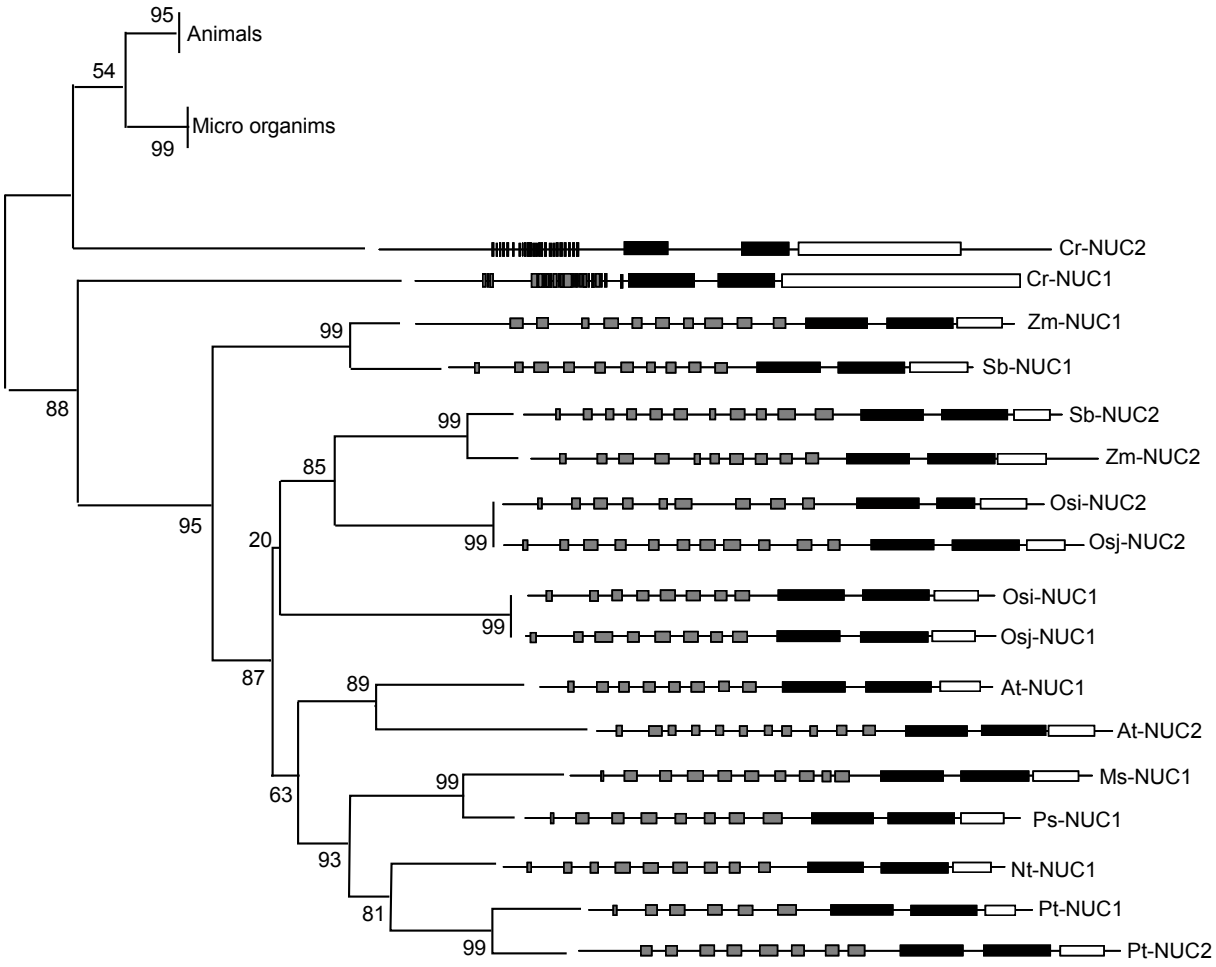
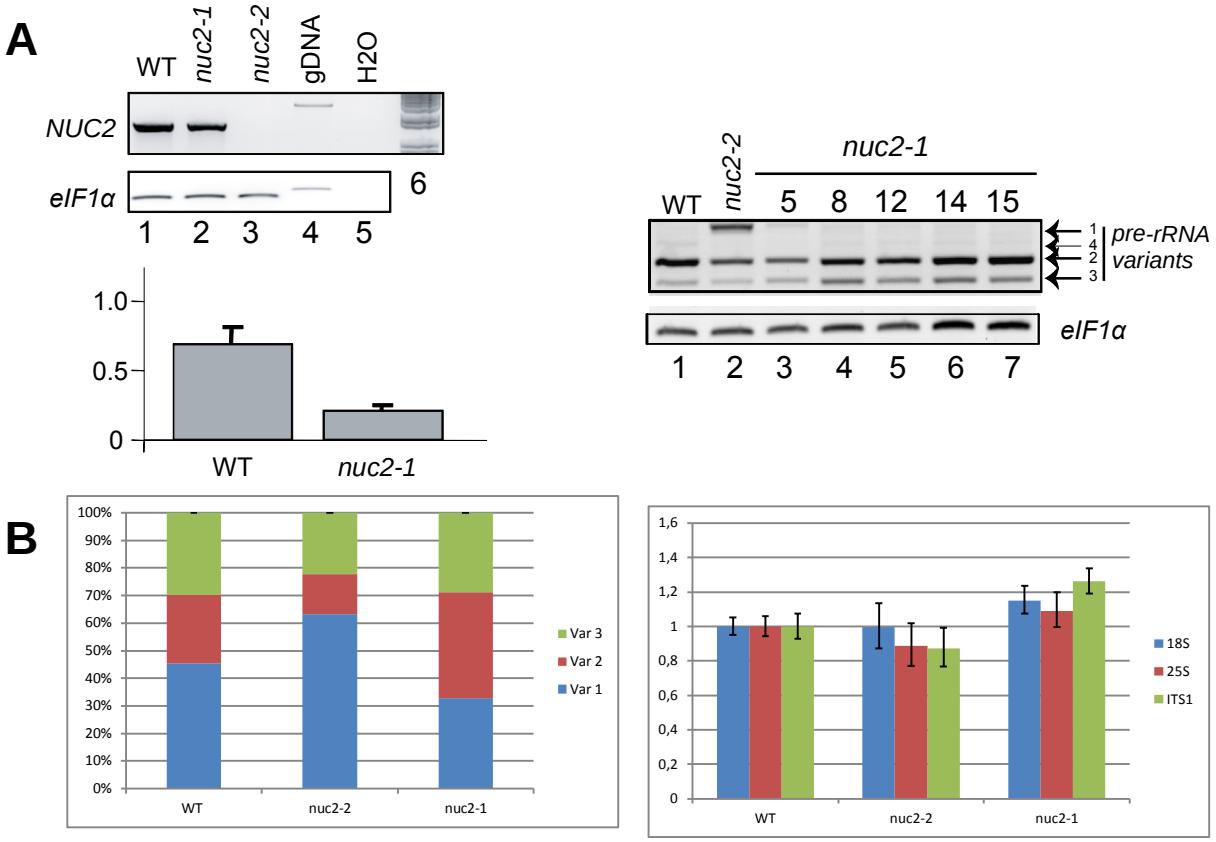
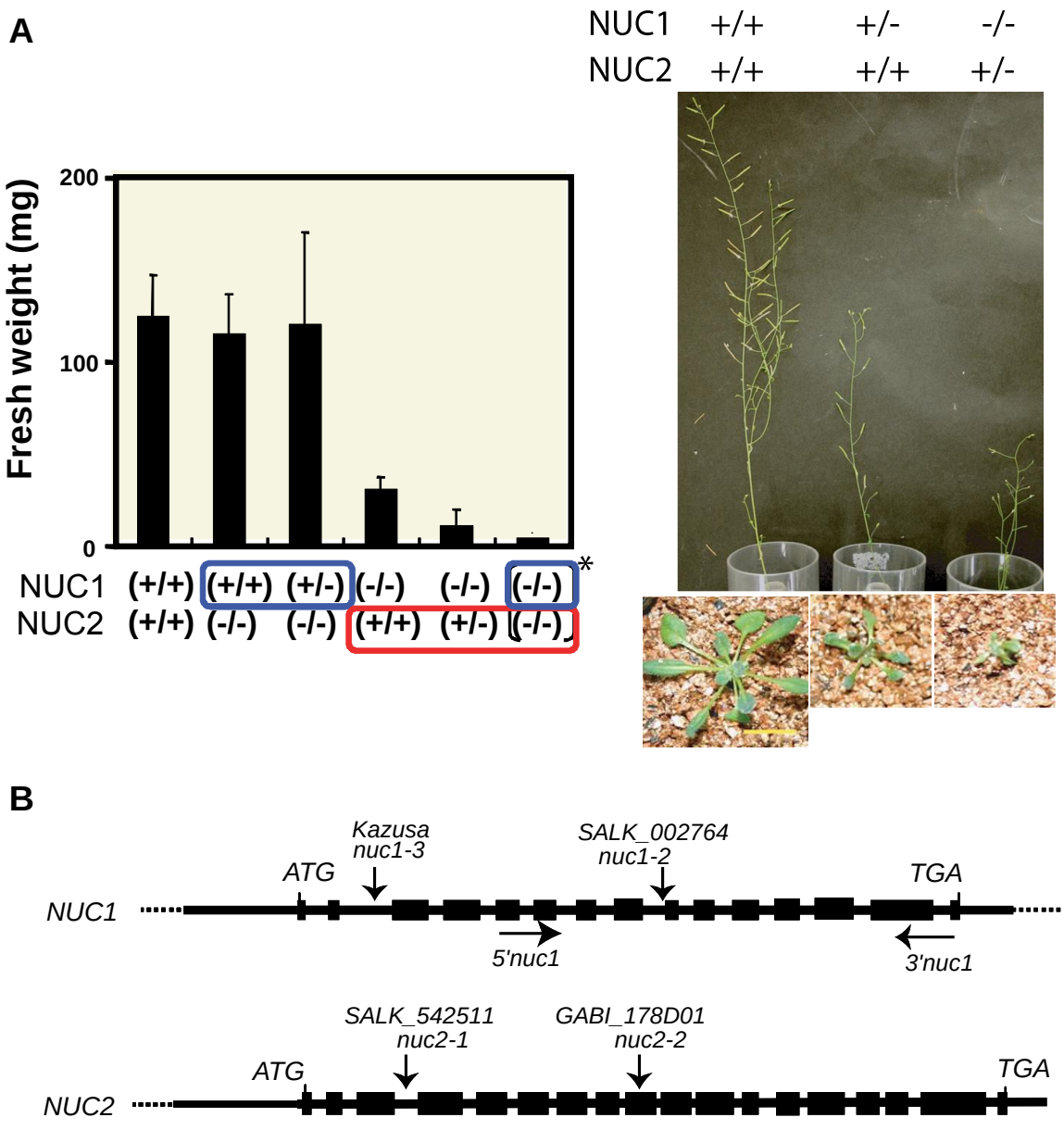


