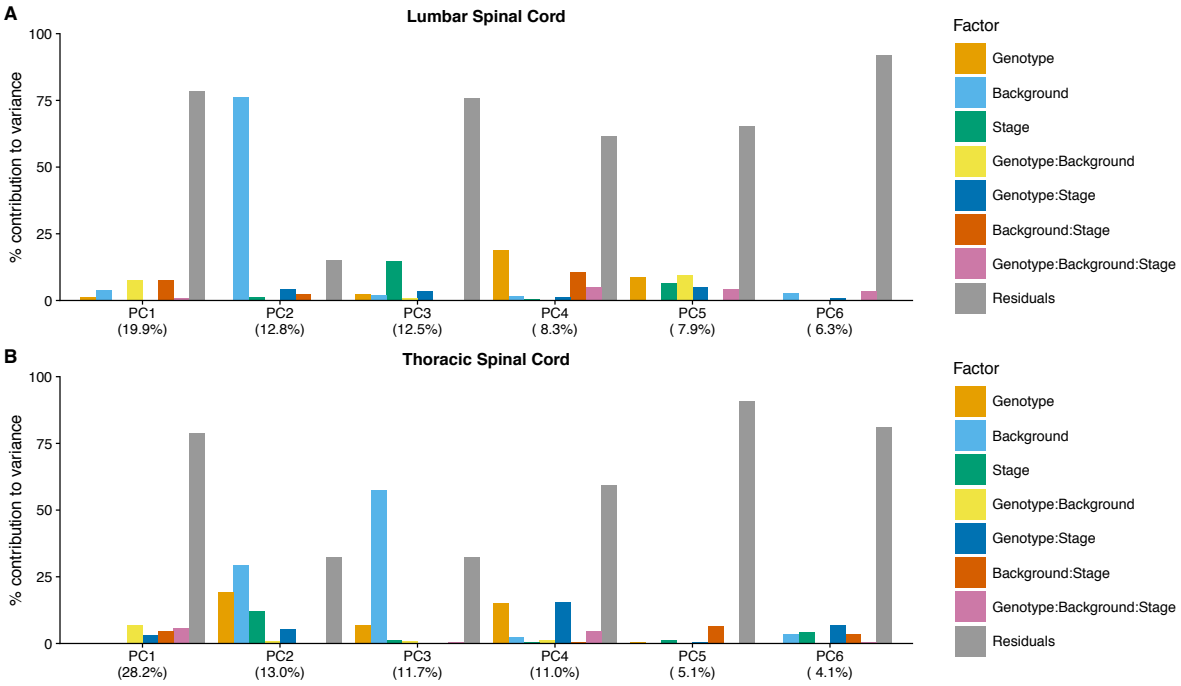
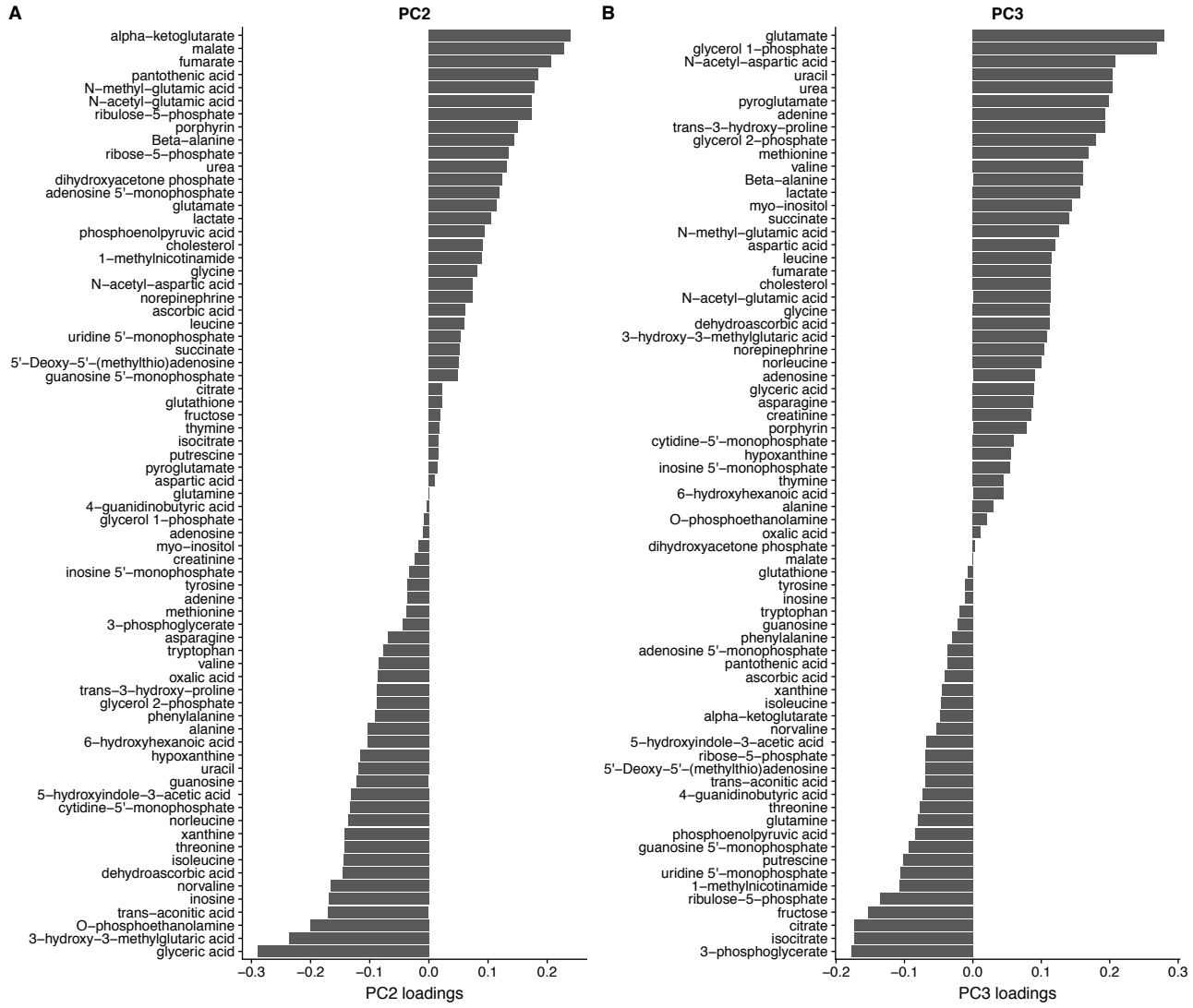
# *Supplementary Material*

