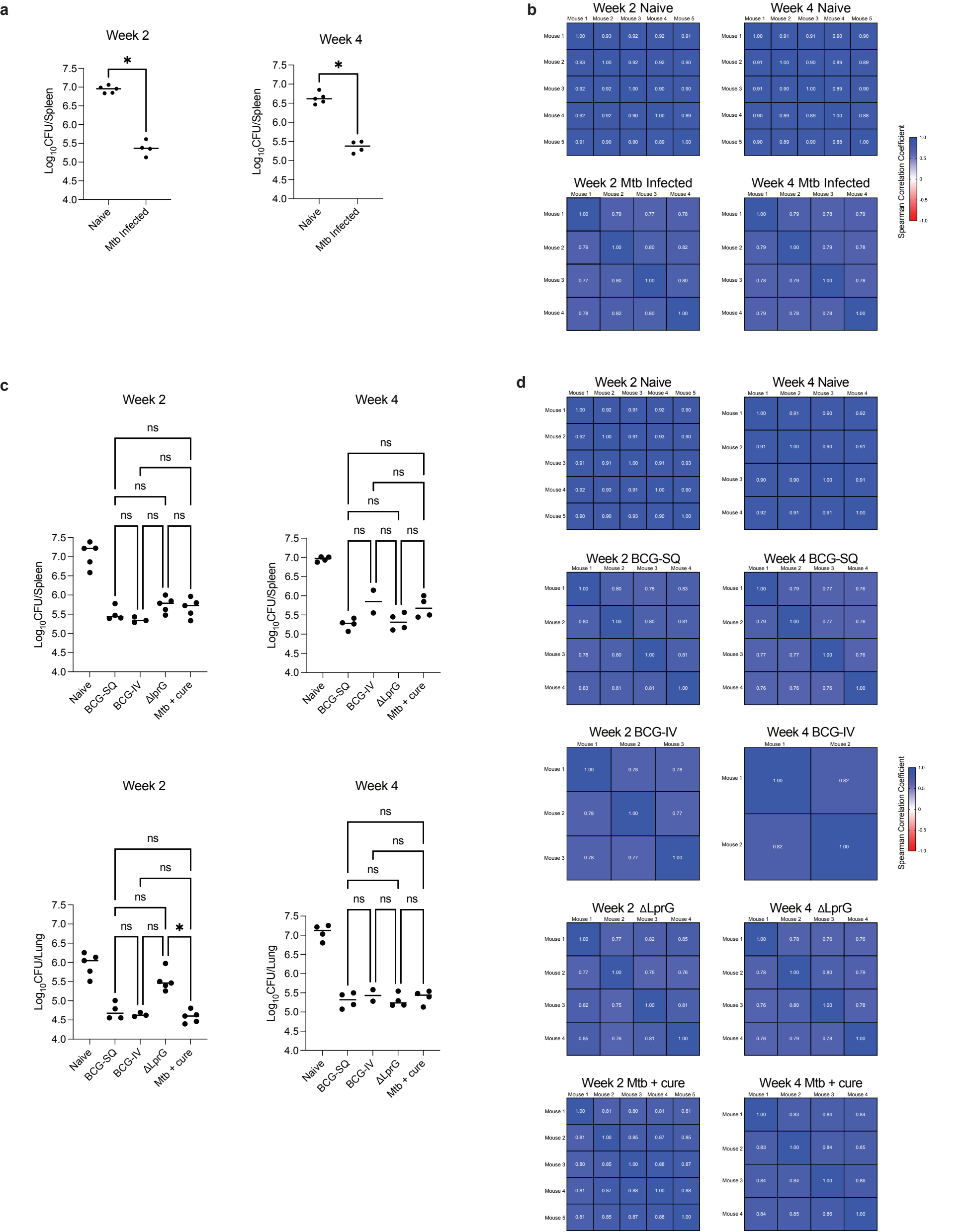




Supplementary Figure 1

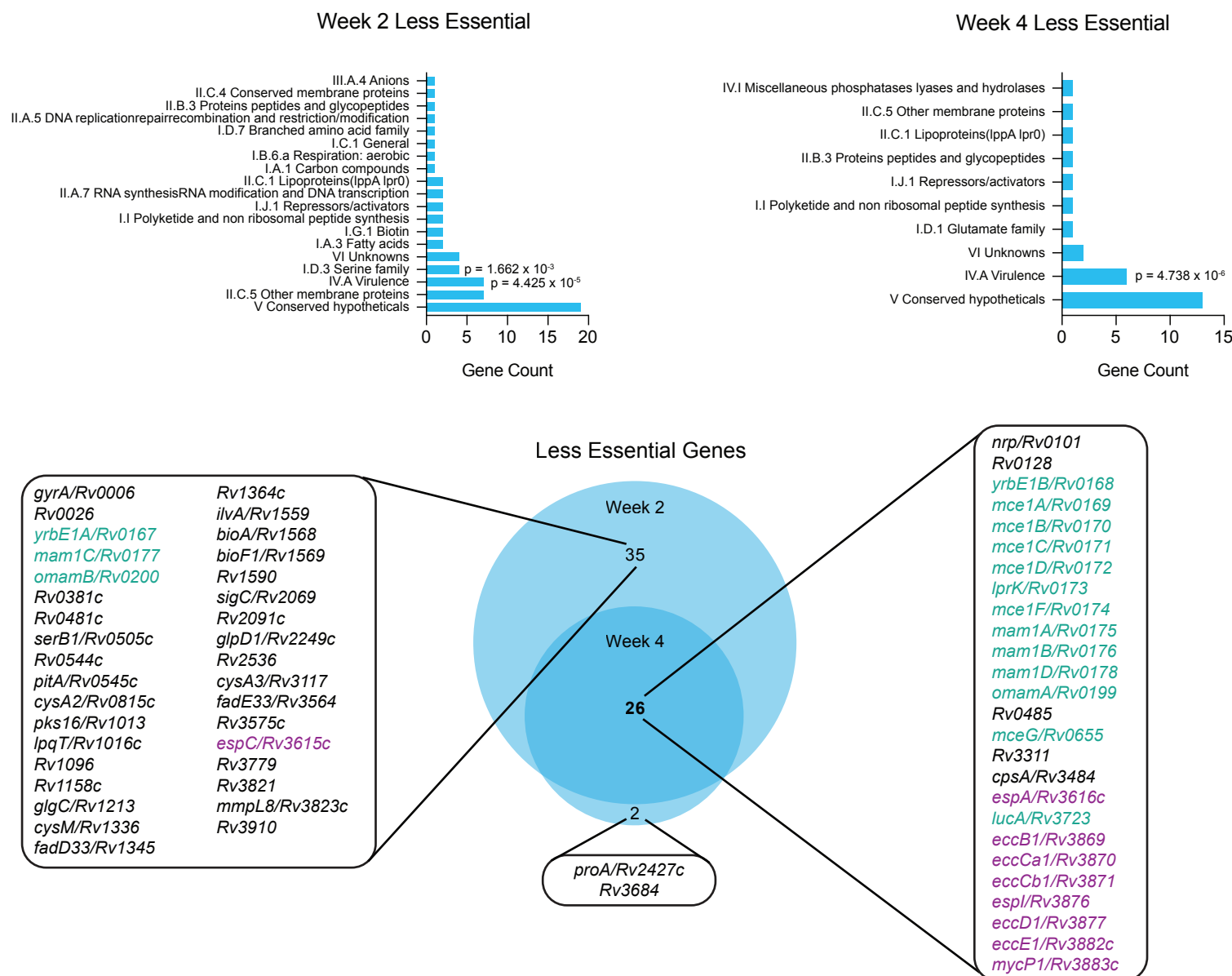
a) Bacterial burden (CFU) from the preliminary transposon challenge experiment.

b) Correlation plots of mean transposon count across each gene from the preliminary experiment. Value in each square is the Spearman correlation coefficient for pairwise comparison between individual mice.

c) Bacterial burden (CFU) from the vaccination transposon library challenge experiments.

