Novel components of the expression-site body and surrounding splicing bodies discovered by TurboID proximity labelling in African Trypanosomes.

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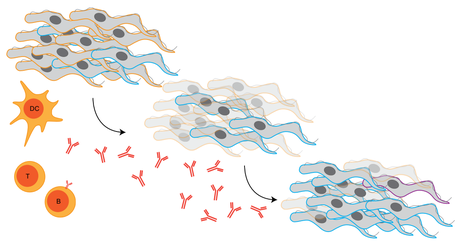
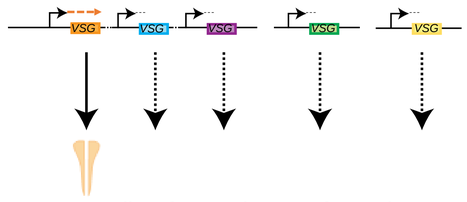
1. Antigenic variation in African Trypanosomes and the role of VEX in monogenic expression

Successful antigenic variation depends on:

1. **Monogenic expression** of a surface-exposed-antigen.
2. Switching of the antigen coat.





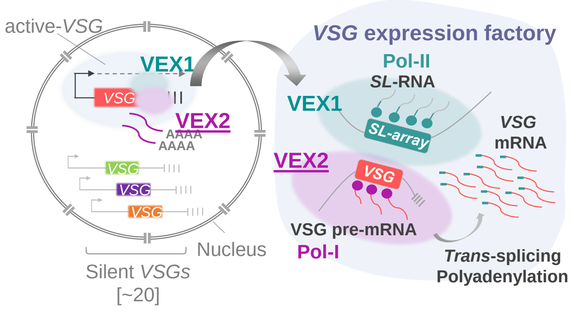
>2,600 genes encoding for variant surface glycoproteins (VSGs) (Müller *et al.*, 2018, PMID: 30333624).

10 million VSG molecules on the surface (Jackson *et al.*, 1985, PMID: 4052037).

10% of total protein.

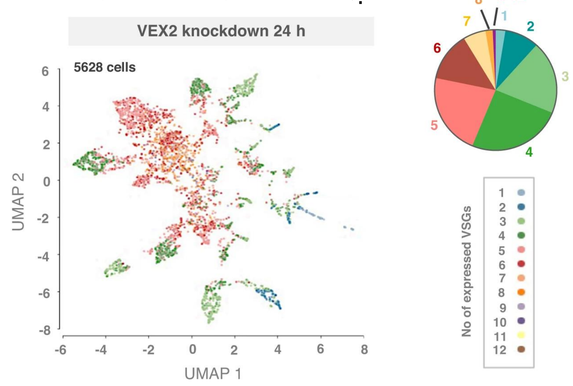
VEX complex bridges the ESB and SLAB

VEX1 and VEX2 form a dynamic complex that brings together the active-*VSG* expression-site and the *SL*-array (Faria *et al.*, 2021, PMID: 33432154). The latter is necessary for trans-splicing and mRNA maturation.



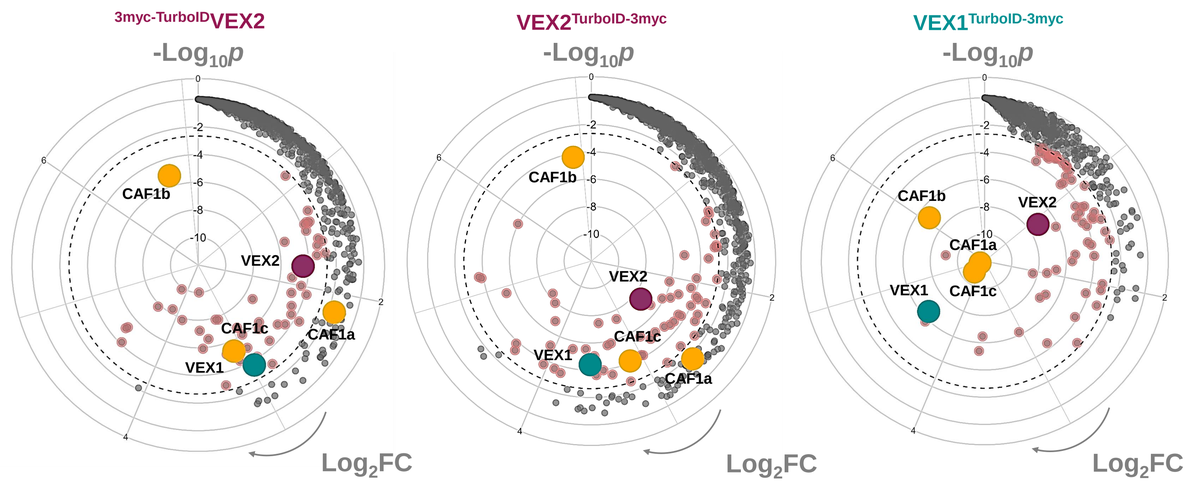
VEX2 KD results in derepression of 'silent' VSGs

