

Early life exposure to environmental contaminants (BDE-47, TBBPA, and BPS) produced persistent alterations in fecal microbiome in adult male mice

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Supplemental Figure Legends

Figure S1. Composition and diversity of CD-1 mice that were maternally exposed to vehicle vs. mice that were not maternally exposed to vehicle or any other chemicals (NT). **A)** Alpha diversity of NT and vehicle-exposed groups via chao1 index (asterisks represent statistically significant differences between NT and vehicle-exposed groups). **B)** Beta diversity of NT and vehicle-exposed groups. **C)** Composition of bacteria (L7) in NT and vehicle-exposed groups. The top 15 most abundant taxa are shown (the remaining taxa are summed and shown in the “Other” category). Asterisks represent statically significant changes as compared to NT group (Student’s t-test, $p < 0.05$).

Figure S2. Microbial biomarkers associated with each exposure group using Lefse.

Figure S3. Two-way hierarchical clustering dendrograms showing differentially regulated taxa by early life vehicle-exposure as compared to NT.

Figure S4. Pearson’s correlation analyses between differentially regulated taxa and BAs **(A)** and short/medium chain fatty acids **(B)**.

Figure S5-S15. Individual microbial pathways predicted by Fishtaco in vehicle- and BDE-47 exposure groups.

Figure S16-S39. Individual microbial pathways predicted by Fishtaco in vehicle- and TBBPA exposure groups.

Supplemental Tables

Supplemental Table 1. Joined reads per sample (16S rDNA Sequencing)	
Sample Name	Number of Reads
No Treatment 1	112,745
No Treatment 2	145,071
No Treatment 3	139,687
No Treatment 4	88,144
No Treatment 5	135,336
No Treatment 6	206,800
No Treatment 7	201,923
No Treatment 8	199,547
No Treatment 9	182,291
No Treatment 10	152,520
No Treatment 11	121,927
No Treatment 12	157,246
No Treatment 13	173,285
No Treatment 14	157,325
No Treatment 15	146,591
No Treatment 16	97,279
No Treatment 17	202,013
No Treatment 18	197,967
No Treatment 19	201,732
Vehicle 1	212,663
Vehicle 2	192,999
Vehicle 3	157,380
Vehicle 4	118,261
Vehicle 5	148,384
Vehicle 6	176,899
Vehicle 7	165,180
Vehicle 8	165,363
Vehicle 9	107,253
Vehicle 10	204,988
Vehicle 11	210,909
Vehicle 12	200,861
Vehicle 13	202,090
Vehicle 14	145,106
Vehicle 15	129,645
Vehicle 16	104,676
Vehicle 17	121,548
Vehicle 18	137,707
BDE47 -1	146,132
BDE47 -2	132,719
BDE47 -3	81,039
BDE47 -4	162,219
BDE-5	N/A
BDE47 -6	3,682
BDE47 -7	213,768
BDE47 -8	211,818
BDE47 -9	181,340
BDE47 -10	121,229
BDE47 -11	126,654

BDE47 -12	164,619
BDE47 -13	144,856
BDE47 -14	169,764
BDE47 -15	129,872
BDE47 -16	105,679
BDE47 -17	177,815
BDE47 -18	209,106
BDE47 -19	214,197
BDE47 -20	196,158
BDE47 -21	198,859
BDE47 -22	204,357
BDE47 -23	200,738
BDE47 -24	213,415
TBBPA 1	201,625
TBBPA 2	202,847
TBBPA 3	203,431
TBBPA 4	201,085
TBBPA 5	50,977
TBBPA 6	205,831
TBBPA 7	3,734
TBBPA 8	136,343
TBBPA 9	201,831
TBBPA 10	165,147
TBBPA 11	177,554
TBBPA 12	210,776
TBBPA 13	195,559
TBBPA 14	201,791
TBBPA 15	186,937
TBBPA 16	141,132
TBBPA 17	211,239
BPS 1	212,935
BPS 2	203,707
BPS 3	207,845
BPS 4	197,791
BPS 5	164,835
BPS 6	182,942
BPS 7	196,858
BPS 8	200,686
BPS 9	205,908
BPS 10	195,259
BPS 11	146,606
BPS 12	206,627
BPS 13	213,364
BPS 14	209,023
BPS 15	203,630

Supplemental Table 2

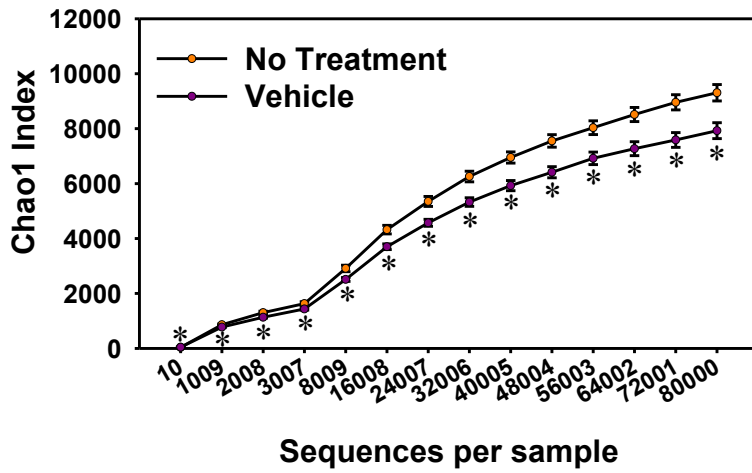
Exposure	Ko ID	Pathways
BDE-47	Ko00120	Primary Bile Acid Biosynthesis
	Ko00121	Secondary bile acid biosynthesis
	Ko03060	Protein Export
	Ko00471	D-Glutamine and D-glutamate metabolism
	Ko00740	Riboflavin metabolism
	Ko04964	Proximal tubule bicarbonate reclamation
	Ko04070	Phosphatidylinositol signaling system
	Ko03010	Ribosome
	Ko00312	Beta-Lactam resistance
	Ko00311	Penicillin and cephalosporin biosynthesis
	Ko00900	Terpenoid backbone biosynthesis
	TBBPA	Ko00945
Ko00281		Geraniol degradation
Ko00740		Riboflavin metabolism
Ko00531		Glycosaminoglycan metabolism
Ko03010		Ribosome
Ko00623		Toluene degradation
Ko00780		Biotin metabolism
Ko00190		Oxidative Phosphorylation
Ko00785		Lipoic acid metabolism
Ko03070		Bacterial Secretion system
Ko00604		Glycosphingolipid metabolism
Ko00130		Ubiquinone and other terpenoid-quinone biosynthesis
Ko00670		One carbon pool by folate
Ko04146		Peroxisome
Ko00511		Other glycan degradation
Ko00910		Nitrogen metabolism
Ko00523		Polyketide sugar unit biosynthesis
Ko00540		Lipopolysaccharide biosynthesis
Ko03018		RNA degradation
Ko00790		Folate biosynthesis
Ko03060		Protein export
Ko00521		Streptomycin biosynthesis
Ko04070		Phosphatidylinositol signaling system
BPS	Ko00364	Fluorobenzoate degradation
	Ko00900	Terpenoid backbone synthesis

Supplemental Table 3.

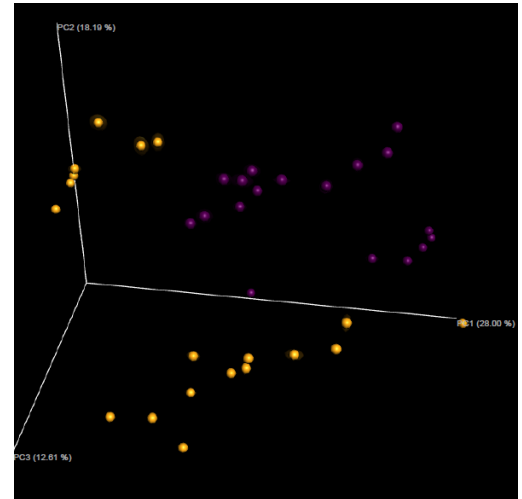
Primers	Sequences
IL-2 Forward	GAGTCAGCAACTGTGGTGGA
IL-2 Reverse	AGGGCTTGTTGAGATGATGC
IL-4 Forward	TCAACCCCCAGCTAGTTGTC
IL-4 Reverse	GGTGTTCTTCGTTGCTGTGA
IL-12p40 Forward	CACACTGGACCAAAGGGACT
IL-12p40 Reverse	ATTTATTCTGCTGCCGTGCT
IL-12p35 Forward	CTCCTGTGGGAGAAGCAGAC
IL-12p35 Reverse	CAGATAGCCCATCACCTGT
Cyp7a1 Forward	CAACGGGTTGATTCCATACC
Cyp7a1 Reverse	ATTTCCCCATCAGTTTGCAG
Gapdh Forward	GGCAAATTCAACGGCACAGT
Gapdh Reverse	GTCTCGCTCCTGGAAGATGG
Nqo1 Forward	TCTGCAGCTTCCAGCTTCTTG
Nqo1 Reverse	TCTGCAGCTTCCAGCTTCTTG
Gapdx Forward	AGTGGGTGAACCCTCACAAG
Gapdx Reverse	AGCTGGGTTTACTGGTGGTG

Figure S1

A

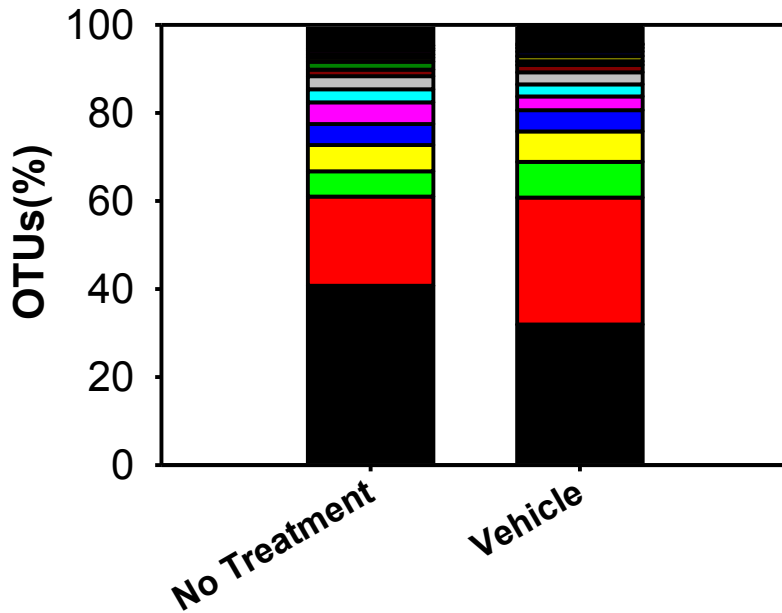


B

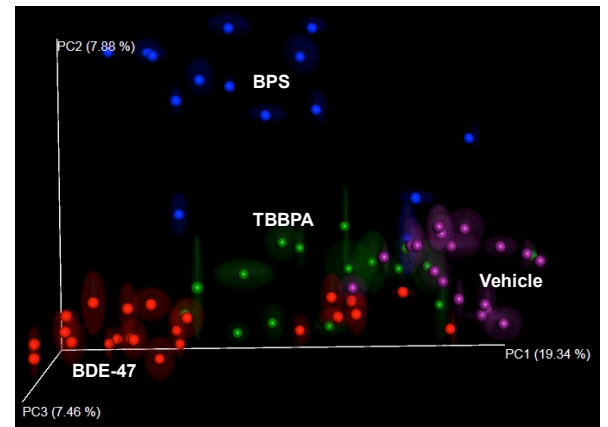


● No Treatment
● Vehicle

C



D



● Vehicle ● BDE-47 ● TBBPA ● BPS

- * *p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__S24-7;g__s__*
- * *p__Firmicutes;c__Clostridia;o__Clostridiales;f__g__s__*
- * *p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__s__*
- * *p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Rikenellaceae;g__s__*
- * *p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;g__Oscillospira;s__*
- * *p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Lactobacillaceae;g__Lactobacillus;s__*
- * *p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;g__s__*
- * *p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;g__s__*
- * *p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;g__Ruminococcus;s__*
- * *p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__*
- * *p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__[Odoribacteraceae];g__Odoribacter;s__*
- * *p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__[Ruminococcus];s__gnavus*
- * *p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Prevotellaceae;g__Prevotella;s__*
- * *p__Tenericutes;c__Mollicutes;o__RF39;f__g__s__*
- * *p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides;s__acidifaciens*
- * Other

Figure S2

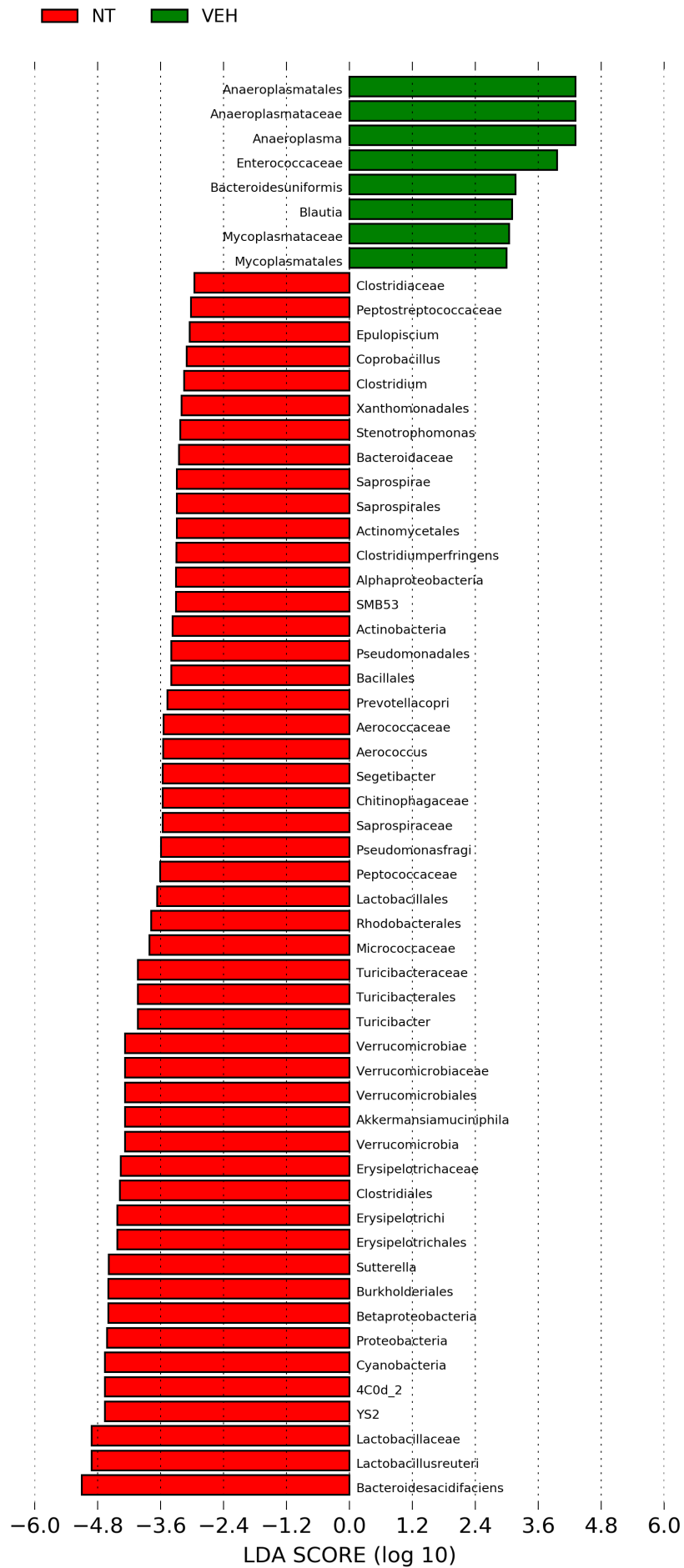
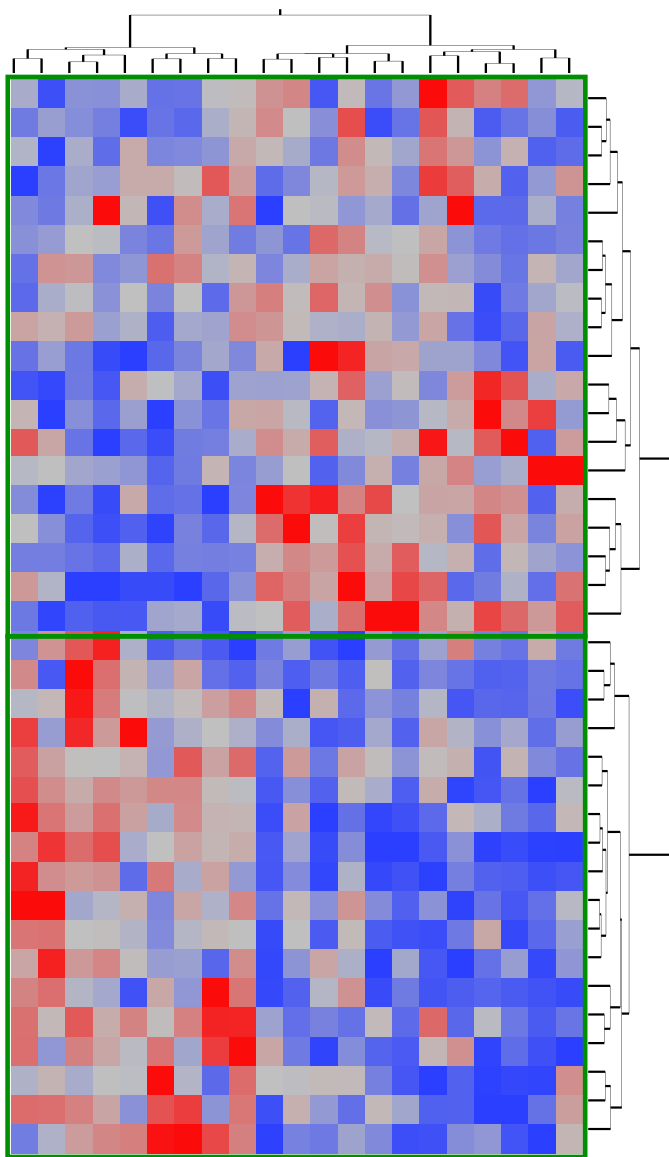


