Supplementary Information for:

Characterization of global gene expression, regulation of metal ions, and infection outcomes in immune competent 129S6 mouse macrophages

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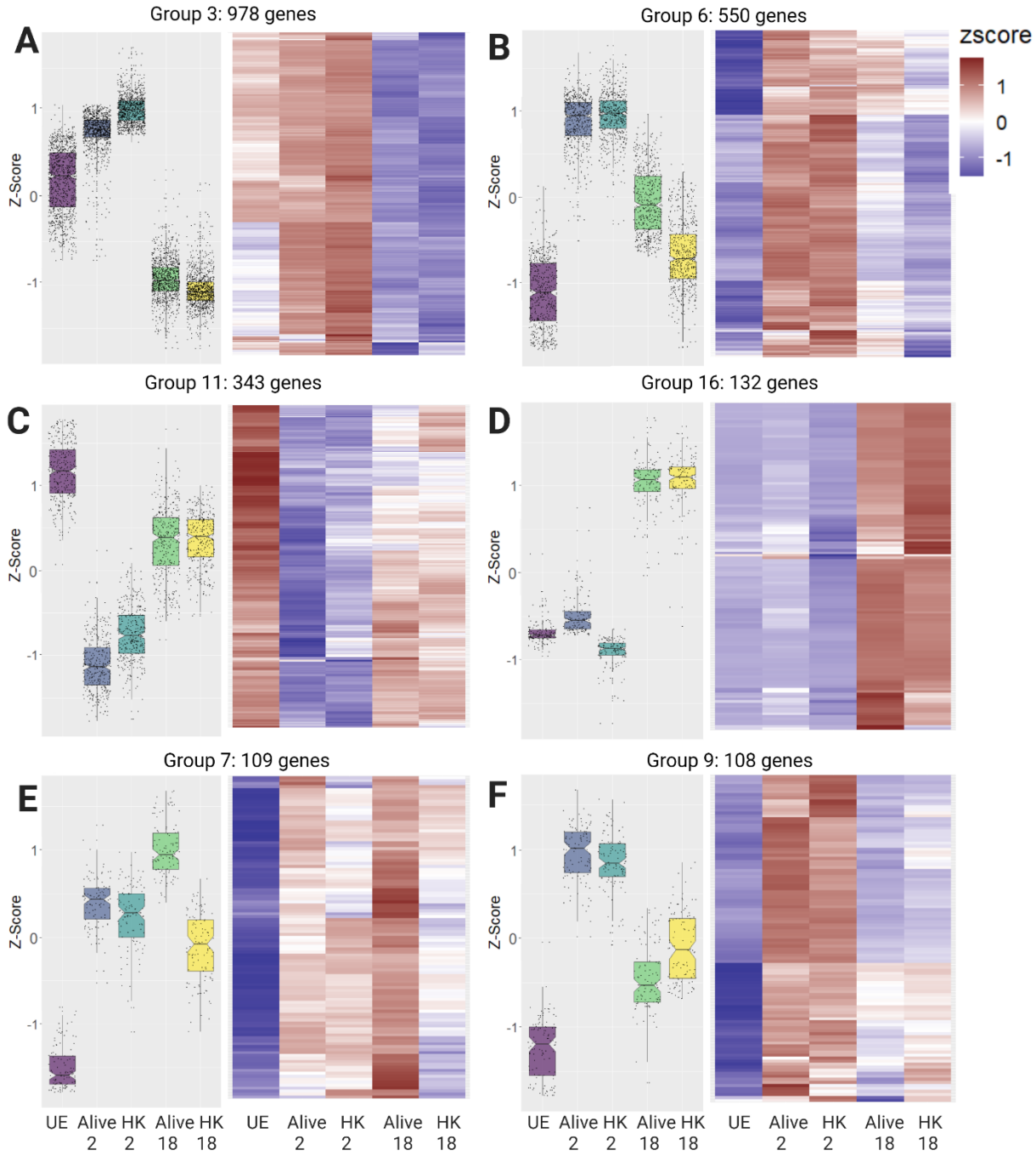
Supplementary Figure S1:



S1: Clustered data exposes multiple distinct trends in gene expression (groups 3, 16, 11, 16, 7, 9). A) Cluster 3 is enriched in genes involved in transcription and translation, cell adhesion, Zn binding (beyond transcription factors), cell cycle, and kinase signaling pathways. B) Cluster 6 is enriched for cell adhesion, actin binding, translation (preinitiation and initiation), immune responses to specific diseases, ATP binding, protein transport (secretion and retrograde transport), and heat shock. C) Cluster 11 is enriched for transcription, Zn binding, DNA repair and autophagy. Transcription and Zn overlap on 40/80 genes. D) Cluster 16 is enriched for

mitochondrial transit peptides, ATPase/kinase function, Golgi proteins, immunity, and fatty acid metabolism. E) Cluster 7 is enriched for cell adhesion, ER protein processing, unfolded protein response, redox, and proteasome proteins. F) Cluster 9 is enriched for genes involved in TNF signaling and response to lipopolysaccharide (LPS), ER functions, and cytokines/inflammatory response.

