

## Supplementary information

# Multi-omics analysis reveals the mechanism of torularhodin accumulation in the mutant *Rhodospiridium toruloides* A1-15 under nitrogen-limited conditions

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**Table S1. Sample sequencing data evaluation results and statistical results of samples mapped to genome**

Sample	Raw Reads	Raw Bases	Clean Reads	Clean Bases	Q30	GC	Total reads	Total mapped reads	Multiple mapped	Uniquely mapped	Non-splice reads	Splice reads	Reads mapped in proper pairs
A01	42.07 M	6.31G	41.47 M	6.08G	0.957 6	0.6324	41466530	40454242 (97.56%)	360699 (0.87%)	40093543 (96.69%)	23357491 (56.33%)	16736052 (40.36%)	39363712 (94.93%)
A02	43.88 M	6.58G	43.25 M	6.33G	0.957 4	0.6327	43245102	42155890 (97.48%)	379109 (0.88%)	41776781 (96.60%)	24880874 (57.53%)	16895907 (39.07%)	41013908 (94.84%)
A03	42.53 M	6.38G	41.93 M	6.14G	0.957 5	0.6325	41934212	40892189 (97.52%)	365537 (0.87%)	40526652 (96.64%)	23738264 (56.61%)	16788388 (40.04%)	39771280 (94.84%)
N01	44.14 M	6.62G	43.52 M	6.38G	0.957 6	0.6317	43521768	42452156 (97.54%)	381427 (0.88%)	42070729 (96.67%)	24621298 (56.57%)	17449431 (40.09%)	41329990 (94.96%)
N02	48.07 M	7.21G	47.37 M	6.94G	0.957	0.6317	47368770	46182815 (97.50%)	423916 (0.89%)	45758899 (96.60%)	26705514 (56.38%)	19053385 (40.22%)	44922038 (94.83%)
N03	47.69 M	7.15G	46.97 M	6.86G	0.957 2	0.6317	46974854	45786480 (97.47%)	415092 (0.88%)	45371388 (96.59%)	26771697 (56.99%)	18599691 (39.59%)	44513678 (94.76%)

**Table S2. Abbreviations in Figure 3c.**

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G-1-P	glucose-1-phosphate
G-6-P	glucose-6-phosphate
F-6-P	fructose-6-phosphate
F-1,6-2P	fructose-1,6-bisphosphate
G-3-P	glyceraldehyde 3-phosphate
G-1,3-2P	glycerate-1,3-bisphosphate
3-PGA	3-phosphoglyceric acid
2-PGA	2-phosphoglyceric acid
PEP	phosphoenolpyruvate
OAA	oxaloacetate
DHAP	dihydroxyacetonephosphate
Glycerol-3-P	glycerol 3-phosphate
Acyl-DHAP	acyl dihydroxyacetonephosphate
Lyso-PA	lysophosphatidic acid
PA	phosphatidic acid
CDP-DAG	cytidine diphosphate-diacylglycerol
PGP	Phosphatidylglycerophosphate
PG	Phosphatidylglycerol
PC	phosphatidylcholine
PE	phosphatidylethanolamine
DG	diacylglycerol
TG	triacylglycerol
FFA	free fatty acid

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**Table S3. Genes used in this work and their expression in NP11 and A1-15 under nitrogen limitation.**

