

**Supplementary data Gainey et al.**

**Table S3. FK181 genetic instability**

| FK181 line | Starve-chunk cycle with first observed non-Rol | Rol Progeny                         |      | non-Rol Progeny |      |
|------------|--|-------------------------------------|------|-----------------|------|
|            |  | GFP-                                | GFP+ | GFP-            | GFP+ |
| 1          | 4  | *                                   |      |                 |      |
| 2          | 4  |                                     |      | +++             |      |
| 3          | 7  |                                     | +    | +++             | +    |
| 4          | 7  |                                     | +++  |                 |      |
| 5          | 7  |                                     | +++  |                 |      |
| 6          | 7  |                                     | +    | +++             | (+)† |
| 7          | 7  |                                     | +    |                 | +    |
| 8          | 8  | 241 progeny   22% non-Rol, 81% GFP- |      |                 |      |
| 9          | 8  | 167 progeny   26% non-Rol, 46% GFP- |      |                 |      |

FK181 was grown to starvation (6 days, 20°C) on a small NG plate and then chunked to a fresh small NG plate and grown to starvation. The first observed non-Rol hermaphrodite (day 3) was picked to a fresh plate the Rol and GFP phenotypes of the progeny recorded. \* Sterile, † weak GFP

**Table S4. Putative endo-siRNA targeted genes implicated in host-pathogen responses.**

| Gene     | Summary description of predicted and/or documented activities   |
|----------|---|
| Y43F8B.2 | Predicted to enable kinase regulator activity and protein kinase A binding activity. Involved in innate immune response.                                |
| ilys-5   | Predicted to enable lysozyme activity. Predicted to be involved in defense response to Gram-positive bacterium.   |
| acdh-1   | Predicted to enable acyl-CoA dehydrogenase activity. Involved in defense response to Gram-negative bacterium and innate immune response.                |
| clec-218 | Involved in defense response to Gram-positive bacterium.  |
| lys-6    | Predicted to enable lysozyme activity. Predicted to be involved in innate immune response and signal transduction.                                      |
| lys-7    | Involved in defense response to other organisms.  |
| clec-52  | Predicted to enable signaling receptor activity. Involved in defense response to Gram-positive bacterium.   |
| spp-1    | Enables pore-forming activity. Involved in defense response to other organism and pore formation in membrane of another organism. Part of pore complex. |

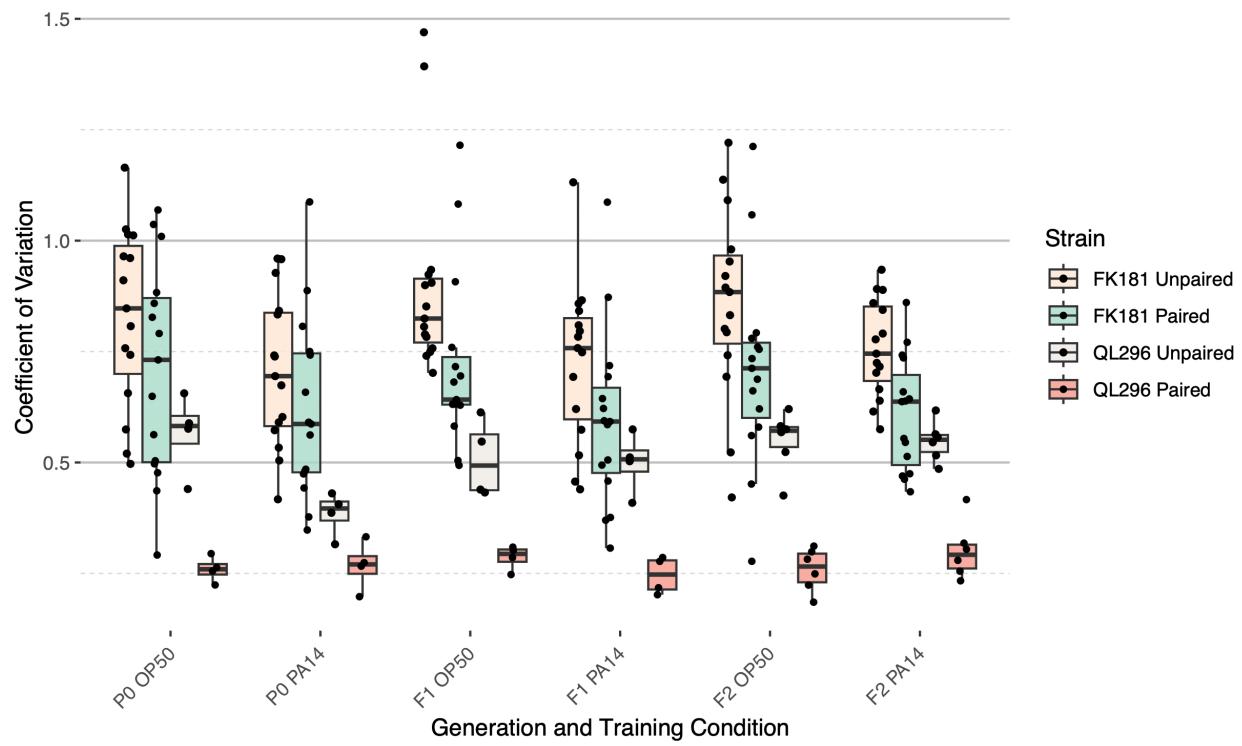
Summarized descriptions from WormBase (WS291).

