

# Olfactory receptor coding sequences cause silencing of episomal constructs in the immortalized OP6 cell line

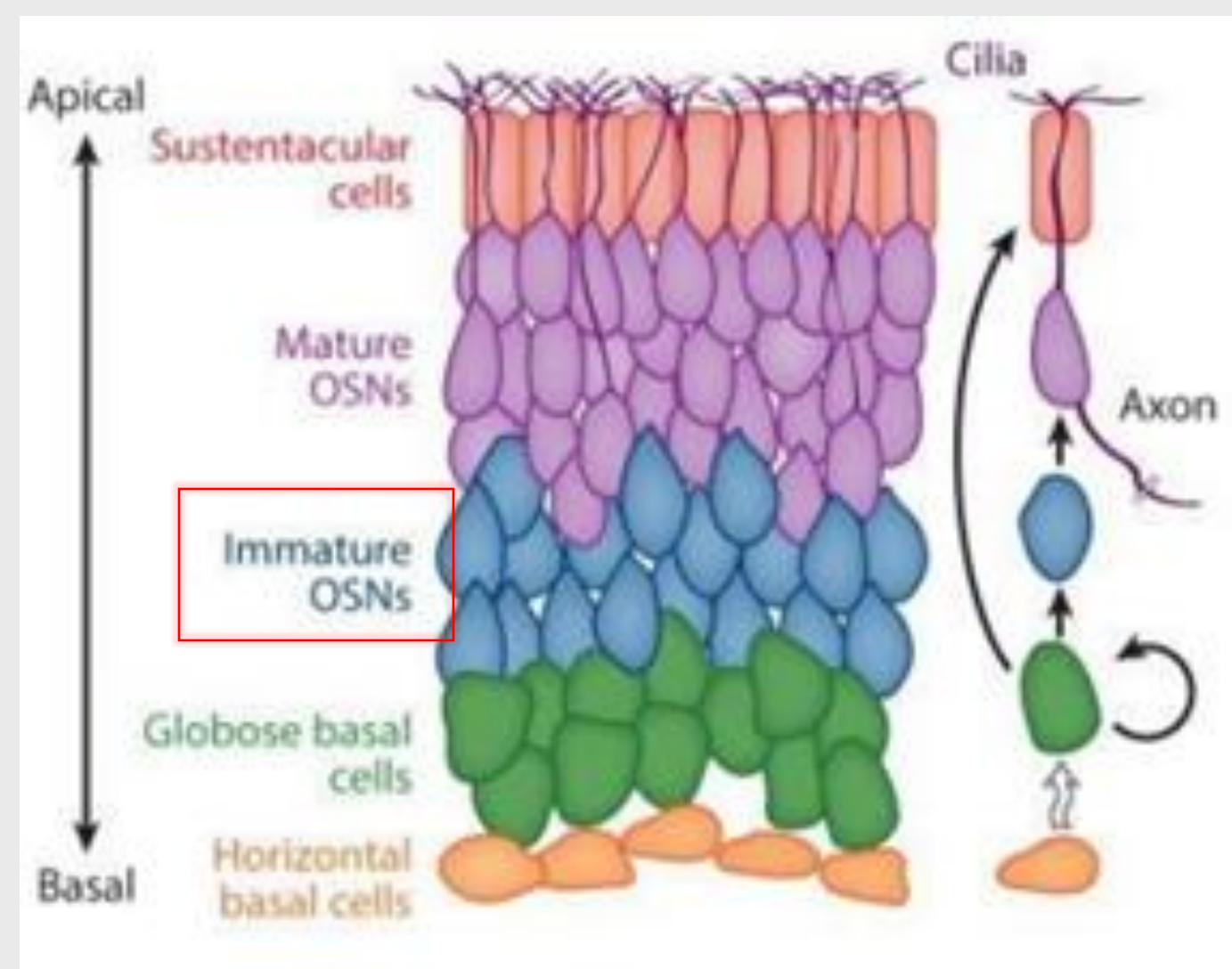
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## 1. Introduction

- An investigation of gene regulation in the mammalian olfactory system
- Each Olfactory Sensory Neuron (OSN) exclusively expresses just one of >1,500 OR genes encoded in the genome (silencing the other ORs)
- Why/How are OR genes mostly repressed?**