Classification	Gene Name	GeneID	Annotation	Raw data						Fold change	Statistics test
				Expression _N01	Expression _N02	Expression _N03	Expression _A01	Expression _A02	Expression _A03	A/N	P-value
Central nitrogen metabolism and amino metabolism	<i>GDH1</i>	RHTO_04650	glutamate dehydrogenase (NADP+)	972.1543	930.9495	945.3012	885.2153	810.0551	847.3380	1.0030	0.9558
	<i>GLN1</i>	RHTO_00673	glutamine synthetase	2174.8045	2308.3914	2086.9063	2251.0520	2170.4779	2218.2720	1.1365	0.0746
	<i>GDH2</i>	RHTO_07718	glutamate dehydrogenase (NAD+)	65.9541	82.1651	72.7710	59.8273	109.6944	64.6140	1.2225	0.3239
	<i>GLT2</i>	RHTO_00025	GOGAT, glutamate synthase	224.1174	204.4633	231.9863	225.6536	265.9902	236.9154	1.2511	0.0329
	<i>GLN2</i>	RHTO_00401	glutamine synthetase	111.0628	114.6364	104.2093	87.9092	62.7818	83.7777	0.7908	0.0009
	<i>MEP1</i>	RHTO_01846	ammonium transmembrane transporter	161.2949	163.8978	165.0777	154.7421	150.9260	151.4269	1.0496	0.4650
	<i>MEP2</i>	RHTO_01680	ammonium transmembrane transporter	1538.5418	1540.2357	1525.2481	1942.3939	1712.5009	1940.9285	1.3626	0.0000
	<i>AAT</i>	RHTO_01882	amino acid transporter	491.6130	480.7860	475.2991	273.8415	229.5760	269.2025	0.5973	0.0000
	<i>NAAP</i>	RHTO_04969	neutral amino acid permease	62.9711	66.5847	64.8391	94.1434	118.6521	99.8907	1.8271	0.0000
	<i>DAAP</i>	RHTO_07825	dicarboxylic amino acid permease	85.5700	85.5951	82.7376	150.8974	136.0753	146.2100	1.9147	0.0000
	<i>AATT1</i>	RHTO_01079	amino acid transmembrane transporter	12.8024	13.3916	12.2065	79.8322	64.6164	79.0022	6.5008	0.0000
	<i>AATT2</i>	RHTO_07915	amino acid transmembrane transporter	511.1890	505.2897	515.7411	611.8914	607.5900	568.7076	1.3162	0.0003
	<i>ILVD</i>	RHTO_08062	dihydroxy-acid dehydratase	2.8637	2.4117	3.0829	1.2313	1.1670	1.3788	0.5075	0.0000
	<i>ACADM</i>	RHTO_04971	medium-chain-acyl-CoA dehydrogenase	81.9498	85.3991	74.8740	147.9645	146.8659	145.8739	2.0478	0.0000
	<i>ACADSB</i>	RHTO_03594	short/branched chain acyl-CoA dehydrogenase	106.4742	116.4739	105.4993	146.5585	175.7246	141.9770	1.6040	0.0001
	<i>UBC</i>	RHTO_06133	ubiquitin C	1334.5282	1541.0297	1203.5526	1479.9325	2282.2610	1601.5290	1.5041	0.0168
	<i>VCP</i>	RHTO_04629	transitional endoplasmic reticulum ATPase	300.7434	322.3281	302.0208	321.9729	442.7580	331.2965	1.3514	0.0335
