Altered DNA methylation profiles in blood from patients with sporadic Creutzfeldt-Jakob disease

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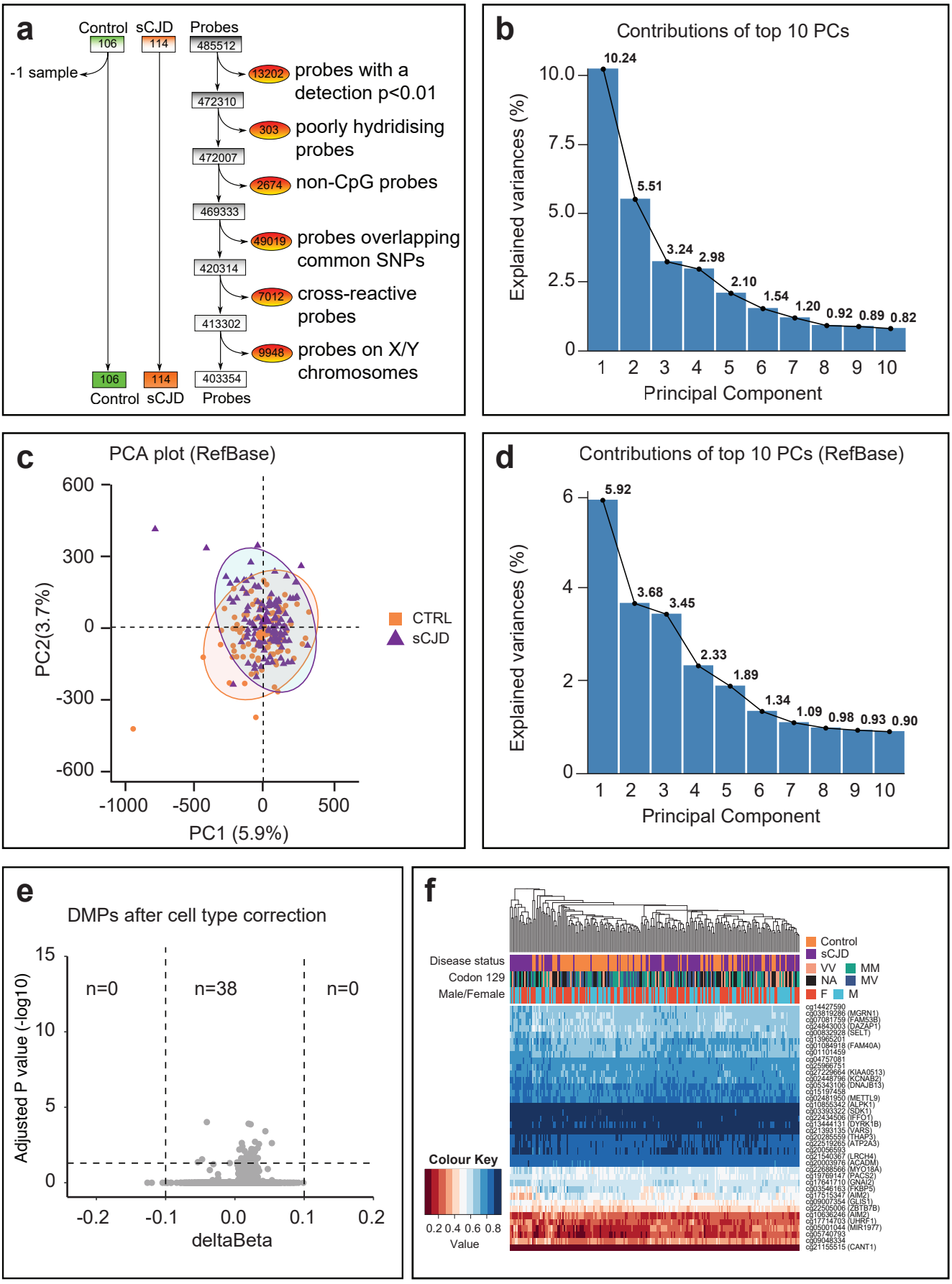
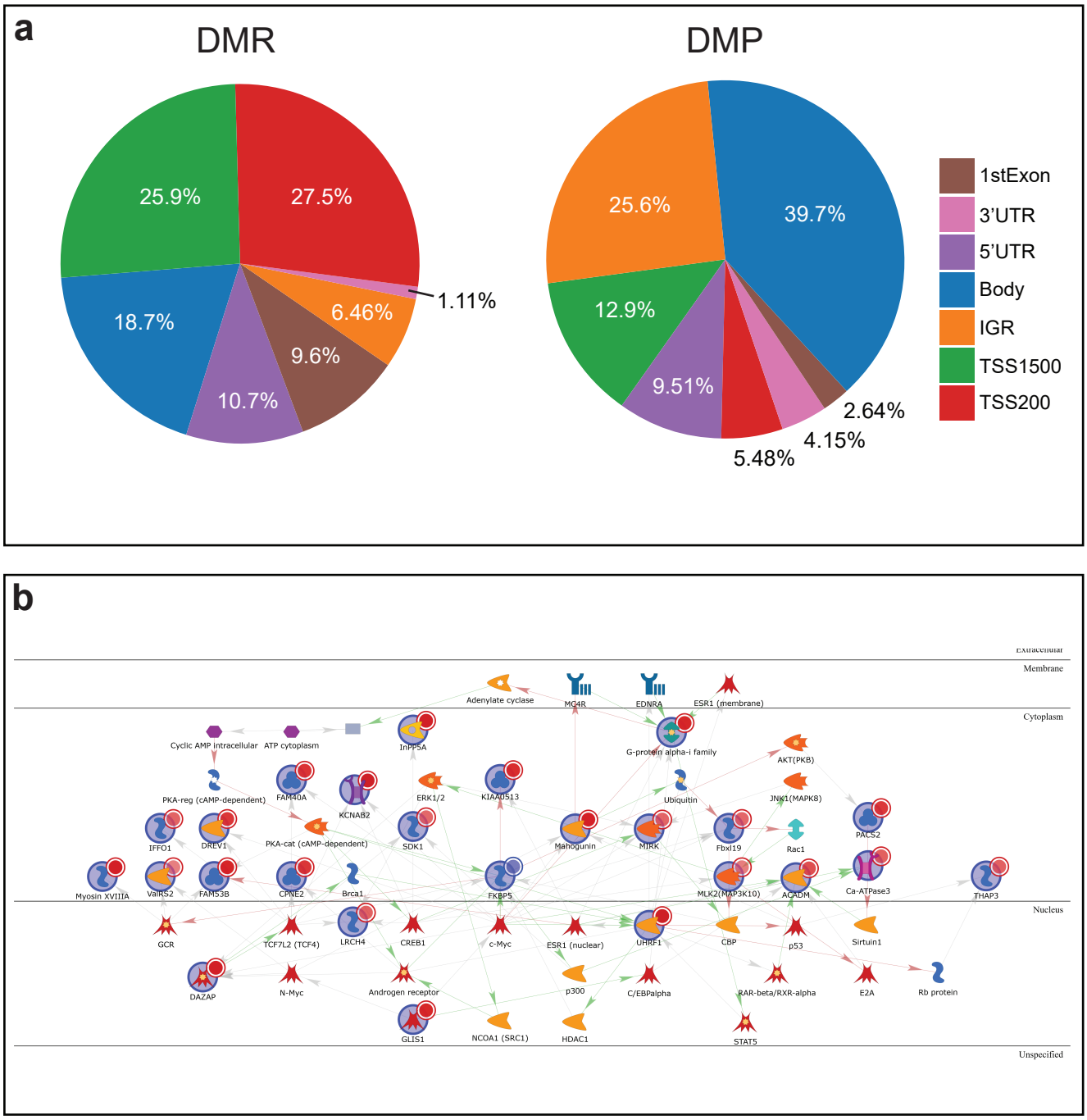