Supplementary Figure S2:

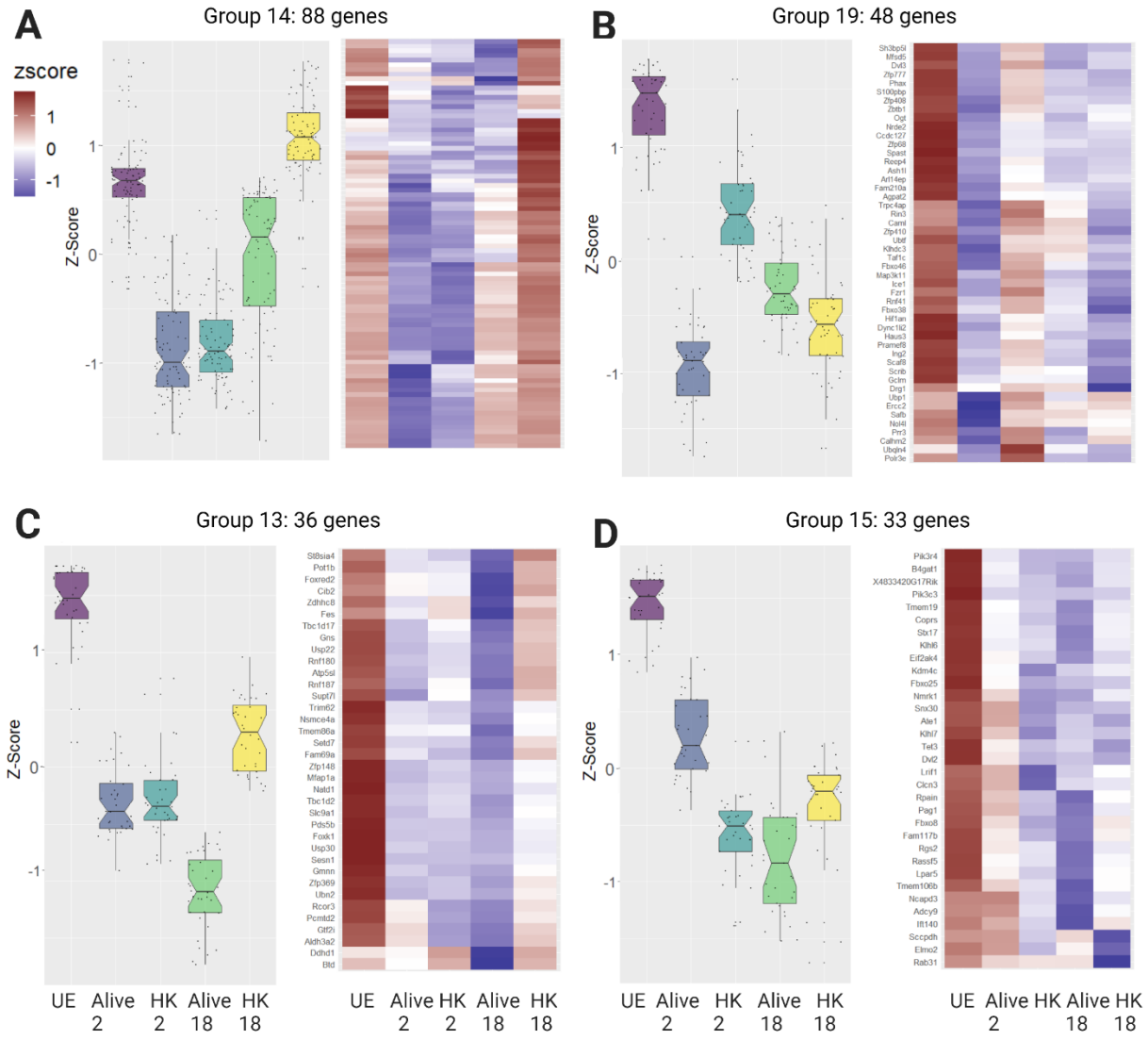


Figure S2: Clustered data exposes multiple distinct trends in gene expression (groups 14, 19, 13, 16). A) Cluster 14 is enriched for genes involved in innate immunity, Zn binding, and transcription. B) Cluster 19 is enriched for transcription and mitosis related genes, and Zn finger motifs. C) Cluster 13 is enriched for genes involved in transcription, Zn binding, ubiquitylation, redox, and Golgi transport. D) Cluster 15 is enriched for genes involved in endosome functioning, redox, Zn binding, and those with transmembrane domains.

