Supplementary Information

Metabolomic description of ivacaftor elevating polymyxin B mediated antibacterial activity in cystic fibrosis *Pseudomonas aeruginosa*

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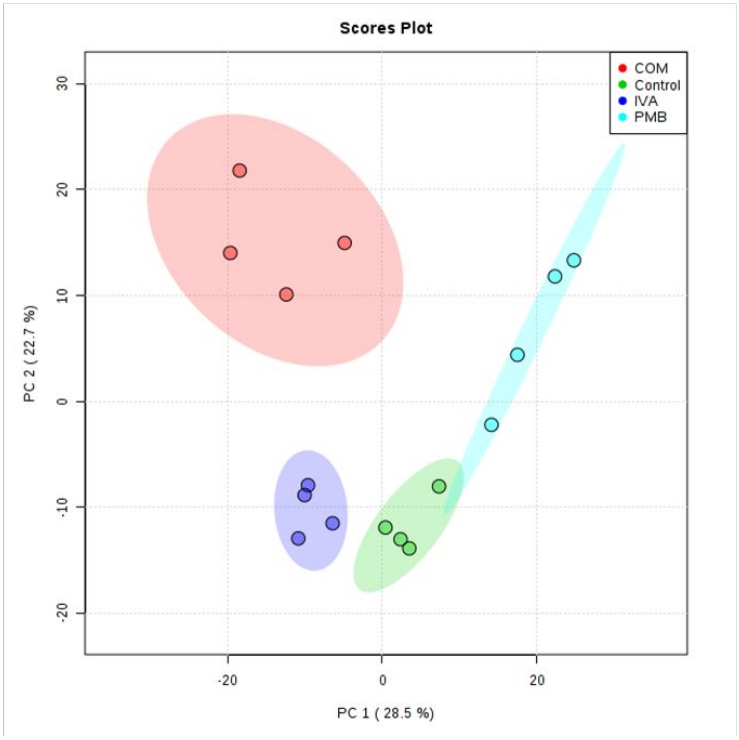
Table S1. Data precision of individual samples represented as the median relative standard deviation (RSD) for all metabolites of *P. aeruginosa* FADDI-PA111 & FADDI-PA006 based on all replicates (n=4) of each group (n=19 for technical replicates of PBQCs).

FADDI-PA111	**Median RSD %**		
	1h	**3h**	**6h**
Control	22	22	19
Polymyxin B	19	21	19
Ivacaftor	27	25	16
COM	27	24	22
FADDI-PA006	**Median RSD %**		
	1h	**3h**	**6h**
Control	22	18	19
Polymyxin B	20	15	18
Ivacaftor	15	18	17
COM	20	22	23

Figure S1. Multivariate analyses of global metabolic changes. PLSDA score plots for metabolite levels of **(A)** FADDI-PA111 and **(B)** FADDI-PA006 samples treated with polymyxin B, ivacaftor and the combination. Each data set represents a total of 16 samples of 4 biological replicates of each condition. Green = control (untreated) ; Cyan = polymyxin B alone (PMB); Purple = ivacaftor (IVA); Red = polymyxin B and ivacaftor combination (COM).

A



B

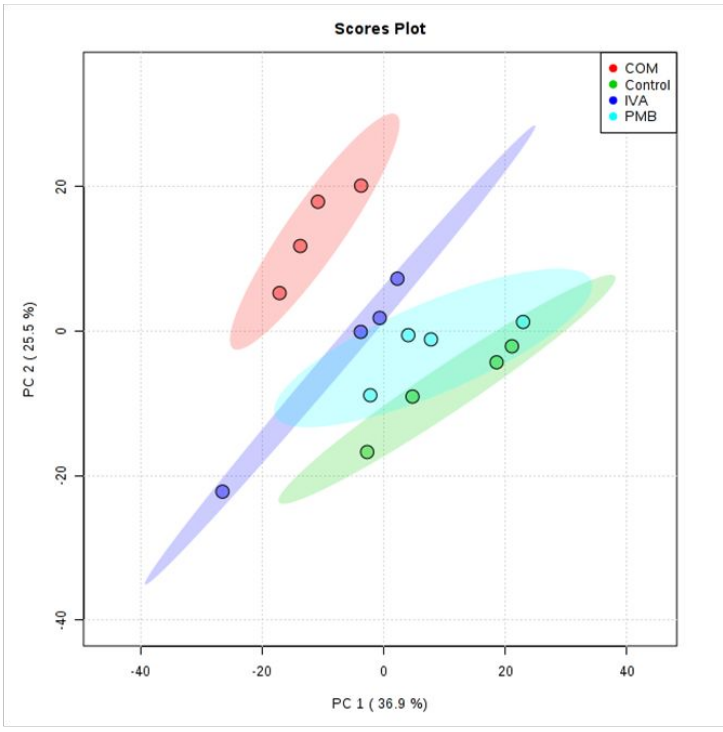


Figure S2. Monotherapy and combination (COM) of polymyxin B (PMB) and ivacaftor (IVA) induce global metabolic changes. Heatmap profiles of all identified metabolites after treatment of **(A)** *P. aeruginosa* FADDI-PA111 and **(B)** FADDI-PA006 with single and combination of polymyxin B and ivacaftor.

