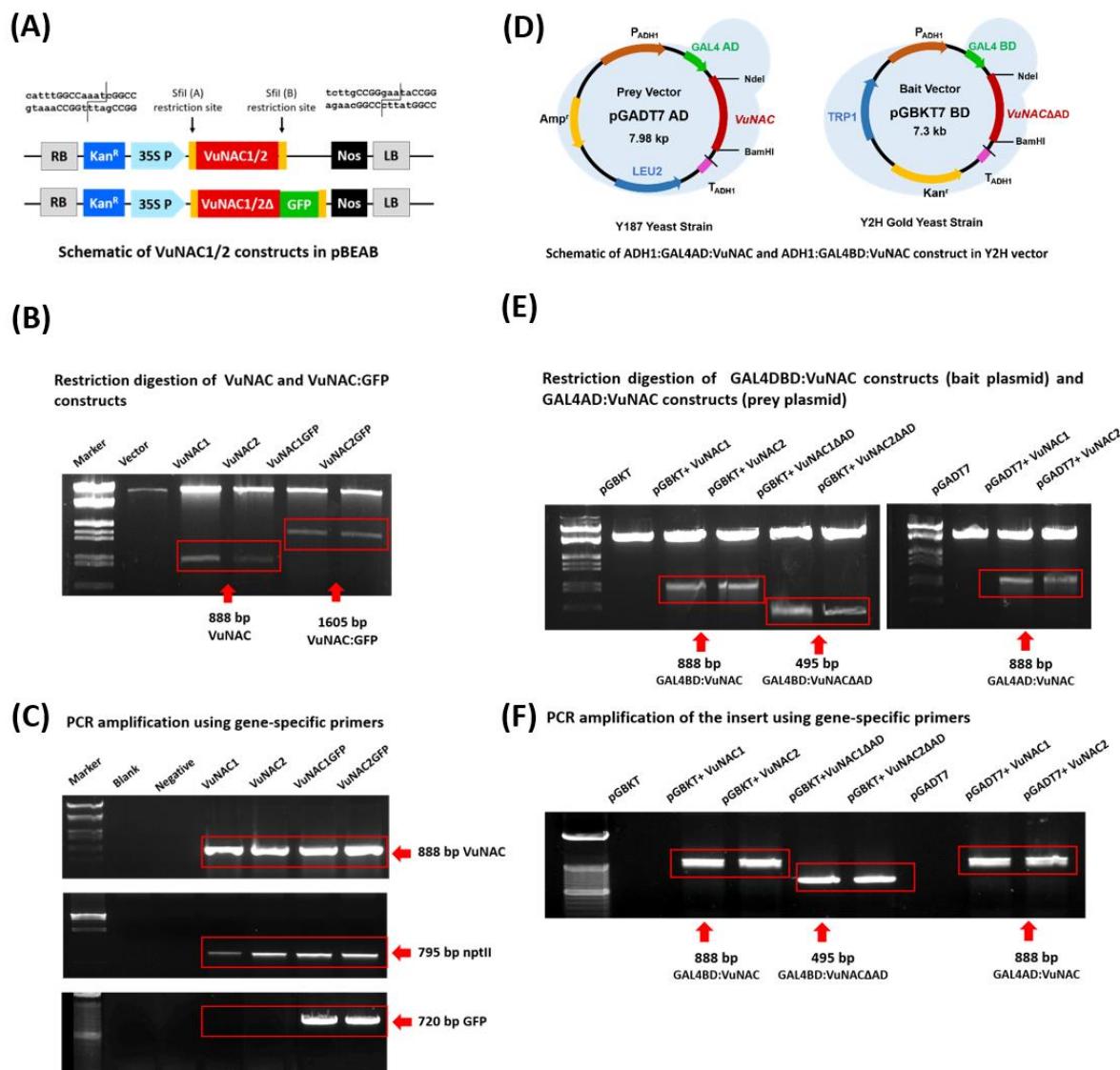


## SUPPORTING INFORMATION

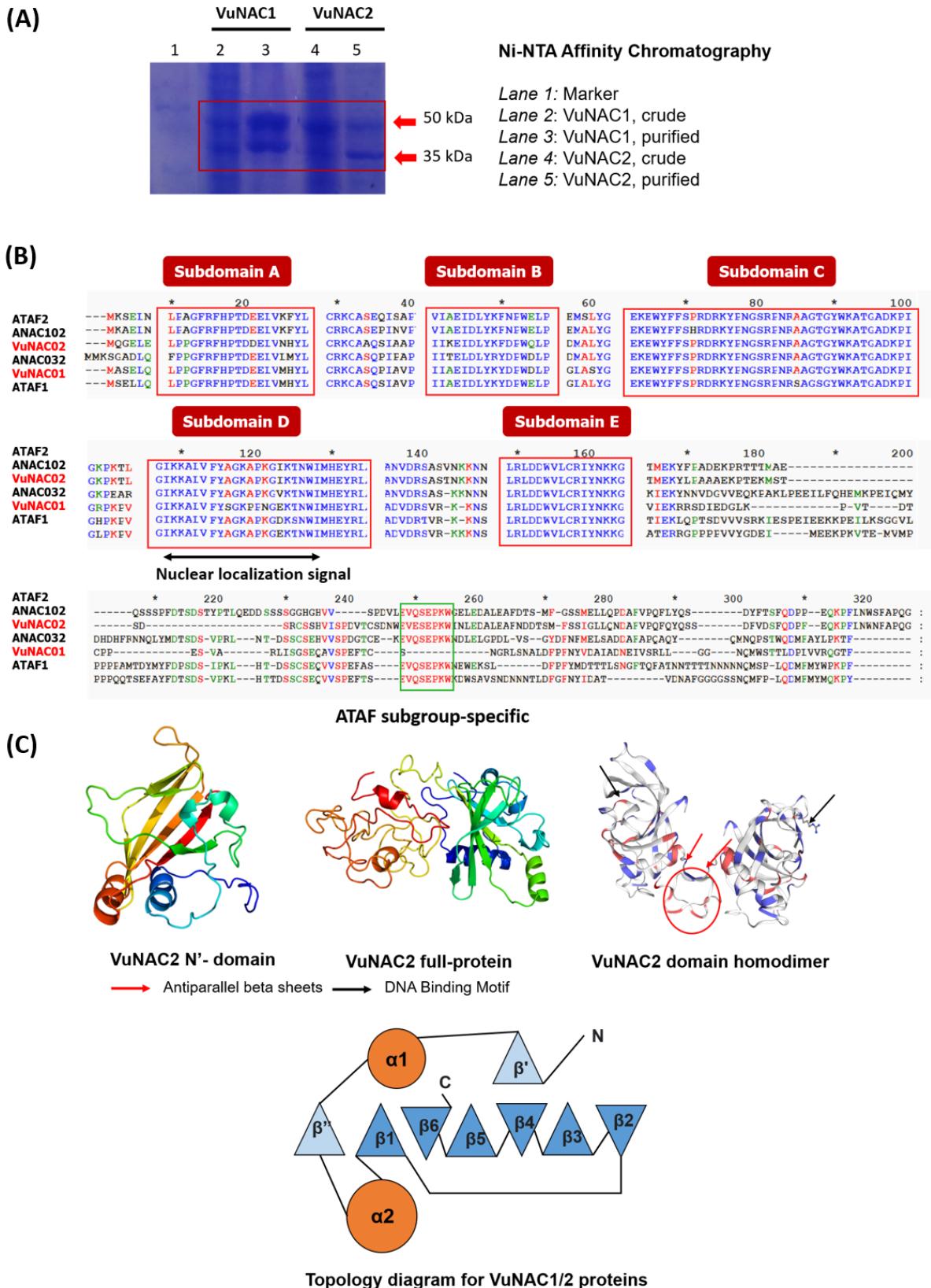
### Cowpea NAC transcription factors positively regulate cellular stress response and balance energy metabolism in yeast via reprogramming of biosynthetic pathways

Richa Srivastava<sup>1</sup> and Lingaraj Sahoo<sup>1\*</sup>

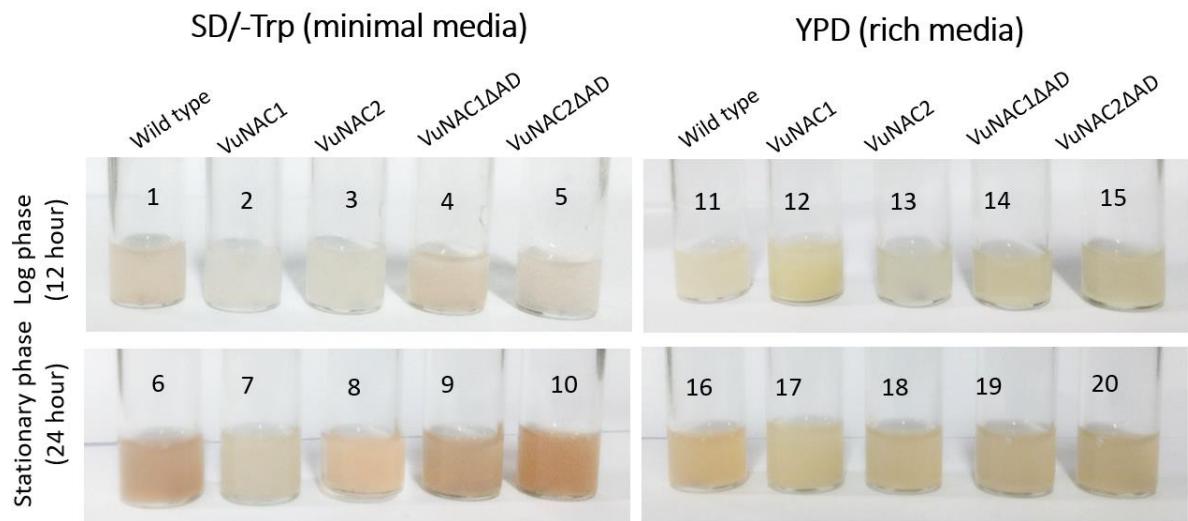
<sup>1</sup>Department of Biosciences and Bioengineering, Indian Institute of Technology Guwahati, Guwahati, 781039, India



