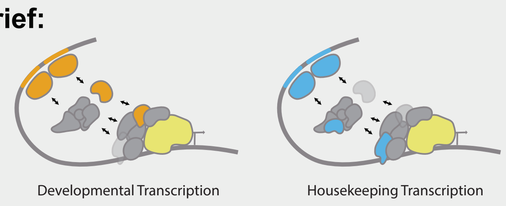
# Identifying cofactor dependencies of Drosophila enhancers by rapid inducible degradation

Oliver Hendy, Felix Muerdter, Katharina Schernhuber, Lukas Huber, Leonid Serebreni & Alexander Stark

Research Institute of Molecular Pathology (IMP), Vienna Biocenter (VBC), Campus Vienna Biocenter 1, 1030 Vienna, Austria

@OliverJHendy

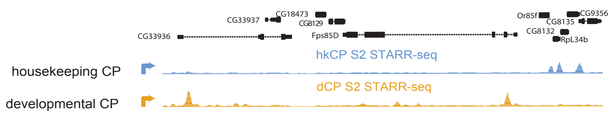
## In Brief:




- Drosophila enhancers and activators show specificty to developmental or housekeeping core promoters
- Using auxin inducible degradation, we test the requirement of several cofactors for enhancer activity.
- Brd9 and Chromator are developmental and housekeeping regulators, resepctively.

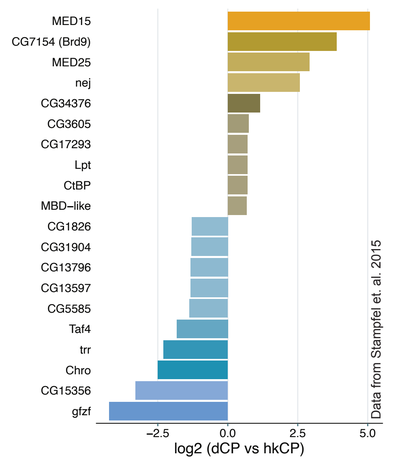
## 1. Introduction

Enhancers are regulatory elements that are bound by transcription factors, which in turn recruit cofactors to activate Pol II transcription at a core promoter. Enhancers cannot indiscriminately activate; in Drosophila, they show remarkable specificity between housekeeping or developmental core promoter types.

