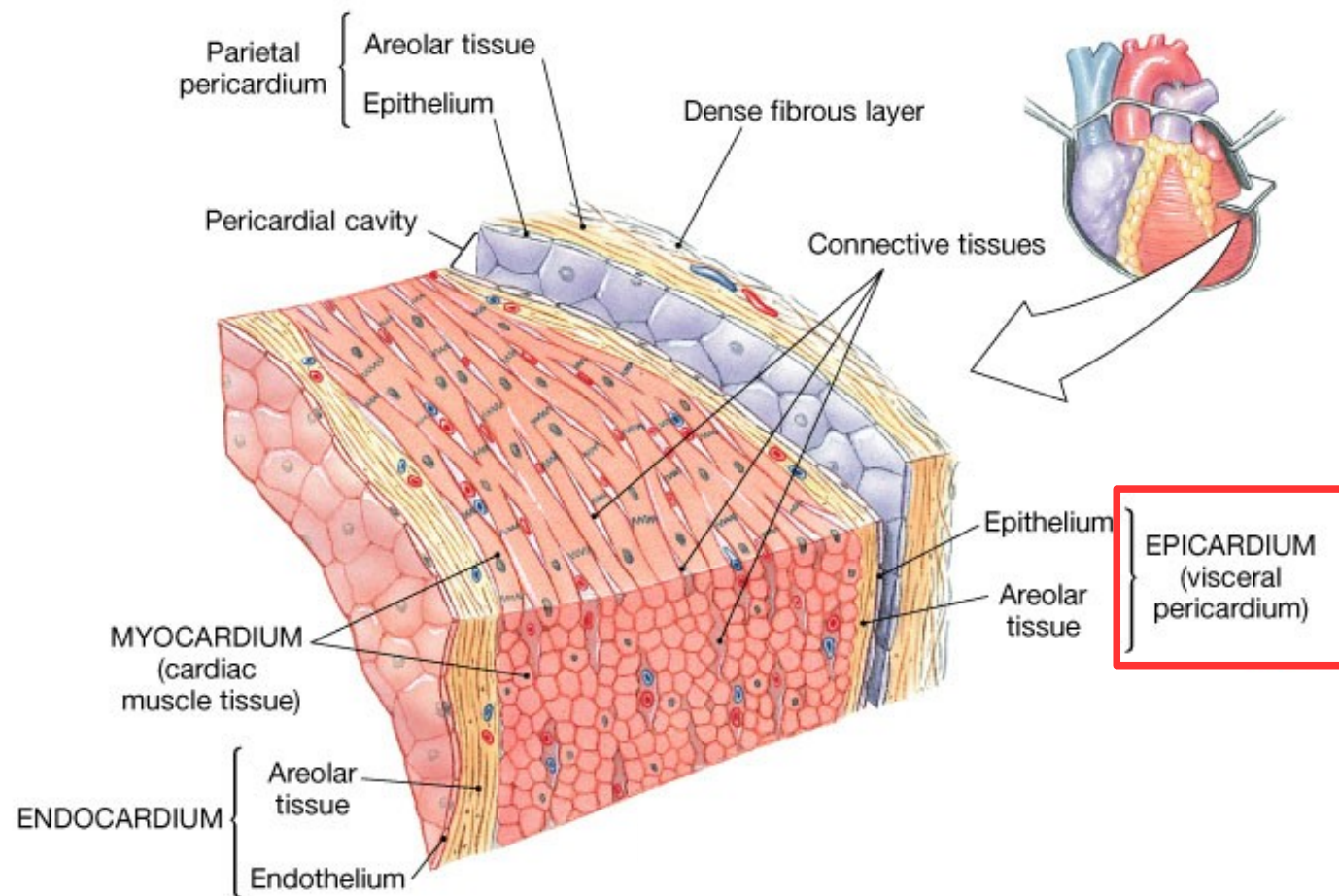
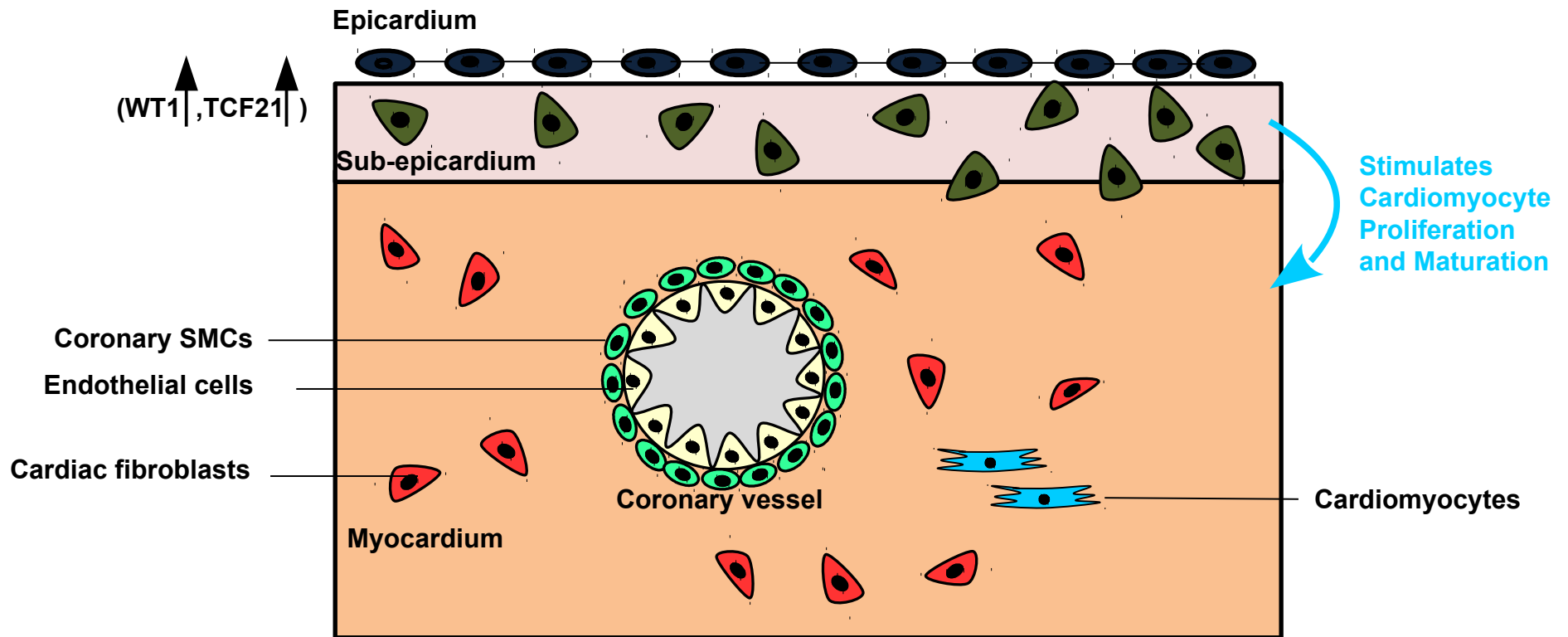
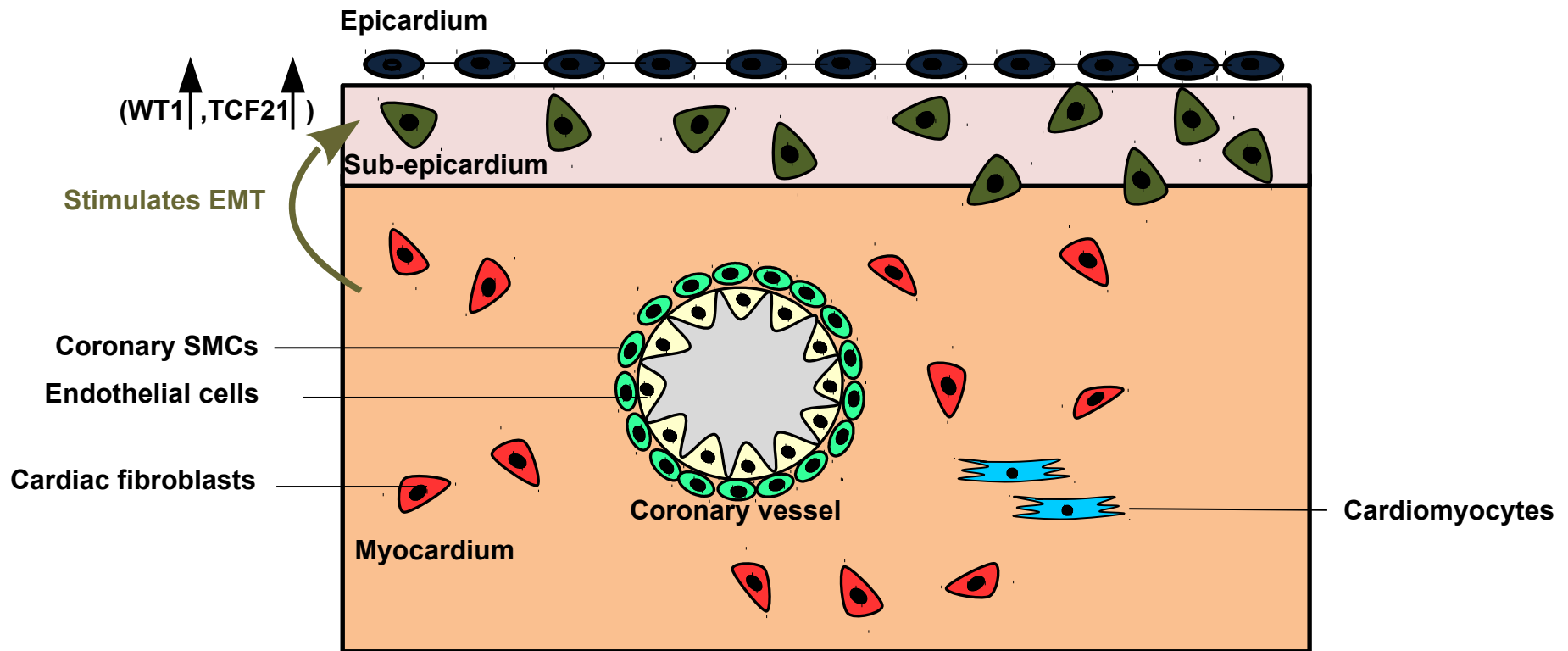




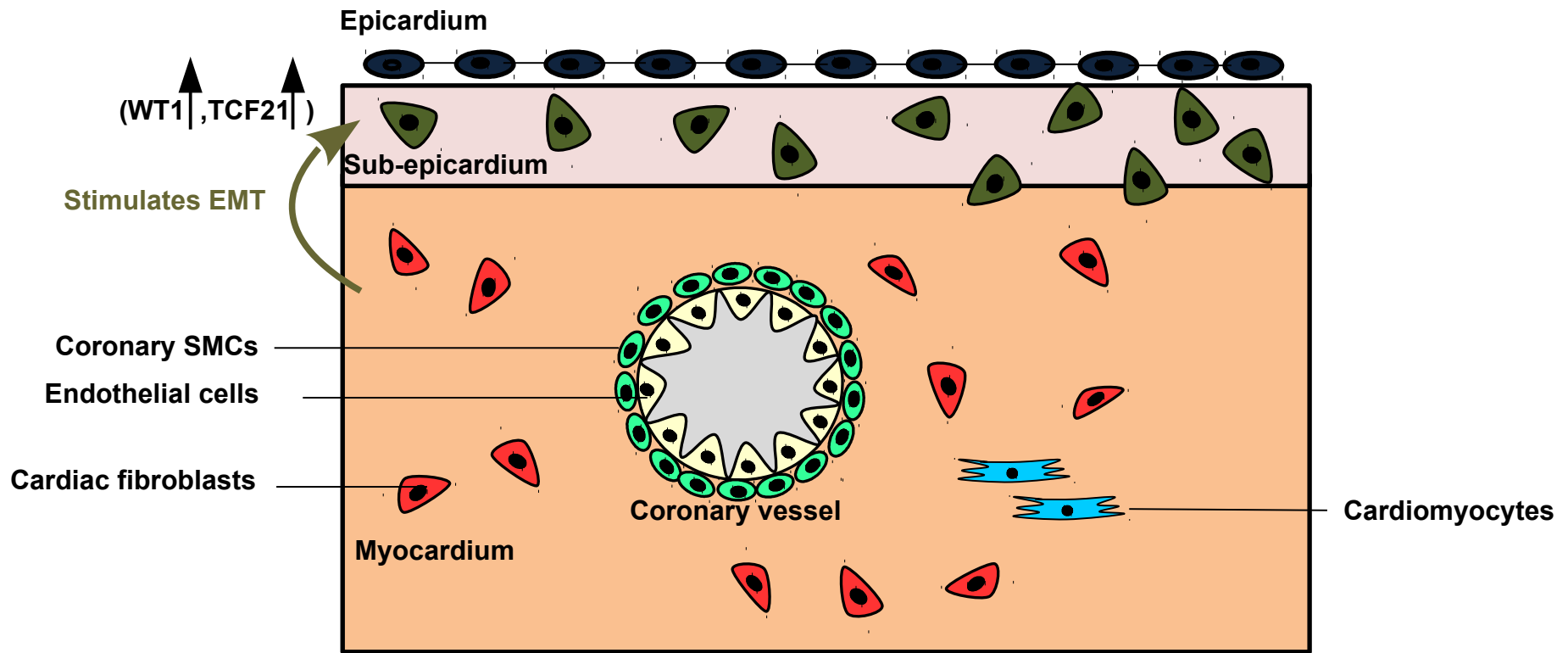
Epicardium undergoes EMT and contributes to coronary vessel development



Epicardium undergoes EMT and contributes to coronary vessel development



Epicardium undergoes EMT and contributes to coronary vessel development



... in fetus during development!

After infarction in adult, epicardium-derived cells produce fibrosis

Approach in the Sinha lab: hPSC-derived epicardium

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RESEARCH ARTICLE

TECHNIQUES AND RESOURCES

Robust derivation of epicardium and its differentiated smooth muscle cell progeny from human pluripotent stem cells

Dharini Iyer, Laure Gambardella, William G. Bernard, Felipe Serrano, Victoria L. Mascetti, Roger A. Pedersen, Amarnath Talasila and Sanjay Sinha*

ABSTRACT

The epicardium has emerged as a multipotent cardiovascular progenitor source with therapeutic potential for coronary smooth muscle cell, cardiac fibroblast (CF) and cardiomyocyte regeneration, owing to its fundamental role in heart development and its potential ability to initiate myocardial repair in injured adult tissues. Here, we describe a chemically defined method for generating epicardium and epicardium-derived smooth muscle cells (EPI-SMCs) and CFs from human pluripotent stem cells (HPSCs) through an intermediate lateral plate mesoderm (LM) stage. HPSCs were initially differentiated to LM in the presence of FGF2 and high levels of BMP4. The LM was robustly differentiated to an epicardial lineage by activation of WNT, BMP and retinoic acid signalling pathways. HPSC-derived epicardium displayed

reciprocal exchange of paracrine factors between the epicardium and the underlying myocardium promote development of coronary vessels and cardiomyocytes (Wessels and Perez-Pomares, 2004). An emerging paradigm proposes that re-activation of the embryonic developmental program in injured adult epicardial tissues by ectopic signals can mediate epicardium differentiation towards cardiogenic fates or enhance the existing level of signalling activity within the epicardium (Smart et al., 2007). Thus, an *in vitro* model that recapitulates the key events regulating early lineage commitment to epicardium and its derivatives would facilitate efficient and reproducible generation of highly enriched vascular cells for potential applications in vascular disease modelling, drug screening and construction of bioengineered cardiac grafts.

The epicardium displays greater capacity for differentiation into

hESC

Lateral Plate
mesoderm

Epicardium

FGF2
Ly294002
BMP4

1.5d

FGF2
BMP4

3.5d

BMP4
WNT3A
RA

10d

Coronary like SMCs

Cardiac fibroblasts

PDGF-BB, TGF- β 1

12d

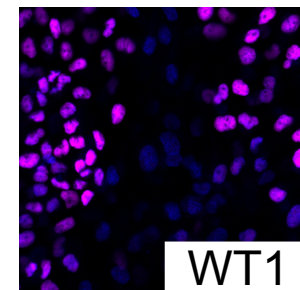
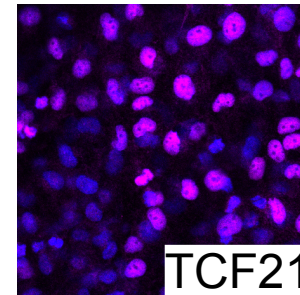
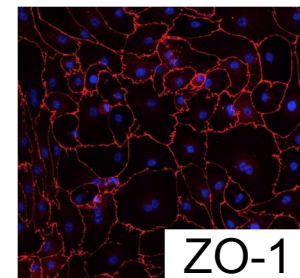
FGF2, VEGF-B

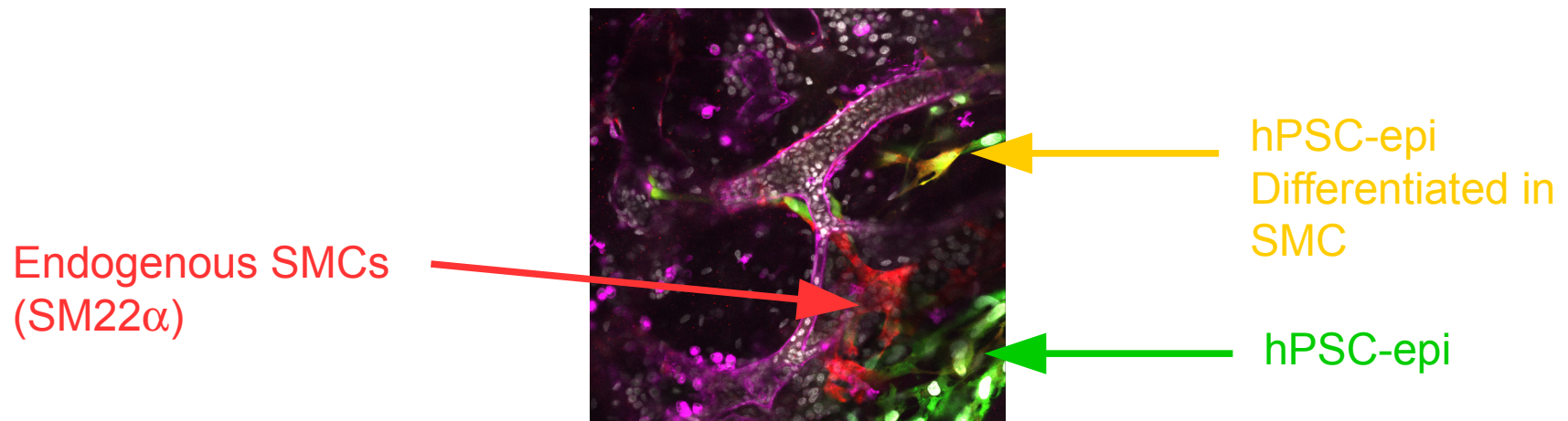
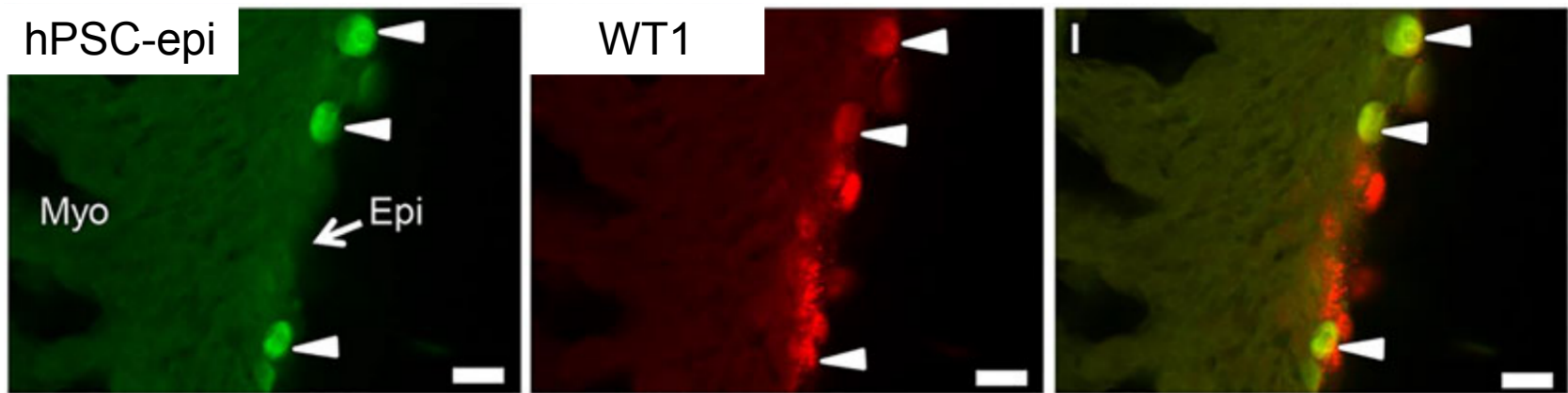
12d

