Two *Nucleoporin 98* homologous genes jointly participate in the regulation of starch degradation to enhance growth in *Arabidopsis*
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Abstract
Background: Starch is synthesized during the day for temporary storage in leaves and then degraded during the subsequent night to support plant growth and development. Impairment of starch degradation leads to stunted growth, even senescence and death. The nuclear pore complex is involved in many cellular processes, but its relationship with starch degradation is unclear until now. We previously identified two Nucleoporin98 (Nup98) genes (Nup98a and Nup98b) redundantly regulated flowering through CONSTANS (CO) independent pathway. in Arabidopsis thaliana. The nup98a nup98b double mutant also showed severe senescence phenotypes.

Results: We found that Nucleoporin 98 (Nup98) participated in the regulation of sugar metabolism in leaves and in turn is involved in senescence regulation in Arabidopsis. We show that Nup98a and Nup98b redundantly function in the different steps of starch degradation, the nup98a nup98b double mutant accumulates more starch than wild type and has a severe early senescence phenotype compared to wild type. The expression of marker genes related to starch degradation was impaired in the nup98a nup98b double mutant, and indicator genes of carbon starvation and senescence expressed earlier in the nup98a nup98b double mutant than that in wild type plants, suggesting abnormality of energy metabolism was the cause of senescence of the nup98a nup98b double mutant. Addition of sucrose to the growth medium can rescue early senescence phenotype of the nup98a nup98b mutant.

Conclusions: Our results provided a line of evidence on a novel role of the nuclear pore complex in energy metabolism related to growth and development, whereas Nup98 functioned in starch degradation conferring growth regulation in Arabidopsis.

Background
The nuclear pore complex (NPC) is the key bridge for communication of macromolecules between the nucleus and cytoplasm and regulates gene expression [1]. NPC is built by at least 30 unique proteins (nucleoporin, Nup) which are highly conserved in eukaryotic cells [2, 3]. Nup98 is a mobile and peripheral FG (for Phe-Gly domain) nucleoporin and spans from the nucleus to cytoplasmic of the central channel of the NPC [4, 5]. Arabidopsis thaliana Nup98a (also known as DRA2) is also found in
different subcellular locations [6]. *Nup98* is involved in regulation of cargo export and import, gene expression, transcriptional memory, and multiple developmental processes in animals and yeast [4-9]. In *Arabidopsis*, DRA2 regulates the shade avoidance syndrome [6]. In rice, the Nup98 homolog APIP12 is involved in basal resistance against the pathogen *Magnaporthe oryzae* and targeted by the *Magnaporthe* effector AvrPiz-t [10]. Recently in *Arabidopsis*, the interaction between Nup98 and Nup88/MOS7 is required for plant immunity against the necrotrophic fungal pathogen *Botrytis cinerea* and mitogen-activated protein kinase signalling [11]. We found Nup98 contributes to flowering regulation in *Arabidopsis* [12].

Senescence is an important cellular progress sensing developmental and environmental cues [13]. Before death, plants remobilize resources in senescing tissues and translocate them to sink tissues to support growth and development [14-16]. To date, at least 200 genes have been identified that regulate or participate in senescence in plants [17]. Starch is synthesized during the day in leaves and then degraded during the subsequent night to support plant growth and development. Impairment of genes functioned in different steps of starch degradation hinders plant growth at different extents [18, 19]. Sugar directly or indirectly regulates senescence: sugar accumulation not only triggers and accelerates, but also delayed senescence [20-22]. In fact, the response to sugar and senescence of plants is in an age- or condition-dependent manner [21, 23, 24]. Within the sugar pathway, three control nodes have been identified; glucose sensor gene *HEXOKINASE 1 (HXK1)*, energy sensor genes *PROTEIN KINASE (KIN10 and KIN11)*, and *TOR* (The target of rapamycin) kinase [22, 25-28].

Currently, the mechanism of NPC in regulation of starch degradation and senescence in plants is still unknown. However, in animals there are several studies reporting the NPC controls cell senescence through modifying chromosome structure, DNA repair and replication or cell division [29-33]. Impaired NPC results in dysfunction of nucleo-cytoplasm transportation [31]. Both Nup107 [34] and Tpr [35, 36] have been linked to cancer cell proliferation and cellular senescence in aging cell, and another Nup98-interacting protein Nup93 was shown to be oxidatively damaged [29].

In this study, we focus on Nup98 in *Arabidopsis thaliana*. In the *Arabidopsis* genome, there are two
Nup98 homologs, Nup98a and Nup98b, respectively. Single mutants of Arabidopsis, nup98a or nup98b, have no obvious phenotypes. However, the double mutant, nup98a nup98b, showed a significant early-senescence phenotype. Gene expression analysis demonstrated that Nup98a and Nup98b participates in starch degradation conferring to growth regulation. Further analysis indicated the early-senescence may result from a defect in the initial steps of starch degradation resulting in dysfunction in energy supply. Interestingly, the early senescence phenotype in the double mutant could be rescued by sugar in the growth media. Our data suggests that Nup98a and Nup98b function redundantly in regulation of starch degradation, which contribute to normal growth and development in Arabidopsis.

Results
Nup98 mutation results in early senescence in plants
Nup98 is a highly conserved nuclear pore protein in eukaryotes. In Arabidopsis thaliana, there are two homologs of the mammalian Nucleoporin 98, Nup98a (At1g10390) and Nup98b (At1g59660), and both share high amino acid sequence similarity in the Phe-Gly (FG)-repeats and autoproteolytic domain (APD, Supplementary Fig. 1) [3, 6]. Nup98a was previously reported as DRACULA2 (DRA2), a regulator of the shade avoidance syndrome (SAS) in Arabidopsis [6] and immune responses to a rice fungal pathogen [11]. To investigate Nup98 functions in plant development, we screened mutants of nup98a (SALK_080083, SALK_090744, SALK_023493, SALK_103803, SALK_015016) and nup98b (CS803848 and GABI_288A08) ordered from ABRC and GABI T-DNA mutant center, respectively. Homozygous lines were isolated for the following insertional mutants of SALK_103803, SALK_015016, GABI_288A08, and among them SALK_103803 and GABI_288A08 were the mutants reported by Parry [9]. The T-DNAs in these homozygous mutants are inserted in the coding regions (Fig. 1A) and RT-PCR results demonstrated that these mutants were null alleles (Fig. 1B) consistent with the Parry’s results [9]. We did not observe any obvious phenotypes in either the nup98a or nup98b single mutants when compared to wild type (WT) under long-day photoperiod conditions (Fig. 1C), as previous studies showed [9]. As Nup98a and Nup98b share high amino acid sequence identity (Supplementary Fig. 1),
we tested the hypothesis that Nup98a and Nup98b acted redundantly with the *nup98a nup98b* double mutants we made by crossing *nup98a* to *nup98b*. Strikingly, both double mutants, *nup98a1 nup98b1* and *nup98a2 nup98b1*, displayed similar early senescence phenotypes when compared to WT (Fig. 1C and 1D). Also, the double mutant plants had additional phenotypes, such as smaller inflorescences, flowers, siliques and short stature and severe sterility when compared to WT (Supplementary Fig. 2 and 3). We recently reported that the *nup98a nup98b* double mutants had early flowering phenotype. As expected, the mutant phenotypes observed in the double mutant plants were rescued by expressing only *Nup98b* (Fig. 1E). Our results demonstrate that *Nup98a* and *Nup98b* act redundantly.

