molecular systems biology

PerturbNet predicts single-cell responses to unseen chemical and genetic perturbations

Hengshi Yu, Weizhou Qian, Yuxuan Song, and Joshua Welch

Corresponding author(s): Joshua Welch (welchjd@umich.edu)

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(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. Depending on transfer agreements, referee reports obtained elsewhere may or may not be included in this compilation. Referee reports are anonymous unless the Referee chooses to sign their reports.)

Reviewer 1

Thank you for the response to my initial review and your efforts to revise the manuscript - in particular the fact that isomers and covariates are now taken into account. However, despite the substantial time since the first submission, the current version still has major structural, methodological, and presentation issues:

- Lack of sufficient effort to address previous comments: The authors have made only minimal revisions to the manuscript, and rather than focusing on improving the main issues with the core of the method, the presentation and validation of its results, chose to expand the application to other domains (protein) without providing robust evidence to give confidence on the new results. The manuscript still lacks the necessary contextualization and supporting data to fully illustrate its advantages and why it may be better than competing baselines. Given the extended time since the initial submission, a more substantial effort was expected.

- Poor presentation: The new figures, in particular the boxplots with R^2 values contain excessive whitespace and compressing the values to a narrow range, making them difficult to interpret. The presentation of different data splits does not add much value (can be left in supplement), but that space could have been better use to make one much clearer plot across splits. The compression of data visualization in the plots prevents an adequate assessment of performance differences between methods, leaving all the interpretation to the stars indicating p-value significance, which as I commented earlier, can be dependent on various parameters and are not a robust way to evaluate the performance competitively. Statement of the numeric performance values for the compared methods are missing (e.g. mentioned in text or in tables), making it impossible to objectively evaluate the effectiveness of the proposed approach. The bottom rows of panels in Figure 2d and 2e present a y-axis that appears meaningless, lacking clear labels and interpretation. The authors have to make a considerably better effort to illustrate the performance of the methods in a objective and quantitative way, and figures should be improved for clarity.

We have updated all main figures to display only a single train/test split per comparison, removed the p-value indicators, and reformatted the plots to allow for better visual comparison of the boxplots. We moved the box plots for the remaining train/test splits to the supplement. To summarize the results across five train/test splits, we included a heatmap illustrating the proportion of cases in which PerturbNet outperformed other methods. Additionally, we provide tables presenting the mean and median values of the evaluation metrics for each dataset to facilitate a clearer comparison between models.

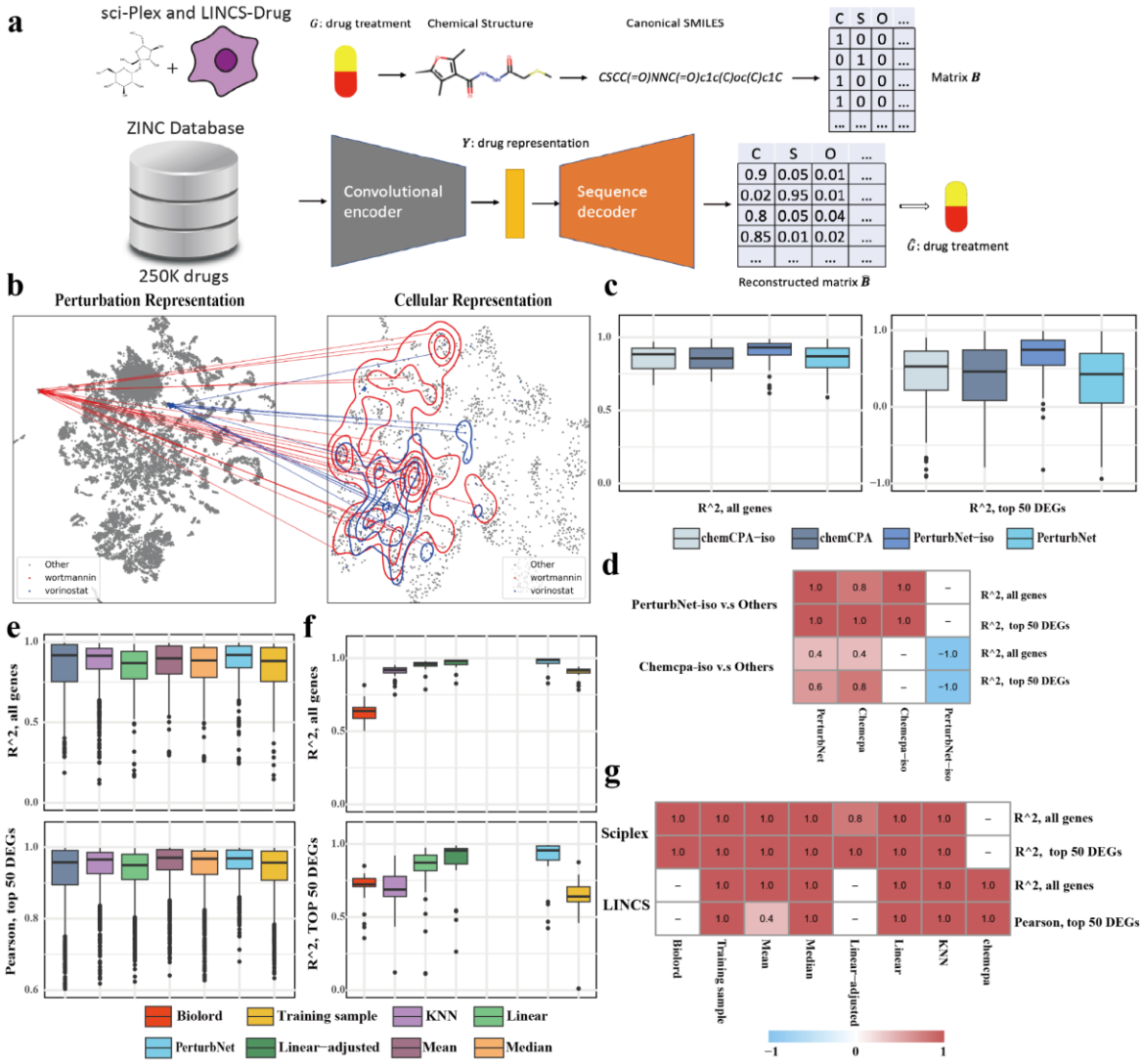


Fig. 2 PerturbNet predicts response to small molecule treatment. ***(a)*** *Diagram of the chemical variational autoencoder (ChemicalVAE) architecture for encoding small molecules represented as SMILES strings. The network was trained on the ZINC dataset, consisting of approximately 250,000 drug-like molecules. No gene expression information is used for training the drug representation network. Note that a cell representation network is also trained on gene expression values (not shown here).* ***(b)*** *Visualization of PerturbNet predictions for two distinct perturbations from the LINCS-Drug dataset. The UMAP coordinates are computed from the latent spaces of the perturbation network (left) and the cell state network (right). The mapping function learned by the cINN is indicated with lines connecting the perturbation and cell state representations. The predicted cell state distributions are also indicated with contour lines.* ***(c)*** *Box plots of R^2 calculated on all genes and the top 50 differentially expressed genes (DEGs) in one test split of the LINCS-Drug dataset for chemCPA and PerturbNet with (-iso) and without stereoisomers shared between train and test sets.* ***(d)*** *Heatmap showing the proportion of train/test splits in which PerturbNet or ChemCPA (trained with stereoisomers) outperforms the same model trained without stereoisomers across five test splits of the LINCS-Drug dataset. "Outperforms" here means a significant difference by a one-sided Wilcoxon test.* ***(e)*** *Box plots of evaluation metrics in one test split of the LINCS-Drug dataset (plots for additional splits are shown in the supplement).* ***(f)*** *Box plots of evaluation metrics in one test split of the sci-Plex dataset (plots for additional splits are shown in the supplement). Some models are not displayed due to out-of-scale values for certain metrics.****(g)*** *Heatmap showing the proportion of train/test splits in which PerturbNet achieves significantly better performance (based*

on one-sided Wilcoxon tests) compared to competing models (columns) across five train/test splits of the sci-Plex and LINCS-Drug datasets.

Model	Median R^2	Mean R^2	Median R^2 (DEG)	Mean R^2 (DEG)
Chemcpa-iso	<u>0.891</u>	<u>0.319</u>	<u>0.563</u>	<u>0.319</u>
Chemcpa-iso-free	0.857	0.192	0.418	0.192
PerturbNet-iso	**0.933**	**0.623**	**0.794**	**0.623**
PerturbNet-iso-free	0.874	0.154	0.394	0.154

Table. 1: Summary of mean and median evaluation metrics on the LINCS-Drug dataset for chemCPA and PerturbNet with (-iso) and without stereoisomers shared between train and test sets. The top-performing values are bolded and the second-best are underlined for each metric.

Model	Median R^2	Mean R^2	Median Pearson (DEG)	Mean Pearson (DEG)
Chemcpa	0.899	0.862	0.956	0.931
KNN	<u>0.912</u>	0.873	0.965	0.940
Linear	0.869	0.847	0.950	0.932
Mean	0.901	<u>0.881</u>	**0.971**	<u>0.954</u>
Median	0.888	0.866	<u>0.968</u>	0.944
PerturbNet	**0.919**	**0.894**	**0.971**	**0.958**
Training sample	0.887	0.853	0.958	0.936

Table. 2: Summary of mean and median evaluation metrics on the LINCS-Drug dataset. The top-performing values are bolded and the second-best are underlined for each metric.

