

Chronic heavy drinking drives distinct transcriptional and epigenetic changes in splenic macrophages

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Contains 6 supplementary figures

Supplementary Fig. S1

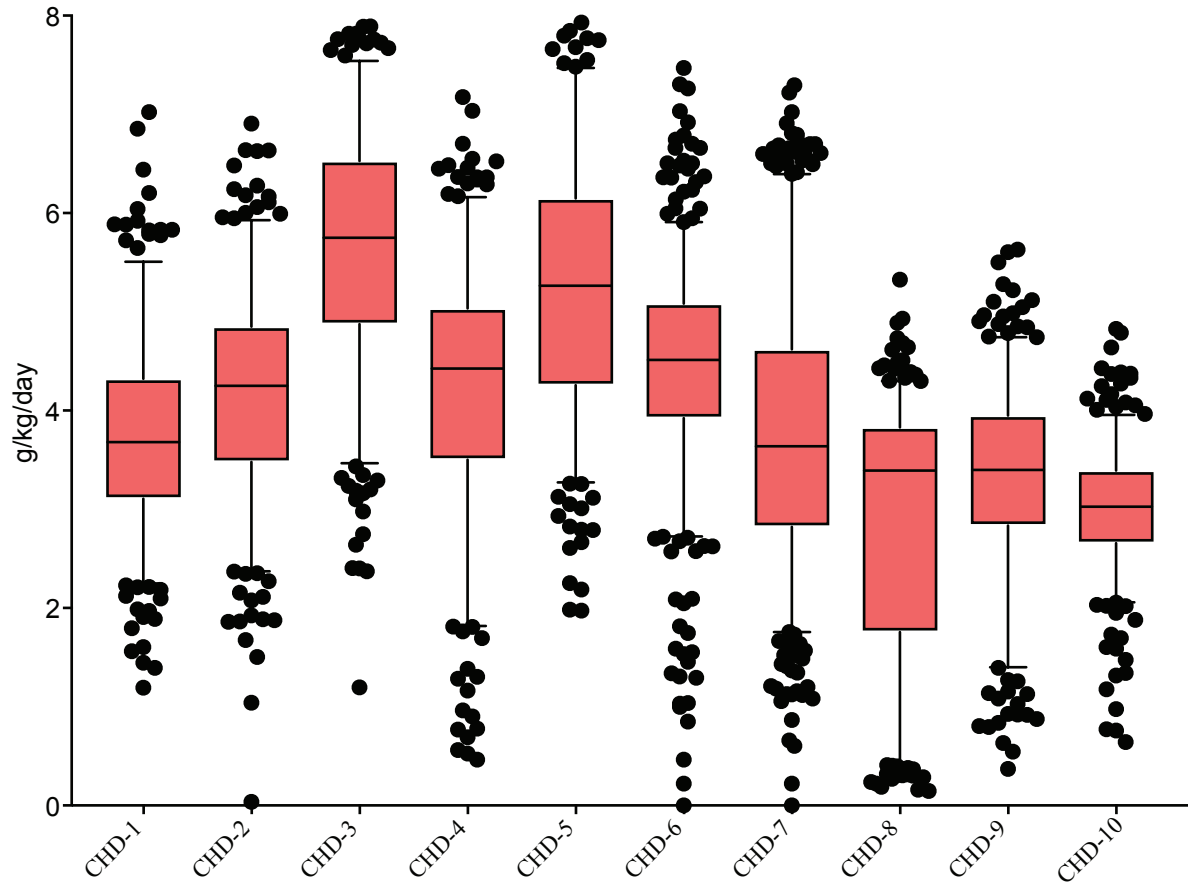


Fig S1: Average daily ethanol consumption over the 12 months open access period

Supplementary Figure S2

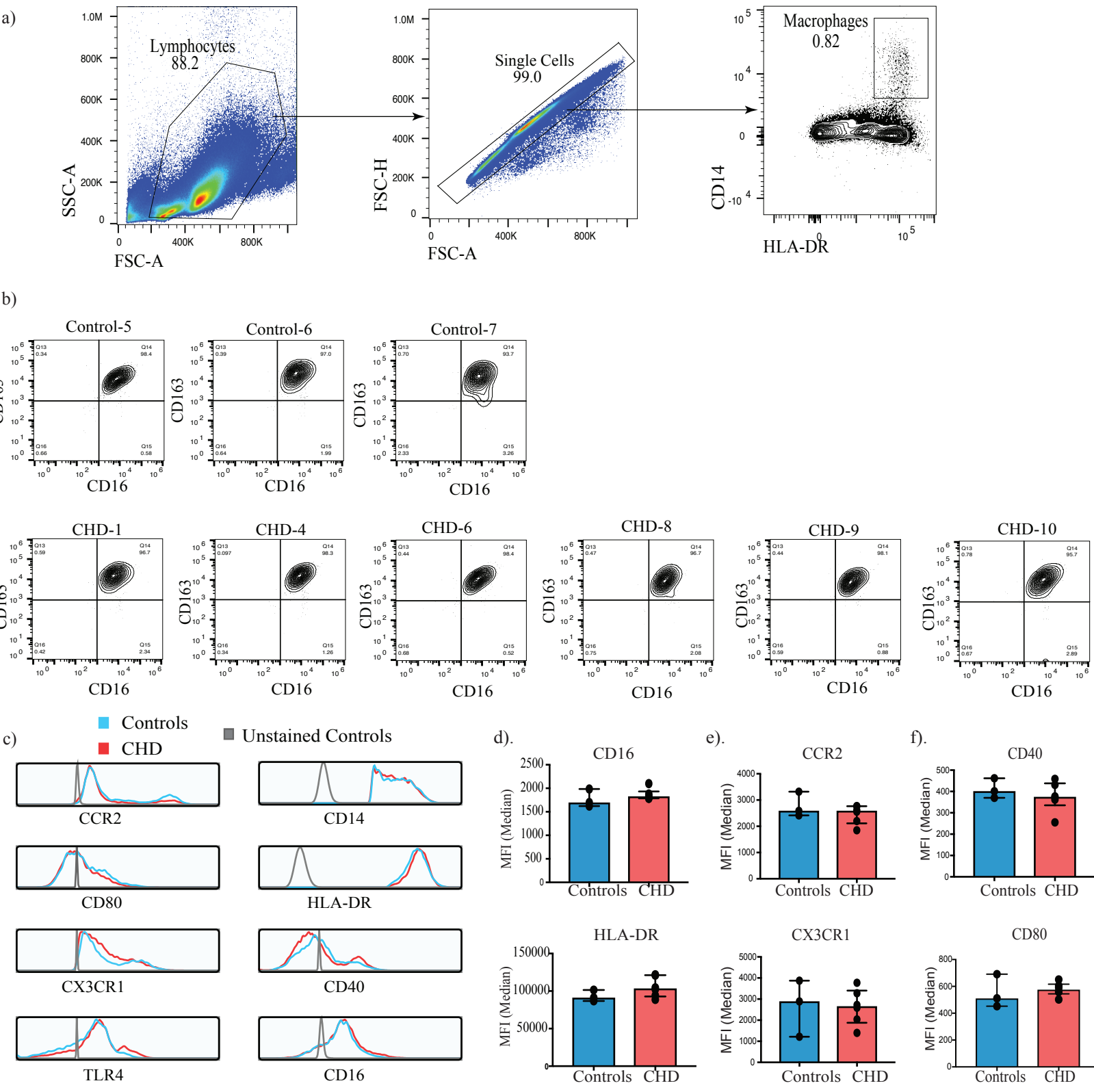


Fig S2: Flow cytometry analysis of splenic macrophages: (a) Gating strategy used in all flow experiments described in this study. Splenic macrophages were identified from the singlet gate using biplots of CD14 and HLA-DR. (b) Contour plots of CD163 vs CD16 expression in gated splenic macrophages (c) Histograms of fluorescent intensities of markers tested relative to unstained controls. Histograms of one control and one CHD sample are shown. (d, e) Bar graphs showing differences in surface expression of canonical monocyte/macrophage markers and (f) activation markers within the macrophage populations of controls (n=3) and CHD (n=6) animals measured using flow cytometry. Median Fluorescence Intensity (MFI) is depicted on the Y-axis. Group differences in surface expression were measured using unpaired t-test with Welch's correction.

