

Supplementary method

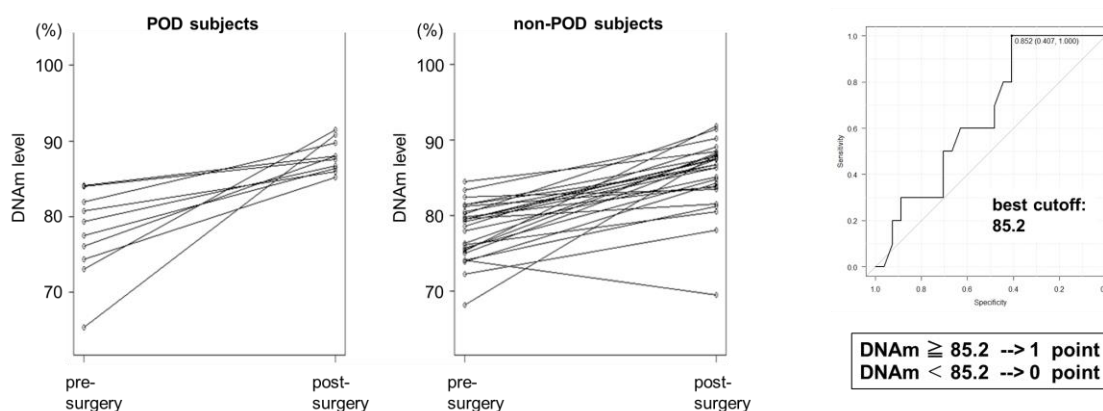
Calculation of Inflammatory methylation Index (IMI)

Score named “Inflammatory Methylation Index (IMI)” was obtained for each subject. First, ROC curves were made and AUCs were calculated based on the DNA methylation levels of each CpG site. We selected several CpG sites with a large AUC as candidates to construct the combined index. Then, we optimized the number and combination of CpG sites to obtain a better ROC curve, and five CpG sites were selected for the IMI. Five CpG sites: one CpG sites from the *TNF* gene (cg08639424), two CpG sites from the *IL1B* gene (cg15836722 and cg23149881), and two CpG sites from the *IL6* gene (cg15703690 and cg17067544) were selected to calculate IMI. We first created an ROC curve for each CpG site to determine the best cutoff value to separate POD from non-POD. For each CpG site, we added 1 point if the post-operative DNAm level met the criteria divided by the cutoff value, 0 points if it did not, and repeated the process for 5 CpGs (**Supplementary Figure 1**). The total score of those points was used as IMI (ranging from 0 to 5). Then, the relationship between IMI and POD was evaluated by a ROC curve. Two examples are shown below. To validate the results, the IMI was calculated using the same CpG sites and the same process with another cohort from our previous study. Then, the relationship between IMI from the previous cohort and delirium was tested.

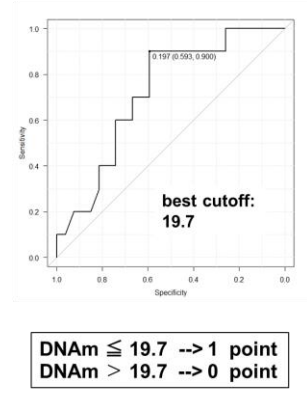
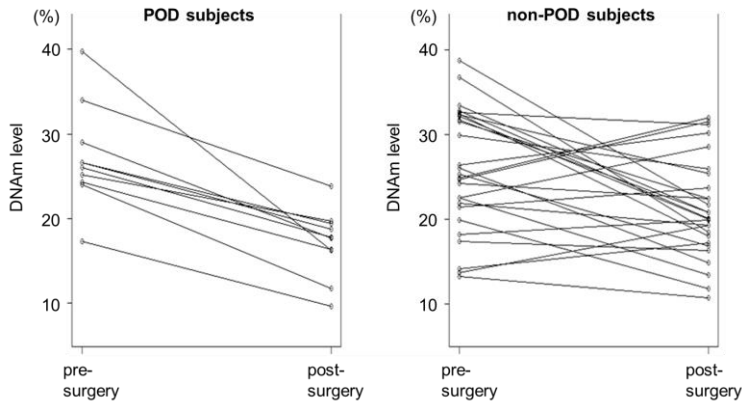
Furthermore, the same approach was used to calculate the IMI from the pre-operative DNAm (pre-IMI) and amount of change in DNAm level after surgery (diff-IMI). Five different CpG sites were selected to optimize the ROC; cg23384708 (*TNF* gene), cg07250315 (*IL1B* gene), cg18635064 (*IL1B* gene), cg03601896 (*IL6* gene), and cg10140158 (*IL6* gene) for pre-IMI and cg19124225 (*TNF* gene), cg07250315 (*IL1B* gene), cg20157753 (*IL1B* gene), cg07998387 (*IL6* gene), and cg13104385 (*IL6* gene) for diff-IMI.

Supplementary Figure 1

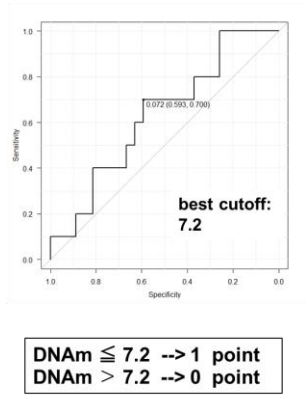
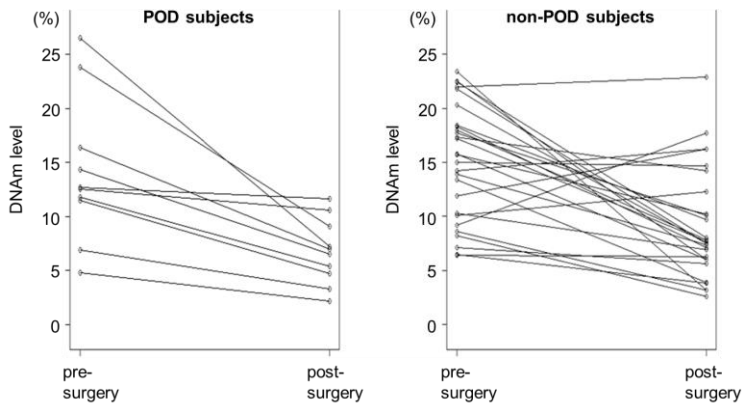
TNF gene: cg08639424



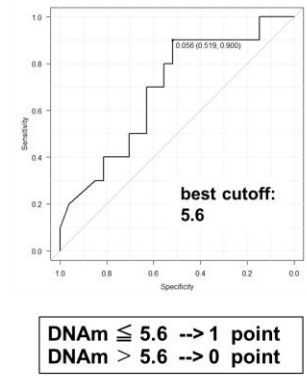
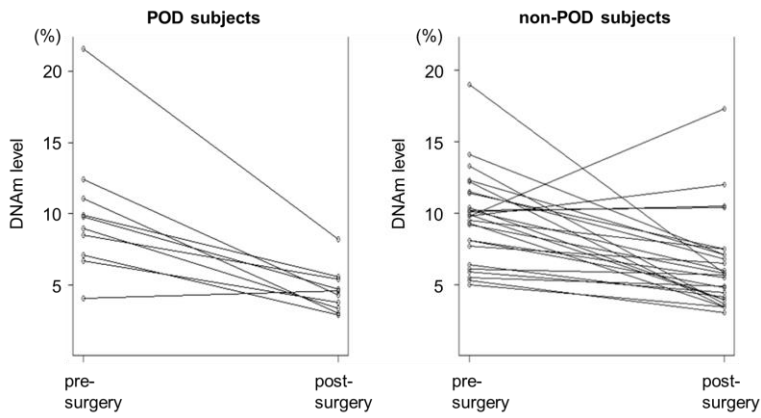
IL1B gene: cg15836722