Supplementary Figure S3:

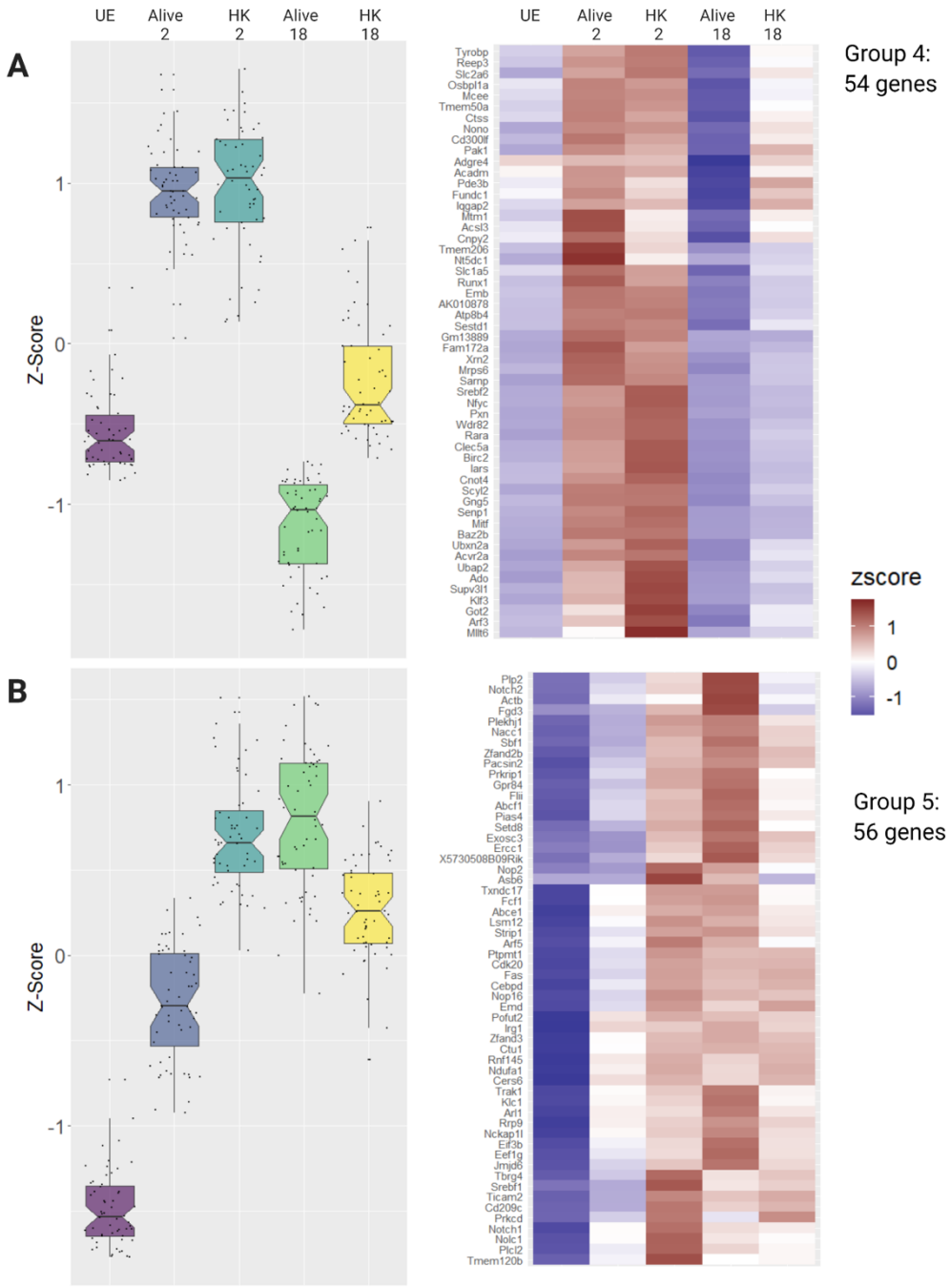


Figure S3: Clustered data exposes multiple distinct trends in gene expression (groups 4 and 5). A) Cluster 4 is enriched for apoptosis regulation and transcription/nuclear functions. B) Cluster 5 is enriched for RNA processing functions.