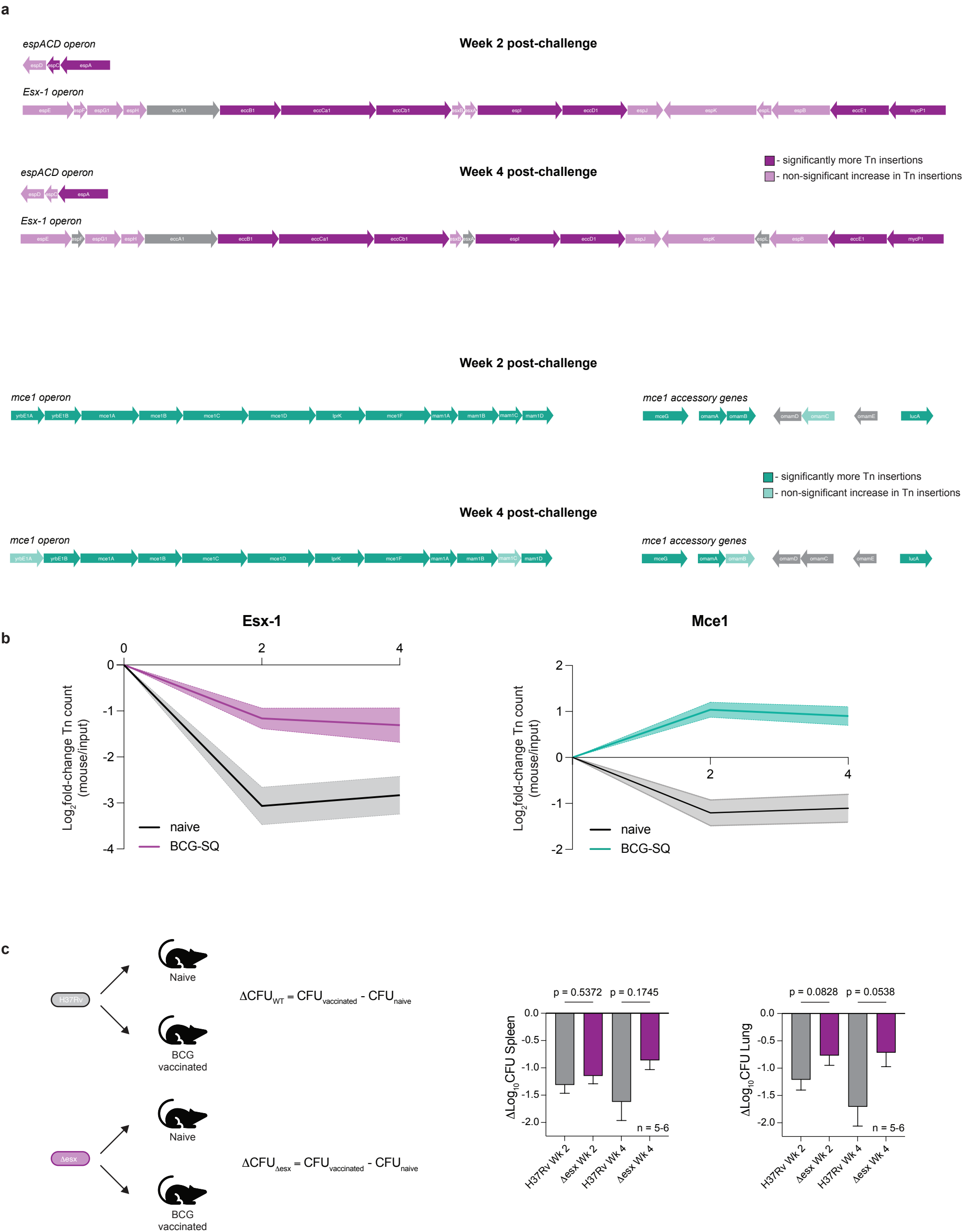
d) Correlation plots of mean transposon count across each gene from the vaccination challenge experiments. Value in each square is the Spearman correlation coefficient for pairwise comparison between individual mice.

b



a) Sanger classification of genes that are significantly more essential in BCG vaccinated mice as compared to naïve mice at the two week and four week time points (top panel), and Venn diagram showing overlap in these gene sets (bottom panel).

b) Sanger classification of genes that are significantly less essential in BCG vaccinated mice as compared to naïve mice at the two week and four week time points; p-values annotate significantly enriched Sanger categories as determined by g:Profiler (top panel). Venn diagram showing overlap in these gene sets (bottom panel). Genes in the overlap set that belong to the Esx-1 or Mce1 pathways are color-coded purple or green, respectively.

