Exploring the mechanisms of haematopoietic lineage progression at the single-cell level

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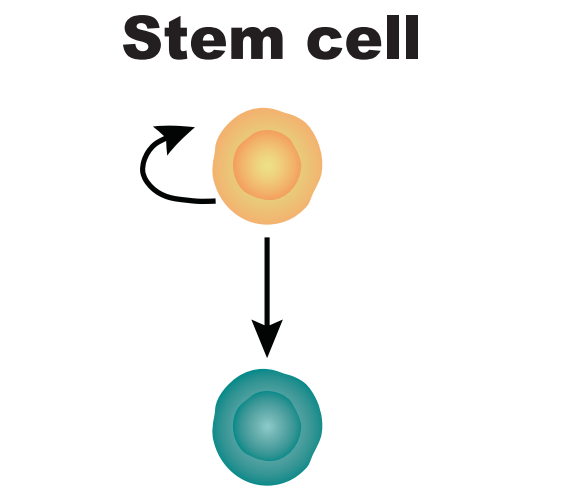
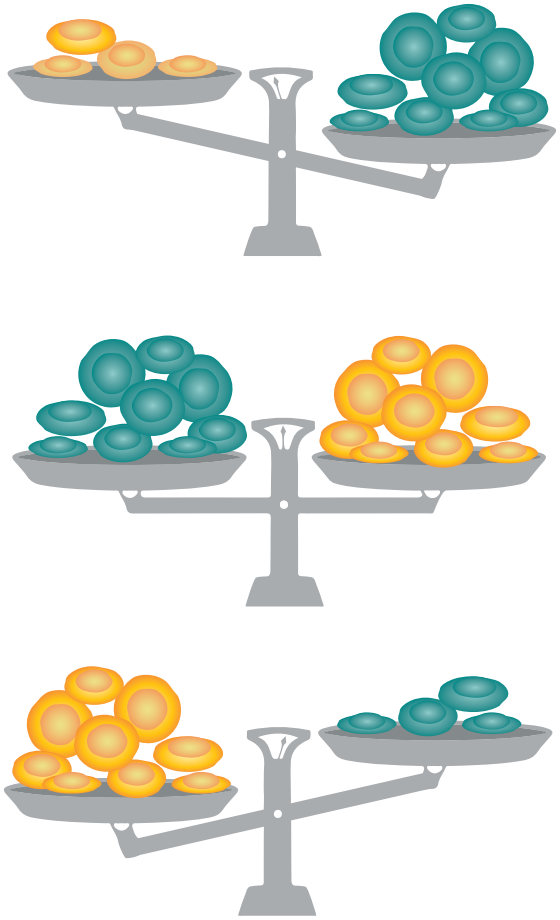
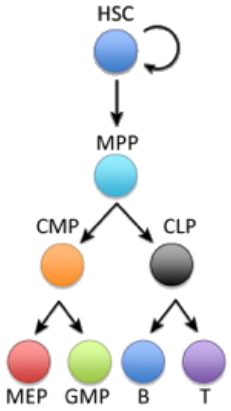
Wellcome Trust – Medical Research Council, Cambridge Stem Cell Institute

How do HSC Differentiate into Diverse Cell Types?

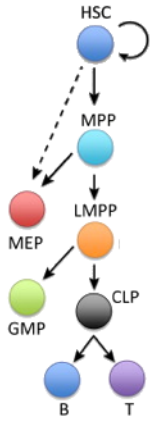




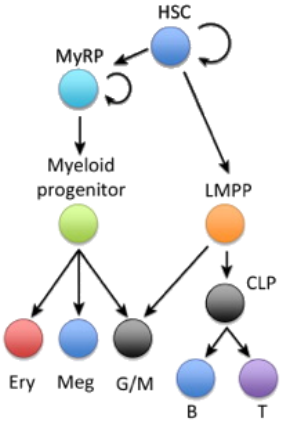
Traditional Haematopoietic Lineage Tree is Incorrect?



