Science Advances
AAAS

Supplementary Materials for

Adipose tissue coregulates cognitive function

Núria Oliveras-Cañellas *et al.*

Corresponding author: Jordi Mayneris-Perxachs, jmayneris@idibgi.org;
José Manuel Fernández-Real, jmfreal@idibgi.org

Sci. Adv. **9**, eadg4017 (2023)
DOI: 10.1126/sciadv.adg4017

The PDF file includes:

Figs. S1 to S7
Legends for tables S1 to S51

Other Supplementary Material for this manuscript includes the following:

Tables S1 to S51

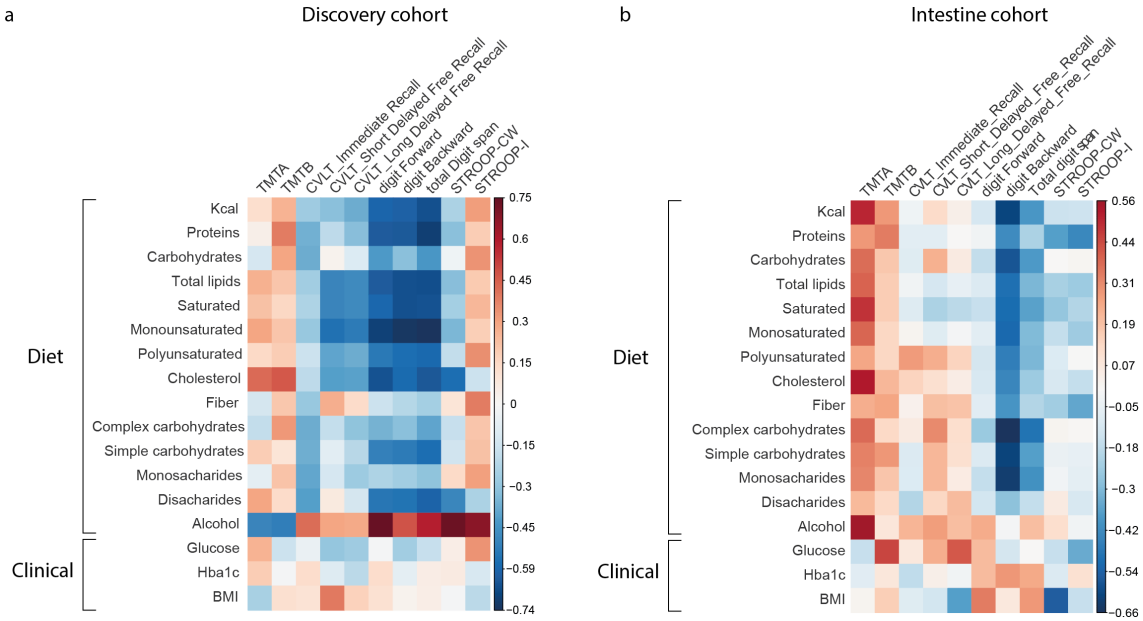

Figure Supplemental 1. Associations between dietary, clinical parameters and cognitive test. Heat map of Spearman's correlations among dietary and clinical parameters and cognitive test after correcting for multiple comparisons (FDR) **a)** in the discovery cohort and **b)** in the intestine cohort.

