Distinct epigenetic signatures between adult-onset and late-onset depression

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Supplementary information

Supplementary Fig. S1 Scatter plots between the array data and the pyrosequencing or the amplicon bisulfite sequencing data using the EZR software (http://www.jichi.ac.jp/saitama-sct/SaitamaHP.files/statmedEN.html).
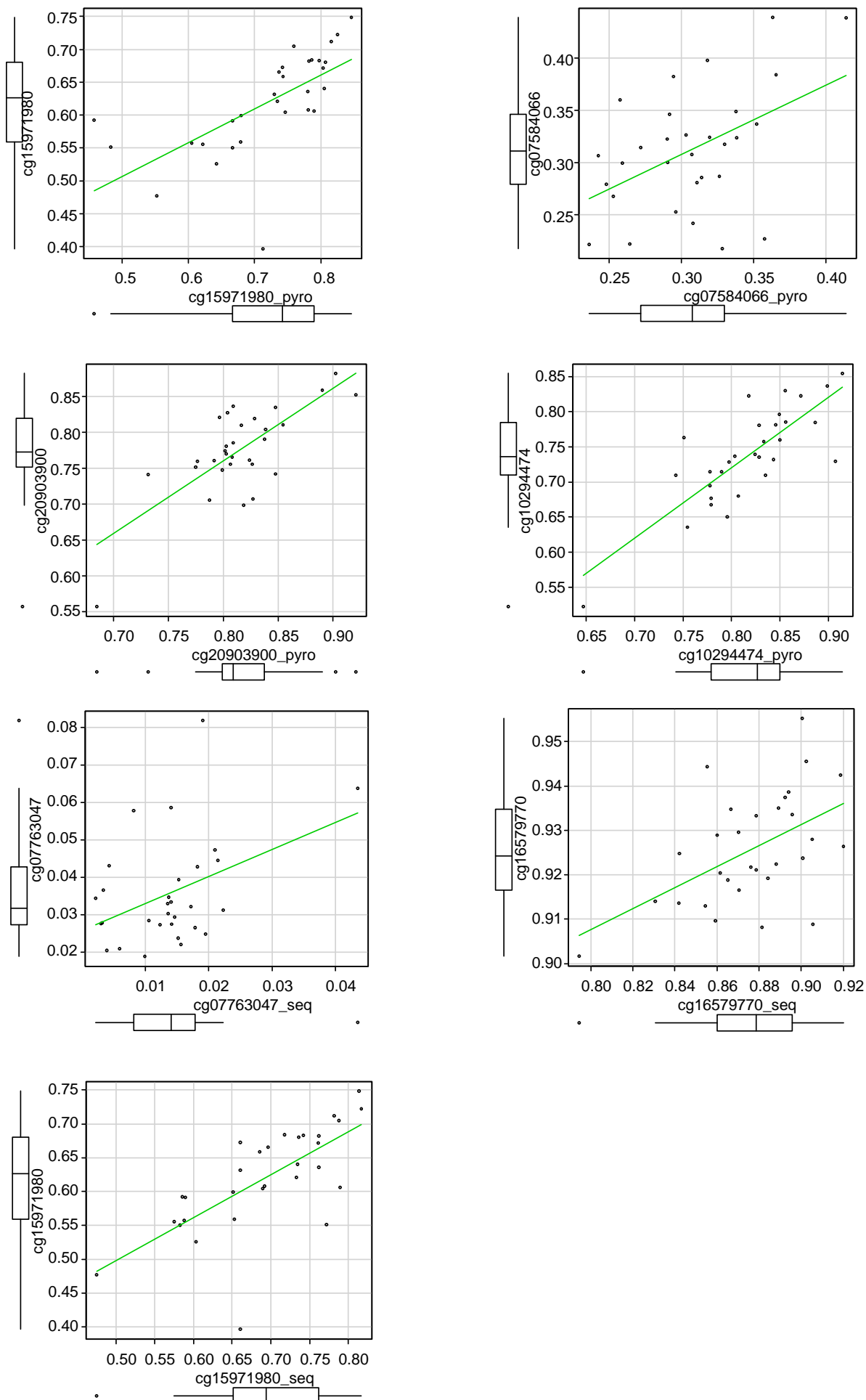
Supplementary Fig. S2 Scatter plots between age and the methylation levels of each site.