Supplementary Fig S3

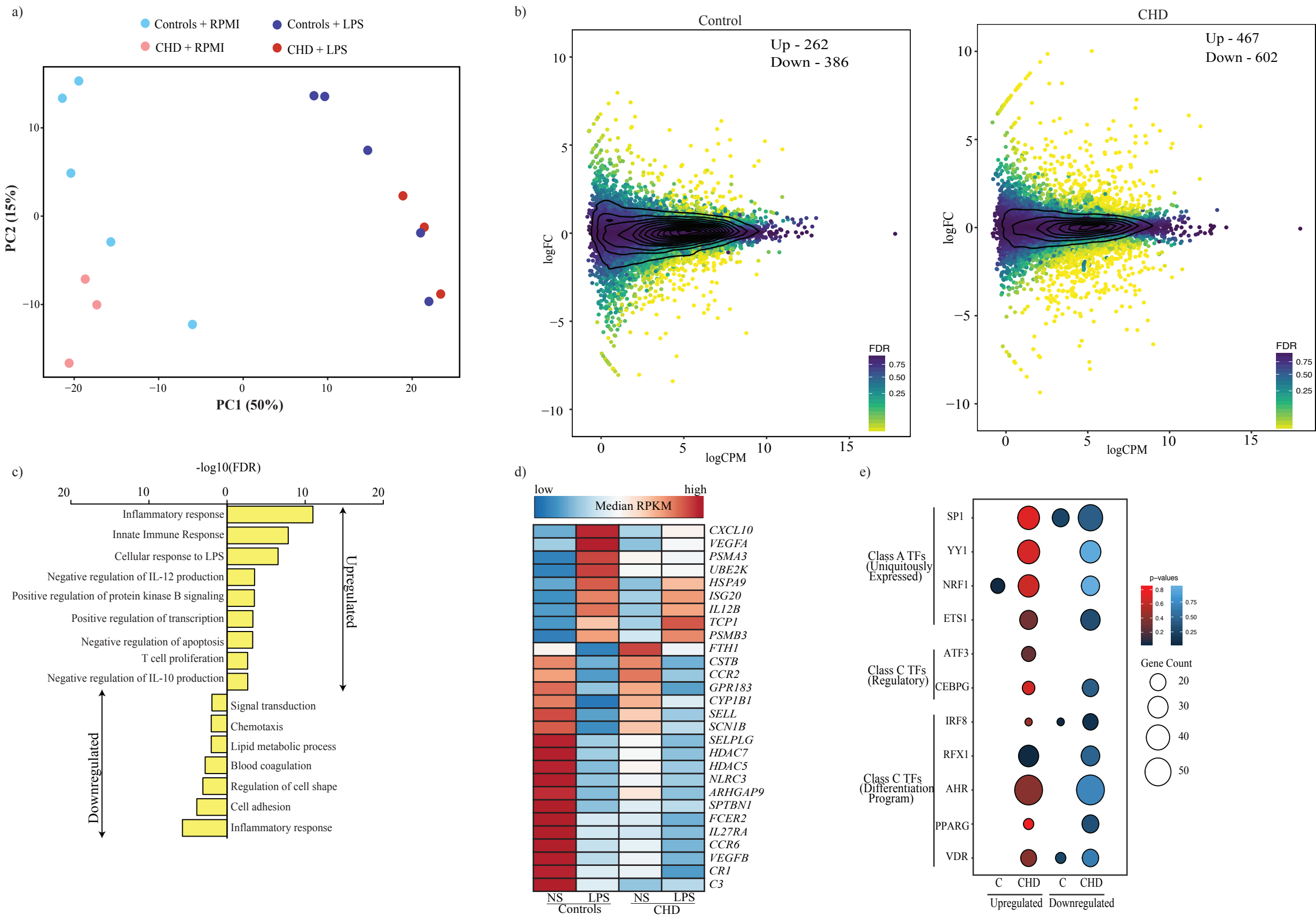


Fig S3: Transcriptional analysis of splenic macrophages following LPS stimulation: (a) PCA of samples included in the RNA-Seq experiment. (b) MA (Log ratio vs Mean Average) plots representing gene fold induction in control (left) and HD (right) splenic macrophages following LPS stimulation. DEG following LPS treatment within each condition is highlighted within each graph. (c) Functional enrichment of up and down-regulated genes detected in control animals only. (d) Clustered heatmap of inflammatory genes up or down regulated at 16 hours post LPS exposure in control animals only. Colours on the heatmap represent scaled median RPKM ranging from low (blue) to high (red). (e) Bubble plots of genes up (red) and down-regulated (blue) by class A transcription factor (TF; ubiquitously expressed in macrophages) and class C TF (regulate macrophage polarization) as recently defined (44). Size and colour of the bubble represent the number of genes mapped and statistical significance respectively.