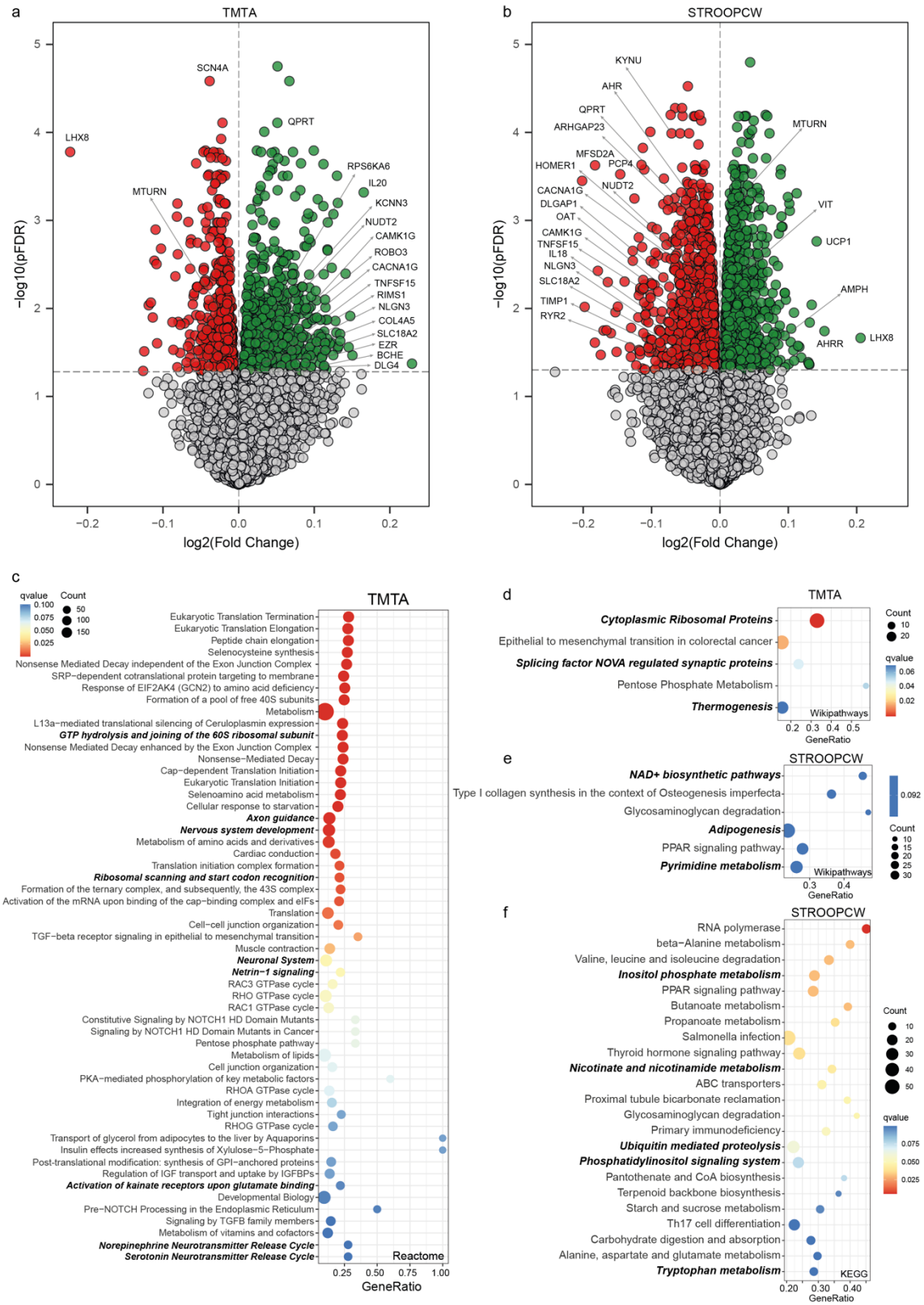

Figure Supplemental 2. Associations of visceral adipose tissue gene expression and other cognitive domains in the discovery cohort. Volcano plots of differentially expressed genes in the visceral adipose tissue associated with **a)** the Trail Making Test

Part A (TMTA), **b)** THE STROOP Colour Word test (STROOPCW) scores in discovery cohort (IRONMET, *n*=17) identified by limma-voom analysis controlling for age, BMI, sex, and education years. The log2 fold change associated with a unit change in the cognitive test score and the log10 *p*-values adjusted for multiple testing (pFDR) are plotted for each gene. Differentially expressed genes (pFDR<0.05) are coloured in red and green indicating down- and up-regulation, respectively. **c)** Dot plot of pathways significantly associated (qvalue<0.1) with the TMTA in the visceral adipose tissue identified from a pathway over-representation analysis mapping significant genes to the Reactome database and **d)** the Wikipathways database. **e)** Dot plot of pathways significantly associated (qvalue<0.1) with the STROOPCW test in the visceral adipose tissue identified from a pathway over-representation analysis mapping significant genes to the Wikipathways database and **f)** the KEGG database. The x-axis in the dot plots and the bubble size in the Manhattan-like plots represents the ratio of input genes that are annotated in a pathway (GeneRatio). Dots are coloured by the qvalue.

