Supplementary Materials for Time-restricted feeding promotes skeletal muscle function in diet- and genetic-induced obesity through shared and unique pathways

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Figure S1

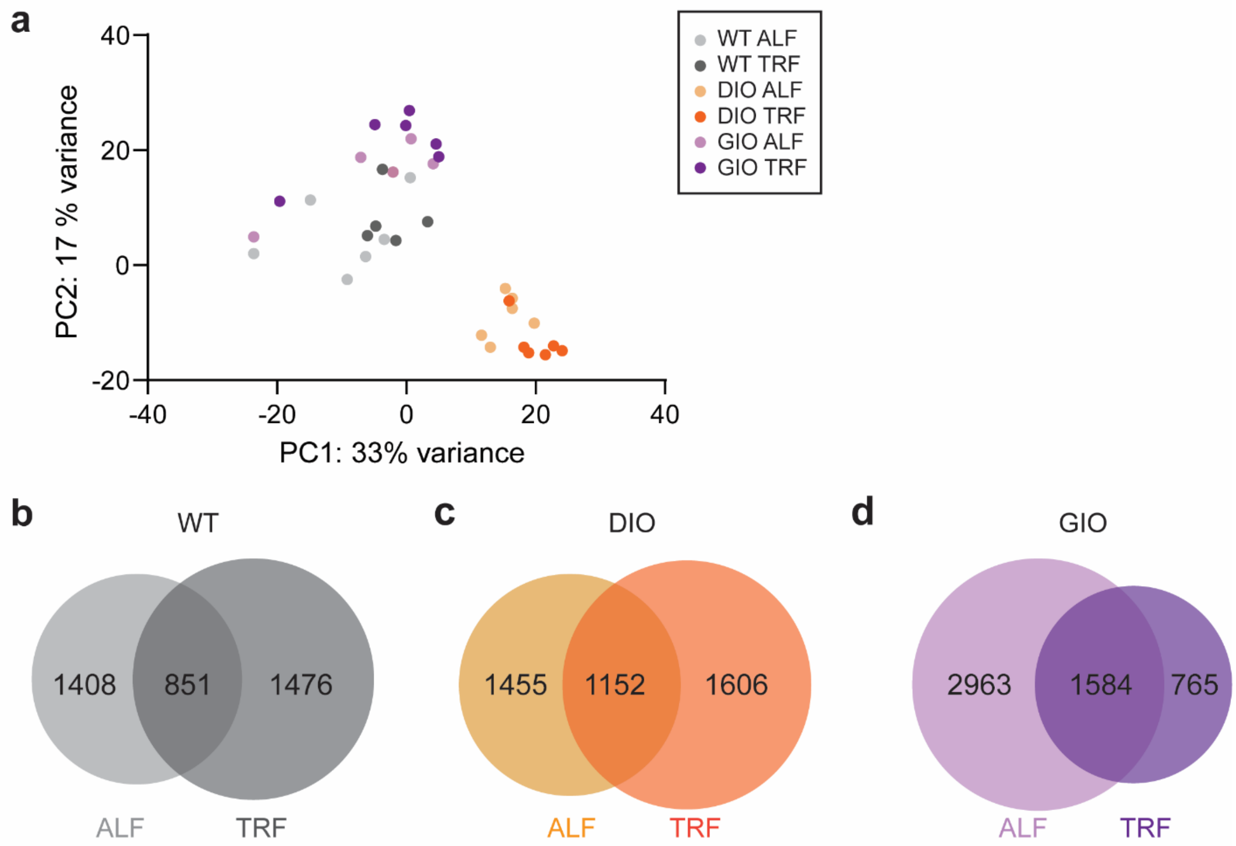

Figure S1: Overview of transcriptome data in WT, DIO and GIO models
(a) PCA plot of skeletal muscle transcriptome data.
(b-d) Periodic skeletal muscle transcripts identified under ALF and TRF in WT (b), DIO (c) and GIO (d).