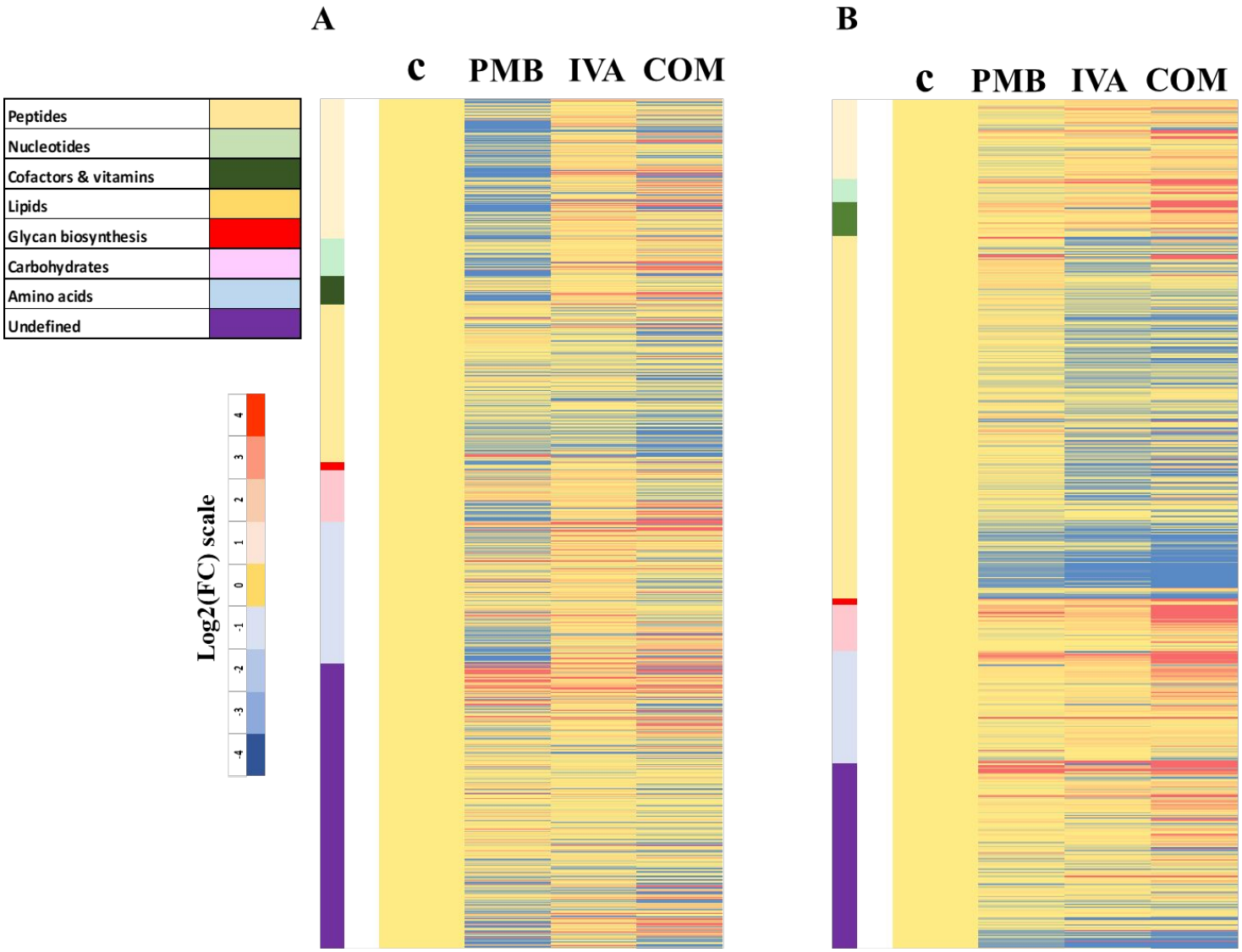


Figure S3. The proportion of different metabolites classes identified in FADDI-PA111 and FADDI-PA006 following treatment with polymyxin B and ivacaftor.

