Lifespan extension by dietary restriction in *Drosophila* is associated with GATA motifs and organ-specific, TOR-dependent transcriptional networks

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Abstract

Healthy lifespan can be extended in eukaryotes by dietary restriction (DR). In *Drosophila,* DR of essential amino acids (EAAs) extends lifespan, which is thought to be dependent on the Target of Rapamycin (TOR) pathway, but the transcriptional bases of these effects are poorly understood. Identifying these transcriptional changes and their regulators offers the prospect of correctly coordinating physiology to mimic the benefits of DR. We have analysed how DR and TOR alter transcriptional networks in adult female *Drosophila*, by specifically diluting EAAs or adding the TOR-suppressive drug rapamycin and analysing the transcriptomes of dissected organs. This network analysis simplified the description of the organ system by two orders of magnitude whilst retaining ~80% of information. The broad transcriptional effects of DR were recapitulated by rapamycin, indicating that DR exerts its transcriptional effects via TOR. At a finer resolution, one particular transcriptional module was associated with transcriptional changes induced by both DR and rapamycin, suggesting a general anti-ageing role for this module. However there were also treatment-specific effects of both DR and rapamycin, with the important implication that not all transcriptional effects of TOR suppression are obligately coupled, which may offer the possibility of separating lifespan benefits from costs of intervention. These changes across organs were mirrored by changes within organs. Lifespan-associated transcriptional changes were strongly associated with binding sites for GATA transcription factors, providing a candidate mechanism to regulate the observed transcriptional effects of diet. Collectively these results show that diet and TOR signaling have extensive effects on transcription across organs, isolate transcriptional changes that may be generally associated with longevity from other effects of DR and of drugs, and show that the genes associated with lifespan extension are enriched in GATA motifs.

Introduction

How can health be maximised throughout life? Answering this question is a major goal, as ever-increasing human lifespans outpace advances in gerontology, at great social, personal and financial cost (Niccoli & Partridge, 2012). Dietary restriction (DR) is an intervention with the evolutionarily conserved capacity to improve lifelong health and extend lifespan via moderate reduction of nutrient intake, but at a cost of reduced biological fitness and vigour in youth (Mair & Dillin, 2008). Despite having been discovered 80 years ago (McCay *et al*, 1935), the molecular mechanisms underpinning lifespan extension by DR remain elusive. Defining these mechanisms could help to ameliorate the burden of ageing without the costs of DR.

Reduced calorie consumption does not fully account for the benefits of DR: specific nutrients and their relative ratios are key (Mair *et al*, 2005; Lee *et al*, 2008; Skorupa *et al*, 2008). In *Drosophila*, the ratio of dietary sugar to yeast modulates lifespan, and this effect is explained by essential amino acids (EAAs) from the yeast (Grandison *et al*, 2009). Crucially, this effect appears to be evolutionarily conserved in mammals, because reducing protein: carbohydrate ratio extends lifespan and improves cardiovascular health in mice, more effectively than simple calorie restriction (Solon-Biet *et al*, 2014 & 2015).

Recent evidence indicates that the effects of EAA restriction on *Drosophila* lifespan and physiology are recapitulated by suppressing the Target of Rapamycin (TOR) pathway (Emran *et al*, 2014; Simpson *et al*, 2015). Understanding of how EAAs and/or TOR assure longevity is incomplete, although maintenance of proteome quality likely plays a role (Taylor & Dillin, 2013; Katewa & Kapahi, 2011; Vilchez et al, 2014). However, TOR also affects transcription (Bülow *et al*, 2010; Robida-Stubbs *et al*, 2011; Tiebe *et al*, 2015), but to date this function has been relatively poorly characterised. In flies, transcriptomic responses to DR and TOR have been characterised at the cellular and organismal levels (e.g. Tiebe *et al*, 2015), but information on organ-specific transcriptomes is a requisite advance for many reasons. First, it is not yet known how organs are coordinated when ageing is ameliorated. There may be multiple possible states of the organ system that extend lifespan, with the important corollary that some of these states may also impart less severe tradeoffs in early life. Second, transcriptional mechanisms affecting lifespan are incompletely described by

whole organism analyses, since tissue-restricted genetic interventions are sufficient to extend lifespan (e.g. overexpression of the orthologous transcription factors FoxO/Daf-16 in flies and nematodes, respectively: Alic *et al*, 2014; Zhang *et al*, 2013). Third, minimising the number of tissues that are manipulated to extend lifespan is likely to minimise organismal side-effects of the intervention. Integrative analyses that account for feedbacks and interdependencies across the organ system are therefore required to further our understanding mechanisms of lifespan extension.

Here, we address a key question: Can the changes in organ-specific transcriptional effects caused by DR be explained by TOR suppression? This study is facilitated by dietary manipulations that offer a more precise toolset to dissect DR than wholesale food dilution. Previously, we developed a semi-defined *Drosophila* diet in which 50% of available essential amino acids (EAA) are provided as a supplement to yeast-based medium, which is optimal for early-life egg laying (Grandison *et al*, 2009; Emran *et al*, 2014). Against this "fully fed" control, lifespan can be extended by two interventions, both with correlated fecundity costs in early life (Bass *et al*, 2007; Emran *et al*, 2014): 1) omission of the EAA supplement (i.e. DR); 2) addition of rapamycin (EAA+rapamycin), which extends lifespan even in the presence of EAAs, by suppressing TOR pharmacologically. We can thus establish the transcriptional effects of TOR associated with diet-induced longevity and drug-induced longevity, and isolate changes that are associated with both long-lived states. Phenotypes associated with these conditions were published by Emran *et al* (2014). Capitalising on orthology between *Drosophila* organs and vertebrate organs, we study transcriptomes in the brain, fat body (the analogue of the vertebrate liver and adipose), gut, ovary and thorax (which largely comprises muscle) of flies fed these diets. By resolving how these diets affect organ-specific transcription, we provide a complete view of organ-resolved physiological states associated with lifespan regulation, and their dependency on nutrition versus signalling.

Results

The effects of DR on ageing are thought to be mediated by the TOR pathway. Here, by characterising the organ-specific and longevity-associated transcriptional changes associated with DR and pharmacological TOR suppression, we address whether molecular changes associated with DR are TOR-dependent, and isolate changes that are associated with longevity in both experimental conditions. We also test for enrichment of *cis*-regulatory elements associated with these changes, to predict transcription factors that may mediate the TOR-dependent effects of DR. The study design is presented in Figure 1. We term EAA restriction as a diet-induced longevity condition, and rapamycin supplementation as a drug-induced longevity condition.

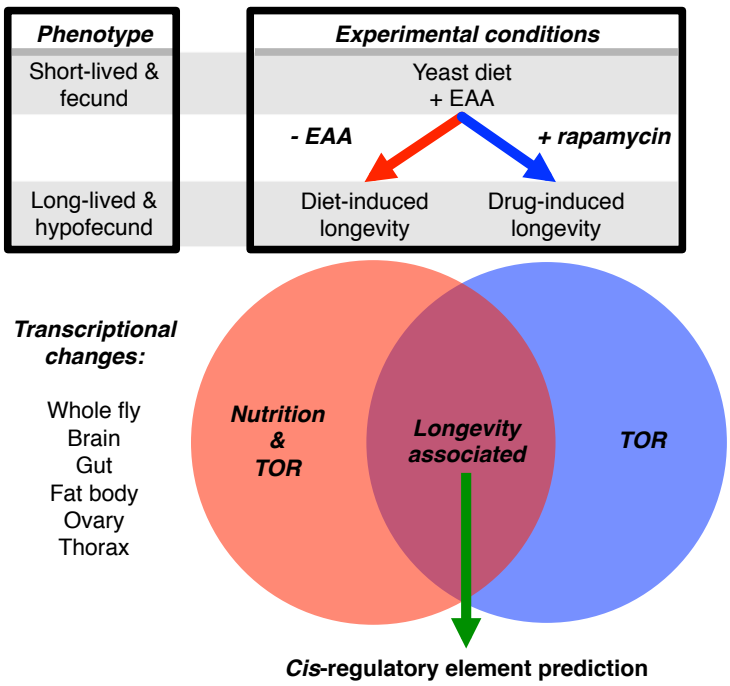


Figure 1. Study design. Against a control in which short lifespan is determined by specific enrichment of essential amino acids (EAA), lifespan is extended by DR of the EAAs (diet-induced longevity), or pharmacologically, by administration of the TOR-suppressive drug rapamycin (drug-induced longevity). This lifespan extension comes at a biological cost of reduced early-life fitness (i.e. fecundity). Tissue-specific transcriptional changes associated with both conditions are therefore longevity-associated, EAA-dependent and TOR-dependent. Identifying *Cis*-regulatory elements associated with these transcriptional networks predicts mechanisms by which DR affects transcription via TOR.

Effects of DR on transcriptome-wide expression are recapitulated by TOR suppression in specific tissues

To assess whether the conditions of diet-induced longevity and drug-induced longevity have equivalent effects on transcription, we compared the changes in expression of all genes in the transcriptome under conditions of diet-induced longevity and drug-induced longevity, with the prediction that their effects would be equivalent and therefore positively correlated. As predicted, in whole flies, brains, guts, ovaries and thoraces, diet-induced and drug-induced longevity conditions had positively correlated effects on gene expression. The transcriptional changes observed in each condition therefore mirror the associated effects on lifespan (Emran *et al*, 2014). However, the two long-lived conditions did not have equivalent effects in the fat body (Figure 2), demonstrating that these two interventions cause changes in expression that are largely coupled across organs, but that this coupling is not obligate.

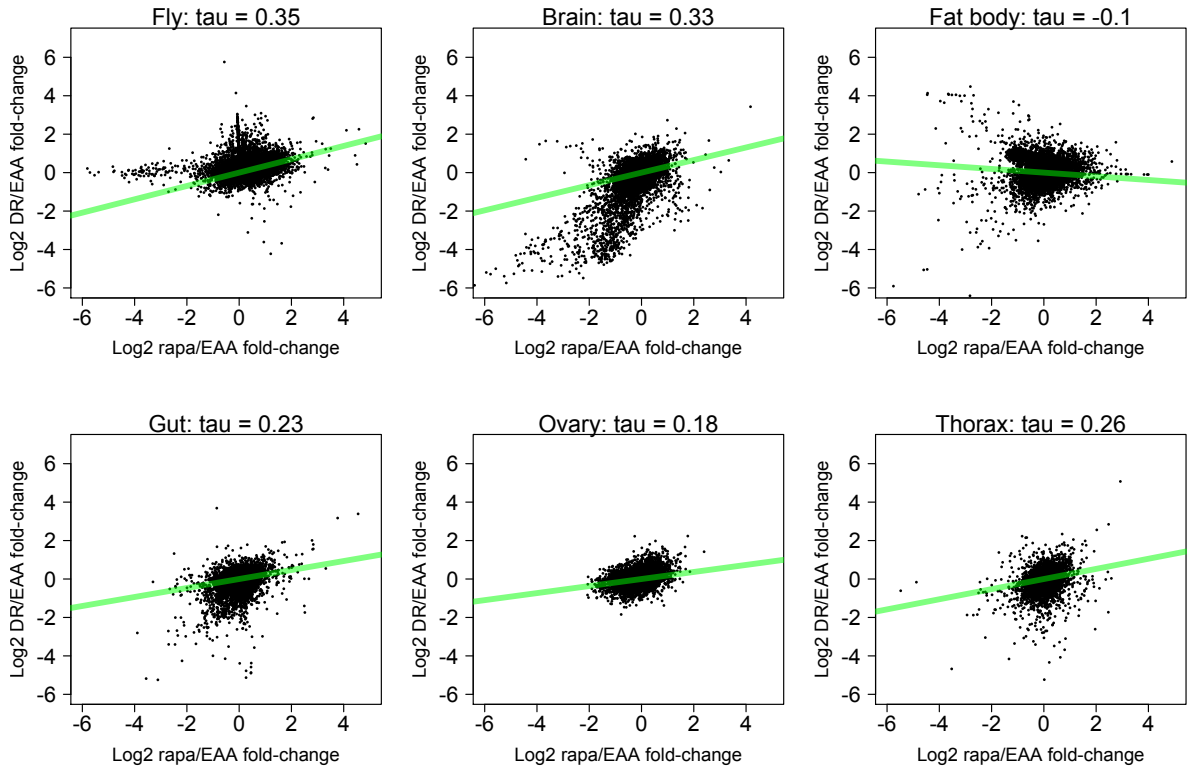


Figure 2. Changes in expression induced by DR are correlated to changes in expression induced by TOR suppression. Log_2 fold-changes in expression in the diet-induced longevity and drug-induced longevity conditions, relative to the EAA-enriched control (DR/EAA fold-change and rapa/EAA fold-change, respectively), were calculated for every gene in the transcriptome. Fold-changes were calculated by DESeq2. Text above panels indicates correlations (Kendall's Tau) between fold-changes induced by the two long-lived conditions. All p-values $< 2.2e^{-16}$

Diet and TOR suppression have overlapping effects on the transcriptional network across organs

A requisite of anti-ageing interventions is to not compromise health in early life, which is possible through diet, by decoupling lifespan from fitness in early life (Grandison *et al*, 2009). Similarly, in the present study, it was evident that the transcriptomes of individual organs were not obligately coupled, because the broad-scale transcriptional effects of the diet-induced and drug-induced longevity conditions were equivalent in multiple organs but not in the fat body (Figure 2) and, accordingly, Principal Components Analysis separated samples by organ and experimental condition, but organ-specific effects of experimental condition were orthogonal (Figure S1). These observations suggested that between-organ coupling of gene expression differs between the two long-lived conditions. This means that the two long-lived conditions cause equivalent quantitative changes within most organs, but qualitatively different changes across all organs. Understanding this decoupling of organ-specific transcription may provide a mechanistic basis to decouple early-life fitness and lifespan, because different long-lived physiological states may be associated with different biological costs. This prompted us to quantify changes in organ coordination (defining "organ coordination" as correspondence between transcriptional changes across organs), by integratively analysing the transcriptional network across the organs under study.

