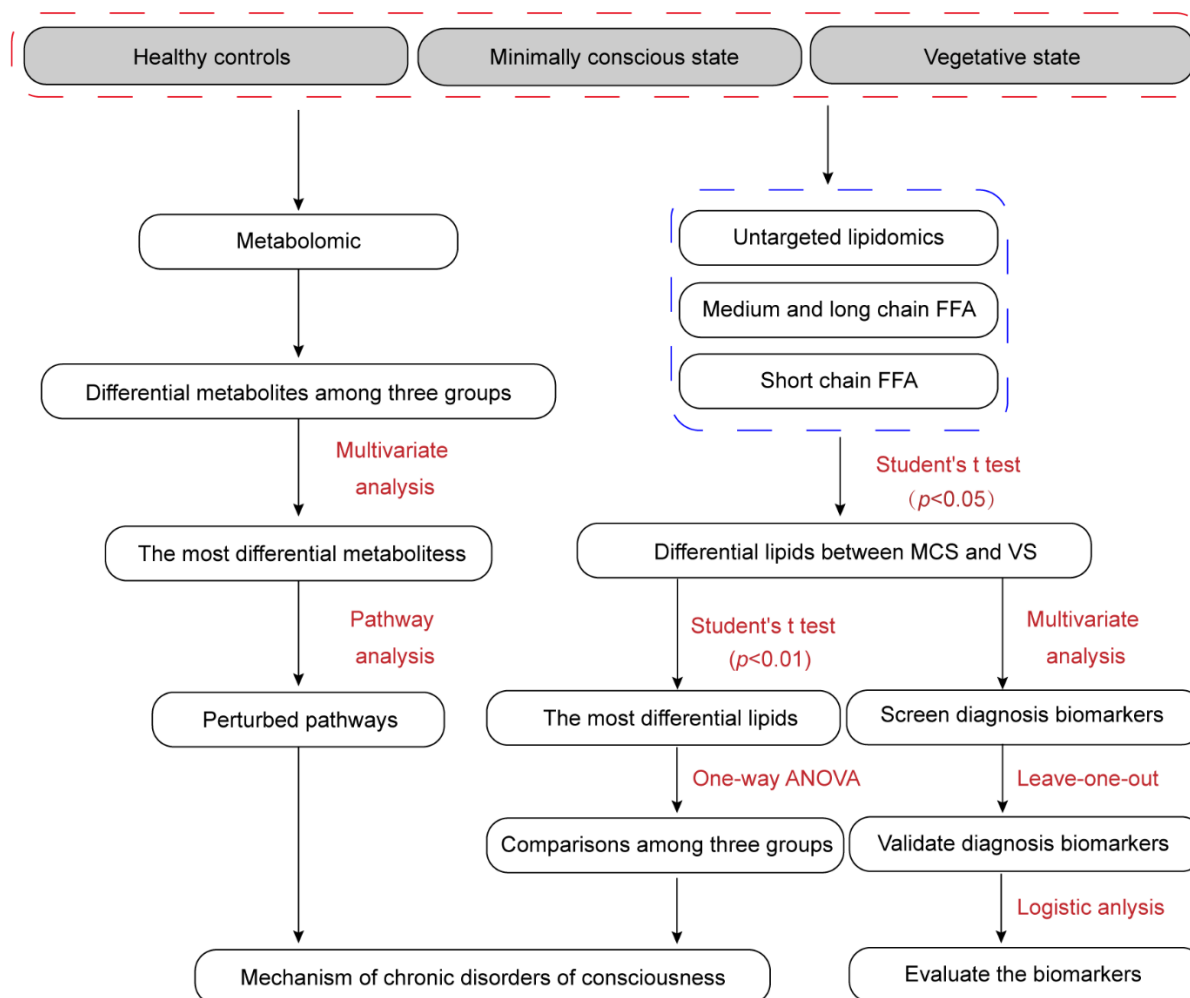SUPPLEMENTARY DATA

# Metabolic Abnormalities in Patients with Chronic Disorders of