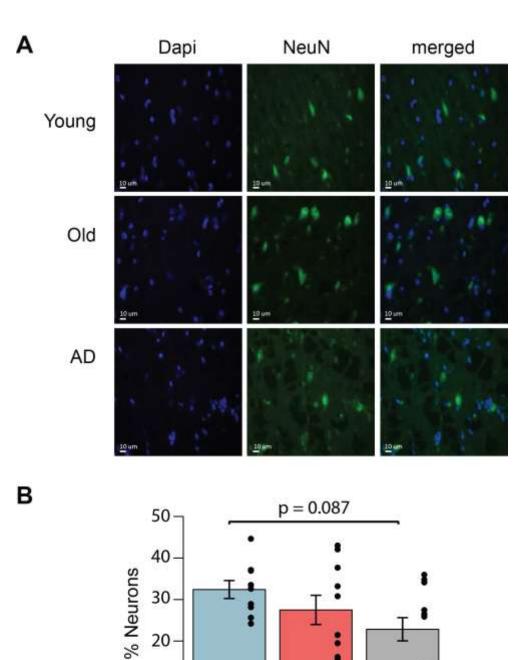
In the format provided by the authors and unedited.

# Dysregulation of the epigenetic landscape of normal aging in Alzheimer's disease

Raffaella Nativio<sup>1</sup>, Greg Donahue<sup>1</sup>, Amit Berson<sup>2</sup>, Yemin Lan<sup>1</sup>, Alexandre Amlie-Wolf<sup>1</sup>, Ferit Tuzer<sup>4</sup>, Jon B. Toledo<sup>1</sup>, Sager J. Gosai<sup>2</sup>, Brian D. Gregory<sup>2</sup>, Claudio Torres<sup>4</sup>, John Q. Trojanowski<sup>3</sup>, Li-San Wang<sup>3</sup>, F. Brad Johnson<sup>3\*</sup>, Nancy M. Bonini<sup>1</sup>, and Shelley L. Berger<sup>1</sup>, <sup>1\*</sup>

<sup>1</sup>Epigenetics Program, Department of Cell and Developmental Biology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA. <sup>2</sup>Department of Biology, University of Pennsylvania, Philadelphia, PA, USA. <sup>3</sup>Department of Pathology & Laboratory Medicine, University of Pennsylvania, Philadelphia, PA, USA. <sup>4</sup>Department of Pathology, Drexel University College of Medicine, Philadelphia, PA, USA. \*e-mail: johnsonb@pennmedicine.upenn. edu; nbonini@sas.upenn.edu; bergers@pennmedicine.upenn.edu



Quantification of neurons in temporal cortex of Young, Old and AD subjects.