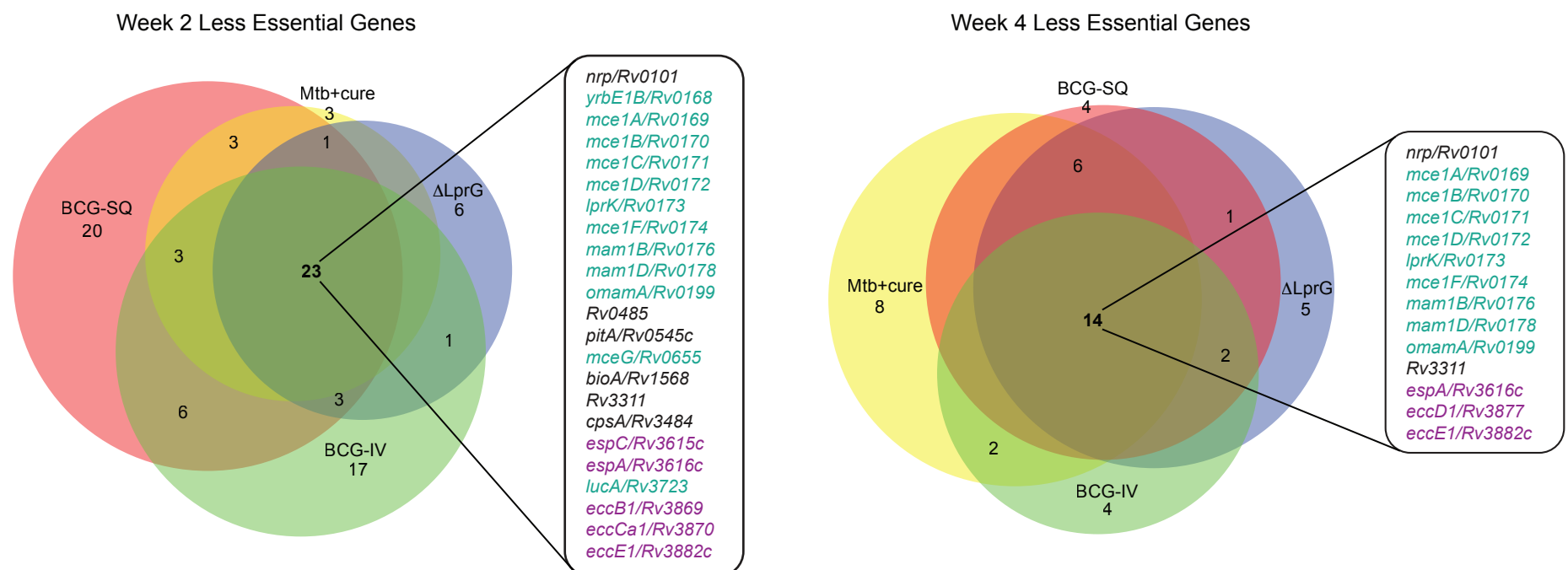
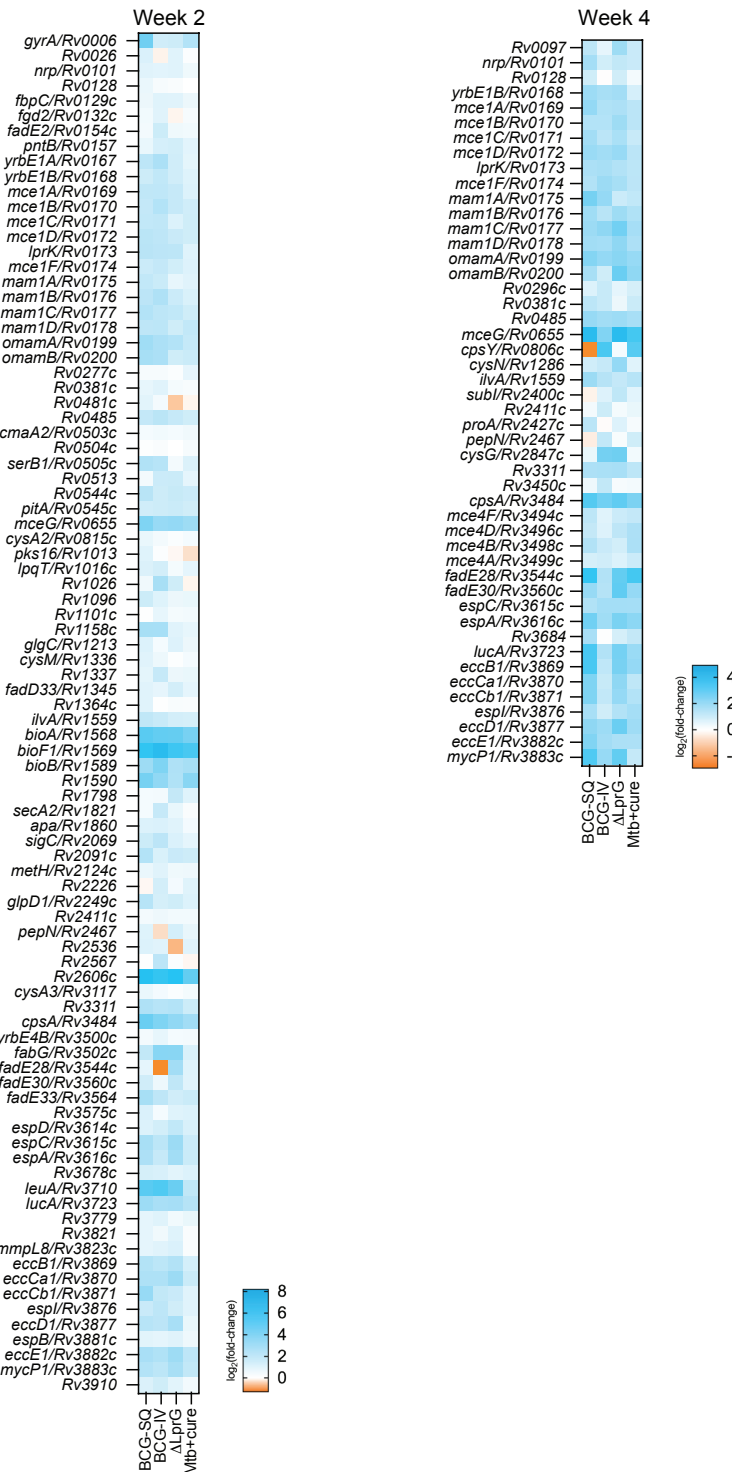
