Modulation of sleep by trafficking of lipids through the Drosophila blood brain barrier

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Abstract

Endocytosis through Drosophila glia is a significant determinant of sleep amount and occurs preferentially during sleep in glia of the blood brain barrier (BBB). To identify metabolites whose trafficking is mediated by sleep-dependent endocytosis, we conducted metabolomic analysis of flies that have increased sleep due to a block in glial endocytosis. We report that acylcarnitines, molecules that conjugate with long chain fatty acids to promote their transport, accumulate in heads of these animals. In parallel, to identify transporters and receptors whose loss contributes to the sleep phenotype caused by blocked endocytosis, we screened genes enriched in barrier glia for effects on sleep. We find that knockdown of lipid transporters *LRP1&2* as well as carnitine transporters *ORCT1&2* increases sleep. In support of the idea that the block in endocytosis affects trafficking through specific transporters, knockdown of LRP or *ORCT* transporters also increases acylcarnitines in heads. We propose that lipid species, such as acylcarnitines, are trafficked through the BBB via sleep-dependent endocytosis, and their accumulation in the brain increases the need for sleep.

Introduction

Although the regulation of sleep is normally studied on a behavioral and circuit level, there is increasing evidence for a role of basic cellular physiology. For instance, we found that disruption of endocytic and trafficking pathways in glia increases sleep in Drosophila (Artiushin, Zhang et al. 2018). Glia of the Drosophila blood/hemolymph brain barrier (BBB) emerged as a new cellular locus of sleep regulation in this study, such that genetically manipulating endocytosis in these cells alone was sufficient to increase sleep. As the increased sleep appeared to reflect higher sleep need, we asked if sleep is typically required for endocytosis through the BBB and found that this was indeed the case. However, the nature of the molecules trafficked through the BBB in a sleep-dependent manner was not known.

Barriers that separate the solutes of blood/hemolymph of the periphery from the interstitial fluid of the central nervous system display a rich profile of transporters, receptors, and trafficking proteins, which often reflect their unique functions. The *Drosophila* barrier glia populations share many conserved features with vertebrate barriers which employ endothelial and astrocytic populations (DeSalvo, Hindle et al. 2014, Weiler, Volkenhoff et al. 2017). For instance, both are capable of moving lipids and carbohydrates, ions, amino

acids and xenobiotics (Weiler, Volkenhoff et al. 2017). Furthermore, the fly barrier populations may serve specialized roles in metabolism, as not only the conduit of energy sources from the periphery, but also by containing the enzymatic machinery necessary for processing energy sources (Volkenhoff, Weiler et al. 2015), and secreting signals in reference to nutritional state (Chell and Brand 2010, Speder and Brand 2014).

Given that much of the traffic through the barrier involves energetic substrates, we conducted metabolomic profiling to identify candidate metabolites whose trafficking may be inhibited by an endocytosis block in glia. To complement this approach, we asked if specific glial proteins mediate this trafficking. As genetic manipulations to block endocytosis can directly impact endocytosis-dependent carrier traffic as well as indirectly affect levels of membrane-associated transporters/receptors by altered recycling, we performed a knockdown screen to broadly search for barrier genes involved in sleep regulation. We report here that specific lipid and carnitine transporters act in barrier glia to affect sleep, and that disrupting expression of these transporters or of endocytosis leads to an accumulation of acylcarnitines in the head.

Results

Expression of *Shi* in glia induces acylcarnitine accumulation in fly heads

Our previous work showed that expression of *shibire (shi*), a dominant negative dynamin that blocks endocytosis, in all or BBB glia increases sleep. To attain an unbiased, global assessment of metabolites that may be relevant to the increased sleep seen in *Repo>20xShi.ts1* flies (hereafter referred to as *Repo>Shi*^{1}), we conducted LC-MS analysis. Heads of male and female *Repo-Gal4 > Shi*^{1} flies as well as Gal4 and UAS controls were collected on dry ice with sieves and immediately frozen at -80 ℃. Each sample contained 200 fly heads (equal male and female), with a total of 5 samples per genetic condition.

As an initial analysis, raw signal was scaled per each metabolite in reference to other samples within the dataset, and comparisons were made between Repo>Shi flies and each control, as well as controls to each other by Welch's t-test (**Supplementary Table 1**).

Sub pathway	Biochemical name	$\frac{Gal4 > UAS}{Gal4Ctrl}$	$\frac{Gal4 > UAS}{UASCtrl}$	$\frac{UASCtrl}{Gal4Ctrl}$
Fatty Acid Metabolism (Acylcarnitine)	acetylcarnitine (C2)	4.25	2.68	1.59
	myristoylcarnitine (C14)	28.81	26.39	1.09
	palmitoylcarnitine (C16)	4.52	1.74	2.60
	palmitoleoycarnitine (C16:1)	26.50	10.35	2.56
	stearoylcarnitine (C18)	1.43	1.06	1.34
	linoleoylcarnitine (C18:2)	6.77	3.14	2.15
	oleoylcarnitine (C18:1)	4.00	1.92	2.08
	arachidoylcarnitine (C20)	1.28	1.17	1.09
	behenoylcarnitine (C22)	1.31	1.09	1.20
	eicosenoylcarnitine (C20:1)	2.28	1.24	1.84
	lignoceroylcarnitine (C24)	0.72	0.83	0.87
	margaroylcarnitine (C17)	4.05	1.47	2.76
	nervonoylcarnitine (C24:1)	2.36	1.26	1.87
	cerotoylcartinine (C26)	0.85	0.97	0.87
	ximenoylcarnitine (C26:1)	0.75	0.88	0.85

Table 1. Fatty acid acylcarnitine accumulation in *Repo>Shi^{1}* fly heads

All samples from *Repo-GAL4>UAS-Shi^{1}*, and both parental controls. Welch's t-test was performed on scaled signal for each metabolite, comparing the conditions shown. Green highlighting marks a significant difference ($p \leq 0.05$) between the groups, where metabolite ratio is < 1.00, while light green is not significant, but close to the threshold ($0.05 < p < 0.10$). Red highlighting marks a significant difference ($p \leq 0.05$) between groups where metabolite ratio is ≥ 1.00, and light red is not significant, but close to the threshold ($0.05 < p < 0.10$).

Metabolites of interest were those for which signal from the experimental samples was significantly different, in the same direction, when compared to both controls, while controls compared to each were not significant. Of secondary interest were metabolites where a difference was seen in controls, but was proportionally smaller than consistent differences of each control to the experimental samples.

In surveying this dataset, the outstanding functional category, which contained multiple metabolites whose signal was consistently different in experimental animals versus controls, were the acyl-carnitines (**Table 1**). Furthermore, the fold changes for given metabolites in this group, which consists of fatty acids conjugated to carnitine, were the highest overall. Carnitinylation occurs on fatty acids of various chain lengths, but only a subset of chain lengths in this dataset had sufficient signal, therefore we statistically compared *Repo>Shi^{1}* flies to both parental controls for metabolites that had signal in at least three of five biological replicates for each genotype. Expression of *Shi^{1}* in glia increased abundance in fly heads of the following acylcarnitine species: C2, C16, C16*1, C17, C18:1, C18:2* (**Figure 1A**). The only metabolite of

this group with less signal in experimental animals was the longer-chain, C24* (**Figure 1B**). Carnitine and deoxycarnitine were not significantly altered as compared to both controls.

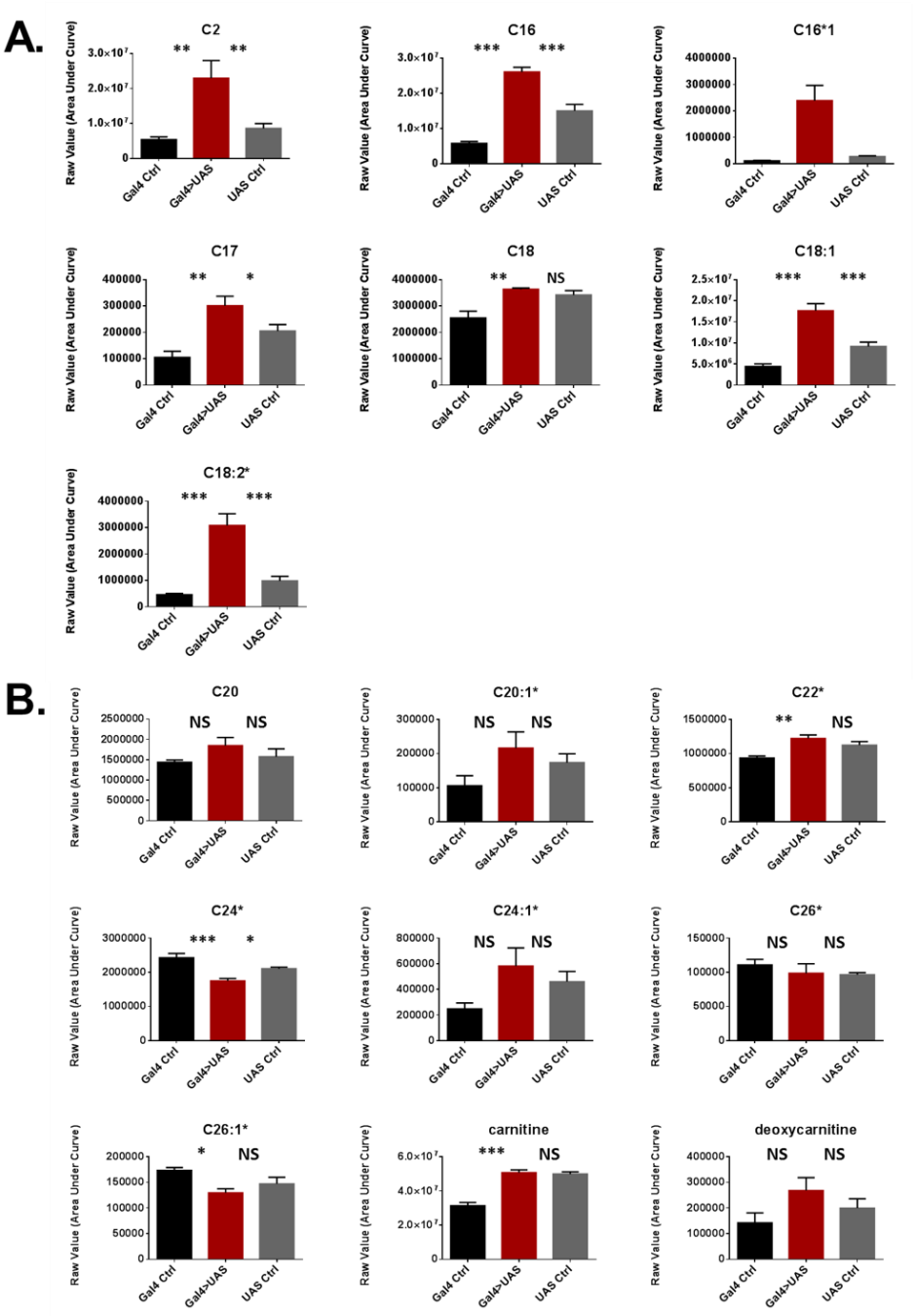

Figure 1: Acylcarnitine levels are increased in *Repo>Shi[1]* fly heads

(**A**) Short and medium chain length or (**B**) long chain length acylcarnitines from Repo-G4 >UAS-Shi[1] fly heads and parental controls. The raw signal from LC/MS is plotted, n=3 – 5 samples, of 200 fly heads each. One-way ANOVA,

with Holk-Sidak post-hoc comparisons. *p < 0.05, **p < 0.01, ***p < 0.001. Error bars represent standard error of the mean (SEM).

Identification of barrier glia genes that affect sleep

In addition to identifying metabolites that accumulate as a result of blocked glial endocytosis, we sought to identify glial molecules whose function might be impacted by the block in endocytosis and thereby contribute to the effect on sleep. As noted above, glia of the BBB are the most relevant glial subtype for sleep-dependent endocytosis. We identified genes enriched in barrier glia by referring to transcriptional profiling that compared expression in the two glial populations that comprise the Drosophila BBB—subperineurial and perineurial glia (SPG + PG)— to all neurons, and all glia (DeSalvo, Hindle et al. 2014). Preference was given to previously studied genes, particularly transporters, receptors and those involved in trafficking, although many genes among the top-50 highly expressed in the barrier glia populations were also tested for effects on sleep. Of the genes enriched in barrier glia, we focused on those that showed low variability in expression from sample to sample. UAS-RNAi constructs for candidate genes were expressed with *Repo/RepoGS* Gal4 drivers and sleep in these lines was compared with that in Gal4 and UAS alone controls. Knockdown of most genes did not produce a significant phenotype, but sleep was increased with knockdown of some transporter genes *CG3036*, *CG6126*, *mnd, VMAT, CG6836, Rh50, CG4462* (**Figure 2- figure supplement 1A**), cytoskeleton/trafficking factors *CG8036*, *Vha16, nuf*, (**Figure 2-figure supplement 1C**) as well as *lsd-2*, *acon* and *MtnA* (**Figure 2- figure supplement 1D**). Meanwhile, knockdown of the transporter gene *CG16700* (**Supp Figure 2A**), cytochrome P450 gene *Cyp6a20* (**Figure 2- figure supplement 1D**) and trafficking factor *Cln7* decreased total sleep (**Figure 2- figure supplement 1C**).

Since the candidate genes were selected based on enrichment within the barrier glia, we chose to examine and secondarily validate promising phenotypes through knockdown with more limited, barrier glia drivers. Thus, we screened a sub-set of the genes suggested by results of the pan-glial screen (**Figure 2-figure supplement 1)** with drivers that target PG or SPG glia. Knockdown of MtnA (105011 KK), CG6386 (108502 KK), CG4462 (105566 KK), or lsd-2 (102269 KK) did not significantly alter sleep when expressed in either of the barrier glial populations alone (data not shown). Reduction of *cyp6a20* in the PG population inconsistently reproduced the pan-glial sleep loss phenotype, so this was not pursued further (data not

shown). The *VMAT* gene produces two isoforms, one of which is thought to be specific to glia (Romero-Calderon, Uhlenbrock et al. 2008). Knockdown of *VMAT* (TRiP HMC02346) in the perineurial glia increased total sleep (**Figure 2A**), but in the subperineurial glia it had no effect (data not shown), which is consistent with protein expression, as the *VMAT-B* antibody specifically marks the perineurial glia (DeSalvo et al., 2014). Pan-glial knockdown of neuronal ceroid lipofuscinosis 7 (*Cln7*), which is expressed in the perineurial glia (Mohammed et al., 2017), decreased total sleep (**Figure 2C**). However, expression of *Cln7* RNAi in the PG increased total sleep time, with no significant change produced by expression in the SPG (**Figure 2B, C**).

