

Cell Fate Plasticity: From Reprogramming to Early Development

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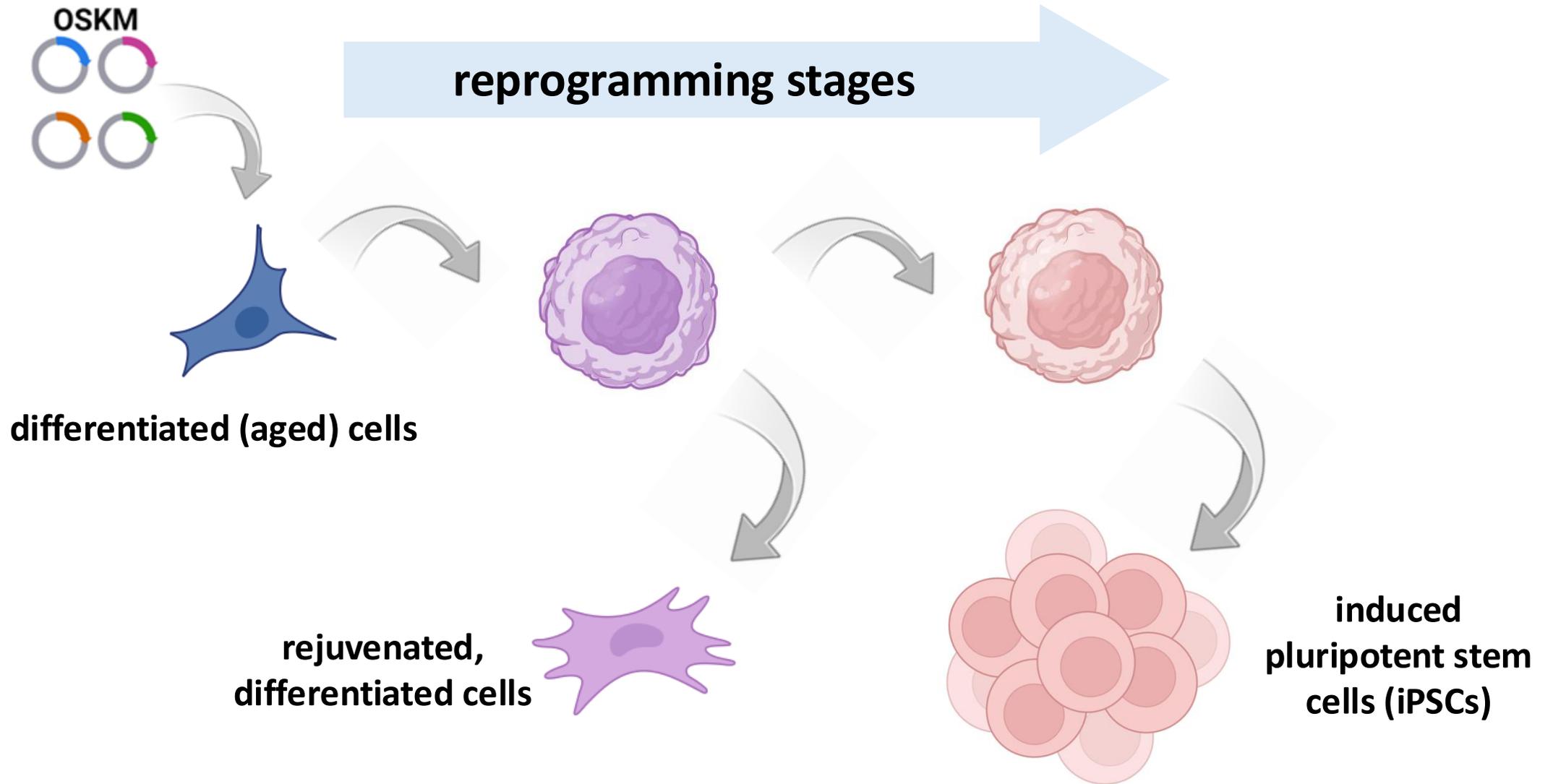
Financial Disclosure

