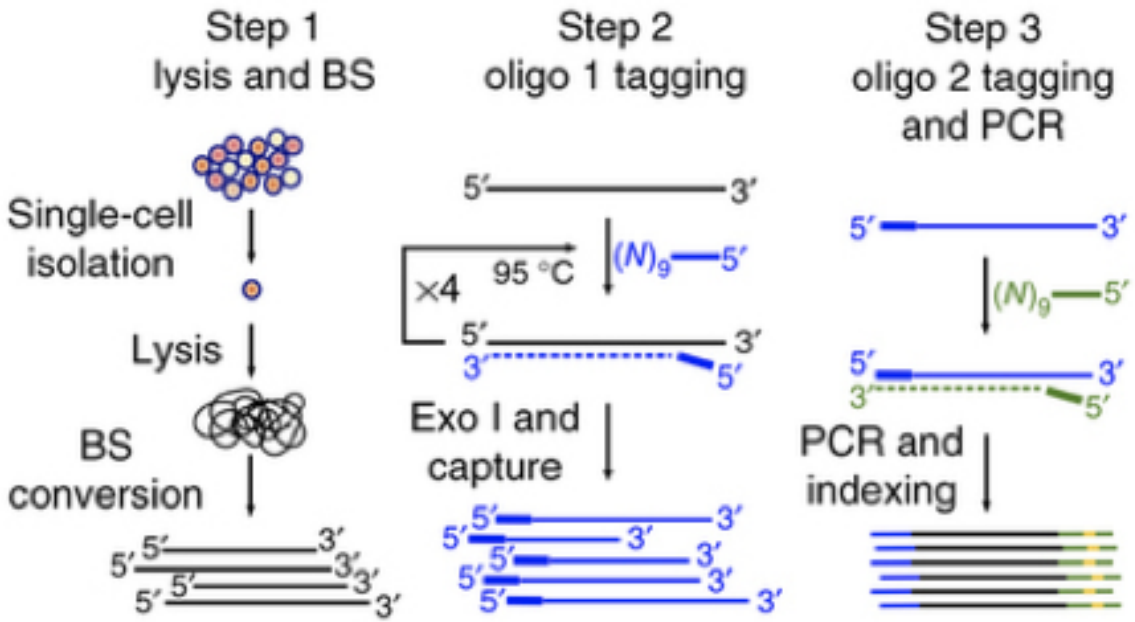
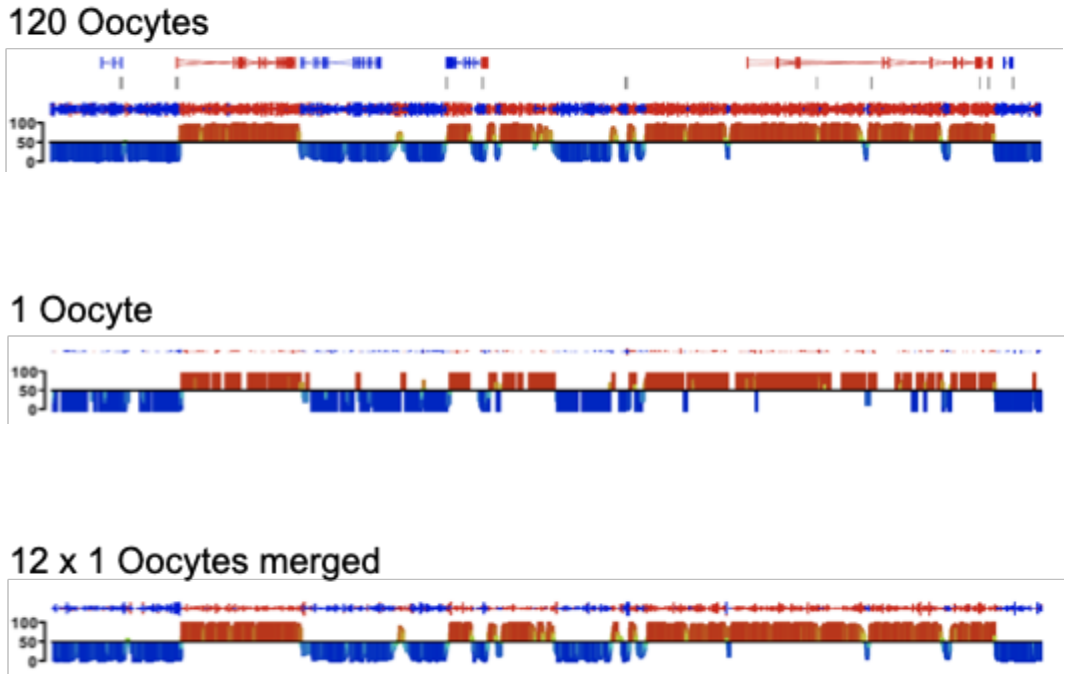
Single-cell multiomics sequencing to investigate the role of the epigenome in cell fate decisions during mouse embryogenesis

Stephen Clark 30 June 2023

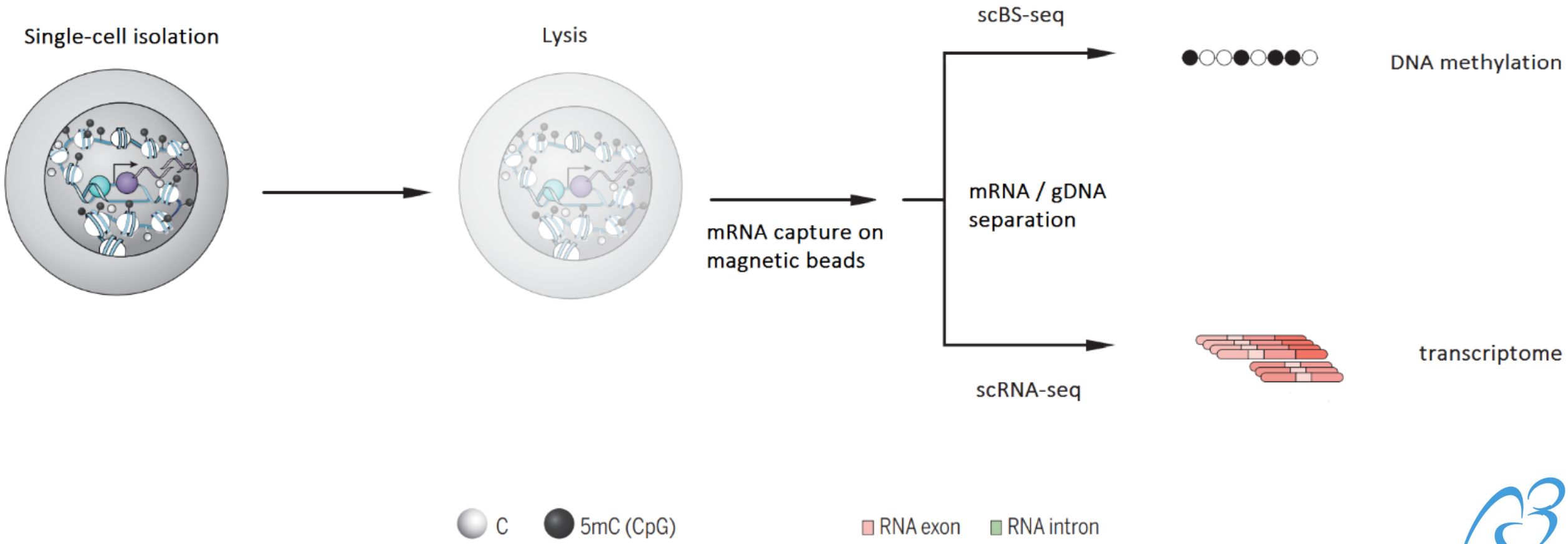
scBS-seq for profiling DNA methylation in single cells





Smallwood, Lee *et al* 2014

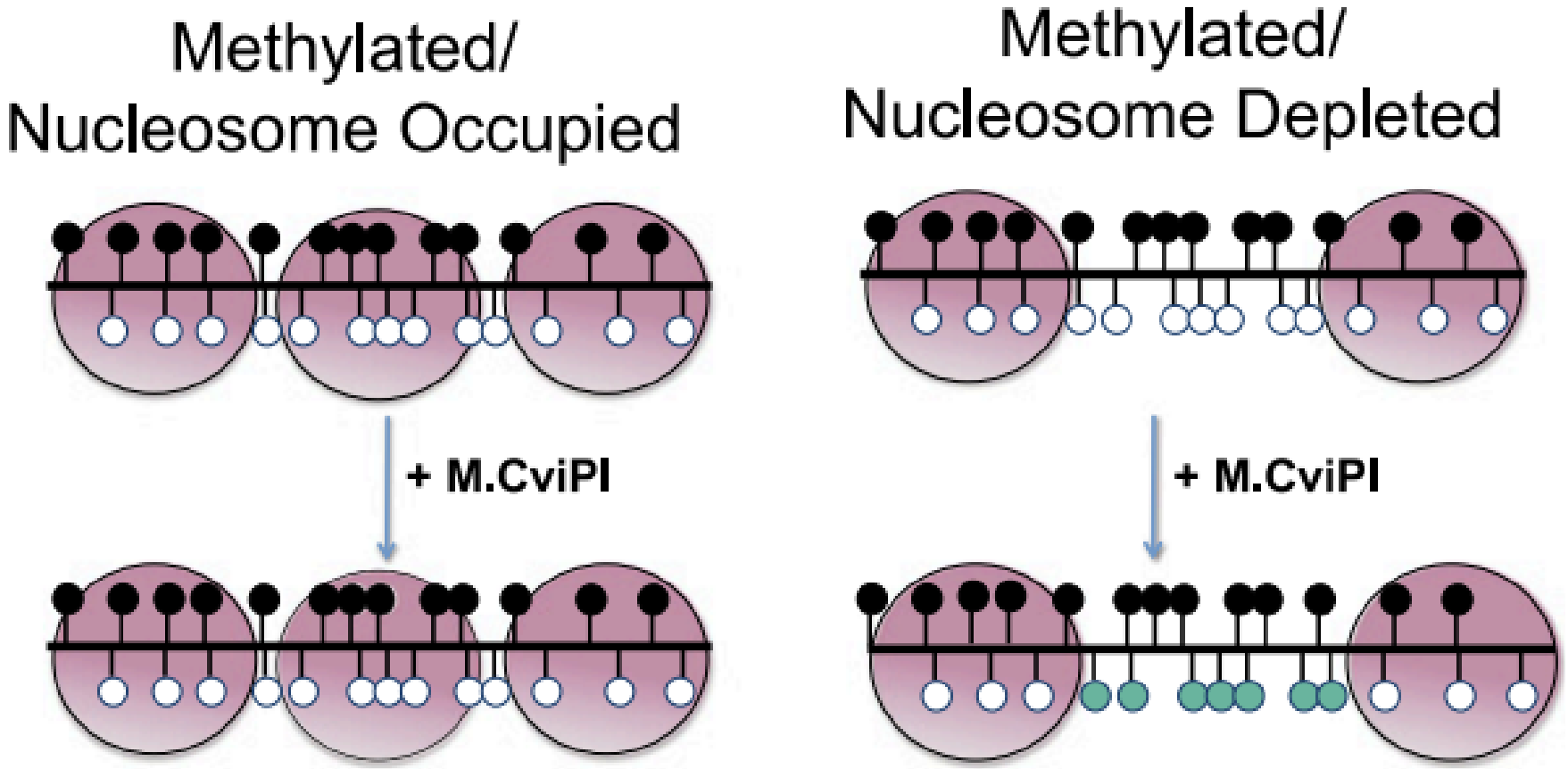
Multi-omics allows epigenomic profiling and cell type classification



