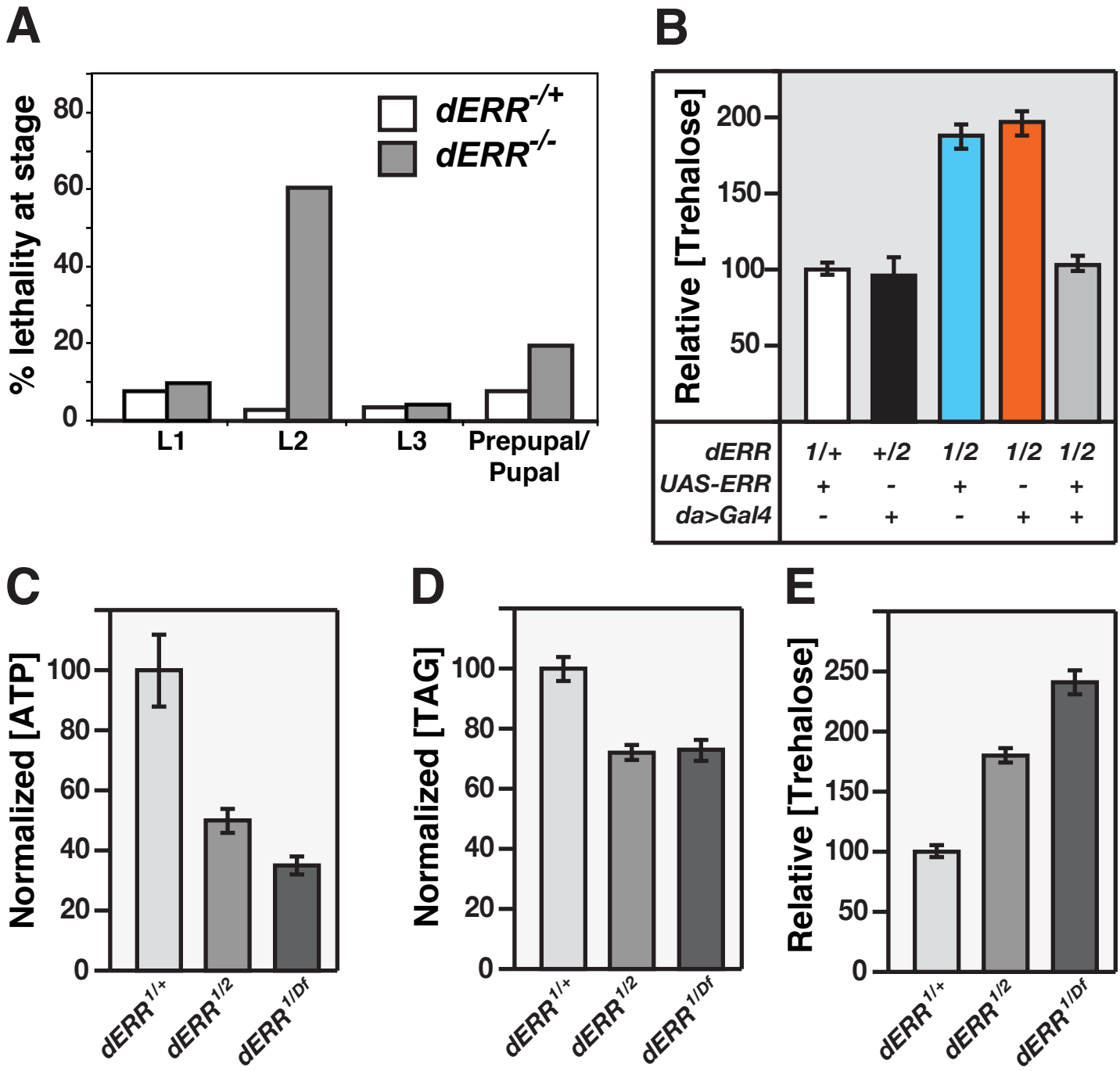
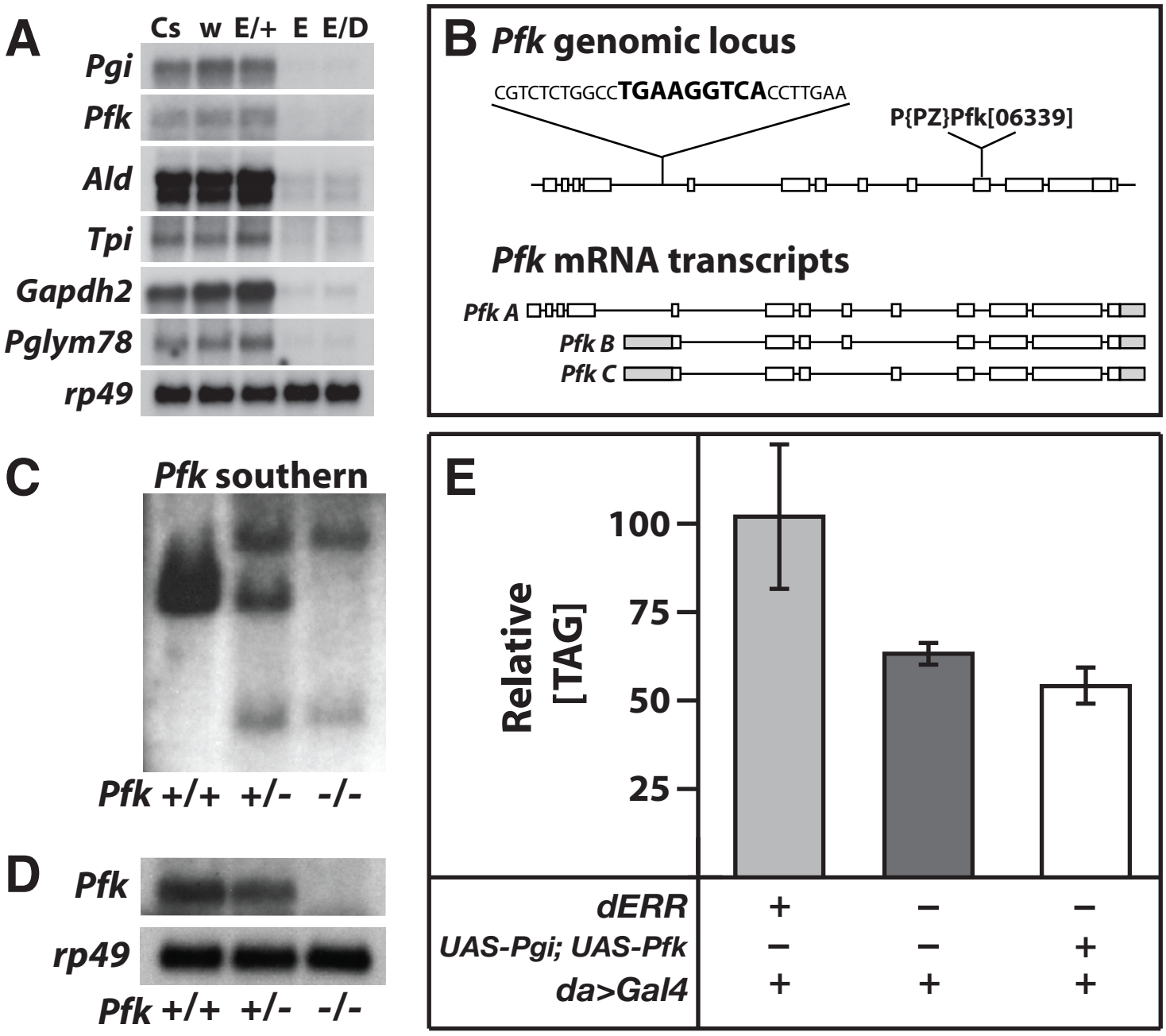
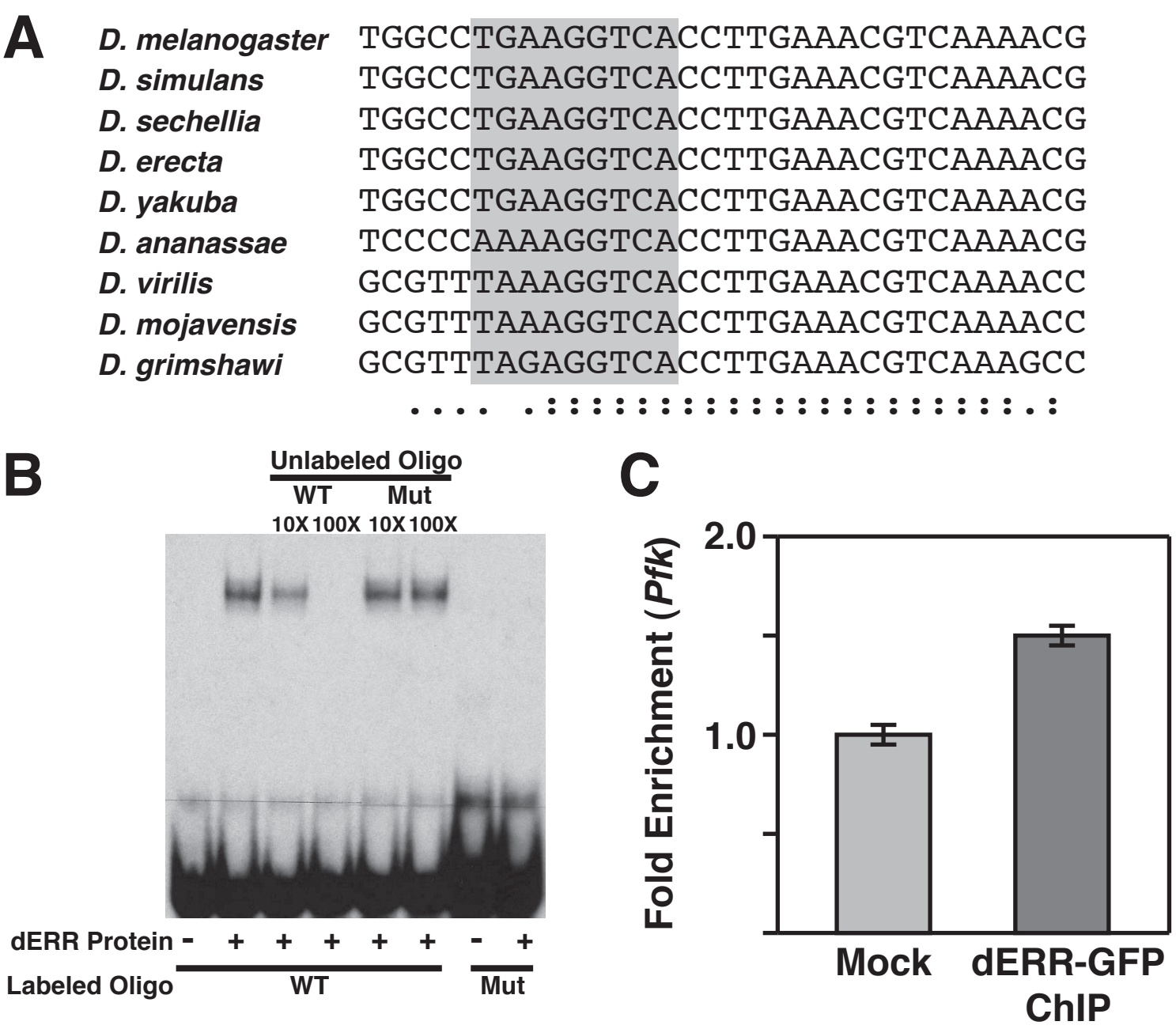


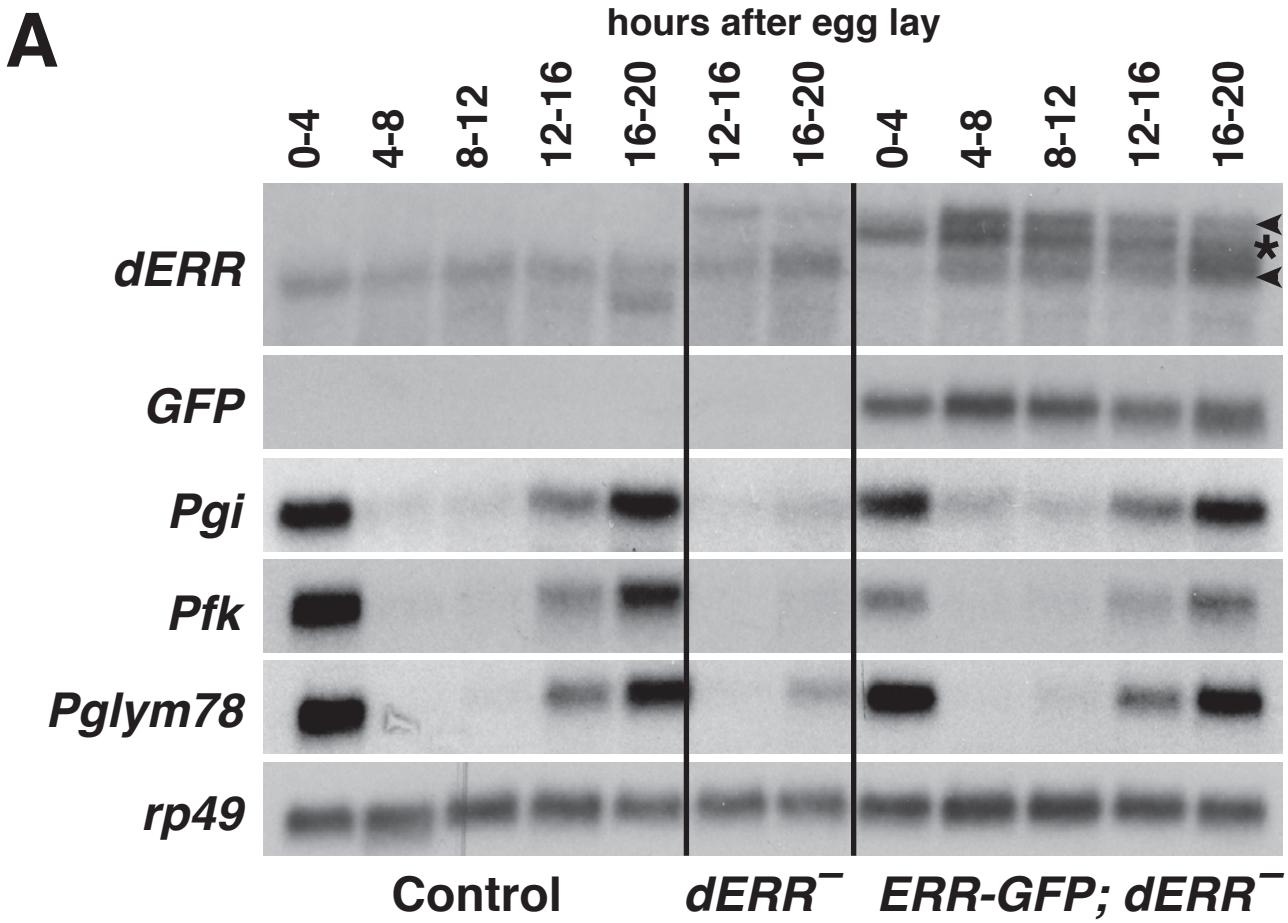
Supplemental Figure 1. Phenotypic characterization of *dERR* mutants. (A) Lethal phase analysis of *dERR* mutants. *dERR*^{1}*/+* controls and *dERR*^{1}*/dERR*^{2} mutants were collected as newly-hatched first instar larvae and followed through development, scoring for the percentage of animals that die as either first instar (L1), second instar (L2), or third instar (L3) larvae, or as prepupae or pupae. Similar results were obtained using *dERR*^{1} homozygous mutants. The *dERR*^{2} mutation deletes two neighboring genes, and homozygous mutants die during