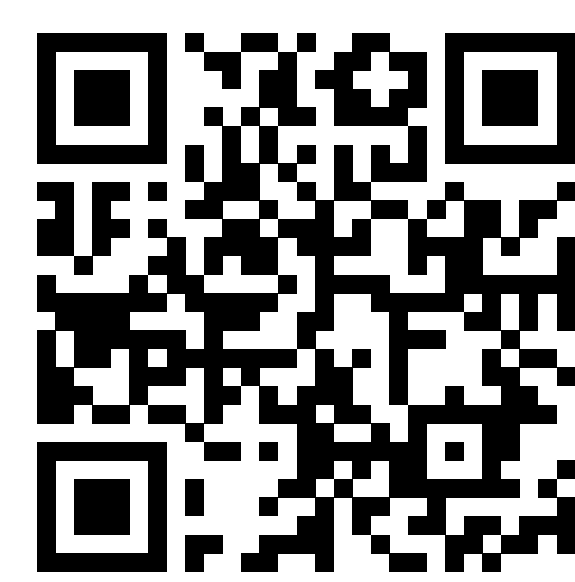


Normalizr: Inferring single-cell differential and co-expression

Package & preprint →

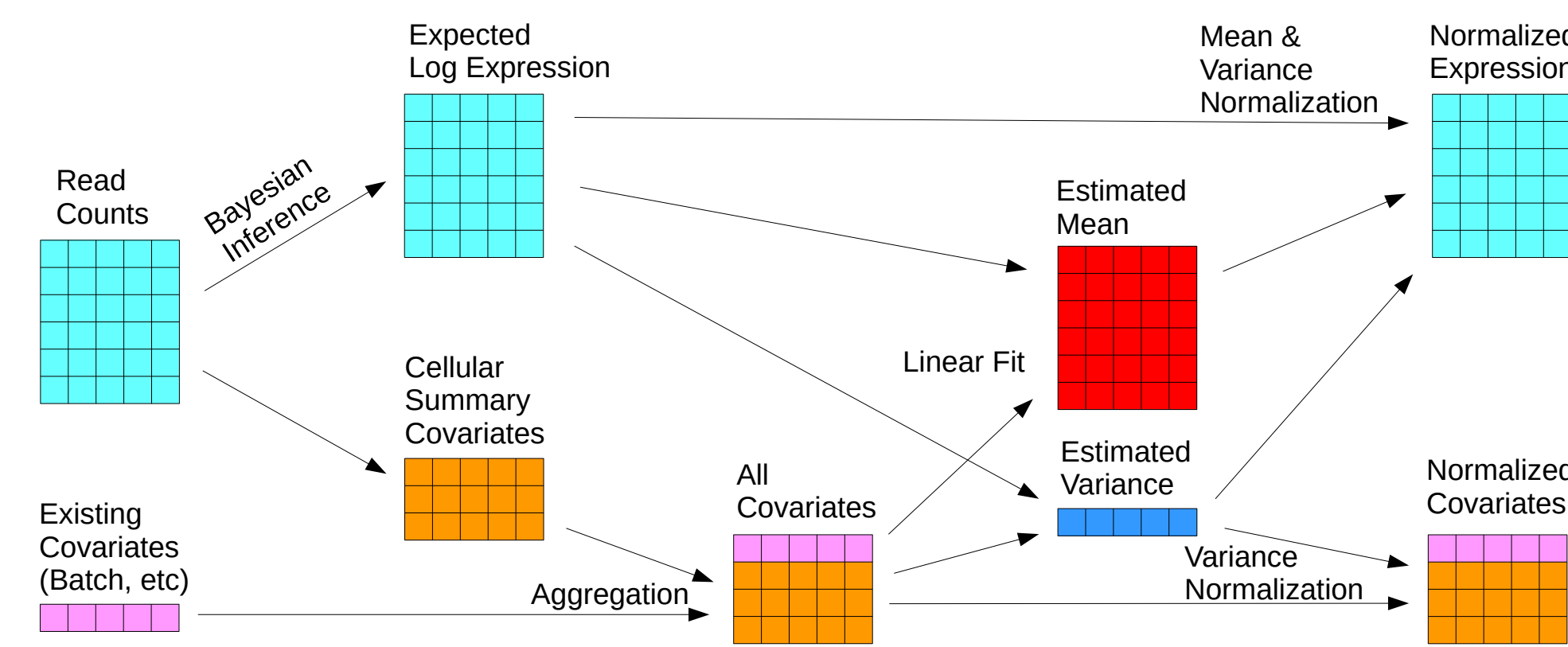


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1. Schematic overview

ScRNA-seq may provide unprecedented statistical power and cell-type specificity for the interrogation of molecular pathways at individual gene or whole-transcriptome levels. We present Normalizr, a two-step normalization-association inferential framework that unifies single-cell differential and co-expression with linear association testing. With posterior mRNA abundances, nonlinear cellular summary covariates, and mean and variance normalization, Normalizr outperforms existing methods and recovers biological insights.

