Mapping single-cell data to reference atlases by transfer learning

Mohammad Lotfollahi, Mohsen Naghipourfar, Malte D. Luecken, Matin Khajavi, Maren Büttner, Marco Wagenstetter, Žiga Avsec, Adam Gayoso, Nir Yosef, Marco Wagenstetter, Žiga Avsec, 1,2,9 and Fabian J. Theis

Large single-cell atlases are now routinely generated to serve as references for analysis of smaller-scale studies. Yet learning from reference data is complicated by batch effects between datasets, limited availability of computational resources and sharing restrictions on raw data. Here we introduce a deep learning strategy for mapping query datasets on top of a reference called single-cell architectural surgery (scArches). scArches uses transfer learning and parameter optimization to enable efficient, decentralized, iterative reference building and contextualization of new datasets with existing references without sharing raw data. Using examples from mouse brain, pancreas, immune and whole-organism atlases, we show that scArches preserves biological state information while removing batch effects, despite using four orders of magnitude fewer parameters than de novo integration. scArches generalizes to multireference mapping, allowing imputation of missing modalities. Finally, scArches retains coronavirus disease 2019 (COVID-19) disease variation when mapping to a healthy reference, enabling the discovery of disease-specific cell states. scArches will facilitate collaborative projects by enabling iterative construction, updating, sharing and efficient use of reference atlases.

Exploiting large reference datasets is a well-established approach to leverage existing conditional neural network models and transfer them to new datasets, called ‘architecture surgery’, as implemented in the scArches pipeline. scArches is a fast and scalable tool for updating, sharing and using reference atlases trained with a variety of neural network models. Specifically, given a basic reference atlas, scArches enables users to share this reference as a trained network with other users, who can in turn update the reference using query-to-reference mapping and partial weight optimization without sharing their data. Thus, users can build their own extended reference models or perform stepwise analysis of datasets as they are collected.

1Helmholtz Center Munich—German Research Center for Environmental Health, Institute of Computational Biology, Neuherberg, Germany. 2School of Life Sciences Weihenstephan, Technical University of Munich, Munich, Germany. 3Department of Computer Science, Technical University of Munich, Munich, Germany. 4Center for Computational Biology, University of California, Berkeley, Berkeley, CA, USA. 5Department of Electrical Engineering and Computer Sciences, University of California, Berkeley, Berkeley, CA, USA. 6Chan Zuckerberg Biohub, San Francisco, CA, USA. 7Ragon Institute of MGH, MIT and Harvard, Cambridge, MA, USA. 8Institute of Medical Informatics, University of Münster, Münster, Germany. 9Department of Mathematics, Technical University of Munich, Munich, Germany. 10Division of Pulmonary and Critical Care Medicine, Feinberg School of Medicine, Northwestern University, Chicago, IL, USA. ✉e-mail: fabian.theis@helmholtz-muenchen.de
which is often crucial for emerging clinical datasets. Furthermore, scArches allows users to learn from reference data by contextualizing new (for example, disease) data with a healthy reference in a shared representation. Due to the flexible choice of the underlying core model that is transferred using scArches, we can learn references with various base models but also train on multimodal data. We demonstrate the features of scArches using single-cell datasets ranging from pancreas to whole-mouse atlases and immune cells from patients with COVID-19. scArches is able to iteratively update a pancreas reference, transfer labels or unmeasured data modalities between reference atlases and query data and map COVID-19 data onto a healthy reference while preserving disease-specific variation.

Results
scArches enables mapping query data to reference. Consider the scenario with N ‘reference’ scRNA-seq datasets of a particular tissue or organism. A common approach to integrate such datasets is to use a conditional variational autoencoder (CVAE) (for example, single-cell variational inference (scVI))\(^3\), train variational autoencoder (trVAE)\(^4\) that assigns a categorical label \(S\) to each dataset that corresponds to the study label. These study labels may index traditional batch IDs (that is, samples, experiments across laboratories or sequencing technologies), biological batches (that is, organs or species when used over the set of orthologous genes), perturbations such as disease or a combination of these categorical variables. Training a CVAE model with reference studies \(S\)\(_{1:N}\) (Fig. 1a) results in a latent space where the effects of condition labels (that is, batch or technology) are regressed out. Thus, we can use this embedding for further downstream analysis such as visualization or identification of cell clusters or subpopulations.

Architectural surgery is a TL approach that takes existing reference models and adapts these to enable query-to-reference mapping. After training an existing autoencoder model on multiple reference datasets, architectural surgery is the process of transferring these trained weights with only minor weight adaptation (fine tuning) and adding a condition node to map a new study into this reference. While this approach is broadly applicable on any deep conditional model, here we apply scArches to three unsupervised models (CVAEs, trVAE, scVI), a semi-supervised (single-cell annotation using variational inference (scANVI))\(^5\) algorithm and a multimodal (total variational inference (totalVI))\(^6\) algorithm (Methods).

To facilitate model sharing, we adapted existing reference-building methods to incorporate them into our scArches package as ‘base models’. Reference models built within scArches can be uploaded to a model repository via our built-in application programming interface for Zenodo (Methods). To enable users to map new datasets on top of custom reference atlases, we propose sharing model weights, which one can download from the model repository and fine tune with new query data. This fine tuning extends the model by adding a set of trainable weights per query dataset called ‘adaptors’. In classical conditional neural networks, a study corresponds to an input neuron. As a trained network has a rigid architecture, it does not allow for adding new studies within the given network. To overcome this, we implement the architecture surgery approach to incorporate new study labels as new input nodes (Methods). These new input nodes with trainable weights are the aforementioned adaptors. Importantly, adaptors are shareable, allowing users to further customize shared reference models by downloading a reference atlas, choosing a set of available adaptors for that reference and finally incorporating the user’s own data by training query adaptors (Fig. 1b). Trainable parameters of the query model are restricted to a small subset of weights for query study labels. Depending on the size of this subset, this restriction functions as an inductive bias to prevent the model from strongly adapting its parameters to the query studies. Thus, query data update the reference atlas.

To illustrate the feasibility of this approach, we applied scArches with trVAE and scVI (see Supplementary Tables 1–7 for detailed parameters) to consecutively integrate two studies into a pancreas reference atlas comprising three studies (Fig. 1c). To additionally simulate the scenario in which query data contain a new cell type absent in the reference, we removed all alpha cells in the training reference data. We first trained different existing reference models within the scArches framework to integrate training data and construct a reference atlas (Fig. 1d,e and Supplementary Fig. 1, first column). Once the reference atlas was constructed, we fine tuned the reference model with the first query data (SMART-seq2 (SS2)) and iteratively updated the reference atlas with this study (Fig. 1d,e, second column) and the second query data (CelSeq2, Fig. 1d,e, third column). After each update, our model overlays data from all shared cell types present in both query and reference while yielding a separate and well-mixed cluster of alpha cells in the query datasets (black dashed circles in Fig. 1d,e). To further assess the robustness of the approach, we held out two cell types (alpha cells and gamma cells) in the reference data while keeping both in the query datasets. Here our model robustly integrated query data while placing unseen cell types into distinct clusters (Supplementary Fig. 2). Additional testing using simulated data showed that scArches is also robust to simultaneously updating the reference atlas with several query studies at a time (Supplementary Fig. 3).