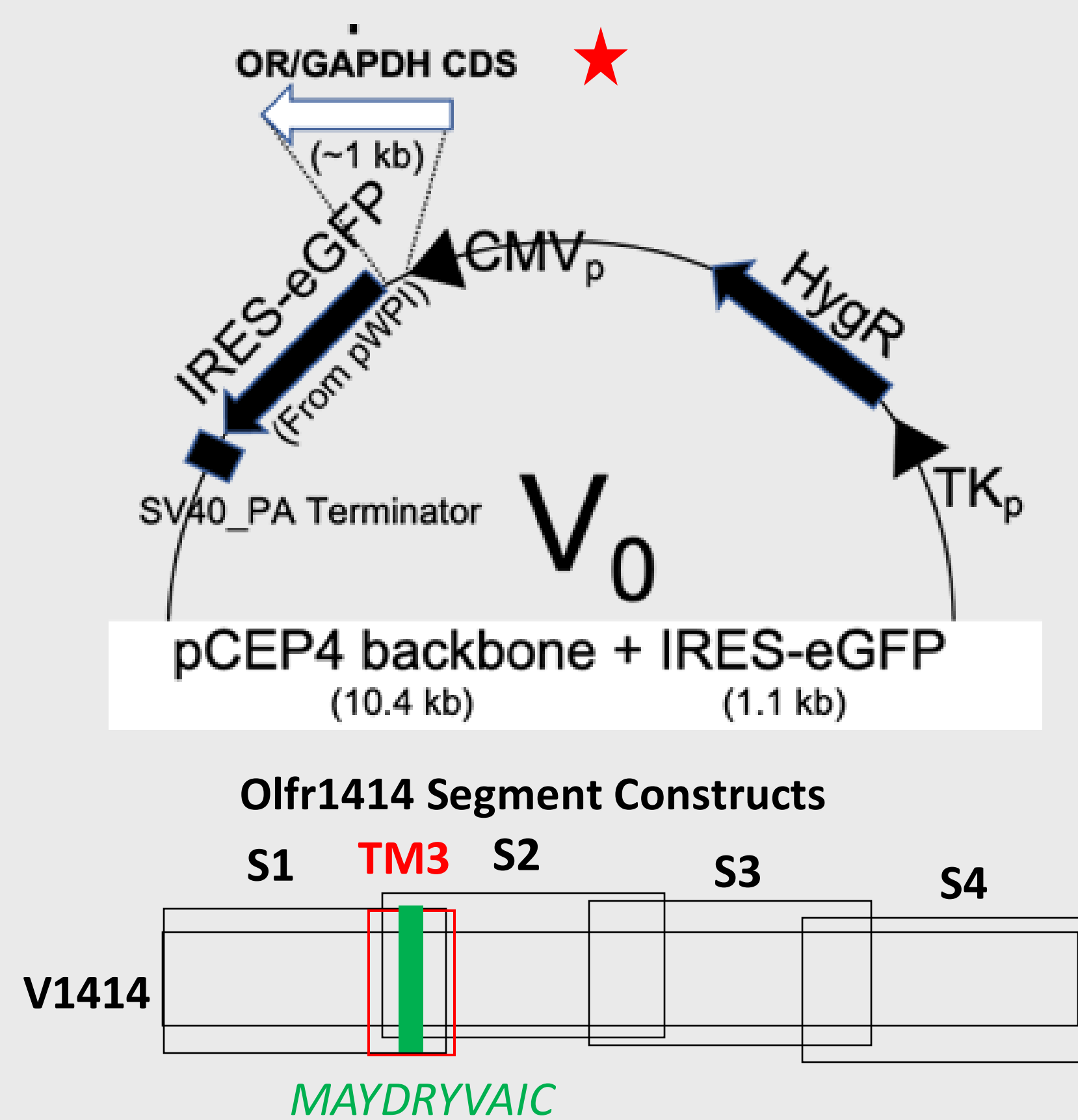
## 2. Methods

### A. OP6 Cell Line



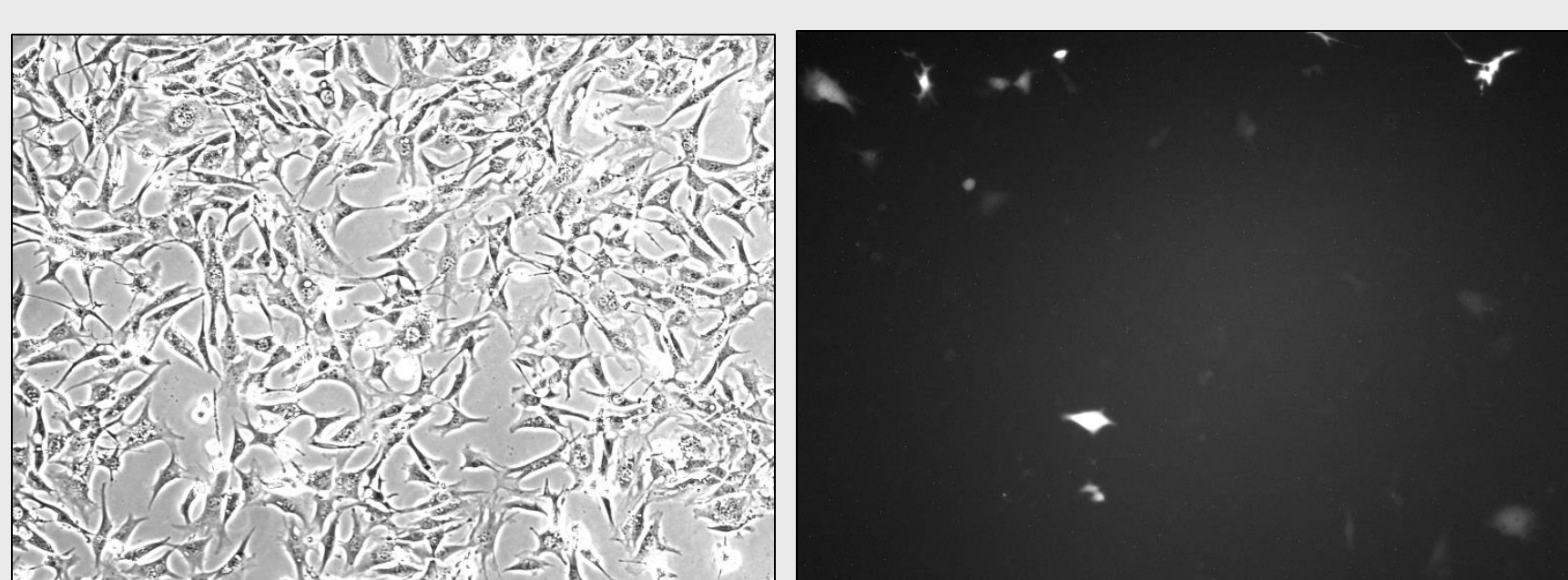
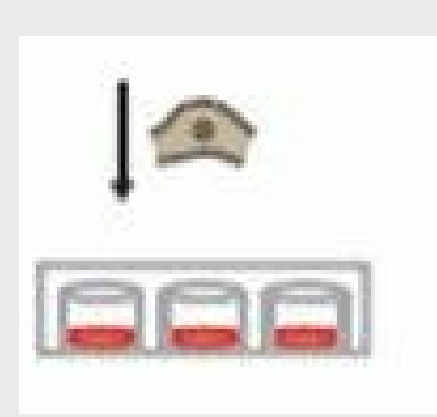
- Immature OSNs in olfactory placode
- Immortalized immature neuron
- Enables study of early gene expression profiles
- Expresses one OR per cell**

