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Transcriptional dynamics in type 2 diabetes progression is linked with circadian, thermogenic, and cellular stress in human adipose tissue.

Supplementary Tables 1, 2

Supplementary Figures 1-14

Supplementary Table 1. Clinical Characteristics of Participants

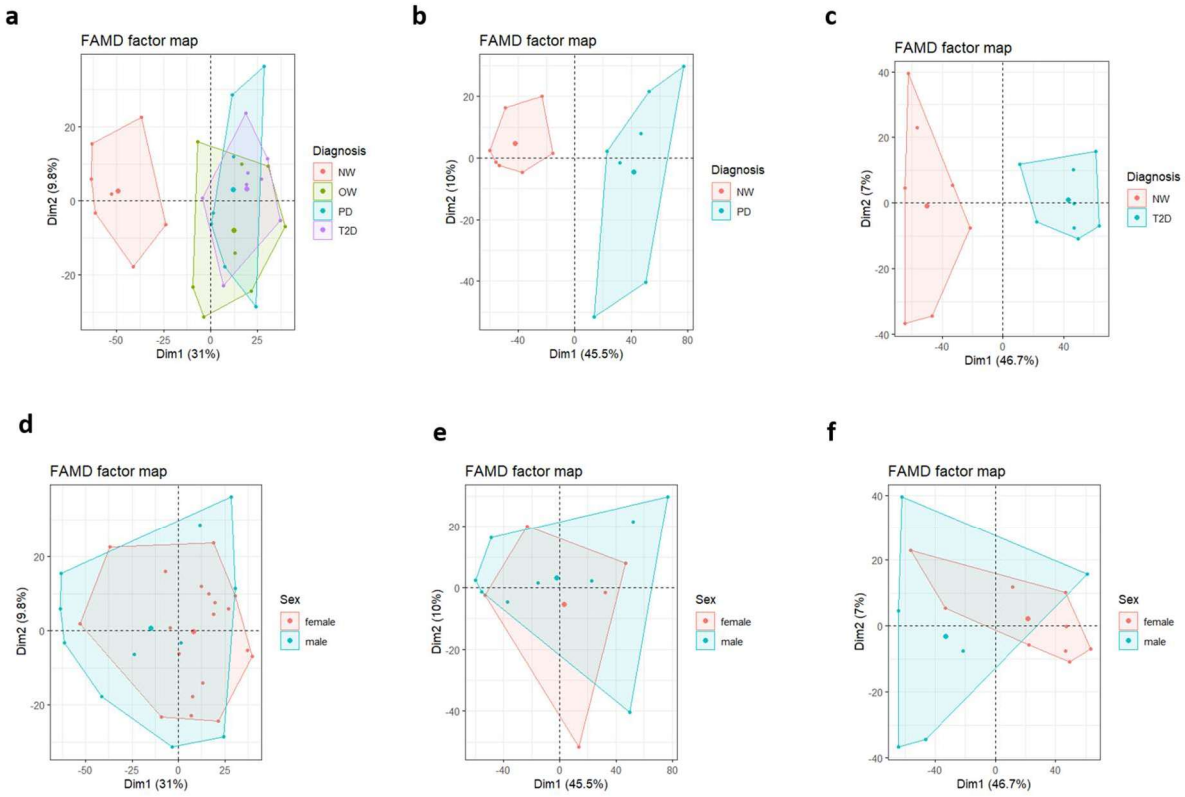
GROUP	NW (a)			OW (b)			PD (c)			T2D (d)		
Parameter	**AVE**	**SEM**	**P**	**AVE**	**SEM**	**P**	**AVE**	**SEM**	**P**	**AVE**	**SEM**	**P**
Weight (kg)	63.24	± 2.03	b^{**},c^{*},d^{**}	72.71	± 1.68	a^{**}	70.81	± 1.98	a^{*}	72.77	± 1.88	a^{**}
Fat mass (kg)	14.79	± 1.13	b^{***},c^{***},d^{***}	29.66	± 1.33	a^{***}	27.14	± 1.13	a^{***}	30.24	± 1.31	a^{***}
Fat mass (%)	24.91	± 2.01	$b^{§§§},c^{§§§},d^{§§§}$	40.45	± 1.08	$a^{§§§}$	38.64	± 1.63	$a^{§§§}$	42.19	± 0.14	$a^{§§§}$
Fat-free mass (%)	75.64	± 2.02	$b^{§§§},c^{§§§},d^{§§§}$	59.50	± 1.10	$a^{§§§}$	61.36	± 1.63	$a^{§§§}$	57.81	± 0.14	$a^{§§§}$
†Muscle mass (%)	20.98	± 1.06	$d^{§§}$	18.78	± 1.02		19.01	± 1.06		17.36	± 1.02	$a^{§§}$
BMI (kg/m^2)	22.03	± 0.47	b^{***},c^{***},d^{***}	28.67	± 0.54	a^{***}	27.49	± 0.51	a^{***},d^{*}	29.57	± 0.55	a^{***},c^{*}
FMI (kg/m^2)	5.30	± 0.43	b^{***},c^{***},d^{***}	11.73	± 0.52	a^{***}	10.97	± 0.66	a^{***}	12.33	± 0.61	a^{***}
FFMI (kg/m^2)	16.80	± 0.42		17.06	± 0.27		17.23	± 0.45		16.93	± 0.27	
Waist Circumference (cm)	77.54	± 2.10	$b^{§§§},c^{§§§},d^{§§§}$	91.72	± 0.96	$a^{§§§},d^{§}$	91.78	± 1.38	$a^{§§§}$	96.77	± 1.49	$a^{§§§}$
Hip Circumference (cm)	94.13	± 0.89	b^{***},c^{**},d^{***}	104.98	± 1.60	a^{***}	100.94	± 1.24	a^{**}	104.68	± 1.21	a^{***}
Wrist (cm)	15.56	± 0.16		16.06	± 0.27		15.96	± 0.27		16.20	± 0.24	
SBP (mmHg)	114.50	± 2.78		115.43	± 2.16		116.90	± 2.62		116.88	± 2.27	
DBP (mmHg)	73.52	± 1.63		76.91	± 1.48		74.14	± 1.38		76.05	± 1.27	
Heart rate (bpm)	64.60	± 1.84	d^{*}	66.43	± 2.33		65.11	± 1.39		72.90	± 2.36	a^{*}
Glucose (mg/dL)	90.76	± 0.96	$c^{§§§},d^{§§§}$	91.11	± 1.14	$c^{§§§},d^{§§§}$	102.00	± 1.57	$a^{§§§},b^{§§§},d^{§§§}$	139.81	± 4.96	$a^{§§§},b^{§§§},c^{§§§}$
HbA1c (%)	5.18	± 0.07	$c^{§§§},d^{§§§}$	5.30	± 0.05	$c^{§§§},d^{§§§}$	5.93	± 0.03	$a^{§§§},b^{§§§},d^{§§§}$	6.77	± 0.14	$a^{§§§},b^{§§§},c^{§§§}$
Insulin (µU/mL)	6.31	± 0.56	$c^{§§},d^{§§§}$	8.08	± 0.67	$d^{§§}$	10.76	± 1.08	$a^{§§}$	15.25	± 1.55	$a^{§§§},b^{§§}$
†Triglyceride (mg/dL)	94.76	± 1.09	c^{*},d^{***}	112.02	± 1.13	d^{**}	146.06	± 1.11	a^{*}	208.47	± 1.08	a^{***},b^{**}
Cholesterol (mg/dL)	181.09	± 7.57	c^{*}	199.17	± 6.65		211.33	± 6.50	a^{*}	188.19	± 6.35	
LDL-C	108.47	± 5.65	c^{*}	124.91	± 5.93		131.80	± 6.00	a^{*},d^{**}	102.97	± 6.41	c^{**}
HDL-C	50.47	± 1.25	d^{*}	45.57	± 2.17		47.67	± 1.83		43.18	± 1.83	a^{*}
Creatinine (mg/dL)	0.77	± 0.03		0.67	± 0.02		0.70	± 0.04		0.71	± 0.03	
†ALT (U/L)	19.27	± 1.06		25.00	± 1.10		22.21	± 1.08		23.45	± 1.11	
AST (U/L)	25.14	± 1.14		26.09	± 1.43		25.89	± 1.39		27.56	± 2.21	
†GGT (U/L)	14.41	± 1.08	d^{**}	16.40	± 1.08		16.40	± 1.07		20.61	± 1.08	a^{**}
‡Fat-free mass (kg)	42.43	± 1.71		40.85	± 1.04		41.10	± 1.87		39.45	± 0.60	
‡Fat mass (g)	16186.95	± 946.44	b^{***},c^{***},d^{***}	29708.45	± 1454.68	a^{***}	27364.14	± 1038.45	a^{***}	29049.76	± 1228.69	a^{***}
‡Fat mass (%)	28.73	± 1.73	$b^{§§§},c^{§§§},d^{§§§}$	41.85	± 0.60	$a^{§§§}$	39.85	± 1.33	$a^{§§§}$	43.01	± 0.89	$a^{§§§}$
‡Visceral adipose tissue (cm^3)	374.28	± 83.03	$b^{###},c^{###},d^{###}$	969.27	± 69.43	$a^{###},d^{##}$	1143.43	± 84.70	$a^{###}$	1345.36	± 88.86	$a^{###}$
‡Visceral adipose tissue (g)	353.06	± 78.34	$b^{###},c^{###},d^{###}$	919.86	± 65.99	$a^{###},d^{#}$	1078.19	± 79.86	$a^{###}$	1268.82	± 83.79	$a^{###}$

