An integrated multi-omics approach identifies epigenetic alterations associated with Alzheimer’s disease

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Protein aggregation is the hallmark of neurodegeneration, but the molecular mechanisms underlying late-onset Alzheimer's disease (AD) are unclear. Here we integrated transcriptomic, proteomic and epigenomic analyses of postmortem human brains to identify molecular pathways involved in AD. RNA sequencing analysis revealed upregulation of transcription- and chromatin-related genes, including the histone acetyltransferases for H3K27ac and H3K9ac. An unbiased proteomic screening singled out H3K27ac and H3K9ac as the main enrichments specific to AD. In turn, epigenomic profiling revealed gains in the histone H3 modifications H3K27ac and H3K9ac linked to transcription, chromatin and disease pathways in AD. Increasing genome-wide H3K27ac and H3K9ac in a fly model of AD exacerbated amyloid-β42-driven neurodegeneration. Together, these findings suggest that AD involves a reconfiguration of the epigenome, wherein H3K27ac and H3K9ac affect disease pathways by dysregulating transcription- and chromatin-gene feedback loops. The identification of this process highlights potential epigenetic strategies for early-stage disease treatment.

Age-related neurodegeneration poses an economic and psychological burden to society, with an estimate of 50 million people affected worldwide. Although there is a strong association between age and neurodegeneration, the molecular mechanisms that cause predisposition to or drive disease during aging are unclear. Late-onset AD is the most common form of dementia and is characterized by accumulation of intercellular β-amyloid plaques and intracellular neurofibrillary tangles, which correlate with neuronal death and loss of cognitive function. The contribution of the genotype to neurodegeneration is complex, with genetic risk variants partially accounting for AD risk2-3. To date, drugs targeting plaques have largely failed in clinical trials4, highlighting the need to identify pathways either upstream of plaque and tangle formation, or distinct mechanisms.

Epigenetic regulation is a critical player in aging of lower organisms; because of its role in integrating environmental stimuli into the genome, it represents a potential therapeutically tractable player in age-related diseases. Studies in mouse and fly models of neurodegeneration have identified epigenetic changes associated with disease pathology, including histone acetylation and methylation changes4-10. In the CK-p25 mouse, knockdown of histone deacetylase 2 (HDAC2) restored normal gene expression and improved cognition11. Although treatment of mouse models with HDAC inhibitors has shown promising results12,13, the unspecific nature of HDAC inhibitors could interfere with RE1 silencing transcription factor (REST)-mediated neuroprotective pathways requiring histone deacetylation14. While mouse models of neurodegeneration are useful to study molecular changes downstream of induction of plaque and tangles, well-designed studies of human postmortem brains can illuminate disease etiology.

The first large-scale studies of epigenetic changes associated with AD involved the study of CpG methylation, a transcriptionally repressive modification when at promoters15-17. While DNA methylation is a relatively stable mark, histone acetylation is more dynamically regulated and is directly involved in memory in model organisms18. We previously undertook a genome-wide analysis of histone-associated epigenetic changes in AD brains compared to the profile of normal aging19. This study, performed in the lateral temporal lobe, uncovered genome-wide enrichment in H4K16ac in normal aging and indicated loss of an age-related protective epigenetic pathway in AD20. Several recent studies have provided additional profiles of histone acetylation changes in brains affected with AD; these studies were performed in different brain regions and did not compare to a normal aging profile19,20.

Given the complexity of the aging and neurodegenerative processes, and their interrelation, we performed a comprehensive multi-omics analysis of brains affected with AD versus the brains of old and younger controls. These studies identify histone
modifications associated with AD and reveal that H3K27ac and H3K9ac gains in AD impact disease pathways by dysregulating transcription- and chromatin–gene feedback loops.

Results

Transcriptomic analysis identifies upregulation of transcription- and chromatin-related genes in AD. To systematically identify the molecular mechanisms underlying AD, we analyzed previous RNA sequencing (RNA-seq) data we generated from a high-quality set of patients with AD (n = 12; mean age = 68), and the brains of cognitively healthy older individuals (n = 10; mean age = 68) and healthy younger individuals (n = 8, mean age = 52) from the lateral temporal lobe, a region affected early in AD (Supplementary Tables 1 and 2). The dataset of brains from healthy younger individuals was included to discriminate changes related to aging from those specific to AD.

Frozen postmortem brains were rigorously selected based on criteria aiming to reduce confounding variables associated with the complex nature of human tissue studies. Brain samples were selected based on Braak and Consortium to Establish a Registry for Alzheimer’s Disease (CERAD) scores and clinically diagnosed AD (Methods and Supplementary Table 1). None of the AD cases had other coincident neurodegenerative diseases and none of the younger or old samples had deposits consistent with other neuropathologies. Samples were further selected based on the level of neuronal loss (cases with severe loss were excluded), postmortem interval (<24h) and sex (mainly male individuals) (Methods and Supplementary Table 1). Neuronal fractions were measured in each sample by immunofluorescence and flow cytometry for neuron-specific NeuN protein, revealing no significant differences between old and AD (immunofluorescence P = 0.30, two-sided Student’s t-test; flow cytometry P = 0.37, two-sided Student’s t-test)\(^\text{18}\).

By comparing gene expression between AD and old samples, we identified 421 genes with significant upregulation in AD, while 434 had significant downregulation (q < 0.05). Gene Ontology (GO) analysis of the upregulated genes using the Database for Annotation, Visualization and Integrated Discovery (DAVID) v6.7 (ref. \(\text{2}^\text{d}\)) revealed ‘regulation of transcription’ as the top GO term in the Biological Process category (false discovery rate (FDR) < 10%) (Fig. 1b). In the top GO terms for genes downregulated, there were categories related to cellular respiration and oxidation (Fig. 1c), which have been previously linked to AD\(^\text{2,23}\). Inspection of genes within the ‘regulation of transcription’ GO term (n = 75) showed several transcription factors and chromatin structure-related genes (Fig. 1d). Transcription factors included zinc finger proteins such as ZNF568, involved in cell proliferation and maintenance of neuronal stem cells\(^\text{14}\), and ZBTB16, which regulates cell cycle progression\(^\text{15}\). Cell cycle activation followed by apoptosis has previously been implicated in AD\(^\text{2,25}\). Other transcription factors included JDP2 and HIF3A, which are involved in apoptosis and adaptive response to low oxygen levels\(^\text{26,27}\).

Among the chromatin genes were those encoding histone acetyltransferases such as CBP (CREBBP), p300 (EP300) and TRRAP (subunit of the SAGA–ATAC complexes), HDACs such as SIRT1 and HDAC4, histone methyltransferases (CXXC\(^\text{2}^\text{d}\)) and histone demethylases (JMJD6) (ref. \(\text{19}\)). HDAC4 levels were increased in mouse models of AD and in brains from patients with AD\(^\text{34–36}\). miR-132-3p, which downregulates p300, is reduced in AD\(^\text{2,27}\), aligning with our results of increased EP300 expression. Comparison of the expression of the 75 transcription- and chromatin-related genes across younger, old and AD datasets revealed selective upregulation in AD with no change in old versus younger (Fig. 1d).

STRING v.11 analysis of the 75 genes revealed an interaction network of 35 proteins, with p300 and CBP located at the center of the network (Fig. 1e, top and Extended Data Fig. 1a). p300 and CBP have high homology and functional redundancy and are mutated in the intellectual disability disorder Rubinstein–Taybi\(^\text{39,40}\). Lysines 18 and 27 on the tail of histone H3 (H3K18 and H3K27) are substrates for CBP/p300 (ref. \(\text{18}\)).

We also detected increased expression of TRRAP, which encodes a subunit of the SAGA–ATAC complexes that acetylate lysine 9 on H3 (H3K9ac)\(^\text{40–42}\). STRING analysis extended to all genes changing in AD versus old (q < 0.05) uncovered additional chromatin factors that interact with CBP/p300 and TRRAP (Extended Data Fig. 1b), thus indicating an even larger and dynamic network of chromatin dysregulation in AD.

Given the potential role of CREBBP, EP300 and other chromatin genes in AD, we compared our data to two published large-scale RNA-seq studies from AD and control samples (temporal cortex) from the Mayo Clinic and the Mount Sinai brain banks\(^\text{44,45}\). Analysis of both datasets revealed the upregulation in AD of several transcription- and chromatin genes identified in our dataset (Supplementary Note 1, Supplementary Fig. 1 and Supplementary Table 3).

Given that changes in cell type proportions across samples could confound our analyses, we further corrected the RNA-seq data for changes in neuronal fractions (Supplementary Note 2). This analysis confirmed and strengthened our observations of increased expression of transcription- and chromatin-related genes in AD (Supplementary Figs. 2 and 3). Similarly, analysis of the RNA-seq data with inclusion of External RNA Controls Consortium (ERCC) spike-in controls, showed no global transcriptional changes between groups and confirmed the same results (Supplementary Note 3, Supplementary Figs. 4 and 5 and Supplementary Table 4).

These transcriptomic analyses, by showing upregulation of transcription and chromatin genes in AD with confirmation in published datasets, indicate an increase of epigenomic plasticity in AD.

