Assessing Functional Efficiency of AtWUS Through Genetic Modifications

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Background in Plant Engineering

Plant transformation through genetic modification has reimagined the way we study the effect of certain genes. However, a major technical challenge facing plant transformation is the development of methods and the difficulty in application, creating a need for increased ease for innovation.[1] Somatic embryogenesis presents itself as an option for major genetic improvement for crops.[6]

The ability to induce somatic embryos with the developmental regulators has revolutionized the way major monocot crops are transformed[3].WUSCHEL (WUS), a developmental regulator, is a complex transcription factors that plays a role in different stages of plant development. Through the alteration of known domains within the WUS protein, the role of WUS goes beyond development and can be utilized for biotechnological implementation.

The genetic architecture of both well-characterized (WUS box, EAR-like, etc.) and less studied domains in WUS proteins are subject for genetic alteration. WUS has been shown as a bifunctional transcription factor that primarily acts as a repressor. By mutating the functional domains (WOX homeobox domain, WUS-box domain, EAR-like domain, acidic domain) of the WUS protein, their role can be determined in the regulation of stem cell identity[2]. The WOX homeobox domain is a helix-loop-helix which is the DNA binding domain that allows for WUS to properly bind its DNA targets. WUS box and EAR-like are domains that allow WUS to function primarily as a repressor. Finally, the acidic region has been shown to be an activation domain that primarily acts during floral patterning. However, if mutated, it can eliminate the activities of WUS including the induction of expression of its downstream targets. Based on the activities of these functional domains WUS can thus be characterized as a repressor or activator. Overexpression of WUS causes uncontrolled cell division that is coordinated through its repressor domains. Ikeda et al. shows the WUS box as an essential feature for cell division functions.

Through the usage of Green Fluorescence Protein tagging, as show through the work of Rodriguez et al., different WUS mutants were tagged and found that certain domains are required to localize WUS in the central zone of the meristem, creating a good mutation target. Through the removal of the dimerization domain or exon 2, the delocalization of WUS produces a variant that can induce more growths and shoots[7]. To test the hypotheses, Fast-TrACC (Fast-Treated Agrobacterium Co-Culture) will be utilized[4] using *A. tumefaciens* culture to deliver a luciferase reporter along with the developmental regulator WUS to promote meristem formation[5].

Figure 1. General Architecture: The genetic architecture of the WUS protein that contains the different domains required for WUS to act as a developmental regulator

Objectives

- Try to enhance the activity/usability of WUS by altering domains and shrinking protein size
- Changing the genetic architecture and how it impacts the ability of AtWUS to promote *de novo* meristem formation
- Gaining information on the optimization of WUS protein for future applications.
- Developing AtWUS variants that can shoot at an increased frequency over the previously utilized ZmWUS2

Citations

1. Altpeter et al. Advancing Crop Transformation in the Era of Genome Editing. *The Plant Cell*, Vol. 28: 1510–1520, July 2016
2. Ikeda, Miho et al. "Arabidopsis WUSCHEL is a bifunctional transcription factor that acts as a repressor in stem cell regulation and as an activator in floral patterning." *The Plant cell* vol. 21,11 (2009): 3493-505. doi:10.1105/tpc.109.069997
3. Lowe, K., Wu, E., Wang, N., Hoerster, G., Hastings, C., Cho, M. J., … Gordon-Kamm, W. (2016). Morphogenic Regulators *Baby boom* and *Wuschel* Improve Monocot Transformation. *The Plant cell*, 28(9), 1998–2015. doi:10.1105/tpc.16.00124
4. Nasti, R. Maher, M. Voytas, D. "Gene editing through *de novo* induction of meristems on dicot seedlings."
5. Nasti, R. Maher, M. Voytas, D. Starker, C. "Supplementary Materials Creating gene edited plants through de novo induction of meristems on seedlings."
6. Osuji,C. Abubakar, S. Mowobi, G. "The place of somatic embryogenesis in crop improvement through genetic transformation" *Journal of Environment and Life Sciences* vol. 1,1 (2016)
7. Rodriguez K, Perales M, Snipes S, Yadav RK, Diaz-Mendoza M, Reddy GV. DNA-dependent homodimerization, sub-cellular partitioning, and protein destabilization control WUSCHEL levels and spatial patterning. *Proc Natl Acad Sci U S A*. 2016;113(41):E6307-E6315. doi:10.1073/pnas.1607673113

