**Supplemental Figure 1: SUVR1, SUVR2 and/or SUVR4 are required for nucleolar dominance in *A. suecica***

(A) Phylogenetic relationships among SUVR proteins, displayed as a Neighbor-Joining tree with bootsrap values.

(B) RNA was extracted from wild-type plants or T1 transformants expressing amiRNAs targeting *SUVR1/2/4* (B, left panel) or *SUVR1* alone (B, right panel). *A. thaliana*-derived rRNA gene transcripts were detected by RT-CAPS. Transgenic *A. suecica* expressing double-stranded RNA that targets *DRM2* and disruopts nucleolar dominance was used as a positive control. After RT-PCR amplification of the ITS1 region, amplicons were subjected to *Hha*I digestion to distinguish *A. thaliana*-derived from *A. arenosa*-derived rRNA genes. Reactions in which reverse transcriptase was omitted serve as negative controls (-RT).

(C) Specificity of *SUVR4* amiRNA targeting. To test for off-target effects, RT-PCR was conducted using primers specific for *SUVH4, SUVH5, SUVH6, SUVR1, SUVR2* or *ACT2* mRNAs. RNA was purified from wild-type (1/8x, 1/2x or 1x amounts) and four *SUVR4* amiRNA lines (1x RNA amounts).

**Supplemental Figure 2: SUVH4, SUVH5 and SUVH6 are not implicated in nucleolar dominance in *A. suecica*.**

A) RT-PCR analysis of *SUVH4* mRNA levels in T2 progeny of two independent *SUVH4* amiRNA lines, compared to wild-type (WT). Actin (*ACT2*) RT-PCR reactions control for the amount of RNA analysed.

B) RT-PCR analysis of *SUVH5* and *SUVH6* mRNA levels in eight transgenic plants expressing an amiRNA that targets both *SUVH5* and *SUVH6*, compared to wild-type (WT) and actin controls.

C) RT-CAPS analysis of *A. arenosa* and/or *A. thaliana*-derived rRNA gene transcripts in *A. suecica* wild-type or *SUVH4* knockdown lines. The derepression of *A. thaliana*-derived rRNA genes (arrow) in a *DRM2* RNAi line serves as a positive control for loss of nucleolar dominance. Actin (*ACT2*) RT-PCR reactions control for the amount of RNA analysed.

D) RT-CAPS analysis of *A. arenosa* and/or *A. thaliana*-derived rRNA gene transcripts in *A. suecica* wild-type or transgenic plants expressing an amiRNA targeting both *SUVH5 and SUVH6*. A *DRM2* RNAi line serves as a positive control for loss of nucleolar dominance. Actin (*ACT2*) RT-PCR reactions control for the amount of RNA analysed.

E) RT-CAPS analysis of *A. arenosa* and/or *A. thaliana*-derived rRNA gene transcripts in *A. suecica* wild-type or *SUVH5 or SUVH6* knockdown lines. A *DRM2* RNAi line serves as a positive control for loss of nucleolar dominance. Actin (*ACT2*) RT-PCR reactions control for the amount of RNA analysed.

**Supplemental Figure 3: Quantitative PCR analyses of *A. thaliana*-derived rRNA genes in wild-type and *SUVR4*-amiRNA plants.**

Specific TaqMan probes were used to evaluate the relative amounts of *A. thaliana* versus *A. arenosa*-derived rRNA gene transcripts by quantitative RT-PCR in wild-type plants or or plants expressing an amiRNA targeting *SUVR4* mRNAs.

**Supplemental Figure 4: H3K27me1 association with rRNA genes**

Data derived from a genome-wide ChIP-sequencing study of H3K27me1- associated regions (Jacob et al. 2010) shows the distribution of H3K27me1 throughout an rRNA gene repeat in wild–type *A. thaliana*, ecotype Col-0.