Model	Median R^2	Mean R^2	Median R^2 (DEG)	Mean R^2 (DEG)
Biolord	0.587	0.554	0.716	0.658
KNN	0.918	0.893	0.664	0.328
Linear	0.954	0.936	0.825	0.569
Linear-adjusted	<u>0.976</u>	<u>0.964</u>	<u>0.917</u>	<u>0.824</u>
Mean	-6.271	-6.156	-10.161	-11.838
Median	-0.636	-0.647	-2.296	-2.364
PerturbNet	**0.984**	**0.968**	**0.951**	**0.865**
Training sample	0.915	0.894	0.596	0.356

Table. 3: Summary of mean and median evaluation metrics on the sci-Plex dataset. The top-performing values are bolded and the second-best are underlined for each metric.

- Inconsistent performance: The performance of PerturbNet in unseen perturbations is not consistently better than other baselines. This is particularly acute when not all genes are considered, but a set of likely more relevant (and higher expressed) genes such differentially expressed or with large effect (Fig. 5d, Figure S4, Figure 3c DEGs). A linear model baseline for Fig. 2 is not shown. This demonstrates that baselines such as linear models or KNNs can do comparatively well.

Following the reviewer's comment, we have added a linear baseline model (both with and without incorporating covariates) to Fig. 2, and PerturbNet significantly outperforms this model. We further added new mean and median baselines following reviewer 2's comments.

We also think that our results are more impressive than what the reviewer suggests. Here is a summary of how the baseline models fare:

- *PerturbNet is statistically significantly better than every baseline model on the SciPlex dataset (Fig. 2). The only exception is that the covariate-adjusted linear model is comparable (not better) on 1 out of 5 train/test splits. If the metrics are averaged across all 5 splits, PerturbNet achieves the best performance.*
- *PerturbNet is statistically significantly better than every baseline model on the LINCS-Drug dataset (Fig. 2). The only exception is that the mean model achieves comparable (not better) performance on 2 out of 5 train/test splits. If the metrics are averaged across all 5 splits, PerturbNet achieves the best performance.*
- *PerturbNet is statistically significantly better than every baseline model on the Norman dataset (Fig. 3). The only exception is that the KNN model achieves comparable (not better) performance on 1 out of 5 train/test splits and the linear model achieves comparable (not better) performance on 2 out of 5 train/test splits. If the metrics are averaged across all 5 splits, PerturbNet achieves the best performance.*
- *The KNN model outperforms PerturbNet on the Ursu dataset in 4 out of 5 train/test splits (Fig. 4). PerturbNet outperforms all baselines on all splits in the Jorge dataset (Fig. 4).*

We note that PerturbNet outperforms current state-of-the-art methods across all of the train-test splits mentioned above (and there are no previous methods that can make predictions for Ursu and Jorge).

We would also like to point out that previous studies have reported only single numbers rather than individual results from multiple train/test splits. The only reason the reviewer can raise this point is because we have been fully rigorous in reporting the results from every train/test split.

We have also updated the visualizations for Fig. 2 , Fig. 3 and Fig. 4 (originally Fig.5) and added tables to facilitate easier comparisons.

Model	Median R^2	Mean R^2	Median Pearson (DEG)	Mean Pearson (DEG)
Chemcpa	0.899	0.862	0.956	0.931
KNN	<u>0.912</u>	0.873	0.965	0.940
Linear	0.869	0.847	0.950	0.932
Mean	0.901	<u>0.881</u>	**0.971**	<u>0.954</u>
Median	0.888	0.866	<u>0.968</u>	0.944
PerturbNet	**0.919**	**0.894**	**0.971**	**0.958**
Training sample	0.887	0.853	0.958	0.936

Table. 2: Summary of mean and median evaluation metrics on the LINCS-Drug dataset. The top-performing values are bolded and the second-best are underlined for each metric.

Model	Median R^2	Mean R^2	Median R^2 (DEG)	Mean R^2 (DEG)
Biolord	0.587	0.554	0.716	0.658
KNN	0.918	0.893	0.664	0.328
Linear	0.954	0.936	0.825	0.569
Linear-adjusted	<u>0.976</u>	<u>0.964</u>	<u>0.917</u>	<u>0.824</u>
Mean	-6.271	-6.156	-10.161	-11.838
Median	-0.636	-0.647	-2.296	-2.364
PerturbNet	**0.984**	**0.968**	**0.951**	**0.865**
Training sample	0.915	0.894	0.596	0.356

Table. 3: Summary of mean and median evaluation metrics on the sci-Plex dataset. The top-performing values are bolded and the second-best are underlined for each metric.

Model	Median R^2	Mean R^2	Median R^2 (DEG)	Mean R^2 (DEG)
Biolord	<u>0.936</u>	<u>0.923</u>	0.540	0.436
GEARS	0.858	0.853	0.513	0.451
KNN	0.918	0.901	0.452	0.409
Mean	-0.097	-0.115	-1.447	-1.549
Median	0.170	0.152	-1.408	-1.439
PerturbNet	**0.942**	**0.928**	**0.629**	**0.535**
Training sample	0.930	0.912	0.430	0.375
Linear	0.891	0.830	<u>0.561</u>	<u>0.489</u>

Table. 4: Summary of mean and median evaluation metrics on the Norman et al. dataset. The top-performing values are bolded and the second-best are underlined for each metric.

Model	Median R^2 (large)	Mean R^2 (large)	Median R^2 (DEG)	Mean R^2 (DEG)
KNN	**0.982**	**0.942**	**0.986**	**0.948**
Linear	-22.932	-27.767	-24.400	-27.518
Mean	-0.473	-0.743	-0.806	-0.650
Median	0.022	-0.006	-0.621	-0.691
PerturbNet	<u>0.870</u>	<u>0.814</u>	<u>0.882</u>	<u>0.823</u>
Training sample	0.697	0.647	0.852	0.787

Table. 5: Summary of mean and median evaluation metrics on the Ursu et al. dataset. The top-performing values are bolded and the second-best are underlined for each metric.

Model	Median R^2 (large)	Mean R^2 (large)	Median R^2 (DEG)	Mean R^2 (DEG)
KNN	-4.031	**-41.873**	0.971	0.955
Linear	-10.186	-139.809	0.837	0.685
Mean	-68.699	-653.178	0.027	-0.208
Median	NA	NA	NA	NA
PerturbNet	**0.2**	-99.65	**0.987**	**0.983**
Training sample	-4.519	-44.121	0.972	0.958

