Supplementary Methods

Vectors

The pWZL-Hygro (H-Ras^{V12}) vector was described previously (1). shRNAs targeting p65 were previously described (2). shRNAs targeting mouse Brd4 were previously described (3). shRNAs targeting human Brd4 were generated using a previously described method (3). Briefly, the 10 top-scoring siRNA predications were obtained using BIOPREDsi, and the siRNA was incorporated into the miR-30 backbone (4). The tandem/polycistronic shRNA vectors were cloned in two steps as described (5). Renilla luciferase shRNA was described before (5). The sequences of all shRNAs can be found in Table S4B. The pT3 transposon vector was a kind gift of Dr. Xin Chen, UCSF San Francisco. To generate the constitutive expression vector, pT3-EF1a, the CpG-free EF1a-promoter from pCpGfree-vitroBmcs (InvivoGen) was inserted into pT3 and an rtTA-miR30 fragment was cloned into pT3-EF1A. For generation of the inducible Transposon vector the TREtight promoter was cloned into pT3 and the mouse N-Ras ^{G12D}-IRES-GFP fragment was cloned into pT3-TREtight. The CMV-SB13 construct was a kind gift of Dr. Mark Kay, Stanford University.

Immunoassays

Immunoassays were performed as previously described (6). The following antibodies were used for IF and IHC: N-Ras (sc-31, Santa Cruz), Cd45 (BD Pharmingen, 550539), GFP (2956, Cell Signaling) and Mcp1 (R&D Systems, AF-479-SP). The following antibodies were used for immunoblotting: p53 (for human: Do-1, Oncogene and for mouse: p53-505, Leica Biosystems), p16 (H-156, Santa Cruz Biotechnologies), Ras (Ab-1, Calbiochem), p21 (C-19, Santa Cruz Biotechnologies), Brd4 (A301-985A, Bethyl Labs), beta-actin (ac-15, Sigma). Cytokine arrays were done using the Proteome Profiler Mouse Cytokine Array Kit, Panel A (ARY006, R&D Systems), following the manufacturer's recommendations.

ChIP-Seq, library construction and Illumina sequencing

ChIP-Seq was performed as previously described (7). Briefly, 5 to 30 million IMR90 cells were crosslinked for 10 minutes using 1 % formaldehyde, followed by quenching with 0.125M glycine for 10 minutes. Notably, to prepare chromatin from crosslinked-senescent cells, aliquots of less than 1 million cells were kept in individual eppendorf tubes for sonication to maximize the chromatin fragmentation efficiency. After purifying immunoprecipitated DNA, a TruSeq ChIP Sample Prep Kit (Illumina) was used to construct the ChIP-Seq library following the manufacturer's protocol except for amplifying the adaptor-ligated library for 15 cycles. ChIP-Seq libraries were sequenced using an Illumina HiSeq 2000 platform with single end reads of 50 bases. Four libraries at equal molarity were pooled to aim for a sequencing depth of 20-40 million aligned reads per sample.

Mapping of ChIP-Seq data

Raw reads were mapped to the reference human genome assembly GRCh37 (hg19) using Bowtie (http://bowtie-bio.sourceforge.net/index.shtml) allowing two-mismatches for only unique alignment; -v 2 -m 1 -best --strata. To avoid clonal artifacts introduced in the library amplification steps, duplicated mapped reads were further discarded using SAMtools (http://samtools.sourceforge.net).

Finding peaks from mapped reads and calculating tag density

To identify ChIP-Seq enriched regions (or peaks), HOMER suite was used (http://homer.salk.edu/homer/). For H3K27Ac and BRD4 ChIP-Seq, the parameter of findPeaks was set to find histone enriched regions (findPeaks.pl -style histone), and also used to identify H3K27Ac-enriched super-enhancers with the default setting (findPeaks.pl -style super). Since H3K27Ac and BRD4 enriched regions are broad in the genome, we avoided redundant detection from peak calling by merging multiple peaks into a single region when the distance between

centers was less than 5000-bp (mergePeaks.pl -d 5000). From given extended regions, the ChIP-Seq signal of tag density was re-calculated by normalizing to a read depth of 10 million tags at a given size of the merged region (annotatePeaks.pl -size given). To create a density plot, +/- 5,000-bp of ChIP-Seq regions were used to count tags with 20-bp bins (annotatePeaks.pl -size 5000 -hist 25 -ghist).

