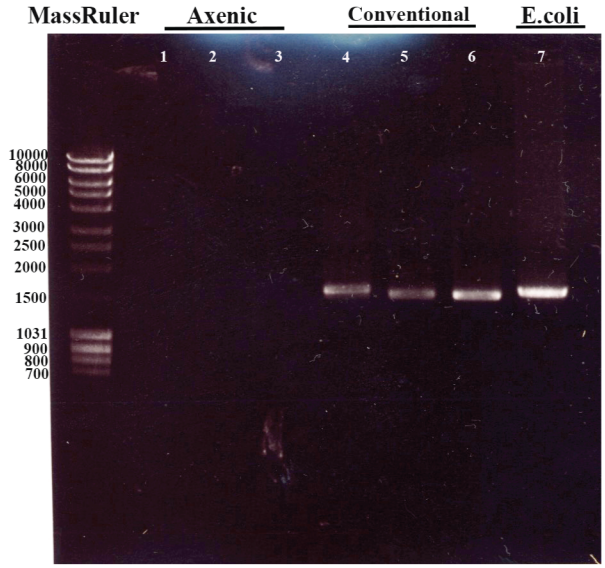
**iScience, Volume *24***

## Supplemental information

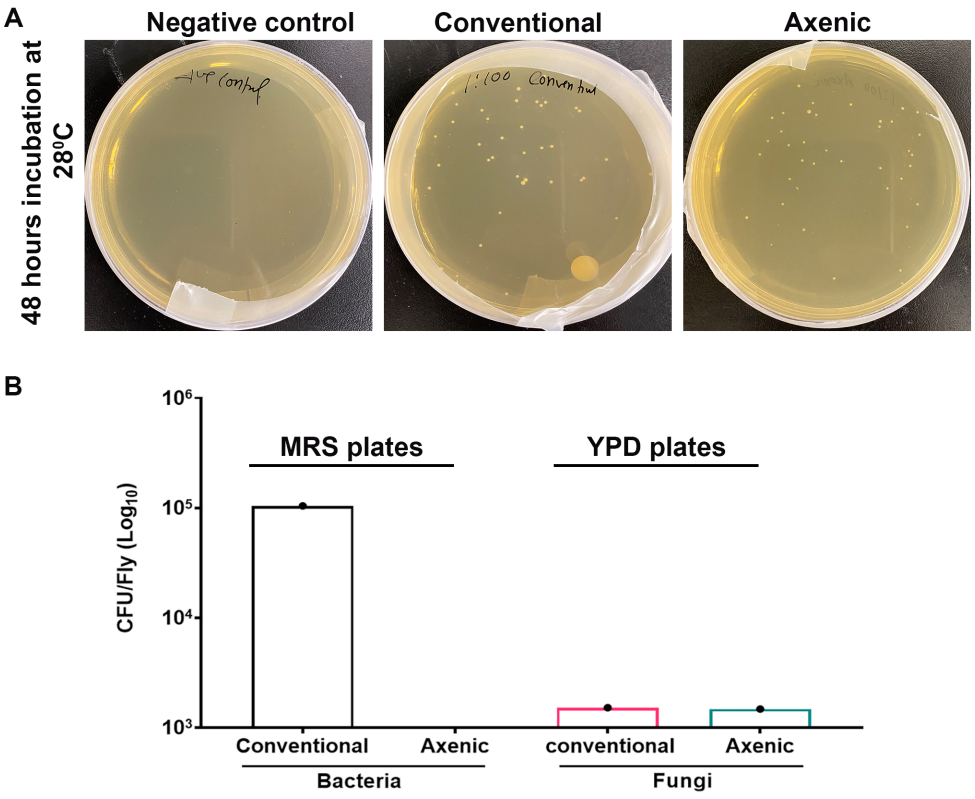
# Common features of aging fail to occur in *Drosophila* raised without a bacterial microbiome