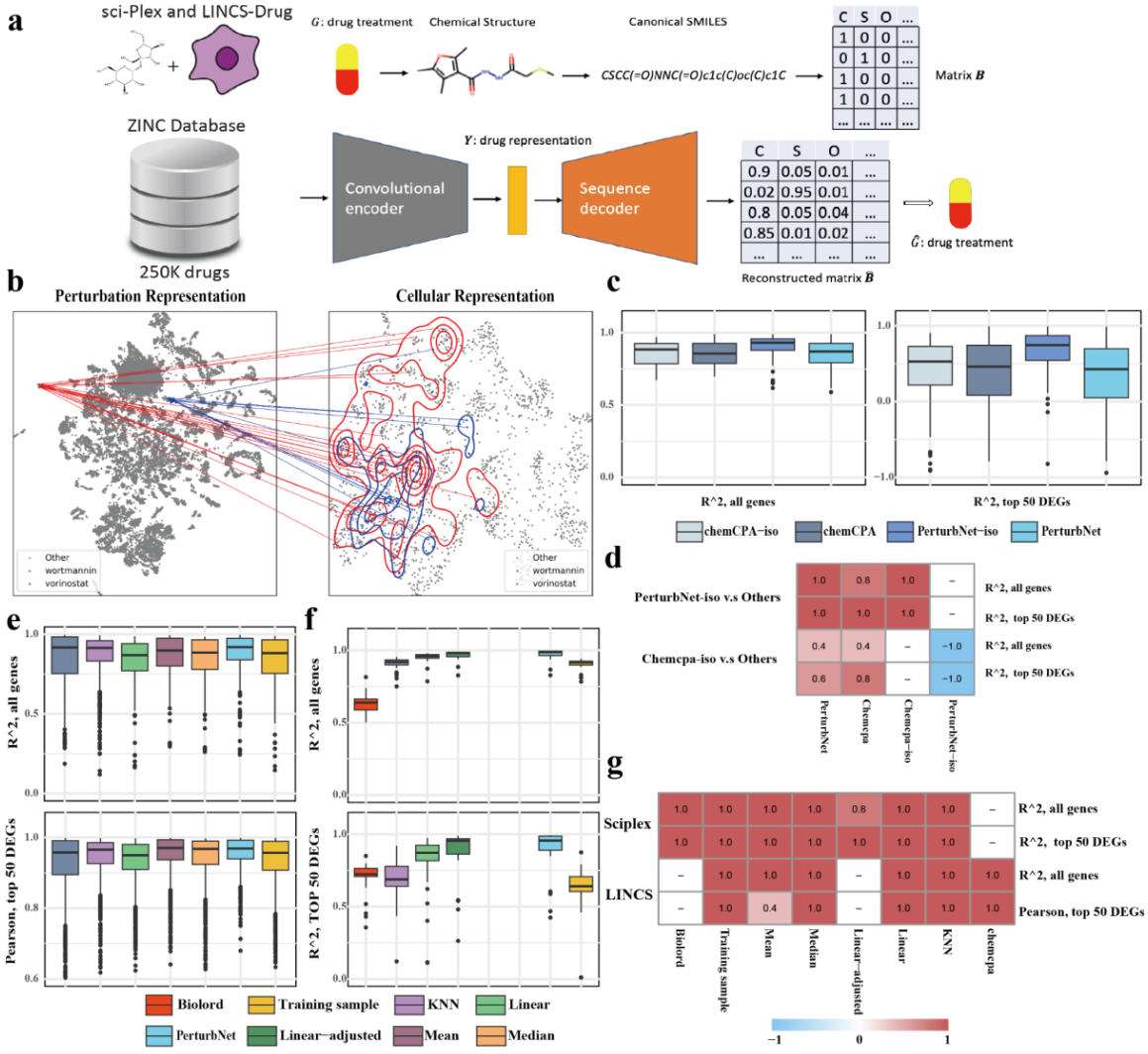


Fig. 2 PerturbNet predicts response to small molecule treatment. (a) *Diagram of the chemical variational autoencoder (ChemicalVAE) architecture for encoding small molecules represented as SMILES strings. The network was trained on the ZINC dataset, consisting of approximately 250,000 drug-like molecules. No gene expression information is used for training the drug representation network. Note that a cell representation network is also trained on gene expression values (not shown here).* ***(b)*** *Visualization of PerturbNet predictions for two distinct perturbations from the LINCS-Drug dataset. The UMAP coordinates are computed from the latent spaces of the perturbation network (left) and the cell state network (right). The mapping function learned by the cINN is indicated with lines connecting the perturbation and cell state representations. The predicted cell state distributions are also indicated with contour lines.* ***(c)*** *Box plots of R^2 calculated on all genes and the top 50 differentially expressed genes (DEGs) in one test split of the LINCS-Drug dataset for chemCPA and PerturbNet with (-iso) and without stereoisomers shared between train and*

test sets. ***(d)*** *Heatmap showing the proportion of train/test splits in which PerturbNet or ChemCPA (trained with stereoisomers) outperforms the same model trained without stereoisomers across five test splits of the LINCS-Drug dataset. "Outperforms" here means a significant difference by a one-sided Wilcoxon test.* ***(e)*** *Box plots of evaluation metrics in one test split of the LINCS-Drug dataset (plots for additional splits are shown in the supplement).* ***(f)*** *Box plots of evaluation metrics in one test split of the sci-Plex dataset (plots for additional splits are shown in the supplement). Some models are not displayed due to out-of-scale values for certain metrics.****(g)*** *Heatmap showing the proportion of train/test splits in which PerturbNet achieves significantly better performance (based on one-sided Wilcoxon tests) compared to competing models (columns) across five train/test splits of the sci-Plex and LINCS-Drug datasets.*

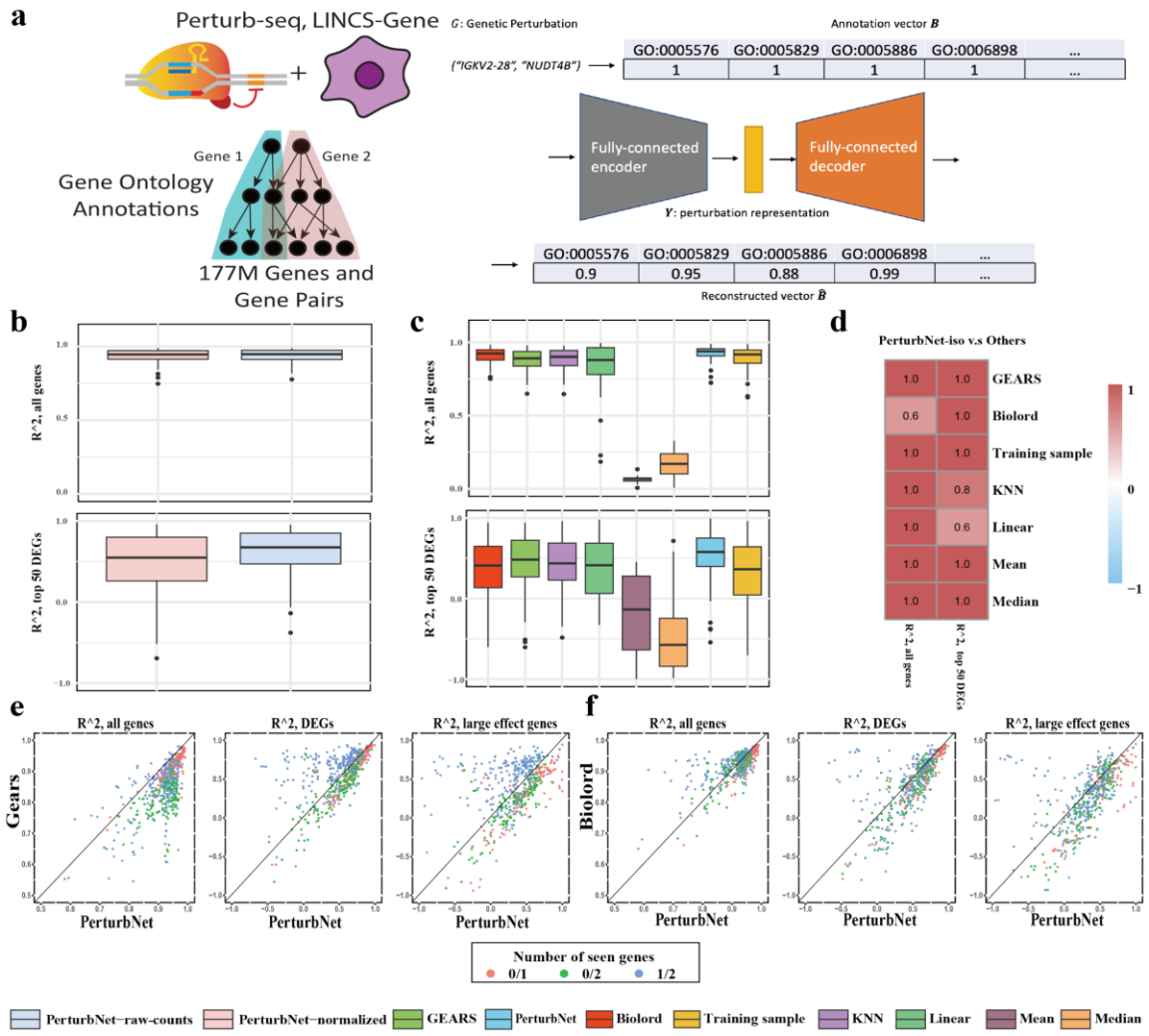


Fig. 3 PerturbNet predicts response to genetic perturbation. ***(a)*** *Diagram of the GenotypeVAE architecture. Perturbations are represented in terms of their gene ontology annotations. The network is trained on all one- and two-gene combinations (approximately 177 million).* ***(b)*** *Box plots of R^2 values for unseen genetic perturbations, calculated on all genes and the top 50 differentially expressed genes (DEGs) in one test split of the Norman et al. dataset (plots for additional splits are in the supplement). "PerturbNet-raw-count" utilizes a zero-inflated negative binomial (ZINB) likelihood for the cell representation network, while "PerturbNet-normalized" employs a Gaussian likelihood.* ***(c)*** *Box plots of R^2 values for unseen genetic perturbations, calculated on all genes and the top 50 DEGs in one test split of the Norman et al. dataset.* ***(d)*** *Heatmap showing the fraction of train/test splits in which PerturbNet achieves significantly better performance (based on one-sided Wilcoxon tests) compared to competing models (columns) on the Norman et al. dataset.* ***(e)–(f)*** *Scatter plots of R^2 values for unseen genetic perturbations, calculated across all genes, the top 50 differentially expressed genes (DEGs), and large-effect genes from the Norman et al. dataset. Data points are aggregated from all five test splits. Different colors represent the "number of seen genes". Labels such as "0/1" indicate that the test perturbation affects one unseen*

gene, while "0/2" indicates two unseen genes are perturbed. "1/2" denotes that two genes are perturbed, but one of the target effects has already been observed independently or in combination with other genetic perturbations.