VEX2 enforces monogenic expression in *T.brucei* parasites. Its depletion leads to simultaneous expression of multiple *VSG* genes (Faria *et al.*, 2019, PMID: 31289266, Faria et al. 2023, PMID: 38081826).



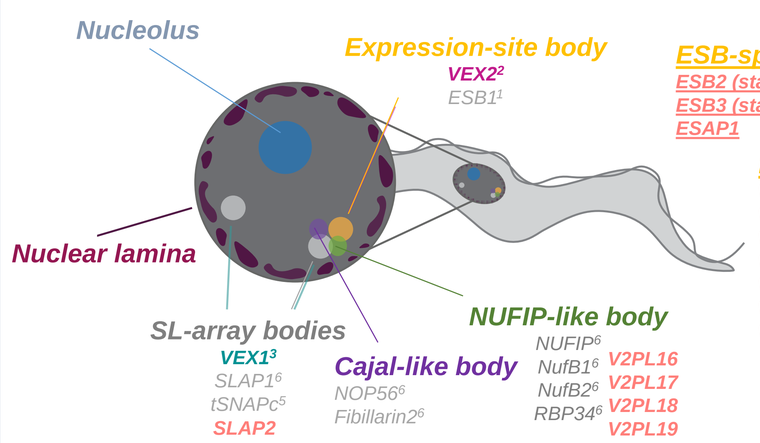
2. Identification of the spatial interactome of the '*VSG* expression factory' using proximity labelling

Two biotin ligases, TurboID (Doerr, 2018, PMID: 30275580) and UltraID (Zhao *et al.*, 2021, PMID: 35788163), were tested. TurboID outperformed UltraID (data not shown).



Proximity labelling data shows an enrichment of previously identified VEX2 interactors, VEX1 and the CAF-1 subunits. Additionally, the dataset revealed a range of previously unidentified hypothetical proteins that form part of the *VSG* expression factory.

VEX2 proximity labelling reveals known proximal proteins and a range of hypothetical proteins



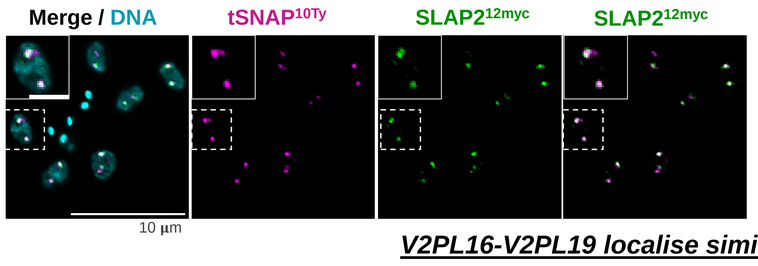
Protein name legend

VEX2 – VSG exclusion protein 2
ESB1 – Expression site-specific Body 1
ESB2 - Expression site-specific Body 2
ESB3 - Expression site-specific Body 3
ESAP1 – Expression site body associated protein 1
VEX1 – VSG exclusion protein 1
SLAP1 – SL-array body protein 1
SLAP2 – SL-array body protein 2
V2PL## - VEX2 Proximity labelling ##.