embryogenesis. (B) Ubiquitous expression of *UAS-dERR* using the *da-GAL4* driver can rescue the *dERR* mutant hypertrehalosemic phenotype. Trehalose concentrations were determined for *dERR*^{1}*/+*; *UAS-dERR* controls (white box), *dERR*^{2}*/+*; *da-GAL4* controls (black box), *dERR*^{1}*/dERR*^{2}; *UAS-dERR* mutants (blue box), *dERR*^{1}*/dERR*^{2}; *da-GAL4* mutants (red box), or rescued *dERR*^{1}*/dERR*^{2}; *UAS-dERR*, *da-GAL4* animals (grey box). (C-E) *dERR*^{1}*/+* controls and both *dERR*^{1}*/dERR*^{2} and *dERR*^{1}*/Df(3L)Exel6112* mutants were collected as mid-second instar larvae and whole animal homogenates were analyzed for concentrations of (C) ATP, (D) TAG, or (E) trehalose. *Df(3L)Exel6112* is a molecularly-defined genetic deficiency that removes the entire *dERR* locus. Amounts of ATP and TAG were normalized to soluble protein levels. For all comparisons between mutant and control larvae, $p < 5 \times 10^{-4}$. n>20 independently collected samples per value with 25 animals per sample. Error bars are ± S.E.

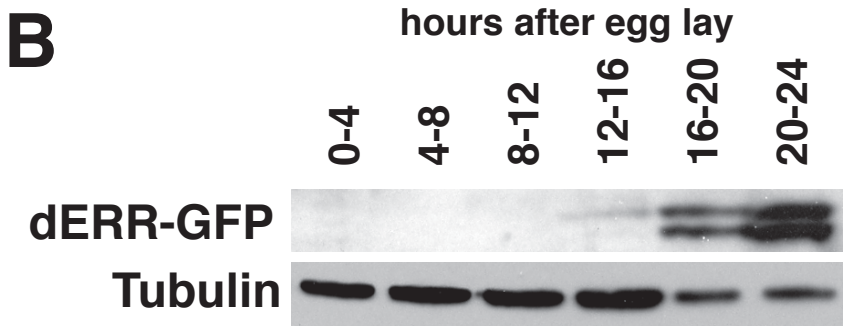


Supplemental Figure 2. Genes involved in carbohydrate metabolism are expressed at reduced levels in *dERR* mutants. (A) Total RNA was collected from mid-second instar larvae of the following genotypes: *CantonS* (Cs), w^{1118} (w), $dERR^{1/+}$ (E/+), $dERR^{1}/dERR^{2}$ (E), or $dERR^{1}/Df(3L)Exel6112$ (E/D). RNA was analyzed by northern blot hybridization for