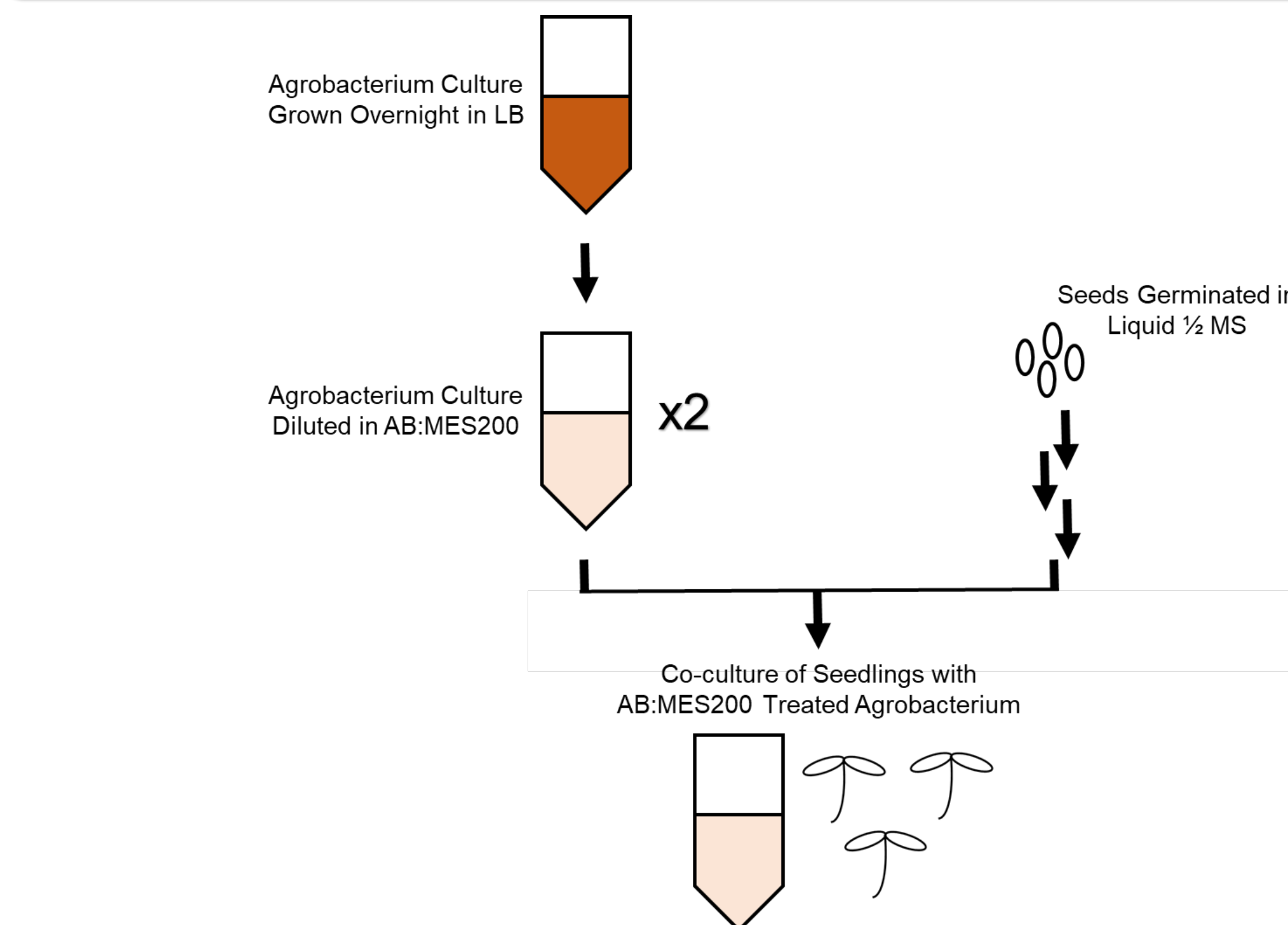## Fast Treated Agrobacterium Co-culture (Fast-TrACC)

