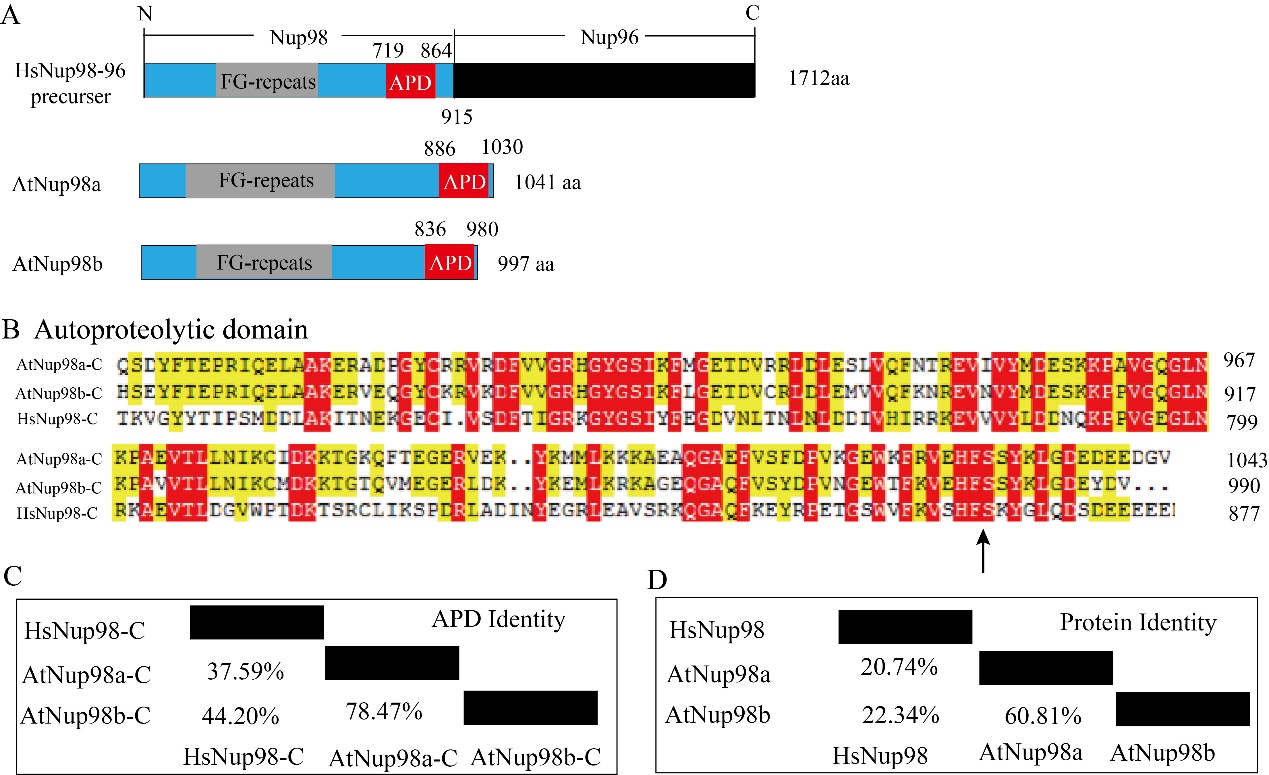
**Two *Nucleoporin98* homologous genes jointly participate in the regulation of starch degradation to repress senescence in *Arabidopsis***

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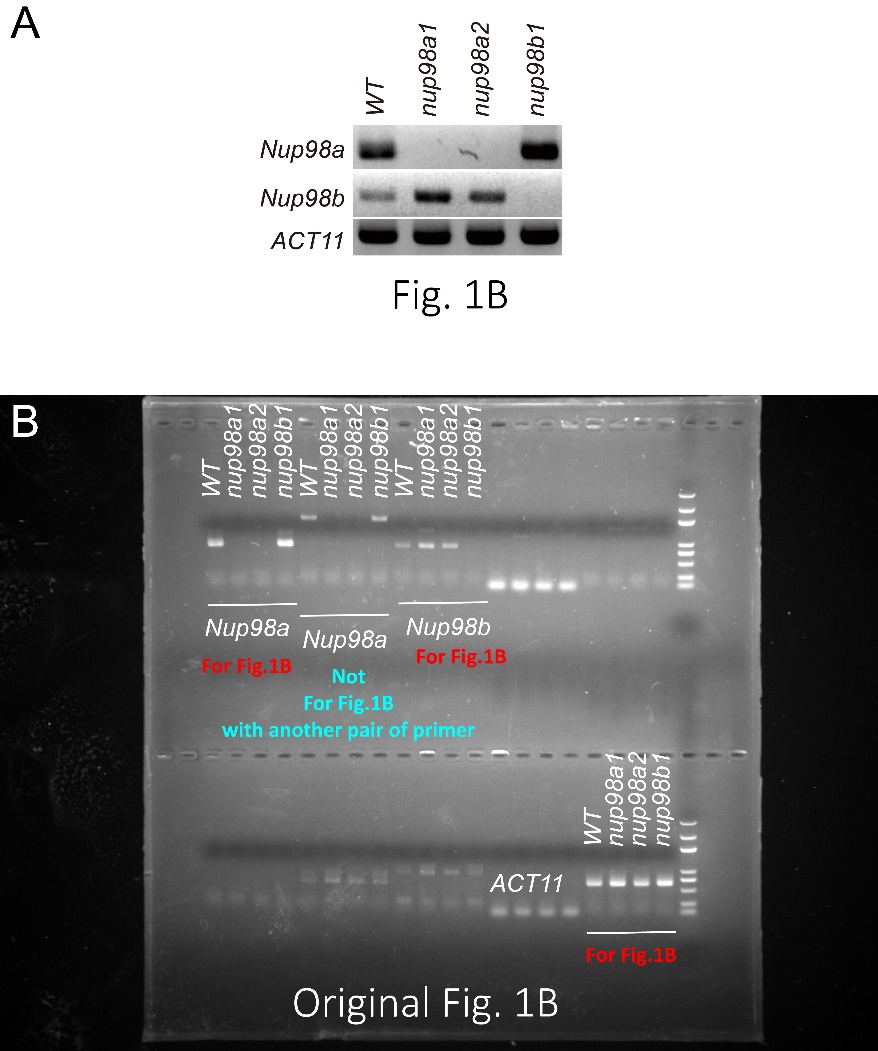
**Additional files**