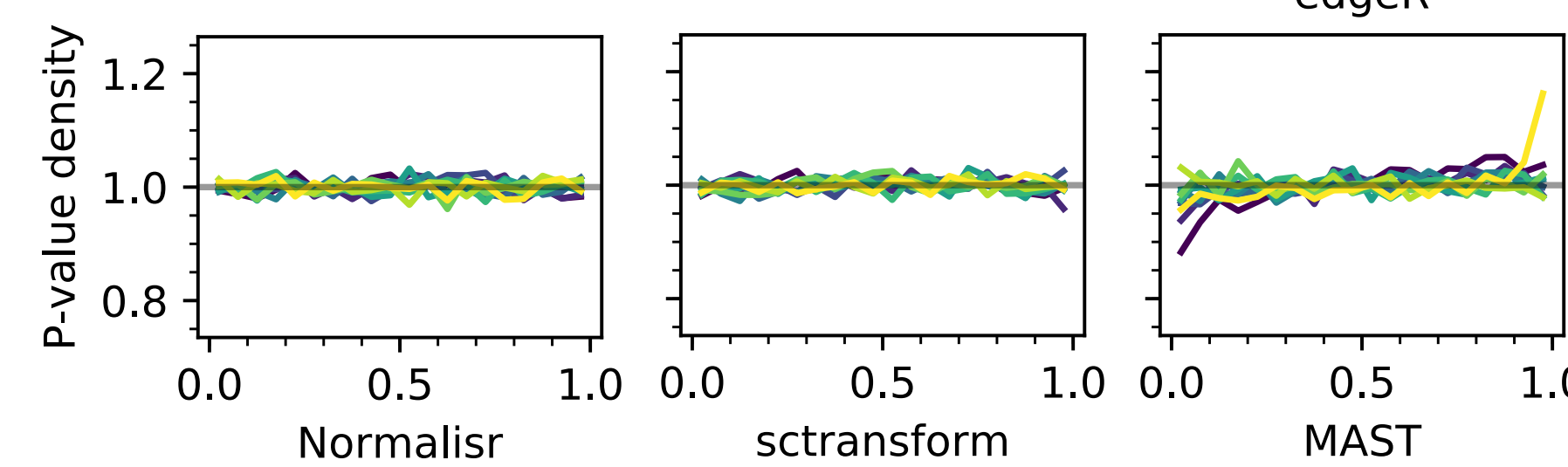


Linear association model: $y = \alpha x + \beta C + \varepsilon$
 y : Normalized expression
 C : Normalized covariates
 x : For differential expression:
 • Grouping
 For co-expression:
 • Normalized expression

2. Normalizr has optimal sensitivity, specificity, scalability, and reproducibility [1]

Differential expression

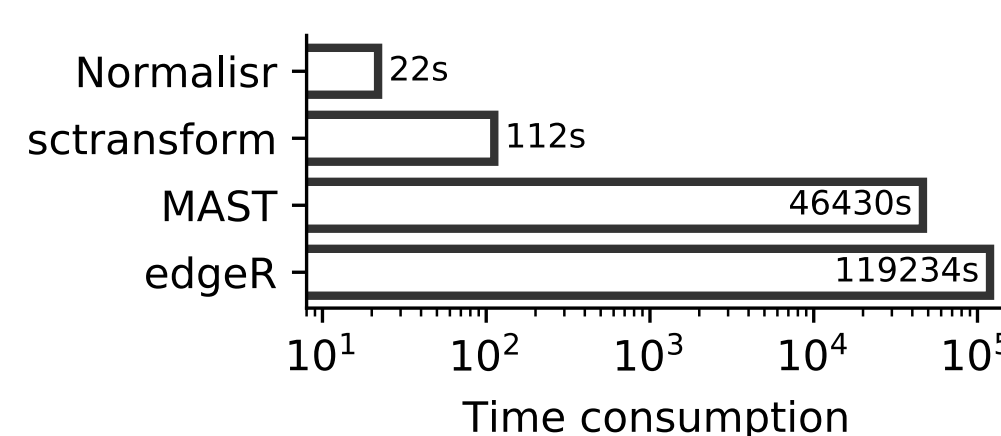
Specificity: Uniform null P-values on random cell splits



Sensitivity:

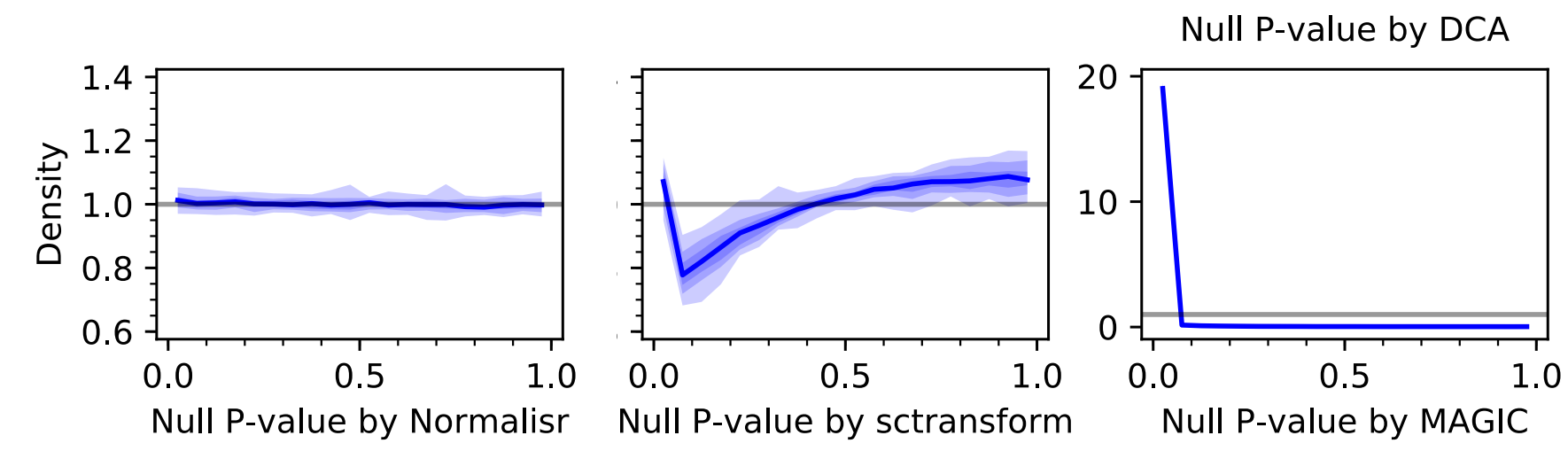
P-value significance in CRISPRi gRNA-targeted genes (positive controls)

Scalability:



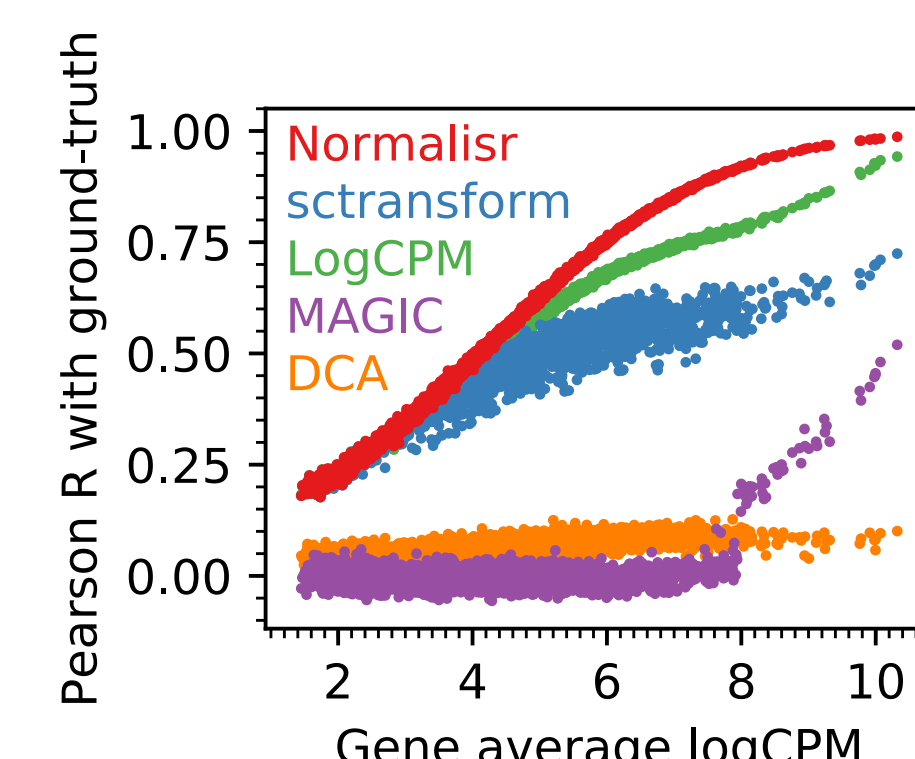
Co-expression

Specificity: Uniform null P-values on synthetic co-expression-less dataset



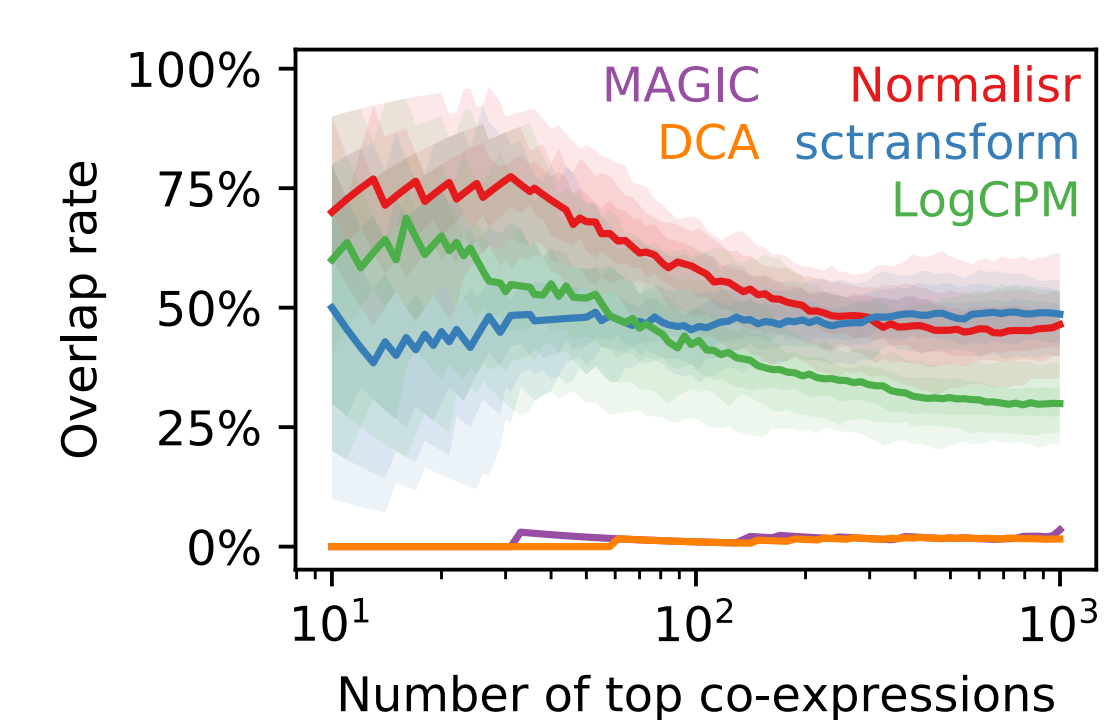
