Profiling cellular processes in adipose tissue during weight loss using time series gene expression

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Supplementary Information

Figures

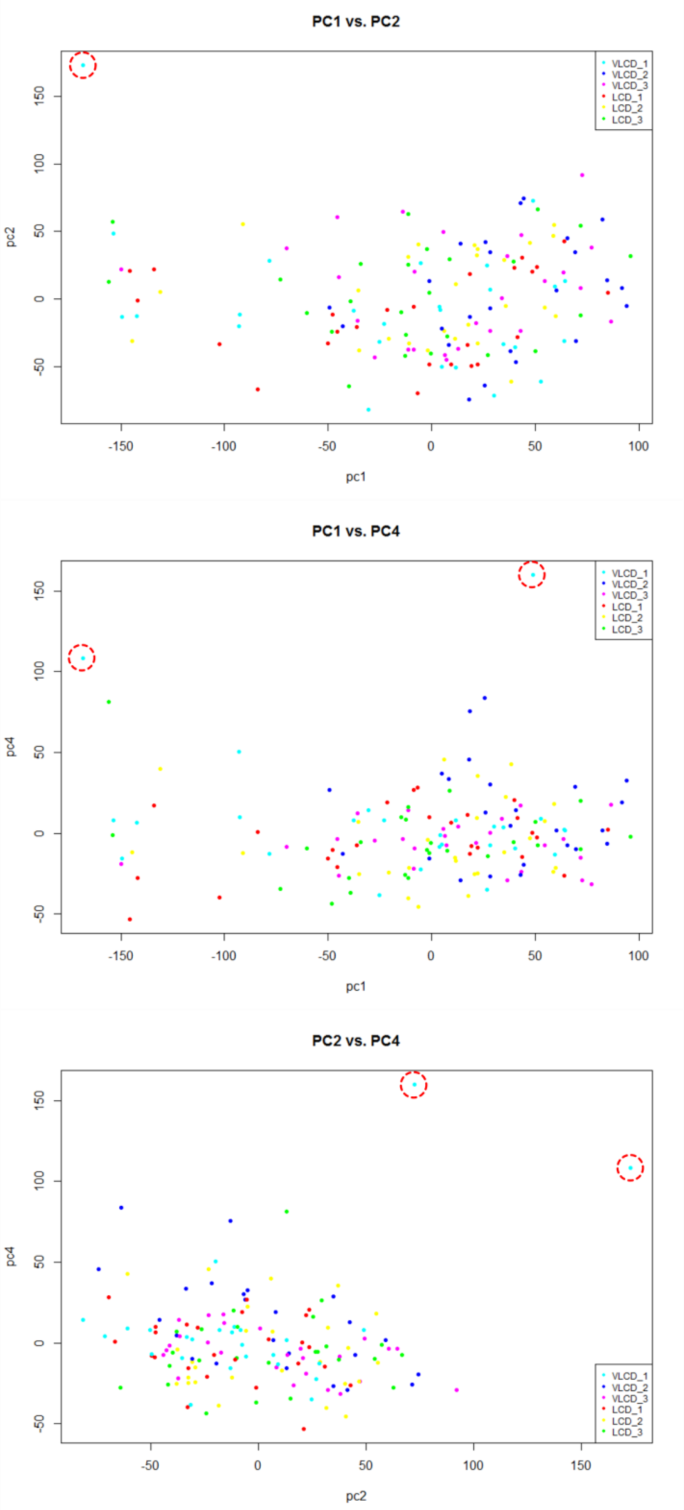

Figure S1: Principal component analysis of the raw gene expression data of the 46 individuals, showing the outlying samples highlighted in red circles.