Overall, TL with architectural surgery enables users to update learnt reference models by integrating query data while accounting for differences in cell type composition.

Minimal fine tuning performs best for model update. To determine the number of weights to optimize during reference mapping, we evaluated the performance of different fine-tuning strategies. Reference mapping performance was assessed using ten metrics recently established to evaluate data-integration performance in terms of removal of batch effects and preservation of biological variation. Batch-effect removal was measured via principal-component regression, entropy of batch mixing, k-nearest neighbor (kNN) graph connectivity and average silhouette width (ASW). Biological conservation was assessed with global cluster matching (adjusted Rand index (ARI), normalized mutual information (NMI)), local neighborhood conservation (kNN accuracy), cell type ASW and rare cell type metrics (isolated label scores). An accurate reference mapping integration should result in both high conservation of biological variation and high batch-removal scores.

Next to fine tuning only the weights connecting newly added studies as proposed above (adaptors), we also considered (1) training input layers in both encoder and decoder while the rest of the weights were frozen and (2) fine tuning all weights in the model. We trained a reference model for each base model using 250,000 cells from two mouse brain studies\(^3\)\(^3\)\(^3\). Next, we compared the integration performance of candidate fine-tuning strategies when mapping two query datasets\(^3\)\(^3\) onto the reference data. Applying scArches trVAE to the brain atlas, the model with the fewest parameters performed competitively with other approaches in integrating different batches while preserving distinctions between different cell types (Fig. 2a–c). Notably, the strongly regularized scArches reduced trainable parameters by four to five orders of magnitude (Fig. 2d). Overall, evaluating integration accuracy for different base models demonstrates the optimal time and integration performance trade-off of using adaptors to incorporate new query datasets compared to that of other approaches (Fig. 2e).

Architectural surgery allows for efficient data integration. To use scArches, one requires a reference atlas model. The quality of reference mapping performed by scArches relies on the parameterization and architecture chosen for the base model as well as the quality and quantity of reference data. To determine the sensitivity of scArches
reference mapping to the reference model used, we investigated how much reference data are needed to enable successful reference mapping. Therefore, we leveraged a human immune cell dataset composed of bone marrow and peripheral blood mononuclear cells (PBMCs). We built reference models of increasing quality by incrementally including more studies in reference building while using the rest of the studies as query data. To further challenge the model, we included a unique cell type for each study while removing it from the rest of the studies. In our experiments, the reference mapping accuracy of scArches scANVI substantially increased until at least 50% (~10,000 cells) of the data were used as reference (Fig. 3a–c). Specifically, we observed distinct clusters of megakaryocyte progenitors, human pluripotent stem cells, CD10^+ B cells and erythroid progenitors only in higher reference ratios (Fig. 3b,c), while these were mixed in the lowest reference fraction (Fig. 3a). This observation held true across other base models (Fig. 3d and Supplementary Fig. 4). We repeated similar experiments on brain and pancreas datasets (Supplementary Figs. 5 and 6). Overall, while performance is both model and data dependent, we observed a robust performance when at least 50% of the data, including multiple study batches, are used in reference training (Fig. 3d and Supplementary Figs. 7–10).

Reference mapping is designed to generate an integrated dataset without sharing raw data and with limited computational resources. Thus, it must be evaluated against the gold standard of de novo data integration, for which these restrictions are not present. To assess this, we performed scArches reference mapping using a reference model containing approximately two-thirds of batches and compared this to existing full integration autoencoder methods and other existing approaches. The overall score for the scArches reference mapping model is similar to that of de novo integration performance (Fig. 3e and Supplementary Figs. 13–15).

We also evaluated the speed of scArches reference mapping compared to full integration strategies. In an scArches pipeline, the reference model must either be built once and can be shared or it can be downloaded directly to map query datasets. Therefore, we...
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consider the time spent by the user to map query datasets as the relevant basis for our comparisons. The running time is also dependent on the base model type. For example, trVAE was much slower than other base models due to the maximum mean discrepancy term, while scVI and scANVI were the fastest (Supplementary Fig. 11a). Overall, scArches can offer a speed-up of up to approximately fivefold and eightfold for scVI and scANVI compared to that of running a de novo autoencoder-based integration for these methods (Supplementary Fig. 16a). This allows mapping of 1 million query cells in less than 1 h (Supplementary Fig. 11b).

scArches is sensitive to nuanced cell states. We further evaluated scArches under a series of challenging cases. A particular challenge for deep learning methods with many trainable parameters is the small data regime. Thus, we first tested the ability of scArches to map rare cell types. For this purpose, we subsampled a specific cell type in our pancreas and immune integration tasks (delta cells and CD16+ monocytes, respectively), such that this population constituted between ~0.1% and ~1.0% of the whole data. Next, we integrated one study as query data and evaluated the quality of reference mapping for the rare cell type. While in all cases the query cells are integrated with reference cells, rare cluster cells can be mixed with other cell types when the fraction is smaller than ~0.5%, and we only observed a distinct cluster for higher fractions (Supplementary Fig. 12).

Second, we evaluated our method on data with continuous trajectories. We trained a reference model using a pancreatic endocrinogenesis dataset from three early time points (embryonic day (E)12.5, E13.5 and E14.5). We integrated the latest time point (E15.5) as query data. Here query data integrated well with reference data, and our velocity analysis on the integrated data confirmed the known differentiation trajectory toward major alpha, beta, delta and epsilon fates (Supplementary Fig. 13).

Fig. 2 | TL and architecture surgery allow fast and accurate reference mapping. a–c, Comparing different granularity levels in the proposed TL strategy by mapping data from two brain studies to a reference brain atlas. The reference model was trained on a subset of 250,000 cells from two brain studies and then updated with data from Zeisel et al.35 and the TM brain subset. Fine-tuning strategies vary from training a few query study label weights (a) to input layers of both the encoder and decoder (b) and retraining the full network (c). d, Number of trained weights across these three granularity levels. e, Comparison of integration accuracy for different fine-tuning strategies on mapping data from two query studies on a brain reference atlas across various base models. Individual scores are minimum–maximum scaled between 0 and 1. Overall scores were computed using a 40:60-weighted mean of batch correction and bio-conservation scores, respectively (see Methods for further visualization details). EBM, entropy of batch mixing; PCR, principal-component regression.
The ultimate goal of query-to-reference mapping is to leverage and transfer information from the reference. This knowledge transfer can be transformative for analyzing new query datasets by transferring discrete cell type labels that facilitate annotation of query data or by imputing continuous information such as unmeasured modalities that are present in reference but absent from query measurements.

