Figure S1

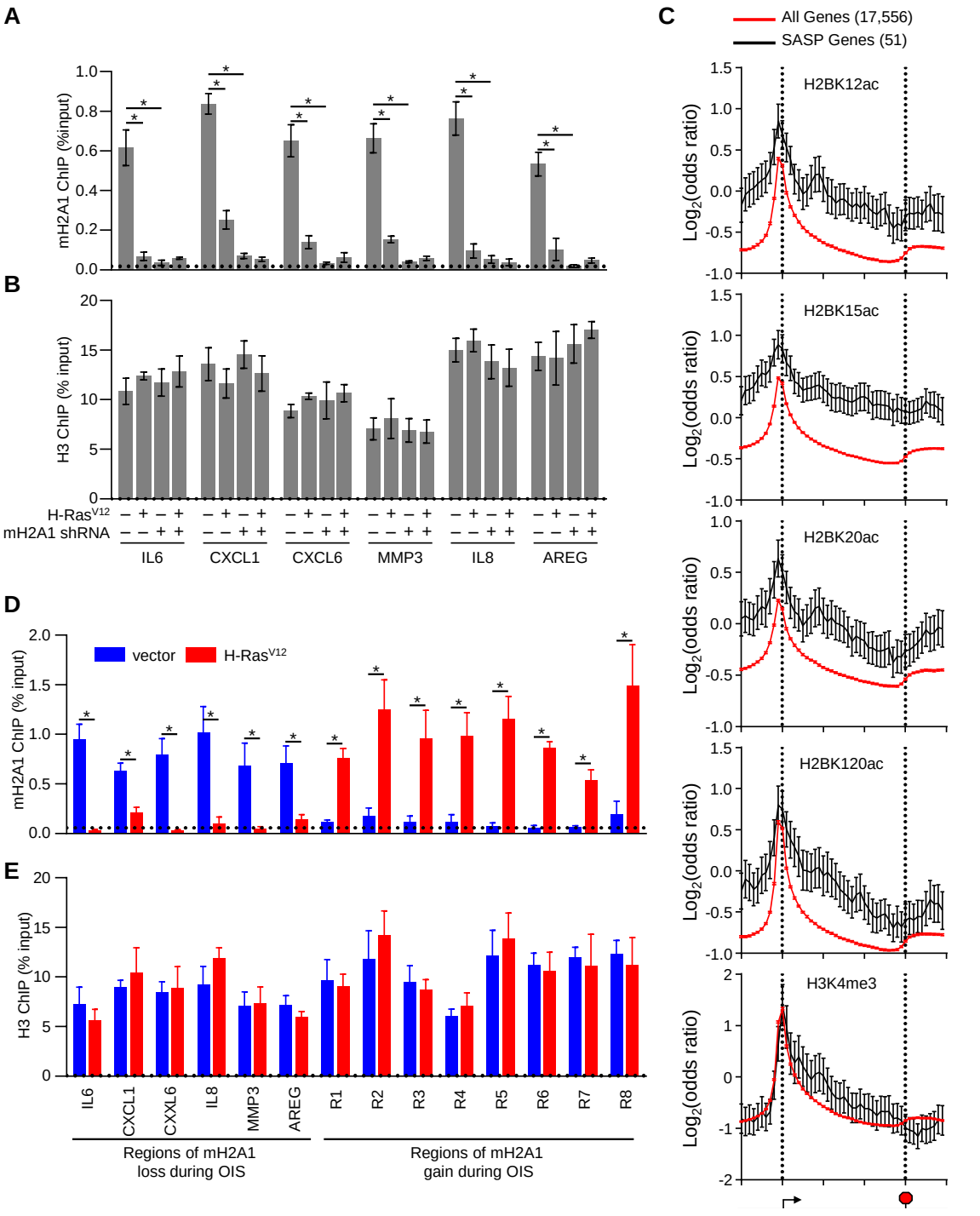
A
mH2A1 ChIP (%input)
B
H3 ChIP (% input)
H-Ras^{V12}
mH2A1 shRNA
IL6
CXCL1
CXCL6
MMP3
IL8
AREG
C
All Genes (17,556)
SASP Genes (51)
H2BK12ac
H2BK15ac
H2BK20ac
H2BK120ac
H3K4me3
Log$_2$(odds ratio)
D
vector
H-Ras^{V12}
mH2A1 ChIP (% input)
E
H3 ChIP (% input)
IL6
CXCL1
CXXL6
IL8
MMP3
AREG
R1
R2
R3
R4
R5
R6
R7
R8
Regions of mH2A1 loss during OIS
Regions of mH2A1 gain during OIS

Figure S1, Related to Figure 1

MacroH2A1 ChIP-seq data is specific and reproducible.

(**A, B**) ChIP-qPCR for macroH2A1 (A) and H3 (B) from IMR90 cells expressing an shRNA against macroH2A1 (+) or luciferase (-), as a control, subjected to retroviral-mediated expression of H-Ras^{V12} (+) or empty vector (-) as a control. The horizontal dotted line indicates upper limit of the 95% confidence interval of the signal from no-antibody control ChIPs. Error bars, s.e.m. (n = 3 independent cell passages and retroviral infections). *p < 0.05 from two-tailed Student's t-tests.

(**C**) Metagene analysis of ChIP-seq data for various histone PTMs in IMR90 cells. Average enrichment for the indicated histone PTMs on either all autosomal protein coding genes (red) or fifty-one SASP genes (black) defined from gene expression microarray data (GSE40349). The data represent the average signal in ten 1-kb windows upstream of the TSS, 30 windows spanning the gene body and ten 1-kb windows downstream of the end of the gene. The location of the TSS and the end of the gene were depicted by the left and right vertical dotted line respectively. Error bars, s.e.m. (n = 17,556 and 51 for red and black curves respectively).