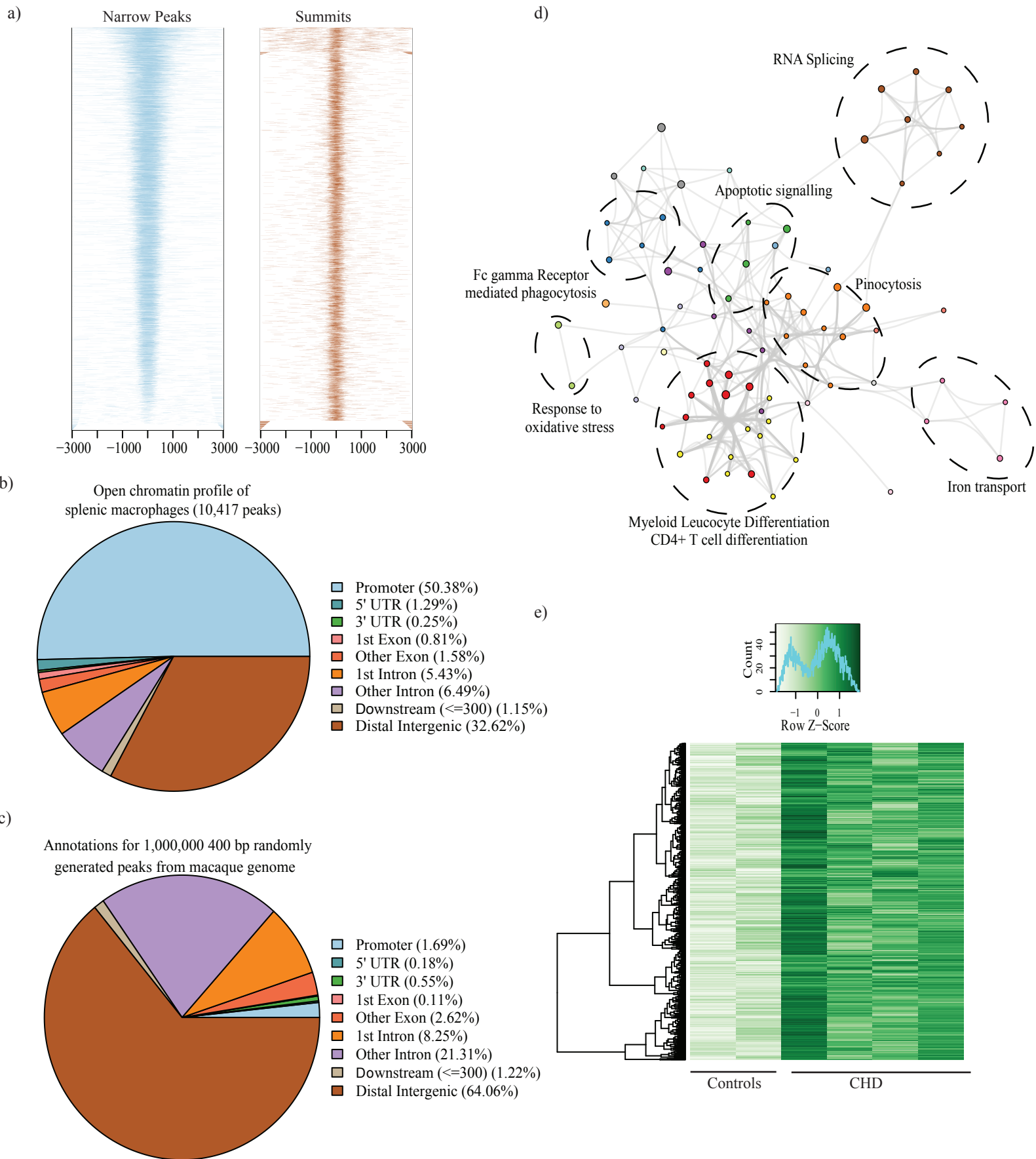


Fig S4: Analysis of chromatic accessibility changes with CHD: (a) Tag heatmaps of narrow peaks and summits centered on the transcription start sites (TSS) suggest a strong signal near promoter regions. (b) Genomic contexts of peaks accessible in splenic macrophages from control animals. (c) Genomic contexts of 1,000,000 randomly generated 400 bp peaks. (d) Functional enrichment of genes with highly accessible promoters (at least two peaks) in splenic macrophages from control animals. (e) Heatmap of accessibility peaks in macrophages from control and HD animals. Each row represents a genomic locus, and the colour intensity is proportional to chromatin accessibility.

