A SWI/SNF Chromatin-Remodeling Complex Acts in Noncoding RNA-Mediated Transcriptional Silencing

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SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Yeast two hybrid
The full length cDNA of IDN2 was generated by PCR and cloned into pAS2 vector (Clontech). Yeast Y190 cells containing pAS2-IDN2 plasmid were transformed with Arabidopsis yeast two hybrid cDNA library, (ABRC stock #CD4-22 (Kim et al., 1997)), and screened on dropout medium lacking leucine, tryptophan, and histidine but containing 50mM 3-aminotriazol. To test the interaction between two proteins in yeast, the full length cDNAs were cloned into pENTR/D-Topo vector (Invitrogen) to produce entry clones according to manufacturer’s instructions. All the entry constructs were subsequently transferred to destination vector pGADT7-GW or pGBKKT7-GW (Lawit et al., 2007), and the pGADT7/pGBKKT7 empty vectors served as negative controls. All the pGBKTK7-based constructs were transformed into yeast strain Y187, and all the pGADT7-based constructs were transformed into yeast strain Y190. Yeast mating of Y187 and Y190 was performed according to Clontech Yeast Protocols Handbook (1999).

Generation of transgenic plants
The full length cDNA or genomic DNA including promoter regions of SWI3B and IDN2 were cloned into pENTR/D-Topo vector (Invitrogen) to produce entry clones according to manufacturer’s instructions. The resulting entry plasmids were incubated with the destination vectors: pMDC107 (Curtis and Grossniklaus, 2003), pEarleyGate103 (Earley et al., 2006), pEarleyGate302 (Earley et al., 2006), or pZY35S302 with the Gateway LR Clonase™ II Enzyme Mix (Invitrogen) to obtain IDN2p:IDN2-GFP (pMDC107-IDN2), IDN2p:IDN2-M8-GFP (pMDC107-IDN2-M8), SWI3Bp:SWI3B-FLAG (pEarleyGate-SWI3B), 35S:SWI3B-GFP (pEarleyGate103-SWI3B), 35S:IDN2-FLAG (pZY35S302-IDN2), 35S:IDN2-GFP (pEarleyGate103-IDN2), 35S:SWI3B-FLAG (pZY35S302-SWI3B), and 35S:IDN2-M8-FLAG (pZY35S302-IDN2-M8). To generate a binary vector pZY35S302 for 35S-driven expression of C-terminally FLAG-tagged proteins, the Gateway cassette, FLAG nucleotide sequence and OCS 3’ were amplified from pEarleyGate302 (Earley et al., 2006) using Pfu DNA Polymerase (Agilent Technologies). Primers used for this and other PCR amplifications are shown in Table S1. After KpnI and HindIII double digestion, the Gateway cassette was inserted into KpnI and HindIII digested pCHF1 vector (Fankhauser et al., 1999). All constructed plasmids were introduced into the GV3101 strain of Agrobacterium tumefaciens and transformed into Arabidopsis plants by the floral dip method (Clough and Bent, 1998) or infiltrated into tobacco leaves (Voinnet et al., 2003).

Protein co-immunoprecipitation
Infiltrated tobacco leaves or 3-week-old Arabidopsis rosette leaves were ground into fine powder in liquid nitrogen, extracted using lysis buffer (50mM Tris-HCl pH 8.0, 150mM NaCl, 10mM EDTA, 10% glycerol, 1mM PMSF, 1% Plant Protease Inhibitor (Sigma), 0.5% Triton-X100 and centrifuged at 8000 rpm at 4°C for 10 min. Resulting protein extracts were incubated with anti-GFP antibody (MBL 598, 1:1000 dilution) and 50μl of 50% slurry of Protein A agarose beads (Invitrogen). Beads were washed 3 times with the lysis buffer, and the bound proteins were eluted with 2x SDS buffer. Gel blots were analyzed using monoclonal anti-GFP antibody (Covance, MMS-118P), or monoclonal anti-FLAG antibody (Stratagene, 200472).