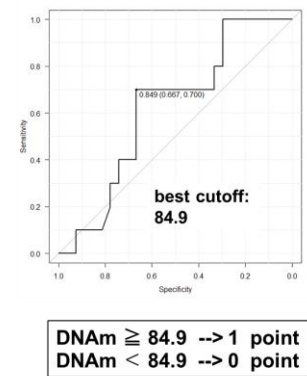
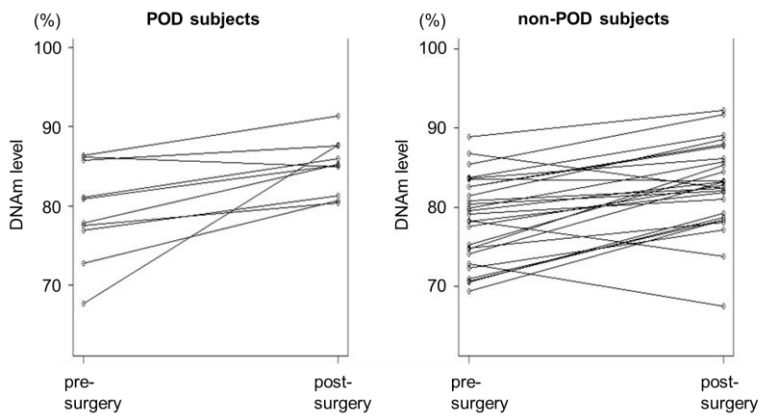
IL1B gene: cg23149881



IL6 gene: cg15703690



IL6 gene: cg17067544



< examples >

| | | subject A | | subject B | | criteria (1 point) |
|-------------|------------|-----------|-------|-----------|-------|--------------------|
| | | DNAm (%) | point | DNAm (%) | point | |
| <i>TNF</i> | cg08639424 | 88.3 | 1 | 80.5 | 0 | DNAm >= 85.2 |
| <i>IL1B</i> | cg15836722 | 13.4 | 1 | 31.2 | 0 | DNAm <= 19.7 |
| <i>IL1B</i> | cg23149881 | 2.6 | 1 | 22.9 | 0 | DNAm <= 7.2 |
| <i>IL6</i> | cg15703690 | 3.4 | 1 | 6.8 | 0 | DNAm <= 5.6 |
| <i>IL6</i> | cg17067544 | 82.4 | 0 | 85.7 | 1 | DNAm >= 84.9 |
| IMI | | 4 | | 1 | | |

Supplementary Tables

Supplementary Table 1. Patient Characteristics

| Classification | All Subjects | EPIC analysis | | p | Statistical test | pyrosequencing | | p | Statistical test |
|----------------------------|--------------|---------------|---------|-------|------------------|----------------|---------|-------|------------------|
| | | POD | non-POD | | | POD | non-POD | | |
| N | 39 | 10 | 27 | | | 7 | 20 | | |
| Mean age — yr | 33.4 | 41.0 | 29.8 | 0.046 | t = -2.07 | 44.9 | 29.5 | 0.01 | t = -2.71 |
| SD | 15.1 | 15.6 | 14.3 | | | 15.1 | 2.1 | | |
| Female sex (n) | 15 | 5 | 8 | 0.44 | $\chi^2 = 0.59$ | 4 | 7 | 0.56 | $\chi^2 = 0.34$ |
| % | 38.5 | 50.0 | 29.6 | | | 57.1 | 35.0 | | |
| Race, White (n) | 36 | 10 | 26 | >0.99 | $\chi^2 < 0.01$ | 7 | 19 | >0.99 | $\chi^2 < 0.01$ |
| % | 97.3 | 100.0 | 96.3 | | | 100.0 | 95.0 | | |
| alcohol (n) | 12 | 3 | 9 | >0.99 | $\chi^2 < 0.01$ | 3 | 8 | >0.99 | $\chi^2 < 0.01$ |
| % | 30.8 | 30.0 | 33.3 | | | 42.9 | 40.0 | | |
| tobacco (n) | 16 | 5 | 10 | 0.74 | $\chi^2 = 0.11$ | 4 | 10 | >0.99 | $\chi^2 < 0.01$ |
| % | 41.0 | 50.0 | 37.0 | | | 57.1 | 50.0 | | |
| Mean ASA-PS | 2.4 | 2.2 | 2.5 | 0.13 | t = 1.56 | 2.1 | 2.5 | 0.16 | t = 1.45 |
| SD | 0.5 | 0.4 | 0.5 | | | 0.4 | 0.5 | | |
| Mean BMI | 29.8 | 29.7 | 29.5 | 0.96 | t = -0.06 | 32.2 | 27.2 | 0.18 | t = -1.38 |
| SD | 10.4 | 8.6 | 11.1 | | | 8.6 | 8.3 | | |
| Mean anesthesia time (min) | 530.6 | 533.8 | 536.7 | 0.93 | t = 0.09 | 535.1 | 525.0 | 0.82 | t = -0.23 |
| SD | 91.0 | 88.4 | 90.0 | | | 85.5 | 104.0 | | |
| mean procedure time (min) | 348.1 | 355.3 | 353.0 | 0.95 | t = -0.07 | 343.1 | 339.5 | 0.93 | t = -0.09 |
| SD | 90.1 | 95.4 | 87.0 | | | 81.1 | 96.2 | | |
| Mean blood loss (ml) | 165.0 | 145.0 | 178.3 | 0.46 | t = 0.74 | 160.7 | 186.5 | 0.69 | t = 0.41 |
| SD | 120.0 | 165.3 | 102.1 | | | 199.9 | 121.2 | | |

Abbreviation: POD; post operative delirium, SD; Standard deviation, ASA: the American Society of Anesthesiologists (ASA) physical status, BMI; Body mass index

Supplementary Table 2. Cell type proportions

| cell type | pre-surgery | | | post-surgery | | | amount of change (post-pre) | | | | | |
|--------------------------|-------------|---------|------|------------------|---------|-----|-----------------------------|------------------|-------|------|------|------------------|
| | POD | non-POD | | POD | non-POD | | POD | non-POD | | | | |
| N | 10 | 27 | p | Statistical test | 10 | 27 | p | Statistical test | 10 | 27 | p | Statistical test |
| CD8 T cells (%) | 6.8 | 8.6 | 0.27 | t = 1.12 | 1.6 | 3.3 | 0.26 | t = 1.16 | -5.2 | -5.3 | 0.95 | t = -0.06 |
| SD | 3.1 | 4.6 | | | 1.6 | 4.4 | | | 3.4 | 4.5 | | |
| CD4-T cells (%) | 12.3 | 11.7 | 0.79 | t = -0.27 | 0.9 | 4.0 | 0.13 | t = 1.55 | -11.4 | -7.7 | 0.21 | t = 1.28 |
| SD | 4.6 | 6.4 | | | 0.8 | 6.2 | | | 4.7 | 8.7 | | |
| natural killer cells (%) | 0.9 | 1.3 | 0.57 | t = 0.57 | 1.1 | 1.5 | 0.64 | t = 0.47 | 0.2 | 0.2 | 0.97 | t = -0.04 |
| SD | 1.5 | 2.1 | | | 1.9 | 2.2 | | | 2.5 | 3.0 | | |
| B cells (%) | 4.0 | 2.9 | 0.31 | t = -1.03 | 0.9 | 1.6 | 0.30 | t = 1.05 | -3.2 | -1.4 | 0.11 | t = 1.65 |
| SD | 3.3 | 2.7 | | | 1.1 | 2.0 | | | 3.2 | 2.8 | | |
| Monocytes (%) | 7.3 | 7.5 | 0.84 | t = 0.20 | 3.0 | 3.6 | 0.50 | t = 0.68 | -4.3 | -3.9 | 0.70 | t = 0.39 |
| SD | 3.2 | 2.9 | | | 1.7 | 2.6 | | | 3.0 | 2.7 | | |

