Supplemental Information

Regulation of Cellular Senescence by Polycomb Chromatin Modifiers through Distinct DNA Damage- and Histone Methylation-Dependent Pathways

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SUPPLEMENTAL INFORMATION

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Lentivirus Vector Procedures
Supernatants collected from 293T cells were filtered through a 0.45 μM filter. Infections of HDF cells were done in the presence of 8 μg/ml polybrene (Stewart et al., 2003). pLKO.1 vectors were used for shRNA-mediated gene knockdowns (Moffat et al., 2006). The control used throughout this study was an shRNA against an irrelevant target (GFP, 5'-TACAACAGCCACAACGTCTAT). The following shRNA vectors were obtained from Open Biosystems: EZH2, TRCN0000018365 (#1 in Figure S1H), TRCN0000040075 (#2 in Figure S1H), TRCN00000040076 (#3 in Figure S1H); WNT2, TRCN0000033373 (#1 in Figure S6D), TRCN0000033372 (#2 in Figure S6D), MYC, TRCN00000010390 (#1 in Figure S6E), TRCN0000039642 (#2 in Figure S6E); and CDKN1A (p21), TRCN0000287021 (#1 in Figure 2F), TRCN0000287091 (#2 in Figure 2F). A pSUPER retrovirus vector was used for the knockdown of ATM (5'-GATACCAGATCCTTGGAGA, #1 in Figure 2H) (Ortega-Atienza et al., 2015) and lentivirus vector for knockdown of ATM was a gift from Didier Trono (Addgene plasmid #14542, shATM#2 in Figure 2H). TRCN0000040076 (EZH2 #3) was used throughout this study. TRCN0000033373 and TRCN0000033372 (WNT2) corresponds to shWnt2-5 and shWnt2-4, respectively, described and validated by (Ye et al., 2007). TRCN00000010390 (MYC) was described and validated by (Guney et al., 2006). For double knockdowns of p21 and EZH2, and ATM and EZH2, early passage LF1 cells were infected with p21 or ATM shRNA vectors, drug selected, grown up as pools, and superinfected with EZH2 shRNA vectors. Full-length EZH2 cDNA was obtained from the ATCC; MYC cDNA was a kind gift of Dr. Michael Cole (Dartmouth Medical School); EED was obtained from Applied Biological Materials. EZH2 and MYC were PCR cloned into pLX303 (plasmid 25897, Addgene) modified by removal of the Gateway Destination sequences and incorporation of multiple cloning sites and sequence verified. EZH2 and EED were also PCR cloned into pLenti-puro, gift from le-Ming Shih (Addgene plasmid #39481). Empty pLX and pLenti-puro vectors were used as controls. For the expression of HRAS(G12V) we used the lentivirus vector pLenti CMV RasV12 Neo (plasmid 22259, Addgene, in Figure 1B) or retrovirus vector pQCXIN CMV ER:RasV12 (kind gift of Dr. Gregory David, New York University School of Medicine, in Figure 4A-4E). ER:RasV12 was activated by continuous treatment with 200 ng/ml of 4-hydroxytamoxifen. The empty vector (plasmid 17485, Addgene) was used as the control. In combined cDNA expression and knockdown experiments, cells were first infected with EZH2 or MYC cDNAs, stable pools were selected as indicated above, and subsequently infected with shRNA vectors.

Immunoblotting
Cells were harvested in Laemmli sample buffer (60 mM Tris pH 6.8, 2% SDS, 10% glycerol, 100 mM DTT). Whole cell extracts were separated by SDS-PAGE and transferred onto immobilon-FL membranes (Millipore). Signals were detected using the LI-COR Odyssey infrared imaging system (LI-COR Biosciences). Immunoblots shown are representative images of three independent experiments.

Antibodies Used
The antibodies used were as follows: Millipore, active β-catenin (05-665, 1:1000), H3K27me3 (07-449, 1:1000), EED (09-774, 1:2000), MYC (06-340, 1:2000), phosphoserine (05-1000,
Chromatin Immunoprecipitation

After crosslinking, formaldehyde was quenched by adding glycine to 125 mM, and the dishes were washed three times in ice-cold phosphate-buffered saline (PBS) containing 1x protease inhibitor (Roche). Composition of the SDS lysis buffer was 1% SDS, 10 mM EDTA, 50 mM Tris pH 8.1, 1x protease inhibitor cocktail. Composition of the ChIP dilution buffer was 0.01% SDS, 1.1% Triton X-100, 1.2 mM EDTA, 16.7 mM Tris-HCl pH 8.1, 167 mM NaCl. After sonication the samples were centrifuged and the supernatants were diluted five-fold with ChIP dilution buffer. An aliquot (1%) of each chromatin preparation was saved as input. Samples were immunoprecipitated with 4 µg of antibody to MYC (Millipore 06-340) or H3K27me3 (Millipore 07-449). After pulldown the magnetic protein A beads were washed once with each of low salt, high salt and LiCl wash buffers, followed by two washes with TE buffer. Final yields of DNA were quantified with the Qubit 2.0 dsDNA HS assay kit (Invitrogen, Q32851). For the Chromatrap Premium ChIP qPCR protocol, the IP slurry containing 1 µg of the sheared chromatin and 2 µg of antibody to H3K27me3 (as above) or H3K27ac (Cell Signaling, 8173S) were diluted in wash buffer 1, transferred into the Chromatrap spin column, and incubated for 6 hrs. at 4° on a rocking platform. The Chromatrap spin columns were washed with wash buffers and the DNA was eluted with 50 µl of elution buffer.