scM&T-seq: Angermueller, Clark, Lee, Macaulay *et al* 2016

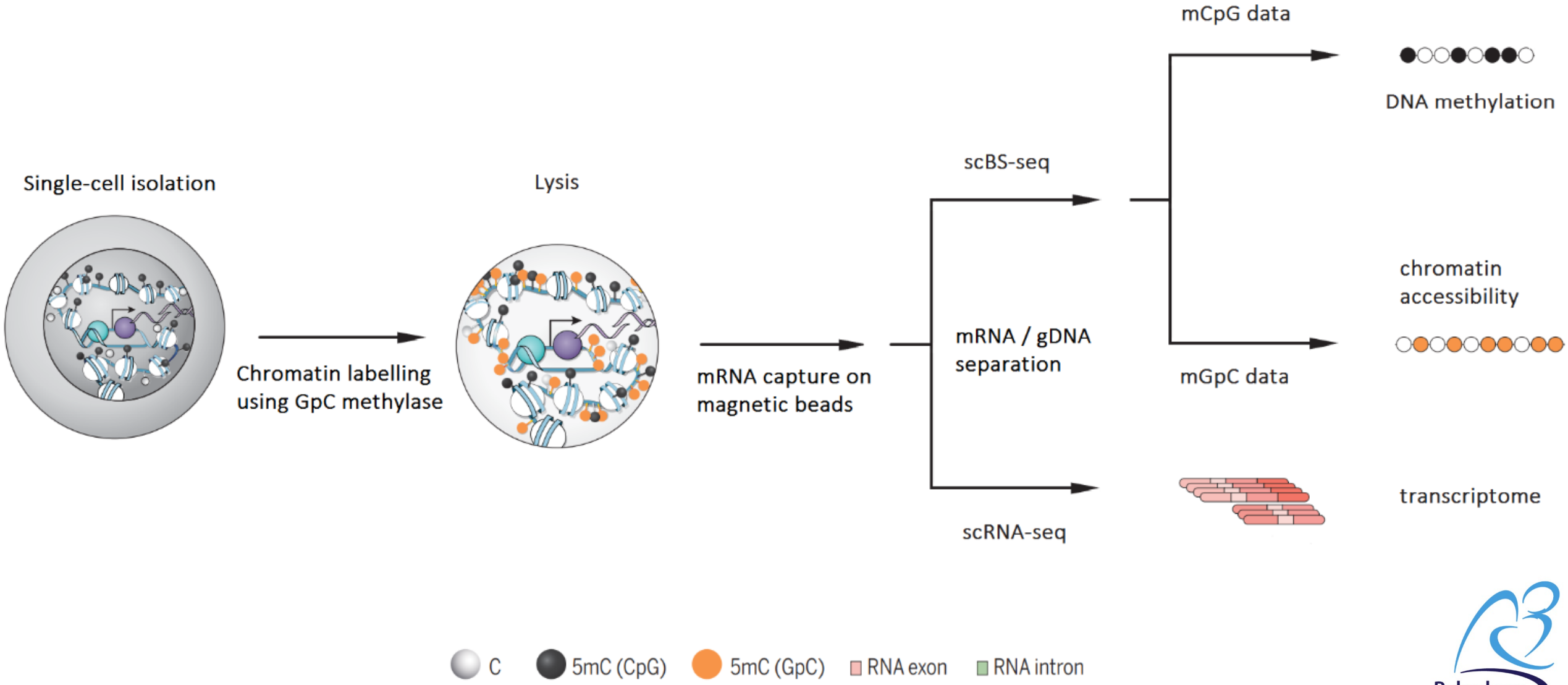
Babraham Institute

scNMT-seq: chromatin state via methylase accessibility



NOMe-seq: Kelly *et al* 2012

scNMT-seq: chromatin state via methylase accessibility

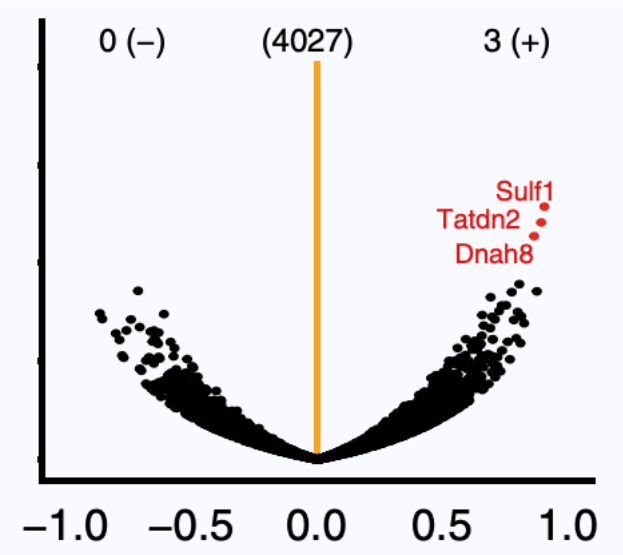


scNMT-seq: Clark, Argelaguet *et al* 2018

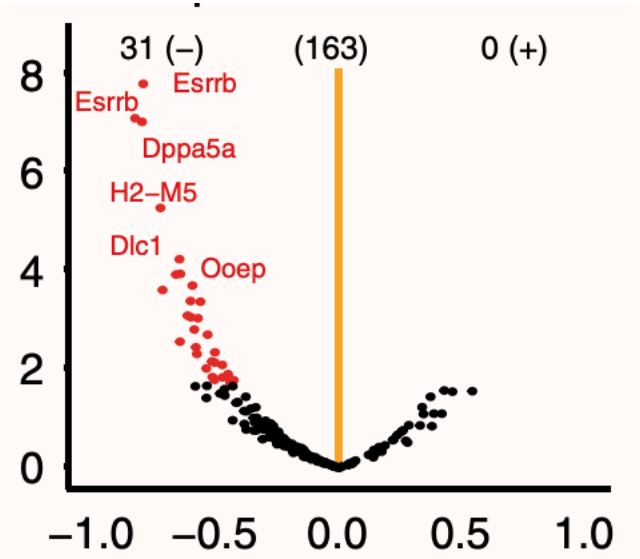
Babraham Institute

scNMT-seq allows discovery of regulatory relationships

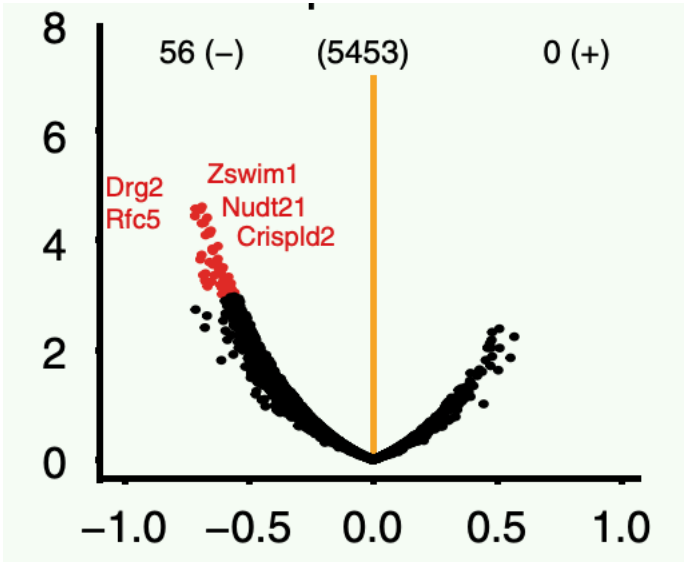
Accessibility vs Expression



Methylation vs Expression



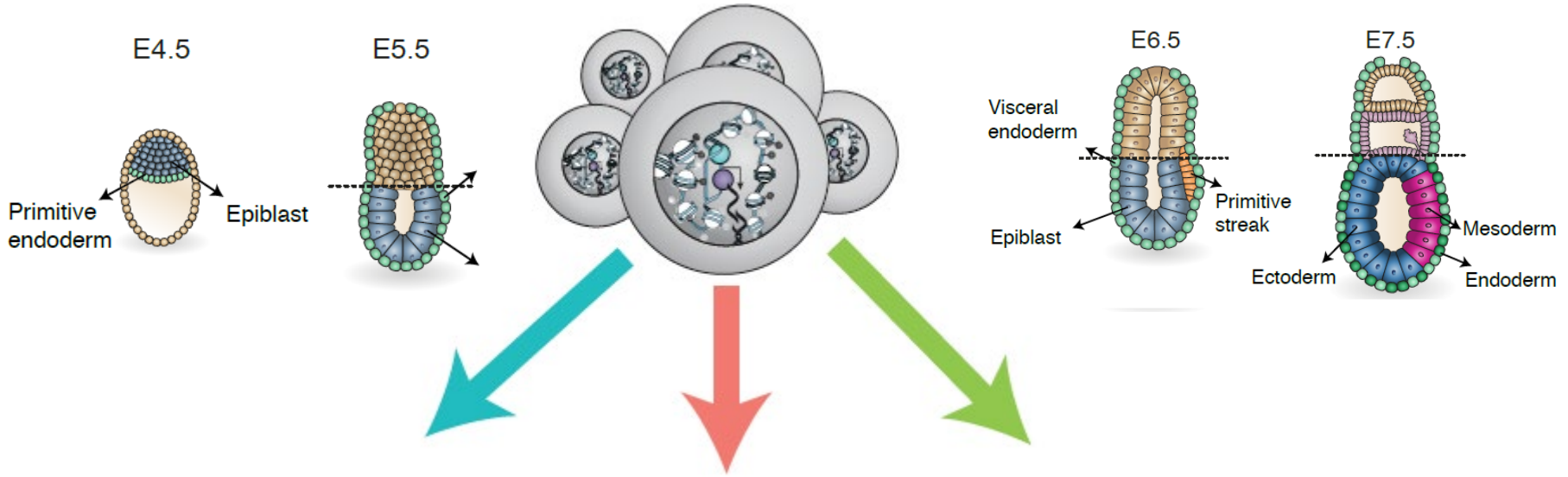
Methylation vs Accessibility

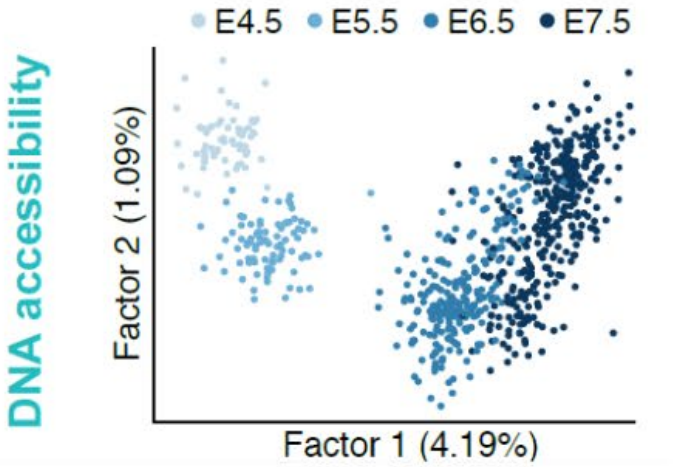


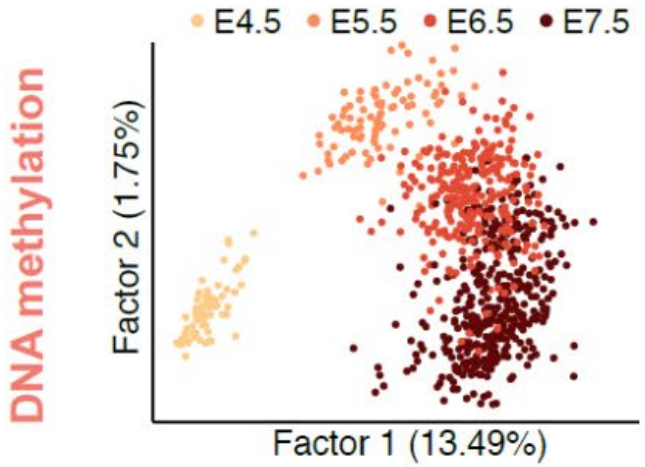
Weighted Pearson correlation

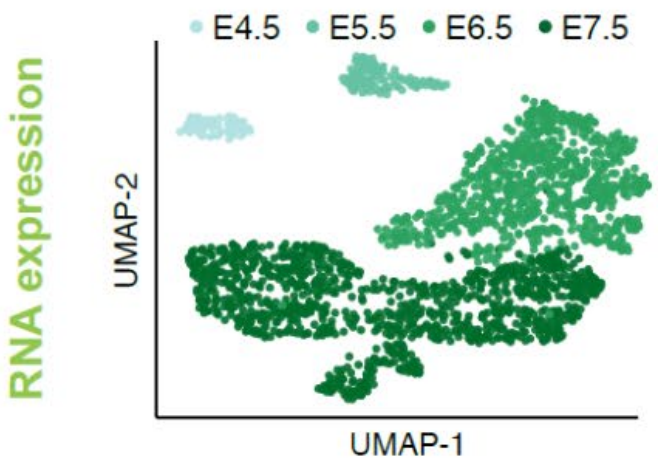
scNMT-seq: Clark, Argelaguet *et al* 2018

