Controlling Cone Cell Subtype Ratios (Red vs. Green. vs. Blue Opsin Expression), by Mutating Cis-Regulators of the *Thrb* Gene

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June 14th, 2023

NOAA-CESSRST NSF-REU, & CUNY HIRES

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TRβ2's Mechanism of Action

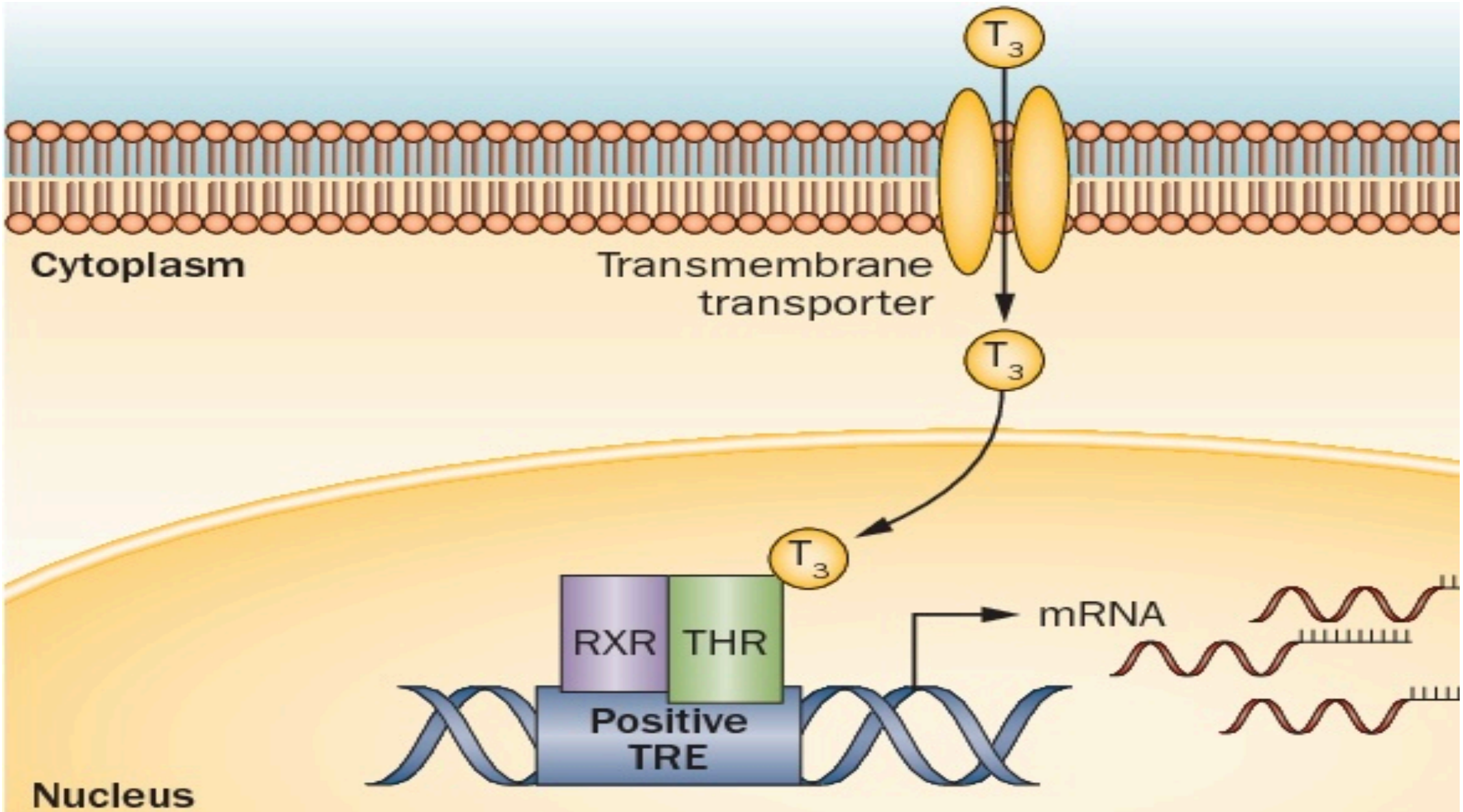

Image Source: Ortiga-Carvalho et. al., 2014

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Knockout of *Thrb* Modifies Cone Subtype Ratios

Thrb deletion in mice, causes a loss of **green cones**, and all cones to be **blue cones** (Ng et. al, 2001)

Mutations in *Thrb* in zebrafish transfate retinal progenitor cells destined to become **red cones** to becomes **ultra-violet cones** and **horizontal cells** (Leo et al., 2020)

In human-derived organoids, knockout of *Thrb* prevents **red** and **green** cone genesis (Eldred et al., 2018)

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ThrbCRM1, ThrbCRM2, ThrbICR cis-Regulate *Thrb* Expression

3 enhancers, are known to control the expression of *Thrb*:

1. Thyroid hormone receptor cis-regulatory module 1 (ThrbCRM1)
2. Thyroid hormone receptor cis-regulatory module 2 (ThrbCRM2)
3. Thyroid horbome receptor beta intron control region (ThrbICR)

Mutating functional cisDNA regions may allow for us to "dial" *Thrb* transcription up or down, as opposed to completely knocking it out >>> thus modifying cone subtype (red, green, blue) ratios

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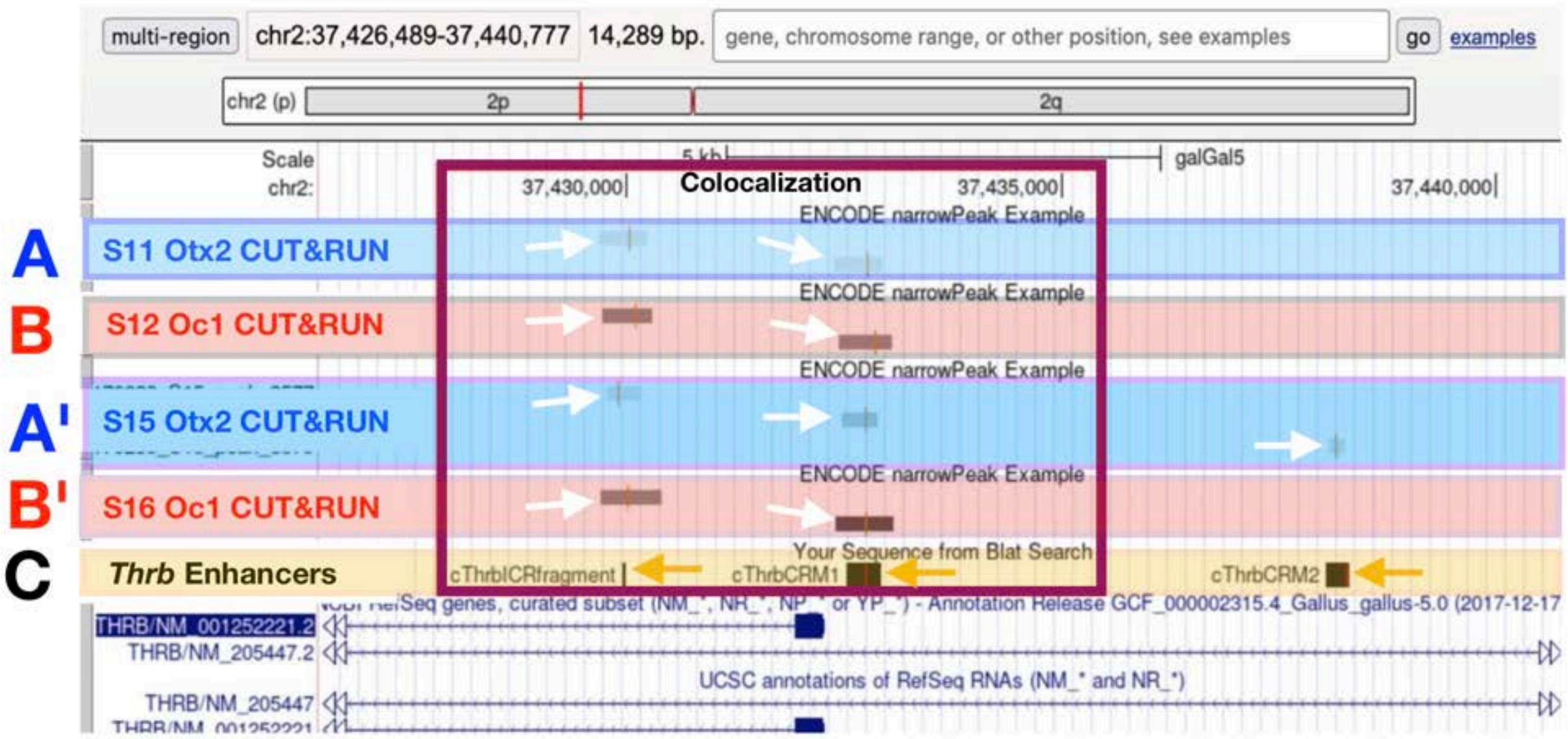
CUT&RUN Data Support Otx2 and Oc1 Bind ThrbCRM1 and ThrbICR Enhancers



