m^6^A-independent genome-wide METTL3 and METTL14 redistribution drives the senescence-associated secretory phenotype

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Methyltransferase-like 3 (METTL3) and 14 (METTL14) are core subunits of the methyltransferase complex that catalyses messenger RNA N^6^-methyladenosine (m^6^A) modification. Despite the expanding list of m^6^A-dependent functions of the methyltransferase complex, the m^6^A-independent function of the METTL3 and METTL14 complex remains poorly understood. Here we show that genome-wide redistribution of METTL3 and METTL14 transcriptionally drives the senescence-associated secretory phenotype (SASP) in an m^6^A-independent manner. METTL14 is redistributed to the enhancers, whereas METTL3 is localized to the pre-existing NF-κB sites within the promoters of SASP genes during senescence. METTL3 and METTL14 are necessary for SASP. However, SASP is not regulated by m^6^A mRNA modification. METTL3 and METTL14 are required for both the tumour-promoting and immune-surveillance functions of senescent cells, which are mediated by SASP in vivo in mouse models. In summary, our results report an m^6^A-independent function of the METTL3 and METTL14 complex in transcriptionally promoting SASP during senescence.

N^6^-methyladenosine (m^6^A) modification plays an important role in dynamic responses to stresses. The recruitment of the m^6^A writer METTL3–METTL14 methyltransferase complex (MTC) generates m^6^A. In addition to the core subunits METTL3 and METTL14, Wilms’ tumour 1-associating protein (WTAP) binds to METTL3–METTL14 and is necessary for the nuclear localization of the METTL3–METTL14 core complex. m^6^A modification is typically coupled with the recruitment of MTC. For example, m^6^A-mediated RNA degradation of heat-shock genes is mediated by localization of METTL3 to these gene loci. In addition, an increase in m^6^A-modified RNA at ultraviolet light-induced DNA damage sites correlate with the recruitment of METTL3 and METTL14 (ref. 7). Furthermore, co-transcriptional m^6^A installation in human pluripotent stem cells is mediated by the interaction between METTL3–METTL14 and the transcription factors SMAD2/3 (ref. 8). Similarly, m^6^A modification of transcription factor CEBPZ target genes in acute myeloid leukaemia is mediated by the interaction between METTL3 and CEBPZ. The m^6^A modification of transcripts from lysine 36 trimethylated histone H3 (H3K36me3)-marked chromatin is associated with the interaction between METTL14 and H3K36me3 (ref. 9). Finally, METTL3 deposits m^6^A modifications on chromosome-associated regulatory RNAs (carRNAs), such as promoter-associated RNAs and enhancer RNAs, to tune chromatin state and transcription10. However, the association of METTL3–METTL14 with these specific chromatin loci is inevitably associated with their m^6^A methyltransferase activity, which results in m^6^A installation on their target transcripts. Notably, the m^6^A-independent function of the METTL3–METTL14 complex remains poorly understood.

Cellular senescence is a stable growth arrest that can be induced by a number of stresses, including activation of oncogenes or chemotherapeutics. Oncogene-induced and therapy-induced senescence underscore the tumour-suppressive role of senescence11. Senescent cells also have non-cell autonomous activities, exemplified by secretion of inflammatory cytokines and chemokines, which is termed the senescence-associated secretory phenotype (SASP)12. SASP plays a context-dependent role in cancer12,13. In addition to its detrimental tumour growth-promoting function14, SASP is critical for immune modulation and surveillance of senescent cells induced by activated oncogenes in pre-malignant lesions15,16. Given that METTL3 and METTL14 regulate m^6^A modification in the stress response17 and cellular senescence is considered a stress response18, we explored the potential role of the METTL3–METTL14 complex during senescence.

Results

METTL3 and METTL14 regulate SASP. We knocked down expression of the core MTC subunit METTL14 in primary embryonic lung fibroblasts (IMR90 cells) undergoing oncogenic RAS-induced senescence to explore the role of MTC during senescence (Fig. 1a and Extended Data Fig. 1a). To limit the potential off-target effects and identify m^6^A-dependent changes, we performed the rescue experiments in METTL14-knockdown cells by expressing wild-type METTL14 or a R298P mutant METTL14 that is defective in mediating RNA m^6^A modification19,20. We performed RNA-sequencing (RNA-seq) analysis in these cells to compare and contrast METTL14-dependent and m^6^A-dependent changes in gene expression. The results show that 93% of the METTL14-dependent changes in gene expression rescued by wild-type METTL14 were also rescued by the R298P mutant (Fig. 1b,c). This result suggests that the vast majority of METTL14-dependent changes in gene expression are m^6^A-independent. Pathway analysis for the genes