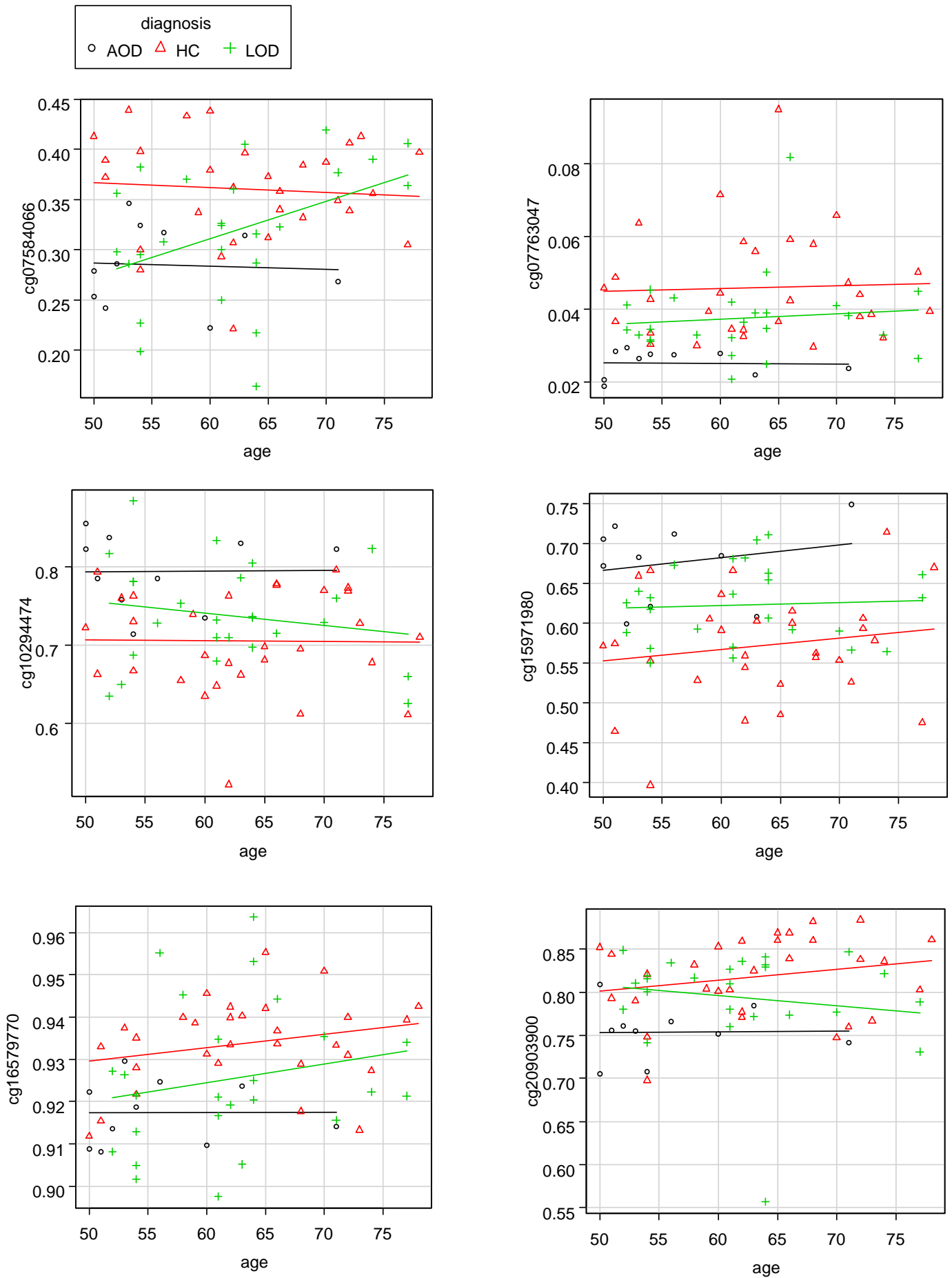
Supplementary Fig. S3 Scatter plots between age at onset and the methylation levels of each site.
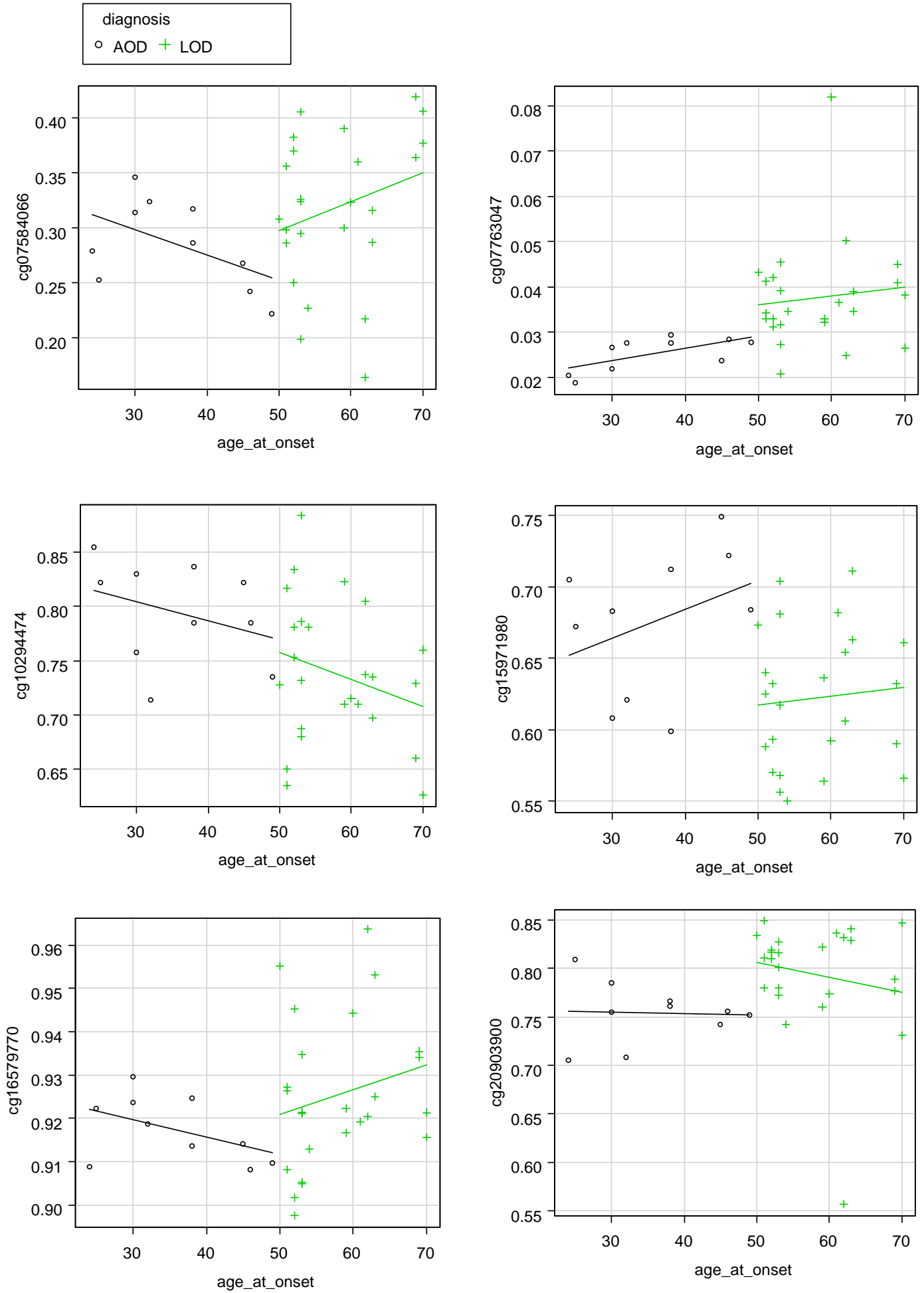
Supplementary Fig. S4 Scatter plots between number of episodes and the methylation levels of each site.