### B. Constructs



### C. Transfections

Seed OP6 cells in 6-well plate and **transfect** with construct



Example brightfield and fluorescence image

## 3. Results

### A. GFP mRNA is repressed by Olfr1414

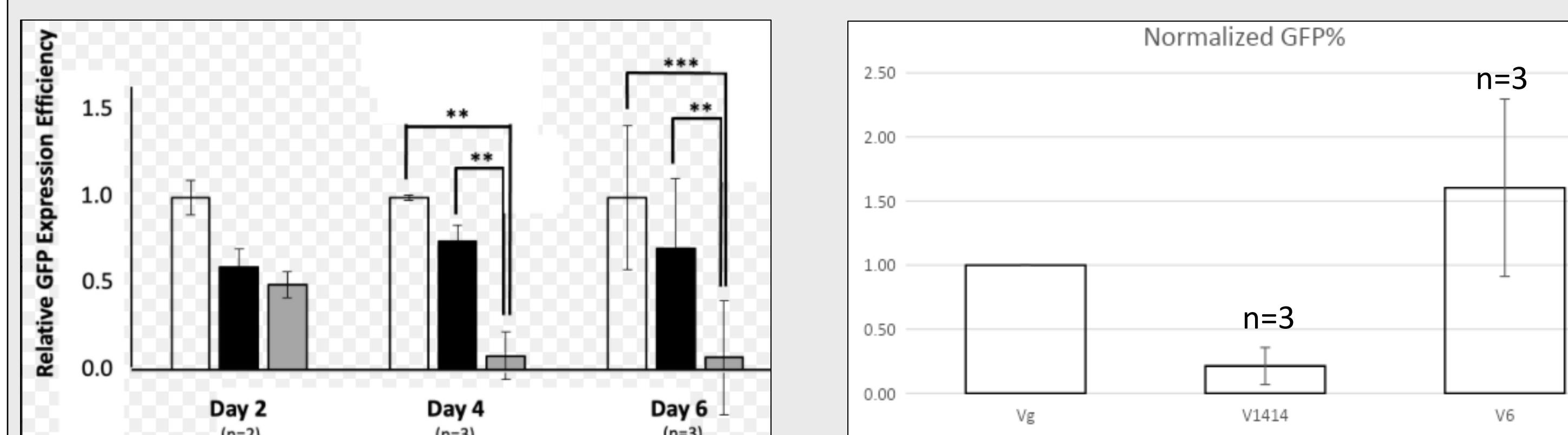


Figure 1. Relative Expression Efficiency of GFP constructs (V0 open, VG black, V1414 gray)

