Electronic Supplementary Material (ESI) for Molecular BioSystems.

Supplementary Information:

Systems-level organization of non-alcoholic fatty liver disease progression network

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Table S1: Patient characteristics of GSE58979: (A) Visceral adipose tissue. (B) Subcutaneous adipose tissue. Data is expressed as mean and range.

A

Characteristics	Group I: <5% steatosis (n = 10)	Group II: NAFLD, 30-50% steatosis (n = 7)	Group III: NASH (n = 8)	Group IV: NASH + fibrosis F2-F3 (n = 5)
Age (years)	43 [31-58]	41 [38-56]	49 [36-58]	31 [23-44]
Gender [Male/Female]	1/9	0/7	2/6	2/3
BMI (kg/cm^2)	46 [35-54]	46 [38-56]	43 [35-59]	40 [39-44]

B

Characteristics	Group I: <5% steatosis (n = 6)	Group II: NAFLD, 30-50% steatosis (n = 6)	Group III: NASH (n = 6)	Group IV: NASH + fibrosis F2-F3 (n = 5)
Age (years)	39 [26-49]	42 [23-53]	48 [36-54]	38 [23-60]
Gender [Male/Female]	1/5	0/6	1/5	1/4
BMI (kg/cm^2)	44 [35-53]	44 [37-56]	51 [35-56]	41 [39-44]

Table S2: Patient characteristics of GSE22070. Visceral adipose tissue data of obese (n=24) and NASH patients without Type 2 Diabetes (T2D) (n=21) were used for the module preservation analysis. Data is expressed as mean and range.

Characteristics	Individuals without T2D and NASH (n = 29)	NASH patients (n = 41)
Age (years)	40[17-54]	46[28-65]
Gender [Male/Female]	7/22	12/29
BMI (kg/m^2)	43 [35-57]	49[35-74]
HbA$_{1c}$	6[5.1-7.2]	6.9[5.2-12.1]

Table S3: Enrichment of Gene ontology (GO) terms and KEGG pathways associated with modules of whole NAFLD network. Benjamini–Hochberg corrected p-values are given except for the values in bold.

Module	KEGG Pathway	P value	GO Term	P value
S1(170) Black	hsa00280: Valine, leucine and isoleucine degradation	1.7E-3	GO: 0006631~fatty acid metabolic process	7.3E-3
	hsa00640: Propanoate metabolism	9.2E-4	GO: 0055114~oxidation-reduction process	5.0E-2
	hsa04923: Regulation of lipolysis in adipocytes	1.6E-3		
S2(289) Blue	hsa04390: Hippo signaling pathway	**9.8E-4**	GO: 0010811~positive regulation of cell-substrate adhesion	**2.6E-4**
S3(168) Cyan	hsa01200: Carbon metabolism	9.4E-4	GO: 0032869~cellular response to insulin stimulus	1.2E-3
	hsa04152: AMPK signaling pathway	8.8E-4		
	hsa03320: PPAR signaling pathway	6.0E-4		
S4(704) Green	hsa04668: TNF signaling pathway	7.0E-11	GO: 0006954~inflammatory response	5.5E-15
	hsa04060: Cytokine-cytokine receptor interaction	1.6E-4	GO: 0032496~response to lipopolysaccharide	3.1E-6
	hsa04064: NF-kappa B signaling pathway	7.8E-4		
S5(112) GreenYellow	hsa04610: Complement and coagulation cascades	1.3E-3	GO: 0050900~leukocyte migration	5.3E-3
S6(70) Gyrey60	hsa04666: Fc gamma R-mediated phagocytosis	**1.5E-3**	GO: 0006954~inflammatory response	5.8E-6
S7(67) LightGreen	hsa04923: Regulation of lipolysis in adipocytes	**1.7E-2**	GO: 0046676~negative regulation of insulin secretion	**7.1E-3**
S8(122) Magenta	hsa04010: MAPK signaling pathway	**5.0E-2**	GO: 0000086~G2/M transition of mitotic cell cycle	**2.1E-3**
	hsa04020: Calcium signaling pathway	**7.4E-2**		
S9(843) Pink	hsa04115: p53 signaling pathway	**1.2E-2**	GO: 0000122~negative regulation of transcription from RNA polymerase II promoter	**9.2E-3**
	hsa00310: Lysine degradation	**1.6E-2**		
	hsa04150: mTOR signaling pathway	**2.5E-2**	GO: 0043161~proteasome-mediated ubiquitin-dependent protein catabolic process	**0.04**
S10(114) Purple	hsa04390: Hippo signaling pathway	2.2E-3	GO: 0070244~negative regulation of thymocyte apoptotic process	**3.7E-2**
	hsa04014: Ras signaling pathway	**7.3E-2**	GO: 0071333~cellular response to glucose stimulus	**3.8E-2**
S11(231) Yellow	hsa04152: AMPK signaling pathway	**8.6E-3**	GO: 0008284~positive regulation of cell proliferation	**4.1E-2**