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Fig. 1] METTL3 and METTL14 regulate SASP. a, IMR90 cells were induced to senesce by oncogenic RAS expressing a non-targeting short interfering RNA (siRNA) control (siControl) or METTL14-targeted siRNA (siMETTL14) with or without the rescue of ectopically expressed wild-type METTL14 or its R298P mutant. The cells were harvested and analysed for expression of the indicated proteins by immunoblot. The experiment was repeated three times independently with similar results.

b-d, RNA-seq analysis of the cells in a. Number of genes with significantly changed expression in the indicated cells (b). c-d, Heatmap of the RNA-seq data of the genes for which expression was significantly changed by METTL14 knockdown and rescued by both wild-type METTL14 or its D394A/W397A mutant.

e-g, IMR90 cells were induced to senesce by oncogenic RAS expressing the non-targeting siControl or METTL3-targeted siRNA (siMETTL3) with or without the rescue of ectopically expressed wild-type METTL3 or its D394A/W397A mutant. h, IMR90 cells were induced to senesce by oncogenic RAS with or without the expression of the indicated short hairpin RNAs (shRNAs). The expression levels of the indicated proteins were determined by immunoblot. The experiment was repeated three times independently with similar results.

i, The secretion of soluble factors under the indicated conditions was detected using antibody arrays. The heatmap indicates the fold change in comparison to the control or RAS-induced senescent condition. The relative expression levels per replicate and average fold change differences are shown (n = 4 biologically independent replicates). P values were calculated using a two-tailed Fisher's exact test. Uncropped blots for a,e,h and numerical source data for i are provided. FC, fold change.
Fig. 2 | SASP is not regulated by m^6^-A. a, Tracks of m^6^-A distribution on the representative SASP genes (top) based on RNA immunoprecipitation using an anti-m^6^-A antibody, followed by sequencing. The m^6^-A signal was normalized to the corresponding input and the relative fold change is shown. The m^6^-A-modified non-SASP genes PHLP2, BCL2A1 and CENPA (bottom) were used as positive controls. The red arrows point to the statistical cut-off in peak calling. b, Levels of m^6^-A in the total RNA of control and RAS-induced senescent cells with or without knockdown of endogenous METTL3 or METTL14 and rescue by the indicated wild-type or mutant METTL3 or METTL14. c, d, The m^6^-A modifications on the indicated SASP genes (c) and the PHLP2 gene used as a positive control (d) were quantified using RNA immunoprecipitation with an anti-m^6^-A antibody, followed by qPCR with reverse transcription. Data represent the mean ± s.d. of three biologically independent experiments. b, d, P values were calculated using a two-tailed Student’s t-test. Numerical source data for b, c, d are provided.
whose expression are regulated by both the wild-type and mutant METTL14 revealed a significant enrichment of SASP-related pathways, such as inflammatory cytokines, NF-κB and p38 MAPK pathways (Extended Data Fig. 1b,c). The SASP genes were indeed significantly enriched among the set of 246 rescued genes (9.9-fold; \( P \approx 1 \times 10^{-13} \), Fisher’s exact test; Fig. 1d).

We next sought to determine whether the observed effects are MTC-complex dependent. Towards this goal, we performed rescue experiments in METTL3-knockdown cells by expressing wild-type METTL3 or a D394A/W397A METTL3 mutant that is enzymatically inactive (Fig. 1e)\(^{17,18}\). Similarly to the METTL14-rescue experiments, we found that 88% of the METTL3-dependent changes in gene expression rescued by wild-type METTL3 were also rescued by the D394A/W397A mutant (Fig. 1f and Extended Data Fig. 1d). Likewise, the SASP genes were enriched by both wild-type and mutant METTL3-regulated genes during senescence (Fig. 1g and Extended Data Fig. 1e). We next knocked down the expression of the core subunits METTL14 and METTL3 as well as the WTAP subunit that is required for the nuclear localization of the METTL3–METTL14 complex\(^\text{3}\) in senescent cells (Fig. 1h). Notably, the expression of SASP genes was reduced by the knockdown of all three subunits of the MTC (Extended Data Fig. 2a). Similar observations were also made in cells undergoing senescence induced by etoposide (Extended Data Fig. 2b). We next measured the changes in gene expression rescued by wild-type METTL3 or METTL14 knockdown, as determined by ChIP-seq analysis. \(\text{d.e, C, control cells; S, senescent cells; TSS, transcription starting site; TES, transcription end site.}\)

