

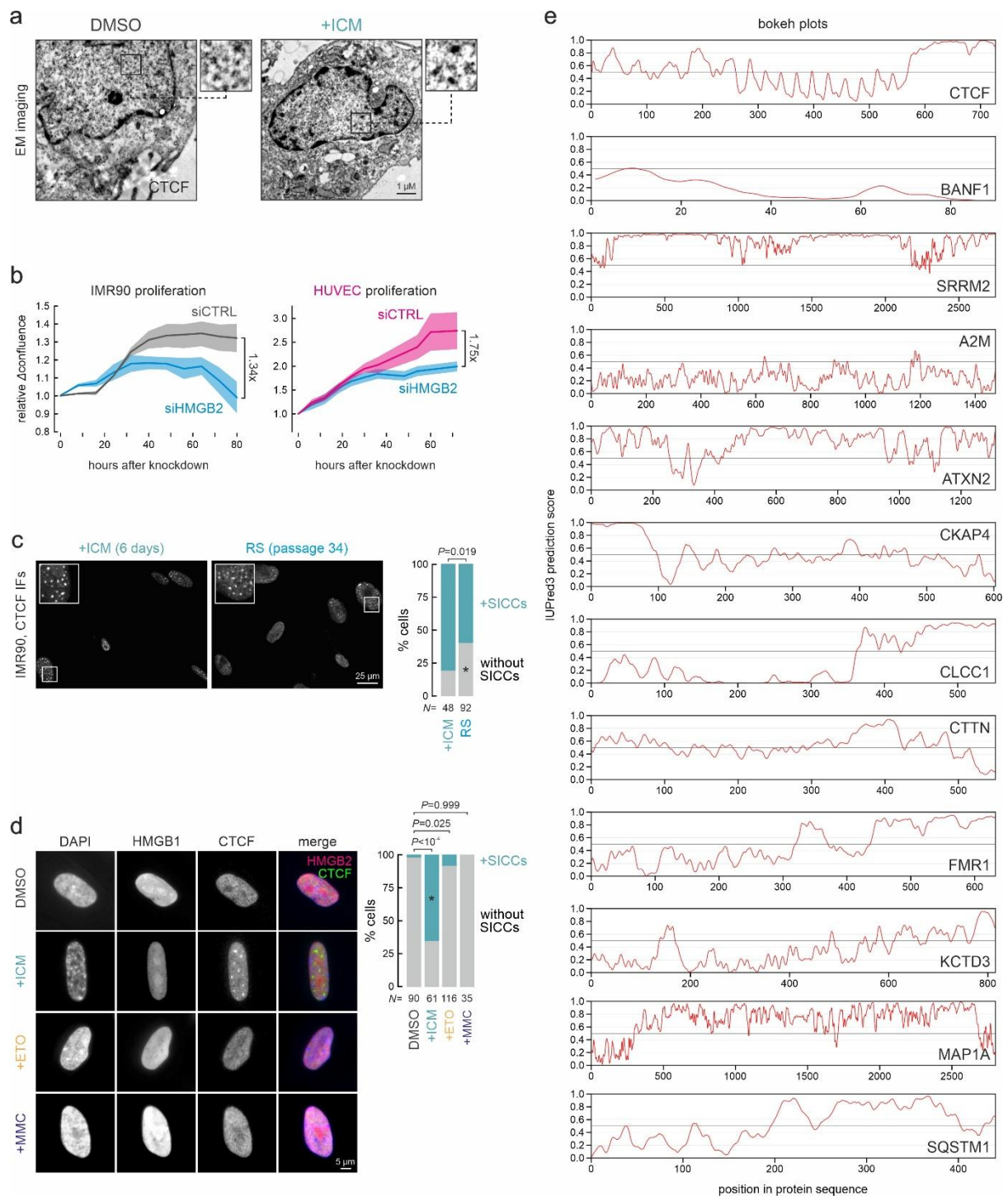
Senescent cells cluster CTCF on nuclear speckles to instruct an alternative splicing program

