SUPPORTING INFORMATION

Quantifying the fitness effects of resistance alleles with and without anthelmintic selection pressure using *Caenorhabditis elegans*

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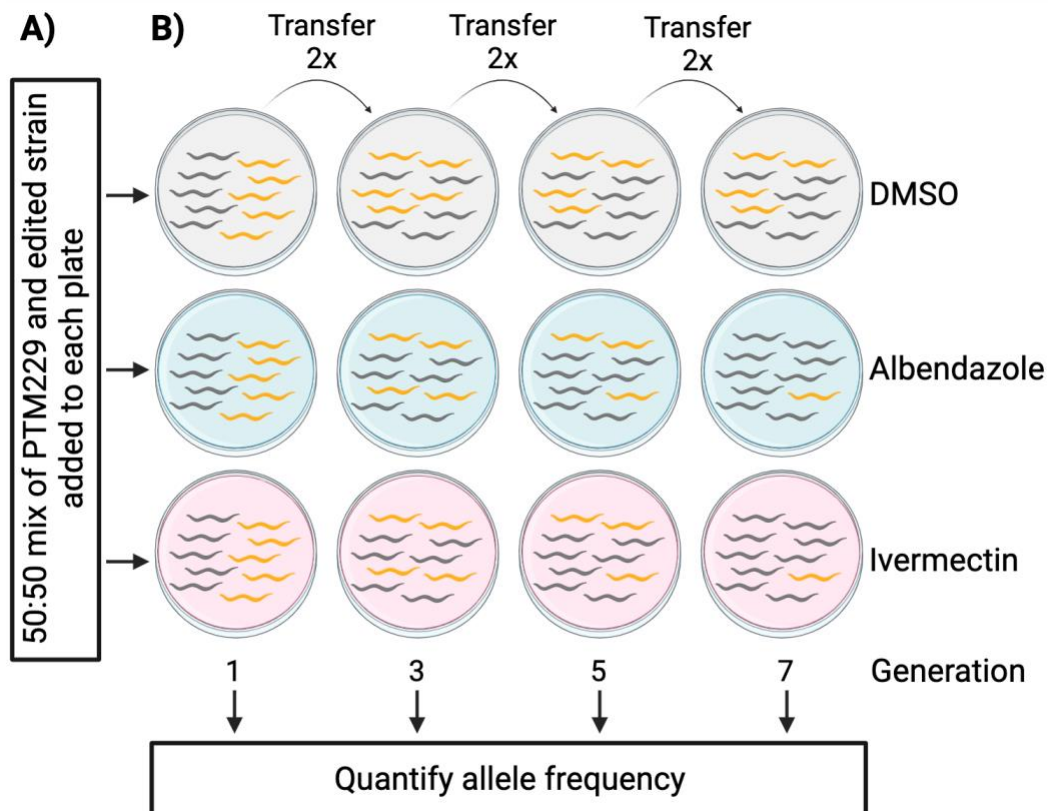
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S1 Fig. The competitive fitness assay allows for the assessment of allele frequency in the presence of DMSO, albendazole, or ivermectin.
**(A)** Equal numbers of the control strain PTM229 were placed on each test plate along with an edited strain. **(B)** Strains were grown on 6 cm NGMA plates for approximately seven days. After seven days, a ~0.5 cm^3 plate chunk of NGMA with animals was transferred to a new 6 cm NGMA plate. Animals were washed off of NGMA plates at each odd generation. After animal collection, DNA extractions, DNA cleanup, and quantification were performed. Allele frequencies were quantified using ddPCR. See *Methods, Competitive fitness assays* for details. Modified from a previous version [65]. Created with Biorender.com.