Expression recovery

Recovery of biological variations in synthetic expression

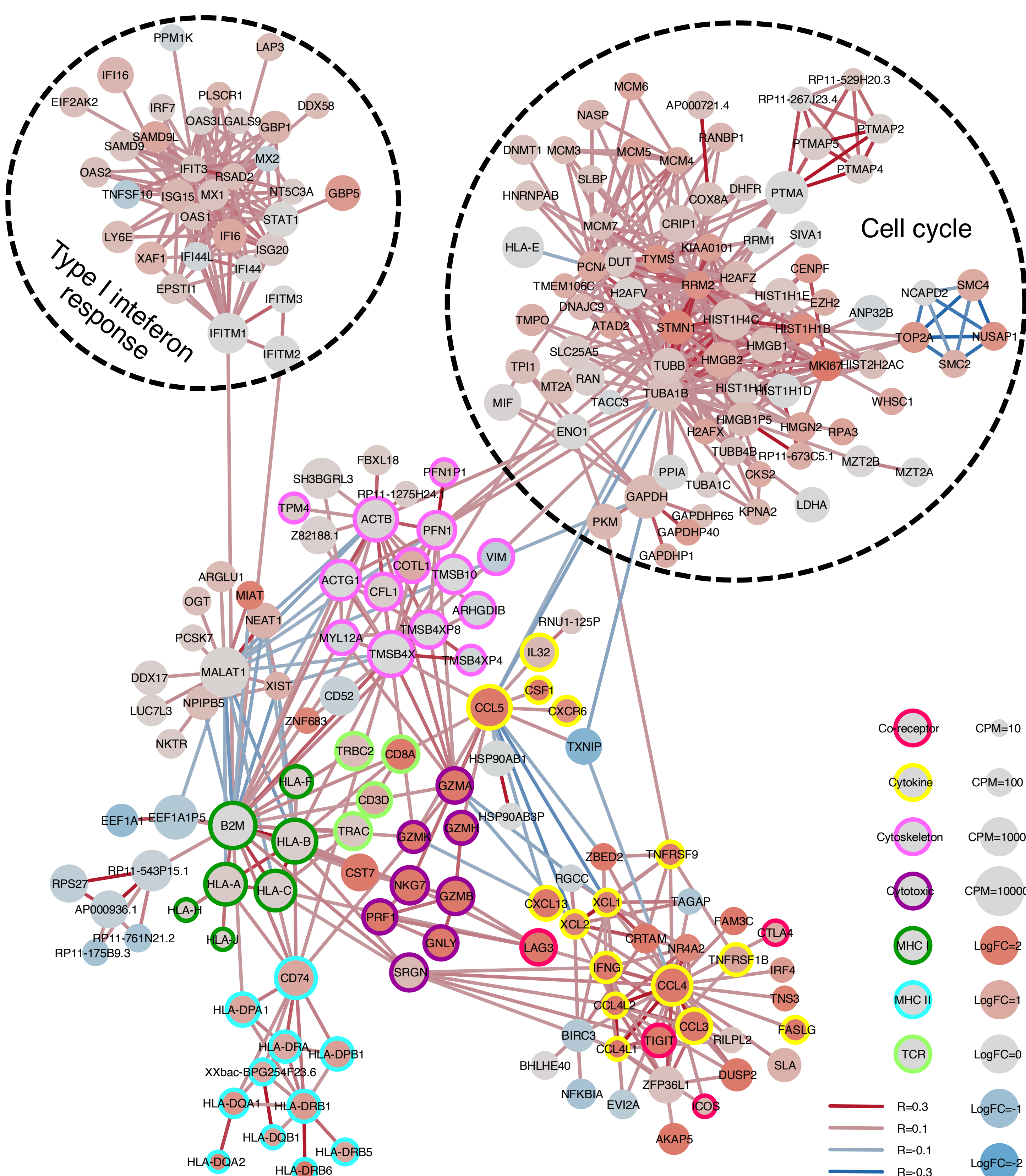


Reproducibility:

Overlap of top co-expressions between sequencing channels



3. Co-expression in dysfunctional T cells in melanoma [2]

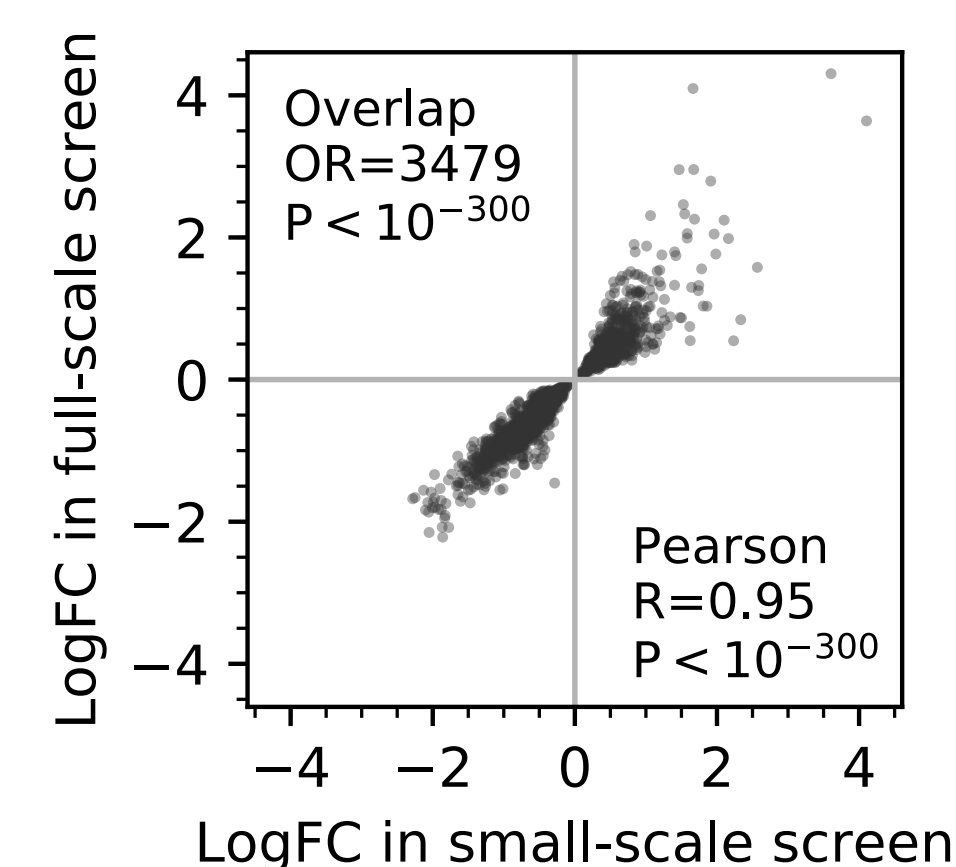


4. Gene regulations from high-MOI CRISPRi scRNA-seq [3]

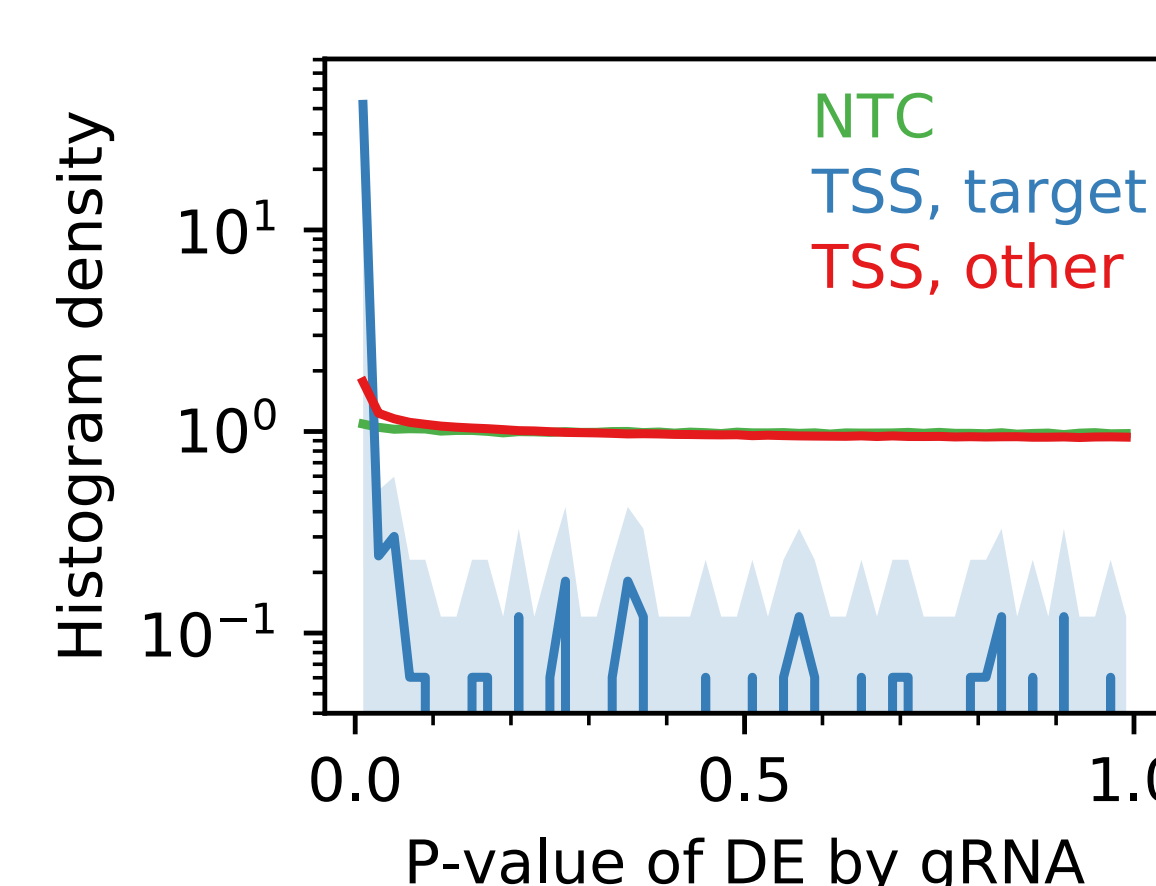
Pooled high-MOI CRISPRi scRNA-seq screen

Each cell contains multiple (e.g. ~20) random CRISPRi gRNAs from pooled transduction. ScRNA-seq obtains CRISPRi and transcriptome information simultaneously for DE analyses.