## Supplemental Materials and Methods:

**Primers for amiRNA production**

| | |
|---|---|
| IFPamiratx12 | GATTATAATCATCGGTCAGCGCCTCTCTCTTTTGTATTCC |
| IIFPamiratx12 | GAGGCGCTGAACTATGATTATAATCAAAGAGAATCAATGA |
| IIIFPamiratx12 | GAGACGCTGACCTAAGATTATTATCACAGGTCGTGATATG |
| IVFPamiratx12 | GATAATAATCTTAGGTCAGCGTCTCTACATATATATTCCT |
| | |
| IFPamiratx345 | GATCAAGCACATGTCACGTGAACTCTCTCTTTTGTATTCC |
| IIFPamiratx345 | GAGTTCACGTGACATGTGCTTGATCAAAGAGAATCAATGA |
| IIIFPamiratx345 | GAGTCCACGTGACATCTGCTTGTTCACAGGTCGTGATATG |
| IVFPamiratx345 | GAACAAGCAGATGTCACGTGGACTCTACATATATATTCCT |
| | |
| ImiRSUVH1 | GATTCTAAATGACCTTAAAGGGGTCTCTCTTTTGTATTCC |
| IImiRSUVH1 | GACCCCTTTAAGGTCATTTAGAATCAAAGAGAATCAATGA |
| IIImiRSUVH1 | GACCACTTTAAGGTCTTTTAGATTCACAGGTCGTGATATG |
| IVmiRSUVH1 | GAATCTAAAAGACCTTAAAGTGGTCTACATATATATTCCT |
| | |
| IFPamlRSUVH2 | GATATATGCAAACTCACGGTTCTCTCTTTTGTATTCC |
| IIFPamlRSUVH2 | GAACCGTGGTGAGTTTGCATATATCAAAGAGAATCAATG |
| IIIFPamIRSUVH2 | GAACAGTGGTGAGTTAGCATATTTCACAGGTCGTGATAT |
| IVFPamlRSUVH2 | GAAATATGCTAACTCACCACTGTTCTACATATATATTCCT |
| | |
| ImiRSUVH3 | GATAATTATATAGTCAATGCCGGTCTCTCTTTTGTATTCC |
| IImiRSUVH3 | GACCGGCATTGACTATATAATTATCAAAGAGAATCAATGA |
| IIImiRSUVH3 | GACCAGCATTGACTAAATAATTTTCACAGGTCGTGATATG |
| IVmiRSUVH3 | GAAAATTATTTAGTCAATGCTGGTCTACATATATATTCCT |
| | |
| IFPamlRSUVH1&3 | GATACATATAAAAGAACCGGCACTCTCCTCTTTGTATTCC |
| IIFpamlRSUVH1&3 | GAGTGCCGGTTCTTTTATATGTATCAAAGAGAATCAATGATGA |
| IIIFPamlRSUVH1&3 | GAGTACCGGTTCTTTAATATGTTTCACGGTCGTGATATG |
| IVFPamlRSUVR1&3 | GAAACATATTAAAGAACCGGTACTCTACATATATATTCCT |
| | |
| lamirH13b | GATTAGGTAAAATAAGCATGCCCTCTCTCTTTTGTATTCC |
| IIamirH13b | GAGGGCATGCTTATTTTACCTAATCAAATAATCATGA |
| IIIamirH13b | GAGGACATGCTTATTATACCTATTCACAGGTCGTGATATG |
| IVamirH13b | GAATAGGTATAATAAGCATGTCCTCTACATATATATTCCT |
| | |
| ImiRSUVH4 | GATGAAAAAAAACGGTGTCCGACTCTCTCTTTTGTATTCC |
| IImiRSUVH4 | GAGTCGGACACCGTTTTTTTTCATCAAAGAGAATCAATGA |
| IIImiRSUVH4 | GAGCCGGACACCGTATTTTTTCATCACAGGTCGTGATATG |
| IVmiRSUVH4 | GATGAAAAAATACGGTGTCCGGC TCTACATATATATTCCT |
| | |
| ImiRSUVH4b | GATACCAGTTAAGTTATGCCGTCTCTCTCTTTTGTATTCC |
| IImiRSUVH4b | GAGACGGCATAACTTAACTGGTATCAAAGAGAATCAATGA |
| IIImiRSUVH4b | GAGAAGGCATAACTTTACTGGTTTCACAGGTCGTGATATG |
| IVmiRSUVH4b | GAAACCAGTAAAGTTATGCCTTCTCTACATATATATTCCT |
| | |
| ImiRSUVH5 | GATTTAACTTTATACCGAGCGCTTCTCTCTTTTGTATTCC |
| IImiRSUVH5 | GAAGCGCTCGGTATAAAGTTAAATCAAAGAGAATCAATGA |

| | |
|---|---|
| IIImiRSUVH5 | GAAGAGCTCGGTATATAGTTAATTCACAGGTCGTGATATG |
| IVmiRSUVH5 | GAATTAACTATATACCGAGCTCTTCTACATATATATTCCT |