Supplemental Figure 1: The phylogenetic tree was generated with MEGA4 software, using Neighbour Joining method coupled with 1000 bootstrap tests. The percentage value of Bootstrap is shown. Schematic of nucleolin and nucleolin-like proteins from *Arabidopsis thaliana*, Q9FVQ1 (At-NUC1) and Q1PEP5 (At-NUC2), *Oryza sativa* ssp. *japonica*, Q6Z1C0 (Osj-NUC1) and Q7XTT4 (Osj-NUC2); *Oryza sativa* ssp. *indica*, BGIOSIBCE026772 (Osj-NUC1) and BGIOSIBCE016635 (Osj-NUC2), *Sorghum bicolor*, Sb07g005510 (Sb-NUC1), and Sb01g019710 (Sb-NUC2); *Zea mays* FGP025 (Zm-NUC1) and FGT019 (Zm-NUC2), *Populus trichocarpa*, 002310655 (Pt-NUC1) and 002307174 (Pt-NUC2), *Medicago sativa*, T09648 (Ms-NUC1), *Nicotiana tabacum* Q8LNZ4 (Nt-NUC1), *Pisum sativum* T06458 (Ps-NUC1), *Chlamydomonas reinhardtii* XP_001689665.1 (CrNUC1) and GO:0003676 (CrNUC2).



Supplemental Figure 2: A) Left: RT-PCR and RT-qPCR reactions using cDNA prepared from 15 day-old WT (lane 1), *nuc2-1* (lane 2) and *nuc2-2* (lane 3) seedlings and primers *5'nuc2*/*3'nuc2* or *5'nuc2q*/*3'nuc2q* to detect *NUC2* transcripts. Amplification of *eIF1α* transcripts was performed to verify similar amounts of cDNA in each sample. Absence of genomic contamination in the cDNA samples was verified by amplification of *eIF1α* genomic DNA that generates higher molecular size bands (lane 4). Right: RT-PCR reactions using cDNA prepared from 15 day-old WT (lane 1), *nuc2-2* (lane 2), and *nuc2-1* (lanes 3-7) seedlings and primers *5'3ets*/*3'3ets* to detect 3'ETS pre-rRNA respectively. Amplification of *eIF1α* transcripts was performed to verify similar amounts of cDNA in each sample. **B)** Left: PCR amplification of 3'ETS sequences using genomic DNA from WT, *nuc2-2*, *nuc2-1* mutant plants. Relative abundance of each rDNA variant was determined using a LabChip GX system. The bar graphs show the percentage of rDNA VAR1 (blue), VAR2 (red) and VAR3 (green). Right: qPCR analysis to amplify 18S, 25S and ITS1 rDNA sequences respectively from WT, *nuc2-2*, *nuc2-1* mutant plants. The bar graphs show relative amounts of rDNA 18S (blue), 25S (red) and ITS1 (green).



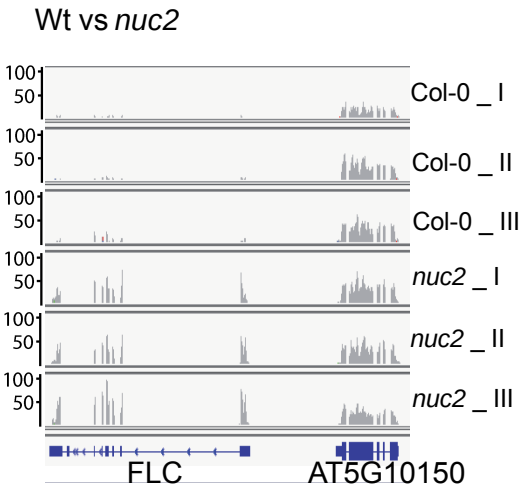
Supplemental Figure 3: A) Analysis of WT, *nuc1-3* (Kojima et al., 2007), *nuc2-1* (SALK_542511) and *nuc1-3nuc2-1* double mutant plants. Left: Bar graph shows fresh weight of WT, *nuc2-1* (with two or one *NUC1* gene), *nuc1-3* (with two or one *NUC2* gene) and *nuc1-3nuc2-1* double mutant plants. Right: WT and *nuc1-3* containing one *NUC2* gene plants grown on soil. **B)** Diagram of *NUC1* and *NUC2* genes from the ATG start to the TGA stop codon is shown. The black boxes correspond to exons separated by introns. The T-DNA insertion in the *NUC1* and *NUC2* mutant plants is indicated.