a
Control
sCJD
Probes
106
114
485512
-1 sample
13202
probes with a detection p<0.01
472310
303
poorly hydridising probes
472007
2674
non-CpG probes
469333
49019
probes overlapping common SNPs
420314
7012
cross-reactive probes
413302
9948
probes on X/Y chromosomes
403354
106
114
Control
sCJD
Probes
b
Contributions of top 10 PCs
Explained variances (%)
10.24
5.51
3.24
2.98
2.10
1.54
1.20
0.92
0.89
0.82
Principal Component
c
PCA plot (RefBase)
PC2(3.7%)
PC1 (5.9%)
CTRL
sCJD
d
Contributions of top 10 PCs (RefBase)
Explained variances (%)
5.92
3.68
3.45
2.33
1.89
1.34
1.09
0.98
0.93
0.90
Principal Component
e
DMPs after cell type correction
Adjusted P value (-log10)
n=0
n=38
n=0
deltaBeta
f
Disease status
Codon 129
Male/Female
Control
sCJD
VV
MM
NA
MV
F
M
Colour Key
Value

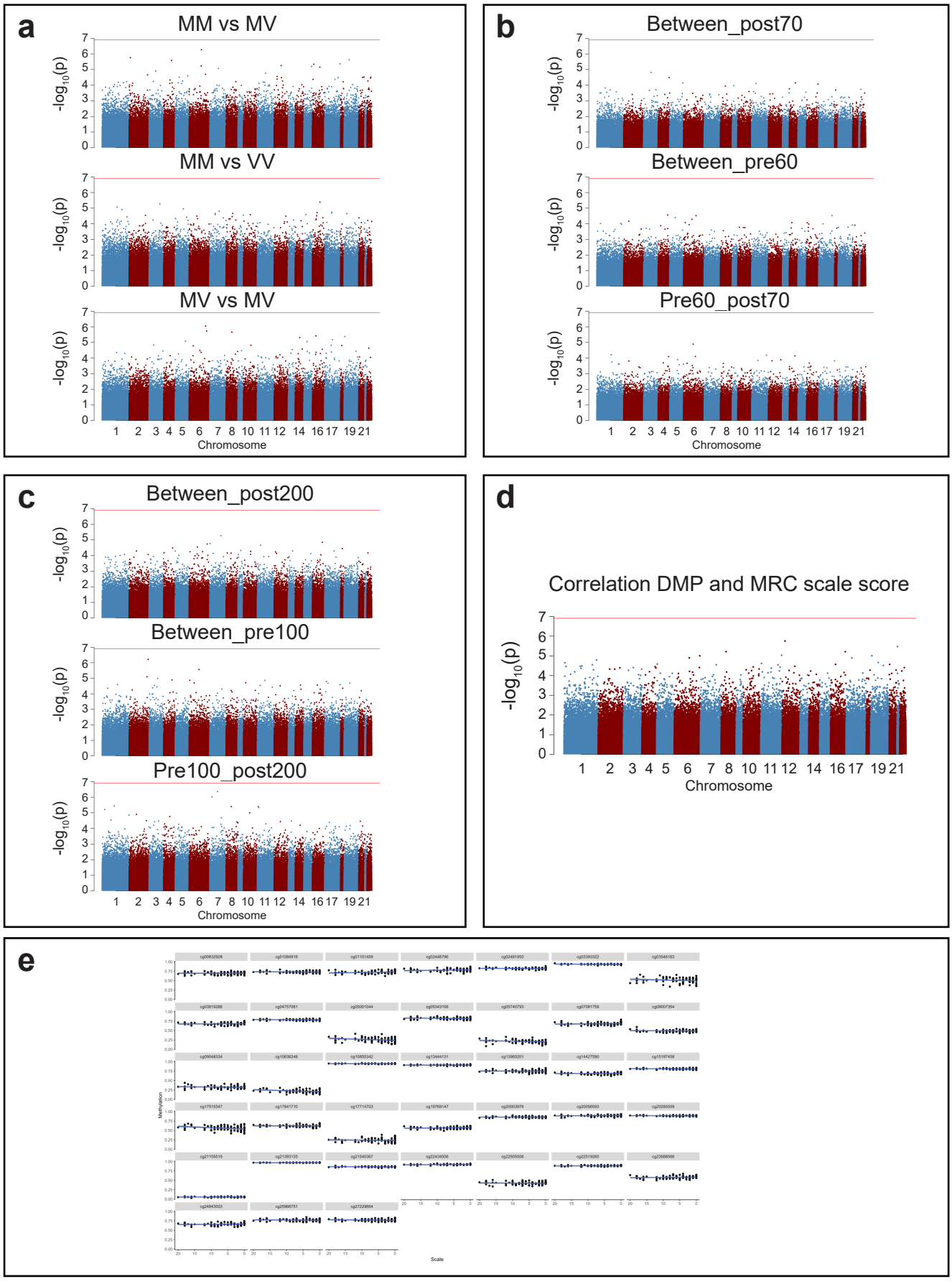
Supplementary Figure 1: Genome-wide methylation profiling in sporadic CJD blood.

