

1 scGPT: Towards Building a Foundation Model for Single-Cell
2 Multi-omics Using Generative AI

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10 **Abstract**

11 Generative pre-trained models have achieved remarkable success in various domains such as nat-
12 ural language processing and computer vision. Specifically, the combination of large-scale diverse
13 datasets and pre-trained transformers has emerged as a promising approach for developing founda-
14 tion models. While texts are made up of words, cells can be characterized by genes. This analogy
15 inspires us to explore the potential of foundation models for cell and gene biology. By leveraging the
16 exponentially growing single-cell sequencing data, we present the first attempt to construct a single-
17 cell foundation model through generative pre-training on over 10 million cells. We demonstrate that
18 the generative pre-trained transformer, scGPT, effectively captures meaningful biological insights
19 into genes and cells. Furthermore, the model can be readily finetuned to achieve state-of-the-art
20 performance across a variety of downstream tasks, including multi-batch integration, multi-omic
21 integration, cell-type annotation, genetic perturbation prediction, and gene network inference. The
22 scGPT codebase is publicly available at <https://github.com/bowang-lab/scGPT>.

23 **1 Main**

24 Generative pre-trained models have recently achieved unprecedented success in many domains. The
25 most well-known applications include computer vision and natural language generation (NLG) [44,
26 43, 45]. These foundation models such as DALL-E2 and GPT-4 follow a similar paradigm of pre-
27 training transformers on large-scale diverse datasets [43, 45]. These foundation models can be
28 readily tailored to a variety of downstream tasks and scenarios. More interestingly, they demon-
29 strate improved performance on multiple tasks compared to task-specific models trained from
30 scratch [22, 58, 47]. This showcases strong evidence of a task-agnostic and “deep” understanding

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31 of knowledge in these domains. Despite the success of foundation models in other domains, currently
32 machine-learning based discovery in single-cell research is rather distributed, with specific
33 models dedicated to specific analysis tasks [32, 42, 27]. Often the breadth and scale of the datasets
34 used in each study are also limited, due to sequencing capacity as well as the scope of the research
35 question [2]. This calls for a foundation model pre-trained on large-scale data to achieve a general
36 understanding of single-cell biology. We expect such a model to serve as a strong foundation and
37 contribute to the discovery of new biological insights with the help of the learned knowledge from
38 millions of sequenced cells.

39 While the feasibility of generative pre-training in single-cell biology remains largely unexplored,
40 we can draw inspirations about modelling and the data-centric perspectives from other domains.
41 From a modelling perspective, the self-attention transformer has been verified as an efficient and
42 effective architecture to model input tokens of words. While texts are made up of words, cells can
43 be characterized by genes. We can learn and extract gene and cell representations simultaneously
44 in a similar fashion as word and sentence embeddings in NLG. The flexible vocabulary structure
45 also allows easy addition of new features and meta information. The attention mechanism of the
46 transformers could be further explored to inform on gene-to-gene and cross-modality associations
47 [60]. From a data perspective, the vast-scale atlases of single-cell RNA sequencing (scRNA-seq),
48 such as the Human Cell Atlas, already encompass tens of millions of cells, and the scale of accessible
49 omic data continues to grow exponentially [26, 51, 23, 2]. This opens ample opportunities to employ
50 self-supervised learning techniques to learn from diverse cell types and tissues, and integrate across
51 different organs and species.

52 In this work, we present the first attempt to build a single-cell foundation model, scGPT, by
53 generative pre-training on over 10 million cells. We introduce several new techniques to address
54 the methodology and engineering challenges of pre-training on large-scale single-cell omic data.
55 To handle the large-scale data, we use an in-memory data structure to store hundreds of datasets
56 that allow fast access. We establish a unified generative pre-training workflow specifically for the
57 non-sequential omic data, and adapt the transformer architecture to simultaneously learn cell and
58 gene representations. We also provide reusable finetuning pipelines and objectives designed for a
59 variety of downstream tasks to help users apply the pre-trained model with ease.

60 We demonstrate that the pre-trained model captures meaningful biological insights on both gene
61 and cell levels. The learned gene embedding maps decode known pathways by grouping together
62 genes that are functionally relevant. With zero-shot learning, the pre-trained model is able to
63 reveal meaningful cell clusters on unseen datasets. With finetuning in a few-shot learning setting,
64 the model achieves state-of-the-art performance on a wide range of downstream tasks, including
65 batch correction, multi-omic integration, cell type annotation, genetic perturbation prediction,
66 pseudo-cell generation, and gene network inference. The release of the scGPT model and workflow
67 aims to facilitate future research in all related areas. We envision that the adoption of pre-trained
68 foundation models will further our understanding of cellular biology, and pave the foundation for
69 future discoveries.

70 2 Results

71 2.1 Single-cell transformer foundation model overview

72 Single-cell sequencing captures the genetic profiles at the individual cell level. For instance, scRNA-
73 seq measures transcriptomic activities from RNA abundance, which informs on cell identity, stage,
74 and functionality. Recent cellular reference maps such as the human cell atlas comprise of millions

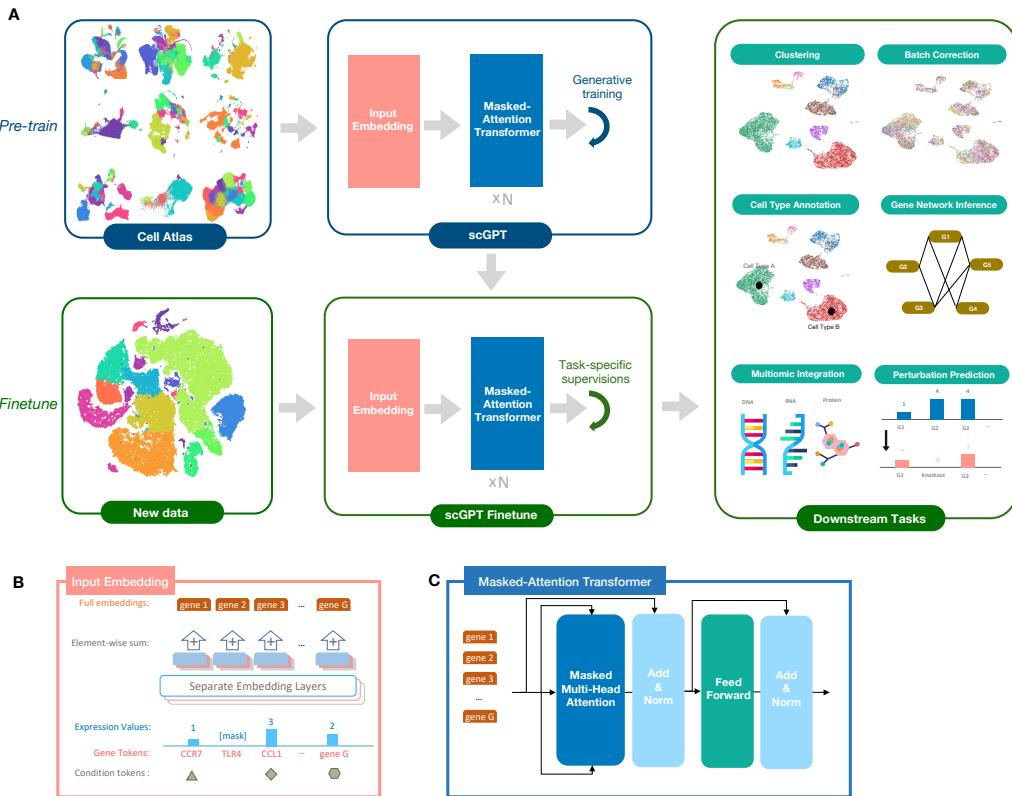


Figure 1: Model Schematic. *(a)* The workflow of scGPT. The model is firstly generatively trained on large-scale scRNA-seq data from cell atlas. For a downstream application, the pre-trained model weights can be finetuned on the new data. The core component of scGPT contains stacked transformer blocks with specialized attention masks for generative training. We applied scGPT on a variety of tasks including clustering, batch correction, cell type annotation, multi-omics, genetic perturbation prediction, and gene network inference. *(b)* The zoom-in view of the input embeddings. The input contains three layers of information, the gene token, expression value, and condition tokens (modality, batch, perturbation condition, et al.). *(c)* The zoom-in view of the scGPT transformer layer. We introduced a specially designed attention mask to conduct generative pre-training on single-cell sequencing data.

75 of single cells from diverse organs and tissues, offering an unparalleled representation of cellular
76 heterogeneity [26, 52]. We introduce scGPT as the generative pre-trained foundation model in the
77 single-cell domain. The core model has stacked transformer layers with multi-head attention that
78 learns cell and gene embeddings simultaneously (See Online Methods 4.2). Figure 1A illustrates a
79 two-stage workflow involving pre-training and fine-tuning of scGPT. In the pre-training stage, we
80 collected over 10.3 million scRNA-seq data of blood and bone marrow cells from the CellXGene
81 portal [11] for training. We introduce a specially designed attention mask and generative training
82 pipeline to train scGPT in a self-supervised manner to jointly optimize cell and gene representations
83 (See Online Methods 4.3). During training, the model gradually learns to generate gene expression
84 of cells based on simple cell or gene expression cues. In the fine-tuning stage, researchers can
85 apply the pre-trained model to new datasets and specific tasks (See online Methods 4.5). We
86 offer flexible finetuning pipelines suitable for a variety of downstream tasks essential in single-cell
87 research, including scRNA-seq integration with batch correction, cell type annotation, multi-omic
88 integration, perturbation prediction, and gene regulatory network inference.

89 scGPT learns cell and gene representations from diverse single-cell data through gene expres-
90 sion modelling. To facilitate gene representation learning, we employed Gene Expression Prediction
91 (GEP) as the generative self-supervised objective to iteratively predict gene expression values of
92 unknown tokens from known tokens in an auto-regressive manner (See Online Methods 4.4). To
93 enhance cell representation learning, we designed Gene Expression Prediction for Cell Modelling
94 (GEPC) objective, where the model predicts gene expression values from cell representations (See
95 Online Methods 4.4). This creates a direct link between the gene expression profile and cellular
96 heterogeneity, allowing for joint optimization within the scGPT framework. Furthermore, scGPT's
97 embedding architecture can easily extend to multiple sequencing modalities, batches, and pertur-
98 bation states. This is achieved by adding new condition tokens for sequencing modalities, batches,
99 and perturbation states. This unique model flexibility enables the pre-trained model to seam-
100 lessly combine with any additional information required for specific downstream tasks. See model
101 architecture illustration in Figure 1B and more details in Online Methods 4.

102 scGPT serves as a powerful single-cell feature extractor on previously unseen datasets. In
103 benchmark experiments, scGPT outperformed recent methods and achieved state-of-the-art results
104 across all downstream tasks. This demonstrates the benefits of pre-training and the transferability
105 of learned knowledge across diverse use cases. By providing a robust and unified framework, scGPT
106 enables single-cell researchers to easily leverage the pre-trained foundation model in related studies.

107 2.2 Integration of multiple scRNA-seq data with batch correction

108 Clustering and visualization of single-cell sequencing data encounter a significant challenge in the
109 presence of batch effects arising from the utilization of multiple datasets or sequencing batches as
110 input. By employing a finetuning workflow, the scGPT framework effectively tackles this challenge
111 by incorporating customized finetuning objectives (refer to Online Methods 4.4). This approach
112 successfully corrects for batch effects while preserving the true biological signals inherent in the
113 data.