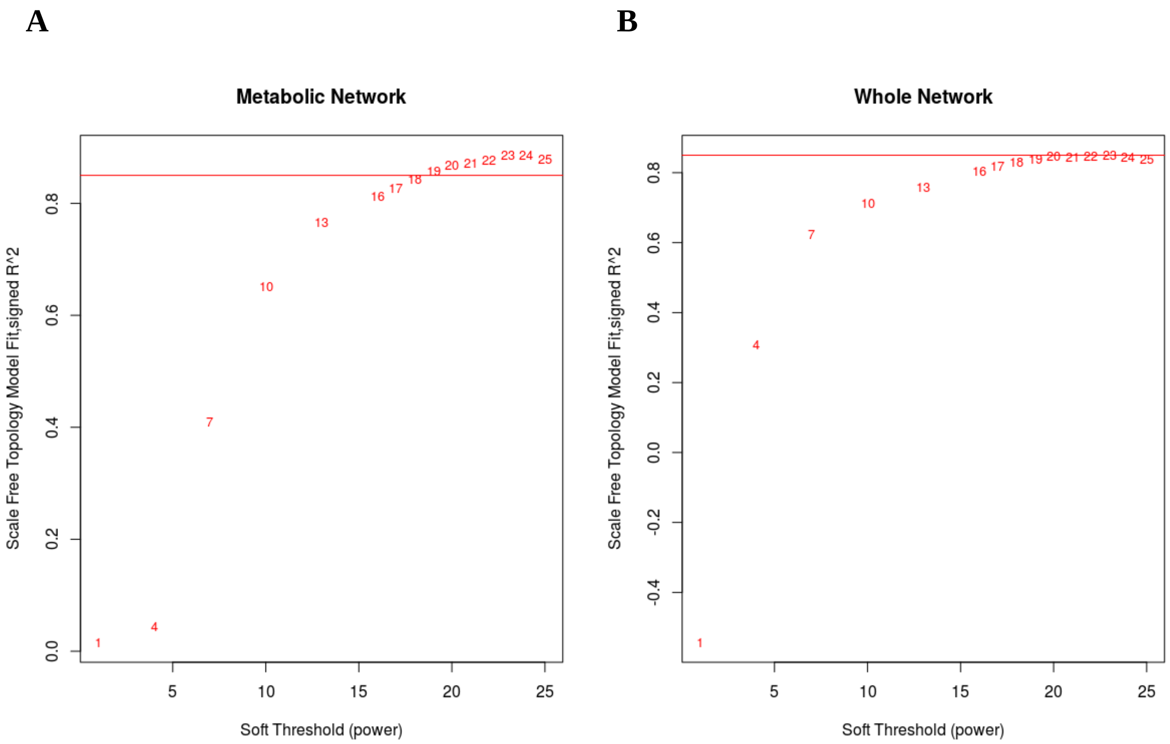


Figure S1: Network topology analysis to identify soft thresholding power (β). Scale free topology fit (R^2) as a function of different powers for **(A)** Metabolic Network and **(B)** Whole Network. R^2 is correlation between log(p(k)) and log(k), where p(k) is the frequency distribution of the connectivity(k). The lowest power β where saturation is reached is taken for the analysis.

