## Editors

**We consider that the scientific questions have been addressed in this revision. However, before accepting the manuscript, we ask that the authors revise the figures, particularly the supplemental ones, for improved readability and accessibility.**

Response

We thank you again for overall positive feedback about our work. We revised all figures, including supplemental ones, for improved readability and accessibility.

**The fonts and markers are often too small to read comfortably even though there appears to be space available. This is particularly true in the supplemental figures, but some of the main figures could also be improved.**

Response

We enlarged fonts and markers in all figures, including supplemental ones, to guarantee a more comfortable reading.

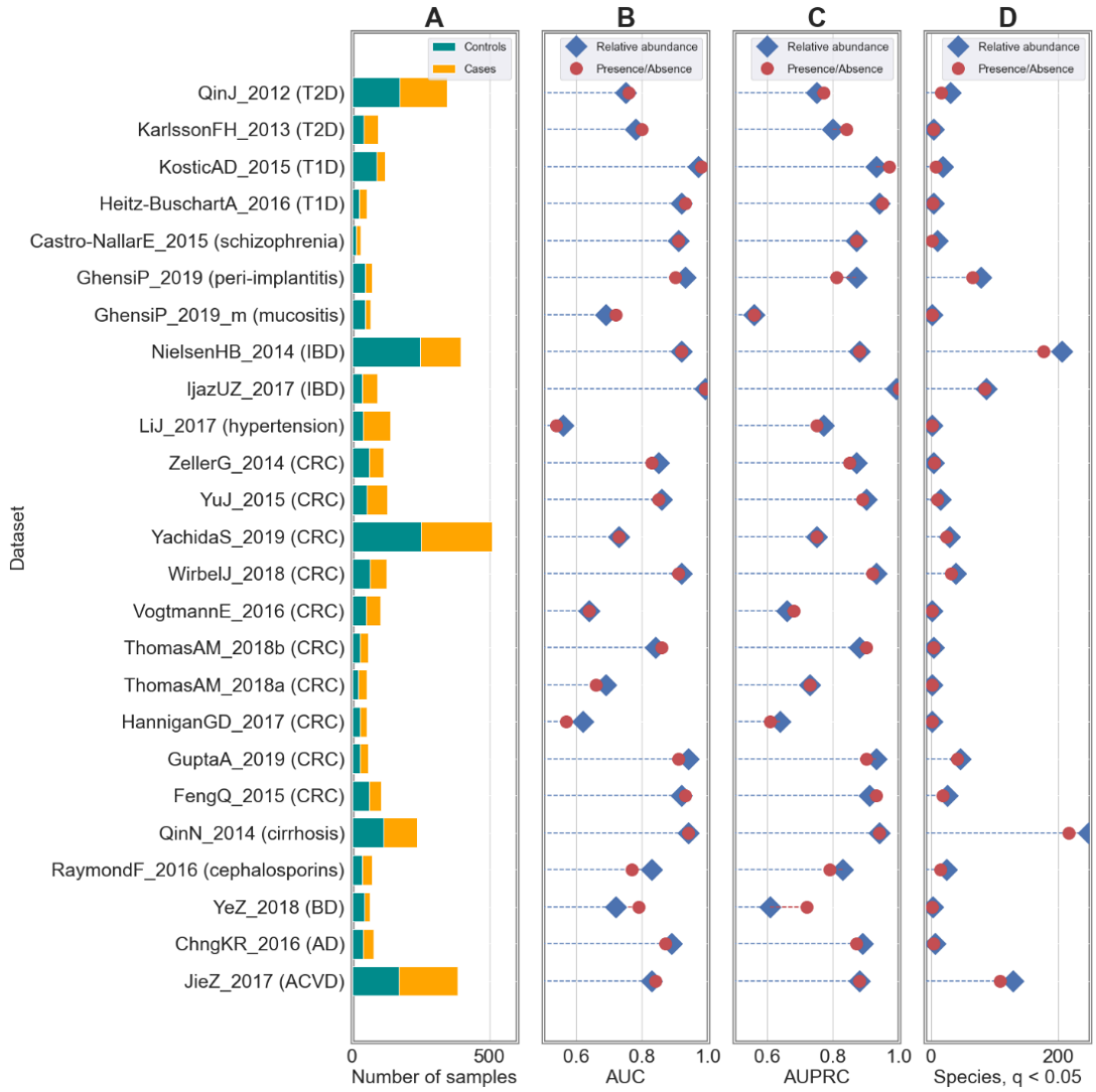



**Fig. 1.**

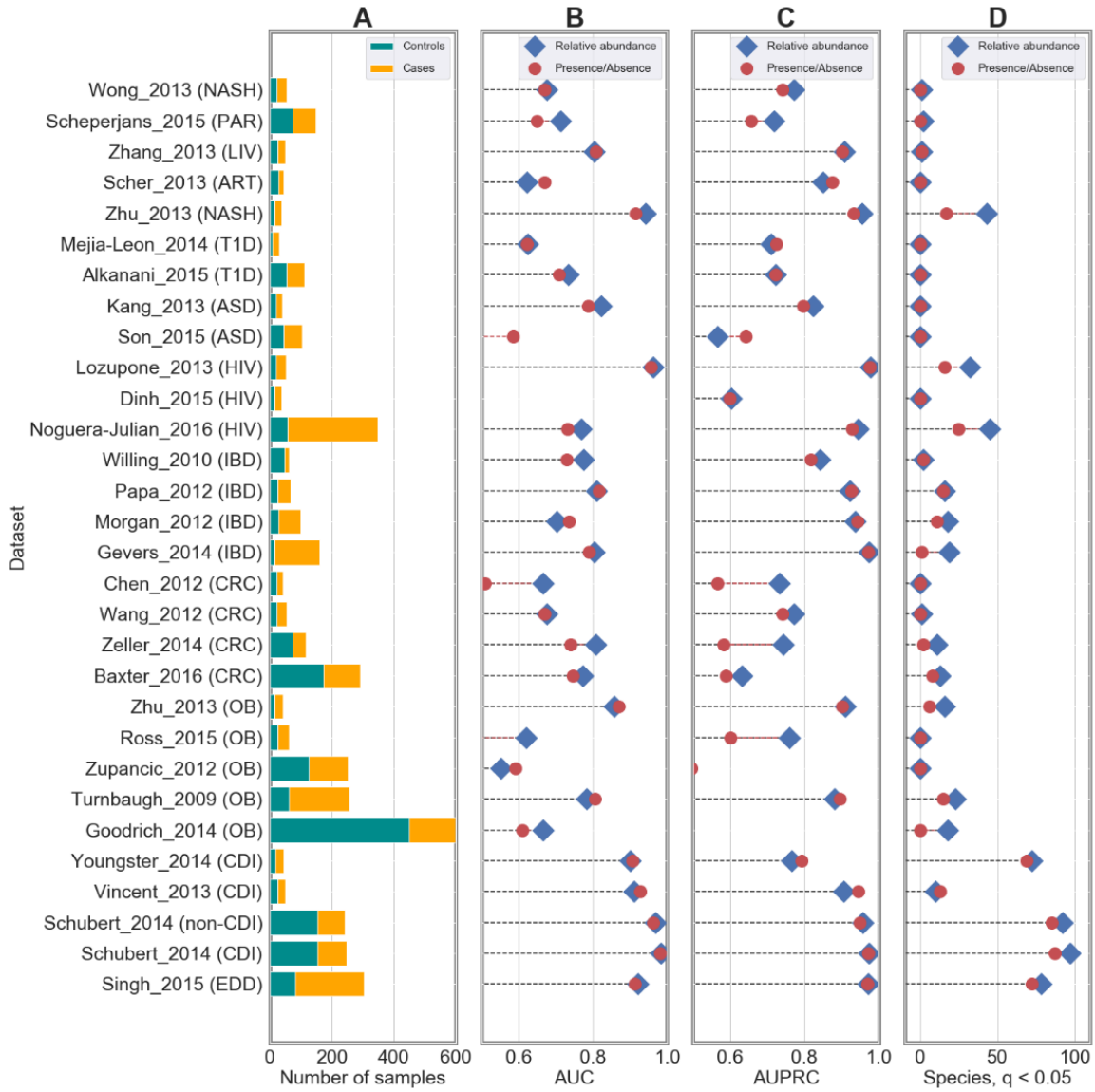

A
B
C
D
Controls
Cases
Relative abundance
Presence/Absence
Dataset
Wong_2013 (NASH)
Scheperjans_2015 (PAR)
Zhang_2013 (LIV)
Scher_2013 (ART)
Zhu_2013 (NASH)
Mejia-Leon_2014 (T1D)
Alkanani_2015 (T1D)
Kang_2013 (ASD)
Son_2015 (ASD)
Lozupone_2013 (HIV)
Dinh_2015 (HIV)
Noguera-Julian_2016 (HIV)
Willing_2010 (IBD)
Papa_2012 (IBD)
Morgan_2012 (IBD)
Gevers_2014 (IBD)
Chen_2012 (CRC)
Wang_2012 (CRC)
Zeller_2014 (CRC)
Baxter_2016 (CRC)
Zhu_2013 (OB)
Ross_2015 (OB)
Zupancic_2012 (OB)
Turnbaugh_2009 (OB)
Goodrich_2014 (OB)
Youngster_2014 (CDI)
Vincent_2013 (CDI)
Schubert_2014 (non-CDI)
Schubert_2014 (CDI)
Singh_2015 (EDD)
0
200
400
600
Number of samples
0.6
0.8
1.0
AUC
0.6
0.8
1.0
AUPRC
0
50
100
Species, q < 0.05