scNMT-seq of 1,105 cells from 4 stages of mouse development



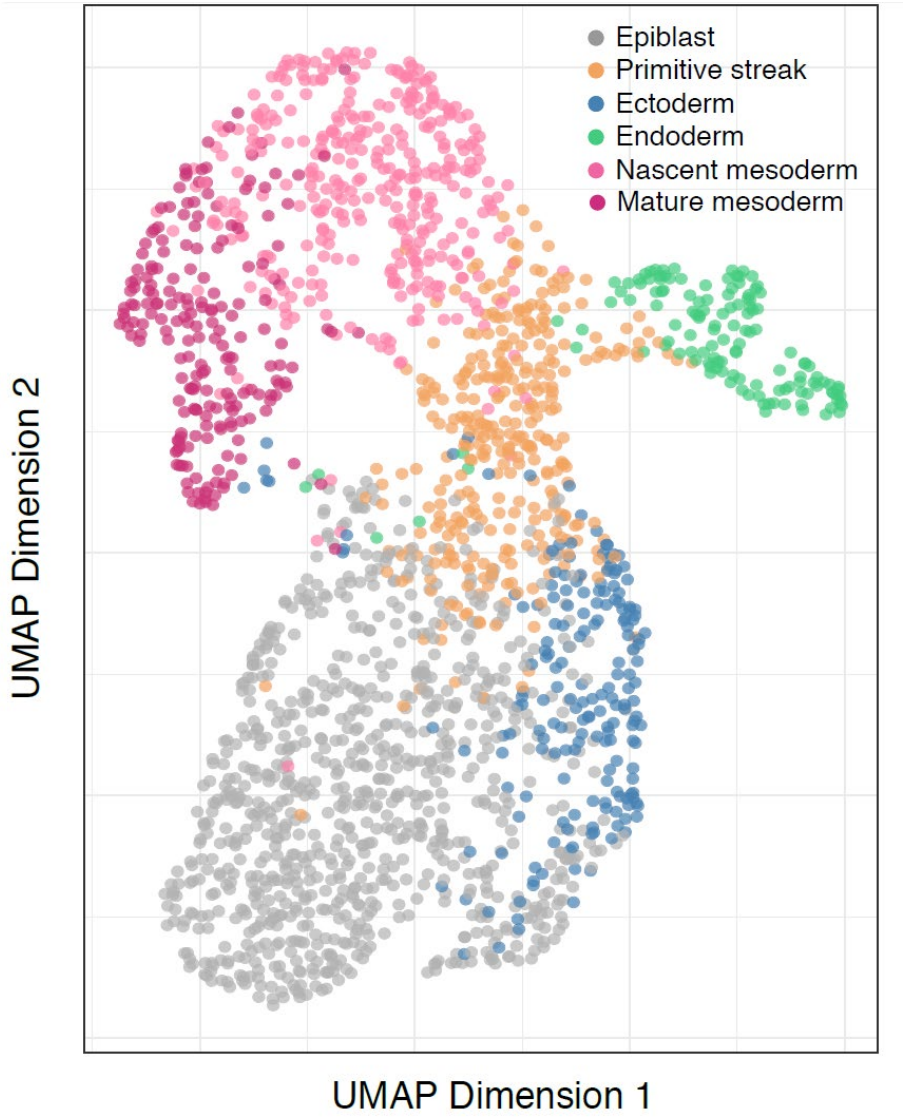




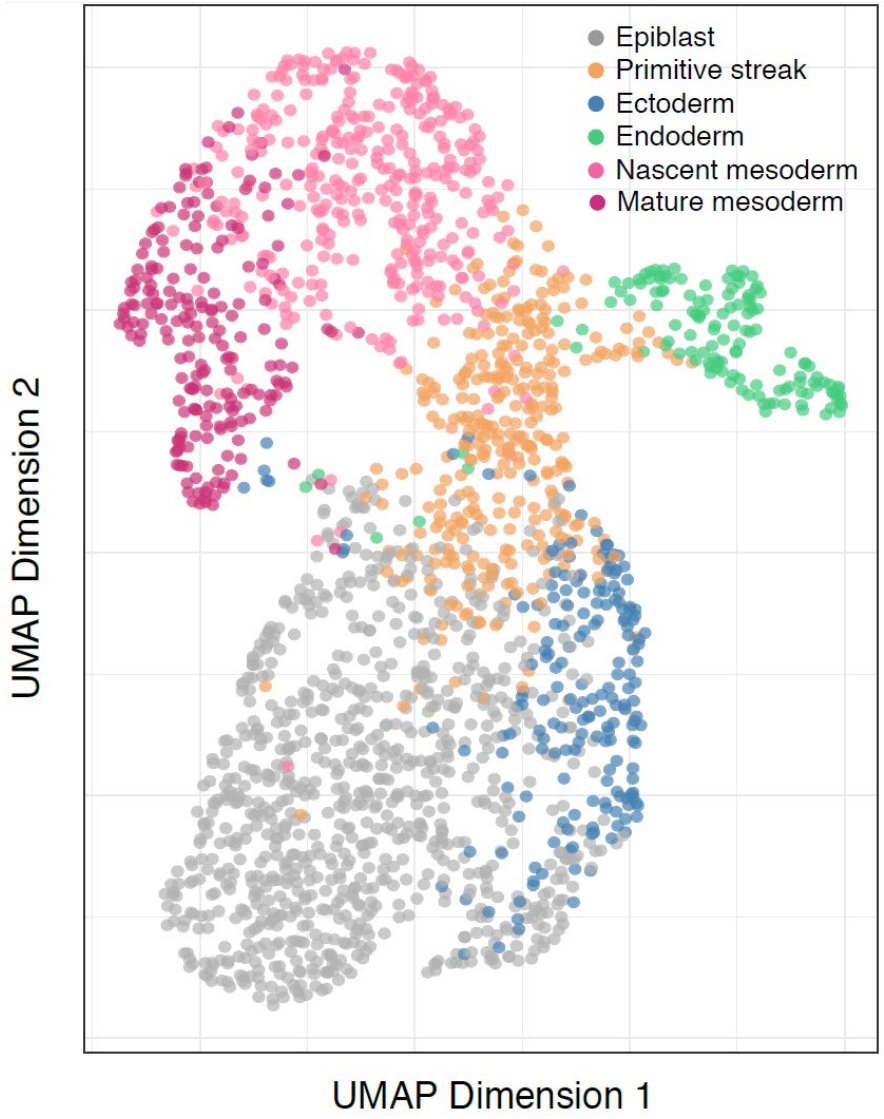


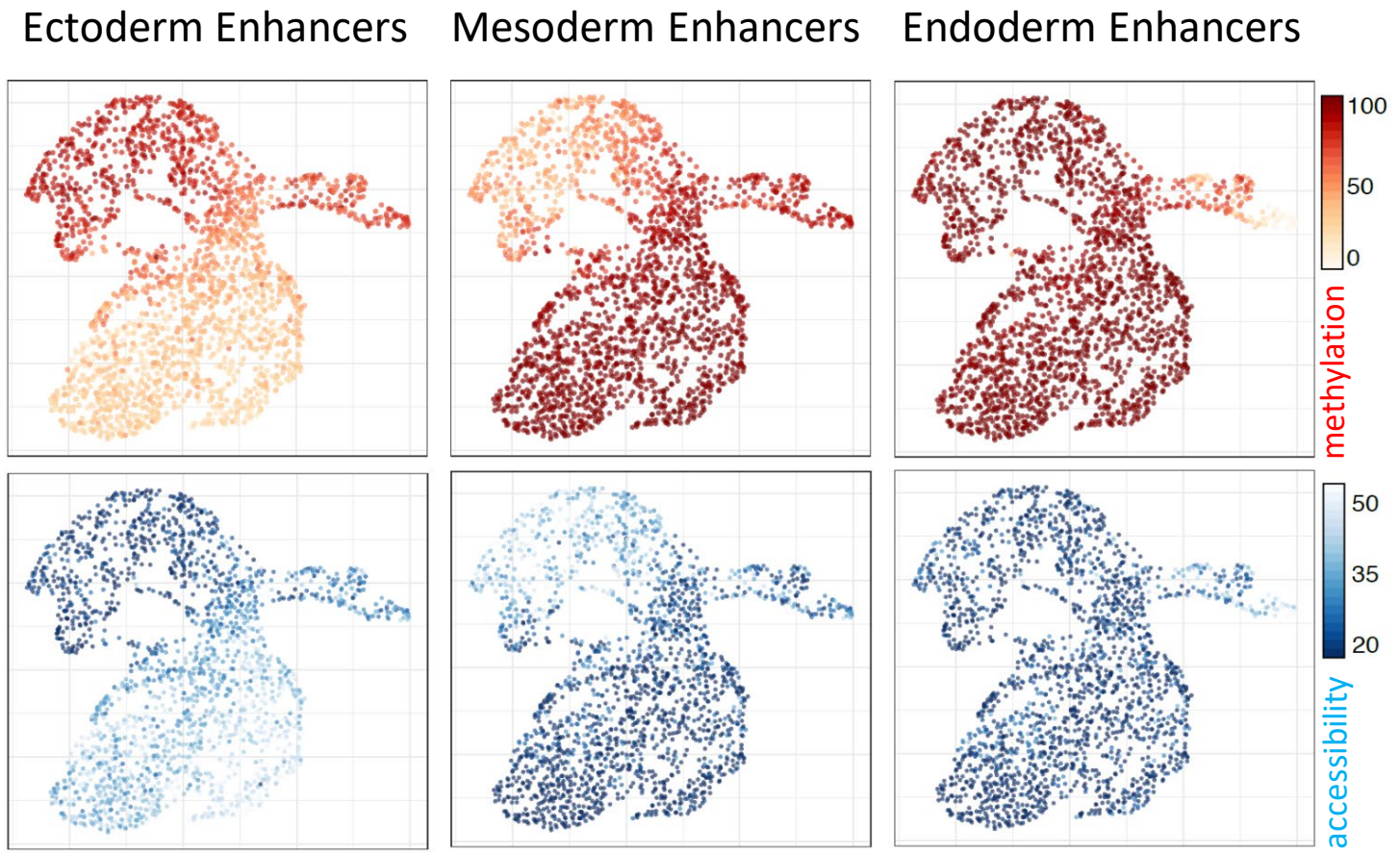
Total 2,524 cells for RNA-seq

Reconstructed developmental trajectory using 3 omics

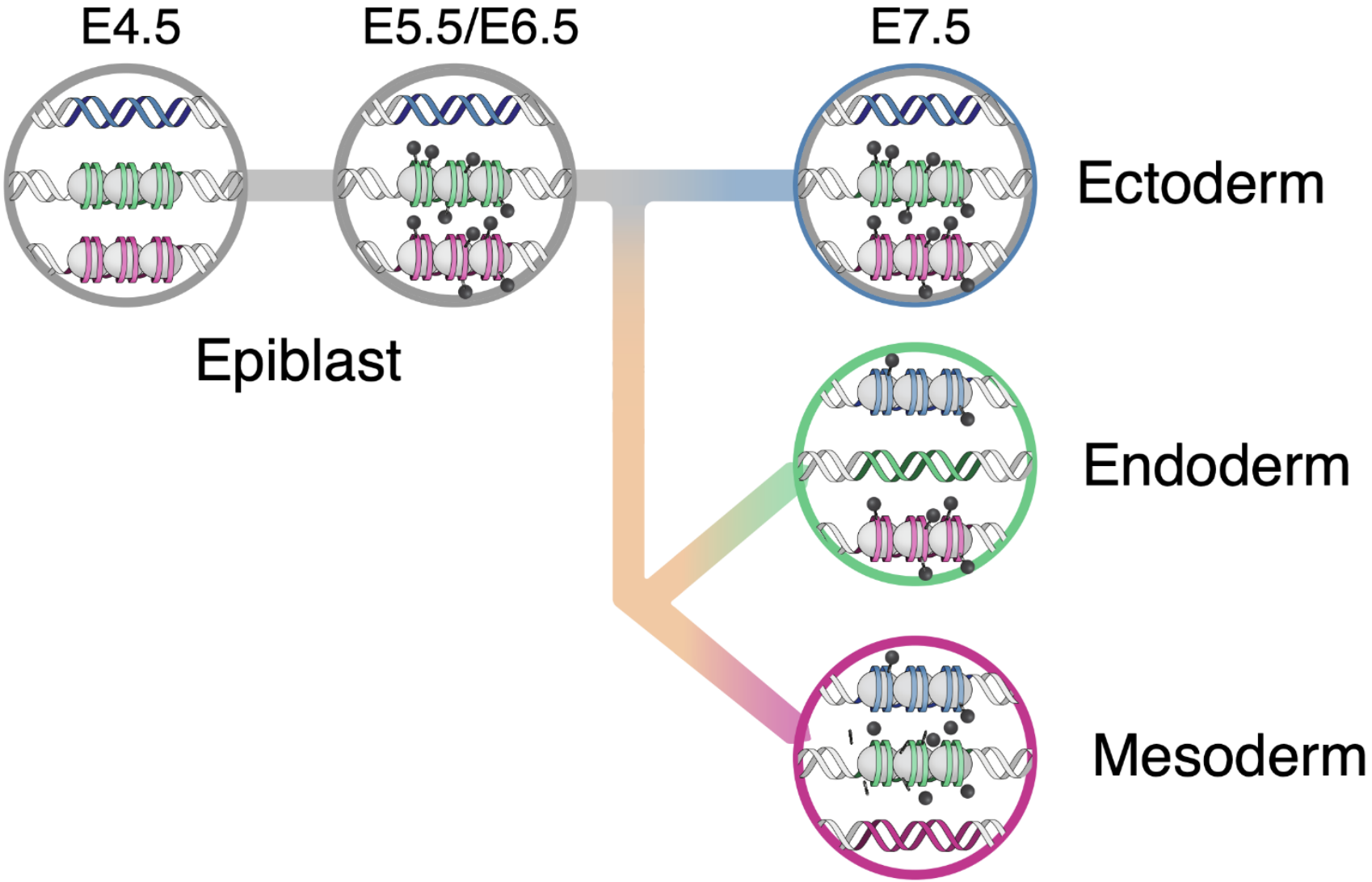


Lineage-specific enhancer hypo-methylation and accessibility





Hierarchical epigenetic model for the primary germ layers



Argelaguet, Clark, Mohammed, Stapel *et al* 2019

Is this the molecular basis of default neuro-ectoderm path?

Neuron. 2001 Apr;30(1):65-78.

Direct neural fate specification from embryonic stem cells: a primitive mammalian neural stem cell stage acquired through a default mechanism.

Tropepe V[1], Hitoshi S, Sirard C, Mak TW, Rossant J, van der Kooy D.

Author information

1 Department of Anatomy & Cell Biology, University of Toronto, Ontario M5S 1A8, Toronto, Canada.

Abstract

Little is known about how neural stem cells are formed initially during development. We investigated whether a default mechanism of neural specification could regulate acquisition of neural stem cell identity directly from embryonic stem (ES) cells. ES cells cultured in defined, low-density conditions readily acquire a neural identity. We characterize a novel primitive neural stem cell as a component of neural lineage specification that is negatively regulated by TGFbeta-related signaling. Primitive neural stem cells have distinct growth factor requirements, express neural precursor markers, generate neurons and glia in vitro, and have neural and non-neural lineage potential in vivo. These results are consistent with a default mechanism for neural fate specificati preceded by a primitive neural stem cell stage during neural linea

PMID: 11343645 DOI: 10.1016/s0896-6273(01)00263-x

Cell Differ Dev. 1989 Dec;28(3):211-7.

Neural differentiation of Xenopus laevis ectoderm takes place after disaggregation and delayed reaggregation without inducer.

Grunz H[1], Tacke L.

Author information

1 Department of Zoophysiology, Universität GHS Essen, F.R.G.

Abstract

When Xenopus blastula or early gastrula ectoderm is disaggregated and cells are kept dispersed for up to 5 h prior to reaggregation, the resulting spheres will differentiate into large neural structures. In contrast, dissociated and immediately reaggregated ectoderm will only differentiate into ciliated epidermis (so-called 'atypical epidermis'). Ectoderm treated with mesoderm-inducing XTC-conditioned medium during the period of reaggregation immediately after disaggregation will only form one- or two-cell types (notochord and somites) only. Ectoderm treated with XTC-factor prior to disaggregation will differentiate into a large variety of cell types.

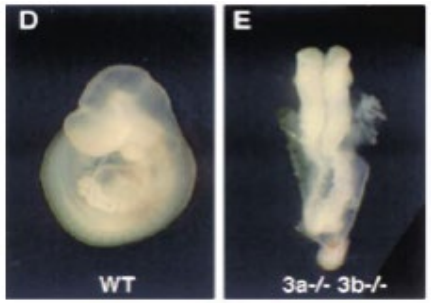
PMID: 2620262

Combining single-cell sequencing with genetic perturbations

Background

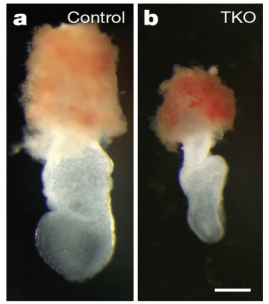
- Methylation mutants are embryonic lethal (usually around gastrulation)

E9.5 *Dnmt3a/b*$^{-/-}$



Okano *et al*., Cell, 1999

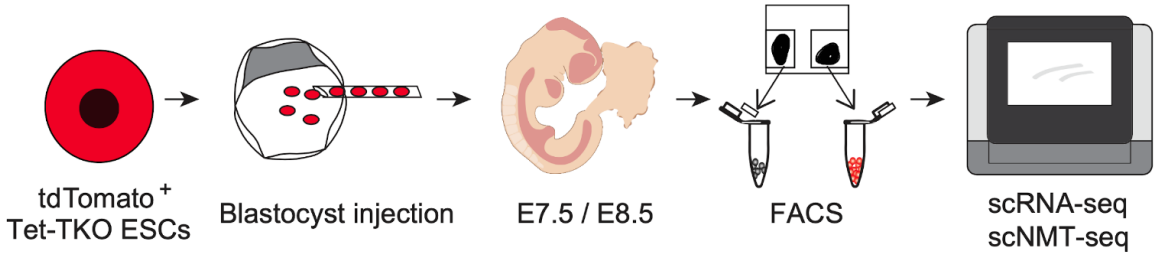
E7.5 *Tet1/2/3*$^{-/-}$



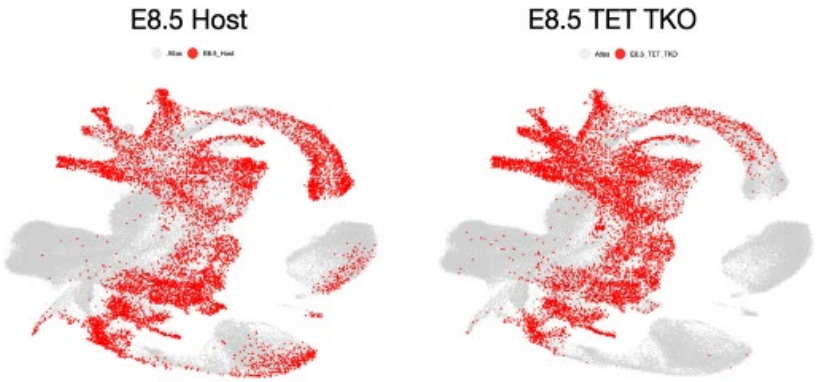
Dai *et al*., Nature 2016

- Precise role of methylation in development / lineage decisions is not well understood
- Use single-cell RNA-seq to characterise cell types at gastrulation (E7.5, E8.5)
- Use scNMT-seq to measure methylation defects in a subset