expression of *Phosphoglucoisomerase* (*Pgi*), *Phosphofructokinase* (*Pfk*), *Aldolase* (*Ald*), *Triose phosphate isomerase* (*Tpi*), *Glyceraldehyde-3-phosphate dehydrogenase 2* (*Gapdh2*), *Phosphoglyceromutase* (*Pglym78*), and the loading control *rp49*. (B) A graphic depiction of the *Pfk* locus with boxes representing exons on top and the resulting three mRNA transcripts below. A canonical dERR binding site is shown in the fourth intron (bold text), along with the location of the Pfk^{06339} P-element insertion in the 10th exon. (C) Confirmation of the P-element insertion in Pfk^{06339} mutants. DNA was isolated from w^{1118} (+/+), $Pfk^{06339}/+$ (+/–), and $Pfk^{06339}/Df(2R)BSC303$ (–/–) second instar larvae, digested with EcoRI, and analyzed by Southern blot hybridization using a probe directed against the 10th exon of *Pfk*. (D) RNA isolated from w^{1118} (+/+), $Pfk^{06339}/+$ (+/–), and $Pfk^{06339}/Df(2R)BSC303$ (–/–) second instar larvae was analyzed by northern blot hybridization to detect *Pfk* and *rp49* mRNAs, showing that there is no detectable *Pfk* mRNA in the Pfk^{06339} mutant. (E) TAG concentrations were determined for $dERR^{2}/+$, *da-GAL4* controls (grey box), $dERR^{1}/dERR^{2}$; *da-GAL4* mutants (black box), and *UAS-Pgi;* $dERR^{1}/dERR^{2}$, *UAS-Pfk*, *da-GAL4* animals (white box). Expression of these transgenes failed to restore normal TAG levels in a mutant background. Error bars represent ± S.E.

