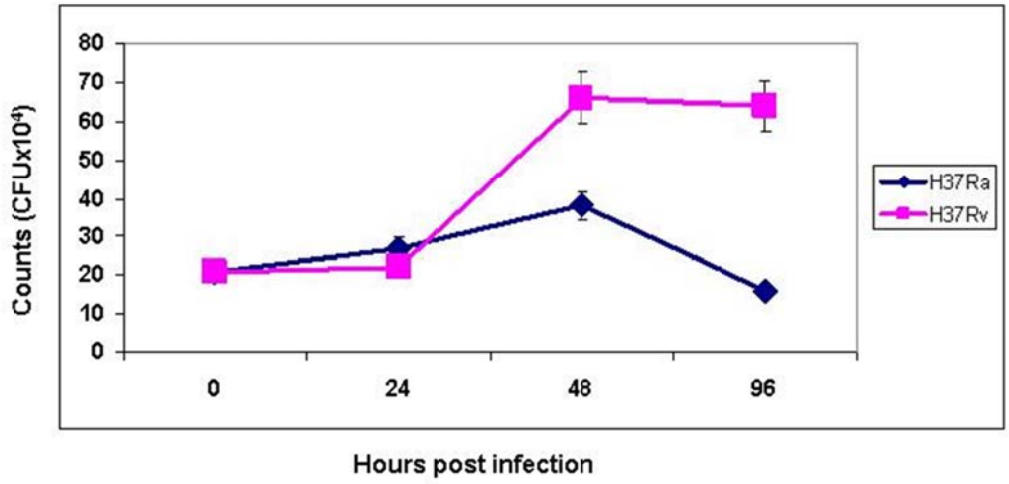
Supplemental Information for the Manuscript: "Express Path Analysis Identifies Tyrosine kinase Src Centric Network Regulating Divergent Host Responses to *Mycobacterium tuberculosis* Infection

Serial No.	Supplemental Information	Title
1	Supplemental Figure S1	Intracellular growth rate of H37Ra and H37Rv in Thp-1 cells
2	Supplemental Table S1	Gene Expression Data of Thp-1 cells infected H37Ra or H37Rv
3	Supplemental Figure S2	Heat Map and clustergram of H37Ra and H37Rv infected Thp-1 cells gene expression data
4	Supplemental Figure S3	k-means clustering and Gene Ontology enrichment of the k-clusters
5	Supplemental Table S2	Shortest paths between source and target nodes and respective expression values in H37Ra and H37Rv infected Thp-1 cells
6	Supplemental Figure S4	Key node to 0 hour time point shortest path network
7	Supplemental Table S3	Upstream analysis of Src for TF binding sites
8	Supplemental Table S4	Expression kinetics of TFs that could bind to Src upstream region in Ra and Rv infected cells
9	Supplemental Figure S5	Comparison of TF expression between Ra and Rv infected cells
10	Supplemental Figure S6	Effect of H37Ra or H37Rv infection on Src protein, phosphorylation levels and Src protein stability in Thp-1 cells
11	Supplemental Figure S7	Effect of Src inhibitor treatment on Thp-1 cells infected with H37Ra or H37Rv