Kathrin Plath, PhD



- Advisory board member

Reprogramming of somatic cells to iPSCs has revolutionized biology



Mapping DNA methylation during human fibroblast to iPSC reprogramming

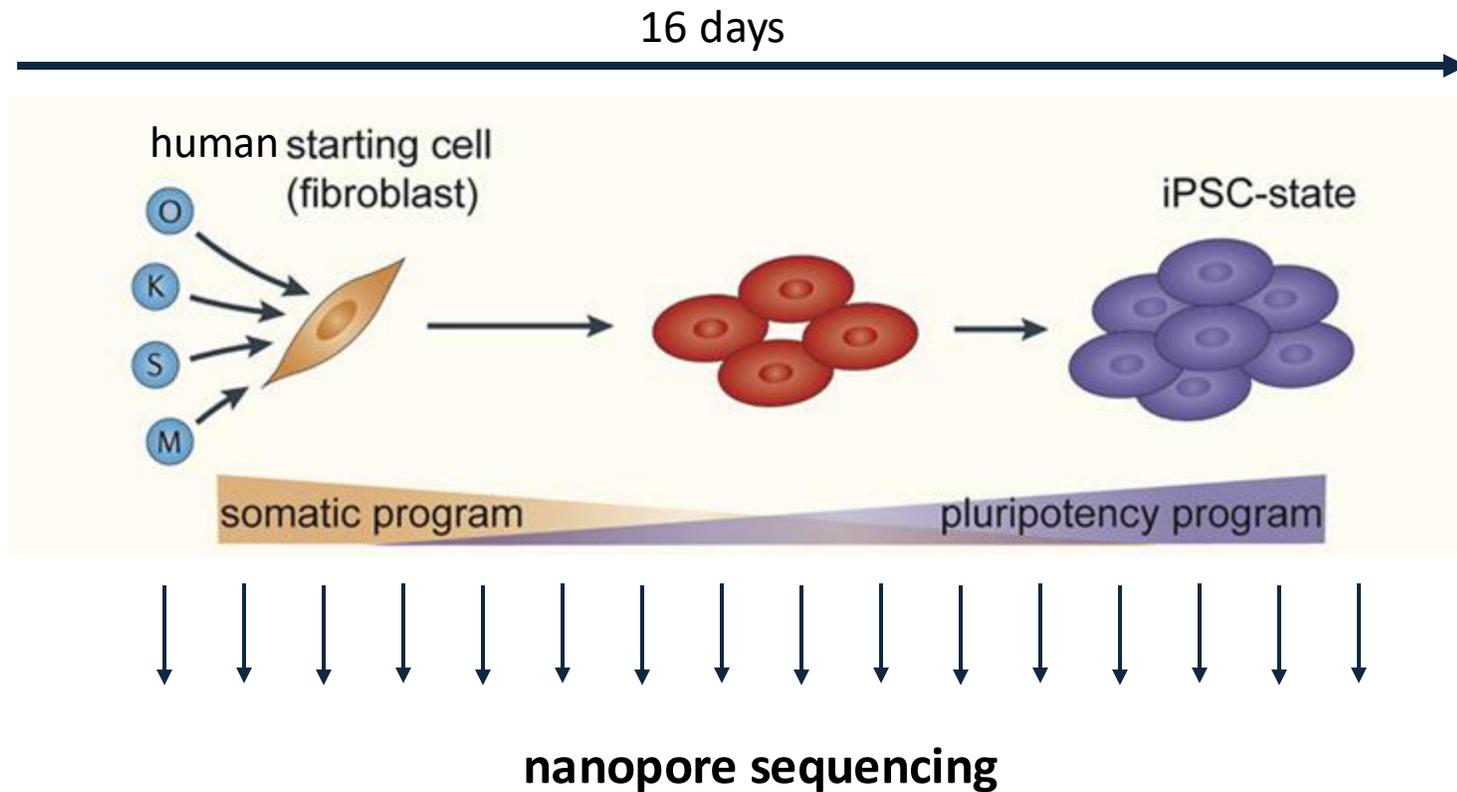
Although it is widely accepted that reprogramming resets the epigenome, we still do not fully understand when and how this happens