The goal of understanding changes in organ coordination called for a transcriptional networking approach. Such approaches reveal interdependencies in gene expression (i.e. gene coexpression), and can therefore identify structural changes in a system. We employed Weighted Gene Coexpression Network Analysis (WGCNA; Langfelder & Horvath, 2008) to determine modules of genes that were similarly coexpressed across all three diets (i.e. consensus modules), and diet-dependent changes in their coregulation across organs. After applying quality controls and removing genes with zero variance, 11164 of 13442 genes clustered into 14 modules (Figure 3a, Supplementary Materials). 2278 genes could not be clustered (and were labelled grey/Module 0). We then summarised the behaviour of these modules across organs and diets with a single vector per module by determining "Eigengene" values, calculated as the first principal component of expression of the genes in each module (Langfelder *et al*, 2007). The Eigengenes accounted for between 72% and 89% of

the variance in each module (weighted average = 79.86%, Figure 3b), thereby simplifying the transcriptional description of organs by two orders of magnitude, from ~$11e^3$ to 14 variables, whilst retaining ~80% of total information. These Eigengenes provided a toolkit by which to isolate changes in the co-regulation of transcriptional modules across organs and diets, and thereby understand changes in organ coordination. Eigengene values are presented in Figure 3c. To isolate pairs of modules that were differentially co-regulated upon DR and/or TOR suppression, we calculated correlations between pairs of Eigengenes, and their changes relative to the EAA-fed control in the diet-induced and drug-induced longevity conditions. We compared these changes to those from 10,000 random permutations of the data to test for statistical significance. As expected, this analysis revealed both similarities and differences in how DR and TOR suppression change the transcriptional network (Figure 4). The diet-induced longevity condition significantly changed the coregulation of three pairs of modules (4 & 13, 4 & 3, 14 & 13), but by contrast all significant changes in organ coordination in the drug-induced longevity condition were associated with just module 11 (relative to modules 1, 4, 7, 8, 9, 12, 13). This approach found changes in coregulation of pairs of modules, but did not isolate which of the pair caused this change. Therefore, by three separate methods, we asked which individual modules' Eigengenes were most strongly perturbed across the experimental conditions (Langfelder *et al,* 2007), which identified modules 4, 11 and 14 (Figure S2). This result suggested that these three modules drove changes in the transcriptional network. Together, these results predict that both DR and TOR suppression change coregulation of module 4 with other modules, and therefore that this module has a role in multiple anti-ageing mechanisms. However, module 11 is associated specifically with drug-induced longevity, whereas module 14 is associated specifically with diet-induced longevity. The differential effects of the two long-lived conditions on these modules demonstrates that TOR-dependent transcription across organs can be partially decoupled, and therefore that longevity can be associated with qualitatively different organismal physiological states.

Combining the network analysis with the preceding analysis of transcriptome-wide effects of DR and TOR suggests that two regulatory processes result from the interplay of EAA availability and its activation of TOR. On a broad scale, changes in expression induced by DR mirror those of rapamycin administration (Figure 2), and accordingly, diet-induced longevity and drug-induced longevity both affect regulation

of one transcriptional module (4), but these two interventions also both affect distinct modules (14 and 11, respectively) (Figure 4). These differences in changes to the transcriptional network suggest distinct organismal physiological states are associated with longevity, depending on whether it is diet-induced or drug-induced. This has the important implication that transcriptional responses to closely-related interventions are not obligately coupled across tissues, and so a beneficial change in one tissue can potentially be decoupled from a costly change in another tissue. It remains to be established how these transcriptional differences affect phenotypes associated with these conditions.

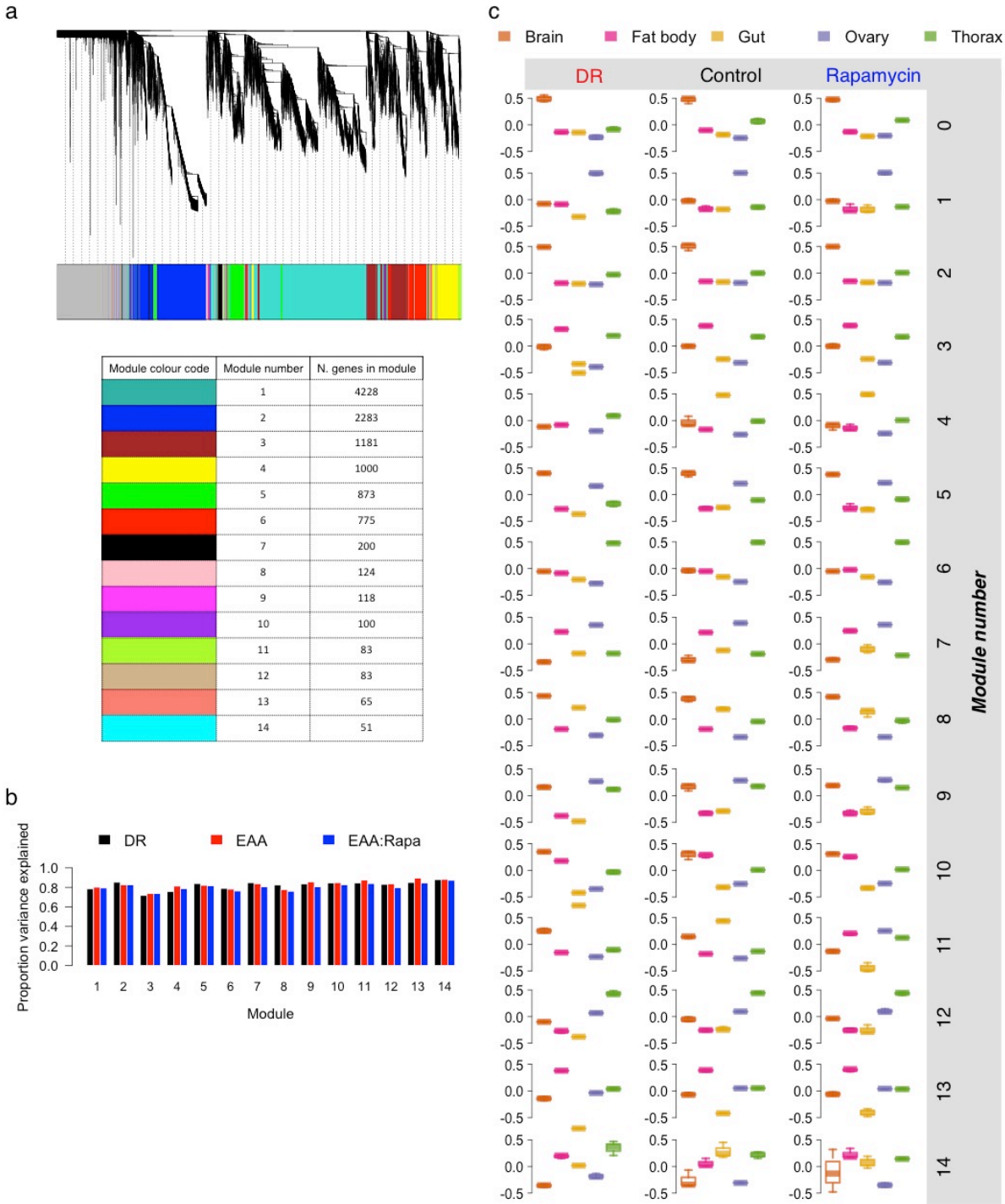


Figure 3. A network description of transcription across *Drosophila* organs and long-lived conditions. (a) Consensus transcriptional modules found across organs in all three experimental conditions. Genes were clustered according to their expression ($\log_2$ RPKM) across organs by Weighted Gene Coexpression Network Analysis (WGCNA), revealing modules of genes showing similar coexpression across organs. Leaves of the tree indicate genes. The tree was cut by hybrid tree cutting to define

transcriptional modules (clusters). Tree cutting assigned genes to one of 14 consensus transcriptional modules, indicated by colour-coded vertical lines that together form the horizontal bar beneath the dendrogram (see Supplementary Spreadsheets for assignments of genes to modules). Genes not assigned to any module are colour-coded grey. **(b)** Bars show variance in gene expression across organs explained by Eigengenes (first principal component of expression) in each module and experimental condition (weighted average = 79.86%). **(c)** Representative expression (Eigengenes) of all modules (y-axis showing Eigengene values, A.U.). These data summarise changes in transcriptional networks across organs and experimental conditions. Data are plotted by experimental condition in columns ("DR" = diet-induced longevity, "Rapamycin" = drug-induced longevity, short-lived control = "EAA"), and transcriptional module in rows. Within each plot, boxes are colour-coded by tissue (see key). Boxplots show medians (horizontal midline), 1st and 3rd quartiles (hinges), and range of data points.

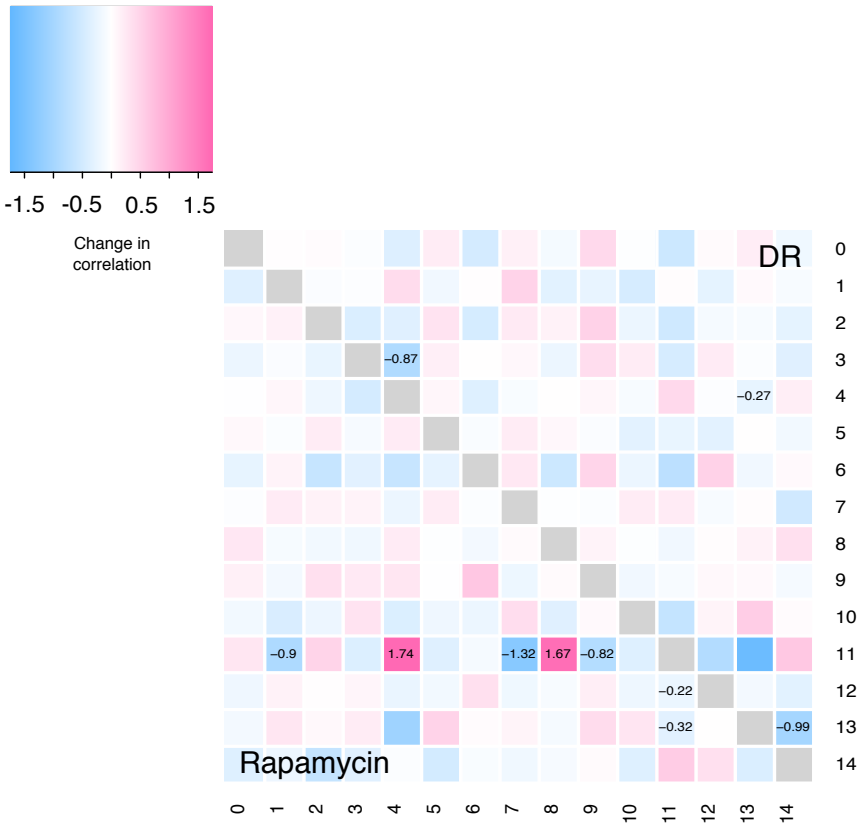


Figure 4. Structure of longevity-associated changes in the transcriptional network. The heatmap shows the observed change in correlation (Spearman's rho) between pairs of modules in response to DR and in response to TOR suppression, relative to the fully EAA-fed condition. Row and column labels represent transcriptional modules. Values are given when the change was significant ($p \leq 0.05$) according to permutation testing.

Diet and TOR suppression have overlapping effects on within-tissue transcriptional changes

To complement the network analysis of transcriptional changes across organs, we used differential expression analysis to identify transcriptional changes within organs in the conditions of diet-induced and drug-induced longevity, relative to the control condition. Differential expression was detected in whole flies, brains, fat bodies, guts and thoraces. In each of these tissues there was significant overlap between the sets of genes that were differentially expressed in both long-lived conditions (Table 1), and both conditions had positively correlated effects (Figure S3), showing that transcriptional effects of DR can be accounted for in part by TOR suppression, in agreement with the preceding network analysis. Surprisingly no genes were differentially expressed in the ovary in the diet-induced longevity condition, despite the robust decreases in egg laying associated with longevity in these conditions (Emran *et al,* 2014). However changes were detectable in the drug-induced longevity condition, and 17% of the differentially expressed genes coded ribosomal proteins, and the full set of down-regulated genes was enriched in ribosome-associated GO terms (Supplementary Files). This may mean that the ovary regulates egg laying post-transcriptionally, consistent with the evolutionary view that selection favours maximal short-term egg production from whatever resources are available.

We also examined overlap between changes observed in different tissues, to ask whether studying specific organs increases insight, and whether we could find molecular signatures of lifespan extension across tissues. The whole-fly samples captured only a small portion of the organ-specific changes associated with lifespan extension Figure 5). Surprisingly, no one gene responded to DR/TOR in all organs (Figure S4), indicating that there is no ubiquitous molecular signature of lifespan extension by DR and/or TOR. Both of these results underline that organismal effects of DR cannot be understood in terms of any one tissue. Together, these differential expression analyses show that, at the level of individual genes, the DR regulon can be accounted for by TOR in all organs but the ovary, but that the identity of the genes whose expression is changed depends on the tissue in question. This likely accounts for the incomplete description of tissue-specific change by the whole-fly samples, because the whole-fly samples take an average of all changes across all organs. This

has the important implication that studying individual organs is likely crucial to elucidate mechanisms of organismal physiological change.