Consciousness

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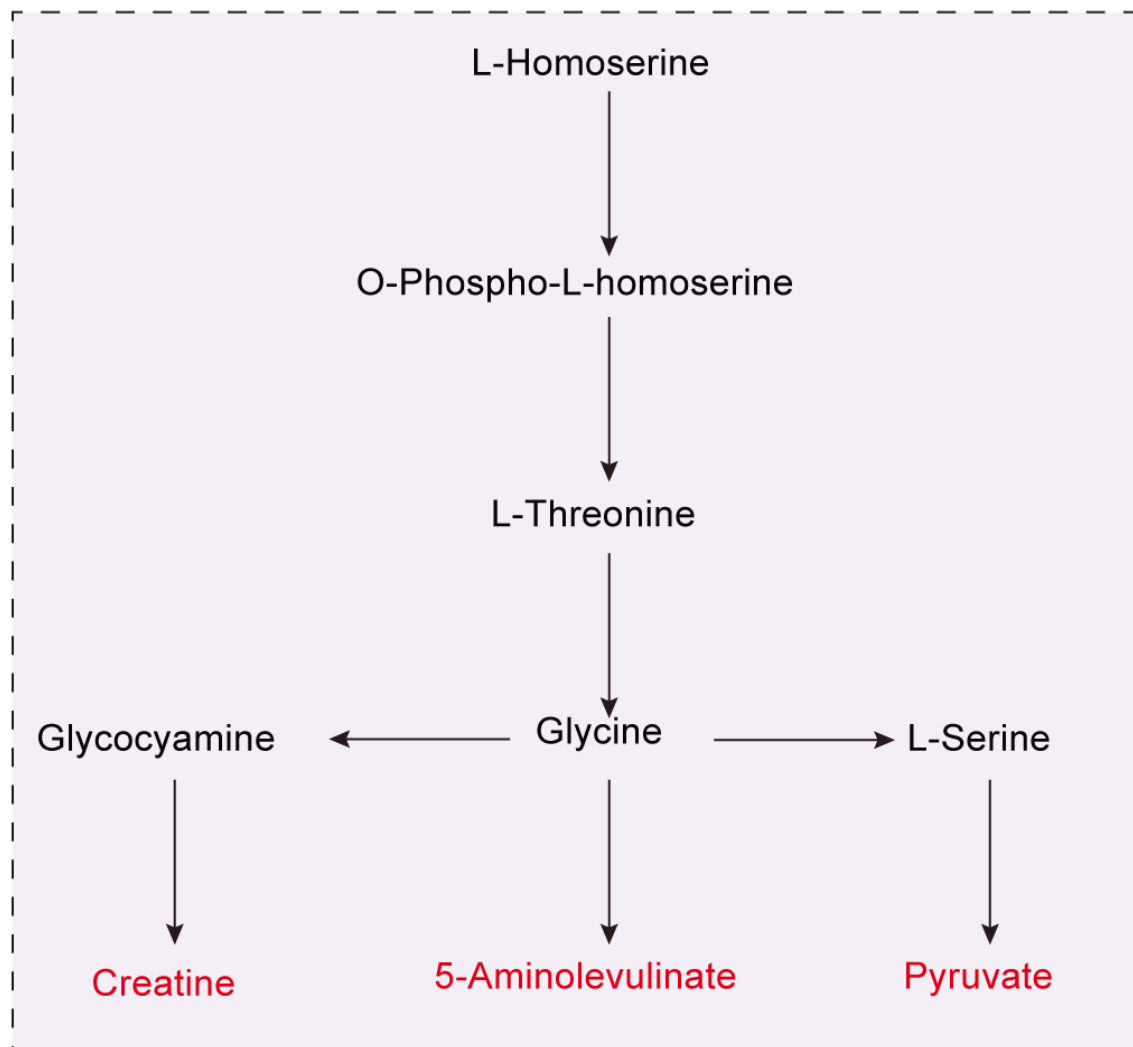
# SUPPLEMENTARY DATA