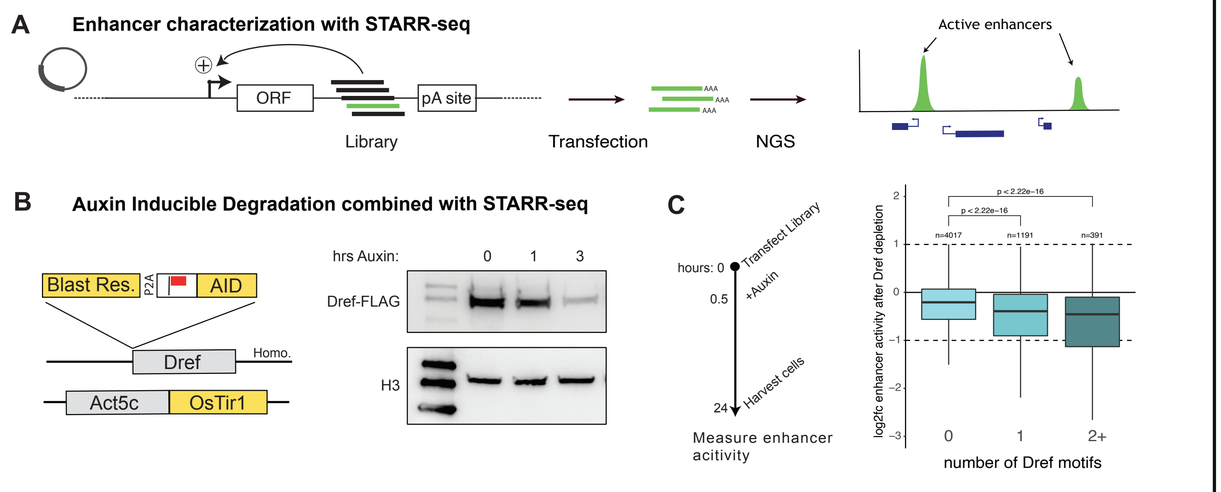



In turn, tethering transcriptional cofactors also activate promoters with selectivity. By fusing cofactors to a Gal4 DNA binding domain, we have shown that several cofactor show preferential core promoter activation when recruited to an upstream 4xUAS site (see right). To test whether these factors are not only sufficient, but also necessary, for specific enhancers, we employed a rapid loss of function approach combined with STARR-seq. We reveal global dependencies for the integral Mediator complex as well as Nejire (p300), and program specific roles of Brd7/9 and Chromator. Finally, investigation of chromatin remodelers uncovers a potential specificity of the SWI/SNF remodeler and the ISWI remodeler to developmental and housekeeping transcription, respectively.

