Bidirectional Translation of Transcriptomic Profiles between Liver and Kidney under Drug Treatment Using Generative Adversarial Networks (GANs)

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Abstract

Translational research in toxicology is essential for understanding how molecular alterations manifest across various biological systems to, for example, decrease reliance on animal models and extrapolation from animals to humans. Toxicogenomics (TGx) significantly contributes to assessing chemical and drug toxicity by providing insights into underlying toxicity mechanisms and developing gene expression-based biomarkers for toxicant classification. Despite the recognized need for a multi-organ approach in evaluating organism-level toxicity, most TGx research has been focused on a limited number of organs, primarily the liver, due to resource-intensive experiments. This paper is the first effort to utilize Generative Adversarial Network (GAN) for bidirectional translation of transcriptomic profiles between organs under chemical treatment. In this study, we developed a novel GAN model, TransTox, to bridge transcriptomic data between the liver and kidney. This model demonstrated robust performance in various evaluations, including external validation on independent datasets from both the training set's source labs and a different lab. The study investigated the concordance between the real data and synthetic data generated by TransTox in elucidating toxicity mechanisms with respect to differential expressed genes (DEGs) and enriched pathways analyses. It showed comparable results in comparison to that obtained from real experimental settings. Moreover, TransTox proved valuable in biomarker applications, where synthetic data could be used to develop valid biomarkers or serve as "digital twins" for diagnostic applications. TransTox holds the potential to extend insights into toxicological effects in other organs by leveraging historical liver-centric TGx experiments, thereby opening avenues for reducing reliance on animal testing in organ toxicity research.

Introduction

Translational science plays a crucial role across diverse applications. In drug development, translational research has specifically emphasized on cross-species translation from preclinical animal investigations to clinical outcomes. On a broad spectrum, various types of translation research have been actively pursued with practical applications. For example, the conversion of genomics data between platforms, such as from microarray to next-generation sequencing, could capitalize on previous investments[1]. Moreover, the translation of data across different biological contexts, such as from non-invasive blood samples to tissue samples, could have significant clinical implications[2]. In toxicology, translational research is essential for understanding how molecular alterations manifest across various biological systems for cross-assay extrapolation, such as translating in vitro findings to in vivo observations, a process known as in vitro to in vivo extrapolation (IVIVE). The recent rapid advancements in artificial intelligence (AI), particularly in the area of generative AI, have introduced novel approaches to enhance translational research such as Generative Adversarial Networks (GANs) and diffusion model. In this study, we developed an AI approach that can predict toxic effects in one organ based on observations from another, thereby enhancing systems toxicology.

Animal models play a crucial role in evaluating the potential toxicity of drugs and chemicals, providing valuable insights into possible toxic effects in humans. The analysis of gene expression profiles from animal studies, a widely employed toxicogenomics (TGx) approach, has significantly contributed to the risk and safety assessment of chemicals and drugs in two key aspects. Firstly, it aids in understanding the underlying biological processes of toxicity in animals and their relevance to humans [3-9]. Secondly, it facilitates the development of gene expression-based biomarkers for classifying toxicants [10-18]. Given the resource-intensive nature of conducting comprehensive TGx studies for every organ, most TGx studies have been centered on a limited number of organs, where the liver TGx experiment is dominant [19]. However, multiple studies have demonstrated that toxicity could manifest across multiple organs under chemical exposure or drug treatment [20, 21]. For example, doxorubicin, used as an anti-neoplastic drug in the treatment of various types of cancers, causes heart, liver, and kidney injury [22]. While it's important to study individual organs, it's equally crucial to consider the interactions and interdependencies between different organs and systems within an organism. Consequently, toxicity assessments often involve evaluating the effects on multiple organs to gain a comprehensive understanding of the overall impact on the organism's health [23, 24]. Particularly, advancements in

systems biology and computational modeling have been focused on exploring the complex interactions between organs and predict potential toxic effects more accurately at an organism level [25-28].

Generative Adversarial Networks (GANs) have gained prominence in biomedicine for applications such as generating new molecules [29-32]. In toxicology, GANs have been used to generate synthetic data that closely mimic the study results from real biological samples [10, 33-36]. Considering the intricate interactions and interdependencies among various organs, we developed a GAN-based model to explore the possibility of translating gene expression profiles between organs of healthy rats [37]. This proof-of-concept study laid the foundation to investigate the transcriptomic translation between different organs under the treatment conditions. Different organs, intricately connected through various feedback mechanisms, likely respond to drug treatment in an interconnected fashion. This suggests that the treatment effects in one organ could be used to predict toxic effects in a different organ. This treatment-centric investigation reflects the real-world practice in toxicology. Such cross-organ translation not only broadens the scope of toxicological investigation but also improves a holistic understanding of the systemic impact of treatments and substances under investigation. Given the fact that most TGx studies have been focused only on the liver, this study may also offer an opportunity to infer transcriptomic responses of other organs based on the historical liver data.

In this study, we developed TransTox to enable the bidirectional translation of transcriptomic profiles between the liver and kidney under drug treatment. TransTox was trained using transcriptomic data from the Toxicogenomics Project-Genomics Assisted Toxicity Evaluation Systems (TG-GATEs) [38], which included varying doses (high/medium/low/control) and treatment durations (3/7/14/28 days). The model was evaluated using three metrics —cosine similarity, root mean square error (RMSE), mean absolute percentage error (MAPE)—and one visualization approach—Uniform Manifold Approximation and Projection for Dimension Reduction (UMAP) [39]. Importantly, the model was assessed on independent datasets from both the training set's source labs and a different lab. To evaluate the synthetic data generated by TransTox in elucidating toxicity mechanisms, we compared the synthetic differential expressed genes (DEGs) and enriched pathways with those obtained from real experimental settings. Additionally, we evaluated the value of TransTox in biomarker applications, where synthetic data could be used to develop valid biomarkers or serve as "digital twins" for diagnostic applications. This comprehensive study demonstrates the potential of TransTox in translating transcriptomic profiles between organs to advance TGx research.

Materials and Methods

Toxicogenomics (TGx) Dataset

The liver and kidney TGx datasets were obtained from the Open TG-GATEs [38], a large-scale TGx database including transcriptomic profiles and pathological findings from rat liver and kidney. The transcriptomic profiles were generated using Affymetrix GeneChip Rat Genome 230 2.0. Data from the Open TG-GATEs were downloaded from the website https://dbarchive.biosciencedbc.jp/en/open-tggates/download.html. Our study used the transcriptomic data from repeated dose experiments on rats, which employed three dose levels (low, middle, and high) and time-matched controls, along with four treatment durations (3, 7, 14, and 28 days).

The robust multi-array average (RMA) method [40] was applied to normalize the TG-GATEs data separately for both the liver and kidney. The expression values of the transcriptomic profiles were transformed to a $\log_2$ scale. To demonstrate the feasibility of organ translation using the TGx data, we focused on the rat S1500+ gene set. This gene set is considered important for toxicity by the Tox21 program and was identified through a hybrid approach comprising five sequential modules [41]. This S1500+ gene set is specifically designed to represent biological diversity, address gene-gene co-expression relationships, and adequately represent known pathways of toxicity. The rat S1500+ gene set was downloaded from the website https://ntp.niehs.nih.gov/whatwestudy/tox21/s1500. Furthermore, we employed the "AnnotationDbi" package with the rat2302.db database to map the rat S1500+ genes to 3,475 microarray probes. From this point onward, the transcriptomic profiles refer to profiles containing the expression values of these 3,475 probes. The dataset had 41 compounds that had the paired transcriptomic data between liver and kidney. For each treatment conditions (combinations of compound, dose, and duration), 3 biological replicates (rats) were used; total of 616 treatment conditions comprised 1,844 profiles for each organ. Additionally, there were 102 compounds that had only liver profiles (no kidney data), with 3,595 liver profiles corresponding to 1,201 treatment conditions. For detailed information regarding the transcriptomic profiles and their associated pathological data, please refer to **Supplementary Table S1**.