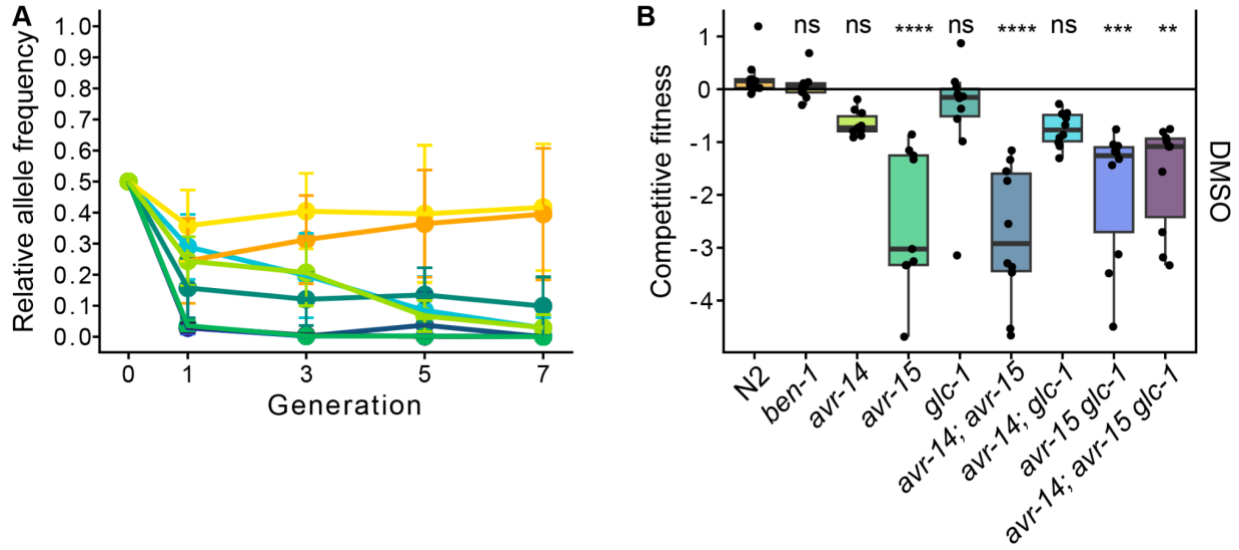
**S2 Fig. Competitive fitness assays across seven generations in DMSO.**
(A) A barcoded N2 wild-type strain, PTM229, was competed with strains that have deletions in either one, two, or three genes that encode for GluCl channels or in the beta-tubulin gene *ben-1* in DMSO. Generation is shown on the x-axis, and the relative allele frequencies of the nine strains with genome-edited alleles and N2 are shown on the y-axis. **(B)** The $\log_2$-transformed competitive fitness of each allele is plotted in DMSO. The gene tested is shown on the x-axis, and the competitive fitness is shown on the y-axis. Each point represents a biological replicate of that competition experiment. Data are shown as Tukey box plots with the median as a solid horizontal line, and the top and bottom of the box representing the 75th and 25th quartiles, respectively. The top whisker is extended to the maximum point that is within the 1.5 interquartile range from the 75th quartile. The bottom whisker is extended to the minimum point that is within the 1.5 interquartile range from the 25th quartile. Significant differences between the wild-type N2 strain and all the other alleles are shown as asterisks above the data from each strain ($p > 0.05$ = ns, $p < 0.001$ = ***, $p < 0.0001$ = ****, Tukey HSD).