| | |
|---|---|
| ImiRSUVH6 | GATTATAATCGATACCGGCCTCGTCTCTCTTTTGTATTCC |
| IImiRSUVH6 | GACGAGGCCGGTATCGATTATAATCAAAGAGAATCAATGA |
| IIImiRSUVH6 | GACGCGGCCGGTATCCATTATATTCACAGGTCGTGATATG |
| IVmiRSUVH6 | GAATATAATGGATACCGGCCGCGTCTACATATATATTCCT |

| | |
|---|---|
| IFPamlRSUVH5&6 | GATTATGGTTTATAAACCATGCCTTCTCTCTTTTGTATTCC |
| IIFPamlRSUV5&6 | GAAGGCAGGTTTATAAACCATAATCAAAGAGAATCAATGA |
| IIIFPamlRSUVH5&6 | GAAGACAGGTTTATATACCATATTCACAGGTCGTGATATG |
| IVFPamlRSUVH5&6 | GAATATGGTATATAAACCTGTCTTCTACATATATATTCCT |

| | |
|---|---|
| lamirH56b | GATTATGGTTTATAAACGTCCCTTCTCTCTTTTGTATTCC |
| IIamirH56b | GAAGGGACGTTTATAAACCATAATCAAAGAGAATCAATGA |
| IIIamirH56b | GAAAGGACGTTTATTAACCATTATCACAGGTCGTGATATG |
| IVamirH56b | GATAATGGTTAATAAACGTCCTTTCTACATATATATTCT |

| | |
|---|---|
| IFPamlRSUVH7&8 | GATATAAAAGTTCCGGCTCGTATTCTCTCTTTGTATTCC |
| IIFPamlRSUV7&8 | GAATACGAGCCGGAACTTTTATATCAAAGAGAATCAATGA |
| IIIFPamlRSUVH7&8 | GAATCCGAGCCGGAAGTTTTATTTCACAGGTCGTGATATG |
| IVFPamlRSUVH7&8 | GAAATAAAACTTCCGGCTCGGATTCTACATATATATCCT |

| | |
|---|---|
| lamirH9 | GATTAATACATACTCCGTTCCACTCTCTCTTTTGTATTCC |
| IIamirH9 | GAGTGGAACGGAGTATGTATTAATCAAAGAGAATCAATGA |
| IIIamirH9 | GAGTAGAACGGAGTAAGTATTATTCACAGGTCGTGATATG |
| IVamirH9 | GAATAATACTTACTCACTCTACATATATATTCCT |

| | |
|---|---|
| ImiRSUVR1 | GATTTTGTACGACTTGGGGCGCTTCTCTCTTTTGTATTCC |
| IImiRSUVR1 | GAAGCGCCCCAAGTCGTACAAAATCAAAGAGAATCAATGA |
| IIImiRSUVR1 | GAAGAGCCCCAAGTCCTACAAATTCACAGGTCGTGATATG |
| IVmiRSUVR1 | GAATTTGTAGGACTTGGGGCTCTTCTACATATATATTCCT |

| | |
|---|---|
| ImiRSUVR2 | GATATATGCAGATTAGGTGCCGTTCTCTCTTTTGTATTCC |
| IImiRSUVR2 | GAACGGCACCTAATCTGCATATATCAAAGAGAATCAATGA |
| IIImiRSUVR2 | GAACAGCACCTAATCAGCATATTTCACAGGTCGTGATATG |
| IVmiRSUVR2 | GAAATATGCTGATTAGGTGCTGTTCTACATATATATTCCT |

| | |
|---|---|
| ImiRSUVR4 | GATTATATACGACCAATTGCCAGTCTCTCTTTTGTATTCC |
| IImiRSUVR4 | GACTGGCAATTGGTCGTATATAATCAAAGAGAATCAATGA |
| IIImiRSUVR4 | GACTAGCAATTGGTCCTATATATTCACAGGTCGTGATATG |
| IVmiRSUVR4 | GAATATATAGGACCAATTGCTAGTCTACATATATATTCCT |

| | |
|---|---|
| IFPamlRSUVR1&2&4 | GATTTTTCAGAACCCTAGTCTGCTCTCTCTTTTGTATTCC |
| IIFPamlRSUVR1&2&4 | GAGCAGACTAGGGTTCTGAAAAATCAAAGAGAATCAATGA |
| IIIFPamIRSUVR1&2&4 | GAGCCGACTAGGGTTGTGAAAATTCACAGGTCGTGATATG |
| IVFPamlRSUVR1&2&4 | GAATTTTCACAACCCTAGTCGGCTCTACATATATATTCCT |

| | |
|---|---|
| IFPamlRSUVR5 | GATTAAATTGAATTTCAGCCCGCTCTCTCTTTTGTATTCC |
| IIFPamlRSUVR5 | GAGCGGGCTGAAATTCAATTTAATCAAAGAGAATCAATGA |