**Table S5.** Worm strains used

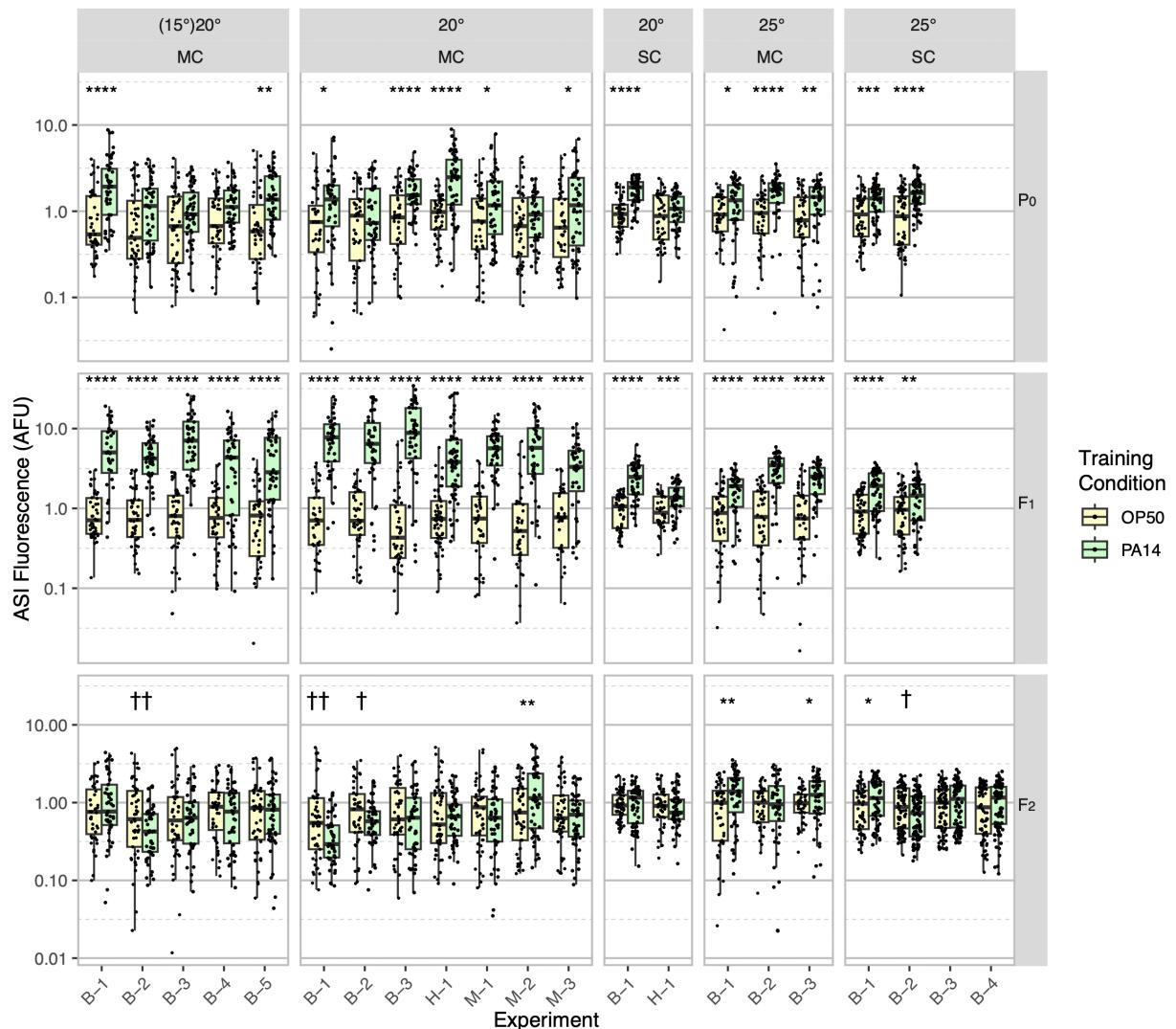
| Strain name | Genotype                                   | Reference             |
|-------------|--|-----------------------|
| N2          | WT   | Brenner, 1973         |
| FK181       | <i>ksls2 [daf-7p::gfp + rol-6(su1006)]</i> | Murakami et al., 2001 |
| QL296       | <i>drcSi89 [daf-7p::GFP; unc-119(+)]</i>   | Zhan et al., 2015     |
| HC445       | <i>sid-1(qt9)</i>                          | Winston et al., 2001  |
| HC306       | <i>sid-2(qt42)</i>                         | Winston et al., 2007  |
| HC1221      | <i>rde-1(ne219); drcSi89</i>               | This study            |
| HC1222      | <i>hrde-1(tm1200); drcSi89</i>             | This study            |
| HC1223      | <i>sid-1(qt158); drcSi89</i>               | This study            |
| HC1218      | <i>sid-2(qt42); drcSi89</i>                | This study            |

