Defining novel multilineage progenitor populations using single-cell RNA-Seq

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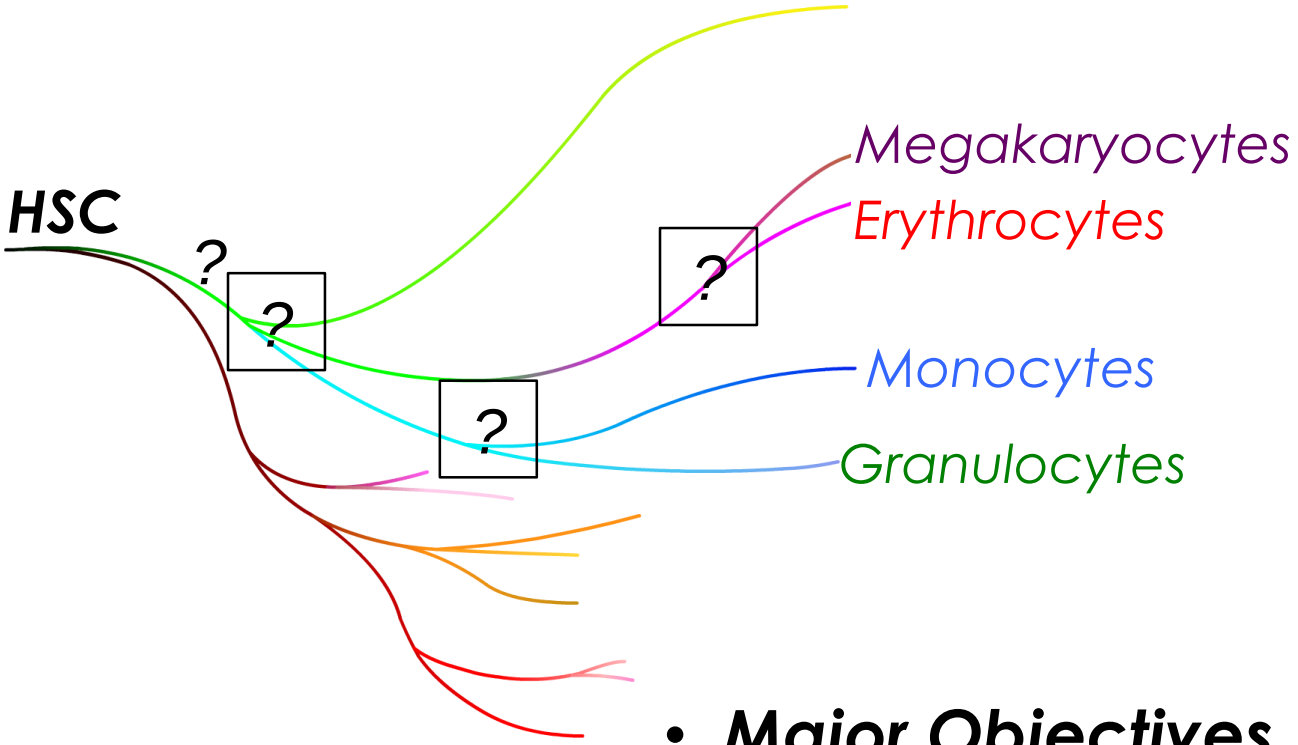
Uncovering Cell Heterogeneity from Single-Cell RNA-Seq



- ***Major Objectives***
  - Define major cell populations.
  - *Identify ultra-rare cells.*
  - *Identify hidden cellular heterogeneity and transition states.*

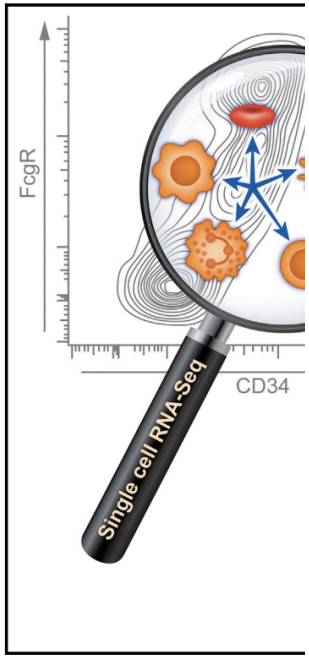
Conflicting Evidence Mixed-Lineage States Exist

Article

Cell

Transcriptiona
Commitment i

Graphical Abstract

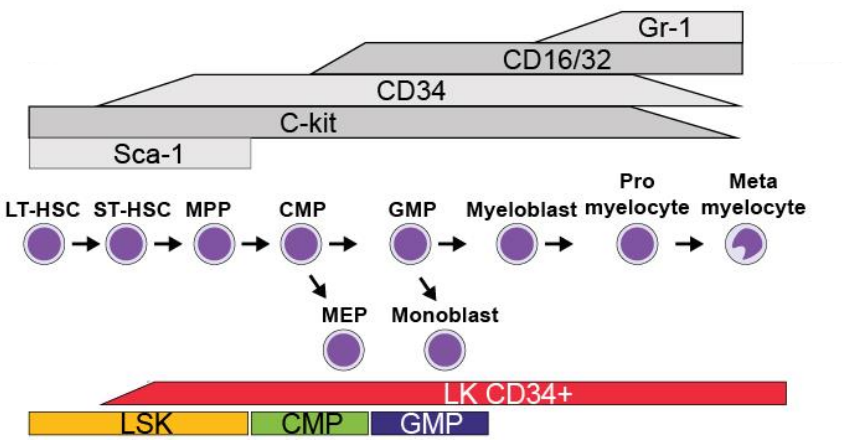


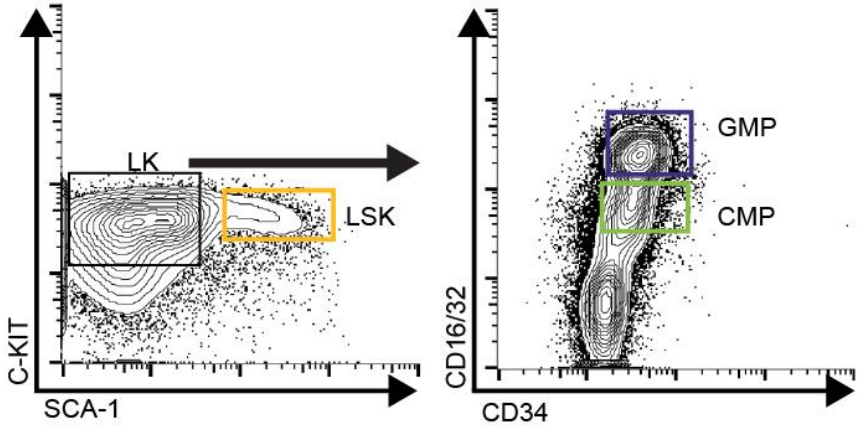
SUMMARY

Within the bone marrow, stem cells differentiate and give rise to diverse blood cell types and functions. Currently, hematopoietic progenitors are defined using surface markers combined with functional assays that are not directly linked with in vivo differentiation potential or gene regulatory mechanisms. Here, we comprehensively map myeloid progenitor subpopulations by transcriptional sorting of single cells from the bone marrow. We describe multiple progenitor subgroups, showing unexpected transcriptional priming toward seven differentiation fates but no progenitors with a mixed state. Transcriptional differ-

