A computational framework to explore cellular response mechanisms from multi-omics datasets

1 Supplementary Information

1.1 Notes

All scripts, Jupyter notebooks, and data are available as part of the supplement, and are also available online at https://github.com/LoLab-VU/MAGINE_Supplement_notebooks. Scripts were created using Python 3.7 and tested on Windows 10 x64. MAGINE can be installed with the command: `pip install magine`.

1.2 Jupyter Notebooks

- **Supplementary File S1**: `supplement_notebook_1_data_exploration.pdf`
  contains examples in data exploration.
- **Supplementary File S2**: `supplement_notebook_2_network_creation_and_exploration.pdf`
  contains examples to generate, visualize, and explore networks.
- **Supplementary File S3**: `supplement_notebook_3_enrichment_analysis.pdf`
  contains examples for running, querying, and visualizing enrichment analysis.
- **Supplementary File S4**: `supplement_notebook_4_agn.pdf`
  contains the example workflow to generate AGN.

1.3 Animations and Videos

- **Supplementary File S5**: `supplement_agn_trajectory.gif`
  Network demonstrating aggregation of ontology terms performed at each time point (time-series animation). The area of each node is proportional to the enrichment of that ontology term at the indicated time point. The thickness of edges is related to the number of edges connecting the molecular nodes underlying the two term nodes (not time-dependent).

Methods

Enriched term compression

We created a method that filters the number of enriched terms based on redundancy in gene content, starting with a ranked array with columns term_name, rank, and set of genes. We then rank all terms based on either their combined score (from EnrichR), number of genes in the term, or p-value. Starting from the highest ranked, we compare all lower ranked terms and remove them if their similarity is above a user defined threshold. We use the Jaccard index[1], which is the size of the intersection divided by the size of the union of any two sets, as our similarity metric.

Using this approach, we are able to minimize the total number of terms while maintaining their enrichment content, as shown in 1 and demonstrated in Figure **S3**. In the bendamustine dataset described in the main manuscript, 84 terms were filtered to 17 terms, a reduction of 80%. It should also be noted that we can take the compressed terms and back calculate from the original enrichment array which terms were removed, as demonstrated in Figure **S3** C.

Algorithm 1 Enrichment compression

```
procedure FILTER
    entry = [term, rank, geneSet]
    array = sorted list of entries                        ▷ EnrichmentResult class
    to_remove = set()
    for i in array do
        for j in array not including i do
            score = jaccard_index(i.geneSet,j.geneSet)
            if score > threshold then                     ▷ i contains more information than j
                to_remove.add(j)                          ▷ remove to_remove from array
```

Generation of Annotated Gene Set networks

Enriched terms provide us with big picture information about processes and when they occur, yet they do not provide us with details about the interconnection among them. *Why is DNA damage repair up regulated at 1 hour and apoptosis up-regulated at 24? Is there a cause and effect? Are they independent?* To answer these questions, we created an algorithm to extract trimmed signaling networks derived from the enriched terms. The terms can be selected based on expert knowledge, rank of enrichment, all compressed terms, or any other criteria.

Once the terms are selected, we find the nodes in the network for each term. From there, we search through all possible combinations of pairs between the terms. For example, if term 1 has genes (A, B, C) and term 2 has (D, E), we count the number of edges from the possible sets ((A, D), (A, E), (B, D), (B, E), (C, D), (C, E)) that are found in the network edges. We then do the reverse (term 2 to term 1). If a node is in both sets, we consider only the edges that connect the other term, not edges that are within the term. This is demonstrated in Figure **S5** (N). This results in one coarse-grained network, where nodes are terms and the edges are frequency of edges between any two terms, and the fine-grained network, which contains the species and the connections between them. By using both, we are able explore at a high level and zoom in on detail when required.

2 Supplementary Tables

identifier	label	type	significant	fold_change	p_value	source	sample_id
BAX	BAX_S(ph)292	gene	True	4	0.01	SILAC	01hr
HMDB00012	Deoxyuridine	metabolite	True	-2	0.01	HILIC	02hr

Supplementary Table S1: Example input data for MAGINE. Data are stored in a comma-separated value (CSV) file.