**Fig. 2.**

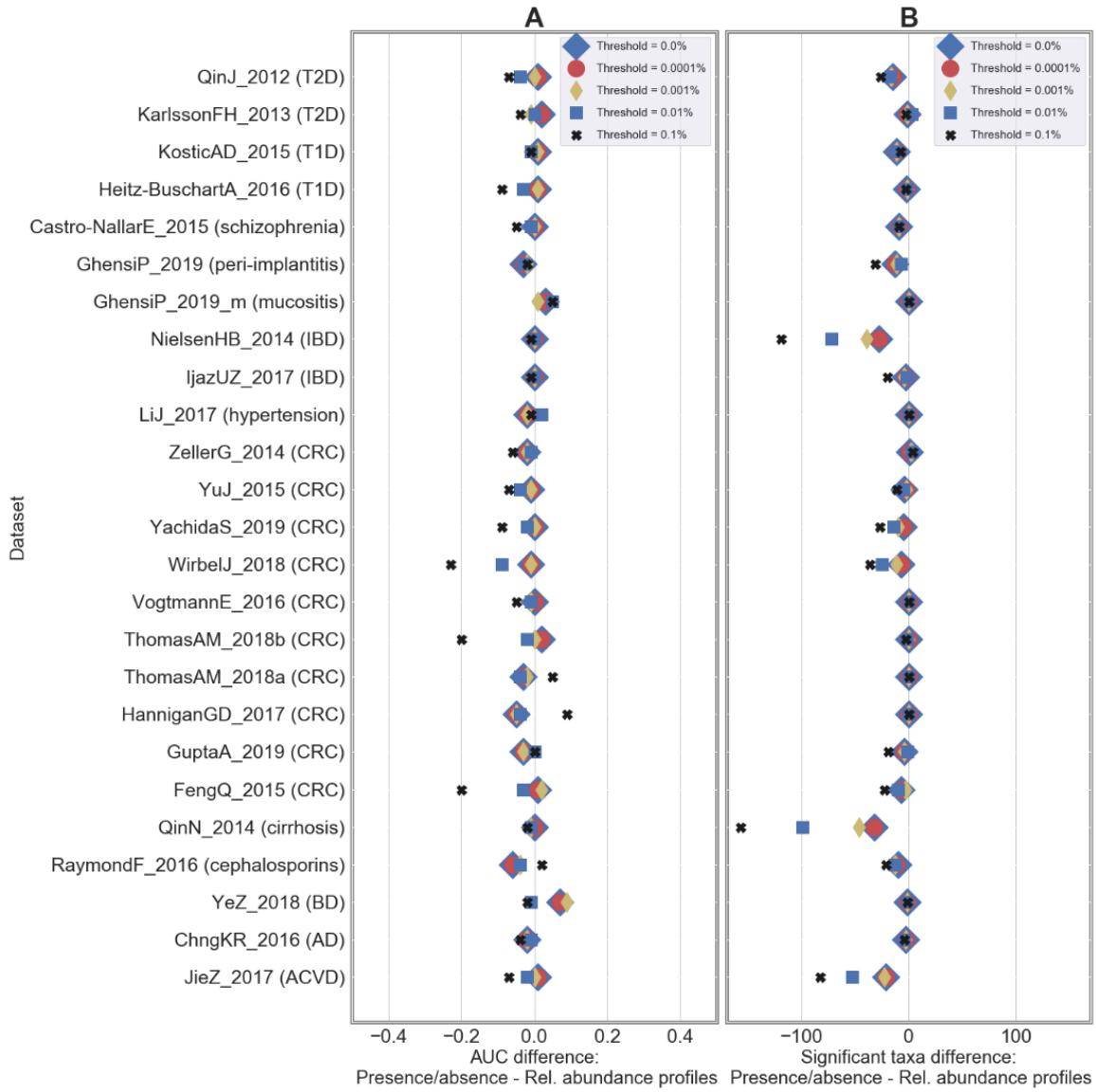

A
B
Threshold = 0.0%
Threshold = 0.0001%
Threshold = 0.001%
Threshold = 0.01%
Threshold = 0.1%
QinJ_2012 (T2D)
KarlssonFH_2013 (T2D)
KosticAD_2015 (T1D)
Heitz-BuschartA_2016 (T1D)
Castro-NallarE_2015 (schizophrenia)
GhensiP_2019 (peri-implantitis)
GhensiP_2019_m (mucositis)
NielsenHB_2014 (IBD)
IjazUZ_2017 (IBD)
LiJ_2017 (hypertension)
ZellerG_2014 (CRC)
YuJ_2015 (CRC)
YachidaS_2019 (CRC)
WirbelJ_2018 (CRC)
VogtmannE_2016 (CRC)
ThomasAM_2018b (CRC)
ThomasAM_2018a (CRC)
HanniganGD_2017 (CRC)
GuptaA_2019 (CRC)
FengQ_2015 (CRC)
QinN_2014 (cirrhosis)
RaymondF_2016 (cephalosporins)
YeZ_2018 (BD)
ChngKR_2016 (AD)
JieZ_2017 (ACVD)
Dataset
−0.4
−0.2
0.0
0.2
0.4
AUC difference:
Presence/absence - Rel. abundance profiles
−100
0
100
Significant taxa difference:
Presence/absence - Rel. abundance profiles

