

# Structural and functional dissection of VSG-Exclusion Protein 2 in African trypanosomes

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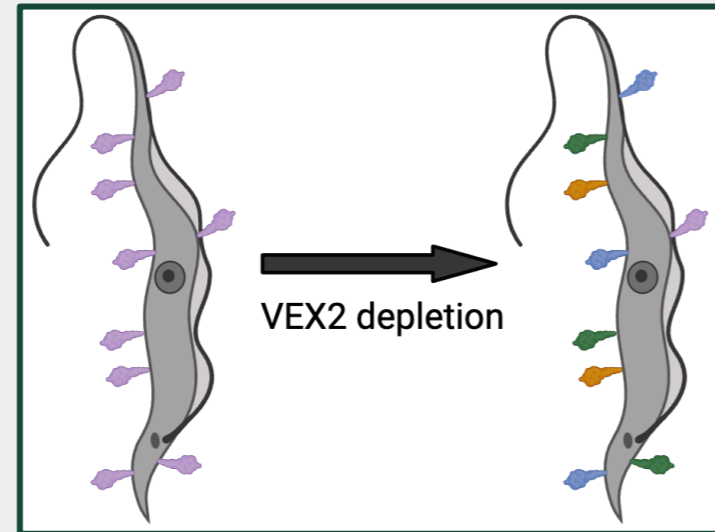
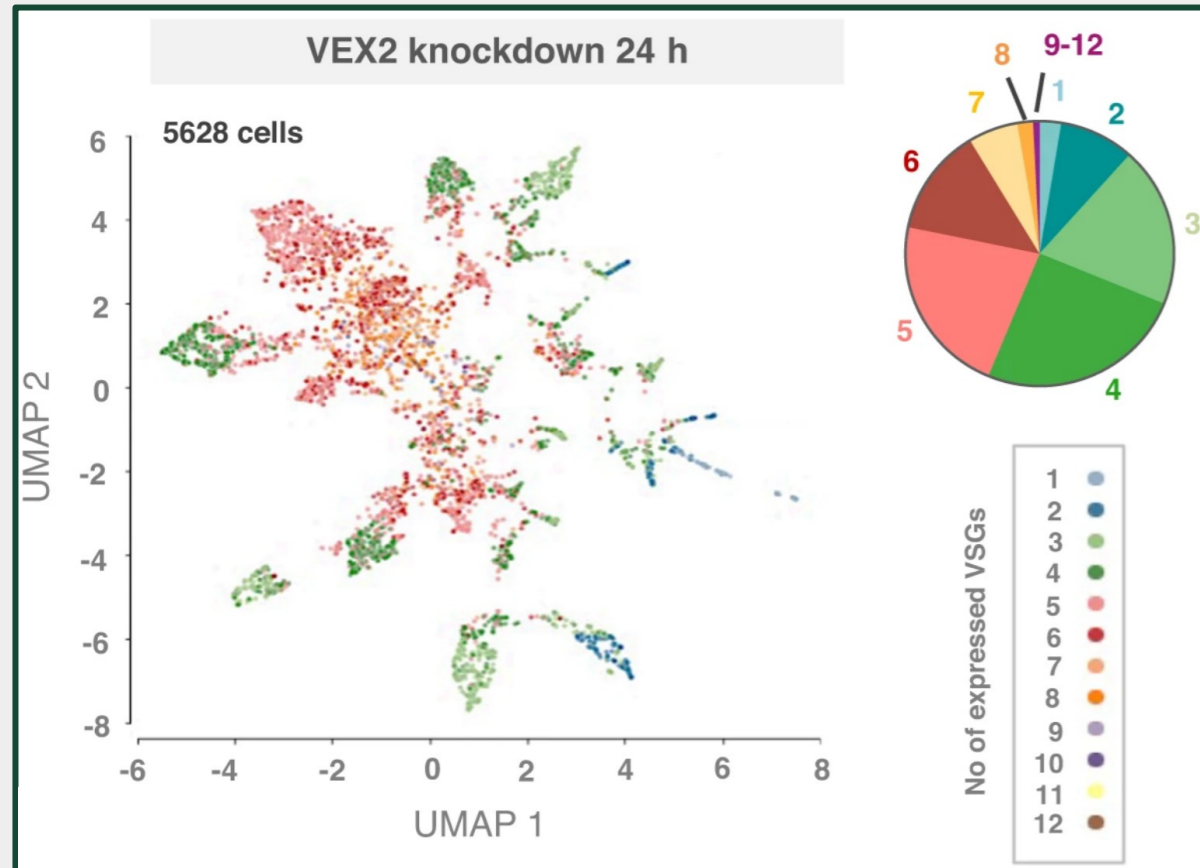
Faria lab



