Aird et al., https://doi.org/10.1083/jcb.201608026

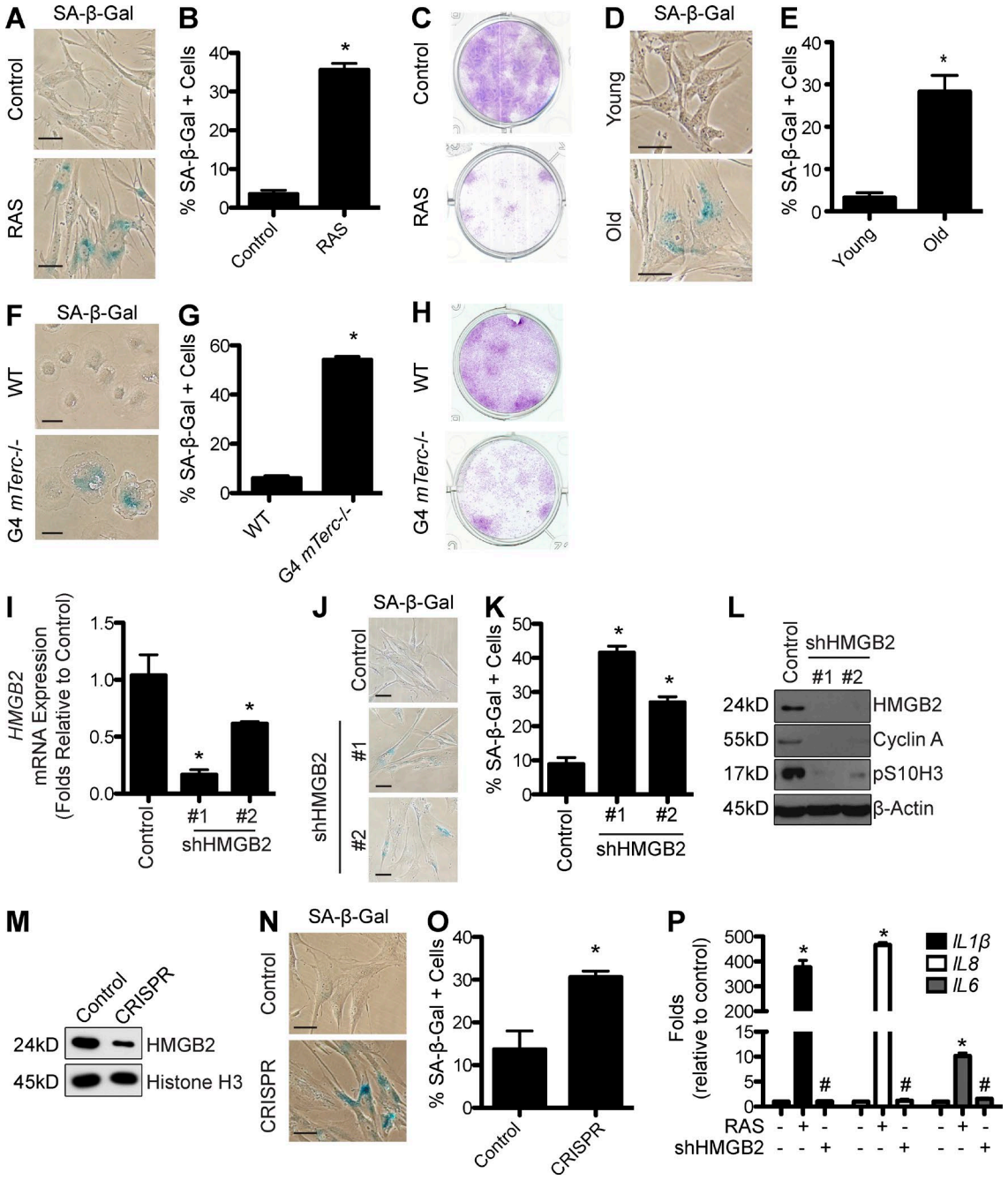

Figure S1. **HMGB2 expression is altered during senescence.** Related to Fig. 1. (A) IMR90 cells were infected with retrovirus encoding oncogenic RAS or control. Cells were selected for 3 d with 1 µg/ml puromycin. 4 d later, cells were stained for SA-β-Gal activity. (B) Quantification of A. (C) Same as A, but an equal number of cells was seeded into 6-well plates, and colonies were stained with crystal violet 14 d later. (D) Young (PD24) and old senescent (PD60) IMR90 fibroblasts were stained for SA-β-Gal activity. (E) Quantification of D. (F) Wild-type (WT) and G4 *mTerc*$^{-/-}$ mouse ear fibroblasts were stained for SA-β-Gal activity. (G) Quantification of F. (H) Same as F, but an equal number of cells was seeded into 6-well plates, and colonies were stained with crystal violet 14 d later. (I) IMR90 cells were infected with lentivirus encoding two individual shHMGB2s or control. Cells were selected for 3 d with 1 µg/ml puromycin. 4 d later, *HMGB2* mRNA expression was determined. *B2M* was used as an internal control. (J) Same as I, but cells were stained for SA-β-Gal activity. (K) Quantification of J. (L) Same as I, but HMGB2, cyclin A, and pS10H3 protein expression was determined by Western blotting. β-Actin was used as a loading control. (M) IMR90 cells were infected with lentivirus expressing Cas9 and an HMGB2 gRNA or control. Cells were selected for 3 d with 1 µg/ml puromycin. 4 d later, HMGB2 protein expression was determined by Western blotting. Histone H3 was used as a loading control. (N) Same as M, but cells were stained for SA-β-Gal activity. Bars, 5 µm. (O) Quantification of N. (P) *IL1β*, *IL6*, and *IL8* mRNA expression was determined in RAS-expressing cells and compared with shHMGB2 cells. *B2M* was used as an internal control. Graphs shown are the mean and SEM of triplicates from a representative experiment that was independently repeated at least three times. *, $P < 0.05$ versus control; #, $P < 0.05$ versus RAS alone.