114 scGPT achieves the state-of-the-art performance in preserving the biological variance of the
115 integrated datasets upon batch correction. We benchmarked scGPT with three popular integration
116 methods, scVI [34], Seurat [55], and Harmony [29] on two integration datasets Immune Human
117 (10 batches) [36] and PBMC 10K (2 batches) [21]. As shown in Figure 2A in the Immune Human
118 dataset, scGPT successfully integrated all batches of CD4+ T cells, CD8+ T cells, and CD14+
119 Monocytes into their individual clusters, whereas Seurat produced a few sub-clusters corresponding
120 to sequencing batches within each of these cell types (See batch visualizations in Supplementary

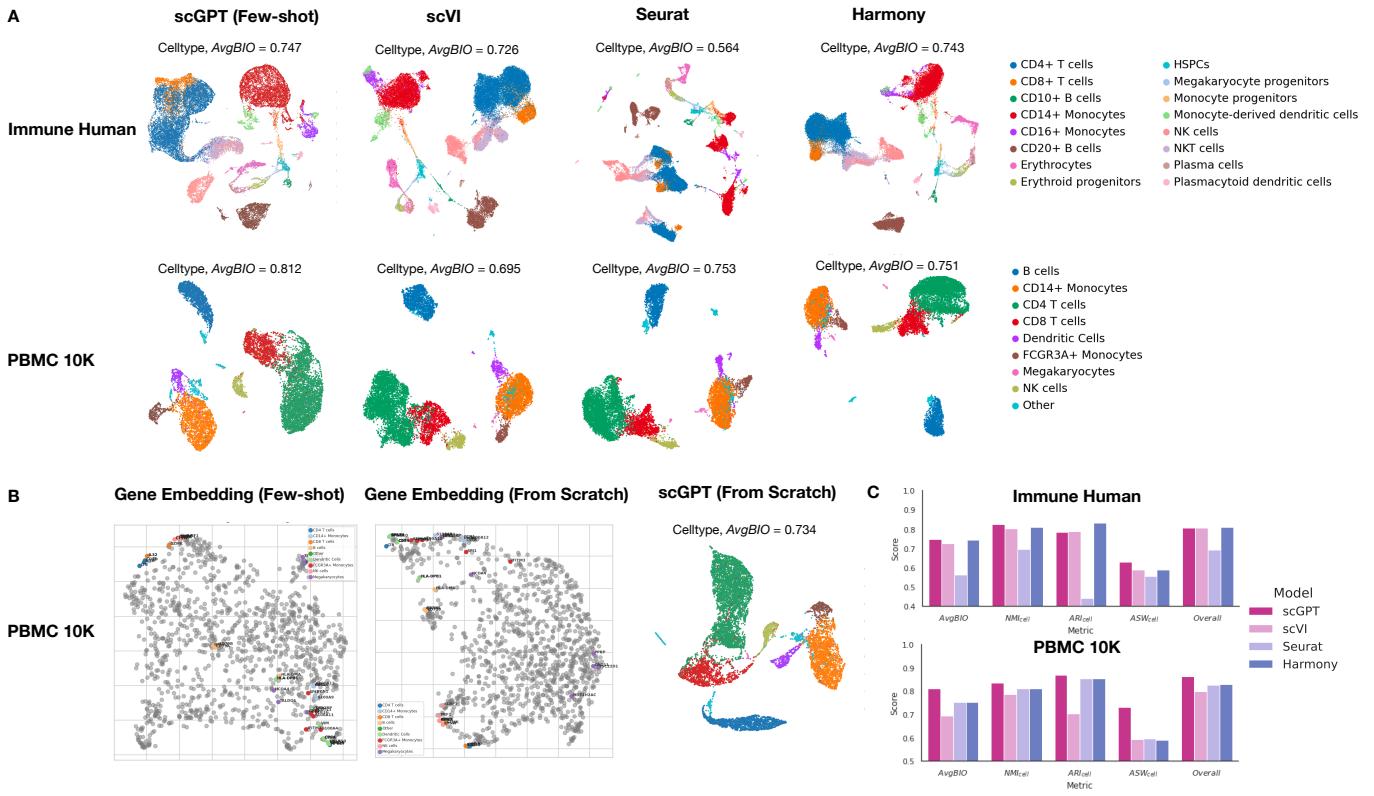


Figure 2: (a) Benchmark of the few-shot scGPT model with scVI [34], Seurat [55], and Harmony [29] on the Immune Human (10 batches) [36] and PBMC 10K (2 batches) [21] datasets for cell type clustering performance upon batch integration. The UMAP plot of learned cell embeddings was colored by *cell types*. (b) Comparison of gene embedding maps of the few-shot and trained-from-scratch scGPT models. The highly variable genes from each *cell type* were highlighted. The UMAP plot of learned cell embeddings from trained-from-scratch scGPT model was visualized. (c) Comparison of the scGPT model with other benchmarked methods on *AvgBIO*, the detailed biological conservation metrics (NMI_{cell} , ARI_{cell} , ASW_{cell}), and the *Overall* score.

121 Figure S3). scGPT also managed to separate the Monocyte-derived dendritic cells from the CD16+
122 Monocytes, but scVI and Harmony both saw a significant overlap of the two clusters. Moreover,
123 in the PBMC 10K dataset, scGPT is the only method that clearly separated out the cell type
124 Other from the annotated clusters. In contrast, scVI, Seurat and Harmony all confused this
125 Other cell type with CD14+ Monocytes and CD8 T cells. This inaccuracy is visualized as these
126 blue dots from cell type Other scattering all over the orange and red clusters. scGPT's superior
127 clustering performance is also reflected in the biological conservation score, where scGPT achieves
128 an *AvgBIO* score of 0.812, which is 5% higher than Seurat and Harmony, and 10% higher than
129 another deep learning method, scVI. In Figure 2C, scGPT presents competitive scores across all
130 cell type clustering metrics attributing to biological conservation. scGPT also ranked top in the
131 Overall metric considering both biological conservation and batch correction performance (See
132 detailed metrics in Supplementary Table S.1, and batch visualizations in Supplementary Figure
133 S3).

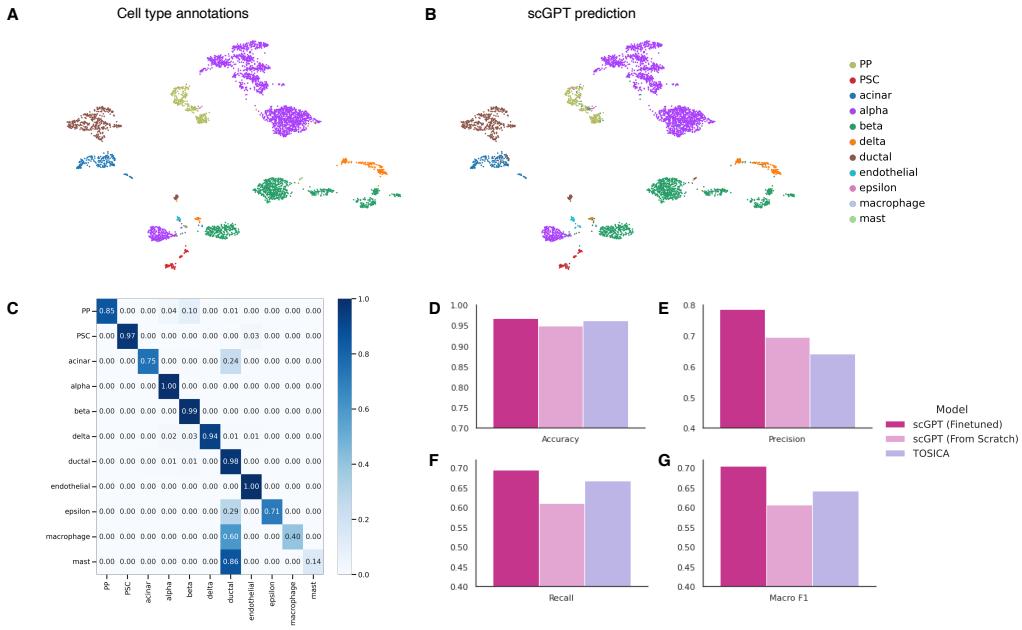


Figure 3: (a) UMAP visualization of cells colored by ground-truth *cell types* in the hPancreas query set. (b) UMAP visualization of cells colored by scGPT predicted *cell types*. (c) Normalized confusion matrix by *cell types*. (d) – (g) Comparison of the finetuned scGPT model with trained-from-scratch model and existing method on *Accuracy*, *Precision*, *Recall* and *MacroF1* scores.

134 We further highlight the benefits of pre-training by showing a significant performance boost in
 135 the finetuned model in comparison to the trained-from-scratch model in the PBMC 10K dataset
 136 (See Figure 2B). The finetuned gene embeddings produced more compact networks for highly variable
 137 genes of the CD4 T cells and Megakaryocytes, compared to the trained-from-scratch model.
 138 We observe similar results in the cell embeddings, as the cell type clusters become more defined in
 139 the finetuned model with a 8% improvement in the *AvgBIO* score. As a sanity check, we validated
 140 that the pre-trained model in zero-shot is also able to produce meaningful cell type clusters with an
 141 *AvgBIO* score of 0.728, on par with the trained-from-scratch model (See Supplementary Figure
 142 S2). This presents the zero-shot model as a generalizable feature extractor that can be readily
 143 applied to unseen datasets. Furthermore, the 8% performance boost from pre-training demon-
 144 strates the benefits of leveraging these pre-trained feature extractors and the knowledge learned.
 145 The foundation model is proven to be not only easily transferable to new datasets but also more
 146 powerful than learning from limited data from scratch.

147 2.3 Cell type annotation

148 Cell type annotation is a crucial step in single-cell analysis after clustering, as it resolves het-
 149 erogeneity in sequenced tissues and lays the foundation for further investigation of cell and gene
 150 functions to gain biological and pathological insights. While several methods have been proposed
 151 for cell annotation, such as cellAssign [64], singleR [3], and Chetah [17], they typically require di-
 152 mension reduction prior to model input, which can lead to information loss. In contrast, scGPT’s
 153 transformer model can directly take in gene expressions in an unbiased manner, with full resolu-
 154 tion on the entire highly variable gene set as input. This approach provides greater reliability and
 155 improved accuracy in cell type classification, as demonstrated in our subsequent analyses.

156 For the cell type annotation task, we finetuned the pre-trained scGPT model using cross-
157 entropy loss against ground-truth labels from a new reference dataset (See Online Methods 4).
158 Using the hPancreas dataset of human pancreas cells as an example, we trained the scGPT model
159 on the reference set and validated the classification performance on a different query set. Figure 3
160 panels A and B present the cell embeddings colored by ground-truth versus predicted cell types,
161 where the scGPT model demonstrates faithful prediction with high accuracy score of 96.7%. The
162 model also achieved high precision in predicting majority of the cell types, except for the rare cell
163 types with extremely low cell numbers in the reference set (See Figure 3C). For example, fewer
164 than 50 cells belong to the mast and macrophage cell types out of the 10.6 thousand cells in the
165 reference set. To benchmark the performance of scGPT, we compared it with TOSICA [12], a
166 recent transformer-based annotation method. scGPT outperforms TOSICA on all classification
167 metrics, including *Accuracy*, *Precision*, *Recall*, and *MacroF1*, as shown in Figure 3D-G. We
168 also trained a separate scGPT model from scratch without the pre-trained model parameters. It
169 achieves reasonable accuracy on the query set. The performance improvement of the finetuned
170 model from the trained-from-scratch model demonstrates the benefits of transfer learning with
171 pre-trained scGPT.

172 2.4 Perturbation Prediction

173 Sequencing and gene editing techniques have recently facilitated high-throughput experiments, en-
174 abling the exploration of cellular responses to multiple genetic perturbations. The approach holds
175 immense promise for uncovering novel gene interactions and advancing regenerative medicine. How-
176 ever, the vast combinatorial space of potential gene perturbations quickly surpasses the practical
177 limits of experimental feasibility. To overcome this limitation, scGPT can be employed to leverage
178 the knowledge gained from cellular responses in known experiments and extrapolate to predict
179 responses in unknown scenarios. The utilization of self-attention mechanisms over the gene dimen-
180 sion enables the encoding of intricate interactions between perturbed genes and the responses of
181 other genes. By leveraging this capability, scGPT can effectively learn from existing experimental
182 data and accurately predict gene expressions following perturbation.