(**a**) Graphical representation of quality control steps; 82158 probes and one sample were excluded after performing the champ.load() function. (**b**) Scree plot showing the percentage of total variance in the methylation profiles as represented by the top 10 most contributing PCs (cell type corrected) before cell type correction. (**c**) Principal component analysis (PCA) showing the first (PC1) and second (PC2) principal components (PC) for 219 DNA methylation profiles after cell type correction with Housemann algorithm. Control samples (orange) and sCJD cases (purple). (**d**) Scree plot showing the percentage of total variance in the methylation profiles as represented by the top 10 most contributing PCs (cell type corrected) after cell type correction with Housemann algorithm. (**e**) Volcano plot showing differences in methylation levels between sCJD and control after cell type correction. (**f**) Dendrogram showing hierarchical clustering of the 38 significant DMPs. Hierarchical clustering analysis was performed using the average clustering method based on Euclidean distance. Disease status: orange (control), purple (sCJD); codon 129 status: MM (green), MV (dark blue), VV (pink), unknown (black); sex: male (blue), female (red).

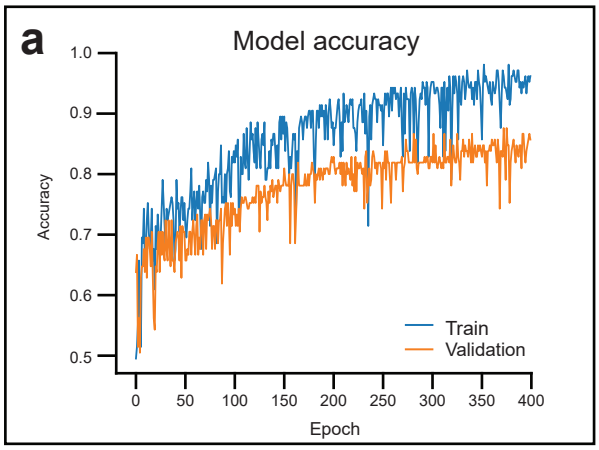


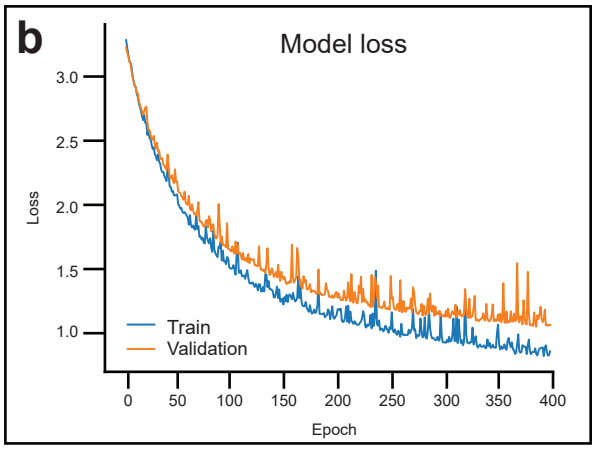
Supplementary Figure 2: Characterization of DMPs and DMRs.