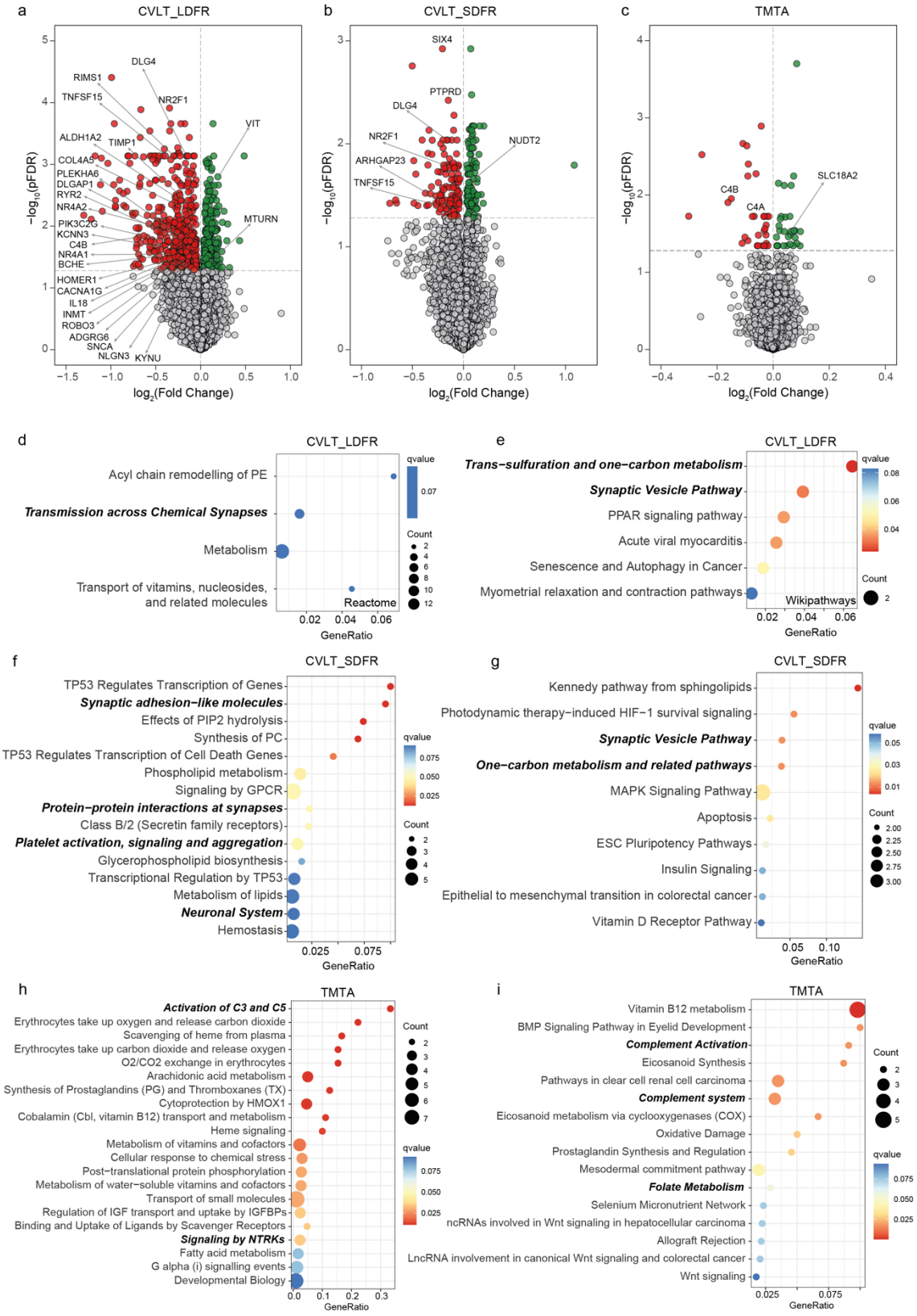

Figure Supplemental 3. Longitudinal associations of subcutaneous adipose tissue (SAT) gene expression at baseline and the scores in different cognitive domains later

in life. Volcano plots of differentially expressed genes in the SAT at baseline associated with **a)** the California Verbal Learning Test Long Delayed Free Recall (CVLT_LDFR), **b)** the California Verbal Learning Test Short Delayed Free Recall (CVLT_SDFR), and **c)** the Trail Making Test part A (TMTA) scores two to three years later in the validation cohort (INTESTINE, *n*=22) identified by limma-voom analysis controlling for age, BMI, sex, and education years. The log2 fold change associated with a unit change in the cognitive test score and the log10 *p*-values adjusted for multiple testing (pFDR) are plotted for each gene. Differentially expressed genes (pFDR<0.05) are coloured in red and green indicating down- and up-regulation, respectively. **d)** Dot plot of pathways significantly associated (qvalue<0.1) with the CVLT_LDFR in the SAT identified from a pathway over-representation analysis mapping significant genes to the Reactome and **e)** Wikipathways databases. **f)** Dot plot of pathways significantly associated (qvalue<0.1) with the CVLT_SDFR in the SAT identified from a pathway over-representation analysis mapping significant genes to the Reactome and **g)** Wikipathways databases. **h)** Dot plot of pathways significantly associated (qvalue<0.1) with the TMTA in the SAT identified from a pathway over-representation analysis mapping significant genes to the Reactome and **i)** Wikipathways databases. The x-axis in the dot plots represents the ratio of input genes that are annotated in a pathway (GeneRatio). Dots are coloured by the qvalue.