**Cofactor preference for developmental (dCP) or housekeeping (hkCP) promoter activation**

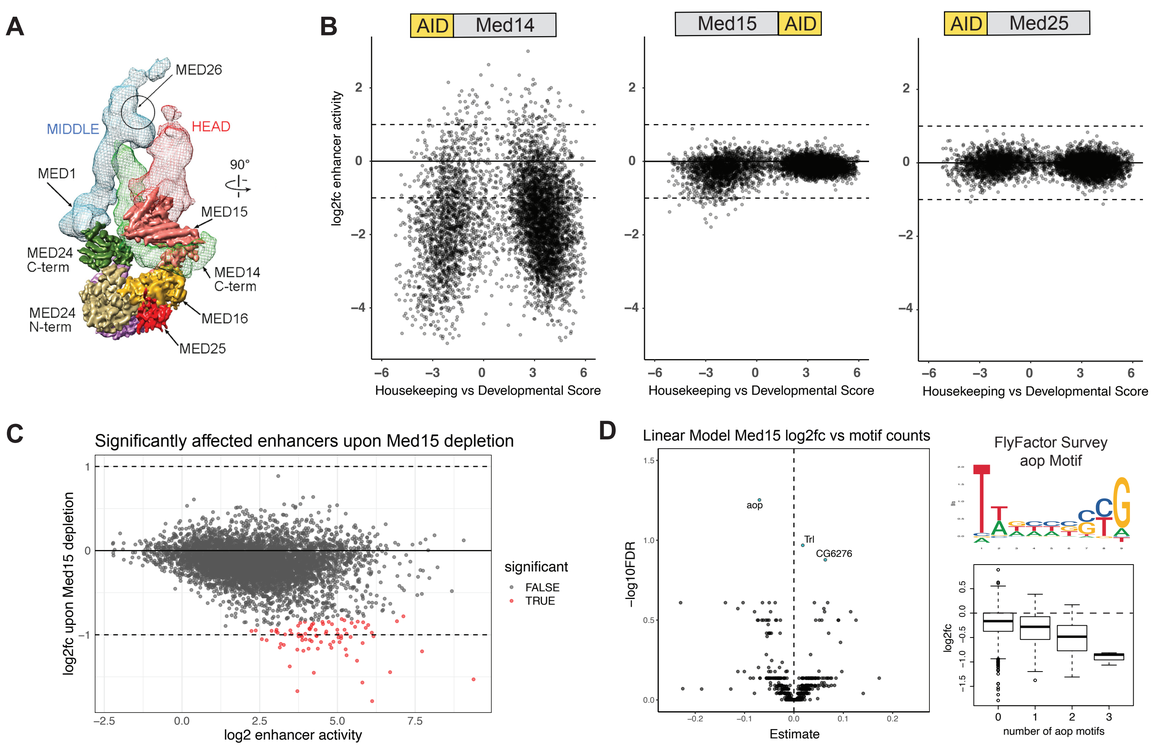



## 2. AID STARR-seq uncovers direct enhancer dependencies



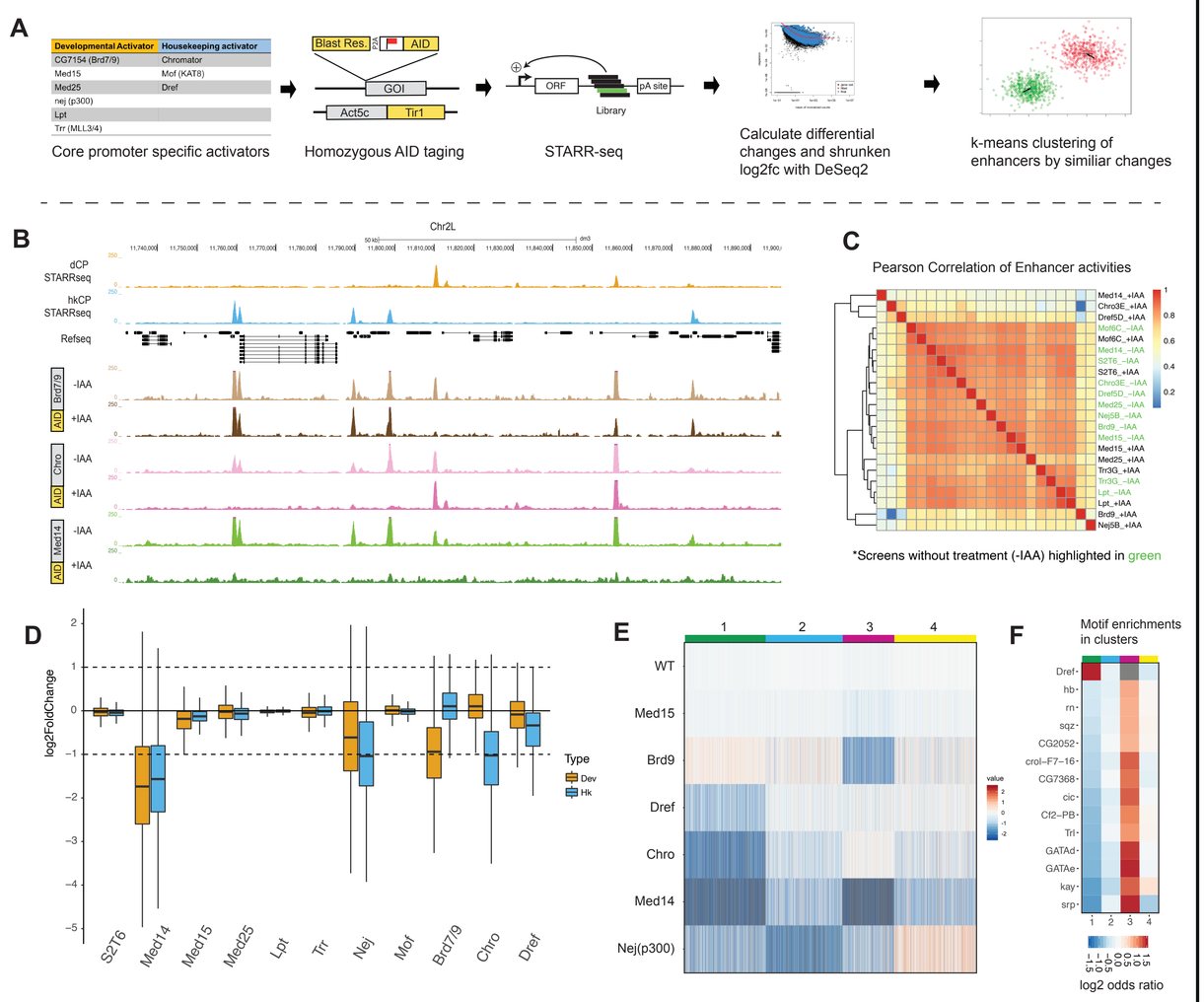

**A)** STARR-seq reporter assay overview. **B)** Auxin Inducible Degradation Strategy. AID is integrated into parental Schneider 2 cell line expressing OsTir1 from a fusion to the endogenous Act5c locus. The AID tag is knocked into the endogenous locus of a gene of interest homozygously, using PITCh Integration system. Treatment with auxin degrades a majority of the protein within 3 hours of treatment **C)** Combining STARR-seq with depletion of Dref selectively deactivates enhancers with 1 or 2 Dref motifs.