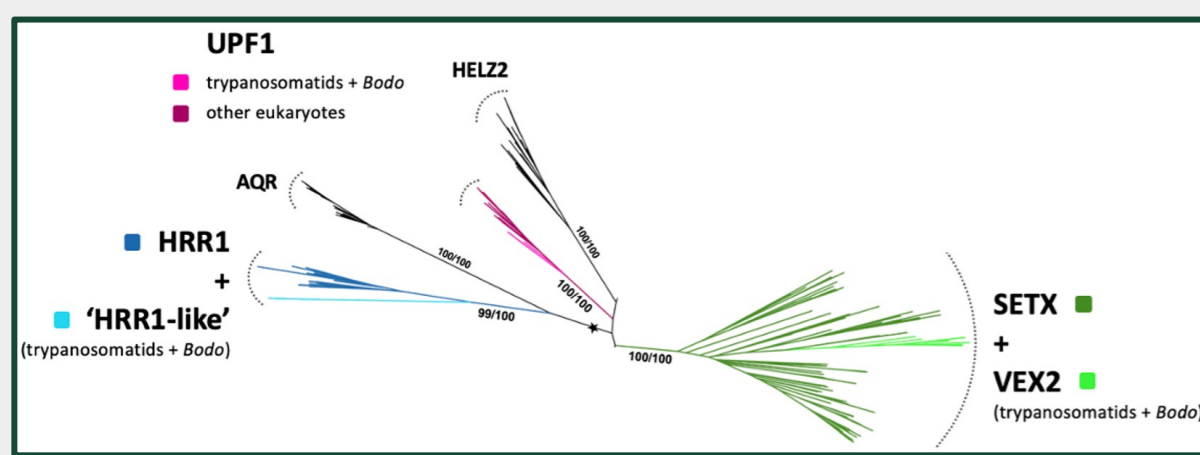
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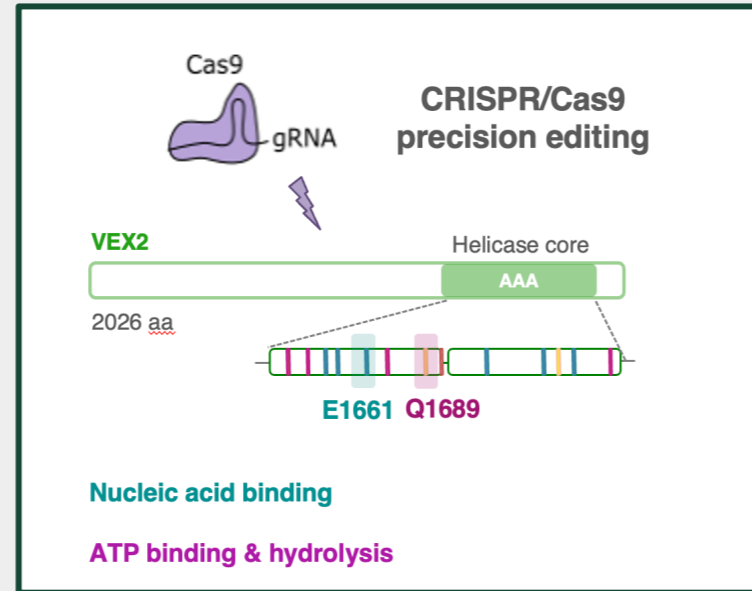
## Introduction



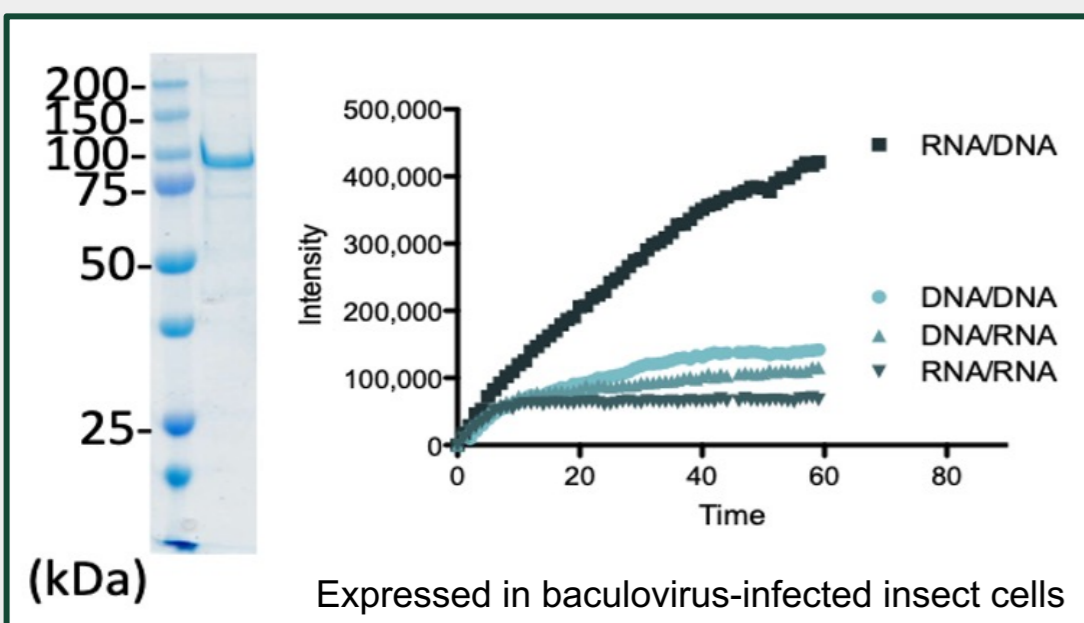
VEX2 depletion disturbs singular Variant Surface Glycoprotein (VSG) expression, an essential mechanism to escape immune response<sup>1</sup>.