**Figure S1.** Cloning of *VuNAC1* and *VuNAC2* genes in plant and yeast expression vector.

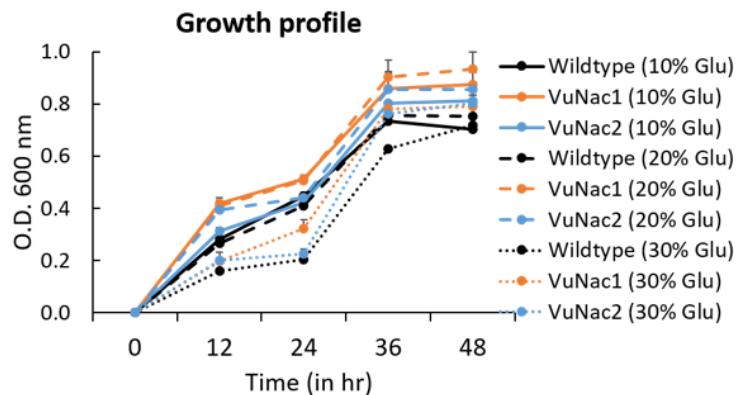


**Figure S2.** Purification and structure prediction of VuNAC1/2 proteins. (A) The IPTG-induced protein expressed in the bacterial system (crude) and its isolation using Ni-NTA agarose affinity chromatography (purified). (B) Identification of structural sub-domains by aligning the VuNAC1/2 proteins with their *Arabidopsis* homologs (ATAF1/2, ANAC032, and ANAC102). (C) The 3D fold of VuNAC2 (top) and the topology diagram of VuNAC1/2 proteins (bottom).

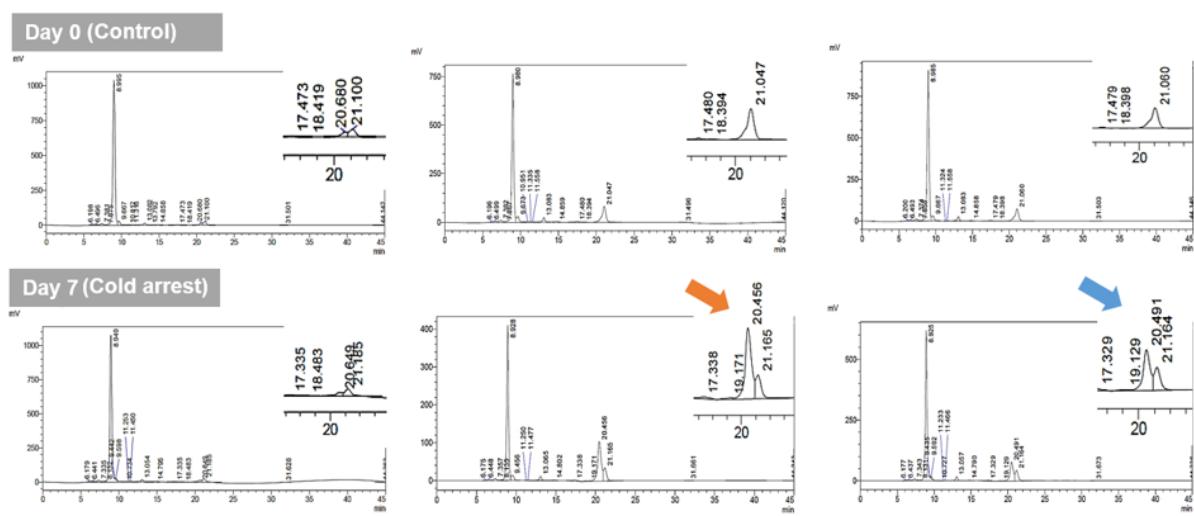


**Figure S3.** Growth in rich *vs.* minimal media. Less accumulation of brown pigment (residual adenine substrate) was observed in the transgenic strains expressing full VuNAC1/2 proteins (mainly VuNAC1) when grown in minimal media SD-/Trp (containing limited adenine) and YPD growth media (rich in adenine) after the nutrient was exhausted during the late-stationary phase. The strains expressing the truncated proteins (VuNAC1/2 $\Delta$ AD) appeared like the wild type.

(A)



(B)



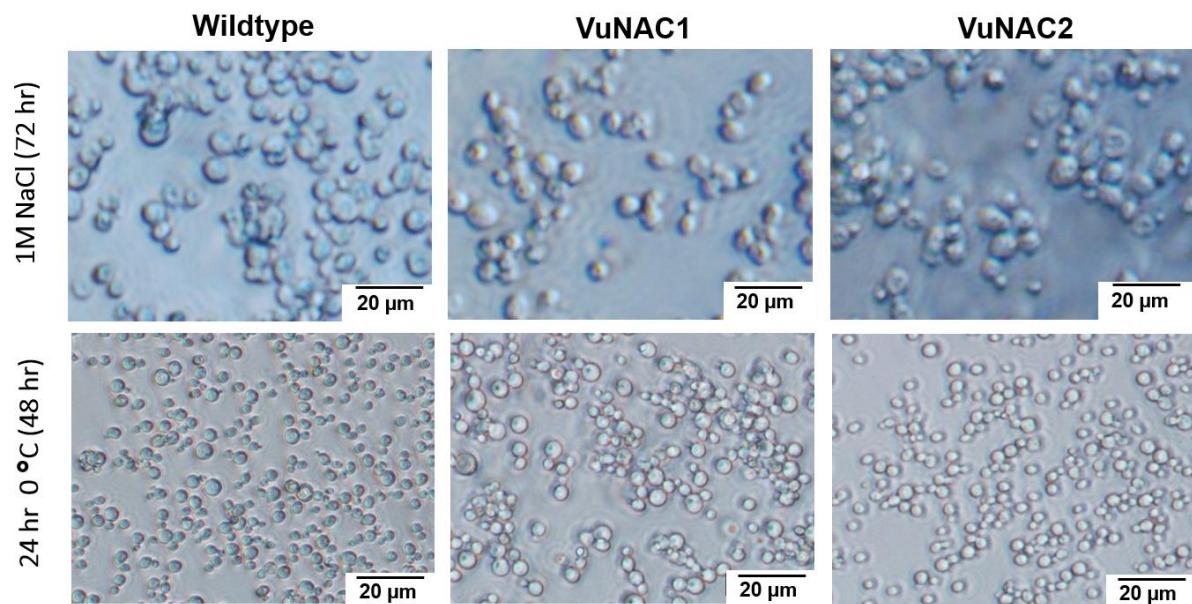
WT	Glucose	Ethanol	Glycerol	VuNAC1	Glucose	Ethanol	Glycerol	VuNAC2	Glucose	Ethanol	Glycerol
Day 0	21.6 %	1.9 %	0.35 %	Day 0	13.9 %	7.2 %	0.54%	Day 0	16.9 %	6.7 %	0.56%
Day 7	21.0 %	2.0 %	0.34 %	Day-7	6.6 %	2.7 %	0.20%	Day 7	10.5 %	3.4 %	0.32%

**Figure S4.** Growth characterization. (A) Growth profile at different glucose concentrations (10%, 20%, and 30%) indicating increased growth of VuNAC1/2-expressing strains in high glucose levels. (B) The HPLC chromatogram showing the ethanol peaks (Day 0) and the peaks for ethanol and its esterified product (the two peaks indicated by the arrow) after a cold-arrest of 4°C (Day 7). The reduced glucose and ethanol level suggested active alcohol fermentation and metabolism in the transgenic strains, while the activity of wild type strain was halted due to freezing.