Figure S2

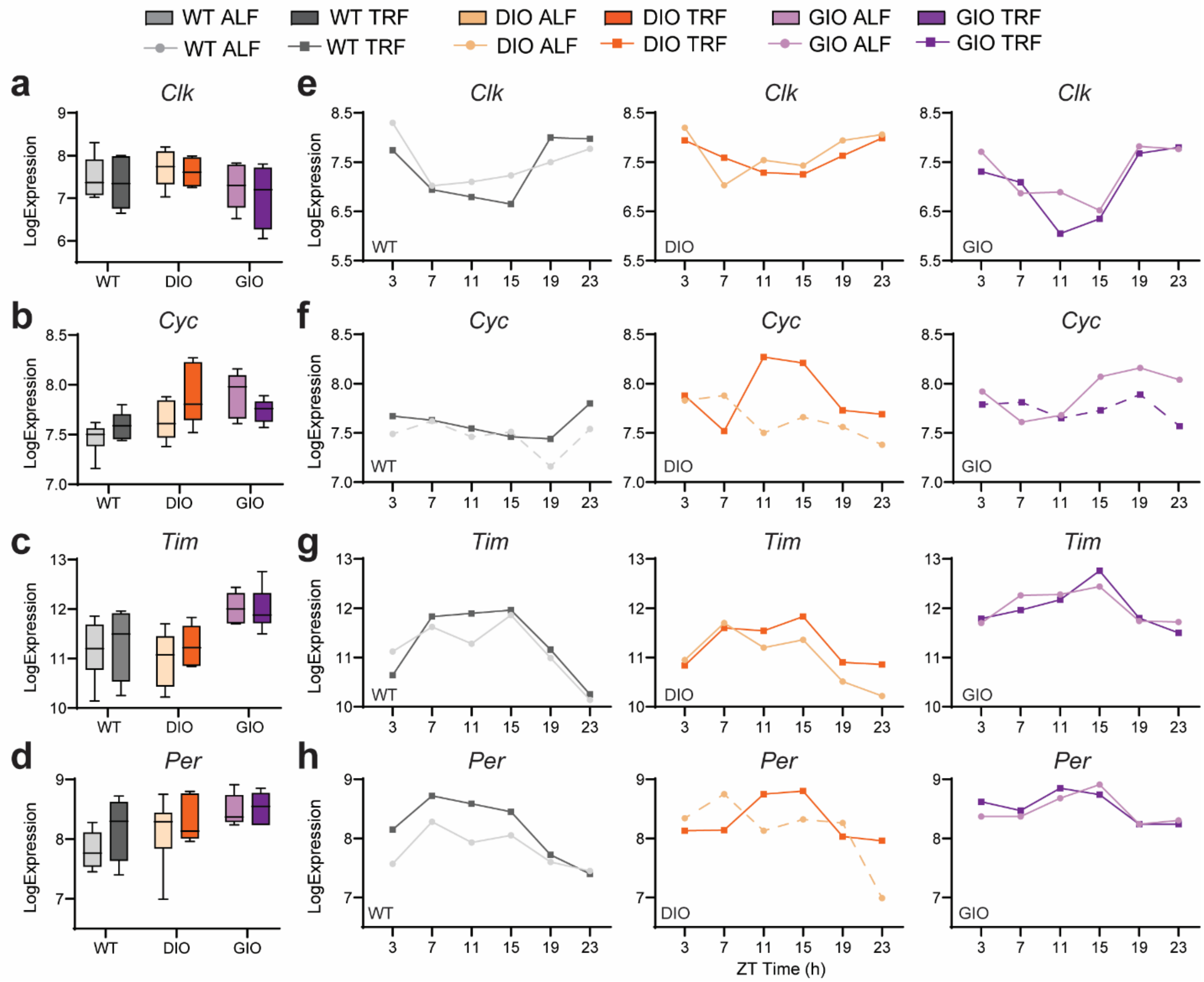

Figure S2: Periodicity of clock genes

(a-d) Expression level (normalized read count, log2) of *Clk* (a), *Cyc* (b), *Tim* (c) and *Per* (d) under ALF and TRF in WT, DIO and GIO.

(e-h) Temporal expressions of *Clk* (e), *Cyc* (f), *Tim* (g) and *Per* (h) under ALF and TRF in WT, DIO and GIO. Empirical_JTK periodicity detection was presented as a solid line (Corrected P-value ≤ 0.05) or dash line (Corrected P-value > 0.05).

Figure S3

a

	Drosophila Homolog	Human Homolog	Fold Change (TRF / ALF)			Annotated Function
			WT	DIO	GIO	
Up Genes	CG6188	Gnmt	2.80	6.40	2.39	Glycine N-methyltransferase
	CG6385	Sardh	1.35	2.47	1.44	Sarcosine dehydrogenase
	CG5955	Tdh	1.60	2.02	1.64	Predicted to have L-threonine 3-dehydrogenase activity
	CG6806	N/A	3.25	3.46	2.23	Larval serum protein 2
	CG5896	N/A	1.31	1.68	1.37	Gram-positive Specific Serine protease
Down Genes	CG7997	Gla	-1.63	-1.73	-1.48	Predicted to have alpha-galactosidase activity
	CG1942	Dgat2	-1.51	-1.93	-1.55	Diacylglycerol O-acyltransferase 2
	CG13992	N/A	-5.4	-5.33	-4.04	Unknown