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**Fig. 3 | Genome-wide redistribution of METTL3 and METTL14.**

a, Heatmap clustering of cut-and-run sequencing profiles of METTL3 and METTL14 in control (left) and senescent (right) cells. The percentages of METTL3 unique sites (top), METTL3 and METTL14 co-localized sites (middle) and METTL14 unique sites (bottom) in control and senescent cells were indicated. b,c, Correlation of the binding signal between METTL3 and METTL14 in control and senescent cells (b), and correlation of the specific binding signal of METTL3 or METTL14 between control and senescent cells (c). d, Distribution of the relative normalized density of the METTL3 and METTL14 cut-and-run sequencing peaks within the gene body context in control and senescent cells. e, Average profiles of the Pol II occupancy on SASP gene loci in control and senescent cells with or without METTL3 or METTL14 knockdown, as determined by ChIP-seq analysis. \(\text{d.e, C, control cells; S, senescent cells; TSS, transcription starting site; TES, transcription end site.}\)
Fig. 4 | METTL3 is localized to the pre-existing NF-κB sites within the promoters of SASP genes. a. Enrichment of the SASP genes among genes whose promoters showed an increased association with METTL3 in senescent cells. b. Heatmap of the binding signal around the METTL3 peaks found in control and RAS-induced senescent cells from cut-and-run sequencing for METTL3 and NF-κB p65. c. Co-immunoprecipitation analysis between METTL3 or METTL14 and the NF-κB p65 subunit and Pol II in control (left) and RAS-induced senescent (right) cells. The experiment was repeated three times independently with similar results. d, e. ChIP-qPCR analysis of the association of METTL3 (d) and NF-κB p65 (e) with the promoters of the SASP gene CXCL3 in control and RAS-induced senescent cells with or without METTL3 or METTL14 knockdown or treated with the IKK inhibitor Bay 11-7082 (5 μM) for 48h. Data represent the mean ± s.e.m. of three biologically independent experiments. a, d, e. P values were calculated using a two-tailed Student’s t-test (d,e) or two-tailed Fisher’s exact test (a). Uncropped blots for c and numerical source data for d,e are provided.

in the levels of secreted SASP factors using an antibody-based array. Indeed, knockdown of all three subunits of the MTC significantly decreased the levels of secreted SASP factors (Fig. 1i). Furthermore, we validated that ectopic expression of both wild-type and mutant METTL3 or METTL14 rescued the expression of the SASP genes that were downregulated by the knockdown of METTL3 or METTL14 (Extended Data Fig. 3). Notably, knockdown of the MTC subunits did not affect senescence-associated growth arrest or expression of markers of senescence, such as senescence-associated beta-galactosidase (SA-β-gal) activity, and upregulation of p16 and p21 (Fig. 1a,c,h and Extended Data Fig. 4). This indicates that the observed decrease in SASP is not an indirect consequence of senescence inhibition. Ectopic expression of METTL3 or METTL14 induced senescence and the associated growth arrest in IMR90 cells, which correlates with an upregulation of the SASP genes (Extended Data Fig. 5a–d). In addition, ectopic expression of both wild-type METTL14 and its R298P mutant further increased the upregulated SASP genes in senescent cells (Extended Data Fig. 5e). Finally, ectopic expression of wild-type or mutant METTL3 and METTL14 upregulated the p53 and p21 senescence-promoting pathway (Extended Data Fig. 5f). Consistent with this, SASP promotes senescence through pathways including the p53 and p21 pathway19. Conditioned media from senescent cells induced by wild-type or mutant METTL3 and METTL14 upregulated SA-β-gal-positive cells and decreased incorporation of bromodeoxyuridine (BrdU), a marker of cell proliferation (Extended Data Fig. 5g,h).