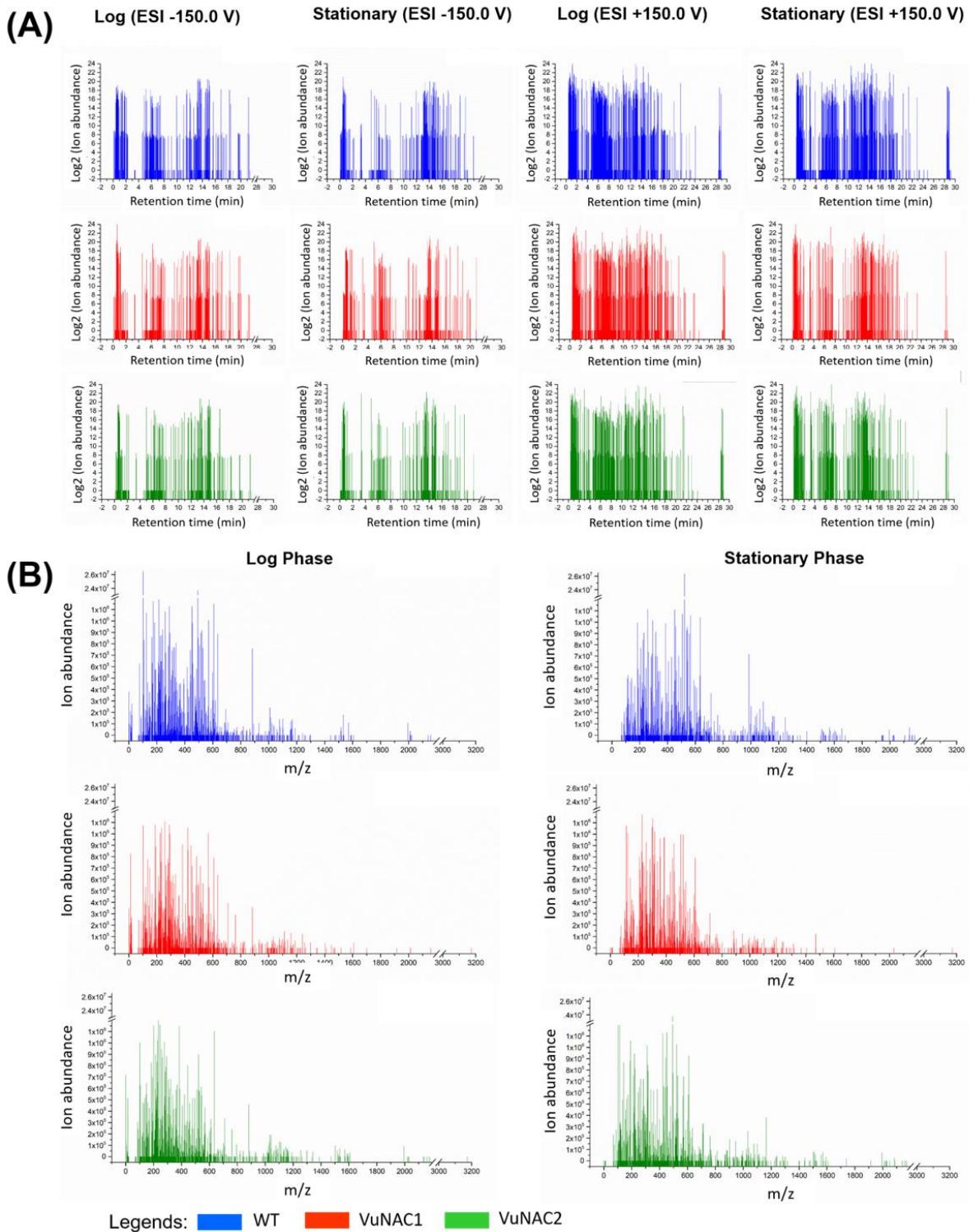
**Table S5. List of FTIR peaks detected in the yeast strains**

Region no.	Assigned peaks ( $\text{cm}^{-1}$ )*	Macromolecule	Functional group
<b>I (3500-3000 <math>\text{cm}^{-1}</math>)</b>	3300 $\text{cm}^{-1}$	polysaccharide alcohol	-OH stretch
	3400-3300 $\text{cm}^{-1}$	aliphatic primary amine	-NH stretch
	3350-3310 $\text{cm}^{-1}$	secondary amine	-NH stretch
	3200-2700 $\text{cm}^{-1}$	alcohol	-OH stretch
	3000-2800 $\text{cm}^{-1}$	amine salt	-NH stretch
	3100 $\text{cm}^{-1}$	amide II	-C=O stretch
<b>II (3000-2820 <math>\text{cm}^{-1}</math>)</b>	2960 $\text{cm}^{-1}$ , 2875 $\text{cm}^{-1}$	lipids	-CH <sub>2</sub> stretch
	2920 $\text{cm}^{-1}$ , 2855 $\text{cm}^{-1}$	lipids	-CH <sub>3</sub> stretch
	2890 $\text{cm}^{-1}$	lipids, proteins, and peptides	-CH deformation
<b>III (2820-1800 <math>\text{cm}^{-1}</math>)</b>	2279-2250 $\text{cm}^{-1}$	isocyanate	-N=C=O stretch
	2239-2225 $\text{cm}^{-1}$	nitrile	-C≡N stretch
	2167-2200 $\text{cm}^{-1}$	thiocyanate	-S-C≡N stretch
	2148-2110 $\text{cm}^{-1}$	azide	-N=N=N stretch
<b>IV (1800-1325 <math>\text{cm}^{-1}</math>)</b>	1747, 1620 $\text{cm}^{-1}$	pectin	-COOH stretch
	1749 $\text{cm}^{-1}$	lipid ester	-C=O stretch
	1652 $\text{cm}^{-1}$	amide I	-C=O stretch
	1620 $\text{cm}^{-1}$	amide I, polypeptides, mannoproteins	-C=O stretch
	1541 $\text{cm}^{-1}$	amide II	-C=O stretch
	1458 $\text{cm}^{-1}$	lipids and proteins	-CH <sub>2</sub> /CH <sub>3</sub> bend
	1419 $\text{cm}^{-1}$	proteins	-COH plane bend
<b>V (1325-750 <math>\text{cm}^{-1}</math>)</b>	1250 $\text{cm}^{-1}$	DNA, RNA, and phospholipids	-PO <sup>-2</sup> stretch
	1215 $\text{cm}^{-1}$	free nucleotides	-CO stretch
	1135 $\text{cm}^{-1}$ , 802 $\text{cm}^{-1}$	mannans and $\beta$ 1,3 glucans	-CO & -C-C stretch
	1001 $\text{cm}^{-1}$	$\beta$ 1,3 glucans	-CO & -C-C stretch
	920 $\text{cm}^{-1}$	monosaccharides (glucose, mannose)	Pyranose ring
	879 $\text{cm}^{-1}$	polysaccharides (starch)	$\beta$ -glycosidic link
	858 $\text{cm}^{-1}$	polysaccharides (starch)	$\alpha$ -glycosidic link

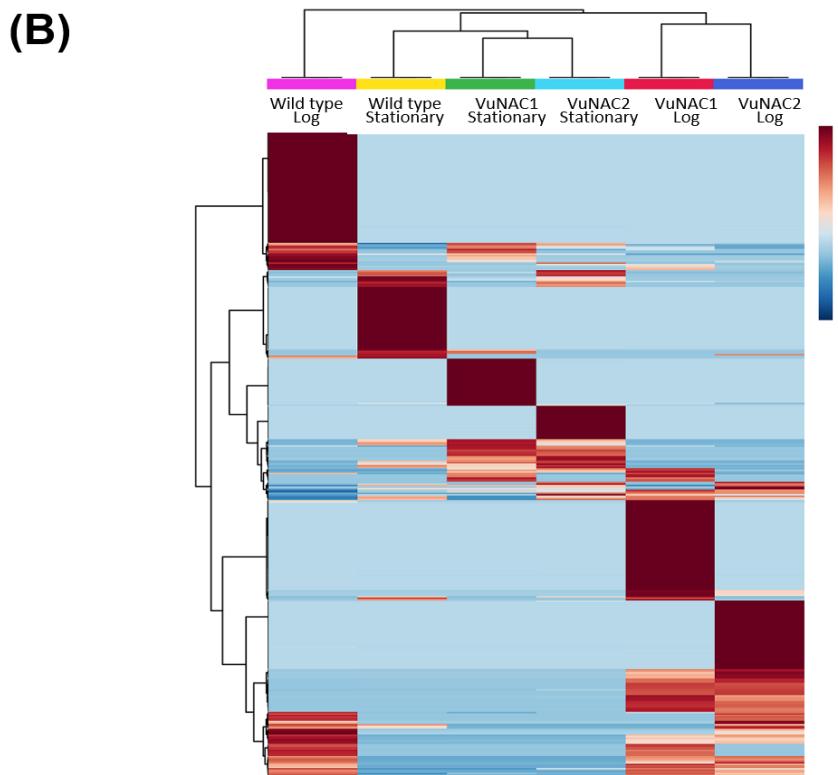
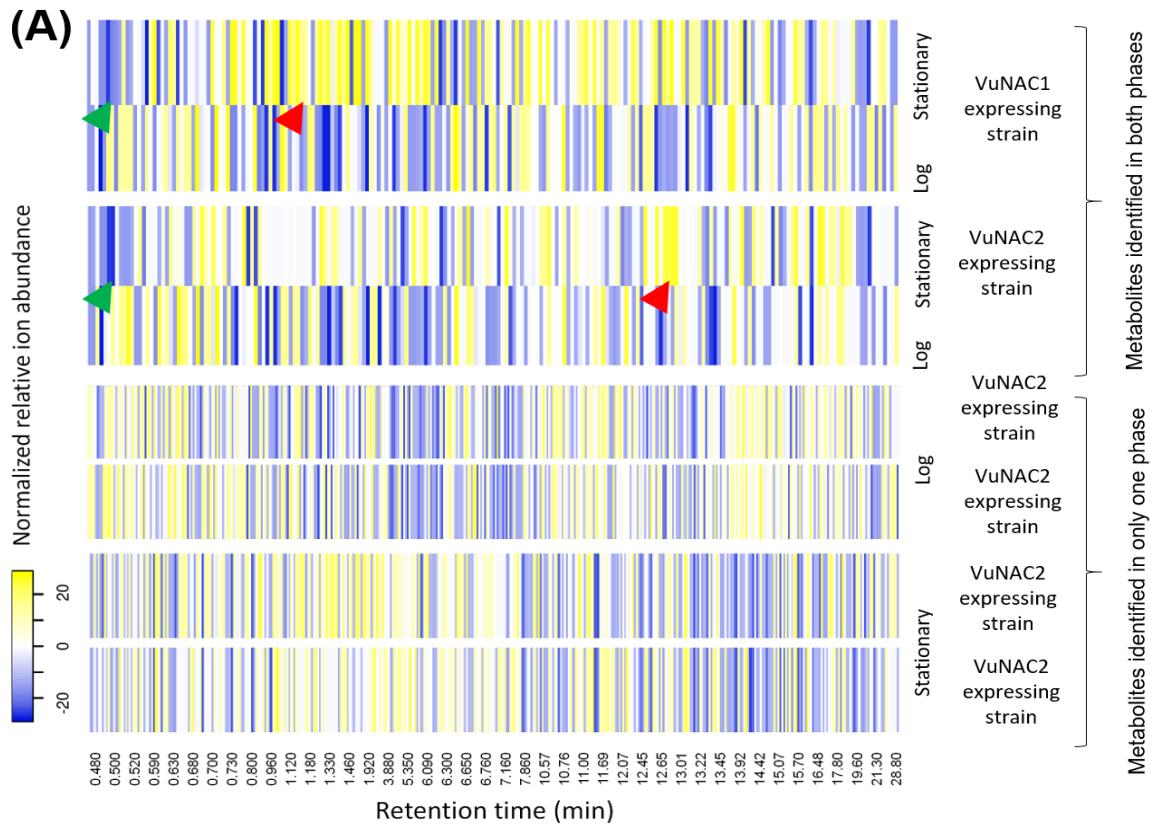
\*Source: Burattini *et al.* 2008, Kochan *et al.* 2018