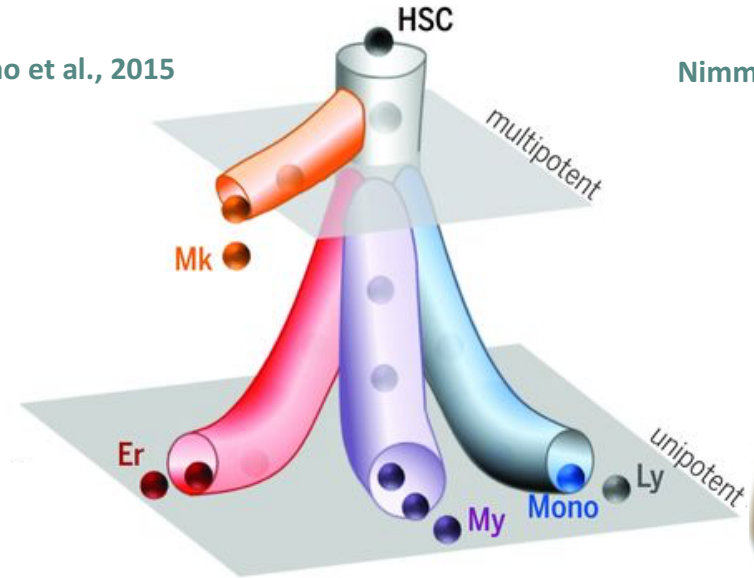
Nimmo et al., 2015



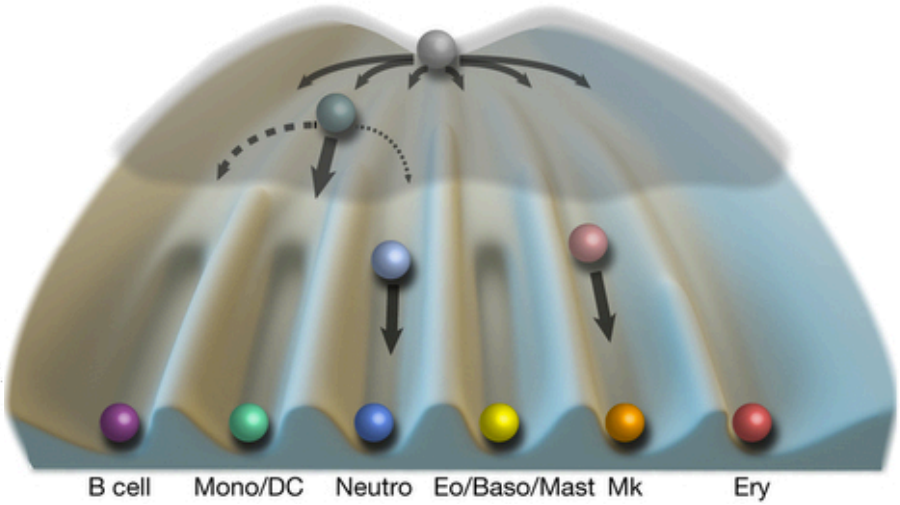
Nimmo et al., 2015



Nimmo et al., 2015

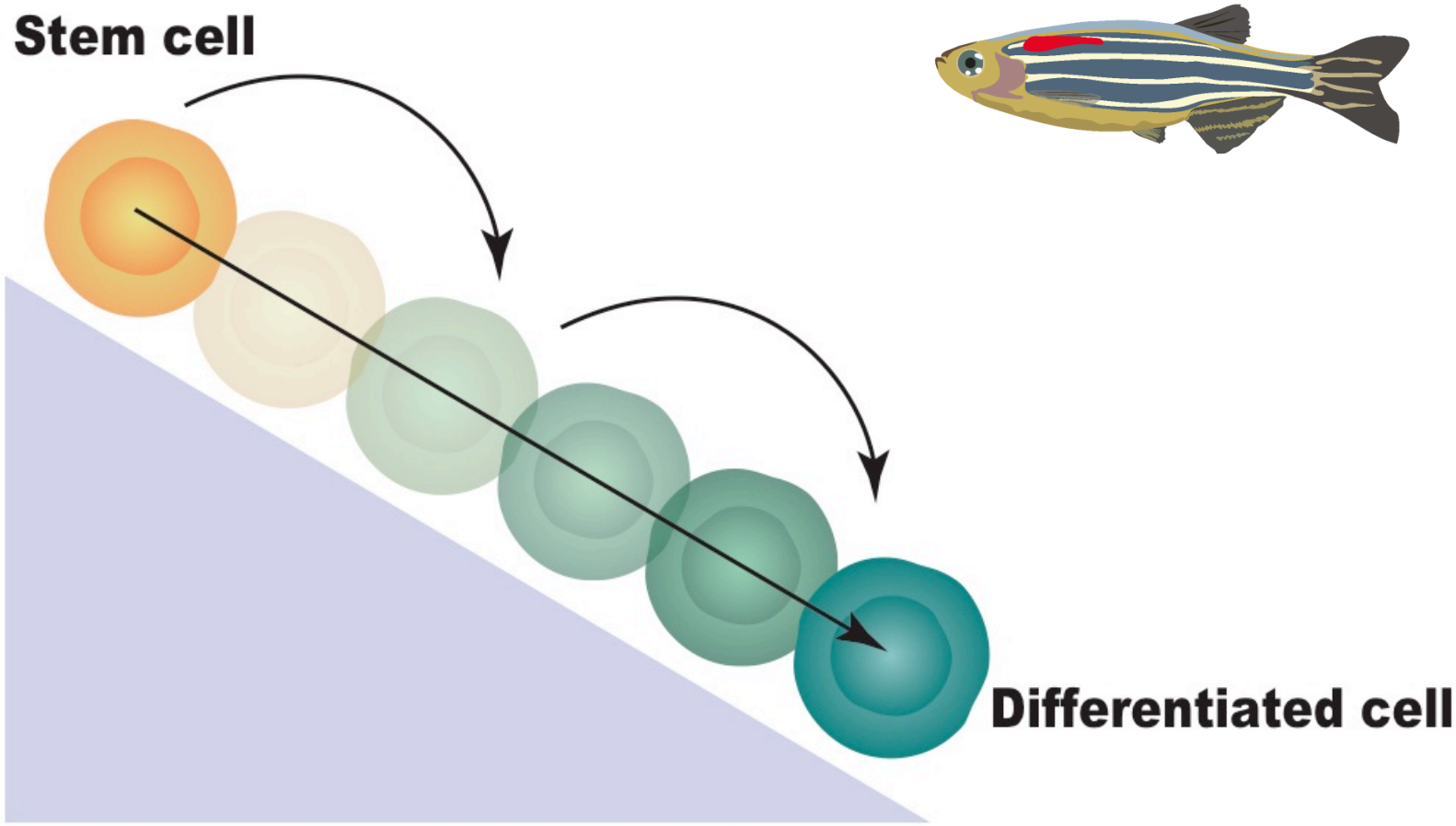


Notta et al., 2016

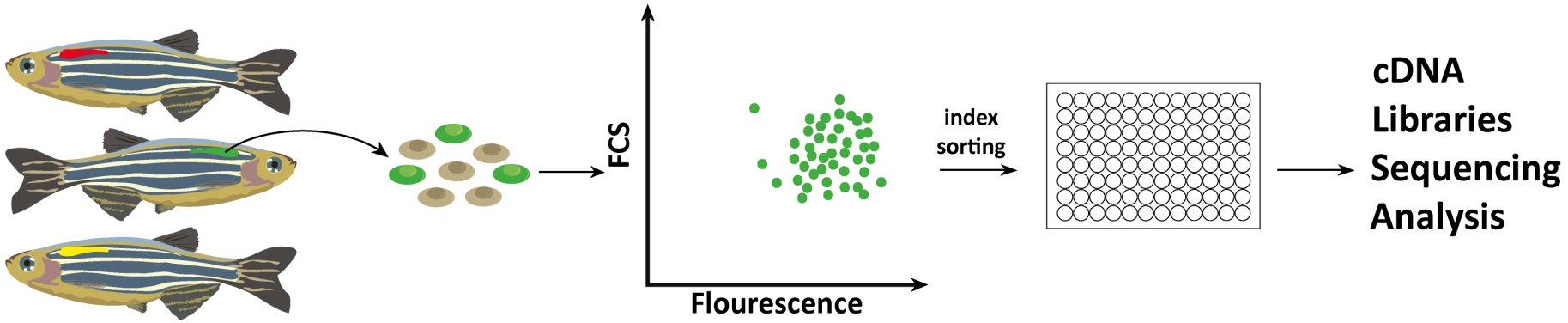


Velten et al., 2017

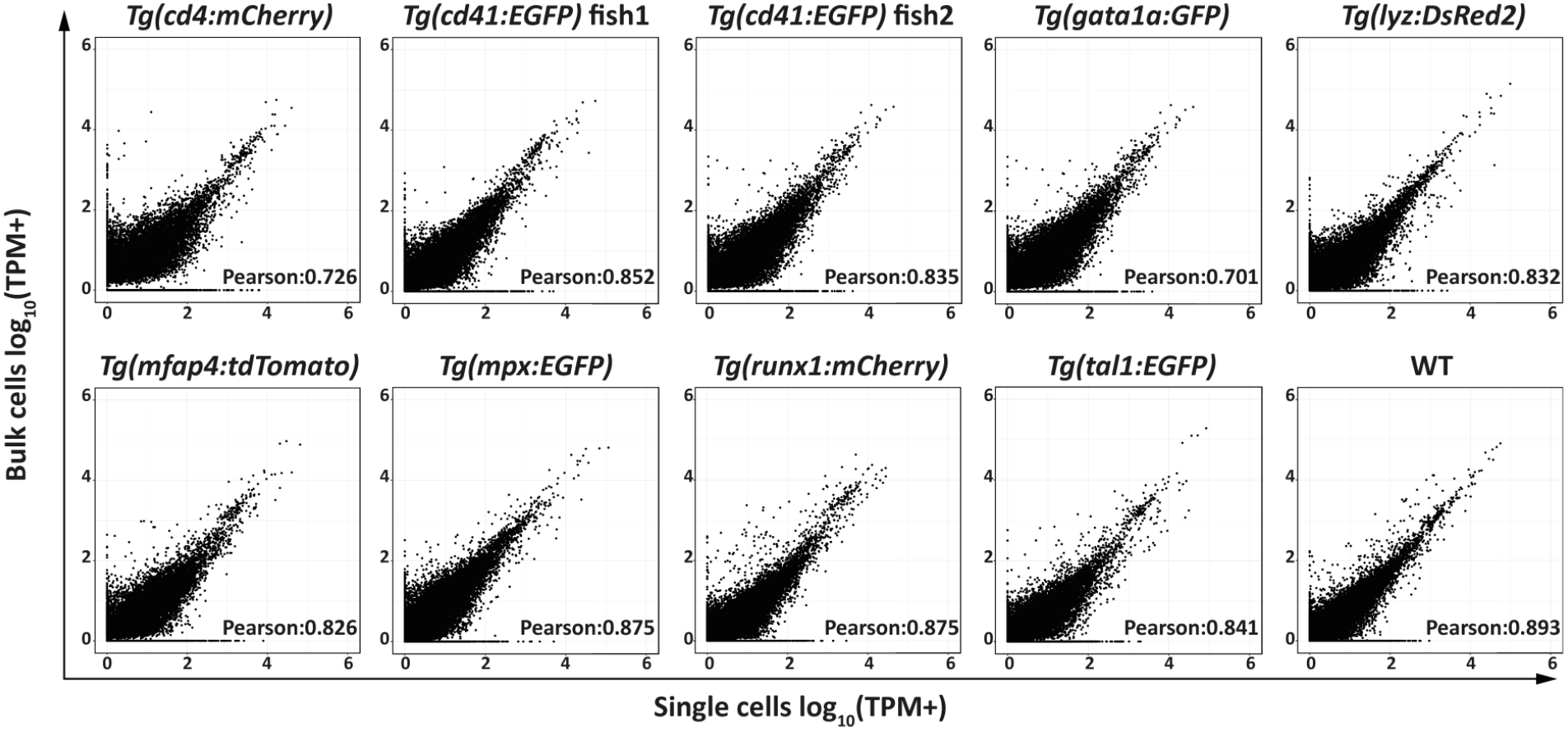
Cellular Decisions: Discrete Steps or a Gradual Process?



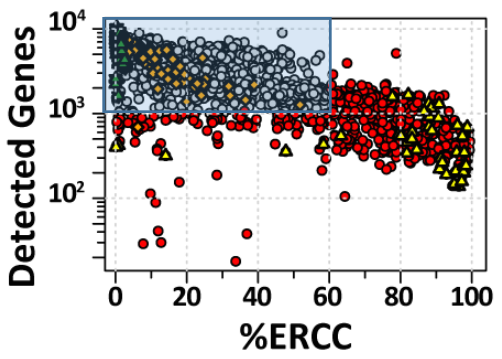
Overview of the Experimental Strategy

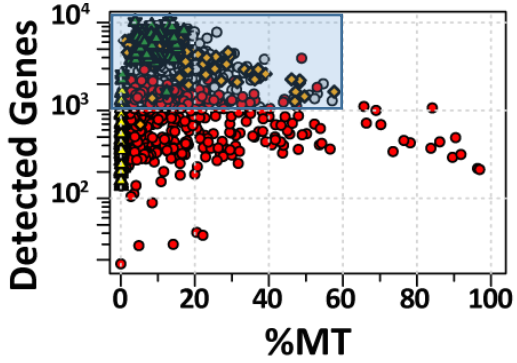


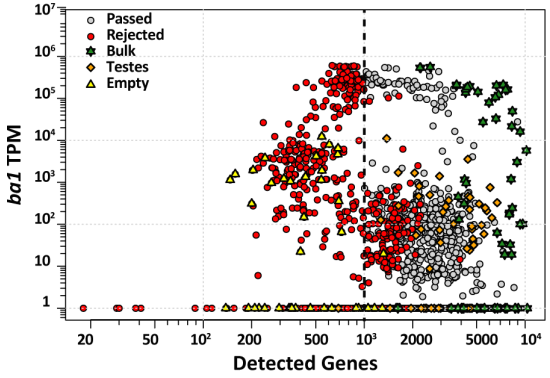
single cell expression profiles were effectively quantified

