



# Discovering which genes protect nematodes from odor-based paralysis

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## Introduction

*P. pacificus* is a free-living nematode in the wild and was first found in Pasadena, California. Unlike the model organism *C. elegans*, it's a necromenic nematode that has a species-beetle host preference, like a parasite. A beetle sex-pheromone was discovered called ZTDO that can cause paralysis in *obi-1* mutant J4 larvae but not the wild-type J4 larvae. *Obi-1* is a lipid binding protein that is specifically susceptible to ZTDO. To determine the genes mediating *obi-1*'s hypersensitivity to volatile ZTDO, we performed a genetic screen for suppressors of *obi-1* and we had arrived at 2 suppressors named as *csu63* and *csu64*. Later, we had gotten potential gene candidates for ZTDO susceptibility and focused on PPA09604, a novel gene found in both *csu63* and *csu64*.

## Questions

- Could PPA09604 be responsible for suppressing *obi-1* J4 hypersensitivity to ZTDO?
- Is it only ZTDO that affects the worm's reaction towards its environment?

## Materials & Methods

Plates containing different worm strains would be grown and be synced to all be at the same developmental J4 stage

Plates to be treated are given 10  $\mu$ L of 0.5% ZTDO or 10  $\mu$ L of 90% ethanol and wait for 2 hours

After 2 hours, all plates are then prepared to undergo RNA extraction

Worms were first grown in preparation for RNA extraction. However, before starting the extraction, they went through one of 3 treatments: no treatment, a mock ethanol treatment, or a ZTDO treatment for 2 hours. The mock treatment was a negative control to verify if the worm could have a different expression level with a chemical other than ZTDO.

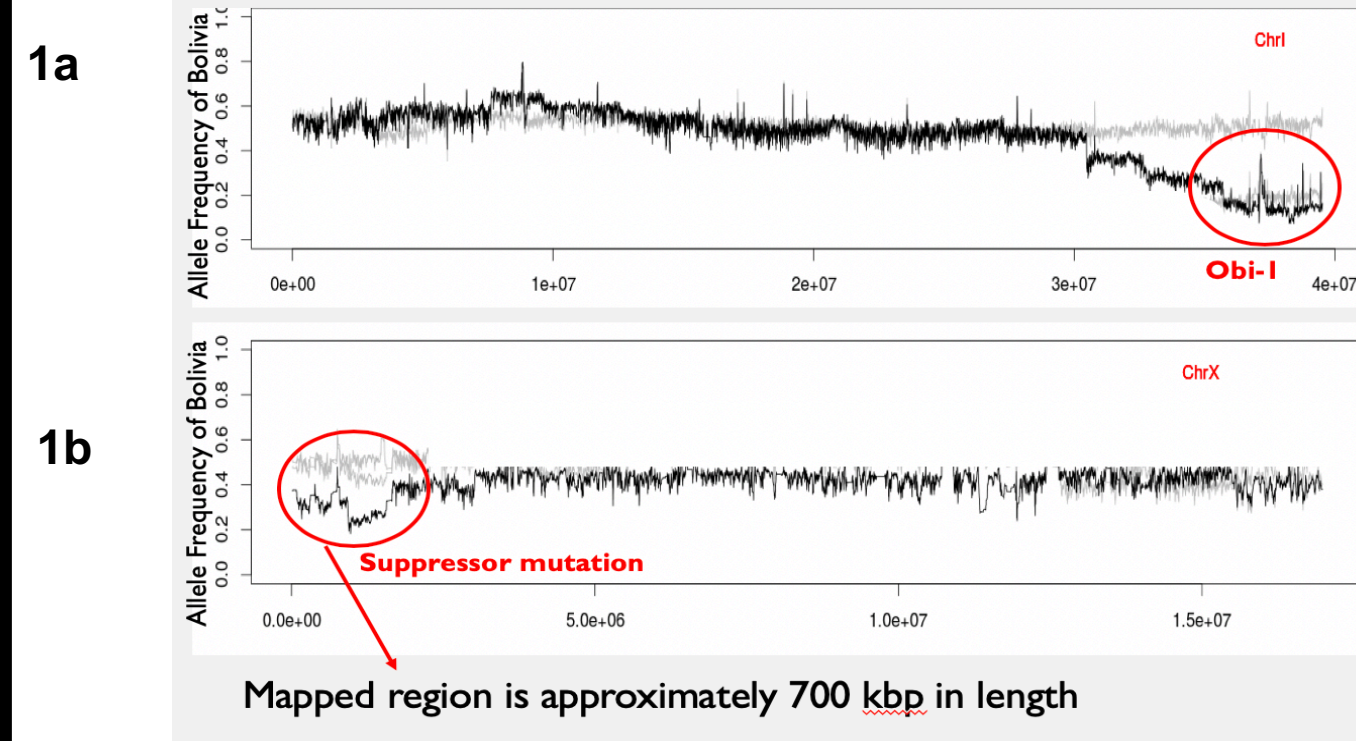
After extraction, RNA was put through cDNA synthesis, with aiming for a concentration of 1ng/ $\mu$ L for all samples. qPCR was done to measure expression levels with beta-tubulin used as the housekeeping gene