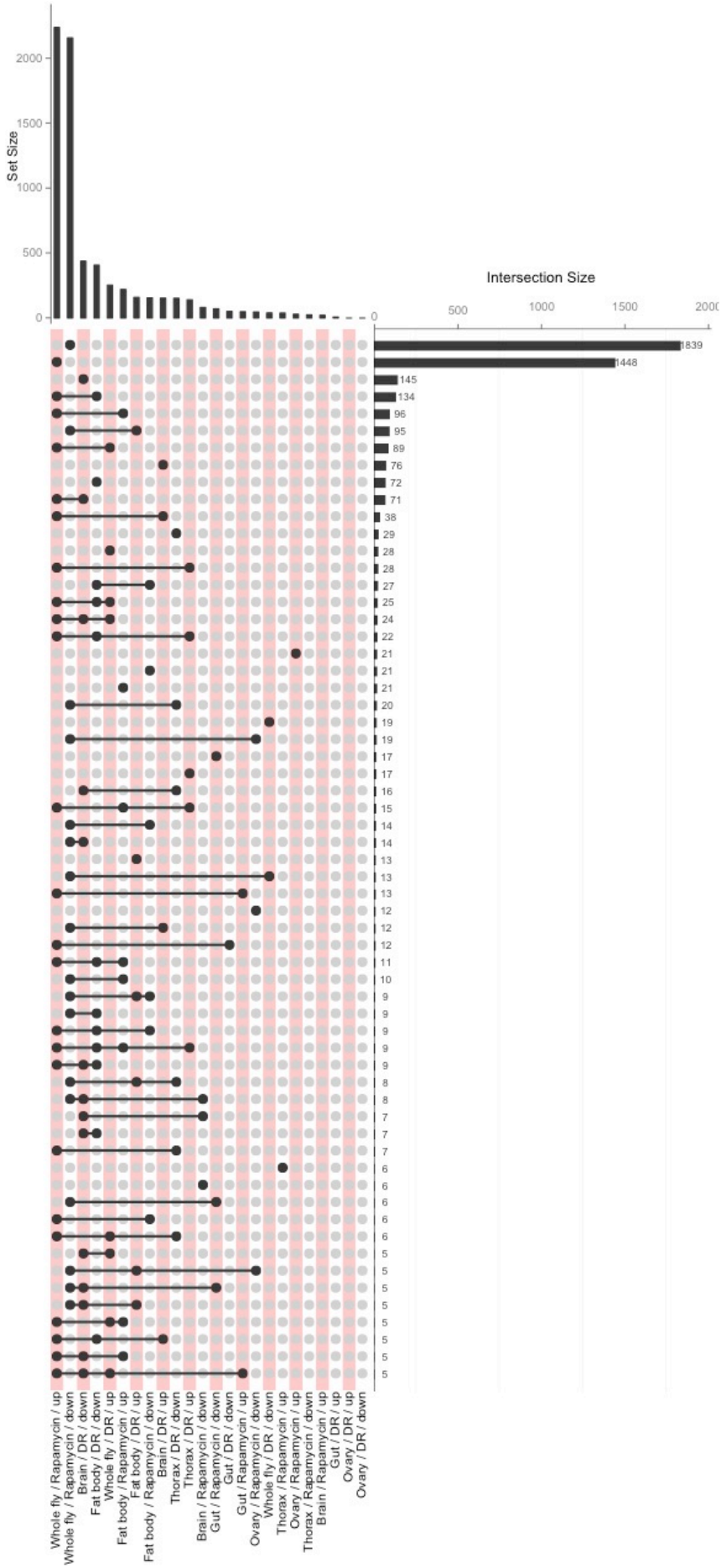
Table I. Overlapping effects of the diet-induced longevity (DR) and drug-induced longevity (rapamycin) conditions on changes in gene expression within specific tissues.

Gene set		Number of genes†			
Tissue	**Expression change***	**DR**	**Rapamycin**	**Overlap**	**P-value§**
Whole fly	Up	254	2239	203	5.55e-121
	Down	40	2159	17	3.99e-06
Brain	Up	154	22	1	0.02
	Down	439	82	63	8.44e-82
Fat body	Up	221	160	6	0.02
	Down	408	156	56	2.75e-46
Gut	Up	8	49	6	7.60e-17
	Down	72	52	6	1.78e-08
Ovary	Up	0	30	NA	NA
	Down	0	47	NA	NA
Thorax	Up	141	39	10	1.32e-13
	Down	153	25	6	8.11e-09

* Relative to EAA diet

† Number of genes showing given change in expression

§ Hypergeometric test


Set Size
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Intersection Size
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Brain / Rapamycin / down
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Gut / DR / down
Gut / Rapamycin / up
Ovary / Rapamycin / down
Whole fly / DR / down
Thorax / Rapamycin / up
Ovary / Rapamycin / up
Thorax / Rapamycin / down
Brain / Rapamycin / up
Gut / DR / up
Ovary / DR / up
Ovary / DR / down

Figure 5. Organ-specific molecular signatures of DR and rapamycin feeding. Effects of the diet-induced and drug-induced longevity conditions on differential expression (FDR $\leq$ 0.01) of individual genes was determined within each organ. Labels at the bottom of the matrix give the tissue, experimental condition and sign of expression change that define each gene set. The bar chart at the top of the matrix gives the size of each gene set. A dot is shown in rows of the matrix for gene sets which do not intersect other sets. Connected dots are shown to indicate intersecting gene sets. The bar chart at the right of the matrix gives the size of each intersecting set. The figure shows that that DR and rapamycin often have complementary effects within the same tissue. Whole-body samples do not fully account for organ-specific transcriptional variation, since not all sets overlap include genes differentially expressed in whole body samples. See Figure S4 for complementary plot showing the same data set ordered by degree of overlap between gene sets, showing that no one gene is differentially expressed in all tissues.

Functional analyses of EAA and TOR-dependent changes in transcription

Both the transcriptional network and differential expression analyses suggested that lifespan extension by DR and by pharmacological TOR suppression are associated with overlapping organ-specific transcriptional changes. This allows us to parse the effects of interventions to extend lifespan to expose transcriptional changes associated with both long-lived conditions, as well as changes specifically associated with DR and rapamycin consumption. To identify gene functions associated with these changes, we analysed enrichment of Gene Ontology (GO) terms (Supplementary Materials). Transcriptional Module 4, which was associated with both diet-induced and drug-induced longevity, was enriched in extracellular metabolic enzymes, particularly peptidases, and also lipases and carbohydrases. Module 14, which was associated with diet-induced longevity only, was enriched in transmembrane sugar transporters and polysaccharide/carbohydrate binding. Module 11, which was associated with drug-induced longevity only, was enriched in ATP-binding cassette (ABC) transporter activity, and also contained the lipases *dob* and *bmm*, which have known roles in lifespan regulation (Grönke *et al*, 2005). This analysis indicated that DR and TOR change the relative contribution of specific organs to these processes. We also interrogated functions of genes found to be differentially expressed within organs. Encouragingly, expression of yolk proteins (*Yp1, Yp2* and *Yp3*) was decreased in the fat body upon DR, and this effect was recapitulated by TOR suppression. This indicates evolutionary conservation of regulation of oogenesis, because the mosquito Yp orthologue *Vitellogenin* is activated by TOR after EAA uptake, in order to initiate egg production (Attardo *et al*, 2003**;** Park *et al*, 2006). GO terms associated with changes in expression within organs are provided in Supplementary Files. Most GO terms (83%) were associated with specific organs: this was consistent with the absence of a ubiquitous molecular signature of DR and TOR across organs (Figure 5), showing that there is also no functional signature. However, we note that the GO analysis indicated that both DR and rapamycin down-regulated immunity in the fat body, but by contrast up-regulated similar GO terms in the gut. This is consistent with known roles of nutrition in immunity (Simpson & Raubenheimer, 2012; Ponton *et al*, 2013; Clark *et al*, 2013; Howick & Lazzaro, 2014; Unckless *et al*, 2015), but suggests that diet alters the contribution of specific organs to overall immunity. In summary, both DR and rapamycin induce organ-specific changes in biological function.

Associations between transcription factor binding sites and longevity-associated transcriptional changes

We sought to identify candidate regulators of transcriptional changes associated with diet-induced longevity and drug-induced longevity. We anticipate that transcription factors (TFs) will constitute powerful means to mimic effects of DR, by correctly coordinating diverse transcriptional targets in multiple tissues. We took an unbiased approach to identifying transcription factor binding site motifs (TFBSs) enriched in association with transcriptional variation across organs and diets, by associating transcriptional modules with TFBSs using *i-Cis target* (Herrmann *et al*, 2012). TFBS enrichment was analysed for each module individually. Our preceding transcriptional network analysis had associated modules 4, 11 and 14 with lifespan extension. Hierarchical clustering suggested that these modules were associated with similar sets of motifs (Figure 6a), consistent with them being regulated by shared mechanisms, and there was substantial overlap between the TFs that were associated with each of these longevity-associated modules (Figure 6b). For each of these three longevity-associated modules, the strongest associations were with the GATA family of TFs (*GATAd, GATAd, grn, pnr, srp*) (Table II). Furthermore, the most significant association observed in the entire analysis was between GATA-binding motifs and Module 4, which is associated with both diet-induced and drug-induced longevity (Figure 4). Other motifs associated with all three longevity-associated modules were annotated with *Bx* - which physically binds *pnr* (Zenvirt *et al*, 2008); *Ham*, which has known roles in cell fate determination (Moore *et al*, 2002, 2004); and *CG10348*. To confirm that the enrichment of GATA sites in longevity-associated modules was not an analytical artefact, we looked at the distribution of GATA element enrichment across all other transcriptional modules. All but two modules (modules 2 and 5) contained some enrichment of GATA factor binding sites, consistent with the known roles of GATA factors in directing tissue-specific transcription (Chlon & Crispino, 2012), and with the fact that all modules were defined by tissue-specific transcription (Figure 3c). However, the strength of enrichment (Escore) for the GATA factors was significantly greater for the longevity-associated modules 14 and 4 (Figure 6c). We also asked whether modules contained the TFs with which they were associated by TFBS analysis, because correlation between expression of a TF and its putative tar-

gets strengthens guilt-by-association (e.g. Potier *et al*, 2014; Dutta *et al*, 2015) and, in our network analysis, expression a TF is by definition correlated to that of the module in which it is found. This approach provided an unbiased way to filter the results of the motif enrichment analysis. Module 4 was not only highly enriched in GATA binding sites but it also contained GATAe (Supplementary Files), further strengthening the association between this TF and this longevity-associated module. Combined with the transcriptional network analysis, these enrichment analyses strongly associated GATA factors with lifespan extension by DR.

To complement the analysis of transcriptional network regulators, we repeated the same approach on gene sets identified by the analysis of transcriptional changes within organs. For each tissue, we analysed enrichment of TFBSs in gene sets that responded to the diet-induced longevity condition or the drug-induced longevity condition, and also in sets that showed overlapping effects of both long-lived conditions. The top-ranked motifs associated with gene sets are presented in Table 3. Most binding sites found by this analysis were associated with organ-specific transcriptional changes, providing candidates TFs to regulate tissue-autonomous responses to systemic diet-dependent signals. However, one cluster of TFs was associated with multiple gene sets across organs, and that cluster contained the GATA factors (Figure 6d). We also quantified the number of gene sets that each TF was associated with, to ask whether specific TFs were associated with multiple DR/TOR-dependent transcriptional changes across tissues. This analysis confirmed what was intutively obvious from the preceding clustering analysis, that GATA-binding motifs were frequently enriched across gene sets (Figure 6e). By this approach, each GATA factor was associated with at least 10 (~32%) of diet/tissue-specific gene sets. These analyses corroborated previous results, associating GATA family TFs with lifespan-associated transcriptional changes across organs.

Motif enrichment analyses strongly linked GATA TFs with lifespan extension by dietary restriction. This is interesting in the light of prior knowledge of the biology of these transcription factors in other organisms. GATA factors play known roles in signalling amino acid availability via TOR in evolutionarily diverse eukaryotes (e.g. yeast: Cooper, 2002; and mosquitos: Attardo *et al*, 2003), as well as coordinating tissue-specific transcriptional programs, and being required for lifespan extension by some tissue-specific genetic lesions (e.g. Zhang *et al*, 2013). Additionally, GATA factors have known roles through development in coordinating tissue-specific transcrip-

tional programs (Chlon & Crispino, 2012), and our data show tissue-specific expression of each of the five *Drosophila* GATA factors (Figure S5), suggesting an ongoing role for these TFs in coordinating tissue-specific expression in adults. Together, our results and previously published work implicate GATA factors as evolutionarily-conserved regulators of transcriptional coordination in response to amino acids, suggesting that these TFs may mediate transcriptional effects of DR.

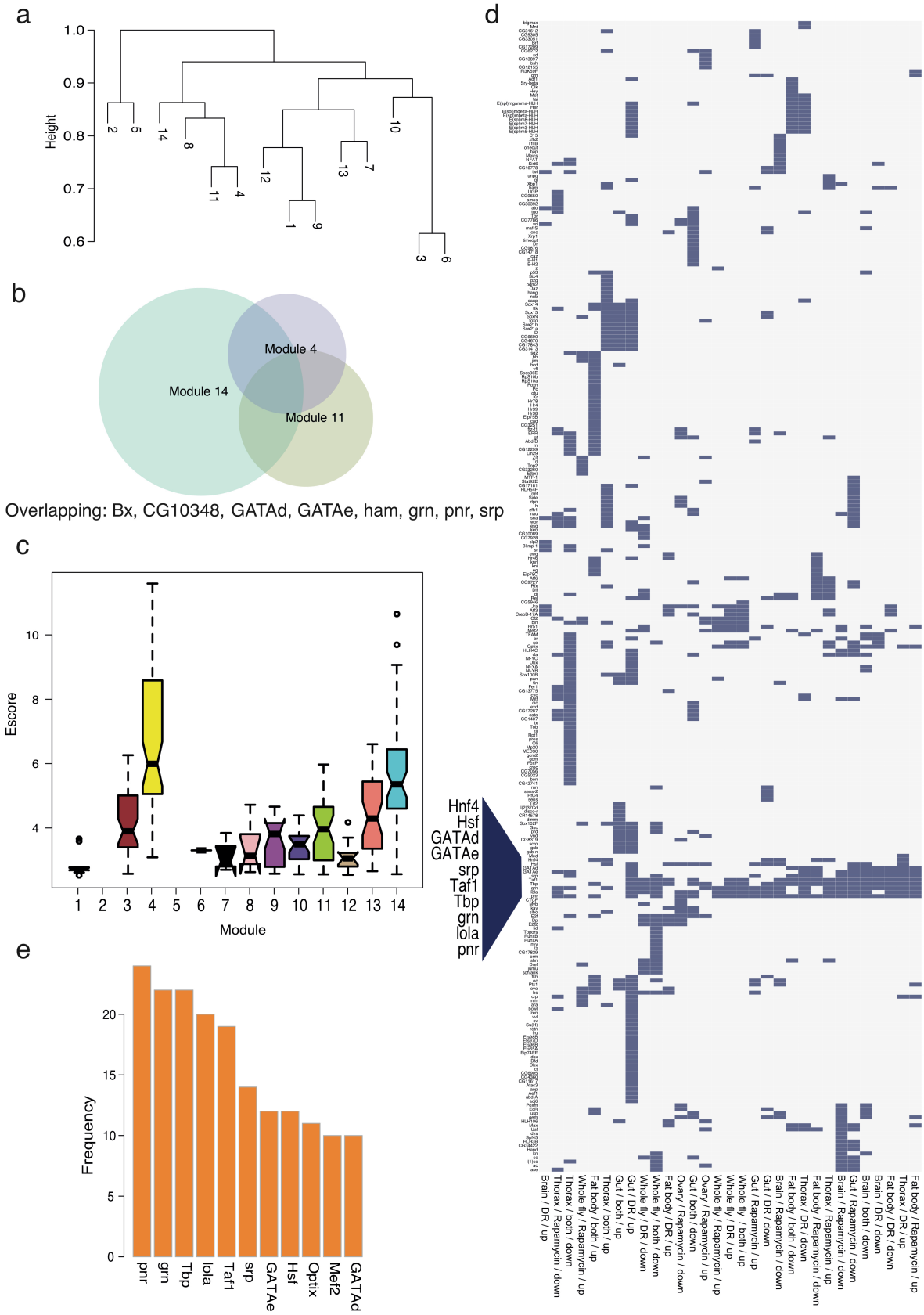

Figure 6. A signature of GATA transcription factors as regulators of the transcriptional response to DR and/or TOR. (a) Lifespan-associated transcriptional modules are grouped by sharing of transcription factor binding site motifs. Each module was tested for enrichment of *cis*-regulatory elements, and clustered by the