IIIFPamIRSUVR5 GAGCAGGCTGAAATTGAATTTATTCACAGGTCGTGATATG
IVFPamIRSUVR5 GAATAAATTCAATTTCAGCCTGCTCTACATATATATTCCT

**Primers used for the RT-CAPS assay:**

ITS1for GCGCTACACTGATGTATTCAACGAG
ITS1Rev CGCACCTTGCGTTCAAAGACTCGA

**Primers used for RT-PCR assays :**

pre-rRNA 3'ETS For CTCGAGGTTAAATGTTATTACTTGGTAAGATTCCGG
pre-rRNA 3'ETS Rev TGGGTTTGTCATATTGAACGTTTGTGTTCATATCACC
5Suvr4CDS For ATGATCAGTCTCTCCGGACTAACC
3Suvr4CDS Rev TCATTTGCGCTTTTTAGACACCTC
5ACT2 for AAGTCATAACCATCGGAGCTG
3ACT2 rev ACCAGATAAGACAAGACACAC

**Taqman primers for qPCR assays:**

ETStaqman For TGACATGGATTCTTCGAGGCCT
ETStaqman Rev CATGACACGCCCATTCTCTTCG
A. arenosa ETS taqman probe Vic-TATATTGGGAAAGCGCATGGT-MBG
A. thaliana ETS taqman probe 6Fam-TATATAACTTGTTCGCATGATATT

Actin F: GAGAGATTCAGATGCCCAGAAGTC
Actin R: TGGATTCCAGCAGCTTCCA
AtSN1 F: CCAGAAATTCATCTTCTTTGGAAAAG
AtSN1 R: GCCCAGTGGTAAATCTCTCAGATAGA
5'ETS-4241F: TTGGAACGATTGATGATTTTGAGT
5'ETS-4316R: GCCTACGAACACTAGCTATCCGATC
25S-10062F: TGTTCACCCACCAATAGGGAA
25S-10132R: TCAGTAGGGTAAAACTAACCTGTCTCAC
IGS-573F: GTCAAACACTTGGTGATATGAACACA
IGS-623R: GCATGGGTTTGTCATATTGAACGT
3'ETS-84F: GAATTCCCAACTTTACACGAGCTC
3'ETS-134R: AAGTAATAACATTTAACCTCGAGAGACGAG

***A. thaliana*-specific probe used for S1 nuclease protection**

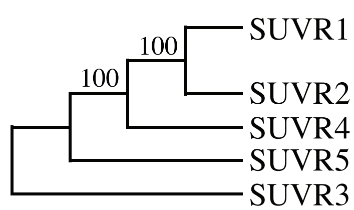
5'-GGGTTCCCCACGGACTGCCCAGACTCCCTCAACACCCACCCCCCTATATAGCTGCC-3'

**PCR primers for generation of FISH probes**
45S rRNA genes
-250 to +250 +1 For CAAGCAAGCCCATTCTCCTC
-250 to +250 +1 Rev CAACTAGACCATGAAAATCC