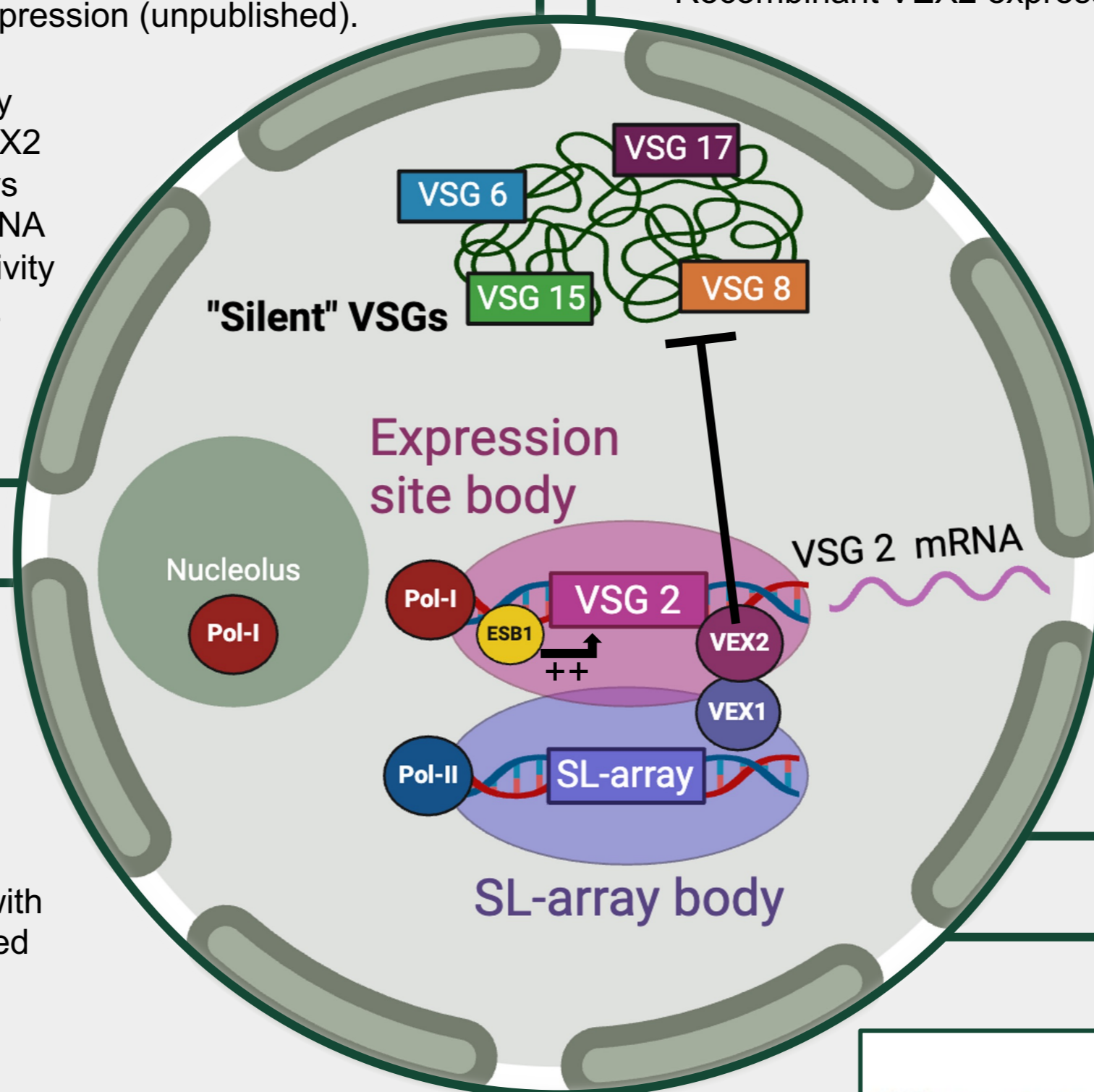
VEX2 is a member of the SF1 helicase family and an orthologue of human Senataxin (SETX) (unpublished).