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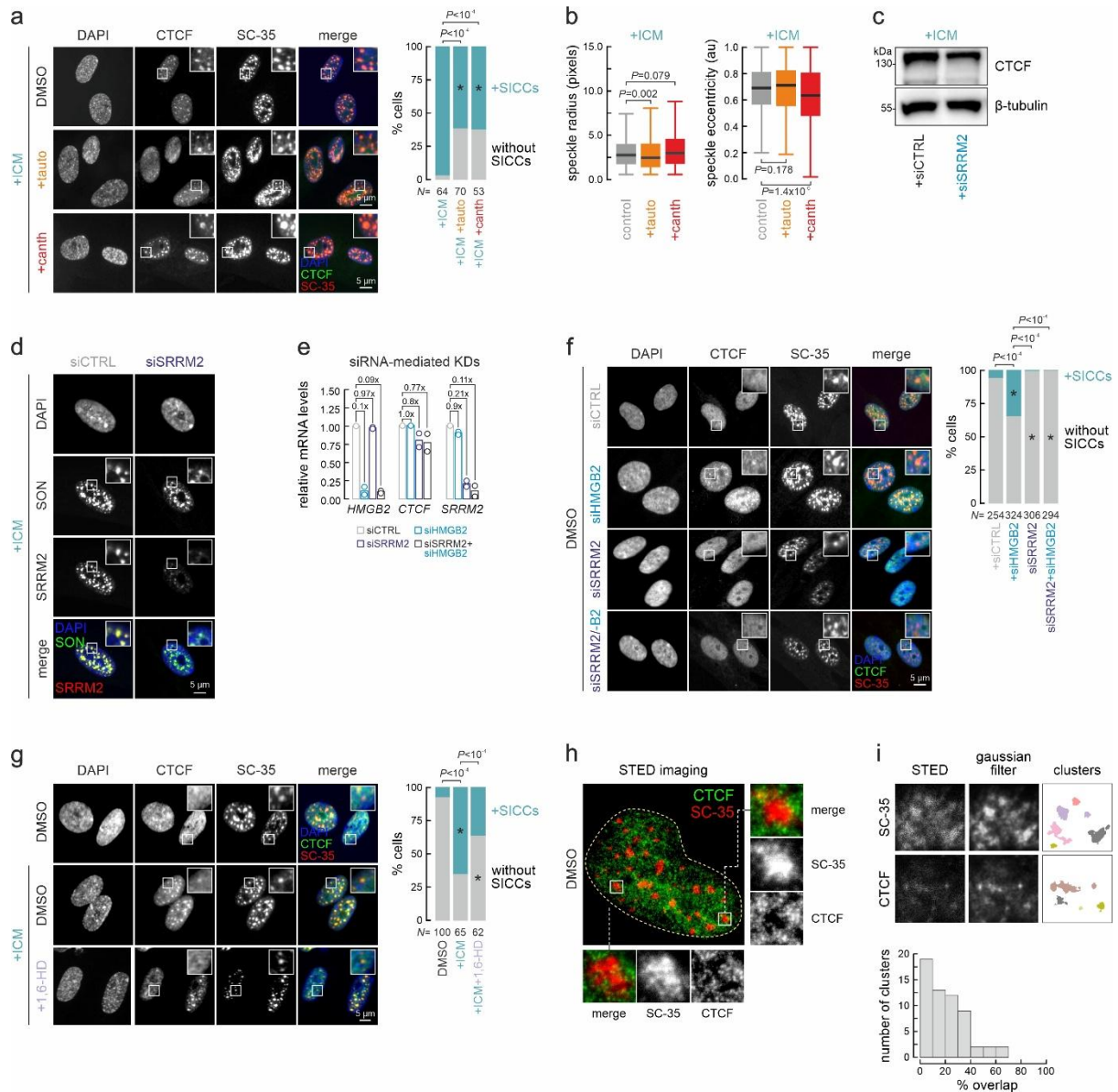
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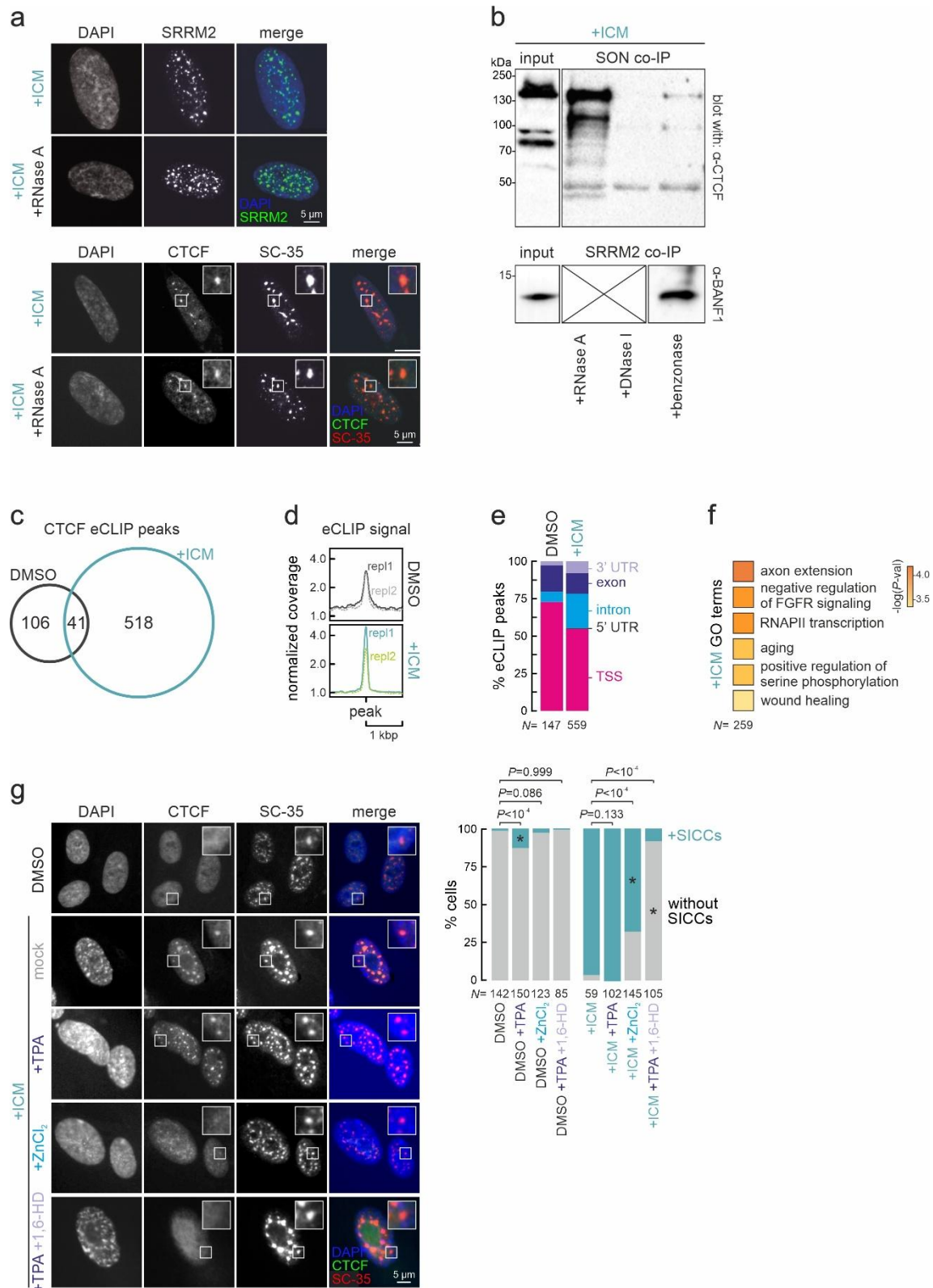
Supplementary Fig 1 | SICC formation and CTCF protein interactors in senescence. **a**, Electron microscopy images from DMSO- and ICM-treated IMR90 immunostained for CTCF. Insets: CTCF clustering in senescence. **b**, Plot showing changes in confluence (mean $\pm$ SEM) of IMR90 (left) or HUVECs (right) treated with non-targeting (siCTRL) or *HMGB2*-targeting siRNAs (siHMGB2; relative to the 0-hour time point) from three replicates. **c**, Left: Representative image (from two independent experiments) of 6-day ICM-treated or replicatively senescent IMR90 (passage 34) immunostained for CTCF. Right: Bar plots of the percent of cells displaying SICC; *N* is the number of cells quantified per condition. *P*-values were deduced via a two-sided Fisher's exact test. **d**, Left: Widefield images (from two independent experiments) of proliferating (DMSO), senescent (+ICM), etoposide- (+ETO; 1 μ M for 24 h) and mitomycin-treated IMR90 (+MMC; 0.1 μ M for 2 h) immunostained for HMGB2 and CTCF and counterstained by DAPI. Right: Plots of the percent of cells displaying SICC; *N* is the number of cells quantified. *P*-values were deduced via two-sided Fisher's exact