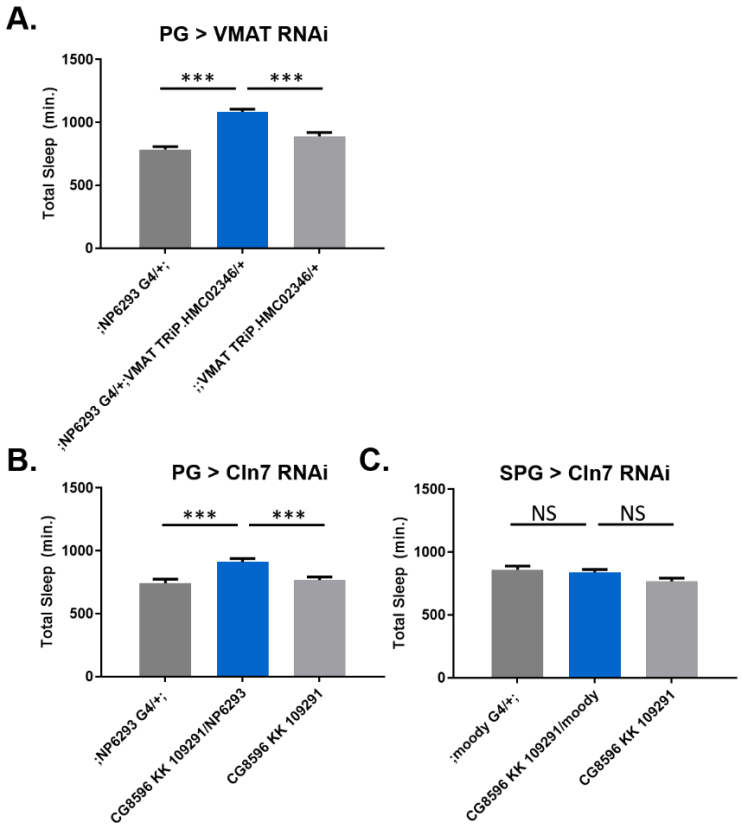

Figure 2: Knockdown of specific barrier-enriched genes with barrier glial drivers

Total sleep in female flies with knockdown of (**A**) VMAT, UAS- HMC02346 driven by (PG) NP6293-Gal4. n=15-16 per genotype. (**B and C**) Cln7 (CG8896), UAS-109291 KK driven by (PG) NP6293-Gal4 or (SPG) moody-Gal4. n = 15-16 per genotype. One-way ANOVA, with Holk-Sidak post-hoc comparisons. *p < 0.05, **p < 0.01, ***p < 0.001. Error bars represent standard error of the mean (SEM).

Simultaneous knockdown of *Lrp1* and *Megalin* in barrier glia increases sleep

Although the candidate screen identified barrier genes whose knockdown increases sleep, as does blocking endocytosis in barrier glia, these genes were not obviously linked to the metabolite profile seen with a block in glial endocytosis. The results of metabolomic screening showed changes in lipid, and particularly carnitine-lipid, trafficking. Therefore, we reassessed our screen candidates to consider transporters and receptors which may function in these pathways and could have been missed due to redundancy/lethality. *Lrp1* and *Megalin* (*Lrp2*) are two LDL receptor-related protein members involved in the transport of lipid carrier proteins at the fly barrier (Brankatschk, Dunst et al. 2014). Expression is likewise found in mammals at the endothelial barrier (Herz 2003).

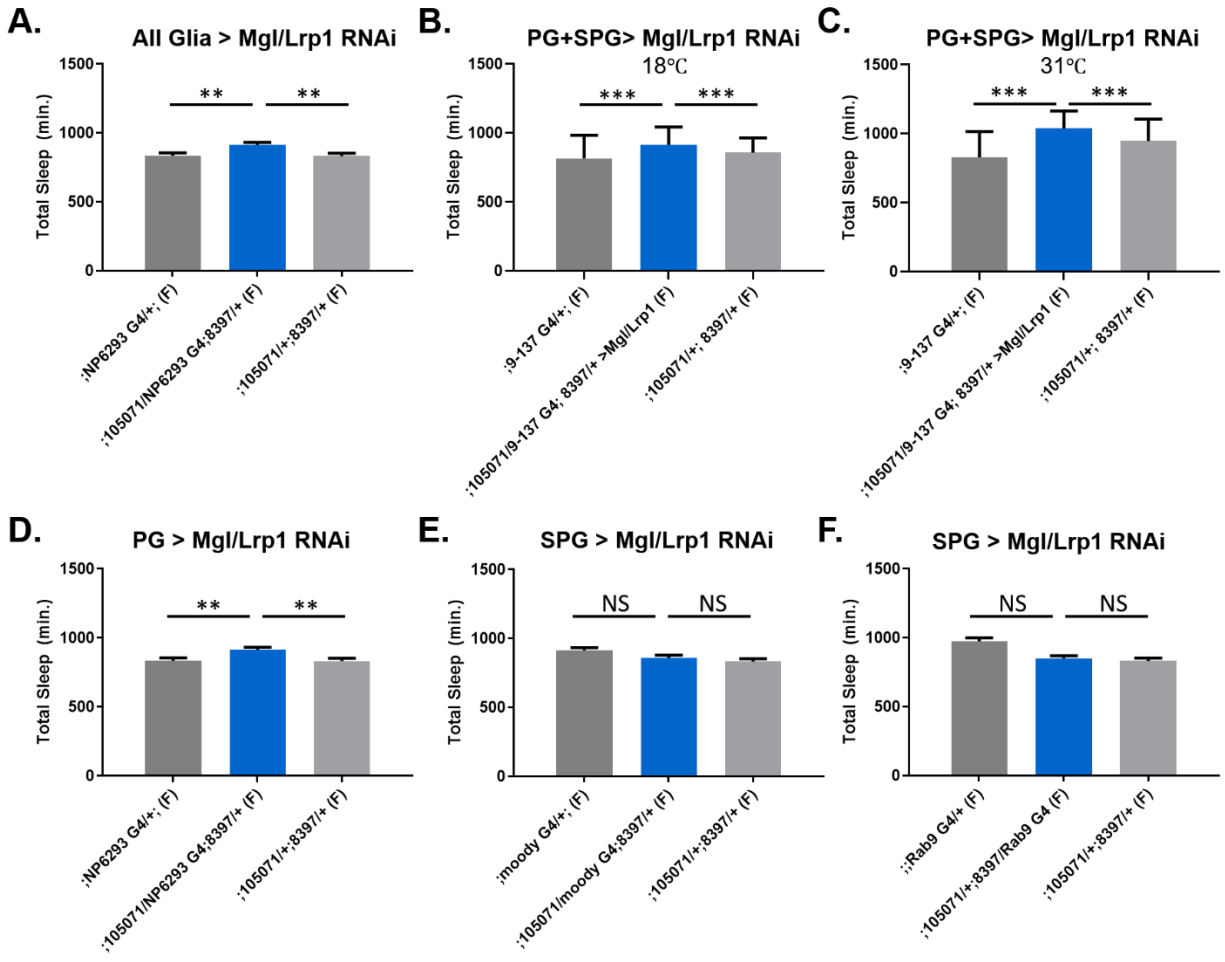

Figure 3. Sleep time changes with knockdown of Lrp genes in all glia or barrier glia

Total sleep in female flies with knockdown of (**A**) *Lrp1* (8397 GD) and *Megalin* (105071 KK) RNAi driven by Repo-GAL4. n = 11-16 per genotype; (**B**) *Lrp1* (8397 GD) and *Megalin* (105071 KK) RNAi driven by 9-137-GAL4 (PG and SPG) with TubGal80^{ts} at the permissive temperature of 18 ℃, n=30- 32 per genotype; (**C**) *Lrp1* (8397 GD) and *Megalin*

(105071 KK) RNAi driven by 9-137-GAL4 (SPG and PG) with TubGal80^{ts} at the restrictive temperature of 31 ℃, n=30-32 per genotype.; (**D, E, F**) *Lrp1* (8397 GD) and *Megalin* (105071 KK) RNAi expressed by: (D) NP6293- Gal4 (**PG**), n = 13-16 per genotype; (**E**) *moody*-GAL4 (SPG), n=16 per genotype; (**F)** Rab9-GAL4 (SPG), n=13-16 per genotype. One-way ANOVA, with Holk-Sidak post-hoc comparisons. *p < 0.05, **p < 0.01. Error bars represent standard error of the mean (SEM).

Knocking down *Lrp1* and *Megalin* (*Lrp2*) individually in the pan-glial screen did not significantly alter total sleep time (**Figure 2- figure supplement 1A**). However, both *Lrp1* and *Megalin* (Lrp2) as well as *Orct* and *Orct2* have been considered to be complementary (Eraly and Nigam 2002, Brankatschk, Dunst et al. 2014), therefore it is possible that inhibition of a single gene is insufficient to appreciably affect transport. Simultaneously knocking down *Lrp1* and *Megalin* in all glia with Repo-Gal4 driver increased total sleep time (**Figure 3A).** To target *Lrp1* and *Megalin* in barrier glia, and also to restrict the knockdown to the adult stage and thereby avoid developmental confounds, we used drivers specific to these glia and coupled them with the temperature-sensitive tubulin-Gal80 (tub-Gal80^{ts}) system that suppresses Gal4 expression at 18 degrees but allows it at 31 degrees. When both *Lrp* genes were knocked down with barrier glia drivers, a significant increase in total sleep was seen with the PG driver (NP6923), but not with either of the SPG drivers (Moody, Rab9) (**Figure 3B, C, D, E**). Knockdown of *Lrp1* and *Megalin* with the 9-137 driver, which expresses in both PG and SPG, also increased total sleep significantly (**Figure 3C and D**). Intriguingly, although the Repo driver did not yield a phenotype with *Lrp1* alone, each of two *Lrp1* RNAi constructs (GD 8397, GD 13913) expressed by the driver 9-137-Gal4 increased total sleep (**Figure 3- figure supplement 1**).

Knockdown of *Orct* and *Orct2* in barrier glia increases sleep

The organic cation (*Orct*) transporters are multi-substrate transporters whose substrates include carnitine (Lahjouji, Mitchell et al. 2001)*,* and perhaps also carnitylated molecules, based on *in vitro* evidence for *Orct2* (Kou, Yao et al. 2017). As with the double knock down of *Lrp1* and *Megalin*, knockdown of both *Orct* genes increased total sleep (**Figure 4A**). In fact, for the *Orct* genes, knockdown in either PG or SPG replicated the increased total sleep phenotype, although this was only true with one line for the SPG (**Figure 4B**). It

is worth noting that the phenotypes with the barrier drivers are considerably more moderate than with knockdown in all glia.

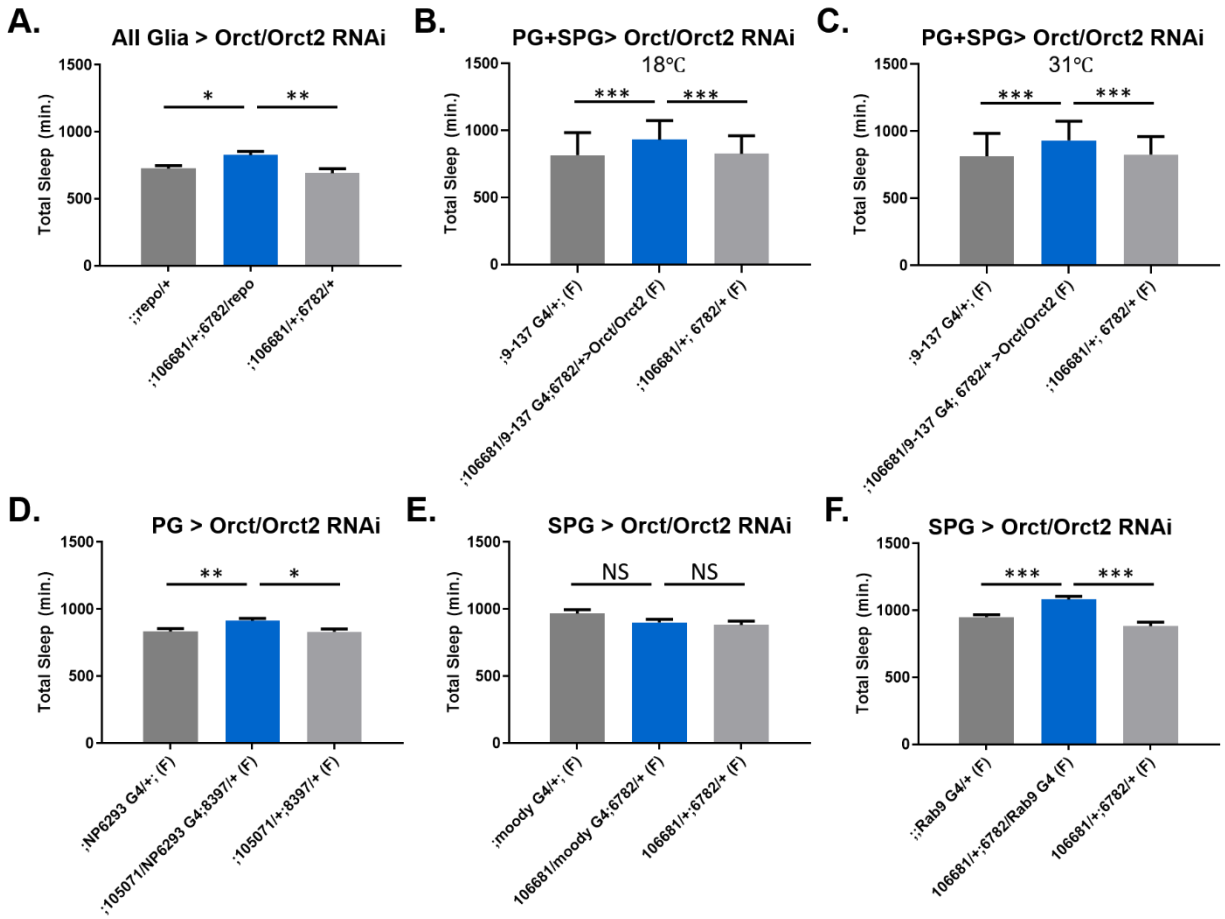

Figure 4. Sleep time changes with knockdown of *Orct* genes in all glia or barrier glia

RNAi constructs of *Orct1* and *Orct2* were expressed as follows: (**A)** *Orct* (6782 GD) and *Orct2* (106681 KK) driven by Repo-GAL4; (**B and C)** *Orct* (6782 GD) and *Orct2* (106681 KK) driven by (PG+SPG) 9-137-Gal4 at 18 ℃(permissive), n=30- 32 per genotype or at 31℃(restrictive), n=30 - 32 per genotype; (**D-F**) *Orct* (6782 GD) and *Orct2* (106681 KK) driven by NP6293-GAL4, n = 13-16 per genotype or by (SPG) *moody*-GAL4, n=15-16 per genotype or by (SPG) Rab9-GAL4, n=15-16 per genotype. One-way ANOVA, with Holk-Sidak post-hoc comparisons. *p < 0.05, **p < 0.01, ***p < 0.001. Error bars represent standard error of the mean (SEM).