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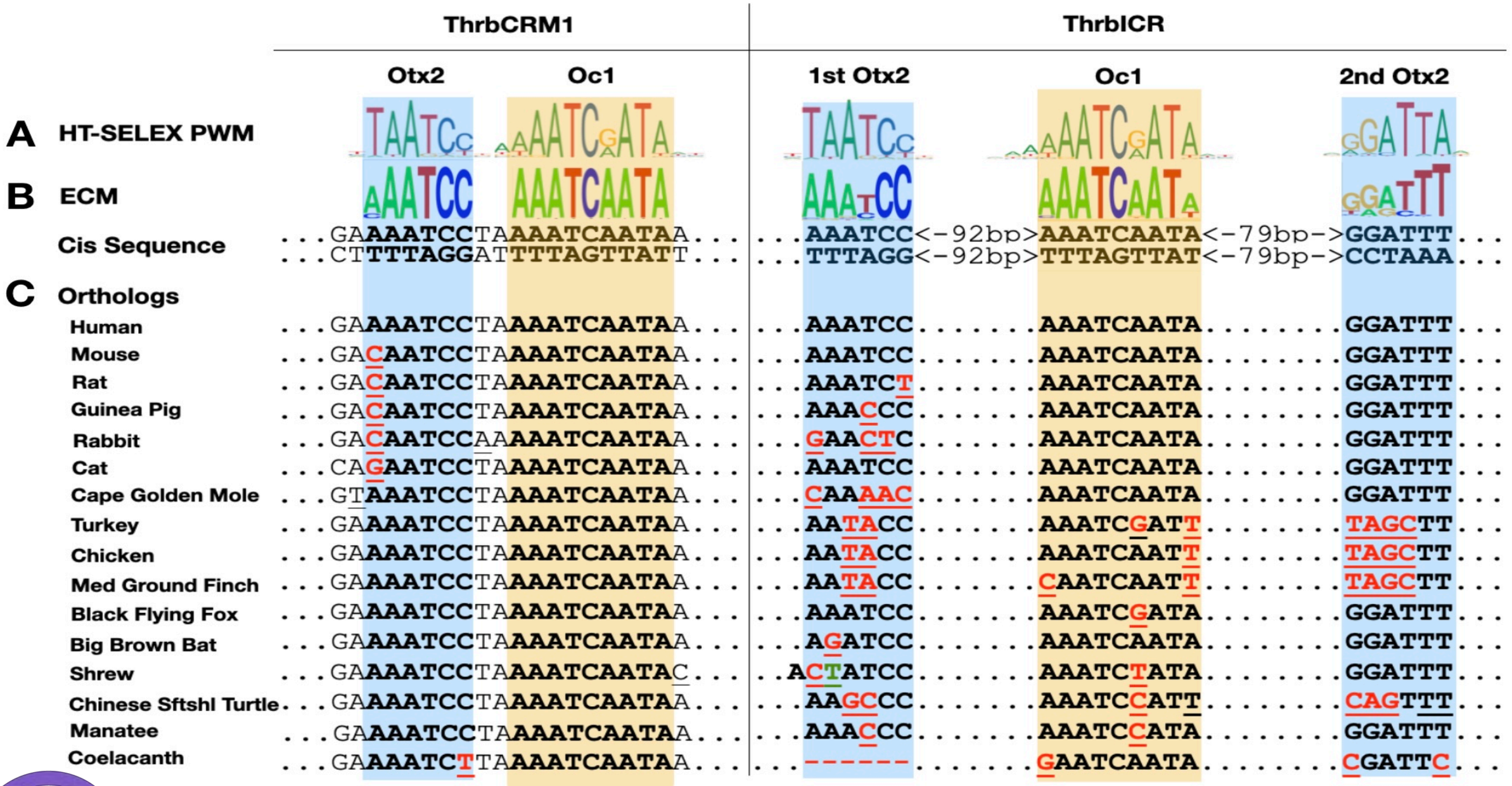
Otx2 and Oc1 Bind Different Sequences *In Vitro* Than They Do in Organism Genomes



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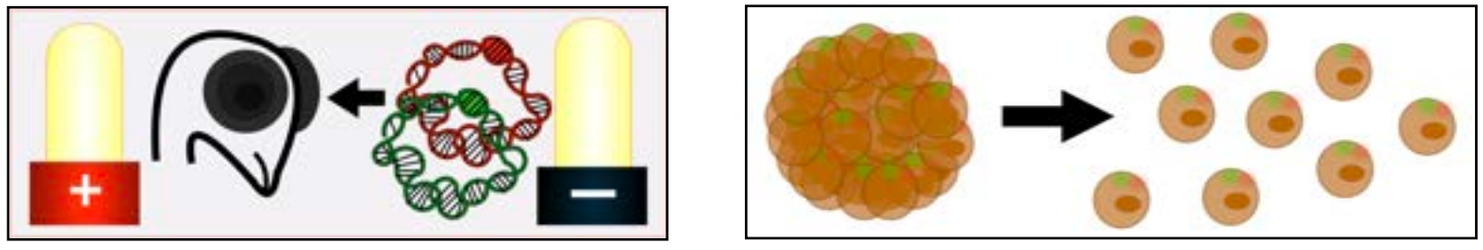
Molecular Methods

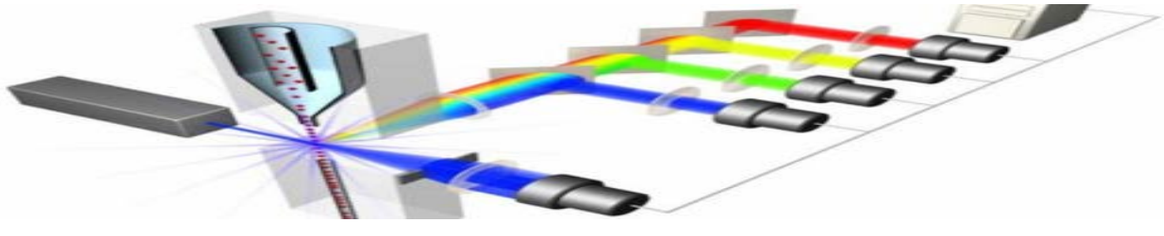
ThrbCRM1 or ThrbICR enhancers were ligated into reporter vectors upstream of GFP or tdT reporter genes

Single base substitutions were made in the Otx2 or Oc1 binding sites within the ThrbCRM1 or ThrbICR enhancers using PCR mutagenesis

Plasmid vectors were coelectroporated into developing chick retinae, cultured, and dissociated using a papain-based protocol



Cells were processed through a flow cytometer to quantitate how altering the Otx2 and Oc1 binding sites affects GFP signal