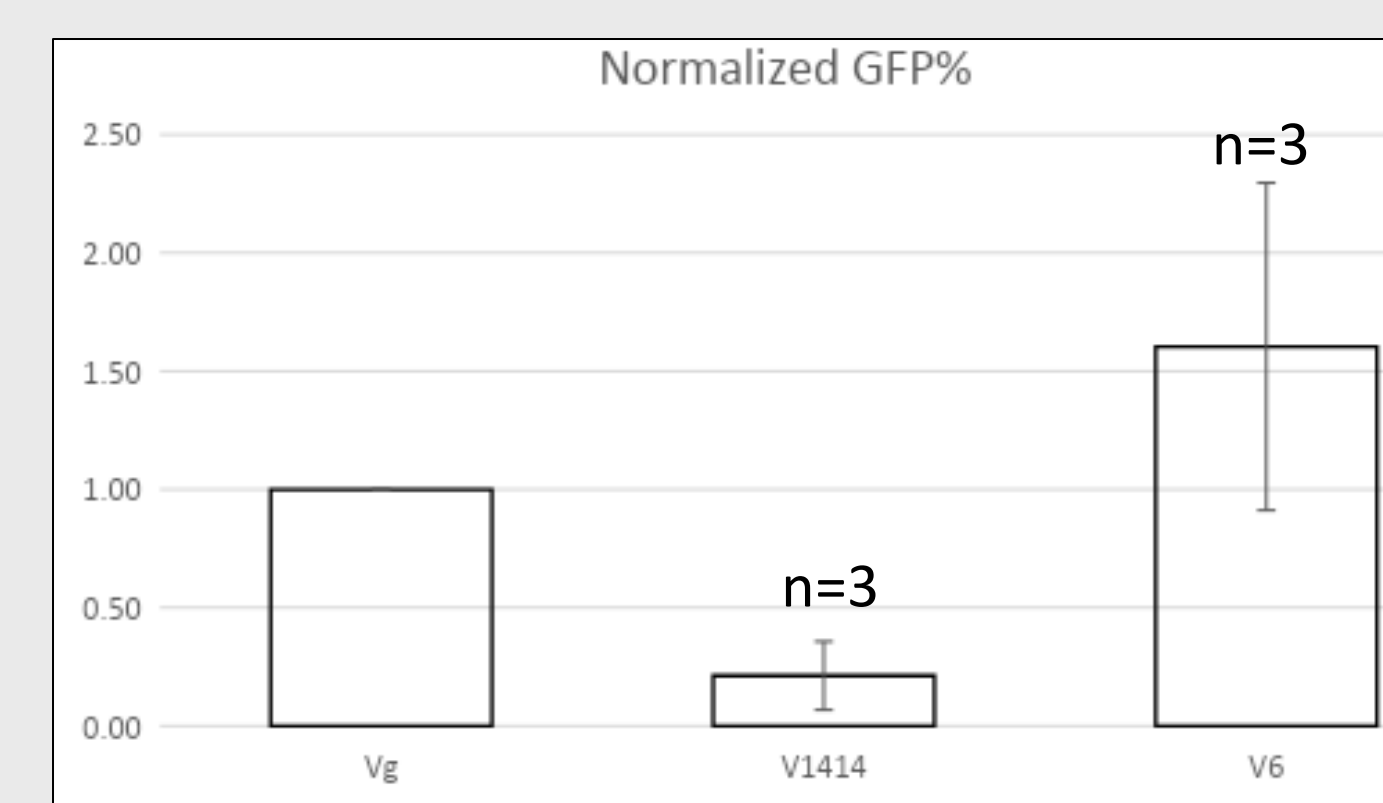


Figure 2. Normalized GFP Expression of GFP constructs (VG, V1414, V6)

