**SUPPLEMENTARY METHODS**

**Comparison of DNF with other methods**

We have searched the literature for drug classification algorithms that are comparable with our integrative similarity-based network approach. Notably, many available methods incorporate ATC and drug target information as input variables for their predictions, which poses an obstacle for comparison, as DNF does not rely on these data types as input but uses them as external benchmarks. As such, we identified a limited number of state-of-the-art methods to perform a comparative study with DNF. These methods comprise two main groups. The first group attempt to decode drugs’ mechanism of action based on drug similarities from CMap perturbation data [(1,2)](https://paperpile.com/c/FA51Ig/eB7aa+VVk7h). The second group comprise mainly supervised machine learning methods applied for ATC or target prediction [(3,4)](https://paperpile.com/c/FA51Ig/vkk3e+Lx1ja).

**Comparison to MoA decoding methods**

Relying only on transcriptomic perturbation data, Iorio et al. and Iskar et al. applied different approaches to first, preprocess CMAP’s perturbation profiles [(5,6)](https://paperpile.com/c/FA51Ig/9IzaC+W36me) and then, to compute the same drug-drug similarity score. In order to calculate similarity between drugs *d*i and *d*j {j=1,..,*n*} (*n* is total number of drugs), first, a *signature* is defined for *d*i, that is, two sets of (*m=*) 250 most significantly up and 250 downregulated genes are selected from the perturbation profile of *d*i. Second, *connectivity scores* [(5,6)](https://paperpile.com/c/FA51Ig/9IzaC+W36me), based on Gene Set Enrichment Analysis (GSEA), between *d*i and all *d*js, are calculated and stored. The computed scores are not necessarily symmetric, i.e., score*d*i,*d*j ≠ score*d*j,*d*i. The final score for each drug pair is calculated as the average of the two scores. Both Iorio et al. and Iskar et al. used the final scores to construct a drug similarity network. We used the same approach to calculate the similarity scores for the set of drugs under study for DNF. However, the aforementioned studies rely on the CMap data, while we used the L1000 profiles in our study. Therefore, due to the smaller number of genes (978) in the L1000 dataset, we had to select a smaller size (e.g., *m*=30) for the drug signatures. Additionally, we had to adapt pre-processing approaches used by the aforementioned studies to be applicable to L1000. The adaptations are described as follows.

**Pre-processing according to Iorio et al.**

Following Iorio’s method, for each drug, we first aggregated all lists of differentially expressed genes computed from treating different cell lines by the drug. For this purpose, we used *RankMerging* function (*GeneExpressionSignature* package: <http://www.bioconductor.org/packages/release/bioc/html/GeneExpressionSignature.html>). The method uses computes Spearman’s foot-rule (distance measure between two ranked lists) between each pair of signatures, and using *Borda* merging method*,* repeatedly, it merges the most similar pair of ranked lists each time, till obtaining one single ranked list for the drug.

After aggregating the signatures, we calculated the drug-pair distances (*connectivity scores* described above). Unfortunately, the corresponding prediction results were not significant. Therefore, in the second attempt, we applied the pre-processed perturbation signatures by PharmacoGx [(7)](https://paperpile.com/c/FA51Ig/zoNd0) package. Then, we computed the scores, and calculated the prediction results (shown in Supplementary Figure 9 as IorioPGX). The prediction results are close to the perturbation layer of DNF, as expected.

**Pre-processing according to Iskar et al.**

Iskar et al. follow as very different approach from Iorio et al. for pre-processing drug signatures. In order to adapt the method to the L1000 dataset, we first, discarded vehicle controls and mean centered the treatment samples within each batch. Then, for each drug, we averaged all its treatment replicates for each cell line. Therefore, we ended up with one single list for each drug for each cell line. L1000 consists of 77 cell lines in contrast to only five cell lines in CMap. In the next step, we calculated *connectivity scores* between drugs within each cell line. For each drug pair, the scores over multiple cell lines were averaged to compute the final scores. Prediction results based on these scores have been demonstrated (Supplementary Figure 9).

