Universality of clone dynamics during tissue development

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The emergence of complex organs is driven by the coordinated proliferation, migration and differentiation of precursor cells. The fate behaviour of these cells is reflected in the time evolution of their progeny, termed clones, which serve as a key experimental observable. In adult tissues, where cell dynamics is constrained by the condition of homeostasis, clonal tracing studies based on transgenic animal models have advanced our understanding of cell fate behaviour and its dysregulation in disease1-5. But what can be learnt from clonal dynamics in development, where the spatial cohesiveness of clones is impaired by tissue deformations during tissue growth? Drawing on the results of clonal tracing studies, we show that, despite the complexity of organ development, clonal dynamics may converge to a critical state characterized by universal scaling behaviour of clone sizes. By mapping clonal dynamics onto a generalization of the classical theory of aerolos, we elucidate the origin and range of scaling behaviours and show how the identification of universal scaling dependences may allow lineage-specific information to be distilled from experiments. Our study shows the emergence of core concepts of statistical physics in an unexpected context, identifying cellular systems as a laboratory to study non-equilibrium statistical physics.

Biological systems, being highly structured and dynamic, function far from thermodynamic equilibrium. This is particularly evident in embryonic development where, through large-scale cellular self-organization, highly complex structures emerge from a group of genetically identical, pluripotent stem cells. To achieve the stereotopic ordering of organs and tissues, the fate of embryonic stem cells and their progeny must be tightly regulated, such that the correct number and type of cells is generated at the right time and place during development. Mechanisms regulating such cell fate decisions are at the centre of research in stem cell and developmental biology6. Efforts to resolve the mechanisms that regulate cell fate behaviour from clonal tracing studies have drawn successfully upon concepts from statistical physics and mathematics4-6. However, in developing tissues, the interpretation of these experiments is complicated by the fact that clonal dynamics is, in principle, less constrained. Moreover, due to large-scale cellular rearrangements as well as stochastic forces from surrounding tissues, labelled clones may fragment into disconnected clusters, or they merge and form larger compounds of labelled cells (Fig. 1b-f).

To understand how complexity at the macroscopic scale translates into coherent collective behaviour at the macro-scale, statistical physics provides a useful theoretical framework. For critical systems, where fluctuations are scale-invariant, successive coarse-graining can yield effective theories describing macroscopic behaviour. In such systems, different ‘microscopic’ interactions can give rise to indistinguishable macroscopic behaviour—a concept known as universality. As a reflection of scale invariance, statistical correlations, such as size distributions, obtain simple scaling forms, which depend only on one or few dimensionless composite variables. But, given the complexity of embryonic development, can such concepts be applied to study cellular behaviour?

At the cellular scale, the patterns of cell fate decisions during embryonic development are reflected in the time-evolution of individual developmental precursors cells and their progeny, which together constitute a clone. While the dynamics of individual clones maybe complex, subject both to intrinsic and extrinsic influences, statistical ensembles of clones may provide robust (predictive) information about the relationship between different cell types and mechanisms regulating cellular behaviour. In mammals, where live-imaging of developing embryonic organs is typically infeasible, efforts to resolve clonal dynamics have relied on cell lineage tracing studies using transgenic animal models7. In this approach, the activation of a reporter gene allows individual cells to be marked with a fluorescent reporter. As a genetic mark, this label is then inherited by all progeny of a marked cell, and allows clone sizes and cell compositions to be recorded at specific times post-labelling (Fig. 1a). Lineage tracing studies therefore provide a ‘two-time’ measure of clonal dynamics in the living embryo. In adult tissues, where cell dynamics is heavily constrained by the steady-state condition of homeostasis, efforts to resolve cell fate behaviour from clonal tracing studies have drawn successfully upon concepts from statistical physics and mathematics4-6. However, in developing tissues, the interpretation of these experiments is complicated by the fact that clonal dynamics is, in principle, less constrained. Moreover, due to large-scale cellular rearrangements as well as stochastic forces from surrounding tissues, labelled clones may fragment into disconnected clusters, or they merge and form larger compounds of labelled cells (Fig. 1b-f).