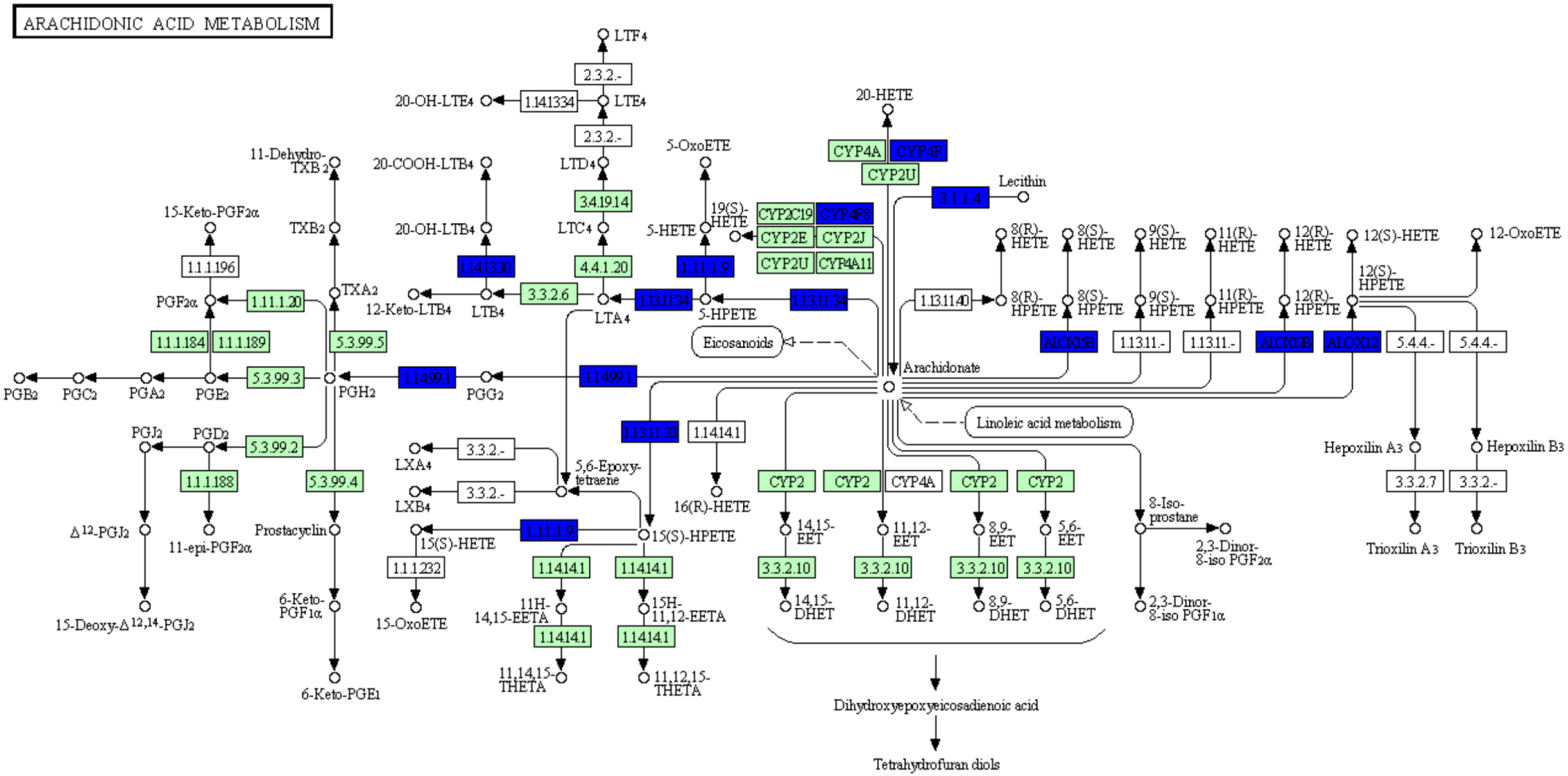


Figure S2: **Arachidonic acid metabolism**. The upregulated genes of the modules, M2 and M8 are shown in blue.