References:

- Brenner, S. (1974). The genetics of *Caenorhabditis elegans*. *Genetics* 77, 71-94.
- Murakami, M., Koga, M., and Ohshima, Y. (2001). DAF-7/TGF-B expression required for the normal larval development in *C. elegans* is controlled by a presumed guanylyl cyclase DAF-11. *Mechanisms of Development* 109, 27-35.
- Winston, W.M., Molodowitch, C., and Hunter, C.P. (2002). Systemic RNAi in *C. elegans* requires the putative transmembrane protein SID-1. *Science* 295, 2456-2459.
- Winston, W. M., Sutherlin, M., Wright, A. J., Feinberg, E. H., & Hunter, C. P. (2007). *Caenorhabditis elegans* SID-2 is required for environmental RNA interference. *Proceedings of the National Academy of Sciences*, 104, 10565-10570.
- Zhan, M., Crane, M.M., Entchev, E.V., Caballero, A., Fernandes de Abreu, D.A., Ch'ng, Q., and Lu, H. (2015). Automated Processing of Imaging Data through Multi-tiered Classification of Biological Structures Illustrated Using *Caenorhabditis elegans*. *PLoS Comput Biol* 11, e1004194.

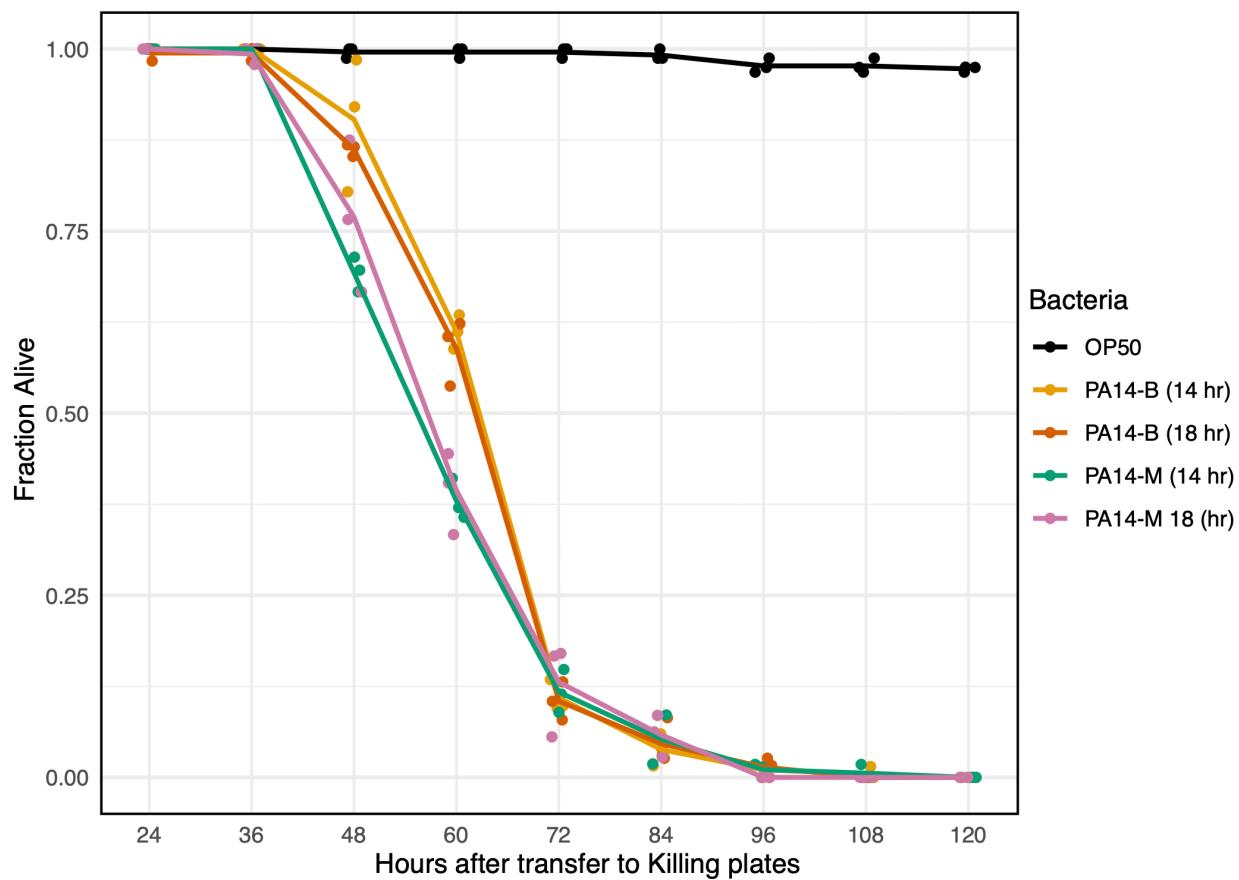


**Figure S1. Coefficient of variation analysis of ASI *daf-7p::gfp* expression levels.**  
The coefficient of variation was calculated for all experiments shown in Figure 2 (average of both ASI neurons - Paired) and Figure S2 (individual neurons - Unpaired) for both FK181 (integrated multicopy array) and QL296 (integrated single copy insertion).



**Figure S2. Box plot display of individual ASI *daf-7p::gfp* expression levels after P0 PA14 exposure.**

Results from 21 independent experiments are normalized to the average OP50 value by generation within each experiment for summary presentation (experiments are named by experimental conditions, see Table 1). This figure includes the exact same data as shown in Figure 2 but plotted for all individual neurons rather than the average of each neuron pair in each animal. MC refers to FK181, which contains an integrated multicopy tandem array composed of the *daf-7p::gfp* reporter and the co-injection marker *rol-6(su1006)*. SC refers to QL296, which is a single copy insert of *daf-7p::gfp* with *unc-119(+)* as the co-selection marker (Zhan et al., 2015). Worms were cultured at either 20° or 25°C and exposed to one of three different PA14 isolates (B, Balskus; H, Hunter; M, Murphy labs). In some experiments worms were grown at 15°C for at least three generations prior to the P0 generation (indicated with parentheses, i.e. (15)20). Statistical significance \*\*\*\* P< 0.0001, \*\*\* < 0.001, \*\* <0.01, \* <0.05, ns > 0.05. † Indicate statistical significance with control higher than experimental. See Methods section for statistical methods.