Finally, we evaluated how well scArches resolves nuanced, transcriptionally similar cell types in the query. We therefore trained a reference model excluding natural killer (NK) cells, while the reference data contained highly similar NKT cells. Integrated query and reference cells resulted in a separate NK cluster in proximity to NKT cells (Supplementary Fig. 14a). Repeating a similar experiment with both NK and NKT cells absent in the reference reproduced distinct clusters for both populations in the vicinity of each other (Supplementary Fig. 14b).

scArches enables knowledge transfer from reference to query. The ultimate goal of query-to-reference mapping is to leverage and transfer information from the reference. This knowledge transfer can be transformative for analyzing new query datasets by transferring discrete cell type labels that facilitate annotation of query data or by imputing continuous information such as unmeasured modalities that are present in reference but absent from query measurements.

We first studied transferring discrete information (for example, cell type labels) to query data. We used the recently published Tabula Seres as our reference, which includes 155 distinct cell types across 23 tissues and five age groups ranging from 1 month to 30 months from plate-based (SS2) and droplet-based (10x Genomics) assays. As query data, we used cells from the 3-month time point (equivalent to Tabula Muris (TM)).
Fig. 4 | scArches successfully transfers knowledge from reference to query. a, b, Querying TM (n=90,120) to the larger reference atlas Tabula Senis (n=264,287) using scArches trVAE (a) across different tissues (b). Tissues were correctly grouped across the two datasets (a,b). c, Location of misclassified and unknown cells after transferring labels from the reference to the query data. The highlighted tissue represents tracheal cells, which we removed from the reference data. d, Reported uncertainty of the transferred labels, which was low in correctly classified cells and high in the incorrect and unknown cells, particularly in the trachea. Box plots indicate the median (center lines) and interquartile range (hinges), and whiskers represents minimum and maximum values. Numbers of cells (n) are denoted above each box plot. e, Numbers of correct, incorrect and unknown cells across different tissues. f, Integration of scRNA-seq data (n=10,315) into the CITE-seq reference. g, Integration of scRNA-seq data (n=10,849) to the larger reference atlas Tabula Senis (reference). h, Imputation of missing proteins for the query dataset using the reference. BAT, brown adipose tissue; GAT, gonadal adipose tissue; MAT, mesenteric adipose tissue; SCAT, subcutaneous adipose tissue.

The query data consists of 90,120 cells from 24 tissues including a previously unseen tissue, trachea, which we excluded from the reference data. scArches trVAE accurately integrates query and reference data across time points and sequencing technologies and creates a distinct cluster of tracheal cells (n=9,330) (Fig. 4a,b and Supplementary Fig. 15a; see Supplementary Fig. 16 for tissue-level data).

We then investigated the transfer of cell type labels from the reference dataset. Each cell in the query TM was annotated using its closest neighbors in the reference dataset. Additionally, our classification pipeline provides an uncertainty score for each cell while reporting cells with more than 50% uncertainty as unknown (Methods). scArches achieved ~84% accuracy across all tissues (Fig. 4c). Moreover, most of the misclassified cells and cells from the unseen tissue received high uncertainty scores (Fig. 4d and Supplementary Fig. 15b). Overall, classification results across tissues indicated a robust prediction accuracy across most tissues (Fig. 4e), while highlighting cells that were not mappable to the reference. Therefore, scArches can successfully merge large and complex query datasets into reference atlases. Notably, we used scArches to map a large query (the mouse cell atlas) onto TM and further onto a recently published human cell landscape (HCL) reference, demonstrating applicability to study similarity of cell types across species (Supplementary Note 1 and Supplementary Figs. 18–21). Overall, scArches-based label projection performs competitively when compared with state-of-the-art methods such as SVM rejection and logistic regression classifiers (Supplementary Fig. 17).

In addition to the label transfer, one can use reference atlases to impute continuous information in the query data such as missing antibody panels in RNA-seq-only assays. Indeed, one can combine scArches with existing multimodal integration architectures such as totalVI, a model for joint modeling of RNA expression and surface protein abundance in single cells. Leveraging scArches totalVI, we
built a cellular indexing of transcriptomes and epitopes by sequencing (CITE-seq)11 reference using two publicly available PBMC datasets (Fig. 4f). Next, we integrated query scRNA-seq data into the reference atlas (Fig. 4g) and used the multimodal reference atlas to impute missing protein data for the query dataset. Using imputed protein abundances, we can distinguish the observed major populations such as T cells (CD3+, CD4+ and CD8+), B cells (CD19+) and monocytes (CD14+) (Fig. 4h) (see Supplementary Fig. 22 for all proteins).

Preserving COVID-19 cell states after reference mapping. In the study of disease, contextualization with healthy reference data is essential. A successful disease-to-healthy data integration should satisfy three criteria: (1) preservation of biological variation of healthy reference and disease query; and (2) preservation of distinct disease variation, such as the emergence of new cell types that are unseen during healthy reference building. To showcase how one can perform disease contextualization with scArches, we created a reference aggregated from bone marrow6, PBMCs7–9 and normal lung tissue10–12 (n = 154,723; Fig. 5a–c) and then mapped onto it a dataset containing alveolar macrophages and other immune cells collected via bronchoalveolar lavage from (1) healthy controls and patients with (2) moderate and (3) severe COVID-19 (n = 62,469)13. As described by Liao and colleagues, this dataset contains immune cells found in the normal lung (for example, tissue-resident alveolar macrophages, TRAMs) as well as unique populations that are absent in the normal lung and emerge only during inflammation (for example, monocyte-derived alveolar macrophages, MoAMs)14. We used a negative binomial (NB) CVAE base model for this experiment (Methods).

