Title: NHX1 participates in tobacco disease defense against *Phytophthora parasitica* var. *nicotianae* by affecting vacuolar pH and priming the anti-oxidative system

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Supplementary Figure S1. Screening distinctive sequence of *NbNHX1* for gene silencing. A, EST information of *NbNHX3* cloned from *N. benthamiana*. The yellow mark indicates the same sequence between unigene of SGN-U521516 and the EST of *NbNHX3*; red mark presents the same sequence between unigene of SGN-U518331 and the EST of *NbNHX3*. B-C, The sequence alignment among *NbNHX1* (Nb1), unigene *NbNHX2* (Nb2, ID: SGN-U515339), and unigene *NbNHX3* (Nb3, ID: SGN-U518331). The underline sequence indicates the distinctive 263 bp sequence from *NbNHX1* used for gene silencing.

Supplementary Figure S2. Virus inducing gene silencing and ectopic expression in *N. benthamiana*. A, TRV inducing gene silencing in *N. benthamiana*. Diagrams illustrate the pTRV2 derivative clone vectors used in this study. B, Phenotypes of *N. benthamiana* at 28 days post inoculation with TRV carrying a conserved fragment of a phytoene desaturase gene. C, Expression of *NbNHX1* in *NbNHX1* silenced tobaccos (N1-N30). D, Wilt spot area in *NbNHX1* silenced tobaccos (N1-N30). D, Wilt spot area in *NbNHX1* silenced tobaccos (N1-N30). E, PEBV mediating ectopic gene expression in *N. benthamiana*. Diagrams illustrate the pCAPE2 derivative clone vectors used in this study. F, Phenotypes of *N. benthamiana* at 28 days post inoculation with PEBV carrying a conserved fragment of a phytoene desaturase gene. G, Expression of *NHX1* in *SeNHX1* transgenic tobaccos (S1-S30) and *AtNHX1* transgenic tobaccos (A1-A30). H, Wilt spot area in *SeNHX1* transgenic tobaccos (S1-S30) and *AtNHX1* transgenic tobaccos (A1-A30).

Supplementary Figure S3. *In situ* calibration curve and pH quantification test. A, The calibration curve was performed by plotting the ratio of emission fluorescence (505-550 nm) excited with 458 or 488 nm, against the pH of equilibration buffers. B,

Vacuolar pH quantification under TEST1 (pH 5.1) and TEST (pH 5.4) treatment. Data are means \pm SE (n = 60 cells from 6 leaves).

Supplementary Figure S4. The relative expression of *NHX1* in different genotype tobacco. A, The relative expression of *NbNHX1* at 0, 24, 36 and 48 hpi. B, The relationship between relative expression of *NbNHX1* and vacuole H^+ net flux. C, The relative expression of *NbNHX1* in NS1-NS6. The relative expression of *NHX1* in S31-S36 (D), A31-A36 (E) and NOE1-NOE6 (F). G, The relative expression of *NbNHX1* in 6 Nb-GFP plants. The relative expression was calculated by actual expression of *NHX1* against that in untreated, or TRV plants or GFP plants.

Supplementary Figure S5. Vacuolar H⁺ net fluxes in *NbNHX1* silenced *N*. *benthamiana* under 0 and 1.5 mM ATP or *PPi* treatment. Dynamic curves of transient H⁺ net fluxes: normal condition plus supply of ATP (A) or *PPi* (B). C-D, Mean of vacuolar H⁺ net fluxes corresponding to (A-B) during the measuring periods. E, The changes of vacuolar H⁺ flux (Δ H⁺ flux) after ATP or *PPi* supply in TRV or TRV-Nb plants. The value obtained from NMT indicates net ion flux; the positive values of H⁺ flux in the figures represent cation efflux or anion influx, and *vice versa*. TRV represents pTRV2 empty vector transformed tobaccos (control plants). TRV-Nb represents pTRV2-NbNHX1 vector transformed tobaccos (*NbNHX1* silenced plants). Data are means \pm SE (n = 6 vacuole per *NbNHX1* silenced line, 6 independent *NbNHX1* silenced lines). The asterisks on the bars indicate significant differences from the TRV plants in the same treatment at $P \le 0.05$.

Supplementary Figure S6. Responses of *NHX1* transgenic tobaccos to ES4326. Infection experiments were performed with 6-week-old plants of Se/At-YFP transgenic and YFP plants mediated by PEBV. 30 leaves were collected to examine the growth of the pathogen at 0, 72, and 96 hpi. YFP means pCAPE2-YFP vector transformed tobaccos. Se/At-YFP represents pCAPE2-At/SeNHX1-YFP vector transformed tobaccos. Data are means \pm SE (n = 30 leaves from 30 independent transgenic lines). The asterisks on the bars indicate significant differences from the YFP plants in the same treatment at P ≤ 0.05 . **Supplementary Table S1.** The primers of ROS responsive genes used in real-time PCR.