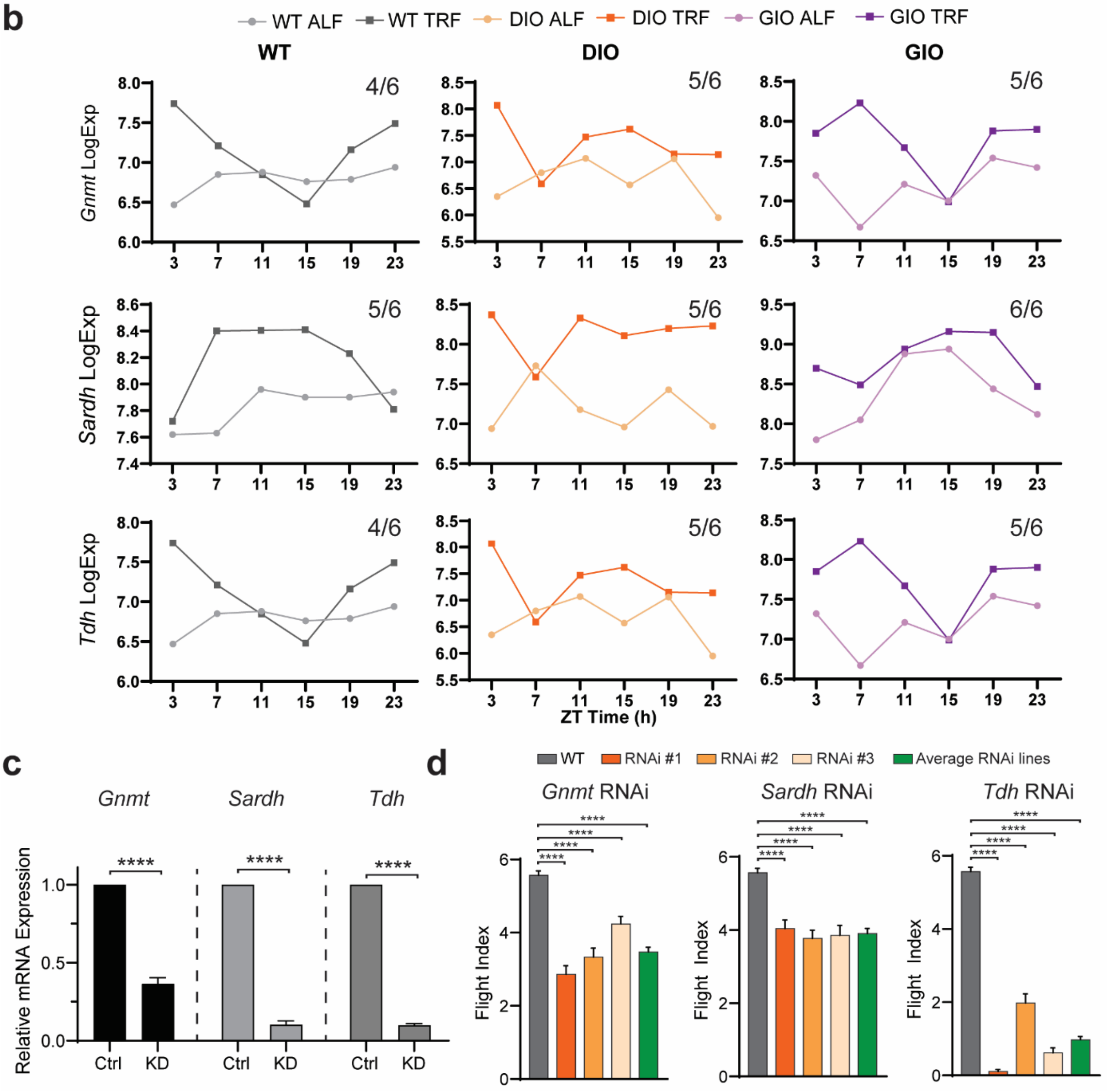

Figure S3: Profiles of the significantly upregulated genes across WT, DIO and GIO models

(a) A table of differentially expressed genes shared across WT, DIO and GIO models.

(b) Temporal expressions of *Gnmt*, *Sardh* and *CG5955* under ALF and TRF in WT, DIO and GIO.

(c) Quantification of RNA levels from thoraces of 3-week-old flies following *Gnmt*, *Sardh* and *CG5955* IFM-specific *KD*.

(d) Flight performance of WT male flies with *Gnmt*, *Sardh* or *CG5955* IFM-specific *KD* at 1 week of age. Three independent RNAi lines per gene were tested. Combined average data were shown in green. N# = 30-50 females for each independent fly line per time point (N# = 100-150 combined). Bar graphs represent mean and SEM, with significance presented as $^{ns}p > 0.05$, $^{*}p \leq 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