(**D, E**) Confirmation analysis ChIP-seq identified regions of macroH2A1 gain and loss during OIS. ChIP-qPCR for macroH2A1 (D) and H3 (E) from IMR90 cells. Cells were transduced by vector only (vector) as control or H-Ras^{V12} and ChIPs were performed 14 days following retroviral infection. ChIP-seq identified regions that undergo macroH2A1 loss and gain during OIS are indicated. The horizontal dotted line indicates upper limit of the 95% confidence interval of the signal from no-antibody control ChIPs. Error bars s.e.m. (n = 3 independent cell passages and retroviral infections). * p < 0.05 from a two-tailed Student's t-test.

Figure S2

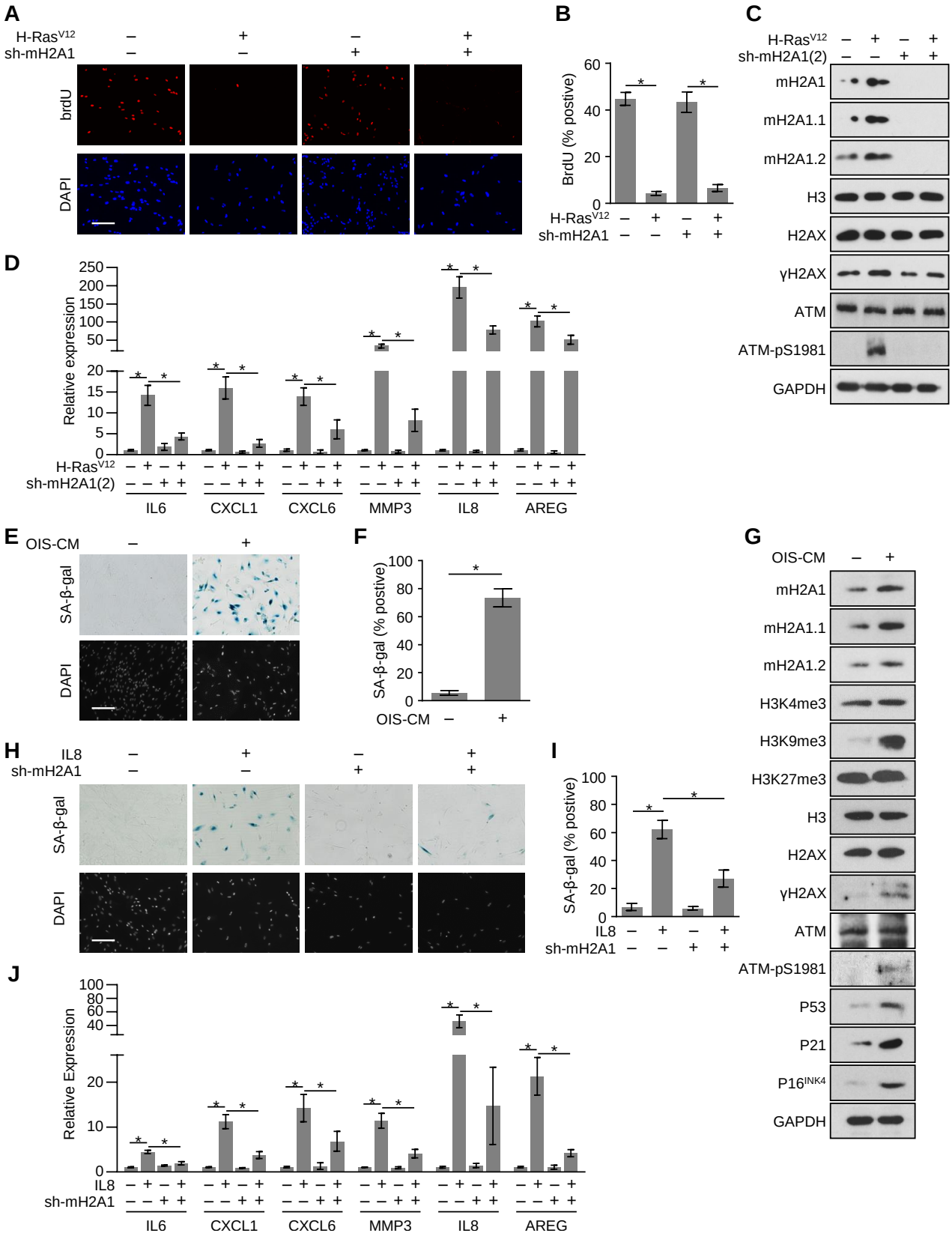
A
H-Ras^{V12}
sh-mH2A1
brdU
DAPI
B
BrdU (% postive)
C
H-Ras^{V12}
sh-mH2A1(2)
mH2A1
mH2A1.1
mH2A1.2
H3
H2AX
γH2AX
ATM
ATM-pS1981
GAPDH
D
Relative expression
IL6
CXCL1
CXCL6
MMP3
IL8
AREG
E
OIS-CM
SA-β-gal
DAPI
F
SA-β-gal (% postive)
G
H3K4me3
H3K9me3
H3K27me3
P53
P21
P16^{INK4}
H
IL8
I
J
Relative Expression

Figure S2, Related to Figure 2

MacroH2A1 is essential for OIS and OIS-CM can trigger paracrine senescence in IMR90 cells.

(**A**) Representative immunofluorescence images for brdU (red) counterstained with DAPI (blue) in brdU-treated IMR90 cells expressing shRNA against luciferase (-) as a control or macroH2A1 (+) subjected to retroviral-mediated expression of H-Ras^{V12} (+) for OIS or vector only (-) as a control for 14 days. Scale bar, 200 μm.

(**B**) Histogram depicting the percentage of brdU positive-staining for cells treated as described in (A). Error bars s.e.m. (n = 3 independent cell passages and retroviral infections). * $p < 0.05$ from a two-tailed Student's t-test.

(**C**) Immunoblots for macroH2A1and DDR factors from IMR90 cells as similar to that described in (A) except that macroH2A1 was depleted by an independent shRNA (sh-mH2A1(2)).

(**D**) RT-qPCR of IMR90 cells described in (C) for the indicated SASP genes. Error bars, s.e.m. (n = 3 independent cell passages and retroviral infections). *$p < 0.05$ from two-tailed Student's t-tests.

