

Supplementary Materials

Supplementary Table 1. Control definitions for each score. The definition of “controls” shown in Figure 3 is different for each score, where all diseases listed for each score were removed from the control pool, along with anyone who reported IBS or its subtypes. The count of comorbidities (n) is shown alongside the minimum size of the control pool (x) available for 1:5 matching, which was determined by subtracting the number of excluded cases (plus all IBS cases) from the size of the validation cohort ($n = 71,220$). For odds ratios based on Rome IV criteria, any positive IBS classification was also excluded from controls.

Score Name (n = comorbidity count; x = minimum control pool)	Comorbidities excluded from controls	References
<i>ButyrateProductionPathways</i> ($n = 37$; $x = 39,021$)	Acne, Atherosclerosis, Atopic dermatitis, <i>C. difficile</i> infection, Cardiovascular condition, Colon polyps, Colorectal cancer, Congestive heart failure, COPD, Coronary heart disease, Crohn's disease, Cystic acne, Depression, Dermatitis (Skin rashes or irritation), Dysglycemia / Irregular blood sugar, Eczema, Emphysema, GI inflammation, Gout, <i>H. pylori</i> infection, High lipids, Hypercholesterolemia, Hypertension, Inflammatory bowel disease, Insomnia, Leaky gut, Liver cirrhosis, Major depressive disorder, Osteoarthritis, Osteoporosis, Prediabetes, Psoriasis, Psoriatic arthritis, Rheumatoid arthritis, Type 1 diabetes, Type 2 diabetes, Ulcerative colitis	[177–203]