Figure S4

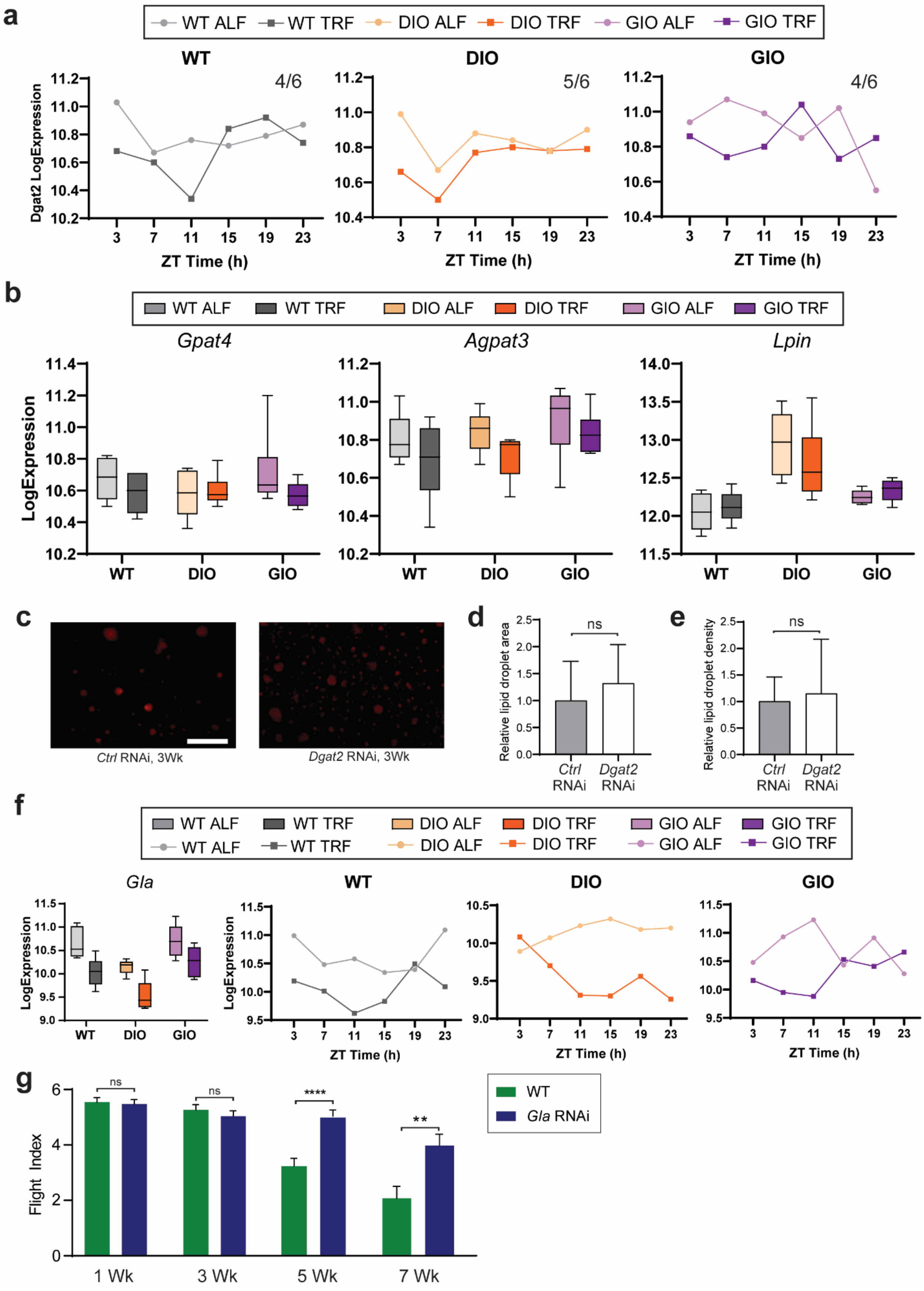
a
WT ALF
WT TRF
DIO ALF
DIO TRF
GIO ALF
GIO TRF
WT
DIO
GIO
4/6
5/6
4/6
Dgat2 LogExpression
11.2
11.0
10.8
10.6
10.4
10.2
3
7
11
15
19
23
ZT Time (h)
b
Gpat4
Agpat3
Lpin
LogExpression
11.4
14.0
13.5
13.0
12.5
12.0
11.5
c
Ctrl RNAi, 3Wk
Dgat2 RNAi, 3Wk
d
Relative lipid droplet area
ns
2.5
2.0
1.5
1.0
0.5
0.0
Ctrl RNAi
Dgat2 RNAi
e
Relative lipid droplet density
f
Gla
LogExpression
11.5
10.5
10.0
9.5
9.0
g
Flight Index
6
4
2
0
1 Wk
3 Wk
5 Wk
7 Wk
ns
****
**
WT
Gla RNAi

Figure S4: Profiles of the significantly downregulated genes across WT, DIO and GIO models

(a) Temporal expressions of *Dgat2* under ALF and TRF in WT, DIO and GIO.
(b) Expression level (normalized read count, log2) of *Gpat4*, *Agpat3*, and *Lpin* from *de novo* triacylglycerol synthesis under ALF and TRF in WT, DIO and GIO.
(c) Fluorescence images of the abdomen from 3-week-old females with IFM-specific *KD* of *Dgat2* upon probing with Nile Red (red puncta). Scale bar is 20 µm.
(d-e) Lipid quantification (size (d) and density (e)) showed no significant differences in lipid droplet area upon IFM-specific *KD* of *Dgat2* compare to age-matched control. *N#* = 9 from three flies' abdomen per genotype.
(f) Expression profiles of *Gla* under ALF and TRF in WT, DIO and GIO.
(g) Flight performance of WT male flies with *Gla* IFM-specific *KD* at 1, 3, 5 and 7 weeks of age. *N#* = 67-99 females for each condition per time point. Bar graphs represent mean and SEM, with significance presented as $^{ns}p > 0.05$, $^{*}p \leq 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