Knockdown of Lrp and *Orct* genes in glia leads to accumulation of acylcarnitines

Given that knockdown of the *Lrp* and *Orct* genes in glia parallels effects of *Shi* expression in terms of increasing total sleep, we asked if it had the same effect on metabolite accumulation in fly heads. Based

on the metabolomic results of *Repo>Shi*[1], we chose to specifically assay acylcarnitines through LC-MS analysis. Metabolomic profiling requires considerable tissue, as so, as in the case of the *Shi*[1] experiment, we collected heads from flies in which either *Lrp1* and *Megalin* or *Orct 1* and *Orct2* were knocked down with *Repo*-Gal4.

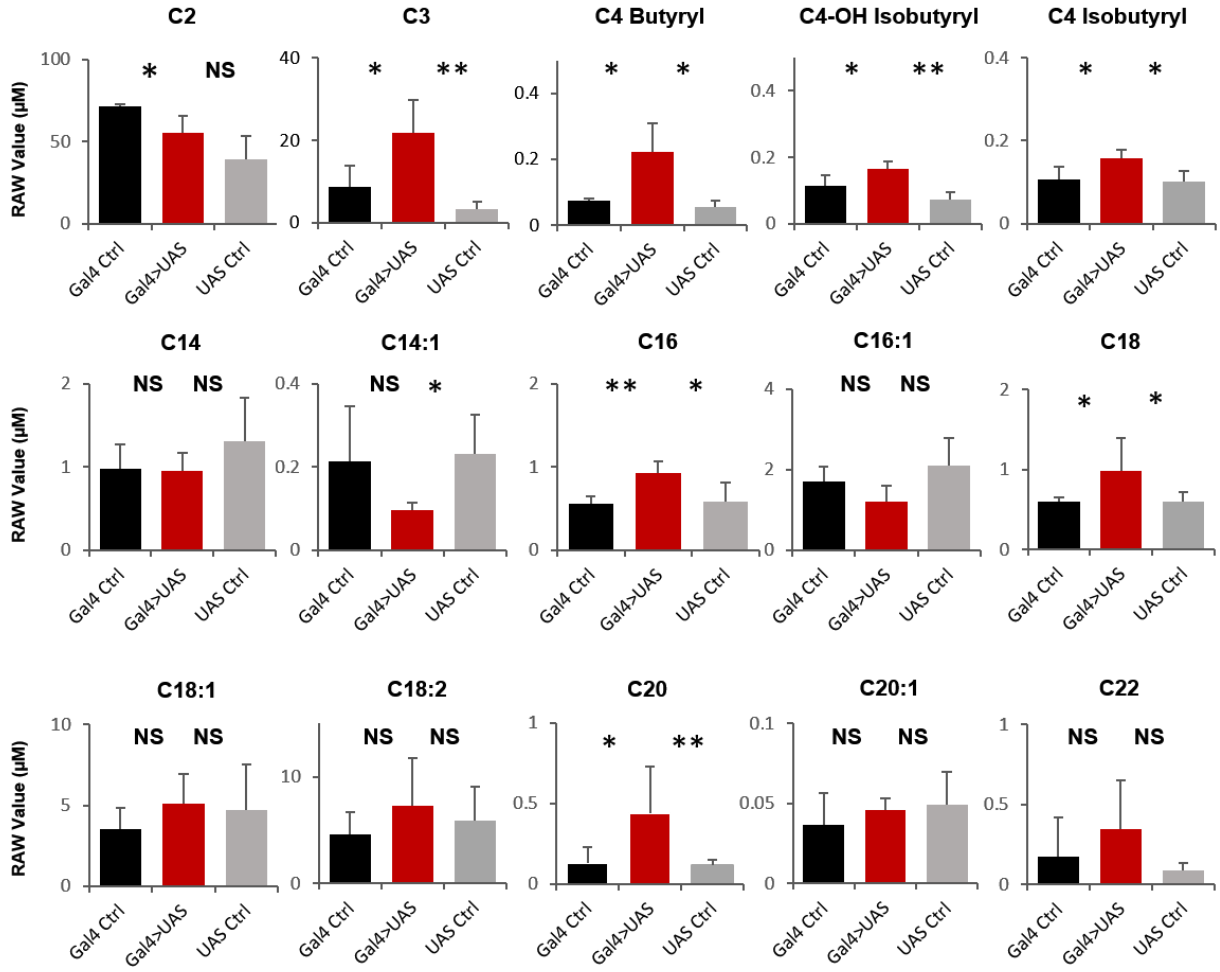

Figure 5. Acylcarnitine levels are increased in Repo>*Lrp1*+ Mgl fly heads

Short and medium chain length acylcarnitines from Repo Gal4 >*Lrp1*+Mgl fly heads and parental controls. The raw signal from LC/MS is plotted, n=3 samples, of 300 fly heads each. Student's t-test with comparison. *p < 0.05, **p < 0.01. Error bars represent standard error of the mean (SEM).

Knockdown of *Lrp1* and *Megalin* in all glia increased abundance in fly heads of the following acylcarnitine species: C3, C4 butyryl, C4-OH isobutyryl, C4 isobutyryl, C16, C18, C20 (**Figure 5**). Longer-chain acylcarnitines, e.g. those over C22, were largely undetected in experimental samples. Knockdown of *Orct* and *Orct2* similarly enriched acylcarnitines in fly heads. In particular, acylcarnitine species C16, C16:1 and

C20 were increased significantly compared to their controls (**Figure 6**). Similar in the case of the *Lrp* samples, longer-chain acylcarnitines over 22 were not detected well.

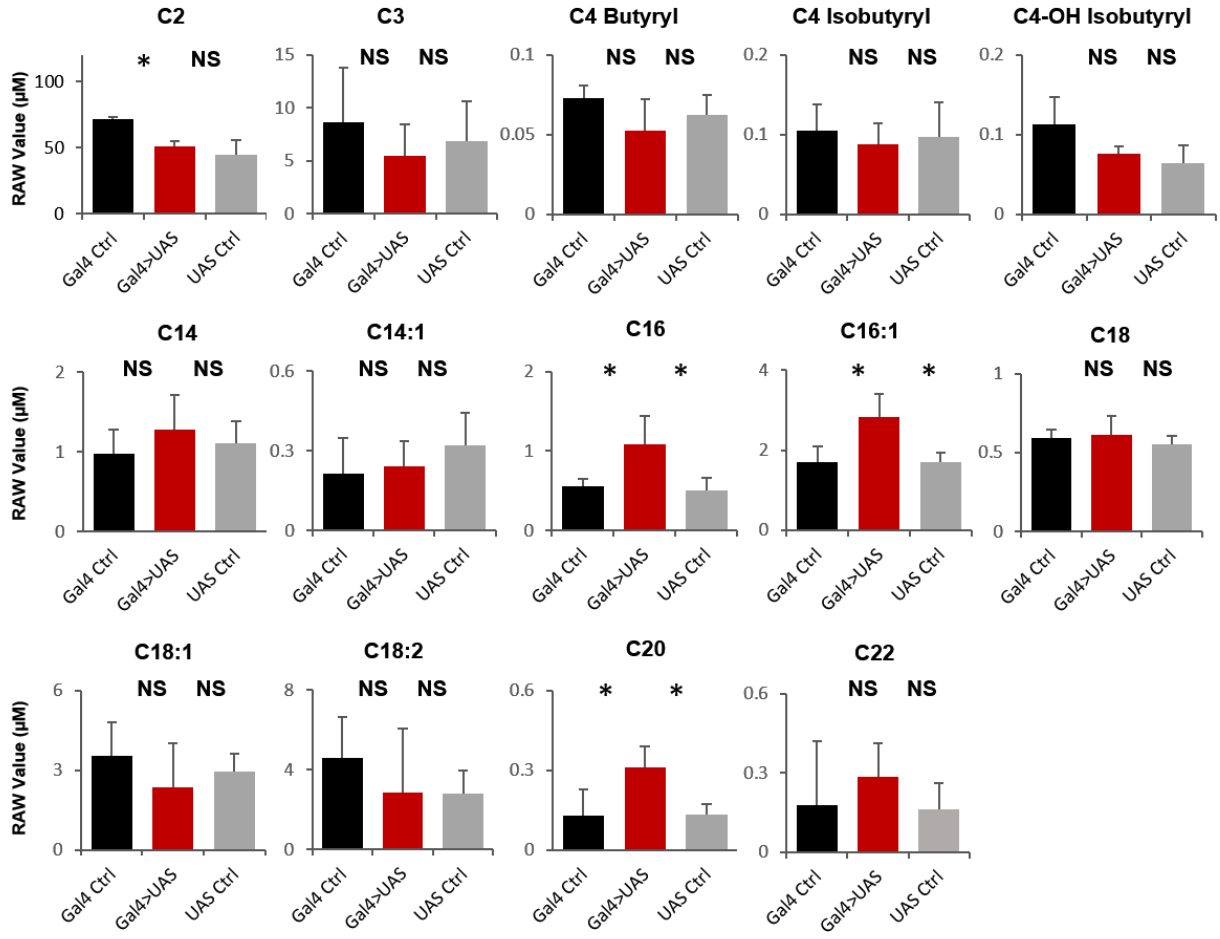

Figure 6. Acylcarnitine levels are increased in Repo>*Orct*+ *Orct2* fly heads

Short and medium chain length acylcarnitines from *Repo-Gal4 >Orct+Orct2* fly heads and parental controls. The raw signal from LC/MS is plotted, n=3 samples, of 300 fly heads each. Student's t-test with comparisons. *p < 0.05, **p < 0.01. Error bars represent standard error of the mean (SEM).

Discussion:

Our previous work suggested that endocytosis through the BBB is a function of sleep, but the nature of the molecules trafficked remained unknown. Using a dual-pronged approach of a targeted genetic screen and unbiased metabolomic analysis, we report here that the passage of lipids through the BBB is important for sleep. Blocking such transport increases sleep in conjunction with an accumulation of acylcarnitines.

Through a pan-glial RNAi knockdown screen of candidate genes expressed in the fly barrier, we identified molecules that affect daily sleep amount. In follow-up experiments, we targeted knockdown to each barrier layer separately, which is subject to the concern that behavioral phenotypes requiring simultaneous knockdown in both barrier populations would be missed. Nevertheless, we consider this scenario to be less probable, as permeability through the populations is quite different, with smaller solutes likely passing through the PG but not the tight barrier of the SPG, lipophilic solutes or xenobiotics potentially passing through each uninhibited, and larger solute requiring endocytic mechanisms that likely have to work in each population in tandem. Therefore, in most cases of knockdown, transport would either be inhibited by the one barrier population essential for it, or would be interrupted by either population. This was indeed the case for the lipid and carnitine transporters we focused on, where knockdown in a specific layer or either layer of the BBB was sufficient for a phenotype. While there was redundancy at the level of the transporters, the two barrier layers did not compensate for each other.

An additional consideration of preliminarily screening with pan-glial drivers is that if knockdown in multiple glial subtypes has opposing effects on sleep, we may have obscured a role for the barrier cells. Again, we attempted to minimize this risk by primarily selecting genes whose expression is both highly abundant and specifically enriched in the barrier populations, as opposed to the set of all glial cells in the transcriptome dataset (DeSalvo et al., 2014). The assumption is that multifold expression in the barrier populations is indicative of prevailing importance in these cells, although this is a caveat.

The vesicular monoamine transporter (VMAT) has previously been identified as a target of reserpine, which promotes sleep in the fly (Nall et al., 2014). VMAT mutants exhibit higher baseline sleep, and also lose less sleep than controls when subject to mechanical sleep deprivation. VMAT can traffic multiple monoamines such as dopamine, serotonin, histamine and octopamine, but no single neuronal population or neurotransmitter system was implicated as responsible for the VMAT sleep phenotype (Nall et al., 2014).

In flies, VMAT exists as two isoforms, VMAT-A, which is expressed in monoaminergic neurons, and VMAT-B, which appears to be specific to perineurial glia (DeSalvo et al., 2014), as it is also found in fenestrated glia in the visual system (Romero-Calderon, Uhlenbrock et al. 2008), which are a specialized form of perineurial glia (Kremer, Jung et al. 2017). It is unknown whether VMAT-B would functional similarly in glia as VMAT-A does in neurons. VMAT-B contains an additional cytoplasmic domain, which has been suggested to promote retention in the plasma membrane as opposed to trafficking to vesicles (Greer, Grygoruk et al. 2005). *VMAT* knockdown increased sleep, as did disrupting endocytic trafficking at the barrier (Artiushin, Zhang et al. 2018). Whole-brain levels of monoamines were not altered in flies that expressed *Shi*^{1} in glia, nevertheless it is possible that this gross analysis would not be sensitive to local changes at the barrier. In the visual system glia, VMAT-B may be necessary for uptake of histamine (Romero-Calderon, Uhlenbrock et al. 2008). Interestingly, histamine is known to alter permeability of the blood-brain barrier in mammals (Lu, Diehl et al. 2010).

Cln7 is a major facilitator superfamily transporter implicated in neuronal ceroid lipofuscinoses, and hence considered to impact lysosomal/autophagal function (Siintola, Topcu et al. 2007). Neither the function, nor what this transporter traffics, are known, but it is thought to be vesicular as well, and is expressed in the perineurial glia in flies (Mohammed, O'Hare et al. 2017). Knockdown of *Cln7* in all glia affected sleep in the opposite direction from knockdown only in the PG. One potential explanation would be that *Cln7* acts on sleep in opposing ways in different glial populations, although protein expression data suggest that *Cln7* is quite limited in the brain, and it is not clear whether it is in other glial populations.

Our metabolomic data indicated that acylcarnitines are elevated in the heads of the long-sleeping Repo>Shi^{1} flies. Acylcarnitines are transported to mitochondria for fatty acid oxidation, but are also secreted as they are found in plasma in mammals (Schooneman, Vaz et al. 2013). Given that circulating acylcarnitines can be taken up by cells, we investigated *Lrp1*/*Megalin* and *Orct*/*Orct2* as candidate transporters for this uptake. Lrp1 and Megalin are lipoprotein carrier receptors known to function in the fly barrier (Brankatschk, Dunst et al. 2014) and although knockdown of each one separately in all glia did not affect sleep, reducing expression of both in all glia or barrier glia increased sleep. Likewise, knockdown of *Orct* and *Orct2,* which are homologs of the human carnitine transporters (Eraly and Nigam 2002) and transport carnitine as well as acylcarnitines (Pochini, Oppedisano et al. 2004), increases sleep. LC-MS

analysis shows that knockdown of these transporters enriches acylcarnitines in fly heads just as blocking endocytosis does, supporting the idea that Lrp and Orct are among the proteins affected by the block in endocytosis. Exactly where the accumulation occurs is not known at this time, but we suggest that it is largely extracellular.