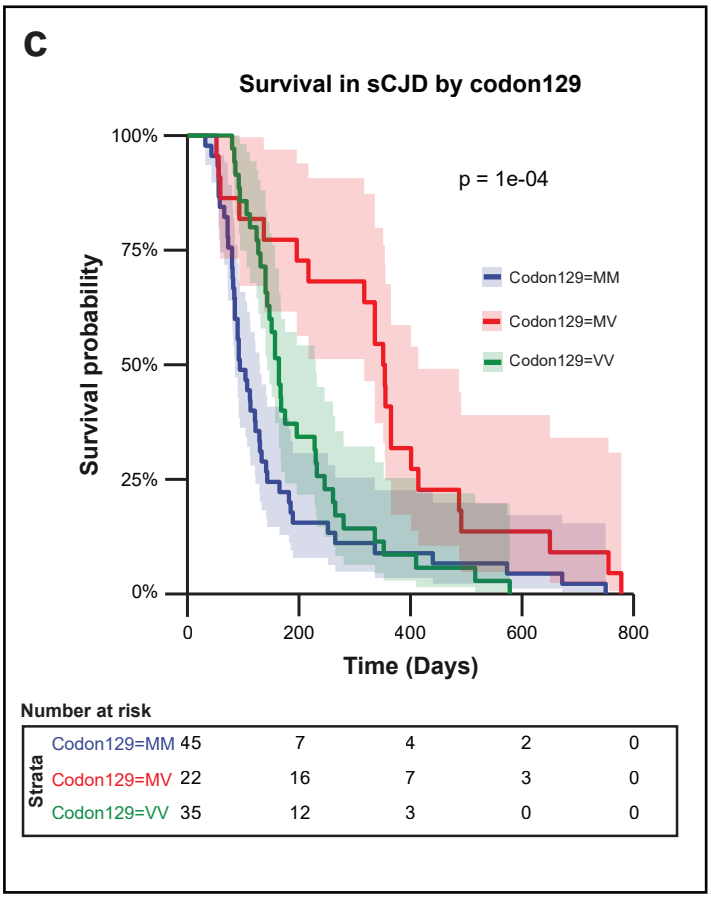
(**a**) Distribution of CpGs in the38 DMPs (right) and 67 DMRs (left). The segments are ordered according to the size of proportions. TSS 1500: located 1500 nucleotides upstream transcriptional start site; TSS 200: located 1500 nucleotides upstream transcriptional start site; Body: body of the gene; IGR: intergenic regions; 5'UTR and 3'UTR are respectively 5' and 3' untranslated regions. (**b**) Pathways affected by changes in DNA methylation in sCJD. Molecules are stratified by subcellular localisation. Green arrows represent activation, red represent inhibition, and grey arrows represent unspecific interaction. Molecules encircled in blue are from the methylation dataset. Upper-right coloured circles represent effect direction, with red circles indicating hypermethylation and blue circles representing hypomethylation in sCJD. Proteins are represented by blue molecular shapes, enzymes are represented by yellow and orange shapes, and transcription factors are represented by red shapes.


a
MM vs MV
MM vs VV
MV vs MV
b
Between_post70
Between_pre60
Pre60_post70
c
Between_post200
Between_pre100
Pre100_post200
d
Correlation DMP and MRC scale score
-log10(p)
Chromosome
1 2 3 4 5 6 7 8 10 11 12 14 16 17 19 21
e
Methylation
Scale

Supplementary Figure 3: Correlation between 38 DMPs and disease-modifying features. (**a**) Manhattan plot of probes associated with *PRNP* codon 129 polymorphism. Red line indicates significance threshold (Bonferroni-adjusted = $1.24x10^{-7}$). X-axis represents ranked chromosomes, Y-axis represents–log10 (p-value). (**b**) Manhattan plot of probes associated with age at onset. Cases are binned in three groups: onset before 60 years old; onset between 60 and 70 years old; onset after 70 years old. Red line indicates significance threshold (Bonferroni-adjusted = $1.24x10^{-7}$). X-axis represents ranked chromosomes, Y-axis represents–log10 (p-value). (**c**) Manhattan plot of probes associated with disease duration. Cases are binned in three groups: less than 100 days, longer than 200 days, or between 100 and 200 days. Red line indicates significance threshold (Bonferroni-adjusted = $1.24x10^{-7}$). X-axis represents ranked chromosomes, Y-axis represents–log10 (p-value). (**d**) Manhattan plot of probes associated with patient's score measured at the time each sample was collected. Red line indicates significance threshold (Bonferroni-adjusted = $1.24x10^{-7}$). X-axis represents ranked chromosomes, Y-axis represents–log10 (p-value). (**e**) Correlation between the patient's score measured at the time each sample was collected with the methylation values at each DMP.