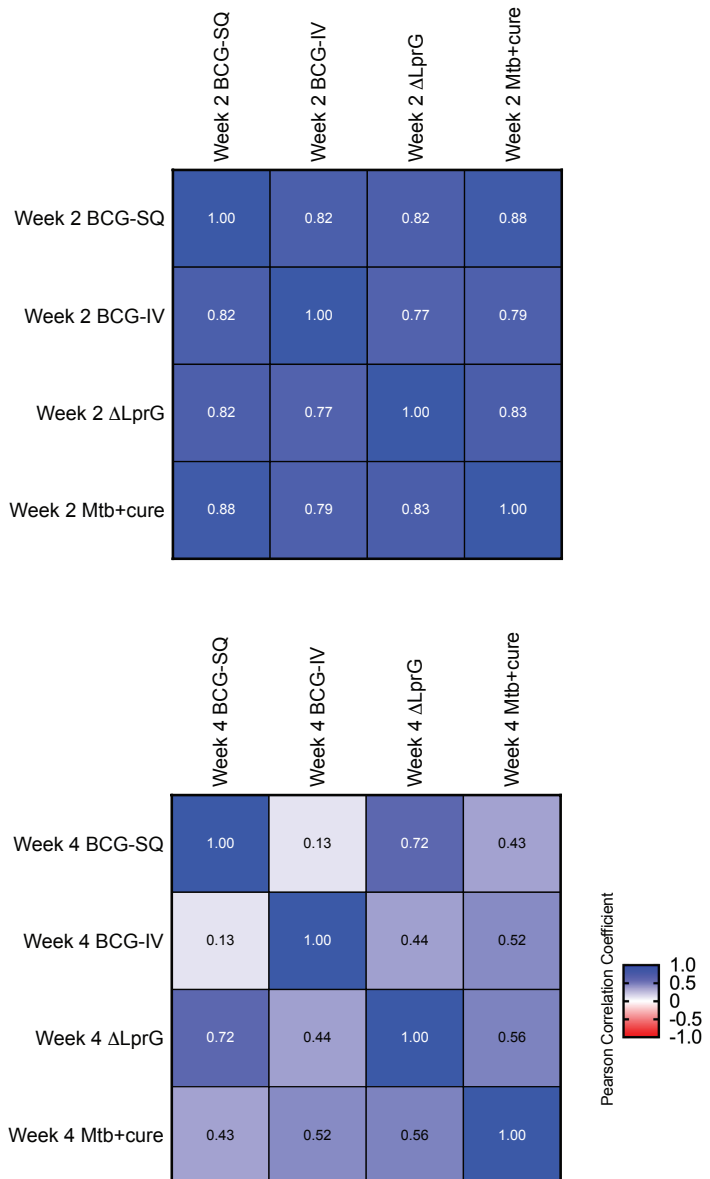