Proteomic analysis identifies increased levels of H3K27ac and H3K9ac in AD. Given the implications of increased expression of chromatin-related genes in AD, we performed a proteomic analysis to investigate changes at the chromatin level. We focused on analysis of histone post-translational modifications (PTMs) since they...
are key determinants of transcriptional regulation. Histones were extracted from the frozen tissue of younger, old and AD brains and were prepared for analysis on a nano liquid chromatography with a tandem mass spectrometry (LC–MS/MS) instrument using a bottom-up approach (Methods and Fig. 2a). The histone PTMs of lysine acetylation and methylation were mapped on canonical

| Regulation of transcription | Transcription | Regulation of RNA metabolic process | Regulation of transcription, DNA-dependent |
|----------------------------|---------------|-------------------------------------|--------------------------------------------|
| AD versus old gains        |               |                                     |                                            |
| AD versus old losses       |               |                                     |                                            |

Transcription

Regulation of RNA

metabolic process

Generation of precursor metabolites and energy

Oxidation reduction

Cellular respiration

Energy derivation by oxidation of organic compounds

![Heatmap of gene expression changes](image)

| Gene       | Younger-5 | Younger-3 | Younger-9 | Younger-8 | Younger-6 | Younger-7 | Old-18 | Old-17 | Old-10 | Old-14 | Old-12 | Old-16 | Old-15 | Old-13 | Old-19 | Old-11 |
|------------|-----------|-----------|-----------|-----------|-----------|-----------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| ZBTB40     | AD-21     | AD-30     | AD-28     | AD-31     | AD-29     | AD-25     | AD-27 |
| ZBTB25     | AD-24     | AD-26     | AD-22     | AD-23     | AD-20     | AD-26     | AD-22 |
| USP21      | AD-24     | AD-26     | AD-22     | AD-23     | AD-20     | AD-26     | AD-22 |
| MAF1       | AD-24     | AD-26     | AD-22     | AD-23     | AD-20     | AD-26     | AD-22 |
| POGK       | AD-24     | AD-26     | AD-22     | AD-23     | AD-20     | AD-26     | AD-22 |
| ZNF202     | AD-24     | AD-26     | AD-22     | AD-23     | AD-20     | AD-26     | AD-22 |
| ZNF568     | AD-24     | AD-26     | AD-22     | AD-23     | AD-20     | AD-26     | AD-22 |
| PROX2      | AD-24     | AD-26     | AD-22     | AD-23     | AD-20     | AD-26     | AD-22 |
| MAJD6      | AD-24     | AD-26     | AD-22     | AD-23     | AD-20     | AD-26     | AD-22 |
| ZNF14      | AD-24     | AD-26     | AD-22     | AD-23     | AD-20     | AD-26     | AD-22 |
| HIF3A      | AD-24     | AD-26     | AD-22     | AD-23     | AD-20     | AD-26     | AD-22 |
| IRE1A       | AD-24     | AD-26     | AD-22     | AD-23     | AD-20     | AD-26     | AD-22 |
| OXOR1      | AD-24     | AD-26     | AD-22     | AD-23     | AD-20     | AD-26     | AD-22 |
| NFKBIA     | AD-24     | AD-26     | AD-22     | AD-23     | AD-20     | AD-26     | AD-22 |
| SIRT1      | AD-24     | AD-26     | AD-22     | AD-23     | AD-20     | AD-26     | AD-22 |
| MXI1       | AD-24     | AD-26     | AD-22     | AD-23     | AD-20     | AD-26     | AD-22 |
| KLF15      | AD-24     | AD-26     | AD-22     | AD-23     | AD-20     | AD-26     | AD-22 |
| ZNF768     | AD-24     | AD-26     | AD-22     | AD-23     | AD-20     | AD-26     | AD-22 |
| HILS1      | AD-24     | AD-26     | AD-22     | AD-23     | AD-20     | AD-26     | AD-22 |
| ZNF790     | AD-24     | AD-26     | AD-22     | AD-23     | AD-20     | AD-26     | AD-22 |
| ZBTB40     | AD-24     | AD-26     | AD-22     | AD-23     | AD-20     | AD-26     | AD-22 |
| ZBTB25     | AD-24     | AD-26     | AD-22     | AD-23     | AD-20     | AD-26     | AD-22 |
| USP21      | AD-24     | AD-26     | AD-22     | AD-23     | AD-20     | AD-26     | AD-22 |
| MAF1       | AD-24     | AD-26     | AD-22     | AD-23     | AD-20     | AD-26     | AD-22 |
| POGK       | AD-24     | AD-26     | AD-22     | AD-23     | AD-20     | AD-26     | AD-22 |
| ZNF202     | AD-24     | AD-26     | AD-22     | AD-23     | AD-20     | AD-26     | AD-22 |
| ZNF568     | AD-24     | AD-26     | AD-22     | AD-23     | AD-20     | AD-26     | AD-22 |
| PROX2      | AD-24     | AD-26     | AD-22     | AD-23     | AD-20     | AD-26     | AD-22 |
| MAJD6      | AD-24     | AD-26     | AD-22     | AD-23     | AD-20     | AD-26     | AD-22 |
| ZNF14      | AD-24     | AD-26     | AD-22     | AD-23     | AD-20     | AD-26     | AD-22 |
| HIF3A      | AD-24     | AD-26     | AD-22     | AD-23     | AD-20     | AD-26     | AD-22 |
| IRE1A       | AD-24     | AD-26     | AD-22     | AD-23     | AD-20     | AD-26     | AD-22 |
| OXOR1      | AD-24     | AD-26     | AD-22     | AD-23     | AD-20     | AD-26     | AD-22 |
| NFKBIA     | AD-24     | AD-26     | AD-22     | AD-23     | AD-20     | AD-26     | AD-22 |
| SIRT1      | AD-24     | AD-26     | AD-22     | AD-23     | AD-20     | AD-26     | AD-22 |
| MXI1       | AD-24     | AD-26     | AD-22     | AD-23     | AD-20     | AD-26     | AD-22 |
| KLF15      | AD-24     | AD-26     | AD-22     | AD-23     | AD-20     | AD-26     | AD-22 |
| ZNF768     | AD-24     | AD-26     | AD-22     | AD-23     | AD-20     | AD-26     | AD-22 |
| HILS1      | AD-24     | AD-26     | AD-22     | AD-23     | AD-20     | AD-26     | AD-22 |
| ZNF790     | AD-24     | AD-26     | AD-22     | AD-23     | AD-20     | AD-26     | AD-22 |
| ZBTB40     | AD-24     | AD-26     | AD-22     | AD-23     | AD-20     | AD-26     | AD-22 |
| ZBTB25     | AD-24     | AD-26     | AD-22     | AD-23     | AD-20     | AD-26     | AD-22 |
| USP21      | AD-24     | AD-26     | AD-22     | AD-23     | AD-20     | AD-26     | AD-22 |
| MAF1       | AD-24     | AD-26     | AD-22     | AD-23     | AD-20     | AD-26     | AD-22 |
| POGK       | AD-24     | AD-26     | AD-22     | AD-23     | AD-20     | AD-26     | AD-22 |
| ZNF202     | AD-24     | AD-26     | AD-22     | AD-23     | AD-20     | AD-26     | AD-22 |
| ZNF568     | AD-24     | AD-26     | AD-22     | AD-23     | AD-20     | AD-26     | AD-22 |
The analysis of histone PTM changes in aging (old versus younger) revealed 5 histone PTMs with significant enrichment in old, while 3 had significant depletion ($P < 0.05$, two-sided Student's $t$-test) (Fig. 2b). These changes involved histone methylation at gene bodies (H3K36me1, H3K36me2, H3K79me1 and H3K23me1) and promoters (H3K27me3, H4K20me2 and H4K20me3), which are associated with activation or repression$^{75-76}$. Among the acetylation changes, H4K20ac, which is involved in transcriptional repression$^{76}$, was reduced in brains from old individuals.

With disease (AD versus old), we found a higher number of histone PTM changes ($n = 14$) (Fig. 2c) than in healthy aging brains ($n = 8$) (Fig. 2b), indicating a stronger effect of disease versus aging processes. Physiological aging may be more heterogeneous across the population, with some individuals aging faster than others, while disease may produce more consistent molecular changes.

Both histone methylation and acetylation changes were detected in comparing AD and old brains (Fig. 2c). While histone methylation changes involved both gains or losses in methylation, acetylation changes exclusively involved gains (Fig. 2c), such as for H3K27ac (on canonical H3 and the H3.3 variant), H3K9ac and H3K4ac ($P < 0.05$, two-sided Student's $t$-test) (Fig. 2c). Increased H3K9ac in AD has been shown to increase in a previous study$^{51}$, which is consistent with these findings. H3K27ac and H3K9ac are transcription-activating PTMs that are enriched at promoters and enhancers$^{59,60}$. H3K4ac is associated with both active transcription$^{44}$ and heterochromatin formation$^{55}$; therefore, its role in chromatin regulation is complex. Intriguingly, H3K27ac is deposited by CBP/p300, which are upregulated in AD (Fig. 1d), while H3K9ac and H3K4ac are deposited by GGN5, which together with TRRAP (upregulated in AD; Fig. 1d) is part of the SAGA–ATAC complexes.

Comparison of histone PTM changes across younger, old and AD (Fig. 2d–f) samples underscored that several histone methylation sites changed in both aging and disease (Fig. 2d) (that is, H4K20me2 and H3K20me3), while the H3 acetyl marks gained in AD did not change significantly with age (Fig. 2e,f). Interestingly, H3.3 levels, a histone variant abundant in nondividing cells$^{56-58}$, remained stable across younger, old and AD samples (Fig. 2g).

Overall, the proteomic analyses revealed discrete histone PTM changes in AD and specifically uncovered increased H3K27ac and H3K9ac. These results are consistent with increased expression of genes encoding CBP/p300 and TRRAP (Fig. 1) and suggest functional relevance for H3K27ac- and H3K9ac-driven pathways in AD.

**Dynamic epigenomic changes in AD, with H3K27ac and H3K9ac gains and H3K122ac losses.** Given that the transcriptomic and proteomic analyses pointed to a specific role for H3K27ac and H3K9ac gains and their modifiers in AD, we performed chromatin immunoprecipitation sequencing (ChIP-seq) to investigate the dynamics of these PTMs. We included H3K122ac and H3K4me1 as additional marks of enhancers$^{59,60}$ and profiled 5-hydroxymethylcytosine (5hmC)$^{61}$, which is highly abundant in the brain and is also enriched at enhancers$^{62,63}$ (Supplementary Table 2). ChIP-seq peaks were called for individual histone PTMs in the younger, old and AD samples (model-based analysis of ChIP-seq (MACS2); FDR $< 1 \times 10^{-7}$) by pooling reads across samples of the same study group (Supplementary Tables 6 and 7). Reads from individual patients were used to assess the statistical significance of peak enrichment differences between groups (Methods).

To further reduce potential confounding variables, such as those due to changes in cell type proportions, the top 10% of peaks with the highest Pearson's correlation with sample neuron fraction were masked from the analysis. (Neuron fractions were measured by flow cytometry of NeuN-stained nuclei$^{18}$.) This step was included to improve the accuracy of the analysis despite having excluded cases with severe neuronal loss from the selection, as discussed above.

H3K27ac, H3K9ac and H3K122ac peaks were detected at both transcriptional start sites (TSS) ($\leq 1$ kilobase (kb)) from the TSS—low H3K4me1 and 5hmC—and enhancers ($> 1$ kb from the TSS—high H3K4me1 and 5hmC) in younger, old and AD samples (Extended Data Fig. 3) (refs. $^{52,53,60,64}$). H3K122ac peaks were enriched closer to the TSS ($\leq 5$ kb), while H3K27ac and H3K9ac peaks were enriched further away ($> 5$ kb from the TSS) (Extended Data Fig. 4a). Peak overlap analysis showed that at least 35% of the three acetyl peaks overlapped (Extended Data Fig. 9b) but that there were also acetyl mark-specific peaks such as at enhancers (Extended Data Fig. 4b–d). Analysis of 5hmC enrichment revealed a positive linear correlation with peak acetylation at enhancers and gene expression at gene bodies (Supplementary Note 4 and Supplementary Fig. 6).