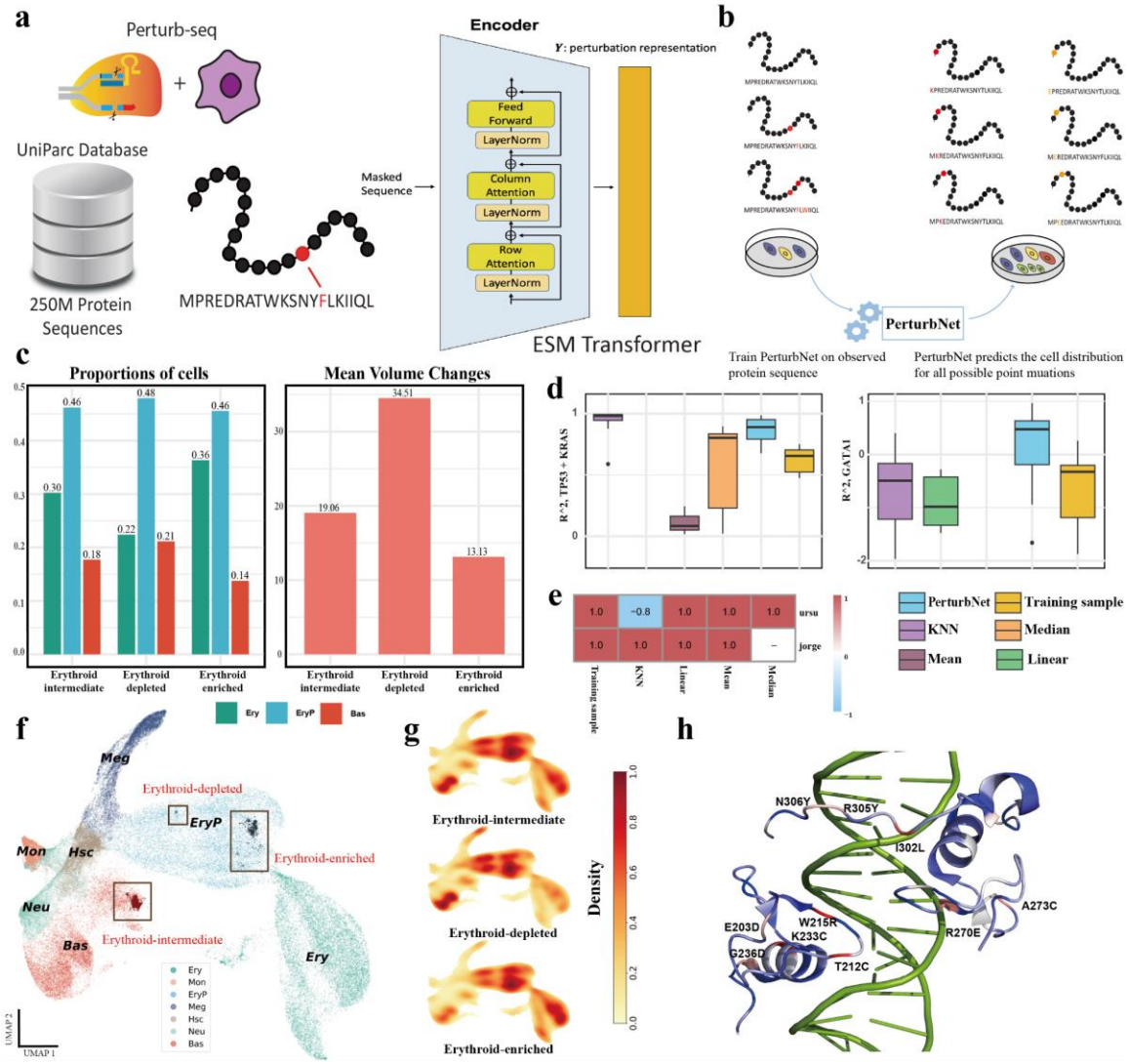


Fig. 4 PerturbNet predicts response to coding sequence mutation. (a) *Diagram of the approach for training the representation network for coding sequence mutations. Each perturbation is an amino acid sequence edited by CRISPR. We used an evolutionary scale modeling (ESM) transformer pre-trained on the UniParc database, which contains 250 million sequences.* ***(b)*** *Workflow for predicting the effects of all possible point coding variants along a specific protein sequence. PerturbNet is trained using all available coding variants, and then used to generate cell state distributions for every possible protein variant at each position within the sequence.* ***(c)*** *Bar plots showing the cell type proportions (left) and the mean volume changes induced by coding variants (right) for the three predicted variant clusters along the GATA1 sequences.* ***(d)*** *Box plots of R^2 values for unseen genetic perturbations (coding variants), calculated on large-effect genes in the datasets from Ursu et al. (TP53 + KRAS) and Jorge et al. (GATA1). The median model is not shown for Jorge et al. because it produces out-of-range values.* ***(e)*** *Heatmap showing the proportion of train/test splits for which PerturbNet significantly outperforms (based on one-sided Wilcoxon tests) other models (columns).* ***(f)*** *UMAP visualization of predicted cell distributions for all possible point coding variants and observed coding variants on the GATA1 protein, with three distinct perturbation clusters highlighted.* ***(g)*** *Cell density of each perturbation cluster in the UMAP.* ***(h)*** *The top 10 predicted large-effect coding variants are located at or near critical GATA1-DNA interacting regions.*

- Insufficient technical details on methodology: While there has been some improvement, the technical descriptions remain vague. Several methodological statements are ambiguous, preventing reproducibility. For example, "Data were preprocessed using Scanpy" lacks details on the specific preprocessing steps, normalization, or parameter choices. "Test set perturbations were at least partially unseen during training" is imprecise and requires clarification on the extent to which perturbations were unseen and how this was ensured. The description of model training, hyperparameter selection, and evaluation criteria is still too high-level and should be expanded with explicit details.

We have added these details to the Methods section, including the specific parameters used in Scanpy and the steps taken to create the split for the Norman et al. dataset. In detail, we add the code we used to preprocessed the dataset in the methods:

```
sc.pp.normalize_total(adata, target_sum = 1e4)

sc.pp.log1p(adata)

sc.pp.highly_variable_genes(adata, min_mean=0.0125, max_mean=5, min_disp=0.5)
```

We also clarify the steps of creating test splits in Norman dataset: "To construct our test set, we randomly selected three different types of perturbations: (1) one gene perturbed and one gene unobserved; (2) two genes perturbed and one gene unobserved; and (3) two genes perturbed and two genes unobserved. This ensures that all test perturbations contain one or more unobserved gene targets."

For model training and hyperparameter selection, in addition to improving the original text descriptions, we also created tables to summarize the parameters for each model, which are shown at the bottom of this section.

For evaluation criteria, we improved the text descriptions and added formulas for each metric. We also included the scanpy function calls used to select differentially expressed genes.

Component	Configuration
Input	Shape: (batch_size, 120, 35)
Conv1D Layer 1	`Conv1d(in=120, out=9, kernel_size=9)` + Tanh + BatchNorm1d
Conv1D Layer 2	`Conv1d(in=9, out=9, kernel_size=9)` + Tanh + BatchNorm1d
Conv1D Layer 3	`Conv1d(in=9, out=10, kernel_size=11)` + Tanh + BatchNorm1d
Flatten + FC (Encoder)	`Linear(90 → 196)` + Tanh + Dropout + BatchNorm1d
Latent Layers	Two `Linear(196 → 196)` layers for mean and log-variance
Decoder FC	`Linear(196 → 196)` + Tanh + Dropout + BatchNorm1d
Repeat Vector	Expand to shape `(batch_size, 120, 196)`
GRU Decoder	`GRU(input_size=196, hidden_size=488, num_layers=3)` + Tanh
Final FC + Output	`Linear(488 → 35)` + Softmax (applied per timestep)
Output Shape	`(batch_size, 120, 35)`
Dropout Rate	0.0828
Latent Dim (`z_dim`)	196
GRU Hidden Size	488
Training Batch Size	128
Learning Rate	1×10^{-4}
Epochs	525
Dataset	ZINC

Table. S2: ChemicalVAE Model Architecture and Training Parameters

Component	Configuration
Input	Shape: (batch_size, 15,988); one-hot annotation vectors from GO
Encoder Layer 1	`Linear(15988 → 512)` + BatchNorm1d + LeakyReLU + Dropout
Encoder Layer 2	`Linear(512 → 256)` + BatchNorm1d + LeakyReLU + Dropout
Latent Mean Layer	`Linear(256 → 10)`
Latent Std Layer	`Linear(256 → 10)`
Decoder Layer 1	`Linear(10 → 256)` + BatchNorm1d + LeakyReLU + Dropout(p=0.2)
Decoder Layer 2	`Linear(256 → 512)` + BatchNorm1d + LeakyReLU + Dropout(p=0.2)
Output Layer	`Linear(512 → 15988)` + Sigmoid
Dropout Rate	0.2
Latent Dim (`z_dim`)	10
Training Batch Size	128
Learning Rate	1×10^{-4}
Epochs	300
Dataset	GO Consortium gene ontology annotations (single and double target genes)

Table. S3: GenotypeVAE Model Architecture and Training Parameters

Component	Configuration
Standard VAE (for normalized data)	
Input	Gene expression vectors (normalized data), shape: (batch_size, x_{dim})
Encoder Layer 1	`Linear(`x_{dim} `→ 512)` + BatchNorm + LeakyReLU + Dropout(p=0.2)
Encoder Layer 2	`Linear(512 → 256)` + BatchNorm + ReLU + Dropout(p=0.2)
Latent Mean Layer	`Linear(256 → 10)`
Latent Scale Layer	`Linear(256 → 10)` + Softplus
Decoder Layer 1	`Linear(10 → 256)` + BatchNorm + LeakyReLU + Dropout
Decoder Layer 2	`Linear(256 → 512)` + BatchNorm + LeakyReLU + Dropout
Output Layer	`Linear(512 →` x_{dim}`)`
Latent Dim (z_{dim})	10
Dropout Rate	0.2
Learning Rate	1×10^{-4}
Batch Size	128
Epochs	150
scVI Model (for count data, using ZINB likelihood)	
Model Type	Variational Autoencoder from `scvi-tools 0.7.1` [32]
Likelihood	Zero-inflated Negative Binomial (ZINB)
Latent Dim (z_{dim})	10
Epochs	700 (default settings)
Sampling Strategy	Library size sampled from training set during generation