Shapiro-Wilk normality test P>0.05. Bartlett test of homogeneity of variances P>0.05. ‡ D'Agostino skewness test. † Log transformation. One-way ANOVA, followed by Tukey's post hoc test, *P<0.05, **P<0.01, ***P<0.001. Welch's ANOVA, followed by Games-Howell post hoc test, §P<0.05, §§P<0.01, §§§P<0.001. Kruskal-Wallis, followed by Wilcoxon post hoc test #P<0.05, ##P<0.01, ###P<0.001. Healthy normal weight (NW) n=22, overweight/obese (OW) n=23, overweight/obese prediabetic (PD) n=21, and overweight/obese diabetic (T2D) n=22.

Supplementary Table 2. Sex distribution of study participants

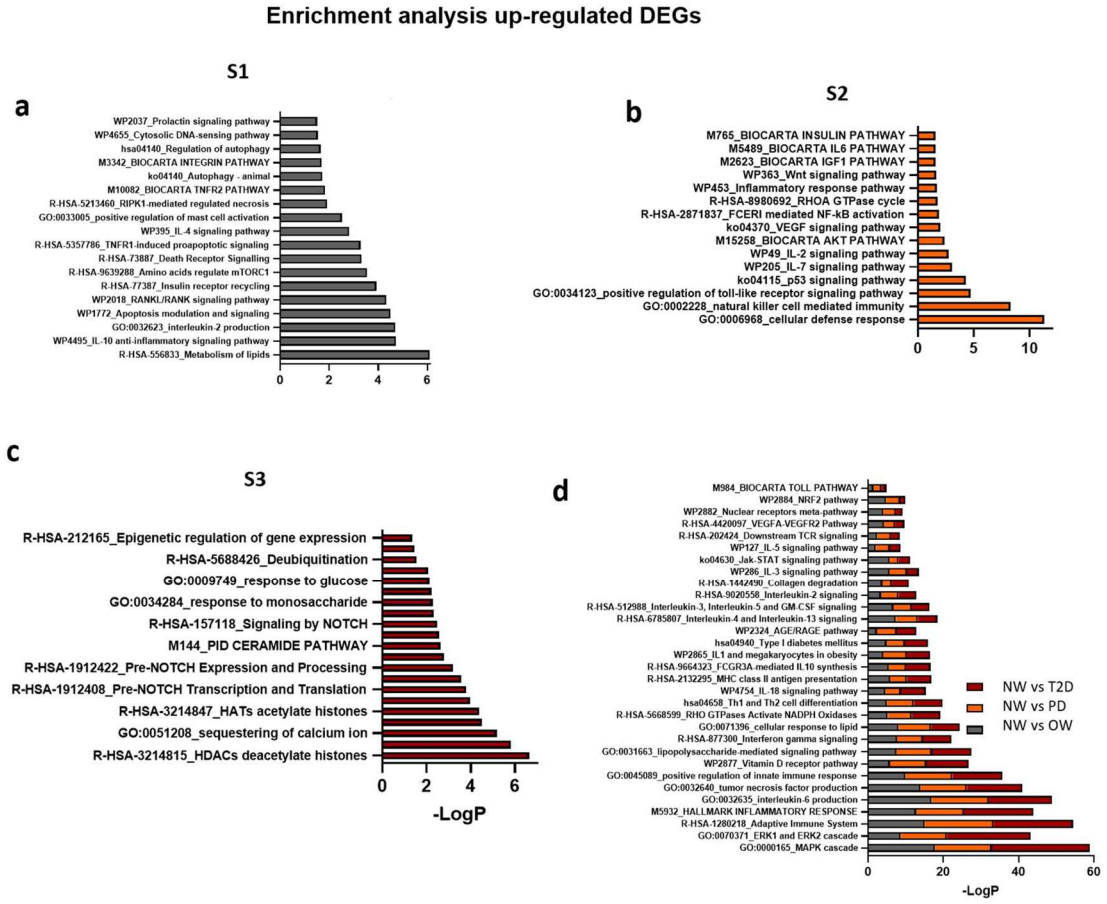
Participants							Transcriptome						
				n	Age AVE	SE	n (W+M)				n	Age AV	SE	n (W+M)
Women	62	NW	Women	10	31.31	±12.66	22	**Women**	19	NW	Women	2	25.6	±6.55	7
Men	26		Men	12				**Men**	11		Men	5			
		OW	Women	20	42.91	±10.64	23			OW	Women	7	43.1	±10	8
			Men	3							Men	1			
		PD	Women	14	48.43	±12.07	21			PTD	Women	3	52.6	±9.57	7
			Men	7							Men	4			
		T2D	Women	18	51.5	±12.21	22			T2D	Women	7	51	±14.15	8
			Men	4							Men	1			
				Total			**88**					Total			**30**

Supplementary Figure 1



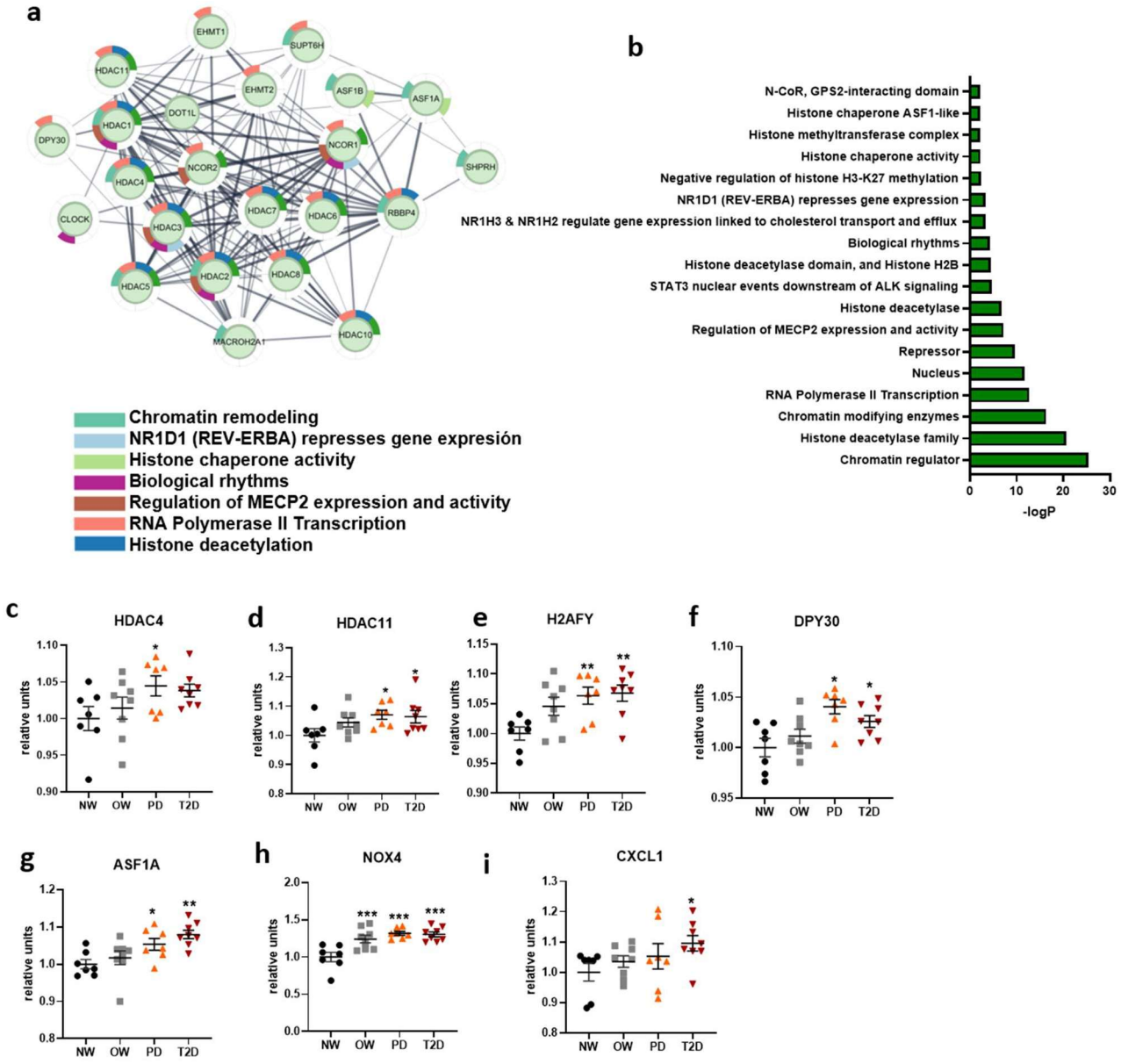
Supplementary Figure 1. Gene expression of DEGs is not influenced by sex. Factor analysis of mixed data (FAMD) was conducted to evaluate the influence of sexual dimorphism on gene expression. Sex (male and female) and diagnosis (NW, OW, PD, and T2D) were used as qualitative variables, and gene expression of DEGs ($P<0.05$, $|\log2(\text{Fold Change})| > 1.1$) as quantitative variables. **a, d** DEGs from S1 (OW vs. NW); **b, e** S2 (PD vs. NW), and **c, f** S3 (T2D vs. NW) were evaluated. NW (n=7), OW (n=8), PD (n=7), T2D (n=8), independent samples.