3. Novel SLAB and NUFIP components

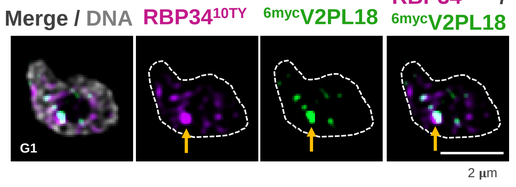
SLAP2 localises specifically to the SLAB

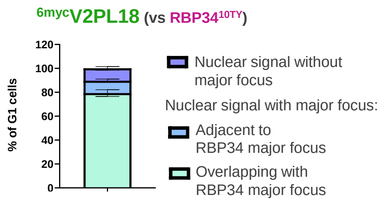
Proximity labelling also revealed a range of components found to localise to other splicing bodies associated with VSG expression. One such new component, SLAP2, overlaps with tSNAP.



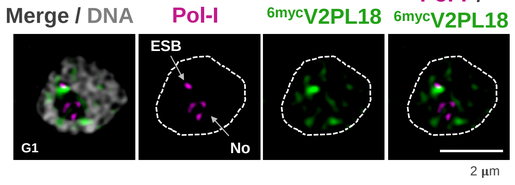
V2PL16-V2PL19 localise similarly to RBP34 (NUFIP component)

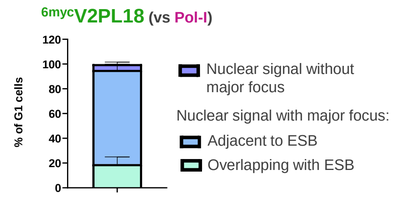
Four proteins (V2PL16-V2PL19) were found to colocalise with RBP34, a marker of the NUFIP 'body'.





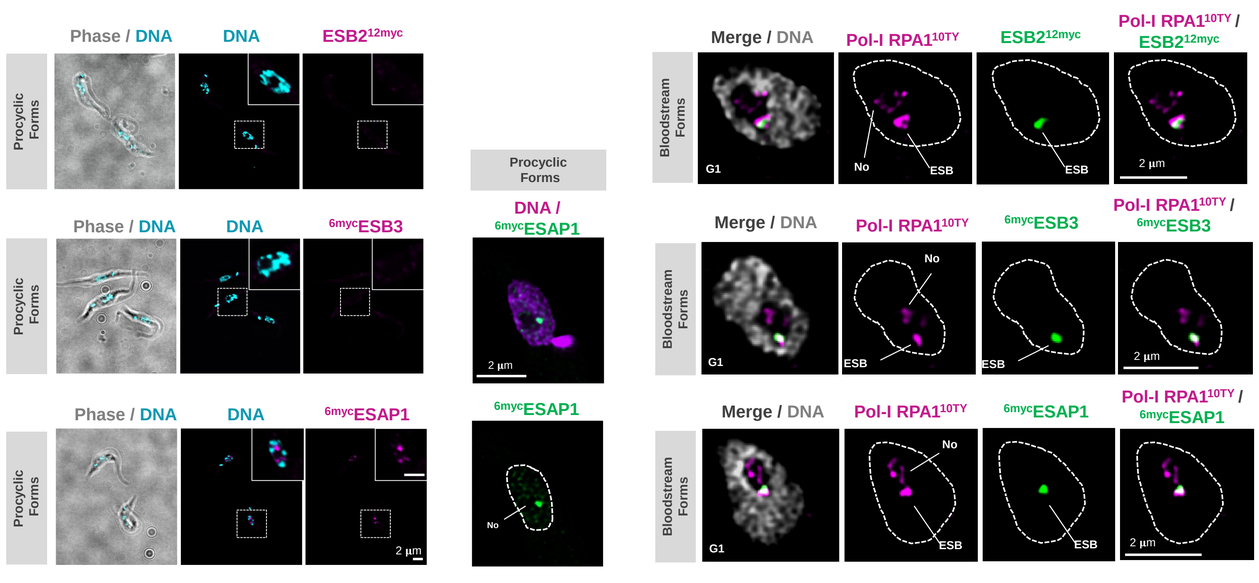
Moreover, these proteins were enriched adjacent to the ESB, similarly to RBP34 (not shown), marked by Pol-I.