A Upregulated

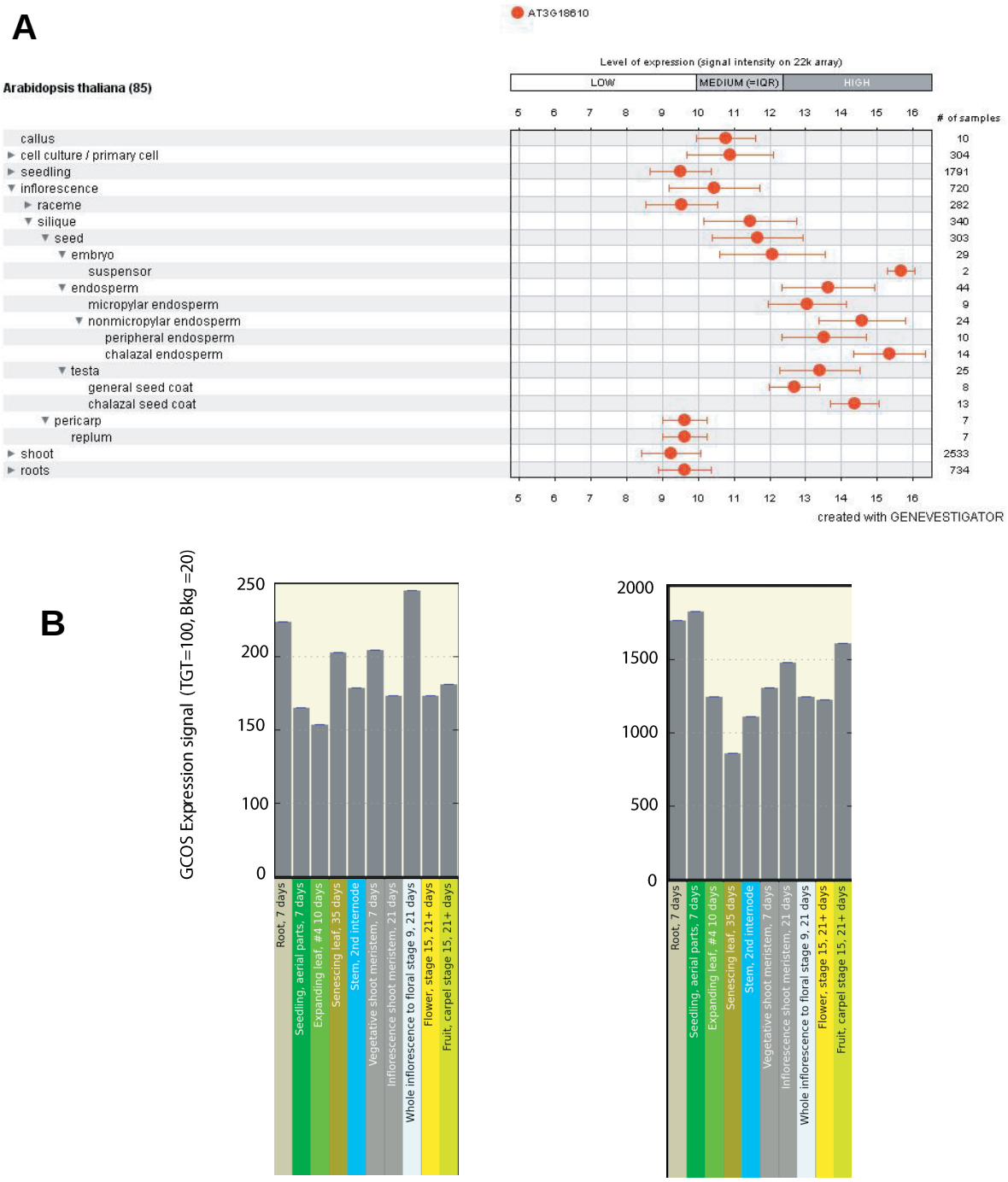
AT1G22160
AT1G27730
AT1G27860
AT1G28370,ATREP10D
AT1G52040
AT1G60190
AT1G74930
AT1G80130
AT2G15400
AT2G25330
AT2G39310
AT3G16360
AT3G18610
AT3G44006
AT4G00250
AT4G00390
AT4G01390
AT4G17490
AT4G22390
AT4G25490
AT4G38340
AT5G04340
AT5G10140 (FLC)
AT5G15500
AT5G44430
AT5G47220
AT5G56370
AT5G58390
AT5G61160
Unassigned
Unassigned
Unassigned
Unassigned

B Downregulated

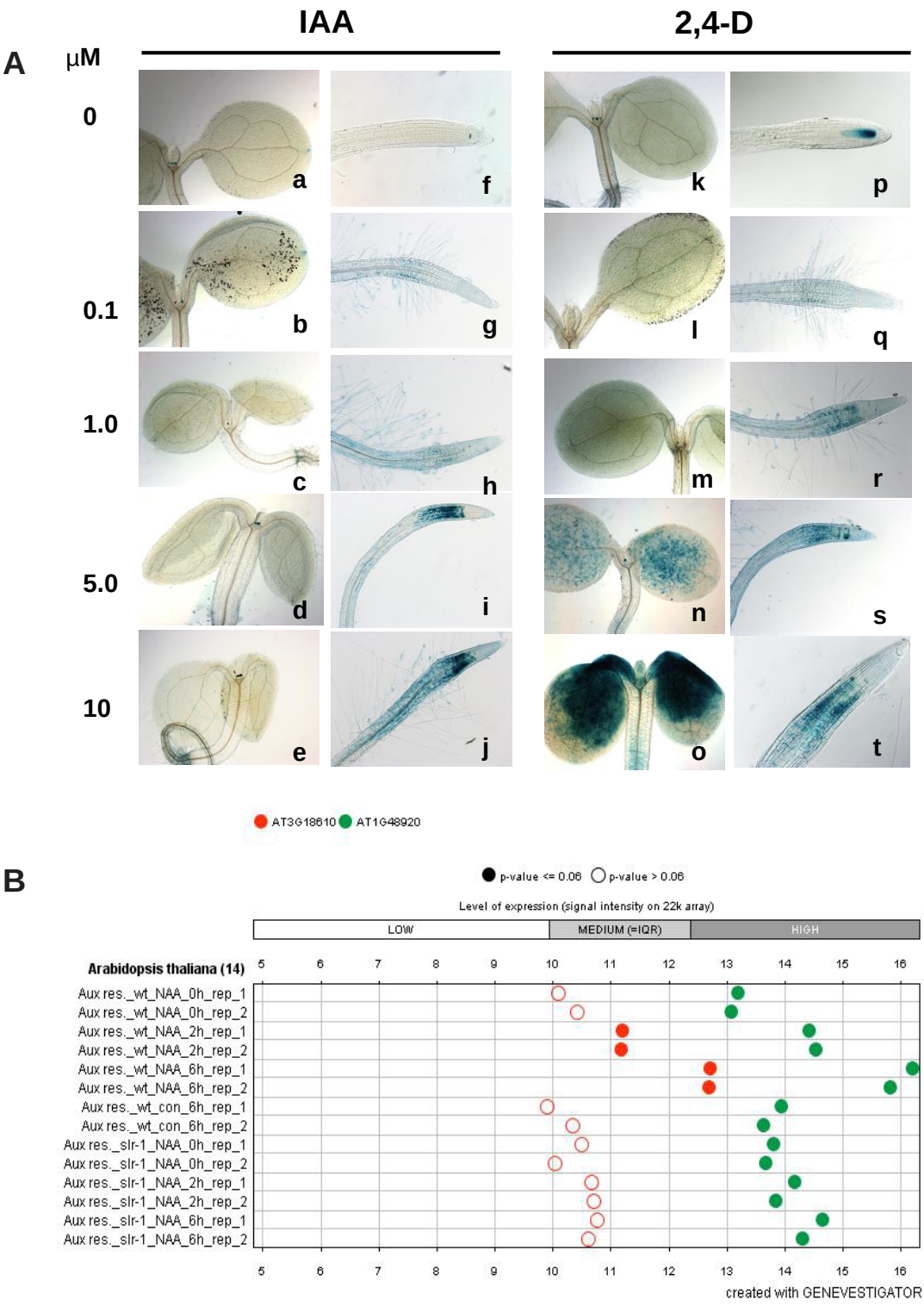
AT1G10585
AT1G14340
AT1G31690
AT1G52770
AT1G75040
AT1G77760
AT2G04066,ATSINE4
AT2G04080
AT2G16660
AT3G11480,HELITRONY1D
AT3G16670
AT4G04620
AT4G04630
AT4G13575
AT4G37990
AT5TE50260,ATCOPIA18A
ATGP8,VANDAL12,VANDAL18NB



