Supplementary Figure Legends for Figures S1 – S8

Figure S1. RNA-Seq biological replicate correlations are strong, and variability is not associated with sample batches.

(A) Pearson correlations for biological replicate sample are strong, and not lower than 0.847 in any case. Sample groups are organized by strain as indicated on the left side and RNAi condition as indicated on the top. All sample groups have at least two biological replicates. Histograms indicate the density of the points in the correlation dot plots. All 17,907 genes considered as expressed in any sample in the dataset are included. All correlations were significant.

(B) Due to the size of the experiment, animals were prepared in six batches, indicated by the shape of the points in the PCA scatterplots. Variability between samples is not associated with these batches. All RNA-Seq libraries were sequenced at the same time to eliminate a common source of technical variation.

Figure S2. A subset of innate immune response-associated genes are upregulated in *eat-2*, but not specific to DR.

We observed enrichment of innate immune response Gene Ontology (GO) terms in *eat-2* animals (Figure 2C), and thus further investigated expression of innate immune response-associated genes in the other samples. **(A)** Venn diagram indicating the number of *C. elegans* genes associated to GO terms for innate immune response, from annotation in WormBase (WS282). 391 genes are represented in the union of the three innate immune response terms. **(B)** The 391 immune response genes from (A) were intersected with the genes significantly up-regulated in any of *eat-2*, *mxl-2*, or N2 *pha-4* RNAi, yielding 85 genes. The heatmap represents foldchanges for the indicated sample group compared to wild-type (N2) for these 85 genes. Red blocks on the left bar indicate GO terms associated with each gene. A subset of these genes is similarly upregulated in *eat-2*, *mxl-2*, and N2 *pha-4* RNAi, and is thus not specific to DR.

Figure S3. Genes with demonstrated or predicted functions in phosphorylation or de-phosphorylation were significantly enriched among transcripts downregulated in *eat-2.*

(A) We found 76 de-phosphorylation genes and 111 phosphorylation genes significantly downregulated in *eat-2* based on Gene Ontology annotation. **(B, C)** We looked at other functions and pathways associated with these genes and represented the relative frequency of the functional terms as a treemap each for the de-phosphorylation genes **(B)** and phosphorylation genes **(C)** downregulated in *eat-2*. The box size represents the number of genes associated with the term relative to all the associations for the group. Only terms for which more than two genes were associated were considered, and phosphorylation cycle terms used to select the genes in (A) were removed. **(D)** Tissue enrichment for the phosphorylation and de-phosphorylation genes down-regulated in *eat-2* from (A). Somatic tissue gene sets are based on the union of tissue-specific and tissue-enriched gene lists from cell type specific bulk RNA-Seq in (Kaletsky et al. 2018). Sperm-specific genes are from (Reinke et al. 2000). Both phosphorylation and de-phosphorylation genes are significantly enriched in sperm, rather than being distinctly regulated in different somatic tissues. Numbers in brackets indicate the size of the gene set or the number of genes in the intersection. Hypergeometric test adjusted p-value significance level: '***' p < 0.001 '**' 0.01 '*' 0.05 '.' 0.1.

Figure S4. DR down-regulation of sperm and muscle genes is dependent on *mxl-2.*

(A.) Tissue enrichment for significantly differentially expressed in genes in *eat-2*, *mxl-2*, and *pha-4 RNAi* all compared to wild-type (N2 EV). We found strong enrichment of muscle- and sperm-associated genes in *eat-2* but not loss of *mxl-2* or *pha-4*. **(B.)** Tissue enrichment for *eat-2* differentially expressed genes that maintained *eat-2*-like expression with *mxl-2* loss ("*mxl-2*-independent", bottom set of rows), and *mxl-2*-dependent genes (middle set of rows). 394 of 395 sperm-associated genes downregulated in *eat-2* were dependent on *mxl-2* to maintain reduced expression in DR. Rather than returning to WT-like-levels, a substantial fraction of sperm-related genes are significantly upregulated in *eat-2;mxl-2* animals (top set of rows). Somatic tissue gene sets are based on the union of tissue-specific and tissue-enriched gene lists from cell-type-specific bulk RNA-Seq from (Kaletsky et al 2018). Sperm-specific genes are from (Reinke et al

2000). Numbers in brackets indicate the size of the gene set, or the number of genes in the intersection. Adjusted p-value significance level: '***' p < 0.001 '**' 0.01 '*' 0.05 '.' 0.1.

Figure S5. Putative high-confidence trans-activation domains (TAD) are predicted on the amino-terminal side of MML-1 and the carboxy-terminal side of MDL-1.