**Fig. 3.**

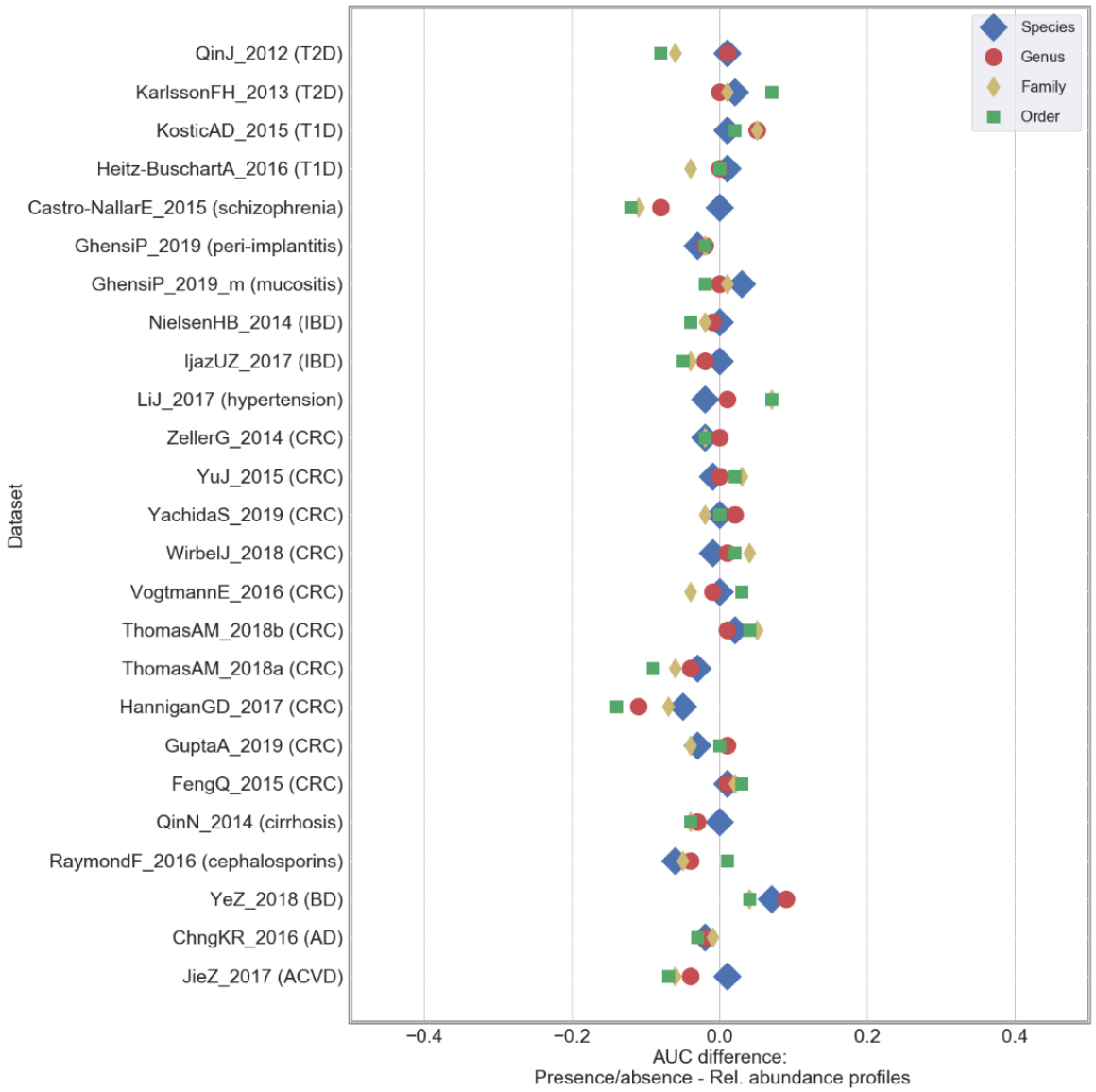

Species
Genus
Family
Order
QinJ_2012 (T2D)
KarlssonFH_2013 (T2D)
KosticAD_2015 (T1D)
Heitz-BuschartA_2016 (T1D)
Castro-NallarE_2015 (schizophrenia)
GhensiP_2019 (peri-implantitis)
GhensiP_2019_m (mucositis)
NielsenHB_2014 (IBD)
IjazUZ_2017 (IBD)
LiJ_2017 (hypertension)
ZellerG_2014 (CRC)
YuJ_2015 (CRC)
YachidaS_2019 (CRC)
WirbelJ_2018 (CRC)
VogtmannE_2016 (CRC)
ThomasAM_2018b (CRC)
ThomasAM_2018a (CRC)
HanniganGD_2017 (CRC)
GuptaA_2019 (CRC)
FengQ_2015 (CRC)
QinN_2014 (cirrhosis)
RaymondF_2016 (cephalosporins)
YeZ_2018 (BD)
ChngKR_2016 (AD)
JieZ_2017 (ACVD)
Dataset
−0.4
−0.2
0.0
0.2
0.4
AUC difference:
Presence/absence - Rel. abundance profiles

**Fig. 4.**

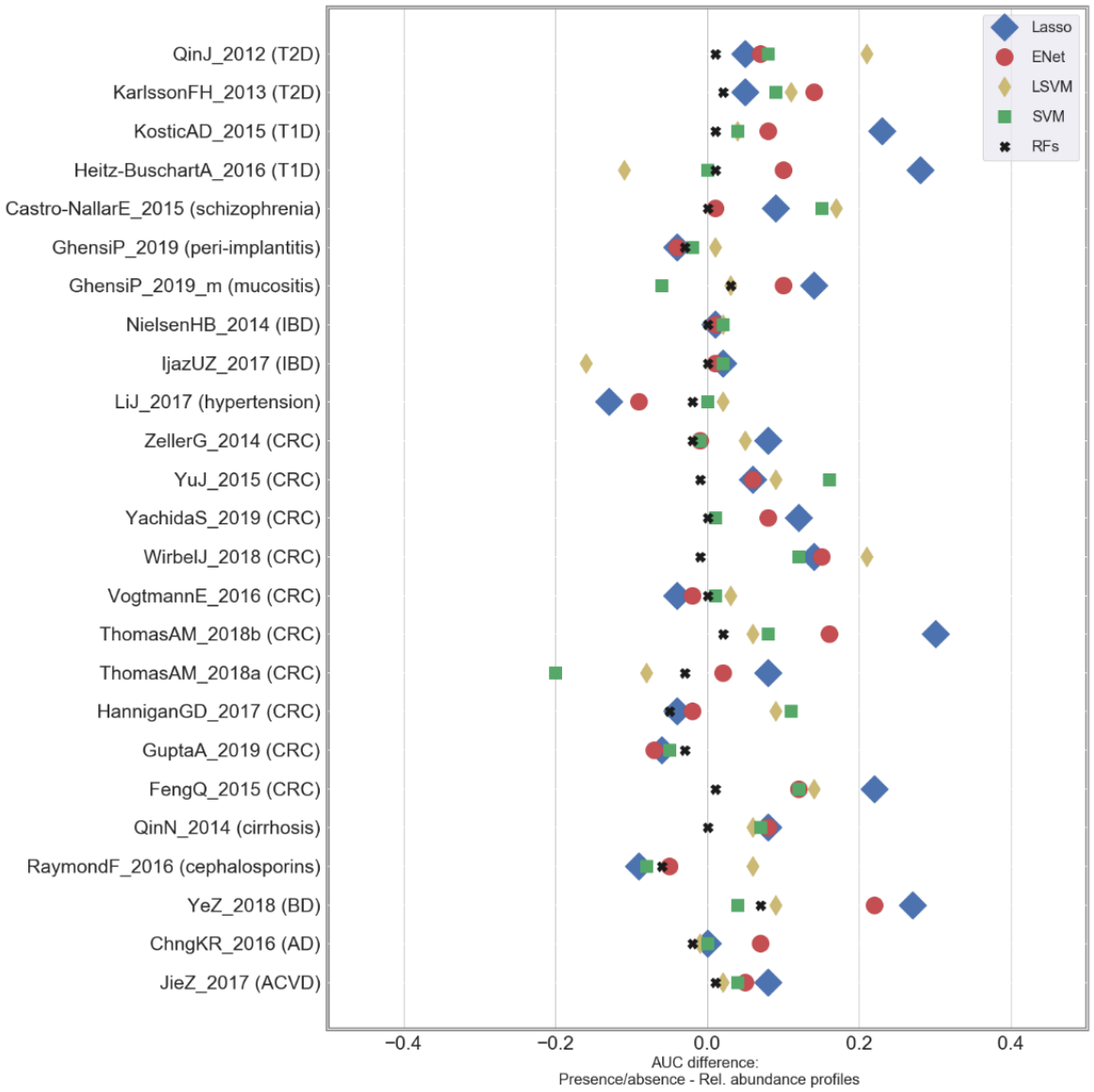

Lasso
ENet
LSVM
SVM
RFs
QinJ_2012 (T2D)
KarlssonFH_2013 (T2D)
KosticAD_2015 (T1D)
Heitz-BuschartA_2016 (T1D)
Castro-NallarE_2015 (schizophrenia)
GhensiP_2019 (peri-implantitis)
GhensiP_2019_m (mucositis)
NielsenHB_2014 (IBD)
IjazUZ_2017 (IBD)
LiJ_2017 (hypertension)
ZellerG_2014 (CRC)
YuJ_2015 (CRC)
YachidaS_2019 (CRC)
WirbelJ_2018 (CRC)
VogtmannE_2016 (CRC)
ThomasAM_2018b (CRC)
ThomasAM_2018a (CRC)
HanniganGD_2017 (CRC)
GuptaA_2019 (CRC)
FengQ_2015 (CRC)
QinN_2014 (cirrhosis)
RaymondF_2016 (cephalosporins)
YeZ_2018 (BD)
ChngKR_2016 (AD)
JieZ_2017 (ACVD)
−0.4
−0.2
0.0
0.2
0.4
AUC difference:
Presence/absence - Rel. abundance profiles

