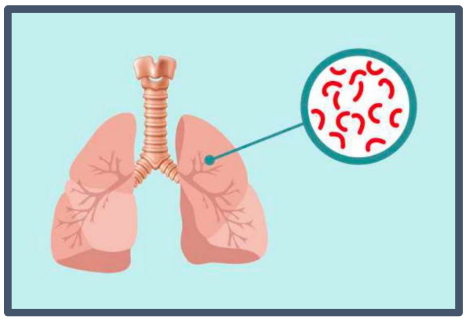
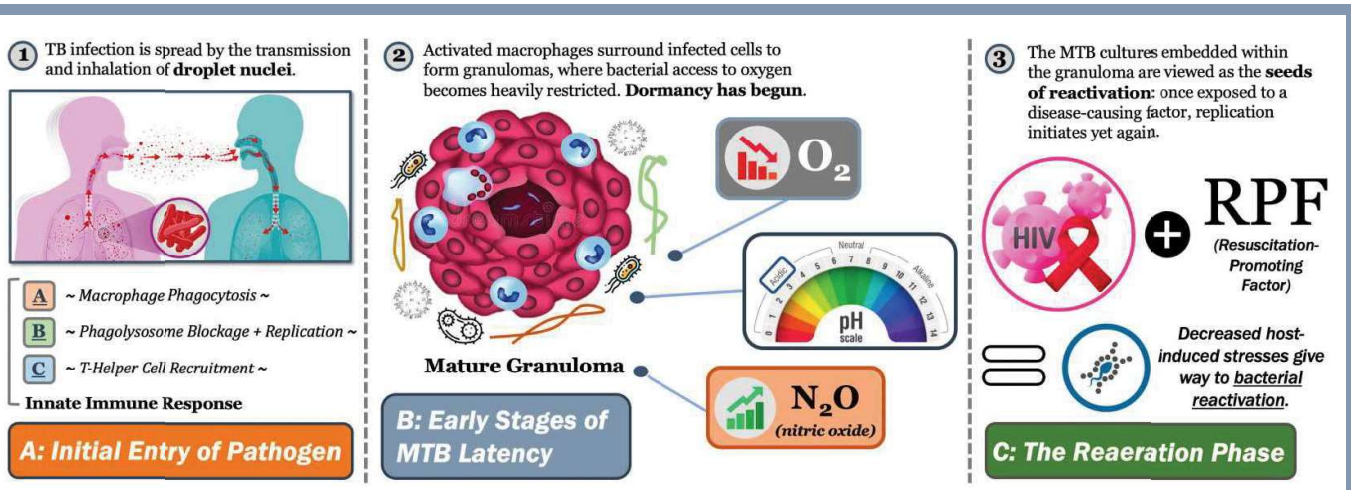
Deciphering a Sleeping Pathogen: Uncovering Novel Transcriptional Regulators of Hypoxia-Induced Dormancy in Mycobacterium Tuberculosis

Background: Introducing Mycobacterium Tuberculosis

Tuberculosis (TB): Bacterial Infection

- Spread through the inhalation of cough/sneeze droplets.
- As a result of this invasion...
  - Foreign bacteria invade host immune system.
  - Trigger airway inflammation in lungs.
  - Spread to other organs in the human body.





Project Premise: Hypoxia-Induced Dormancy

Purpose: Outlining the Research Problem

#3

Deletion of TFs thought crucial to dormancy only conferred **mild growth defects**.

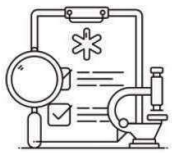
Several modeling techniques have been used to simulate oxygen depletion, which makes **synthesizing findings** considerably difficult.

Experimental attempts at directed gene disruption and protein localization give way to **questionable results**.

Current understanding of the MTB genetic architecture is **highly insufficient**.

Modeling TB infection can be more rigorously achieved with a **computational approach**.

Goal: To uncover transcriptional agents and regulatory mechanisms that control the transition of MTB in and out of dormancy.



#1: Literature Review	#2: Key Takeaways	#3: Research Objective

High-Level Methodology

1

Compose an **aggregate hypoxia dataset** from several RNA-seq and microarray experiments in vivo.