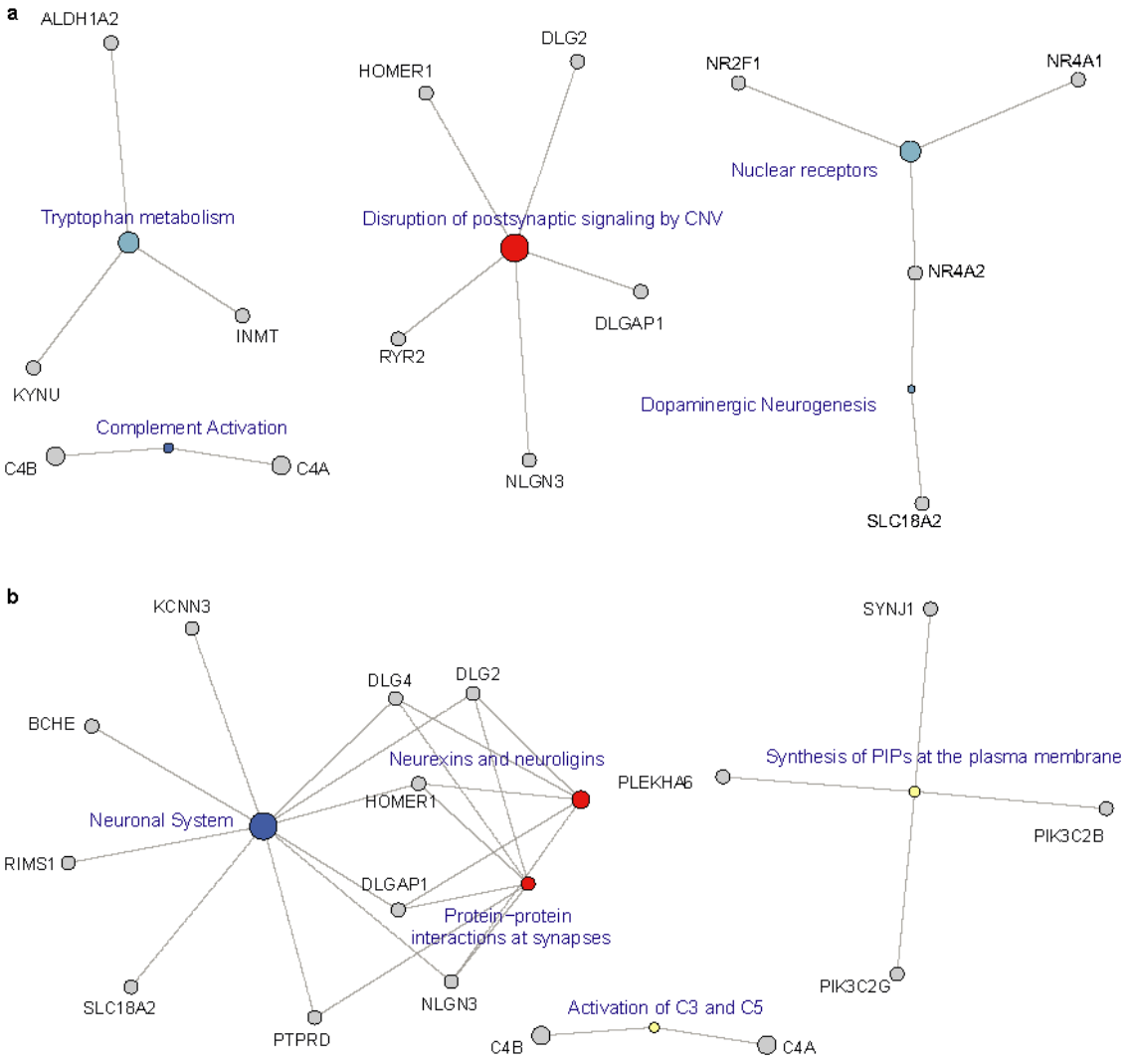

Figure Supplemental 4. Gen-concept networks depicting pathways and genes with key roles in synaptic function a) Gene-concept network depicting significant genes involved in selected enriched pathways from the Wikipathways and b**)** Reactome databases.