**Supplementary Table 1. Primers used in this study.**

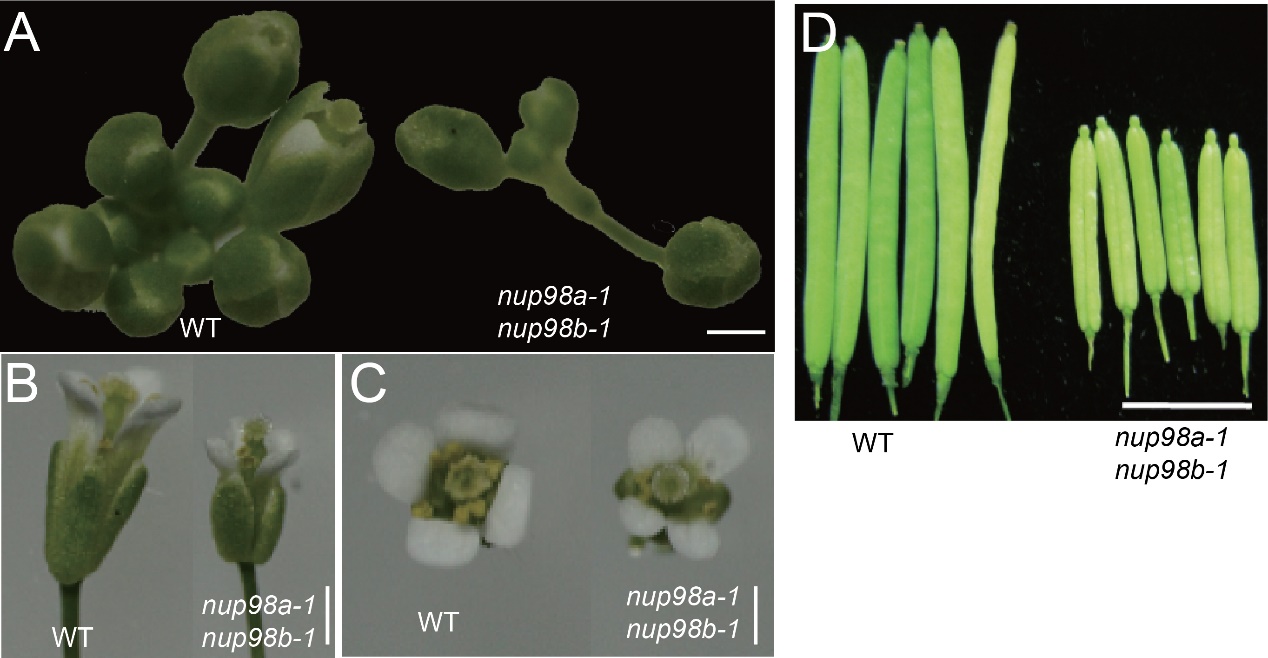
|  |  |  |  |
| --- | --- | --- | --- |
| Primer Name | Gene ID | Nucleotide Sequence (5’-3’) | Purpose |
| At4g34270-F | At4g34270  Reference gene | gtgaaaactgttggagagaagcaa | qRT-PCR |
| At4g34270-R | tcaactggataccctttcgca | qRT-PCR |
| AGL15-F | AT5G13790 | ttccttctaccttcttctc | qRT-PCR |
| AGL15-R | agtgacttgtctgctatt | qRT-PCR |
| AMY1-F | At4g25000 | cttggctatgattgatgat | qRT-PCR |
| AMY1-R | ttatgcgttacttcttctc | qRT-PCR |
| AMY2-F | At1g76130 | ctcggttggagaatgttg | qRT-PCR |
| AMY2-R | ctgattatacgctgtctatgg | qRT-PCR |
| AMY3-F | At1g69830 | tgagatattatgccaaggt | qRT-PCR |
| AMY3-R | gaacagtgaatccaagtg | qRT-PCR |
| ARF2-F | At5g62000 | gatgatgataaggttgac | qRT-PCR |
| ARF2-R | aatctgtgtaagtaggtt | qRT-PCR |
| ARP4-F | [AT1G18450](http://www.arabidopsis.org/servlets/TairObject?id=29135&type=gene) | gtctgagtatgaggaaca | qRT-PCR |
| ARP4-R | ctaataatcttcaaggaacaac | qRT-PCR |
| BAM1-F | At3g23920 | accattgaagaacagagaa | qRT-PCR |
| BAM1-R | tcatcacaaacacaggaa | qRT-PCR |
| BAM2-F | At4g00490 | agattcaggttgttatgt | qRT-PCR |
| BAM2-R | aagtagatgtcaggattg | qRT-PCR |
| BAM3-F | At4g17090 | ggaatagcgagtatggaa | qRT-PCR |
| BAM3-R | ctgaagataggagttggt | qRT-PCR |
| BAM4-F | At5g55700 | tgatgatgccgattgatac | qRT-PCR |
| BAM4-R | gctaacttgagtgccttaa | qRT-PCR |
| BAM5-F | At4g15210 | aagataccgacaatacag | qRT-PCR |
| BAM5-R | gcaacttctataccttct | qRT-PCR |
| BAM6-F | At2g32290 | gtcatcacgaacgataacg | qRT-PCR |
| BAM6-R | accatcactccatctacttg | qRT-PCR |
| BAM7-F | At2g45880 | tggatgtgaaggaagaag | qRT-PCR |
| BAM7-R | actcgtcattactactactg | qRT-PCR |
| BAM8-F | At5g45300 | cagagatggttgtatgagat | qRT-PCR |
| BAM8-R | aggagaaggttgacgata | qRT-PCR |
| BAM9-F | At5g18670 | cttccgttagacacagtt | qRT-PCR |
| BAM9-R | ctcaataccttctacaccaa | qRT-PCR |
| CAT1 | At1g20630 | tattcttcgtccgtgatg | qRT-PCR |
| CAT1 | cagtatcctccagttctc | qRT-PCR |
| COI1-F | AT2G39940 | atggcggtgtatgtctcag | qRT-PCR |
| COI1-R | gcggaagtcacagaggtt | qRT-PCR |
| DPE1-F | At5g64860 | gcaaggagttgatataatgg | qRT-PCR |
| DPE1-R | cgctaacaagaagaggaa | qRT-PCR |
| DPE2-F | At2g40840 | ggacttagactcaactatg | qRT-PCR |
| DPE2-R | gattaccaccagattcaa | qRT-PCR |
| EBP1 | At3g51800 | ccaatcgctcctccttaa | qRT-PCR |
| EBP1 | gaactcatctctgataactctc | qRT-PCR |
| GWD1/SEX1-F | At1g10760 | aagttggcaggttattagt | qRT-PCR |
| GWD1/SEX1-R | ttgtaggtctatcgtaggt | qRT-PCR |
| GWD2-F | At4g24450 | agtgtgataatggatgaag | qRT-PCR |
| GWD2-R | caatcgctgagaatagac | qRT-PCR |
| GWD3/PWD-F | At5g26570 | gacacatcaggagaatcaa | qRT-PCR |
| GWD3/PWD-R | cagtatcaggagcatcatt | qRT-PCR |
| HXK1-F | [AT4G29130](http://www.arabidopsis.org/servlets/TairObject?type=locus&id=127303) | ctacagaatgcgaagact | qRT-PCR |
| HXK1-R | taccagaggacagagaag | qRT-PCR |
| ISA3-F | At4g09020 | tggcatagacaacaaggt | qRT-PCR |
| ISA3-R | tggatggttacagttcagt | qRT-PCR |
| KIN10-F | At3g01090 | cagatggtatgctcagtaac | qRT-PCR |
| KIN10-R | tgcttcgttctctattatgc | qRT-PCR |
| KIN11-F | At3g29160 | gtttactttgccgggttac | qRT-PCR |
| KIN11-R | ccatcaaaaagaagaaagga | qRT-PCR |
| LDA-F | At5g04360 | aatgatgagactgttgag | qRT-PCR |
| LDA-R | aatgttggaagaagatgta | qRT-PCR |
| LHCA1-F | At3g54890 | tctcttcttccaagtctaa | qRT-PCR |
| LHCA1-R | attctgatacgaccaaca | qRT-PCR |
