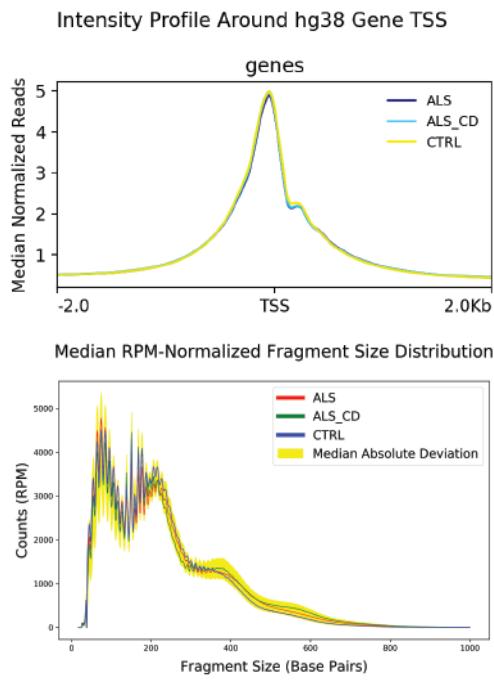
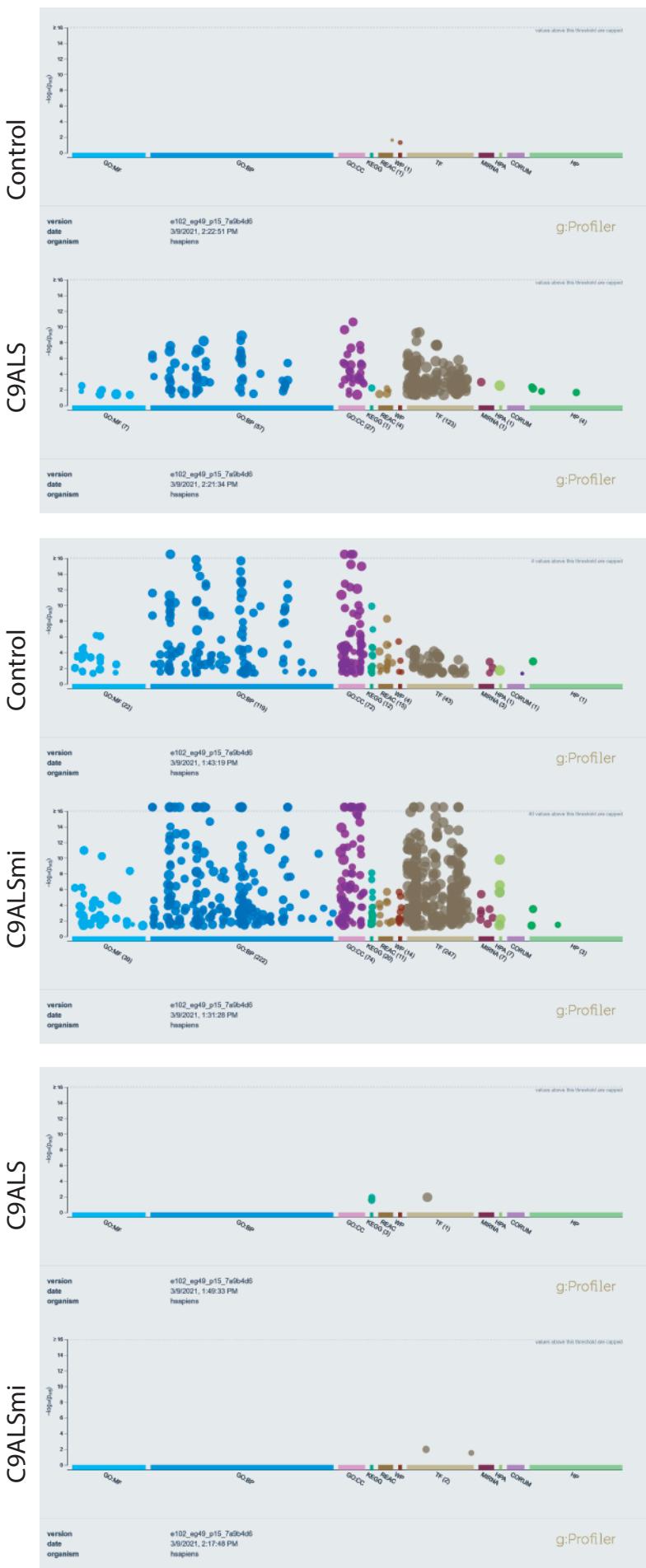


FigureXX: Comparison of open chromatin regions show more differential hits in C9ALSmi compared to control, than C9ALS compared to control.