Abbreviation: POD; post operative delirium, SD; Standard deviation

Supplementary Table 3. Pre-surgery DNAm in the *TNF* gene

| cg | location | EPIC | | | | | pyrosequencing | | | | |
|------------|----------------|---------------------|-------------------------|------|-------------------|---------------------|--------------------|-------------------------|------|-------------------|---------------------|
| | | POD N=10 mean | non-POD N=27 mean | diff | t-test p-value | adjusted p-value | POD N=7 mean | non-POD N=20 mean | diff | t-test p-value | adjusted p-value |
| | chr6: 31542460 | | | | | | 72.7 | 74.5 | -1.7 | 0.30 | 0.98 |
| cg08639424 | chr6: 31542556 | 77.7 | 78.0 | 0.3 | 0.85 | 0.90 | | | | | |
| cg19978379 | chr6: 31542671 | 73.0 | 71.3 | -1.7 | 0.37 | 0.24 | | | | | |
| cg24452282 | chr6: 31542741 | 73.8 | 73.3 | -0.5 | 0.83 | 0.79 | | | | | |
| | chr6: 31543170 | | | | | | 6.5 | 7.2 | -0.7 | 0.59 | 0.49 |
| | chr6: 31543176 | | | | | | 8.0 | 9.2 | -1.2 | 0.44 | 0.70 |
| | chr6: 31543178 | | | | | | 2.0 | 3.8 | -1.8 | 0.08 | 0.56 |
| | chr6: 31543193 | | | | | | 4.6 | 5.5 | -0.9 | 0.47 | 0.53 |
| cg21370522 | chr6: 31543219 | 13.6 | 13.0 | -0.6 | 0.75 | 0.73 | 3.8 | 4.4 | -0.5 | 0.50 | 0.53 |
| cg19648923 | chr6: 31543266 | 10.3 | 10.6 | 0.3 | 0.84 | 0.47 | 2.7 | 3.0 | -0.4 | 0.56 | 0.71 |
| cg01569083 | chr6: 31543290 | 19.0 | 18.8 | -0.2 | 0.94 | 0.47 | 2.9 | 3.5 | -0.6 | 0.38 | 0.62 |
| cg03037030 | chr6: 31543300 | 5.6 | 6.6 | 1.0 | 0.38 | 0.92 | 8.5 | 9.1 | -0.7 | 0.61 | 0.61 |
| | chr6: 31543430 | | | | | | 1.5 | 2.1 | -0.6 | 0.15 | 0.70 |
| | chr6: 31543487 | | | | | | 5.3 | 6.6 | -1.3 | 0.20 | 0.81 |
| cg12681001 | chr6: 31543541 | 8.8 | 9.1 | 0.4 | 0.79 | 0.39 | 9.1 | 10.9 | -1.8 | 0.20 | 0.67 |
| cg21222743 | chr6: 31543546 | 5.8 | 6.9 | 1.1 | 0.39 | 0.49 | 7.7 | 9.2 | -1.5 | 0.22 | 0.80 |
| cg10717214 | chr6: 31543558 | 8.3 | 9.7 | 1.4 | 0.33 | 0.46 | 33.6 | 33.5 | 0.1 | 0.96 | 0.83 |
| cg04425624 | chr6: 31543566 | 17.2 | 17.5 | 0.3 | 0.92 | 0.30 | 20.8 | 21.3 | -0.5 | 0.86 | 0.44 |
| cg21467614 | chr6: 31543638 | 17.1 | 17.9 | 0.8 | 0.66 | 0.18 | | | | | |
| cg08553327 | chr6: 31543647 | 15.4 | 16.9 | 1.6 | 0.53 | 0.31 | | | | | |
| cg26729380 | chr6: 31543655 | 10.6 | 11.9 | 1.3 | 0.49 | 0.14 | | | | | |
| cg10650821 | chr6: 31543686 | 11.4 | 11.7 | 0.3 | 0.86 | 0.58 | | | | | |
| | chr6: 31544695 | | | | | | 63.2 | 66.1 | -2.9 | 0.59 | 0.60 |
| | chr6: 31544752 | | | | | | 78.2 | 76.8 | 1.4 | 0.70 | 0.46 |
| | chr6: 31544800 | | | | | | 24.0 | 22.3 | 1.8 | 0.26 | 0.17 |
| | chr6: 31544822 | | | | | | 58.1 | 56.0 | 2.1 | 0.40 | 0.30 |
| | chr6: 31544848 | | | | | | 14.6 | 12.8 | 1.7 | 0.09 | <u>0.01</u> |
| cg01360627 | chr6: 31544931 | 69.8 | 70.0 | 0.2 | 0.93 | 0.97 | 27.4 | 25.3 | 2.1 | 0.23 | 0.10 |
| cg23384708 | chr6: 31544934 | 67.4 | 63.0 | -4.4 | <u>0.04</u> | 0.77 | 77.3 | 72.8 | 4.5 | 0.16 | 0.11 |
| cg20477259 | chr6: 31544960 | 62.5 | 61.3 | -1.2 | 0.61 | 0.78 | 68.9 | 67.5 | 1.4 | 0.63 | 0.65 |
| | chr6: 31545253 | | | | | | 87.9 | 88.2 | -0.3 | 0.88 | 0.91 |
| | chr6: 31545258 | | | | | | 88.7 | 87.4 | 1.3 | 0.52 | 0.31 |
| | chr6: 31545267 | | | | | | 84.1 | 81.3 | 2.8 | 0.29 | 0.14 |
| cg15989608 | chr6: 31545322 | 72.2 | 70.9 | -1.3 | 0.54 | 0.35 | 92.1 | 89.8 | 2.3 | 0.21 | 0.12 |
| cg26736341 | chr6: 31545343 | 71.4 | 69.5 | -1.8 | 0.43 | 0.35 | 78.0 | 79.2 | -1.1 | 0.65 | 0.21 |
| | chr6: 31545432 | | | | | | 89.9 | 85.9 | 3.9 | 0.11 | 0.09 |
| cg04472685 | chr6: 31545474 | 82.0 | 81.2 | -0.7 | 0.66 | 0.56 | 83.4 | 84.2 | -0.8 | 0.68 | 0.77 |
| cg19124225 | chr6: 31545836 | 94.8 | 95.9 | 1.1 | 0.10 | 0.45 | | | | | |
| cg02137984 | chr6: 31545899 | 93.7 | 95.1 | 1.4 | 0.10 | 0.27 | | | | | |
| cg06825478 | chr6: 31546068 | 91.0 | 89.9 | -1.2 | 0.47 | 0.28 | 91.8 | 91.8 | 0.1 | 0.93 | 0.64 |
| | chr6: 31546086 | | | | | | 85.2 | 85.1 | 0.1 | 0.96 | 0.97 |

Notes:

underline nominal significance (P < 0.05)

adjusted p-value (EPIC analysis); multiple regression analysis results corrected by age, sex, and cell proportion.

adjusted p-value (pyrosequencing); multiple regression analysis results corrected by age and sex.

