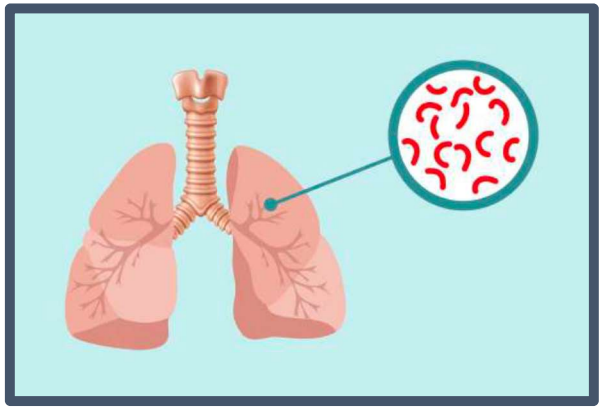
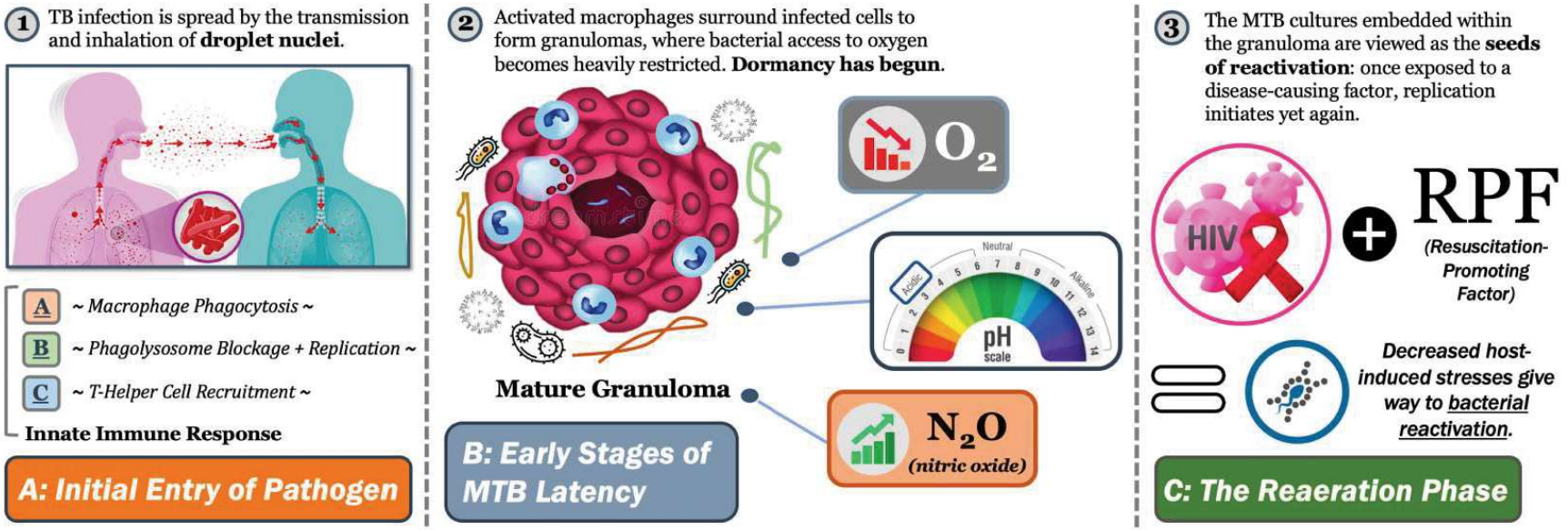
Deciphering a Sleeping Pathogen: Uncovering Novel Transcriptional Regulators of Hypoxia-Induced Dormancy in Mycobacterium Tuberculosis

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

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

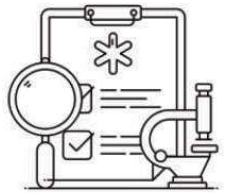
Several modeling techniques have been used to simulate oxygen depletion, which makes **synthesizing findings** considerably difficult.

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.

