

Supplementary information

An insulin, AMPK, and steroid hormone-mediated metabolic switch regulates the transition between growth and diapause in *C. elegans*

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Contains supplementary figures complemented with figure legends.

Legends to supplementary figures

Supplementary Figure 1. Effect of DA on the heat production of *daf-2* and *daf-7*

Heat flow of *daf-2* and *daf-7* grown at 25°C in the presence or absence of DA.

Inhibition of DAF-12 suppresses the switch to low heat production in *daf-7* but not in *daf-2*. n=2 for each condition. DA – dafachronic acid.

Supplementary Figure 2. Arrested larvae of *daf-2* grown on dafachronic acid resemble growing L3 larvae.

Electron micrograph of a *daf-2* arrested L3 larva grown at 25°C in the presence of dafachronic acid. The body is not radially constricted but multiple lipid droplets are visible (left panel, arrowheads). Alae are absent (left panel). The gut lumen is elongated with multiple microvilli (central panel, big rectangle on left panel) and the cuticle has no striated layer (right panel, small rectangle on left panel). Representative images of five worms. Scale bars 5 μm (left panel), 1 μm (central panel) and 0.5 μm (right panel).

Supplementary Figure 3. DAF-16 controls the catabolism of energy reserves in dauer larvae

(A) TLC of lipids and sugars in *daf-2* dauers and *daf-2;daf-12* arrested L3 larvae grown at 25°C, and wild-type (N2) dauers and *daf-16* dauer-like larvae grown on 4-MS in the period after the developmental arrest is completed. While the *daf-2*, *daf-2;daf-12* and wild-type animals show conservation of TGs, trehalose and glucose, in *daf-16* only traces of these compounds are visible within 2 days of arrest. This is not the case with phospholipids, which are preserved to a good extent in all larvae.

(B) 2D-TLC of amino acids from the same types of animals as in (A). *daf-2*, *daf-2;daf-12* and wild-type animals are able to preserve the bulk amino acids, while in *daf-16* larvae after 2 days of arrest the amino acid levels are very low.

TG – triglycerides, GlcCer – glucosylceramides, Mar – maradolipids, PE – phosphatidylethanolamine, PS – phosphatidylserine, PI – phosphatidylinositol, PC – phosphatidylcholine, Glc – glucose, Tre – trehalose, 1 – arginine, 2 – lysine, 3 – glutamate, 4 – glycine/serine, 5 – glutamate, 6 – alanine/threonine. Representative images of at least 2 experiments.

Supplementary Figure 4. Dauers of *daf-2;aak-2(gt33)* do not spontaneously exit to reproductive growth.

(A) Scheme of the experiment. Dauer larvae of *daf-2* and *daf-2;aak-2* were obtained by incubation at the restrictive temperature (25 °C) and subjected to treatment with 1% SDS immediately after the completion of the dauer formation (Day 0) or after prolonged incubation at 25 °C (Day 2 and 5) in the presence of food. After SDS treatment, the survival of the animals was scored. Separately, dauer larvae were allowed to exit by a shift to permissive temperature (15 °C) until day 2, when a mixture of reproductive stages (L3, L4 larvae and young adults (YA)) and not recovered dauers was formed. This population was also treated with SDS.

(B) Survival after SDS treatment. Both *daf-2* and *daf-2;aak-2* larvae show almost 100% survival even after five days at the restrictive temperature (25 °C). In contrast, the dauers shifted to permissive temperature (15 °C) display substantial sensitivity to SDS.

In (B), means + SD of 2 experiments performed in triplicates.

Supplementary Figure 5. AAK-2 is required for preservation of sugars and amino acids in *daf-2* dauers.

(A) 2D-TLC of ¹⁴C-acetate labelled sugars and amino acids from *daf-2* dauers measured at different time points after the arrest. The depicted compounds are well preserved over time.

(B) 2D-TLC of ¹⁴C-acetate labelled sugars and amino acids from *daf-2;aak-2* dauers.

Unlike *daf-2*, *daf-2;aak-2* are depleted of sugars and amino acids very fast.

1 – trehalose, 2 – glucose, 3 – glutamate, 4 – glycine/serine, 5 – glutamate, 6 – alanine/threonine. Representative images from 2 experiments.