Supplementary Table 4. Pre-surgery DNAm in the *IL1B* and *IL6* gene

| cg | POD non-POD | | diff | t-test p-value | adjusted p-value |
|--------------------|--------------|--------------|------|-------------------|---------------------|
| | N=10 mean | N=27 mean | | | |
| <i>IL1B</i> | | | | | |
| cg01290568 | 26.2 | 26.5 | 0.3 | 0.92 | 0.59 |
| cg02596281 | 84.4 | 84.2 | -0.2 | 0.93 | 0.78 |
| cg07250315 | 39.9 | 36.9 | -3.0 | 0.16 | 0.02 |
| cg07935264 | 8.8 | 9.2 | 0.4 | 0.70 | 0.78 |
| cg10486274 | 93.9 | 93.6 | -0.3 | 0.65 | 0.64 |
| cg14117934 | 86.7 | 86.1 | -0.6 | 0.73 | 0.30 |
| cg15218327 | 92.4 | 92.0 | -0.4 | 0.69 | 0.36 |
| cg15836722 | 27.3 | 26.0 | -1.3 | 0.61 | 0.28 |
| cg18635064 | 93.2 | 95.1 | 1.9 | 0.14 | 0.10 |
| cg18773937 | 6.8 | 7.3 | 0.6 | 0.65 | 0.57 |
| cg19890119 | 15.9 | 15.5 | -0.5 | 0.83 | 0.97 |
| cg20157753 | 5.9 | 5.2 | -0.7 | 0.19 | 0.17 |
| cg20983042 | 87.5 | 86.9 | -0.6 | 0.77 | 0.85 |
| cg23149881 | 14.1 | 15.0 | 0.9 | 0.67 | 0.52 |
| <i>IL6</i> | | | | | |
| cg00087425 | 6.3 | 6.5 | 0.2 | 0.72 | 0.96 |
| cg00175482 | 88.6 | 89.1 | 0.6 | 0.62 | 0.78 |
| cg01770232 | 7.7 | 6.9 | -0.8 | 0.44 | 0.98 |
| cg02335517 | 93.3 | 93.4 | 0.2 | 0.87 | 0.46 |
| cg03601896 | 4.0 | 3.7 | -0.4 | 0.57 | > 0.99 |
| cg05265849 | 21.4 | 19.7 | -1.7 | 0.56 | 0.62 |
| cg07998387 | 69.8 | 69.4 | -0.5 | 0.80 | 0.79 |
| cg10140158 | 86.8 | 88.0 | 1.2 | 0.34 | 0.37 |
| cg13104385 | 41.1 | 39.5 | -1.6 | 0.67 | 0.66 |
| cg15703690 | 10.0 | 9.5 | -0.5 | 0.69 | 0.42 |
| cg17067544 | 79.3 | 78.5 | -0.8 | 0.69 | 0.53 |
| cg20509117 | 62.2 | 62.3 | 0.1 | 0.98 | 0.83 |
| cg21785978 | 5.2 | 5.5 | 0.3 | 0.68 | 0.84 |
| cg23731304 | 38.7 | 37.2 | -1.6 | 0.48 | 0.11 |

Notes:
adjusted p-value; multiple regression analysis results corrected by age, sex, and cell proportion.

Supplementary Table 5. Correlations between age and pre-surgery DNAm in the *IL1B* and *IL6* gene

| cg | all N=37 | | POD N=10 | | non-POD N=27 | |
|--------------------|-------------|-------------|-------------|-------------|-----------------|-------------|
| | r | p | r | p | r | p |
| <i>IL1B</i> | | | | | | |
| cg01290568 | -0.16 | 0.35 | -0.08 | 0.84 | -0.19 | 0.35 |
| cg02596281 | -0.11 | 0.53 | -0.12 | 0.74 | -0.12 | 0.56 |
| cg07250315 | -0.02 | 0.91 | 0.05 | 0.89 | -0.16 | 0.43 |
| cg07935264 | -0.25 | 0.14 | -0.33 | 0.35 | -0.19 | 0.34 |
| cg10486274 | -0.05 | 0.75 | -0.29 | 0.42 | 0.01 | 0.96 |
| cg14117934 | -0.22 | 0.20 | 0.48 | 0.16 | -0.40 | <u>0.04</u> |
| cg15218327 | -0.27 | 0.10 | -0.75 | <u>0.01</u> | -0.21 | 0.30 |
| cg15836722 | -0.14 | 0.42 | -0.16 | 0.65 | -0.18 | 0.36 |
| cg18635064 | -0.11 | 0.53 | -0.17 | 0.65 | 0.05 | 0.79 |
| cg18773937 | -0.22 | 0.20 | 0.34 | 0.34 | -0.42 | <u>0.03</u> |
| cg19890119 | 0.06 | 0.73 | 0.14 | 0.71 | 0.02 | 0.93 |
| cg20157753 | 0.00 | 0.98 | -0.40 | 0.26 | 0.04 | 0.86 |
| cg20983042 | -0.13 | 0.45 | -0.17 | 0.64 | -0.15 | 0.46 |
| cg23149881 | -0.25 | 0.13 | -0.22 | 0.53 | -0.25 | 0.21 |
| <i>IL6</i> | | | | | | |
| cg00087425 | -0.02 | 0.90 | -0.18 | 0.62 | 0.05 | 0.81 |
| cg00175482 | -0.24 | 0.16 | -0.33 | 0.35 | -0.16 | 0.42 |
| cg01770232 | 0.14 | 0.39 | 0.48 | 0.16 | -0.17 | 0.41 |
| cg02335517 | 0.08 | 0.64 | 0.50 | 0.14 | 0.01 | 0.95 |
| cg03601896 | 0.29 | 0.08 | 0.49 | 0.15 | 0.18 | 0.37 |
| cg05265849 | 0.14 | 0.39 | 0.16 | 0.65 | 0.10 | 0.63 |
| cg07998387 | -0.06 | 0.73 | 0.24 | 0.50 | -0.18 | 0.38 |
| cg10140158 | -0.20 | 0.25 | -0.26 | 0.46 | -0.11 | 0.59 |
| cg13104385 | 0.04 | 0.84 | 0.13 | 0.73 | -0.04 | 0.85 |
| cg15703690 | 0.38 | <u>0.02</u> | 0.55 | 0.10 | 0.29 | 0.14 |
| cg17067544 | -0.24 | 0.15 | -0.24 | 0.50 | -0.30 | 0.14 |
| cg20509117 | 0.14 | 0.41 | 0.47 | 0.17 | 0.07 | 0.75 |
| cg21785978 | -0.38 | <u>0.02</u> | -0.11 | 0.77 | -0.49 | <u>0.01</u> |
| cg23731304 | -0.33 | <u>0.04</u> | -0.32 | 0.37 | -0.43 | <u>0.03</u> |

Notes:
underline nominal significance (P < 0.05)

