Supplemental material

Metabolomics studies to decipher stress responses in *Mycobacterium smegmatis* point to a putative pathway of methylated amines biosynthesis

Arshad Rizvi[1]*, Saleem Yousf[2]*, Kannan Balakrishnan[1], Harish Kumar Dubey[1], Shekhar C. Mande[4,5], Jeetender Chugh[2,3#], Sharmistha Banerjee[1#]

[1]Department of Biochemistry, School of Life Sciences, University of Hyderabad, Hyderabad, Telangana 500046, India

[2]Department of Chemistry, Indian Institute of Science Education and Research (IISER), Pune, Maharashtra 411008, India

[3]Department of Biology, Indian Institute of Science Education and Research (IISER), Pune, Maharashtra 411008, India

[4]National Centre for Cell Science, Pune 411007, Maharashtra, India

[5]Present affiliation: Council of Scientific & Industrial Research (CSIR), Anusandhan Bhawan, 2 Rafi Marg, New Delhi-110001, India

*Authors contributed equally

[#]To whom correspondence may be addressed

Sharmistha Banerjee, PhD

Professor

Room No. F-60

Department of Biochemistry, School of Life Sciences

University of Hyderabad, Gachibowli, Hyderabad
Telangana, India
PIN - 500046
Phone (Office): +91-40-23134573
Phone (Lab): +91-40-23134673
Email ID: sbsl@uohyd.ac.in

and

JeetenderChugh, PhD
Assistant Professor
C-115, Main Building
Indian Institute of Science Education & Research,
Dr. Homi Bhabha Road, Pashan, Pune
Maharashtra, India
PIN – 411008
Phone (Office): +91-20-25908121
Phone (Lab): +91-20-25908231, +91-20-25909264
Email ID: cjeet@iiserpune.ac.in

Running title: *Stress-induced metabolic changes in mycobacteria*

Differential metabolites associated with stresses

Fifty-six metabolites, including the standard DSS (4,4-dimethyl-4-silapentane-1-sulfonic acid), were thus identified and are marked on the ^{1}H NMR spectrum as shown in Fig S1a in the supplemental material. Five metabolites (GTP, CDP, tryptophan, fructose-1-6 biphosphate, fumarate) were excluded from analysis as their representative peaks were not clear in all spectra. All the fifty metabolites and their respective ^{1}H chemical shifts (in reference to DSS) have been listed in Table S1 in the supplemental material and were considered for further analysis. The normalized concentration data matrix obtained from each of the 10 experimental replicates for each experimental condition, namely acidic stress, oxidative stress, nutrient starvation and untreated (control), were analyzed using MetaboAnalyst software to assess the influence of these stresses on *M. smegmatis*. Both univariate and multivariate approaches with the required and recommended statistical analyses (1) were performed for listing the differential metabolic profiles. While Table S2 in the supplemental material lists all the metabolites identified, only the metabolites with a minimum cutoff of 1.2-fold change (FC >1.2), with statistical significance [p-value ($p<0.05$), and false discovery rate (FDR<0.05)] are considered to be differential in a stress condition as compared to the control. These metabolites are indicated in boldface in Table S2. A fold change of >1.2 (with $p<0.05$) was considered because immediate adaptive changes to stresses were expected to cause small differences at metabolite level rather than large differences. Similar cutoffs have been used and reported in earlier studies (2-5). With these parameters, univariate analysis showed an overall 31, 20, and 46 metabolites with differential levels in acidic stress, oxidative stress, and nutrient starvation respectively as compared to the control (Table S2a-c in the supplemental material). Analysis of variance (ANOVA) was used to determine statistically significant differences in the means between all the four groups (Table

S2d in the supplemental material). A graphical representation of individual metabolite levels in different stresses is represented as a heat map in Fig 1.

Further, multivariate analysis such as principal component analysis (PCA) and partial least squares-discriminate analysis (PLS-DA) was performed to examine the intrinsic variation in groups. The total variance explained by five components PCA analyses were 94.1% for acidic stress, 80.1% for oxidative stress, and 94.4% for nutrient starvation stress. The scores plot of the first two principal components PC1 and PC2 (Fig S2 in the supplemental material) showed visually distinct metabolite profiles of each stress as compared to the control. Percentage variance explained by PC1 and PC2 for the different stress conditions has been mentioned in their respective plots (Fig S2a-c in the supplemental material). When all the stresses were compared together with the control in a 3D-PCA plot (Fig S2d in the supplemental material), clear segregations were observed amongst stresses pointing to the distinctness in the metabolite profiles of different stresses as compared to the control. The 2D-PCA scores plot for a pairwise comparison of the three stress conditions *versus* control revealed that the maximum discriminatory features are in control *versus* nutrient starvation (Fig S2c in the supplemental material) followed by control *versus* acidic stress (Fig S2a in the supplemental material) and control *versus* oxidative stress (Fig S2b in the supplemental material). In the PLS-DA model, which has a higher discriminatory potential that is ideal for the classification of groups, showed further segregation between the groups over PCA analysis (Fig S3 in the supplemental material).To validate the PLS-DA model and avoid overfitting, 5-fold cross-validated scores from the model were used to calculate cross-validation parameter Q^2, which indicates the goodness of predictability and R^2 that indicates goodness of fit of the model. A value of Q^2 closer to 1 indicates a high prediction accuracy of the model. The values of R2 and Q^2 in Table S3 in

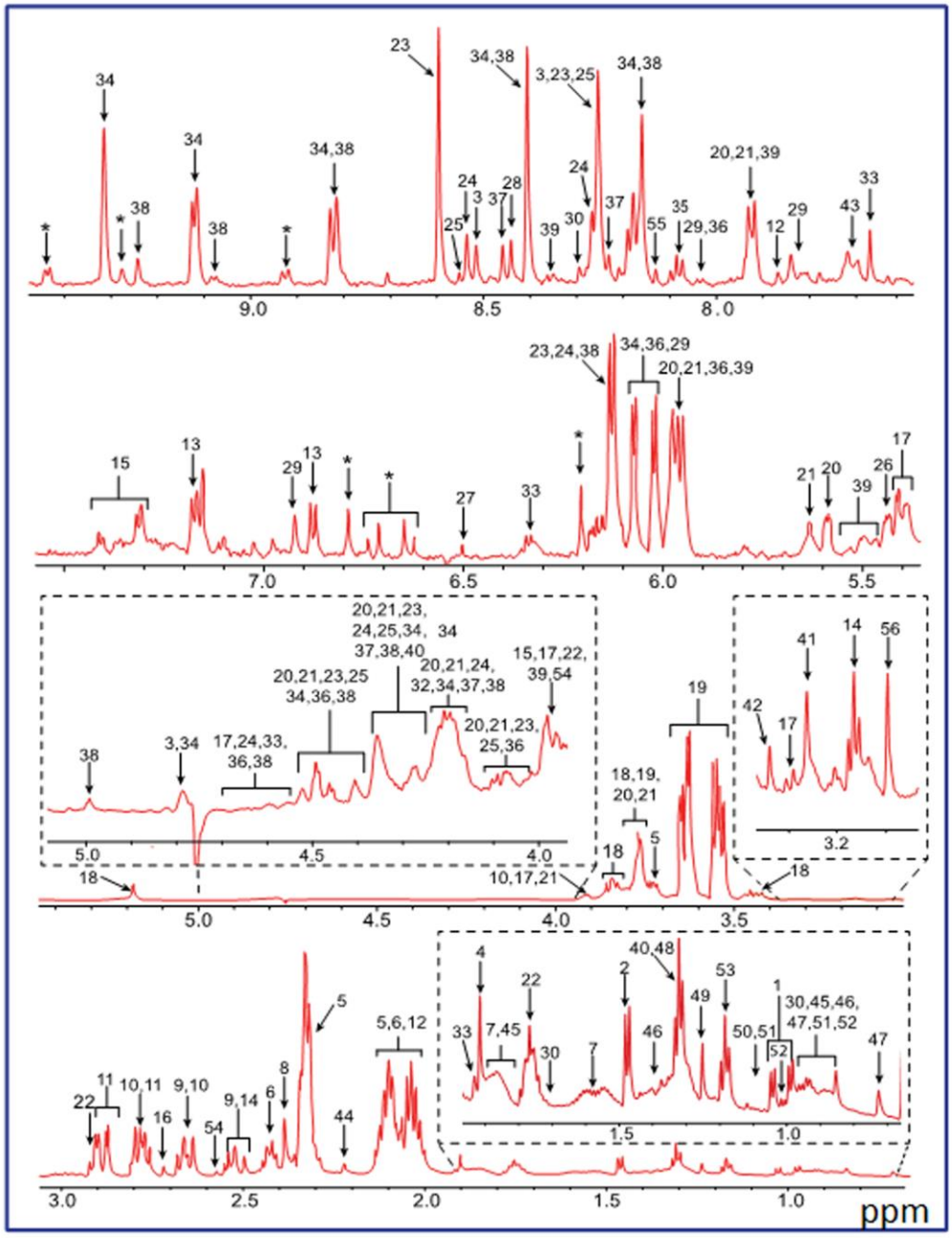
the supplemental material indicated that models generated from our data had high predictive accuracy and this model is representative of true differences in metabolic profiles of respective stress conditions compared to control.