ZO-1

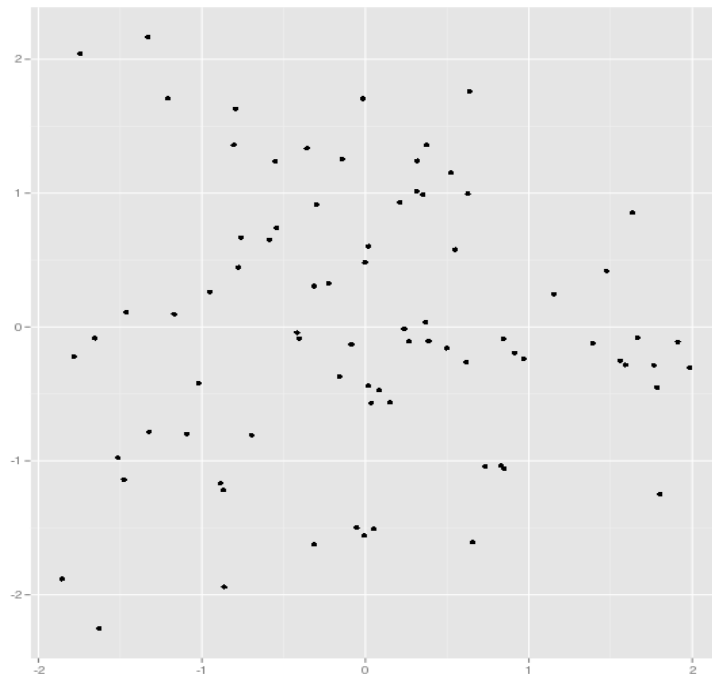
TCF21

WT1

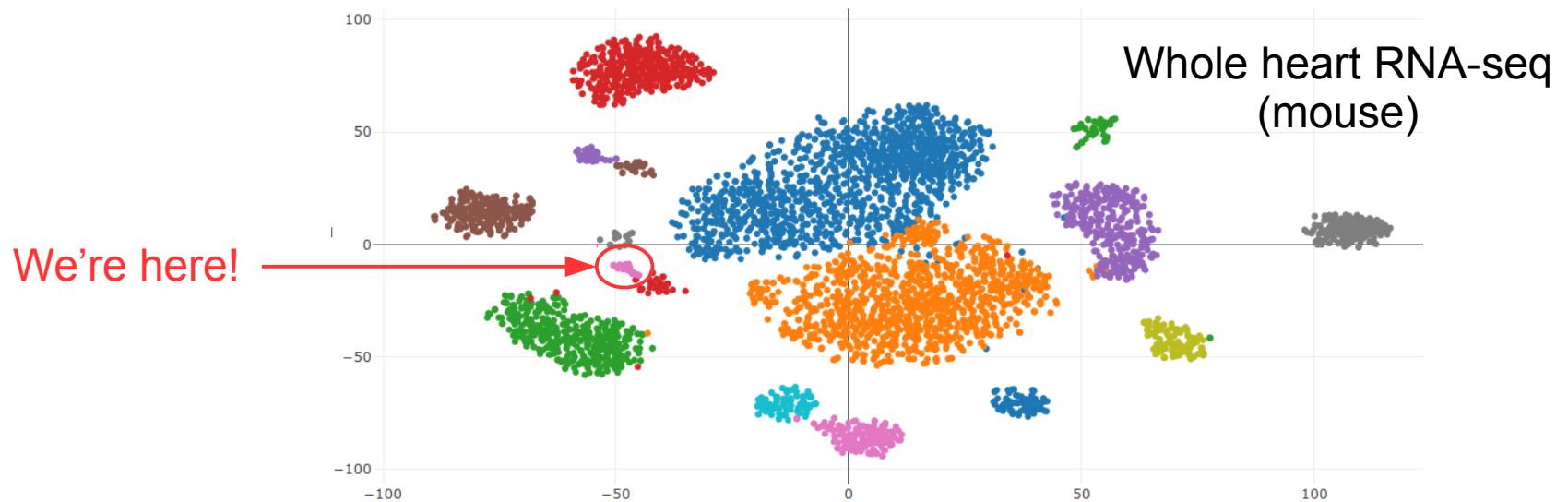
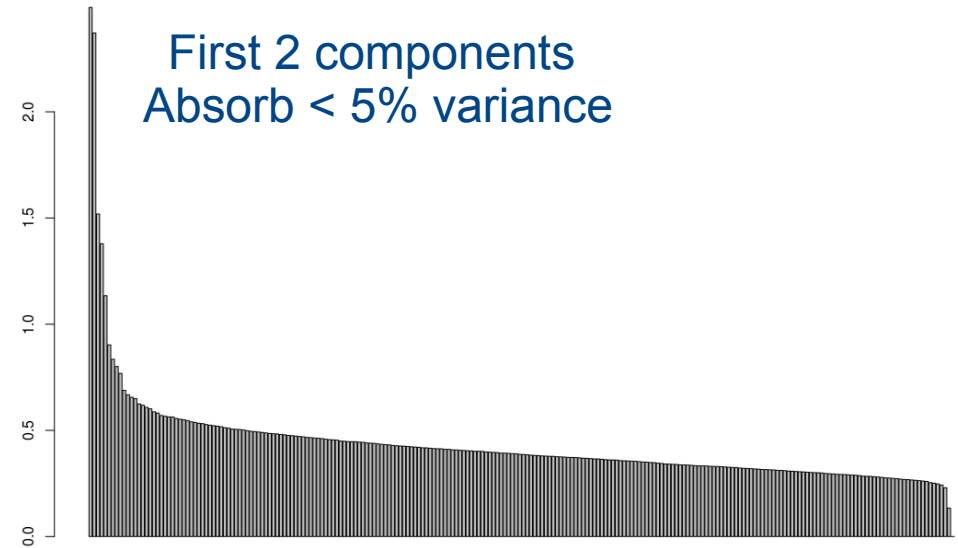
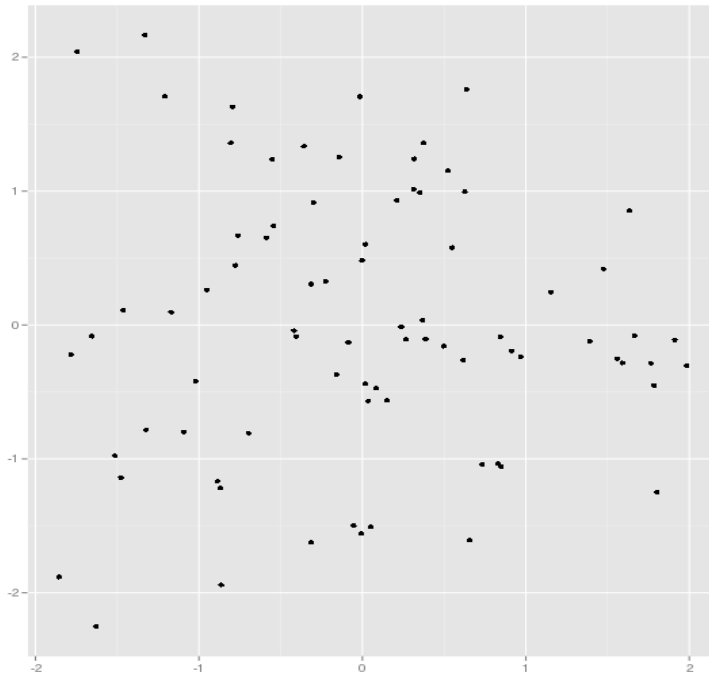




First unbiased PCA showed no structure

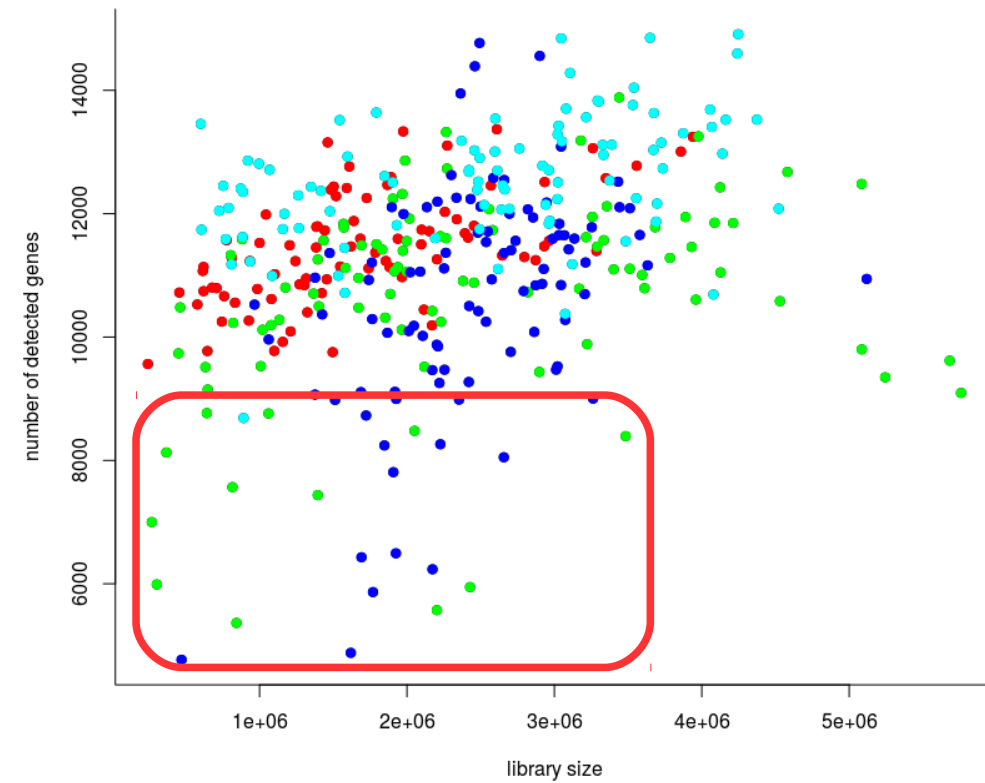


First unbiased PCA showed no structure

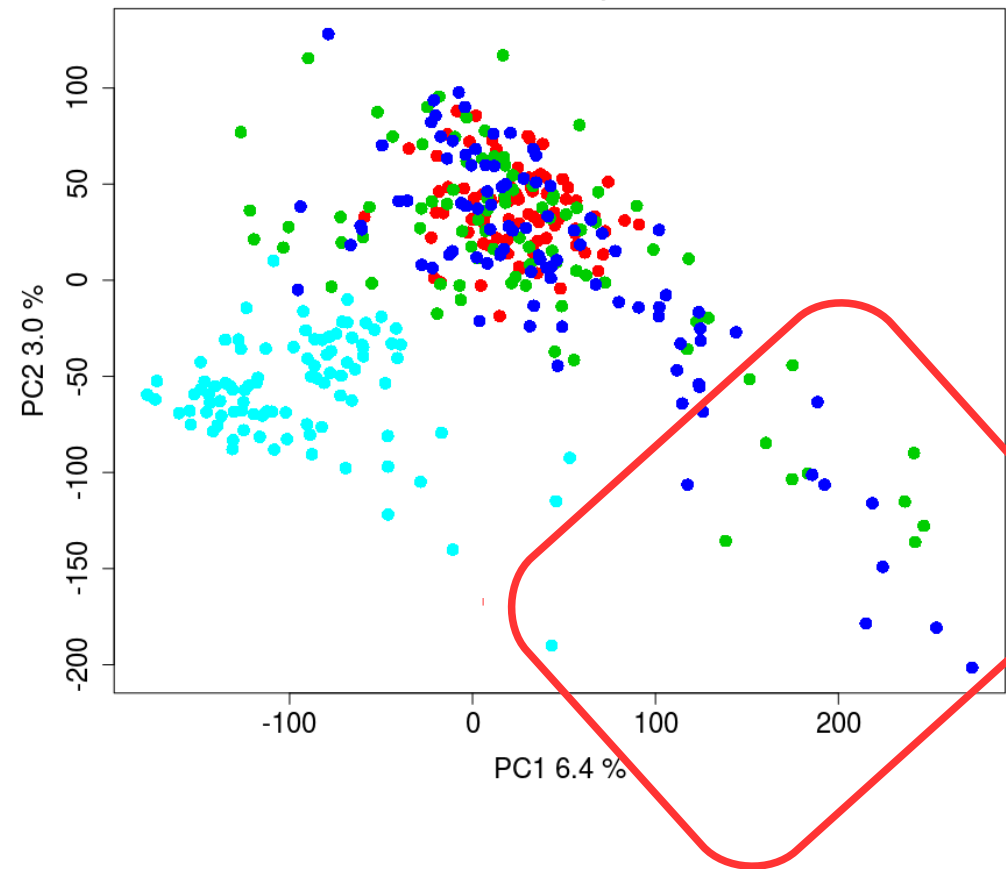


Cells with low coverage muddle the picture

Non-zero gene vs. read counts

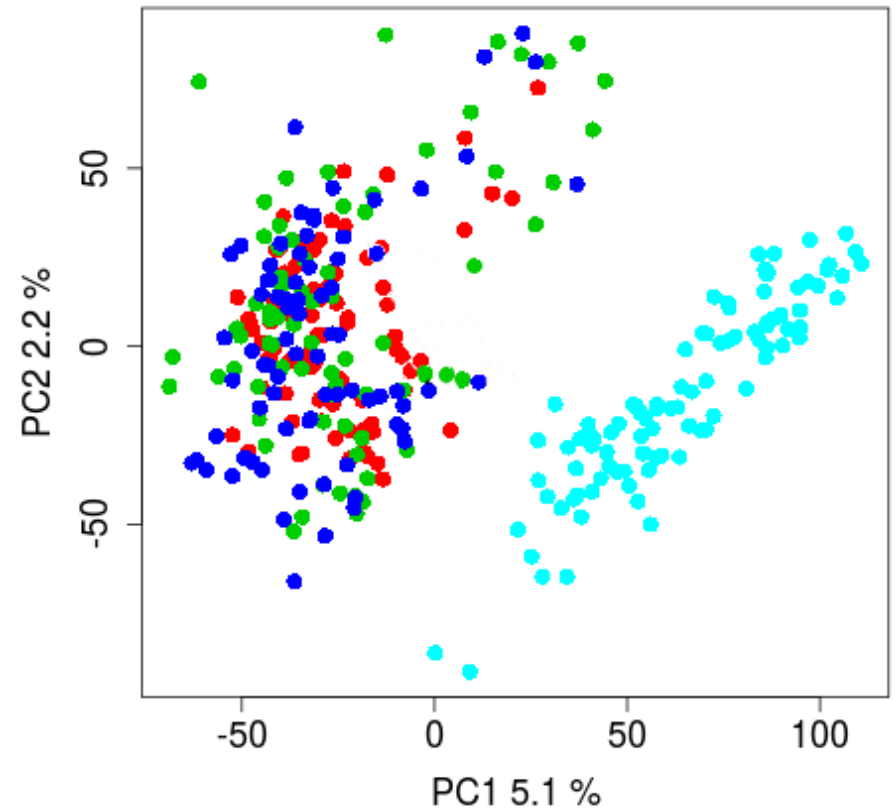
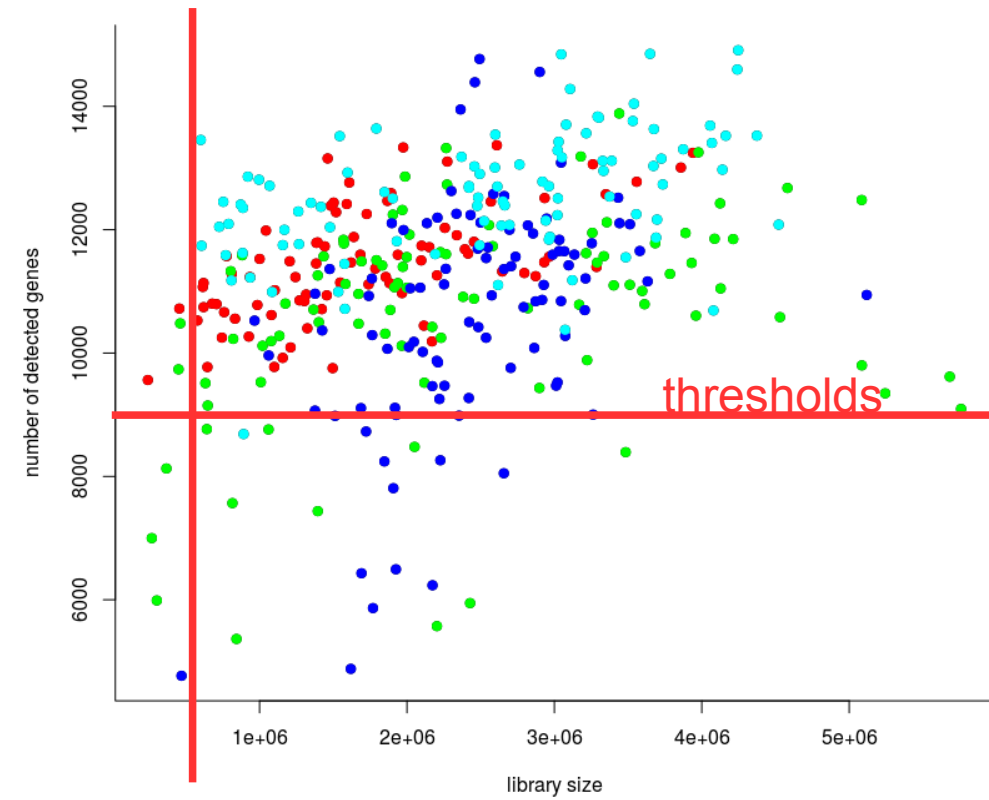


PCA all samples



● LPM1 ● EPI1 ● EPI2 ● EPI3

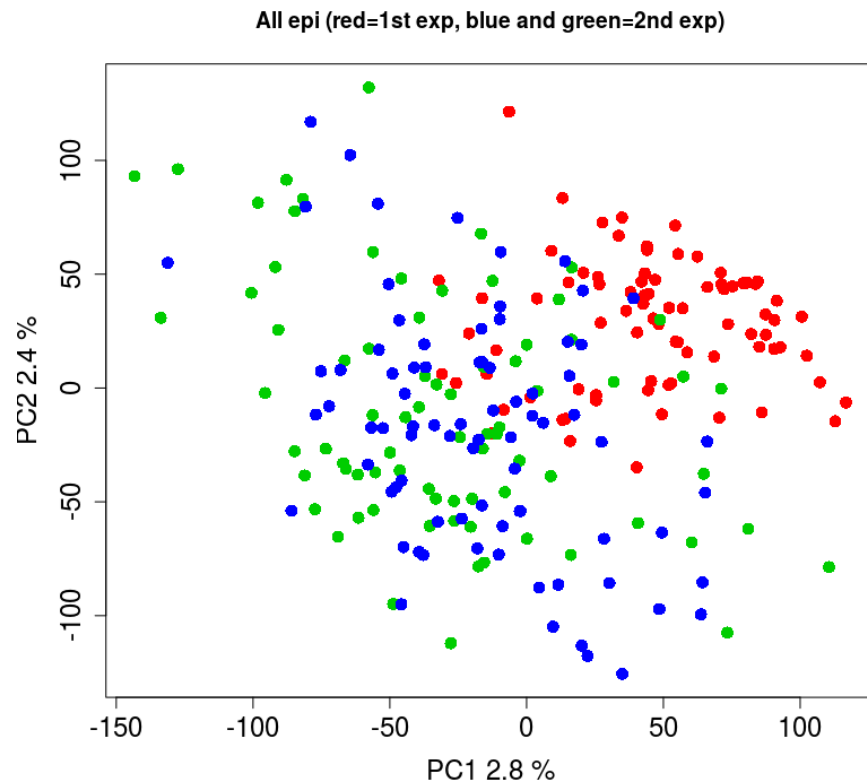
After removing low information cells



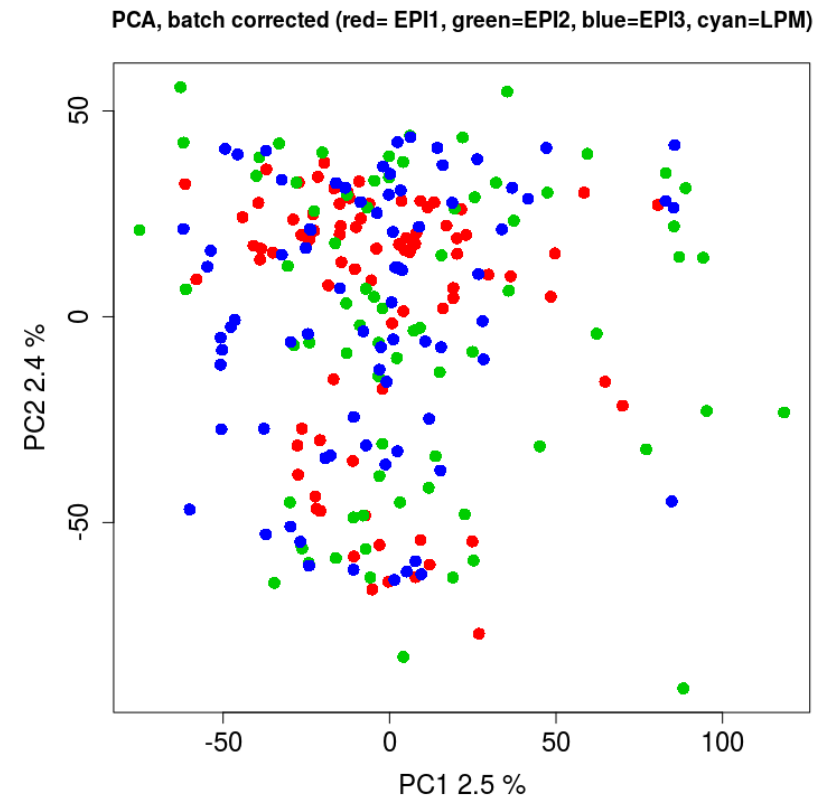
● LPM1 ● EPI1 ● EPI2 ● EPI3

Batch effect is the main factor of variation

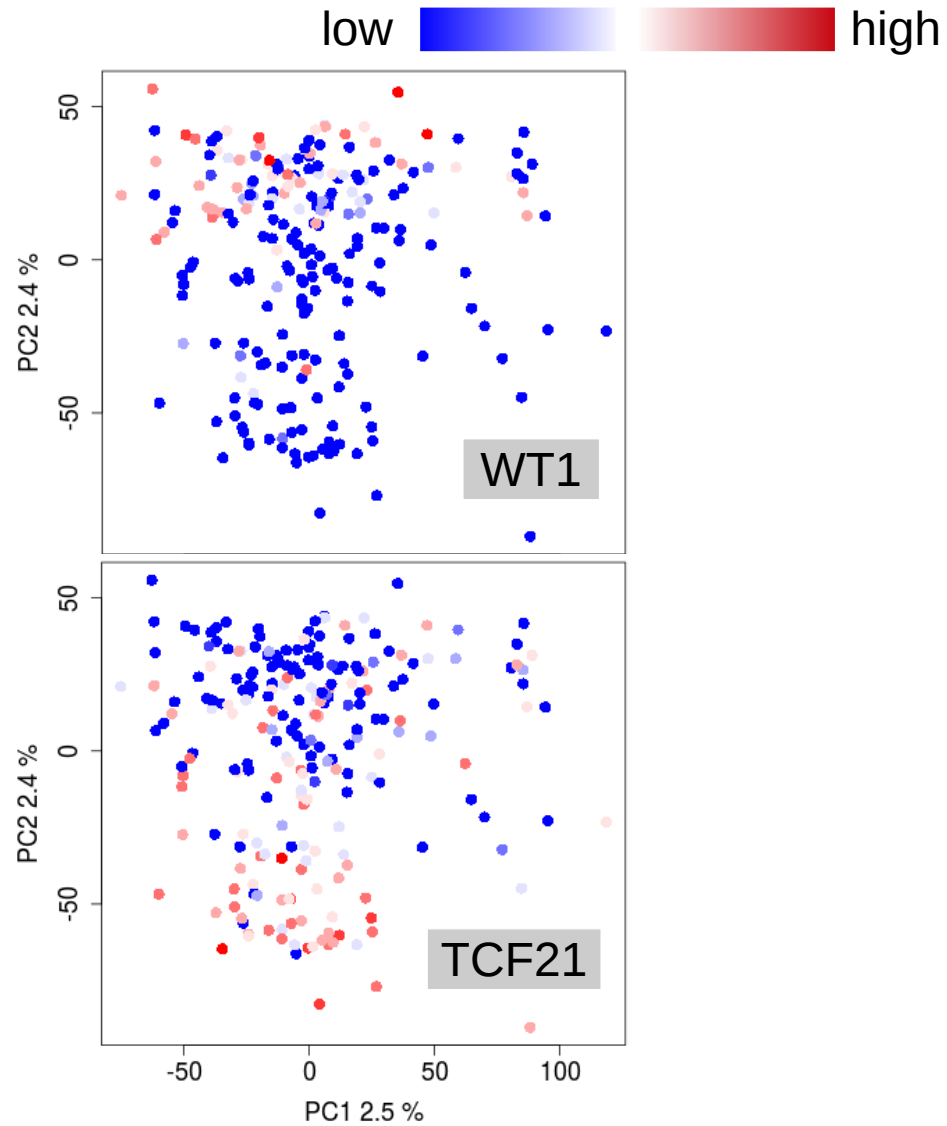
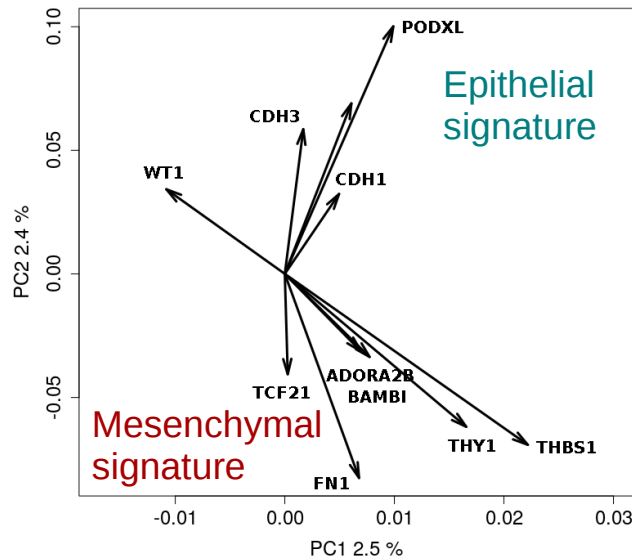
Before batch correction