Analysis of histone acetyl PTM changes between AD and old samples (on a per acetyl mark basis) (Fig. 3a–c) revealed a higher number of H3K27ac and H3K9ac peaks with significant gains in acetylation than losses in AD (Fig. 3d,e): $3.8 \times 10^3$ peaks with H3K27ac gains versus $2.8 \times 10^3$ with losses in AD (Fig. 3d) and $3.2 \times 10^3$ peaks with H3K9ac gains versus $1.9 \times 10^3$ losses in AD ($P < 0.05$, two-sided Wilcoxon rank-sum test) (Fig. 3e). In contrast, an opposite trend was detected for the H3K122ac changes, that is, a higher number of H3K122ac peaks with significant acetylation losses ($5.5 \times 10^3$) than gains ($1.6 \times 10^3$) in AD ($P < 0.05$, two-sided Wilcoxon rank-sum test) (Fig. 3f). The higher number of H3K27ac and H3K9ac peaks with acetylation gains in AD is consistent with the proteomic results of increased H3K27ac and H3K9ac in AD (Fig. 2). The preferential losses of H3K122ac in AD, not observed in the proteomic analysis, could represent redistribution of H3K122ac enrichment in the genome without change in overall histone mark abundance.

Analysis of the distribution of peaks with acetylation changes relative to their distance from the closest TSS (Fig. 3g–i) showed that H3K27ac or H3K9ac changes were enriched $> 5$ kb from the TSS (Fig. 3g,h), while H3K122ac changes were enriched closer to the TSS ($\leq 5$ kb) (Fig. 3i).

There was a significant positive linear correlation between gene expression and acetylation of the closest peak (on a per acetyl mark basis) in both old and AD ($r = 0.40–0.43; P < 2.2 \times 10^{-16}$) (Extended Data Fig. 5a–f). We also found a significant positive linear correlation between the amplitude of gene expression changes ($q < 0.05$, DESeq2) and corresponding H3K27ac ($r = 0.16; P = 3.2 \times 10^{-3}$) and H3K9ac ($r = 0.13; P = 5.1 \times 10^{-4}$) changes in AD versus old (Extended Data Fig. 5g,h). When considering the total acetylation level at each site (by summing H3K27ac, H3K9ac and H3K122ac enrichments), there was a higher correlation with gene expression...
than when considering individual acetyl marks (Extended Data Fig. 5j–l), suggesting that the three marks cooperate in regulating gene expression. After detecting preferential H3K27ac and H3K9ac gains and preferential H3K122ac losses in AD, we investigated how these changes related to the normal aging profile. For each histone PTM,
we cross-compared peak enrichment in younger, old and AD (a one-way analysis) and classified the significant changes (P<0.05, one-way analysis of variance (ANOVA)) into three major classes: age-regulated (gains or losses with aging (old versus younger) but not in disease (AD versus old)); age-disregulated (gains or losses with aging and in disease); and disease-specific (gains or losses with disease but not in aging) (Fig. 3j–l). All three acetyl PTMs were preferentially enriched with disease-specific changes (peak numbers listed in Fig. 3j–l). Notably, H3K27ac and H3K9ac changes had predominant disease-specific gains (1.8 × 10^4 gains versus 1.6 × 10^3 losses for H3K27ac and 1.8 × 10^4 gains versus 0.8 × 10^3 losses for H3K9ac) (Fig. 3j,k), whereas H3K122ac had predominant disease-specific losses (2.8 × 10^3 losses versus 1 × 10^4 disease-specific gains) (Fig. 3l). Analysis of the H3K4me1 changes revealed preferential disease-specific losses in AD (Supplementary Note 5 and Supplementary Fig. 7). Furthermore, comparison of the three acetyl PTMs at sites with disease-specific changes for each modification revealed that they trended in the same direction of change (Extended Data Fig. 6a–f). H3K4me1, which is a mark of enhancers, was also reduced at the sites with acetylation losses (Extended Data Fig. 6d–f). Decreased H3K4me1, in addition to acetylation, indicates a further and more profound remodeling of the losses versus gains in AD. Notably, because the gains do not involve methylation changes, it implies that they may be amenable targets of epigenetic drugs.

Taken together, the three-way analysis confirmed the trends observed by comparing AD versus old samples, and, importantly, identified changes specifically associated with disease.

H3K27ac and H3K9ac disease-specific gains are associated with transcription, chromatin and disease pathways. Given the predominance of H3K27ac and H3K9ac disease-specific gains and H3K122ac disease-specific losses in AD, we investigated the functional pathways associated with these changes. GO using the Genomic Regions Enrichment of Annotations Tool (GREAT) v:3.0.0 (ref. 55) was used to consider genes within 100 kb from the disease-specific gains or losses for distant regulatory elements such as enhancers. Genes associated with H3K27ac or H3K9ac disease-specific gains had top GO terms related to transcription (for H3K27ac) (Fig. 4a,d) and nucleic acid metabolic processes (for H3K9ac) (Fig. 4b,e) (FDR < 5%). Other top GO terms (for H3K27ac) included 'leukocyte differentiation' and 'Wnt signaling pathway', which are involved in neurodegeneration66–68 (Extended Data Fig. 8a,c) and synaptic transmission and cell–cell signaling (for H3K9ac) (Extended Data Fig. 8b,d and Supplementary Table 8). GO analysis using DAVID v.6.7 confirmed the GREAT results including transcriptional regulation in the top terms for the H3K27ac and H3K9ac disease-specific gains (Extended Data Fig. 9 and Supplementary Table 9).

DNA motif enrichment analysis of sites with H3K27ac or H3K9ac disease-specific gains using Hypergeometric Optimization of Motif EnRichment (HOMER) v.4.6 (ref. 56) revealed enrichment for the transcription factors NRF1 and CTCF (Fig. 4g,h). NRF1 regulates mitochondrial genes, the cell cycle and the DNA damage response69–71, while CTCF is a chromatin architecture and insulator factor72–74. Among other top DNA motifs for H3K9ac gains were E2F1, involved in cell proliferation and apoptotic processes, and E2F7 (Fig. 4h), which is involved in cell cycle progression and the DNA damage response75–77. Cell cycle reactivation coupled to apoptosis has been implicated in AD78; thus, remodeling of the epigenome with upregulation of chromatin genes could mediate this process. In contrast, analysis of the H3K122ac disease-specific losses revealed top motifs for transcription factors involved in neuronal processes, immune response and oxidative stress78–80 (Fig. 4i), indicating a different role of the H3K122ac losses in AD.

We compared our epigenomic results to published epigenomic studies of other brain regions affected by tau pathology81–83 (Supplementary Notes 6 and 7). These analyses revealed that H3K27ac and H3K9ac changes correspond to similar changes in other brain regions affected by AD (Supplementary Figs. 8 and 9) and identified histone acetyl changes that overlapped with CpG methylation associated with AD (Supplementary Tables 10 and 11). In contrast, epigenomic comparison with mouse models of AD showed only a modest overlap (Supplementary Note 8 and Supplementary Fig. 10).

These epigenomic analyses identified H3K27ac and H3K9ac disease-specific gains associated with both epigenetic- and disease-related pathways, supporting a role for aberrant epigenetic activation in AD.

H3K27ac disease-specific gains are enriched in AD SNPs and expression quantitative trait loci (eQTLs). Genome-wide association studies (GWAS) of SNPs have revealed over 20 loci linked to AD84,85. These SNPs may pinpoint regions involved in AD with which they are in linkage disequilibrium or themselves could harbor regulatory elements participating in disease. We considered the overlap between the six classes of epigenetic changes identified by the three-way analysis (Fig. 3j–l) and the AD-associated
SNPs. We used a curated list of AD-associated SNPs \((P < 1 \times 10^{-5})\) from the International Genomics of Alzheimer’s Project (IGAP) meta-analysis study that passed two stages of clinical testing and involved >74,000 individuals\(^8\). SNPs in linkage disequilibrium were merged using PLINK v.1.9. INRICH analysis\(^9\) revealed a significant overlap between the AD SNPs and the H3K27ac disease-specific gains \((P < 2 \times 10^{-4})\) (Fig. 4j) (Supplementary Table 12). This result was confirmed when performing INRICH analysis using data from the newest IGAP meta-analysis study\(^8\) (H3K27ac disease-specific gains, \(P = 0.01\)) (Extended Data Fig. 10).

To further explore the relationship between epigenetic changes and genetic risk variants in AD, we considered the overlap with...
AD-associated eQTLs. eQTLs are SNPs that correlate with gene expression and represent potential gene regulatory elements. We used a highly powered dataset with approximately 400 individuals containing eQTLs from the temporal cortex (TX) of AD cases (TX_AD) (eQTLs, n = 85,359), eQTLs from non-AD cases (other types of dementia; TX_CTL) (eQTLs, n = 156,134) and the two combined (TX_ALL; eQTLs, n = 156,134). The imputation scheme of this dataset (HapMap2) allowed the analysis of more SNPs for eQTL activity. By performing the overlap analysis, we found a significant enrichment of H3K27ac disease-specific gains for TX_AD and TX_ALL eQTLs (TX_AD, P = 1.08 × 10^−4; TX_ALL, P = 1.08 × 10^−2) and of H3K9ac disease-specific gains for TX_ALL eQTLs (P = 1.08 × 10^−10) (Fig. 4). We also found a significant enrichment between H3K122ac disease-specific losses and TX_ALL eQTLs, as well as H3K122ac age-regulated losses and TX_AD and TX_ALL eQTLs (TX_AD, P = 2.16 × 10^−8; TX_ALL, P = 1.08 × 10^−2) (Fig. 4k and Supplementary Table 13). The enrichment of the acetyl peaks with both TX_AD and TX_ALL eQTLs suggests that they harbor regulatory elements involved in AD and more general neurodegenerative processes, while enrichment for TX_ALL implies involvement only with general neurodegenerative processes but not AD specifically.

Overall, the enrichment of disease-specific changes, and particularly of H3K27ac disease-specific gains, with AD GWAS SNPs and eQTLs underscores their potential involvement in AD.