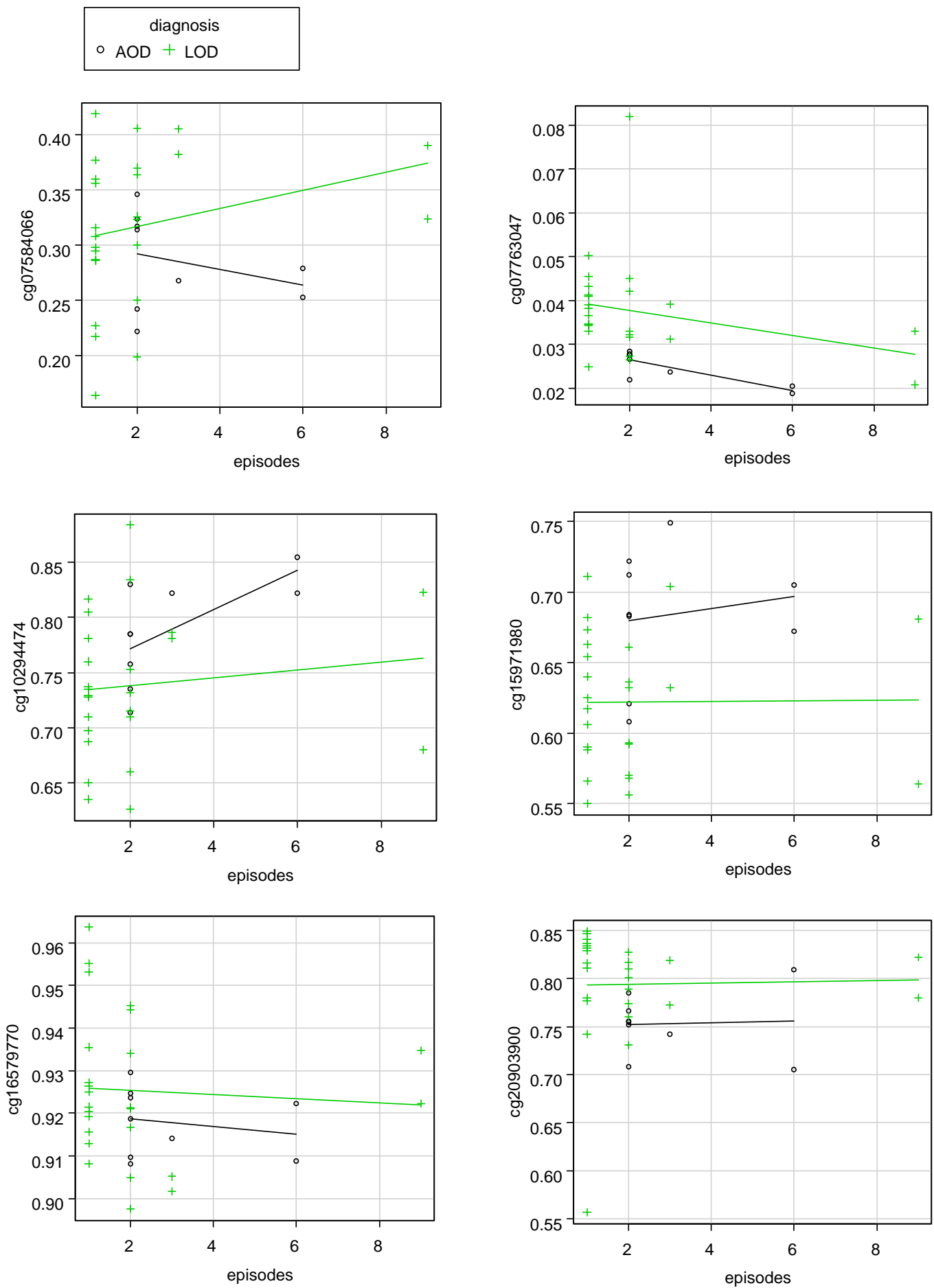
Supplementary Fig. S5 Scatter plots between WBCs and the methylation levels of each site.
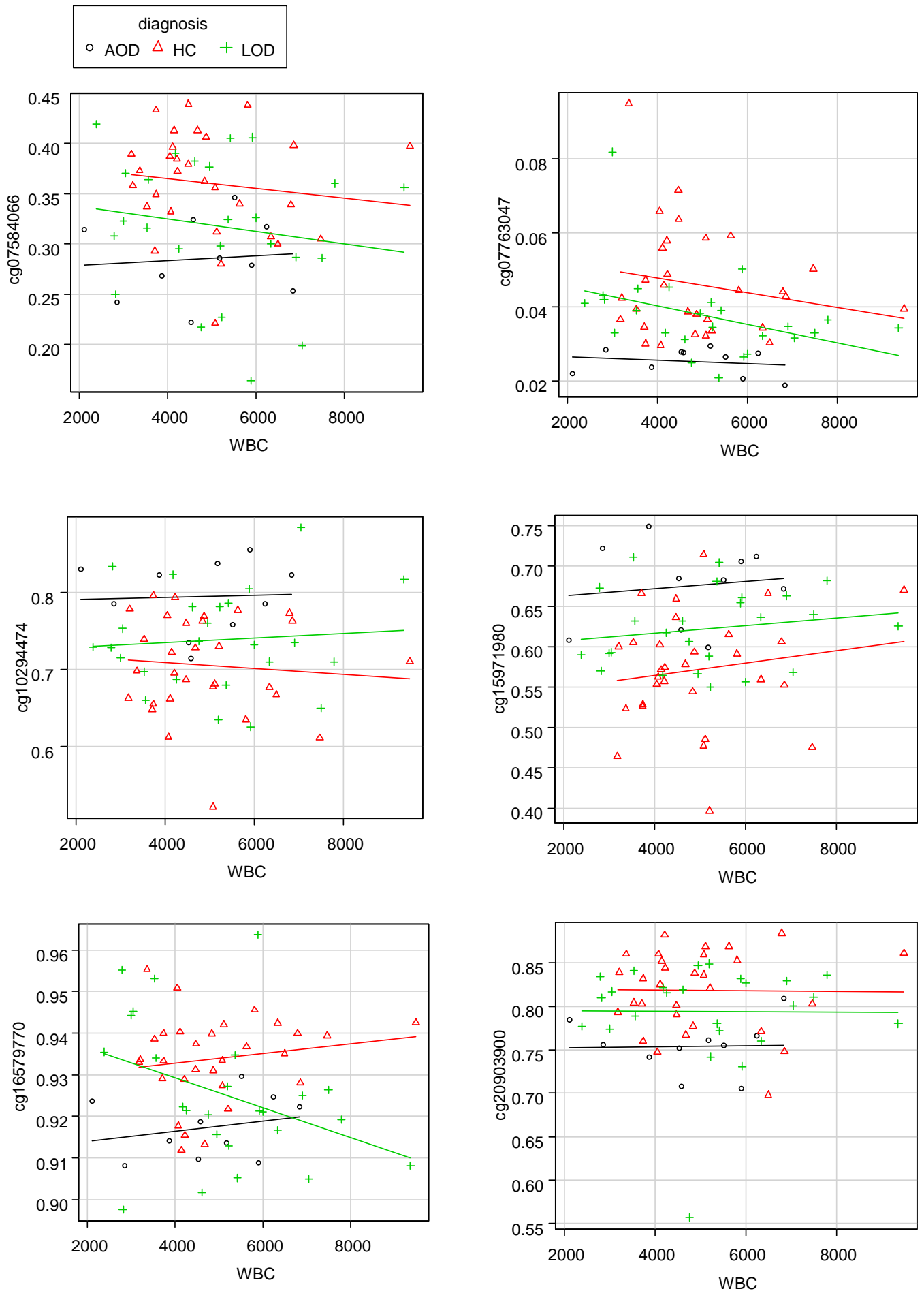
Supplementary Fig. S6 Scatter plots between neutrophils (%) and the methylation levels of each site.
