Supplementary Information

Supplementary Tables

Supplementary Table 1. Primer/oligo sequences used for MPRA library construction.
Primer/oligo sequences used to generate the lentiviral MPRA backbone, to integrate AD-associated genetic elements into the lentiviral MPRA backbone, to generate MPRA libraries, and to perform EMSAs.

Supplementary Table 2. RNA/DNA ratios for all elements tested.
RNA/DNA ratios for all AD-associated genetic elements, positive controls, and negative controls for each biological replicate.

Supplementary Table 3. MPRA summary statistics for transcriptional activity of AD-associated genetic elements.
Transcriptional activity of AD-associated genetic elements over negative controls in both resting and proinflammatory macrophages. MPRA-active elements are specified.

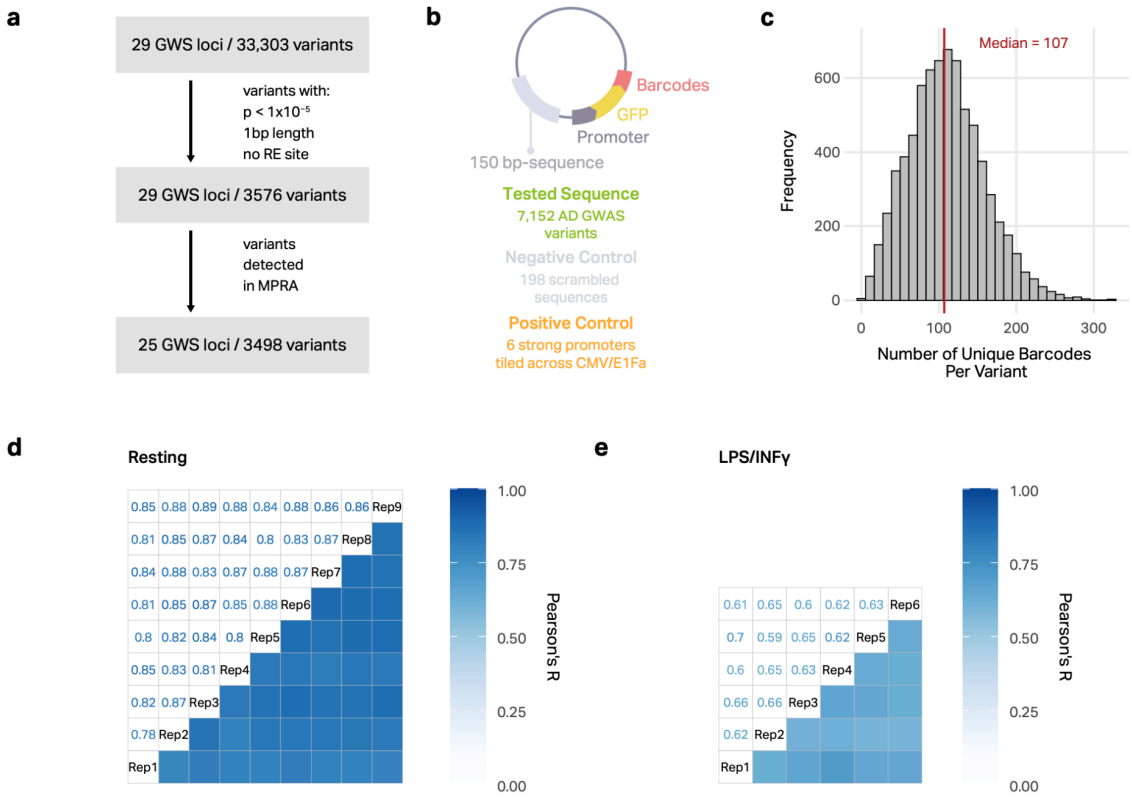
Supplementary Table 4. MPRA summary statistics for allelic regulatory activity of AD-associated variants.
Allelic regulatory activity of AD-associated genetic variants in both resting and proinflammatory macrophages. MPRA-allelic variants and emVars are specified.