**Arvind Kumar Shukla, Kory Johnson, and Edward Giniger**

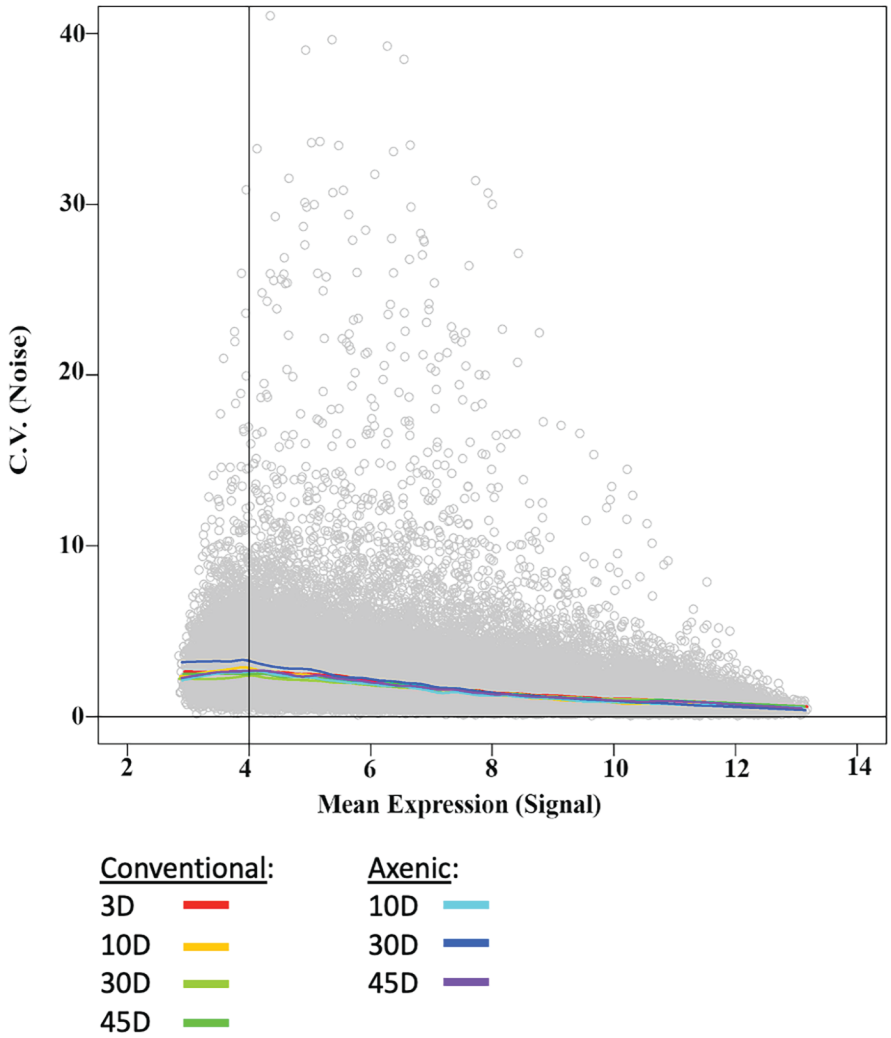
## Supplemental Figures



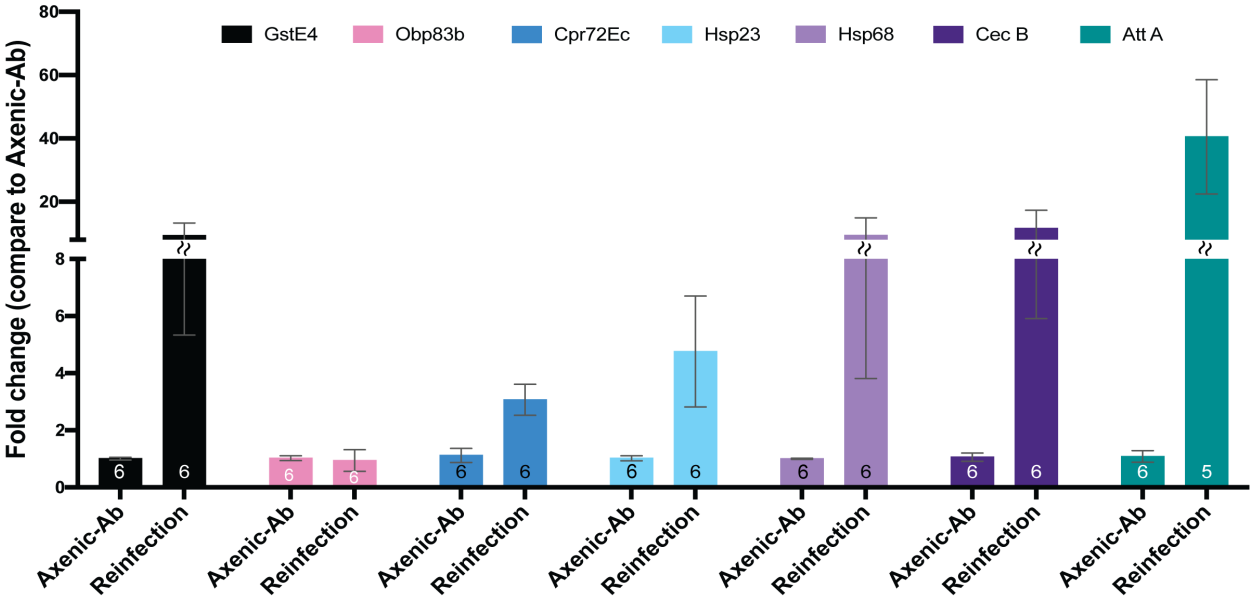

**Supplemental Figure S1**: **Validation that axenic flies do not have bacterial contamination, related to Figure 1**. Microbial 16S rDNA was amplified from whole fly extract using universal primers (27F, 1492R) and separated by agarose gel electrophoresis. E.coli DNA was used as positive control. MassRuler$^{TM}$ DNA ladder mix was used as size standard.