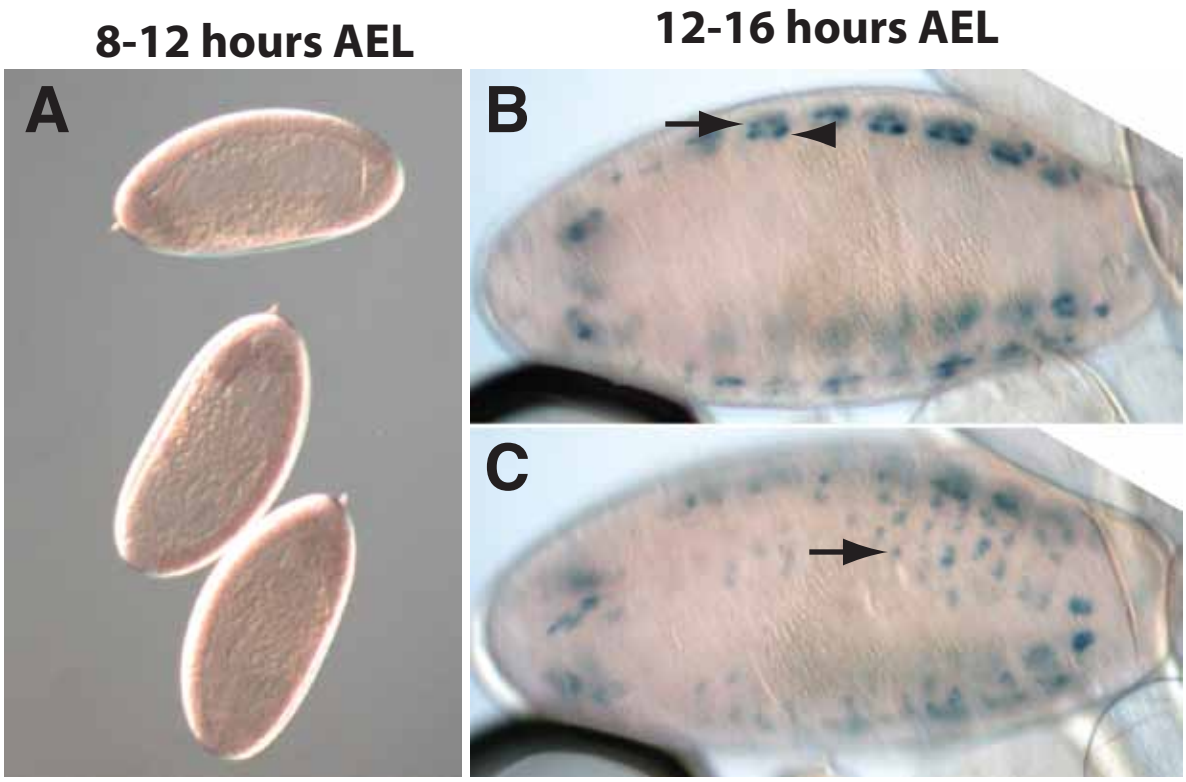


Supplemental Figure 3. dERR binds to *Pfk in vitro* and *in vivo*.