Supplementary Figure 2



Supplementary Figure 2. Enrichment analysis up-regulated DEGs. Identified significantly enriched biological functions from the up-regulated DEGs in each stage: **a** S1 (OW vs. NW), **b** S2 (PD vs. NW), **c** S3 (T2D vs. NW), and **d** all stages. NW (n=7), OW (n=8), PD (n=7), T2D (n=8), independent samples.

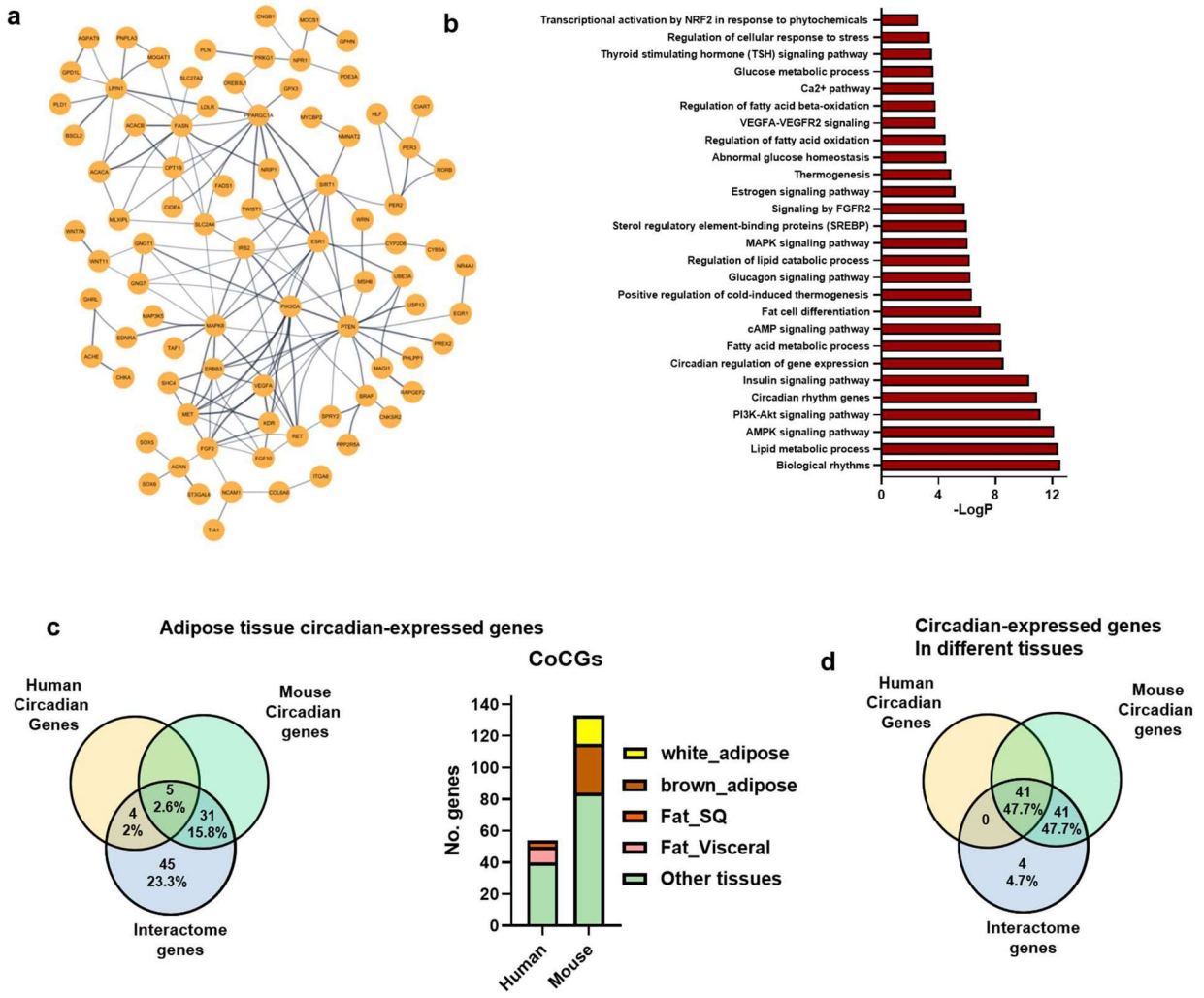
Supplementary Figure 3



Supplementary Figure 3. Chromatin-modifying enzymes are involved in metabolic control during the development of T2D. a Protein-protein interactome network from histone-modifying enzymes, **b** Identified significantly enriched biological functions. Relative gene expression of several histone-modifying enzymes and cofactors, **c** Histone Deacetylase 4 (HDAC4), **d** Histone Deacetylase 11, **e** Histone Family Member Y (H2AFY), **f** Histone Methyltransferase Complex Regulatory Subunit (DYP30), **g** Anti-Silencing Function 1A Histone Chaperone (ASF1A), **h** NADPH Oxidase 4, **i** C-X-C Motif Chemokine Ligand 1 (CXCL1), ANOVA with ebayes. Significant difference vs. NW group

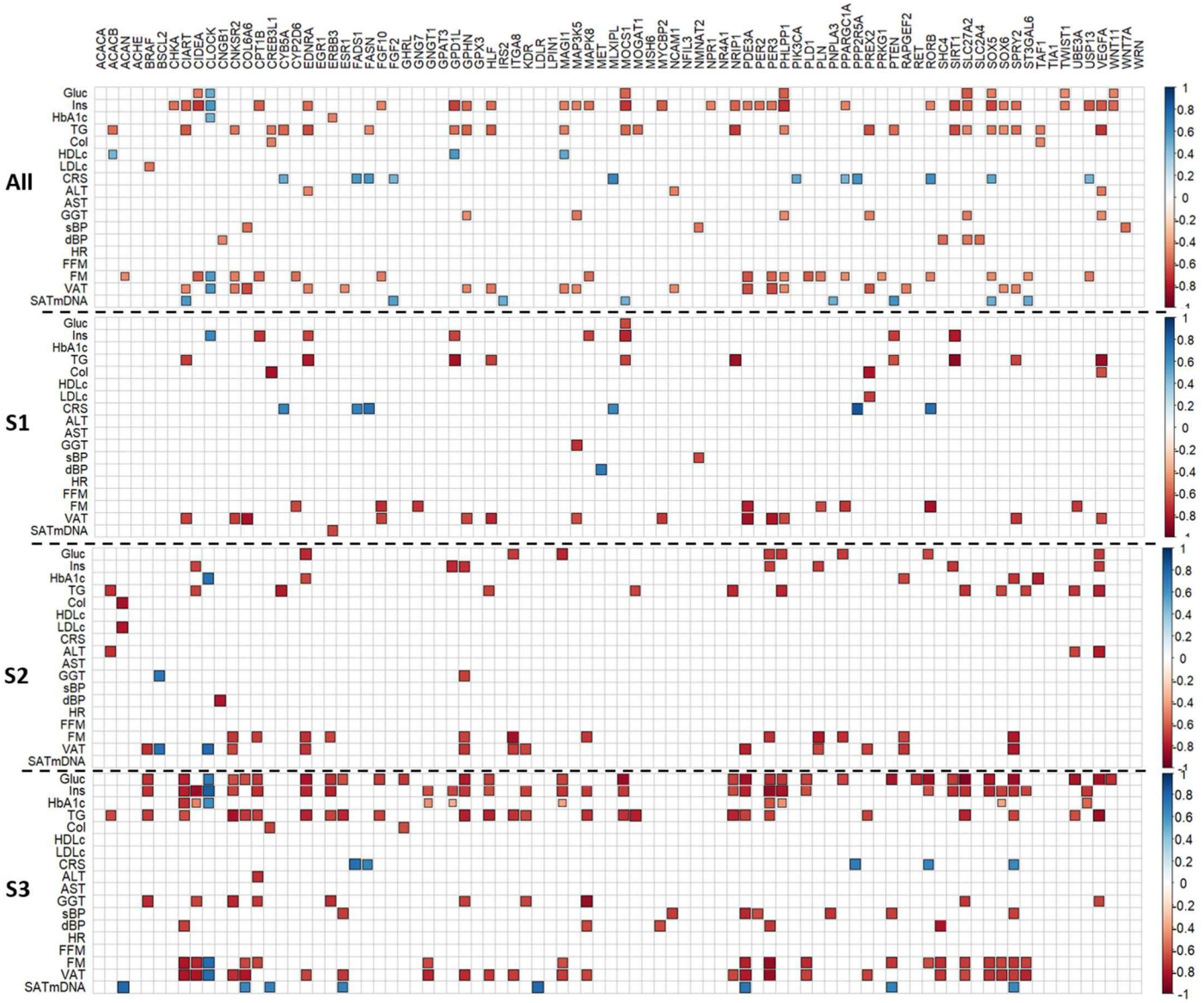
*P< 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001. Significant difference vs.OW group ##P< 0.01. NW (n=7), OW (n=8), PD (n=7), T2D (n=8), independent samples. Error bars as mean ± SEM.