	Name	Description
Function	load_data	Load MAGINE formatted csv
	create_summary_table	Create summary of counts per experimental method and sample_id
	subset	Filter data based on list of species
	require_n_sig	Filter data to include species that were measured at least N times
Plots	heatmap	Create heatmap or clustermap of species
	volcano_plot	Create volcano plot
	volcano_by_sample	Create a series of volcano plots for each sample
	plot_species	Plot scatter plots of selected species
	plot_histogram	Create histogram of fold change values
Properties	sig	Filter data to include only significant flagged entries
	up	Filter data to include fold change >0
	down	Filter data to include fold change <0
	id_list	Compile a list of unique species from sample
	by_sample	Generate list of unique species for each sample

Supplementary Table S2: Categorized list of key functions in MAGINE's `data` module.

	Name	Description
Functions	load_enrichment	Load MAGINE created enrichment analysis csv
	run	Run enrichment analysis for list of genes across selected gene sets
	run_samples	Run enrichment analysis for multiple lists of genes across selected gene sets
	run_enrichment_for_project	Run enrichment for entire ExperimentalData instance
	require_n_sig	Filter data to include species that were measured at least N times
	filter_multi	Filter by multiple criteria (p_value, combined_score, database, etc.)
	find_similar_terms	Rank order terms by similar gene sets
	filter_based_on_words	Filter by key words
	all_genes_from_df	Generate list of all genes in current EnrichmentResult instance
	term_to_genes	Generate list of genes for given term
	remove_redundant	Remove terms that are less enriched but highly similar
Plots	heatmap	Find all paths between list
	dist_matrix	Plot distance matrix of all terms

Supplementary Table S3: Categorized list of key functions in MAGINE's `enrichment` module

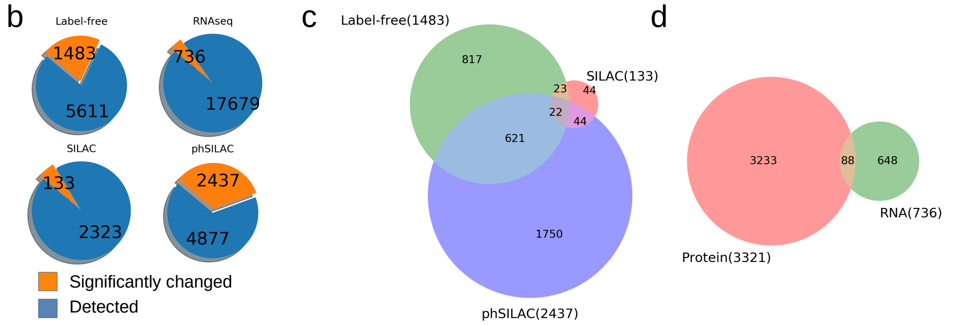
Name	**Description**
build_network	Generate network centered around provided species
create_subnetwork	Create annotated set network from enriched terms and background network
paths_between_pair	Find shortest path(s) between pair
paths_between_list	Find all paths between list
paths_between_two_lists	Find paths between two lists
neighbors	Find neighbors (upstream and/or downstream) of node
expand_neighbors	Add neighbors of node to network
draw	Draw using igraph, matplotlib, graphviz, cytoscape.js
RenderModel	Create Cytoscape instance of network through py2cytoscape

Supplementary Table S4: Categorized list of key functions in MAGINE's `network` module