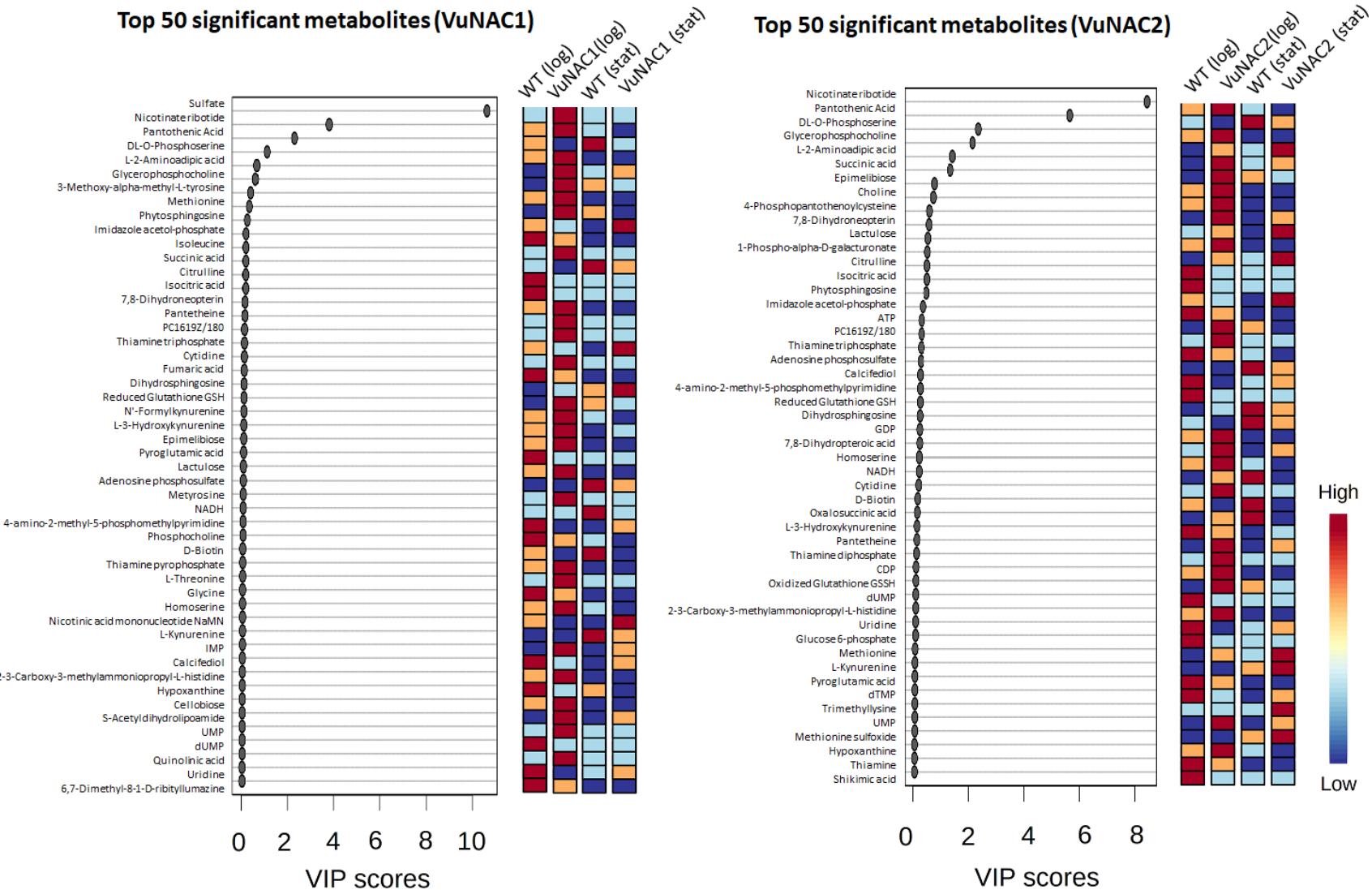
**Figure S6.** Phenotypic analysis in response to salt and cold stress. The transgenic strains exhibited tolerance to NaCl and cold stress as indicated by the viable and dividing cells. Although, after NaCl treatment, the strains showed a reduction in cell size but retained the viability, unlike the wild type strain displaying severe cell death. Also, after the cold arrest, the transgenic strains displayed less cell shrinking than the wild type.



**Figure S7.** LCMS Spectral Plot. (A) Ion abundance vs. retention time. (B) Ion abundance vs.  $m/z$  ratio.

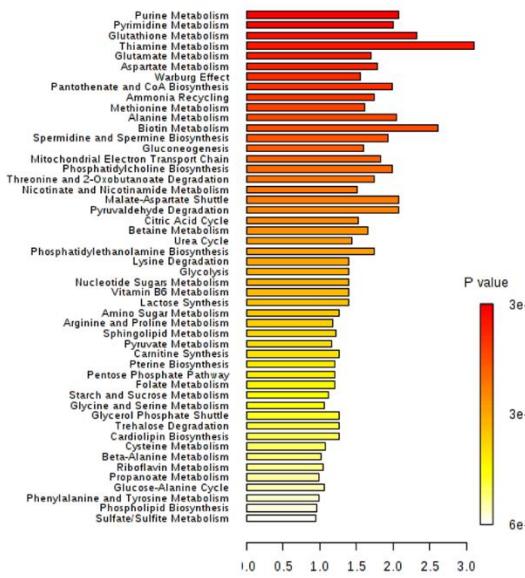


**Figure S8.** Metabolite clustering. (A) Heatmap representing the relative ion abundance (transgenic vs. wild type strain) in the late-log and late-stationary phases, indicating altered regions in the LCMS spectra, increase (red arrow), and decrease (green arrow). (B) Metabolome clustering indicating a close relationship between the profile of VuNAC-expressing strains in different growth phases and between the stationary-phase profile of VuNAC-expressing strains and log-phase profile of the wild type strain.



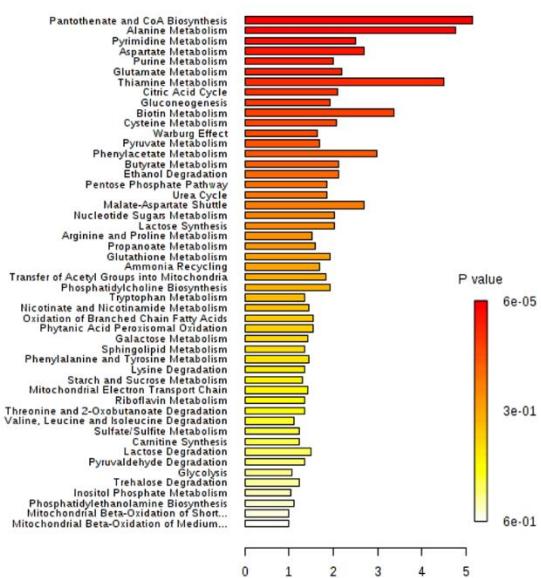
**Figure S9.** Top 50 differentially accumulated metabolites (DAMs) listed in the order of their VIP scores.

### Top 50 pathways altered in log phase

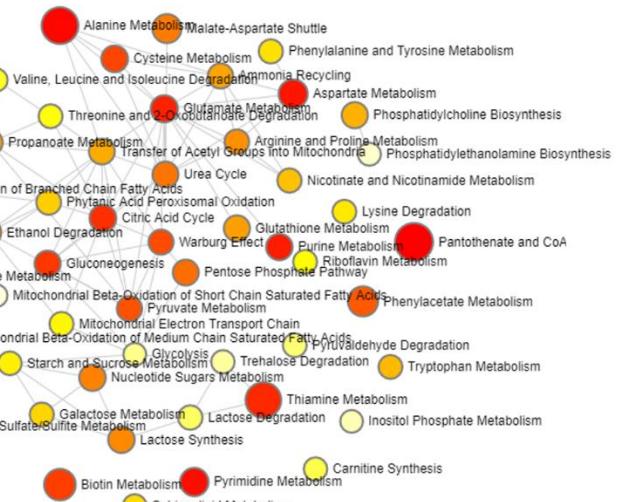
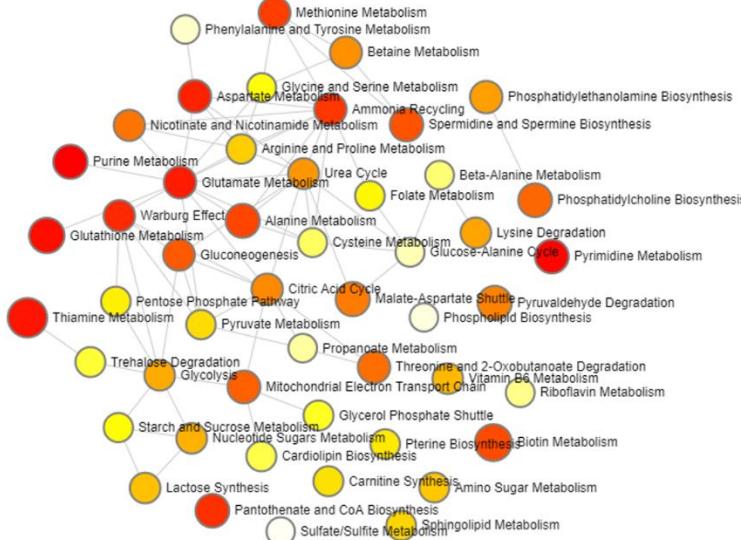


Fold enrichment

### Top 50 pathways altered in stationary phase



Fold enrichment



**Figure S10.** Pathway Enrichment Analysis. Major altered pathways and their networks resulted from the differential accumulation of metabolites in the VuNAC1/2 expressing strains in different growth phases.