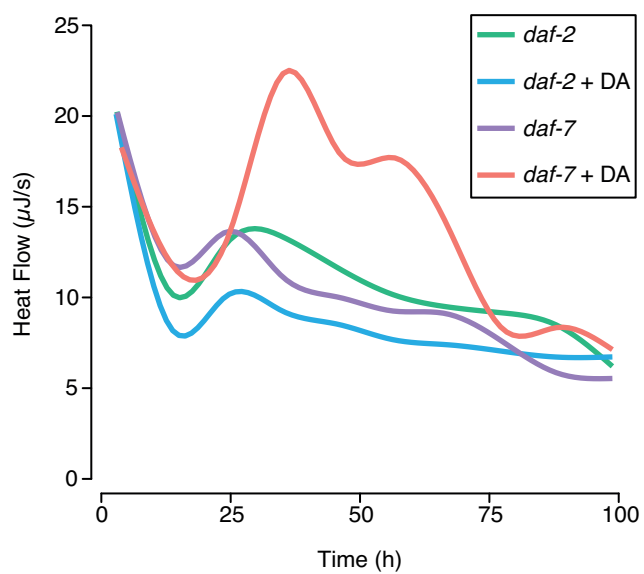
Supplementary Figure 6. FIB-1 shows nucleolar localization in dauer larvae and in *daf-2;aak-2* animals grown at 25°C on DA.

(A) FIB-1::eGFP localizes to nucleoli in both reproductive and dauer larvae. The outlines of the nuclei are indicated by dashed lines.

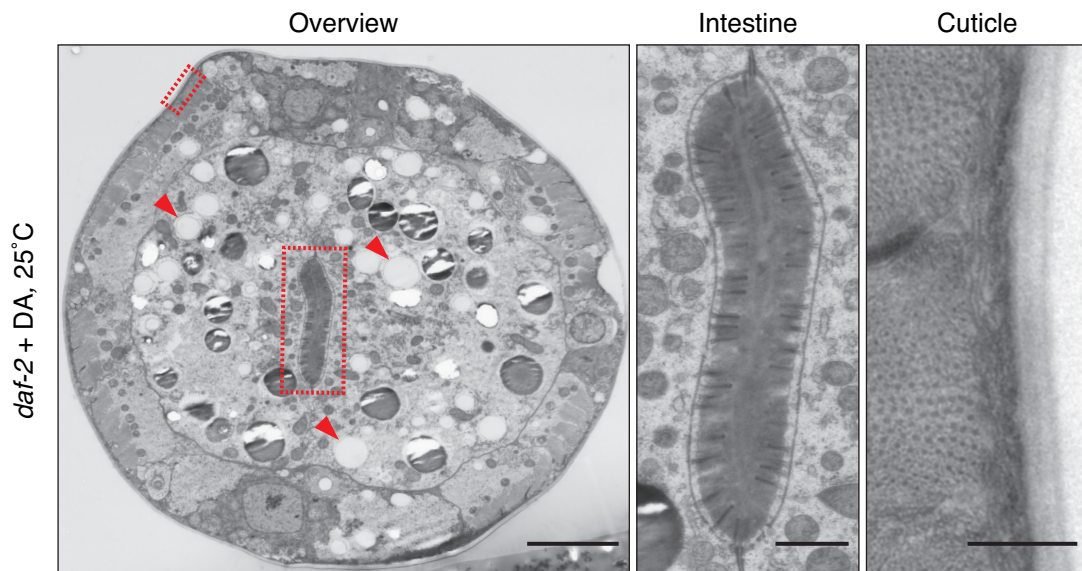
(B) FIB-1::eGFP localization in *daf-2;aak-2* animals grown at 25°C in the presence or absence of DA. After 48 hours of postembryonic development, L2d and L3 larvae displayed clear nucleolar FIB-1 (left panels). When the worms arrested as dauers or grew to adults after 72 hours of postembryonic development, they still displayed FIB-1 localized to the nucleoli (right panels).

Scale bars – 5 μm. Representative images of at least 2 experiments with at least 7 animals.

