

Figure S1. Wolbachia does not increase the longevity of flies of the w^{1118} background line infected with several filamentous fungal pathogens. Flies of each given background and sex were systemically infected with the indicated pathogen. Infections were performed with either (a) *Aspergillus fumigatus*, (b) *Aspergillus flavus*, (c) *Fusarium oxysporum*, or (d) *Fusarium graminaerum*. Infections of all groups were performed side-by-side, along with those of the w^k background line (Figure 1), with at least two blocks of infections performed on different days. Each line represents a total of 60 flies. Sham controls were performed with sterile 20% glycerol. Full statistics, available in Table S1, were done with a Cox mixed effects model. Controls are the same in all panels and in panel S2a because they were performed concurrently in the same background.



Figure S2. Wolbachia increases the longevity of w¹¹¹⁸ background line flies infected with certain filamentous fungal

entomopathogens. Flies of each given background and sex were systemically infected with the indicated pathogen. Infections were performed with either (a) *Beauveria bassiana*, (b) *Metarhizium anisopliae*, (c) *Clonostachys rosea*, or (d) *Trichoderma atroviride*. Infections of all groups were performed side-by-side, along with those of the w^k background line (Figure 2), with at least two blocks of infections performed on different days. Each line represents a total of 60 flies. Sham controls were performed with sterile 20% glycerol. Full statistics, available in Table S1, were done with a Cox mixed effects model. Controls for panel S2a are the same for Figure S1, and the panels in S2b-d are the same because they were performed concurrently in the same background.



Figure S3. *Wolbachia* increases the longevity of flies of the w^{1118} background line infected with yeast pathogens. Flies of each given background and sex were systemically infected with the indicated pathogen. Infections were performed with either (a) *Candida auris*, (b) *Candida glabrata*, or (c) *Galactomyces pseudocadidus*. Infections of all groups were performed side-by-side, along with those of the w^{1118} background line (Figure 3), with at least two blocks of infections performed on different days. Each line represents a total of 60 flies. Sham controls were performed with sterile 20% glycerol. Full statistics, available in Table S1, were done with a Cox mixed effects model. Controls are the same in all panels and because they were performed concurrently in the same background.



Figure S4. Wolbachia increases the number of eggs laid but not the percentage of eggs hatched post-*B. bassiana* infection in the w^{1118} background line. Female flies were systemically infected with *B. bassiana* or treated with a sham control. The flies then laid eggs for 3 days post-infection. (a) Numbers of eggs laid. (b) Proportion of eggs hatched. Each dot represents the total offspring of a single female, with an overall mean of 48 eggs laid. The boxes indicate the interquartile range. Outer edges of the box indicate 25th (lower) and 75th (upper) percentiles and the middle line indicates 50th percentile (median). Whiskers represent maximum and minimum ranges of data within 1.5 times the interquartile range of the box. Statistics are based on a logistic regression (Table S1). The entire experiment was performed twice, and graphs represent a combination of data from both blocks.



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Figure S5. *Wolbachia* associates with reduced pathogen titer after infection with no significant change in *Wolbachia* titer in w^{1118} flies. Female flies were systemically infected with the indicated fungal pathogen and pathogen titers were measured both immediately after infection and 24 h post-infection. Dots represent pools of 3 infected females. (a) *Wolbachia* titers. (b) *B. bassiana* titers. The boxes indicate the interquartile range. Outer edges of the box indicate 25^{th} (lower) and 75^{th} (upper) percentiles and the middle line indicates 50^{th} percentile (median). Whiskers represent maximum and minimum ranges of data within 1.5 times the interquartile range of the box. Statistics are based on a logistic regression (Table S1). The entire experiment

59 was performed twice, and graphs represent a combination of data from both blocks.

523 Table S2. Microorganisms used in this study.

| Species (strain) | Microbial | Isolation Source | Stock Number or |
|------------------------|----------------|-------------------------|---------------------------|
| | Classification | | Isolated/Gifted By |
| Candida glabrata (CBS | Yeast | Feces | ATCC 2001 |
| 138) | | | |
| Candida auris | Yeast | Clinical isolate | CDC B11903 |
| Galactomyces | Yeast | Drosophila | Isolated by I. |
| pseudocandidus | | | Nevarez-Saenz |
| Fusarium oxysporum (f. | Filamentous | Tomato | FGSC 9935 |
| sp. Lycopersici) | fungus | | |

| Beauveria bassiana | Filamentous | Locusta migratoria | Gift from P. |
|--------------------------|-------------|--------------------|----------------------|
| (GHA) | fungus | _ | Shahrestani |
| Aspergillus fumigatus | Filamentous | Clinical isolate | FGSC 1100 |
| | fungus | | |
| Aspergillus flavus (NRRL | Filamentous | Peanut | FGSC A1446 |
| 3357) | fungus | | |
| Metarhizium anisopliae | Filamentous | Insect | ARSEF 23 |
| (recently renamed | fungus | | |
| Metarhizium robertsii) | | | |
| Clonostachys rosea | Filamentous | Aedes albopictus | Isolated by P. |
| | fungus | (mosquito) L4 | Tawidian & gifted by |
| | | larvae, Manhattan, | K. Michel |
| | | KS | |
| Trichoderma viride | Filamentous | Aedes albopictus | Isolated by P. |
| | fungus | (mosquito) L4 | Tawidian & gifted by |
| | | larvae, Manhattan, | K. Michel |
| | | KS | |

594 Table S3. Primers used in this study.

| Gene | Primer Name | Sequence |
|----------------------------------|-------------|---------------------------|
| Wolbachia groEL | groEL_F | CTAAAGTGCTTAATGCTTCACCTTC |
| | groEL_R | CAACCTTTACTTCCTATTCTTG |
| Drosophila rp49 | Rp49_F | CGGTTACGGATCGAACAAGC |
| | Rp49_R | CTTGCGCTTCTTGGAGGAGA |
| Beauveria bassiana gamma-tubulin | Bbas_F | CAGAGCGACGACACACGC |
| | Bbas_R | CCCACGCCATTCTTGCCAATG |