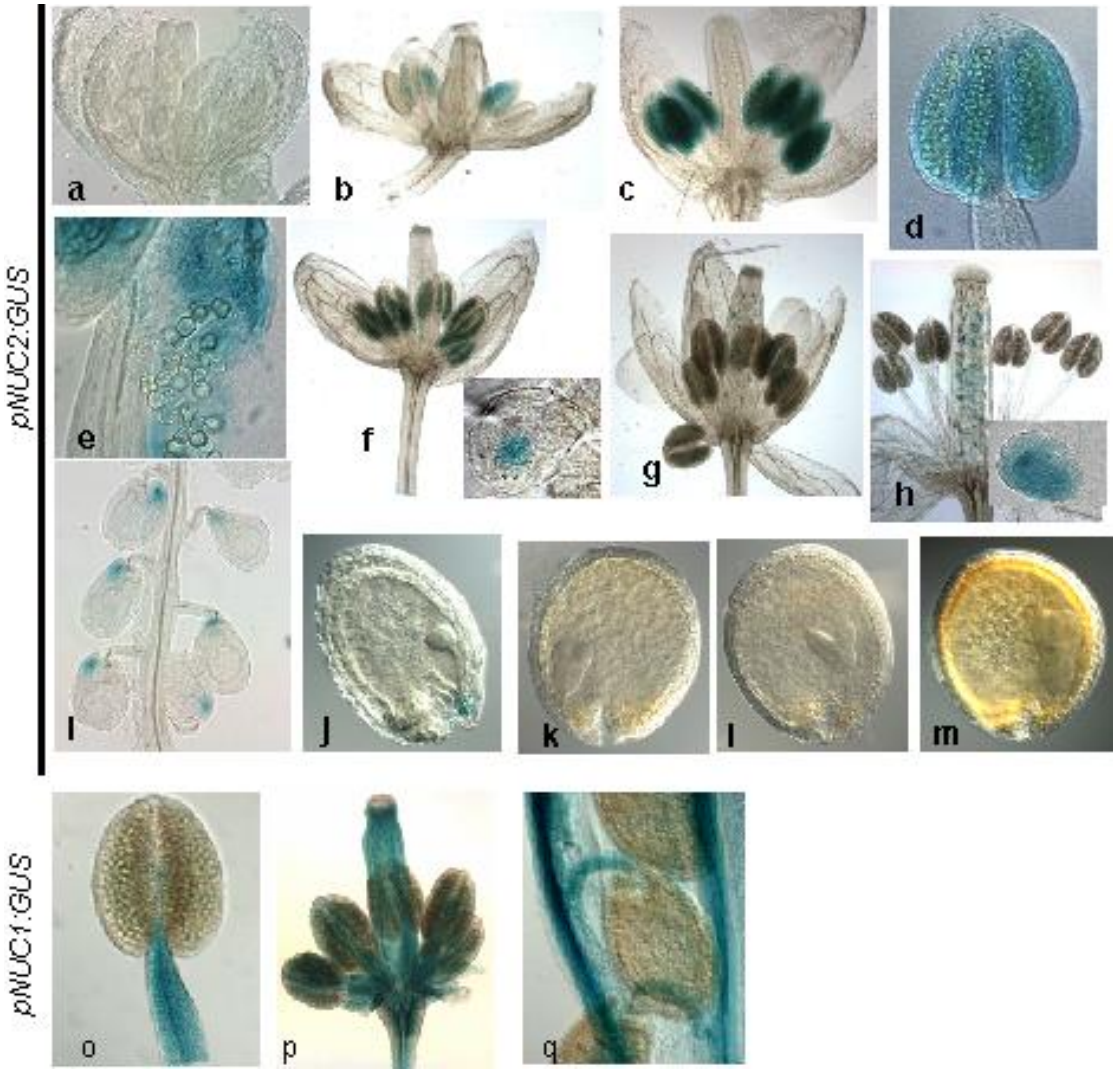
Supplemental Figure 4: RNAseq analysis of *nuc2-2* plants. List of up and down-regulated genes in *nuc2-2* mutant compared to WT plants. FLC (green) is highlighted. The graph shows expression of *FLC* in three WT and *nuc2-2* samples. The identification of At3g18610 (*NUC2*) among the up-regulated genes is due to the accumulation of truncated (upstream, but not downstream of the T-DNA insertion) and reversed transcripts originated from 35S promoter located in the LB border of the T-DNA insertion.