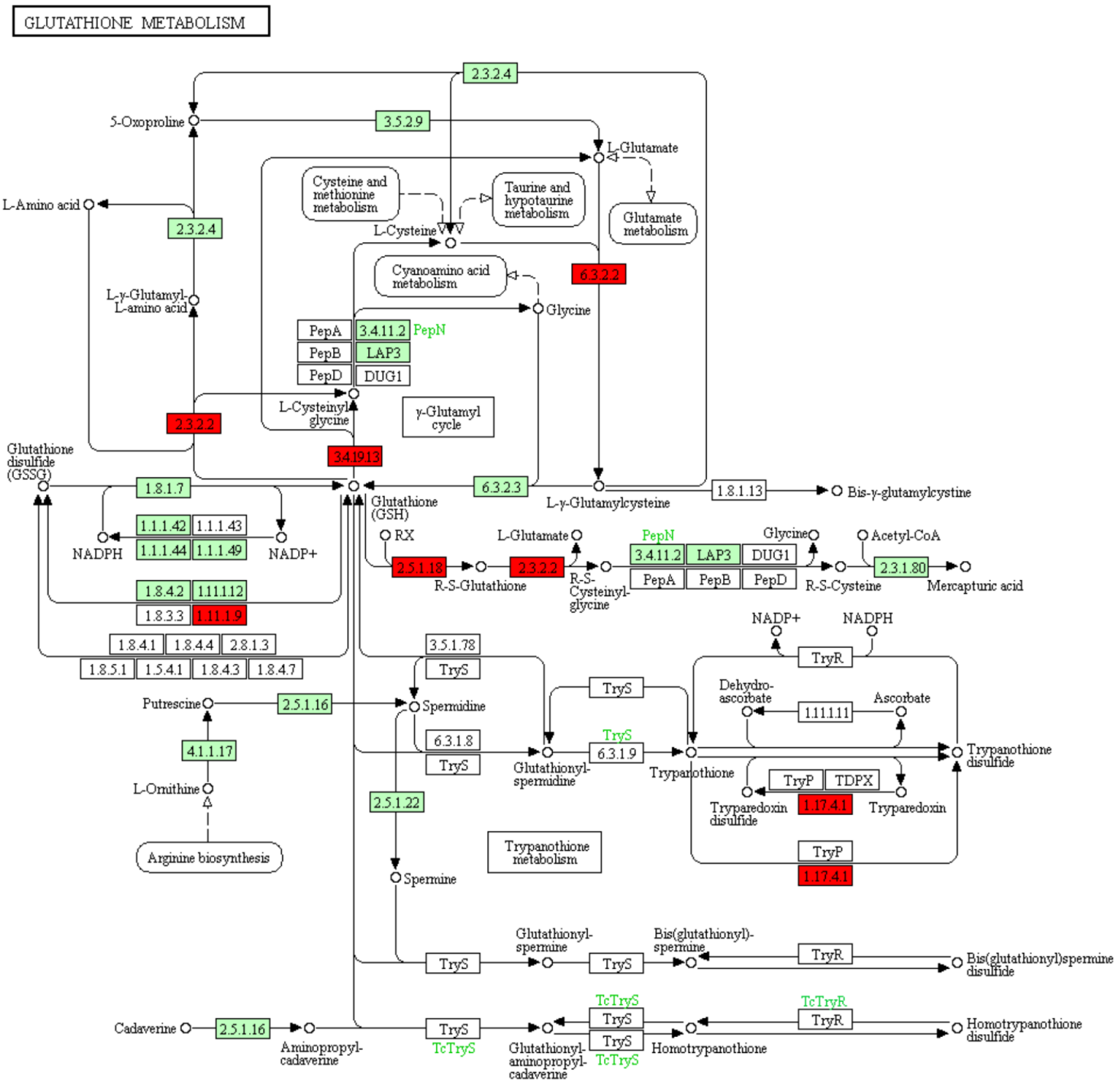


Figure S3: Glutathione metabolism. The downregulated genes of the module M7 are shown in red.