Isolation and Analysis of Diverse Mouse Hematopoietic Progenitors

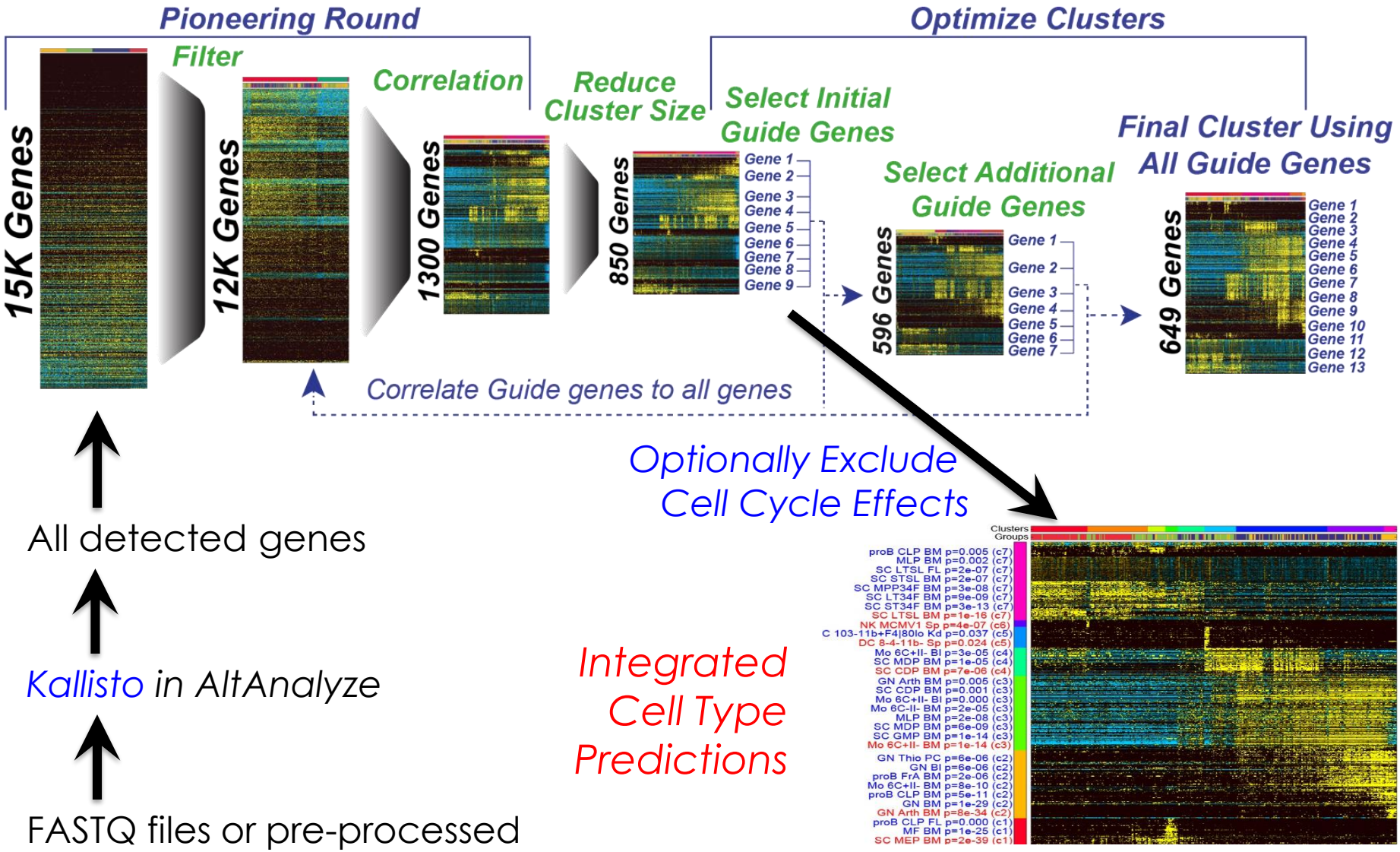
Olsson et al. Nature 2016



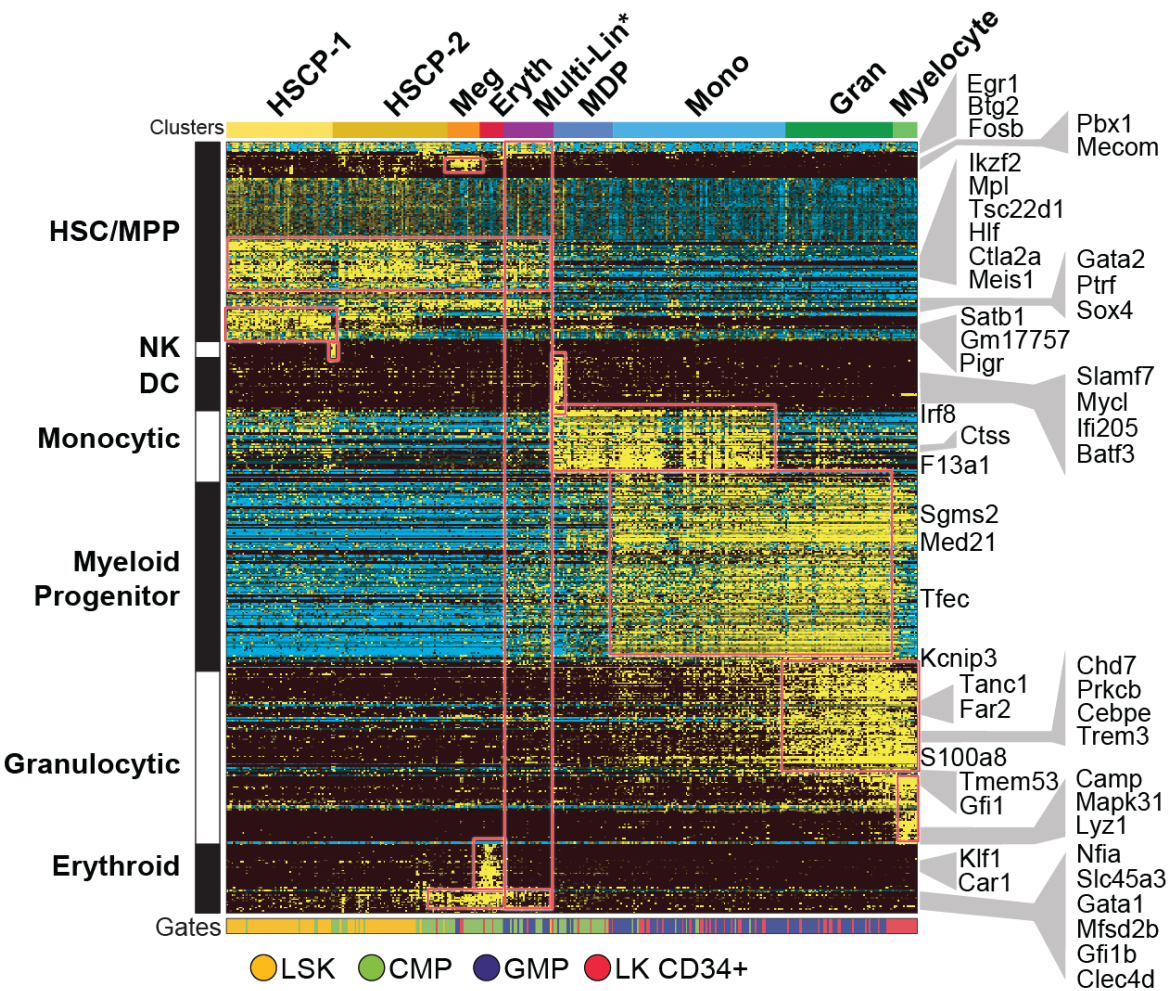


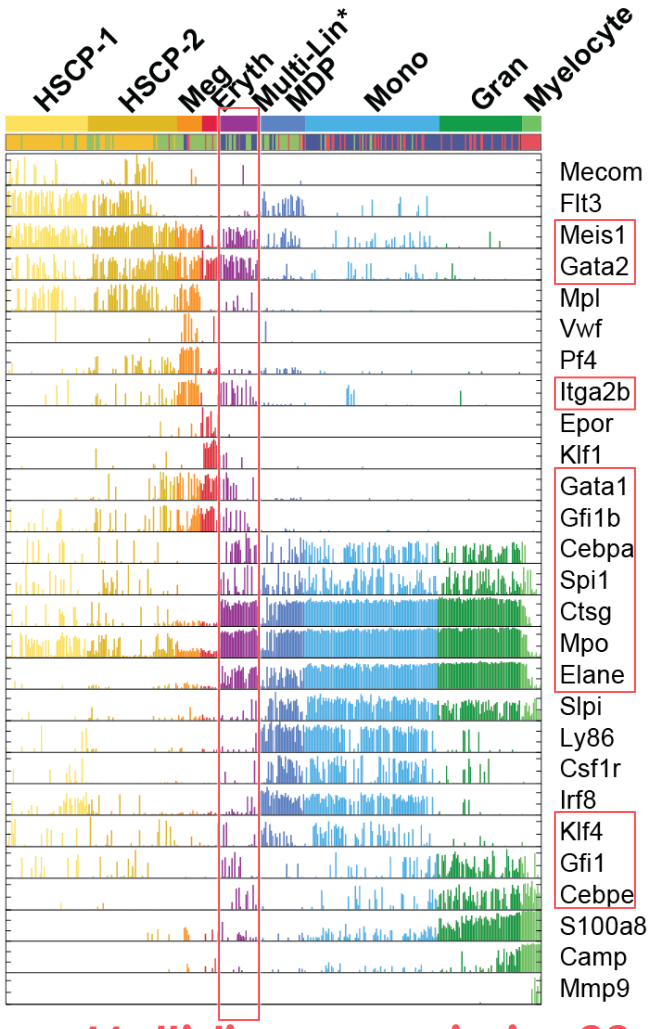
- Analyze using the 96-cell Fluidigm platform.
- Average 3 million reads of PE 75nt reads.
- Exclude outliers (depth, alignment %).

