Supplementary Information:

Network structure and fluctuation data improve inference of metabolic interaction strengths with the inverse Jacobian

Contents

Supplementary Note 1

Evaluation literature models

To evaluate our algorithm and the potential effect of non-diagonal fluctuations on the differential Jacobian, we consider the four literature models that have also been used in our previous study [1]. We describe how to generate the two conditional Jacobians as following:

1, A model of carbohydrate energy metabolism [2]: One condition is obtained from the nominal parameter values; for the other condition we increased the reaction rate parameter in R2, Pyrute+NADHc→Lactose+NADc, five-fold.

2, AMPK-mTOR pathway model [3]: The paper describes a wild type and an mTOR knockout model based on time-series experimental data; these two variants are used to define the differential Jacobian.

3, Hepatic glucose metabolism model [4]. The first condition is from nominal parameters. We applied a two-fold parameter change for the reaction rate of the second reaction R2 for the second condition.

4, Large-scale blood cell metabolism model [5]. We introduced a five-fold increase to several components of the Jacobian matrix directly.

These Jacobian matrixes are available in Supplementary data 1.

References

1.   Li, J., S. Waldherr, and W. Weckwerth, *COVRECON: Automated Integration of Genome- and Metabolome-Scale Network Reconstruction and Data-driven Inverse Modeling of Metabolic Interaction Neworks.* Bioinformatics, 2023.
2.   Nazaret, C. and J.-P. Mazat, *An old paper revisited:"A mathematical model of carbohydrate energy metabolism. Interaction between glycolysis, the Krebs cycle and the H-transporting shuttles at varying ATPases load" by VV Dynnik, R. Heinrich and EE Sel'kov.* Journal of theoretical biology, 2008. **252**(3): p. 520-529.
3.   Dalle Pezze, P., et al., *A systems study reveals concurrent activation of AMPK and mTOR by amino acids.* Nature communications, 2016. **7**(1): p. 1-19.
4.   Bulik, S., H.-G. Holzhütter, and N. Berndt, *The relative importance of kinetic mechanisms and variable enzyme abundances for the regulation of hepatic glucose metabolism–insights from mathematical modeling.* BMC biology, 2016. **14**(1): p. 1-22.
5.   Holzhütter, H.G., *The principle of flux minimization and its application to estimate stationary fluxes in metabolic networks.* European journal of biochemistry, 2004. **271**(14): p. 2905-2922.
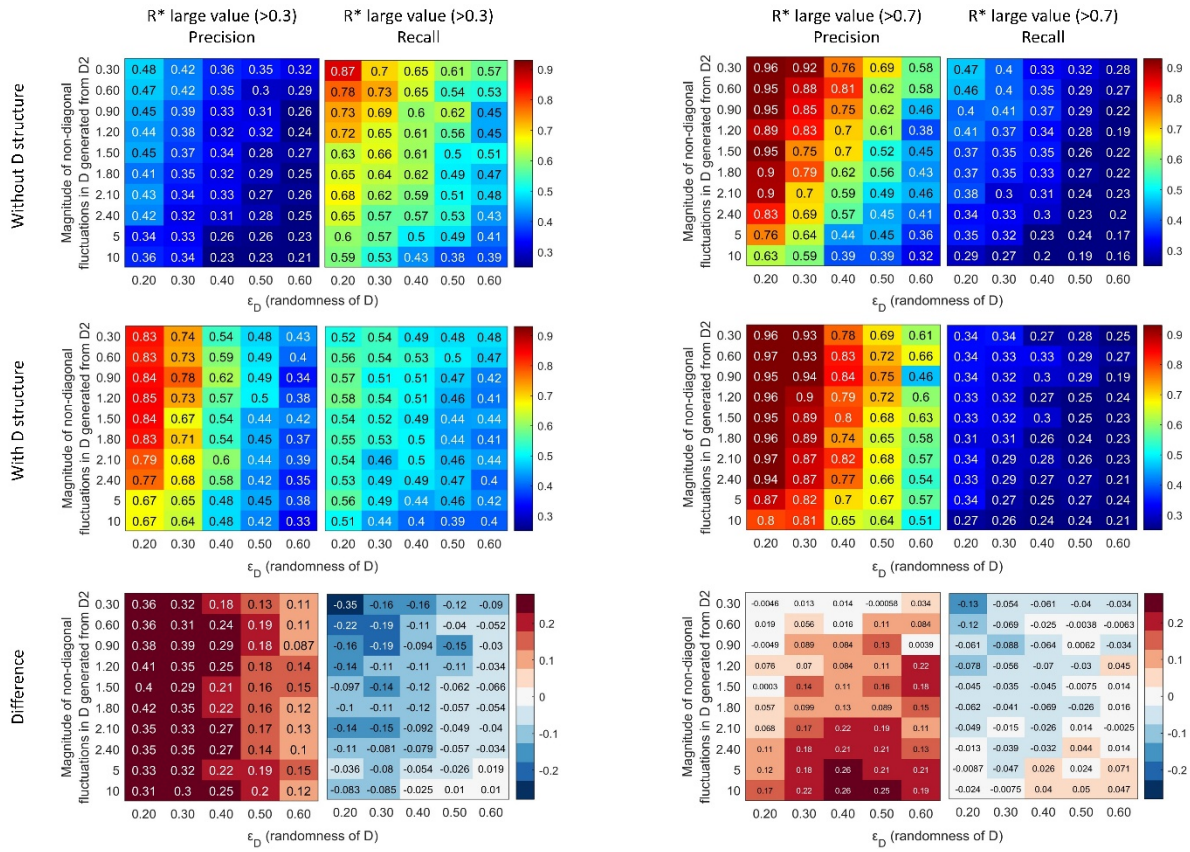
Supplementary Figure 1. Precision and Recall of the large values in R^* based on different thresholds (left: 0.3, right: 0.7). Color code refers to Figure 4A.
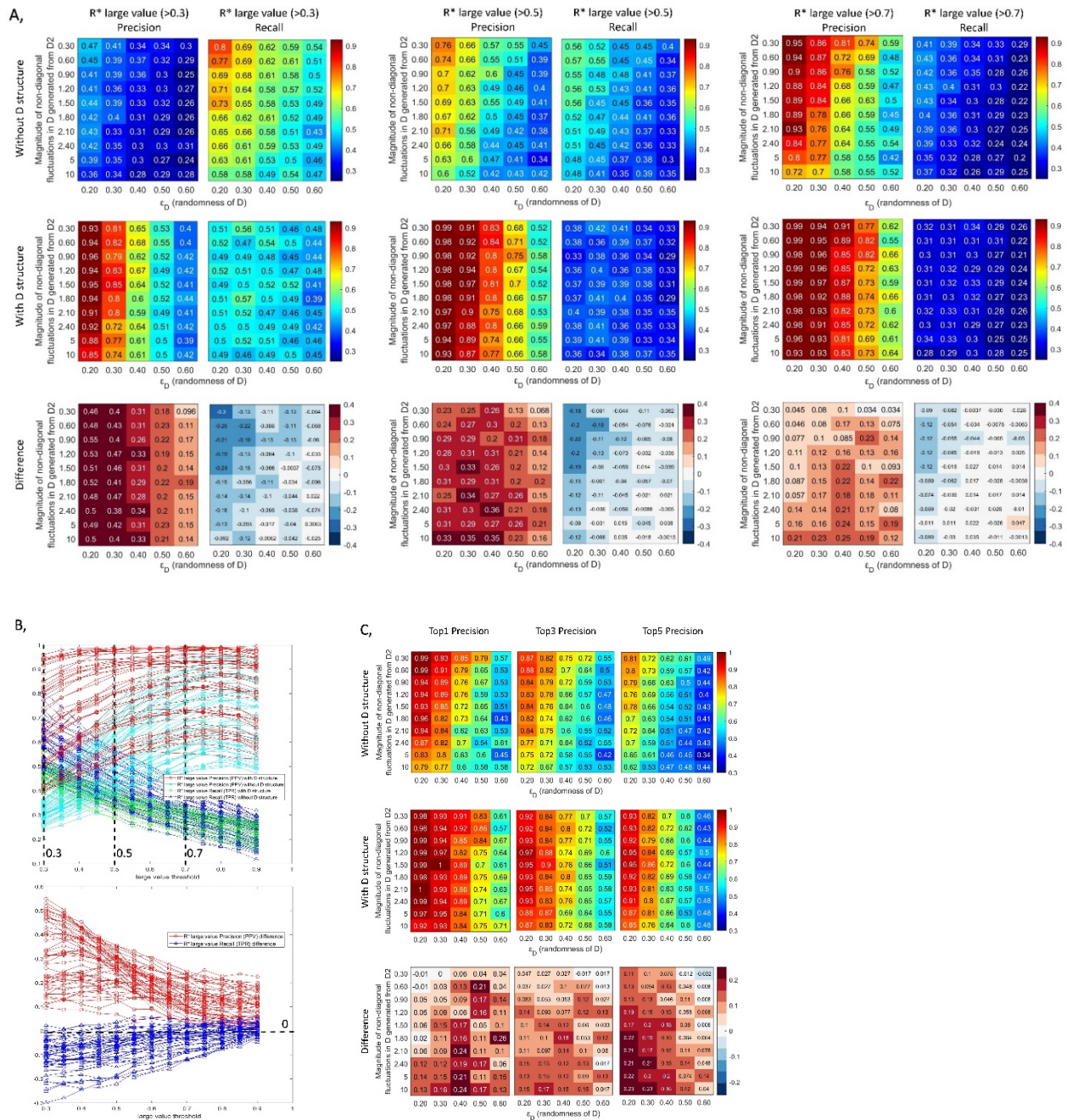
Supplementary Figure 2. Inverse differential Jacobian algorithm with/without D structure evaluation using various randomness of D ε_D and the magnitude of off-diagonal fluctuations. The evaluation is conducted using the first model in method section with 200 repeats and 6 random enzyme fluctuations applied. A, Precision and Recall of the large values (above 0.5) in R^* over 200 repeats with/without D structure information; B, the line plots of Precision and Recall of the large values in R^* with/without D structure information based on different large value thresholds (0.3-0.9), where the snapshots of value 0.3,

