

EM-analysis

Importing packages...

There are functions hiding here...

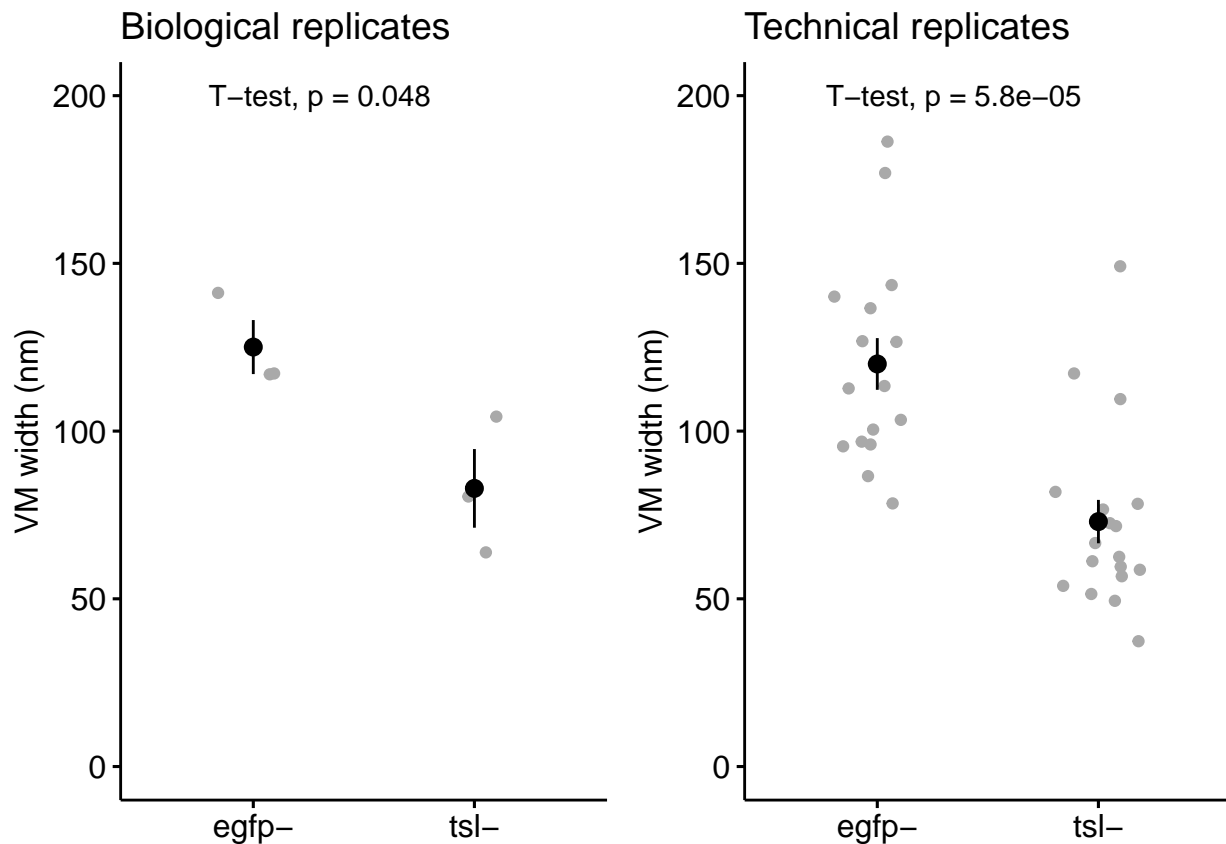
And a script hiding here...

Is there a difference in VM width between *tsl*- and *egfp*- wasps?

To determine if the VM is thinner in *tsl*- versus WT wasps, I measured the VM width across multiple oocytes and 3 wasps per treatment, using an ImageJ macro. I then calculated the mean VM width within each image (technical replicate), and averaged this to produce the mean width for each individual wasp. I excluded all images of the termini of the oocytes, as the *tsl*- ones have a very thick vitelline membrane in places, which was throwing out my data.