(A) An alignment comparing the genomic sequence surrounding the dERR binding site in the fourth intron of *D. melanogaster Pfk* with the same region in eight related *Drosophila* species using Vista. The 9 bp that comprise the dERR binding site are highlighted in grey. (.) indicates that a sequence is conserved in seven of the nine species and (:) indicates that the sequence is perfectly conserved. (B) EMSA assays were conducted using dERR protein synthesized *in vitro* using a rabbit reticulocyte lysate. dERR protein was incubated with either a radiolabeled oligonucleotide that contained the conserved dERR binding site in *Pfk* (WT), or with a mutant oligonucleotide in which two bp within the core dERR-binding site were mutated (Mut). Additionally, either a 10- or 100-fold excess of unlabeled wild-type (WT) or mutant (Mut) oligonucleotide were tested as competitors. dERR protein can bind to the wild-type *Pfk* binding site, but not the mutant binding site, and this interaction is effectively inhibited by adding a 10-fold or 100-fold excess of wild-type, but not mutant, oligonucleotide. (B) *dERR-GFP; dERR*^{1} embryos were collected 12-24 hours after egg laying, homogenized, and subjected to formaldehyde crosslinking followed by sonication. GFP antibodies were used to immunoprecipitate protein-DNA complexes. The presence of sequences corresponding to the fourth intron of Pfk were assayed by q-PCR. Results were combined from two independent cross-linking experiments. Error bars represent ± S.E. $p<0.01$.

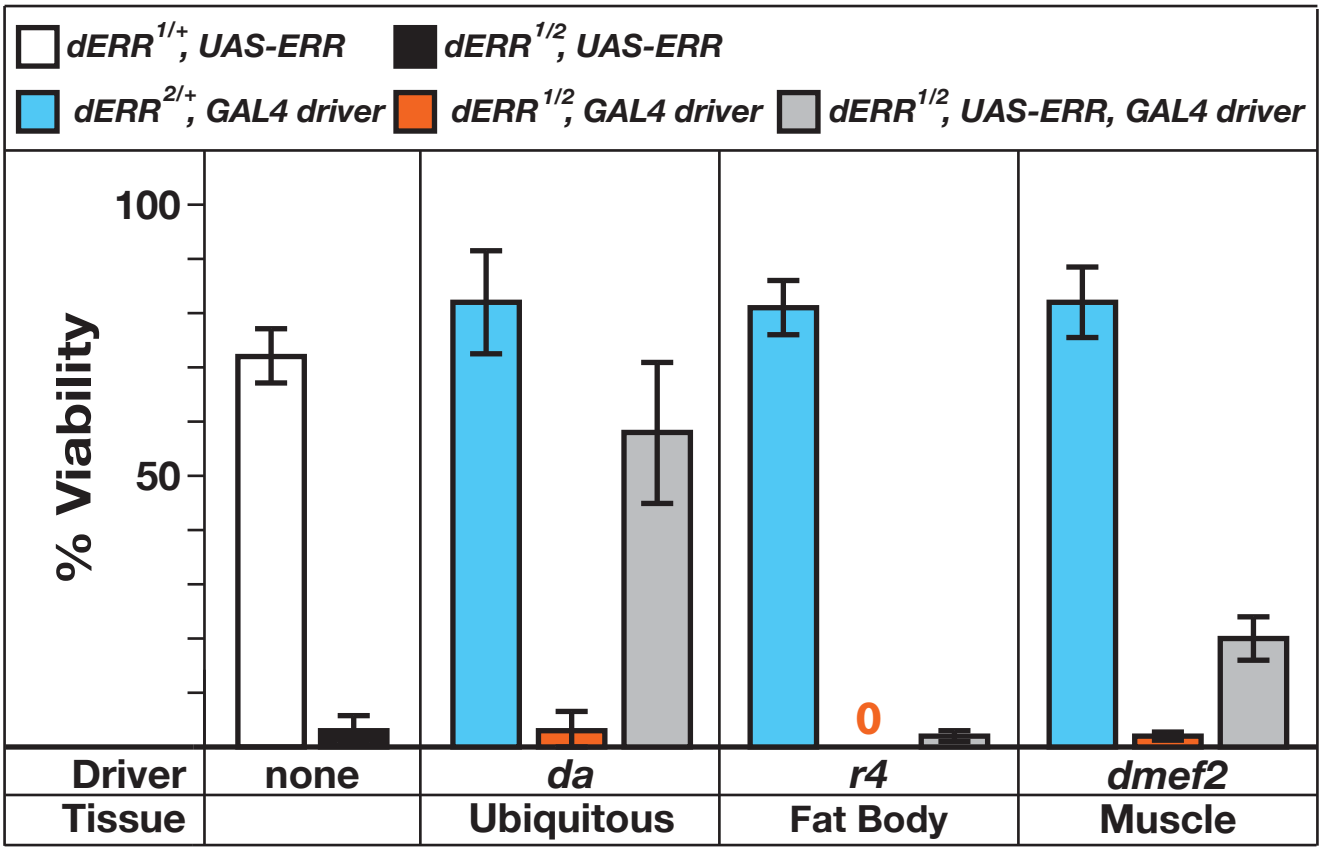




Supplemental Figure 4. dERR-GFP is post-transcriptionally regulated during embryogenesis.