Figure S3

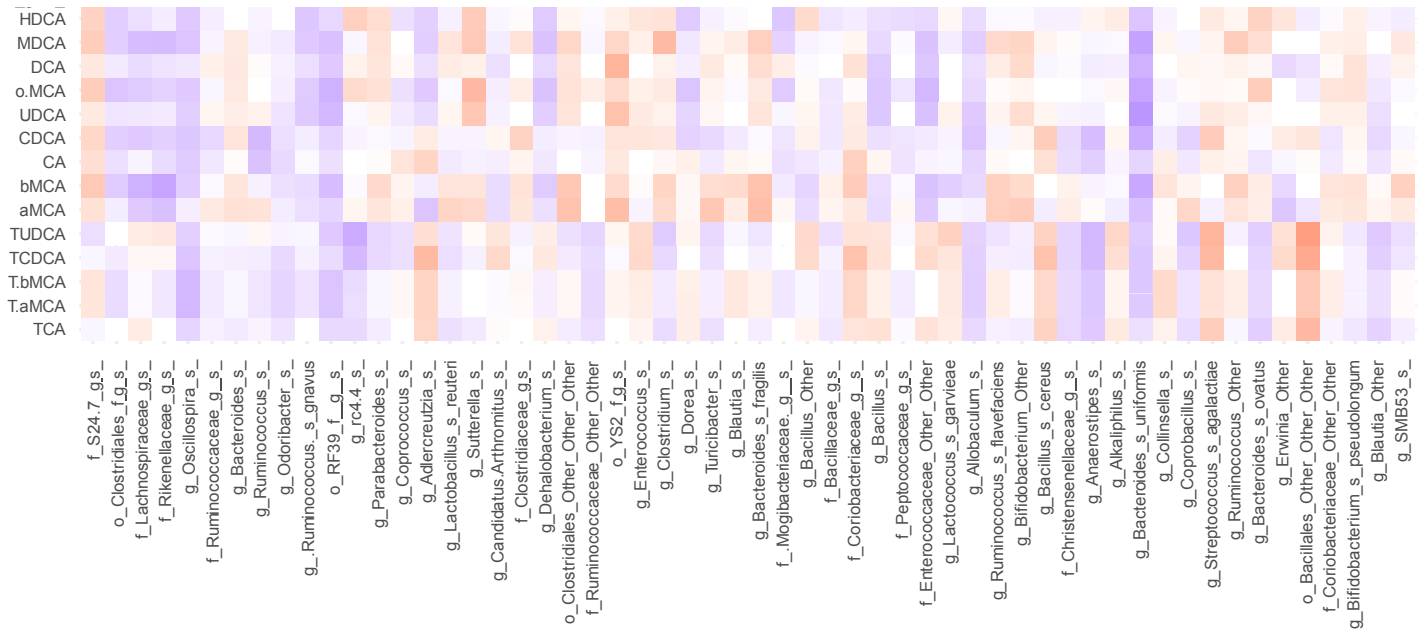
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 Vehicle13
 No treatment16
 No treatment19
 No treatment9
 No treatment18
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 No treatment15
 No treatment12
 No treatment4
 No treatment7
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 No treatment10
 No treatment2
 No treatment3
 No treatment8
 No treatment17
 No treatment6
 No treatment11
 Vehicle16
 Vehicle17
 Vehicle15
 Vehicle1
 Vehicle2
 Vehicle3
 Vehicle6
 Vehicle4
 Vehicle8
 Vehicle12
 Vehicle10
 Vehicle5
 Vehicle14
 Vehicle18
 Vehicle7
 Vehicle11
 Vehicle9



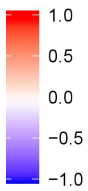
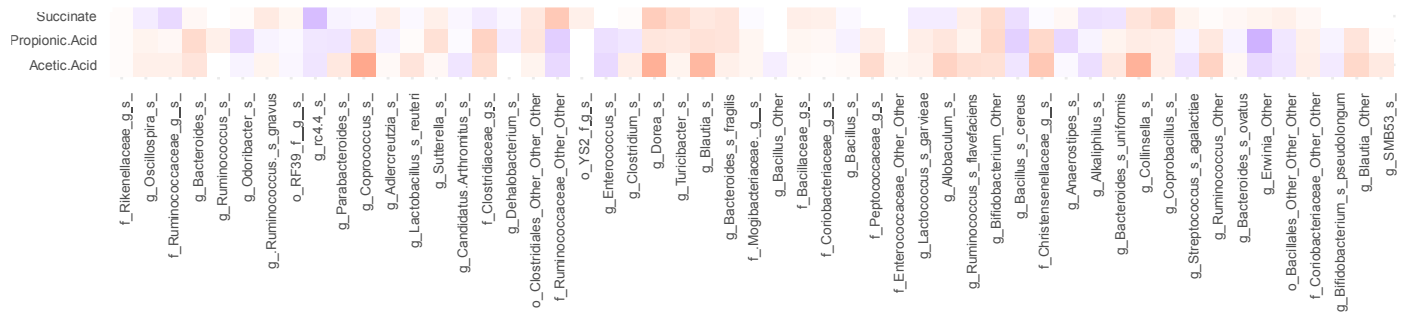
p_Firmicutes;Clostridia;Clostridiales;Peptococcaceae;rc4-4;s
 p_Firmicutes;Clostridia;Clostridiales;Ruminococcaceae;Other;Other
 p_Firmicutes;Clostridia;Clostridiales;g_s
 p_Firmicutes;Clostridia;Clostridiales;Lachnospiraceae;g_s
 p_Firmicutes;Clostridia;Clostridiales;Lachnospiraceae;Other;Other
 p_Tenericutes;Mollicutes;Anaeroplasmatales;Anaeroplasmataceae;g_s
 p_Firmicutes;Clostridia;Clostridiales;Lachnospiraceae;g_s
 p_Tenericutes;Mollicutes;RF39;g_s
 p_Bacteroidetes;Bacteroidia;Bacteroidales;[Odoribacteraceae];g
 p_Bacteroidetes;Bacteroidia;Bacteroidales;Bacteroidaceae;Bacteroides;acidifaciens
 p_Firmicutes;Erysipelotrichia;Erysipelotrichales;Erysipelotrichaceae;g_s
 p_Bacteroidetes;Bacteroidia;Bacteroidales;Bacteroidaceae;Bacteroides;g_s
 p_Bacteroidetes;Bacteroidia;Bacteroidales;S24-7;g_s
 p_Firmicutes;Bacillio;Lactobacillales;Lactobacillaceae;Lactobacillus;reuteri
 p_Proteobacteria;Betaproteobacteria;Burkholderiales;Alcaligenaceae;Sutterella;s
 p_Firmicutes;Bacillio;Bacillales;Bacillaceae;Bacillus;Other
 p_Firmicutes;Clostridia;Clostridiales;Lachnospiraceae;g_s
 p_Firmicutes;Clostridia;Clostridiales;Other;Other;Other
 p_Firmicutes;Clostridia;Clostridiales;Clostridiaceae;g_s
 p_Cyanobacteria;4C0d-2o;Y52;f;g_s
 p_Firmicutes;Bacillio;Lactobacillales;Lactobacillaceae;Lactobacillus;g_s

Figure S4

A



B



Pearson's correlation

Figure S5

ko00120

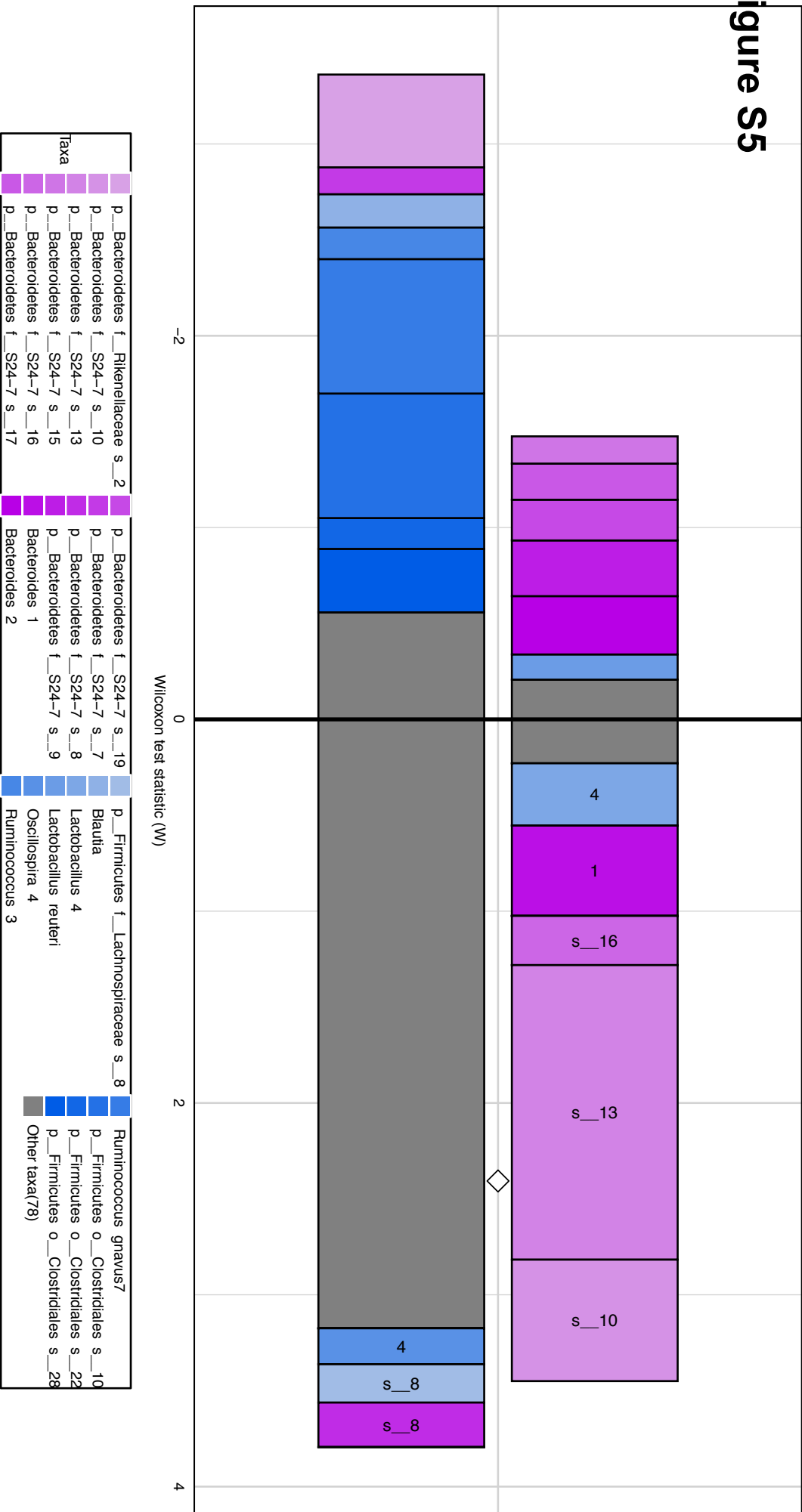
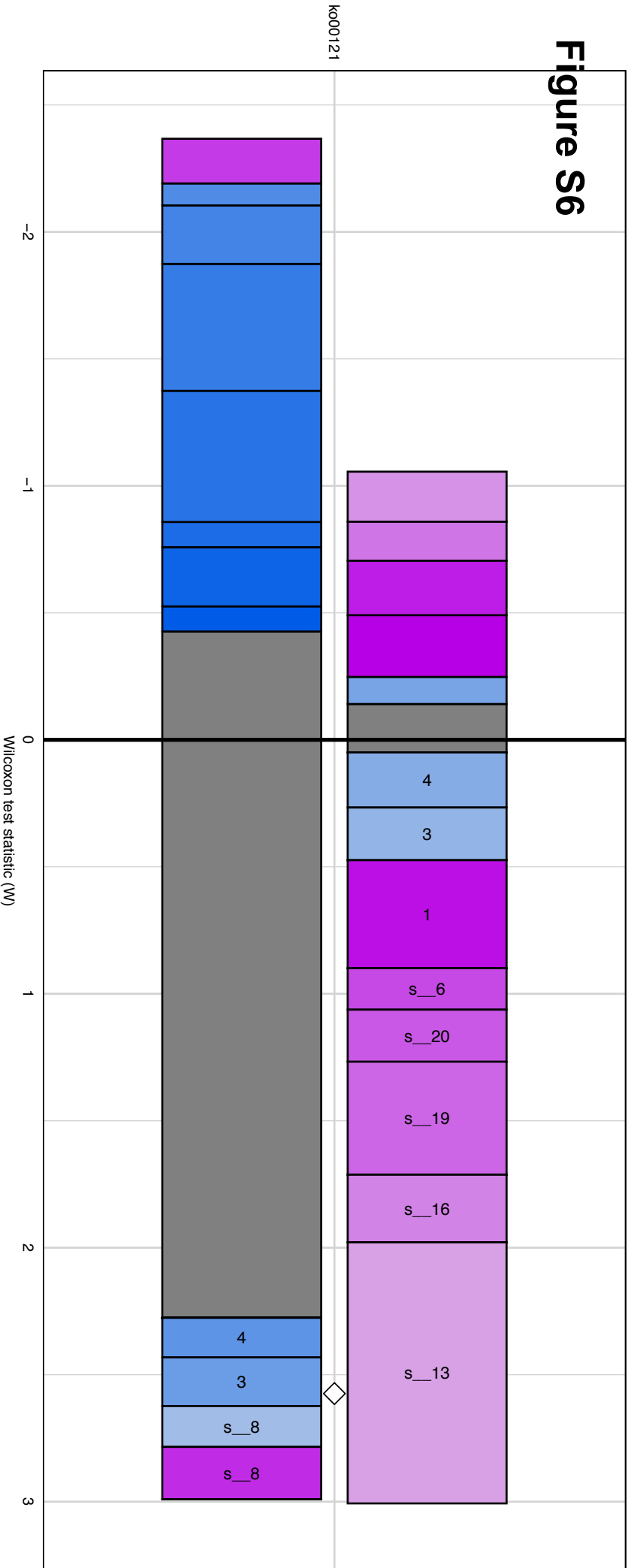