Supplementary Table 5. ABC-defined enhancer-gene pairs
ABC pairs in resting and proinflammatory macrophages with an ABC score > 0.02 in its given condition.

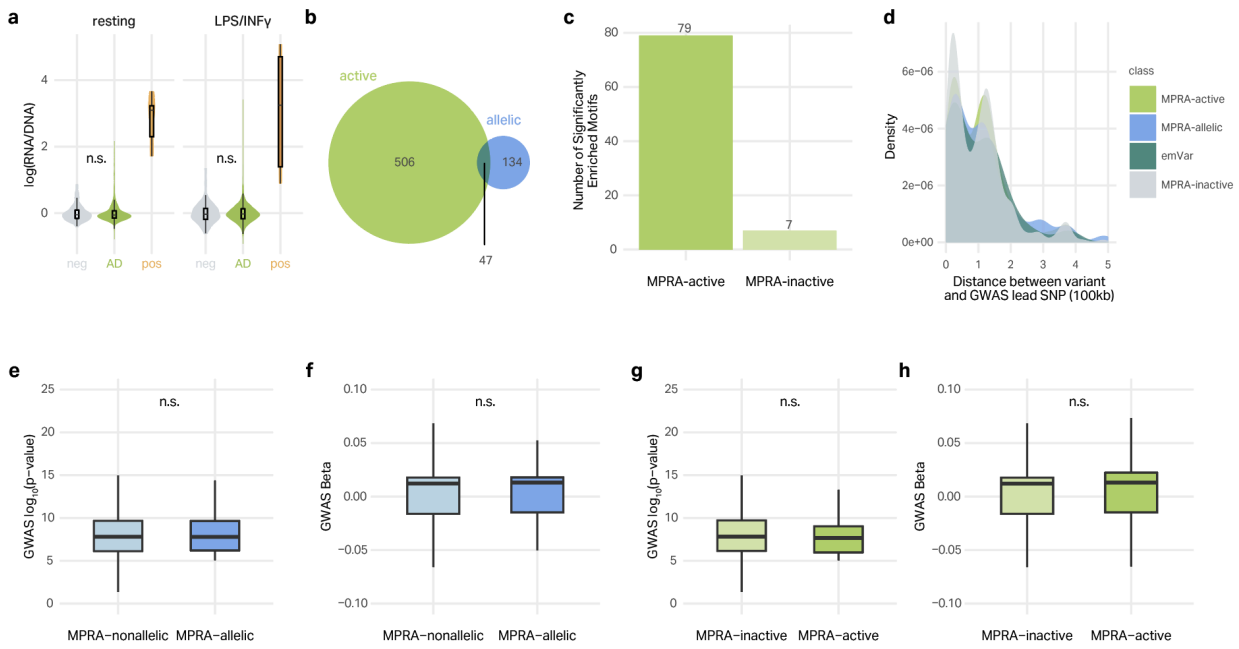
Supplementary Table 6. TF motif disruption by emVars.
Results from emVar motifbreakR analysis showing which emVars disrupt which TF motifs, pre-filtered for FDR < 0.05 and strong effects.

Supplementary Table 7. emVar-gene relationship.
Target genes of emVars from ABC and eQTL overlap.

Supplementary Figures



Supplementary Figure 1. Overview of MPRA library design and quality control.
a, Workflow of variant filtering from GWAS to final MPRA library design.
b, Schematic showing MPRA construct and the number of tested variants, scrambled negative controls, and positive controls.
c, Histogram showing the number of barcodes per variant detected in MPRA analysis.
d-e, Reproducibility analysis for all replicates from the resting macrophages (d) and LPS+INFγ-treated macrophages (e)



Supplementary Figure 2. Characterization of MPRA-active elements and MPRA-allelic variants.

a, Violin plots showing the activity of all AD tested elements before separating into MPRA-active and MPRA-inactive elements (AD), negative controls (neg), and positive controls (pose) for resting (left) and LPS+INFγ-treated (right) macrophages. Violins represent the distribution of the data, box plots show the median and 25th to 75th quartile with whiskers extending to the most extreme non-outliers. (n.s. represents p > 0.05 from two-sided Wilcoxon rank-sum test between negative controls and AD tested elements).