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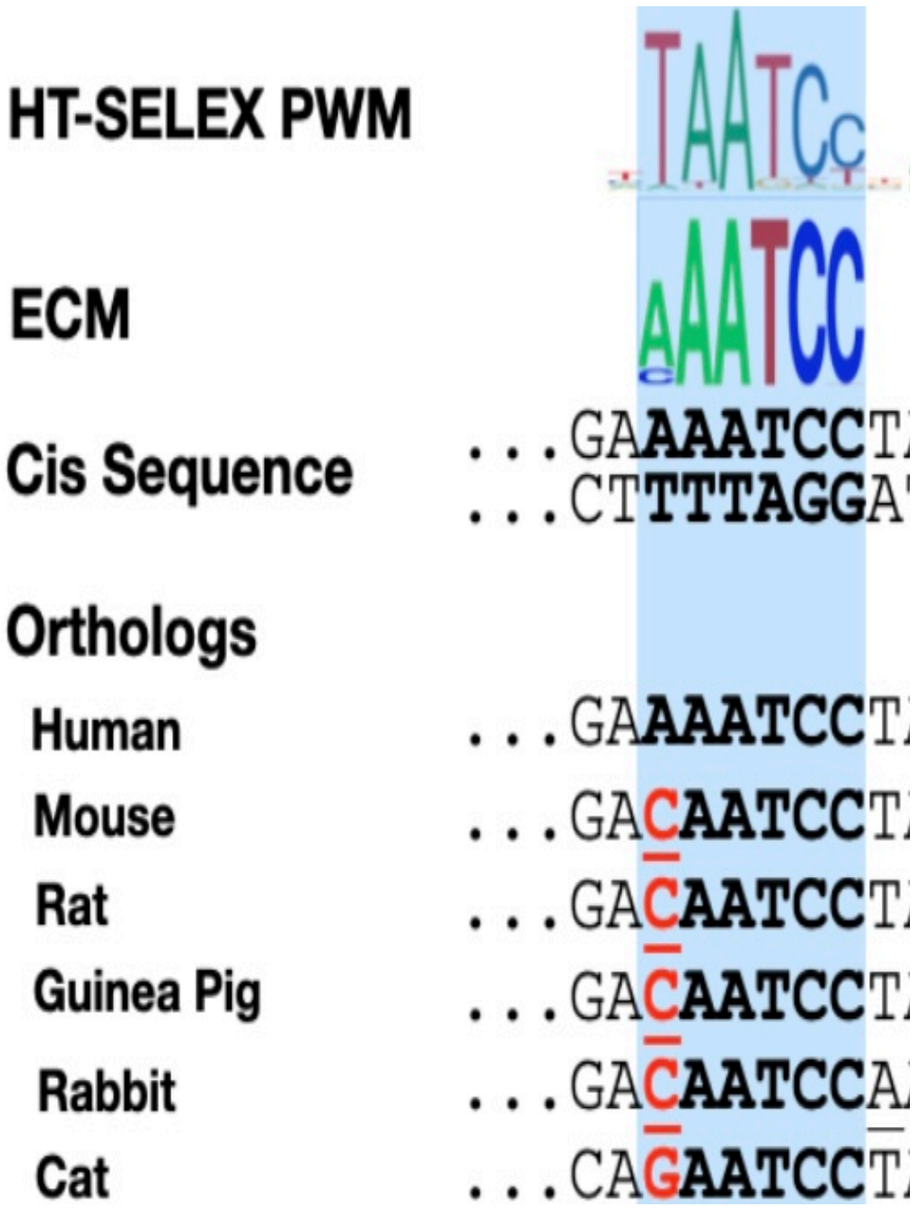
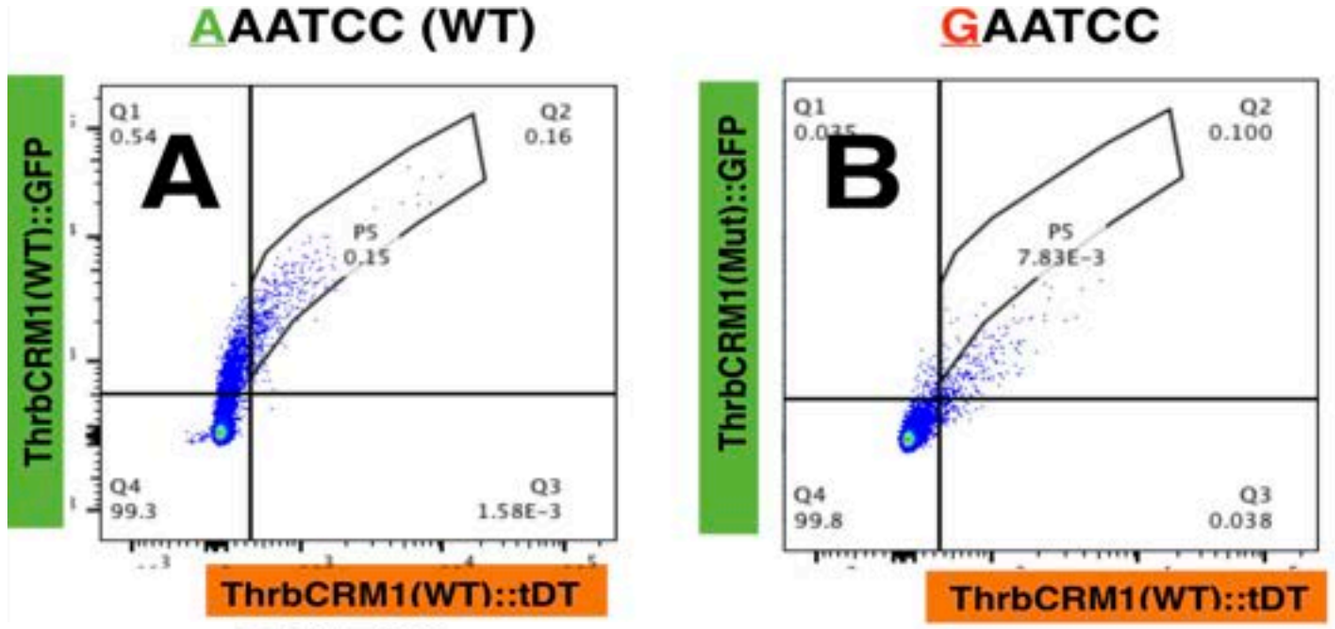
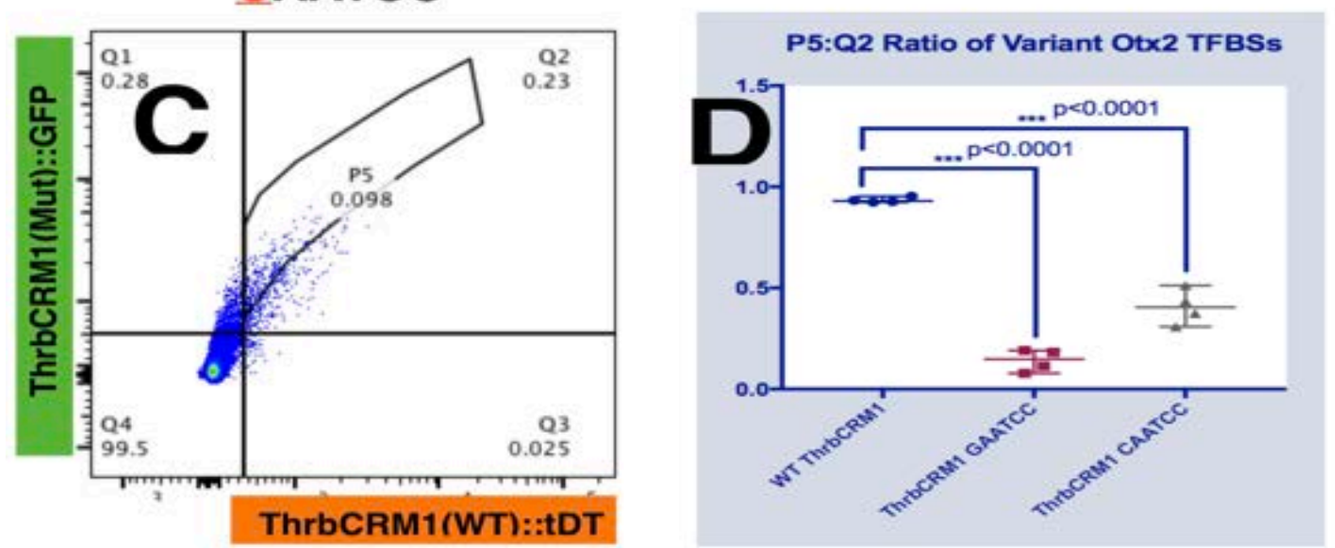
Rodent (CAATCC) and Feline (GAATCC) Otx2 Site Variants Alter ThrbCRM1 Activity