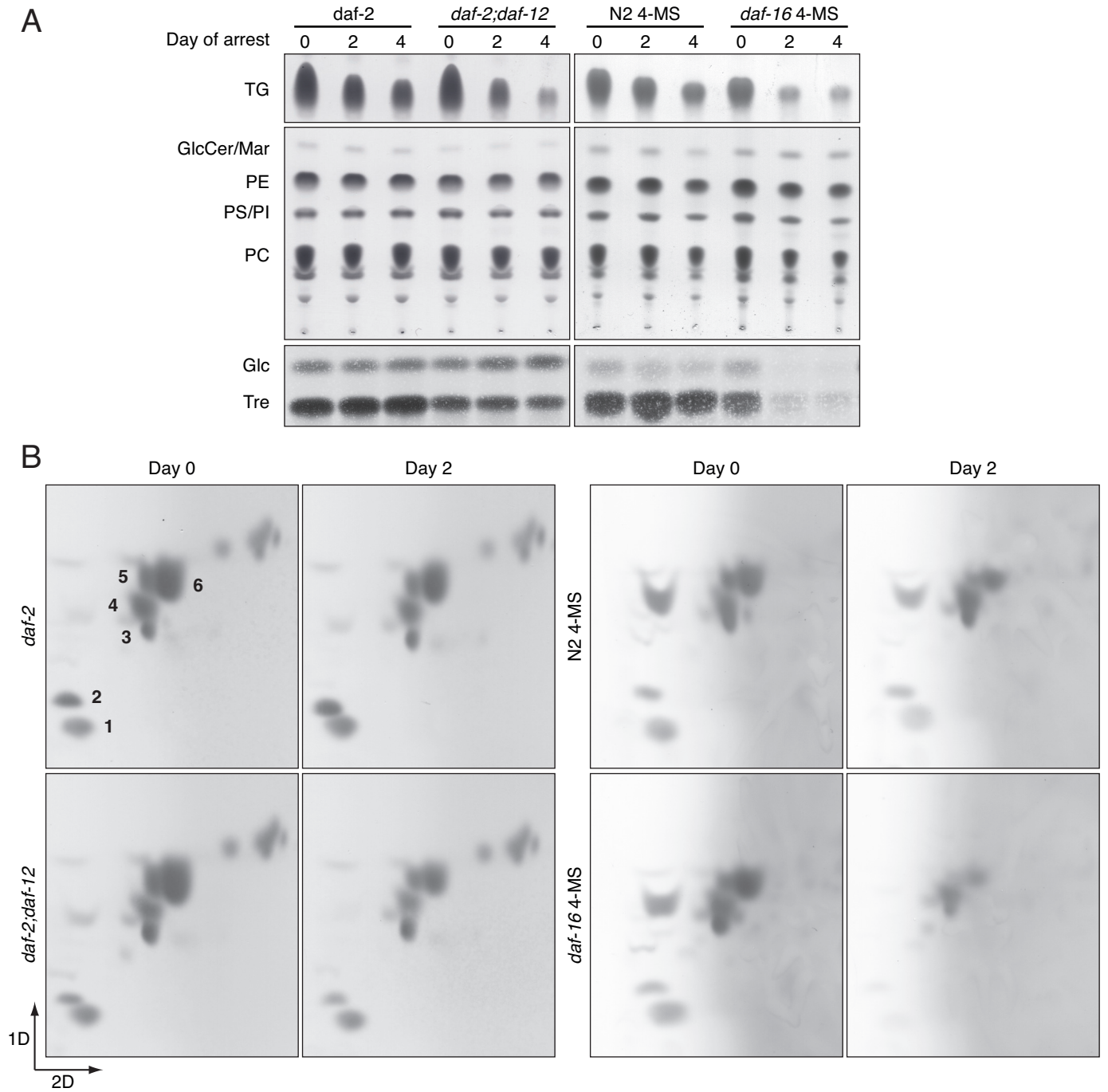
Supplementary Figure 1.