0.5 and 0.7 refer to A; C, the accuracy of the top 1, top 3 and top 5 large values in R^* over 200 repeats with/without D structure information.

Real differential Jacobian matrix DJ

All reactions perturbed with 0.3 times magnitude compared with D1 magnitude

a,
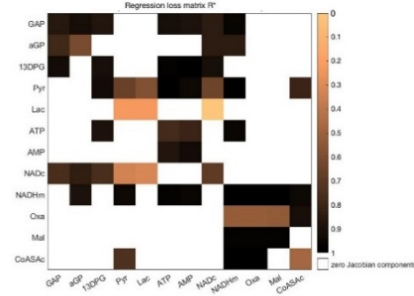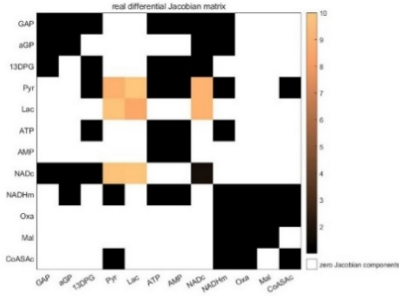
b,
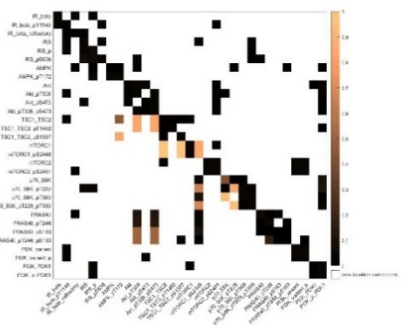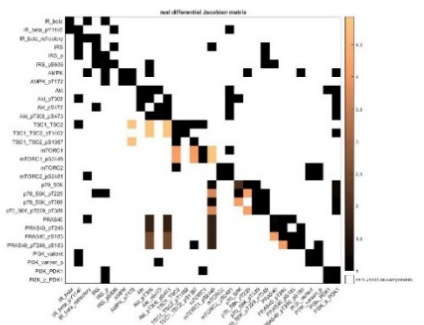
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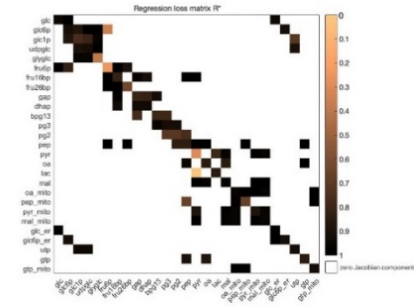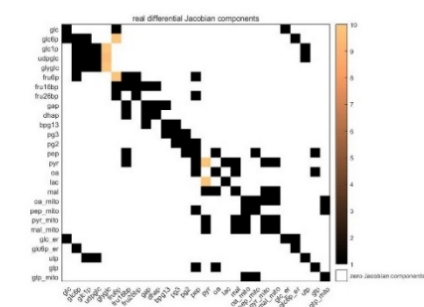
d,
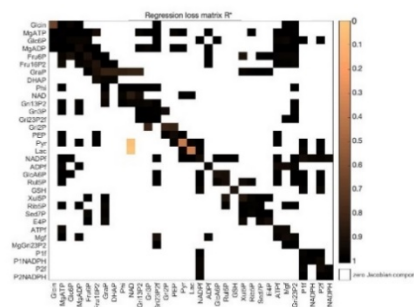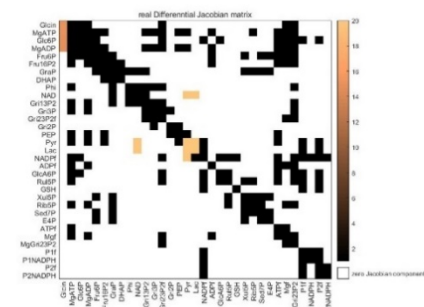
Supplementary Figure 3. The regression loss Jacobian algorithm evaluation assuming a diagonal fluctuation matrix D with $\varepsilon_D = 0.4$. For each test, all reactions are perturbed using a 0.3 magnitude fluctuation compared the metabolites fluctuation.