Figure S6



K000121

Figure S7

ko03060

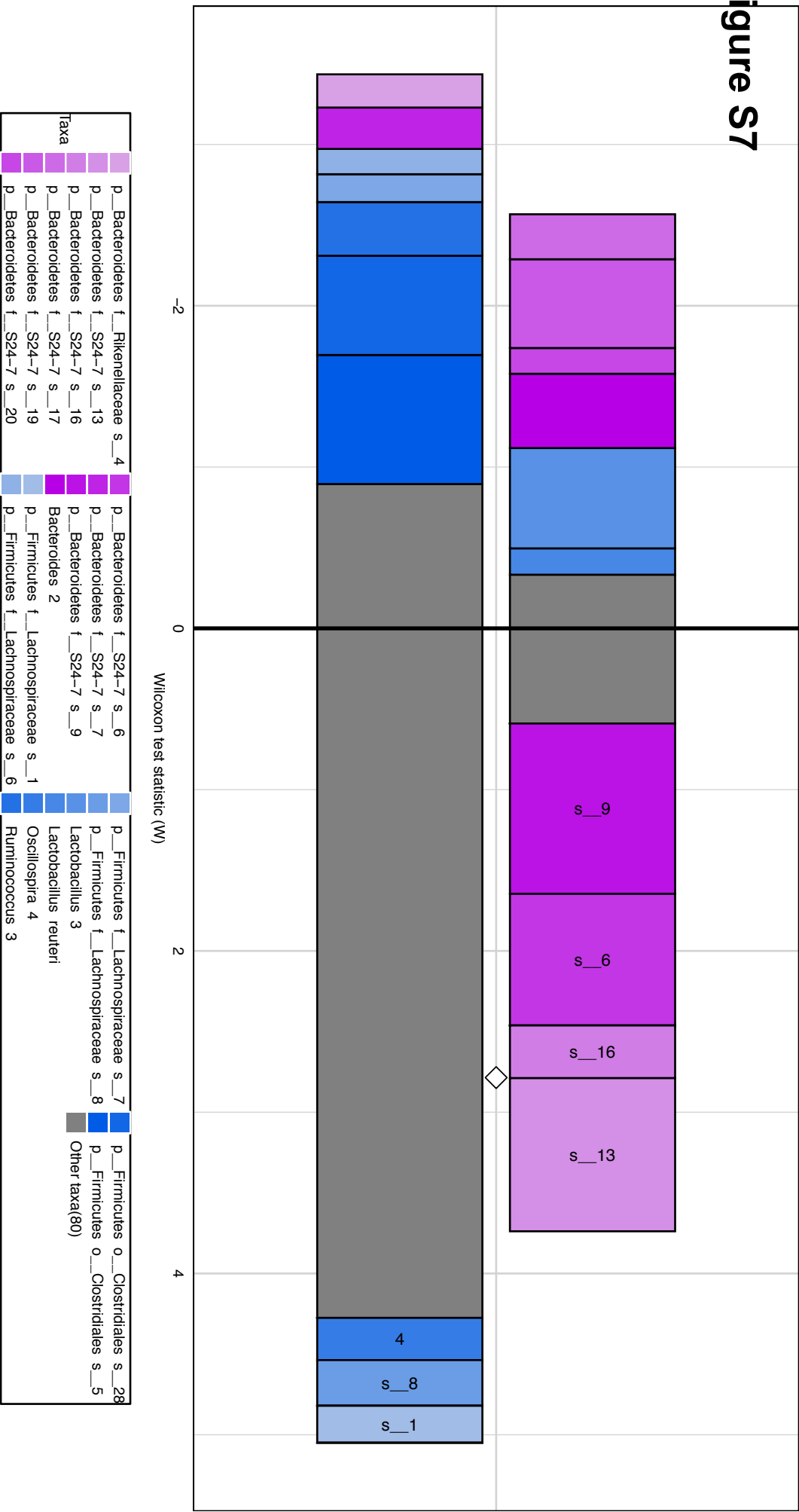


Figure S8

k000471

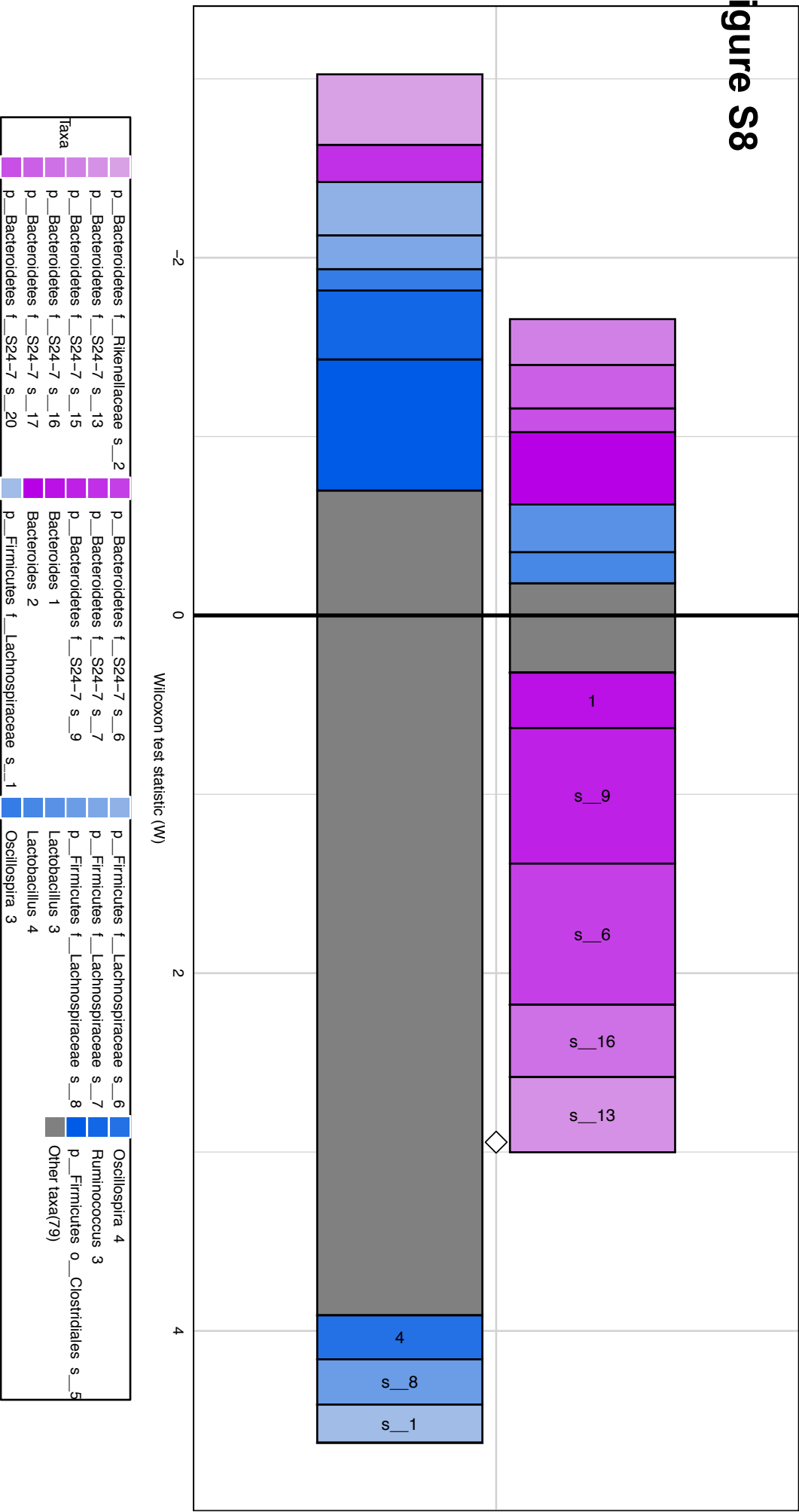


Figure S10

K004964

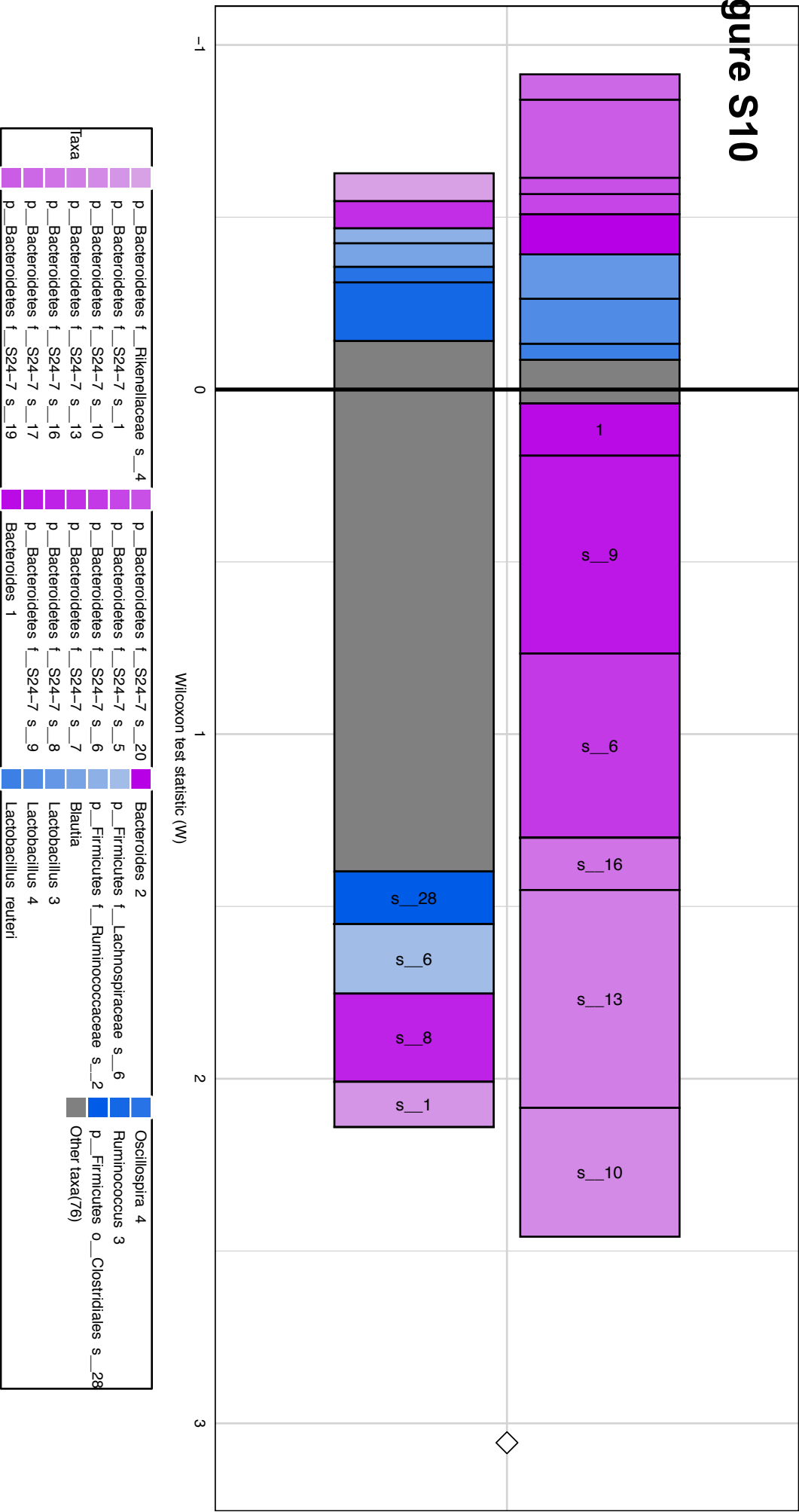


Figure S11

ko04070