Immunoprecipitations

Cells were lysed in cell lysis buffer (20 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton, 2.5 mM sodium pyrophosphate, 1 mM beta-glycerophosphate, 1 mM NaVO₃, 1 µg/ml leupeptin, 1 mM PMSF) for 30 min. on ice and then passed through a 26.5-gauge needle three times. Cell lysates were incubated with anti-EZH2 antibody (Cell Signaling, 5246, 1:300) or control IgG overnight at 4°C. Pre-washed magnetic beads (Thermo Fisher Scientific, 11203D) were added to each sample and incubated for 6 hrs. at 4 °C. The beads were washed five times with cell lysis buffer, boiled at 100°C for 5 min. in Laemmli sample buffer and processed for immunoblotting.

RNA-seq

Cells were infected with a lentivirus vector expressing shRNA #3 against EZH2 (Figure 1E-H) and cells were harvested at 4 and 8 days after infection. Total RNA was harvested from cells using Trizol reagent (Invitrogen) and further purified using the Purelink RNA Mini kit (Invitrogen) with DNase I digestion. RNA library preparation with polyA selection and Illumina HiSeq 2x150bp sequencing was performed by GeneWiz Inc. Paired-end reads were quality trimmed using Trim galore v0.4.0 and subsequently aligned to the human reference genome, hg19, using HISAT2 v2.1.0. Reads mapping to annotated genes were quantified using featureCounts (Liao et al., 2014). Differential gene expression was determined using DESeq2 v1.12.4 (Love et al., 2014) and significance was defined as FDR-corrected p-values of <0.05. The log2 fold change for each gene was used to rank the list of genes for GSEAPreranked analysis (Subramanian et al., 2005). FPKM values were calculated using DESeq2 and Z-scores were generated from FPKMs. Upstream regulators were identified using Ingenuity Pathway Analysis (IPA, Qiagen) with default settings. The cutoffs used were p <0.05 and absolute
fold-change >1.75. The differential expression and alignment rates are shown in Table S4, and the DESeq2 output for the entire dataset in Table S5.

**Immunofluorescence and ImmunoFISH**
Nonspecific binding solution contained 4% bovine serum albumin (BSA; Thermo Fisher Scientific), 2% donkey serum, and 0.1% Triton X-100 in PBS. The antibodies used were as follows: Santa Cruz, p16 (sc-756, 1:100), p21 (sc-397; 1:100); Millipore, γH2AX (05-636, 1:100); Becton Dickinson, 53BP1 (NB100-304, 1:100); Cell Signaling, phospho-ATM (Ser1981, 4526, 1:100), EZH2 (5246, 1:100). The rabbit polyclonal antibody against human mH2A was a kind gift from Dr. Peter Adams (University of Glasgow) and was used as indicated before (Kreiling et al., 2011). For the TIF assay (Herbig et al., 2004), cells were first fixed, permeabilized and immuno-stained for 53BP1 as described above. The samples were then dehydrated in a 70%, 90%, and 100% ethanol (3 min. each) and air dried. Nuclear DNA was denatured for 5 min. at 80°C in hybridization buffer containing 0.5 µg/ml Cy5-conjugated peptide nucleic acid (PNA) telomere C probe (PNA bio, #F1003), 70% formamide, 12 mM Tris HCl (pH 8), 5 mM KCl, 1 mM MgCl₂, 0.001% Triton X-100, and 2.5 mg/ml acetylated BSA. After denaturation, incubation was continued for 14 hrs. at room temperature in a humidified chamber. Cells were washed two times for 15 min. with 70% formamide in 2x SSC (0.3 M NaCl, 30 mM Na-citrate), followed by a 10 min. wash with 2x SSC, and a 10 min. wash with PBS. For EdU incorporation, cells were treated for 24 hours with EdU and processed as instructed by the manufacturer (Life Technologies, #C10337).

**Cytokine Array**
Cultures were washed three times with PBS and incubated in serum-free medium for 24 hrs. The conditioned media (CM) were collected and centrifuged at 1000 x g for 10 min. at 4°C. The CM were diluted in proportion to the cell number and analyzed using the Quantibody Human Cytokine Array 1 (QAH-CYT-1, RayBiotech) as per manufacturer’s instructions. The signals were detected with a GenePix 4200B microarray scanner.

