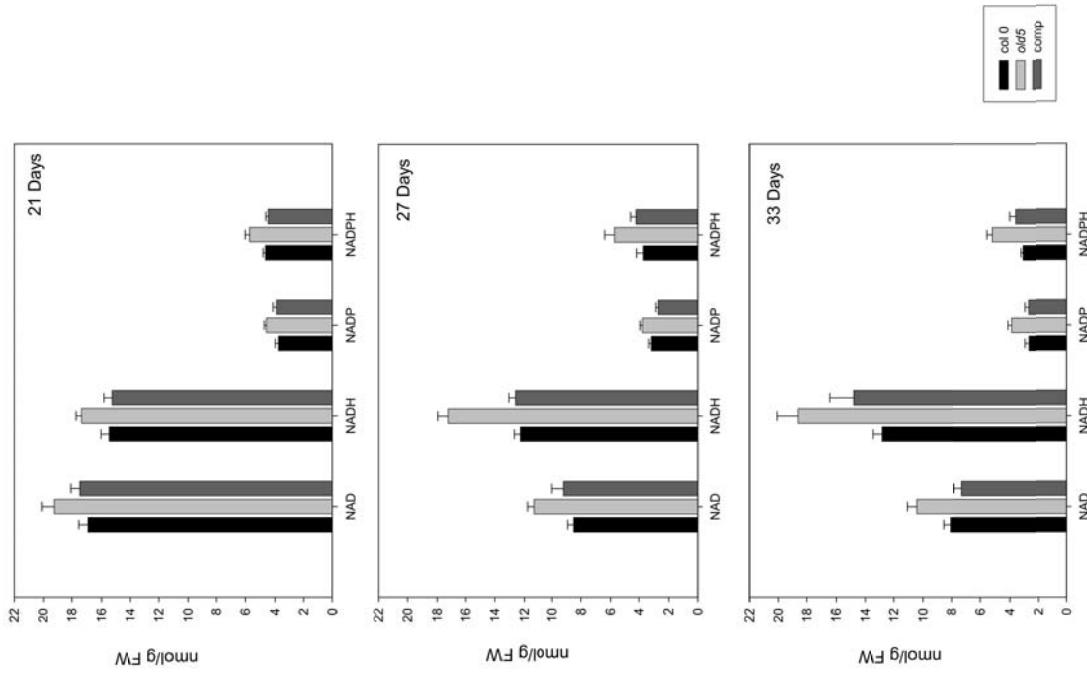


Supplemental Data Schippers et al. (2008) The *Arabidopsis* onset of *leaf death 5* mutation of quinolinate synthase affects NAD biosynthesis and causes early ageing.

Supplemental Figure 1:

Complementation of the *o/d5* mutation reverses the increased pyridine nucleotide content. (A) Pyridine nucleotide content in *o/d5* and wild type at day 21, day 27 and day 33 (*o/d5* gray bars, wild type solid black bars, complemented line dark grey). The values shown are the means of six repeats \pm SD (indicated by error bars).



Supplemental table 1. Metabolite levels in the first leaf pair of 21 and 27 day old plants of *o/d5* relative to wild type. Leaves were harvested 5h into the photoperiod and irrespective of the method of analysis. Values presented are the mean of relative values ± standard error of 6 measurements per genotype.