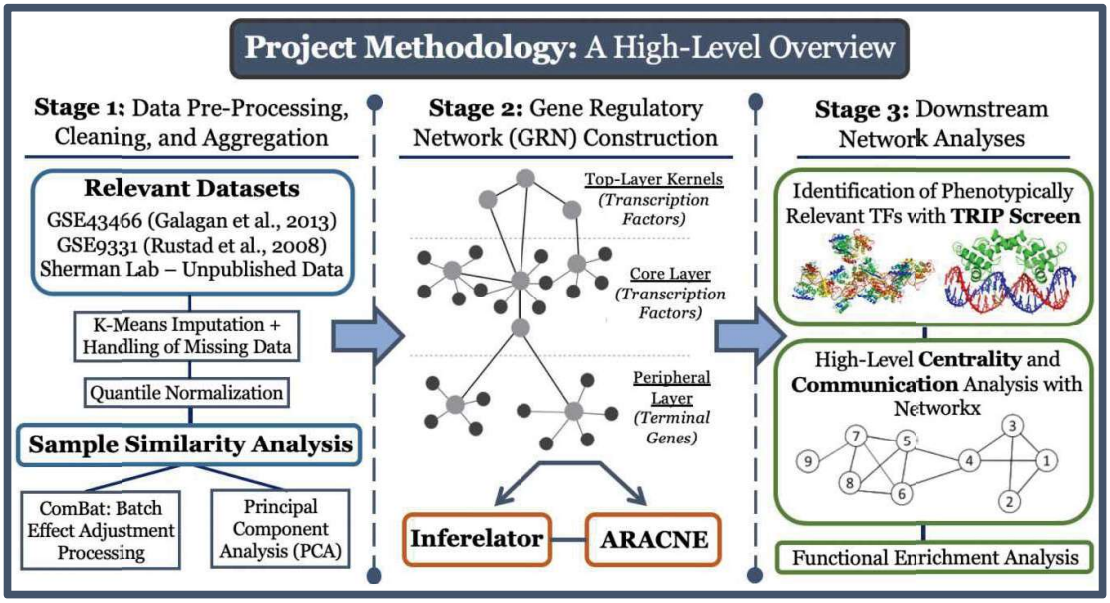
2

Infer a **gene regulatory network (GRN)** based on these observations.

3

Apply **downstream analyses** to unearth interesting transcriptional dynamics.

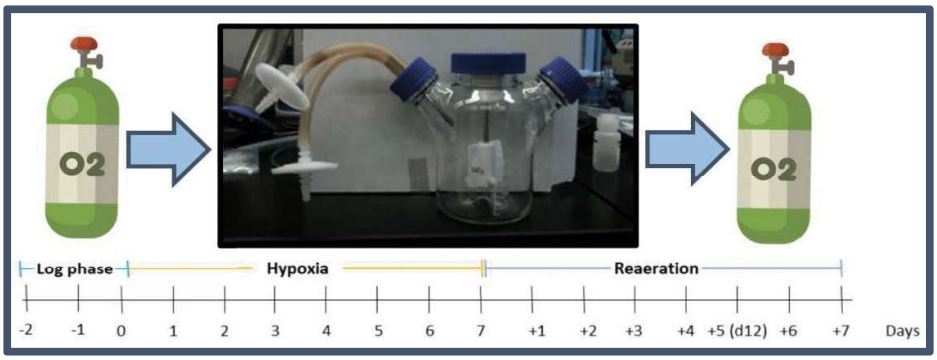
The Three-Phase Approach

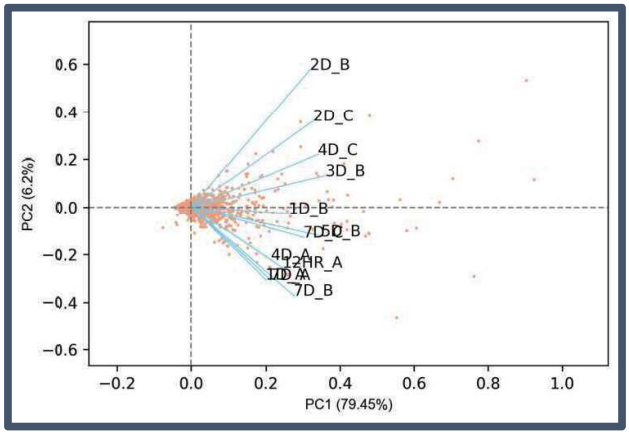


Data Collection

Three Transcriptome Datasets (Hypoxic Time Course Experiments):