183 For the perturbation prediction task, we evaluated our model using two perturbation datasets
184 pre-processed by Roohani, Huang, and Leskovec [53]: (1) the Pertub-seq dataset of K562 leukemia
185 cell line [1], which comprises 87 one-gene perturbations, with approximately 100 cells per pertur-
186 bation and a minimum of 7,000 unperturbed cells, and (2) the other Norman Perturb-Seq dataset
187 [41], consisting of 131 two-gene perturbations and 105 one-gene perturbations.

188 We assessed the perturbation prediction by calculating the Pearson correlation (*corr*) between
189 the predicted and the corresponding ground-truth expression values after perturbation. In addition,
190 we introduced a variant of the Pearson metric, denoted as *corr*(Δ), which measures the correlation
191 based on the magnitude of expression change post-perturbation compared to the control. We have
192 presented Pearson metrics for various gene sets, namely all genes (*ALL*) and the top 20 differentially
193 expressed genes (*DE*). For detailed information on how these metrics were calculated, refer to
194 Supplementary Online Methods S.2.

Table 1: Results of perturbation prediction

Model	Norman et al. [41]				Adamson et al. [1]			
	DE		ALL		DE		ALL	
	corr	corr(Δ)	corr	corr(Δ)	corr	corr(Δ)	corr	corr(Δ)
MLP	0.909	0.428	0.987	0.408	0.948	0.729	0.991	0.656
GEARS	0.917	0.508	0.986	0.387	0.961	0.726	0.991	0.652
scGPT	0.923	0.546	0.988	0.459	0.971	0.775	0.992	0.647

195 We conducted a performance comparison between scGPT, the recent GEARS method [53], and
196 the multi-layer perception (MLP) baseline. Our results demonstrate that scGPT achieves the high-
197 est correlation across seven out of eight evaluation metrics. It is worth noting that approximately
198 50% of the gene expression counts are zero in the raw scRNA-seq data. Therefore, we contend that
199 evaluating differentially expressed genes, specifically the DE columns in Table 1, provides more
200 compelling evidence. Notably, scGPT exhibits significant improvements in the correlation of the
201 (Δ) change of the top 20 differentially expressed genes, which is arguably the pivotal metric.

202 **2.5 scGPT facilitates multi-omic integration and multi-modal represen-**
203 **ta**
2.5.1 scGPT facilitates multi-omic integration

204 Single-cell multi-omic (scMultiomic) data presents multiple views of genetic regulation all at once,
205 including epigenetic, transcriptomic, and translation activities [57, 37]. It provides an ample oppor-
206 tunity to enhance feature and cell representation learning beyond gene expressions. However, the
207 challenge lies in how to reliably aggregate cell representations from multiple views while preserving
208 biological signals.

209 The scGPT framework can be readily extended to integrate multiple sequencing data modal-
210 ities. Each omic type in scMulti-omic data (e.g., gene expression, chromatin accessibility, and
211 protein abundance) is similar to a different language in NLG. Analogously, scGPT supports joint
212 optimization of multi-omic tokens from diverse sequencing modalities. This framework also allows
213 seamless addition of new sequencing modalities to existing pre-trained network by extending the
214 “vocabulary”. In the benchmark experiments, scGPT demonstrates outstanding performance in
215 cell representation learning and multiomic batch integration tasks compared to existing state-of-
216 the-art methods (See Figure 4).

217 scGPT effectively extracts integrated cell embeddings from paired scMultiomic data. In this
218 paired data integration setting, each sequenced cell contains all the data modalities. We used the
219 10X Multiome PBMC [14] dataset with joint gene expression and chromatin accessibility mea-
220 surements as an example. We benchmarked scGPT with two state-of-the-art methods scGLUE[9]
221 and Seurat v4[24] on cell type clustering performance. As shown in Figure 4, scGPT is the only
222 method that produced a clear separate cluster for CD8 Naive cells, whereas the other two methods
223 failed. scGPT also differentiated Memory B cells from Naive B and Intermediate B cell clusters,
224 yet scGLUE produced a merged cluster of all three types of B cells. scGPT separated the CD4 and
225 CD8 cell groups into two distinct groups of clusters, surpassing the results of Seurat v4. scGPT
226 demonstrates superior cell type clustering performance overall ($AvgBIO=0.767$) and robustness
227 across the diverse biological conservation metrics benchmarked (See Figure 4 and Supplementary
228 Table S.1).

229 scGPT simultaneously integrates multi-modal batches from mosaic scMultiomic data via joint
230 representation learning. In the mosaic data integration setting, sequenced samples share a few
231 but not all data modalities. We used the ASAP human PBMC dataset [38] with four sequencing
232 batches and three data modalities as an example. The first two sequencing batches contain gene
233 expression and protein abundance data from CITE-seq, and the second two batches have chromatin
234 accessibility and protein abundance measurements from ASAP-seq. In the benchmark experiment
235 with scMoMat[65], scGPT demonstrates superior batch correction performance as shown in Figure
236 4, especially in the rarer cell group NK cell. In comparison, scMoMat produced two distinct
237 clusters for each cell type corresponding to the first two and second two batches, indicating failure
238 to mitigate modality differences. scGPT achieves a overall batch correction score $AvgBATCH$
239 of 0.948, with a close-to-perfect *GraphConn* score of 0.992 and a significantly higher ASW_{batch}
240 score of 0.904 compared to scMoMat ($ASW_{batch} = 0.849$). scGPT’s biological conservation metrics

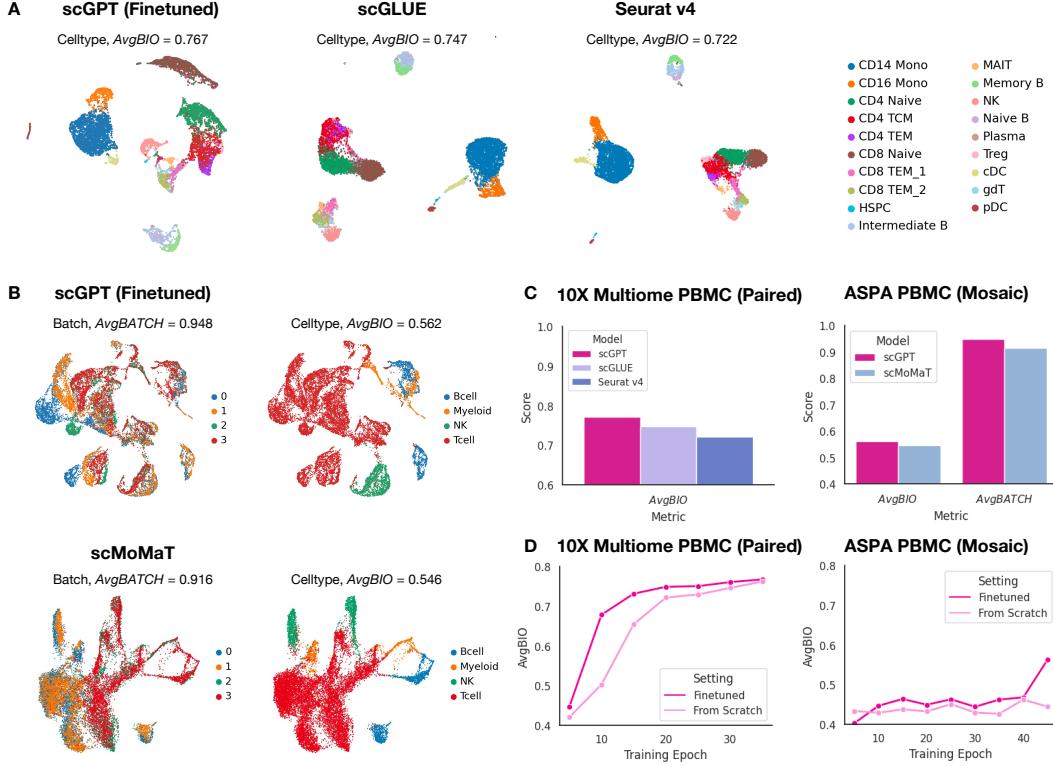


Figure 4: (a) Benchmark of the few-shot scGPT model with scGLUE[9] and Seurat v4[24] on the 10x Multiome PBMC [14] dataset (paired data setting) for cell type clustering task. The UMAP plot of learned cell embeddings was colored by *cell types*. (b) Benchmark of scGPT with scMoMat[65] on the ASAP PBMC [38] dataset (mosaic data setting) for batch correction and cell type clustering tasks. The UMAP plots of learned gene embeddings were colored by *sequencing batches* (left) and *cell types* (right). (c) Comparison of the scGPT model with other benchmarked methods on *AvgBIO* and *AvgBATCH* scores. (d) Comparison of training progress curves of the few-shot and trained-from-scratch models with *AvgBIO* scores evaluated every five epochs.

also compare favorably to scMoMat's, which further indicates the robustness of multi-modal batch correction without interfering with the biological signals (See Figure 4 and Supplementary Table S.1).

scGPT readily supports the addition of new data modalities to existing pre-trained models. We compared scGPT's training progress curves in two settings, finetuned on the pre-trained model and trained from-scratch, to demonstrate the benefits of pre-training in the multi-omic integration task. As shown in Figure 4, for the 10X Multiome PBMC dataset, the finetuned model reached the best AvgBIO scores in the 70% range 5 epochs earlier than the trained-from-scratch model. This demonstrates the benefits of the pre-trained model in leading training progress and faster convergence. In the more challenging mosaic integration setting with the ASAP PBMC dataset, the finetuned model's AvgBIO scores increased steadily as training proceeded, whereas the trained-from-scratch model did not see much progress with training. At epoch 45, the pre-trained model finished at an AvgBIO score of 0.562, which is more than 10% higher than the trained-from-scratch model with a score of 0.444. This suggests that the model leverages the learned gene embeddings from large atlases to guide the learning of peak and protein embeddings in the pre-trained setting.

2.6 Gene embeddings for Gene Regulatory Network inference

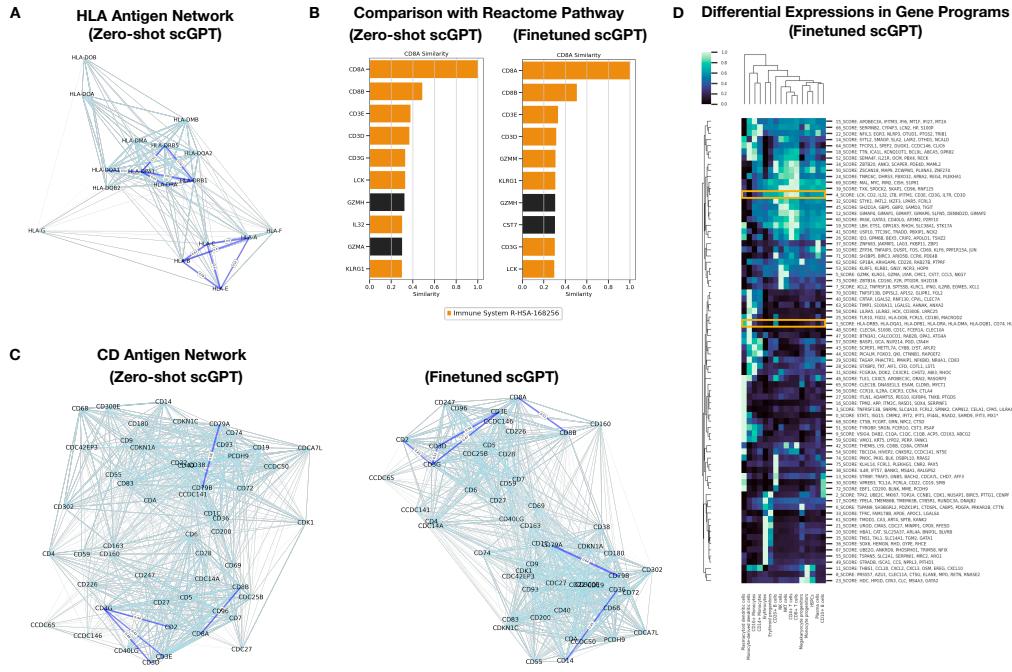


Figure 5: (a) HLA antigen network from zero-shot scGPT. (b) CD8A gene neighbors from zero-shot and finetuned scGPT models, ranked by embedding similarity colored by ground-truth signalling pathway from Reactome. (c) CD antigen network from zero-shot and finetuned scGPT on the Immune Human dataset. (d) Differential expressions among scGPT-extracted gene programs by cell types in the Immune Human dataset.