To investigate if senescence phenotype was specific to the *nup98a nup98b* mutant, we selected another three nucleoporin mutants, *nup96-1*, *nup160-1*, and *nup107-1*, which showed flowering phenotypes in our previous report [37], to analyze the effect of other nuclear pore components on senescence. To our surprise, there is no early senescence phenotype observed in these mutants (Supplementary Fig. 4), suggesting that some of nucleoporins may not be involved in the regulation of senescence and *Nup98* had more or less specific functions on this developmental event.

*Nup98* gene may be involved at multiple pathways of senescence initiation

While the early senescence observed in the *nup98a nup98b* double mutant could be a secondary effect of altered development, we further explored the role of *Nup98a* and *Nup98b* in plant senescence. To date, at least 200 genes have been identified as regulating or participating in senescence in plants [17]. We investigated and summarized literatures in Supplementary Fig. 5 focusing the main genes, which showed that various endogenous and environmental cues, such as different hormones, sugar signalling, light and photoperiod conditions, and multiple stresses, trigger plant senescence and that many genes regulate senescence in multiple cross-talking pathways. To identified the potential link to early senescence of the *nup98a1 nup98b1* double mutant plants, we measured mRNA abundance of important senescence-associated genes in different pathways (Supplementary Fig. 5) at ZT0 (the time point of light on) and ZT16 (the time point of light off) in plants grown under long day conditions by quantitative RT-PCR. As shown in Fig. 2, many of the tested genes had different transcript abundances in the *nup98a nup98b* mutant when compared to
WT, although different patterns were observed. In the first category, *WRKY53, SAG13, WRKY6, NAC1* and *NAC2* displayed higher transcript abundances at both ZT0 and ZT16 (Fig. 2A), suggesting that stress (*WRKY53, WRKY6, and NAC1*) and SA (*WRKY53, WRKY6, and NAC2*) pathways were related to the *nup98a1 nup98b1* double mutant phenotype. In the second category, *SAG12, NAP, SAG2* and *CAT1*, also stress and SA pathway genes, were only increased at either ZT0 or ZT16 (Fig. 2B). In contrast, in the third category, *SAUR36, WRKY70, ARP4, SEN1*, and *COI1* were decreased in abundance at either ZT0 and/or ZT16 (Fig. 2C), indicating that auxin (*SAUR36*) and jasmonate (*COI1*) may be negatively related to the *nup98* phenotypes. The abundance of *AGL15, EBP1, RPS6a*, and *NPR1* had opposite changes at ZT0 and ZT16 (Fig. 2D), suggesting the function of these genes on *Nup98* was dependent on circadian rhythm.

This expression profile suggested that there were at least three characteristics of senescence in the *nup98* mutant. Firstly, several pathways, mainly stress and SA pathways, were involved in regulation of senescence in the *nup98a nup98b* double mutant. Secondly, different genes functioned in their own special modes, positively or negatively at different phases (morning or afternoon phases). Thirdly, some of genes, such as *WRKY53, WRKY6, NAP1, SAG2*, may play roles in multiple pathways.

The results indicated that the mechanism of senescence regulation in the *nup98* double mutant was much complicated. Many genes showed circadian expression pattern, consistent with our previous report that *Nup98* participated in regulation circadian clock [12].

**Starch metabolism is impaired in the *nup98a nup98b* double mutant**

During photosynthesis, glucose is synthesized and stored as starch, which is degraded at night.

Starch synthesis, degradation and metabolism pathways involve a number of genes in plants [18, 19]. Firstly, we checked starch homeostasis in the *nup98a1 nup98b1* double mutant plants that were grown under 12 h light/12 h dark conditions (at ZT0, dawn, and ZT12, dusk) (Fig. 3). All plants accumulated starch at dusk, however, double mutant plants accumulated much more starch when compared to wild type plants as determined by iodine staining (Fig. 3A) and starch quantitative assay (Fig. 3B). The more intense signals in older, 21 and 28-day, double mutant leaves may be a consequence of starch accumulating over time. Therefore, the double mutant displayed much more
starch not only at dawn but also at dusk when compared to wild type plants and this was much clearer in 14 and 21-day old seedlings (Fig. 3A and 3B). Together, these results suggested that starch metabolism was impaired in the nup98a nup98b double mutant.

We measured the transcript abundance of genes involved in starch metabolism [18] by quantitative RT-PCR (Fig. 4) and found that many genes had significantly lower abundant transcripts at least one time point in the double mutant when compared to wild type. These genes encoded enzymes for the degradation of starch not only in the early steps of starch degradation in chloroplasts [18], such as GWD1 (SEX1), β-BAM1, BAM3, BAM5, BAM6, BAM7, BAM8, SEX4, and LSF1, but also in the later steps of starch degradation, such as limit-dextrinase (LDA), α-amylase (AMY1 and AMY2), disproportioning enzyme (DPE1 and DPE2), α-glucan phosphorylase (PHS1 and PHS2). Time-dependent low-expression of these genes suggested they were in the control of circadian clock, since Nup98 is involved in circadian regulation in Arabidopsis [12]. These genes function at different steps [18]. GWD1 is α-glucan water, dikinase (also called SEX1) and phosphorylates glucosyl residues of amylopectin at the C-6 position, the initiation step for starch degradation. BAMs are a family of β-amylase breaking down the α-1,4-linked glucose chains. LSF1 (Like Sex Four 1) and SEX4 (Starch excess 4) releases phosphate bound at C-6 and C-3 of glucosyl residues. Both ISA and LDA hydrolyze α-1,6 branch points but show different substrate specificities. AMYs act on α-1,4-linkages releasing linear α-1,4-linked oligosaccharides and branched α-1,4- and α-1,6-linked oligosaccharides. DPEs Transfer glucose/α-1,4-linked glucan moiety from a donor glucan to an acceptor, releasing the non-reducing end glucose/glucan moiety. PHSs acts on the non-reducing end of α-1,4-linked glucose. Lower abundance of these genes would be expected to results in a starch-excess phenotype in the nup98a nup98b double mutant (Fig. 3) as these enzyme mutants [18]. We also found that there were some genes, which did not show a significant change in mRNA abundance. These genes included GWD2, GWD3, ISA3 and AMY3, suggesting the effect of Nup98a/b on starch metabolism was more or less specific.