**Table S11. Metabolites detected by LCMS and their relative intensities**

S.N.	Compounds	Description	m/z ratio	RT (min)	Fold-change (log phase)		Fold-change (stationary phase)	
					VuNAC1	VuNAC2	VuNAC1	VuNAC2
<b>PYRIMIDINE METABOLISM<sup>1</sup></b>								
1	L-Dihydroorotic acid	de novo pyrimidine precursors	218.1	0.5	-6.71	-6.71	-	-
2	Orotidylic acid		390.2	5.9	-7.74	-7.74	-	-
3	UMP	Salvage pyrimidine and RNA precursors	307.2	10.7	16.23	15.60	0.00	7.69
4	CMP	RNA precursors	345.0	0.7	0.00	7.64	-	-
5	2',3'-Cyclic CMP	Secondary messengers	343.1	0.8	8.22	0.00	-	-
6	CDP	DNA & RNA precursors	445.0	0.7	0.55	8.12	8.14	0.00
7	dUMP	Thymidine precursors	289.2	1.1	-17.80	-17.80	-	-
8	dUDP		388.2	6.5	-15.80	-15.80	7.04	6.95
9	dCDP	DNA precursors (Cytidine)	386.2	11.0	-	-	0.00	15.09
10	dTMP	DNA precursors (Thymidine)	342.2	0.7	-1.99	-9.51	15.68	0.00
11	dTDP		406.2	5.9	-8.02	-8.02	-	-
12	Cytidine	Salvage pyrimidine precursors	281.2	7.3	18.09	17.25	-	-
13	Deoxycytidine		231.1	5.2	7.57	0.00	-	-
14	2'-Deoxyuridine		270.2	14.4	-	-	15.52	7.87
15	Uridine		264.2	4.9	-17.00	-17.00	0.71	0.00
16	Thymidine		288.2	12.5	6.94	0.00	7.12	0.00
<b>PURINE METABOLISM<sup>2,3</sup></b>								
17	ADP-D-ribose	Purine precursor	605.3	5.7	-7.70	-7.70	-	-
18	AIR	de novo purine precursor	333.1	2.9	-8.59	-8.59	-	-
18	AICAR		321.2	0.7	-7.16	-7.16	-	-
19	FAICAR <sup>4</sup>		317.2	6.7	-16.54	-16.54	15.15	0.00
20	Phosphoribosyl formamido carboxamide		404.2	5.9	-7.98	-7.98	15.12	0.00
21	IMP	Salvage purine precursor	408.2	13.7	16.75	15.19	7.83	0.00
22	Hypoxanthine		136.0	0.8	-8.60	0.23	-15.69	-15.69
23	XMP		363.2	14.9	7.35	-0.04	-	-
24	GDP	RNA precursor (Guanidine)	465.2	5.1	0.17	8.99	-	-
25	Adenosine	Salvage purine precursor	287.2	5.4	-8.25	-8.25	-	-
26	ATP	RNA precursor, energy currency	489.2	0.5	7.47	17.89	-9.00	-9.00
27	dATP	DNA precursor (Adenosine)	508.2	6.2	-7.75	-0.62	-7.35	-7.35
28	dADP		410.2	1.1	-8.08	-8.08	16.18	0.00
29	dAMP	Secondary messengers	333.2	2.9	-1.42	-1.31	-	-
<b>VITAMIN B6<sup>5</sup>, THIAMINE<sup>6</sup> &amp; BIOTIN METABOLISM</b>								
30	2-Oxo-3-hydroxy-4-phosphobutanoic acid	Vitamin B6 precursor	214.1	1.4	-8.12	-8.12	-	-
31	Pyridoxine 5'-phosphate	Vitamin B6	269.1	9.6	7.76	14.90	-	-
32	Pyridoxamine 5'-phosphate		230.2	1.9	0.00	8.39	-0.07	-0.16
33	Pyridoxal 5'-phosphate (PLP) <sup>5</sup>		246.2	5.4	-16.47	-16.47	-	-
34	4-amino-2-methyl-5-phosphomethylpyrimidine	Thiamine precursor	218.1	1.2	-19.21	-19.21	16.84	0.00
35	Thiamine <sup>6</sup>	TPP co-enzyme precursor	264.1	0.5	-1.30	-0.72	-	-
36	Thiamine diphosphate	Thiamine derivative, Cofactor	423.3	11.6	15.49	16.55	-	-
37	Thiamine pyrophosphate	TPP co-enzyme	442.3	16.8	8.42	-0.09	-	-
38	Thiamine triphosphate	Thiamine derivative	505.3	5.1	-1.53	-3.23	4.21	-15.72
39	7,8-Diaminononanoate	Biotin precursor	234.2	14.7	-	-	15.76	7.83
40	D-Biotin	Cofactor in carboxylation	244.1	6.0	-7.75	-7.75	-16.98	-16.98
<b>FOLIC ACID METABOLISM<sup>7</sup></b>								
41	2,5-Diamino-6-(5'-phosphoribosylamino)-4-pyrimidineone	Biopterin & Flavin precursor	336.2	16.4	8.71	0.00	-7.41	-0.08
42	2,5-Diamino-4-hydroxy-6-(5'-phosphoribosylamino) pyrimidine		335.2	12.5	0.00	14.14	-	-
43	7,8-Dihydronopterin	Biopterin precursor	254.2	16.5	9.12	9.07	0.00	18.58
44	7,8-Dihydropteroic acid		352.3	18.0	-0.13	8.45	0.00	15.85
45	Tetrahydrofolic acid	Folate derivative	467.4	15.8	7.28	0.00	-	-
46	5,10-Methenyltetra-hydrofolic acid		460.4	18.3	-7.90	-7.90	-8.47	-8.47
<b>RIBOFLAVIN METABOLISM<sup>8</sup></b>								