The interactivity between transcription factors, cofactors and target genes underlying a Gene Regulatory Network (GRN) mediates important biological processes. Existing GRN inference methods often rely on correlation in static gene expressions or pseudo-time estimates as a proxy for causal graphs [46]. scGPT, optimized by the generative training of gene tokens, implicitly encodes such relationships in its gene embeddings. The gene embeddings can therefore be applied to construct similarity networks that entail gene-gene interactions. We hereby validate the scGPT's gene embedding network against known biology, and then explore its applicability to gene program discovery.

scGPT demonstrates its ability to group the functionally related genes and differentiate functionally different genes from its gene embedding network. In Figure 5A, we visualized the similarity network of the human leukocyte antigens (HLA) antigens from the pre-trained gene embeddings. In the zero-shot setting, the scGPT model highlights two clusters corresponding to the two well-characterized HLA classes that trigger different immune responses, namely HLA class I and HLA class II. The HLA class I antigens HLA-A, -C, and -E are recognized by CD8+ T cells to mediate cell killing, whereas HLA class II antigens HLA-DR, -DP, and -DQ are recognized by CD4+T cells to trigger broader helper functions [13]. For the finetuned scGPT model on the Immune Human dataset, we explored the CD antigen network specific to the immune cell types present in this dataset (See Figure 5C). The pre-trained scGPT is able to identify CD3E, D, and G genes as a group that encodes the T3 complex for T-cell activation, CD79A and B for B-cell signalling, and CD8A and B as co-receptors for HLA class 1 molecules [40]. The finetuned scGPT further

277 highlights the connection between CD36 and CD14 as markers for monocytes and macrophages.
278 This demonstrates scGPT’s ability to generalize from the knowledge learned in pre-training and
279 extract specific information related to the dataset at hand.

280 scGPT reconstructs meaningful gene programs in a purely unsupervised workflow. In Figure 5D,
281 we visualized the gene programs extracted by the finetuned scGPT model on the Immune Human
282 dataset, and their differential expressions by cell types. These gene programs are selected in an
283 unsupervised manner by first clustering the gene embeddings and then thresholding on clusters
284 that consist of 5 or more genes, following Ceglia et al. [10]’s proposed pipelines (See Online Methods
285 4). We observed that the same HLA antigen cluster was identified as group 1. Similarly, the CD3
286 genes involved in T3 complex were identified as group 4, with highest expressions in T cells. This
287 confirms that scGPT’s inferred gene programs correspond to function groups that are biologically
288 meaningful. We further validated the gene similarity relationships encoded by the scGPT model
289 against the known Reactome database [50]. Using the CD8A gene as an example, we demonstrate
290 that its closest neighbors are more likely to be part of the Immune System R-HSA-168256 pathway
291 than genes that are further away, from both zero-shot and finetuned scGPT models (See Figure
292 5B). The top three genes closest to CD8A remain consistent before and after finetuning. The
293 finetuned model highlighted one additional gene GZMM that is commonly enriched in NK, NKT
294 and T cell subclusters [4] which are major cell types found in the Immune Human dataset. We
295 further validated this correlation on the entire gene set across the Reactome database. Among
296 pairs of genes, there exists a positive correlation between the cosine similarity score of the gene
297 embeddings and the number of common pathways shared by these genes, with a Pearson correlation
298 score of 0.316.

299 While a more comprehensive evaluation pipeline is to be established, these findings showcase
300 that scGPT has learned meaningful biological patterns from generative pre-training in the zero-
301 shot setting. More specifically, we demonstrate its ability to perform unsupervised gene program
302 discovery on new datasets along with other cell-level analysis tasks by leveraging the pre-trained
303 model. We envision this attempt as one of the first steps towards knowledge discovery in the
304 single-cell domain assisted by foundation models.

305 3 Discussion

306 We hereby present scGPT, the first foundation model that leverages pre-trained transformers
307 learned from over 10 million single-cell data. The self-supervised pre-training paradigm with
308 increasingly large amount of training data has created powerful language models such as chatGPT
309 and GPT4 [44, 43]. These successes inspired us to apply the same pre-training paradigm to
310 the single-cell domain, with the aim of decoding complex biological interactions with the pre-
311 trained transformers. The transformer models naturally support the joint learning of gene and
312 cell embeddings, analogous to word and sentence embeddings in NLG. These technical advantages
313 create a solid foundation for modelling these different aspects of cellular processes together at once.

314 We demonstrate the benefits of pre-training with comprehensive experiments in both zero-shot
315 and finetuning settings. The pre-trained model itself is a universal feature extractor. It showcases
316 strong capabilities of extrapolating to unseen datasets, presenting meaningful cell clustering in
317 zero-shot experiments. The learned gene networks also reflect known gene programs and their
318 functional roles. These abilities give us confidence in the pre-trained model that it has not only
319 memorized but also synthesized the patterns from the large-scale single-cell data. We also observed
320 a consistent contribution of the pre-trained model in multiple downstream tasks via transfer learn-
321 ing. For example, in both multi-batch and multi-omic integration tasks, the finetuned model

322 has demonstrated superior performance in cell-type clustering, with an 8 to 12% increase in the
323 biological conservation score compared to the trained-from-scratch models.

324 To implement generative pre-training for the non-sequential single-cell data, we introduced
325 specialized attention masking to support generation and joint gene and cell representation learning.
326 In the finetuning pipeline, we offer setups for a diverse range of downstream tasks such as batch
327 correction, cell type assignment, multi-omic integration, perturbation prediction, and gene network
328 inference. We hereby release the scGPT codebase and the pre-trained model. We hope that this
329 provides a unified framework to help researchers easily adapt the pre-trained models to their own
330 tasks at hand.

331 For future directions, we plan to pre-train on a larger-scale dataset with more diversity, includ-
332 ing multi-omic data, spatial omics, and diseased conditions. It is also interesting to incorporate
333 perturbation and temporal data in the pre-training stage for causal discovery. More importantly,
334 we would like to validate the pre-trained model on a wider range of biologically meaningful tasks
335 to understand and interpret what the pre-trained model has learned. We also aim to explore
336 in-context instruction learning for single-cell data. The goal is to have a pre-trained model that
337 understands different tasks and contexts in the zero-shot setting without having to finetune. scGPT
338 thus serves as the first step to use large-scale pre-trained foundation model to understand the con-
339 text and nuances in cell biology. We envision that the pre-training paradigm be readily integrated
340 into single-cell research, and serve as a foundation to leverage the existing knowledge from the
341 exponentially growing cell atlases for new discoveries.

342 4 Methods

343 4.1 Input embeddings

344 The single-cell sequencing data is processed into a cell-gene matrix, $\mathbf{X} \in \mathbb{R}^{N \times G}$, where each element
345 $X_{i,j} \in \mathbb{R}^+$ represents the read count of a RNA for scRNA-seq or a peak region if scATAC-seq. For
346 example in scRNA-seq, the element denotes the RNA abundance for gene $j \in 0, 1, \dots, G$ in cell
347 $i \in 0, 1, \dots, N$. In subsequent sections, we will refer to this matrix as the raw matrix. The input to
348 scGPT consists of three main components: (1) gene (or peak) tokens, (2) expression values, and (3)
349 condition tokens. For each modeling task, the gene tokens and expression values are pre-processed
350 from the raw count matrix \mathbf{X} accordingly:

351 **Gene Tokens** In the scGPT framework, each gene is considered the smallest unit of information,
352 equivalent to a word in natural language generation (NLG). We therefore use gene names as tokens,
353 and assign each gene g_j a unique integer identifier $id(g_j)$ within the complete vocabulary of tokens.
354 This approach offers great flexibility to harmonize multiple studies with different gene sets (i.e.,
355 generated by distinct sequencing technologies or pre-processing pipelines). Specifically, different
356 sets of gene tokens can be integrated into a common vocabulary by taking the union set of all genes
357 across studies. Additionally, we incorporate special tokens in the vocabulary, such as $<cls>$ for
358 aggregating all genes into a cell representation, and $<pad>$ for padding the input to a fixed
359 length. Conceptually, we draw parallels between gene tokens and word tokens in NLG. The input
360 gene tokens of each cell i are hence represented by a vector $\mathbf{t}_g^{(i)} \in \mathbb{N}^M$:

$$\mathbf{t}_g^{(i)} = [id(g_1^{(i)}), id(g_2^{(i)}), \dots, id(g_M^{(i)})], \quad (1)$$

361 where M is a pre-defined input length, and usually equals to the number of selected highly variable
362 genes.

363 **Expression Values** The gene expression matrix X requires additional processing before being
364 used as input for modeling. A fundamental challenge in gene expression modeling is the variability
365 in absolute magnitudes across different sequencing protocols [54]. Due to variations in sequencing
366 depths and the presence of sparsely expressed genes, the data scales differ significantly among
367 different batches of sequencing samples. These differences are not easily mitigated with common
368 preprocessing techniques such as transcripts per million (TPM) normalization and \log_{10} trans-
369 formation [25]. To make it more clear, the same absolute value can convey different "semantic"
370 meanings across sequencing batches. To address this scale difference, we propose the ***value bin-***
371 ***ning*** technique to convert all expression counts into relative values. For each non-zero expression
372 count in each cell, we calculate the raw absolute values and divide them into B consecutive inter-
373 vals $[b_k, b_{k+1}]$, where $k \in \{1, 2, \dots, B\}$. Each interval represents an equal portion of all expressed
374 genes ($1/B$). It is important to note that a new set of bin edges is computed for each cell, so the
375 interval edges b_k may vary among cells. The binned expression value $x_j^{(i)}$ for cell i is defined as:

$$x_j^{(i)} = \begin{cases} k, & \text{if } X_{i,j} > 0 \text{ and } X_{i,j} \in [b_k, b_{k+1}], \\ 0, & \text{if } X_{i,j} = 0. \end{cases} \quad (2)$$

376 Through this binning technique, the semantic meaning of $x_j^{(i)}$ is consistent across sequencing
377 batches. For instance, a value of $x_j^{(i)} = B$ consistently indicates the highest expression among
378 genes. Before applying the value binning step, we performed \log_{10} transformation, and highly
379 variable gene selection [35]. To simplify the notation, we use $X_{i,j}$ to represent both the raw and
380 preprocessed data matrices prior to binning. Therefore, the final input vector of binned expression
381 values for cell i is denoted as

$$\mathbf{x}^{(i)} = [x_1^{(i)}, x_2^{(i)}, \dots, x_M^{(i)}]. \quad (3)$$

382 **Condition Tokens** The condition tokens encompass diverse meta information associated with
383 individual genes, such as functional pathways (represented by pathway tokens) or perturbation
384 experiment alterations (indicated by perturbation tokens). To represent position-wise condition
385 tokens, we utilize an input vector that shares the same dimension as the input genes. This vector
386 is denoted as:

$$\mathbf{t}_c^{(i)} = [t_{c,1}^{(i)}, t_{c,2}^{(i)}, \dots, t_{c,M}^{(i)}], \quad (4)$$

387 where $t_{c,j}^{(i)}$ represents an integer index corresponding to a condition.