Chromatin-immunoprecipitation (ChIP)
ChIP was performed as described (Wierzbicki et al., 2008) with the following modifications: proceeding washes with Honda buffer, nuclei were washed once in 1 ml MNase reaction buffer (10 mM Tris-Cl pH 8, 15 mM NaCl, 60
Mm KCl, 1 mM CaCl$_2$; centrifugation at 1900 g, 5 minutes at 4°C) and resuspended in 1 ml MNase reaction buffer. 250 µl aliquots of nuclei were incubated with 600 Kunitz units of Micrococcal Nuclease (NEB) at 37°C for 10 min, then sonicated with two 10 second long pulses (1 minute intervals) with a Fisher Scientific Sonic Dismembrator Model 100 (power setting 1). Immunoprecipitation was performed using 50 µl Dynabeads protein A (Invitrogen) and 2.5 µl anti-histone H3 antibody (ab1791, Abcam) or affinity purified anti-SWI3B antibody at 4°C over-night. After reversion of crosslinking, samples were incubated with 20 µg proteinase K (Invitrogen) at 65°C for 2 hours. Rabbit polyclonal anti-SWI3B antibody was raised against a C-terminal portion of the SWI3B protein (aa 248-469) expressed in bacteria and affinity purified. H3 ChIP-seq samples were treated similarly, but without MNase treatment and were sonicated eight times with 10 second long pulses. Library generation and Illumina sequencing were performed by the University of Michigan Sequencing Core.

RNA analysis
For RT-PCR total RNA from inflorescences was extracted using RNeasy Plant Mini kit (Qiagen), and treated with DNase I (Invitrogen). Real time RT-PCR was performed using One-Step qRT-PCR kit (Invitrogen) according to the manufacturer’s instructions. Pol V-dependent transcripts were assayed in RNA digested with 1 unit of Turbo DNase (Ambion) and reverse transcribed with Superscript III reverse transcriptase (Invitrogen) using random primers (Invitrogen) followed by real time PCR. For RNA-seq total RNA was extracted from 2.5 weeks old seedlings using RNeasy Plant Mini kit (Qiagen). rRNA was depleted from 8µg total RNA using RiboMinus Plant Kit for RNA-seq (Invitrogen). Library generation and Illumina sequencing was performed by the University of Michigan Sequencing Core.

DNA methylation analysis
DNA methylation tests using methylation sensitive restriction endonucleases were performed as described (Rowley et al., 2011) and analyzed by PCR or real-time PCR.

MNase-seq
2g of 2.5-weeks old seedlings were ground to a fine powder in liquid nitrogen and resuspended in 15 ml Honda buffer (0.44 M Sucrose, 1.25 % Ficoll, 2.5 % Dextran T40, 20 mM HEPES-KOH pH 7.4, 10 mM MgCl$_2$, 0.5% Triton X-100, 5 mM DTT, 1 mM PMSF, 1 % plant protease inhibitors (Sigma)). After filtering through two layers of Miracloth, the filter was washed in 10 ml Honda buffer. This washing buffer was then filtered through two fresh layers of Miracloth and the combined filtrates were centrifuged (2500 g, 15 minutes at 4°C). The pellet was washed four times in 1 ml Honda buffer (centrifugation at 2500 g, 15 minutes at 4°C) and 1 ml MNase reaction buffer (10 mM Tris-Cl pH 8, 15 mM NaCl, 60 mM KCl, 1 mM CaCl$_2$; centrifugation at 3000 g, 5 minutes at 4°C) and finally resuspended in 660 µl MNase reaction buffer. 100 µl aliquots of nuclei were incubated with Micrococcal Nuclease (NEB) at 20°C for 10 minutes. To terminate the reaction, 10 µl STOP buffer (100 mM EDTA, 100 mM EGTA), 10 µl 10 % SDS and 40 µg proteinase K (Invitrogen) were added followed by an incubation at 60°C for one hour. DNA was extracted with phenol-chloroform-isoamyl alcohol and chloroform-isoamyl alcohol and precipitated with ethanol. The pellet was washed in 70% ethanol, resuspended in 30 µl TE and incubated with 1 U RNase cocktail (Ambion) at 37°C for one hour and then at 4°C over-night. DNA corresponding to the mononucleosomal fraction was purified (QIAEX II gel extraction kit, Qiagen) after separation on a 2% agarose gel and 20 ng of DNA was used for library generation. Library generation and Illumina sequencing was performed by the University of Michigan Sequencing Core.
Figure S1. IDN2 interacts with SWI3B, Related to Figure 1