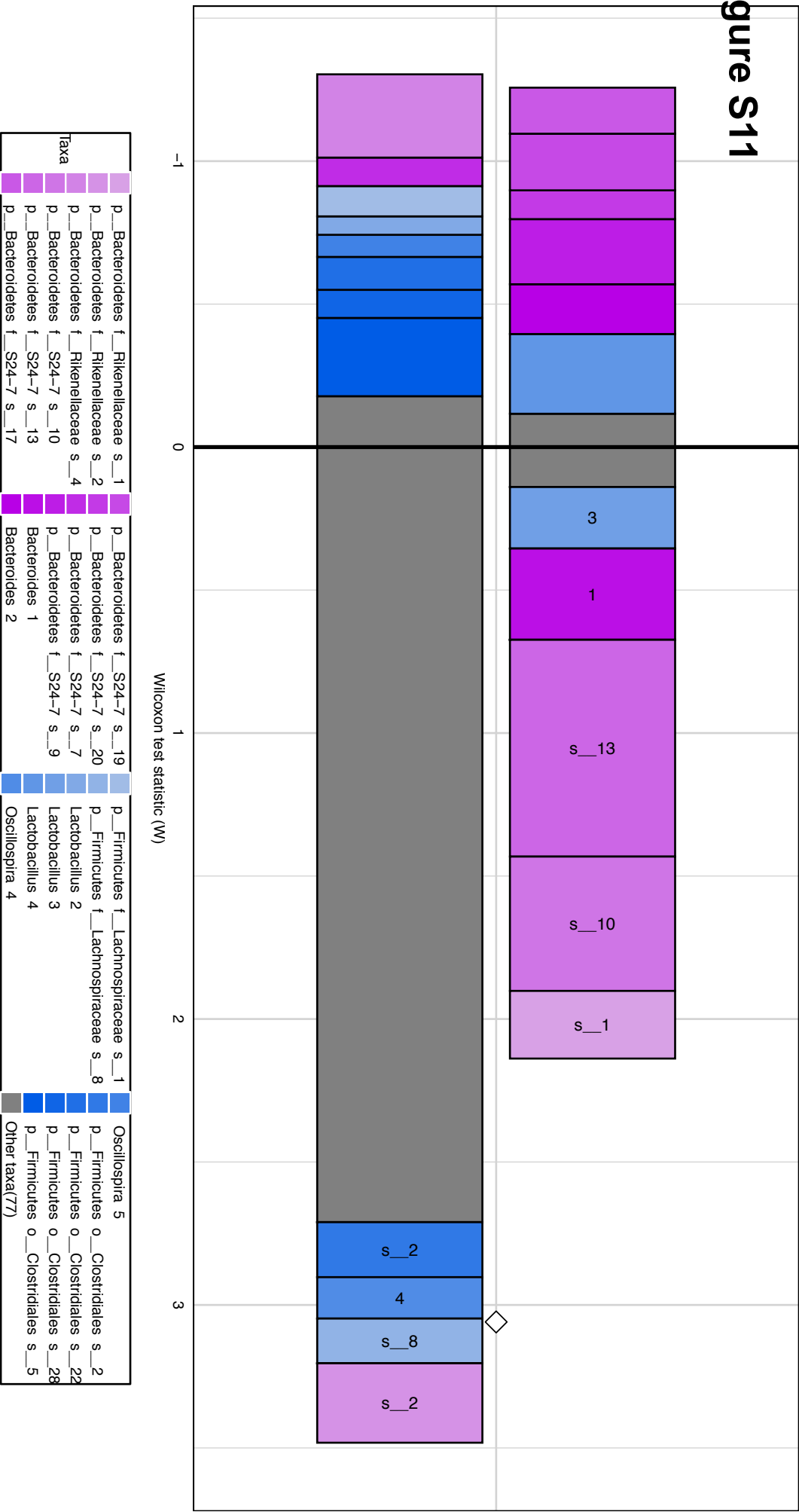


Figure S12

ko03010

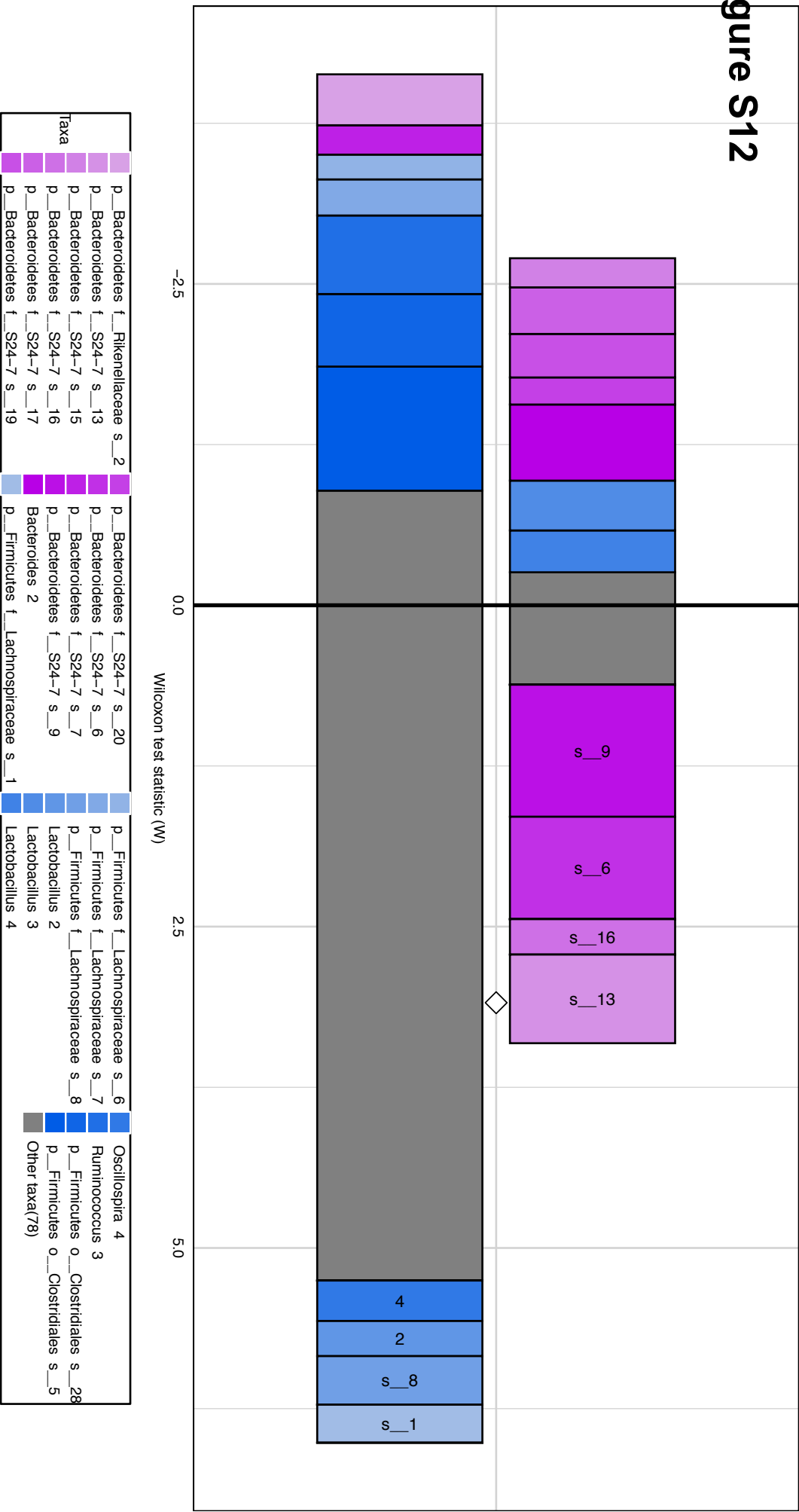


Figure S13

ko00312

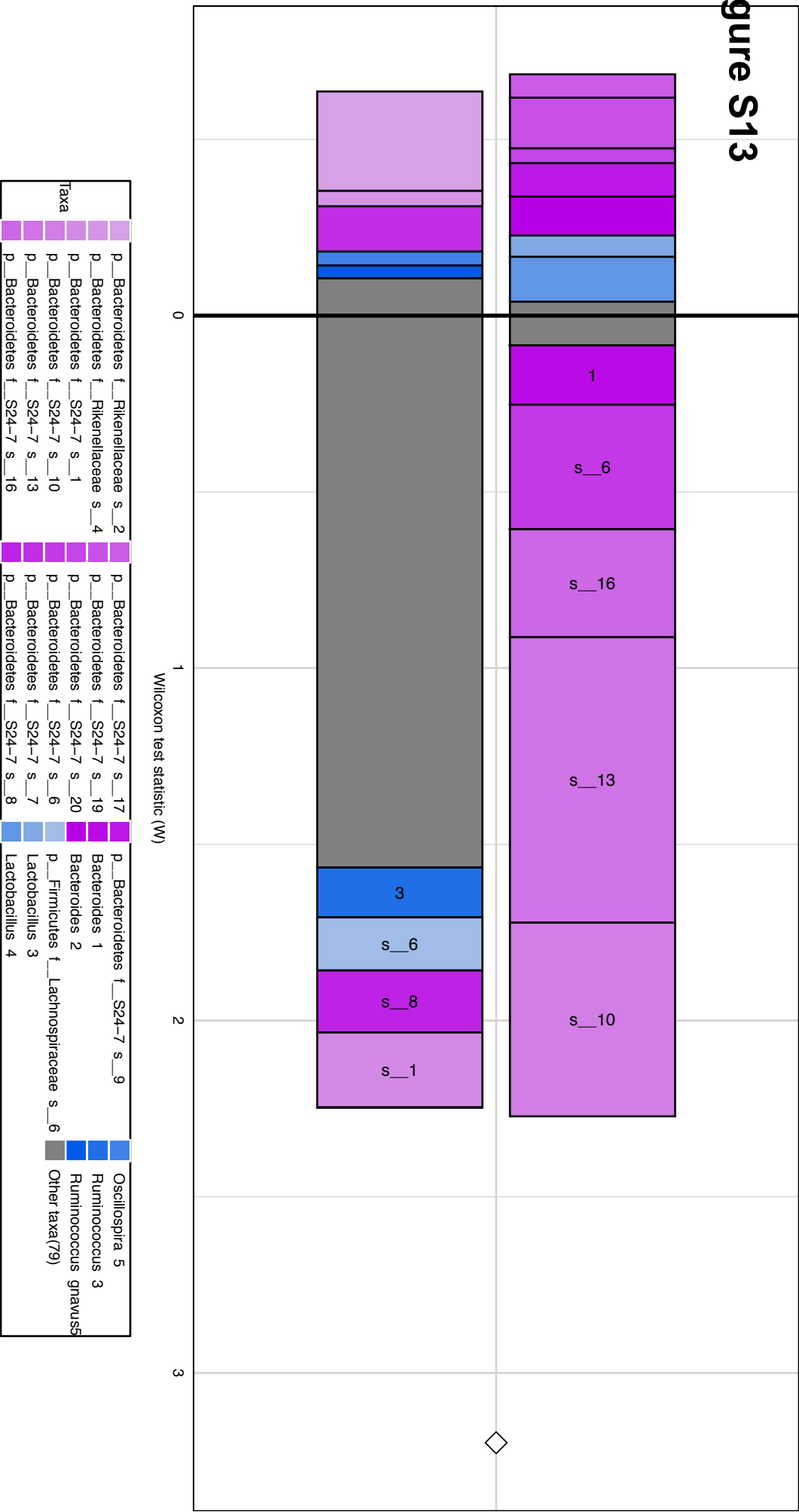


Figure S14

ko00311

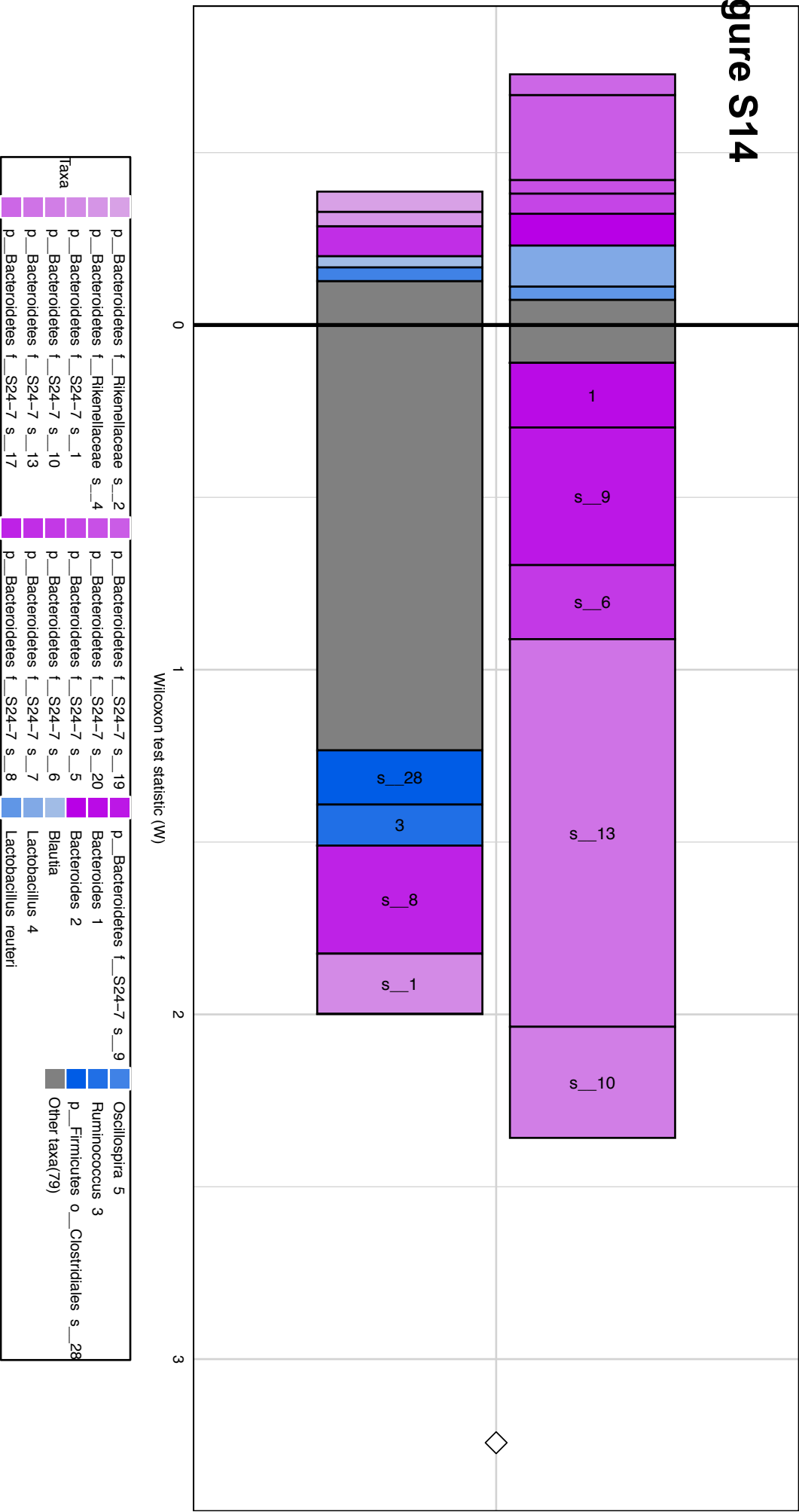
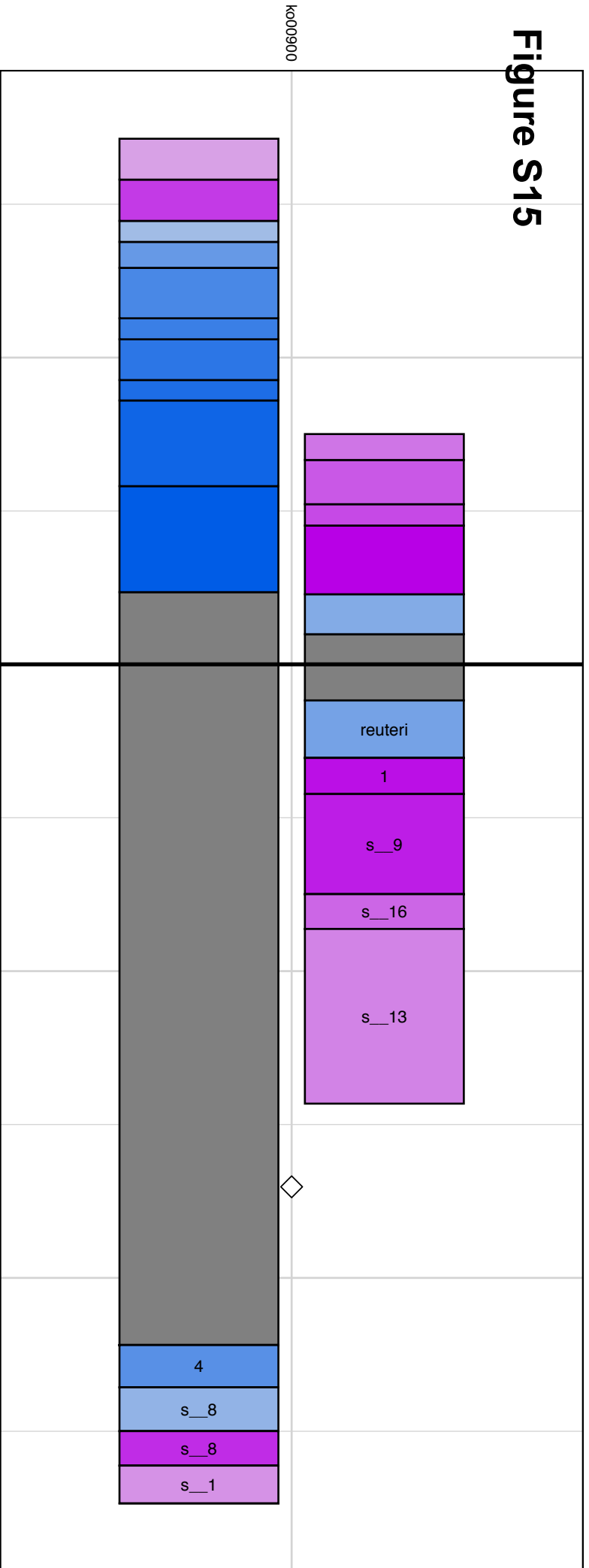