Training and Test Sets

To study organ translation between the liver and kidney, we employed drug-specific pairwise samples to train TransTox where each pairwise sample consisted of a liver profile and a kidney profile associated with a drug. For example, when considering acetaminophen as the drug, a liver profile under treatment condition A (e.g., dose=low and duration=7 days) was paired with a kidney profile under treatment condition B (dose=middle, duration=3 days), serving as a training sample for the kidney generator (using liver to generate kidney) in TransTox. Simultaneously, the kidney profile under treatment condition B was paired with the liver profile under treatment condition A, providing a training sample for the liver generator (using kidney to generate liver) in TransTox.

To prevent information leakage, we divided the training and test sets based on compounds. This approach allocated 32 compounds to the training set and 9 compounds to the test set out of the total 41 compounds. In addition to the three-dose treatments, we included control treatment profiles, enabling TransTox to thoroughly understand the underlying relationships between the liver and kidney. In this setup, a control treatment organ profile (e.g., liver) could only be paired with the control treatment profile of the same compound in the other organ (e.g., kidney). In total, there were 45,402 pairwise samples for each generator in the training set, while there were 11,250 pairwise samples in the test sets to evaluate the performance of both the liver and kidney generators.

TransTox Framework

TransTox was used to translate transcriptomic profiles under treatments from one organ (e.g., liver) to another (e.g., kidney), leveraging our previous model [37], which was developed using CycleGAN [42]. Specifically, TransTox consisted of two pairs of generator and discriminator, one for liver and the other for kidney, interacting with each other as depicted in **Figure 1**. The liver Generator ((*G(liver)*) generated liver profiles from kidney profiles, while the Kidney Generator (*G(kidney)*) generated kidney profiles from liver profiles. Correspondingly, the Liver Discriminator (*D(liver)*) assessed the difference between synthetic and real data for the liver, while the Kidney Discriminator (*D(kidney)*) performed a similar function for the kidney. We developed a revised version of CycleGAN, implementing three key modifications to tailor it for organ translation using transcriptomic profiles. Firstly, we replaced the convolutional neural layers with dense layers to handle transcriptomic profiles as input data instead of images. Secondly, we enhanced the input layer by including additional labels to specify the origin and target attributes of the input profiles (e.g., organ, dose, time, etc.). Thirdly, we expanded the generator

architecture with additional neurons to hold the labels of the target profile, thereby guiding the translation towards the desired direction.

Within the TransTox architecture, the generator input layer is designed to incorporate four essential components. The first component was the profile of the organ used for generating another organ—a 3,475-dimensional vector of transcriptomic values. The second component consisted of a 14-dimensional vector containing binary source label information, specifying details such as the organ, dose level, and time points. The third component was a 14-dimensional vector containing binary target label information. The fourth component involved a 3,475-dimensional Gaussian noise, sampled from a normal distribution, intended to enhance the model's robustness. These four components were concatenated into a 6,978-dimensional vector and scaled to the range between 0 and 1. The generator was a fully connected neural network with five hidden layers, featuring 8,192, 7,168, 7,168, 4,096, and 4,096 nodes, respectively. Each hidden layer was followed by a dropout layer, except for the last one, with dropout rates of 0.8, 0.8, 0.8, and 0.4, respectively. The Leaky ReLU activation function, with an alpha value of 0.2, was applied across all hidden layers. The output layer was activated by a sigmoid activation function with 3,475 neurons, aligning with the dimensionality of features in the transcriptomic profiles.

The discriminator was a fully connected neural network consisting of four layers. The input layer comprised 3,475 neurons corresponding to the 3,475 probes, which received either the synthetic or real profile. Following this, there were two hidden layers with 256 and 64 neurons, respectively. The ReLU activation function was applied in each layer, followed by a dropout layer with a rate of 0.5. The output layer, consisting of a single neuron indicating whether the profile is real or synthetic, was activated by a sigmoid activation function. For optimization purposes, the discriminator model utilized Stochastic Gradient Descent (SGD) with a learning rate of 0.0001 and a momentum of 0.90.

Assessing Synthetic Transcriptomic Profile from TransTox

In evaluating TransTox, we employed a comprehensive approach to assess its effectiveness in generating synthetic profiles in the test set. The first part of the assessment involved both visualization and quantitative analysis using three metrics. The UMAP technique [39] was selected for its ability to reduce high-dimensional data into a 2D representation, providing a visual overview of the distribution of synthetic and real profiles. Cosine similarity was applied to measure the likeness between synthetic and

corresponding real profiles. The RMSE quantified the absolute differences between two profiles, offering insights into the accuracy of TransTox's predictions. Additionally, the MAPE provided a percentage-wise measure of the errors between synthetic and real profiles.

External Validation of TransTox Predictions using DrugMatrix

DrugMatrix [43] was used as an external validation set to assess the accuracy of TransTox predictions for data generated from a different laboratory but with a similar study design. DrugMatrix is a large TGx database that includes transcriptomic profiles from primary tissues of male rats, including liver and kidney, profiled on the same Affymetrix Rat 230.2 platform as applied in TG-GATEs. The DrugMatrix data was downloaded from the Gene Expression Omnibus (https://www.ncbi.nlm.nih.gov/geo/, last accessed October 3, 2023) with accession numbers GSE57815 and GSE57811 for liver and kidney, respectively. Two sequential criteria were applied to select compounds. Firstly, compounds with treatments lasting 3, 7, and 14 days were chosen to align with the TG-GATEs experimental time. Secondly, compounds needed to have both liver and kidney profiles, allowing for comparison between the generated profiles and the corresponding real profiles. In total, 39 compounds were selected, corresponding to 388 profiles (216 for liver and 172 for kidney). Five out of the 39 compounds were tested in both TG-GATEs and DrugMatrix, providing a matched reference for the analysis results. The remaining 34 compounds had incomplete profiles (i.e., either liver or kidney profiles, but not both) or no data in TG-GATEs, thus lacking a matched reference for the analysis results. Additionally, the treatment dose was converted to three levels (low, middle, and high) to maintain consistency with TG-GATEs dose information. The strategy for assigning doses was detailed in our previous study [10]. The same microarray data preprocessing approach, RMA normalization, was employed to process the transcriptomic profiles from DrugMatrix. The processed liver profiles, including treatment information such as dose and time, were used as inputs for TransTox to generate the kidney profiles for the same compound, and vice versa. Subsequently, cosine similarity was applied to assess the similarity between the generated profiles from TransTox and the actual corresponding profiles from DrugMatrix.

TransTox-Generated Transcriptomic Profiles in Elucidating Toxicity Mechanisms in Comparison with Real Experimental Settings

One important TGx application is to elucidate toxicity mechanisms. For that, we compared DEGs between synthetic profiles and their corresponding real profiles. Specifically, we calculated the average transcriptomic values of three biological replicates (rats) for a given treatment condition and compared them to that of the time-matched real control (i.e., three rats) to determine the fold change. For the real transcriptomic data, DEGs were defined as those with absolute fold changes greater than 1. The thresholds used to define DEGs for the synthetic profiles in the test set were based on the training set; the threshold was selected to ensure a similar average number of DEGs compared to the real data. In addition, we conducted KEGG pathway enrichment analyses separately for DEGs derived from synthetic profiles and real ones in each treatment condition using GSEApy [44]. Enriched pathways with p-values < 0.05 were extracted for comparative analysis. This approach aimed to assess the concordance of DEGs and enriched pathways, providing insights into the potential of TransTox in elucidating toxicity mechanisms.