<i>GABAProductionPathways</i> ($n = 17$; $x = 46,045$)	Anxiety, Autism, Behavioral / Mood disorder, Colon polyps, Colorectal cancer, Crohn's disease, Depression, Dysmotility, Epilepsy, Inflammatory bowel disease, Irritable bowel syndrome-Diarrhea, Insomnia, Leaky gut, Metabolic syndrome, NAFLD, Type 1 diabetes, Type 2 diabetes, Ulcerative colitis	[204–206]
<i>LacticAcidProductionPathways</i> ($n = 16$; $x = 51,028$)	Chronic fatigue syndrome, Colon polyps, Colorectal cancer, Crohn's disease, Dysbiosis, Gastritis, Infections, Irritable bowel syndrome-Diarrhea, Inflammatory bowel disease, Insulin resistance, Ischemic colitis, Metabolic syndrome, Prediabetes, Short bowel syndrome, Type 2 diabetes, Ulcerative colitis	[93,207–230]
<i>LPSBiosynthesisPathways</i> ($n = 39$; $x = 38,062$)	Alcohol addiction, Allergies, ALS, Alzheimer's disease, Anxiety, Atherosclerosis, Autism, Cardiovascular condition, Celiac disease, Cognitive dysfunction / Cognitive impairment, Colon polyps, Colorectal cancer, Coronary heart disease, Crohn's disease, Depression, Dysbiosis, GI inflammation, HIV, Inflammatory bowel disease, Influenza, Insulin resistance, Kidney disease, Leaky gut, Liver cirrhosis, Metabolic syndrome, Mild cognitive impairment, NAFLD, Obesity, Osteoarthritis, Pancreatic Cancer, Pancreatitis, Parkinson's disease, Peripheral artery disease, Prediabetes, Rheumatoid arthritis, Sepsis, SIBO, Type 2 diabetes, Ulcerative colitis	[62,231–244]
<i>MethanogenesisPathways</i> ($n = 32$; $x = 44,552$)	Abdominal weight, Abnormal fasting glucose, Anorexia, Colon polyps, Colorectal cancer, Crohn's disease, Diverticulitis, Diverticulosis, Dysbiosis, Dysglycemia / Irregular blood sugar, Encopresis, Gastritis, Gastroparesis, GI inflammation, <i>H. pylori</i> , High lipids, Hypercholesterolemia, Infections, Irritable bowel syndrome-Constipation, Inflammatory bowel disease, Insulin resistance, Leaky gut, Metabolic syndrome, Multiple sclerosis, NAFLD, Obesity, Parkinson's disease, Prediabetes, SIBO, Type 1 diabetes, Type 2 diabetes, Ulcerative colitis	[126,245–295]