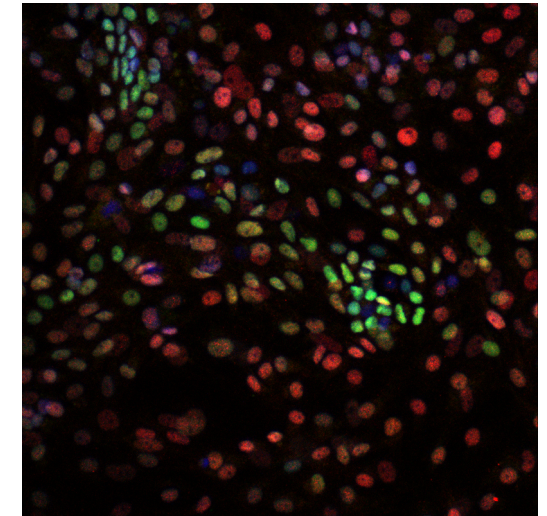
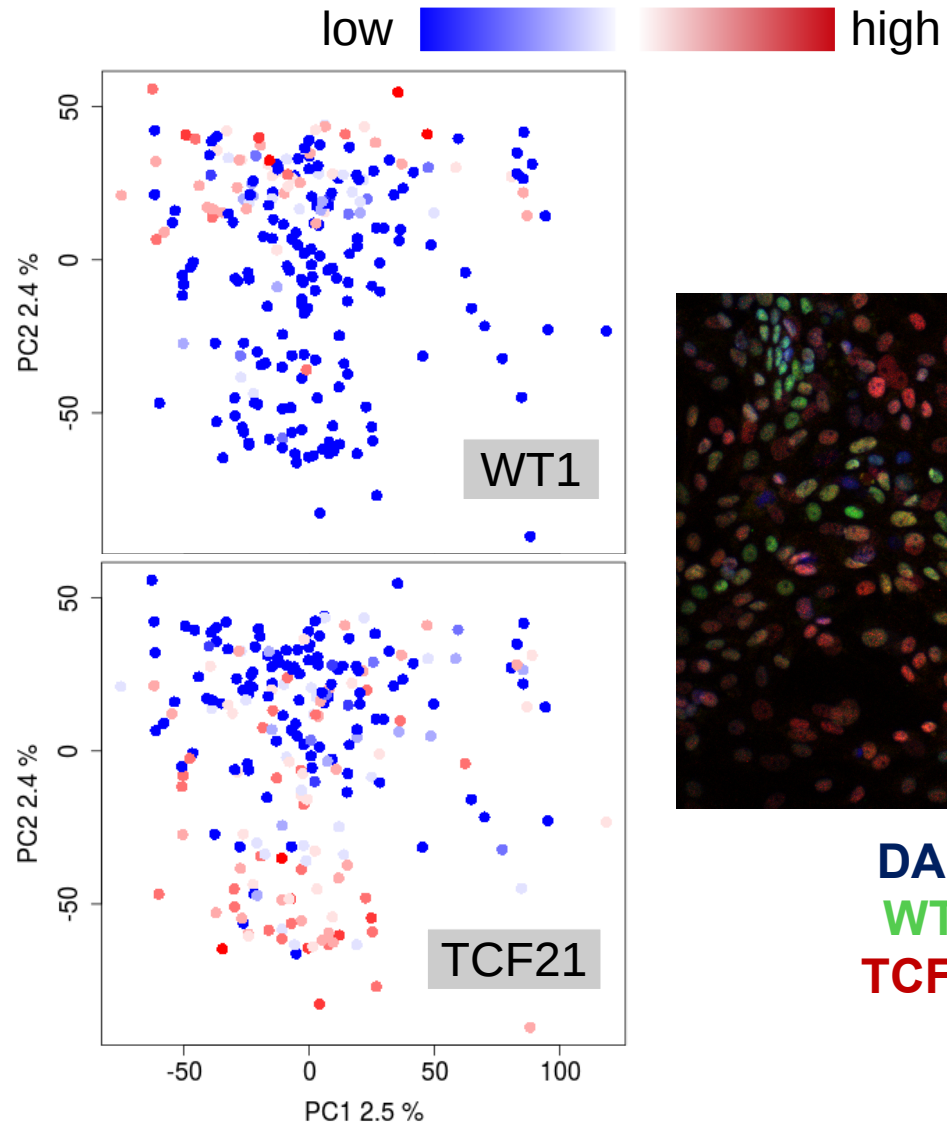
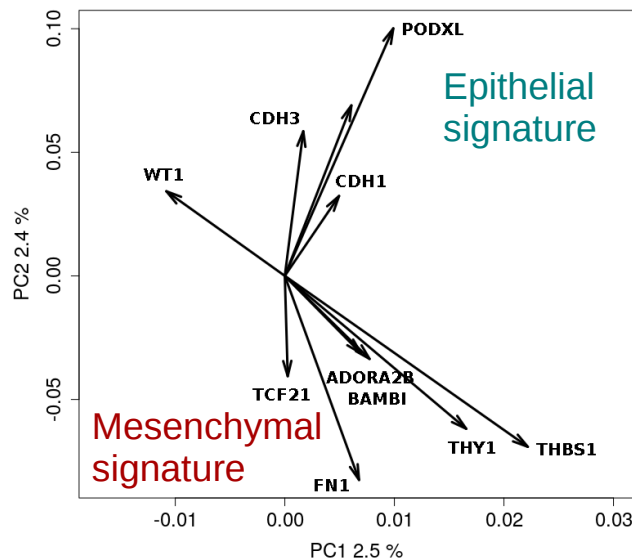
After batch correction



Engineered epicardium contains two subpopulations

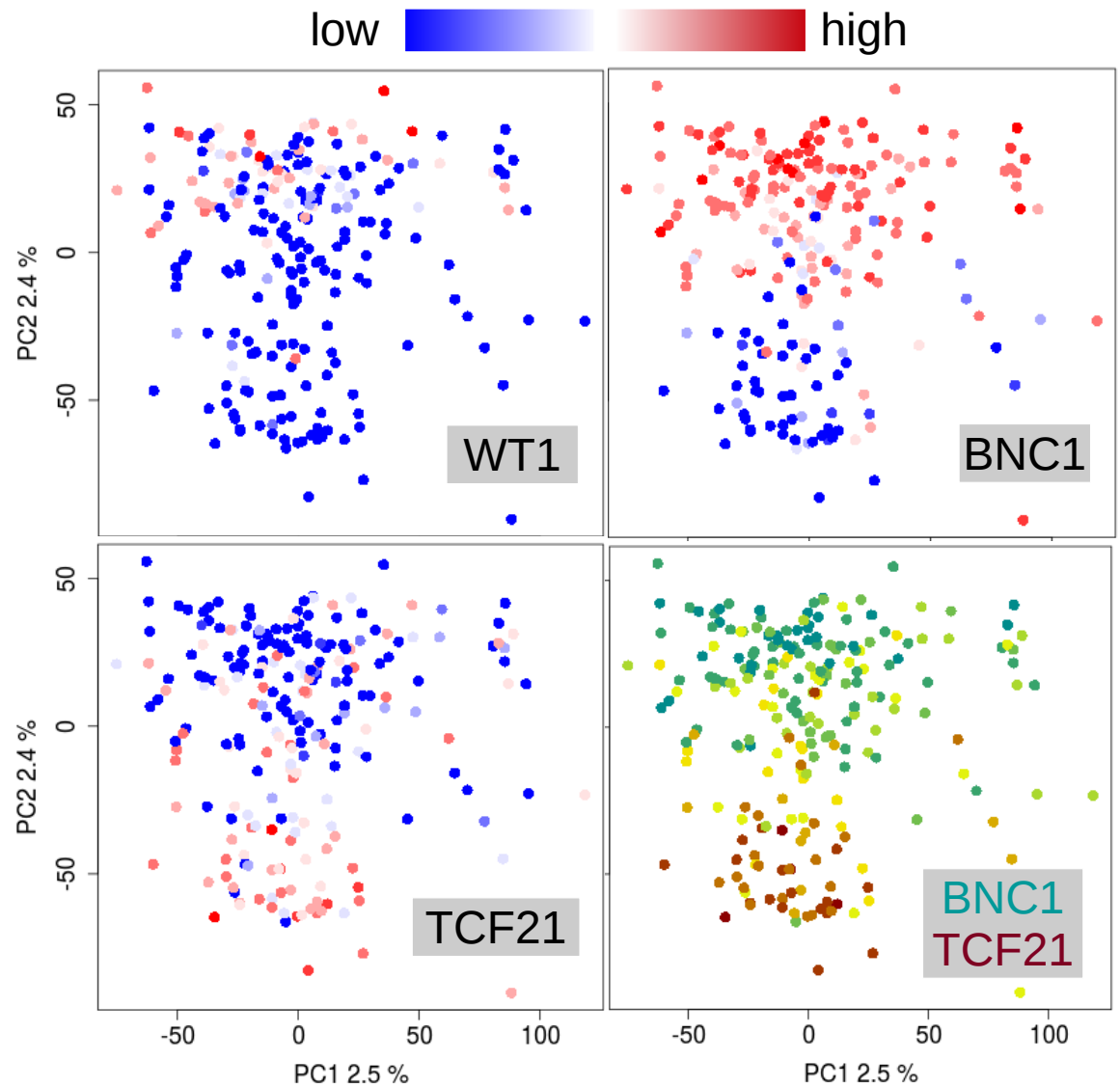
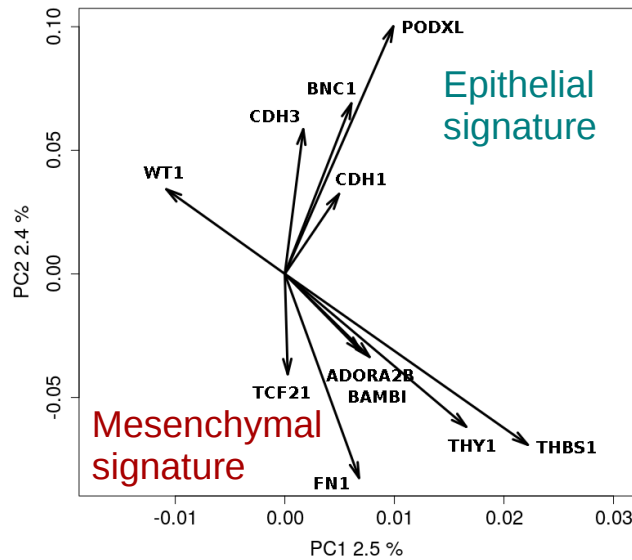


Engineered epicardium contains two subpopulations

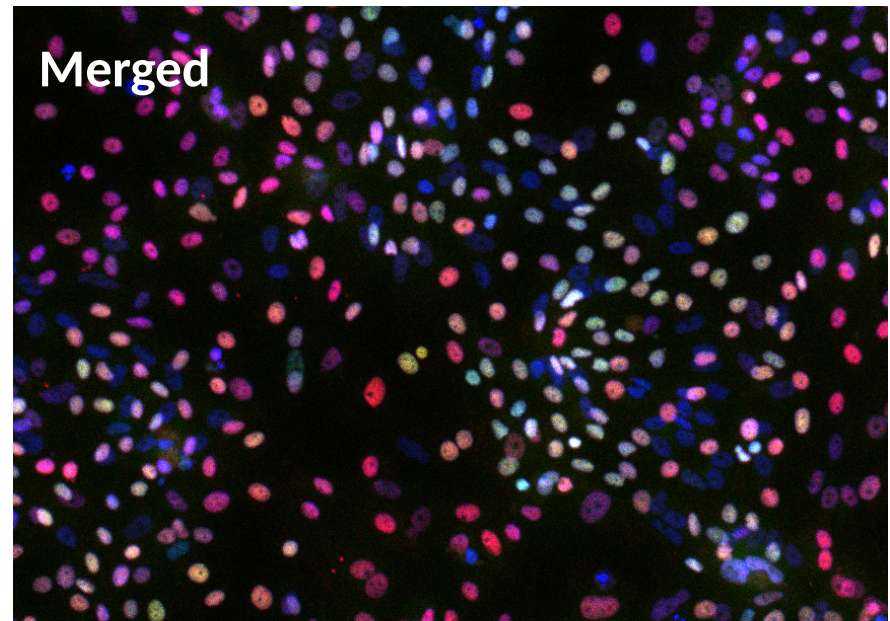
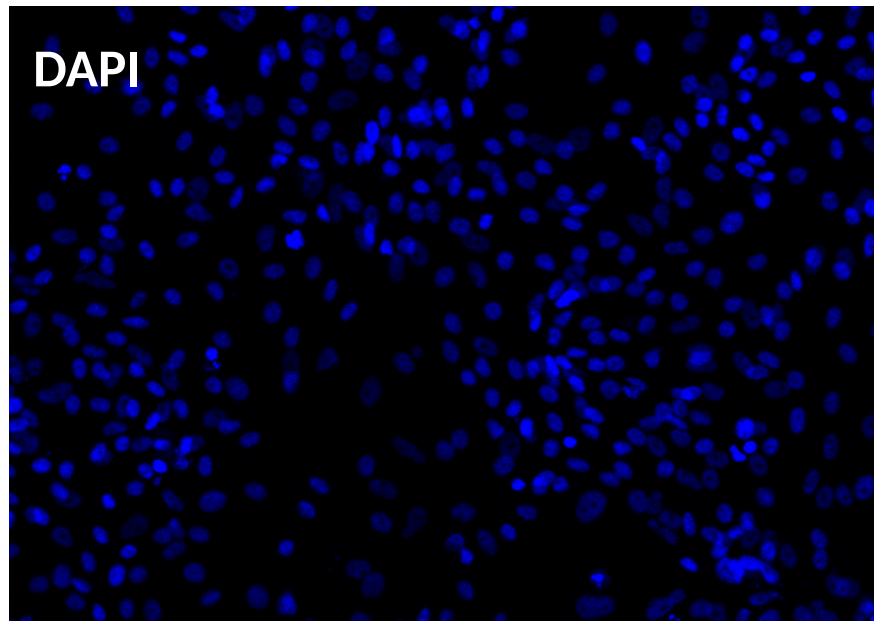
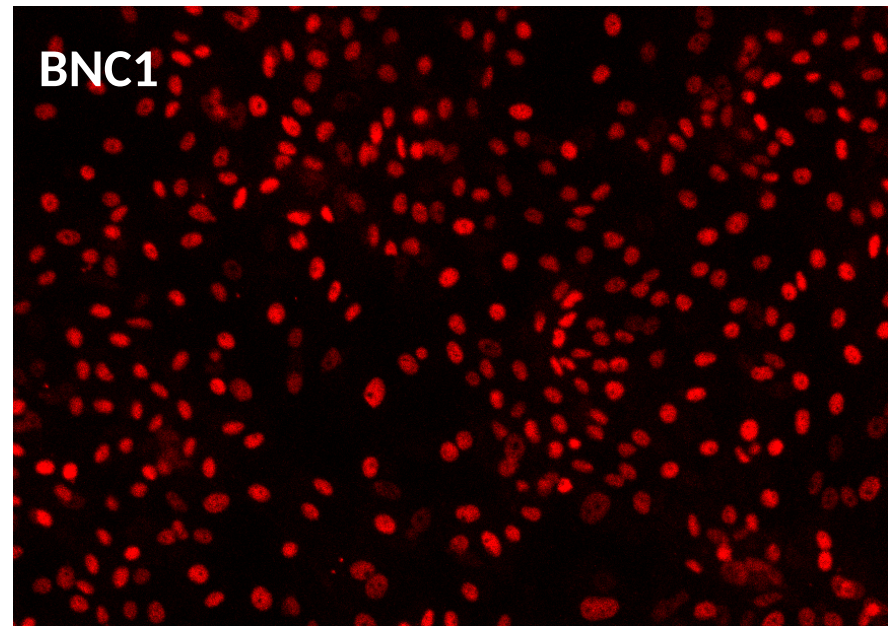
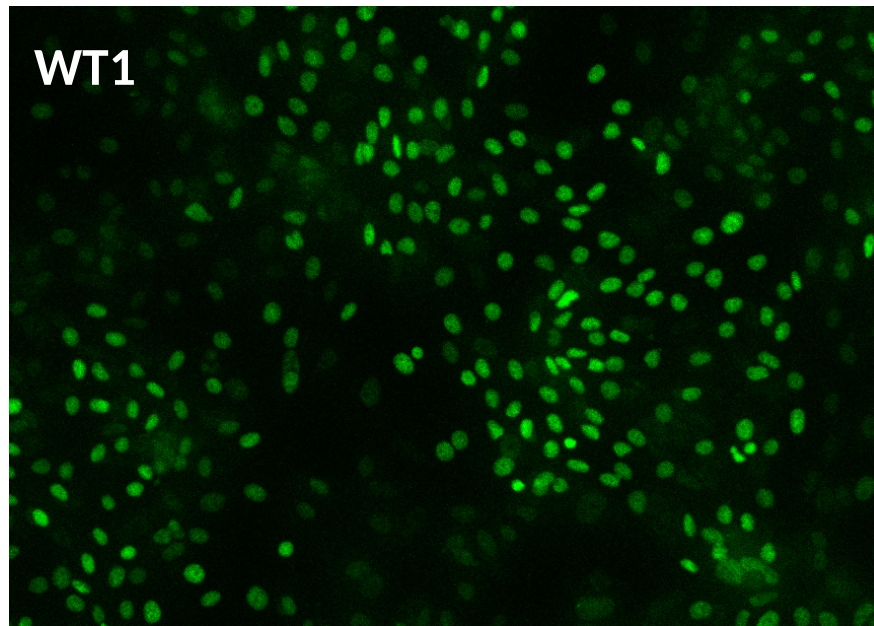


DAPI
WT1
TCF21

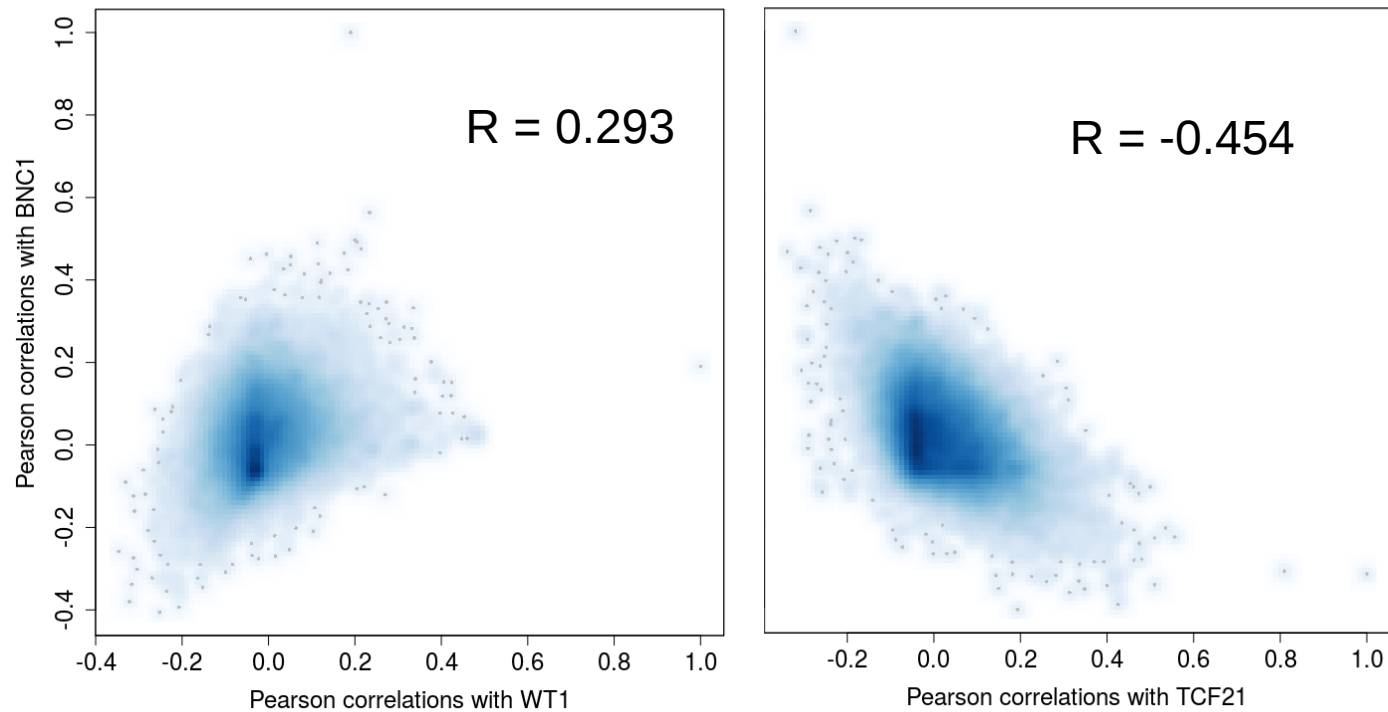
Engineered epicardium contains two subpopulations