Supplemental text References

1. Xia J, Mandal R, Sinelnikov IV, Broadhurst D, Wishart DS.2012. MetaboAnalyst 2.0--a comprehensive server for metabolomic data analysis. Nucleic Acids Res 40:W127-33.
2. Feng Q, Liu Z, Zhong S, Li R, Xia H, Jie Z, Wen B, Chen X, Yan W, Fan Y, Guo Z, Meng N, Chen J, Yu X, Zhang Z, Kristiansen K, Wang J, Xu X, He K, Li G.2016. Integrated metabolomics and metagenomics analysis of plasma and urine identified microbial metabolites associated with coronary heart disease. Sci Rep 6:22525.
3. Hou W, Meng X, Zhao A, Zhao W, Pan J, Tang J, Huang Y, Li H, Jia W, Liu F.2018. Development of Multimarker Diagnostic Models from Metabolomics Analysis for Gestational Diabetes Mellitus (GDM). Mol Cell Proteomics 17:431-441.
4. Abdelrahman M, Sawada Y, Nakabayashi R, Sato S, Hirakawa H, El-Sayed M, Hirai MY, Saito K, Yamauchi N, Shigyo M.2015. Integrating transcriptome and target metabolome variability in doubled haploids of Allium cepa for abiotic stress protection. Molecular Breeding 35.
5. Wu H, Chen Y, Li ZG, Liu XH.2018. Untargeted metabolomics profiles delineate metabolic alterations in mouse plasma during lung carcinoma development using UPLC-QTOF/MS in MSE mode. Royal Society Open Science 5.

Supplemental material figures and tables

Figure S1



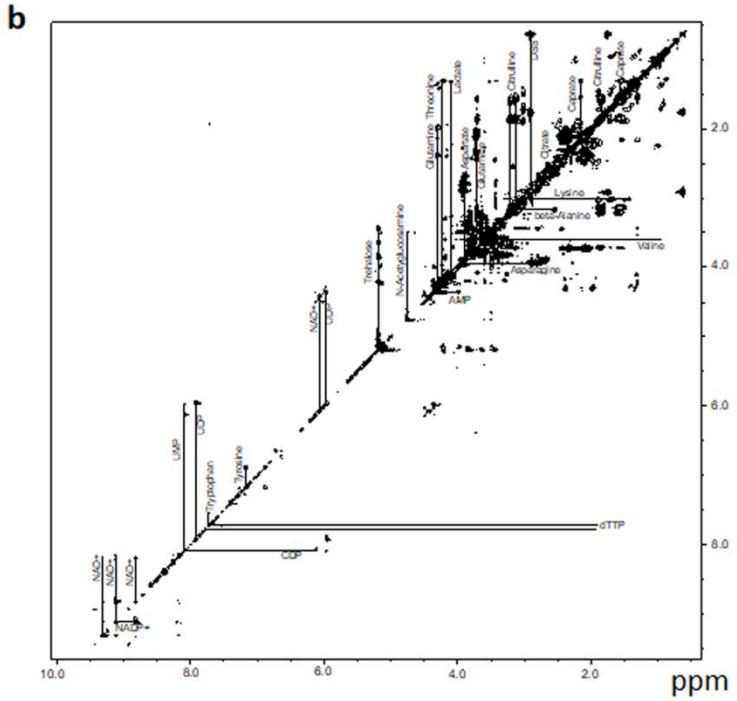


Fig S1 a) Representative ^{1}H NMR and two-dimensional (2D) spectrum of *M. smegmatis*. The ^{1}H NMR spectrum was obtained for each sample as described. Key: 1, Valine; 2, Alanine; 3,ATP; 4, Acetate; 5, Glutamate; 6, Glutamine; 7, Citrulline; 8, Succinate; 9, Citrate; 10, Aspartate; 11, Asparagine; 12, Homoserine; 13, Tyrosine; 14, beta-alanine; 15, Phenylalanine; 16, Dimethylamine; 17, Maltose; 18, Trehalose ; 19, Glycerol; 20, UDP-glucose; 21, UDP-galactose; 22, DSS (standard); 23, AMP; 24, ADP; 25, IMP; 26, Glucose-1-phosphate; 27, fumarate ; 28, Formate; 29, CDP; 30, Leucine; 31, Lysine; 32, Fructose 1-6, bisphosphate; 33, dTTP; 34, NAD^{+}; 35, N-acetyl glucosamine; 36, UMP; 37, NADPH; 38, NADP+; 39, UDP-N-acetylglucosamine; 40, Threonine; 41, Betaine; 42, Methanol; 43, Tryptophan; 44, Acetone; 45, 2-aminobutyrate; 46, Caprate; 47, Cholate; 48, Lactate 49, 3-hydroxyisovalerate; 50, 3-methyl-2-oxovalerate; 51, 2-hydroxy-3-methylvalerate; 52, Isoleucine; 53, Ethanol; 54, Methylamine; 55, GTP; and 56, Malonate. **b)** Representative two-dimensional (2D) ^{1}H-^{1}H TOCSY NMR spectrum of *M. smegmatis*.