We first evaluated the integration of query batches in the reference. scArches successfully integrated alveolar macrophages from different datasets and preserved biological variability between them, although some ambient RNA signals remained (Supplementary Note 2 and Supplementary Fig. 23). For example, activated TRAMs (FABP4+IL1B+CXCL5+) that originate from a single individual (donor 2 in the Travaglini et al.52 dataset) formed a distinct subcluster within TRAMs (Fig. 5a–d). We then evaluated the projection of COVID-19 query data onto the reference model. The dataset from Liao and colleagues contains the following cell types: airway epithelial cells, plasma cells and B cells, CD4+ and CD8+ T cells, NK cells, neutrophils, mast cells, dendritic cells, monocytes and alveolar macrophages (Fig. 5b,c and Supplementary Fig. 24)15. Within the macrophage cluster (characterized by the expression of C1QA), two distinct populations dominated the structure of the embedding (Fig. 5c,d): TRAMs (FABP4+C1QA+CCL2+) and inflammatory MoAMs (FABP4+C1QA+CCL2-). As expected, query TRAMs from healthy controls integrated well with TRAMs from the reference dataset. While TRAMs from patients with moderate COVID-19 integrated with TRAMs from control lung tissue, they did not mix with normal TRAMs completely, as they were activated and characterized by increased expression of IFI27 and CXCL10. MoAMs are predominantly found in samples from patients with severe COVID-19.
and to a lesser extent in samples from patients with moderate COVID-19. MoAMs originate from monocytes that are recruited to sites of infection (as illustrated by the gradient of C1QA expression) and thus do not appear in healthy reference tissue. Indeed, MoAMs were embedded in closer proximity to monocytes than to TRAMs in our embedding, reflecting their ontological relationship (see Supplementary Fig. 25 for partition-based graph abstraction proximity analysis).

We then evaluated CD8+ T cells. While the reference bone marrow and blood cells predominantly contained naive CD8+ T cells (CCL5+), lung and bronchoalveolar lavage fluid contained cytotoxic memory CD8+ T cells (GZMA+GZMH+; Fig. 5e and Supplementary Fig. 26). Moreover, cytotoxic memory CD8+ T cells from patients with COVID-19 were characterized by the expression of interferon-response genes ISG15, MX1 and others, which is in agreement with a recent report that the interferon response is a feature separating severe acute respiratory syndrome coronavirus 2 pneumonia from other viral and non-viral pneumonias (Fig. 5e, Supplementary Note 2 and Supplementary Fig. 26).

Overall, the scArches joint embedding was dominated by nuanced biological variation, for example, macrophage subtypes, even when these subtypes were not annotated in reference datasets (for example, activated TRAMs from patients with moderate COVID-19 or a patient with a lung tumor). Although disease states were absent in the reference data, scArches separated these states from the healthy reference and even preserved biological variation patterns. Hence, disease-to-healthy integration with scArches met all three criteria for successful integration.

Discussion

We introduced architectural surgery, an easy-to-implement approach for TL, reusing neural network models by adding input nodes and weights (adaptors) for new studies and then fine tuning only those parameters. Architectural surgery can extend any conditional neural network-based data-integration method to enable decentralized reference updating, facilitate model reuse and provide a framework for learning from reference data.

In applications, we demonstrated how integration of whole-species atlases enables the transfer of cell type annotations from a reference to a query atlas. We further showed that COVID-19 query data can be mapped on top of a healthy reference while retaining variation among both disease and healthy states, which we promote in scArches by avoiding showing the method the disease effect during training. In general, different effects such as disease states are assumed to be orthogonal in high-dimensional space; thus, if a batch-confounded effect (for example, any donor-level covariate when donor is used as batch) is not seen during training, we would not expect it to be removed. We observe this phenomenon in our COVID-19 example and in multiple experiments: biologically meaningful variations from held-out alpha cells in the pancreas (Fig. 1d,e) or unseen nuanced cell identities in immune cell data (Supplementary Fig. 14) are mapped to a new location when they are unseen during training.

The reduction in model training complexity by training adaptors moreover leads to an increase in speed while preserving integration accuracy when compared to full integration methods. It also improves usability and interpretability, because mapping a query dataset to a reference requires no further hyperparameter optimization and keeps reference representation intact. Adaptors only impact the first network layer and therefore ‘commute’: application order is irrelevant for iteratively expanding a reference, arriving always at the same result due to the frozen nature of the network and independence of adaptor weights. With scArches, one can therefore use pre-trained neural network models without computational expertise or graphics processing unit power to map, for example, disease data onto stored reference networks prepared from independent atlases. We make use of these features by providing a model database on Zenodo (Methods).

Model sharing in combination with reference mapping via scArches allows users to create custom reference atlases by updating public ones and paves the way for automated and standardized analyses of single-cell studies. Especially for human data, sharing expression profiles is often difficult due to data-protection regulations, size, complexity and other organizational hurdles. With scArches, users can obtain an overview of the whole dataset to validate harmonized cell type annotation. By sharing a pre-trained neural network model that can be locally updated, international consortia can generate a joint embedding without requiring access to the full gene sets. In turn, users can quickly build upon this by mapping their own typically much smaller data into the reference, acquiring robust latent spaces, cell type annotation and identification of subtle state-specific differences with respect to the reference.

scArches is a tool that leverages existing conditional autoencoder models to perform reference mapping. Thus, by design, it inherits both benefits and limitations of the underlying base models. For example, a limitation of these models is that the integrated output is a low-dimensional latent space instead of a corrected feature matrix as provided by mmnCorrect or Scanorama. While generating a batch-corrected input matrix is possible, this may lead to spurious and false signals similar to denoising methods. Similarly, imputation of modalities not measured in query data (for example, scArches totalVI) performs better for more abundant features, which has already been outlined in the original totalVI publication. A further limitation is the need for a sufficiently large and diverse set of samples for reference building. Deep learning models typically have more trainable parameters than other integration methods and thus often require more data. This constraint translates directly to the performance of scArches reference mapping (Fig. 3a–d): using a small reference along with a low number of studies leads to poor integration of query data while removing biological variation such as nuanced cell types. Furthermore, even with equal training data, reference model performance will differ, affecting reference mapping via scArches. As robust and scalable reference building is still ongoing research in the scRNA-seq field, the choice of reference model is a central challenge when using scArches. Yet we demonstrate that even imperfect reference models (Supplementary Note 3) can be used for meaningful analyses as demonstrated by our data analysis of patients with COVID-19. Finally, one must consider the limitations of the base model on batch-effect removal during reference mapping, in which it is unlikely to remove stronger batch effects than those seen in the training data. In our cross-species experiments, reference mapping performs well mostly in immune cell populations, which appear to contain the smallest batch effect across species (Supplementary Figs. 18, 20 and 21).

While scArches is applicable in many scenarios, it is best suited when the query data consists of cell types and experimental protocols similar to the reference data. Then, the query data may easily contain new cell types or states such as disease or other kinds of perturbations, which are preserved after mapping. Additionally, we advise against using scArches for integrating query data with a reference created out of a single study and recommend integration with full sample access instead. Further, the number of overlapping genes between query and reference data can also influence integration quality. We generally recommend using a larger set of highly variable genes (HVGs) in the reference-building step to guarantee a bigger feature overlap between reference and query, which increases the robustness of reference mapping in the presence of missing genes (Methods and Supplementary Fig. 28).

