## Functional heterogeneity in the developing zebrafish epicardium

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## Introduction

The epicardium is a layer of cells enveloping the heart muscle and plays a vital role during heart development and regeneration<sup>1</sup>. The transcription factors *tbx18*, *tcf21* and *wt1b* have often been used interchangeably to identify epicardial cells in the zebrafish<sup>2-4</sup>. However, work in our lab showed that the developing zebrafish epicardium expressed these markers in a heterogeneous manner (Figure 1). To better profile epicardial heterogeneity, we performed Smart-seq2 based single cell RNA sequencing and identified three subpopulations of epicardial cells (Epi1-3) that were distinct in their transcriptomic profile and localization in the heart. Functional perturbation uncovered roles for Epi1-3 markers both within the epicardium and in the interplay between epicardial cells and other cardiac cell types. Our aim is to better understand epicardial form and function during zebrafish heart development as it may inform on the reactivation of the epicardium during adult heart regeneration.

## The developing zebrafish epicardium is heterogeneous

Figure 1: 5dpf tcf21tbx18+ wt1b+ tcf21+ tbx18+

Tg(tbx18:myr-eGFP; tcf21:myr-tdTomato; wt1b:H2B-Dendra2)

Heterogeneity in the zebrafish epicardium at 5 days post fertilization (dpf). V = ventricle, BA =bulbus arteriosus. Scale bars are 50µm (**A**,**A**'), 10µm (**A**", **A**"").



The larval zebrafish epicardium consists of multiple cell 2 populations (Epi1-3) with distinct gene expression patterns



## Loss of Epi1-3 markers disrupts epicardium formation and 4 epicardial interactions with other cell types in the heart Tg(tbx18:myr-Citrine; tcf21:H2B-Dendra2) Α *tcf21* positive tgm2b Epi1 Control, 5dpf **C** KO *tgm2b*, 5dpf B • mutant 1 (38bp ins) • mutant 2 (7bp del + 1bp sub) KO tgm2b Control 2 4 6 8 10 Log2 tgm2b"2b" Ε sema3fb *Tg(tbx18:myr-Citrine)* Celle 50 Control, 5dpf **G** KO sema3fb, 5dpf Epi2 tbx BA 30 BA of .**⊆** 20 BA Numbe KO sema3fb Control Log2 FPKM

cxcl12a Epi3

Tg(tcf21:myr-eGFP; ptprc/CD45:D J Control, 5dpf J' s in ium KO cxcl12a,

Figure 2: Single cell RNA-seq on isolated larval heart cells (A). Pagoda<sup>5</sup> analysis identified three distinct clusters of epicardial cells (Epi1-3) in a total of 366 cells (B). Cell differentiation, such as that from Epi1 towards Epi2 (grey shade in **C**), was analyzed with Velocyto<sup>6</sup> ( $\mathbf{C}$ ) and Monocle<sup>7</sup> ( $\mathbf{D}$ ). Epi1-3 showed distinct transcriptomic signatures, as represented by e.g. adrenomedullin a (adma, Epi1), myosin light chain kinase a (*mylka*, Epi2) and *claudin 11a* (*cldn11a*, Epi3) (**E**). MC = mesenchymal cells, m/eHC = myeloid/erythroid haematopoietic cells, CM = cardiomyocytes, NC = neural cells.



Figure 4: Cas9-mediated transient knockouts and stable mutants of Epi1-3 markers. Loss of the Epi1 marker transglutaminase 2b (tgm2b) (A) resulted in reduced cell numbers in the epicardial sheet (**B**,**C**, quantification in **D**). Loss of the Epi2 marker semaphorin 3fb (sema3fb) (E) increased the number of tbx18<sup>+</sup> cells in the BA (arrows in **F**,**G**, quantification in **H**). Loss of the Epi3 marker *cxcl12a* (I) decreased the number of *ptprc/CD45:DsRed*<sup>+</sup> cells that were in contact with the epicardium (J,K, arrows in J',J'', quantification in L). BA = bulbus arteriosus. Scale bars are 50µm (**B**,**C**,**J**,**K**), 10µm (**F**,**G**, **J**',**J**'').







Figure 3: Localization of Epi1-3 in the larval zebrafish heart. In situ detection of the Epi1 marker adma labelled the epicardial cell layer (A-A"). Immune-fluorescence identified Mylka<sup>+</sup>/tbx18-myr-Citrine<sup>+</sup> cells in the bulbus arteriosus (BA) (**B-B**"). The Epi3 marker *cldn11a* labelled a subset of the epicardial cell layer (C-C"). Scale bars are 20µm (A,B,C), 5μm (**A**',**A**",**B**',**B**",**C**',**C**").

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- The larval zebrafish epicardium consists of three distinct subpopulations of cells (Epi1-3)
- *tgm2b* (Epi1) regulates the cell numbers in the main epicardial cell sheet
- sema3fb (Epi2) restricts the number of tbx18<sup>+</sup> cells in the BA
- cxcl12a (Epi3) guides ptprc/CD45<sup>+</sup> myeloid cells to the developing heart



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