tests. **e**, Line plots showing the predicted IUPred3 disorder score along CTCF and eleven of its interactors from co-IP mass spectrometry data.



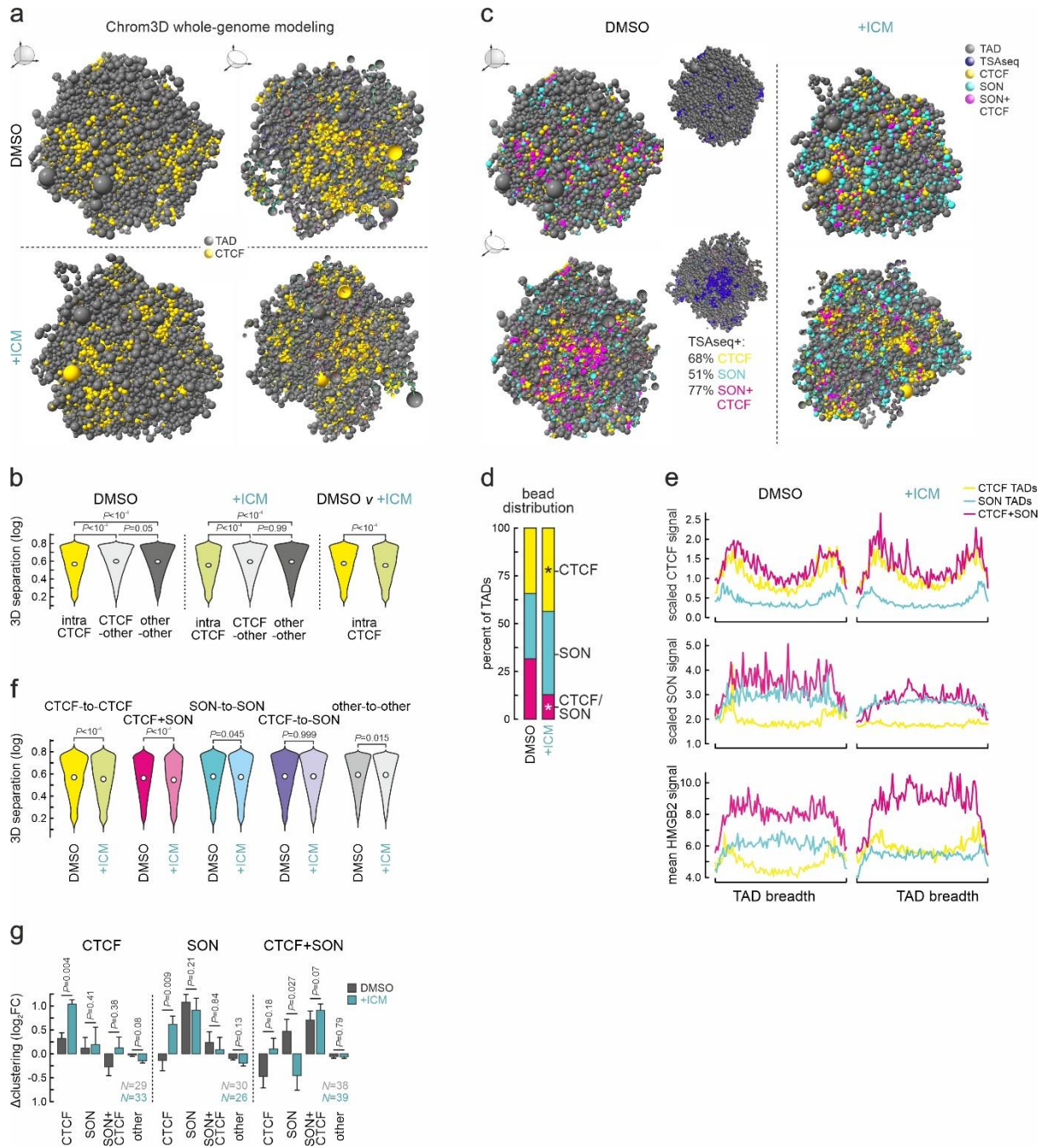
Supplementary Fig 2 | Properties of SICCs in respect to nuclear speckles. **a**, Left: Representative images (from two independent experiments with similar results) of senescent IMR90 (+ICM) immunostained for CTCF and SC-35, and counterstained by DAPI following treatment or not with tautomycin or cantharidin to induce morphological changes to nuclear speckles. Insets: Characteristic nuclear CTCF and SC-35 signal foci. Right: Plots showing the percentage of cells displaying SICCs; N is the number of cells in each condition. P -values were deduced via two-sided Fisher's exact tests. **b**, Box plots (lines show medians; box limits indicate the 25th and 75th percentiles; whiskers extend 1.5x the 25th-75th interquartile range) showing changes in speckle radius (left) and eccentricity (right) upon tautomycin (orange) or cantharidin treatment (red). P -values were deduced via two-sided Mann-Whitney U-tests. **c**, Western blot of CTCF levels in senescent IMR90 (+ICM) treated or not with *SRRM2*-targeting siRNAs; β -tubulin levels provide a loading control (from two independent experiments with similar results). **d**, Representative images (from two independent experiments with similar results) of senescent IMR90 (+ICM) immunostained for SRRM2 and SON, and counterstained by DAPI 48 h after transfection with non-targeting (siCTRL) or *SRRM2* siRNAs (siSRRM2). **e**, Plots showing relative changes in mRNA levels (mean $\pm$ SD from two independent replicates) upon *HMGB2*- and/or *SRRM2*-knockdown in proliferating IMR90 (DMSO) normalized to *HSC70* controls. **f**, Left: Representative images (from two independent experiments with similar results) of proliferating IMR90 (DMSO) immunostained for CTCF and SC-35, and counterstained by DAPI at 48 h after transfection with non-targeting (siCTRL), *HMGB2*- (siHMGB2), *SRRM2*- (siSRRM2) or *HMGB2*- plus *SRRM2*- targeting siRNAs