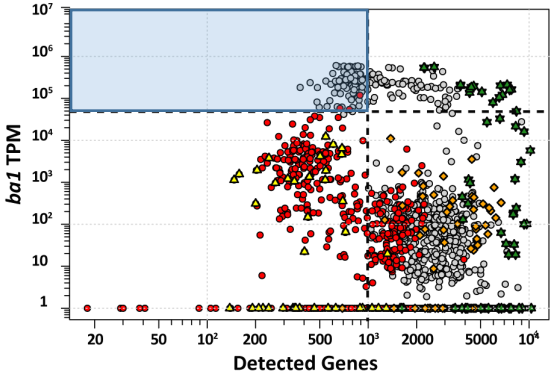


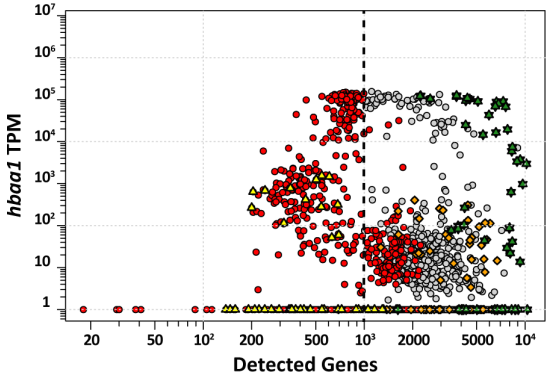
Quality Control of Single Cells

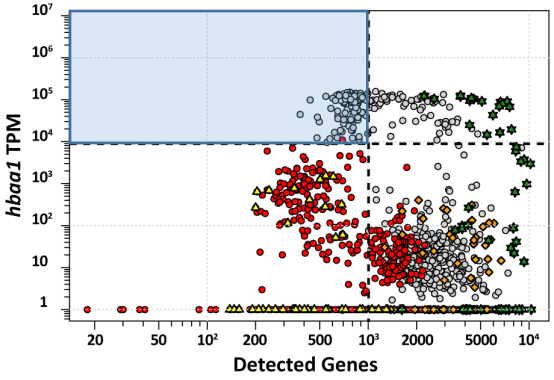








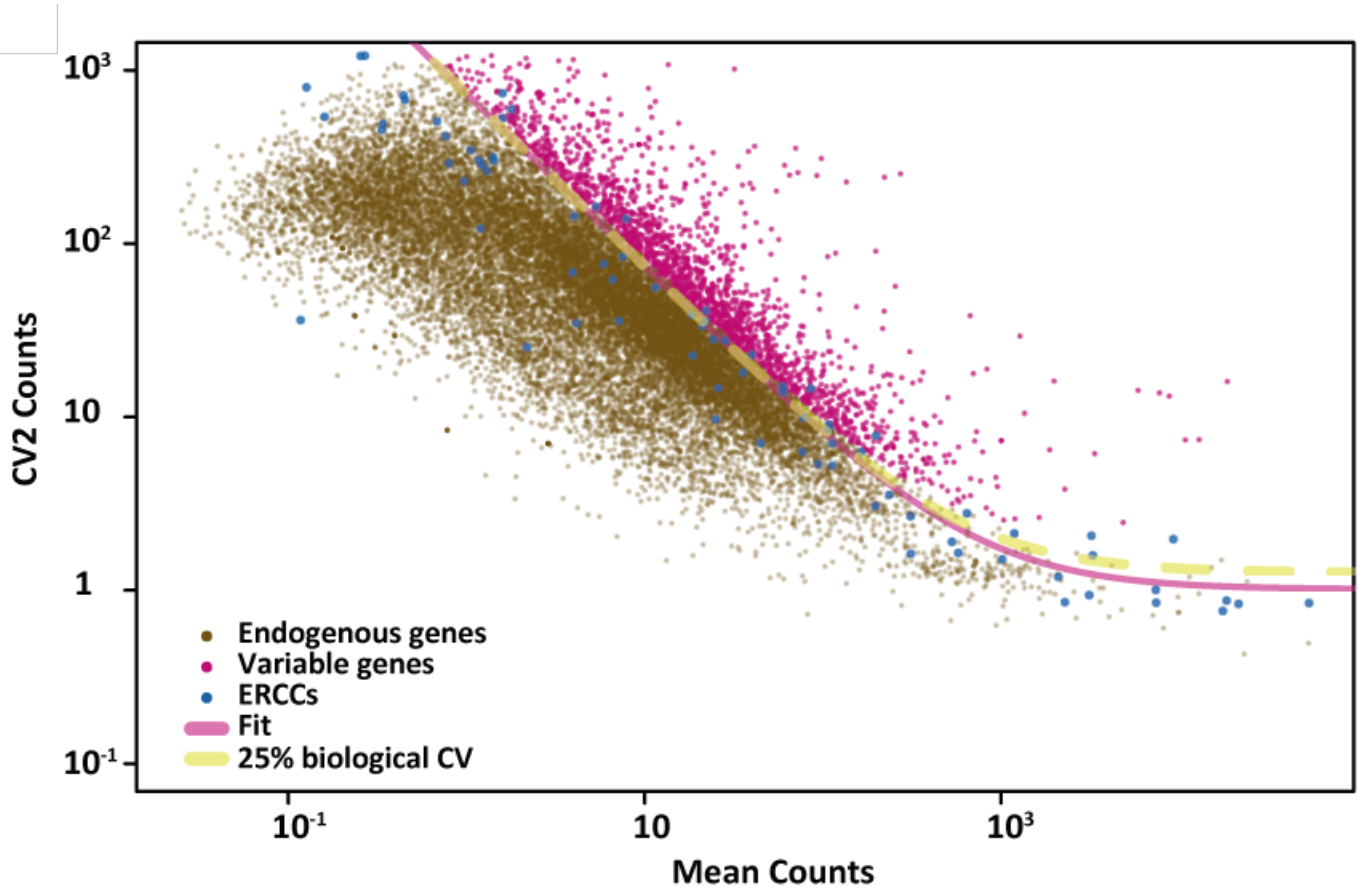




1,422 single cells

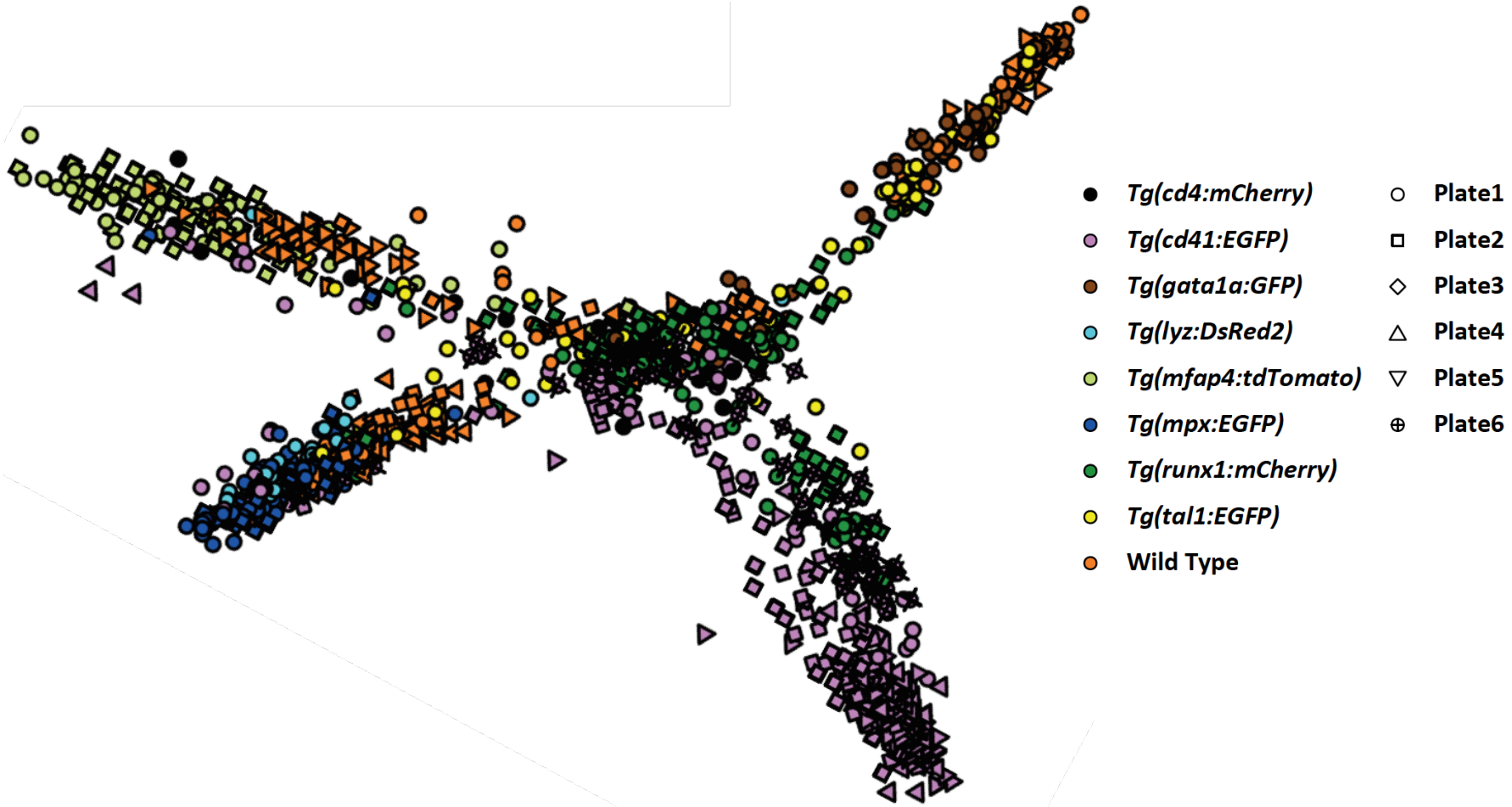
Accounting for technical noise in single-cell RNA-seq experiments