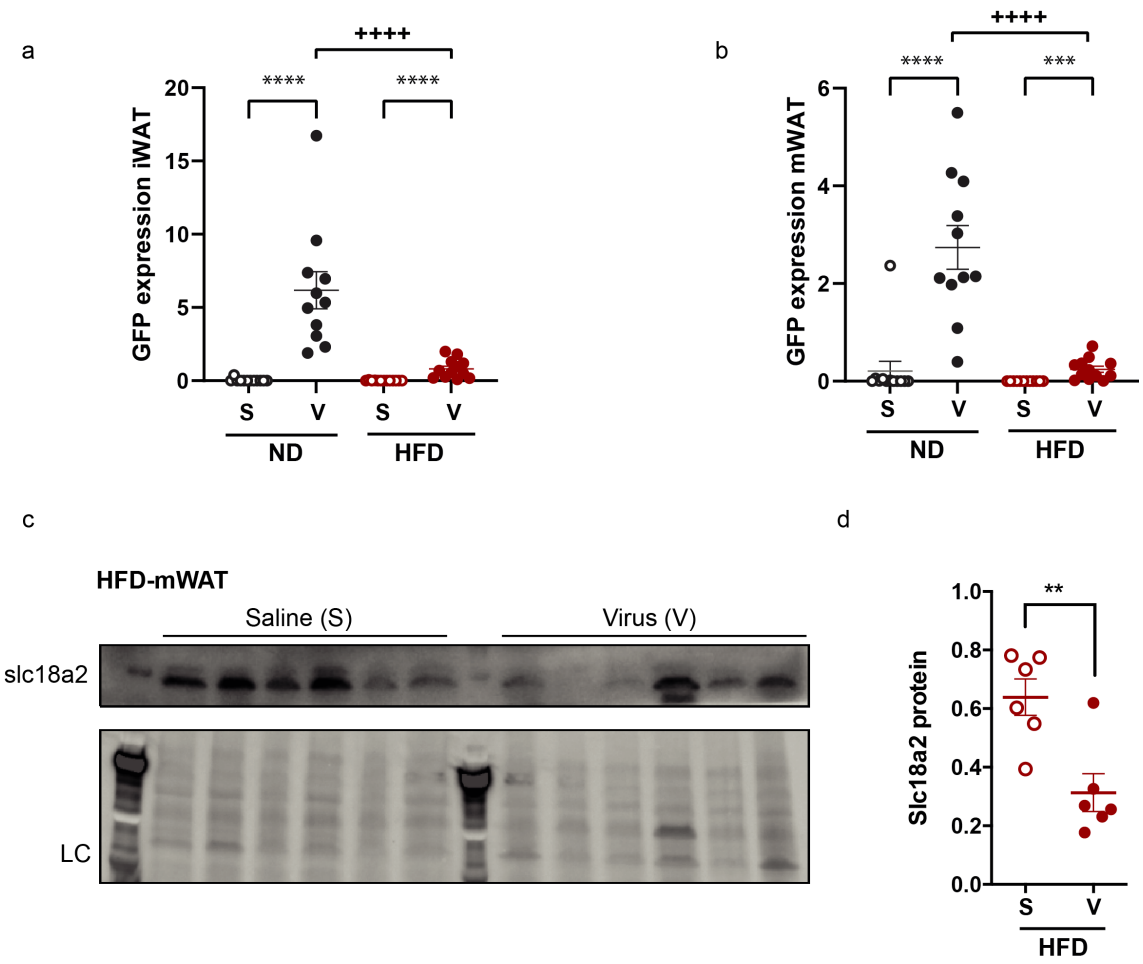

Figure Supplemental 5. Validation of downregulation of *slc18a2* in adipose tissue. a) Expression of green fluorescence protein (GFP) in the inguinal white adipose tissue (iWAT) and **b)** the mesenteric white adipose tissue (mWAT). **c)** Western-Blot for the protein levels of slc18a2 in the mWAT of mice fed a HFD. **d)** slc18a2 protein levels of the groups fed a HFD. Data is shown as dots with the mean ± SEM; n=12 normal diet + saline (ND-S), n=11 normal diet + virus (ND-V), n=13 high fat diet + saline (HFD-S), n=12 high fat diet + virus (HFD-V). ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$ for the comparison S *vs* V; $^{++++}$ $P < 0.0001$ diet effect. Calculated with two-way ANOVA.

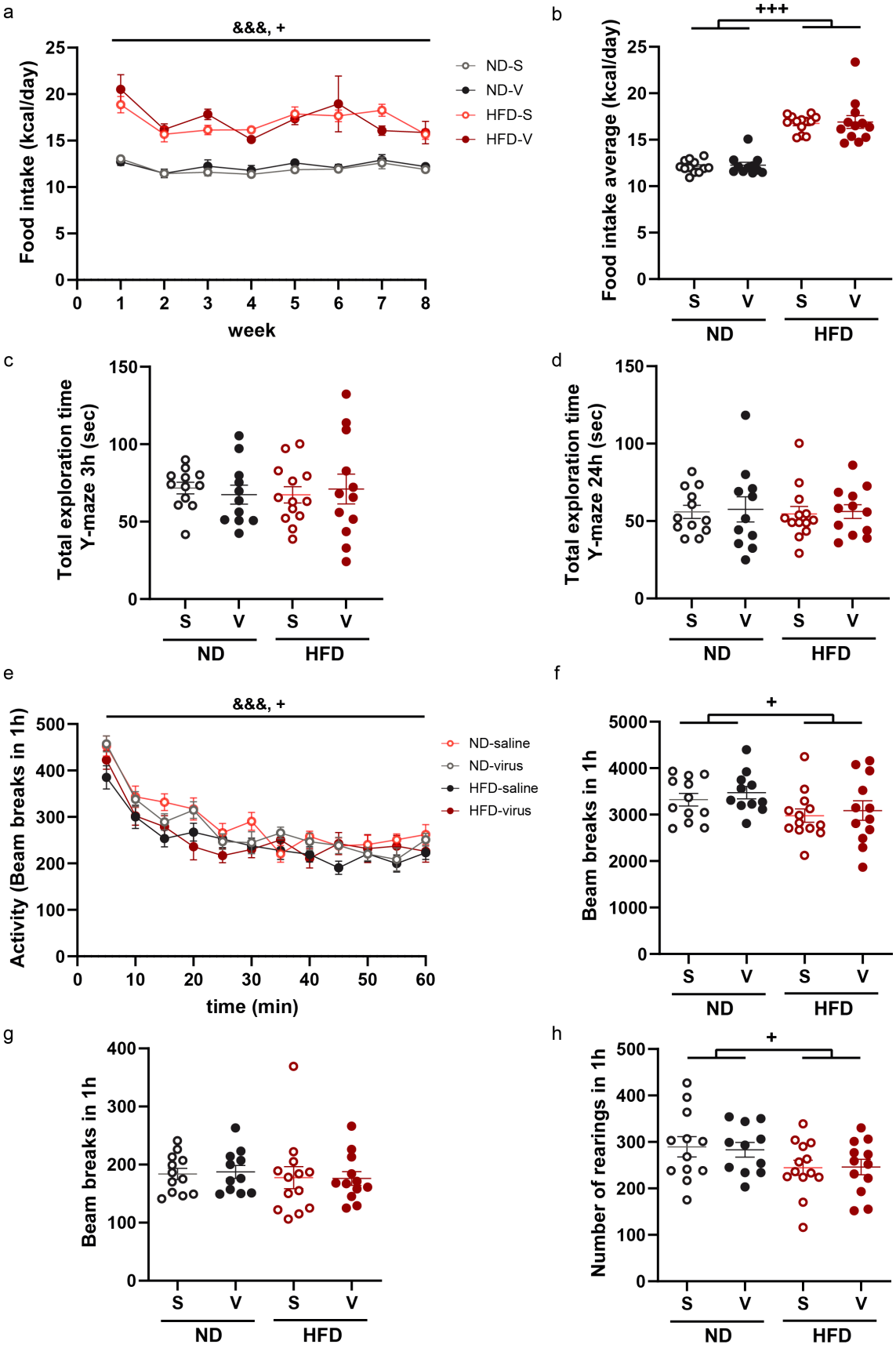
a
&&&, +
Food intake (kcal/day)
week
ND-S
ND-V
HFD-S
HFD-V
b
+++
Food intake average (kcal/day)
S
V
S
V
ND
HFD
c
Total exploration time Y-maze 3h (sec)
S
V
S
V
ND
HFD
d
Total exploration time Y-maze 24h (sec)
S
V
S
V
ND
HFD
e
&&&, +
Activity (Beam breaks in 1h)
time (min)
ND-saline
ND-virus
HFD-saline
HFD-virus
f
+
Beam breaks in 1h
S
V
S
V
ND
HFD
g
Beam breaks in 1h
S
V
S
V
ND
HFD
h
+
Number of rearings in 1h
S
V
S
V
ND
HFD

