Profiling epigenetic age in single cells

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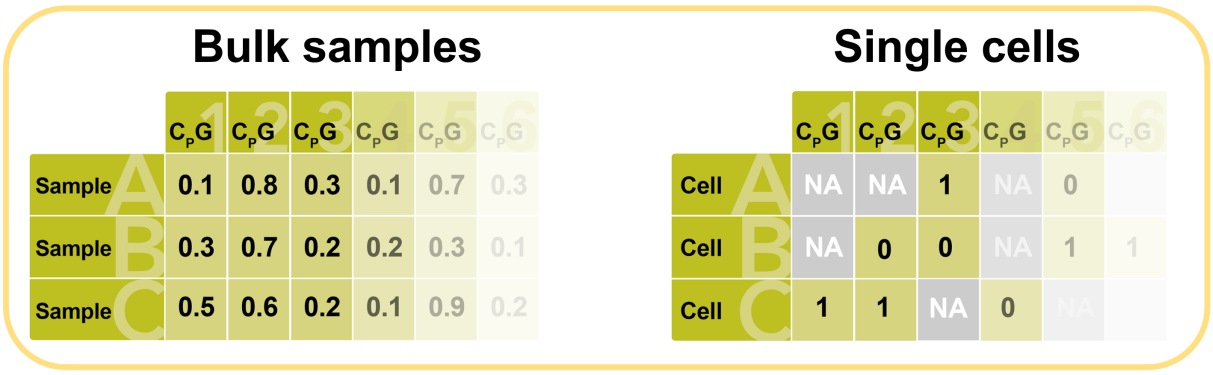
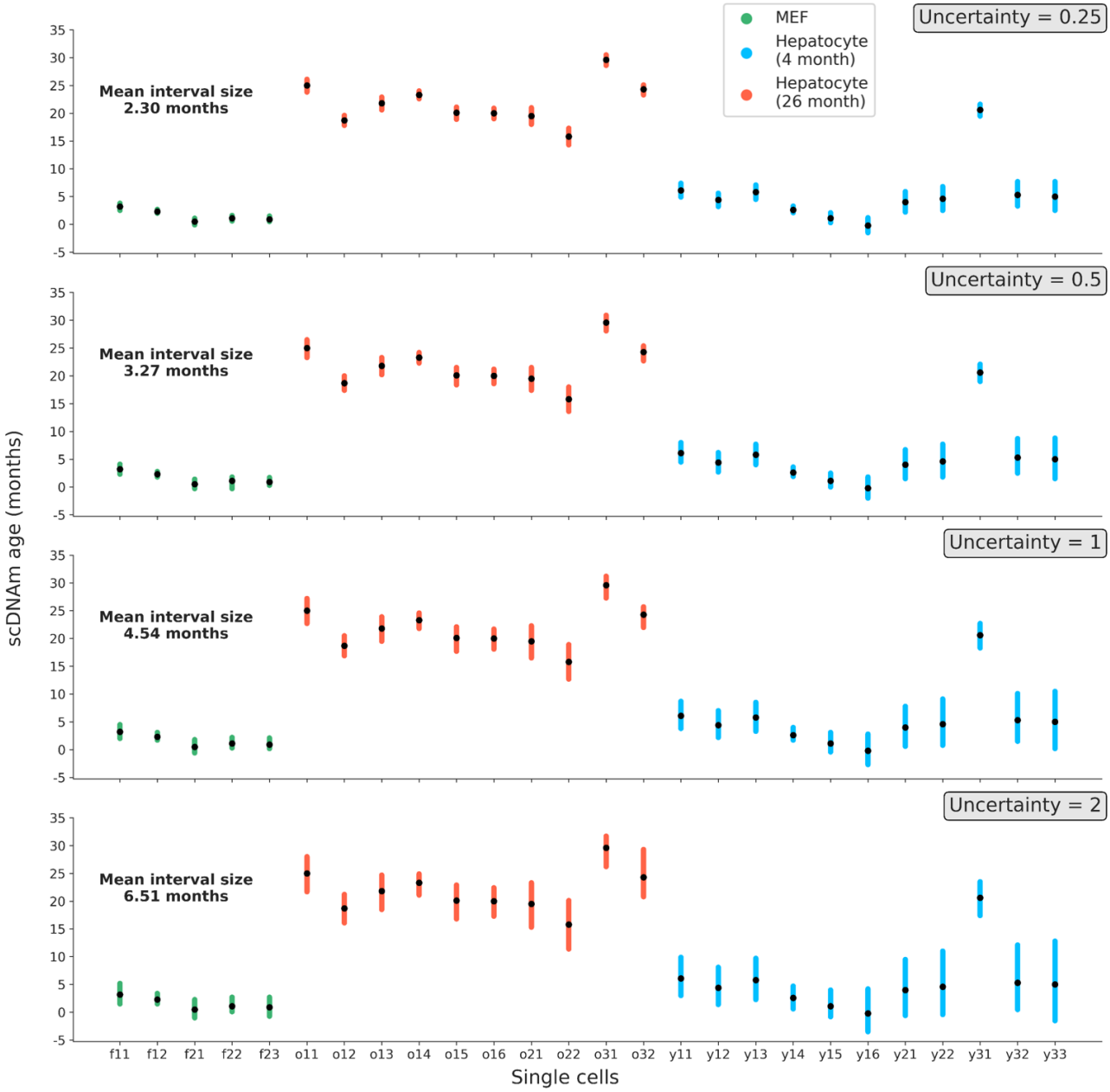
SUPPLEMENTARY INFORMATION