(**E**) Representative SA-β-gal and DAPI counterstaining of IMR90 cells subjected to paracrine senescence with cultured media from H-Ras^{V12}-meidated senescent IMR90 cells (OIS-CM, +) or cultured media from IMR90 cells expressing empty vector (-) as a control for 14 days. Scale bar, 200 μm.

(**F**) Histogram depicting the percentage of SA-β-gal positive-staining for cells described in (E). Error bars, s.e.m. (n = 3 independent cell passages and retroviral infections). *$p < 0.05$ from two-tailed Student's t-tests.

(**G**) Immunoblots for macroH2A1 (mH2A1), DDR factors and cell cycle inhibitors from IMR90 cells described as (E).

(**H**) Representative SA-β-gal and DAPI counterstaining of IMR90 cells expressing an shRNA against luciferase (-) or macroH2A1 (+, sh-mH2A1) subjected to 10nM IL8 (+) treatment or BSA (-) control for 14 days. Scale bar, 200 μm.

(**I**) Histogram depicting the percentage of SA-β-gal positive-staining cells described in (H). Error bars, s.e.m. (n = 3 independent cell passages and retroviral infections). *$p < 0.05$ from two-tailed Student's t-tests.

(**J**) RT-qPCR for the indicated SASP genes from IMR90 cells treated as described in (H). Error bars, s.e.m. (n = 3 independent cell passages and retroviral infections). *$p < 0.05$ from two-tailed Student's t-tests.

Figure S3

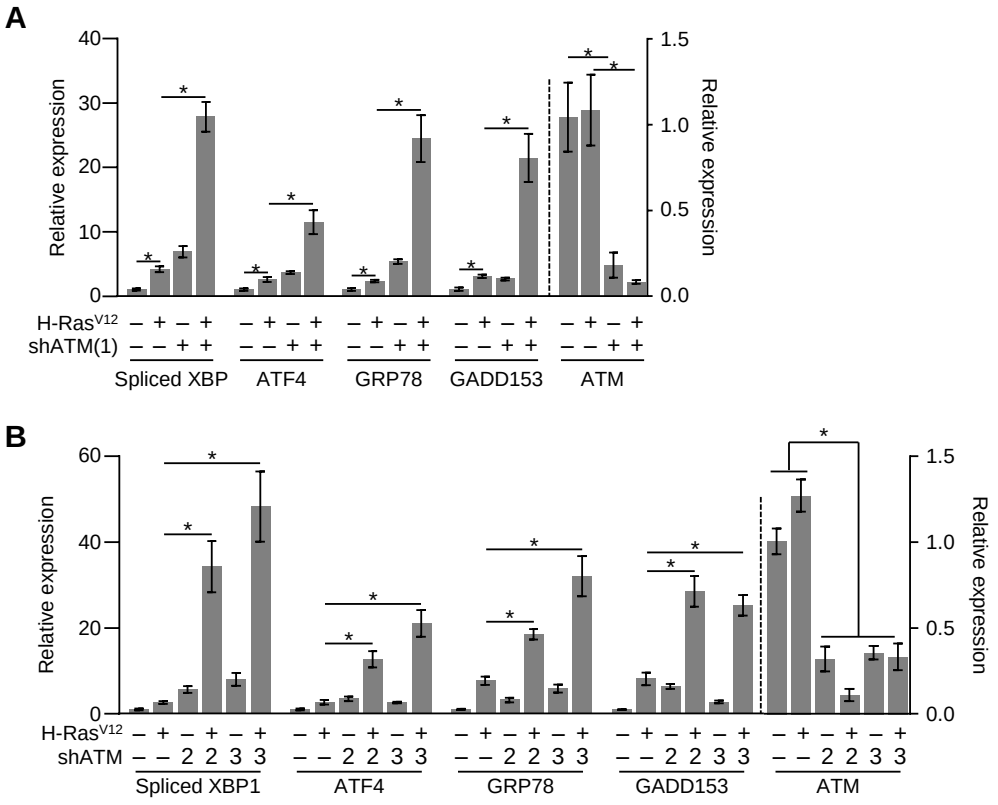
A
Relative expression
40
30
20
10
0
1.5
1.0
0.5
0.0
H-Ras^{V12} – + – + – + – + – + – + – + – + – + – +
shATM(1) – – + + – – + + – – + + – – + + – – + +
Spliced XBP
ATF4
GRP78
GADD153
ATM
B
Relative expression
60
40
20
0
1.5
1.0
0.5
0.0
H-Ras^{V12} – + – + – + – + – + – + – + – + – + – + – + – + – + – +
shATM – – 2 2 3 3 – – 2 2 3 3 – – 2 2 3 3 – – 2 2 3 3 – – 2 2 3 3
Spliced XBP1
ATF4
GRP78
GADD153
ATM

Figure S3, Related to Figure 4

ATM suppresses senescence-associated ER stress.

(**A**) RT-qPCR for the indicated UPR genes from IMR90 cells stably expressing an shRNA against ATM (shATM(1), +) or luciferase (-) as a control subjected to retroviral-mediated expression of HRasV12 (+) for OIS or empty vector (-) as a control. The dotted line separates the genes plotted on the left and right axes, respectively. Error bars, s.e.m. (n = 3 independent cell passages and retroviral infections). *p < 0.05 from two-tailed Student's t-tests.

(**B**) RT-qPCR for the indicated UPR genes from IMR90 cells. The indicated cells were subjected to retroviral-mediated expression of H-Ras^{V12} (+) for OIS or empty vector (-) as a control for 3 days. Then, shRNA against luciferase as a control or either of two independent shRNAs against ATM (shATM(2) or shATM(3)) was infected by lentiviral-mediated expression. ATM depletion was also confirmed by RT-qPCR. The dotted line separates the genes plotted on the left and right axes, respectively. Error bars, s.e.m. (n = 3 independent cell passages and retroviral infections). *p < 0.05 from two-tailed Student's t-tests.