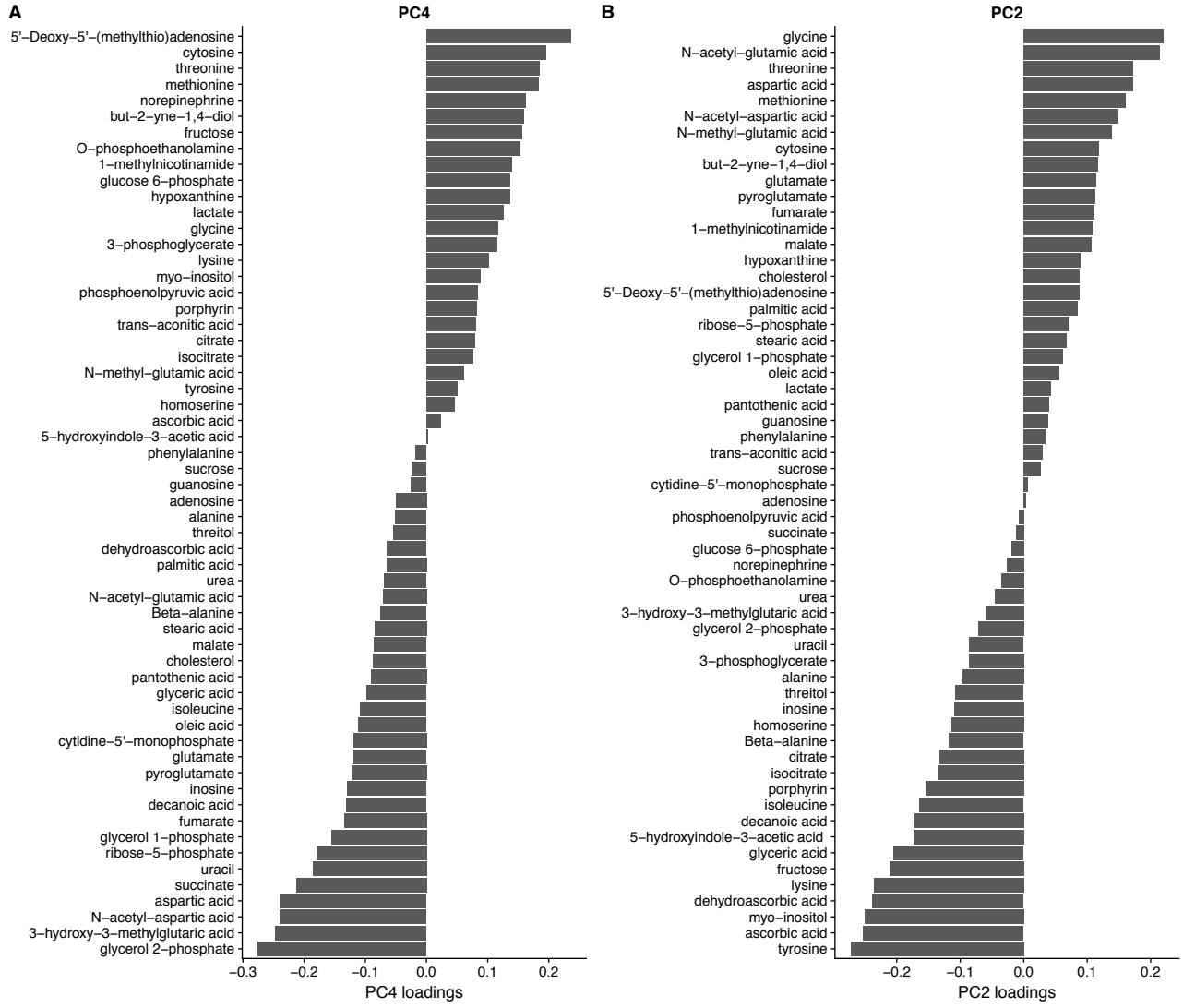
## 1 Supplementary Figures



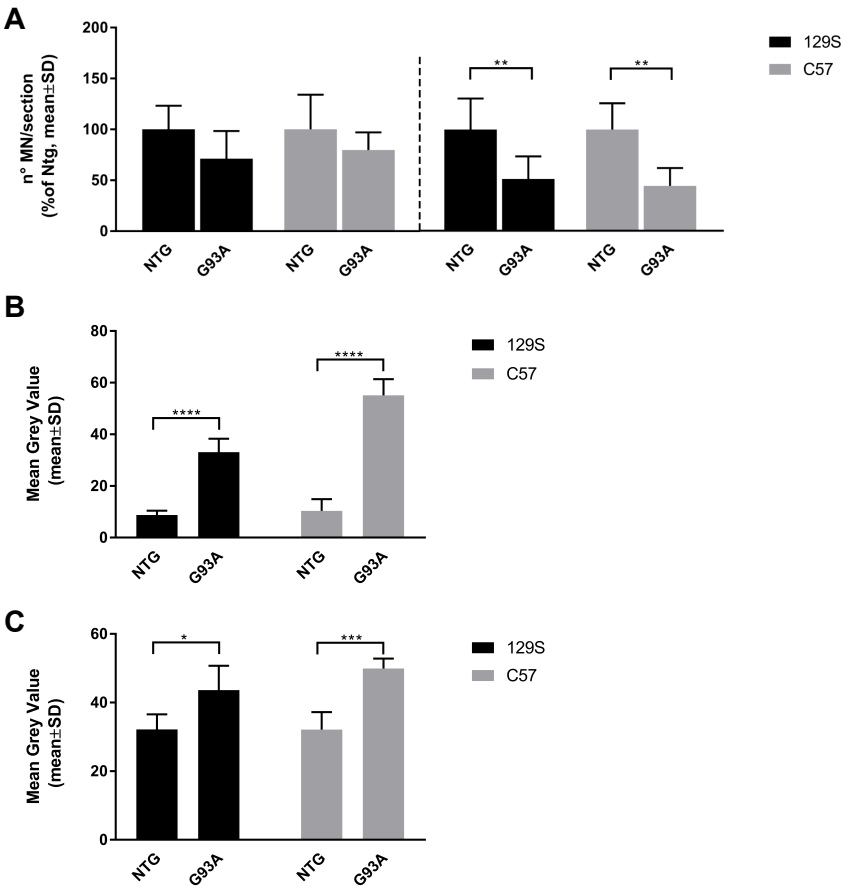

**Supplementary Figure 1. Percent contribution to variance of the experimental factors examined in the first 6 principal components of (A) lumbar spinal cord and (B) thoracic spinal cord metabolic profiles.** Effects of the three experimental factors SOD1 genotype, mouse background, and disease stage, as well as their two-way and three-way interaction effects were examined using a linear model.