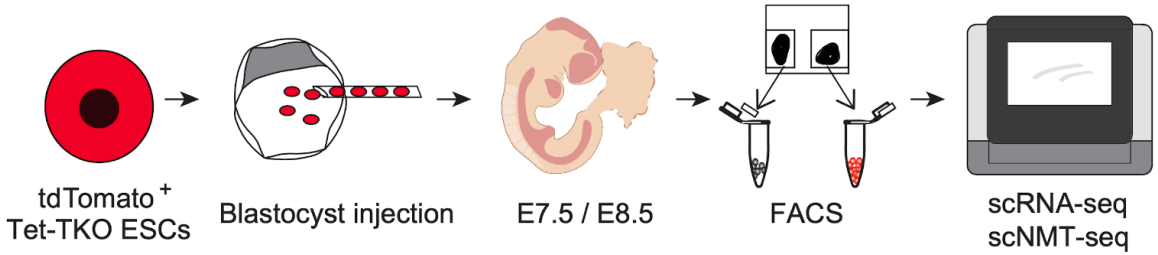
scRNA-seq of Tet TKO chimaeras



Map cell types to comprehensive scRNA-seq atlas (Pijuan-Sala *et al* 2019)



scRNA-seq of Tet TKO chimaeras



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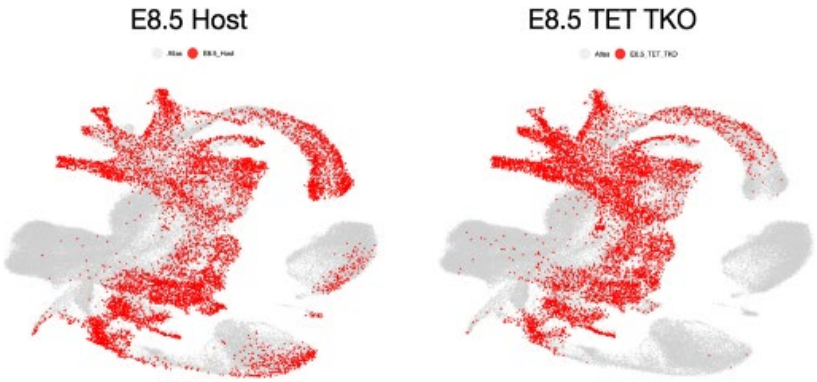
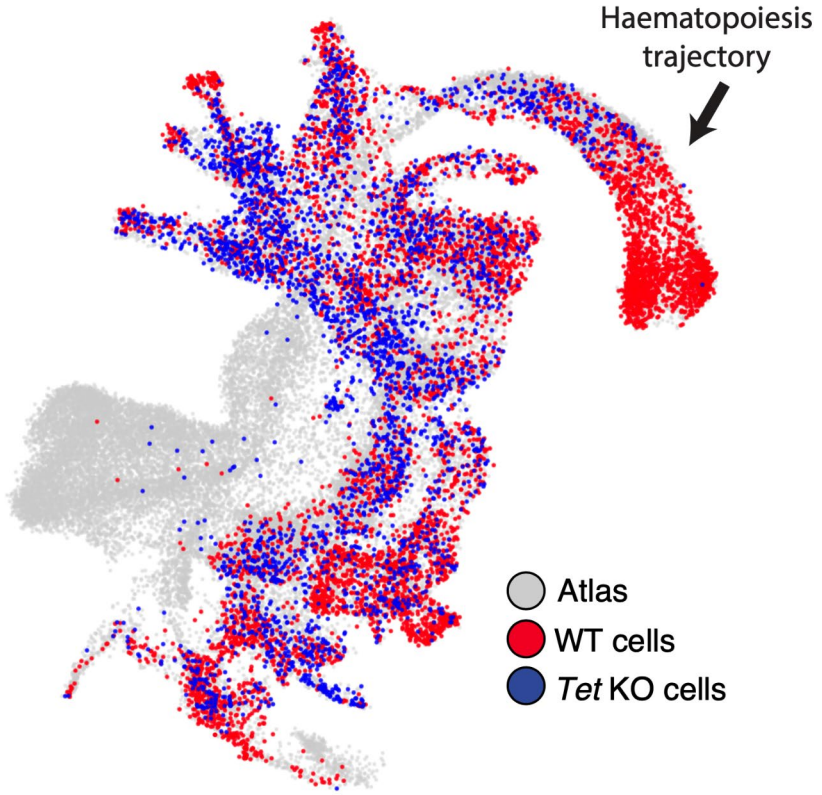
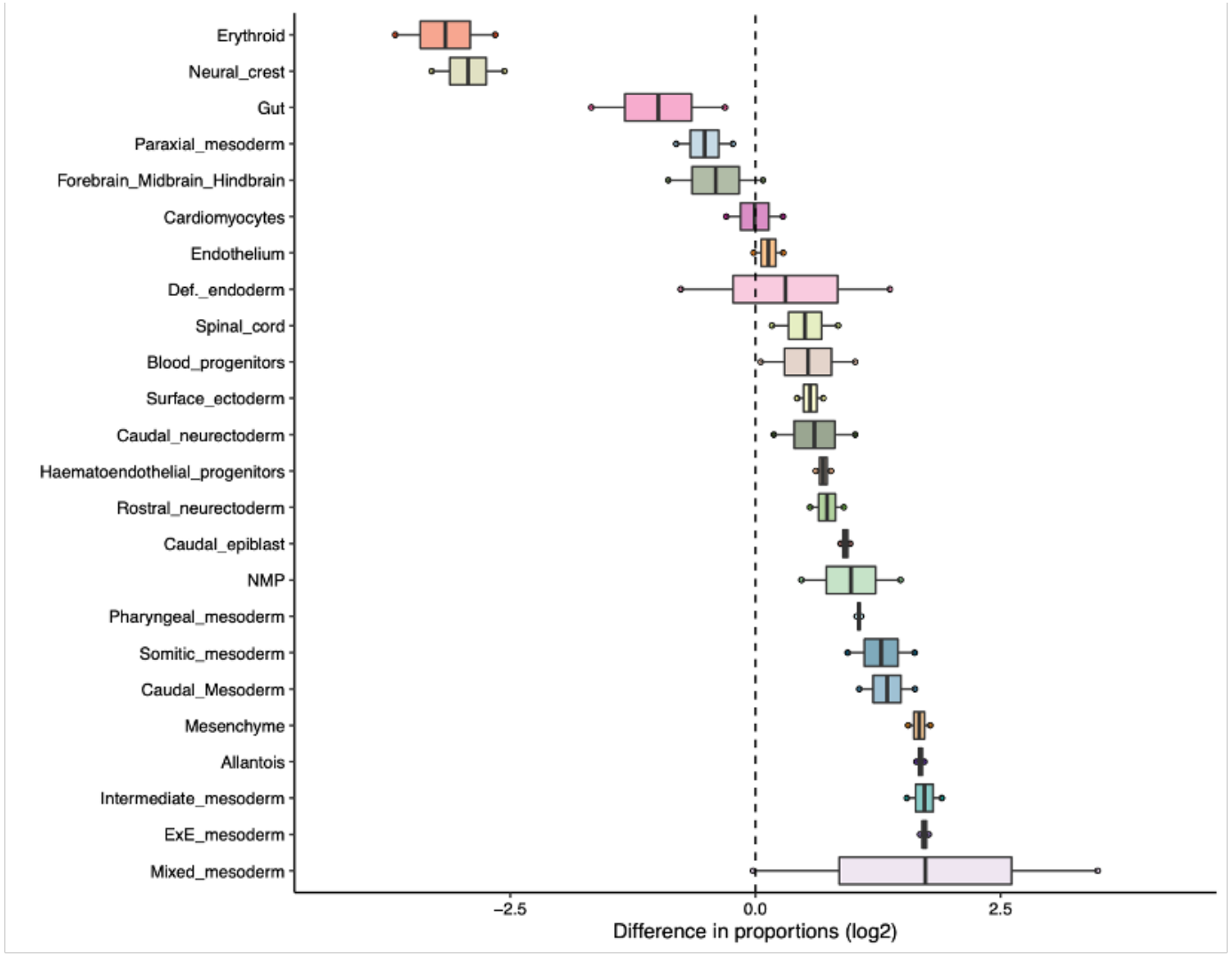
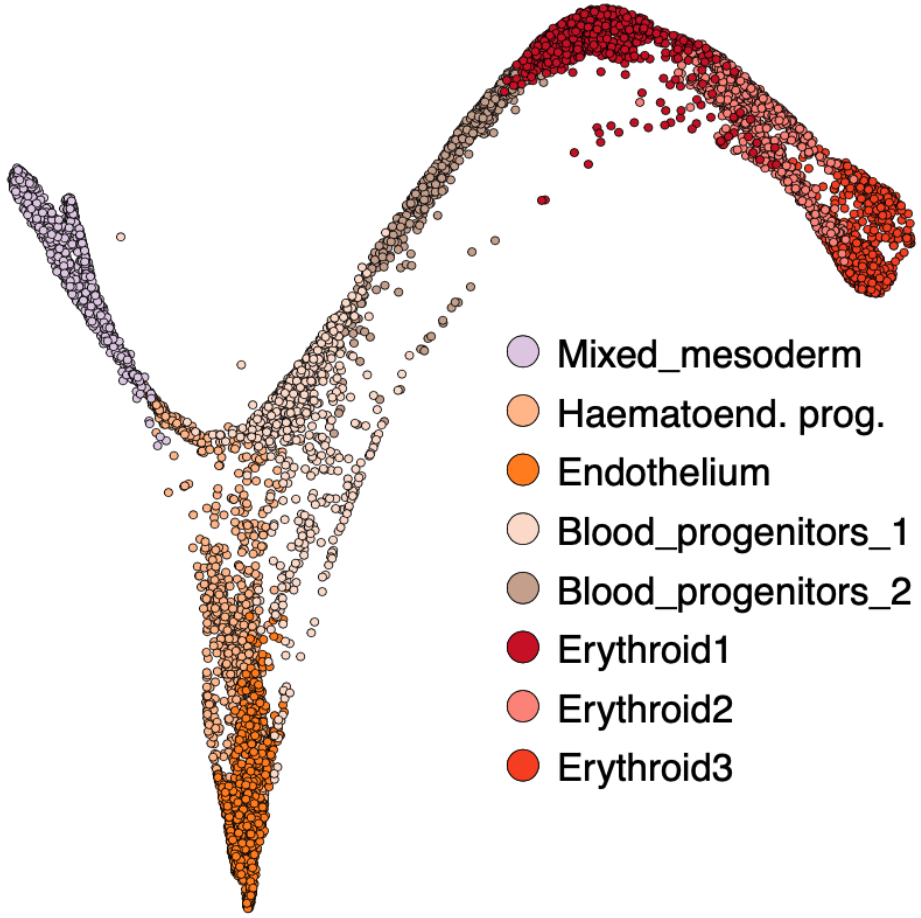


