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Potential roles of gut microbiome and metabolites in modulating ALS in mice

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Supplementary table 1. A vivarium-affected dysbiosis in the SOD1-Tg mouse model. Abundance percentage summary of the top 20 highly abundant microbiome genera in WT animals at the two facilities and their corresponding abundances in SOD1-Tg animals. The comparison has performed once, (N=5-8) mice in each group.

Taxa	**Vivarium A (%)**		**Vivarium B (%)**	
	WT	**SOD1-Tg**	**WT**	**SOD1-Tg**
Akkermansia	34.17	12.35	0.00	0.01
S24-7	26.18	36.96	0.04	0.03
Lactobacillus	10.15	2.90	17.01	32.76
Clostridiales	9.29	5.52	18.18	12.06
Prevotella	7.52	3.38	7.83	6.00
Parabacteroides	1.85	2.45	0.35	0.37
Ruminococcaceae	1.68	1.03	4.98	2.75
Oscillospira	1.56	0.97	6.68	3.95
Bacteroides	1.56	2.29	3.24	2.36
Rikenellaceae	1.44	1.58	4.22	2.98
Lachnospiraceae	1.03	0.47	16.76	9.74
YS2	0.76	1.20	3.20	2.50
Erysipelotrichaceae	0.44	0.06	0.00	0.00
Adlercreutzia	0.37	0.22	0.00	0.00
Ruminococcus	0.22	0.84	2.68	1.36
RF32	0.18	0.20	0.06	0.08
Coprococcus	0.16	0.24	1.24	0.80
Desulfovibrio	0.14	0.27	1.91	1.01
Dorea	0.11	0.76	0.06	0.15
Helicobacteraceae	0.02	0.41	0.00	0.00

Supplementary table 2. AM treatment is associated with higher abundance of microbial genes of Nicotinamide synthesis. Increased abundance of microbiome-derived nicotinamide producing genes in AM treated SOD1-Tg fecal samples measured by metagenomic shotgun sequencing (N=7 mice in each group), two-sided Mann Whitney U ranksum test.

Gene name	Enzyme name	q value
Amuc_0922	5'-nucleotidase	1.7E-5
Amuc_0430	Nicotinamide-nucleotide amidase	0.003
Amuc_0390	Nicotinate-nucleotide adenylyltransferase	0.007
Amuc_0924	NAD^+ kinase	0.015
Amuc_0620	NAD^+ synthase	$1.01X10^{-5}$
Amuc_1263	Nicotinate-nucleotide pyrophosphorylase	0.193
Amuc_2150	Quinolinate synthase	0.033
Amuc_1079	L-aspartate oxidase	0.013

Supplementary table 3. Top-changed bacterial species between ALS patients (N=37) and healthy control individuals (N=29). Two-sided FDR-corrected Mann-Whitney ranksum test.

Bacterial species	**ALS Median (log)**	**Healthy Median (log)**	**Directed p values**	**p values**	**q values**
Anaerostipes hadrus	-3.30	-2.71	2.89	0.00	0.09
Bacteroidales bacterium ph8	-2.91	-2.57	2.47	0.00	0.09
Bifidobacterium pseudocatenulatum	-3.30	-2.91	2.58	0.00	0.09
Clostridium leptum	-3.30	-3.30	-2.56	0.00	0.09
Escherichia coli	-3.05	-3.30	-2.83	0.00	0.09
Eubacterium hallii	-2.02	-1.87	2.21	0.01	0.13
Lachnospiraceae bacterium 5 1 63FAA	-3.12	-2.55	2.12	0.01	0.14
Ruminococcus gnavus	-3.30	-3.30	-1.95	0.01	0.17
Clostridium nexile	-3.30	-3.30	-1.84	0.01	0.20
Bacteroides fragilis	-3.30	-3.30	-1.57	0.03	0.22
Bacteroides plebeius	-3.30	-3.30	1.57	0.03	0.22
Bifidobacterium dentium	-3.30	-3.30	-1.40	0.04	0.22
Burkholderiales bacterium 1 147	-3.30	-3.30	-1.37	0.04	0.22
Catenibacterium mitsuokai	-3.30	-3.30	-1.49	0.03	0.22
Clostridium bolteae	-3.30	-3.30	-1.58	0.03	0.22
Clostridium ramosum	-3.30	-3.30	-1.37	0.04	0.22
Coprococcus sp ART55 1	-3.30	-3.30	1.65	0.02	0.22
Escherichia unclassified	-3.30	-3.30	-1.49	0.03	0.22
Eubacterium rectale	-1.35	-1.25	1.40	0.04	0.22
Eubacterium ventriosum	-3.30	-2.69	1.67	0.02	0.22
Megasphaera unclassified	-3.30	-3.30	-1.47	0.03	0.22
Oscillibacter unclassified	-2.90	-3.01	-1.38	0.04	0.22
Parasutterella excrementihominis	-3.30	-3.30	-1.38	0.04	0.22
Roseburia hominis	-3.17	-2.84	1.65	0.02	0.22
Streptococcus anginosus	-3.30	-3.30	-1.36	0.04	0.22
Faecalibacterium prausnitzii	-1.23	-1.15	1.34	0.05	0.22