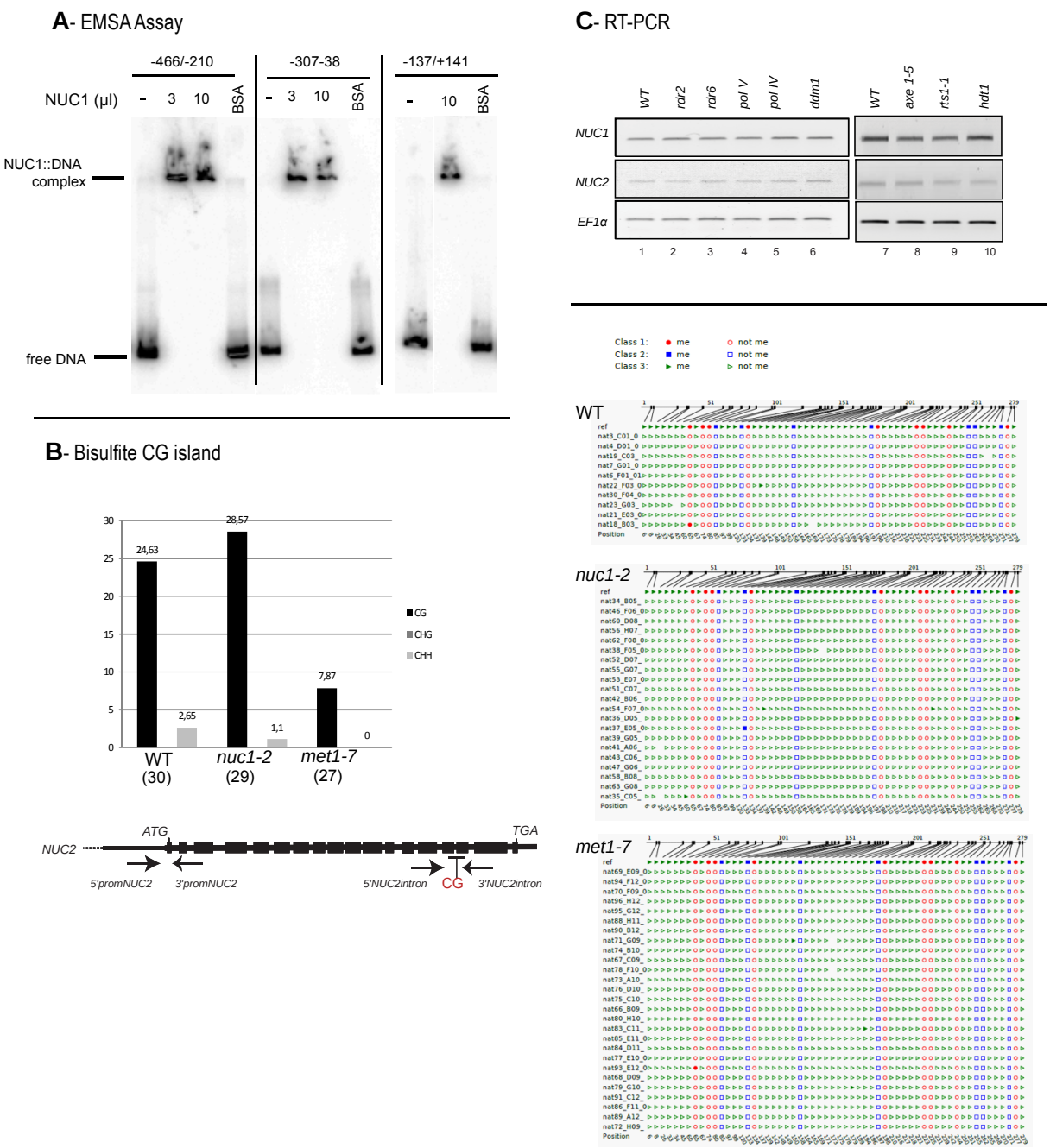
Supplemental Figure 5: Analysis *in silico* to compare relative expression level of *NUC1* and *NUC2* genes. Data were obtained from microarray available (www.bar.utoronto.ca/welcome.htm and www.genevestigator.com/gv/).



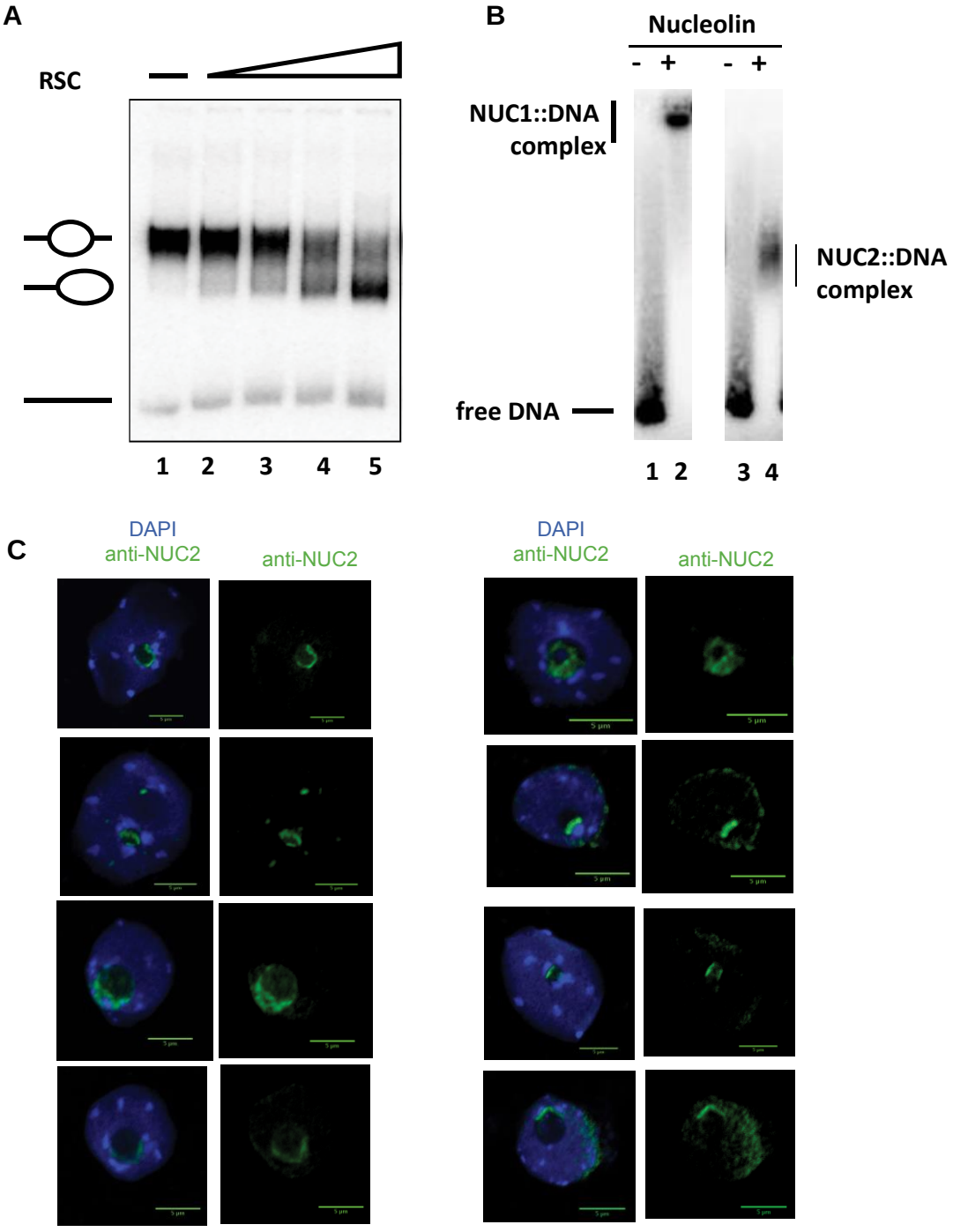
Supplemental Figure 6: A) Five day-old *A. thaliana* seedlings grown on Wattman/MS medium were transferred for 24h to MS liquid medium containing 0, 0.1, 1.0, 5.0 and 10 µM of IAA (a-j) or 2,4 D (k-t). **B)** Analysis *in silico* to compare relative expression levels of *NUC1* (green dots) and *NUC2* (red dots) genes in response to auxin. Data were obtained from a microarray available on www.genevestigator.com/gv/).



