Sci-fate characterizes the dynamics of gene expression in single cells

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Gene expression programs change over time, differentiation and development, and in response to stimuli. However, nearly all techniques for profiling gene expression in single cells do not directly capture transcriptional dynamics. In the present study, we present a method for combined single-cell combinatorial indexing and messenger RNA labeling (sci-fate), which uses combinatorial cell indexing and 4-thiouridine labeling of newly synthesized mRNA to concurrently profile the whole and newly synthesized transcriptome in each of many single cells. We used sci-fate to study the cortisol response in >6,000 single cultured cells. From these data, we quantified the dynamics of the cell cycle and glucocorticoid receptor activation, and explored their intersection. Finally, we developed software to infer and analyze cell-state transitions. We anticipate that sci-fate will be broadly applicable to quantitatively characterize transcriptional dynamics in diverse systems.

During development of organisms, as well as during myriad physiological and pathophysiological processes, individual cells traverse a manifold of molecularly and functionally distinct states. However, although experimental methods for profiling various aspects of single-cell biology have recently proliferated, almost all such methods deliver only a static snapshot of each cell. To address this in part, 'pseudotime' methods computationally place individual cells along a continuous trajectory based on their transcriptomes1–5. However, pseudotime infers rather than directly measures transcriptional dynamics, is dependent on sufficient representation along the trajectory and may fail to capture the detailed dynamics of individual cells (for example, directionality, multiple superimposed potentials)7. In contrast, time-lapse microscopy can experimentally measure transcriptional dynamics, but is limited to visualization of a few marker genes in a few cells, and as such may be insufficient to decipher the complexity of many biological systems.

In the present study, we describe a technique, sci-fate, for measuring the dynamics of gene expression in large numbers of single cells and at the level of the whole transcriptome. In brief, we integrated protocols for labeling newly synthesized mRNA with 4-thiouridine (4sU)8,9 with single-cell combinatorial indexing RNA-sequencing (sci-RNA-seq)10. As a proof of concept, we applied sci-fate to a model system of cortisol response, characterizing expression dynamics in >6,000 single cells. From these data, we quantify the dynamics of the transcription factor (TF) modules that underpin the cell cycle, glucocorticoid receptor (GR) activation and other processes, and develop a framework for inferring the distribution of cell-state transitions. The methods described in the present study may be broadly applicable to quantitatively characterize transcriptional dynamics in diverse systems.

Results

Overview of sci-fate. Briefly, sci-fate relies on the following steps (Fig. 1a): (1) cells are incubated with 4sU, a thymidine analog, to label newly synthesized RNA11–17. (2) Cells are harvested, fixed with 4% paraformaldehyde (PFA), and then subjected to a thiol (SH)-linked alkylation reaction, which covalently attaches a carboxymethyl group to 4sU by nucleophilic substitution1. (3) Cells are distributed by dilution to four 96-well plates. The first sci-RNA-seq molecular index is introduced via in situ reverse transcription (RT) with a poly(T) primer bearing both a well-specific barcode and a degenerate unique molecular identifier (UMI). During first-strand complementary DNA synthesis, modified 4sU is a template for guanine rather than adenine incorporation. (4) Cells from all wells are pooled and then redistributed by FACS to multiple 96-well plates. (5) Double-stranded complementary DNA is synthesized. After Tn5 transposition, cDNA is PCR-amplified via primers recognizing the Tn5 adaptor on the 5′-end and the RT primer on the 3′-end. These primers also bear a well-specific barcode that introduces the second sci-RNA-seq molecular index. (6) PCR amplicons are subjected to massively parallel DNA-sequencing. As with other sci-methods15,16–25, most cells pass through a unique combination of wells, such that their contents are marked by a unique combination of barcodes that can be used to group reads derived from the same cell. (7) The subset of each cell's transcriptome corresponding to newly synthesized transcripts is distinguished by T→C conversions in reads mapping to mRNAs (see Methods).

For quality control, we first tested sci-fate with a mixture of HEK293T (human) and NIH/3T3 (mouse) cells under four conditions: with versus without 4sU labeling (200 nM, 6 h), and with versus without the SH-linked alkylation reaction. The resulting transcriptomes were overwhelmingly species coherent (>99% purity for both human and mouse cells, 2.7% collisions; see Supplementary Fig. 1a,b) with similar mRNA recovery rates (overall median 21,342 UMIs per cell; see Supplementary Fig. 1c). However, only with 4sU labeling and SH-linked alkylation did we observe a substantial proportion of reads bearing T→C conversions, that is, newly synthesized transcripts (46% and 31% for treated human and mouse cells, respectively, versus 0.8% for untreated cells; see Supplementary Fig. 1d). The aggregated transcriptomes of cells derived from sci-fate and conventional sci-RNA-seq were highly correlated (Spearman's correlation r = 0.99; see Supplementary Fig. 1e,f), suggesting that short-term labeling and conversion do not substantially bias transcript counts.
To investigate the transcriptional dynamics of cortisol response, we applied sci-fate to an in vitro model wherein dexamethasone (DEX), a synthetic mimic of cortisol, activates glucocorticoid receptor (GR), which binds to thousands of locations across the genome and rapidly alters gene expression. Specifically, we treated lung adenocarcinoma-derived A549 cells for 0, 2, 4, 6, 8 or 10 h with 100 nM DEX. In each condition, cells were incubated with 4sU (200 nM) for the 2 h immediately preceding harvest. We then performed a 384×192 sci-fate experiment (Fig. 1b). Each of the 6 conditions was
represented by 64 wells during the first round of indexing, such that all samples could be processed in a single sci-RNA-seq experiment to minimize batch effects.

After filtering out low-quality cells, potential doublets and a small subgroup of differentiated cells (see Methods), we obtained single-cell profiles for 6,680 cells (median 26,176 UMI's corresponding to mRNAs detected per cell). A median of 20% mRNA UMIs were labeled per cell (Fig. 1c; see Supplementary Fig. 2a–c). The proportion of newly synthesized mRNAs was markedly higher in reads mapping to intronic (65%) versus exonic (13%) regions (FDR < 2.2 × 10^{-16}, two-sided Wilcoxon's signed-rank test; Fig. 1d, and see Supplementary Fig. 2d,e), consistent with the expectation that the intronic reads are more likely to have been recently synthesized. We also compared intronic reads and newly synthesized mRNA for RNA-velocity analysis and observed a subjectively consistent picture, suggesting that they capture similar information (see Supplementary Fig. 2f).