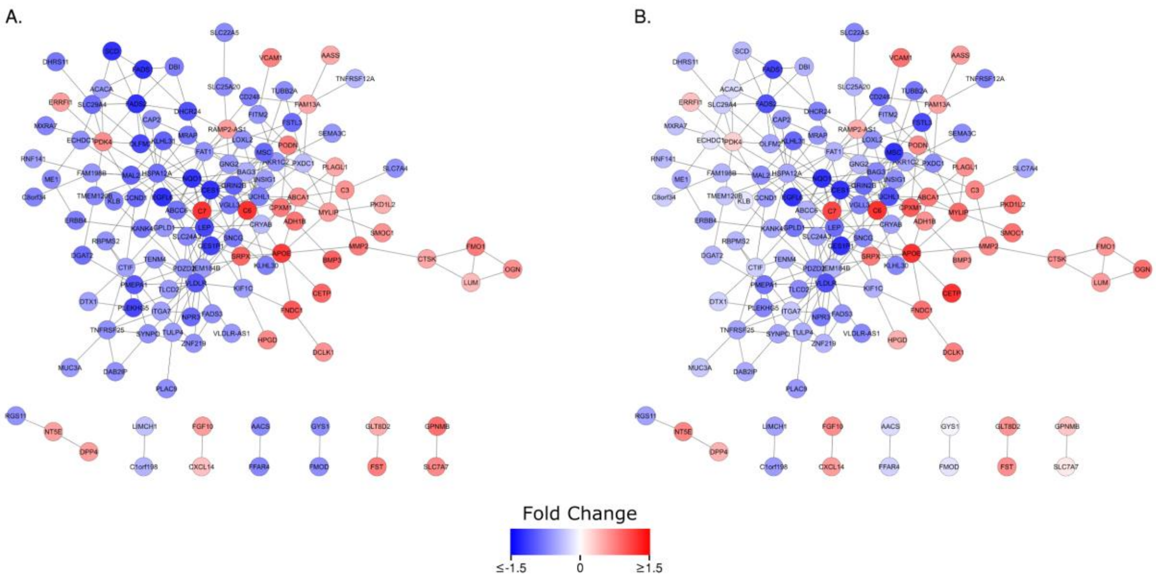

Figure S2: Correlation network generated for the low calorie diet (LCD).

A) Fold change for time points 2-1
B) Fold change for time points 3-1

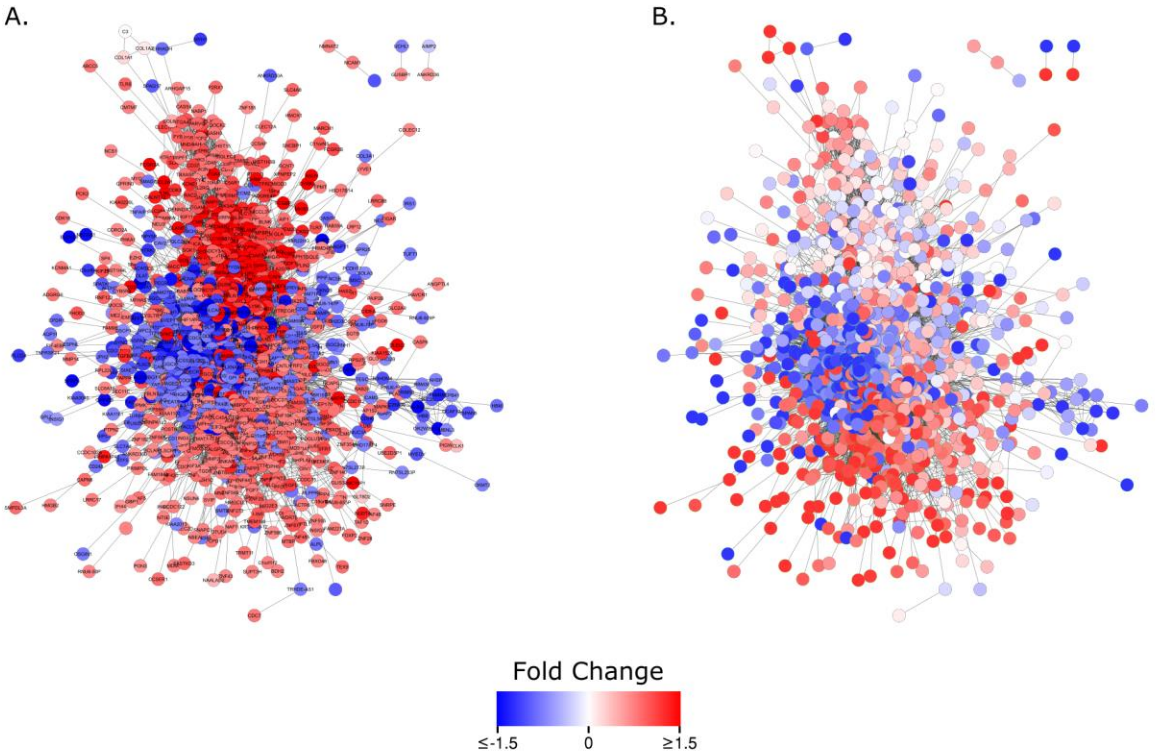

Figure S3: Correlation network generated for the very low calorie diet (VLCD).