Figure S15



k000900

Figure S16

ko00945

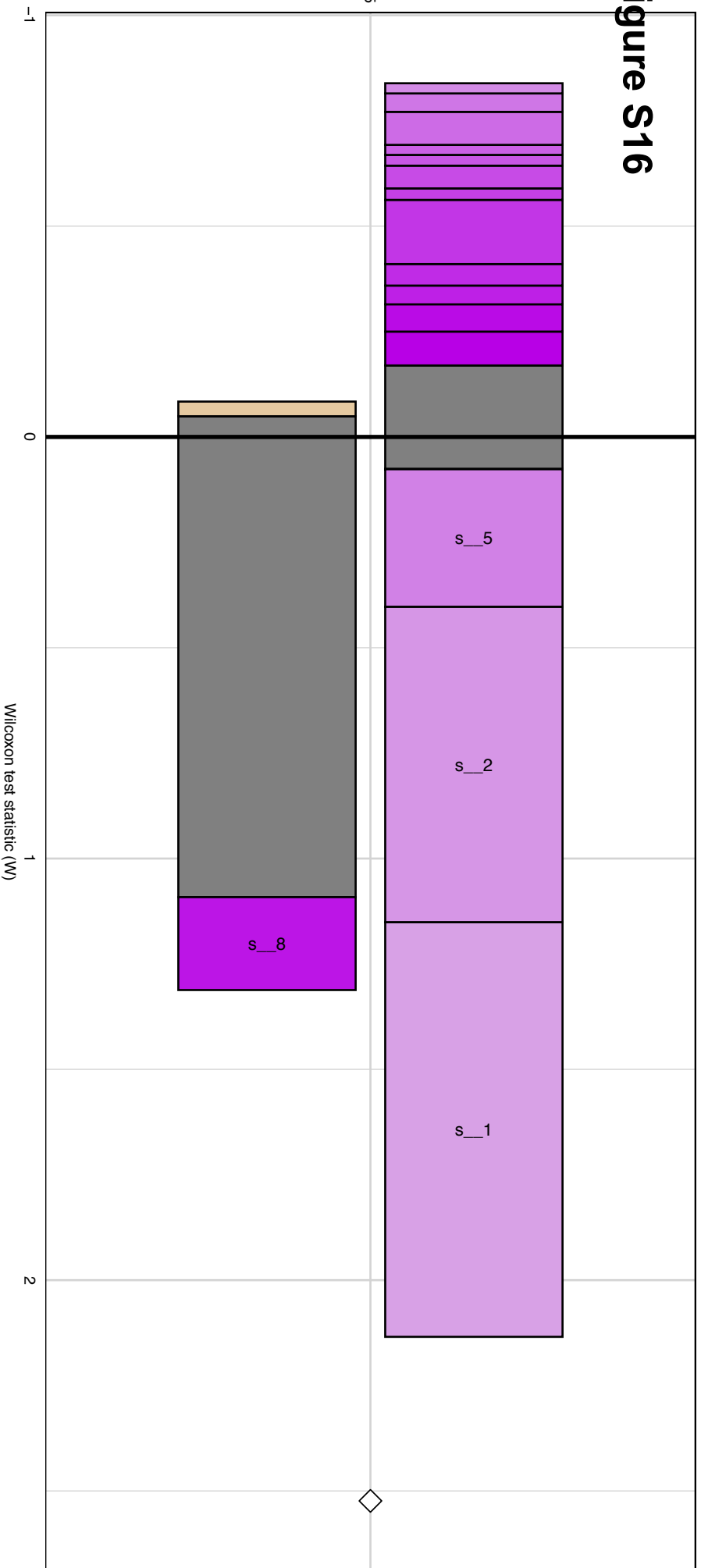


Figure S17

ko00281

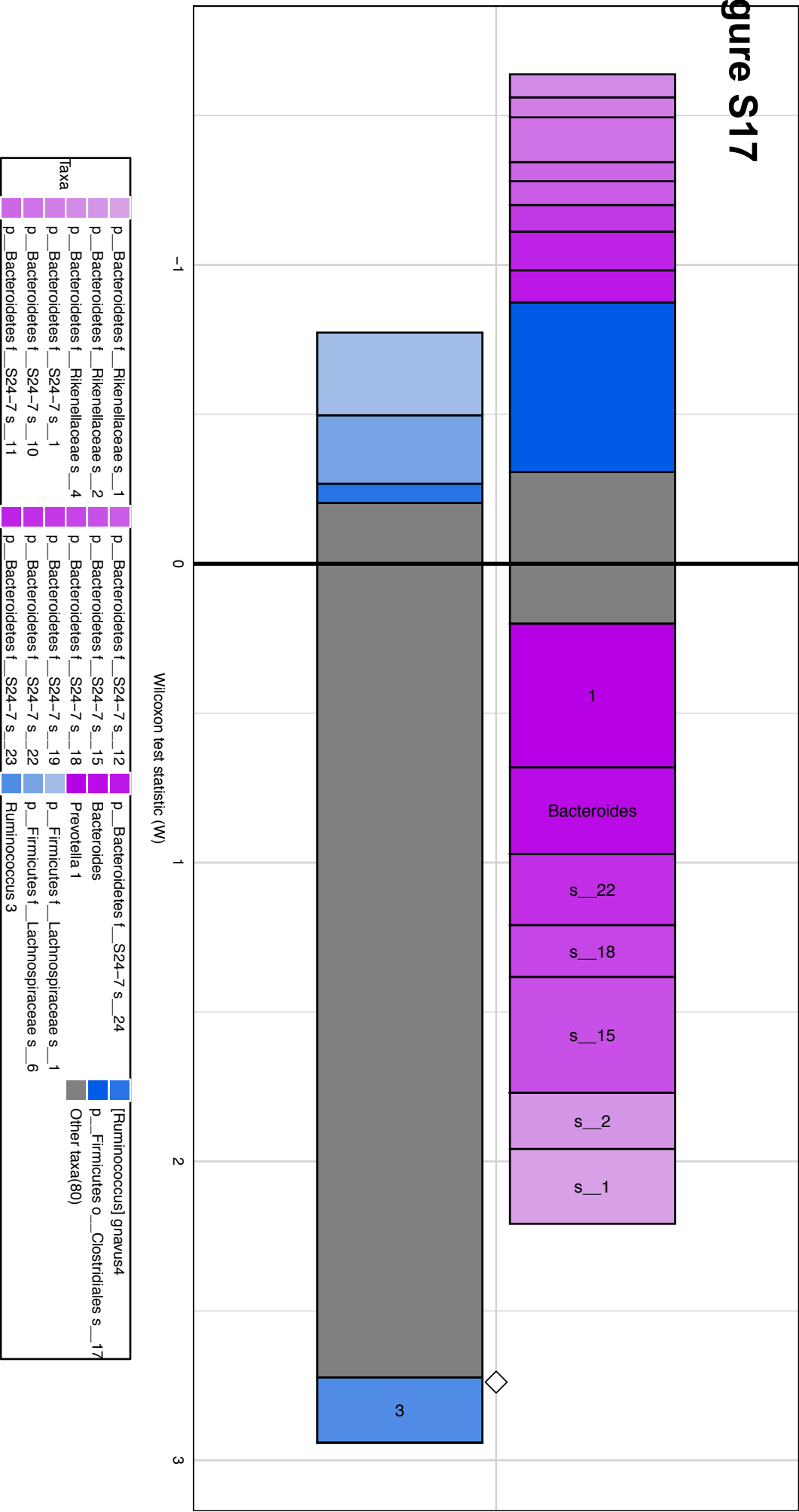


Figure S18

k000740

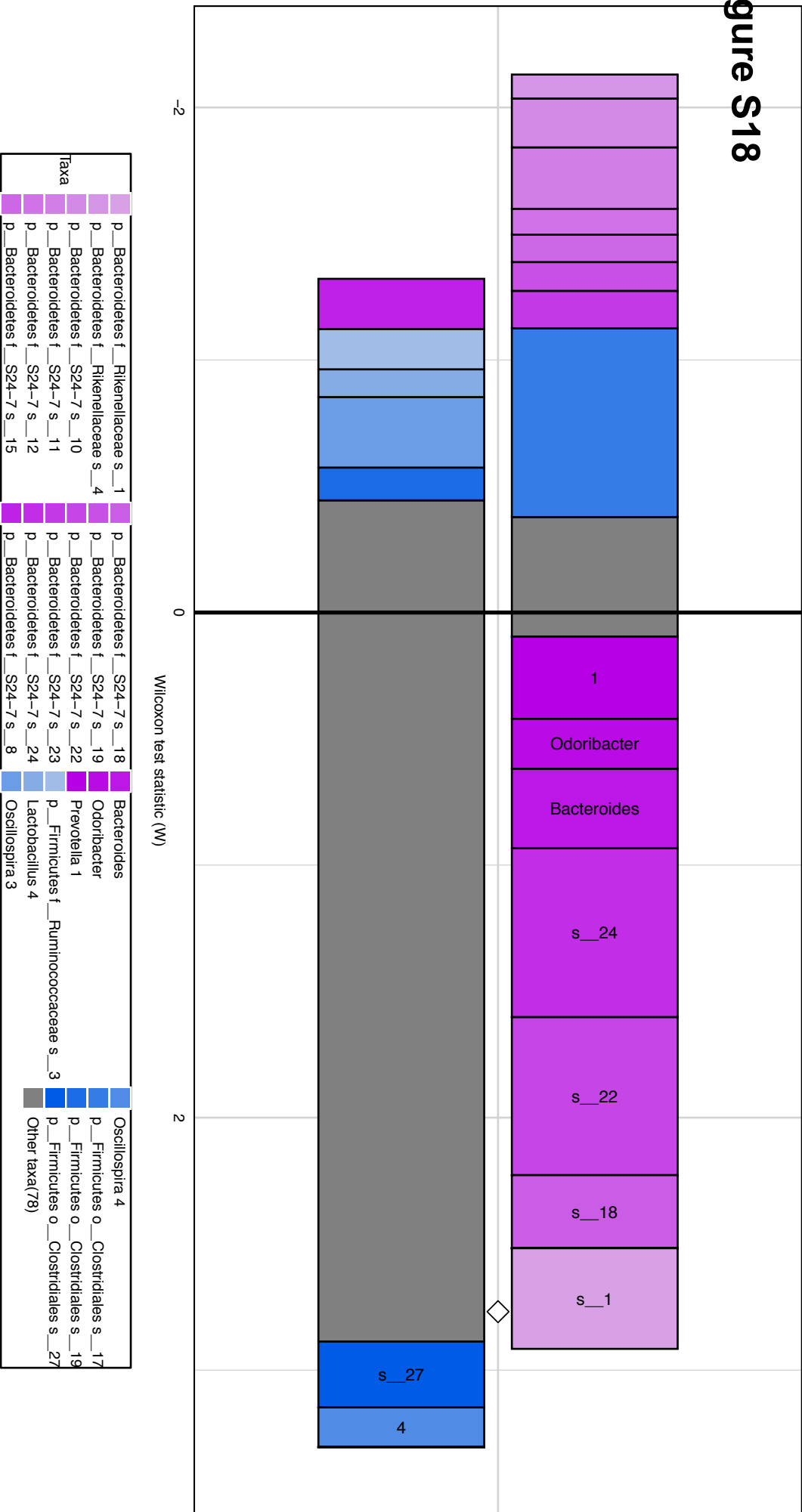


Figure S19

ko00531

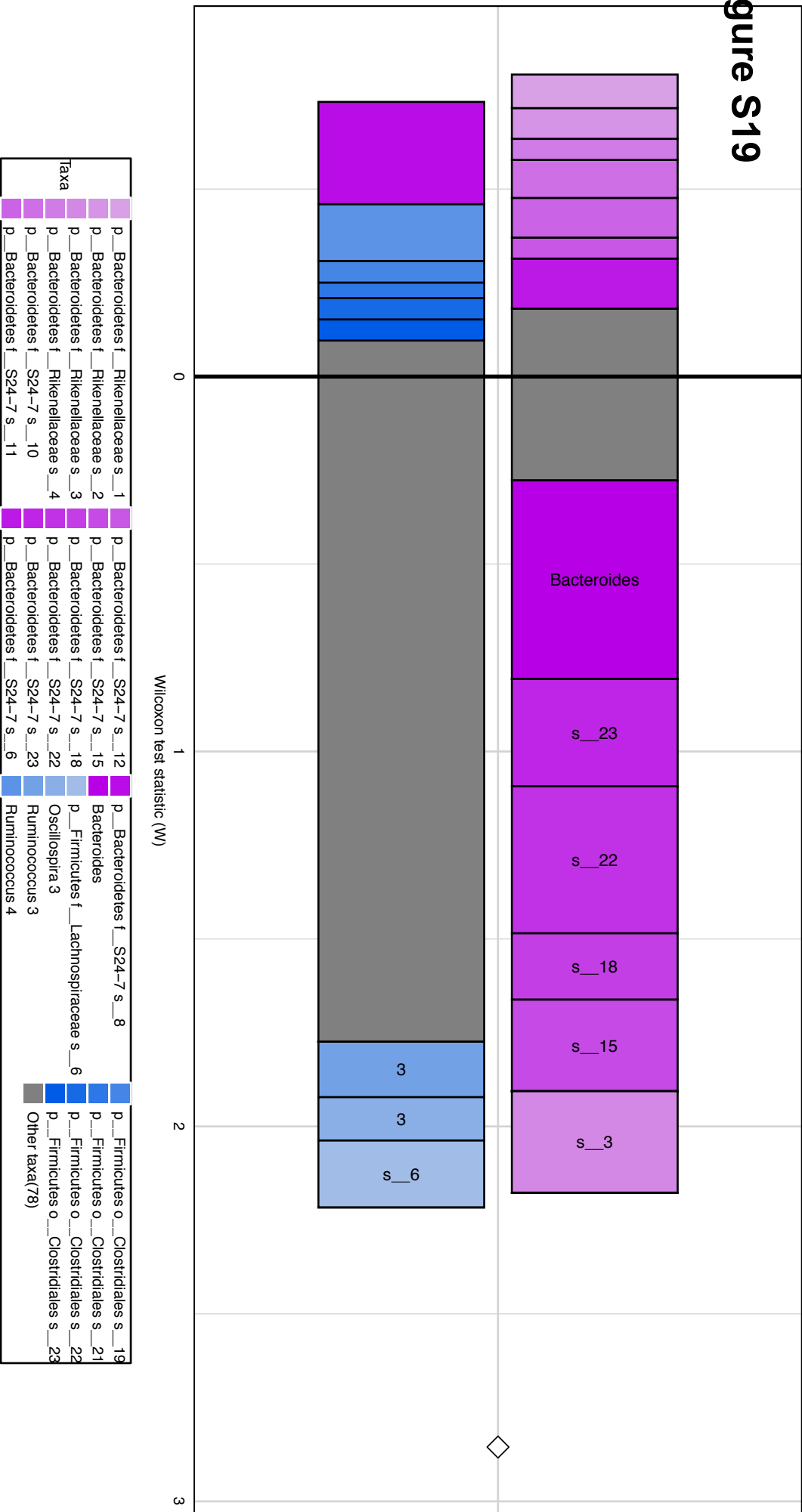


Figure S21

K000623

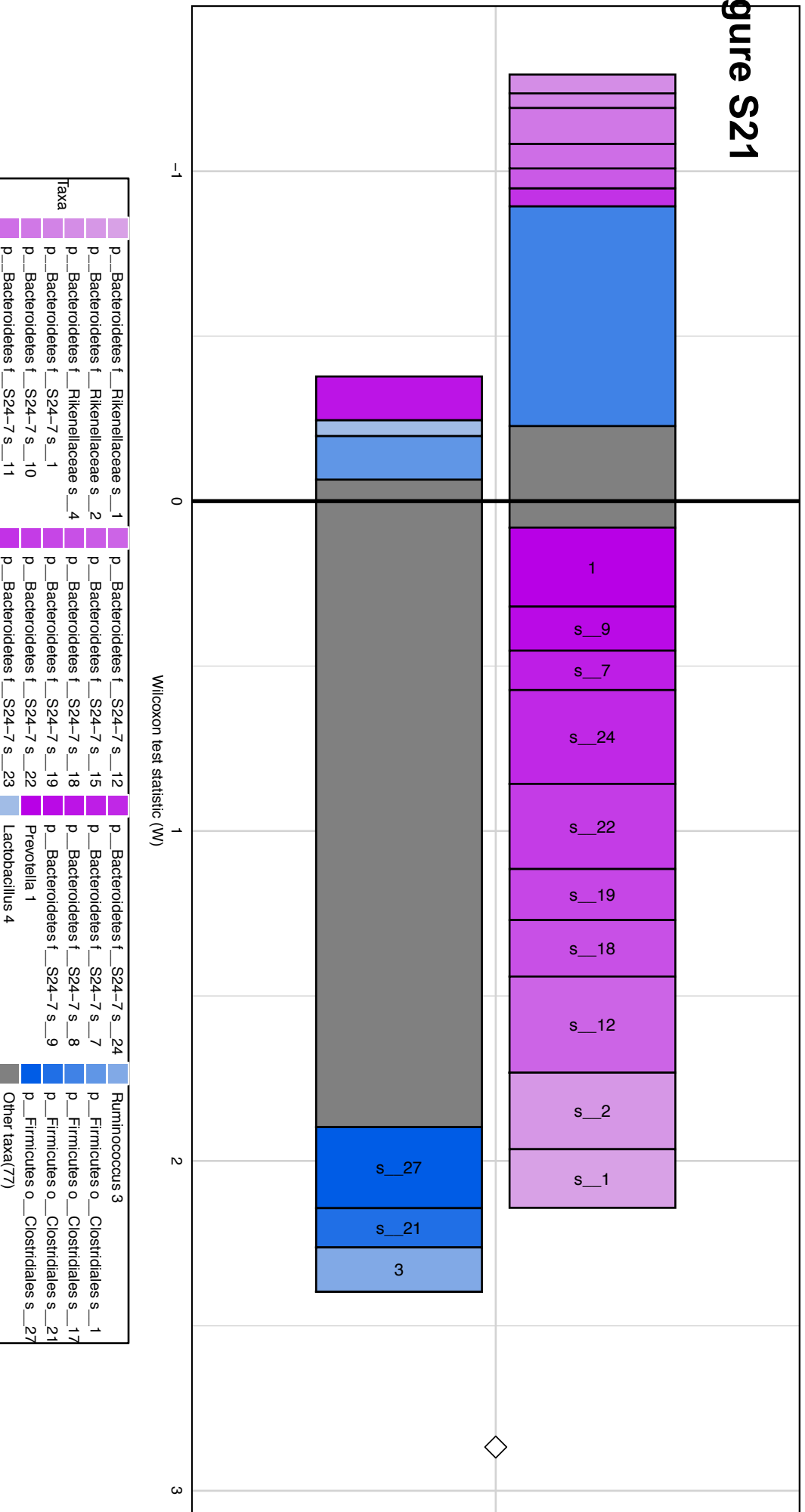


Figure S22

K000604

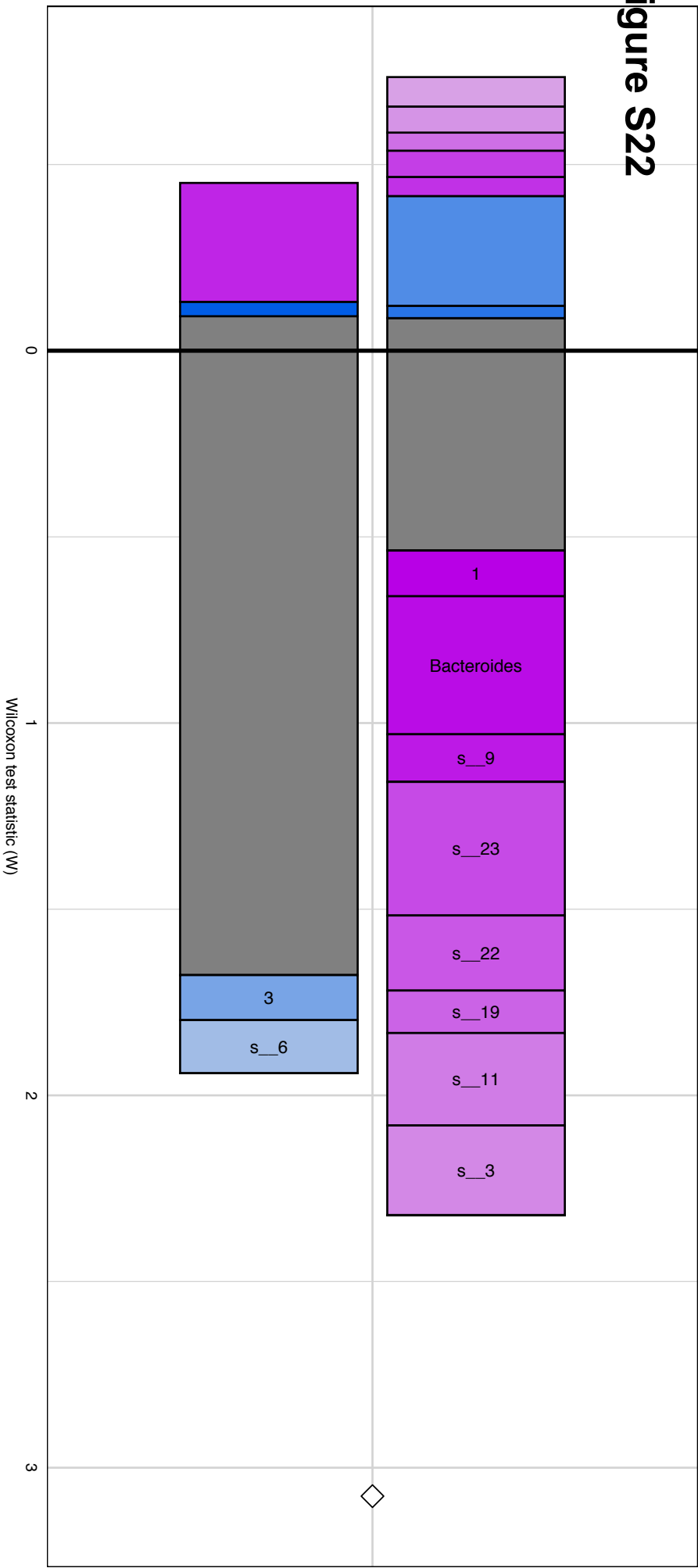


Figure S23

k000780

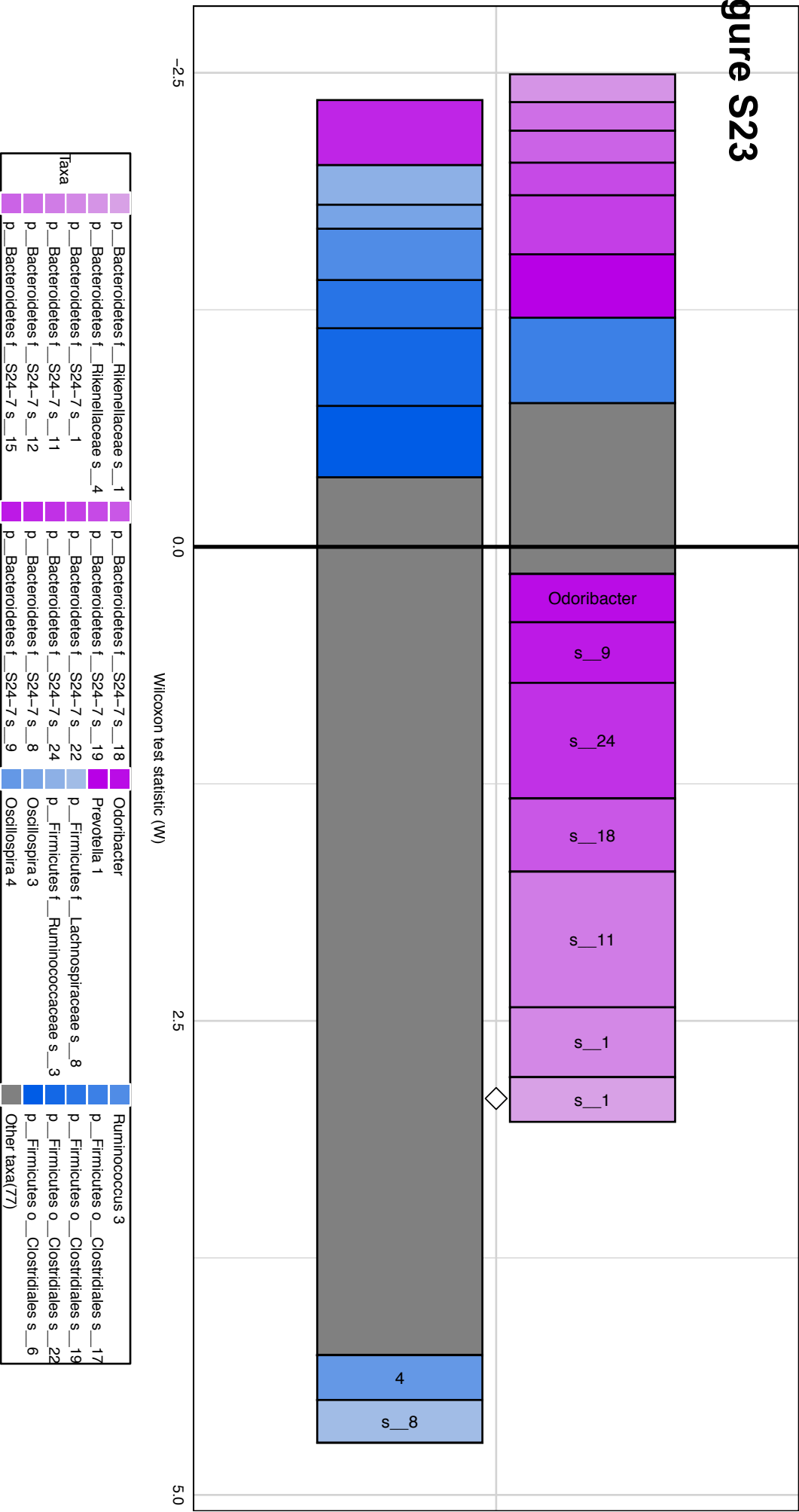


Figure S25

k000785

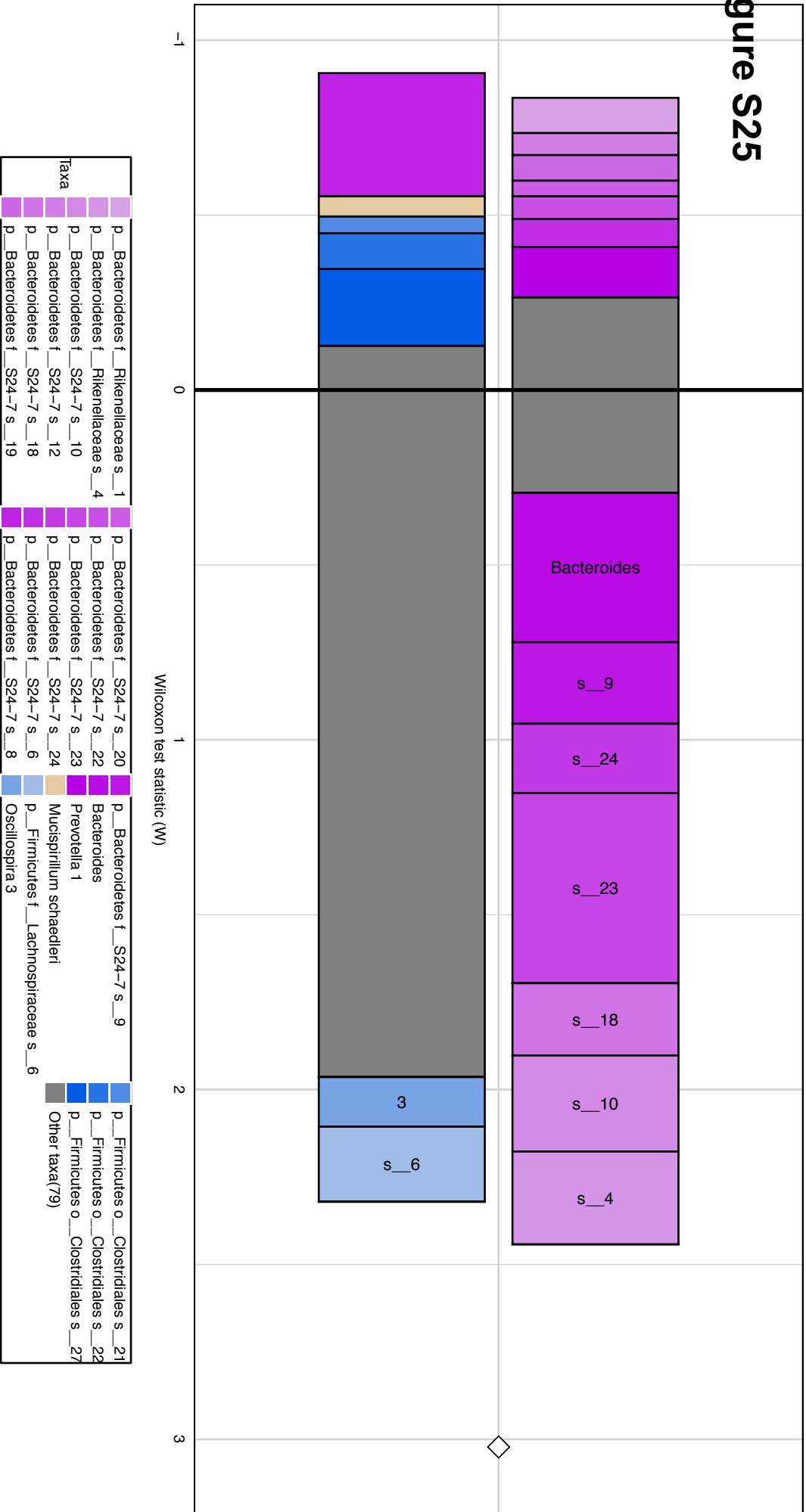


Figure S26

ko03070

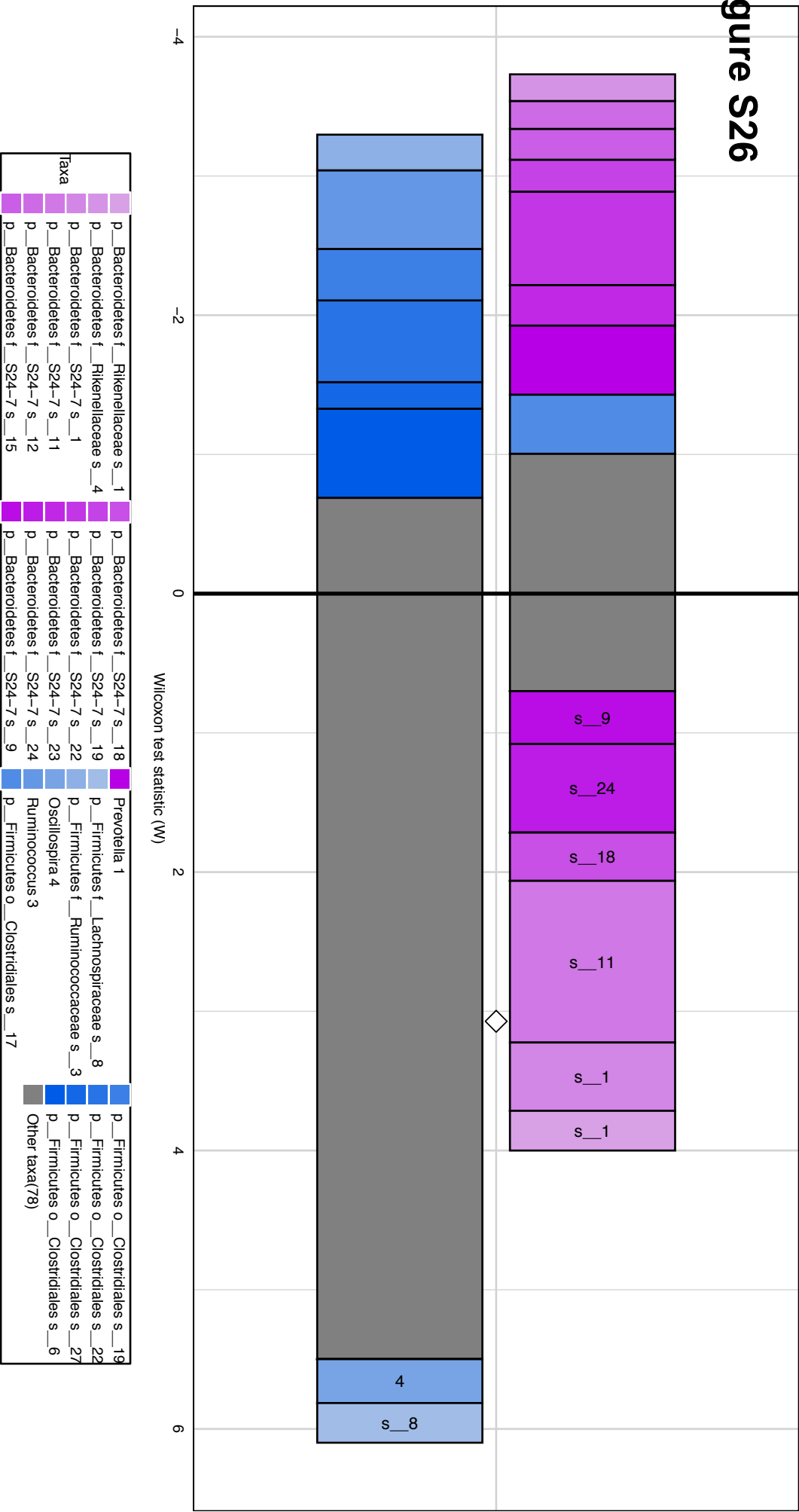


Figure S29

k000670

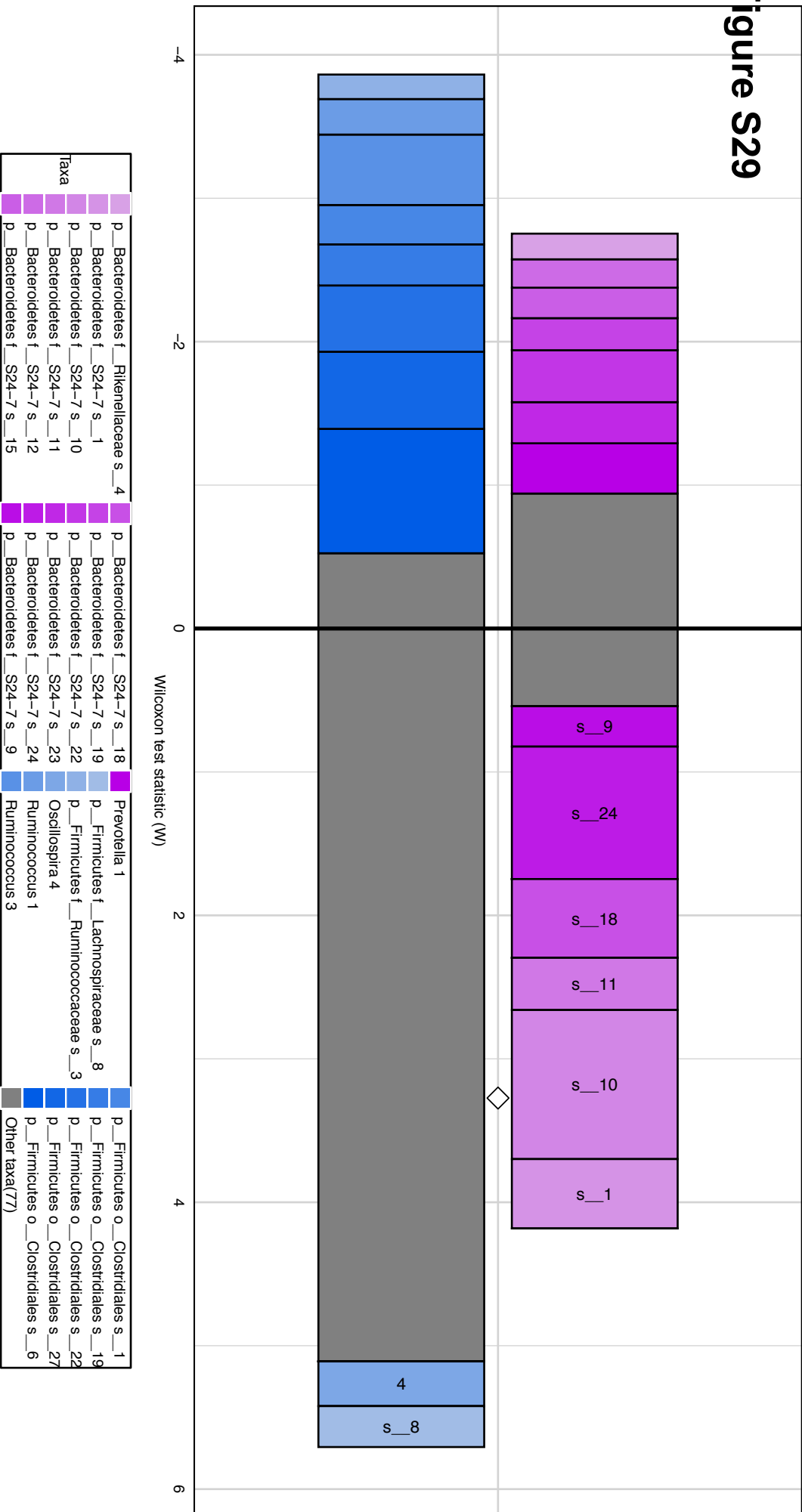


Figure S30

ko04146

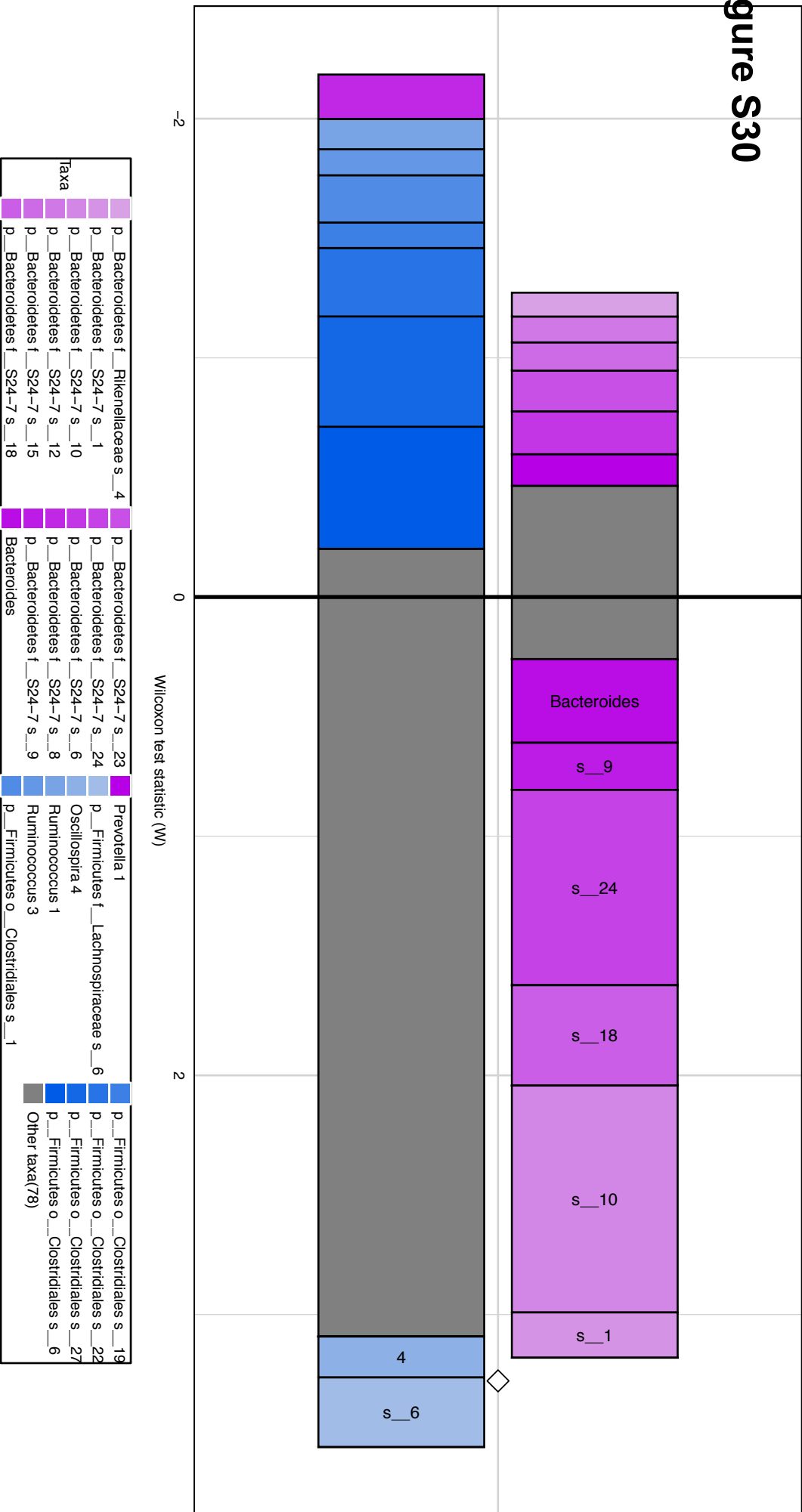
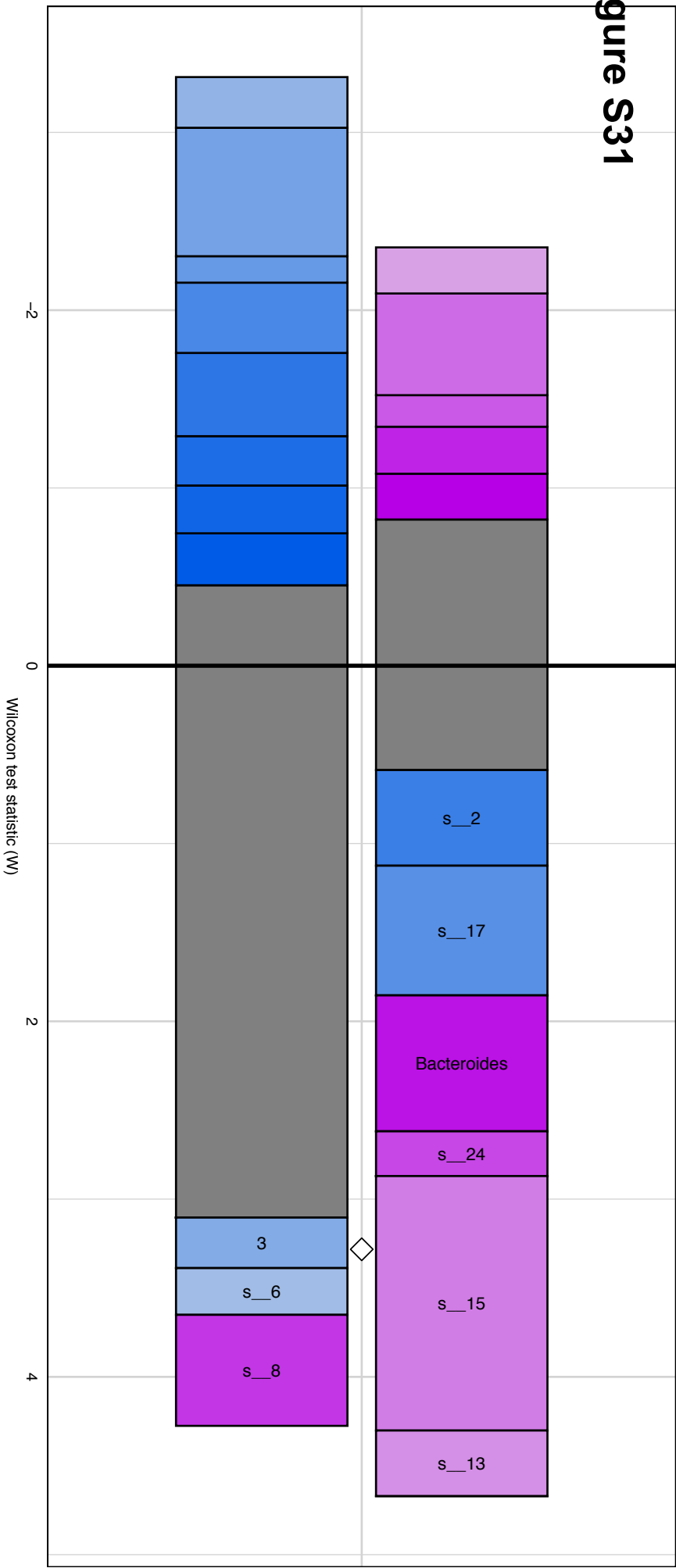


Figure S31

ko00511



Taxa	Color
p__Bacteroidetes f__S24-7 s__11	Light Purple