(A) IDN2 interacts with Pol V-produced lncRNA. Input, no antibody and no RT controls as well as RNA IP results shown in Figure 1A were normalized to wild type input. No RT control was performed using ACTIN2 primers. Bars show averages normalized to wild type inputs and standard deviations from four biological repeats.

(B) IDN2 protein levels. Equal amounts of total protein extracts from idn2 mutants, nrpe1 mutant and corresponding wild type controls were assayed using western blot with affinity purified rabbit polyclonal anti-IDN2 antibody.
(C) IDN2 interacts with SWI3B in tobacco. FLAG-tagged SWI3B was coexpressed in tobacco leaves with GFP-tagged IDN2. After immunoprecipitation with anti-GFP antibody the sample was analyzed using western blot with anti-FLAG antibody. Plants expressing only single construct were used as controls. Total protein extracts (inputs) were assayed using western blot to demonstrate comparable protein expression levels. Asterisks indicate non-specific bands. Reciprocal co-immunoprecipitation is shown in Figure 1E.

(D) IDN2 interacts with SWI3B by its coiled-coil domain. Truncated IDN2 was assayed for interactions with SWI3B using yeast two hybrid. A series of three 10x dilutions is shown. Yeast growth on a plate with His is shown as a loading control.

(E) The XS domain within IDN2 is not required for interaction with SWI3B in yeast two hybrid assay. A deletion mutant in the XS domain of IDN2 corresponding to the idn2-1 mutant (Ausin et al., 2009) was tested for interaction with SWI3B using yeast two hybrid. A series of three 10x dilutions is shown. Yeast growth on a plate with His is shown as a loading control.

(F) The XS domain within IDN2 is not required for interaction with SWI3B in tobacco leaves. A FLAG-tagged deletion mutant in the XS domain of IDN2 corresponding to the idn2-1 mutant (Ausin et al., 2009) was coexpressed with GFP-tagged SWI3B in tobacco leaves. After immunoprecipitation with anti-GFP antibody the sample was analyzed using western blot with anti-FLAG antibody.
Figure S2. Characterization of IDN2 dimerization domain and its functional significance, Related to Figure 2

(A) Octuple mutations in subdomain B of the coiled-coil region disrupt IDN2 dimerization. Interaction of wild type IDN2, IDN2 with subdomain B deleted, IDN2 with octuple mutations (M8) and IDN2 with triple mutations were assayed for interaction with wild type IDN2 using yeast two-hybrid. A series of three 10x dilutions is shown. Yeast growth on a plate with His is shown as a loading control.

(B) IDN2 dimerization is required for its function – additional independent transgenic lines extending the result shown in Figure 2E. *idn2-2* knock out mutant *Arabidopsis* plants were transformed with wild type IDN2 or IDN2 M8. Obtained transgenic plants were assayed for changes in DNA methylation by digesting with DNA methylation-sensitive restriction endonucleases (*Hae*III for *AtSN1* and *IGN5*, *Sau3A*I for *MEA-ISR*) followed by PCR. Transformation of the *idn2-2* knock out mutant with wild type IDN2 restored DNA methylation to wild type levels at all tested loci. IDN2 M8 was unable to restore DNA methylation at any of the tested loci. Sequences with no restriction sites were used as controls (*ACTIN2* for *Hae*III and *JA35/JA36* for *Sau3A*I).