We also measured the abundance of genes related to photosynthesis and sugar metabolism by RT-qPCR in the nup98a1 nup98b1 double mutant and WT plants (Supplementary Fig. 6). In terms of photosynthesis related genes, the decrease of mRNA abundance of LHCA and LHCB was observed in
the nup98a1 nup98b1 double mutant at different time points, e.g., LHCA1/2 and LHCB1.1 at ZT0 and
LHCA1 and LHCB1.4 at ZT16. We also observed the decrease of mRNA abundances of KIN10 and
KIN11, two sugar signaling genes in the double mutant when compared to WT. Both genes delay plant
senescence [22, 27, 28] and therefore the reduced abundance may be associated with earlier
senescence (Supplementary Fig. 5). We also observed slightly-increased mRNA abundance of HXX1 at
dusk (ZT16) and this may contribute to earlier senescence via the cytokinin signaling pathway [25].
Unexpectedly, mRNA abundance of TPS1, a senescence activator [21], was reduced in the nup98a
nup98b double mutant compared to wild type, suggesting that T6P (trehalose-6-phosphate) was not
related to senescence of the nup98a1 nup98b1 double mutant. The results indicated that starch
synthesis and sugar signalling were impaired in the double mutant.

Exogenous sugar rescues the early senescence in the nup98a nup98b double mutant

Based on our results above, we interpreted that the carbon or energy supply was impaired in nup98a
nup98b double mutant plants. We tested the idea by supplying exogenous carbon in the form of
sucrose in growing medium to see if the early senescence phenotype in the nup98a nup98b double
mutant plants could be rescued. Our results showed that sucrose and MS nutrients can support the
double mutant plants growing well even though they were weak compared to WT (Supplementary Fig.
7). Both plants can complete their life cycles on medium containing agarose supplement with sucrose
and nutrients. Then, we allowed double mutant and control plants grown in MS medium until
inflorescence emergence and then transferred them to soil. As expected, the double mutant grew well
as WT did on MS medium (Fig. 5). However, after transferring to soil, senescence symptoms on
mutant plants’ leaves quickly appear at day 6, and the mutant plants wilted at day 30 (Fig. 5). If
seeds were sown on medium containing only MS nutrients or sucrose, both WT and nup98 cannot
survive as plants grow agarose medium without any supplements (Supplementary Fig. 7).
To rule out the potential effect of soil on senescence phenotypes observed above, we carried out
another experiment to test if exogenous macro and micronutrients would complement the
phenotypes observed in the mutant by continuously growing plants on medium at different strengths
of sucrose and macro- and micro-nutrients (Supplementary Fig. 8). To our surprise, not only did sucrose suppress the early senescence phenotype in the double mutant but also macro- and micro-nutrients in the presence of sucrose. The lower strength nutrients (½ MS) enhanced the lower sucrose effect on suppressing senescence, suggesting that both energy supply and nutrients metabolism were impaired in the *nup98a nup98b* double mutant. We also tested if sucrose could recue the *nup98a nup98b* double mutant phenotype in soil. However, such an experiment failed and both the *nup98a nup98b* double mutant and WT seedlings died, because sucrose enhanced pathogen growing (Supplementary Fig. 9).

**Misexpression of starvation and senescence marker genes in the *nup98a nup98b* double mutant**

Results above implied that the *nup98a nup98b* double mutant may suffer from sugar starvation or/and senescence. Next, we asked when starvation or senescence initiated in the *nup98a nup98b* double mutant. *DORMANCY-ASSOCIATED PROTEIN-LIKE 1* (*DRM1/DYL1*, At1g28330) and *DARK INDUCIBLE 6* (*DIN6*, At3g47340) [38, 39] are two well-studied sugar starvation gene markers, whereas *SAG12* (At5g45890) and *WRKY53* (At4g23810) are well-characterized senescence markers [40-42]. Autophagy is an important event occurring during sugar starvation and senescence [38, 43, 44], and *AUTOPHAGY8a* (*ATG8a*, At4g21980) and *ATG8e* (At2g45170) are two typical molecular indicators for autophagy in plants [45]. Therefore, we investigated expression changes of these genes in the *nup98a nup98b* double mutant compared to that in WT, and the results showed that they had different changes in a time- and developmental-dependent mode (Fig. 6). In the double mutant, *DRM1* had significantly higher expression at ZT0, but lower at ZT12 from very early stage (day 5 after germination) (Fig. 6A). *DIN* is a light-repressed and dark-induced gene [46], and its high level of expression at ZT0 in the *nup98a nup98b* double mutant became obvious at day 15, but at ZT12 higher abundancy appeared earlier from day 10 (Fig. 6A). Compared to WT, the senescence marker *WARKY53* in the *nup98a nup98b* double mutant expressed higher at the early stage when *DRM1* expression was in disorder (day 5) (Fig. 6B). *SAG12* is a developmental controlled indicator for the later stage of senescence [47, 48]. We found that there was no much difference of *SAG12* expression
in the early stage (day 5) between the nup98a nup98b double mutant and wild type. However, SAG12 had a higher expression level in the nup98a nup98b double mutant at both ZT0 and ZT12 from day 10 (Fig. 6B). In the meanwhile, the two markers of autophagy, ATG8a and ATG8e, also had higher abundancy of mRNA in most of samples of the double mutant from day 10. Token together, our results showed that the nup98a nup98b double mutant appeared the sign of energy starvation, at least at molecular level, in early developmental stage when plants did not display visible senescence phenotypes. And these expression changes may have circadian and developmental characters. A previous report shows that different sugars (such as sucrose, glucose, and fructose) have different effects on the regulation of senescence [39]. It may be a cue to study the function of Nup98 on senescence regulation.

**Nup98 proteins mainly localize to the nuclear membrane and nucleoplasm**

Nup98 is one of the mobile and peripheral FG (Phe-Gly domain) nucleoporins and is located at both the nuclear and cytoplasmic sides of the NPC central channel [4, 5]. *Arabidopsis* Nup98a (also known as DRA2) is also found distributing in different subcellular compartments [6]. We constructed transgenic *Arabidopsis* plants expressing 35S::GFP:Nup98a and 35S::GFP:Nup98b and analyzed the subcellular localization of both translation fusion proteins. Not surprisingly, both proteins were distributed in the cytoplasm, the nucleoplasm and at the nuclear periphery (Fig. 7). We also observed no significant difference in the subcellular distribution of Nup98a and Nup98b and this is consistent with our observations of genetic redundancy. In conclusion, our combined results demonstrated that Nup98a and Nup98b proteins were localized at both the nucleus and cytoplasm similar to their homologs in other organisms.