Supplementary table 4. Microbiome-derived nicotinamide metabolism is impaired in ALS patients. KO relative abundances of microbiome-associated genes of the nicotinamide pathway in ALS (N=36) and healthy (N=28) stool samples. *q=0.03-0.04, two-sided FDR corrected Mann Whitney U ranksum test.

KEGG entry	Gene name	Enzyme name	q value
KO0969	nadD	nicotinate-nucleotide adenylyltransferase	0.04
KO3783	punA	Purine-nucleoside phosphorylase	0.05
KO3742	Amuc_0430	Nicotinamide-nucleotide amidase	0.11
KO0278	Amuc_1079	L-aspartate oxidase	0.05
KO1950	Amuc_0620	NAD^+ synthase	0.05
KO0164	OGDH	2-oxoglutarate dehydrogenase E1 component	0.03
KO3782	katG	catalase-peroxidase	0.05
KO0767	Amuc_1263	nicotinate-nucleotide pyrophosphorylase	0.04
KO1782	fadJ	enoyl-CoA hydratase	0.03
KO1825	fadB	3-hydroxyacyl-CoA dehydrogenase	0.05
KO3517	Amuc_2150	quinolinate synthase	0.11
KO0858	Amuc_0924	NAD^+ kinase	0.06

Supplementary Table 5. Primers used in qPCR analysis.

Primer ID	Sequence	Target & reference	reference
AM1	CAGCACGTGAAGGTGGGGAC	AM 16S rDNA gene	[46]
AM2	CCTTGCGGTTGGCTTCAGAT	AM 16S rDNA gene	[46]
F-S	CAACGCGMARAACCTTACC	16S rDNA gene	https://vamps2.mbl.edu
F-AQ	CTAACCGANGAACCTYACC	16S rDNA gene	https://vamps2.mbl.edu
F-UC3	ATACGCGARGAACCTTACC	16S rDNA gene	https://vamps2.mbl.edu
F-PP	CNACGCGAAGAACCTTANC	16S rDNA gene	https://vamps2.mbl.edu
R-S	CGACRRCCATGCANCACCT	16S rDNA gene	https://vamps2.mbl.edu