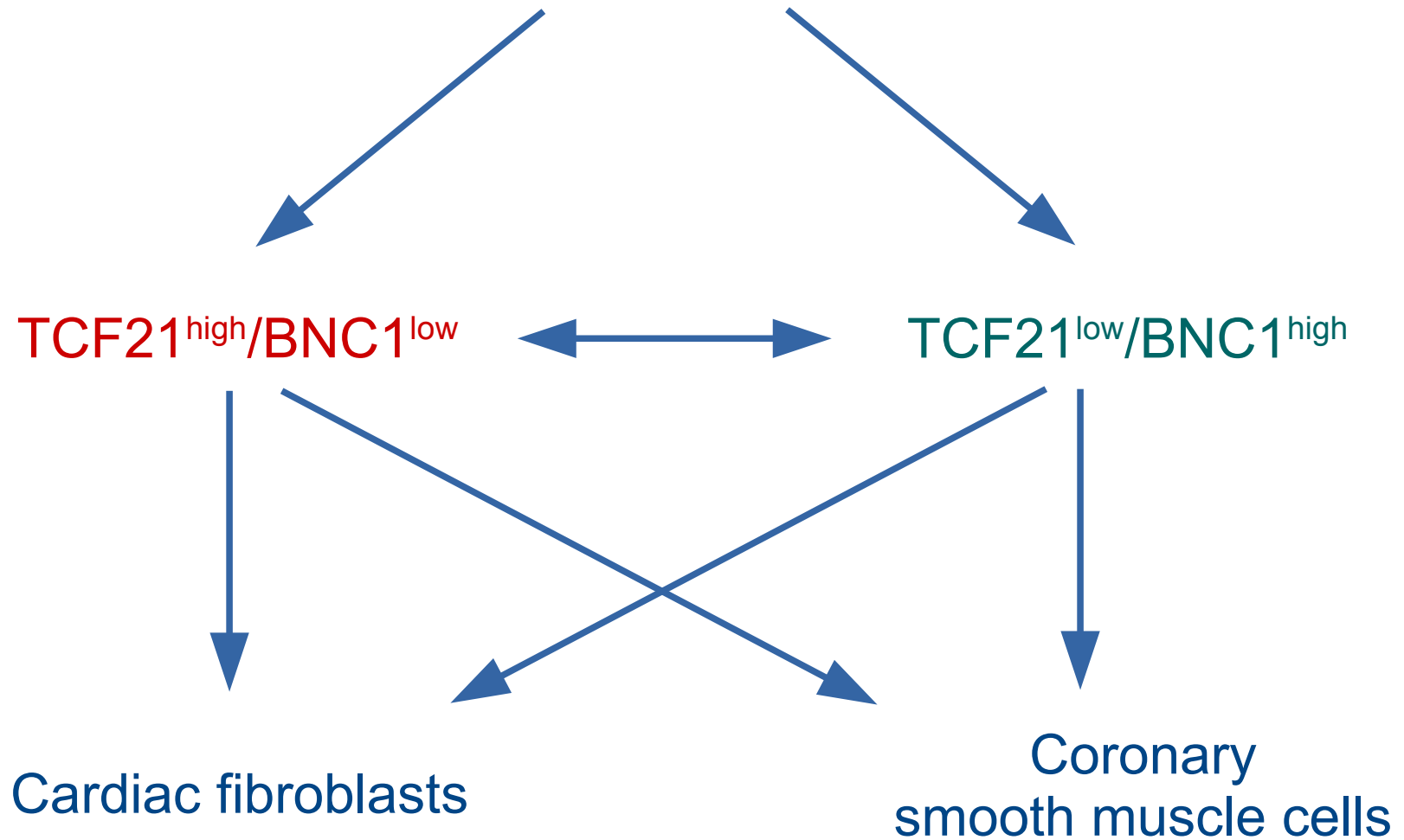
WT1^{high} population included in the BNC1^{high} at protein level



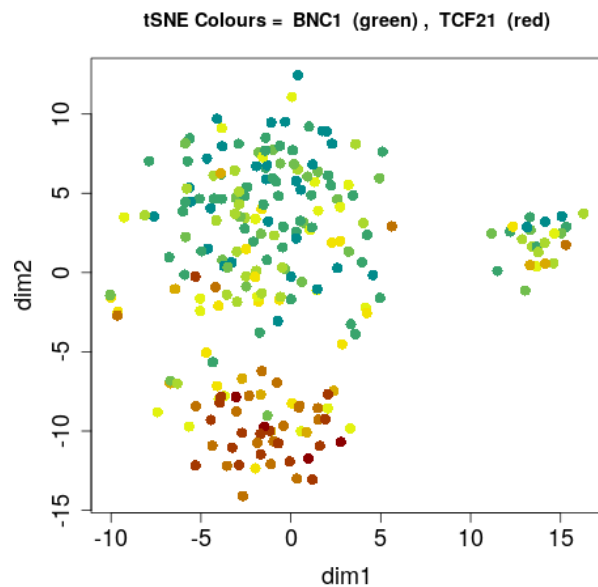
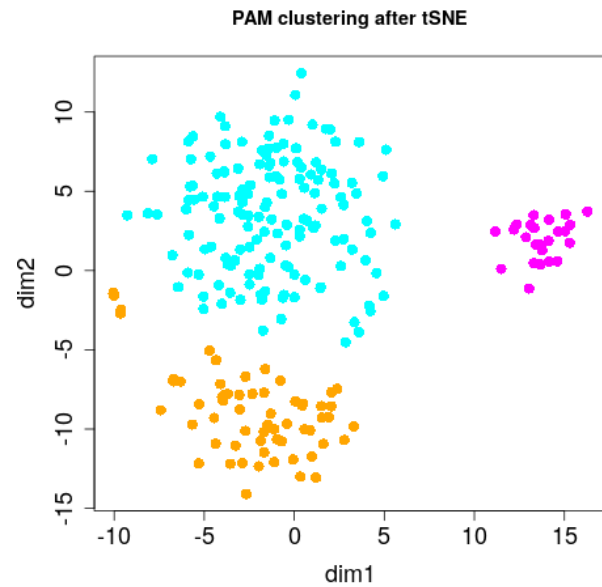
Genes correlating with BNC1 anti-correlate with TCF21



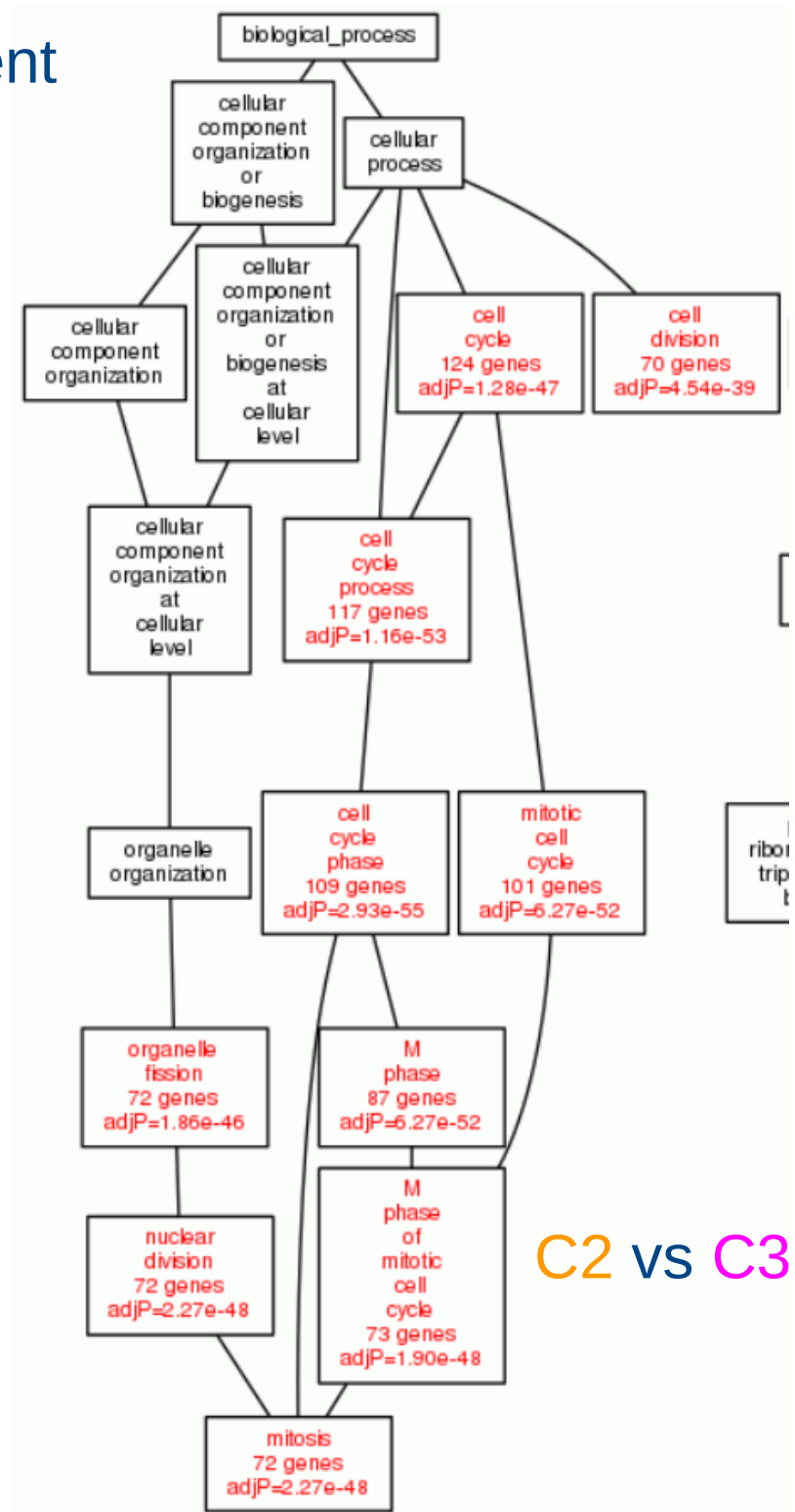
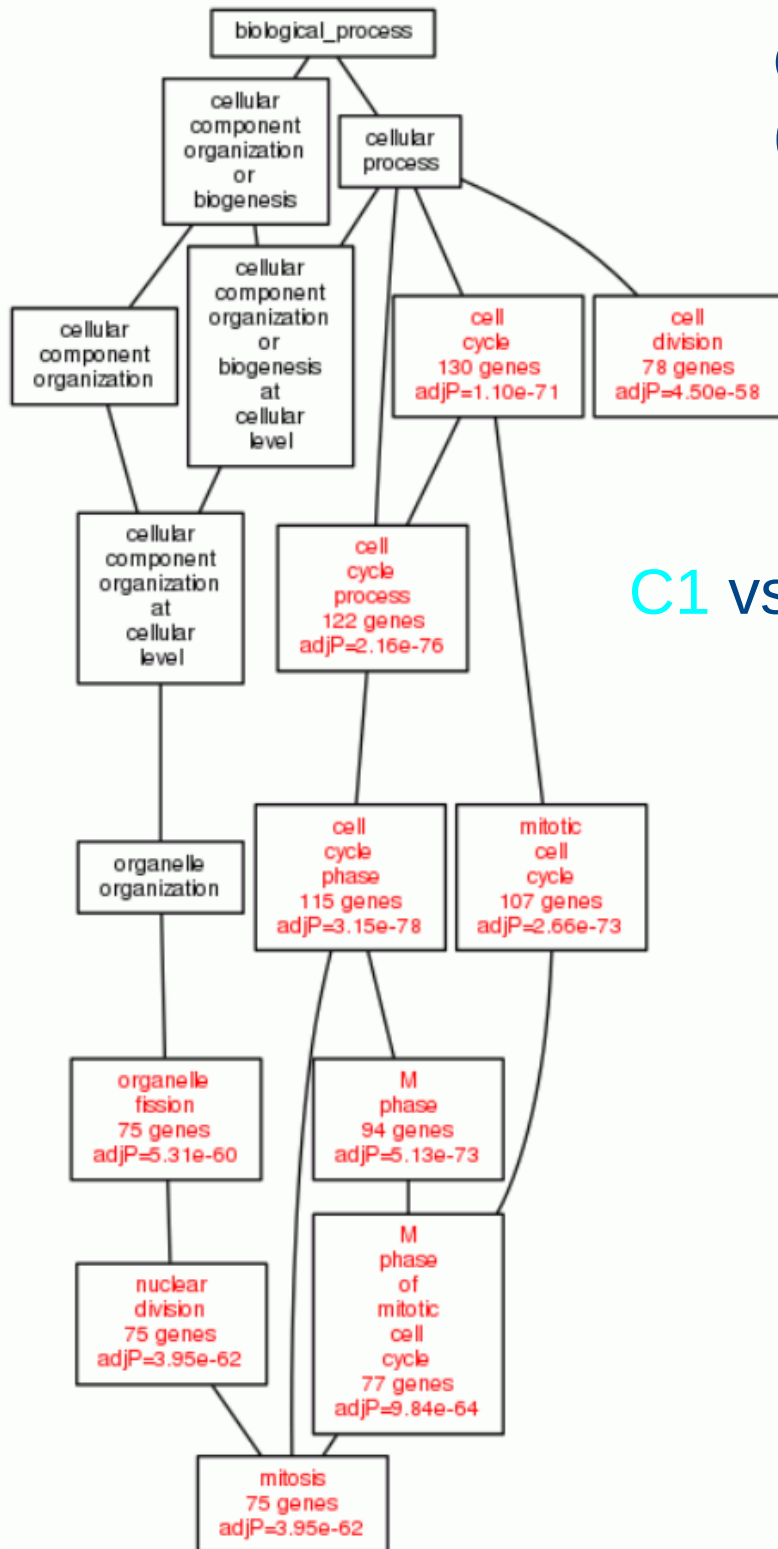
Lateral Plate Mesoderm



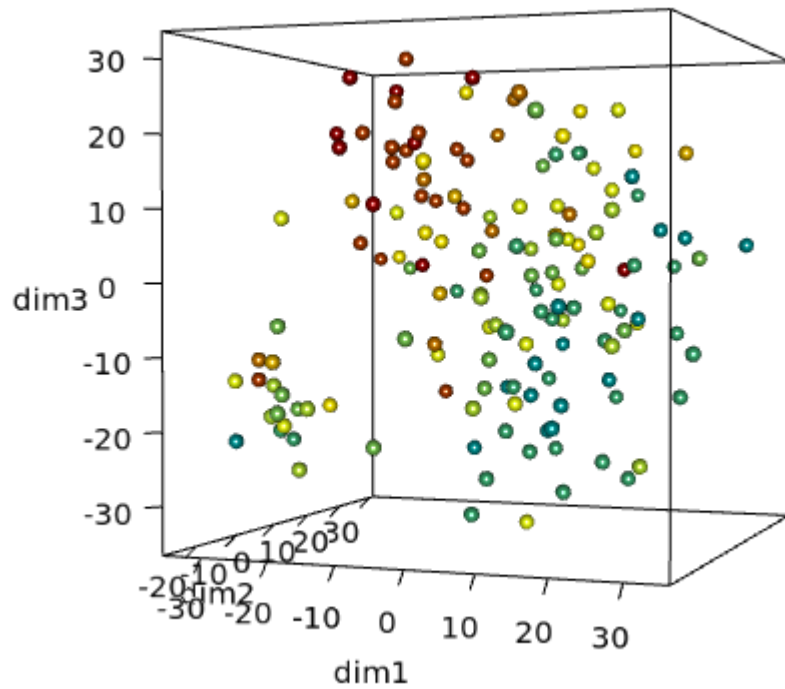
tSNE and clustering to bring similar cells together



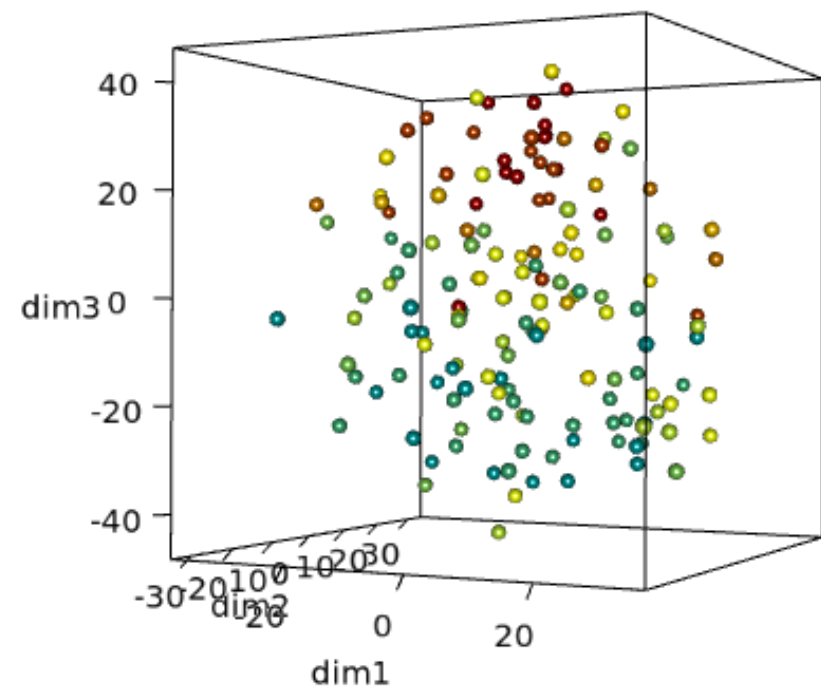
GO enrichment (WebGestalt)

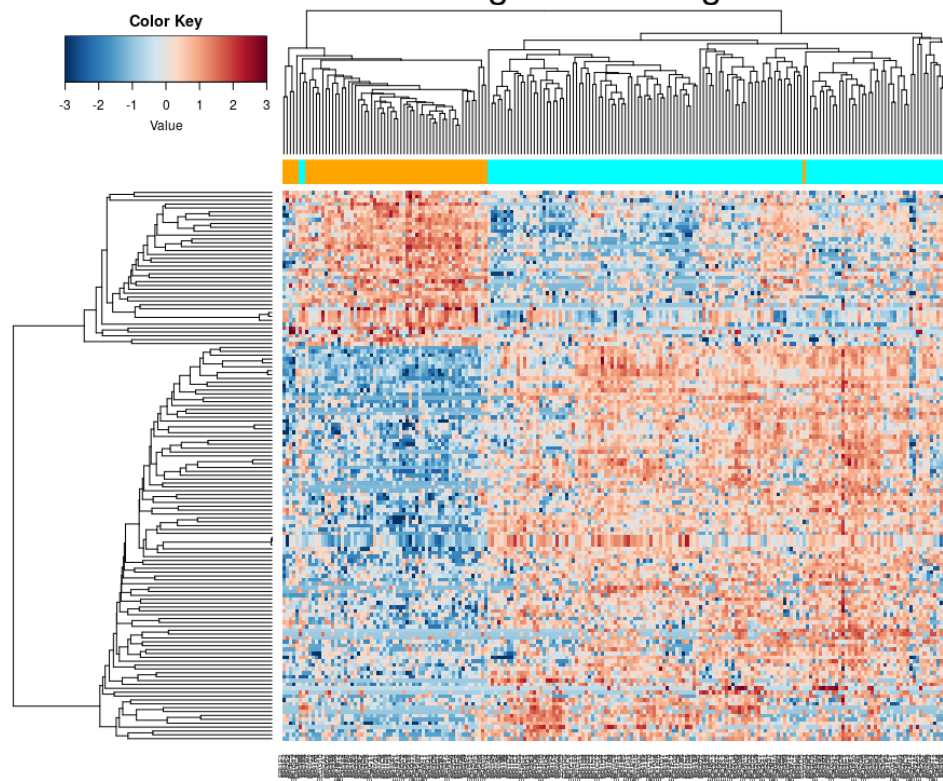
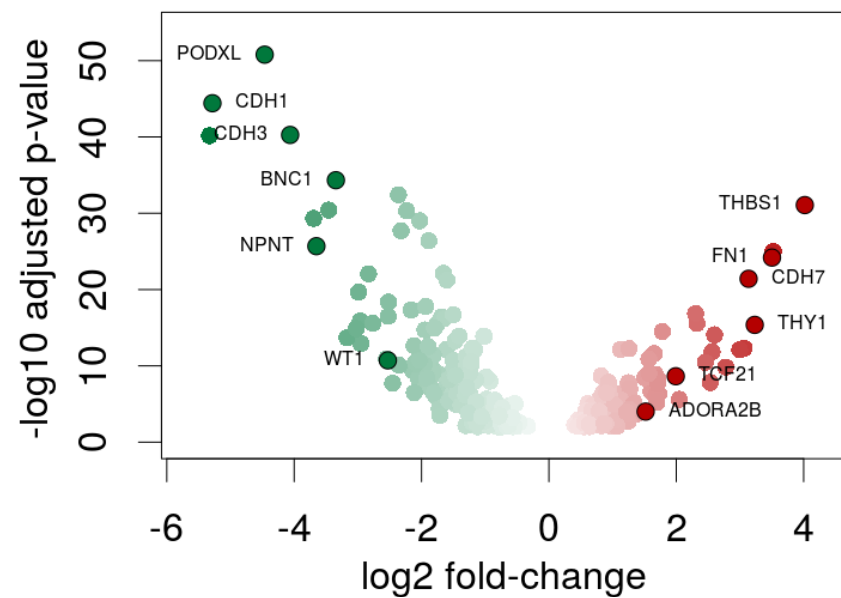
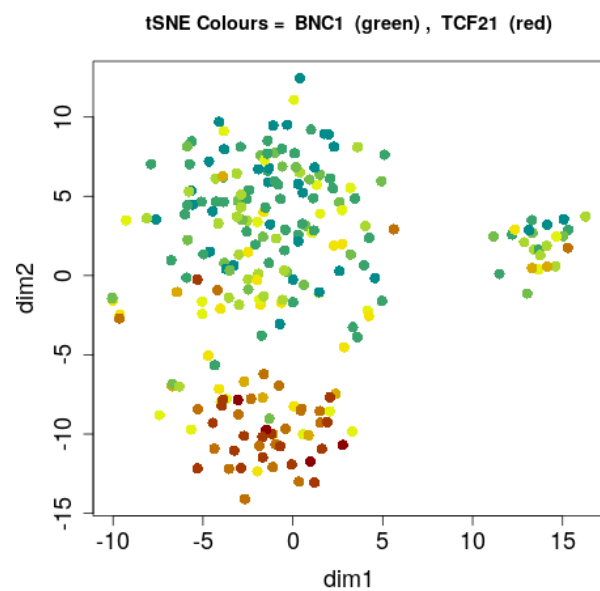
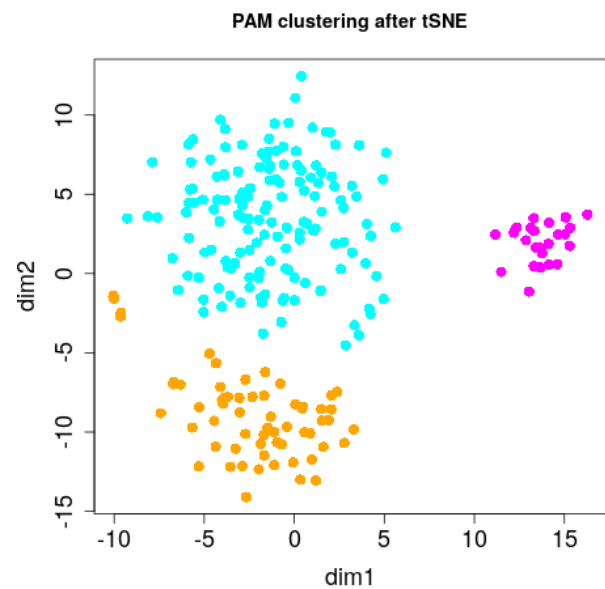