To evaluate the relevance of findings generated by TransTox (i.e., DEGs and pathways) to real experimental settings, we compared the TransTox results with a TGx experiment involving entacapone and tolcapone, which employed a study design similar to that of TG-GATEs [45]. This dataset was curated from the US Food and Drug Administration's (FDA's) National Center for Toxicological Research (NCTR) ArrayTrack database [46]. The experimental design, animal study execution, and the collection of the liver samples have been detailed in a prior study [45]. In total, we collected 120 transcriptomic profiles sampled from male Sprague-Dawley rat liver tissue, including 10 treatments derived from the combination of two compounds (entacapone, tolcapone), four dose levels (control, low, middle, and high), and two dosing time periods (3- and 28-days). Specifically, the 3-day experiment used only control and high doses, resulting in 36 profiles (2 compounds x 12 rats + 1 control x 12 rats). In contrast, all treatment conditions were included in the 28-day study, producing 84 profiles (2 compounds x 12 rats x 3 doses + 1 control x 12 rats). The 120 transcriptomic profiles were processed with RMA normalization. Each treatment group comprised 12 biological replicates (i.e., 12 rats), which were further divided into four random subgroups, with three rats in each subgroup, consistent with the TG-GATEs study for a comparable comparison. Essentially, each subgroup represented an independent experiment, and the identical experiment was conducted four times by the same investigators in the same lab, concurrently, with only difference are the rats used. Subsequently, we analyzed the overlap in DEGs and pathways between any two subgroups, resulting in six comparisons for each treatment. These analyses serve as reference points for evaluating the performance of TransTox results within the context of the same experiment conducted multiple times with different rats.

Necrosis Biomarker Development

To explore the potential of TransTox in biomarker development, we focused on necrosis, a common pathological finding in both drug-induced liver and kidney injury. Necrosis also manifests in various acute and chronic liver diseases [47], as well as renal disorders [48]. Distinguishing necrosis is fundamental for accurate disease diagnosis and injury assessment. A treatment is categorized as necrosis positive if at least one sample exhibits the necrosis pathological finding under that treatment. Conversely, a treatment is classified as necrosis negative if there are no pathological findings for all the rats in that treatment.

The model development focused on 102 compounds selected for specific reasons: (1) their liver profiles were available in TG-GATEs, and (2) their profiles were not used to develop or test TransTox. These 102 compounds consisted of 113 necrosis positives and 963 necrosis negatives in the liver, along with 26 necrosis positives and 664 necrosis negatives in the kidney.

In the liver, we first randomly selected 113 out of 963 treatment conditions without liver necrosis (negatives) to pair with the 113 treatment conditions with liver necrosis (positives) to construct a balanced training set. For each treatment condition, the average value across biological replicates were used. The liver necrosis model was developed using a logistic regression (LR) method. For the test set, all the treatment conditions with necrosis positive/negative data (real and synthetic) from the 9 different compounds were used to evaluate the liver LR model.

For the kidney, we were unable to develop a necrosis model based on real kidney profiles due to the absence of real data for modeling. Therefore, we generated synthetic kidney profiles using the kidney generator with real liver profiles and using these synthetic profiles to develop the kidney necrosis prediction model. Firstly, we randomly selected 26 out of 664 treatment conditions without kidney necrosis (negatives). Secondly, these were combined with the 26 treatment conditions with kidney necrosis (positives) to create a balanced training set. Thirdly, the average value across biological replicates was calculated for each treatment condition and further utilized to train the kidney LR model for predicting kidney necrosis. Finally, the model was tested on the treatment conditions with necrosis positive/negative data (real and synthetic) from the 9 different compounds.

To quantitatively assess the models' performance on two different test datasets, we utilized four performance metrics: Matthews correlation coefficient (MCC), accuracy, sensitivity, and specificity. The formulas for calculating these measurements are as follows:

$$MCC = \frac{TP*TN - FP*FN}{\sqrt{(TP+FP)*(TP+FN)*(TN+FP)*(TN+FN)}} \quad (1)$$

$$accuracy = \frac{TP+TN}{TP+TN+FN+FP} \quad (2)$$

$$sensitivity = \frac{TP}{TP+FN} \quad (3)$$

$$specificity = \frac{TN}{TN+FP} \quad (4)$$

In these formulas, TP, TN, FP, and FN represent true positive, true negative, false positive, and false negative, respectively. Further elaboration on these definitions can be found elsewhere [49].

Results

TransTox Training Results

The training set consisted of 45,402 pairs of profiles between the liver and kidney, derived from 32 compounds (approximately 80% of the total 41 compounds) that consisted of 510 treatment conditions, and 1,526 rats. Both liver and kidney generators were selected at 1,000 epochs, where the loss function was stabilized. High agreement was observed between synthetic and real profiles for both the liver and kidney, as visualized with UMAP (**Figure 2a and 2e**).

To objectively assess the training results, we included a negative control based on the experimental data. The negative controls characterized the distribution of pairwise samples within an organ, providing a baseline distribution against which the differences between real and synthetic data need to surpass with statistical significance. In the liver, the agreement between synthetic and real data was 0.9995 in cosine similarity, significantly higher than the mean of the negative control group (0.9986) with a t-test p-value <2.225e-308 (**Figure 2b**). Similarly, there was a small RMSE of 0.247, significantly lower than the mean of the negative control group (0.405) with a p-value <2.225e-308 (**Figure 2c**). The MAPE was 0.025, also significantly smaller than the negative control group's mean of 0.041 with a p-value <2.225e-308 (**Figure 2d**). Similar results were obtained in kidney, with a high cosine similarity of 0.9996 (against the negative control of 0.9990 with a p-value <2.225e-308, **Figure 2f**), RMSE of 0.218 (compared to the negative control of 0.347 with a p-value <2.225e-308, **Figure 2g**), and MAPE of 0.019 (against the mean of the negative control of 0.032 with a p-value <2.225e-308, **Figure 2h**). These results suggest that the high agreement between the synthetic and real profiles for both liver and kidney.

Validation of TransTox Predictions with a Test Set from the Same Lab

The TransTox model was evaluated with a TG-GATEs test set that consisted of 11,250 pairs of profiles, including 9 compounds (approximately 20% of the total 41 compounds), 106 treatment conditions, and 318 rats. We employed the same four approaches as the training set to evaluate the test results. For both the liver and kidney, the predicted profiles showed a high degree of agreement with the experimental results, as illustrated in the UMAP visualization and quantitively measured by cosine similarity, RMSE, and MAPE (**Figure S1**).

Furthermore, we conducted a comparison at the gene level, examining the treatment-based synthetic profiles alongside their corresponding real profiles for each organ and between organs. We first calculated the difference in gene expression values between the synthetic and real data (normalized to the real value) for the liver, presented in a histogram plot. As depicted in **Figure 3a**, 95% of synthetic gene expression values fall within a range of less than 10% variation compared with real values. We then conducted the same analysis for the kidney, where 98% of synthetic gene expression values were within less than 10% variation in comparison to real values (**Figure 3b**). Lastly, to ensure the relative expression between two organs for each gene remained unchanged, we calculated the difference between synthetic and real data with respect to the between-organ gene distance (normalized to the average of the real value of the two organs) for all treatment conditions. As shown in **Figure 3c**, the majority (93%) of the synthetic between-organ gene distances were maintained with variations of less than 10% in comparison to real between-organ gene distances.

Given that not all genes are equally important with respect to mechanistic study and biomarker development, we defined a subset of genes, called Important Genes, which had an absolute fold change greater than 1 in at least one treatment condition. As shown in **Figure 3**, similar trends were observed for these genes.