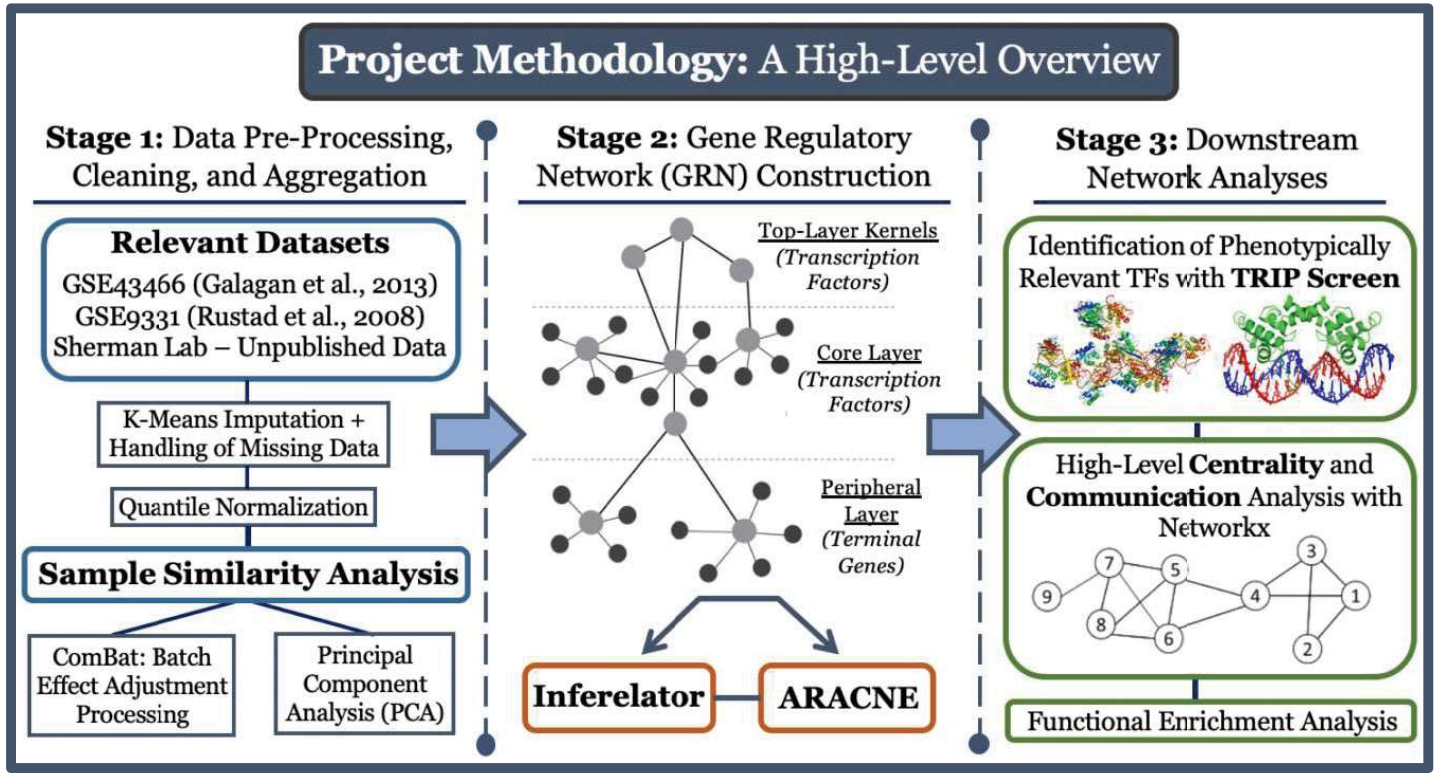
2

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

3

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

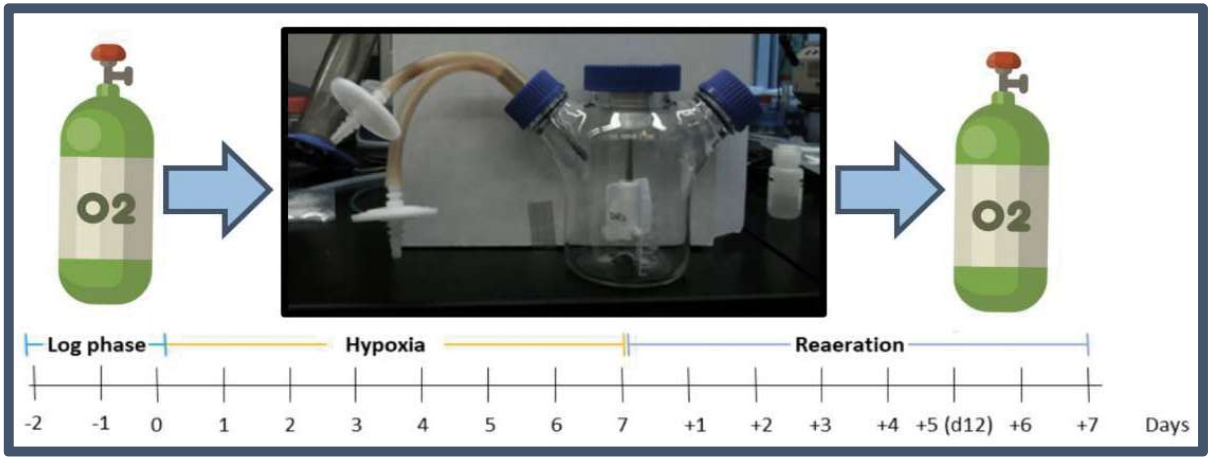
The Three-Phase Approach

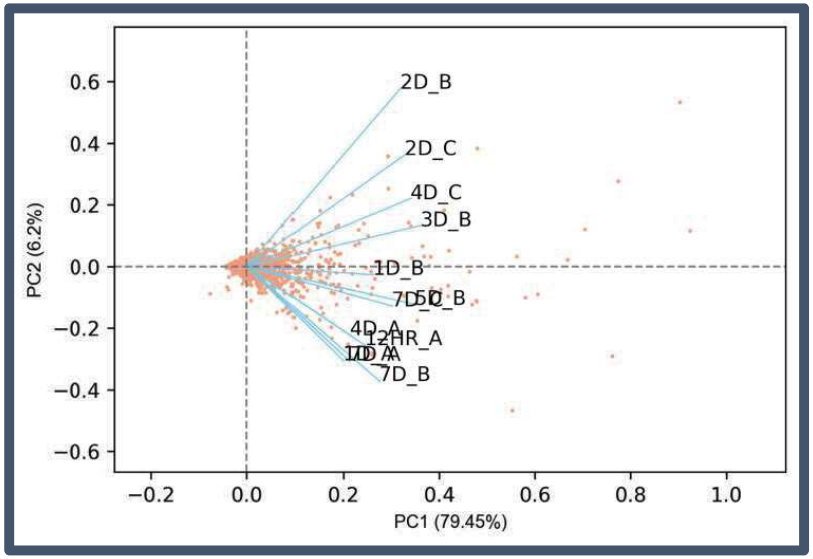


Data Collection

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

Quantile Normalization → **Batch Effect Adjustment with the R ComBat Package** → **PCA Analysis** → **Biplot** / **PCA Plot** / **Dendrogram**

Making the Aggregate Data

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 <u>phenotypically relevant TFs</u> 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.*