1,422 single cells
1,845 highly variable genes

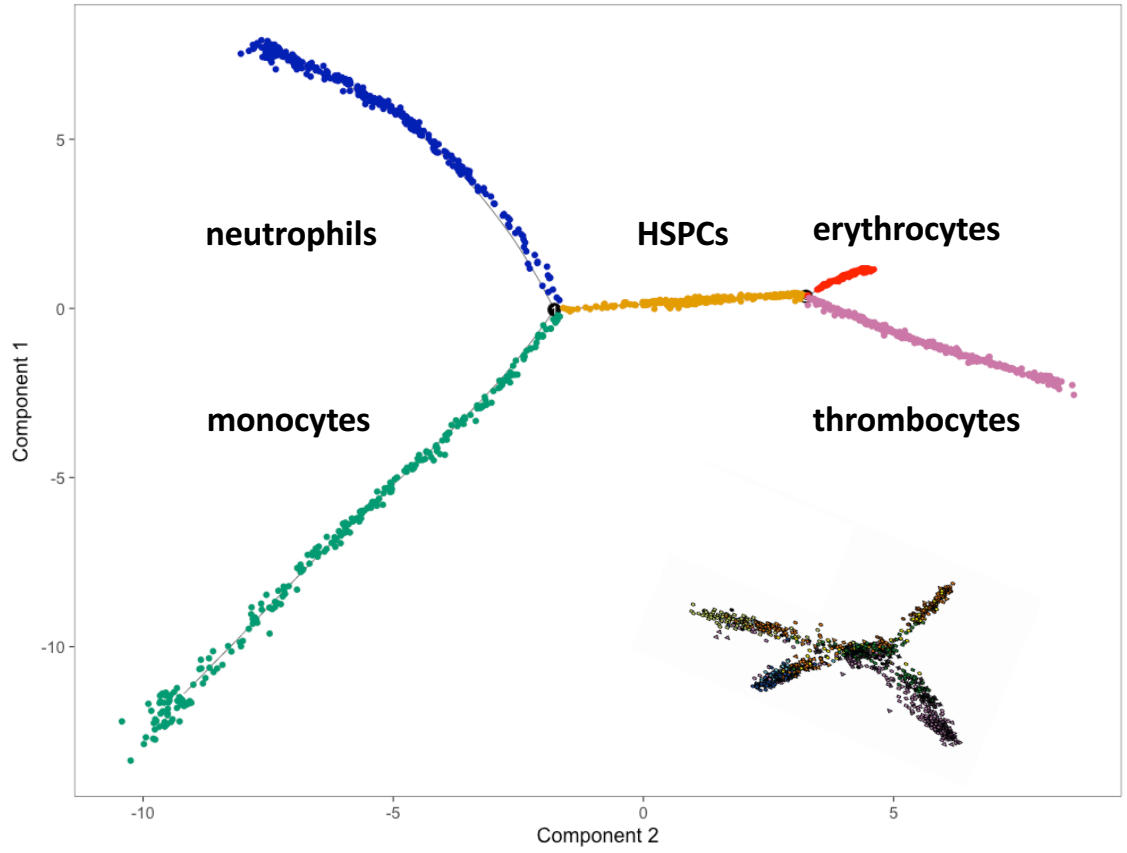


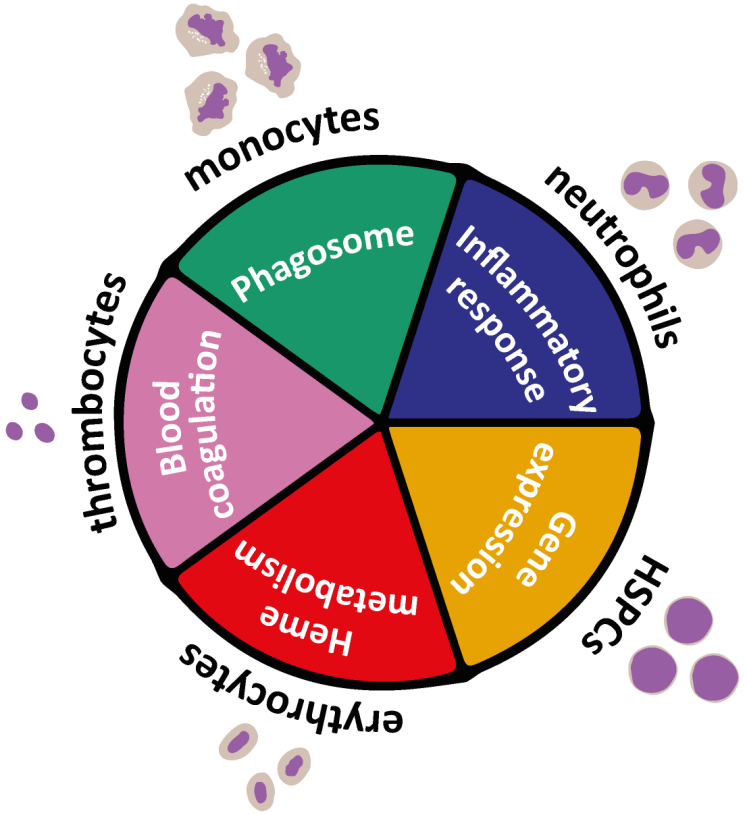
Method proposed by Brennecke et al. 2013

Cells Separated Based on their Biological Differences Rather than Batch Induced Biases