## Acknowledgements

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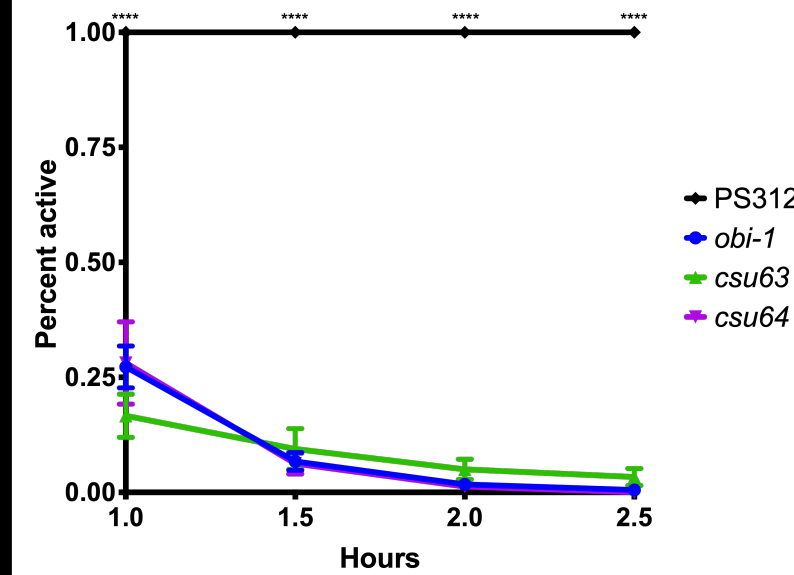
J. Cardenas is supported by BUILD PODER.

## Results



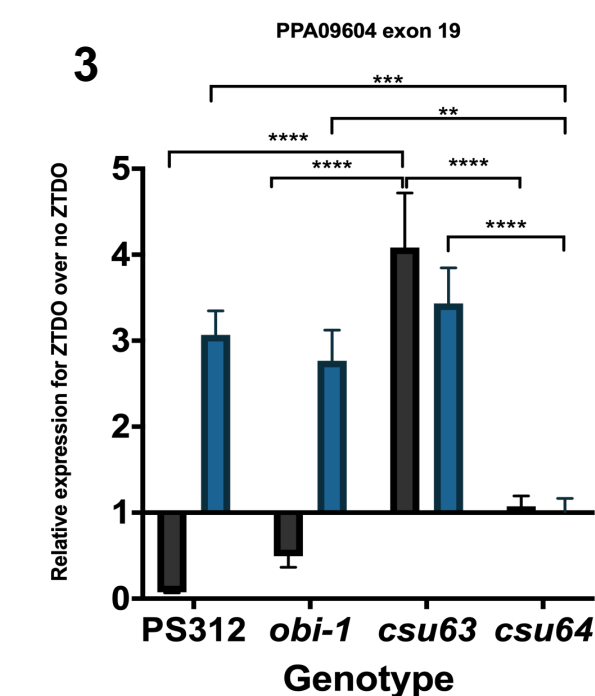
**Fig 1. *csu63* Mapping.** We crossed *csu63*, our best suppressor line, with the polymorphic *Bolivia* strain, and obtained 53 mapping strains used to determine the loci of the phenotype-causing mutation by whole genome sequencing. The graph depicts allele frequency of *Bolivia* on the y-axis (0.5 being the neutral range with even representation of California and *Bolivia*) and position on the chromosome on the x-axis. (a) The notable dip on Chromosome I confirms that the lines are in the *obi-1* background. (b) The dip on the left arm of Chromosome X depicts the locus in which the suppressor mutation may reside.