Young

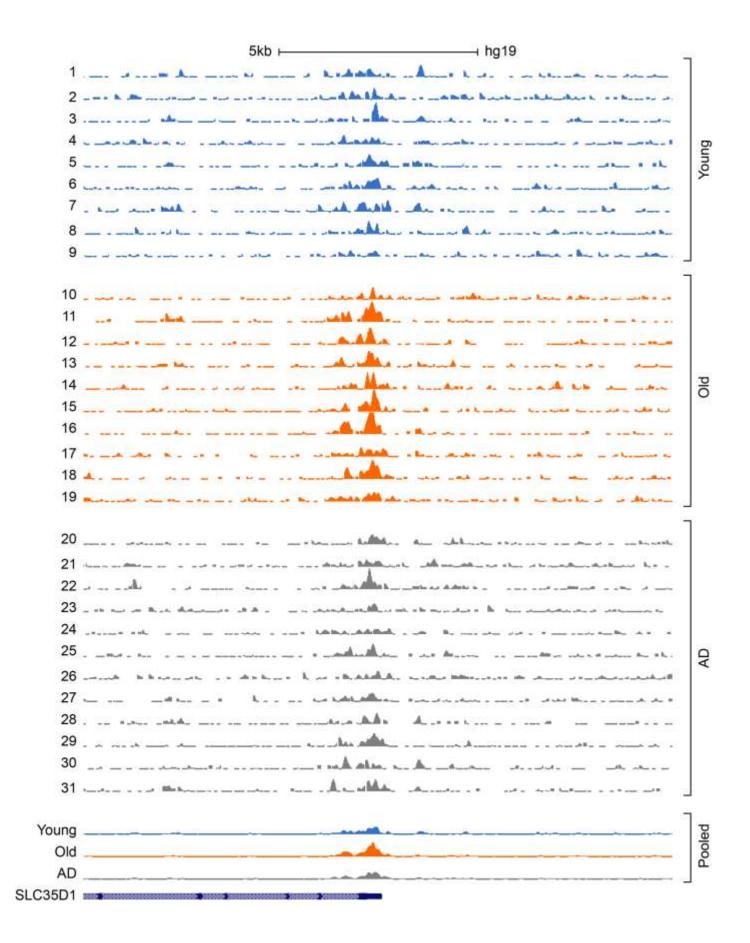
10

0

(A) Representative IF images showing total nuclei (DAPI-blue, left column), NeuN positive nuclei (green, central column) and merged images (right column) for Young (top row), Old (middle row), AD (bottom row). (B) Barplot (with data points) representing mean  $\pm$  SEM of neuron percentages quantified by IF (n° Young = 9; n° Old = 10; n° AD = 12). Sample size (n°) represents individual brain samples. Differences are not significant (*P*=0.087, 1-way ANOVA; F (2,28)=2.669).

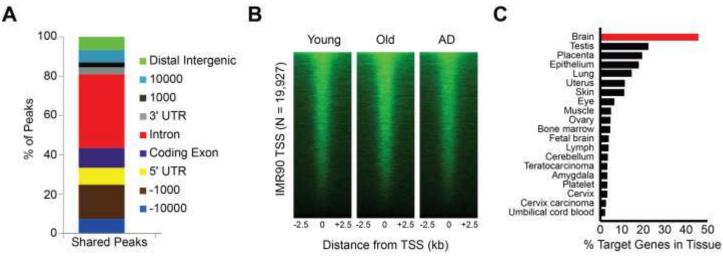
AD

Old



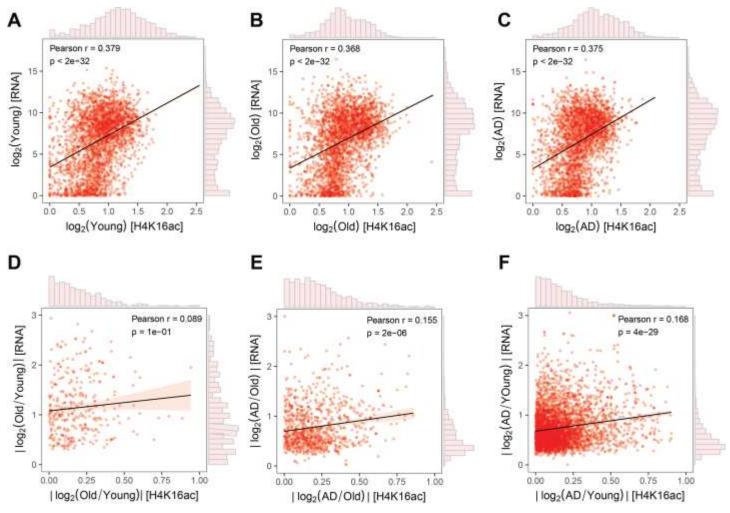
H4K16ac enrichment in individual and pooled samples.

UCSC Genome browser track views of H4K16ac enrichment at the promoter of *SLC35D1* gene in individual Young (blue), Old (orange), AD (gray) samples and corresponding pooled samples. The numbers on the right of each track refer to the sample ID as reported in Table S1.



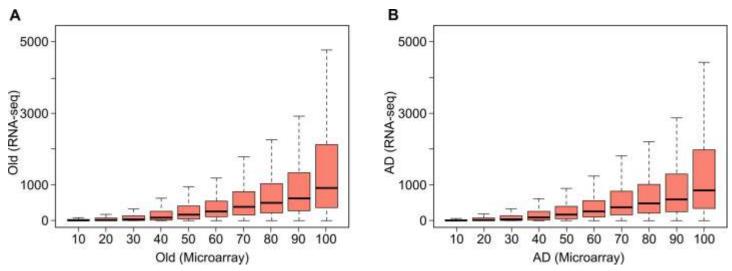
Comparison between H4K16ac peaks detected in the brain and other cell types.

(A) Genomic compartment analysis showing % of H4K16ac shared peaks (in common between Young, Old and AD) is similar to Taylor et al. [20] in mouse ESC and NPC cells. (B) Heatmap showing H4K16ac enrichment in Young, Old and AD over previously mapped H4K16ac peaks in human IMR90 cells [15]. Peaks are sorted in decreasing order of Young brain enrichment. (C). Barplot showing % of tissue-specific expressed genes targeted by H4K16ac (top 10% of Young peaks and up to 1 kb from TSS) for H4K16ac shared peaks (UniProt tissue expression database, DAVID). Tissue terms were filtered by gene count > 200 and FDR < 1%.