(A) Total RNA from staged *w*^{1118} control, *dERR*^{1} mutant, and *dERR-GFP; dERR*^{1} rescued embryos was analyzed by northern blot hybridization to detect the expression of transcripts encoding dERR, GFP, and glycolytic enzymes. Similar to endogenous *dERR* transcripts, the *dERR-GFP* reporter is expressed throughout embryogenesis (***** indicates the transcript corresponding to *dERR-GFP*), and does not induce expression of transcripts encoding glycolytic enzymes until 12-16 hours after egg-laying (AEL). The additional *dERR* transcripts present in *dERR-GFP; dERR*^{1} larvae are aberrant mRNAs produced from the *dERR*^{1} locus (indicated by arrowheads). Hybridization to detect *rp49* mRNA is included as a control for loading and transfer. (B) Western blot analysis of protein extracts from staged *dERR-GFP; dERR*^{1} rescued embryos using antibodies to detect GFP and tubulin. dERR-GFP protein is first detectable 12-16 hours AEL and reaches maximal expression 20-24 hours AEL, similar to the timing of endogenous dERR protein expression in wild-type embryos (Figure 5).



Supplemental Figure 5. The GAL4-dERR ligand sensor is activated during mid-embryogenesis.
(A-C) Staged embryos carrying the *GAL4-dERR* and *UAS-nlacZ* transgenes were heat-treated for 30 minutes, allowed to recover for 4 hours at 25ºC, and then fixed and stained with X-gal. (A) Embryos that had developed for 8-12 hrs after egg laying (AEL) prior to heat treatment exhibited minimal X-gal staining. (B-C) In contrast, strong staining was seen in the muscle (arrowhead) and epidermis (arrow) of embryos at 12-16 hrs AEL. All X-gal stains were performed in parallel for the same amount of time. A few older embryos in the 8-12 hr sample stained with X-gal, as expected, providing an internal positive control.



Supplemental Figure 6. *dERR* function is required in multiple tissues for completion of larval development

The tissue specific requirements of *dERR* during larval development were examined in the following genotypes: *dERR*^{1}/+; *UAS-dERR* controls (white boxes), *dERR*^{1}/*dERR*^{2}; *UAS-dERR* mutants (black boxes), *dERR*^{2}/+; *GAL4* controls (blue boxes), *dERR*^{1}/*dERR*^{2}; *GAL4* mutants (red boxes), and rescued *dERR*^{1}/*dERR*^{2}; *UAS-dERR*, *GAL4* animals (grey boxes). Three *GAL4* transgenes were used to drive *UAS-dERR* expression: *da-GAL4* (ubiquitous expression), *r4-GAL4* (fat body), and *dmef2-GAL4* (muscle). Larvae were placed in vials containing standard media and scored for their ability to form pupae. n>100 larvae for each genotype. Error bars are ± S.E.