Figure S4

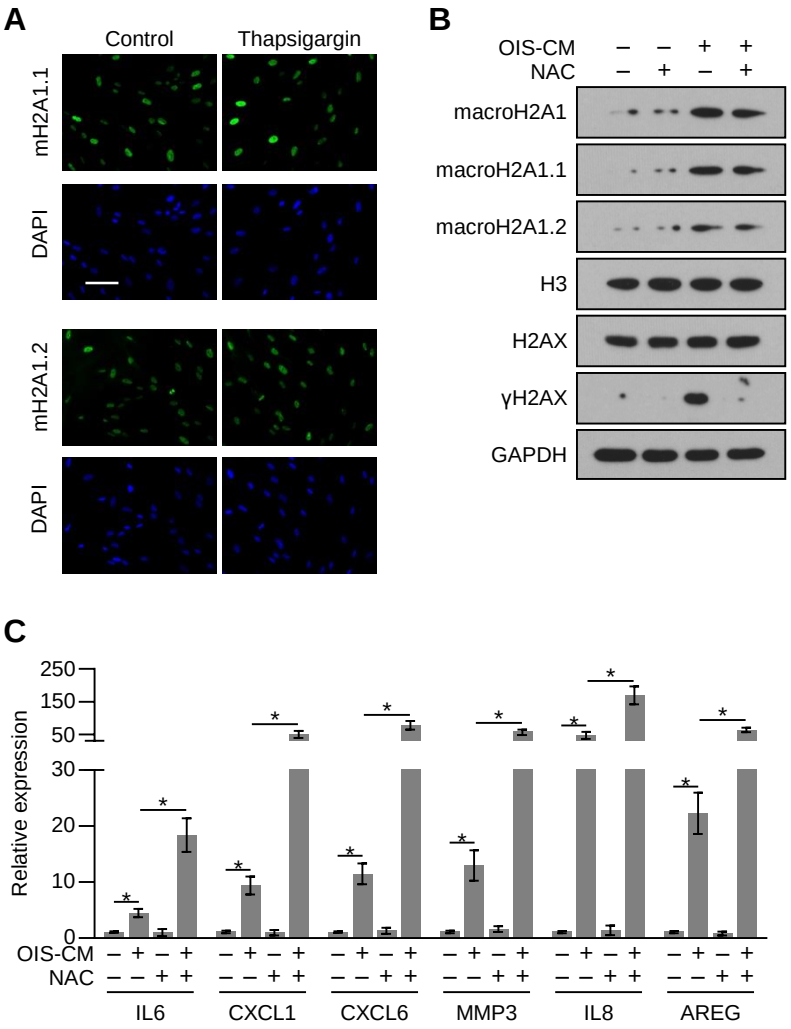
A
Control
Thapsigargin
mH2A1.1
DAPI
mH2A1.2
DAPI
B
OIS-CM – – + +
NAC – + – +
macroH2A1
macroH2A1.1
macroH2A1.2
H3
H2AX
γH2AX
GAPDH
C
Relative expression
250
150
50
30
20
10
0
OIS-CM – + – + – + – + – + – + – + – + – + – + – +
NAC – – + + – – + + – – + + – – + + – – + + – – + +
IL6
CXCL1
CXCL6
MMP3
IL8
AREG

Figure S4, Related to Figure 5

ER stress represses SASP gene expression in a ROS dependent manner.

(**A**) Representative immunofluorescence images for macroH2A1.1 or macroH2A1.2 (green) and counterstained with DAPI (blue) from IMR90 cells treated with 0.25 μM of the ER stress inducing agent, thapsigargin, for 3 days. Scale bar, 100 μm.

(**B**) Immunoblots for indicated macroH2A1 subtypes and DDR factors from IMR90 cells subjected to paracrine senescence with cultured media from H-Ras^{V12}-meidated senescent IMR90 cells (OIS-CM, +) or cultured media from IMR90 cells expressing empty vector (-) as a control for 3 days. Where indicated, cells were additionally treated with 10 mM NAC (+) or water only (-) as a control.

(**C**) RT-qPCR for the indicated SASP genes from IMR90 cells treated as described in (B). Error bars, ± s.e.m. (n = 3 independent cell passages and retroviral infections). *p < 0.05 from two-tailed Student's t-tests.