**Discussion**

Senescence is a physiological process during the plant life cycle, eventually leading to cell and tissue disintegration and death in plants. In such a physiological process, various nutrients are redistributed from senescing organs, such as leaves, to reproductive organs, for example seeds [13]. However, irregular or premature senescence could lead to organ failure or even whole plant death [49]. Fine tuning senescence could benefit plants by avoiding the deleterious effects of abiotic stresses and
thereby lead to an optimal reproductive outcome. A significant number of factors including hormones, developmental age, abiotic stress and light participate in regulation of plant senescence [22, 23, 50-59]. While these factors play important and clear roles in plant senescence, the role of sugar is unclear as different groups have published opposing results [20-22, 60]. The NPC, is an important gatekeeper for both macromolecular transportation between the nucleus and cytoplasm and gene transcription, and therefore plays an important role in different plant developmental processes [29-33]. Our study provides some additional insight into the plant senescence field as we found that the NPC participates in senescence regulation. Our investigation confirmed that Nup98 genes participated in starch degradation, then conferring to senescence initiation in Arabidopsis.

We identified two homologs of Nup98, Nup98a and Nup98b, in the Arabidopsis genome and both showed high protein sequence similarity and highly similar cellular protein-localization patterns. We observed no obvious phenotypes of senescence in the nup98 single mutants under our growth conditions even though previously nup98a mutant plants, also known as dra2, showed a shade avoid phenotype of longer hypocotyls [6]. However, in the nup98a nup98b double mutant, we observed senescence phenotypes in very early developmental stage and severely reduced seed production. Molecular evidence showed that key marker genes of plant senescence, such as SAG12, NAP1, WRAY53, WRKY6, WRKY70, NAC1, NAC2 and HXK1, were detected having significant changes in expression, these genes were related to different senescence pathways, for example, ethylene, salicylic acid, ABA, cytokinin, and stress pathways. These genes may play a role in a temporal (circadian) manner as their significant changes only at a specific time point, for example only at dawn or dusk, in the double mutant when compared to wild type plants. Our results suggested that senescence of the nup98a nup98b double mutant was the consequence of impairment of multiple senescence pathways.

We also observed that genes in sugar signaling pathways (TOR, KIN10, KIN11, TPS1, SnRK1) and carbon starvation genes (DRM1 and DIN6) were all mis-regulated in nup98a nup98b double mutant plants. What’s more, sucrose could rescue senescence phenotype of the nup98a nup98b double mutant, indicating that sugar availability was hindered in the double mutant.
In plants, sugar is derived from photosynthesis and stored as starch. Starch accumulates in the chloroplast during the day and is degraded at night [18, 19]. The nup98a nup98b double mutant accumulated much higher levels of starch that was unlikely due to higher photosynthesis efficiency but impaired starch degradation. This was evident by 1) lower expression levels of genes related to photosynthesis, LHCA1, LHCA2, LHCBI.1 and LHCBI.4, which would lead to reduced starch synthesis in the double mutant; 2) lower expression of starch degradation genes embracing many steps of starch degradation [18], that would lead to starch accumulation in the nup98a nup98b double mutant. A gradual accumulation of starch over time was also contributed by a partial reduction in degradation pathway gene expression. Impairment of starch degradation directly lead to sugar starvation, and subsequently stunted growth, senescence, finally death.

Beyond this, Nup98a and Nup98b may also have functions in other nutrient metabolism as the concentration of nutrients in the growth medium had a significantly impact on the growth and senescence in nup98a nup98b double mutants. In this case, components (sugar or MS) in growth medium should be taken into consideration, especially for senescence study in future.

Previous studies show that the circadian clock regulate starch metabolism in plants [61, 62]. EARLY FLOWERING3 (ELF3) positively regulates starch accumulation and degradation of starch was significantly slower in elf3 mutant plants than in the corresponding wild types [63]. ELF3 and other clock evening genes (ELF4 and LUX ARRHYTHMO) also affect leaf senescence [64, 65]. Many genes studied in this study showed time point-dependent changes. We recently found that the expression level of ELF3 and other clock genes significantly reduced in the nup98a nup98b double mutant [12].

Token together, clock genes may participate in signalling pathway of Nup98a/Nup98b regulating starch degradation and senescence in Arabidopsis.

We just reported that the nup98a1 nup98b1 ft-10 triple mutant displayed the late flowering character as the ft-10 mutant but maintained early senescence phenotypes of the nup98a1 nup8b1 double mutant [12], suggesting that Nup98a and Nup98b were involved in regulation of both flowering and senescence, two developmental processes individually regulated by Nup98a and Nup98b.

It is obvious that the function of Nup98 in senescence regulation are indirect. Mutation of Nup98
genes leads to starch degradation hindered firstly, then sugar starvation, and finally senescence. In the future, it will be interesting to determine how Nup98 controls the function of genes related to starch degradation.

Conclusion
Our findings identified a novel function of nuclear pore complex on starch metabolism and verified that Nup98a and Nup98b overlappingly controlled starch degradation and indirectly regulate senescence in Arabidopsis.

Methods
Plant materials and growth conditions
Seeds of the T-DNA insertion mutants of nup98a (SALK_080083, SALK_090744, SALK_023493, SALK_103803, and SALK_015016) and nup98b (CS803848 and GABI_288A08) were ordered from ABRC and GABI T-DNA mutant center, respectively. Homozygous screening was according to the protocol provided by SALK (http://signal.salk.edu/). All mutants were identified by Dr. Long Xiao. Arabidopsis thaliana Columbia wild type and its derived mutants were grown under long day (16 h/8 h, light/dark, except where indicated in the text) conditions, with 100 µmol m⁻² s⁻¹ lighting provided by fluorescent lamps. Plants were grown on soil in pots with a diameter of 10 cm or on medium in petri dishes containing different strengths of sucrose and nutrients as indicated in the text.

Gene and promoter cloning, plasmid construction
Standard GATEWAY (Invitrogen) methods were employed for cloning and plasmid construction. Most vectors are made by our lab [66]. The full-length of Nup98a and Nup98b open reading frames were PCR amplified with specific primers (Table S1), and then cloned into Fu30 [66] which contains N-terminal GFP marker. The gene entry vectors (Fu30-GFP:Nup98a or Fu30-GFP:Nup98b), the 35S promoter entry vector (Fu76-35S) and the binary vector (Fu39-2) [66] were applied to LR reaction (Invitrogen). The resulting Fu39-2-35S:GFP:Nup98a and Fu39-2-35S:GFP:Nup98b binary vectors were introduced into Agrobacterium tumefaciens strain GV3101 pMP90RK, and then transformed into A. thaliana using the floral dipping method [67]. Homozygous transgenic plants were used for phenotypic and molecular characterization.

Semi-quantitative PCR, quantitative real time RT-PCR, and subcellular localization
The whole seedlings were harvested at ZT0 (the time point of light on) and ZT16 (the time point of light off) at day 14 after germination. RNA preparation, cDNA synthesis and both quantitative real-time and semi-quantitative RT-PCRs were carried out following Xiao et al. [68], except for the use of At4g34270 as a reference gene in triplicate [69, 70]. All gene accession numbers and relevant primer sequences are listed in Table S1. GFP fluorescent signals were visualized by confocal microscopy, and propidium iodide (PI) is for cell wall staining [66].