(siSRRM2/-B2). Characteristic nuclear CTCF and SC-35 signal foci are shown magnified (insets). Right: Bar plots showing the percent of cells displaying SICCs. *N*, the number of cells quantified in each condition; *P*-values were deduced via two-sided Fisher's exact tests. **g**, As in panel f, but for proliferating (DMSO) and senescent IMR90 (+ICM) treated or not with 6% 1,6-hexanediol for 1 min (+1,6-HD). *P*-values were deduced via two-sided Fisher's exact tests. **h**, Super-resolution imaging of a proliferating (DMSO-treated) IMR90 nucleus stained for CTCF and SC-35. Exemplary regions around nuclear speckles (white rectangles) are shown magnified. **i**, Colocalization analysis of nuclear speckles overlapping SICCs in gSTED images from senescent IMR90. A gaussian filter ($\sigma=1$ pixel) is applied to raw SC-35 and CTCF images to deduce clusters before overlap is calculated (top). The plot (below) shows the number of clusters overlapping to different extents.



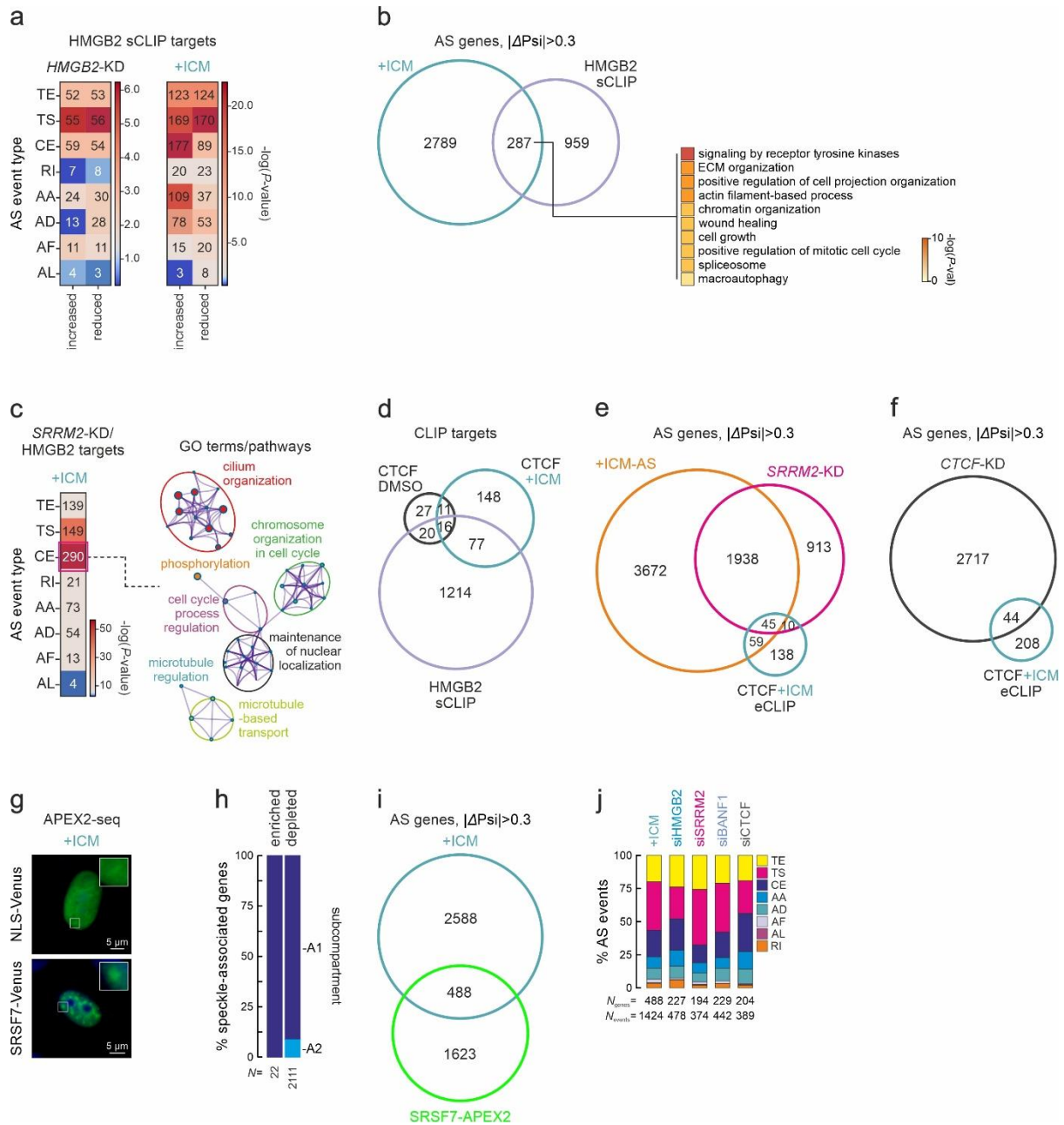
Supplementary Fig 3 | SICCs are resistant to RNA degradation and Zn^{2+} chelation. **a**, Widefield images of senescent IMR90 (+ICM) treated or not with RNase A for 20 min and immunostained for SRRM2 (top) or for CTCF and SC-35 (bottom) and counterstained by DAPI. Insets: magnified CTCF and SC-35 foci. **b**, Western blot analysis of SON (top row) and SRRM2 co-IP experiments (bottom row) in senescent IMR90 performed using benzonase, (RNase-free) DNase I or RNase A on lysates before blotting for CTCF or BANF1. Input lanes provide a control; from two independent experiments with similar results. **c**, Venn diagram of CTCF eCLIP peaks from