External Validation of TransTox Predictions using DrugMatrix: Comparative Analysis of Synthetic Profiles Against Real Profiles from Different Labs with Similar Study Designs

We evaluated TransTox performance using data from the DrugMatrix dataset, which originated from a different laboratory with a similar but not identical study design (refer to the Materials and Methods section). The generated profiles were compared with the actual profiles in the DrugMatrix using cosine similarity (**Figure 4**).

There were five compounds for which both liver and kidney profiles were available and matched between TG-GATEs and DrugMatrix. This provided an opportunity to assess the accuracy of synthetic data compared to the differences observed between the two labs using real experimental data. **Figure 4a** comprises four violin plots: two for the kidney (left panel) and two for the liver (right panel). For each organ, the left violin plot represents the correlation of experimental data, while the right box plot represents the correlation between synthetic and real profiles. For both organs, no statistical difference

was observed in the correlation between real and generated profiles compared to the correlation of real profiles between TG-GATEs and DrugMatrix.

There were 34 compounds from DrugMatrix that had both liver and kidney profiles, but they had incomplete or no data in TG-GATEs. TransTox was applied to generate synthetic profiles for both the liver and kidney, as shown in the right violin plots of each panel in **Figure 4b**. Similar to **Figure 4a**, we employed two reference sets (one for the liver and the other for the kidney) to evaluate the accuracy of the synthetic results. For the liver, 29 compounds had profiles available in both TG-GATEs and DrugMatrix, with 9 compounds overlapping the 34 compounds. Therefore, we used the liver profiles of these 29 compounds as a reference point. However, for the kidney, only 11 compounds had profiles available in both datasets, and none overlapped with the 34 compounds. In summary, the similarity of the real and generated data was slightly lower (0.9955) than that of the reference data (0.9963) in the liver, while comparable for the kidney where the similarity of the generated group and real group (reference data) was 0.9974 and 0.9973, respectively.

TransTox-Generated Transcriptomic Profiles in Elucidating Toxicity Mechanisms in Comparison with Real Experimental Settings

To assess the ability of TransTox to recapitulate results from the real toxicogenomics studies, particularly regarding mechanistic elucidation, we compared synthetic profiles with their corresponding real profiles, focusing on DEGs and enriched pathways (refer to the Materials and Methods section). The comparison was conducted with the test set.

Figure 5a depicted the percentage of overlapped DEGs between synthetic and real profiles across three dose levels and four time points in the liver test set. Variations were observed for different dose and time points. The average overlapped DEGs ratios were 0.426, 0.436, and 0.364 for the low, middle, and high dose groups, respectively, and ratios of 0.410, 0.396, 0.381, and 0.454 for the 3-, 7-, 14-, and 28-days treatments, respectively. When comparing the percentage of overlapped KEGG pathways between two sets of DEGs of synthetic and real profiles, the ratio of overlapped pathways (with p-value < 0.05) as presented in **Figure 5b** was 0.349, 0.422, and 0.437 for the low, middle, and high dose groups, respectively, and 0.297, 0.435, 0.389, and 0.488 for the 3-, 7-, 14-, and 28-days treatments, respectively.

Similar analyses were performed for synthetic profiles in the kidney test set, and variations were also observed among different dose and time points. The average overlapped DEGs ratios were 0.597, 0.553,

and 0.368 for the low, middle, and high dose groups, respectively, and 0.529, 0.547, 0.478, and 0.478 for the 3-, 7-, 14-, and 28-days treatments, respectively (**Figure 5c**). Additionally, KEGG pathway enrichment analyses yielded an average overlapped ratio of enriched KEGG pathways (with a p-value < 0.05) of 0.369, 0.323, and 0.258 for low, middle, and high dose groups, respectively, and 0.306, 0.435, 0.286, and 0.231 for the 3-, 7-, 14-, and 28-days treatments, respectively (**Figure 5d**).

To contextualize the above results within real experimental settings, we selected a TGx study involving two drugs (entacapone and tolcapone) with a similar study design and transcriptomics experiment as TG-GATEs. This study used 12 rats for each treatment group, allowing them to be divided into four random groups of 3 rats each, aligning with TG-GATEs. We assumed each group represented an independent experiment, and the identical experiment was conducted four times, with the only difference being the rats used (refer to the Materials and Methods section). Subsequently, we analyzed the overlap in DEGs and pathways between any two groups to serve as reference points for evaluating the performance of TransTox results. As presented in **Figure 5e**, the overlap in DEGs between two identical experiments was in a similar range as observed in the comparison between synthetic and real profiles presented in **Figure 5a**. Similarly, the overlap in enriched KEGG pathways for any two identical experiments relating to entacapone and tolcapone (**Figure 5f**) was also in a similar range as observed in the comparison between synthetic and real profiles depicted in **Figure 5b**.

TransTox for Biomarker Development – Necrosis Prediction

As depicted in **Figure 6a** for the liver, we constructed a logistic regression (LR) model using 226 real profiles, which were subsequently assessed on two test sets: one with real liver profiles and the other with matched synthetic profiles (see Materials and Methods section for details). Both test sets included 14 liver necrosis positives and 82 liver necrosis negatives. Results presented in **Table 1** indicate that the test set with synthetic profiles exhibited comparable performance to the test set with real profiles across all statistical measures.

For the kidney, instead of utilizing real profiles as in the case of the liver, we developed an LR model using 52 synthetic profiles and tested it on two datasets: one with real kidney treatment profiles and the other with synthetic kidney treatment profiles (**Figure 6b**; see Materials and Methods section). Both datasets included 3 kidney necrosis positives and 65 kidney necrosis negatives. Similar performance was

observed in the kidney, with the test set using synthetic profiles showing comparable performance to the test set with real profiles (**Table 1**).

Discussion

Since its inception approximately 25 years ago, TGx has played a critical role in assessing chemical and drug safety. The majority of TGx studies have primarily concentrated on the liver due to (1) liver injury has been the top reasons for drugs failing in the clinical settings and (2) TGx experiments are costly to evaluate many organs. TransTox marks the initial AI effort in translating transcriptomic data between the liver and kidney under chemical treatment, providing a glimpse into the potential to extrapolate molecular expression to other organs based on the historical liver TGx studies. In a broader context, this investigation creates an opportunity to diminish the reliance on animal experimentation in future research focusing on organ toxicity. This is particularly applicable in cases where a wealth of liver data exists, thereby contributing to broader initiatives dedicated to reducing, refining, and replacing animal studies, as outlined in the 3Rs Principle [50, 51].

TransTox was evaluated in multiple ways, most of which were focused on the results from the data that were not used in the training process (i.e., the test set and DrugMatrix data) and the scenarios that are relevant to the TGx applications (i.e., mechanistic interpretation and biomarker development). The results demonstrated TransTox's robust performance across all these tests.

DrugMatrix was applied to assess TransTox to utilize TGx data generated by other labs and predict compounds not seen in the training process. Selecting a validation dataset poses challenges due to diverse experimental designs, especially concerning TGx data, which exhibits significant variations not only in the choice of study design but also across different labs. It is crucial to choose an external dataset that allows an objective evaluation of TransTox results based on the model performance not due to the impact of differences in applied study designs. The study designs of DrugMatrix and TG-GATEs share commonalities but also have key differences, particularly regarding to dose range findings that affect dose definition (i.e., low, medium, and high). Fortunately, several compounds were tested in both TG-GATEs and DrugMatrix, serving as reference points to evaluate TransTox predictions. As depicted in **Figure 4**, the predicted results (correlation between synthetic and real data) aligned with experimental results (correlation between two datasets for common compounds), demonstrating its robustness for potential TGx applications.