Automated Single Cell Analysis in AltAnalyze:
Iterative Clustering and Guide-gene Selection



Molecular Dissection of Hematopoiesis from scRNA-Seq using ICGS

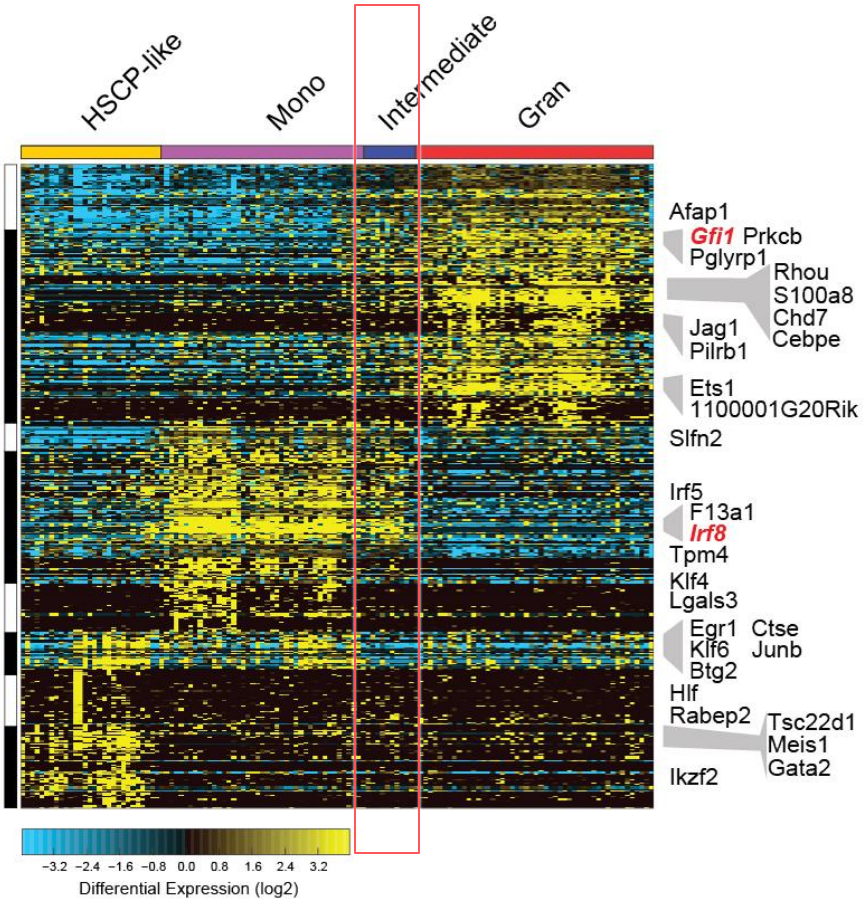


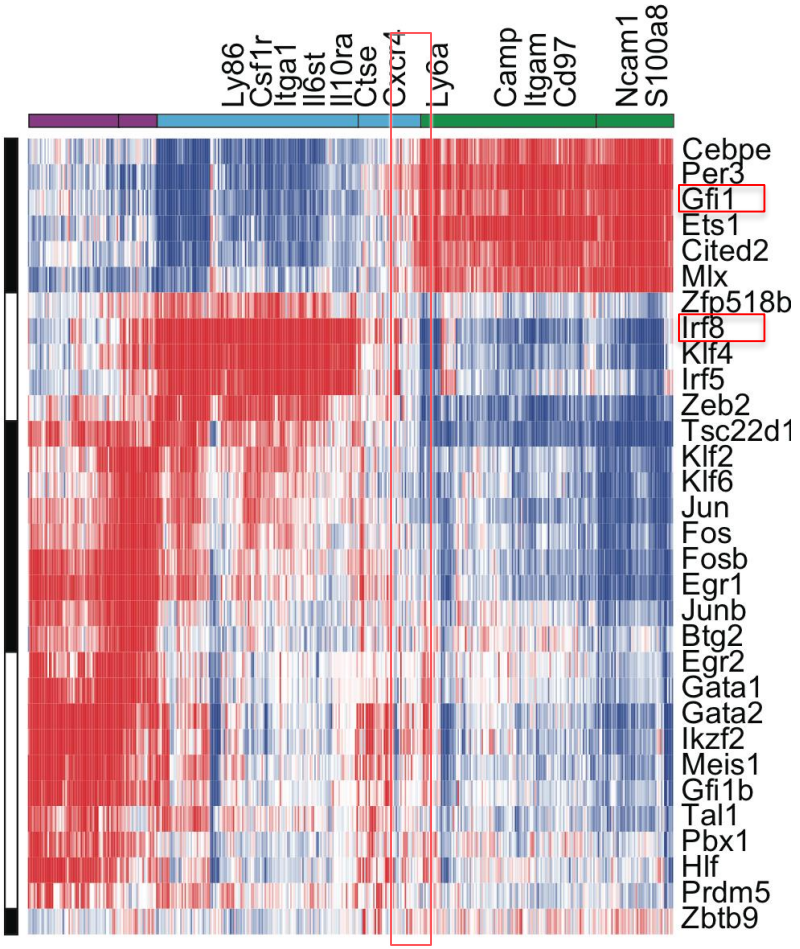


Multi-lineage priming??

Olsson et al. Nature 2016

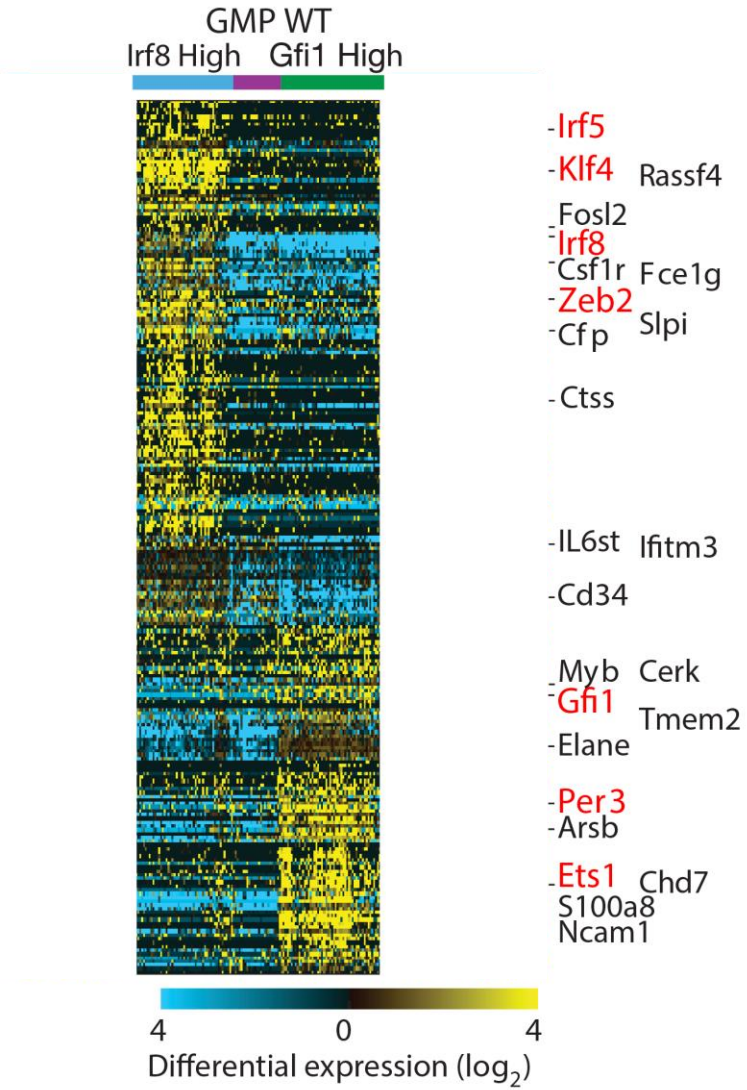
Monocyte and Granulocyte Progenitors Defined by Opposing Transcription Factors