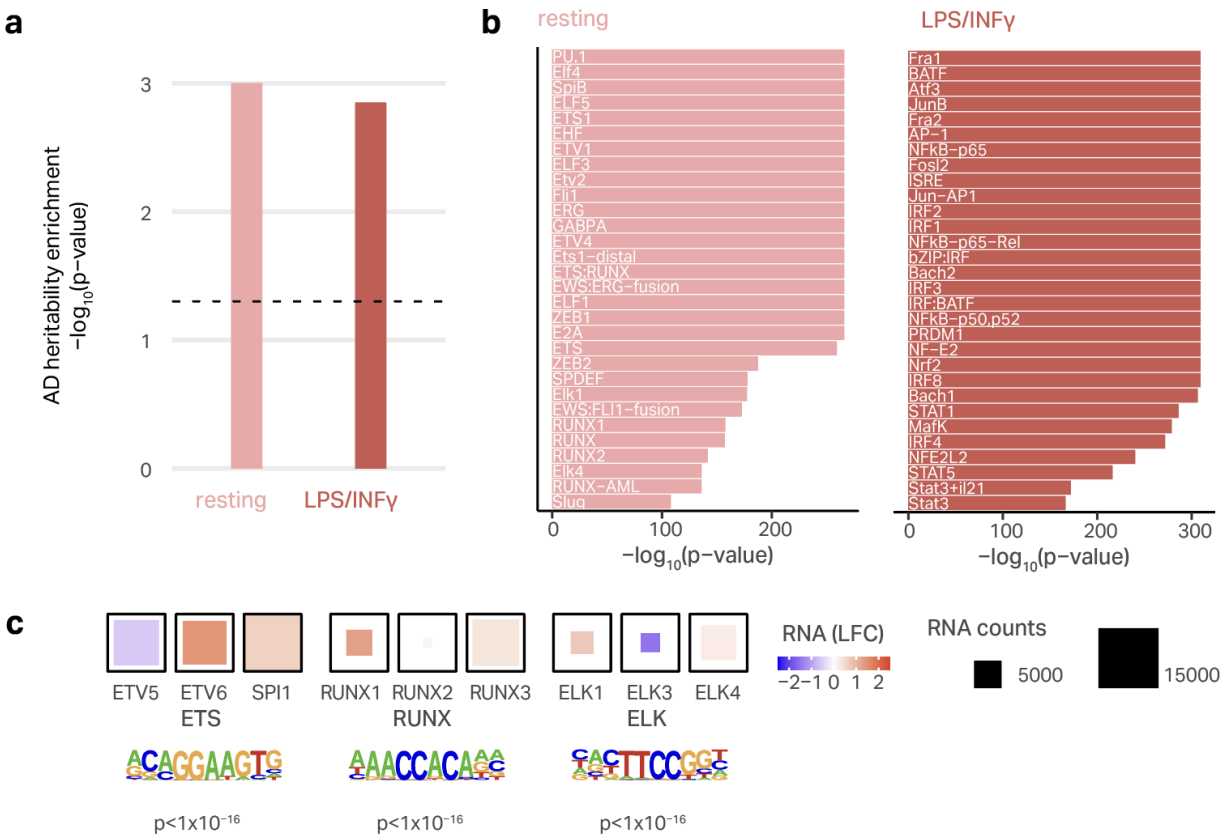
b, Venn diagram showing the overlap between MPRA-active elements and MPRA-allelic variants (emVars).

c, Barplot showing the number of significantly enriched TF motifs at MPRA-active and MPRA-inactive elements.

d, Density plot showing the distance between MPRA-active, MPRA-allelic, emVars, and MPRA-inactive/nonallelic elements and their respective lead GWAS variants. P-values calculated with a two-sided Wilcoxon rank-sum test (n.s. represents p > 0.05).

e-f, Box plots showing the difference in GWAS p-values (e) and beta values (f) for MPRA-allelic vs MPRA non-allelic variants. Box plots show the median and 25th to 75th quartiles with whiskers extending to the most extreme non-outliers (n.s. represents p > 0.05).

g-h, Boxplots showing the difference in GWAS p-values (g) and beta values (h) for MPRA-active vs MPRA-inactive elements. Box plots show the median and 25th to 75th quartiles with whiskers extending to the most extreme non-outliers (n.s. represents p > 0.05).