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ACATGGGGACATACACTAGCGTGTCTATGACACACCACTTGGGTGTCTACATCACACCA	Nb2	AAACCT
AGTAGGATCTCCGGACCTTGCGAGTTTGAGCTGGAAGCACCTGCTTATACAGTAGGTT	Nb1	ACCAGCACCATTACCATTGTTCTTTCAGCACAGTGGTATTTGGTTTATTGACAAAACCT
GTCTACATCATACCTACTTTTTGAGGGTTGCATATCAGGGACAATAATACTTTTCATAAGC		
AAAGGAAAAAGTTCTCACATACTTAGATTTAATGAAGAGGTGTTCTTCATTTATCTACTT	Nb2	TTAATTAGATTTATGCTACCCTCACCAAAACACTTGACCAGAATGATCTCTTCTGAACCA
CCACCAATAATATTTAATGCAGGATTCCAGGTGAAGAAGAAGCAGTTCTTCCACAACTT	Nb1	CTTATAAGTCTCCTGCTGCCACCACAGAGGCAGTTAAGTACAGTTTCATCAGATGCA
CTTAACGATTATGTCGTTTGGAGTTATCGGGGTTTTCATATCATCAAGTATTATCACAGCT		*:**:**: * .****.**. ****. ** .** .
GGTAGCTGGTGGCTGTTTCCCAAGTTAAATTTCGACGGATTGAGTGTTCGCGACTACCT	Nh2	ACGACCCCABABATCCTTCATTGTGCCACTTCTTGACAGTGCACAAGACTCAGAAGCTGAT
TGGTATTGGAGCAATATTTTCGTCGACAGATACTGTTTGTACGCTGCAGGTTCTTCATCA	Nb1	AATACTCCAAAATCTCTTACAGCGCCACTTCTAGCCAGTCAAGAGGGTTCTGAAGTCGAT
AGACGAGACTCCGTTACTATACAGCCTCGTCTTTGGGGAAGGAGTAGTAAACGATGCT		*. ** ******** * * :* ********:*.**** .* *.*. **.**
ACATCAGTTGTTCTCTTCAATGCAGTGCAAAAACCTTGATGTTGATAGATTCAACGGCTG		
GTCGGCTGTCCATAICTTTCTAGATTTTCTGTACCTCTTCTCCATAAGCACTGCTCTTGG	Nb2	CTGGGCCAACATATACCCCGTCCCAACAGTTTGCGGATGCTCCTATCAACCCCATCT CTDAATAGTCAAGATCTCCTCCCCCCCCCCCCCCGGTTGCGGGATGCTCCTATCAACCCCCATCT
AGTTGCTGTCAGTTTTAAACTCTTTTTAATCCCGTAATAAGTGGTAGAAATAGGGACTAA	NOT	** *** **.** *. ** ******* *******
CTATTTCAGTATTTCCTATTGAATACGGATTTAGTACTCGAAAACATACTCTTGTCCTTTC		
TATCTATTGATGAGAGGAAAGCCATACCTTTCCATGGCAAGACTTGGAAAAAATGAAA	Nb2	CACACTGTGCATCGTTACTGGAGAAAATTTGACAATGCATTCATGCGTCCCGTTTTTGGT
AGACTGTTTACGTTGCGTTTAAAATAAAGGATGGTGTAATTGATGAATTTATTGTCGTCT	Nb1	CATAAGGTGCATAGGTACTGGCGCAAGTTTGATGATTCATTC
AAGCAAATATACTACAAAATTCACTATTAAAGATGCCATTTTCATCAGGTTGCGTTTAAA		
ATAAAGGATGGTGTAATTGATGAATTATTGTCGTCTAAGCAAATATACTACAAATTCACT	Nb2	GGAAGAGGTTTTGTACCTTTTGTTCCAGGATCACCAACTGAACCAAGTGATCATTAATCG
ATTAAGATGCCATTTCATCACGCATAAGATTGAGCTCAGTTTCAAGATTAACAAACTTTT	Nb1	GGTCGGGGGTTTACTCCTCTTGCTCC AACGGAACAGGAA
TGTGTTTCTCTGGGGATAATGAAATCAAATACTATATATTTTGGTTGATGATGATGATGTCTT		**:.*.** ***. **** *** ***:**:**:**:**:**:**:
TCGCATGTTATTTTTACTCTTGAATAATGAATTTTTTTAGTTAG	Nb2	састса
ATAGTGCTTTATCTGAAAATGTGTCTCGCTCCTTGGAATACGAGCCTTACTGAGTATGTC	Nb1	CCATGA
CAATTCTTCGTGCAGGCTGGACTTCTAACATCGTACATTCTGAAGGGCTTATACTTTGG		****