Here, by establishing a formal mapping between clonal dynamics and a generalization of the theory of aerolos, we show that, during embryonic development, clonal dynamics converges to a critical state, giving rise to universal scaling behaviour of the size distributions of labelled clusters. Further, we explore how understanding
the origins of scaling and universality can form the quantitative basis for recovering information on cell fate behaviour during development. We thus find the emergence of core concepts of statistical physics in the unexpected context of embryonic development. As well as being of interest in the study of tissue development, these findings have important implications for the study of tissue regeneration and tumour growth.

To develop this programme, we begin with an example of clonal evolution during the development of mouse heart. The gene *Mesp1* is transiently expressed between embryonic day (E)6.5 and E7.5 in...
mice in the earliest precursor cells of the heart. Quantitative analysis of hearts labelled at low density (one to two clones per heart) have established the temporal progression in differentiation and proliferative capacity of these precursors. However, with just one or two clones per embryo, and inherent variability in the efficiency of labelling, low-density labelling is highly inefficient in probing evolutionary processes during development. By contrast, at high (mosaic) labelling density, each embryo provides a potentially rich dataset. The situation is exemplified in Fig. 1e, which shows mouse hearts at E12.5 and postnatal day P1 after mosaic labelling between E6.5 and E7.5 using the multicolour Mesp1-Cre/Rosa-Confetti reporter construct (with 50% of the cardiac surface being fluorescently labelled with each of three colours, cyan, yellow and red, roughly equally represented). However, at this density of labelling, a single contiguous cluster of labelled cells can be derived from the chance fusion of two or more independent clones induced with the same colour. Given that clone sizes are not constrained by tissue size, and the ambiguity arising from clone merger and fragmentation, to what extent can information on cell fate behaviour be recovered?

To address this question, we quantified the surface area (SA) covered by each cluster in a given heart compartment at different developmental time points. From the SAs, we then determined their distributions in each heart region (Fig. 1f). Although cardiac development involves complex cell fate decisions, with regional and temporal variations in proliferation, we found that the resulting cluster size distribution was remarkably conserved: After rescaling the SA of each cluster by the ensemble average for each compartment size distribution, low-density labelling is highly inefficient in probing evolutionary processes during development. At long times and larger cluster sizes, the time evolution of the cluster size distribution becomes controlled by three fixed points (dependent on the details of the merging and fragmentation processes), where it acquires a universal scaling dependence (Supplementary Information). The inset shows a schematic of the renormalization process, with the largest cluster sizes converging more rapidly onto the universal distribution than the smallest cluster sizes. This result implies that, despite the complexity and variable histories, the resulting SA distribution is fully characterized by the average alone, the defining property of scaling.

The simplicity of the cluster size distribution that is reflected in scaling behaviour suggests that its origin may not rely on details of the morphogenic programme in heart. Rather, to uncover its origin, we began by considering the simplest set of processes that could determine cluster size: first, as labelled cells divide, clusters may grow at a rate proportional to their size. Second, in expanding tissues, clones may fragment into disconnected clusters as cells disperse or the tissue deforms. If the rate of growth and fragmentation increase in proportion to cluster size, the SA distribution would be predicted to become stationary. However, although clonal tracing studies indicate that growth and fragmentation occur on a similar timescale during the early phase of heart development (E6.5 and E12.5), average cluster sizes at E12.5 and P1 differ by a factor of 2.7, showing that steady state is not reached. More importantly, such a simple line of argument neglects the possibility that clusters of the same colour can merge into larger, cohesively labelled regions. Yet the number of clusters varies only marginally between E12.5 and P1, indicating that merger and fragmentation could be equally abundant.

To resolve the origin of scaling, it is instructive to leave temporarily the realm of biology and consider the growth dynamics of ‘inanimate’ compounds. Indeed, processes involving merger and fragmentation occur in multiple contexts in physics, including the nucleation of nano-crystals, amyloid fibrils, polymerization, endocytosis and the dynamics of aerosols. In common with clonal evolution in tissues, droplets in aerosols may merge (coagulate) or they may fragment (Fig. 2a). By analogy with clonal growth due to cell division, droplets may also expand by condensation of free molecules, while cell loss due to death or migration out of the imaging window is mirrored in the evaporation and shrinking of droplet sizes. Finally, by analogy with the migration of cells into the field of view, new droplets may nucleate from free molecules. Through this correspondence, can the statistical physics of aerosols provide insight into the dynamics of cell clusters in tissues and the emergence of scaling?