Supplementary Table 6. Post-surgery DNAm in the *TNF* gene

| cg | location | EPIC | | | | | pyrosequencing | | | | |
|------------|----------------|---------------------|-------------------------|------|-------------------|---------------------|--------------------|-------------------------|------|-------------------|---------------------|
| | | POD N=10 mean | non-POD N=27 mean | diff | t-test p-value | adjusted p-value | POD N=7 mean | non-POD N=20 mean | diff | t-test p-value | adjusted p-value |
| | chr6: 31542460 | | | | | | 78.1 | 78.1 | 0.0 | 0.99 | 0.84 |
| cg08639424 | chr6: 31542556 | 88.0 | 85.5 | 2.5 | 0.11 | 0.28 | | | | | |
| cg19978379 | chr6: 31542671 | 81.9 | 80.6 | 1.3 | 0.55 | 0.11 | | | | | |
| cg24452282 | chr6: 31542741 | 86.9 | 83.6 | 3.3 | 0.23 | 0.53 | | | | | |
| | chr6: 31543170 | | | | | | 6.4 | 5.4 | 1.0 | 0.30 | <u>0.03</u> |
| | chr6: 31543176 | | | | | | 7.5 | 7.6 | -0.1 | 0.90 | 0.27 |
| | chr6: 31543178 | | | | | | 2.3 | 2.7 | -0.4 | 0.50 | 0.84 |
| | chr6: 31543193 | | | | | | 3.5 | 4.1 | -0.6 | 0.45 | 0.62 |
| cg21370522 | chr6: 31543219 | 14.4 | 13.9 | 0.5 | 0.84 | 0.60 | 3.6 | 3.4 | 0.2 | 0.63 | 0.11 |
| cg19648923 | chr6: 31543266 | 7.8 | 8.2 | -0.4 | 0.76 | 0.59 | 2.4 | 2.3 | 0.1 | 0.82 | 0.32 |
| cg01569083 | chr6: 31543290 | 16.8 | 17.7 | -0.9 | 0.65 | 0.98 | 2.5 | 2.7 | -0.2 | 0.61 | 0.46 |
| cg03037030 | chr6: 31543300 | 4.5 | 5.0 | -0.5 | 0.59 | 0.77 | 7.6 | 7.8 | -0.3 | 0.74 | 0.65 |
| | chr6: 31543430 | | | | | | 1.9 | 2.0 | -0.1 | 0.80 | 0.34 |
| | chr6: 31543487 | | | | | | 4.9 | 5.4 | -0.5 | 0.64 | 0.61 |
| cg12681001 | chr6: 31543541 | 6.7 | 7.5 | -0.7 | 0.30 | 0.93 | 9.5 | 9.0 | 0.5 | 0.75 | 0.50 |
| cg21222743 | chr6: 31543546 | 3.4 | 4.2 | -0.9 | 0.26 | <u>0.01</u> | 7.1 | 7.4 | -0.3 | 0.74 | 0.60 |
| cg10717214 | chr6: 31543558 | 6.0 | 6.5 | -0.5 | 0.62 | <u>0.02</u> | 35.0 | 32.0 | 3.1 | 0.35 | 0.90 |
| cg04425624 | chr6: 31543566 | 12.0 | 13.5 | -1.5 | 0.42 | 0.64 | 21.6 | 20.5 | 1.1 | 0.70 | 0.86 |
| cg21467614 | chr6: 31543638 | 14.1 | 15.0 | -0.9 | 0.47 | 0.23 | | | | | |
| cg08553327 | chr6: 31543647 | 11.6 | 13.7 | -2.1 | 0.24 | 0.65 | | | | | |
| cg26729380 | chr6: 31543655 | 6.4 | 8.4 | -2.1 | 0.13 | 0.44 | | | | | |
| cg10650821 | chr6: 31543686 | 10.0 | 10.2 | -0.2 | 0.86 | 0.63 | | | | | |
| | chr6: 31544695 | | | | | | 69.4 | 73.5 | -4.0 | 0.50 | 0.43 |
| | chr6: 31544752 | | | | | | 86.2 | 85.9 | 0.3 | 0.92 | 0.96 |
| | chr6: 31544800 | | | | | | 26.3 | 23.8 | 2.5 | 0.08 | 0.21 |
| | chr6: 31544822 | | | | | | 62.5 | 62.1 | 0.4 | 0.88 | 0.75 |
| | chr6: 31544848 | | | | | | 14.0 | 13.0 | 1.0 | 0.14 | 0.06 |
| cg01360627 | chr6: 31544931 | 80.2 | 78.1 | 2.1 | 0.37 | 0.69 | 28.9 | 26.7 | 2.2 | 0.22 | 0.34 |
| cg23384708 | chr6: 31544934 | 74.0 | 71.6 | 2.4 | 0.32 | 0.47 | 83.9 | 82.0 | 1.8 | 0.56 | 0.90 |
| cg20477259 | chr6: 31544960 | 73.4 | 71.3 | 2.1 | 0.44 | 0.42 | 76.4 | 74.1 | 2.4 | 0.37 | 0.74 |
| | chr6: 31545253 | | | | | | 92.3 | 91.8 | 0.5 | 0.78 | 0.93 |
| | chr6: 31545258 | | | | | | 92.2 | 91.3 | 0.9 | 0.73 | 0.72 |
| | chr6: 31545267 | | | | | | 87.8 | 87.8 | 0.0 | >0.99 | 0.68 |
| cg15989608 | chr6: 31545322 | 80.8 | 79.1 | 1.7 | 0.43 | 0.49 | 91.9 | 94.2 | -2.3 | 0.10 | <u>0.03</u> |
| cg26736341 | chr6: 31545343 | 80.6 | 79.0 | 1.7 | 0.48 | 0.61 | 82.4 | 80.4 | 2.0 | 0.37 | 0.39 |
| | chr6: 31545432 | | | | | | 91.4 | 89.1 | 2.2 | 0.57 | 0.66 |
| cg04472685 | chr6: 31545474 | 87.9 | 87.4 | 0.5 | 0.73 | 0.14 | 87.1 | 86.7 | 0.4 | 0.82 | 0.62 |
| cg19124225 | chr6: 31545836 | 96.5 | 96.1 | 0.3 | 0.50 | 0.98 | | | | | |
| cg02137984 | chr6: 31545899 | 96.8 | 96.5 | 0.2 | 0.69 | 0.28 | | | | | |
| cg06825478 | chr6: 31546068 | 94.7 | 94.1 | 0.6 | 0.59 | 0.77 | 94.5 | 94.2 | 0.3 | 0.70 | 0.70 |
| | chr6: 31546086 | | | | | | 89.4 | 89.3 | 0.1 | 0.91 | 0.73 |

Notes:

underline nominal significance (P < 0.05)

adjusted p-value (EPIC analysis); multiple regression analysis results corrected by age, sex, and cell proportion.

adjusted p-value (pyrosequencing); multiple regression analysis results corrected by age and sex.

Supplementary Table 7. Post-surgery DNAm in the *IL1B* and *IL6* gene

| cg | POD | non-POD | diff | t-test | adjusted |
|--------------------|------|---------|------|-------------|-------------|
| | N=10 | N=27 | | p-value | p-value |
| | mean | mean | | | |
| <i>IL1B</i> | | | | | |
| cg01290568 | 12.7 | 17.3 | -4.6 | 0.09 | 0.58 |
| cg02596281 | 81.8 | 82.9 | -1.1 | 0.47 | 0.82 |
| cg07250315 | 33.0 | 33.3 | -0.3 | 0.88 | 0.17 |
| cg07935264 | 5.5 | 6.8 | -1.2 | 0.20 | 0.73 |
| cg10486274 | 93.1 | 93.1 | 0.1 | 0.91 | 0.71 |
| cg14117934 | 85.8 | 85.4 | 0.3 | 0.76 | 0.87 |
| cg15218327 | 89.6 | 88.2 | 1.4 | 0.18 | <u>0.02</u> |
| cg15836722 | 17.1 | 21.2 | -4.1 | 0.05 | 0.12 |
| cg18635064 | 92.4 | 95.0 | -2.6 | <u>0.04</u> | 0.05 |
| cg18773937 | 4.2 | 5.1 | -0.9 | 0.27 | 0.91 |
| cg19890119 | 6.7 | 9.6 | -2.9 | 0.16 | 0.69 |
| cg20157753 | 4.2 | 5.0 | -0.8 | 0.13 | 0.61 |
| cg20983042 | 86.8 | 87.1 | -0.3 | 0.80 | 0.74 |
| cg23149881 | 6.8 | 9.1 | -2.3 | 0.19 | 0.69 |
| <i>IL6</i> | | | | | |
| cg00087425 | 6.2 | 6.8 | -0.6 | 0.40 | 0.58 |
| cg00175482 | 85.7 | 87.3 | -1.6 | 0.22 | 0.48 |
| cg01770232 | 7.7 | 7.5 | 0.2 | 0.77 | 0.50 |
| cg02335517 | 93.0 | 92.5 | 0.4 | 0.63 | 0.88 |
| cg03601896 | 2.7 | 3.1 | -0.4 | 0.41 | 0.60 |
| cg05265849 | 16.9 | 17.9 | -1.0 | 0.72 | 0.68 |
| cg07998387 | 68.6 | 70.7 | -2.2 | 0.29 | 0.50 |
| cg10140158 | 87.0 | 88.2 | -1.2 | 0.20 | 0.38 |
| cg13104385 | 38.4 | 39.4 | -0.9 | 0.81 | 0.63 |
| cg15703690 | 4.6 | 6.2 | -1.7 | 0.13 | 0.31 |
| cg17067544 | 85.0 | 82.7 | 2.3 | 0.22 | 0.59 |
| cg20509117 | 62.5 | 61.6 | 0.8 | 0.78 | 0.96 |
| cg21785978 | 4.4 | 5.3 | -0.9 | 0.15 | 0.08 |
| cg23731304 | 33.5 | 34.9 | -1.4 | 0.49 | 0.98 |