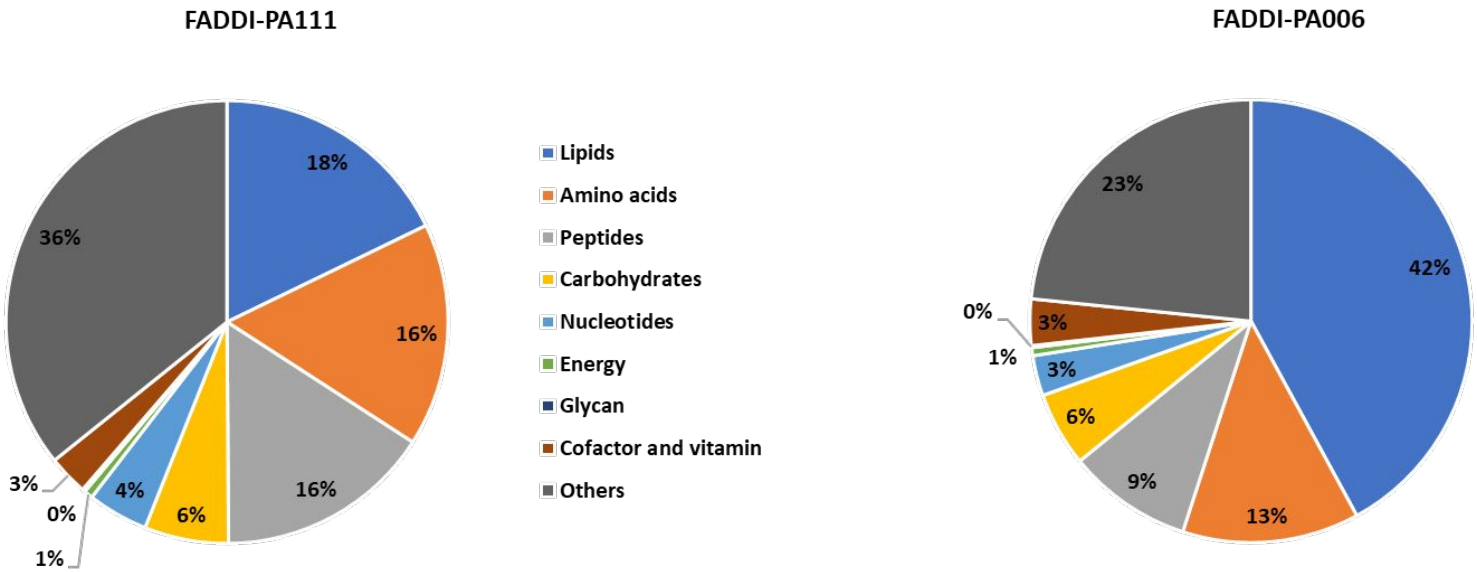


Figure S4. Summary number of significant metabolites after antibiotic monotherapy and combination (COM) treatment of FADDI-PA111 and FADDI-PA006. Changes (≥ 0.59-log2-fold, $p \leq 0.05$; FDR ≤ 0.1).