Figure S2

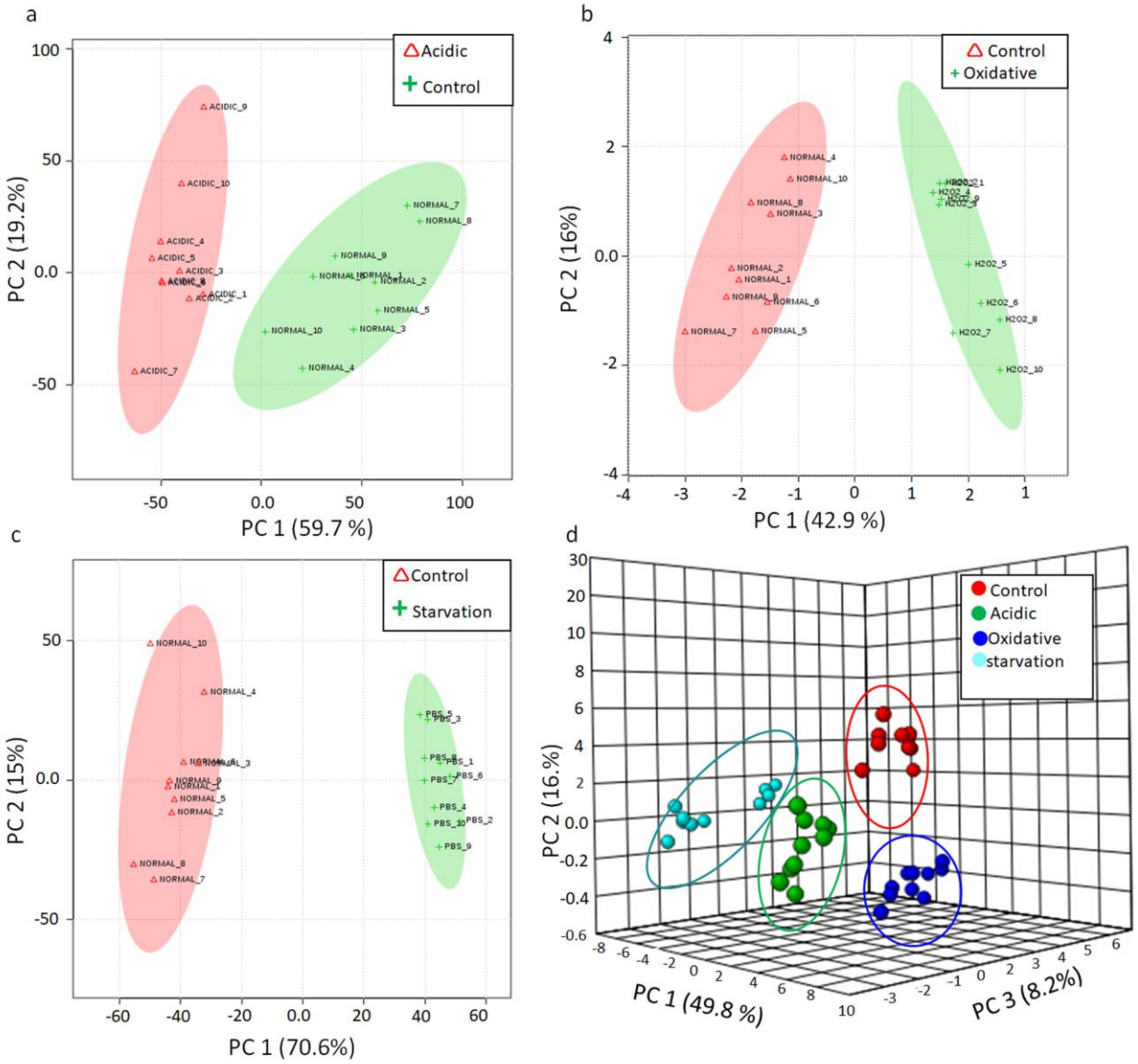


Fig S2: Principal component analyses (PCA) score plots showing distinctive metabolic profiles between different stress conditions. 2D plots of a) acidic stress *versus* control. b) oxidative stress *versus* control. c) nutrient starvation *versus* control. d) 3D plot of acidic stress, oxidative stress, nutrient starvation, and control, suggesting all groups could be distinctly categorized. The shaded area indicated the 95% confidence region. The dots inside the plots correspond to biological replicates under each category.

Figure S3

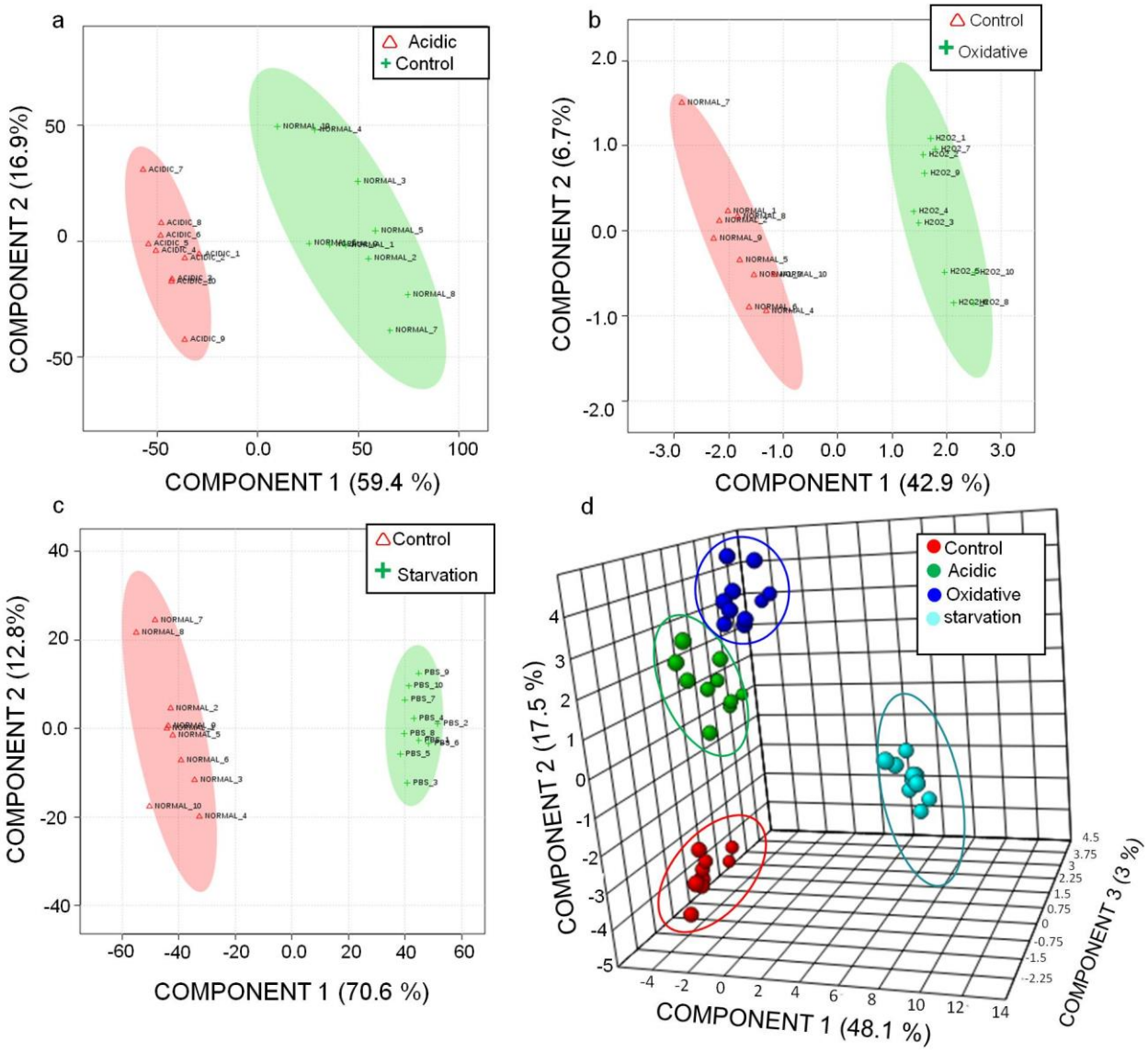


Fig S3: Partial Least Squares - Discriminant Analysis (PLS-DA) score plots showing distinctive metabolic profiles between different stress conditions. 2D plots of a) acidic stress *versus* control. b) oxidative stress *versus* control. c) nutrient starvation *versus* control. d) 3D plot of acidic stress, oxidative stress, nutrient starvation, and control. The shaded area indicated the 95% confidence region. The dots inside the plots correspond to biological replicates under each category.

Figure S4

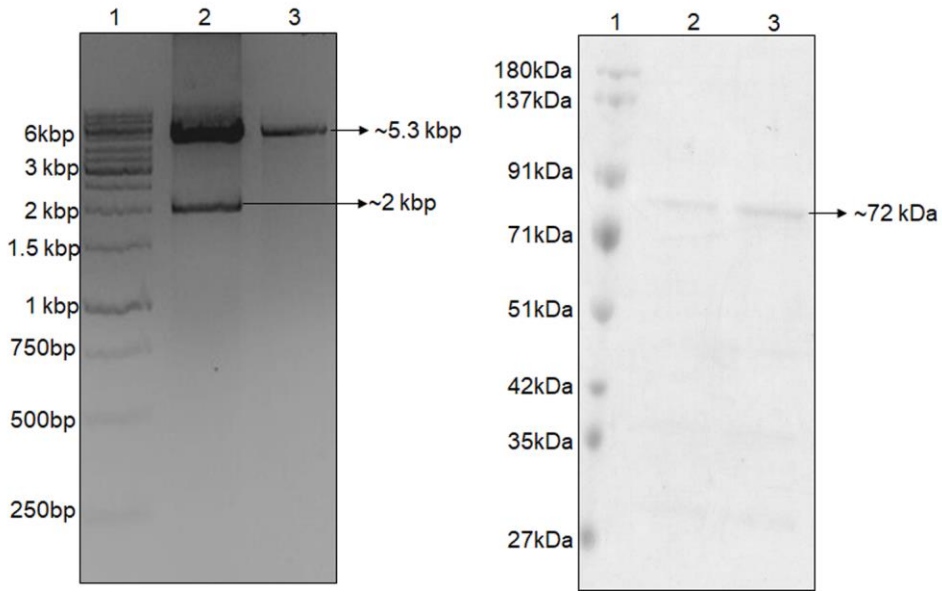


Fig S4: Cloning and purification of MSMEG_5124: (a) 1 % agarose gel showing the clone confirmation of pET-MS_5124 plasmid by restriction digestion using BamHI and HindIII. Lane 1: 1kb ladder; lane 2: A fall out of ~2kbp; lane 3: digested pET28a vector. (b) 10 % SDS-PAGE showing purified protein (MSMEG_5124) lane 1: Molecular weight marker (in kDa). Lane 2 and lane 3: purified MSMEG_5124 with 6X histidine tag (~72.8 kDa).