Metabolites	WT 21 days	Old5 21 days	WT 27 days	OLD5 27 days	WT 27/21	OLD5 27/21
Amino acids						
Alanine	1.00 ± 0.08	1.15 ± 0.19	1.00 ± 0.20	1.32 ± 0.26	0.67 ± 0.13	0.77 ± 0.15
Asparagine	1.00 ± 0.16	1.51 ± 0.13	1.00 ± 0.10	2.39 ± 0.21	0.40 ± 0.04	0.63 ± 0.05
Aspartic acid	1.00 ± 0.14	1.36 ± 0.05	1.00 ± 0.09	1.89 ± 0.36	0.81 ± 0.07	1.12 ± 0.21
β-Alanine	1.00 ± 0.12	0.95 ± 0.07	1.00 ± 0.09	4.30 ± 0.27	0.56 ± 0.05	2.55 ± 0.16
Cysteine	1.00 ± 0.06	1.04 ± 0.07	1.00 ± 0.06	1.27 ± 0.14	0.90 ± 0.05	1.10 ± 0.12
GABA	1.00 ± 0.06	1.01 ± 0.05	1.00 ± 0.08	2.95 ± 0.40	1.07 ± 0.08	3.14 ± 0.43
Glutamate	1.00 ± 0.07	2.25 ± 0.26	1.00 ± 0.10	1.95 ± 0.20	0.62 ± 0.06	0.54 ± 0.05
Glutamine	1.00 ± 0.06	1.43 ± 0.09	1.00 ± 0.06	1.65 ± 0.22	0.46 ± 0.03	0.53 ± 0.07
Glycine	1.00 ± 0.10	0.74 ± 0.18	1.00 ± 0.16	0.61 ± 0.05	0.54 ± 0.08	0.44 ± 0.03
Homoserine	1.00 ± 0.04	0.91 ± 0.04	1.00 ± 0.05	1.26 ± 0.17	0.88 ± 0.05	1.23 ± 0.17
Isoleucine	1.00 ± 0.06	0.93 ± 0.03	1.00 ± 0.08	1.50 ± 0.14	0.87 ± 0.07	1.41 ± 0.13
Lysine	1.00 ± 0.33	0.61 ± 0.04	1.00 ± 0.33	1.51 ± 0.10	0.65 ± 0.22	1.60 ± 0.11
Methionine	1.00 ± 0.03	1.25 ± 0.15	1.00 ± 0.37	1.04 ± 0.09	0.87 ± 0.33	0.73 ± 0.06
O-acetyl-serine	1.00 ± 0.06	0.89 ± 0.04	1.00 ± 0.07	1.18 ± 0.16	0.93 ± 0.07	1.25 ± 0.17
Ornithine	1.00 ± 0.19	1.46 ± 0.17	1.00 ± 0.15	1.66 ± 0.12	0.65 ± 0.10	0.74 ± 0.06
Phenylalanine	1.00 ± 0.08	1.23 ± 0.08	1.00 ± 0.16	3.97 ± 0.48	0.60 ± 0.09	1.95 ± 0.23
Proline	1.00 ± 0.06	1.73 ± 0.17	1.00 ± 0.10	2.03 ± 0.32	0.73 ± 0.07	0.85 ± 0.13
Putrescine	1.00 ± 0.07	0.93 ± 0.04	1.00 ± 0.11	2.48 ± 0.27	0.67 ± 0.07	1.77 ± 0.19
Serine	1.00 ± 0.12	1.69 ± 0.22	1.00 ± 0.08	2.62 ± 0.65	0.50 ± 0.04	0.78 ± 0.19
Tyrosine	1.00 ± 0.05	1.15 ± 0.06	1.00 ± 0.08	1.15 ± 0.05	1.12 ± 0.09	1.12 ± 0.05
Valine	1.00 ± 0.07	1.14 ± 0.06	1.00 ± 0.08	2.13 ± 0.15	0.62 ± 0.05	1.17 ± 0.08
Organic acids						
α-Ketoglutarate	1.00 ± 0.06	1.22 ± 0.10	1.00 ± 0.05	1.80 ± 0.12	0.42 ± 0.02	0.62 ± 0.04
Citrate	1.00 ± 0.26	0.59 ± 0.13	1.00 ± 0.16	0.69 ± 0.27	3.48 ± 0.57	4.07 ± 1.09
Fumarate	1.00 ± 0.08	1.03 ± 0.09	1.00 ± 0.05	1.16 ± 0.10	1.31 ± 0.06	1.47 ± 0.13
Glycerate	1.00 ± 0.11	0.70 ± 0.10	1.00 ± 0.10	0.54 ± 0.06	0.65 ± 0.07	0.50 ± 0.05
Malate	1.00 ± 0.24	1.50 ± 0.22	1.00 ± 0.06	1.54 ± 0.17	2.33 ± 0.14	2.39 ± 0.26