**Starch staining and quantitative analysis**

Starch in leaves of 14- 21-, and 28-day-old wild type and mutant Arabidopsis plants were stained with iodine [71]. Plants were grown under a 12-h-light/12-h-dark photoperiod, harvested at ZT0 (the time point of light on) and ZT12 (the time point of light off), decolorized with hot 80% (v/v) ethanol, and stained with iodine-potassium iodide solution. Representative plants were shown. Starch quantitative analysis was carried out according to the instruction of Starch Determination Kit (Solarbio, Beijing, Cat#BC0700) on Spectrophotometer (RAYLEIGH, VIS-7220N; Beijing Beifen-Ruili Analytical Instruments (Group) Co., Ltd) with standard curve method, which was generated with different concentration of glucose (0.2, 0.1, 0.05, 0.025, 0.0125, 0.00625, 0.003125, 0.00156 mg/mL).

**Statistical analysis**

Each experiment has at least three biological replicates and got similar results. For photographs, we selected one representative plant to make figures. For digital statistical analysis, all data were analyzed and determined using SPSS software package (*P < 0.05 and **P <0.01, compared to the control). Error bars indicate ± SD of the mean.

**Abbreviations**

NPC, NUCLEAR PORE COMPLEX, NUP, NUCLEOPORIN; CO, CONSTANS; ABA, ABSCISIC ACID; ABI, ABA INSENSITIVE; ACS, AMINOACYCLOPROPANE-1-CARBOXYLATE (ACC) SYNTHASE; AGL15, AGAMOUS-LIKE 15AHK, ARABIDOPSIS HISTIDINE KINASE; AHP, ARABIDOPSIS THALIANA HISTIDINE PHOSPHOTRANSFER PROTEINS; AMY3, ALPHA-AMYLASE-LIKE 3APD, AUTOPROTEOLYTIC DOMAIN; ARF, AUXIN RESPONSE FACTOR; ARR, ARABIDOPSIS RESPONSE REGULATOR; C2H4, ETHYLENE ; AUTOPHAGY8A (ATGBAM, BETA-AMYLASE;CAT, CATALASE; CK, CYTOKININ; COI1, CORONATINE INSENSITIVE 1; CWINV, CELL WALL INVERTASE; DIN6, DARK INDUCIBLE 6; DRM1/DYL1, DORMANCY-ASSOCIATED PROTEIN-LIKE 1;
EBP1, ERBB-3 BINDING PROTEIN; EEL, ENHANCED EM LEVEL; EIN, ETHYLENE INSENSITIVE; ELF, EARLY FLOWERING; ETR, ETHYLENE RESPONSIVE; G6P, 6-PHOSPHORIC ACID GLUCOSE; GLK, GOLDEN 2-LIKE TRANSCRIPTION FACTOR; GWD1, A-GLUCAN WATER, DIKINASE; HXK1, HEXOKINASE1; ISA3, ISOAMYLASE 3; KIN, PROTEIN KINASE; LDA, LIMIT-DEXTRINASE; LHCA, PHOTOSYSTEM I LIGHT HARVESTING COMPLEX GENE ALHCB, PHOTOSYSTEM I LIGHT HARVESTING COMPLEX GENE BLSF1, LIKE SEX FOUR 1; N, NITROGEN; NAC, NAM, ATAF, AND CUC; NAP, NAC-LIKE PROTEIN; NPR1, NONEXPRESSER OF PR GENES 1NYC, NONYELLOW COLORING; ORE, ORESARA; PHS, A-GLUCAN PHOSPHORYLASE; PHYB, PHYTOCHROME B; PIF, PHYTOCHROME INTERACTING FACTOR; PYR/PYL/RCAR, PYRABACTIN RESISTANCE/PYR1-LIKE ORREGULATORY COMPONENT OF ABA RECEPTOR; RPS6A, RIBOSOMAL PROTEIN S6ASAG, SENESCENCE-ASSOCIATED GENE; SAS, SHADE AVOIDANCE SYNDROME, SAUR, SMALL AUXIN UPREGULATED; SAUR36, SMALL AUXIN UPREGULATED; SEX4, STARCH EXCESS 4; SGR, STAYGREEN; T6P, TREHALOSE-6-PHOSPHATE; TOR, TARGET OF RAPAMYCIN; TPS1, TREHALOSE-6-PHOSPHATE SYNTHASE 1; WRKY, WRKY DNA-BINDING PROTEIN;

Declarations

**Ethics approval and consent to participate**

Not applicable.

**Consent to publish**

All Authors read and approved the manuscript.

**Plant specimens**

Not applicable.

**Availability of data and materials**

All data generated or analyzed during this study are included in this published article (and its Supplementary Information files).

**Competing Interests**

The authors declare no competing interests.

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**Author Contributions**

Conceptualization, Y.-F.F. and X.Z.; Methodology, L.X. and Y.-F.F.; Investigation, L.X., F.C., S.J., Z.C., P.H, Y.M., C.L., and L.L.; Formal analysis, L.X., F.C., X.W., and Q.C.; Visualization, L.X. and Y.-F.F.; Writing -Original Draft, Y.-F.F., and Q.C. Writing -Review & Editing, I.S. and Y.-F.F.; Funding Acquisition, Y.-F.F. and X.Z.; Project administration, Y.-F.F. and X.Z.; Resources, X.Z, Y.M., X.W., and Q.C; Supervision, Y.-F.F. All Authors read and approved the manuscript.

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**References**

1. Weis K: Regulating access to the genome: nucleocytoplasmic transport throughout the cell cycle. *Cell* 2003, 112(4):441-451.

2. Strambio-De-Castillia C, Niepel M, Rout MP: The nuclear pore complex: bridging nuclear transport and gene regulation. *Nat Rev Mol Cell Biol* 2010, 11(7):490-501.

3. Tamura K, Fukao Y, Iwamoto M, Haraguchi T, Hara-Nishimura I: Identification and characterization of nuclear pore complex components in Arabidopsis thaliana. *The Plant cell* 2010, 22(12):4084-4097.

4. Capitanio JS, Montpetit B, Wozniak RW: Nucleoplasmic Nup98 controls gene expression by regulating a DExH/D-box protein. *Nucleus* 2018, 9(1):1-8.

5. Franks TM, McCloskey A, Shokirev MN, Benner C, Rathore A, Hetzer MW: Nup98 recruits the Wdr82-Set1A/COMPASS complex to promoters to regulate H3K4
trimethylation in hematopoietic progenitor cells. *Genes Dev* 2017, 31(22):2222-2234.

6. Gallemi M, Galstyan A, Paulisic S, Then C, Ferrandez-Ayela A, Lorenzo-Orts L, Roig-Villanova I, Wang X, Micol JL, Ponce MR et al: DRACULA2 is a dynamic nucleoporin with a role in regulating the shade avoidance syndrome in Arabidopsis. *Development* 2016, 143(9):1623-1631.