Supplementary Figure S4:

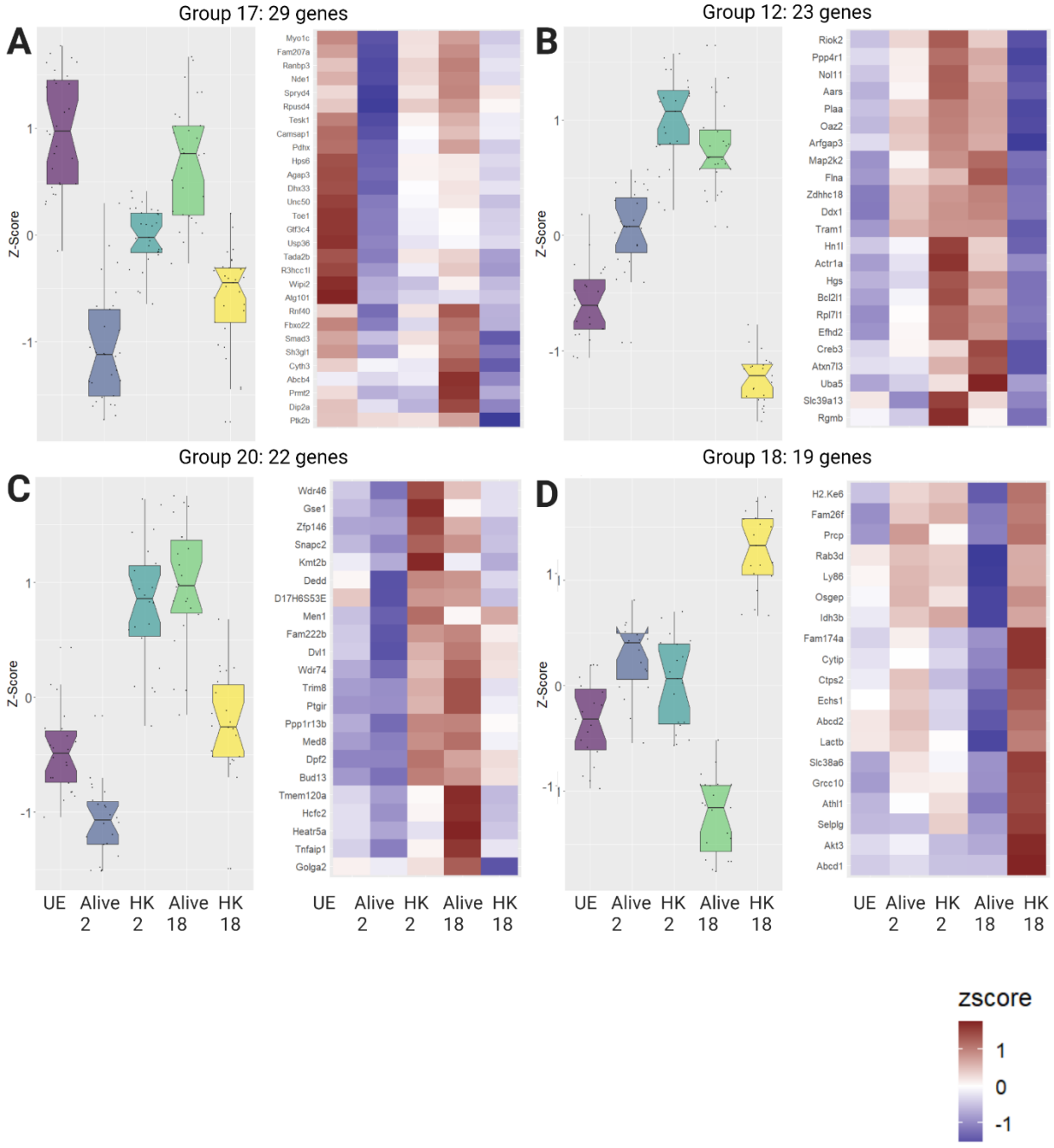


Figure S4: Clustered data exposes multiple distinct trends in gene expression (groups 17, 12, 20, 18). A) Cluster 17 is enriched for protein transporters and Zn binding. **B)** Cluster 12 is enriched for ATP binding, Zn and metal binding, transcription and protein transport. **C)** Cluster 20 is enriched for transcription, apoptosis, and Zn binding. **D)** Cluster 18 is enriched for ATP binding, glycoprotein/disulfide modifications, and membrane functions.