**Supplementary Figure 1**. **Flowchart of the experimental workflow for the study.** Healthy controls (HS), patients in vegetative state (VS) and minimally conscious state (MCS) were recruited for metabolomic and lipidomic studies. Both metabolite and lipid change specific to different levels of consciousness were examed. Potential lipids or metabolites that are responsible for distinguishing VS and MCS groups were identified in our analysis. FFA: free fatty acid. VS: vegetative state; MCS: minimally conscious state.
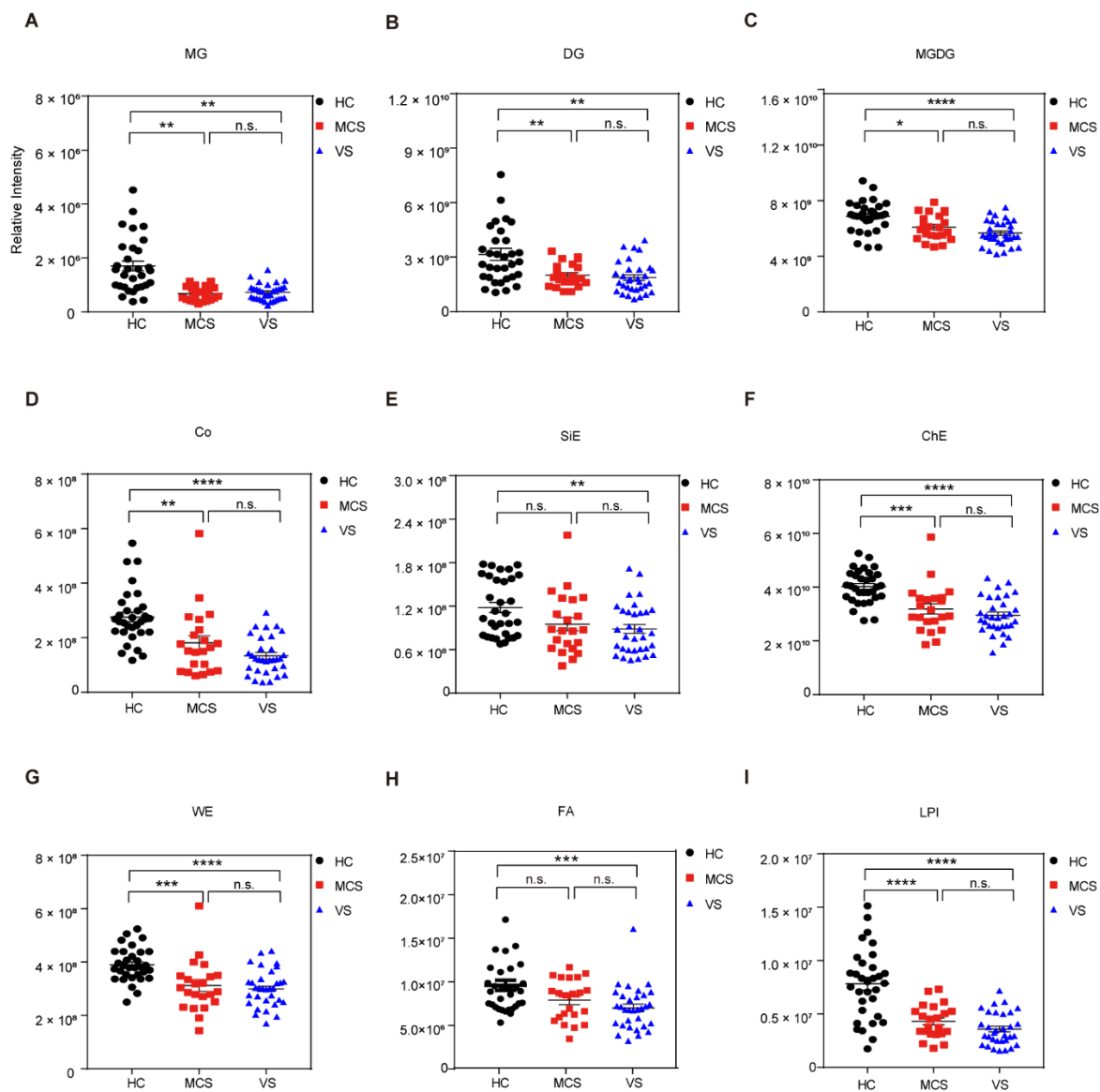
# SUPPLEMENTARY DATA

## Glycine, serine and threonine metabolism pathway



**Supplementary Figure 2. Glycine, serine and threonine metabolism pathways in HC, MCS, and VS groups.** Metabolites highlighted in red color were the ones that increased in these pathways in MCS and VS groups compared to HC group. HC: healthy controls; VS: vegetative state; MCS: minimally conscious state.

**Supplementary Figure 3. Different levels of lipid subclasses among the HC, MCS and VS groups.** Data represent as the mean ± SEM; *$P<0.05$, **$P<0.01$, ***$P<0.001$, ****$P<0.0001$, n.s.: no significant difference, one-way ANOVA. PE: phosphatidylethanolamine; PS: phosphatidylserine; PG: phosphatidylglycerol; PI: phosphatidylinositol; Cer: ceramides; CerG1: simple glc series; HC: healthy controls; VS: vegetative state; MCS: minimally conscious state.