| LHCA2-F | At3g61470 | aataatggcatcatctctt | qRT-PCR |
| LHCA2-R | cagtcaacttcttcttca | qRT-PCR |
| LHCB1.1-F | At1g29920 | tgagccaagttctatctgt | qRT-PCR |
| LHCB1.1-R | ctctaccatccaccacaa | qRT-PCR |
| LHCB1.4-F | At2g34430 | gagtgaagttcggagaag | qRT-PCR |
| LHCB1.4-R | ccaagtagtccaatcctc | qRT-PCR |
| LSF1-F | At3g01510 | ggcattcagcaacttcac | qRT-PCR |
| LSF1-R | agcaacatcaccaactaca | qRT-PCR |
| LSF2-F | At3g10940 | gagaacgatgagtgtgatt | qRT-PCR |
| LSF2-R | agaagtgaagcaagaagatt | qRT-PCR |
| NAC1-F | AT1G56010 | aggagttatatgtagagac | qRT-PCR |
| NAC1-R | tgatgatgtagtgatgat | qRT-PCR |
| NAP-F | AT1G69490 | gaagaagagataatgatgatg | qRT-PCR |
| NAP-R | caggttgatgaagatgat | qRT-PCR |
| NPR1-F | AT1G64280 | attgccaaggattacgaagt | qRT-PCR |
| NPR1-R | tctcactctgctgctgta | qRT-PCR |
| ORE1/NAC2-F | At5g39610 | tacgaggcatcaagaatc | qRT-PCR |
| ORE1/NAC2-R | aggtagtgagttatgagttc | qRT-PCR |
| PHS1-F | At3g29320 | ggattggtggagaagacat | qRT-PCR |
| PHS1-R | gagccgcagattgatagt | qRT-PCR |
| PHS2-F | At3g46970 | agagattgacaagaggtt | qRT-PCR |
| PHS2-R | acacataagttagccattc | qRT-PCR |
| RPS6A | At4g31700 | agaagccaagaatgagag | qRT-PCR |
| RPS6A | ggtaagtgttgacataggt | qRT-PCR |
| SAG12-F | AT5G45890 | caagcactgatgaaggcagt | qRT-PCR |
| SAG12-R | tgcactctccagtgaacaca | qRT-PCR |
| SAG13 | AT2G29350 | gacaacataaggacgaactctg | qRT-PCR |
| SAG13 | tacgcaccgcttctttct | qRT-PCR |
| SAG2-F | AT5G60360 | tcttcttcttcttcttcttct | qRT-PCR |
| SAG2-R | aactactgatgataggattgt | qRT-PCR |
| SAUR36 | AT2g45210 | atgtctcgtctcactctc | qRT-PCR |
| SAUR36 | atcttcctcatcttcttatagc | qRT-PCR |
| SEN1-F | At4g35770 | gcttggacagagaatgag | qRT-PCR |
| SEN1-R | tgattgatacttgcgttga | qRT-PCR |
| SEX4-F | At3g52180 | tcttcttcttcttcttct | qRT-PCR |
| SEX4-R | gtcttggctatattcatc | qRT-PCR |
| TPS1-F | AT1G78580 | catcaggagaccgaagac | qRT-PCR |
| TPS1-R | tgttgttgttgttagagttagag | qRT-PCR |
| WRKY53-F | At4g23810 | catcatcgccaagattacat | qRT-PCR |
| WRKY53-R | ggtccttctaagcctctc | qRT-PCR |
| WRKY6-F | At1g62300 | ttctccttcgttaatctc | qRT-PCR |
| WRKY6-R | atcatcttcttcgtcaat | qRT-PCR |
| WRKY70-F | At3g56400 | aatgaaggaagaagacaa | qRT-PCR |
| WRKY70-R | gcctgatgatgataatct | qRT-PCR |
| RT98a-F | At1g10390 | atccgttgtcaaactacagagg | RT-PCR |
| RT98a-R | ggagacgagccaaaactaaagg | RT-PCR |
| RT98b-F | At1g59660 | ccaactcctgttacaaacccat | RT-PCR |
| RT98b-R | gtccaatctctcgccttccatc | RT-PCR |
| DRM1-qRT-F | At1g28330 | AGGAAGGAACATGTGGCACT | qRT-PCR |
| DRM1-qRT-R | GAGTCACCGCTGTACAACCA | qRT-PCR |
| DIN6-qRT-F | AT3G47340 | TCTCGACCCTGAATCCAAGATGAT | qRT-PCR |
| DIN6-qRT-R | GTCGTCAAAGGCTCTCCTTAGA | qRT-PCR |
| ATG8a-qRT-F | AT4G21980 | CAATTTGTATACGTGGTTCGT | qRT-PCR |
| ATG8a-qRT-R | AGCAACGGTAAGAGATCCAA | qRT-PCR |
| ATG8E-qRT-F | AT2G45170 | TCTTTAAGATGGACAACGATTTC | qRT-PCR |
| ATG8E-qRT-R | CTCAGCCTTTTCCACAATCA | qRT-PCR |
| SAG12-qRT-F1 | At5g45890 | TATTACAGGTTATGAGGATGTCCC | qRT-PCR |
| SAG12-qRT-R1 | ACCACATAGTCCTTGTTTATCC | qRT-PCR |
| WRKY53-qRT-F1 | AT4G23810 | CTTCTGCTAAACTGGTCATCC | qRT-PCR |
| WRKY53-qRT-R1 | CTCTCTCTGGGCTTATTCTCA | qRT-PCR |
| ACT2-F | At5g09810 | CTCATGAAGATTCTCACTGAG | RT-PCR |
| ACT2-R | ACAACAGATAGTTCAATTCCCA | RT-PCR |
| TIP41-F | At4g34270 | GTGAAAACTGTTGGAGAGAAGCAA | qRT-PCR |
| TIP41-R | TCAACTGGATACCCTTTCGCA | qRT-PCR |