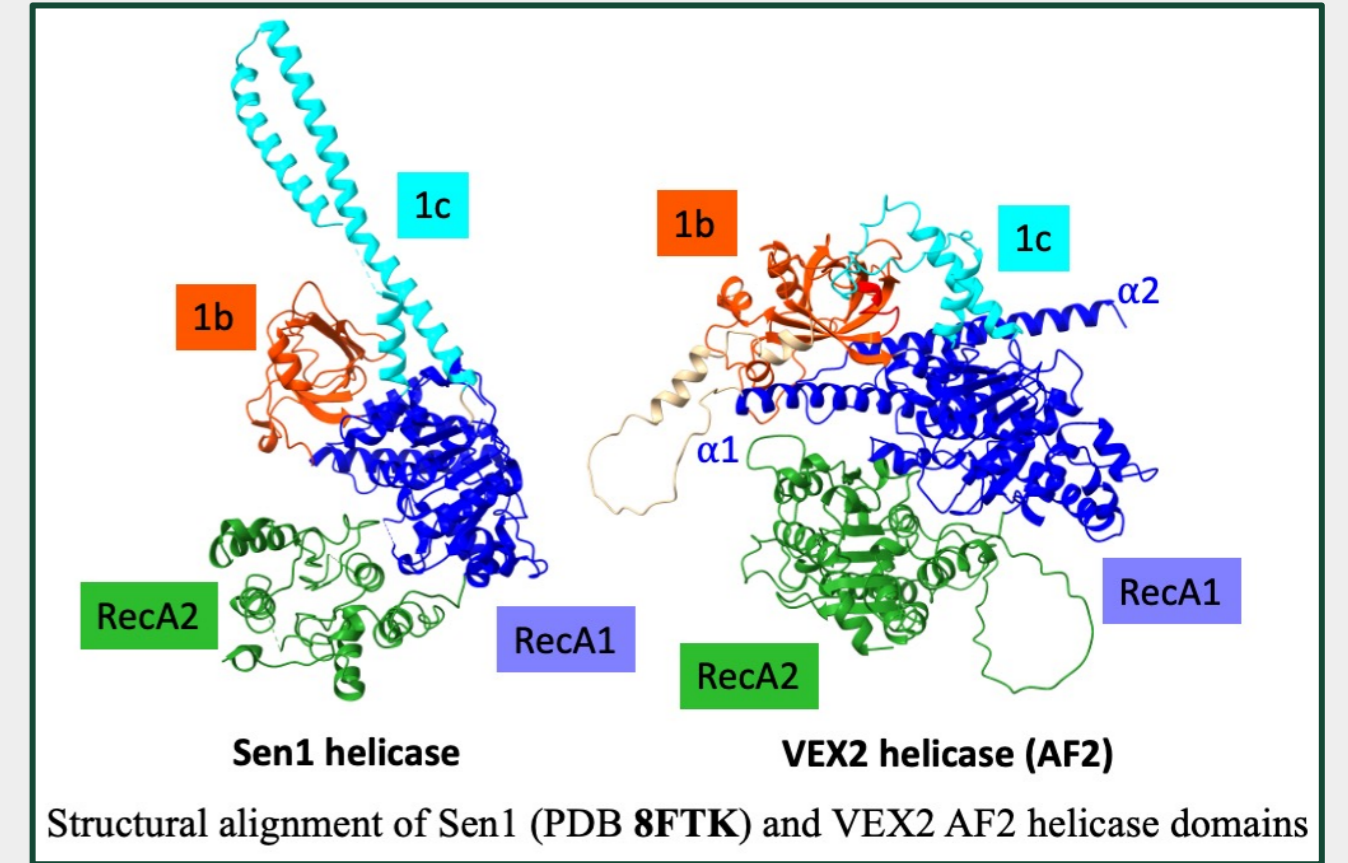
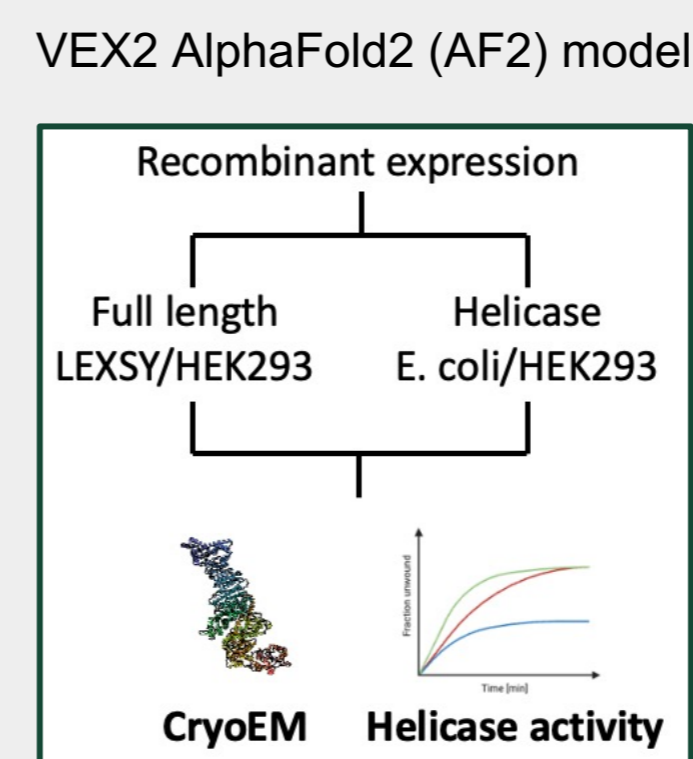
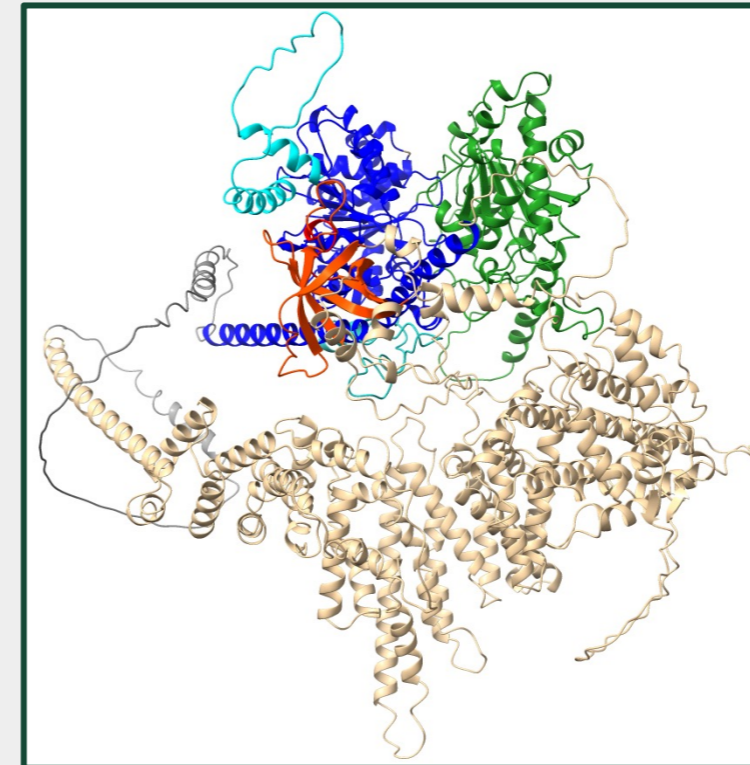
VEX2 helicase activity is essential for maintaining singular VSG expression (unpublished).