R* large value (>0.3)
Precision

R* large value (>0.3)
Recall

R* large value (>0.7)
Precision

R* large value (>0.7)
Recall

Without D structure

With D structure

Difference

Magnitude of non-diagonal fluctuations in D generated from D2

Number of non-diagonal fluctuations in D generated from D2
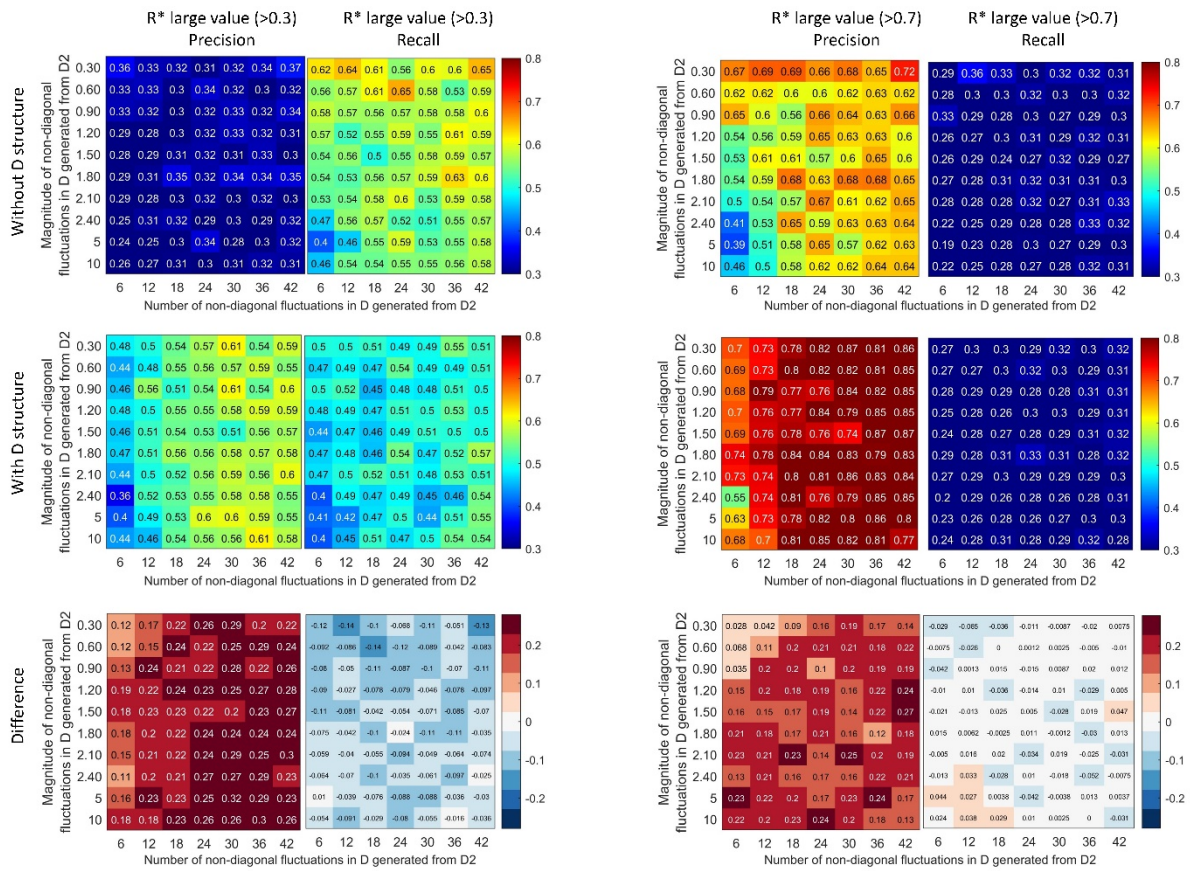
Supplementary Figure 4. Precision and Recall of the large values in R^* based on different thresholds (left: 0.3, right: 0.7). Color code refers to Figure 5A.
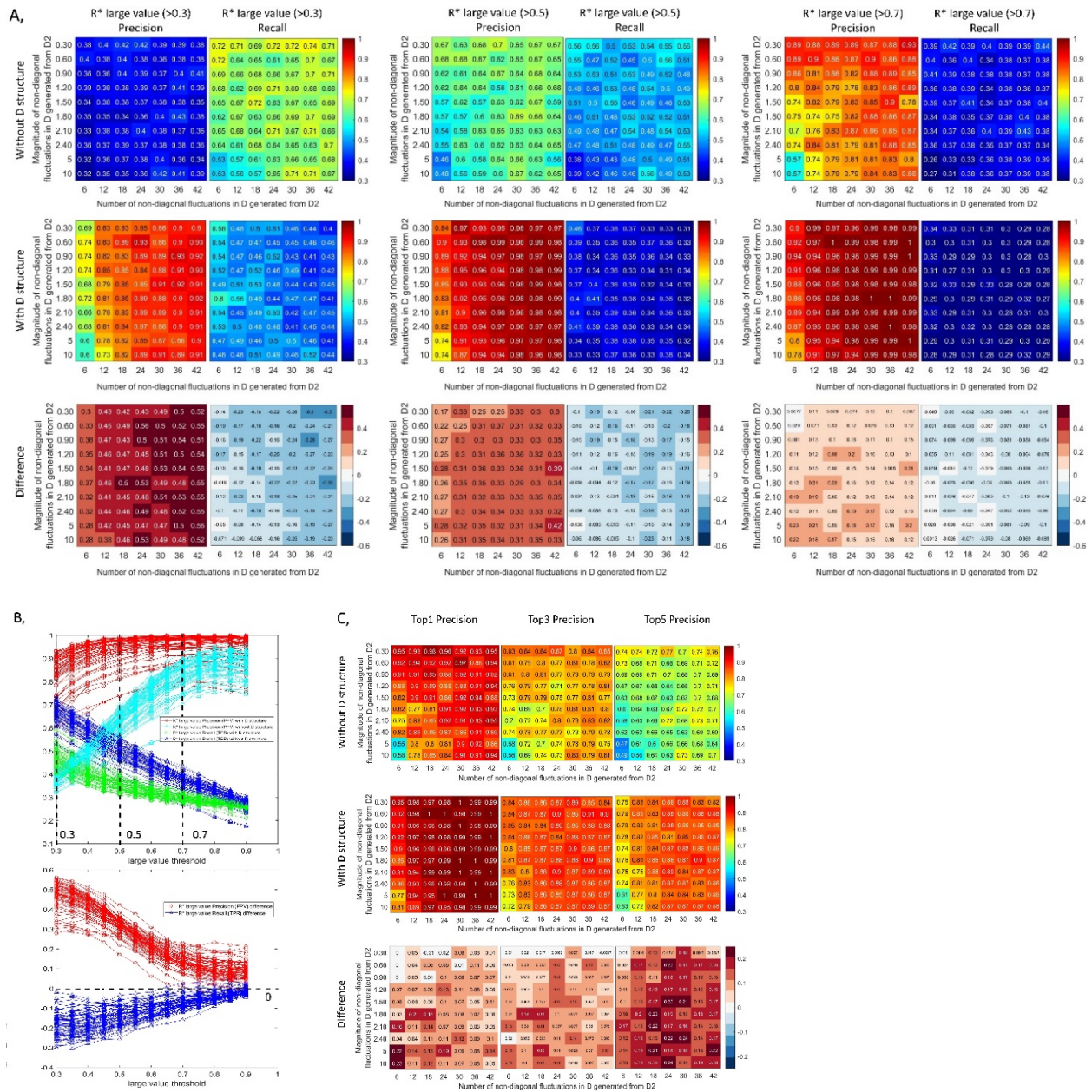
Supplementary Figure 5. Inverse differential Jacobian algorithm with/without D structure evaluation using various number and magnitude of off-diagonal fluctuations. The evaluation is conducted using the first model in method section with 200 repeats and $\varepsilon_D = 0.2$. A, Precision and Recall of the large values (above 0.5) in $\boldsymbol{R}^*$ over 200 repeats with/without D structure information; B, the line plots of Precision and Recall of the large values in $\boldsymbol{R}^*$ with/without D structure information based on different large value thresholds (0.3-0.9), where the snapshot of value 0.5 refers to A; C, the accuracy of the top 1, top 3 and top 5 large values in $\boldsymbol{R}^*$ over 200 repeats with/without D structure information.

