Supplementary Material

A Two-way Proteome Microarray Strategy to Identify Novel Mycobacterium tuberculosis-human Interactors

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1 Supplementary Figures and Tables

1.1 Supplementary Figures

Supplementary Figure 1. *Mtb* SP-unique, CP-unique and SP-CP-common interactors screened on human proteome microarray. (A) *Mtb* SP-unique interactors (green), (B) *Mtb* CP-unique interactors (red) and (C) *Mtb* SP-CP-common interactors were showed with their SNR≥2.0. The colors shades represent the numerical value.
Supplementary Figure 2. NRF1 and SMAD2 are potential *Mtb* interactors. Potential *Mtb* interactors NRF1 and SMAD2 were set as representative proteins for further study. The SNR and images were shown. Experiments were performed in two replicates.

| Protein | Image | SNR532 | Image | SNR647 |
|---------|-------|--------|-------|--------|
| NRF1    | ![Image](image1.png) | 6.53   | ![Image](image2.png) | 8.98   |
| SMAD2   | ![Image](image3.png) | 4.24   | ![Image](image4.png) | 5.90   |

Supplementary Figure 3. Purification of His tagged Rv0577, Rv3153, Rv2117 and Rv2423. His tagged *Mtb* proteins were purified and detected by SDS-PAGE followed by coomassie brilliant blue staining.

Supplementary Figure 4. PPI based on the experimental validation. Network plot was visualized by Cytoscape software. Nodes depict proteins (rectangle: human proteins, ellipse: *Mtb* proteins) by various colors and edges represent interactions.
1.2 Supplementary Tables

Table S1 The host interacting proteins with *Mtb* that have been identified from previous studies.

| Host Protein Name | Mtb binding partners | Uniprot ID | Method       | Reference                          |
|-------------------|----------------------|------------|--------------|-----------------------------------|
| TRIM27            | PtpA                 | P14373     | Y2H          | Wang J. et al., 2016, *Sci Rep*   |
| IL8               | Rv3248c,Rv0296c,Rv1018c | P10145     | AP/MS        | Wang J. et al., 2015, *Nat Immunol* |
| Ub                | PtpA                 |            | Y2H          | Dziadek B. et al., 2016, *Plos One* |
| β2M               | ESAT-6               | P61769     | Y2H          | Sreejit G. et al., 2014, *Plos Pathog* |
| Hrs               | EsxH,EsxG            | O14964     | Y2H          | Mehra A. et al., 2013, *Plos Pathog* |
| DUSP16/MKP-7      | Eis                  | Q9BY84     | acetylation assays *in vitro* | Kim K.H. et al., 2012, *PNAS* |
| TLR2              | Rv0577               | O60603     | Pulldown     | Byun E.H. et al., 2012, *FASEB J* |
| V-ATPase          | PtpA                 | Q15904     | Pulldown     | Wong D. et al., 2011, *PNAS*       |
| Rab5/Rab7         | NdkA                 | P20339/P51149 | Pulldown       | Sun J. et al., 2010, *Plos One*   |
| ALO17             | Rv3655c              | Q63HN8     | Pulldown     | Danelishvili L. et al., 2010, *Plos One* |
| PSF               | Rv3654c              | P23246     | Pulldown     | Danelishvili L. et al., 2010, *Plos One* |
| VPS33B            | PtpA                 | Q9H267     | substrate trapping | Bach H. et al., 2008, *Cell Host Microbe* |
| Coronin-1A        | LpdC                 | P31146     | Pulldown and 2D-gel | Deghmane A-E. et al., 2007, *J Cell Sci* |
| PI3P              | SapM                 | Q13496     | phosphatase assay *in vitro* | Vergne I. et al., 2005, *PNAS* |
| Ingenuity Canonical Pathways                                      | -log(p-value) | Ratio | Molecules                                      |
|-----------------------------------------------------------------|---------------|-------|-----------------------------------------------|
| TGF-β Signaling                                                 | 3.47          | 0.046 | SMAD2, MAPK3, SMAD4, HNF4A                    |
| Transcriptional Regulatory Network in Embryonic Stem Cells      | 2.94          | 0.0556| CDX2, HNF4A, FOXD3                            |
| Role of NANOG in Mammalian Embryonic Stem Cell Pluripotency     | 2.92          | 0.0328| MAPK3, CDX2, SMAD4, FOXD3                     |
| Osteoarthritis Pathway                                         | 2.89          | 0.0236| SMAD2, GLIS2, PPARD, SMAD4, SLC39A8           |
| Melatonin Signaling                                            | 2.59          | 0.0417| RORA, MAPK3, MAP2K5                           |
| Ephrin B Signaling                                             | 2.57          | 0.0411| MAPK3, ACP1, GNB1L                            |
| Regulation of IL-2 Expression in Activated and Anergic T Lymphocytes | 2.46          | 0.0375| SMAD2, MAPK3, SMAD4                           |
| IL-22 Signaling                                                | 2.43          | 0.0833| MAPK3, STAT5B                                |
| Role of JAK family kinases in IL-6-type Cytokine Signaling     | 2.39          | 0.08  | MAPK3, STAT5B                                |
| Antiproliferative Role of TOB in T Cell Signaling              | 2.36          | 0.0769| SMAD2, SMAD4                                 |
| PPARα/RXRα Activation                                          | 2.31          | 0.0222| SMAD2, MAPK3, SMAD4, STAT5B                  |
| Sirtuin Signaling Pathway                                      | 2.29          | 0.0171| PCK2, MAPK3, NDUFB6, LDHA, NFE2L2             |