Acylcarnitine accumulation in flies with blocked glial endocytosis or lipid transport could occur as a consequence of the high sleep in these animals. We believe this is unlikely as an increase in acylcarnitines appears to generally occur under conditions of sleep deprivation i.e. conditions that would promote sleep. Thus, carnitine conjugation of long chain fatty acids was reported in cortical metabolites of sleep-deprived mice, while short and medium chain fatty acids were reduced (Hinard, Mikhail et al. 2012). Changes in acylcarnitines were also noted in the peripheral blood of sleep-deprived or sleep-restricted humans (Davies, Ang et al. 2014, Weljie, Meerlo et al. 2015) as well as over a day:night cycle (Dallman etal (Ang, Revell et al. 2012, Dallmann, Viola et al. 2012). We find too that a common feature of short-sleeping fly mutants, which are models for chronic sleep deprivation, is an increase in acylcarnitines, regardless of the mechanism that causes the sleep loss (Bedont, Kolesnik et al. 2021). Thus, acylcarnitines are generally associated with sleepiness. While this does not necessarily mean that they promote sleep, we suggest that acylcarnitines are a key marker of sleep need across species, and could be exploited for this purpose.

Methods

Fly Stocks

The initial screen was performed with lab stock drivers: ;; Repo-GAL4/TM6c, Sb and UAS-*Dicer*; *Repo*GeneSwitch. SPG driver *Moody*-GAL4 and surface driver 9-137-Gal4 was shared by Roland Bainton, while PG driver NP6293-GAL4 was a gift of Marc Freeman. ;;UAS-*20xShi.ts1* (referred to as UAS-*20xShi*^{1}) was shared by Gerald Rubin. *Rab9*-GAL4 (#51587) was acquired from Bloomington. RNAi lines were ordered from VDRC (KK and GD collection) and Bloomington (TRiP collection) stock centers, with the stock number provided in Figure 1 and supplement Figure 1. For control genotypes, GAL4 and UAS lines were crossed to iso31.

Behavior

Flies were crossed and raised on standard food in bottles. Offspring were kept at 25 ℃, in LD12:12 conditions until at least 6 days post-eclosion, before age-matched flies which were group housed in bottles were used in sleep assays. Mated females were loaded into glass locomotor tubes with 2% agar 5% sugar. Sleep was quantified by the Drosophila Activity Monitor (DAM) system, by the established minimum definition of 5 minutes of inactivity. Data was analyzed in PySolo (Gilestro et al., 2009).

Metabolomics

Entire flies were quickly frozen in Falcon tubes chilled on dry ice, and placed at -80°C. Each tube contained 50 flies. Heads were subsequently removed from the body by briefly vortexing the tube. Heads were then separated from the rest of the body by an array of copper sieves, whose housing was buried in dry ice to keep the preparation cool. For each sample, 200 fly heads, of equal parts from males and females, were collected in 1.5 mL tubes which were quickly refrozen. Samples were shipped on dry ice to Metabolon, Inc., where they were assessed by LC-MS (Evans...Milgram 2009). For metabolomic analysis of *Lrp* and *Orct* knockdown fly lines, each sample contained 300 fly heads, of equal parts from males and females. Samples were processed by LC-MS at the Penn Metabolomics Core.

Statistics:

For both behavioral and acylcarnitine metabolomics results, the experimental group was compared to two parental controls by One-Way ANOVA with Holm-Sidak post-hoc tests. For the initial comparisons of metabolomics data, Metabolon performed Welch's t-tests on scaled signal data for each metabolite,

between all conditions. Raw signal was scaled so that the median would be equal to 1, using all samples that had been concurrently run. Missing values were filled in with the lowest value of run samples for that metabolite. Additional details of statistics tests are listed in the figure legends.

Acknowledgement

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Sub Pathway	Biochemical Name	Platform	Comp ID	KEGG	HMDB	PubChem	Gal4>UAS / GAL4 Ctrl	Gal4>UAS / UAS Ctrl	UAS Ctrl / GAL4 Ctrl
Glycine, Serine and Threonine Metabolism	glycine	LC/MS pos early	58	C00037	HMDB00123	750	0.73	0.90	0.81
	N-acetylglycine	LC/MS polar	27710		HMDB00532	10972	0.77	0.96	0.80
	sarcosine	LC/MS pos early	1516	C00213	HMDB00271	1088	0.36	1.12	0.33
	betaine	LC/MS pos early	3141	C00719	HMDB00043	247	0.56	0.56	1.01
	serine	LC/MS pos early	1648	C00065	HMDB00187	5951	1.09	1.01	1.08
	N-acetylserine	LC/MS pos early	37076		HMDB02931	65249	0.87	1.02	0.85
	2-methylserine	LC/MS pos early	53229	C02115		94309	0.75	0.50	1.49
	threonine	LC/MS pos early	1284	C00188	HMDB00167	6288	0.72	0.94	0.77
	N-acetylthreonine	LC/MS polar	33939			152204	0.56	0.90	0.63
	homoserine	LC/MS pos early	18351	C00263	HMDB00719	12647	1.27	0.79	1.61
Alanine and Aspartate Metabolism	alanine	LC/MS pos early	1126	C00041	HMDB00161	5950	0.98	0.96	1.03
	N-acetylalanine	LC/MS polar	1585	C02847	HMDB00766	88064	0.73	0.89	0.82
	N-methylalanine	LC/MS pos early	37069	C02721	HMDB01906	5288725	0.59	0.63	0.93
	N-acetylaspartate (NAA)	LC/MS polar	22185	C01042	HMDB00812	65065	1.46	1.12	1.30
	asparagine	LC/MS pos early	512	C00152	HMDB00168	6267	1.11	0.81	1.37
	N-acetylasparagine	LC/MS pos early	33942		HMDB06028	99715	0.81	0.89	0.91
Glutamate Metabolism	glutamate	LC/MS pos early	57	C00025	HMDB00148	611	1.41	1.06	1.33
	glutamine	LC/MS pos early	53	C00064	HMDB00641	5961	1.32	0.87	1.51
	alpha-ketoglutaramate*	LC/MS polar	62101				1.84	0.96	1.92
	N-acetylglutamate	LC/MS polar	15720	C00624	HMDB01138	70914	1.27	0.61	2.08
	N-acetylglutamine	LC/MS pos early	33943	C02716	HMDB06029	182230	0.68	0.92	0.74
	glutamate, gamma-methyl ester	LC/MS pos early	33487		HMDB61715	68662	0.80	0.86	0.93
	pyroglutamine*	LC/MS pos early	46225			134508	0.34	0.95	0.36
	gamma-aminobutyrate (GABA)	LC/MS pos early	1416	C00334	HMDB00112	119	1.14	1.03	1.11
	carboxyethyl-GABA	LC/MS pos early	40007		HMDB02201	2572	0.76	1.15	0.66
	N-methyl-GABA	LC/MS pos early	39577	C15987		70703	0.82	0.64	1.29
	propionylglutamine	LC/MS pos early	54909				0.65	1.05	0.62
Histidine Metabolism	histidine	LC/MS neg	59	C00135	HMDB00177	6274	0.84	0.81	1.04
	1-methylhistidine	LC/MS pos early	30460	C01152	HMDB00001	92105	1.11	0.73	1.51
	3-methylhistidine	LC/MS pos early	15677	C01152	HMDB00479	64969	1.91	0.26	7.23
	N-acetylhistidine	LC/MS pos early	33946	C02997	HMDB32055	75619	0.82	0.81	1.00
	imidazole propionate	LC/MS pos early	40730		HMDB02271	70630	0.46	0.77	0.60
	imidazole lactate	LC/MS pos early	15716	C05568	HMDB02320	440129	0.57	0.75	0.75
	histamine	LC/MS pos early	1574	C00388	HMDB00870	774	1.05	0.93	1.13
	4-imidazoleacetate	LC/MS pos early	32349	C02835	HMDB02024	96215	0.86	0.92	0.94
	N-acetylhistamine	LC/MS pos early	48679	C05135	HMDB13253	69602	1.92	1.64	1.17
Lysine Metabolism	lysine	LC/MS pos early	1301	C00047	HMDB00182	5962	1.31	1.10	1.19
	N6,N6,N6-trimethyllysine	LC/MS pos early	1498	C03793	HMDB01325	440120	0.96	1.96	0.49
	5-(galactosylhydroxy)-L-lysine	LC/MS pos early	43582				1.05	0.88	1.19
	saccharopine	LC/MS polar	1495	C00449	HMDB00279	160556	1.53	1.37	1.11
	pipecolate	LC/MS pos early	1444	C00408	HMDB00070	849	0.74	1.22	0.61
	N-trimethyl 5-aminovalerate	LC/MS pos early	57687				3.87	1.87	2.07

Sub Pathway	Biochemical Name	Platform	Comp ID	KEGG	HMDB	PubChem	Gal4>UAS / GAL4 Ctrl	Gal4>UAS / UAS Ctrl	UAS Ctrl / GAL4 Ctrl
Phenylalanine Metabolism	phenylalanine	LC/MS pos early	64	C00079	HMDB00159	6140	0.81	0.90	0.90
	N-acetylphenylalanine	LC/MS neg	33950	C03519	HMDB00512	74839	0.58	1.37	0.42
Tyrosine Metabolism	tyrosine	LC/MS pos early	1299	C00082	HMDB00158	6057	2.61	1.29	2.03
	dihydoxyphenylalanine (L-DOPA)	LC/MS pos early	1576	C00355	HMDB00181	6047	1.41	1.04	1.36
	N-formylphenylalanine	LC/MS neg	48433			759256	1.50	1.31	1.15
Tryptophan Metabolism	tryptophan	LC/MS pos early	54	C00078	HMDB00929	6305	1.17	1.02	1.15
	kynurenine	LC/MS pos early	15140	C00328	HMDB00684	161166	2.29	1.44	1.59
	kynurenate	LC/MS neg	1417	C01717	HMDB00715	3845	3.21	2.14	1.50
	3-hydroxykynurenine	LC/MS pos early	22110	C02794	HMDB00732	89	4.59	1.17	3.94
	xanthurenate	LC/MS neg	15679	C02470	HMDB00881	5699	2.05	1.07	1.91
	N-acetylserotonin	LC/MS neg	1500	C00978	HMDB01238	903	0.81	0.99	0.82
Leucine, Isoleucine and Valine Metabolism	leucine	LC/MS pos early	60			5246661	0.64	0.95	0.68
	N-acetylleucine	LC/MS neg	1587	C02710	HMDB11756	70912	0.61	1.14	0.53
	4-methyl-2-oxopentanoate	LC/MS neg	22116	C00233	HMDB00695	70	0.61	1.17	0.52
	beta-hydroxyisovalerate	LC/MS polar	12129		HMDB00754	69362	0.81	1.05	0.78
	isoleucine	LC/MS pos early	1125	C00407	HMDB00172	6306	0.71	0.94	0.76
	3-methyl-2-oxovalerate	LC/MS neg	15676	C00671	HMDB03736	47	0.75	1.16	0.65
	alpha-hydroxyisovalerate	LC/MS polar	46537		HMDB00407	99823	0.81	0.96	0.84
	ethylmalonate	LC/MS polar	15765		HMDB00622	11756	0.81	0.83	0.98
	methylsuccinate	LC/MS polar	15745		HMDB01844	10349	0.96	0.82	1.18
	valine	LC/MS neg	1649	C00183	HMDB00883	6287	0.62	0.87	0.72
	3-methyl-2-oxobutyrate	LC/MS polar	44526	C00141	HMDB00019	49	0.88	1.28	0.68
	3-hydroxyisobutyrate	LC/MS polar	1549	C06001	HMDB00336	87	0.72	1.09	0.66
Methionine, Cysteine, SAM and Taurine Metabolism	methionine	LC/MS pos early	1302	C00073	HMDB00696	6137	0.75	1.08	0.70
	N-acetylmethionine	LC/MS neg	1589	C02712	HMDB11745	448580	0.54	0.96	0.57
	N-formylmethionine	LC/MS neg	2829	C03145	HMDB01015	439750	0.76	1.53	0.50
	methionine sulfoxide	LC/MS pos early	18374	C02989	HMDB02005	158980	0.57	0.75	0.77
	N-acetylmethionine sulfoxide	LC/MS pos early	45428			193368	0.60	0.90	0.67
	S-adenosylhomocysteine (SAH)	LC/MS neg	42382	C00021	HMDB00939	439155	1.14	1.22	0.93
	cystathionine	LC/MS pos early	15705	C02291	HMDB00099	439258	0.97	1.14	0.85
	cysteine	LC/MS pos early	1868	C00097	HMDB00574	5862	0.49	0.73	0.67
	N-acetylcysteine	LC/MS pos early	1586	C06809	HMDB01890	12035	0.87	0.89	0.97
	S-methylcysteine sulfoxide	LC/MS pos early	43378		HMDB29432	82142	1.12	0.71	1.58
	cystine	LC/MS neg	56	C00491	HMDB00192	67678	0.63	0.64	1.00
	lanthionine	LC/MS pos early	42002			98504	0.75	1.48	0.50
	cysteine sulfinic acid	LC/MS pos early	37443	C00606	HMDB00996	109	1.84	0.87	2.12
	taurine	LC/MS neg	2125	C00245	HMDB00251	1123	0.96	0.88	1.09
	N-acetyltaurine	LC/MS neg	48187			159864	1.02	0.85	1.20
	cyano-alanine	LC/MS polar	35660	C02512		13538	1.00	0.77	1.31
Urea cycle; Arginine and Proline Metabolism	arginine	LC/MS pos early	1638	C00062	HMDB00517	232	0.99	0.96	1.03
	argininosuccinate	LC/MS pos early	15497	C03406	HMDB00052	828	1.11	1.23	0.90
	ornithine	LC/MS pos early	1493	C00077	HMDB03374	6262	1.38	1.38	1.00
	2-oxoarginine*	LC/MS pos early	55072	C03771	HMDB04225	558	0.73	1.00	0.74
	citrulline	LC/MS pos early	2132	C00327	HMDB00904	9750	0.73	0.97	0.75
	proline	LC/MS pos early	1898	C00148	HMDB00162	145742	1.31	1.02	1.28
	dimethylarginine (SDMA + ADMA)	LC/MS pos early	36808	C03626	HMDB01539	123831	1.38	1.09	1.27
	N-acetylarginine	LC/MS pos early	33953	C02562	HMDB04620	67427	1.72	1.72	1.00
	N-delta-acetylornithine	LC/MS pos early	43249			9920500	1.35	1.09	1.24
	N-alpha-acetylornithine	LC/MS pos early	32984	C00437	HMDB03357	439232	1.54	1.48	1.04
	trans-4-hydroxyproline	LC/MS pos early	32306	C01157	HMDB00725	5810	0.77	0.62	1.23
	argininate*	LC/MS pos early	57461		HMDB03148	160437	0.75	1.15	0.65