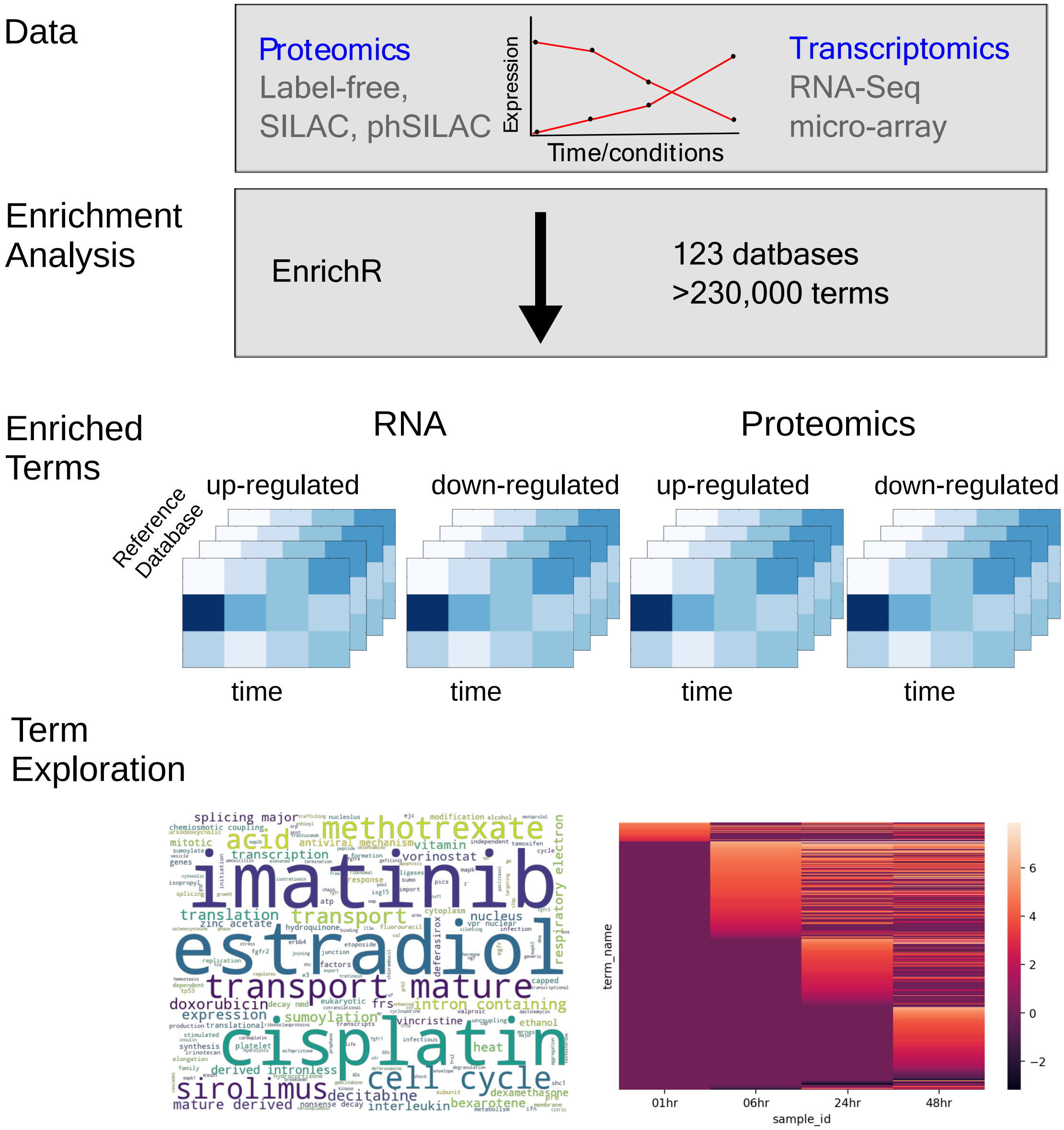
3 Supplementary Figures

a

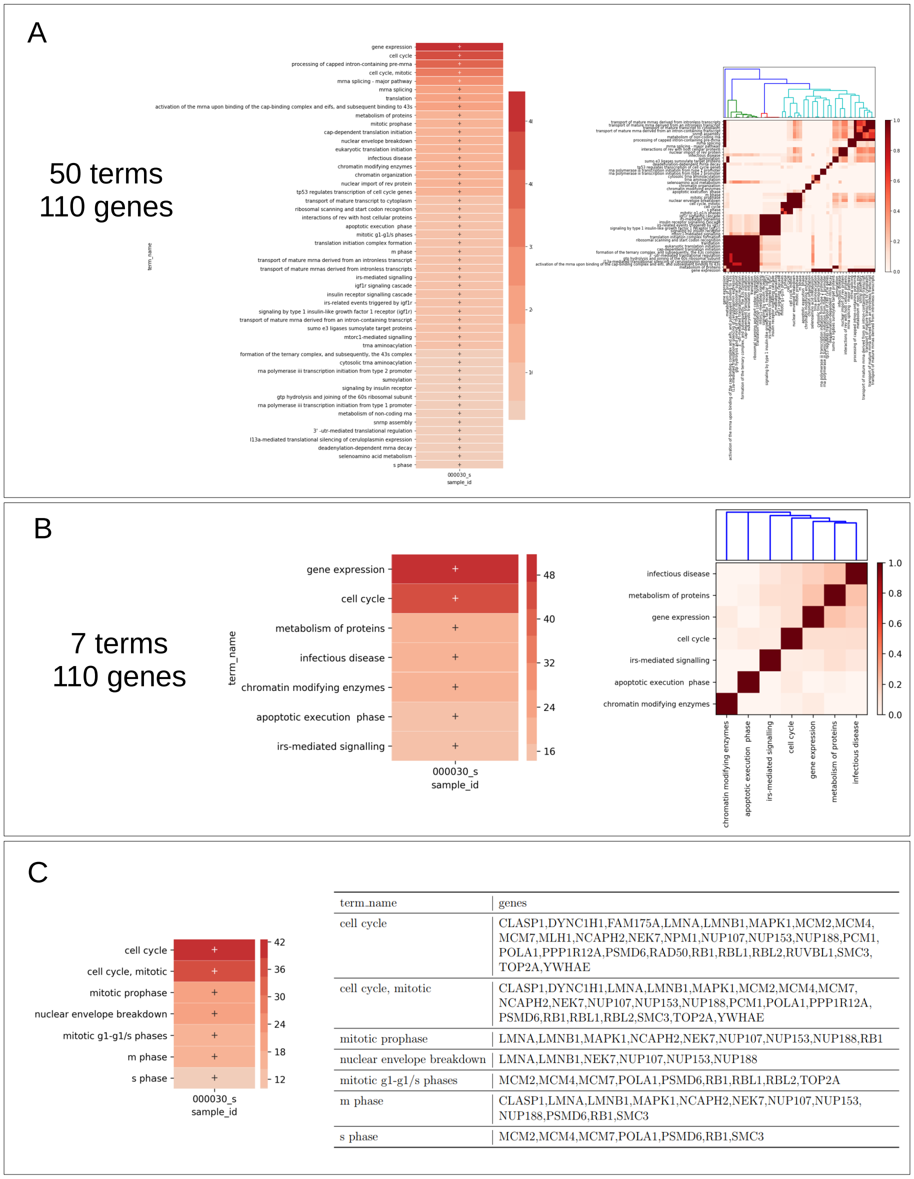
	30s	30m	01h	03h	06h	12h	18h	24h	36h	48h	60h	72h
Label-free	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
phSILAC	✓	-	✓	-	✓	✓	-	✓	-	-	-	-
SILAC	✓	-	✓	-	✓	✓	-	✓	-	-	-	-
RNAseq	-	-	✓	-	✓	✓	-	✓	-	-	-	-



Supplementary Fig. S1: **Overlap between experimental measurements.** a.) Table of time points per experimental platform that were measured for the bendamustine study. b.) Summary of number of significant changed species vs detected. c.) Venn diagram comparing phSILAC, label-free, and SILAC. d.) Venn diagram comparing proteomics (phSILAC, label-free, SILAC) vs RNAseq.