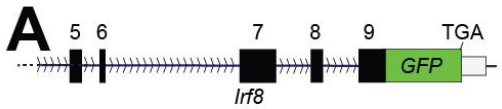
Olsson et al. Nature 2016

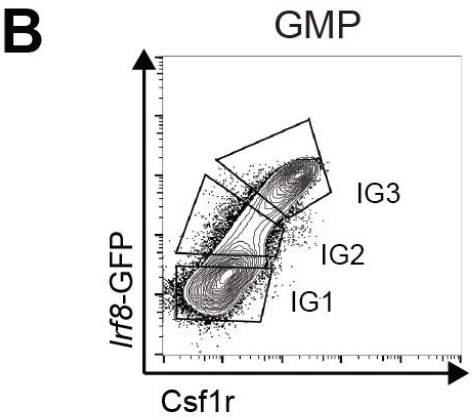
Gfi1and Irf8 Interact on Promoters to Regulate Myeloid Specification

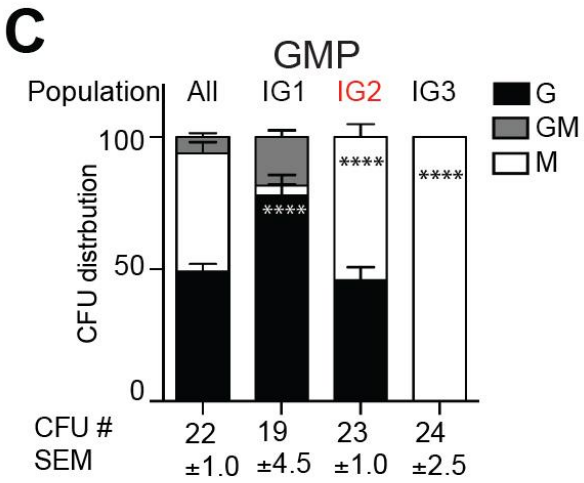


Olsson et al. Nature 2016

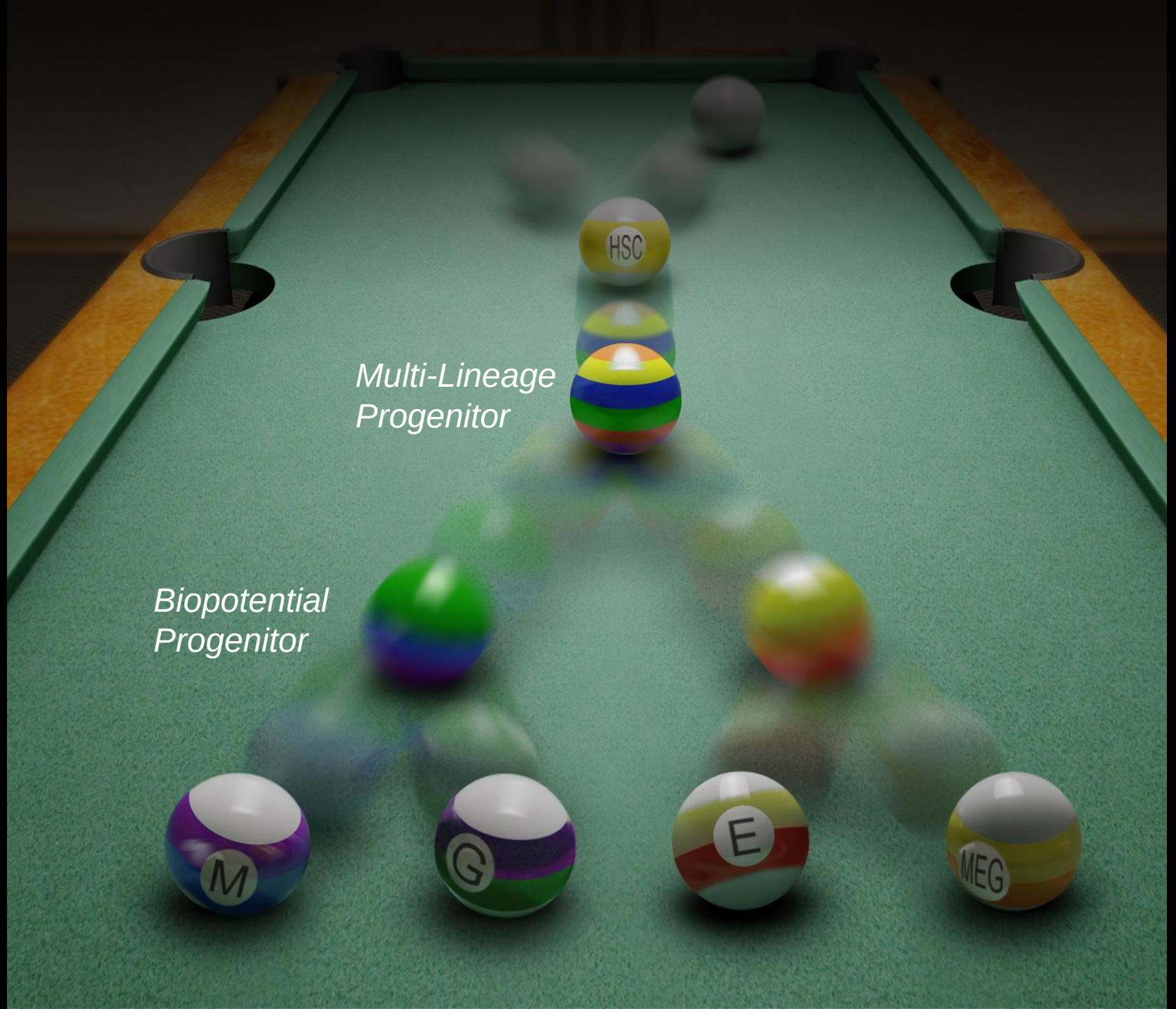
Intermediate Gfi1 and Irf8 Define a Metastable Bipotential Progenitor Population