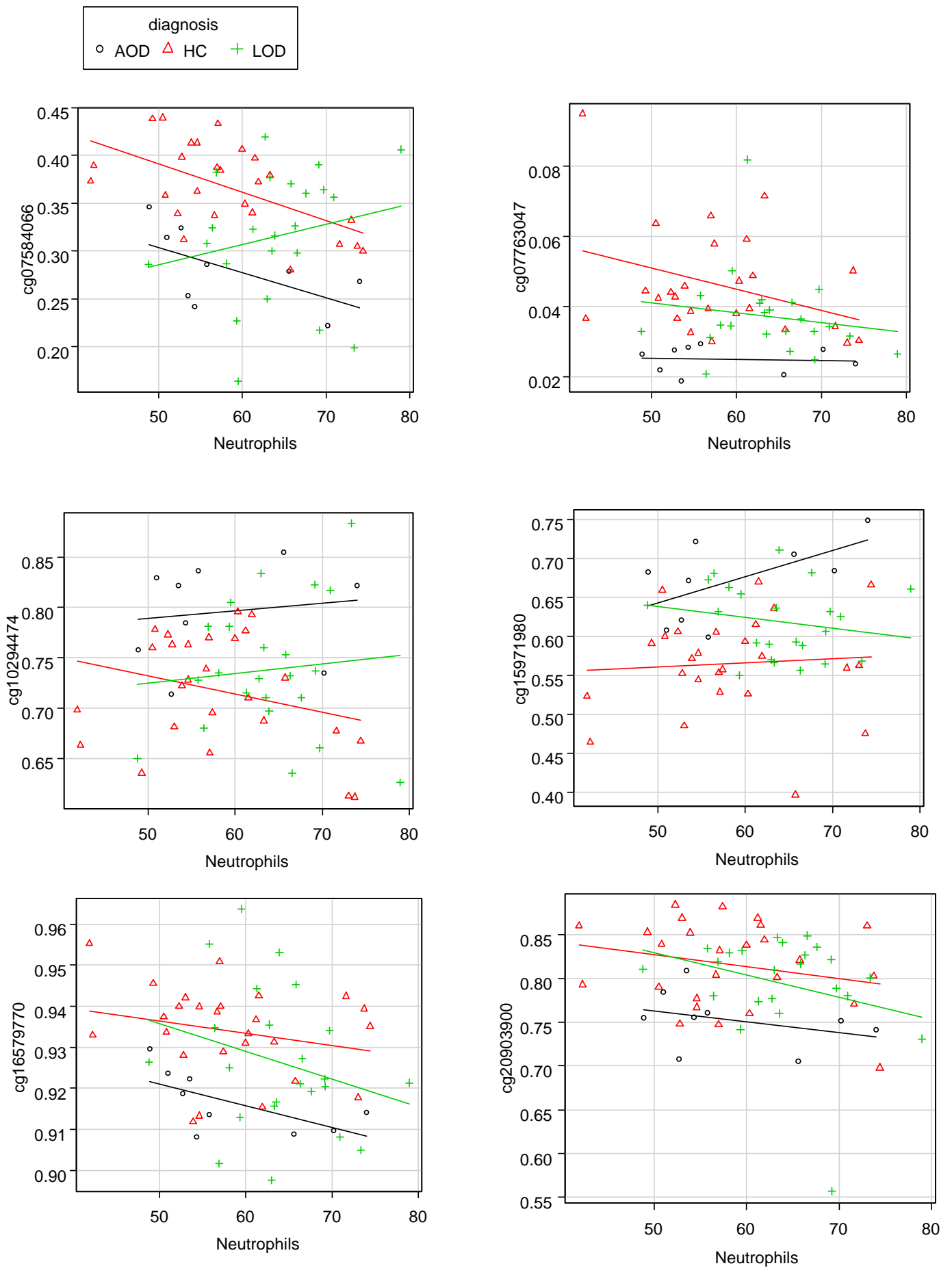
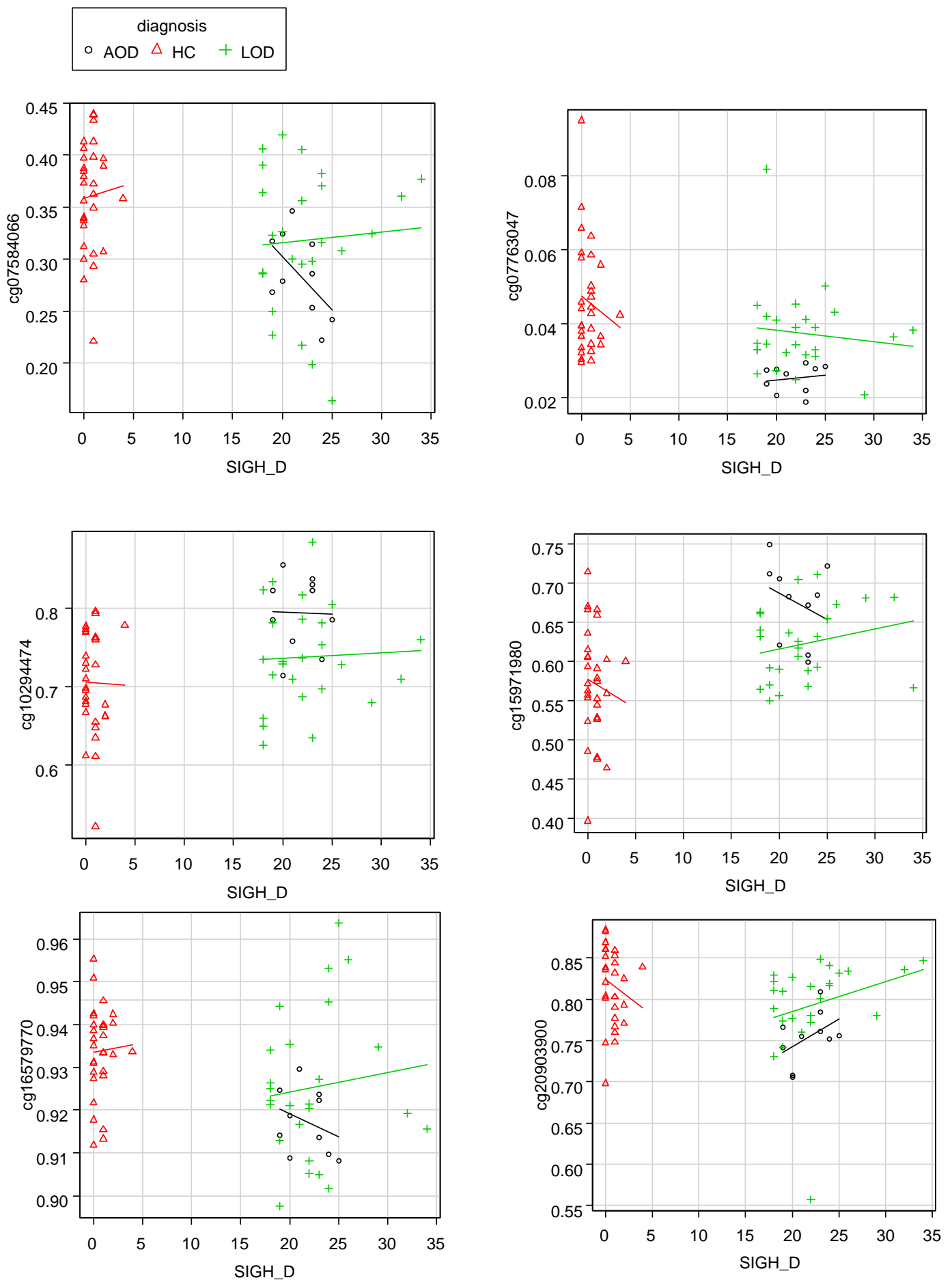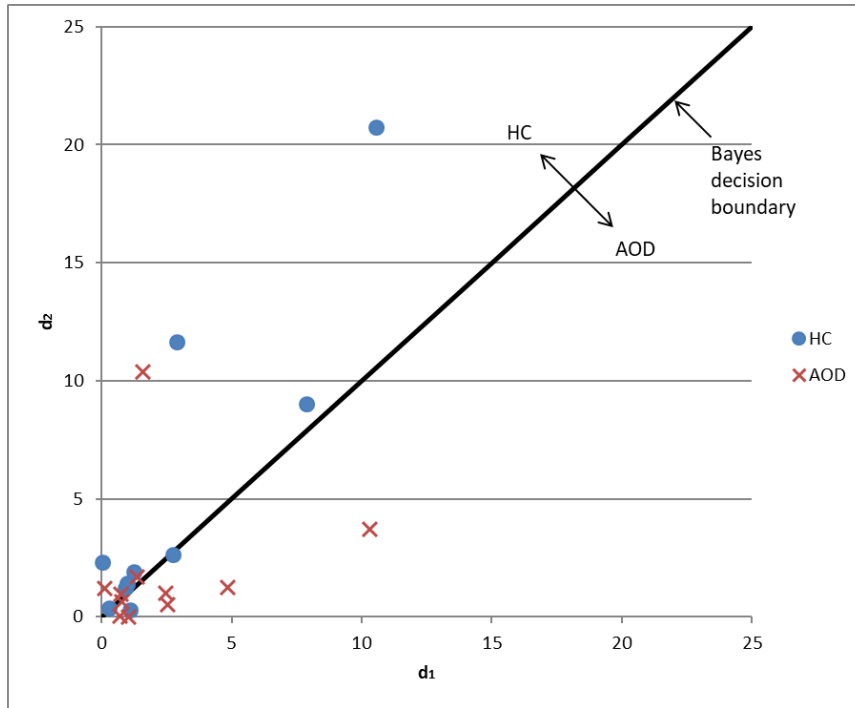**Supplementary Fig. S7** Scatter plots between SIGH-D scores and the methylation levels of each site.