R* large value (>0.3)
Precision

R* large value (>0.3)
Recall

R* large value (>0.7)
Precision

R* large value (>0.7)
Recall

Enzyme-activity Integrative D sampling

Number of small non-diagonal fluctuations (magnitude 0.3)

Precision (>0.3):

y \ x	3	6	9	12	15
5	0.67	0.79	0.75	0.8	0.83
10	0.74	0.78	0.78	0.86	0.85
15	0.79	0.83	0.8	0.87	0.84
20	0.82	0.83	0.82	0.84	0.88
25	0.86	0.82	0.87	0.87	0.86
30	0.88	0.83	0.91	0.85	0.9
35	0.87	0.88	0.87	0.92	0.92
40	0.88	0.87	0.82	0.9	0.9

Recall (>0.3):

y \ x	3	6	9	12	15
5	0.54	0.52	0.45	0.48	0.48
10	0.55	0.54	0.52	0.5	0.55
15	0.5	0.49	0.51	0.42	0.46
20	0.53	0.5	0.53	0.49	0.47
25	0.5	0.5	0.49	0.47	0.47
30	0.49	0.53	0.47	0.45	0.47
35	0.5	0.48	0.47	0.45	0.48
40	0.47	0.47	0.5	0.52	0.47

Precision (>0.7):

y \ x	3	6	9	12	15
5	0.89	0.9	0.9	0.94	0.94
10	0.9	0.89	0.94	0.97	0.98
15	0.94	0.95	0.93	0.96	0.96
20	0.94	0.94	0.94	0.96	0.98
25	0.98	0.96	0.98	0.99	0.99
30	0.99	0.95	0.99	0.97	0.99
35	0.98	0.99	0.98	0.98	1
40	0.99	0.97	0.94	0.99	0.99

Recall (>0.7):

y \ x	3	6	9	12	15
5	0.35	0.33	0.29	0.3	0.3
10	0.3	0.32	0.32	0.31	0.31
15	0.3	0.31	0.33	0.29	0.29
20	0.29	0.33	0.31	0.3	0.3
25	0.3	0.33	0.3	0.3	0.31
30	0.3	0.34	0.29	0.3	0.32
35	0.3	0.3	0.31	0.29	0.31
40	0.31	0.29	0.3	0.33	0.3

Number of large non-diagonal fluctuations (magnitude 10)

Difference to without D structure

Number of small non-diagonal fluctuations (magnitude 0.3)

Precision (>0.3):

y \ x	3	6	9	12	15
5	0.31	0.38	0.38	0.42	0.47
10	0.38	0.39	0.38	0.5	0.49
15	0.42	0.49	0.45	0.49	0.47
20	0.44	0.46	0.43	0.49	0.52
25	0.51	0.43	0.5	0.48	0.5
30	0.52	0.44	0.53	0.49	0.51
35	0.48	0.52	0.47	0.52	0.52
40	0.5	0.51	0.47	0.53	0.5

Recall (>0.3):

y \ x	3	6	9	12	15
5	-0.11	-0.11	-0.18	-0.19	-0.13
10	-0.087	-0.12	-0.12	-0.13	-0.089
15	-0.14	-0.13	-0.15	-0.22	-0.22
20	-0.1	-0.17	-0.13	-0.18	-0.19
25	-0.15	-0.16	-0.18	-0.21	-0.17
30	-0.19	-0.17	-0.19	-0.27	-0.21
35	-0.19	-0.19	-0.19	-0.24	-0.2
40	-0.21	-0.19	-0.16	-0.2	-0.22

Precision (>0.7):

y \ x	3	6	9	12	15
5	0.1	0.073	0.14	0.12	0.18
10	0.096	0.13	0.11	0.17	0.18
15	0.12	0.21	0.17	0.16	0.14
20	0.062	0.15	0.14	0.15	0.13
25	0.12	0.16	0.19	0.17	0.12
30	0.15	0.15	0.16	0.17	0.15
35	0.17	0.16	0.12	0.1	0.13
40	0.14	0.2	0.14	0.11	0.13

Recall (>0.7):

y \ x	3	6	9	12	15
5	-0.029	-0.06	-0.057	-0.08	-0.052
10	-0.054	-0.039	-0.066	-0.052	-0.053
15	-0.069	-0.015	-0.044	-0.081	-0.084
20	-0.069	-0.04	-0.07	-0.068	-0.06
25	-0.071	-0.069	-0.071	-0.052	-0.041
30	-0.083	-0.025	-0.074	-0.084	-0.055
35	-0.064	-0.071	-0.071	-0.064	-0.08
40	-0.057	-0.096	-0.079	-0.096	-0.078

Number of large non-diagonal fluctuations (magnitude 10)

Difference to with topological D structure

Number of small non-diagonal fluctuations (magnitude 0.3)

Precision (>0.3):

y \ x	3	6	9	12	15
5	0.13	0.026	0.038	0.021	0.018
10	0.17	0.088	0.056	0.059	0.035
15	0.17	0.13	0.073	0.028	-0.0023
20	0.28	0.086	0.076	0.053	0.044
25	0.28	0.12	0.11	0.096	0.04
30	0.29	0.19	0.073	0.077	0.066
35	0.28	0.19	0.095	0.09	0.047
40	0.29	0.18	0.097	0.075	0.078

Recall (>0.3):

y \ x	3	6	9	12	15
5	-0.043	-0.034	-0.058	-0.011	-0.0012
10	-0.021	0.033	-0.039	-0.038	0.0062
15	-0.075	-0.072	-0.036	-0.036	-0.015
20	-0.019	-0.031	-0.0062	-0.005	-0.018
25	-0.073	-0.056	-0.026	-0.035	-0.027
30	-0.088	-0.024	-0.005	-0.023	-0.03
35	-0.017	-0.06	-0.0075	-0.044	-0.03
40	-0.054	-0.053	-0.035	0.0087	-0.011