Notes:

underline nominal significance (P < 0.05)

adjusted p-value; multiple regression analysis results corrected by age, sex, and cell proportion.

Supplementary Table 8. Correlations between age and post-surgery DNAm in the *IL1B* and *IL6* gene

| cg | all N=37 | | POD N=10 | | non-POD N=27 | |
|--------------------|-------------|-------------|-------------|------|-----------------|--------------|
| | r | p | r | p | r | p |
| <i>IL1B</i> | | | | | | |
| cg01290568 | -0.08 | 0.65 | -0.21 | 0.57 | 0.04 | 0.83 |
| cg02596281 | -0.09 | 0.58 | 0.06 | 0.86 | -0.11 | 0.58 |
| cg07250315 | -0.09 | 0.61 | -0.03 | 0.94 | -0.10 | 0.63 |
| cg07935264 | -0.06 | 0.72 | -0.53 | 0.11 | 0.16 | 0.44 |
| cg10486274 | -0.14 | 0.42 | 0.02 | 0.96 | -0.19 | 0.33 |
| cg14117934 | -0.05 | 0.75 | 0.67 | 0.03 | -0.33 | 0.09 |
| cg15218327 | -0.11 | 0.52 | 0.10 | 0.79 | -0.28 | 0.15 |
| cg15836722 | 0.00 | 0.98 | 0.11 | 0.77 | 0.12 | 0.55 |
| cg18635064 | -0.10 | 0.55 | 0.16 | 0.66 | -0.10 | 0.63 |
| cg18773937 | -0.13 | 0.46 | 0.06 | 0.87 | -0.11 | 0.59 |
| cg19890119 | 0.10 | 0.56 | -0.01 | 0.98 | 0.24 | 0.23 |
| cg20157753 | -0.09 | 0.59 | -0.55 | 0.10 | 0.22 | 0.27 |
| cg20983042 | -0.07 | 0.69 | 0.68 | 0.03 | -0.32 | 0.10 |
| cg23149881 | -0.12 | 0.46 | -0.25 | 0.50 | -0.01 | 0.95 |
| <i>IL6</i> | | | | | | |
| cg00087425 | 0.34 | <u>0.04</u> | 0.02 | 0.95 | 0.56 | <u>0.003</u> |
| cg00175482 | -0.22 | 0.19 | 0.03 | 0.94 | -0.32 | 0.11 |
| cg01770232 | 0.26 | 0.12 | 0.36 | 0.30 | 0.22 | 0.28 |
| cg02335517 | 0.06 | 0.71 | 0.59 | 0.07 | -0.08 | 0.70 |
| cg03601896 | 0.28 | 0.09 | 0.21 | 0.55 | 0.40 | 0.04 |
| cg05265849 | 0.09 | 0.59 | 0.11 | 0.75 | 0.12 | 0.54 |
| cg07998387 | -0.20 | 0.23 | -0.19 | 0.59 | -0.14 | 0.48 |
| cg10140158 | -0.07 | 0.70 | -0.23 | 0.52 | 0.08 | 0.70 |
| cg13104385 | 0.05 | 0.77 | 0.10 | 0.79 | 0.05 | 0.79 |
| cg15703690 | 0.14 | 0.39 | 0.18 | 0.61 | 0.28 | 0.16 |
| cg17067544 | -0.23 | 0.17 | -0.23 | 0.52 | -0.36 | 0.07 |
| cg20509117 | 0.15 | 0.38 | 0.29 | 0.41 | 0.06 | 0.75 |
| cg21785978 | 0.11 | 0.51 | 0.47 | 0.17 | 0.13 | 0.53 |
| cg23731304 | -0.25 | 0.13 | 0.15 | 0.68 | -0.32 | 0.10 |

Notes:
underline nominal significance (P < 0.05)

Supplementary Table 9. Comparisons degree of DNAm change by neurosurgery between POD and non-POD in the *TNF* gene