**Supplementary Figure 2. Lumbar spinal cord principal component analysis loadings for PC2 and PC3.**




**Supplementary Figure 3. Thoracic spinal cord principal component analysis loadings for PC4 and PC2.**




**Supplementary Figure 4. Neuropathological alterations in the thoracic segment of the spinal cord of two SOD1$^{G93A}$ mouse strains and their respective non-transgenic mice. (**A) Motor neuron count in thoracic spinal cord at different disease stages. Neurons were labeled with Neurotrace and motor neurons identified by the soma dimension (area ≥ 400 μm$^2$). A slight but not significant decrease in the number of motor neurons was observed in both SOD1$^{G93A}$ mouse strains compared to their respective non-transgenic (NTG) mice at the onset of symptoms (left) while this effect becomes more evident and significant in the late symptomatic stage (right). Quantification of (B) GFAP and (C) IBA-1 immunostaining in the ventral thoracic spinal cord of both SOD1$^{G93A}$ mouse strains at the onset of the symptoms. In both strains, a marked increase of reactive astrocytosis (GFAP) and microglia (IBA-1) were observed compared to Ntg mice (two-way ANOVA, n=4-5, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$).

## 2 Supplementary Tables

### Supplementary Table 1. Shapiro-Wilk normality test results and skewness measures for the lumbar spinal cord data

| metabolite | Untransformed data | | $log_{10}$-transformed data | |
|---|---|---|---|---|
| | Shapiro-Wilk p-value | Skewness | Shapiro-Wilk p-value | Skewness |
| lactate | 0.02040102 | 0.44774212 | 0.00355528 | -0.6788974 |
| norvaline | 0.02464713 | -0.4483184 | 4.00E-07 | -1.5736805 |
| oxalic acid | 0.03182764 | 0.73002326 | 0.15238701 | -0.3740703 |
| norleucine | 0.22865667 | 0.29792803 | 0.08599242 | -0.3844879 |
| isoleucine | 0.07870787 | -0.194213 | 0.00043436 | -0.7691025 |
| valine | 0.01442641 | 0.87469199 | 0.5125091 | 0.43932992 |
| urea | 0.00209265 | 0.18477593 | 2.50E-05 | -1.2983575 |
| glycine | 0.55609293 | -0.3125919 | 0.03186714 | -0.7058836 |
| succinate | 0.72650947 | -0.2178467 | 0.06231515 | -0.7016515 |
| glyceric acid | 3.97E-08 | 0.08127738 | 7.41E-08 | -0.1194463 |
| porphyrin | 0.00022435 | 1.2741266 | 0.7901108 | 0.16837924 |
| uracil | 0.38417161 | 0.4016033 | 0.64881809 | -0.2395524 |
| fumarate | 0.64582708 | 0.168718 | 0.26970727 | -0.3495089 |
| threonine | 0.00069051 | 0.89336327 | 0.00413958 | -0.4034829 |
| thymine | 0.06173438 | 0.14990475 | 0.00244254 | -0.9002933 |
| 6-hydroxyhexanoic acid | 0.15555482 | 0.44086269 | 0.20398432 | -0.497403 |
| Beta-alanine | 2.63E-05 | 1.5661566 | 0.45093345 | 0.39933794 |
| trans-3-hydroxy-proline | 0.0003028 | 1.22474793 | 0.92550157 | 0.15237982 |
| 1-methylnicotinamide | 1.71E-05 | 1.55691341 | 0.53396489 | 0.18708315 |
| malate | 0.22805966 | 0.10450474 | 0.10857986 | -0.4573087 |
| pyroglutamate | 0.00075429 | -1.1266884 | 1.63E-06 | -1.9948463 |
| 4-guanidinobutyric acid | 3.51E-07 | 1.92177285 | 0.60895133 | -0.2123755 |
| glutamine | 0.63885483 | -0.2750856 | 0.0049358 | -1.0176923 |
| phenylalanine | 0.21478261 | -0.4785612 | 0.00021901 | -1.3340476 |
| creatinine | 0.00497969 | 0.31149342 | 0.00555907 | -0.0821925 |
| alpha-ketoglutarate | 0.00256776 | 0.53439296 | 0.02108272 | -0.0188888 |
| phosphoenolpyruvic acid | 6.32E-05 | 1.2353991 | 0.66915803 | 0.02396782 |
| 3-hydroxy-3-methylglutaric acid | 0.00049511 | 0.48511189 | 0.01957794 | 0.00291231 |
| N-acetyl-aspartic acid | 0.01357195 | -0.7379926 | 9.00E-05 | -1.2114178 |
| asparagine | 0.01008885 | 0.39618716 | 0.07668982 | -0.3252969 |
| N-methyl-glutamic acid | 0.51435831 | 0.06729656 | 0.00050828 | -0.9631502 |
| glycerol 2-phosphate | 0.67180874 | -0.2605035 | 0.04287739 | -0.7051435 |
| putrescine | 7.98E-08 | 2.61618422 | 0.01309887 | 0.48321562 |
| trans-aconitic acid | 0.72658647 | 0.17391183 | 0.26853147 | -0.4373365 |