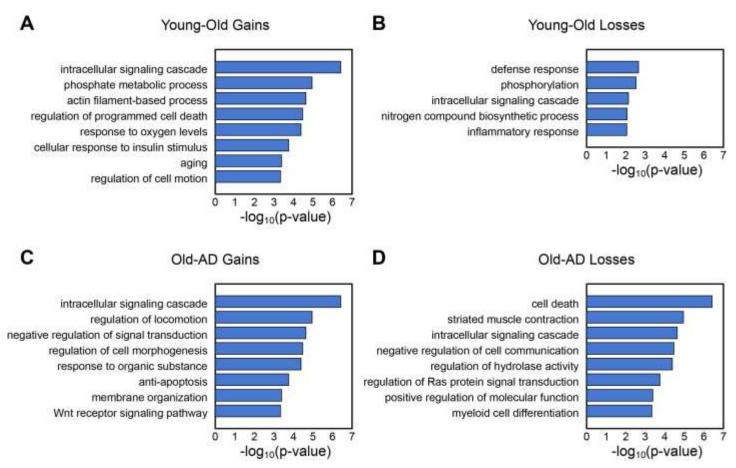
Correlation between H4K16ac and gene expression in Young, Old and AD subjects.

(A-C) Scatter plot of H4K16ac peak enrichment ( $\log_2 AUC + 1$ ) vs gene expression ( $\log_2 DESeq$ -score +1) of genes expressed in (A) Young, (B) Old and (C) AD considering the closest peak from the TSS. For graphical representation, 3000 randomly chosen points are shown in each panel. (D-F) Absolute H4K16ac fold-changes (closest peak from the TSS) vs absolute gene expression changes for the significantly (P < 0.05, FDR< 0.05) differentially expressed genes for the comparisons (D) Young to Old, (E) Old to AD and (F) Young to AD. Linear regression trendlines and Pearson correlation coefficients are indicated.



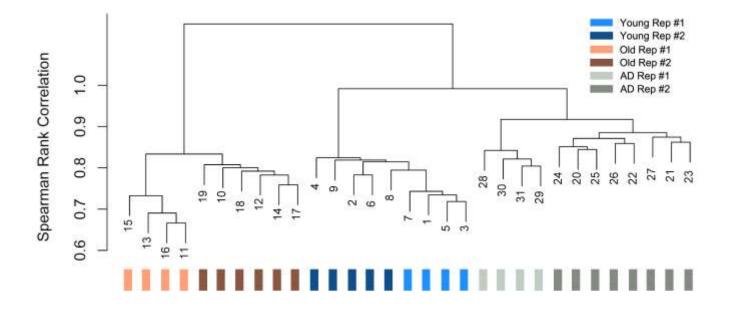
Comparison between RNA-seq and published microarray data (Blalock et al. 2011) in Old and AD subjects.

Boxplot showing RNA-seq gene expression (DEseq-scores) in Old (A) or AD (B) for genes identified in a published AD microarray study [21] from human hippocampus. All expressed genes from the published microarray data (control Old and AD) were sorted into ten groups of increasing expression and compared to Old (A) or AD (B) in our RNA-seq data.



GO analysis of genes with H4K16ac changes in aging and AD subjects.

(A-D) Bar plot for top eight most significantly enriched GO terms (BP; DAVID) for genes with significant (P < 0.05, Welch's t-test, twosided) H4K16ac (A) gains or (B) losses during aging (Young-Old comparison) and H4K16ac (C) gains or (D) losses in AD (Old-AD comparison). Reported GO terms contain > 20 genes and FDR < 10%.



Correlation across patients at the three classes of H4K16ac changes.

Dendrogram of Spearman Rank Correlation across patients (rep1 and rep2) for the three classes of peaks (Age-regulate, Agedysregulated and Disease-specific; P < 0.05, 1-way Anova) showing patients clustering according to study group (Young, Old and AD). The numbers for each patient refer to the sample ID as reported in Table S1.