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GAGGCATTCTTCCGTTCGTGAAATAGCTCTCATGCTTCTGATGGCATATTTGTCCTACAT GTTGGCTGAGCTTTGCAGCCTAGTGGATTTTGACCGTCTTCTTCTCGAGGCAATTTGA TGTCTCACTTGCATGGCATAATGTGACTGATAGTTCAGAAGCACTACGAGGCATGCA	C Nb1 Nb3	GCATATAATAAGTTCACTCGTTTGGGTCACACTCAGTTGCAAGACAATGCAATAATGATT
GAGGCATTCTTCCGTTCGTGAAATAGCTCTCATGCTTCTGATGGCATATTTGTCCTACAT GTTGGCTGA6CTTTGCAGCCTCAGTGGGATTTTGACCGTCTTCTTTCCGGAATTTTGA TGTCTCACTATGCATGGCATAATGTGACTGATAGTTCAAGAATCACTACGAGGCATGCA TTTGAAGCTATGCTTTCATTGCCGAAACGTTCATATTTTTATACGTGGGGATGGACGCG	C Nb1 Nb3	GCATATAATAAGTTCACTCGTTTGGGTCACACTCAGTTGCAAGACAATGCAATAATGAT
GAGGCATTCTTCCGTTCGTGAAATAGCTCTCATGCTTCTGATGGCATATTTGTCCTACAT GTTGGCTGAGCTTTGCAGCCTCAGTGGGGATTTTGACCGTCTTCTTTTCCGGAATTTTGA TGTCTCACTATGCATAGCGATAATGGACTGATAGTTCAAGAATCACTACGAGGGATGGACGGC TTTGAAGCTATGTCTTTCATTGCCGAAACGTCATATTTTATACGTGGGGATGGACGGC CTGGACATTGAGAAGTGGAAAATGACCAAACGAGTGTTTGGACCTCCAATGGCAATA	C Nb1 Nb3 Nb1	GCATATAATAAGTTCACTCGTTTGGGTCACACTCAGTTGCAAGACAATGCAATAATGAT TAATGGT **********************************
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GAGGCATTCTTCCGTTCGTGAAATAGCTCTCATGCTTCTGATGGCATAITTGTCCTACAT GTTGGCTGAGCTTTGCAGCCTCAGTGGGATTTTGACCGTCTTCTTTCCGGAATTTTGA TGTCTCACTATGCATGGCATAATGTGACTGATAGTTCAAGAATCACTACGAGGCATGCA TTTGAAGCTATGTCTTTCATTGCCGAAACGTTCATAITTTTATACGTGGGGATGGACCCG CTGGACATTGAGAAGTGGAAAATGAGCAAACAGAGTGTTTGGATCTCAATGGGAATAT ATGGCACCGTGCTCATGTTGATAGCTGTCGGGGGGTGCTGCTTTTGTGTCCCTCTCTCT	C Nb1 Nb3 Nb1 Nb3	GCATATAATAAGTTCACTCGTTTGGGTCACACTCAGTTGCAAGACRATGCAATAATGAT TAATGGTT
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GAGGCATTCTTCCGTTCGTGAAATAGCTCTCATGCTTCTGATGGCATATTTGTCCTACAT GTTGGCTGAGCTTTTGCAGCCTAGTGGGATTTTGACCGTCTTTTTCCGAGATTTGA IGTCTCACTTGCAGGCATAATGGACTGATAGTTCAAGAATCACTACGAGGCATGCA TTTGAAGCTATGTCTTTCATTGCCGAAACGTTCATATTTTTATACGTGGGGAGTGGACGG CTGGACATTGAGAAGTGGAAAATGAGCAAACAGAGTGTTTGGACTTCAATGGGGAGAG ATGGCACCGTGCTCATTGTGATAGCTGCGGGCGTGCTGCTTTGTGTGTCCCTCTCTG GCTCTTTCCAATTTGATGAGCGGCGTGTTATGAGAGGACCCCTCAATAGGTTCAAACA TCAGATAGTAATTTGGTGGGCTGGTCTTATGAGAGGACCCTCAATAGGTTCAACA CCAGCAGTTACTTGGTGGCCTGGTCTTATGAGAGGACCCTCTAATGGTTACCA CAACCAGTTACTTGGTGGCCTGGTCTTATGAGAGGACCCTCTAATGGTTACCA CTACAGTAATCGTTGTGCTCTTCAGTGAGTGCTCCAGTGCATTCGCTTGGCATTGCTCCTCTG AACCTCACTCACTTGGTGTCTCAGTAGAGTGCCCCCAAATGGTCCACCGAG GCTCCCCCCCCCC	C Nb1 Nb3 Nb1 Nb3 Nb1 Nb3 Nb1 Nb3 Nb1 Nb3	CCATATAATAAGTTCACACCCCTTTGGGTCACACCCAGTGCAAGACAATGCAATAATGAT TAATGGTT *********************