We envision two major directions for further applications and development. First, scArches can be applied to generate context-specific large-scale disease atlases. Large disease reference datasets are increasingly becoming available. By mapping
between disease references, we can assess the similarity of these diseases at the single-cell level and thus inform for finding mechanisms, reversing disease state or studying perturbations, for example, for drug repurposing. The suitability of model organisms for disease research can be directly translated into the human context: for example, projecting mouse single-cell tumor data on a reference human patient tumor atlas may help to identify accurate tumor models that include desired molecular and cellular properties of a patient's microenvironment. Incorporating additional covariates as conditional neurons in the reference model will allow modeling of treatment response with a certain perturbation or drug. Secondly, we envision assembling multimodal single-cell reference atlases to include epigenomic, chromosome conformation, proteome and spatially resolved measurements.

In summary, with the availability of reference atlases, we expect sCArches to accelerate the use of these atlases to analyze query datasets.

Online content
Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41587-021-01001-7.

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Methods

Architecture surgery. Our method relies on a concept known as TL. TL is an approach in which weights from a model trained on one task are taken and used as weight initialization or fine tuning for another task. We introduce an architecture surgery, a strategy to apply TL in the context of conditional generative models and single-cell data. Our proposed method is general and can be used to perform TL on both CVAEs and conditional generative adversarial nets.

We assume that we want to train a reference CVAE model with a d-dimensional dataset (x ∈ Rd) from n different studies (x ∈ Rd), where R denotes real number space. We further assume that the bottleneck z with layer size is k ∈ ℜd. Then, an input for a single cell will be x′ = x - x′, where x and s are the d-dimensional gene expression profile and n-dimensional one-hot encoding of study labels, respectively. The symbol denotes the row-wise concatenation operation. Therefore, the model receives (d + n) -dimensional and (k + n) -dimensional vectors as inputs for encoder and decoder, respectively. Assuming m query datasets, the target model will be initialized with all the parameters from the reference model. To incorporate m new study labels, we add m new dimensions to s in both encoder and decoder networks. We refer to these new added study labels as s′. Next, m newly randomized initialized weight vectors are also added to the first layer of the encoder and decoder. Finally, we fine tune the new model by only training the weights connected to the last m dimensions of s′ that correspond to the condition labels. Let us assume that p and q are the number of neurons in the first layer of the encoder and decoder; then, during the fine tuning, only (m)(p + q) parameters will be trained. Let us parameterize the first layer of the encoder and decoder part of the scArches as φ and g0, respectively. Let us further assume that ReLU activations are used in the layers. Therefore the equations for f1, and g1, are

\[ f_1(x, s, \phi_1, s_1, \phi_1) = \max(0, \theta_1 x + \phi_1^T s + \theta_1^T s) \]

\[ g_1(z, s, \theta_1, \phi_1, \theta_1) = \max(0, \theta_1 z + \phi_1 s + \theta_1^T s) \]

where \( \phi \) and \( \theta \) are parameters of encoder and decoder, and \( T \) denotes transpose operation. Therefore, the gradients of \( f_1 \) with respect to \( \phi \) and \( \theta \) are

\[ \nabla_{\phi, f_1} = \begin{cases} 0 & \text{if } \theta_1^T x + \phi_1^T s + \theta_1^T s < 0 \\ s' & \text{otherwise} \end{cases} \]

\[ \nabla_{\theta, g_1} = \begin{cases} 0 & \text{if } \theta_1^T z + \phi_1 s + \theta_1^T s < 0 \\ s' & \text{otherwise} \end{cases} \]

Finally, because all other weights except \( \phi \) and \( \theta \) are frozen, we only compute the gradient of scArches' cost function with respect to \( \phi \) and \( \theta \):

\[ \nabla_{\phi, L_{\text{VAE}}}(X, S, \theta) = \nabla_{\phi, L_{\text{VAE}}}(X, S, \theta) \cdot \nabla_{\phi, f_1}(x, s, \phi_1, s_1, \phi_1) \]

\[ \nabla_{\theta, L_{\text{VAE}}}(X, S, \theta) = \nabla_{\theta, L_{\text{VAE}}}(X, S, \theta) \cdot \nabla_{\theta, g_1}(x, s, \theta_1, \phi_1, \theta_1) \]

scArches base models. Conditional variational autoencoders. Variational autoencoders (VAEs) were shown to learn the underlying complex structure of data. VAEs were proposed for generative modeling of the underlying data leveraging variational inference and neural networks to maximize the following equation:

\[ p_{\theta}(X | S) = \int p_{\gamma}(X | Z, S) p_{\delta}(Z | S) dZ \]

where \( X \) is a random variable representing the model’s input, \( S \) is a random variable indicating various conditions, \( \theta \) is the neural network parameters, and \( p_{\phi}(X | Z, S) \) is the output distribution that we sample \( Z \) to reconstruct \( X \). In the following equation, we exploit notations from ref. 29 and a tutorial from ref. 69. We further assume that the bottleneck \( z \) with layer size \( k \in \mathbb{R}^d \). Then, an input for a single cell will be \( x' = x - x' \), where \( x \) and \( s \) are the \( d \)-dimensional gene expression profile and \( n \)-dimensional one-hot encoding of study labels, respectively. The symbol denotes the row-wise concatenation operation. Therefore, the model receives \((d + n)\)-dimensional and \((k + n)\)-dimensional vectors as inputs for encoder and decoder, respectively. Assuming \( m \) query datasets, the target model will be initialized with all the parameters from the reference model. To incorporate \( m \) new study labels, we add \( m \) new dimensions to \( s \) in both encoder and decoder networks. We refer to these new added study labels as \( s' \). Next, \( m \) newly randomized initialized weight vectors are also added to the first layer of the encoder and decoder. Finally, we fine tune the new model by only training the weights connected to the last \( m \) dimensions of \( s' \) that correspond to the condition labels. Let us assume that \( p \) and \( q \) are the number of neurons in the first layer of the encoder and decoder; then, during the fine tuning, only \((m)(p + q)\) parameters will be trained. Let us parameterize the first layer of the encoder and decoder part of the scArches as \( \phi \) and \( g_0 \), respectively. Let us further assume that ReLU activations are used in the layers. Therefore the equations for \( f_1 \) and \( g_1 \) are

\[ f_1(x, s, \phi_1, s_1, \phi_1) = \max(0, \phi_1^T x + \phi_1^T s + \theta_1^T s) \]

\[ g_1(z, s, \theta_1, \phi_1, \theta_1) = \max(0, \theta_1 z + \phi_1 s + \theta_1^T s) \]

where \( \phi \) and \( \theta \) are parameters of encoder and decoder, and \( T \) denotes transpose operation. Therefore, the gradients of \( f_1 \) with respect to \( \phi \) and \( \theta \) are

\[ \nabla_{\phi, f_1} = \begin{cases} 0 & \text{if } \phi_1^T x + \phi_1^T s + \theta_1^T s < 0 \\ s' & \text{otherwise} \end{cases} \]

\[ \nabla_{\theta, g_1} = \begin{cases} 0 & \text{if } \theta_1^T z + \phi_1 s + \theta_1^T s < 0 \\ s' & \text{otherwise} \end{cases} \]