Trans-activation domains (TADs) in *C. elegans* Myc-family TFs (blue headers) and their human homologs (grey headers) were predicted with ADpred; the red horizontal line in each plot indicates an ADpred score of 0.8, which was suggested as a threshold of high-confidence TAD prediction in the ADpred manuscript (Erijman et al 2020). Scores are predicted along the protein sequence with black lines: raw scores per amino acid, and blue lines: smoothed to highlight broader trends. **(A.)** ADpred analysis identified a high-confidence TAD from 245-276 on the amino terminal side of MML-1 between the "Mondo-conserved" domains IV and V (Ceballos, Esse, Grishok 2021) that parallels similar predicted TADs in the human homologs MLXIP and MLXIPL. **(B.)** There are TAD regions of lower confidence on the C-terminal side of MXL-2, which also correspond to a region of similar confidence on the C-terminal side of human homolog MLX. **(C.)** *C. elegans* MDL-1, thought to function primarily as a transcriptional repressor, harbors a high confidence predicted TAD on the C-terminal end, indicating that MDL-1 may have roles in transcriptional activation in some contexts, unlike its human counterparts. **(D.)** Similar to mammalian MAX, there were no predicted TADs in MXL-1 but we found a predicted TAD of lower confidence on the C-terminal side of MXL-3. Protein sequences were from WormBase for *C. elegans* and UniProt for human: MLXIP (Q9HAP2), MXLIPL (Q9NP71), MLX (Q9UH92), MXI1 (P50539), MXD1/MAD (Q05195), MXD3/MAD3 (Q9BW11), MXD4/MAD4 (Q14582), MAX (P61244).

Figure S6. Plot for additional trial for brood size assay. Loss of MXL-2 in DR but not *ad libitum* animals compromises fecundity and embryo viability.

Additional trial data for brood size assay, analogous to the representative trial in Figure 4. Brood size assays were performed to determine how reproduction-associated gene expression changes with *mxl-2* loss in *eat-2* affected reproductive fitness. **(A)** Viable progeny from each day over the course of the reproductive lifespan were counted for singled parent hermaphrodites for **(i)** N2 and *mxl*-2, and **(ii)** *eat*-2 and *eat-2;mxl-2* animals. **(B)** Total brood size aggregated from across the

observations in (A) for each strain. **(C)** Unhatched eggs were counted for each strain, but only found for *eat-2;mxl-2* animals. Data is shown from a representative trial. See Table S4 for complete results and statistical analysis. Stars indicate FDR-corrected p-values: *< 0.05, **< 0.01, ***< 0.005.

Figure S7. Key autophagy-associated genes were not differentially expressed in *eat-2* but are dysregulated in *eat-2;mxl-2*.

Heatmap of $\log_2$ foldchanges for the indicated sample group compared to wild-type (N2) for key genes involved in autophagy, with their canonical role indicated by the labeled sidebar on the left. Autophagy-related gene expression was not up-regulated in *eat-2* animals but was dysregulated by loss of *mxl-2* in the *eat-2* background. Columns are ordered by hierarchical clustering. Significantly differentially expressed genes are indicated by red stars, adjusted p-value significance level: '***' p < 0.001 '**' 0.01 '*' 0.05 '.' 0.1.

Figure S8. Enrichment for predicted transcription factor binding in promoters of genes differentially expressed in *eat-2*, m*xl-2*, *daf-16* (RNAi) or *pha-4* (RNAi).

Heatmaps representing enrichment of transcription factor motifs in promoters of genes **(A)** down-regulated or **(B)** up-regulated compared to wild-type. **(A)** Consistent with the expected binding motif of MML-1::MXL-2, the most enriched motifs for genes down-regulated under DR correspond to canonical CACGTG E-box sites and the motif for MDL-1. DAF-16 motifs are most significantly enriched for genes down-regulated with RNAi for *daf-16*, but we do not observe enrichment for PHA-4 predicted sites in samples with RNAi for *pha-4*, or in *eat-2* DR conditions. E-box sites or motifs are not enriched in the genes downregulated in *mxl-2* mutants, however MML-1::MXL-2 activity is low under such conditions (Johnson et al 2014). **(B).** An invariant set of transcription factor motifs represent those most significantly enriched across all single-perturbation conditions for up-regulated genes, corresponding to GATA-binding factors. Numbers in brackets are the size of the gene set, or the size of the intersection. Stars indicate significant enrichment results. Adjusted p-value significance level: '***' p < 0.001 '**' 0.01 '*' 0.05 '.' 0.1.