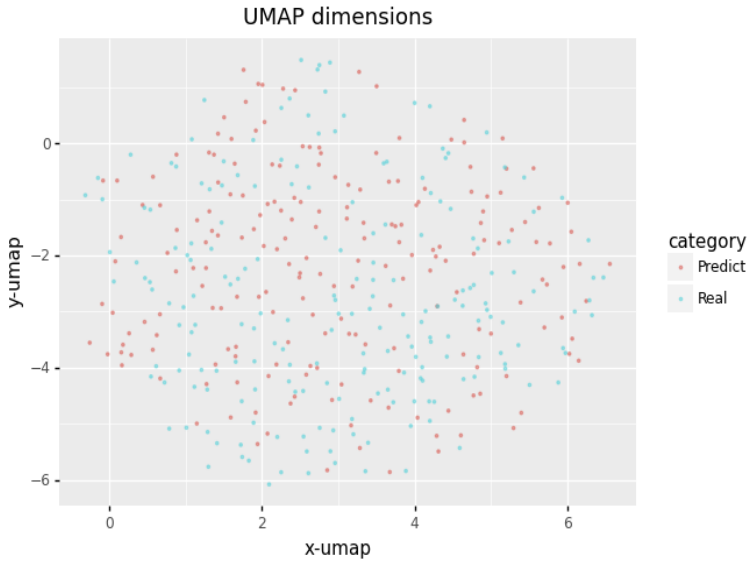
Table. S4: Cell Representation Network Architecture and Training Parameters

Component	Configuration
Flow Architecture	20 invertible blocks, each with: - Alternating affine coupling layer - ActNorm layer - Fixed permutation layer
Embedding Module	- Input: conditioning vector - 2 hidden layers, hidden dim = 256 - Output dim = 10 - Activation: LeakyReLU - Optional BatchNorm (enabled)
Shared Model Parameters	`in_channels` = 10, `embedding_dim` = 10, `hidden_dim` = 1024, `hidden_depth` = 2, `activation` = none, `conditioner_use_bn` = True
Dataset-specific Configuration	
Norman et al.	`conditioning_dim` = 10, `epochs` = 50
Ursu et al.	`conditioning_dim` = 1280, `epochs` = 50
Jorge et al.	`conditioning_dim` = 1280, `epochs` = 50
LINCS-Drug	`conditioning_dim` = 196, `epochs` = 100
sci-Plex	`conditioning_dim` = 200, `epochs` = 100
Training Parameters	Batch size = 128, Learning rate = 4.5×10^{-6}

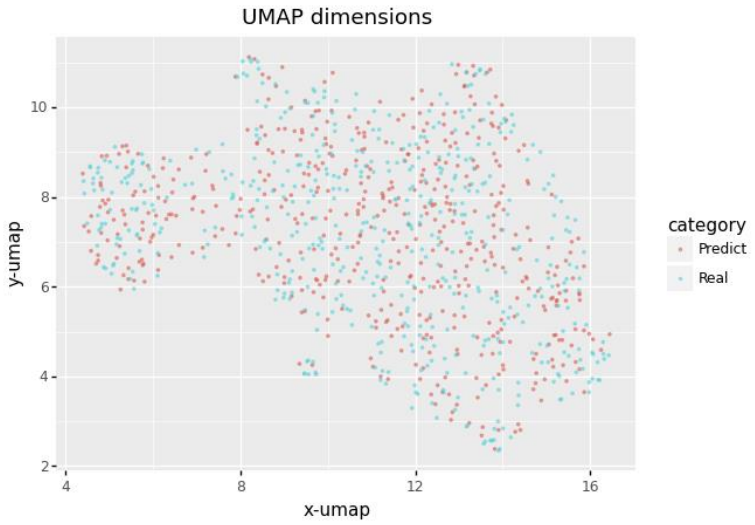
Table. S5: Conditional Invertible Neural Network (cINN) Architecture and Training Parameters

- Generalization of the generated profiles: Thank you for the clarification on how the sampling is performed. In the notebooks provided (https://github.com/welch-lab/PerturbNet/blob/main/notebooks/Tutorial_PerturbNet_coding_variants.ipynb, https://github.com/welch-lab/PerturbNet/blob/main/notebooks/Tutorial_PerturbNet_Genetic.ipynb) it is possible to see areas of real data (in the UMAP plots) which are not well covereed by the generated profiles. Investigating these could give us a better understanding of the limitations of the method.

The UMAPs of predicted and observed data are in fact very well-mixed, indicating that PerturbNet is accurately modeling the data. See these plots:

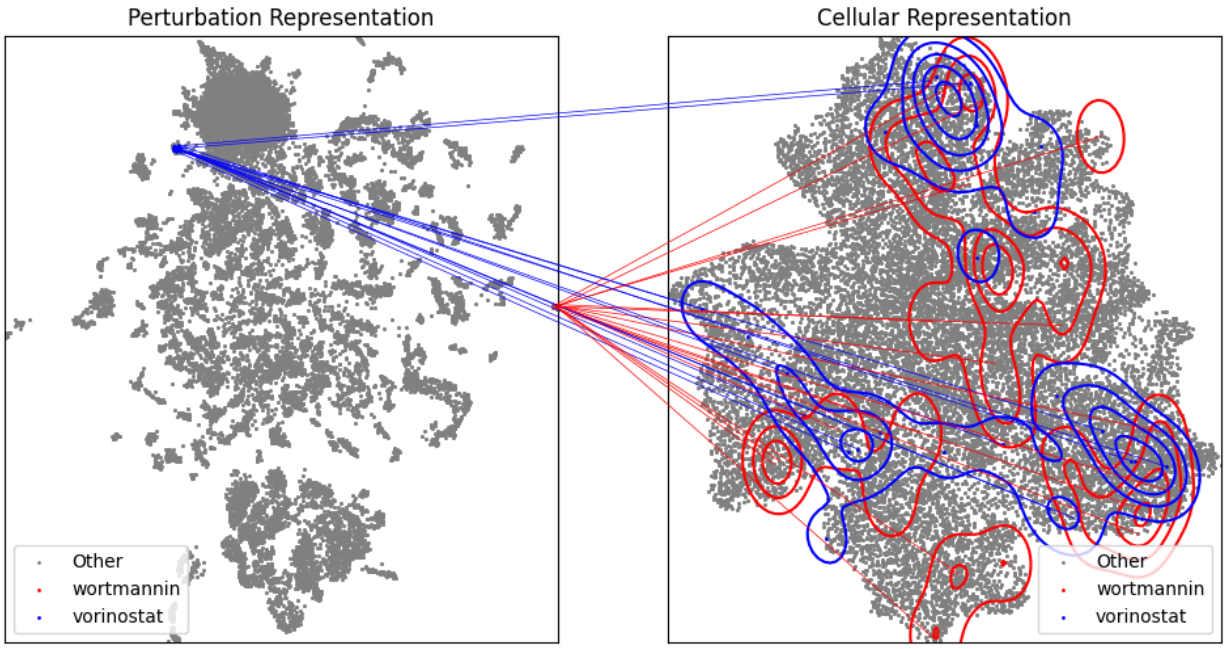


Umap in genetic perturbation notebook



Umap in coding variants perturbation notebook

Perhaps the reviewer is referring to the plot showing different perturbation effects (such as the one below). This plot is supposed to show different distributions to highlight perturbation-induced cell state shifts. The real cells (shown in gray on the background) represent the full spectrum of cell states in the dataset, while an individual perturbation (blue or red in this figure) occupies a subset of the cell state landscape.



Example visualization of PerturbNet predictions for two distinct perturbations from the LINCS-Drug dataset.

- Organization and writing: The manuscript remains poorly structured, with key methodological details dispersed in a way that makes comprehension challenging. The flow of information should be improved to guide the reader more effectively through the motivation, methodology, results, and implications.

We modified the presentation of the methodology in the main text to summarize the architectures used in a single place and in a top-down fashion, referring to the methods section for further details. This makes it easier to parse the presentation of the subsequent result sections.

Reviewer #1 (Remarks on code availability):

Python 3.7 is no longer supported as of June 27, 2023. To maximize the adoption and maintainability of source code, I would recommended updating to a later version of Python (3.13 is the latest released version, but anything above 3.10 is still relatively

modern). Docunmentation of the programmatic interface beyond the notebooks would also provide a better way to increase adoption of the method.

We rewrote all of the tensorflow parts of the code in PyTorch, upgraded to a recent Python version, and added Sphinx autodocs. The ReadTheDocs page is here: https://perturbnet.readthedocs.io/en/latest/

Reviewer 2

I think the authors have strengthened their case that this method is currently the state-of-the-art for perturbation prediction, which is proving to be a difficult problem. This includes addressing comparison to published methods and addressing some of the concerns raised by recent preprints that have highlighted that these models are often defeated by simple baselines. The paper is also quite easy to read (and in fact could probably be shortened without losing much).

One challenge is that the absolute improvements relative to simpler baselines are not that large. E.g., a k-nn model performs well in Figs. 2d and 6d and a linear model performs well in Fig. 3c, in line with the preprints. This is despite these models having no access to the side information implicit in the representations used in PerturbNet. Nevertheless, it does seem to outperform them most of the time, which does seem to move the needle in the field.

Given these modest improvements relative to baselines, it's not entirely clear what new capabilities PerturbNet unlocks in its current form. As the authors state, this may be partly due to the limited existing datasets for training, which I do suspect will improve quickly. A separate advantage is that the architecture here is simple and easily extendable in the future, so perhaps learning better representations or encoding more information at this step is a path to better predictive power. I do see and acknowledge the potential, and it's possible this architecture is the right direction to focus.

We would like to emphasize that we are the first to show that effects of protein-coding mutations on global gene expression can be predicted from the mutated protein sequence. This is an important qualitative contribution beyond the quantitative bump in performance that we show.