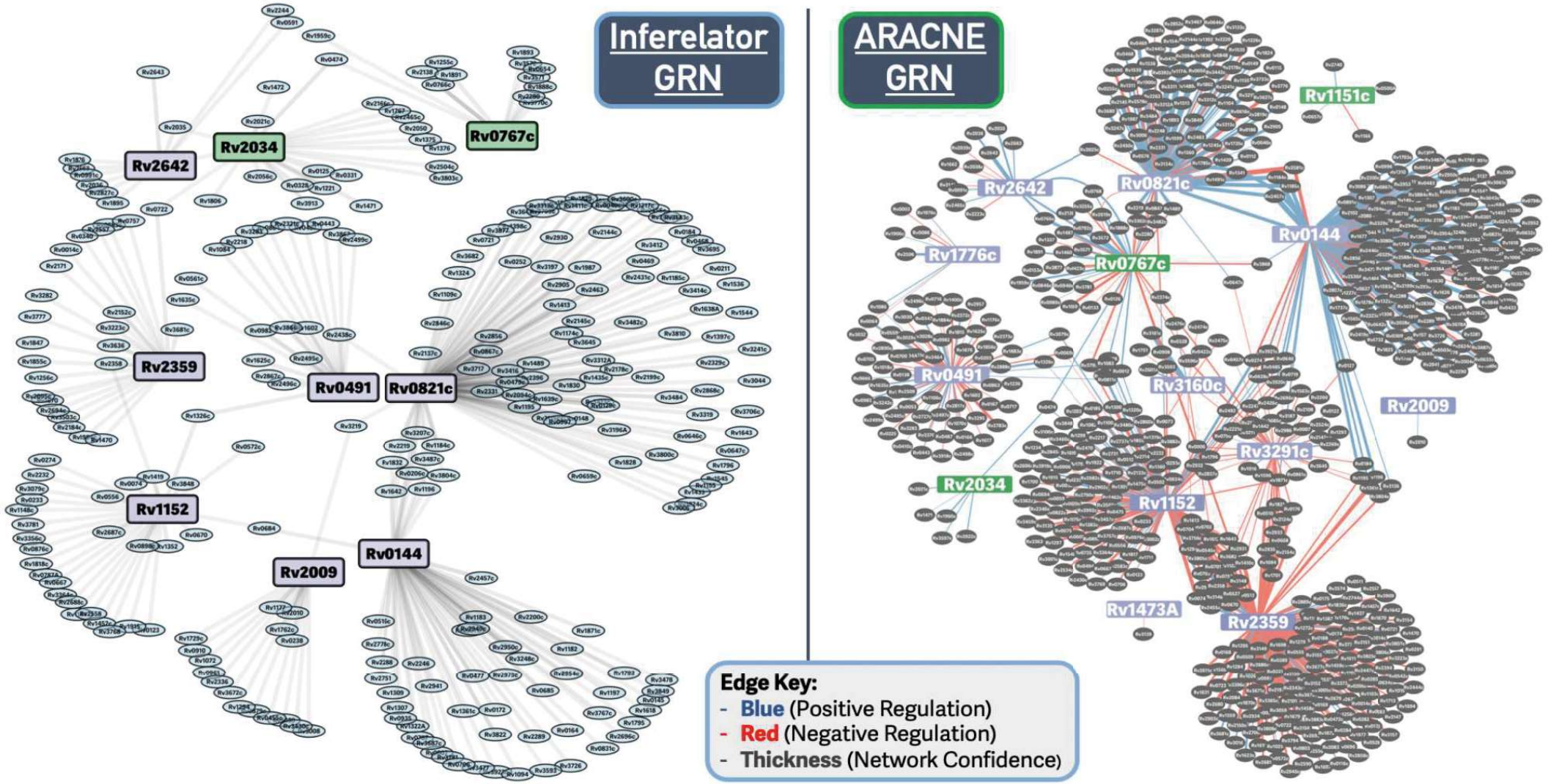
TRIP Data Analysis

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

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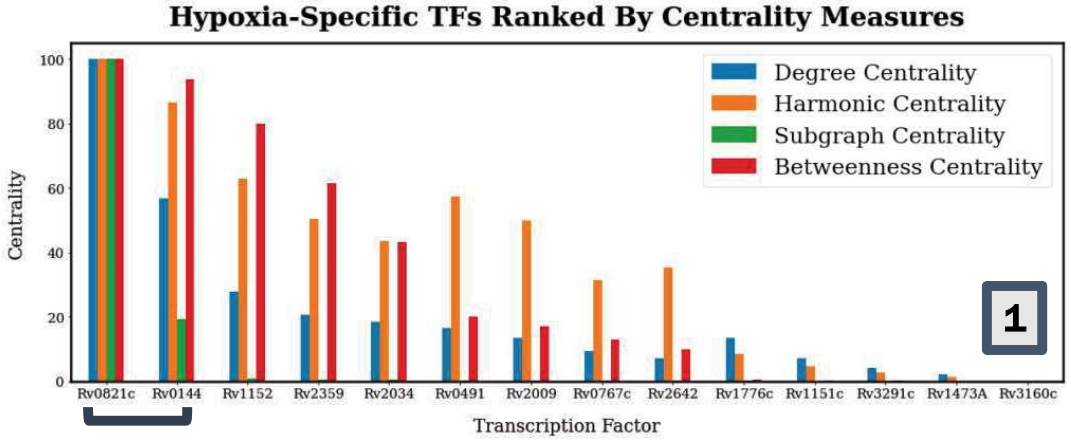
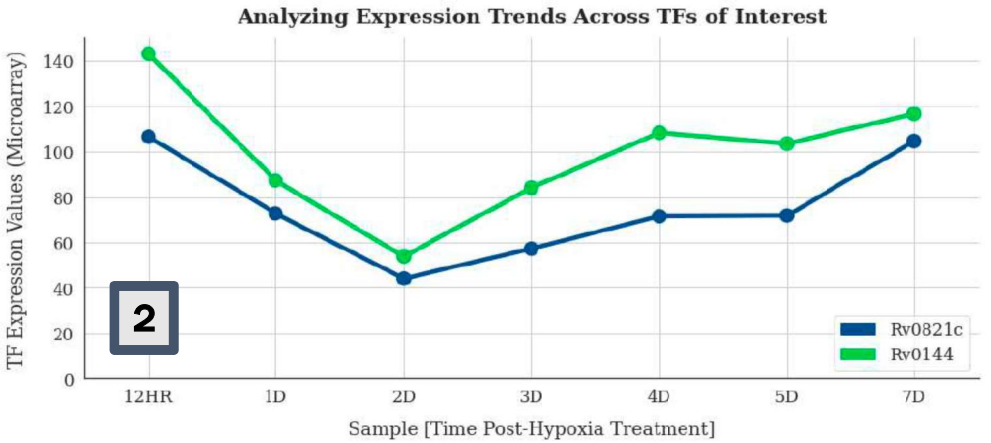




