Supporting Information

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SI Methods

Comparative Methods. Comparison of expression profiles among species included expression data from *D. melanogaster* and *C. elegans*. The *D. melanogaster* data set consisted of competitive hybridizations of pools of reproductive females to nonreproductive, diapause females comparable to our experimental design, whereas the *C. elegans* data set consisted of four two-channel hybridizations comparing dauer pools to a control mRNA sample and four comparing nondauer (12 h after dauer exit) to the same control. For subsequent clustering we used only log(2) fold changes of genes that are orthologous across all species. Orthology was established by (*i*) selecting FlyBase BLAST hits at $e < 10^{-5}$ for *S. crassipalpis* ESTs to *D. melanogaster* and (*ii*) identifying *D. melanogaster–C. elegans* orthologues in the InParanoid database (http://inparanoid.sbc.su.se/), retaining only orthologues in which a single *D. melanogaster* gene produced an Inparalog score of 1.0 only when paired with a single *C. elegans* gene ($N = 1{,}925$). For *C. elegans* we calculated log(2) fold change as the difference of log fold changes between the dauer versus control and nondauer versus control arrays. Because which array values are compared is arbitrary (no competitive hybridizations, all compared with a common control), we calculated fold change differences using all possible ($n = 24$) pair-wise comparisons of dauer arrays to nondauer arrays. Hierarchical clustering analysis yielded quantitatively similar results with identical topologies across permutations. For simplicity, we present clustering data for only one permutation (see *Results and Discussion* in the main text).

Based on a list of orthologous genes, we also tested whether the genes most differentially regulated between the dormant and nondormant phenotype within each species were also genes whose expression patterns were most similar among the species. We first performed discriminate function analysis on the expression data (using pdmClass in R) and produced a ranked list of genes from those best able to discriminate among the species to the least discriminatory. Next, we calculated Kolmogorov-Smirnov statistics to test whether ranked lists containing only the twofold differentially regulated genes (three lists, one for each species) exhibited significantly different distributions than the ranked list of all genes. For all three species, twofold differentially regulated genes were significantly overrepresented at the top end (i.e., the most discriminating) of the rank list (Fig. S6).

Of genes that were at least twofold differentially regulated between dormant and nondormant phenotypes in each species, only 10 were twofold differentially regulated in all species (Table S2). Of these, two were differentially regulated in different directions across species. Only two genes were twofold up-regulated across species: phosphoenolpyruvate carboxykinase and pyruvate carboxylase, the irreversible members of the gluconeogenesis pathways discussed in the main text.