Oxalate	1.00	±	0.10	0.95	±	0.06	1.00	±	0.09	1.53	±	0.14	1.28	±	0.11	2.06	±	0.19
Phosphorate	1.00	±	0.12	1.03	±	0.10	1.00	±	0.14	1.93	±	0.50	0.77	±	0.11	1.44	±	0.37
Pyruvate	1.00	±	0.08	1.04	±	0.09	1.00	±	0.12	1.37	±	0.18	0.76	±	0.09	1.00	±	0.13
Saccharate	1.00	±	0.13	0.90	±	0.07	1.00	±	0.09	1.22	±	0.16	1.35	±	0.12	1.83	±	0.24
Succinate	1.00	±	0.04	1.19	±	0.06	1.00	±	0.05	3.04	±	0.27	1.34	±	0.07	3.42	±	0.31
Fatty acids																		
Nonanoic acid	1.00	±	0.11	0.79	±	0.05	1.00	±	0.07	1.14	±	0.15	0.96	±	0.06	1.38	±	0.18
Stearate	1.00	±	0.06	0.97	±	0.05	1.00	±	0.07	1.15	±	0.16	0.96	±	0.06	1.14	±	0.16
Sugars																		
Fructose	1.00	±	0.05	1.01	±	0.04	1.00	±	0.05	1.05	±	0.10	1.09	±	0.05	1.13	±	0.11
Maltose		ND		ND			1.00	±	0.08	2.62	±	0.09	ND		ND			
Sucrose	1.00	±	0.08	1.30	±	0.13	1.00	±	0.13	1.59	±	0.09	0.77	±	0.10	0.95	±	0.05
Threhalose	1.00	±	0.30	1.50	±	0.29	1.00	±	0.16	0.90	±	0.09	0.71	±	0.11	0.43	±	0.05
Xylose	1.00	±	0.06	0.67	±	0.10	1.00	±	0.11	3.22	±	0.47	0.49	±	0.05	2.36	±	0.34
Sugar alcohols																		
Erythritol	1.00	±	0.05	0.91	±	0.05	1.00	±	0.05	1.03	±	0.06	0.49	±	0.03	0.55	±	0.03
Galactitol	1.00	±	0.04	1.34	±	0.08	1.00	±	0.09	2.23	±	0.11	0.63	±	0.06	1.04	±	0.20
Glycerol	1.00	±	0.10	0.81	±	0.06	1.00	±	0.22	0.93	±	0.06	1.06	±	0.23	1.21	±	0.08
Mannitol	1.00	±	0.03	0.81	±	0.06	1.00	±	0.06	0.83	±	0.10	0.69	±	0.04	0.71	±	0.13
Other metabolites																		
Guaniidine	1.00	±	0.15	1.42	±	0.16	1.00	±	0.09	1.69	±	0.29	0.70	±	0.06	0.84	±	0.14
Spermidine	1.00	±	0.11	1.55	±	0.17	1.00	±	0.11	3.08	±	0.17	0.57	±	0.06	1.12	±	0.06

Supplemental table 2. Metabolite levels in the first leaf pair of 21, 27 and 33 day old plants of complemented *o/d5* relative to wild type. Leaves were harvested 5h into the photoperiod and irrespective of the method of analysis. Values presented are the mean of relative values ± standard error of 6 measurements per genotype.