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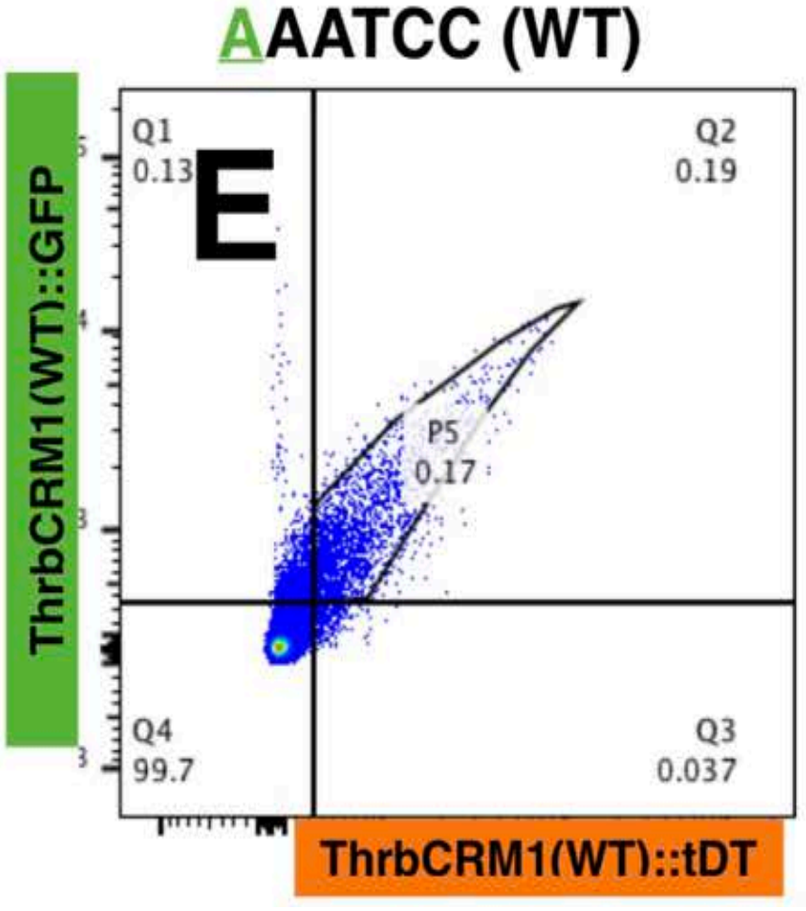
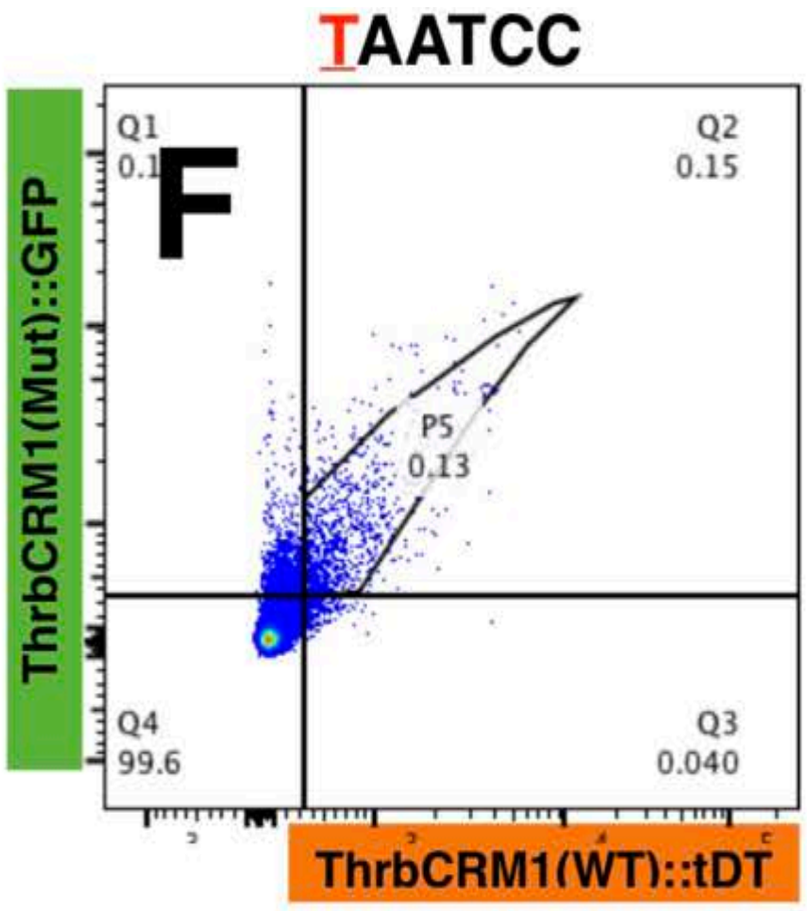
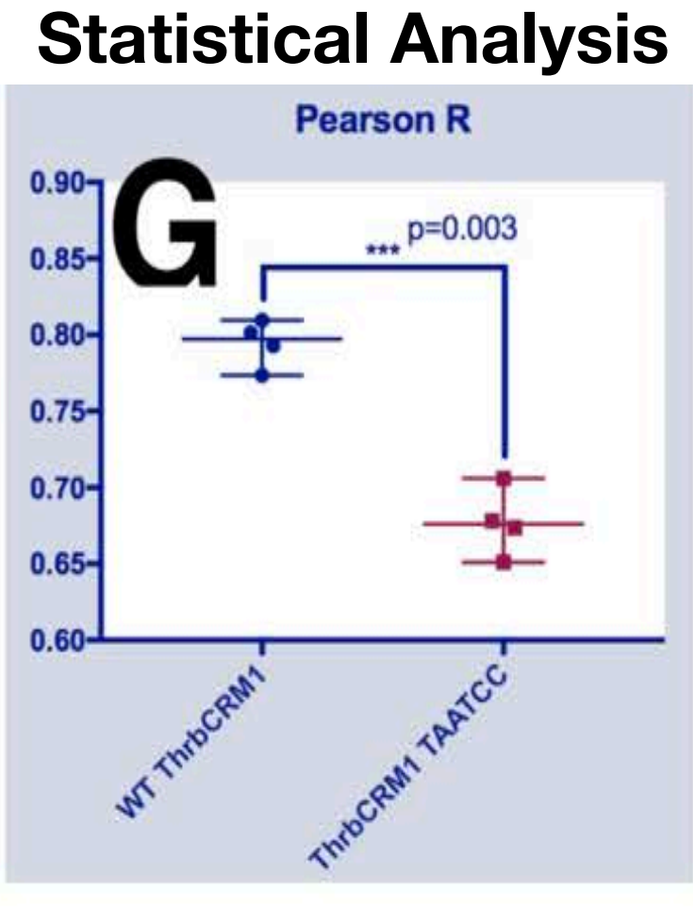
Optimal TAATCC Otx2 Site Drives A More Variant Pattern of ThrbCRM1 Activity