(C) The XS domain of IDN2 is not required for dimerization in yeast two hybrid. A mutated IDN2 corresponding to the *idn2-1* mutant was tested for interaction with wild type IDN2 using yeast two hybrid. A series of three 10x dilutions is shown. Yeast growth on a plate with His is shown as a loading control.

(D) The XS domain of IDN2 is not required for dimerization in tobacco leaves. A FLAG-tagged deletion mutant in the XS domain of IDN2 corresponding to the *idn2-1* mutant (Astin et al., 2009) was coexpressed with GFP-tagged wild type IDN2 in tobacco leaves. After immunoprecipitation with anti-GFP antibody the sample was analyzed using western blot with anti-FLAG antibody.
Figure S3. SWI3B contributes to RNA-mediated transcriptional silencing, Related to Figure 3

(A) SWI3B expression is reduced in the swi3b/+ line. RNA accumulation of SWI3B was assayed using real time RT-PCR in swi3b/+ mutant compared to Col-0 wild type. Graphs show averages and standard deviations from three biological repeats.

(B-E) Silencing targets are derepressed in swi3b/+ mutant. RNA accumulation from At1TE58825, At4TE27915 and At3TE51910 was assayed using real time RT-PCR in idn2-2 mutant compared to Ws wild type and in swi3b/+ and nrpe1 mutants compared to Col-0 wild type. UBQ10 was tested as a control (E). Graphs show averages normalized to ACTIN2 and wild type and standard deviations from three biological repeats.

(F) SWI3B controls the expression levels of a significant subset of Pol V and IDN2 targets. Venn diagram showing genes identified using RNA-seq to be downregulated in nrpe1, idn2-1 and/or swi3b/+ mutants. RNA-seq was performed in three independent biological repeats. * denotes statistically significant enrichment of overlaps (see text and Figure S3G for details).

(G) Overlaps between genes identified by RNA-seq to be upregulated or downregulated in the analyzed mutants. p-values correspond to the observed overlap compared to overlap expected by chance and were obtained using chi-square test.
Figure S4. RNA-mediated transcriptional silencing involves the SWI/SNF complex, which works downstream of IncRNA production, Related to Figure 4

(A-D) Silencing targets are derepressed in mutants defective in SWI/SNF subunits. RNA accumulation from At3TE47400 (A), At4TE27915 (B) or At5G27845 (C) was assayed using real time RT-PCR in swi3a/+ , swi3b/+ , swi3c, swi3d, syd and brm mutants compared to Col-0 wild type. ROC3 was tested as a control (D). Graphs show averages normalized to ACTIN2 and wild type and standard deviations from three biological repeats.
(E-I) IDN2 and SWI3B function downstream of lncRNA production. Pol V-produced lncRNAs IGN20 (F), IGN25 (G), IGN26 (H) and IGN28 (I) were assayed using real-time RT-PCR in idn2-1 and swi3b/+ mutants compared to Col-0 wild type. nrpe1 mutant was used as a negative control. To check for potential DNA contaminations no RT control was performed on ACTIN2 (E) and additionally no RNA controls were performed for all primer pairs tested. Graphs show averages normalized to wild type and standard deviations from three biological repeats.

(J) SWI3B binding to chromatin is reduced in the nrpe1 mutant. ChIP with anti-SWI3B antibody was performed in Col-0 wild type and nrpe1 mutant. Bars show averages and standard deviations from three biological repeats, normalized to inputs and Col-0. Western blot showing antibody specificity is in (K).