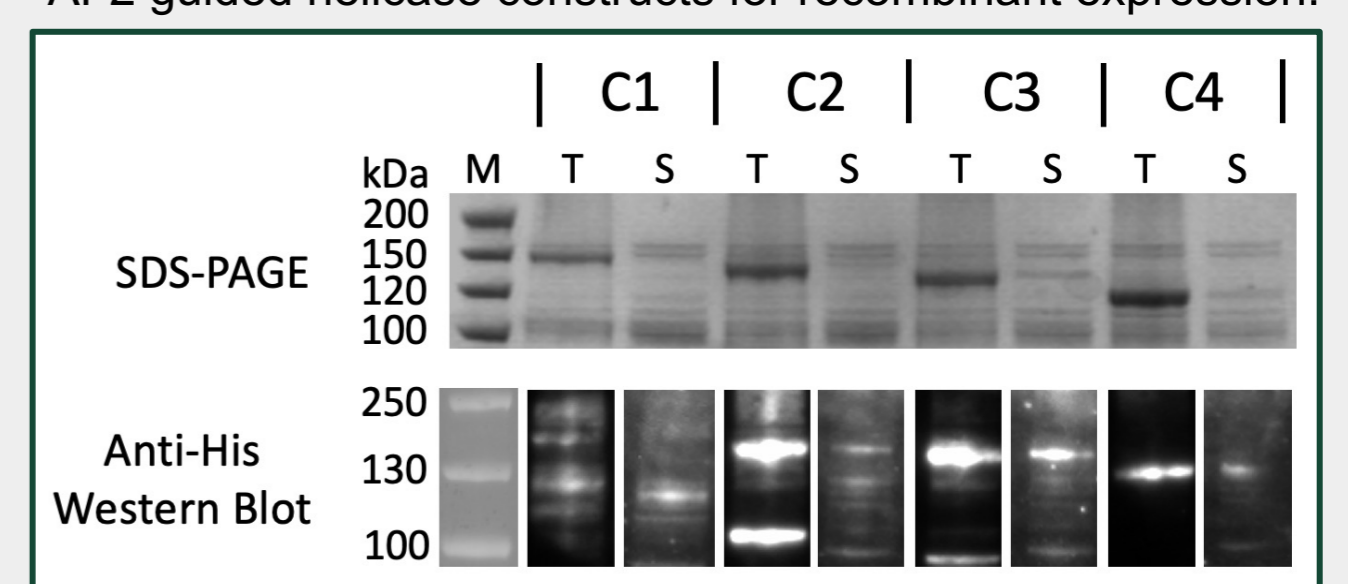
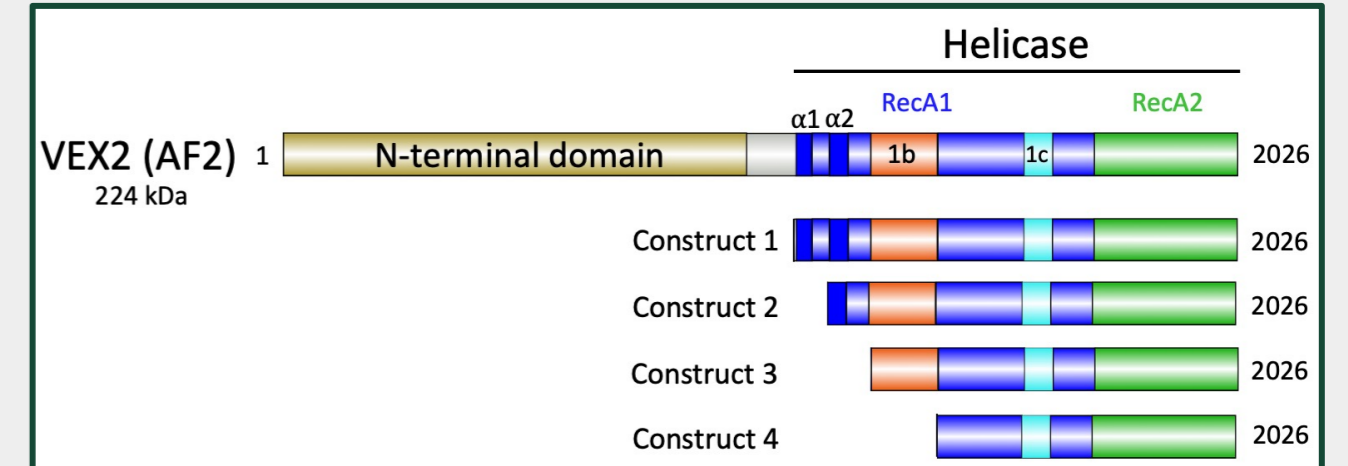
Recombinantly expressed VEX2 helicase shows strong RNA:DNA unwinding activity (unpublished).