Figure 2. Fast-TrACC Delivery. Fast-TrACC delivery involves three days of treatment to an *Agrobacterium tumefaciens* culture of interest and subsequent co-culture with seedlings germinated in liquid. There are two dilutions in AB:MES200 media after periods of overnight growth. After the second dilution the Agrobacterium culture is added to the germinated seedlings so that the liquid media composition in 50% AB:MES/50% ½ MS (v/v). The AB:MES200 media in the treatment plan is composed of salts and chemicals that increase vir gene expression and promotes increased transfer of the desired T-DNA cargo. This transfer occurs specifically between the tissues that are co-cultured with the Agrobacterium.

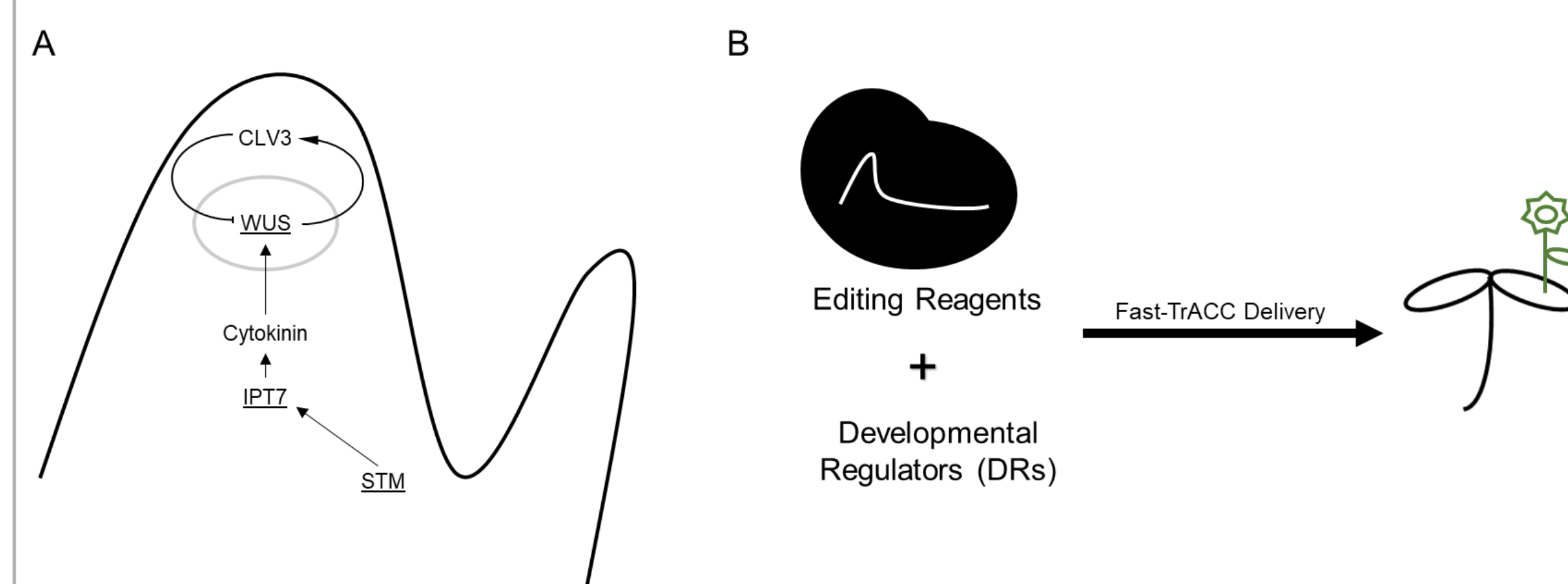## Using Developmental Regulators to Induce *de novo* meristems

Figure 3. Application of Developmental Regulators (DRs) for Meristem Induction. There are key transcription factors known to pattern plant development. These developmental regulators (DRs) have important roles in meristem maintenance and stem cell proliferation (a). Combinations of these DRs, such as WUSCHEL (WUS) and SHOOT MERISTEMLESS (STM), have been implicated in the patterning and formation of *de novo* shoot meristems when ectopically overexpressed. Co-opting these types of candidate DRs (a, underlined) and adding gene editing reagents there is the potential to generate meristems with an edit of interest. Using Fast-TrACC combinations of DRs were delivered to determine their efficacy to generate gene edited *de novo* meristems (b).