(K) Western blot showing specificity of anti-SWI3B antibody. Total proteins from tobacco leaves expressing epitope-tagged SWI3B and from Col-0, nrpe1 and swi3b/+ Arabidopsis plants were assayed using affinity-purified anti-SWI3B antibody. Asterisks indicate non-specific bands detectable only in Arabidopsis extracts.
Figure S5. Pol V mediates nucleosome positioning, Related to Figure 5

(A) Micrococcal Nuclease (MNase) digestion of nuclei from Col-0 wild type and nrpe1 mutant. MNase activity is shown in Kunitz Units. Mononucleosomal DNA was later sequenced using Illumina sequencing.

(B) Genome browser screenshots showing regions of Pol V-stabilized nucleosomes selected for validation (Figure 5A). Shown data include from top: annotation, MNase-seq in Col-0 wild type and nrpe1 mutant, Pol V ChIP-seq in Col-0 wild type and nrpe1 mutant (Wierzbicki et al., 2012), CHH methylation in Col-0 wild type and nrpe1 mutant (Zhong et al., 2012).
(C-E) Nucleosomes stabilized by Pol V are enriched in Pol V-dependent DNA methylation. Profiles of CHH methylation (C), CHG methylation (D) and CG methylation (E) were calculated and plotted on nucleosomes identified using MNase-seq and H3 ChIP-seq with nucleosome center in the middle of each graph. Nucleosomes unaffected in nrpe1 were tested as controls. Published DNA methylation data were used (Zhong et al., 2012).

(F) Nucleosomes stabilized by Pol V are enriched on gene promoters. Nucleosomes identified using MNase-seq were overlapped with gene promoters and transcribed regions.

(G) Nucleosomes stabilized by Pol V are distributed throughout the chromosomes but nucleosomes destabilized by Pol V are enriched at the centromere. Differential nucleosomes identified using MNase-seq were plotted on the chromosome 5 with the fold value of the change in nrpe1 mutant compared to Col-0 wild type. Pol V-stabilized nucleosomes have negative and Pol V-destabilized nucleosomes have positive enrichment values.
Table S1. Oligonucleotides used in this study, Related to the Experimental Procedures