A) Fold change for time points 2-1
B) Fold change for time points 3-1

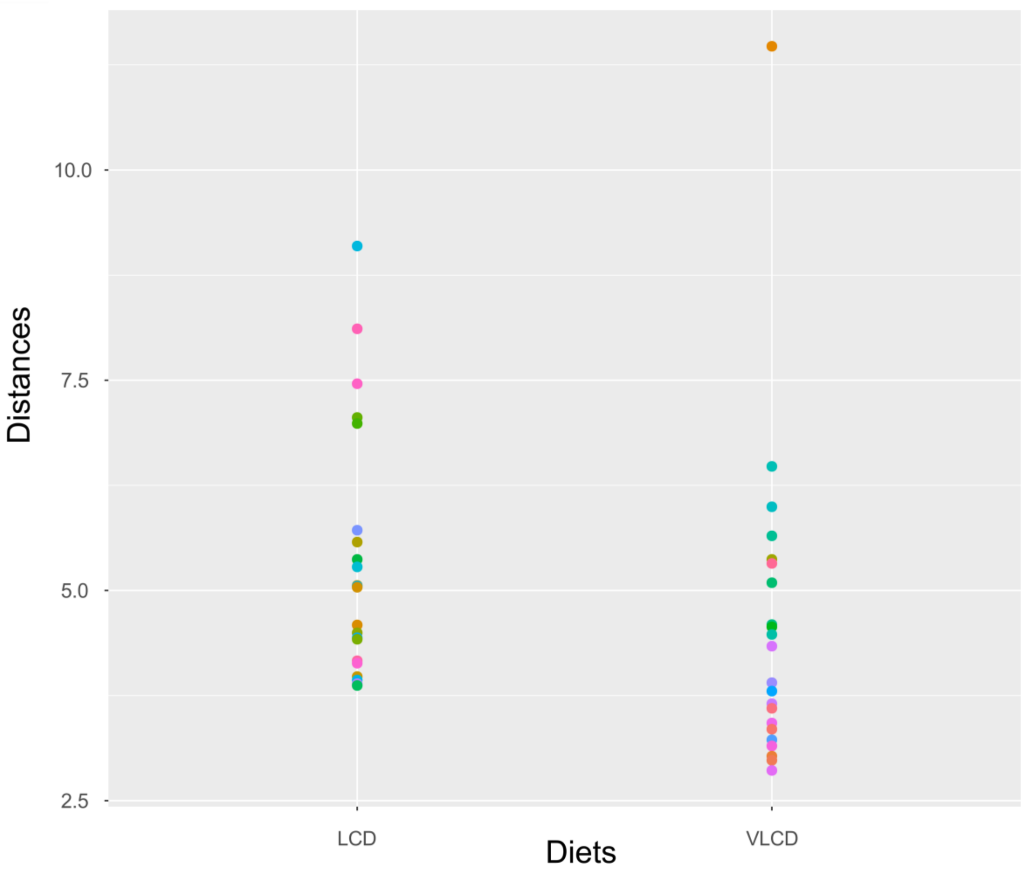

Figure S4: Frobenius norm plot showing the norm distance of the correlation network of the 44 individuals with the respective diet-wide correlation network.