Sub Pathway	Biochemical Name	Platform	Comp ID	KEGG	HMDB	PubChem	Gal4>UAS / GAL4 Ctrl	Gal4>UAS / UAS Ctrl	UAS Ctrl / GAL4 Ctrl
Polyamine Metabolism	putrescine	LC/MS pos early	1408	C00134	HMDB01414	1045	5.09	0.83	6.14
	N-acetyl-isoputreanine*	LC/MS pos early	62309				1.00	1.00	1.00
	spermidine	LC/MS pos early	485	C00315	HMDB01257	1102	1.23	0.99	1.25
	5-methylthioadenosine (MTA)	LC/MS pos early	1419	C00170	HMDB01173	439176	1.48	1.17	1.26
	4-acetamidobutanoate	LC/MS pos early	1558	C02946	HMDB03681	18189	0.89	0.86	1.03
	(N(1) + N(8))-acetylspermidine	LC/MS pos early	57814				0.79	0.91	0.86
Guanidino and Acetamido Metabolism	4-guanidinobutanoate	LC/MS pos early	15681	C01035	HMDB03464	500	1.00	1.14	0.87
Glutathione Metabolism	glutathione, reduced (GSH)	LC/MS pos early	2127	C00051	HMDB00125	124886	4.09	0.83	4.94
	cysteine-glutathione disulfide	LC/MS pos early	35159		HMDB00656	4247235	1.72	0.82	2.10
	cysteinylglycine	LC/MS pos early	35637	C01419	HMDB00078	439498	0.36	0.49	0.73
	cysteinylglycine disulfide*	LC/MS pos early	62103		HMDB00709		1.89	0.88	2.15
	5-oxoproline	LC/MS neg	1494	C01879	HMDB00267	7405	0.64	0.79	0.80
Gamma-glutamyl Amino Acid	gamma-glutamylalanine	LC/MS pos early	37063		HMDB29142	440103	0.88	0.91	0.97
	gamma-glutamylcysteine	LC/MS pos early	1778	C00669	HMDB01049	842	0.25	0.58	0.43
	gamma-glutamylglutamate	LC/MS pos early	36738	C05282	HMDB11737	92865	0.90	0.83	1.08
	gamma-glutamylglutamine	LC/MS pos early	2730	C05283	HMDB11738	150914	1.66	1.13	1.47
	gamma-glutamylglycine	LC/MS pos early	33949		HMDB11667	165527	0.37	0.63	0.59
	gamma-glutamylhistidine	LC/MS pos early	18245			7017195	0.55	0.75	0.73
	gamma-glutamylisoleucine*	LC/MS neg	34457		HMDB11170	14253342	0.40	1.01	0.39
	gamma-glutamylleucine	LC/MS neg	18369		HMDB11171	151023	0.45	0.87	0.52
	gamma-glutamyl-alpha-lysine	LC/MS pos early	55015			65254	1.29	1.32	0.98
	gamma-glutamylmethionine	LC/MS pos early	44872		HMDB29155	7009567	0.43	1.40	0.31
	gamma-glutamylthreonine	LC/MS pos early	33364		HMDB29159	76078708	0.61	0.94	0.65
	gamma-glutamylvaline	LC/MS pos early	43829		HMDB11172	7015683	0.48	0.70	0.69
Dipeptide	alanylalanine	LC/MS pos early	15129		HMDB28680	5484352	1.13	0.91	1.24
	alanylglutamate	LC/MS pos early	37064			656476	0.84	0.84	1.00
	alanylproline	LC/MS pos early	37083		HMDB28695	418040	0.83	1.21	0.69
	alanylthreonine	LC/MS pos early	37085		HMDB28697	426318	1.05	0.98	1.06
	alpha-glutamylalanine	LC/MS pos early	41369		HMDB03764	100098	0.90	0.93	0.97
	alpha-glutamylglutamate	LC/MS pos early	22166	C01425	HMDB28818	439500	0.87	1.07	0.81
	asparaginylalanine	LC/MS pos early	54731		HMDB28724		1.12	0.98	1.14
	glutaminylglutamate	LC/MS pos early	43025				0.71	0.94	0.76
	serylthreonine	LC/MS pos early	54732		HMDB29049		0.69	0.92	0.74
	glycylglycine	LC/MS pos early	21029	C02037	HMDB11733	11163	0.86	1.05	0.82
	glycylisoleucine	LC/MS pos early	36659		HMDB28844	88079	1.04	0.88	1.18
	glycylleucine	LC/MS pos early	34398	C02155	HMDB00759	92843	0.89	0.83	1.07
	glycylphenylalanine	LC/MS neg	33954		HMDB28848	92953	1.24	1.19	1.04
	glycylproline	LC/MS pos early	22171		HMDB00721	3013625	0.69	0.75	0.92
	glycylvaline	LC/MS pos early	18357		HMDB28854	97417	0.67	0.83	0.81
	isoleucylalanine	LC/MS pos early	40046		HMDB28900	5246009	1.72	0.99	1.73
	isoleucylglutamate	LC/MS pos early	40057				0.96	1.23	0.79
	isoleucylglutamine	LC/MS pos early	40019			7020102	1.60	1.05	1.52
	isoleucylglycine	LC/MS neg	40008		HMDB28907	342532	1.15	1.09	1.05
	isoleucylthreonine	LC/MS pos early	42968		HMDB28917	16122515	1.46	0.95	1.53
	leucylalanine	LC/MS pos early	40010		HMDB28922	259321	4.22	1.01	4.19
	leucylglycine	LC/MS pos early	40045		HMDB28929	79070	1.67	0.96	1.73
	leucylleucine	LC/MS pos early	36756	C11332	HMDB28933	76807	5.53	0.92	6.02
	leucylproline	LC/MS pos early	35663		HMDB11175	80817	0.52	0.94	0.56
	leucylthreonine	LC/MS pos early	42969		HMDB28939	10353878	2.16	0.91	2.37
	phenylalanylglutamate	LC/MS neg	41432			4422358	0.87	0.91	0.96
	prolylalanine	LC/MS pos early	40705		HMDB29010	418041	0.87	0.96	0.90
	prolylglutamine	LC/MS pos early	40659				1.11	0.92	1.21
	prolylglycine	LC/MS pos early	40703		HMDB11178	6426709	0.86	1.01	0.85
	prolylleucine	LC/MS pos early	31914			3527720	0.94	0.92	1.03
	prolylproline	LC/MS pos early	40731		HMDB11180	11622593	0.71	0.89	0.80
	prolylthreonine	LC/MS pos early	44551		HMDB29027		0.74	1.12	0.67
	prolylvaline	LC/MS pos early	40720			152307	0.72	0.81	0.89
	serylalanine	LC/MS pos early	42049		HMDB29032	17958834	1.07	0.85	1.26
	serylleucine	LC/MS pos early	40066		HMDB29043	7015695	1.15	1.11	1.04
	serylproline	LC/MS pos early	42055		HMDB29047	4369021	1.04	0.90	1.16
	serylserine	LC/MS pos early	42053			138784	0.94	0.83	1.13
	serylvaline	LC/MS pos early	42058		HMDB29052	7020159	1.45	0.95	1.54
	valylaspartate	LC/MS pos early	40650		HMDB29123	9964657	0.91	0.92	0.99
	valylglycine	LC/MS neg	40475		HMDB29127	136487	1.07	1.01	1.06
	valylproline	LC/MS pos early	40485		HMDB29135	5003412	0.70	0.95	0.74
	isoleucylleucine/leucylisoleucine	LC/MS pos early	52322				2.09	0.79	2.65
	alpha-glutamylproline*	LC/MS pos early	57731				1.00	1.00	1.00
Modified Peptides	pyroglutamylleucine*	LC/MS neg	62096				0.94	1.26	0.75

Sub Pathway	Biochemical Name	Platform	Comp ID	KEGG	HMDB	PubChem	Gal4>UAS GAL4 Ctrl	Gal4>UAS UAS Ctrl	UAS Ctrl GAL4 Ctrl
Glycolysis, Gluconeogenesis, and Pyruvate Metaboli	glucose	LC/MS polar	48152	C00031	HMDB00122	79025	1.03	1.01	1.01
	fructose 1,6-diphosphate/glucose 1,6-diphosphate/n	LC/MS neg	46896	C00354			1.62	0.84	1.93
	dihydroxyacetone phosphate (DHAP)	LC/MS neg	15522	C00111	HMDB01473	668	1.48	1.68	0.88
	3-phosphoglycerate	LC/MS neg	1414	C00597	HMDB00807	724	0.86	0.81	1.06
	phosphoenolpyruvate (PEP)	LC/MS neg	597	C00074	HMDB00263	1005	0.77	0.73	1.06
	pyruvate	LC/MS polar	48990	C00022	HMDB00243	1060	1.01	0.86	1.17
	lactate	LC/MS polar	527	C00186	HMDB00190	612	0.63	0.85	0.74
	glycerate	LC/MS polar	1572	C00258	HMDB00139	752	1.04	1.12	0.93
Pentose Phosphate Pathw ay	6-phosphogluconate	LC/MS neg	15442	C00345	HMDB01316	91493	0.60	0.71	0.86
	sedoheptulose-7-phosphate	LC/MS neg	35649	C05382	HMDB01068	616	0.87	0.77	1.13
Pentose Metabolism	ribose	LC/MS polar	1471	C00121	HMDB00283	5779	0.94	1.01	0.94
	ribitol	LC/MS polar	15772	C00474	HMDB00508	6912	0.01	0.87	0.02
	ribonate	LC/MS polar	27731	C01685	HMDB00867	5460677	0.78	0.91	0.86
	arabitol/xylitol	LC/MS polar	48885	C01904		6912	1.10	0.96	1.15
	ribulose/xylulose	LC/MS polar	54671			5289590	0.74	0.94	0.79
	arabonate/xylonate	LC/MS polar	48255				0.93	0.93	1.00
	sedoheptulose	LC/MS polar	53237		HMDB03219	5459879	0.84	0.75	1.13
	ribulonate/xylulonate*	LC/MS polar	61858				1.01	1.00	1.01
Glycogen Metabolism	maltotetraose	LC/MS polar	15910	C02052	HMDB01296	446495	0.68	0.74	0.91
	maltotriose	LC/MS polar	44688	C01835	HMDB01262	439586	1.04	0.83	1.25
	maltose	LC/MS polar	15586	C00208	HMDB00163	10991489	1.26	0.90	1.40
	isomaltose	LC/MS polar	39777	C00252	HMDB02923	439193	1.02	1.13	0.91
Fructose, Mannose and Galactose Metabolism	fructose	LC/MS polar	48195	C00095	HMDB00660	5984	0.85	0.90	0.94
	mannitol/sorbitol	LC/MS polar	46142	C00794	HMDB00247	5780	0.66	0.93	0.71
	mannose	LC/MS polar	48153	C00159	HMDB00169	18950	0.71	0.62	1.15
	galactitol (dulcitol)	LC/MS polar	1117	C01697	HMDB00107	11850	0.81	0.64	1.27
	galactonate	LC/MS polar	27719	C00880	HMDB00565	128869	0.66	0.64	1.03
Nucleotide Sugar	UDP-N-acetylglucosamine/galactosamine	LC/MS neg	46148				0.81	0.81	1.00
Aminosugar Metabolism	glucuronate	LC/MS polar	15443	C00191	HMDB00127	444791	0.75	0.95	0.79
	N-acetylglucosamine 6-phosphate	LC/MS polar	15107	C00357	HMDB02817	439219	2.39	0.90	2.67
	N-acetylglucosaminylasparagine	LC/MS pos early	48149	C04540	HMDB00489	123826	1.26	0.93	1.36
	erythronate*	LC/MS polar	42420		HMDB00613	2781043	0.90	0.98	0.91
	N-acetylglucosamine/N-acetylgalactosamine	LC/MS pos early	46539		HMDB00215	24139	0.87	0.93	0.94
Advanced Glycation End-product	N6-carboxymethyllysine	LC/MS pos early	36713			123800	1.06	0.97	1.09
TCA Cycle	citrate	LC/MS neg	1564	C00158	HMDB00094	311	0.85	1.18	0.72
	aconitate [cis or trans]	LC/MS neg	46173				0.95	0.92	1.03
	isocitric lactone	LC/MS polar	54724			98259	1.59	1.85	0.86
	alpha-ketoglutarate	LC/MS polar	528	C00026	HMDB00208	51	1.35	1.07	1.26
	succinate	LC/MS polar	1437	C00042	HMDB00254	1110	1.24	0.87	1.41
	fumarate	LC/MS polar	1643	C00122	HMDB00134	444972	0.71	1.04	0.69
	malate	LC/MS neg	1303	C00149	HMDB00156	525	0.76	0.97	0.78
	itaconate	LC/MS polar	18373	C00490	HMDB02092	811	0.92	0.59	1.58
	tricarballylate	LC/MS polar	15729	C19806	HMDB31193	14925	0.90	0.92	0.98
	2-methylcitrate	LC/MS neg	37483	C02225	HMDB00379	439681	1.09	1.03	1.06
	mesaconate (methylfumarate)	LC/MS polar	18493	C01732	HMDB00749	638129	0.91	0.62	1.46
Oxidative Phosphorylation	acetylphosphate	LC/MS polar	15488	C00227	HMDB01494	186	1.13	0.71	1.60
	phosphate	LC/MS pos early	42109	C00009	HMDB01429	1061	1.00	0.99	1.01