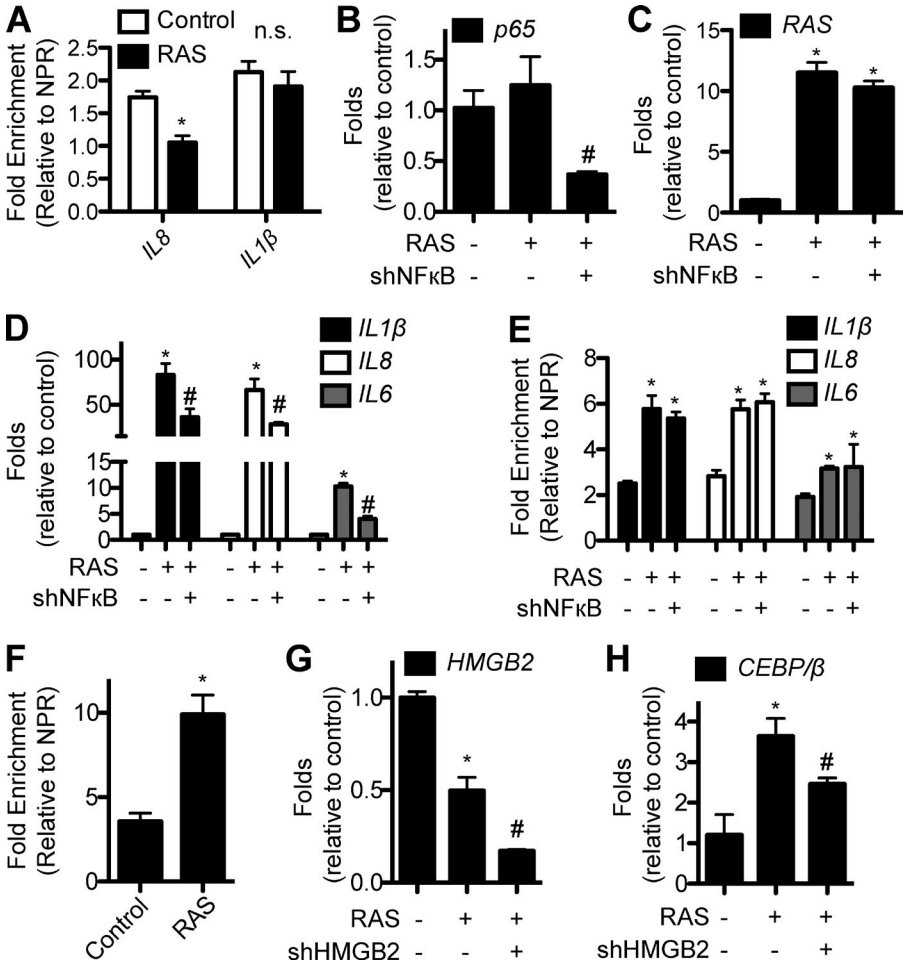

Figure S2. **HMGB1 does not bind to SASP gene loci; HMGB2 binding is independent of NF-κB and C/EBP-β.** Related to Fig. 2. (A) IMR90 cells were infected with retrovirus encoding oncogenic RAS or control. Cells were selected for 3 d with 1 µg/ml puromycin, and 4 d later HMGB1 ChIP was performed. HMGB1 binding to *IL1β* and *IL8* was determined by quantitative PCR and normalized to a nonpeak region (NPR) control. Note that HMGB1 binding is not enriched at SASP gene loci. n.s., not significant. (B) Normal diploid IMR90 cells were infected with retrovirus encoding oncogenic RAS alone or in combination with a lentivirus expressing an shRNA to the p65 subunit of NF-κB (shNF-κB). Cells were selected for 3 d with 3 µg/ml puromycin. 4 d later, total mRNA was isolated, and *p65* mRNA expression was determined. (C) Same as B, but *RAS* mRNA expression was determined. (D) Same as B, but *IL1β*, *IL8*, and *IL6* mRNA expression was determined. (E) Same as B, but HMGB2 ChIP was performed, and HMGB2 binding to *IL1β*, *IL8*, and *IL6* was determined by quantitative PCR and normalized to a nonpeak region control. (F) Same as A, but HMGB2 ChIP was performed, and HMGB2 binding to *C/EBP-β* was determined by quantitative PCR and normalized to a nonpeak region control. (G) Normal diploid IMR90 cells were infected with retrovirus encoding oncogenic RAS alone or in combination with a lentivirus expressing an shHMGB2. Cells were selected for 3 d with 3 µg/ml puromycin. 4 d later, total mRNA was isolated, and *HMGB2* mRNA expression was determined. (H) Same as G, but *C/EBP-β* mRNA expression was determined. (B–D, G, and H) *B2M* was used as an internal control. For all panels, graphs shown are the mean and SEM of triplicates from a representative experiment that was independently repeated at least three times. *, $P < 0.05$ versus control; #, $P < 0.05$ versus RAS alone.