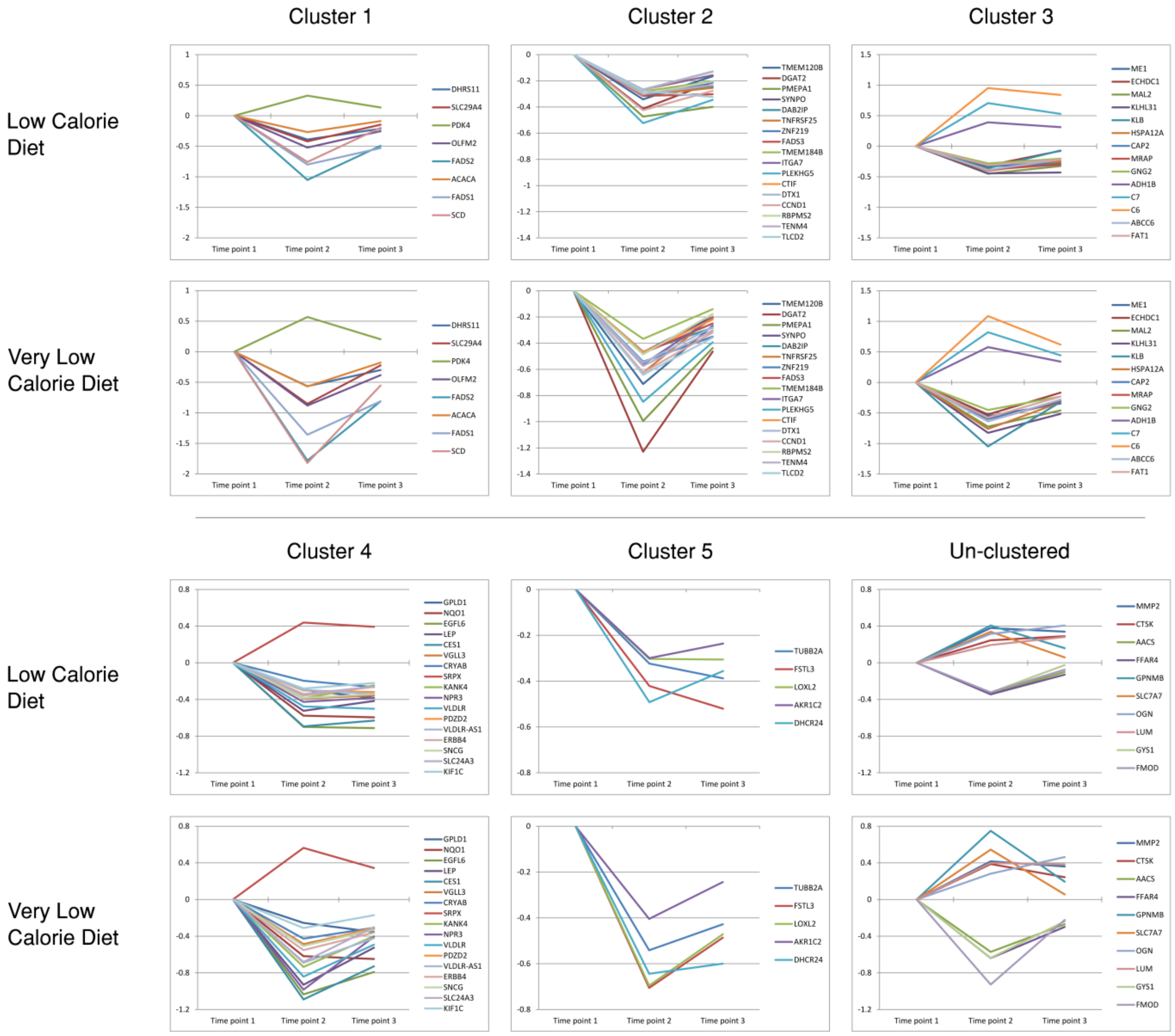

Figure S5: Gene expression patterns of the respective genes in each topological cluster of the overlap network, based on the fold change in the respective diets. Also shows the five un-clustered paired correlated genes.