Figure S5

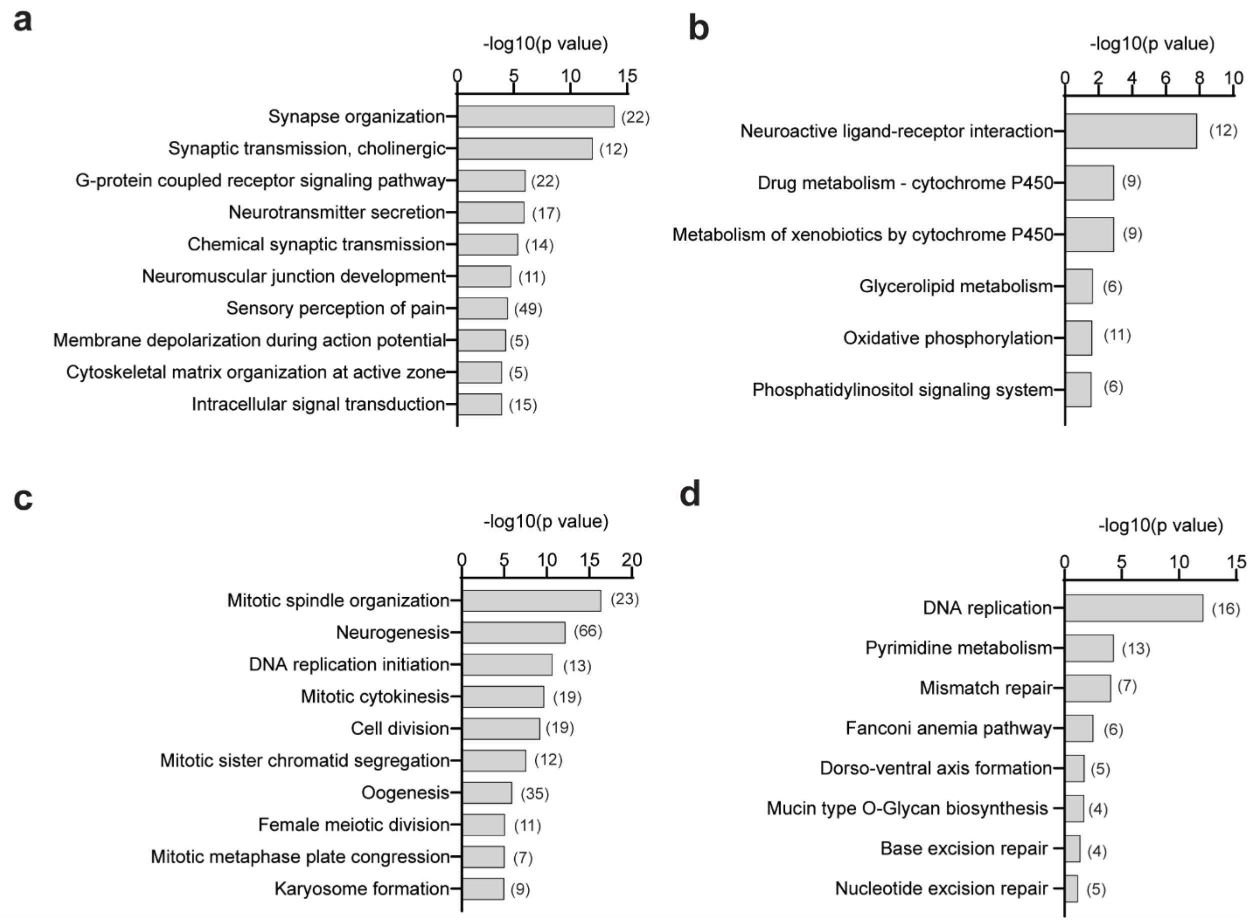

Figure S5: GO and Reactome analyses of significantly downregulated genes under TRF versus ALF in obesity models.

(a) GO analysis and (b) Reactome pathway analysis of 636 genes that were significantly downregulated under TRF in the DIO model. Bar charts represent the - Log10 (*p* value) of each enriched GO term and pathway. The number of genes identified in each GO term and pathway is shown in parenthesis.

(c) GO analysis and (d) Reactome pathway analysis of 579 genes that were significantly downregulated under TRF in the GIO model. Bar charts represent the -Log10 (*p* value) of each enriched GO term and pathway. The number of genes identified in each GO term and pathway is shown in parenthesis.