Finally, because all other weights except \( \phi \) and \( \theta \) are frozen, we only compute the gradient of scArches’ cost function with respect to \( \phi \) and \( \theta \):

\[ \nabla_{\phi, L_{\text{VAE}}}(X, S, \theta) = \nabla_{\phi, L_{\text{VAE}}}(X, S, \theta) \cdot \nabla_{\phi, f_1}(x, s, \phi_1, s_1, \phi_1) \]

\[ \nabla_{\theta, L_{\text{VAE}}}(X, S, \theta) = \nabla_{\theta, L_{\text{VAE}}}(X, S, \theta) \cdot \nabla_{\theta, g_1}(x, s, \theta_1, \phi_1, \theta_1) \]

scArches scVI. Lopez et al.67 developed a fully probabilistic approach, called scVI, for normalization and analysis of scRNA-seq data. scVI is also based on a CVAE, described in detail above. But, in contrast to the trVAE architecture, the decoder assumes a zero-inflated negative binomial (ZINB) distribution; and therefore the reconstruction loss differs to the MSE loss of trVAE. Another major difference is that scVI explicitly models the library size, which is needed for the ZINB loss calculation with another shallow neural network called the library encoder. Therefore, with similar notation as above, we have the output distribution \( p(X | Z, S, L) \), where \( L \) is the scaling factor that is sampled by the outputs of the library encoder, namely the empirical mean \( L_s \) and the variance \( L_v \) of the log library per batch:

\[ L \sim \text{lognormal}(L_s, L_v^2) \]

When we now separate the outputs of the decoder \( g_0 \) into \( g_0^L \), the decoded mean proportion of the expression data, and \( g_0^D \), the decoded dropout effects, we can write the ZINB mass function for \( p(X | Z, S, L) \) in the following closed form:

\[ p(X = 0 | Z, S, L) = g_0^D(Z, S) \cdot \left( \frac{\gamma}{\gamma + g_0^L(Z, S)} \right)^X \]

\[ p(X = Y | Z, S, L) = \frac{1}{Y} \Gamma(Y + X) \cdot \left( \frac{\gamma}{\gamma + g_0^L(Z, S)} \right)^X \cdot \left( \frac{X}{\gamma + g_0^L(Z, S)} \right)^Y \]

where \( \Sigma \) is the gene-specific inverse dispersion, \( \Gamma \) is the gamma function, and \( Y \) represents non-zero entries drawn from a ZINB distribution. Because the evidence lower bound and therefore the optimization objective can be calculated by applying the reparameterization trick and supposing Gaussians, which is possible here because of the proposed ZINB distribution, we can write the scVI cost function as follows:

\[ L_{\text{scVI}}(X, S, \theta) = L_{\text{VAE}}(X, S, \theta) - \alpha \cdot D_{\text{KL}}(q_\theta(L | X, S) || p (L)) \]

Furthermore, because of the applied reparameterization trick, an automatic differentiation operator can be used, and the cost function can be optimized by applying stochastic gradient descent. For the application in scArches, we removed

\[ \alpha \]

\[ \alpha \]

\[ \alpha \]
the library encoder and computed the library size for each batch in a closed form by summing up the counts. This does not decrease the performance of the model and accelerates the surgery step. The resulting network can then be used similarly to the tVAE network by simply retraining only the condition weights corresponding to the new batch annotations in S.

scArches scANVI. scANVI is a semi-supervised method that builds up on the scVI model and was proposed in detail by Xu et al.1. By constructing a mixture model, it is able to make use of any cell type annotations during autoencoder training to improve latent representation of the data. In addition to this, scANVI is capable of labeling datasets with only some marker gene labels as well as transferring labels from a labeled dataset to an unlabeled dataset. For the training of scANVI, the authors proposed an alternating optimization of the cost function $L_{scANVI}(X, S, C, \theta)$ and the classification loss $C$, which results from a shallow neural network that serves as a classifier with a cross-entropy loss after the last softmax layer. In more detail, the cost function can be formulated in the following manner:

$$L_{scANVI}(X, S, C, \theta) = L_{latent}(X, S, C, \theta) + L_{unlabeled}(X, S, \theta),$$

where $C$ is the cell types in the annotated datasets, and both cost function summands $L_{total}$ and $L_{unlabeled}$ are obtained by similar calculations as in the case of scVI. The major difference here, however, is that the Kullback–Leibler divergence is applied to an additional latent encoder that takes cell type annotations into account. For the unlabeled case, each sample is broadcasted into every available cell type. As scANVI builds up on scVI, we use the same adjustments here to apply common scArches totalVI. For the purpose of combining paired measurement of RNA and surface proteins from the same cells, such as for scITE-seq data, Gayoso et al.3 presented a deep generative model called totalVI. totalVI learns a joint low-dimensional probabilistic representation of RNA and protein measurements. For the RNA portion of the data, totalVI uses an architecture similar to that of scVI, which we discussed in detail above; but, for proteins, a new model is introduced that separates protein information into background and foreground components. With the surgery functionality of scArches added to totalVI, it is now possible to learn a joint latent space of RNA and protein data. The CV AE reference dataset and do surgery on a query dataset with only RNA data to impute protein data for that query dataset as well. To accomplish this goal, we again only retrain the weights that correspond to the new batch labels.