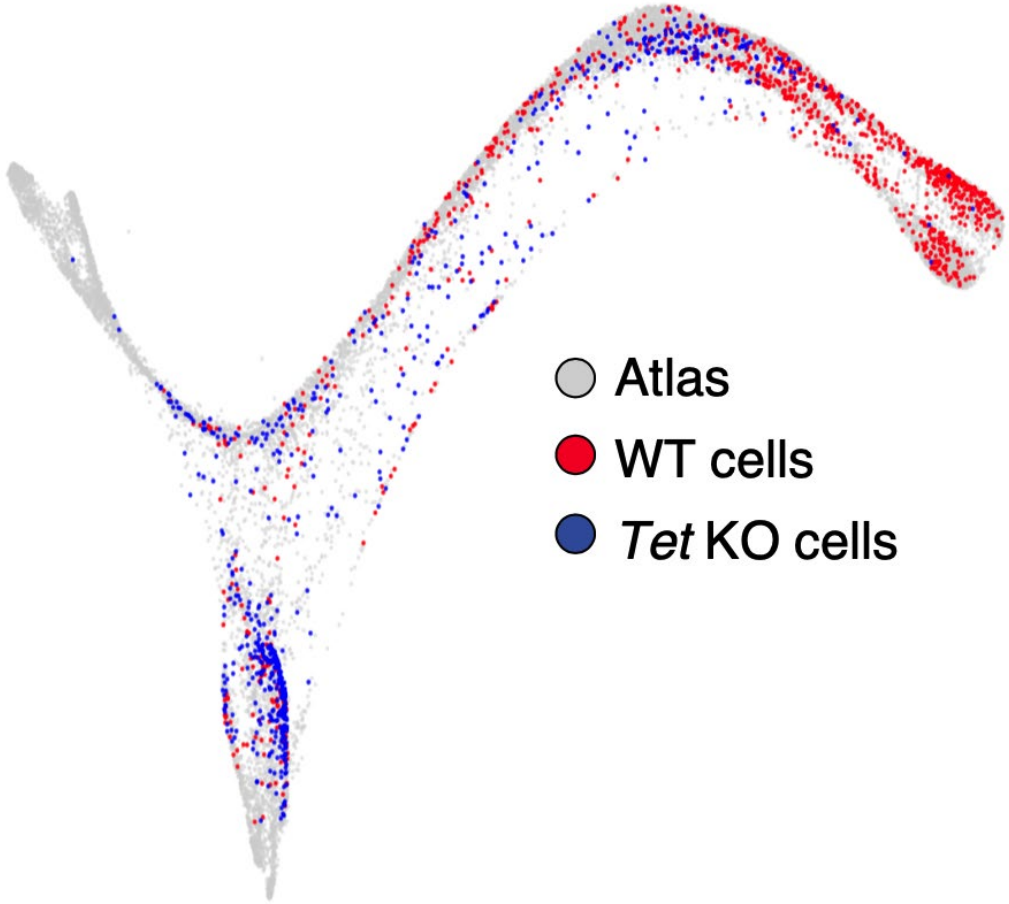


Blood and neural crest are depleted in TKO



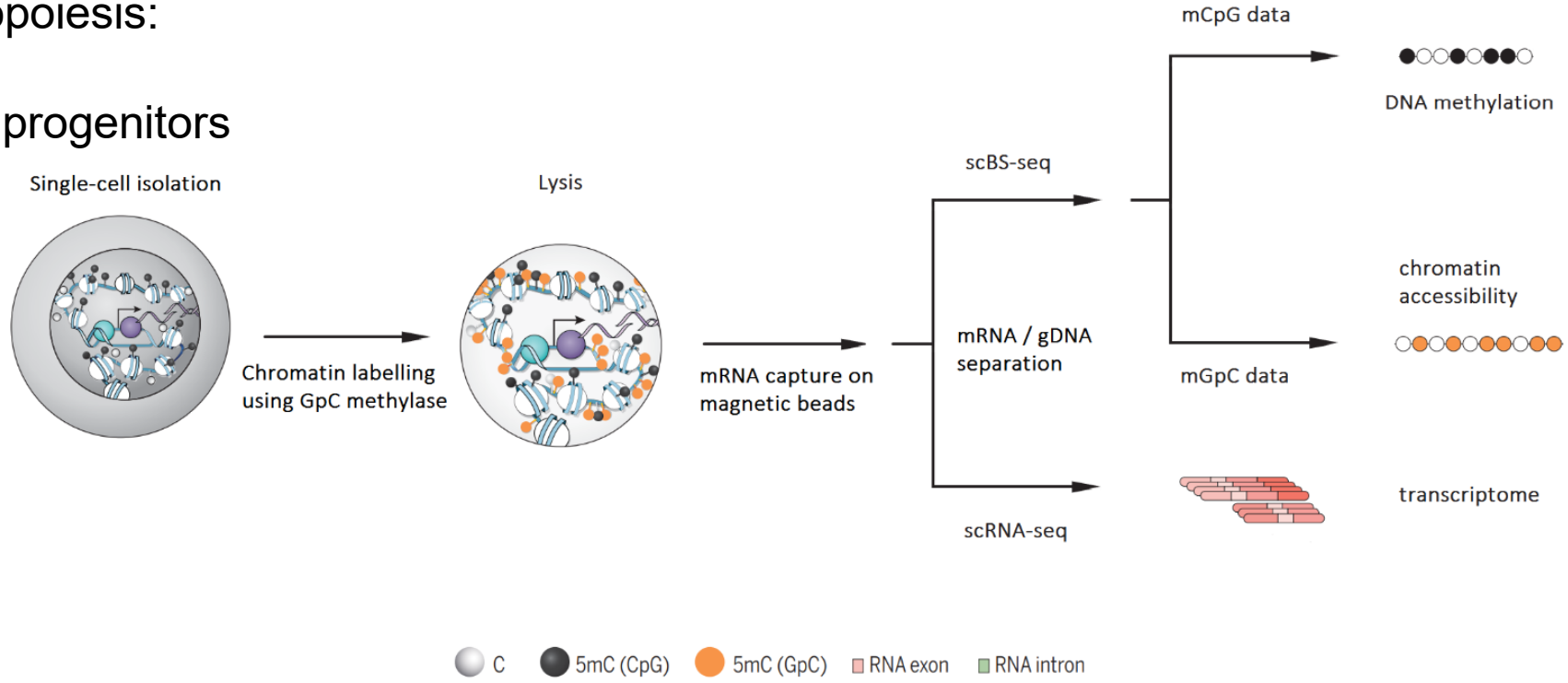
Blood trajectory is perturbed in TKO



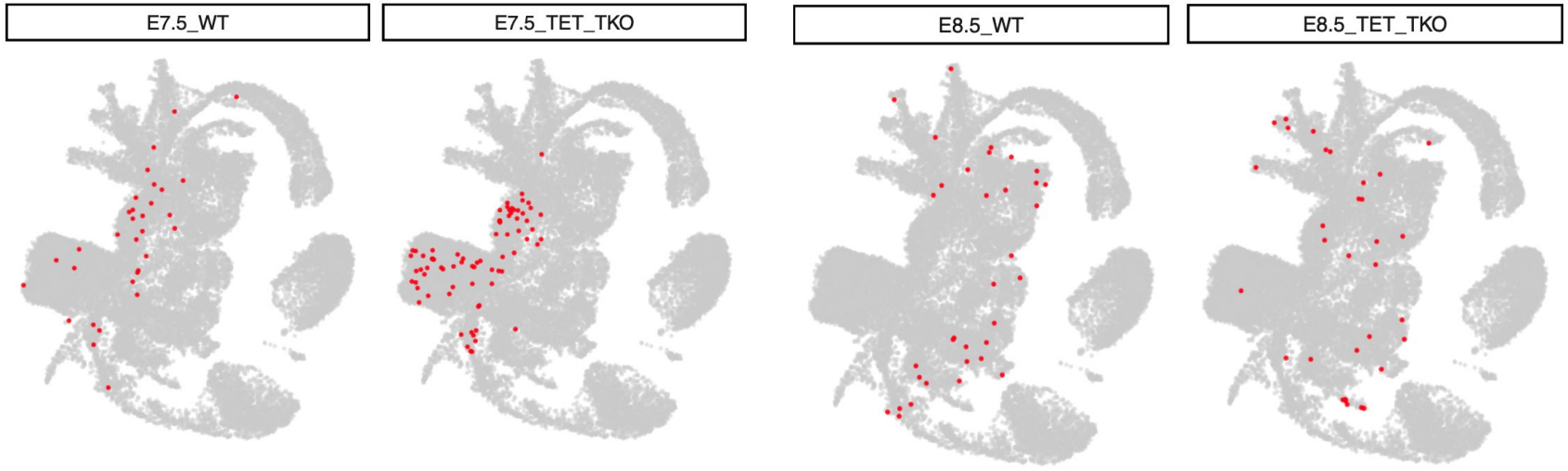


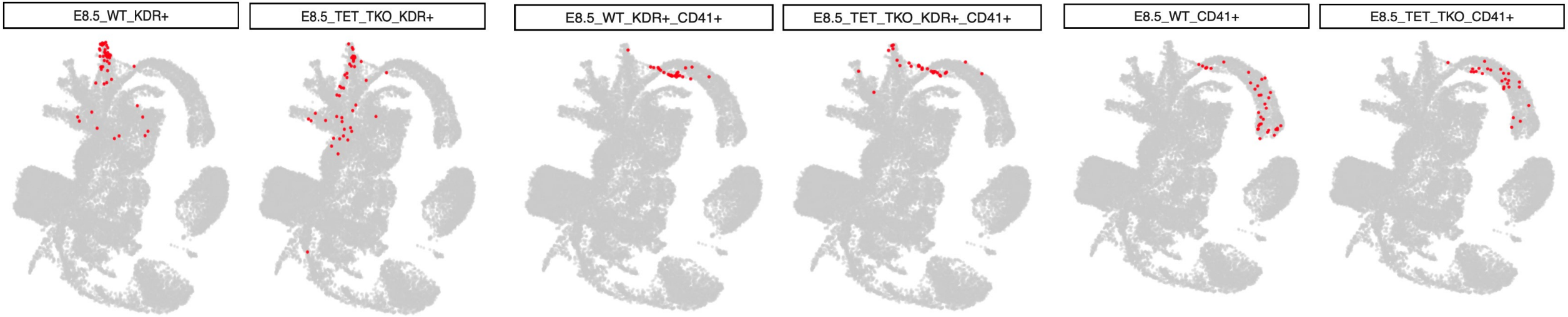
scNMT-seq of embryo cells flow-sorted for blood

Stain for markers of haematopoiesis:
CD41+ = blood cells
KDR+ = haematoendothelial progenitors

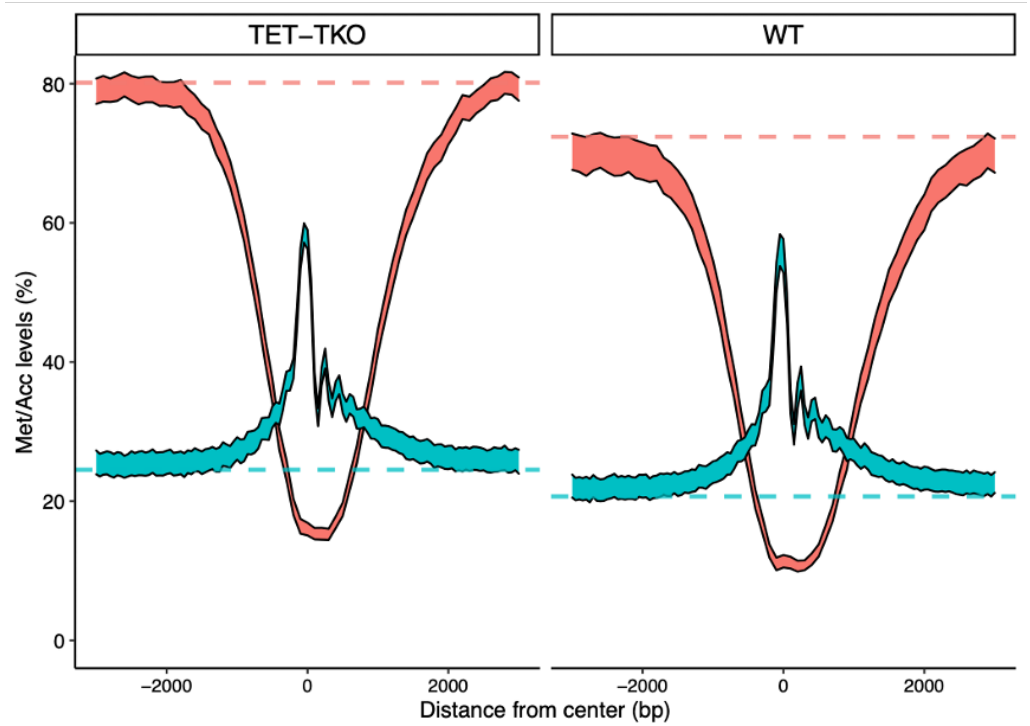


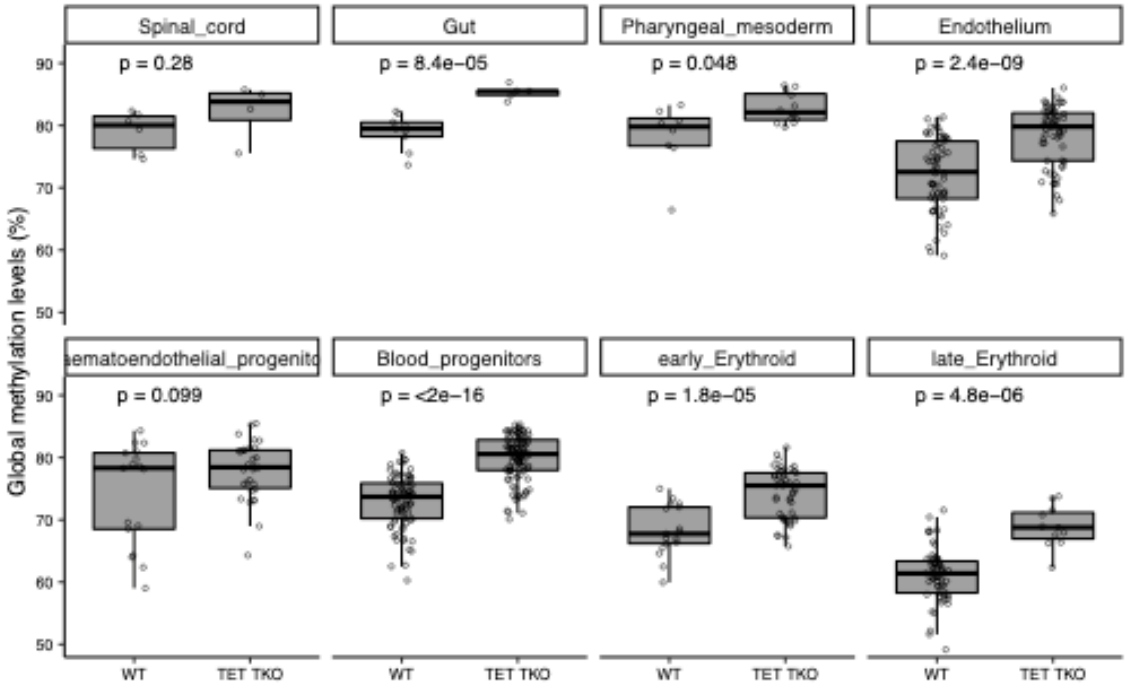
scNMT-seq of blood enriched WT and TKO cells