Figure Supplemental 6. Effect of diet and *slc18a2* downregulation in the adipose tissue of mice on food intake, exploration time and locomotion. a) Food intake (kcal/day) was monitored weekly during the entire protocol (8 weeks). **b)** Average of food intake in kcal/day. **c,d)** Exploration time on the short-term (3h) and long-term (24h) novel object-recognition tests. **e)** Kinetics of total activity measured as beam breaks in activity chambers for 1 hour. **f)** Total horizontal activity, **g)** back or forth movements, and **h)** rearings measured in 1 hour. In **a**,**e** individual data is shown as the mean ± SEM and in **b-d,f-h** as dots with the mean ± SEM; .n=12 normal diet + saline (ND-S), n=11 normal diet + virus (ND-V), n=13 high fat diet + saline (HFD-S), n=12 high fat diet + virus (HFD-V). $^{\&\&\&}$ $P < 0.001$ week effect; **** $P < 0.0001$ ND-S *vs* ND-V or HFD-S *vs* HFD-V; $^{+}$ $P < 0.05$, $^{+++}$ $P < 0.001$ diet effect; $ $P < 0.05$ treatment effect; $^{@@@}$ $P < 0.001$ week x diet interaction; $^{\wedge\wedge\wedge}$ $P < 0.001$ week x treatment interaction; **a,e** calculated with three- or **b-d,f-h** two-way ANOVA.