Increase of H3K27ac and H3K9ac promotes amyloid-β42 (Aβ42)-induced neurodegeneration in vivo. These dynamic epigenetic changes in AD brains prompted us to examine functional interactions between H3K27ac and H3K9ac and AD-associated pathologies. We explored whether manipulating H3K27ac and H3K9ac levels in a Drosophila model could modulate Aβ42 toxicity. Because manipulation of CBP can lead to broad phenotypic effects due to numerous nonhistone target substrates, we instead manipulated individual histone residues and determined the effect of expression of the altered histones on Aβ42 toxicity.

To manipulate histone PTM levels, we utilized Drosophila lines expressing histone H3.3 with either a lysine-to-methionine substitution at lysine 27 (H3.K327M) or a lysine-to-methionine substitution at lysine 9 (H3.K39M) (Supplementary Table 14). Expression of H3.K327M globally reduces H3K27me3 and increases H3K27ac, while expression of H3.K9M reduces H3K9me3 and increases H3K9ac. Expression of H3.K327M or H3.K39M enhanced Aβ42-induced eye degeneration (Fig. 5a,b), while expression of the mutant histones on their own, or wild-type H3.3 alone, had no significant effect on the eye (Supplementary Fig. 11). Given our focus on CBP, which modulates H3K27ac in Drosophila, we expanded the analysis to additional substitutions: H3.K327M to mimic acetylation and H3.K327Q to mimic acetylation; and H3.K327Q to change the lysine to a small nonpolar amino acid that mimics the absence of acetylation. We found that expression of H3.K327Q exacerbated Aβ42 eye toxicity, which is consistent with our previous data, whereas H3.K327A, as predicted, had no effect (Fig. 5a,b).

These functional data of Aβ42 toxicity worsened by increased H3K27ac and H3K9ac further indicate that gains in H3K27ac and H3K9ac are central players in AD.

Discussion
We report a combined multi-omic analysis of AD-affected brains, which uncovered epigenomic alterations associated with AD.

Our transcriptomic analyses of younger, old and AD samples revealed upregulation of transcription- and chromatin-related genes in AD, including the histone acetyl transferases CBP/p300 and TRAPP (a component of the SAGA complex), which mediate deposition of H3K27ac and H3K9ac, respectively (Fig. 1). Studies of CBP in animal models point to CBP requirement for memory-related processes. Studies of p300 in neurodegeneration models show that p300 acetylates tau and promotes its aggregation, thus revealing a pro-disease role for p300, although uncoupled from its chromatin-related function. A proteomic analysis revealed striking increases of H3K27ac and H3K9ac in AD (Fig. 2). Genome-wide analyses of H3K27ac and H3K9ac, together with H3K122ac and H3K4me1, revealed predominant H3K27ac and H3K9ac disease-specific gains in AD (Fig. 3), which were associated with functional categories related to transcription and chromatin genes (Fig. 4). Dysregulation of epigenetic pathways, also highlighted by the RNA-seq changes, may be upstream of the activation of pro-disease pathways in AD (model in Fig. 5c). H3K9ac disease-specific gains at CREBBP (probably by SAGA) indicated a potential positive feedback loop promoting sustained CBP expression in AD and consequent H3K27ac. We underscore that previous AD studies did not identify aberrant epigenetic activation as a functional pathway in AD, probably because of the lack of comparison between the disease state and healthy brain aging.

In contrast to these gains, analysis of H3K122ac, deposited by a different histone acetyltransferase, revealed preferential disease-specific losses associated with different functional pathways. This comparison highlights the complexity of the AD epigenome, where different histone acetyl modifications drive different functional pathways. Our previous findings of H4K16ac protective pathways in AD further highlights this scenario. As is the case with cancer, where a multistep process selects for inactivation of tumor suppressors followed by activation of oncogenes, it is possible that in AD, inactivation of protective pathways (for example, H4K16ac driven) is followed by activation of pro-disease pathways (for example, H3K27ac and H3K9ac driven). The dynamic trend of histone acetylation uncovered in AD suggests that the use of general HDAC inhibitors might not be effective. Indeed, increases of H3K27ac and H3K9ac in a fly model of AD promoted Aβ42-induced neurodegeneration (Fig. 5a), thereby confirming a negative effect of increased acetylation. Enrichment of H3K27ac disease-specific gains for AD SNPs and eQTLs (Fig. 4j,k) further support a role for H3K27ac gains in AD.

Overall, these multi-omic analyses have identified H3K27ac and H3K9ac as potential epigenetic drivers of AD, which spur disease pathways through dysregulation of transcription and
chromatin–gene feedback loops. Importantly, these findings are confirmed when comparing our data to published datasets performed on other brain regions affected by AD\textsuperscript{19,20}. Given the several pathways with which CBP and p300 are involved, approaches aiming to epigenetically inactivate pro-disease pathways and activate/reinforce protective pathways may be a promising therapeutic strategy for AD. Our findings provide mechanistic insights on AD progression and highlight alternative avenues for potential intervention.
Fig. 5 | Increased H3K27 and H3K9 acetylation enhances Aβ42 toxicity in Drosophila. a, Drosophila eye showing that the histone mutants H3.3K9M (lysine (K) to methionine (M) alteration at residue 9), H3.3K27M (K to M alteration at residue 27) and H3.3K27Q (K to glutamine (Q) alteration at residue 27) independently enhance Aβ42 toxicity both in external (top) and internal eye tissue (bottom). The histone mutant H3.3K27A (K to alanine (A) alteration at residue 27) has no effect on Aβ42 toxicity. Expression of H3.3K27M and HK9M globally increases H3K27ac⁹², while H3.3K27Q mimics acetylation⁹⁴ and H3.3K27A mimics the absence of acetylation (Supplementary Table 14 for fly genotypes). GFP, green fluorescent protein; GMR, glass multimer reporter. b, Bar graph (with individual data points) representing the mean ± s.d. of the internal retinal depth in n = 4–6 individual animals per genotype (***P < 0.0001, one-way ANOVA (F,6,28) = 83.24) with Tukey’s multiple comparisons test. Aβ42 + GFP versus Aβ42 + H3.3WT (P = 0.9962) and Aβ42 + H3.3K9M (P = 7.839 × 10⁻⁷), Aβ42 + H3.3K27M (P = 7.839 × 10⁻⁷) and Aβ42 + H3.3K27Q (P = 6.913 × 10⁻⁶). NS, not significant. c, Model of aberrant activation of chromatin and pro-disease pathways in AD. Increased H3K27ac and H3K9ac (by CBP/p300 and SAGA) drive the activation of chromatin (left) and pro-disease pathways (right) in AD. Increased expression of transcription and chromatin genes (left), including CBP/p300 and TRAPP in the SAGA complex (left), may be upstream and reinforce activation of pro-disease pathways. H3K9ac at CREBBP (potentially by SAGA) leads to a positive feedback loop of sustained CBP expression and downstream histone acetylation in the AD epigenome. Environmental stressors may be upstream of the activation of chromatin pathways.
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Methods

Brain tissue samples. Postmortem human brain samples from the lateral temporal lobe (Brodmann area 21 or 20) were obtained from the Center for Neurodegenerative Disease Research (CNDR) brain bank at the University of Pennsylvania. Informed consent for autopsy was obtained for all patients and this was approved by the Pennsylvania Institutional Review Board. The CNDR autopsy brain bank protocols were exempted from full human research. (Research on tissue derived from an autopsy is not considered human research; see https://grants.nih.gov/policy/humansubjects/research.htm). A detailed description of the brain bank standard operating procedures has been reviewed elsewhere26. A neuropathological diagnosis of AD was established based on the presence of plaques and tangles using the CERAD scores and Braak stages, respectively27,28. The CERAD plaque score assesses the burden of neocortical plaques (0 and A–C in order of increasing frequency) in the neocortex. The Braak staging is based on the progression of neurofibrillary tangles from the entorhinal cortex (stage I) to widespread neocortical pathology including the primary visual cortex (stage VI).

Selection of brain tissue samples. The brain tissue samples were selected based on the presence of plaques and neurofibrillary tangles using the CERAD scores and Braak stages, respectively27,28. All selected AD cases had high level of AD neuropathological changes (Braak V–VI and CERAD D–E; Supplementary Table 1). The younger and older control brains had no or minimal neocortical amyloid plaques (Braak 0 or I/II) or neurofibrillary tangles (CERAD 0). None of the AD cases had other coincident neurodegenerative diseases. Control individuals had no deposits consistent with frontotemporal lobar degeneration or other neurodegenerative diseases. Selection of AD cases was also controlled for sex (mainly male individuals), neuronal loss (excluding cases with severe loss) and postmortem interval (≤24 h). Neuronal loss was assessed through semiquantitative measurements by hematoxylin and eosin (H&E) staining by board-certified neuropathologists of the CNDR. The H&E scoring for neuronal loss ranges from 0 to 3 where 0 signifies no neuronal loss and 3 is severe neuronal loss. Only cases with neuronal loss of 0 or 1 (mild or moderate) were included. In addition, neuronal fractions were measured for each sample by flow cytometry of NeuN-stained nuclei and used for ChIP–seq analysis.

RNA-seq analysis. RNA-seq data were previously generated and processed for the same brain samples (lateral temporal lobe) analysed in this study29. Briefly, total RNA was isolated using the RNeasy Mini Kit (Qiagen) and treated with an RNase-free DNase step (Qiagen). Ribosomal RNA was removed using the rRNA Depletion Kit (New England Biolabs) and multiplexed RNA-seq libraries were generated using the NEBNext Ultra Directional RNA Library Prep Kit for Illumina (New England Biolabs). Libraries were sequenced (75-base pairs (bp), single-end) on a NextSeq 500 Platform (Illumina) in accordance with the manufacturer's protocol. RNA-seq reads were aligned to the human reference genome (assembly GRCh37.75, hg19) using STAR v.2.3.0e with default parameters. FeatureCounts v.1.6.2 was used to generate a matrix of mapped fragments per RefSeq annotated gene. Analysis for differential gene expression between the AD and old samples was performed using the DESeq2 R package v.1.16.1 with FDR < 0.05.

For the correlation between the ChIP–seq and RNA-seq data, the DESeq2-normalized read count over each gene was normalized per kb of gene length and then transformed to log2 (log2(count DESeq2-normalized read count over each gene was normalized per kb of gene length)) using C18 Stage Tips. Histone PTM preparation and analysis by MS. Histone PTM preparation and analysis by MS were performed as described previously30. Briefly, nuclei were isolated from 100-μm frozen brain tissue (lateral temporal lobe) and histones extracted with 0.2 M of H2SO4 for 2h and precipitated with 33% trichloroacetic acid overnight. The pellets containing histone proteins were dissolved in 30 μl of 50 mM of NH4HCO3 (pH 8.0). Two rounds of histone derivatization were performed by mixing the histone samples with propionic anhydride and acetic anhydride (previously mixed in a ratio of 1:3 (v/v)) in the ratio of histone samples to mix of 1:4 (v/v) for 20 min at room temperature. Histones were then digested with trypsin (trypsin to sample ratio, 1:20) in 50 mM of NH4HCO3, for 6h at room temperature. The derivatization procedure was repeated after digestion to derivatize peptide N termini. Samples were desalted before LC–MS analysis by using C18 Stage Tips.