Figure S5

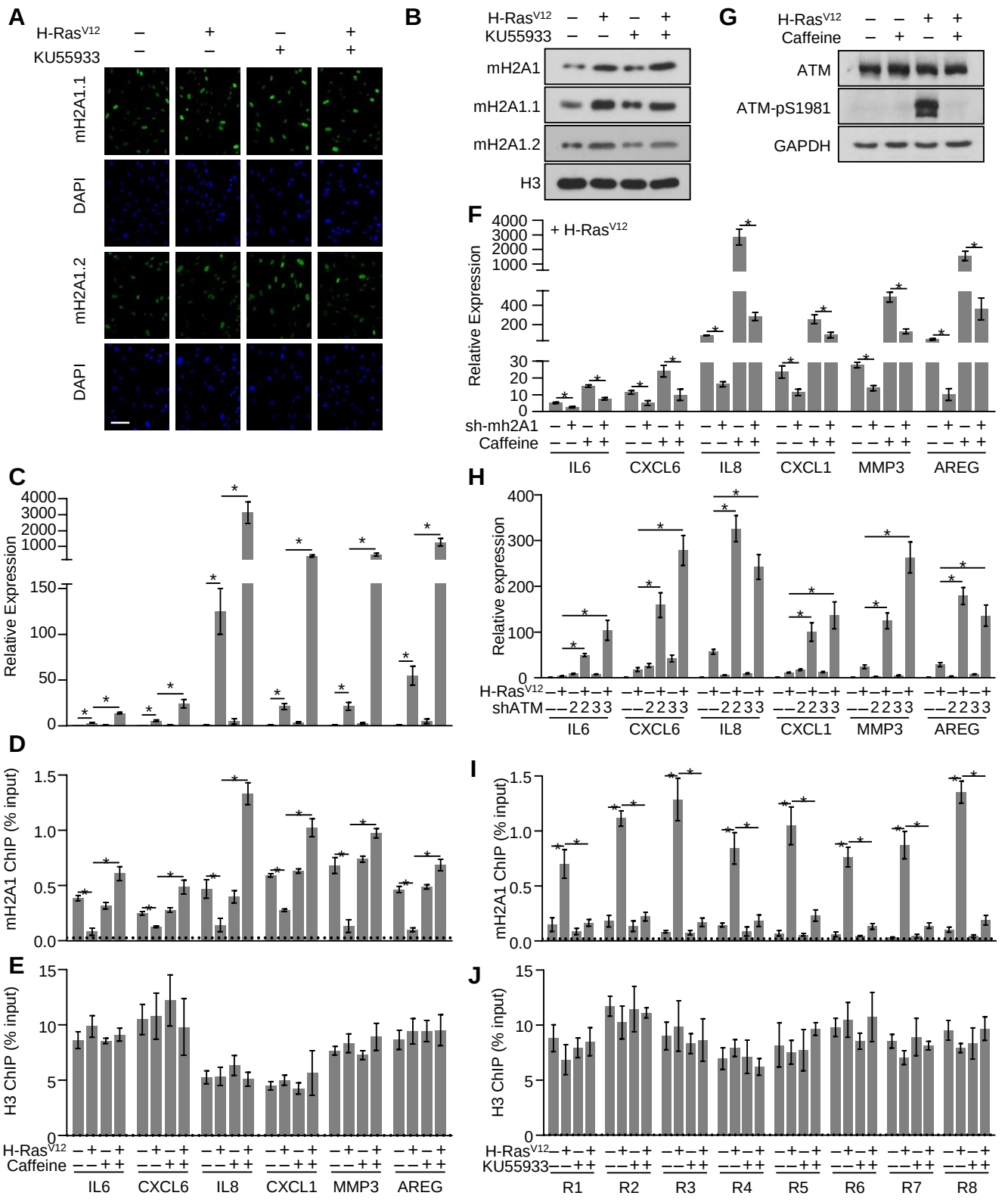
A
H-Ras^{V12}
KU55933
mH2A1.1
DAPI
mH2A1.2
DAPI
B
H-Ras^{V12}
KU55933
mH2A1
mH2A1.1
mH2A1.2
H3
G
H-Ras^{V12}
Caffeine
ATM
ATM-pS1981
GAPDH
F
+ H-Ras^{V12}
Relative Expression
sh-mh2A1
Caffeine
IL6
CXCL6
IL8
CXCL1
MMP3
AREG
C
Relative Expression
D
mH2A1 ChIP (% input)
E
H3 ChIP (% input)
H-Ras^{V12}
Caffeine
IL6
CXCL6
IL8
CXCL1
MMP3
AREG
H
Relative expression
H-Ras^{V12}
shATM
IL6
CXCL6
IL8
CXCL1
MMP3
AREG
I
mH2A1 ChIP (% input)
J
H3 ChIP (% input)
H-Ras^{V12}
KU55933
R1
R2
R3
R4
R5
R6
R7
R8

Figure S5, Related to Figure 6

ATM represses SASP expression by inducing the mobilization of macroH2A1 away from SASP genes.

(**A**) Representative immunofluorescence images for macroH2A1.1 or macroH2A1.2 (green) and counterstained with DAPI (blue) from IMR90 cells subjected to retroviral-mediated expression of H-Ras^{V12} (+) for OIS or empty vector (-) as a control for 3 days. Where indicated cells were treated with 10 μM of the ATM inhibitor KU55933 (+) or DMSO (-) as a control. Scale bar, 100 μm.

(**B**) Immunoblots for indicated macroH2A subtypes from IMR90 cells described as (A).

(**C**) RT-qPCR for the indicated SASP genes from IMR90 cells treated similarly to that described in (A) except 100 μM caffeine was used instead of KU55933. Error bars, s.e.m. (n = 3 independent cell passages and retroviral infections). *p < 0.05 from two-tailed Student's t-tests.

(**D,E**) ChIP-qPCR for macroH2A1 (D) and H3 (E) from IMR90 cells treated as described in (C). Error bars, s.e.m. (n = 3 independent cell passages and retroviral infections). *p < 0.05 from two-tailed Student's t-tests.

(**F**) RT-qPCR for the indicated genes from IMR90 expressing an shRNA against macroH2A1 (+, sh-mH2A1l) or luciferase (-) as a control subjected to retroviral-mediated expression of H-Ras^{V12} for 3 days. Error bars, ± s.e.m. (n = 3 independent cell passages and retroviral infections). *p < 0.05 from two-tailed Student's t-tests.

(**G**) Immunoblots for ATM and ATM-pS1981 from IMR90 cells treated as described in (C).

(**H**) RT-qPCR for the indicated SASP genes from IMR90 cells. The indicated cells were subjected to retroviral-mediated expression of H-Ras^{V12} (+) for OIS or empty vector (-) as a control for 3 days. Then, shRNA against luciferase as a control or either of two independent shRNAs against ATM (shATM(2) or shATM(3)) was infected by lentiviral-mediated expression 5 days prior to harvesting the cells. ATM depletion was also confirmed by RT-qPCR. Error bars, s.e.m. (n = 3 independent cell passages and retroviral infections). *p < 0.05 from two-tailed Student's t-tests.

(**I, J**) ChIP-qPCR of loci that gain macroH2A1 occupancy upon OIS for macroH2A1 (I) and H3 (J) from IMR90 cells treated as described in (A). The horizontal dotted line indicates upper limit of the 95% confidence interval of the signal from no-antibody control ChIPs. Error bars, s.e.m. (n = 3 independent cell passages and retroviral infections). *p < 0.05 from two-tailed Student's t-tests.

Supplementary Table 1. Antibodies used in this study.