The distribution of cluster sizes, \( f(x,t) \), is the result of different sources of variability, including merger, fragmentation, cell division and loss. Formally, the time evolution of the cluster size distribution can be cast (symbolically) as a sum of operators \( L \) that describe the effect of these contributions on the time evolution:

\[
\partial_t f(x,t) = L_{\text{growth}} f(x,t) + \mu L_{\text{merger}} f(x,t) + \psi L_{\text{fragmentation}} f(x,t) + \ldots
\]
where the parameters, $\varphi$, $\mu$, and so on, characterize the relative strength of these processes against that of growth (for details, see Supplementary Theory). To investigate the origin of scaling, we questioned what determines the long-term, large-scale dependence of the cluster size distribution. In statistical physics this question is typically answered by successively coarse-graining the dynamics and monitoring changes in the relative contributions of different processes. Under this renormalization, when a cell divides, cluster sizes are rescaled by the resulting increase in tissue size, $x \rightarrow x/(1+\delta X) \equiv \rho$. Simultaneously, time is rescaled in such way that the total rate of merging and fragmentation events remains constant in this process. Notably, after repeated rounds of dynamic renormalization, the kinetic equation converges to a self-similar (critical) form, where the fluctuations in cluster sizes are dominated solely by a balance between merger and fragmentation events (Supplementary Theory), while the influence of other processes becomes vanishingly small,

$$\partial_x f(\rho, \tau) \approx \varphi' L_{\text{fragmentation}}[f(\rho, \tau)] + \mu' L_{\text{merging}}[f(\rho, \tau)].$$

Here, $\varphi'$ and $\mu'$ are rescaled parameters and $\tau$ is a rescaled time (Supplementary Theory). Intuitively, this means that, as the organ grows, different sources of variance contribute to the cluster size distribution by different degrees (Fig. 2b and Supplementary Fig. S1A). Crucially, in the long term, contributions relating to cell fate behaviour (for example, cell division or loss) become dominated by merger and fragmentation processes, resulting in information on the former becoming erased (Supplementary Theory). Therefore, while cell fate decisions affect the mean cluster size, the shape of the distribution is determined entirely by merger and fragmentation events (Fig. 2c), leading to the emergence of scaling behaviour observed in heart development (Fig. 1h).

Important results suggest not only that the cluster size distribution is entirely determined by its average (scaling), but also that the shape of the distribution is independent of the biological context (universality). The form of the scaling function, $\psi$, relies on the dependence of the merging and fragmentation rates on cluster size. In a uniformly growing tissue, clone merger and fragmentation events are the result of the slow diffusive motion of clusters originating from random forces exerted by the surrounding tissue. In this case, the resulting scaling form is well-approximated by a log-normal size dependence (Fig. 2c, Supplementary Theory). Indeed, such distributions are typical of merging and fragmentation processes and describe the empirical distribution of droplet sizes in
aerosols\textsuperscript{14,19}. Similar universal behaviour is recapitulated by a simple lattice-based Monte Carlo simulation of uniform tissue growth, where the stochastic nature of cell division alone leads to merger and fragmentation (Supplementary Fig. S1B and Supplementary Theory).

Importantly, this analysis provides an explanation for the observed scaling behaviour of labelled cluster sizes of mouse heart, where the distribution indeed follows a strikingly log-normal size dependence (Fig. 3a,b and Supplementary Fig. S2A,B). To further challenge the universality of the scaling dependencies, we used a similar genetic labelling strategy to trace the fate of early developmental precursors in mouse liver and pancreas as well as the late-stage development of zebrafish heart\textsuperscript{20}. In all cases, cluster size distributions showed collapse onto a log-normal size dependence (Fig. 3c-1 and Supplementary Fig. S2C-E), with the notable exception of a subpopulation of pancreatic precursors (see below).