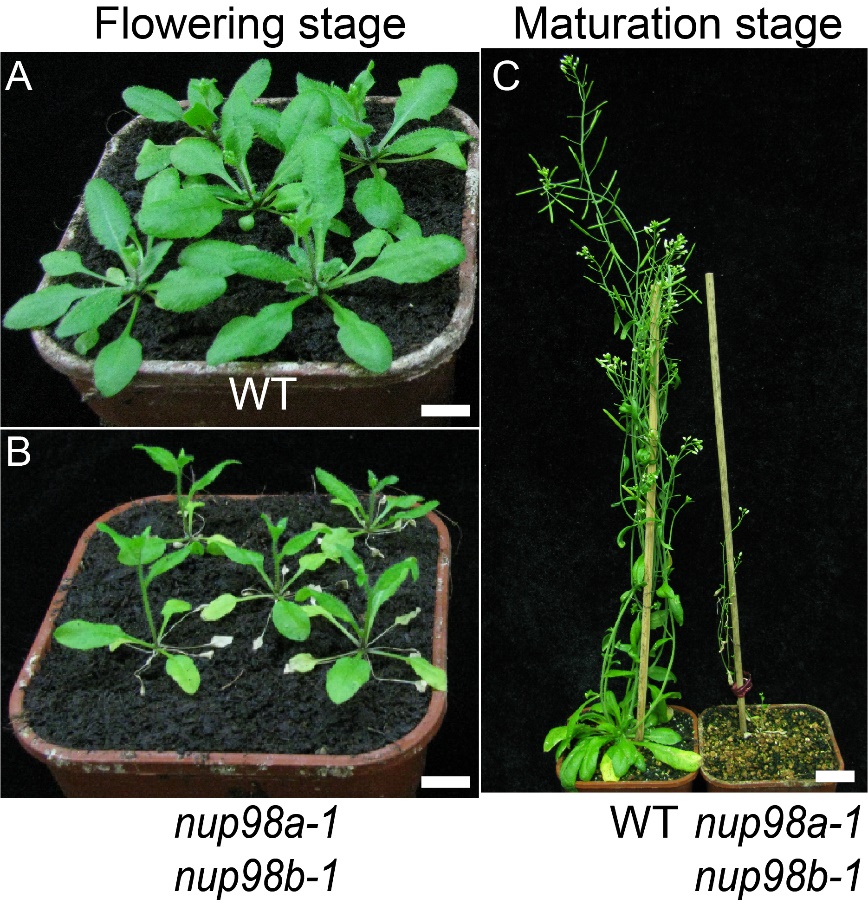
**Figure S1. Protein domains in Nup98a and Nup98b of *Arabidopsis thaliana*.** A, Sketches of Nup98-96 proteins from *Homo sapiens* HsNup98-96 (NM\_016320.4) and two homologs from *Arabidopsis* *thaliana* Nup98a (At1g10390) and Nup98b (At1g59660). APD, autoproteolytic domains. FG, phenylalanine and glycine repeats. B, Alignment of the autoproteolytic domains of HsNup98-96, At Nup98a and At Nup98b. The arrow indicates the conserved HFS cleavage site. C, Overall identity of the autoproteolytic domains of HsNup98-96, At Nup98a and At Nup98b. D, Overall identity of the proteins of HsNup98-96, At Nup98a and At Nup98b.