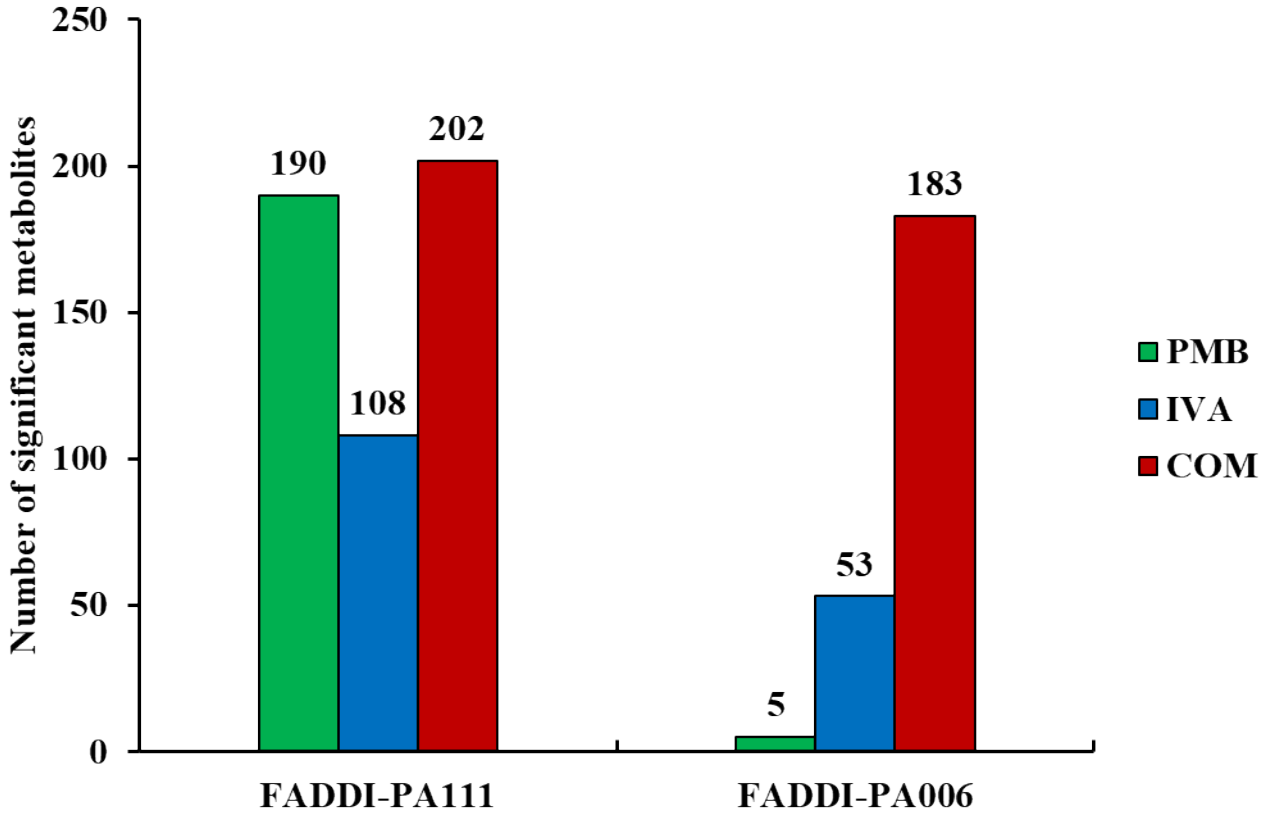
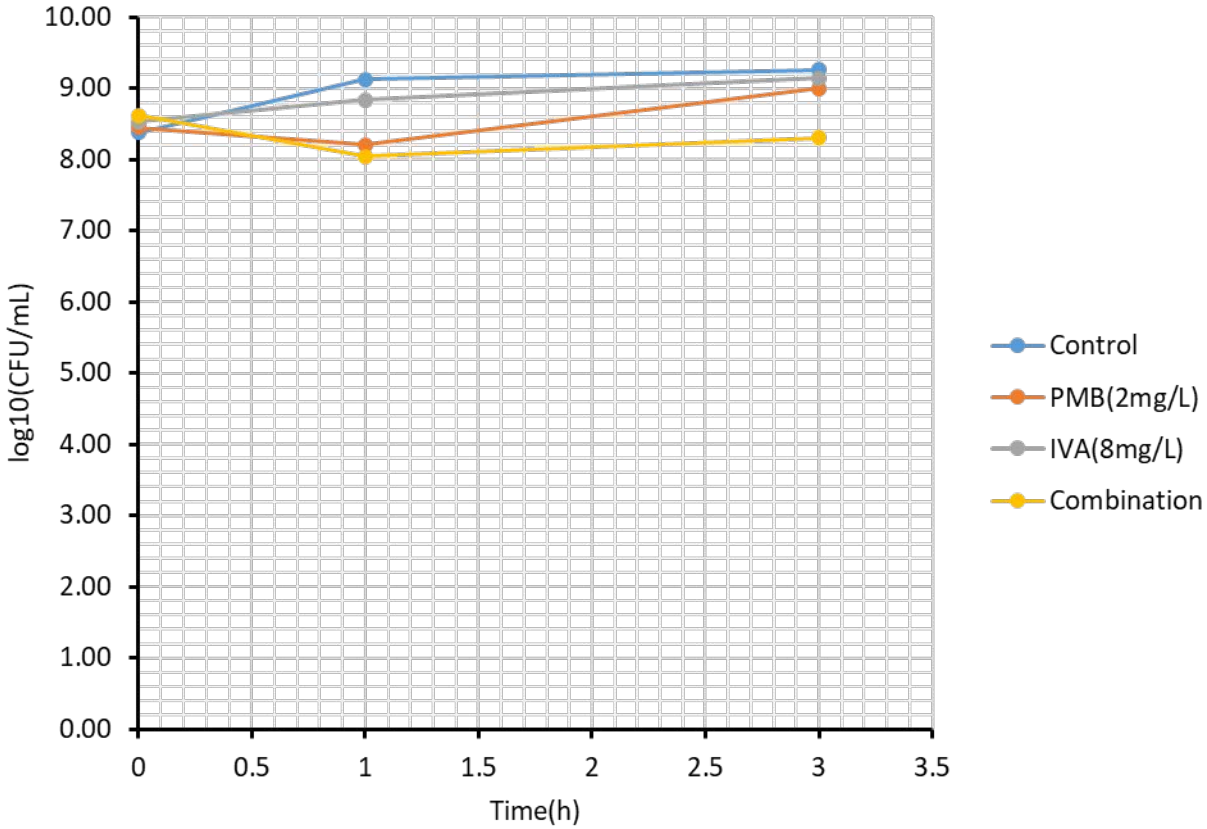