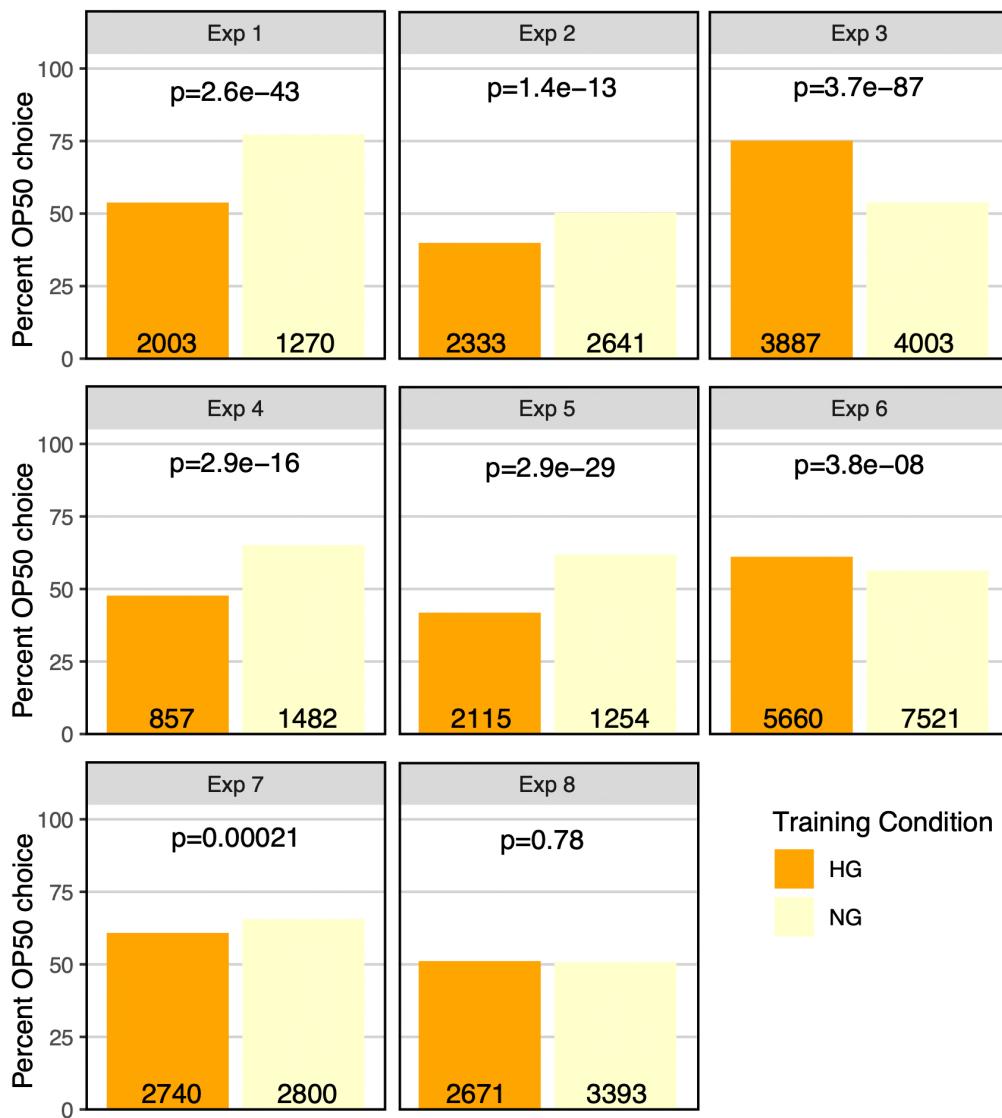
**Figure S3. Pathogenicity of PA14 isolates.**

Day 1 N2 adults were transferred to plates seeded with the indicated 14 hr or 18 hr bacterial cultures and then scored for viability at 12-hour intervals. Data points were jittered along the X-axis for display. n= 38 – 80 per replicate, 3 replicates per condition. Data for this figure is presented in Table S6.