Factor or PTM	Vendor	Cat Number	Dilution For blotting	Dilution For IF	Vol.for ChIP (µl)
macroH2A1	Millipore	07-219	1:1,000		8
macroH2A1.1	Cell signaling	4160S	1:1,000	1:200	
macroH2A1.2	Cell signaling	4827S	1:1,000	1:100	
H2B Ac K12	Abcam	ab40883	1:1,000		
H3K4me3	Millipore	04-745	1:1,000		
H3K9me3	abcam	ab8898	1:1,000		
H3K27me3	Millipore	07-449	1:3,000		
H2AX	abcam	ab124781	1:1,000		
γH2AX	Millipore	05-636	1:1,000		
H3	Abcam	ab1791	1:3,000		4
ATM	GeneTex	GTX70103	1:1,000		
ATM-pS1981	Millipore	05-740	1:1,000		
P53	SANTA CRUZ	sc-126	1:1,000		
P21	SANTA CRUZ	sc-397	1:1,000		
$P16^{INK4}$	BD Pharmingen	554079	1:1,000		
Anti-Flag M2	Sigma	F3165	1:5,000		
Anti- BrdU	BD	555627		1:250	
Anti-8-oxo-dG	abcam	ab62623		1:100	
GAPDH	Cell signaling	2118L	1:1,000		
HRP-conjugated goat anti-rabbit secondary antibody	Jackson Labs	111035003	1:30,000		
HRP-conjugated goat anti-mouse secondary antibody	Jackson Labs	115036003	1:30,000		
Mouse Anti Rabbit IgG, Light Chain Specific Secondary antibody	Jackson Labs	112692	1:30,000		

Table S2. ChIP primers used in this study.

Primer set name	Location in the genome	Forward sequence	Reverse sequence
IL6 –P	chr7:22767668+22767893	GATTCCTCAAAGCCATTCCA	TGGAGTCCAGAGGTGGTAGG
IL8 -2kb	chr4:74604071+74604193	AGGCAACCGTTAGGGAAAAG	GGACACAACCTGGCTTGACT
CXCL1 -1.6kb	chr4:74733260+74733377	TGGAAACTGAGCTTTTGGTG	TGCTACCCAACTACCCCTAATG
CXCL6 -3kb	chr4:74699511+74699633	GCAATTTGCTGGGTGATTTT	AATTGGCCCCAAAGTGAAAT
MMP3 -1.8kb	chr11:102715943+102716042	GGGGGAAAAACCATGTCTTG	ATTCACATCACTGCCACCAC
AREG +1kb	chr4:75481819+75481968	GGACAGCTGAATTTGCTTGC	AAGTAGGGTGCCTTTCGAGTC
R1	chr7:25256321+25256519	TGTGCTGATTCCTCACTGCT	AACACCACCAGCACAACAAA
R2	chr4:75717898+75718092	GCATCGCCAACCCTTTACTA	GCAGGGTAACCTGTGGAAAA

R3	chr4:78182313+78182401	ACGGACAGCTGCAGATTCTT	CTCAGCTTACCCAGCCTGAC
R4	chr4:152762165+152762260	CCAATAAATCCAGCCCAGAA	GGGTGCTTTTGATGGTTTGT
R5	chr4:75717898+75718092	GCATCGCCAACCCTTTACTA	GCAGGGTAACCTGTGGAAAA
R6	chr4:52992369+52992650	AAGGAAAAATGAAGCCCAAA	CTCAAAGTTGCCCAGGAATC
R7	chr11:82442652+82442723	TACAGTTTGCCCACCCTCTC	TGGGTTCCATTTCTCTCCTG
R8	chr11:79197477+79197721	ACTGGGAGGGGCTTAGTCAT	CATCCACTGTGCCTCAGAGA

Table S3. RT-PCR primers used in this study.

Gene name	Forward sequence	Reverse sequence
ACTB	AGCTACGAGCTGCCTGAC	AAGGTAGTTTCGTGGATGC
IL6	TTCTGCGCAGCTTTAAGGAG	AGGTGCCCATGCTACATTTG
IL8	ATGACTTCCAAGCTGGCCGTG	TGTGTTGGCGCAGTGTGGTC
CXCL1	CACCCCAAGAACATCCAAAG	TAACTATGGGGGATGCAGGA
CXCL6	TGTTTACGCGTTACGCTGAG	AACTTGCTTCCCGTTCTTCA
MMP3	AGGGAACTTGAGCGTGAATC	TCACTTGTCTGTTGCACACG
AREG	AGCTGCCTTTATGTCTGCTG	TTTCGTTCCTCAGCTTCTCC
Spliced XBP1	TGCTGAGTCCGCAGCAGG	GTCCAGAATGCCCAACAGGA
ATF4	CCAGGTGTTCTCTGTGGGTC	TGGCTGCTGTCTTGTTTTGC
GRP78	TCACATGTCTTTGGGTGGGG	CCAGATGCACATGACCCAGT
GADD153	GCCTTTCTCCTTTGGGACACTGTCCAGC	CTCGGCGAGTCGCCTCTACTTCCC
ATM	CGAAAAGAATCTGGGGTTTG	ACAAAGTAGGGTGGGAAAGC

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Cell culture

IMR90 primary human fetal lung fibroblast cells (ATCC), hTERT-immortalized IMR90 (IMR90-hTERT) cells (Novikov et al., 2011) and IMR90-hTERT cells depleted of macroH2A1 with either of two independent shRNAs or luciferase (as a control) (Novikov et al., 2011) were cultured in MEM supplemented with 10% FBS. Transformed human embryonic kidney HEK293T cells (ATCC) were cultured in DMEM supplemented with 10% FBS. Cell lines in culture were routinely tested for mycoplasma contamination. Retroviral packaging and infections were performed as previously described (Novikov et al., 2011).