presence/absence of associated transcription factor binding site motifs. Dendrogram labels represent module numbers. This analysis clusters together transcriptional modules associated with longevity (4, 11, 14, also clustered with module 8). **(b)** Longevity-associated modules are associated with overlapping sets of transcription factor binding sites. Text below the Venn diagram names the transcription factors associated with binding sites enriched in all three longevity-associated modules. **(c)** Distribution per module of enrichment (Escore) of motifs annotated as binding GATA transcription factors. The box plots show medians (horizontal midline), 1st and 3rd quartiles (hinges), and range of data points. The notches equate to ~95% confidence intervals for the medians [(1.58 * interquartile range) / (square root *n*)]. The plot shows that enrichment of GATA binding sites is particularly strong for modules 4 and 14, both of which are associated with lifespan extension by DR. **(d)** GATA factor binding sites are enriched across multiple gene sets regulated by DR/TOR. Columns represent sets of genes according to tissue and the sign of expression change per treatment. Rows represent TFs associated with the sets of differentially expressed genes by enrichment of binding site motifs in the gene set. The axes are ordered by clustering the presence (blue) and absence (grey) of TF binding sites, using binary distance. Names of transcription factors occurring in a cluster across multiple gene sets have been magnified. **(e)** The bar plot shows the frequency of associations with longevity-associated transcriptional changes within organs, for the most frequently associated TFs. TFs associated with <10 gene sets are excluded.

Table II. Annotations for top five ranked motifs enriched in association with transcriptional modules. Unannotated motifs are excluded.

Longevity-associated	Module number	TF*	Escore†
Diet-induced & Drug-induced	4	grn srp pnr GATAd GATAe	11.58
		grn srp pnr GATAd GATAe	11.21
		grn srp pnr GATAd GATAe	10.07
		grn srp pnr GATAd GATAe	10.07
		grn srp pnr GATAd GATAe	10.07
Drug-induced	11	grn srp pnr GATAd GATAe	5.95
		grn srp pnr GATAd GATAe	5.88
		CG10348 ham	5.68
		grn srp pnr GATAd GATAe	5.56
		CG10348 ham	5.56
Diet-induced	14	grn GATAd pnr GATAe	10.63
		grn srp pnr GATAd GATAe	9.67
		grn GATAd pnr GATAe	9.05
		grn srp pnr GATAd GATAe	8.69
		grn GATAd pnr GATAe	8.60
No	1	Dref	8.50
		Dref	7.28
		Dref	5.66
		crp	4.78
		CG7928	4.47
No	2	lola	4.57
		lola	4.44
		Trl	4.43
		Trl	4.43
		Trl	4.42
No	3	grh	7.30
		grh	6.64
		GATAe srp pnr GATAd	6.24
		grn srp pnr GATAd GATAe	5.91
		grn srp pnr GATAd GATAe	5.91
No	5	CG9906 CG1924 Cnx99A	4.94
		jim	4.21
		nerfin-1 nerfin-2	4.01
		slp1 slp2 foxo fd19B	3.88
		Dref	3.87
No	6	vri	6.88
		gt	5.73
		Mef2	5.54
		Mef2	5.43
		vri	5.18
No	7	crp	5.08
		pnr	3.82
		HLH106	3.80
		CrebB-17A	3.61
		Hnf4	3.43
No	8	CG10348 ham	5.63
		CG10348 ham	5.44
		scrt	5.35
		scrt	5.00
		CG12605	4.91
No	9	crp	6.92
		Trl	5.44
		Trl	5.44
		Trl	4.96
		Trl CG33260	4.68

		Atf6	5.60
		grn GATAd pnr GATAe	4.37
No	10	CG11071	4.35
		grn GATAd pnr GATAe	4.34
		srp	4.31
		crp	9.27
		CG6792	6.20
No	12	kay Atf3	5.58
		Dref	4.91
		vri	4.74
		HLH106	8.86
		HLH106	7.99
No	13	GATAd	6.58
		srp	6.50
		GATAe	6.37

* Genes annotated as associated with the motif
† Escore for the motif

Table III. Annotations for top five ranked motifs enriched in association with differentially expressed gene sets. Unannotated motifs are excluded.

Gene set				
Tissue	**DR***	**Rapamycin***	**TFs****	**Escore†**
Whole fly	-	-	Tbp,Taf1	11.11
			E2f,E2f2,Dp	10.22
			Tbp	8.98
			E2f,E2f2,Dp	8.17
			E2f,E2f2,Dp	7.96
Whole fly	+	+	Cf2	6.25
			Mef2	5.41
			bin	5.40
			Cf2	5.37
			Cf2	5.22
Whole fly	-		E2f,E2f2,Dp	6.55
			Tbp,Taf1	6.44
			lola	6.27
			Blimp-1	5.98
			Blimp-1	5.98
Whole fly	+		Cf2	5.84
			Mef2	5.23
			Mef2	5.22
			Optix	5.19
			bin	5.07
Whole fly		-	Top2	4.32
			Dref	4.05
			Trl,CG33260	3.93
			Dref	3.92
			Dref	3.87
Whole fly		+	lola	8.29
			lola	8.27
			lola	7.61
			Optix	4.70
			bin	4.45
Brain	-	-	Tbp	8.40
			Tbp,Taf1	8.35
			grn,pnr	6.85
			pnr	6.21
			lola	6.13
Brain	-		pnr	11.03
			grn	10.04
			ham	10.01
			grn,pnr	9.95
			pnr	9.55
Brain	+		Jra	4.31
			Jra	4.29
			Atf3	4.02
			sna	3.77
			ato	3.68
Brain		-	Tbp,Taf1	9.58
			Tbp	7.63
			srp	5.33
			lola	5.02
			grn,pnr	4.76
			Tbp	8.07
			Tbp,Taf1	7.42

Brain		+	gem	4.89
			pnr	4.63
			tin	4.57
Fat body	-	-	Rel	8.75
			Rel	7.10
			Tbp	6.94
			pnr	5.50
			Tbp,Taf1	4.99
Fat body	+	+	hb	6.89
			hb	6.34
			sqz,rn,Lin29	5.90
			hb	5.83
			sqz,rn,Lin29	5.70
Fat body	-		pnr	5.45
			grn	5.34
			pnr	4.85
			grn	4.77
			GATAe	4.65
Fat body	+		Hsf	6.54
			Hsf	5.75
			Hsf	5.10
			ovo	4.77
			bs	4.49
Fat body		-	GATAd	6.61
			srp	5.77
			pnr	5.53
			GATAe	5.46
			Tbp	5.38
Fat body		+	lola	6.46
			grn	6.45
			lola	6.15
			grn	5.87
			pnr	5.62
Gut	-	-	cic	10.44
			Tbp	8.89
			Tor	7.88
			Tbp,Taf1	6.90
			Tbp	6.81
Gut	+	+	scro	13.34
			oc	12.11
			scro	10.44
			ttk	10.16
			vnd	9.38
Gut	-		gem	6.79
			grn	6.49
			pnr	6.37
			pnr	5.53
			grn,pnr	4.92
Gut	+		oc	8.97
			fru	7.82
			scro	7.61
			fru	7.51
			ken	7.28
Gut		-	zfh1	8.56
			Tbp,Taf1	8.19
			da	8.18
			Tbp	7.93

			da	7.41
Gut		+	Hsf	4.58
			pnr	4.46
			ERR	4.11
			br	3.77
			grh	3.71
Ovary		-	E2f,E2f2,Dp	4.56
			Myb	4.55
			Myb	4.46
			vri	4.41
			vri	4.26
Ovary		+	twi	5.19
			HLH106	4.18
			CG13897	3.89
			bin	3.87
			foxo	3.81
Thorax	-	-	grn	9.63
			grn	8.02
			pnr	7.72
			pnr	7.71
			pnr	7.63
Thorax	+	+	zfh1	6.02
			sna	5.55
			sna	5.41
			ham	5.32
			Rfx	4.24
Thorax	-		Tbp	9.37
			grn,pnr	9.02
			Tbp,Taf1	8.47
			GATAd	7.43
			GATAe	7.07
Thorax	+		Tbp,Taf1	10.54
			Tbp	8.63
			lola	7.43
			grn	6.99
			Hnf4	6.86
Thorax		-	ttk	7.43
			crp	7.19
			ttk	6.93
			Fer1	6.46
			ase	6.34
Thorax		+	Rel	7.19
			Tbp,Taf1	7.02
			Tbp	6.95
			Rel	5.03
			Rfx	4.68

* sign of expression change, relative to EAA diet. The gene set is defined by only the effect of one treatment when only one symbol is given.
** Genes annotated as associated with the motif
† Escore for the motif

Discussion

Dietary restriction improves lifelong health and extends lifespan in a range of organisms, from yeast to mammals. A growing body of evidence shows the particular importance of changes in dietary nutrient balance, in particular, lowered protein: carbohydrate ratio (Solon-Biet *et al,* 2015; Skorupa *et al*, 2008). These reports heavily implicate the importance of amino acid-sensitive TOR signaling in the control of diet-mediated longevity (Simpson *et al*, 2015), as was recently demonstrated in *Drosophila* by Emran *et al.* (2014), who showed that active TOR signalling was required for enrichment of EAAs to shorten lifespan. Our results show that EAA restriction has effects that are congruent with a number of the effects of pharmacological TOR suppression, indicating that the transcriptional effects of DR are indeed mediated at least in part by TOR. The fact that both of these interventions extend lifespan suggests that the overlapping transcriptional changes they induce may have important anti-ageing effects. The evolutionary conservation of TOR, and of the effect of DR to extend lifespan, suggest that DR likely extends lifespan by regulating conserved signalling mechanisms via TOR.

We have modeled how conditions that enhance lifelong health coordinate transcription across *Drosophila* organs, specifically evaluating the transcriptional effects of EAA restriction and the extent to which these effects are recapitulated by pharmacological TOR suppression. To our knowledge, this is the first organ-resolved transcriptional analysis of dietary interventions to extend *Drosophila* lifespan. The results show that at a broad scale the effects of the diet-induced longevity condition are equivalent to those of the drug-induced longevity condition and, accordingly, our transcriptional network analysis associated one specific transcriptional module (module 4) with both long-lived states. Furthermore, there was significant overlap between the within-organ changes associated with the conditions of diet-induced longevity and drug-induced longevity. However, we also show that DR and rapamycin differ in some of the large transcriptional changes that they induce: this shows that even with two similarly TOR-suppressive interventions, transcription across organs is not obligately coupled, and therefore that longevity can be associated with distinct physiological states. This is an important result because it associates two related means of lifespan assurance with specific genes whose regulation is separable from other

transcriptional effects. If it is possible to target these genes specifically to promote lifespan, without inducing other transcriptional effects, it may be possible to decouple the lifespan benefits of DR from the costs in early life. This differential coordination of transcription between tissues is suggestive of a mechanism by which the trade-off relaxation could be mediated.

We have uncovered a compelling association between longevity-associated transcriptional change and the GATA family of transcription factors. Whilst there is some existing evidence of a role for GATA factors in lifespan regulation, to our knowledge they have not previously been associated with DR. One transcriptional module revealed by our analysis, module 4, is associated with both diet-induced and drug-induced longevity, is significantly more enriched in GATA binding sites than other modules, and contains a GATA TF, *GATAe*: These results predict that this module receives signals from TOR via *GATAe* to ameliorate ageing. The GATA factors are an ancient family of transcriptional regulators, which have well-characterised and essential roles in coordinating development and growth in organisms from yeast to mice. In multicellular differentiated organisms, GATA factors are required in the formation of multiple tissue types, which in *Drosophila* includes the heart (Sorrentino *et al*, 2005), fat body (Sam *et al*, 1996) and gut (Murakami *et al*, 2005). GATA factors are also expressed during adulthood and are each expressed with their own range of tissue specificity (Figure S5), but their ongoing roles in coordinating functions of adult organs are not well characterised. In *Drosophila*, recent studies have shown a role of *GATAe* in maintenance of cell identity and tissue function in the adult gut, but it is not yet clear from which molecular pathways *GATAe* integrates information (Dutta *et al*, 2015; Buchon *et al*, 2013). One of the better-described roles for GATA TFs in adult animals is in nutrient regulation of oogenesis in mosquitos (Attardo *et al*, 2003; Hansen *et al*, 2004; 2005; Park *et al*, 2006). In *Aedes aegypti*, oogenesis requires a blood meal, which contains the mosquito's only source of protein. Egg production is suppressed before feeding, due in part to GATA-mediated repression in the fat body of the major yolk precursor protein gene, Vitellogenin (*Vg*) (Martin *et al*, 2001). After the blood meal, *Vg* expression is activated by TOR enhancing expression of the transcriptional activator AaGATAa (Park *et al*, 2006). In *Drosophila*, there is evidence that this regulatory circuit may be conserved, since one of the GATA TFs, *srp*, is involved in regulating expression of the yolk protein genes (Lossky & Wensink, 1995).