**Detection of SA-β-Gal Activity**
Cells grown on coverslips were fixed with 0.2% glutaraldehyde and 2% formaldehyde for 5 min., washed twice with PBS, and incubated overnight at 37°C in a staining solution (Debacq-Chainiaux et al., 2009) containing 5-bromo-4-chloro-3-indolyl-β-galactopyranoside.

**Microscopy**
Imaging was done on either a Zeiss Axiovert 200M fluorescence microscope equipped with a Roper CoolSnap HQ monochrome camera controlled by MetaMorph software 6.1 (Molecular Devices), or a Zeiss LSM 710 Confocal Laser Scanning Microscope. Image analysis was performed using ImageJ open source software from the NIH (http://rsbweb.nih.gov/ij/).

**Site-directed mutagenesis**
EZH2 mutant (S652A and S734A), EZH2 deletion (EZH2 Δ SET) and EED mutant (F97A, W364A, and Y365A) were generated by using the QuikChange Lightning Site-Directed Mutagenesis kit (Agilent Technologies). All constructs were confirmed by sequencing.
Figure S1, Related to Figure 1

Expression and Knockdown of EZH2 and Phenotypes of Senescent Cells

(A) Cells were serially propagated under standard culture conditions. Onset of senescence was designated as the zero time point, at which >95% of the cells in the culture were SA-β-Gal positive. The points in the graph correspond to the lanes shown in Figure 1D.

(B) Levels of EZH2 protein and the H3K27me3 mark were examined by immunoblotting in HDF strains WI-38 and IMR90 at early passage (E) and 5 weeks after the onset of senescence (S).

(C) LF1 early passage, replicatively senescent, and serum-starved quiescent cells were assayed for SA-β-Gal activity and EdU incorporation (**p<0.01, n=3).

(D) EZH2 protein levels were examined by immunoblotting in samples from panel (C).

(E) Cells were treated with etoposide (Eto, 40 µM) at the zero time point and levels of EZH2 protein and H3K27me3 marks were examined by immunoblotting. EZH2 and H3K27me3 levels were normalized to GAPDH (green and red plots, respectively).

(F) SA-β-Gal positive cells were scored in the etoposide treatment experiment shown in panel (E) (*p<0.05, **p<0.01, n=3).

(G) Schematic overview and timeline of lentivirus shRNA infection experiments. Cells were infected with lentiviral particles for a total of 2 days. After a wash and 12 hrs further incubation the cultures were replated, and this time point was designated as t=0 for all the time courses presented in this study. Cells were treated with puromycin for 2 days, at which time all sensitive cells had been effectively removed. Cells were harvested at successive time points as indicated in individual experiments.

(H) LF1 cells were infected with lentivirus vectors expressing three different shRNAs against the EZH2 transcript. The effectiveness of knockdowns was examined by RT-qPCR for the EZH2 mRNA 2 days after infection (**p<0.01, n=3). shRNA against GFP was used as the control. The shRNAs used are listed in the Extended Experimental Procedures.

(I) EZH2 was knocked down as described in Figure 1E. Representative images of cells displaying SAHF and SA-β-Gal activity are shown. SAHF formation was visualized by DAPI staining. Images were acquired 2 days after infection with EZH2 shRNA.

(J) EZH2 was knocked down as in panel I and mH2A expression was examined using IF microscopy.

(K) mH2A nuclear signals were quantified in the IF images using ImageJ software and plotted as histograms of cell number (% of total) against fluorescent intensity in arbitrary units (a.u.). At least 260 nuclei were observed for each condition. Error bars represent SD.
RNA-seq Analysis of Global Gene Expression Changes Elicited by Knockdown of EZH2

(A-B) EZH2 was knocked down as described in Figure 1E and RNA-seq was performed on RNA harvested on day 4 and 8 after infection. The data were analyzed using GSEA and the top 5 upregulated and downregulated GO process pathways, ranked by normalized enrichment score (NES), are shown for 4 days (A) and 8 days (B) after infection.

(C) Upstream Regulator analysis was performed using IPA and the 8 most activated or inhibited regulators are shown at 8 days after EZH2 knockdown.