Precision (>0.7):

y \ x	3	6	9	12	15
5	0.089	-0.014	0.027	0.0054	8.5e-05
10	0.13	0.0044	0.029	0.0012	0.014
15	0.09	0.052	0.025	0.0076	0.004
20	0.14	0.041	0.031	-0.0059	0.012
25	0.2	0.049	0.049	0.023	0.023
30	0.23	0.1	-0.0005	0.025	0.031
35	0.15	0.12	0.028	0.0015	0.013
40	0.18	0.087	0.03	0.011	0.03

Recall (>0.7):

y \ x	3	6	9	12	15
5	0.012	-0.0025	-0.0062	-0.0063	-0.0075
10	-0.016	0.024	-0.018	-0.016	-0.015
15	-0.028	-0.013	0.032	-0.0013	0.0037
20	-0.01	0.018	-0.0025	-0.0037	-0.014
25	-0.023	-0.015	-0.0063	-0.036	0.0062
30	0.017	0.014	-0.0088	-0.019	-0.0025
35	-0.011	-0.015	0.011	-0.041	-0.012
40	0.0012	-0.031	-0.015	-0.019	-0.0038

Number of large non-diagonal fluctuations (magnitude 10)

Supplementary Figure 6. Precision and Recall of the large values in R^* based on different thresholds (left: 0.3, right: 0.7). Color code refers to Figure 6A.