Figure S5

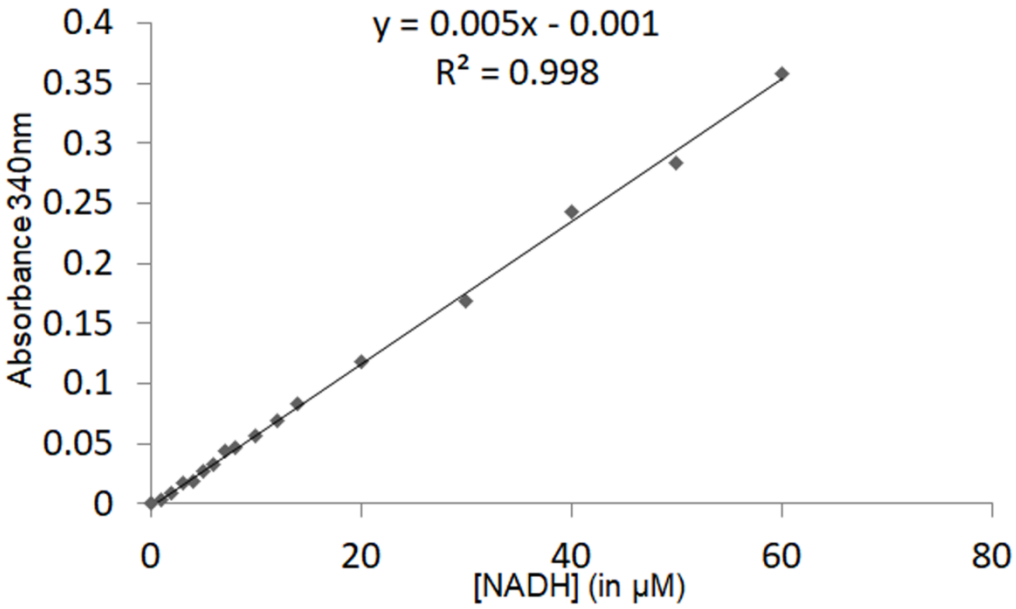


Fig S5: The standard plot for NADH quantification: Concentration of NADP (in µM) *versus* absorbance (at 340nm) plot used as the standard for quantification of NADH produced in the enzyme assays for MSMEG_5124.

Table S1: List of *M. smegmatis* metabolites that were assigned to their respective ^{1}H chemical shifts (ppm) in the ^{1}H NMR spectrum.

S.No	Metabolites	PPM
1	2-Aminobutyrate	3.706(t), 1.887(m), 0.968(t)
2	2-Hydroxy-3-methylvalerate (HMVA)	3.88(d), 1.705(m), 1.351(m), 1.162(m), 0.932(d), 0.872(t)
3	3-Hydroxyisovalerate	1.233(s), 2.35(s)
4	3-Methyl-2-Oxovalerate	0.88(t), 1.086(d), 1.444(m), 1.687(m), 2.922(m)
5	Acetate	1.90(s)
6	Acetone	2.221(s)
7	ADP	8.534(s), 8.261(s), 6.122(d), 4.52(m), 4.35(m), 4.00(m)
8	Alanine	1.46(d), 3.805(q)
9	AMP	8.596(s), 8.25(s), 6.12(d), 4.49(t), 4.355(m), 4.02(m)
10	Aspargine	2.777(dd), 2.89(dd),3.98(dd)
11	Aspartate	2.66(dd), 2.79(dd), 3.916(dd)
12	ATP	8.52(s),8.25(s), 6.128(d), 4.56(t), 4.41(m), 4.23(m), 4.30(m)
13	Beta-alanine	3.16(t), 2.54(t)
14	Betaine	3.263(s), 3.885(s)
15	Caprate	0.839(t), 1.281(m), 1.528(m), 2.519(m)
16	Cholate	0.711(s), 0.905(s), 0.960(d), 1.00(m), 1.16(m), 1.130(m), 1.140(m), 1.491(m), 1.568(m), 1.623(m),
		1.75(m),1.89(m), 2.01(m), 2.05(m), 2.23(m), 3.501(m), 3.39(m),4.06(m)
17	Citrate	2.53(d), 2.666(d)
18	Citrulline	1.526(m), 1.59(m), 1.84(m), 1.889(m),

		3.147(m), 3.126(m),3.74(m)
19	Dimethylamine	2.718(s)
20	DSS (Standard)	0.0(s), 0.62(t),1.75(m), 2.91(t)
21	dTTP	7.68(s), 6.333(m), 4.618(m), 4.22(m), 4.17(m), 2.38(m), 1.918(s)
22	Ethanol	1.185(t), 3.664(q)
23	Formate	8.44(s)
24	Fumarate	6.50(s)
25	Glucose-1-Phosphate	5.45(dd), 3.908(m), 3.86(m), 3.76(m), 3.487(m), 3.398(t)
26	Glutamate	2.03(m),2.10(m), 2.34(m), 3.75(dd)
27	Glutamine	2.141(m), 2.459(m), 3.76(t)6
28	GTP	8.110(s), 6.12(s),5.96(s), 4.54(m), 4.35(m4.24(m)
29	Homoserine	2.01(m), 2.16(m), 3.77(m), 3.85(dd)
30	IMP	8.553(s), 8.231(s), 6.136(d), 4.51(m), 4.36(m), 4.01(m)
31	isoleucine	0.926(t), 0.992(d), 1.248(m), 1.457(m), 1.968(m), 3.661(d)
32	Lactate	4.096(q), 1.313(d)
33	Leucine	3.721(m), 1.701(m), 0.94(m)
34	Lysine	3.74(t), 3.02(t), 1.89(m), 1.71(m), 1.46(m)
35	Malonate	3.09(s)
36	Maltose	5.41(d), 5.39(d), 5.211(d), 3.96(m), 3.93(m), 3.84(m), 3.76(m), 3.70(m), 3.66(m),3.62(m),
		3.58(m), 3.421(m),3.27(m)
37	Methylamine	2.573(s)
38	N-Acetyl-glucosamine	
39	NAD	9.314(s), 9.121(d), 8.824(d), 8.406(s), 8.184(m), 8.154(s), 6.07(d), 6.021(d),

		4.522(m),
40	NADP+	9.281(s), 9.08(d), 8.80(d), 8.41(s), 8.18(m), 8.13(s), 6.112(d), 6.022(d), 4.99(q), 4.60(t),
41	Phenyl alanine	7.42(m), 7.36(m), 7.32(m), 3.98(dd), 3.27(m), 3.13(m)
42	Succinate	2.385(s)
43	Threonine	4.244(m), 3.582(d), 1.313(d)
44	Trehalose	5.18(d), 3.84(m), 3.80(m), 3.76(m), 3.64(dd),3.43(t)
45	Tryptophan	7.72(d), 7.53(d), 7.32(s), 7.24(m), 7.19(m0, 4.04(dd), 3.47(dd), 3.29(dd)
46	Tyrosine	7.17(d), 6.87(d), 3.93(dd), 3.18(dd),3.04(dd)
47	UDP Galactose	7.93(d), 5.97(m), 5.63(dd), 4.36(m), 4.25(m), 4.15(m),4.02(d), 3.90(dd), 3.805(dt), 3.75(m)
48	UDP-Glucose	7.925(d), 5.977(m), 5.593(dd), 4.36(m), 4.27(m), 4.24(m), 4.189(m), 3.877(m), 3.77(m),
49	UDP-N-Acetylglucosamine	8.177(d), 8.077(m), 5.19(d), 4.70(d), 3.904(m), 3.841(m), 3.784(m), 3.74(m),
50	UMP	8.07(d), 5.98(m), 4.41(t), 4.34(t), 4.26(m), 3.97(m)
51	Valine	0.996(d), 1.047(d), 2.281(m),3.617(d)

180

181

Table S2: Fold change and VIP score of a) acidic stress *versus* control. b) oxidative stress *versus* control. c) nutrient starvation *versus* control. d) Analysis of variance (ANOVA) table showing significantly ($p<0.05$) changed metabolites in comparison to acidic stress, oxidative stress, and nutrient starvation groups and control.

a) Fold changes and VIP scores of **acidic stress** *versus* **control**.