In exploring these data, we first asked whether the newly synthesized versus whole-transcriptome data convey identical or distinct information with respect to cell state. Performing dimensionality reduction with Uniform Manifold Approximation and Projection (UMAP) on whole transcriptomes failed to separate DEX-untreated (0 h) versus DEX-treated (2 h) cells (Fig. 1e, left), and see Supplementary Fig. 2g). In contrast, applying UMAP to the newly synthesized subset of the single-cell transcriptomes readily separated DEX-untreated versus -treated cells (Fig. 1e, center). These patterns are probably a consequence of the fact that, in DEX-treated cells, the newly synthesized transcriptome more faithfully reflects the GR response itself. Illustrating this, the classic markers for GR response, FGD4 and FKBP5 (ref. 25) and the expression levels of TFs such as E2F1, FOXO1 (ref. 41) and JUNB (ref. 26) (see Supplementary Fig. 3c,d). This group also included several TFs not previously implicated in the GR response, including YOD1 and GTF2IRD1, both of which exhibited greater expression and activity in DEX-treated cells (see Supplementary Fig. 3e,f). The main TFs driving cell-cycle progression were also identified, for example E2F1, E2F2, E2F7, BCR1 and MYBL2 (ref. 47). Notably, the expression levels of TFs such as E2F1 were more highly correlated with the levels of newly synthesized than overall target gene mRNAs (see Supplementary Fig. 3g). We also observed regulatory links corresponding to induced cell differentiation such as GATA3 (ref. 48), mostly expressed in a subset of quiescent cells, as well as TFs involved in oxidative stress response such as NRF1 (ref. 49) and NFE2L2 (NRF2) (ref. 50).

We calculated a measure of each of these 29 TFs' activities in each cell, based on the normalized aggregation of the levels of newly synthesized mRNA for all its target genes. We then computed the absolute correlation coefficient between each pair of TFs with respect to their activity across the 6,680 cells. Hierarchical clustering of these pairwise correlations identified several major TF modules, that is sets of TFs that appear to be regulating the same process (Fig. 2b). A first large TF module corresponds to all cell cycle-related TFs in the set, for example E2F1 and FOXM1 (ref. 43). A second large TF module corresponds to GR response-related TFs, for example FOXO1, CEBPB, JUNB and RARB (ref. 47). The other modules include one corresponding to GR-activated G1/G2/M-phase cells (KLF6, TED1 and YOD1; see Supplementary Fig. 3h), and another corresponding to predominantly differentiating, GR-activated, G1-phase cells (GATA3 and AR; see Supplementary Fig. 3i) (ref. 39). Additional TFs or TF modules appear to capture other processes that are heterogeneous in this population of cells, including NRF1 and NFE2L2 for stress response/apoptosis (top enriched pathway of NFE2L2-linked genes: ferroptosis, adjusted P = 1 × 10^{-5}) (ref. 49), KL5 for DNA-damage repair (top pathway: ATM signaling, adjusted P = 0.018) (ref. 47) and SREBF2 for cholesterol homeostasis (top pathway: SREBF and miR33 in cholesterol and lipid homeostasis, adjusted P = 9 × 10^{-5}) (ref. 50).

To assign cell-cycle states to individual cells, we first ordered cells by their cell cycle-linked TF module activity. This resulted in a smooth, almost circular trajectory, in which the levels of newly synthesized mRNA corresponding to known cell-cycle markers was dynamic (Fig. 2c) (ref. 44). We observed a gap between late G2/M phase and early G1 phase, consistent with the dramatic cell-state change during cell division. By unsupervised clustering of the activities of individual TFs within the cell cycle-linked TF module, we identified nine cell-cycle states spanning the early, middle and late cell-cycle

**TF module activity decomposes cellular processes.** Multiple, dynamic gene, regulatory processes are concurrently under way in this in vitro system—minimally, the GR response and the cell cycle. We speculated that these might be disentangled, and their intersection probed, by first identifying the TF modules driving new mRNA synthesis in relation to each process.

TF modules, comprising candidate links between TFs and their regulated genes, were identified as follows: for each gene, across the 6,680 cells, we computed correlations between the levels of newly synthesized mRNA for that gene and the overall expression level of each of the 859 expressed TFs, using LASSO (least absolute shrinkage and selection operator) regression. Out of 1,086 links involving TFs characterized by ENCODE (ref. 51), 807 were validated by TF-binding sites near the genes' promoters (ref. 52), a 4.3-fold enrichment relative to background expectation (odds ratio for validation = 2.89 for LASSO-identified links versus 0.67 for background, P < 2.2 × 10^{-16}, two-sided Fisher's exact test). These covariance links were further filtered by chromatin immunoprecipitation–sequencing (ChIP-seq) binding (ref. 53), and supplemented with additional covariance links validated by motif enrichment analysis (Fig. 2a; see Methods). Altogether, we identified 986 links between 29 TFs and 532 genes (see Supplementary Fig. 3a,b and Supplementary Table 2). As a control, we permuted the cell order of the cell × TF expression matrix (T) (see Methods), and then repeated the analysis. No links were identified after permutation. Some of the identified TF–gene regulatory relationships are supported by a manually curated database of TF networks (TRRUST) (ref. 54), for example E2F1 (top enriched TF of E2F1-linked genes = E2F1, adjusted P = 8 × 10^{-7}) (ref. 29), NFE2L2 (top enriched TF of NFE2L2-linked genes = NFE2L2, adjusted P = 0.0003) (ref. 49) and SREBF2 (top enriched TF of SREBF2-linked genes = SREBF2, adjusted P = 0.0006) (ref. 50).
Fig. 2 | Characterizing TF modules driving concurrent, dynamic gene, regulatory processes in populations of single cells. a, Schematic of approach used to identify links between TFs and their regulated genes. b, Heatmap showing the absolute Pearson’s correlation coefficient between the activities of pairs of TFs (cell number, n = 6,680). c, UMAP visualization of A549 cells (n = 6,680) based on the activity of cell cycle-related TF module, colored by levels of newly synthesized mRNA corresponding to S-phase markers (top left), G2/M-phase markers (top right) and E2F1 activity (bottom left). The bottom right panel is colored by pseudotime based on the point position on the principal curve estimated using the princurve package. d, Same as c, but colored according to nine cell-cycle states defined by unsupervised clustering analysis. In broad terms, cell-cycle states 1–3 correspond to G1 phase, 4–6 to S phase and 7–9 to G2/M phase. e, Scatter plot showing the changes in the fraction of newly synthesized mRNA in each cell (n = 6,680) along cell-cycle progression. The red line is the smoothed curve estimated using the geom_smooth function. f, Similar to e, but showing smoothed activity of selected TF modules as a function of cell cycle pseudotime. g, UMAP visualization of A549 cells (n = 6,680) based on the activity of GR response-related TF module, colored by DEX treatment time (left), CEBPB or FOXO1 activity (middle panels), or cluster ID from unsupervised clustering (right). Throughout the figure, to calculate the TF module activity, newly synthesized UMI counts for genes linked to module-assigned TFs are scaled by library size, log(transformed), aggregated and then mapped to Z-scores.
phases (Fig. 2d). Early G1- and late G2/M-phase cells exhibited decreased synthesis of new RNA relative to other parts of the cell cycle, possibly due to chromosomal condensation during mitosis (Fig. 2e)\textsuperscript{31-33}. Other (that is, non-cell-cycle) TF modules exhibited different dynamics in relation to cell-cycle progression (Fig. 2f). For example, GATA3 activity peaks in the early G1 phase, potentially reflecting a cell-differentiation pathway distinct from cell-cycle reentry\textsuperscript{41}. In contrast, the modules of KLF5 and SREBF2, associated with DNA repair and lipid homeostasis, respectively, exhibited greater activity from S phase to G2 phase, possibly related to roles in DNA replication and cell division, respectively\textsuperscript{41}.