Supplementary Table 6. DNA oligos used for rRNA depletion

Oligo name	Sequence
AG9327_18_1	TAATGATCCTTCCGCAGGTTCACCTACGGAAACCTTGTTACGACTTTTAC
AG9328_18_2	TTCCTCTAGATAGTCAAGTTCGACCGTCTTCTCAGCGCTCCGCCAGGGCC
AG9329_18_3	GTGGGCCGACCCCGGCGGGGCCGATCCGAGGGCCTCACTAAACCATCCAA
AG9330_18_4	TCGGTAGTAGCGACGGGCGGTGTGTACAAAGGGCAGGGACTTAATCAACG
AG9331_18_5	CAAGCTTATGACCCGCACTTACTCGGGAATTCCCTCGTTCATGGGGAATA
AG9332_18_6	ATTGCAATCCCCGATCCCCATCACGAATGGGGTTCAACGGGTTACCCGCG
AG9333_18_7	CCTGCCGGCGTAGGGTAGGCACACGCTGAGCCAGTCAGTGTAGCGCGCGT
AG9334_18_8	GCAGCCCCGGACATCTAAGGGCATCACAGACCTGTTATTGCTCAATCTCG
AG9335_18_9	GGTGGCTGAACGCCACTTGTCCCTCTAAGAAGTTGGGGGACGCCGACCGC
AG9336_18_10	TCGGGGGTCGCGTAACTAGTTAGCATGCCAGAGTCTCGTTCGTTATCGGA
AG9337_18_11	ATTAACCAGACAAATCGCTCCACCAACTAAGAACGGCCATGCACCACCAC
AG9338_18_12	CCACGGAATCGAGAAAGAGCTATCAATCTGTCAATCCTGTCCGTGTCCGG
AG9339_18_13	GCCGGGTGAGGTTTCCCGTGTTGAGTCAAATTAAGCCGCAGGCTCCACTC
AG9340_18_14	CTGGTGGTGCCCTTCCGTCAATTCCTTTAAGTTTCAGCTTTGCAACCATA
AG9341_18_15	CTCCCCCCGGAACCCAAAGACTTTGGTTTCCCGGAAGCTGCCCGGCGGGT
AG9342_18_16	CATGGGAATAACGCCGCCGCATCGCCGGTCGGCATCGTTTATGGTCGGAA
AG9343_18_17	CTACGACGGTATCTGATCGTCTTCGAACCTCCGACTTTCGTTCTTGATTA
AG9344_18_18	ATGAAAACATTCTTGGCAAATGCTTTCGCTCTGGTCCGTCTTGCGCCGGT
AG9345_18_19	CCAAGAATTTCACCTCTAGCGGCGCAATACGAATGCCCCCGGCCGTCCCT
AG9346_18_20	CTTAATCATGGCCTCAGTTCCGAAAACCAACAAAATAGAACCGCGGTCCT
AG9347_18_21	ATTCCATTATTCCTAGCTGCGGTATCCAGGCGGCTCGGGCCTGCTTTGAA
AG9348_18_22	CACTCTAATTTTTTCAAAGTAAACGCTTCGGGCCCCGCGGGACACTCAGC
AG9349_18_23	TAAGAGCATCGAGGGGGCGCCGAGAGGCAAGGGGCGGGGACGGGCGGTGG