| Target | Name                  | Sequence (5'-3')                                      | Application                  |
|--------|-----------------------|------------------------------------------------------|-----------------------------|
|        | **Primers for plasmid constructs** |                                                       |                             |
| **IDN2** | proIDN2-F             | cacctgttgtaagctgcgtgtgatac                       | amplify genomic IDN2        |
| **IDN2** | proIDN2-R             | agccatccaccgctggtttgagc                         |                             |
| **IDN2** | IDN2-cacc-F            | caccatgggaagcactgtgatttta                       |                             |
| **IDN2** | IDN2-R                | agccatccaccgctggtttgagc                         |                             |
| **IDN2** | IDN2-pAS2-EcoRI-F      | tggaggccgaattcatgggaagacgagatggatt               | generate pAS2-IDN2 construct |
| **IDN2** | IDN2-pAS2-PstI-R       | tagcttggctgcaagctccatccgctggt                   |                             |
| **SWI3B** | SWI3b-cacc-F           | caccatgggaagcactgtgatttta                       | amplify SWI3B cDNA          |
| **SWI3A** | SWI3a-cacc-F           | caccatgggaagcactgtgatttta                       | amplify SWI3A cDNA          |
| **SWI3D** | SWI3d-cacc-F           | caccatgggaagcactgtgatttta                       | amplify SWI3D cDNA          |
| **SWI3C** | SWI3C-cacc-F           | caccatgggaagcactgtgatttta                       |amozylymph SWI3C cDNA        |
| **SWI3B** | SWI3B-Pro-CACC-F      | caccatgggaagcactgtgatttta                       |                             |
| **SWI3B** | SWI3B-R               | acacttcatatctctctcagttttgc                     |                             |
|        | **Gateway**            |                                                       |                             |
| **pEG300F** |                      | gctacgctgtgcctgcaaggtttgc                      | generate pZ35S302 vector    |
| **pEG300R** |                      | gctacgctgtgcctgcaaggtttgc                      |                             |
|        | **Truncations and site-directed mutagenesis** |                                                       |                             |
| **IDN2** | IDN2-274F              | cacccatggaacatctcaacaaagaatctgggcttgc          | generate IDN2-ΔE deletion   |
| **IDN2** | IDN2-274R              | cacccatggaacatctcaacaaagaatctgggcttgc          | generate IDN2-ΔE deletion   |
| **IDN2** | IDN2-721F              | cacccatggaacatctcaacaaagaatctgggcttgc          | generate IDN2-ΔF deletion   |
| **IDN2** | IDN2-721R              | cacccatggaacatctcaacaaagaatctgggcttgc          | generate IDN2-ΔG deletion   |
| **IDN2** | IDN2-cacc-F            | caccatgggaacatctcaacaaagaatctgggcttgc          | generate IDN2-ΔH deletion   |
| **IDN2** | IDN2-2512StopR         | caccatgggaacatctcaacaaagaatctgggcttgc          | generate IDN2-ΔI deletion   |
| **IDN2** | IDN2-cacc-F            | caccatgggaacatctcaacaaagaatctgggcttgc          | generate IDN2-ΔJ deletion   |
| **IDN2** | IDN2-cacc-F            | caccatgggaacatctcaacaaagaatctgggcttgc          | generate IDN2-ΔK deletion   |
| **IDN2** | IDN2-2512StopR         | caccatgggaacatctcaacaaagaatctgggcttgc          | generate IDN2-ΔL deletion   |
| **IDN2** | IDN2-1153-1320(385-440)F | gctacgctgtgcctgcaaggtttgc                      | generate IDN2-ΔM deletion   |
| **IDN2** | IDN2-1153-1320(385-440)R | gctacgctgtgcctgcaaggtttgc                      | generate IDN2-ΔN deletion   |
| **IDN2** | IDN2-1405-1512(469-504)F | gctacgctgtgcctgcaaggtttgc                      | generate IDN2-ΔO deletion   |
| **IDN2** | IDN2-1405-1512(469-504)R | gctacgctgtgcctgcaaggtttgc                      | generate IDN2-ΔP deletion   |
| **IDN2** | IDN2-1325R/V329G/H332R-F | gctacgctgtgcctgcaaggtttgc                      | generate IDN2-ΔQ deletion   |
| **IDN2** | IDN2-1325R/V329G/H332R-R | gctacgctgtgcctgcaaggtttgc                      | generate IDN2-ΔR deletion   |
| **IDN2** | MM5on3-F               | gatgatctgtaagctgcgtgtgatac                       | generate IDN2 triple mutant  |
| **IDN2** | MM5on3-R               | gatgatctgtaagctgcgtgtgatac                       | generate IDN2 octuple mutant (M8) |
| Gene     | Primer 1                      | Primer 2                      | Method            | RNA detection |
|----------|--------------------------------|-------------------------------|-------------------|---------------|
| SWI3B    | Swi3b-qRT-F2                 | cgggaagtagtgcgttagtttaaaca   | real time RT-PCR  |               |
|          | Swi3b-qRT-R2                 | ctcctcaggcacttttgctgaaagaga | real time RT-PCR  |               |
| ACTIN2   | Actin2-A118                   | gaggagattcagatcggcagacttc    | real time RT-PCR  |               |
|          | Actin2-A119                   | tggatttactctcagctttca       | RNA IP-qPCR       |               |
