SUPPLEMENTARY INFORMATION

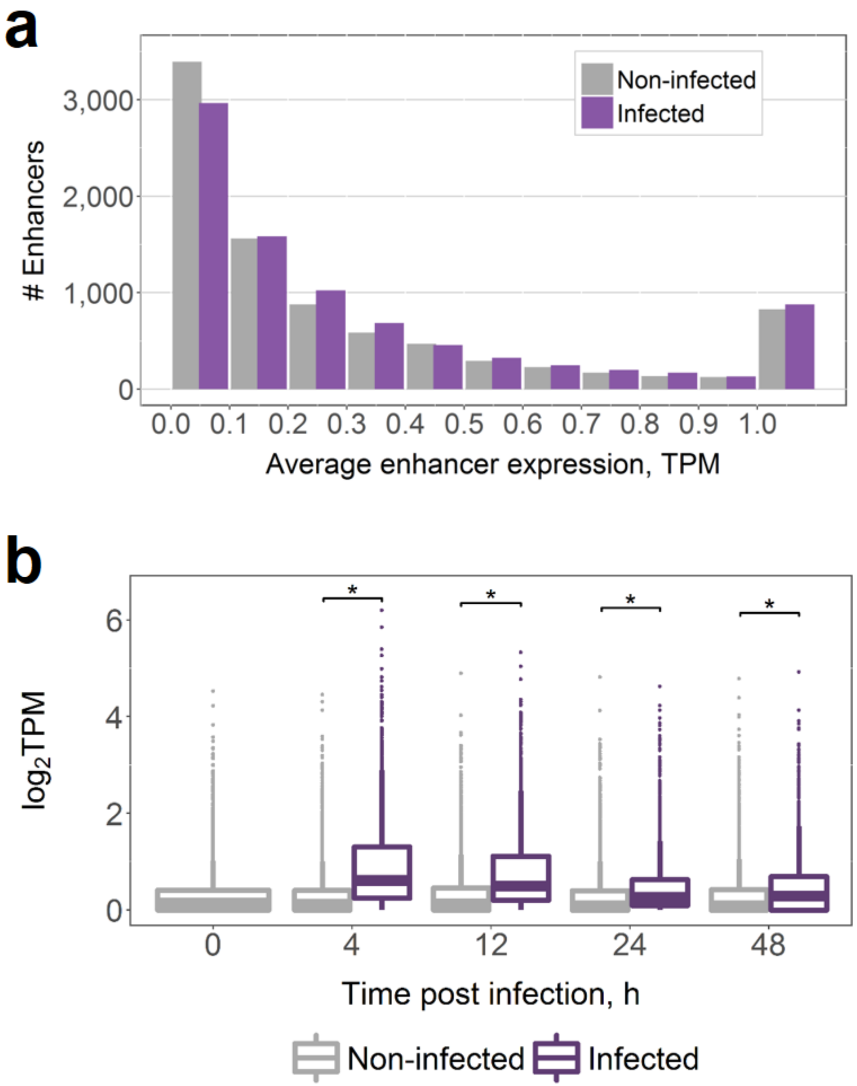

Figure S1. Many enhancers respond to *M.tb* infection with increased eRNA expression. a Expression of all 8,667 macrophage enhancer eRNA in non-infected and infected macrophages; each bin includes the left edge. **b** Expression of 2,999 enhancer eRNA associated with up-regulated DEGs; expression in TPM was averaged across replicates, (*) indicate paired two-sided Wilcoxon signed-rank test p-value $< 2.2*10^{-16}$.

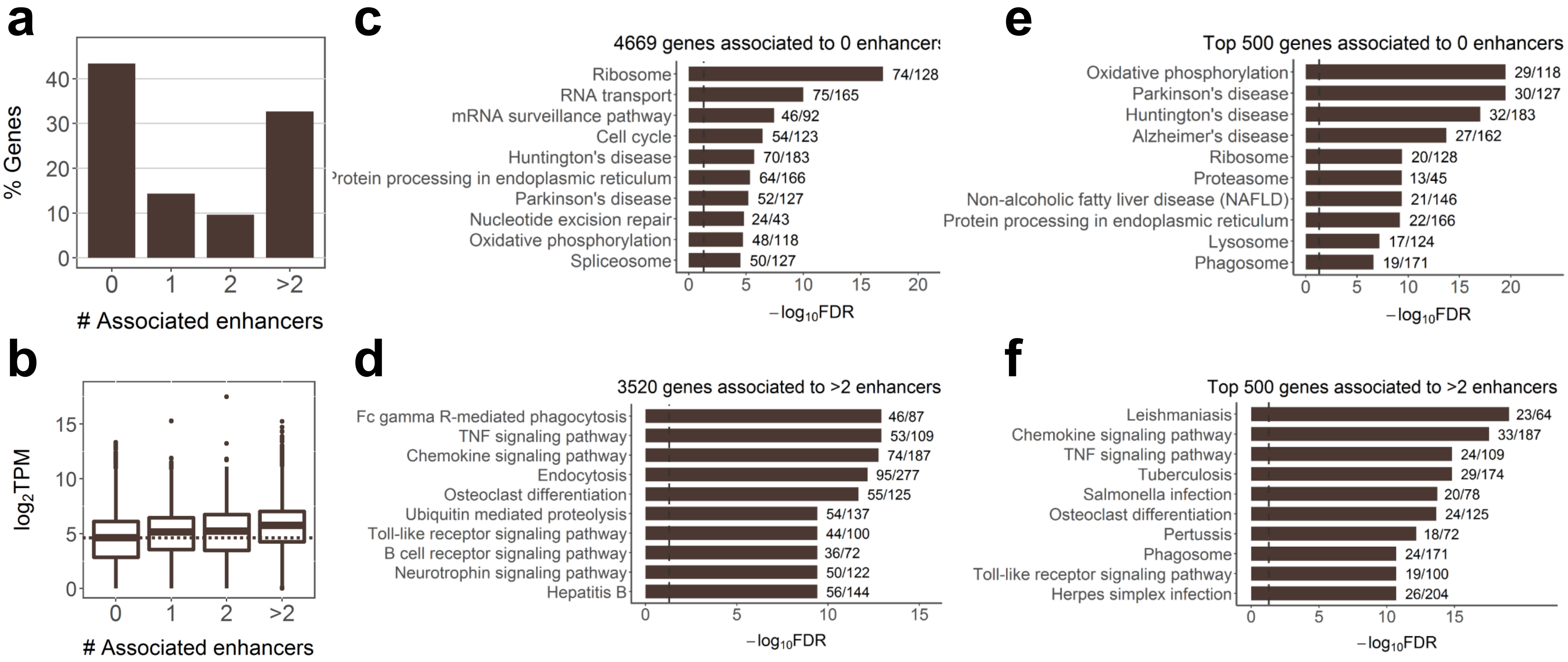

Figure S2. Higher number of associated enhancers is a concomitant of higher gene expression and immune functions in infected macrophages. a, b Percentage and expression of genes associated with different number of enhancers in infected macrophages; expression in TPM was averaged across infected samples, dashed line shows median expression of genes not associated with any enhancer. **c** KEGG pathway maps enriched for genes associated with no transcribed enhancers. **d** KEGG pathway maps enriched for genes associated with more than two transcribed enhancers. **e** KEGG pathway maps enriched for top 500 genes with the highest average expression in infected macrophages among genes associated with no transcribed enhancers. **f** KEGG pathway maps enriched for top 500 genes with the highest average expression in infected macrophages among genes associated with more than two transcribed enhancers. In **c-f,** top 10 maps with the lowest FDR are shown; next to the bars are the numbers of genes in the map covered by our gene list; dashed lines indicate FDR = 0.05.