CVAEs for single-cell genomics. CVAEs were first applied to scRNA-seq data in CVAEs for single-cell genomics. retrain the weights that correspond to the new batch labels. reference dataset and do surgery on a query dataset with only RNA data to impute scArches totalVI. be near to the corresponding reference cells in the latent representation. As scANVI builds up on scVI, we use the same adjustments here to apply account. For the unlabeled case, each sample is broadcasted into every available cell type. As scANVI builds up on scVI, we use the same adjustments here to apply common scArches totalVI. For the purpose of combining paired measurement of RNA and surface proteins from the same cells, such as for scITE-seq data, Gayoso et al.3 presented a deep generative model called totalVI. totalVI learns a joint low-dimensional probabilistic representation of RNA and protein measurements. For the RNA portion of the data, totalVI uses an architecture similar to that of scVI, which we discussed in detail above; but, for proteins, a new model is introduced that separates protein information into background and foreground components. With the surgery functionality of scArches added to totalVI, it is now possible to learn a joint latent space of RNA and protein data. The CV AE reference dataset and do surgery on a query dataset with only RNA data to impute protein data for that query dataset as well. To accomplish this goal, we again only retrain the weights that correspond to the new batch labels.

Next, we define $U$, a uniform random variable on the cell population. Let $B_i$ be the frequencies of 15 nearest neighbors for the cell $U$ in batch $x$. We report the entropy of this variable and then average across $T=100$ measurements of $U$. To normalize the entropy of the batch mixing score between 0 and 1, we set the base of the logarithm to the number of batches $c$.

Average silhouette width. Silhouette width measures the relationship between within-cluster distances of a cell and between-cluster distances of that cell to the closest cluster. In general, an ASW score of 1 implies clusters that are well separated, an ASW score of 0 implies overlapping clusters, and an ASW score of −1 implies strong misclassification. When we use the ASW score as a measure of biological variance, we calculate it on cell types in the following manner:

$$ASW = \frac{ASW + 1}{2},$$

where the final score is already scaled between 0 and 1. Therefore larger values correspond to denser clusters. In contrast to the ASW score, we also calculate an ASW score on batches within cell clusters to obtain a measure for batch-effect removal. In this case, we again scale but also invert the ASW score to have a consistent metric comparison:

$$ASW_{\pi} = 1 - \text{abs}(ASW).$$

A higher final score here implies better mixing and therefore a better batch-removal effect.

Normalized mutual information. We use NMI to compare the overlap of two different cell type clusterings. In detail, we computed a Louvain clustering on the latent representation of the data and compared it to the latent representation itself in a cell type-wise manner. To obtain scores between 0 and 1, the overlap was scaled using the mean of entropy terms for cell type and cluster labels. Therefore an NMI score of 1 corresponds to a perfect match and good conservation of biological variance, whereas an NMI score of 0 corresponds to uncorrelated clustering.

Adjusted Rand index. This metric considers correct clustering overlaps as well as counting correct disagreements between two clusterings. Again, similar to NMI, cell type labels in the integrated dataset are compared with Louvain clustering. The adjusted Rand index score is normalized between 0 and 1, where 1 corresponds to good conservation of biological variance and 0 corresponds to random labeling.

Principal-component regression. In contrast to principal-component analysis (PCA), we calculate a principal $R$ regression with $R$ to the batch label onto each principal component. The total variance (Var) explained by the batch variable can then be formulated as follows:

$$\text{Var}(X|B) = \sum_{i=1}^{N} \text{Var}(X|PC_i) - R^2(\text{PC}_i|B),$$

where $\text{pi}=\text{no. cells with batch }i \text{ in the region}$,

$$\text{no. cells in the region}.$$
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where $X$ is the data matrix, $B$ is the batch label, and $N$ is the number of principal components (PC).

Graph connectivity: For this metric, we calculate a subset kNN graph $G(N, E)$ for each cell type label $c$, such that each subset only contains cells from the given label. The total graph connectivity score can then be calculated as follows:

$$g_c = \frac{1}{|C|} \sum_{c \in C} \frac{|\text{LCC}(G(N_c, E_c))|}{|N_c|},$$

where $C$ is the set of cell type labels, $|\text{LCC}()|$ is the number of nodes in the largest connected component of the graph, and $|N_c|$ is the number of nodes with the given cell type label. This means that we check if the graph representation of the latent representation connects all cells with the same cell type label. Therefore, a score of 1 would imply that all cells with the same cell type label are connected, which would further indicate good batch mixing. A graph in which no cells are connected would result in a score of 0.

Isolated label silhouette. For this metric, we use $ASW_c$, defined above, but only on the isolated label subset of the latent representation. Scaling and meaning of the score are the same as described for ASW. If there are multiple isolated labels, we average over each score similar to the isolated labeled $F_c$ score.

kNN accuracy. We first compute the 15 nearest neighbors of each cell in the data. We then compute the ratio of the correct cell type annotations inside those 15 neighbors. This cell-wise score is then averaged over all cell types separately and then averaged over all remaining scores again to obtain a single kNN-accuracy score between 0 and 1. A higher kNN-accuracy score corresponds to better preservation of local cell type purity. This metric was inspired by a similar metric used in scANVI.

Visualization of integration scores. To compare performances of different models, we designed an overview table (inspired by Saalens et al.24) that displays individual integration scores as circles and aggregated scores as bars. Each individual score is minimum–maximum scaled to improve visual comparison of different models and then averaged into aggregated scores by category (batch correction and biological conservation). Finally, an overall score is calculated as a weighted sum of batch correction and bio-conservation, considering a ratio of 40:60, respectively. When shown, reference and query times are not considered in the calculation of aggregated scores. Moreover, these time values are scaled together to allow direct comparison. The overall ranking of each model, for each score, is represented by the color scheme.

Datasets. All cell type labels and metadata were obtained from original publications unless specifically stated otherwise below.

Brain data. The mouse brain dataset is a collection of four publicly available single-cell RNA-seq mouse brain studies25-27, for which additional information on cerebral regions was provided. We obtained the raw count matrix from Rosenberg et al.28, under GEO accession ID GSE110823, the annotated count matrix from Zeisel et al.29 from https://mousebrain.org (file name 1.5_allloom, downloaded on 9 September 2019) and count matrices per cell type from Saunders et al.30 from http://dropviz.org (DGE by region section, downloaded on 30 August 2019). Data from mouse brain tissue sorted by flow cytometry (myeloid and non-myeloid cells, including the annotation file annotations_FACS.CSV) from TM were obtained from https://figshare.com (retrieved 14 February 2019). We harmonized cluster labels via fuzzy string matching and attempted to preserve the original annotation as far as possible. Specifically, we annotated ten major cell types (neuron, astrocyte, oligodendrocyte, oligodendrocyte precursor cell, endothelial cell, brain pericyte, ependymal cell, olfactory ensheathing cell, macrophage and microglia). In the case of Saunders et al.30, we facilitated the additional annotation data table for 585 reported cell types (annotation.BrainCellAtlasSaundersversion2018.04.01.TXT retrieved from http://dropviz.org on 30 August 2019). Among these, some cell types were annotated as 'endothelial tip', 'endothelial stalk' and 'mural'. We examined the subset of the Saunders et al.30 dataset as follows: we used Louvain clustering (default resolution parameter, 1.0) to cluster, followed by gene expression profiling via the rankgene groups function in scanny. Using marker gene expression, we assigned microglia (Glia), oligodendrocytes (Plip), astrocytes (Glap, Gia) and endothelial cells (Htl) to the subset. Finally, we applied mean normalization and log(counts + 1) to transform count matrices. In total, the dataset consists of 978,734 cells.