The data is *just* significant, using our three biological replicates. If we treat each image as a biological replicate, the data becomes very significant. (I did this as an idiocy check.) I also calculated the effect size using the Cohen's d test. (Unsure if this is appropriate.) The effect size is large: -2.4179685 for biological replicates and -1.6155525 for technical replicates. Mean VM width *tsl*:- 82.9179933 Mean VM width control: 125.0452509 .

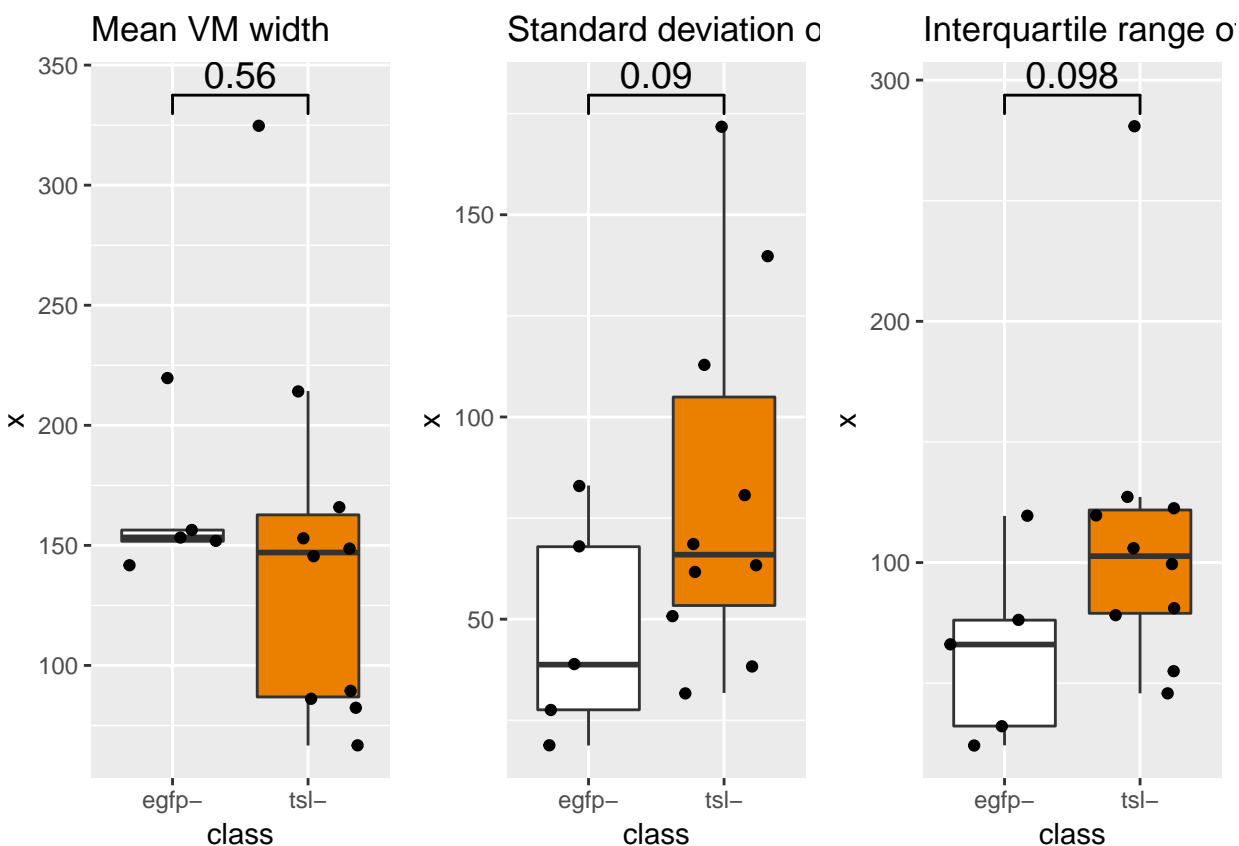
I had hoped for a more convincing result, given this was obtained by excluding data. But the large effect size is reassuring, and the small sample size does give a low statistical power.