### B. GFP expression is generally repressed by ORs

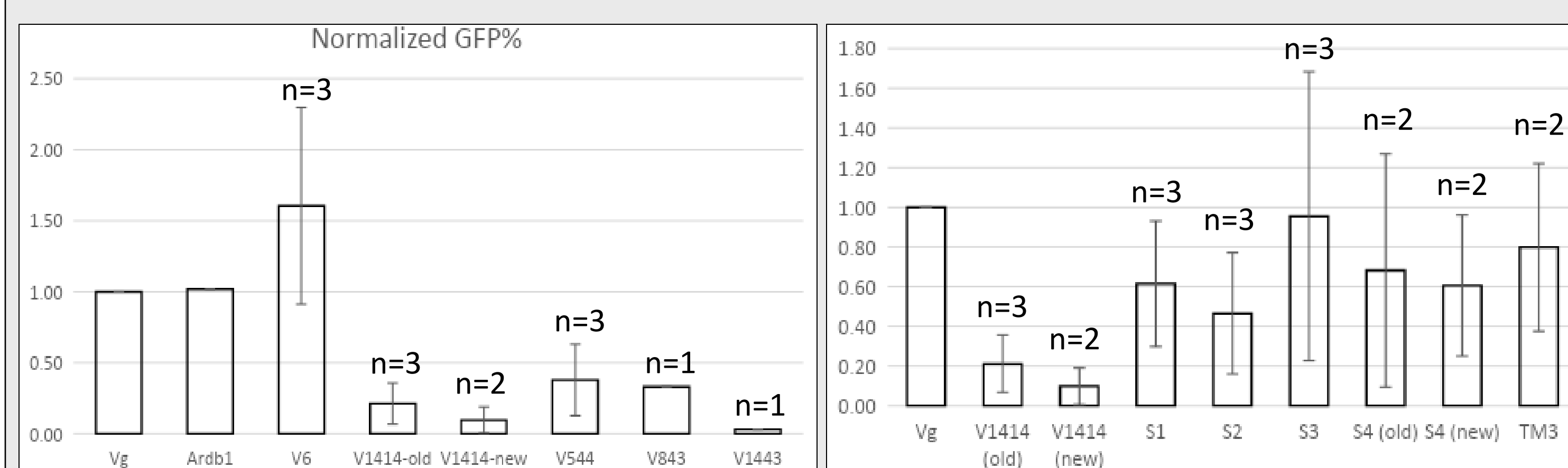


Figure 3. Normalized GFP Expression of ORs vs. Controls

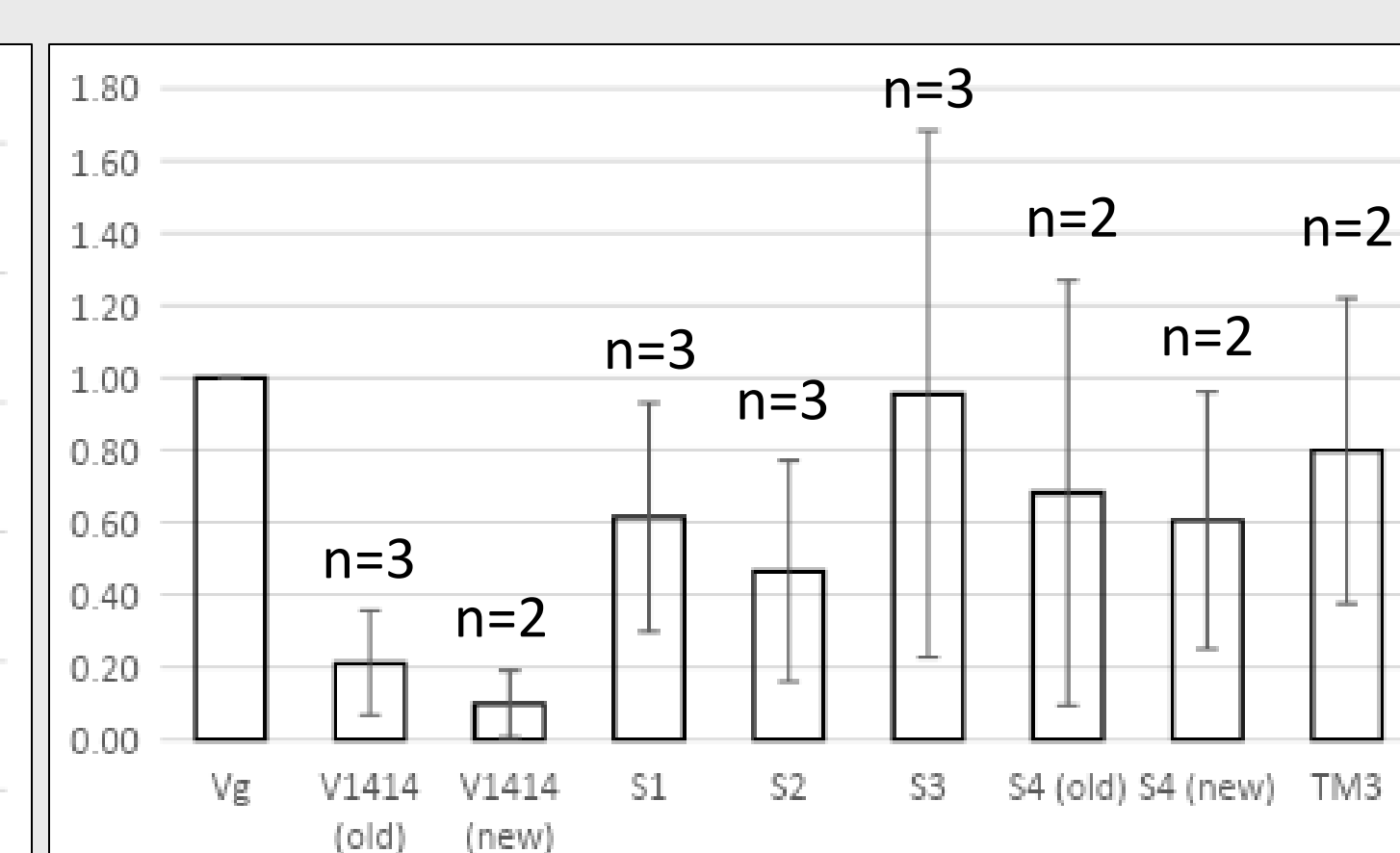