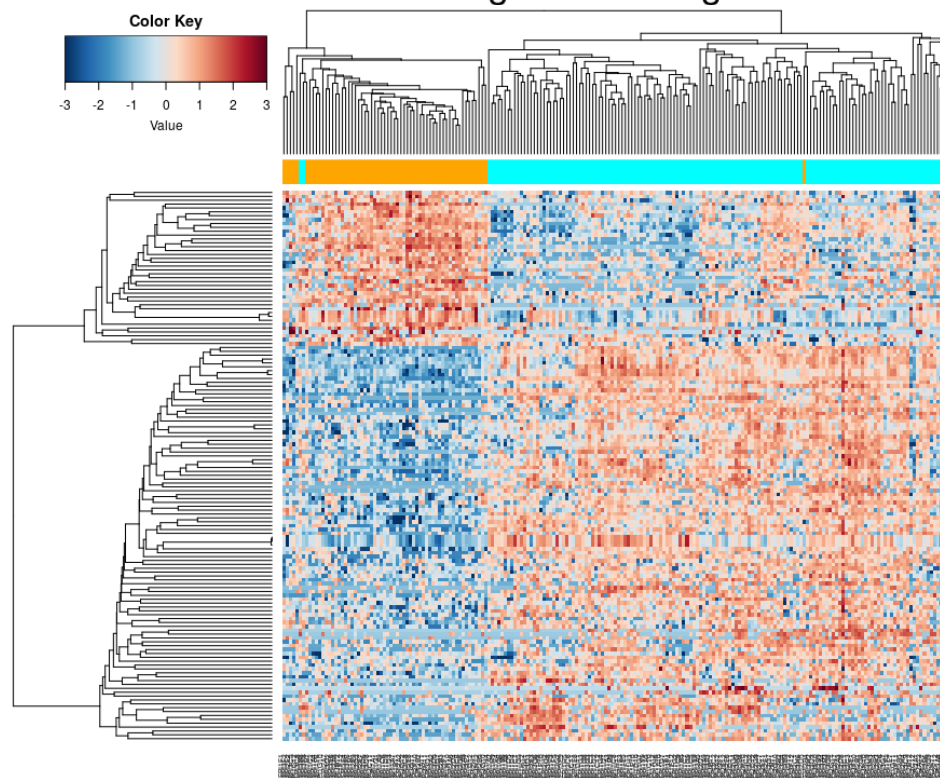
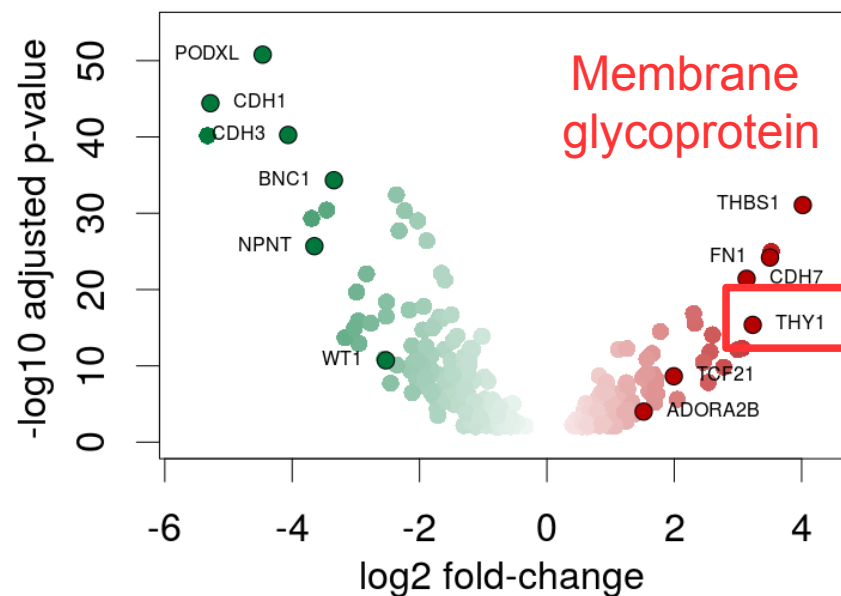
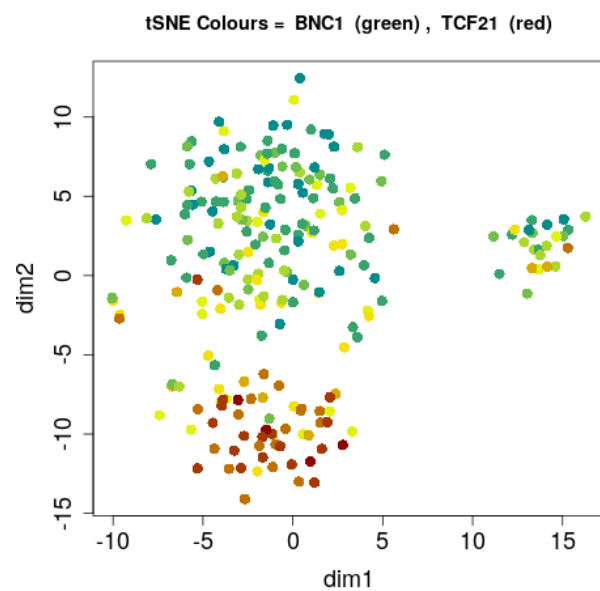
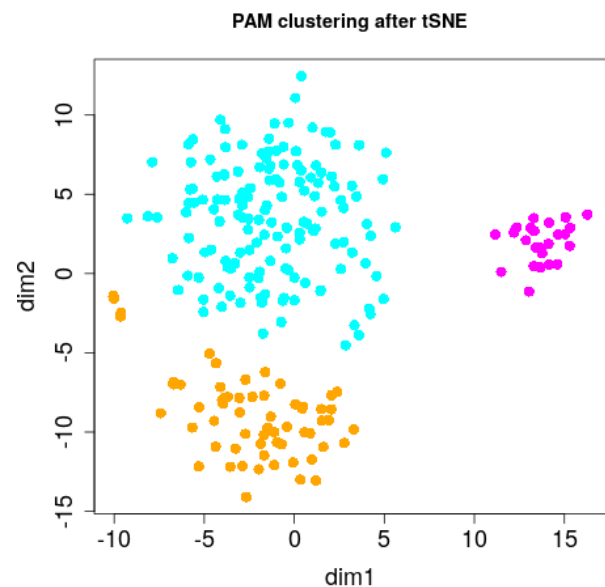
tSNE EPI23
before scLVM correction



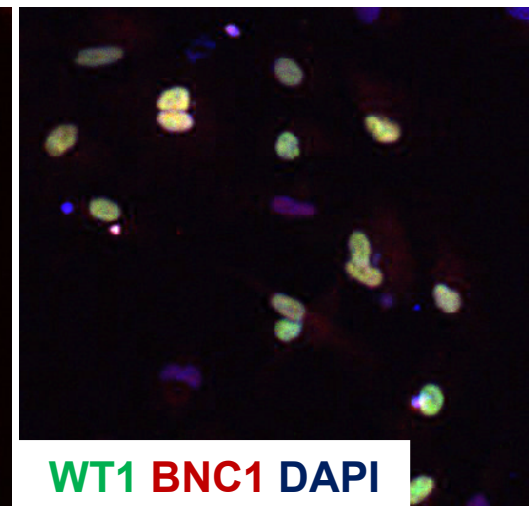
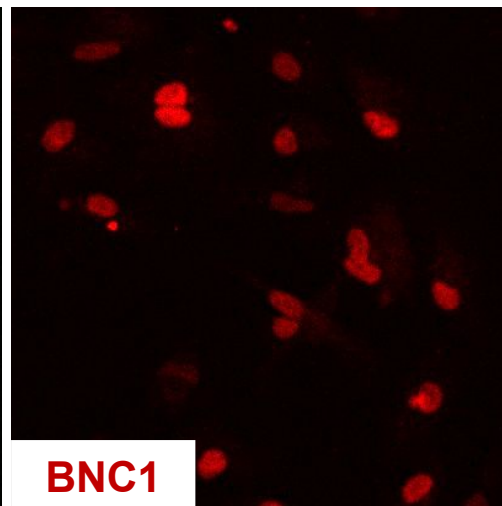
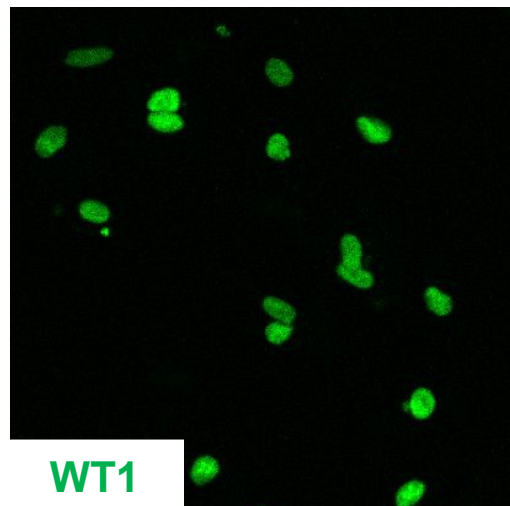
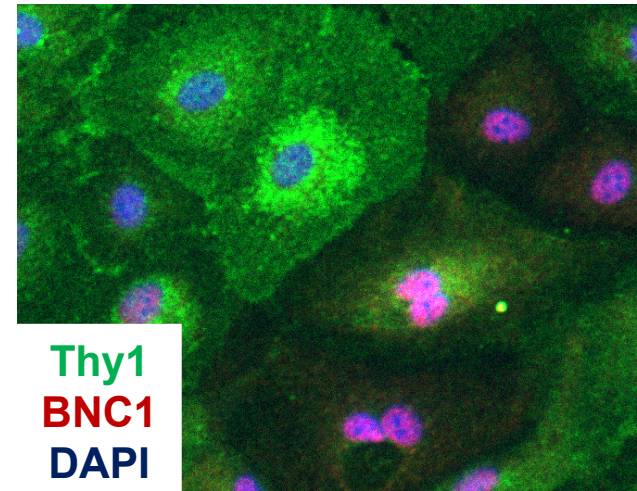
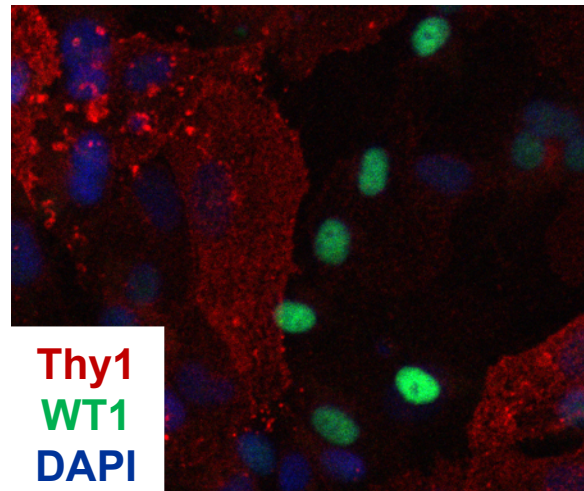
tSNE EPI23
after scLVM correction
for cell cycle







Validation in human samples (fetal 8 weeks)



**63% GFP⁺ Thy1⁺ hPSC-epi cells +
37% GFP⁻ unsorted hPSC-epi cells**

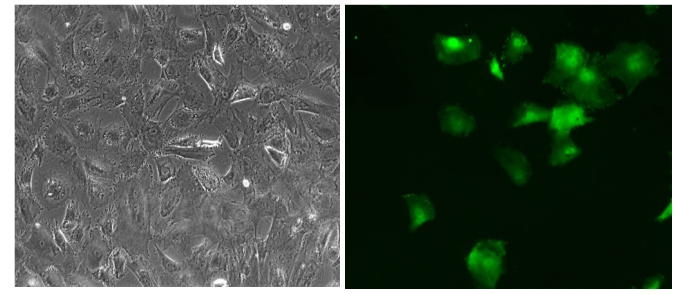
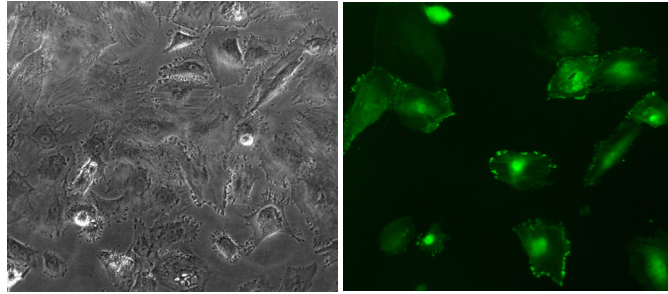
OR

**72% GFP⁺ Thy1⁻ hPSC-epi cells +
28% GFP⁻ unsorted hPSC-epi cells**

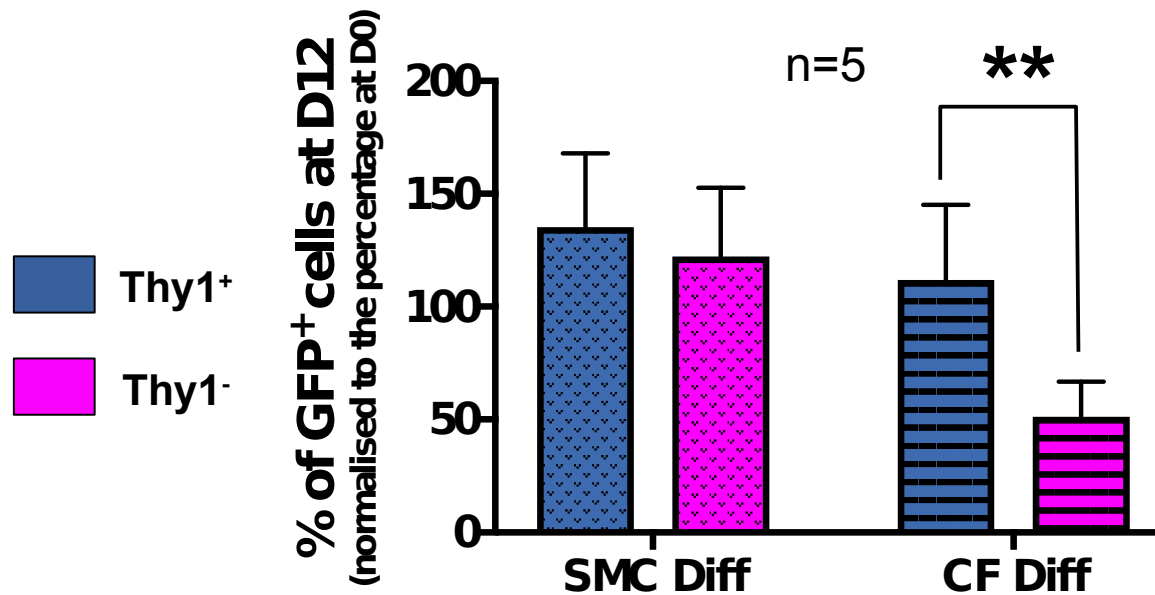
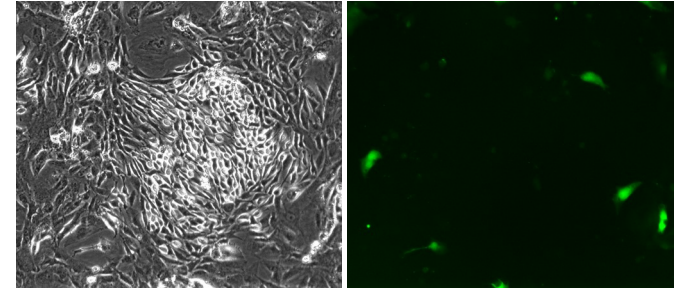
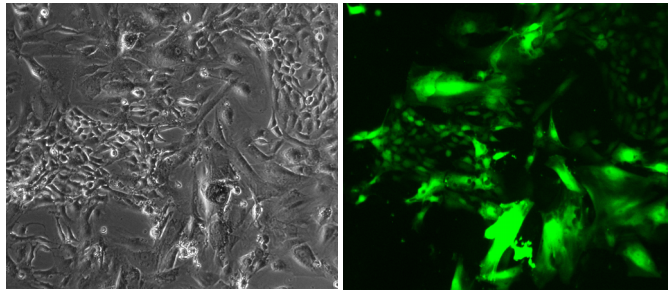
12 days ↓