Olsson et al. Nature 2016


HSC
Multi-Lineage
Progenitor
Biopotential
Progenitor
M
G
E
MEG

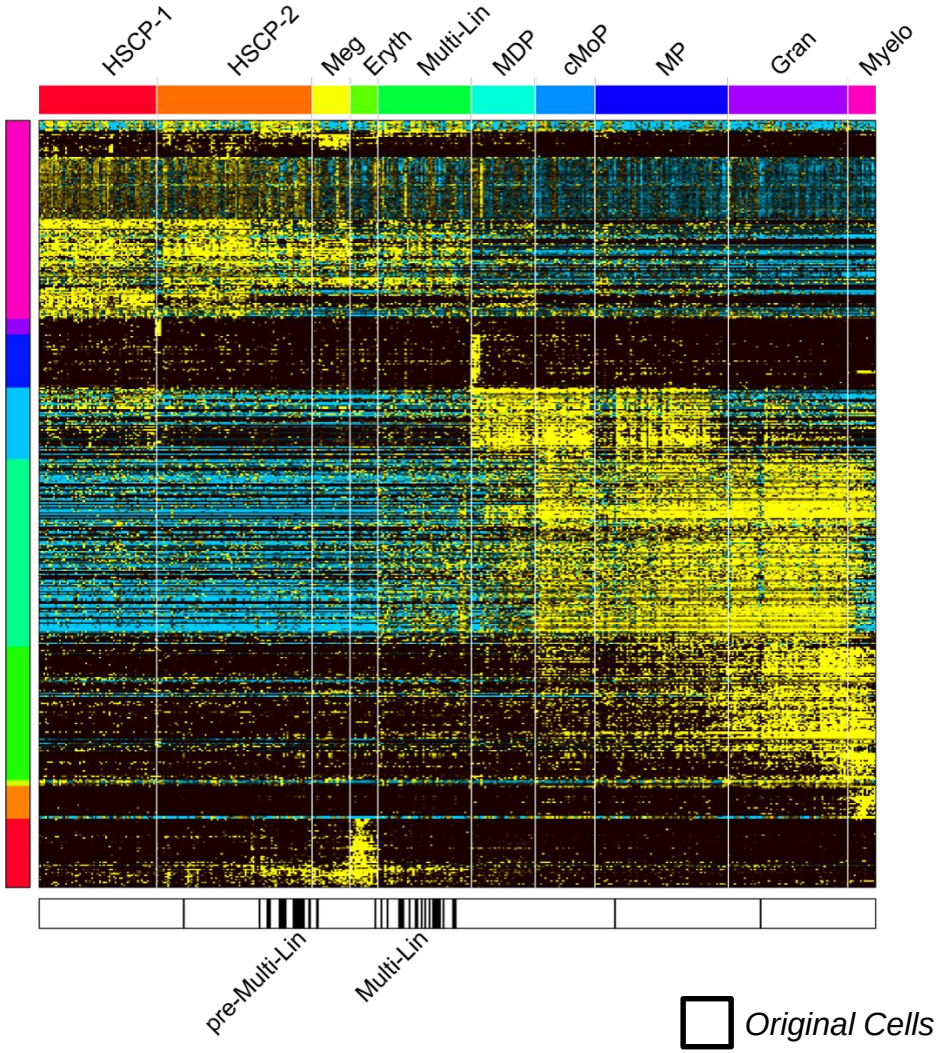
Initial Conclusions

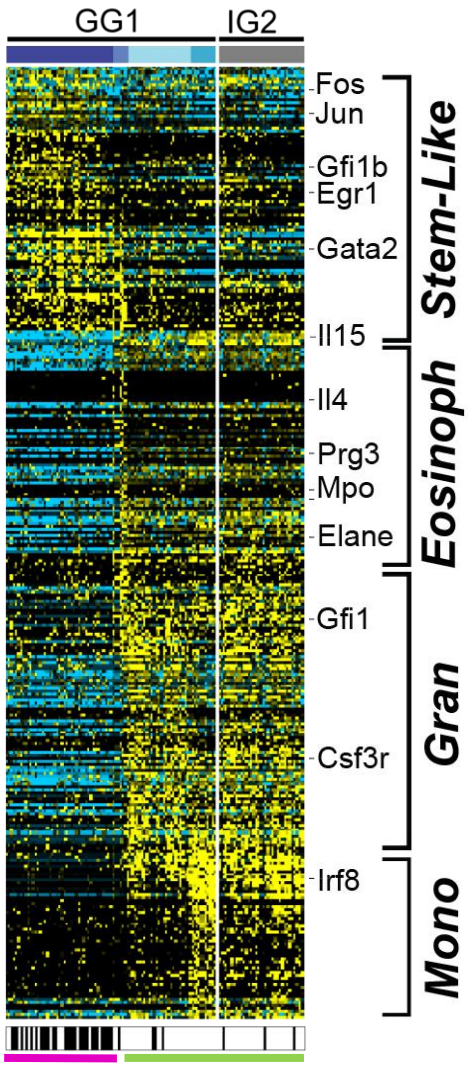
1. scRNA-Seq combined with TF-deletion and ChIP-Seq can define transcriptional regulatory networks.
2. Appears to require deep RNA-Seq.
3. Non-HSC, Multi-Lineage progenitors are frequently found in CMP and GMP gated cell populations.
4. These cells are primarily defined by multi-lineage gene priming and only weakly defined by unique marker genes.
5. Multi-Lin's can be captured and enriched by sorting for progenitors with dual lineage programs (Gfi1 and Irf8 expression).
6. Genetic deletion of these factors traps cells in an undecided state.

Controversies and Questions from scRNA-Seq Predictions

1. Other myeloid biologists argue Multi-Lin's are technical artifacts (doublets).
2. Bi-Potential progenitors enriched but not purified.
3. Multi-Lineage progenitors and MEPs not identified.