Gene Ontology (GO) analysis of ChIP-Seq region

GREAT tools were used to predict GO terms of ChIP-Seq enriched regions (http://bejerano.stanford.edu/great/public/html/index.php). Senescence-activated or -inactivated regions identified above were tested with default settings to define associated genes (basal plus extension; proximal 5,000-bp upstream and 1,000-bp downstream). From the results, GO terms from the GO consortium (http://geneontology.org) were plotted as bar graphs with binomial -log10 p-values. Importantly, as the confidence of ChIP-Seq enriched regions is critical for GO analysis, H3K27Ac tag counts greater than 40 in the senescent condition (4,440 highly confident enhancers from 6,555 senescence-activated typical enhancers) or in the proliferating condition (4,288 highly confident enhancers from 7,095 senescence-inactivated typical enhancers) were tested with GREAT tools. There was no further filtering for testing super-enhancers with GREAT, since all of the super-enhancers were highly enriched with H3K27Ac.

Gene Set Enrichment Analysis (GSEA) of ChIP-Seq peak associated genes

Gene set enrichment analyses were performed according to the instructions (http://www.broadinstitute.org/gsea/index.jsp). To generate custom gene sets from ChIP-Seq data, genes with the closest TSS from each ChIP-Seq peak were assigned as peak-associated genes.

Motif enrichment analysis of 37 BRD4-gained enhancers

Motif enrichment was identified with 1,000-bp window centered on 37 BRD4-associated enhancers using the HOMER suite (findMotfisGenome.pl -size 1000).

Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)-based expression analysis and high throughput RNA sequencing (RNA-Seq)

Total RNA was isolated using the RNeasy minikit (Qiagen), and cDNA was obtained using the TaqMan reverse transcription reagents (Applied Biosystems). qRT-PCR data analyses were performed as previously described (2). Gene-specific primer sets for mouse and human sequences were designed using NCBI's qPrimerDepot (http://primerdepot.nci.nih.gov) or described elsewhere (Supplementary Table S4A). β2-microglobulin or β-actin served as endogenous normalization controls. qRT-PCR was carried out in triplicate using SYBR Green PCR Master Mix (Applied Biosystems) on the ViiA 7 Real-Time PCR System (Life technologies). For high throughput RNA sequencing, total RNA from three independent experiments was extracted using an RNeasy minikit (Qiagen). Cells transduced with empty vector or with pWZL-Hygro (H-Ras^{V12}) and the indicated shRNAs were collected 12 days post-infection. Quiescent cells were generated by serum starvation (following 4 days in DMEM 0.1% FBS) as performed for the H3K27Ac and BRD4 Chip-sequencing analyses. RNA-Seq libraries construction and sequencing were performed at the integrated genomics operation (IGO) Core at MSKCC according to standard protocols. Poly-A selection was performed. For sequencing approximately 10 million 50bp paired-end reads were acquired per replicate condition. Resulting RNA-Seq data was analyzed by removing adaptor sequences using Trimmomatic (8). RNA-seq reads were then aligned to GRCh37.75(hg19) with STAR (9) and genome-wide transcript counting was performed by HTSeq (10) to generate a matrix of fragments per kilobase of exon per million fragments mapped (RPKM).

Clustering and correlation analysis of RNA-Seq data

Gene expressions of RNA-Seq data were clustered using hierarchical clustering based on one minus pearson correlation test. Principal component analysis was performed using the R/Bioconductor package. For pathway enrichment analysis, the weighted GSEA Preranked mode was used a set of curated signatures in the molecular signatures database (MSigDB v5.0) (http://www.broadinstitute.org/gsea/msigdb/index.jsp). From 10,348 signatures, signatures with 15-500 genes were only considered for the further analyses. From the results, enriched signatures with a false discovery rate (FDR) less than 0.25 were considered as statistically significant.