Metabolites	WT 21 days	Comp 21 days	WT 27 days	Comp 27 days	WT 33 days	Comp 33 days	WT 27/21	WT 33/21
Amino acids								
Alanine	1.00 ± 0.10	1.02 ± 0.12	1.00 ± 0.23	0.87 ± 0.13	1.00 ± 0.15	1.14 ± 0.12	0.49 ± 0.10	0.23 ± 0.11
Aspartic acid	1.00 ± 0.13	0.88 ± 0.14	1.00 ± 0.07	0.83 ± 0.12	1.00 ± 0.12	1.26 ± 0.19	0.47 ± 0.03	0.42 ± 0.05
β-Alanine	1.00 ± 0.04	0.96 ± 0.06	1.00 ± 0.14	1.03 ± 0.03	1.00 ± 0.06	0.96 ± 0.05	0.77 ± 0.10	0.90 ± 0.05
GABA	1.00 ± 0.12	1.06 ± 0.06	1.00 ± 0.13	1.11 ± 0.04	1.00 ± 0.19	0.85 ± 0.09	0.96 ± 0.12	1.54 ± 0.28
Glutamate	1.00 ± 0.13	0.78 ± 0.09	1.00 ± 0.13	0.92 ± 0.15	1.00 ± 0.20	0.88 ± 0.15	0.27 ± 0.04	0.15 ± 0.03
Glycine	1.00 ± 0.15	0.84 ± 0.06	1.00 ± 0.16	0.95 ± 0.15	1.00 ± 0.04	0.94 ± 0.09	0.72 ± 0.11	0.55 ± 0.02
Isoleucine	1.00 ± 0.07	1.01 ± 0.09	1.00 ± 0.13	1.00 ± 0.06	1.00 ± 0.08	0.75 ± 0.04	1.28 ± 0.16	1.93 ± 0.16
Lysine	1.00 ± 0.07	0.90 ± 0.11	1.00 ± 0.15	1.02 ± 0.15	1.00 ± 0.17	1.11 ± 0.22	0.89 ± 0.13	1.82 ± 0.32
Methionine	1.00 ± 0.36	0.72 ± 0.11	1.00 ± 0.08	1.22 ± 0.12	1.00 ± 0.26	0.85 ± 0.20	0.41 ± 0.03	4.56 ± 1.18
Ornithine	1.00 ± 0.11	0.75 ± 0.14	1.00 ± 0.13	0.87 ± 0.17	1.00 ± 0.30	1.06 ± 0.30	0.34 ± 0.03	0.93 ± 0.27
Phenylalanine	1.00 ± 0.09	0.88 ± 0.08	1.00 ± 0.09	0.96 ± 0.10	1.00 ± 0.17	0.88 ± 0.10	0.81 ± 0.07	1.63 ± 0.28
Proline	1.00 ± 0.11	1.08 ± 0.03	1.00 ± 0.24	0.73 ± 0.08	1.00 ± 0.15	1.09 ± 0.11	0.59 ± 0.09	0.51 ± 0.05
Serine	1.00 ± 0.08	0.93 ± 0.06	1.00 ± 0.08	0.99 ± 0.04	1.00 ± 0.05	0.83 ± 0.06	0.96 ± 0.08	1.09 ± 0.05
Threonine	1.00 ± 0.07	0.86 ± 0.03	1.00 ± 0.05	1.01 ± 0.04	1.00 ± 0.05	0.85 ± 0.12	0.69 ± 0.03	0.88 ± 0.05
Tryptophan	1.00 ± 0.04	1.12 ± 0.04	1.00 ± 0.05	0.96 ± 0.02	1.00 ± 0.05	0.99 ± 0.04	1.40 ± 0.06	1.36 ± 0.06
Tyrosine	1.00 ± 0.20	0.91 ± 0.19	1.00 ± 0.18	1.09 ± 0.29	1.00 ± 0.27	1.11 ± 0.30	1.52 ± 0.27	5.40 ± 1.48
Valine	1.00 ± 0.08	1.04 ± 0.10	1.00 ± 0.13	0.88 ± 0.11	1.00 ± 0.06	0.76 ± 0.11	0.74 ± 0.09	0.84 ± 0.05
Organic acids								
Citrate	1.00 ± 0.21	1.64 ± 0.49	1.00 ± 0.26	0.70 ± 0.11	1.00 ± 0.18	1.16 ± 0.28	1.08 ± 0.28	0.55 ± 0.10
Fumarate	1.00 ± 0.11	1.09 ± 0.06	1.00 ± 0.06	0.97 ± 0.23	1.00 ± 0.04	0.93 ± 0.04	1.15 ± 0.07	1.32 ± 0.06
Glycerate	1.00 ± 0.20	1.01 ± 0.15	1.00 ± 0.11	0.86 ± 0.10	1.00 ± 0.17	0.96 ± 0.08	0.57 ± 0.06	0.32 ± 0.06
Iso-citrate	1.00 ± 0.07	0.91 ± 0.04	1.00 ± 0.08	0.72 ± 0.11	1.00 ± 0.19	1.31 ± 0.50	0.75 ± 0.06	1.05 ± 0.20
Malate	1.00 ± 0.15	1.19 ± 0.20	1.00 ± 0.17	0.95 ± 0.08	1.00 ± 0.18	0.96 ± 0.12	1.99 ± 0.34	2.36 ± 0.42
Phosphorate	1.00 ± 0.17	1.04 ± 0.22	ND	ND	1.00 ± 0.18	1.29 ± 0.42	0.42 ± 0.08	0.83 ± 0.15
Succinate	1.00 ± 0.12	1.06 ± 0.12	1.00 ± 0.10	1.08 ± 0.15	1.00 ± 0.07	1.04 ± 0.11	0.93 ± 0.10	1.15 ± 0.08
Sugars								
Glucose	1.00 ± 0.07	0.89 ± 0.04	1.00 ± 0.08	0.73 ± 0.11	1.00 ± 0.18	1.26 ± 0.46	0.73 ± 0.06	0.94 ± 0.15