The SASP genes are differentially expressed at the different stages of senescence. We next determined the dynamics of METTL3- and METTL14-regulated SASP genes. Towards this goal, we performed time-course studies using the well-characterized IMR90 cells transduced with an oestrogen receptor, H-RASG12V fusion protein (ER:RAS), that can be induced with 4-hydroxytamoxifen (Extended Data Fig. 6a). Notably, oncogenic RAS induction did not affect the overall m6A levels (Extended Data Fig. 6b). The expression of SASP genes regulated by METTL3 and METTL14 coincided with induction of formation of the cytoplasmic chromatin fragments that promote SASP genes through activation of NF-κB (Extended Data Fig. 6c–e)20. This correlated with the association of METTL3 with
SASP is not regulated by m6A. Our results suggest that the upregulation of SASP genes is METTL3- and METTL14-dependent but m6A-independent. To directly test this possibility, we profiled the m6A distribution at the transcriptome level in control and senescent cells by RNA immunoprecipitation, followed by sequencing. The m6A distribution was not significantly altered at the transcriptome level during senescence (Extended Data Fig. 7a,b). Notably, the SASP genes regulated by METTL3–METTL14 were not subjected to m6A regulation, as evidenced by background signal in the analysis (Fig. 2a). We next sought to validate this finding by knocking down endogenous METTL3 or METTL14 with or without rescue with wild-type or mutant METTL3 or METTL14 in senescent cells. The levels of m6A were indeed reduced by METTL3 or METTL14 knockdown, which could be rescued by wild-type, but not mutant, METTL3 or METTL14 (Fig. 2b). In addition, we validated that the mRNA of the SASP genes were not subjected to m6A modification, as evidenced by the comparable levels between anti-m6A antibody and a control IgG in the RNA immunoprecipitation analysis (Fig. 2c). In contrast, as a positive control, m6A modification on PHLP2 mRNA was decreased by endogenous METTL3 or METTL14 knockdown, which was rescued by wild-type, but not the mutant, METTL3 or METTL14 (Fig. 2d). Given the role of m6A modification on carRNAs in regulating transcription, we profiled carRNAs in senescent cells with or without METTL3 or METTL14 knockdown (Extended Data Fig. 7c). We validated previous reports that m6A modification on carRNAs is regulated by METTL3 and METTL14 (for example, Extended Data Fig. 7d). However, none of the METTL3 and METTL14-regulated SASP genes were subjected to regulation of m6A modification on their associated carRNAs (for example, Extended Data Fig. 7e). Together, these results support that the METTL3–METTL14 complex regulates SASP genes independently of its m6A function.

Genome-wide METTL3 and METTL14 redistribution. We next sought to determine the mechanism by which MTC regulates SASP by mapping the association of METTL3 and METTL14 with chromatin using cut-and-run in control and senescent cells. In control cells, the two proteins showed overwhelming co-localization (Fig. 3a–c). In contrast, both METTL3 and METTL14 redistributed in senescent cells, but with different patterns (Fig. 3a–c). Specifically, METTL3 showed an increase in binding upstream near the transcription start sites (TSS) of genes, whereas METTL14 showed an increase in binding at least 10 kb away from the gene bodies (Fig. 3d and Extended Data Fig. 8a). Given the redistribution patterns of the METTL3–METTL14 complex and their roles in regulating SASP, we next directly explored the role of METTL3 and METTL14 in gene transcription during senescence by performing RNA polymerase II (Pol II) chromatin immunoprecipitation (ChIP) with sequencing (ChIP–seq). Interestingly, although knockdown of METTL3 or METTL14 did not affect the global Pol II distribution (Extended Data Fig. 8b), the association of Pol II with the SASP gene loci was decreased by their knockdown (P = 1 × 10^{−29} for METTL3 knockdown and P = 1 × 10^{−42} for METTL14 knockdown, paired Student’s t-test; Fig. 3e). This is consistent with the findings that knockdown of METTL14 or METTL3 suppresses the expression of SASP genes (Fig. 1).

METTL3 is localized to the pre-existing NF-kB sites within the promoters of the SASP genes. We next cross-referenced the METTL3 cut-and-run with RNA-seq datasets comparing control and senescent cells. Analysis of the genomic loci specifically bound by METTL3 in senescent cells within 500 base pairs (bp) of the TSS of a gene with significantly upregulated expression in senescent cells (false discovery rate (FDR) < 5% with a METTL3 cut-and-run signal increase of more than twofold) revealed a significant enrichment of SASP genes (P = 2 × 10^{−8}, Fisher’s exact test; Fig. 4a). Analysis of the enrichment of transcription-factor binding sites of the direct target genes of METTL3 revealed NF-kB—a known regulator of SASP genes—as the top transcription factor (Extended Data Fig. 8c). Accordingly, we performed a cut-and-run analysis for the regulatory NF-kB p65 subunit. The analysis revealed a significant correlation between the redistribution of METTL3 (change of signal in senescent versus control cells) and NF-kB p65 distribution in senescent cells (Fig. 4b and Extended Data Fig. 8d). Compared with controls, a significant number of METTL3 was redistributed to the pre-existing NF-kB p65 sites (Fig. 4b). Consistent with these results, we observed an interaction between METTL3–METTL14 and NF-kB p65 in both control and senescent cells through co-immunoprecipitation analysis (Fig. 4c and Extended Data Fig. 8e). When the translocation of NF-kB into the nucleus was blocked by a small molecule inhibitor, Bay 11-7082, the association of METTL3 with the promoters of SASP genes such as CXCL5 and CXCL5, but not the negative control regions, was significantly reduced (Fig. 4d and Extended Data Fig. 8f,g). Conversely, knockdown of METTL3 (but not METTL14) decreased the association of NF-kB p65 with the promoters of the SASP genes but not the negative control regions, whereas knockdown of both METTL3 and METTL14 reduced the activity of the NF-kB reporter in senescent cells (Fig. 4e and Extended Data Fig. 8h–j). Notably, the METTL3 mutant that is deficient for its m6A methyltransferase activity rescued p65 phosphorylation, its nuclear chromatin association and its association with the promoters of SASP genes with an equivalent efficiency to wild-type METTL3 in METTL3-knockdown senescent cells (Extended Data Fig. 8k–m). Similar to a previous report, the analysis also revealed an interaction between METTL3–METTL14 and Pol II (Fig. 4c). This is consistent with the findings that the knockdown of METTL14 or METTL3 decreased the Pol II association with the SASP genes (Fig. 3e).