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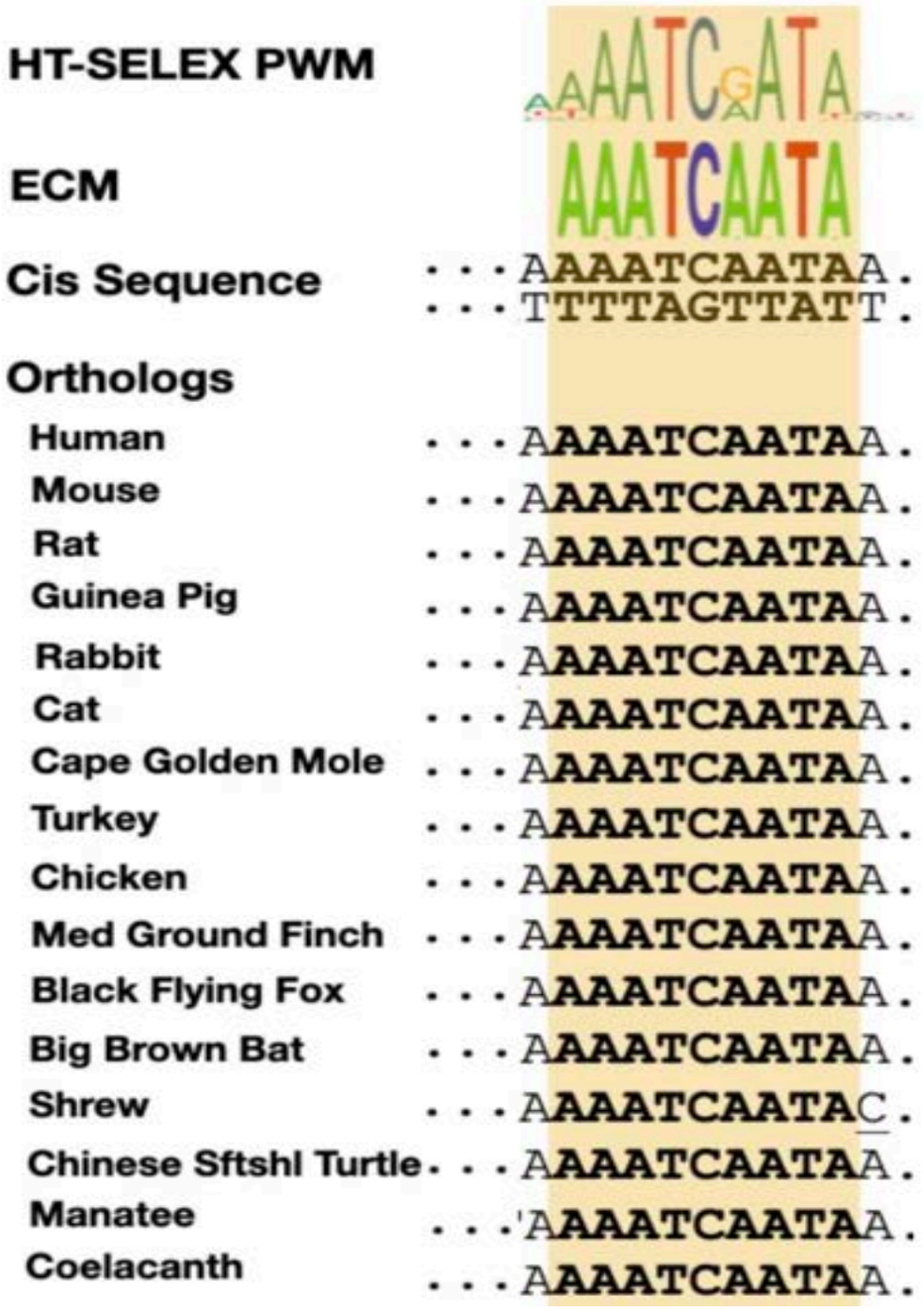
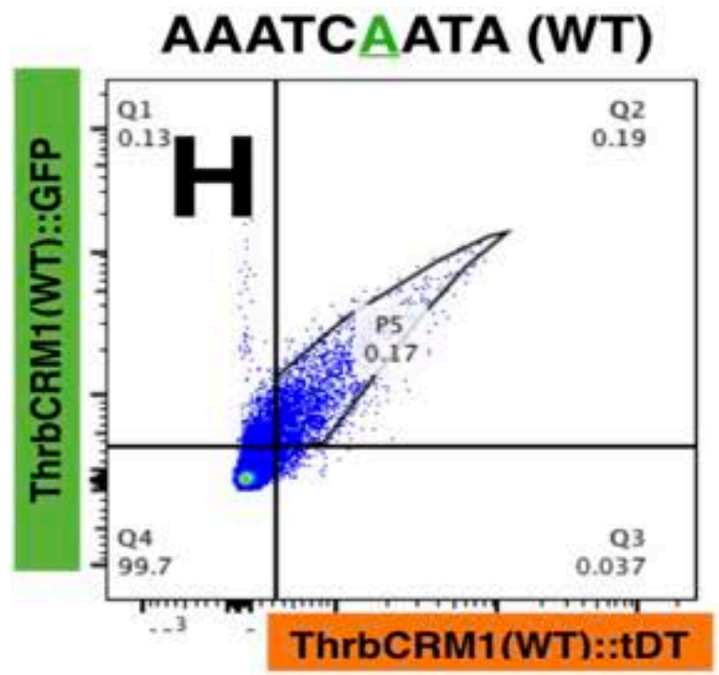
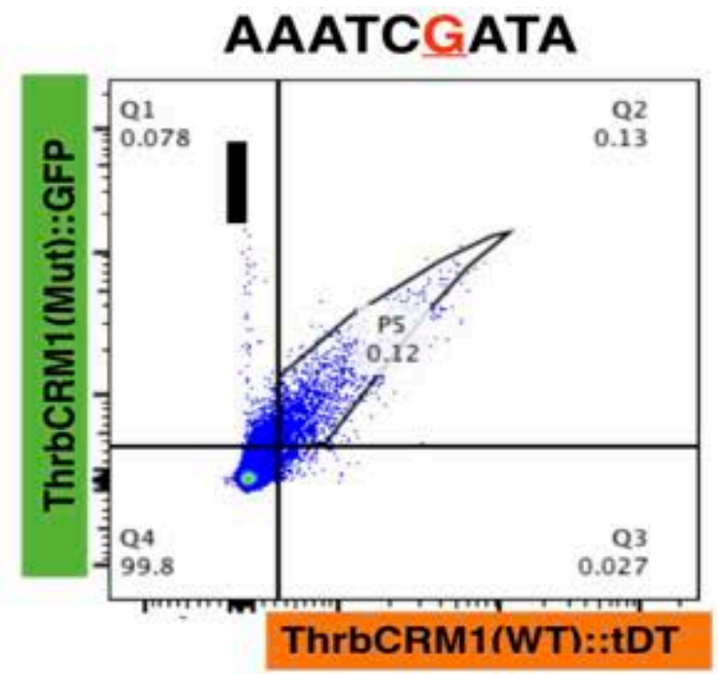
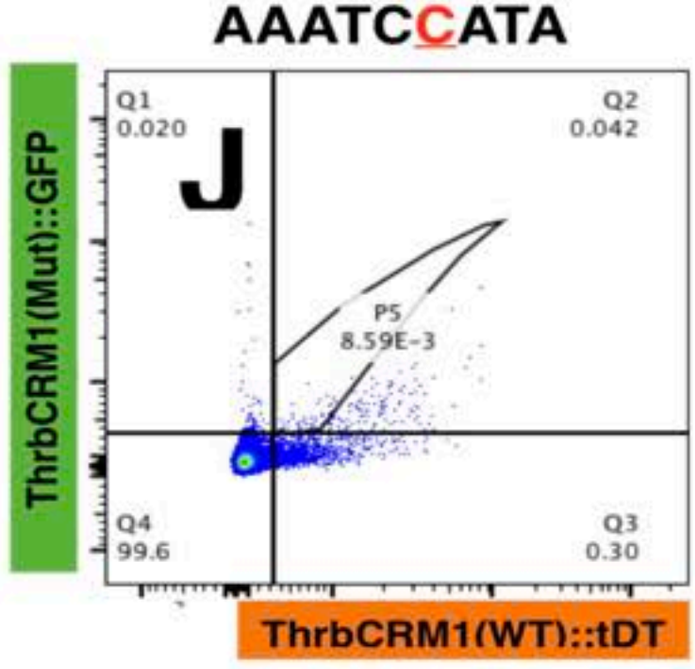
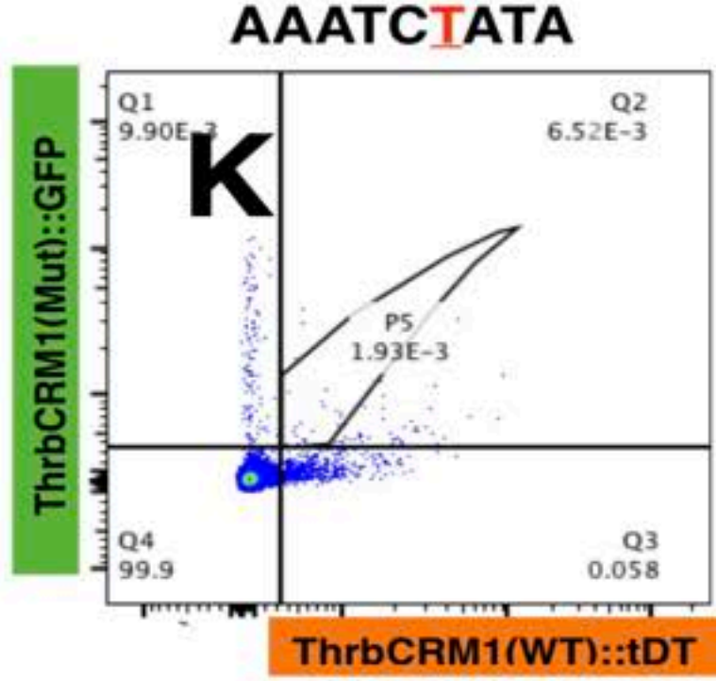
Oc1 Site Variants Alter ThrbCRM1 Activity