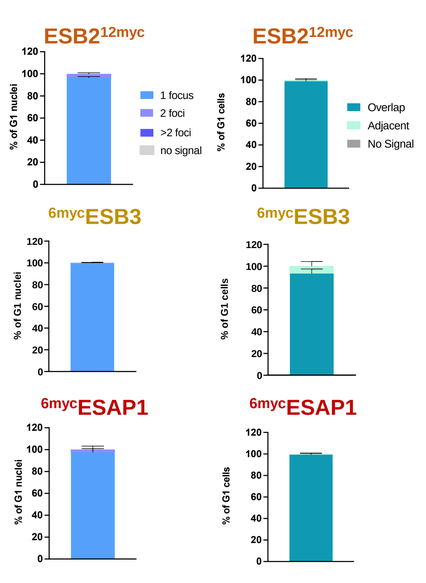


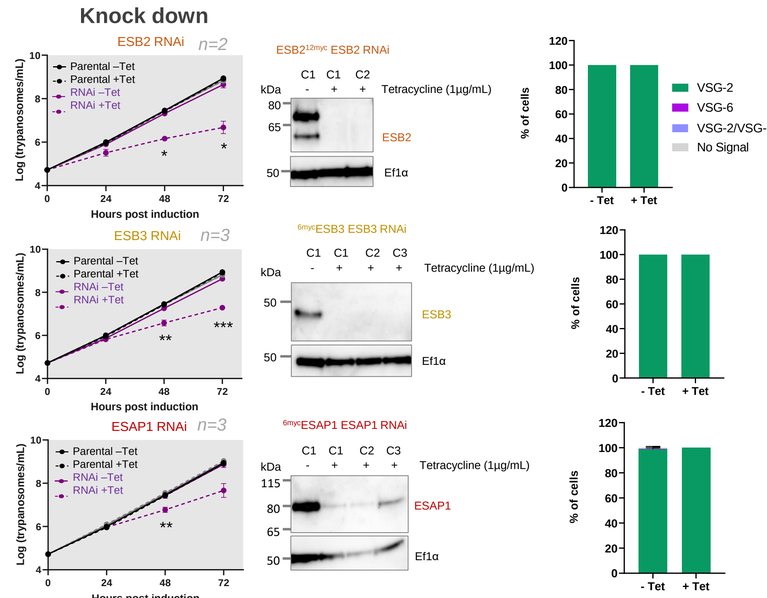


4. Novel ESB components: ESB2, ESB3 and ESAP1

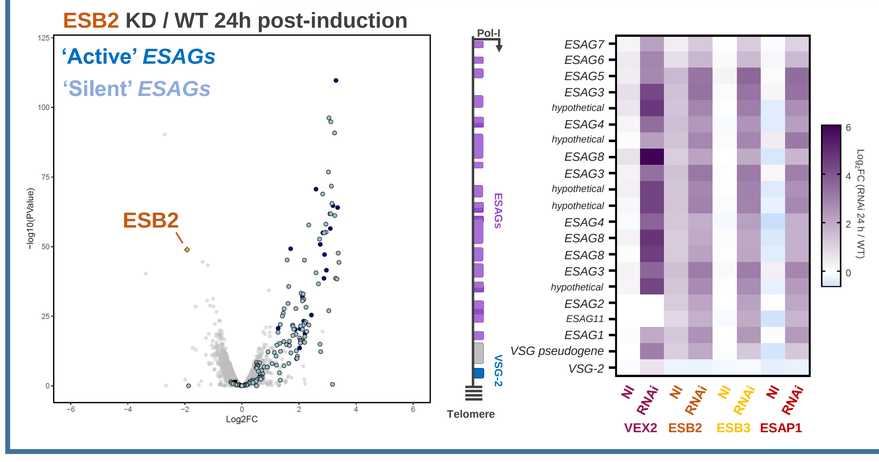
ESB2, ESB3 and ESAP1, identified by VEX2 proximity labelling, were all found to specifically localise to the ESB, marked by Pol-I expression, in bloodstream forms. RNAi knock down of each protein resulted in significant fitness loss.