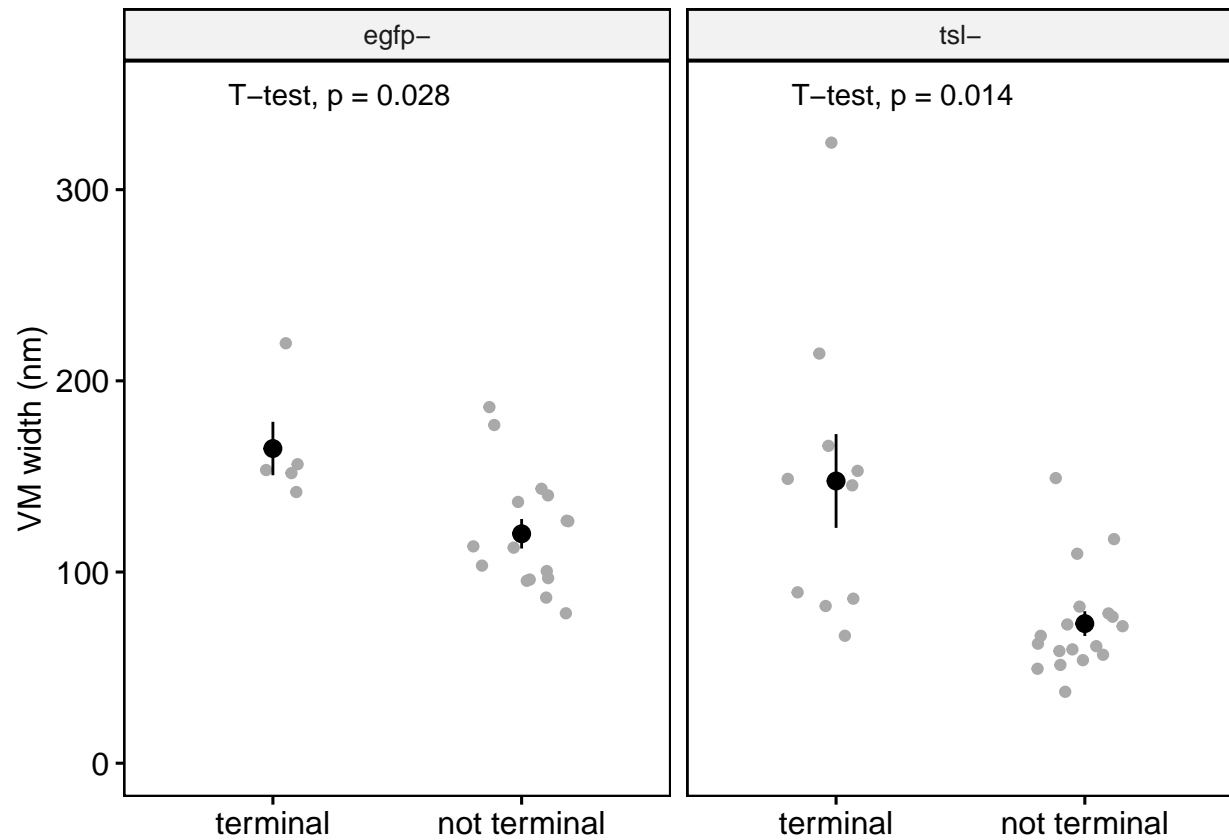
What does the excluded terminal data look like?

When plotting the excluded terminal values, the *tsl-* values are highly variable, but there doesn't seem to be an absolute difference in mean VM width. Note that for these data, I had to treat technical replicates as biological replicates, as some biological replicates completely lacked data points. This is acceptable, as I'm only trying to get a feel for the data.

I thought there might be a difference in measures of range- standard deviation, and interquartile range. This doesn't seem to be the case.