AG9350_18_24	CTCGCCTCGCGGCGGACCGCCCGCCCGCTCCCAAGATCCAACTACGAGCT
AG9351_18_25	TTTTAACTGCAGCAACTTTAATATACGCTATTGGAGCTGGAATTACCGCG
AG9352_18_26	GCTGCTGGCACCAGACTTGCCCTCCAATGGATCCTCGTTAAAGGATTTAA
AG9353_18_27	AGTGGACTCATTCCAATTACAGGGCCTCGAAAGAGTCCTGTATTGTTATT
AG9354_18_28	TTTCGTCACTACCTCCCCGGGTCGGGAGTGGGTAATTTGCGCGCCTGCTG
AG9355_18_29	CCTTCCTTGGATGTGGTAGCCGTTTCTCAGGCTCCCTCTCCGGAATCGAA
AG9356_18_30	CCCTGATTCCCCGTCACCCGTGGTCACCATGGTAGGCACGGCGACTACCA
AG9357_18_31	TCGAAAGTTGATAGGGCAGACGTTCGAATGGGTCGTCGCCGCCACGGG
AG9358_18_32	GCGTGCGATCGGCCCGAGGTTATCTAGAGTCACCAAAGCCGCCGGCGCCC
AG9359_18_33	GCCCCCCGGCCGGGGCCGGAGAGGGGCTGACCGGGTTGGTTTTGATCTGA
AG9360_18_34	TAAATGCACGCATCCCCCCCGCGAAGGGGGTCAGCGCCCGTCGGCATGTA
AG9361_18_35	TTAGCTCTAGAATTACCACAGTTATCCAAGTAGGAGAGGAGCGAGCGACC
AG9362_18_36	AAAGGAACCATAACTGATTTAATGAGCCATTCGCAGTTTCACTGTACCGG
AG9363_18_37	CCGTGCGTACTTAGACATGCATGGCTTAATCTTTGAGACAAGCATATGCT
AG9364_18_38	TGGCTTAATCTTTGAGACAAGCATATGCTACTGGCAGGATCAACCAGGTA
AG9466_5.8_1	AAGCGACGCTCAGACAGGCGTAGCCCCGGGAGGAACCCGGGGCCGCAAGT
AG9467_5.8_2	GCGTTCGAAGTGTCGATGATCAATGTGTCCTGCAATTCACATTAATTCTC
AG9468_5.8_3	GCAGCTAGCTGCGTTCTTCATCGACGCACGAGCCGAGTGATCCACCGCTA
AG9469_16_1	AAACCCTGTTCTTGGGTGGGTGTGGGTATAATACTAAGTTGAGATGATAT
AG9470_16_2	CATTTACGGGGGAAGGCGCTTTGTGAAGTAGGCCTTATTTCTCTTGTCCT
AG9471_16_3	TTCGTACAGGGAGGAATTTGAANGTAGATAGAAACCGACCTGGATTACTC
AG9472_16_4	CGGTCTGAACTCAGATCACGTAGGACTTTAATCGTTGAACAAACGAACCT
AG9473_16_5	TTAATAGCGGCTGCACCATCGGGATGTCCTGATCCAACATCGAGGTCGTA
AG9474_16_6	AACCCTATTGTTGATATGGACTCTAGAATAGGATTGCGCTGTTATCCCTA
AG9475_16_7	GGGTAACTTGTTCCGTTGGTCAAGTTATTGGATCAATTGAGTATAGTAGT
AG9476_16_8	TCGCTTTGACTGGTGAAGTCTTAGCATGTACTGCTCGGAGGTTGGGTTCT

AG9477_16_9	GCTCCGAGGTCGCCCCAACCGAAATTTTTAATGCAGGTTTGGTAGTTTAG
AG9478_16_10	GACCTGTGGGTTTGTTAGGTACTGTTTGCATTAATAAATTAAAGCTCCAT
AG9479_16_11	AGGGTCTTCTCGTCTTGCTGTGTTATGCCCGCCTCTTCACGGGCAGGTCA
AG9480_16_12	ATTTCACTGGTTAAAAGTAAGAGACAGCTGAACCCTCGTGGAGCCATTCA
AG9481_16_13	TACAGGTCCCTATTTAAGGAACAAGTGATTATGCTACCTTTGCACGGTTA
AG9482_16_14	GGGTACCGCGGCCGTTAAACATGTGTCACTGGGCAGGCGGTGCCTCTAAT
AG9483_16_15	ACTGGTGATGCTAGAGGTGATGTTTTTGGTAAACAGGCGGGGTAAGATTT
AG9484_16_16	GCCGAGTTCCTTTTACTTTTTTTAACCTTTCCTTATGAGCATGCCTGTGT
AG9485_16_17	TGGGTTGACAGTGAGGGTAATAATGACTTGTTGGTTGATTGTAGATATTG
AG9486_16_18	GGCTGTTAATTGTCAGTTCAGTGTTTTAATCTGACGCAGGCTTATGCGGA
AG9487_16_19	GGAGAATGTTTTCATGTTACTTATACTAACATTAGTTCTTCTATAGGGTG
AG9488_16_20	ATAGATTGGTCCAATTGGGTGTGAGGAGTTCAGTTATATGTTTGGGATTT
AG9489_16_21	TTTAGGTAGTGGGTGTTGAGCTTGAACGCTTTCTTAATTGGTGGCTGCTT
AG9490_16_22	TTAGGCCTACTATGGGTGTTAAATTTTTTACTCTCTCTACAAGGTTTTTT
AG9491_16_23	CCTAGTGTCCAAAGAGCTGTTCCTCTTTGGACTAACAGTTAAATTTACAA
AG9492_16_24	GGGATTTAGAGGGTTCTGTGGGCAAATTTAAAGTTGAACTAAGATTCTA
AG9493_16_25	TCTTGGACAACCAGCTATCACCAGGCTCGGTAGGTTTGTCGCCTCTACCT
AG9494_16_26	ATAAATCTTCCCACTATTTTGCTACATAGACGGGTGTGCTCTTTTAGCTG
AG9495_16_27	TTCTTAGGTAGCTCGTCTGGTTTCGGGGGTCTTAGCTTTGGCTCTCCTTG
AG9496_16_28	CAAAGTTATTTCTAGTTAATTCATTATGCAGAAGGTATAGGGGTTAGTCC
AG9497_16_29	TTGCTATATTATGCTTGGTTATAATTTTTCATCTTTCCCTTGCGGTACTA
AG9498_16_30	TATCTATTGCGCCAGGTTTCAATTTCTATCGCCTATACTTTATTTGGGTA
AG9499_16_31	AATGGTTTGGCTAAGGTTGTCTGGTAGTAAGGTGGAGTGGGTTTGGGGCT
AG9500_12_1	GTTCGTCCAAGTGCACTTTCCAGTACACTTACCATGTTACGACTTGTCTC
AG9501_12_2	CTCTATATAAATGCGTAGGGGTTTTAGTTAAATGTCCTTTGAAGTATACT
AG9502_12_3	TGAGGAGGGTGACGGGCGGTGTGTACGCGCTTCAGGGCCCTGTTCAACTA