Supplementary Figure 4



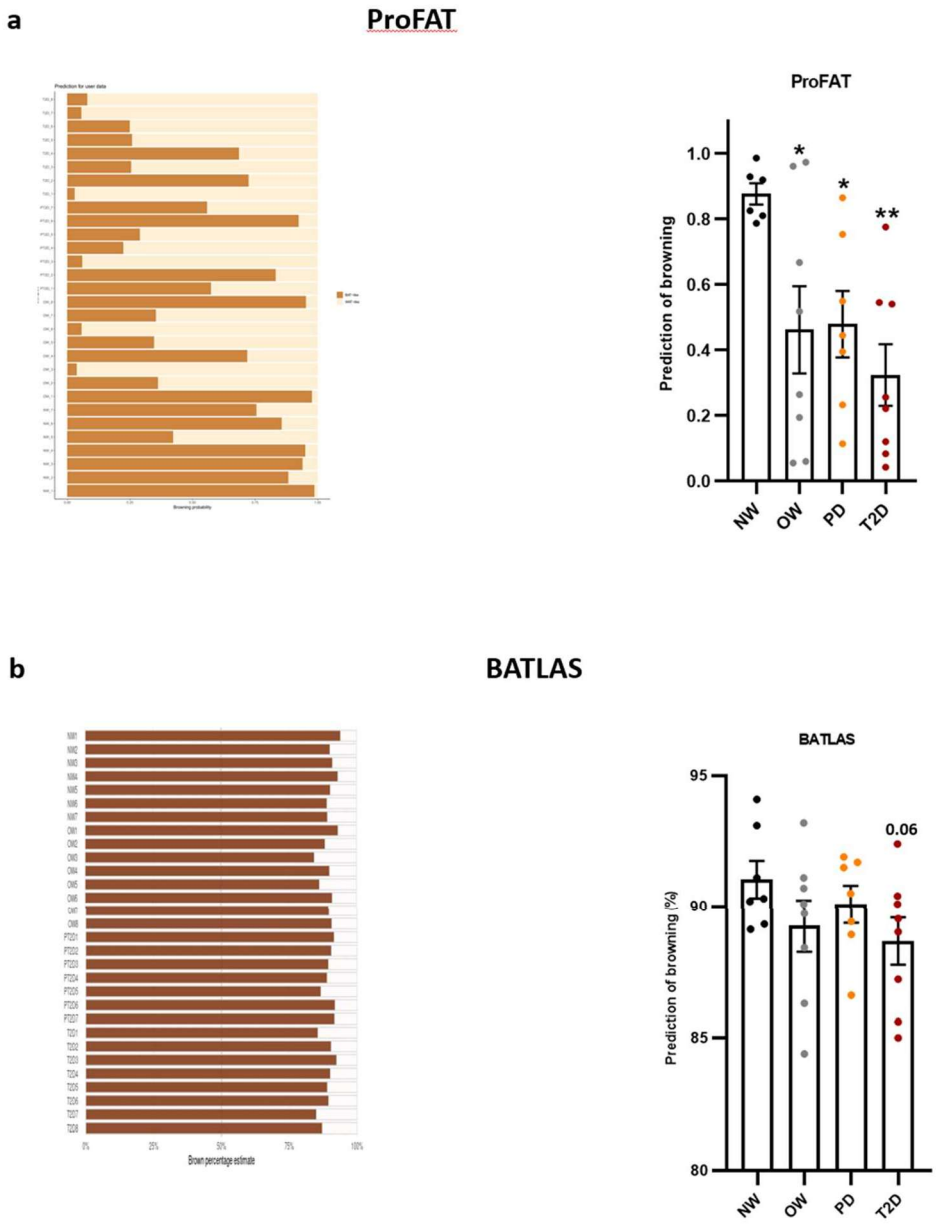
Supplementary Figure 4. Reduced expression of catabolic-related genes in type 2 diabetes linked to the circadian clock: a Interactome network from downregulated DEGs at S3 (NW vs. T2D), and **b** Significantly enriched biological functions within the interactome, **c- d** Number of circadian-expressed genes identified from the CircaDB database (mouse, Jonckheere-Terpstra-Kendall (JTK); human CYCLOPS; P<0.05) [1].

Supplementary Figure 5



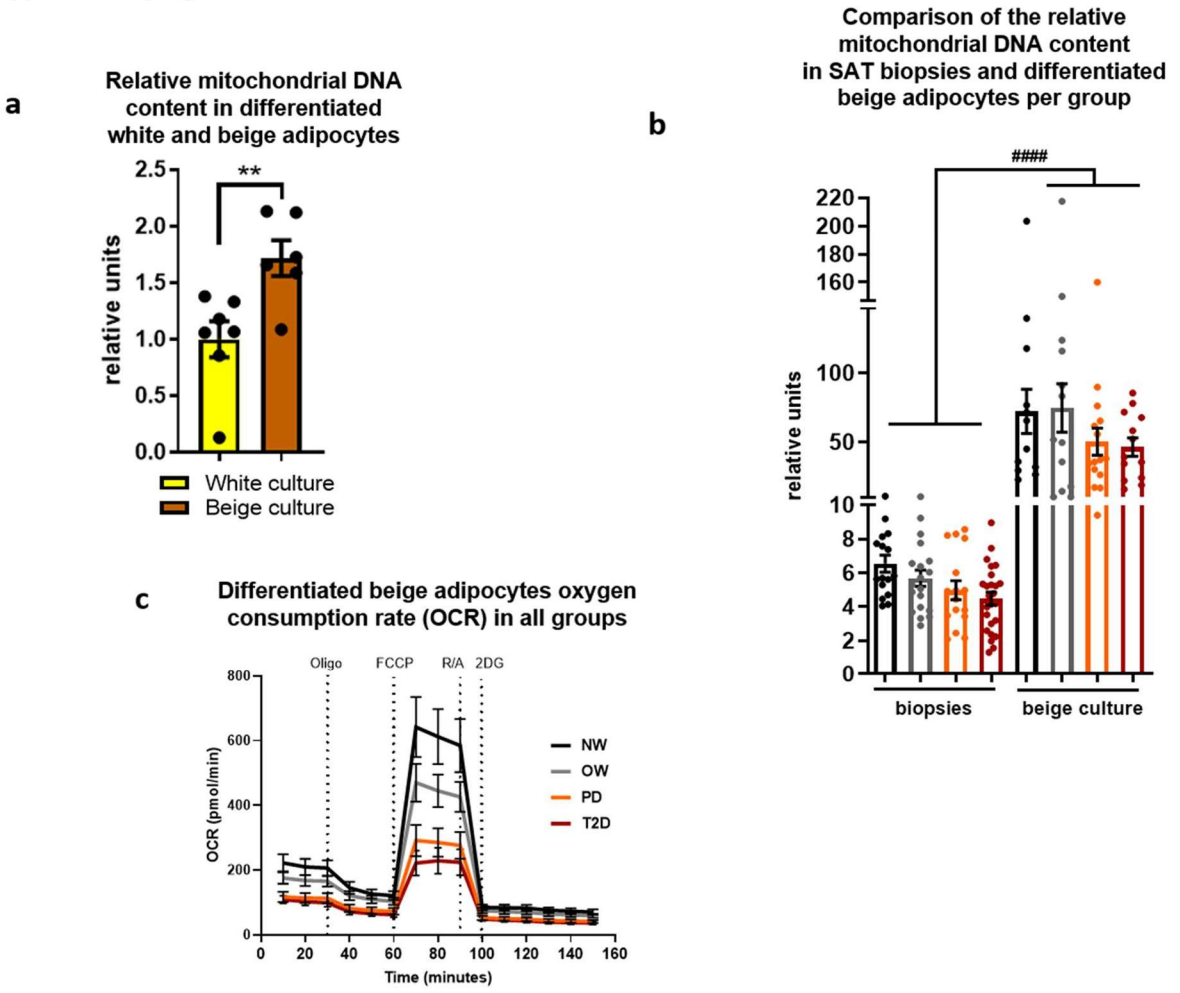
Supplementary Figure 5. Correlation analysis of protein-protein interacting gene expression vs. metabolic and body composition characteristics. Significant Spearman's correlation coefficients (rho) were colored blue (positive) and red (negative), P<0.001. NW (n=7), OW (n=8), PD (n=7), T2D (n=8), independent samples.