Similarly, the cells can also be ordered into a smooth trajectory based on GR response-linked TF module activity. As expected, this trajectory correlates well with DEX treatment time, as well as the activity of GR response-related TFs (Fig. 2g). By unsupervised clustering of the activities of individual TFs within the GR response-linked TF module, we identified GR response states corresponding to no, low and high levels of activation (Fig. 2g).

We next sought to explore the intersection of the nine cell-cycle states (Fig. 2d) and the three GR response states (Fig. 2g). Each of 27 possible state combinations was represented by some cells, with the smallest group corresponding to 1.1% of the overall dataset (n = 74 cells, intersection of ‘early G2/M’ cell-cycle state and ‘no GR activation’ state; see Supplementary Fig. 4a,b). Although we observe several TF modules that appear specific to certain intersections of the cell cycle and GR response (KLF6/TEAD1/YOD1 and GATA5/AR; Fig. 2b), several observations support the conclusion that the dynamics of the cell cycle and GR response operate largely independently. First, we observe minimal correlation between the activities of the primary TF modules for the cell-cycle and the GR response across the 6,680 cells (Pearson’s correlation r = 0.004; Fig. 2b). Second, the relative proportions of each of the 27 possible state combinations are readily predicted by proportions of cell-cycle and GR response states, that is, with no interaction term (see Supplementary Fig. 4b).

**Inferring single-cell transcriptome dynamics with sci-fate.** We next sought to develop a strategy to use sci-fate data to infer the past transcriptional state of each cell, that is at the onset of 4S U labeling, which might in turn allow us to relate cells derived from different time points. The inference of past transcriptional state requires knowledge of two parameters—first, the detection rate of newly synthesized transcripts (that is, the proportion of newly synthesized transcripts containing one or more detected T\textrightarrow{}C mutations) and, second, the degradation rate of each mRNA species. In this section, we discuss how each of these parameters can be estimated directly from the sci-fate data generated for this experiment. A more detailed consideration is provided in Methods.

Under the assumption that mRNA degradation rates are not affected by DEX treatment (this assumption is validated in the following paragraph), it is relatively straightforward to estimate sci-fate’s detection rate for newly synthesized transcripts. Each sci-fate transcriptome in this dataset consists of two components—the newly synthesized transcriptome, the detection rate of which we hope to estimate, and the ‘leftover’ transcriptome, that is transcripts that were present at the onset of 4S U labeling, minus any degradation over the course of the 2h. Comparing the 0-h (untreated) and 2-h DEX treatment groups, we expect that their leftover bulk transcriptomes (at the onset of 2-h 4S U labeling) should be identical, as should sci-fate’s detection rate for newly synthesized transcripts. As such, an equation can be constructed relating the transcriptomes of these treatment groups to each other (see Methods). For each of the 186 genes exhibiting the largest differences in new transcription between the two conditions, we solved this equation to estimate sci-fate’s detection rate. As these estimates were largely consistent across genes and robust to sequencing depth (see Supplementary Fig. 5a–e), we used their median value (82%) as sci-fate’s estimated detection rate for all subsequent analyses.

We next sought to estimate the degradation rate of each mRNA species. As noted above, the bulk transcriptome at each time point in our experiment can be broken down into the newly synthesized transcriptome and the leftover transcriptome. Furthermore, the leftover transcriptome should equal the bulk transcriptome from the time point 2h earlier, provided that we correct for mRNA degradation over that interval. From these assumptions, an equation can be constructed and solved to estimate the mRNA half-life of each gene, which we did independently for each 2-h interval of the experiment (see Methods and Supplementary Table 3). As a first quality check, we simply compared these estimated mRNA degradation rates between time points, and found them to be both consistent and robust to sequencing depth (see Supplementary Fig. 5g; median Pearson’s r = 0.92). As a second quality check, we compared them with orthogonally generated estimates of mRNA half-lives from the literature\textsuperscript{43}. Despite the fact that different technologies were used on different cell lines (A549 versus K562), the estimates of mRNA half-lives were reasonably consistent (see Supplementary Fig. 5h; Pearson’s r = 0.76). Of note, the absolute differences in estimated mRNA half-lives between sci-fate and previous techniques could be due to the use of different cell lines or systematic differences between the techniques.

With these parameters in hand, we next estimated the past transcriptional state of each cell in our dataset (see Methods and Supplementary Fig. 6a,b), and sought to use these estimated states to link individual cells to each other across time points (Fig. 3a). Specifically, for each cell B (for example, a cell from the 2-h time point), we used a recently developed alignment method\textsuperscript{34} to identify a cell A profiled at an earlier time point (for example, a cell from the 0-h time point), wherein A’s current state was closest to B’s estimated past state. In this framework, A can be regarded as the parent state of B. Applying this strategy to each of the five intervals comprising our experiment, we constructed a set of linkages spanning the entire dataset and time course (Fig. 3b).

A key contrast with conventional pseudotime is that, with sci-fate, each cell is now characterized not only by its present state, but also by specific linkages to a series of distinct cells matching its predicted past and/or future states (Fig. 3c). To evaluate whether these mini-trajectories contain structure, we applied UMAP and unsupervised clustering, which resulted in three distinct trajectory clusters (Fig. 3d). To annotate these, we checked the proportions of each of the aforementioned three GR response states and nine cell-cycle states in each of them, as a function of time. As expected, all three trajectories exhibited a rapid transition from no GR activation to low/high GR activation (Fig. 3e). However, each trajectory appears to correspond to a different starting point with respect to the cell cycle (Fig. 3f). Trajectory 1 corresponds to cells that transition from G2/M to G1 phase over the course of the 10-h experiment. Trajectory 2 corresponds to cells that transition from late S phase to G2/M phase over the course of the experiment. Trajectory 3 corresponds to cells that transition from G1 to either S phase or G1 arrest over the course of the experiment. The inference of G1 arrest subsequent to DEX treatment is consistent with the dynamics of cell-state proportions in this experiment as well as with previous research\textsuperscript{55,56}. As a control, we clustered the cell-state transition trajectories by simply aligning neighboring time points without knowledge of newly synthesized mRNA; this failed to recover the expected cell-cycle dynamics (see Supplementary Fig. 6c).

