SUPPORTING INFORMATION

Supplementary Fig 1: Proximal effects of long-term dauer on brood size, embryo length, and body size. A-C. Each point represents the mean of individual worms for the indicated trait within a biological replicate. Paired replicates are connected with a gray line. Grand means are connected with a black line.

Supplementary Fig 2: Long-term dauer diapause promotes variability in F1 progeny number and size. A. Brood size data were pooled across 7 biological replicates including 115 post-dauer worms and 120 control worms, $p = 2.2 \times 10^{-8.}$

B. Embryo length data were pooled across 6 replicates including 256 post-dauer embryos and 235 control embryos, $p = 0.00033$.

C. Worm body lengths following recovery following 1 or 8 days of L1 arrest in F1 progeny of post-dauers and controls were pooled from 8 biological replicates including 748 controls starved for 1 day, 897 post-dauers starved for 1 day, 421 controls starved for 8 days, and 337 post-dauers starved for 8 days. For 1 day, $p = 0.13$; for 8 days, $p = 0.02$.

A-C. Trait values for individual post-dauer and control worms were mean-normalized to the mean trait value for the corresponding condition and replicate. Data were pooled across biological replicates. Levene's test was used to assess differences in variance.

$*p<0.05$, $***p<0.001$

Supplementary Fig 3: Transgenerational effects of long-term dauer diapause in the F3 generation. A-B. Each point represents the mean of individual worms in the F3 generation for the indicated trait within a biological replicate. Paired replicates are connected with a gray line. Grand means are connected with a black line.

778   C. Replicate 1 of post-dauer and control F3 lifespan. 47 control and 66 post-dauer F3 worms

779   assayed. Control mean: 12.1 days; post-dauer mean: 15 days

780   D. Replicate 2 of post-dauer and control F3 lifespan. 64 control and 64 post-dauer F3 worms

781   assayed. Control mean: 13.6 days; post-dauer mean: 15.5 days

782   E. Replicate 3 of post-dauer and control F3 lifespan. 63 control and 64 post-dauer F3 worms

783   assayed. Control mean: 14.4 days; post-dauer mean: 16.2 days

784   F. Worm body length measurements were pooled across replicates to test for differences in

785   variance (same data as 3B), For 1 day, p = 0.089; for 8 days, p = 0.82

786   G. Brood sizes data were pooled across replicates to test for differences in variance (same data

787   as Fig 3C). For 0 d: p = 0.18; for 8 d: p = 0.48.

788   F-G. Trait values for individual post-dauer and control worms were mean-normalized to the

789   mean trait value for the corresponding condition and replicate. Data were pooled across

790   biological replicates. Levene's test was used to assess differences in variance.

791   n.s. not significant

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793   **Supplementary Fig 4: Individual replicates separate on PCA**. A. Principal component

794   analysis (PC1 and PC2) of all biological replicates included in differential expression analysis.

795   B. Biological replicates plotted in PC1 only. Considering both current environment (fed vs.

796   starved) and ancestral environment (control, short-term dauer, and long-term dauer) as factors,

797   both effects are significant in a multivariate ANOVA using Wilks' Lambda for PC1. Effect of

798   current environment, $p < 2.2 \times 10^{-16}$; effect of ancestral environment, p = 0.00022.

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800   **Supplementary Fig 5: Additional RNA-seq analysis**. A. Class I and Class II targets defined

801   as genes positively or negatively regulated by DAF-16. Class I and Class II targets were filtered

802   to include those with FDR < 0.05 that were also in the background set of 8649 genes included in

803   starved / fed differential expression analysis. Class I targets are over-enriched in genes up in

804   starved / fed (hypergeometric p = 1 x 10^{-49}). Class II targets are over-enriched among genes in

805   starved / fed (hypergeometric p = 2.6 x 10^{-34}). Class I targets can explain 23.1% of starvation

806   upregulation; class II targets can explain 21.9% of starvation downregulation.

807   B. Log2 fold change of all genes in ST post-dauer starved / ST post-dauer fed compared to log2

808   fold change in LT post-dauer starved / LT post-dauer fed. Red line indicates simple linear

809   regression through all points, and thickness of line indicates 95% confidence interval. Slope is

810   0.98, with confidence interval ranging from 0.973 to 0.981. Slope is significantly different from 0,

811   p < 2.2 x 10^{-16}. summary.lm() was used in R to generate slope and standard error estimates,

812   and a t-test was used to calculate significance.

813   C. Log2 fold changes of all genes in control starved / control fed plotted against log2 fold

814   changes of ST post-dauer fed F3 / control fed plotted in black.

815   D. Log2 fold changes of all genes in control starved / control fed plotted against log2 fold

816   changes of ST post-dauer starved F3 / control starved plotted in black.

817   C-D. Genes up in control starved / control fed using FDR < 1 x 10^{-10} are plotted in green. Genes

818   down in control starved / control fed are plotted in tan. Blue line is a simple linear regression

819   through all points, and thickness of line indicates confidence interval. The summary.lm() function

820   was used in R to generate t-values and their corresponding p-values.

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822   **Supplementary Table 1: Starvation survival and lifespan statistics**

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824   **Supplementary Table 2: RNA-seq reads and mapping efficiency**

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826   **Supplementary Table 3: Pearson correlation coefficient matrix of 8649 genes used in**

827   **mRNA-seq differential expression analysis of F3 worms**

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829   **Supplementary File 1: edgeR output for pairwise RNA-seq comparisons (.xlsx file)**

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831   **Supplementary File 2: Raw data for Figure 2**

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833   **Supplementary File 3: Raw data for Figure 3A-D**

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835   **Supplementary File 4: Raw data for Figure 3E-F**