47	5-Amino-6-(5'-phosphoribitylaminouracil	Riboflavin precursor	338.2	17.8	-0.19	-0.62	-	-
48	2-Hydroxy-3-oxobutyl phosphate		166.1	5.5	-7.77	-7.77	-	-
49	6,7-Dimethyl-8-(1-D-ribityl) lumazine	Riboflavin intermediate	325.3	17.2	-9.04	-0.25	-	-
50	FMN	Cofactor, Riboflavin derivative	456.4	11.3	6.79	0.00	-	-
51	FMNH2			13.0				
			462.3	1	-	-	0.00	15.90
<b>PANTOTHENATE &amp; COENZYME A METABOLISM<sup>9</sup></b>								
52	Pantothenic Acid <sup>9</sup>	Coenzyme A precursor	218.1	1.2	-19.21	-19.21	-5.05	-2.24
53	4-Phosphopantethenoyl cysteine	Coenzyme A intermediate	406.1	3.4	9.22	18.78	0.00	14.70
54	Pantetheine		278.1	6.2	18.31	16.71	0.00	8.46
<b>NAD/NADH BIOSYNTHESIS<sup>10</sup></b>								
55	NaMN	Vitamin B3/NAD precursor	374.2	5.3	-8.65	-8.65	18.39	0.00
56	NADH <sup>10</sup>	Cofactor	665.1	0.8	0.00	8.02	-17.38	-17.38
57	Nicotinamide ribotide	NAD/B3 Salvage precursor	273.3	10.6	0.51	0.33	-7.80	-7.80
58	Nicotinamide	Vitamin B3	103.1	0.5	9.06	0.00	-	-
<b>OTHER VITAMINS</b>								
59	Calcifediol	Involved in UV protection	357.4	14.4	-1.41	-2.15	1.80	1.16
60	Retinylphosphate mannose	Lipid-linked oligosaccharide synthesis	528.2	6.8	-	-	14.02	7.22
<b>GLUTAMATE, ASPARTATE &amp; ALANINE METABOLISM</b>								
61	Glutamate	TCA intermediate, nucleotides & amino acid precursor	147.1	0.6	-8.42	-7.32	-	-
62	L-Aspartic acid	TCA intermediate & nucleotide precursor	132.1	17.0	-8.88	-0.23	-	-
63	L-Aspartyl-4-phosphate	Aspartate derivative, amino acid precursor	273.1	0.5	7.53	0.00	-	-
64	L-aspartic 4-semialdehyde		137.1	2.8	0.00	8.60	-	-
65	Homoserine	Lysine, threonine & cysteine precursor	119.1	0.5	7.03	7.62	-8.76	-8.76
<b>GLUTATHIONE METABOLISM<sup>11</sup></b>								
66	Pyroglutamic acid	Glutathione precursor	167.1	0.8	-19.27	-2.00	-	-
67	Reduced Glutathione (GSH) <sup>11</sup>	Anti-oxidant	307.1	0.7	20.68	20.27	-2.96	-0.17
68	Oxidized Glutathione (GSSH) <sup>11</sup>		612.2	0.7	2.32	2.34	-2.06	-0.44
<b>HISTIDINE, ARGININE &amp; LYSINE METABOLISM</b>								
69	Phosphoribosyl-AMP	Amino acid & secondary metabolite precursor	559.3	12.2	-7.81	-7.93	14.83	0.00
70	Phosphoribulosylformimino-AICAR-P	Histidine Precursor	637.4	16.3	8.58	0.00	-7.24	9.64
71	Imidazole acetol-phosphate		201.1	1.5	-1.03	-1.18	-	-
72	2-(3-Carboxy-3-(methylammonio)propyl)-L-histidine	Histidine derivative	251.1	11.7	8.25	7.96	-	-
73	Ornithine	Arginine biosynthesis, NO	132.1	0.5	-8.81	-8.81	-	-
74	Citrulline	homeostasis	174.1	0.4	-20.16	-20.16	-	-
75	Nitro-L- Arginine <sup>12</sup>	Arginine derivative, NO inhibitor	236.1	1.9	8.30	0.00	-	-
76	L-Glutamic acid 5-phosphate	Proline precursor	228.1	1.5	-7.96	-7.96	-	-
77	1-Pyrroline-5-carboxylic acid	Proline & arginine precursor	117.1	0.7	-0.34	-9.61	-	-
78	L-2-Aminoadipic acid	Lysine precursor	143.1	0.7	20.65	20.32	0.29	0.78
79	Allysine	Lysine catabolism	145.1	0.7	-8.00	-7.69	-	-
80	Saccharopine	Lysine precursor	257.3	11.9	-0.50	-8.47	8.36	0.00
81	Lysine	Nitrogen nutrient, Histone component	146.1	0.5	7.46	8.44	-	-
82	Hydroxylysine	Lysine analog, collagen biosynthesis	222.1	10.9	-	-	7.20	14.44
83	Trimethyllysine <sup>4</sup>	Histone component, Carnitine biosynthesis	188.2	0.5	-	-	8.47	17.21
<b>LINEAR &amp; BRANCHED AMINO ACID<sup>13</sup> METABOLISM</b>								
84	Glycine	Thiamine & amino acid precursor	103.1	0.5	-0.59	0.05	-	-
85	DL-O-Phosphoserine	Serine intermediate, Cysteine Precursor	207.1	0.7	0.79	0.54	-	-
86	AZASERINE	Purine antagonist, glutamine analog	215.1	0.5	-	-	7.63	8.20
87	L-Threonine	Isoleucine precursor	157.1	1.4	16.94	0.00	-	-
88	Isoleucine <sup>13</sup>	Promote fermentation duration	113.1	2.7	18.61	9.24	-	-
89	Homolanthionine	Isoleucine precursor	250.1	0.7	9.11	7.99	-8.65	0.41
90	2-Isopropylmalic acid	Leucine precursor	204.1	0.5	-7.38	-7.38	-8.39	-8.39
91	3-Hydroxyisobutyric acid	Valine metabolite	86.0	0.6	-8.16	-8.20	0.00	16.50
<b>SULFUR AMINO ACID METABOLISM</b>								
92	3-mercaptopyruvate	Cysteine metabolite	124.0	28.9	7.87	0.00	8.40	0.00
93	Methionine <sup>14, 15</sup>	Amino acid, Prolongs log phase	148.1	4.7	19.42	9.28	-8.50	8.91
94	Methionine sulfoxide <sup>16</sup>	Anti-oxidant, Effects life-span	165.1	0.7	-	-	0.41	0.75
95	Sulfate	Sulfur amino acid biosynthesis	98.0	0.6	24.34	0.00	-	-
96	Adenosine phosphosulfate	Sulfur reduction pathway	427.0	0.6	-	-	-1.28	-9.41
<b>AROMATIC AMINO ACID METABOLISM</b>								
97	Shikimic acid	Aromatic amino acid precursor	173.1	1.4	-16.89	-16.89	8.77	0.00
98	Shikimate 3-phosphate		236.1	2.0	-7.62	-7.62	-	-
99	3-Methoxy-alpha-methyl-L-tyrosine	Phenylalanine derivative	225.1	6.5	0.81	-0.09	-	-

Dopamine precursor Inhibition of respiratory growth and survival in yeast								
100	Levodopa <sup>17</sup>		243.1	0.5	-	-	-7.09	-7.09
101	Metyrosine	Tyrosine derivative	177.1	3.1	17.45	0.00	-	-
102	N-Formyl-L-tyrosine		231.2	9.0	0.00	14.01	-	-
103	L-3,5-Diodotyrosine		432.9	0.2	-	-	-6.86	-6.86
104	N'-Formylkynurenine	Tryptophan catabolite	217.2	7.6	9.06	-0.16	-7.05	-7.05
105	L-Kynurenine		254.2	13.4	-	-	-8.38	0.64
106	L-3-Hydroxykynurenine		223.2	9.7	0.08	-0.51	2.03	1.57
107	Quinolinic acid	Folate and pterin precursor	187.1	0.5	16.21	8.35	-	-
108	4-(2-Aminophenyl)-2,4-dioxobutanoic acid	Tryptophan catabolite	189.0	3.9	-	-	0.56	-0.79
PYRUVATE METABOLISM								
109	Pyruvaldehyde	Pyruvate intermediate	71.1	2.7	0.00	8.37	-	-
110	S-Lactoylglutathione		401.4	14.4	7.01	0.00	-	-
111	L-Lactic acid	Pyruvate metabolite	132.1	0.5	-8.81	-8.81	-	-
112	Acetic acid		102.1	6.2	-	-	-0.32	-7.88
113	S-Acetyl dihydrolipoamide	Converts pyruvate into acetyl-CoA	248.1	0.6	16.24	0.00	7.98	7.56
TCA & GLYOXYLATE CYCLE								
114	Oxalacetic acid	TCA cycle metabolite, Carboxylic acid derivatives	114.1	6.2	-	-	-0.59	0.34
115	trans-Aconitic acid		212.1	6.0	0.00	8.70	-9.29	-9.29
116	Isocitric acid		174.1	0.4	-20.16	-20.16	-	-
117	2-Oxoglutaric acid	TCA cycle metabolite, Keto acids derivatives	188.1	7.8	-	-	7.41	0.00
118	Oxalosuccinic acid	TCA cycle metabolite, Carboxylic acid derivatives	189.1	1.0	16.76	8.22	-16.83	-16.83
119	Succinic acid		117.1	0.6	-0.28	10.44	-0.39	-0.65
120	Fumaric acid		115.1	0.6	-9.52	0.13	-	-
121	4-hydroxy-2-oxoglutaric acid	Glyoxylate Precursor, Glutaric acid derivative	190.1	6.1	7.65	0.00	-6.91	-6.91
122	Glyoxylic acid	Glyoxalate	102.0	0.5	-	-	-7.92	-7.92
123	Glycolic acid	Glyoxalate derivative	118.1	1.9	9.34	0.00	-	-
GLYCOLYSIS/ GLUCONEOGENESIS & PENTOSE PHOSPHATE PATHWAY								
124	Beta-D-Glucose	Hexose sugar	202.1	1.4	-9.11	-1.94	-	-
125	Glucose 6-phosphate	Hexose sugar derivative	260.1	0.8	-17.70	-17.70	17.54	0.00
126	D-fructose 1,6-bisphosphate		322.1	0.7	0.00	7.25	-	-
127	D-Glyceraldehyde 3-phosphate	Glycolysis end product	151.0	5.1	-8.63	-8.63	-	-
128	L-Sorbose	Hexose sugar	222.2	12.0	7.50	7.26	-	-
129	D-Glucoside	Hexose sugar derivative	198.1	3.4	9.43	0.00	-	-
130	D-Sedoheptulose 7-phosphate	Heptose sugar derivative	328.0	12.0	0.00	7.93	-	-
131	Cellobiose	Disaccharides	342.3	11.2	8.10	-0.16	-	-
132	Lactulose	Disaccharides, Synthetic Sugar	342.1	0.5	9.92	11.01	-	-
133	D-Ribose 5-phosphate	Pentose sugar, Purine precursor	276.1	6.6	-7.16	-7.16	-	-
134	6-Phosphogluconic acid	Hexose sugar derivative	276.1	0.5	-	-	-7.76	-0.77
GALACTOSE METABOLISM								
135	Melibitol	Disaccharide, Galactose metabolism	361.2	1.1	9.07	0.00	-	-
136	Epimelibiose		388.1	0.5	9.82	11.26	-9.69	-9.69
137	1-Phospho-alpha-D-galacturonate	Galactose derivative	216.1	0.6	9.41	18.29	-15.84	2.85
AMINO SUGAR & NUCLEOTIDE SUGAR METABOLISM								
138	Glucosamine 6-phosphate	Lipid & Nucleoside sugar precursor	259.2	1.3	-10.10	-1.53	-	-
139	Glucosamine	Nucleoside sugar precursor	161.1	0.6	-7.22	-7.19	-	-
140	UDP-glucose	Glycogen precursor, cell-wall beta-glucan synthesis	583.3	6.4	7.37	0.00	15.68	0.00
141	UDP-GlcNAc	Cell-wall glycan synthesis, Coenzyme precursor	607.1	0.6	9.44	9.37	-	-
142	UDP-D-Xylose	Nucleoside sugar	596.1	0.7	-	-	7.67	0.00
143	UDP-N-acetyl-D-galactosamine	Nucleoside sugar, Cell wall precursor					0.00	16.57
LIPID METABOLISM								
144	Choline	Phosphatidyl choline precursor	103.1	0.5	-8.01	1.74	-	-
145	Phosphocholine	Represses Phospholipid biosynthesis	183.1	13.0	-1.66	-0.25	-0.73	0.08
146	Glycerophosphocholine <sup>18</sup>	Membrane lipid homeostasis, salt tolerance	257.1	0.6	2.84	2.31	-0.26	1.89
147	PC(16:1(9Z)/18:0)	Glycerophosphocholines, C16, C18	761.1	0.7	18.15	17.87	-	-
148	GPCho(O-16:0/2:0)	C16, C2, Membrane phospholipid, activator	523.4	15.9	10.71	0.78	8.78	9.64
149	GPCho(16:0/0:0)	Glycerophosphocholines, C16	495.3	14.0	-	-	-3.36	-1.59
150	GPEtn(18:0/0:0)	Glycerophosphorylethanolamine, C18	541.3	14.4	8.11	8.08	-7.73	-7.73
151	GPEtn(16:0/0:0)	Glycerophosphorylethanolamine, C16	513.3	13.2	8.88	8.13	-10.30	-0.73
152	GPEtn(18:1(9Z)/0:0)	Glycerophosphorylethanolamine, C18	479.3	13.2	-1.56	-1.11	-9.27	-9.27
153	GPSer(16:0/0:0)	Glycerophosphoserine, C16	497.3	14.4	10.33	7.77	18.63	18.38
154	GPSer(18:1(9Z)/0:0)	Glycerophosphoserine, C18	523.3	14.9	-7.88	0.60	-8.58	-8.42
155	GGPGro(18:0/0:0)	Glycerophosphoglycerol, C18	529.3	17.0	-0.19	-8.80	-7.24	-7.24