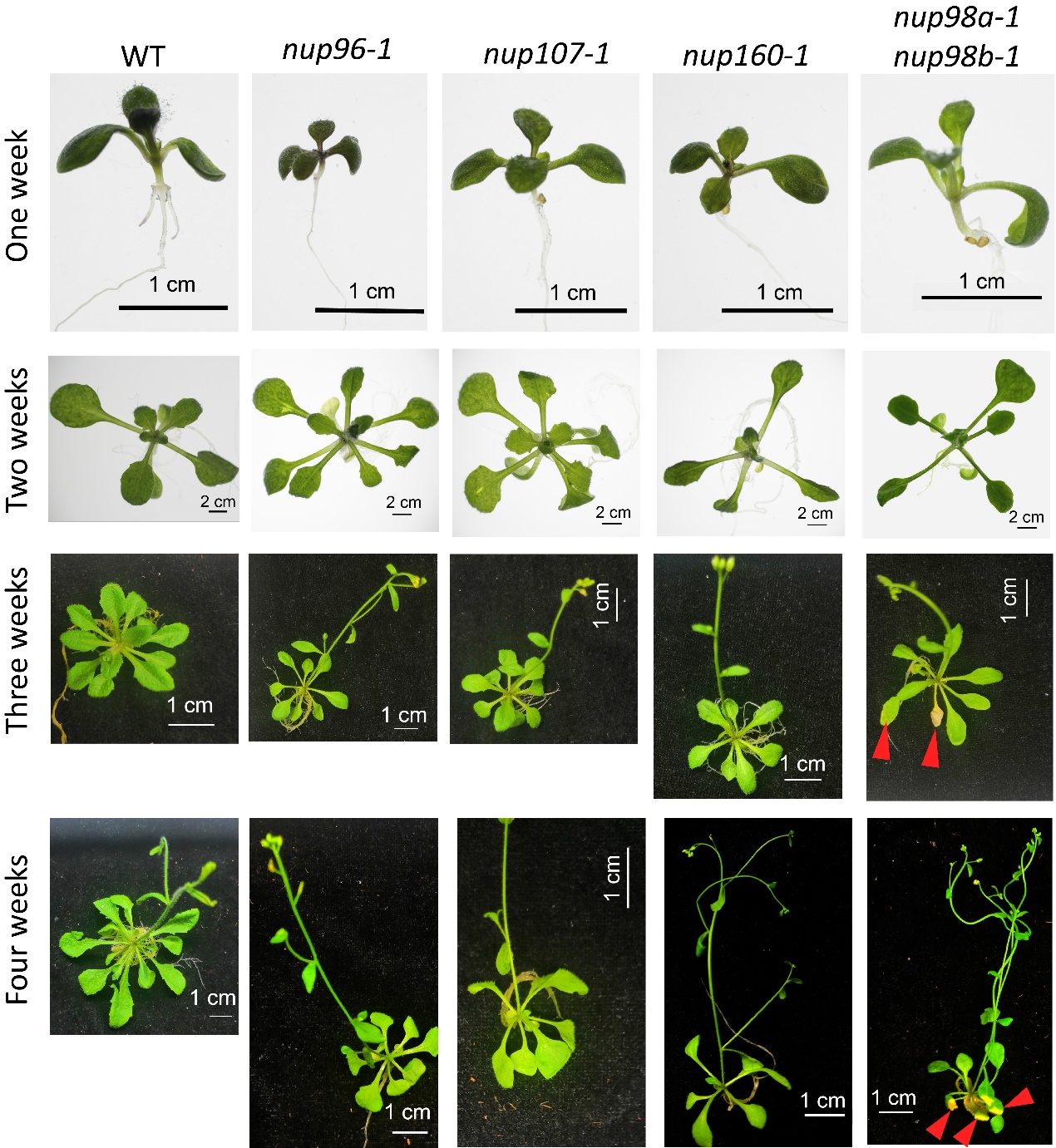
**Figure S2. The original photograph of the gel in Figure 1B.** A, Fig.1B; B, The original photo.