Supplementary Figure 6



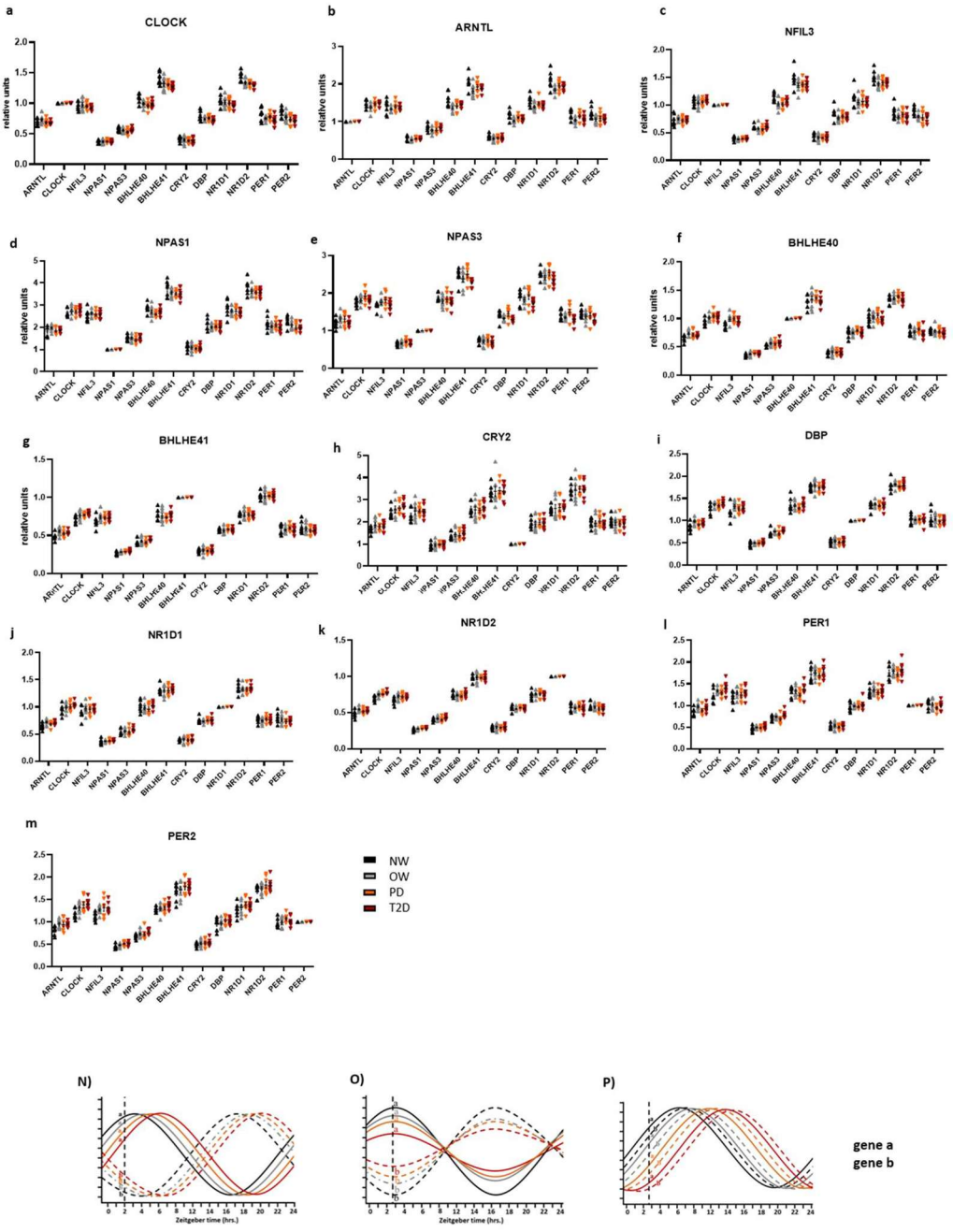
Supplementary Figure 6. ProFat and BATLAS computational analyses of the transcriptomic profile in each sample. **a** Browning probability values from the ProFat server for each sample [2]. **b** Brown percentage estimated values from the BATLAS server [3]. Bar graph showing mean ± SEM. One-way ANOVA followed by Dunnett's post hoc test. Significant difference vs. NW group *P < 0.05, **P < 0.01. NW (n=7), OW (n=8), PD (n=7), T2D (n=8), independent samples. Error bars as mean ± SEM.

Supplementary Figure 7



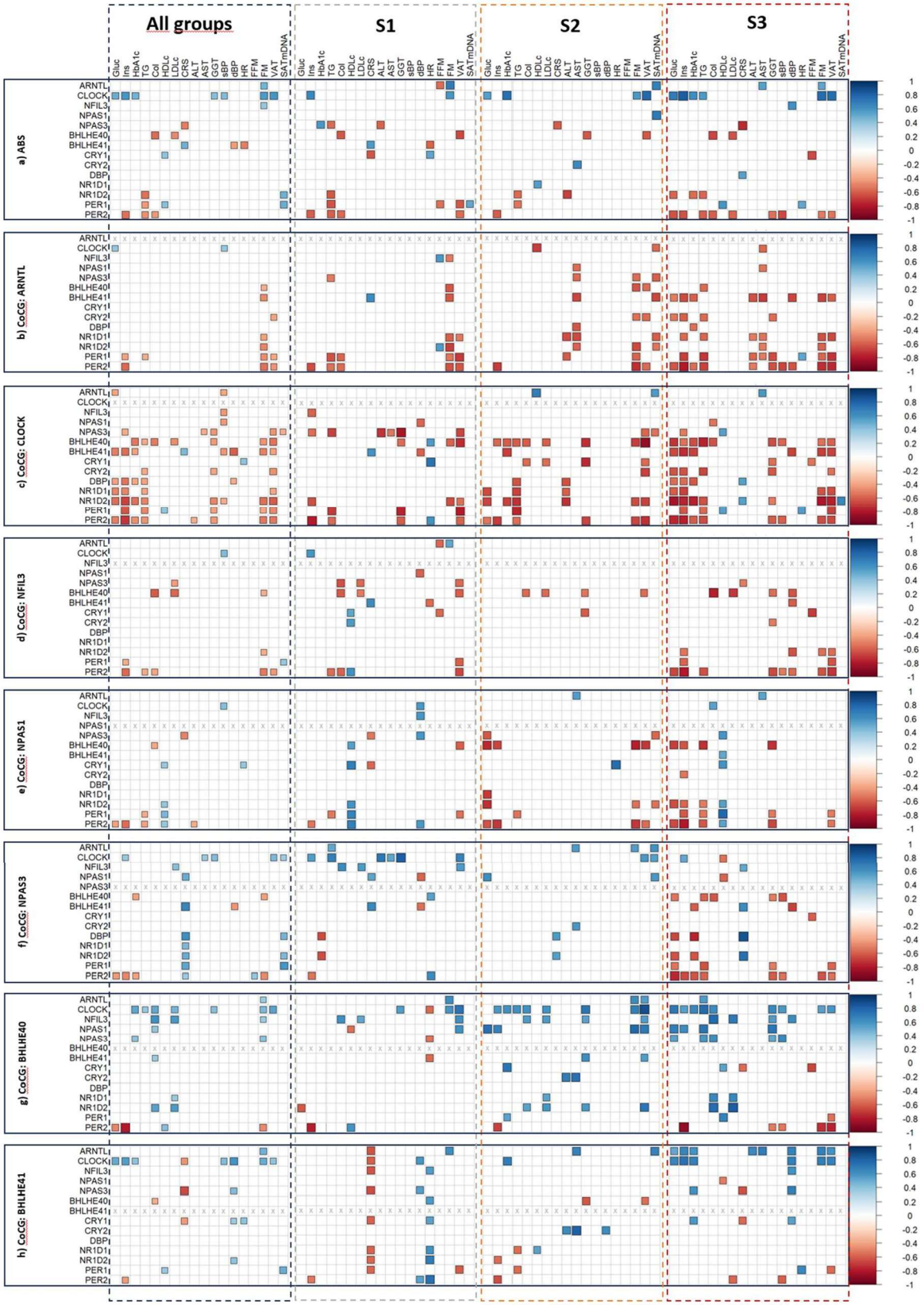
Supplementary Figure 7a Relative mitochondrial DNA content in white (n=7, yellow bar) and beige (n=6, beige bar) differentiated adipocytes, **P<0.01, unpaired t-test. **b** Comparison of the relative mitochondrial DNA content in SAT biopsies and differentiated beige adipocytes per group, **c** Differentiated beige adipocytes oxygen consumption rate (OCR) in all groups, data are the mean ± SEM, experimental conditions: Oligomycin (Oligo), carbonyl cyanide4-(trifluoromethoxy) phenylhydrazone (FCCP), rotenone/actinomycin A (R/A), 2-deoxy-d-glucose (2DG), NW (n=11), OW (n=13), PD (n=16), T2D (n=17). Error bars as mean ± SEM.