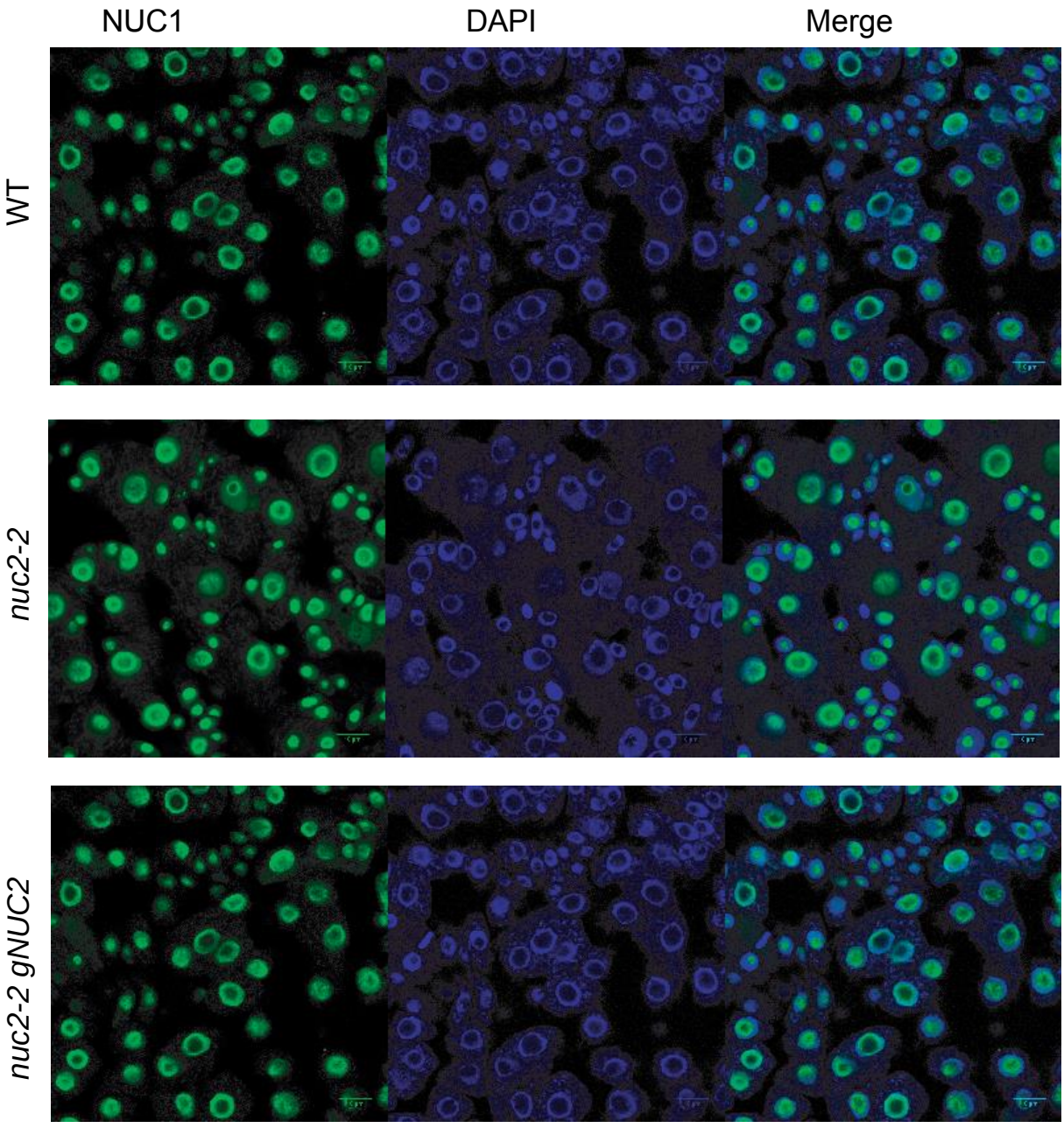
Supplemental Figure 7: GUS staining is not detected at stage 8 (a) or before (not shown), but it appears continually in anthers from stage 9 to stage 11 (b-c and f). GUS staining is detected in the pollen grain sacs, mainly in the tapetum cells and in the walls or parenchyma cells (d) but not in young pollen grains (e). At stage 11, GUS staining decreases in anthers but appears early in nucellus and/or embryo sac (f and insert). Then, at stage 12, the GUS staining increases in pistils (g) and it is clearly observed in embryo sacs at stage 15 (h and insert). Finally, GUS staining is detected at globular stage in the mycropilar (i-j) to disappear at heart (k), torpedo (l) and linear cotyledon (m). Interestingly, *NUC1* promoter activity, is detected in filament (o), pistil at stage 12 (p) and mycropilar at globular stage (q). In these tissues and organs we do not observe GUS staining activity when reporter gene is fused to *NUC2* promoter sequences (compare o, p, q with d, g and i).



Supplemental Figure 8: **A)** NUC1 binds *NUC2* sequences. Analysis of NUC1 binding activity on 4% polyacrylamide gel. EMSA was performed with 3 and 10 µL of His-NUC1 recombinant protein (20 ng/µL). BSA was used as control protein for binding activity. **B)** Bisulfite sequencing analysis of *NUC2* gene in WT, *nuc1-2* and *met1-7* plants. Left: The Bar graph shows the representation (%) of methylated sites in the intron sequence. Right: Graphical representation of bisulfite sequencing in the promoter. The images were generated by using CyMATE. **C)** RT-PCR reactions to detect *NUC1* and *NUC2* transcripts in *rdr2*, *rdr6*, *pol IV*, *pol V*, *ddm1*, *axe1-5*, *rts1-1* and *hdt1* mutant plants.

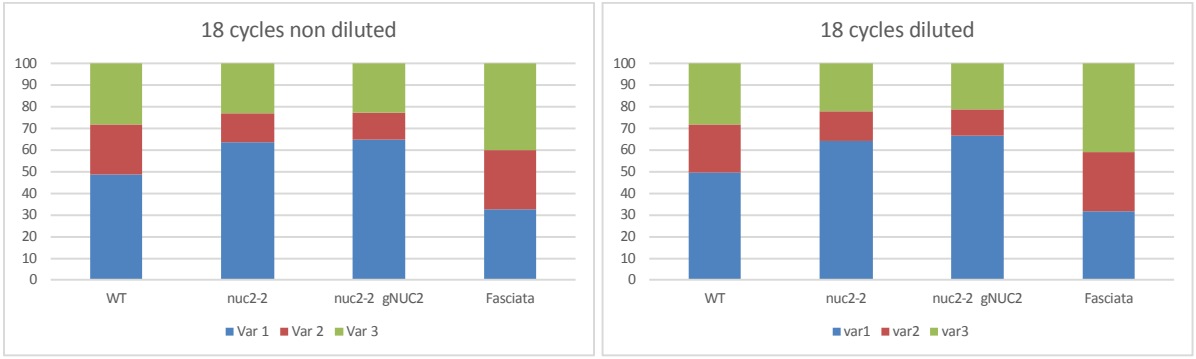


Supplemental Figure 9: A) Titration of RSC in nucleosome remodeling assay. Nucleosomes incubated with increasing amount of RSC (lanes 2-5) result in an efficient mobilization and formation of end-positioned nucleosomes **B)** Incubation of NUC1 (lane 2) or NUC2 (lane 4) with radiolabelled DNA. Lanes 1 and 3; *NUC2* sequence (-307/-38) alone. Protein::DNA complexes are resolved in a 1% agarose gel in 0.5X TBE buffer conditions. Note that NUC1 protein forms a protein-DNA complex that migrates more slowly compared with the protein-DNA complex formed by NUC2 protein. **C)** Immunolocalization of NUC2 in leaves from *nuc1* plants. The NUC2 signal (green) practically co-localizes with chromatin counterstained with DAPI (blue) situated in the periphery of the nucleolus. This is observed consistently in different *nuc1* nuclei.