## Structural Biology



VEX2 AF2 model shows strong differences with Sen1 1c subdomain, which is essential for regulatory interaction with the N-terminus in Sen1<sup>4</sup>.

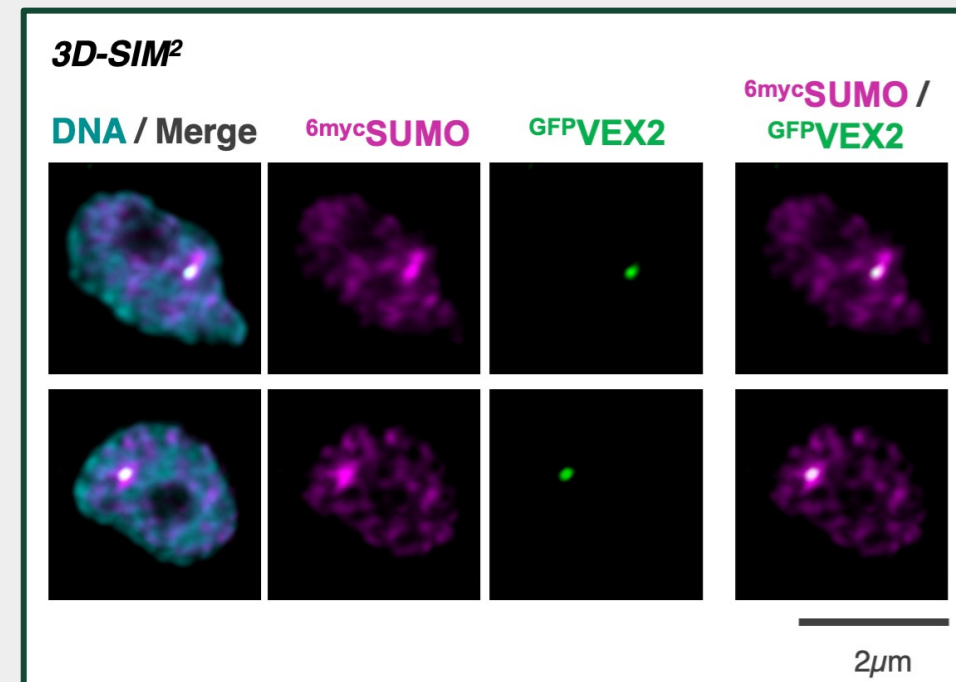


SDS-PAGE and Western Blot of total (T) and soluble (S) fractions of *E. coli* BL21 DE3 expression of four VEX2 helicase constructs with MBP-tag.

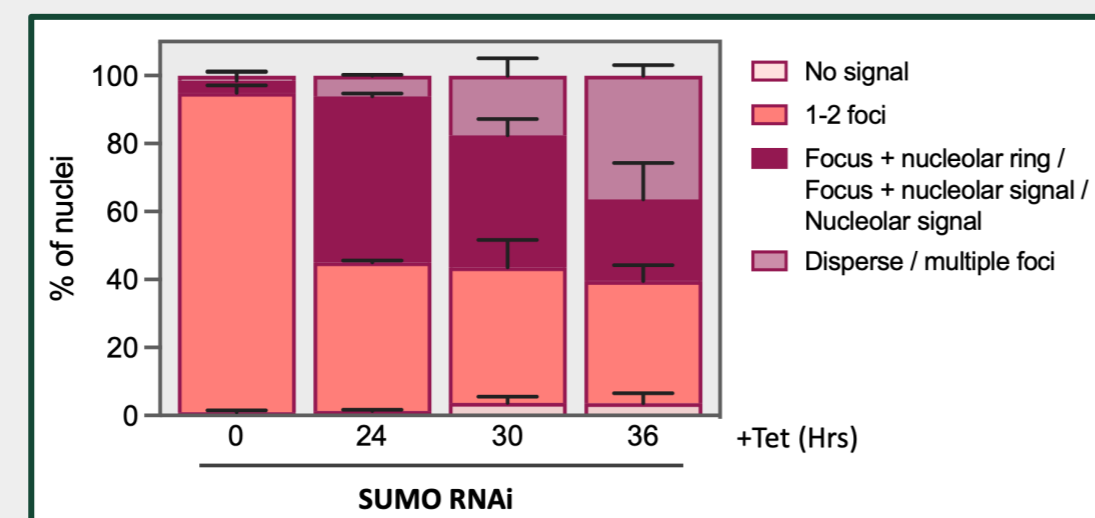
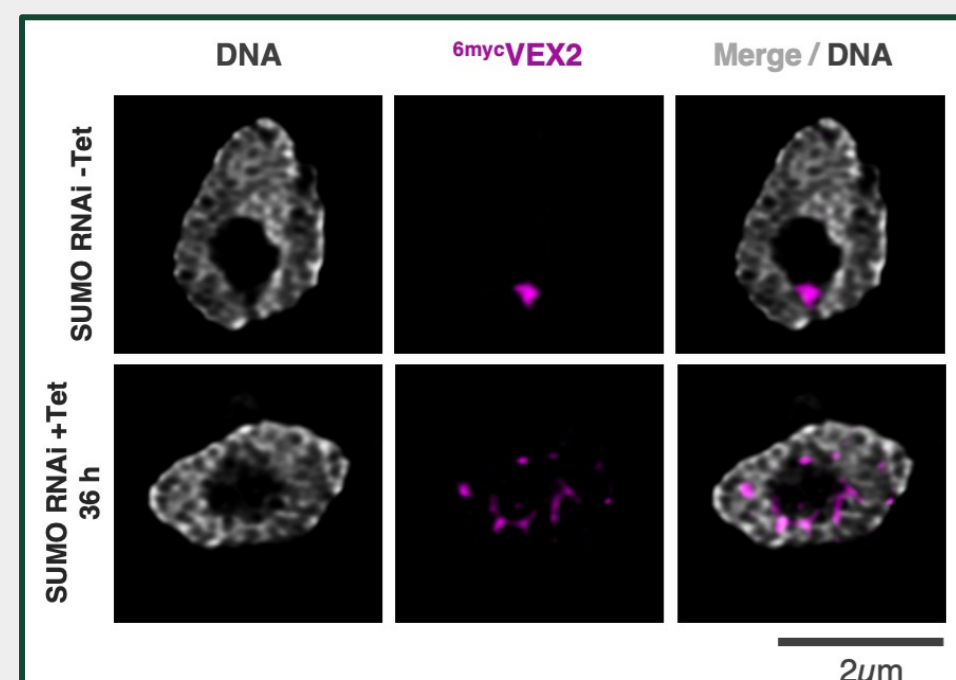
### Main goals:

- Understand substrate specificity of VEX2.
- Functional characterization of the different helicase subdomains, linker and N-terminus.
- Structure of a potential multiprotein complex.

## SUMOylation

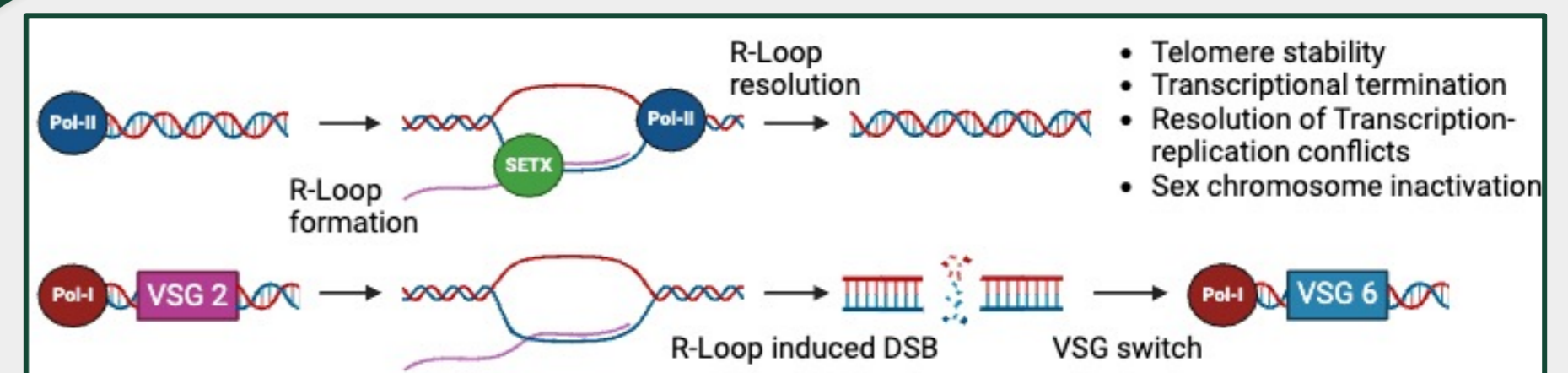


VEX2 colocalizes with a highly SUMOylated focus at the ESB.



SUMO depletion causes a significant accumulation of VEX2 in the nucleolar periphery.