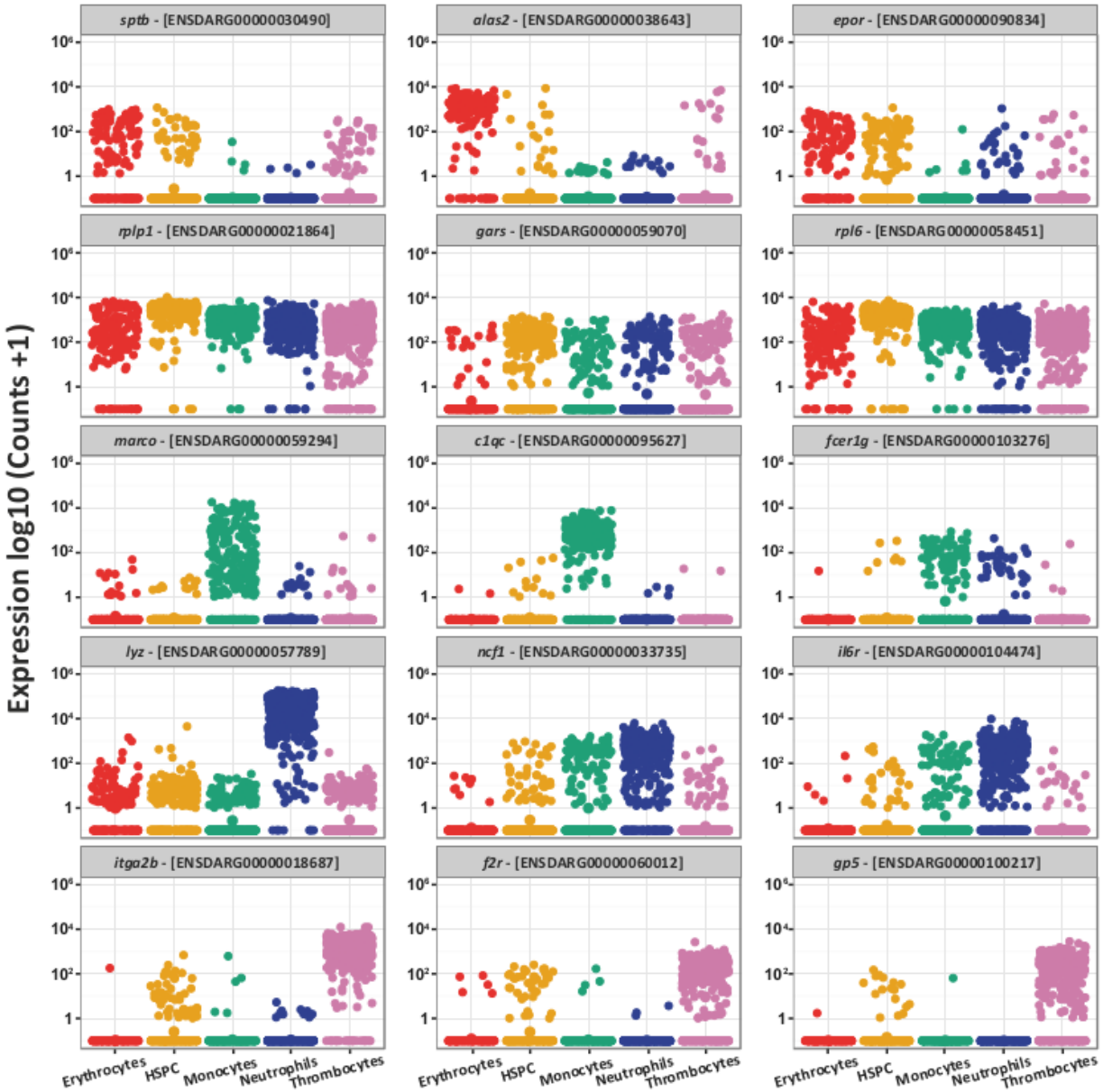


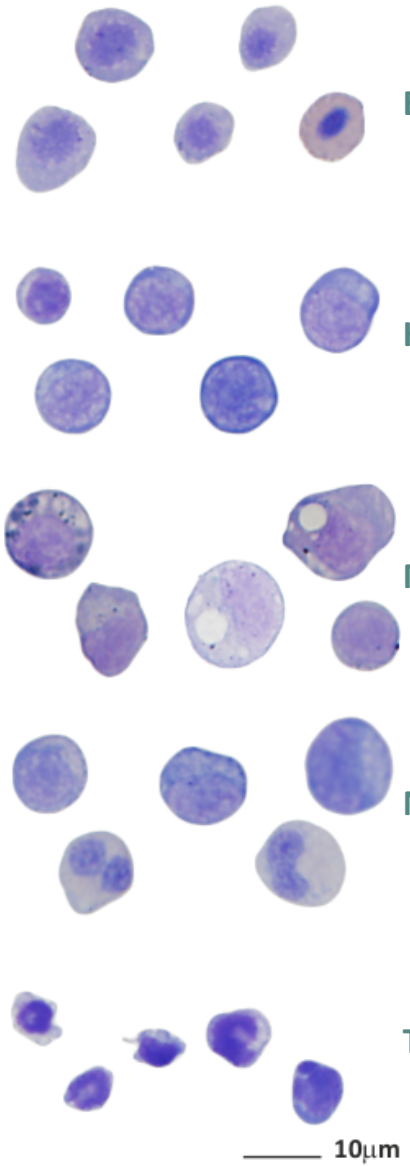
Pseudotime Ordering Revealed Five Distinct Cell States



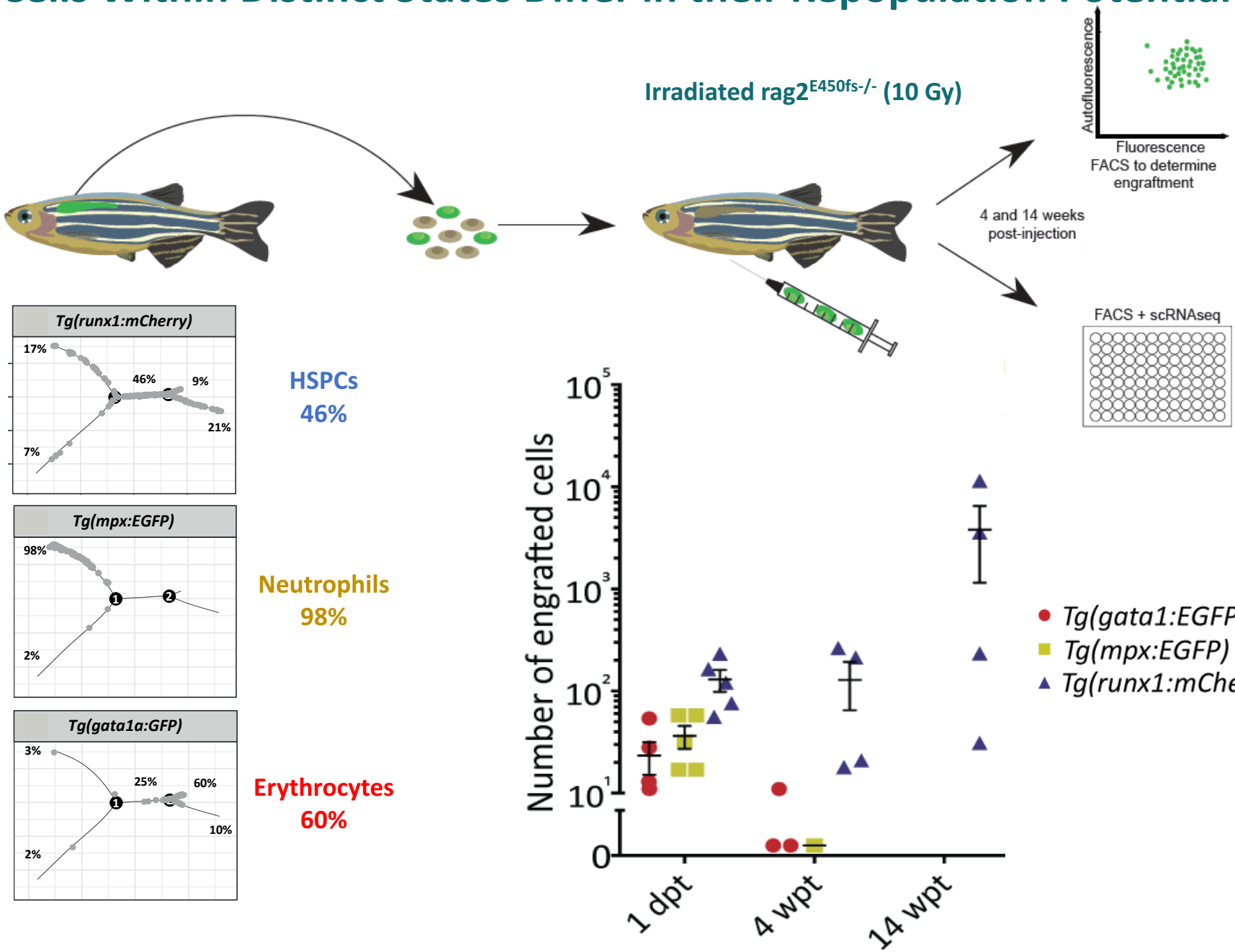


Morphological Properties of Cells Confirm Computational Predictions

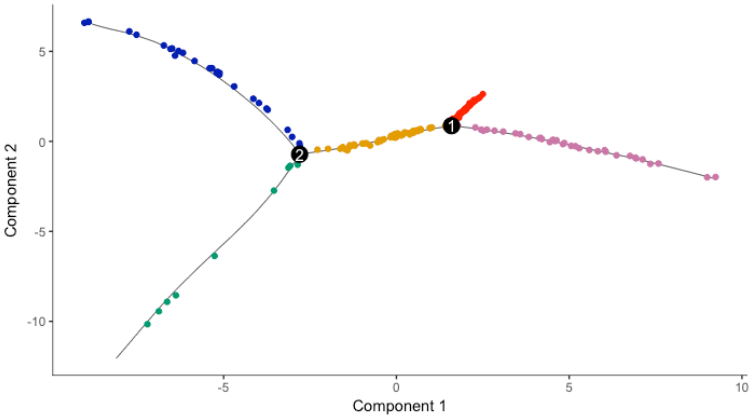




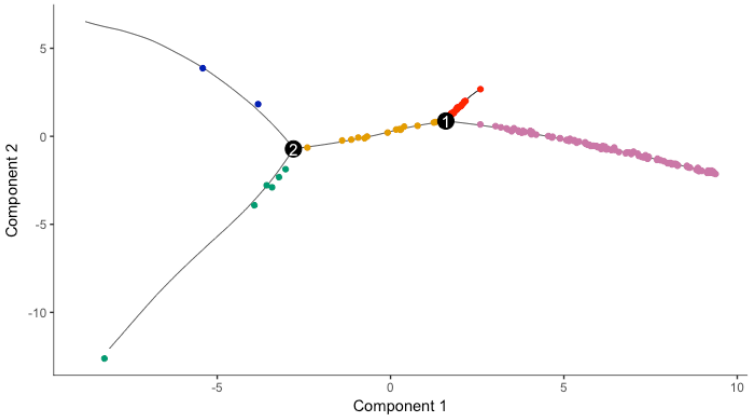
Cells Within Distinct States Differ in their Repopulation Potential



Cells Within Distinct States Differ in their Repopulation Potential

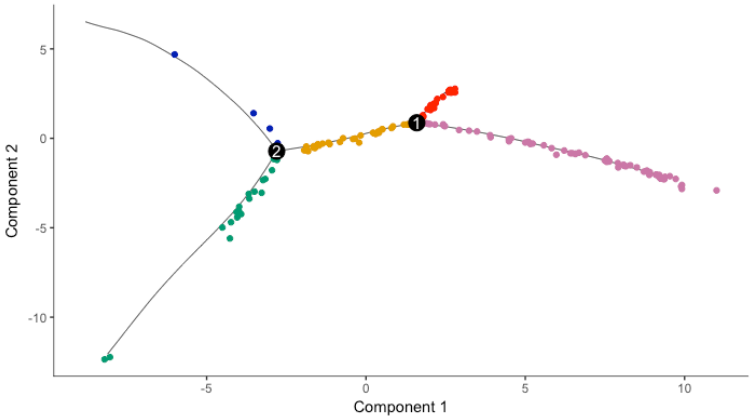


Non-transplanted Tg(*runx1*:mCherry)