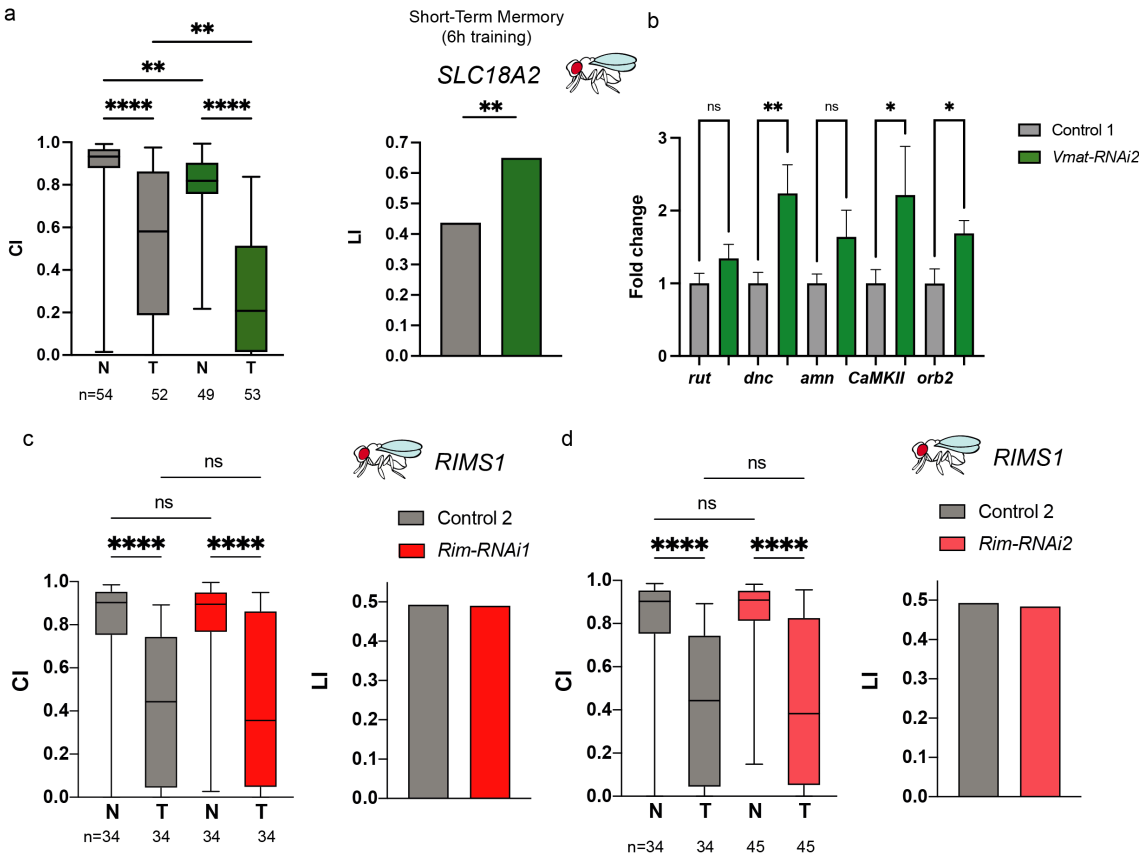

Figure Supplemental 7. Courtship and learning indexes of flies of with SLC18A2 and Rim knockdown in fat body. a) *Vmat* downregulation in the *Drosophila* fat body and associations with short-term memory. Results display male short-term memory in the courtship conditioning paradigm performed 6h after training and 1 hour of isolation. Control-1 (*w; C7*-GAL4/+) and *Vmat-RNAi2* fat body-specific knockdown flies (*w; C7*-GAL4/+*; UAS-Dcr-2/ Vmat -RNAi2*). **b)** Relative gene expression assessed by qRT-PCR of *rutabaga(rut), dunce (dnc), amnesiac (amn), homer, CAMKll,* and *orb2* in fly brains of *UAS-Rim* fat body-specific overexpression flies and their corresponding genetic background control. Error bars represent normalized S.E.M. *P*-values were determined using the *t*-test (* $P<0.05$, ** $P<0.01$, *** $P<0.001$, **** $P<0.0001$). Data are derived from a minimum of five biological and two technical replicates. **c,d)** *Rim* downregulation in the *Drosophila* fat body and associations with learning. Results display male learning in the courtship conditioning paradigm performed immediately after 2.5h training.

Control-1 and 2 (*w; C7*-GAL4/+*; UAS-Dcr-2/*+) and *Rim-RNAi1 and Rim-RNAi2* fat body-specific knockdown flies (*w; C7*-GAL4/*Rim-RNAi1; UAS-Dcr-2/*+ and *w; C7*-GAL4/+*; UAS-Dcr-2/ Rim-RNAi2*). Error bars represent normalized S.E.M. *P*-values were determined using the *t*-test (* $P<0.05$, ** $P<0.01$, *** $P<0.001$, **** $P<0.0001$). Data are derived from a minimum of five biological and two technical replicates.

Table S1. General lineal model, controlling by age, sex and years of education (ANCOVA) depicting the efects of physical exercise in the 10 cognitive test

Table S2. Cohorts clinical and neuropsychological characteristics.

Table S3. Gene transcripts associated with the **CVLT Immediate Recall** fitting a robust linear regression model controlling for age, BMI, sex and education years in the **Discovery cohort (IRONMET, n=17)**

Table S4. Gene transcripts associated with the **CVLT Long Delayed Free Recall** fitting a robust linear regression model controlling for age, BMI, sex and education years in the Discovery cohort (IRONMET, n=17)

Table S5. Gene transcripts associated with the **CVLT Short Delayed Free Recall** fitting a robust linear regression model controlling for age, BMI, sex and education years in the Discovery cohort (IRONMET, n=17)

Table S6. Gene transcripts associated with the **Backaward Digit Span** fitting a robust linear regression model controlling for age, BMI, sex and education years in the Discovery cohort (IRONMET, n=17)

Table S7. Gene transcripts associated with the **Forward Digit Span** fitting a robust linear regression model controlling for age, BMI, sex and education years in the Discovery cohort (IRONMET, n=17)

Table S8. Gene transcripts associated with the **Total Digit Span** fitting a robust linear regression model controlling for age, BMI, sex and education years in the Discovery cohort (IRONMET, n=17)

Table S9. Gene transcripts associated with the **STROOPCW** fitting a robust linear regression model controlling for age, BMI, sex and education years in the Discovery cohort (IRONMET, n=17)

Table S10. Gene transcripts associated with the **STROOPI** fitting a robust linear regression model controlling for age, BMI, sex and education years in the Discovery cohort (IRONMET, n=17)

Table S11. Gene transcripts associated with the **TMTA** fitting a robust linear regression model controlling for age, BMI, sex and education years in the Discovery cohort (IRONMET, n=17)

Table S12. Gene transcripts associated with the **TMTB** fitting a robust linear regression model controlling for age, BMI, sex and education years in the Discovery cohort (IRONMET, n=17)

Table S13. Over-representation analysis (**REACTOME**) of the **VAT genes** significantlly associated with the **CVLT Immediate Recall** in the **discovery cohort (IRONMET, n=17)**

Table S14. Over-representation analysis (**REACTOME, WIKIPATHWAYS**) of the **VAT genes** significantlly associated with the **TMTB** in the **discovery cohort (IRONMET, n=17)**

Table S15. Over-representation analysis (**REACTOME, WIKIPATHWAYS**) of the **VAT genes** significantlly associated with the **STROOPI** in the **discovery cohort (IRONMET, n=17)**

Table S16. Over-representation analysis (**REACTOME, WIKIPATHWAYS**) of the **VAT genes** significantlly associated with the **TMTA** in the **discovery cohort (IRONMET, n=17)**

Table S17. Over-representation analysis (**WIKIPATHWAYS, KEGG**) of the **VAT genes** significantlly associated with the **STROOPCW** in the **discovery cohort (IRONMET, n=17)**

Table S18. VAT Gene transcripts associated with the **CVLT Immediate Recall** fitting a robust linear regression model controlling for age, BMI, sex and education years in the **Validation cohort (INTESTINE, n=22)**

Table S19. VAT Gene transcripts associated with the **CVLT Long Delayed Free Recall** fitting a robust linear regression model controlling for age, BMI, sex and education years in the **Validation cohort (INTESTINE, n=22)**

Table S20. VAT Gene transcripts associated with the **CVLT Short Delayed Free Recall** fitting a robust linear regression model controlling for age, BMI, sex and education years in the **Validation cohort (INTESTINE, n=22)**

Table S21. VAT Gene transcripts associated with the **Backward Digit Span** fitting a robust linear regression model controlling for age, BMI, sex and education years in the **Validation cohort (INTESTINE, n=22)**