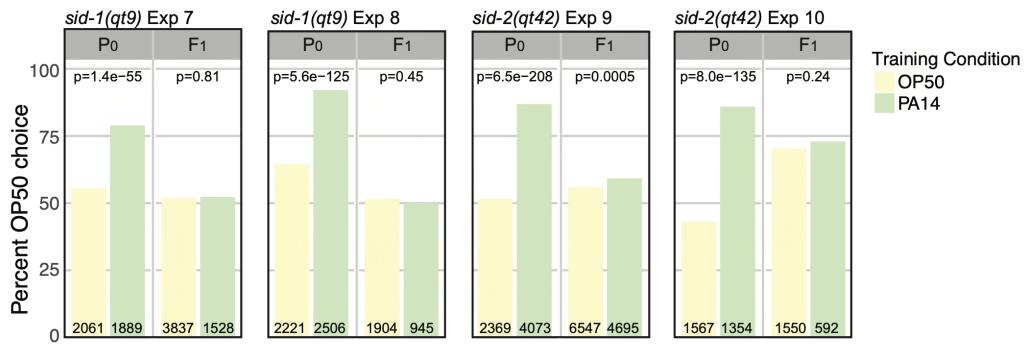
**Figure S4. P0 PA14 exposure fails to reproducibly induce F2 PA14 avoidance behavior.**

The same data as presented in Figure 1 and Figure 3 choice index plots. At each generation and for both PA14 training and OP50 control animals the total number of OP50 and PA14 choices were summed across all choice plates and analyzed using a 2X2 contingency table and Fisher's exact test. Plotted is the percent OP50 choice for each condition at each generation (the PA14 choice is 100% minus the OP50 choice). The number of OP50 choice and PA14 choice animals scored for each experiment, generation, and training condition is indicated on each bar plot. The p-values represent the probability that the two observations came from identical, binomially-distributed populations.