Supplementary Figure S5:

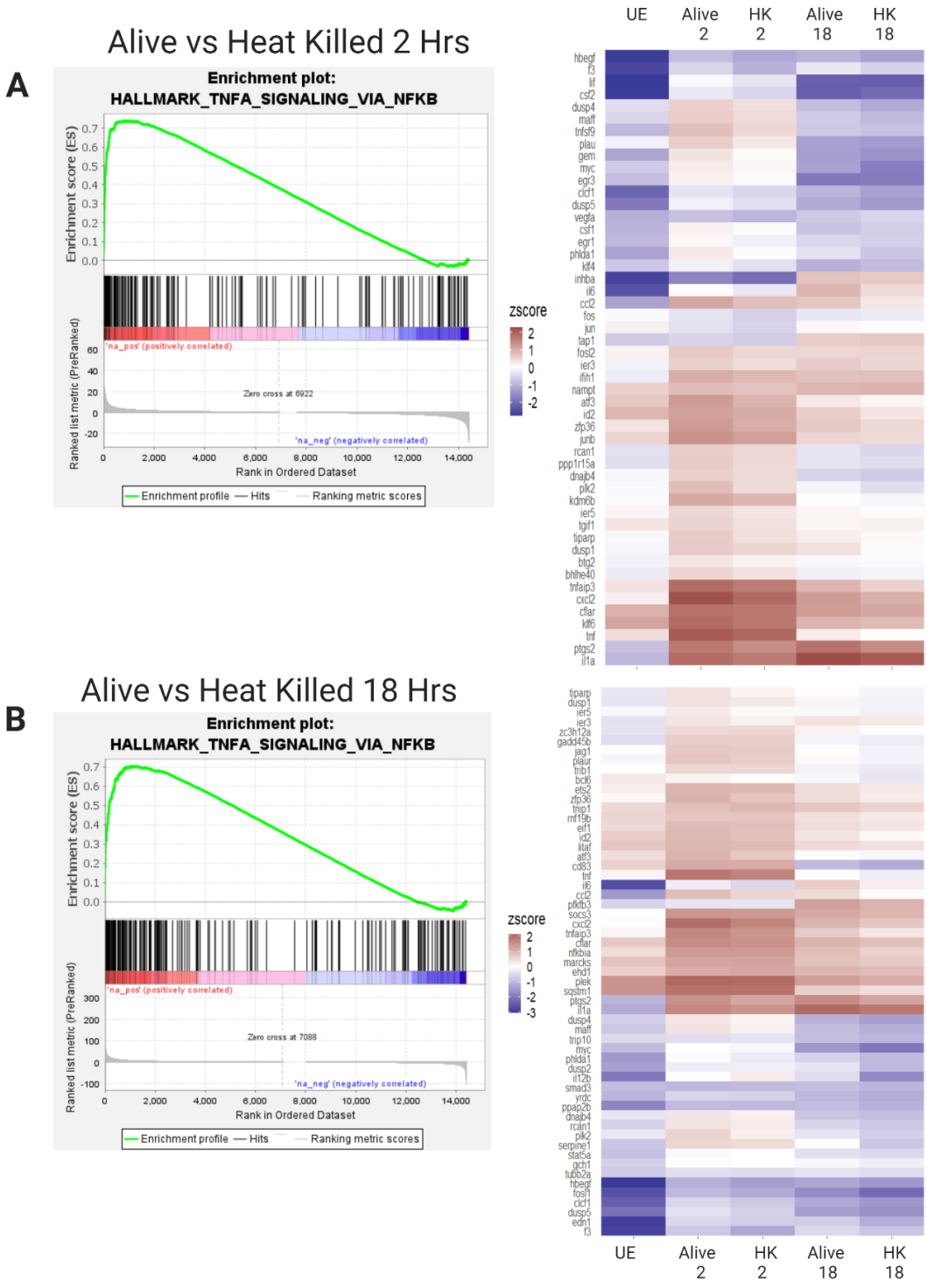


Figure S5: GSEA analysis of Hallmark gene set TNFα signaling via NF-kB. Each panel shows a GSEA enrichment plot and a hierarchically clustered expression heat map of the leading edge genes from that GSEA plot. The leading edge genes are all vertical black lines on the positive end of the spectrum, down to the point of maximum Enrichment Score. Enrichment plots were