	<i>PEAP</i>	RHTO_06300	pepsinogen A2 precursor	69.7921	67.0134	66.8421	98.7585	87.7564	105.9765	1.6091	0.0000
	<i>CTSD</i>	RHTO_06029	aspartic-type endopeptidase	31.9159	31.8148	32.7802	43.6260	31.6372	42.4967	1.3595	0.0000
	<i>CYC1</i>	RHTO_03214	cyclin	65.5856	84.4208	75.3995	185.6903	338.9104	150.0311	3.4693	0.0000
<i>CYC2</i>	RHTO_03715	cyclin	46.5475	44.5625	48.5517	87.8704	90.7758	96.9194	2.2233	0.0000	
<i>CPLC1</i>	RHTO_04254	G1/S-specific cyclin PLC1	187.3548	180.4238	178.8758	258.0211	498.3947	269.9128	2.1737	0.0002	
<i>CPHO80</i>	RHTO_08156	protein of Cyclin PHO80-like family	33.2761	31.5015	34.3722	36.8932	54.4126	42.6796	1.5440	0.0033	
Lipid metabolism	<i>GPD1</i>	RHTO_08013	glycerol-3-phosphate dehydrogenase complex	40.0447	36.8237	37.8821	43.5414	38.2444	38.0771	1.1730	0.0212
	<i>GPD2</i>	RHTO_02273	glycerol-3-phosphate dehydrogenase (NAD+)	84.2417	86.0734	81.5150	110.4809	91.0125	110.1786	1.3837	0.0000
	<i>GPD3</i>	RHTO_07665	glycerol-3-phosphate dehydrogenase	47.0044	47.8223	47.1454	53.3141	93.6148	59.3641	1.6731	0.0059
	<i>GNPAT1</i>	RHTO_06391	glyceronephosphate O-acyltransferase	23.5573	22.8437	24.6652	21.5173	19.2865	21.5831	0.9848	0.7758
	<i>GAT1</i>	RHTO_03058	glycerol-3-phosphate O-acyltransferase / dihydroxyacetone phosphate acyltransferase	235.7290	238.9775	242.5696	287.3374	296.1247	279.2324	1.3575	0.0001
	<i>AYR1</i>	RHTO_03193	1-acylglycerone phosphate reductase	20.5670	17.7116	16.9012	14.1669	10.3616	17.4528	0.8461	0.1768
	<i>SLC1</i>	RHTO_06718	1-acylglycerol-3-phosphate acyltransferase	111.6805	105.8472	111.8162	129.7742	96.8213	121.9597	1.1810	0.0013
	<i>GPSGA</i>	RHTO_04998	CDP-diacylglycerol-glycerol-3-phosphate 3- phosphatidyltransferase	24.9630	25.1103	26.0179	29.9602	46.7802	30.9398	1.6235	0.0044
	<i>GEP4</i>	RHTO_07538	protein of HAD-superfamily phosphatase, subfamily IIIA	75.7830	88.0527	76.3342	98.2009	222.7285	102.4141	2.0532	0.0038
	<i>FAS1</i>	RHTO_02032	fatty acid synthase subunit beta, fungi type	162.5456	145.8220	157.6016	192.6041	188.8712	197.6613	1.3994	0.0000
	<i>FAS2</i>	RHTO_02139	fatty acid synthase subunit alpha, fungi type	168.2326	155.8607	174.7382	226.5294	244.8430	238.7609	1.6089	0.0000
	<i>ACP1</i>	RHTO_02545	[acyl-carrier-protein]	123.9665	139.3824	127.3351	164.4029	251.5114	177.8251	1.7395	0.0006
	<i>ACP2</i>	RHTO_01048	acyl-carrier protein	671.0330	689.9716	643.1398	601.2855	541.7157	614.5920	0.9832	0.7619