**Fig. 5.**

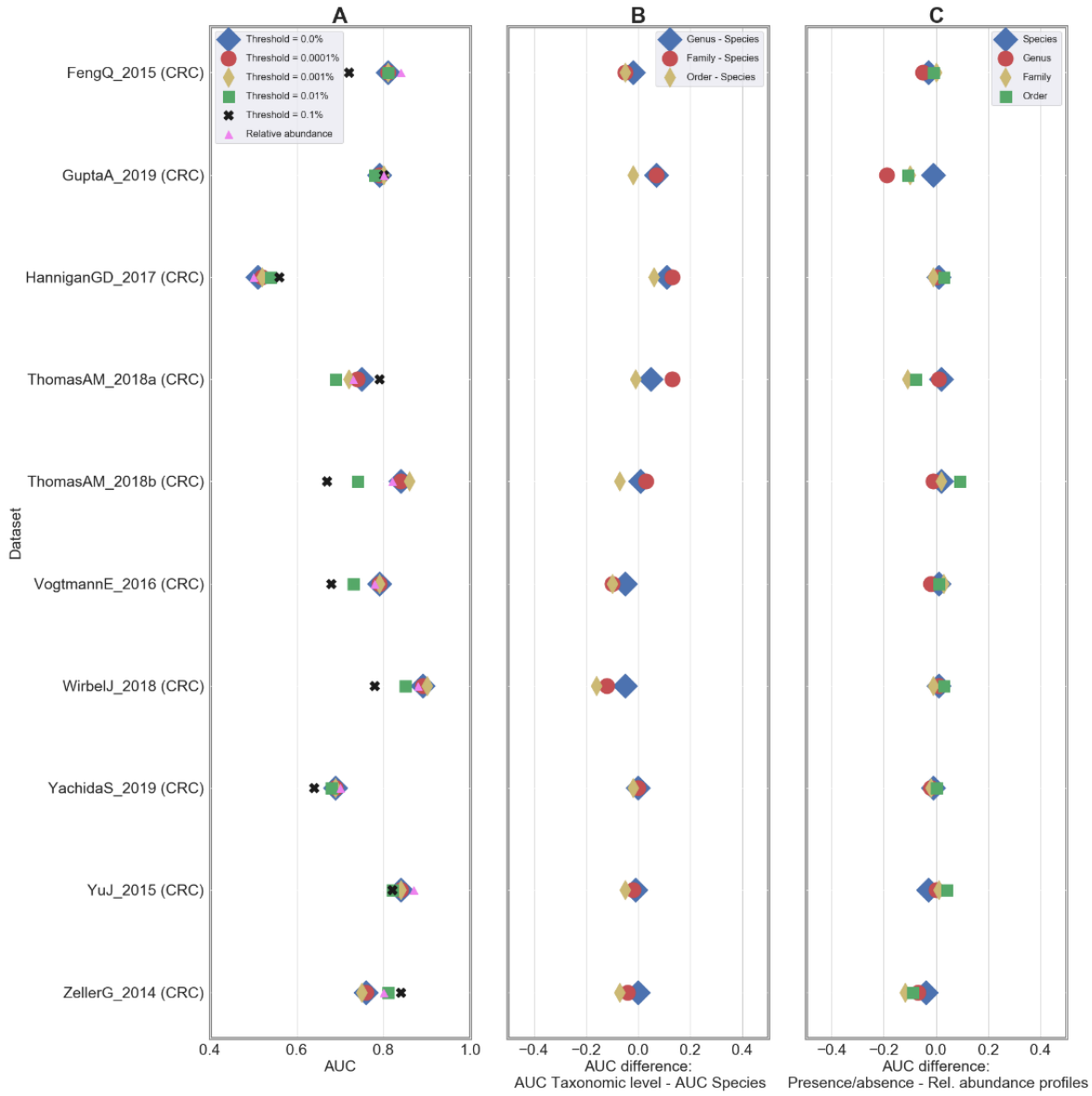
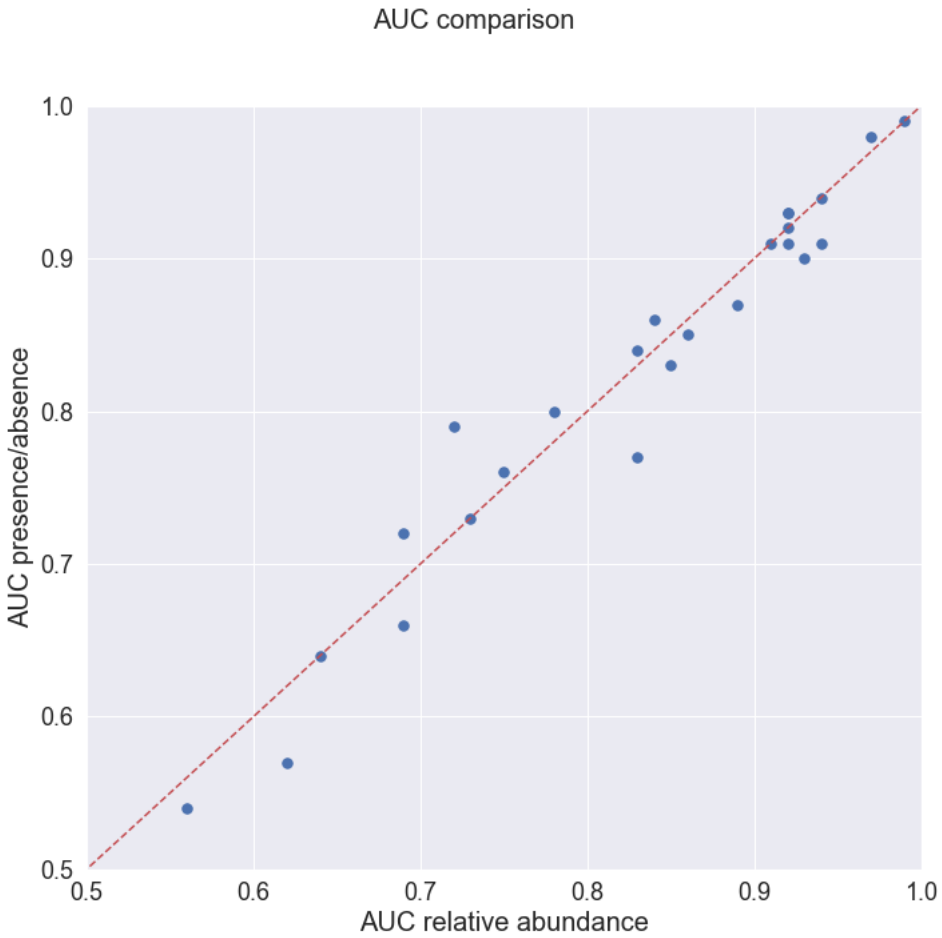
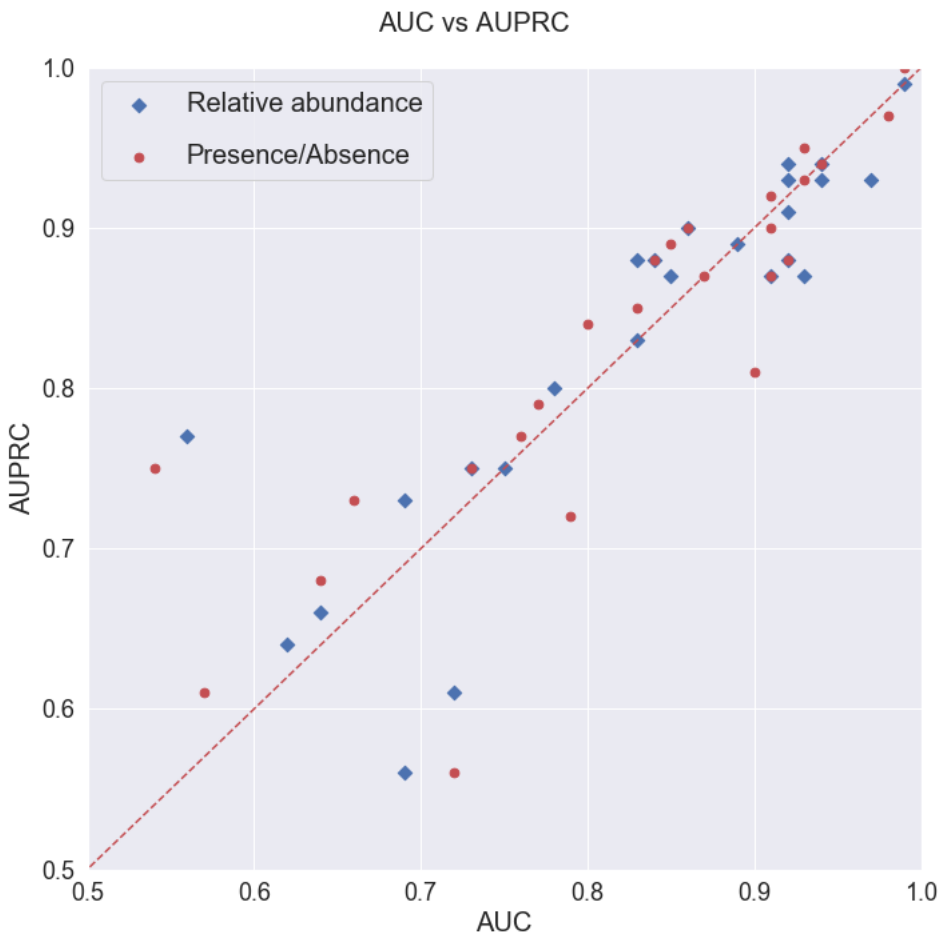

A
B
C
Threshold = 0.0%
Threshold = 0.0001%
Threshold = 0.001%
Threshold = 0.01%
Threshold = 0.1%
Relative abundance
Genus - Species
Family - Species
Order - Species
Species
Genus
Family
Order
FengQ_2015 (CRC)
GuptaA_2019 (CRC)
HanniganGD_2017 (CRC)
ThomasAM_2018a (CRC)
ThomasAM_2018b (CRC)
VogtmannE_2016 (CRC)
WirbelJ_2018 (CRC)
YachidaS_2019 (CRC)
YuJ_2015 (CRC)
ZellerG_2014 (CRC)
Dataset
0.4
0.6
0.8
1.0
AUC
−0.4
−0.2
0.0
0.2
0.4
AUC difference:
AUC Taxonomic level - AUC Species
AUC difference:
Presence/absence - Rel. abundance profiles