Figure 4. Normalized GFP Expression in segments of V1414

### C. Olfr1414 also represses nearby HygR gene

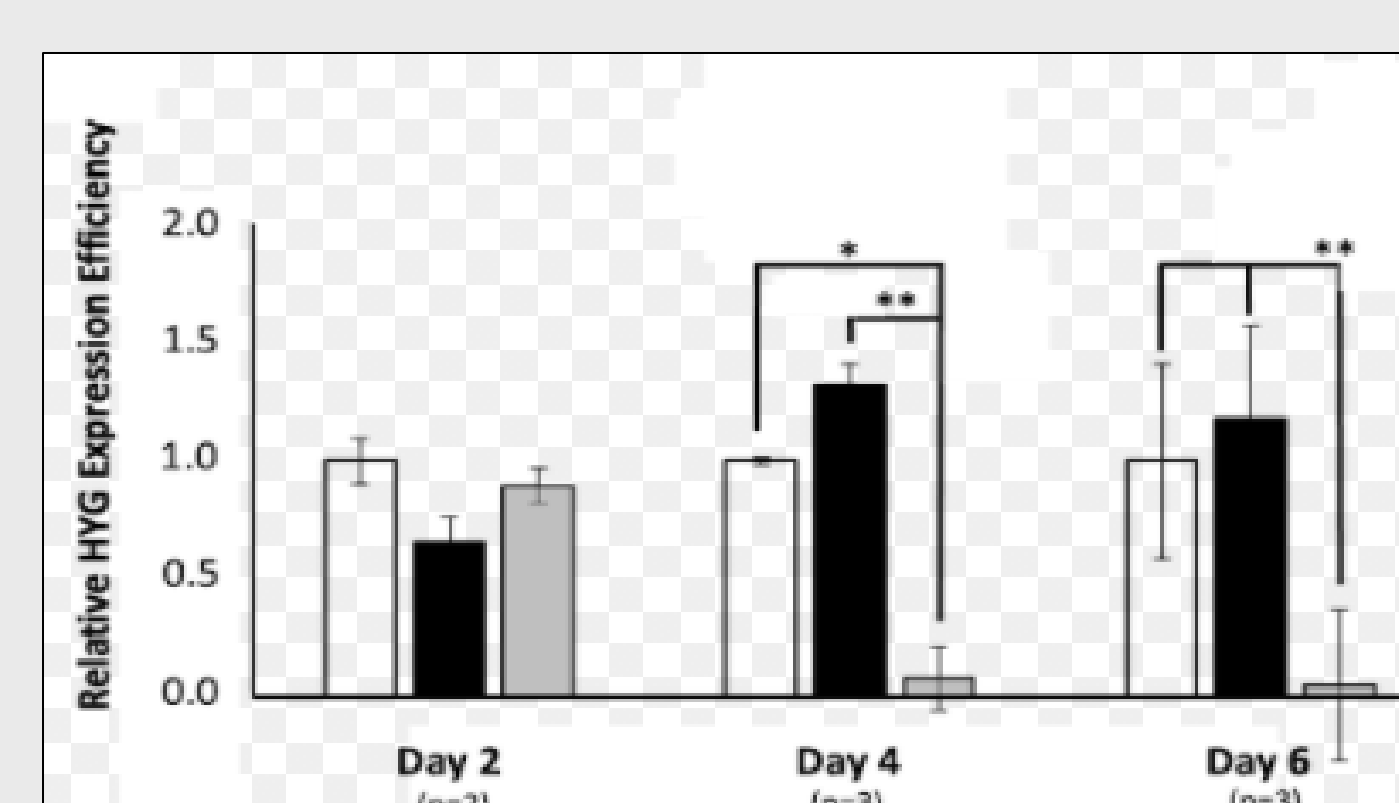


Figure 5. Relative Hyg Expression Efficiency of GFP constructs (V0 open, VG black, V1414 gray)