	<i>ACCI</i>	RHTO_02004	acetyl-CoA carboxylase	191.2040	173.7774	196.6000	243.2968	243.5509	249.2246	1.4775	0.0000
	<i>TGL1</i>	RHTO_00993	triacylglycerol lipase	54.2179	50.4508	54.8184	58.5481	44.4611	51.4110	1.0821	0.1455
	<i>TGL2</i>	RHTO_06751	lipase 2	30.1829	29.5103	28.8881	25.9857	30.1486	26.6715	1.0586	0.5868
	<i>ATG15</i>	RHTO_00361	triacylglycerol lipase ATG15-like protein	103.9339	99.5988	106.0200	96.4925	79.5869	92.6941	0.9719	0.4676
	<i>FAA1</i>	RHTO_00058	long-chain acyl-CoA synthetase	103.8543	104.6253	99.7944	123.6782	110.8905	120.2137	1.2911	0.0000
	<i>FAA2</i>	RHTO_04350	long-chain acyl-CoA synthetase	19.7578	17.0641	18.5169	25.7741	18.7174	24.4724	1.3900	0.0000
	<i>FAA3</i>	RHTO_04957	long-chain acyl-CoA synthetase	44.0916	44.3488	42.0134	49.6622	43.5869	47.6065	1.2104	0.0006
	<i>CRAT</i>	RHTO_00095	carnitine O-acetyltransferase	46.3890	48.2254	47.7145	81.2943	90.9673	77.8222	1.9897	0.0000
	<i>CACT</i>	RHTO_01354	carnitine acyl carnitine carrier, mitochondrial	60.3271	63.7753	58.0731	105.3601	90.4076	97.1787	1.8015	0.0000
	<i>ABCT1</i>	RHTO_06195	ABC fatty acid transporter	69.7029	71.5407	73.9882	112.9540	114.0465	110.4471	1.7679	0.0000
	<i>ABCT2</i>	RHTO_04105	peroxisomal ABC transporter	80.0420	73.7057	77.2565	101.1996	84.0272	97.1552	1.3687	0.0000
	<i>ACOX1</i>	RHTO_03890	acyl-CoA oxidase	25.7386	23.6892	26.1424	43.7321	46.9431	45.2056	2.0314	0.0000
	<i>ACOX2</i>	RHTO_03776	acyl-CoA oxidase	64.5442	79.8492	68.3700	115.3287	231.7014	129.8031	2.5926	0.0000
	<i>FOX2</i>	RHTO_06581	multifunctional beta-oxidation protein	61.4052	62.3069	61.2147	90.8876	92.5501	97.6859	1.7117	0.0000
	<i>ACADSB</i>	RHTO_03594	short/branched chain acyl-CoA dehydrogenase	106.4742	116.4739	105.4993	146.5585	175.7246	141.9770	1.6040	0.0001
	<i>ACDM</i>	RHTO_04971	medium-chain-acyl-CoA dehydrogenase	81.9498	85.3991	74.8740	147.9645	146.8659	145.8739	2.0478	0.0000
	<i>ACADVL</i>	RHTO_00397	acyl-CoA dehydrogenase	160.6708	171.4257	153.9891	261.7228	259.9650	247.1596	1.7818	0.0000
	<i>ECH1</i>	RHTO_05407	enoyl-CoA hydratase	85.6731	91.6327	82.1567	88.5138	65.0956	85.9628	1.0282	0.6957
	<i>ECH2</i>	RHTO_02517	enoyl-CoA hydratase	12.5322	9.0900	12.0771	13.6634	6.1341	11.6739	1.0293	0.8700
	<i>ECH3</i>	RHTO_04298	enoyl-CoA hydratase/isomerase family protein	30.4464	35.0598	31.9089	33.6203	33.1216	36.7467	1.1928	0.0400
	<i>HCD1</i>	RHTO_05520	3-hydroxyacyl-CoA dehydrogenase	78.7839	81.7910	79.5735	117.1546	86.9735	106.4595	1.4425	0.0000
	<i>ACA1</i>	RHTO_00476	3-ketoacyl-CoA thiolase, mitochondrial	198.4777	202.9607	200.9464	249.5323	213.0191	230.8891	1.2902	0.0000
	<i>POT1</i>	RHTO_02848	3-ketoacyl-CoA thiolase (POT1)	77.4384	82.7779	71.3698	80.8206	112.9705	80.7977	1.3531	0.0476