Table S2 Top twenty IPA pathways for *Mtb* interactors
### Table S3 Top ten IPA “Diseases and Bio Functions” items for *Mtb* effectors

| Diseases or Functions | Annotation | p-Value | Molecules | # Molecules |
|-----------------------|------------|---------|-----------|-------------|
| **Acute Myeloid Leukemia Signaling** | 2.27 | 0.0323 | MAPK3,STAT5B,MAP2K5 | |
| **PPAR Signaling** | 2.25 | 0.0316 | PPARD,MAPK3,STAT5B | |
| **Regulation of the Epithelial-Mesenchymal Transition Pathway** | 2.23 | 0.0212 | SMAD2,MAPK3,SMAD4,MAP2K5 | |
| **Oncostatin M Signaling** | 2.13 | 0.0588 | MAPK3,STAT5B | |
| **DNA Methylation and Transcriptional Repression Signaling** | 2.13 | 0.0588 | MTA2,MBD2 | |
| **Chronic Myeloid Leukemia Signaling** | 2.12 | 0.0283 | MAPK3,SMAD4,STAT5B | |
| **Mouse Embryonic Stem Cell Pluripotency** | 2.12 | 0.0283 | MAPK3,SMAD4,FOXD3 | |
| **Glucocorticoid Receptor Signaling** | 2.03 | 0.0147 | SMAD2,PCK2,MAPK3,SMAD4,STAT5B | |

**Gene Expression**

- **Activation of DNA endogenous promoter**
  - p-Value: 2.54E-20
  - Molecules: ENTX,ZNF136
  - # Molecules: 39

- **Transcription of DNA**
  - p-Value: 1.61E-19
  - Molecules: OXB9,IKZF3,IKZF4,INSM1,IRF2,KLF11,KLF8,MAP2K5,MAPK3,MBD2,MTA2,NFE2L2,NRF1,OVO1,PPAR,D,RORA,SKOR2,SMAD2,SMAD4,OX10,SOX4,SP6,SRCAP,STAT5B,V
  - # Molecules: 42
Gene Expression Transcription of RNA 1.06E-16

Cell Death and Survival Necrosis 42

Gene Transactivation of RNA 0.000000

Cellular Movement Invasion of breast cancer cell lines 14

Cellular Movement Invasion of cells 18

Embryonic Development Patterning of rostrocaudal axis 6

Cellular Development, Cellular Cell proliferation of Growth and tumor cell lines Proliferation 24
| n       | ENTX                                                                 |
|---------|----------------------------------------------------------------------|
|         | BCL2L14,CDX2,FOXD3,FOXM1,GLO1,HNF4A,HOXB9,IRF2,KLF8,MAP2K5,MAPK3,MTA2,NFE2L2,NRF1,PARVA,PCK2,PPARD,SMAD2,SMA |
| Cell Death and Survival lines | Cell death of tumor cell lines 0.000010 ARVA,PCK2,PPARD,SMAD2,SMA    |
|         | D4,SOX10,SOX4,SRCA,STAT5B 23                                       |
Table S4 Top three networks in diseases and functions for *Mtb* interactors

| Top network functions                                                                 | Score | Focus Molecules                                                                 |
|---------------------------------------------------------------------------------------|-------|---------------------------------------------------------------------------------|
| Gene Expression, Embryonic Development, Organismal Development                        | 32    | AKIRIN2, CDX2, BCL2L14, CYP39A1, FOXD3, HOXB4, HOXB9, MAP2K5, NFE2L2, PCK2, RORA, SF3B2, SK OR2, SMAD2, SMAD4, SRCAP |
| Cellular Assembly and Organization, Endocrine System Disorders, Gastrointestinal Disease | 32    | ACP1, DDX39B, GLO1, GNB1L, HNF4A, KLF11, LAPT M4A, NDUFB6, NRF1, OVL1, PARVA, SOX4, SOX10, STAT5B, ZNF410, ZNF557 |
| Gene Expression, Carbohydrate Metabolism, Small Molecule Biochemistry                 | 27    | ARID3A, ESRRG, FOXM1, IKZF3, IKZF4, INSM1, IRF2, KAT14, LDHA, MAPK3, MBD2, MTA2, PPARD, VPS8 |

Table S5 Top five tox functions list for *Mtb* interactors identified

| Name                                      | p-value | overlap |
|-------------------------------------------|---------|---------|
| TGF- Signaling                            | 3.84E-04| 4.4 %   |
| Liver Proliferation                       | 2.13E-03| 2.1 %   |
| Renal Necrosis/Cell Death                 | 4.84E-03| 1.3 %   |
| PPAR/RXR Activation                      | 5.20E-03| 2.2 %   |
| Mechanism of Gene Regulation by Peroxisome Proliferators via PPAR | 5.67E-03| 3.2 %   |
1.3 Supplementary Original Figures

Supplementary Original Figure 1. His pull-down of His-Rv0577 with endogenous NRF1. ** represented specific band, * represented non-specific band
Supplementary Original Figure 2. His pull-down of His-Rv0577, His-Rv3153, His-Rv2117 and His-Rv2423 with endogenous SMAD2. ** represented specific band
Supplementary Original Figure 3. Purification of His tagged Rv0577, Rv3153, Rv2117 and Rv2423.