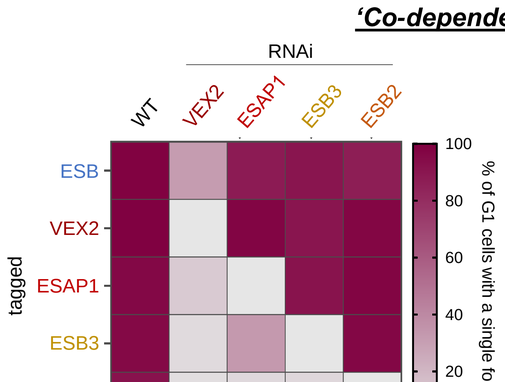


ESB2, ESB3 and ESAP1 KDs lead to specific upregulation of ESAGs



ESB2, ESB3 and ESAP1 knock down each result in the upregulation of both active and 'silent' *ESAG* genes, but do not significantly affect either active or 'silent' *VSG* gene expression despite sharing the same promotor(s) per polycistron. Patterns of upregulation per *ESAG* are similar between the knockdowns of ESB2, ESB3 and ESAP1, but dissimilar to the VEX2 knock down pattern.

'Co-dependencies' between novel ESB factors



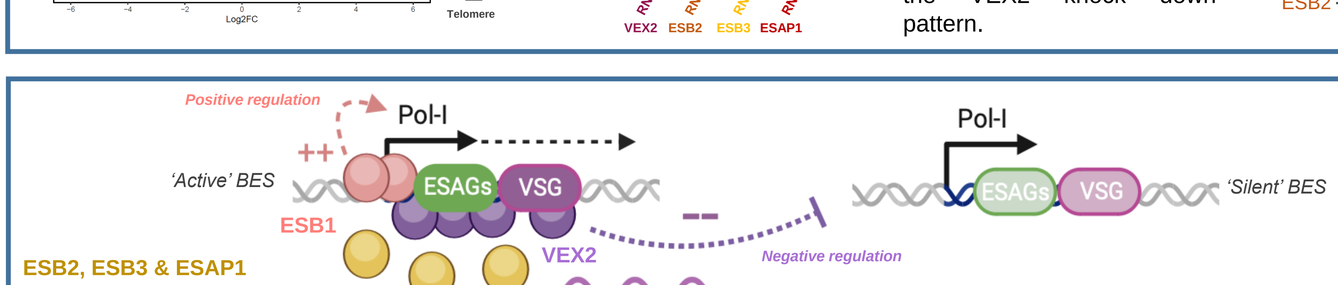
Proposed 'Hierarchy'

VEX2: RNA:DNA helicase
↓
ESAP1: RNA-binding protein?
↓
ESB3: Unknown function
↓
ESB2: RNA nuclease?

Expressed in: PCFs + BSFs

Expressed in: **BSFs only**

We hypothesise that the effect on *ESAGs* seen after VEX2 knock down are due to the essentiality of VEX2 to the integrity of the ESB, an indirect effect. This is supported by the loss of focal ESB2, ESB3 and ESAP1 signal after VEX2 knock down. Moreover, a hierarchy seems to exist within the group of three newly discovered ESB components.



5. Summary & Future directions

Uncovering molecular mechanisms behind ESB2-mediated *ESAG* expression regulation.

- Is the ESB2-KD phenotype dependent on the nuclease activity?
- Does ESB2 bind (*ESAG*) mRNA?
- Does ESB2 affect transcription, splicing and/or degradation of *ESAG* transcripts?

References

1. López-Escobar et al., 2022, PMID: 35879525. 2. Faria et al., 2019, PMID: 31289266. 3. Glover et al., 2016, PMID: 27226299. 4. Das et al., 2005, PMID: 16055739. 5. Budzak et al., 2022, PMID: 35013170. 6. Jehi et al., 2014, PMID: 24810301.