Samples were run on a nano LC–MS/MS setup. Nano LC was configured with a two-column system including a 100-μm ID×2-cm trap column and a 75-μm ID×25-cm analytical column mounted onto an Eksigent NanoLC Ultra 2D Plus. The high-performance LC gradient was as follows: 2% to 28% solvent B (A = 0.1% formic acid; B = 99% acetonitrile, 0.1% formic acid) over 45 min, from 28 to 80% solvent B in 5 min, 80% B for 10 min at a flow rate of 300 nl/min29. Nano LC was coupled to an Orbitrap Fusion Tribrid mass spectrometer (Thermo Fisher Scientific). Data were acquired using a data-independent acquisition method30. Specifically, a full-scan MS spectrum (m/z 300–1,100) was acquired in the Orbitrap with a resolution of 120,000 (at 200 m/z) and an automatic gain control (AGC) limit of 5×10⁶. MS/MS was performed with an AGC target of 3×10⁹. MS/MS was acquired with an AGC target of 3×10⁹ using an injection time limit of 30 or 60 ms. MS/MS was acquired using higher-collision dissociation with normalized collision energy of 27.

Data were analyzed using EpiProfile v.2.0 (ref. 31). EpiProfile extracts the ion chromatogram of the (un)modified histone peptides. The peptide relative ratio was calculated using the total area under the extracted ion chromatograms of all peptides with the same amino acid sequence (including all of its modified forms) as 100%. For isobaric peptides, the relative ratio of two isobaric forms was estimated by averaging the ratio for each fragment ion with different mass between the two species. Statistical regulation of histone marks was assessed using a t-test (heteroscedastic, 2-tailed). Statistical significance was assessed when P < 0.05.

ChIP–seq. ChIP–seq was performed as described previously31. Briefly, nuclei were isolated from 200 μg of frozen brain tissue (lateral temporal lobe) by Dounce homogenization in nuclear isolation buffer (50 mM of Tris-HCl at pH 7.5, 25 mM of KC1, 5 mM of MgCl2, 0.25 M of sucrose and freshly added protease inhibitors

String analysis. STRING v.11 (ref. 32) analysis was performed to identify possible interaction networks among the genes upregulated in AD. The threshold for confidence was set to medium. STRING networks were visualized with Cytoscape v.3.6 (ref. 33) where node size represents gene expression in AD, color intensity represents the gene expression change of AD versus old and the thickness of lines represents the strength of the STRING interaction.

Comparison to published RNA-seq. RNA-seq data were downloaded from Synapse’s AMP-AD Knowledge Portal for the Mount Sinai Brain Bank (MSBB) (syn16793954, RNA-seq of tissue from Brodmann area 22 with expression values adjusted for sex, ancestry, age, RNA integrity number, postmortem interval, exon rate, RNA rate and batch effects) and the Mayo Clinic (syn6126176, RNA-seq of tissue from temporal cortex with counts per million expression values as reported in TCX gene counts). MSBB patients were deemed to have AD with a neuropathology scoring of 2 or 3 while patients with a score of 1 were grouped as controls according to the clinical annotation provided by the MSBB. Mayo Clinic patients were categorized as AD or control depending on the diagnosis given by the Mayo Clinic study (TCX covariates). Decile plots were assessed by averaging gene expression scores from all patients across study groups and dividing the genes into ten tiers by the average expression in the published datasets. Box-and-whisker plots were then assessed for this study’s RNA-seq data in the tiered genes. Heat maps of normalized expression values for all genes (eight tiers) per patient or gene clustering was performed. Box-and-whisker plots for individual genes were created to show the distribution of expression scores between AD and control patients in the published datasets; significance was assessed using Mann–Whitney U-tests.

Comparison to published microarray data. Supplementary Table 1 from Blalock et al.34 was filtered for genes in the list of 75 chromatin genes upregulated in AD in our data. The resulting table gives the Pearson’s correlation coefficient of gene expression in laser-capture microdissected hippocampus from patients with varying degrees of AD pathology to two indexes for pathology severity, the Mini-Mental State Examination and neurofibrillary tangle scores. Correlation coefficients (r) from the filtered table are presented in the column plot in Supplementary Fig. 1f.
and sodium butyrate) followed by ultracentrifugation on a 1.8 M sucrose cushion. Nuclear pellets were resuspended and cross-linked in 1% formaldehyde for 10 min at room temperature, quenched with 125 mM of glycine for 5 min and washed twice in cold PBS. Then, 2 × 10^6 nuclei were lysed in nuclear lysis buffer (10 mM of Tris-HCl at pH 8.0, 100 mM of NaCl, 1 mM of EDTA, 0.5 mM of EGTA, 0.1% sodium deoxycholate, 0.5% N-lauroylsarcosine and freshly added protease inhibitors and sodium butyrate) and chromatin was sheared using a Covaris S220 sonicator to a final approximate 250 bp. Equal aliquots of sonicated chromatin were used per immunoprecipitation reaction with either 3 µl of H3K27ac antibody (catalog no. 4729; Abcam), or 5 µl of H3K9ac antibody (catalog no. 39137; Active Motif) or 3 µl of H3K122ac antibody (catalog no. 33309; Abcam) or 4 µl of H3K4me1 antibody (catalog no. 8895; Abcam) preconjugated to Protein G Dynabeads (Thermo Fisher Scientific); 10% of each immunoprecipitation was used for the inputs. ChIP reactions were incubated overnight at 4°C followed by ChIP washes in 1% SDS, 0.5% N-lauroyl sarcosine and protease inhibitor mixture (10 mM of Tris-HCl at pH 8, 10 mM of EDTA) at 65°C. Eluted DNA from ChIP and input samples was reverse-cross-linked and purified using PCR columns (QIAGEN). Then, 5 µg of ChIP and input DNA was used to generate ChIP–seq libraries using the NEBNext Ultra DNA Library Prep Kit for Illumina (New England Biolabs). Libraries were multiplexed using the NEBNext Multiplex Oligos for Illumina (dual index primers) and sequenced (75 bp, single-end) on a NextSeq 500 platform in accordance with the manufacturer's protocol.

ChIP–seq analysis. ChIP–seq analysis was performed as in Nakatani et al. with modifications to demultiplexed reads. After DNA sequencing, ChIP–seq tags (approximately 20 million reads per sample per histone mark) were aligned to the human reference genome (assembly NCBI37, hg19) using Bowtie v1.1.1 (ref. 1) allowing up to two mismatches per sequencing tag (parameters --m 1 --best). Reads mapped to mitochondria or Encyclopedia of DNA Elements (ENCODE) blacklist regions were removed from the analysis. There were no statistically significant differences in sequencing coverage of the younger, old and AD samples. The ChIP–seq signal in the MTLL (unit reads per kilobase of transcript per million mapped reads (RPKM)) was quantified for each individual sample using the Bwttool package v.1.0 (‘bwttool summary’). To reduce the confounding variables due to changes in neuronal fractions across the samples, the top 10% MTLs with the highest Pearson’s correlation with sample neuron fraction were masked from the analysis. (Neuron fractions for these samples were measured on our previous study using CEPH histone acetyl marks, masked MTLs for each H3K27ac, H3K9ac and H3K122ac were merged into a union set of acetylation sites (called ‘multiMTL’) and the ChIP–seq signal for each histone mark and each individual patient was evaluated for these multiMTL sites. (The signal is only accounted for over each individual ̣ mark on genes targeted by H3K27ac, H3K9ac or H3K122ac, or 300,000 peak changes (P < 0.05, one-way ANOVA) were compared between the peak and control ChIP–seq tracks for the AD and control groups were downloaded from the UCSC Genome Browser in Marzi et al. 14. Peaks with H3K27ac significant changes in AD were downloaded from Supplementary Tables 4 and 5 in the same paper. To compare our ChIP–seq data with published human frontotemporal cortex H3K9ac ChIP–seq data, published H3K27ac ChIP–seq data for the AD and control groups were downloaded from Supplementary Table 3 in Klein et al. 15. Comparison to published human ChIP–seq data. To compare our ChIP–seq data with published human cortical H3K9ac ChIP–seq data, published H3K27ac ChIP–seq data for the AD and control groups were downloaded from Supplementary Table 3 in Klein et al. 15. According to the same paper. To compare our ChIP–seq data with published human cortical H3K9ac ChIP–seq data, published H3K27ac ChIP–seq data for the AD and control groups were downloaded from Supplementary Table 3 in Klein et al. 15. Comparison to published human mouse ChIP–seq data. To compare our ChIP–seq data with published mouse cortical H3K9ac ChIP–seq data, published H3K27ac ChIP–seq track files were downloaded from the Gene Expression Omnibus (GSE6519). H3K27ac peaks with changes in acetylation were downloaded from Supplementary Table 3 in Klein et al. 15, which provides both mouse genome coordinates and the lifted-over human genome coordinates. For comparison with the tau mouse model, H3K9ac ChIP–seq track files and peaks with H3K9ac changes at either 6 or 11 months were obtained by the Bioinformatics, Babraham UK) projects/trim_galore/) to remove adapter sequences and poor-quality nucleotides. High-quality reads were then mapped to the hg19 reference genome by Bowtie v1.1.1 with uniquely mapped reads retained for the following analyses. PCR duplicates were removed by Samtools and normalization was performed by deepTools. Reads of samples from the same study group were pooled for 3umCh track visualization. To compare 3umCh with RNA-seq, fragments per kilobase of transcript per million mapped reads values for 3umCh over each ReSeq gene were computed using FeatureCounts and correlation plots with corresponding gene RNA-seq expression levels were plotted in R v.3.2.2. To compare 3umCh with ChIP–seq data, metabolites of 3umCh signal over the ChIP–seq-defined regions of the same time and number were chosen. Davis was assessed with a modified Fisher's exact test (EASE) and FDR correction by the Yekutieli procedure. An FDR < 10% represents the threshold of significance in DAVID. The whole genome was used as the background for these analyses.