# SUPPLEMENTARY DATA
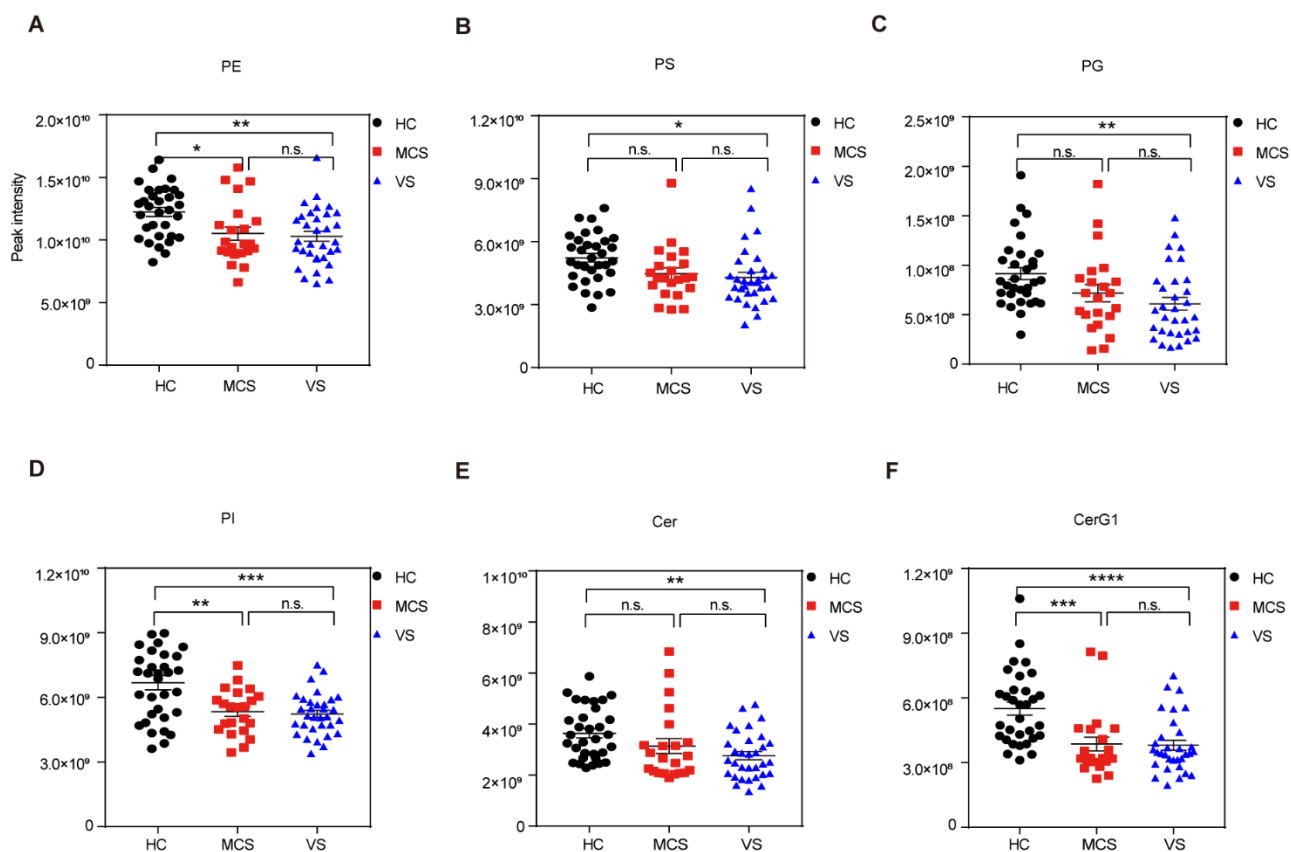


**Supplementary Figure 4. Different levels of lipid subclasses among the HC, MCS and VS groups.** Data represent as the mean ± SEM; *$P<0.05$, **$P<0.01$, ***$P<0.001$, ****$P<0.0001$, n.s.: no significant difference, one-way ANOVA. MG: monoglyceride; DG: diglyceride; MGDG: monogalactosyldiacylglycerol; Co: coenzyme; SiE: sitosterol ester; ChE: cholesterol ester; WE: wax esters; FA: fatty acid; LPI: lysophosphatidylinositol; HC: healthy controls; VS: vegetative state; MCS: minimally conscious state.