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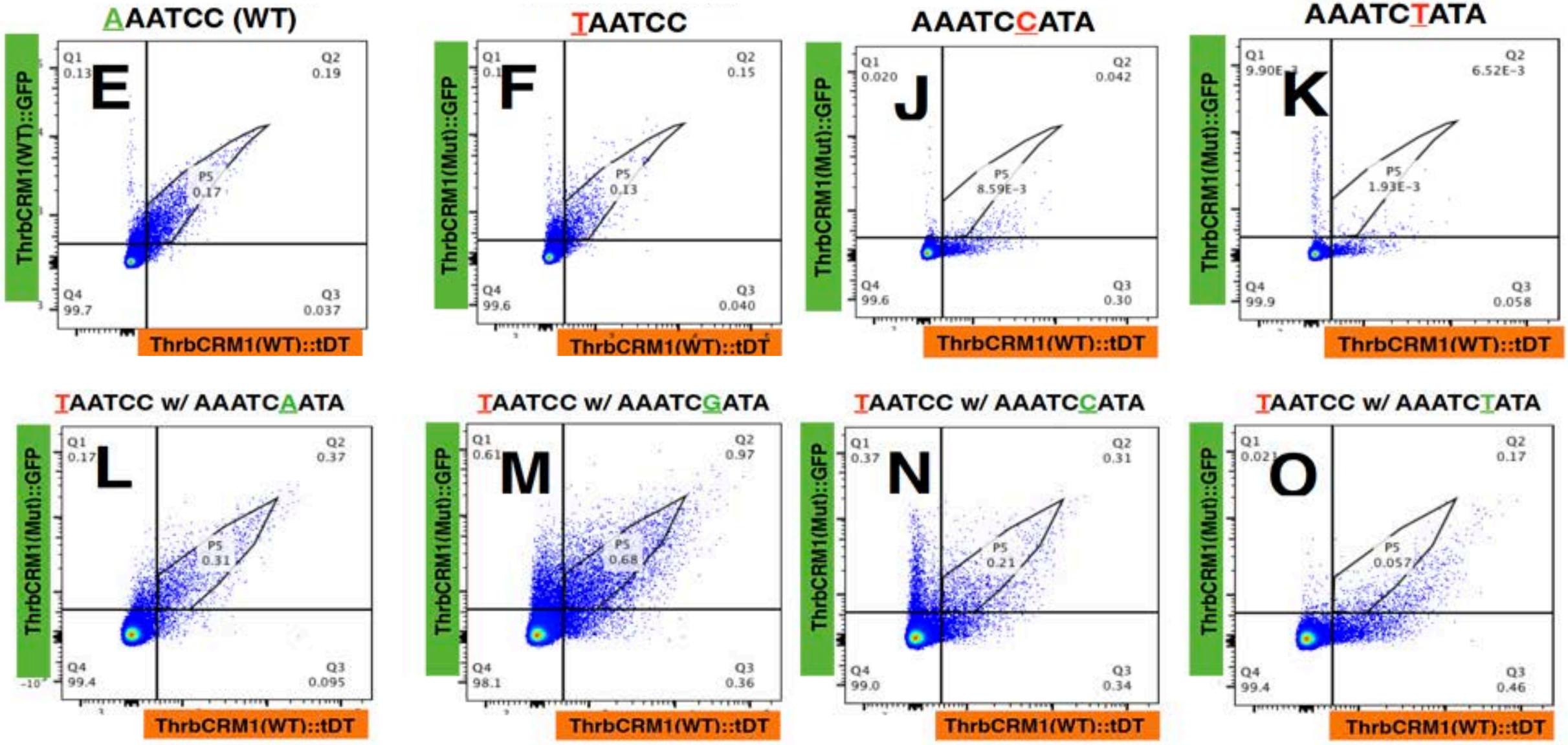
Dual Otx2-Oc1 ThrbCRM1 Mutants Reveal TAATCC Confers Cell-type Specificity