388 **Embedding layers** We utilize the conventional embedding layers¹ \mathbf{emb}_g and \mathbf{emb}_c for the gene
389 tokens and condition tokens, respectively, to facilitate the mapping of each token to a fixed-
390 length embedding vector of dimension D . We employ fully connected layers, denoted as \mathbf{emb}_x ,
391 for the binned expression values to enhance expressivity. This choice enables the modeling of the
392 continuum of gene expression values. Consequently, the final embedding $h^{(i)} \in \mathbb{R}^{M \times D}$ for cell i is
393 defined as,

¹pytorch embedding layer

$$\mathbf{h}^{(i)} = \text{emb}_g(\mathbf{t}_g^{(i)}) + \text{emb}_x(\mathbf{x}^{(i)}) + \text{emb}_c(\mathbf{t}_c^{(i)}). \quad (5)$$

394 **4.2 Cell and gene expression modeling by transformers**

395 **4.2.1 scGPT Transformer**

396 We employ the self-attention transformer [60, 18] to encode the complete input embedding $\mathbf{h}^{(i)}$
397 in equation 5. The self-attention mechanism operates on the sequence of M embedding vectors,
398 making it particularly suitable for capturing interactions between genes. The output of the stacked
399 transformer blocks can be defined as follows:

$$\begin{aligned} \mathbf{h}_0^{(i)} &= \mathbf{h}^{(i)} \\ \mathbf{h}_l^{(i)} &= \text{transformer_block}(\mathbf{h}_{l-1}^{(i)}) \quad \forall l \in [1, n] \end{aligned} \quad (6)$$

400 We utilize the resulting representation $\mathbf{h}_n^{(i)} \in \mathbb{R}^{M,D}$ for both gene-level and cell-level tasks.
401 Gene-level finetuning objectives (See Online Methods 4.4) are directly applied. Examples in-
402 clude the gene expression prediction (GEP) objective and the perturbed expression prediction
403 task (perturb-GEP). For cell-level tasks, we first integrate $\mathbf{h}_n^{(i)}$ into a cell embedding vector. (See
404 Online Methods 4.2.2). An example would be the cell type assignment task, where the cell embed-
405 dings are used to predict cell type labels by an added classifier in the CLS training objective.

406 The input dimension M can reach tens of thousands of genes, significantly exceeding the in-
407 put length of conventional transformers commonly used in NLG. To address this challenge and
408 ensure efficient self-attention mechanisms, we leverage more advanced approaches such as Flash-
409 Attention [16]. This implementation effectively enhances the model capacity and enables effective
410 processing of large-scale input dimensions. Other efficient transformers can also be utilized, such
411 as Transformers with linear complexity (Linformer) [61] and Kernelized Self-Attention (KSA) [28].

412 **4.2.2 Cell representation**

413 Each cell is considered a "sentence" composed of genes, and its representation $\mathbf{h}_c^{(i)} \in \mathbb{R}^D$ is obtained
414 by aggregating the learned gene-level representations $\mathbf{h}_n^{(i)}$. Various pooling operations, such as
415 element-wise mean-pooling or weighted-pooling, can be readily employed in this context. In this
416 study, we opt to employ a special token $<\text{cls}>$ for the cell representation, enabling the model
417 to learn the pooling operation within transformer blocks. The $<\text{cls}>$ token is appended to the
418 beginning of the input tokens, and the final embedding at this position is extracted as the cell
419 representation. Consequently, we have the cell embedding $\mathbf{h}_c^{(i)}$ equals to a row in the stacked
420 final-layer embeddings $\mathbf{h}_n^{(i)}[<\text{cls}>]$, where the $[<\text{cls}>]$ operation retrieves the row at the index
421 corresponding to the $<\text{cls}>$ token in the input.

422 **4.2.3 Condition tokens for batch and modality**

423 We use additional sets of tokens to represent different batches and sequencing modalities, specif-
424 ically for the scRNA-seq and scMultiomic integration tasks. This is similar to condition tokens

425 introduced in Online Methods 4.1, and implemented similarly using the standard embedding lay-
426 ers. The modality tokens $t_m^{(i)}$ are associated with individual input features g_j (e.g., to indicate
427 whether it is a gene, region or protein). The batch tokens are on the cell level originally but can
428 be propagated to all features of a single cell as well. In other words, the same batch token $t_b^{(i)}$ can
429 be repeated up to the length M of input features of single cell i :

$$\mathbf{t}_b^{(i)} = \left[t_{b,1}^{(i)}, t_{b,2}^{(i)}, \dots, t_{b,M}^{(i)} \right] = \left[t_b^{(i)}, t_b^{(i)}, \dots, t_b^{(i)} \right]. \quad (7)$$

430 The difference between the tokens described in Online Methods 4.1 and the batch and modal-
431 ity tokens is that these embeddings are not used as input to the transformer blocks. Instead,
432 they are concatenated with the transformer output on either feature or cell level prior to entering
433 specific fine-tuning objectives. This is to prevent the transformer from amplifying the attention
434 within features of same modalities while underestimating those of different modalities. Further-
435 more, knowing the modality and/or batch identities facilitates gene expression modelling in the
436 downstream fine-tuning objectives. As the model learns to predict expression values conditioned
437 on modality and/or batch identities, such biases are implicitly removed from the gene and cell
438 representations themselves. This serves as a technique to facilitate batch correction.

439 As an example, in the scMultiomic integration task, we concatenate the transformer output with
440 the sum of batch and modality embeddings. This serves as input to the downstream fine-tuning
441 objectives for expression modelling:

$$\mathbf{h}'^{(i)}_n = concat(\mathbf{h}_n^{(i)}, \mathbf{emb}_b(t_b^{(i)}) + \mathbf{emb}_m(t_m^{(i)})), \quad (8)$$

442 where \mathbf{emb}_b and \mathbf{emb}_m denote the batch and modality embedding layers respectively.

443 Alternatively, in the scRNA-seq integration task, concatenation of batch embedding with the
444 cell representation yields the following representation:

$$\mathbf{h}'^{(i)}_c = concat(\mathbf{h}_c^{(i)}, \mathbf{emb}_b(t_b^{(i)})), \quad (9)$$

445 where $t_b^{(i)}$ denotes the batch identity of cell i .

446 4.3 Generative pre-training

447 4.3.1 Foundation model pre-training

448 The foundation model is designed to be a generalizable feature extractor that can benefit a diverse
449 range of downstream tasks. It contains the entire set of genes in the human genome. The expression
450 values were normalized, log-transformed, and binned prior to model training (See Online Methods
451 4.1). To speed up the training, we restrict the input to only genes with non-zero expressions for
452 each input cell. This strategy provides useful pre-trained results contributing to the subsequent
453 finetuning stage, where we include all genes with zero expressions as well by default. To efficiently
454 train the model to capture gene-gene relation and gene-cell relation, we introduce a new generative
455 training strategy with specialized attention masks as detailed in the following section.

456

4.3.2 Attention mask for generative pre-training

457 Self-attention has been widely used to capture the co-occurrence patterns among tokens. In natural
458 language processing, this has been achieved mainly in two ways: (1) masked token prediction used
459 in transformer encoder models such as BERT [18] and Roberta [33], where randomly masked
460 tokens in the input sequence are predicted in the model’s output; (2) auto-regressive generation
461 with sequential prediction in causal transformer decoder models such as the OpenAI GPT series [48,
462 49, 6, 43]. The generative pre-training used in OpenAI GPT3 [6] and GPT4 [43] employs a unified
463 framework in which the model predicts the most likely next token from a “prompt” consisting
464 of known input tokens. This framework offers great flexibility to be utilized in various natural
465 language generation (NLG) applications and demonstrates new capabilities such as contextual
466 awareness in zero-shot and few-shot settings [7]. We believe that the generative training can be
467 beneficial to single-cell models in a similar manner. Specifically, we are interested in two tasks: (1)
468 generating unknown gene expression values based on known gene expressions, i.e., generation by
469 “gene prompts”, and (2) generating whole genome expressions given an input cell type condition,
470 i.e., generation by “cell prompts”.

471 Despite similar usage of tokens and prompts, modelling genetic reads is inherently different
472 from natural language due to the non-sequential nature of the data. Unlike words in a sentence,
473 the order of genes within a cell is interchangeable, and there is no equivalent concept of “next gene”
474 to predict. This makes it challenging to apply the causal masking formulation from GPT models
475 directly in single-cell domain. To address this challenge, we developed a specialized attention
476 masking mechanism for scGPT that defines the order of prediction based on attention scores.

477 The scGPT’s attention mask supports both gene-prompt and cell-prompt generations in a
478 unified way. The binary attention mask is applied on the self-attention map in the transformer
479 blocks. For an input $\mathbf{h}_l^{(i)} \in \mathbb{R}^{M \times D}$ of M tokens (See Online Methods 4.2.1), the transformer block
480 will generate M query and key vectors to compute the attention map, $\mathbf{A} \in \mathbb{R}^{M \times M}$. The attention
481 mask is of the same size $M \times M$. We visualize the attention mask in Supplementary Figure S1A,
482 where queries are organized in rows and keys in columns. The token identity associated with each
483 column of the mask is annotated at the bottom of the figure, namely $<cls>$, known genes, and
484 unknown genes. Each token in the input embedding $\mathbf{h}_l^{(i)}$ can be one of these three groups: (1)
485 the reserved $<cls>$ token for cell embedding (introduced in Online Methods 4.2.2), (2) known
486 genes with token embeddings and expression value embeddings, and (3) unknown genes whose
487 expression values are to be predicted. The rule of thumb for scGPT’s attention-masking is to only
488 allow attention computation between embeddings of the “known genes” and the query gene itself.
489 In each generation iteration, scGPT predicts the gene expression values of a new set of genes, and
490 these genes in turn become the “known genes” in the next iteration for attention computation.
491 This approach reflects the casual masking design with next token prediction in the conventional
492 transformer decoders by making sequential predictions in the non-sequential single-cell data.

493 As illustrated in Supplementary Figure S1A, during training, we randomly pick a ratio of the
494 genes as unknown so their expression values are omitted in the input. The queries on the positions
495 of these unknown genes are only allowed with attention computation on the known genes and the
496 query gene itself. For example, the last gene to predict at position M has attention scores with the
497 cell embedding, known genes and itself only, but not the other unknown genes, as illustrated in the
498 last row of the attention mask. The scGPT model predicts the expressions for these unknown genes
499 via the stacked transformer blocks with the masked attention map described above. The inference
500 steps are illustrated in Supplementary Figure S1B. During the inference for cell-prompt generation,
501 scGPT generates all genome-wide gene expressions conditioned on the specific cell types. A trained
502 cell embedding is inputted at the first position representing the cell type condition. The whole
503 generation process of thousands of gene expressions is conducted in K iterative steps (i.e., $K = 3$

504 steps in Supplementary Figure S1B). For example, in one iteration $i \in \{1, 2, \dots, K\}$, the attention
505 masking mechanism allows attention with all predicted genes from previous 0 to $i-1$ iterations. In
506 each iteration, scGPT selects the top $1/K$ genes from the unknown set with the highest prediction
507 confidence to be included as known genes in the next iteration $i+1$. Intuitively, this workflow
508 streamlines the generation of large groups of gene expressions in an auto-regressive manner, where
509 gene expressions with highest prediction confidence are first generated and used to help subsequent
510 rounds of generation. The gene-prompt generation works similarly in an iterative manner. The
511 difference is that it starts with a set of known genes with observed expression values, instead of a
512 cell embedding.

513 The scGPT attention masking unifies the encoding process of known genes and the generation
514 on unknown genes. It also stands as one of the first transformer schemes to conduct auto-regressive
515 generation for non-sequential data.

516 4.4 Fintuning objectives

517 scGPT leverages various fine-tuning objectives to facilitate the learning of biologically valid repre-
518 sentations of cells and genes, as well as for regularization purposes such as batch correction.

519 **Gene Expression Prediction (GEP)** To encourage the learning of gene-gene interactions,
520 scGPT incorporates gene expression prediction. Within each cell, a subset of genes and their
521 corresponding expression values $\mathbf{x}^{(i)}$ are randomly masked. scGPT is optimized to accurately
522 predict the expression values at the masked positions. This fine-tuning objective benefits the
523 model in effectively encoding co-expressions among the genes in the dataset. Specificcally, we
524 employ a fully connected MLP to estimate the expression value for M genes, on the transformer
525 output. The optimization of this objective involves utilizing the cross entropy loss at the masked
526 positions, denoted as \mathcal{M}_{mask} . The GEP works as follows,

$$\begin{aligned}\tilde{\mathbf{x}}^{(i)} &= \text{MLP}(\mathbf{h}_n^{(i)}), \\ \mathcal{L}_{GEP} &= \frac{1}{|\mathcal{M}_{mask}|} \sum_{j \in \mathcal{M}_{mask}} \text{ce}(\tilde{x}_j^{(i)}, x_j^{(i)}).\end{aligned}\tag{10}$$

527 Here, $\tilde{\mathbf{x}}^{(i)} \in \mathbb{N}^M$ represents the row of expression estimates for cell i , and ce denotes the cross
528 entropy function. It is worth noting that in integration tasks, we use $\mathbf{h}'_n^{(i)}$ in Equation 8 instead
529 of $\mathbf{h}_n^{(i)}$.