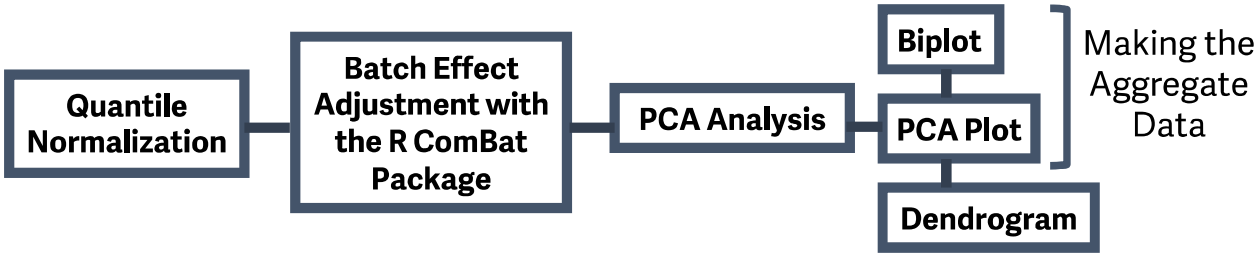
- GSE43466 [Rustad et al., 2008]
- GSE9331 [Galagan et al., 2013]
- Unpublished Study @ UW Sherman Lab





Aggregation Results

The Data Processing Workflow



Incorporating the TRIP Dataset

Hypoxia TRIP Screen:

- ❑ Tracked 207 TFI Strains under several forms of environmental stress.
- ❑ Abundance Fold Change (Uninduced v. Induced).
- ❑ **Method:** Comparisons between log-phase abundance FCs to those at hypoxia and reaeration treatment.

Goal: Identify phenotypically relevant TFs that undergo significant growth abundances or defects in the transition from steady-state to hypoxic conditions.