Supplementary Fig. S8 Discrimination performance using combinations of two cytosine methylation sites. Comparison of the discrimination ability using two (cg16579770 and cg07763047) (**a**) or three (cg07584066, cg16579770, and cg07763047) (**b**) DNA methylation sites.

Supplementary Fig. S9 Comparison of the spread among AOD, LOD, and HC distributions using cg16579770 and cg07763047.

The ratio of the determinants of AOD or LOD to HC are shown. The statistical test between cg16579770 and cg07763047 for discriminating AOD from HC.
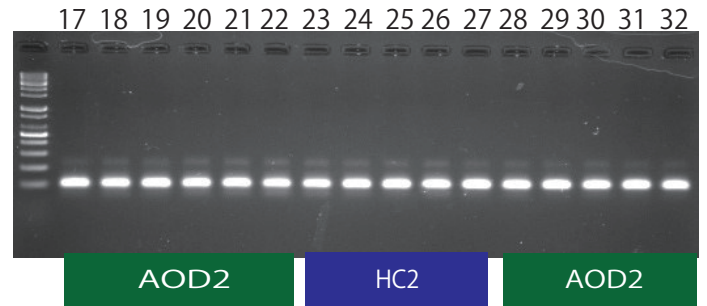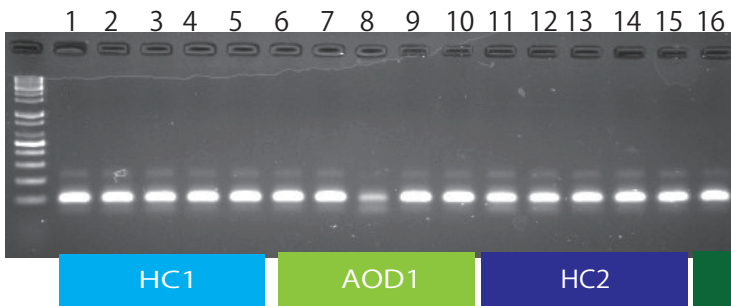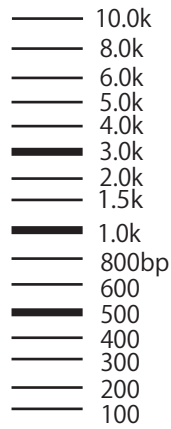
Supplementary Fig. S10 Gel images of PCR products for pyroseqencing. Five micro-litters of DNA Ladder 1kb plus (BioFact) were used for loading controls and markers.

Supplementary Fig. S11 Gel images of PCR products for amplicon bisulfite sequencing. Gel imaging was performed ATTO printgraph and ATTO imageSaver AE-6905C. Five micro-litters of GeneRuler 100 bp Plus DNA Ladder (Thermo Fisher Scientific) were used for loading controls and markers.

Supplementary Fig. S12 A summary of statistical strategy.

Supplementary Table S1 The 100 top-ranked DNA methylation sites in AOD and LOD samples.

Supplementary Table S2 The 100 top-ranked RefSeq genes in AOD and LOD samples.