Supplemental Figure S1



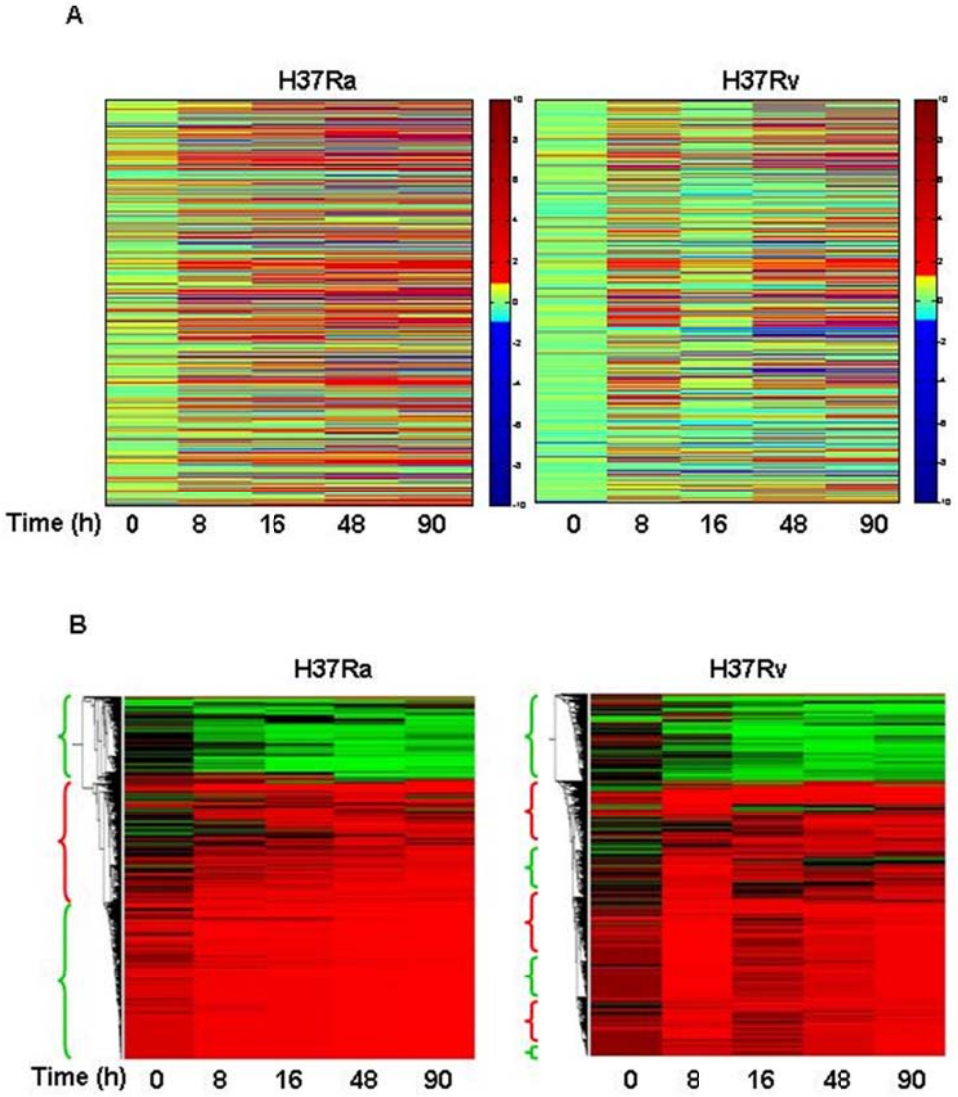
Supplemental Figure S1: Intracellular growth rate of H37Ra and H37Rv in Thp-1 cells

Thp-1 cells were infected with H37Ra or H37Rv at 1:10 MOI, samples were collected, lysed and plated at 24, 48 and 96 hours after infection. Colony counts were done at 14 and 21 days after plating, corresponding CFU is represented in the plot.

Supplemental Table S1: Gene Expression Data of Thp-1 cells infected H37Ra or H37Rv

Gene expression data for H37Ra and H37Rv infected Thp-1 cells across all the time points. Data is represented as log2 ratio from the uninfected control. Column headers represent infecting strain (H37Ra or H37Rv) and the time after infection when samples for expression data were generated.

Supplemental Figure S2

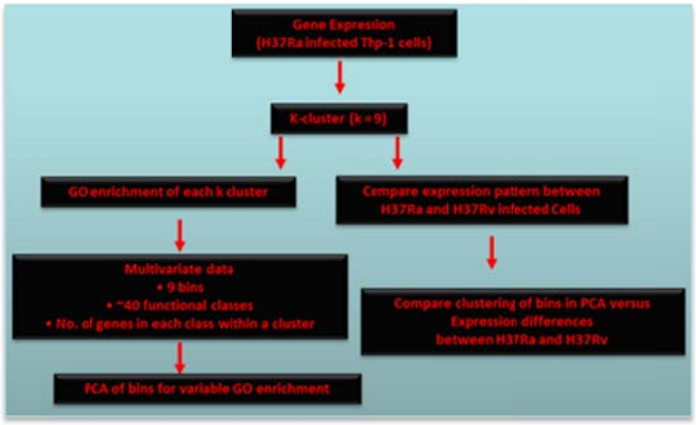


Supplemental Figure S2: Heat Map and clustergram of H37Ra and H37Rv infected Thp-1 cells gene expression data