Optimized Isolation of CMP Multi-Potential Progenitors



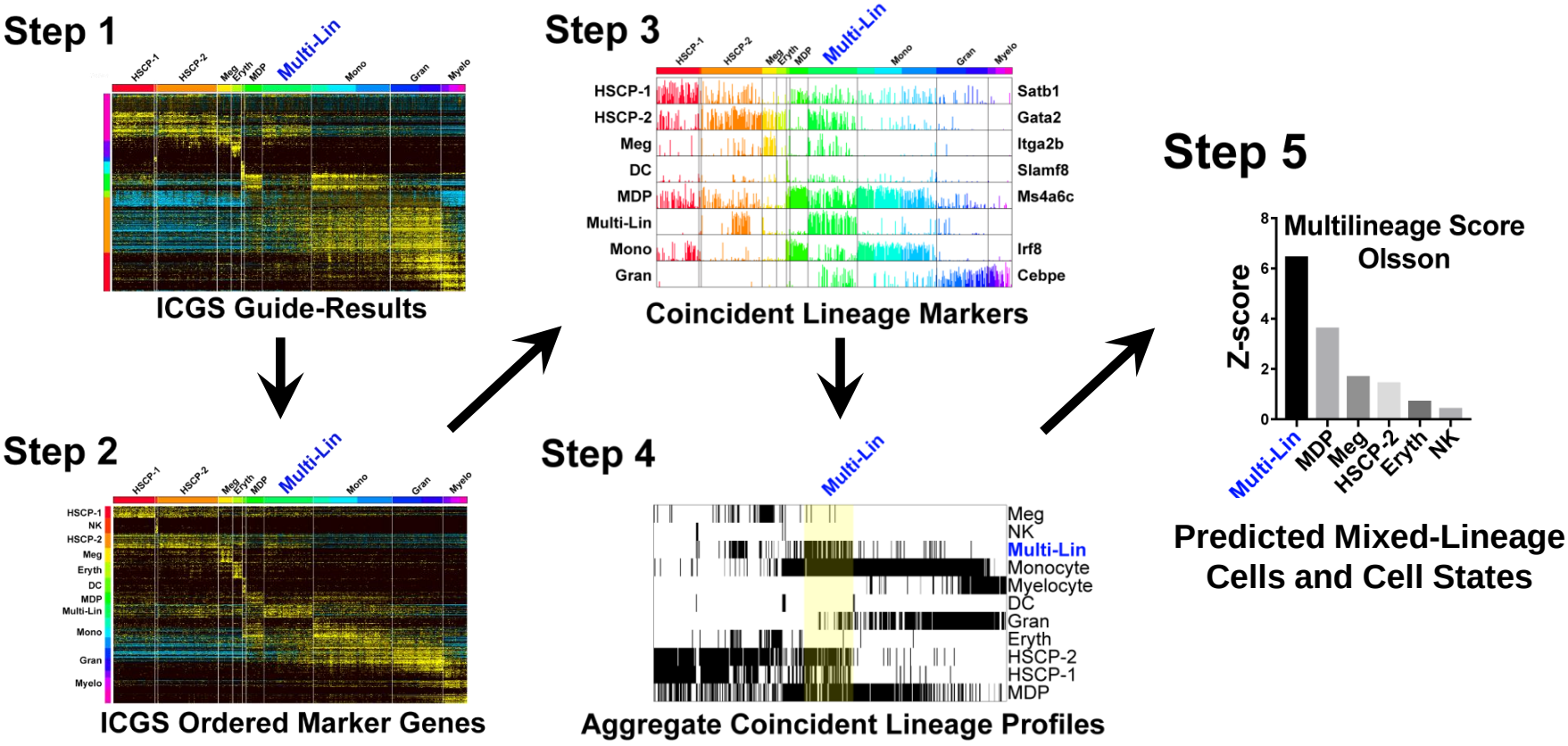


Original Cells

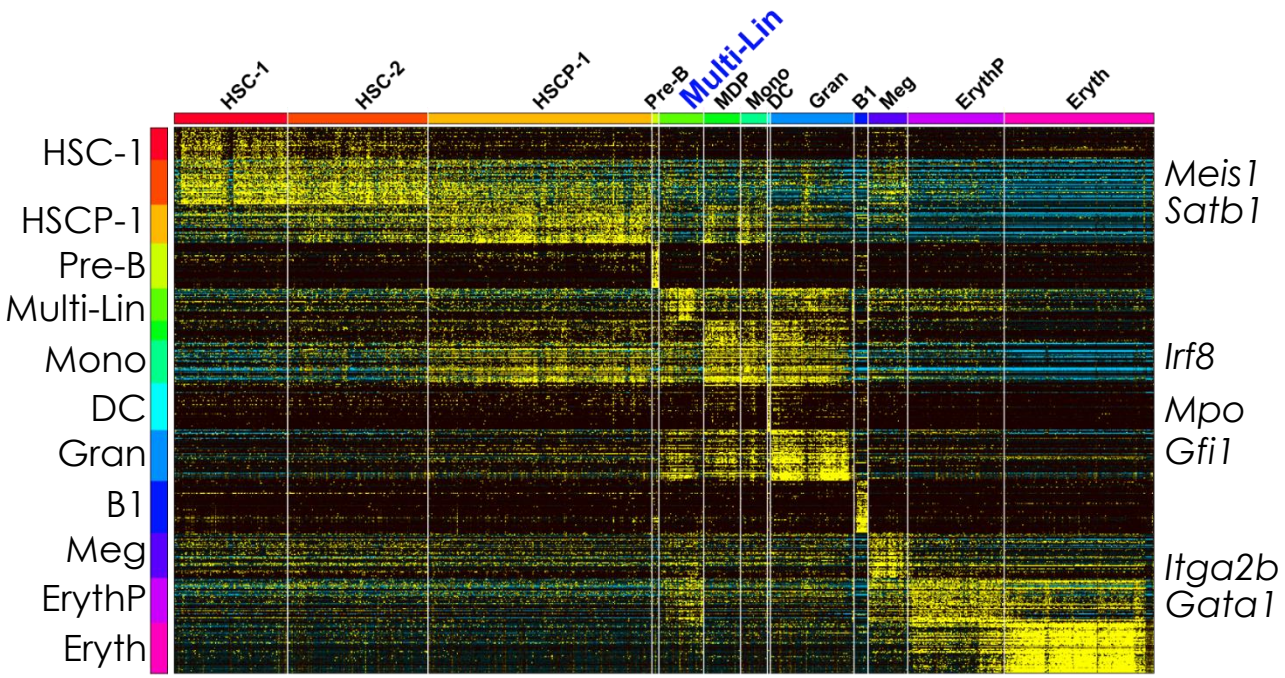
Optimized Markers

Manuscript in preparation

New Algorithms to Predict Multi-Lineage States from scRNA-Seq (Schrodinger)



Predicted Multi-Lineage Progenitors from Other scRNA-Seq Datasets



- Identification of similar Multi-lineage states from other scRNA-Seq datasets (ICGS + examination of lineage markers).

>200k reads/library
1.8k cells

HEMATOPOIESIS AND STEM CELLS

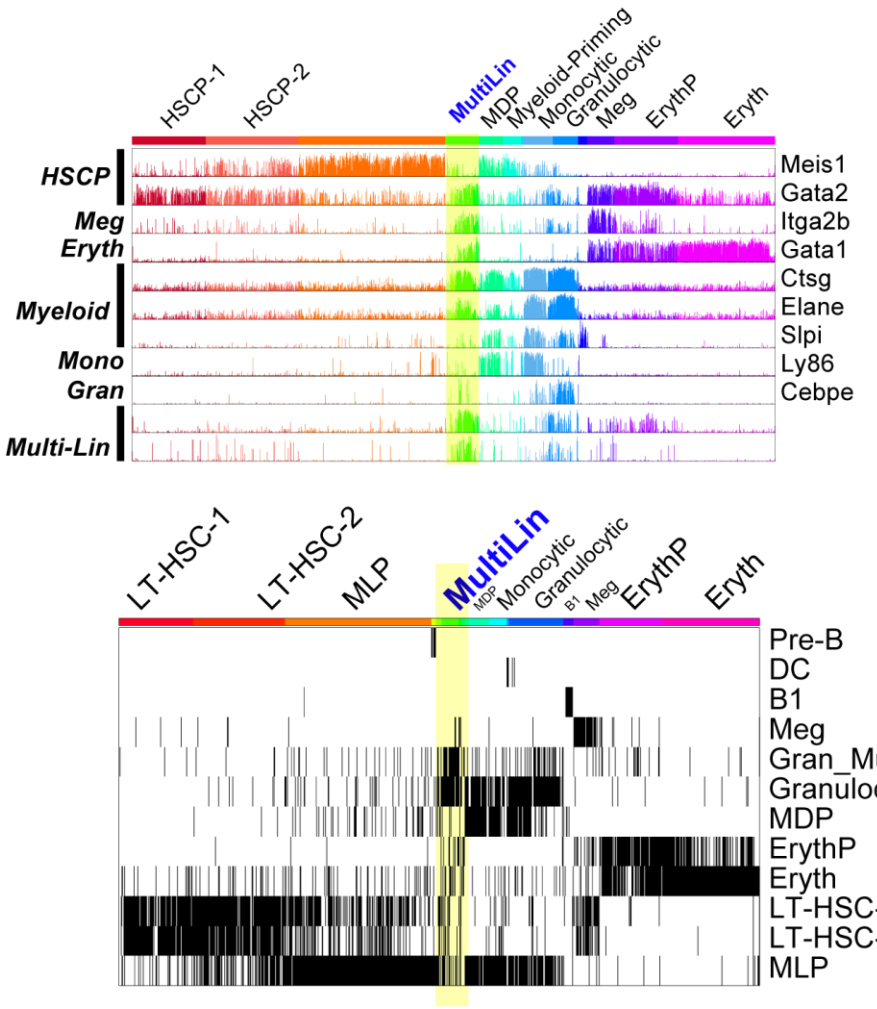
e-*Blood*

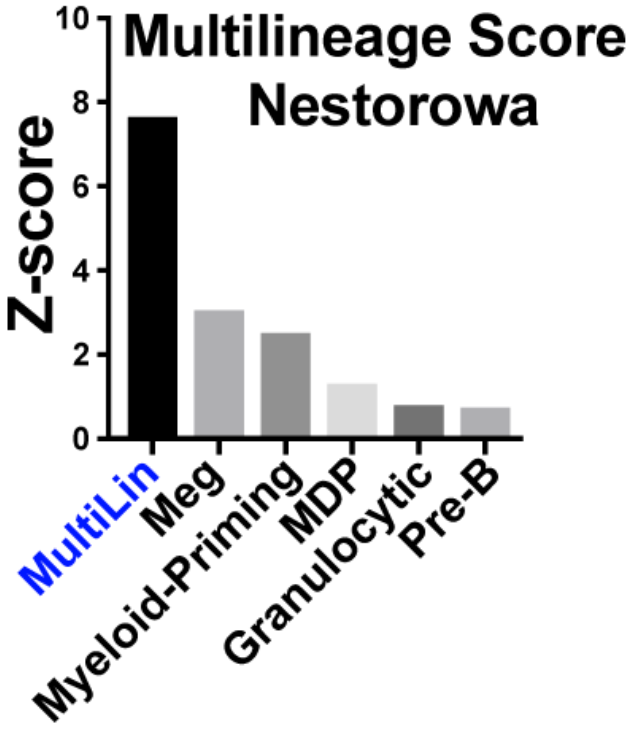
A single-cell resolution map of mouse hematopoietic stem and progenitor cell differentiation

Sonia Nestorowa,* Fiona K. Hamey,* Blanca Pijuan Sala, Evangelia Diamanti, Mairi Shepherd, Elisa Laurenti, Nicola K. Wilson, David G. Kent, and Berthold Göttgens

Department of Haematology and Wellcome Trust-Medical Research Council Cambridge Stem Cell Institute, University of Cambridge, Cambridge, United Kingdom