Graphical representation. Scatter plots and box plots of the ChIP–seq data were visualized using the R package ggplot2 v.3.3.1. Metaplots and signal heatmaps centered around peaks were generated using deepTools (‘computeMatrix’ and ‘plotHeatmap’) v.2.5.7.

DNA motif enrichment analysis. DNA motif enrichment analysis of sequences underneath the H3K27ac or H3K9ac or H3K122ac peaks with acetylation changes (P < 0.05, one-way ANOVA) was performed using HOMER v.4.6 (ref. 16), with default parameters and DNA motif scanning windows equal to the size of each individual peak. Background DNA was defined by an equivalent number of reads, 500,000 reads, from each sample in the same paper.

5umCh–library construction. Nano-5umCh–Seal was performed as described previously in Han et al. 17. Briefly, 100 ng of genomic DNA was fragmented in 10% fragmentation buffer at 55°C. Fragmented DNA was purified by Zymo DNA Clean and Concentration Kit (Zymo Research). Then, selective 5umCh–chemical labeling was performed in glycosylation buffer (50 mM of HEPES buffer, pH 8.0, 25 mM of MgCl2 containing the fragmented DNA, BGT and N-UDP-Glc, and incubated at 37°C for 10 min) followed by glycosylation reaction in double-distilled H2O, DBCO–PEG4 Biotin (Click Chemistry Tool) was added and incubated at 37°C for 2 h. The biotin-labeled DNA was pulled down by C1 Streptavidin beads (Thermo Fisher Scientific) for 15 min at room temperature. Next, the captured DNA fragments were subjected to PCR amplification using the Nextera DNA Sample Preparation Kit. The resulting amplified product was purified by 1.0× AMPure XP beads. The input library was made by the same procedure directly without enrichment. The libraries were quantified by a Qubit Fluorometer (Life Technologies Invitrogen). Qubit 2.0 DNA RNA Protein Fluorometer, Q32866 and sequenced on a NextSeq 500 platform in accordance with the manufacturer's protocol.

5umCh–Seal data analysis. Raw sequencing reads were first trimmed by Trim Galore! v.0.44 (https://www.bioinformatics.babraham.ac.uk/projects/trim_galore/) to remove adapter sequences and poor-quality nucleotides. High-quality reads were then mapped to the hg19 reference genome by Bowtie v1.1.1 with uniquely mapped reads retained for the following analyses. PCR duplicates were removed by Samtools and normalization was performed by deepTools. Reads of samples from the same study group were pooled for 3umCh track visualization. To compare 3umCh with RNA-seq, fragments per kilobase of transcript per million mapped reads values for 3umCh over each ReSeq gene were computed using FeatureCounts and correlation plots with corresponding gene RNA-seq expression levels were plotted in R v.3.2.2. To compare 3umCh with ChIP–seq data, metabolites of 3umCh signal over the ChIP–seq-defined regions of the same time and number were chosen. Davis was assessed with a modified Fisher's exact test (EASE) and FDR correction by the Yekutieli procedure. An FDR < 10% represents the threshold of significance in DAVID. The whole genome was used as the background for these analyses.
Authors upon request. We used the UCSC liftOver tool to convert mouse genomic coordinates to human coordinates using similar parameters as those in the original paper. Venn diagrams and box plots of peak comparisons were generated in the same way as described above for the comparison with the published human data.

Comparison to published DNA methylation. Seventy-one CpG sites whose methylation levels were associated with neuritic amyloid plaques in the frontalateral cortex were identified in De la Iglesia et al. We examined the significance of the overlap (defined as a CpG site within 1 kb from a peak) between the differentially enriched H3K27ac or H3K9ac or H3K122ac changes in AD (gains or losses) and the 71 CpG sites using the Fisher's exact test implemented in bedtools.

Association between histone acetyl changes and AD GWAS SNPs. For the enrichment analysis of the differential acetylation peaks with AD GWAS SNPs, from Lambert et al.14, a set of 2,371 SNPs passing stage I and stage II GWAS meta-analysis with a P value of \( p < 1 \times 10^{-8} \) were downloaded from the IGAP46. INRICH was used to infer the relationship between H3K27ac, H3K9ac and H3K122ac changes and PLINK-joined AD GWAS SNP intervals (linkage due to HapMap release 23) using standard parameters. The set of all changed peaks \( (P < 0.05, \text{one-way ANOVA}) \) was the background for the experiment.

IGAP is a large two-stage study based on GWAS of individuals of European ancestry. In stage 1, IGAP used genotyped and imputed data on 7,055,881 SNPs to meta-analyze 4 previously published GWAS datasets consisting of 17,008 AD cases and 37,154 controls (the European Alzheimer Disease Initiative, the Alzheimer Disease Genetics Consortium, the Cohorts for Heart and Aging Research in Genomic Epidemiology Consortium, the Genetic and Environmental Risk in AD Consortium). In stage 2, 11,632 SNPs were genotyped and tested for association in an independent set of 8,572 AD cases and 11,312 controls. Finally, a meta-analysis was performed combining the results from stages 1 and 2.

INRICH was used to examine the intersection of differential acetylation peaks with the Kunkle et al.17 stage 1 AD GWAS SNPs in the following way. Summary statistics for 11,480,632 SNPs were downloaded from the National Institute on Aging Genetics of Alzheimer's Disease Storage Site (NIAGADS). Reference data from the 1000 Genomes Project was downloaded for volume 1, release 05-02-2013. Per-chromosome VCF files were concatenated with BCTools v1.19, then converted to PLINK format using PLINK v1.9 with the parameters, --split-x b37 no-fail --allow-extra-chr 0 --const-vid --vcf-idspace-to _ --keep-allele-order.

Duplicate/multiallelic and reference-mismatched variants were identified using PLINK v2.0 with the parameters --rn-dup-list --set-missing-vars @ [P<0.001] a concatenated blacklisted of those IDs was prepared. To find SNP clumps, 3,592 stage 1 SNPs with a GWAS P value \( < 1 \times 10^{-8} \) were input to PLINK v1.9 with the parameters --exclude Blacklist.txt --clump Kunkle.1E-5.assoc --clump-p1 0.00001 --clump-p2 0.05 --clump-r2 0.5 --clump-range-border 20. The clumped ranges file was parsed with gawk v4.0.2 to produce chromosome, start and stop coordinates for 540 clumped regions and the resulting file was used as input to PLINK, which was run as described previously.

eQTL data processing and sampling analysis. For the Zhou et al. data,18 eQTL data tables were downloaded from the NIAGADS at the University of Pennsylvania (U2A-AG041689-01), which is funded by the National Institute on Aging. The original quality controlled samples from the cerebellum in addition to temporal cortex, but we only used the temporal cortex data due to the cortical origin of our H4K16ac measurements and because regulatory elements are variable across brain regions.10 Custom awk-based bash scripts, available by request, were used to convert eQTL data tables into BED format, using the liftOver utility from the UCSC Genome Browser to convert annotations from the hg18 genome build to hg19 to overlap the H3K27ac, H3K9ac and H3K122ac peaks. Twelve AD, 10 non-AD and 18 combined condition eQTLs were unmapped by liftOver. We then used the intersect tool from the bedtools suite to overlap the H3K27ac, H3K9ac and H3K122ac changed peaks \( (P < 0.05, \text{one-way ANOVA}) \) with the eQTL BED files.

For the sampling analysis, the shuffle tool from bedtools was used to generate 10,000 sets of matched control intervals, where unmappable regions as defined by the DAC blacklisted regions downloaded from the UCSC Genome Browser and ENCODE46. For each dataset, custom scripts, also available by request, were used to summarize the overlap intersections in easy-to-parse files that were then read into the R programming language, which was used to perform the empirical enrichment analyses.

Fly husbandry and fly lines. Stocksm maintained on standard cornmeal–molasses medium. All flies were maintained at 26°C. Standard fly lines were from the Bloomington Drosophila Stock Center (Bloomington, IN) (http://stocksblast.igv.org/Drosophilaaccessible.html). The fly husbandry and fly lines were performed as described below. The P[UAS-attB-H3.3-FLAG-HA construct (courtesy of H.-M. Herz, St. Jude Children's Research Hospital) was mutagenized to make point mutations encoding H3.3K27A and H3.3K27Q using the QuickChange II XL Site-Directed Mutagenesis Kit (Agilent Technologies): the β at position 27 (AAG) was changed to H3.3K27A (AAG>GGC) and H3.3K27Q (AAG>CAG). The constructs were sequenced after mutagenesis and were then used to make the transgenic fly lines, inserting into the attP site 53R2 (BestGene) (see Supplementary Table 14 for the oligonucleotide sequences).

Drosophila eye imaging. Expression of UAS-AJ42, UAS-mCD8:GFP and UAS-H3.3 variants (H3.3WT, H3.3K27M, H3.3K9M, H3.3K27A and H3.3K27Q) were driven by GMR-GAL4 (U). To image the external eyes, adult 2–3-day-old Drosophila females were anesthetized with ether, placed on microscope slides using double-sided tape and imaged with a Leica Z16 APOA (Leica Biosystems). For the internal sections, fly heads were fixed in Bouin's solution (Sigma-Aldrich) for 120 h. Heads were rinsed in leaching buffer (50 mM of Tris/130 mM of NaCl) overnight and processed with increasing concentrations of ethanol (70, 80, twice with 95 and twice with 100%) for 30 min each, twice in xylene for 30 min each and embedded in paraffin at 63°C for 2 h each. Paraffin blocks were made using Shandon Histocentre2 (Thermo Fisher Scientific) and 8-μm sections were cut using a Leica RM2255 microtome. Sections were deparaffinized with HistoClear (National Diagnostics) and mounted on SuperFrost Plus Slides (Thermo Fisher Scientific) using Cytoseal XYL mounting medium (Thermo Fisher Scientific). Images of endogenous autofluorescence were taken with a Leica DM6000B fluorescence microscope. Images of retina sections were analyzed with ImageJ v2.0.0-rc-69.152 (NIH) to measure the retinal depth across a consistent plane of the brain at the point of the optic chiasm. For each fly, four sections in the region of interest were measured and the average was used as the retinal depth for that animal; 4–6 animals per genotype were analyzed.