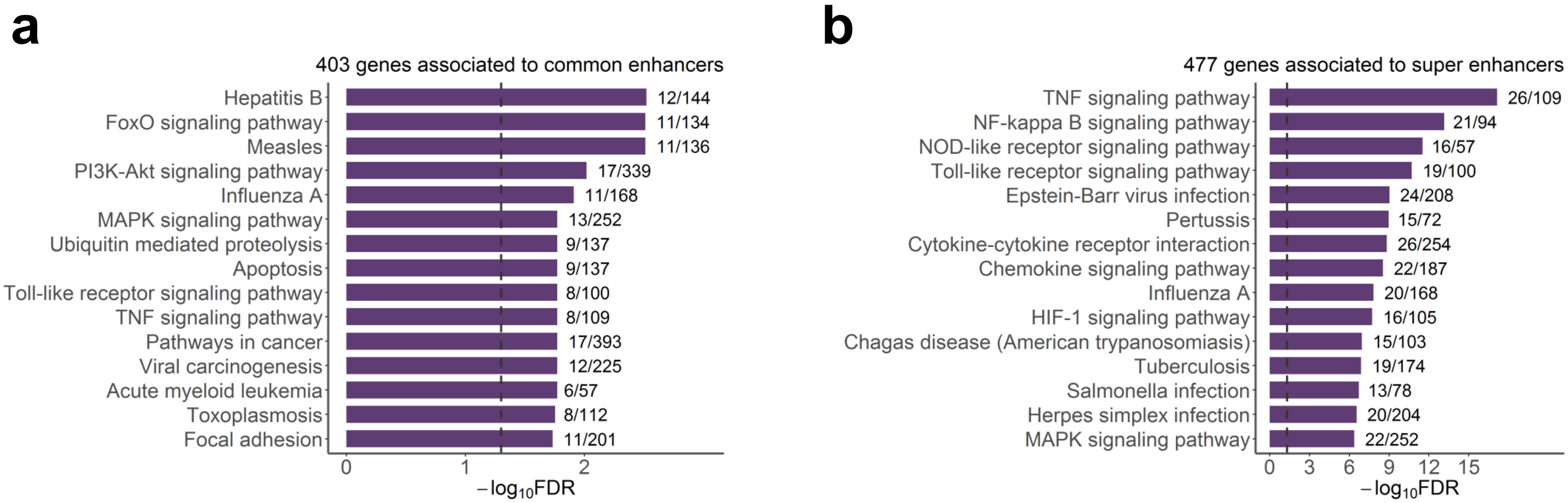

Figure S3. Up-regulated DEGs associated with super enhancers show more infection-specific functions. a KEGG pathway maps enriched for 403 genes associated with transcribed enhancers that do not overlap super enhancer regions. **b** KEGG pathway maps enriched for 477 genes associated with transcribed enhancers overlapping super enhancer regions. In **a** and **b**, top 15 maps with the lowest FDR are shown; next to the bars are the numbers of genes in the map covered by our gene list; dashed lines indicate FDR = 0.05.

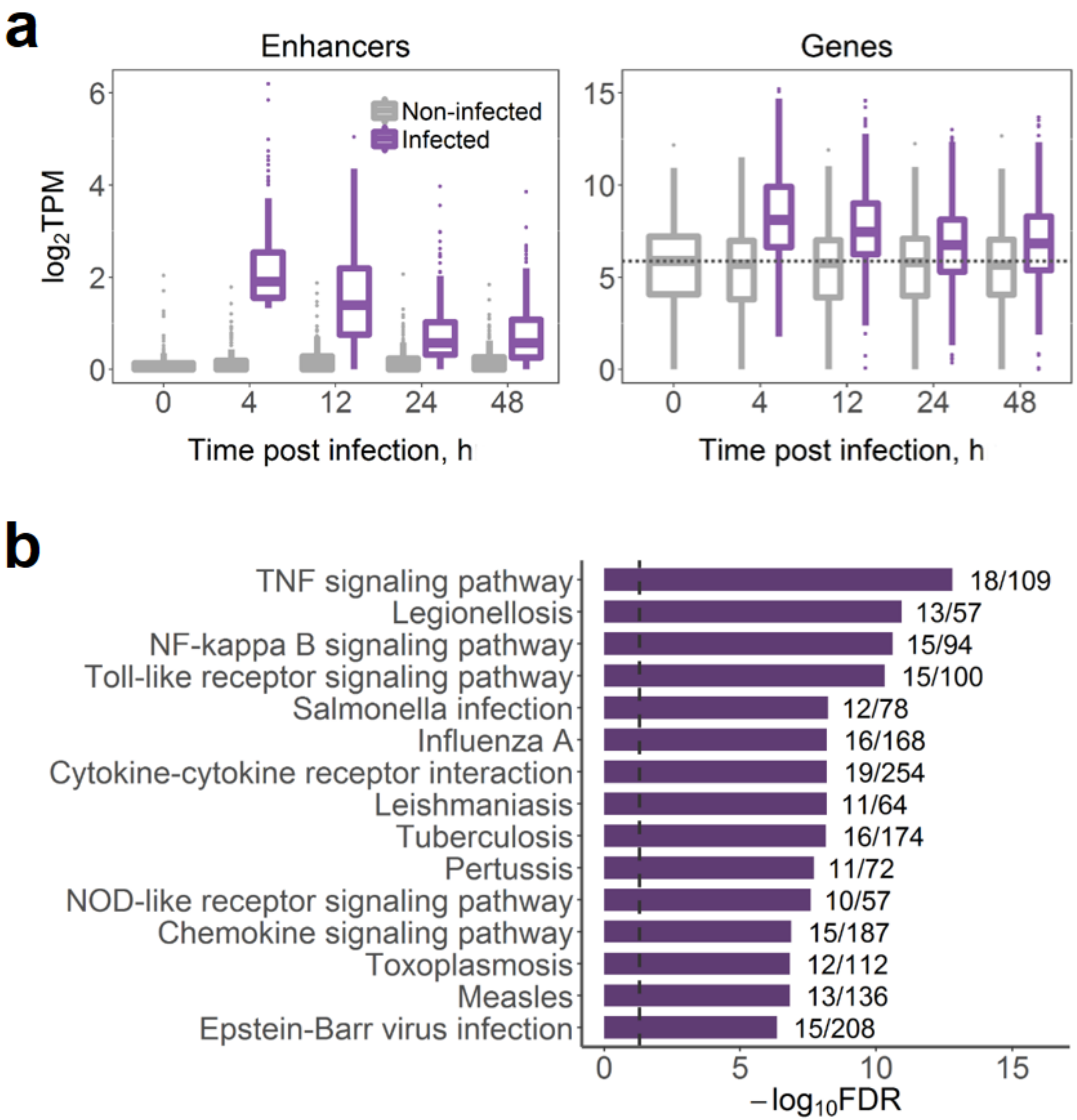

Figure S4. 257 induced enhancers associated with 263 DEGs up-regulated at 4 h post infection. a Expression of enhancer eRNA and genes; dashed line shows median gene expression prior to the infection, expression in TPM was averaged across replicates. **b** Top 15 KEGG pathway maps with the lowest FDR enriched for the genes; next to the bars are the numbers of genes in the KEGG term covered by our gene list; dashed line indicates FDR = 0.05.