| | | | | |
|---|---|---|---|---|
| glycerol 1-phosphate | 0.01282367 | -0.4965239 | 0.00023376 | -1.0986612 |
| O-phosphoethanolamine | 0.02209108 | -0.2583668 | 0.00224189 | -0.5195303 |
| N-acetyl-glutamic acid | 0.53780941 | -0.2453589 | 0.00473258 | -0.9031522 |
| citrate | 0.00976504 | 0.22069631 | 0.00050769 | -1.0082123 |
| isocitrate | 0.01233934 | 0.21062277 | 0.00054206 | -1.0199078 |
| adenine | 0.02718839 | -0.6319295 | 0.00065156 | -1.1663856 |
| fructose | 7.77E-06 | 1.69792933 | 0.12990974 | 0.38651585 |
| dehydroascorbic acid | 0.28201975 | 0.45131139 | 0.31670763 | -0.3552899 |
| tyrosine | 0.00036111 | 1.03889042 | 0.02135616 | 0.60295468 |
| ascorbic acid | 0.04477959 | 0.62498373 | 0.47374722 | -0.3957052 |
| pantothenic acid | 0.56080716 | 0.33297749 | 0.75020579 | -0.24484 |
| xanthine | 0.01102294 | 0.87466317 | 0.87894989 | 0.21124864 |
| cytidine-5'-monophosphate | 0.11455722 | 0.65833491 | 0.69385748 | -0.165582 |
| myo-inositol | 0.00830092 | -0.7775153 | 5.50E-06 | -1.8797719 |
| ribose-5-phosphate | 0.01889108 | 0.85869809 | 0.91002644 | -0.1198442 |
| ribulose-5-phosphate | 0.00451063 | 0.88954007 | 0.59350212 | 0.07388209 |
| norepinephrine | 0.54534728 | -0.1697139 | 0.07993152 | -0.5536569 |
| glutathione | 0.00346837 | -0.4618695 | 2.16E-05 | -0.9943321 |
| tryptophan | 2.99E-06 | 1.64830312 | 0.23277442 | 0.33238321 |
| 5-hydroxyindole-3-acetic acid | 0.12365573 | 0.60737575 | 0.74893846 | -0.0344799 |
| inosine | 0.0223292 | 0.65371995 | 0.73814891 | -0.1032131 |
| adenosine | 0.00253102 | 1.04159204 | 0.97090648 | 0.02421294 |
| guanosine | 0.06175975 | 0.28783915 | 0.06950303 | -0.4048177 |
| 5'-Deoxy-5'-(methylthio)adenosine | 0.00358778 | -0.6534773 | 2.72E-05 | -1.2359627 |
| uridine 5'-monophosphate | 0.81084782 | 0.18233267 | 0.08355543 | -0.6625947 |
| inosine 5'-monophosphate | 0.06964811 | 0.24637823 | 0.12931126 | -0.2657276 |
| adenosine 5'-monophosphate | 0.5188134 | 0.05366184 | 0.1510513 | -0.4701019 |
| guanosine 5'-monophosphate | 0.30649007 | 0.4150819 | 0.20468945 | -0.4982112 |
| cholesterol | 0.0029408 | 0.92322202 | 0.85748265 | -0.1047519 |
| alanine | 0.67331689 | -0.106838 | 0.15436266 | -0.5234367 |
| leucine | 1.35E-05 | 1.42286619 | 0.07287744 | 0.61958219 |
| methionine | 0.00292055 | 0.94984167 | 0.12459749 | 0.55635668 |
| aspartic acid | 0.05443855 | 0.06704621 | 0.00749326 | -0.5719846 |
| glutamate | 0.43861664 | -0.154033 | 0.0065909 | -0.939417 |
| dihydroxyacetone phosphate | 0.02955529 | 0.67350542 | 0.52236933 | -0.0830992 |
| hypoxanthine | 0.00505988 | 0.87834761 | 0.67246834 | 0.22446778 |
| 3-phosphoglycerate | 0.65294438 | 0.29041497 | 0.54690206 | -0.4257234 |