Table S22. VAT Gene transcripts associated with the **Forward Digit Span** fitting a robust linear regression model controlling for age, BMI, sex and education years in the **Validation cohort (INTESTINE, n=22)**

Table S23. VAT Gene transcripts associated with the **Total Digit Span** fitting a robust linear regression model controlling for age, BMI, sex and education years in the **Validation cohort (INTESTINE, n=22)**

Table S24. VAT Gene transcripts associated with the **STROOPCW** fitting a robust linear regression model controlling for age, BMI, sex and education years in the **Validation cohort (INTESTINE, n=22)**

Table S25. VAT Gene transcripts associated with the **STROOPI** fitting a robust linear regression model controlling for age, BMI, sex and education years in the **Validation cohort (INTESTINE, n=22)**

Table S26. VAT Gene transcripts associated with the **TMTA** fitting a robust linear regression model controlling for age, BMI, sex and education years in the **Validation cohort (INTESTINE, n=22)**

Table S27. VAT Gene transcripts associated with the **TMTB** fitting a robust linear regression model controlling for age, BMI, sex and education years in the **Validation cohort (INTESTINE, n=22)**

Table S28. SAT Gene transcripts associated with the **CVLT Immediate Recall** fitting a robust linear regression model controlling for age, BMI, sex and education years in the **Validation cohort (INTESTINE, n=22)**

Table S29. SAT Gene transcripts associated with the **CVLT Long Delayed Free Recall** fitting a robust linear regression model controlling for age, BMI, sex and education years in the **Validation cohort (INTESTINE, n=22)**

Table S30. SAT Gene transcripts associated with the **CVLT Short Delayed Free Recall** fitting a robust linear regression model controlling for age, BMI, sex and education years in the **Validation cohort (INTESTINE, n=22)**

Table S31. SAT Gene transcripts associated with the **Forward Digit Span** fitting a robust linear regression model controlling for age, BMI, sex and education years in the **Validation cohort (INTESTINE, n=22)**

Table S32. SAT Gene transcripts associated with the **Backward Digit Span** fitting a robust linear regression model controlling for age, BMI, sex and education years in the **Validation cohort (INTESTINE, n=22)**

Table S33. SAT Gene transcripts associated with the **Total Digit Span** fitting a robust linear regression model controlling for age, BMI, sex and education years in the **Validation cohort (INTESTINE, n=22)**

Table S34. SAT Gene transcripts associated with the **STROOPCW** fitting a robust linear regression model controlling for age, BMI, sex and education years in the **Validation cohort (INTESTINE, n=22)**

Table S35. SAT Gene transcripts associated with the **STROOPI** fitting a robust linear regression model controlling for age, BMI, sex and education years in the **Validation cohort (INTESTINE, n=22)**

Table S36. SAT Gene transcripts associated with the**TMTA** fitting a robust linear regression model controlling for age, BMI, sex and education years in the **Validation cohort (INTESTINE, n=22)**

Table S37. SAT Gene transcripts associated with the**TMTB** fitting a robust linear regression model controlling for age, BMI, sex and education years in the **Validation cohort (INTESTINE, n=22)**

Table S38. Over-representation analysis (**REACTOME,WKIPATHWAYS**) of the **VAT genes** significantlly associated with the **TMTA** in the **validation cohort (INTESTINTE, n=22)**

Table S39. Over-representation analysis (**REACTOME**) of the **VAT genes** significantlly associated with the Digit Forwards Span in the **validation cohort (INTESTINTE, n=22)**

Table S40. Over-representation analysis (**REACTOME**) of the **VAT genes** significantlly associated with the STROOPCW in the **validation cohort (INTESTINTE, n=22)**

Table S41. Over-representation analysis (**REACTOME, WIKIPATHWAYS**) of the S**AT genes** significantlly associated with the CVLT Long Delayed Free Recall in the **validation cohort (INTESTINTE, n=22)**

Table S42. Over-representation analysis (**REACTOME, WIKIPATHWAYS**) of the S**AT genes** significantlly associated with the CVLT Short Delayed Free Recall in the **validation cohort (INTESTINTE, n=22)**

Table S43. Over-representation analysis (**REACTOME, WIKIPATHWAYS**) of the S**AT genes** significantlly associated with the TMTA in the **validation cohort (INTESTINTE, n=22)**

Table S44. Genes form the Veen Diagram associated at least with one cognitive test.

Table S45. Over-representation analysis (**REACTOME, WIKIPATHWAYS**) of the **common significant genes (n=188)** assocaited with at least one tests in the **VAT and SAT from the discovery (IRONMET, n=17) and validation cohort (INTESTINTE, n=22).**

Table S46. Results from network-oriented over-representation analysis after hierarchial clustering of redundant terms

Table S47. Resume results of learning capabilities assessed with the courtship conditioning paradigme

Table S48. FAT BODY Gene transcripts for the comparison CONTROL vs RIM overexpression in Drosophila fat body

Table S49. BRAIN Gene transcripts for the comparison CONTROL vs RIM overexpression in Drosophila heats

Table S50. Over-representation analysis (**REACTOME**) of differentially expressed BRAIN genes for the comparison CONTROL vs RIM overexpression in Drosophila fat body

Table S51. Primer sequences for the Drosophila qRT-PCR analyses