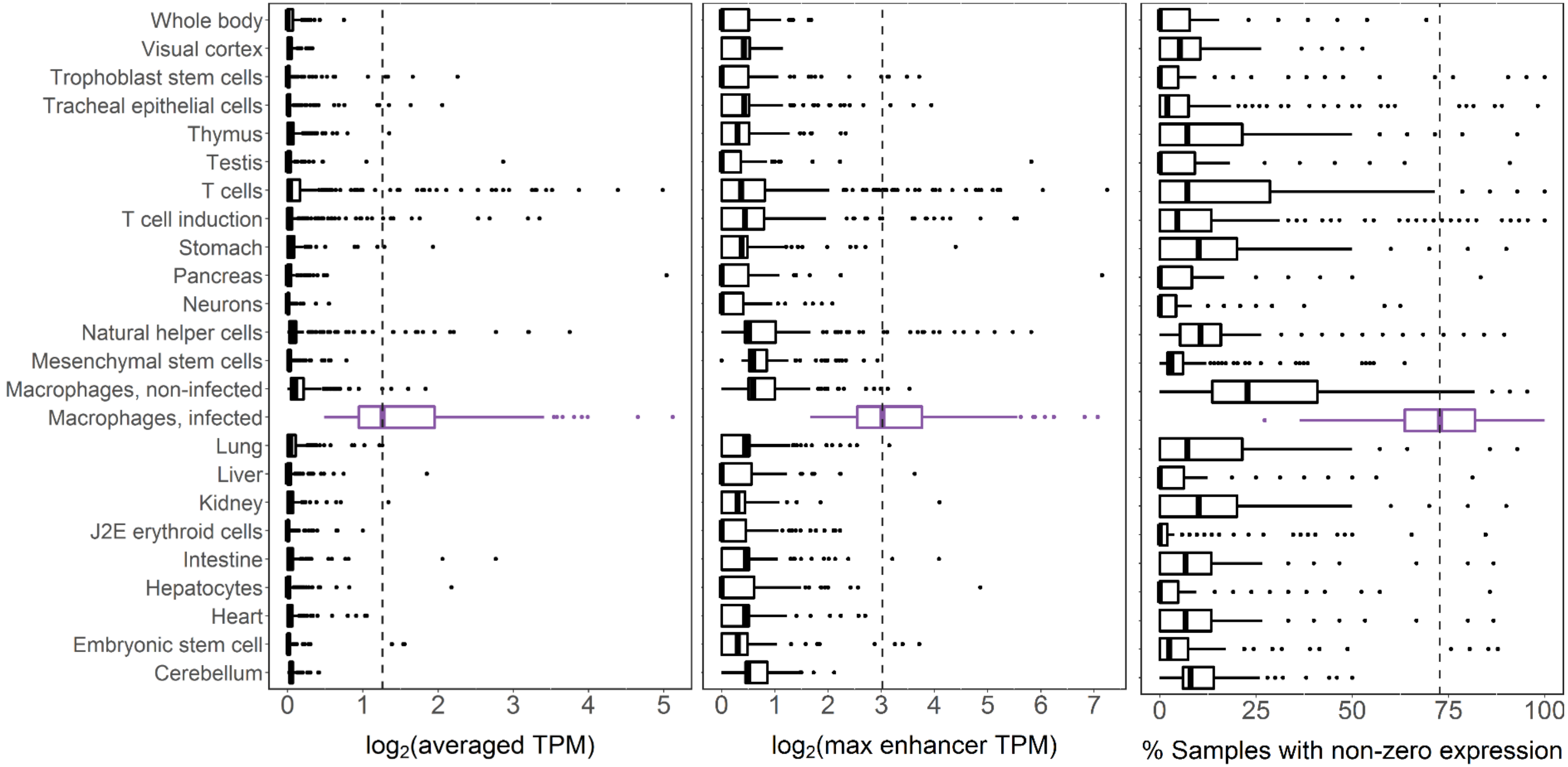

Figure S5. Expression of the induced enhancers in mouse tissues. See Table S4 for the list of non-macrophage samples used. Left panel: expression of each enhancer was averaged across tissue samples. Middle panel: maximum enhancer expression value in each tissue is used. Right panel: percentage of samples with nonzero expression was calculated for each enhancer in each tissue.