constructed based on a ranked gene set of differential expression data (either A2 vs HK2 or A18 vs HK18). **A)** At 2 Hrs the TNFα signaling via NF-κB gene set is strongly enriched in response to live vs HK *Salmonella*. (q = 0.000, NES = 2.42) **B)** At 18 Hrs the TNFα signaling gene set has been dampened somewhat, though it is still enriched in live vs HK conditions (q = 0.002, NES = 1.88), and while there is a core set of 32 genes upregulated at both time points, there are 15 fewer leading edge genes at 18 Hrs than at 2 Hrs. UE: unexposed control; HK: heat killed; q: false discovery rate; NES: Normalized Enrichment Score from GSEA.

Supplementary Figure S6:

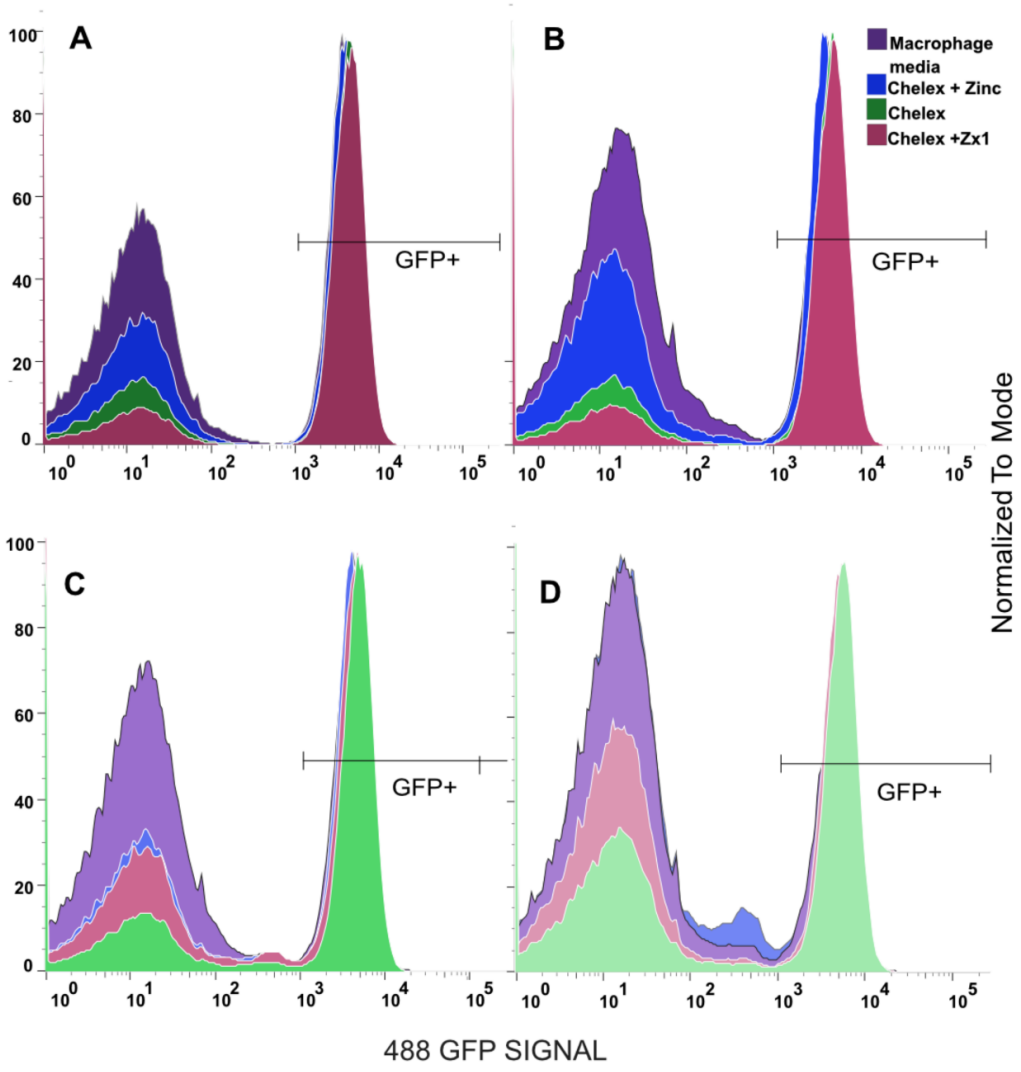


Figure S6: FACS analysis of infected 129S6 BMDMs clustered by time post infection. These histograms of flow cytometry data indicate the GFP signal from macrophages infected with *Salmonella* expressing pDiGc at 2, 10, 18 or 24 hours post infection. At each time point, Zn replete medias (normal macrophage growth media, serving as a positive control, and Media with Chelex-treated FBS + 30μM Zn) showed more robust bacterial clearance than Zn depleted media (Media with Chelex-treated FBS) or Zn deficient media (Media with Chelex-treated FBS + ZX1 extracellular Zn chelator). A) 2 Hrs post infection. B) 10 Hrs post infection. C) 18 Hrs post infection. D) 24 Hrs post infection. Blue 'chelex+zinc' curve is directly behind the purple macrophage media curve, both showing almost 100% bacterial clearance.

Supplemental Figure S7:

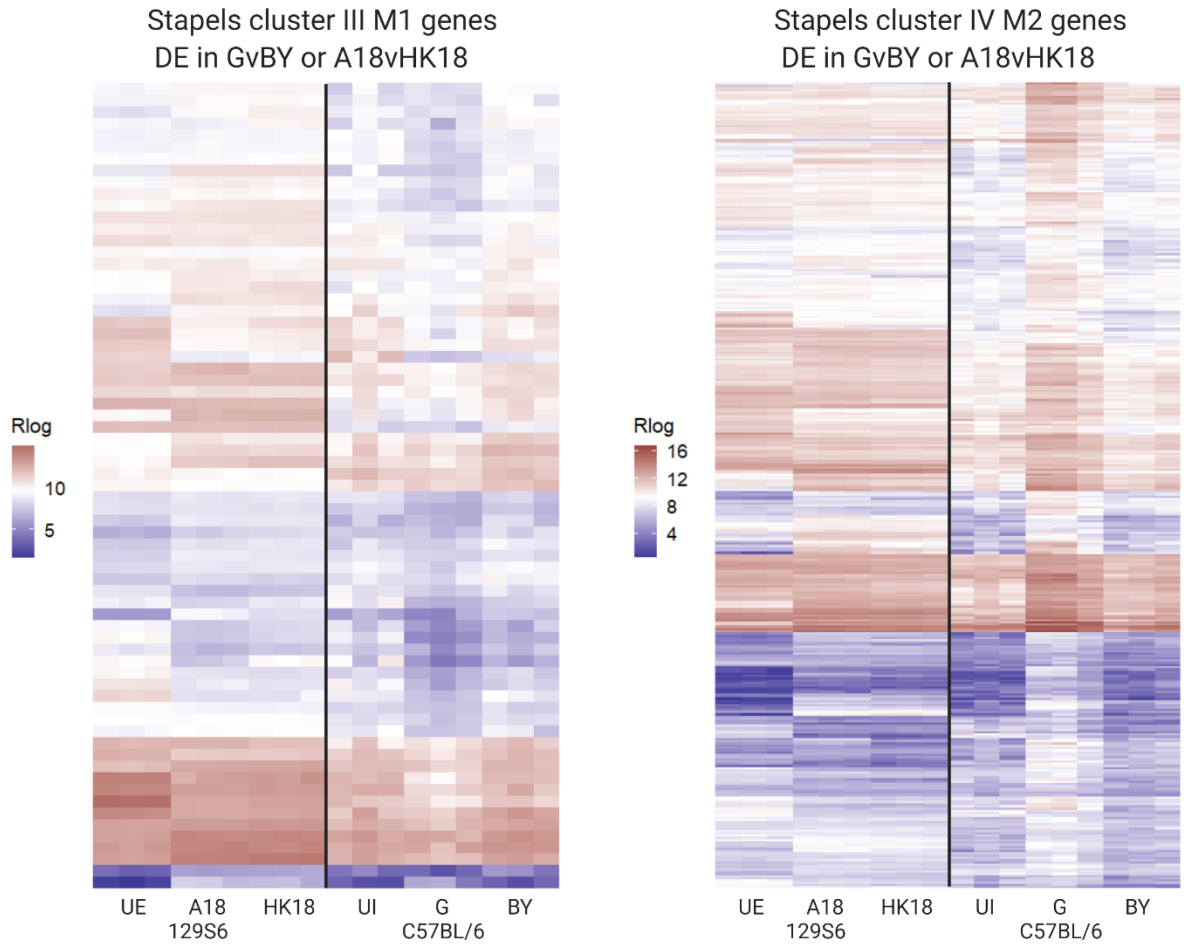


Figure S7: Heatmaps of the gene clusters III and IV that were defined in the Stapels' paper. After processing Stapels' data and normalizing it with our own, we mapped the expression values of the genes in Stapels' cluster III (M1 in character) and IV (M2 in character). Genes in each cluster that were differentially expressed ($q < 0.01$) in binary comparisons of A18 vs HK18 or G vs BY and expressed above background are included here. Staples et al hypothesize that this differential expression indicates an M2 phenotype that is being induced by the presence of