GAGGCATTCTTCCGTTCGTGAAATAGCTCTCATGCTTCTGATGGCATATTTGTCCTACAT GTTGGCTGAGCTTTGCAGCCTCAGTGGGATTTTGACCGTCTTTTCTTTTCCGGAATTTGA TTTGAAGCTATGTCTTTCCATGCCTAATGTGACTGATAGTTCAAGAATCACTACGAGGCATGCA TTTGAAGCTATGTCTTTCATTGCCGAAACGTTCATATTTTTTATACGTGGGGATGGACGCG CTGGACATTGAGAAGTGGAAAATGACCAAACAGAGTGTTTGGACTTCAATGGGACATG ATGGCACCGTGCTCATGTGATAGCTGCGGGCGTGCTGCTTTGTGTTCCCCTCTCT GCTCTTTCCAATTCATGAGCGCGCGTGTTGACAGGAGCCCTCCAATAACGTTCAAACA CCAGATAGTATTTGGTGGGGCTGGTTTATGAGAGGACGCGTCTTGACTAGCGTTACAA CCAGCGATTCCATTTCGTGGTGTTACAGTGTGAGCGCGCGC	C Nb1 Nb3 Nb1 Nb3 Nb1 Nb3 Nb1 Nb3 Nb1 Nb3	CATATAATAAGTTCACTCGTTTGGGTCACACTCAGTTGCAAGACAATGCAATAATGAT TAATGGTT ACCAGCACCATTACCATTGTTTTTGCTTTTGGGTCATGGTTATTGGCCAAAAACCA ACCACTACAGTAATCGTTGTGTGTCAGTACAAGTGGTTTATTGACCAAAAACCA CTTATAAGTCTCTGCGCACCACAGASGCAGTTAAGTACGGTTTCTGTCGTGAGAGGGAGTAAGAGCA CTTATAAGTCTCTGCGCACCACAGASGCAGTTAAGTAGGGTTCCTGAAGAGGAGGAGTAAGAG CTTATAAGTCTCTGCGACACCACGAGGAGCAATAAGGAGGTTCCTGAAGTCGATGAAATGGGC CTTATAAGTCTCTGCGACACCACGAGGCAGTTGAGGGGTCCTGAGAGGGTCCTGAAGATGGAG ACCACTATCTCAAAAGTAGGCCGCCTTGCTTTGCT
GAGGCATTCTTCCGTTCGTGAAATAGCTCTCATGCTTCTGATGGCATATTTGTCCTACAT GTTGGCTGAGCTTTTGCAGCTCATAGTGGATTTTGACCGTCTTTTTCCGAGGATTTGA TGTCTCACTTGCATGGCATAATGTGACTGATAGTTAGACAGAATCTCATGGAGGATTGA TGTCTACTTGCATGGCATAATGTGACTGATAGTTTGACCGTCGGGATGGGAGCGG CTGGACATTGGAGAGTGGAAAATGGGCAAACGTCGTATTTTTATACGTGGGGATGGGACGG CTGGACATTGGAGAAGTGGAAAATGGGCCAAACGGTCGTTTTGTGTTCCCCTCTCT GCTCTTTCCATTTCATGACGGAAATGCTGCAGGGCGTGCTCCTTTGTGTTCCCCTCTCT CCCGTTTCCATTTCATGACGGAAATGCTACAAGAGGACCTCCAATAGGTTCAAACG CAAGCAGTTCACTTTCTGGTGGTGTTAGGAGGGCGTGCTCCTTTGCCTTGCCTTGGCAT CCAAGTAGTATTTGGTGGGCGTGTTTGAGAGGAGCTGCTCTTGCCTTGCCTTGGCAT CAAGCAGTTCACTTATTCTGGTGTGTCAGGTGACCCGGCAGTCGCTCAATGGTAACG CTACAGTACCTTTGGTGGGCCGTCTTTGGCTTGCCTGCGCGAGCCCGTAATGGTACCA CTACCAGTCACTTCTGGTGTCTCCAGTGCCAACTGCTAACGTTCTTAGGGCCACCTGTA AACCTCACTCCCTTTGCTGTGCTCTCAGTGCCAACTGCTAACTTCTCTACGGCGA CTTCGATGACACTTCTACGCCCCAATTGGCGAACTCCTCTTG CGCGTTGGCAAAGTCACCCCGTATTGGCGCAACTACTTCTACTGGCGAACTCAGT CGGCTGGGCAAGAGCACCGATATTGGCGAACCTCACTTCTACTGCGCAACTTCTACTGGCGAACTCACTTCTACTGGCCAACTTCTACTGCCCACAATCCACTCCCCACAATCGCCCAAATGCCCCCACTACTTGGCCAACTTCTACTGCCCACAATCGCCACAATCGCCACTCACT	