**Supplementary Table 2. Shapiro-Wilk normality test results and skewness measures for the thoracic spinal cord data**

| metabolite | Untransformed data | | $log_{10}$-transformed data | |
|---|---|---|---|---|
| | Shapiro-Wilk p-value | Skewness | Shapiro-Wilk p-value | Skewness |
| lactate | 0.00103379 | 1.05339731 | 0.69415938 | 0.25179422 |
| alanine | 0.00735564 | 0.7498704 | 0.4241388 | 0.22380023 |
| isoleucine | 0.00902094 | 0.97468241 | 0.79362335 | 0.22874703 |
| but-2-yne-1,4-diol | 1.56E-06 | 1.13961873 | 0.00070159 | 0.42476312 |
| urea | 0.37492863 | 0.39270642 | 0.80549331 | -0.1209646 |
| threonine | 1.99E-06 | 1.52165647 | 0.04236388 | 0.46762387 |
| glycine | 1.74E-05 | 1.68067649 | 0.68504592 | 0.24298625 |
| succinate | 0.81674464 | 0.01967431 | 0.33271641 | -0.3921392 |
| glyceric acid | 1.13E-07 | 0.10065179 | 1.15E-07 | -0.0529294 |
| porphyrin | 0.0225825 | 0.67975201 | 0.17895658 | -0.0556902 |
| uracil | 0.01304499 | 0.64992778 | 0.07069526 | -0.1991665 |
| fumarate | 0.96235452 | 0.18759787 | 0.90898562 | -0.2733364 |
| methionine | 6.27E-12 | 3.12073146 | 8.58E-07 | 1.68519544 |
| aspartic acid | 0.95376795 | 0.02241364 | 0.25248719 | -0.5241439 |
| Beta-alanine | 5.32E-08 | 1.96812002 | 0.05310032 | 0.65913551 |
| homoserine | 4.61E-16 | 4.49694867 | 1.32E-14 | 3.90106115 |
| decanoic acid | 0.07447819 | -0.177188 | 0.02789525 | -0.4022251 |
| malate | 0.06701654 | 0.63739183 | 0.8461334 | 0.13875674 |
| 1-methylnicotinamide | 3.14E-09 | 2.376629 | 0.00393005 | 0.92504824 |
| threitol | 1.54E-05 | -1.6660659 | 3.66E-12 | -4.8243021 |
| glutamate | 0.0386482 | 0.68017333 | 0.28864605 | 0.2969733 |
| pyroglutamate | 0.1428269 | 0.50525941 | 0.48051248 | 0.17446407 |
| cytosine | 5.53E-08 | 2.04261499 | 6.00E-05 | 1.29368578 |
| phenylalanine | 0.12623879 | 0.52685874 | 0.82092098 | -0.0121978 |
| phosphoenolpyruvic acid | 0.00151762 | 1.00933 | 0.59301599 | 0.34488983 |
| 3-hydroxy-3-methylglutaric acid | 0.1784219 | -0.0501328 | 0.01469032 | -0.5217882 |
| N-acetyl-aspartic.acid | 0.29642219 | 0.33925689 | 0.43162884 | -0.1655918 |
| N-methyl-glutamic acid | 0.00016299 | 1.4441075 | 0.52276049 | -0.0833892 |
| glycerol 2-phosphate | 0.02528366 | -0.0908842 | 0.01445822 | -0.2710657 |
| trans-aconitic acid | 8.11E-08 | 1.76259298 | 0.00049528 | 0.99526882 |
| glycerol 1-phosphate | 0.0638909 | 0.62145339 | 0.46175349 | -0.1399812 |
| O-phosphoethanolamine | 0.00073611 | 0.99772177 | 0.0291486 | -0.1319156 |
| N-acetyl-glutamic acid | 9.93E-07 | 2.21121056 | 0.11275192 | 0.62598278 |
| hypoxanthine | 1.33E-12 | 3.12954232 | 0.00034524 | 1.08934277 |
| 3-phosphoglycerate | 0.35351395 | 0.09395988 | 0.1245176 | -0.4833503 |