**Supplemental Figure S2**: **Assessment of fungal load in axenic flies, related to Figure 1.** Axenic flies were surface washed using 70% Ethanol and homogenized in 1X PBS. 100 µl homogenate was plated on yeast extract peptone dextrose (YPD) media in 1:100, 1:1000, and 1:10000 dilution, and plates were incubated at 280C for 48 hours. (A) YPD plates showing fungal colonies in the plate with 1:100 dilution, (B) Bacterial and fungal CFU per fly presented in one graph. For comparison of bacterial and fungal load, CFU from 48 hours incubation of MRS plates at 28°C was also included.

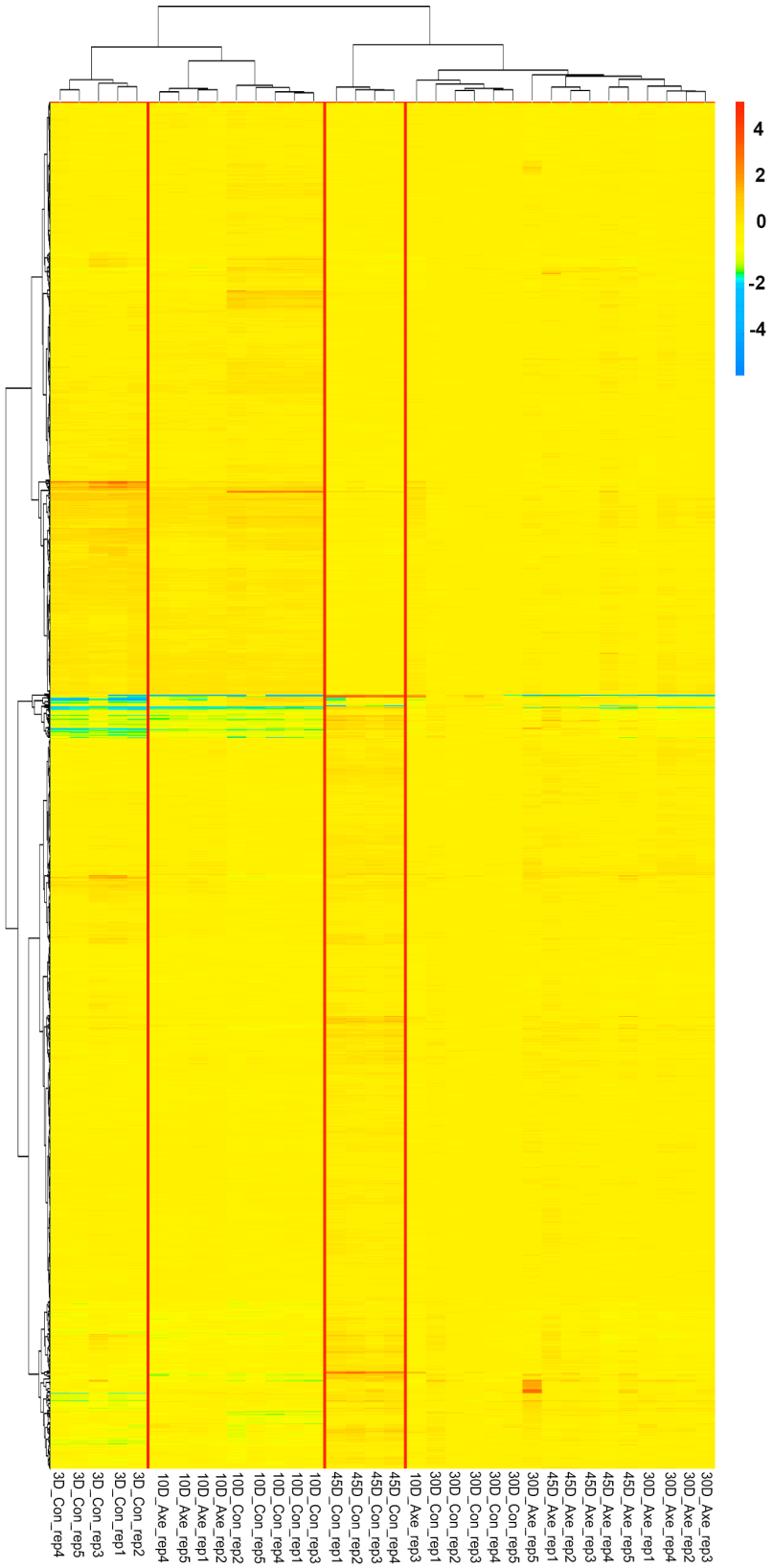



**Supplemental Figure S3**: **Expression variance is consistent among microarray samples, related to Figure 1**. Coefficient of variation (C.V.) was plotted against mean expression level for each gene queried in microarrays (gray circles). LOWESS fit line for mean C.V. is shown for each sample type (color-coded as indicated) and are not substantially different between samples.

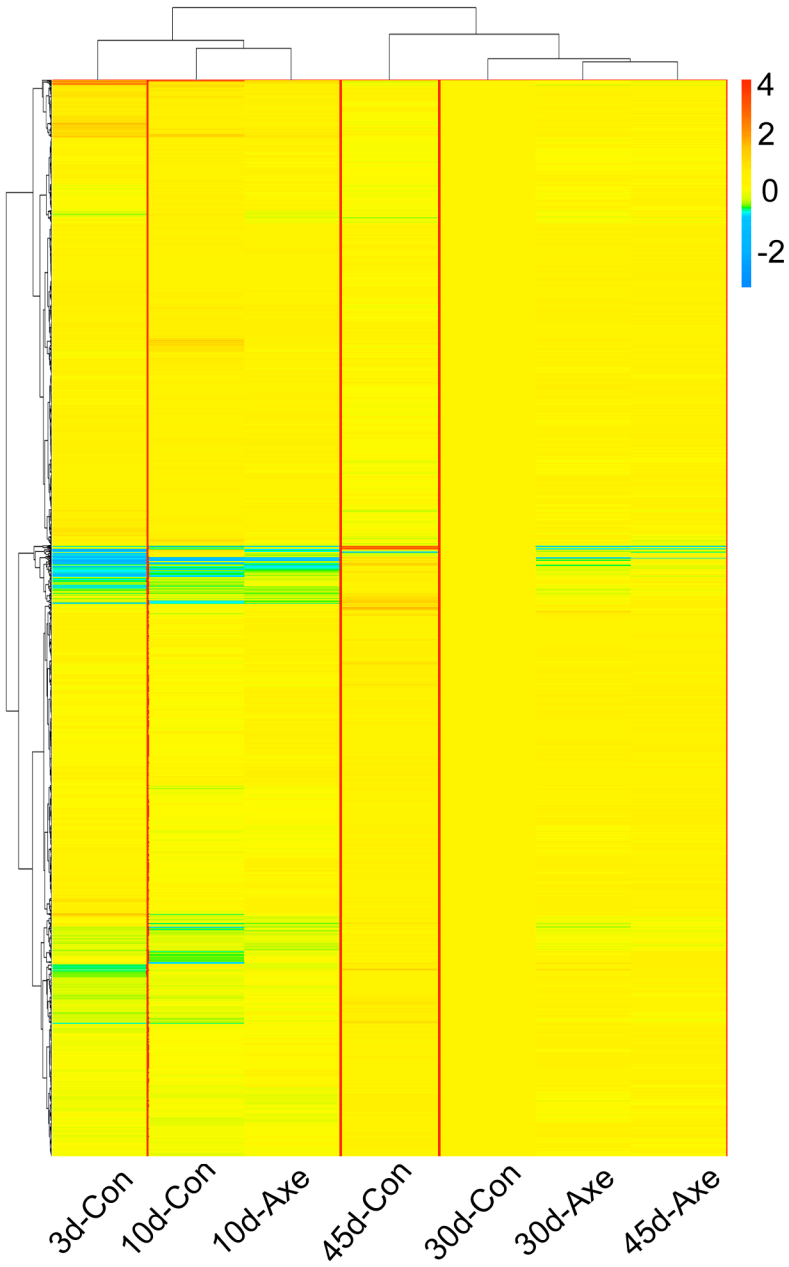



**Supplemental Figure S4**: **Quantification of axenic gene expression without antibiotic and after reinfection, related to Figure 2.** (A) Seven representative genes were selected for qPCR validation from among five biological processes (metabolism, immunity, cuticle, olfaction, and stress response). Fold change was calculated compared to 10d-old axenic without antibiotics sample after normalization with Rpl32. Bars represent mean ± SEM and number of replicates is shown at bottom of each bar. Axenic-Ab: Bleached embryo were transferred aseptically to food without antibiotic supplementation and raised in the same vials for 10 days after fly emergence; Reinfection: Five day old Axenic-Ab flies were transferred to vials previously used for growing conventional flies and raised an additional five days.