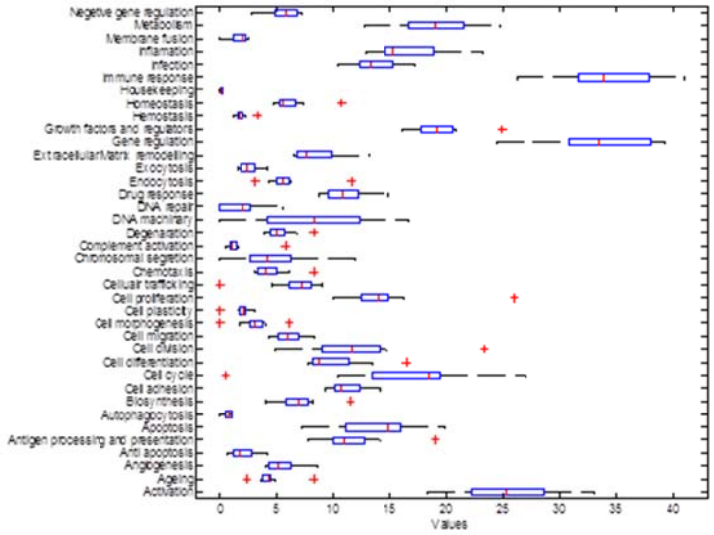
Figure S2A represents heat map of gene expression data of each of the ~25000 genes in H37Ra or H37Rv infected Thp-1 cells. Clustergrams showing pattern specific clustering of time dependent gene expression data in H37Ra or H37Rv infected cells are shown in Figure S2B. Note the differences in the number of clusters between H37Ra or H37Rv infected cells.

Supplemental Figure S3

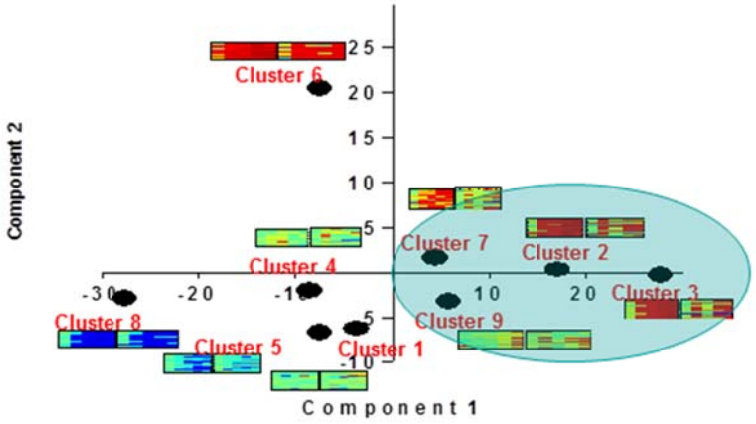
A



B



C



Supplemental Figure S3: k-means clustering and Gene Ontology enrichment of the k-clusters

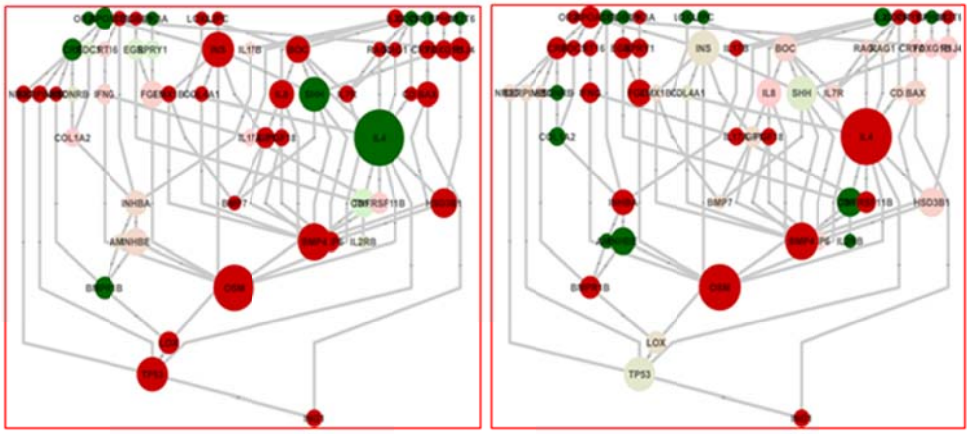
Figure S3A shows flow chart for the k-means clustering exercise of the expression data. K-clustering was done only in H37Ra infected Thp-1 cells data. Genes were clustered based on the response to avirulent infection. Comparison of genes from these clusters between H37Ra and H37Rv infected cells allowed us to identify clusters that are contrastingly regulated between the two cases. Gene-Ontology enrichment was performed using Bio-interpreter online software tool. Functional GO classes and their relative distribution across nine different k-clusters are represented in the box-plot in Figure S3B. Functional classes like gene regulation and immune response show highest variation in their distribution between the k-clusters.