**Supplementary Table 1.** Clinical characteristics of AD patients, EMCS patients and healthy controls.

| Metabonomics | | | | *P* value | | |
|---|---|---|---|---|---|---|
| Characteristics | HCs | AD | EMCS | HCs-EMCS | HCs-AD | EMCS-AD |
| Patients(n) | 6 | 19 | 15 | / | / | / |
| Male/Female(n) | 2/4 | 11/8 | 10/5 | 0.163 | 0.294 | 0.601 |
| Age(years) | 55.8 ± 4.8 | 70.5 ± 8.3 | 55.1 ± 8.7 | 0.639 | <0.001 | <0.001 |
| GCS | / | / | 7.2±1.3 | / | / | / |
| CRS-R | / | / | 21.9 | / | / | / |
| Cause | / | / | TBI | / | / | / |

Continuous variables are expressed as the mean ± standard deviation (SD); TBI: traumatic brain injury; GCS: Glasgow Coma Scale; CRS-R: Coma Recovery Scale-Revised scores; HCs: healthy controls; AD: Alzheimer's disease; EMCS: Emerged from Minimally Conscious State.

# SUPPLEMENTARY DATA

**Supplementary Table 2.** List of putatively identified lipid molecules differing between MCS and VS groups. The eleven lipids in the table are those with significant differences between these two groups ($P<0.01$, Student's $t$-test).

| Lipids | Class | RT (min) | Fold change | $P$ value |
|---|---|---|---|---|
| AcCa(10:1)+H | AcCa | 1.613 | 0.550 | 0.0005 |
| AcCa(16:1)+H | AcCa | 2.568 | 0.681 | 0.0012 |
| PC(34:4)+H | PC | 10.237 | 0.579 | 0.0013 |
| AcCa(12:1)+H | AcCa | 1.820 | 0.473 | 0.0024 |
| PC(16:1/16:1)+HCOO | PC | 10.455 | 0.613 | 0.0045 |
| PC(16:0/22:5)+HCOO | PC | 11.439 | 0.794 | 0.0053 |
| AcCa(14:0)+H | AcCa | 2.408 | 0.699 | 0.0054 |
| PC(14:0/20:4)+HCOO | PC | 10.248 | 0.601 | 0.0062 |
| SM(d41:0)+HCOO | SM | 15.524 | 0.715 | 0.0069 |
| AcCa(14:2)+H | AcCa | 1.881 | 0.662 | 0.0094 |
| So(d18:1)+H | So | 3.023 | 0.582 | 0.0099 |

RT: Retention time; AcCa: acylcarnitine; PC: phosphatidylcholine; SM: sphingomyelin; So: sphingosine. VS: vegetative state; MCS: minimally conscious state

**Supplementary Table 3.** List of putatively identified lipid molecules differing between VS and MCS groups based on VIP >1 (PLSDA) and $P <0.05$ (Student's $t$-test).

| Lipids | Class | RT (min) | Fold change | $P$ value | VIP |
|---|---|---|---|---|---|
| PC(14:0/20:4)+HCOO | PC | 23.608 | 0.858 | 0.026 | 8.407 |
| ChE(18:1)+NH$_4$ | ChE | 10.439 | 0.690 | 0.017 | 4.498 |
| Cer(d18:1/23:0)+HCOO | Cer | 13.519 | 0.794 | 0.005 | 4.168 |
| PC(38:5)+H | PC | 11.727 | 0.775 | 0.035 | 3.016 |
| PC(16:0/22:5)+HCOO | PC | 13.107 | 0.801 | 0.014 | 2.703 |
| SM(d40:2)+H | SM | 12.747 | 0.786 | 0.046 | 2.126 |
| PC(18:0/20:4)+HCOO | PC | 21.796 | 0.833 | 0.044 | 1.669 |
| PC(38:3)+H | PC | 16.800 | 0.723 | 0.031 | 1.539 |
| PC(32:2)+H | PC | 10.248 | 0.787 | 0.025 | 1.473 |
| PC(16:0p/22:1)+HCOO | PC | 14.933 | 1.376 | 0.014 | 1.280 |
| PC(40:5)+H | PC | 11.439 | 0.741 | 0.028 | 1.218 |
| PC(20:0/20:4)+HCOO | PC | 12.385 | 0.601 | 0.006 | 1.217 |
| TG(16:0/18:1/18:3)+NH$_4$ | TG | 13.091 | 0.710 | 0.025 | 1.151 |