12 days ↓

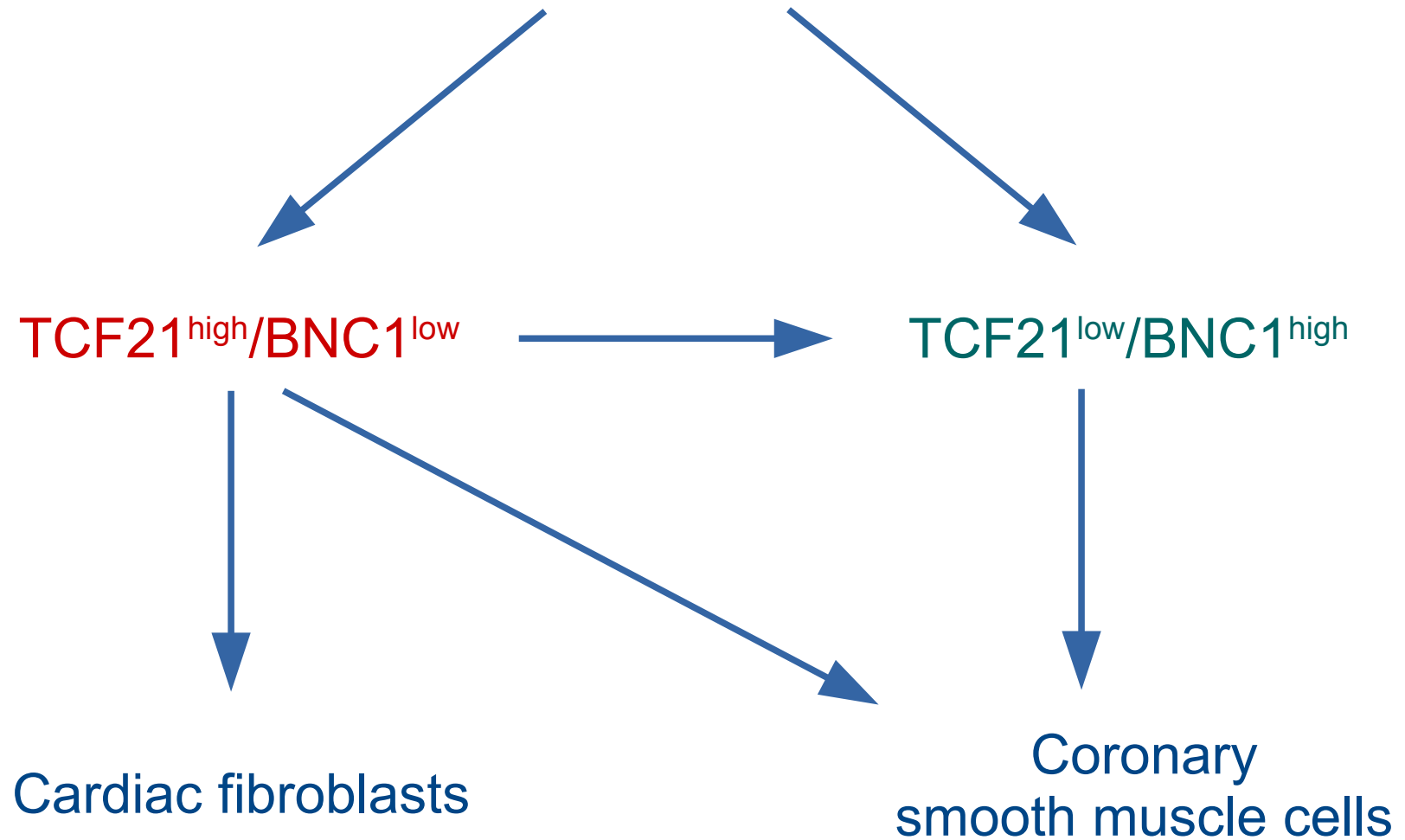
SMC
differentiation



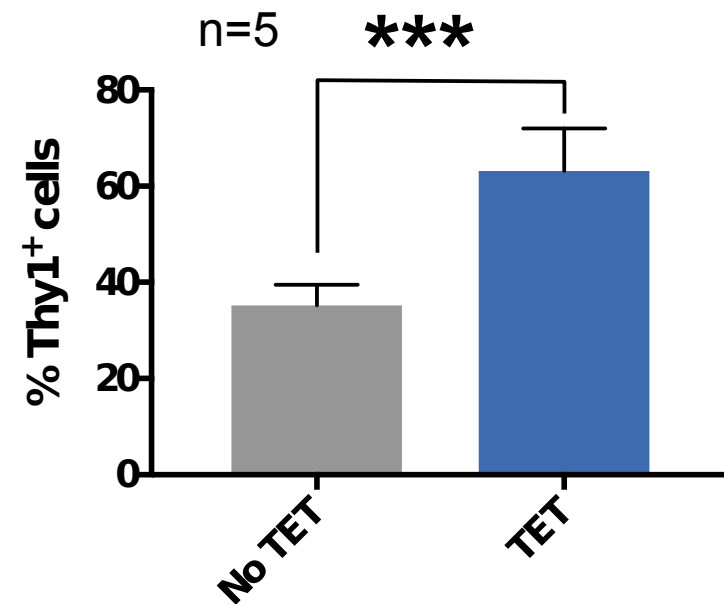
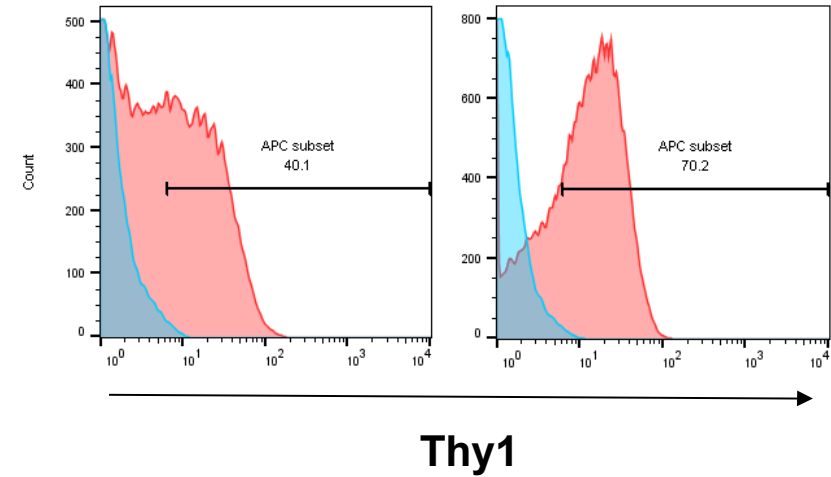
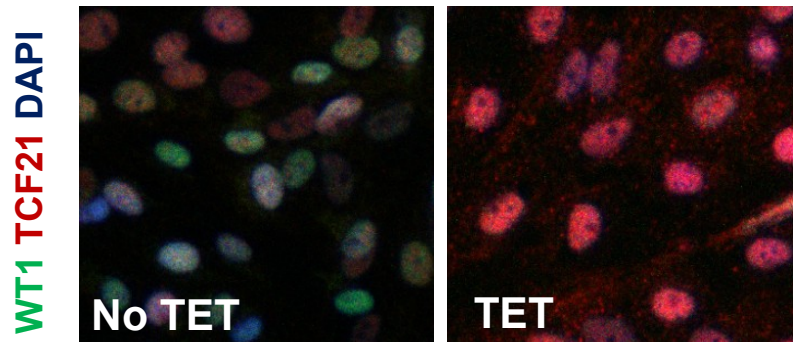
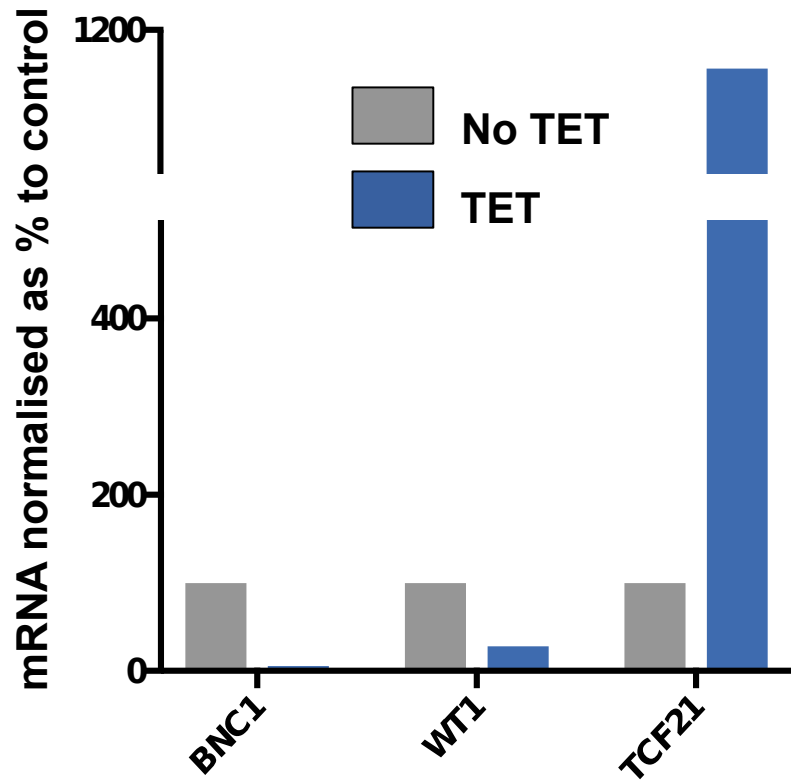
CF
differentiation



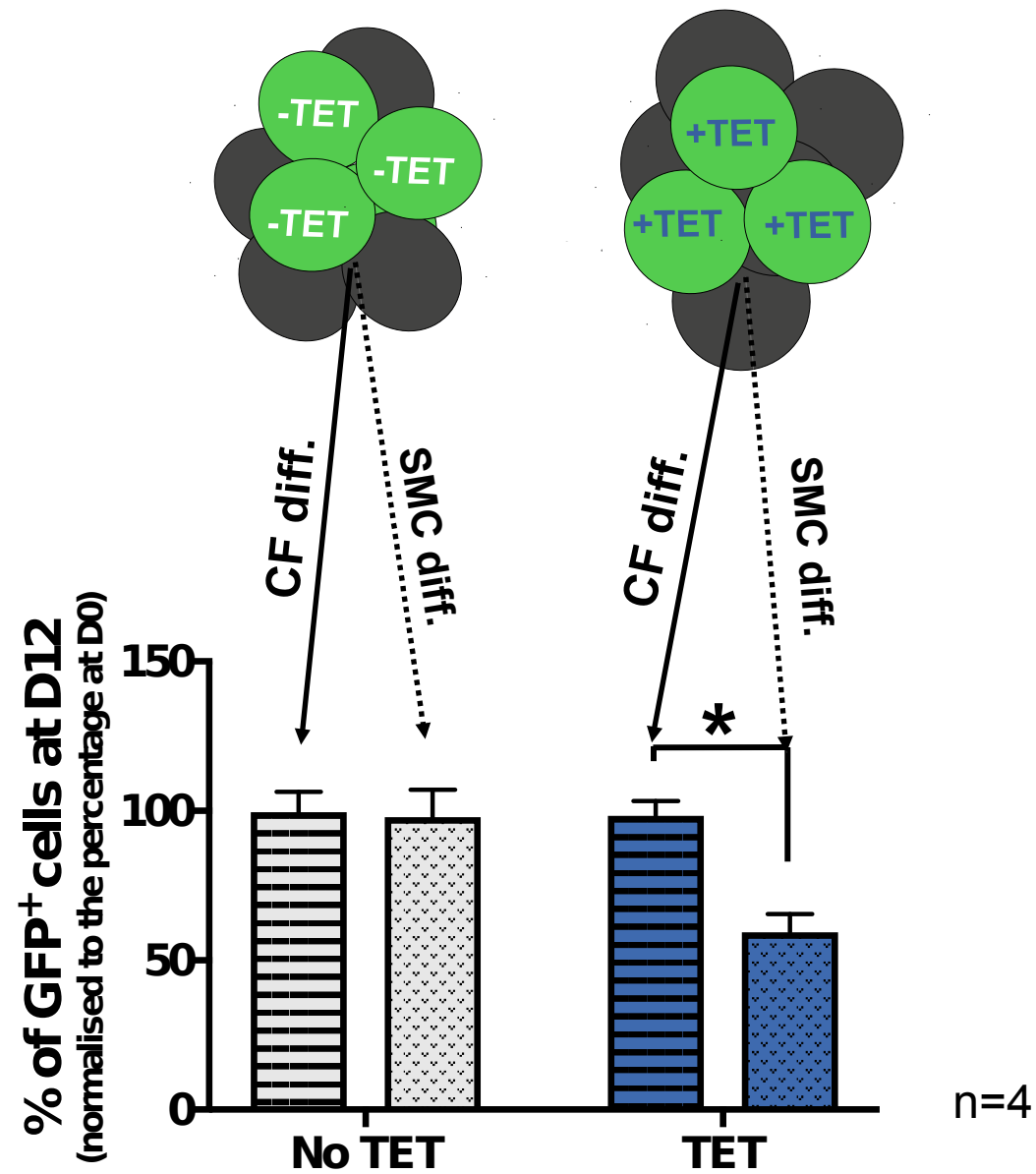
Lateral Plate Mesoderm



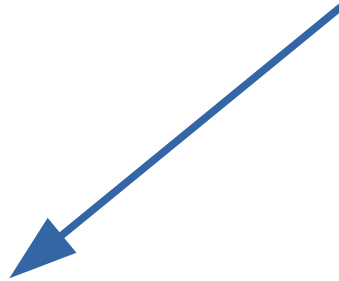
BNC1 KD favours TCF21 population



BNC1 KD favours CF differentiation



Lateral Plate Mesoderm



TCF21^{high}/BNC1^{low}



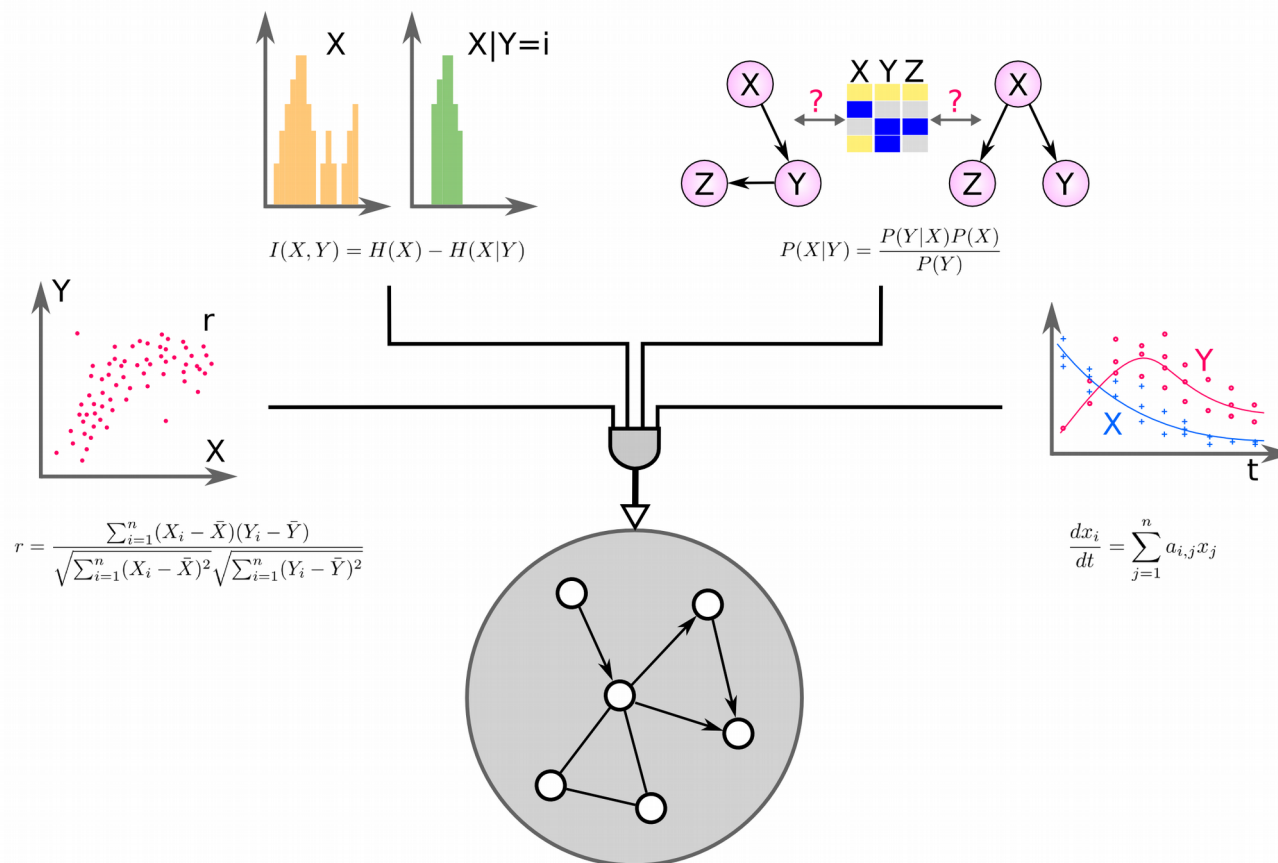
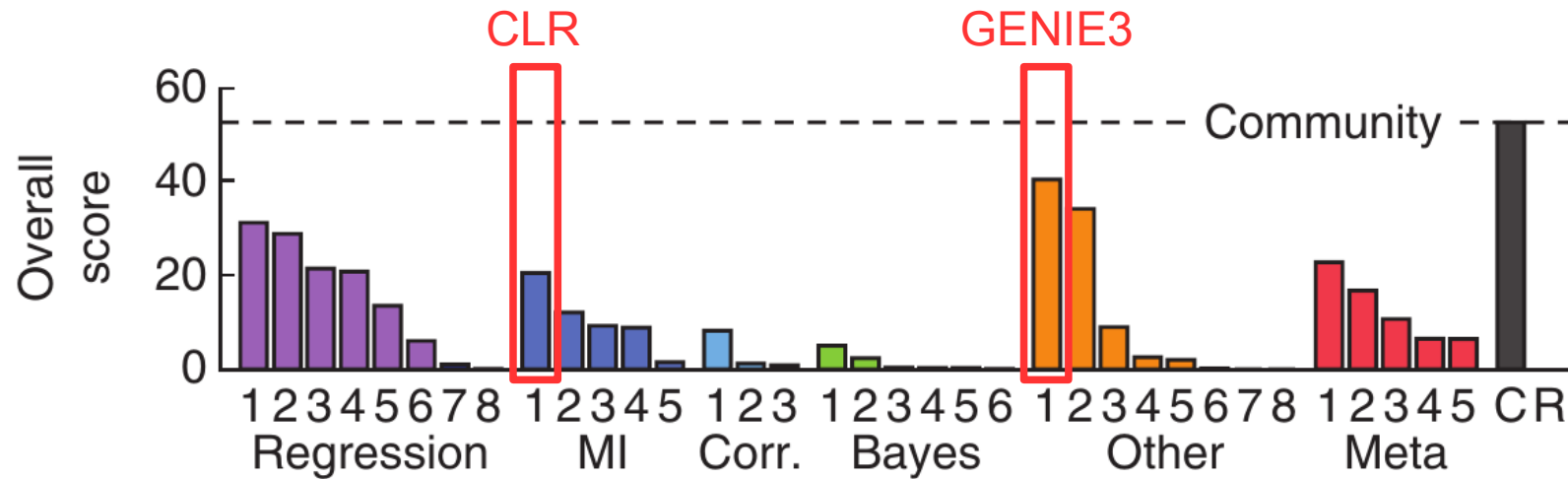
TCF21^{low}/BNC1^{high}



Cardiac fibroblasts



Coronary
smooth muscle cells



CLR (minet)

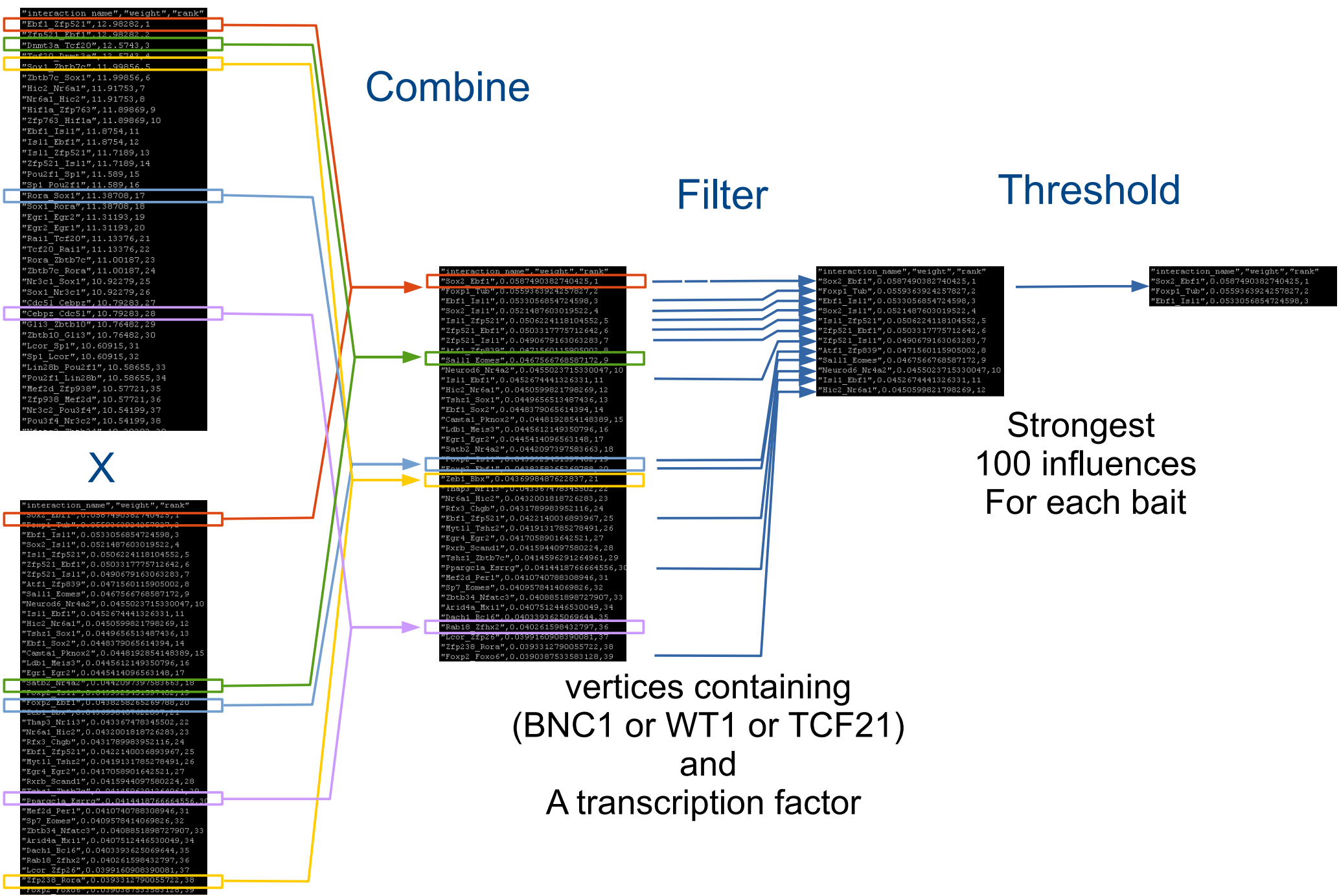
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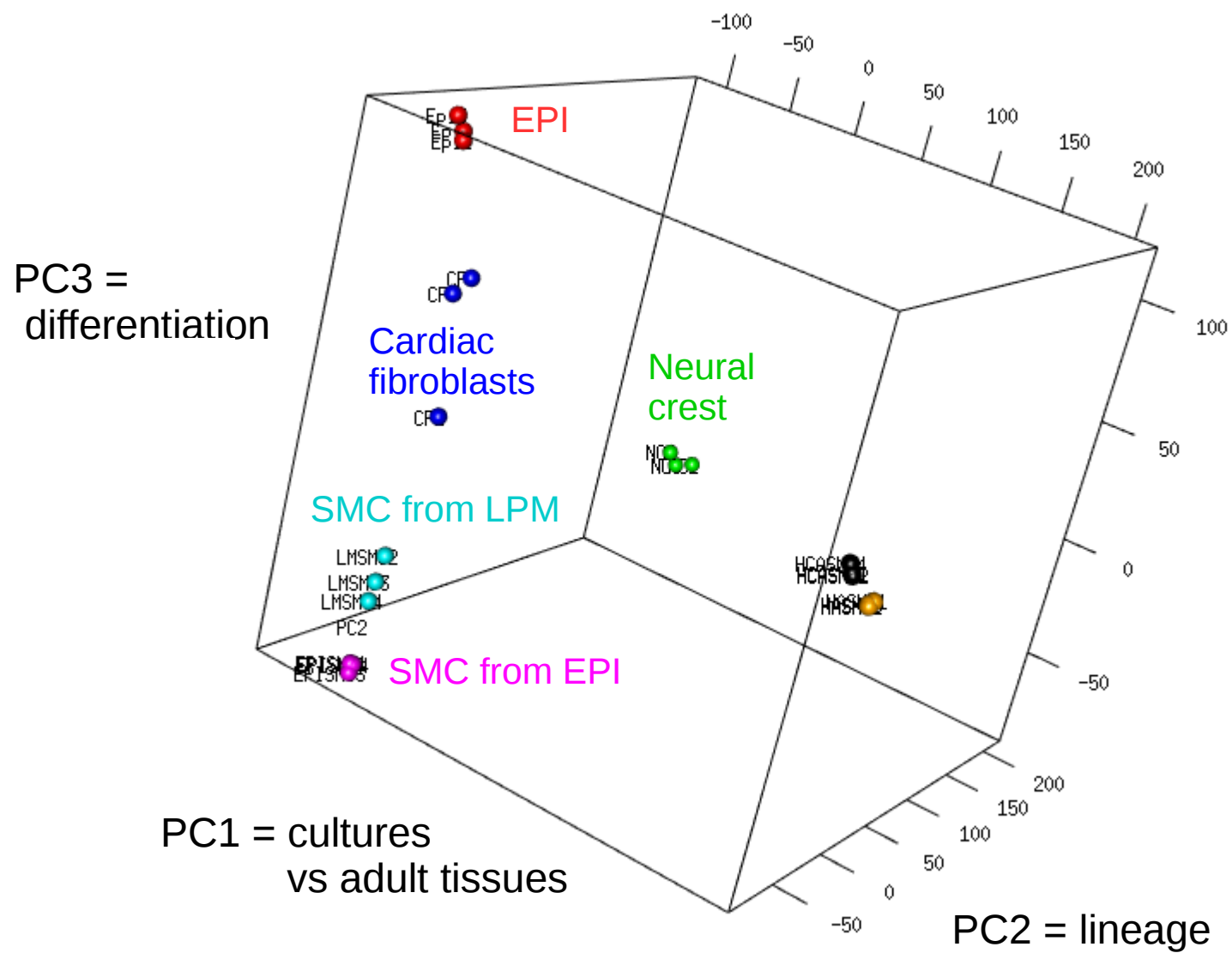
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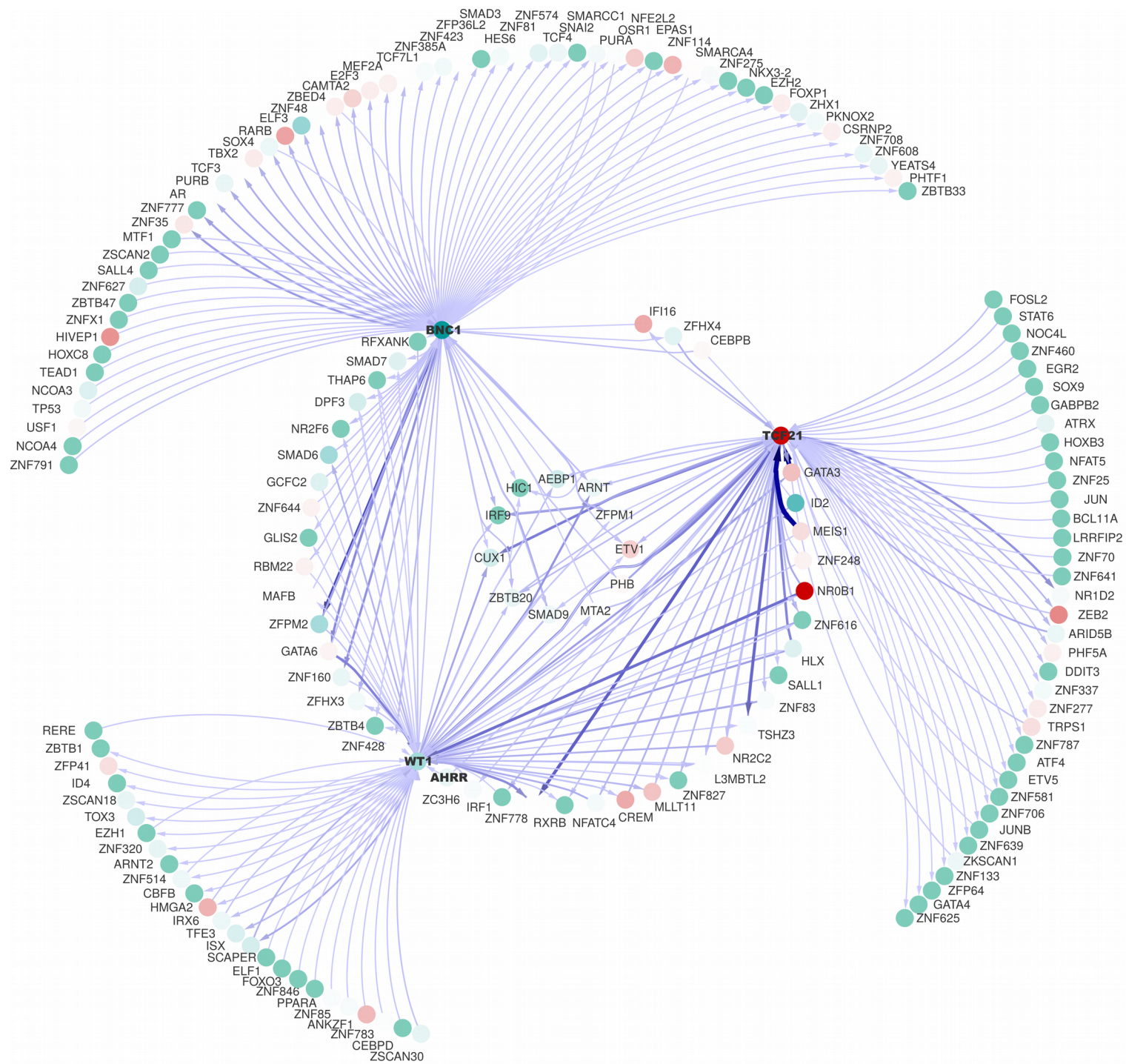
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directed