| Adipose_Subcutaneous -<br>Adipose_Visceral_Omentum -<br>Adrenal_Gland -<br>Artery_Aorta -<br>Artery_Coronary -<br>Artery_Tibial - | 0.6731<br>1.0000<br>1.0000 | 0.2376<br>1.0000 | 1.0000<br>1.0000 |
|---|----------------------------|------------------|------------------|
| Adipose_Visceral_Omentum -<br>Adrenal_Gland -<br>Artery_Aorta -<br>Artery_Coronary -  | 1.0000<br>1.0000           | 1.0000           |                  |
| Artery_Aorta -<br>Artery_Coronary -   | 1.0000                     |                  |                  |
| Artery_Coronary -   | V                          | 1.0000           | 1.0000           |
|   | 1.0000                     | 0.8711           | 1.0000           |
| Artery Tibial   | 1.0000                     | 1.0000           | 1.0000           |
| Artery_riviar 7   | 0.7787                     | 0.1320           | 1.0000           |
| Brain_Anterior_cingulate_cortex_BA24 -  | 1.0000                     | 1.0000           | 1.0000           |
| Brain_Caudate_basal_ganglia -   | 1.0000                     | 1.0000           | 1.0000           |
| Brain_Cerebellar_Hemisphere   | 1.0000                     | 1.0000           | 1.0000           |
| Brain_Cerebellum  | 1.0000                     | 1.0000           | 1.0000           |
| Brain_Cortex -  | 1.0000                     | 1.0000           | 1.0000           |
| Brain_Frontal_Cortex_BA9  | 1.0000                     | 1.0000           | 1.0000           |
| Brain_Hippocampus -   | 1.0000                     | 1.0000           | 1.0000           |
| Brain Hypothalamus  | 1.0000                     | 1.0000           | 1.0000           |
| rain_Nucleus_accumbens_basal_ganglia -  | 1.0000                     | 1,0000           | 1.0000           |
| Brain_Putamen_basal_ganglia -   | 1.0000                     | 1.0000           | 1.0000           |
| Breast_Mammary_Tissue -   | 1.0000                     | 1.0000           | 1.0000           |
| Cells_EBV-transformed_lymphocytes -   | 1.0000                     | 1.0000           | 1.0000           |
| Cells_Transformed_fibroblasts -   | 0.0132                     | 0.0132           | 0.1584           |
| Colon_Sigmoid -   | 1.0000                     | 1.0000           | 1.0000           |
| Colon Transverse  | 1.0000                     | 0.8447           | 1.0000           |
| Esophagus_Gastroesophageal_Junction -   | 1.0000                     | 1.0000           | 1.0000           |
| Esophagus_Mucosa -  | 1.0000                     | 0.3300           | 0.8315           |
| Esophagus_Muscularis -  | 1.0000                     | 0,1320           | 1.0000           |
| Heart_Atrial_Appendage -  | 1.0000                     | 1.0000           | 1.0000           |
| Heart Left Ventricle -  | 1.0000                     | 0.7259           | 1.0000           |
| Liver -   | 1.0000                     | 1.0000           | 1.0000           |
| Lung -  | 1.0000                     | 0.3564           | 1.0000           |
| Muscle_Skeletal -   | 1.0000                     | 0.1716           | 0.8975           |
| Nerve_Tibial -  | 0.7787                     | 0.0528           | 0.8183           |
| Ovary -   | 1.0000                     | 1.0000           | 1.0000           |
| Pancreas -  | 1.0000                     | 0.2244           | 1.0000           |
| Pituitary -   | 1.0000                     | 1.0000           | 1.0000           |
| Prostate -  | 1.0000                     | 1.0000           | 1.0000           |
| Skin Not Sun Exposed Suprapubic   | 1.0000                     | 1.0000           | 1.0000           |
| Skin_Sun_Exposed_Lower_leg -  | 1.0000                     | 0.1320           | 0.7787           |
| Small_Intestine_Terminal_Ileum -  | 1.0000                     | 1.0000           | 1.0000           |
| Spleen -  | 1.0000                     | 1.0000           | 1.0000           |
| Stomach -   | 1.0000                     | 1,0000           | 1.0000           |
| Testis -  | 1.0000                     |                  |                  |
| Thyroid -   |                            | 0.1980           | 1.0000           |
| Uterus -  | 0.5939                     | 0.0132           | 0.3696           |
| Vagina -  | 1.0000                     |                  | 1.0000           |
| Whole_Blood -   | 1.0000<br>1.0000           | 1.0000           | 1.0000           |

# Heatmap of Bonferroni adjusted p-values

1.00

p-value

0.05

# **Supplementary Figure 8**

H4K16ac overlap analysis with tissue eQTLs from the GTEx project.

Heatmap of Bonferroni adjusted p-values for sampling-based analysis of H4K16ac overlap (the three classes of changes) with tissue eQTLs from the GTEx project.