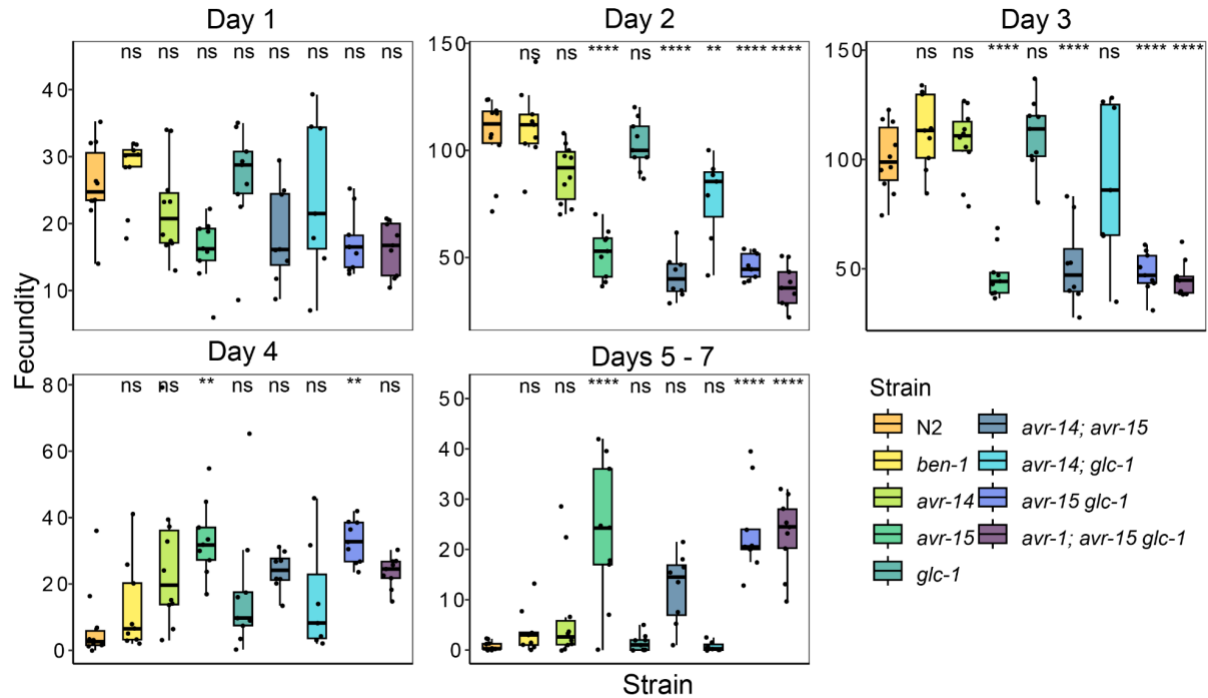
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S3 Fig. Variation in daily fecundity of *C. elegans* deletion strains in DMSO.
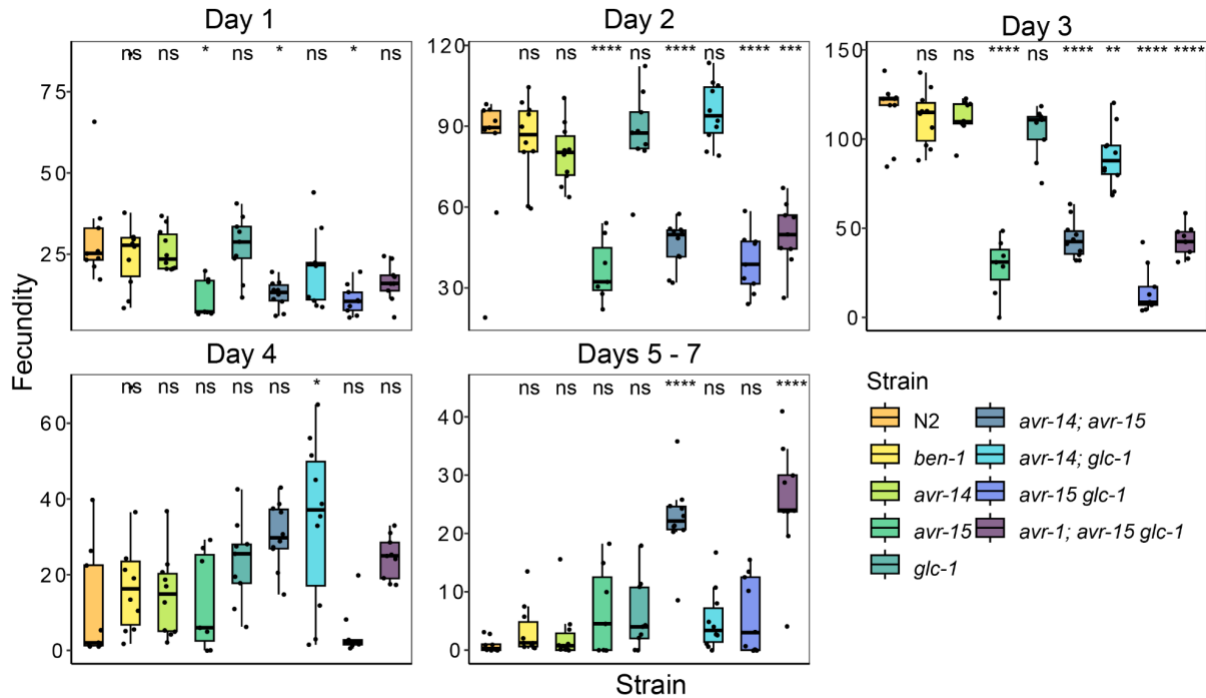Boxplots for daily fecundity when exposed to DMSO on the y-axis, for each deletion strain on the x-axis. Each point represents the daily fecundity count for one biological replicate. Error bars show the standard deviation of lifetime fecundity among 7 - 10 biological replicates. Data are shown as Tukey box plots with the median as a solid horizontal line, and the top and bottom of the box represent the 75th and 25th quartiles, respectively. Significant differences between the wild-type strain, N2, and all other deletions are shown as asterisks above the data from each strain ($p >$ 0.05 = ns, $p < 0.05$ = *, $p < 0.01$ = **, $p < 0.001$ = ***, $p < 0.0001$ = ****, Tukey HSD).