* ***Log2FC >= 1.5*** *used as the cutoff for statistical significance.*

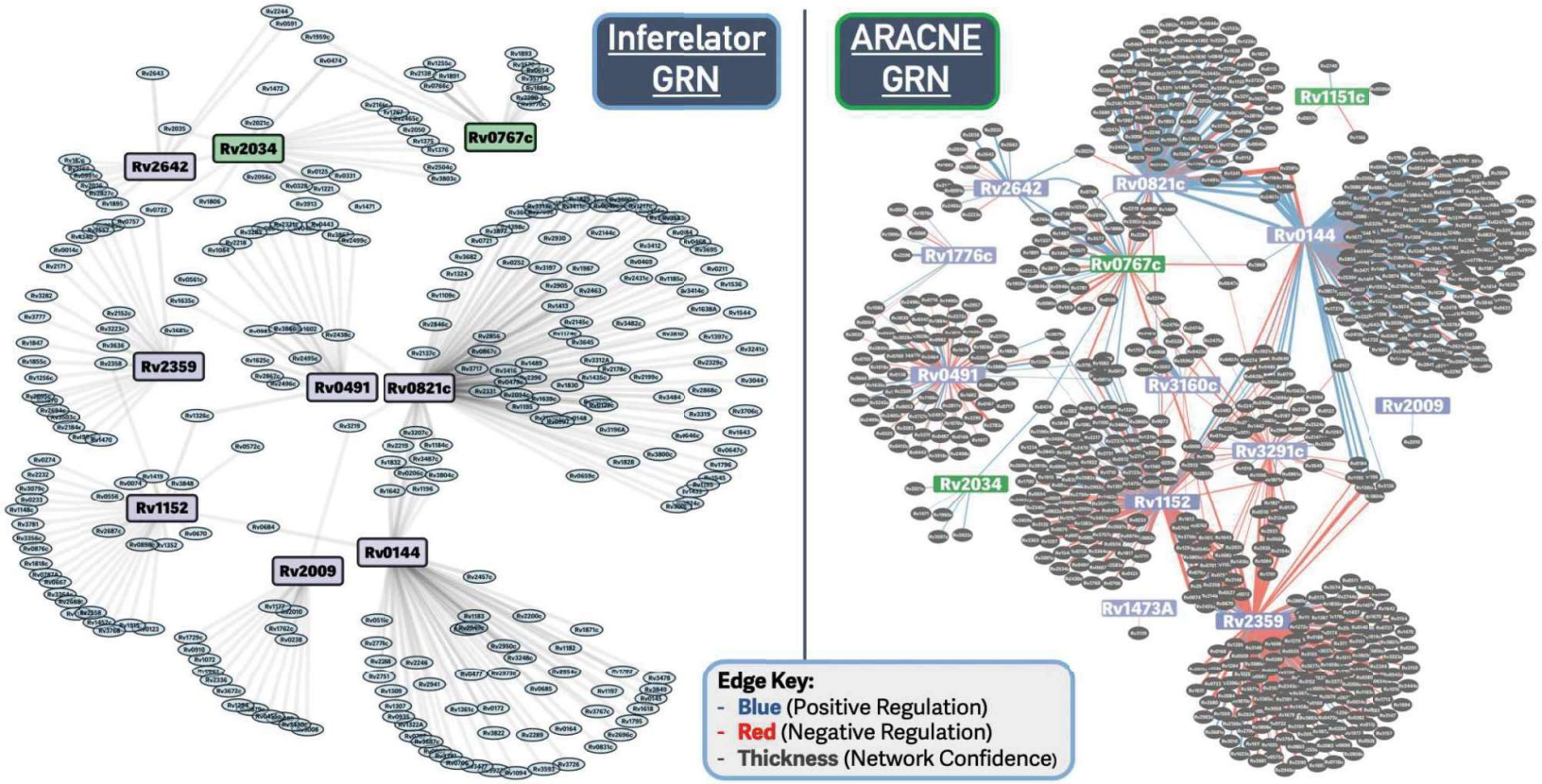
TRIP Data Analysis

The set of growth abundance (GA) and growth defect (GD) TFs associated with hypoxia.

Regulator	UT_FC	HYP_FC	OVR_FC	Class
Rv0767c	-6.02	1.909	7.929	GA
Rv2034	-2.31	1.88	4.19	GA
Rv1151c	-0.17	1.62	1.79	GA
Rv1776c	-6.09	-1.61	4.479	GD
Rv2642	1.13	-1.689	2.819	GD
Rv2009	0.6	-1.81	2.41	GD
Rv2359	0.45	-1.909	2.359	GD
Rv1152	0.49	-1.869	2.359	GD
Rv1473A	0.77	-1.57	2.34	GD
Rv0821c	0.56	-1.57	2.13	GD
Rv3291c	0.46	-1.609	2.069	GD
Rv0491	0.37	-1.63	2.0	GD
Rv0144	0.5	-1.5	2.0	GD
Rv3160c	0.23	-1.65	1.88	GD

UT=Untreated, **HYP**=Hypoxia, **OVR**=ABS(UT-HYP)

Visualizing the Hypoxia-Specific Gene Regulatory Networks



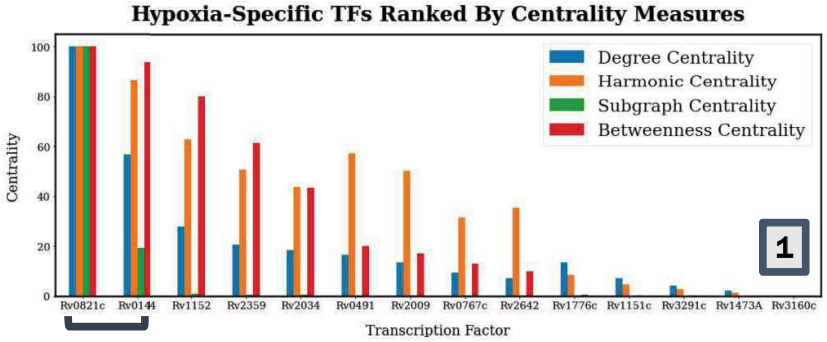
Looking into the Functional Roles of Network Genes