Source Code

```
#####
###
#Modified DCeN algorithm for the creation of correlation networks.
#
#Credit for creating the original algorithm goes to:
#        Elo LL, Schwikowski B (2013) Analysis of Time-Resolved Gene Expression
Measurements across Individuals. PLoS ONE 8(12): e82340.
doi:10.1371/journal.pone.0082340
#
#Modified by Samar Tareen for use with single group/stimulus data over multiple
individuals
#Submitted with Manuscript ID: as Appendix material
#
#For complete details of each section of the algorithm, we refer to the original
publication
#
```

```
###



#Function name 'tsCorr' (time series correlation); Takes
#       1. dataframe as input data
#       2. filter.corr=0.7 as default minimum correlation cutoff for 1-filter.corr in
positive and -(1-filter.corr) in negative correlations
#       3. filter.n=0.25 as minimum percentage of individuals in which the
correlations should hold

tsCorr <- function(data, filter.corr=0.7, filter.n=0.25) {

    ## Count significant correlations and determine prefiltering for positive
correlations
    posFilt<- matrix(nrow=nrow(data[[1]]), ncol=nrow(data[[1]]))
    posFilt[]<- 0
    negFilt<- matrix(nrow=nrow(data[[1]]), ncol=nrow(data[[1]]))
    negFilt[]<- 0
    avgCorr<- matrix(nrow=nrow(data[[1]]), ncol=nrow(data[[1]]))
    avgCorr[]<- 0

    for(i in 1:length(data)) {
        C1<- cor(t(data[[i]]))
        C1<- C1 * sqrt(ncol(data[[i]])-2) / sqrt(1 - C1*C1)
        C1<- (1 - 2 * pt(-abs(C1), ncol(data[[i]])-2, lower.tail = TRUE, log.p =
FALSE))*sign(C1)

        avgCorr<- avgCorr + C1

        posTemp <- C1
        posTemp[posTemp >  1-filter.corr]<- 1
        posTemp[posTemp <= 1-filter.corr]<- 0
        diag(posTemp)<- 0
        posFilt<- posFilt + posTemp
        rm(posTemp)

        negTemp <- C1
        negTemp[negTemp <  -(1-filter.corr)]<- -1
        negTemp[negTemp >= -(1-filter.corr)]<- 0
        diag(negTemp)<- 0
        negFilt<- negFilt + negTemp
        rm(negTemp)
    }
    rm(C1)
    gc()

    cutoff1<- ceiling(filter.n*length(data))
    posFilt[posFilt<cutoff1]<- 0
    A.pos<- pmax(posFilt)
    A.pos[A.pos>0]<- 1
    rm(posFilt)
    gc()

    negFilt<- abs(negFilt)
    cutoff1<- ceiling(filter.n*length(data))
    negFilt[negFilt<cutoff1]<- 0
    A.neg<- pmax(negFilt)
    A.neg[A.neg>0]<- 1
    rm(negFilt)
    gc()

    avgCorr<- avgCorr/length(data)
```

```
    C1<- avgCorr
    rm(avgCorr)
    gc()

    A<- pmax(A.pos,A.neg)
    C1[A==0]<- 0
    a<- rowSums(A)
    rm(A.pos)
    rm(A.neg)
    rm(A)
    gc()

    #in case the correlation network needs to be generated as output (tab delimited
text file)
    #write.table(C1, paste(getwd(), "/tsCorr.txt", sep=""), sep="\t", row.names = T,
col.names = T, quote=F)
    return(C1)
}

#####
```

Tables

Table S1: Results from the robustness analysis using the minimum %age individuals having the same correlation direction (for |corr.| ≥ 0.6).

%age minimum individuals		**LCD**	**VLCD**
25	Total edges	127	127
	%age	100.00	100.00
30	Conserved edges	127	126
	%age	100.00	99.21
35	Conserved edges	127	126
	%age	100.00	99.21
40	Conserved edges	125	126
	%age	98.43	99.21
45	Conserved edges	119	125
	%age	93.70	98.43
50	Conserved edges	108	120
	%age	85.04	94.49

Table S2: Results from the leave-n-out cross-validation analysis for n= 1 and 2 (max 10% of total samples) in the overlap network. The analysis here represents loss of sensitivity. The correlation criteria is |corr.| ≥ 0.6 in at least 25% of the individuals having the same direction of the correlation.

Leave-n-out, n=	**# of combinations in each diet**	**Criteria: |corr.| ≥ 0.6 and min. 25% ind.s**	**LCD**	**VLCD**
0	1	Total edges	127	127
		%age	100.00	100.00
1	22	Conserved edges	85	117
		%age	66.93	92.13
2	231	Conserved edges	52	104
		%age	40.94	81.89

Table S3: Results from the leave-n-out cross-validation analysis for n= 1 and 2 (max 10% of total samples) in the correlation networks of the respective diets. The analysis here represents loss of specificity. The correlation criteria is |corr.| ≥ 0.6 in at least 25% of the individuals having the same direction of the correlation.