See also Tables S4, S5.
Figure S3, Related to Figure 2

Cellular Phenotypes Elicited by Knockdown of EZH2
(A) EZH2 was knocked down as in Figure 1E and expression of p21 mRNA was measured by RT-qPCR at 4 and 8 days after infection (**p<0.01, n=3).
(B) Expression of p16 was determined in the experiment shown in (A).
(C) Cells grown continuously in the presence of DZNep (5 µM) for 9 days were assayed for EdU incorporation (**p<0.01, n=3).
(D) Expression of the indicated genes was determined by RT-qPCR in cells treated with DZNep for 9 and 18 days. The data are represented as heat maps relative to vehicle (DMSO) control (n=3).
(E) A double knockdown of EZH2 and p21 was performed as described in Figure 2F, and the levels of p21 protein were determined by immunoblotting. The control for EZH2 knockdown was shGFP, and for p21, the empty vector.
(F-G) A double knockdown of EZH2 and p21 was performed as above and proliferation was assessed by cell number (F) or EdU staining (G) (*p<0.05, **p<0.01, n=3).
(H-I) A double knockdown of EZH2 and ATM was performed and proliferation was assessed by cell numbers (H) or EdU staining (I) (*p<0.05, **p<0.01, n=3).
(J) Replicatively senescent cells or cells made senescent by knockdown of EZH2 were processed for the simultaneous visualization of 53BP1 DNA damage foci (by IF) and telomeres (by FISH). Colocalization of 53BP1 and telomere signals are scored as Telomere dysfunction Induced Foci (TIFs). DNA was counterstained with DAPI (blue). Arrows point to representative enlarged images of 53BP1 DNA damage foci.
(K) The experiment in (J) above was quantified by counting DNA damage foci and TIFs in >150 nuclei per condition. A cell with at least 1 clearly visible 53BP1 focus was scored as a 53BP1-positive cell, and a cell in which ≥50% of 53BP1 foci colocalized with a telomere was scored as a TIF-positive cell. (**p<0.01, n=3). The increase in TIFs in cells infected with shEZH2 was not significant compared to the control (cells infected with shGFP).
(L) EZH2 was knocked down as in (A) in LF1 cells immortalized with human telomerase (hTERT) and SA-β-Gal-positive cells were scored 4 days after infection (**p<0.01, n=3).
(M-N) EZH2 was knocked down as in Figure 2I, and p21, p16 and EZH2 protein levels were determined by immunoblotting (M) or assayed for SA-β-Gal activity (N) 4 days after infection (**p<0.01, n=3).
Error bars represent SD.
**Figure S4, Related to Figure 2**

**Inhibition of H3K27me3 Mark Recognition by EED Phenocopies EZH2 Knockdown**

(A) Cells were continuously treated with the drug EED226 (10 μM) or vehicle (DMSO) for 7 days and assayed for SA-β-Gal activity (**p<0.01, n=3).**

(B-C) Cells were treated with EED226 (10 μM) or DMSO and expression of p21 (B) and p16 (C) mRNA was measured by RT-qPCR at 7 and 14 days (**p<0.01, n=3).**

(D) Cells were treated with varying concentrations of EED226 and p16, p21 and H3K27me3 levels were determined by immunoblotting.

(E) Cells were treated as in (A) and 53BP1 foci were visualized by IF after 7 days of drug treatment (**p<0.01, n=3).**

(F) Expression of the indicated genes was determined by RT-qPCR in cells treated with EED226 (10 μM) or DMSO control at 7 and 14 days. The data are represented as heat maps relative to the DMSO control (n=3).

(G) Cells were infected with lentivirus vectors to ectopically express WT EED cDNA or EED(F97A/W364A/Y365A) mutated cDNA and p16, p21 and H3K27me3 levels were scored for 53BP1 foci as in (E) (**p<0.01, n=3).**

(H) Cells ectopically expressing WT EED or EED(F97A/W364A/Y365A) cDNAs (experiment in (G) above) were assessed for the expression of the indicated genes as in (F). Error bars represent SD.
Figure S5, Related to Figure 3

Inhibition of EZH2 Activity Induces Senescence by Depleting H3K27me3 Marks and Activating p16 and SASP Genes

(A) Cells were continuously treated with the drug GSK126 (5 µM) or vehicle (DMSO) for 20 days and then assayed for EdU incorporation (** p<0.01, n=3).
(B) Cells were infected with lentivirus vectors expressing EZH2 or EZH2ΔSET cDNAs, or empty vector as control (as in Figure 3G), and p16, p21 and H3K27me3 levels were determined by immunoblotting 9 days after infection.
(C-D) In the experiment shown in (B) above, expression of p16 and p21 mRNAs was determined by RT-qPCR (C) and proliferation was assessed by EdU incorporation (D) (** p<0.01, n=3).
(E) In the experiment shown in (B) above, 53BP1 foci were visualized by IF (n=3).
(F) Cells were made quiescent by incubation in medium supplemented with 0.25% FBS and then treated with GSK126 (5 µM) for the indicated times. Levels of the H3K27me3 mark were determined by immunoblotting.
(G) Cells were treated with GSK as in (F) above for 21 days and immunostained with antibodies to p16. Nuclei were counterstained with DAPI. The frequency of p16-expressing cells is shown in the right panel (% of total cells, random fields, >200 cells per condition, **p<0.01).
(H) Cells were treated with GSK126 (5 µM) or DMSO for 10 days and H3K27me3 and H3K27ac enrichment at the enhancer between the MMP3 and MMP12 loci was determined by ChIP-qPCR. Normal rabbit IgG was used as the IP control (** p<0.01, n=3). Location of the primer pair is indicated in the schematic on right. The IMR90 H3K27ac and H3K4me1 tracks were obtained from ENCODE (GEO ID: GSM469966 and GSM521895).
(I) Cells were treated with GSK126 (5 µM), JQ1 (100 nM) or both, and levels of the p16 protein were determined by immunoblotting after 10 days.
(J) Cells were treated as in (I) and the frequency of SA-β-Gal positive cells was determined (* p<0.05, ** p<0.01, n=3).
(K) Cells were treated with GSK126 for 10 days, followed by 4 days of combined treatment with GSK126 and rapamycin (12.5 nM). Expression of the indicated SASP genes was determined by RT-qPCR (n=3). The data are expressed as heat maps relative to control (vehicle-treated) cells.
(L) Cells were treated as in (K) and IκBα protein levels were determined by immunoblotting. Error bars represent SD.
Figure S6, Related to Figure 5 and Figure 6