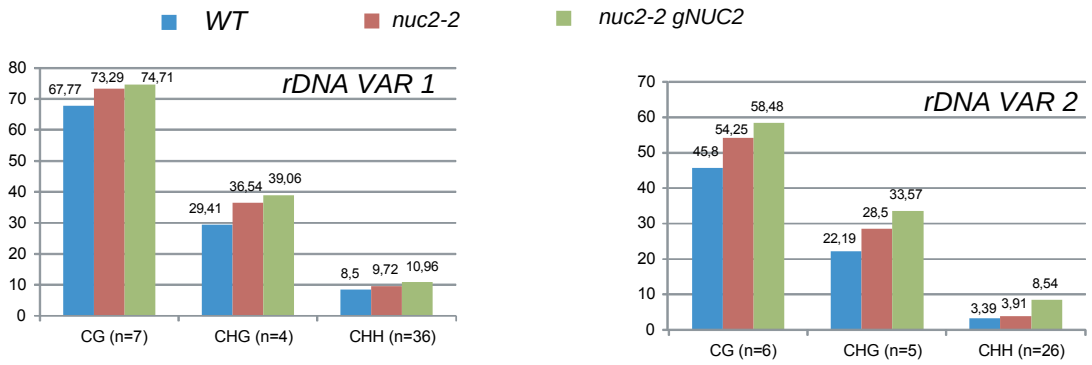


Supplemental Figure 10: Immunolocalization of NUC1 in WT, *nuc2-2* and *nuc2-2 gNUC2* plants. Nucleolin was detected in the nucleolus of WT and mutated plants. Immunostaining was performed using antibodies against NUC1 peptide described previously and Alexa 488 (Roche).

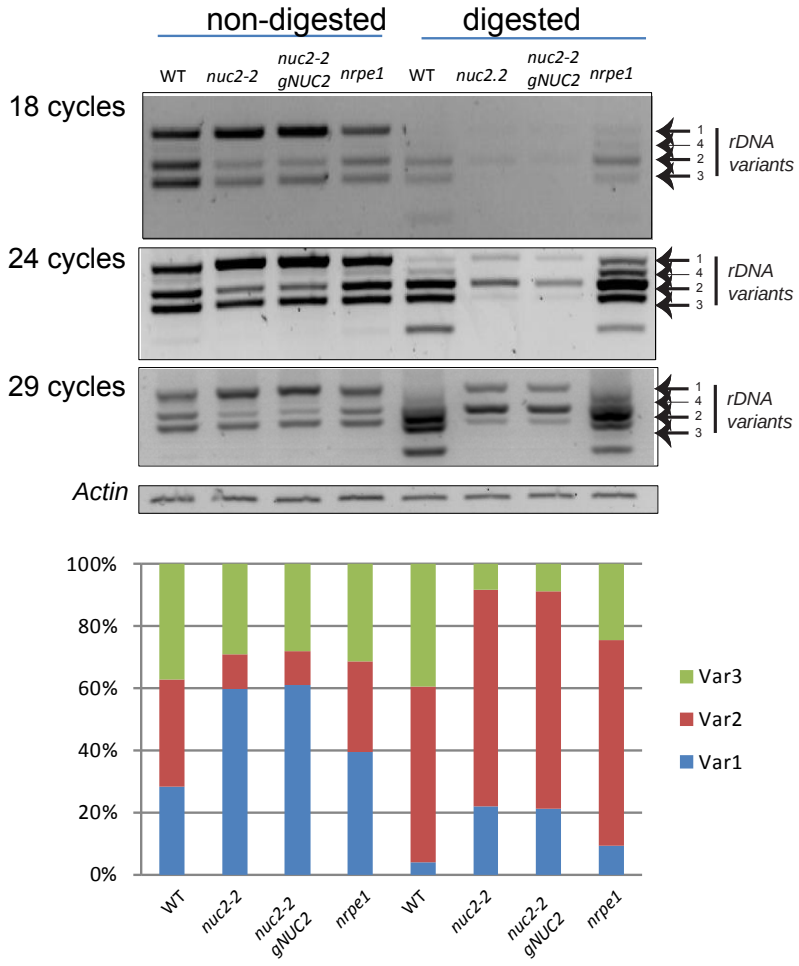
	24 Cycles			24 Cycles (diluted samples)		
	VAR1	VAR2	VAR3	VAR1	VAR2	VAR3
WT	45,43	24,8	29,77	45,85	24,55	29,6
nuc2-2	61,42	13,67	24,91	62,59	12,31	25,1
nuc2-2 gNUC2	64,6	11,07	23,04	64,03	11,84	24,13
nuc1-2	44,64	29,51	25,85	44,54	27,99	27,47
fas2-4	27,28	31,47	41,25	26,46	31,36	42,17
	18 cycles			18 cycles (diluted samples)		
	VAR1	VAR2	VAR3	VAR1	VAR2	VAR3
WT	48,66	23,15	28,19	49,69	22,18	28,13
nuc2-2	63,62	13,62	22,76	64,3	13,33	22,37
nuc2-2 gNUC2	64,63	12,71	22,66	66,59	12,07	21,34
fas2-4	32,56	27,46	39,98	31,85	27,4	40,75



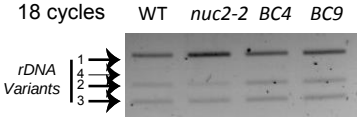
Supplemental Figure 11: PCR and LabChip experiments to determine relative abundance of rDNA variants in WT, *nuc1*, *nuc2*, *nuc2-2 gNUC2* and *fas2* mutant plants. LabChip experiment was realized using 18 or 24 cycles and diluted (1:4) or undiluted samples.



Supplemental Figure 12: Bisulfite sequencing analysis. The bar graphs show the percentage of methylated sites in the 3'ETS rRNA gene sequences (VAR1 and VAR2) from WT (79), *nuc2-2* (81) and *nuc2-2 gNUC2* (78) mutant plants in a CG, CHG and CHH context. *n*= number of potentially methylated sites.



Supplemental Figure 13: Top, Genomic DNA from WT, *nuc2-2, nuc2-2 gNUC2* and *nrpe1* plants digested or not with McrBC. Amplifications of 3'ETS were performed with 18, 24 and 29 cycles. Actin amplification was performed to verify similar amount of DNA. Bottom, Bar graph shows LabChip experiments (using PCR with 29 cycles) to determine relative abundance (%) of rDNA variants in McrBC treated or untreated genomic DNA from WT, *nuc2-2, nuc2-2 gNUC2* and *nrpe1* plants.



	24 Cycles			24 Cycles diluted		
	VAR1	VAR2	VAR3	VAR1	VAR2	VAR3
WT	46,55	20,25	33,19	45,55	21,25	33,2
nuc2-2	62,83	11,94	25,23	62,1	12,39	25,51
BC4	44,86	23,52	31,61	44,9	23,78	31,33
BC9	47,06	22,16	30,78	46,99	22,82	30,19

Supplemental Figure 14. PCR and LabChip experiments to determine relative abundance of rDNA variants in WT, *nuc2-2* and WT x *nuc2-2* backcrossed plants. Top, PCR reactions with 18 cycles amplification. Bottom, LabChip experiment using 24 cycles PCR reaction samples (undiluted or 1:4 fold diluted) to determine relative abundance (%) of rDNA variants

Supplemental Table 1

N° signals/Nucleolus	**WT** ***n=63***	***nuc2-2*** ***n= 68***	***nuc2-2 gNUC2*** ***n=44***
≤2	100,00	86,76	86,36
3≥	0,00	13,24	13,64
Total	100,00	100,00	100,00

Supplemental Table 1: Percentage of nucleolus associated with ≤2 or ≥3 rDNA signals in WT, *nuc2-2* and *nuc2-2 gNUC2* plants. The ***n*** indicates number of nuclei analyzed in each sample.