Our new data now show that rapamycin abrogates higher egg laying caused by EAA enrichment (Emran *et al*, 2014), corresponding to changes in the fat body in expression of yolk proteins (Supplementary Materials). This association between dietary nitrogen and GATA transcriptional control is also consistent with mechanisms in the yeast *S. cerevisiae*, in which selective amino acid catabolism is controlled by a circuit known as Nitrogen Catabolite Repression (NCR) (Cooper, 2002). In this system, when the available nitrogen sources only support poor growth, TOR-dependent nuclear localisation of a GATA transcription factor triggers the expression of genes involved in the transport and metabolism of less-preferred nitrogen sources (Cooper, 2002). Together, these data point to connections between protein uptake, growth and reproduction, TOR signaling and GATA-mediated transcriptional control. Theory suggests that loss of function with age results from deleterious pleiotropic effects of mechanisms that promote growth and reproduction in the young (Williams, 1957). GATA factors now fit these criteria, as nutrient-responsive regulators of growth that we associate with molecular responses to lifespan-extending regimes. Testing the role of GATA factors should be a goal of future DR research.

Tissue-targeted interventions to lower insulin signalling are sufficient to extend lifespan in worms and flies (reviewed in Rera *et al*, 2013), and GATA factors are required for some such effects. It is well-established that the balance of dietary nutrients has the evolutionarily conserved capacity to determine lifelong health (Simpson *et al*, 2015), and to recognise this balanced supply, insulin and TOR signalling must coordinate. Reducing either insulin or TOR signalling is sufficient to extend lifespan, suggesting that the lifespan-extending effects of these two interventions may be mediated by the nexus of their signalling effects. In *C. elegans*, the GATA factor *ELT-2* is required for longevity following dietary restriction or mutation of the insulin receptor (Zhang *et al*, 2013), and GATA factor overexpression extends lifespan (Budovskaya *et al*, 2007). Additionally, previous transcriptional studies in both flies and worms have uncovered enrichment of GATA motifs in the insulin regulon (e.g. Murphy *et al*, 2003; Alic *et al*, 2011), and our study has now additionally uncovered an association between two different TOR-suppressive interventions, lifespan extension (Emran *et al*, 2014), and the GATA element. Together these results suggest that signals from TOR and other nutrient sensing pathways are mediated at least in part by GATA factors. In light of these data, our demonstration that diet-responsive regulons are largely tissue-specific suggests that TOR and the GATA factors mediate cell-autonomous

interpretations of global signals (e.g. insulins or bioamines), into a local language that dictates physiological change appropriate to the tissue in question.

The transcription factors *FoxO* and the *REPTOR/REPTOR-BP* complex are also candidates signals for interpretation by GATA factors in *Drosophila. Repressed by TOR* (*REPTOR*) and its binding partner (*REPTOR-BP*) have recently been discovered as novel TOR-dependent transcription factors (Tiebe *et al*, 2015). Given the complementary nature of Tiebe *et al*'s cell culture experiments and our study of dissected tissues, an emergent hypothesis is that GATA factors determine organ-specific responses by modulating the function of systemic *REPTOR* signalling. On the other hand, molecular interactions between *FoxO* and TOR are already established: TOR lies in a network of interacting signaling pathways including insulin (Teleman, 2010), and *FoxO* activation is required for lifespan extension by reduced insulin signalling (Slack *et al*, 2011). However, *FoxO* binds to TOR's promoter and is required for normal TOR expression, whilst GATA motifs are associated with genes that are not bound by *FoxO* but show *FoxO*-dependent regulation by IIS (Alic *et al,* 2011), suggesting a regulatory circuit from IIS to GATA factors via *FoxO* and TOR. Given that *FoxO* is not required for the lifespan-extending effects of DR (Min *et al*, 2008) and that different insulin-like peptides are produced in response to different nutritional stimuli (Kim & Neufeld, 2015), we suggest that signals from IIS via *FoxO* may be modified in peripheral tissues by TOR-modulated GATA activity, which shapes tissue-specific responses to diet that ultimately modify physiology and longevity (Figure 7).

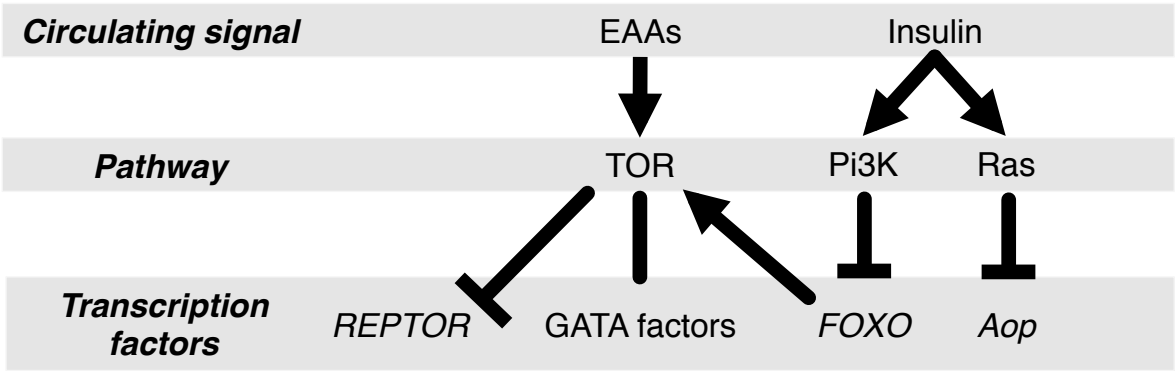

Figure 7. Predicted molecular integration of GATA factors in nutrient-sensing and lifespan-relevant transcriptional networks in *Drosophila*. Our transcriptomic analysis associates transcriptional effects of EAA restriction with TOR, and the TOR-dependent EAA regulon with GATA factors. Previous studies have shown that (1) TOR directly represses *REPTOR* (Tiebe *et al*, 2015), (2) The insulin pathway bifurcates via Pi3K and Ras to regulate lifespan via *FOXO* and *Aop* (Slack *et al* 2011; Slack *et al* 2015), and (3) GATA binding sites are associated with genes that are differentially expressed in *FOXO* mutants but not bound by *FOXO*, that *FOXO* both binds *TOR*'s promoter and *TOR* transcription is reduced in *FOXO* mutants (Alic *et al*, 2011). Together, these results suggest that *TOR* integrates signals from IIS/Pi3K signalling via *FOXO*, that regulons of DR, TOR and *FOXO* are all enriched in GATA factor binding sites, and therefore that GATA factors mediate transcriptional effects of both EAAs and *FOXO* via TOR.

The gut appears to be a particularly effective target for tissue-specific interventions to extend lifespan (reviewed in Rera *et al*, 2013). The adult gut has a critical role in the ongoing health of organisms, balancing the passage of nutrients whilst resisting environmental stresses (Buchon & Osman, 2015). In flies, there are complex relationships between age, gut maintenance, diet, metabolism, resident microbiota and expression of antimicrobial genes (Ren *et al*, 2007; Biteau *et al*, 2010; Rera *et al*, 2011; Rera *et al*, 2012; Ayyaz *et al*, 2015; Carlson *et al*, 2015; Petkau *et al*, 2014; Wong *et al*, 2014). Failure of gut integrity is associated with changes in microbiota and greater antimicrobial peptide expression, and appears to be a marker of imminent death (Rera *et al*, 2012; Clark *et al*, 2015). In worms, the GATA factor ELT-2 interacts with p38 transcriptional regulators to modify adult gut immunity (Block *et al*, 2015), whilst GATA factors are required for normal gut development and maintenance in *Drosophila* and mice (Dutta *et al*, 2015; Aronson *et al*, 2014). It is thus tempting to speculate that age-related death could be brought about by loss of gut integrity enhancing exposure to environmental microbes and toxins, and that GATA factors might play a role in this process. However, fly lifespan is not necessarily extended by enhancing expression of components of the immune system (Libert *et al*, 2006), antibiotic treatment (Ren *et al*, 2007) or genetically reducing gut dysplasia (Ayyaz *et al*, 2015). Thus, although compromised gut integrity appears to be a marker of frailty in late life, it is not obligately linked to death. Our results are directly relevant to these issues, because coordination across organs of a transcriptional module (module 4) that was perturbed under long-lived conditions strongly corresponded to GATAe expression, and GATAe and paracrine insulin signalling have each been shown to be required for intestinal stem cell proliferation (Dutta *et al,* 2015; O'Brien *et al*, 2011). It will be interesting in future studies to test how GATA factor function in the gut changes with age, if modulating GATA factor expression in the gut can improve age-related health outcomes, and if gut-restricted genetic manipulation of GATA function recapitulates effects of diet on organismal physiology

In conclusion, we have shown that the large-scale effects of EAA restriction on transcription are likely mediated by the TOR pathway. At a finer scale, transcriptional changes associated with conditions of diet-induced and drug-induced longevity are partially but incompletely overlapping, indicating differences between these conditions in organ coordination to impart distinct physiological states. Exploring these dif-

ferent states is a promising route to understand how to ameliorate ageing at a reduced biological cost. We have demonstrated a strong signal that GATA factors may mediate these changes in gene expression. This signal is consistent with known evolutionarily conserved roles of GATA factors in regulation of tissue identity, nitrogen metabolism and lifespan, but it is the first time that this family of transcription factors has been associated with lifespan extension by DR. Our study provides a clearly defined set of genes to be targeted by further experimental studies, which may include potential therapeutic targets. The evolutionary conservation of GATA factors and of the capacity of TOR to mediate lifespan extension suggests that GATA factors may be relevant to ameliorating ageing by DR in a broad range of organisms, including humans.

Materials & methods

Flies and diets

Drosophila melanogaster and diets were prepared according to Emran et al, 2014. The study used three experimental diets. 1SY medium is a dietary restriction (DR: diet-induced longevity) regime that extends lifespan in wild-type and laboratory-maintained flies (Bass *et al*, 2007; Metaxakis & Partridge, 2013), containing 100 g/l autolysed yeast (MP Biomedicals, OH, USA), 50 g/l sucrose (Tate & Lyle, London, UK), 15 g/l agar (Sigma-Aldrich, Dorset, UK), 30 ml/l nipagin (Chemlink Specialities, Dorset, UK), and 3 ml/l propionic acid (Sigma-Aldrich, Dorset, UK). EAA food comprised 1SY with the addition of cocktail of essential amino acids dissolved in water (final concentrations: L-arginine 0.43 g/l, L-histidine 0.21 g/l, L-isoleucine 0.34 g/l, L-leucine 0.48 g/l, L-lysine 0.52 g/l, L-methionine 0.1 g/l, L-phenylalanine 0.26 g/l, L-threonine 0.37 g/l, L-tryptophan 0.09 g/l, L-valine 0.4 g/l). EAA+rapamycin (drug-induced longevity) food consisted of EAA food with the addition of Rapamycin (LC laboratories, MA, USA) dissolved in ethanol, to a final concentration of 200 μM in the diet. The use of DR medium as a substrate ensures that lifespan modulation by EAA or EAA+rapamycin food is in the healthiest possible context with regard to ageing, rather than rescuing or exacerbating poor condition.

Outbred wild-type Dahomey flies bearing the endosymbiont *Wolbachia* were cultured on a 12:12 light cycle at 25°C and 60% humidity on 1SY medium. Parents of experimental flies oviposited onto grape juice agar for 18h. Eggs were washed from this agar, added to 1SY and cultured to adulthood. Newly emerged flies were allowed to mate *ad libitum* for 48h before being lightly anaesthetised with CO_2. Males were removed, and female flies were allocated to one of the three experimental diets. Females were maintained on these diets for six days. The experiment was independently replicated three times, generating three samples per organ or per whole fly, per diet.

RNA preparation and sequencing

RNA was collected 6-10h into the flies' light cycle. To prepare RNA for sequencing, whole flies were flash-frozen. Brains, abdominal fat bodies, ovaries, guts and thoraces were micro-dissected in ice-cold RNAlater solution and frozen at -80°C.

RNA was extracted using the QIAGEN total RNA isolation kit and quantified on an Agilent 2100 bioanalyser. Sequencing was performed by the high throughput genomics services center at the Huntsman Cancer Institute (University of Utah). Sample concentration and purity of RNA was measured on a NanoDrop spectrophotometer, and RNA integrity was assessed on an Agilent 2200 TapeStation. Illumina TruSeq libraries were prepared from this RNA with the Illumina TruSeq Stranded mRNA Sample Prep kit and sequenced on an Illumina HiSeq2000 101 v3 platform using paired-end sequencing.

Data analysis

Reads were aligned to the *D. melanogaster* genome annotation 5.57 using TopHat2 2.0.14 and counted using HTSeq 0.5.4p3 (Kim *et al*, 2013; Anders *et al*, 2013). Non-protein coding genes were retained. Unmapped reads were discarded. Enumerated reads were then analysed in R (3.0 & 3.1) using BioConductor. RPKM was calculated from read counts generated by HTSeq, using the EdgeR library.

Principal Components Analysis of log2-transformed RPKM were performed using the prcomp R function with scaled variance. Genes with zero variance were excluded from the analysis. This analysis indicated that two gut samples reared on the SYA diet were clear outliers from the other gut samples (Figure S1), consequently these two samples were excluded from further analysis (see Supplementary Text for further information). Remaining samples separated clearly by organ type on two PCs, which together explained 53% total variance, therefore differential expression was determined in separate analyses of each organ.

Differential expression across the three experimental conditions was determined with a negative binomial GLM fitted by DESeq2 (1.8.1, Love *et al* 2014), without rejection based on Cook's distance, calculating P-values with a two-sided Wald test, and calculating false discovery rate by Storey's method. Intersections between gene sets were visualised with the upset package for R (Lex *et al*, 2014).