**Inferred cell transitions recapitulate expected dynamics.** We next sought to evaluate whether the distribution of cell-state transitions inferred by sci-fate are consistent with the expected dynamics. We assigned each cell into one of the 27 states (3 GR response×9 cell-cycle states) and computed a cell-state transition network (Fig. 4a),
with the assumption that the cell-state transitions in this experiment follow a Markov process, with transition probabilities that do not change over time. This assumption is validated in part by the observation that the distributions of predicted cell-state transitions, estimated from the last three time intervals (4–6 h, 6–8 h, 8–10 h), are highly correlated with each other (see Supplementary Fig. 7a) despite varied cell-state proportions at 4 h versus the later time points (see Supplementary Fig. 7b). Consistent with DEX treatment, transitions are highly biased from the G1 to S, S to G2/M and G2/M to G1 phase of the cell cycle (Fig. 4a). As a control analysis, cell-state transition networks were similarly derived, but based either on randomly permuted cell-state transition links or on links derived from mature mRNAs only; these both failed to recapitulate the expected pattern of cell-cycle transitions (see Supplementary Fig. 7c).

The 27 states shown in Fig. 4a each correspond to subsets of cells, the transcriptomes of which are similar, making use of the joint information provided by distinguishing between old (>2 h) and new (<2 h) transcripts. It corresponds to expected phenomena, for example irreversible progression through the GR response, as well as irreversible progression through the cell cycle. As examples, S-phase cells without GR activation (0-h treatment) mostly transit into S-phase cells with GR activation (2-h treatment), whereas G2/M-phase cells with no GR activation (0-h treatment) mostly transit to G2/M- or G1-phase cells with GR activation (2-h treatment) (Fig. 4b). For comparison, overlaying the same UMAP coordinates with RNA-velocity vectors recovered similar patterns, but only when treatment time information was incorporated into the RNA-velocity analysis (see Supplementary Fig. 7d).

Can we use this framework for a better understanding of the characteristics of transcriptional states that govern their dynamics? As a first approach, we calculated pairwise Pearson’s distance between the aggregated transcriptomes of each of the 27 states. As expected, the greater the distance between any pair of states, the lower the proportional representation of that transition in the
network (Spearman's correlation coefficient $= -0.38$; Fig. 4c). As a second approach, we computed ‘instability’ as the proportion of cells inferred to be moving out of a given state between time points (Fig. 4d). As expected, states corresponding to no GR activation were the least stable by this metric. Furthermore, among high GR activation states, states corresponding to early G1 were the most stable. These representations of the data are consistent with the transition network, wherein the states corresponding to high GR activation and early G1 are a frequent ‘destination’ of all nearby states (purple triangles in Fig. 4a).

Discussion

Sci-fate captures information analogous to RNA velocity\(^{31}\), which distinguishes ‘older’ and ‘newer’ transcripts based on their splicing status. On the one hand, RNA velocity is more straightforward than sci-fate, because it makes use of information that is indirectly captured by many single-cell-profiling technologies, whereas sci-fate requires 4sU-labeling steps that cannot necessarily be used in all contexts. On the other hand, sci-fate lends itself to experimental control in a way that RNA velocity does not, as the timing and length of 4sU labeling can be specified whereas, with RNA velocity, it is a product of endogenous splicing dynamics. Furthermore, as we show, an experimental design that couples the labeling of newly synthesized mRNA to a time series enables the quantitative analysis of cells with complex transcriptional histories and futures.

While our manuscript was under review, two methods directed at the same goal, scSLAM-seq and NASC-seq, were reported\(^{15,16}\). Although there are similarities including the labeling strategy, we note major differences with respect to performance, accuracy and scalability: (1) as sci-fate uses combinatorial indexing, we successfully measured newly synthesized mRNA in $>$6,000 cells in one experiment, compared with $<$200 cells for scSLAM-seq or NASC-seq. Given that sci-fate is easily adaptable to three-level combinatorial indexing\(^{-15}\), it should already be possible to profile newly synthesized mRNA as $>$1 million cells per experiment. (2) Sci-fate costs $<$US$0.20 per cell for library preparation with two-level indexing, and $<$US$0.01 per cell with three-level indexing\(^{-15}\). By comparison, both scSLAM-seq and NASC-seq utilize smart-seq which costs $\sim$US$11$ per cell for library preparation\(^{-15}\). On a related point, sci-fate required an order of magnitude fewer raw reads per cell ($\sim$200,000 sci-fate versus $\sim$2 million with scSLAM-seq), but achieved a greater number of genes detected per cell. (3) A key feature of sci-fate is that...
we performed in situ 4sU chemical conversion in bulk fixed cells, resulting in a high reaction efficiency and low mRNA loss. In contrast, scSLAM-seq and NASC-seq require extraction of mRNA from each cell, followed by bead-based purification and chemical conversion. As a result, sci-fate exhibits higher efficiency to detect low abundance transcripts (median 6,500 genes detected per cell with sci-fate versus ~4,000 with scSLAM-seq, despite 1/10th of the raw sequencing depth). Furthermore, sci-fate exhibits a higher detection rate of newly synthesized mRNA (82% in sci-fate versus <50% in scSLAM-seq). (4) The signal-to-noise ratio (labeled versus unlabeled cells) of sci-fate is 20–50 fold, compared with only ~10-fold for scSLAM-seq or NASC-seq. This is partly due to the fact that the sci-fate library preparation is strand specific, whereas smart-seq is not. (5) Sci-fate enables direct counting of newly synthesized versus pre-existing mRNA via 3′-tagged UMIs, which are used by neither scSLAM-seq nor NASC-seq. Additional advantages of sci-fate include compatibility with fixed cells and the ability to concurrently process multiple independent biological samples within a single experiment. Finally, it is notable that in situ 4sU chemical conversion requires cell permeabilization and, at least in our experience, PFA fixation, neither of which is straightforward to introduce on droplet-based single-cell RNA-sequencing (scRNA-seq) platforms such as 10x Genomics.

We note that, although sci-fate enables quantification of mRNA synthesis in single cells, we remain in need of methods for measuring mRNA degradation rates in single cells. Related to this, our simplifying assumption that gene-specific degradation rates are constant across our DEX time course might not be a good choice in other systems. Specifically, in systems where the gene-specific degradation rates are expected or observed to vary substantially over time, these should be estimated for each time interval separately.