S.No	Metabolites	Fc	p.value	FDR	VIP score
1	**D-Maltose**	**3.08**	**1.00E-09**	**1.30E-08**	0.65
2	**Capric acid**	**2.17**	**7.50E-05**	**2.00E-04**	0.47
3	**Taurine**	**1.77**	**3.60E-05**	**1.00E-04**	0.62
4	**Methylamine**	**1.59**	**1.30E-01**	**1.60E-01**	0.22
5	**2-Hydroxy-3-**	**1.46**	**3.30E-03**	**6.40E-03**	0.25
	methylpentanoic acid				
6	**Dimethylamine**	**1.45**	**1.20E-01**	**1.40E-01**	0.25
7	**Malonic acid**	**1.34**	**1.50E-02**	**2.50E-02**	0.73
8	**3-Methyl-2-oxovaleric acid**	**1.34**	**1.30E-02**	**2.20E-02**	0.09
9	**Citric acid**	**1.29**	**2.30E-01**	**2.60E-01**	0.63
10	**L-Asparagine**	**1.28**	**1.80E-02**	**3.00E-02**	**1.78**
11	Uridinediphosphate glucose	1.11	3.40E-01	3.70E-01	0.11
12	3-Hydroxyisovaleric acid	1	9.80E-01	1.00E+00	0
13	L-Lactic acid	1	1.00E+00	1.00E+00	0
14	Betaine	0.96	6.30E-01	6.90E-01	0.04
15	NADP	0.92	6.60E-01	7.00E-01	0.03
16	ADP	0.84	8.90E-02	1.20E-01	0.15
17	NAD	0.83	2.00E-02	3.20E-02	0.39
18	Nicotinamide N-oxide	0.83	8.50E-02	1.20E-01	0.13
19	Acetone	0.83	1.90E-05	6.30E-05	0.25
20	Trehalose	0.82	1.70E-01	2.00E-01	**1.15**
21	Citrulline	0.8	1.00E-01	1.30E-01	0.42
22	Uridinediphosphate-N-acetylglucosamine	0.8	3.00E-02	4.60E-02	0.13

23	Formic acid	0.78	1.00E-03	2.20E-03	0.28
24	Acetic acid	0.74	4.30E-02	6.10E-02	0.41
25	Adenosine monophosphate	0.72	6.00E-04	1.40E-03	0.55
26	Sucrose	0.7	1.40E-01	1.70E-01	0.17
27	Adenosine triphosphate	0.7	9.90E-02	1.30E-01	0.2
28	Inosinic acid	0.7	9.10E-03	1.60E-02	0.12
29	L-Lysine	0.67	1.30E-03	2.70E-03	0.29
30	L-Leucine	0.66	1.70E-03	3.50E-03	0.28
31	**Uridinediphosphategalactose**	**0.66**	**2.50E-05**	**7.70E-05**	0.2
32	**L-Phenylalanine**	**0.65**	**9.10E-11**	**1.50E-09**	0.21
33	**Glucose 1-phosphate**	**0.62**	**4.00E-02**	**5.80E-02**	0.25
34	**Uridine 5'-monophosphate**	**0.61**	**1.10E-04**	**2.80E-04**	0.3
35	**Succinic acid**	**0.6**	**9.50E-06**	**3.40E-05**	0.85
36	**Cholic acid**	**0.6**	**3.90E-03**	**7.20E-03**	0.27
37	**Thymidine 5'-triphosphate**	**0.59**	**8.70E-06**	**3.40E-05**	0.28
38	**L-Glutamine**	**0.59**	**2.30E-06**	**1.00E-05**	**1.73**
39	**L-Alpha-aminobutyric acid**	**0.57**	**6.00E-04**	**1.40E-03**	0.31
40	**L-Aspartic acid**	**0.56**	**7.00E-05**	**2.00E-04**	**1.73**
41	**D-Glutamic acid**	**0.55**	**1.20E-08**	**9.00E-08**	**4.71**
42	**L-Homoserine**	**0.49**	**1.30E-08**	**9.00E-08**	**2.61**
43	**Isoleucine**	**0.37**	**3.90E-08**	**2.40E-07**	0.32
44	**1-Methylnicotinamide**	**0.33**	**8.20E-08**	**4.50E-07**	0.42
45	**Beta-Alanine**	**0.31**	**2.20E-06**	**1.00E-05**	**1.34**
46	**L-Threonine**	**0.31**	**4.50E-06**	**1.90E-05**	**1.25**
47	**L-Valine**	**0.3**	**4.40E-09**	**4.40E-08**	0.77
48	**L-Tyrosine**	**0.28**	**7.60E-11**	**1.50E-09**	0.54
49	**Alanine**	**0.26**	**5.70E-11**	**1.50E-09**	**1.19**

187

188 Fc: fold change; VIP score: variable of importance score obtained from PLS-DA plot, p-value: p
189 values obtained after performing a t-test, FDR: value obtained after performing false discovery
190 test. Note: - Metabolite (in bold) indicate statistically differential metabolite. Metabolites with

191 VIP score >1 were considered to be contributing to the model significantly.

192 **b)** Fold changes and VIP scores of **oxidative stress** *versus* **control**

S.no	Metabolites	Fc	p.value	FDR	VIP score
1	**L-Lysine**	**3.33**	**1.80E-07**	**1.10E-06**	**1.96**
2	**Methylamine**	**2.85**	**1.90E-05**	**6.60E-05**	**1.57**
3	**Uridinediphosphate-N-acetylglucosamine**	**2.36**	**3.50E-07**	**1.80E-06**	**1.4**
4	**Betaine**	**2.36**	**3.20E-09**	**8.10E-08**	**1.7**
5	**L-Aspartic acid**	**2.2**	**1.30E-07**	**9.00E-07**	**2.69**
6	**Capric acid**	**2.1**	**8.00E-06**	**3.10E-05**	**1.4**
7	**D-Maltose**	**2.08**	**2.50E-08**	**4.20E-07**	**1.37**
8	**2-Hydroxy-3-methylpentanoic acid**	**1.9**	**4.50E-05**	**1.50E-04**	**1.22**
9	**3-Methyl-2-oxovaleric acid**	**1.83**	**2.30E-07**	**1.30E-06**	0.94
10	**Isoleucine**	**1.5**	**1.20E-03**	**3.60E-03**	0.83
11	**Acetic acid**	**1.43**	**7.30E-02**	**1.40E-01**	0.8
12	**Dimethylamine**	**1.43**	**2.40E-03**	**6.60E-03**	0.89
13	**Taurine**	**1.36**	**3.50E-02**	**8.10E-02**	0.71
14	L-Lactic acid	1.22	9.70E-02	1.60E-01	0.59
15	L-Glutamine	1.17	3.40E-03	9.00E-03	0.94
16	3-Hydroxyisovaleric acid	1.13	1.40E-01	2.20E-01	0.34
17	Uridinediphosphate glucose	1.13	8.50E-02	1.50E-01	0.41
18	Acetone	1.11	2.00E-01	2.90E-01	0.3
19	L-Leucine	1.1	3.60E-01	4.70E-01	0.25
20	L-Asparagine	1.1	1.70E-01	2.60E-01	0.61
21	Formic acid	1.1	2.20E-01	3.20E-01	0.3
22	L-Homoserine	1.09	2.90E-01	4.00E-01	0.48
23	Thymidine 5'-triphosphate	1.09	6.70E-01	7.00E-01	0.12
24	Citric acid	1.08	5.40E-01	6.20E-01	0.3
25	Citrulline	1.04	6.50E-01	6.90E-01	0.16
26	L-Phenylalanine	1.03	5.70E-01	6.40E-01	0.1

27	L-Threonine	0.99	9.80E-01	9.80E-01	0.01
28	Beta-Alanine	0.98	8.10E-01	8.30E-01	0.09
29	Succinic acid	0.95	4.90E-01	5.80E-01	0.24
30	Trehalose	0.93	6.30E-01	6.90E-01	0.28
31	Cholic acid	0.92	4.00E-01	4.90E-01	0.21
32	Alanine	0.91	9.40E-02	1.60E-01	0.42
33	L-Valine	0.91	3.40E-01	4.40E-01	0.3
34	Uridinediphosphategalactose	0.9	1.20E-01	1.90E-01	0.3
35	L-Alpha-aminobutyric acid	0.89	4.10E-01	5.10E-01	0.24
36	L-Tyrosine	0.89	6.30E-02	1.30E-01	0.41
37	ADP	0.83	4.10E-02	8.90E-02	0.52
38	Uridine 5'-monophosphate	0.82	5.40E-02	1.10E-01	0.51
39	Sucrose	0.8	3.00E-01	4.00E-01	0.34
40	Glucose 1-phosphate	0.76	7.40E-02	1.40E-01	0.56
41	**D-Glutamic acid**	**0.71**	**8.90E-08**	**7.40E-07**	**2.16**
42	**Inosinic acid**	**0.7**	**1.60E-02**	**3.90E-02**	0.6
43	**Malonic acid**	**0.69**	**1.30E-02**	**3.10E-02**	**1.08**
44	**Nicotinamide N-oxide**	**0.67**	**7.80E-05**	**2.40E-04**	0.9
45	**NAD**	**0.59**	**1.70E-06**	**7.50E-06**	**1.47**
46	**Adenosine monophosphate**	**0.52**	**6.40E-08**	**6.60E-07**	**1.62**
47	**Adenosine triphosphate**	**0.44**	**4.60E-06**	**1.90E-05**	**1.35**
48	**1-Methylnicotinamide**	**0.42**	**6.60E-08**	**6.60E-07**	**1.4**
49	**NADP**	**0.13**	**2.10E-12**	**1.10E-10**	**1.7**

Fc: fold change; VIP score: variable of importance score obtained from PLS-DA plot, p-value: p values obtained after performing a t-test, FDR: value obtained after performing false discovery test. Note: - Metabolite (in bold) indicate statistically differential metabolite. Metabolites with VIP score >1 were considered to be contributing to the model significantly.