Unsigned gene coexpression networks were determined using data from all organs, excluding the whole-fly samples, using the WGCNA package in R (Langfelder & Horvath, 2008). Consensus modules were determined automatically using the *blockwiseConsensusModules* function with default settings and a power of 26, stipulating a minimum module size of 50 genes. Eigengene determination, variance ex-

plained by Eigengenes and clustering were performed using internal WGCNA functions, as per the package tutorial (Langfelder & Horvath, 2008, http://labs.genetics.ucla.edu/horvath/CoexpressionNetwork/Rpackages/WGCNA/Tutorials/index.html). Changes in between-module correlations by experimental condition were calculated by custom R functions: correlation matrices (Spearman's rho) for module Eigengenes were calculated in each experimental condition, and changes were calculated by subtracting the DR matrix and the EAA+rapamycin matrix from the EAA matrix, to calculate observed changes in correlations between pairs of Eigengenes under DR and under rapamycin administration, respectively. To generate null distributions for changes in correlations for each pair of modules in each of the two long-lived conditions, the same procedure was repeated 10,000 times, permuting each Eigengene and calculated changes in correlation for each pair. Observed changes were considered significant when they did not fall between the 2.5^{th} and 97.5^{th} percentiles of their respective null distribution.

The most likely acyclic network between modules was determined by Additive Bayesian Network analysis of module Eigengenes using the R package ABN with 10000 iterations. This approach determines the likelihood of inter-dependencies between variables by randomly simulating data and comparing to the observed inter-dependencies. ABN found a consensus structure to the data after ~1000 iterations, indicating that the structure is robust to further simulation. The ABN was plotted in Cytoscape, using hierarchical network ordering. Meta-module analysis and Eigengene perturbation analysis were performed according to Langfelder & Horvath (2007).

Enrichment of cis-regulatory motifs was analysed using i-Cis target (Hermann *et al*, 2012), excluding genes for which DEseq2 models did not converge. Unannotated CRMs were excluded from further analysis. Samples were hierarchically clustered according to the presence/absence of transcription factor binding sites with the R hclust function, using a binary distance metric. Venn diagrams were plotted using the venneuler package in R. Notches on boxplots, approximating 95% confidence intervals of medians were produced using the "notch" argument to the R boxplot function. Heatmaps were plotted using the heatmap.2 function from the R gplots package, and ordered by heirarchical clustering using binary distance.

Bibliography

Alic N, Andrews DT, Giannakou ME, Papatheodorou I, Slack C, Hoddinott MP, Cochemé HM, Schuster EF, Thornton JM & Partridge L (2011) Genome wide dFOXO targets and topology of the transcriptomic response to stress and insulin signalling. *Molecular Systems Biology* **7:** 502

Alic N, Tullet JM, Niccoli T, Broughton S, Hoddinott MP, Slack C, Gems D & Partridge L (2014) Cell-nonautonomous effects of dFOXO/DAF-16 in aging. *Cell reports* **6:** 608–616

Anders S, McCarthy D, Chen Y, Okoniewski M, Smyth G, Huber W & Robinson M (2013) Count-based differential expression analysis of RNA sequencing data using R and Bioconductor. *Nat Protoc* **8:** 1765–1786

Aronson BE, Stapleton KA & Krasinski SD (2014) Role of GATA factors in development, differentiation, and homeostasis of the small intestinal epithelium. *American journal of physiology. Gastrointestinal and liver physiology* **306:** 474–490

Attardo G, Higgs S, Klingler K, Vanlandingham D & Raikhel A (2003) RNA interference-mediated knockdown of a GATA factor reveals a link to anautogeny in the mosquito Aedes aegypti. *Proceedings of the National Academy of Sciences* **100:** 13374–13379

Ayyaz A, Li H & Jasper H (2015) Haemocytes control stem cell activity in the Drosophila intestine. *Nature Cell Biology* **17:** 736–748

Bass TM, Grandison RC, Wong R, Martinez P, Partridge L, Piper MD (2007) Optimization of dietary restriction protocols in *Drosophila*. The Journals of Gerontology Series A, Biological Sciences and Medical Sciences **62**: 1071-1081

Biteau B, Karpac J, Supoyo S, DeGennaro M, Lehmann R & Jasper H (2010) Lifespan Extension by Preserving Proliferative Homeostasis in Drosophila. *PLoS Genetics* **6:** e1001159

Block DH, Twumasi-Boateng K, Kang H, Carlisle JA, Hanganu A, Lai T & Shapira M (2015) The Developmental Intestinal Regulator ELT-2 Controls p38-Dependent Immune Responses in Adult C. elegans. *PLOS Genetics* **11:** e1005265

Buchon N, Osman D, David F, Fang H, Boquete J-P, Deplancke B & Lemaitre B (2013) Morphological and Molecular Characterization of Adult Midgut Compartmentalization in Drosophila. *Cell Reports* **3**

Buchon N & Osman D (2015) All for one and one for all: Regionalization of the Drosophila intestine. *Insect Biochemistry and Molecular Biology*

Budovskaya YV, Wu K, Southworth LK, Jiang M, Tedesco P, Johnson TE & Kim SK (2007) An elt-3/elt-5/elt-6 GATA Transcription Circuit Guides Aging in C. elegans. *Cell* **134:** 1–13

Bülow MH, Aebersold R, Pankratz MJ & Jünger MA (2010) The Drosophila FoxA Ortholog Fork Head Regulates Growth and Gene Expression Downstream of Target of Rapamycin. *PLoS ONE* **5:** e15171

Carlson KA, Gardner K, Pashaj A, Carlson DJ, Yu F, Eudy JD, Zhang C & Harshman LG (2015) Genome-Wide Gene Expression in relation to Age in Large Laboratory Cohorts of Drosophila melanogaster. *Genet Res Int* **2015:** 835624

Chlon TM, Crispino JD (2012) Combinatorial regulation of tissue specification by GATA and FOG factors. *Development* **139:** 3905-3916

Clark R, Salazar A, Yamada R, Fitz-Gibbon S, Morselli M, Alcaraz J, Rana A, Rera M, Pellegrini M, Ja W & Walker D (2015) Distinct Shifts in Microbiota Composition during Drosophila Aging Impair Intestinal Function and Drive Mortality. *Cell Reports*

Clark RI, Tan S, Péan CB, Roostalu U, Vivancos V, Bronda K, Pilátová M, Fu J, Walker DW, Berdeaux R, Geissmann F & Dionne MS (2013) MEF2 Is an In Vivo Immune-Metabolic Switch. *Cell* **155:** 1–13

Cooper TG (2002) Transmitting the signal of excess nitrogen in Saccharomyces cerevisiae from the Tor proteins to the GATA factors: connecting the dots. *FEMS microbiology reviews* **26:** 223–238

Dutta D, Dobson AJ, Houtz P, Gläßer C, Revah J, Korzelius J, Patel P, Edgar B & Buchon N (2015) Regional Cell-Specific Transcriptome Mapping Reveals Regulatory Complexity in the Adult Drosophila Midgut. *Cell Reports* **12**: 346-358

Emran S, Yang M, He X, Zandveld J & Piper M (2014) Target of rapamycin signalling mediates the lifespan-extending effects of dietary restriction by essential amino acid alteration. *Aging* **6:** 390–398

Grandison R, Piper M & Partridge L (2009) Amino-acid imbalance explains extension of lifespan by dietary restriction in Drosophila. *Nature* **462:** 1061–1064

Grönke S, Mildner A, Fellert S, Tennagels N & Petry S (2005) Brummer lipase is an evolutionary conserved fat storage regulator in Drosophila. *Cell metabolism* **5**: 323-330

Hansen I, Attardo G, Park J-H, Peng Q & Raikhel A (2004) Target of rapamycin-mediated amino acid signaling in mosquito anautogeny. *Proceedings of the National Academy of Sciences of the United States of America* **101:** 10626–10631

Hansen I, Attardo G, Roy S & Raikhel A (2005) Target of Rapamycin-dependent Activation of S6 Kinase Is a Central Step in the Transduction of Nutritional Signals during Egg Development in a Mosquito. *Journal of Biological Chemistry* **280:** 20565–20572

Herrmann C, Sande B de, Potier D & Aerts S (2012) i-cisTarget: an integrative genomics method for the prediction of regulatory features and cis-regulatory modules. *Nucleic Acids Research* **40:** e114–e114

Howick VM & Lazzaro BP (2014) Genotype and diet shape resistance and tolerance across distinct phases of bacterial infection. *BMC Evol. Biol.* **14:** 56

Katewa & Kapahi (2011) Role of TOR signaling in aging and related biological processes in Drosophila melanogaster. *Experimental Gerontology* **5**: 382-390

Kim D, Pertea G, Trapnell C, Pimentel H, Kelley R & Salzberg S (2013) TopHat2: accurate alignment of transcriptomes in the presence of insertions, deletions and gene fusions. Genome Biol 14: R36

Kim J & Neufeld TP (2015) Dietary sugar promotes systemic TOR activation in Drosophila through AKH-dependent selective secretion of Dilp3. **6:** 6846

Langfelder, P., and Horvath, S. (2007). Eigengene networks for studying the relationships between co-expression modules. BMC Systems Biology *1*, 54.

Langfelder P & Horvath S (2008) WGCNA: an R package for weighted correlation network analysis. *BMC Bioinformatics* **9:** 559

Lee K, Simpson S, Clissold F, Brooks R, Ballard W, Taylor P, Soran N & Raubenheimer D (2008) Lifespan and reproduction in Drosophila: New insights from nutritional geometry. *Proceedings of the National Academy of Sciences* **105:** 2498–2503

Lex A, Gehlenborg N, Strobelt H, Vuillemot R, Pfister H (2014) UpSet: Visualization of intersecting sets. IEEE Transactions on Visualization and Computer Graphics **20**: 1983-1992

Libert S, Chao Y, Chu X, Pletcher SD (2006) Trade-offs between longevity and pathogen resistance in Drosophila melanogaster are mediated by NFκB signaling. Aging Cell **5**: 533-544

Lossky M & Wensink PC (1995) Regulation of Drosophila yolk protein genes by an ovary-specific GATA factor. *Molecular and cellular biology* **15:** 6943–6952
Love M, Huber W & Anders S (2014) Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol.* **15:** 550
Mair W & Dillin A (2008) Aging and Survival: The Genetics of Life Span Extension by Dietary Restriction. *Biochemistry* **77:** 727–754
Mair W, Piper MD & Partridge L (2005) Calories Do Not Explain Extension of Life Span by Dietary Restriction in Drosophila. *PLoS Biology* **3:** e223
Martín D, Piulachs MD & Raikhel AS (2001) A novel GATA factor transcriptionally represses yolk protein precursor genes in the mosquito Aedes aegypti via interaction with the CtBP corepressor. *Molecular and Cellular Biology* **21:** 164-174
McCay CM, Crowell MF & Maynard LA (1935) The effect of retarded growth upon the length of life span and upon the ultimate body size. *Nutrition* **5:** 155–172
Metaxakis A & Partridge L (2013) Dietary Restriction Extends Lifespan in Wild-Derived Populations of Drosophila melanogaster. *PLoS ONE* **8:** e74681
Min K, Yamamoto R, Buch S, Pankratz M & Tatar M (2008) Drosophila lifespan control by dietary restriction independent of insulin like signaling. *Aging cell* **7:** 199–206
Moore, A.W., Jan, L.Y., Jan, Y.N. (2002). hamlet, a binary genetic switch between single- and multiple- dendrite neuron morphology. *Science* **297**: 1355-1358
Moore, A.W., Roegiers, F., Jan, L.Y., Jan, Y.N. (2004). Conversion of neurons and glia to external-cell fates in the external sensory organs of Drosophila hamlet mutants by a cousin-cousin cell-type respecification. *Genes & Development* **18**: 623-628
Murakami R, Okumura T & Uchiyama H (2005) GATA factors as key regulatory molecules in the development of Drosophila endoderm. *Development, growth & differentiation* **47:** 581–589
Murphy CT, McCarroll SA, Bargmann CI, Fraser A, Kamath RS, Ahringer J, Li H & Kenyon C (2003) Genes that act downstream of DAF-16 to influence the lifespan of Caenorhabditis elegans. *Nature* **424:** 277–284
Niccoli T & Partridge L (2012) Ageing as a Risk Factor for Disease. *Current Biology* **22:**
O'Brien LE, Soliman SS, Li X & Bilder D (2011) Altered Modes of Stem Cell Division Drive Adaptive Intestinal Growth. *Cell* **147:** 603–614
Park J-H, Attardo G, Hansen I & Raikhel A (2006) GATA Factor Translation Is the Final Downstream Step in the Amino Acid/Target-of-Rapamycin-mediated Vitellogenin Gene Expression in the Anautogenous Mosquito Aedes aegypti. *Journal of Biological Chemistry* **281:** 11167–11176
Petkau K, Parsons BD, Duggal A & Foley E (2014) A deregulated intestinal cell cycle program disrupts tissue homeostasis without affecting longevity in Drosophila. *Journal of Biological Chemistry* **289:** 28719–28729
Ponton F, Wilson K, Holmes A, Cotter S, Raubenheimer D & Simpson S (2013) Integrating nutrition and immunology: A new frontier. *Journal of Insect Physiology* **59:** 130-137
Potier D, Davie K, Hulselmans G, Sanchez M, Haagen L, Huynh-Thu V, Koldere D, Celik A, Geurts P, Christiaens V & Aerts S (2014) Mapping Gene Regulatory Networks in Drosophila Eye Development by Large-Scale Transcriptome Perturbations and Motif Inference. *Cell Reports*
Ren C, Webster P, Finkel S & Tower J (2007) Increased internal and external bacterial load during Drosophila aging without life-span trade-off. *Cell metabolism* **6:** 144–152