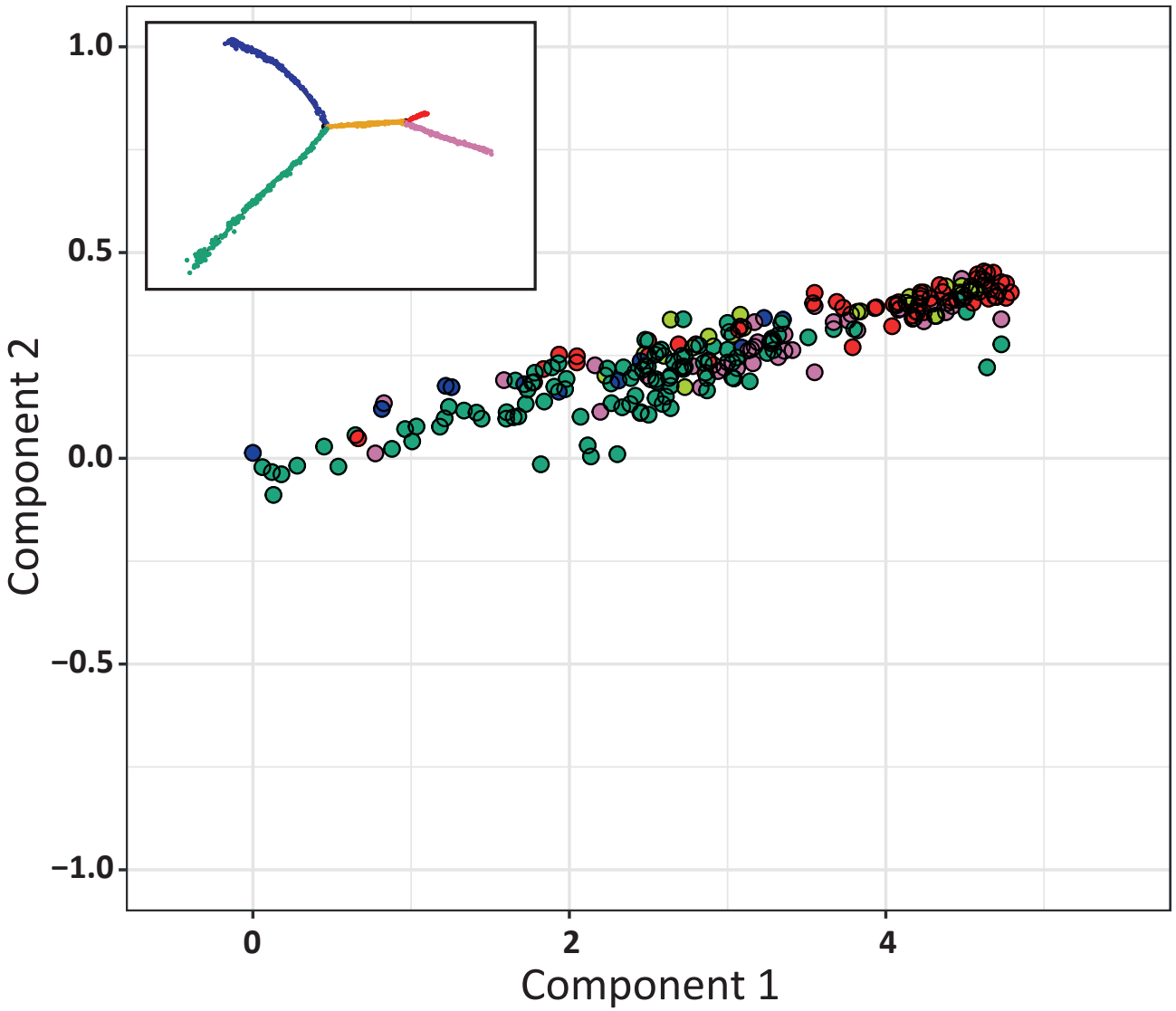
Transplanted Tg(*runx1*:mCherry)
4 wpt

Erythrocytes
HSPCs
Monocytes
Neutrophils
Thrombocytes



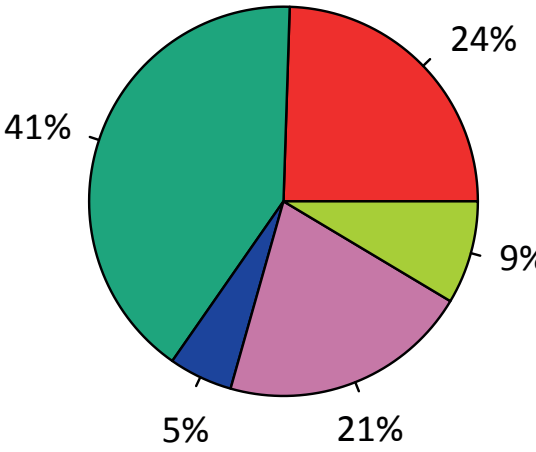
Transplanted Tg(*runx1*:mCherry)
14 wpt

Transcriptional Changes at the HSPC Branch

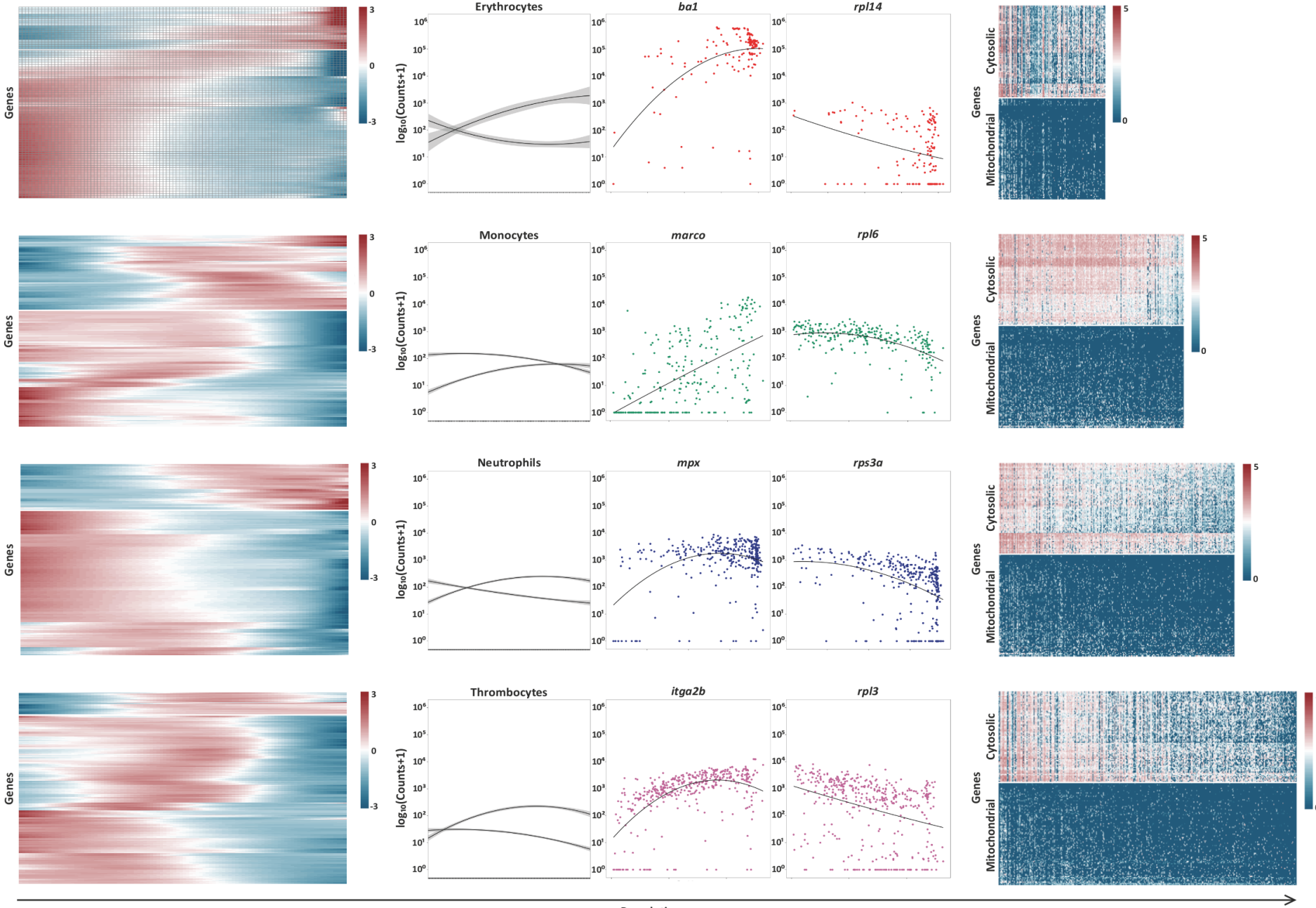


Predicted State

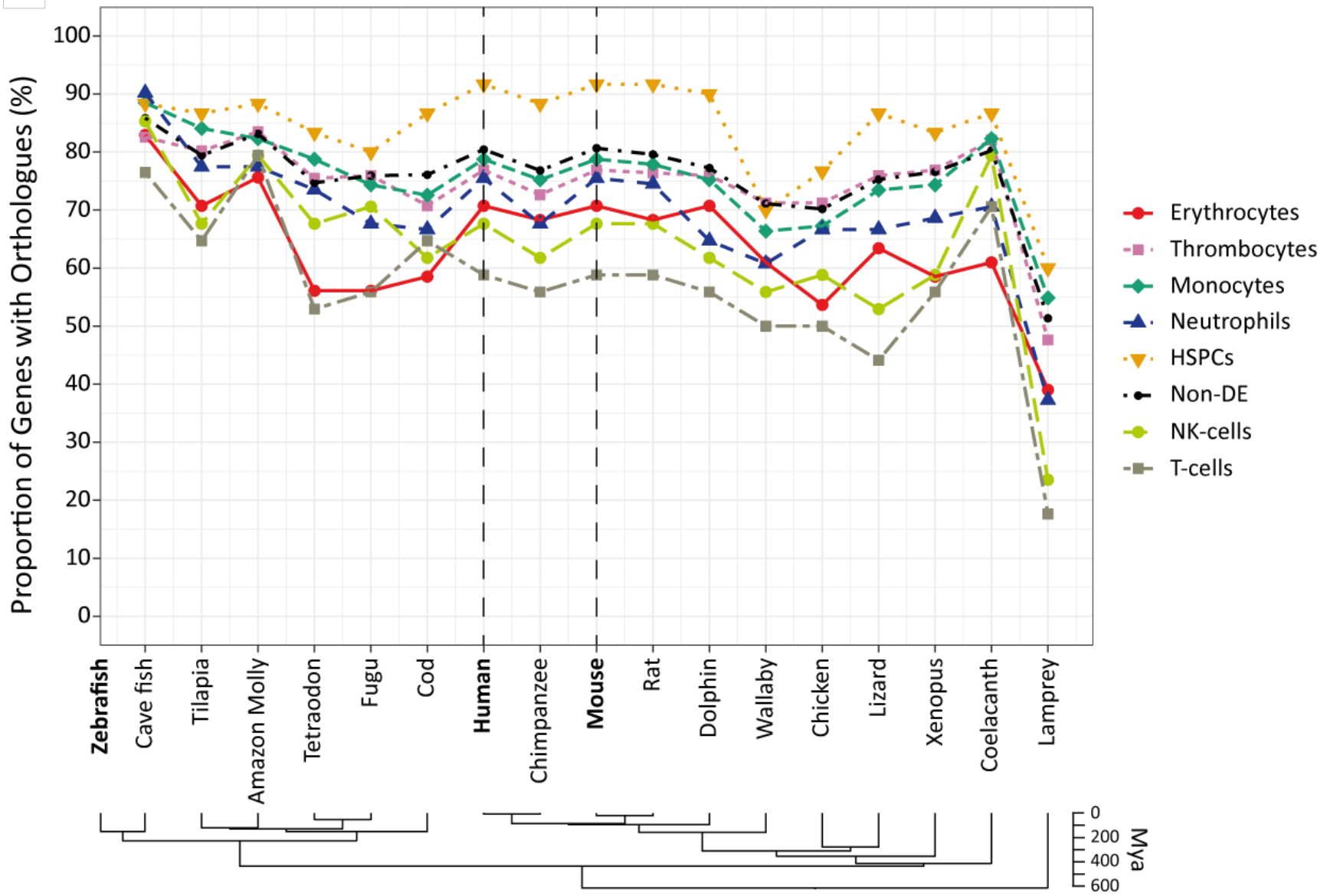
- Erythrocytes
- Monocytes