Supplementary Table S3 Validation of specific DNA methylation sites.
(**a**) Correlation between the methylation levels in pyrosequencing and the genome-wide DNA methylation array. (**b**) Correlation between the methylation levels in amplicon bisulfite sequencing and the genome-wide DNA methylation array. Q-values were calculated using the false discovery rate method (the Benjamini-Hochberg adjustment method).

Supplementary Table S4 Correlation between the methylation levels and each parameter.
Q-values were calculated using the false discovery rate method (the Benjamini-Hochberg adjustment method).

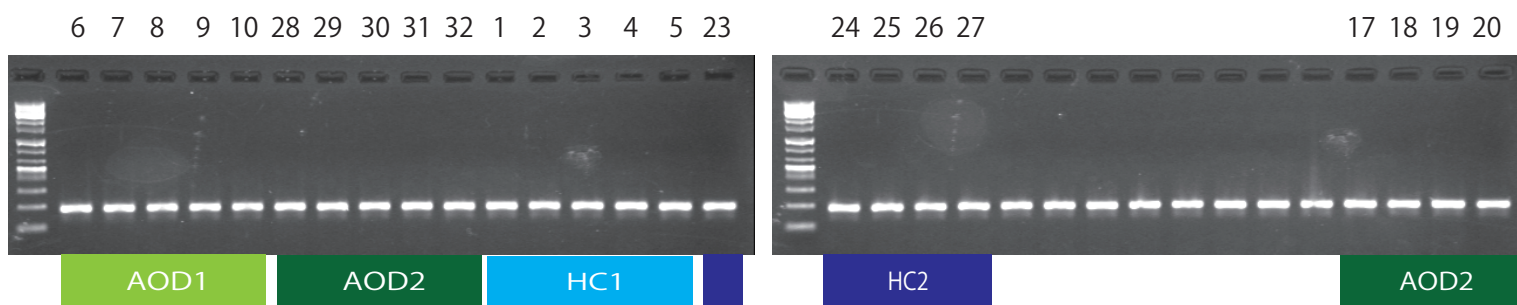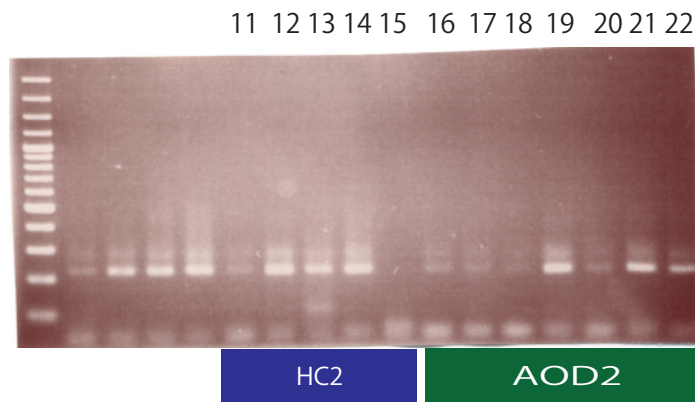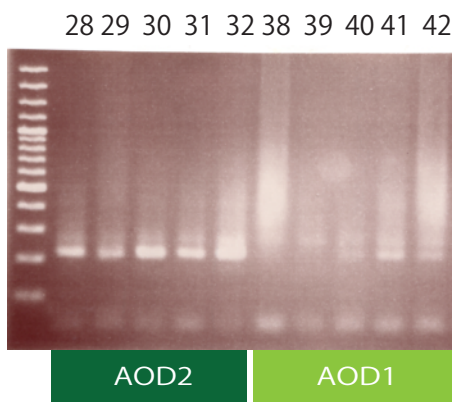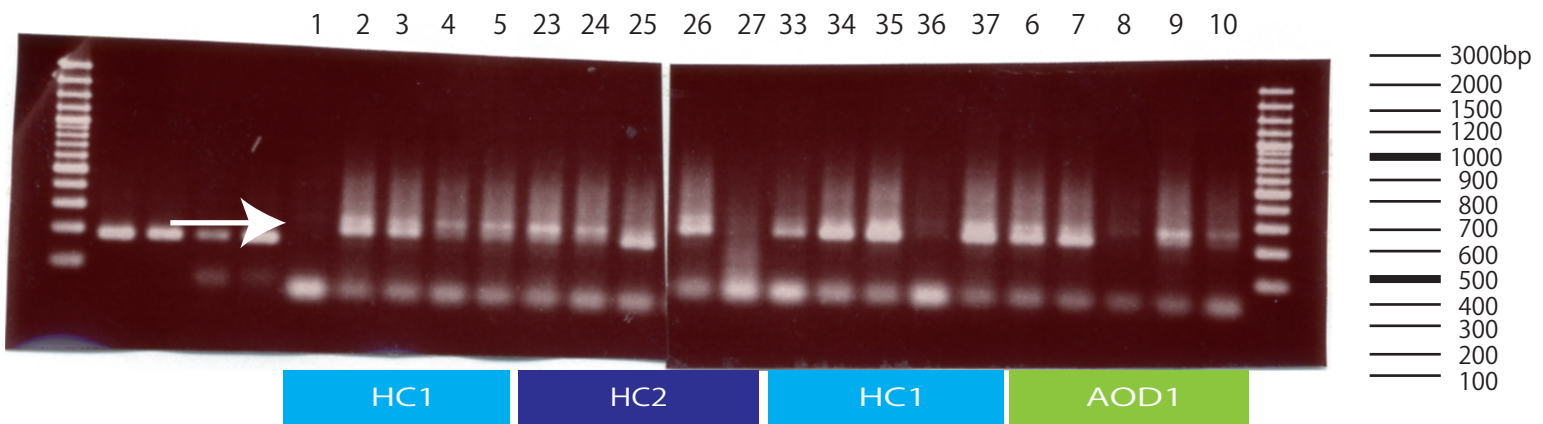**Supplementary Table S5** Primer list for pyrosequencing. PCR and sequencing primers were designed using Pyrosequencing Assay Design Software v2.0 (Qiagen).