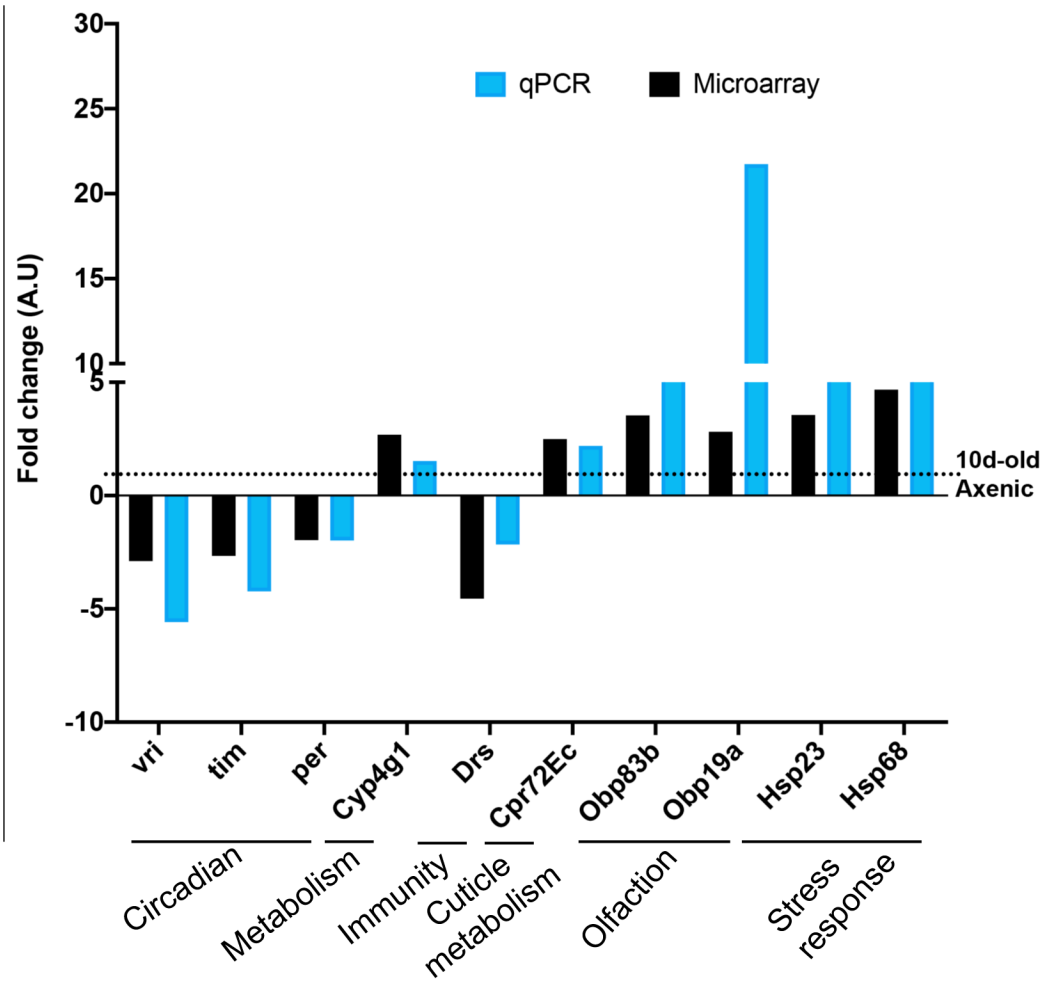
## Supplemental Figure S5



**Supplemental Figure S5. Sample-level heat map of relative expression of age-correlated genes, related to Figure 3.** Heat map visualization of relative expression of all age-correlated genes, as selected by polyserial correlation of data from conventionally raised flies. Sample-level fold changes per gene ($log_2$) were calculated relative to the mean value for 30 day-conventional samples and then organized by unsupervised clustering (pheatmap). Sample identifiers are shown at the bottom of each column, and Y-axis represent 4577 genes. Heat map has been pseudocolored with a multicolor LUT to improve discrimination of small differences. Color code for fold-change is shown at upper right.

