Bromodomain Factor 5 is an essential regulator of transcription in Leishmania

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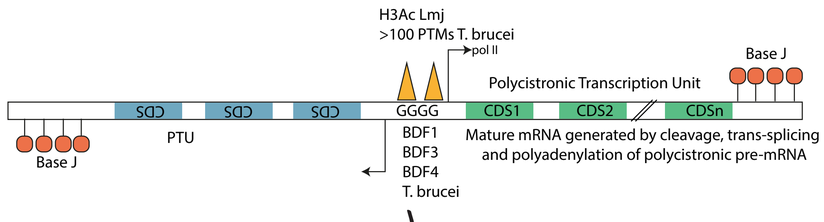
Overview

Gene expression in Leishmania is different from the mammalian host due to the arrangement of the genome. Transcriptional start sites are marked by histone acetylation but it is unclear how this is interpreted into control of expression of polycistronic transcription units (PTUs). We are investigating bromodomain (BD) containing proteins, or bromodomain factors (BDFs), as a potential regulators of transcription in Leishmania.

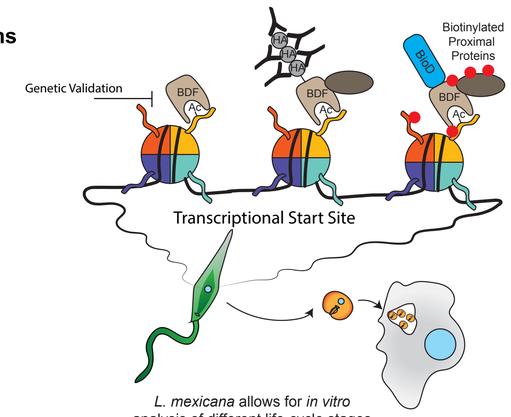
Bromodomains are small protein domains that bind to acetylated lysine, a modification often found in histone tails, resulting in changes to gene expression through further histone modification, chromatin remodelling, or recruitment of polymerase complexes. They are poorly characterised in Leishmania but we hypothesise they could orchestrate constitutive gene transcription, and would be essential for parasite survival.

We genetically validated the BDFs in *Leishmania mexicana,* a robust, genetically tractable species. After identifying BDF5 as essential in both promastigotes and amastiogtes we determined the genomic distribution of the protein to be mostly at transcriptional start sites. Proximal proteomics identified many complexes involved in processes require for transcription. We then used spike-in controlled, total RNA-seq to show that BDF5 is required for optimal transcription of RNA polymerase II transcribed PTUs.

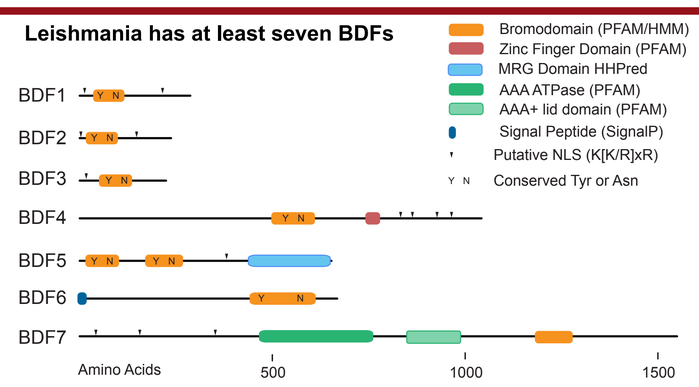
Histone Acetylation and BDFs at Transcriptional Start Sites