Rera M, Azizi MJ & Walker DW (2013) Organ-specific mediation of lifespan extension: More than a gut feeling? **12:** 436–444
Rera M, Bahadorani S, Cho J, Koehler CL & Ulgherait M (2011) Modulation of longevity and tissue homeostasis by the Drosophila PGC-1 homolog. *Cell Metabolism* **14:** 623–634
Rera M, Clark RI & Walker DW (2012) Intestinal barrier dysfunction links metabolic and inflammatory markers of aging to death in Drosophila. *Proceedings of the National Academy of Sciences* **109:** 21528–21533
Robida-Stubbs S, Glover-Cutter K, Lamming DW, Mizunuma M, Narasimhan S, Neumann-Haefelin E, Sabatini DM & Blackwell KT (2011) TOR Signaling and Rapamycin Influence Longevity by Regulating SKN-1/Nrf and DAF-16/FoxO. *Cell Metabolism* **15:** 713–724
Sam S, Leise W & Hoshizaki D (1996) The serpent gene is necessary for progression through the early stages of fat-body development. *Mechanisms of Development* **60:** 197–205
Simpson S, Couteur D & Raubenheimer D (2015) Putting the Balance Back in Diet. *Cell* **161:** 1823
Simpson SJ & Raubenheimer D (2012) The Nature of Nutrition: a unifying framework from animal adaptation to human obesity. Princeton University Press
Skorupa D, Dervisefendic A, Zwiener J & Pletcher S (2008) Dietary composition specifies consumption, obesity, and lifespan in Drosophila melanogaster. *Aging Cell* **7:** 478–490
Slack C, Giannakou M, Foley A, Goss M & Partridge L (2011) dFOXO-independent effects of reduced insulin-like signaling in Drosophila. *Aging Cell* **10:** 735–748
Slack C, Alic N, Foley A, Cabecinha M, Hoddinott M & Partridge L (2015) The Ras-Erk-ETS-Signaling Pathway Is a Drug Target for Longevity. *Cell* **162**
Solon-Biet S, Mitchell S, Coogan S, Cogger V, Gokarn R, McMahon A, Raubenheimer D, Cabo R, Simpson S & Couteur D (2015a) Dietary Protein to Carbohydrate Ratio and Caloric Restriction: Comparing Metabolic Outcomes in Mice. *Cell Reports* **11:** 1529-1534
Solon-Biet S, Walters K, Simanainen U, McMahon A, Ruohonen K, Ballard J, Raubenheimer D, Handelsman D, Couteur D & Simpson S (2015b) Macronutrient balance, reproductive function, and lifespan in aging mice. *Proceedings of the National Academy of Sciences* **112:** 3481–3486
Solon-Biet SM, McMahon AC, Ballard WJ, Ruohonen K, Wu LE, Cogger VC, Warren A, Huang X, Pichaud N, Melvin RG, Gokarn R, Khalil M, Turner N, Cooney GJ, Sinclair DA, Raubenheimer D, Couteur DG & Simpson SJ (2014) The Ratio of Macronutrients, Not Caloric Intake, Dictates Cardiometabolic Health, Aging, and Longevity in Ad Libitum-Fed Mice. *Cell Metabolism* **19:** 418–430
Sorrentino R, Gajewski KM & Schulz RA (2005) GATA factors in Drosophila heart and blood cell development. **16:** 107–116
Taylor & Dillin (2013) XBP-1 is a cell-nonautonomous regulator of stress resistance and longevity. *Cell* **153:** 1435-1447
Teleman AA (2010) miR-200 De-FOGs Insulin Signaling. *Cell Metabolism* **11:** 8–9
Tiebe M, Lutz M, Garza A, Buechling T, Boutros M & Teleman AA (2015) REPTOR and REPTOR-BP Regulate Organismal Metabolism and Transcription Downstream of TORC1. *Developmental Cell* **33:** 272–284
Unckless RL, Rottschaefer SM & Lazzaro BP (2015) The Complex Contributions of Genetics and Nutrition to Immunity in Drosophila melanogaster. *PLOS Genetics* **11:**

e1005030
Vilchez D, Saez I & Dillin A (2014) The role of protein clearance mechanisms in organismal ageing and age-related diseases. *Nature Communications* **5:** 5659
Williams G (1957) Pleiotropy, natural selection, and the evolution of senescence. *Evolution* **11:** 398–411
Wong AC-N, Dobson AJ, Douglas AE (2014). Gut microbiota dictate the metabolic response of *Drosophila* to diet. *Journal of Experimental Biology* **217**: 1894-1901
Zenvirt, S., Nevo-Caspi, Y., Rencus-Lazar, S., Segal, D. (2008). Drosophila LIM-only is a positive regulator of transcription during thoracic bristle development. *Genetics* **179**: 1989-1999
Zhang P, Judy M, Lee S-J & Kenyon C (2013) Direct and Indirect Gene Regulation by a Life-Extending FOXO Protein in C. elegans: Roles for GATA Factors and Lipid Gene Regulators. *Cell Metabolism* **17:** 85-100

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Authors' Contributions

Designed the study: MP, AD, MY, E Blanc. Performed experiments: MY, XH, AD, E Bolukbasi. Analysed data: AD, E Blanc. Wrote the manuscript: AD, MP.

Supplementary Figures

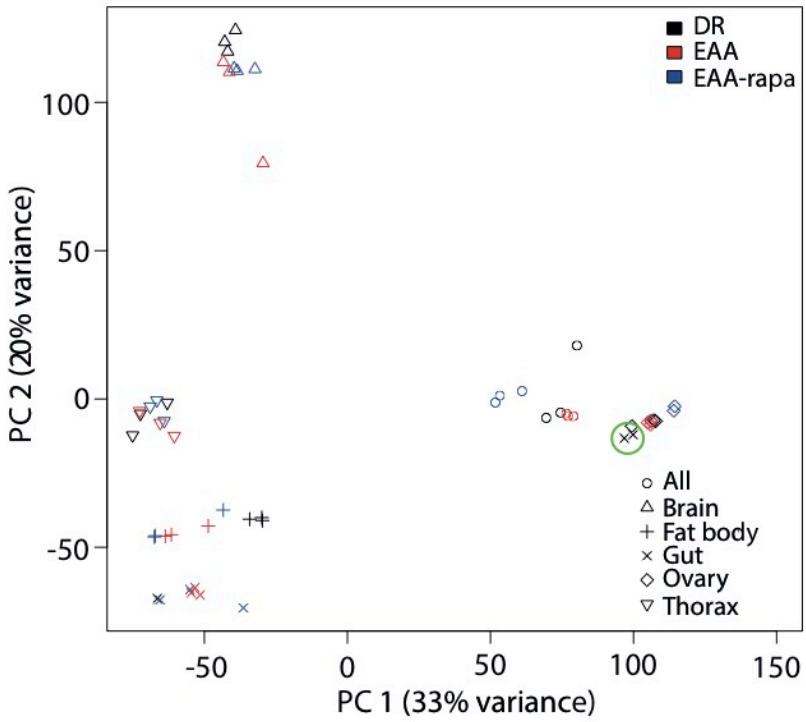
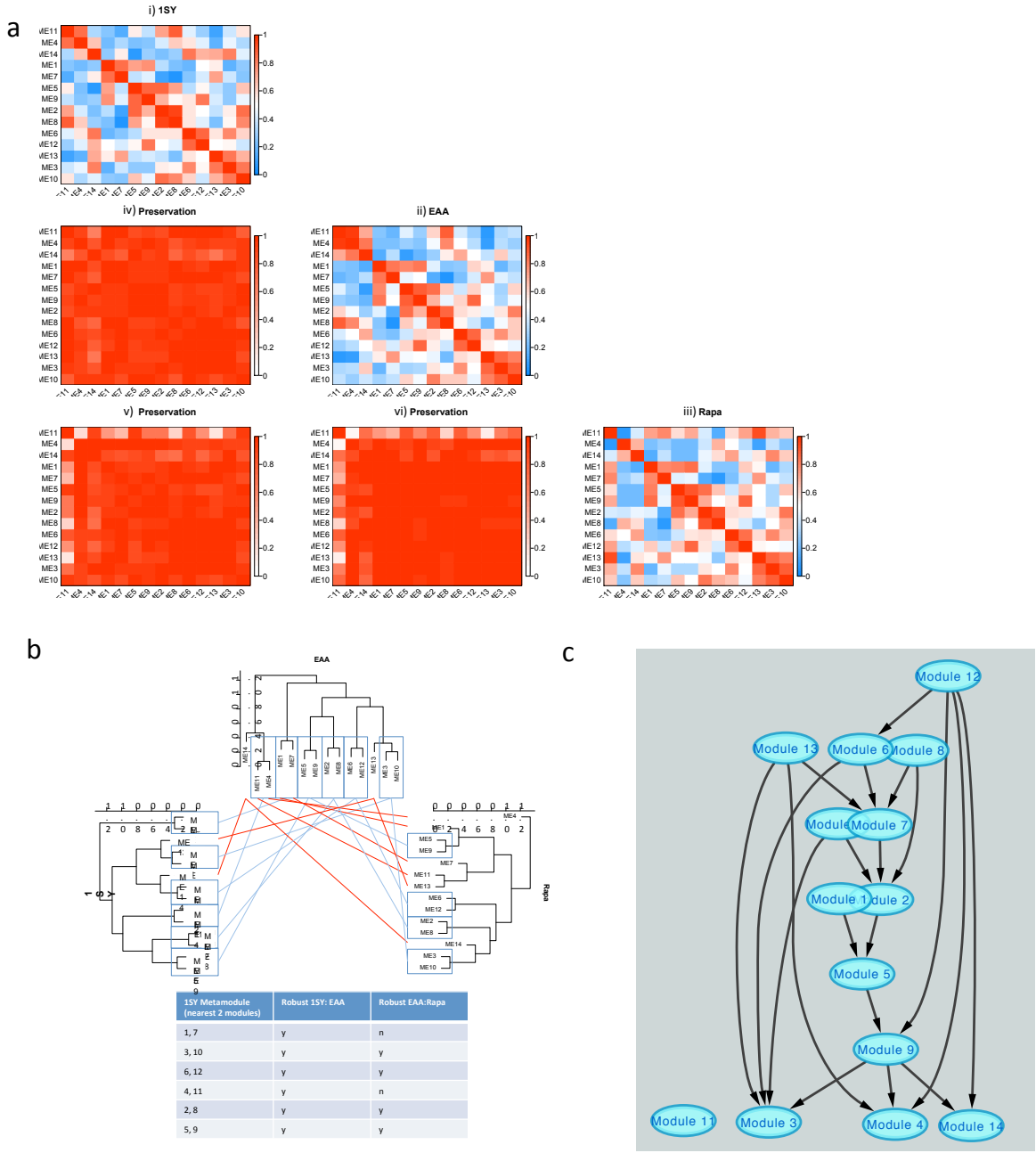


Figure S1. Effects of DR and TOR are orthogonal across organs. Principal components of whole-transcriptome expression ($\log_2$ RPKM) separated samples and showed that effects of DR and TOR suppression are non-equivalent across organs. Samples highlighted in green are gut samples which were excluded from the network and differential expression analyses (see Materials & Methods and Supplementary Text).



1SY Metamodule (nearest 2 modules)	Robust 1SY: EAA	Robust EAA:Rapa
1, 7	y	n
3, 10	y	y
6, 12	y	y
4, 11	y	n
2, 8	y	y
5, 9	y	y

Figure S2. Identification of DR/TOR-responsive transcriptional modules by Eigengene analysis. **(a)** Adjacency (correlation) between module Eigengenes identifies modules 11, 4 and 14 as showing the strongest changes in correlations to the rest of the network. Panels i-iii show module-module correlations within diets (i, DR (1SY); ii, EAA; iii, EAA+rapamycin). Panels iv-vi show changes in module-module correlations between treatments (iv, DR (1SY); v, EAA; vi, EAA+rapamycin). White colouration indicates reduced preservation of module-module correlations between diets. Rows and columns of all plots are ordered by the sum strength of perturbation across the three conditions. **(b)** Clustering of modules by Eigengenes changes across diets. Modules are hierarchically clustered by Eigengenes within each diet,

and nearest neighbours are identified when pairs are clearly apparent (blue boxes). Some of these pairings are not robust to dietary changes (red lines), including modules 11 and 4. Module 14 does not have a near neighbour on any diet, and clusters most closely with different modules on each diet. Thus the structure of the transcriptional network, summarised by Eigengenes, changes according to diet, particularly in terms of the relationships of modules 11, 4, 14 to the rest of the network. **(c)** Structure of EAA/TOR-dependent changes in the transcriptional network. Additive Bayesian Network (ABN) analysis was used to find structure in the representative expression values (Eigengenes) for the full set of transcriptional modules. Eigengenes are defined as the first principal component of expression values for a module. These explained ~80% of total transcriptomic variance across the organ system. This analysis indicates that perturbations by EAAs/TOR of the organ system's transcriptional architecture most strongly affects coregulation of modules 3, 4, 11 and 14. Changes in the relationship of module 11 to the rest of the network were sufficiently strong that it could not be related to expression of other modules.