**Comparison to supervised ATC/target prediction methods**

The ATC (target) prediction methods decode ATC codes (targets) for unknown drugs according to their similarities to a set of drugs with known ATC codes (targets). We selected SuperPred [(3)](https://paperpile.com/c/FA51Ig/vkk3e) and DrugE-Rank [(4)](https://paperpile.com/c/FA51Ig/Lx1ja) methods for comparison with DNF, as these methods are freely available. Therefore, these methods do not aim at providing drug similarity scores and therefore they are not directly comparable to DNF. To address this issue, we post-processed their outputs generated from the corresponding web-based applications.

**SuperPred: ATC prediction Method**

SuperPred computes and integrates three structural, i.e., 2D, fragment and 3D, similarities between an input drug di and a dataset of drugs with known ATC codes.

The following steps were performed to collect SuperPred’s output and compare to DNF:

1. We retrieved SMILES for the set of drugs under study by DNF (di) and submitted them one-by-one to the SuperPred website and obtained the predictions. SuperPred retrieves the top five similar drugs (d’j) to the input drug. A prediction score is assigned to each retrieved drug (scoredi,d’j). Each d’j is a drug with a set of *k* known ATC codes (atckd’j). In some cases, the top prediction (i.e., the most similar drug) retrieved by SuperPred is the same as the input drug. We discarded such predictions, and collected the rest of the predictions for each drug (see “superPredResultsFinal.xlsx”). We defined the set of ATC predictions for di based on ATCs of d’js, i.e., ATCdi = {atckd’j}, and assigned a weight to each code according to the prediction score, i.e., watc,dik = scoredi,d’j.
2. We used Kendall's *tau* distance between partial rankings [(8)](https://paperpile.com/c/FA51Ig/QpwsD) on SuperPred’s predictions to compute pairwise drug-drug similarities.
3. Finally, we processed this matrix using “generateDrugPairs.R”, “generateRocPlot.R” and “generatePRPlot.R” functions (https://github.com/bhklab/DNF) along with predictions from the four layers of DNF. Please refer to Supplementary Figure 9 for results.

**DrugE-Rank: Target prediction Method**

DrugE-Rank [(4)](https://paperpile.com/c/FA51Ig/Lx1ja), a state-of-the-art target prediction method that uses an ensemble of a few efficient computational methods, i.e., k-nearest neighbor (k-NN), Bipartite Local Model with support vector classification (BLM-svc) [(9)](https://paperpile.com/c/FA51Ig/p1jPf), Bipartite Local Model with support vector regression (BLM-svr) [(9)](https://paperpile.com/c/FA51Ig/p1jPf), Laplacian regularized least squares (LapRLS) [(10)](https://paperpile.com/c/FA51Ig/W1EKr), Network based Laplacian regularized least squares (NetLapRLS) [(10)](https://paperpile.com/c/FA51Ig/W1EKr), and Weighted Nearest Neighbor-based Gaussian Interaction Profile classifier (WNN-GIP) [(11)](https://paperpile.com/c/FA51Ig/4zvEK) [(12)](https://paperpile.com/c/FA51Ig/Y1GbT). The predictions for our drug set were kindly provided by Dr. Shanfeng Zhu. For each drug, *d*i, a ranked list of 20 targets was provided. Then, for each pair of drugs we followed steps 2 and 3 from the SuperPred’s post-processing algorithm, described above, to compute the pairwise similarities and to compare with DNF’s results.

**Cautionary Note**

Although the adaptation of these methods was challenging due to the use of different perturbation data (L1000 instead of CMAP for Iorio and Iskar) and the limitations of the SuperPred website (predictions restricted to the top five hits), we provided the results of our comparison in Supplementary Figure 9. While DNF outperforms the published methods in all cases, we acknowledge that these results should be cautiously interpreted due to the differences in both data pre-processing and modification of the algorithms to make them comparable with our similarity-based networks.

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