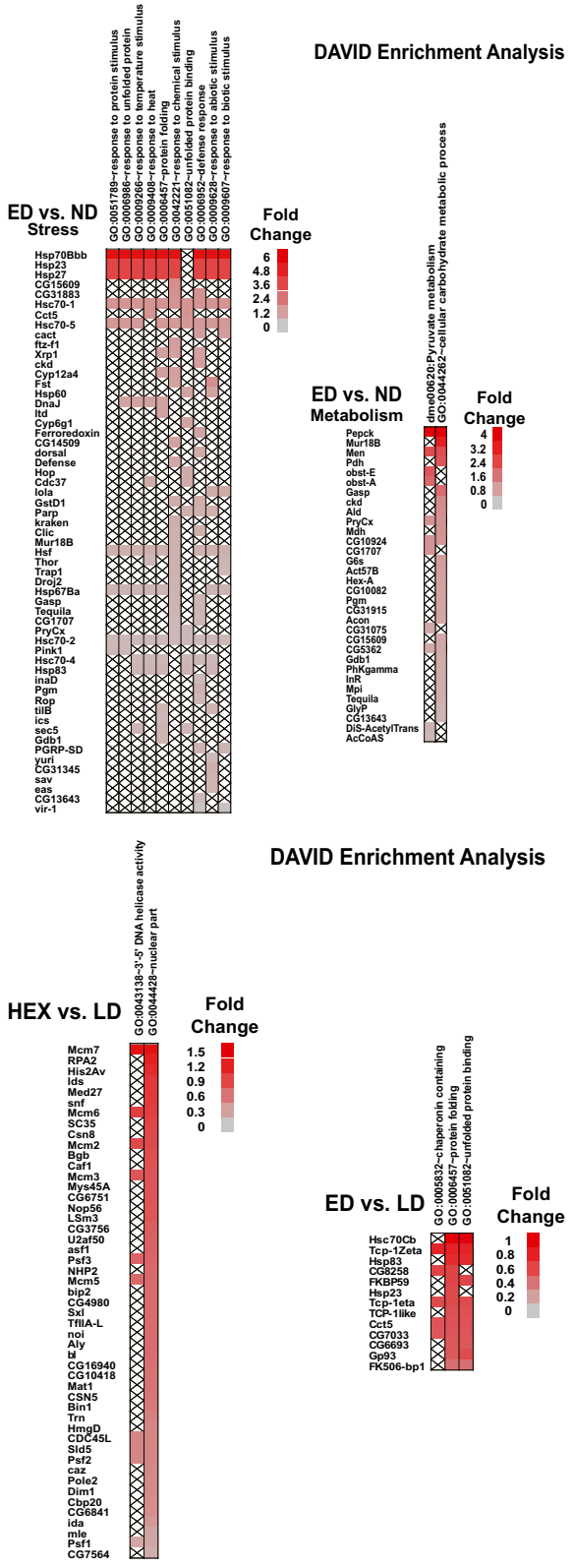

Fig. S1. Lists of ESTs up-regulated in DAVID analysis. ESTs not enriched in any category are marked with an X, the relative log twofold change of ESTs up-regulated in any category are shown in red.