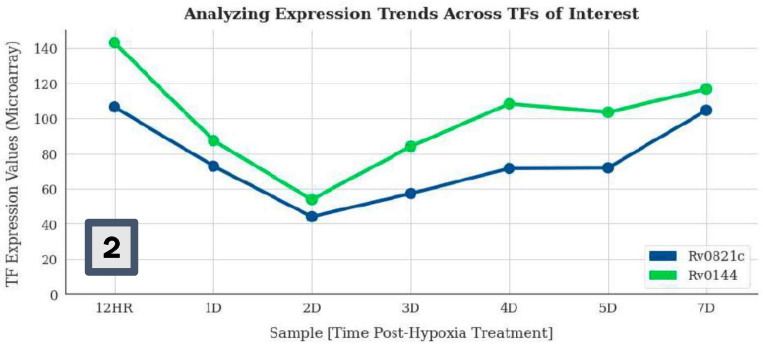
GO Term	Overlap	P-Value	Genes	Phenotypic Relevance
Peptidoglycan Biosynthetic Process	8/15	0.003567	Rv2154c; Rv1086; Rv3682; Rv3794; Rv2152c; Rv0483; Rv0050; Rv1018c.	The peptidoglycan layer is essential for maintaining cellular integrity and forming a permeability barrier.
Proton-Transporting ATP Synthase Activity	6/8	0.034982	Rv1309; Rv1311; Rv1307; Rv1310; Rv1308; Rv1306.	Protonmotive force is required for maintaining ATP homeostasis and viability of hypoxic MTB.
Cell Redox Homeostasis	5/12	0.002969	Rv1470; Rv1471; Rv0688; Rv1324; Rv1677.	Preservation of an appropriate redox balance is critical to the persistence of MTB.
Fatty Acid Biosynthetic Process	7/17	0.048612	Rv3825c; Rv1484; Rv2524c; Rv0533c; Rv1094; Rv2244; Rv2246.	Macrophage fatty acid metabolism is needed to supplement MTB survival in hypoxia.
Response to Stress	8/14	0.013853	Rv3223c; Rv2028c; Rv3134c; Rv2374c; Rv2624c; Rv0576; Rv0982; Rv2035.	An indicator that bacteria are sensing and adapting to the anaerobic environment.

** Enrichment analysis was performed with the Enrichr API of GSEAPy; an adjusted **P-Value cutoff of <= 0.05** was used to determine statistical significance.*

Investigating the Rv0821c-Rv0144 Crosstalk

#9





Shared Target Components Between Rv0821c and Rv0144

Target	Functional Description	Category
Rv1184c	Essential for PAT lipid biosynthesis, which is a significant constituent of the **mycobacterial cell wall**.	*Cell Wall and Cell Processes*
Rv0206c	MmpL3 protein is a transmembrane transporter of mycolic acid; long chain fatty acids found in the **lipid-rich cell walls** of tuberculosis bacterium.	*Cell Wall and Cell Processes*
Rv3804c	Refers to proteins of the antigen 85 complex that contribute to the biogenesis of trehalose dimycolate, a dominant structure required for **cell wall integrity**.	*Lipid Metabolism*
Rv3487c	Lipolytic enzyme LipF involved in cellular metabolism.	*Intermediary Metabolism and Respiration*
Rv2219	Probable conserved transmembrane protein.	*Cell Wall and Cell Processes*
Rv1832	Glycine cleavage system that catalyzes the degradation of glycine, which has been implicated in the biosynthesis of peptidoglycan and other **cell wall structural components**.	*Intermediary Metabolism and Respiration*
Rv1196	Resembles PPE18, a **cell wall associated protein** that is involved in inflammatory response and cytokine manipulation.	*PE/PPE*

3

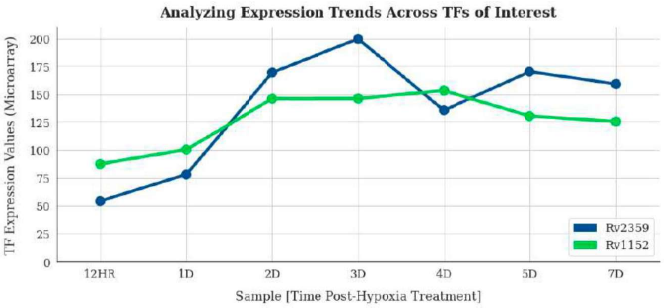
Rv0821c (PhoY2): Inactivation leads to antibiotic resistance; maintains inorganic phosphate homeostasis; stress response.