Supplementary Figure 8

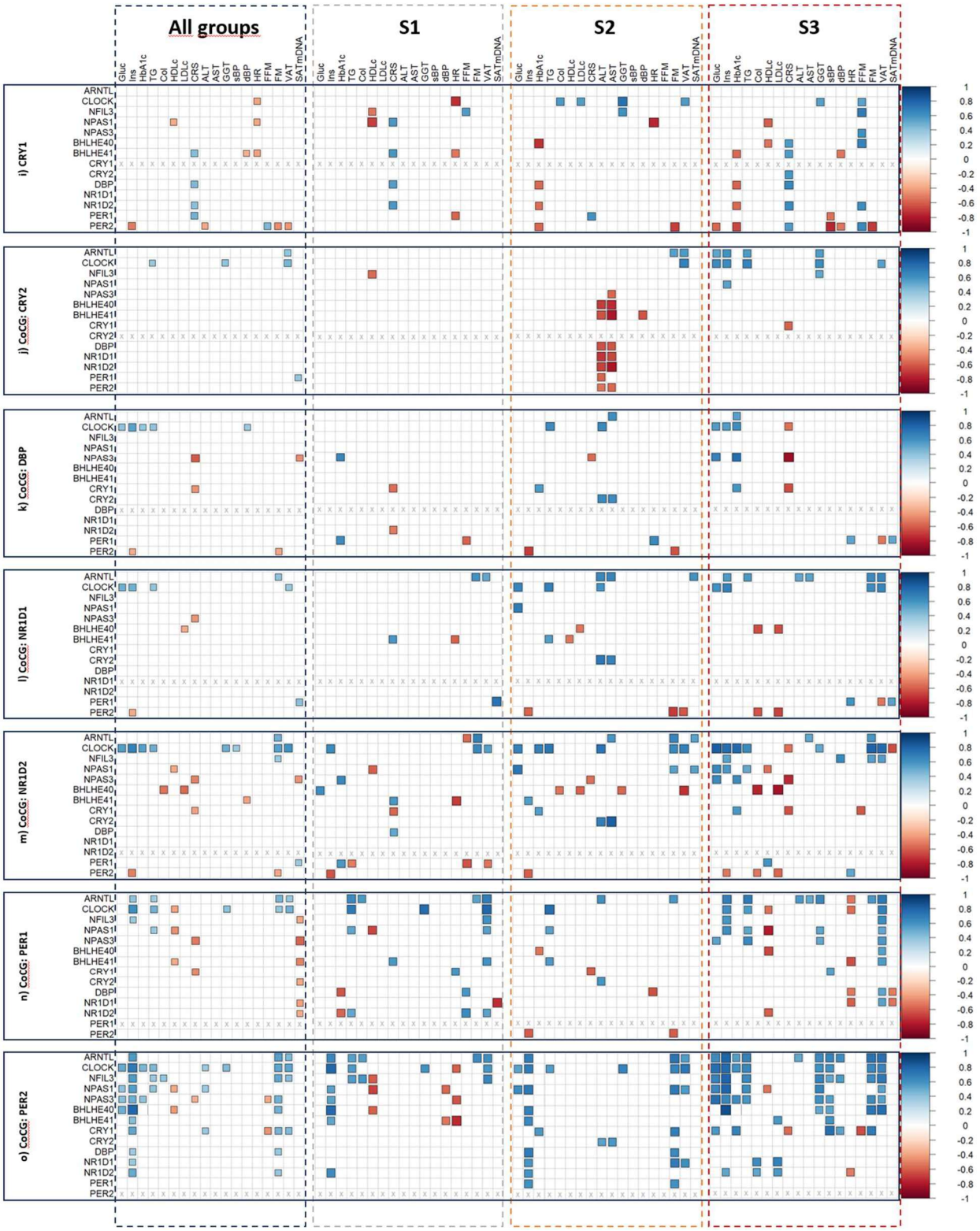


Supplementary Figure 8. a-m, Ratios from gene expression values (CoCG:CoCG). Representative plot, illustrating how ratio values can be affected by **n** Antiphase acrophases and different amplitude, **o** Antiphase acrophases and phase shift, and **p** Phaseshift with similar acrophases. Continuous lines represent gene “a”, and dotted lines gene “b”. Colored lines match experimental groups. NW (n=7), OW (n=8), PD (n=7), T2D (n=8), independent samples. Error bars as mean ± SEM.

Supplementary Figure 9



-Continue Supplementary Figure 9-



Supplementary Figure 9. Correlation between core-clock gene expression in SAT biopsies vs. clinical parameters from , S1 (OW and NW), S2 (PD and NW), S3 (T2D and

NW), and all stages: **a** Absolute, **b** COCG:ARNTL, **c** COCG:CLOCK, **d** COCG:NFIL3, **e** COCG:NPAS1, **f** COCG:NPAS3, **g** COCG:BHLHE40, **h** COCG: BHLHE41, **i** COCG:CRY1, **j** COCG:CRY2, **k** COCG:DBP, **l** COCG:NR1D1, **m** COCG:NR1D2, **n** COCG: PER1, and **o** COCG:PER2 ratios. Significant Spearman's correlation coefficients (rho) were colored blue (positive) and red (negative), $P<0.05$, NW (n=7), OW (n=8), PD (n=7), T2D (n=8), independent samples.

Supplementary Figure 10

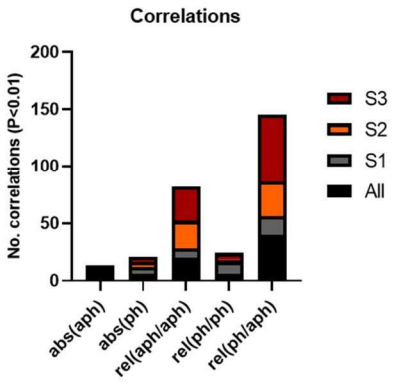
abs(aph)				
Parameter	ALL	S1	S2	S3
ALT	0	0	1	0
AST	0	0	0	0
Col	1	0	0	1
CRS	1	0	0	0
dBP	0	0	0	0
FFM	0	0	0	1
FM	0	0	0	0
GGT	0	0	0	0
Glu	0	0	0	0
HbA1c	0	0	0	0
HDLc	0	0	0	0
HR	1	0	0	0
Ins	1	0	0	0
LDLc	1	0	0	0
SATmDNA	2	0	0	0
sBP	0	0	0	1
TG	1	0	0	0
VAT	0	1	0	0

abs(ph)				
Parameter	ALL	S1	S2	S3
ALT	0	0	0	0
AST	0	0	0	0
Col	0	0	0	0
CRS	1	0	0	1
dBP	0	0	0	0
FFM	0	0	0	0
FM	1	1	0	1
GGT	0	0	0	0
Glu	1	0	0	1
HbA1c	1	0	1	0
HDLc	0	0	0	0
HR	0	0	0	0
Ins	1	1	0	1
LDLc	0	0	0	0
SATmDNA	0	0	2	0
sBP	0	0	0	0
TG	0	0	0	0
VAT	1	0	1	1

aph/aph				
Parameter	ALL	S1	S2	S3
ALT	0	0	6	0
AST	0	0	8	0
Col	2	0	0	4
CRS	2	0	0	0
dBP	0	0	0	0
FFM	0	0	0	4
FM	4	0	4	4
GGT	0	0	0	0
Glu	2	0	0	0
HbA1c	0	0	2	2
HDLc	0	0	0	2
HR	0	4	0	2
Ins	6	2	2	2
LDLc	2	0	0	6
SATmDNA	2	2	0	0
sBP	0	0	0	2
TG	0	0	0	0
VAT	0	0	2	2