METTL14 regulates the SASP gene enhancers. We next cross-referenced the METTL14 cut-and-run with RNA-seq datasets comparing control and senescent cells with or without METTL14 knockdown and rescued with wild-type METTL14 or its R298P

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**Fig. 5 | METTL14 regulates the SASP gene enhancers.** a, Overlap between genes with expression rescued by both wild-type METTL14 and its R298P mutant in senescent cells with METTL14 knockdown and genes with ≥ twofold increase in METTL14 binding in senescent compared with control cells. RNA-seq analysis, n = 3 biologically independent experiments; FC, fold change. b, Average profiles of the cut-and-run signal for METTL14 and the ChIP–seq signal for H3K27ac for genomic loci with increased association for both METTL14 and H3K27ac in senescent compared with control cells (≥ twofold). a,b,c, Control cells; S, senescent cells. c, Representative cut-and-run peaks for METTL3, NF-kB p65, METTL14 and H3K27ac on the indicated SASP genes loci in control and RAS-induced senescent cells. d, ChIP-qPCR analysis of the association of H3K27ac with the enhancers of the indicated SASP gene loci in control and RAS-induced senescent cells with or without METTL14 knockdown. Data represent the mean ± s.d. of three biologically independent experiments. a,d, P values were calculated using a two-tailed Student’s t-test (d) or two-tailed Fisher exact test (a). Numerical source data for d are provided.
mutant (Fig. 5a). Specifically, we focused on genes with increased METTL14 peaks in senescent cells (> twofold within 100 kb from the TSS) and overlapped those genes with genes for which expression was suppressed by METTL14 knockdown and rescued by both wild-type and mutant METTL14. The analysis revealed a significant enrichment (\(P = 2 \times 10^{-4}\), Fisher’s exact test) of such overlapped

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**Table:**

| Treatment          | METTL14 Signal |
|--------------------|----------------|
| Control + METTL14  | 0              |
| S + METTL14        | 0.5            |
| C + IgG            | 1.0            |
| S + IgG            | 1.5            |
| C + H3K27ac        | 2.0            |
| S + H3K27ac        | 2.5            |

**Legend:**

- C: Control
- S: METTL14
- C + METTL14
- S + METTL14
- C + IgG
- S + IgG
- C + H3K27ac
- S + H3K27ac
**Fig. 6 | METTL3 and METTL14 mediate the formation of the promoter-and-enhancer loop of the SASP genes.**

**a.** Analysis by 3C-qPCR of the frequency of the promoter–enhancer interaction on the indicated SASP gene loci in control and RAS-induced senescent cells with or without METTL14 knockdown (middle and bottom). The schematics illustrate the 3C-qPCR primers targeting the enhancer and promoter of the SASP gene loci according to the cut-and-run seq peaks for H3K27ac and METTL14 in control and RAS-induced senescent cells (top). NPC, non-peak control. **b.** Model for the mechanism by which the redistributed METTL3 and METTL14 promote SASP gene expression in senescent cells by mediating promoter-and-enhancer looping. 

