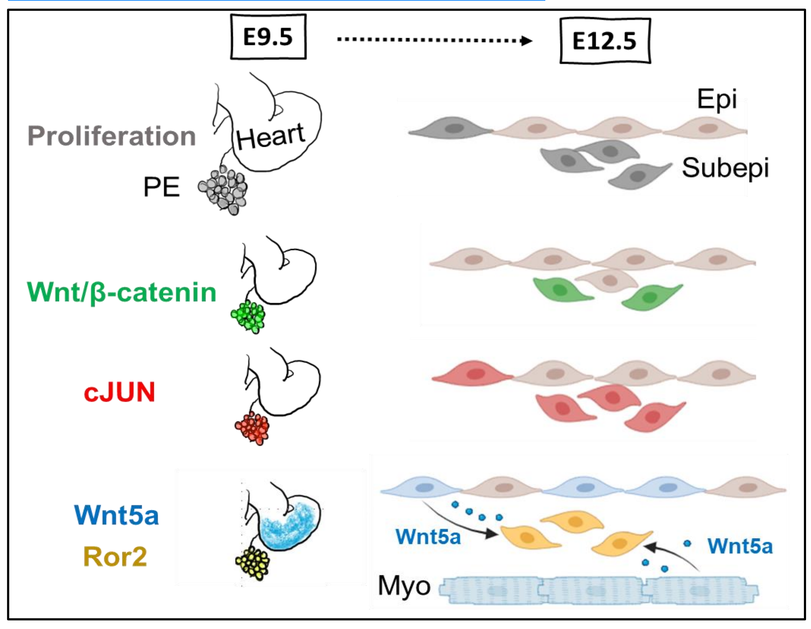


Figure 5: ***Wnt5a* is primarily expressed in the myocardium and epicardial cells at E12.5 as shown by RNA-scope analysis. Its main receptor *Ror2* is expressed in proepicardial cells and particularly in epicardial-derived subepicardial cells**. Scale bars: 100 µm (A), 50 µm (B), 25 µm (A', B'), 20 µm (C-D')

Figure 6: **Primary cultures of proepicardia and primitive epicardial cells, incubated with CHIR. This canonical Wnt agonist modulates cell proliferation via β-catenin signalling, whereas non-canonical Wnt5a enhances cell proliferation via cJun**. Scale bars: 50 µm (G-J').

CONCLUSIONS



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