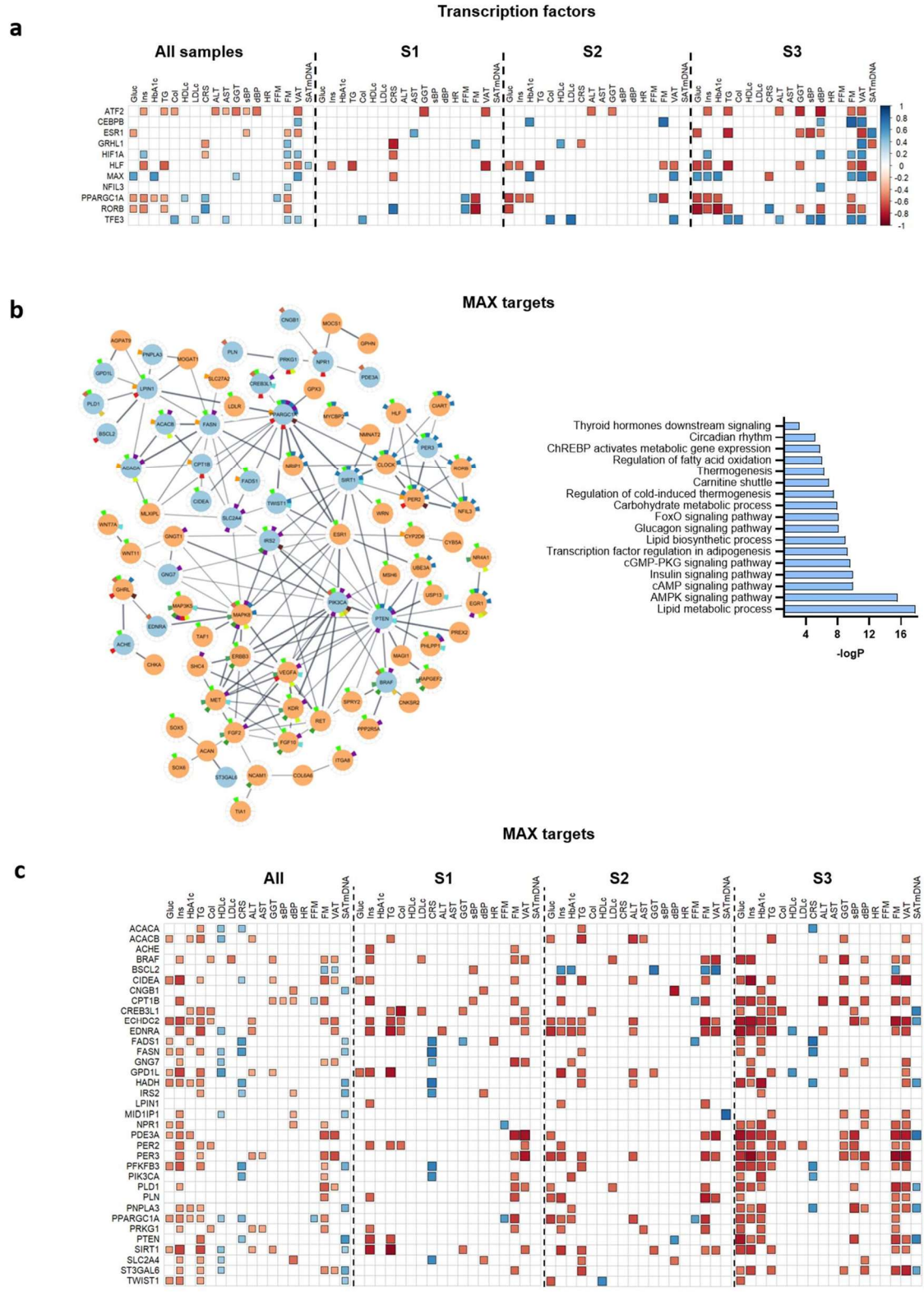
ph/ph				
Parameter	ALL	S1	S2	S3
ALT	0	2	0	0
AST	0	0	0	0
Col	0	0	0	0
CRS	4	0	0	2
dBP	0	0	0	0
FFM	0	0	0	0
FM	0	0	0	0
GGT	0	2	0	0
Glu	0	0	0	2
HbA1c	0	2	0	2
HDLc	0	0	2	0
HR	0	0	0	0
Ins	2	0	0	0
LDLc	0	0	0	0
SATmDNA	0	0	0	0
sBP	0	0	0	0
TG	0	2	0	0
VAT	0	2	0	0

ph/aph				
Parameter	ALL	S1	S2	S3
ALT	0	0	1	0
AST	0	0	1	1
Col	2	0	0	1
CRS	3	1	0	2
dBP	1	0	0	3
FFM	0	0	0	1
FM	9	2	4	5
GGT	1	1	3	5
Glu	4	0	4	9
HbA1c	2	1	2	3
HDLc	0	3	0	1
HR	0	1	1	0
Ins	9	2	5	10
LDLc	2	0	0	1
SATmDNA	1	0	1	1
sBP	0	0	0	1
TG	2	1	3	6
VAT	4	4	5	9



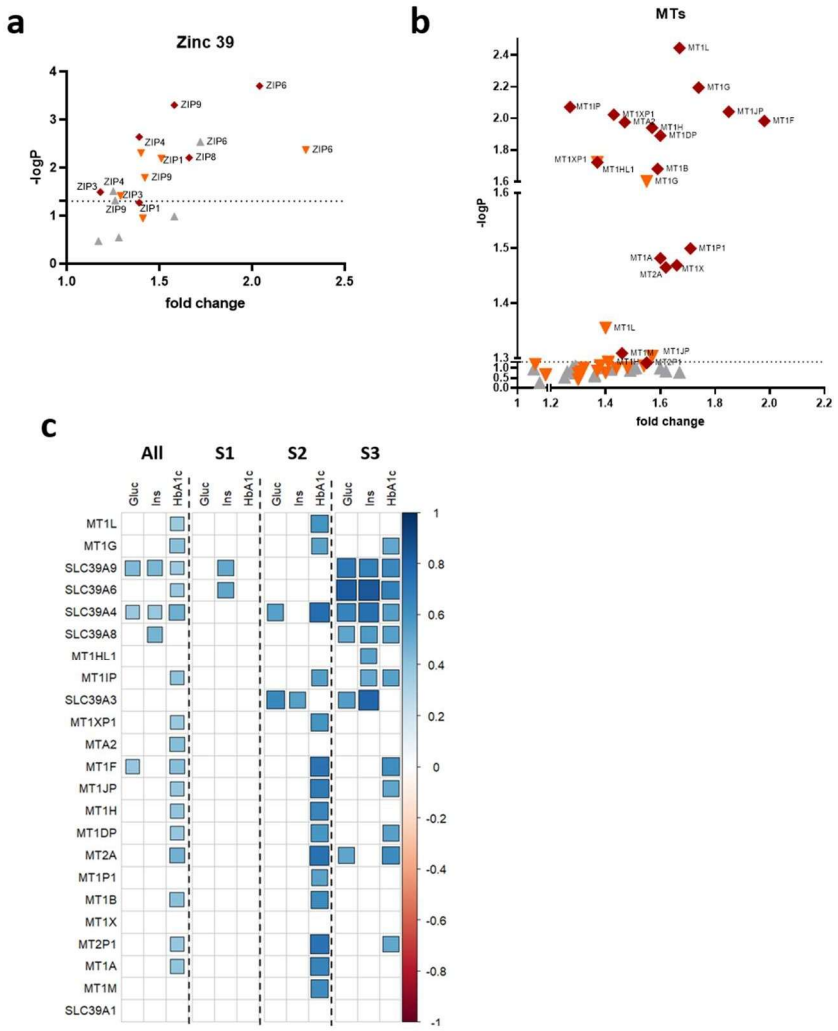
Supplementary Figure 10. Quantification of significant correlations (only P<0.01) from Supplementary Figure 9.