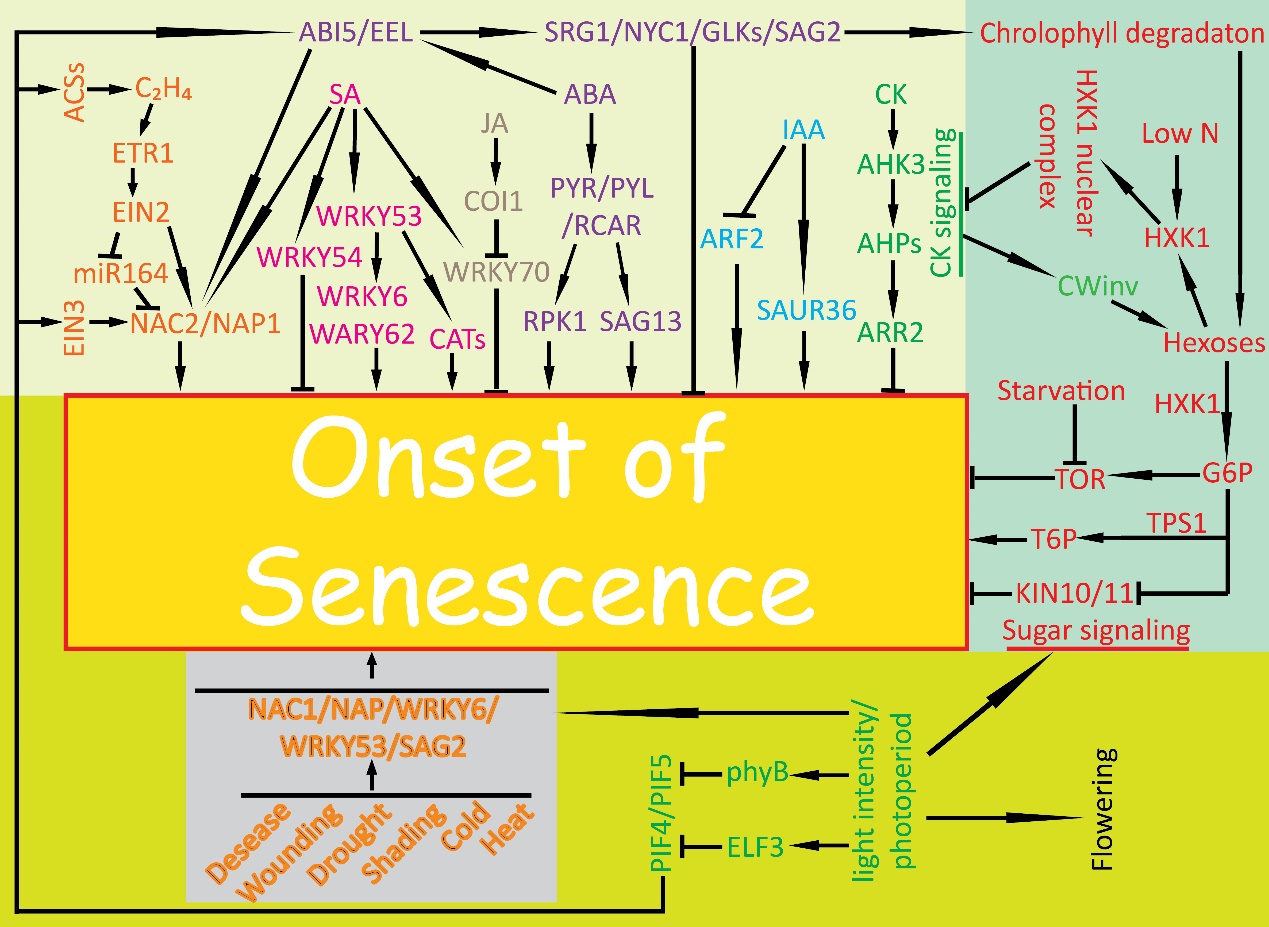
**Figure S3. The *nup98a1*, *nup98b1* double mutant showed pleiotropic phenotypes in various organs.** The seeds of mutant and WT were sown in soil after low temperature treatment for 3 days on wet filter paper and grew in long day conditions. **A**, A top view of Inflorescence; B, A side view of flowers; C, A top view of flowers; D, Siliques. Scale bars = 2 mm. All the images are our own data.



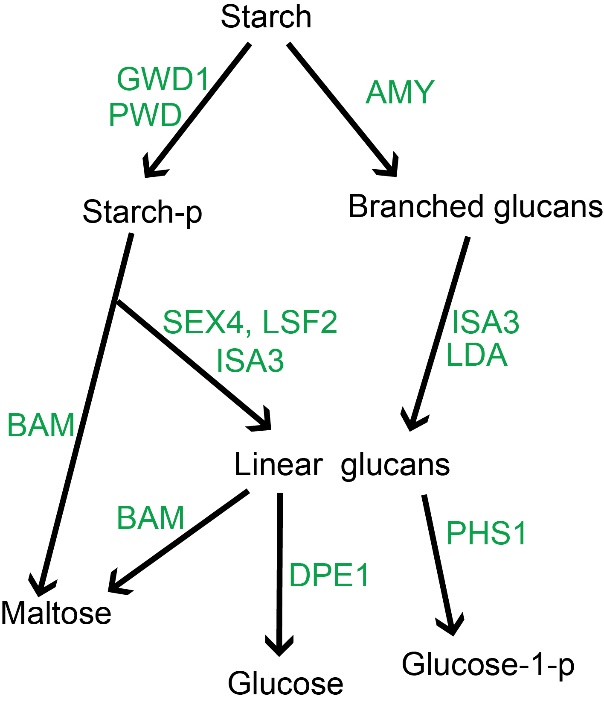
**Figure S4. The *nup98a1*, *nup98b1* double mutant showed a senescent and sterile phenotype.** The seeds of mutant and WT were sown in soil after low temperature treatment for 3 days on wet filter paper and grew in long day conditions. **A** and **B**, Plants at early stage of flowering. Plants grew in dishes supplemented with MS and sucrose for one week, and then transplanted in soil. **C**, Plants at mature stage. All the images are our own data.