**c.** Representative images of 3D DNA-FISH for the IL1β locus in control and RAS-induced senescent cells with or without knockdown of METTL3 or METTL14. Dual-labelled DNA-FISH was performed with a probe for the promoter and another probe for the enhancer. Inset, magnified views of regions in the white boxes in the main images. Scale bars, 5 µm. **d.** The distance between the IL1β promoter and enhancer probes was determined using the ImageJ software. At least 40 loci were quantified for each of the indicated groups. **e,f.** ChIP-qPCR analysis of the association of haemagglutinin (HA)-tagged wild-type and mutant METTL3 (e) and FLAG-tagged wild-type and mutant METTL14 (f) with the promoters and enhancers of the indicated SASP genes in control and senescent cells with or without endogenous METTL3 or METTL14 knockdown, and rescued with HA-tagged wild-type or mutant METTL3 or FLAG-tagged wild-type or mutant METTL14. **a.e.f.** Data represent the mean ± s.d. of three biologically independent experiments. P values were calculated using a two-tailed Student’s t-test. Numerical source data are provided.
putative METTL14 target genes (Fig. 5a and Extended Data Fig. 9a) that were also enriched for SASP genes (Extended Data Fig. 9b). Given that METTL14 showed a redistribution from the TSS to at least 10 kb away from the gene body in senescent cells (Fig. 3d), we explored whether these redistributed sites represent distal enhancers. Cross-referencing of co-localized binding sites for METTL14 and lysine 27 acetylated histone H3 (H3K27ac), an enhancer marker\(^\text{23}\), indeed revealed that the METTL14 and H3K27ac bindings were increased more than twofold in 195 genes in senescent cells within 100 kb from the TSS (Fig. 5b). SASP genes such as CXCL1, CXCL3,
CXCL5, CXCL6, IL1α, IL1β and IL6 (Fig. 5c) were also enriched among these genes (Extended Data Fig. 9c). Notably, acute-phase serum amyloids A1 and A2 (SAA1 and SAA2) were only weakly regulated by METTL3 and METTL14, while only the regulation of SAA2, but not SAA1, by METTL3 and METTL14 can be validated (Extended Data Figs. 3c and 9d)[4]. The association of H3K27ac with distal enhancers of SASP genes was impaired by METTL14 knockdown (Fig. 5d and Extended Data Fig. 9e). Notably, METTL14 expression positively correlated with the expression of a number of SASP genes in human pancreatic intra-epithelial neoplasia, precursors to malignancy, which contain oncogenic RAS-induced senescent cells[21] (Extended Data Fig. 9f). In contrast to decreasing the association of METTL3 with the promoters of SASP genes, NF-κB inhibition did not affect the association of METTL14 with the distal enhancers of SASP genes (Extended Data Fig. 9g).

The redistribution pattern of METTL3 and METTL14 suggest that they may regulate SASP gene expression through the formation of chromatin looping, allowing promoter-and-enhancer interaction through the interaction between METTL3 and METTL14. We directly examined chromatin looping between the METTL14-redistributed enhancer and METTL3-enriched promoter of SASP genes, such as CXCL3, CXCL5 and IL1β, using in situ chromosome conformation capture quantitative PCR (3C-qPCR) assays in control and senescent cells[22]. We observed a robust association between the promoters and enhancers of these SASP genes (Fig. 6a). Notably, METTL14 knockdown reduced the interaction to the levels observed in control cells (Fig. 6a). This supports the idea that the interaction between METTL3 and METTL14 mediates the promoter-and-enhancer looping in senescent cells to promote the expression of SASP genes (Fig. 6b). Consistent with these results, three-dimensional DNA fluorescence in situ hybridization (3D DNA-FISH) analysis of the IL1β locus revealed that the distance between its promoter-and-enhancer was reduced in senescent cells and the knockdown of either METTL3 or METTL14 increased their distances (Fig. 6c,d). Finally, enzymatically inactive mutant METTL3 did not reduce its association with the promoters of the SASP genes (Fig. 6c). Similarly, mutant METTL14 did not negatively affect its association with enhancers of SASP genes (Fig. 6f). Together, these findings support that the METTL3–METTL14 complex regulates the promoter-and-enhancer interaction of SASP genes in an enzymatic activity-independent manner.

METTL3 and METTL14 are required for the tumour-promoting function of SASP. SASP factors play a context-dependent role in cancer[22,23]. For example, the SASP promotes the growth of tumour cells both in vitro and in vivo in immunocompromised mice[4]. To further establish the role of MTC-regulated SASP in a physiological context, we treated ovarian cancer cells with conditioned media collected from senescent cells with or without knockdown of METTL14, METTL3 or WTAP subunits of the MTC. The growth-promoting effects of the conditioned media from the senescent cells were significantly reduced by the knockdown of all three subunits of MTC (Fig. 7a). Consistent with this, the tumour growth-stimulating effects of the co-injected senescent fibroblasts were significantly impaired by the in vivo knockdown of all three MTC subunits in xenograft models (Fig. 7b,c).