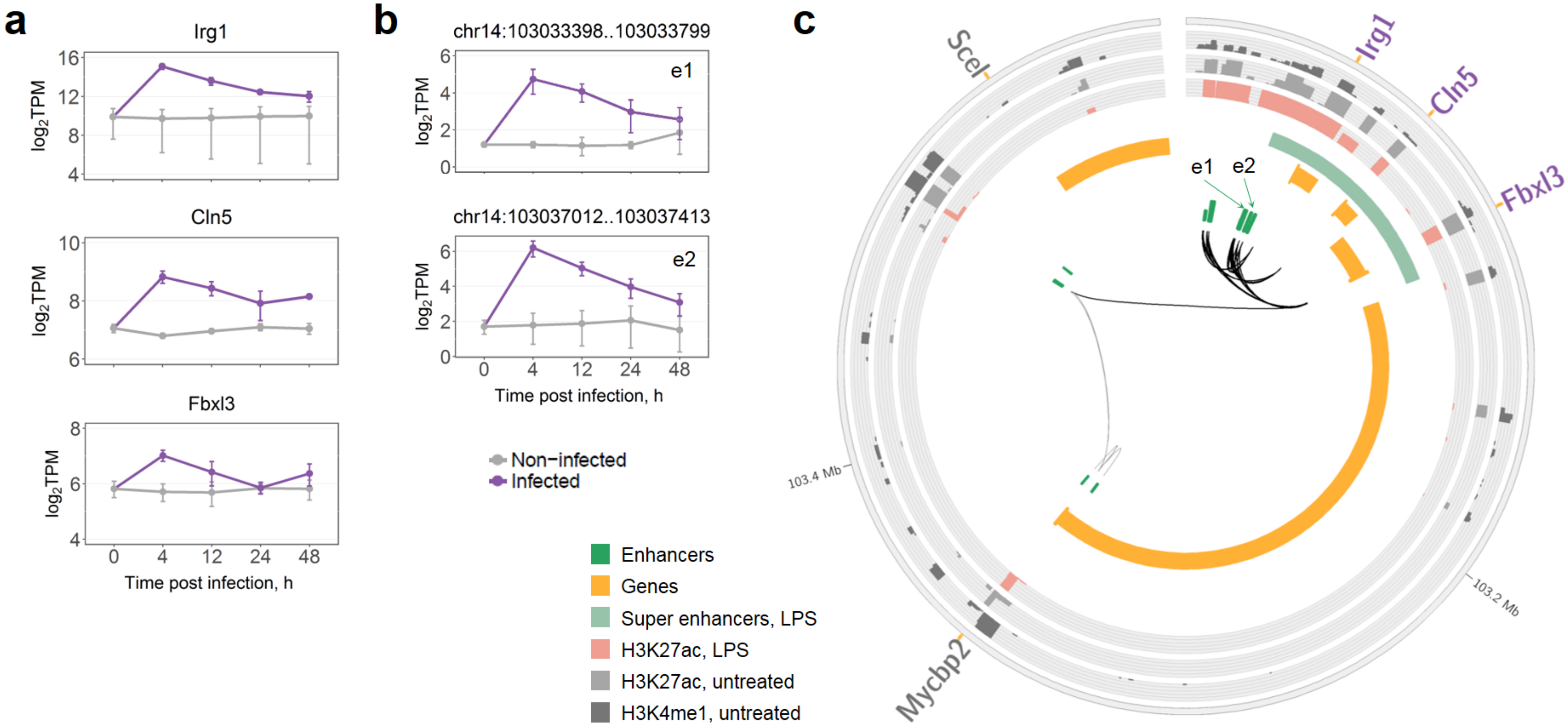

Figure S6. Regulation of *Irg1*, *Cln5*, and *Fbxl3* genes. a Time course expression of the genes. **b** Time course eRNA expression of associated induced enhancers with the highest average expression at 4 h. In **a** and **b,** data were averaged over replicates and log-transformed, error bars are the SEM. **c** TAD containing the genes and associated enhancers; induced enhancers are shown as longer green blocks. Genes are split into two tracks based on the strand, wide orange marks denote gene promoters. DEGs up-regulated at 4 h are shown in purple and their associations with enhancers are shown as thicker black connections. Super enhancers shown as defined by Hah et al. in LPS-treated macrophages. Histone marks are shown as defined by Ostuni et al. in LPS-treated and untreated macrophages.

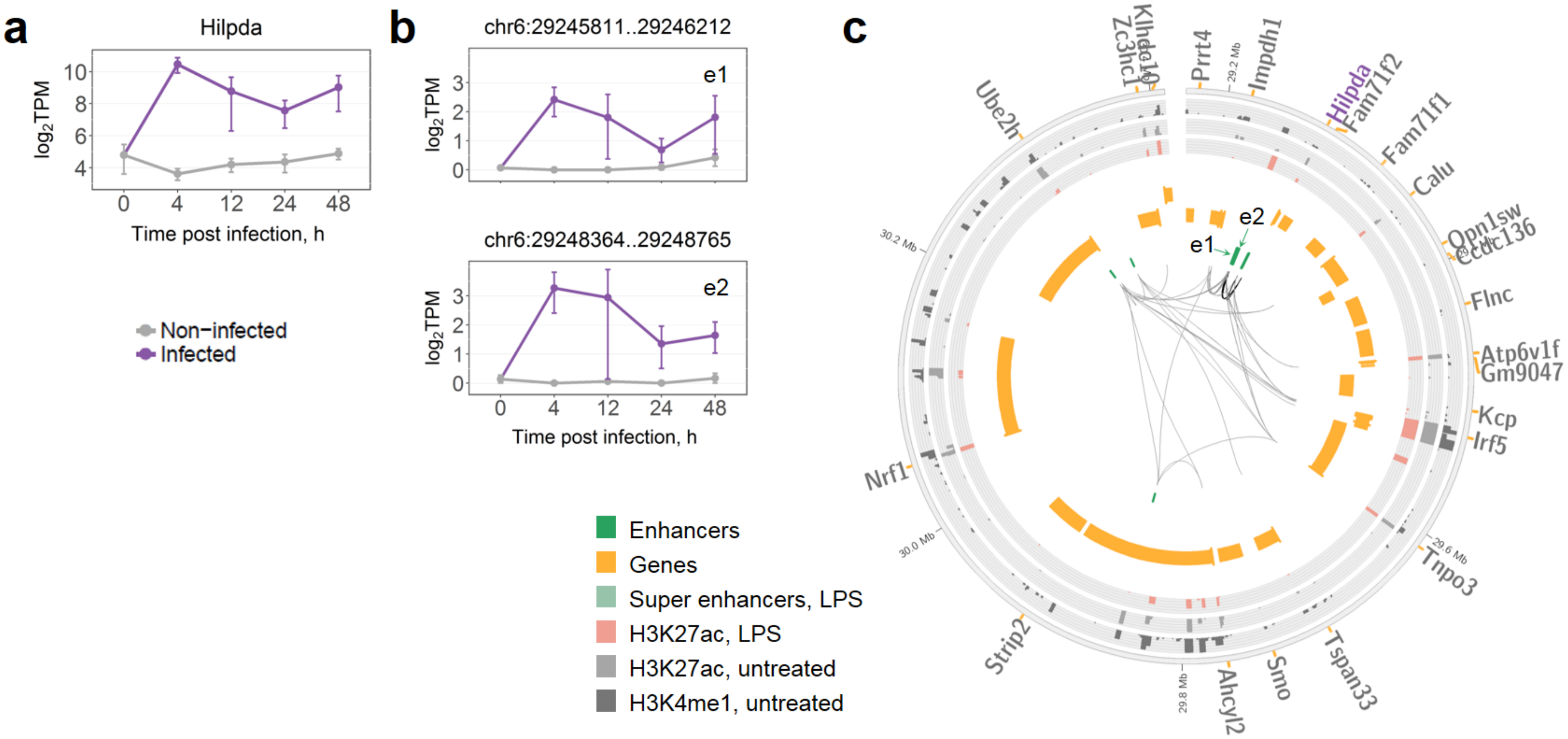

Figure S7. Regulation of *Hilpda* gene. a Time course expression of the gene. **b** Time course eRNA expression of associated induced enhancers with the highest average expression at 4 h. In **a** and **b,** data were averaged over replicates and log-transformed, error bars are the SEM. **c** TAD containing the gene and associated enhancers; induced enhancers are shown as longer green blocks. Genes are split into two tracks based on the strand, wide orange marks denote gene promoters. The *Hilpda* gene up-regulated at 4 h is shown in purple and its associations with enhancers are shown as thicker black connections. Super enhancers shown as defined by Hah et al. in LPS-treated macrophages. Histone marks are shown as defined by Ostuni et al. in LPS-treated and untreated macrophages.