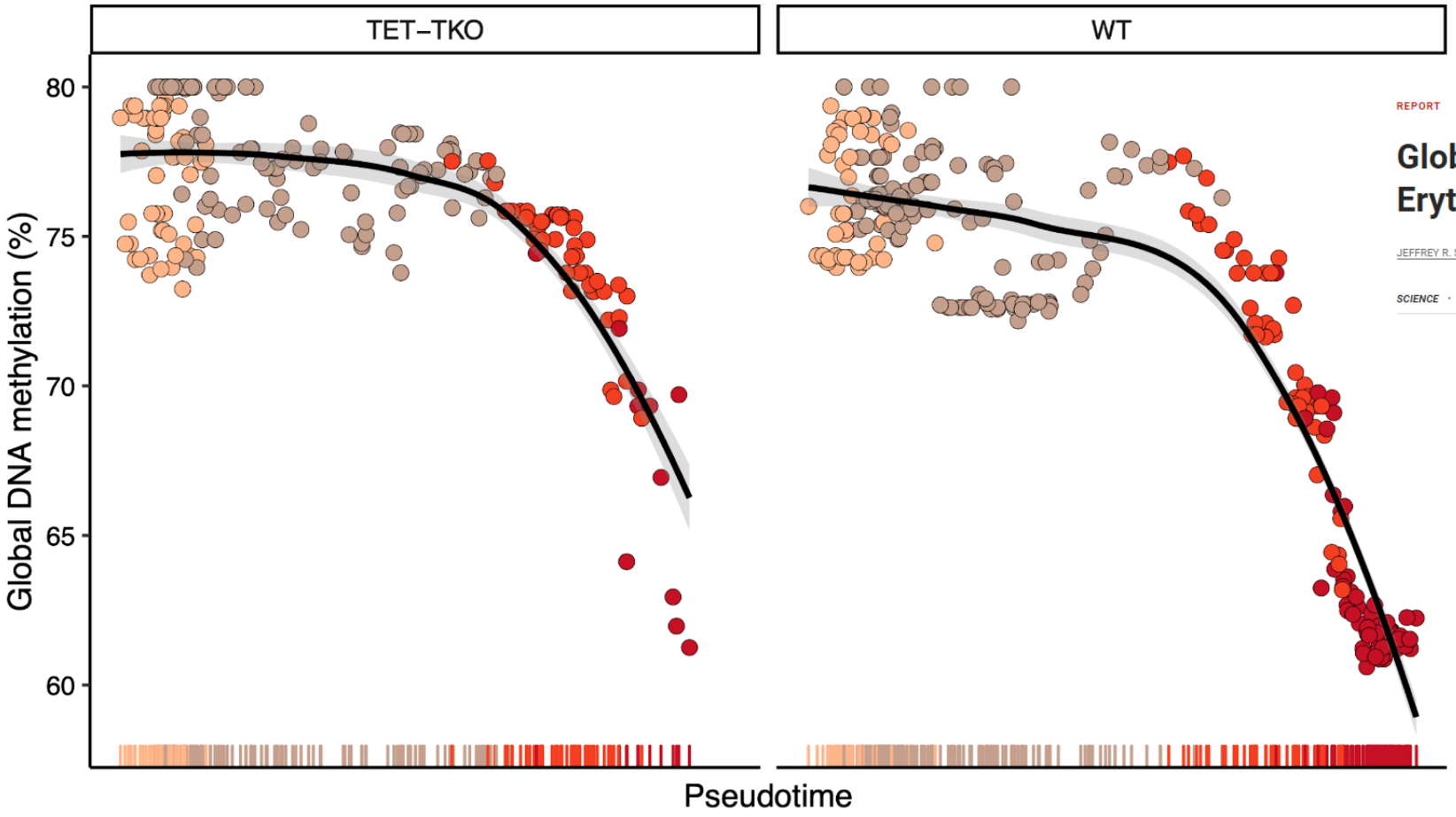


Global methylation is modestly increased in Tet TKO





Blood cells are de-methylated independent of Tet enzymes



REPORT

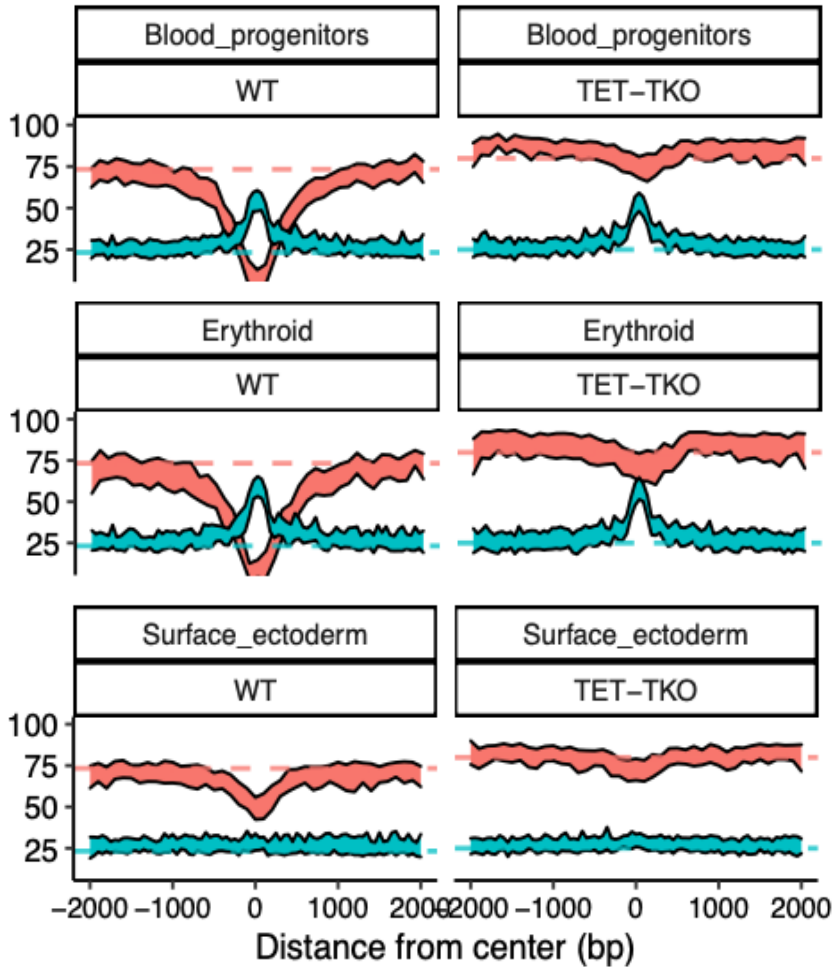
Global DNA Demethylation During Mouse Erythropoiesis in Vivo

JEFFREY R. SHEARSTONE, RAMONA POP, CHRISTOPH BOCK, PATRICK BOYLE, ALEXANDER MEISSNER, AND , MERAV SOCOLOVSKY Authors Info & Affiliations

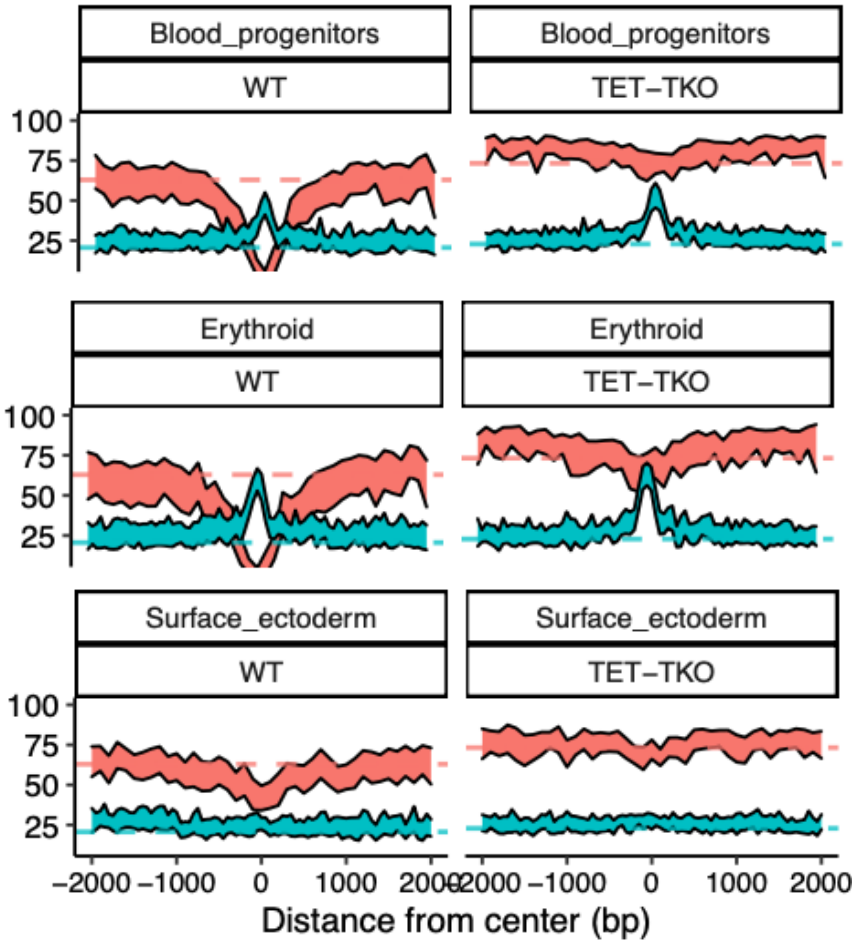
SCIENCE · 11 Nov 2011 · Vol 334, Issue 6057 · pp. 799-802 · DOI: 10.1126/science.1207306

Tet dependent de-methylation of lineage specific ATAC peaks

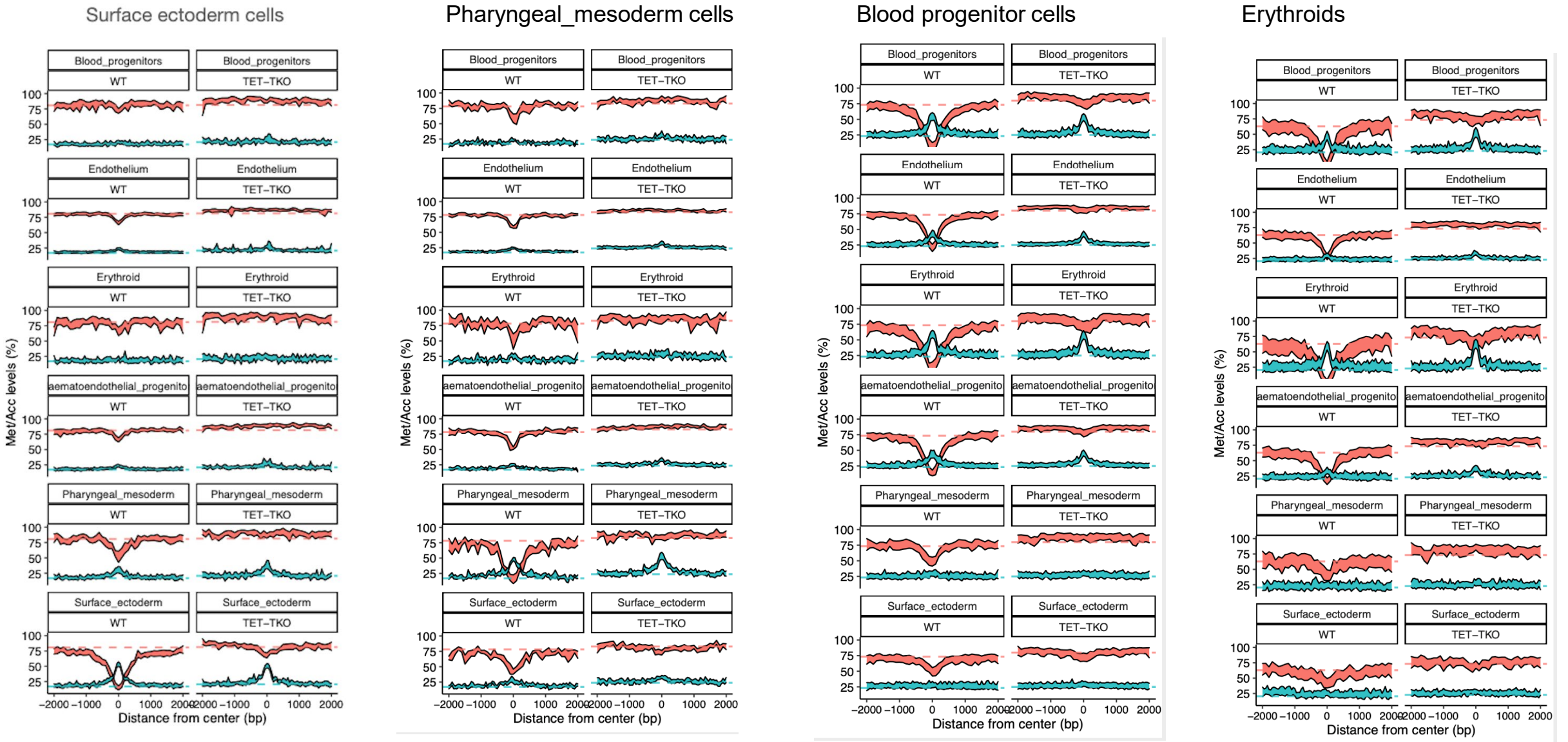
Blood progenitor cells



Erythroids



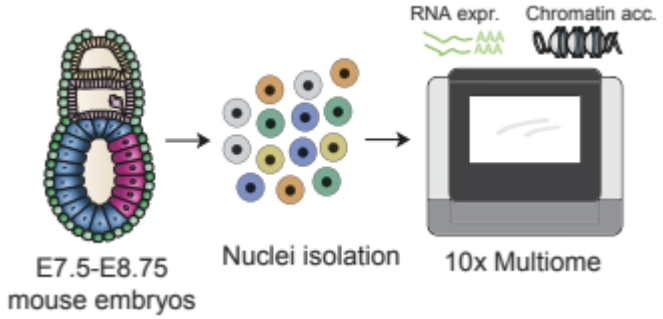
Tet dependent de-methylation of lineage specific ATAC peaks is universal



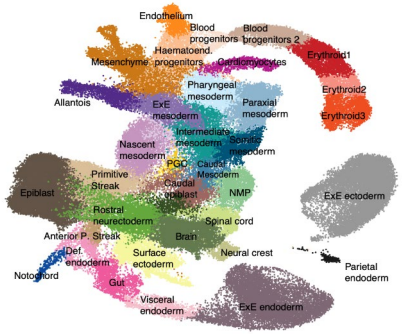
Clark, Argelaguet, Lohoff *et al* 2022

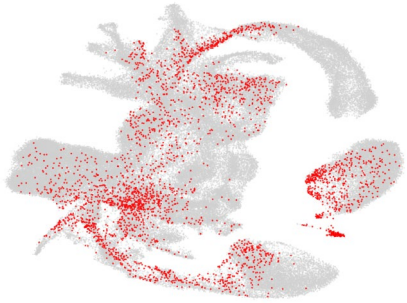
Expanding the embryo multi-omic atlas

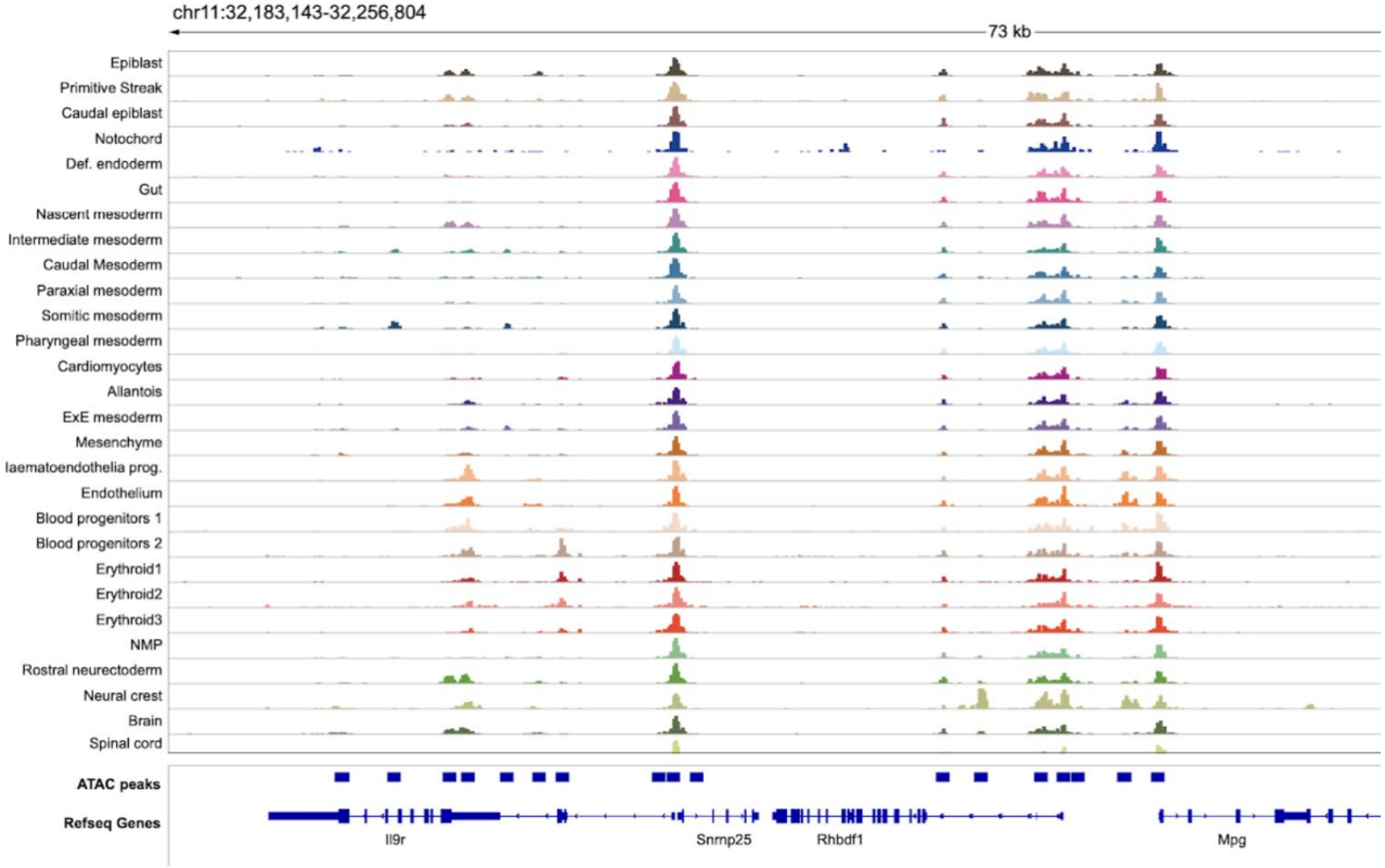
A multi-omics atlas of mouse early organogenesis