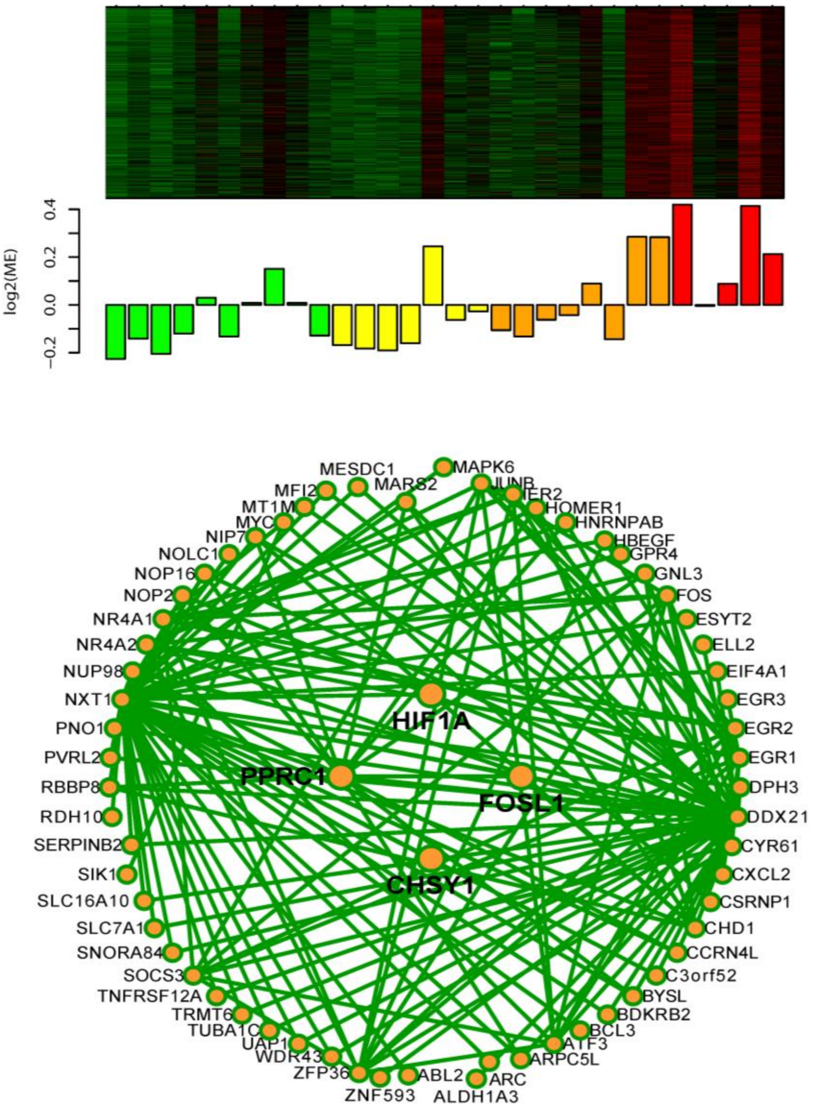


Figure S4: Inflammatory module associated with NAFLD. Heatmap of expression of module S4 genes are shown in the top panel (rows-genes, columns-samples). The red and green colours in the heatmap represent over-expression and under-expression, respectively. The middle panel shows the corresponding eigengene values across the samples for the module S4: Green, obese; yellow, NAFL; dark yellow, NASH; red, NASH with fibrosis. The top 150 interactions within the module S4 are shown in the bottom panel. The genes with high intramodular connectivity are shown in larger size.