Supplemental Table 2

Oligo	**Sequence**
5'nuc2	GACGAGGAAACTGTCCCTATG
3'nuc2	CTACTCTTCATCATTAAAGACCG
5'nuc2α	GAACACATTGGCGAGAAATG
3'nuc2α	GAACCGTCCACGTTTCAAGCG
5'nuc2b	GTCAAGTGTCTAGAGAATCAC
3'nuc2b	CAGCTAGGTTTACGAGAAGC
5'nuc2c	CAGAAGCCAATCTCTCTCTAC
3'nuc2c	GAAGCAGGTGTTTCAACCTG
5'nuc2q	TTACTAGGCCGCGATG
3'nuc2q	GTCTCACGATCTGTTGG
5'nuc1	CCAAGAAGCCCGCAGCTGCTG
3'nuc1	CTACTCGTCACCGAAGGTAGTC
5'eIf1α	CTAAGGATGGTCAGACCCG
3'eIf1α	CTTCAGGTATGAAGACACC
5'3ets	GACAGACTTGTCCAAAACGCCCACC
3'3ets	CTGGTCGAGGAATCCTGGACGATT
5'flcq	CCGAACTCATGTTGAAGCTTGTTGAG
3'flcq	CGGAGATTTGTCCAGCAGGTG
5'actineq	GGTAACATTGTGCTCAGTGGTGG
3'actineq	AACGACCTTAATCTTCATGCTGC
5'u3	CGACCTTACTTGAACAAGATCTGTTG
3'u3	CTGTCAGACCGCCGTGCGA

tis	CCTCGTGCCGATATCCGATACCATCCC
p	CCGATCACCACTCATACGCCGAAC
5'-315	GGATATGATGYAATGTTTTGTGATYG
3'+243	CCCATTCTCCTCRACRATTCARC
5' -250	CAAGCAAGCCCATTCTCCTC
3'+250	CAACTAGACCATGAAAATC
5'18S	CGTAGTTGAACCTTGGGATG
3'18S	CACGACCCGGCCAATTA
5'25S	GCATCAGGTCTCCAAGGTG
3'25S	AGCCCTCAGAGCCAATC
5'its1	GTATCGGCATGCTCGGG
3'its1	TTCGTTTGCATGTTCCTTGAC
5's	CTTTTCGGGCNTTTTNGTG
3's	CGAAAAGGTATCACATGCC
5'AtLINE	CCGATGGTGACCAAGAGTTT
3'AtLINE	TCAATGTCGGAGACCTCCTC
5'AtSN1	TGTCTTGGAAAGGATATTGGAAG
3'AtSN1	AAGTGGTGGTTGTACAAGCC
5NUC2intron	GATGATTGGATTYATTTTTGG
3NUC2intron	CAAAAACATACATAATCCCATC
5promNUC2	YTTGGGAGTYAAGTGTYTAG
3promNUC2	CTTRCCCATARATCCTRATC
bis3'ets_fwd	TGGATAGTGAGAATAATAAGTGAAGAG
bis3'ets_rev	TCATCCATCATTTAATACTAATTCT

Supplemental methods

Immunostaining

4 week-old leaves were fixed in cold 4% formaldehyde in Tris buffer (10 mM Tris-HCl pH 7.5, 10 mM NaEDTA, 100 mM NaCl) for 20 minutes and wash for 2 x 10 minutes with cold Tris buffer. Leaves were then chopped with a razor blade in LB01 buffer (15 mM Tris-HCl pH 7.5, 2 mM NaEDTA, 0.5 mM spermine, 80 mM KCl, 20 mM NaCl and 0.1% Triton X-100). The cell slurry was then filtered through a falcon cell strainer cap of 30µm. 5 µl of nuclei suspension were added to 10 µl of sorting buffer (100 mM Tris-HCl pH 7.5, 50 mM KCl, 2 mM $MgCl_2$, 0.05% Tween-20 and 5% sucrose) and spread on a polylysine slide. After air drying, samples were post-fixed in 2% formaldehyde in Phosphate Buffer (PBS) for 5 minutes and then washed twice with water before being air-dried.

In a moist chamber, 1X PBS + 0.5% triton were added directly on the slides for 15 min at RT, before being washed 3 times in 1X PBS for 5 min at RT. 1:500 dilution of purified α-At-NUC2 antibody (Pontvianne et al 2007), were applied on the slide in 1x PBS containing 3% BSA and 0.05% tween 20. After overnight at 4 °C, 3 washes in 1X PBS were performed, and the secondary antibody was added (Anti-Rabbit coupled with Alexa 488 from Invitrogen). After 3 hours at RT, the samples were washed 3 times in 1X PBS. Slides were then mounted in Vectashield at 1 µg/ml of DAPI and seal them with nail polish. Observation and imaging was performed using a confocal microscope LSM 700 from Zeiss.

Bisulfite analysis of *Arabidopsis NUC2* sequences

For bisulfite analysis, 500 ng of DNA was treated using the Epitect Bisulfite Kit (Qiagen). For *NUC2* sequences, primers *5'promnuc2*/*3'promnuc2* and *5'nuc2intron*/*3'nuc2intron* were used to amplify the *NUC2* promoter and transcribed (14^{th} intron) sequences. For 3'ETS rDNA sequences, primers *bis3ets_fwd/bis3'ets_rev* were used.

EMSA assay:

Recombinant NUC1-His and NUC2-His proteins were purified from *E. coli* cells. *NUC2* promoter sequences used for ChIP experiments (-466/-210, -307/-38 and -

137/+141) were amplified by PCR, purified and end labeled using T4- polynucleotide kinase in presence of γP^{32}-ATP. For EMSA assay, equal amounts of NUC1-His and NUC2-His proteins were incubated for 30 minutes on ice with radioactive DNA probe in a buffer containing 10 mM Tris HCl pH7.5, 5% glycerol, 1mM DTT, 100 mM NaCl and 2.5 mM $MgCl_2$. Then nucleolin:DNA complex reactions were resolved on a 4% native polyacrylamide gel or 1% agarose gel and run in 0.5X TBE buffer. Complexes were analyzed using Phosphor Imager Technology.

RNA-seq and bioinformatics analyses

Total RNA was extracted from three week-old *Arabidopsis* plant leaf tissues using TRIzol reagent (MRC, Inc.). Total RNA from three different pools of wild-type Col-0 or *nuc2-2* mutant plants was prepared independently to generate three biological replicates per sample. Sequencing was performed by the MGX facility using a Hiseq 2000 to generate 1X 51bp long reads. Illumina reads from non-stranded, polyA+ RNA-seq libraries were aligned to the *A. thaliana* TAIR10 annotated genome reference using Tophat2, Cufflinks, Cuffmerge and Cuffdiff (Langmead et al., 2009).

Name	Replicate number	Mapped	Platform	Facility	Source
Col-0	1	43534749	Hiseq 2000	MGX	This study
Col-0	2	38143538	Hiseq 2000	MGX	This study
Col-0	3	45482777	Hiseq 2000	MGX	This study
nuc2-2	1	39905299	Hiseq 2000	MGX	This study
nuc2-2	2	44147023	Hiseq 2000	MGX	This study
nuc2-2	3	40600021	Hiseq 2000	MGX	This study