proliferating (DMSO) and senescent IMR90 (+ICM). **d**, Mean eCLIP signal from two biological replicates in the 2 kbp around proliferating or senescent peaks. **e**, Plots showing CTCF eCLIP peak distribution from DMSO- and ICM-treated IMR90. **f**, GO term enrichment for the 259 CTCF-bound mRNAs in senescence. **g**, Left: As in panel a, but for proliferating (DMSO) and senescent IMR90 (+ICM) treated with 50 μ M of a zinc-chelating agent (+TPA), with 30 μ M ZnCl₂ or with TPA plus 1,6-hexanediol. Characteristic nuclear CTCF and SC-35 signal foci are shown magnified (insets). Right: Plots showing percent of cells displaying SICCs. *N*, the number of cells quantified; *P*-values were deduced via two-sided Fisher's exact tests.



Supplementary Fig 4 | Chrom3D modeling of 3D chromatin reorganization in senescence. **a**, Chrom3D diploid genome models from proliferating (DMSO) and senescent IMR90 (+ICM) showing TADs (beads) that carry the top 20% of CTCF signal (yellow). **b**, Bottom: Violin plots (circles show medians; plots extend 1.5x the 25th-75th interquartile range) showing mean pairwise 3D distances of indicated beads. *P*-values were deduced via two-sided Mann-Whitney U-tests. **c**, As in panel a, but showing TADs (beads) that carry the top 20% of CTCF (yellow), SON (light blue), or CTCF+SON signal (magenta). Insets: DMSO models with TADs carrying the top 20% of TSA-seq signal (blue) and their per cent overlap to other beads (bottom). **d**, Plots showing the percent of TADs in the models from panel c marked by top 20% CTCF (yellow), SON (light blue) or CTCF+SON signal (magenta). **P*<10⁻⁴, two-sided Fisher's exact tests. **e**, Plots showing scaled CTCF, SON or HMGB2 occupancy within CTCF- (yellow), SON- (light blue) and CTCF+SON-enriched TADs (magenta) in the proliferating (DMSO) and senescent Chrom3D models (+ICM) from panel c. **f**, Violin plots (circles show medians; plots extend 1.5x the 25th-75th interquartile range) showing mean pairwise 3D distances among the indicated groups in the models from panel c. *P*-values were deduced via two-sided Mann-Whitney U-tests. **g**, Plots quantifying clustering ($\pm$ SEM) of CTCF, SON or CTCF+SON TADs relative to randomized controls

in models from DMSO- or ICM-treated IMR90. N , number of clusters in each case; P -values were deduced via unpaired two-tailed Student's t -tests.