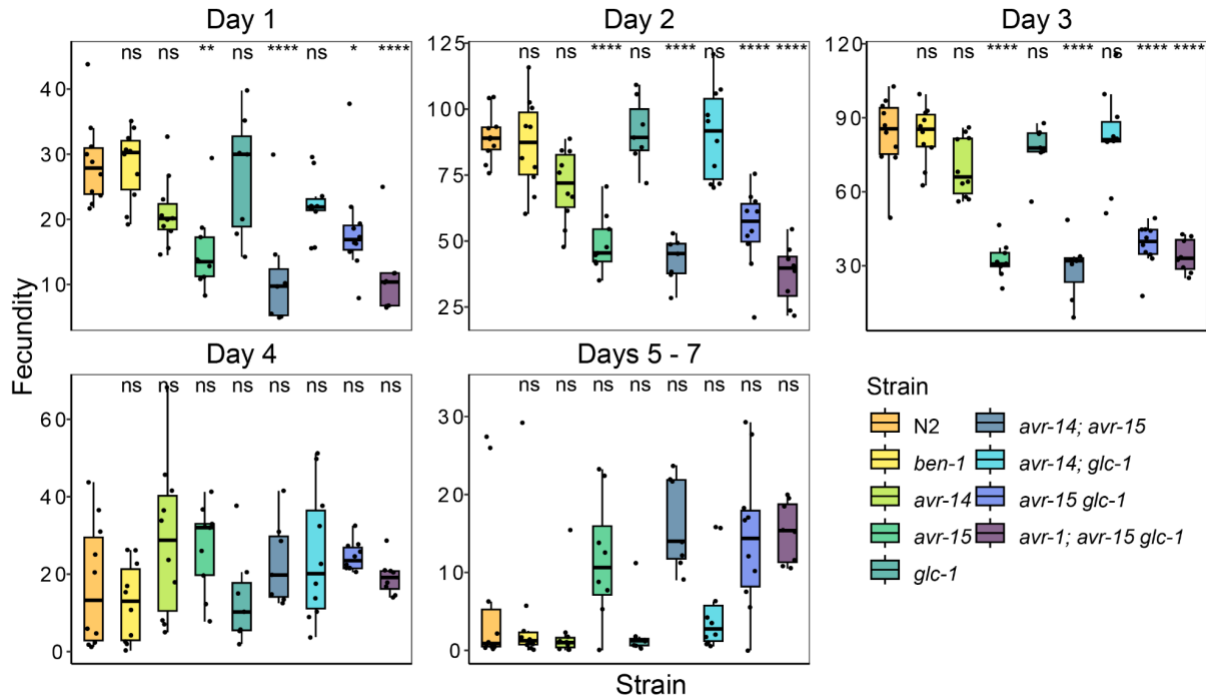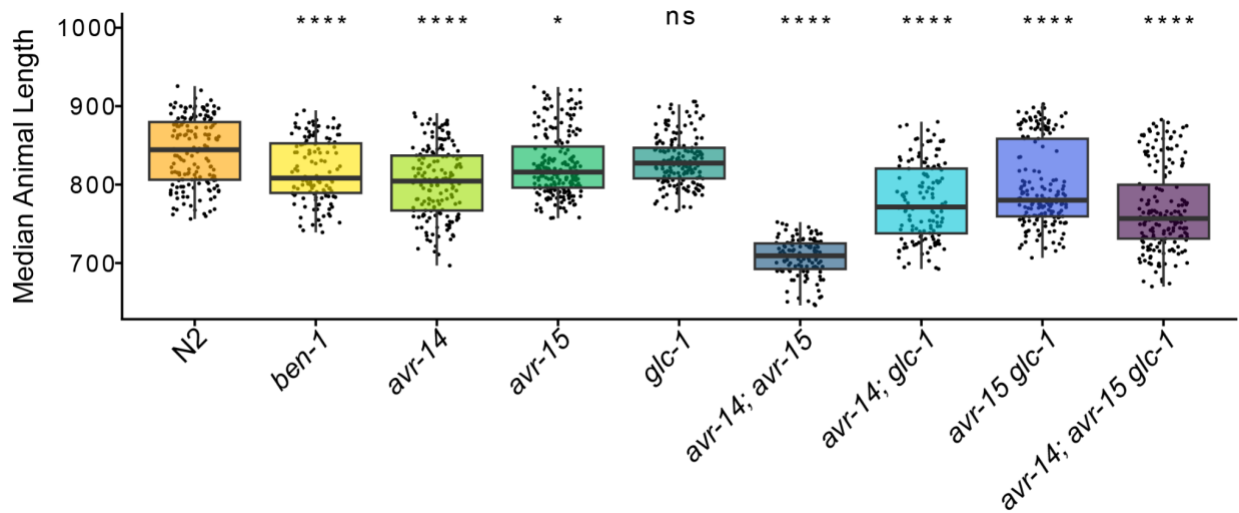
S4 Fig. Variation in daily fecundity of *C. elegans* deletion strains in albendazole.
Boxplots for daily fecundity when exposed to albendazole on the y-axis, for each deletion strain on the x-axis. Each point represents the daily fecundity count for one biological replicate. Error bars show the standard deviation of lifetime fecundity among 7 - 10 biological replicates. Data are shown as Tukey box plots with the median as a solid horizontal line, and the top and bottom of the box represent the 75th and 25th quartiles, respectively. Significant differences between the wild-type strain, N2, and all other deletions are shown as asterisks above the data from each strain ($p > 0.05$ = ns, $p < 0.05$ = *, $p < 0.01$ = **, $p < 0.001$ = ***, $p < 0.0001$ = ****, Tukey HSD).

S5 Fig. Variation in daily fecundity of *C. elegans* deletion strains in ivermectin
Boxplots for daily fecundity when exposed to ivermectin on the y-axis, for each deletion strain on the x-axis. Each point represents the daily fecundity count for one biological replicate. Error bars show the standard deviation of lifetime fecundity among 7 - 10 biological replicates. Data are shown as Tukey box plots with the median as a solid horizontal line, and the top and bottom of the box represent the 75th and 25th quartiles, respectively. Significant differences between the wild-type strain, N2, and all other deletions are shown as asterisks above the data from each strain ($p > 0.05$ = ns, $p < 0.05$ = *, $p < 0.01$ = **, $p < 0.001$ = ***, $p < 0.0001$ = ****, Tukey HSD).