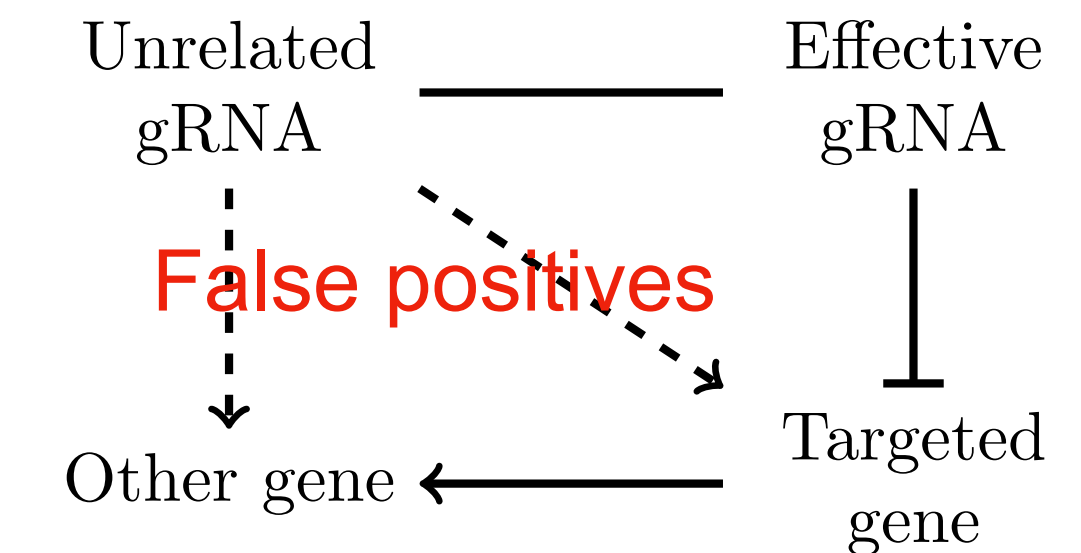
Reproducible gRNA-gene associations



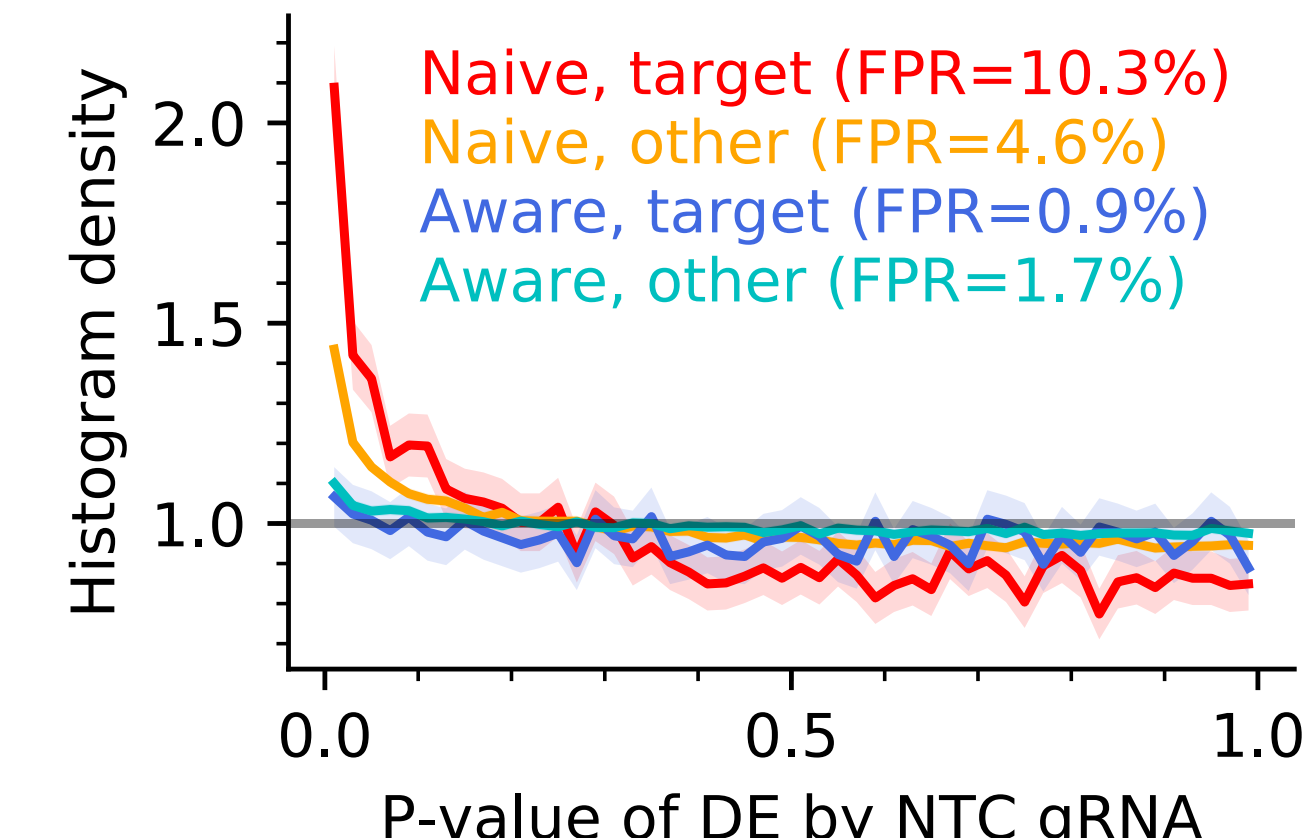
Inferring gene regulations as gRNA trans-effects (TSS, other)