## 3. Global and enhancer specific roles of Mediator Complex subunits



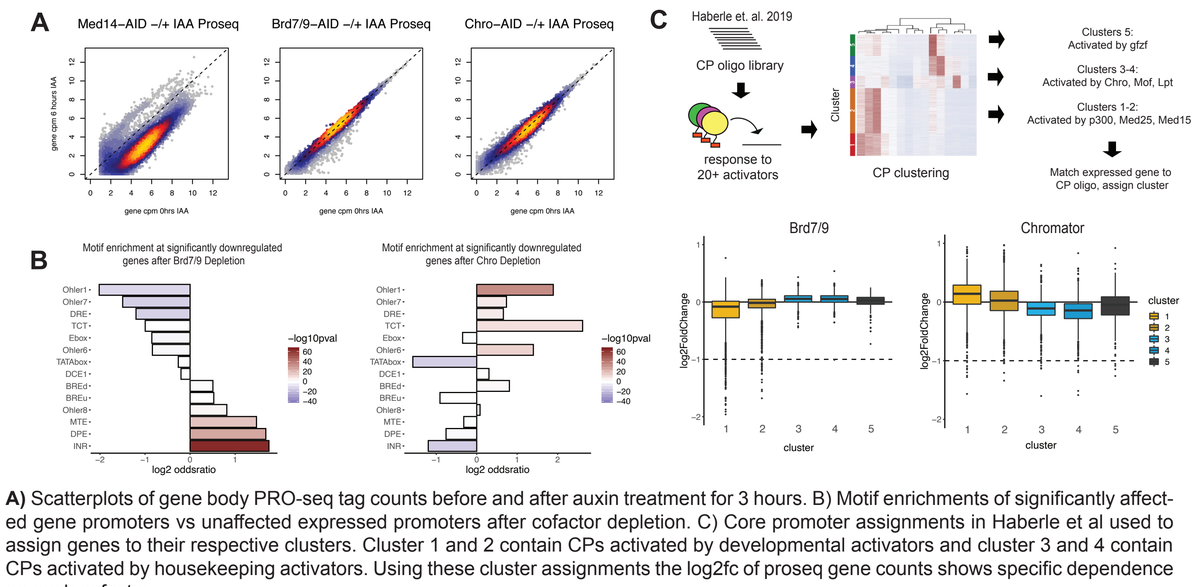

A) Location of AID tagged mediator subunits on the human mediator complex. CryoEM structure adapted from Khattabi et. al. Cell 2019 B) Changes in enhancer activity after depletion of the indicated mediator subunits. Shrunken log2fc from DeSeq2 used. C) Differential analysis of Med15 after auxin treatment with significantly affected enhancers colored. (padj<0.05) D) Linear model of log2fc after Med15 depletion vs motif counts from 468 FlyFactorSurvey Motifs. log2fc after Med15 depletion of enhancers binned by number of aop motifs