C Nb1 Nb3 Nb1 Nb3 Nb1 Nb3 Nb1 Nb3 Nb1 Nb3	GCATATAATAAGTTCACTCGTTTGGGGTCACACCAGTGCAAGACAATGCAATAATGAT TAATGGTT ACCAGCACCACTACCATTGTCTTTTCAGCACAGTGGTATTTGGTTATTGGCTACAGACAAAGCA ACCACTACAGTAATCGTTGTGCTCTTCAGTACAGTGTCTTGGCTAAGACAAAGCA CTTATAAGTCTCCTGCTGCCACCACGAGGCAGTAAGTACGGTTCTGCTCAGCAGAAGCA CTTATAAGTCTCCTGCTGCCACCACGAGGCAGTAAGTAGGTCGTCGACGTCGAAGTCGAAGT CTGATAACCACTCACTCCCACCACGAGCAGCAGTAAGTAGGTCGTCGAAGTCGAAGTCGACTACT ACCACAACTACTCTTCACGGCCACCTTCAGCCAGCAGAGAGGGTCTGAAGTCGATCTA ACCCCAACTACTCTCTACAGGGCCACTTCAGCCAGCAGAGAGGGTCTGAAGTCGATCTA ACCCCAACTACTCTTACAGGGCCCCTTTGCTCAAGTCGAGGTCTGAAGTCGATCTAC ACTCCAACTATCTCTCACAGCGCCCCTTTGCGCCAAGAGGGTCTGCAAGTCGACTCTA ACCCCAACTACTCTCTCACGCCCCCCGCGTTGGAGGTCGTCTTTTGGCCCCACCACCACTACT ACCCCAACTACTTCTCACGCCACCCAGCTTGAGGAGGTCTGCTTTTGGCGCCACCACTCAT ACCCCAACTACTTCTCACGCGCACCACGCAGTTGAGGAGCTTGTCTATGTTTGAGGAGCCTGTTTTGAGGAGCCGTGTTT
GAGGCATTCTTCCGTTCGTGAAATAGCTCTCATGCTTCTGATGGCATATTTGTCCTACAT GTTGGCTGAGCTTTTGCAGCCTAATGGACTCTTGATCGTCTTTTCCGGAATTTGA GTTGGCTGATGTCATTGCAGCGATATGGACTGATAGTGACATACGAGCAATTGA ITGAAGCTATGTCTTTCATTGCCGAAACGTTCATATTTTATACGTGGGGAGTGGACGG CTGGACATTGAGAAGTGGAAAATGAGCAAACAGAGTGTTTGGACTTCAATGGGGCATGGA ATGGCACCGTGCTCATTGTGATAGTGCGGGCGTGCTGTGTGTG	C Nb1 Nb3 Nb1 Nb3 Nb1 Nb3 Nb1 Nb3 Nb1 Nb3 Nb1 Nb3	GCAT ATAATAAGTTCACCTCOTTTGGGTCACACTCAGTTGCAGGACAATGCAATAATGATT TAATGGTT TAATGGTT ACCAGCACCATTACCATTG TCTTTTCAGCACAG TGGTATTTGGTTTTGCATTGACAAAACCT ACCACTACAGTAATCGTTGTGCTCTTGG ACCTTGTTTTGGCTTCTTGACAAAACCT ACCACTACAGTAATCGTGTGCCCCTCAGTACAAGGAGT CTTATAAGTCTCCTGCCGCCCACCTCAGGCAGCTAAGGAGGTTCTGACATAGGA CTCAAAAACTCTTACTCCCACTCAGCAGTCAAGGAGGTTCTGAAGGAGGTCTTA ACCACTACAGTACTCCTACAGCGCCCCTTCCTTCATTGCAGGGGGTCTGTACTGGAAGTCGACTTA ACCTACTAAGTCTCCTCACGCCCCCCCCCCCCTCAGTCAAGGAGGTCTCGTGAGGGGTCTACTTCGAGGATCTGCC ACTCCAAAATCTCTTCCAAGGCGCCCTTCTCTTCTTGT ACTCCAAAATCTCTTCCAAGGCGCCCCTCCTTCCATTCGAGGGGTCTGCTGCGAGCCACTCTAT ACTACTAAGTCTCTCCACACCACCCACCCAGCTGAGGGGTCGTGTTCTTCGGGGCCCACTCCTT ACTACTAAGTCTCTCCAAGGCGCCCCTTCCTTCGAGGGTCGTCTTCTTCGGGGCCCACTCTT ACTCCAAAATCTCTCTCCCCCCCCCCCCC