Figure S6

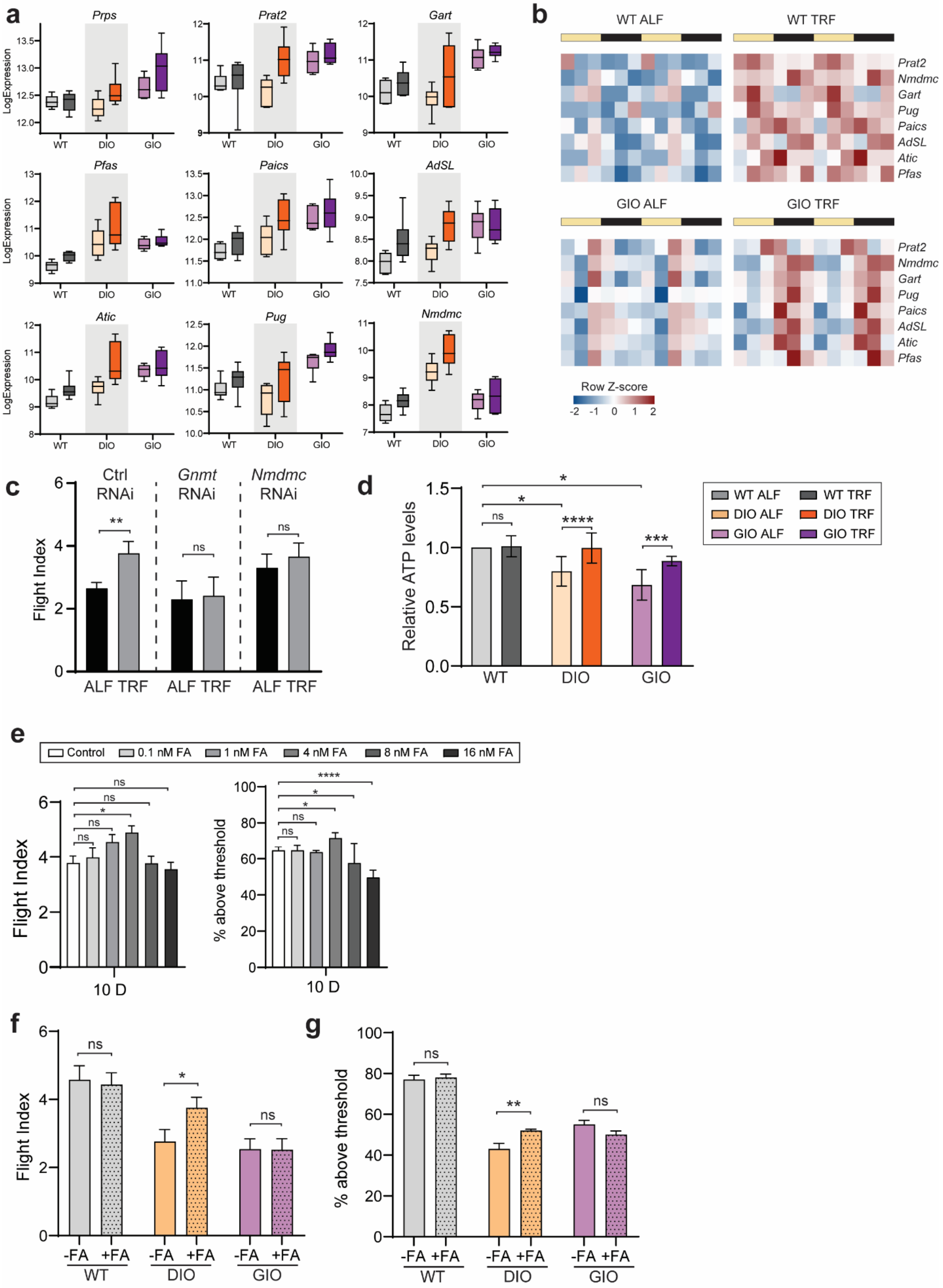
a
Prps
Prat2
Gart
Pfas
Paics
AdSL
Atic
Pug
Nmdmc
LogExpression
WT
DIO
GIO
b
WT ALF
WT TRF
GIO ALF
GIO TRF
Prat2
Nmdmc
Gart
Pug
Paics
AdSL
Atic
Pfas
Row Z-score
-2 -1 0 1 2
c
Ctrl RNAi
Gnmt RNAi
Nmdmc RNAi
Flight Index
**
ns
ns
ALF TRF
ALF TRF
ALF TRF
d
Relative ATP levels
*
*
ns
****
***
WT
DIO
GIO
WT ALF
WT TRF
DIO ALF
DIO TRF
GIO ALF
GIO TRF
e
Control
0.1 nM FA
1 nM FA
4 nM FA
8 nM FA
16 nM FA
Flight Index
% above threshold
ns
*
****
10 D
10 D
f
Flight Index
ns
*
ns
-FA +FA
WT
DIO
GIO
g
% above threshold
ns
**
ns
-FA +FA
WT
DIO
GIO

Figure S6: De novo purine biosynthesis and folate cycle genes under ALF and TRF in WT, DIO and GIO models

(a) Expression levels of genes from Fig. 4d in WT, DIO and GIO

(b) Heatmap representation of genes from Fig. 4e in WT and GIO models.

(c) Flight performance of 3-week-old female flies with IFM-specific *KD* of *Gnmt* and *Nmdmc.* N# = 30-50 females for each condition. Bar graphs represent mean and SEM, with significance presented as $^{ns}p > 0.05$, $^{*}p \leq 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

(d) Relative ATP levels of 3-week-old WT, DIO and GIO flies under ALF and TRF with *N#* = 4.

(e) Flight performance of 10--day-old (left) and climbing performance of 10-day-old (right) DIO-ALF female flies with supplement of 0.1 mM, 1 mM, 4 mM, 8 mM, and 16 mM folic acid.

(f) Flight performance of 3-week-old WT, DIO and GIO female flies with or without folic acid supplement. *N#* = 64-111. Bar graphs represent mean and SEM, with significance presented as $^{ns}p > 0.05$, $^{*}p \leq 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

(g) Climbing performance of 3-week-old WT, DIO and GIO female flies with or without folic acid supplement. *N#* = 60-70. Bar graphs represent mean and SEM, with significance presented as $^{ns}p > 0.05$, $^{*}p \leq 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