Senescence of IMR90 and IMR90-hTERT cells was induced using three independent methods, OIS, paracrine senescence, and macroH2A1.1 ectopic expression. OIS was induced

by retroviral-mediated ectopic expression of H-Ras^{V12} or empty vector (as a control). The retroviral expression constructs, pBABE-puro and pBABE-puro-H-Ras^{V12}, were gifts from William Hahn (Addgene plasmid #9051). Paracrine senescence was induced either by using conditioned media from H-Ras^{V12} expressing senescent IMR90-hTERT cells (or normally growing IMR90-hTERT cells as a control) or by treating IMR90-hTERT cells with or without 10 nM recombinant human IL8 (R&D systems, 618-IL-101) reconstituted at 100 µg/ml in PBS containing 0.1% bovine serum albumin. For paracrine senescence by conditioned media, media was collected from H-Ras^{V12}-induced senescent cells or normally growing cells as a control. Fourteen days post-infection, the cultured medium in the dish was collected every 3 days, centrifuged at 3,000 rpm × 10min, then the supernatant was filtered through a 0.2 µm pore filter (GE Helthcare). The resulting filtered medium was then mixed with an equal volume of MEM 20% FBS and used as media for normal IMR90-hTERT cells. Senescence was also monitored in IMR90-hTERT cells after retroviral-mediated ectopic expression of either green fluorescent protein (GFP), macroH2A1.1, macroH2A1.1 point mutants G224E, G314E, macroH2A1.2 as described previously (Chen et al., 2014).

Both stable and transient depletion of ATM was performed with lentivirus-mediated expression of shRNA obtained from the TRC genome-wide shRNA collection. For stable depletion of ATM, IMR90 cells were subjected to lentiviral-mediated expression of either an shRNA against luciferase (shLuc, sequence: 5'-CCTAAGGTTAAGTCGCCCTCG-3') or an shRNA against ATM (shATM(1), target sequence: 5'-CCTGCCAACATACTTTAAGTA-3', TRCN0000038654). Three days post-infection, the cells were selected with 1 µg/ml puromycin for three days. In the case of transient depletion of ATM, IMR90 cells subjected to retroviral-mediated expression of H-Ras^{V12} or empty vector as a control for three days were infected with either shLuc, shATM(2) (target sequence: 5'-CCTTTCATTCAGCCTTTAGAA-3',

TRCN0000039948) or shATM(3) (target sequence: 5'-CCTGCCAACATACTTTAAGTA-3', TRCN0000038654). The cells were then used five days post-infection for RT-qPCR assays.

Where indicated, cells were treated with 100 µM Caffeine (Sigma, C0750), 10 µM KU55933 (R&D systems, 3544), a selective ATM kinase inhibitor and/or 250 nM thapsigargin (Fisher, 328570010) and 10 mM NAC (Sigma, A7250).

SA-β-Gal staining

Cells were plated in cell culture chambers with culture slides affixed on the bottom (BD Falcon) to provide a consistent surface for cell growth at 5×10^4 cells/chamber and allowed to attach overnight. Cells in the chamber were washed in PBS once, and then fixed for 20 min at room temperature in fixing solution (2% formaldehyde and 0.2% glutaraldehyde in PBS). The fixed cells were washed with PBS twice and incubated at 37°C for 16 h with freshly prepared SA-β-Gal staining solution (1 mg/ml 5-bromo-4-chloro-3-indolyl β-D-galactoside (X-Gal), 40 mM citric acid/sodium phosphate pH 6.0, 5 mM potassium ferrocyanide, 5 mM potassium ferricyanide, 150 mM NaCl and 2 mM $MgCl_2$). Following staining, the cells were washed with PBS three times. The cells were then mounted to coverslips with a DAPI-containing mounting solution (SouthernBiotech, 0100-20) to label nuclei. The cells were imaged with a Zeiss Axio Observer microscope. Stained and unstained cells were quantified over four separate fields containing at least 80 cells.

Bromodeoxyuridine (BrdU) incorporation assay

Cells were plated in coverslips in 6 wells plate format for 24 hours. After addition of 10 µM brdU (Sigma Aldrich, B5002) for 2 hr, cells were fixed in cold 70% ethanol for 5 min at room temperature. Then, the cells were treated for 30 min with 1.5 N HCl and blocked for 1 hr with blocking buffer (1x PBS / 5% fetal bovine serum / 0.3% Triton X-100) at room temperature. The cells were then Incubated in Brdu primary antibody solution (BD, 555627, 1:250) followed by

incubation in Alexa Fluor® 568-conjugated secondary antibody diluted in 1x PBS with 1% BSA for 1.5 hours at room temperature in the dark. Coverslips were mounted onto slides using DAPI-containing mounting solution (SouthernBiotech, 0100-20). The cells were imaged with a Zeiss Axio Observer microscope. Images were quantified over four separate fields containing at least 100 cells.

Immunoblots and acid-extraction of histones

Immunoblots and acid-extraction of histones were performed as previously described (Chen et al., 2014). Briefly, cells from a 10-cm dish were lysed in 100 ul of 0.1% Triton X-100 lysis buffer. After incubation on ice for 30 min, the lysate was centrifuged at 14,000 r.p.m. for 10 min at 4 °C. The supernatant was collected as the detergent lysate. The pellets containing the histones were resuspended in 80 ul of 0.5 M HCl at 4 °C for 2 hours under constant agitation. The sample was spun at 14,000 r.p.m. for 10 min at 4 °C. The resulting supernatant containing the histones was neutralized with 20 ul of 2 M Tris base. The acid-extracted fraction was used for immunoblots of histones and the detergent lysates were used for immunoblots of all other factors. The detergent lysates and acid extracts were subjected to SDS-PAGE and immunoblotting (Antibodies information are listed in Table S1. HRP-conjugated goat anti-mouse or anti-rabbit secondary antibody (Jackson Labs) was used for detection by ECL chemiluminescence according to the manufacturer's instructions (Thermo, Super Signal West Pico). All immunoblots have been repeated at least twice with independent biological samples.