a

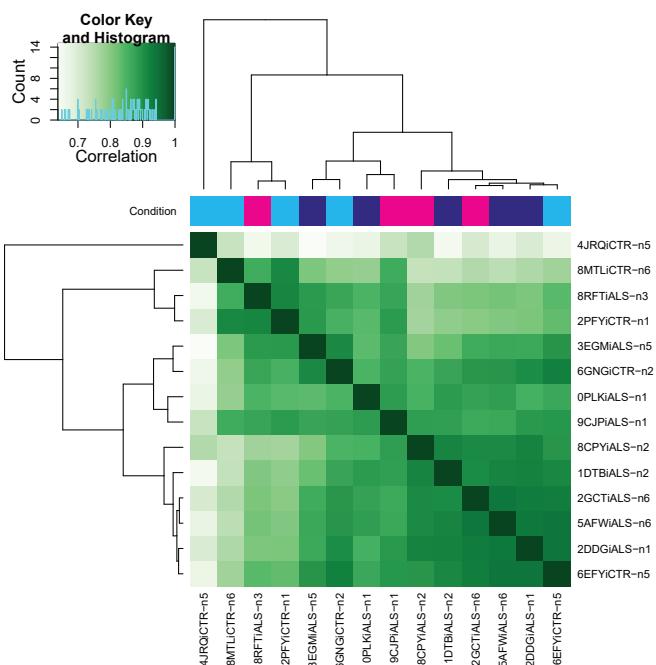
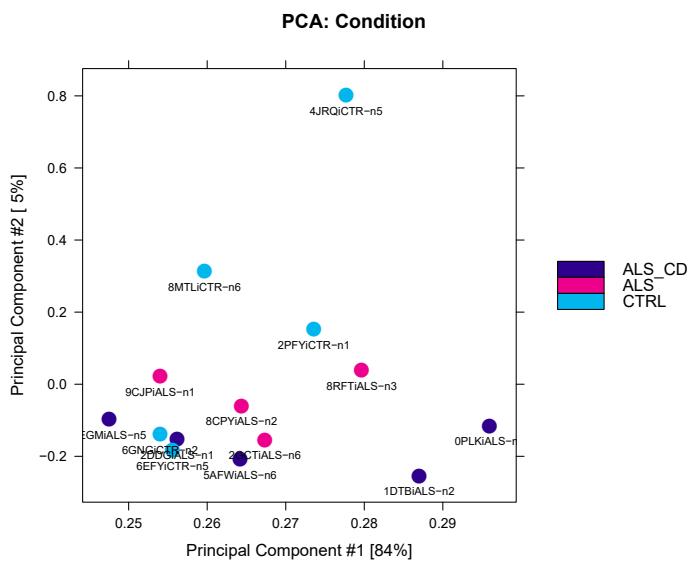


c

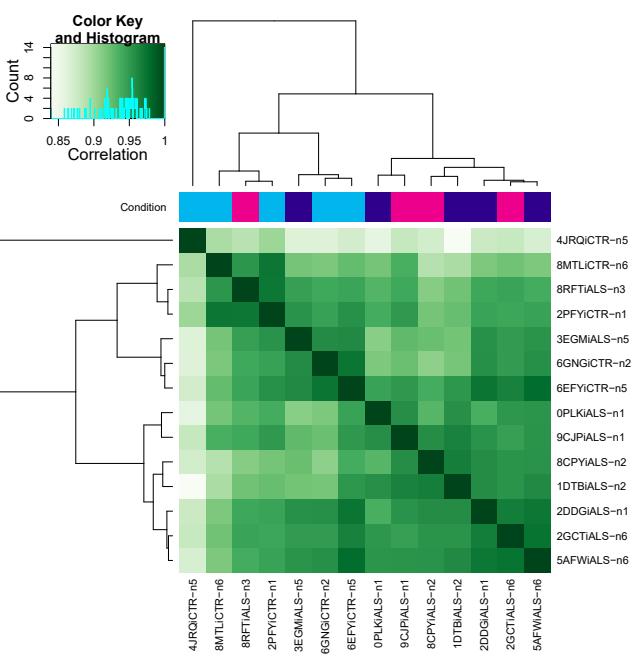
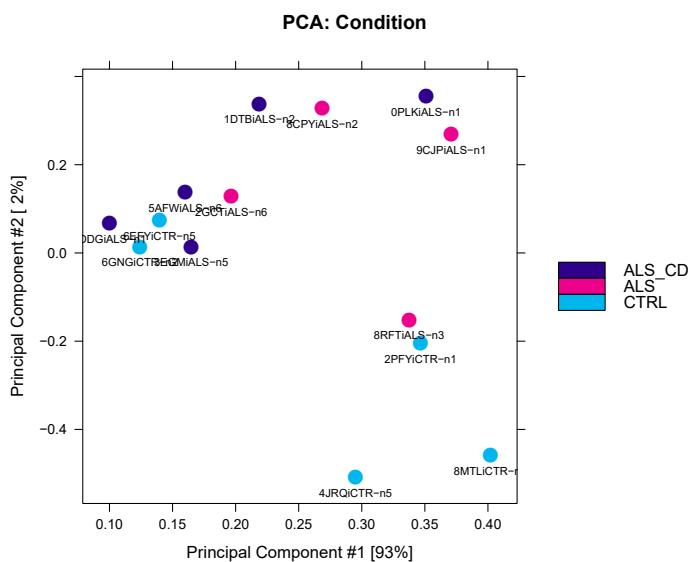