Statistics and reproducibility. Statistical analysis of the ChiP–seq data was performed with a two-sided Wilcoxon rank-sum test or one-way ANOVA as reported in the text and corresponding figure legends. Statistical analysis of RNA-seq data was performed using DESeq2 (Wald test) with an FDR \( < 0.05 \) controlled by Benjamini–Hochberg correction. MS analysis was performed using a two-sided Student's t-test. Samples used in each experiment are reported in Supplementary Table 2. No statistical method was used to predetermine sample sizes but our sample sizes are similar to those reported in published studies. For replicability, data were compared to published epigenomic and transcriptomic studies of AD47,48. Statistical analyses of Drosophila internal eyes were performed with a one-way ANOVA followed by a Tukey's multiple comparison test; 4–5 animals were used for each genotype. Both epigenomic and fly data collection and analysis were not performed blind to the conditions of the experiments. No data points were excluded from the analyses.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability. The epigenomic and transcriptomic data that support the findings of this study are available through the National Center for Biotechnology Information Gene Expression Omnibus repository under accession no. GSE133875. Part of the input libraries were previously generated and are available under accession no. GSE84618. The proteomic data are available through the repository Chorus under accession no. 1684.

Code availability. The code developed for the analyses performed in this study is available at https://github.com/yemilana/ADePgenetics.

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Author contributions
R.N., N.M.B. and S.L.B. conceived the project. R.N. performed the ChIP-seq, RNA-seq and mass spectrometry experiments and supervised most of the analyses. Y.L. performed the ChIP-seq and RNA-seq analyses. G.D. performed the comparisons with published RNA-seq data and AD SNP enrichment analysis. S.S. performed the mass spectrometry and STRING analysis. A.B., A.R.S. and O.S. performed the fly experiments. R.N. extracted genomic DNA and J.N. performed 5hmC-Seal. X.C. processed the 5hmC data. A.A.-W. performed the AD eQTL enrichment analysis. C.H., L.W., B.A.G., J.Q.T., N.M.B. and S.L.B. contributed to the methodology and resources. R.N., N.M.B. and S.L.B. wrote the manuscript. All authors reviewed the manuscript and discussed the work.

Competing interests
C.H. holds a patent on the technology used (no. US8741567) and is a shareholder in Shanghai Epican Genetech.

Additional information
Extended data is available for this paper at https://doi.org/10.1038/s41588-020-0696-0. Supplementary information is available for this paper at https://doi.org/10.1038/s41588-020-0696-0. Correspondence and requests for materials should be addressed to N.M.B. or S.L.B. Reprints and permissions information is available at www.nature.com/reprints.
Extended Data Fig. 1 | STRING network analysis for genes changing in AD. a, Barplot showing the number of STRING (v11) interactions for genes with the top number of interactions in Fig. 1e. b, STRING interaction network for genes changing in AD vs Old (q < 0.05) that interact with EP300, CREBBP and TRAPP. Interactions that were identified in Fig. 1e are not shown in this network. The gene network was visualized with Cytoscape (v3.6)104. Size of nodes represents RNA expression values, the color represents gene expression changes (log2 fold-change) in the AD vs Old comparison (red for upregulated in AD; blue for downregulated in AD) and the thickness of the line is the confidence of the interaction calculated by STRING. Nodes circled in red or blue represent known transcription and chromatin genes.
Extended Data Fig. 2 | Histone posttranslational modifications in Younger, Old and AD. a, Amino acid sequence of canonical histone H3 (H3.1 and H3.2) tail and globular domain, and its H3.3 variant. The residue that differs between canonical H3 and H3.3 is highlighted in red. b, Amino acid sequence of histone H4 tail and globular domain. Bars below the amino acid sequence in panels a-b represent peptides generated in the trypsinization process that were identified on the mass spectrometer (LC-MS/MS). Grey bars represent peptides not reliably detected and therefore excluded from the analysis. c-e, Stacked bar plots showing relative abundance of histone modifications (methylation and acetylation) on histones H3, H3.3 and H4 in (c) Younger, (d) Old and (e) AD. The lysine residues (K) analyzed are listed below the stacked bar plots.
Extended Data Fig. 3 | Histone acetyl marks are enriched at both TSS and enhancers. Metaplots showing peak enrichment of H3K27ac, H3K9ac and H3K122ac and corresponding 5hmC and H3K4me1 enrichments for peaks at transcriptional start sites (TSSs) (≤1 Kb from TSS) and enhancer (Enh) sites (>1 Kb from TSS) in (a–f) Younger, (g–l) Old and (m–r) AD brains. Histone acetyl-peaks are enriched at both TSSs and enhancers, while 5hmC and H3K4me1 mark enhancer sites.
Extended Data Fig. 4 | H3K27ac, H3K9ac and H3K122ac peak distribution in Younger, Old and AD. a. Histogram of peak density for H3K27ac (light green), H3K9ac (light blue) and H3K122ac (light red), based on their distance from the transcriptional start site (TSS) for peaks detected in Younger, Old and AD. Grey vertical lines demark (from left to right): 5, 25, 50 and 100 Kb distance from TSS. b–d. Venn Diagram showing the overlap between H3K27ac, H3K9ac and H3K122ac peaks for (b) All peaks, (c) TSS peaks (≤1Kb from TSS) and (d) enhancer (Enh) peaks (>1Kb from TSS) detected in Younger, Old and AD.
Extended Data Fig. 5 | See next page for caption.
Extended Data Fig. 5 | Correlation between ChIP-seq and RNA-seq data. a-c, Scatterplot of (a) H3K27ac, (b) H3K9ac and (c) H3K122ac peak enrichment vs gene expression for genes expressed in Old. d-f, Scatterplot of (d) H3K27ac, (e) H3K9ac and (f) H3K122ac peak enrichment vs gene expression for genes expressed in AD. For graphical representation in a-b, 3000 randomly chosen points are shown in each panel. g-l, Scatterplot of (g) H3K27ac, (h) H3K9ac and (i) H3K122ac absolute peak fold-change vs absolute gene expression change for significantly ($q < 0.05$) differentially expressed genes in AD vs Old. j,k, Scatterplot of total acetyl-peak enrichment (H3K-total-ac; sum of H3K27ac, H3K9ac and H3K122ac peak enrichment at the same site) vs gene expression for genes expressed in (j) Old and (k) AD. l, Scatterplot of H3K-total-ac absolute peak fold-change vs absolute gene expression change for significantly ($q < 0.05$) differentially expressed genes in AD vs Old. The closest peak to the TSS was chosen for these analyses. Linear regression trendlines, Pearson’s correlation coefficients and p-values (test for association using Pearson’s product moment correlation coefficient implemented by R stats package, two-sided) are indicated in each panel (a-l).
Extended Data Fig. 6 | Comparison between histone marks enrichments at sites with disease-specific changes. a–c, Boxplots showing H3K27ac, H3K9ac, H3K122ac and H3K4me1 peak enrichment at sites with (a) H3K27ac, (b) H3K9ac, (c) H3K122ac (highlighted in blue) disease-specific gains. d–f, Boxplots showing H3K9ac, H3K122ac and H3K4me1 peak enrichment at sites with (d) H3K27ac, (e) H3K9ac and (f) H3K122ac (highlighted in blue) disease-specific losses. Asterisks in (a–f) denote level of significance comparing peak enrichment across Younger (N = 11-12), Old (N = 10) and AD (N = 9–11) (* P < 0.05; ** P < 0.01, 1-way ANOVA) (Supplementary Table 2). Boxplots show minimum, first quartile, median (center line), third quartile and maximum.
Extended Data Fig. 7 | H3K9ac disease-specific gain at CREBBP but not EP300. a–c, Boxplot showing (a) H3K9ac, (b) H3K27ac and (c) H3K122ac peak enrichment at the CREBBP gene in Younger, Old and AD. A H3K9ac disease-specific gain is observed at CREBBP (highlighted in blue in a). d–f, Boxplot showing (d) H3K9ac, (e) H3K27ac (f) H3K122ac peak enrichment at the EP300 gene in Younger, Old and AD showing no disease-specific changes. The closest peak to the gene was considered for this analysis. P-values comparing peak enrichment across Younger (N = 8–9), Old (N = 10) and AD (N = 9–11) (Supplementary Table 2) (1-way ANOVA) are reported in each panel. Boxplots show minimum, first quartile, median (center line), third quartile and maximum. Dots overlaid on boxplots represent individual data points.
Extended Data Fig. 8 | Functional analysis of H3K27ac and H3K9ac disease-specific losses. **a,b,** Barplot showing top GO terms (Biological Processes; GREAT, FDR < 5%, % by both the binomial and the hypergeometric tests) for (a) H3K27ac disease-specific losses and (b) H3K9ac disease-specific losses for terms with at least 20 genes. **c,d,** UCSC genome browser view showing an example of (c) H3K27ac disease-specific loss at the PCSK1 gene and (d) H3K9ac disease-specific loss at the SVOP gene. H3K27ac, H3K9ac, H3K122ac, H3K4me1 ChIP-seq and RNA-seq tracks are showed for Younger, Old and AD. **e,f,** Top DNA motifs (HOMER v4.6) for (e) H3K27ac disease-specific losses and (f) H3K9ac disease-specific losses in AD. Enrichment results are shown for known motifs (q < 0.05, Benjamini-Hochberg).
Extended Data Fig. 9 | Functional analysis of disease-specific changes using DAVID. a,b, Barplot showing top GO terms (Biological Processes, DAVID v6.7, FDR <10%, Yekutieli) for genes targeted by (a) disease-specific gains (H3K27ac or H3K9ac) and (b) disease-specific losses (either H3K27 or H3K9ac or H3K122ac) for terms with at least 20 genes.
Extended Data Fig. 10 | H3K27ac disease-specific gains are enriched with AD GWAS SNPs from Kunkle et al. Bar plot showing the significance (-log_{10} p-value) of the association between each of the six classes of H3K27ac, H3K9ac and H3K122ac changes (age-regulated gains or losses, age-dysregulated gains or losses and disease-specific gains or losses) and AD SNP-regions from Kunkle et al. using INRICH. Red dashed horizontal line represents the threshold of significance (P < 0.05).
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- A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted
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Software and code

Policy information about availability of computer code

Data collection

ChIP-seq, RNA-seq (with ERCC spike-in) and 5hmC-Seal libraries were sequenced on a NextSeq 500 and 550 platforms (Illumina) in accordance with the manufacturer’s protocol. Demultiplexed ChIP-seq or 5hmC-Seal tags were downloaded from BaseSpace (Illumina). Mass spec sample were run on a nanoLC-MS/MS setup.

Data analysis

Analysis of ChIP-seq data:
- sequencing tags alignment: Bowtie v1.1.1 (human reference genome, assembly NCBI37/hg19)
- peak calling: MACS2
- comparison of peak enrichment across study groups: MTL method (Chen et al. 2008)
- Gene Ontology analysis: DAVID v6.6 and GREAT v3.0.0
- Motif enrichment analysis: HOMER v4.6
- Graphical representation: R package ggplot2 (version 3.3.1) for scatterplots and boxplots; Deeptools ("computeMatrix" and "plotHeatmap", version 2.5.7) for metaplots and signal heatmaps; UCSC toolkit ("bedGraphToBigWig") for bigWig files generation.