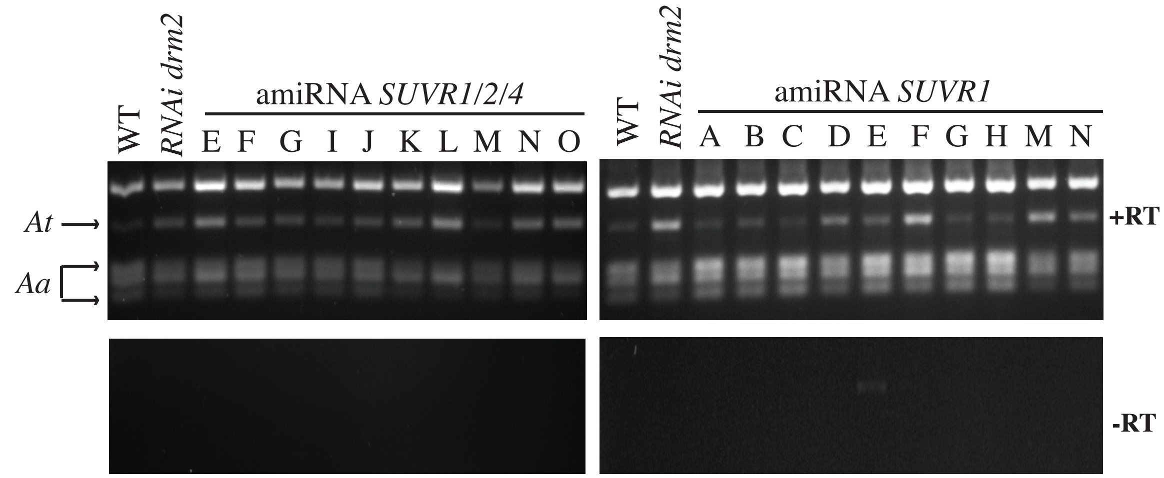
5S rRNA genes
5Sfor GGATGCGATCATACCAG
5Srev CGAAAAGGTATCACATGCC