Sub Pathway	Biochemical Name	Platform	Comp ID	KEGG	HMDB	PubChem	Gal4>UAS / GAL4 Ctrl	Gal4>UAS / UAS Ctrl	UAS Ctrl / GAL4 Ctrl
Fatty Acid Synthesis	malonate	LC/MS polar	15872	C00383	HMDB00691	867	0.85	0.86	1.00
Medium Chain Fatty Acid	caprate (10:0)	LC/MS neg	1642	C01571	HMDB00511	2969	1.45	1.04	1.40
	laurate (12:0)	LC/MS neg	1645	C02679	HMDB00638	3893	0.79	1.08	0.73
	5-dodecenoate (12:1n7)	LC/MS neg	33968		HMDB00529	5312378	1.17	1.14	1.03
Long Chain Fatty Acid	myristate (14:0)	LC/MS neg	1365	C06424	HMDB00806	11005	1.18	1.04	1.14
	myristoleate (14:1n5)	LC/MS neg	32418	C08322	HMDB02000	5281119	0.99	1.38	0.72
	pentadecanoate (15:0)	LC/MS neg	1361	C16537	HMDB00826	13849	1.40	0.98	1.43
	palmitate (16:0)	LC/MS neg	1336	C00249	HMDB00220	985	1.21	0.99	1.22
	palmitoleate (16:1n7)	LC/MS neg	33447	C08362	HMDB03229	445638	1.19	1.01	1.18
	margarate (17:0)	LC/MS neg	1121		HMDB02259	10465	1.41	0.91	1.55
	10-heptadecenoate (17:1n7)	LC/MS neg	33971		HMDB60038	5312435	1.54	0.89	1.72
	stearate (18:0)	LC/MS neg	1358	C01530	HMDB00827	5281	1.10	0.95	1.16
	oleate/vaccenate (18:1)	LC/MS neg	52285				1.16	1.04	1.11
	nonadecanoate (19:0)	LC/MS neg	1356	C16535	HMDB00772	12591	1.25	1.08	1.16
	10-nonadecenoate (19:1n9)	LC/MS neg	33972		HMDB13622	5312513	1.16	0.72	1.62
	arachidate (20:0)	LC/MS neg	1118	C06425	HMDB02212	10467	1.34	0.92	1.45
	eicosenoate (20:1)	LC/MS neg	33587	C16526	HMDB02231	5282768	1.55	1.16	1.33
	erucate (22:1n9)	LC/MS neg	1552	C08316	HMDB02068	5281116	1.16	0.82	1.41
Polyunsaturated Fatty Acid (n3 and n6)	hexadecatrienoate (16:3n3)	LC/MS neg	57651			5312428	0.88	0.96	0.91
	stearidonate (18:4n3)	LC/MS neg	33969	C16300	HMDB06547	5312508	0.89	0.66	1.34
	eicosapentaenoate (EPA; 20:5n3)	LC/MS neg	18467	C06428	HMDB01999	446284	1.25	1.45	0.86
	linoleate (18:2n6)	LC/MS neg	1105	C01595	HMDB00673	5280450	1.10	0.96	1.14
	linolenate [alpha or gamma; (18:3n3 or 6)]	LC/MS neg	34035	C06426	HMDB03073	5280934	1.08	0.97	1.11
	dihomo-linolenate (20:3n3 or n6)	LC/MS neg	35718	C03242	HMDB02925	5280581	0.80	0.87	0.92
	arachidonate (20:4n6)	LC/MS neg	1110	C00219	HMDB01043	444899	1.30	1.19	1.10
	dihomo-linoleate (20:2n6)	LC/MS neg	17805	C16525	HMDB05060	6439848	1.61	1.87	0.86
Fatty Acid, Branched	13-methylmyristate (i15:0)	LC/MS neg	38293			151014	1.20	0.86	1.40
	15-methylpalmitate (i17:0)	LC/MS neg	38768			17903417	1.18	0.77	1.54
	17-methylstearate (i19:0)	LC/MS neg	38296		HMDB37397	3083779	1.27	0.80	1.59
Fatty Acid, Dicarboxylate	glutarate (C5-DC)	LC/MS polar	396	C00489	HMDB00661	743	0.90	1.00	0.90
	2-hydroxyglutarate	LC/MS pos early	37253	C02630	HMDB00606	43	1.10	1.04	1.06
	adipate (C6-DC)	LC/MS polar	21134	C06104	HMDB00448	196	0.90	1.38	0.65
	suberate (C8-DC)	LC/MS polar	15730	C08278	HMDB00893	10457	1.46	1.51	0.97
	azelate (C9-DC)	LC/MS neg	18362	C08261	HMDB00784	2266	2.01	2.14	0.94
	sebacate (C10-DC)	LC/MS polar	32398	C08277	HMDB00792	5192	0.72	0.94	0.76
	dodecanedioate (C12-DC)	LC/MS neg	32388	C02678	HMDB00623	12736	0.59	0.94	0.62
	tetradecanedioate (C14-DC)	LC/MS neg	35669		HMDB00872	13185	0.92	1.04	0.89
	hexadecanedioate (C16-DC)	LC/MS neg	35678	C19615	HMDB00672	10459	1.30	1.18	1.10
	hexadecenedioate (C16:1-DC)*	LC/MS neg	61862				1.09	1.32	0.82
	octadecadienedioate (C18:2-DC)*	LC/MS neg	61860				1.00	1.00	1.00
Fatty Acid, Amino	2-aminooctanoate	LC/MS pos late	43343		HMDB00991	69522	2.29	0.97	2.36
	N-acetyl-2-aminooctanoate*	LC/MS neg	62059		HMDB59745	95555	1.15	0.90	1.28
Fatty Acid Metabolism (also BCAA Metabolism)	propionylglycine	LC/MS polar	31932		HMDB00783	98681	0.65	1.12	0.58
	methylmalonate (MMA)	LC/MS polar	1496	C02170	HMDB00202	487	0.70	0.96	0.73
Fatty Acid Metabolism(Acyl Glycine)	hexanoylglycine	LC/MS neg	35436		HMDB00701	99463	1.19	1.10	1.08
Fatty Acid Metabolism(Acyl Carnitine)	acetylcarnitine (C2)	LC/MS pos early	32198	C02571	HMDB00201	1	4.25	2.68	1.59
	myristoylcarnitine (C14)	LC/MS pos late	33952		HMDB05066	6426854	28.81	26.39	1.09
	palmitoylcarnitine (C16)	LC/MS pos late	44681	C02990	HMDB00222	461	4.52	1.74	2.60
	palmitoleoylcarnitine (C16:1)*	LC/MS pos late	53223			71464547	26.50	10.35	2.56
	stearoylcarnitine (C18)	LC/MS pos late	34409		HMDB00848	6426855	1.43	1.06	1.34
	linoleoylcarnitine (C18:2)*	LC/MS pos late	46223		HMDB06469	6450015	6.77	3.14	2.15
	oleoylcarnitine (C18:1)	LC/MS pos late	35160		HMDB05065	6441392	4.00	1.92	2.08
	arachidoylcarnitine (C20)*	LC/MS pos late	57513		HMDB06460		1.28	1.17	1.09
	behenoylcarnitine (C22)*	LC/MS pos late	57514				1.31	1.09	1.20
	eicosenoylcarnitine (C20:1)*	LC/MS pos late	57519				2.28	1.24	1.84
	lignoceroylcarnitine (C24)*	LC/MS pos late	57515				0.72	0.83	0.87
	margaroylcarnitine (C17)*	LC/MS pos late	57512		HMDB06210		4.05	1.47	2.76
	nervonoylcarnitine (C24:1)*	LC/MS pos late	57531				2.36	1.26	1.87
	cerotoylcarnitine (C26)*	LC/MS pos late	57516		HMDB06347		0.85	0.97	0.87
	ximenoylcarnitine (C26:1)*	LC/MS pos late	57517				0.75	0.88	0.85
Carnitine Metabolism	deoxycarnitine	LC/MS pos early	36747	C01181	HMDB01161	134	1.77	1.17	1.52
	carnitine	LC/MS pos early	15500	C00318	HMDB00062	10917	1.62	1.01	1.59

Sub Pathway	Biochemical Name	Platform	Comp ID	KEGG	HMDB	PubChem	Gal4>UAS / GAL4 Ctrl	Gal4>UAS / UAS Ctrl	UAS Ctrl / GAL4 Ctrl
Fatty Acid, Monohydroxy	3-hydroxypropanoate	LC/MS polar	1556	C01013	HMDB00700	68152	0.96	1.20	0.80
	3-hydroxydecanoate	LC/MS neg	22053		HMDB02203	26612	0.90	0.71	1.25
	3-hydroxysebacate	LC/MS polar	31943		HMDB00350	3017884	0.62	0.57	1.09
	3-hydroxylaurate	LC/MS neg	32457		HMDB00387	94216	1.07	1.30	0.82
	3-hydroxymyristate	LC/MS neg	21158			16064	1.63	1.02	1.59
	3-hydroxyoleate*	LC/MS neg	61843				1.51	0.88	1.72
	13-HODE + 9-HODE	LC/MS neg	37752			43013	1.19	1.05	1.13
Fatty Acid, Dihydroxy	12,13-DiHOME	LC/MS neg	38395	C14829	HMDB04705	10236635	0.63	0.99	0.63
	9,10-DiHOME	LC/MS neg	38399	C14828	HMDB04704	9966640	0.83	1.37	0.61
Endocannabinoid	N-myristoyltaurine*	LC/MS neg	61825			3810823	1.24	0.63	1.97
	N-oleoyltaurine	LC/MS neg	39732			6437033	1.16	0.81	1.42
	N-palmitoleoyltaurine*	LC/MS neg	61824				0.97	0.82	1.18
	linoleoyl ethanolamide	LC/MS neg	52608		HMDB12252	5283446	1.02	0.73	1.40
Inositol Metabolism	myo-inositol	LC/MS polar	1124	C00137	HMDB00211	892	1.03	0.94	1.10
Phospholipid Metabolism	choline	LC/MS pos early	15506	C00114	HMDB00097	305	1.01	1.04	0.97
	choline phosphate	LC/MS pos early	34396	C00588	HMDB01565	1014	1.08	1.14	0.95
	cytidine 5'-diphosphocholine	LC/MS pos early	34418	C00307	HMDB01413	13804	1.13	0.92	1.23
	glycerophosphorylcholine (GPC)	LC/MS pos early	15990	C00670	HMDB00086	71920	0.97	0.99	0.97
	phosphoethanolamine	LC/MS pos early	1600	C00346	HMDB00224	1015	0.93	1.05	0.88
	cytidine-5'-diphosphoethanolamine	LC/MS polar	34410	C00570	HMDB01564	123727	0.97	0.85	1.14
	glycerophosphoethanolamine	LC/MS polar	37455	C01233	HMDB00114	123874	0.89	0.97	0.91
	glycerophosphoserine*	LC/MS pos early	57404			3081457	1.33	1.48	0.90
	glycerophosphoinositol*	LC/MS pos early	52307			167572	1.23	1.31	0.93
Phosphatidylcholine (PC)	1-myristoyl-2-palmitoyl-GPC (14:0/16:0)	LC/MS pos late	19258		HMDB07869	129657	0.79	0.92	0.87
	1,2-dipalmitoyl-GPC (16:0/16:0)	LC/MS pos late	19130		HMDB00564	452110	0.71	0.90	0.79
	1-palmitoyl-2-palmitoleoyl-GPC (16:0/16:1)*	LC/MS pos late	52470		HMDB07969		1.04	0.92	1.12
	1-palmitoyl-2-stearoyl-GPC (16:0/18:0)	LC/MS pos late	52616		HMDB07970		0.71	0.92	0.77
	1-palmitoyl-2-oleoyl-GPC (16:0/18:1)	LC/MS pos late	52461		HMDB07972	6436017	0.95	0.97	0.98
	1,2-dipalmitoleoyl-GPC (16:1/16:1)*	LC/MS pos late	52472				0.95	0.83	1.15
	1-palmitoleoyl-2-linolenoyl-GPC (16:1/18:3)*	LC/MS pos late	53180		HMDB08008		0.92	1.03	0.90
	1,2-distearoyl-GPC (18:0/18:0)	LC/MS pos late	19132		HMDB08036	94190	0.72	0.93	0.78
	1-stearoyl-2-oleoyl-GPC (18:0/18:1)	LC/MS pos late	52438		HMDB08038		0.85	1.01	0.84
	1-oleoyl-2-linoleoyl-GPC (18:1/18:2)*	LC/MS pos late	52453				0.90	0.88	1.02
	1,2-dilinoleoyl-GPC (18:2/18:2)	LC/MS pos late	52603		HMDB08138	5288075	0.78	0.81	0.97
	1-linoleoyl-2-linolenoyl-GPC (18:2/18:3)*	LC/MS pos late	53176		HMDB08141		0.79	0.88	0.90
	1,2-dilinolenoyl-GPC (18:3/18:3)*	LC/MS pos late	53179		HMDB08206		0.77	0.89	0.87
Phosphatidylethanolamine (PE)	1,2-dipalmitoyl-GPE (16:0/16:0)*	LC/MS pos late	57341		HMDB08923	445468	0.84	0.97	0.87
	1-palmitoyl-2-stearoyl-GPE (16:0/18:0)*	LC/MS pos late	57388		HMDB08925	5326793	0.64	0.78	0.82
	1-palmitoyl-2-oleoyl-GPE (16:0/18:1)	LC/MS pos late	19263		HMDB05320	5283496	0.96	0.94	1.02
	1,2-dipalmitoleoyl-GPE (16:1/16:1)*	LC/MS pos late	52688		HMDB05342	9546809	0.84	0.86	0.98
	1-stearoyl-2-oleoyl-GPE (18:0/18:1)	LC/MS pos late	42448		HMDB08993		0.96	1.03	0.93
	1-oleoyl-2-linoleoyl-GPE (18:1/18:2)*	LC/MS pos late	52687		HMDB05349	9546753	0.93	0.91	1.03
	1,2-dilinoleoyl-GPE (18:2/18:2)*	LC/MS pos late	53174		HMDB09093	9546812	0.85	0.85	0.99
Phosphatidylserine (PS)	1,2-dioleoyl-GPS (18:1/18:1)	LC/MS pos late	19191			6438639	1.29	0.95	1.35
Phosphatidylglycerol (PG)	1-palmitoyl-2-palmitoleoyl-GPG (16:0/16:1)*	LC/MS pos late	53213				1.32	0.78	1.68
	1-palmitoyl-2-oleoyl-GPG (16:0/18:1)	LC/MS pos late	52448			5283509	0.91	0.81	1.11
Phosphatidylinositol (PI)	1,2-dipalmitoleoyl-GPI (16:1/16:1)*	LC/MS pos late	52721				1.06	0.90	1.18
	1-palmitoyl-2-oleoyl-GPI (16:0/18:1)*	LC/MS polar	52669		HMDB09783		0.91	0.77	1.18
	1-oleoyl-2-linoleoyl-GPI (18:1/18:2)*	LC/MS polar	52451		HMDB09838		0.89	0.90	0.99