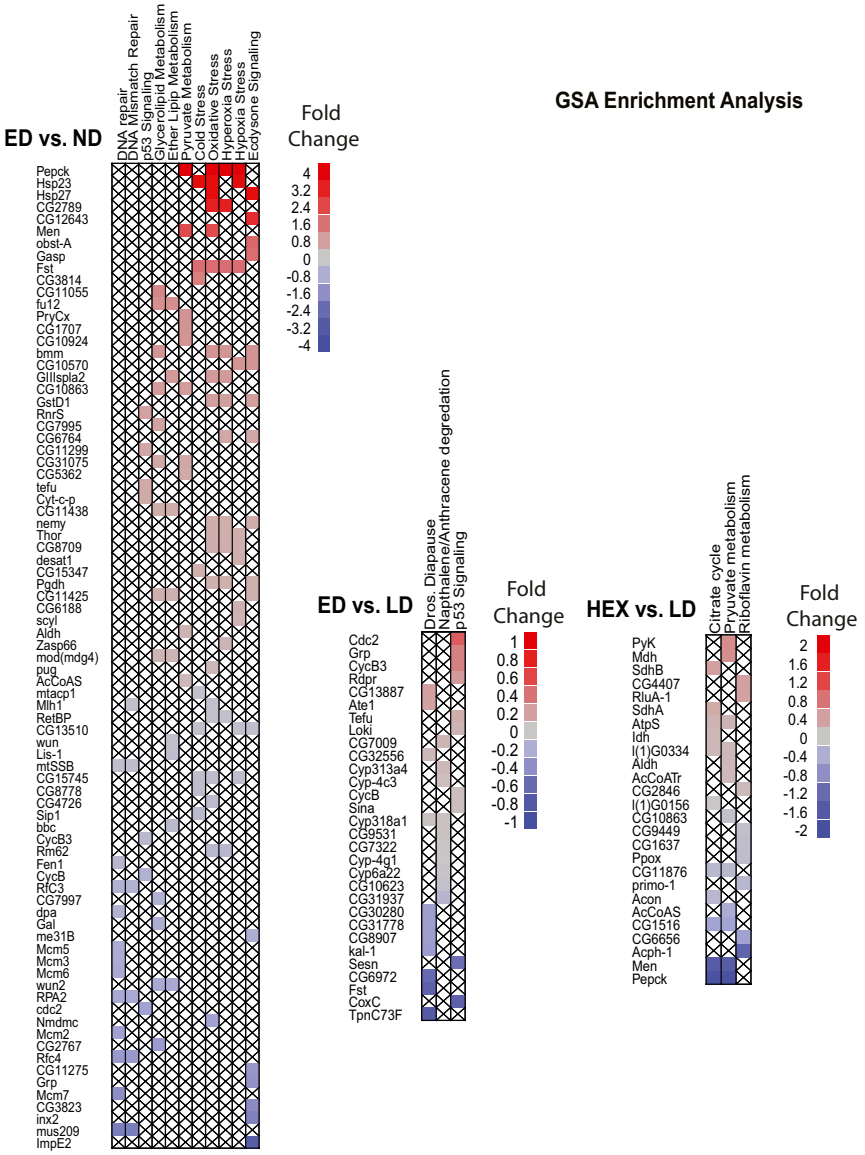

Fig. S2. Lists of ESTs enriched in GSA analyses across KEGG pathways and a priori lists from the literature. ESTs not enriched in any list are marked with an X, the relative $\log_2$ fold change of ESTs enriched in any list are shown in color.

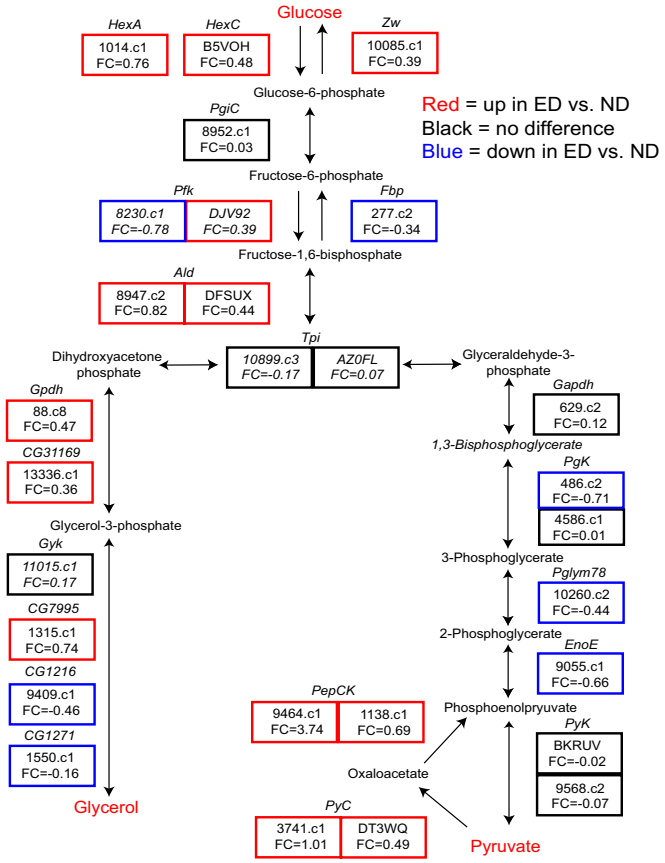

Fig. S3. Glycolysis/gluconeogenesis pathway diagram modified from the KEGG database for *D. melanogaster*. The name for each enzyme is listed above the box, and the *S. crassipalpis* EST database number and $\log_2$ fold change value comparing early diapausing pupae to nondiapausing pupae is listed within the box. Red boxes designate ESTs that had significantly greater abundance in early diapause pupae than nondiapause pupae after FDR correction $q < 0.01$. Blue boxes denote ESTs that had significantly lower abundance in early diapausing pupae compared with nondiapausing pupae after FDR correction. Black boxes denote no difference between early diapausing and nondiapausing pupae. Metabolites in red text were determined to accumulate in greater abundance in early-diapausing pupae relative to nondiapause pupae by metabolomics (1).

1. Michaud MR, Denlinger DL (2007) Shifts in the carbohydrate, polyol, and amino acid pools during rapid cold-hardening and diapause-associated cold-hardening in flesh flies (*Sarcophaga crassipalpis*): A metabolomic comparison. *J Comp Physiol B* 177:753–763.