Supplementary Table S6 Primer list and PCR conditions for amplicon sequencing. Each primer was designed using the Methyl Primer Express software v1.0 (Thermo Fisher Scientific Inc.).

Figure S1 Scatter plots between the array data and the pyrosequencing or the amplicon bisulfite sequencing data

Figure S2 Scatter plots between age and the methylation levels of each site

Figure S3 Scatter plots between age at onset and the methylation levels of each site

Figure S4 Scatter plots between number of episodes and the methylation levels of each site

Figure S5 Scatter plots between WBCs and the methylation levels of each site

Figure S6 Scatter plots between neutrophils (%) and the methylation levels of each site

Figure S7 Scatter plots between SIGH-D scores and the methylation levels of each site

Figure S8a

Use of cg16579770 and cg07763047

Figure S8b

Use of cg07584066, cg16579770 and cg07763047

Supplementary Fig. S8 Discrimination performance using combinations of two cytosine methylation sites
Comparison of the discrimination ability using two (cg16579770 and cg07763047) (a) or three (cg07584066, cg16579770, and cg07763047) (b) DNA methylation sites

Figure S9

Supplementary Fig. S9 Comparison of the spread among AOD, LOD, and HC distributions using cg16579770 and cg07763047.
The ratio of the determinants of AOD or LOD to HC are shown. The statistical test between cg16579770 and cg07763047 for discriminating AOD from HC.

Figure S10

Gel images of PCR products

Samples of pyrosequencing

HC1	Group 1b HC
HC2	Group 2 HC
AOD1	Group 1b AOD
AOD2	Group 2 AOD

cg15971980

cg20903900

cg10294474

cg07584066

Figure S11

Gel images of PCR products

Samples of amplicon bisulfite sequencing

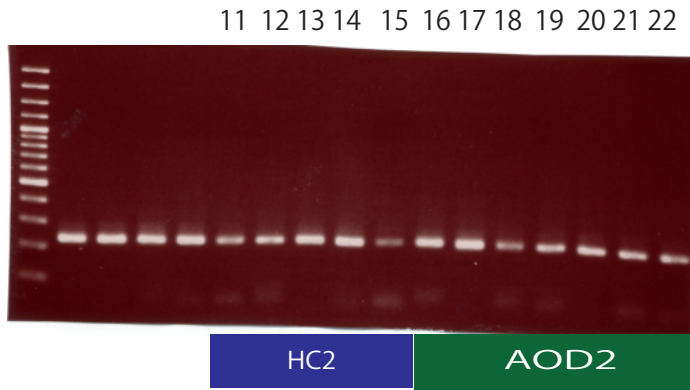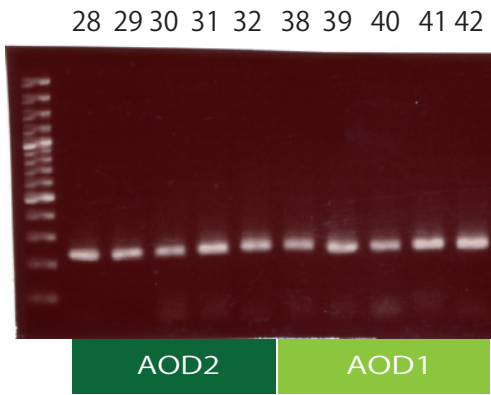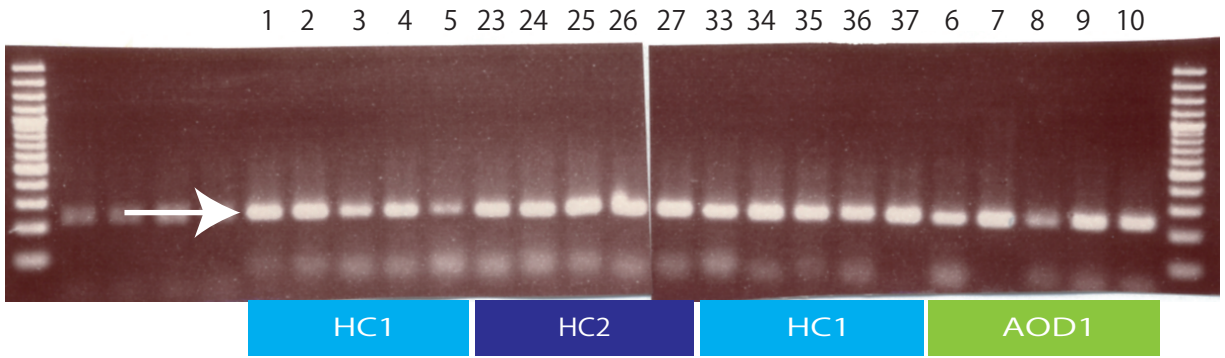# cg07763047

cg14567489

cg17458347

cg15871980

cg15794987

Figure S12 A summary of statistical strategy

Group1

Group1a
(discovery
samples)

Group1b
(training
samples)

the values of
determinants
cg16579770
cg07763047

Fisher ratio
(selection of
methylation
sites)

validation by
pyrosequencing
amplicon
bisulfite
sequencing

Bayes classifier
AOD vs HC

Group2
(test samples
accuracy 77%)