## 2 ZTDO partial suppressors



## Fig 2. Effectivity of found partial suppressors.

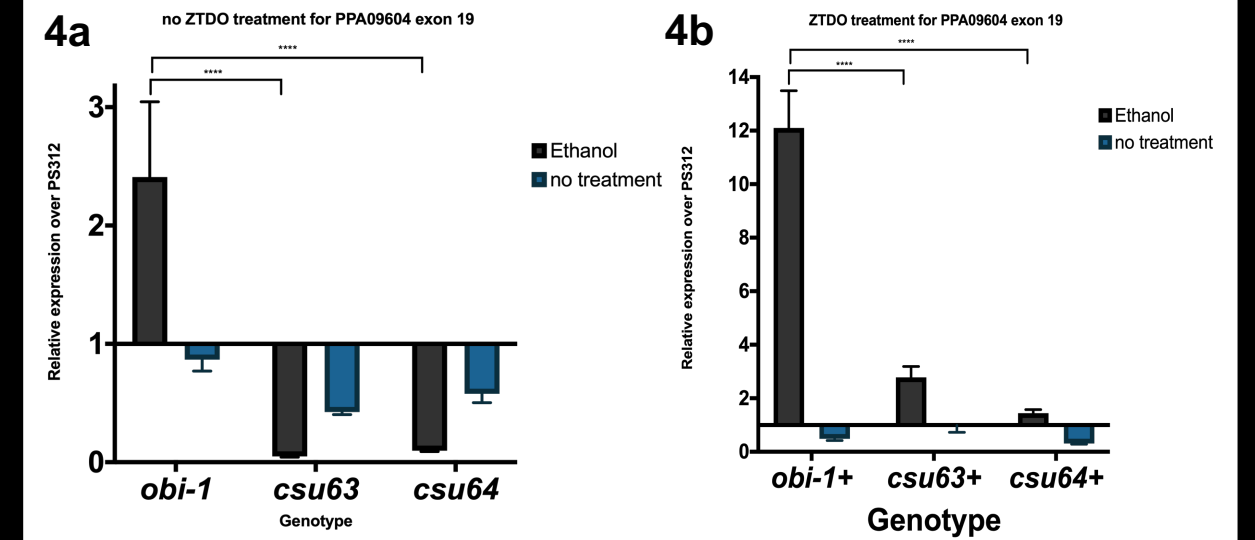
We had tested out our two suppressors to see how much of an improvement there is for ZTDO resistance. While *csu63* and *csu64* don't have a significant improvement, *csu63* performed better and was the suppressor that we focused on the most. One-way ANOVA with Dunnett's multiple comparison test was used to look for statistical differences between means against PS312 \*\*\*\*P < 0.0001.



