Supplementary information

Supplementary methods

Cell culture and Western blot analysis

The early passage normal human diploid fetal lung fibroblast WI38 cells were purchased from American Type Culture Collection. WI38 and 293T cells were cultured in DMEM supplemented with 10% fetal bovine serum (FBS). Senescence-associated β-galactosidase (SA-β-gal) assays were performed by using Senescence Detection Kit (BioVision). Western blot analysis was performed as previously described (Kotake et al., 2007). Antibodies to SUZ12 (Millipore) and α-tubulin (Sigma) were purchased commercially.

Retroviral transduction and RNA interference

WI38 cells were infected with retroviruses expressing human papillomavirus oncoprotein E7 (kindly provided by Dr. D. Galloway, Fred Hutchinson Cancer Research Center, Seattle, WA) or H-Ras^{G12V} (Kindly provided by Dr. CJ. Der, University of North

Carolina at Chapel Hill, Chapel Hill, NC). Retroviruses encoding shRNAs silencing *ANRIL*, *EZH2* and control *GFP* were constructed by ligating respective oligonucleotides (*ANRIL*, GGTCATCTCATTGCTCTATCC; *EZH2*, AGACTCTGAATGCAGTTGC; *GFP*, GCTACGTCCAGGAGCGCAC) into a pSuper-retro vector. The retroviral production and transduction were performed as previous described (Kotake et al., 2007). WI38 cells were transfected with siRNA oligonucleotides using Lipofectamine RNAiMAX (Invitrogen), according to the manufacturer's protocol. The nucleotide sequences of siRNA for *ANRIL* and *SUZ12* were (*ANRIL*, GGUCAUCUCAUUGCUCUAU'; *SUZ12*, GUCGCAACGGACCAGUUAA) with 3' dTdT overhang.

Quantitative RT-PCR (Q-RT-PCR)

Total RNA was extracted by RNeasy Plus (QIAGEN), and 1 μg was used for cDNA synthesis primed with Oligo$(dT)_{20}$ primers (Invitrogen). The cDNA was added to a qRT-PCR mixture that contained 1X SYBR Green PCR master mix (Applied Biosystems or Qiagen) and 500 nM gene-specific primers. Assays were performed in triplicate on a 7900 HT sequence detection system (Applied Biosystems) or Rotor-Gene 3000 system

(Corbett Research). The expression level of each gene was normalized with glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The specific PCR pairs for *p15*^{INK4B}, *p16*^{INK4A}, *ARF* and *GAPDH* have been described (Kotake et al., 2007b). The specific PCR pairs for *EZH2* and *ANRIL* were as follows: *EZH2*, 5'-TGCACATCCTGACTTCTGTG-3' and 5'-AAGGGCATTCACCAACTCC-3'; *ANRIL*, 5'-TGCTCTATCCGCCAATCAGG-3' and 5'- GGGCCTCAGTGGCACATACC-3'.

Chromatin immunoprecipitation assay

3X10^6 WI38 cells were treated with 1% formaldehyde. The crosslinking was stopped by the addition of 0.125 M glycine. The cells were lysed with cell lysis buffer on ice (10 mM Hepes/pH7.9, 0.5% NP-40, 1.5 mM $MgCl_2$, 10 mM KCl, 0.5 mM DTT and protease inhibitor cocktail). After centrifugation, the cell pellets were lysed by sonication on ice with nuclear lysis buffer (20 mM Hepes/pH7.9, 25% glycerol, 0.5% NP-40, 0.42 M NaCl, 1.5 mM $MgCl_2$, 0.2 mM EDTA and protease inhibitor cocktail). After centrifugation, the lysates were diluted with equal volume of dilution buffer (1% Triton X-100, 2 mM EDTA, 50 mM NaCl, 20 mM Tris-HCl/pH7.9 and protease inhibitor cocktail).

Immunoprecipitation was performed with an antibody specific to SUZ12 (Abcam) and normal rabbit IgG as a control. After immunoprecipitation, 20μl salmon sperm DNA/protein G agarose (Millipore) was added and followed by 1 h incubation. The eluates were incubated at 65ºC to reverse the formaldehyde cross-linking. DNA fragments were purified by using PCR purification Kit (Qiagen). PCR was performed using Platinum Taq polymerase (Invitrogen) and the following pairs of primers: *p15*^{INK4B} (a), 5'- CTCTTGGTGGGAAGGTGTG -3' and 5'- CCCCAAGAAGGTCAAATAAGG'; *p15*^{INK4B} (b), 5'- TCTGGTAAGGGTGTGCTGTG -3' and 5'- AAAACTCCTCTGTGGCATGTG -3'; *p15*^{INK4B} (c), 5'- TCACCTAGGAAGATTAGGAAGG -3' and 5'- GCAACCTGGCACACATAAGAC -3'; *p15* (d), 5'- GGGGCTGGAACCTAGATCG -3' and 5'- CGTTGAAAGCAGACAGACAAG -3'; *p16*^{INK4A} (e), 5'- AGGGGAAGGAGAGAGCAGTC -3' and 5'- GGGTGTTTGGTGTCATAGGG-3'; *GAPDH* (f), 5'- GGTAGGGAGTTCGAGACCAG -3' and 5'- TCAACGCAGTTCAGTTAGGC -3'.

RNA immunoprecipitation assay

RNA immunoprecipitation (RIP) assays were carried out as described previously (Rinn

et al., 2007) with some modification. 5-8 x 10^6 WI38 cells were harvested by trypsinization and resuspended in 2 ml PBS, 2ml nuclear isolation buffer (1.28 M sucrose; 40 mM Tris-HCl ph 7.5; 20 mM $MgCl_2$; 4% Triton X-100) and 6 ml water on ice for 20 min. Nuclei were then pelleted by centrifugation at 2,500 g for 15 min. Nuclear pellets were resuspended in 1 ml RIP buffer [150 mM KCl, 25 mM Tris pH 7.4, 5 mM EDTA, .5 mM DTT, .5% NP40, 25 µg/ml leupeptin, 1 mM benzamidine, 10 µg/ml trypsin inhibitor, 25 µg/ml aprotinin, 100 U/ml SUPERASin (Ambion)]. Resuspended nuclei were split into two fractions of 500 µl each and were mechanically sheared using a 25 G needle with 4 strokes. Nuclear membrane and debris were pelleted by centrifugation at 16,000 g for 10 min. Antibody to rabbit IgG (NeoMarkers), YY1 (Santa Cruz Biotechnology) or SUZ12 (Abcam) were added to supernatant (3 µg each) together with 20 µl of 50% protein G beads (Thermoscientific) and incubated overnight at 4°C with gentle rotation. After pelleting the beads at 2,300 g for 1 min, the supernatant was removed and beads were resuspended in 500 µl RIP buffer and repeated for a total of 3 RIP washes and followed by 1 wash in PBS. Co-precipitated RNAs were isolated by RNeasy Plus (QIAGEN) and qRT-PCR for *U1* (5'-ATACTTACCTGGCAGGGGAG-3'and

5’-CAGGGGAAAGCGCGAACGCA-3’) or *ANRIL* (5’-TGCTCTATCCGCCAATCAGG-3’ and 5’-GGGCCTCAGTGGCACATACC-3’) were performed as described (Kotake et al., 2007)