Sci-fate can be broadly applied to most in vitro systems to quantitatively characterize cell-state dynamics within short time windows (for example, one to several hours). For even shorter time frames, a concern is that the signal-to-noise ratio will drop as the rate of labeling falls toward the background rate of 0.8%. For longer time frames, a time-series approach can be adopted as in the main experiment described in the present study.

A major limitation of sci-fate is that 4sU-labeling experiments are generally performed within in vitro cell culture models. However, recent studies have shown that 4sU can be used together with transgenic UPRT-expressing mice to stably label cell type-specific nascent RNA transcription in vivo[14–16], suggesting that sci-fate, with further optimizations to enhance 4sU incorporation and detection rate, can potentially be used to profile single-cell transcriptional dynamics in vivo and at scale.

Online content
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Methods
Mammalian cell culture. All mammalian cells were cultured at 37°C with 5% CO₂, and were maintained in high-glucose Dulbecco's modified Eagle's medium ( Gibco catalog no. 11965) for HEK293T and NIH/3T3 cells or Dulbecco's modified Eagle's medium (Gibco catalog no. 12600) for A549 cells, both supplemented with 10% fetal bovine serum and 1% penicillin–streptomycin (Gibco, catalog no. 15140122; 100 U ml⁻¹ of penicillin, 100 µg ml⁻¹ of streptomycin). Cells were trypsinized with 0.25% trypsin–ethylenediaminetetraacetic acid (EDTA) (Gibco, catalog no. 25200-065) and split 1:10 three times a week.

Sample processing for sc-fate. For HEK293T and NIH/3T3 cells, cells were incubated with 200 µM 4SU for 6 h before cell harvest. A549 cells were treated with 100 nM DEX for 0, 2, 4, 6, 8, or 10 h. Cells in all treatment conditions were incubated with 200 µM 4SU for the last 2 h before cell harvest. Of note, an excessively long labeling time (the extent of which in all treatments was labeled) resulted in a significant increases in background signal. Through a test experiment on HEK293T cells, we found that the transcriptional degradation rate is around 10% per h for each cell. We then selected 2 h as the labeling time window, such that about 80% of detected transcriptomes per cell would be previously synthesized and usable to infer the previous cell state. In theory, a shorter labeling time enables more accurate cell past state inference but also requires sampling of more time points to cover a continuous process with a given time interval (that is, 10 h in our DEX-treatment experiment). A short labeling time would also potentially be affected by greater noise. In our data, the background labeling rate (‘labeled’ reads ratio in non-labeled cells) is 0.8%. Given that 2 h of labeling results in detection of ~20% of transcriptomes, to keep at least a 10:1 signal-to-noise ratio, the maximum labeling period should be at least 50 min.

All cell lines (A549, HEK293T and NIH/3T3 cells) were trypsinized, spun down at 300 g for 5 min (4°C) and washed once with 1 ml PBS (1×10⁶ cells/ml PBS, pH 7.4, 0.2 mg ml⁻¹ bovine serum albumin (New England Biolabs), 1% SuperRnasein (Thermo) and 10 mM Dithiothreitol (DTT)). After washing, cells were resuspended in PBST at 2–10 million cells ml⁻¹, flash frozen and stored in liquid nitrogen. PFA-fixed cells were thawed in a 37°C water bath, spun down at 500 g for 5 min, and incubated with 500 µl PBST including 0.2% Triton X-100 for 3 min on ice. Cells were pelleted and resuspended in 500 µl nuclelease-free water including 1% SuperRnasein. Then, 3 ml of 0.1 N HCl was added into the cells for a 5-min incubation on ice; 5.35 ml Tris-HCl pH 8.0, and 35% of 10% Triton X-100 were added into cells to neutralize the HCl. Cells were pelleted and washed with 1 ml PBST. Cells were resuspended in 100 µl PBST (without DTT), and 100 µl PBST (without DTT) with fixed cells was incubated with a mixture including 40 µl iodocetic acid (100 mM), 30 µl sodium phosphate buffer (500 µM, pH 8.0), 200 µl dimethylsulfoxide and 20 µl H₂O, at 50°C for 15 min. The reaction was quenched by 8 µl DTT (1 M) and 8.5 µl PBS⁻¹. Of note, the cell loss rate is high (>95%) in the chemical conversion and centrifugation steps. Cells were pelleted and resuspended in 100 µl PBS (1x PBST, pH 7.4, 0.2 mg ml⁻¹ bovine serum albumin, 1% SuperRnasein). For all later washes, cells were pelleted by centrifugation at 950 g for 5 min (“4°C”).

The following steps are similar for the sci-RNA-seq protocol with PFA-fixed nuclei. Briefly, cells were distributed into four 96-well plates. For each well, 500–5,000 cells (2 µl) were mixed with 1 µl of 25 µM anchored oligo(dT) primer (5′-ACACTCTTCTCCCTACACGCTTCTTCCGATCT-3′) and 20 pM of 10 µl P7 primer (5′-CAAGCAGAAGACGGCATACGATGTTGCTCTTGAGGTCAAGCT-3′), and 20 µl NEBNext High-Fidelity 2x PCR Master Mix (New England Biolabs). Amplification was carried out using the following program: 72°C for 5 min, 98°C for 30s, and 18–22 cycles of 98°C for 10 s, 66°C for 30 s, 72°C for 1 min and 72°C for 5 min. After PCR, amplification products were pooled together as one sample, purified with AMPure XP beads. Library concentrations were determined using Qubit (Invitrogen) and the libraries were visualized by electrophoresis on a 6% tris-borate–EDTA–polyacrylamide gel electrophoresis (PAGE) gel. Libraries were sequenced on the NextSeq 500 platform (Illumina) using a v2.150-cycle kit (read 1: 1 cycle, read 2: 150 cycles, index 1: 1 cycles, index 2: 10 cycles).

Read alignment and downstream processing. Read alignment and gene count matrix generation for the scRNA-seq was performed using the pipeline that we developed for sci-RNA-seq with minor modifications. Reads were first mapped to a reference genome with STAR v.2.5.2b, with gene annotations from GENCODE v.19 for humans and GENCODE v.11 for mice. For experiments with HEK293T and NIH/3T3 cells, we used an index combining chromosomes from both humans (hg19) and mice (mm10). For the A549 experiment, we used human genome build hg19.