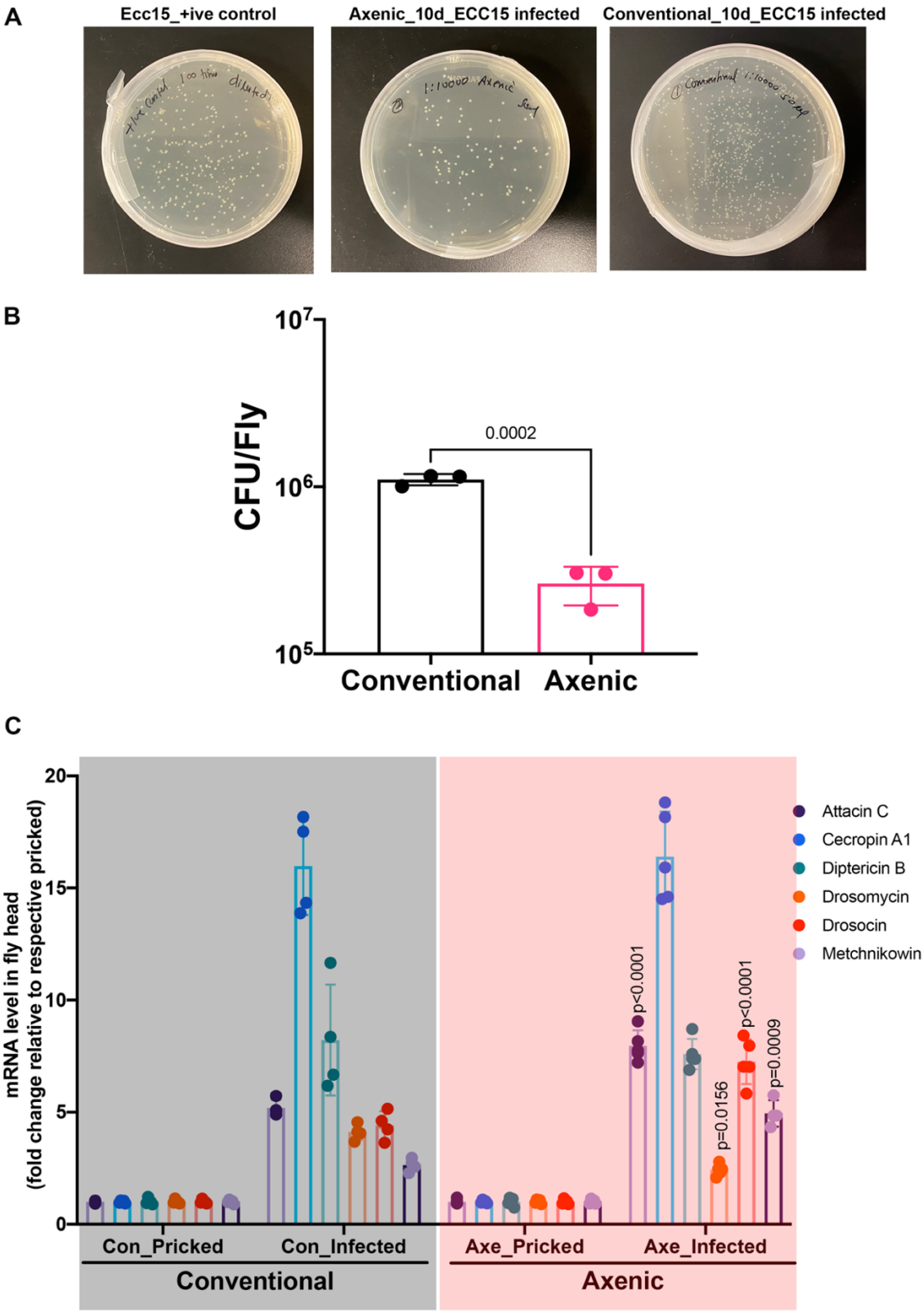



**Supplemental Figure S6. Heat map of relative expression of age-correlated genes, pseudocolored to discriminate small differences, related to Figure 3**. Same heat map as presented in Figure 3, pseudocolored with a multicolor LUT that offers better discrimination of small differences.