One of the primary applications of TGx is to gain an understanding of underlying toxicity mechanisms through DEGs analysis and pathway extension. The concordance of DEGs across various gene expression platforms and labs has been extensively studied [52] and is largely dependent on chemical treatment and transcript abundance [53]. The synthetic data produced by TransTox under specific treatment conditions can be likened to data obtained from the same experiment in a laboratory setting. In essence, the concordance of DEGs between synthetic and real data can be relatively evaluated by comparing it to that observed in experiments repeated multiple times. By following this logic, we selected a TGx experiment as reference points to evaluate the synthetic DEGs from TransTox. The experiment applied the similar study design as TG-GATEs including the microarray platforms to study two drugs (tolcapone and entacapone) with each experiment conditions involving 12 rats (4 groups of 3 rats to align with TG-GATEs design). We found that the percentage of agreement in DEGs between synthetic and real data is comparable to what is observed in experimental settings that were carried out in the same lab and by the same investigators but with different rats. A similar conclusion was drawn in pathway analysis, suggesting that TransTox could provide a synthetic approach for mechanistic studies in toxicology.

The other important TGx application is to develop predictive biomarkers for toxicity prediction. We evaluated the potential utility of synthetic profiles for biomarker development, addressing two primary questions: (1) whether an existing biomarker can be directly applied to synthetic profiles as “digital twin”, and (2) whether synthetic profiles could be used directly to develop biomarkers. For questions 1, the liver model using real data achieved similar classification of necrosis in both real and synthetic profiles. For questions 2, the synthetic kidney biomarkers achieved comparable performance to both real and synthetic profiles in distinguishing necrosis positives from negatives. These findings highlighted a possibility to use the synthetic data as “digital twin” to augment data size in biomarker development and evaluation in toxicology.

It is noted that TransTox was constructed using only 32 drugs which covered a limited chemistry space. In our previous work of assessing applicability domain in microarray-based genomic research [54], we noticed that gene expression data is less sensitive compared to QSARs approaches with respect to applicability domain. In this study, two external datasets were used to validate the model, including 9 drugs from TG-GATEs and 39 compounds from DrugMatrix, and the results are comparable to the experimental findings. With that said, our current evaluation in applicability domain was limited and the extensive application of TransTox to the diverse chemical universe required further studies. Therefore,

the next iteration of TransTox will be focused on expanding the data size for both training, testing and validation. Due to computational constraints when this work was conducted, exhaustive testing of all hyperparameter combinations and model structures had not been extensively explored, which should also be carried out in the next iteration of the model. Based on several GANs model developed for the TGx data including this one, we felt that the integration of existing biological knowledge, such as pathways, could be a valuable approach to improve the learning quality and, subsequently, improving the model's overall performance.

A significant challenge in toxicological research is the scarcity of available data samples and concerning animal usage, manifesting in three distinct scenarios. Firstly, regarding specific experimental technologies like transcriptomic methods, the absence of systematic evaluations across all organs impedes a systemic study of toxicity at the organism level. Secondly, regarding the animal use and 3Rs principle, in vitro experiments has been extensively evaluated in replacing in vivo studies, posing a challenge of in vitro-in vivo extrapolation (IVIVE). The third scenario involves multi-omics studies, where acquiring a complete set of omics data for an extensive collection of drugs or chemicals becomes especially challenging for comprehensive analysis. This study provided a glimpse of utilizing the advanced AI models for experimental data translation, offering a potential solution to extend the data diversity and to translate the data between experimental platforms.

Table 1: Performance of the liver and kidney necrosis predictive models on the test sets with synthetic or real transcriptomic data. TN: true negative; FP: false positive; FN: false negative; TP: true positive.

Training data	Test data	TN	FP	FN	TP	Accuracy	MCC	Sensitivity	Specificity
Liver model using real profiles	Real data	73	9	5	9	0.85	0.48	0.64	0.89
	Synthetic data	72	10	5	9	0.84	0.46	0.64	0.88
Kidney model using synthetic profiles	Real data	58	7	1	2	0.88	0.34	0.67	0.89
	Synthetic data	56	9	1	2	0.83	0.29	0.67	0.86

Figure Caption

Figure 1: TransTox Framework: The architecture includes a liver generator and a kidney generator, each paired with a liver discriminator and a kidney discriminator. The generator's input comprises four components: the real transcriptomic profile from the source organ, label information for the source organ, label information for the target organ, and a noise vector. **G**: generator; **D**: Discriminator; Source label indicates the organ, time, dose of the source (initial) transcriptomic profile (like kidney, 3-day, low); Target label indicates organ, time, dose for the target transcriptomic profile (like liver, 28-day, middle).

Figure 2: Training performance of TransTox on liver (2a to 2d) and kidney (2e to 2h): Evaluation through UMAP, cosine similarity, RMSE, and MAPE.

Figure 3: Evaluation of TransTox's performance on the test set of TG-GATEs with a gene-level comparison between the treatment-based synthetic profiles and their corresponding real profiles for each organ and between organs, where x-axis is the normalized difference while the y-axis is the number of gene-treatment combinations. (3a) Difference in liver profiles between synthetic and real profiles. (3b) Difference in kidney profiles between synthetic and real profiles. (3c) Between-organ gene difference between synthetic profiles compared to that in real profiles. "All genes" refer to the studied S1500 genes, while "Important Genes" are those with an absolute fold change greater than 1 in at least one treatment.

Figure 4: TransTox's performance on the external validation set of DrugMatrix. In Figure 4a, the cosine similarity distribution is shown for the five common compounds between DrugMatrix and TG-GATEs. The "Real group" represents values calculated between the real DrugMatrix and TG-GATEs transcriptomic profiles, while the "Generated group" indicates values calculated between the real DrugMatrix and synthetic transcriptomic profiles generated by TransTox. In Figure 4b, the cosine similarity distribution is presented for the 34 compounds for which DrugMatrix had both liver and kidney profiles, but TG-GATEs had data available for only one organ or neither.

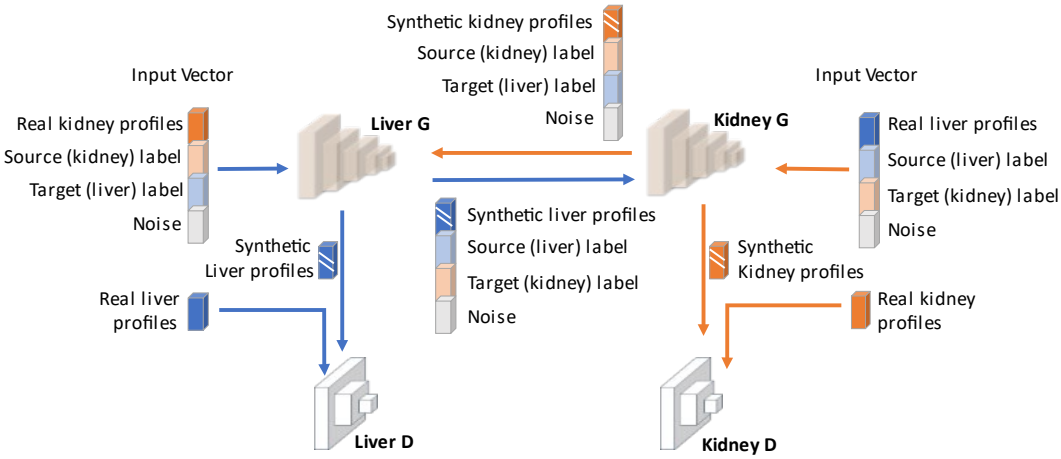
Figure 5: Mechanism Analysis: Comparison of differentially expressed genes (DEGs) identified by synthetic profiles with those defined by real transcriptomic profiles. DEGs from both synthetic and real profiles were independently subjected to KEGG pathway enrichment analyses. The Violin plots illustrate the overlap ratio of DEGs and enriched KEGG pathways in comparison to real profiles under different dose and time conditions. Liver results are depicted in panels 5a and 5b, while kidney results are

presented in panels 5c and 5d. Panels 5e and 5f illustrate the overlap ratio of DEGs and pathways for real experimental settings subjected to the same treatments for two compounds, Entacapone and Tolcapone, while the generated group presents the overlap ratio of synthetic and real liver profiles under the corresponding treatment condition.