7. Light WH, Brickner JH: Nuclear pore proteins regulate chromatin structure and transcriptional memory by a conserved mechanism. *Nucleus* 2013, 4(5):357-360.

8. Cross MK, Powers MA: Nup98 regulates bipolar spindle assembly through association with microtubules and opposition of MCAK. *Mol Biol Cell* 2011, 22(5):661-672.

9. Parry G: Components of the Arabidopsis nuclear pore complex play multiple diverse roles in control of plant growth. *J Exp Bot* 2014, 65(20):6057-6067.

10. Tang MZ, Ning YS, Shu XL, Dong B, Zhang HY, Wu DX, Wang H, Wang GL, Zhou B: The Nup98 Homolog APIP12 Targeted by the Effector AvrPiz-t is Involved in Rice Basal Resistance Against Magnaporthe oryzae. *Rice* 2017, 10.

11. Genenncher B, Wirthmueller L, Roth C, Klenke M, Ma L, Sharon A, Wiermer M: Nucleoporin-Regulated MAP Kinase Signaling in Immunity to a Necrotrophic Fungal Pathogen. *Plant Physiology* 2016, 172(2):1293-1305.

12. Jiang S, Xiao L, Huang P, Cheng Z, Chen F, Miao Y, Fu YF, Chen Q, Zhang XM: Nucleoporin Nup98 participates in flowering regulation in a CONSTANS-independent mode. *Plant cell reports* 2019, 38(10):1263-1271.

13. Gan S, Amasino RM: Making Sense of Senescence (Molecular Genetic Regulation and Manipulation of Leaf Senescence). *Plant Physiol* 1997, 113(2):313-319.

14. Buchanan-Wollaston V: The molecular biology of leaf senescence. *J Exp Bot* 1997, 48(307):181-199.

15. Kroger R, Holland MM, Moore MT, Cooper CM: Plant senescence: a mechanism for
nutrient release in temperate agricultural wetlands. *Environ Pollut* 2007, 146(1):114-119.

16. Himelblau E, Amasino RM: Nutrients mobilized from leaves of Arabidopsis thaliana during leaf senescence. *J Plant Physiol* 2001, 158:1317-1323.

17. Balazadeh S, Riano-Pachon DM, Mueller-Roeber B: Transcription factors regulating leaf senescence in Arabidopsis thaliana. *Plant Biol (Stuttg)* 2008, 10 Suppl 1:63-75.

18. Streb S, Zeeman SC: Starch metabolism in Arabidopsis. *The Arabidopsis book / American Society of Plant Biologists* 2012, 10:e0160.

19. Ruan YL: Sucrose Metabolism: Gateway to Diverse Carbon Use and Sugar Signaling. *Annual Review of Plant Biology* 2014, 65:33-67.

20. van Doorn WG: Is the onset of senescence in leaf cells of intact plants due to low or high sugar levels? *J Exp Bot* 2008, 59(8):1963-1972.

21. Wingler A, Delatte TL, O'Hara LE, Primavesi LF, Jhurreea D, Paul MJ, Schluepmann H: Trehalose 6-phosphate is required for the onset of leaf senescence associated with high carbon availability. *Plant Physiol* 2012, 158(3):1241-1251.

22. Sheen J: Master Regulators in Plant Glucose Signaling Networks. *J Plant Biol* 2014, 57(2):67-79.

23. Thomas H: Senescence, ageing and death of the whole plant. *New Phytol* 2013, 197(3):696-711.

24. Wingler A, Purdy S, MacLean JA, Pourtau N: The role of sugars in integrating environmental signals during the regulation of leaf senescence. *J Exp Bot* 2006, 57(2):391-399.

25. Cho JI, Ryoo N, Eom JS, Lee DW, Kim HB, Jeong SW, Lee YH, Kwon YK, Cho MH, Bhoo SH et al: Role of the rice hexokinases OsHXX5 and OsHXX6 as glucose sensors. *Plant Physiol* 2009, 149(2):745-759.
26. Baena-Gonzalez E, Rolland F, Thevelein JM, Sheen J: A central integrator of transcription networks in plant stress and energy signalling. *Nature* 2007, 448(7156):938-942.

27. Xiong Y, Sheen J: Rapamycin and glucose-target of rapamycin (TOR) protein signaling in plants. *J Biol Chem* 2012, 287(4):2836-2842.

28. Xiong Y, Sheen J: Moving beyond translation: glucose-TOR signaling in the transcriptional control of cell cycle. *Cell cycle* 2013, 12(13):1989-1990.

29. D'Angelo MA, Raices M, Panowski SH, Hetzer MW: Age-dependent deterioration of nuclear pore complexes causes a loss of nuclear integrity in postmitotic cells. *Cell* 2009, 136(2):284-295.

30. Webster BM, Colombi P, Jager J, Lusk CP: Surveillance of nuclear pore complex assembly by ESCRT-III/Vps4. *Cell* 2014, 159(2):388-401.

31. Fichtman B, Harel A: Stress and aging at the nuclear gateway. *Mech Ageing Dev* 2014, 135:24-32.

32. Fernandez-Martinez J, Rout MP: Nuclear pore complex biogenesis. *Curr Opin Cell Biol* 2009, 21(4):603-612.

33. Hetzer MW: The role of the nuclear pore complex in aging of post-mitotic cells. *Aging (Albany NY)* 2010, 2(2):74-75.

34. Kim SY, Kang HT, Choi HR, Park SC: Reduction of Nup107 attenuates the growth factor signaling in the senescent cells. *Biochem Biophys Res Commun* 2010, 401(1):131-136.

35. Snow CJ, Paschal BM: Roles of the nucleoporin Tpr in cancer and aging. *Advances in experimental medicine and biology* 2014, 773:309-322.

36. David-Watine B: Silencing nuclear pore protein Tpr elicits a senescent-like phenotype in cancer cells. *PloS one* 2011, 6(7):e22423.
37. Xiao L, Liu W, Chen F, Zhang X, Chen Q, Fu Y-F: The Phenotype Analysis of NUP107-160 Subcomplex Mutants in Arabidopsis. *J Agri Sci Tech* 2016, 18(5):54-61.

38. Contento AL, Kim SJ, Bassham DC: Transcriptome profiling of the response of Arabidopsis suspension culture cells to Suc starvation. *Plant Physiol* 2004, 135(4):2330-2347.

39. Gonzali S, Loreti E, Solfanelli C, Novi G, Alpi A, Perata P: Identification of sugar-modulated genes and evidence for in vivo sugar sensing in Arabidopsis. *J Plant Res* 2006, 119(2):115-123.

40. Miao Y, Smykowski A, Zentgraf U: A novel upstream regulator of WRKY53 transcription during leaf senescence in Arabidopsis thaliana. *Plant Biol (Stuttg)* 2008, 10 Suppl 1:110-120.