GAGGCATTCTTCCGTTCGTGAAATAGCTCTCATGCTTCTGATGGCATATTTGTCCTACAT GTTGGCTGAGCTTTGCAGCCTCAGTGGATTTTGACCGTCTTTTCTTTTCCGGAATTTTGA IGTCTCACTTGCATGCCTAATGTGACTGATAGTTCAAGAATCACTACGAGGCATGCA TTTGAAGCTATGTGTTTCATTGCCGAAACGTTCATATTTTTTATACGTGGGGGATGGACGCG CTGGACATTGAGAAGTGGAAAATGCACAAACGATCATATTTTTTATACGTGGGGGATGGACGCG CTGGACATTGAGAAGTGGAAAATGCACAAACGATGCTTTGGACTTCAATGGGACATT ATGGCACCGTGCTCATGGATAGCTGCGGGGCGGG	C Nb1 Nb3 Nb1 Nb3 Nb1 Nb3 Nb1 Nb3 Nb1 Nb3 Nb1 Nb3	GCATATAATAAGTTCACTCCTTTTGGGTCACACTCAGTTGCAAGACAATGCAATAATGATT TAATGGTT ACCAGCACCACTACCATTGTTCTTTCAGCACAGTGGTATTTGGTTTATGACAAAACCA ACCACTACAGTAATCGTTGTGTGTCTTCAGTACAGTGGTTATTGACCAAAAACCA CTTATAAGTCTCCTGCTGCCACCACAGAGGCAGTTAAGTACGGTTTCTTGTCAAGAGCAA CTTATAAGTCTCCTGCTGCCACCACAGAGGCAGTTAAGTAGGGTTCTCTAAGTAAG
GAGGCATTCTTCCGTTCGTGAAATAGCTCTCATGCTTCTGATGGCATATTTGTCCTACAT GTTGGCTGAGCTTTTGCAGCCTAATGGACTCTTTGACCGTCTTTTTCCGAGGATTTGA TGTCTCACTTGCATGCCTAATGGACTAATGGACTGATAGTTAGACATACGAGACATTGA TGTGCTCACTATGCATGGCATAATGGACATATGTATGTTCAGAGACATCACGAGGCATGCA TTGAAGCTATGCTGTGCAATGGAAATGGACAAACGTCATATTTTATACGTGGGGATGGACGGG CTGGACATGAGAAGTGAAAATGGACAAACGTCATATTTTTATACGTGGGGATGGACGG CTGGACATGAGAAGTGAAAATGGACAAACGTCATATTTTTATACGTGGGGATGGACCGG TGGACATGAGAATGGAAAATGGACAAACGGACACCTCCATTAGGTTCGATGCCTCCTCT GCTCTTTCCATTTCATGAACGGAAATGCTCAAGAGGACCTCCCTATTGGCTTGCCTCCCTC	C Nb1 Nb3 Nb1 Nb3 Nb1 Nb3 Nb1 Nb3 Nb1 Nb3 Nb1 Nb3	GCATATAATAAGTTCACTCOTTTGGGTCACACCACGAGAGACAATGCAATAATGATT TAATGGTT ACCAGCACCATTACCATTG TCTTTTCAG CACAGTGGTATTTGGTTTATTGACAAAGCCA ACCACTACAGTAATCOTTGTGTCTTTCAGGAGCAGTTGAGTAGAGTTTTGACAAGACCA ACCACAAAATCCTCTGCCCCCCACGAGGGCGGTTAAGTACAGGAGCTAGCATAGATGACA CTGATTAACCATCTACTGCCACCAGAGGCGGTTAAGTACAGGAGCAGCATAGATGAG ACCCCAAAATCCTCTTACAGCGCCCATCATGACCAGGCAGTCAAGAGGAGCAGCATAGATGACA ACCCCAAAATCCTCTTACAGCGCCCATCATGACCAGGCAGTCCAAGAGGAGCTAGCATAGATGAC ACCCCAAAATCCTCTTCCACGCCACCAGGAGCGGATGCAAGAGGATGCTACTACGGCCACCACCACCACCACGAGTTGAGGAGGCTGATGAGGAGCTCATTCCAGGGCGCAAGTTGGAGGCCGATGATGGCCCATCCAT
GAGGCATTCTTCCGTTCGTGAAATAGCTCTCATGCTTCTGATGGCATATTTGTCCTACAT GTTGGCTGAGCTTTGCAGCCTAATGGACTCTAGTGGCTCTTTTCCGGAGATTTGA GTTGGCTGAGCTTTGCATGGCTAATGGACTGATAGTCAGAGACCTACGAGGCATGCA TTGAAGCTATGCATGGCATAATGGACCTGATAGTTCAAGAGTCACTACGAGGCATGCA TTGAAGCTATGTCTTTCATTGCCGAAACGTCGATATTTTATACGTGGGGGATGGACGG CTGGACATTGAGAAGTGGAAAATGAGCAAACAGAGTGTTTTTATACGTGGGGGATGGACGG CTGGACATTGAGAAGTGGAAAATGAGCAAACAGAGTGTTTTTATACGTGGGGGATGGACGG CTGGACATTGAGAAGTGGAAAATGAGCAAACAGAGTGTTTTTATACGTGGGGGATGGACCGC CTGGACATTGAGAAGTGGACAATGAGCGAACAGAGGCTCTCCATTGGCTTGCCCTCTTGT