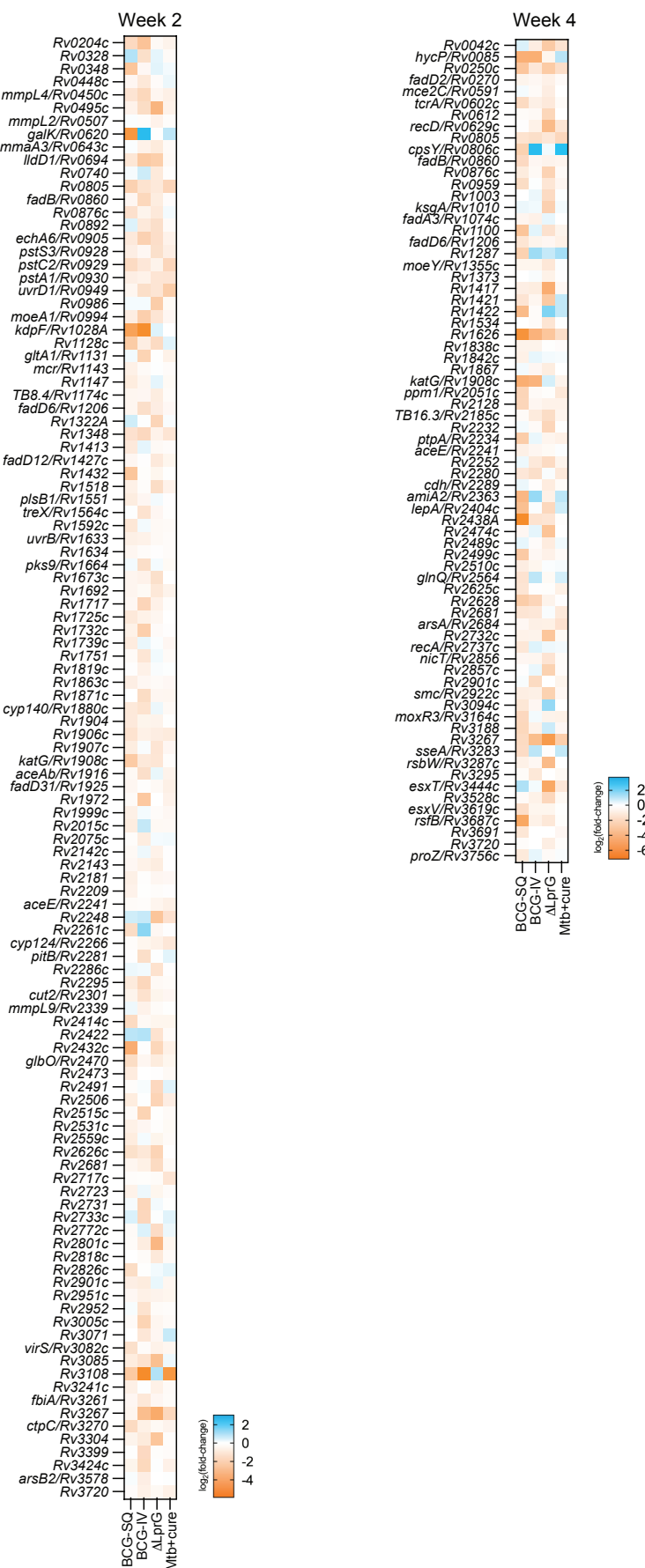
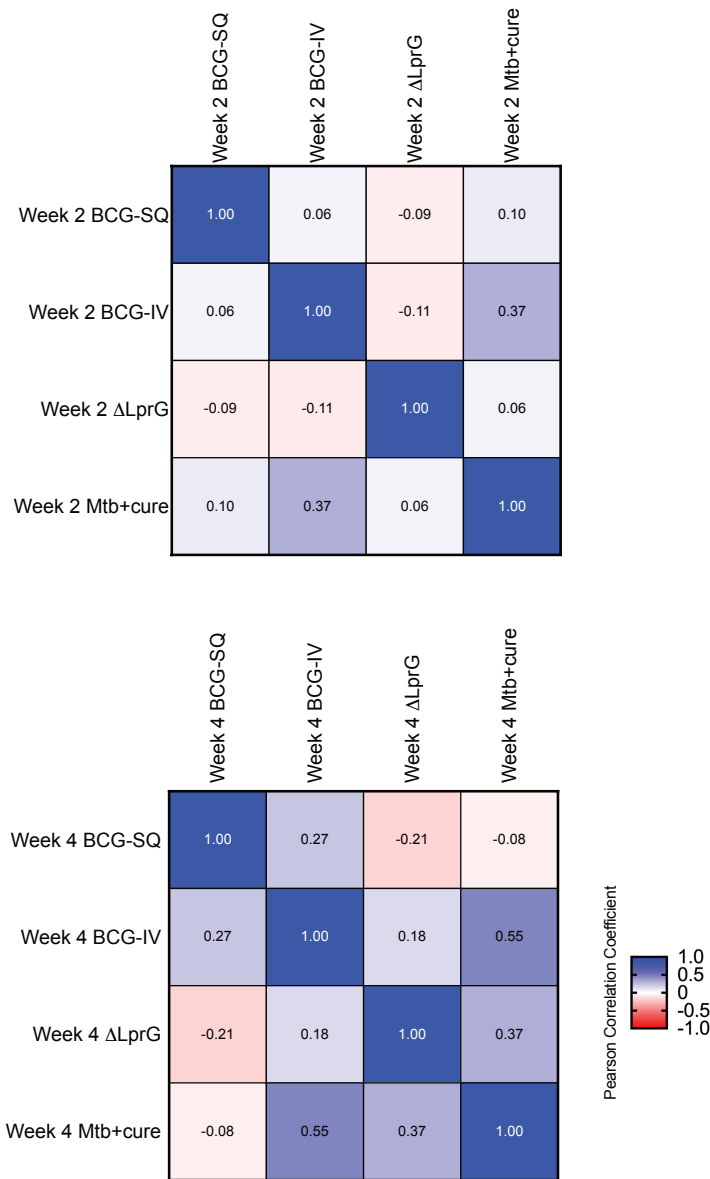