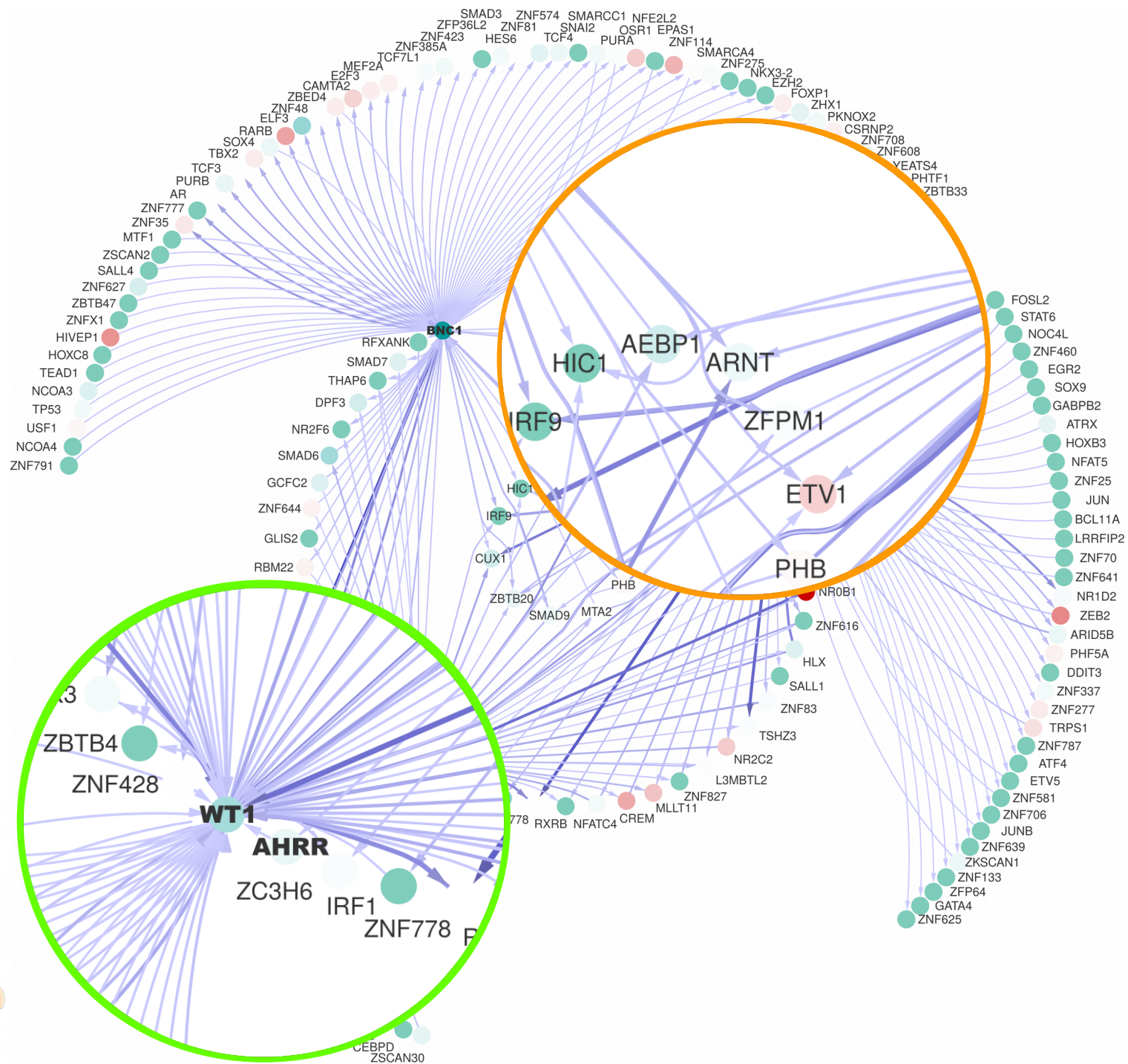
May 2017

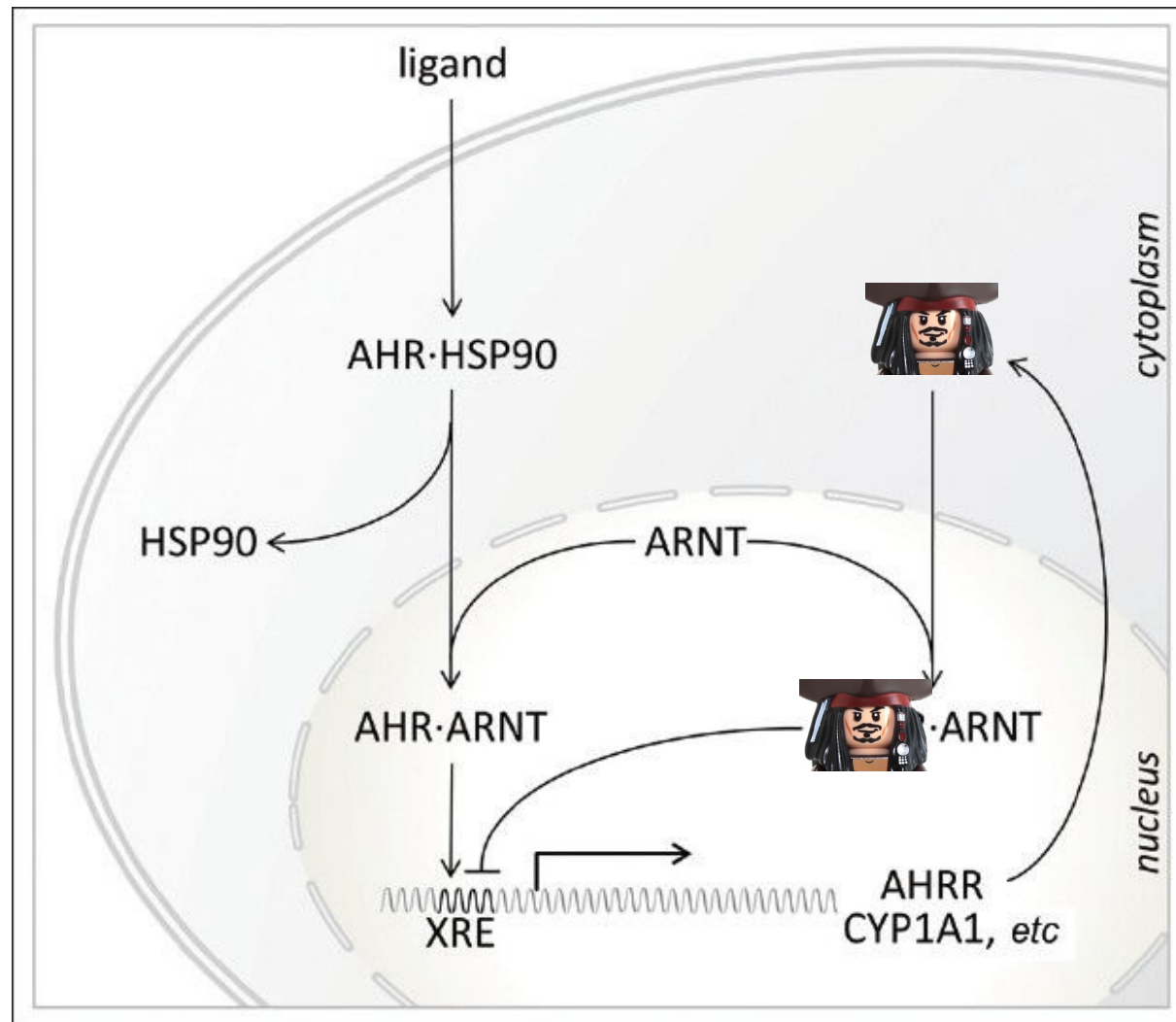
RESEARCH ARTICLE

TCF21 and the environmental sensor aryl-hydrocarbon receptor cooperate to activate a pro-inflammatory gene expression program in coronary artery smooth muscle cells

Juyong Brian Kim^{1,2}, Milos Pjanic^{1,2}, Trieu Nguyen¹, Clint L. Miller^{1,2}, Dharini Iyer^{1,2}, Boxiang Liu³, Ting Wang¹, Olga Sazonova¹, Ivan Carcamo-Orive¹, Ljubica Perisic Matic⁴, Lars Maegdefessel⁴, Ulf Hedin⁴, Thomas Quertermous^{1,2*}

Ahr = dioxin receptor

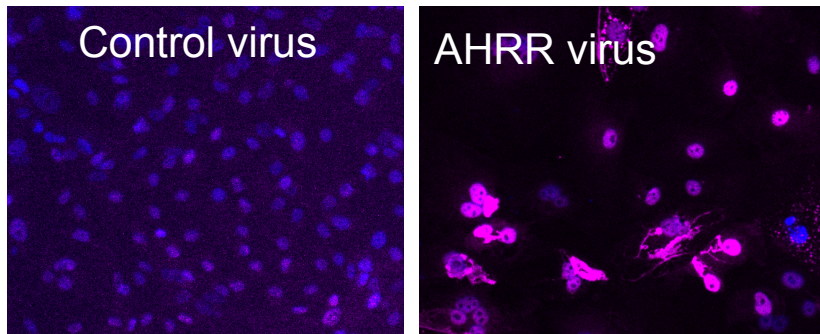




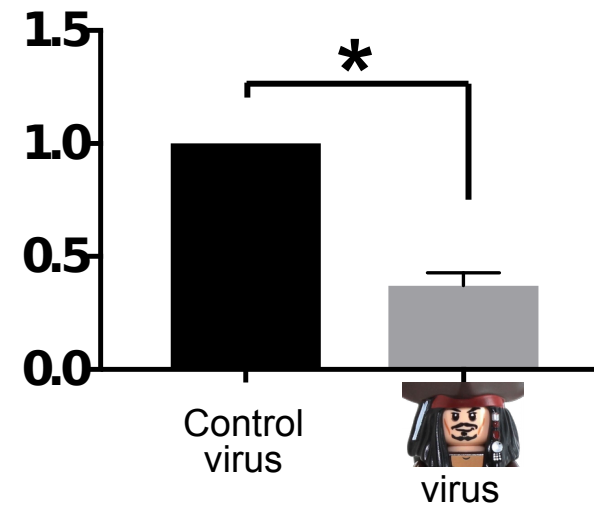
Brokken *et al* (2014)

Overexpression of inhibits TCF21

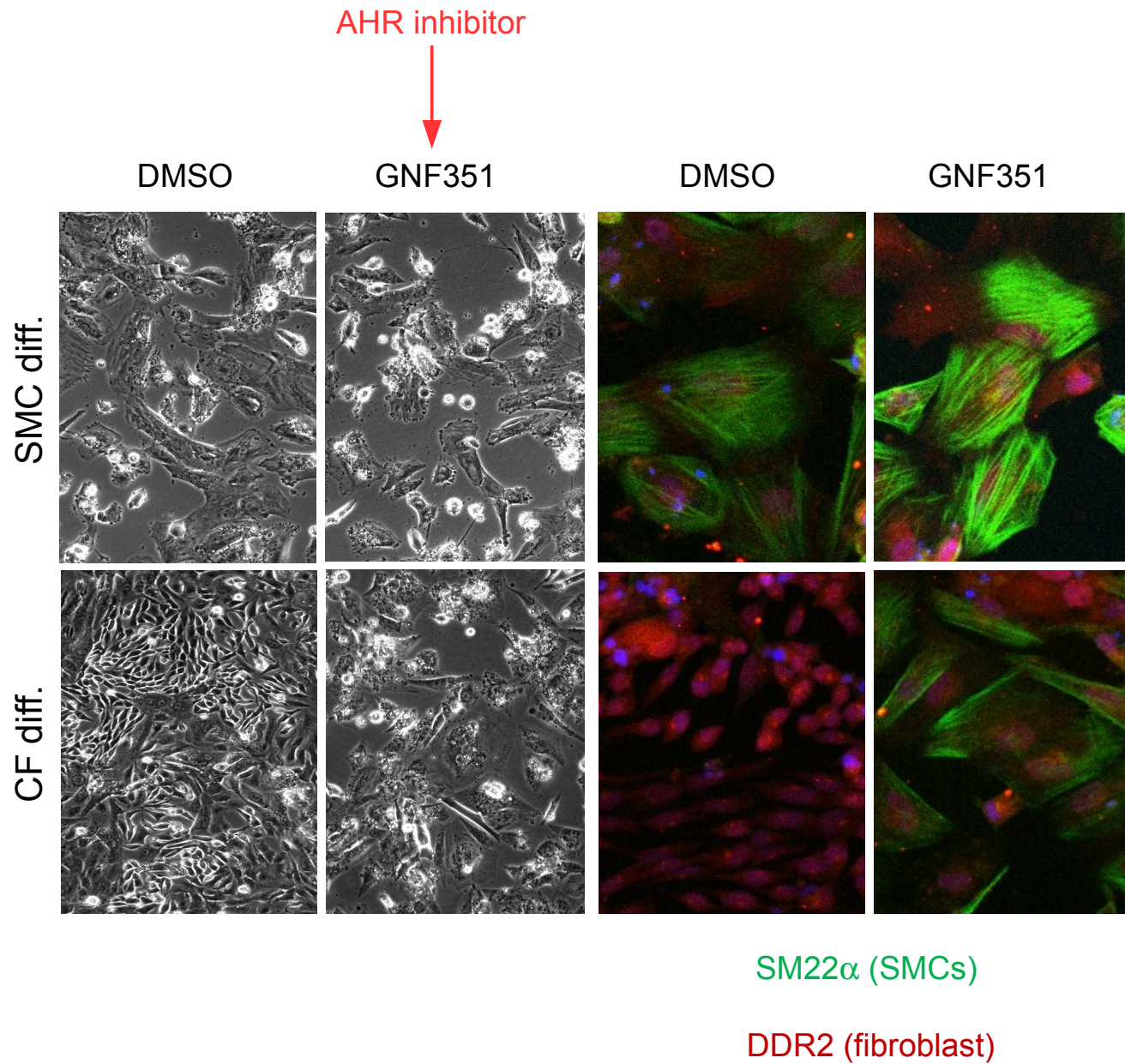
Overexpression of 
detected by an anti- (purple)



TCF21 level of expression
(compared to GAPDH and
normalised to the control virus)



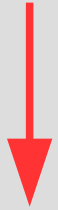
AHR inhibition suppresses CF differentiation



Lateral Plate Mesoderm



TCF21^{high}/BNC1^{low}



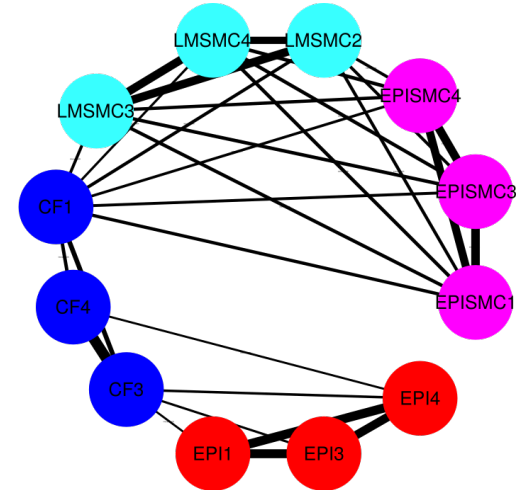
Cardiac fibroblasts



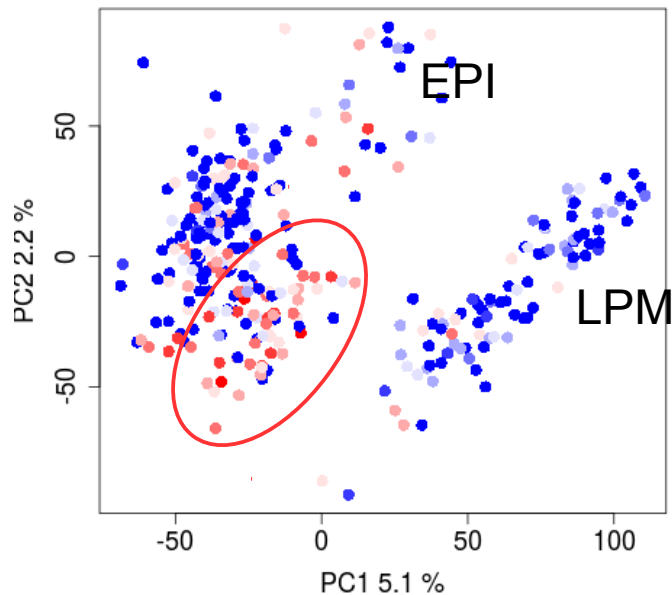
TCF21^{low}/BNC1^{high}



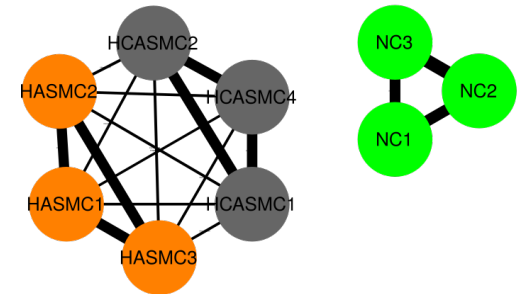
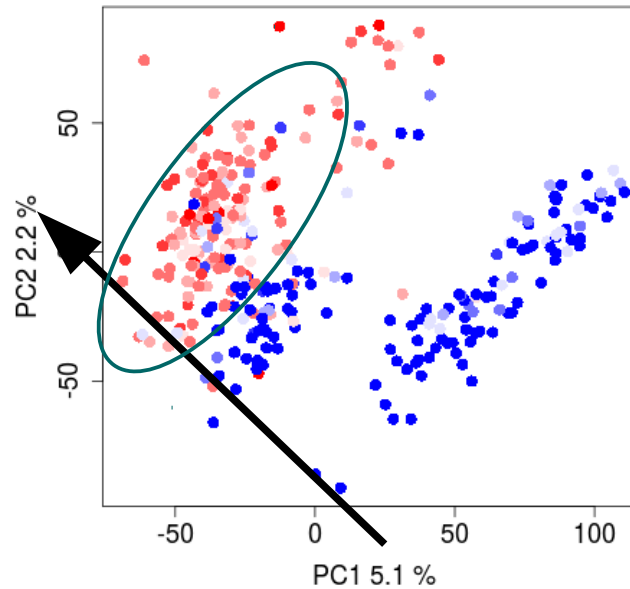
Coronary
smooth muscle cells