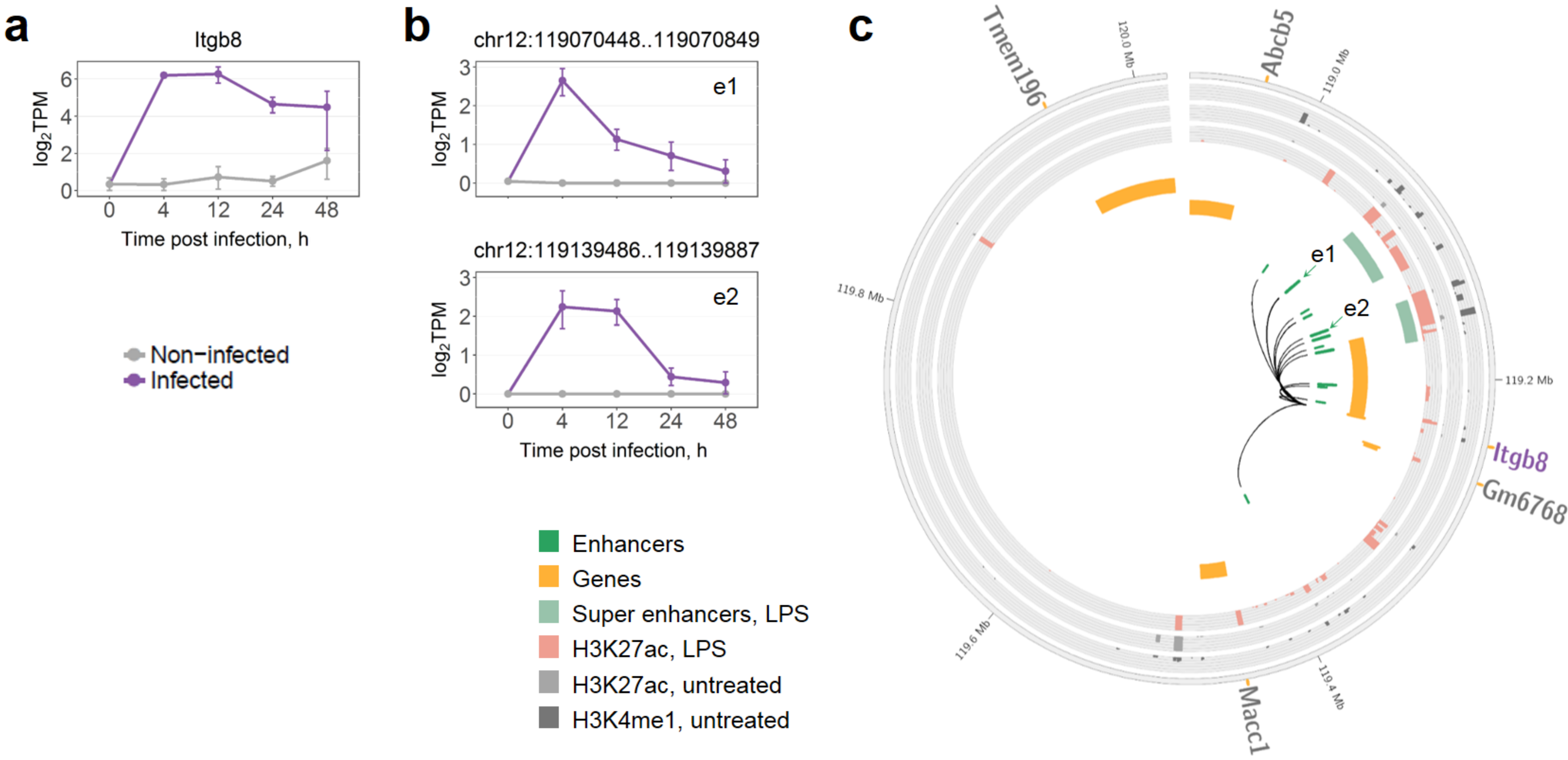

Figure S8. Regulation of *Itgb8* gene. a Time course expression of the gene. **b** Time course eRNA expression of associated induced enhancers with the highest average expression at 4 h. In **a** and **b,** data were averaged over replicates and log-transformed, error bars are the SEM. **c** TAD containing the gene and associated enhancers; induced enhancers are shown as longer green blocks. Genes are split into two tracks based on the strand, wide orange marks denote gene promoters. DEGs up-regulated at 4 h are shown in purple and their associations with enhancers are shown as thicker black connections. Super enhancers shown as defined by Hah et al. in LPS-treated macrophages. Histone marks are shown as defined by Ostuni et al. in LPS-treated and untreated macrophages.