GCTCTTTCCAATTTCATGAACCGAAATGAGCAACAGAGGCGCTCTCAATAGCGTTCAACA CCAAGCAGTTACTTTGGTGGCCTGGTCTTATGAGAGGAGCTGCTCTTATGCCTTGGCTT CCAAGCAGTTCCCTTTGGTGTCTCAGTGACACTGGTATTGGTGCTCCTTGGCATTGCTAGCA CTACAGTAATCGTTGTGCCTCTTGGTGTCAAGAGTGGCCACGACAAGCCCCTAATGGTTACCA CTACAGTAATCGTTGTGGCCTGTTTGGAGCAGCCGCACAACGTCTGTAGCAA CCCCCCACTCCCTTGCTTTGGAGGCCGCCCACACCTCTCTCT	C Nb1 Nb3 Nb1 Nb3 Nb1 Nb3 Nb1 Nb3 Nb1 Nb3 Nb1 Nb3 Nb1	CATATAATAAGTTCACCTCTTTGGGTCACACCAGTGCAAGACAATGCAATAATGATT TAATGGTT TAATGGTT TAATGGTT TAATGGTT COCAGCACCACTACAGTAGTGCTCTTTGGCTCTTGGCAAGTCAAAAACCT ACCACTACAGTAATCGTTGTGCTCTTGG AGCTGTTTGGCAAGTCAAAAACCT ACCACTACAGTAATCGTTGTGCGCTCTTGG AGCTGTCTGGCAAGAGCCA TCCAAAAACCATCTACTACGCCGCCACTCCAGGCGAGTAAGGGGTCTCATCAGAAGTCGACTGA ACCACTACAGTACTCCTACGCCGCCACTCCAGCCAGTGCAAGGGGGTCTGATCGGAAGTCGGCAGTCAG ACCACTACAAGTCCTCTCCAGCCACCCAGCTGAGGGAGTAAGGGGTCTCTTGGAAGTCGGACGTCTA ACCACTACAGTACTCCTACGCGCCACTCCTAGCCAGTCGAGGGGGTCTGCTGCGAGCCAGTCAT ACCACTACAGTCCTCCACACCCAGCGGTTGAGGGAGTCAGTTCTGCGGCCACTCCT ACTACTAAGTCCTCTCCCACCCCAC
GAGGCATTCTTCCGTTCGTGAAATAGCTCTCATGCTTCTGATGGCATATTTGTCCTACAT GTTGGCTGAGCTTTGCAGCCTCAGTGGATTTTGACCGTCTTTTCTTTTGCGAATTTTGA IGTCTCACTTGCATGCCTAATGTGACTGATAGTTCAAGAATCACTACGAGGCATGCA TTTGAAGCTATGTGTTTCATTGCCGAAACGTTCATATTTTTATACGTGGGGATGGACGG CTGGACATTGAGAAGTGGAAAATGCACCAAACGGTGTTTGGACTTCAATGGGGACATG ATGGCACCGTGCTCATTGTGATAGCTGCGGGCGTGCTGCTTTTGTTCCCTCTCT GCTCTTTCCATTTCATGAACGGAATGCTACAGAGGACCCTCCAATAAGTTCCAATCA CCAGATAGTAATTGGTGGGCTGGTGTTATGAGAGGACCCTCCAATAAGGTTCCAAC CAAGCAGTTCACTTATTCTGGTGGTGTTACGAGGAGCCTGCTGCGCGTGATGGTACCA CAAGCAGTTCACTTATTCTGGTGGTGTTACGAGTGCAGGCAG	C Nbl Nb3 Nb1 Nb3 Nb1 Nb3 Nb1 Nb3 Nb1 Nb3 Nb1 Nb3 Nb1 Nb3	CATATAATAAGTCACCCCTTTTGGGTCACACCCAGTGCAAGACAATGCAATAATGAT TAATGGTT ACCAGCACCATTACCATTGTTCTTTCAGCACAGTGGTATTGGTTTATGACAAAACCA ACCACTACAGTAATCGTTGTGTGTCTTCAGTACAGGGTTTATGACCAAAAACCA CTTATAAGTCTCCTGCTGCCACCACAGAGCAGTTAAGTACAGGGTTTCTCAAGAAAAACCA CTTATAAGTCTCCTGCTGCCACCACAGAGCAGTTAAGTACAGGGTTTCTCAAGAGCAATGCAAA CTTATAAGTCTCCTGCTGCCACCACAGAGCAGTTAAGTACAGGGTTCTCTAAGTACAGAGAGT CTTATAAGTCTCCTGCGCCACCACAGAGCAGTTAAGTACAGAGGTTTCTCAAGTACAAGAGAA CTTATAAGTCCCACAGAGCACTTCAAGCAGTCAAGAGGGTTCTCTAAGTACAGAGAGT CTTATAAGTCCAAGAGTTCCACCACCACGAGCAGTTAAGTAGAGGTTCTCTAAGTACAGCACACTAACTA













Supplementary Table S1.