The single-cell sam files were first converted into alignment tsv files using the sam2tsv function in yarkit. Next, for each single-cell alignment file, mutations matching the background SNP reference were filtered out. For background SNP reference of A549 cells, we downloaded the paired-end, bulk RNA-seq data for A549 cells from ENCODE (sampled names: ENCF5842FVG, ENCF5382ZTA, ENC2F144JEZ, ENC6F629L0I, ENCF1F49CDJ, ENCF0606WNO, ENCF828WTU, ENC58F80VGD). Each paired-end fastq files was first adaptor clipped using trim_galore (ref.) with default hightm aligned to hg19 genome with STAR v.2.5.2b. Unmapped and multiple mapped reads were removed using samtools v.1.3. Duplicated reads were filtered out by MarkDuplicates function in picard 1.105. De-duplicated reads from all samples were combined and sorted with sambv 1.3. Background SNPs were called using the mpileup function in samtools v.1.3 and the mpileup2snm function in VarScan 2.3.9. For the HEK293T and NIH/3T3 test experiment, a background SNP reference was generated in a similar pipeline, with the aggregated single-cell sam data from control condition (no 4SU labeling and no iodocetic acid treatment condition).

For each single-cell alignment file, all mutations with quality score ≤13 were removed. Mutations at both ends of each read were mostly due to sequencing errors, and thus were also filtered out. Mutations mapping to the background SNP reference were filtered out for each read. For each read, we checked where there were T→C mutations for sense strand or A→G mutations for antisense strand, and labeled these mutated reads as newly synthesized.

Each cell was characterized by two digital gene expression matrices from the full sequencing data and newly synthesized RNA data as described in the read alignment and downstream processing steps. Genes with expression in fewer than five cells were filtered out. Cells with <2,000 UMIs or >8,000 UMIs were discarded. Cells with doublet score >0.2 by doublet analysis pipeline Scratch v0.2 were removed.

The dimensionality of the data was first reduced using principal components analysis (after selecting the top 2,000 genes with the highest variance) on a digital gene expression matrix on either full gene expression data or the newly synthesized gene expression data by Monocle 3 alpha. The top ten PCs were selected for dimensionality reduction analysis with UMAP v0.3.2, a recently proposed algorithm based on Riemannian geometry and algebraic topology to perform dimensionality reduction and data visualization. For joint analysis, we combined the top ten PCs calculated on the whole transcriptome and the top ten PCs on the newly synthesized transcriptome for each single cell before dimension reduction with UMAP. Cell clusters were done via the densityPeak algorithm implemented in Monocle 3 alpha. We first performed UMAP analysis on joint information of all processed cells, and identified an outlier cluster (724 of 7,404 cells). These cells were marked as high-level expression outlier and a marker of differentiated cells; and were filtered out before downstream analysis.

Linking TFs to their regulated genes. We sought to identify links between TFs and their regulated genes based on expression covariance. Cells with >17,000 TFs (gene sets) with high expression (or gene sets) on a digital gene expression matrix were mapped to the background gene set expression matrix on either full or newly synthesized gene expression data by Monocle 3 alpha. We first performed UMAP analysis on joint information of all processed cells, and identified an outlier cluster (724 of 7,404 cells). These cells were marked as high-level expression outlier and a marker of differentiated cells; and were filtered out before downstream analysis.

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size factor (SG) estimate using estimateSizeFactors in Monocle 3/alpha=10 on the full expression matrix of each cell, and log(transformed):

\[ G_i = \ln \left( \frac{G_i}{SG} \right) + 0.1 \]

To simplify downstream comparison between genes, we standardize the response \( G_i \) before fitting the model for each gene with the scale() function in R. Similar to \( G_i, T_i \) is the adjusted TF expression value for each cell. It is calculated using the full TF expression count (\( t \)) for each cell, normalized by the SG estimate using estimateSizeFactors in Monocle 3/alpha=10 on the full expression matrix of each cell, and log(transformed):

\[ T_i = \ln \left( \frac{t_i}{SG} \right) + 0.1 \]

Before fitting, \( T_i \) is standardized with the scale() function in R, \( \beta \), and \( \beta \) are regression coefficients in LASSO regression.

Although negative correlations between a TF's expression and a gene's new synthesis rate could reflect the activity of a transcriptional repressor, we felt that the more probable explanation for negative links reported by glmnet was mutually exclusive patterns of cell-state-specific expression and TF activity. Thus, during prediction, we excluded TFs with negatively correlated expression with a potential target gene's new synthesis rate, and also low regression coefficient (\( \leq 0.03 \)) links.

We identified a total of 6,103 links between TFs and regulated genes. A modified strategy without filtering negatively correlated TF-gene synthesis rate pairs identified 47 additional repressive TF-gene links between 9 TFs and 46 genes (see Supplementary Table 2).

Our approach aims to identify TFs that may regulate each gene, by finding the subset that can be used to predict its expression in a regression model. However, a TF with expression correlated with a gene's expression does not definitively mean that it is directly regulating that gene. To identify putatively direct targets within this set, we intersected the links with TFs profiled in ENCODE ChIP-seq experiments\(^\text{36} \). Of the 6,103 links between TFs and genes by LASSO regression, 1,086 links have TFs characterized in ENCODE, 807 of which were validated by target-binding sites near gene promoters from ENCODE\(^\text{37} \), a 4.3-fold enrichment relative to background expectation (odds ratio for validation = 2.89 for links identified in LASSO regression versus 0.67 for background; \( P = 2.2 \times 10^{-16} \), two-sided Fisher's exact test). Only genes set with significant enrichment and based on the correct TF ChIP-seq binding sites were retained (two-sided, Fisher's exact test, false discovery rate of 5%), and further pruned to remove indirect target genes without TF-binding data support. Ultimately, 591 links were retained using this approach.

To expand the set of validated TF–gene links, we further applied the package SCENIC\(^\text{38} \), a pipeline to construct gene-regulatory networks based on the enrichment of target TF motifs in the 10-kb window around genes' promoters. Each co-expression module identified by LASSO regression was analyzed using cis-regulatory motif analysis with RcisTarget v.1.2.137. Only modules with substantial motif enrichment (normalized enrichment score reported by RcisTarget > 3) of the correct TF regulator were retained, and pruned to remove indirect target genes without motif support. We filtered the TF–gene links using three correlation coefficient thresholds (0.3, 0.4 and 0.5), and combined all links validated by RcisTarget\(^\text{37} \). In total, 509 links were validated using this motif-based approach.

Combining both approaches, we identified a total of 986 TF–gene regulatory links using the covariance between TF expression and gene synthesis rate, validated by DNA-binding data or motif analysis. To evaluate the possibility that the links were artifacts of regularized regression, we permuted the order of single-cell TF expression (were artifacts of regularized regression, we permutated the order of single-cell TF by DNA-binding data or motif analysis. To evaluate the possibility that the links identified using the covariance between TF expression and gene synthesis rate, we segmented each of these two conditions by k-means clustering (\( k = 3 \)), and recovered a total of nine cell-state classes. Three GR response states were identified using the densityPeak algorithm implemented in Monocle 3/alpha=10\(^\text{32} \), on the UMAP coordinates ordered by cell-cycle TF modules. As each main cell-cycle phase still showed variable TF activity and cell-cycle marker expression, we segmented each of these two conditions by k-means clustering (\( k = 3 \)), and recovered a total of nine cell-state classes. Three GR response states were identified using the densityPeak algorithm implemented in Monocle 3/alpha=10\(^\text{32} \).