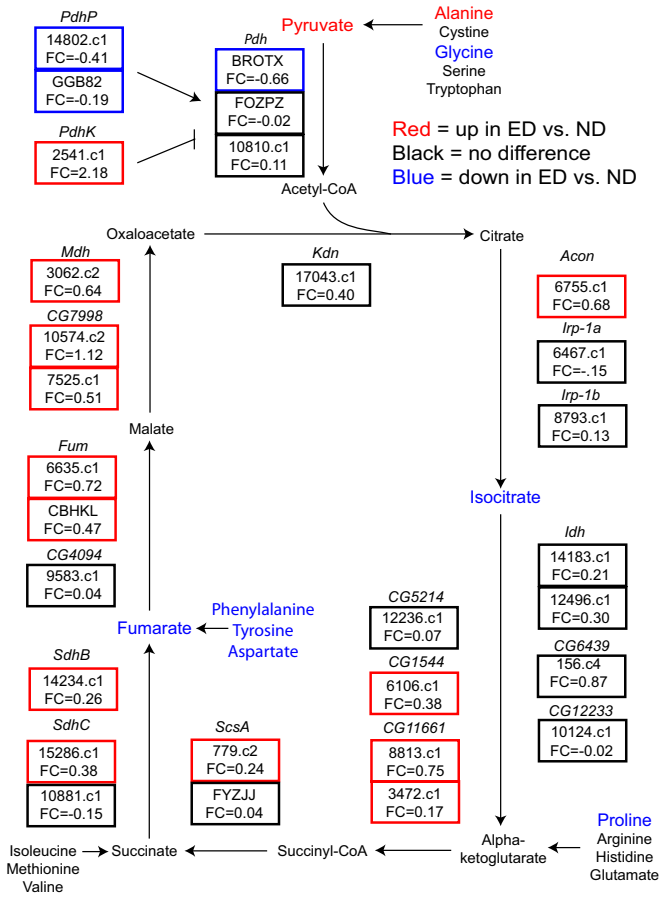

Fig. S4. TCA cycle diagram modified from the KEGG database for *D. melanogaster*. The name for each enzyme is listed above the box, and the *S. crassipalpis* EST database number and $\log_2$ fold change value comparing early diapausing pupae to nondiapausing pupae is listed within the box. Red boxes designate ESTs that had significantly greater abundance in early diapause pupae than nondiapause pupae after FDR correction $q < 0.01$. Blue boxes denote ESTs that had significantly lower abundance in early diapausing pupae compared with nondiapausing pupae after FDR correction. Black boxes denote no difference between early diapausing and nondiapausing pupae. Metabolites in red text were determined to accumulate in greater abundance in early-diapausing pupae relative to nondiapause pupae by metabolomics (1), whereas metabolites in blue text were less abundant in early diapause pupae compared with nondiapause pupae.

1. Michaud MR, Denlinger DL (2007) Shifts in the carbohydrate, polyol, and amino acid pools during rapid cold-hardening and diapause-associated cold-hardening in flesh flies (*Sarcophaga crassipalpis*): A metabolomic comparison. *J Comp Physiol B* 177:753–763.