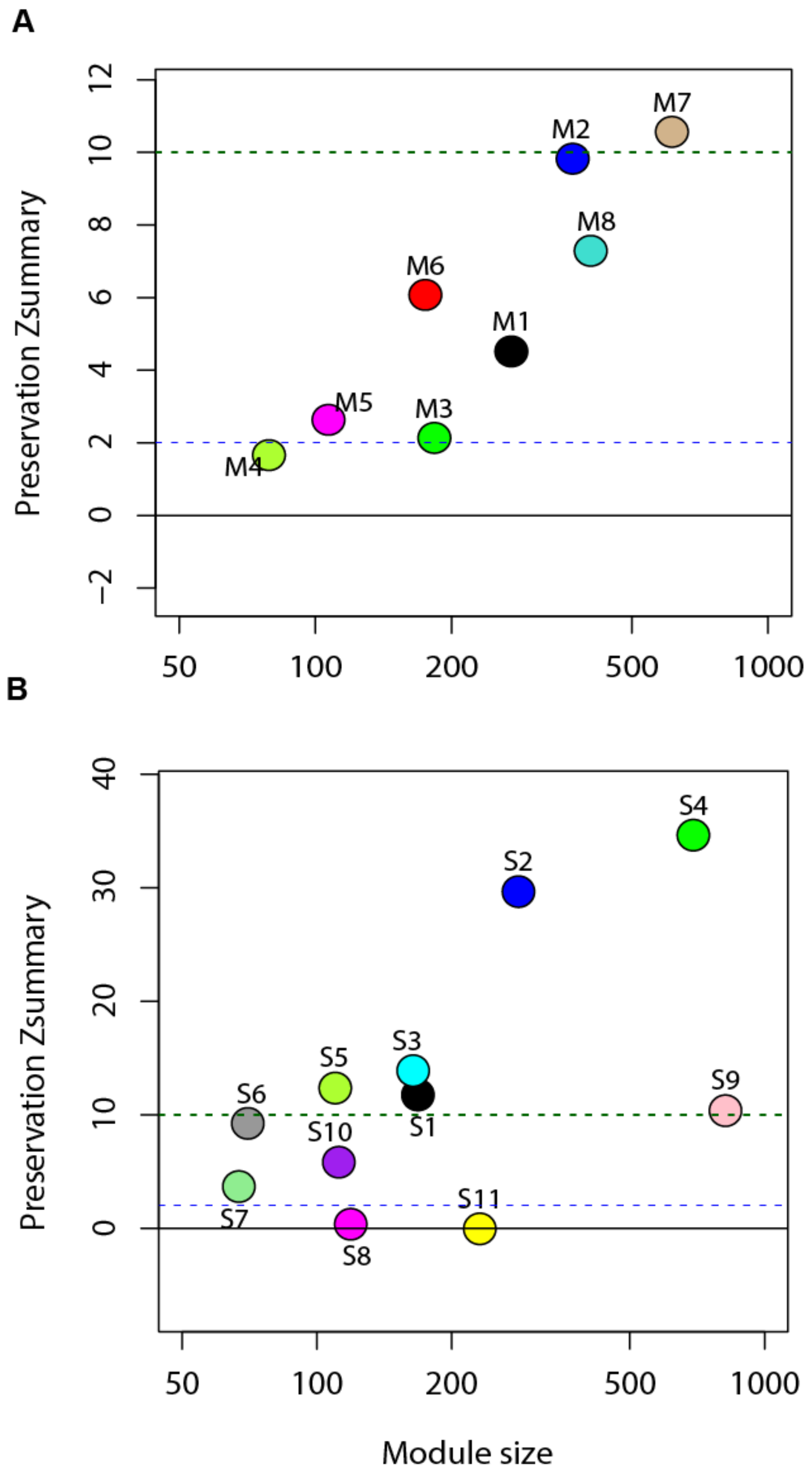
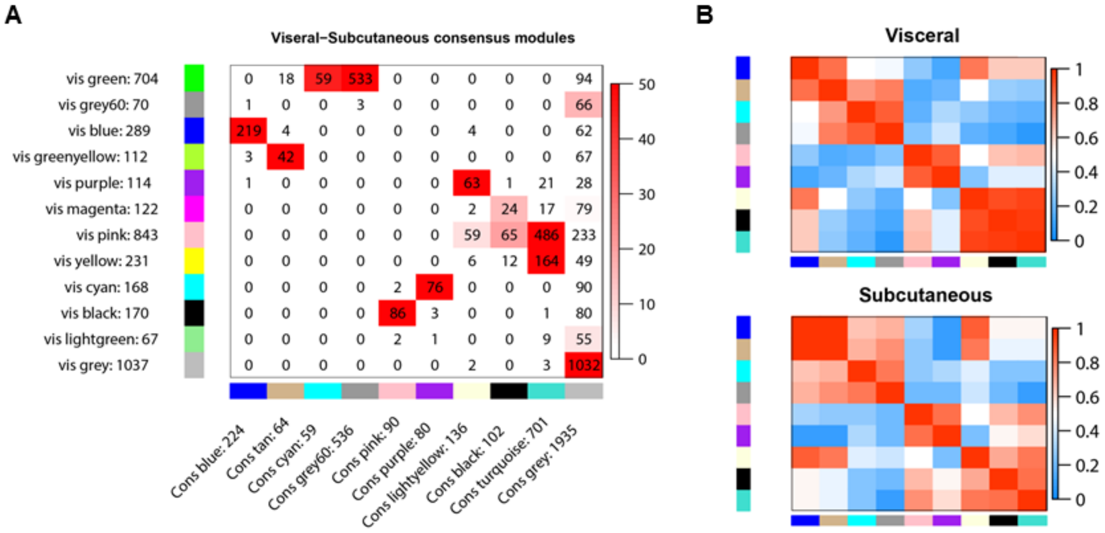