Leave-n-out, n=	**# of combinations in each diet**	**Criteria: |corr.| ≥ 0.6 and min. 25% ind.s**	**LCD**	**VLCD**
0	1	Total edges	149	1125
1	22	New edges (avg. of all combinations)	20	54
2	231	New edges (avg. of all combinations)	~36	~82

Table S4: Results from the ConsensusPathDB analysis on the overlap network. BioCarta leptin pathway discussed in the discussion section of the article is highlighted in red.

pathway	**p-value**	**q-value**	**source**	**members_input_overlap**	**members_input_overlap_geneids**	**size**	**effective_size**
Omega-3 fatty acid metabolism	4.84E-05	0.00781987	EHMN	ENSG00000221968; ENSG00000134824; ENSG00000149485	3995; 3992; 9415	16	16
Fatty Acid Biosynthesis	0.000130585	0.00781987	Wikipathways	ENSG00000099194; ENSG00000093144; ENSG00000278540	55862; 31; 6319	22	22
Biosynthesis of unsaturated fatty acids - Homo sapiens	0.000149689	0.00781987	KEGG	ENSG00000099194; ENSG00000134824;	3992; 6319; 9415	23	23

(human)				ENSG00000149485			
AMPK signaling pathway - Homo sapiens (human)	0.000235714	0.00781987	KEGG	ENSG00000104812; ENSG00000099194; ENSG00000174697; ENSG00000110092; ENSG00000278540	6319; 31; 595; 3952; 2997	124	123
Omega-6 fatty acid metabolism	0.000272466	0.00781987	EHMN	ENSG00000221968; ENSG00000134824; ENSG00000149485	3992; 3995; 9415	28	28
Keratan sulfate biosynthesis	0.000272466	0.00781987	Reactome	ENSG00000139329; ENSG00000106809; ENSG00000122176	4060; 4969; 2331	28	28
Alpha Linolenic Acid and Linoleic Acid Metabolism	0.000304106	0.00781987	SMPDB	ENSG00000134824; ENSG00000149485	9415; 3992	6	6
Terminal pathway of complement	0.000424502	0.008930523	Reactome	ENSG00000112936; ENSG00000039537	730; 729	7	7
Keratan sulfate/keratin metabolism	0.000446526	0.008930523	Reactome	ENSG00000139329; ENSG00000106809; ENSG00000122176	4060; 4969; 2331	33	33
Nuclear Receptors Meta-Pathway	0.000514786	0.009234772	Wikipathways	ENSG00000065833; ENSG00000099194; ENSG00000198848; ENSG00000110092; ENSG00000004799; ENSG00000112186; ENSG00000181019	4199; 595; 1066; 6319; 1728; 10486; 5166	314	313
Linoleic acid (LA) metabolism	0.000564347	0.009234772	Reactome	ENSG00000134824; ENSG00000149485	3992; 9415	8	8
reversal of insulin resistance by leptin	0.000723467	0.010852004	BioCarta	ENSG00000174697; ENSG00000278540	3952; 31	9	9
alternative	0.000901689	0.012484929	BioCarta	ENSG000001	730; 729	10	10

complement pathway				12936; ENSG00000039537			
Extracellular matrix organization	0.001202898	0.015465837	Reactome	ENSG00000135424; ENSG00000143387; ENSG00000134013; ENSG00000139329; ENSG00000087245; ENSG00000122176	4017; 3679; 2331; 4060; 4313; 1513	264	263
lectin induced complement pathway	0.001314758	0.01549266	BioCarta	ENSG00000112936; ENSG00000039537	730; 729	12	12
alpha-linolenic acid (ALA) metabolism	0.001549266	0.01549266	Reactome	ENSG00000134824; ENSG00000149485	3992; 9415	13	13
alpha-linolenic (omega3) and linoleic (omega6) acid metabolism	0.001549266	0.01549266	Reactome	ENSG00000134824; ENSG00000149485	3992; 9415	13	13
icosapentaenoate biosynthesis II (metazoa)	0.001549266	0.01549266	HumanCyc	ENSG00000134824; ENSG00000149485	3992; 9415	13	13
classical complement pathway	0.001802198	0.017073456	BioCarta	ENSG00000112936; ENSG00000039537	730; 729	14	14
ECM proteoglycans	0.002000476	0.018004287	Reactome	ENSG00000139329; ENSG00000135424; ENSG00000122176	4060; 3679; 2331	56	55
Complement Activation, Classical Pathway	0.00266988	0.022884683	Wikipathways	ENSG00000112936; ENSG00000039537	730; 729	17	17
AMPK Signaling	0.003665435	0.026814921	Wikipathways	ENSG00000104812; ENSG00000174697; ENSG00000278540	3952; 31; 2997	68	68
PPAR signaling pathway - Homo sapiens (human)	0.003819792	0.026814921	KEGG	ENSG00000065833; ENSG00000099194; ENSG00000134824	6319; 9415; 4199	69	69
Linoleate metabolism	0.004306497	0.026814921	EHMN	ENSG00000221968;	9415; 3992; 3995	72	72