<i>OxalateMetabolismPathways</i> ($n = 19$; $x = 43,222$)	Abdominal weight, Cardiovascular condition, Colon polyps, Colorectal cancer, Coronary heart disease, Crohn's disease, Hashimoto's disease, Hypertension, Hyperthyroidism, Hypothyroidism, Inflammatory bowel disease, Kidney disease, Kidney stones, Obesity, Short bowel disease, Stroke, Thyroid cancer, Thyroid nodule(s), Ulcerative colitis	[296–307]
<i>SulfideProductionPathways</i> ($n = 16$; $x = 53,736$)	Colon polyps, Colorectal cancer, Crohn's disease, Diverticulitis, Diverticulosis, GI inflammation, <i>H. pylori</i> infection, Infections, Inflammatory bowel disease, Irritable bowel syndrome-Constipation, Irritable bowel syndrome-Diarrhea, Irritable bowel syndrome, Leaky gut, Parkinson's disease, SIBO, Ulcerative colitis	[308–314]
<i>UricAcidProductionPathways</i> ($n = 14$; $x = 49,983$)	Colon polyps, Colorectal cancer, Crohn's disease, Gout, Hypertension, Inflammatory bowel disease, Insulin resistance, Kidney disease, Kidney stones, Metabolic syndrome, Obesity, Prediabetes, Type 2 diabetes, Ulcerative colitis	[315–324]

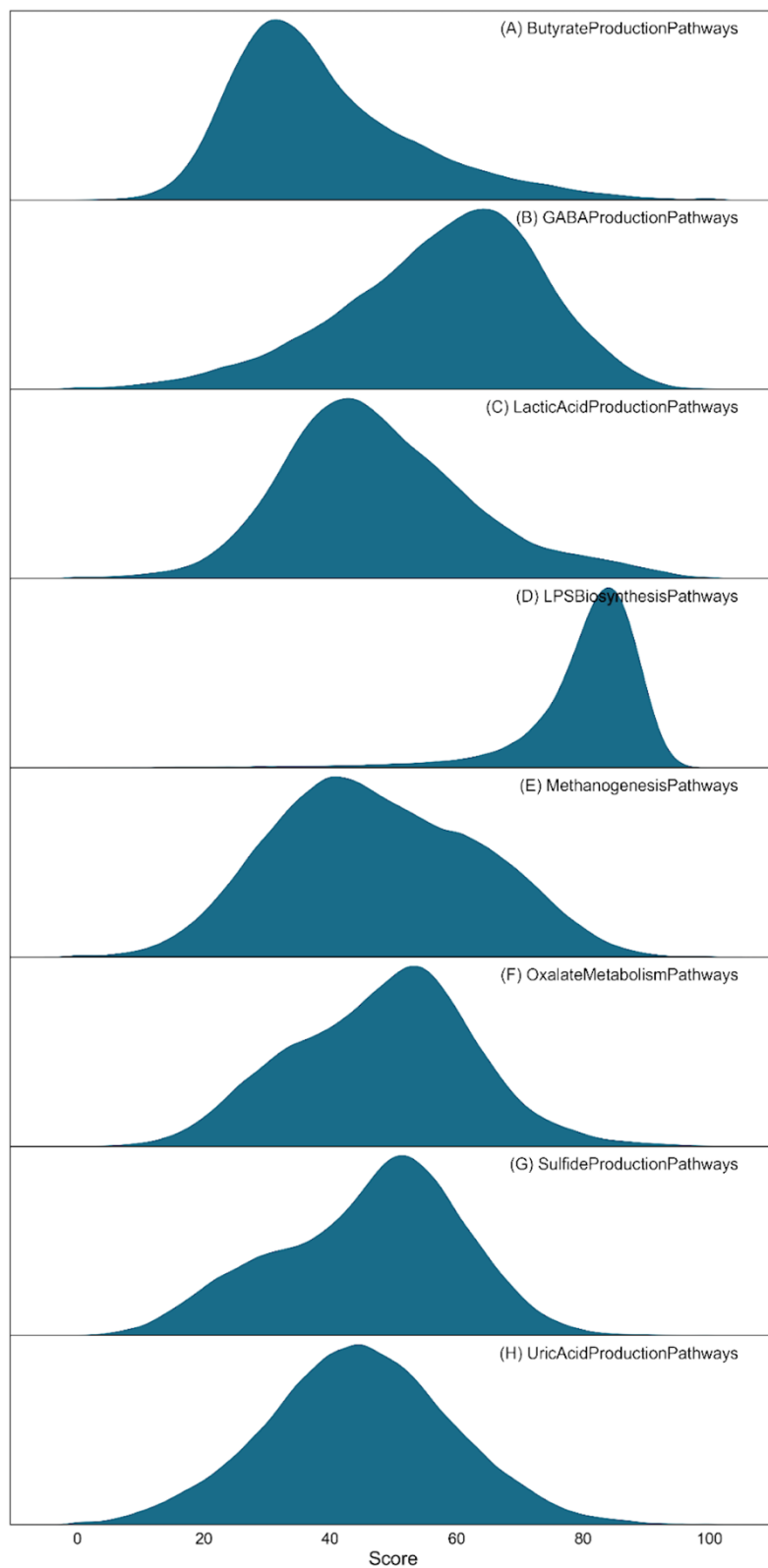
Supplementary Table 2. Odds Ratio and Confidence Intervals shown in Figure 3. IBS cases are the composite of IBS and all IBS subtypes while “control” definitions are score-specific (Supplementary Table 1). Values represent Odds Ratios. Significance levels of the p-values are shown in superscript, with 95% Confidence Interval in parentheses. $p \leq 0.05$ (*); $p \leq 0.01$ (**); $p \leq 0.001$ (**); not significant (ns).