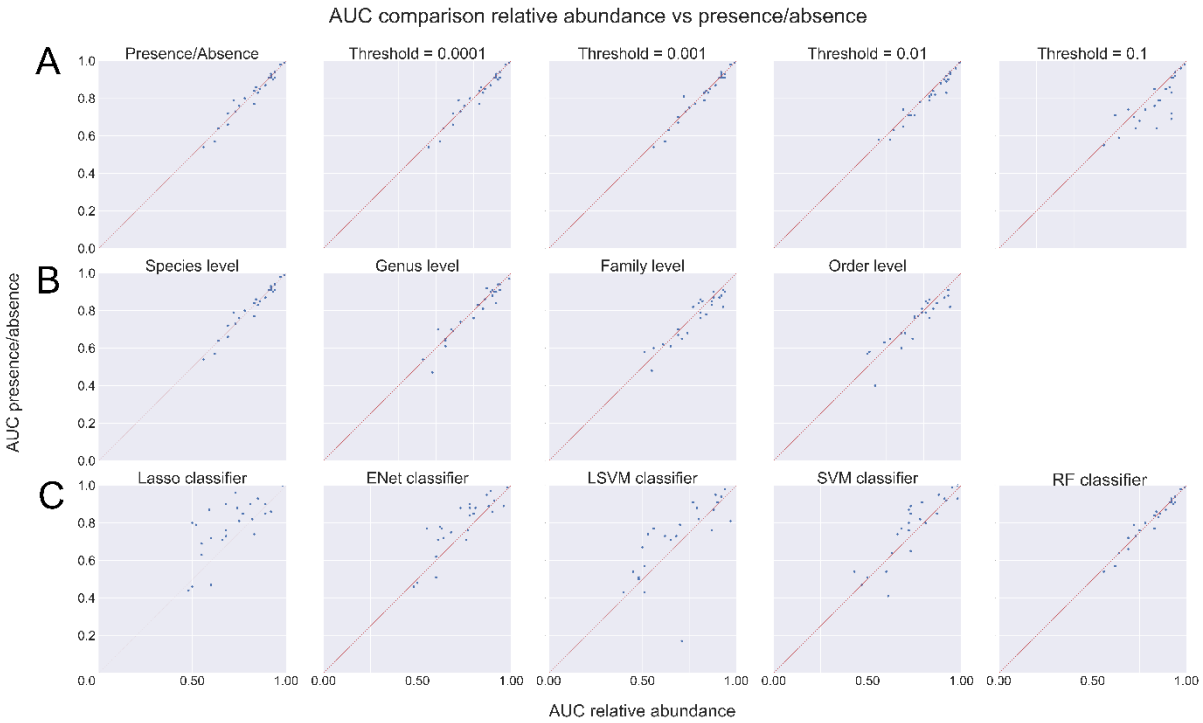
**Fig. 6.**


AUC comparison
AUC presence/absence
AUC relative abundance
1.0
0.9
0.8
0.7
0.6
0.5
0.5
0.6
0.7
0.8
0.9
1.0

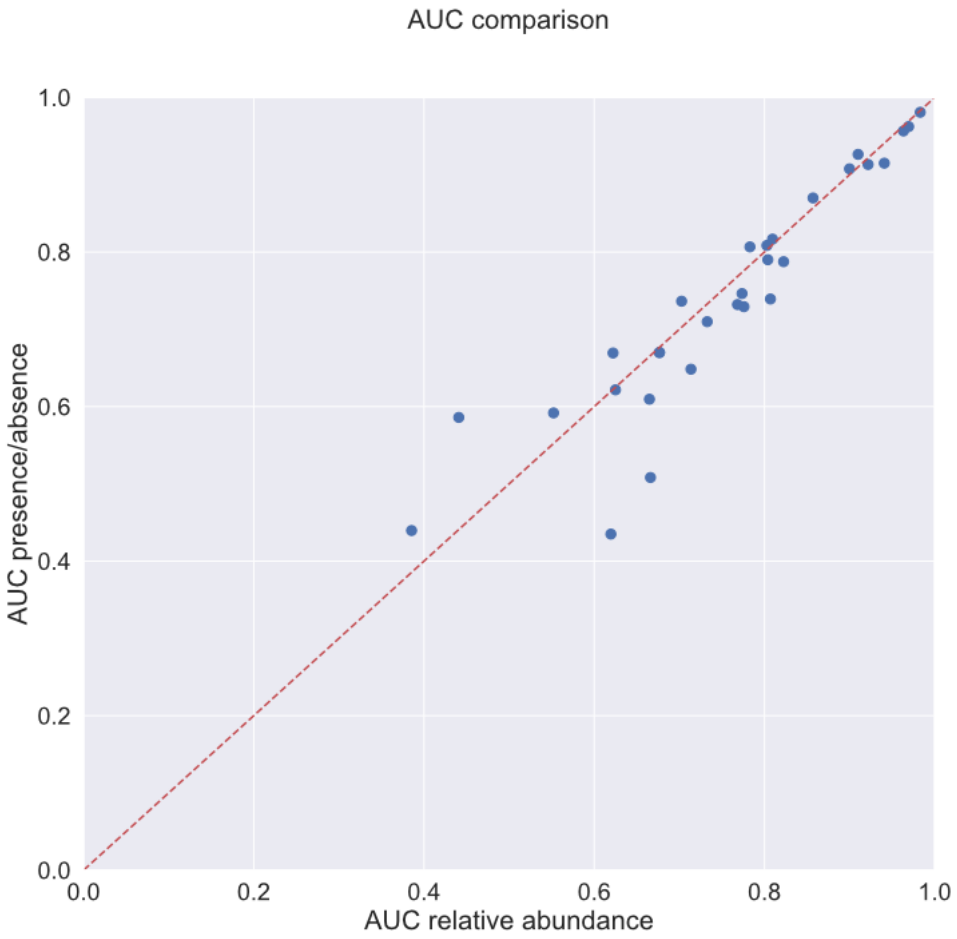
**S1 Fig.**


AUC vs AUPRC
Relative abundance
Presence/Absence
AUPRC
AUC
1.0
0.9
0.8
0.7
0.6
0.5
0.5
0.6
0.7
0.8
0.9
1.0

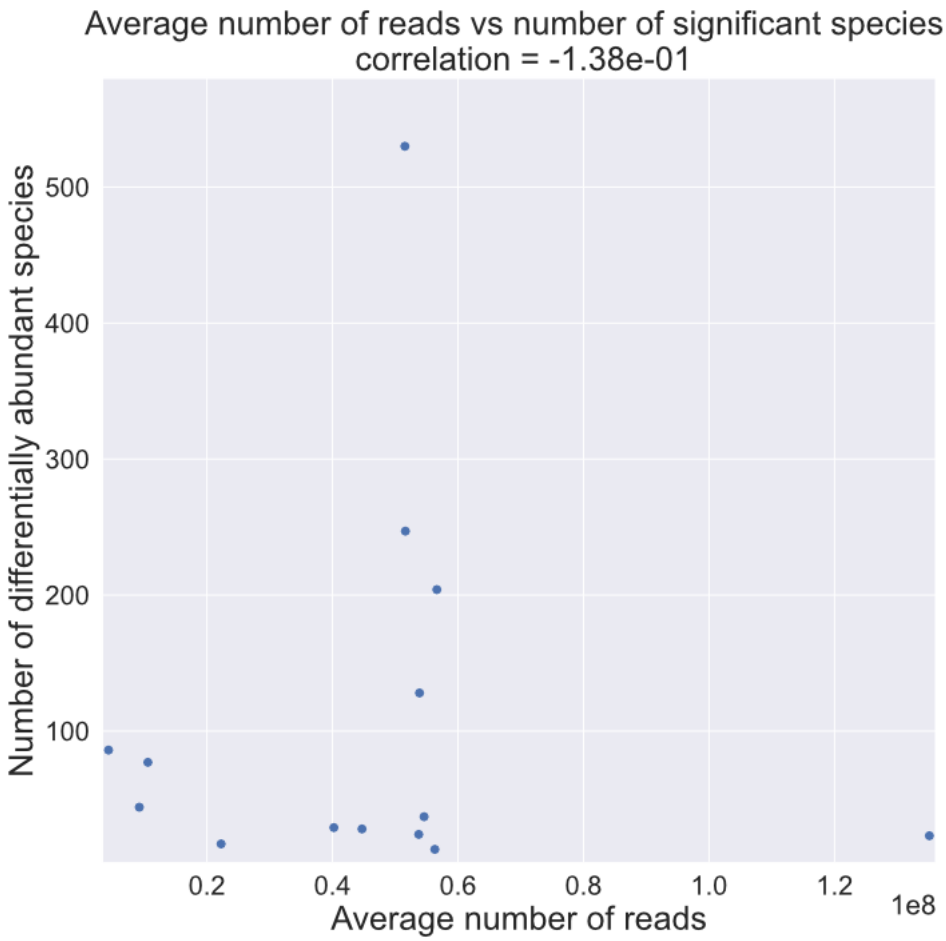
**S2 Fig.**


AUC comparison relative abundance vs presence/absence
A
Presence/Absence
Threshold = 0.0001
Threshold = 0.001
Threshold = 0.01
Threshold = 0.1
B
Species level
Genus level
Family level
Order level
C
Lasso classifier
ENet classifier
LSVM classifier
SVM classifier
RF classifier
AUC presence/absence
AUC relative abundance
0.0
0.2
0.4
0.6
0.8
1.0
0.00
0.50
1.00