				ENSG00000134824; ENSG00000149485			
Pyruvate Dehydrogenase Complex Deficiency	0.004469153	0.026814921	SMPDB	ENSG00000065833; ENSG00000278540	4199; 31	22	22
Primary hyperoxaluria II, PH2	0.004469153	0.026814921	SMPDB	ENSG00000065833; ENSG00000278540	4199; 31	22	22
Pyruvate kinase deficiency	0.004469153	0.026814921	SMPDB	ENSG00000065833; ENSG00000278540	4199; 31	22	22
Leigh Syndrome	0.004469153	0.026814921	SMPDB	ENSG00000065833; ENSG00000278540	4199; 31	22	22
Pyruvate Metabolism	0.004469153	0.026814921	SMPDB	ENSG00000065833; ENSG00000278540	4199; 31	22	22
Pyruvate Decarboxylase E1 Component Deficiency (PDHE1 Deficiency)	0.004469153	0.026814921	SMPDB	ENSG00000065833; ENSG00000278540	4199; 31	22	22
Leptin signaling pathway	0.005011674	0.028826678	Wikipathways	ENSG00000174697; ENSG00000110092; ENSG00000278540	595; 3952; 31	76	76
Metabolism	0.005124743	0.028826678	Reactome	ENSG00000151632; ENSG00000062282; ENSG00000106809; ENSG00000116133; ENSG00000196616; ENSG00000149485; ENSG00000004799; ENSG00000134824; ENSG00000139329; ENSG00000104812; ENSG00000278540; ENSG00000122176; ENSG00000186469; ENSG000001	1728; 2331; 4060; 1718; 2997; 31; 125; 5166; 4969; 1646; 54331; 9415; 84649; 3992	1481	1472

				81019			
Regulation of Complement cascade	0.005308714	0.02895662	Reactome	ENSG00000112936; ENSG00000039537	730; 729	24	24
Propanoate metabolism - Homo sapiens (human)	0.007186387	0.038045578	KEGG	ENSG00000093144; ENSG00000278540	55862; 31	28	28
Activation of Matrix Metalloproteinases	0.008222042	0.042284788	Reactome	ENSG00000143387; ENSG00000087245	1513; 4313	30	30
Collagen degradation	0.009893155	0.049465773	Reactome	ENSG00000143387; ENSG00000087245	1513; 4313	33	33

Table S5: List of genes in the respective clusters from the overlap network, represented here in HGNC symbols.

Clusters	**Genes**
1	ACACA, DHRS11, FADS1, FADS2, OLFM2, PDK4, SCD, SLC29A4
2	CCND1, CTIF, DAB2IP, DGAT2, DTX1, FADS3, ITGA7, PLEKHG5, PMEPA1, RBPMS2, SYNPO, TENM4, TLCD2, TMEM120B, TMEM184B, TNFRSF25, ZNF219
3	ABCC6, ADH1B, C6, C7, CAP2, ECHDC1, FAT1, GNG2, HSPA12A, KLB, KLHL31, MAL2, ME1, MRAP
4	GPLD1, KANK4, KIF1C, LEP, NPR3, NQO1, PDZD2, SLC24A3, SNCG, SRPX, VGLL3, VLDLR, VLDLR-AS1
5	AKR1C2, DHCR24, FSTL3, LOXL2, TUBB2A
Un-clustered	AACS, CTSK, FFAR4, FMOD, GPNMB, GYS1, LUM, MMP2, OGN, SLC7A7