| cg | location | EPIC | | | | | pyrosequencing | | | | | | |
|------------|----------------|------|------|---------|---------|-------------|----------------|------|---------|---------|-------------|--------|--------|
| | | POD | | non-POD | | t-test | adjusted | POD | | non-POD | | t-test | t-test |
| | | N=10 | N=27 | N=7 | N=20 | | | | | | | | |
| post-pre | post-pre | | | p-value | p-value | post-pre | post-pre | diff | p-value | p-value | | | |
| | | mean | mean | diff | | | mean | mean | | | | | |
| | chr6: 31542460 | | | | | | 5.4 | 3.6 | 1.8 | 0.45 | 0.87 | | |
| cg08639424 | chr6: 31542556 | 10.3 | 7.5 | 2.8 | 0.18 | 0.57 | | | | | | | |
| cg19978379 | chr6: 31542671 | 8.9 | 9.3 | -0.4 | 0.86 | 0.17 | | | | | | | |
| cg24452282 | chr6: 31542741 | 13.1 | 10.3 | 2.8 | 0.31 | 0.86 | | | | | | | |
| | chr6: 31543170 | | | | | | -0.1 | -1.8 | 1.7 | 0.19 | 0.40 | | |
| | chr6: 31543176 | | | | | | -0.5 | -1.6 | 1.1 | 0.43 | 0.81 | | |
| | chr6: 31543178 | | | | | | 0.3 | -1.1 | 1.3 | 0.23 | 0.72 | | |
| | chr6: 31543193 | | | | | | -1.1 | -1.3 | 0.2 | 0.83 | 0.80 | | |
| cg21370522 | chr6: 31543219 | 0.8 | 1.0 | -0.2 | 0.94 | 0.58 | -0.2 | -0.9 | 0.7 | 0.29 | 0.72 | | |
| cg19648923 | chr6: 31543266 | -2.5 | -2.4 | -0.1 | 0.96 | 0.69 | -0.3 | -0.8 | 0.5 | 0.42 | 0.73 | | |
| cg01569083 | chr6: 31543290 | -2.2 | -1.2 | -1.1 | 0.63 | 0.48 | -0.4 | -0.8 | 0.4 | 0.56 | 0.97 | | |
| cg03037030 | chr6: 31543300 | -1.1 | -1.6 | 0.5 | 0.66 | 0.90 | -0.9 | -1.3 | 0.4 | 0.74 | 0.80 | | |
| | chr6: 31543430 | | | | | | 0.4 | -0.1 | 0.6 | 0.26 | 0.38 | | |
| | chr6: 31543487 | | | | | | -0.4 | -1.2 | 0.8 | 0.48 | 0.52 | | |
| cg12681001 | chr6: 31543541 | -2.0 | -1.7 | -0.4 | 0.78 | 0.51 | 0.4 | -1.9 | 2.3 | 0.11 | 0.29 | | |
| cg21222743 | chr6: 31543546 | -2.4 | -2.7 | 0.2 | 0.83 | 0.47 | -0.6 | -1.8 | 1.2 | 0.36 | 0.56 | | |
| cg10717214 | chr6: 31543558 | -2.2 | -3.1 | 0.9 | 0.47 | 0.07 | 1.1 | -1.5 | 2.6 | 0.38 | 0.32 | | |
| cg04425624 | chr6: 31543566 | -5.2 | -3.9 | -1.3 | 0.61 | 0.51 | 1.6 | -0.8 | 2.3 | 0.46 | 0.55 | | |
| cg21467614 | chr6: 31543638 | -3.0 | -2.9 | -0.1 | 0.94 | 0.50 | | | | | | | |
| cg08553327 | chr6: 31543647 | -3.8 | -3.2 | -0.5 | 0.81 | 0.85 | | | | | | | |
| cg26729380 | chr6: 31543655 | -4.2 | -3.5 | -0.7 | 0.69 | 0.69 | | | | | | | |
| cg10650821 | chr6: 31543686 | -1.4 | -1.5 | 0.1 | 0.97 | 0.64 | | | | | | | |
| | chr6: 31544695 | | | | | | 6.2 | 7.4 | -1.2 | 0.84 | 0.73 | | |
| | chr6: 31544752 | | | | | | 8.0 | 9.0 | -1.1 | 0.81 | 0.53 | | |
| | chr6: 31544800 | | | | | | 2.3 | 1.5 | 0.8 | 0.59 | 0.78 | | |
| | chr6: 31544822 | | | | | | 4.4 | 6.1 | -1.7 | 0.61 | 0.28 | | |
| | chr6: 31544848 | | | | | | -0.6 | 0.2 | -0.7 | 0.29 | 0.04 | | |
| cg01360627 | chr6: 31544931 | 10.3 | 8.0 | 2.3 | 0.36 | 0.58 | 1.5 | 1.4 | 0.1 | 0.93 | 0.47 | | |
| cg23384708 | chr6: 31544934 | 6.7 | 8.6 | -2.0 | 0.43 | <u>0.01</u> | 6.6 | 9.2 | -2.6 | 0.56 | 0.22 | | |
| cg20477259 | chr6: 31544960 | 10.9 | 10.0 | 0.9 | 0.73 | 0.27 | 7.5 | 6.6 | 0.9 | 0.82 | 0.89 | | |
| | chr6: 31545253 | | | | | | 4.4 | 3.6 | 0.8 | 0.77 | 0.89 | | |
| | chr6: 31545258 | | | | | | 3.5 | 3.9 | -0.4 | 0.89 | 0.72 | | |
| | chr6: 31545267 | | | | | | 3.7 | 6.5 | -2.8 | 0.41 | 0.16 | | |
| cg15989608 | chr6: 31545322 | 8.7 | 8.2 | 0.4 | 0.86 | 0.10 | -0.7 | 4.4 | -5.1 | 0.06 | <u>0.02</u> | | |
| cg26736341 | chr6: 31545343 | 9.3 | 9.4 | -0.1 | 0.95 | 0.09 | 4.6 | 2.1 | 2.6 | 0.40 | 0.31 | | |
| | chr6: 31545432 | | | | | | 1.5 | 3.2 | -1.7 | 0.71 | 0.65 | | |
| cg04472685 | chr6: 31545474 | 6.0 | 6.2 | -0.2 | 0.90 | 0.12 | 3.1 | 3.0 | 0.2 | 0.94 | 0.87 | | |
| cg19124225 | chr6: 31545836 | 1.7 | 0.2 | 1.5 | 0.11 | 0.52 | | | | | | | |
| cg02137984 | chr6: 31545899 | 3.1 | 1.4 | 1.7 | 0.09 | 0.92 | | | | | | | |
| cg06825478 | chr6: 31546068 | 3.6 | 4.2 | -0.6 | 0.77 | 0.17 | 2.6 | 2.4 | 0.2 | 0.84 | 0.96 | | |
| | chr6: 31546086 | | | | | | 4.3 | 4.2 | 0.1 | 0.97 | 0.81 | | |

Notes:

underline nominal significance (P < 0.05)

adjusted p-value (EPIC analysis); multiple regression analysis results corrected by age, sex, and cell proportion.

adjusted p-value (pyrosequencing); multiple regression analysis results corrected by age and sex.

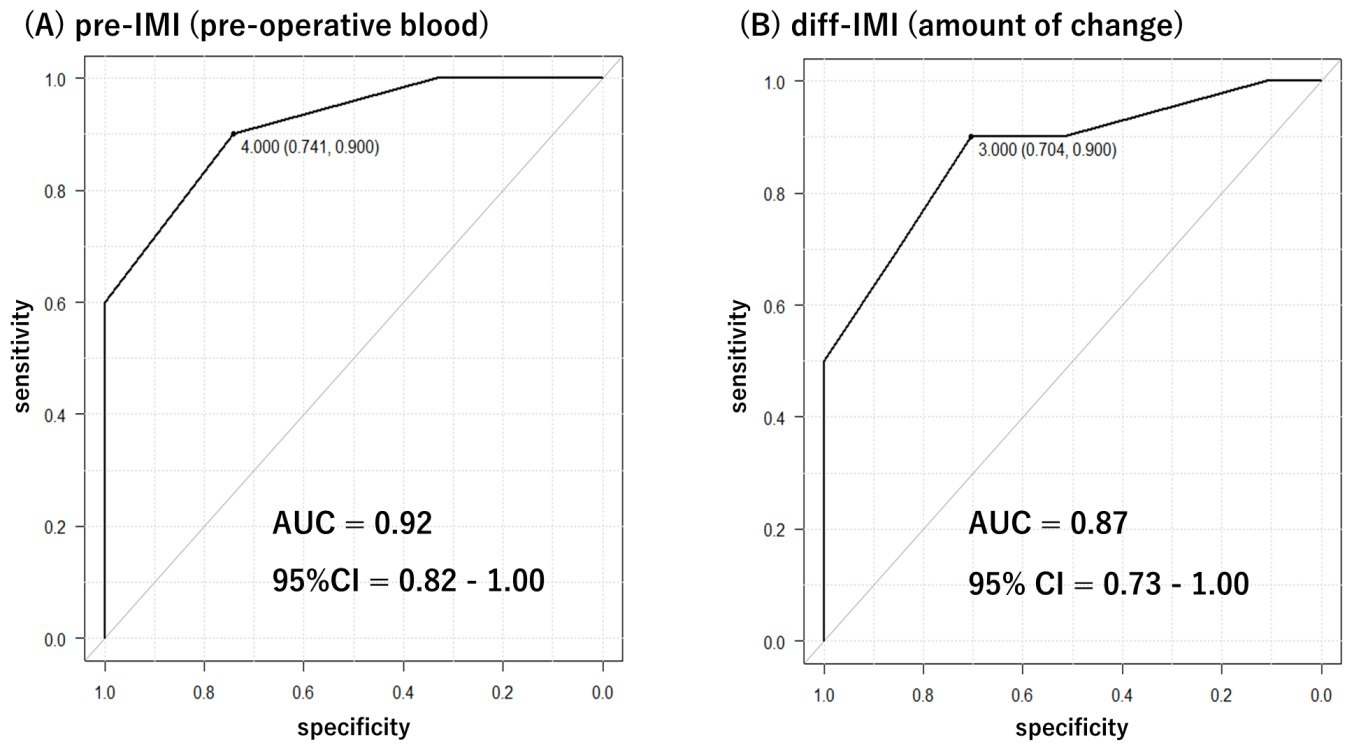
Supplementary Table 10. Comparisons of degree of DNAm change by neurosurgery between POD and non-POD in the IL1B and IL6 gene