530 GEP presents a general self-supervised finetuning objective, which aims to forecast gene ex-
531 pression values. In certain downstream tasks, such as the perturbation prediction, the model is
532 required to predict perturbed gene expression values instead of the original values. We refer to this
533 variation as **perturb-GEP**. We maintain the MLP estimator in equation 10, but utilize the gene
534 expressions post-perturbation as the target $x_j^{(i)}$. In perturb-GEP, the predicted expression values
535 are simply altered to apply to all valid target positions instead of solely the masked positions in
536 GEP.

537 **Gene Expression Prediction for Cell Modelling (GEPC)** This finetuning objective oper-
538 ates similarly to GEP, but predicts gene expression values based on the cell representation $\mathbf{h}^{(i)}_c$ to

539 explicitly foster cell representation learning. For each gene j in an input cell i , we create a query
 540 vector \mathbf{q}_j and utilize the parameterized inner product of \mathbf{q}_j and the cell representation $\mathbf{h}^{(i)}c$ as the
 541 predicted expression value.

$$\begin{aligned}\mathbf{q}_j &= \text{MLP}(\text{emb}_g(t_g^{(i)})), \\ \tilde{x}_j^{(i)} &= \mathbf{q}_j \cdot \mathbf{W} \mathbf{h}_c^{(i)}, \\ \mathcal{L}_{GEPC} &= \frac{1}{|\mathcal{M}_{mask}|} \sum_{j \in \mathcal{M}_{mask}} \text{ce}(\tilde{x}_j^{(i)}, x_j^{(i)})\end{aligned}\quad (11)$$

542 GEP inherits the gene token embedding, $\text{emb}_g(t^{(i)}g)$, from Equation equation 5. In integration
 543 tasks, we utilize $\mathbf{h}^{(i)}$ from Equation 9 instead of $\mathbf{h}_c^{(i)}$, and we also concatenate the modality and/or
 544 batch embeddings to emb_g . In our experiments, we observed that combining GEP and GEPC leads
 545 to significantly improved performance compared to using either method individually.

546 **Elastic Cell Similarity (ECS)** This finetuning objective enhances cell representations through
 547 the utilization of a similarity learning loss [31]:

$$\mathcal{L}_{ECS} = -(\text{sim}(\mathbf{h}_c^{(i)}, \mathbf{h}_c^{(i')}) - \beta)^2, \quad (12)$$

548 where sim represents the cosine similarity function, while i and i' refer to two cells within the mini-
 549 batch. Additionally, β denotes a predefined threshold. The underlying idea behind this approach is
 550 to enhance the similarity between pairs exhibiting cosine similarity values above β , thereby making
 551 them even more similar. Conversely, dissimilar pairs are encouraged to be further apart.

552 **Domain Adaptation via Reverse Back-propagation (DAR)** Cell representation learning
 553 is hindered by the presence of batch effects, which result from non-biological batch differences
 554 introduced by sequencing technologies [19, 59]. To mitigate this problem, we employ a distinct
 555 multi-layer perceptron (MLP) classifier to predict the sequencing batch associated with each input
 556 cell, and modify the back-propagation process by reversing the gradients within the model. This
 557 approach leverages insights from the robust domain adaptation method proposed by Ganin and
 558 Lempitsky [20].

559 **Cell Type Classification (CLS)** This finetuning objective is designed to leverage the learned
 560 cell representations to annotate single cells. We use a separate MLP classifier to predict the cell
 561 types from their cell representations $\mathbf{h}_c^{(i)}$. This finetuning objective is optimized with cross entropy
 562 loss ce between the predicted cell type probabilities and ground-truth labels.

563 4.5 Finetuning on downstream tasks

564 **Batch correction on integrating multiple scRNA-seq datasets** Batch effects can be a
 565 major confounder in cell type clustering when the input contains multiple datasets from different
 566 sequencing batches or technologies. Therefore, we aim to correct batch effects while preserving bio-
 567 logical variances when integrating multiple scRNA-seq datasets. For finetuning on this integration
 568 task, the common set of gene tokens between the pre-trained foundation model and the current

569 dataset were retained. We further selected a subset of highly variable genes from the common
570 set as input. The gene expression values were normalized, log-transformed and binned prior to
571 model training. All pre-trained model weights were used to initialize the finetuned model. All gene
572 tokens with both zero and non-zero expression values were used in training. In addition to GEP
573 and GEPC, the ECS, DAR and DSBN finetuning objectives were optimized simultaneously for
574 enhanced cell contrastive learning and explicit batch correction through reverse back-propagation
575 and domain-specific normalization.

576 **Cell type annotation** For the cell type annotation task, we finetuned the model on a reference
577 set with ground-truth labels, and validated the annotation performance on an external query set.
578 The common set of gene tokens between the pre-trained foundation model and the reference set was
579 retained. We pre-processed the expression values prior to model training similar to the integration
580 task. All pre-trained model weights were used to initialize the finetuned model. All gene tokens
581 with both zero and non-zero expression values were used in training. The CLS finetuning objective
582 was used to minimize the classification loss.

583 **Perturbation prediction** Gene editing techniques applied to scRNA-seq experiments have re-
584 vealed cellular responses to various genetic perturbations. However, the vast combinatorial space
585 of potential gene perturbations quickly surpasses the limits of feasible experimentation. Conse-
586 quently, machine learning approaches have been employed to leverage known perturbations and
587 predict unknown ones. To fine-tune the perturbation prediction task, we initially selected highly
588 variable genes and pre-processed the expression values prior to model training. All pre-trained
589 model weights were utilized for initializing the fine-tuned model. During training, all gene tokens
590 with both zero and non-zero expression values were included. To achieve this, we adopted the
591 perturb-GEP finetuning objective with two modifications to the training setup. Firstly, instead
592 of utilizing the masked and unmasked versions of the same cell as input and learning target, we
593 employed a control cell as the input and the perturbed cell as the target. This was achieved by ran-
594 domly pairing a non-perturbed control cell with each perturbed cell to construct the input-target
595 pairs. Secondly, rather than randomly masking gene positions as in the original GEP setting, we
596 employed the target perturbed genes as positions for prediction. The input values consisted of the
597 non-perturbed gene expression values rather than mask values. Consequently, the model learned
598 to predict the post-perturbation responses based on the control gene expressions.

599 **Integrative representation learning for scMultiomic data** scMultiomic data may contain
600 different sequencing modalities in each batch, which presents a more challenging scenario for inte-
601 grative analysis. We examined two data integration settings, paired and mosaic, for scMultiomic
602 data. In the paired setting, all samples (cells) share all the data modalities sequenced. In the
603 mosaic setting, some batches share a few common data modalities but not all. Due to the presence
604 of additional ATAC and/or protein tokens, we inherited the trained gene embeddings for RNA
605 data only, and trained the additional token embeddings and rest of the model from scratch. All
606 tokens with both zero and non-zero expression values were used in training. We used an additional
607 set of modality tokens to indicate the data type of each token (i.e., gene, region, or protein) and
608 to facilitate the masked gene and value prediction in GEP and GEPC finetuning objectives (See
609 Online Methods 4.2.3). In the paired setting, the model was optimized with GEP and GEPC
610 finetuning objectives. In the mosaic setting, DAR was included to facilitate multi-modal batch
611 correction.

612 **Gene Regulatory Network Inference** In the zero-shot setting, we extracted the gene similarity
613 network from scGPT models's gene embeddings based on cosine similarities. In the finetuned
614 setting, we constructed the gene networks in a similar manner from the scGPT model finetuned
615 on the Immune Human dataset. Following Ceglia et al. [10]'s pipelines, we further extracted gene
616 programs from the gene embedding clusters that consist of five or more genes. See Online Methods
617 4.7 for more details on gene network analysis and validation.

618

4.6 Datasets

619 **CELLxGENE scRNA-seq human PBMC Collection** We retrieved over 10.3 million human
620 PBMC scRNA-seq samples from the CELLxGENE portal [11] for foundation model pre-training.
621 A total of 65 datasets were collected from CELLxGENE by filtering on Organism (i.e., Homo
622 sapiens), Tissue (i.e., blood, bone marrow), and Disease (i.e., normal, COVID-19, influenza).

623 **PBMC 10K** The PBMC 10K dataset comprises two scRNA-seq batches of human peripheral
624 blood mononuclear cells (PBMCs) obtained from a healthy donor. The dataset was re-processed
625 by Gayoso et al. [21], resulting in the identification of 3,346 differentially expressed genes. The
626 first batch encompasses 7,982 cells, while the second batch encompasses 4,008 cells. The cell
627 groups annotated using Seurat [55] consist of 9 categories, namely B cells, CD4 T cells, CD8 T
628 cells, CD14+ Monocytes, Dendritic Cells, NK cells, FCGR3A+ Monocytes, Megakaryocytes, and
629 Other.

630 **Immune Human** The Immune Human dataset encompasses five scRNA-seq datasets: one de-
631 rived from human bone marrow and four from human peripheral blood. Various sequencing tech-
632 nologies were employed, including 10X Genomics, 10X Genomics v2, 10X Genomics v3, and Smart-
633 seq2. The dataset comprises a total of 33,506 cells and includes 12,303 genes. The ten distinct
634 batches were defined based on the origin of the donors. The harmonized data encompass 16 cell
635 groups. We used the data re-processed and the annotations provided by Luecken et al. [36].

636 **hPancreas** The hPancreas dataset contains five scRNA-seq datasets of human pancreas cells,
637 re-processed by Chen et al. [12] for the cell type assignment task. The five datasets were split into
638 reference and query sets by data sources. The reference set consists of Braon[5] and Muraro[39]
639 datasets, and the inference set consists of Xin[63], Segerstolpe[56], and Lawlor[30] datasets. The
640 reference and query sets both have 3,000 genes, and ground-truth annotations retained from their
641 original publications. The reference set contains 10,600 cells of 13 cell groups (alpha, beta, ductal,
642 acinar, delta, PSC, PP, endothelial, macrophage, mast, epsilon, schwann, and t_cell). The query
643 set contains 4,218 cells of 11 cell groups (alpha, beta, ductal, PP, acinar, delta, PSC, endothelial,
644 epsilon, mast, MHC class II). Note that MHC class II is a new cell type in the query set not
645 previously seen in the reference set.

646 **Adamson** The Adamson perturbation dataset contains gene expression data from the K562
647 leukemia cell line perturbed by Pertub-seq [1]. This dataset includes 87 unique one-gene pertur-
648 bations, each replicated in around 100 cells.

649 **Norman** The Norman perturbation dataset contains gene expression data from the K562 leukemia
650 cell line perturbed by Pertub-seq [41]. This dataset has 131 two-gene perturbations and 105 one-

651 gene perturbations. Each perturbation is replicated in around 300-700 cells.

652 **10X Multiome PBMC** The 10X Multiome PBMC dataset [14] contains paired single-cell RNA
653 and ATAC data on human PBMC cells sequenced by the 10X Single Cell Multiome protocol. In this
654 dataset, all samples came from the same healthy donor. Each cell contains both gene expression
655 and chromatin accessibility measurements. The processed data by Cao and Gao [9] contains 9,631
656 cells with counts from 29,095 genes and 107,194 regions. The annotations include 19 cell groups
657 (CD14 Mono, CD16 Mono, CD4 Naive, CD4 TCM, CD4 TEM, CD8 Naive, CD8 TEM_1, CD8
658 TEM_2, HSPC, Intermediate B, MAIT, Memory B, NK, Naive B, Plasma, Treg, cDC, gdT, and
659 pDC).