Figure S5

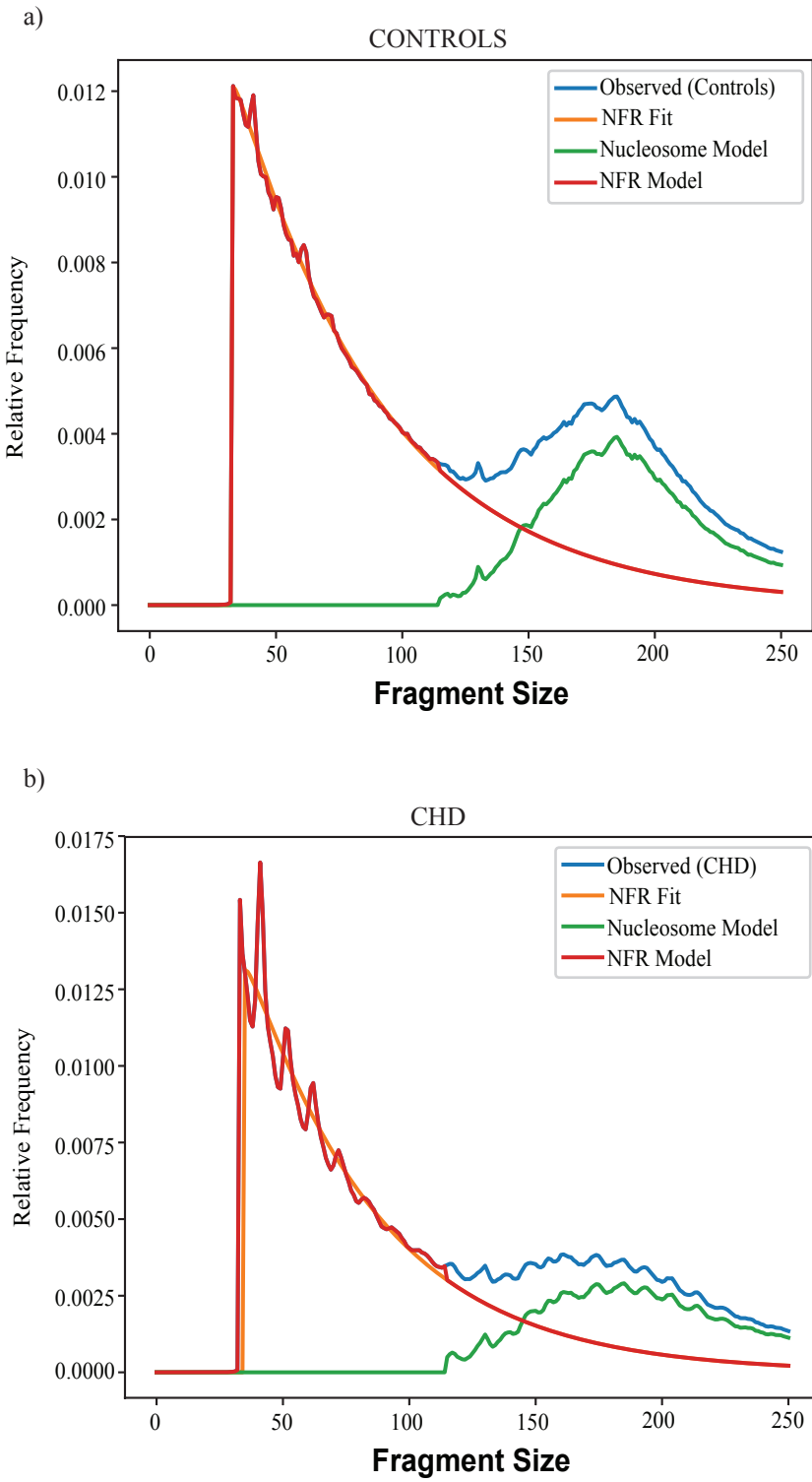


Figure S5: Nucleosome changes with CHD: Histograms of Nucleosome-free regions and nucleosomes detected in macrophages from (a) controls and (b) CHD animals.

Supplementary Fig S6