Score Name	IBS (max $n = 6,373$)	IBS-C (max $n = 1,732$)	IBS-D (max $n = 1,006$)	IBS-M (max $n = 1,091$)
<i>ButyrateProductionPathways</i>	1.3*** (1.2 - 1.5)	0.8* (0.7 - 0.9)	2.6*** (2.1 - 3.3)	1.5*** (1.2 - 1.9)
<i>GABAProductionPathways</i>	1.7*** (1.5 - 1.8)	2.1*** (1.8 - 2.6)	1.6*** (1.3 - 2.0)	1.5*** (1.2 - 1.9)
<i>LacticAcidProductionPathways</i>	1.4*** (1.3 - 1.5)	0.9 ^{ns} (0.8 - 1.1)	2.3*** (1.8 - 2.9)	1.2 ^{ns} (1.0 - 1.5)
<i>LPSBiosynthesisPathways</i>	1.2** (1.1 - 1.3)	1.3** (1.1 - 1.5)	0.9 ^{ns} (0.7 - 1.1)	1.1 ^{ns} (0.9 - 1.4)
<i>MethanogenesisPathways</i>	1.2*** (1.1 - 1.4)	1.6*** (1.4 - 2.0)	1.2 ^{ns} (1.0 - 1.5)	1.3* (1.0 - 1.5)
<i>OxalateMetabolismPathways</i>	1.3*** (1.2 - 1.4)	0.9 ^{ns} (0.7 - 1.0)	2.1*** (1.6 - 2.6)	1.2* (1.0 - 1.5)
<i>SulfideProductionPathways</i>	1.1 ^{ns} (1.0 - 1.2)	0.7*** (0.6 - 0.9)	1.8*** (1.5 - 2.3)	1.1 ^{ns} (0.9 - 1.3)
<i>UricAcidProductionPathways</i>	1.5*** (1.4 - 1.6)	1.0 ^{ns} (0.8 - 1.1)	2.3*** (1.8 - 2.9)	1.3* (1.1 - 1.6)

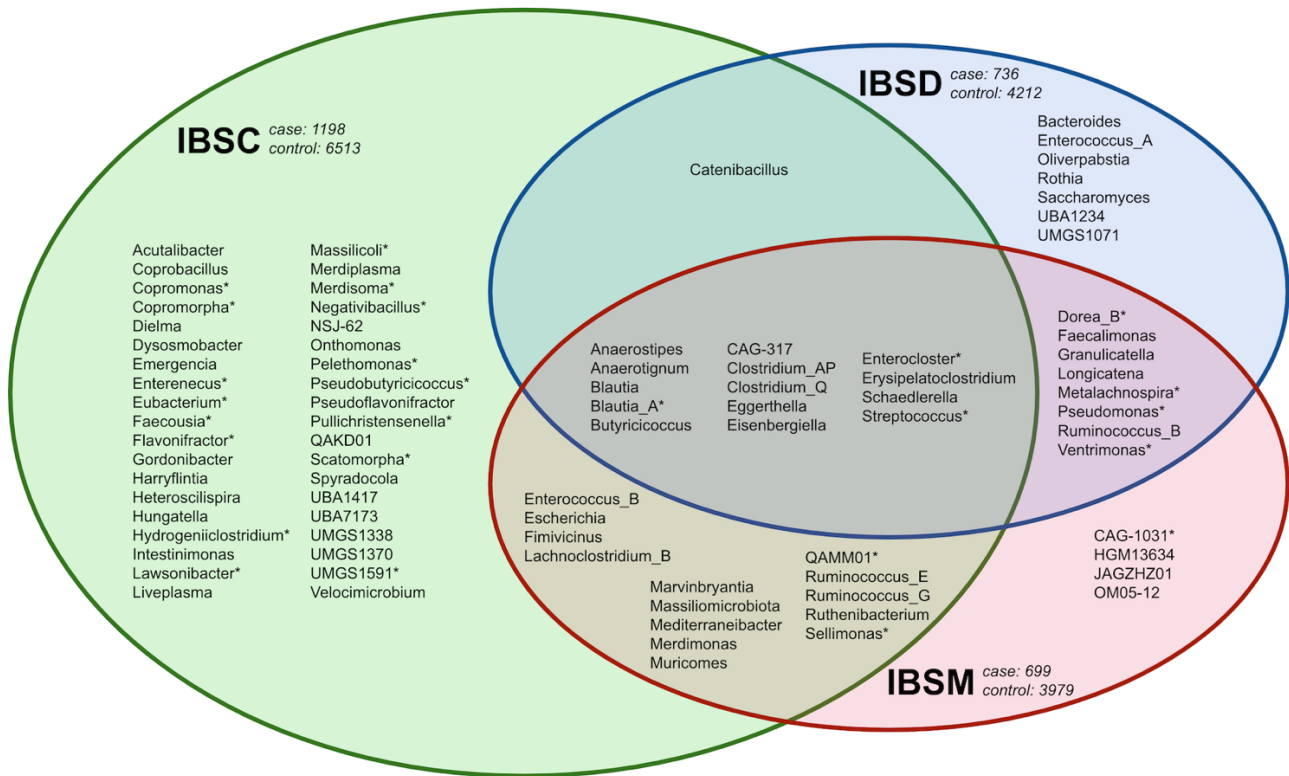
Supplementary Table 3. Odds Ratio and Confidence Intervals shown in Figure 4. IBS cases are the composite of any IBS subtype classification, and cases for each IBS subtype classification are defined by the Rome IV Diagnostic Questionnaire. “Control” definitions are score-specific (Supplementary Table 1). Values represent Odds Ratios. Significance levels of the p-values are shown in superscript, with 95% Confidence Interval in parentheses. $p \leq 0.05$ (*); $p \leq 0.01$ (**); $p \leq 0.001$ (***); not significant (ns).