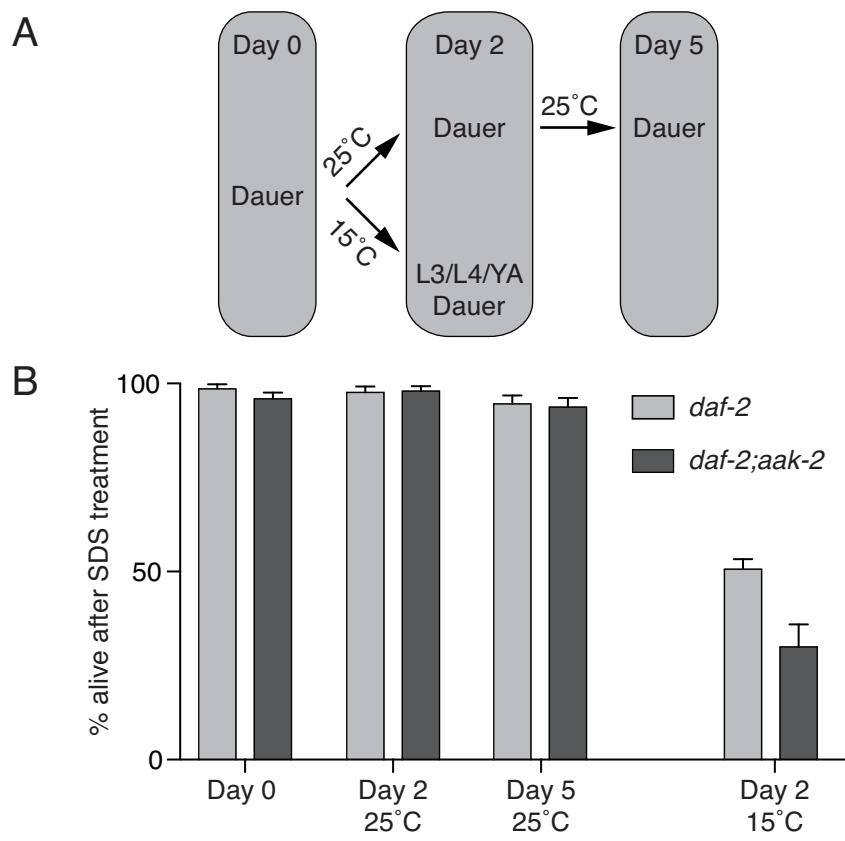
Supplementary Figure 2.



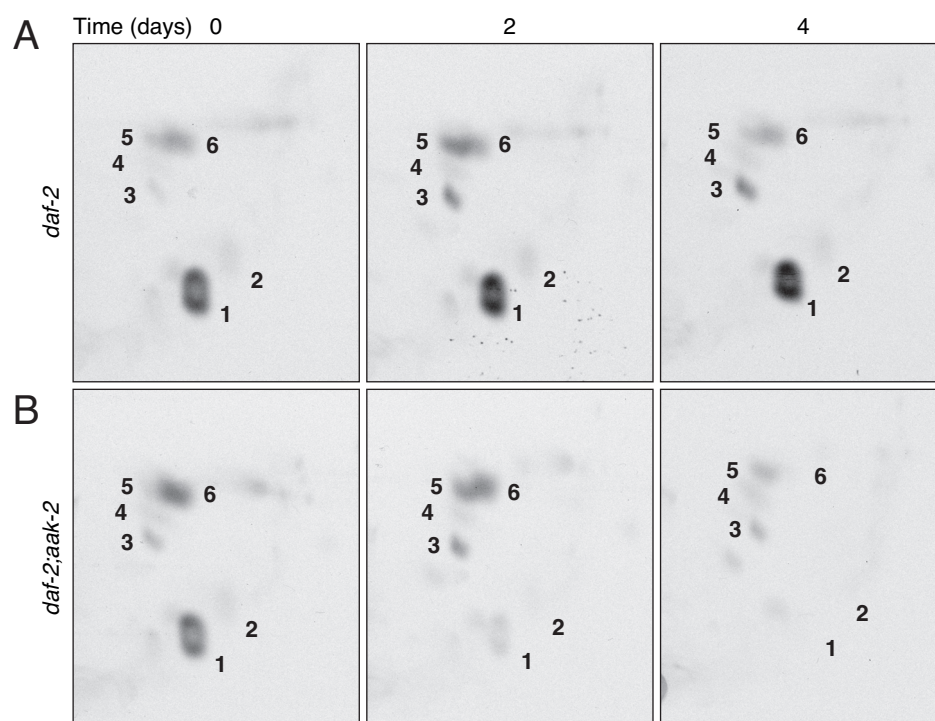
Supplementary Figure 3.



Supplementary Figure 4.



Supplementary Figure 5.



Supplementary Figure 6.