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1131 **S6 Fig. High-throughput assays for each deletion strain in control conditions.** Median
1132 animal length values from populations of nematodes grown in DMSO are shown on the y-axis.
1133 Each point represents the median animal length from a well containing approximately 5 - 30
1134 animals. Data are shown as Tukey box plots with the median as a solid horizontal line, the top
1135 and bottom of the box representing the 75th and 25th quartiles, respectively. The top whisker is
1136 extended to the maximum point that is within 1.5 interquartile range from the 75th quartile. The
1137 bottom whisker is extended to the minimum point that is within 1.5 interquartile range from the
1138 25th quartile. Significant differences between the wild-type strain and all other strains are shown
1139 as asterisks above the data from each strain ($p > 0.05$ = ns, $p < 0.05$ = *, $p < 0.001$ = ***, $p <$
1140 0.0001 = ****, Tukey HSD).
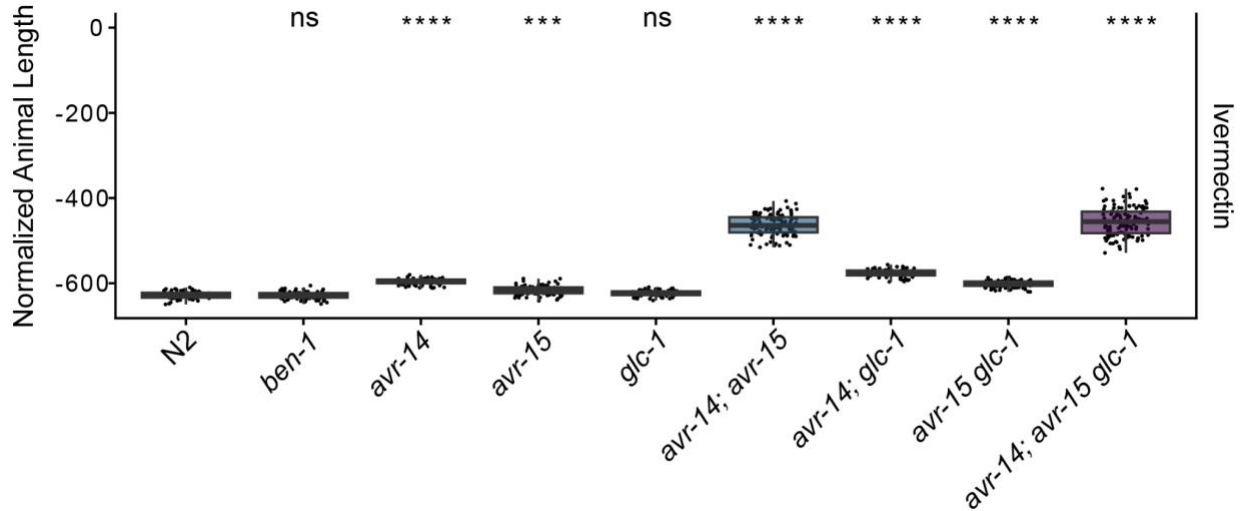
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1143 **S7 Fig. High-throughput assays for each deletion strain in 500 nM of ivermectin.** The
1144 regressed median animal length values for populations of nematodes growth in 500 nM ivermectin
1145 are shown on the y-axis. Each point represents the regressed median animal length value of a
1146 well containing approximately 5-30 animals. Data are shown as Tukey box plots with the median
1147 as a solid horizontal line, and the top and bottom of the box representing the 75th and 25th
1148 quartiles, respectively. The top whisker is extended to the maximum point that is within the 1.5
1149 interquartile range from the 75th quartile. The bottom whisker is extended to the minimum point
1150 that is within the 1.5 interquartile range from the 25th quartile. Significant differences between the
1151 wild-type strain and all other deletions are shown as asterisks above the data from each strain (p
1152 > 0.05 = ns, $p < 0.001$ = ***, $p < 0.0001$ = ****, Tukey HSD).