Figure S5. Time-kill kinetics of polymyxin B and ivacaftor alone and in combination against *P. aeruginosa* **(A)** FADDI-PA111 and **(B)** FADDI-PA006.

A



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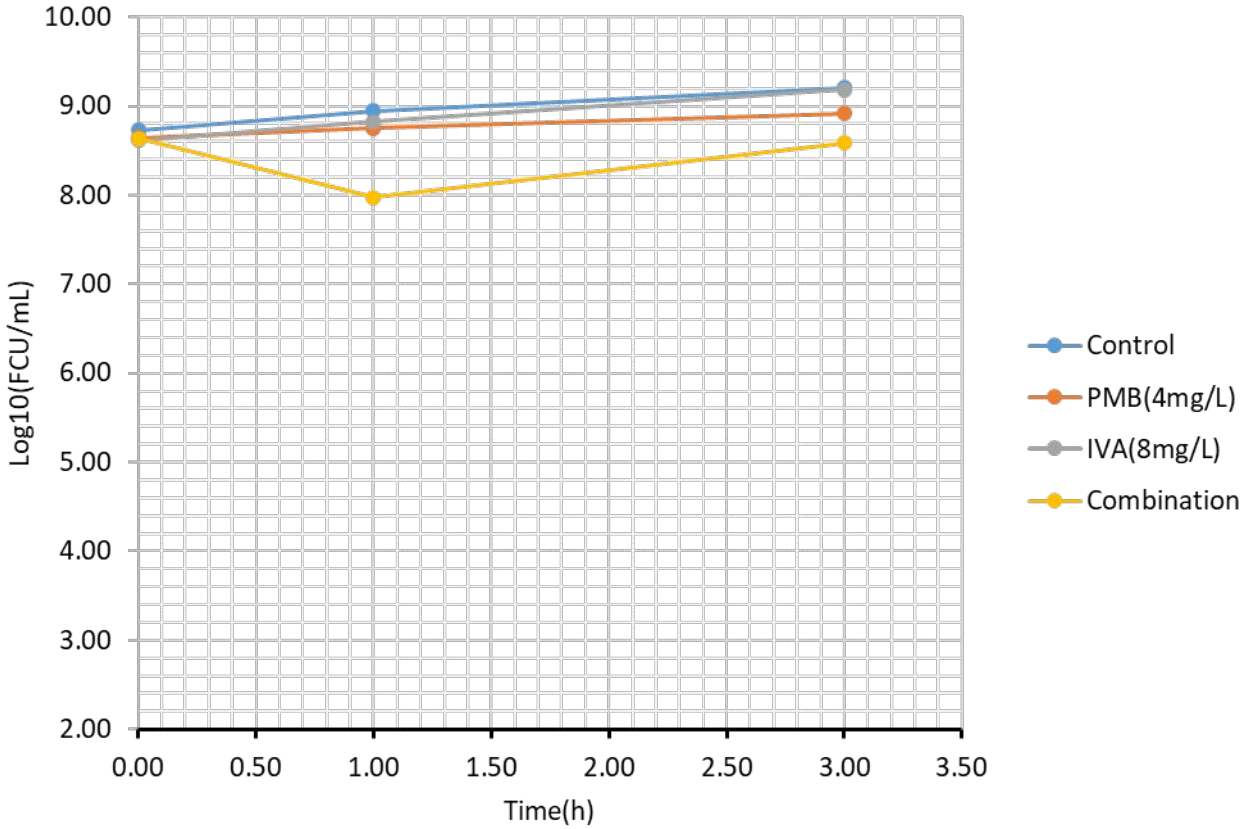
10.00
9.00
8.00
7.00
6.00
5.00
4.00
3.00
2.00
Log10(FCU/mL)
0.00
0.50
1.00
1.50
2.00
2.50
3.00
3.50
Time(h)
Control
PMB(4mg/L)
IVA(8mg/L)
Combination