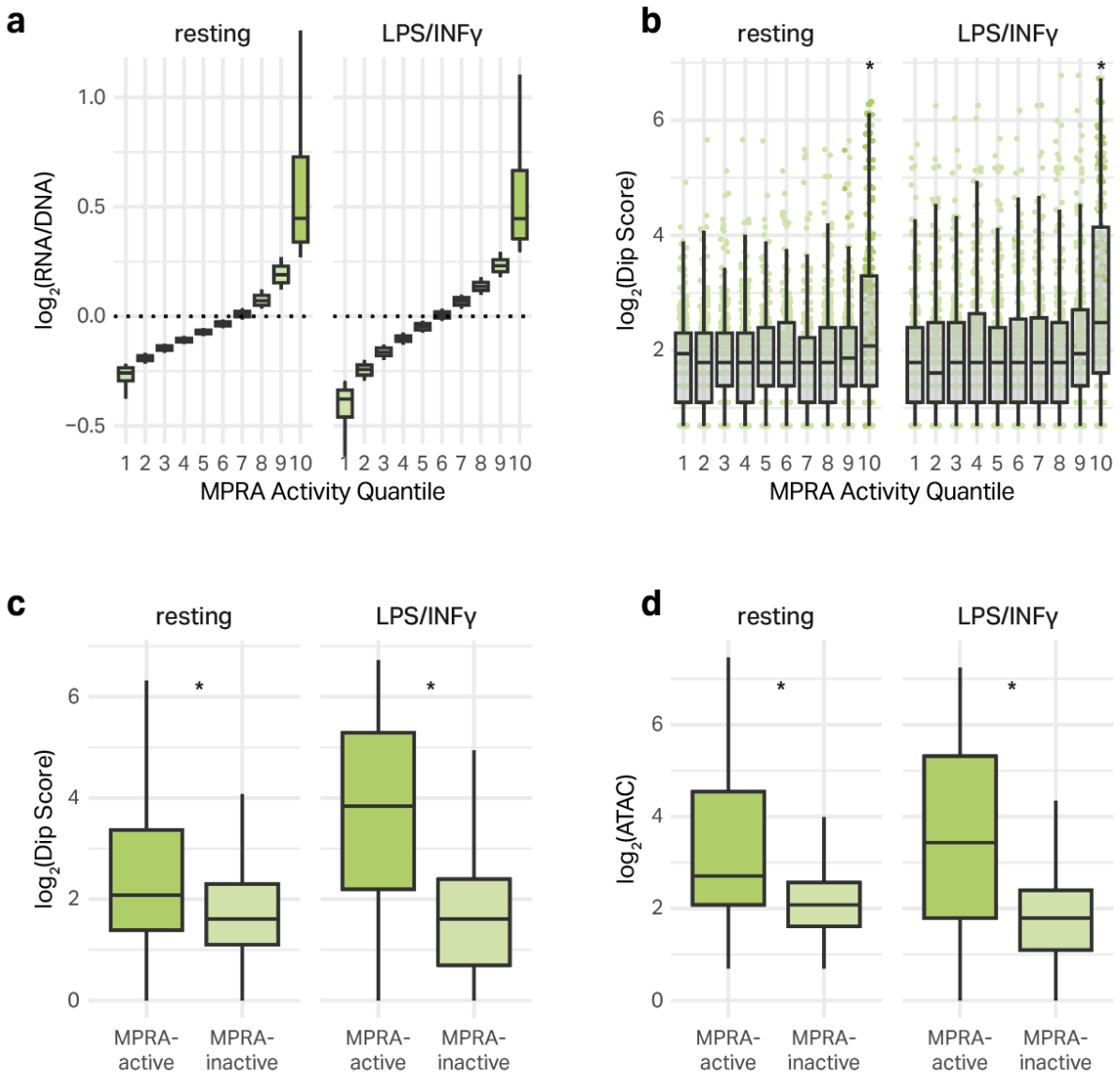


Supplementary Figure 3. Additional epigenetic characterization of differential macrophage regulatory architecture.