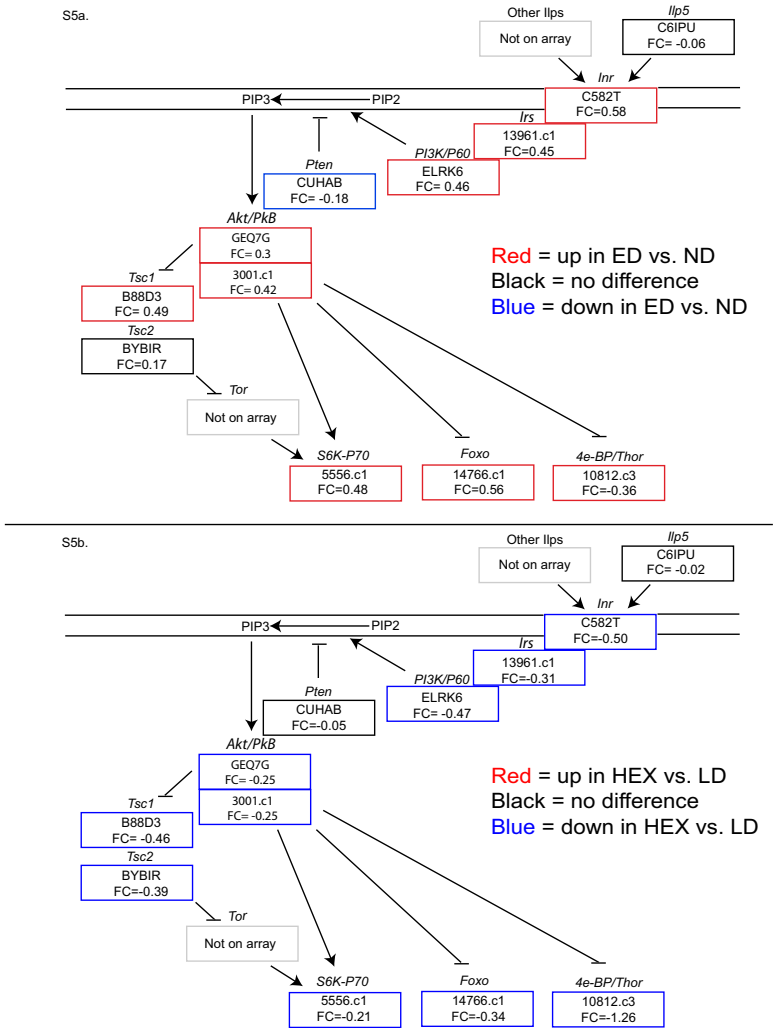

Fig. S5. Insulin-signaling pathway modified from Wu and Brown (1) shows that insulin signaling pathway members mostly have higher relative transcript abundance during diapause than in nondiapause pupae or pupae terminating diapause. (*A*) Log_2FC change in early diapause pupae versus nondiapause pupae. Red boxes denote significantly greater abundance in early diapause compared with nondiapause. Blue boxes denote significantly lower abundance. Black boxes indicate no change. (*B*) Log_2FC change in hexane treated pupae versus late diapause pupae. Red boxes denote significantly greater abundance in hexane treated late diapause pupae relative to late diapause pupae, blue boxes denote significantly lower abundance, and black boxes indicate no change.

1. Wu Q, Brown MR (2006) Signaling and function of insulin-like peptides in insects. *Annu Rev Entomol* 51:1–24.

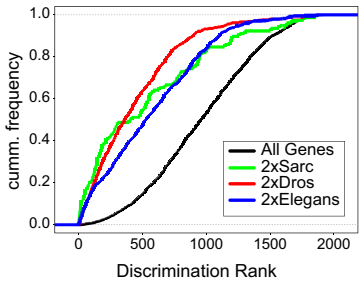

Fig. S6. Empirical cumulative distribution functions of ranked gene lists. The dormancy versus nondormancy expression pattern of a high-ranking gene is better able to discriminate among *S. crassipalpis*, *D. melanogaster*, and *C. elegans* than that of a low-ranking gene. The plot includes distribution functions for all orthologous genes (black line) and lists of genes twofold differentially regulated between dormant and nondormant phenotypes of *S. crassipalpis* (green line), *D. melanogaster* (red line), and *C. elegans* (blue line).

Table S1. A priori gene lists constructed from studies of gene expression responses in *Drosophila* used in GSA analysis of diapause responses

List description	Reference	ED vs. ND	ED vs. LD	LD vs. HEX
Response to dFOXO	1	–	–	–
Response to TOR	2	–	–	–
Response to ecdysone	3	*	–	–
Response to hypoxia	4	*	–	–
Response to hyperoxia	5	*	–	–
Response to oxidative stress	6	*	–	–
Response to cold stress	7	*	–	–
Response to cold stress	8	–	–	–
Reproductive diapause	9	–	*	–

*Significant enrichment (differential regulation) of the set within a phenotypic comparison in *S. crassipalpis*.