Statistical analyses Pooled data is presented as means ± standard deviation (SD) values of duplicate or triplicate biological replicates, as indicated in corresponding figure legends. Asterisks (*) indicate statistically significant differences compared to S/shRen (for shRNA samples) or S/veh (for JQ1), as determined by an unpaired Student's T-test. In figures, "*" stands for $p < 0.05$, "**" for $p < 0.01$, and "***" for $p < 0.001$.

Supplementary References

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Supplementary Figures

Supplementary Figure S1. Senescence-activated SASP enhancers are marked by H3K4Me1 in proliferating IMR90 cells. **(**A) Genomic regions for *IL1A/B*, *IL8* and *MMP8* genes, showing that

H3K27Ac-gained-enhancers in senescence are marked by H3K4Me1 in proliferating cells. (B) Pie charts showing percentages of H3K4Me1-occupied regions in proliferating cells, in senescence-activated H3K27Ac TEs (left panels) and SEs (right panel).

Supplementary Figure S2. Reproducibility analyses of biological replicates for H3K27Ac and BRD4 ChIP-Seq. (A) Genome-wide correlation plots for ChIP-Seq signal at individual H3K27Ac (top panel) and BRD4 (bottom panel) peaks between two biological replicates. Correlation was tested by Cistrome tool box with normalized bigWig tracks of each sample. Pearson correlation coefficients are indicated at the upper right quadrant of each plot.

(B) H3K27Ac (top panels) and BRD4 ChIP-Seq (bottom panels) occupancy profiles with biological replicates. *IL1A/B* (left panels) and *FOSL2* (right panels) are shown as representative loci for senescence-activated and senescence-inactivated SEs respectively. Data is shown for two independent biological replicates (Rep).

(C) Table for the total number of raw read counts, number of reads successfully aligned to the reference genome (hg19) and percentage of mapped reads for the H3K27Ac (top) and BRD4 (bottom) ChIP-Seq replicates. Numbers of aligned and unaligned reads were counted by Samtools flagstat command (http://samtools.sourceforge.net).

Supplementary Figure S3. Enhancer remodeling during oncogene-induced senescence parallels gene expression changes.

(A-D) Gene Set Enrichment Analyses (GSEA) of each enhancer-signature with proliferating and quiescent conditions, as described in Figs. 2B-2E for the proliferating vs. senescent comparison.

(E) Tables showing representative genes from the indicated GO categories associated with TEs that are significantly activated (top panel) or inactivated (bottom panel) in senescent versus proliferating IMR90 cells.

(F, G) GSEA plot for the publically available E2F target gene set

(HALLMARK_E2F_TARGETS, M5925 from MSigDB) in proliferating versus quiescent (F), or proliferating vs senescent (G) IMR90 cells.

(H, I) Box plots of H3K27Ac signals of each condition relative to proliferating for E2F targets-associated TEs (H) and SEs (I). Fold changes with log2 scale were calculated by dividing H3K27Ac tag counts from quiescent (blue) and senescent (red) conditions to proliferating condition. Significance of differences was determined using a two-tailed t test.

(J) Area-proportional Venn diagrams showing the overlap between genes associated with TEs (top panels) or SEs (bottom panels) that show significant activation (left panels) or inactivation (right panels) in senescent versus proliferating IMR90 cells (red), or in quiescent versus proliferating IMR90 cells (blue).

(K) Tables showing representative genes from the indicated GO categories associated with SEs that are significantly activated (top panel) or inactivated (bottom panel) in senescent versus proliferating IMR90 cells.

Supplementary Figure S4. Genetic and pharmacologic inhibition of BRD4 impairs SASP gene expression during oncogene-induced senescence (OIS).

(A) Table showing GO categories and representative genes associated with BRD4-gained SEs in senescence.

(B) Heat map showing the relative expression of BRD4 signature GO category genes (see Fig. 4C) in proliferating or senescent IMR90 cells expressing shRNAs against BRD4 or Renilla (Ren) (left panels), or treated with JQ1 or vehicle (DMSO) (right panels).