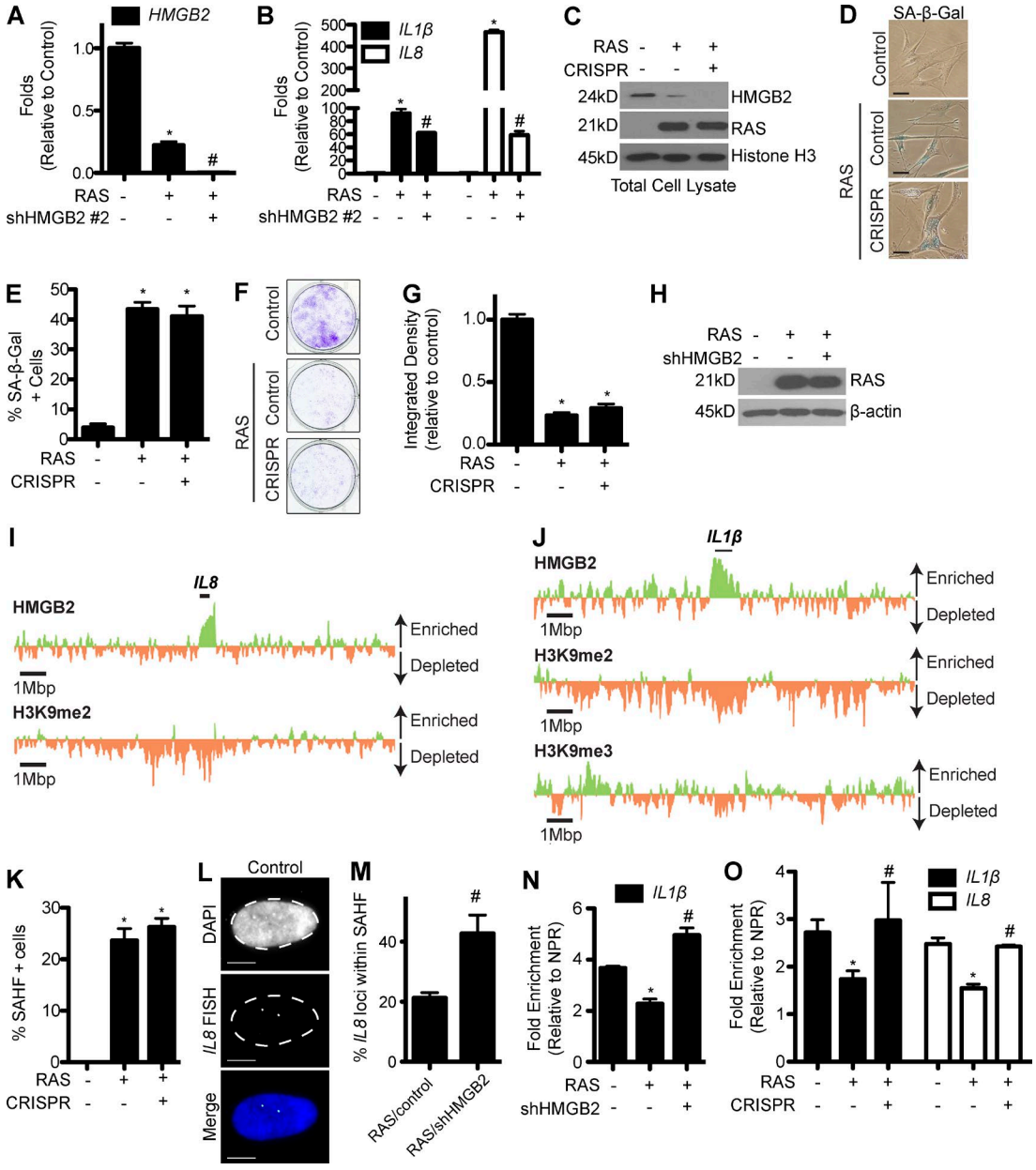

Figure S3. **Loss of HMGB2 blunts SASP gene expression while maintaining the senescence-associated cell cycle exit, and loss of HMGB2 allows for spreading of heterochromatin.** Related to Figs. 3 and 4. (A) IMR90 cells were infected with retrovirus encoding oncogenic RAS alone or in combination with a lentivirus encoding shHMGB2 (#2) or control. Cells were selected for 3 d with 3 µg/ml puromycin. 4 d later, *HMGB2* mRNA expression was determined. (B) Same as A, but *IL1β* and *IL8* mRNA expression was determined. (A and B) *B2M* was used as an internal control. (C) IMR90 cells were infected with retrovirus encoding oncogenic RAS alone or in combination with a Cas9-expressing lentivirus expressing a gRNA to HMGB2. Cells were selected for 3 d with 3 µg/ml puromycin. 4 d later, total protein was isolated, and HMGB2 and RAS protein expression was determined by immunoblotting. Histone H3 was used as a loading control. (D) Same as C, but cells were stained for SA-β-Gal activity. (E) Quantification of D. (F) Same as C, but an equal number of cells was seeded into 6-well plates, and colonies were stained with crystal violet 14 d later. (G) Quantification of F. (H) IMR90 cells were infected with retrovirus encoding oncogenic RAS alone or in combination with a lentivirus expressing a shHMGB2 (#1). Cells were selected for 3 d with 3 µg/ml puromycin. 4 d later, total protein was isolated, and RAS protein expression was determined by immunoblotting. β-Actin was used as a loading control. (I) Cross-referencing of HMGB2 ChIP-Seq data and H3K9me2 ChIP-Seq data at the *IL8* locus. Green indicates enrichment of HMGB2 or H3K9me2 binding in senescent cells, whereas orange indicates depletion of HMGB2 or H3K9me2 binding in senescent cells. (J) Same as I, but enrichment or depletion of HMGB2, H3K9me2, or H3K9me3 at the *IL1β* gene locus. (K) IMR90 cells were infected with retrovirus encoding oncogenic RAS alone or in combination with a Cas9-expressing lentivirus expressing a gRNA to HMGB2. Cells were selected for 3 d with 3 µg/ml puromycin. 