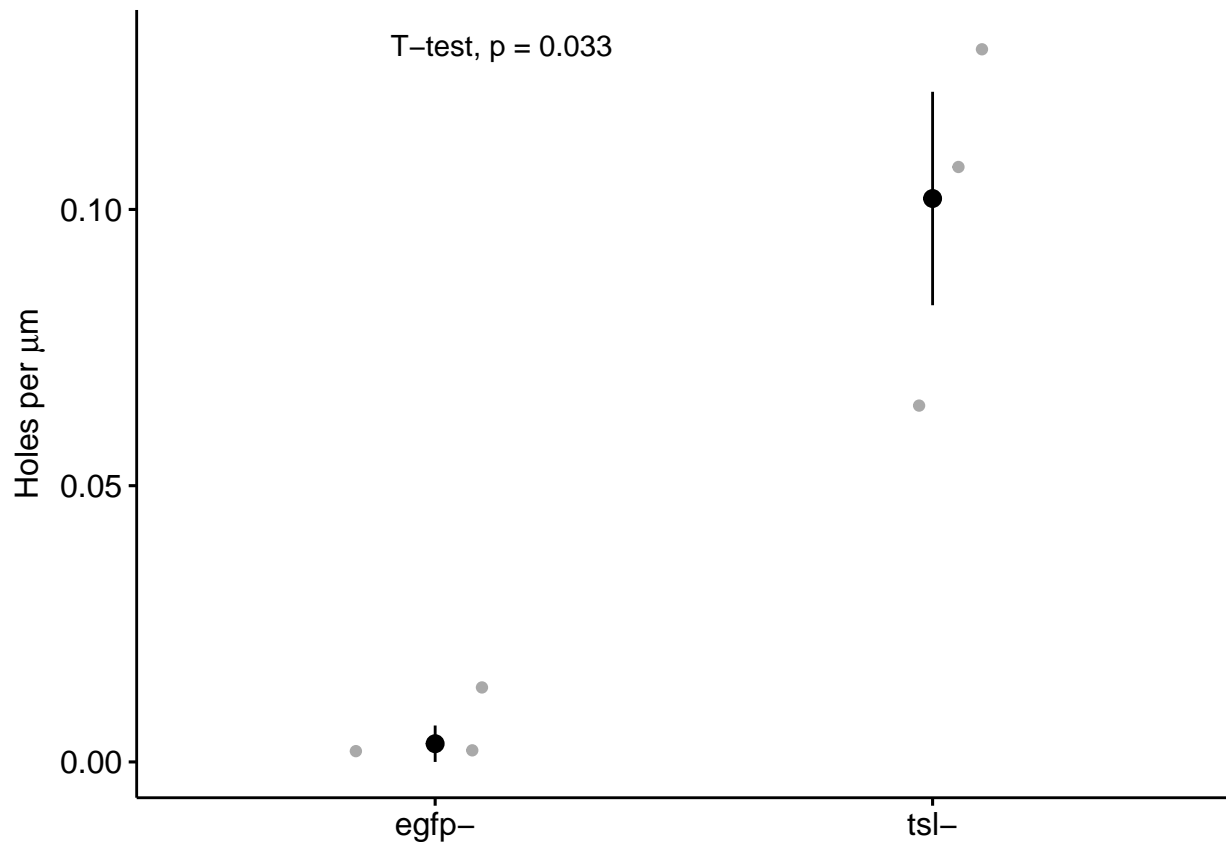
For both the control and *tsl*- wasps, the termini of the oocytes is thicker than the middle. This suggests that excluding the data is valid. BOOM. Overall, the difference is also statistically detectable: $p = 0.0044868$ (student's t-test); effect size is large = 1.2312672 (cohen's test). However, note that for this analysis I treated technical replicates as biological replicates.



Quantifying holes in the vitelline membrane

The *tsl*- wasps also have clear gaps in their vitelline membranes. I counted the number of these per unit length (to correct for different magnifications).

This does come out as “statistically significant”- see plot below. The effect size is large: 4.1116166. The measurement we get out of this is somewhat daft: about 0.1 holes per μm .



In conclusion...

- we can detect a (barely) significant difference in VM width in *tsl*- vs control wasps, when data from the termini are excluded.
- when treating technical replicates as biological replicates (for reasons of sample size), the termini of both treated and control oocytes are thicker than the middles. This validates the decision to exclude these data.
- there is a detectable difference in number of holes per micron between the two treatments.

The termini of the *tsl*- oocytes do look different to the wild types. Therefore I wonder if it's worth looking at those further, as we are investigating the terminal patterning system.