(C, D) qRT-PCR analyses of the indicated SASP factors in proliferating (P) or senescent (S) IMR90 fibroblasts. IL6, CXCL1 and MMP10 are shown as examples of BRD4 and p65 commonly regulated genes, while VEGFA and VEGFC are regulated by BRD4 only (extension of Figs. 4F and 4G). Data are presented as means ± SD of two independent experiments.

(E, F) qRT-PCR analyses of the indicated SASP factors in proliferating (P) or senescent (S)_BJ

foreskin fibroblasts (E) or WI38 lung fibroblasts (F) expressing the indicated shRNAs, or treated with JQ1 or vehicle (veh). Senescence was induced by H-Ras^{V12} expression. Data are presented as means ± SD of two independent experiments.

Supplementary Figure S5. BRD4 couples enhancer remodeling to SASP gene expression during etoposide-induced senescence.

(A) Genome-wide correlation between oncogene- and etoposide-induced senescence. Fold enrichments of H3K27Ac occupancy were calculated by comparing the tag counts in OIS or etoposide conditions to proliferating or DMSO-treated controls, respectively. Pearson correlation was used to test the strength of the correlation.

(B) Representative H3K27Ac profiles for the *IL8* genomic region in OIS and etoposide induced-senescence. The OIS *IL8* H3K27Ac mark plot is data partly from Fig. 3G.

(C) qRT-PCR analyses of BRD4, IL6, CXCL1 and BMP2 expression in proliferating (P) and etoposide-induced senescent (S) cells expressing control shRNA (shRen) or two independent BRD4 hairpins (shBRD4-1 and -2) or treated with JQ1 or vehicle (veh). Data are presented as means ± SD of two independent experiments.

Supplementary Figure S6. Systemic and cell-autonomous suppression of Brd4 impairs the immune surveillance of senescent hepatocytes.

(A) SA-β-Gal staining on frozen tissue sections of livers harvested at day 12 from mice injected with transposon-based vectors encoding N-Ras^{G12D}-IRES-GFP and a control shRNA and treated with vehicle (top panel) or iBET (bottom panel).

(B) Representative co-IF micrograph showing foci of N-Ras^{G12D}-IRES-GFP-expressing hepatocytes (visualized by GFP IF, green) being differentially targeted by immune cells (visualized by Cd45 IF, red) in livers from mice treated with vehicle (top panels) compared to iBET (bottom panels), at day 12 post-transduction. In merge image, nuclei are counterstained

with DAPI.

(C) SA-β-gal staining on frozen tissue sections of livers harvested at day 6 (top panels) and day 12 (bottom panels) post-transduction from animals injected with transposon-based vectors encoding N-Ras^{G12D}-IRES-GFP and the indicated shRNAs.

Supplementary Table S1. Lists of senescence-activated and senescence-inactivated TEs and SEs. (A) Senescence-activated TEs. (B) Senescence-inactivated TEs. (C) Senescence-activated SEs. (B) Senescence-inactivated SEs.

Supplementary Table S2. Lists of BRD4-gained TEs and SEs. (A) BRD4-gained TEs. (B) BRD4-gained SEs.

Supplementary Table S3. Lists of gene signatures from RNA-Seq analysis. (A) Senescence-induced genes. (B) Senescence-repressed genes. (C) Senescence signature genes (top 100 induced + repressed genes). (D) BRD4 signature genes. (E) p65 signature genes. (F) JQ1 signature genes. (G) BRD4 and p65 gene regulation comparison.

Supplementary Table S4. Sequences of shRNAs and qRT-PCR primers. (A) Sequences of qRT-PCR primers. (B) Sequences of shRNAs.

Supplementary Movie S1. Co-culture of NK cells and P/shRen IMR90 cells.

Proliferating IMR90 cells expressing control shRNA (green) are not substantially targeted by NK cells (red).

Supplementary Movie S2. Co-culture of NK cells and S/shRen IMR90 cells.

Senescent IMR90 cells expressing control shRNA (green) are substantially targeted by NK cells (red).

Supplementary Movie S3. Co-culture of NK cells and S/shRen IMR90 cells.

Senescent IMR90 cells expressing BRD4 shRNA (green) are not substantially targeted by NK cells (red).