## Fig 3. Relative expression of ZTDO treatment vs no treatment.

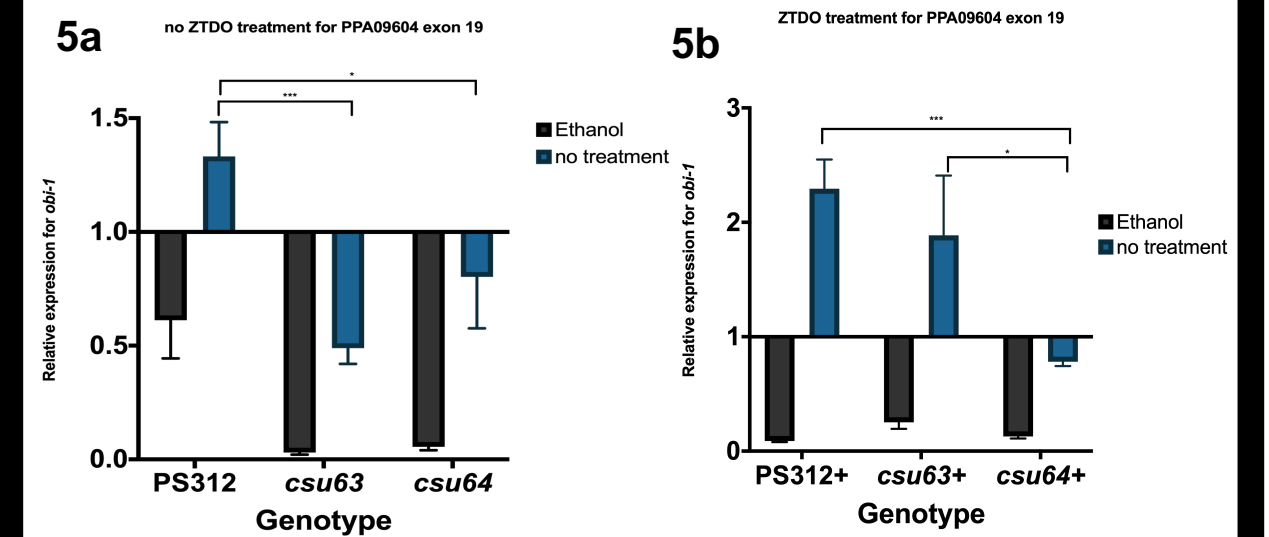
qPCR was done on PS312, *obi-1*, *csu63* and *csu64* in order to test expression of the PPA09604 gene when worms were treated with ZTDO against worms that weren't treated with ZTDO. PS312 and *obi-1* had more of a change between replicates, while *csu63* had prominent overexpression. 2-way ANOVA with Tukey's multiple comparisons test was used to for statistical differences with at least 6 replicates per genotype between means of ZTDO against no ZTDO \*\*P < 0.01, \*\*\*P < 0.001 \*\*\*\*P < 0.0001

## Results



## Fig 4. Relative expression of *obi-1*, *csu63*, and *csu64* over PS312.

(a) PS312 was prominent in its expression of PPA09604 except against *obi-1* undergoing an ethanol treatment. (b) All genotypes are expressing PPA09604 higher than PS312 when treated with ethanol. 2-way ANOVA with Tukey's multiple comparisons test was used to for statistical differences with at least 6 replicates per genotype between means of PS312 \*\*\*\*P < 0.0001



## Fig 5. Relative expression of PS312, *csu63*, and *csu64* over *obi-1*.

(a) *obi-1* was prominent in its expression of PPA09604 except against PS312 with no treatment. (b) All genotypes are expressing PPA09604 higher than *obi-1* when not treated. 2-way ANOVA with Tukey's multiple comparisons test was used to for statistical differences with at least 6 replicates per genotype between means of *obi-1* \*P < 0.05, \*\*\*P < 0.001

## Conclusions

- More replicates will be needed to help outline a difference between treatments as for some genotypes, ethanol surprisingly resulted in worms having higher expression levels compared to being treated with ZTDO
- However, *csu63* has shown that no matter the treatment, it's always expressing PPA09604 the most when treated with ZTDO, while *csu64*'s expression is about the same whether it's treated with ZTDO or not

## References

(1) Cinkornpumin, J. K., Wisidagama, et al. (2014). A host beetle pheromone regulates development and behavior in the nematode *Pristionchus pacificus*. *eLife*. 10.7554/eLife.03229.