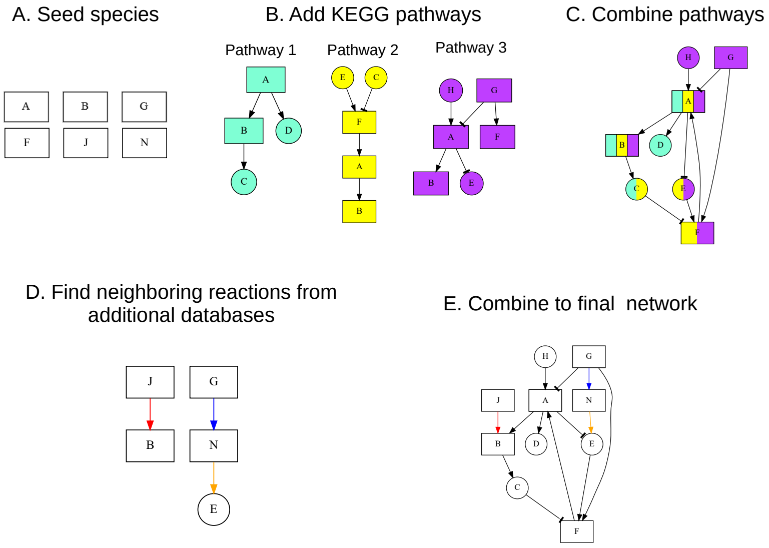


Supplementary Fig. S2: **Time series enrichment analysis pipeline**. Abstract representation of the automation of running enrichment analysis on multiple time points and experimental platforms. We utilize EnrichR to access various databases. We then organize the data. This allows us to explore the output in various ways. We can use word clouds to compress various databases to see common terms across them all. We can also plot enriched terms over times to see trends of biological processes.