198 **c)** Fold changes and VIP scores of **nutrient starvation** *versus* **control.**

S.no	Metabolites	Fc	p.value	FDR	VIP score
1	**2-Hydroxy-3-methylpentanoic acid**	**10.45**	**2.80E-07**	**5.30E-07**	**1.05**
2	**L-Leucine**	**4.91**	**1.80E-05**	**2.70E-05**	0.84
3	**Capric acid**	**3.88**	**8.10E-08**	**2.00E-07**	0.62
4	**Taurine**	**3.88**	**1.90E-10**	**8.80E-10**	**1.04**
5	**Methylamine**	**3.45**	**2.10E-03**	**2.60E-03**	0.48
6	**L-Lysine**	**2.88**	**1.20E-07**	**2.70E-07**	0.62
7	**L-Tyrosine**	**2.83**	**1.90E-08**	**5.30E-08**	0.66
8	**Malonic acid**	**2.68**	**7.70E-08**	**2.00E-07**	**1.65**
9	**Acetone**	**1.93**	**7.50E-12**	**4.20E-11**	0.5
10	**3-Hydroxyisovaleric acid**	**1.87**	**4.30E-16**	**2.10E-14**	0.4
11	**3-Methyl-2-oxovaleric acid**	**1.83**	**3.50E-04**	**4.80E-04**	0.13
12	**Betaine**	**1.75**	**7.40E-06**	**1.20E-05**	0.4
13	**Acetic acid**	**1.43**	**5.50E-03**	**6.30E-03**	0.48
14	**Formic acid**	**1.38**	**4.80E-04**	**6.30E-04**	0.29
15	Dimethylamine	1.31	4.40E-01	4.50E-01	0.12
16	**L-Lactic acid**	**1.3**	**4.60E-02**	**4.90E-02**	0.32
17	**Citrulline**	**1.28**	**4.40E-02**	**4.70E-02**	0.43
18	D-Glutamic acid	1.22	5.00E-04	6.40E-04	**2.27**
19	L-Alpha-aminobutyric acid	1	1.00E+00	1.00E+00	0.0001
20	**L-Homoserine**	**0.79**	**4.60E-03**	**5.40E-03**	**1.06**
21	Cholic acid	0.73	1.50E-01	1.50E-01	0.13
22	**L-Valine**	**0.7**	**2.60E-02**	**2.90E-02**	0.29
23	**L-Threonine**	**0.63**	**4.90E-03**	**5.70E-03**	0.61
24	**Nicotinamide N-oxide**	**0.57**	**2.90E-04**	**4.10E-04**	0.23
25	**Trehalose**	**0.56**	**2.00E-03**	**2.40E-03**	**2.02**
26	**NAD**	**0.5**	**2.60E-07**	**5.30E-07**	0.7

27	**ADP**	**0.46**	**4.20E-06**	**7.20E-06**	0.31
28	**Uridinediphosphate glucose**	**0.45**	**7.00E-07**	**1.30E-06**	0.4
29	**Inosinic acid**	**0.43**	**7.70E-06**	**1.20E-05**	0.15
30	**Isoleucine**	**0.41**	**1.50E-05**	**2.30E-05**	0.23
31	**Alanine**	**0.4**	**7.80E-09**	**2.30E-08**	0.83
32	**Succinic acid**	**0.35**	**2.40E-07**	**5.10E-07**	0.88
33	**Adenosine triphosphate**	**0.33**	**1.50E-05**	**2.30E-05**	0.35
34	**L-Phenylalanine**	**0.3**	**4.10E-15**	**1.00E-13**	0.24
35	**L-Glutamine**	**0.28**	**5.30E-09**	**1.70E-08**	**1.87**
36	**Uridine 5'-monophosphate**	**0.21**	**1.50E-09**	**5.80E-09**	0.38
37	**L-Aspartic acid**	**0.18**	**1.90E-09**	**6.90E-09**	**2.06**
38	**NADP**	**0.11**	**1.00E-07**	**2.40E-07**	0.23
39	**Uridinediphosphategalactose**	**0.08**	**8.90E-14**	**8.90E-13**	0.29
40	**Adenosine monophosphate**	**0.08**	**6.40E-13**	**4.60E-12**	0.93
41	**Uridinediphosphate-N-acetylglucosamine**	**0.08**	**4.30E-10**	**1.80E-09**	0.29
42	**D-Maltose**	**0.06**	**2.40E-14**	**4.00E-13**	0.35
43	**Thymidine 5'-triphosphate**	**0.05**	**7.80E-13**	**4.80E-12**	0.37
44	**Sucrose**	**0.04**	**7.20E-05**	**1.00E-04**	0.36
45	**1-Methylnicotinamide**	**0.04**	**1.10E-10**	**5.60E-10**	0.4
46	**Glucose 1-phosphate**	**0.04**	**3.80E-06**	**6.70E-06**	0.41
47	**Beta-Alanine**	**0.004**	**3.50E-09**	**1.20E-08**	**1.32**
48	**Citric acid**	**0.0024**	**1.40E-07**	**3.10E-07**	**1.65**
49	**L-Asparagine**	**0.0006**	**4.00E-14**	**5.00E-13**	**3.6**

199

200 Fc: fold change; VIP score: variable of importance score obtained from PLS-DA plot, p-value: p
201 values obtained after performing a t-test, FDR: value obtained after performing false discovery
202 test. Note: - Metabolite (in bold) indicate statistically differential metabolite. Metabolites with
203 VIP score >1 were considered to be contributing to the model significantly.

204 **d) Analysis of variance (ANOVA) table** showing significantly ($p<0.05$) changed metabolites
205 in comparison to acidic stress, oxidative stress, and nutrient starvation groups and control.

Metabolites	F.value	p.value	FDR	VIP Score
D-Glutamic acid	362.5	6.1E-27	3.1E-25	**1.16**
Adenosine monophosphate	229.2	1.7E-23	4.2E-22	**1.52**
L-Asparagine	222.5	2.8E-23	4.6E-22	**1.49**
Taurine	134.8	1.3E-19	1.6E-18	0.93
Uridinediphosphategalactose	127.5	3.1E-19	2.8E-18	**1.21**
Alanine	127.0	3.3E-19	2.8E-18	0.23
1-Methylnicotinamide	119.5	9.0E-19	5.7E-18	0.93
L-Aspartic acid	118.7	1.0E-18	5.7E-18	0.08
Acetone	118.6	1.0E-18	5.7E-18	**1.21**
Malonic acid	114.4	1.8E-18	9.1E-18	0.74
L-Tyrosine	112.4	2.4E-18	1.1E-17	**1.35**
D-Maltose	106.3	6.0E-18	2.5E-17	**1.30**
3-Hydroxyisovaleric acid	96.4	2.9E-17	1.0E-16	**1.04**
L-Glutamine	93.5	4.7E-17	1.6E-16	0.68
2-Hydroxy-3-methylpentanoic acid	83.6	2.7E-16	8.3E-16	**1.20**
Uridinediphosphate-N-acetylglucosamine	83.5	2.8E-16	8.3E-16	0.34
Beta-Alanine	78.8	6.9E-16	1.9E-15	0.54
Capric acid	61.2	3.3E-14	8.7E-14	0.85
NAD	57.2	8.9E-14	2.2E-13	**1.39**
L-Phenylalanine	55.1	1.6E-13	3.7E-13	0.95
NADP	49.9	6.7E-13	1.5E-12	**1.39**
Betaine	45.3	2.6E-12	5.7E-12	**1.17**
L-Leucine	42.4	6.7E-12	1.4E-11	**1.17**
Thymidine 5'-triphosphate	40.9	1.1E-11	2.2E-11	0.81