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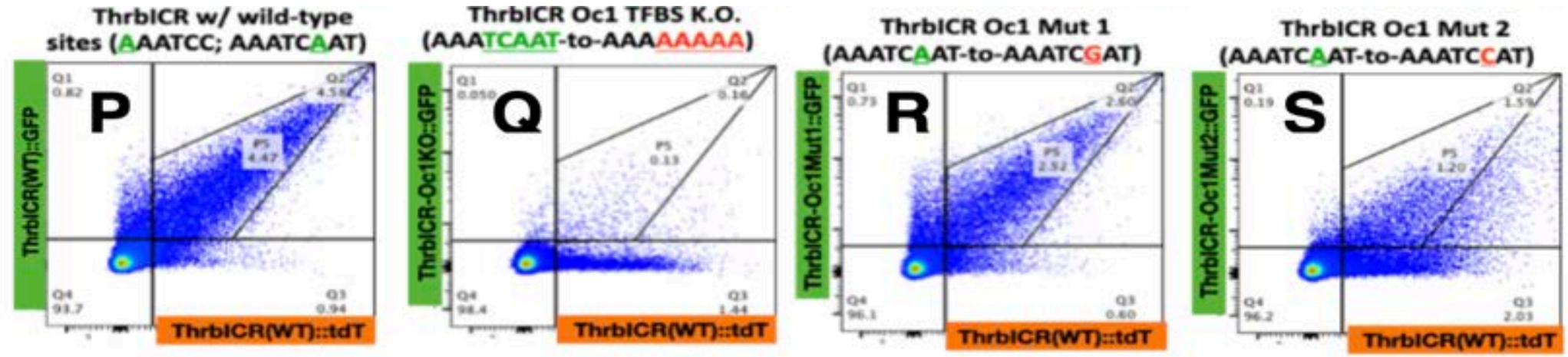
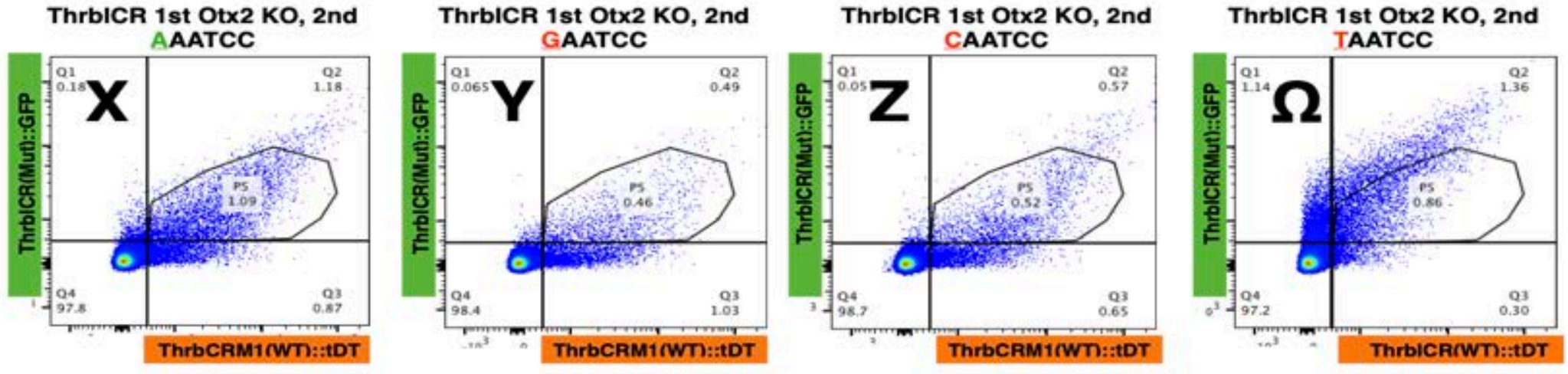
Identification of Oc1 and Otx2 Sites in ThrbICR and Affects of Variants





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Conclusions and Significance

Mutating Otx2 and Oc1 TFBS permutations in ThrbCRM1 and ThrbICR alters *Thrb* expression and likely cone subtype ratios

These findings paint a more vivid picture of the evolution of color vision

They additionally advance understandings of enhanceropathies

Our data support the potential of engineering designer enhancer elements that quantitatively and spatially control the transcription of delivered therapeutic, and/or endogenous, genes, to fall within narrow, precisely-defined desired windows

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Acknowledgements

Thanks to:

The NIH, the NSF,

all members of the Emerson Lab for their prodigious support, fruitful discussions and training,