Regulation of EZH2 Expression by ATM and WNT-MYC Signaling

(A) Cells were treated with etoposide (40 µM, left panel), etoposide plus the ATM inhibitor KU-55933 (10 µM, middle panel), or with etoposide and infected with shRNA against ATM (right panel), and immunostained with antibodies to phospho-ATM (S1981). Nuclei were counterstained with DAPI.

(B) Cells were treated with etoposide (40 µM) with or without the proteasome inhibitor MG132 (10 µM) and levels of EZH2 protein were determined by immunoblotting. GAPDH was used as the loading control (* p<0.05, calculated relative to t=0 time point, n=2).

(C) Levels of active β-catenin (ABC) and MYC protein were examined by immunoblotting in early passage cells and 2 weeks after the onset of replicative senescence.

(D-E) Cells were infected with lentivirus vectors expressing two different shRNAs against the WNT2 or MYC transcripts. The effectiveness of knockdowns was examined by RT-qPCR for the WNT2 (D) or MYC (E) mRNAs 2 days after infection (**p<0.01, n=3). shRNA against GFP was used as the control. The shRNAs used are listed in the Extended Experimental Procedures.

(F) WNT2 or MYC were knocked down as in (D) and (E) above and proliferation was assessed by counting cell numbers (**p<0.01, n=3).

(G) Ectopic expression of EZH2 cDNA was combined with a shRNA knockdown of WNT2 or MYC and proliferation was assessed by counting cell numbers (**p<0.01, n=3).

(H) Ectopic expression of MYC cDNA was combined with a shRNA knockdown of WNT2. Controls were empty pLX lentivirus vector for MYC (–) and shGFP for WNT2. Levels of p16, p21, and MYC proteins were determined by immunoblotting. Note that while endogenous MYC expression is effectively downregulated by shWNT2 (lane 3), ectopic expression is maintained by the pLX-MYC vector (lane 4).

(I) p21 mRNA expression was determined by RT-qPCR in the experiment shown in (H) (*p<0.05, n=3).

(J) The presence of SA-β-Gal positive cells was scored in the experiment shown in (H) (**p<0.01, n=3).

Error bars represent SD.
Table S1, Related to Experimental Procedures

List of primer sequences for RT-qPCR

| Gene          | Orientation | Sequences (5’ to 3’)          |
|---------------|-------------|-------------------------------|
| **EZH2**      | Sense       | GCAACACCCCAACACTTTAAGC        |
|               | Antisense   | CTCCCCTCCAAATGCTGGTA          |
| **WNT2**      | Sense       | TGGCAGGAGGCTGTAAGAGC          |
|               | Antisense   | ATGGCCAGCCAGCATGTC            |
| **MYC**       | Sense       | GGATTCTCTGCTCTCCTCGAC         |
|               | Antisense   | TTTTCTCTCCTCAGAGTCGC          |
| **IL1A**      | Sense       | GAGAGCATGGTGTTGTTGCAAA        |
|               | Antisense   | AGGCTTGATGTATTTCTCCTCTGA      |
| **IL1B**      | Sense       | GGCAGGCGAGCAGCCTGTCATCTGT     |
|               | Antisense   | CCCACCTACATACAGGATTGTA        |
| **MMP3**      | Sense       | CCCACCTACATACAGGATTGTA        |
|               | Antisense   | CCCAGACTTTTCAGAGCTTTCTCA      |
| **IL6**       | Sense       | CACTCGCGACAAAACAACCTGAA       |
|               | Antisense   | ACCAGGCCAAGTCTCCTCATTGA       |
| **CXCL8 (IL8)** | Sense     | GACTGGCAGAAAAACACCTGAA       |
|               | Antisense   | ACCAGGCCAAGTCTCCTCATTGA       |
| **CCL2**      | Sense       | AAGACCATTGTGCGGCAAGGA         |
|               | Antisense   | TTCGAGTTTTGGTTGTCT            |
| **CXCLI**     | Sense       | CAATCCTGACATCCCCCATAG        |
|               | Antisense   | CAGCCACAGTACAGCCTCCTT         |
| **CDKN2A (p16-INK4A)** | Sense  | CGGACAGTCCCTACAGCCTCAGACATC |
|               | Antisense   | CCCTGTAGGACCTTCGGTGTA        |
| **CDKN1A (p21-CIP1)** | Sense  | GAGACTCTCAGGGTGAACAC        |
|               | Antisense   | TTCTGTGGGCGGATTAGG           |
| **GAPDH**     | Sense       | GGAGTCAACGGATTTGGTCTG         |
|               | Antisense   | GGTGAGGCATGGAAGGGTGCA         |
### Table S2, Related to Experimental Procedures