Justin Langerman



Yu Sun

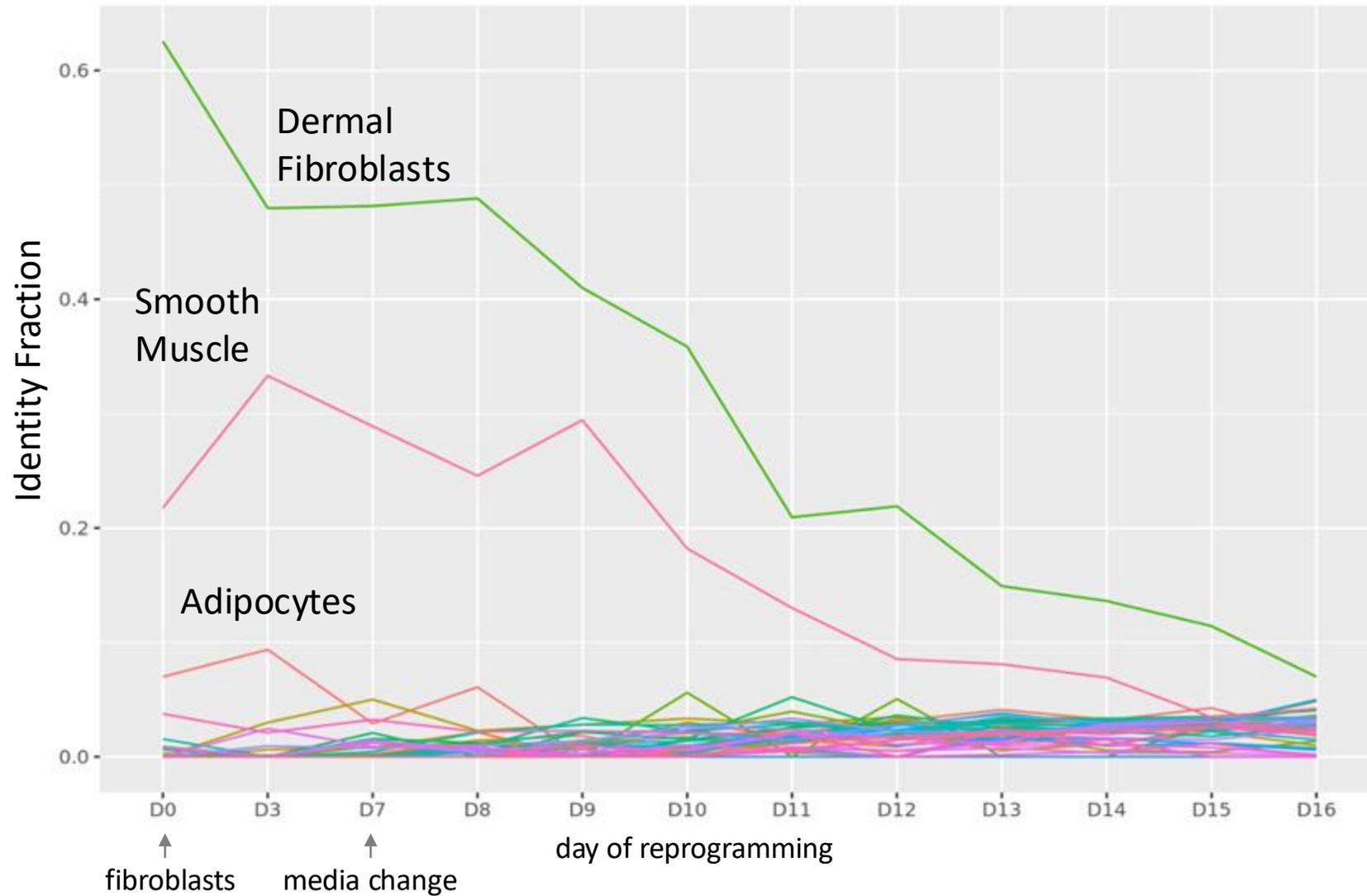


Nanopore sequencing:

(i) can **directly read DNA base modifications** (like 5mC, 5hmC) without chemical treatment and amplification

(ii) produces **long reads** (tens of kbs), allowing capture of methylation across entire regulatory regions through **fragment level analyses**