live, growing *Salmonella*. Overwhelmingly though, these genes are DE in Stapels' data and not between our alive and HK conditions, supporting the idea that 129S6 macrophages represent a distinct model system.

Supplementary Figure S8

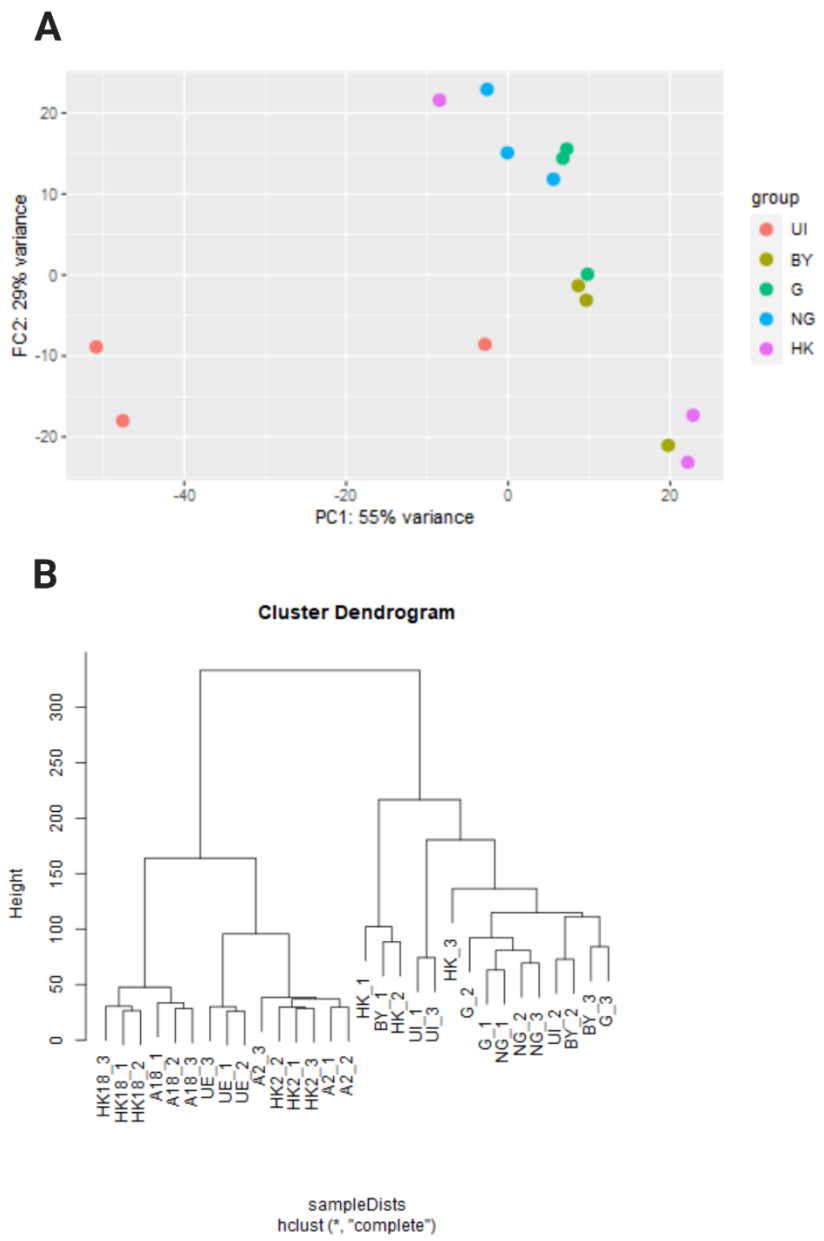


Figure S8: PCA and dendrogram showing Stapels' data analyzed with our pipeline. A) PCA of all 15 of Stapels' samples after their FASTQ files were trimmed, mapped and counted using our pipeline. PCA produced with EdgeR. B) Dendrogram demonstrating hierarchical clustering of Stapels' samples and ours, normalized together in DESeq2.