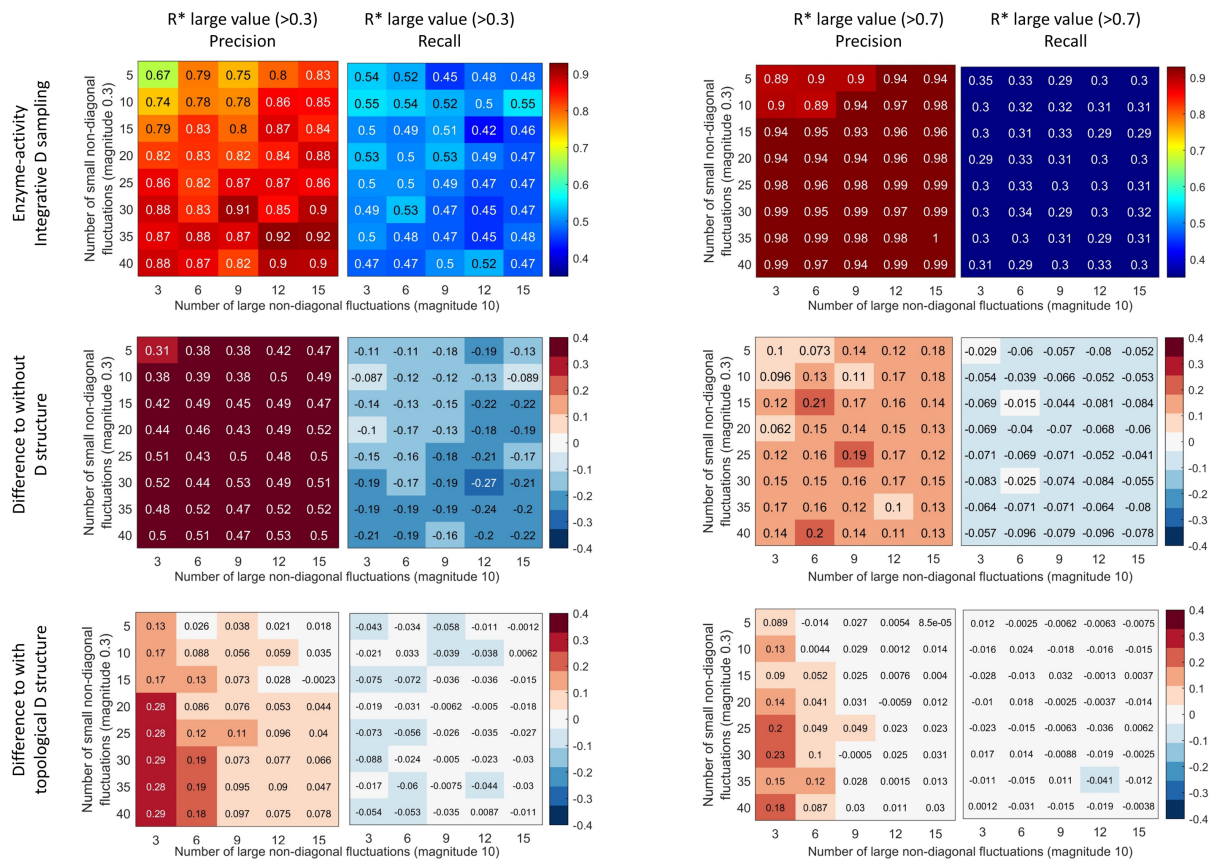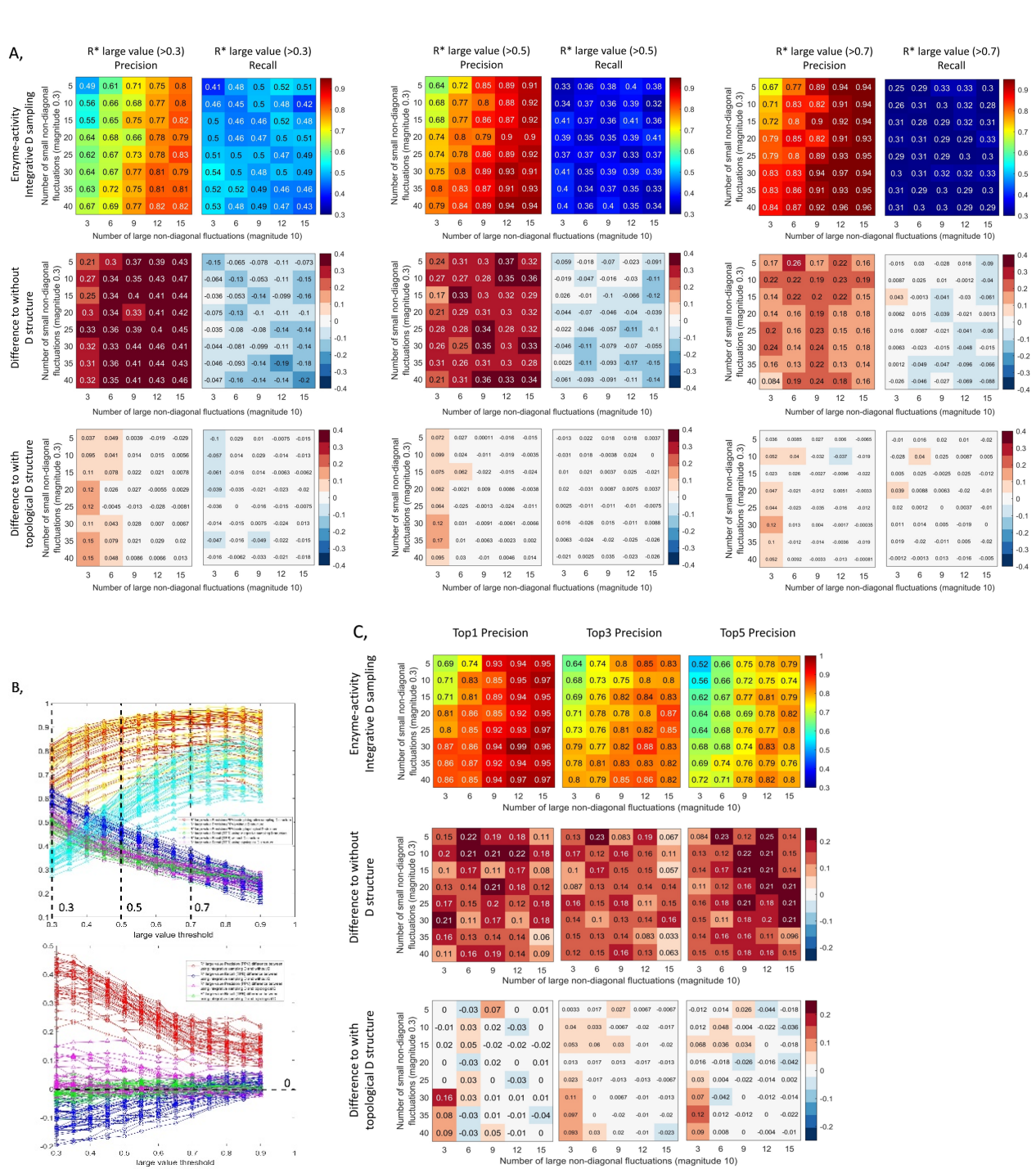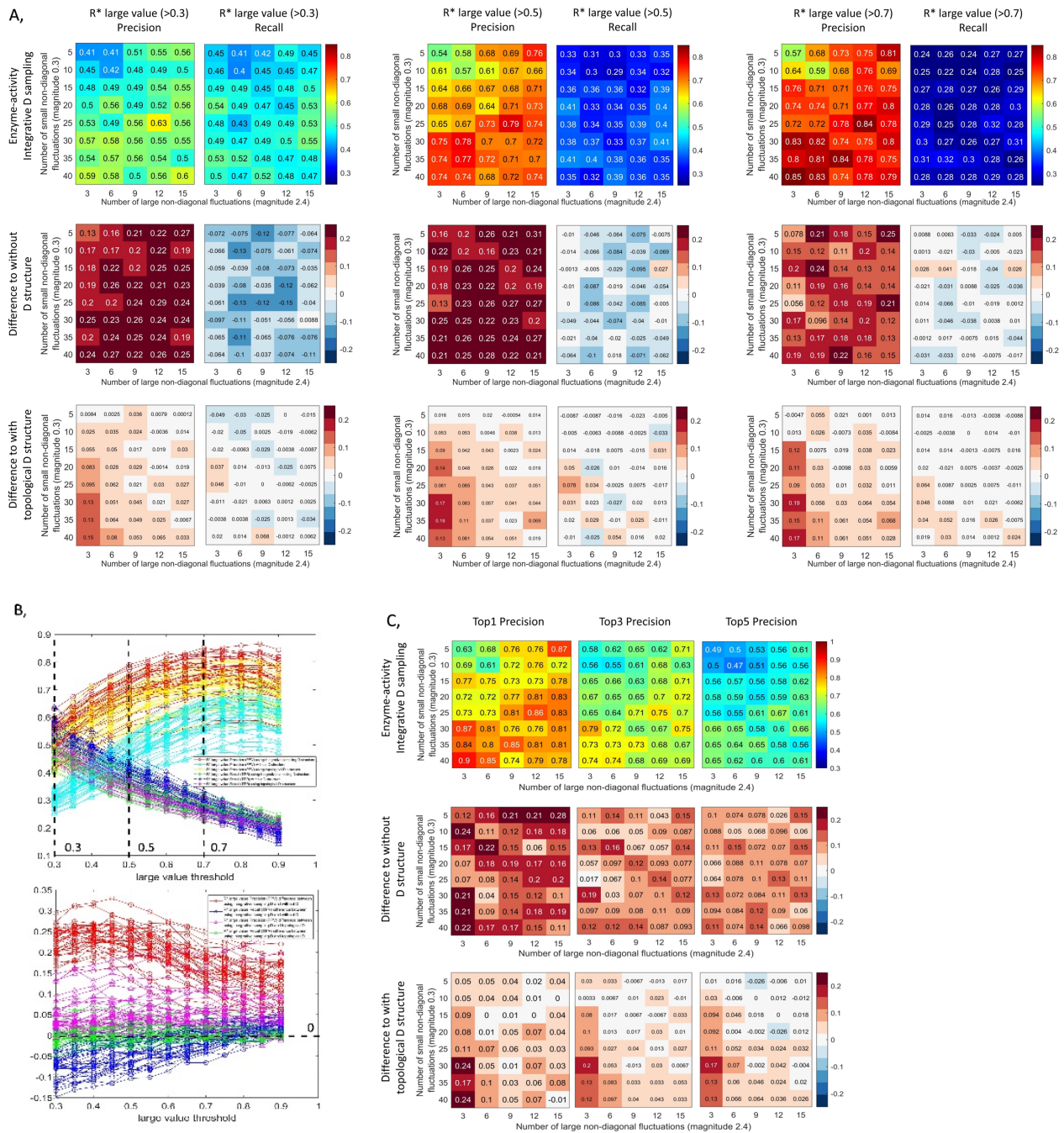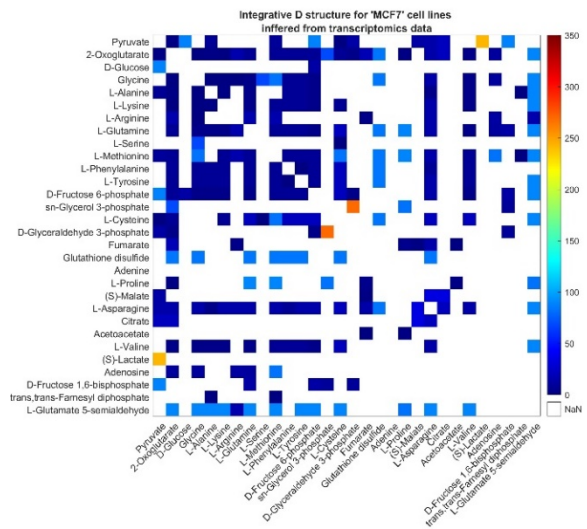**Supplementary Figure 7. Inverse differential Jacobian algorithm evaluation using various number of large (magnitude: 10) and small (magnitude: 0.3) non-diagonal fluctuations using three strategies: integrative D sampling, topological D and without D structure.** The evaluation is conducted using the first model in method section with 200 repeats and $\varepsilon_D = 0.2$. A, Precision and Recall of the large values (above 0.5) in $\boldsymbol{R}^*$ over 200 repeats with/without D structure information; B, the line plots of Precision and Recall of the large values in $\boldsymbol{R}^*$ with/without D structure information based on different large value