Loss of cell identity occurs in the late stages of reprogramming



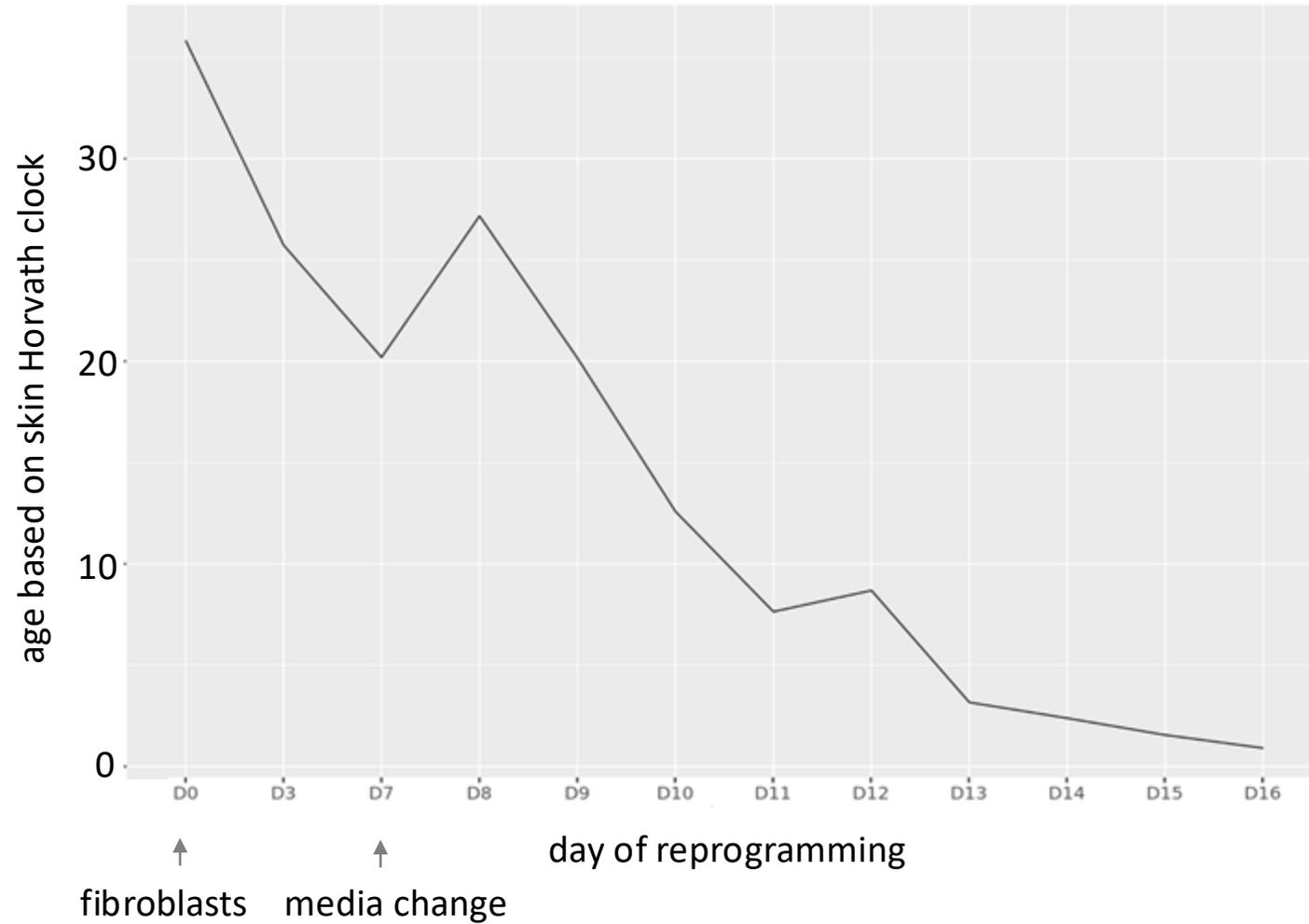
Cell identity deconvolution using cell type-specific methylation states (Loyfer, et al. Nature, 2023)

CellType

Adipocytes	Heart-Cardio
Bladder-Ep	Heart-Fibro
Blood-B	Kidney-Ep
Blood-Granul	Liver-Hep
Blood-Mono+Macro	Lung-Ep-Alveo
Blood-NK	Lung-Ep-Bron
Blood-T	Megakaryocytes
Bone-Osteob	Neuron
Breast-Basal-Ep	Oligodend
Breast-Luminal-Ep	Ovary-Ep
Colon-Ep	Pancreas-Acinar
Colon-Fibro	Pancreas-Alpha
Dermal-Fibro	Pancreas-Beta
Endothel	Pancreas-Delta
Epid-Kerat	Pancreas-Duct
Eryth-prog	Prostate-Ep
Fallopian-Ep	Skeletal-Musc
Gallbladder	Small-Int-Ep
Gastric-Ep	Smooth-Musc
Head-Neck-Ep	Thyroid-Ep

These regions serve as markers to quantify the presence of chromatin from a specific cell type even in a heterogenous mixture