Pancreas. Five publicly available pancreatic islet datasets31-33, with a total of 15,681 cells in raw count matrix format were obtained from the Scanorama34 dataset, which has already assigned its cell types using batch-corrected gene expression by Scanorama. The Scanorama dataset was downloaded from http://scanorama.cslai.mit.edu/data.tar.gz. In the preprocessing step, raw count datasets were normalized and log transformed by scanny preprocessing methods. Preprocessed data were used directly for the pipeline of scArches. One thousand HVGs were selected for training model.

The human cell landscape. The HCL dataset was obtained from https://figshare.com/articles/HCL_DGE_Data/7235471. RAW count matrix data for all tissues were aggregated. A total of 277,909 cells were selected and processed using the scanny Python package. Homologous genes were selected using BioMart 100 before merging with HCL data. Data were normalized together with HCL as explained before.

Immune data. The immune dataset consists of ten human samples from two different tissues: bone marrow and peripheral blood. Data from bone marrow samples were retrieved from Oetjen et al.35, while data from peripheral blood samples were obtained from 10x Genomics (https://support.10xgenomics.com/single-cell-gene-expression/datasets/3.0.0/plmc_10k_v3) and Freytag et al.36, Sun et al.37 and Villani et al.38. Details on the retrieval location of datasets, the different protocols used and ways in which samples were chosen for analysis can be found in Luecken et al.39. We performed quality control separately for each sample but adopted a common strategy for normalization: all samples for which count data were available were individually normalized by scan pooling40. This excludes data from Villani et al.38, which included only TPM values. All datasets were log+1 transformed in scanny. Cell type labels were harmonized starting from existing annotations (Oetjen et al.35) to create a consistent set of cell identities. Well-known markers of cell types were collected and used to extend annotation to samples for which they were not previously available. When necessary, subclustering was performed to derive more precise labeling. Finally, cell populations were removed if no label could be assigned. Four thousand HVGs were selected for training.

Endocrine pancreas. The raw dataset of pancreatic endocrinogenesis (n = 22,163)41 is available at the GEO under accession number GSE132188. We considered a subset of 2,000 HVGs for training. Cell type labels were obtained from an adad object provided by the authors of scVelo42.

CITE-seq. We obtained three publicly available datasets from 10x Genomics, already curated and preprocessed as described in the totalVI study43. These datasets include 10k PBMCs from a Healthy Donor—Gene Expression and Cell Surface Protein (PBMC, 10k (CITE-seq))44, 5k PBMCs from a healthy donor with cell surface proteins (v3 chemistry)45 (PBMC, 5k (CITE-seq))46 and 10k PBMCs from a Healthy Donor (v3 chemistry) (PBMC, 10k (RNA-seq))47. Reference data included 14 proteins, and 4,000 HVGs were selected for training.

COVID-19. The COVID-19 dataset along with its metadata was downloaded from https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE1459261 and https://github.com/zhangleib/COVID_b alf. The dataset that was used in this paper includes n = 62,469 cells. Data from lungs48,49, bone marrow50,51 and bone marrow52,53 were later merged with those from COVID-19 samples. Data were normalized using scanny, and 2,000 HVGs were selected for training the model. Cell type labels were obtained from the original study.

Tabula Muris Senis. The TM Senis dataset with GEO accession number GSE132042 is publicly available at https://figshare.com/projects/Tabula_Muris_Senis/645982. The dataset contains 356,213 cells with cell type, tissue and method annotation. We normalized the data using size factor normalization with 10,000 counts for each cell. Next, we log+1 transformed the dataset and selected 5,000 HVGs according to their average expression and dispersion. All preprocessing steps were carried out using the scanny Python package. In this study, we used a combination of sequencing technology and time point as batch covariates.
Therefore, we labeled each cell in the query dataset as follows: out-of-distribution cells with new labels, which do not exist in the training data. We calculated the uncertainty where \( \text{dist}(y, \text{neighbors}) \) for each cell \( y \) and its neighbors \( n \) in the latent space. Next, we applied the Gaussian kernel to distances using the nearest distances:

\[
d_{A,N} = \frac{\text{dist}(c, n)}{\text{std}(\text{dist}(c, n))}
\]

where \( \text{dist}(c, n) \) is the Euclidean distance of the query cell \( c \) and its neighbors \( n \) in the latent space. Next, we applied the Gaussian kernel to distances using

\[
d_{A,N} = \frac{\text{dist}(c, n)}{\text{std}(\text{dist}(c, n))}
\]

Next, we computed the probability of assigning each label \( y \) to the query cell \( c \) by normalizing across all adjusted distances using

\[
p(Y = y | X = c, N) = \frac{\sum_{n \in N} R(y, n) = y \cdot D_{A,N}}{\sum_{n \in N} D_{A,N}}
\]

where \( R(y, n) \) is the label of \( n \)’s nearest neighbor and \( l \) is the indicator function. Finally, we calculated the uncertainty \( u \) for each cell \( c \) in the query dataset using its set of closest neighbors in the reference dataset \( N \). We defined the uncertainty \( u(c, N) \) for a query cell \( c \) with label \( y \) and \( N \) as its set of nearest neighbors as

\[
u(c, N) = 1 - p(Y = y | X = c, N)
\]

We reported cells with more than 50% uncertainty as unknown to detect out-of-distribution cells with new labels, which do not exist in the training data. Therefore, we labeled each cell in the query dataset as follows:

\[
y' = \text{argmin}_{y \in N} u(c, N)\]

\[
y' = \begin{cases} y' & \text{if } u(c, N) \leq 0.5 \\ \text{unknown} & \text{otherwise} \end{cases}
\]

### Code availability
Software is available at https://github.com/theislab/scarches. The code to reproduce the results is available at https://github.com/theislab/scarches-reproducibility.

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M.I. designed visualizations and curated the immune dataset. M.I. and M.D.L. analyzed the COVID-19 dataset with help from A.V.M. A.G. and N.Y. contributed by adapting scArches with scvi-tools. F.J.T. supervised the research. All authors wrote the manuscript.

Competing interests
F.J.T. reports ownership interest in Cellarity. N.Y. is an advisor and/or has equity in Celsius Therapeutics and Rheos Medicines. The remaining authors declare no competing interests.

Additional information
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