AG9503_12_4	AGCACTCTACTCTTAGTTTACTGCTAAATCCACCTTCGACCCTTAAGTTT
AG9504_12_5	CATAAGGGCTATCGTAGTTTTCTGGGGTAGAAAATGTAGCCCATTTCTTG
AG9505_12_6	CCACCTCATGGGCTACACCTTGACCTAACGTCTTTACGTGGGTACTTGCG
AG9506_12_7	CTTACTTTGTAGCCTTCATCAGGGTTTGCTGAAGATGGCGGTATATAGGC
AG9507_12_8	TGAGCAAGAGGTGGTGAGGTTGATCGGGGTTTATCGATTACAGAACAGGC
AG9508_12_9	TCCTCTAGAGGGATATGAAGCACCGCCAGGTCCTTTGAGTTTTAAGCTGT
AG9509_12_10	GGCTCGTAGTGTTCTGGCGAGCAGTTTTGTTGATTTAACTGTTGAGGTTT
AG9510_12_11	AGGGCTAAGCATAGTGGGGTATCTAATCCCAGTTTGGGTCTTAGCTATTG
AG9511_12_12	TGTGTTCAGATATGTTAAAGCCACTTTCGTAGTCTATTTTGTGTCAACTG
AG9512_12_13	GAGTTTTTTACAACTCAGGTGAGTTTTAGCTTTATTGGGGAGGGGGTGAT
AG9513_12_14	CTAAAACACTCTTTACGCCGGCTTCTATTGACTTGGGTTAATCGTGTGAC
AG9514_12_15	CGCGGTGGCTGGCACGAAATTGACCAACCCTGGGGTTAGTATAGCTTAGT
AG9515_12_16	TAAACTTTCGTTTATTGCTAAAGGTTAATCACTGCTGTTTCCCGTGGG
AG9516_12_17	TGTGGCTAGGCTAAGCGTTTTGAGCTGCATTGCTGCGTGCTTGATGCTTG
AG9517_12_18	TTCCTTTTGATCGTGGTGATTTAGAGGGTGAACTCACTGGAACGGGGATG
AG9518_12_19	CTTGCATGTGTAATCTTACTAAGAGCTAATAGAAAGGCTAGGACCAAACC
AG9519_5_1	AAAGCCTACAGCACCCGGTATTCCCAGGCGGTCTCCCATCCAAGTACTAA
AG9520_5_2	CCAGGCCCGACCCTGCTTAGCTTCCGAGATCAGACGAGATCGGGCGCGTT
AG9521_5_3	TTCCGAGATCAGACGAGATCGGGCGCGTTCAGGGTGGTATGGCCGTAGAC