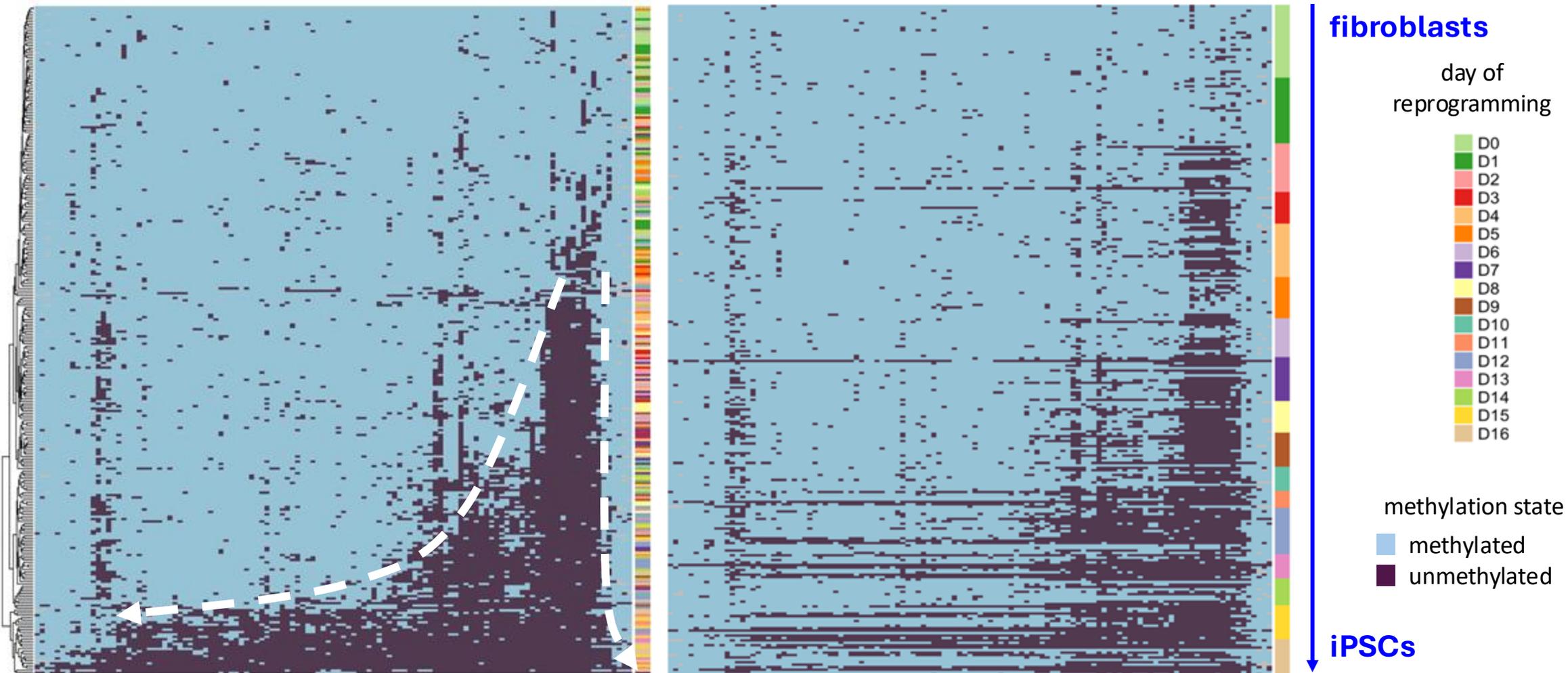
Cellular rejuvenation occurs across the entire reprogramming process



- beginning age is very close to chronological age of fibroblast donor (46 years)
- gradual decline from the beginning, ~3 years / day