RT: Retention time; VIP: variable importance in the projection; VS: vegetative state; MCS: minimally conscious state

**Supplementary Table 4.** The diagnostic performance of the lipids in 14-lipid panel.

| Lipids name | AUC | Specificity | Sensitivity | $P$ value | 95%CI |
|---|---|---|---|---|---|
| ChE(18:1)-NH$_4$ | 0.609 | 0.219 | 1.000 | 0.175 | 0.454- 0.765 |
| PC(32:2)-H | 0.669 | 0.781 | 0.591 | 0.036 | 0.513- 0.825 |
| SM(d40:2)-H | 0.676 | 0.875 | 0.455 | 0.029 | 0.525- 0.827 |
| PC(38:5)-H | 0.690 | 0.875 | 0.591 | 0.018 | 0.536- 0.844 |
| PC(38:3)-H | 0.690 | 0.500 | 0.909 | 0.018 | 0.548- 0.833 |
| PC(40:5)-H | 0.682 | 0.438 | 0.909 | 0.024 | 0.539- 0.824 |
| TG(16:0/18:1/18:3)-NH$_4$ | 0.696 | 0.625 | 0.773 | 0.015 | 0.555- 0.838 |
| Cer(d18:1/23:0)-HCOO | 0.663 | 0.375 | 0.909 | 0.043 | 0.517- 0.810 |
| PC(14:0/20:4)-HCOO | 0.702 | 0.813 | 0.591 | 0.012 | 0.553- 0.850 |
| PC(16:0p/22:1)-HCOO | 0.695 | 0.844 | 0.591 | 0.016 | 0.545- 0.844 |
| PC(16:0/22:5)-HCOO | 0.706 | 0.750 | 0.636 | 0.011 | 0.558- 0.854 |
| PC(18:0/20:4)-HCOO | 0.697 | 0.875 | 0.500 | 0.014 | 0.551- 0.844 |
| PC(20:0/20:4)-HCOO | 0.648 | 0.344 | 0.955 | 0.067 | 0.499- 0.797 |
| Arachidonic acid | 0.701 | 0.778 | 0.565 | 0.010 | 0.564- 0.837 |

AUC: area under the ROC curve; ROC: Receiver-Operating Characteristic.