Fructose	1.00	±	0.09	0.95	±	0.10	1.00	±	0.15	0.73	±	0.09	1.00	±	0.22	1.17	±	0.47	0.91	±	0.13	2.02	±	0.44	
Fructose-6-phosphate	1.00	±	0.15	0.90	±	0.04	1.00	±	0.08	1.16	±	0.19	1.00	±	0.06	1.19	±	0.12	1.53	±	0.13	1.64	±	0.10	
Fucose	1.00	±	0.04	0.98	±	0.03	1.00	±	0.05	0.97	±	0.04	1.00	±	0.03	0.96	±	0.05	0.87	±	0.04	0.96	±	0.03	
Maltose	1.00	±	0.06	1.08	±	0.07	1.00	±	0.08	1.05	±	0.05	1.00	±	0.08	1.01	±	0.07	1.33	±	0.11	0.96	±	0.08	
Mannose	1.00	±	0.06	1.02	±	0.06	1.00	±	0.11	1.03	±	0.11	1.00	±	0.15	0.84	±	0.15	1.30	±	0.14	2.16	±	0.32	
Raffinose	1.00	±	0.10	0.94	±	0.07	1.00	±	0.12	0.89	±	0.11	1.00	±	0.14	1.14	±	0.20	2.73	±	0.34	0.93	±	0.13	
Sucrose	1.00	±	0.11	1.00	±	0.07	1.00	±	0.09	0.99	±	0.06	1.00	±	0.05	1.02	±	0.04	0.77	±	0.07	0.76	±	0.03	
Trehalose	1.00	±	0.05	0.95	±	0.03	1.00	±	0.09	0.97	±	0.03	1.00	±	0.04	0.95	±	0.07	0.85	±	0.07	0.59	±	0.02	
Xylose	1.00	±	0.07	0.87	±	0.08	1.00	±	0.08	0.91	±	0.14	1.00	±	0.03	0.98	±	0.09	0.94	±	0.03	1.05	±	0.01	
Sugar alcohols																									
Erythritol	1.00	±	0.06	0.93	±	0.03	1.00	±	0.03	0.88	±	0.04	1.00	±	0.10	0.93	±	0.04	0.52	±	0.01	0.33	±	0.03	
Galactitol	1.00	±	0.10	1.06	±	0.19	1.00	±	0.10	1.00	±	0.13	1.00	±	0.16	1.30	±	0.21	2.12	±	0.22	2.61	±	0.40	
Other metabolites																									
Spermidine	1.00	±	0.04	0.86	±	0.05	1.00	±	0.15	1.13	±	0.06	1.00	±	0.13	1.03	±	0.08	0.54	±	0.08	0.62	±	0.08	
Urea	1.00	±	0.17	0.87	±	0.05	1.00	±	0.18	0.98	±	0.11	1.00	±	0.23	0.75	±	0.09	1.10	±	0.20	1.52	±	0.35	