Rv0144: Shown to be regulated by RelA, critical for establishing persistent infection in mice.

Takeaway: A dual mechanism of mycobacterial persistence linked to cell wall synthesis and intracellular transport.

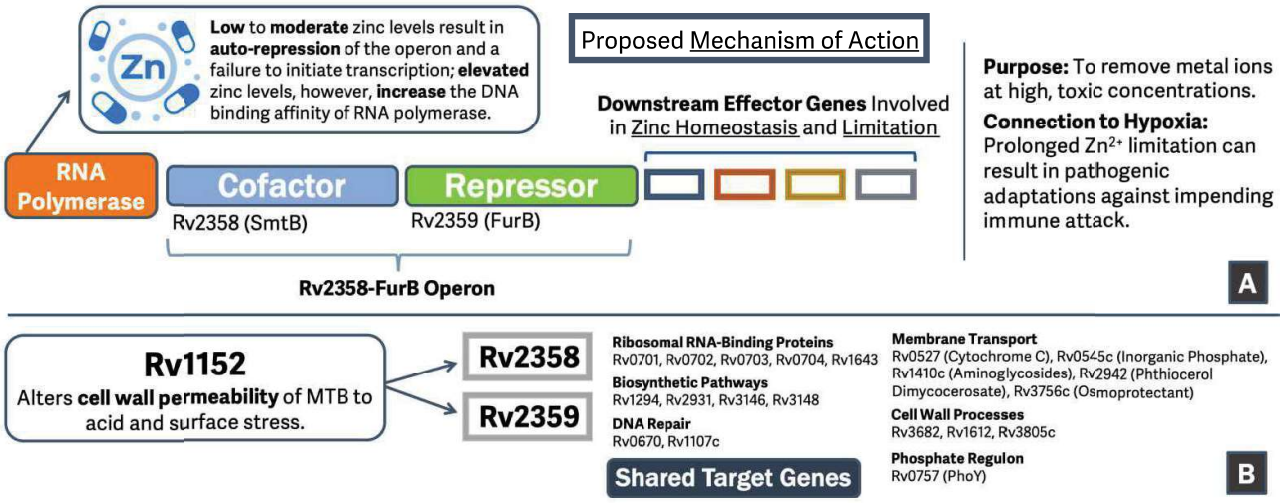
Characterizing the Rv2359-Rv1152 Relationship

#10



Regulator	UT_FC	HYP_FC	OVR_FC	Class
Rv2359	0.45	-1.909	2.359	GD
Rv1152	0.49	-1.869	2.359	GD

Strong expression- and phenotypic-based correlations, along with GRN connectivity, indicate a **potential relationship**.



The Rv1152-Rv2359 connection could function as a **metal ion-respondent homeostasis mechanism** that is effectively downregulated during hypoxia.

As a result, pathogen would have more time to make **anticipatory adaptations** to future host immune response and build resistance to oxidative stress.

Discussion & Conclusion

Results

- MTB dormancy in hypoxia shown to be functionally associated with stress response, cell redox homeostasis, metal ion cycling, and cell wall metabolism – all of which modulate critical **host-pathogen interactions**.
- Unraveling Transcriptional Regulatory Mechanisms
  - **Rv0821c-Rv0144:** Dual System of Persistence (Via Cell Wall Synthesis)
  - **Rv1152-Rv2359:** Delayed Zinc Limitation Enables Anticipatory Adaptations
- Investigating Key Factors of Interest
  - Nutritional Immunity
  - Defense Antioxidants Counter Pro-Inflammatory Cytokines

Future Directions

- **Incorporate reaeration data** (7D to 12D) to catalog other physiological adjustments during reintroduction to the stationary phase.
- Apply the **DREM 2.0 approach**, which identifies bifurcation points that track transitions between coordinated regulatory programs and gene states.