Supplementary Fig 5 | HMGB2 and CTCF mRNA targets alternatively spliced in senescence. **a**, Heat map showing the number and relative enrichment [$-\log(P\text{-value})$] of AS events from HMGB2 target-mRNAs in *HMGB2*-KD and ICM-treated IMR90; $-\log(P\text{-value})$ calculated by a two-sided Fisher's exact test. **b**, Left: Venn diagram showing the overlap of AS genes in senescence (+ICM) with HMGB2 sCLIP targets in proliferating IMR90. Right: The top 10 GO terms enriched for the 287 shared targets. **c**, Left: As in panel a, but for *SRRM2*-KD AS genes bound by HMGB2. Right: GO term analysis of genes undergoing core exon (CE) skipping; $-\log(P\text{-value})$ calculated by a two-sided Fisher's exact test. **d**, Venn diagram of the overlap of CTCF- and HMGB2-bound mRNAs from CLIP data. **e**, As in panel d, but for the overlap of AS genes in senescence (+ICM) and *SRRM2*-knockdown (KD) with CTCF eCLIP targets in ICM-treated IMR90. **f**, As in panel e, but for AS genes upon *CTCF*-knockdown. **g**, Widefield images from ICM-treated IMR90 expressing NLS-GFP (top) or SRSF7-GFP fusions (bottom) from two independent experiments with similar results. **h**, Plot showing the percent of speckle-enriched or-depleted genes in A1/A2 subcompartments. **i**, Venn diagram showing the overlap of speckle-depleted mRNAs with AS genes in senescence (+ICM). **j**, Plot showing the percent of AS event types linked to speckle-depleted mRNAs from APEX2-seq data. The numbers of genes (N_{genes}) and AS events (N_{events}) for each dataset are shown.

Supplementary Table 1 | List of qPCR primers used in this study.

Target gene	Forward primer (5'-3')	Reverse primer (5'-3')
<i>HSC70</i>	TTA TTG GAG CCA GGC CTA CAC	GCG ACA TAG CTT GGA GTG GT
<i>ATXN2</i>	CCC TTC AGT ACA AGC CCA CC	GCA GTA GAA GGG AGG AGG GA
<i>CTCF</i>	CTCF CAG AGG TTA ATG CAG AGA AAG TG	AAT GCC ATG CCA CAG AGA TG
<i>FMR1</i>	TGT CTC TGG GAC TTT CTG CAA	TCC TGA ATC AGC TTT CCA TTT T
<i>SRRM2</i>	TAC GAA ACA GCC TAG CAG CC	GGC TAG GTC GAG TTG CAG ATT
<i>BANF1</i>	TCC CAA AAG CAC CGA GAC TTC	ACT GGC CAA GGA CAA CAT AGG
<i>SQSTM1</i>	CTG CAC AAG AAC CTG GCT TT	CAC TGG AAA AGG CAA CCA AG

Supplementary Table 2 | List of siRNAs used in this study.

siRNA target	Manufacturer	Catalogue No.
<i>BANF1</i>	Dharmacon	E-011536-00-0050
<i>HMGB2</i>	Dharmacon	E-011689-00-0010
<i>SRRM2</i>	Sigma-Aldrich	NM_016333
<i>CTCF</i>	ThermoFischer	4392420
<i>ATXN2</i>	Sigma-Aldrich	NM_002973
<i>MR1</i>	Sigma-Aldrich	NM_002024
<i>SQSTM1</i>	Sigma-Aldrich	NM_003900
non-targeting control	Dharmacon	D-001910-10-50
universal negative control	Sigma-Aldrich	SIC001

Supplementary Source Data | Uncropped western blot scans.