a, AD heritability enrichment in H3K27ac peaks from resting and LPS+INFγ-treated macrophages. P-values calculated by S-LDSC.

b, TF motifs enriched in resting-specific (left) and LPS+INFγ-specific (right) ATAC peaks.

c, TF motif enrichment for select motifs enriched in resting-specific ATAC peaks and the gene expression of the top three most highly expressed family members.

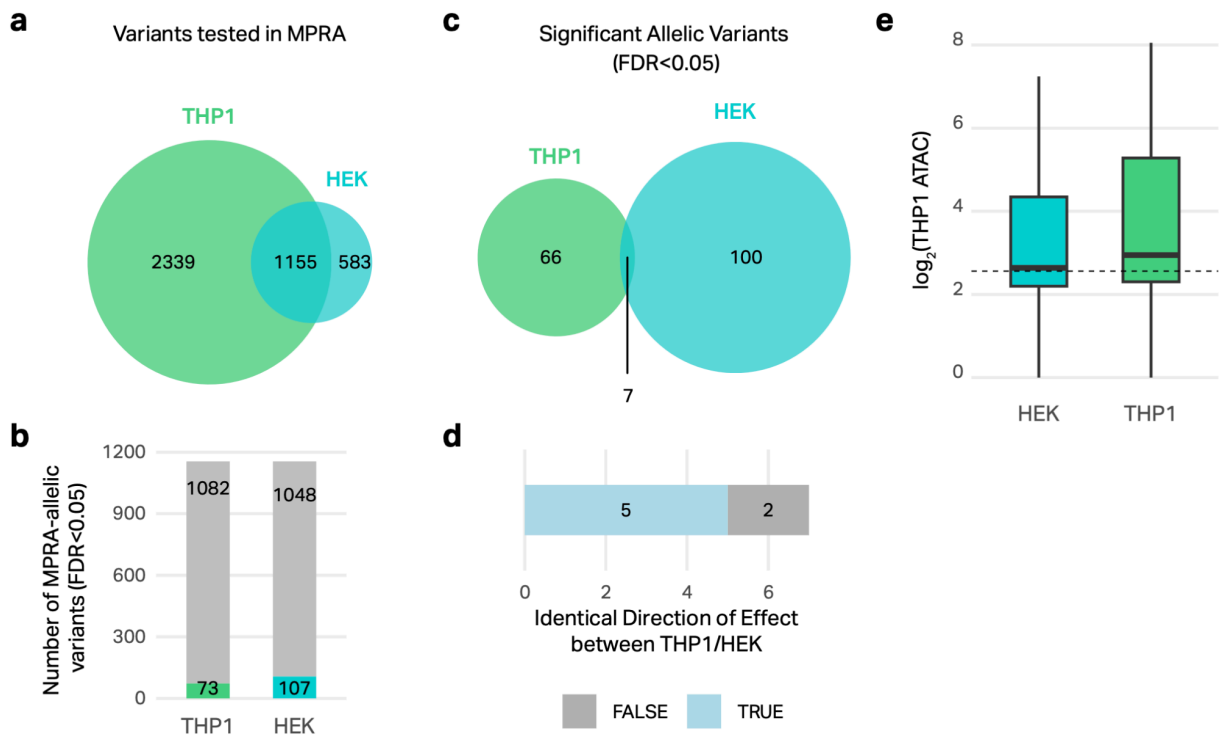


Supplementary Figure 4. MPRA-active elements are more accessible and have higher H3K27ac signals than MPRA inactive elements