UNZIPPING: Demethylation begins focally and expands to broad domains

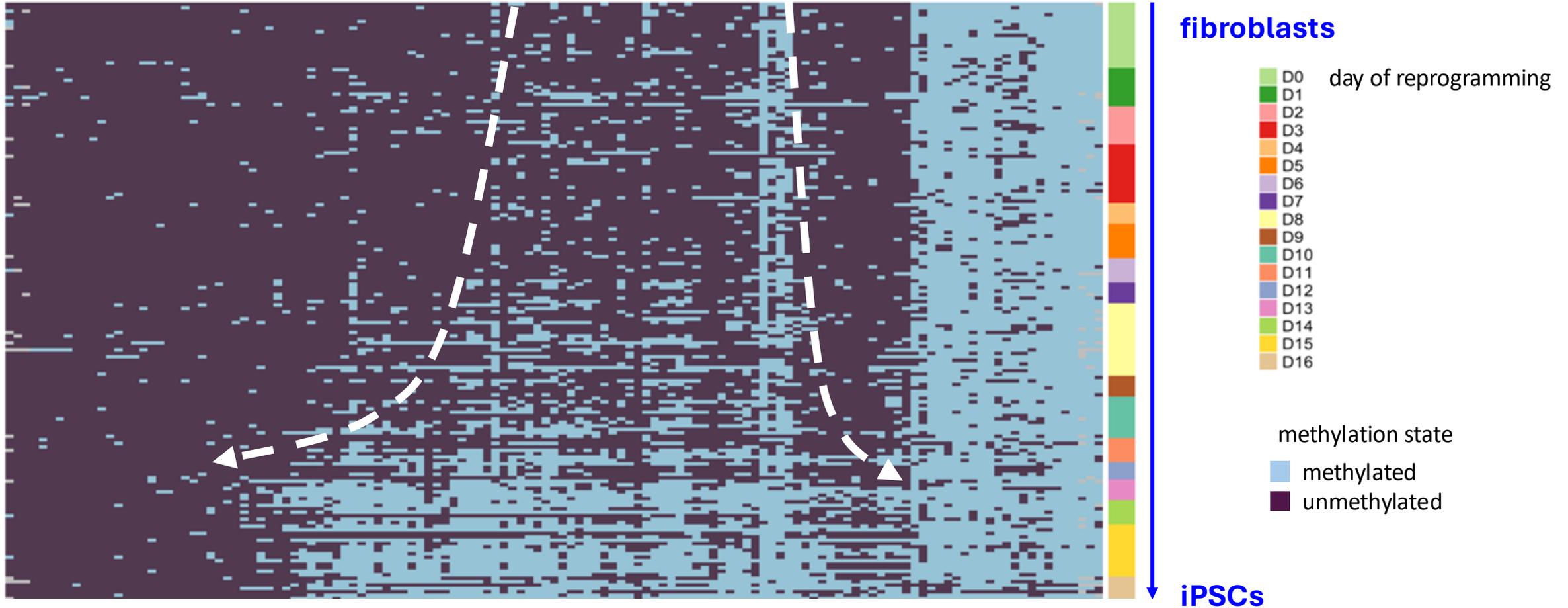
Fragment level analysis reveals widening of demethylated region



chr4: 6975687 - 6980546
117 CpGs, 278 fragments

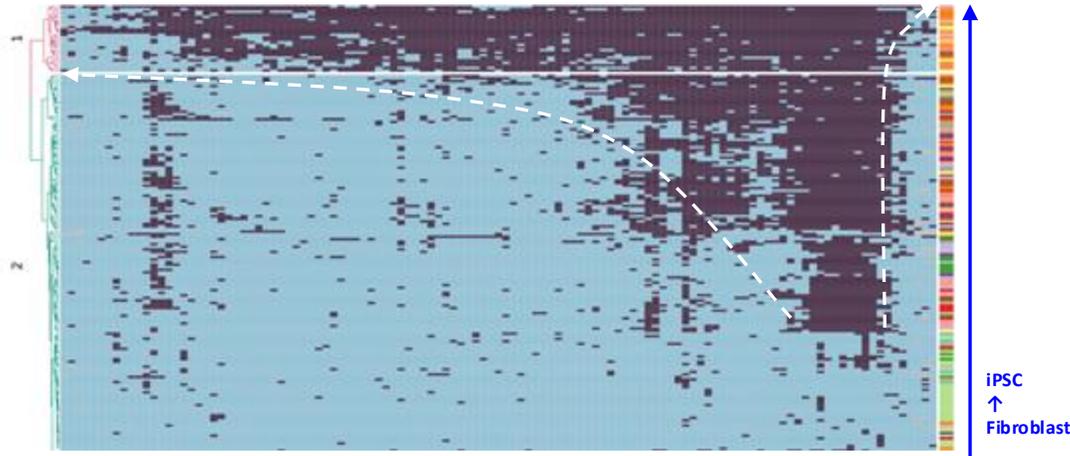
ZIPPING: Methylation expands to broader domains over time

Fragment level analysis reveals widening of regions with de novo methylation



chr10: 17230493 – 17241847 (VIM gene)
131 CpGs, 173 fragments

Summary I - Somatic cell-to-iPSC reprogramming and DNA methylation



- long-read sequencing of reprogramming generated high resolution maps of DNA methylation at the **single cell level**

- uncovered unexpected dynamics in methylation with ~24,000 “**unzipping**” and “**zipping**” regions

zipping and unzipping mechanisms likely play a key role in the resetting of cell identity and rejuvenation during reprogramming

dynamics of epigenetic reprogramming: what comes first – changes in methylation or chromatin accessibility?

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Thank you!

Methylation and demethylation events initiate throughout reprogramming