- Neutrophils
- Thrombocytes
- Unspecified



Common Developmental Events Direct Lineage Differentiation



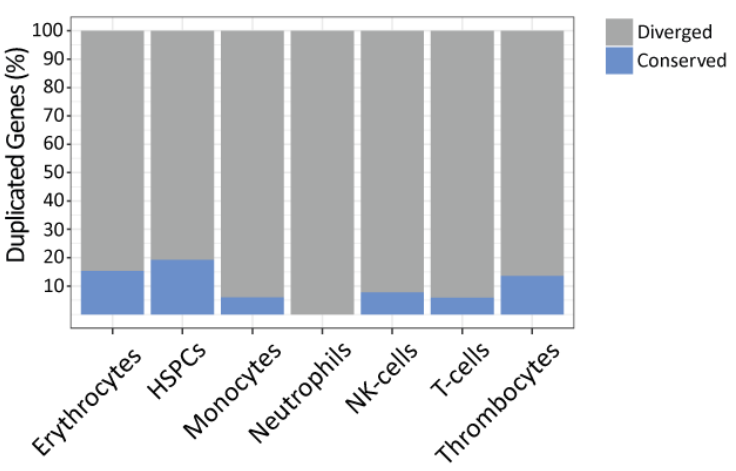
Zebrafish Have a Highly Conserved HSPC Transcriptome

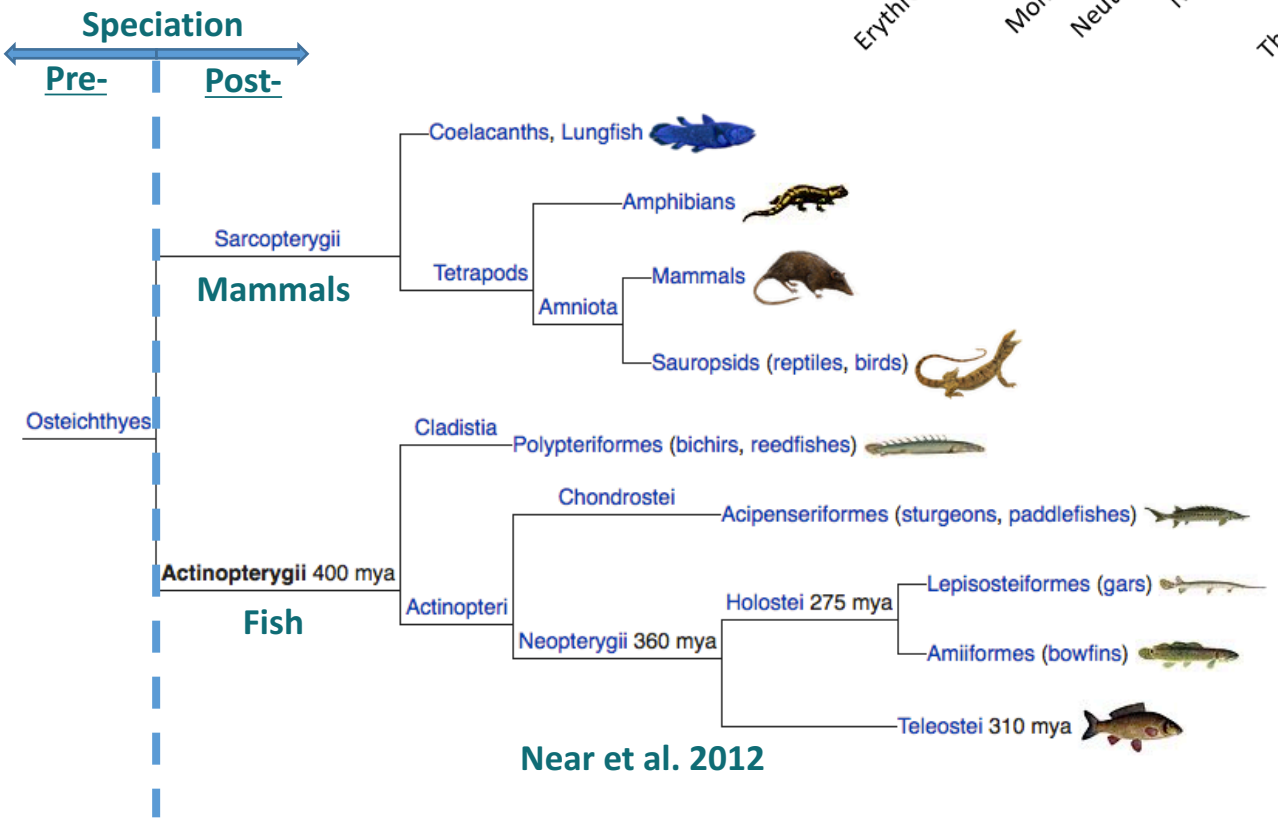


Zebrafish Have a Highly Conserved HSPC Transcriptome

Out of 7,424 paralogs
- 79% were duplicated pre- and
- 21% were duplicated post-speciation