**List of primer sequences for ChIP-qPCR**

| Gene/location | Orientation | Sequences (5’ to 3’) | Notes |
|---------------|-------------|-----------------------|-------|
| *ARF* Exon 1β | Sense       | GTGGGTCCAGGCTCTGCAGTA | Fig. 2A, #1 |
|               | Antisense   | CCTTTCGGCACCAGAGTGAG  |       |
| 15 kb downstream of *ARF* promoter | Sense | GCACTTGCCCCCTCCAGGTATA | Fig. 2A, #2 |
|               | Antisense   | TGAATGTTCAAAGGCCCTATGCC |       |
| 200 bp upstream *p16-INK4A* TSS | Sense | ACCCCGATTCAATTTGGCAG | Fig. 2A, #3 |
|               | Antisense   | AAAAGAAATACCGCACCAG |       |
| *p16-INK4A*, exon 1α | Sense | AGAGGCTCTGCAGGG | Fig. 2A, #4 |
|               | Antisense   | TCGAAGCGCTACCTGATTC |       |
| 200 bp downstream of *p16-INK4A* exon 1α | Sense | GCCAAGTCAAACGCCATA | Fig. 3J, #2 |
|               | Antisense   | AGTTTTGACGACGCACTTGA |       |
| 678 bp upstream of *IL1A* | Sense | CCTTCAGATCTTCTAGCATGTC | Fig. 3J, #3 |
|               | Antisense   | TGGCATCCAAAACGAGGTCA |       |
| 11490 bp upstream of *IL1A* | Sense | AGAGGCTCTGCAGGG | Fig. 3J, #4 |
|               | Antisense   | TCGAAGCGCTACCTGATTCC |       |
| 20187 bp upstream of *IL1A* | Sense | GCCAAGTCAAACGCCATA | Fig. 3J, #5 |
|               | Antisense   | TGGCATCCAAAACGAGGTCA |       |
| 28917 bp upstream of *IL1A* | Sense | CCTTCAGATCTTCTAGCATGTC | Fig. 3J, #6 |
|               | Antisense   | TGGCATCCAAAACGAGGTCA |       |
| 5567 bp downstream of *IL1B* | Sense | CACATGCTGCAATTCTGCGTG | Fig. 3J, #7 |
|               | Antisense   | ATGAGTCAGCTTACCCCTTAC |       |
| 3756 bp upstream of *IL1B* | Sense | TGGCATCCAAAACGAGGTCA | Fig. 3J, #8 |
|               | Antisense   | TGGCATCCAAAACGAGGTCA |       |
| 32953 bp upstream of *IL1B* | Sense | AGTGAAGAAGAGTTGATGTTG | Fig. 3J, #9 |
|               | Antisense   | ACCAGTCAGCTGTTGCTTCTG |       |
| 45347 bp upstream of *IL1B* | Sense | TGGCATCCAAAACGAGGTCA | Fig. 3J, #10 |
|               | Antisense   | TGGCATCCAAAACGAGGTCA |       |
| 6898 bp upstream of *MMP3* | Sense | TATCTGGGCTGATGATGGGA | Fig. S5H, #1 |
|               | Antisense   | TATCTGGGCTGATGATGGGA |       |
| *EZH2*, 1034 bp upstream of TSS | Sense | ACATTGCTGCCATTTTCAGAC | Fig. 6C, #1 |
|               | Antisense   | AGTGAAGAAGAGTTGATGTTG |       |
| *EZH2*, 156 bp upstream of TSS | Sense | TGGCATCCAAAACGAGGTCA | Fig. 6C, #2 |
|               | Antisense   | TGGCATCCAAAACGAGGTCA |       |
| *EZH2* TSS | Sense | GCCATCGCCATCGGTTTAT | Fig. 6C, #3 |
|               | Antisense   | GGGCCCTGATGTCAG |       |
Table S3, Related to Figure 3