41. Hensel LL, Grbic V, Baumgarten DA, Bleecker AB: Developmental and age-related processes that influence the longevity and senescence of photosynthetic tissues in arabidopsis. *The Plant cell* 1993, 5(5):553-564.

42. Lohman KN, Gan S, John MC, Amasino RM: Molecular analysis of natural leaf senescence in Arabidopsis thaliana. *Physiol Plant* 1994, 92(2):322-328.

43. Usadel B, Nagel A, Thimm O, Redestig H, Blaesing OE, Palacios-Rojas N, Selbig J, Hannemann J, Piques MC, Steinhauser D et al: Extension of the visualization tool MapMan to allow statistical analysis of arrays, display of corresponding genes, and comparison with known responses. *Plant Physiol* 2005, 138(3):1195-1204.

44. Bassham DC, Laporte M, Marty F, Moriyasu Y, Ohsumi Y, Olsen LJ, Yoshimoto K: Autophagy in development and stress responses of plants. *Autophagy* 2006, 2(1):2-11.

45. Bassham DC: Methods for analysis of autophagy in plants. *Methods* 2015, 75:181-188.
46. Fujiki Y, Yoshikawa Y, Sato T, Inada N, Ito M, Nishida I, Watanabe A: Dark-inducible genes from Arabidopsis thaliana are associated with leaf senescence and repressed by sugars. *Physiol Plant* 2001, 111(3):345-352.

47. Noh YS, Amasino RM: Regulation of developmental senescence is conserved between Arabidopsis and Brassica napus. *Plant Molecular Biology* 1999, 41(2):195-206.

48. Weaver LM, Gan S, Quirino B, Amasino RM: A comparison of the expression patterns of several senescence-associated genes in response to stress and hormone treatment. *Plant Mol Biol* 1998, 37(3):455-469.

49. Klimesova J, Nobis MP, Herben T: Senescence, ageing and death of the whole plant: morphological prerequisites and constraints of plant immortality. *New Phytol* 2015, 206(1):14-18.

50. Lim PO, Kim HJ, Nam HG: Leaf senescence. *Annu Rev Plant Biol* 2007, 58:115-136.

51. Khan M, Rozhon W, Poppenberger B: The role of hormones in the aging of plants - a mini-review. *Gerontology* 2014, 60(1):49-55.

52. Guo Y: Towards systems biological understanding of leaf senescence. *Plant Mol Biol* 2013, 82(6):519-528.

53. Guiboileau A, Sormani R, Meyer C, Masclaux-Daubresse C: Senescence and death of plant organs: nutrient recycling and developmental regulation. *C R Biol* 2010, 333(4):382-391.

54. Zhang H, Zhou C: Signal transduction in leaf senescence. *Plant Mol Biol* 2013, 82(6):539-545.

55. Jibran R, Hunter DA, Dijkwel PP: Hormonal regulation of leaf senescence through integration of developmental and stress signals. *Plant Mol Biol* 2013, 82:547-561.

56. Bakshi M, Oelmuller R: WRKY transcription factors: Jack of many trades in plants. *Plant signaling & behavior* 2014, 9(2):e27700.
57. Koyama T: The roles of ethylene and transcription factors in the regulation of onset of leaf senescence. *Frontiers in plant science* 2014, 5:650.

58. Hinderhofer K, Zentgraf U: Identification of a transcription factor specifically expressed at the onset of leaf senescence. *Planta* 2001, 213(3):469-473.

59. Miao Y, Laun T, Zimmermann P, Zentgraf U: Targets of the WRKY53 transcription factor and its role during leaf senescence in Arabidopsis. *Plant Mol Biol* 2004, 55(6):853-867.

60. Wingler A, Masclaux-Daubresse C, Fischer AM: Sugars, senescence, and ageing in plants and heterotrophic organisms. *J Exp Bot* 2009, 60(4):1063-1066.

61. Kim JA, Kim HS, Choi SH, Jang JY, Jeong MJ, Lee SI: The Importance of the Circadian Clock in Regulating Plant Metabolism. *International journal of molecular sciences* 2017, 18(12).

62. Seluzicki A, Burko Y, Chory J: Dancing in the dark: darkness as a signal in plants. *Plant Cell and Environment* 2017, 40(11):2487-2501.

63. Flis A, Mengin V, Ivakov AA, Mugford ST, Hubberten HM, Encke B, Krohn N, Hohne M, Feil R, Hoefgen R et al: Multiple circadian clock outputs regulate diel turnover of carbon and nitrogen reserves. *Plant, cell & environment* 2019, 42(2):549-573.

64. Kim H, Kim HJ, Vu QT, Jung S, McClung CR, Hong S, Nam HG: Circadian control of ORE1 by PRR9 positively regulates leaf senescence in Arabidopsis. *Proc Natl Acad Sci U S A* 2018, 115(33):8448-8453.

65. Kikis EA, Khanna R, Quail PH: ELF4 is a phytochrome-regulated component of a negative feedback loop involving the central oscillator components CCA1 and LHY. *Plant J* 2005, 44(2):300-313.

66. Wang X, Fan C, Zhang X, Zhu J, Fu YF: BioVector, a flexible system for gene specific-expression in plants. *BMC Plant Biol* 2013, 13(1):198.
67. Clough SJ, Bent AF: Floral dip: a simplified method for Agrobacterium-mediated transformation of Arabidopsis thaliana. *Plant J* 1998, 16(6):735-743.

68. Xiao C, Chen F, Yu X, Lin C, Fu YF: Over-expression of an AT-hook gene, AHL22, delays flowering and inhibits the elongation of the hypocotyl in Arabidopsis thaliana. *Plant Mol Biol* 2009, 71(1-2):39-50.

69. Gutierrez L, Mauriat M, Guenin S, Pelloux J, Lefebvre JF, Louvet R, Rusterucci C, Moritz T, Guerineau F, Bellini C et al: The lack of a systematic validation of reference genes: a serious pitfall undervalued in reverse transcription-polymerase chain reaction (RT-PCR) analysis in plants. *Plant biotechnology journal* 2008, 6(6):609-618.

70. Czechowski T, Stitt M, Altmann T, Udvardi MK, Scheible WR: Genome-wide identification and testing of superior reference genes for transcript normalization in Arabidopsis. *Plant Physiol* 2005, 139(1):5-17.

71. Caspar T, Lin TP, Kakefuda G, Benbow L, Preiss J, Somerville C: Mutants of Arabidopsis with altered regulation of starch degradation. *Plant Physiol* 1991, 95(4):1181-1188.