660 **ASAP PBMC** The ASAP PBMC dataset contains four sequencing batches with three data
661 modalities (gene expression, chromatin accessibility, and protein abundance) [38]. The four batches
662 each contain 5,023, 3,666, 3,517, and 4,849 cells respectively. In batches 1 and 2, all samples have
663 4,768 genes and 216 protein measurements from CITE-seq. In batches 3 and 4, all samples have
664 17,742 regions and the same 216 protein measurements from ASAP-seq. The annotations by [38]
665 contain 4 cell groups (Bcell, Myeloid, NK, and Tcell).

666 4.7 Experiment Setup

667 **scRNA-seq batch integration** In this work, we compared the performance of scGPT with
668 three other methods, namely Seurat [55], Harmony [29], and scVI [34]. The evaluation covers
669 batch correction and cell type clustering on two integration datasets: PBMC 10K [21] and Immune
670 Human [36]. Harmony and scVI are highlighted as the top-performing methods in the recent
671 integration benchmark conducted by Luecken et al. [36]. To ensure a fair comparison, all methods
672 were provided with the same number of 1,200 highly variable genes as input. Gene expression
673 values were normalized per cell by considering the total counts across all genes and subsequently
674 log-transformed. The integrated cell embeddings were obtained after the completion of training
675 and were used for evaluation.

676 The evaluation of the integrated cell embeddings was performed using biological conservation
677 metrics proposed by Luecken et al. [36]. These metrics include the normalized mutual information
678 (NMI_{cell}), adjusted Rand index (ARI_{cell}), and average silhouette width (ASW_{cell}). These
679 scores measure the consistency between the derived cell type clusters and the ground truth labels.
680 For easier comparison, we also computed the average of these metrics, referred to as $AvgBIO$.
681 Additionally, we reported the batch correction metrics proposed by Luecken et al. [36] to assess
682 batch mixing. The batch correction performance was quantified using the inverse of the average
683 silhouette width for batch clustering, denoted as ASW_{batch} , and the graph connectivity measure,
684 denoted as $GraphConn$. We computed $AvgBATCH$ as the average of ASW_{batch} and $GraphConn$
685 to summarize the batch mixing performance. Furthermore, we introduced an *Overall* score, which
686 is a weighted sum of $AvgBIO$ and $AvgBATCH$, consistent with the approach taken by [36]. See
687 Supplementary Online Methods S.2 for details of metric calculations.

688 **scRNA-seq cell type annotation** We benchmarked scGPT against the recent transformer-
689 based cell type annotation method TOSICA [12] on the hPancreas dataset. We used the same
690 pre-processed reference and query sets by Chen et al. [12] for model training and validation. The
691 predicted cell type labels on the query set were retrieved for evaluation.

692 We evaluated cell type assignment performance based on four standard classification metrics,
693 *Accuracy*, *Precision*, *Recall*, and *MacroF1*. *Accuracy*, *Precision*, and *Recall* are calculated
694 globally for overall performance, whereas *MacroF1* is averaged per class to increase the weighing
695 of rare cell types. We also reported a normalized confusion matrix with *Precision* by cell type for
696 additional details. See Supplementary Online Methods S.2 for details on metric calculations.

697 **scRNA-seq perturbation** We compare scGPT against the recent perturbation prediction method
698 GEARS [53]. To ensure consistency, we followed the pre-processing steps outlined by Roohani,
699 Huang, and Leskovec [53] in their benchmark. Initially, gene expression values were normalized
700 per cell using the total counts across all genes, and a logarithmic transformation was applied. Sub-
701 sequently, we selected 5,000 highly variable genes and incorporated any perturbed genes that were
702 not initially considered into the gene set. In the experiments, for one-gene perturbations in both
703 datasets Adamson et al. [1] and Norman et al. [41], the perturbations are split to ensure that test
704 perturbations are not seen in training, i.e., no cells in training set has undergone any of the test
705 perturbations. For two-gene perturbations in the Norman et al. [41] dataset, the train-test split
706 consists of three scenarios with increasing difficulty: (1) 0/2 unseen genes, (2) 1/2 unseen genes,
707 and (3) 2/2 unseen genes in the training set.

708 To evaluate the accuracy of perturbation prediction, we employed the Pearson correlation co-
709 efficient (*corr*) between the predicted gene expressions and the ground-truth expression values.
710 Additionally, we calculated a variant of the Pearson metric based on the amount of change in ex-
711 pression post-perturbation compared to the control, denoted as *corr*(Δ). Furthermore, we reported
712 these Pearson metrics for different gene sets, including all genes (*ALL*), and the top 20 differen-
713 tially expressed genes (*DE*). Thus, we presented four evaluation metrics in total, namely *corr* and
714 *corr*(Δ) for the *ALL* and *DE* conditions, respectively. See Supplementary Online Methods S.2 for
715 details of metric calculation.

716 **scMultiomic integration** We benchmarked scGPT in two integration settings, paired and mo-
717 saic, against the recent scMultiomic integration methods Seurat v4 [24], scGLUE [9] and scMoMat
718 [65] respectively. In the paired data integration experiment, we benchmarked scGPT with scGLUE
719 [9] and Seurat v4 [24] on the 10X Multiome PBMC [14] dataset. The same 1,200 highly variable
720 genes and 4,000 highly variable peaks were used as input to all methods. In the mosaic data inte-
721 gration experiment, we benchmarked scGPT with scMoMat [65] on the ASAP PBMC [38] dataset.
722 The same 1,200 highly variable genes, 4,000 highly variable peaks, and all 216 protein features
723 were used as input to both methods. While keeping the input feature set consistent, we used each
724 method's custom pre-processing pipeline to normalize the expression values. The integrated cell
725 embeddings were retrieved for evaluation after training.

726 In both paired and mosaic data integration settings, we evaluated cell embedding quality on
727 the four biological conservation metrics *NMI_cell*, *ARI_cell*, *AWS_cell*, and *AvgBIO*. In the
728 mosaic data integration setting, we further evaluated mixing of different omic batches with the
729 three batch correction metrics *AWS_batch*, *GraphConn*, and *Avg_BATCH*. An overall score was
730 also reported on the mosaic integration experiment. See Supplementary Online Methods S.2 for
731 details on metric calculation.

732 **Gene Regulatory Network Inference** We validated scGPT's gene similarity network against
733 the known HLA and CD antigen networks. For each network, we first defined the related gene set by
734 filtering on gene names with set prefixes (i.e., HLA- and CD-). We then filtered on genes involved
735 in the Immune System R-HSA-168256 pathway from the Reactome 2022 database [50]. For the CD

736 antigens, we used the common genes with the selected HVG set from the Immune Human dataset
737 for the ease of comparison between pre-trained and finetuned models. We then extracted gene
738 embeddings of these selected genes from the scGPT model and constructed a similarity network
739 based on cosine similarity. We highlighted sub-networks of strong connections by selecting edges
740 with cosine similarities greater than a certain threshold (i.e., 0.5 for HLA and 0.4 for CD antigen
741 network). We then compared the sub-networks against known functional groups in immunology.
742 Furthermore, we evaluated the gene similarity relationships encoded by the scGPT model with
743 Reactome. Following Ceglia et al. [10]’s pipelines, we first evaluated whether the neighbors of a
744 gene involved in a known pathway belong to that pathway. Using CD8A gene as an example,
745 we ranked its 10 nearest neighbors (including itself) by cosine similarity in the selected HVG set
746 from the Immune Human dataset, and examined their membership in the Immune System R-HSA-
747 168256 pathway. Subsequently, on a system level, we examined the relationship between cosine
748 similarity of pairs of genes and the number of common pathways that a gene pair is involved in.
749 We reported the Pearson correlation score between cosine similarity score and pathway coverage
750 on the entire gene set across all pathways in Reactome.

751 4.8 Implementation Details

752 The pretrained foundation model has an embedding size of 512. It consists of 12 stacked transformer
753 blocks with 8 attention heads each. The fully connected layer has hidden size of 512. In pre-training,
754 we randomly split the data and used 97% (10 million) of the data for training and 3% (0.3 million)
755 for validation. We set the ratio of genes to generate to be uniformly sampled from three options of
756 0.25, 0.50 and 0.75. The model was optimized by the Adam optimizer, using a mini-batch size of
757 32, at a starting learning rate of 0.0001 and a 0.9 weight decay after each epoch. The model was
758 trained for a total of 6 epochs.

759 For the tasks of scRNA-seq batch integration, cell type annotation, and perturbation prediction,
760 we utilized the same model configuration inherited from the pre-trained model. During the fine-
761 tuning process, we initiated with a learning rate of 0.0001, which decayed to 90% after each epoch.
762 The mask ratio for GEP and GEPC was set to 0.4, while the parameter β in ECS was set to 0.6.
763 When combined with other losses, ECS was assigned a weighting of 10. To divide the datasets
764 into training and evaluation sets, we employed a ratio of 9:1. The model was trained for a fixed
765 duration of 30 epochs, and after each epoch, the GEP loss value was evaluated on the validation
766 set. The reported results correspond to the model with the best validation score. Notably, for the
767 perturbation task (refer to Section section 2.4), we observed that the model typically converged
768 within 3 epochs, and we report the best-validated model accordingly.

769 For the multi-omic integration task, we loaded the gene embeddings from the pre-trained model
770 and used the same embedding size of 512 for all tokens (i.e., including gene, ATAC-peak, and/or
771 protein). The main model is set to have 4 stacked transformer blocks with 8 attention heads each,
772 and a hidden layer size of 512. Each dataset is split into train and evaluation sets at 9:1 ratio.
773 We used DAR weighing of 1.0 for batch integration. We used a starting learning rate of 0.001 and
774 a weight decay of 0.95 after each epoch. We trained the model for fixed 60 epochs and similarly
775 reported the best-validated model.

776 We used the SCANPY python library [62] for gene expression pre-processing, including nor-
777 malization, log-transformation and highly variable gene selection. We used the EpiScanpy python
778 library [15] on chromatin accessibility data for highly variable peak selection. In the scRNA-seq
779 batch integration and scMultiomic integration tasks, the evaluation metrics are calculated using the
780 implementation in `scib.metrics` by Luecken et al. [36]. In the cell-annotation task, the evaluation
781 metrics are implemented using the `scikit-learn` package.

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- 922

923 **S Supplementary**

924 **S.1 Benchmarking results on downstream tasks**

Dataset	Model	Biological Conservation				Batch Correction			Overall
		AvgBIO	<i>NMI_{cell}</i>	<i>ARI_{cell}</i>	<i>ASW_{cell}</i>	AvgBATCH	<i>ASW_{batch}</i>	<i>GraphConn</i>	
Immune Human [36]	scGPT	0.746	0.824	0.784	0.630	0.894	0.817	0.970	0.804
	scVI [34]	0.726	0.803	0.787	0.587	0.923	0.871	0.975	0.805
	Seurat [55]	0.564	0.697	0.441	0.555	0.883	0.858	0.908	0.692
	Harmony [29]	0.743	0.810	0.832	0.588	0.914	0.859	0.968	0.811
PBMC 10K [21]	scGPT	0.812	0.834	0.869	0.732	0.940	0.949	0.931	0.863
	scVI	0.695	0.786	0.704	0.593	0.950	0.971	0.930	0.797
	Seurat	0.753	0.810	0.854	0.595	0.934	0.937	0.931	0.826
	Harmony	0.751	0.810	0.855	0.589	0.945	0.967	0.923	0.829

Table 2: scRNA-seq Integration Benchmark Results. scGPT was benchmarked with scVI [34], Seurat [55], and Harmony [29] on the Immune Human (10 batches) [36] and PBMC 10K (2 batches) [21] datasets for cell type clustering and batch correction performance. We present three aggregate scores *AvgBIO*, *AvgBATCH*, and *Overall*. These aggregate scores were calculated from three detailed biological conservation metrics (NMI_{cell} , ARI_{cell} , ASW_{cell}) and two batch correction metrics (ASW_{batch} , $GraphConn$). See metric details in Supplementary Online Methods S.2

Dataset	Model	Classification Metrics			
		<i>Accuracy</i>	<i>Precision</i>	<i>Recall</i>	<i>MacroF1</i>
hPancreas [12]	scGPT	0.967	0.785	0.694	0.705
	TOSICA [12]	0.961	0.641	0.667	0.641

Table 3: Cell Type Annotation Benchmark Results. scGPT was benchmarked with TOSICA [12] on the hPancreas [12] dataset for cell type annotation performance. We present four classification evaluation metrics *Accuracy*, *Precision*, *Recall*, and *MacroF1*. See metric details in Supplementary Online Methods S.2.