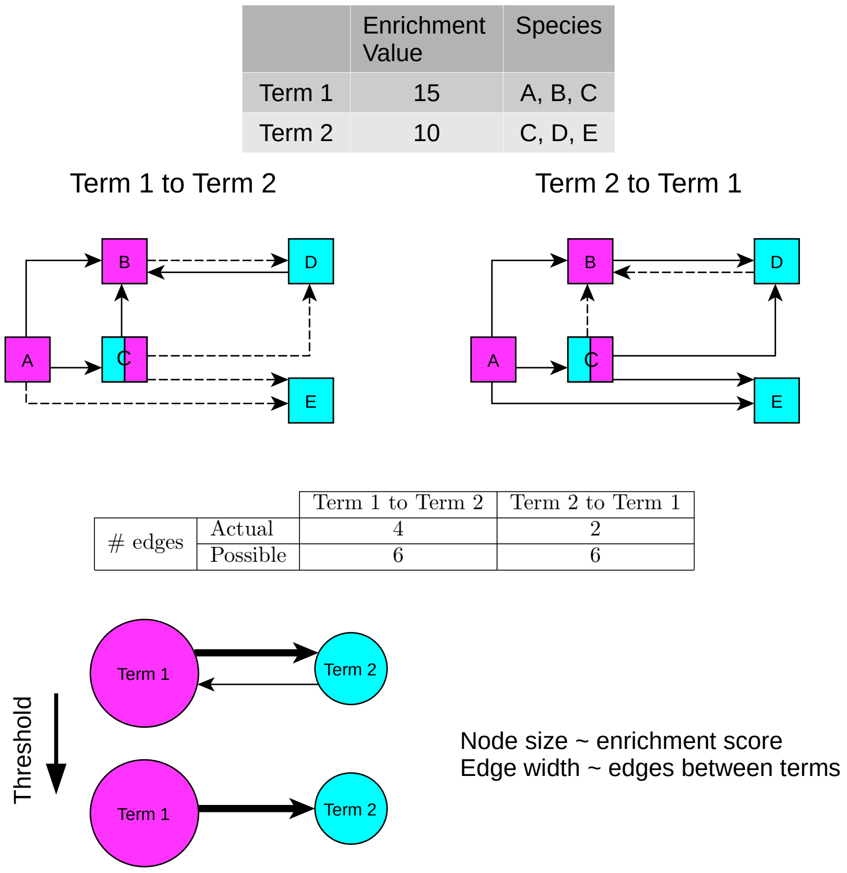


term_name	genes
cell cycle	CLASP1,DYNC1H1,FAM175A,LMNA,LMNB1,MAPK1,MCM2,MCM4, MCM7,MLH1,NCAPH2,NEK7,NPM1,NUP107,NUP153,NUP188,PCM1, POLA1,PPP1R12A,PSMD6,RAD50,RB1,RBL1,RBL2,RUVBL1,SMC3, TOP2A,YWHAE
cell cycle, mitotic	CLASP1,DYNC1H1,LMNA,LMNB1,MAPK1,MCM2,MCM4,MCM7, NCAPH2,NEK7,NUP107,NUP153,NUP188,PCM1,POLA1,PPP1R12A, PSMD6,RB1,RBL1,RBL2,SMC3,TOP2A,YWHAE
mitotic prophase	LMNA,LMNB1,MAPK1,NCAPH2,NEK7,NUP107,NUP153,NUP188,RB1
nuclear envelope breakdown	LMNA,LMNB1,NEK7,NUP107,NUP153,NUP188
mitotic g1-g1/s phases	MCM2,MCM4,MCM7,POLA1,PSMD6,RB1,RBL1,RBL2,TOP2A
m phase	CLASP1,LMNA,LMNB1,MAPK1,NCAPH2,NEK7,NUP107,NUP153, NUP188,PSMD6,RB1,SMC3
s phase	MCM2,MCM4,MCM7,POLA1,PSMD6,RB1,SMC3

Supplementary Fig. S3: **Demonstration of enrichment compression** A. Top 50 terms from Reactome for the 30 second phSILAC timepoint. The left figure shows the terms and their enriched values while the right shows the Jaccard index between all pairs. B. After filtering based on Jaccard index similarity. The resulting left figure has 7 terms, retaining all 110 genes that were contained in the terms from A. The right figure demonstrates that we have minimal overlap between corresponding terms. C. Demonstration of the collapsing of terms to a parent term. The left shows all terms that were collapsed from the term "cell cycle". The table on the right provides the genes that make up each term. Note that if a term was too broad ("cell cycle"), the user could remove it and repeat the process to maintain the level of detail which they require.