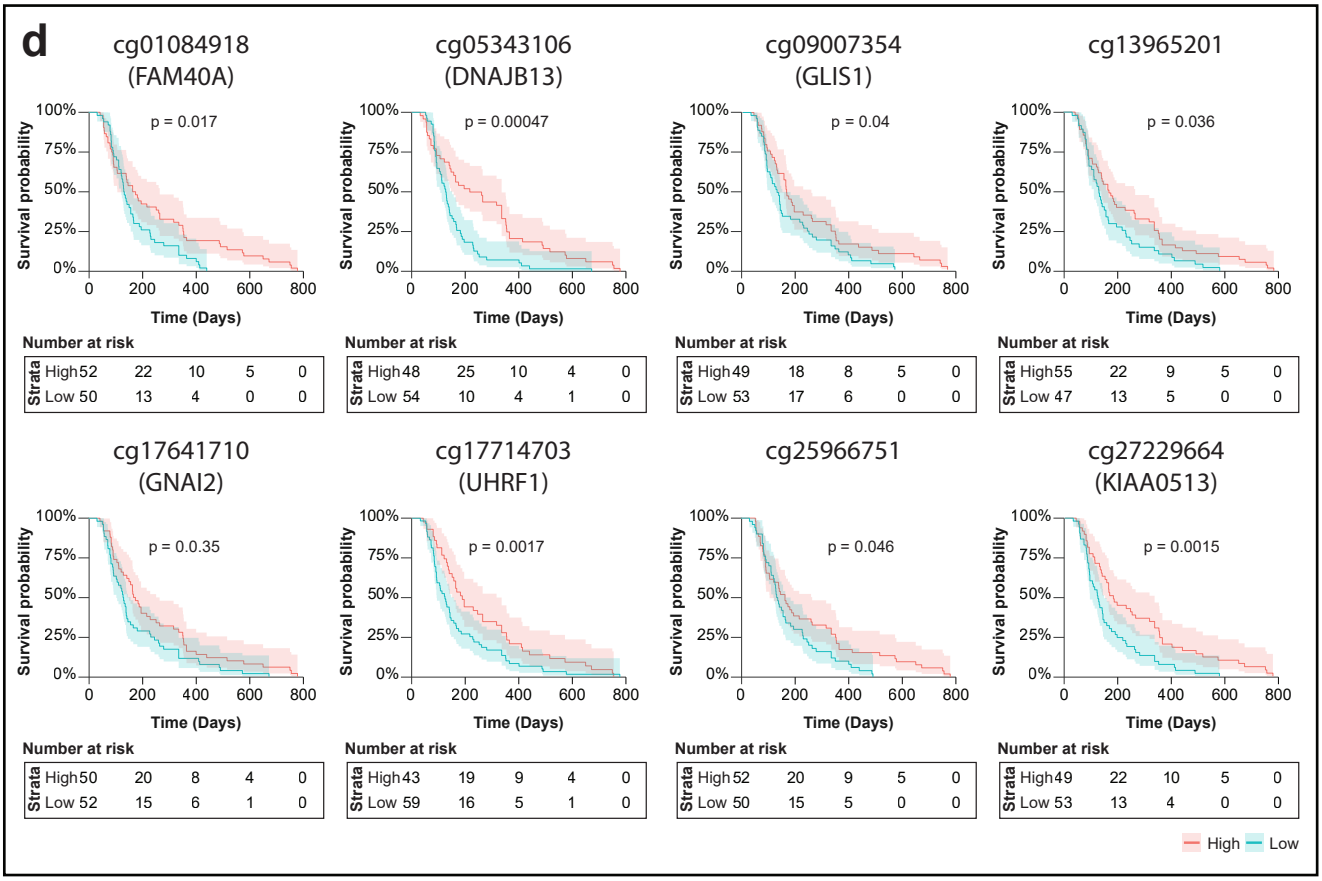






Number at risk

Strata					
Codon129=MM	45	7	4	2	0
Codon129=MV	22	16	7	3	0
Codon129=VV	35	12	3	0	0



Number at risk

Strata					
High	52	22	10	5	0
Low	50	13	4	0	0

Number at risk

Strata					
High	48	25	10	4	0
Low	54	10	4	1	0

Number at risk

Strata					
High	49	18	8	5	0
Low	53	17	6	0	0

Number at risk

Strata					
High	55	22	9	5	0
Low	47	13	5	0	0

Number at risk

Strata					
High	50	20	8	4	0
Low	52	15	6	1	0

Number at risk

Strata					
High	43	19	9	4	0
Low	59	16	5	1	0

Number at risk

Strata					
High	52	20	9	5	0
Low	50	15	5	0	0

Number at risk

Strata					
High	49	22	10	5	0
Low	53	13	4	0	0

Supplementary Figure 4: DNA methylation array profiles to refine sCJD diagnosis and disease duration.

Performance of DNA methylation-based neural network classifier in sCJD. (**a**) Model accuracy during training process over 400 epochs. (**b**) Model loss during the training process. The loss is defined as binary cross-entropy. (**c**) Kaplan-Meier survival analysis. sCJD patients were divided into 3 groups based on the genotype at PRNP codon 129 (MM, VV or MV). (**d**) Survival analysis for 8 sites. Patients were divided into high (above median; red) and low (below media; green) DNA methylation values at each DMP. *p*-values were calculated using the log-rank test.