This analysis shows that, in the long term, the collective cellular dynamics leads to a critical state dominated by a balance between merging and fragmentation events. The emerging universal scaling distributions progressively become void of information on underlying biological processes on a timescale determined by the merging and fragmentation rates. But how can such information be recovered? In analogy to the turnover of adult homeostatic tissues, such as interfollicular epidermis or intestine\textsuperscript{21,22}, the behaviour of the size distribution under renormalization (Fig. 2b and Supplementary Theory) shows how lineage-specific information can be recovered: first, it is preserved in the non-universal cluster size dependences at short times post-labelling, prior to convergence to the scaling regime. Second, convergence onto universal scaling dependences is the slowest for small cluster sizes ($\propto \lambda(x)$). Third, if the rate of clone merger is negligibly small, different cluster size distributions can emerge according to the mode of cell division. The range of possible behaviours is summarized in Table 1. Finally, as merging and fragmentation are emergent properties of cell fate decisions, deviations from the scaling form can inform on structural properties of organ formation. As an example, in the developing pancreas, acinar cells initiate from precursors localized at the tips of a complex ductal network and aggregate as cohesive cell clusters, thereby suppressing clonal fragmentation. This results in a departure from scaling behaviour of the cluster size distribution (Fig. 3f and Supplementary Fig. S2F).

In recent years, there has been a growing emphasis on genetic lineage tracing as a tool to resolve the proliferative potential and fate behaviour of stem and progenitor cells in normal and diseased tissues\textsuperscript{1}. Here, we have shown that the collective cellular dynamics in tissue growth and turnover lead to universal clone dynamics, where cluster size distributions become independent of the fate behaviour of cell populations. As well as highlighting the benefit of low-density labelling and the dangers of making an unguarded assessment of clonality in lineage-labelled systems, these findings identify quantitative strategies to unveil cell fate-specific information from short-term or small-cluster-size dependences, with potential applications to studies of clonal dynamics in both healthy and diseased states. At the same time, by highlighting the unexpected emergence of core concepts of statistical physics in a novel context, this study provides a model of how the cellular dynamics of living tissues can serve as a laboratory for statistical physics.

### Methods

Methods, including statements of data availability and any associated accession codes and references, are available at [https://doi.org/10.1038/s41567-018-0055-6](https://doi.org/10.1038/s41567-018-0055-6).

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### Table 1 | Non-universal dependencies of the cluster size distribution

| Growth mode | Clonal | Fragmentation | Merging and fragmentation |
|-------------|--------|--------------|--------------------------|
| Exponential | $(x)^{-1} \exp(-x/x)$ \(x = \exp(t)\) | $\varphi \exp(-\varphi^2 x) \langle x \rangle = \varphi^{-1}$ | $(x/\langle x \rangle)^a \langle x \rangle = \exp(t)$ |
| Linear      | $\frac{1}{\sqrt{2\pi \alpha}} \exp \left( -\frac{x^2}{2\alpha^2} \right) \langle x \rangle = t$ | $\varphi (2 + \sqrt{\alpha}) \exp \left( -\sqrt{\alpha} \frac{x}{2+\sqrt{\alpha}} \right) \langle x \rangle = \varphi^{-1/2}$ | $(x/\langle x \rangle)^a \langle x \rangle = \exp(t)$ |
| Homeostasis | $(x)^{-1} \exp(-x/x) \langle x \rangle \propto t$ | $J(x) \langle x \rangle \propto t$ | $(x/\langle x \rangle)^a \langle x \rangle = \exp(t)$ |

Analytical expressions for the cluster size distribution (top row in each cell) and average cluster size (bottom row). Shown are expressions in situations where labelling density is clonal, where labelling density is almost clonal but clones are subject to fragmentation, and where both merging and fragmentation of clones occur (left to right). As merging and fragmentation both result from tissue rearrangements, merging should always imply fragmentation. Time is measured in units of the cell cycle time. Expressions are valid after convergence to the scaling regime, when the typical cluster size is much larger than the size of single cells, and in the mean-field limit, which is a good approximation for two- and three-dimensional tissues. In addition, it is assumed that the full spectrum of cluster sizes can be experimentally resolved. If clones fragment but do not merge, fragmentation and growth ultimately compensate to lead to a stationary distribution. In the case of clonal merging and fragmentation, expressions give empirical approximations, where $a$ depends on the details of the merging and fragmentation processes (see Supplementary Theory).
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Author contributions