Chromatin Immunoprecipitation (ChIP)

Chromatin Immunoprecipitation (ChIP) was performed as previously described (Chen et al., 2014). Briefly, cells were grown to 90% confluence in 15-cm dishes, cross-linked with 1% formaldehyde in PBS at room temperature for 10 min and then quenched in 125 mM glycine for 5 min. The cells were washed with cold PBS once and were collected by centrifugation and then

sonicated in lysis buffer (50 mM Tris, pH 7.9, 10 mM EDTA, 1% SDS, protease-inhibitor cocktail and 1 mM DTT) to generate chromatin fragments of ~500 bp in length. The material was clarified by 10 min centrifugation at 14,000 r.p.m. and 4 °C, and 20 µl supernatant was used as input for quantitation. The remaining supernatant was diluted ten-fold in dilution buffer (20 mM Tris, pH 7.9, 2 mM EDTA, 150 mM NaCl, 0.5% Triton X-100, protease-inhibitor cocktail and 1 mM DTT) and precleared with 20 µl protein A–agarose beads at 4 °C for 3 h. The supernatant was used in immunoprecipitations at 4 °C overnight with antibodies against histone H3 (4 µl), macroH2A1 (8 µl), as indicated (catalog numbers, dilution factors and validation information in Table S1) and was then incubated with 40 µl protein A–agarose beads at 4 °C for 2 h. No-antibody (NA) controls were always included. The immunoprecipitated DNA was cleared of protein by digestion with 0.4 mg/ml glycogen and proteinase K (0.45 mg/ml, Roche) in Txn stop buffer at 37 °C for 1 h. The DNA was then extracted with phenol/chloroform/isoamyl alcohol (25:24:1) and ethanol precipitated. Quantitative real-time PCR with SYBR Green (Invitrogen) was used to determine enrichment of immunoprecipitated material relative to input with gene-specific primers to the specified regions (Table S2).

ChIP-Seq

Retrovirus encoding constitutive active H-Ras^{V12} was infected into primary IMR90 cells to trigger OIS. In parallel, IMR90 cells were infected by empty vector as a control. Following 14 days selection in 1 µg/ml puromycin, a portion of the cells were stained for SA-β-Gal activity to confirm senescence. The cells were then processed for ChIP-seq as previously described (Chen et al., 2014). All genomic coordinates use human genome version hg19. Mapped reads and ISOR processed data were uploaded to GEO (GSE64601). Our previous macroH2A1 ChIP-seq from normally growing IMR90 cells is available from GEO (GSE54847). ChIP-seq data for the 26 additional histone marks in IMR90 cells were downloaded from the GEO website (GSE16256).

Expression microarray analysis

SASP genes were identified from previously published publically available expression microarray data (GSE40349) (Aksoy et al., 2012). Genes with altered expression between the growing and H-Ras^{V12}-mediated senescent IMR90 cells were determined by analysis using the GEO2R web tool (http://www.ncbi.nlm.nih.gov/geo/info/geo2r.html) using default parameters. SASP genes were defined as secreted genes (using Gene Ontology accession GO_0005576) that were upregulated at least 4-fold with a Benjamini and Hochberg corrected p value less than 0.01.

RT-qPCR

RNA purification and RT-qPCR were performed as described previously (Chen et al., 2014). mRNA levels were analyzed by reverse transcription followed by quantitative PCR (RT-qPCR). RNA was isolated with TriPure (Roche) according to the manufacturer's protocol. The RNA was reverse transcribed with Moloney murine leukemia virus (MMLV) reverse transcriptase (Invitrogen) and a dT18 primer. cDNA, SYBR Green PCR master mix, and forward and reverse primers were used in 45 cycles of amplification (95 °C for 15 s, 60 °C for 1 min) following 10 min incubation at 95 °C, with a LightCycler 480 (Roche). The efficiency-corrected threshold cycle (ΔCT) method was used to determine the relative levels of RNA. For transcription analysis, the expression was normalized to ACTB. Melting-curve analysis was performed to ensure specificity. Expression primer sequences are listed in Table S3.

Dihydroethidium (DHE) staining for ROS detection

To measure the total superoxide level in living cells, cells were plated in coverslips in 6 wells plates for 24 hours. Then the cells were stained with 10 μM DHE (Santa Cruz, sc-204724) in cell culture medium and incubated for 30 min. Cell-permeable dye Hoechest 33342 (Life

Technologies, H-3570) was added for counterstaining the nuclei of the IMR90 cells. Images were acquired on a Zeiss Axio Observer microscope. Images were quantified over four separate fields containing at least 100 cells.

Immunofluorescence

Cells (5 X 10^4) were plated on coverslips in 6-well plates. After 24 hours, the cells were fixed with 4% paraformaldehyde for 15 min at room temperature. Washed by PBS twice, the cells were then permeabilized with PBS with 0.1% Triton X-100 for 10 min at room temperature. In the case of 8-oxo-dG, the fixed cells were incubated with 2 M HCl for 20 min at room temperature, followed by, 0.1 M sodium borate, pH 8.5. Cells were blocked for 1 hr at room temperature in 5% fetal bovine serum (in PBS). The cells were then incubated with primary antibodies (anti-macroH2A1.1 (Cell Signaling, 12455S, 1:200), macroH2A1.2 (Cell Signaling, 4827S, 1:100), anti-8-oxo-dG (abcam, ab62623, 1:100) diluted in PBS with 1% calf serum overnight at 4 °C. Following washing, the cells were incubated with either goat anti-rabbit IgG conjugated to Alexa Fluor® 488 (Life Technologies, A-11034, 1:1000) or goat anti-mouse IgG conjugated to Alexa Fluor® 594 conjugate (Life Technologies, A-11005, 1:1000) at room temperature for 1 hr, washed, and mounted onto slides using DAPI-containing mounting solution (SouthernBiotech, 0100-20). Images were acquired on a Zeiss Axio Observer microscope. Images were quantified over four separate fields containing at least 100 cells.

Statistical analysis

All SA-β-Gal staining, ROS detection, immunofluorescence, ChIP and RT-qPCR experiments have been repeated at least 3 times. All western blots were performed at least twice using independent biological samples. Results are presented as means ± s.e.m. Two-tailed Student's t-tests were used to determine the significance of differences between samples

indicated in figures. For ChIP-Seq results analysis, Fisher exact tests have been applied to determine the significance of enrichment in count data.

ACCESSION NUMBERS

ChIP-seq data have been deposited in the Gene Expression Omnibus database under accession code GSE64601.

SUPPLEMENTAL REFERENCES

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