Supplementary Figure 3

a) Schematic of Esx-1 and Mce1 operons illustrating essentiality of each gene at two or four weeks post-infection, relative to naïve animals.

b) Plots of transposon junction read count across Esx-1 and Mce1 genes relative to the input library over time in naïve and BCG vaccinated mice.

c) Schematic illustrating the single strain infection experiment (left) and the difference in bacterial burden in naïve as compared to vaccinated animals for wild-type H37Rv and an Esx-1 H37Rv deletion strain in spleen and lung tissues (right). Statistical significance determined by unpaired T-test. n = 5-6 mice per group.

a**b****c****d****e****Supplementary Figure 4**

- a)** Venn diagrams showing overlap in the less essential genes at two weeks (left panel) and four weeks (right panel) post-infection in immune relative to naïve animals. Genes in the overlap set that belong to the Esx-1 or Mce1 pathways are color-coded purple or green, respectively.
- b)** Heat maps showing $\log_2(\text{fold-change})$ values for all genes that are significantly less essential in at least one of the immune conditions, relative to naïve animals, at two weeks (left) and four weeks (right) post-infection.
- c)** Heat maps showing Pearson correlation coefficients among immune conditions for the less essential genes at two weeks (top) and four weeks (bottom) post-infection.
- d)** Heat maps showing $\log_2(\text{fold-change})$ values for all genes that are significantly more essential in at least one of the immune conditions, relative to naïve animals, at two weeks (left) and four weeks (right) post-infection.
- e)** Heat maps showing Pearson correlation coefficients among immune conditions for the more essential genes at two weeks (top) and four weeks (bottom) post-infection.

Supplementary Data 1. Transit analysis of transposon junction sequencing data.

Supplementary Data 2. List of conditions from MtbTnDb included in Figure 2b and 4b, and citations.

Supplementary Data 3. CFU values in the spleen and average $\log_2$ (fold-change) values for transposon read count averaged across the Esx-1 or Mce1 genes across a panel of genetically diverse mice. CFU data from Reference 35 were downloaded from https://github.com/sassettilab/Smith_et_al_CC_TnSeq, and TnSeq data were downloaded from MtbTnDb in order to use standardized data.