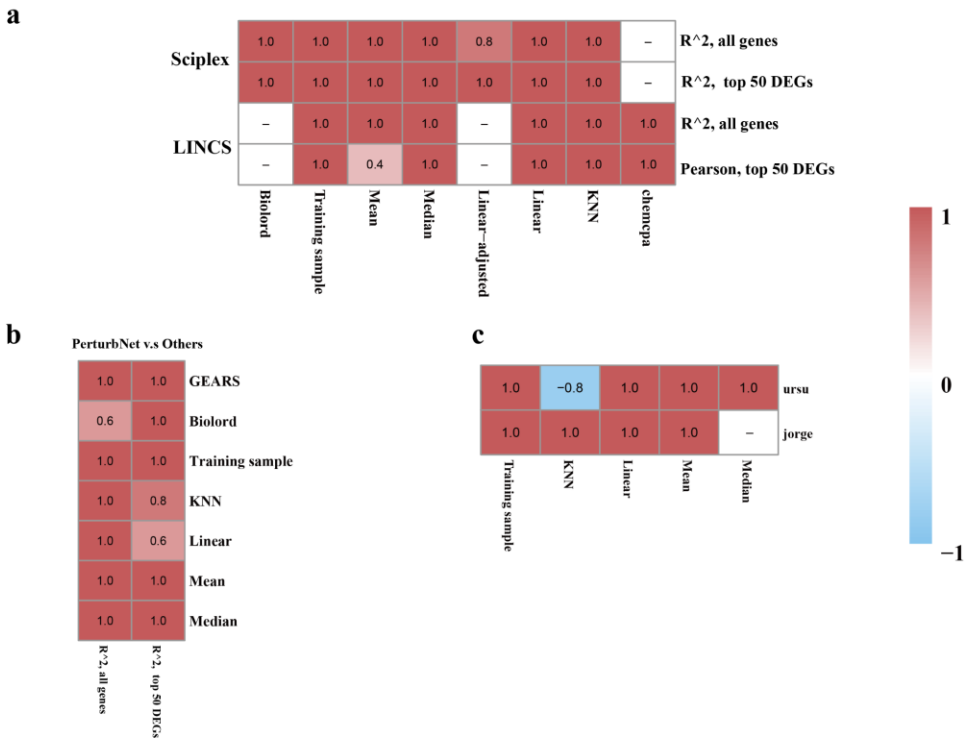
I think if the authors can truly establish that their method is the state-of-the-art then it is a valuable advance, as defeating simple baselines has proven difficult. This requires a handful of additional comparisons that I don't think are particularly onerous to carry out.

Otherwise, I think this work is suitable for a more technical journal as this field continues to develop.

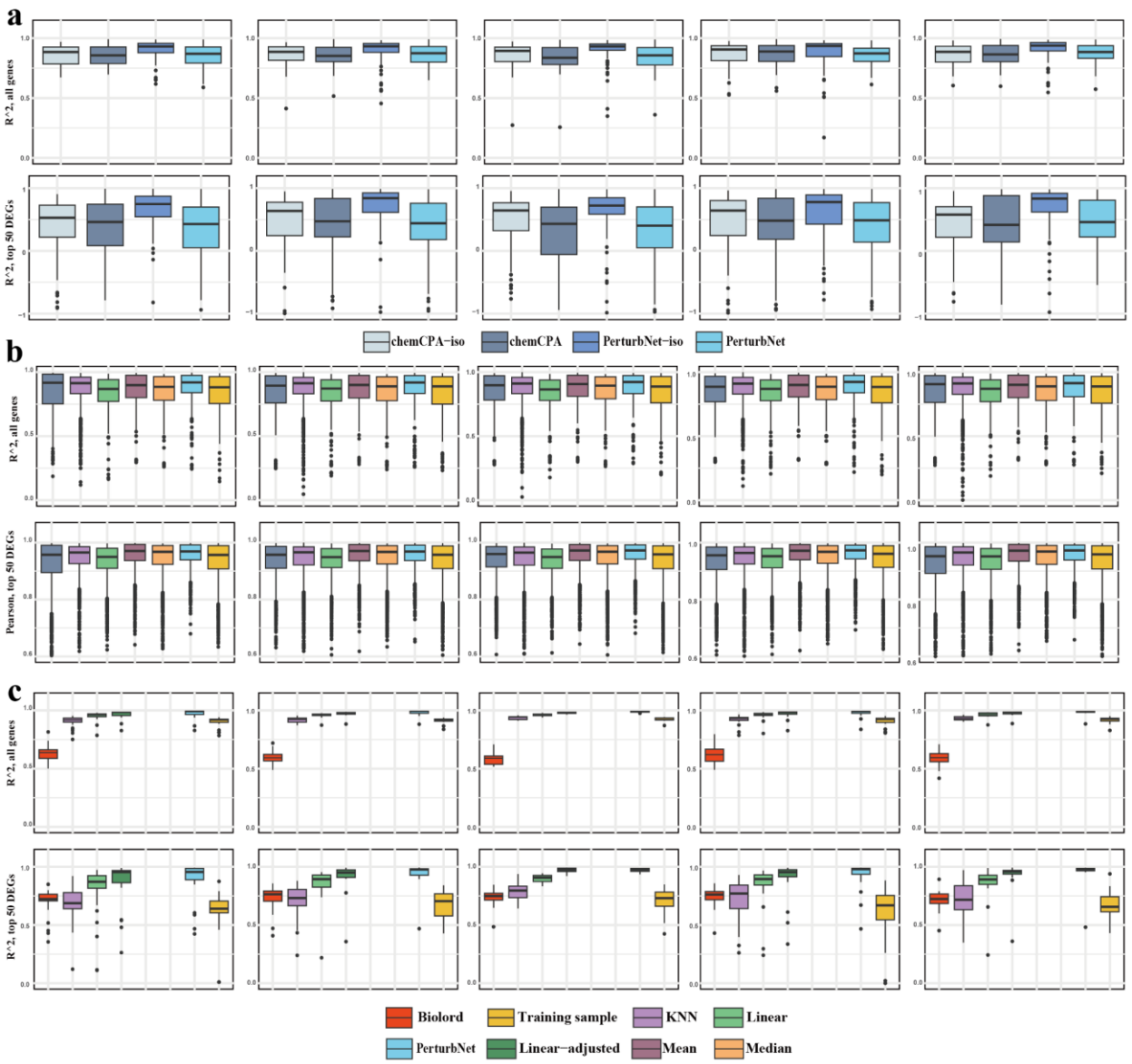
Specific comments:

A recent preprint (A systematic comparison of computational methods for expression forecasting, https://doi.org/10.1101/2023.07.28.551039) introduced new baselines that outperform the linear one used here, including mean and median. It is also packaged in a github repository, which I think should make comparisons easy to perform: https://github.com/ekernf01/perturbation_benchmarking/. Can you please show improvement of PerturbNet relative to the mean/median baselines?

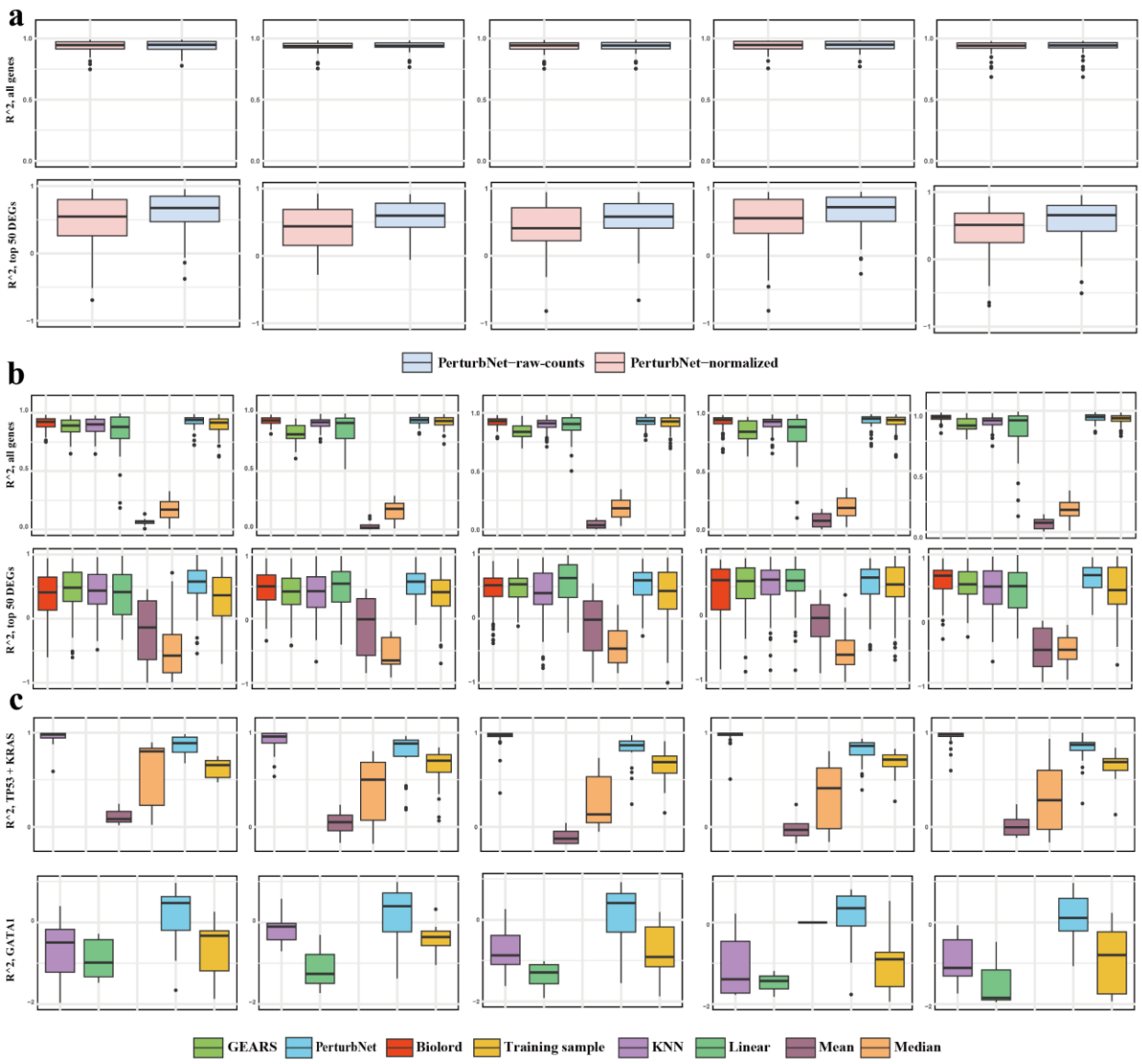
We have computed these additional baselines, and PerturbNet outperforms them.