1. Gershman B, et al. (2007) High-resolution dynamics of the transcriptional response to nutrition in *Drosophila*: A key role for *dFOXO*. *Physiol Genomics* 29:24–34.
2. Guertin DA, Guntur KVP, Bell GW, Thoreen CC, Sabatini DM (2006) Functional genomics identifies *TOR*-regulated genes that control growth and division. *Curr Biol* 16:958–970.
3. Beckstead RB, Lam G, Thummel CS (2005) The genomic response to 20-hydroxyecdysone at the onset of *Drosophila* metamorphosis. *Genome Biol* 6:R99.
4. Liu GW, Roy J, Johnson EA (2006) Identification and function of hypoxia-response genes in *Drosophila melanogaster*. *Physiol Genomics* 25:134–141.
5. Landis GN, et al. (2004) Similar gene expression patterns characterize aging and oxidative stress in *Drosophila melanogaster*. *Proc Natl Acad Sci USA* 101:7663–7668.
6. Girardot F, Monnier FV, Tricoire H (2004) Genome wide analysis of common and specific stress responses in adult *Drosophila melanogaster*. *BMC Genomics* 5:16.
7. Qin W, Neal SJ, Robertson RM, Westwood JT, Walker VK (2005) Cold hardening and transcriptional change in *Drosophila melanogaster*. *Insect Mol Biol* 14:607–613.
8. Telonis-Scott M, Hallas R, McKechnie SW, Wee CW, Hoffmann AA (2009) Selection for cold resistance alters gene transcript levels in *Drosophila melanogaster*. *J Insect Physiol* 55: 549–555.
9. Baker DA, Russell S (2009) Gene expression during *Drosophila melanogaster* egg development before and after reproductive diapause. *BMC Genomics* 10:242.

Table S2. List of genes twofold or more differentially regulated between dormant and nondormant phenotypes in *S. crassipalpis*, *D. melanogaster*, and *C. elegans*

			Direction of regulation				
SarcEST ID(s)	Flybase ID	CG no.	*D. melanogaster*	*S. crassipalpis*	*C. elegans*	Name	Symbol
EUA37Q301EIXA6	FBgn0001078	CG4059	Down	Up	Down	ftz transcription factor 1	ftz-f1
FLY.5376.C1	FBgn0001197	CG5499	Down	Down	Down	Histone H2A variant	His2Av
FLY.9464.C1	FBgn0003067	CG17725	Up	Up	Up	Phosphoenolpyruvate carboxykinase	Pepck
FLY.10670.C1	FBgn0005655	CG9193	Down	Down	Down	mutagen-sensitive 209	mus209
FLY.9953.C1	FBgn0011327	CG3431	Down	Down	Down	Ubiquitin C-terminal hydrolase	Uch-L3
FLY.5928.C1	FBgn0011704	CG8975	Down	Down	Down	Ribonucleoside diphosphate reductase small subunit	RnrS
FLY.10602.C1, EUA37Q301DL8MD	FBgn0026170	CG4494	Down	Down	Down	smt3	smt3
FLY.37.C22	FBgn0027560	CG4104	Up	Down	Down	Trehalose-6-phosphate synthase 1	Tps1
FLY.3741.C1	FBgn0027580	CG1516	Up	Up	Up	Pyruvate carboxylase (predicted)	CG1516
FLY.10882.C1	FBgn0034405	CG15102	Up	Down	Up	Juvenile hormone epoxide hydrolase 2	Jheh2

Other Supporting Information Files

Dataset S1. Results of a discriminant analysis listing each EST that was changed at least twofold (up or down) in any comparison along with the F-ratio and P values relative to that EST's ability to separate each of the four phenotypic classes (nondiapause pupa, early diapause pupa, late diapause pupa, and late diapause pupa treated with hexane to terminate diapause) from each other.

Dataset S1 (XLSX)