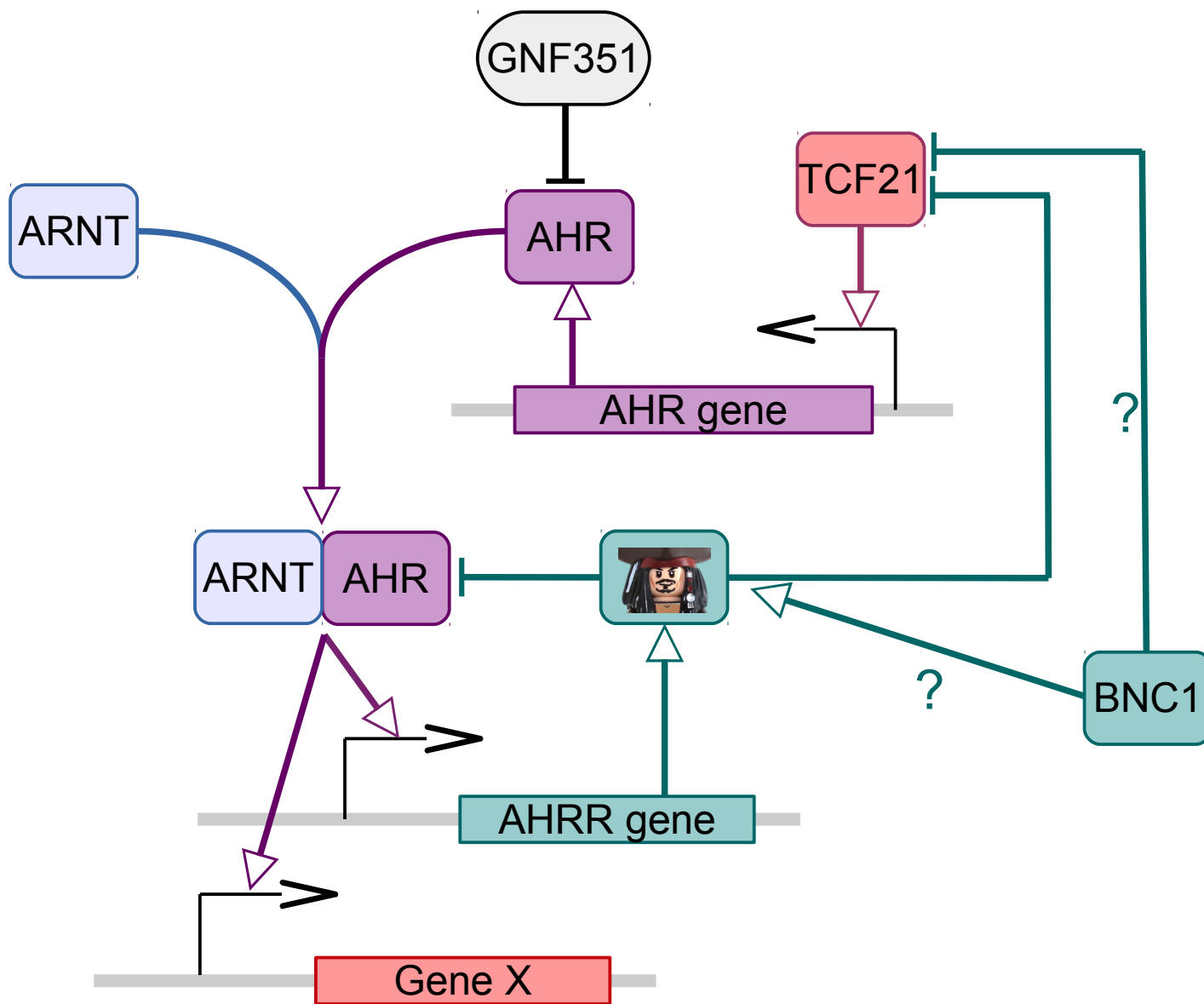


TCF21

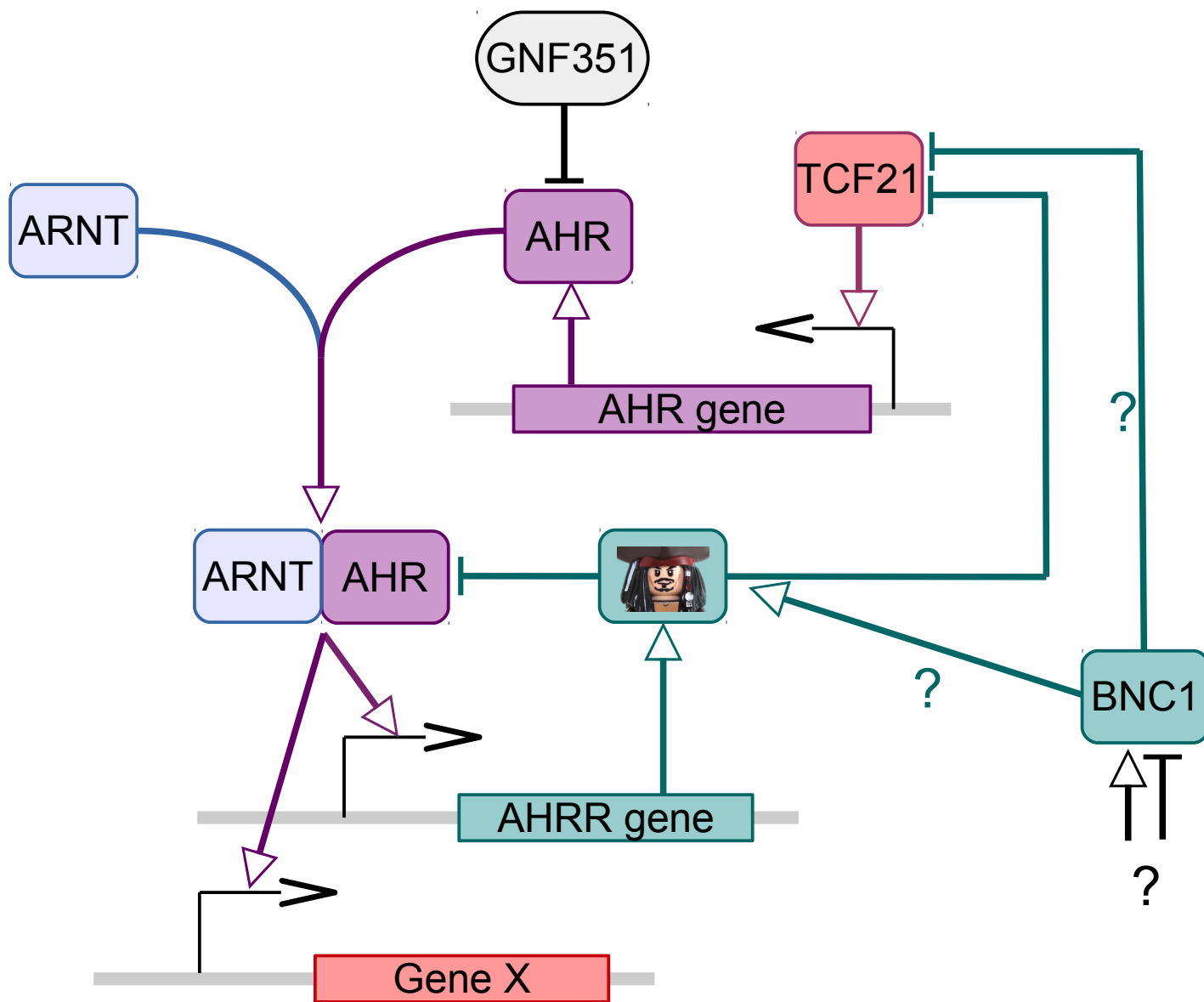


BNC1





Important for fibroblast differentiation



Important for fibroblast differentiation

Collaborators

Sanjay Sinha
(Cambridge Stem Cell Institute)

Laure Gambardella

Sophie McNamus
William Bernard

Paul Riley (Oxford University)

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Vicky Moignard

Le Novere Lab

Agathe Delaune

Derya Sebukhan

Vincent Knight-Shrijver

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Sequencing

Kristina Tabadda

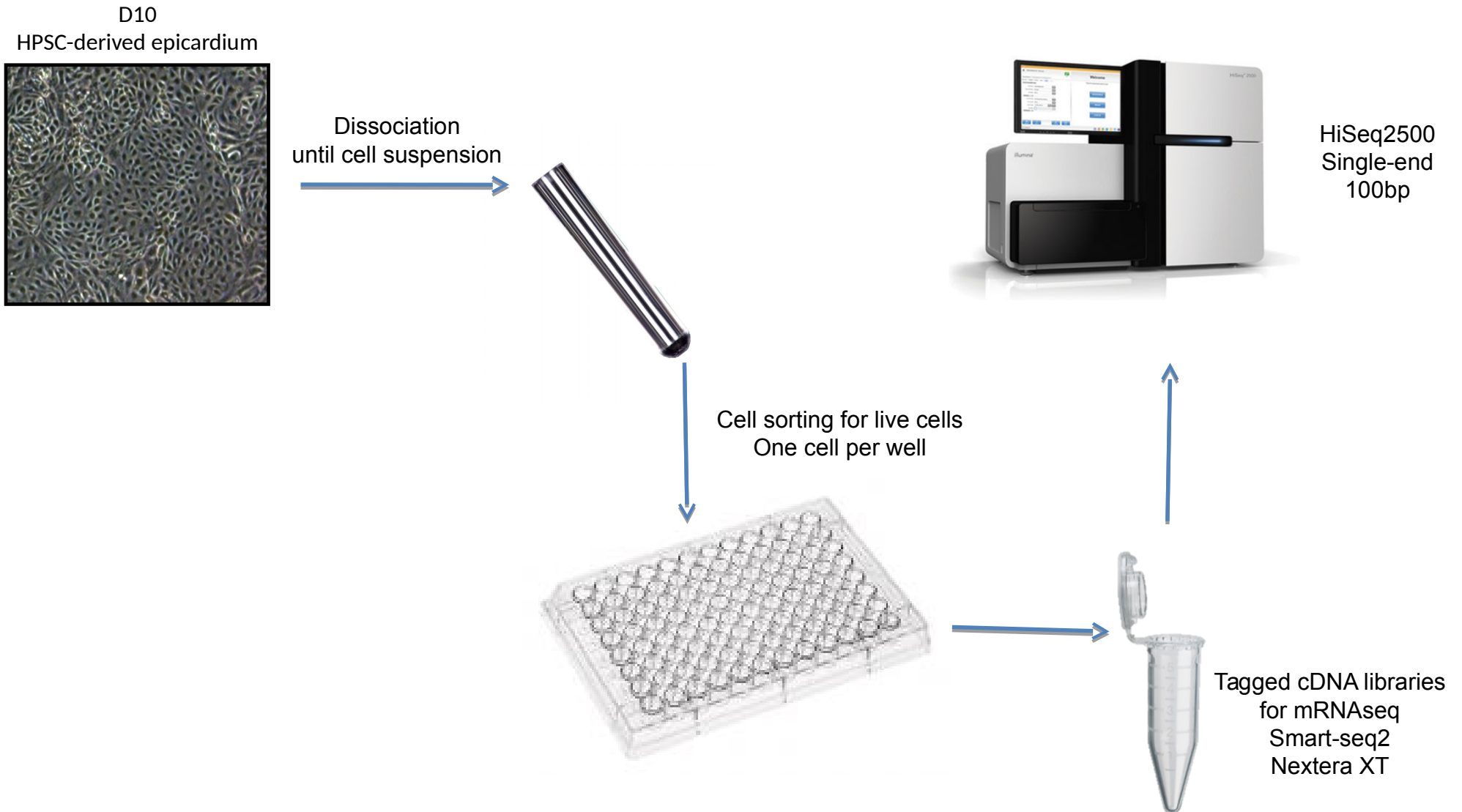
Clare Murnane

Bioinformatics

Simon Andrews



Single Cell RNA-Seq



Revealing New Mouse Epicardial Cell Markers through Transcriptomics

Lars Bochmann^{1,2,3*}, Padmini Sarathchandra², Federica Mori³, Enrique Lara-Pezzi^{1,4}, Domenico Lazzaro³, Nadia Rosenthal^{1,2}

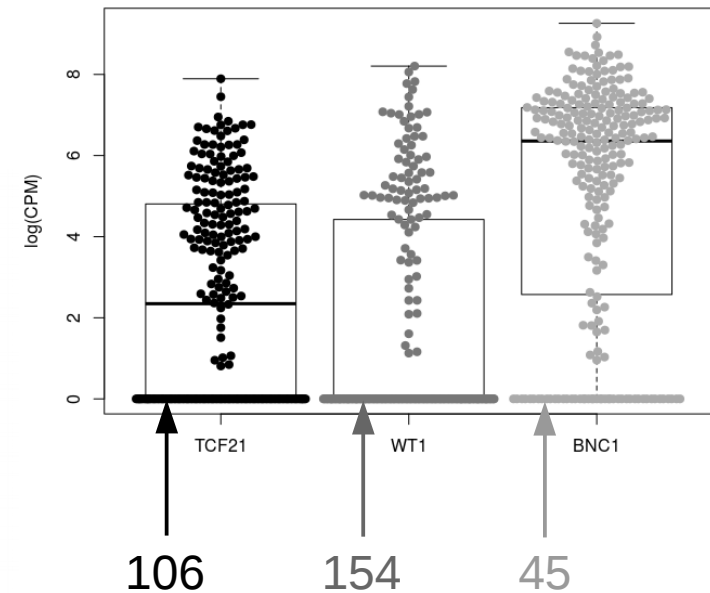
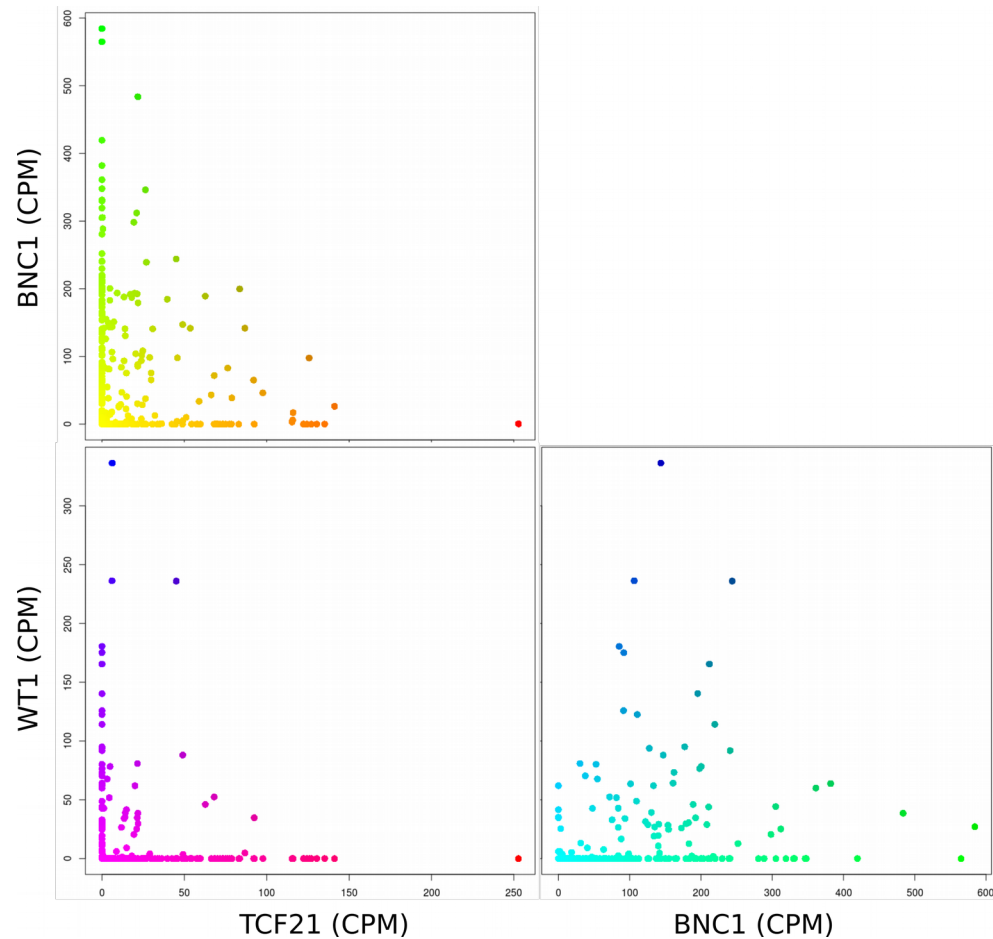
1 Mouse Biology Unit, European Molecular Biology Laboratory, Monterotondo, Italy, **2** Harefield Heart Science Centre, Imperial College London, London, United Kingdom, **3** Istituto di Ricerche di Biologia Molecolare P. Angeletti SpA, Pomezia, Italy, **4** Cardiovascular Development Biology Department, Centro Nacional de Investigaciones Cardiovasculares – CNIC, Madrid, Spain

Abstract

Background: The epicardium has key functions during myocardial development, by contributing to the formation of coronary endothelial and smooth muscle cells, cardiac fibroblasts, and potentially cardiomyocytes. The epicardium plays a morphogenetic role by emitting signals to promote and maintain cardiomyocyte proliferation. In a regenerative context, the adult epicardium might comprise a progenitor cell population that can be induced to contribute to cardiac repair. Although some genes involved in epicardial function have been identified, a detailed molecular profile of epicardial gene expression has not been available.

Methodology: Using laser capture microscopy, we isolated the epicardial layer from the adult murine heart before or after cardiac infarction in wildtype mice and mice expressing a transgenic IGF-1 propeptide (mIGF-1) that enhances cardiac repair, and analyzed the transcription profile using DNA microarrays.

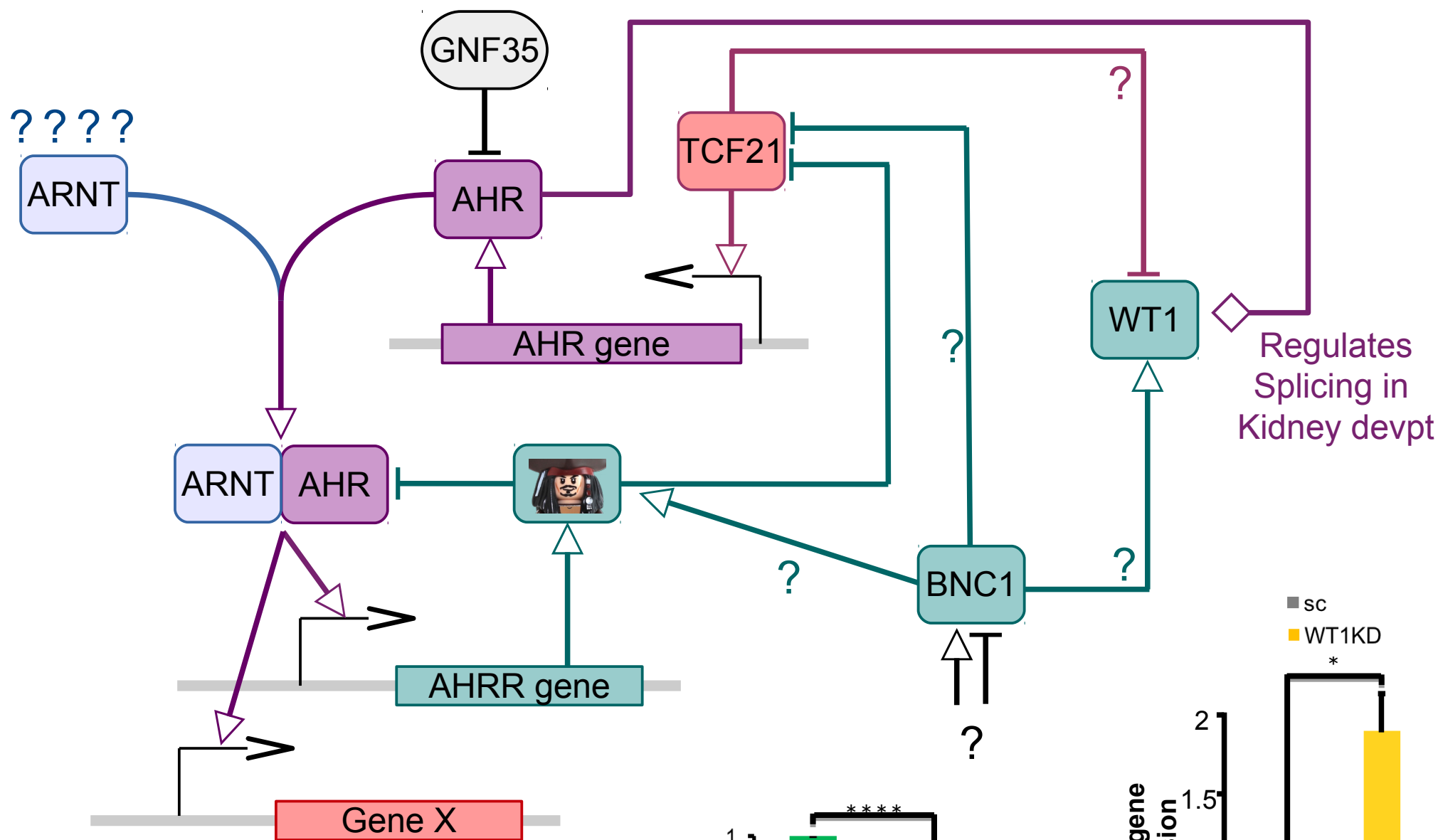
Dropouts => We cannot separate cells based on single gene expressions



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"Zfp238_Rora",	0.0393312790055722,	38
"Foxp2_Foxo6",	0.0390387533583128,	39

Consistent results!!!



Important for fibroblast differentiation