Shared Target Components Between Rv0821c and Rv0144

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*

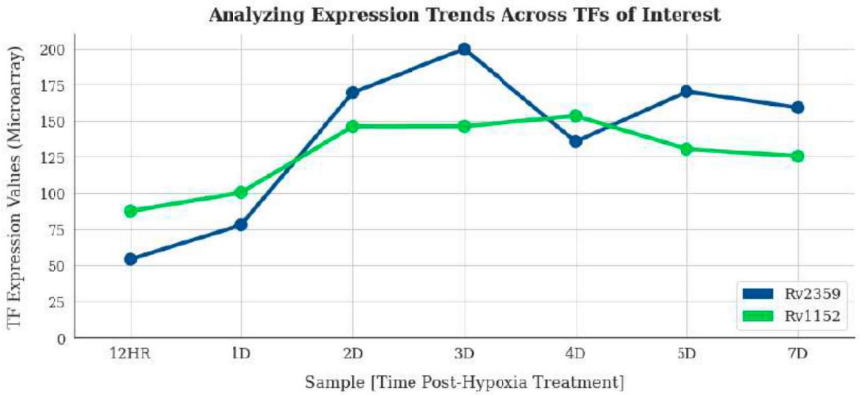
3

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.

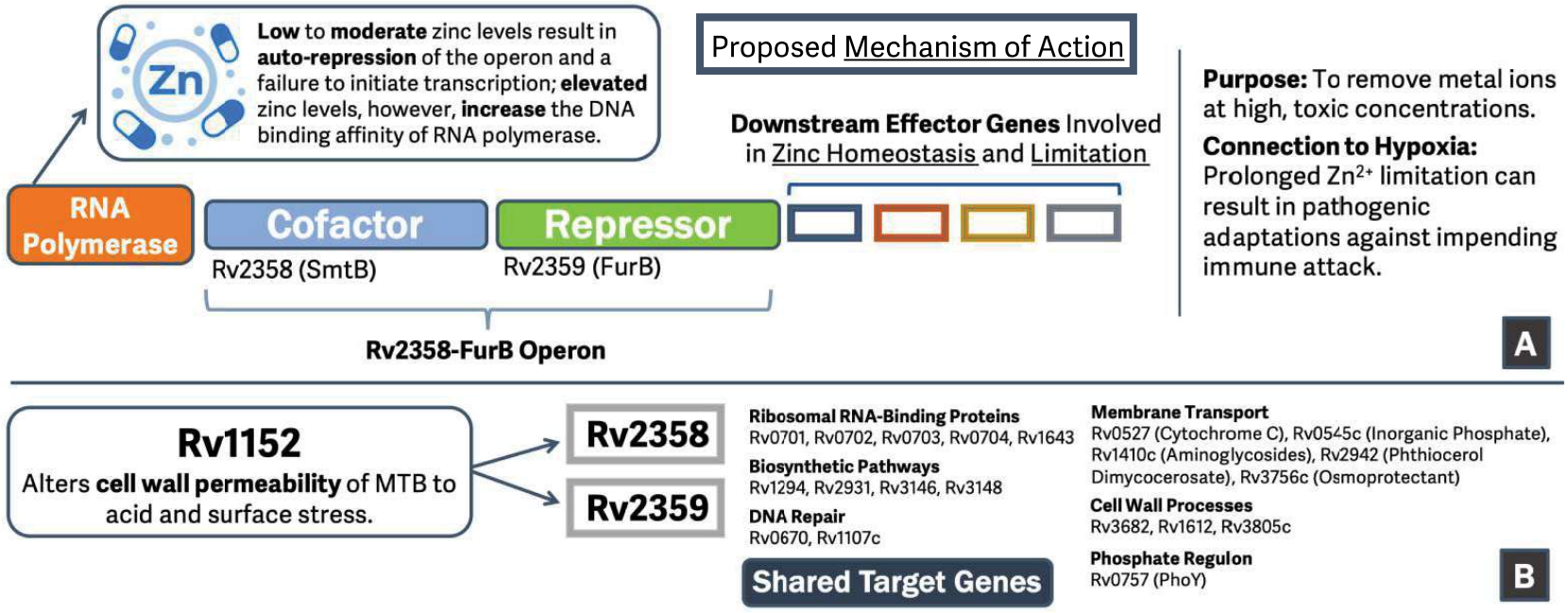
Takeaway: A dual mechanism of mycobacterial persistence linked to cell wall synthesis and intracellular transport.

Characterizing the Rv2359-Rv1152 Relationship



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

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