Supplementary Fig. S4: **Construction of data seeded network.** Example set of seed species to build network: (a) User provides seed species list; (b) KEGG pathways containing seed species are downloaded and merged; (c) Edges between seed species and nodes added by the KEGG pathways are identifier; (d) All nodes and edges are combined into a single network, (e).

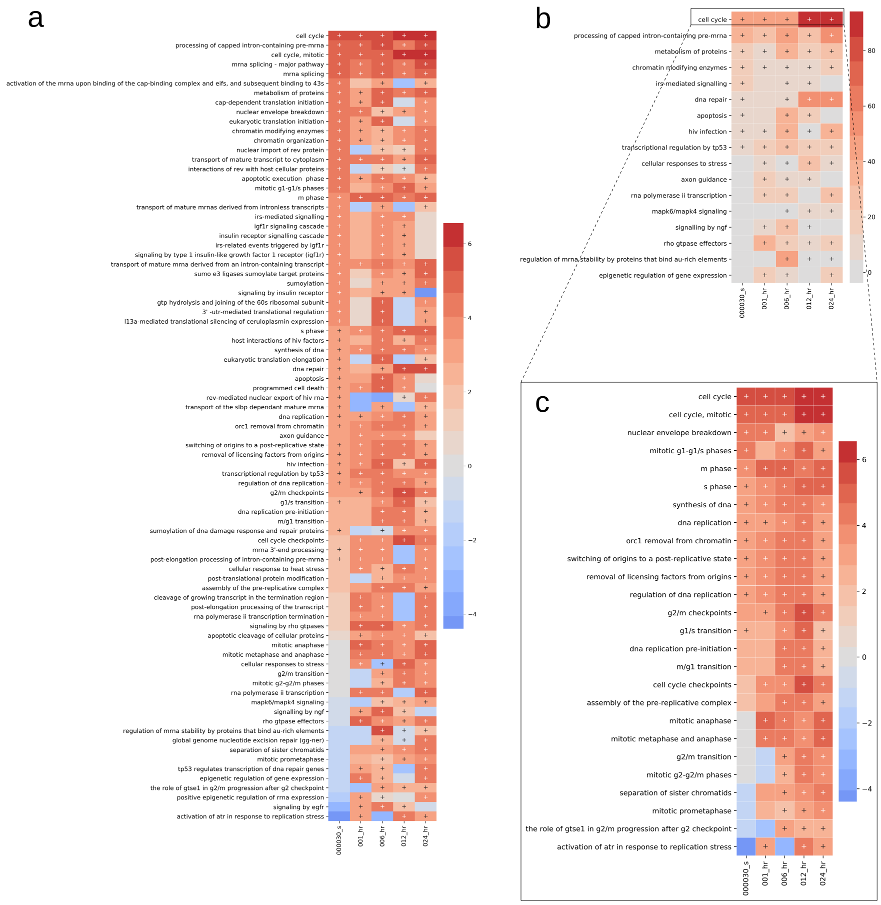


	Enrichment Value	Species
Term 1	15	A, B, C
Term 2	10	C, D, E

		Term 1 to Term 2	Term 2 to Term 1
# edges	Actual	4	2
	Possible	6	6

Node size ~ enrichment score
Edge width ~ edges between terms

Supplementary Fig. S5: **Annotated gene set network construction** Nodes belonging to Term 1 are labeled as pink, Term 2 as blue, and nodes in both are colored both. We calculate the number of edges between nodes in Term 1 to Term 2 and Term 2 and Term 1 (dashed lines), shown in middle table. We then create nodes for each Term, with the size of the node corresponding to the enrichment value. Edges are created between the terms and width set according to the number of edges between the terms. Finally, we applied a minimum of 3 edges threshold between terms to arrive at our final network.



Supplementary Fig. S6: **MAGINE provides enrichment compression** A. Enrichment results for the Reactome gene set. The left figure shows the terms and their enriched values, while the right shows the Jaccard index between all pairs. B. Resulting array after filtering based on Jaccard index similarity. The resulting left figure has 17 terms. C. Terms that have high overlap with *cell cycle*. MAGINE not only provides a method to compress the information of the enrichment array, it also provides methods to extract more detailed, but less enriched terms.

References

[1] Gilbert, G. Distance between sets. *Nature* **239**, 174 (1972).