Figures
Figure 1. Mutation of Nup98 leads to an early senescence phenotype. A, T-DNA insertion alleles in Nup98a and Nup98b. Black bars indicate exons, thin black lines indicate introns or UTR, open line in the 5′-UTR indicates an intron in 5′-UTR, triangles shows T-DNA insertions and the mutant name and T-DNA identifier above the symbol. B, RT-PCR confirmed the single mutants of nup98a1, nup98a2, and nup98b1. The position of primers of F and R were indicated in A (black arrows). ACT2 was used as a control. C, Single mutants of nup98a1, nup98a2, and nup98b1 displayed similar phenotypes to wild type however the double mutants of nup98a1 nup98b1 and nup98a2 nup98b1 showed severe early senescence. The photos were taken on day 20 after germination. D, The leaf senescence phenotype of the nup98a nup98b double mutant rosette leaves harvested at inflorescence emergence. E, Ectopic expression of Nup98b rescued the leaf senescence phenotype of the nup98a1 nup98b1 double mutant. All plants grew in soil from germination. The photos were taken on day 30 after germination. An * indicates measurements that were significantly (*P < 0.05; **P < 0.01) different from the control. Error bars indicate ± SD of the mean.
Mutation of Nup98 leads to an early senescence phenotype. A, T-DNA insertion alleles in Nup98a and Nup98b. Black bars indicate exons, thin black lines indicate introns or UTR, open line in the 5′-UTR indicates an intron in 5′-UTR, triangles shows T-DNA insertions and the mutant name and T-DNA identifier above the symbol. B, RT-PCR confirmed the single mutants of nup98a1, nup98a2, and nup98b1. The position of primers of F and R were indicated in A (black arrows). ACT2 was used as a control. C, Single mutants of nup98a1, nup98a2, and nup98b1 displayed similar phenotypes to wild type however the double mutants of nup98a1 nup98b1 and nup98a2 nup98b1 showed severe early senescence. The photos were taken on day 20 after germination. D, The leaf senescence phenotype of the nup98a nup98b double mutant rosette leaves harvested at inflorescence emergence. E, Ectopic expression of Nup98b rescued the leaf senescence phenotype of the nup98a1 nup98b1 double mutant. All plants grew in soil from germination. The photos were taken on day 30 after germination. An * indicates measurements that were significantly (*P < 0.05; **P < 0.01) different from the control. Error bars indicate ± SD of the mean.
Figure 2. The expression of genes related to senescence are altered in the nup98a1 nup98b1 double mutant. A, Genes up-regulated both at dawn (ZT0) and dusk (ZT16). B, Genes up-regulated at dawn (ZT0) or dusk (ZT16). C, Genes down-regulated at dawn (ZT0) and/or dusk (ZT16). D, Genes up-regulated or down-regulated at dawn (ZT0) and dusk (ZT16), respectively. E, Genes with no significant changes both at dawn (ZT0) and dusk (ZT16). The nup98a1 nup98b1 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 RT-PCR measurements were repeated at least three times, in triplicate. All RT-PCR gene expression measurements were normalized to the control TIP41 (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.)
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Figure 3. The nup98a1 nup98b1 double mutant has abnormal starch degradation. Arabidopsis seeds were sown on 1/2 MS medium and placed at 4°C for 3 days, then transferred to 12 h light/12 h dark conditions. Plants were grown for an additional 7, 14, 21, or 28 days and were harvested at ZT0 and ZT12 for starch staining by iodine-potassium iodide (A) and starch quantitative assay by using Starch Determination Kit (Solarbio, Beijing, Cat#BC0700) on Spectrophotometer (B).
Figure 3

The nup98a1 nup98b1 double mutant has abnormal starch degradation. Arabidopsis seeds were sown on 1/2 MS medium and placed at 4°C for 3 days, then transferred to 12 h light/12 h dark conditions. Plants were grown for an additional 7, 14, 21, or 28 days and were harvested at ZT0 and ZT12 for starch staining by iodine-potassium iodide (A) and starch quantitative assay by using Starch Determination Kit (Solarbio, Beijing, Cat#BC0700) on Spectrophotometer (B).
Figure 4. The expression of genes related to starch metabolism are altered in the *nup98a1 nup98b1* double mutant. The seeds of mutant and WT were sown on MS medium. After low temperature treatment for 3 days, plants were grown under long day conditions. Samples were harvested at ZT16, 20 and 24 during the dark phase. All RT-PCR measurements were repeated at least three times, in triplicate. All RT-PCR gene expression measurements were normalized to the control *TIP41* (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) different from the control. Error bars indicate ± SD of the mean.
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Figure 5. Increased nutrients and sucrose delays senescence in nup98a1 nup98b1 double mutants. The seeds of mutant and WT were sown on medium with different strengths of MS and sucrose. After stratification, plants were grown under long day conditions. Both nutrients and sucrose delayed senescence phenotypes in the nup98a nup98b double mutant plants. Bars = 5 cm.
Figure 5

Increased nutrients and sucrose delays senescence in nup98a1 nup98b1 double mutants.

The seeds of mutant and WT were sown on medium with different strengths of MS and sucrose. After stratification, plants were grown under long day conditions. Both nutrients and sucrose delayed senescence phenotypes in the nup98a nup98b double mutant plants.

Bars = 5 cm.
Figure 6. Marker genes of starvation and senescence mis-express in the nup98a1
nup98b1 double mutant. A, Sugar starvation genes. B, Senescence genes. C,
Autophagy genes. The seeds of the mutant and WT were sown on MS medium. After low
temperature treatment for 3 days, the seedlings were grown for 7 days under long day
conditions, then seedlings were transplanted in soil. From that, samples were harvested
in day 5, 10, and 15 at both ZT0 and ZT12. All qPCR measurements were repeated at
least three times, normalized to the reference gene TIP41 (At4g34270) and expressed as
a relative expression value. Student’s t test was used to statistically analyze the data.
Error bars indicate ± SD of the mean.
Marker genes of starvation and senescence mis-express in the nup98a1 nup98b1 double mutant. A, Sugar starvation genes. B, Senescence genes. C, Autophagy genes. The seeds of the mutant and WT were sown on MS medium. After low temperature treatment for 3 days, the seedlings were grown for 7 days under long day conditions, then seedlings were transplanted in soil. From that, samples were harvested in day 5, 10, and 15 at both ZT0 and ZT12. All qPCR measurements were repeated at least three times, normalized to the reference gene TIP41 (At4g34270) and expressed as a relative expression value. Student’s t test was used to statistically analyze the data. Error bars indicate ± SD of the mean.

Figure 7. Nup98a and Nup98b proteins are localized to the nuclear membrane and in the nucleoplasm. The green fluorescent protein (GFP) was fused to either Nup98a or Nup98b to generate N-terminal translational fusional proteins, GFP-Nup98a and GFP-Nup98b, and were driven by the CaMV 3SS promoter after stable transformation into Arabidopsis thaliana plants. Arrow heads indicated that both GFP-Nup98a and GFP-Nup98b enriched near the nuclear periphery when compared to the cytoplasm. PI (propidium iodide) is for cell wall staining.
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