Score Name	IBS (max $n = 8,629$)	IBS-C (max $n = 1,942$)	IBS-D (max $n = 2,952$)	IBS-M (max $n = 3,309$)
<i>ButyrateProductionPathways</i>	1.5*** (1.3 - 1.6)	0.7*** (0.6 - 0.8)	2.7*** (2.4 - 3.1)	1.3*** (1.1 - 1.4)
<i>GABAProductionPathways</i>	1.8*** (1.6 - 1.9)	2.5*** (2.1 - 3.0)	1.6*** (1.4 - 1.8)	1.8*** (1.6 - 2.1)
<i>LacticAcidProductionPathways</i>	1.4*** (1.3 - 1.5)	0.8** (0.7 - 0.9)	2.2*** (2.0 - 2.5)	1.3*** (1.2 - 1.5)
<i>LPSBiosynthesisPathways</i>	1.1* (1.0 - 1.2)	1.3** (1.1 - 1.6)	1.0 ^{ns} (0.9 - 1.1)	1.1 ^{ns} (0.9 - 1.2)
<i>MethanogenesisPathways</i>	1.2*** (1.1 - 1.3)	1.8*** (1.5 - 2.1)	1.0 ^{ns} (0.9 - 1.1)	1.3*** (1.2 - 1.5)
<i>OxalateMetabolismPathways</i>	1.3*** (1.2 - 1.5)	0.7*** (0.6 - 0.8)	2.1*** (1.8 - 2.4)	1.2** (1.1 - 1.4)
<i>SulfideProductionPathways</i>	1.1** (1.0 - 1.2)	0.6*** (0.5 - 0.7)	1.7*** (1.5 - 1.9)	1.1 ^{ns} (0.9 - 1.2)
<i>UricAcidProductionPathways</i>	1.5*** (1.4 - 1.6)	0.9 ^{ns} (0.8 - 1.0)	2.3*** (2.0 - 2.7)	1.4*** (1.3 - 1.6)

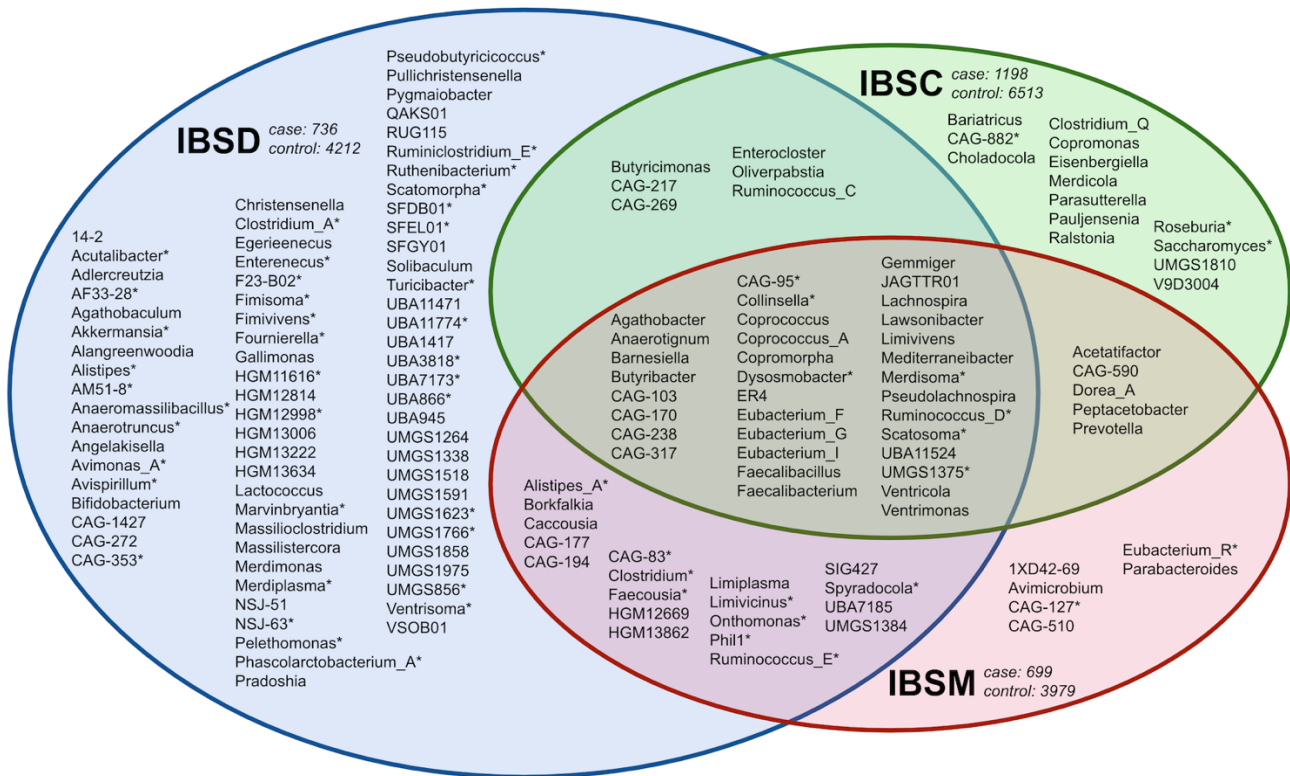
Supplementary Figure 1. Landscape of score distribution. The distribution of eight functional pathway scores (A-H) in an independent cohort ($n = 71,220$ samples). The distribution of each pathway score across the entire cohort is expected to follow a Gaussian normal distribution, where a high score is “Good”. Some scores deviate significantly from this normal distribution, which can be attributed to missing molecular signatures in the reference cohort, resulting in extremely high or low scores. Scores exceeding 100 or falling below 0 were clipped.