Sub Pathway	Biochemical Name	Platform	Comp ID	KEGG	HMDB	PubChem	Gal4>UAS / GAL4 Ctrl	Gal4>UAS / UAS Ctrl	UAS Ctrl / GAL4 Ctrl
Lysophospholipid	1-linolenoyl-GPG (18:3)*	LC/MS neg	62368				1.17	0.98	1.20
	1-palmitoyl-GPC (16:0)	LC/MS pos late	33955		HMDB10382	86554	**0.79**	0.98	**0.81**
	2-palmitoyl-GPC (16:0)*	LC/MS pos late	35253		HMDB61702	15061532	0.98	0.92	1.07
	1-palmitoleoyl-GPC (16:1)*	LC/MS pos late	33230		HMDB10383	24779461	1.06	1.07	0.99
	2-palmitoleoyl-GPC (16:1)*	LC/MS pos late	35819		HMDB10383		1.14	0.99	1.15
	1-stearoyl-GPC (18:0)	LC/MS pos late	33961		HMDB10384	497299	0.75	1.01	0.75
	1-oleoyl-GPC (18:1)	LC/MS pos late	48258		HMDB02815	16081932	1.06	1.07	0.99
	1-linoleoyl-GPC (18:2)	LC/MS neg	34419	C04100	HMDB10386	11988421	1.15	0.95	1.20
	1-linolenoyl-GPC (18:3)*	LC/MS pos late	45951		HMDB10388		**0.74**	0.98	**0.75**
	1-palmitoyl-GPE (16:0)	LC/MS pos late	35631		HMDB11503	9547069	0.85	0.93	0.92
	1-stearoyl-GPE (18:0)	LC/MS pos late	42398		HMDB11130	9547068	0.76	0.91	0.84
	2-stearoyl-GPE (18:0)*	LC/MS neg	41220		HMDB11129		0.90	1.05	0.86
	1-oleoyl-GPE (18:1)	LC/MS pos late	35628		HMDB11506	9547071	0.98	0.94	1.04
	1-linoleoyl-GPE (18:2)*	LC/MS pos late	36600		HMDB11507	52925130	0.95	1.00	0.95
	1-palmitoyl-GPS (16:0)*	LC/MS neg	46130			9547100	1.57	1.17	1.35
	1-stearoyl-GPS (18:0)*	LC/MS neg	45966			9547101	1.23	0.87	1.42
	1-oleoyl-GPS (18:1)	LC/MS neg	19260		HMDB61694	9547099	1.49	1.00	1.49
	1-linoleoyl-GPS (18:2)*	LC/MS neg	43676				1.11	0.81	1.38
	1-palmitoyl-GPG (16:0)*	LC/MS neg	45970			3300276	1.03	0.77	1.34
	1-stearoyl-GPG (18:0)	LC/MS neg	34437				0.96	0.93	1.03
	1-oleoyl-GPG (18:1)*	LC/MS neg	45968				1.27	1.16	1.09
	1-linoleoyl-GPG (18:2)*	LC/MS neg	54885				1.11	0.91	1.22
	1-palmitoyl-GPI (16:0)	LC/MS neg	35305		HMDB61695		1.11	0.95	1.17
	1-stearoyl-GPI (18:0)	LC/MS neg	19324		HMDB61696		0.83	0.66	1.26
	1-oleoyl-GPI (18:1)*	LC/MS neg	36602				1.65	1.12	1.48
	1-linoleoyl-GPI (18:2)*	LC/MS neg	36594				1.20	0.99	1.21
Glycolipid Metabolism	1,2-dilinoleoyl-galactosylglycerol (18:2/18:2)*	LC/MS pos late	54899			6535011	1.05	0.90	1.16
Plasmalogen	1-(1-enyl-palmitoyl)-2-oleoyl-GPE (P-16:0/18:1)*	LC/MS pos late	52477		HMDB11342		1.23	1.06	1.15
	1-(1-enyl-stearoyl)-2-oleoyl-GPE (P-18:0/18:1)	LC/MS pos late	52614		HMDB11375		0.94	0.86	1.09
	1-(1-enyl-stearoyl)-2-linoleoyl-GPE (P-18:0/18:2)*	LC/MS pos late	52748		HMDB11376		0.75	1.02	0.74
Lysoplasmalogen	1-(1-enyl-stearoyl)-GPE (P-18:0)*	LC/MS pos late	39271				0.67	1.01	0.67
Glycerolipid Metabolism	glycerol	LC/MS neg	15122	C00116	HMDB00131	753	1.19	1.09	1.09
	glycerol 3-phosphate	LC/MS polar	43847	C00093	HMDB00126	754	1.09	1.15	0.95
	glycerophosphoglycerol	LC/MS polar	48857	C03274		439964	1.02	1.26	0.81
Monoacylglycerol	1-myristoylglycerol (14:0)	LC/MS neg	35625	C01885	HMDB11561	79050	1.26	0.76	1.65
	1-palmitoylglycerol (16:0)	LC/MS neg	21127		HMDB31074	14900	1.04	0.93	1.12
	1-palmitoleoylglycerol (16:1)*	LC/MS neg	52431		HMDB11565		2.31	1.29	1.79
	1-oleoylglycerol (18:1)	LC/MS neg	21184		HMDB11567	5283468	1.85	1.31	1.41
	1-linoleoylglycerol (18:2)	LC/MS neg	27447			5283469	1.95	1.40	1.40
	2-myristoylglycerol (14:0)	LC/MS neg	34383		HMDB11530	137938	1.06	0.75	1.41
	2-palmitoylglycerol (16:0)	LC/MS neg	33419		HMDB11533	123409	1.49	1.09	1.37
	2-palmitoleoylglycerol (16:1)*	LC/MS neg	52432		HMDB11565		2.25	1.15	1.96
	2-oleoylglycerol (18:1)	LC/MS neg	21232		HMDB11537	5319879	1.66	0.87	1.90
	2-linoleoylglycerol (18:2)	LC/MS neg	32506		HMDB11538	5365676	1.61	1.64	0.98
Diacylglycerol	diacylglycerol (12:0/18:1, 14:0/16:1, 16:0/14:1) [2]*	LC/MS pos late	55001				1.23	1.19	1.03
	diacylglycerol (14:0/18:1, 16:0/16:1) [2]*	LC/MS pos late	54954				**1.39**	1.07	1.30
	diacylglycerol (16:1/18:2 [2], 16:0/18:3 [1])*	LC/MS pos late	54966				1.18	1.10	1.07
	palmitoyl-oleoyl-glycerol (16:0/18:1) [2]*	LC/MS pos late	54942	C13861	HMDB07102		1.45	0.93	1.56
	palmitoyl-linoleoyl-glycerol (16:0/18:2) [2]*	LC/MS pos late	52634		HMDB07103		1.55	0.93	1.67
	palmitoleoyl-oleoyl-glycerol (16:1/18:1) [2]*	LC/MS pos late	52631				1.12	1.07	1.04
	palmitoleoyl-linoleoyl-glycerol (16:1/18:2) [1]*	LC/MS pos late	54967		HMDB07132		1.22	1.13	1.08
	oleoyl-oleoyl-glycerol (18:1/18:1) [2]*	LC/MS pos late	54946		HMDB07218		1.08	1.04	1.03
	oleoyl-linoleoyl-glycerol (18:1/18:2) [2]	LC/MS pos late	46799		HMDB07219		1.00	1.17	0.86
	oleoyl-linolenoyl-glycerol (18:1/18:3) [2]*	LC/MS pos late	54970		HMDB07220		0.93	**1.81**	**0.51**
	linoleoyl-linolenoyl-glycerol (18:2/18:3) [2]*	LC/MS pos late	54964		HMDB07250		**0.70**	1.22	**0.57**

Sub Pathway	Biochemical Name	Platform	Comp ID	KEGG	HMDB	PubChem	Gal4>UAS / GAL4 Ctrl	Gal4>UAS / UAS Ctrl	UAS Ctrl / GAL4 Ctrl
Sphingolipid Synthesis	sphinganine	LC/MS pos late	17769	C00836	HMDB00269	3126	0.55	1.00	0.55
	tetradecasphinganine (d14:0)*	LC/MS pos late	57543				1.15	1.08	1.07
	hexadecasphinganine (d16:0)*	LC/MS pos late	57544	C13915		656816	1.21	1.04	1.16
Dihydroceramides	N-arachidoyl-tetradecanoylsphinganine (d14:0/20:0)*	LC/MS pos late	57499				0.89	1.07	0.84
	N-behenoyl-tetradecanoylsphinganine (d14:0/22:0)*	LC/MS pos late	57501				0.81	0.94	0.86
Ceramides	N-stearoyl-tetradecanoylsphingosine (d14:1/18:0)*	LC/MS pos late	57500				1.02	1.09	0.94
	N-arachidoyl-tetradecanoylsphingosine (d14:1/20:0)*	LC/MS pos late	57505				0.95	1.17	0.81
	N-behenoyl-tetradecanoylsphingosine (d14:1/22:0)*	LC/MS pos late	57494				0.94	0.97	0.97
Hexosylceramides (HCER)	glycosyl-N-arachidoyl-tetradecanoylsphingosine (d1	LC/MS pos late	57496				1.33	1.04	1.28
	glycosyl-N-behenoyl-tetradecasphingosine (d14:1/22	LC/MS pos late	57849				2.56	0.90	2.84
	glycosyl ceramide (d14:1/24:0, d16:1/22:0)*	LC/MS pos late	57495				5.01	0.70	7.14
Lactosylceramides (LCER)	lactosyl-N-arachidoyl-tetradecanoylsphingosine (d14	LC/MS pos late	57498				1.44	1.44	1.00
Sphingosines	tetradecanoylsphingosine (d14:1)*	LC/MS pos late	57493				1.15	0.98	1.17
	hexadecasphingosine (d16:1)*	LC/MS pos late	57428				1.04	1.03	1.01
Mevalonate Metabolism	3-hydroxy-3-methylglutarate	LC/MS polar	531	C03761	HMDB00355	1662	1.07	1.08	1.00
	mevalonate	LC/MS polar	39583	C02104	HMDB00227	439230	0.89	1.91	0.46
Sterol	beta-sitosterol	LC/MS pos late	27414	C01753	HMDB00852	222284	1.08	1.14	0.94
	campesterol	LC/MS pos late	33997	C01789	HMDB02869	173183	1.00	1.19	0.84
	ergosterol	LC/MS pos late	27553	C01694	HMDB00878	444679	0.93	0.99	0.94
Purine Metabolism, (Hypo)Xanthine/Inosine containing	inosine 5'-monophosphate (IMP)	LC/MS pos early	2133	C00130	HMDB00175	8582	1.19	0.70	1.70
	inosine	LC/MS neg	1123	C00294	HMDB00195	6021	0.95	1.02	0.93
	hypoxanthine	LC/MS polar	3127	C00262	HMDB00157	790	0.54	1.17	0.46
	xanthine	LC/MS polar	3147	C00385	HMDB00292	1188	0.98	1.55	0.63
	xanthosine 5'-monophosphate (xmp)	LC/MS neg	12024	C00655	HMDB01554	73323	0.75	1.00	0.75
	xanthosine	LC/MS neg	15136	C01762	HMDB00299	64959	1.69	2.14	0.79
	N1-methylinosine	LC/MS pos early	48351		HMDB02721	65095	2.71	1.36	2.00
	2'-deoxyinosine	LC/MS neg	15076	C05512	HMDB00071	65058	2.82	2.51	1.12
	urate	LC/MS neg	1604	C00366	HMDB00289	1175	0.73	0.98	0.74
	uric acid ribonucleoside*	LC/MS neg	62102			164933	1.08	1.56	0.70
	allantoin	LC/MS polar	1107	C02350	HMDB00462	204	2.88	0.93	3.09
Purine Metabolism, Adenine containing	adenosine 5'-diphosphate (ADP)	LC/MS neg	3108	C00008	HMDB01341	6022	1.08	0.56	1.93
	adenosine 5'-monophosphate (AMP)	LC/MS pos early	32342	C00020	HMDB00045	6083	1.13	0.65	1.74
	adenosine 3'-monophosphate (3'-AMP)	LC/MS neg	35142	C01367	HMDB03540	41211	0.76	0.75	1.00
	adenosine-2',3'-cyclic monophosphate	LC/MS neg	37467	C02353	HMDB11616	2024	1.01	0.87	1.17
	adenosine	LC/MS pos early	555	C00212	HMDB00050	60961	1.00	0.98	1.02
	adenine	LC/MS pos early	554	C00147	HMDB00034	190	0.59	0.85	0.70
	1-methyladenine	LC/MS pos early	1527	C02216	HMDB11599	78821	0.46	1.11	0.41
	N1-methyladenosine	LC/MS pos early	15650	C02494	HMDB03331	27476	0.87	1.03	0.85
	N6-succinyladenosine	LC/MS pos early	48130		HMDB00912	165243	0.96	0.72	1.32
Purine Metabolism, Guanine containing	guanosine 5'- diphosphate (GDP)	LC/MS neg	2848	C00035	HMDB01201	8977	0.80	0.69	1.16
	guanosine 5'- monophosphate (5'-GMP)	LC/MS neg	2849	C00144	HMDB01397	6804	0.98	0.75	1.31
	guanosine-2',3'-cyclic monophosphate	LC/MS neg	37139	C06194	HMDB11629	92823	1.01	0.75	1.35
	guanosine	LC/MS neg	1573	C00387	HMDB00133	6802	1.26	1.18	1.07
	guanine	LC/MS pos early	32352	C00242	HMDB00132	764	1.05	0.99	1.06
	7-methylguanine	LC/MS pos early	35114	C02242	HMDB00897	11361	0.99	1.20	0.82
	2'-O-methylguanosine	LC/MS neg	36811	C04545			1.53	1.18	1.30
	7-methylguanosine	LC/MS pos early	31580	C20674			1.15	0.91	1.26
	N2,N2-dimethylguanosine	LC/MS neg	35137		HMDB04824	92919	1.86	1.11	1.68
	2'-deoxyguanosine	LC/MS neg	1411	C00330	HMDB00085	187790	1.36	1.22	1.12