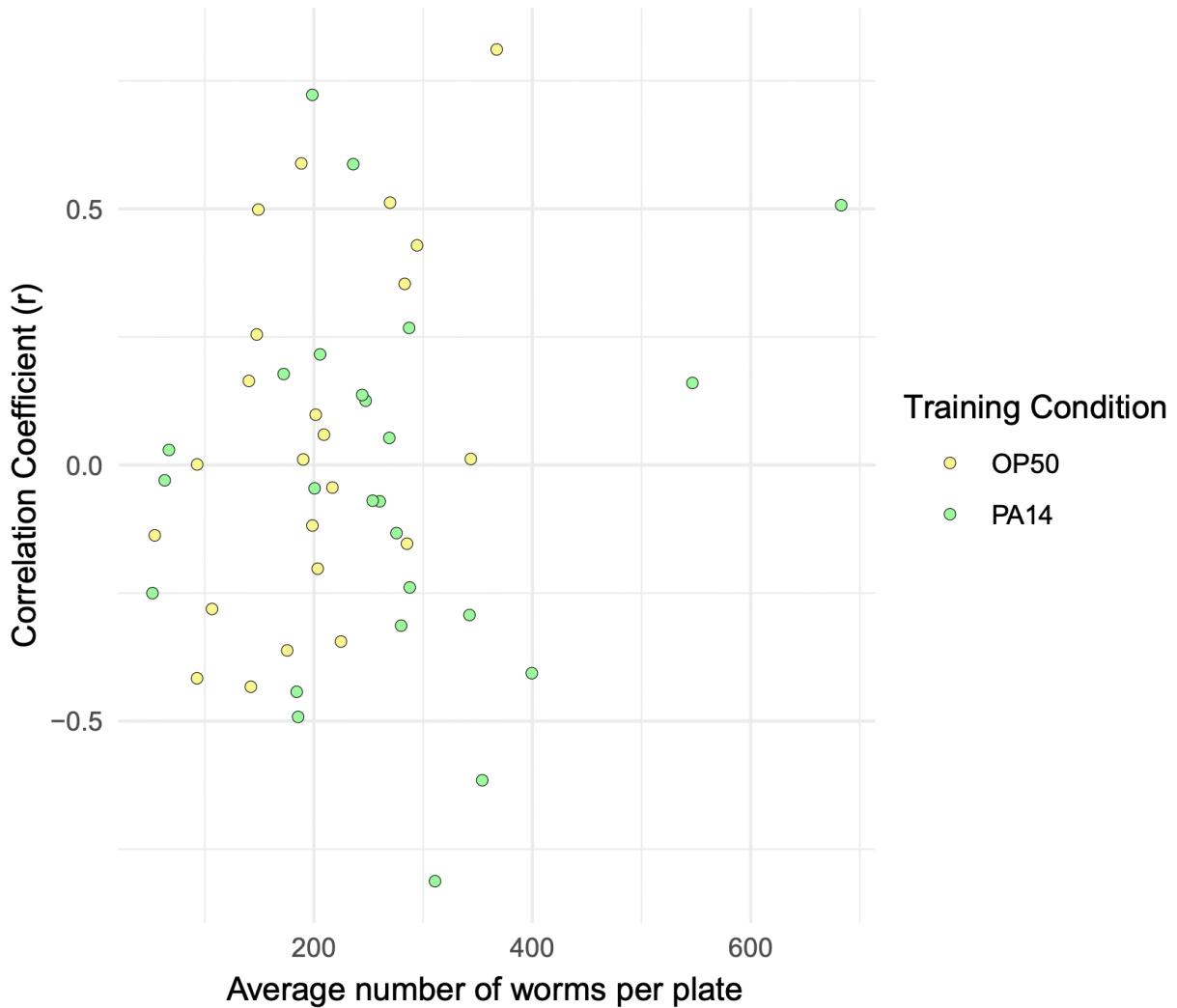
**Figure S5. OP50 growth conditions, independent of PA14 exposure, affect OP50 vs PA14 choice.**

The same data as presented in Figure 4 choice index plots. N2 worms grown to adulthood on HG plates were “trained” on either HG OP50 or NG OP50 plates for 24 hours and then assayed on OP50 vs PA14 choice plates. For each of eight experiments the total number of OP50 and PA14 choices were summed across all choice plates and analyzed using a 2X2 contingency table and Fisher’s exact test. Plotted is the percent OP50 choice for each condition (the PA14 choice is 100% minus the OP50 choice). The number of OP50 choice and PA14 choice animals scored for each experiment, generation, and training condition is indicated on each bar plot. The p-values represent the probability that the two observations came from identical, binomially-distributed populations. Data for this figure is presented in Table S7.



**Figure S6. *sid-1* and *sid-2* are required for intergenerational (F1) inheritance of avoidance behavior.**

The same data as presented in Figure 5 choice index plots. At each generation and for both PA14 training and OP50 control animals the total number of OP50 and PA14 choices were summed across all choice plates and analyzed using a 2X2 contingency table and Fisher's exact test. Plotted is the percent OP50 choice for each condition at each generation (the PA14 choice is 100% minus the OP50 choice). The number of OP50 choice and PA14 choice animals scored for each experiment, generation, and training condition is indicated on each bar plot. The p-values represent the probability that the two observations came from identical, binomially-distributed populations.



**Figure S7. Sample size does not correlate with choice index scores.**

The correlation coefficient ( $r$ ) calculated from sample size vs choice index for each experiment (generation, training condition) shown in Figure 3 is plotted against the average number of worms in each experiment. Experiments with six or fewer choice plates were excluded. Data for this figure is presented in Table S8.