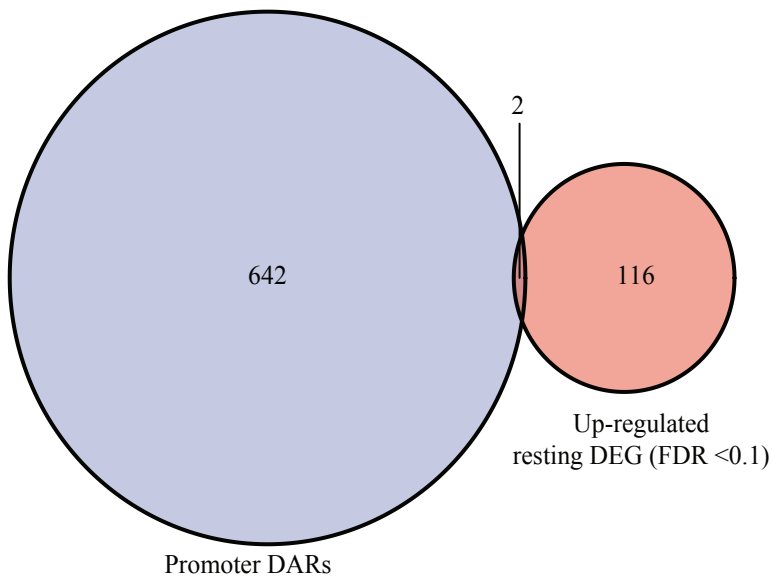


Fig S6: Comparing gene expression changes with chromatin accessibility: Venn diagram comparing differentially expressed genes with CHD (FDR<0.1) and genes with promoter associated differentially accessible regions (DAR)