Figure S7

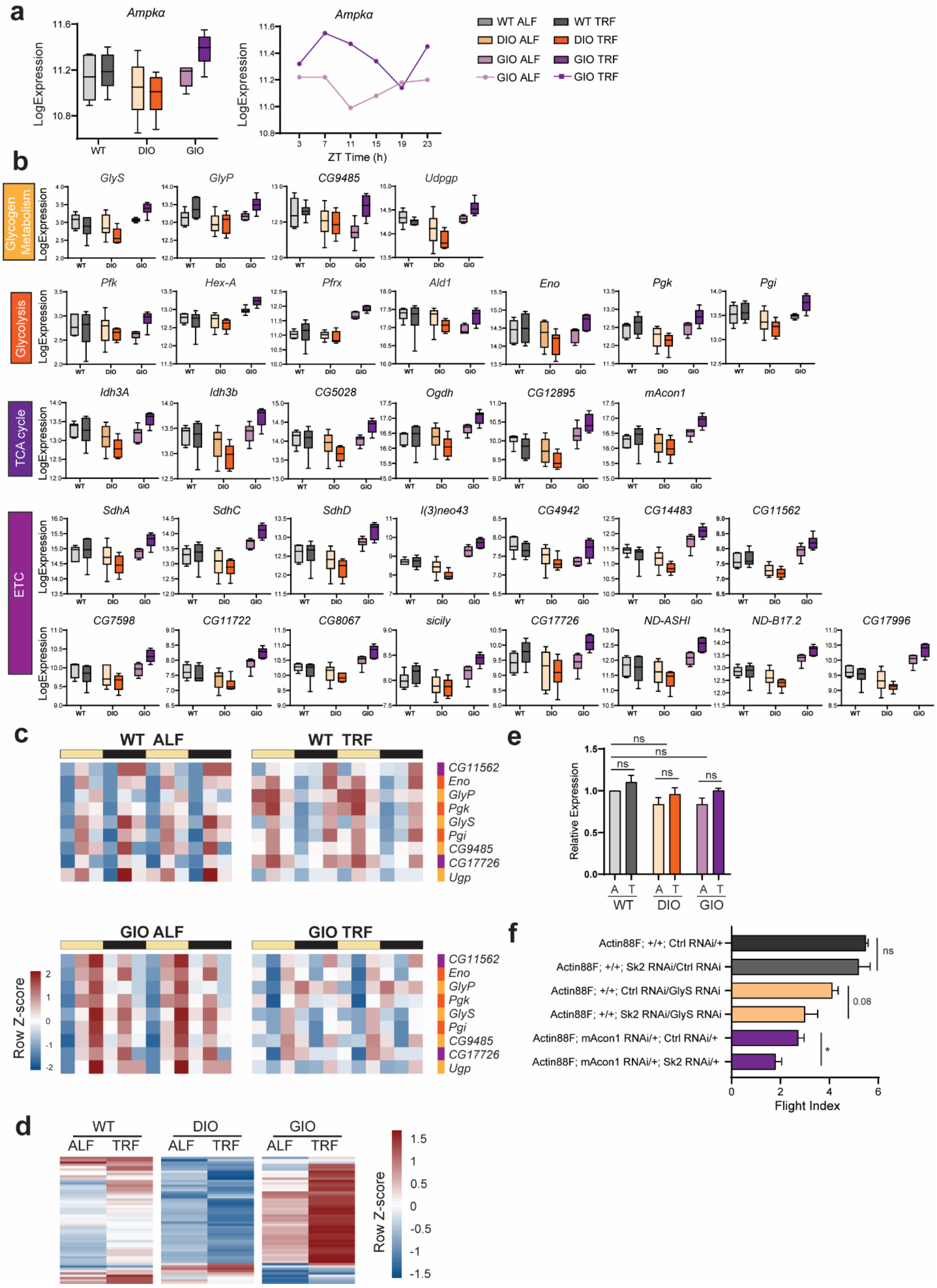
a
Ampkα
LogExpression
11.6
11.2
10.8
WT
DIO
GIO
Ampkα
11.6
11.4
11.2
11.0
10.8
3
7
11
15
19
23
ZT Time (h)
WT ALF
WT TRF
DIO ALF
DIO TRF
GIO ALF
GIO TRF
GIO ALF
GIO TRF
b
Glycogen Metabolism
GlyS
GlyP
CG9485
Udpgp
Glycolysis
Pfk
Hex-A
Pfrx
Ald1
Eno
Pgk
Pgi
TCA cycle
Idh3A
Idh3b
CG5028
Ogdh
CG12895
mAcon1
ETC
SdhA
SdhC
SdhD
l(3)neo43
CG4942
CG14483
CG11562
CG7598
CG11722
CG8067
sicily
CG17726
ND-ASHI
ND-B17.2
CG17996
LogExpression
WT
DIO
GIO
c
WT ALF
WT TRF
GIO ALF
GIO TRF
CG11562
Eno
GlyP
Pgk
GlyS
Pgi
CG9485
CG17726
Ugp
Row Z-score
2
1
0
-1
-2
d
WT
DIO
GIO
ALF
TRF
ALF
TRF
ALF
TRF
Row Z-score
1.5
1
0.5
0
-0.5
-1
-1.5
e
Relative Expression
1.5
1.0
0.5
0.0
ns
ns
ns
ns
ns
A T
A T
A T
WT
DIO
GIO
f
Actin88F; +/+; Ctrl RNAi/+
Actin88F; +/+; Sk2 RNAi/Ctrl RNAi
Actin88F; +/+; Ctrl RNAi/GlyS RNAi
Actin88F; +/+; Sk2 RNAi/GlyS RNAi
Actin88F; mAcon1 RNAi/+; Ctrl RNAi/+
Actin88F; mAcon1 RNAi/+; Sk2 RNAi/+
ns
0.08
*
0
2
4
6
Flight Index