L-Lysine	40.7	1.2E-11	2.3E-11	**1.35**
Citric acid	38.4	2.6E-11	4.8E-11	**1.34**
Uridine 5'-monophosphate	36.7	4.7E-11	8.5E-11	**1.09**
Isoleucine	35.2	8.5E-11	1.5E-10	0.19
Uridinediphosphate glucose	31.5	3.5E-10	5.9E-10	**1.27**
Glucose 1-phosphate	24.4	8.5E-09	1.4E-08	**1.07**
Formic acid	24.2	9.7E-09	1.5E-08	**1.08**
Succinic acid	23.4	1.4E-08	2.1E-08	0.83
Sucrose	23.3	1.5E-08	2.2E-08	**1.18**
Citrulline	20.9	5.2E-08	7.5E-08	0.96
ADP	20.1	8.1E-08	1.1E-07	**1.30**
L-Threonine	19.6	1.1E-07	1.5E-07	0.69
3-Methyl-2-oxovaleric acid	18.1	2.5E-07	3.3E-07	0.75
L-Homoserine	15.2	1.5E-06	2.0E-06	0.94
Adenosine triphosphate	13.4	4.9E-06	6.1E-06	**1.04**
L-Valine	13.4	5.2E-06	6.3E-06	0.76
Inosinic acid	9.9	6.6E-05	7.8E-05	**1.01**
Acetic acid	9.0	1.4E-04	1.7E-04	**1.01**
Trehalose	6.8	9.1E-04	1.0E-03	0.99
Nicotinamide N-oxide	6.5	1.3E-03	1.4E-03	0.88
Methylamine	5.5	3.1E-03	3.4E-03	0.71
L-Lactic acid	4.4	1.0E-02	1.1E-02	0.44
L-Alpha-aminobutyric acid	4.3	1.0E-02	1.1E-02	0.72
Dimethylamine	1.8	1.7E-01	1.7E-01	0.10
Cholic acid	0.5	6.9E-01	6.9E-01	0.15

206

207 Note: F value indicates the ratio of the variance of the group means to that of the pooled within-
208 group variance. The larger the F value, the greater the relative variance among the group means.
209 The p-value tells you the probability of obtaining an F value as extreme or more extreme as the

one observed under the assumption that the null hypothesis is true. FDR: value obtained after performing a false discovery test. VIP score: variable of importance score obtained from PLS-DA plot. VIP score >1 metabolites were considered to be contributing to the model significantly.

Table S3: R^2 and Q^2 values of Partial Least Squares - Discriminant Analysis (PLS-DA).

Conditions	Measure	1 comps	2 comps	3 comps	4 comps	5 comps
Acidic-Control	R^2	0.9	0.96	0.97	0.98	0.99
	Q^2	0.87	0.92	0.92	0.86	0.78
Oxidative-Control	R^2	0.95	0.98	0.99	0.99	0.99
	Q^2	0.92	0.92	0.93	0.92	0.88
Starvation-control	R^2	0.99	0.99	1	1	1
	Q^2	0.98	0.97	0.98	0.98	0.98
Acidic-Oxidative-Starvation-Control	R^2	0.8	0.95	0.98	0.99	0.99
	Q^2	0.77	0.93	0.95	0.97	0.97

Note: The R^2 value indicated goodness of fit, and Q^2 indicates the goodness of predictability. A value closer to 1 indicates the accuracy of the prediction model. Comps: component

Table S4: Metabolic pathway impact analysis with metabolic pathways significantly influenced a) acidic stress *versus* control. b) oxidative stress *versus* control. c) nutrient starvation *versus* control.

a) Metabolic pathway impact analysis: acidic stress *versus* control.

Acidic stress *versus* control	Total Cmpd[a]	Hits[b]	-log(P) [c]	FDR [d]	Impact [e]
Phenylalanine, tyrosine and tryptophan biosynthesis	22	2	25.318	4.35E-10	0.02
D-Alanine metabolism	5	1	23.584	7.84E-10	0.00
Novobiocin biosynthesis	3	1	23.302	7.84E-10	0.00
Thiamine metabolism	21	1	23.302	7.84E-10	0.00
Phenylalanine metabolism	3	1	23.118	7.84E-10	0.00
Glycine, serine and threonine metabolism	28	4	20.306	1.09E-08	0.21
Taurine and hypotaurine metabolism	7	3	19.457	2.18E-08	0.00
Valine, leucine and isoleucine degradation	37	1	19.252	2.34E-08	0.00
D-Glutamine and D-glutamate metabolism	7	2	18.675	0.000000037	0.38
Lysine biosynthesis	15	3	18.542	3.73E-08	0.00
Cysteine and methionine metabolism	37	2	18.469	3.73E-08	0.06
Sulfur metabolism	13	2	18.169	4.61E-08	0.07
Selenoamino acid metabolism	16	2	17.287	0.000000103	0.01
Pantothenate and CoA biosynthesis	21	3	16.581	0.000000193	0.02
beta-Alanine metabolism	9	2	15.166	0.000000728	1.00
Alanine, aspartate and	20	5	15.122	0.000000728	0.69

glutamate metabolism					
Arginine and proline metabolism	40	3	14.979	0.000000791	0.05
Valine, leucine and isoleucine biosynthesis	26	2	14.326	0.00000142	0.02
Tyrosine metabolism	11	2	14.281	0.00000142	0.00
Aminoacyl-tRNA biosynthesis	66	8	13.426	0.00000318	0.00
Propanoate metabolism	21	2	13.23	0.00000368	0.00
Pyrimidine metabolism	37	2	13.044	0.00000423	0.11
Purine metabolism	66	5	12.754	0.00000541	0.14
Nitrogen metabolism	14	4	12.576	0.00000619	0.00
Butanoate metabolism	22	1	11.567	0.0000157	0.05
Benzoate degradation via CoA ligation	10	1	11.567	0.0000157	0.00
Nicotinate and nicotinamide metabolism	13	3	9.8877	8.1E-05	0.15
Peptidoglycan biosynthesis	19	2	9.0395	1.8E-04	0.08
Cyanoamino acid metabolism	8	2	7.5334	7.9E-04	0.00
Methane metabolism	13	1	6.9018	1.4E-03	0.10
Lysine degradation	13	1	6.6492	1.8E-03	0.00
Amino sugar and nucleotide sugar metabolism	38	4	3.9977	0.02	0.21
Galactose metabolism	26	4	3.3779	0.04	0.10
Streptomycin biosynthesis	10	1	3.2292	0.05	0.22
Polyketide sugar unit biosynthesis	5	1	3.2292	0.05	0.00
Citrate cycle (TCA cycle)	20	2	3.1543	0.05	0.12
Pentose and	26	2	2.6266	0.08	0.01

glucuronateinterconversions					
Starch and sucrose metabolism	30	5	2.173	0.13	0.39
Pyruvate metabolism	21	2	2.1177	0.13	0.08
Glycolysis or Gluconeogenesis	30	4	1.8442	0.17	0.02
Glyoxylate and dicarboxylate metabolism	22	2	1.6469	0.20	0.43
Ascorbate and aldarate metabolism	8	1	1.0865	0.35	0.00
Glutathione metabolism	17	1	0.4176	0.66	0.00

Note: [a] Total number of metabolites in the pathway,[b] Number of matched metabolites, [c]-log(P) is the negative natural log of the P value for each pathway, [d] False Discovery Rate (Benjamini-Hochberg),[e] Impact: The Impact is the pathway impact value calculated from pathway topology analysis. Pathways with pathway-impact values ≥ 0.1, p-value ($p<0.05$), and false discovery rate (FDR) ($FDR<0.05$) were considered to be perturbed significantly

b) Metabolic pathway impact analysis: oxidative stress *versus* control.

oxidative *versus* **control**	Total Cmpd[a]	Hits[b]	-log(P) [c]	FDR [d]	Impact [e]
Glutathione metabolism	17	1	26.8	9.4814E-11	0.00
Nicotinate and nicotinamide metabolism	13	3	21.6	8.6862E-09	0.15
Nitrogen metabolism	14	4	19.8	3.6832E-08	0.00
Glycine, serine and threonine metabolism	28	4	19.1	4.6296E-08	0.21
Lysine biosynthesis	15	3	19.0	4.6296E-08	0.00