p__Bacteroidetes f__S24-7 s__13	Light Purple
p__Bacteroidetes f__S24-7 s__15	Light Purple
p__Bacteroidetes f__S24-7 s__22	Light Purple
p__Bacteroidetes f__S24-7 s__23	Light Purple
p__Bacteroidetes f__S24-7 s__24	Light Purple
p__Bacteroidetes f__S24-7 s__8	Light Purple
p__Bacteroidetes f__S24-7 s__9	Light Purple
Bacteroides	Light Purple
Prevotella 1	Light Purple
p__Firmicutes f__Lachnospiraceae s__6	Light Purple
p__Firmicutes f__Ruminococcaceae s__1	Light Purple
Ruminococcus 3	Light Purple
Ruminococcus 4	Light Purple
Ruminococcus 5	Light Purple
p__Firmicutes o__Clostridiales s__17	Light Purple
p__Firmicutes o__Clostridiales s__19	Light Purple
p__Firmicutes o__Clostridiales s__2	Light Purple
p__Firmicutes o__Clostridiales s__22	Light Purple
p__Firmicutes o__Clostridiales s__23	Light Purple
p__Firmicutes o__Clostridiales s__27	Light Purple
p__Firmicutes o__Clostridiales s__8	Light Purple
Other taxa(78)	Light Purple
p__Bacteroidetes f__S24-7 s__11	Blue
p__Bacteroidetes f__S24-7 s__13	Blue
p__Bacteroidetes f__S24-7 s__15	Blue
p__Bacteroidetes f__S24-7 s__22	Blue
p__Bacteroidetes f__S24-7 s__23	Blue
p__Bacteroidetes f__S24-7 s__24	Blue
p__Bacteroidetes f__S24-7 s__8	Blue
p__Bacteroidetes f__S24-7 s__9	Blue
Bacteroides	Blue
Prevotella 1	Blue
p__Firmicutes f__Lachnospiraceae s__6	Blue
p__Firmicutes f__Ruminococcaceae s__1	Blue
Ruminococcus 3	Blue
Ruminococcus 4	Blue
Ruminococcus 5	Blue
p__Firmicutes o__Clostridiales s__17	Blue
p__Firmicutes o__Clostridiales s__19	Blue
p__Firmicutes o__Clostridiales s__2	Blue
p__Firmicutes o__Clostridiales s__22	Blue
p__Firmicutes o__Clostridiales s__23	Blue
p__Firmicutes o__Clostridiales s__27	Blue
p__Firmicutes o__Clostridiales s__8	Blue
Other taxa(78)	Blue