	<i>POT2</i>	RHTO_07118	3-ketoacyl-coA thiolase peroxisomal A precursor	144.3345	155.3776	148.0164	172.4144	198.2737	152.5541	1.3262	0.0190
	<i>POT3</i>	RHTO_00300	peroxisomal 3-ketoacyl-CoA thiolase	44.6690	51.9757	40.9008	56.7338	59.2274	51.4341	1.3730	0.0060
	<i>ACOX1</i>	RHTO_03890	acyl-CoA oxidase	25.7386	23.6892	26.1424	43.7321	46.9431	45.2056	2.0314	0.0000
	<i>ACOX2</i>	RHTO_03776	acyl-CoA oxidase	64.5442	79.8492	68.3700	115.3287	231.7014	129.8031	2.5926	0.0000
ROS generation	<i>FTR1</i>	RHTO_00148	iron permease FTR1	301.4806	312.0726	299.0079	524.6837	647.7574	520.0769	2.1076	0.0000
	<i>SIT1</i>	RHTO_04727	MFS transporter, siderochrome-iron transporter	201.5423	216.4817	189.1932	353.3032	400.7994	373.3573	2.0989	0.0000
	<i>FERB</i>	RHTO_00765	ferric-chelate reductase	132.6650	134.1094	132.1583	227.5871	212.6958	218.0789	1.8552	0.0000
	<i>ARN</i>	RHTO_01307	MFS transporter, siderophore-iron:H <sup>+</sup> symporter	76.6370	80.5255	75.6464	158.8036	223.6071	170.8844	2.7114	0.0000
	<i>FOX2</i>	RHTO_06581	multifunctional beta-oxidation protein	61.4052	62.3069	61.2147	90.8876	92.5501	97.6859	1.7117	0.0000
	<i>AOX</i>	RHTO_07747	alternative oxidase	1251.3892	1329.0272	1207.8544	1445.2288	2148.4537	1603.4705	1.5677	0.0032
	<i>CAT</i>	RHTO_01370	catalase A	2.6398	2.7433	2.1755	4.9694	4.9974	4.7322	2.1881	0.0000
ROS degradation	<i>SOD1</i>	RHTO_00206	superoxide dismutase, Fe-Mn family	839.0318	849.4277	799.1885	922.6614	797.7976	944.7385	1.1994	0.0003
	<i>SOD2</i>	RHTO_06854	superoxide dismutase, Fe-Mn family	144.7967	132.9024	130.9181	127.8257	102.7930	114.9965	0.9464	0.3402
	<i>SOD3</i>	RHTO_08091	superoxide dismutase, Fe-Mn family	10.3082	14.5561	15.2581	8.9422	9.9365	8.4538	0.7694	0.0912
	<i>CPO</i>	RHTO_00268	chloroperoxidase-like protein	39.1027	38.9693	39.6087	31.1498	33.5491	30.3678	0.9126	0.3255
	<i>CYTCl</i>	RHTO_06193	cytochrome-c peroxidase	350.1928	343.9367	338.2036	368.4970	285.5695	353.4281	1.0895	0.0599
	<i>CYTC2</i>	RHTO_07451	cytochrome-c peroxidase	266.3230	289.0901	264.4046	318.8791	336.5888	313.5179	1.3340	0.0014
Terpenoids skeleton extension and carotenoids biosynthesis	<i>ACAT</i>	RHTO_02048	acetyl-CoA C-acetyltransferase	165.9370	162.3392	171.0256	165.4551	181.1392	161.4674	1.1514	0.1319
	<i>HMGCS</i>	RHTO_02305	hydroxymethylglutaryl-CoA synthase	139.3315	140.7862	130.2692	139.4639	140.6413	130.3869	1.1278	0.1584
	<i>HMGA</i>	RHTO_04045	hydroxymethylglutaryl-CoA reductase (NADPH)	41.6582	37.0881	41.3199	41.3811	40.9416	42.6466	1.1724	0.0232
	<i>MVAK2</i>	RHTO_02073	phosphomevalonate kinase	55.4857	55.7992	55.5693	57.9357	51.9063	53.0425	1.0961	0.1275
	<i>MVD</i>	RHTO_06005	diphosphomevalonate decarboxylase	40.9683	38.9102	41.0231	38.1669	19.4880	33.9972	0.8359	0.1643