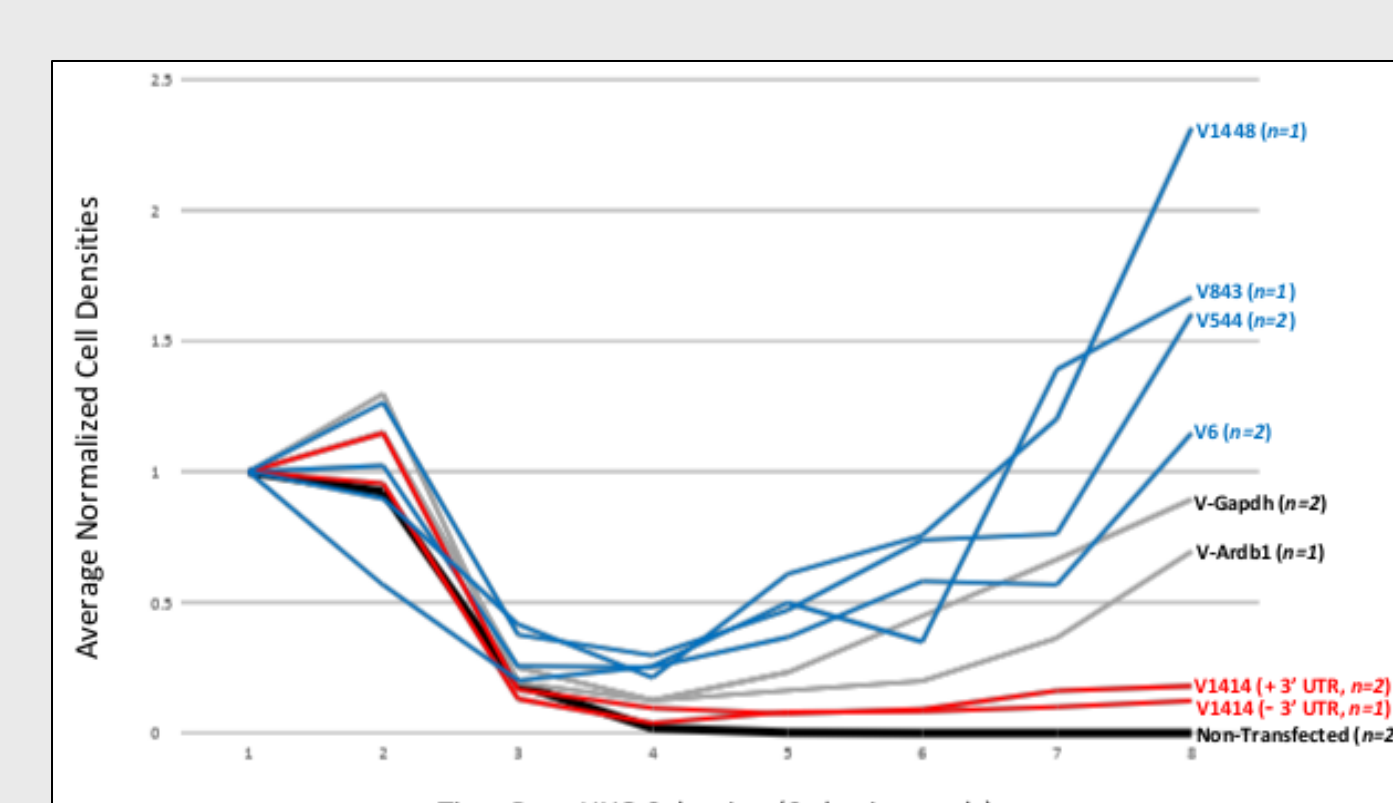


Figure 6. Average Normalized Cell Densities using Hygromycin Selection of Various Constructs

## 4. Conclusions

- All but one OR gene was silenced suggesting self-induced suppression, either due to transcriptional repression or mRNA instability
- Olfr6 is exceptional among tested ORs (robust mRNA levels). This OR is advantaged in vivo (frequently selected). Is there a correlation between self-suppression and selection probability?
- No single Olfr1414 subsequence is sufficient for observed level of silencing, suggesting a distributed property. Multiple elements? mRNA structure?
- Olfr1414 also extends silencing effect onto nearby HygR gene/promoter. This suggests a transcriptional/chromatin effect.

## 5. Future Studies

- Need additional samples to improve statistical rigor: for example, are Olfr1414 segments partially or fully rescued?
- Need additional ORs to test whether self-suppression is inversely correlated with in vivo selection probability
- Is the 5' end of Olfr1414 (including TM3/MAYDRYVAIC) particularly important for silencing? (D/M constructs)
- Is OR mRNA structure important for silencing: addition of introns?
- What is the mechanism of silencing? Histone ChIP/DNA methylation assays, trans factors? mRNA half-life studies?

## Acknowledgements

I would like to thank Spencer Tang for his data and constructs, Ghazia Abbas for her assistance and training, and Robert Lane for his guidance, support, and teachings