| soloLTR  | soloLTR-F4                    | tcaatggaaaccagttgacacatt    | real time RT-PCR  |               |
| (At1G27845) | soloLTR-R4                 | caaaaattaggatcttgtttgccagct  | real time RT-PCR  |               |
| At1TE51360 | LTRCO1-F3                  | gccccaaatggctcatatagtcctg  | real time RT-PCR  |               |
|          | LTRCO1-R3                    | aaggggttctgctggagaaaaaga    | real time RT-PCR  |               |
| At3TE51910 | LTRCO3-F2                   | attaatctccccacgctgattaga   | real time RT-PCR  |               |
|          | LTRCO3-R2                    | tctgagcgttagagattgttgag    | real time RT-PCR  |               |
| At2TE78930 | 78930F1                     | tgttaatagtagcggaaagagla    | real time RT-PCR  |               |
|          | 78930FR1                     | ttaattagtgtagcggaaagagga   | real time RT-PCR  |               |
| At1TE58825 | 58825F1                     | acttacgcatccatctgtgccgtg   | real time RT-PCR  |               |
|          | 58825R1                      | atcctctctctctcatgatgatc    | real time RT-PCR  |               |
| At4TE27915 | 27915F1                     | attcaactgctcggaaaaatctct   | real time RT-PCR  |               |
|          | 27915R1                      | agatcggtggctcggtttttccc    | real time RT-PCR  |               |
| At3TE47400 | IG12F1                      | cgaagaccctcaaaaaatactgct    | real time RT-PCR  |               |
|          | IG12R1                       | ggggagggagagagacgacgagacctc | real time RT-PCR  |               |
| TUBULIN8 | JR147                         | gcttactaatcagagatcggaga    | real time RT-PCR  |               |
|          | JR148                         | cttggtatctcctcgtcggaa      | real time RT-PCR  |               |
| UBQ10    | GB473_UBQ10s_fw              | ccaatccctctgtaaggggaa      | real time RT-PCR  |               |
|          | GB474_UBQ10s_rv              | gattgggggcgtgaagcggtg     | real time RT-PCR  |               |
| ROC3     | GB469_ROC3s_fw               | aagggttgtagctgcatctggaa    | real time RT-PCR  |               |
|          | GB470_ROC3s_rv               | gtcgaccaacatcagatgga    | real time RT-PCR  |               |
| 25S rRNA | JR41                          | tgttacaccacccaataagggaa    | RNA IP-qPCR       |               |
|          | JR42                          | tcagtaagagaacaactcctgcct   | real time RT-PCR  |               |
| IGN5     | GB268_IGN5-A                 | acaataaagaagcccaaaaacc    | real time RT-PCR  |               |
|          | GB269_IGN5-A                 | gccaattacagcgaacgctt      | real time RT-PCR  |               |
| IGN20    | GB280_IGN20                   | aagaacgccaccaatacgg    | real time RT-PCR  |               |
|          | GB281_IGN20                   | ccaccgcctcctatgtaaatg     | real time RT-PCR  |               |
| IGN22    | GB282_IGN22                   | tgttcagatgctggaattt    | real time RT-PCR  |               |
|          | GB283_IGN22                   | gcctggtttttatgtatcaggag    | real time RT-PCR  |               |
| IGN25    | GB288_IGN25                   | aacccccacccccatgcctca    | real time RT-PCR  |               |
|          | GB289_IGN25                   | gcctggagaacgaccatcaat    | real time RT-PCR  |               |
| IGN26    | GB290_IGN26                   | cgtgtttccttcacctatgtc     | real time RT-PCR  |               |
|          | GB291_IGN26                   | ggcagaaaccccttaacttc   | real time RT-PCR  |               |
| IGN27    | JA13                          | gattttaaagcacttctccccctca | real time RT-PCR  |               |
|          | JA14                          | ggtctgagccqcccaacaaaat   | real time RT-PCR  |               |
| IGN28    | JA17                          | atgtggtattgatgtttacccctc | real time RT-PCR  |               |
|          | JA18                          | gaagagcagcaacatatgttgagc  | real time RT-PCR  |               |
| IGN29    | JA227                         | cgttgttttagtaggggcagaga    | real time RT-PCR  |               |
|          | JA228                         | taaaacctttgtcccaccaacca    | RNA IP-qPCR       |               |
| IGN30    | GB402_PV-3                    | gtgtgtatgtgatcatttatgtaggag | real time RT-PCR  |               |
|          | GB403_PV-3                    | atatagttaaatagggctcacaacttc | real time RT-PCR  |               |
| IGN31    | GB416                         | ccaatcggacaccagaaac    | real time RT-PCR  |               |
|          | GB417                         | caggggtgtgactgtgacga    | real time RT-PCR  |               |
| IGN32    | GB424                         | cccaaaccacagccatgaat    | real time RT-PCR  |               |
|          | GB425                         | tgaattttgccctcacaacca    | RNA IP-qPCR       |               |
| IGN33    | GB418                         | ttctctaggttccacccggatt    | real time RT-PCR  |               |
|          | GB419                         | cgggtttcatgctcctcct    | RNA IP-qPCR       |               |
### DNA methylation assays