# SUPPLEMENTARY DATA

**Supplementary Table 5.** The cause of each patient with VS, MCS or EMCS.

| No. | State | Gender | Age | Cause |
|-----|-------|--------|-----|-------|
| **Lipidomics** | | | | |
| 1 | MCS-13 | Male | 51 | Fall |
| 2 | MCS-56 | Male | 70 | Traffic accident |
| 3 | MCS-72 | Male | 35 | Traffic accident |
| 4 | MCS-80 | Female | 49 | Traffic accident |
| 5 | MCS-98 | Female | 53 | Traffic accident |
| 6 | MCS-104 | Female | 60 | Fall |
| 7 | MCS-110 | Male | 21 | Traffic accident |
| 8 | MCS-134 | Male | 44 | Traffic accident |
| 9 | MCS-157 | Female | 51 | Traffic accident |
| 10 | MCS-158 | Male | 60 | Traffic accident |
| 11 | MCS-162 | Female | 56 | Fall |
| 12 | MCS-166 | Male | 56 | Traffic accident |
| 13 | MCS-167 | Male | 68 | Traffic accident |
| 14 | MCS-170 | Male | 44 | Fall |
| 15 | MCS-171 | Male | 31 | Traffic accident |
| 16 | MCS-180 | Female | 40 | Traffic accident |
| 17 | MCS-199 | Female | 65 | Traffic accident |
| 18 | MCS-201 | Male | 55 | Fall |
| 19 | MCS-202 | Female | 59 | Fall |
| 20 | MCS-221 | Female | 58 | Traffic accident |
| 21 | MCS-222 | Male | 53 | Traffic accident |
| 22 | MCS-227 | Female | 70 | Traffic accident |
| 23 | VS04 | Male | 64 | Fall |
| 24 | VS12 | Female | 49 | Traffic accident |
| 25 | VS14 | Male | 68 | Fall |
| 26 | VS28 | Male | 63 | Traffic accident |
| 27 | VS35 | Male | 27 | Traffic accident |
| 28 | VS53 | Male | 67 | Traffic accident |
| 29 | VS59 | Male | 44 | Traffic accident |
| 30 | VS66 | Male | 55 | Fall |
| 31 | VS67 | Male | 23 | Traffic accident |
| 32 | VS68 | Female | 55 | Traffic accident |
| 33 | VS78 | Female | 49 | Traffic accident |
| 34 | VS81 | Male | 30 | Fall |
| 35 | VS99 | Female | 43 | Traffic accident |
| 36 | VS107 | Female | 24 | Traffic accident |
| 37 | VS133 | Male | 21 | Traffic accident |
| 38 | VS154 | Female | 53 | Traffic accident |
| 39 | VS155 | Male | 43 | Traffic accident |
| 40 | VS160 | Male | 52 | Traffic accident |
| 41 | VS161 | Female | 67 | Traffic accident |
| 42 | VS163 | Male | 62 | Traffic accident |
| 43 | VS164 | Male | 42 | Fall |
| 44 | VS165 | Male | 49 | Traffic accident |
| 45 | VS168 | Male | 16 | Traffic accident |
| 46 | VS187 | Male | 60 | Traffic accident |
| 47 | VS189 | Male | 67 | Traffic accident |
| 48 | VS200 | Male | 52 | Traffic accident |
| 49 | VS214 | Male | 35 | Traffic accident |
| 50 | VS220 | Male | 72 | Traffic accident |
| 51 | VS226 | Male | 49 | Fall |
| 52 | VS228 | Male | 55 | Traffic accident |
| 53 | VS237 | Female | 68 | Fall |
| 54 | VS244 | Male | 44 | Traffic accident |
| **Metabolomics** | | | | |
| 1 | VS1 | Male | 43 | Traffic accident |
| 2 | MCS2 | Male | 43 | Traffic accident |
| 3 | VS3 | Female | 63 | Traffic accident |

# SUPPLEMENTARY DATA

| 4 | VS4 | Female | 63 | Traffic accident |
|---|---|---|---|---|
| 5 | VS5 | Female | 63 | Traffic accident |
| 6 | VS6 | Male | 56 | Traffic accident |
| 7 | VS7 | Male | 64 | Fall |
| 8 | VS8 | Male | 69 | Traffic accident |
| 9 | MCS9 | Male | 70 | Traffic accident |
| 10 | MCS10 | Male | 70 | Traffic accident |
| 11 | VS11 | Male | 77 | Fall |
| 12 | VS13 | Male | 77 | Fall |
| 13 | MCS14 | Male | 61 | Fall |
| 14 | MCS15 | Male | 66 | Traffic accident |
| 15 | MCS16 | Male | 66 | Traffic accident |
| 16 | MCS17 | Female | 63 | Traffic accident |
| 17 | MCS18 | Female | 63 | Traffic accident |
| 18 | MCS19 | Female | 54 | Traffic accident |
| 19 | VS20 | Male | 68 | Fall |
| 20 | VS21 | Male | 68 | Fall |
| 21 | VS22 | Male | 68 | Fall |
| 22 | MCS23 | Female | 61 | Traffic accident |
| 23 | MCS24 | Female | 61 | Traffic accident |
| 24 | EMCS5 | Male | 44 | Traffic accident |
| 25 | EMCS14 | Male | 58 | Traffic accident |
| 26 | EMCS23 | Male | 69 | Traffic accident |
| 27 | EMCS25 | Female | 55 | Fall |
| 28 | EMCS36 | Male | 53 | Traffic accident |
| 29 | EMCS39 | Female | 50 | Fall |
| 30 | EMCS43 | Female | 54 | Traffic accident |
| 31 | EMCS301 | Male | 60 | Traffic accident |
| 32 | EMCS203 | Female | 46 | Traffic accident |
| 33 | EMCS209 | Female | 46 | Traffic accident |
| 34 | EMCS210 | Male | 57 | Fall |
| 35 | EMCS4 | Male | 45 | Traffic accident |
| 36 | EMCS110 | Male | 69 | Fall |
| 37 | EMCS207 | Male | 69 | Traffic accident |
| 38 | EMCS3 | Male | 51 | Fall |

VS: vegetative state; MCS: minimally conscious state; EMCS: emerged from a minimally conscious state.