- ~60k cells:
  - E7.5, E7.75, E8.0, E8.25, E8.5



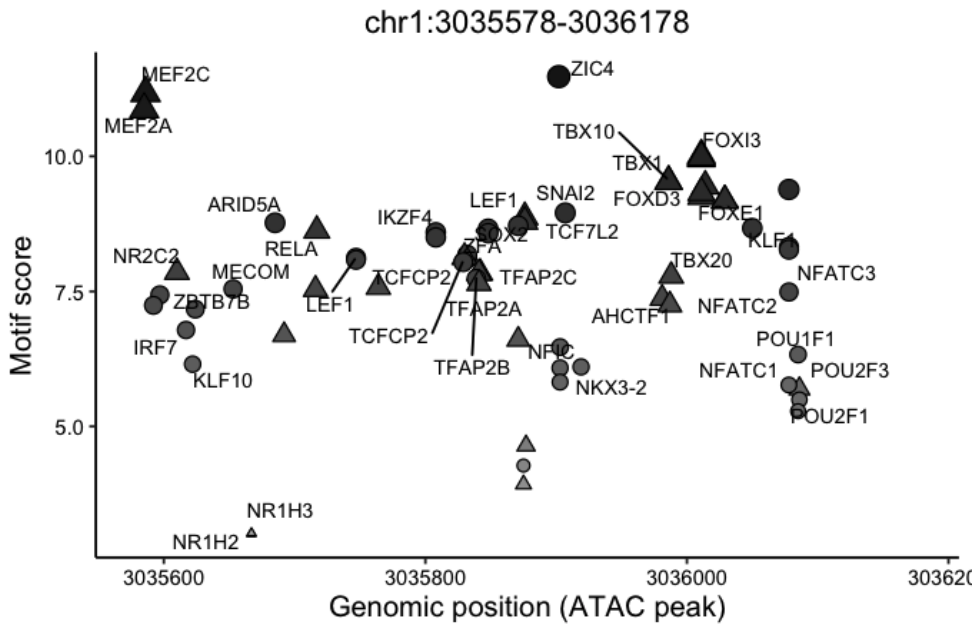




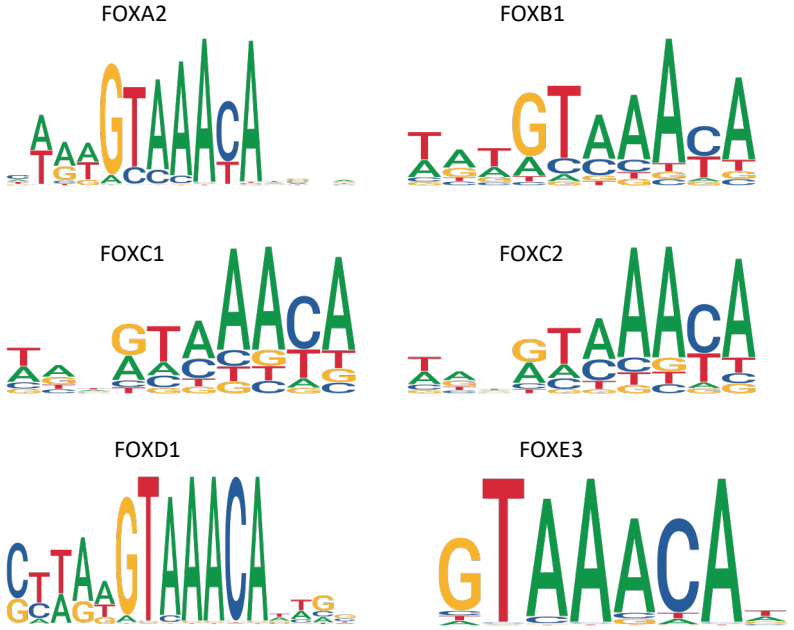
Celltype specific cis-regulatory regions - useful for DNAme analysis!

Challenges of linking TFs to cis-regulatory elements

Many TF motifs observed within individual ATAC peaks (600 bp)



Motif redundancy within the same family of TFs



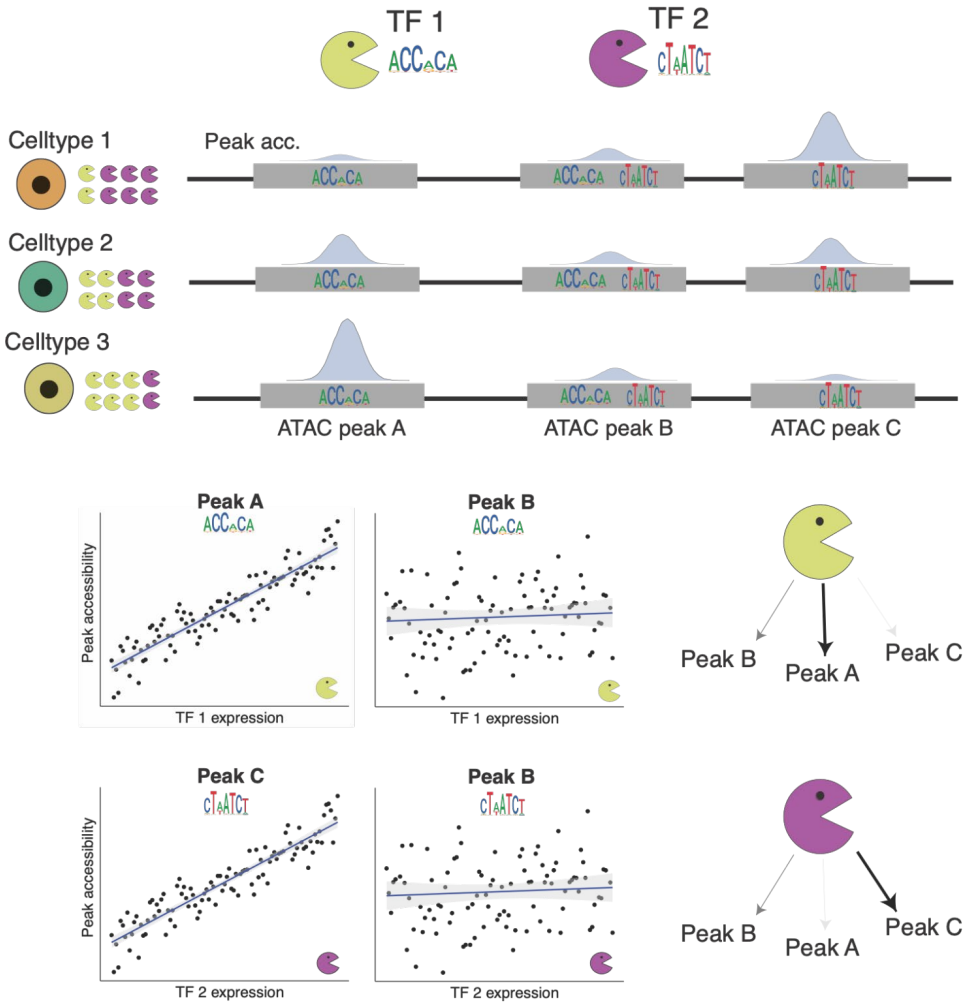
Prediction of TF binding sites (*in silico* ChIP-seq library)

A simple model that combines three sources of information to predict TF binding sites:

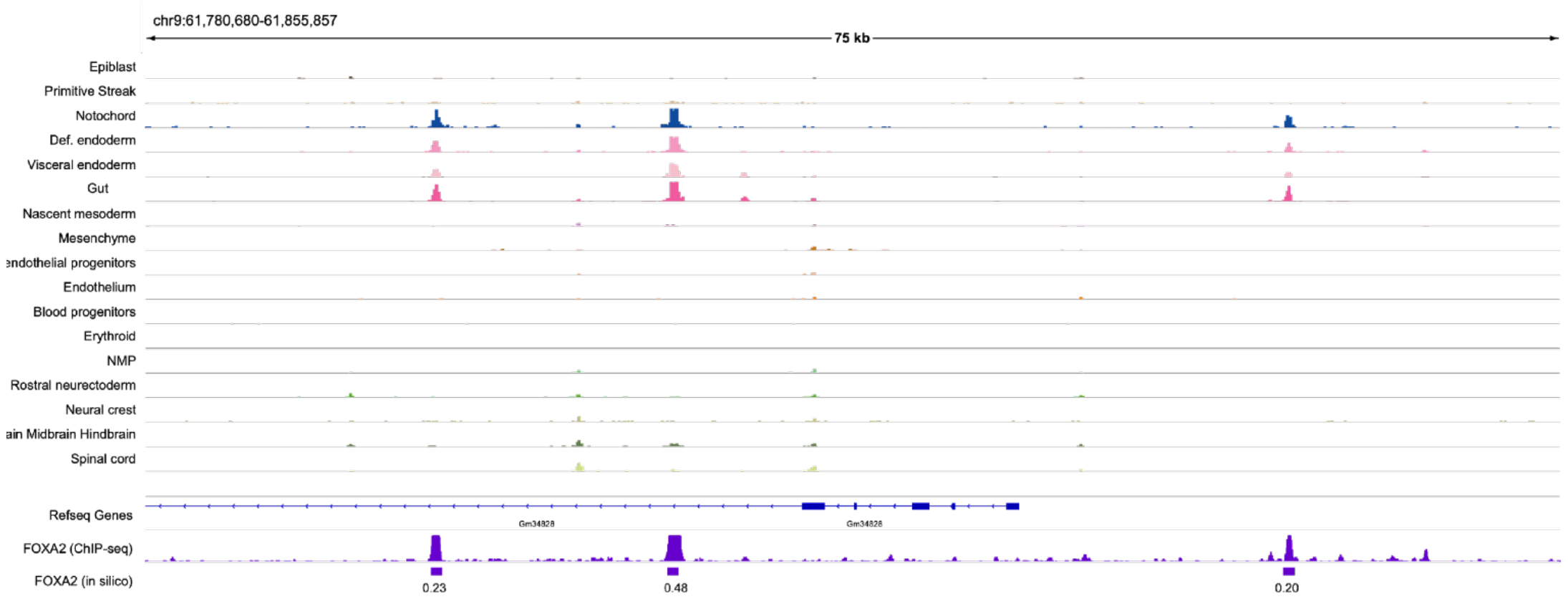
(1) Overall peak accessibility

(2) Motif score

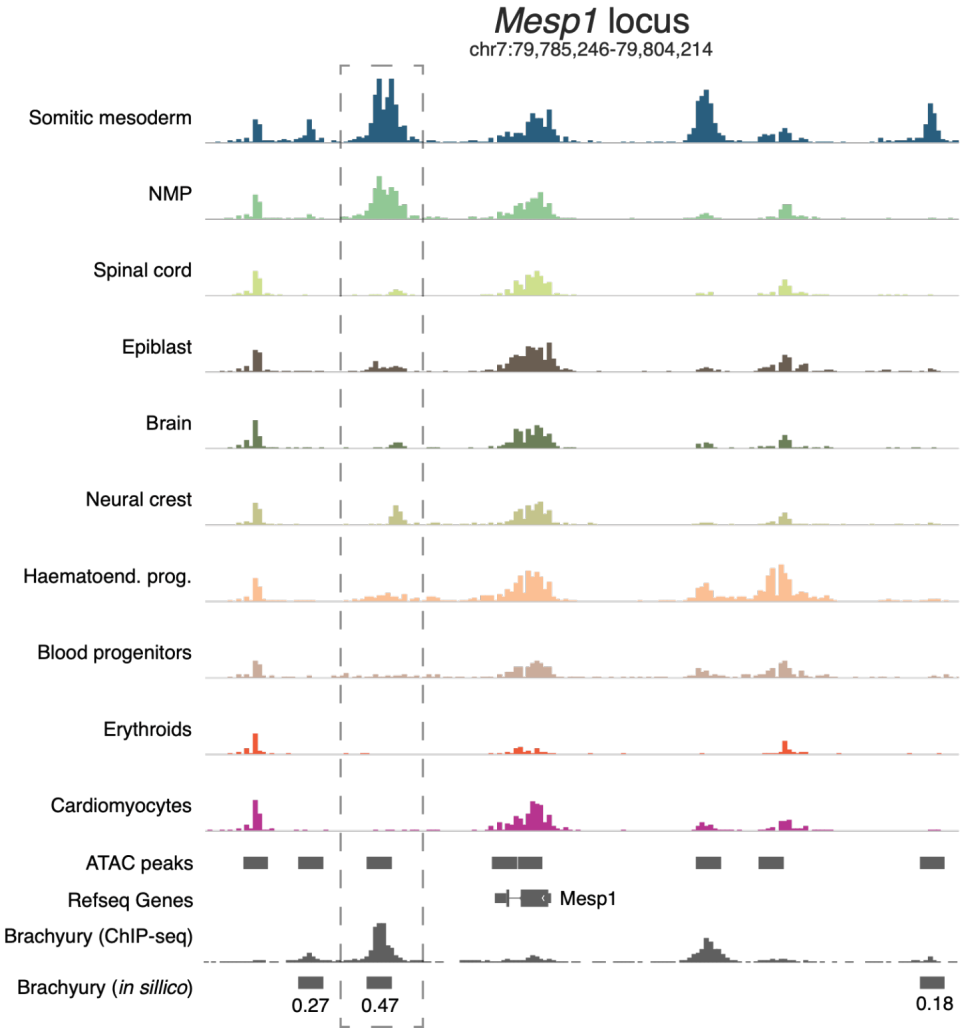
(3) Correlation between TF RNA expression and peak accessibility