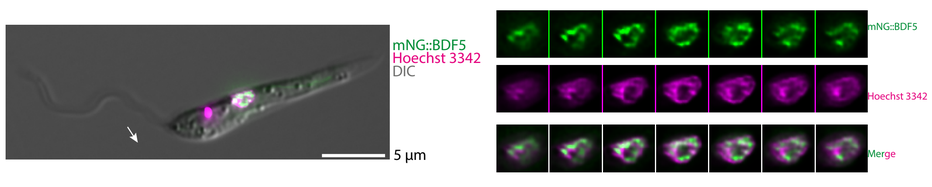
Graphical Aims



Leishmania has at least seven BDFs

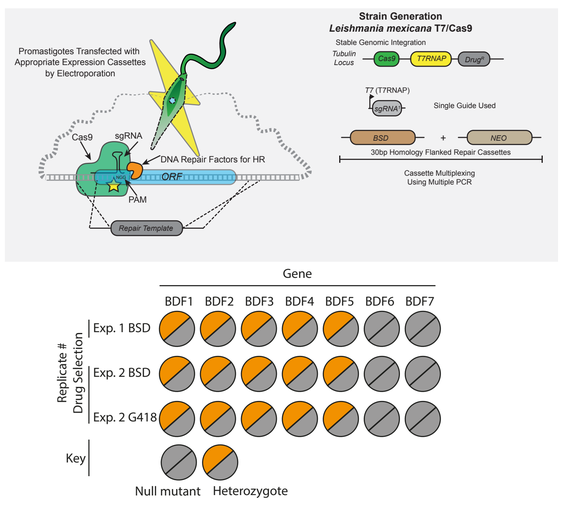


BDF5 is found in foci at the periphery of the nucleus



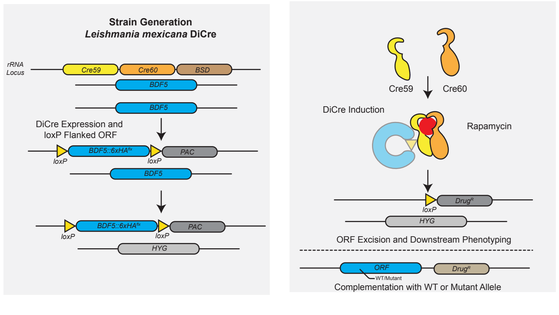
Assessment of LmxBDF5 Essentiality

Cas9 gene deletion attempts indicate BDF essentiality

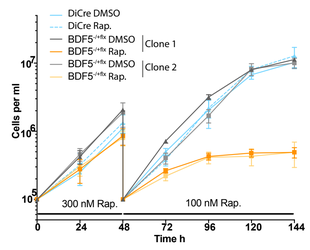


BDF1-5 likely to be essential. ∆bdf7 mutants under investigation as they cannot differentiate to amastigote forms

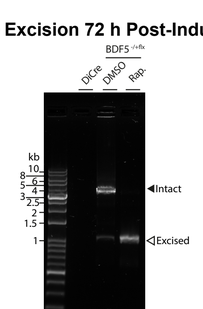
DiCre inducible gene deletion shows BDF5 is essential for promastigotes