Pontvianne_Fig.S1

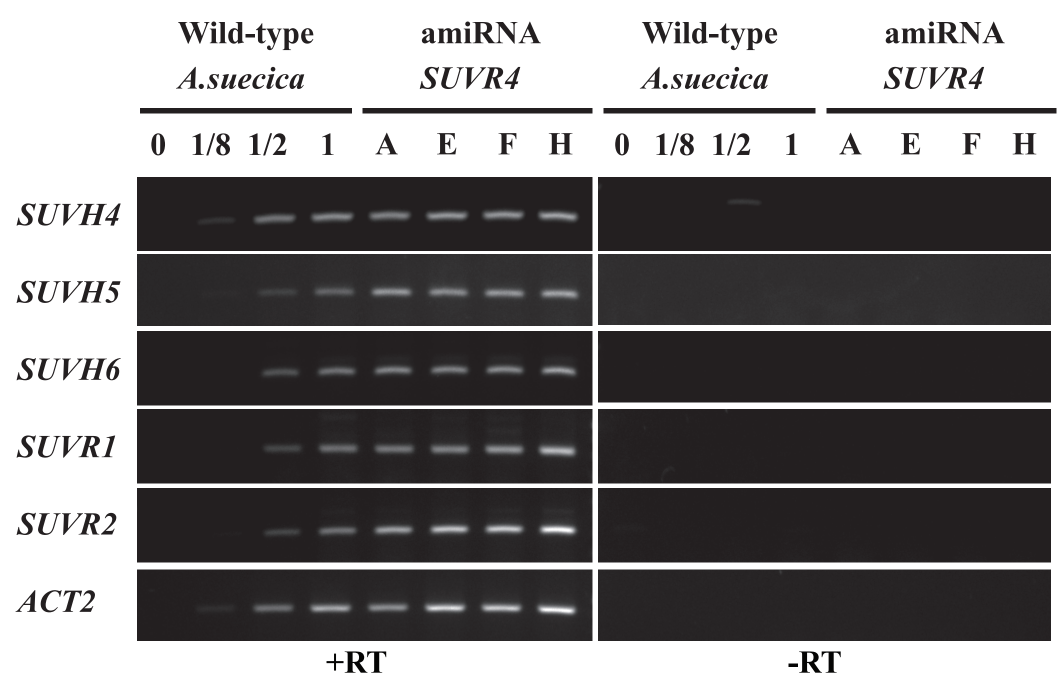
A. Phylogenetic tree



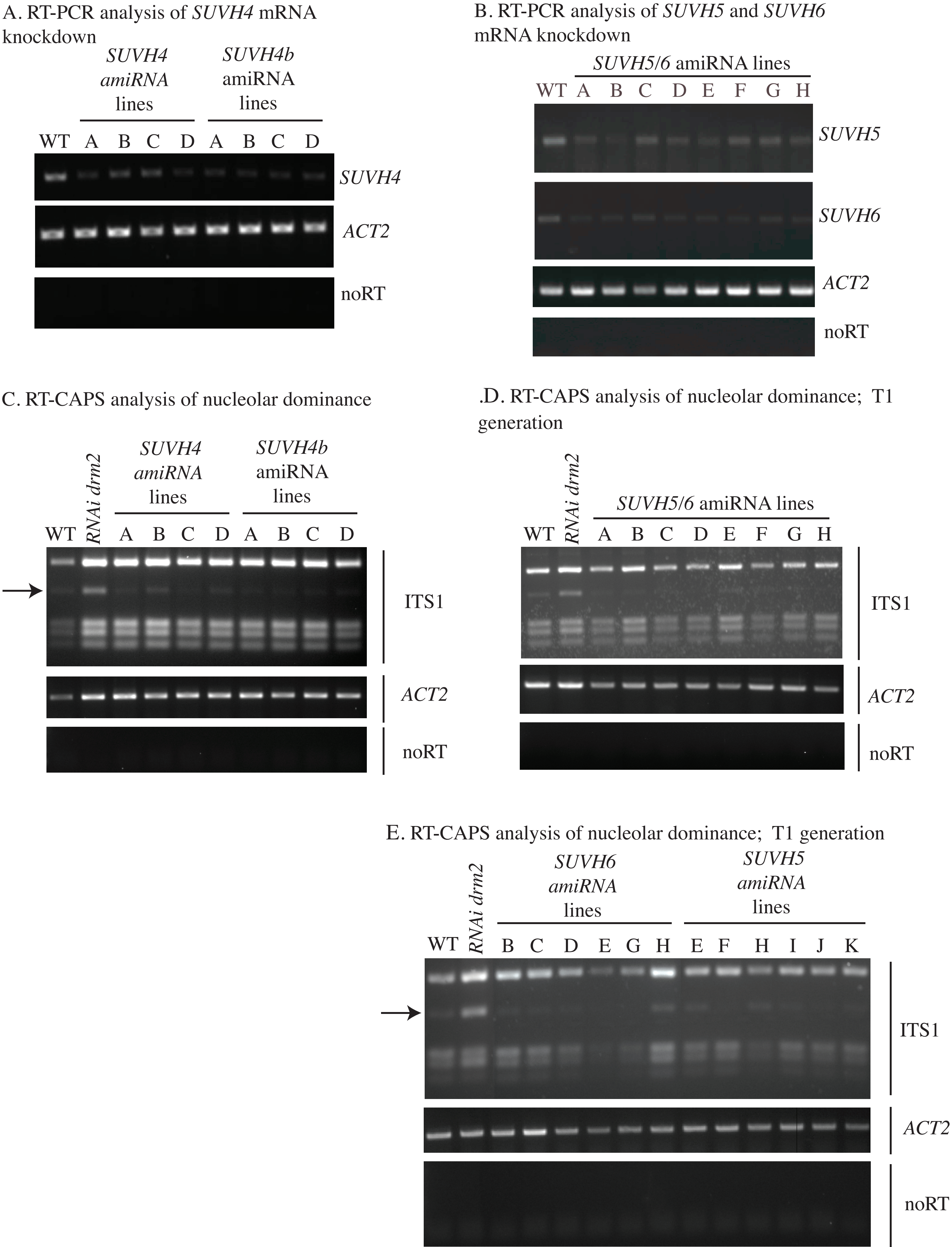

B. RT-CAPS assay, T1 generation transgenic lines



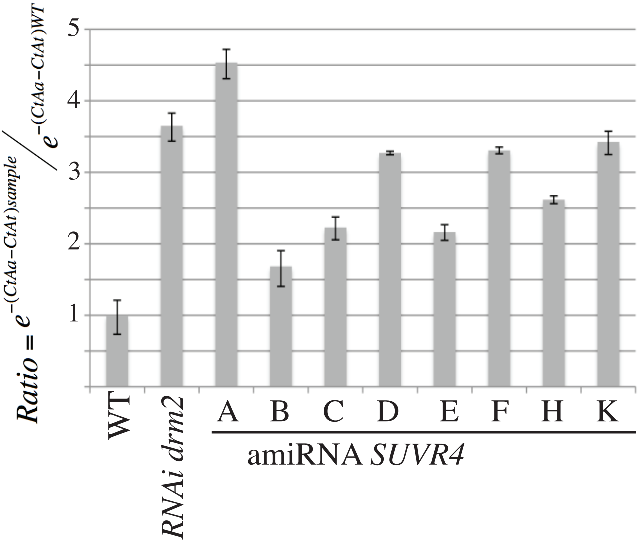

C. RT-PCR amplification of *SUVR* or *SUVH* mRNAs in amiRNA lines targeting *SUVR4*.



Pontvianne_Fig.S2



qPCR analyses



H3K27me1 and bulk H3 association with rRNA genes, determined by ChIP-seq