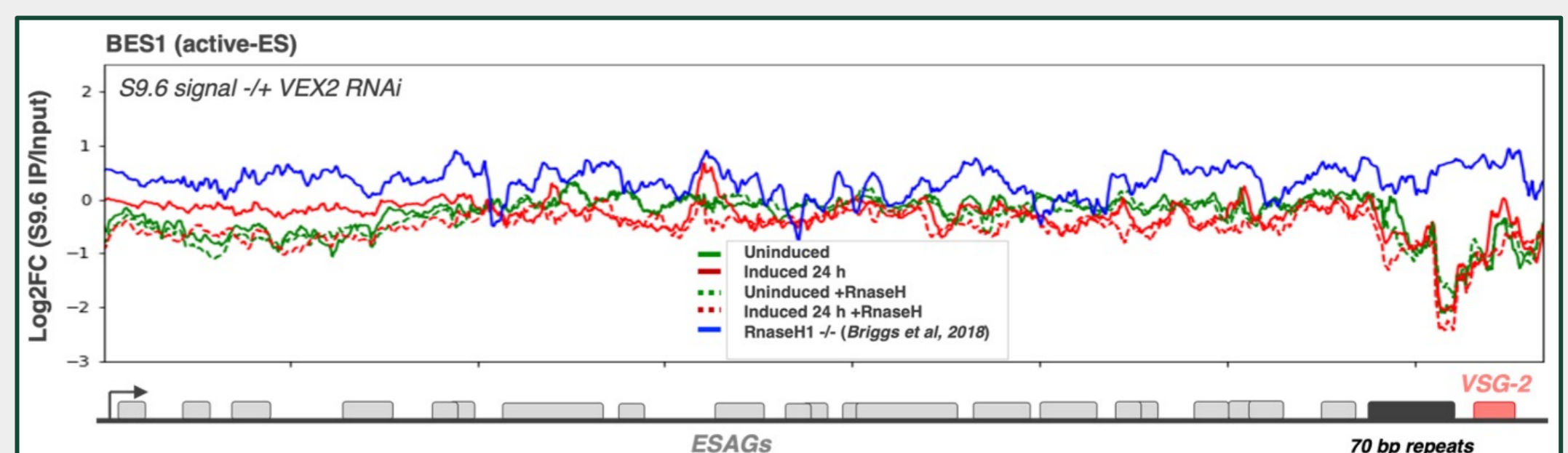
## R-Loops



Senataxin, the human orthologue of VEX2, is involved in human R-Loop metabolism<sup>5</sup>. R-Loops are also implicated with the induction of VSG switching in *T. brucei*<sup>6</sup>.

### Hypothesis:

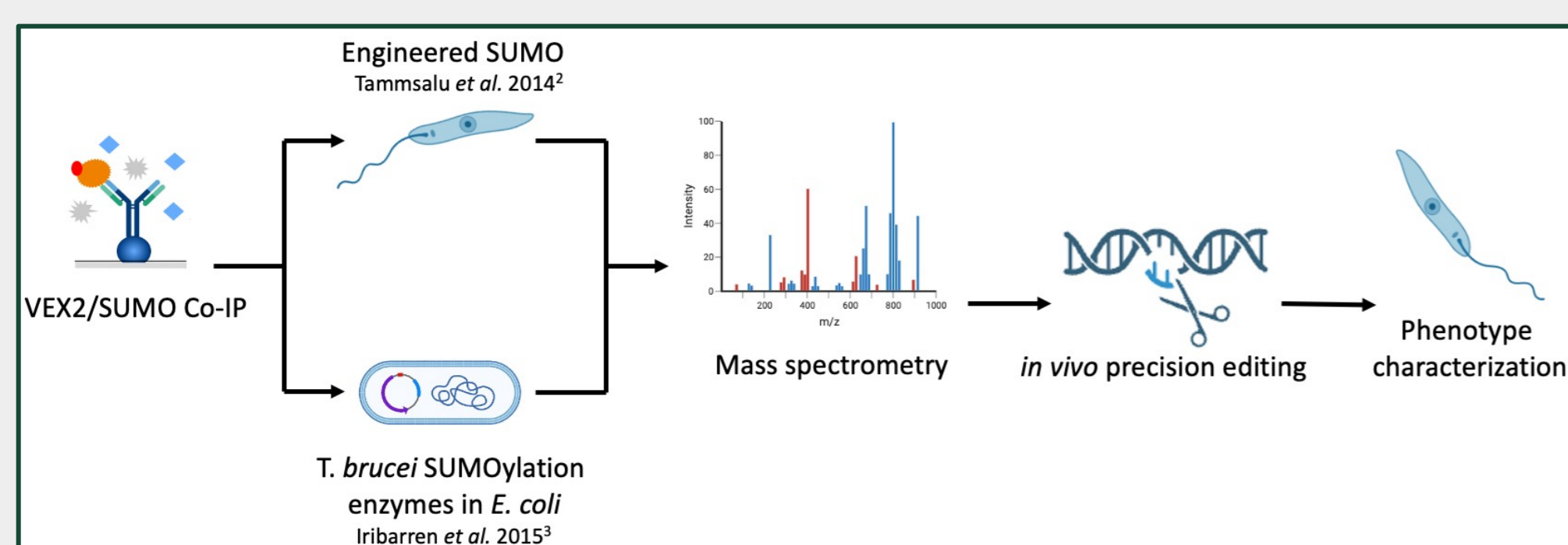
**VEX2 helicase activity is essential in regulating R-Loop levels at the active VSG expression site.**



DRIP-Seq analysis of VEX2-depleted cells shows an increase in the level of R-Loops at the beginning of BES1 (where VEX2 accumulates the most, based on previous ChIP-Seq data<sup>1</sup>) – ongoing research aims to investigate whether this is directly dependent on VEX2 helicase activity.

### Hypothesis:

1. VEX2 is SUMOylated.
2. SUMOylation of VEX2 confers ESB specific localization.
3. SUMOylation is stage/cell cycle dependent.



Future approach to study the SUMOylation of VEX2.

## References

- <sup>1</sup>Faria et al. 2023, PMID: 38081826; <sup>2</sup>Tammsalu et al. 2014, PMID: 24782567; <sup>3</sup>Iribarren et al. 2015, PMID: 26258470; <sup>4</sup>Appel et al. 2023, PMID: 37832548; <sup>5</sup>Groh et al. 2017, PMID: 27771483; <sup>6</sup>Saha et al. 2020, PMID: 31682833; Illustrations were created with BioRender.com