Figure S5: Module preservation analysis. The $Z_{summary}$ statistics of each module (labelled by colour) within metabolic network (top panel) and whole network (below panel) are shown. Weak to moderate evidence of preservation: $2< Z_{summary} <10$ and strong evidence of module preservation: $Z_{summary} \geq 10$.



	Cons blue: 224	Cons tan: 64	Cons cyan: 59	Cons grey60: 536	Cons pink: 90	Cons purple: 80	Cons lightyellow: 136	Cons black: 102	Cons turquoise: 701	Cons grey: 1935
vis green: 704	0	18	59	533	0	0	0	0	0	94
vis grey60: 70	1	0	0	3	0	0	0	0	0	66
vis blue: 289	219	4	0	0	0	0	4	0	0	62
vis greenyellow: 112	3	42	0	0	0	0	0	0	0	67
vis purple: 114	1	0	0	0	0	0	63	1	21	28
vis magenta: 122	0	0	0	0	0	0	2	24	17	79
vis pink: 843	0	0	0	0	0	0	59	65	486	233
vis yellow: 231	0	0	0	0	0	0	6	12	164	49
vis cyan: 168	0	0	0	0	2	76	0	0	0	90
vis black: 170	0	0	0	0	86	3	0	0	1	80
vis lightgreen: 67	0	0	0	0	2	1	0	0	9	55
vis grey: 1037	0	0	0	0	0	0	2	0	3	1032

Figure S6: Consensus module analysis of VAT and SAT whole networks. (A) Overlap of VAT-SAT consensus modules with VAT modules is shown. Rows represent VAT modules, columns represent consensus modules and numbers in the table indicate the number of genes intersecting between consensus and VAT modules. Fisher's exact test p-value for the overlap of the two modules is indicated within the table in red colour (-log(p)). The more significant overlap is represented by dark red colour. (B) Eigengene adjacencies in the consensus network of VAT and SAT are shown as heatmaps. Red denotes high adjacency (positive correlation) and blue denotes low adjacency (negative correlation). Each row and column represents a consensus module eigengene. The strongest relationships between the eigengenes are observed in the VAT compared to SAT.