Assay of Conditioned Media for Cytokine Production

| CONDITION | GENE SYMBOL | CONCENTRATION (pg/ml) |
|-----------|-------------|-----------------------|
| CTR #1    | IL1A        | 117.875               |
| CTR #1    | IL1B        | 140.9496              |
| CTR #1    | IL2         | 15.0576               |
| CTR #1    | IL4         | 39.3584               |
| CTR #1    | IL5         | 24.906                |
| CTR #1    | IL6         | 22.4068               |
| CTR #1    | IL8         | 3.4572                |
| CTR #1    | IL10        | 96.278                |
| CTR #1    | IL12        | 8.7362                |
| CTR #1    | IL13        | 6.1605                |
| CTR #1    | CSF2        | 27.6012               |
| CTR #1    | CXCL1       | 77.3528               |
| CTR #1    | IFNG        | 153.7699              |
| CTR #1    | CCL2        | 90.0836               |
| CTR #1    | CCL3        | 314.4603              |
| CTR #1    | CCL4        | 12.4705               |
| CTR #1    | MMP9        | 131.8152              |
| CTR #1    | CCL5        | 27.0336               |
| CTR #1    | TNF         | 16.115                |
| CTR #1    | VEGF        | 918.7487              |
| CTR #2    | IL1A        | 115                   |
| CTR #2    | IL1B        | 136.6784              |
| CTR #2    | IL2         | 14.1165               |
| CTR #2    | IL4         | 39.3584               |
| CTR #2    | IL5         | 24.906                |
| CTR #2    | IL6         | 20.6832               |
| CTR #2    | IL8         | 3.0444                |
| CTR #2    | IL10        | 89.999                |
| CTR #2    | IL12        | 8.0465                |
| CTR #2    | IL13        | 5.8867                |
| CTR #2    | CSF2        | 27.6012               |
| CTR #2    | CXCL1       | 66.3024               |
| CTR #2    | IFNG        | 147.2265              |
| CTR #2    | CCL2        | 74.1396               |
| CTR #2    | CCL3        | 304.9312              |
| CTR #2    | CCL4        | 12.1142               |
| CTR #2    | MMP9        | 127.8208              |
| CTR #2    | CCL5        | 27.0336               |
| CTR #2    | TNF         | 16.4373               |
| CTR #2    | VEGF        | 680.2715              |
| GSK126+JQ1 #1 | IL1A      | 117.875               |
| GSK126+JQ1 #1 | IL1B      | 136.6784              |
| GSK126+JQ1 #1 | IL2       | 15.9987               |
| GSK126+JQ1 #1 | IL4       | 40.516                |
| GSK126+JQ1 #1 | IL5       | 25.499                |
| GSK126+JQ1 #1 | IL6       | 96.5216               |
| GSK126+JQ1 #1 | IL8       | 7.0692                |
| GSK126+JQ1 #1 | IL10      | 96.278                |
| GSK126+JQ1 #1 | IL12      | 8.9661                |
| GSK126+JQ1 #1 | IL13      | 6.4343                |
| GSK126+JQ1 #1 | CSF2      | 30.1104               |
| GSK126+JQ1 #1 | CXCL1     | 103.5975              |
| GSK126+JQ1 #1 | IFNG      | 153.7699              |
| GSK126+JQ1 #1 | CCL2      | 217.6356              |
| GSK126+JQ1 #1 | CCL3      | 323.9894              |
| Group          | Protein | Value       |
|---------------|---------|-------------|
| GSK126+JQ1 #1 | CCL4    | 12.8268     |
| GSK126+JQ1 #1 | MMP9    | 135.8096    |
| GSK126+JQ1 #1 | CCL5    | 27.0336     |
| GSK126+JQ1 #1 | TNF     | 17.7265     |
| GSK126+JQ1 #1 | VEGF    | 524.3441    |
| GSK126+JQ1 #2 | IL1A    | 115         |
| GSK126+JQ1 #2 | IL1B    | 145.2208    |
| GSK126+JQ1 #2 | IL2     | 15.3713     |
| GSK126+JQ1 #2 | IL4     | 50.9344     |
| GSK126+JQ1 #2 | IL5     | 25.499      |
| GSK126+JQ1 #2 | IL6     | 109.8795    |
| GSK126+JQ1 #2 | IL8     | 5.16        |
| GSK126+JQ1 #2 | IL10    | 104.65      |
| GSK126+JQ1 #2 | IL12    | 8.7362      |
| GSK126+JQ1 #2 | IL13    | 7.2557      |
| GSK126+JQ1 #2 | CSF2    | 29.4831     |
| GSK126+JQ1 #2 | CXCL1   | 95.3097     |
| GSK126+JQ1 #2 | IFNG    | 150.4982    |
| GSK126+JQ1 #2 | CCL2    | 235.174     |
| GSK126+JQ1 #2 | CCL3    | 314.4603    |
| GSK126+JQ1 #2 | CCL4    | 12.8268     |
| GSK126+JQ1 #2 | MMP9    | 131.8152    |
| GSK126+JQ1 #2 | CCL5    | 27.8784     |
| GSK126+JQ1 #2 | TNF     | 17.4042     |
| GSK126+JQ1 #2 | VEGF    | 454.0239    |
| GSK126 #1     | IL1A    | 132.25      |
| GSK126 #1     | IL1B    | 140.9496    |
| GSK126 #1     | IL2     | 17.8809     |
| GSK126 #1     | IL4     | 42.6312     |
| GSK126 #1     | IL5     | 30.243      |
| GSK126 #1     | IL6     | 10455.3576  |
| GSK126 #1     | IL8     | 278.2788    |
| GSK126 #1     | IL10    | 119.301     |
| GSK126 #1     | IL12    | 9.6558      |
| GSK126 #1     | IL13    | 8.8985      |
| GSK126 #1     | CSF2    | 43.911      |
| GSK126 #1     | CXCL1   | 1164.4359   |
| GSK126 #1     | IFNG    | 173.4001    |
| GSK126 #1     | CCL2    | 2134.1044   |
| GSK126 #1     | CCL3    | 343.0476    |
| GSK126 #1     | CCL4    | 12.8268     |
| GSK126 #1     | MMP9    | 131.8152    |
| GSK126 #1     | CCL5    | 27.8784     |
| GSK126 #1     | TNF     | 17.4042     |
| GSK126 #1     | VEGF    | 3321.8651   |
| GSK126 #2     | IL1A    | 115         |
| GSK126 #2     | IL1B    | 166.5768    |
| GSK126 #2     | IL2     | 16.9398     |
| GSK126 #2     | IL4     | 40.516      |
| GSK126 #2     | IL5     | 23.72       |
| GSK126 #2     | IL6     | 5046.2699   |
| GSK126 #2     | IL8     | 224.1504    |
| GSK126 #2     | IL10    | 100.464     |
| GSK126 #2     | IL12    | 8.7362      |
| GSK126 #2     | IL13    | 8.4878      |
| GSK126 #2     | CSF2    | 37.0107     |
| GSK126 #2     | CXCL1   | 868.8377    |
| GSK126 #2     | IFNG    | 170.1284    |
| GSK126 #2     | CCL2    | 1387.128    |
| GSK126 #2     | CCL3    | 323.9894    |
| GSK126 #2     | CCL4    | 13.1831     |
| Gene  | Value       |
|-------|-------------|
| MMP9  | 135.8096    |
| CCL5  | 28.7232     |
| TNF   | 18.3711     |
| VEGF  | 1872.6575   |
| IL1A  | 140.875     |
| IL1B  | 149.492     |
| IL2   | 20.3905     |
| IL4   | 45.1464     |
| IL5   | 31.429      |
| IL6   | 24071.7976  |
| IL8   | 758.8296    |
| IL10  | 198.835     |
| IL12  | 10.5754     |
| IL13  | 12.4579     |
| CSF2  | 148.6701    |
| CXCL1 | 16943.0258  |
| IFNG  | 199.5737    |
| CCL2  | 5057.4368   |
| CCL3  | 371.6349    |
| CCL4  | 17.1024     |
| MMP9  | 159.776     |
| CCL5  | 32.9472     |
| TNF   | 22.561      |
| VEGF  | 4073.9855   |
| IL1A  | 126.5       |
| IL1B  | 158.0344    |
| IL2   | 19.4494     |
| IL4   | 48.6192     |
| IL5   | 27.871      |
| IL6   | 12237.9909  |
| IL8   | 617.7552    |
| IL10  | 182.091     |
| IL12  | 10.5754     |
| IL13  | 10.8151     |
| CSF2  | 124.2054    |
| CXCL1 | 13926.2666  |
| IFNG  | 179.9435    |
| CCL2  | 3494.1276   |
| CCL3  | 343.0476    |
| CCL4  | 15.3209     |
| MMP9  | 143.7984    |
| CCL5  | 30.4128     |
| TNF   | 19.9826     |
| VEGF  | 2432.1617   |