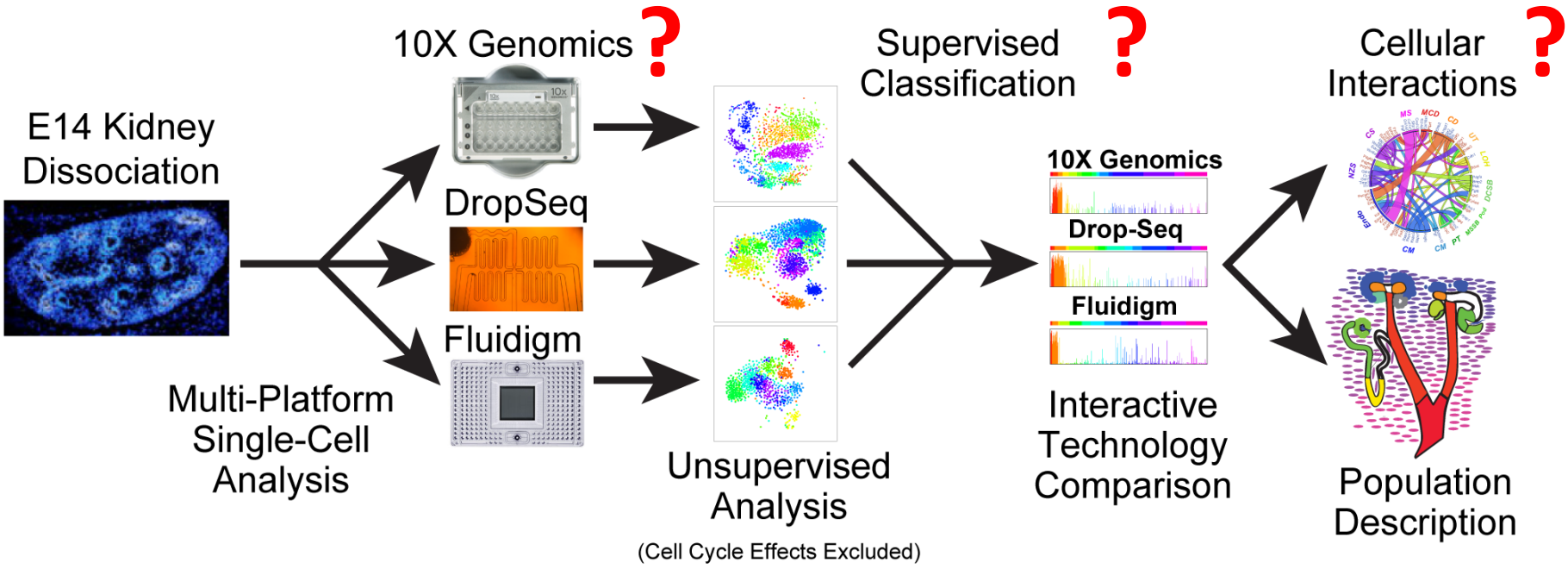
Schrodinger Detection of Multi-Lineage States in ~500 Mouse Bone Marrow Progenitors (Nestorowa et al.)





Also validated in data from:
Tusi BK, Klein AM, Socolsovsky M et al. Nature. 2018 Mar 1;555(7694):54-60

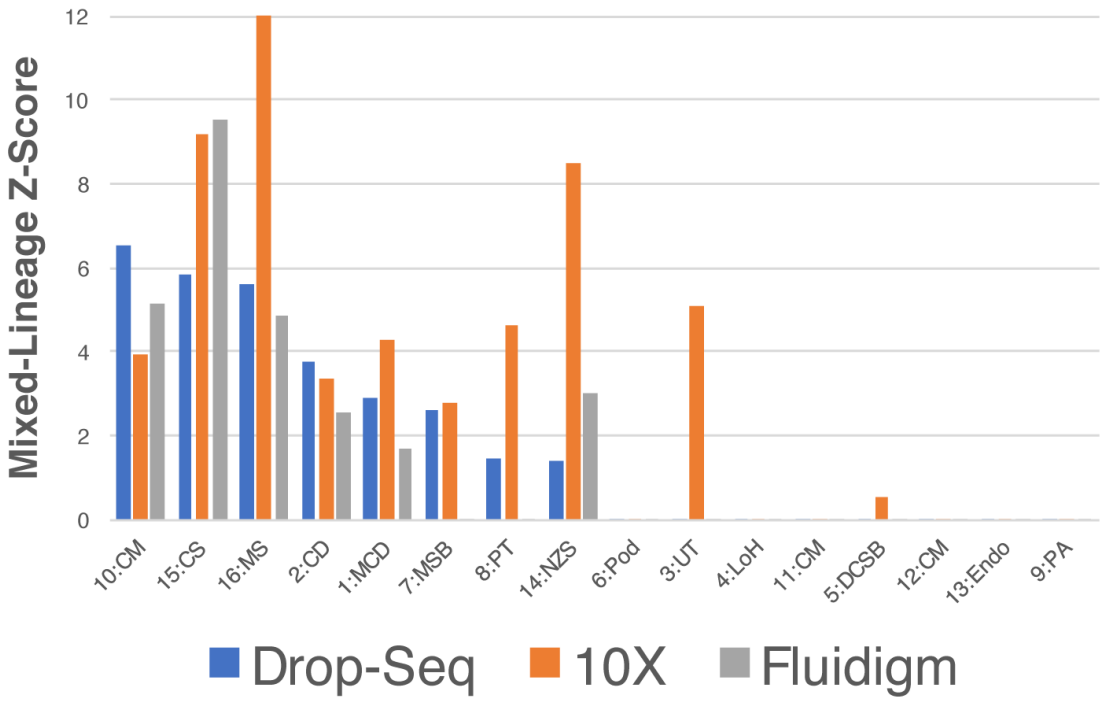
Can This Approach Be Used to Find Similar Cell Populations Across Technologies?



Magela et al. 2017 Developmental Biology

Consistent Schrodinger Prediction of Multi-Lineage States in Embryonic Kidney

- 1 Medullary Collecting Duct
- 2 Collecting Duct
- 3 Uteric Tip
- 4 Loop of Henle
- 5 Distal Comma Shaped Body
- 6 Podocytes
- 7 Mid S-Shaped Body
- 8 Proximal Tubule
- 9 Pretubular Aggregate
- 10 Cap Mesenchyme
- 11 Cap Mesenchyme
- 12 Cap Mesenchyme
- 13 Endothelium
- 14 Nephrogenic Zone Stroma
- 15 Cortical Stroma
- 16 Medullary Stroma



Acknowledgements

Salomonis Lab

Meenakshi Venkatasubramanium
Kashish Chetal

Grimes Lab (CCHMC)

H. Leighton Grimes
Andre Olsson

Singh Lab (CCHMC)

Harinder Singh
Virendra Chaudhri

Aronow Lab (CCHMC)

Bruce Aronow
Phil Dexheimer

Potter Lab (CCHMC)