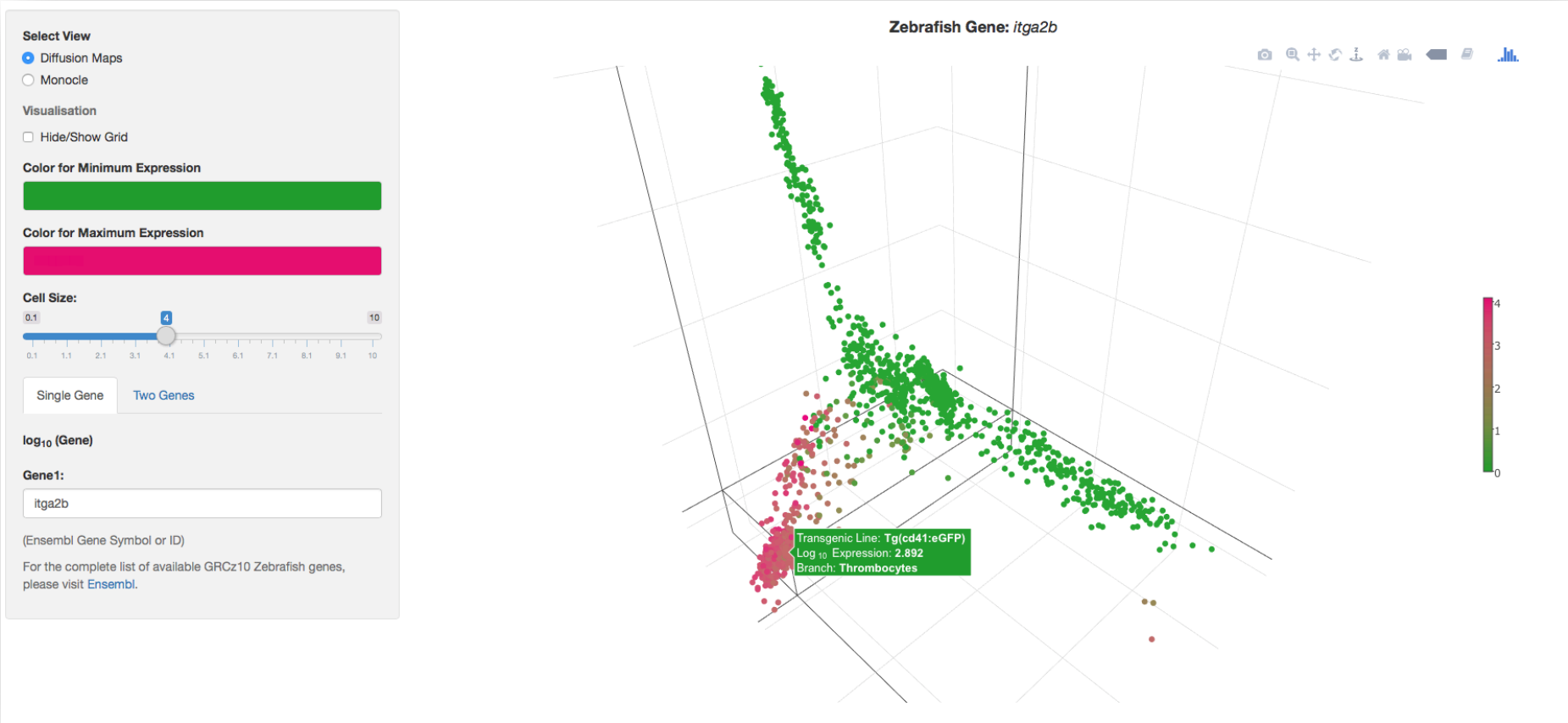
paralogs were more likely to functionally
- Diverge (88%) than to
- Conserved (12%)





Near et al. 2012

BASiCz - Blood Atlas of Single Cells in zebrafish



BASiCz

Summary

- Here we used a marker-free approach to computationally reconstruct the blood lineage tree in zebrafish and order cells based on their global transcriptional differences

- Within the population of transcriptionally similar stem and progenitor cells our analysis revealed considerable cell-to-cell differences in their probability to transition to another committed state

- Once fate decision was executed, the suppression of transcription of ribosomal genes and up-regulation of lineage specific factors coordinately controlled lineage differentiation

- Evolutionary analysis further demonstrated that this haematopoietic program was highly conserved between zebrafish and higher vertebrates

Acknowledgement

Lauren Ferreira

Ana Cvejic

Jan Botthof

Helena Andres

Pietro Lio

Shwethaa Raghunathan

Animal Facility, Sanger Institute
Cytometry Core Facility, Sanger Institute

UNIVERSITY OF CAMBRIDGE

Wellcome Trust - Medical Research Council
Cambridge Stem Cell Institute

CANCER RESEARCH UK

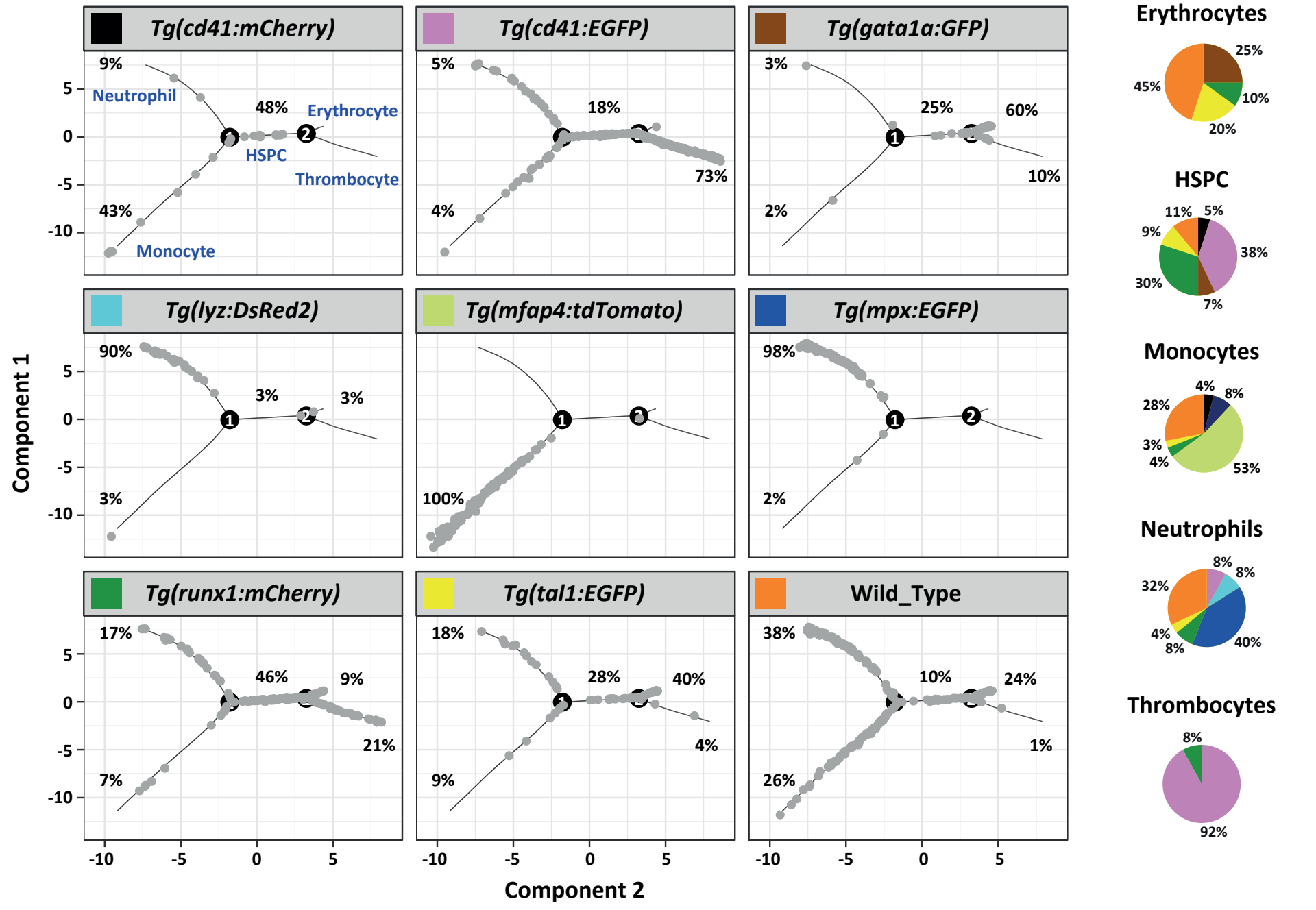
erc

Fate Decisions

From Nafplio (Greece)

To Cambridge (UK)

Thank you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Tg(cd41:EGFP)
Tg(gata1a:GFP)
Tg(lyz:DsRed2)
Tg(mfap4:tdTomato)
Tg(mpx:EGFP)
Tg(runx1:mCherry)
Tg(tal1:EGFP)
Wild_Type
Neutrophil
Erythrocyte
HSPC
Thrombocyte
Monocyte
9%
48%
43%
5%
18%
73%
4%
3%
25%
60%
10%
2%
90%
3%
3%
3%
100%
98%
2%
17%
46%
9%
21%
7%
18%
28%
40%
4%
9%
38%
10%
24%
1%
26%
Component 1
Component 2
-10
-5
0
5
Erythrocytes
25%
10%
20%
45%
HSPC
11%
5%
38%
7%
30%
9%
Monocytes
4%
8%
28%
3%
4%
53%
Neutrophils
8%
8%
32%
40%
4%
8%
Thrombocytes
8%
92%