Figure 6: Framework of Necrosis Predictive Models: Liver Necrosis Predictive Model (6a) - Developed using real liver profiles from 102 new compounds, the model was evaluated on two test sets: one with real liver profiles and the other with synthetic liver data generated by kidney generator. Kidney Necrosis Predictive Model (6b) - Utilizing synthetic kidney profiles generated from the kidney generator with the liver profiles of 102 new compounds, the model was assessed on two test sets: real kidney data and synthetic kidney data generated from the liver generator.

Supplementary Figure Caption

Figure S1: Evaluation of TransTox's performance on the external validation set of TG-GATEs for liver (S1a to S1d) and kidney (S1e to S1h): Evaluation through UMAP, cosine similarity, RMSE, and MAPE. Positive control is each measurement was calculated from the pairwise samples of biological replicates within an organ, providing a reference point for the achievable boundaries in an experiment.



G: generator; D: Discriminator
Source label: indicates the organ, time, dose of the source transcriptomic profile (like kidney, 3 day, low)
Target label: indicates organ, time, dose for the target transcriptomic profile (like liver, 28 day, middle)

1

Figure 1

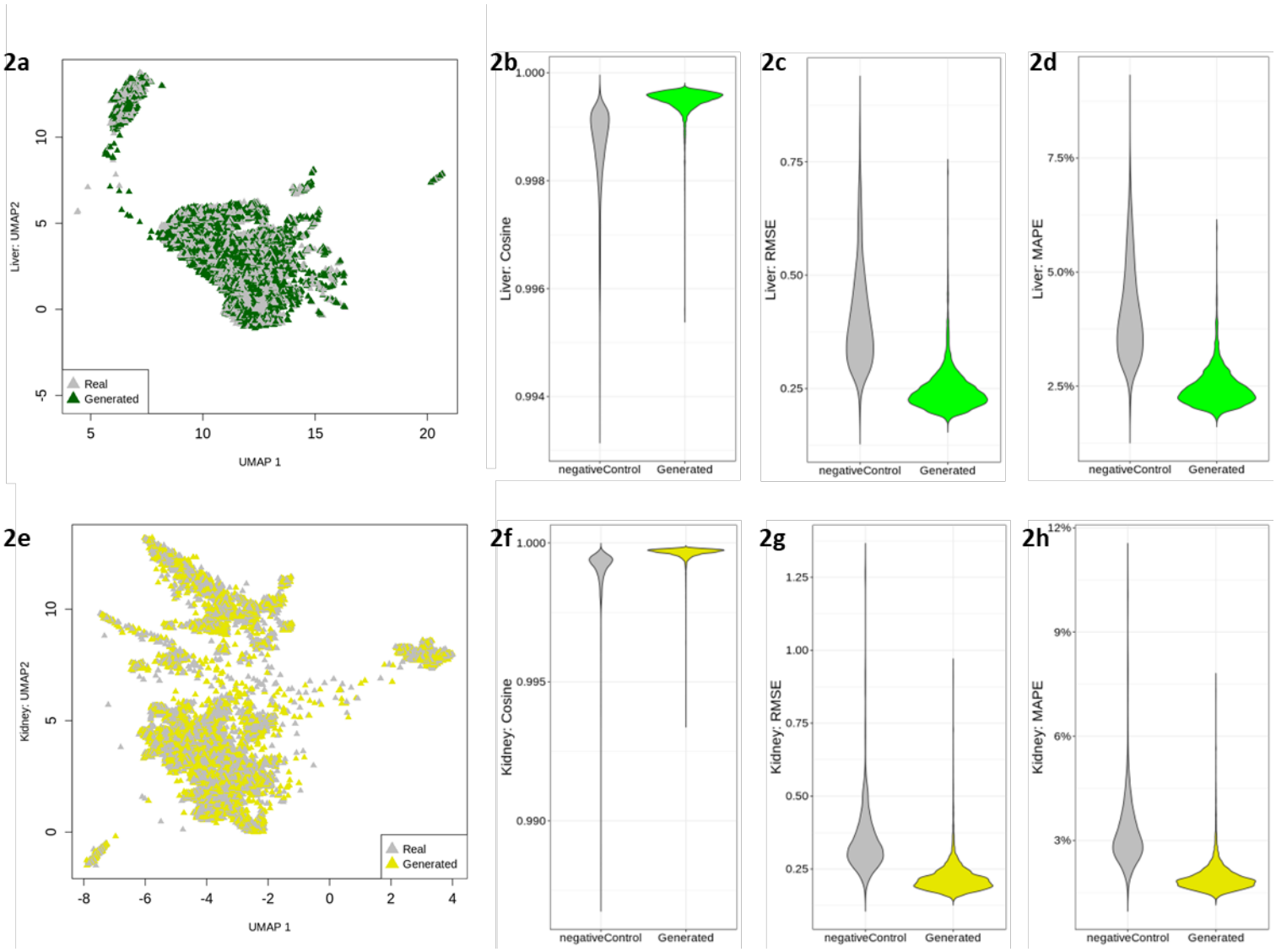

2a
Liver: UMAP2
UMAP 1
Real
Generated
2b
Liver: Cosine
negativeControl
Generated
2c
Liver: RMSE
negativeControl
Generated
2d
Liver: MAPE
negativeControl
Generated
2e
Kidney: UMAP2
UMAP 1
Real
Generated
2f
Kidney: Cosine
negativeControl
Generated
2g
Kidney: RMSE
negativeControl
Generated
2h
Kidney: MAPE
negativeControl
Generated