Supplementary Table 7. Human study participant details

#Participant	Sex	Group	Age (y)	Weight (kg)	Height (cm)	ALS FRS	Relation
ALS_728	F	ALS	33	39.3	170	19	
ALS_747	M	ALS	61	84.5	181	28	
ALS_1890	M	ALS	67	83.5	171	42	
ALS_1447	M	ALS	68	80.2	170	37	
ALS_1633	M	ALS	40	69.4	175	25	
ALS_1640	M	ALS	76	92	170	39	
ALS_1641	M	ALS	51	68.3	181	27	
ALS_1659	F	ALS	55	48.7	165	8	
ALS_1671	F	ALS	55	51.3	163	28	
ALS_1680	F	ALS	53	55.7	170	38	
ALS_1717	M	ALS	39	69.9	173	29	
ALS_1730	F	ALS	70	54.9	150	19	
ALS_1731	M	ALS	68	102.3	178	42	
ALS_1739	M	ALS	61	66.4	165	26	
ALS_1745	M	ALS	47	64	175	24	
ALS_1753	F	ALS	72		156	18	
ALS_1764	M	ALS	49	79.5	180	37	
ALS_1781	M	ALS	53	83.2	176	39	
ALS_1784	F	ALS	51	73.5	165	28	
ALS_1787	M	ALS	55	63.5	171	32	
ALS_1789	M	ALS	60	80.2	160	38	
ALS_1799	M	ALS	57	78	167	40	
ALS_1814	M	ALS	59	64.3	178	43	
ALS_1841	M	ALS	47	69.7	186	37	
ALS_1779	M	ALS	58	86.2	179	38	
ALS_1825	M	ALS	56	100	174	22	
ALS_1851	M	ALS	65	67	174	23	
ALS_1869	M	ALS	64	62.4	160	38	
ALS_1883	M	ALS	71		180	27	
ALS_1857	F	ALS	75	72.7	154	41	
ALS_1823	M	ALS	67	74	176	36	
ALS_1888	M	ALS	57	89.8	187	42	
ALS_1896	M	ALS	61	65	163	45	
ALS_1897	F	ALS	70	59.5	156	43	

ALS_1898	M	ALS	36	82.3	176	38	
ALS_1899	M	ALS		178		40	
ALS_1894	M	ALS	62	83.5	187	40	
C_728	F	Healthy	46	73	160	48	Wife
C_747	F	Healthy	56	65	163	48	Wife
C_1742	F	Healthy	43	75	164	48	Mother
C_1633	F	Healthy	42	80	167	48	Wife
C_1640	F	Healthy	72	54	158	48	Wife
C_1641	M	Healthy				48	Husband
C_1659	M	Healthy	58	88	187	48	Husband
C_1671	M	Healthy	55	99	185	48	Husband
C_1680	M	Healthy	59	87	193	48	Husband
C_1717	F	Healthy	46	75	155	48	Wife
C_1730	M	Healthy	74	80	176	48	Husband
C_1731	F	Healthy	67	50	162	48	Wife
C_1739	F	Healthy	59	71	141	48	Wife
C_1745	F	Healthy	46	65	175	48	Wife
C_1753	M	Healthy	50	70	172	48	Husband
C_1764	F	Healthy	44	85	163	48	Wife
C_1781	F	Healthy	50	90	160	48	Wife
C_1784	M	Healthy	50	104	174	48	Husband
C_1799	F	Healthy	56	65	165	48	Wife
C_1814	F	Healthy	55	71	163	48	Wife
C_1851	F	Healthy	66	68.4	167	48	Wife
C_1833	F	Healthy				48	Wife
C_1857	M	Healthy	66	74	170	48	Husband
C_1888	F	Healthy	59.5	78	160	48	Wife
C_1890	F	Healthy	67	73	164	48	Wife
C_1894	F	Healthy	69	75	168	48	Wife
C_1896	F	Healthy	62	50	160	48	Wife
C_1897	M	Healthy	70	91	178	48	Husband
C_1891	F	Healthy	72	64	150	48	Wife