CTR, Control.
Table S4, Related to Figures 1 and S2.

Alignment Rates of RNA-seq Data

| Sample             | Number of reads | Reads after trimming | % of reads remaining | HISAT2 alignment % | % aligned concordantly exactly 1 time | # aligned concordantly exactly 1 time | FeatureCount gene count |
|--------------------|-----------------|----------------------|----------------------|--------------------|---------------------------------------|----------------------------------------|-------------------------|
| shGFP_1            | 39,544,533      | 39,493,427           | 99.87                | 96.17              | 87.73                                 | 34,647,571                            | 32,177,852              |
| shGFP_2            | 33,331,456      | 33,293,441           | 99.89                | 96.1               | 88.73                                 | 29,541,575                            | 27,440,073              |
| shGFP_3            | 31,621,901      | 31,581,385           | 99.87                | 96.01              | 88.33                                 | 27,894,550                            | 25,876,556              |
| shEZH2_4hr_1       | 30,174,636      | 30,145,492           | 99.90                | 95.72              | 88.56                                 | 26,696,692                            | 24,665,826              |
| shEZH2_4hr_2       | 27,559,330      | 27,532,883           | 99.90                | 95.33              | 87.76                                 | 24,163,287                            | 22,260,670              |
| shEZH2_4hr_3       | 32,452,763      | 32,420,262           | 99.90                | 96.2               | 88.15                                 | 28,578,966                            | 26,016,533              |
| shEZH2_8hr_1       | 34,542,173      | 34,496,893           | 99.87                | 94.39              | 86.3                                  | 29,771,809                            | 27,425,389              |
| shEZH2_8hr_2       | 34,805,705      | 34,770,894           | 99.90                | 94.27              | 86.55                                 | 30,094,531                            | 27,588,381              |
| shEZH2_8hr_3       | 33,358,345      | 33,326,622           | 99.90                | 94.86              | 88.64                                 | 29,540,951                            | 27,126,907              |
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