Figure 2

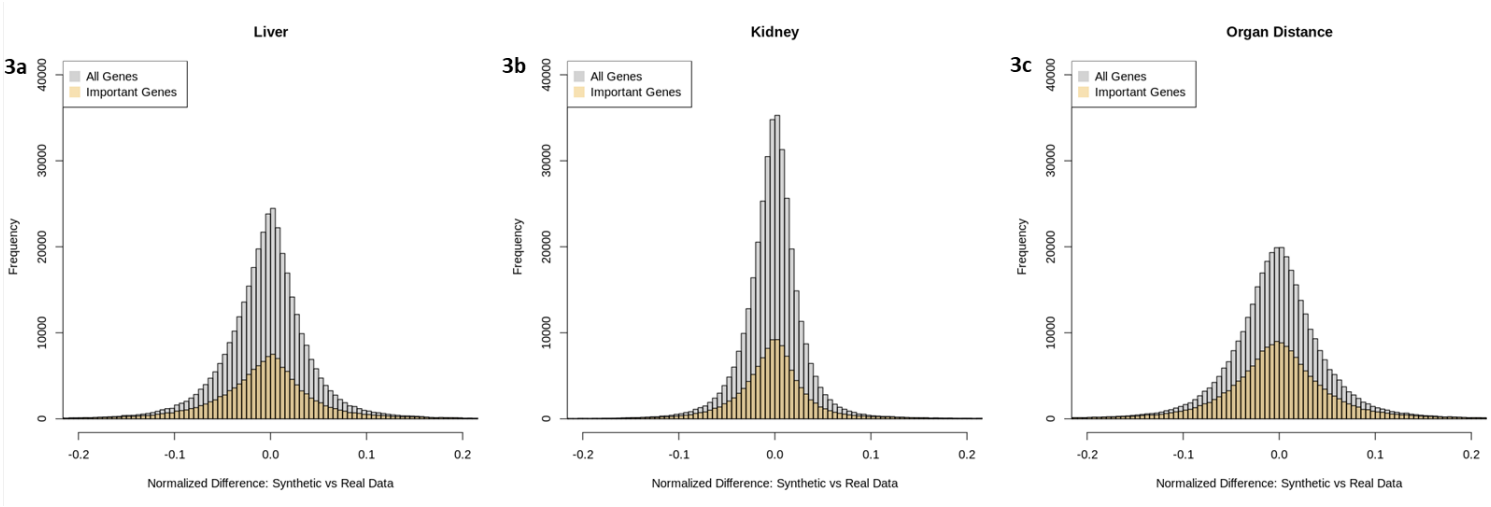

3a
Liver
All Genes
Important Genes
Frequency
0
10000
20000
30000
40000
-0.2
-0.1
0.0
0.1
0.2
Normalized Difference: Synthetic vs Real Data
3b
Kidney
All Genes
Important Genes
Frequency
0
10000
20000
30000
40000
-0.2
-0.1
0.0
0.1
0.2
Normalized Difference: Synthetic vs Real Data
3c
Organ Distance
All Genes
Important Genes
Frequency
0
10000
20000
30000
40000
-0.2
-0.1
0.0
0.1
0.2
Normalized Difference: Synthetic vs Real Data

Figure 3

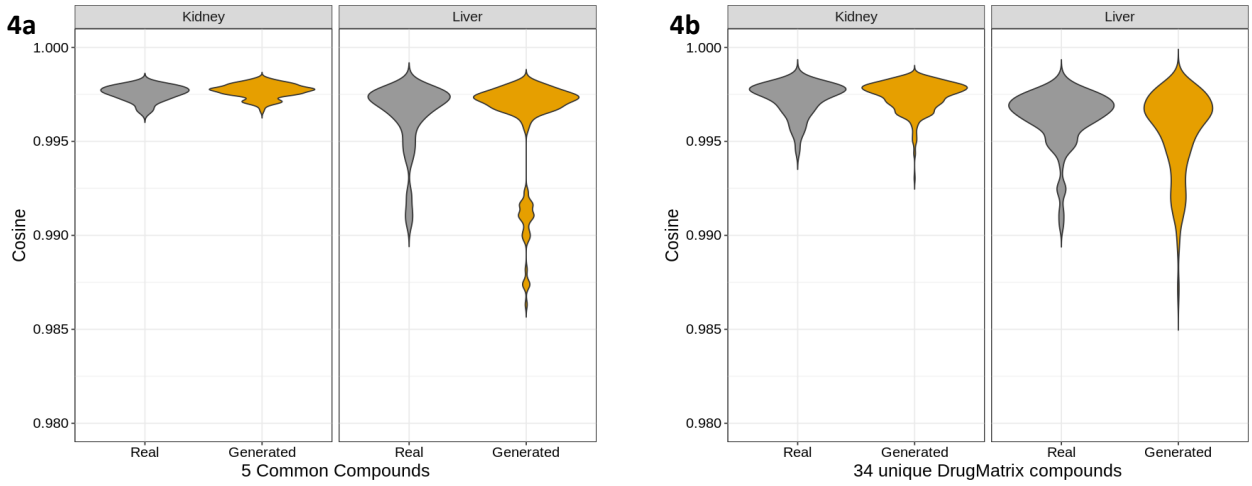

4a
Kidney
Liver
1.000
0.995
0.990
0.985
0.980
Cosine
Real
Generated
Real
Generated
5 Common Compounds
4b
Kidney
Liver
1.000
0.995
0.990
0.985
0.980
Cosine
Real
Generated
Real
Generated
34 unique DrugMatrix compounds

Figure 4

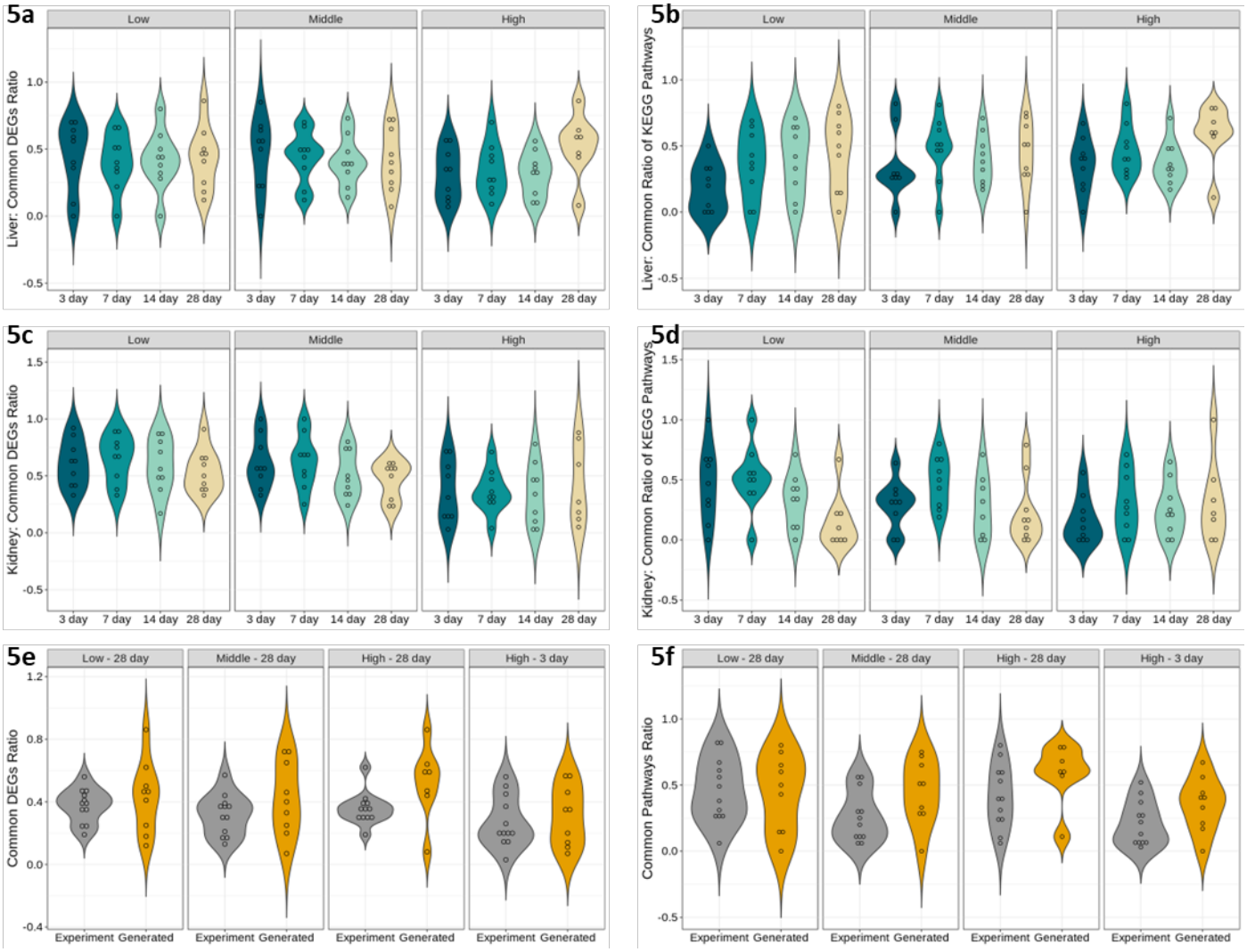

5a
Low
Middle
High
Liver: Common DEGs Ratio
3 day
7 day
14 day
28 day
5b
Liver: Common Ratio of KEGG Pathways
5c
Kidney: Common DEGs Ratio
5d
Kidney: Common Ratio of KEGG Pathways
5e
Low - 28 day
Middle - 28 day
High - 28 day
High - 3 day
Common DEGs Ratio
Experiment Generated
5f
Common Pathways Ratio