## 4. Cofactor Dependencies differ between Housekeeping and Developmental transcription



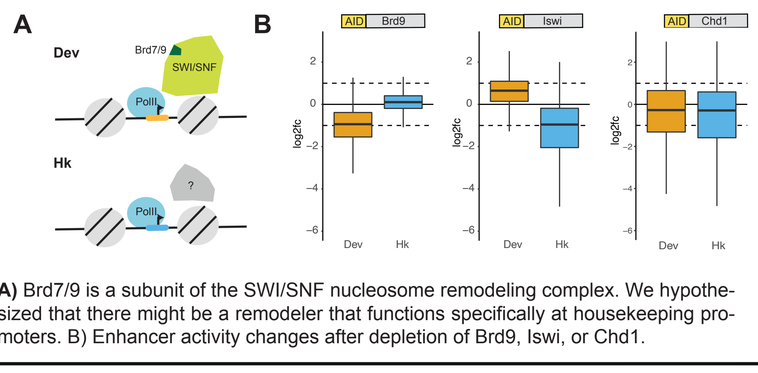

**A)** Overview of the cofactor dependency screen. B) Screenshot of selected STARRseq tracks. C) Pearson correlation between enhancer enrichments and hierarchical clustering of replicate pooled cofactor screens. D) Changes in housekeeping and developmental enhancer activity after cofactor depletion. Shrunken log2fc from DeSeq2 differential analysis used. E) K-means clustering of enhancers by log2fc across cofactor screens with at least 50 significantly affected enhancers. F) Motif enrichment of each cluster.

## 5. Endogenous gene dependencies revealed by Precision Run On Sequencing (PRO-seq)




**A)** Scatterplots of gene body PRO-seq tag counts before and after auxin treatment for 3 hours. B) Motif enrichments of significantly affected gene promoters vs unaffected expressed promoters after cofactor depletion. C) Core promoter assignments in Haberle et al used to assign genes to their respective clusters. Cluster 1 and 2 contain CPs activated by developmental activators and cluster 3 and 4 contain CPs activated by housekeeping activators. Using these cluster assignments the log2fc of proseq gene counts shows specific dependence on each cofactor.

## 6. Remodeler dependencies in the Hk vs Dev program




**A)** Brd7/9 is a subunit of the SWI/SNF nucleosome remodeling complex. We hypothesized that there might be a remodeler that functions specifically at housekeeping promoters. B) Enhancer activity changes after depletion of Brd9, Iswi, or Chd1.

## 7. Summary

**A.** Combining AID with STARRseq allows identification of primary transcriptional targets of TFs/cofactors

**B.** Mediator Complex Integrity is required for transcription genome wide. Med15 is required at a subset of developmental enhancers

**C.** Brd9 and Chromator are specifically required for Developmental and Housekeeping transcription, respectively

**D.** Depletion of chromatin remodelers suggests that SWI/SNF and Iswi act on distinct sets of promoters/enhancers.

## References

Stampfel, Gerald, et al. "Transcriptional regulators form diverse groups with context-dependent regulatory functions." Nature 528.7580 (2015): 147-151.
El Khattabi, L., Zhao, H., Kalchschmidt, J., Young, N., Jung, S., Van Blerkom, P., ... & Krebs, J. (2019). A pliable mediator acts as a functional rather than an architectural bridge between promoters and enhancers. Cell, 178(5), 1145-1158.
Haberle, V., Arnold, C. D., Pagani, M., Rath, M., Schernhuber, K., & Stark, A. (2019). Transcriptional cofactors display specificity for distinct types of core promoters. Nature, 570(7759), 122-126.
Sakuma, T., Nakade, S., Sakane, Y., Suzuki, K. I. T., & Yamamoto, T. (2016). MMEJ-assisted gene knock-in using TALENs and CRISPR-Cas9 with the PITCh systems. Nature protocols, 11(1), 118.
Nishimura, K., Fukagawa, T., Takisawa, H., Kakimoto, T., & Kanemaki, M. (2009). An auxin-based degron system for the rapid depletion of proteins in nonplant cells. Nature methods, 6(12), 917.
Zabidi, M. A., Arnold, C. D., Schernhuber, K., Pagani, M., Rath, M., Frank, O., & Stark, A. (2015). Enhancer–core-promoter specificity separates developmental and housekeeping gene regulation. Nature, 518(7540), 556-559.

## Acknowledgements

MaxCyte, Inc. (Jason Marks, Erik Jans, Peer Heine) for support with efficient transfection. Deep sequencing was performed at the VBCF Next-Generation Sequencing Unit. F.M. was supported by an EMBO long-term fellowship (EMBO ALTF 491–2014). Research in the Stark group is supported by the European Research Council (ERC) (grant agreement no. 647320) and by the Austrian Science Fund (FWF, F4303-B09). Basic research at the IMP is supported by Boehringer Ingelheim GmbH and the Austrian Research Promotion Agency