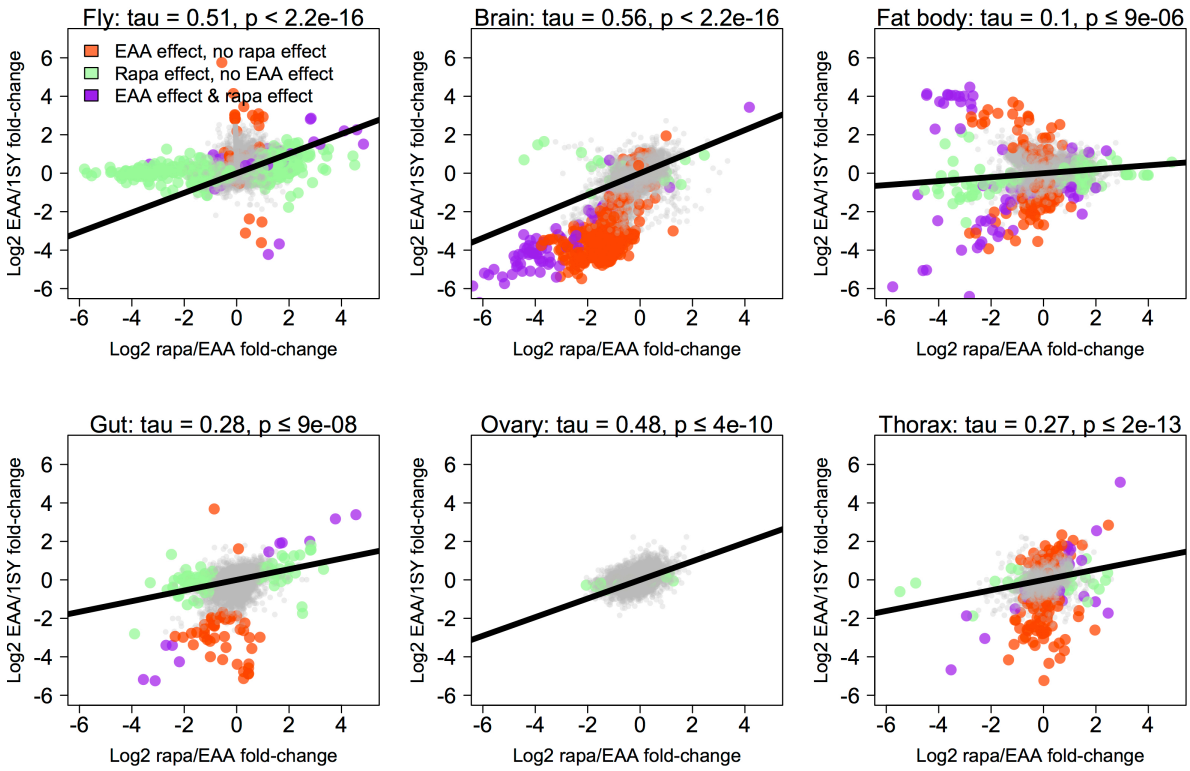


Figure S3. **Fold-changes in expression genes differentially-expressed after DR and rapamycin supplementation are positively correlated.** Points representing genes are coloured according to whether they responded to DR, rapamycin, or both. Genes which were not differentially expressed are coloured grey. Text above panels indicates results of rank correlations (Kendall's Tau) for just differentially expressed, genes, and lines indicate the slope of the correlation for the differentially expressed genes. Note that this figure is a re-colouration of Figure 1, with correlation analysis specific to the differentially expressed genes.

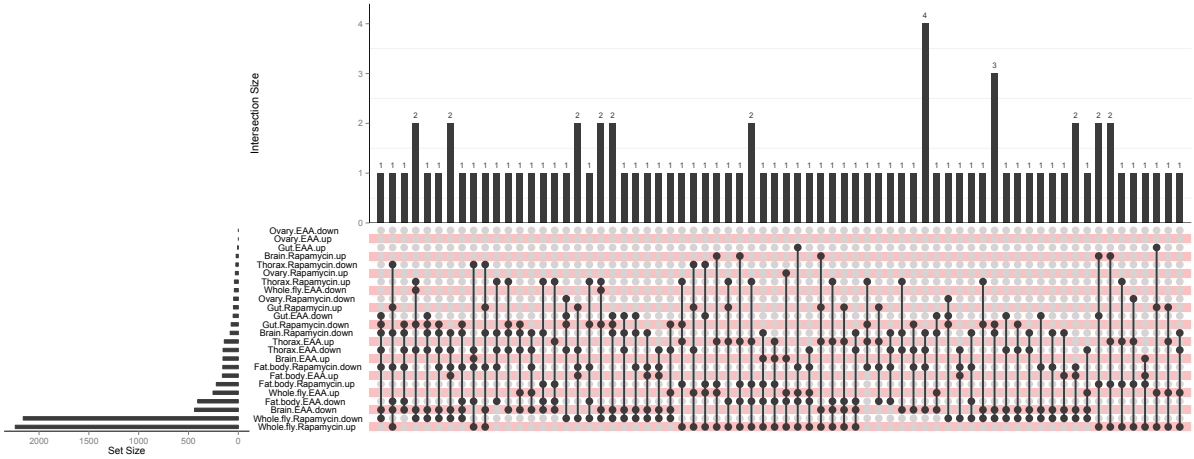


Figure S4. Sets of differentially expressed genes and their intersections across tissues. Effects of the diet-induced and drug-induced longevity conditions on differential expression (FDR $\leq$ 0.01) of individual genes was determined within each organ. Labels to the left of the matrix give the tissue, experimental condition and sign of expression change that define the gene set. The bar chart at the left of the figure gives the total number of genes that were differentially expressed in gene set. Columns of the matrix show a point for a given set of genes that share differential expression in one tissue, diet and showing a shared sign of expression change. Rows of the matrix show connected points for sets of genes that show intersecting differential expression across >1 gene set. Sets of <5 genes are excluded. The bar chart at the top of the matrix gives the size of the intersection between sets, or the size of the set for non-overlapping genes. The figure shows that DR and rapamycin often have complementary effects within the same tissue. Whole-body samples do not fully account for organ-specific transcriptional variation, since not all sets overlap include genes differentially expressed in whole body samples. See Figure 5 for complementary figure showing the same data set ordered by gene set size.

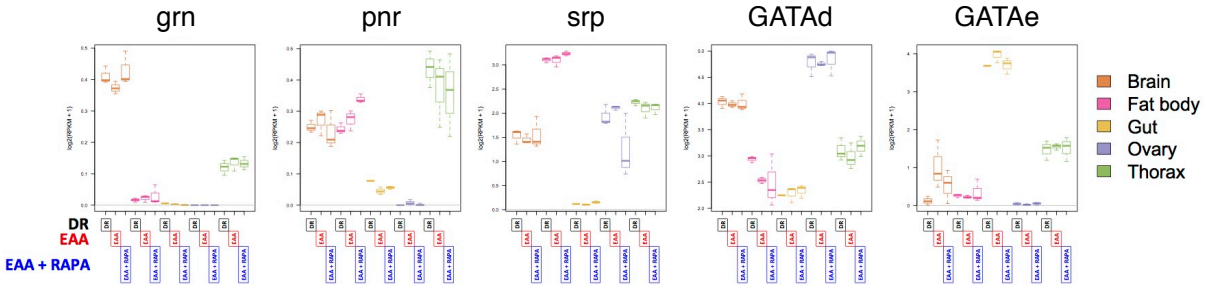


Figure S5. Expression of GATA factors across organs and dietary conditions. Each panel shows normalized expression (log_2 RPKM) of each of the five GATA family transcription factors In the brain, fat body, gut, ovary and thorax, per each experimental diet DR, EAA, EAA+rapa. Plots show medians (horizontal midline), 1st and 3rd quartiles (hinges), and range of data points.

Supplementary Text

Supplementary text 1 – exclusion of gut samples

For each tissue under study per each of the three experimental diets, three samples were originally collected. PCA analysis showed that the gut samples clustered together and were transcriptionally distinct from other tissues, with the exception of two samples from the DR condition (Figure S1). These two samples showed expression which correlated neither other gut samples from the present study, nor gut transcriptomes from external sources (*unpublished data*; D. Dutta, *Pers. Comm.*; and (Dutta *et al*, 2015)). We were able to run all our analyses without including these two samples, and so they were excluded.

Supplementary text 2 – Functional analysis of differentially expressed genes

There is now a wealth of analyses of changes in gene expression in response to genetic (e.g. Alic *et al*, 2011) and dietary (e.g. Whitaker *et al*, 2014) inteventions to extend lifespan in *Drosophila*, as well as descriptions of transcriptomic change throughout the lifecourse (e.g. Carlson et al, 2015). Parallel studies have also been conducted in a number of other model organisms (e.g. Selman *et al*, 2006; Murphy *et al*, 2003). Our goal in the present work was to identify putative regulators of TOR-dependent transcriptional change within organs, and regulators of structural changes to the transcriptome across the organ system. Genes that showed organ-specific differential expression are identified and discussed below.

At the organismal level, gene expression was generally higher under DR than under EAA-enriched conditions, consistent with fly physiology being primed for immediate exploitation of resources as they become available, to increase fitness in the short term. Such genes included a suite of known or predicted metabolic enzymes - particularly enzymes involved in lipid metabolism - growth regulators (e.g. *dally, dawdle*), transcription factors (e.g. *doublesex),* amino acid transporters (*Eaat1, NAAT1*), genes responsive to the growth-stimulatory insect hormone ecdysone (*Eip63F-1, DOR*), 8 peptidases of the *Jonah* family, and humoral antimicrobials (*Lysozyme P, Defensin, PGRP-SC2*). Conversely, the 17 genes that were downregulated DR and by TOR included the metabolic regulator *Ilp8* and *Gapdh2*. In addition to these reciprocally regulated genes, expression of some genes responded to EAAs but not to ra-

pamycin, i.e. they were EAA-dependent but without evidence of TOR-dependence. Such genes include the immune genes *cactus* and *insensitive*, and the transcription factors *double-parked* and *E2f2*. Of the genes that were downregulated by EAA enrichment, 25 (18%) were not responsive to rapamycin, including enzymes involved in glutathione and glutamate metabolism and in glycine/serine/threonine metabolism, *Eip-71CD*, two heat shock proteins (*Hsp60c*, *Hsp70Bb*), and immunity genes (*Lysozyme B*, *PGRP-SC1a*). In a few cases, the effects of DR and of rapamycin were not equivalent: 6 genes (*Antigen-5 related-2, CG31869, Metallothionein-D, traffic jam, Uhg5, Vitelline membrane 26Ab*) that were upregulated by DR were downregulated by rapamycin, and *Osiris-6* and *Osiris-7* were downregulated by DR but upregulated by rapamycin. Full statistical analyses and enrichment of GO terms are presented in Supplementary Spreadsheets. Collectively, these results demonstrate that EAAs have extensive effects on the whole-fly transcriptome, which are largely TOR-dependent.

The three fly yolk proteins (*Yp1, Yp2, Yp3*) had lower expression in the fat body after DR, and after rapamycin feeding. These changes correspond to egg laying on the same diets (Emran et al., 2014). A curious feature of the data was the differential expression of multiple genes annotated as chorion proteins (*Cp*) and vitelline membrane proteins (*Vm*) in organs other than the ovary. This corresponds to other data (Dobson, Chaston & Douglas, *în prep*) which show that genes annotated as vitelline membrane proteins show altered expression after nutritional alteration in whole-body samples of *Drosophila* males, implying nutrient-dependent roles for these genes in processes other than egg production. In the present dataset, these genes changed expression in concert with a number of other genes involved in oogenesis and reproduction, but the changes were not always equivalent across organs (eg *Cp36* and *Cp38*). We note that a number of chorion proteins have recently been shown to exhibit changes in expression across *Drosophila* lifespan (Carlson et al, 2015). Together these results indicate unappreciated roles for these genes outside of the chorion. We also noted that some genes with roles in reproduction were not TOR-dependent, or that TOR suppression and DR had opposite effects on expression.

Many genes with metabolic functions were regulated by DR, but these effects were not necessarily TOR-dependent, and responses across organs were not equivalent. For example, in the brain, expression of maltases decreased upon DR, and whilst expression of some of these enzymes (*Mal-A7, Mal-A8*) was TOR-

dependent, expression of others (*Mal-A1, MalA2, Mal-A3*) was not. In the thorax, other maltases (*Mal-B1, Mal-A8, Mal-i6*) were downregulated by DR in a way that was complemented by TOR suppression.

We also noted that expression of secreted antimicrobials responded to DR/TOR, in a highly organ-specific fashion. Immunity is a major fitness component which is regulated by diet (Ponton *et al*, 2013; Simpson & Raubenheimer, 2012; Clark *et al*, 2013; Unckless *et al*, 2015), therefore our data can shed light on how diet changes the contribution of specific organs to organismal immunity. The fat body is a major source of antimicrobials, and expression of *Cecropin*, *Diptericin*, *Lysozyme X*, *Metchnikowin*, *Drosomycin-like 5*, *Attacin B* and *Attacin C* decreased after DR or rapamycin feeding. However, in the brain and thorax, immunity genes were downregulated by DR, but not by rapamycin. This suggests that systemic immunity may be determined by interplay of both nutrient availability and signalling, but immunity in specific tissues is determined by nutrient availability alone, and that some organs are selectively spared the immunosuppressive effect of reduced TOR signalling.

Bibliography (Supplementary Text)

Alic N, Andrews DT, Giannakou ME, Papatheodorou I, Slack C, Hoddinott MP, Cochemé HM, Schuster EF, Thornton JM & Partridge L (2011) Genome wide dFOXO targets and topology of the transcriptomic response to stress and insulin signalling. *Molecular Systems Biology* **7:** 502

Carlson KA, Gardner K, Pashaj A, Carlson DJ, Yu F, Eudy JD, Zhang C & Harshman LG (2015) Genome-Wide Gene Expression in relation to Age in Large Laboratory Cohorts of Drosophila melanogaster. *Genet Res Int* **2015:** 835624

Clark RI, Tan S, Péan CB, Roostalu U, Vivancos V, Bronda K, Pilátová M, Fu J, Walker DW, Berdeaux R, Geissmann F & Dionne MS (2013) MEF2 Is an In Vivo Immune-Metabolic Switch. *Cell* **155:** 1–13

Dutta D, Dobson A, Houtz P, Gläßer C, Revah J, Korzelius J, Patel P, Edgar B & Buchon N (2015) Regional Cell-Specific Transcriptome Mapping Reveals Regulatory Complexity in the Adult Drosophila Midgut. *Cell Reports*

Emran S, Yang M, He X, Zandveld J & Piper MD (2014) Target of rapamycin signalling mediates the lifespan-extending effects of dietary restriction by essential amino acid alteration. *Aging* **6:** 390–8

Murphy CT, McCarroll SA, Bargmann CI, Fraser A, Kamath RS, Ahringer J, Li H & Kenyon C (2003) Genes that act downstream of DAF-16 to influence the lifespan of Caenorhabditis elegans. *Nature* **424:** 277–284

Ponton F, Wilson K, Holmes A, Cotter S, Raubenheimer D & Simpson S (2013) Integrating nutrition and immunology: A new frontier. *Journal of Insect Physiology* **59:** 130-137

Simpson SJ & Raubenheimer D (2012) The Nature of Nutrition: a unifying framework from animal adaptation to human obesity. Princeton University Press

Unckless RL, Rottschaefer SM & Lazzaro BP (2015) The Complex Contributions of Genetics and Nutrition to Immunity in Drosophila melanogaster. *PLOS Genetics* **11:** e1005030
Whitaker R, Gil, Ding F, Tatar M, Helfand S & Neretti N (2014) Dietary switch reveals fast coordinated gene expression changes in Drosophila melanogaster. *Aging* **6:** 355–68