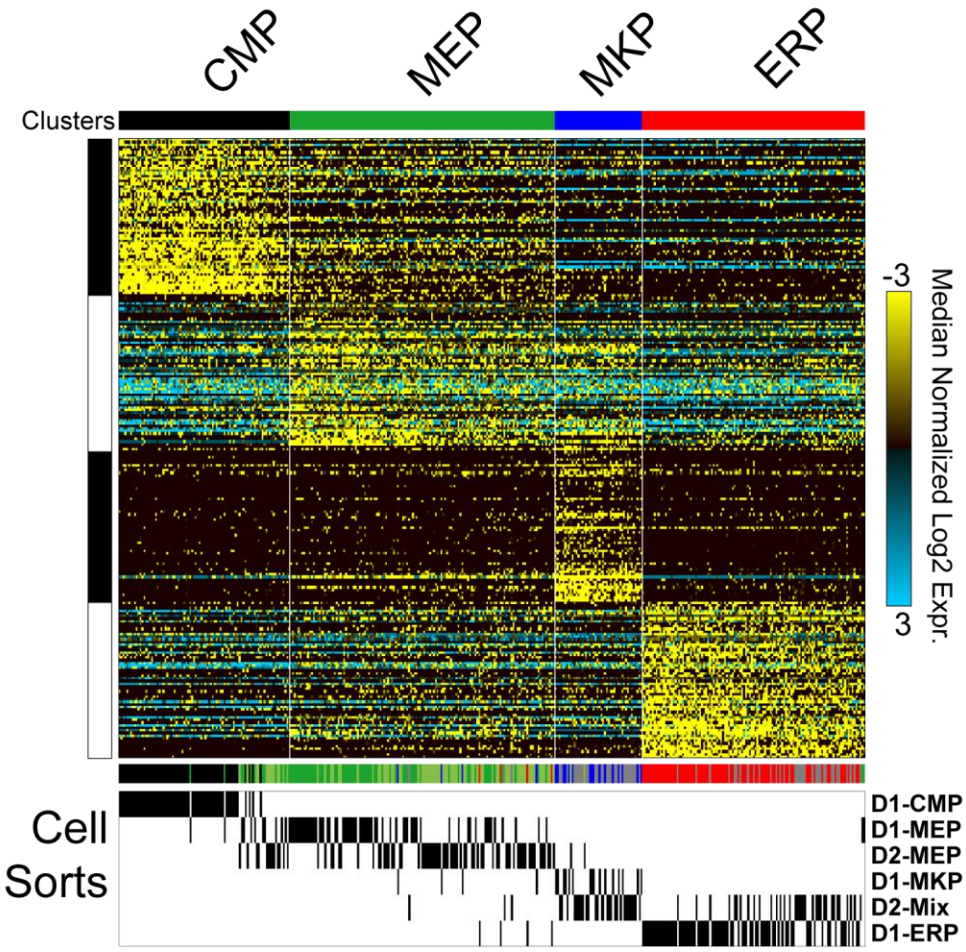
Steve Potter
Mike Adam
Bliss Magella

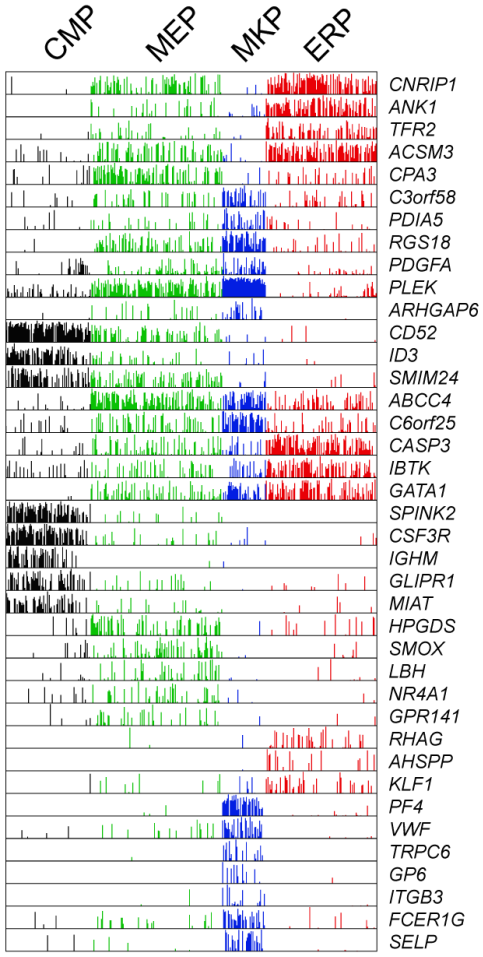
Funding

NIH (R01HL122661 – Grimes

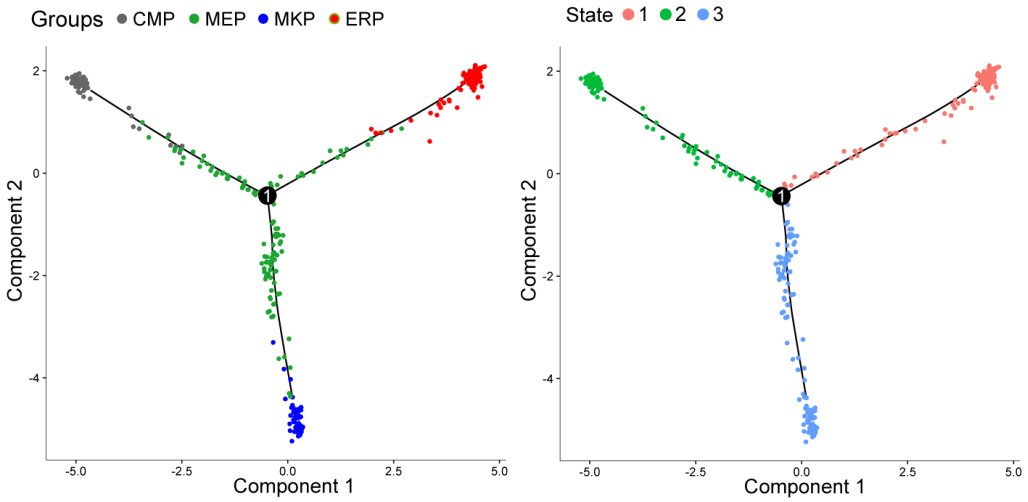
CCHMC Center for Pediatric Genomics Award

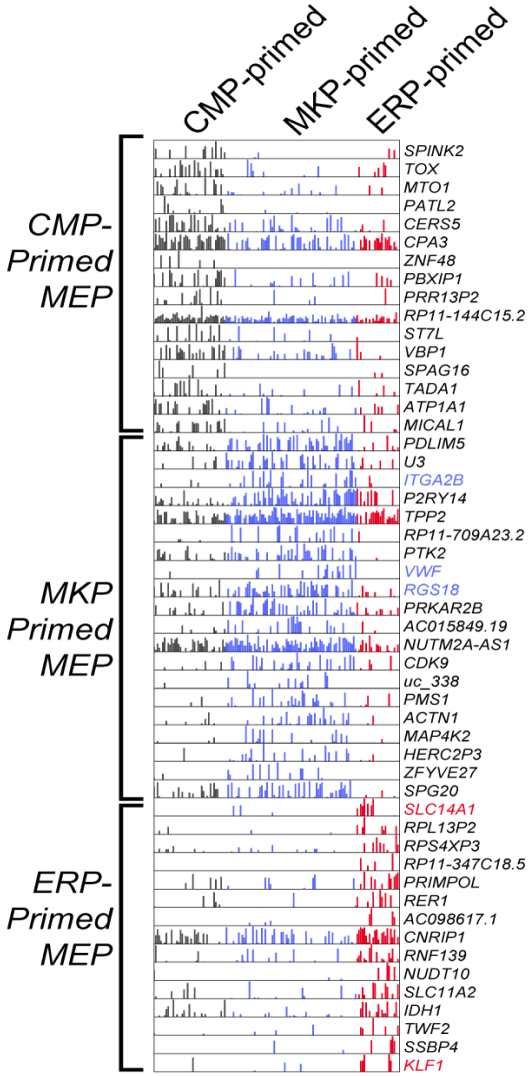
Identifying Bi-Potential Megakaryocyte-Erythroid Intermediates from Human scRNA-Seq



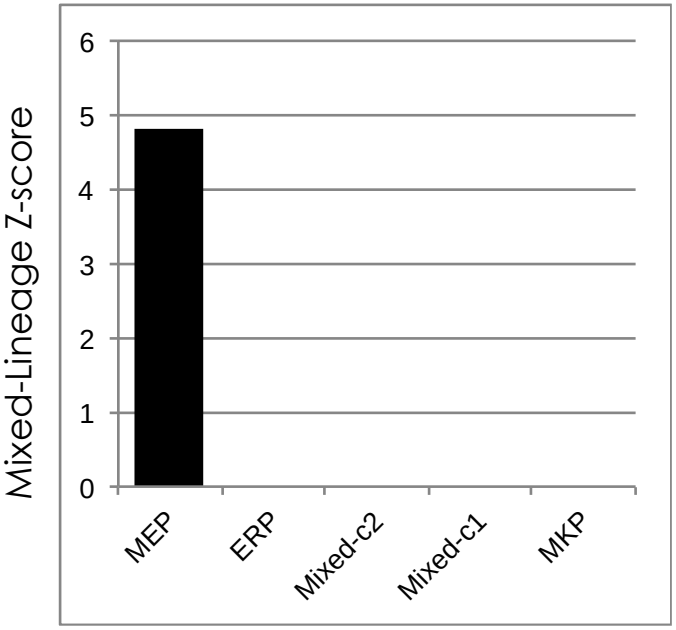


Monocle Trajectory Analysis of MarkerFinder Results Finds Distinct MEP Subsets





Schrodinger Accurately Predicts that MEPs are Mixed-Lineage Progenitors



Mixed-c1 : SLC44A1, ACTN1, TTC27, PDLIM1, FERMT3, TMSB4X, ITGA2B, ABCB1, MYH9, PTGS1, STOM, CTR9, SH2B3, C6orf25, CXCL8, TUBA4A, PKM, PLEK, CD9, GSN

Mixed-c2 : CA1, MPC2, SF3B3, CD55, FBXO7, PRDX2, CD36, SDCBP, SPTA1, ANXA2, APOC1, RB1CC1, CALM2, WDR48, ACSM3, GOLGA4, ELL2, HBS1L, FAM45A, IARS, DLD, AHI1, SLC39A8, SKIL, ACSM1, BLVRB, SEC22C, CXADR, IRF1, ZDHHC2, USP12, RHOBTB3, EZR, KIT, FBXO34, ANK1, YBX1, STARD7, PDZD8

ERP : CNRIP1, TMEM14C, SLC40A1, FAM118A, RYR3, CASP3, U1, ZFP36L1, ELOVL6, HERC2P2, TRIB2, MYC, RREB1, P2RX5, SNORD3A

MEP : HSD17B11, FCER1A, RPS3AP47, CPA3, TESPA1, MEIS1, FREM1, SERPINB1, PBX1

MKP : CD52, CD74, FNBP1, IDS, CD37, KIAA0125, SORL1, AJ006998.2, AHNAK, EGR1, KLF4, KLF2, NPR3, CRHBP, PROM1, ADAM28, SMIM24, CLEC2B, ID2, ID3, VIM, ATP8B4