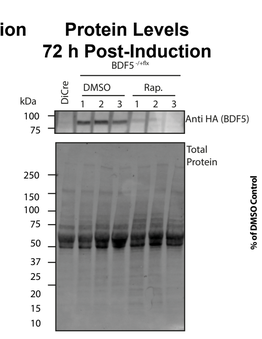
Promastigote Growth 98% Reduced



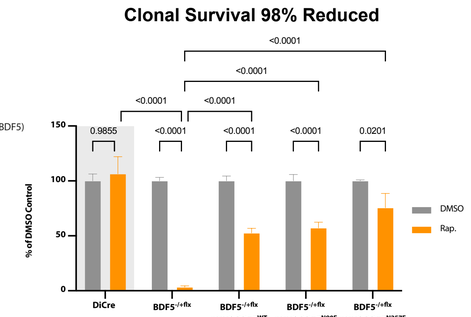
Gene Excision 72 h Post-Induction



Protein Levels 72 h Post-Induction

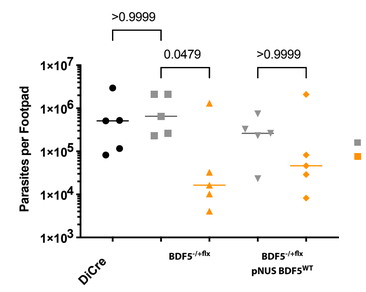


Clonal Survival 98% Reduced

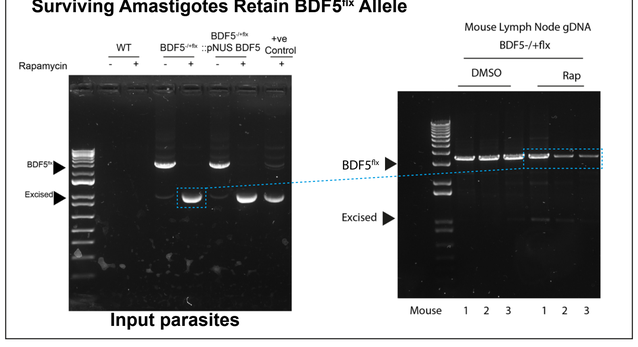


BDF5 is essential for amastigote survival in mice

Parasite Burden is Reduced 50-fold After 8-Week Infection

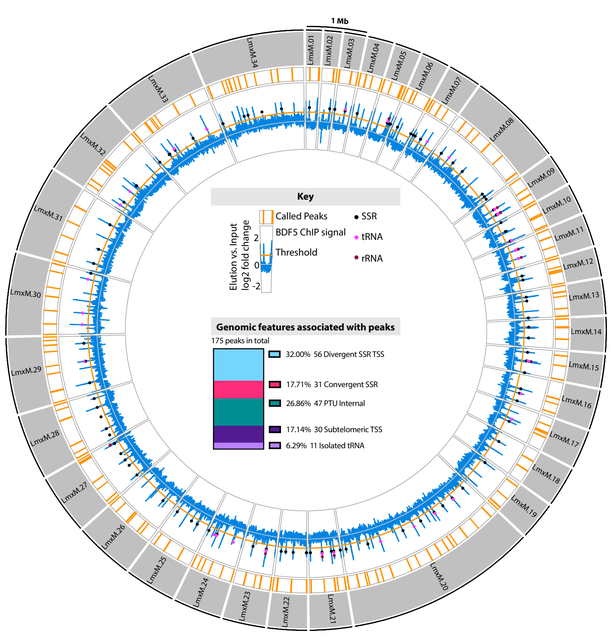


Surviving Amastigotes Retain BDF5^{flx} Allele

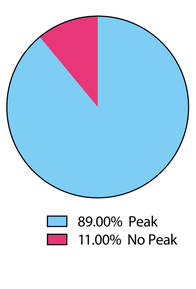


BDF5 ChIP-Seq

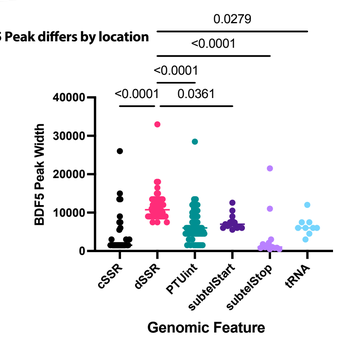
BDF5 is located predominantly at transcriptional start sites