Supplemental table 3. Primers used in this study.

Map Based Cloning	Forward	Reverse	Product
K6A12-23K	GGATTGTTAACCTGAGATATCTTATGGTTTAGT	TGGGAGACCAAAATACTGTTACAATAG	329
MXI22-12K	ACGTATGTGATCTTAGTAACCAAATAAAGTG	TAGCCAGAATGGATGACTGGG	357
Y2H			
QS/OLD5	GAATT <u>CCCCGGGG</u> ATGGCGTTAGCTCTCCGTC	ATCATCGTCGACGTCATCTCTTGCCTCACAACT	2182
SufE	GAATT <u>CCCCGGGG</u> ATGGCAGCAGGATGTCCTCTCT	ATCATCGTCGACGTCAAACCTCAGCAGGAGTCCTTGC	1141
CpNifS1	GAATT <u>CCCCGGGG</u> ATGGAAAGGTGTGGCTATGAAACTC	ATCATCGTCGACGTTATTGAAAGAGTTGAAGAAAGCT	1417
CpNifS2	GAATT <u>CCCCGGGG</u> ATGTTGTCGAGATTGTTCAAGGCCAG	ATCATCGTCGACGCTAGACTCTGATAGTTGGACCAGT	874
AO	GAATT <u>CCCCGGGG</u> ATGGCGGCATGTTCTACTGGA	ATCATCGTCGACGTTAGCAATCAATAAGTGAAGCTGCT	1981
CpNifS3	GAATT <u>CCCCGGGG</u> ATGGCGTCATCTCCACCGGA	ATCATCGTCGACGTCACAAAGATAGAGACGTGATTGA	1453
Real Time PCR			
ACT2	TCTCCGCTTGAATTGTCTC	TATGAGCTTGGAAAGAAAGAGC	185
QS/OLD5	TCATGAGCTTGTACCTACCA	GGTTCCCTTCTGGAAATAGTG	249
SAG12	TATTACAGGTTATGGGGATGTCCC	ACCCACATAGTCCTTGTATTATCC	300
SAG13	GAACCTCAAGGATGGAGTCTTGG	ATTGTTGACGAGGGATGTGAG	279
QPRT	AAATGGATGTAGAGGTGGAGAC	TCTGTCCGATCTTGTGTAECTG	236
GDH2	GCAACAAACAGAAACCTTCCGT	TCGTACACTCAACCTTGTATTCTC	105
NaPRT2	CATACGATGTGATGAAGAGTGG	CATTACTAGCAGTGTACGGACC	203
AO	TGAAGAAACCGTAAGAGTTGTG	CAAGCCCATCCTGAGAAGTGTGAG	267
NaPRT1	CATTAACGGACCCACTAACCC	ACAGATCAAACACAGATCGCTC	118
NADS	AAGGTATAAGGTTGATGGAGGA	CTATGAACCC/AAGGCAAGAG	116
DEFL	CTTAGTCATTTCGGATGTGCC	GCATCTTCCACCTTTAGCTC	159
OxiReductase	TGATATCTGCAGGAATGAAACG	GGGTAGTAGTTAAGGTTGACTC	161
NIC1	GATCAGCTAAAGAAGCAGATTC	TTTGTGGAGCCATATTGCC	136