| Assay | Forward Primer | Reverse Primer | Method | Enzyme  |
|-------|----------------|----------------|--------|---------|
| soloLTR (At5TE35950) | soloLTR-C-F(A211)(AluI) | soloLTR-C-R(A212)(AluI) | Chop qPCR, AluI |  |
| siR02 | siR02chop-qPCR-F(AluI) | siR02chop-qPCR-R(AluI) | Chop qPCR, AluI |  |
| Al2TE78930 | 78930M1-F(A211)(AluI) | 78930M1-R(A211) | Chop qPCR, AluI |  |
| JA35/JA36 | JA35 | JA36 | Chop qPCR |  |
| IGN6 | IGN6-A30(AluI) | IGN6-A31(AluI) | Chop qPCR, AluI, (Wierzbicki et al., 2008) |  |
| IGN22 | IGN22-A413(AvalII) | IGN22-A414(AvalII) | Chop qPCR, AvalII, (Rowley et al., 2011) |  |
| At3TE51910 | 51910M-F1(NlaIII) | 51910M-R1(NlaIII) | Chop qPCR, AvalII, (Wierzbicki et al., 2008) |  |
| AtSN1 | AtSN1-A32(HaeIII) | AtSN1-A33(HaeIII) | Chop qPCR, HaeIII, (Herr et al., 2005) |  |
| IGN5 | IGN5-A28(HaeIII) | IGN5-A29(HaeIII) | Chop qPCR, HaeIII, (Wierzbicki et al., 2008) |  |
| MEA-ISR | MEA-ISR-F(Sau3AI) | MEA-ISR-R(Sau3AI) | Chop qPCR, Sau3AI |  |
| ACTIN2 | Actin2g-qPCR-F | Actin2g-qPCR-R | Chop qPCR, non cutting control for AluI, AvalII, NiAlll |  |
| ACTIN2 | Actin2g-qPCR-F2 | Actin2g-qPCR-R2 | Chop qPCR, non cutting control for Sau3AI |  |
| ACTIN2 | ACTIN2-A65 | ACTIN2-A66 | Chop qPCR, non cutting control for HaeIII, (Wierzbicki et al., 2008) |  |

### Nucleosome validation

| Assay | Forward Primer | Reverse Primer | Method | Reference |
|-------|----------------|----------------|--------|-----------|
| PVS1 | JR339 | gaaaaagtagaggaagagagagca | ChIP-qPCR |  |
| PVS2 | JR339 | gaaaaagtagaggaagagagagca | ChIP-qPCR |  |
| PVS3 | JR339 | gaaaaagtagaggaagagagagca | ChIP-qPCR |  |
| PVS4 | JR339 | gaaaaagtagaggaagagagagca | ChIP-qPCR |  |
| PVS5 | JR339 | gaaaaagtagaggaagagagagca | ChIP-qPCR |  |
| PVS6 | JR339 | gaaaaagtagaggaagagagagca | ChIP-qPCR |  |
| ACTIN2 | Actin2-A118 | Actin2-A119 | Chop qPCR, (Wierzbicki et al., 2008) |  |
| HSP70 | A512 | A513 | Chop qPCR (Kumar and Wigge, 2010) |  |
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