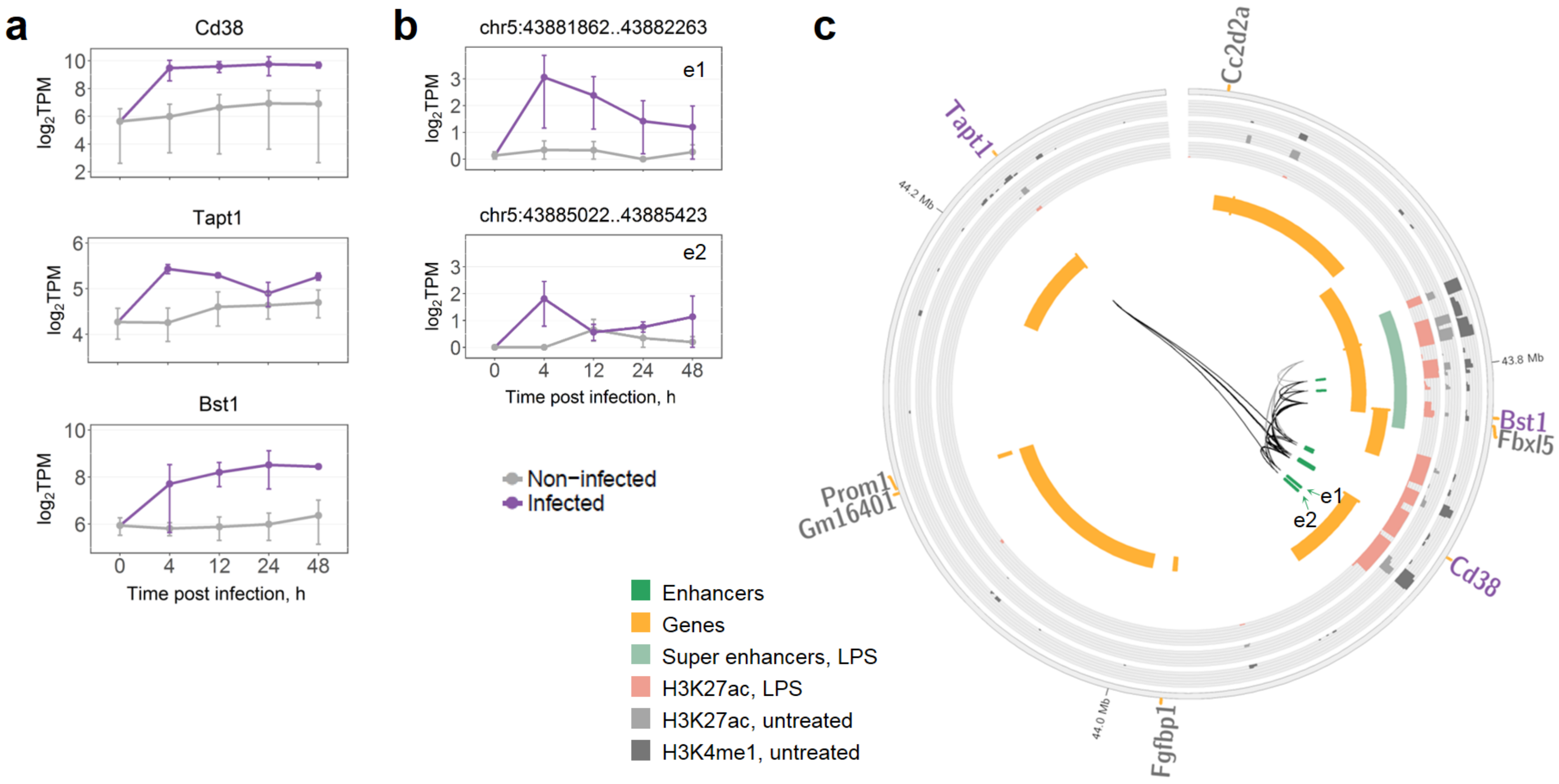

Figure S9. Regulation of *Cd38*, *Bst1*, and *Tapt1* genes. a Time course expression of the genes. **b** Time course eRNA expression of associated induced enhancers with the highest average expression at 4 h. In **a** and **b,** data were averaged over replicates and log-transformed, error bars are the SEM. **c** TAD containing the genes and associated enhancers; induced enhancers are shown as longer green blocks. Genes are split into two tracks based on the strand, wide orange marks denote gene promoters. DEGs up-regulated at 4 h are shown in purple and their associations with enhancers are shown as thicker black connections. Super enhancers shown as defined by Hah et al. in LPS-treated macrophages. Histone marks are shown as defined by Ostuni et al. in LPS-treated and untreated macrophages.

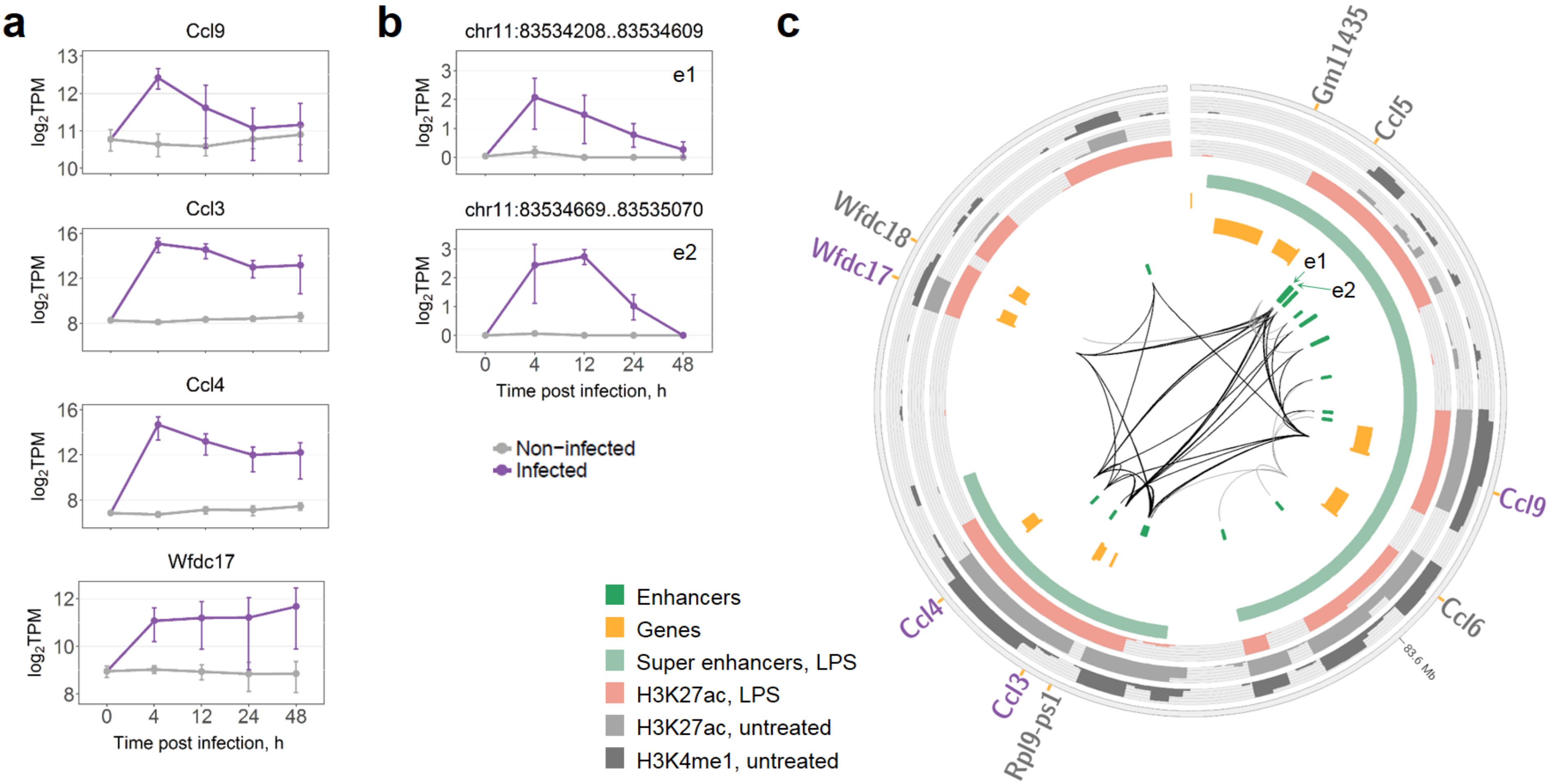

Figure S10. Regulation of *Ccl9*, *Ccl3*, *Ccl4*, and *Wfdc17* genes. a Time course expression of the genes. **b** Time course eRNA expression of associated induced enhancers with the highest average expression at 4 h. In **a** and **b,** data were averaged over replicates and log-transformed, error bars are the SEM. **c** TAD containing the genes and associated enhancers; induced enhancers are shown as longer green blocks. Genes are split into two tracks based on the strand, wide orange marks denote gene promoters. DEGs up-regulated at 4 h are shown in purple and their associations with enhancers are shown as thicker black connections. Super enhancers shown as defined by Hah et al. in LPS-treated macrophages. Histone marks are shown as defined by Ostuni et al. in LPS-treated and untreated macrophages.