Heatmap showing the proportion of train/test splits in which PerturbNet achieves significantly better performance (based on the one-sided Wilcoxon tests) compared to competing models (columns) across five train/test splits of (a) sci-Plex and LINCS-Drug dataset. (b) Norman et al. dataset. (c) Ursu et al. and Jorge et al. dataset.



Boxplots of evaluation metrics on the LINCS-Drug and sci-Plex datasets. (a) Comparison between PerturbNet and ChemCPA trained with and without stereoisomers. (b) Benchmark results on the LINCS-Drug dataset. (c) Benchmark results on the sci-Plex dataset.



Boxplots of evaluation metrics on the genetic perturbation datasets. (a) Comparison between PerturbNet trained on raw counts and on normalized expression. (b) Benchmark results on the Norman et al. dataset. (c) Benchmark results on the Ursu et al. and Jorge et al. datasets.

I am still concerned about Figure 4. As noted in my previous review, I don't have technical expertise to comment deeply here, but I just don't think it makes sense to decompose the function of small molecules atom by atom in this manner. What is the physical interpretation of these scores supposed to be? Most small molecules interact non-specifically with many binding partners. Are predictions for stereoisomers or structurally similar molecules concordant? I am wondering if the authors would be willing to discard or deemphasize this portion of the aper. Otherwise, I think it should be evaluated by a chemist. Alternatively, it seems like a similar case for interpretability

could instead be made with the data in Figure 6 where at least there is a structural interpretation.

We have significantly de-emphasized the discussion of the interpretability analysis and moved the main figure to a supplementary figure.

21st May 2025

Manuscript Number: MSB-2025-13097
Title: PerturbNet predicts single-cell responses to unseen chemical and genetic perturbations

Dear Dr Welch,

Thank you for the submission of your reviewed and revised manuscript to Molecular Systems Biology. As discussed, we consulted an expert advisor to assess whether the reviewer concerns had been sufficiently addressed and if they agreed that the manuscript was suitable for Molecular Systems Biology. I am pleased to inform you that the advisor was supportive, finding the overall goal of the manuscript and method very important, and we will be able to accept your manuscript pending the following final amendments:

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https://www.embopress.org/page/journal/17444292/authorguide
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"The datasets and computer code produced in this study are available in the following databases:
- Chip-Seq data: Gene Expression Omnibus GSE46748 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE46748)
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11) All Materials and Methods need to be described in the main text using our 'Structured Methods' format. According to this format, the Methods section includes a Reagents and Tools Table (listing key reagents, experimental models, software and relevant equipment and including their sources and relevant identifiers) followed by a Methods and Protocols section describing the methods, ideally using a step-by-step protocol format. The aim is to facilitate adoption of the methodologies across labs. Please download and fill our Reagents and Tools Table template (.docx), which you can find in our author guidelines:
https://www.embopress.org/page/journal/14693178/authorguide#structuredmethods.
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14) Appendix file: Please upload the Appendix as a single PDF containing all of the supplementary figures and tables (no separate image files are needed) including a title page with "Appendix for + manuscript title" and a Table of Contents with page numbers. Please also ensure the word "Appendix" is included in all labels for Appendix Figures and Appendix Tables including in the Table of Contents and in the callouts in the main manuscript.
15) Funding: Please ensure that all funding sources included in the manuscript are entered the same into the manuscript submission system. Currently there is a mismatch between grant number R01HG101883 in the manuscript versus R01HG010883 in our submission system. In addition, U01HG011952 is missing from our submission system.
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I look forward to reading a new revised version of your manuscript as soon as possible.

Yours sincerely,

Poonam Bheda, PhD
Scientific Editor
Molecular Systems Biology

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3. three to four 'bullet points' highlighting the main findings of your study

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Thank you for pointing that out. The data were actually used when generating the figure; we were simply pointing out that all of the values for some samples fall outside the axis limits. We have rephrased the figure legends to avoid the impression that the data are not shown. For the figures from page 8 and page 17, we replaced the legend with “The y axis is truncated at 0.” We have also included these data in our source data files.

11) All Materials and Methods need to be described in the main text using our 'Structured Methods' format. According to this format, the Methods section includes a Reagents and Tools Table (listing key reagents, experimental models, software and relevant equipment and including their sources and relevant identifiers) followed by a Methods and Protocols section

describing the methods, ideally using a step-by-step protocol format. The aim is to facilitate adoption of the methodologies across labs.
Please download and fill our Reagents and Tools Table template (.docx), which you can find in our author guidelines:
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- Please note that the box plots need to be defined in terms of minima, maxima, centre, bounds of box and whiskers, and percentile in the legends of figures 2C, E, F; 3B, C; 4D
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We have added the sentences below to the figure legends for Fig. 2, 3, and 4 to define the box plots: “for all box plots in this panel, the box plots show the median (center line), the 25th and 75th percentiles (box bounds), and 1.5× the interquartile range (whiskers). Points beyond the whiskers are plotted as outliers.”
We have added sample size n for each boxplot in the legends.
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We have added the word “Appendix” for all of the supplementary figures and tables and in the callouts in the main manuscript. We have separated the appendix to the main text and added the tables of content contents with page numbers.

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We have updated the funding listed in the paper and submission system. The grants are now correctly cited.

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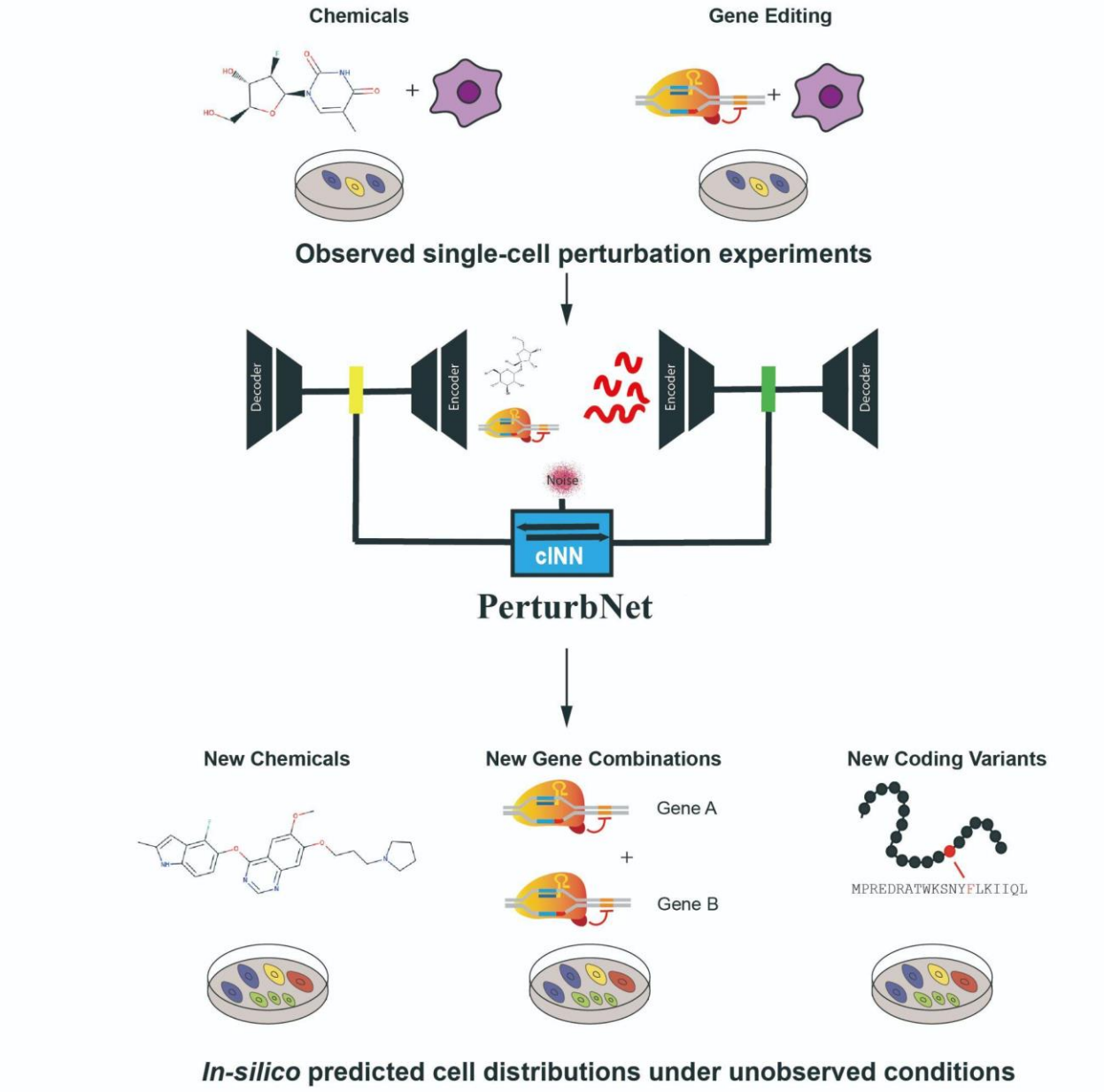

PerturbNet is a generative AI model that can predict shifts in cell state—changes in overall gene expression—in response to multiple types of unseen cellular perturbations.