Sub Pathway	Biochemical Name	Platform	Comp ID	KEGG	HMDB	PubChem	Gal4>UAS GAL4 Ctrl	Gal4>UAS UAS Ctrl	UAS Ctrl GAL4 Ctrl
Pyrimidine Metabolism, Orotate containing	dihydroorotate	LC/MS polar	601	C00337	HMDB03349	648	4.52	0.68	6.61
	orotate	LC/MS polar	1505	C00295	HMDB00226	967	1.34	0.92	1.45
Pyrimidine Metabolism, Uracil containing	uridine-2',3'-cyclic monophosphate	LC/MS neg	37137	C02355	HMDB11640	439715	0.78	0.79	0.98
	uridine	LC/MS neg	606	C00299	HMDB00296	6029	1.50	1.53	0.98
	uracil	LC/MS polar	605	C00106	HMDB00300	1174	0.90	0.91	1.00
	pseudouridine	LC/MS neg	33442	C02067	HMDB00767	15047	1.49	1.74	0.85
	2'-O-methyluridine	LC/MS neg	57655			102212	1.69	2.76	0.61
	3-ureidopropionate	LC/MS pos early	3155	C02642	HMDB00026	111	1.00	1.29	0.77
	beta-alanine	LC/MS pos early	55	C00099	HMDB00056	239	1.21	1.00	1.21
	N-acetyl-beta-alanine	LC/MS polar	37432	C01073		76406	1.07	1.20	0.90
Pyrimidine Metabolism, Cytidine containing	cytidine 5'-monophosphate (5'-CMP)	LC/MS pos early	2372	C00055	HMDB00095	6131	1.55	1.00	1.55
	cytidine 2' or 3'-monophosphate (2' or 3'-CMP)	LC/MS pos early	61705				0.96	0.93	1.03
	cytidine 2',3'-cyclic monophosphate	LC/MS neg	37465	C02354	HMDB11691	417654	0.76	0.82	0.92
	cytidine	LC/MS neg	514	C00475	HMDB00089	6175	1.82	1.47	1.24
	cytosine	LC/MS pos early	573	C00380	HMDB00630	597	1.05	1.39	0.76
	3-methylcytidine	LC/MS pos early	35132			159649	1.28	1.21	1.06
	5-methylcytidine	LC/MS pos early	22119		HMDB00982	92918	2.98	1.09	2.72
	2'-O-methylcytidine	LC/MS pos early	57554			150971	2.78	1.94	1.43
Pyrimidine Metabolism, Thymine containing	3-aminoisobutyrate	LC/MS pos early	1566	C05145	HMDB03911	64956	0.56	0.96	0.59
Purine and Pyrimidine Metabolism	methylphosphate	LC/MS pos early	37070		HMDB61711	13130	1.17	0.94	1.24
Dinucleotide	(3'-5')-adenylyluridine	LC/MS neg	52740			112074	1.00	1.00	1.00
Nicotinate and Nicotinamide Metabolism	nicotinate	LC/MS pos early	1504	C00253	HMDB01488	938	0.93	1.21	0.77
	nicotinamide ribonucleotide (NMN)	LC/MS pos early	22152	C00455	HMDB00229	14180	1.11	1.05	1.06
	nicotinamide riboside	LC/MS pos early	33013	C03150	HMDB00855	439924	1.37	2.01	0.68
	nicotinamide adenine dinucleotide (NAD+)	LC/MS pos early	5278	C00003	HMDB00902	5893	0.90	0.76	1.19
	nicotinate adenine dinucleotide (NAAD+)	LC/MS neg	15725			25246170	0.88	0.75	1.18
	trigonelline (N'-methylnicotinate)	LC/MS pos early	32401	C01004	HMDB00875	5570	1.11	0.90	1.23
Riboflavin Metabolism	riboflavin (Vitamin B2)	LC/MS pos early	1827	C00255	HMDB00244	493570	2.72	0.71	3.84
	flavin adenine dinucleotide (FAD)	LC/MS neg	2134	C00016	HMDB01248	643975	0.73	0.74	0.99
Pantothenate and CoA Metabolism	pantothenate	LC/MS neg	1508	C00864	HMDB00210	6613	0.75	0.91	0.82
	pantetheine	LC/MS pos early	57555	C00831		439322	0.52	0.75	0.70
Ascorbate and Aldarate Metabolism	ascorbate (Vitamin C)	LC/MS pos early	32354	C00072	HMDB00044		1.63	1.24	1.31
	dehydroascorbate	LC/MS polar	1659	C05422	HMDB01264	835	0.58	0.70	0.82
	threonate	LC/MS polar	27738	C01620	HMDB00943	151152	0.97	1.11	0.87
	gulonate*	LC/MS polar	46957	C00257	HMDB03290	9794176	0.75	1.01	0.74
Tocopherol Metabolism	alpha-tocopherol	LC/MS pos late	1561	C02477	HMDB01893	14985	1.09	1.23	0.89
	gamma-tocopherol/beta-tocopherol	LC/MS pos late	52473				1.58	1.51	1.04
Biotin Metabolism	biotin	LC/MS pos early	568	C00120	HMDB00030	171548	1.31	1.20	1.09
Tetrahydrobiopterin Metabolism	biopterin	LC/MS neg	12358	C06313	HMDB00468	445040	0.67	0.85	0.79
	dihydrobiopterin	LC/MS pos early	35129	C00268	HMDB00038	1879	0.52	0.78	0.66
Pterin Metabolism	isoxanthopterin	LC/MS pos early	27732	C03975	HMDB00704	10729	0.82	0.76	1.09
	pterin	LC/MS neg	43023	C00715	HMDB00802	73000	1.23	1.12	1.09
	sepiapterin	LC/MS pos early	48139	C00835	HMDB00238	65253	0.54	0.83	0.65
	xanthopterin	LC/MS polar	54728			8397	0.88	0.70	1.26
Hemoglobin and Porphyrin Metabolism	5-aminolevulinate	LC/MS pos early	2290	C00430	HMDB01149	137	0.72	1.67	0.43
Thiamine Metabolism	thiamin (Vitamin B1)	LC/MS pos early	5341	C00378	HMDB00235	1130	0.61	0.56	1.09
	thiamin monophosphate	LC/MS pos early	15798	C01081	HMDB02666	3382778	0.92	0.61	1.51
Vitamin A Metabolism	carotene diol (1)	LC/MS pos late	57635				0.88	1.10	0.80
	carotene diol (2)	LC/MS pos late	57636				0.78	1.25	0.62
	carotene diol (3)	LC/MS pos late	57637				0.52	1.09	0.48
Vitamin B6 Metabolism	pyridoxal	LC/MS pos early	1651	C00250	HMDB01545	1050	1.10	0.94	1.16
	pyridoxate	LC/MS neg	31555	C00847	HMDB00017	6723	1.48	0.96	1.55
Benzoate Metabolism	4-hydroxyhippurate	LC/MS neg	35527		HMDB13678	151012	0.34	1.36	0.25
	4-hydroxybenzoate	LC/MS neg	21133	C00156	HMDB00500	135	0.69	1.06	0.65
Food Component/Plant	2,3-dihydroxyisovalerate	LC/MS polar	38276	C04039	HMDB12141	677	0.39	1.13	0.35
	2-isopropylmalate	LC/MS polar	15667	C02504	HMDB00402	77	0.87	0.84	1.04
	gluconate	LC/MS polar	587	C00257	HMDB00625	10690	0.88	0.87	1.01
	ergothioneine	LC/MS pos early	37459	C05570	HMDB03045	3032311	1.10	1.17	0.94
	erythritol	LC/MS polar	20699	C00503	HMDB02994	222285	0.76	1.03	0.74
	kojibiose	LC/MS polar	21040	C19632	HMDB11742	164939	1.20	1.14	1.06
	panose	LC/MS polar	37284	C00713	HMDB11729	5288421	1.23	0.87	1.42
	quinate	LC/MS polar	18335	C00296	HMDB03072	6508	0.80	1.03	0.78
	stachydrine	LC/MS pos early	34384	C10172	HMDB04827	115244	0.52	0.55	0.95
	methyl glucopyranoside (alpha + beta)	LC/MS pos early	46144				1.10	0.98	1.13
	2-keto-3-deoxy-gluconate	LC/MS polar	48141	C00204	HMDB01353	161227	0.68	0.43	1.58
Drug - Topical Agents	salicylate	LC/MS polar	1515	C00805	HMDB01895	338	0.59	0.67	0.88
Chemical	succinimide	LC/MS polar	41888	C07273		11439	1.26	1.11	1.14
	thioproline	LC/MS pos early	53231			93176	0.77	1.00	0.77

Table S1: Metabolomics of *Repo*>20x*Shibire* fly heads

All measured metabolites and their respective categories are listed for samples from *Repo*-GAL4>UAS-20x*Shibire*, and both parental controls. Welch's t-test was performed on scaled signal for each metabolite, comparing each condition. Green highlighting marks a

significant difference (p≤0.05) between the groups, where metabolite ratio is < 1.00, while light green is not significant, but close to the threshold (0.05<p<0.10). Red highlighting marks a significant difference (p≤0.05) between groups where metabolite ratio is ≥ 1.00, and light red is not significant, but close to the threshold (0.05<p<0.10).

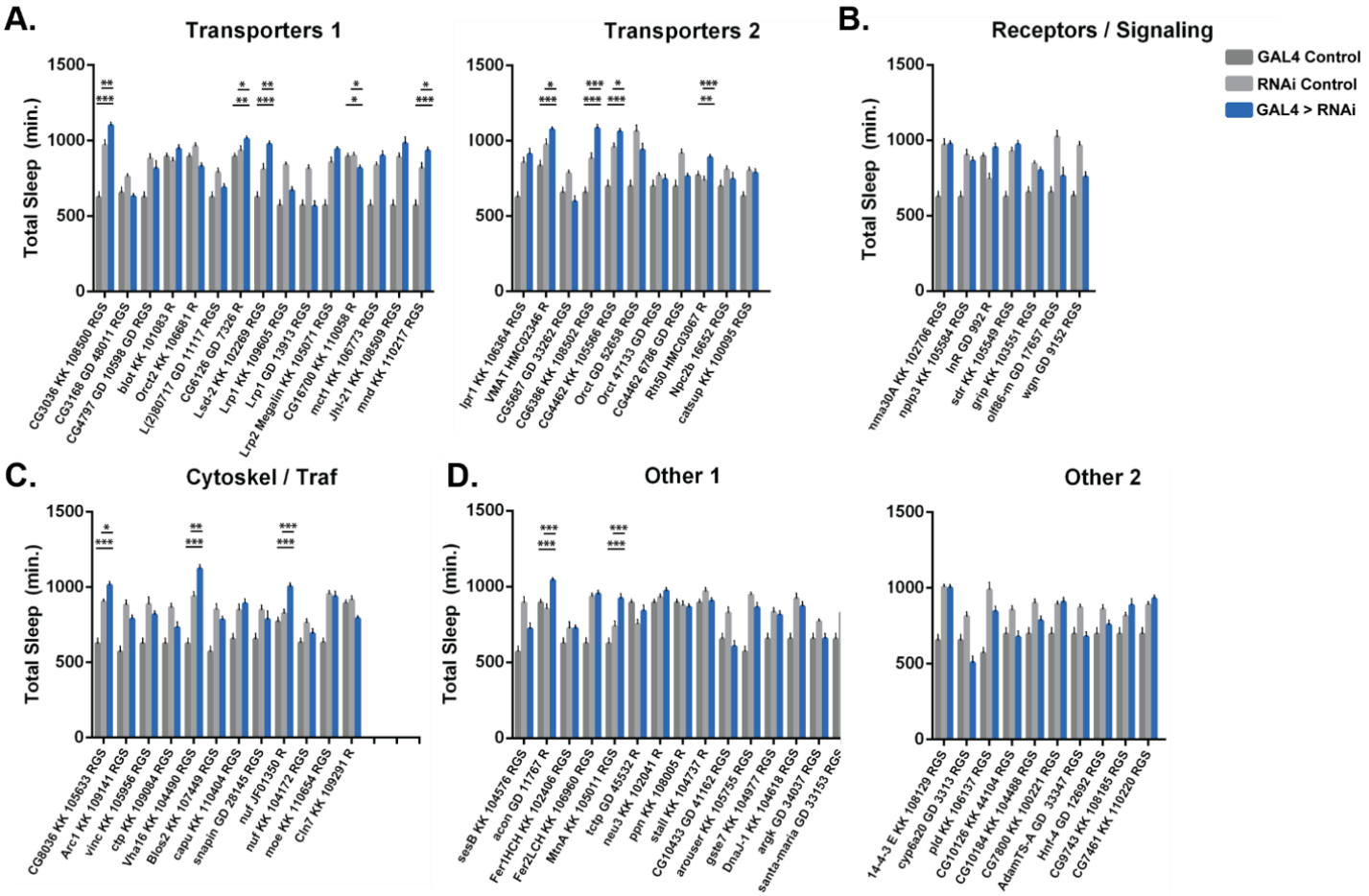

Figure 2 supplement 1: Pan-glial RNAi knockdown screen of candidate genes enriched in the barrier glia

Total sleep in female flies with RNAi knockdown of listed genes (KK and GD are VDRC collections, TRiP lines are from Bloomington Stock Center) (**A**)Transporters, (**B**) Receptors/Signaling pathway factors, (**C**) Cytoskeleton/Trafficking factors and (**D**) other genes by either Repo-Gal4 (labeled R) or UAS-Dicer; RepoGeneSwitch on RU+ food (labeled RGS). n = 9 – 16 flies per genotype, median = 16. One-way ANOVA, with Holk-Sidak post-hoc comparisons. *p < 0.05, **p < 0.01, ***p < 0.001. Error bars represent standard error of the mean (SEM). Significance values only marked for genes in which experimental flies were different from both parental controls. Certain experiments were performed simultaneously and therefore share a Gal4 control, re-plotted per each gene.

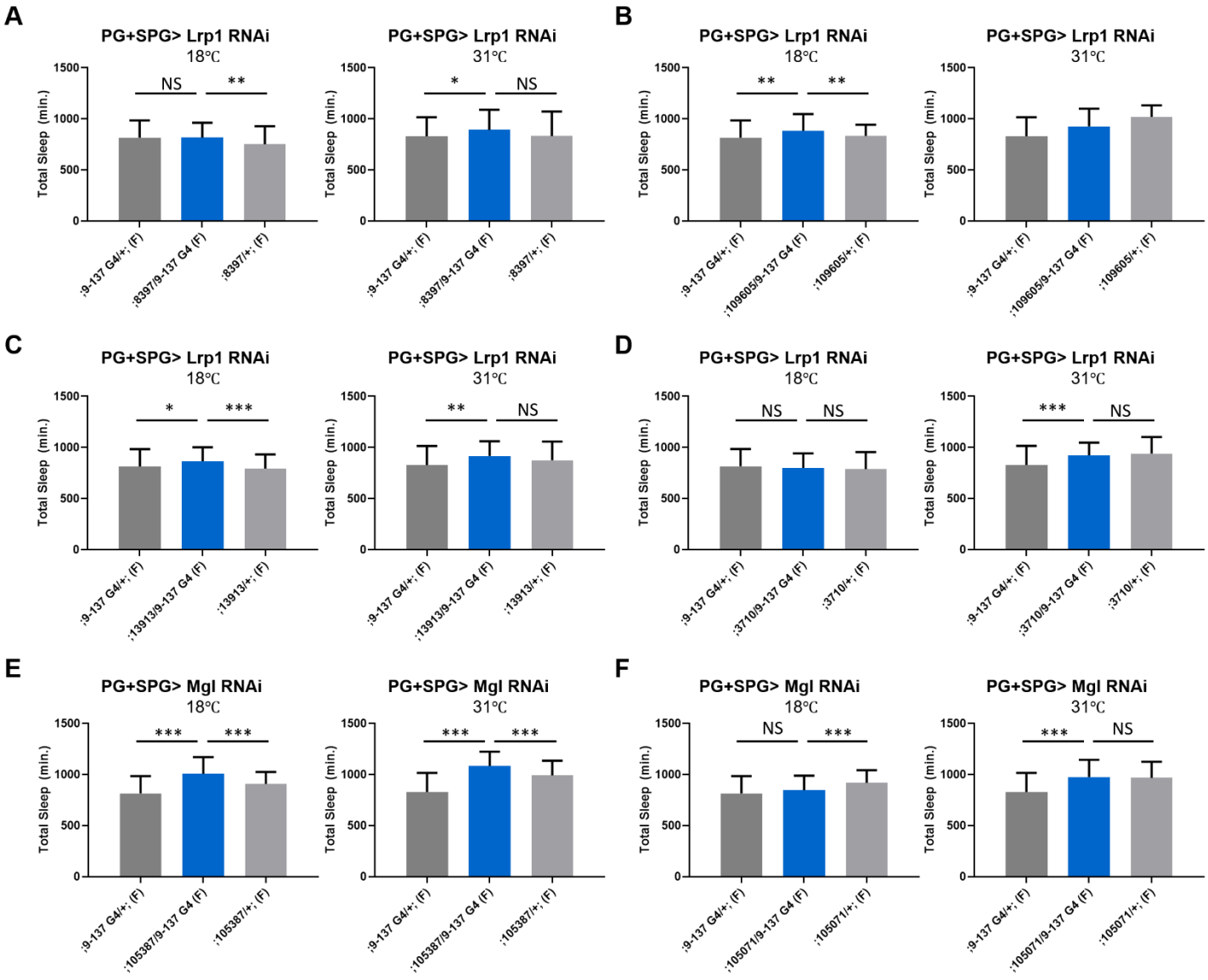

Figure 3 supplement 1. Sleep time changes with knockdown of Lrp genes in surface glia

Total sleep in female flies with knockdown of (**A**) Lrp1 (8397 GD) **(B)**Lrp1(109605 KK) **(C)** Lrp1 (13913 KK) **(D)** Lrp1 (3710 GD) (**E**) Megalin (105387 KK) (F) Megalin (105071 KK) driven by (PG+SPG) driver 9-137-GAL4, n = 40-48 per genotype at 18 ℃(permissive) and at 31 ℃ (restrictive). One-way ANOVA, with Holk-Sidak post-hoc comparisons. *p < 0.05, **p < 0.01, ***p < 0.001. Error bars represent standard error of the mean (SEM).