METTL3 and METTL14 are required for SASP-mediated immune surveillance. In addition to the detrimental tumour-promoting effects, SASP plays a key role in the immune modulation and surveillance of pre-malignant oncogene-induced senescent cells during tumour initiation[22,23]. To explore the role of METTL3/METTL14-regulated SASP in immune surveillance, we used a sleeping beauty transposase-based mouse model of oncogene-induced senescence in vivo, in which senescence can be acutely induced and the fate of senescent cells can be monitored[24]. Specifically, hydrodynamic tail-vein injection of a vector expressing sleeping beauty transposase and transposon vector expressing both oncogenic NRasG12V and shMETTL14, shMETTL3 or a negative control shRenilla (shRen) caused stable integration of the transposon selectively into hepatocytes (Fig. 7d and Extended Data Fig. 10a,b). Oncogenic NRasG12V acutely triggers senescence and SASP in hepatocytes, which activates immune surveillance and the clearance of pre-malignant hepatocytes[25]. At day 6 post injection, NRasG12V induced senescence and the formation of immune-cell clusters around the NRas-positive cells (Fig. 7e,f). Clusters of immune cells were in close proximity to the NRas-expressing cells in shRen controls (Fig. 7g). As a control, the NRasG12V/ΔD39A mutant, which is incapable of inducing senescence, failed to trigger senescence or induce the formation of immune-cell clusters (Fig. 7e and Extended Data Fig. 10c). Notably, similar numbers of NRas-expressing and SA-β-gal-positive cells were observed in both the control shRen and the shMETTL14- or shMETTL3-expressing groups (Fig. 7e–i). This suggests a similar efficacy in delivering the transposon vectors in these groups. Consistent with our in vitro findings, expression of shMETTL14 or shMETTL3 did not affect the number of SA-β-gal-positive cells (Fig. 7e,f), indicating that senescence was induced at a comparable level in the different groups. However, shMETTL14 and shMETTL3 significantly decreased the number of immune-cell clusters (Fig. 7g,h and Extended Data Fig. 10d). By day 14, the livers from the shRen control-expressing mice showed a significant reduction in the NRas-expressing and SA-β-gal-positive hepatocytes (Fig. 7e–h), which is consistent with immune-mediated clearance of NRas-expressing senescent cells[25]. In contrast, the shMETTL14- and shMETTL3-expressing groups retained significantly more NRas- and SA-β-gal-positive cells (Fig. 7e–h). This correlates with a significantly lower number of immune-cell clusters in the shMETTL14 and shMETTL3 groups compared with the shRen-expressing groups (Fig. 7i). Together, these findings support that METTL3 and METTL14 are required for the SASP-mediated immune clearance of senescent cells in vivo.

**Discussion**

Our study establishes an m6A-independent function of the METTL3–METTL14 complex. The redistribution of METTL3 to the promoters and METTL14 to the enhancers of SASP genes suggests that the METTL3–METTL14 complex plays an important role in regulating transcription independently of its m6A function. Notably, METTL3- and METTL14-regulated SASP genes are primarily NF-κB target genes, which is consistent with our findings that METTL3 and METTL14 interact with NF-κB to regulate its activity. Although SASP is critical for immune surveillance and clearance of senescent cells in pre-malignant lesions induced by activation of oncogenes, it is detrimental in established tumours by promoting tumour growth[22,23]. Therefore, our findings are consistent with the emerging evidence that MTC subunits such as METTL3 and METTL14 predominantly play an oncogenic role in cancers[23]. In addition, independently of METTL14, METTL3 interacts with EIF3 to regulate looping of mRNA, including those with m6A modifications, to control mRNA translation during tumorigenesis[26,27]. Together, these findings suggest that compared with targeting the m6A methyltransferase activity of the MTC complex, degradation of the subunits of the MTC complex might be advantageous because it will simultaneously inhibit both the m6A-dependent and -independent functions of the MTC. A limitation of our study is that we focused on the m6A-independent transcription-regulating function of METTL3 and METTL14 in the context of SASP during senescence. However, we envision that the m6A-independent transcription-regulating function of METTL3 and METTL14 may be involved in many biological processes beyond our current study.
Online content
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Methods

Cells and culture conditions. IMR90 primary human diploid lung embryonic fibroblasts were cultured under low oxygen tension (2%) in DMEM medium supplemented with 10% fetal bovine serum (FBS), l-glutamine, sodium pyruvate, non-essential amino acids and sodium bicarbonate. All experiments were performed on IMR90 cells between the population doublings of 25 and 35. The ovarian cancer TOV21G cell line was cultured in RPMI 1640 medium with 10% FBS and 1% penicillin–streptomycin under 5% CO2. The viral packaging cells 293FT, Phoenix cells and mouse NIH 3T3 fibroblasts were cultured in DMEM medium supplemented with 10% FBS and 1% penicillin–streptomycin under 5% CO2. All of the cell lines were authenticated at The Wistar Institute’s Genomics Facility using short- tandem-repeat DNA profiling. Regular mycoplasma testing was performed using the LookOut mycoplasma PCR detection kit (Sigma, cat. no. M00035).