Past transcriptome state recovery from sci-fate. To infer the past transcriptome state (that is, the cell state before 4U labeling commenced), we assumed that mRNA half-lives are consistent across different DEX-treatment durations. This assumption is further validated by a self-consistency check later. Under this assumption, the partly degraded bulk transcriptome before the 2-h 4U labeling should be the same between both DEX and 2-h DEX-treated cells. Thus, for any given gene, differences in whole transcriptomes (bulk) between these time points should be equal to differences in the newly synthesized transcriptomes (bulk), corrected by technique's detection rate:

\[ A_{\alpha} = \frac{A_{\alpha}S_{\alpha} - \left( N_{\alpha}S_{\alpha}/\alpha \right)}{\alpha} = A_{\alpha}S_{\alpha} - \left( N_{\alpha}/\alpha \right)/\alpha \]

where \( A_{\alpha} \) is the aggregated UMI count for all cells in the no DEX treatment group; \( S_{\alpha} \) is the library size (total UMI count of cells) at no DEX treatment; \( N_{\alpha} \) is the aggregated newly synthesized UMI count for all cells in the no DEX treatment group; \( A_{\alpha}S_{\alpha} \) is the aggregated UMI count for all cells in the 2-h DEX treatment group; \( S_{\alpha} \) is the library size (total UMI count of cells) in the 2-h DEX treatment group; \( N_{\alpha} \) is the aggregated newly synthesized UMI count for all cells in the 2-h DEX treatment group; and \( \alpha \) is the detection rate for each gene in sci-fate. As cells from different time points were profiled in the same experiment and the UMI counts detected per cell were similar across conditions (see Supplementary Fig. 2a, b), we assume the same overall RNA amount in the 0- and 2-h samples, and normalize the aggregated gene count reads by total counts of each time point. For experiments where this assumption may not hold, spike-in standards could be used to control for differences in the overall amount of mRNA between conditions.

In theory, one detection rate can be calculated for each gene. However, for genes with minor differences of the newly synthesized rate between two conditions, the estimated \( \alpha \) is dominated by noise. We thus selected genes showing higher differences in the normalized, newly synthesized rate between two conditions: we first tested a series of thresholds for gene filtering and calculated the \( \alpha \) for each gene. We then plotted the relationship between threshold and the ratio of genes with out-of-range \( \alpha \) values (<0 or >1). We selected the threshold that was at the knee point of the plot, resulting in 186 genes being selected (see Supplementary Fig. 5a). The differences in newly synthesized mRNA of these genes correlate highly with the differences in mRNA expression level (Pearson's \( r = 0.93 \); see Supplementary Fig. 5b), suggesting that the new RNA detection rate is stable across genes (see Supplementary Fig. 5c). In the present study we use the median detection rate across 186 selected genes to estimate \( \alpha \).

We next computed the mRNA degradation rate across each 2-h interval. As the A549 cell population can be regarded as stable without external perturbation, for 2-h DEX-treated cells, its past state (before 2-h 4U labeling) should be the same as the 0-h DEX-treated cells. Expanding on this logic, the past state (before 4U labeling) for \( T = 0/-2/-4/-6/-8/-10\)-h DEX-treated cells should be similar to the profiled 0-h/2-h/4-h/6-h/8-h/10-h cells, respectively:

\[ \alpha_{\beta} = \frac{N_{\beta}S_{\beta}}{N_{\beta}S_{\beta}/\alpha} = A_{\beta}S_{\beta}/\alpha \]

where \( A_{\beta} \) is the aggregated UMI count for all cells in 1; \( S_{\beta} \) is the library size (total UMI count of cells) at 1; \( N_{\beta} \) is the aggregated newly synthesized UMI count for all cells at 1; \( \beta \) is the estimated detection rate for sci-fate; \( A_{\beta} \) is the aggregated UMI count for all cells in 0; \( S_{\beta} \) is the library size (total UMI count of cells) at 0; and \( \beta \) is 1 – gene-specific degradation rate between 0 and 1, and is related to the mRNA half-life \( \beta \) by:

\[ \beta = \left( \frac{1}{2} \right)^{1/(10^{-\beta}/r)} \]

The \( \beta \) was calculated for each of the 14,587 genes across each 2-h interval of DEX treatment. As with the self-consistency check, the gene degradation rates are highly correlated across different DEX-treatment times (see Supplementary Fig. 5g). We therefore used the average degradation rate for each gene for downstream analysis.

With the overall sci-fate detection rate as well as per-gene degradation rates estimated, the past transcriptome state of each cell can be estimated using:

\[ a_{\beta} = n_{\beta}/(a_{\beta} = a_{\beta} \times \beta) \]

\( a_{\beta} \) is the single-cell UMI count in 1; \( n_{\beta} \) is the single-cell newly synthesized UMI count at 1; and \( \alpha \) is the detection rate for each gene in sci-fate. In the present
study, we use the median detection rate across 186 selected genes as its estimates; \( \beta \) is 1 – gene-specific degradation rate between \( t_0 \) and \( t_1 \). The \( a_{ij} \) is the estimated single-cell transcription in a past time point \( t_0 \), with all negative values (15.6% of values on average) converted to 0.

The detection rate (\( \alpha \)) of the newly synthesized transcriptome is experiment specific and depends mainly on the 4sU-labeling concentration. Generally, a lower 4sU concentration will lead to a lower 4sU incorporation rate into newly synthesized mRNA, which will reduce the detection rate of newly synthesized transcripts. In addition, the length of sequencing reads may affect the detection rate, as short sequencing reads will reduce the possibility of detecting incorporated 4sU. In the case of our experiments and as noted earlier, we used relatively long reads (on average, 75bp of transcript-derived sequence obtained per read) to potentially increase detection rate. We also designed the experiments such that all treatment conditions share the same 4sU treatment concentration and incubation time in the same cell type. Furthermore, all cells were sequenced in the same sequencing run, so that all conditions were expected to share a similar \( \alpha \). For sci-fate experiments with different labeling conditions or sequencing settings, the \( \alpha \) would need to be re-estimated for each part of the experiment. Of note, our simplifying assumption that gene-specific degradation rates are constant across our DEX time course might not be a good choice in other systems. Specifically, in systems where the gene-specific degradation rates are expected or observed to substantially vary over time, these should be estimated for each time interval separately.