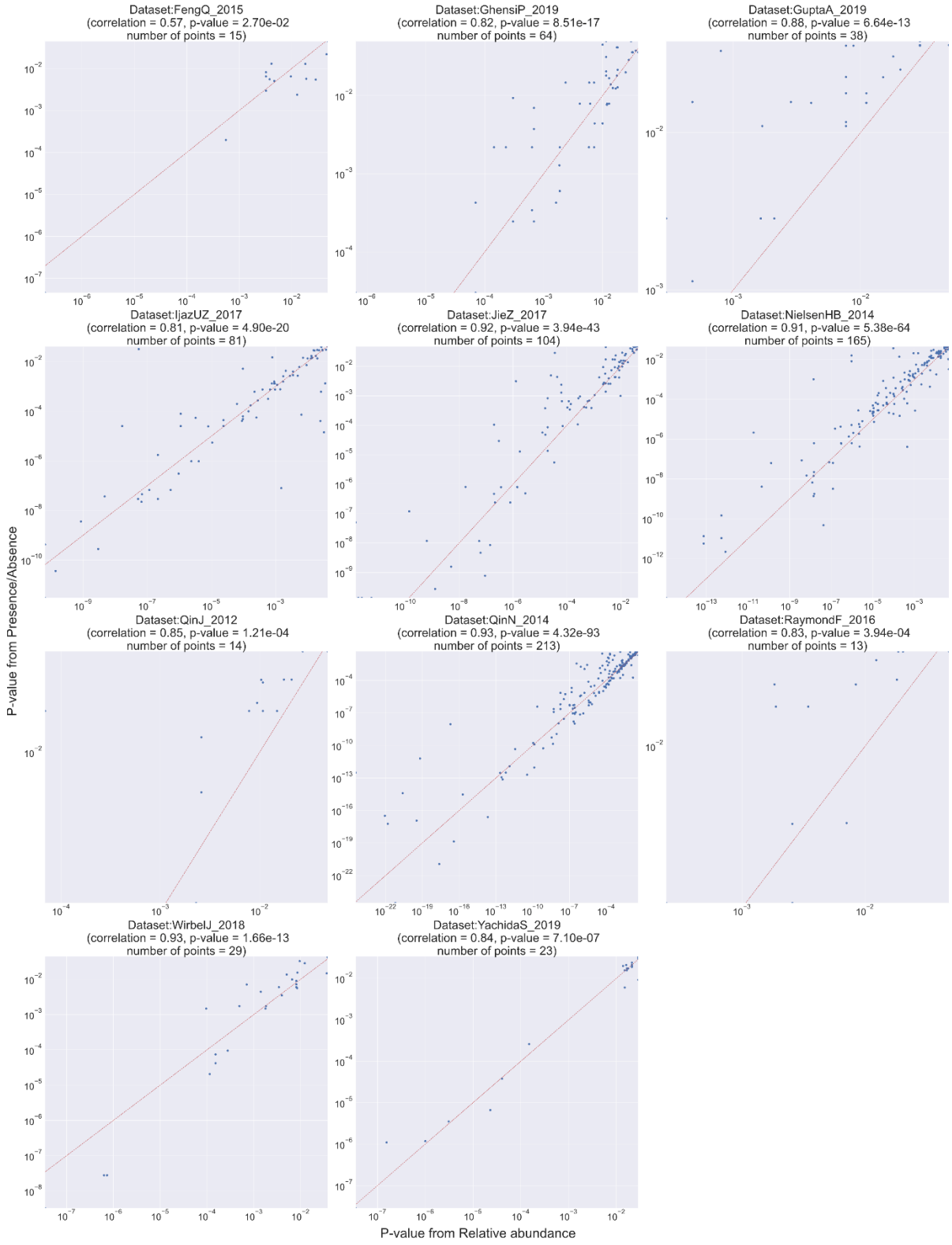
**S3 Fig.**


AUC comparison
AUC presence/absence
AUC relative abundance
0.0
0.2
0.4
0.6
0.8
1.0

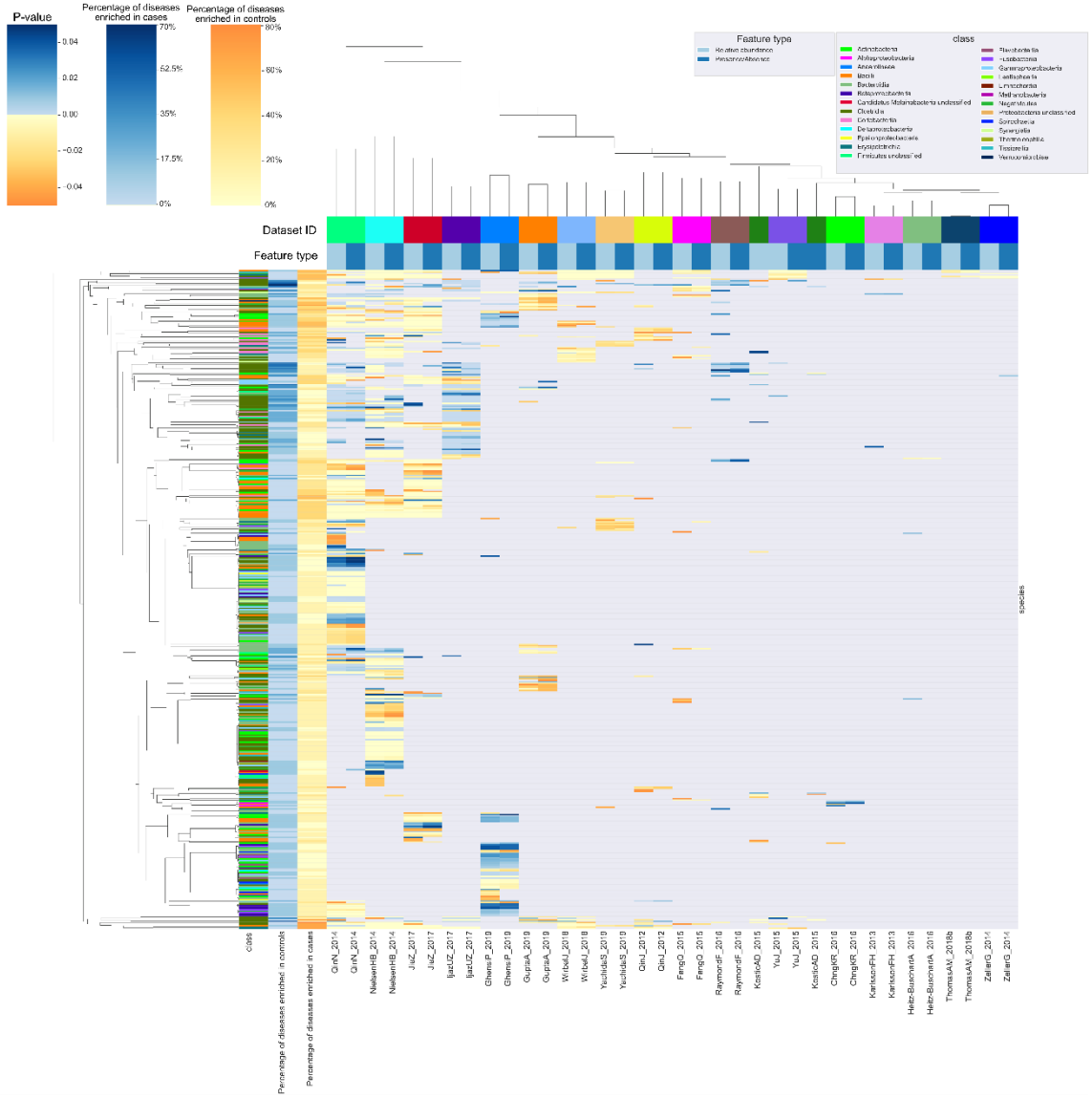
**S4 Fig.**


Average number of reads vs number of significant species
correlation = -1.38e-01
Number of differentially abundant species
100
200
300
400
500
0.2
0.4
0.6
0.8
1.0
1.2
1e8
Average number of reads

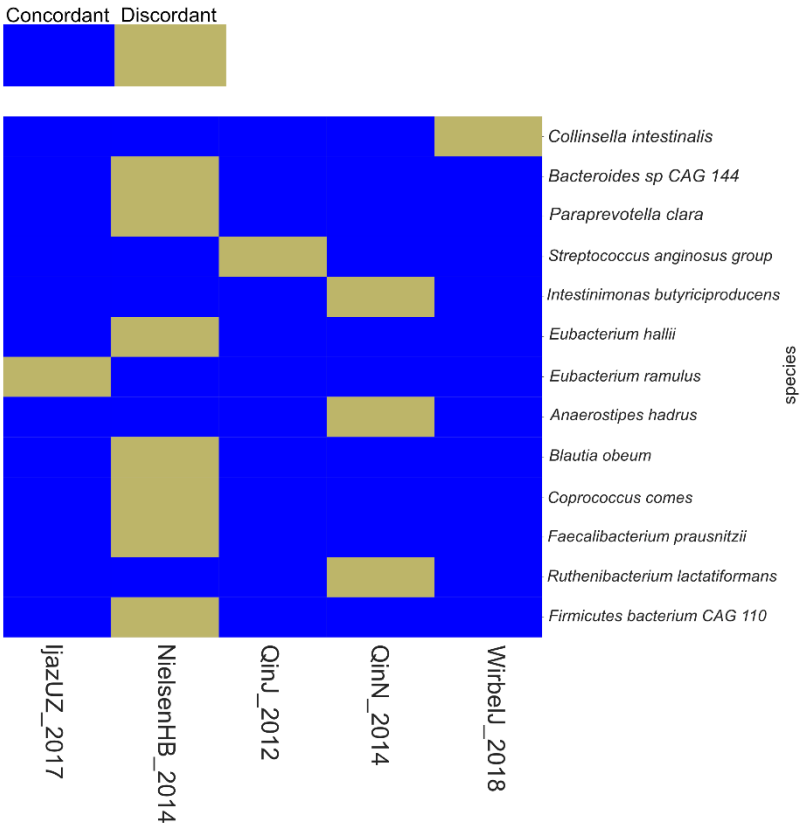
**S5 Fig.**


Dataset:FengQ_2015
(correlation = 0.57, p-value = 2.70e-02
number of points = 15)
Dataset:GhensiP_2019
(correlation = 0.82, p-value = 8.51e-17
number of points = 64)
Dataset:GuptaA_2019
(correlation = 0.88, p-value = 6.64e-13
number of points = 38)
Dataset:IjazUZ_2017
(correlation = 0.81, p-value = 4.90e-20
number of points = 81)
Dataset:JieZ_2017
(correlation = 0.92, p-value = 3.94e-43
number of points = 104)
Dataset:NielsenHB_2014
(correlation = 0.91, p-value = 5.38e-64
number of points = 165)
Dataset:QinJ_2012
(correlation = 0.85, p-value = 1.21e-04
number of points = 14)
Dataset:QinN_2014
(correlation = 0.93, p-value = 4.32e-93
number of points = 213)
Dataset:RaymondF_2016
(correlation = 0.83, p-value = 3.94e-04
number of points = 13)
Dataset:WirbelJ_2018
(correlation = 0.93, p-value = 1.66e-13
number of points = 29)
Dataset:YachidaS_2019
(correlation = 0.84, p-value = 7.10e-07
number of points = 23)
P-value from Presence/Absence
P-value from Relative abundance