156	GPA(17:1(9Z)/0:0)	Glycerophospholipid, C17	485.3	11.6	-1.29	-9.42	-	-
157	GPA(21:4(6Z,9Z,12Z,15Z)/0:0)	Glycerophospholipid, C21	531.3	14.7	-	-	0.21	1.57
158	Dihydrosphingosine	Sphingolipid precursor	301.3	11.9	0.15	-1.06	1.92	-0.40
159	Phytosphingosine	Sphingolipid precursor	317.3	12.0	-0.82	-1.07	19.96	19.57
160	C17 Sphinganine	Sphingoid, toxic to growth	287.3	10.9	-8.43	-2.24	8.14	0.23
161	Ganglioside GM3 (d18:1/12:0)	Sphingolipid	1078.6	7.9	14.66	14.79	-	-
162	N-Acetyl-D-glucosaminylidiphosphatidichol	Glycan precursor, Prenol lipid	587.2	14.3	15.48	0.00	7.71	7.55

#### FATTY ACID METABOLISM

163	13,14-dihydroxy-docosanoic acid	Hydroxy FA, C22, Behenic acid derivative	371.3	14.8	-0.83	-0.51	0.00	7.27
164	3-hydroxy behenic	Hydroxy FA, C22, Behenic acid derivative	373.4	11.1	-	-	1.01	9.42
165	18-hydroxy-9S,10R-dihydroxy-stearic acid	Hydroxy FA, C18, Membrane lipid precursor	314.3	16.9	7.83	-0.20	0.00	8.86
166	11-methyl-octadecanoic acid	Methyl FA, C18, membrane lipid precursor	297.3	20.1	-7.81	-7.83	-	-
167	13-hydroxy stearic acid	Hydroxy FA, C18	282.3	14.6	-	-	-8.81	-8.41
168	Hexadecanedioic acid	FA, C16, Membrane lipid precursor	285.2	13.8	8.40	8.74	-	-
169	7-palmitoleic acid	Unsaturated FA, C16	236.2	16.5	8.26	0.00	16.65	8.35
170	3-hydroxy-hexadecanoic acid	Hydroxy FA, C16, intermediate in FA synthesis	254.2	13.2	-0.82	7.28	-	-
171	1-Hexadecyl-2-O-methyl-glycerol	Methyl FA, C16, Inhibits growth	329.3	13.1	-0.89	-1.64	1.63	0.88
172	Isopentadecylic acid	FA derivative, C15	241.2	10.8	-	-	8.73	0.63
173	1-tetradecanol	FA alcohol, C14	213.3	11.1	-	-	1.54	1.67
174	2-methyl-tridecanedioic acid	Methyl FA, C13	257.2	12.1	10.07	10.15	0.00	15.35
175	13-amino-tridecanoic acid	Amino FA, C13	228.2	13.3	-	-	9.38	10.29
176	3-Hydroxydodecanedioic acid	Hydroxy FA, C12	268.1	13.6	8.56	-0.07	8.13	8.09
177	6-methyl-dodecanedioic acid	FA derivative, C12	226.2	10.0	7.13	7.13	-	-
178	4,8-dimethyl dodecanoic acid	Methyl FA, C12	245.2	9.3	-	-	1.08	0.02
179	4-hydroxy lauric acid	Hydroxy FA, C12	233.2	7.9	-	-	0.77	-1.21
180	4-hydroxy-undecanoic acid	Hydroxy FA, C11	224.1	11.0	-8.54	-8.54	-	-
181	3-Hydroxysuberic acid	Hydroxy FA, C8	232.1	0.5	-16.10	-16.10	-	-
182	a-hydroxybutyrate	Hydroxy acid, C4, Alternate energy source	104.1	0.5	8.77	0.00	-	-
183	Carnitine <sup>19</sup>	Carnitine, FA oxidation	143.1	1.0	7.81	8.62	-16.49	-16.49

#### OTHER METABOLITES

184	N-Acetyl-D-mannosamine 6-phosphate	Sialic acid precursor	347.1	0.5	8.42	7.71	-	-
185	N-Acetyl-9-O-lactoyl neuraminic acid <sup>20</sup>	Sialic acid, protects from phagocytosis reduces cell-adhesion, Increases (-) charge	363.1	5.2	8.43	0.00	1.17	0.45
186	N-Acetyl-8-O-methyl-neuraminic acid		305.1	1.4	-0.29	6.96	-	-
187	Peonidin	Anthocyanin, Dephinidin derivative	305.2	9.3	-6.88	-6.88	16.49	8.37
188	Peonidin 3-O-glucoside	Anthocyanin	467.4	16.4	6.09	-9.66	-	-
189	4-Vinylguaiacol		172.2	9.8	6.98	-0.63	-	-
190	Petunidin		317.3	10.7	0.01	9.77	-	-
191	Alloxanthin	Carotenoid, Anti-oxidant & vitamin precursor	584.3	17.0	7.94	7.55	-	-
192	Engeletin	Flavonols, Inhibits ROS, NO & hypoxia signalling anti-inflammatory influence energy metabolism	456.4	11.5	15.12	0.00	-	-
193	Kaempferol <sup>21</sup>	Flavonols, anti-inflammatory and antioxidant	324.2	13.4	9.55	9.68	-	-
194	Quercetin	Flavonols, Anti-oxidant	285.2	14.3	0.00	7.94	8.27	-8.15
195	Catechin <sup>22</sup>	Flavanols, Alcohol tolerance, Toxin resistance	289.3	10.8	-0.07	-0.51	1.66	1.30
196	Piceatannol	Stilbenes	272.2	14.2	16.05	15.83	-	-
197	benzil		256.2	19.5	-	-	0.12	8.88
198	Resveratrol	Stilbenes, Promote lipid metabolism	227.3	11.2	-0.31	-8.08	-	-
199	Cucurbitacin E	Protects from autophagy & oxidative stress	598.3	15.1	-1.98	-10.51	-	-
200	Epinephrine-like	Hormone, promote glycogen break	165.1	1.2	22.23	22.02	0.90	0.42
201	Indole-3-carboxylic acid-like	Auxin, promotes sporulation	143.0	3.9	0.65	-8.80	18.18	8.20
202	1H-IAA, 5-[[[methylamino]sulfonyl]methyl]- glucuronide-like	Auxin derivative	440.1	0.6	1.56	-8.24	-	-
203	Kinetin riboside-like	Kinetin derivative promotes cell proliferation	347.1	5.9	8.06	7.97	-	-
204	Kinetin-like	Kinetin, promotes cell proliferation	215.1	3.7	-	-	19.02	8.30
205	Abscisic acid-like	ABA	246.1	1.0	3.29	-5.16	-	-
206	Tuberonic acid-like	JA derivative, defense and signaling	208.1	13.6	8.08	-0.10	-	-
207	2-phenylethanol	Aromatic compounds, Inhibits growth	142.1	1.3	-17.81	-17.81	-	-
208	Gallic acid	Gallic acid, Secondary-metabolite	192.1	1.4	-18.02	-18.02	-	-
209	4-Hydroxyphenylethanol	Aromatic compounds, Antioxidant	120.1	6.3	16.44	0.00	-	-
210	Indole-3-ethanol	Aromatic compounds, Quorum sensing	161.1	7.7	15.73	0.00	-	-
211	Terephthalic acid	Aromatic compounds, Fermentation product	148.0	11.3	8.72	9.06	-	-
212	Benzyl alcohol		90.1	1.1	-1.22	8.04	-	-
213	Benzanthrone	Aromatic compounds, Yellowish color	212.1	6.2	18.93	18.47	-	-