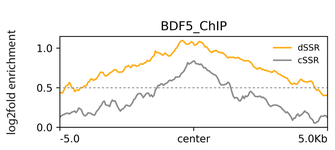
Proportion of SSRs with Called Peak



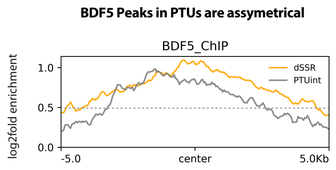
Size of BDF5 Peak differs by location



BDF5 Peaks are broader and more intense at TSSs



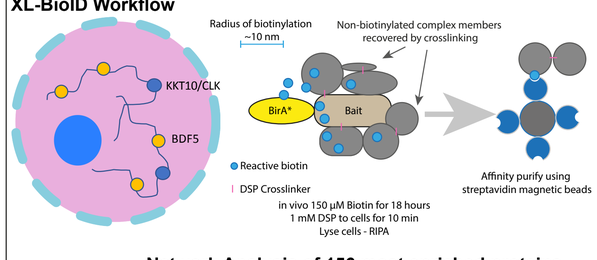
BDF5 Peaks in PTUs are assymetrical



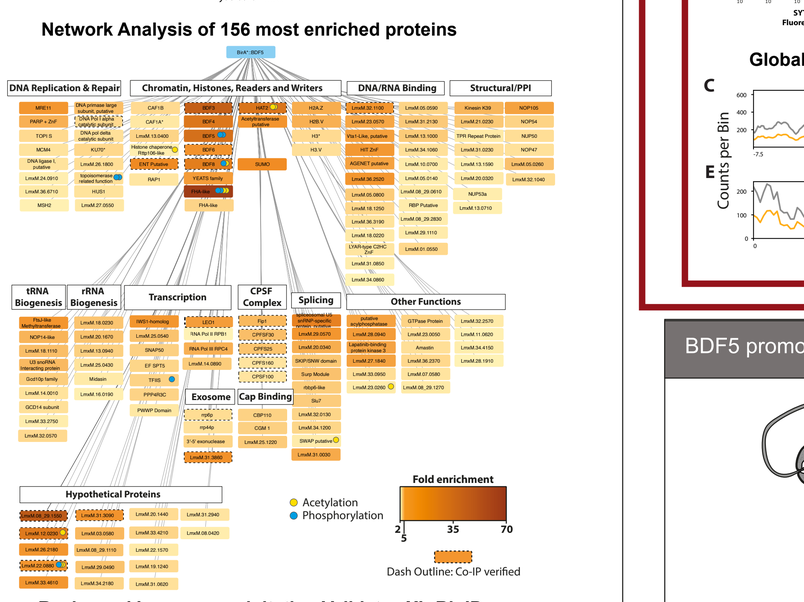
Proximity Proteomics

XL-BioID used to determine proximal proteome of BDF5

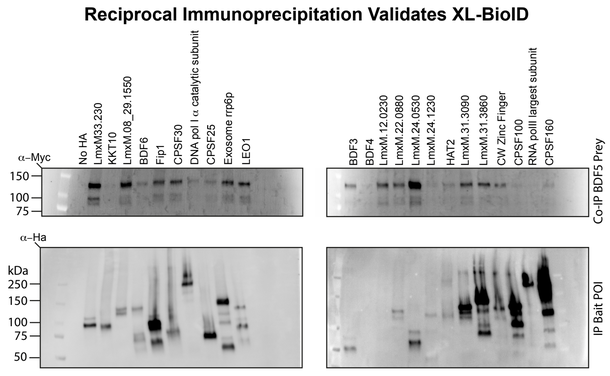
XL-BioID Workflow



Network Analysis of 156 most enriched proteins



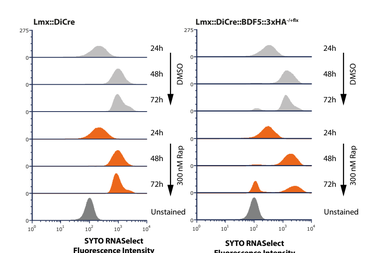
Reciprocal Immunoprecipitation Validates XL-BioID



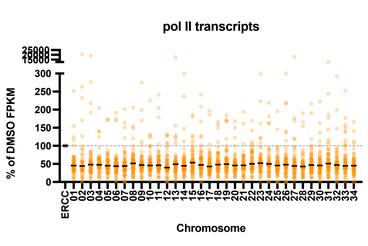
Total RNA-seq

Total RNA levels and transcription from pol II PTUs decreases after BDF5 deletion

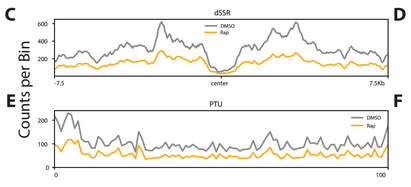
Total RNA levels reduced:SYTO RNA Select Stain



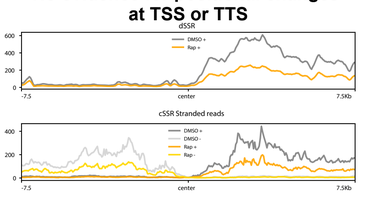
Spike-in normalised total RNA seq



Global reduction of pol II PTUs



No evidence for positional changes at TSS or TTS



BDF5 promotes pol II activity in a transcriptionally active "neighbourhood"