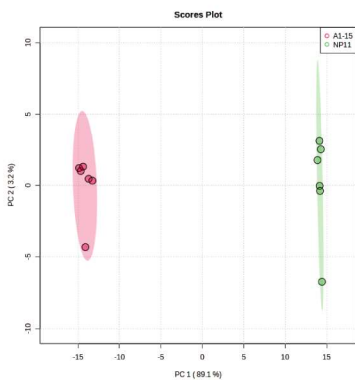
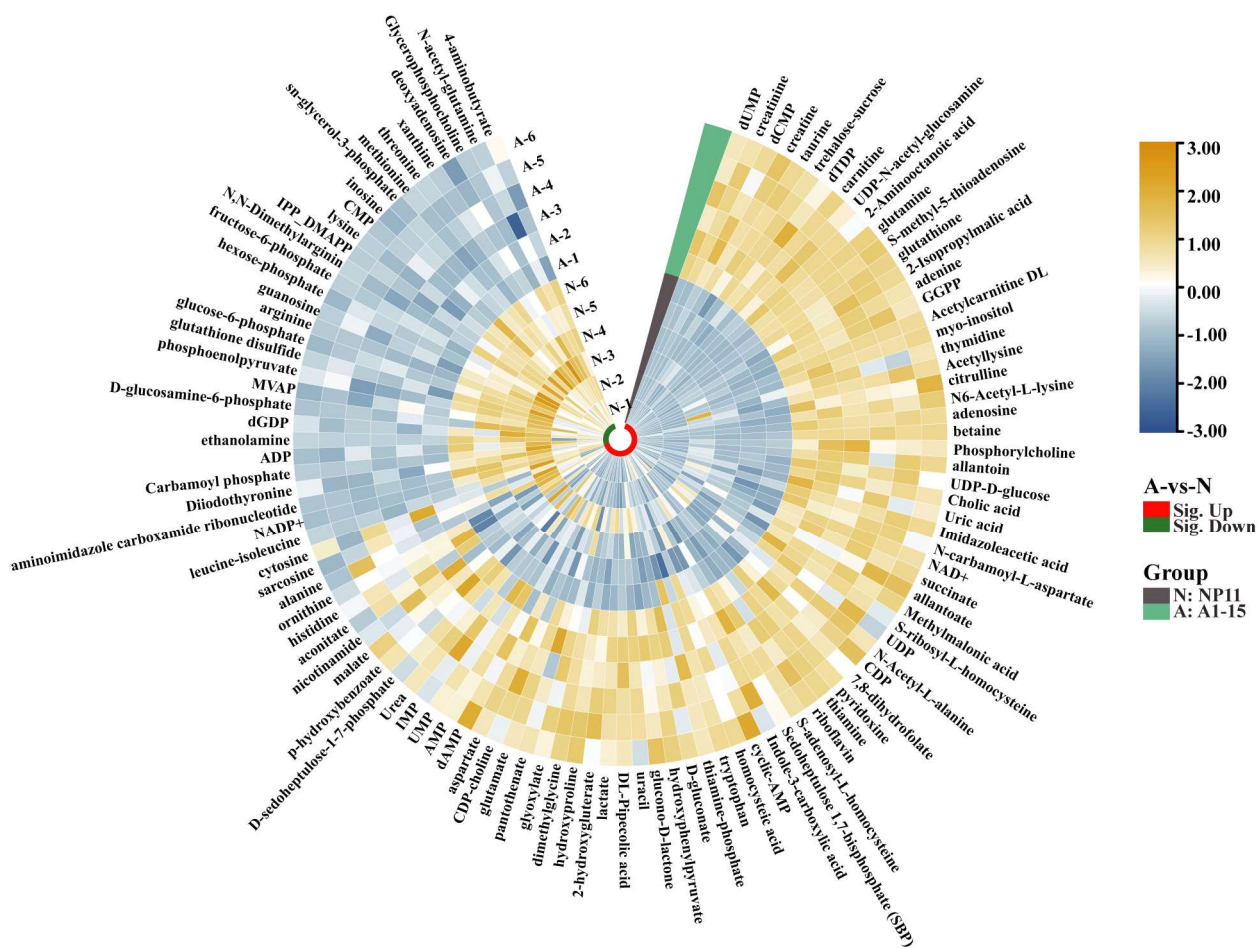
<i>FDPS</i>	RHTO_01660	farnesyl diphosphate synthase	58.8020	53.3836	54.8537	52.8678	33.4713	49.0055	0.8993	0.2364
<i>GGPS</i>	RHTO_02504	geranylgeranyl diphosphate synthase, type III	72.7102	68.3254	68.7630	105.3020	123.2319	108.6778	1.8215	0.0000
<i>CRTY</i>	RHTO_04605	phytoene synthase	42.8421	43.5711	46.6221	77.9883	79.8084	76.3016	1.9854	0.0000
<i>CRTI</i>	RHTO_04602	phytoene dehydrogenase	120.2258	122.1750	127.2721	345.2831	443.3679	356.3384	3.5250	0.0000
<i>FNTB1</i>	RHTO_05830	protein farnesyltransferase/geranylgeranyltransferase type-1 subunit alpha	45.5079	43.5820	44.4032	28.0030	19.1551	27.4693	0.6220	0.0000
<i>FNTB2</i>	RHTO_06679	protein farnesyltransferase/geranylgeranyltransferase type-1 subunit alpha	91.4103	84.2579	90.6765	52.4449	40.2231	51.2268	0.6033	0.0000