thresholds (0.3-0.9), where the snapshot of value 0.5 refers to A; C, the accuracy of the top 1, top 3 and top 5 large values in $\boldsymbol{R}^*$ over 200 repeats with/without D structure information.
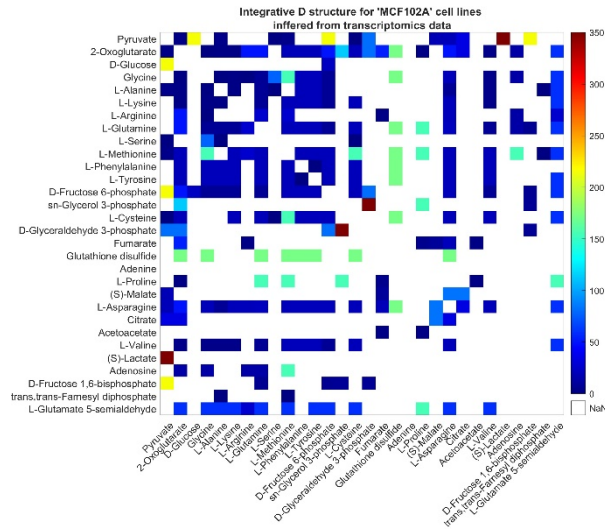
Supplementary Figure 8. Inverse differential Jacobian algorithm evaluation using various number of large (magnitude: 2.4) and small (magnitude: 0.3) non-diagonal fluctuations using three strategies: integrative D sampling, topological D and without D structure. The evaluation is conducted using the first model in method section with 200 repeats and $\varepsilon_D = 0.4$. A, Precision and Recall of the large values (above 0.5) in $\boldsymbol{R}^*$ over 200 repeats with/without D structure information; B, the line plots of Precision and Recall of the large values in $\boldsymbol{R}^*$ with/without D structure information based on different large value
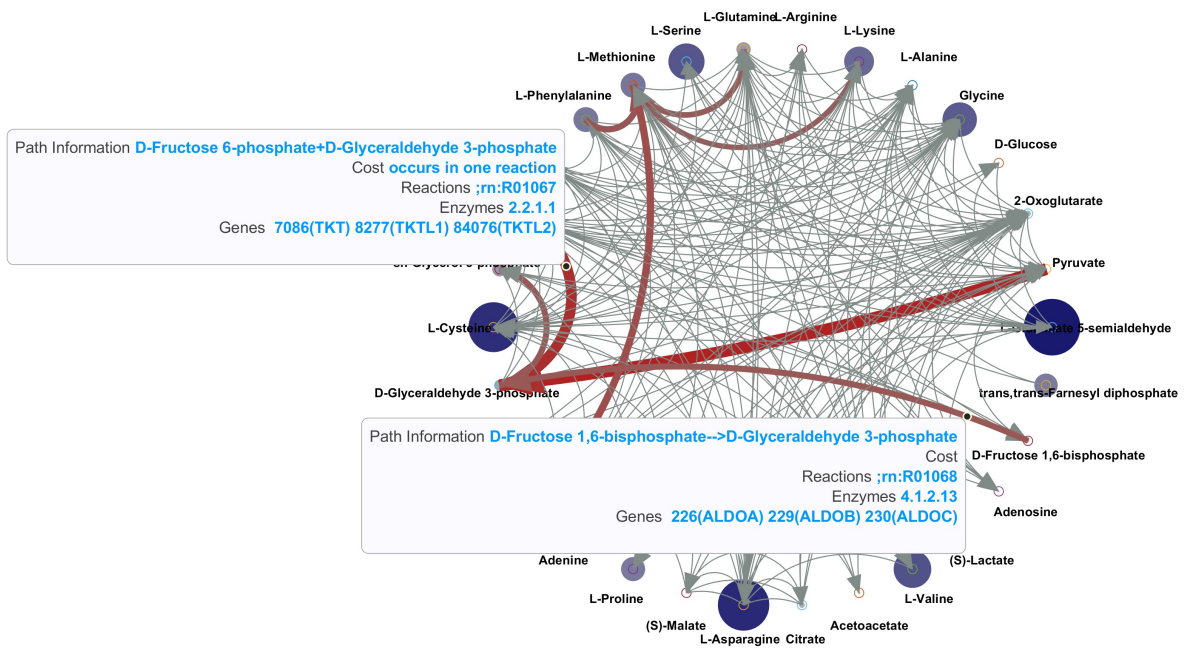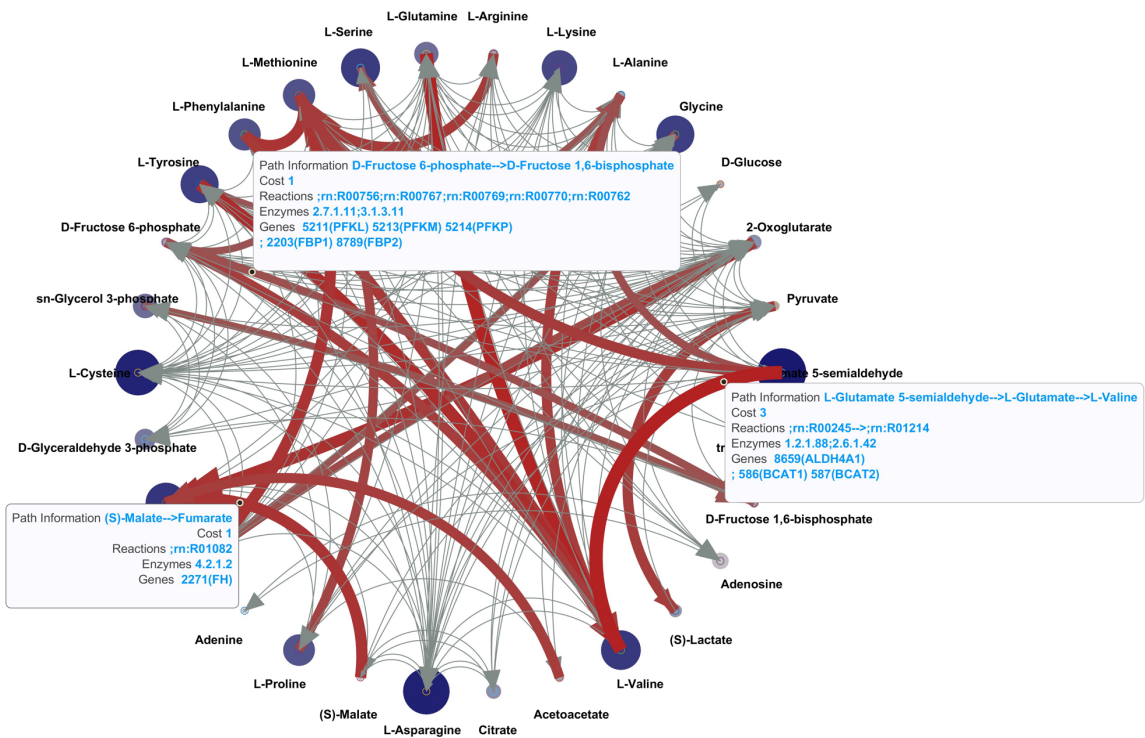
thresholds (0.3-0.9), where the snapshot of value 0.5 refers to A; C, the accuracy of the top 1, top 3 and top 5 large values in $\boldsymbol{R}^*$ over 200 repeats with/without D structure information.
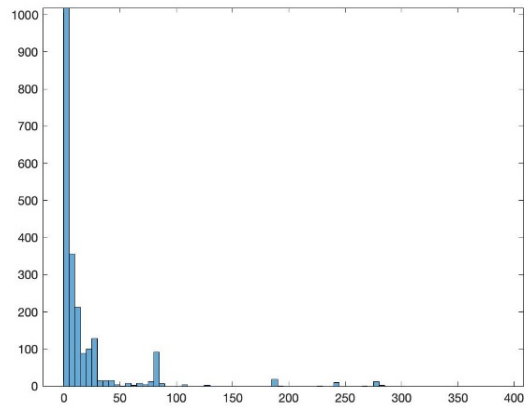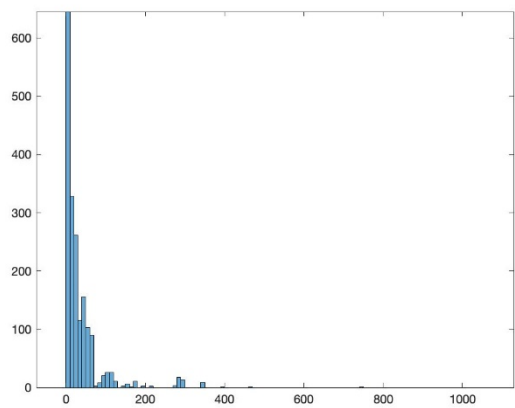
**Supplementary Figure 9. The histogram plots of the activities variances of the two cell lines enzymes**.