D-Glutamine and D-glutamate metabolism	7	2	18.4	7.413E-08	0.38
Amino sugar and nucleotide sugar metabolism	38	4	18.1	8.2151E-08	0.21
Aminoacyl-tRNA biosynthesis	66	8	17.4	1.5592E-07	0.00
Arginine and proline metabolism	40	3	17.0	2.0177E-07	0.05
Purine metabolism	66	5	16.2	3.5045E-07	0.14
Alanine, aspartate and glutamate metabolism	20	5	16.2	3.5045E-07	0.69
Pantothenate and CoA biosynthesis	21	3	15.8	5.0472E-07	0.02
beta-Alanine metabolism	9	2	15.6	5.0554E-07	1.00
Cysteine and methionine metabolism	37	2	15.6	5.0554E-07	0.06
Lysine degradation	13	1	15.5	5.0675E-07	0.00
Cyanoamino acid metabolism	8	2	15.0	8.0805E-07	0.00
Peptidoglycan biosynthesis	19	2	12.7	0.00000772	0.08
Pyrimidine metabolism	37	2	7.1	0.00	0.11
Starch and sucrose metabolism	30	5	4.8	0.02	0.39
Taurine and hypotaurine metabolism	7	3	4.1	0.04	0.00
Pentose and glucuronateinterconversions	26	2	3.2	0.08	0.01
Pyruvate metabolism	21	2	3.2	0.08	0.08
Glycolysis or Gluconeogenesis	30	4	3.0	0.09	0.02

Selenoamino acid metabolism	16	2	3.0	0.09	0.01
Novobiocin biosynthesis	3	1	2.8	0.11	0.00
Thiamine metabolism	21	1	2.8	0.11	0.00
Streptomycin biosynthesis	10	1	2.6	0.11	0.22
Polyketide sugar unit biosynthesis	5	1	2.6	0.11	0.00
Galactosemetabolism	26	4	2.5	0.12	0.10
Ascorbate and aldarate metabolism	8	1	2.5	0.12	0.00
D-Alanine metabolism	5	1	2.4	0.13	0.00
Phenylalanine, tyrosine and tryptophan biosynthesis	22	2	2.4	0.13	0.02
Sulfur metabolism	13	2	2.2	0.14	0.07
Methane metabolism	13	1	1.5	0.28	0.10
Tyrosine metabolism	11	2	1.4	0.31	0.00
Valine, leucine and isoleucine degradation	37	1	1.1	0.40	0.00
Butanoate metabolism	22	1	0.7	0.55	0.05
Benzoate degradation via CoA ligation	10	1	0.7	0.55	0.00
Glyoxylate and dicarboxylate metabolism	22	2	0.7	0.56	0.43
Phenylalanine metabolism	3	1	0.6	0.61	0.00
Citrate cycle (TCA cycle)	20	2	0.4	0.68	0.12
Valine, leucine and isoleucine biosynthesis	26	2	0.4	0.69	0.02
Propanoate metabolism	21	2	0.3	0.75	0.00

Note: [a] Total number of metabolites in the pathway,[b] Number of matched metabolites, [c]-log(P) is the negative natural log of the P value for each pathway, [d] False Discovery Rate (Benjamini-Hochberg),[e] Impact: The Impact is the pathway impact value calculated from pathway topology analysis. Pathways with pathway-impact values ≥ 0.1, p-value ($p<0.05$), and false discovery rate (FDR) ($FDR<0.05$) were considered to be perturbed significantly

c) Metabolic pathway impact analysis: nutrient starvation *versus* control.

nutrient starvation *versus* control	Total Cmpd[a]	Hits[b]	-log(P) [c]	FDR [d]	Impact [e]
Aminoacyl-tRNA biosynthesis	66	8	37.035	2.2896E-15	0.00
Cyanoamino acid metabolism	8	2	36.778	2.2896E-15	0.00
Nitrogen metabolism	14	4	35.437	5.8368E-15	0.00
Alanine, aspartate and glutamate metabolism	20	5	34.855	7.8364E-15	0.69
Phenylalanine metabolism	3	1	33.132	3.5122E-14	0.00
Taurine and hypotaurinemetabolism	7	3	28.881	2.0525E-12	0.00
Arginine and proline metabolism	40	3	28.064	3.9833E-12	0.05
Pantothenate and CoA biosynthesis	21	3	26.985	1.025E-11	0.02
beta-Alanine metabolism	9	2	26.768	1.1328E-11	1.00
Glycolysis or Gluconeogenesis	30	4	25.904	2.4188E-11	0.02
Peptidoglycan biosynthesis	19	2	24.457	9.34E-11	0.08
Nicotinate and nicotinamide	13	3	21.797	1.224E-09	0.15

metabolism					
Purine metabolism	66	5	21.231	1.9908E-09	0.14
Selenoamino acid metabolism	16	2	20.546	3.6656E-09	0.01
Propanoate metabolism	21	2	19.606	8.7585E-09	0.00
Phenylalanine, tyrosine and tryptophan biosynthesis	22	2	19.39	1.0194E-08	0.02
Pyrimidine metabolism	37	2	19.329	1.0195E-08	0.11
Amino sugar and nucleotide sugar metabolism	38	4	19.114	1.1938E-08	0.21
Tyrosine metabolism	11	2	18.768	1.5985E-08	0.00
D-Alanine metabolism	5	1	18.664	1.6856E-08	0.00
Glycine, serine and threonine metabolism	28	4	18.275	2.3685E-08	0.21
Citrate cycle (TCA cycle)	20	2	18.082	2.7415E-08	0.12
Lysine biosynthesis	15	3	17.871	3.2389E-08	0.00
Novobiocin biosynthesis	3	1	17.783	3.2549E-08	0.00
Thiamine metabolism	21	1	17.783	3.2549E-08	0.00
Cysteine and methionine metabolism	37	2	16.842	8.0151E-08	0.06
Glyoxylate and dicarboxylate metabolism	22	2	16.204	1.4619E-07	0.43
Glutathione metabolism	17	1	16.099	1.5653E-07	0.00
Lysine degradation	13	1	15.943	1.7658E-07	0.00
Pentose and glucuronateinterconversions	26	2	15.885	1.8096E-07	0.01
Galactose metabolism	26	4	15.317	3.0892E-07	0.10
Butanoate metabolism	22	1	15.232	3.1595E-07	0.05
Benzoate degradation via CoA ligation	10	1	15.232	3.1595E-07	0.00

Ascorbate and aldarate metabolism	8	1	14.17	8.8741E-07	0.00
D-Glutamine and D-glutamate metabolism	7	2	13.302	2.0523E-06	0.38
Streptomycin biosynthesis	10	1	12.493	4.3623E-06	0.22
Polyketide sugar unit biosynthesis	5	1	12.493	4.3623E-06	0.00
Methane metabolism	13	1	7.6449	5.4E-04	0.10
Starch and sucrose metabolism	30	5	6.8585	1.2E-03	0.39
Sulfur metabolism	13	2	6.3757	1.8E-03	0.07
Pyruvate metabolism	21	2	6.0924	2.4E-03	0.08
Valine, leucine and isoleucine biosynthesis	26	2	5.3424	4.9E-03	0.02
Valine, leucine and isoleucine degradation	37	1	3.6576	0.02580	0.00

Note: [a] Total number of metabolites in the pathway,[b] Number of matched metabolites, [c]-log(P) is the negative natural log of the P value for each pathway, [d] False Discovery Rate (Benjamini-Hochberg),[e] Impact: The Impact is the pathway impact value calculated from pathway topology analysis. Pathways with pathway-impact values ≥ 0.1, p-value ($p<0.05$), and false discovery rate (FDR) ($FDR<0.05$) were considered to be perturbed significantly

Table S5: Pre-processing method to perform standard statistical analysis

Pre-Processing Method	Acidic - Control	Oxidative-Control	Starvation-Control	Acidic-Oxidative-Control
Normalization	----	Sum	----	Sum

Transformation	----	Cube Root	----	----
scaling	Pareto	Pareto	Pareto	Auto

247

248

249 **Table S6: Primers used in the study**

Gene name	Sequence (5'-3')	Annealing Temperature (°C)
TrimethylamineBiosynthesis synthesis pathways in *M. smegmatis* RT primers		
2,4-DCA MSMEG_5124FP	AGATCGTCTGTTTGGCAAC	58
2,4-DCAMSMEG_5124RP	GATCACTCAGCAGCGGAC	58
YeaXMSMEG_4371RTFP	AGAGATCCACGAGCAGTT	57
YeaXMSMEG_4371RTRP	CTGCGGGAGCACCTCGAC	57
TR2Fe-2SMSMEG_0657FP	TGCTCGTCCATCCATCCCG	57
TR2Fe-2SMSMEG_0657RP	GACGGCCAGCGGTGTCAT	57
Endogenous controls *M. smegmatis*		
MSMEG_2758 sigAFP	GAAGACACCGACCTGGAACT	55
MSMEG_2758 sigA RP	GACTCTTCCTCGTCCCACAC	55
Primers for cloning of *M. smegmatis*MSMEG_5124 gene.		
MSMEG_5124FP	5´-GTGACATACCCGAACCTG	58
MSMEG_5124RP	3´-CTACAGGCGCGCGGCGAG	58

250

251