Analysis of RNA-seq with ERCC spike-in mixes:
- sequencing tags alignment: STAR with default parameters (human reference genome, assembly GRCh37.75/hg19 concatenated with ERCC sequences)
- count of ERCC spike-in reads: Samtools idxstat
- matrix of mapped fragments per RefSeq annotated gene: FeatureCounts
- normalization of RefSeq read counts with ERCC spike-in transcripts: RUVg R package

Analysis of 5hmC-Seal data:
- sequencing tags alignment: Bowtie v1.1.1 (human reference genome, assembly NCBI37/hg19)
- FPKM quantification over RefSeq: FeatureCounts
- metaplots of 5hmC enrichment over ChIP-seq peaks: Deeptools
Gene interaction network: STRING v11 (Cytoscape v3.6 for network visualization)

Mass spec data analysis: EpiProfile v2.0

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

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- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
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ChIP-seq and RNA-seq data are available through the NCBI Gene Expression Omnibus (GEO) repository under accession number GSE130746. Part of the input libraries were previously generated and are available under GSE153875. Mass spec data are available through the repository Chorus under accession number 1684.

Field-specific reporting

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Life sciences study design

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Sample size
For epigenomic and transcriptomic analyses sample size was determined by the availability of brain samples (CNDR brain bank at UPenn) that matched the selection criteria (brain region, age, gender, Braak and CERAD stages, level of neuronal loss, comorbidity and postmortem interval). Sample size is comparable to published studies from postmortem brain tissue (Nativio et al, Nature Neuroscience, 2018; Moreno-Jimenez et al, Nature Medicine, 2019).

For Drosophila experiments, sample size was based on previously published work (Berson et al. Current Biology, 2017). n=20 animals for all external eye experiments. n=4-6 animals for all internal eye experiments.

Data exclusions
No data were excluded from the analyses

Replication
Transcriptomic and epigenomic data were compared to published AD studies from different brain banks. Transcriptomic data were compared to AD RNA-seq studies from the Mayo Clinic (Allen et al, Scientific Data, 2016) and Mount Sinai (Wang et al, Scientific Data, 2018) brain banks. Epigenomic data were compared to published epigenomic studies of AD from entorhinal (Marzi et al, Nature Neuroscience, 2018) and frontolateral cortex (Klein et al, Nature Neuroscience, 2019).

For Drosophila eye imaging, retinal depth for each animal was measured by taking the average eye depth from 4 internal sections.

Randomization
Brain samples were allocated into three study groups based on age and disease: healthy Younger, healthy Old and AD affected.

For Drosophila experiments, animals were grouped by genotype. For each experiment, all animals being compared were aged and processed at the same time to prevent batch effects.

Blinding
Data collection and analyses were not performed blind to the conditions of the experiments. Samples for each epigenomic and transcriptomic experiment were processed and sequenced at the same time to prevent batch effects. For Drosophila experiment, all samples within each compared experiment were imaged at the same time. For quantification, internal eye images were all measured using the same quantification approach (ImageJ).

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.
### Materials & experimental systems

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- Animals and other organisms
- Human research participants
- Clinical data

### Methods

- Antibodies
- ChIP-seq
- Flow cytometry
- MRI-based neuroimaging

### Antibodies

- **Antibodies used**
  - For ChIP-seq:
    - H3K27ac antibody: Abcam, cat # 4729; 3μl per IP
    - H3K9ac antibody: Active Motif, cat # 39137; 5μl per IP
    - H3K122ac antibody: Abcam, cat # 33309; 3μl per IP
    - H3K4me1 antibody: Abcam, cat # 8895; 4μl per IP
  - Validation - H3K27ac antibody, ChIP grade: [https://www.abcam.com/histone-h3-acetyl-k27-antibody-chip-grade-ab4729.html?productWallTab=ShowAll](https://www.abcam.com/histone-h3-acetyl-k27-antibody-chip-grade-ab4729.html?productWallTab=ShowAll)
  - H3K9ac antibody: ChIP grade: [https://www.activemotif.com/catalog/details/39137/histone-h3-acetyl-lys9-antibody-pab](https://www.activemotif.com/catalog/details/39137/histone-h3-acetyl-lys9-antibody-pab)
  - H3K122ac antibody: [https://www.abcam.com/histone-h3-acetyl-k122-antibody-ab33309.html](https://www.abcam.com/histone-h3-acetyl-k122-antibody-ab33309.html)
  - H3K4me1 antibody, ChIP grade: [https://www.abcam.com/histone-h3-mono-methyl-k4-antibody-chip-grade-ab8895.html](https://www.abcam.com/histone-h3-mono-methyl-k4-antibody-chip-grade-ab8895.html)

### Animals and other organisms

- **Policy information about studies involving animals**: ARRIVE guidelines recommended for reporting animal research

  - **Laboratory animals**
    - Genotype, gender, and temperature for the fly experiments are reported below and in Supplementary Table 14:
      - fly gmr-GAL4/+;UAS-mCD8::GFP/+; Female; 1-3d; 26ºC
      - fly gmr-GAL4,UAS-Aβ42/+;UAS-mCD8::GFP/+; Female; 1-3d; 26ºC
      - fly gmr-GAL4,UAS-Aβ42/UAS-H3.3WT-FLAG-HA; Female; 1-3d; 26ºC
      - fly gmr-GAL4,UAS-Aβ42/UAS-H3.3K27M-FLAG-HA; Female; 1-3d; 26ºC
      - fly gmr-GAL4,UAS-Aβ42/UAS-H3.3K9M-FLAG-HA; Female; 1-3d; 26ºC
      - fly gmr-GAL4,UAS-Aβ42/UAS-H3.3K27Q-FLAG-HA; Female; 1-3d; 26ºC
  - **Wild animals**: No wild animals were used in the study.
  - **Field-collected samples**: No field-collected samples were used in the study.
  - **Ethics oversight**: No ethical approval was required for Drosophila experiments.

  Note that full information on the approval of the study protocol must also be provided in the manuscript.

### Human research participants

- **Policy information about studies involving human research participants**

  - **Population characteristics**: Postmortem brain samples were categorized accordingly to age, gender, disease stage and neuronal loss. Supplementary Table 1 reports detailed patient information.

  - **Recruitment**: Postmortem human brain samples were obtained from the Center for Neurodegenerative Disease Research (CNDR) brain bank at the University of Pennsylvania (Penn). Informed consent for autopsy was obtained for all patients and it was approved by the Penn Institutional Review Board (Penn IRB). The CNDR autopsy brain bank protocols were exempted from full human research (research on tissue derived from an autopsy is not considered human research – see https://humansubjects.nih.gov/human-specimens-cell-lines-data). A detailed description of the brain bank standard operating procedures has been reviewed elsewhere (Toledo et al, Alzheimer’s & Dementia, 2014).

  - **Ethics oversight**: Identify the organization(s) that approved the study protocol.

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## ChIP-seq

### Data deposition

- Confirm that both raw and final processed data have been deposited in a public database such as [GEO](https://www.ncbi.nlm.nih.gov/geo).
- Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

| Data access links | Access link to GEO: [https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE153875](https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE153875) |
|-------------------|----------------------------------------------------------------------------------------------------------------------------------|
| Files in database submission | H3K27ac, H3K9ac, H3K122ac and H3K4me1 ChIP-seq data from Younger, Old and AD samples: fastq (on SRA), BED and bigWig files  
Input data from Younger, Old and AD: fastq files on SRA  
RNA-seq data with ERCC spike-in controls from Younger, Old and AD: fastq files on SRA |
| Genome browser session (e.g. UCSC) | [https://genome.ucsc.edu/cgi-bin/hgTracks?db=hg19&lastVirtModeType=default&lastVirtModeExtraState=&virtModeType=default&virtMode=0&nonVirtPosition=&position=chr6%3A5777270%2D6058501&hgsid=869046015_CHffqqpqO632fryWP9j20CKcGZy](https://genome.ucsc.edu/cgi-bin/hgTracks?db=hg19&lastVirtModeType=default&lastVirtModeExtraState=&virtModeType=default&virtMode=0&nonVirtPosition=&position=chr6%3A5777270%2D6058501&hgsid=869046015_CHffqqpqO632fryWP9j20CKcGZy) |

### Methodology

#### Replicates
For ChIP-seq: Younger (N=9); Old (N=10); AD (N=12) brain samples from lateral temporal lobe

#### Sequencing depth
In average 20 million reads were sequenced per ChIP-seq sample (Supplementary Table 6)

#### Antibodies
For ChIP-seq:
- H3K27ac antibody: Abcam; cat # 4729; lot # GR312658-1
- H3K9ac antibody: Active Motif; cat # 39137; lot # 09811002
- H3K122ac antibody: Abcam; cat # 33309; lot # GR284790-2
- H3K4me1 antibody: Abcam, cat # 8895; lot # GR3206758-1

#### Peak calling parameters
Peaks for H3K27ac, H3K9ac, H3K122ac and H3K4me1 ChIP-seq pooled tracks (based on study group - Younger, Older and AD) were called using MACS2 broad parameters with FDR < 1x10^-2.

#### Data quality
For Data quality we report sequencing alignment statistics in Supplementary Table 6. Average ChIP-seq uniquely aligned tags is 83%. Reads mapped to mitochondria or ENCODE blacklist regions were removed from the analysis. There were no statistically significant differences in sequencing coverage across the three study groups (H3K9ac P = 0.3; H3K27ac P = 0.84; H3K122ac P = 0.82; H3K4me1 P = 0.94; 1-way ANOVA). Only peaks detected in at least one sample were used for downstream analysis.

#### Software
Code developed for the ChIP-seq analyses is available at [https://github.com/yeminlan/ADEpigenetics](https://github.com/yeminlan/ADEpigenetics)

Analysis of ChIP-seq data:
- sequencing tags alignment: Bowtie v1.1.1 (human reference genome, assembly NCBI37/hg19)  
- peak calling: MACS2  
- comparison of peak enrichment across study groups (Younger, Old and AD): MTL method (Chen et al. 2008)  
- Graphical representation: R package ggplot2 (version 3.3.1) for scatterplots and boxplots; Deeptools (“computeMatrix” and “plotHeatmap”, version 2.5.7) for metaplots and signal heatmaps; UCSC toolkit (“bedGraphToBigWig”) for bigWig files generation.