Supplementary Figure 1: Comparison of bulk and single-cell methylation profiling outputs Schematic comparison of bulk (left) and single-cell (right) methylation sequencing approaches. Using bulk tissue ensures high and consistent CpG coverage across samples, while genome-wide single-cell profiles suffer from effectively random and sparse coverage of reads. In turn, bulk samples produce continuous fractional methylation values ranging from 0 to 1 (due to the presence of reads from many different cells), while single-cell samples exhibit primarily binarized methylation (0 or 1), with many missing values for each single cell. Consequently, dense feature tables used to train elastic net regression models for traditional epigenetic clock construction are currently unfeasible to create in single cells.



Supplementary Figure 2: Confidence interval computation for scDNAm predictions
Confidence interval estimation for hepatocytes and embryonic fibroblasts. Predicted epigenetic ages for mouse embryonic fibroblasts (green, n = 5), young (blue, n = 11) and old (red, n = 10) hepatocytes are shown in black, based on the liver model sampling the top 1% age-associated CpGs per cell. A confidence interval based on the cell-specific likelihood distribution is shown for 4 different uncertainty parameters. A lower uncertainty parameter value is indicative of a more stringent interval. The mean size of the confidence interval across all cells for each parameter is shown on the left side of each panel. Refer to Extended Data Fig. 4 for additional details regarding cell-specific likelihood distributions.

Study	Accession	Cell types and number analyzed
Gravina et al. (2016)	SRA344045	5 embryonic fibroblasts 11 hepatocytes from 4-month-old mice 10 hepatocytes from 26-month-old mice
Hernando-Herraez et al. (2019)	GSE121436	116 muscle stem cells from 1.5-month-old mice 89 muscle stem cells from 26-month-old mice
Angermueller et al. (2016)	GSE68642	16 2i embryonic stem cells 65 serum embryonic stem cells
Smallwood et al. (2014)	GSE56879	12 2i embryonic stem cells 20 serum embryonic stem cells
Argelaguet et al. (2019)	GSE121690	94 E4.5 embryonic cells 101 E5.5 embryonic cells 145 E6.5 embryonic cells 155 E7.5 embryonic cells

Supplementary Table 1: Single-cell datasets analyzed in this study

Table of studies/datasets of single-cell methylation profiles analyzed in this study. The first author of the study and year of publication are shown in the first column, GEO/SRA accession in the second column, and the cell type and number of cells analyzed in the third column (after coverage filtration, see Extended Data Fig. 9a for additional details).