Gene name	Unigene No.	Primers
TPX1	SGN-U505985	F 5' GCCGTTGGTGACATCATTCCC 3'
		R 5' CCTTTAGATTTCAACTCGTCAGC 3'
COX6B	SGN-U511126	F 5' CAGGTTCCCAACAACCAATCAAA 3'
		R 5' CCCATTCTCCCTCTGCTCGTT 3'
CSD1	SGN-U514519	F 5' GCAGCAGTGAAGGTGTTAGTGGCA 3'
		R 5' GGATTGTGGACCAGCAAGAGGAAT 3'
CAT1	SGN-U518840	F 5' CATACAAGTACCGTCCGTCAAGTGC 3'
		R 5' GAAAAACCCTTTGGCACTAGCACCTC 3'
TAPX	SGN-U506407	F 5' TCCCTCAAATGCCTCCAATCCT 3'
		R 5' GCATTTCGGCGAGGCTAACG 3'
PER21	SGN-U506123	F 5' TTGACAATGAAGCCCAGGTGA 3'
		R 5' CTCCTCCGCAGATTGATTGG 3'
PER12	SGN-U515270	F 5' CTGCTAGGGACTCTGTTGTCTTGACT 3'
		R 5' CGACGGAGGACGACTGTGGTATT 3'
NOS	SGN-U518155	F 5' TAAGACAACACGCCTTGGATT 3'
		R 5' GCCTGCCTTACTATTTGCCTC 3'
APX6	SGN-U521022	F 5' GGGTTTGGGAATCCAGTTGTC 3'
		R 5' TGTCAACAAGTTTGGTATAGGCATT 3'
PRXR1	SGN-U509102	F 5' TTTGCGGAGGACAATTCTGGC 3'
		R 5' CCTGGACACTCCCTTTCAACAG 3'

APX3	SGN-U515717	F 5' GGAAGGACGGTTGCCCGATGCT 3'
		R 5' TGTCTGTGGGAAGTTTCAGTAGGC 3'
FSD2	SGN-U508368	F 5' CTGGTTCTGGCACAATAACGGCT 3'
		R 5' TCCTCCTCCGTTGGGCTTCA 3'
FSD1	SGN-U508369	F 5' TGGCTCGCATACAAACCTGAA 3'
		R 5' CCACGACACGAGCTTCTCCATAA 3'
MSD1	SGN-U508400	F 5' GTGAGCAGACGGACCTTAGCAACA 3'
		R 5' CGTGACCTCCGCCGTTGAATT 3'
GST8	SGN-U513459	F 5' GCAGTATGTATGGGATGAGGGC 3'
		R 5' TTCACTGCATATTCATGTACGGTATT 3'
GST21	SGN-U513460	F 5' GGTTCTGGGCTGATTACACTGAC 3'
		R 5' CCAAAGTAAGGTTTGTCTCCCAAT 3'
GST29	SGN-U511196	F 5' CAGCCAAGGGCAATGTCCAAGTA 3'
		R 5' GTTCTGGTCCATCTATTCTCCCTTCT 3'
PR1	SGN-U512904	F 5' TCAACTCAACGCCGCTGGTG 3'
		R 5' GAATCAAAGGGCTGTTGCTCTTCA 3'
PR4	SGN-U508784	F 5' TTTCTGTGATGCTGCTGGACC 3'
		R 5' GCCTTGCTGATAGCCCACTCC 3'
Gns1	SGN-U515120	F 5' GAAAGCCTGGACCTATTGAAACC 3'
		R 5' GCAGTAGCATTAGTTTCAACCGAAC 3'