Comparing ZmWUS2 variant vs. AtWUS

Figure 4. ZmWUS2 Variant: The genetic architecture of WUS maize variant, which contains the different domains required to act as a developmental regulator. ZmWUS2 acts as the control group.
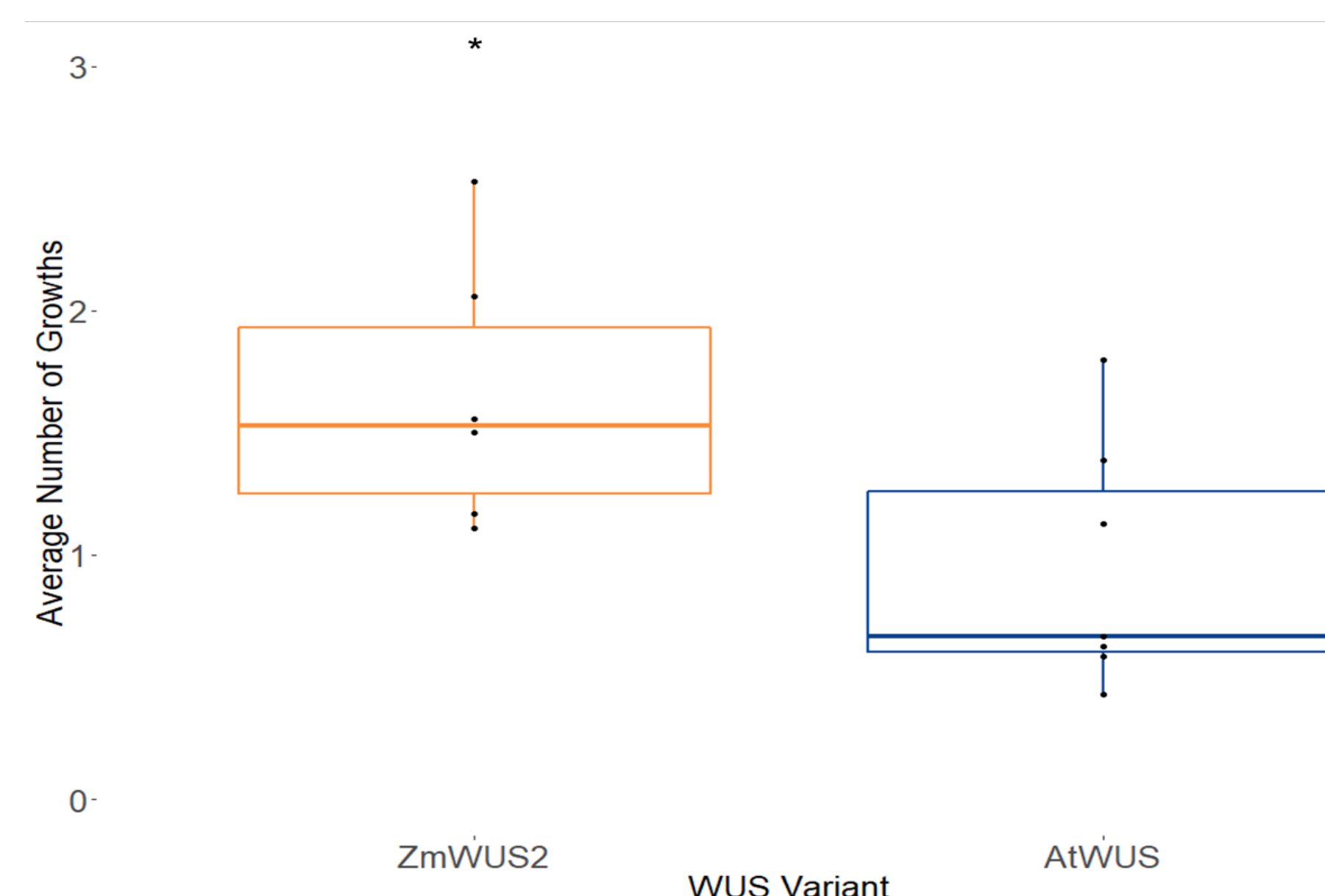

Figure 5. Comparing ZmWUS2 to AtWUS. WUS is a complex transcription factor that plays different roles in development. By examining the different variants that play a role in maintaining homeostasis, the different restraints can be seen when producing ectopic growths. Through the comparison of ZmWUS2 variants with AtWUS, it can be seen that ZmWUS2 has a significantly higher growth count than the latter, producing a more optimal variant.