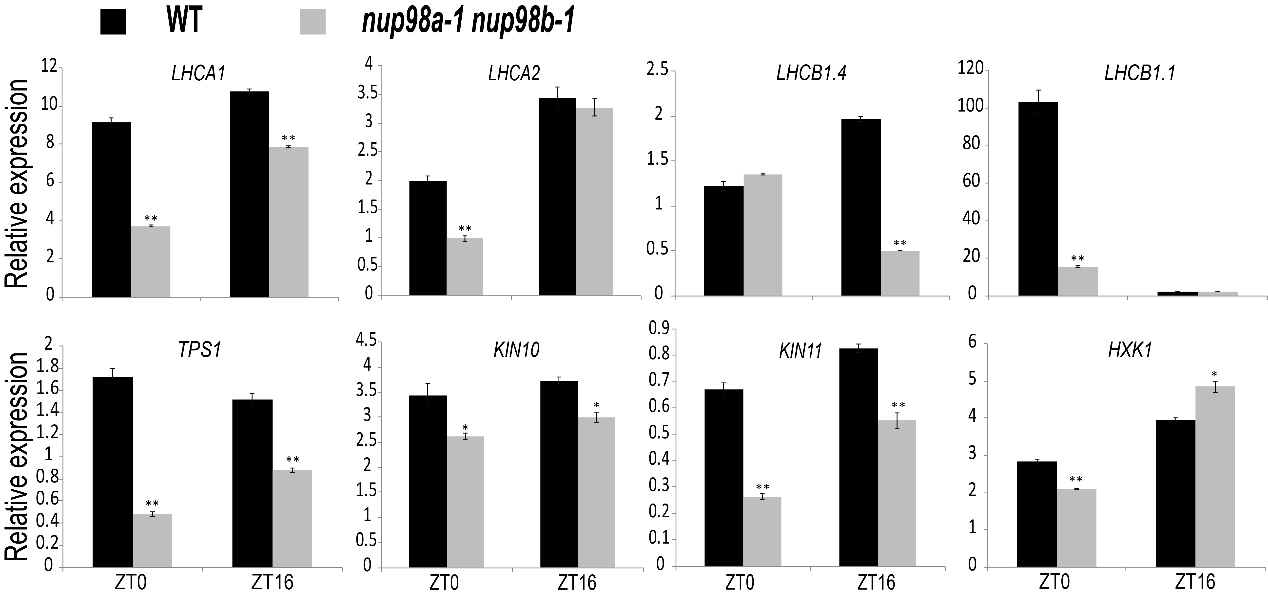
**Figure S5. Senescent phenotypes were specific to the *nup98a1*, *nup98b1* double mutant compared with mutants of other nucleoporins.** The data showed that the mutants of nucleoporin genes, *Nup96*, *Nup107*, and *Nup160*, did not exhibit senescence characteristics even though plants were in bloom, while the *nup98a-1 nup98b-1* mutant appeared senescence symptom (yellow leaves, red arrows) from three weeks after germination. All plants grew in long day conditions. All the images are our own data.



**Figure S6. Molecular network of senescence initiation in plants.** The summary is based on previous publications [1-12](#_ENREF_1), but not limited these publications. Generally, initiation of senescence includes the hormone (light yellow background), sugar metabolism (light green background), and light and photoperiod pathway (green-yellow background), and stress pathways (grey background). These pathways can induce senescence at different developmental stages (yellow background). Genes and metabolites in the same pathway are indicated by the same color. Due to space limitations, this summary model does not include all genes identified to date. Abbreviations: ABA, abscisic acid; ABI, ABA INSENSITIVE; AHK, *ARABIDOPSIS* HISTIDINE KINASE; ACS, AMINOCYCLOPROPANE-1-CARBOXYLATE (ACC) SYNTHASE; AHP, *ARABIDOPSIS THALIANA* HISTIDINE PHOSPHOTRANSFER PROTEINS; ARF, AUXIN RESPONSE FACTOR; ARR, ARABIDOPSIS RESPONSE REGULATOR; C2H4, ethylene ; CAT, CATALASE; CK, cytokinin; COI1, CORONATINE INSENSITIVE 1; CWINV, CELL WALL INVERTASE; EEL, ENHANCED EM LEVEL; EIN, ETHYLENE INSENSITIVE; ELF, EARLY FLOWERING; ETR, ETHYLENE RESPONSIVE; G6P，6-phosphoric acid glucose; GLK, Golden 2-like Transcription factor; HXK1, HEXOKINASE 1; KIN, PROTEIN KINASE10; N, nitrogen; NAC，NAM, ATAF, AND CUC; NAP, NAC-LIKE PROTEIN; NYC, NONYELLOW COLORING; ORE, ORESARA; PHYB, phytochrome B; PIF, PHYTOCHROME INTERACTING FACTOR; PYR/PYL/RCAR, PYRABACTIN RESISTANCE/PYR1-LIKE ORREGULATORY COMPONENT OF ABA RECEPTOR; SAG, SENESCENCE-ASSOCIATED GENE; SAUR, SMALL AUXIN UPREGULATED; SGR, STAYGREEN; T6P, TREHALOSE-6-PHOSPHATE; TOR, TARGET OF RAPAMYCIN; TPS1, TREHALOSE-6-PHOSPHATE SYNTHASE 1; WRKY, WRKY DNA-BINDING PROTEIN. Arrowheads indicate promoting effect while short bars indicate inhibiting effect on senescence. All the images are our own data.