Figure S7: Glycolysis, glycogen metabolism, TCA cycle and ETC genes under ALF and TRF in WT, DIO and GIO models

(a) Expression of *Ampkα* mRNA levels in WT, DIO and GIO models

(b) Expression levels of genes from Fig. 5d in WT, DIO and GIO

(c) Heatmap representation of the expression levels of purine biosynthesis associated genes in ALF and TRF in the WT and GIO model.

(d) Heatmap representation of the average expression levels of genes related to glycolysis, glycogen metabolism, TCA cycle, and ETC with increased but not significant expression under TRF versus ALF in the GIO model.

(e) Quantification of relative expression of western blot of AMPKα protein levels of WT, DIO and GIO under ALF and TRF, with *N#* = 3.

(f) Flight index of 3-week-old flies with indicated genotypes.

Figure S8

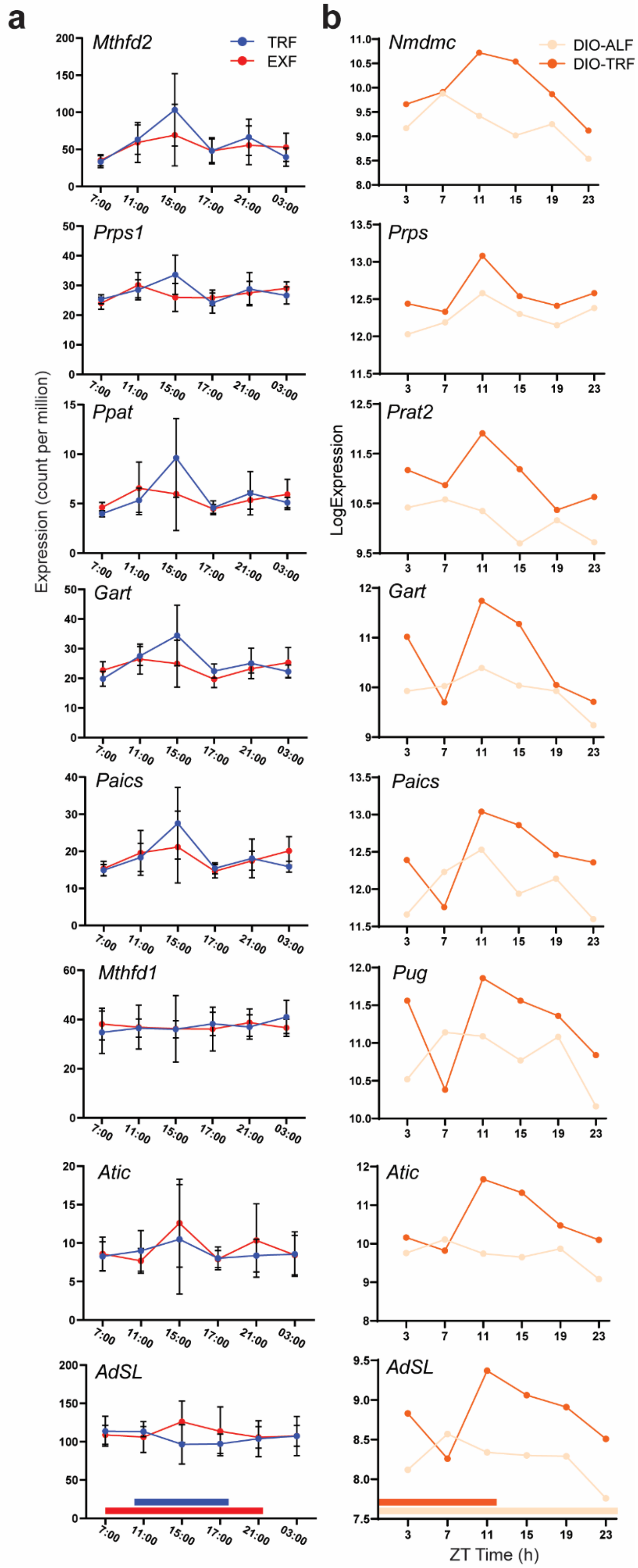
a
b
Mthfd2
Prps1
Ppat
Gart
Paics
Mthfd1
Atic
AdSL
TRF
EXF
Expression (count per million)
7:00
11:00
15:00
17:00
21:00
03:00
Nmdmc
Prps
Prat2
Gart
Paics
Pug
Atic
AdSL
DIO-ALF
DIO-TRF
LogExpression
3
7
11
15
19
23
ZT Time (h)

Figure S8: Comparison with skeletal muscle transcriptomic data from obese men under TRF.
(a) Expression levels of genes associated with purine synthesis and folate cycle from Lundell et al., 2020 RNA-seq dataset under EXF and TRF.
(b) Expression levels of the same genes from (a) in our Drosophila RNA-seq dataset under ALF and TRF.