Limitations

Experimental Data Restricted to the **Defined Hypoxic Model**

Lack of **Gold Standard Data** to Supplement GRN Construction

References

Abdi, H., & Williams, L. J. (2010). Principal component analysis. *Wiley interdisciplinary reviews: computational statistics*, *2*(4), 433-459.

Balázsi, G., Heath, A. P., Shi, L., & Gennaro, M. L. (2008). The temporal response of the Mycobacterium tuberculosis gene regulatory network during growth arrest. *Molecular systems biology*, *4*(1), 225.

Brennan, P. J. (2003). Structure, function, and biogenesis of the cell wall of Mycobacterium tuberculosis. *Tuberculosis*, *83*(1-3), 91-97.

Canneva, F., Branzoni, M., Riccardi, G., Provvedi, R., & Milano, A. (2005). Rv2358 and FurB: two transcriptional regulators from Mycobacterium tuberculosis which respond to zinc. *Journal of bacteriology*, *187*(16), 5837-5840.

Castro, M. A., Wang, X., Fletcher, M. N., Markowetz, F., & Meyer, K. B. (2013). Vignette for RTN: reconstruction of transcriptional networks and analysis of master regulators.

Dahl, J. L., Kraus, C. N., Boshoff, H. I., Doan, B., Foley, K., Avarbock, D., ... & Barry III, C. E. (2003). The role of RelMtb-mediated adaptation to stationary phase in long-term persistence of Mycobacterium tuberculosis in mice. *Proceedings of the National Academy of Sciences*, *100*(17), 10026-10031.

Dow, A., Sule, P., O'Donnell, T. J., Burger, A., Mattila, J. T., Antonio, B., ... & Prisic, S. (2021). Zinc limitation triggers anticipatory adaptations in Mycobacterium tuberculosis. *PLoS pathogens*, *17*(5), e1009570.

Emmert-Streib, F., Dehmer, M., & Haibe-Kains, B. (2014). Gene regulatory networks and their applications: understanding biological and medical problems in terms of networks. *Frontiers in cell and developmental biology*, *2*, 38.

Fang, Z., Liu, X., & Peltz, G. (2022). GSEApy: a comprehensive package for performing gene set enrichment analysis in Python. *Bioinformatics*.

Galagan, J. E., Minch, K., Peterson, M., Lyubetskaya, A., Azizi, E., Sweet, L., ... & Schoolnik, G. K. (2013). The Mycobacterium tuberculosis regulatory network and hypoxia. *Nature*, *499*(7457), 178-183.

Harper, J., Skerry, C., Davis, S. L., Tasneen, R., Weir, M., Kramnik, I., ... & Jain, S. K. (2012). Mouse model of necrotic tuberculosis granulomas develops hypoxic lesions. *Journal of Infectious Diseases*, *205*(4), 595-602.

Johnson, W. E., Li, C., & Rabinovic, A. (2007). Adjusting batch effects in microarray expression data using empirical Bayes methods. *Biostatistics*, *8*(1), 118-127.

Kapopoulou, A., Lew, J. M., & Cole, S. T. (2011). The MycoBrowser portal: a comprehensive and manually annotated resource for mycobacterial genomes. *Tuberculosis*, *91*(1), 8-13.

Ma, S., Morrison, R., Hobbs, S. J., Soni, V., Farrow-Johnson, J., Frando, A., ... & Sherman, D. R. (2021). Transcriptional regulator-induced phenotype screen reveals drug potentiators in Mycobacterium tuberculosis. *Nature microbiology*, *6*(1), 44-50.

Manjelievskaia, J., Erck, D., Piracha, S., & Schrager, L. (2016). Drug-resistant TB: deadly, costly and in need of a vaccine. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, *110*(3), 186-191.

Margolin, A. A., Nemenman, I., Basso, K., Wiggins, C., Stolovitzky, G., Favera, R. D., & Califano, A. (2006, March). ARACNE: an algorithm for the reconstruction of gene regulatory networks in a mammalian cellular context. In *BMC bioinformatics* (Vol. 7, No. 1, pp. 1-15). BioMed Central.