Each of the nine k-clusters, together represented enrichment of nearly 40 different functional classes. However representation of various functional classes among the clusters varied significantly. In order to identify closely related clusters in terms of functional class enrichment, PCA was performed. The distribution of clusters on PC1 and PC2 axes is represented in Figure S3C. Closely placed clustered in the PC1-PC2 plot are enriched with similar GO classes. Important to note here is the cluster shown in shaded area, where clusters 2, 7, 3 and 9 are placed very close to each other. Therefore they are enriched with similar functional classes. Interestingly they were also the clusters that showed maximum differences in terms of expression between H37Ra and Rv infected cells.

Supplemental Table S2: Shortest paths between source and target nodes and respective expression values in H37Ra and H37Rv infected Thp-1 cells

Table lists each of the Express paths (see text and methods for details) identified for Key nodes to 0 hr, 0hr to 8hr, 8hr to 16 hr, 16hr to 48hr and 16hr to 90hr source-target sets. Table also contains expression value for each of the nodes in the shortest path in H37Ra or H37Rv infected Thp-1 cells.

Supplemental Figure S4

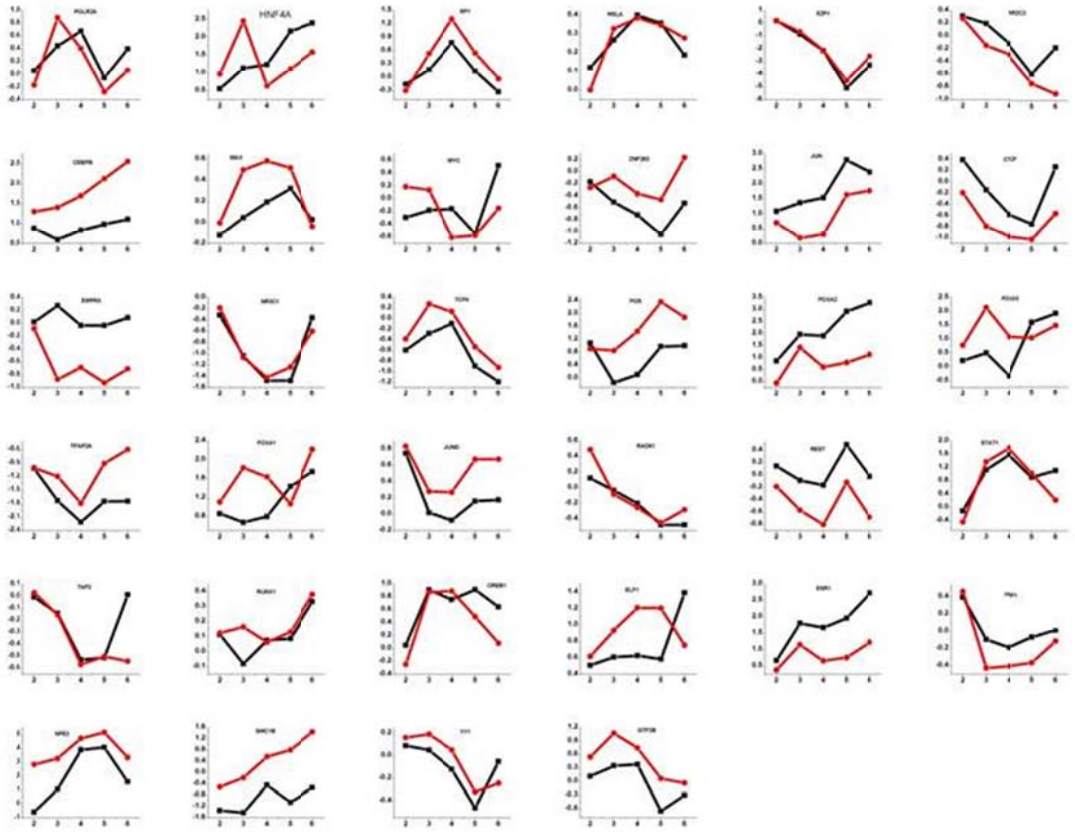


Supplemental Figure S4: Key node to 0 hour time point Express path network
Express path network between key nodes and 0hr nodes in H37Ra or H37Rv infected cells are shown in the figure. See text for details.