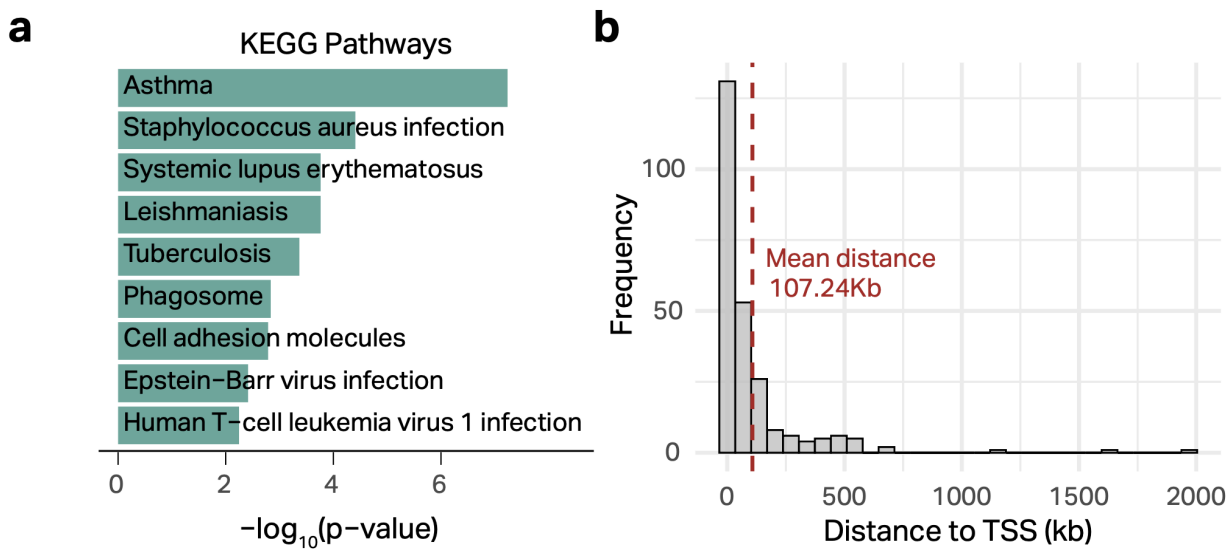
a, Activity of MPRA elements in each of the ten quantiles (divided by MPRA-activity) in resting and LPS+INFγ-treated macrophages. Box plots show the median and IQR with whiskers extending to the most extreme non-outliers.

b, H3K27ac Dip Score for variants in each of the ten MPRA quantiles in resting and LPS+INFγ-treated macrophages. Box plots show the median and IQR with whiskers extending to the most extreme non-outliers. Asterisks represent two-sided Wilcoxon rank-sum test $p < 0.05$.

c-d, H3K27ac Dip Score (c) and ATAC counts (d) are higher in MPRA-active elements when compared to MPRA-inactive elements in resting and LPS+INFγ-treated macrophages. Box plots show the median and IQR with whiskers extending to the most extreme non-outliers. Asterisks represent two-sided Wilcoxon rank-sum test $p < 0.05$.



Supplementary Figure 5. Comparison of THP-1 and HEK293 MPRA results.
a, Venn diagram showing the variants tested in our THP-1 macrophage MPRA (THP1) compared to the AD-associated variants tested in the previously published HEK293T MPRA (HEK).
b, Barplot showing the number of tested variants that are MPRA-allelic in either THP-1 macrophages and HEK293T cells.
c, Venn diagram showing the overlap of MPRA-allelic variants in both THP-1 macrophages and HEK293T cells.
d, Barplot showing the direction of effect between the risk and protective alleles for the MPRA-allelic variants in both THP-1 macrophages and HEK293T cells.
e, ATAC-seq counts from THP-1 resting macrophages for HEK MPRA-active elements versus THP-1 MPRA-active elements. Box plots show the median and IQR with whiskers extending to the most extreme non-outliers (two-sided Wilcoxon rank-sum test, $p = 5.5 \times 10^{-4}$).



Supplementary Figure 6. Additional characterization of emVar target genes.
a, KEGG pathways enriched for the 76 emVar target genes.
b, Distances between emVars and their target gene TSS.