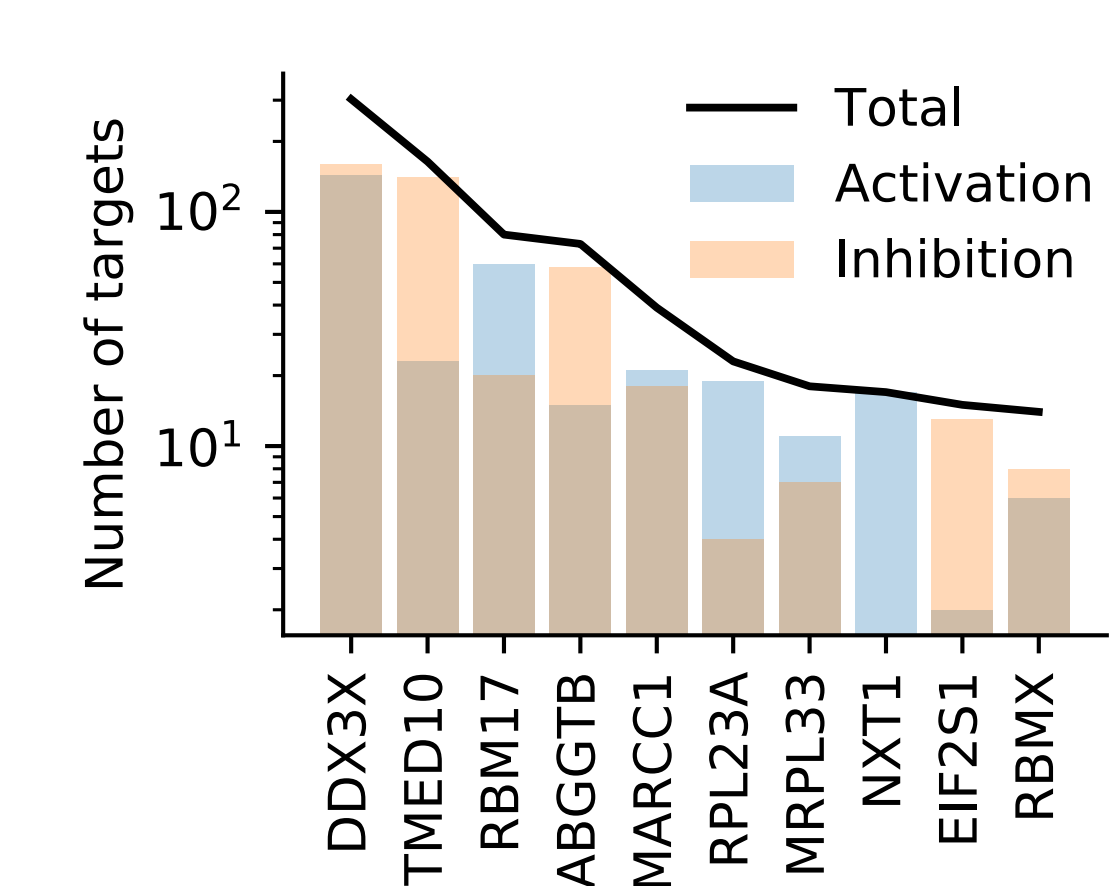
False positives from gRNA competition: Hypothesis



Confirmation with competition-naïve method and accounting for them with competition-aware method



Functional preferences of top regulators



5. Conclusion

Normalizr unified single-cell differential expression and co-expression with linear association testing with optimal sensitivity, specificity, and speed. Normalizr reliably revealed co-regulations and functional roles in high-quality, gene-level co-expression networks and inferred gene regulations in CRISPRi screen of single-cell differential expression. High-MOI CRISPRi screens suffer from false positives from gRNA competition that can be statistically accounted for. ScRNA-seq has the potential to become a partial but better substitute for bulk RNA-seq from *in vitro* to cohort levels.

Contact

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References

[1] Adamson et al. Cell (2016); [2] Li et al. Cell (2019); [3] Gasperini et al. Cell (2019).