S.R. and B.D.S. conceived the project. S.C., F.L., C.J.H., N.P. and M.S. performed the experiments and collected the raw data. M.H. supervised the liver experiments. S.R. developed the theory, and performed the modelling and statistical analysis. S.R. and B.D.S drafted the manuscript. All authors edited and approved the final manuscript.

Competing interests

The authors declare no competing interests.

Ethical approval

We have complied with all relevant ethical regulations. *Mesp1-Cre* mice colonies were maintained in a certified animal facility in accordance with European guidelines. These experiments were approved by the local ethical committee under the reference #LA1230332(CEBEA). Research using mice for pancreas and liver samples has been regulated under the Animal (Scientific Procedures) Act 1986 Amendment Regulations 2012 following ethical review by the University of Cambridge Animal Welfare and Ethical Review Body (AWERB). These experimental data sets were obtained as by-products from other research projects undertaken by the respective laboratories.

Additional information

Supplementary information is available for this paper at https://doi.org/10.1038/s41567-018-0055-6.

Correspondence and requests for materials should be addressed to S.R. or B.D.S.
Methods

Surface area analysis of mosaically labelled hearts. To generate mosaically labelled hearts at high density, Mesp1-Cre reporter mice were crossed with the Rosa-Confetti reporter mice kindly provided by Hans Clevers, Hubrecht Institute, Utrecht. Hearts collected at embryonic days E12.5 and P1 were fixed in 4% paraformaldehyde for 1 hr at room temperature. Nuclei were counterstained with Topro3 (1/500, Invitrogen). The surface images were acquired with a confocal microscope (LSM780 Carl Zeiss). The surface area (SA) of each independent cluster was measured using Fiji software on the maximum intensity projection.

Pancreas. R26R-CreERT2; R26-Confetti mice were intraperitoneally injected with Tamoxifen (from Sigma) at 0.030 mg per gram of female at E12.5 of pregnancy under Home Office guidelines, Animal Scientific Procedure Act (ASPA) 1986. P14 pancreas was fixed in 4% paraformaldehyde overnight, and then washed in phosphate-buffered saline (PBS). Samples were sucrose-treated (30%) and mounted in optimal cutting temperature compound (OCT), and subsequently thick 100 μm cryostat sectioned. Sections were rehydrated in PBS, blocked overnight in PBS, 2% donkey serum and 0.5% Triton X-100. The samples were incubated in Dolichos biflorus agglutinin (DBA), biotinylated (from Vectorlabs) for three days at 4 °C, and AF647-Streptavidin (from Life Technologies) was applied for two days at 4 °C. Next, sections were cleared with RapiClear 1.52 (from SunJin Lab). Images were acquired with a Leica TCS SP5 confocal microscope, using the tiling mode. The images were analysed with Velocity, and volumes and coordinates of centres of clonal clusters quantified. To obtain three-dimensional reconstructions from Z stacks obtained with a Leica SP5 microscope, Imaris (v8, Bitplane) was used.