Dataset	Model	Biological Conservation				Batch Correction			Overall
		AvgBIO	<i>NMI_{cell}</i>	<i>ARI_{cell}</i>	<i>ASW_{cell}</i>	AvgBATCH	<i>ASW_{batch}</i>	<i>GraphConn</i>	
10X Multiome PBMC [14] (Paired)	scGPT	0.767	0.818	0.822	0.661	-	-	-	-
	scGLUE [9]	0.747	0.815	0.806	0.619	-	-	-	-
	Seurat v4 [24]	0.722	0.784	0.691	0.691	-	-	-	-
ASAP PBMC [38] (Mosaic)	scGPT	0.562	0.601	0.472	0.614	0.948	0.904	0.992	0.716
	scMoMat [65]	0.546	0.448	0.557	0.633	0.916	0.849	0.983	0.667

Table 4: scMultiomic Integration Benchmark Results. For the paired 10X Multiome PBMC [14] dataset, scGPT was benchmarked with scGLUE [9] and Seurat v4 [24] for cell type clustering performance evaluated on four biological conservation metrics. Batch correction metrics are not applicable to this setting. For the mosaic ASAP PBMC [38] dataset, scGPT was benchmarked with scMoMat [65] for cell type clustering and multiomic integration performance, evaluated on eight biological conservation and batch correction metrics.

925 **S.2 Evaluation Metric Calculations**

926 **S.2.1 Single-cell integration**

927 We adopted the evaluation metric calculations outlined by Luecken et al. [36] in their benchmark
928 study. Each metric is described below.

929 **Normalized Mutual Information**

930 To quantify the concurrence between the cell type labels based on ground truth and the Lou-
931 vain cluster labels obtained from integrated cell embeddings, we computed the normalized mutual
932 information (NMI) score. The Louvain clustering was conducted across resolutions ranging from
933 0.1 to 2, with increments of 0.1. The best score will be selected. The NMI score for cell types,
934 referred to as NMI_{cell} , ranges between 0 and 1, where a higher score indicates a better match of
935 cell types.

936 **Adjusted Rand Index**

937 The adjusted rand index (ARI) was employed to assess both the agreement between the anno-
938 tated labels and the MNI-optimized Louvain clusters. Furthermore, the rand index was adjusted
939 to account for randomly correct labels. The ARI score for cell types, denoted as ARI_{cell} , ranges
940 from 0 to 1, where 0 corresponds to random labeling and 1 represents a perfect match.

941 **Average Silhouette Width**

942 The silhouette width assesses the relationship between a cell's within-cluster distances and its
943 distances to the closest cluster boundaries. By averaging the silhouette widths of all cells, we
944 calculate the average silhouette width (ASW) score. This score ranges from -1 to 1, where a score
945 of 1 indicates well-separated clusters, while scores from -1 to 0 suggest overlapping clusters and
946 misclassification.

947 For evaluating cell type clustering, we compute the ASW score based on cell type labels,
948 represented as ASW_{cell} . To obtain this score, we utilize the following formula:

$$ASW_{cell} = (ASW_C + 1)/2$$

949 Here, C represents the cell types.

950 Regarding batch mixing evaluation, we calculate the ASW score considering batch labels and
951 adjust it by subtracting 1. This score is denoted as ASW_{batch} . The calculation is as follows:

$$ASW_{batch} = 1 - |ASW_B|$$

952 Both ASW_{cell} and ASW_{batch} have values between 0 and 1. Higher scores indicate better
953 cell-type clustering or batch-mixing performance.

954 **Graph Connectivity**

955 The graph connectivity metric quantifies the average proportion of cells within each cell type
956 that are connected through a kNN (k-nearest neighbors) graph. For every cell identity c in the
957 set C , we compute the size of the largest connected component using kNN among cells exclusively

958 belonging to identity c . This value is divided by the total number of cells with identity c to obtain
 959 a normalized measure. The ***GraphConn*** score is then reported as the average across all cell
 960 types:

$$\text{GraphConn} = \frac{1}{|C|} \sum_{c \in C} \frac{|LCC(G_c^{kNN})|}{N_c}$$

961 Here, LCC represents the largest connected component, and N denotes the number of cells of
 962 each celltype.

963 Aggregated Metrics

964 The aggregated metric ***AvgBIO*** calculates the average of biological conservation metrics:

$$\text{AvgBIO} = (\text{ARI}_{cell} + \text{NMI}_{cell} + \text{ASW}_{cell})/3$$

965 Similarly, the aggregated metric ***AvgATCH*** computes the average of batch mixing metrics:

$$\text{AvgATCH} = (\text{ASW}_{batch} + \text{GraphConn})/2$$

966 In accordance with the convention established in [36], an ***Overall*** metric is derived as the
 967 weighted average of ***AvgBIO*** and ***AvgATCH***:

$$\text{AvgATCH} = 0.6 * \text{AvgBIO} + 0.4 * \text{AvgATCH}$$

968 S.2.2 Cell Type Assignment

969 We used the standard classification metrics *Accuracy*, *Precision*, *Recall*, and *MacroF1* to evaluate
 970 cell type assignment performance. The *Accuracy*, *Precision*, *Recall*, and *MacroF1* scores are
 971 calculated from true positives (tp), false positives (fp), true negatives (tn), and false negatives
 972 (fn) globally or averaged per class.

973 The *Accuracy*, *Precision* and *Recall* scores are calculated globally:

$$\text{Accuracy} = \frac{tp}{tp + fp + tn + fn}, \quad \text{Precision} = \frac{tp}{tp + fp}, \quad \text{Recall} = \frac{tp}{tp + fn} .$$

974 The *MacroF1* score is calculated per cell type c first and averaged across cell types:

$$\text{MacroF1} = \sum_{c \in C} \frac{F1_c}{N_c}, \quad \text{where} \quad F1_c = \frac{2 \times \text{Precision}_c \times \text{Recall}_c}{\text{Precision}_c + \text{Recall}_c} .$$

975 The above metrics are calculated using `scikit-learn`'s implementations [8].

976

Supplementary Figures

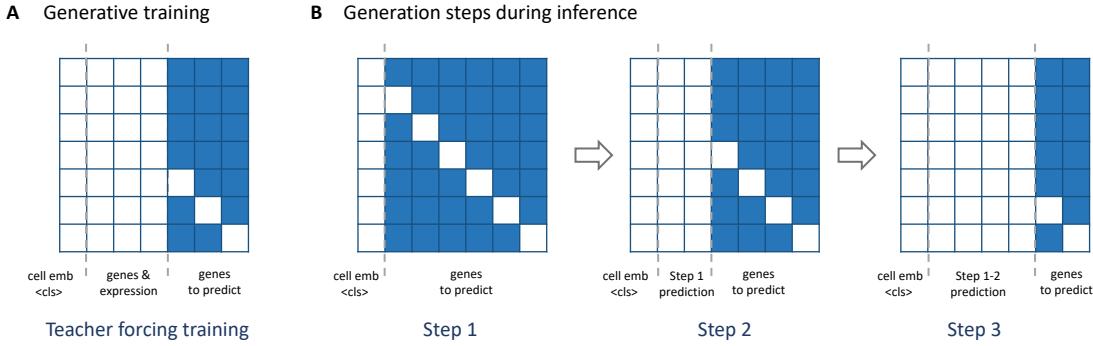


Figure S1: The scGPT Attention Mask. The masked positions are colored in blue, and the allowed positions in white. These masked and unmaksed positions correspond to the $M \times M$ attention map for M input tokens. The row indices correspond to queries and columns correspond to keys. In the self-attention computation of transformers, the attention scores on the marked positions will be removed. The token identity associated with each column is annotated below, namely cell emb $<cls>$ for cell embedding, genes & expressions for known genes, and genes to predict for unknown genes. (a) scGPT attention mask in training where only query gene and the known genes participate in attention computation. (b) At inference time, the attention mask at each step during the iterative process of scGPT cell-prompt generation.

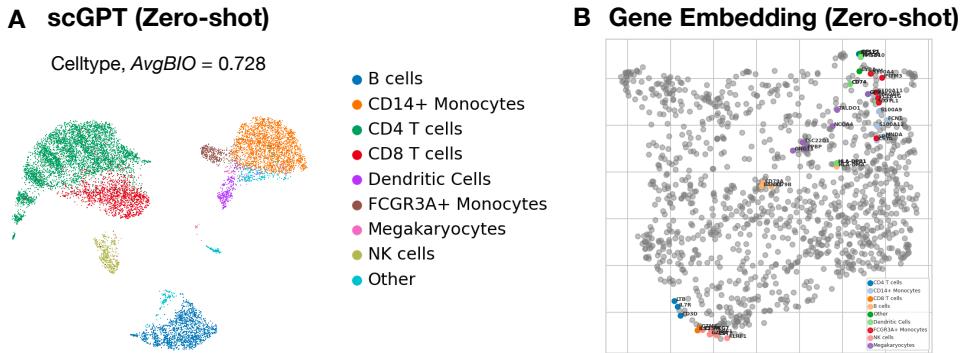


Figure S2: Visualization of Zero-shot scGPT Learned Cell and Gene Embeddings on the PBMC 10K Dataset. (a) UMAP visualization of cell embeddings colored by *cell types*. (a) UMAP visualization of gene embeddings. The highly variable genes corresponding to each *celltype* were colored accordingly.

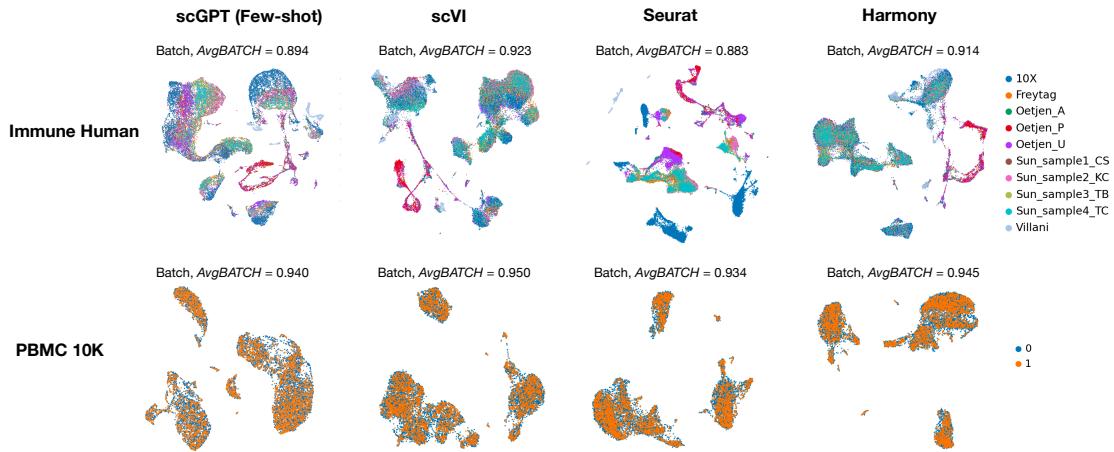


Figure S3: Benchmark of scGPT with scVI [34], Seurat [55], and Harmony [29] on the Immune Human (10 batches) [36] and PBMC 10K (2 batches) [21] Datasets for Batch Correction. UMAP visualization of cell embeddings colored by *sequencing batches*.