and collaborators in Chemistry

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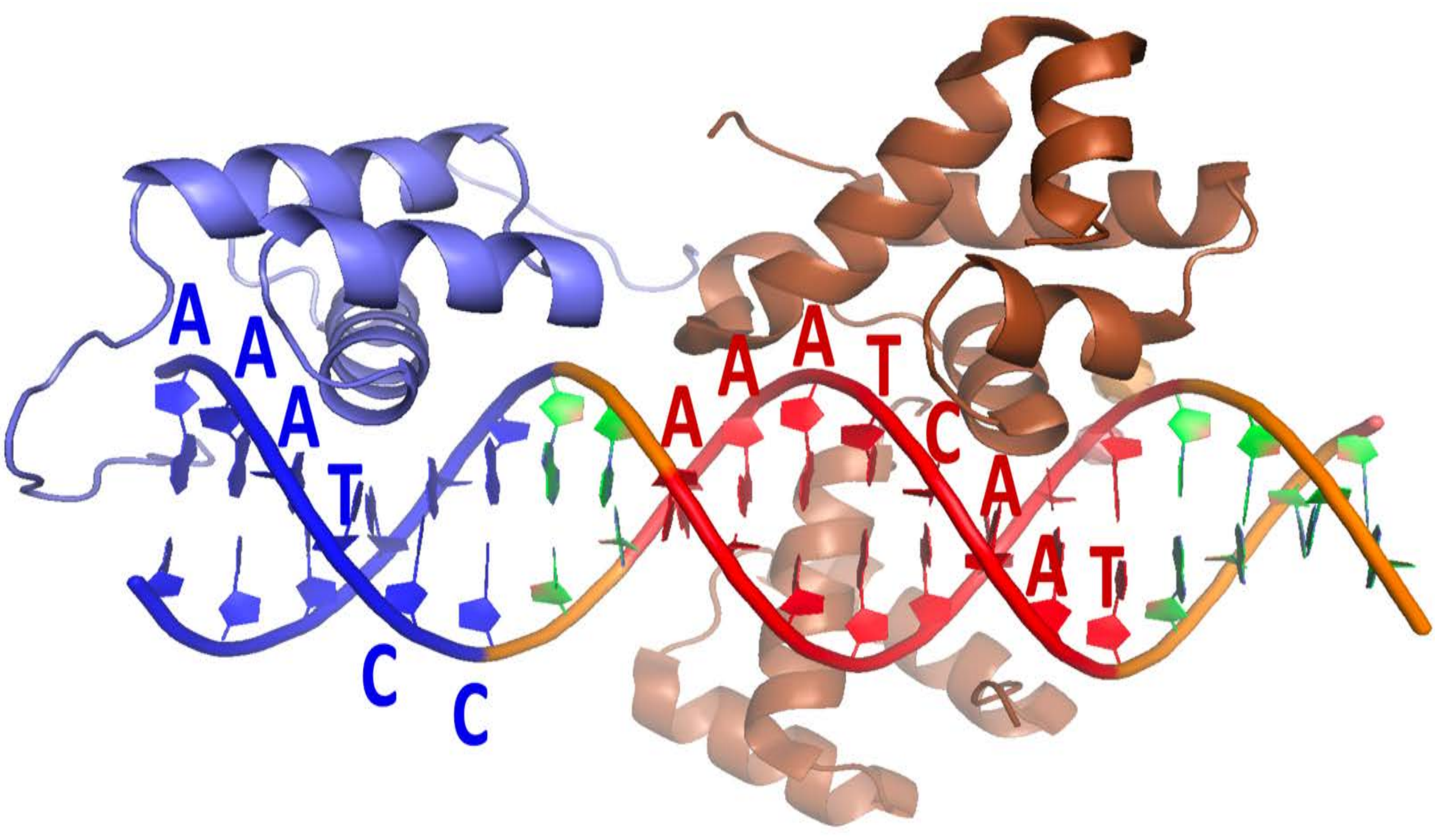
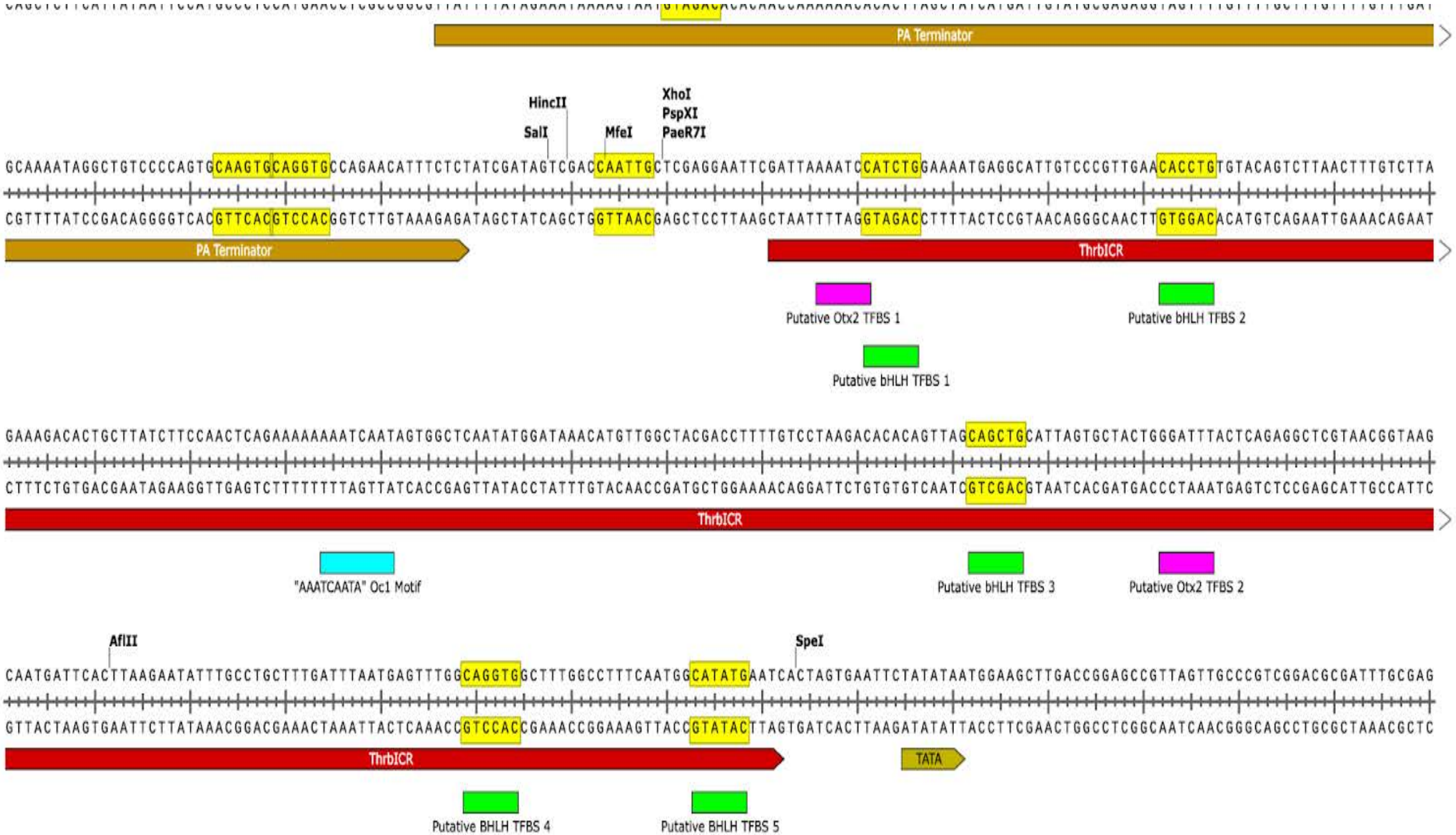
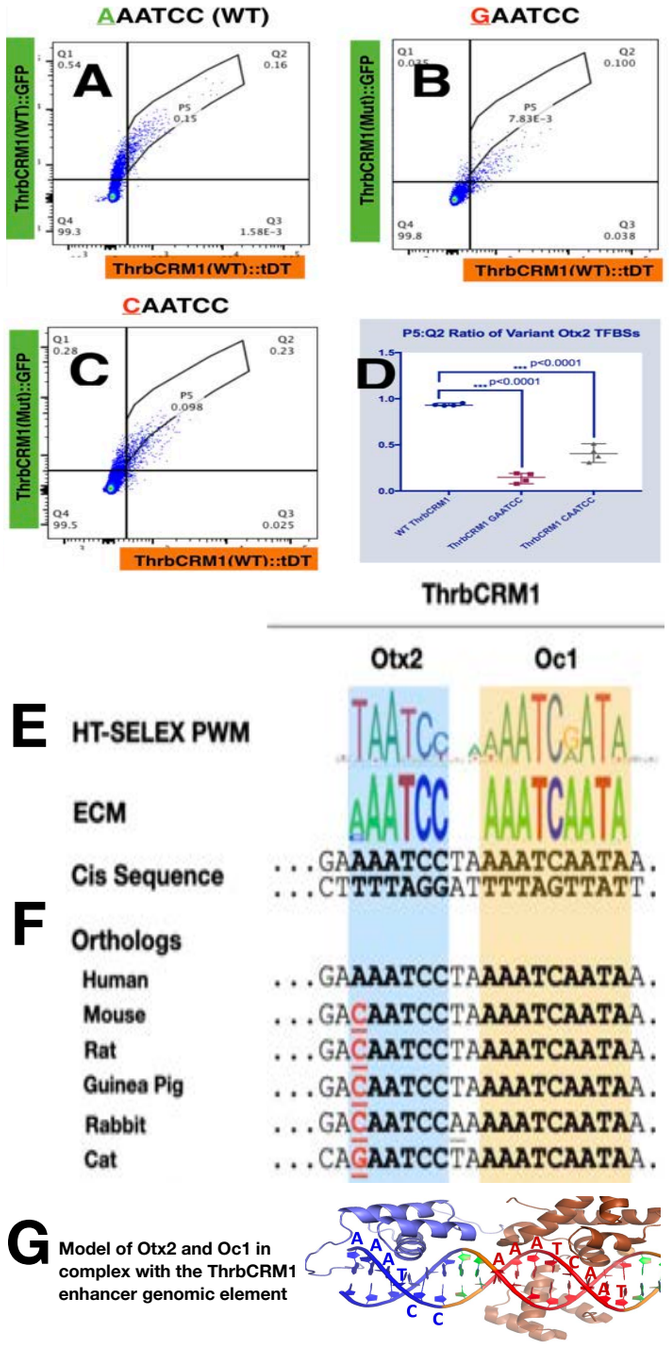
Analyzing Mutant-cisDNA TF Interactions *in silico*



Mutating "CANNTG" NeuroD1 TFBSs in ThrbICR





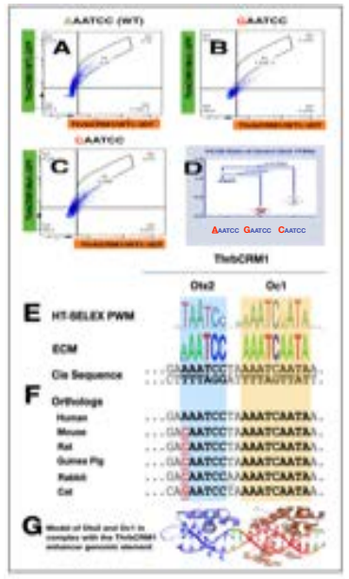


Figure 1. TFBS Sequence Discord, Affect of TFBS Mutations and Model of Oc1:Otx2:ThrbCRM1 Complex [A] Flow cytometry plot of *G. gallus* retinal progenitor cells (RPCs) co-electroporated at embryonic day 5 with plasmids reporter constructs harboring wild-type (WT) versions of the ThrbCRM1 enhancer driving the expression of green fluorescent protein (GFP) (y-axis), and tDTomato (tDT) (x-axis). **[B]** Flow cytometry plot of RPCs co-electroporated with a feline ortholog of ThrbCRM1 containing a "GAAATC" Otx2 TFBS variant driving GFP (y-axis), and WT ThrbCRM1 driving tDT (internal control). **[C]** Flow cytometry plot of RPCs co-electroporated with a rodent/lagomorph ortholog of ThrbCRM1 containing a "CAAATC" Otx2 TFBS variant driving GFP (y-axis), and WT ThrbCRM1 driving tDT (internal control). **[D]** Statistical analysis of flow cytometry geometry (P5 to Q2 cell population ratios). **[E]** *in vitro* HT-SELEX derived TFBS motifs compared with sequence logos derived from evolutionary conservation of ThrbCRM1. **[F]** ThrbCRM1 orthologs from different species. **[G]** 3D structural model of Otx2 and Oc1 bound to the ThrbCRM1 element created in PyMOL.