- PerturbNet uses a flexible framework to “mix and match” neural networks and predict effects of chemical, gene knockdown, gene overexpression, and DNA sequence mutations.
- Despite being an all-in-one model, PerturbNet shows competitive performance with previous methods tailored to predict either chemical or genetic effects
- PerturbNet accurately predicts gene expression changes induced by coding sequence mutations in TP53, KRAS, and GATA1.
- An *in silico* screen of all possible single amino acid substitutions in GATA1 identifies candidates likely to modify erythroid cell differentiation.

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17th Jun 2025
Manuscript number: MSB-2025-13097R
Title: PerturbNet predicts single-cell responses to unseen chemical and genetic perturbations

Dear Dr Welch,

Thank you again for sending us your revised manuscript. We are now satisfied with the modifications made and I am pleased to inform you that your paper has been accepted for publication.

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Poonam Bheda, PhD
Scientific Editor
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Molecular Systems Biology - Author Guidelines
EMBO Molecular Medicine - Author Guidelines

Reporting Checklist for Life Science Articles (updated January
This checklist is adapted from Materials Design Analysis Reporting (MDAR) Checklist for Authors. MDAR establishes a minimum set of requirements in transparent reporting in the life sciences (see Statement of Task: 10.31222/osf.io/9sm4x). Please follow the journal's guidelines in preparing your
Please note that a copy of this checklist will be published alongside your article.

Abridged guidelines for figures
1. Data
The data shown in figures should satisfy the following conditions:
- the data were obtained and processed according to the field's best practice and are presented to reflect the results of the experiments in an accurate and unbiased manner.
- ideally, figure panels should include only measurements that are directly comparable to each other and obtained with the same assay.
- plots include clearly labeled error bars for independent experiments and sample sizes. Unless justified, error bars should not be shown for technical
- if n<5, the individual data points from each experiment should be plotted. Any statistical test employed should be justified.
- Source Data should be included to report the data underlying figures according to the guidelines set out in the authorship guidelines on Data

2. Captions
Each figure caption should contain the following information, for each panel where they are relevant:
- a specification of the experimental system investigated (eg cell line, species name).
- the assay(s) and method(s) used to carry out the reported observations and measurements.
- an explicit mention of the biological and chemical entity(ies) that are being measured.
- an explicit mention of the biological and chemical entity(ies) that are altered/varied/perturbed in a controlled manner.
- the exact sample size (n) for each experimental group/condition, given as a number, not a range;
- a description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
- a statement of how many times the experiment shown was independently replicated in the laboratory.
- definitions of statistical methods and measures:
  - common tests, such as t-test (please specify whether paired vs. unpaired), simple χ2 tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section;
  - are tests one-sided or two-sided?
  - are there adjustments for multiple comparisons?
  - exact statistical test results, e.g., P values = x but not P values < x;
  - definition of 'center values' as median or average;
  - definition of error bars as s.d. or s.e.m.

Please complete ALL of the questions below.
Select "Not Applicable" only when the requested information is not relevant for your study.

Materials

Newly Created Materials	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
New materials and reagents need to be available; do any restrictions apply?	Not Applicable	

Antibodies	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
For **antibodies** provide the following information: - Commercial antibodies: RRID (if possible) or supplier name, catalogue number and or/clone number - Non-commercial: RRID or citation	Not Applicable	

DNA and RNA sequences	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Short novel DNA or RNA including primers, probes: provide the sequences.	Not Applicable	

Cell materials	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Cell lines: Provide species information, strain. Provide accession number in repository **OR** supplier name, catalog number, clone number, and/**OR** RRID.	Not Applicable	
Primary cultures: Provide species, strain, sex of origin, genetic modification status.	Not Applicable	
Report if the cell lines were recently **authenticated** (e.g., by STR profiling) and tested for mycoplasma contamination.	Not Applicable	

Experimental animals	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Laboratory animals or Model organisms: Provide species, strain, sex, age, genetic modification status. Provide accession number in repository **OR** supplier name, catalog number, clone number, **OR** RRID.	Not Applicable	
Animal observed in or captured from the field: Provide species, sex, and age where possible**.**	Not Applicable	
Please detail **housing and husbandry conditions**.	Not Applicable	

Plants and microbes	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Plants: provide species and strain, ecotype and cultivar where relevant, unique accession number if available, and source (including location for collected wild specimens).	Not Applicable	
Microbes: provide species and strain, unique accession number if available, and source.	Not Applicable	

Human research participants	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
If collected and within the bounds of privacy constraints report on age, sex and gender or ethnicity for all study participants.	Not Applicable	

Core facilities	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
If your work benefited from core facilities, was their service mentioned in the acknowledgments section?	Not Applicable	

Design

Study protocol	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
If study protocol has been **pre-registered, provide DOI in the manuscript**. For clinical trials, provide the trial registration number **OR** cite DOI.	Not Applicable	
Report the **clinical trial registration number** (at ClinicalTrials.gov or equivalent), where applicable.	Not Applicable	

Laboratory protocol	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Provide DOI OR other citation details if **external detailed step-by-step protocols** are available.	Not Applicable	

Experimental study design and statistics	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Include a statement about **sample size** estimate even if no statistical methods were used.	Yes	It's available in the methods section
Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. **randomization procedure**)? If yes, have they been described?	Not Applicable	
Include a statement about **blinding** even if no blinding was done.	Not Applicable	
Describe **inclusion/exclusion criteria** if samples or animals were excluded from the analysis. Were the criteria pre-established? If sample or data points were omitted from analysis, report if this was due to attrition or intentional exclusion and provide justification.	Yes	It's available in the methods section
For every figure, are **statistical tests** justified as appropriate? Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it. Is there an estimate of variation within each group of data? Is the variance similar between the groups that are being statistically compared?	Yes	We used the wilcoxon rank-sum test, which is non-parametric and thus does not assume and distribution. We also performed linear regression and checked normality using quantile-quantile plots.

Sample definition and in-laboratory replication	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
In the figure legends: state number of times the experiment was **replicated** in laboratory.	Yes	It's available in the methods section and figure legends
In the figure legends: define whether data describe **technical or biological replicates**.	Yes	It's available in the methods section and figure legends

Ethics

Ethics	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Studies involving **human participants**: State details of **authority granting ethics approval** (IRB or equivalent committee(s), provide reference number for approval.	Not Applicable	
Studies involving **human participants**: Include a statement confirming that **informed consent** was obtained from all subjects and that the experiments conformed to the principles set out in the WMA Declaration of Helsinki and the Department of Health and Human Services Belmont Report.	Not Applicable	
Studies involving **human participants:** For publication of **patient photos**, include a statement confirming that consent to publish was obtained.	Not Applicable	
Studies involving experimental **animals**: State details of **authority granting ethics approva**l (IRB or equivalent committee(s), provide reference number for approval. Include a statement of compliance with ethical regulations.	Not Applicable	
Studies involving **specimen and field samples:** State if relevant **permits** obtained, provide details of authority approving study; if none were required, explain why.	Not Applicable	

Dual Use Research of Concern (DURC)	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Could your study fall under dual use research restrictions? Please check biosecurity documents and list of **select agents and toxins** (CDC): https://www.selectagents.gov/sat/list.htm	Not Applicable	
If you used a select agent, is the security level of the lab appropriate and reported in the manuscript?	Not Applicable	
If a study is subject to dual use research of concern regulations, is the name of the **authority granting approval and reference number** for the regulatory approval provided in the manuscript?	Not Applicable	

Reporting

The MDAR framework recommends adoption of discipline-specific guidelines, established and endorsed through community initiatives. Journals have their own policy about requiring specific guidelines and recommendations to complement MDAR.

Adherence to community standards	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
State if relevant guidelines or checklists (e.g., **ICMJE, MIBBI, ARRIVE, PRISMA**) have been followed or provided.	Not Applicable	
For **tumor marker prognostic studies,** we recommend that you follow the **REMARK** reporting guidelines (see link list at top right). See author guidelines, under 'Reporting Guidelines'. Please confirm you have followed these guidelines.	Not Applicable	
For **phase II and III randomized controlled trials**, please refer to the **CONSORT** flow diagram (see link list at top right) and submit the CONSORT checklist (see link list at top right) with your submission. See author guidelines, under 'Reporting Guidelines'. Please confirm you have submitted this list.	Not Applicable	

Data Availability

Data availability	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Have **primary datasets** been deposited according to the journal's guidelines (see 'Data Deposition' section) and the respective accession numbers provided in the Data Availability Section?	Not Applicable	
Were **human clinical and genomic datasets** deposited in a public access-controlled repository in accordance to ethical obligations to the patients and to the applicable consent agreement?	Not Applicable	
Are **computational models** that are central and integral to a study available without restrictions in a machine-readable form? Were the relevant accession numbers or links provided?	Yes	It's available in the data availability section
If publicly available data were reused, provide the respective **data citations in the reference list**.	Yes	Marked as [DATASET] in the reference list