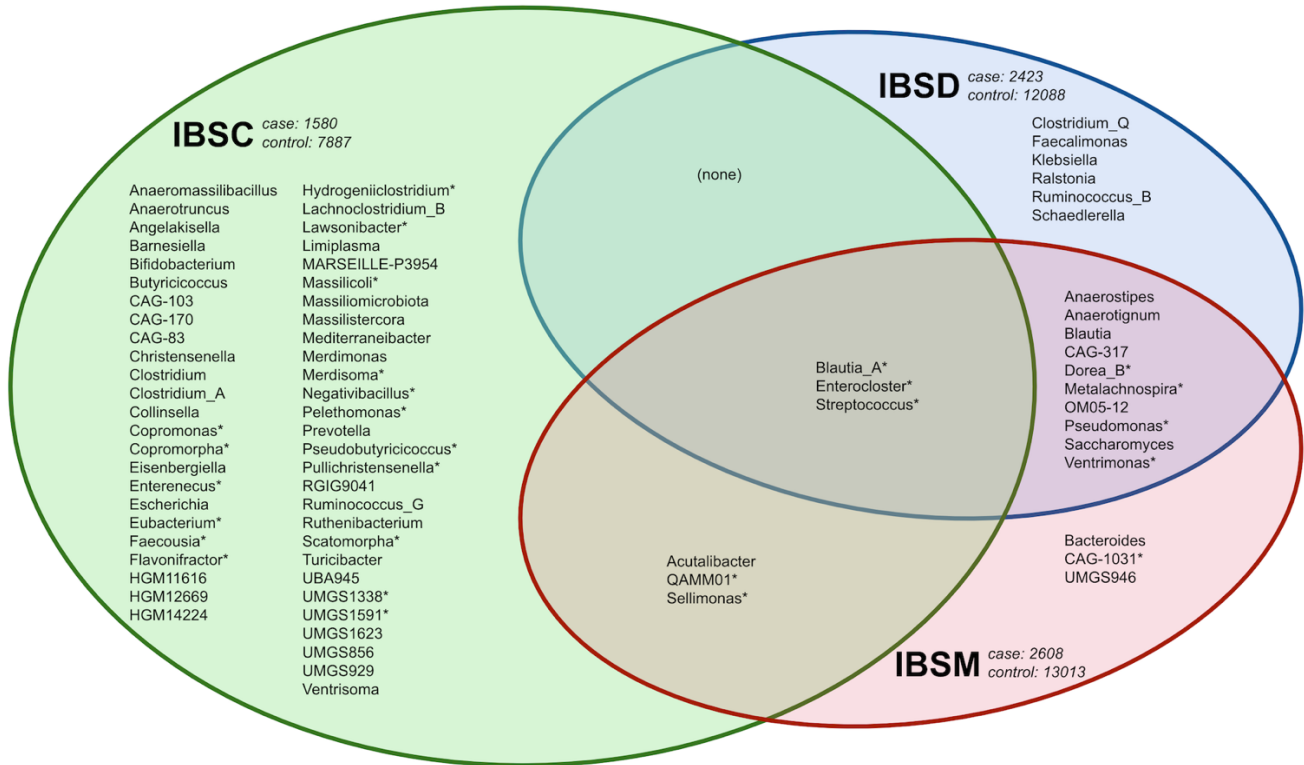
Supplementary Figure 2. Genera of significantly increased species (differentially expressed) between self-reported IBS phenotypes and individuals with no comorbidities. Controls are defined as individuals with zero self-reported comorbidities. At the highest level, cases and controls were matched 1:5 for “IBS-C” (green; $n = 1,198$), “IBS-D” (blue; $n = 736$), and “IBS-M” (red; $n = 699$). All significantly increased species ($p \leq 0.05$ and $\log$ “Fold Change” ≥ 0.6) were combined into respective genera before constructing the Venn Diagram. All genera with differential expression consistent to Rome IV-determined IBS phenotypes (Supplementary Figure 4) are indicated with an asterisk (*).



Supplementary Figure 3. Genera of significantly decreased species (differentially expressed) between self-reported IBS phenotypes and individuals with no comorbidities. Controls are defined as individuals with zero self-reported comorbidities. At the highest level, cases and controls were matched 1:5 for “IBS-C” (green; $n = 1,198$), “IBS-D” (blue; $n = 736$), and “IBS-M” (red; $n = 699$). All significantly decreased species ($p \leq 0.05$ and $\log$ “Fold Change” ≤ -0.6) were combined into respective genera before constructing the Venn Diagram. All genera with differential expression consistent to Rome IV-determined IBS phenotypes (Supplementary Figure 5) are indicated with an asterisk (*).