Linkage analysis to build single-cell state trajectory. The goal of what we call here 'linkage analysis' is to associate each cell with parent and child cells at different time points, that is, single-cell state trajectories. Our approach is based on the fact that the past transcriptome states (before 2 h 4sU labeling) of cells at \( t_1 \) should share the same cell population distribution with the profiled transcriptome states of cells at \( t_0 \) (2 h earlier than \( t_1 \)), assuming that there is no cell apoptosis. We thus applied a published manifold alignment strategy to identify common cell states between two datasets, based on common sources of variation\(^{31} \). As a result, whole transcriptomes from \( t_0 \) cells and recovered past transcriptomes from \( t_1 \) cells are aligned in the same UMAP space. This analysis is based on an assumption that, for intermediate time points, we are oversampling the space of physiologically distinct states in this time course. Violation of this and other assumptions can be detected by outliers during alignment of the two datasets. For each cell \( A \) from \( t_0 \), we selected its nearest neighbor in \( t_0 \) as its parent state in the alignment UMAP space. Similarly, for each cell from \( t_1 \), we selected its nearest neighbor in \( t_1 \) as its child cell state. Of note, it is not necessary for the link to be bidirectional: the parent state of one cell can be linked to a different child cell. After the parent and child cells were identified for each cell (except cells at the start and end time points), we then extend each cell trajectory by searching for the linked parent cell of each cell's parent, and similarly the linked child cell of each cell's child. Thus, each single cell can be characterized by a single-cell state transition path across all six time points spanning 10h. As multiple cells (>50) are profiled for each of the 27 defined cell states, stochastic cell-state transition processes can also potentially be captured.

Dimensionality reduction and clustering analysis. For dimensionality reduction on single-cell transcriptomes, the top five PCs for full transcriptomes and the top five PCs for newly synthesized transcriptomes were selected for each state, and combined in temporal order along a single-cell state trajectory for UMAP analysis. The main cell trajectory types were identified using a density peak-clustering algorithm\(^{70} \).

With cell-state proportion at the beginning time point (0-h treatment) and cell-state transition probabilities estimated from the data, we first predicted the cell-state distribution after 2 h, assuming that the cell-state transitions in DEX treatment are cell-autonomous, time-independent, Markovian processes. Similarly, the cell-state distribution at later time points can be predicted from the cell-state distribution 2h earlier.

For RNA-velocity analysis of these same data, single-cell spliced/unspliced expression matrices were generated using the command line interface of velocyto v0.17\(^{71} \) with the default run _smartseq2 mode on single-cell bam files. Cell transition direction inference was performed with an optimized, scalable, RNA-velocity analysis toolkit scVel0 v0.1.17 and scanny v1.4.1 with default settings\(^{42,72} \). To integrate treatment time information into the RNA-velocity analysis of the 0-h and 2-h time points, we prohibited transitions of cells within 0h, and instead selected the future state as the cell at the 2-h time point with the highest transition probability.

Cell-state instability and cell-state distance calculations. We defined cell-state instability as the proportion of cells in a given state ‘moving’ to any other state at the next time point. To calculate cell-state distances, we first sampled an equal number (n = 50) of cells from each state, and separately aggregate the full transcriptome and newly synthesized transcriptome of sampled cells of that state (that is, in this ‘joint transcriptome’, each gene is represented by two columns, one

for the whole transcriptome and one for the newly synthesized transcriptome). The cell-state distance is calculated as (1 – Pearson’s correlation coefficient between the joint transcriptomes of two different states).

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

The data generated by this study can be downloaded in raw and processed forms from the National Center for Biotechnology Information Gene Expression Omnibus (GSE131351).

Code availability

Scripts for processing sci-fate sequencing were written in Python and R with code available at https://github.com/JunyueC/sci-fate_analysis.

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Author contributions

J.S. and J.C. designed the research. J.C. developed the technique and performed the experiments with the assistance of F.S. J.C. performed the computation analysis with S.A. and W.Z. and C.T. J.S. and J.C. designed the research. J.C. developed the technique and performed the experiments with the assistance of F.S. J.C. performed the computation analysis with S.A. and W.Z. and C.T. J.S. and C.T. also contributed to the writing of the paper.

Competing interests

FSJ declares competing financial interests in the form of stock ownership and paid employment by Illumina, Inc. One or more embodiments of one or more patents and patent applications filed by Illumina may encompass the methods, reagents and data disclosed in this article.

Additional information

Supplementary information is available for this paper at https://doi.org/10.1038/s41587-020-0480-9.

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- For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted
- Give P values as exact values whenever suitable.
- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- Estimates of effect sizes (e.g. Cohen's d, Pearson's r), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection

No software was used except for Illumina RTA basecalling.

Data analysis

Common, freely available DNA sequencing data analysis software was used to analyze data, as described in Methods: bc12fastq/v2.16, python/v2.7.13, trim_galore/v0.4.1, samtools/v1.3, picard/1.105, STAR/v 2.5.2b, R/3.5.0, VarScan/2.3.9, scanpy/v1.4.1, scrublet/v0.2, EnrichR/v1.0, UMAP/v0.3.2, reticulate/v1.10, Monocle3/alpha, Seurat/2.3.4, pheatmap/1.0.12, RcisTarget/v1.2.1, glmnet/v.2.0, velocyto/v.0.1.17, and scVelo/v.0.1.17.

Scripts for processing sci-fate sequencing were written in python and R with code available at https://github.com/JunyueC/sci-fate_analysis.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

The data generated by this study can be downloaded in raw and processed forms from the NCBI Gene Expression Omnibus (GSE131351).
Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

- Life sciences
- Behavioural & social sciences
- Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size

No explicit calculations were performed to determine sample size.

Data exclusions

No data were excluded from the study.

Replication

The technique was tested and validated in two independent experiments using different cell lines. For A549 cell experiment, each of the six treatment conditions was represented by 64 replicate wells during the first round of processing in sci-fate. All attempts at replication were successful.

Randomization

The order of samples are randomized during drug treatment, and during sample processing in sci-fate.

Blinding

Investigators were blinded to group allocation during data collection (sequencing) and analysis.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

| Materials & experimental systems | Methods |
|----------------------------------|---------|
| n/a | Involved in the study |
| ☒ | Antibodies |
| ☒ | Eukaryotic cell lines |
| ☒ | Palaeontology |
| ☒ | Animals and other organisms |
| ☒ | Human research participants |
| ☒ | Clinical data |
| ☒ | Involved in the study |
| ☒ | ChIP-seq |
| ☒ | Flow cytometry |
| ☒ | MRI-based neuroimaging |

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

HEK293T, NIH/3T3 and A549 cells were from ATCC

Authentication

None of the cell lines were authenticated.

Mycoplasma contamination

Cell lines were not tested for Mycoplasma contamination.

Commonly misidentified lines

No commonly misidentified cell lines were used.

(See ICLAC, register)