Figure 5

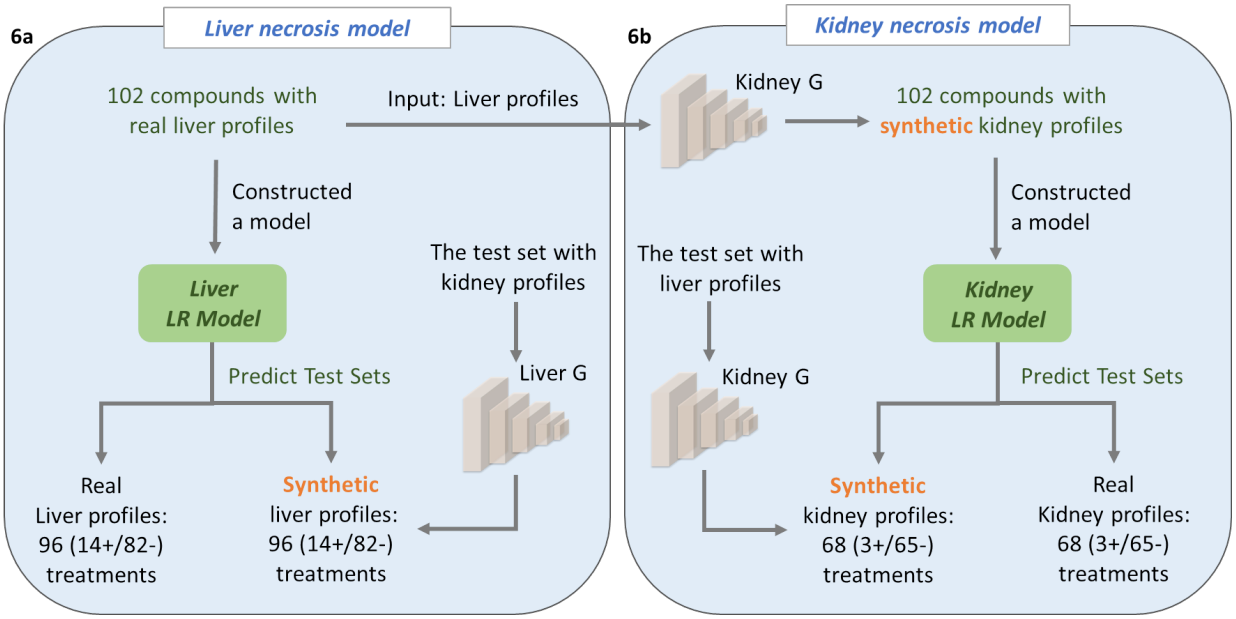

6a
Liver necrosis model
102 compounds with
real liver profiles
Input: Liver profiles
Constructed
a model
Liver
LR Model
The test set with
kidney profiles
Liver G
Predict Test Sets
Real
Liver profiles:
96 (14+/82-)
treatments
Synthetic
liver profiles:
96 (14+/82-)
treatments
6b
Kidney necrosis model
Kidney G
102 compounds with
synthetic kidney profiles
Constructed
a model
The test set with
liver profiles
Kidney
LR Model
Kidney G
Predict Test Sets
Synthetic
kidney profiles:
68 (3+/65-)
treatments
Real
Kidney profiles:
68 (3+/65-)
treatments

Figure 6

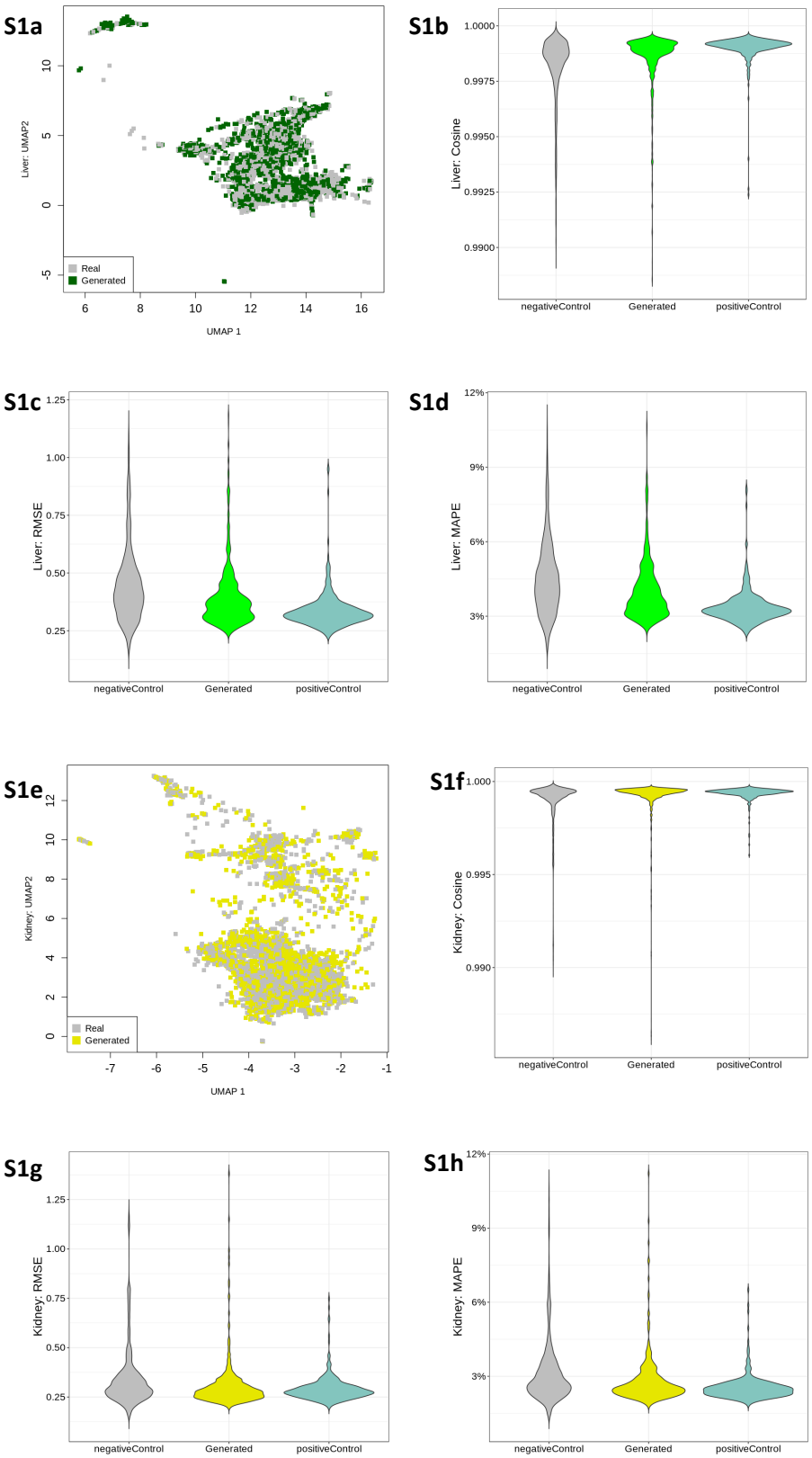

S1a
Liver: UMAP2
UMAP 1
Real
Generated
S1b
Liver: Cosine
negativeControl
Generated
positiveControl
S1c
Liver: RMSE
negativeControl
Generated
positiveControl
S1d
Liver: MAPE
negativeControl
Generated
positiveControl
S1e
Kidney: UMAP2
UMAP 1
Real
Generated
S1f
Kidney: Cosine
negativeControl
Generated
positiveControl
S1g
Kidney: RMSE
negativeControl
Generated
positiveControl
S1h
Kidney: MAPE
negativeControl
Generated
positiveControl

Figure S1

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