| | | | | |
|---|---|---|---|---|
| citrate | 0.02929924 | 0.65182437 | 0.70297506 | 0.20787004 |
| isocitrate | 0.03314647 | 0.6463238 | 0.72094896 | 0.20662518 |
| dehydroascorbic acid | 0.00222611 | 0.17407475 | 0.00134397 | -0.4153103 |
| fructose | 8.42E-08 | 2.15137802 | 0.00114342 | 1.06642477 |
| lysine | 5.01E-10 | 2.99524522 | 0.01466412 | 0.84084986 |
| tyrosine | 2.24E-05 | 1.06077351 | 0.02207511 | 0.44155422 |
| ascorbic acid | 5.35E-10 | 1.93218434 | 0.5761648 | 0.04665431 |
| pantothenic acid | 0.14802669 | 0.47392104 | 0.76992519 | 0.063144 |
| cytidine-5'-monophosphate | 0.54582839 | 0.18660501 | 0.39166289 | -0.2863956 |
| palmitic acid | 0.00039965 | 1.12812855 | 0.03730601 | 0.60340087 |
| myo-inositol | 0.0006933 | 1.09129241 | 0.01024614 | 0.84003518 |
| ribose-5-phosphate | 0.54810443 | -0.1480995 | 0.00014226 | -1.437473 |
| norepinephrine | 2.99E-06 | 1.5987109 | 0.01262846 | 0.43267124 |
| 5-hydroxyindole-3-acetic acid | 0.01695962 | 0.75991055 | 0.72221248 | 0.10595959 |
| oleic acid | 3.18E-05 | 1.50818689 | 0.24663216 | 0.3142163 |
| stearic acid | 1.35E-07 | 2.44299905 | 0.00032749 | 1.32640923 |
| glucose 6-phosphate | 1.74E-08 | 2.06391493 | 0.92507777 | 0.09146722 |
| inosine | 0.03763147 | 0.7585465 | 1.39E-06 | -1.8377256 |
| adenosine | 2.83E-06 | 1.78126616 | 0.85277155 | 0.01798009 |
| sucrose | 1.60E-13 | 4.64824812 | 1.40E-06 | 1.77803571 |
| 5'-Deoxy-5'-(methylthio)adenosine | 2.05E-06 | 1.74925288 | 0.05461372 | 0.6162538 |
| guanosine | 6.94E-05 | 1.39443975 | 0.00041203 | -0.9303759 |
| cholesterol | 7.45E-13 | 4.7433487 | 0.07131249 | 0.68735738 |

**Supplementary Table 3. Age of mice at different points in the disease course.** The onset of symptoms is defined as the time when mice show the first signs of limb muscle force deficit on grip strength (when they fall from the inverted grid before 90 seconds). Paralysis is defined as the time when mice are completely unable to stay on the inverted grid. Survival is defined when mice are not able to right themselves within 10 seconds when laid on their side.

| Stage of disease | Weeks of age | |
|---|---|---|
| | 129S-G93A | C57-G93A |
| Onset | 14.3 ± 1.1 | 17.4 ± 1.0 |
| Paralysis | 16.1 ± 0.7 | 22.7 ± 1.5 |
| Survival | 17.8 ± 0.8 | 25.8 ± 1.6 |