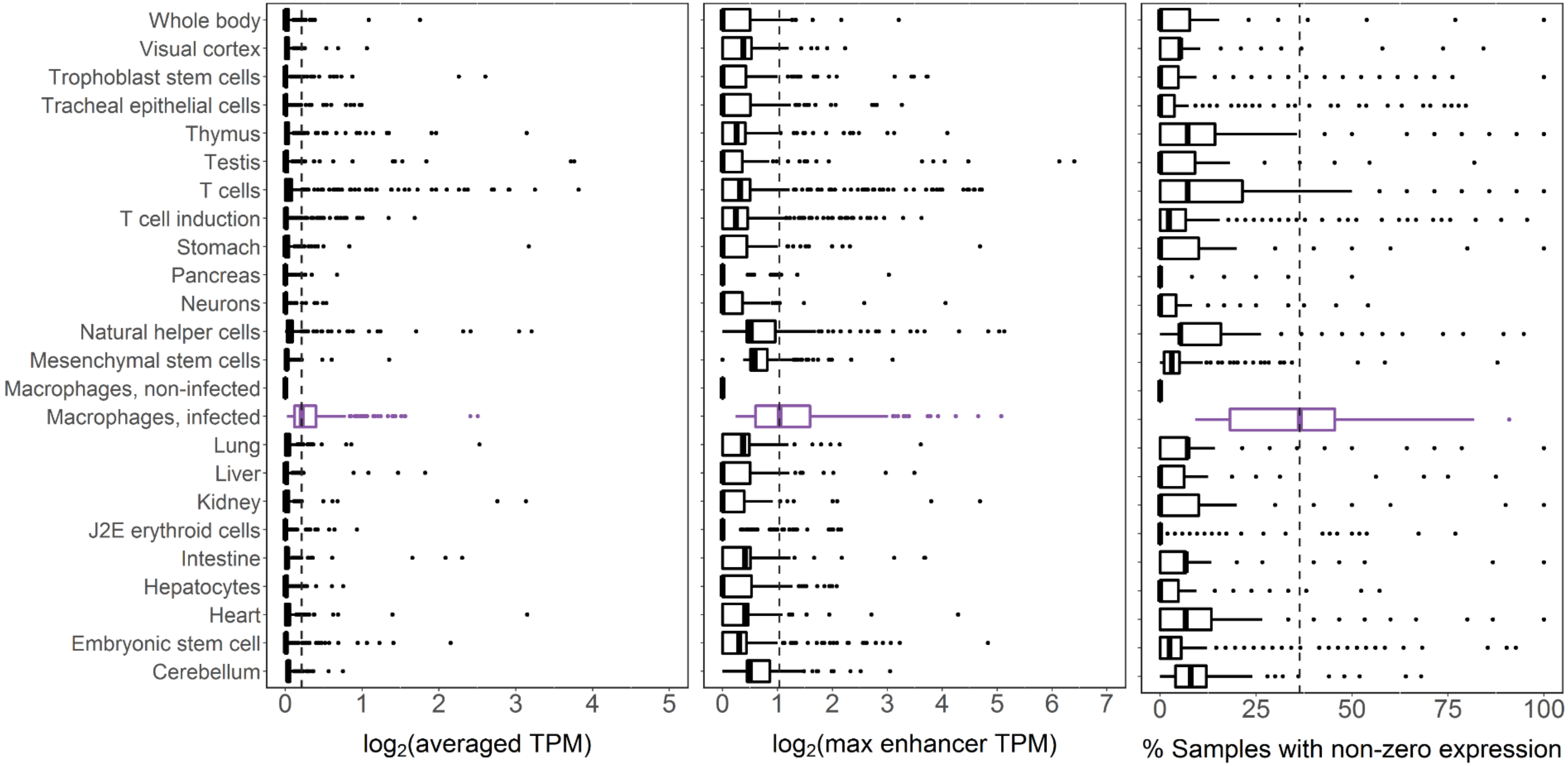

Figure S11. Expression of the acquired enhancers in mouse tissues. See Table S4 or the list of non-macrophage samples used. Left panel: expression of each enhancer was averaged across tissue samples. Middle panel: maximum enhancer expression value in each tissue samples is used. Right panel: percentage of samples with nonzero expression was calculated for each enhancer in each sample group.

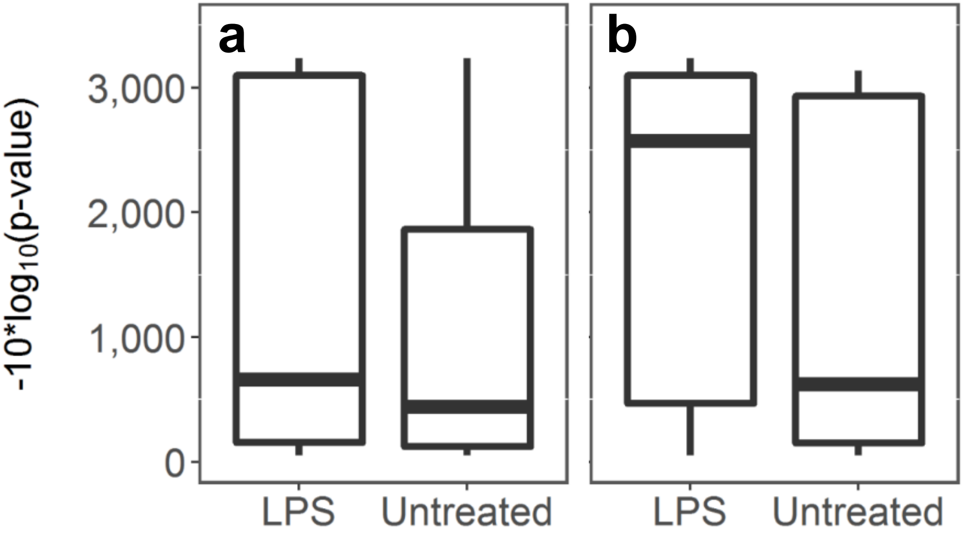

Figure S12. H3K27ac ChIP-seq peaks. Data from Ostuni et al. (Latent Enhancers Activated by Stimulation in Differentiated Cells. Cell. 2013;152:157-171). **a** All significant H3K27ac peaks detected in untreated and LPS-treated samples. **b** A subset of peaks overlapping acquired enhancers.