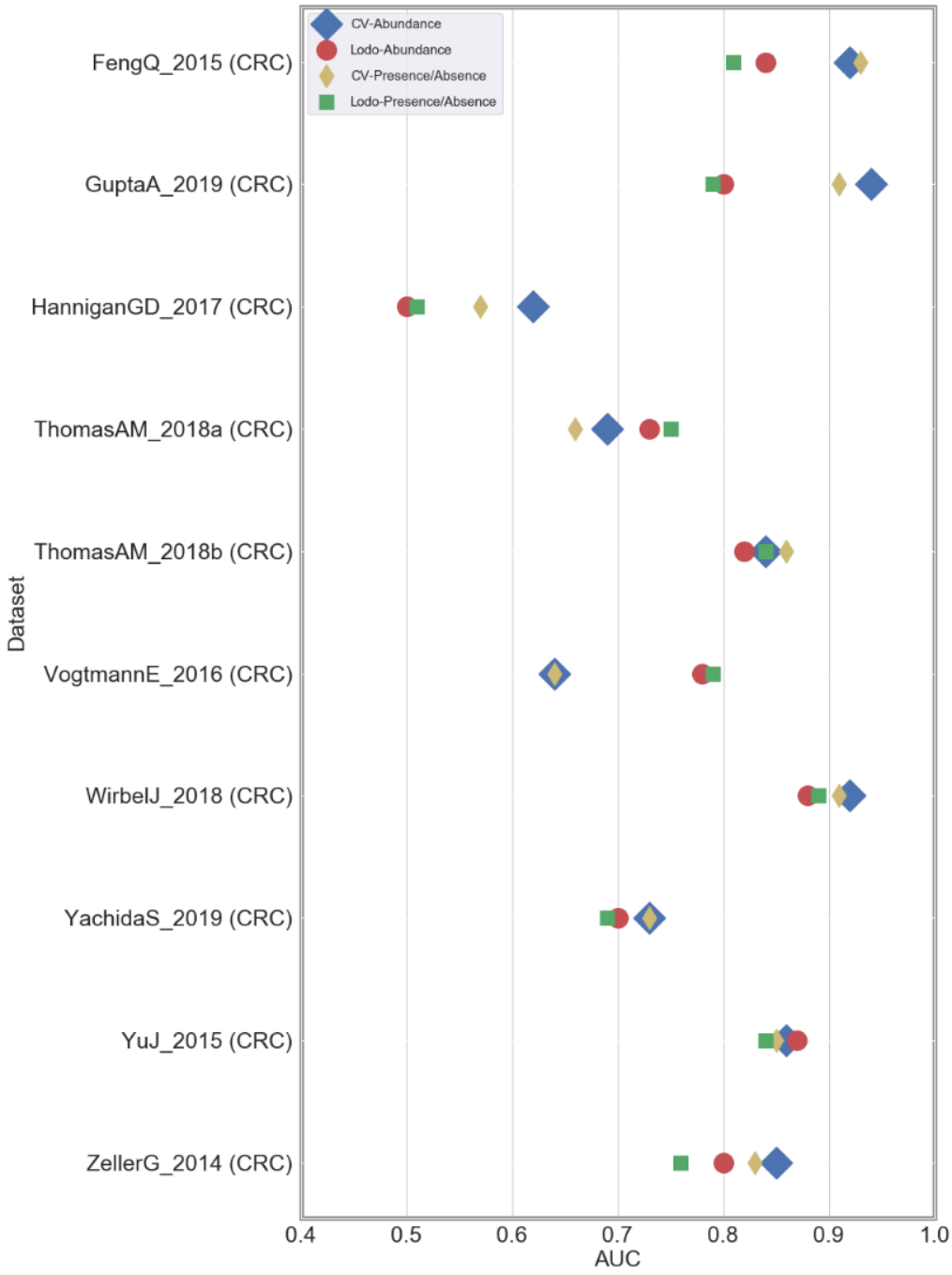
**S6 Fig.**


P-value
Percentage of diseases enriched in cases
Percentage of diseases enriched in controls
Feature type
class
Dataset ID
Feature type
species
Class
Percentage of diseases enriched in controls
Percentage of diseases enriched in cases

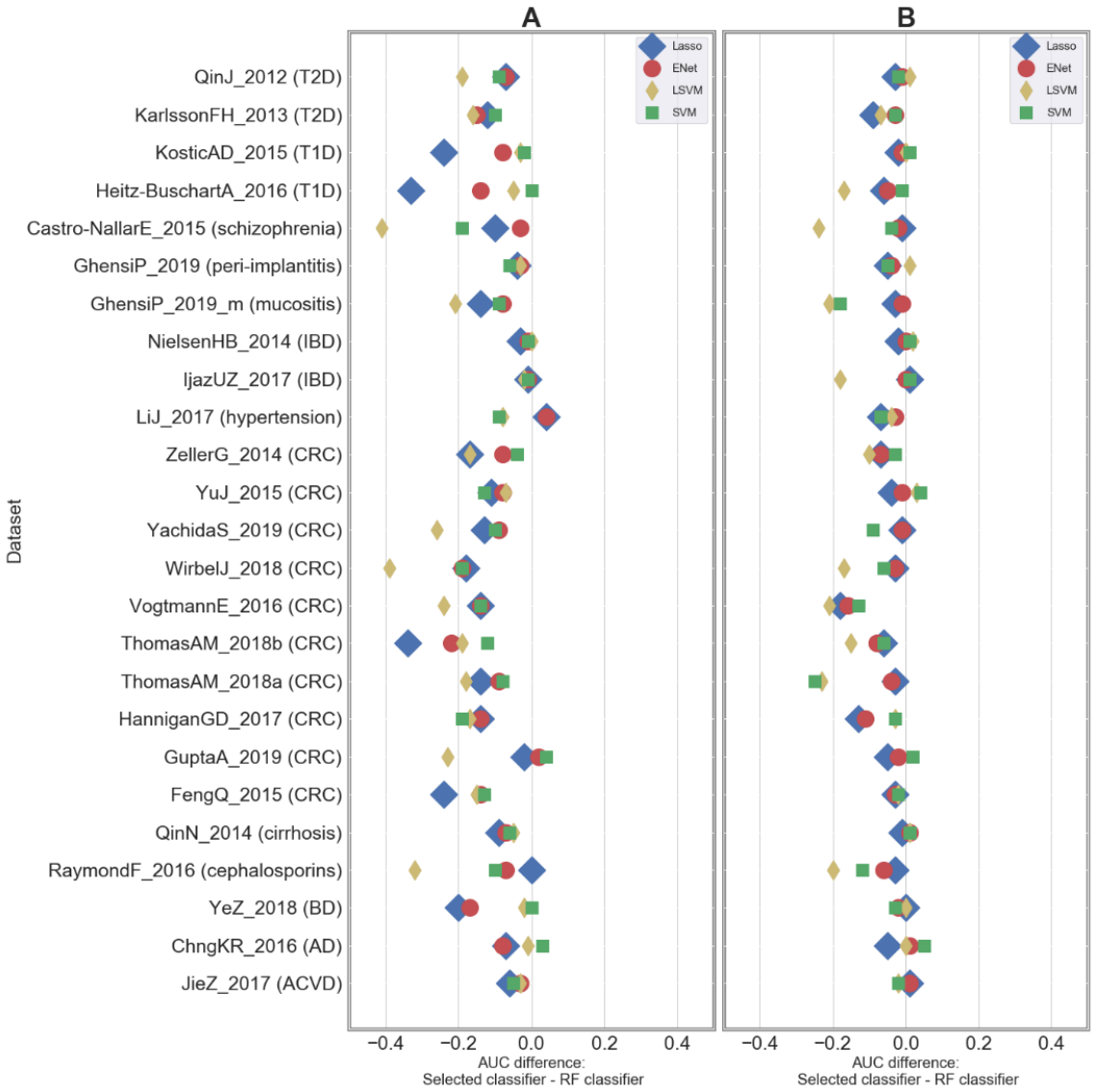
**S7 Fig.**


Concordant
Discordant
Collinsella intestinalis
Bacteroides sp CAG 144
Paraprevotella clara
Streptococcus anginosus group
Intestinimonas butyriciproducens
Eubacterium hallii
Eubacterium ramulus
Anaerostipes hadrus
Blautia obeum
Coprococcus comes
Faecalibacterium prausnitzii
Ruthenibacterium lactatiformans
Firmicutes bacterium CAG 110
species
IjazUZ_2017
NielsenHB_2014
QinJ_2012
QinN_2014
WirbelJ_2018