Liver. R26R-CreERT2;Rainbow mice were kindly provided by Magdalena Zernicka-Goetz (University of Cambridge, UK). R26R-CreERT2;Rainbow male mice were crossed with wild-type MF1 females and labelling induced by intraperitoneal injection of pregnant dams with Tamoxifen (Sigma). Tamoxifen was prepared at 10 mg ml−1 in sunflower oil and induction performed using 0.025 mg Tamoxifen per gram of pregnant dam. Pregnant dams were induced at E9.5 and the resulting pups had livers collected at postnatal day P30–P45. Livers were divided into pieces of thickness ~10 mm, washed at least three times in PBS to remove blood and fixed in 4% paraformaldehyde overnight before being washed twice in PBS. Liver pieces were mounted in 4% Low Melt Agarose (Bio-Rad) and 100-μm-thick sections cut using a vibratome (Leica VT1000 S). Thick sections were stored in PBS at 4 °C before immunostaining. Briefly, sections were blocked in PBS + 5% dimethyl sulfoxide (DMSO), Sigma) + 2% donkey serum (Sigma) + 1% Triton X-100 (Sigma) overnight before incubation in PBS + 1% DMSO + 2% donkey serum + 0.5% Triton X-100 + 1:40 goat anti-Osteopontin (R&D Systems, AF808) for three days at 4 °C. Following several washes in PBS + 1% DMSO + 0.5% Triton X-100 at 4 °C for 24 h, sections were incubated in PBS + 1% DMSO + 2% donkey serum + 0.5% Triton X-100 + 1:250 donkey anti-goat antibody conjugated to AF647 (Life Technologies) for two days at 4 °C. Following the staining, sections were cleared by increasing glycerol gradient before incubation with PBS + 1:1000 Hoechst 33342 (Sigma) for 1 h at 4 °C to counterstain nuclei and mounted with Vectashield (Vector Laboratories). Images of liver sections were acquired using a Leica TCS SP5 confocal microscope and processed using LAS AF Lite software (Leica). Cell numbers for each labelled cluster were counted manually from acquired images.

Life Sciences Reporting Summary. Further information on experimental design is available in the Life Sciences Reporting Summary.

Code availability. Custom code used to in this study is available from the corresponding authors upon reasonable request.

Data availability. The data that support the plots within this paper and other findings of this study are available from the corresponding author upon request.

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▸ Experimental design

1. Sample size
   Describe how sample size was determined.
   Sample sizes were chosen to be sufficient to resolve the shape of the cluster size distribution.

2. Data exclusions
   Describe any data exclusions.
   GFP clones in confetti labeling are rare and were discarded from the analysis in heart and liver.

3. Replication
   Describe whether the experimental findings were reliably reproduced.
   All attempts of replication were successful.

4. Randomization
   Describe how samples/organisms/participants were allocated into experimental groups.
   N/A

5. Blinding
   Describe whether the investigators were blinded to group allocation during data collection and/or analysis.
   Blinding was not relevant as all mice were wild-type phenotypically

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.

6. Statistical parameters
   For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

   n/a Confirmed
   □ □ The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
   □ □ A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
   □ □ A statement indicating how many times each experiment was replicated
   □ □ The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)
   □ □ A description of any assumptions or corrections, such as an adjustment for multiple comparisons
   □ □ The test results (e.g. P values) given as exact values whenever possible and with confidence intervals noted
   □ □ A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
   □ □ Clearly defined error bars

See the web collection on statistics for biologists for further resources and guidance.
Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study.

To solve mean-field Master equations and to perform lattice simulations we used custom code written in Matlab. To analyse cluster sizes generated from lattice simulations we used Matlab’s Image Processing toolbox. Further data analysis was performed using custom code in R. Fiji software was used to analyse cluster sizes in mouse heart. Pancreas images were analysed using Volocity and Imaris. LAS AF Lite software was used to process images from Liver samples.

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). Nature Methods guidance for providing algorithms and software for publication provides further information on this topic.

Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.

No unique materials were used.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

No antibodies were used.

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

No eukaryotic cell lines were used.

b. Describe the method of cell line authentication used.

No eukaryotic cell lines were used.

c. Report whether the cell lines were tested for mycoplasma contamination.

No eukaryotic cell lines were used.

d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.

No eukaryotic cell lines were used.

Animals and human research participants

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11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study.

To generate mosaically labelled hearts at high density, Mesp1-Cre mice (Saga et al. 1999) were crossed with the Rosa-Confetti reporter mice (Snippert et al. 2010) kindly provided by Hans Clevers. R26R-CreT2; R26-Confetti mice were used for the pancreas samples. For the liver samples, R26R-CreERT2;Rainbow+ male mice were crossed with wild-type MF1 females.

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12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

The study did not involve human research participants.