Supplemental Table S3: Upstream analysis of Src for TF binding sites
Genomic stretch ~3kb upstream to the Src transcription start site was scanned for potential binding site for different TFs. The analysis was performed using standard Transfac analysis tool (Biobase database). The table here shows genomic location and the corresponding TF for each of them. Note that many of the TFs have more than one potential binding region in the Src upstream sequence.

Supplemental Table S4: Expression kinetics of TFs that could bind to Src upstream region in Ra and Rv infected cells
The TFs identified through the promoter analysis was then looked for their expression in Ra and Rv infected cells across the time points in the microarray data. We could find expression value for 34 out of 35 TFs identified. The table shows corresponding expression value of the TF genes in the microarray experiments.

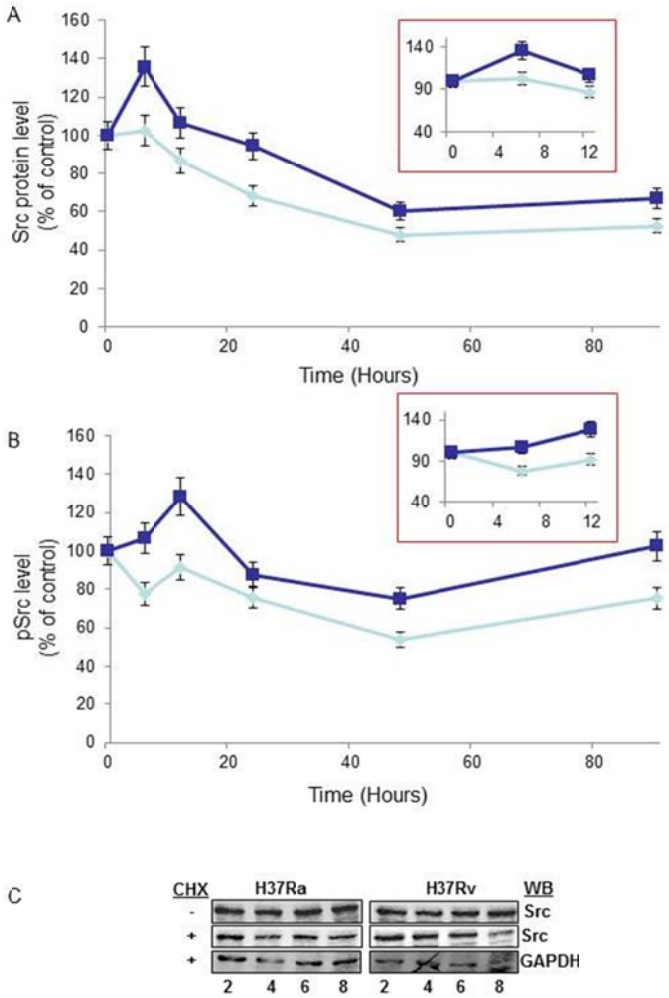
Supplemental Figure S5



Supplemental Figure S5: Comparison of TF expression between Ra and Rv infected cells

The expression profile of TFs listed in Table S4 are represented here as line plots, comparing expression of the TF genes in Ra (black) or Rv (red) infected cells. It is interesting to observe how different TFs show wide range of profile between Ra and Rv infected cells varying from exactly similar to contrastingly opposite pattern.