**S8 Fig.**


CV-Abundance
Lodo-Abundance
CV-Presence/Absence
Lodo-Presence/Absence
FengQ_2015 (CRC)
GuptaA_2019 (CRC)
HanniganGD_2017 (CRC)
ThomasAM_2018a (CRC)
ThomasAM_2018b (CRC)
VogtmannE_2016 (CRC)
WirbelJ_2018 (CRC)
YachidaS_2019 (CRC)
YuJ_2015 (CRC)
ZellerG_2014 (CRC)
Dataset
0.4
0.5
0.6
0.7
0.8
0.9
1.0
AUC

**S9 Fig.**

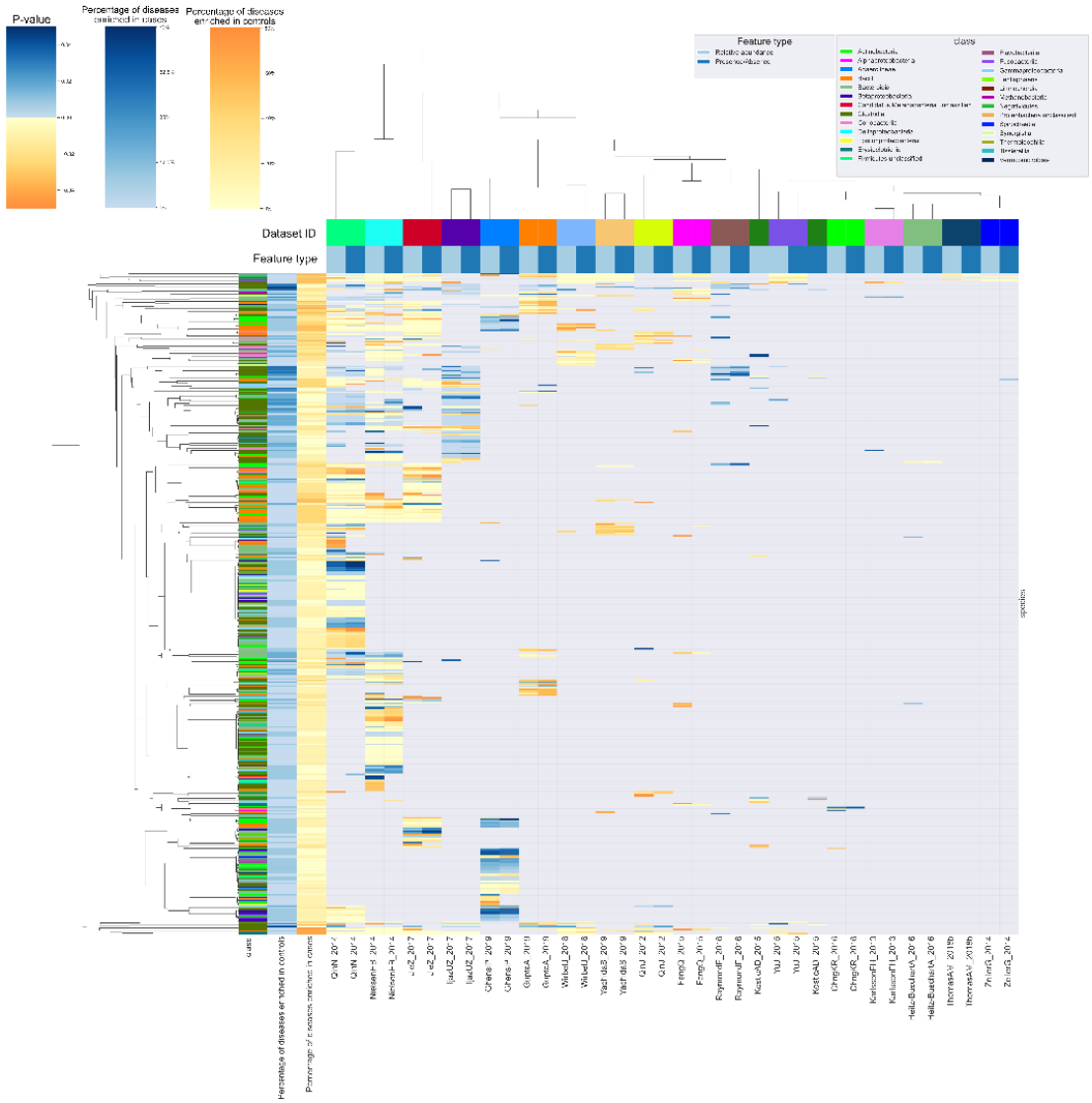

A
B
Lasso
ENet
LSVM
SVM
QinJ_2012 (T2D)
KarlssonFH_2013 (T2D)
KosticAD_2015 (T1D)
Heitz-BuschartA_2016 (T1D)
Castro-NallarE_2015 (schizophrenia)
GhensiP_2019 (peri-implantitis)
GhensiP_2019_m (mucositis)
NielsenHB_2014 (IBD)
IjazUZ_2017 (IBD)
LiJ_2017 (hypertension)
ZellerG_2014 (CRC)
YuJ_2015 (CRC)
YachidaS_2019 (CRC)
WirbelJ_2018 (CRC)
VogtmannE_2016 (CRC)
ThomasAM_2018b (CRC)
ThomasAM_2018a (CRC)
HanniganGD_2017 (CRC)
GuptaA_2019 (CRC)
FengQ_2015 (CRC)
QinN_2014 (cirrhosis)
RaymondF_2016 (cephalosporins)
YeZ_2018 (BD)
ChngKR_2016 (AD)
JieZ_2017 (ACVD)
Dataset
−0.4
−0.2
0.0
0.2
0.4
AUC difference:
Selected classifier - RF classifier

**S10 Fig.**

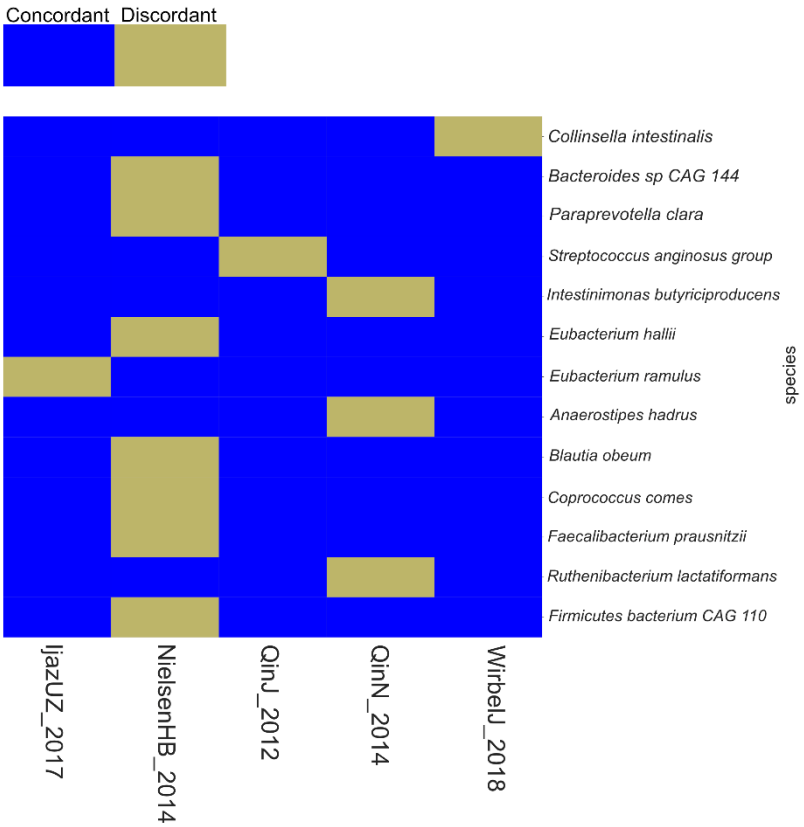
**We also ask that Figs S7 and S8 be redone using a coulorblind-safe color scheme rather than rely on red/green distinction.**

Response

We updated Figs S7 and S8 by using a colourblind-safe color scheme:




**S7 Fig.**




**S8 Fig.**

**We ask the authors to consider pointing to Figs. S1 and S2 from the caption of Fig 1.**

Response

We pointed the two supplementary figures in the caption of **Fig 1**:

**Fig 1. Classification accuracies are robust to degradation from species-level relative abundance to presence/absence profiles in shotgun datasets.** Results obtained on 25 case-control studies for host phenotype classification from human microbiomes. (**A**) Number of case and control samples across the different studies. (**B**) AUC and (**C**) AUPRC scores using RF as back-end classifiers on species-level taxonomic profiles. Comparison between relative abundance (in blue) and presence/absence (in red) profiles highlighted negligible differences and no statistical differences in none of the studies (see **S1 Fig** for AUC scores and **S2 Table** for p-values). Metrics of comparison in terms of AUC, AUPRC, precision, recall, and F1 are summarized in **S2 Table** and **S2 Fig**. (**D**) Number of statistically significant taxa from relative abundance (in blue) and presence/absence (in red) profiles.