Figure S32

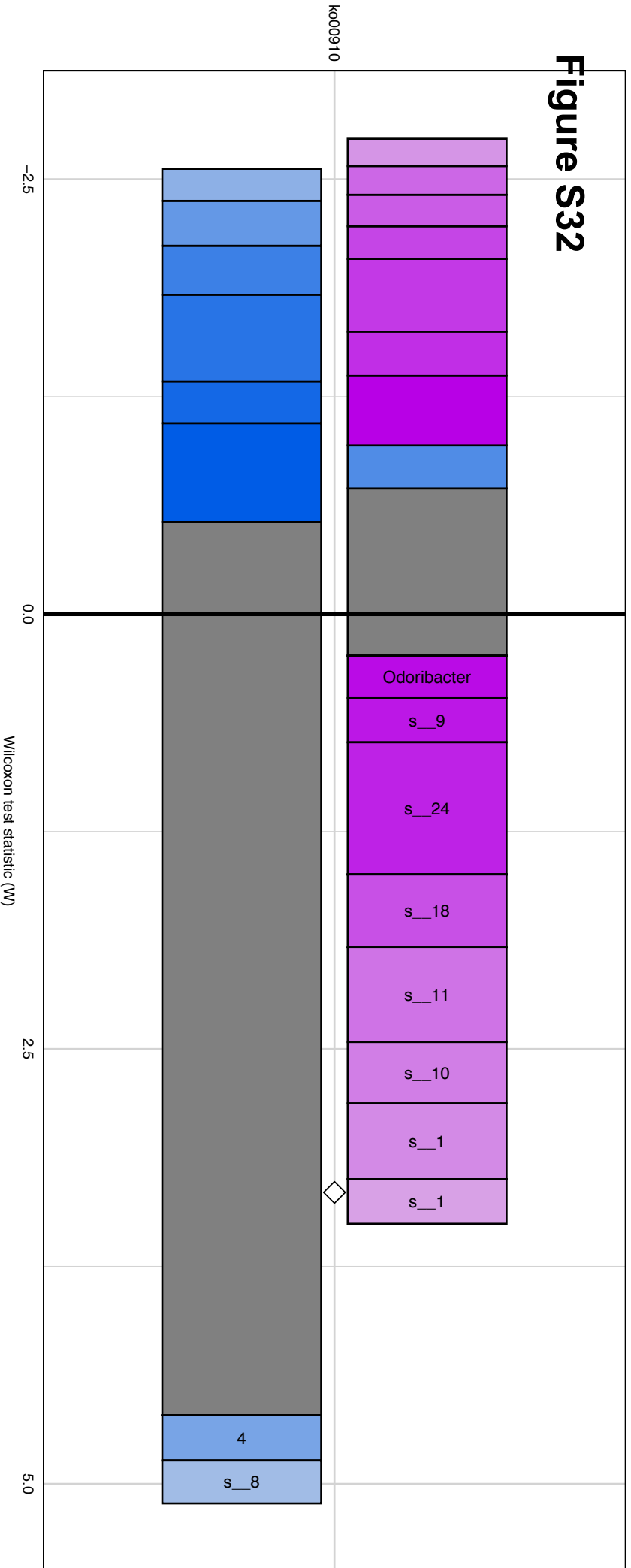


Figure S34

K000540

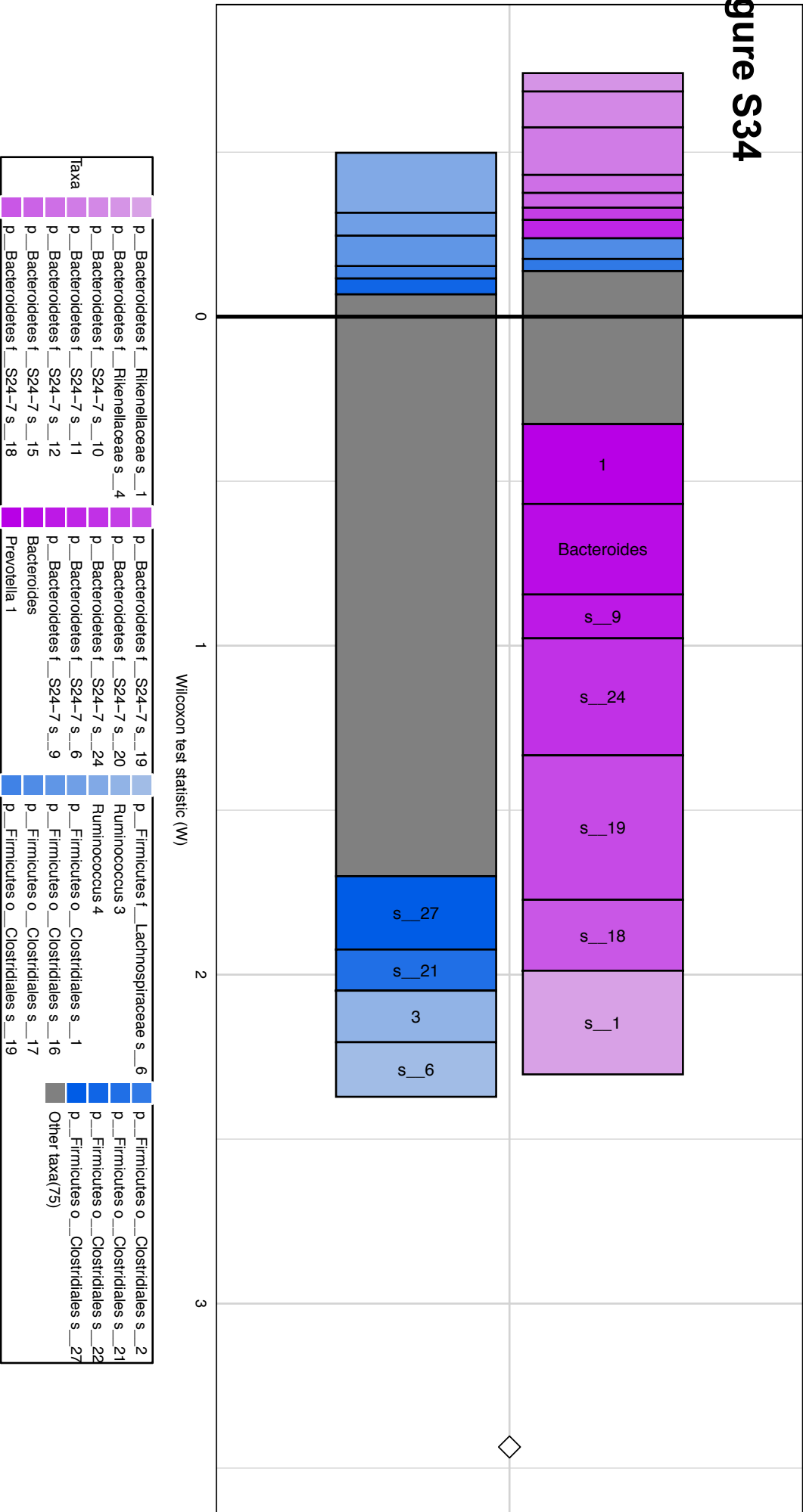


Figure S35

ko03018

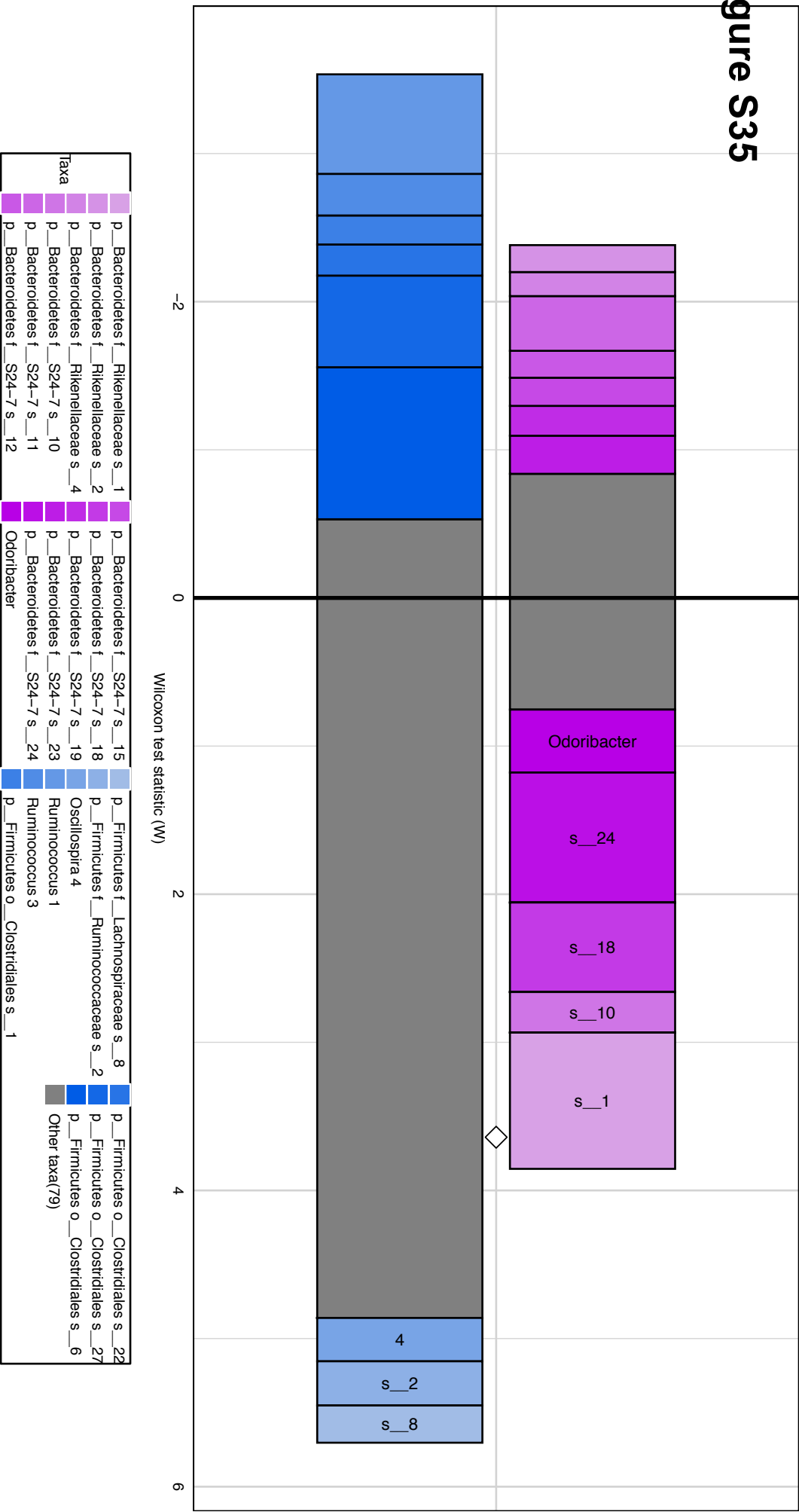


Figure S36

k000790

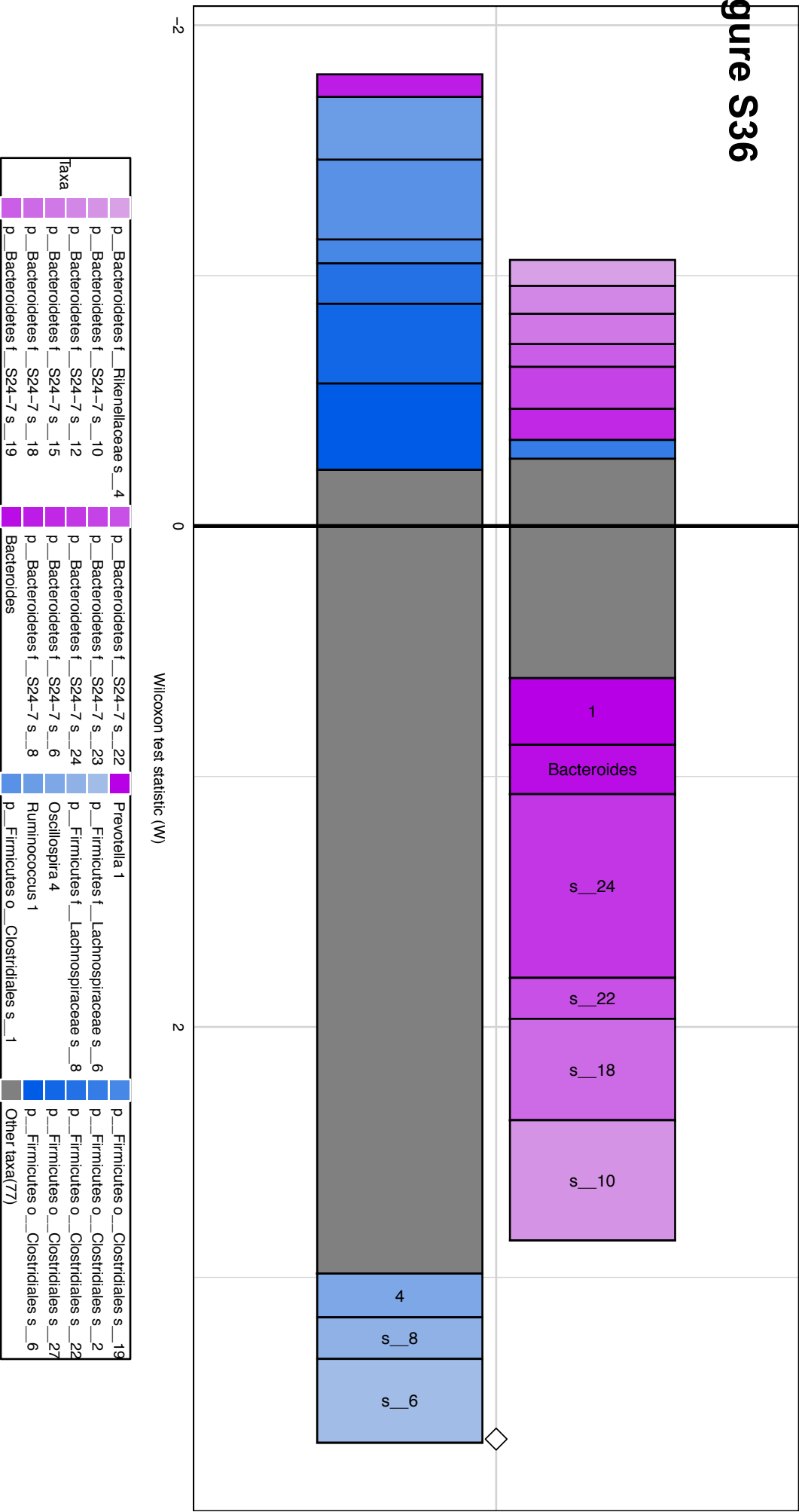


Figure S38

ko00521

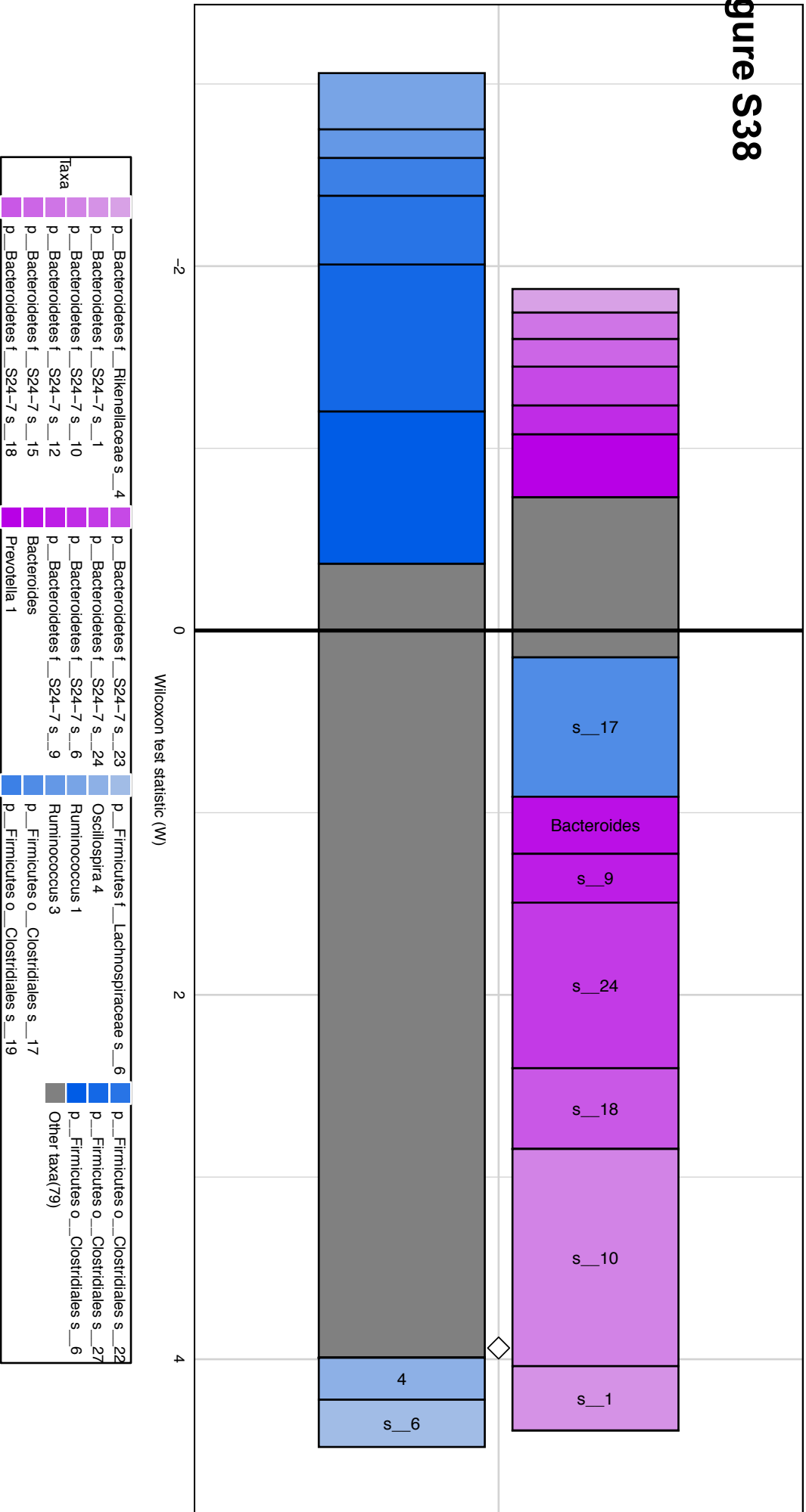


Figure S39

ko04070