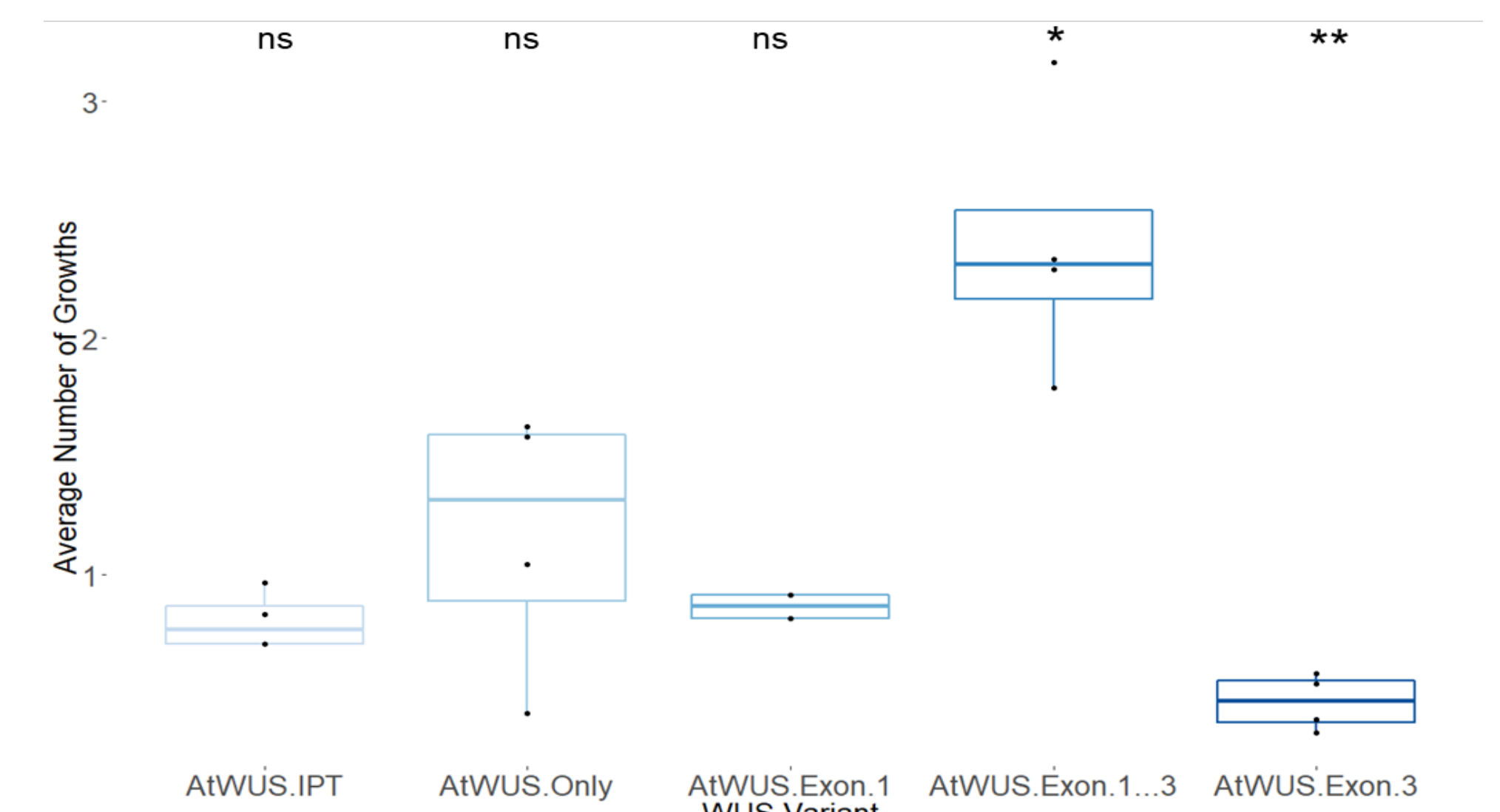## Removing the Dimerization Domain of AtWUS