Supplementary Figure 10. The inferred fluctuation matrix structure for the two different cell lines (left: D_h for MCF 102A and right: D_d for MCF 7).

Path Information **D-Fructose 6-phosphate-->D-Fructose 1,6-bisphosphate**
Cost **1**
Reactions **;rn:R00756;rn:R00767;rn:R00769;rn:R00770;rn:R00762**
Enzymes **2.7.1.11;3.1.3.11**
Genes **5211(PFKL) 5213(PFKM) 5214(PFKP)**
; 2203(FBP1) 8789(FBP2)

Path Information **L-Glutamate 5-semialdehyde-->L-Glutamate-->L-Valine**
Cost **3**
Reactions **;rn:R00245-->;rn:R01214**
Enzymes **1.2.1.88;2.6.1.42**
Genes **8659(ALDH4A1)**
; 586(BCAT1) 587(BCAT2)

Path Information **(S)-Malate-->Fumarate**
Cost **1**
Reactions **;rn:R01082**
Enzymes **4.2.1.2**
Genes **2271(FH)**

Path Information **D-Fructose 6-phosphate+D-Glyceraldehyde 3-phosphate**
Cost **occurs in one reaction**
Reactions **;rn:R01067**
Enzymes **2.2.1.1**
Genes **7086(TKT) 8277(TKTL1) 84076(TKTL2)**

Path Information **D-Fructose 1,6-bisphosphate-->D-Glyceraldehyde 3-phosphate**
Cost
Reactions **;rn:R01068**
Enzymes **4.1.2.13**
Genes **226(ALDOA) 229(ALDOB) 230(ALDOC)**

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Supplementary Figure 11. The interactive plots of breast cancer inverse Jacobian analysis. Top plot uses a diagonal D assumption and bottom plot uses enzyme-activity integrative D sampling. The circular plot can be interactively checked by clicking the interactions (lines) through the matlab figure format result available in Supplementary material S2.
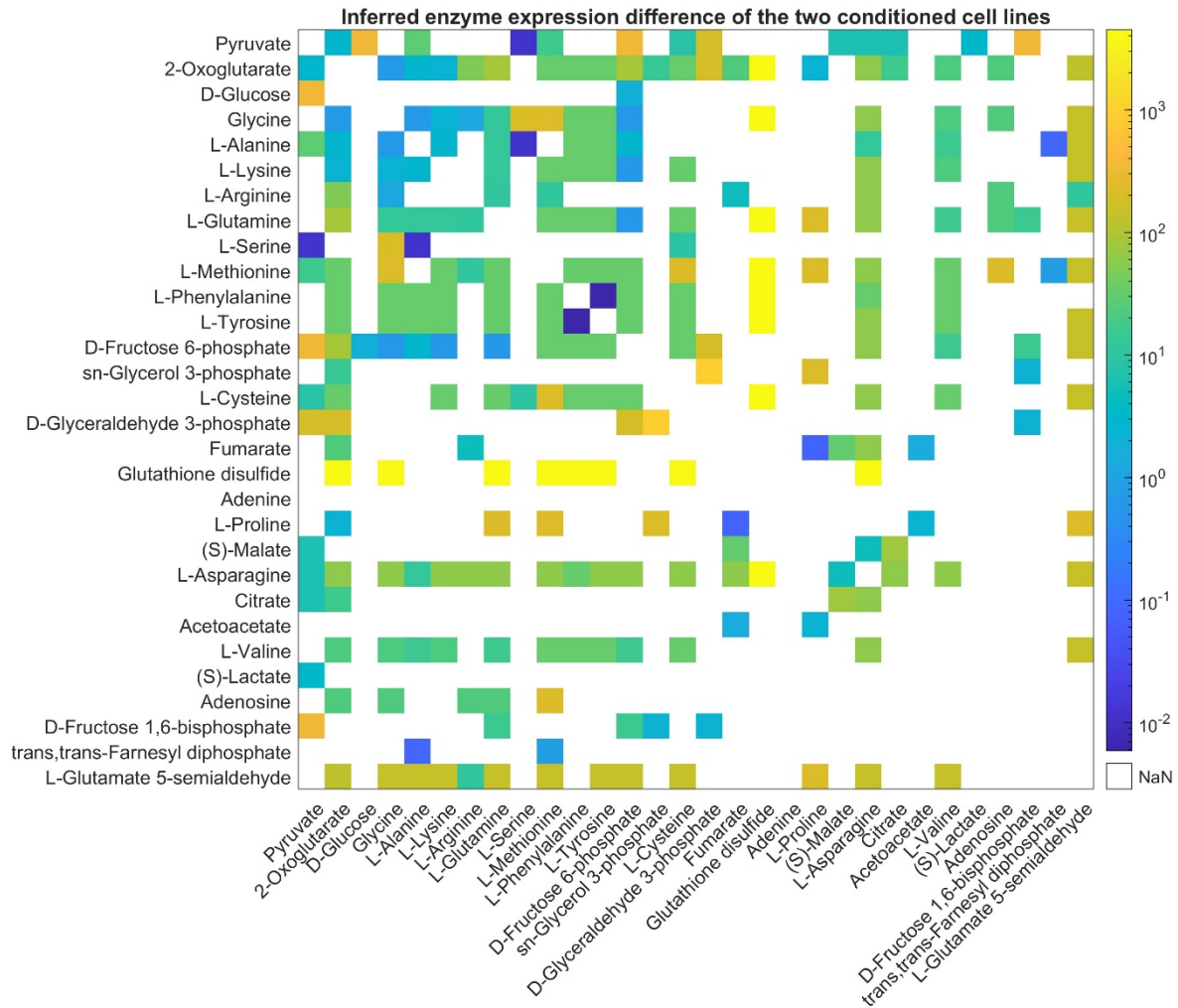
Supplementary Figure 12. The inferred enzyme expression difference of the two conditioned cell lines.