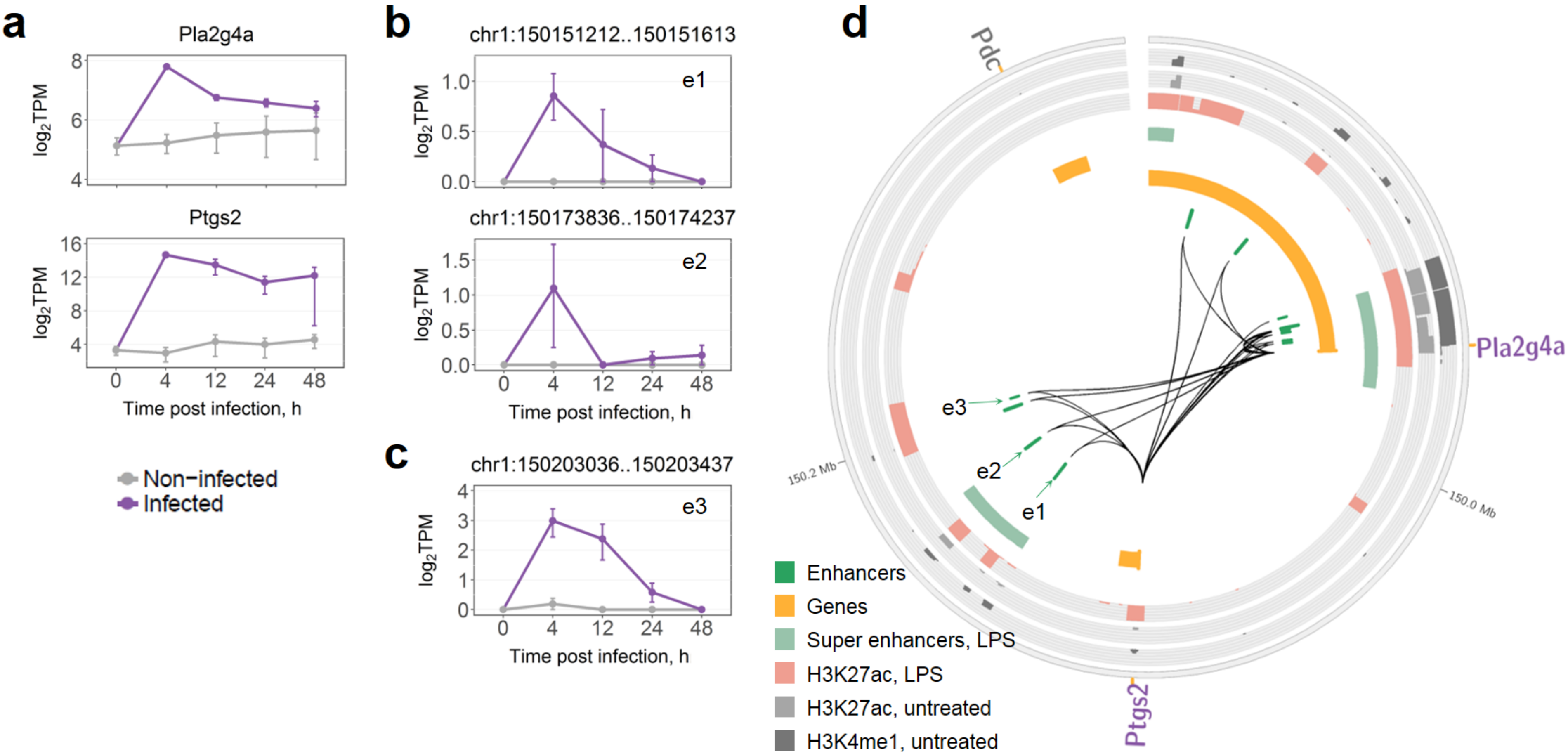

Figure S13. Regulation of *Pla2g4a* and *Ptgs2* genes. a Time course expression of the genes. **b** Time course eRNA expression of associated acquired enhancers with the highest average expression at 4 h. **c** Time course eRNA expression of associated induced enhancer. In **a, b,** and **c,** data were averaged over replicates and log-transformed, error bars are the SEM. **d** TAD containing the genes and associated enhancers; acquired enhancers are shown as longer green blocks. Genes are split into two tracks based on the strand, wide orange marks denote gene promoters. DEGs up-regulated at 4 h are shown in purple. Super enhancers shown as defined by Hah et al. in LPS-treated macrophages. Histone marks are shown as defined by Ostuni et al. in LPS-treated and untreated macrophages.

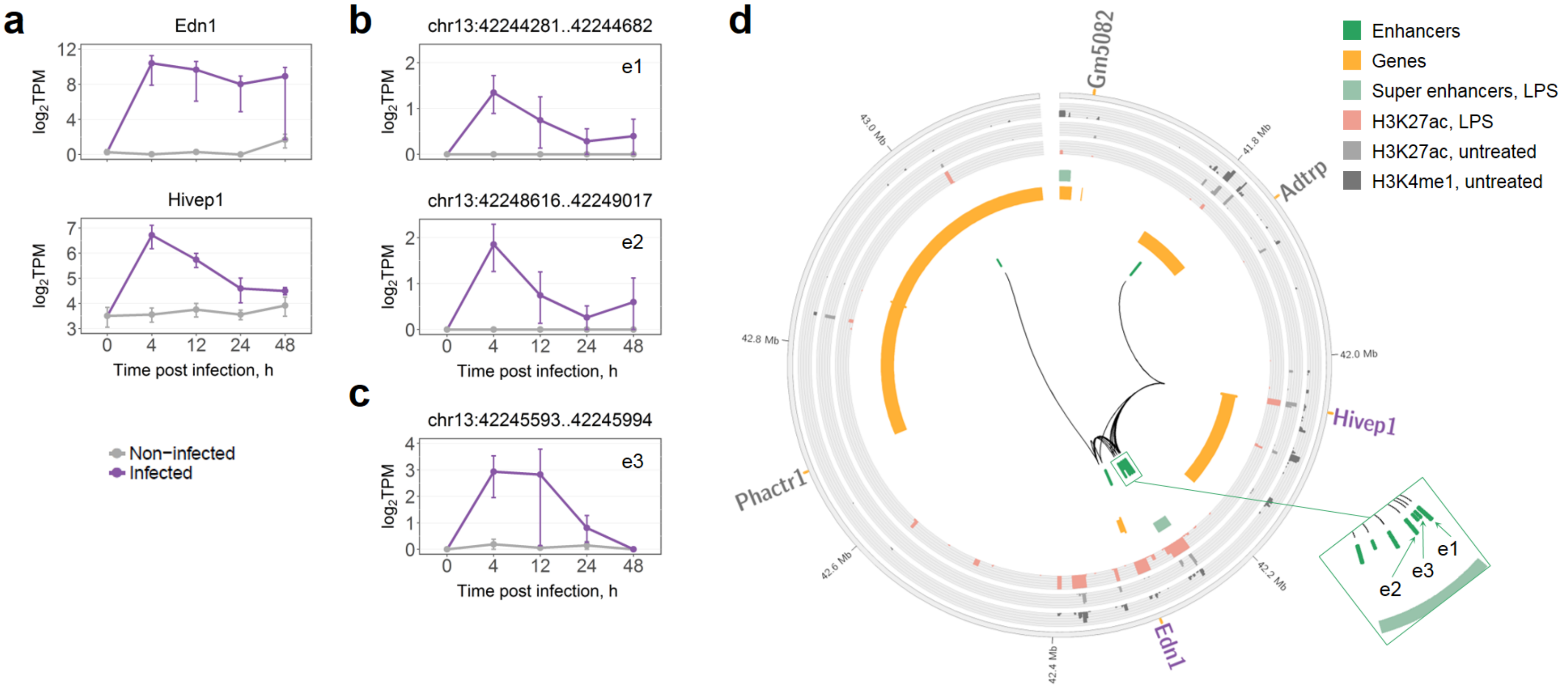

Figure S14. Regulation of *Edn1* and *Hivep1* genes. a Time course expression of the genes. **b** Time course eRNA expression of associated acquired enhancers with the highest average expression at 4 h. **c** Time course eRNA expression of associated induced enhancer with the highest expression at 4 h. In **a, b,** and **c,** data were averaged over replicates and log-transformed, error bars are the SEM. **d** TAD containing the genes and associated enhancers; acquired enhancers are shown as longer green blocks. Genes are split into two tracks based on the strand, wide orange marks denote gene promoters. DEGs up-regulated at 4 h are shown in purple. Super enhancers shown as defined by Hah et al. in LPS-treated macrophages. Histone marks are shown as defined by Ostuni et al. in LPS-treated and untreated macrophages.