Figure 6. AtWUS Domains. Looking to test different domains in a single WUS variant, deletion variants of the AtWUS were made to isolate specific domains of the WUS protein and determine their effect on growth formation. When delivering full length AtWUS with or without IPT there is a low level of growth initiation formed. The same trend occurs with just the DNA binding domain (Exon 1), presumably due to its binding functioning as a basal repressor. By delivering the corepressor binding domains (Exon 3), WUS-Box and EAR-Like, there is essentially no growth formation likely due to no binding to the target sequences. When the homodimerization domain is deleted (No Exon 2), there is a significant increase in growth formation presumably due to great propensity to move between cells.
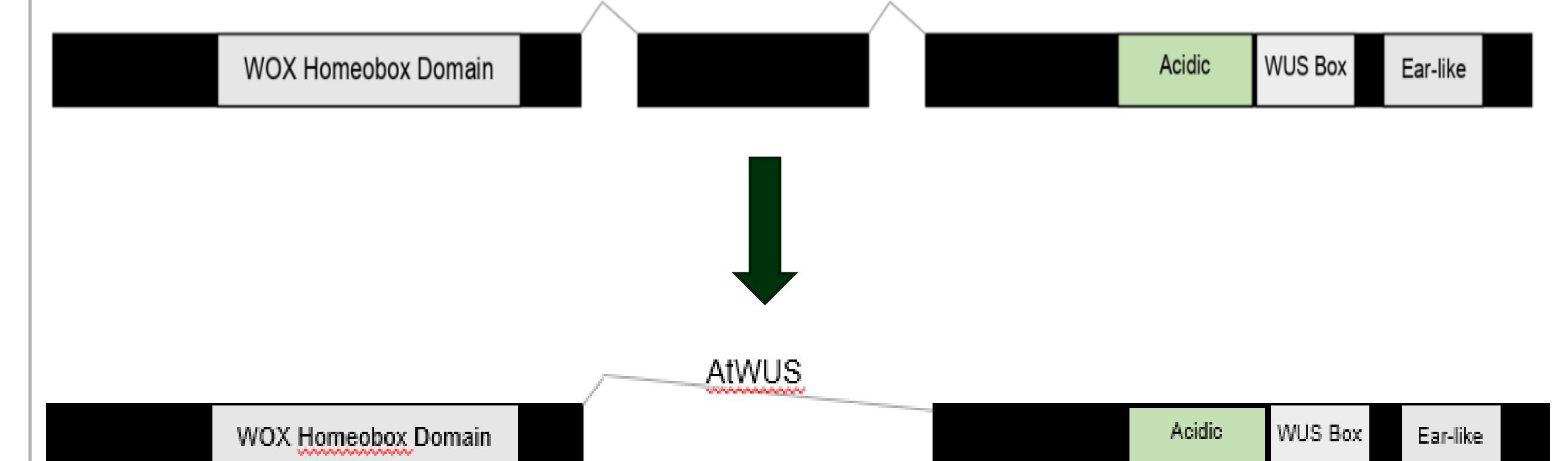