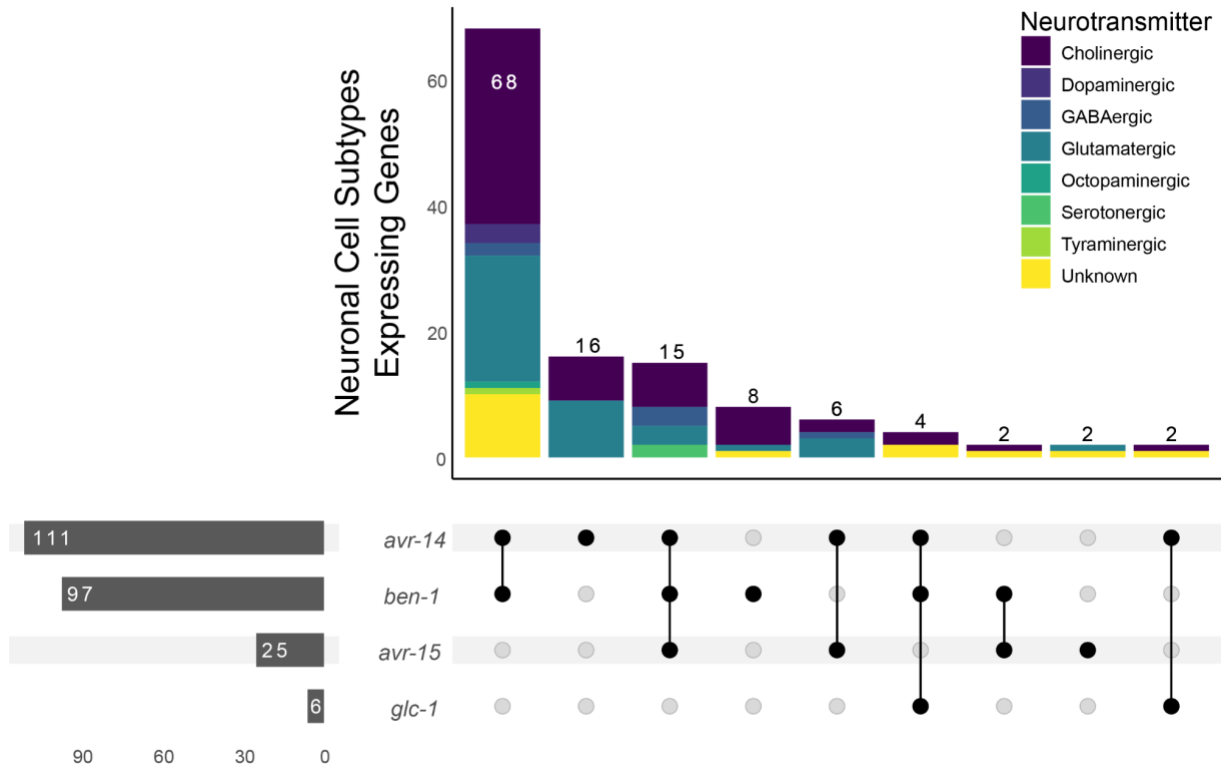
S8 Fig. Upset plot of the neuronal expression patterns for *ben-1*, *avr-14*, *avr-15*, and *glc-1*. Upset plot of single-cell RNA-sequencing data obtained from CeNGEN. Horizontal bar plots sum the total number of neurons where the gene is expressed. Vertical bar plots sum overlap where genes are expressed in the neuronal cell subtypes. Black dots directly under vertical bar plots signify the gene(s) that overlap in the neuronal cell subtypes indicated in the vertical bar plot.

REFERENCES

1. Zhao Y, Long L, Xu W, Campbell RF, Large EE, Greene JS, et al. Changes to social feeding behaviors are not sufficient for fitness gains of the Caenorhabditis elegans N2 reference strain. eLife. 2018. doi:10.7554/elife.38675