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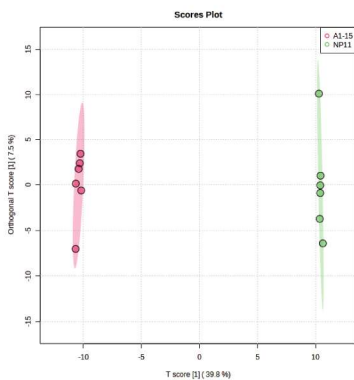
\* N:NP11, A:A1-15

Figure S1

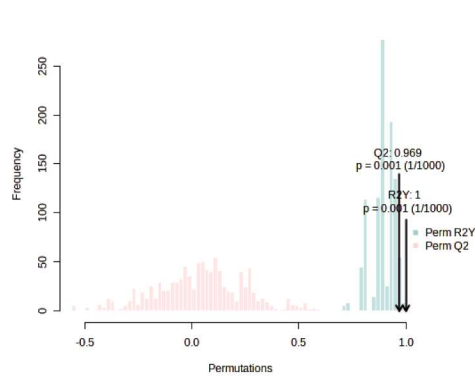
a



b



c

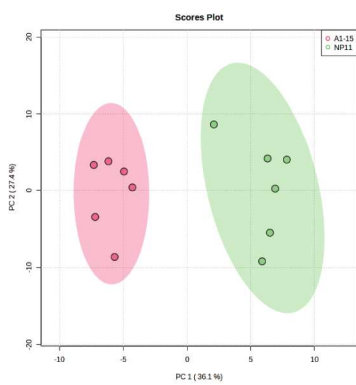
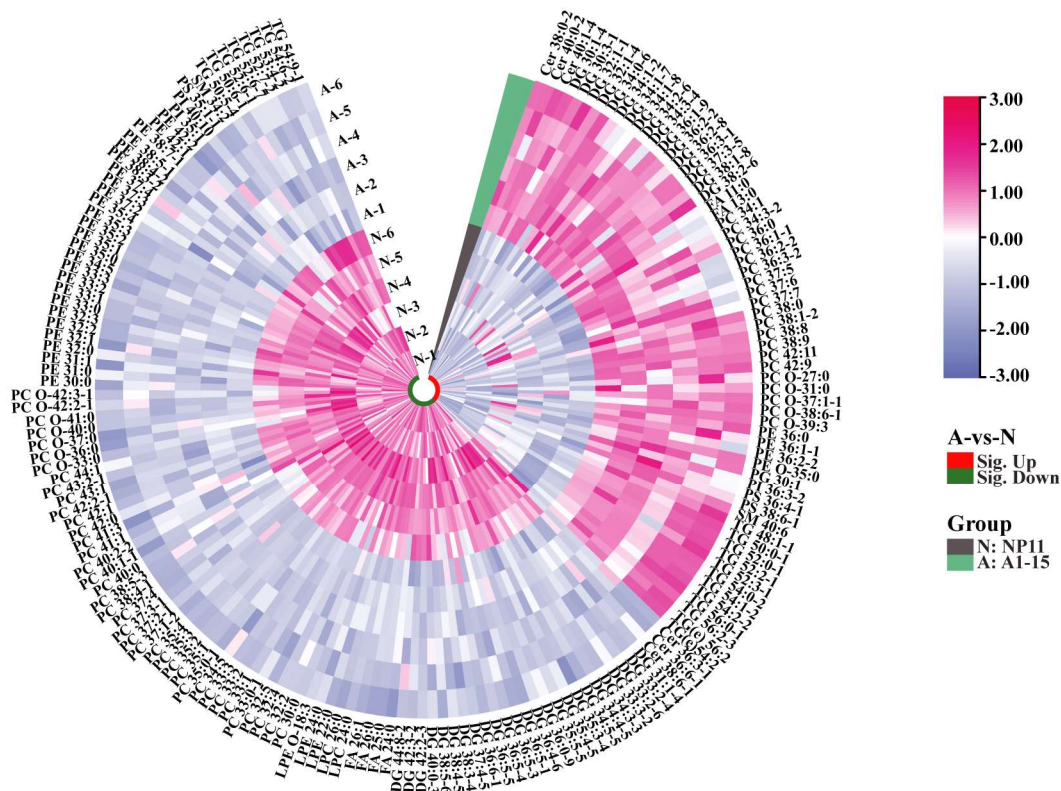


d

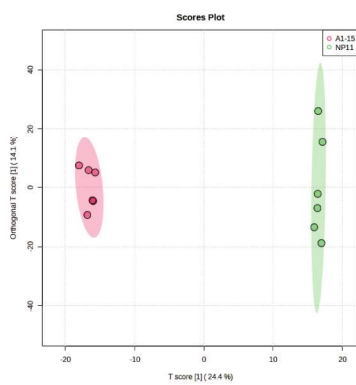
Figure S1. (a) Heat map of differential metabolites in A1-15 compared to NP11 under nitrogen limitation. (b) PCA score plot of all metabolite features. (c) OPLS-DA score plot of all metabolite features, (d) Permutation analysis, showing the observed and cross-validated R2Y and Q2 coefficients

Figure S2

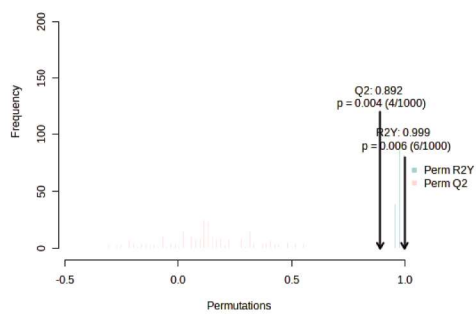
a



b



c



d

Figure S2. (a) Heat map of the differential lipids in A1-15 compared to NP11 under nitrogen limitation. (b) PCA score plot of all lipid features. (c) OPLS-DA score plot of all lipid features. (d) Permutation analysis, showing the observed and cross-validated R2Y and Q2 coefficients