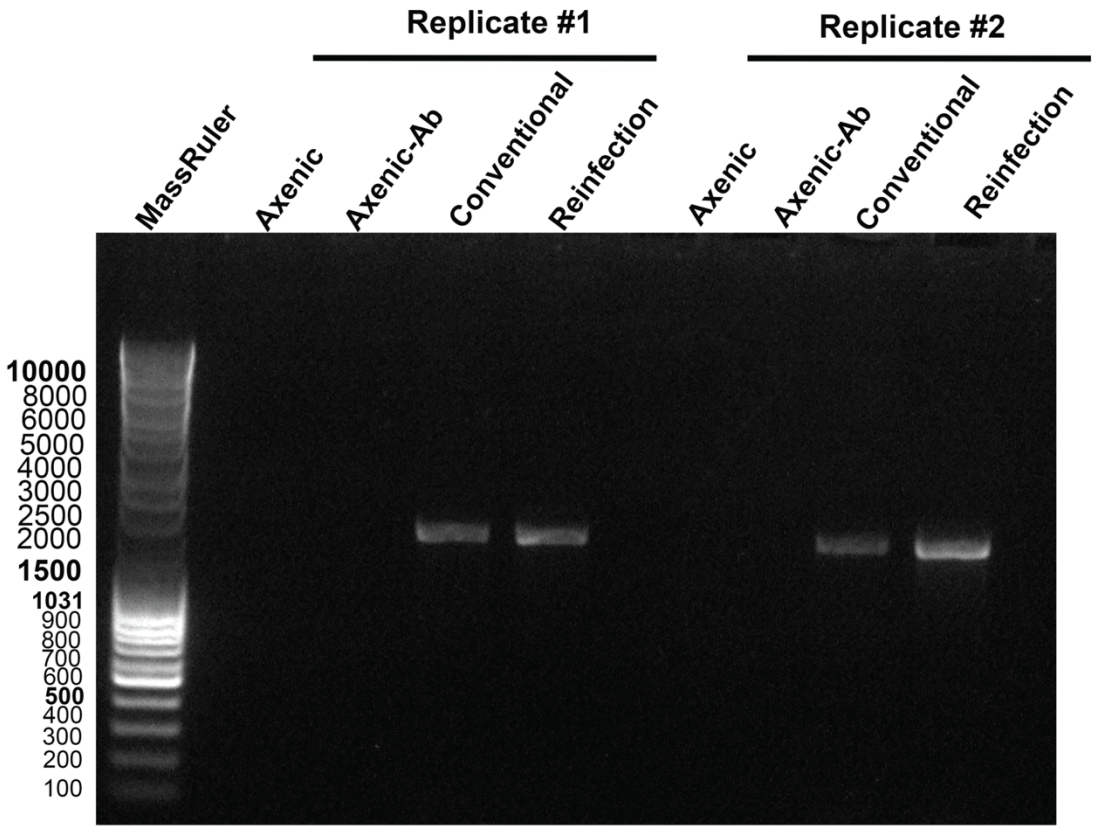



**Supplemental Figure S7**: **qPCR validation of microarray expression data, related to Figure 4.** Ten representative genes were selected for validation from among five biological processes (Circadian Rhythm, Immunity, Metabolism, Olfaction and Stress Response). Expression level relative to a normalization standard was calculated for each gene from microarray data and from qRT-PCR measurements. Bar graph presents the mean expression of the indicated gene in 10d conventional samples relative to 10d axenic (set to 1.0; dotted line), using the two measurement methods. Normalization standard was *rpl32*. Five biological replicates were performed for each measurement.

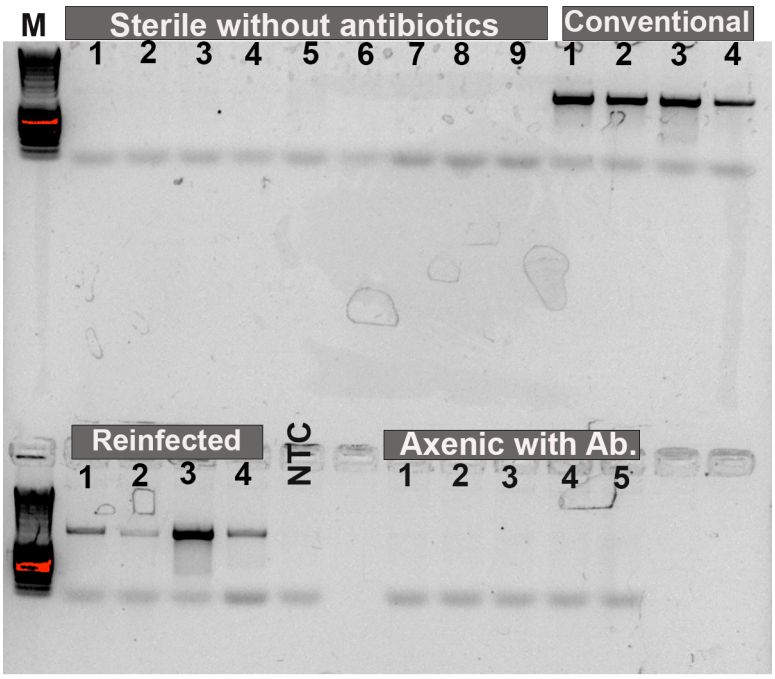
**Supplemental Figure S8**



**Supplemental Figure S8**: **Assessment of immunogenic response of conventional and axenic flies, related to Figure 6.** 10d-old conventional and axenic flies were infected using lancet dipped in ECC15 culture along with pricked control. (A) After 16 hours of infection, live flies were surfaced washed and homogenized in 1X PBS. Nutrient agar plates showing bacterial colonies after 48 hours incubation at 28$^{o}$C. (B) Graph showing CFU per fly. The black bar is conventional and the red is axenic. Bars indicate mean values, with SD shown; circles are individual data points. p value is shown (ANOVA). (C) qPCR quantification of AMP expression, 16 hours post-infection. Fold change was calculated compared to respective pricked control after normalization with rp49 and presented as mean $\pm$ SD. Four-five replicates were used in each case.