Milano, A., Branzoni, M., Canneva, F., Profumo, A., & Riccardi, G. (2004). The mycobacterium tuberculosis rv2358--furb operon is induced by zinc. *Research in microbiology*, *155*(3), 192-200.

Mora, B. B., Cirtwill, A. R., & Stouffer, D. B. (2018). pymfinder: a tool for the motif analysis of binary and quantitative complex networks. *BioRxiv*, 364703.

Muttucumaru, D. N., Roberts, G., Hinds, J., Stabler, R. A., & Parish, T. (2004). Gene expression profile of Mycobacterium tuberculosis in a non-replicating state. *Tuberculosis*, *84*(3-4), 239-246.

Namugenyi, S. B., Aagesen, A. M., Elliott, S. R., & Tischler, A. D. (2017). Mycobacterium tuberculosis PhoY proteins promote persister formation by mediating Pst/SenX3-RegX3 phosphate sensing. *MBio*, *8*(4), e00494-17.

Park, H. D., Guinn, K. M., Harrell, M. I., Liao, R., Voskuil, M. I., Tompa, M., ... & Sherman, D. R. (2003). Rv3133c/dosR is a transcription factor that mediates the hypoxic response of Mycobacterium tuberculosis. *Molecular microbiology*, *48*(3), 833-843.

Peddireddy, V., Doddam, S. N., & Ahmed, N. (2017). Mycobacterial dormancy systems and host responses in tuberculosis. *Frontiers in immunology*, *8*, 84.

Peterson, E. J., Reiss, D. J., Turkarslan, S., Minch, K. J., Rustad, T., Plaisier, C. L., ... & Baliga, N. S. (2014). A high-resolution network model for global gene regulation in Mycobacterium tuberculosis. *Nucleic acids research*, *42*(18), 11291-11303.

Rao, Y., Lee, Y., Jarjoura, D., Ruppert, A. S., Liu, C. G., Hsu, J. C., & Hagan, J. P. (2008). A comparison of normalization techniques for microRNA microarray data. *Statistical applications in genetics and molecular biology*, *7*(1).

Russell, D. G. (2001). Mycobacterium tuberculosis: here today, and here tomorrow. *Nature reviews Molecular cell biology*, *2*(8), 569-578.

Rustad, T. R., Harrell, M. I., Liao, R., & Sherman, D. R. (2008). The enduring hypoxic response of Mycobacterium tuberculosis. *PloS one*, *3*(1), e1502.

Rustad, T. R., Sherrid, A. M., Minch, K. J., & Sherman, D. R. (2009). Hypoxia: a window into Mycobacterium tuberculosis latency. *Cellular microbiology*, *11*(8), 1151-1159.

Shi, W., & Zhang, Y. (2010). PhoY2 but not PhoY1 is the PhoU homologue involved in persisters in Mycobacterium tuberculosis. *Journal of Antimicrobial Chemotherapy*, *65*(6), 1237-1242.

Skok Gibbs, C., Jackson, C. A., Saldi, G. A., Tjärnberg, A., Shah, A., Watters, A., ... & Bonneau, R. (2022). High-performance single-cell gene regulatory network inference at scale: the Inferelator 3.0. *Bioinformatics*, *38*(9), 2519-2528.

Voskuil, M. I., Bartek, I. L., Visconti, K., & Schoolnik, G. K. (2011). The response of Mycobacterium tuberculosis to reactive oxygen and nitrogen species. *Frontiers in microbiology*, *2*, 105.

Wang, C., Mao, Y., Yu, J., Zhu, L., Li, M., Wang, D., ... & Gao, Q. (2013). PhoY2 of mycobacteria is required for metabolic homeostasis and stress response. *Journal of bacteriology*, *195*(2), 243-252.

Wang, X., Wang, H., & Xie, J. (2011). Genes and regulatory networks involved in persistence of Mycobacterium tuberculosis. *Science China Life Sciences*, *54*(4), 300-310.

Zeng, J., Deng, W., Yang, W., Luo, H., Duan, X., Xie, L., ... & Xie, J. (2016). Mycobacterium tuberculosis Rv1152 is a novel GntR family transcriptional regulator involved in intrinsic vancomycin resistance and is a potential vancomycin adjuvant target. *Scientific reports*, *6*(1), 1-12