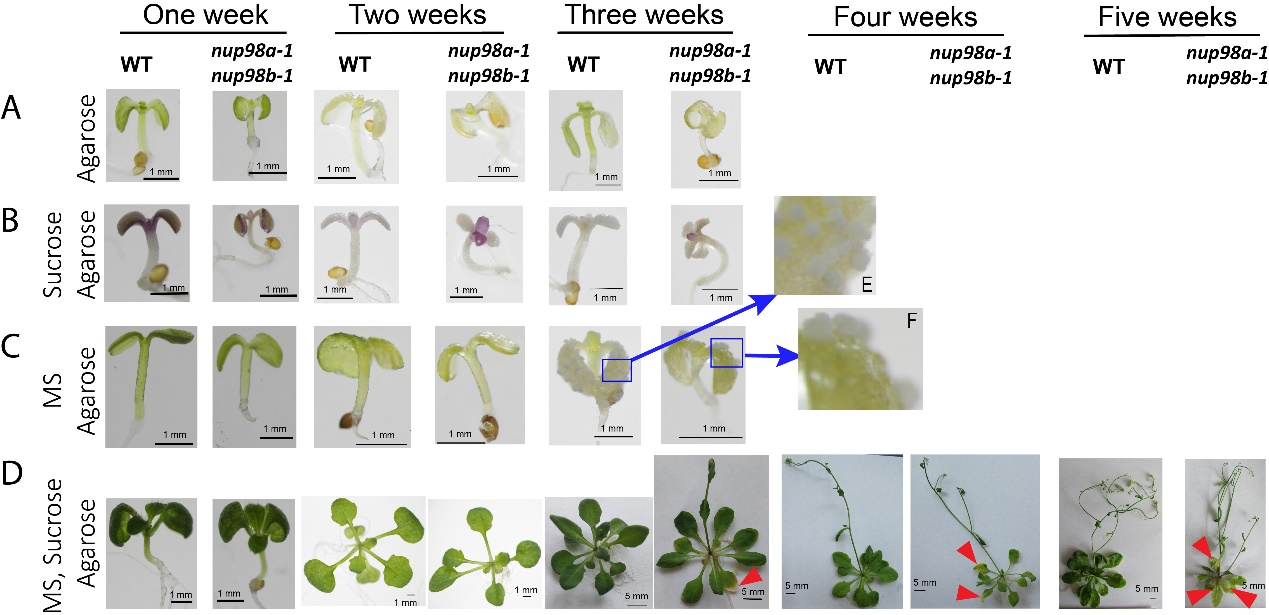
**Figure S7. A simplefied pathway of starch degradation in chloroplasts.** Starch is degraded into maltose, glucose, or glucose -1-phospholate in chloroplast, and these three monosaccharides are exported from chloroplast into cytoplasm to provide energy source for cells. Black words indicate saccharides, while green words represent enzymes degrading starch (only main enzymes showed). GWD, α-glucan, water dikinase; PWD, phosphoglucan, water dikinase; AMY, α-amylase; BAM, β-amylase; SEX4, STARCH EXCESS 4 (phosphoglucan phosphatase); LSF2, LIKE SEX FOUR2 (phosphoglucan phosphatase); DPE, disproportionating enzyme; ISA3, iso-amylase3; LDA, limit-dextrinase; PHS, α-glucan phosphorylase. These degradation steps happen in chloroplasts, and the products of maltose, glucose and glucose-1-p would be exported from chloroplasts into cytoplasm.



**Figure S8. Expression analysis of genes related to photosynthesis and sugar metabolism in the *nup98a1*, *nup98b1* double mutant.** The *nup98a-1 nup98b-1* double mutant and WT plants were grown in petri dishes in long day conditions for 14 days, and then harvested at ZT0 and ZT16 for gene expression analysis. All qPCR measurements were repeated at least three times, in triplicate. Gene expression measurements were normalized to the control At4g34270 and expressed as a relative expression value. Student’s t test was used to statistically analyze the data. An \* indicates measurements that were significantly (\**P* < 0.05; \*\**P* <0.01) different from the control. Error bars indicate ± SD of the mean.



**Figure S9. Exogenous sucrose reduces early senescence in the *nup98a1*, *nup98b1* double mutant plants.** Medium is MS (Murashige & Skoog) basal nutrients and 3% sucrose. The seeds of WT and the *nup98a-1 nup98b-1* double mutant were sown on MS medium with sucrose. After stratification, plants were grown under long day conditions. After inflorescences emerged, plants were transplanted to soil. Senescence symptoms appeared 4 days later in the double mutant leaves, about 12 days earlier than the wild type control. All the images are our own data.



**Figure S10.** **Phenotype analysis of the *nup98a1*, *nup98b1* double mutant compared with WT grown on different mediums.** Medium is MS (Murashige & Skoog) basal nutrients and 3% sucrose. Row A, agarose medium. Row B, agarose plus sucrose. Row C, agarose plus MS basal nutrients. Row D, agarose plus sucrose and MS basal nutrients. Plants cannot fulfil the life cycle and die in about three weeks after germination in medium A, B, and C, whereas plants in medium D can go through the life cycle. There were white callus-like structures found on the leaf surfaces of three week-seedlings on medium C (blue arrow, E and F). The*nup98a-1* *nup98b-1* double mutant displayed senescence leaves (red arrow heads) early than WT (from week three) as Figure 5 showed. We also observed that the seedlings of 25% of WT or 8% of the *nup98a-1* *nup98b-1* double mutant on medium C can grow normal more or less until three weeks after germination. All experiments were repeated at least twice, and one representative photo each was showed here. All the images are our own data.

**Reference:**

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