Figure 7. AtWUS Variant: The genetic architecture of Arabidopsis thaliana WUS variant, which contains the different domains required to act as a developmental regulator is shown in the top portion of the figure. However, after removal of different dimerization domains in a single WUS variant, the heterodimerization domain which is located in exon 2 showed to produce a significant amount of growths due to the ability to move between cells. In the bottom half of the figure, a revised genetic scheme is shown for a hypothetically more optimal AtWUS Variant.
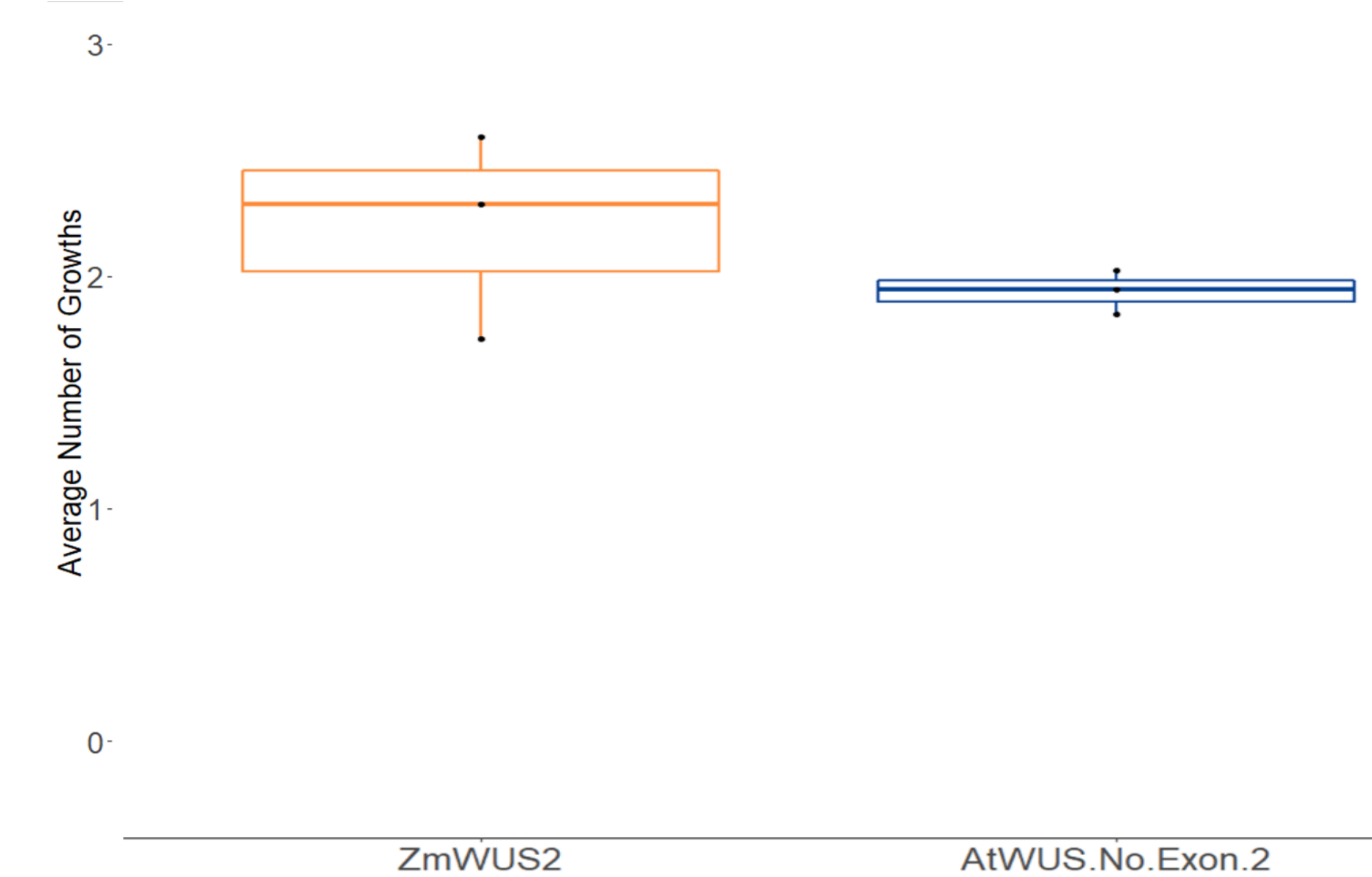
AtWUS Optimization

Figure 8. Results of AtWUS without Exon 2. Through a cross-comparison analysis of ZmWUS2 with AtWUS no exon 2, ectopic growth counts show that when the dimerization domain is missing the activity of the variant is not significantly different than the ZmWUS2 variant. This essentially rescues the function from the full length AtWUS which is significantly worse at inducing growths than the ZmWUS2 variant.

Future Applications

- Determine if different WUS variants induce shooting similarly to how they induce *de novo* growth formation
- Build and test ZmWUS2 with no dimerization domain and test functionality purposes
- Test EAR-like variants to see if any promote growth formations and shooting at a greater frequency
- If improvements are observed with both dimerization and EAR-like mutants combine the two to see if further improvements can be made.

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