| cg | POD | non-POD | diff | t-test p-value | adjusted p-value |
|---|--------------------------|--------------------------|------|-------------------|---------------------|
| | N=10 post-pre mean | N=27 post-pre mean | | | |
| IL1B | | | | | |
| cg01290568 | -13.4 | -9.1 | -4.3 | 0.23 | 0.90 |
| cg02596281 | -2.6 | -1.3 | -1.2 | 0.41 | 0.90 |
| cg07250315 | -6.9 | -3.6 | -3.3 | 0.11 | 0.59 |
| cg07935264 | -3.2 | -2.4 | -0.9 | 0.52 | 0.36 |
| cg10486274 | -0.7 | -0.5 | -0.2 | 0.77 | 0.63 |
| cg14117934 | -0.9 | -0.7 | -0.2 | 0.91 | 0.64 |
| cg15218327 | -2.8 | -3.7 | 1.0 | 0.45 | 0.66 |
| cg15836722 | -10.1 | -4.7 | -5.4 | <u>0.046</u> | <u>0.04</u> |
| cg18635064 | -0.8 | -0.1 | -0.7 | 0.36 | 0.63 |
| cg18773937 | -2.5 | -2.3 | -0.3 | 0.82 | 0.25 |
| cg19890119 | -9.2 | -5.9 | -3.4 | 0.19 | 0.96 |
| cg20157753 | -1.7 | -0.2 | -1.5 | <u>0.03</u> | 0.25 |
| cg20983042 | -0.6 | 0.2 | -0.8 | 0.60 | 0.98 |
| cg23149881 | -7.4 | -6.0 | -1.4 | 0.57 | 0.27 |
| IL6 | | | | | |
| cg00087425 | -0.1 | 0.3 | -0.4 | 0.64 | 0.28 |
| cg00175482 | -2.9 | -1.9 | -1.0 | 0.50 | 0.66 |
| cg01770232 | 0.1 | 0.6 | -0.6 | 0.37 | 0.67 |
| cg02335517 | -0.3 | -0.9 | 0.6 | 0.35 | 0.29 |
| cg03601896 | -1.3 | -0.6 | -0.8 | 0.28 | 0.76 |
| cg05265849 | -4.5 | -1.8 | -2.7 | <u>0.04</u> | 0.28 |
| cg07998387 | -1.3 | 1.4 | -2.7 | <u>0.04</u> | 0.31 |
| cg10140158 | 0.2 | 0.2 | 0.0 | >0.99 | 0.93 |
| cg13104385 | -2.6 | -0.1 | -2.5 | <u>0.04</u> | 0.13 |
| cg15703690 | -5.4 | -3.2 | -2.2 | 0.15 | 0.85 |
| cg17067544 | 5.7 | 4.2 | 1.5 | 0.39 | 0.75 |
| cg20509117 | 0.2 | -0.7 | 0.9 | 0.70 | 0.40 |
| cg21785978 | -0.8 | -0.1 | -0.6 | 0.45 | 0.18 |
| cg23731304 | -5.2 | -2.3 | -3.0 | 0.08 | 0.23 |
| Notes: underline nominal significance (P < 0.05) adjusted p-value; multiple regression analysis results corrected by age, sex, and cell proportion. | | | | | |

Supplementary Figures

Supplementary Figure 1 is shown in “Supplementary method” section.

Supplementary Figure 2



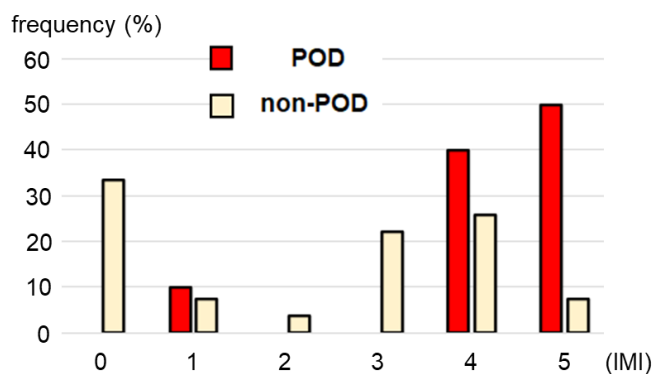
Supplementary Figure 2

(A) ROC curve based on pre-IMI from pre-operative blood

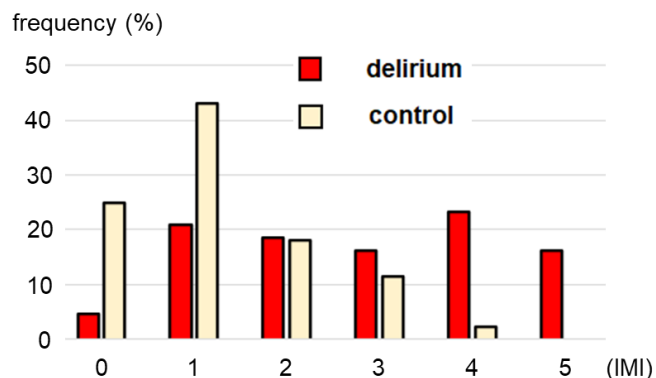
(B) ROC curve based on diff-IMI from amount of change of DNAm

Supplementary Figure 3

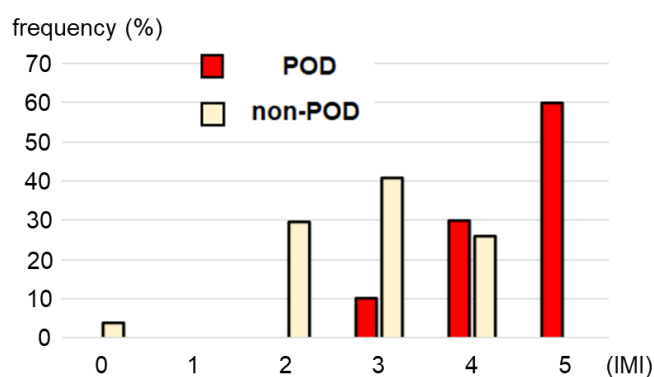
(A) IMI (post-operative blood)



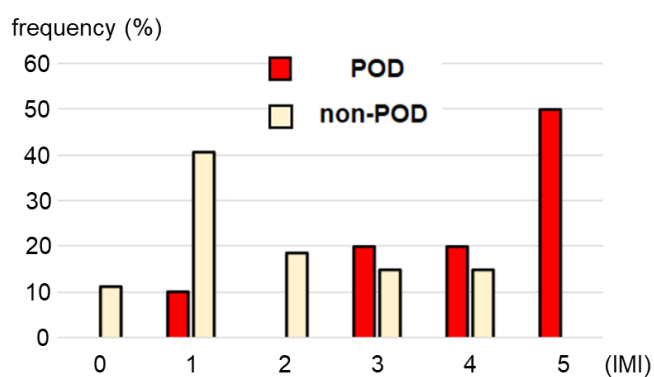
(B) IMI (the validation dataset)



(C) pre-IMI (pre-operative blood)



(D) Distribution of diff-IMI (amount of change)



Supplementary Figure 3

- (A) Distribution of IMI (post-operative blood)
- (B) Distribution of IMI (the validation dataset)
- (C) Distribution of pre-IMI (pre-operative blood)
- (D) Distribution of diff-IMI (amount of change)