214	4-hydroxystyrene	Aromatic compounds, Fermentation product	102.1	1.2	-	-	11.33	8.71
215	P-hydroxycinnamaldehyde	Apoptosis inducer, NO synthase inhibitor	148.1	1.2	-	-	18.03	17.48
216	Cinnarizine	Drug, Anti-histamine, calcium signal blocker	410.2	14.8	-	-	7.86	7.91
217	Flumazenil	Drug, Benzodiazepine antagonist, inhibits GABA signaling by shifting carbon metabolism toward respiration, as calorie restriction does.	303.1	0.5	-	-	7.03	7.76
218	5-Hydroxydopamine <sup>23</sup>	Drug, Inhibits respiratory growth, counteracts with ascorbate and GSH	151.1	0.7	-8.99	-8.89	-	-
219	Dihydrolevobunolol glucuronide	Drug, beta-adrenergic signal blocker	469.2	13.3	-	-	7.25	14.70
220	Penbutolol glucuronide	Drug, beta-adrenergic signal blocker	509.3	13.8	-	-	16.13	7.50
221	Miglitol <sup>19</sup>	Drug, Inhibit breakdown of complex carbohydrate	189.1	1.2	-	-	-10.17	-0.63
222	Sulfasalazine	Drug, Inhibits biopterin biosynthesis	440.1	0.5	-	-	-3.05	-1.74
223	Sulfamerazine	Drug, Inhibits folic acid synthesis	246.1	0.5	-	-	-9.98	-9.98
224	Flumequine	Drug, Anti-lipase, growth inhibition	307.1	0.7	-	-	-8.14	-8.14
225	Methacholine	Drug, Inhibits autophagy, promotes log growth	141.1	1.0	7.83	7.94	-	-
226	N-(3-oxo-hexanoyl)-homoserine lactone <sup>24</sup>	Quorum sensing, Oxidative stress tolerance	212.1	0.7	8.35	16.27	-	-
227	Metergoline	Alkaloid, Induces cell death	423.2	1.9	-8.62	-9.48	-	-

**Table S12. The CDS sequences encoding the VuNAC1 and VuNAC2 TF**

<b>VuNAC1</b>	
<b>NCBI Gene ID</b>	<b>114163399</b>
<b>&gt;VuNAC01 [Vigna unguiculata] cultivar Kanannado NAC1 protein gene, complete mRNA</b>	
ATGGCATCAGAGCTTCATTGCCCCAGGCTCAGATTCCATCCAACGGACGAGGAGCTGTGATGCACCA CCTCTGCCCAAATGCGCGTGCAGCCATGCCGTTCCATCATGCCGAAATGACCTCTACAAATACGA CCCTTGGGACCTCCCAGGATTGGCTTATGGAGAGAAAGAGTGGTACTTCTTTACCACGGGACCGGA AGTACCCCTAACGGTCAGGCCAACCGGGCGGGAACCGTTACTGGAAGGCAACCGGGCGGATA AGCCCATTGGTCACCCGAAACCGGTTGGATCAAGAAAGCTTGGTGTACGCTGGAAAGCTCGAAA GGGGACAAAAGCAATTGGATCATGCACGAGTATCGTCTGGCTGATGTAGATCGCACCGTCGAAAAAGA ACAGCTTAAGGTTGGATGATTGGTGCTGTGCGTATCTACAACAAGAAGGGCACGATCGAGAAATTACA ACCGACCAGCGACGTAGCTGAGCCGAAAATCGAATCCCCGAGATCGAAGAGAAGAACCGGAGAT TCTGAAAAGCGGAGGAGTTCTCCGCCGCCGGCGATGACGGACTACATGTACTCGATCCGTCGGATT CAATCCGAAAGCTGACACGGACTCGAGCTGTTGGAGCAGGTGGTTGCCGGAATTGCTAGCGAGGT GCAGAGCGAGCCGAAGTGGAACGAGTGGAGAAGAGCCTGGATTTCTACATGGACACCACCACT CTGAGCAACGGCTTCACCCATTGCGACCAATAACACCACCAATAATAATAATCAGATGTCG CCGCTGCAGGACATGTTACTGGCCAAGCCCTTTGA	
<b>VuNAC2</b>	
<b>NCBI Gene ID</b>	<b>114178342</b>
<b>&gt;VuNAC02 [Vigna unguiculata] cultivar Kanannado NAC2 protein gene, complete mRNA</b>	
ATGCAAGGAGAATTGGATTACCAACCCGGGTTAGATTCCACCCACTGACGATGAACTCGTAATCACTA CTTGTGTAGAAAGTGTGCTGCTCAATCCATTGCCGCTCCATCATCAAAGAAATGATTGTATAAGTTGA TCCATGGCAGCTTCAGACATGGCTTTACGGTGAGAAAGAGTGGTACTTTCTCCCTCGTGACCGAAA ATACCCTAACGGTCACGACCGAACCGGGCGCGGGAACCGGCTACTGGAAGGCCACCGGAGCGGATAAA CCGATTGGAAAACCGGAAGCCGTGGAATTAGAAAGCCCTGTGTTACGCCGGAAAGCCCCGAAAG GAGTCAGACAAATTGGATCATGCACGAATATCGCTGGCCAACGTCGATAGATCTGCCCTCAAGAAAAC AACAAATTGAGGCTTGATGATTGGTGCTATGTCGGATTACAACAAGAAAGGGAAAGATTGAGAAGTACA ATAATGTCGACGGGGTGGTGGAACAGAAACCGCGAAATTACCGGAGGAGATTCTGTTCCAGCACGAGAT GAAGCCTGAGATCCAGATGTACGACCACGATCATTCAAGAACCAATTGTACATGGACACGTCGGATT CGGTGCCGAGGTTAACACGGACTCTAGCTGCTCCGAGCACGTGGTTGCCGGACGGCACCTGCGAGAA GGAGGTGCAGAGCGAGCCAAAGTGGAACGACCTGGAGTTGGGCCGGACCTGGTTGGCTACGATTT AACTTCATGGAGCTATCAGCAGATGACGCTTTGCTCTCAGGCCAATACCAAATGAACCAGCCCTCGACC TGGCAAGATATGTCGCGTACCTTCCGAAGACATTAA	

**Table S13. List of primers used in the study**

Purpose		Name	Sequence
<b>Gene isolation</b>	Forward	NAC_Degenerate_1	5'-GGIGsIAsIGAIyTICArYTIICCICC-3'
	Reverse	NAC_Degenerate_1	5'-TCIyTICwyTTIsGyTCIGwyTGIACyTC-3'
	Forward	NAC_Degenerate_2	5'-CCIGGITTymGITTyCAyCCIACIGAyGA-3'
	Reverse	NAC_Degenerate_2	5'-TTIsGyTCIGwyTGIACyTCrswrGCrAAITC-3'
<b>3' RACE PCR</b>	Reverse	Adapter primer	5'-CTGATCTAGAGGTACCGGATCCTTTTTTTTTTTTT-3'
	Forward	VuNAC1-partial-1	5'-CGTCTGGCTGATGTAGATCGCA5'-3'
	Forward	VuNAC1-partial-2	5'-TTGGTACCTCGCACCGTCGCAAAAAGAAC-3'
<b>5' RACE PCR</b>	Forward	Universal primer	5'-CTGATCTAGAGGTACCGGATCC-3'
	Reverse	VuNAC2-partial	5'-CGGGTGGTAATTCCAATTCTCC-3'
	Reverse	VuNAC2-partial	5'-TTCCAATTCTCCTTGCATT CGC-3'
<b>Full-length gene cloning</b>	Forward	VuNAC1-FL-SfiA	5'-catttGGCCaaatcGGCATGGCAGAGCTTCATT-3'
	Reverse	VuNAC1-FL-SfiB	5'-AGAACGGCCtttatGGCCTCAAAGGGCTGGGCCA-3'
	Forward	VuNAC2-FL-SfiA	5'-catttGCCaaatcGCCATGCAAGGAGAATTGGAATTACC-3'
	Reverse	VuNAC2-FL-SfiB	5'-AGAACGGCCtttatGGCCTAAATGTCTCGGAAGGTACG-3'
<b>Over-lapping PCR GFP fusion</b>	Reverse	VuNAC1ΔstopGFP	5'-GCCCTTGCTCACCATAAAGGGCTGGGCCAGTACA-3'
	Reverse	VuNAC2ΔstopGFP	5'-GCCCTTGCTCACCATAAATGTCTCGGAAGGTACG-3'
	Forward	GFP-FL	5'-ATGGTGAGCAAGGGCGAGGAGC-3'
	Reverse	GFP-FL-SfiB	5'-AGAACGGCCtttatGGCCTTACTGTACAGCTCGTCCA-3'
<b>Yeast expression</b>	Forward	VuNAC1_Y2H_NdeI	5'-GGTGGTCATATGATGGCATCAGAGCTTCATT-3'
	Reverse	VuNAC1_Y2H_BamHI	5'-TTATATGGATCCTCAAAGGGCTGGGCCAGTA-3'
	Forward	VuNAC2_Y2H_NdeI	5'-GGTGGTCATATGATGCAAGGAGAATTGGAATTACC-3'
	Reverse	VuNAC2_Y2H_BamHI	5'-GGTGGTGGATCCTAAATGTCTCGGAAGGTAC-3'
	Reverse	VuNAC1Δ_Y2H_BamHI	5'-TTATATGGATCCTCATAATTCTCGATCGTGCCCT-3'
	Reverse	VuNAC2Δ_Y2H_BamHI	5'-GGTGGTGGATCCTTACTCTCAATCTCCCTTCTGT-3'
<b>Protein expression</b>	Forward	VuNAC1_pET_BamHI	5'-TTATATGGATCCATGGCATCAGAGCTTCATT-3'
	Reverse	VuNAC1_pET_Sall	5'-TTATATGTCGACTCAAAGGGCTGGGCCAGTA-3'
	Forward	VuNAC2_pET_BamHI	5'-TTATAAGGATCCATGCAAGGAGAATTGGAATTACC-3'
	Reverse	VuNAC2_pET_Xhol	5'-TTATATCTCGAGTTAAATGTCTCGGAAGGTACG-3'

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