Sup Fig _: Quality control metrics from ATACseq data

a. Counts PCA and Heatmap



b. Occupancy PCA and Heatmap



ATAC-Seq Sample Preparation

ATAC-Seq was carried out as described¹. Briefly, cells were lysed in cell lysis buffer (10 mM Tris-HCl, pH 7.4, 10 mM NaCl, 3 mM MgCl₂, 0.1% IGEPAL CA-630, protease inhibitors) on ice for 5 min and centrifuged at 230 rcf for 5 min at 4 °C. The pellet, containing the nuclei, was re-suspended in 25 ml of 1X Tagment DNA Buffer (Illumina). 50K nuclei were subjected to transposase reaction (Nextera - Illumina) followed by DNA purification. The tagmented DNA was PCR amplified using Nextera indexing primers (Illumina). Nucleosome-free fragments (175-250 bp) were size selected using AMPure XP magnetic beads (Beckman Coulter) and further amplified by PCR to obtain the final libraries. The libraries were sequenced using the Illumina NextSeq platform (paired end, 75nt). All samples passed quality control checks that included morphological evaluation of nuclei, assessment of size distribution via Agilent Bioanalyzer, and real-time qPCR to assess the enrichment of open-chromatin sites.

ATAC-Seq Bioinformatics Analysis

Read trimming, alignment to genome using trimmed reads, calling of peaks, and reporting of single-sample QC metrics were all completed with The Encyclopedia of DNA Elements (ENCODE)² Data Coordination Center's ATAC-Seq pipeline (version 1.7.1)³. Raw FASTQ reads were trimmed with cutadapt⁴ (version 2.5). Trimmed reads were aligned to the GRCh38 genome with Bowtie2⁵ (version 2.3.4.3). PCR duplicate reads were marked with Picard⁶ MarkDuplicates (version 2.20.7) and removed. Peaks were called with macs2⁷ (version 2.2.4). Differential analysis was conducted with DiffBind⁸ (version 2.10.0) using the DESEQ2 method⁹ and False Discovery Rate (FDR)¹⁰ correction with a threshold of less than 0.1.

¹Milani, P, et al. Cell freezing protocol suitable for ATAC-Seq on motor neurons derived from human induced pluripotent stem cells. Sci Rep 6, (2016). <https://doi.org/10.1038/srep25474>

²The ENCODE Project Consortium., Overall coordination (data analysis coordination)., Dunham, I. et al. An integrated encyclopedia of DNA elements in the human genome. Nature 489, (2012). <https://doi.org/10.1038/nature11247>

³<https://github.com/ENCODE-DCC/atac-seq-pipeline/tree/v1.7.1>

⁴Martin, M. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet.journal 17, (2011). <https://doi.org/10.14806/ej.17.1.200>

⁵Langmead, B., Salzberg, S. Fast gapped-read alignment with Bowtie 2. Nat Methods 9, (2012). <https://doi.org/10.1038/nmeth.1923>

⁶<http://broadinstitute.github.io/picard/>

⁷Feng, J., Liu, T., Qin, B. et al. Identifying ChIP-seq enrichment using MACS. Nat Protoc 7, (2012). <https://doi.org/10.1038/nprot.2012.101>

⁸Ross-Innes, C., Stark, R., Teschendorff, A. et al. Differential oestrogen receptor binding is associated with clinical outcome in breast cancer. *Nature* 481, (2012).

<https://doi.org/10.1038/nature10730>

⁹Love, M.I., Huber, W. & Anders, S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol* 15, 550 (2014). <https://doi.org/10.1186/s13059-014-0550-8>

¹⁰Benjamini, Y., Hochberg, Y. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. *J. R. Statis. Soc. B* 57, (1995).