Supplementary Figure 4. Genera of significantly increased species (differentially expressed) between Rome IV-defined IBS phenotypes and individuals with no comorbidities. Controls are defined as individuals with zero self-reported comorbidities. At the highest level, cases and controls were matched 1:5 for “IBS-C” (green; $n = 1,198$), “IBS-D” (blue; $n = 736$), and “IBS-M” (red; $n = 699$). All significantly increased species ($p \leq 0.05$ and $\log$ “Fold Change” ≥ 0.6) were combined into respective genera before constructing the Venn Diagram. All genera with differential expression consistent to self-reported IBS phenotypes (Supplementary Figure 2) are indicated with an asterisk (*).



Supplementary Figure 5. Genera of significantly decreased species (differentially expressed) between Rome IV-defined IBS phenotypes and individuals with no comorbidities. Controls are defined as individuals with zero self-reported comorbidities. At the highest level, cases and controls were matched 1:5 for “IBS-C” (green; n = 1,198), “IBS-D” (blue; n = 736), and “IBS-M” (red; n = 699). All significantly increased species ($p \leq 0.05$ and log “Fold Change” ≥ 0.6) were combined into respective genera before constructing the Venn Diagram. All genera with differential expression consistent to self-reported IBS phenotypes (Supplementary Figure 3) are indicated with an asterisk (*).