4 d later, cells were stained for SAHF using DAPI, and the number of SAHF-positive cells was quantified. (L) FISH was performed with a BAC containing the *IL8* gene locus in control IMR90 cells. The dashed white line indicates the nucleus. Bars, 5 µm. (M) The percentage of *IL8* gene loci that were fully within SAHF (distance of 0 from gene locus to SAHF) was calculated. (N) IMR90 cells were infected with retrovirus encoding oncogenic RAS alone or in combination with a lentivirus expressing an shHMGB2. Cells were selected for 3 d with 3 µg/ml puromycin. 4 d later, H3K9me2 ChIP was performed, and H3K9me2 binding to *IL1β* was determined by quantitative PCR and normalized to a nonpeak region (NPR) control. (O) Same as N, but using HMGB2 CRISPR cells. H3K9me2 ChIP was performed, and H3K9me2 binding to *IL1β* and *IL8* was determined by quantitative PCR and normalized to a nonpeak region of control. For all panels, graphs shown are the mean and SEM of triplicates from a representative experiment that was independently repeated at least three times. *, $P < 0.05$ versus control; #, $P < 0.05$ versus RAS alone.

Table S1. **Primers used for qRT-PCR**

Gene	Forward primer (5′–3′)	Reverse primer (5′–3′)
p65	CCAGACCAACAACAACCCCT	TTGGGGGCACGATTGTCAAA
RAS	CCGTTTGATCTGCTCCCTGT	AGCAGGTGGTCATTGATGGG
C/EBP-β	GCACAGCGACGAGTACAAGA	TTGAACAAGTTCCGCAGGGT
B2M	GTGCTCGCGCTACTCTCTCT	TCTCTGCTGGATGACGTGAG
Mouse *HMGB2*	CGCGGAGAACTCTGCAAAAC	GTCTGTCTACCTGCTGCGAG
Mouse *B2M*	AGTTAAGCATGCCAGTATGGCCGA	ACATTGCTATTTCTTTCTGCGTGC
Mouse *IL6*	GACAAAGCCAGAGTCCTTCAGAGAG	CTAGGTTTGCCGAGTAGATCTC
Mouse *IL1β*	CAACCAACAAGTGATATTCTCCATG	GATCCACACTCTCCAGCTGCA

Table S2. **Primers used for ChIP quantitative PCR**

Gene	Forward primer (5′–3′)	Reverse primer (5′–3′)
Nonpeak region	TGCCCTTGGCACAGATTCTT	GGGGTGAGGTCAAGGTCAAG
IL6	TGCAATAACCACCCCTGACC	AGCTGCGCAGAATGAGATGA
IL1β	ACTACCAGTCCTGACTCCCT	GCCACCGAAGACTATCCTCC
IL8	GTGTCCTCCACAGAATGTTGG	TCCAGCTATGCTAAAGCGCA
C/EBP-β	GGGAGAAAGCCGGTCATGTT	ACAGTGTCAGTGTGGAACCG
Mouse nonpeak region	TAGCCAATGGCACACCTGAG	GCAGTGAATGCTGCTTCCTT
Mouse *IL6*	GCTCCAAAAGATAAAAGCGCCA	GAGGGACCCATATGCTAGTTGG
Mouse *IL1β*	ACATTCGGCCATGAATTCCTTTGG	GAAAAAGCAAGTGCGCCACC

Table S3 is an Excel spreadsheet showing chromatin regulatory genes that are significantly altered in all four GEO datasets.

Table S4 is an Excel spreadsheet showing enrichment analysis of genes regulated by HMGB2 in senescent cells.