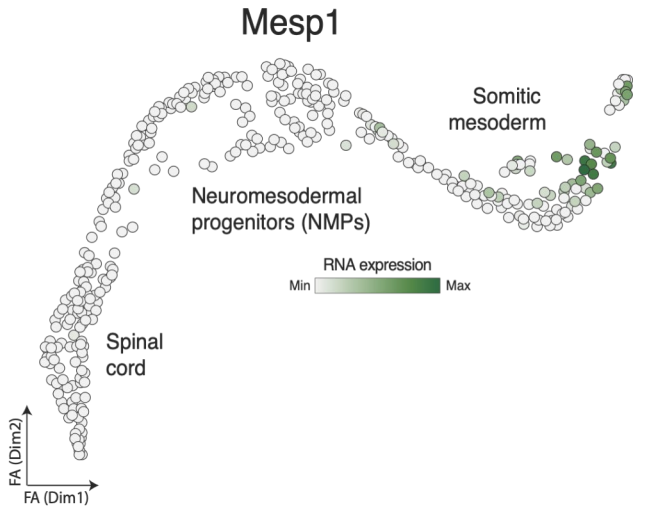


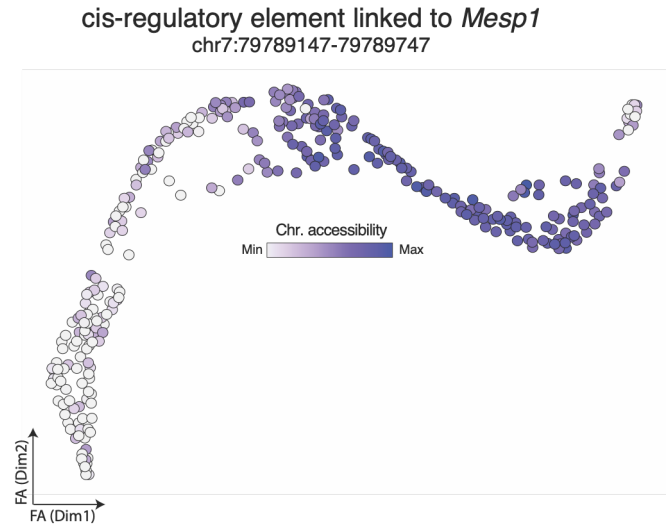
In silico ChiP-seq example FOXA2



Dynamics of cis-regulatory regions bound by Brachyury suggests priming



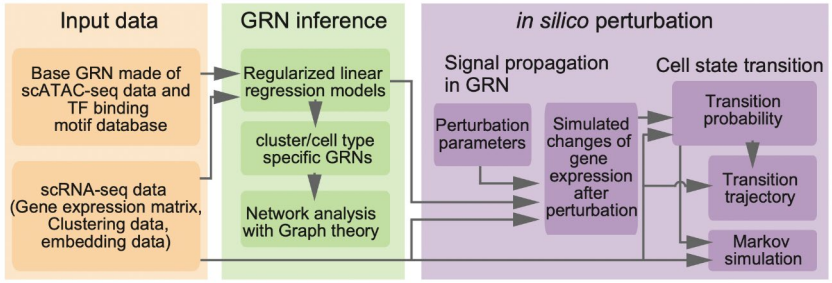


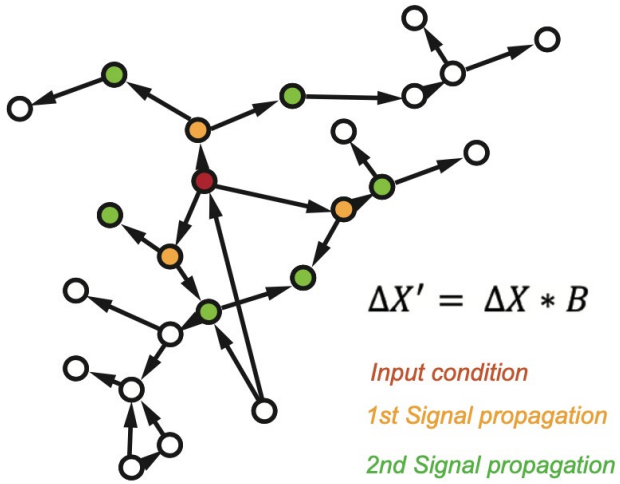


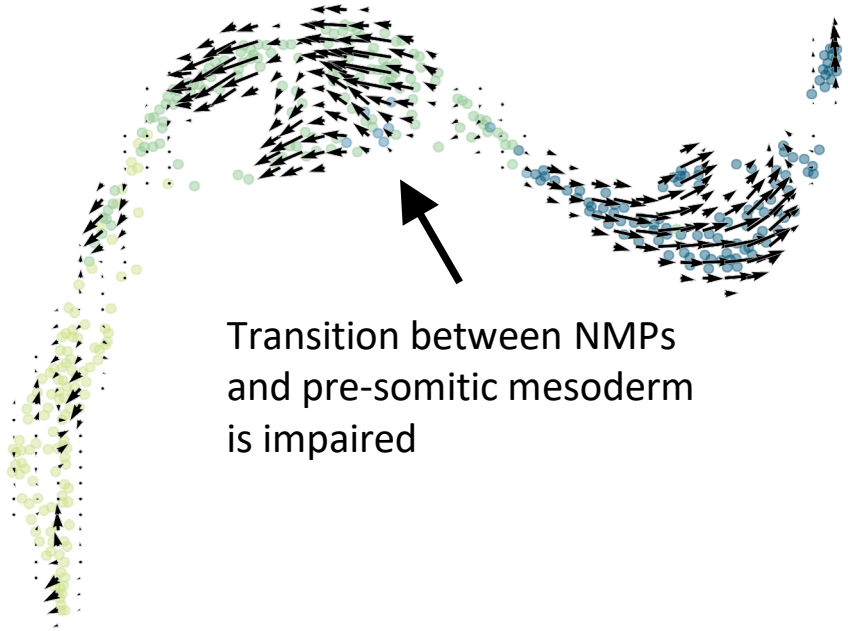
in silico KO of Brachyury

CellOracle: Dissecting cell identity via network inference and in silico gene perturbation

Kenji Kamimoto, Christy M. Hoffmann, Samantha A. Morris

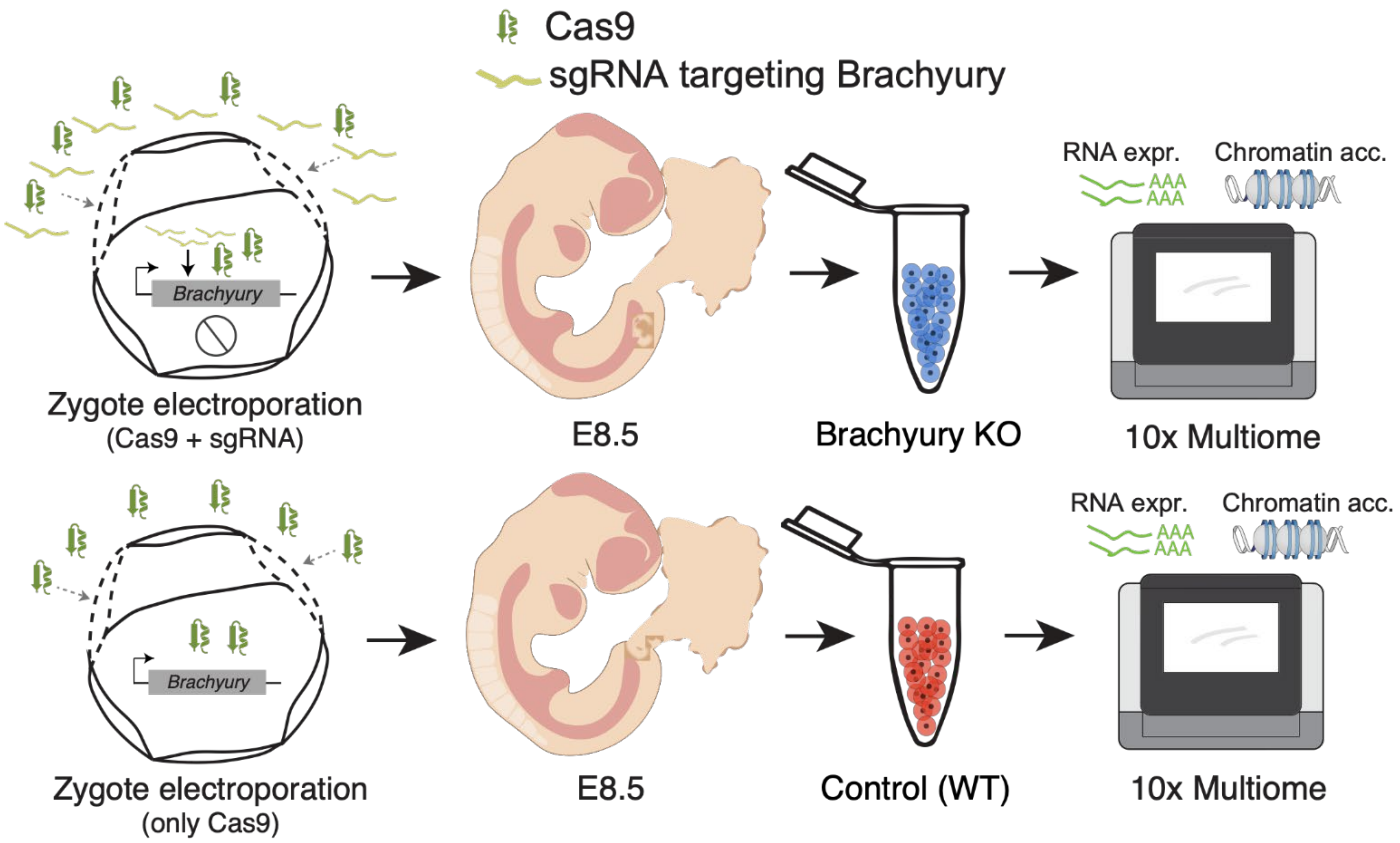




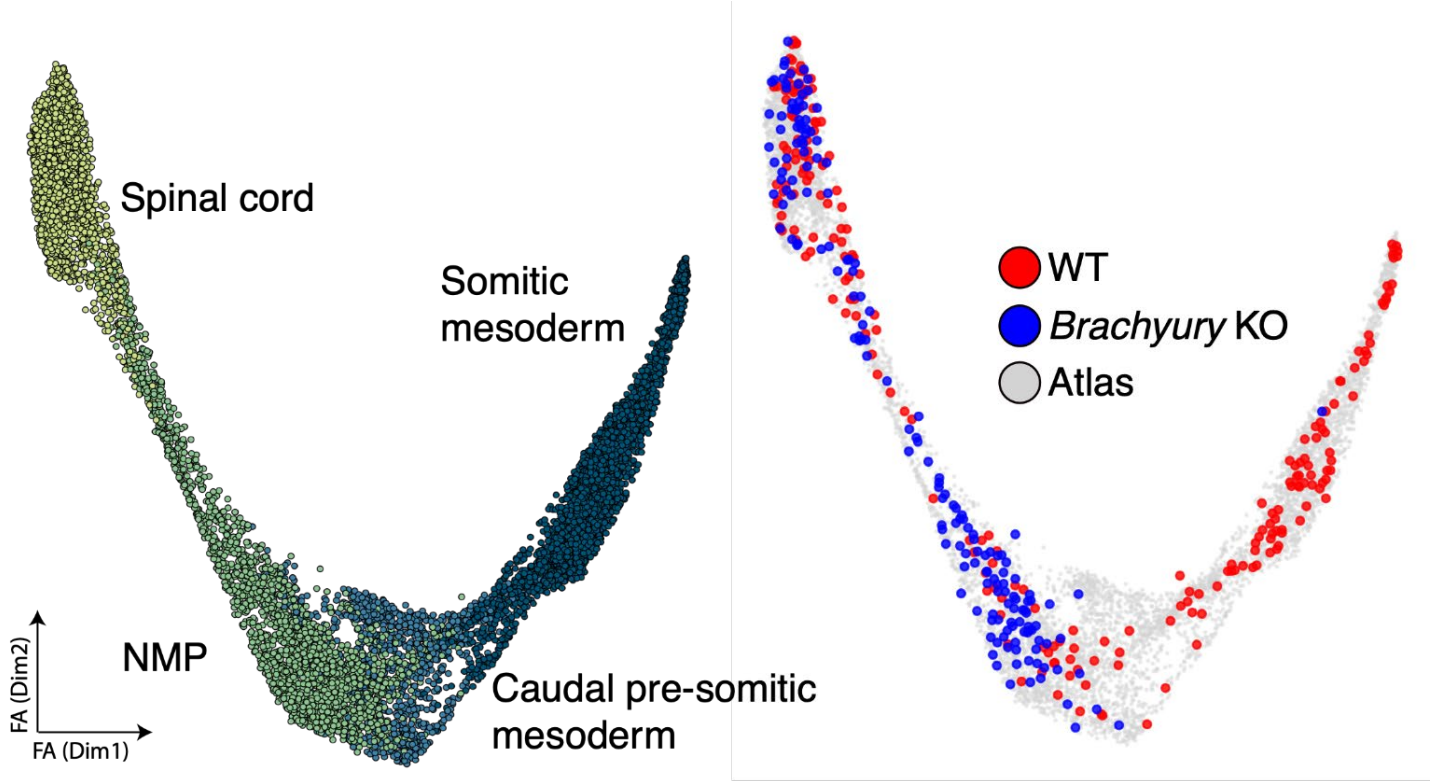


Transition between NMPs and pre-somitic mesoderm is impaired

Experimental validation: Brachyury KO profiled with multiome



Brachyury KO NMPs unable to move to pre-somitic mesoderm



Pijuan-Sala *et al.* 2019

Argelaguet *et al* 2022 (BioRxiv)

Summary

- We can use single-cell multi-omics to learn new biology
- Epigenomic profiles associated with the ectoderm fate are established as early as the E4.5 epiblast. In contrast, profiles associated with meso/endoderm are only established on differentiation into these cell types
- Tet-dependent de-methylation is required for lineage-specific enhancer de—methylation. And formation of the primitive blood lineage.
- Atlas of transcriptome and chromatin accessibility for > 60,000 cells in organogenesis and cell type specific annotation of cis-regulatory elements
- In silico ChIP-seq by combining transcription factor expression and motif accessibility
- Priming of mesodermal genes by Brachyury shown in silico and validated experimentally

Acknowledgements

Ricard Argelaguet
Tim Lohoff
Hisham Mohammed
Carine Stapel
Gavin Li
Christel Krueger
Felix Krueger

Wolf Reik
Gavin Kelsey
John Marioni
Oliver Stegle
Jenny Nichols

Babraham facilities

Flow cytometry 

Sequencing 

Bioinformatics 

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TET knockout: https://doi.org/10.1186/s13059-022-02762-3
Multiome atlas: https://doi.org/10.1101/2022.06.15.496239