Supplementary Figure 11



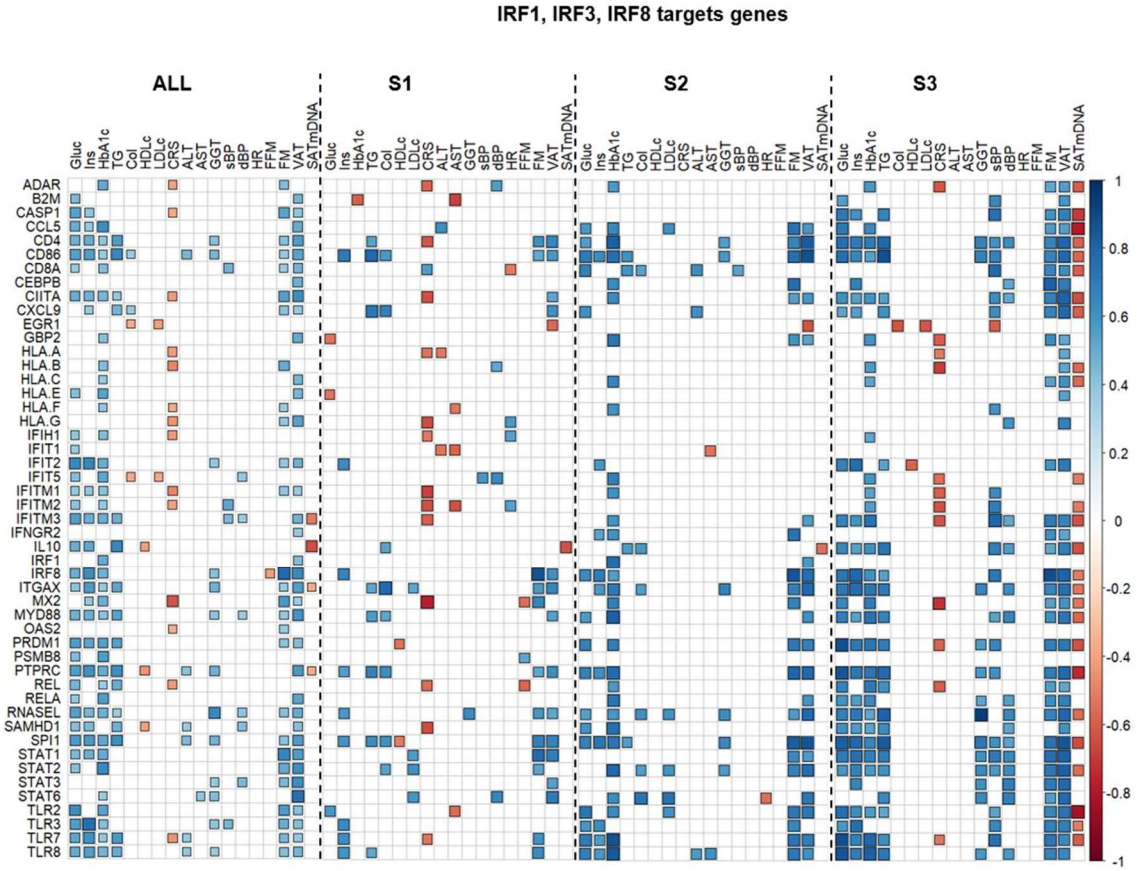
Supplementary Figure 11. a Correlation analysis of transcription factor expression with metabolic and body composition characteristics. **b** Interactome network depicting the predicted MAX targets (blue circles) and their predicted biological processes. **c** Correlation analysis of MAX target gene expression with metabolic and body composition characteristics. Significant Spearman's correlation coefficients (rho) were colored blue (positive) and red (negative), $P<0.05$, NW (n=7), OW (n=8), PD (n=7), T2D (n=8), independent samples.

Supplementary Figure 12



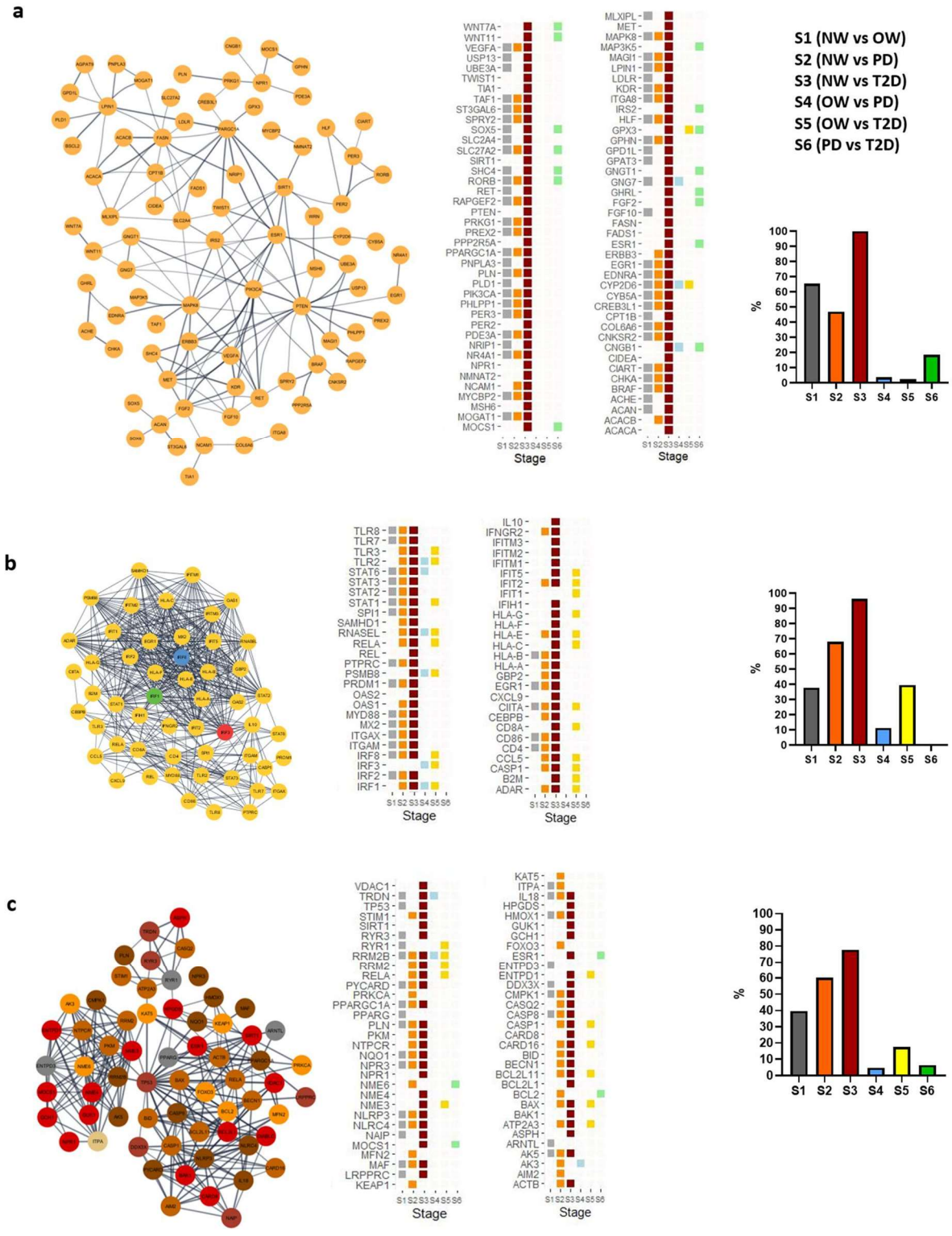
Supplementary Figure 12. Scatter plots showing the fold change and -logP values of **a** Solute Carrier Family (SLC) 39 family genes in each stage, **b** Metallothioneins (MT) in each stage, **c** Correlation Analysis of SLC39 and MT gene expression with metabolic characteristics. Significant Spearman's correlation coefficients (rho) were colored blue (positive) and red (negative), P<0.05, NW (n=7), OW (n=8), PD (n=7), T2D (n=8), independent samples.

Supplementary Figure 13



Supplementary Figure 13. Correlation analysis of IRF target gene expression with metabolic and body composition characteristics. Significant Spearman's correlation coefficients (rho) were colored blue (positive) and red (negative), P<0.05, NW (n=7), OW (n=8), PD (n=7), T2D (n=8), independent samples.

Supplementary Figure 14



Supplementary Figure 14. Identification and quantification of DEGs from the interactomes, **a** Supplementary Figure 4A, **b** Main Figure 6E and **c** Main Figure 7A. Heatmaps show significant DEGs: grey S1 (NWvsOW), orange S2 (NWvsPD), red S3 (NWvsT2D), S4 blue (OWvsPD), S5 yellow (OWvsPD), S6 green (PDvsT2D). Bar graphs show the count of interactome DEGs (percentage) identified in each combination ($P<0.05$). NW (n=7), OW (n=8), PD (n=7), T2D (n=8), independent samples.

Supplementary References

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3 Perdikari, A. *et al.* BATLAS: Deconvoluting Brown Adipose Tissue. *Cell Reports* **25**, 784-797.e784 (2018). https://doi.org:https://doi.org/10.1016/j.celrep.2018.09.044