**Supplemental Figure S9: Validation that axenic flies do not have bacterial contamination after withdrawing antibiotics, and reinfection of axenic flies restored bacteria, related to Figure 6.** Microbial 16S rDNA was amplified from whole fly extract using universal primers (27F, 1492R) and separated by agarose gel electrophoresis. MassRuler DNA ladder mix was used as a size standard. Axenic: Flies raised on antibiotic-containing food; Axenic-Ab: After five days of axenic culture, flies were aseptically transferred to food without antibiotics in the laminar hood and raised an additional five days before testing; Conventional: Flies raised on regular food; Reinfection: After five days of axenic culture, axenic flies were transferred to food vials that had previously been contaminated with bacteria by being used to culture conventionally raised flies.




**Supplemental Figure S10: Validation that acutely sterilized flies do not have bacterial contamination and reinfection of sterile flies restored bacteria, related to Figure 6.** Similar to Supplemental Figure S9, microbial 16S rDNA was amplified from flies raised under the indicated conditions and separated by agarose gel electrophoresis. MassRuler DNA ladder mix was used as a size standard. Sterile without antibiotics: Flies hatched from bleached embryos, grown on sterile food without ever being exposed to antibiotics. Conventional: Flies hatched from embryos without bleaching. Reinfection: Flies hatched from bleached embryos but transferred to vials that had previously housed flies from unbleached embryos. Axenic with Ab: Flies hatched from bleached embryos, transferred to food supplemented with antibiotics by our standard protocol.

| Human Process using aging classifer | Normal | Axenic |
|---|---|---|
| **Muscle degeneration** | | |
| late-onset proximal muscle weakness | 1.31E-03 | -------- |
| accumulation of muscle fiber desmin | 1.31E-03 | -------- |
| abnormal muscle fiber desmin | 1.31E-03 | -------- |
| distal upper limb muscle weakness | 7.92E-03 | -------- |
| muscle fiber inclusion bodies | 2.20E-02 | -------- |
| muscle fiber splitting | 3.25E-02 | -------- |
| distal muscle weakness | 3.24E-02 | -------- |
| **Peripheral nerve dysfunction** | | |
| paresis of extensor muscles of the big toe | 1.31E-03 | -------- |
| decreased number of large peripheral myelinated nerve fibers | 6.24E-03 | -------- |
| abnormal peripheral myelination | 7.92E-03 | -------- |
| decreased Achilles reflex | 1.58E-02 | -------- |
| abnormal peripheral action potential amplitude | 2.51E-02 | -------- |
| decreased amplitude of sensory action potentials | 2.51E-02 | -------- |
| decreased number of peripheral myelinated nerve fibers | 4.54E-02 | -------- |
| **Cataract** | | |
| capsular cataract | 1.31E-03 | -------- |
| posterior capsular cataract | 1.31E-03 | -------- |
| posterior polar cataract | 1.58E-02 | -------- |
| polar cataract | 2.51E-02 | -------- |
| **Skin barrier** | | |
| palmoplantar keratoderma | 3.24E-02 | -------- |
| hyperkeratosis | 3.71E-02 | -------- |
| **Metabolism** | | |
| abnormal circulating carboxylic acid concentration | 1.31E-03 | 8.00E-04 |
| abnormality of serum amino acid level | 2.45E-03 | 7.00E-04 |
| autophagic vacuoles | 7.92E-03 | -------- |
| abnormal circulating metabolite concentration | 3.40E-02 | -------- |
| abnormal circulating methionine concentration | 3.65E-02 | -------- |
| megaloblastic anemia | -------- | 4.04E-02 |

**Supplemental Figure S11**: **GO analysis of aging classifier genes using gProfiler, related to Figure 7.** GO analysis was performed using gProfiler to identify human disease processes linked to the human orthologs of *Drosophila* age classifier genes, selected based on conventional or axenic-raised flies, as indicated. Human processes were grouped manually based on broad biological function. Adjusted threshold p-values from gProfiler are shown for all significantly enriched categories.