Figure S3

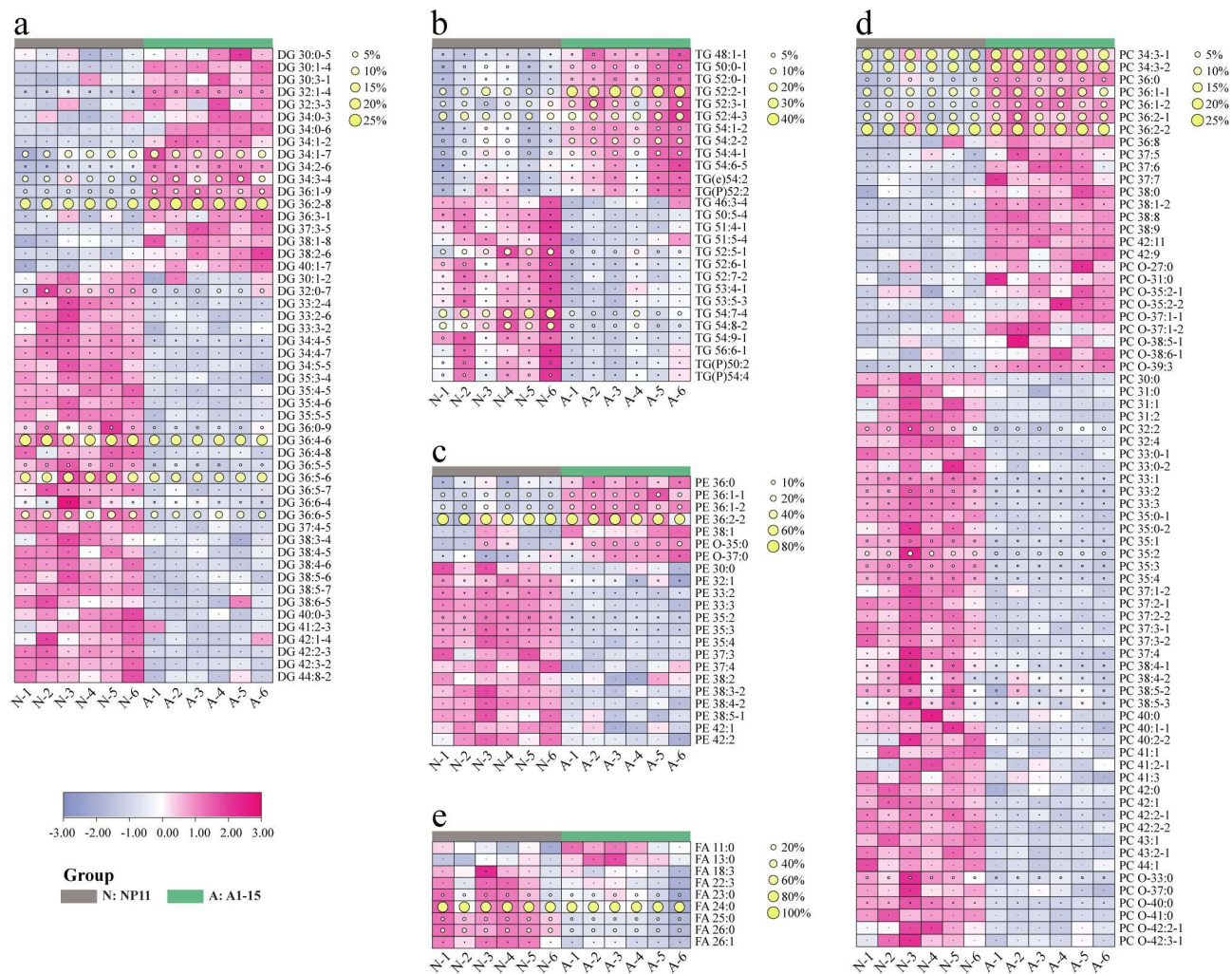


Figure S3. Heat map of five classes differential lipids of NP11 and A1-15 according to lipidomics analysis (complete version). (a)DGs, (b)TGs, (c)PEs, (d)PCs, (e)FAs. The size of the circle indicates the percentage of its corresponding lipid in that lipid class in that sample.