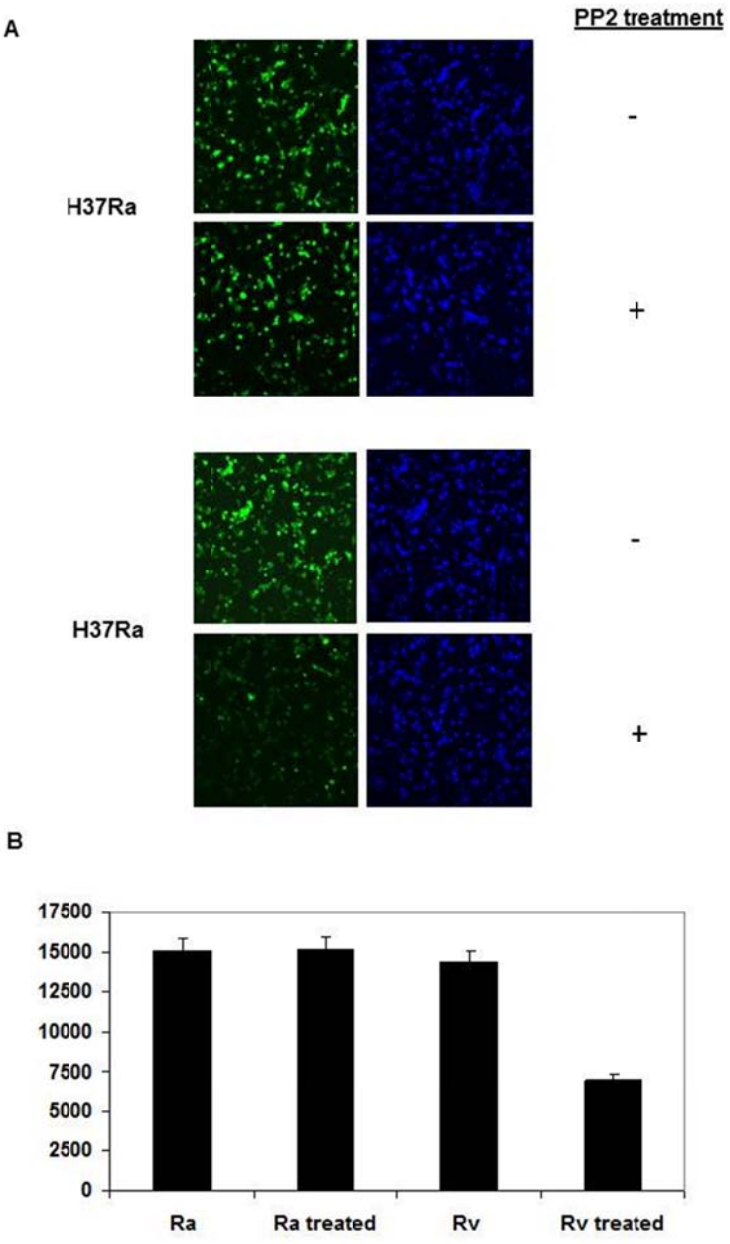
Supplemental Figures S6



Supplemental Figure S6: Effect of H37Ra or H37Rv infection on Src protein, phosphorylation levels and Src protein stability in Thp-1 cells

Panel A shows Src protein level in cells infected with Ra (light blue) or Rv (dark blue). Data represents average of four independent experiments. Inset is to highlight the remarkable differences observed at early time points. Data are normalized for 0 hour levels as 100 percent. Similarly Panel B shows relative phosphorylation kinetics of Src in Ra and Rv infected cells. Here again data represents average of four separate experiments and was normalized against the 0 hour phosphorylation level of Src (hence called % of control). Inset shows significant differences during the early phase of infection in Src phosphorylation. For Panel C, Src degradation rate was calculated in cells where do novo synthesis was inhibited using cycloheximide (CHX, inhibitor of eukaryotic protein synthesis). Cells infected with H37Ra or H37Rv were treated with cycloheximide or mock at 16 hours after infection, and sample were generated every two hours up to 8 hours after infection. Samples were lysed and probed for Src proteins through western blots.

Supplemental Figure S7



Supplemental Figure S7: Effect of Src inhibitor treatment on Thp-1 cells infected with H37Ra or H37Rv

PKH67 labeled H37Ra or H37Rv infected Thp-1 cells were treated either with PP2 (Src inhibitor) or negative control (DMSO) till 96 hours post-infection. Cells were fixed, stained with DAPI for cell count and observed under laser confocal microscope at 20X magnification (S7A). Green fluorescence as a function of number of cells were quantitated as described in methods and plot is shown in figure S7B.