Reagents, plasmids and antibodies. Etoposide was purchased from Sigma (E1383) and Bay 11 7082 was purchased from Selleckchem (S2913). Non-targeting siControl (D-001810-01), and siMETTL14 (J-014169-18) and siMETTL3 (J-005170-20) were purchased from Dharmacon. The pBBA-puro-H-RAS(V12) and pBBA-puro-Empty plasmids were obtained from Addgene. The METTL3 (53739) and METTL14-expressing (53740) plasmids were obtained from Addgene and subcloned into the pCDH vector. Site-specific mutuation of FLAG-tagged METTL14 was generated using a Q5 site-directed mutagenesis kit (New England Biolabs) according to manufacturer’s instructions using the following primers: Forward, 5′-TGAACACGTACCAGAAGCTTCT-3′ and Reverse, 5′-CCTTTTGATCCCCATCAGG-3′. Site-specific mutation of HA-tagged METTL3 was generated using a Q5 site-directed mutagenesis kit (New England Biolabs) according to manufacturer’s instructions using the following primers: Forward, 5′-AGCTCTTAGAGGACATGTAATGCAAG-3′ and Reverse, 5′-AGCTCTTAGAGGACATGTAATGCAAG-3′. Site-specific mutation of FLAG-tagged METTL14 was performed using the following primers: Forward, 5′-TTGGGAATCCATTGCAATCTCTGGGATTTG-3′ and Reverse, 5′-CTGAGATTTGCGGCCGCGG-3′. Site-specific mutation of FLAG-tagged METTL14 was performed using the following primers: Forward, 5′-TGCCACCATGGAATCTTTAGG-3′ and Reverse, 5′-TTGGGAATCCATTGCAATCTCTGGGATTTG-3′. All mutation sites were confirmed by Sanger sequencing at The Wistar Institute’s Genomics Facility. The scramble control shRNA (1864), and the viral packaging plasmids pMD2.G (12259) and psPAX2 (12260) were obtained from Addgene. The following TRC lentiviral vectors encoding shRNAs against human METTL3, METTL14 and WTAP were obtained from the Molecular Screening Facility at the Wistar Institute: shMETTL3-1, TRCN0000034715; shMETTL3-2, TRCN0000034716; shWTAP-1, TRCN0000034717; and shWTAP-2, TRCN0000034718. The following transposon-based plasmids were used: NRas(V12) expression transposon vector pKt2/Luc-PGK-PGK-EF1-V12 was provided by Broad, U, at a final concentration of 3 μg ml−1, at 37 °C for 3 h and fixed with 4% paraformaldehyde (PFA) for 30 min at room temperature; 2N HCl was used to denature the DNA for the access to visualize the incorporated BruD. NIH 3T3 transfectants were obtained by transfection of NIH 3T3 cells with siRNA against human METTL3, METTL14 and WTAP using a Q5 site-directed mutagenesis kit (New England Biolabs) following two weeks of selection with G418 (400 μg ml−1, Gibco), the cells were then maintained with a lower dose of G418 (200 μg ml−1) and treated with 4-hydroxymethylxammaten at a final concentration of 100 nM; cells were harvested to examine the expression of RAS and other markers at the indicated time points. For etoposide-induced senescence, IMR90 cells at approximately 60–70% confluence were treated with 100 μM etoposide for 48 h, washed with PBS and then cultured in normal medium. The cells were harvested for analysis at day 8 as described. For SA-β-gal staining, cells were fixed using 2% formaldehyde and 0.2% glutaraldehyde in PBS and washed twice with PBS. The cells were then incubated overnight in X-gal solution (150 μM 5-iodo-2′-deoxyuridine (IodU), 1 mM MgCl₂, 50 μM KFe(CN)₆, 50 μM KFe(CN)₄, and 1 mg ml⁻¹ X-gal) at 37°C in a non-CO2 incubator. For mouse liver tissue, frozen sections were prepared using a KAPA RNA HyperPrep kit and sequenced in a 75-bp single-end run. For the METTL3-rescue experiment, libraries were prepared using a KAPA RNA HyperPrep kit and sequenced in a 75-bp paired-end run. The libraries were sequenced at the Wistar Genomics Facility using an Illumina NextSeq 500 system.

Retrovirus and lentivirus production and infection. Retrovirus was produced using Phoenix cells as previously described. Lentivirus was produced by transfection of 293FT cells with FuGENE 6 (Promega). The supernatant virus was harvested 48 h post transfection and concentrated by ultracentrifugation. For infection of oncogenic H-RAS(V12)-induced senescent cells, IMR90 cells infected with oncogenic H-RAS(V12)-encoding or empty vector-expressing retrovirus were selected by 1 μg ml⁻¹ puromycin at day 4 post infection; the cells were infected with shRNA-expressing lentivirus and then selected with 1 μg ml⁻¹ puromycin.

Senescence induction and SA-β-gal staining. Senescence induction by oncogenic H-RAS(V12) was performed as described previously. For ER-RAS senescence induction, IMR90 cells were infected with retrovirus encoding a 4-hydroxymethylxammaten-inducible ER-RAS construct (pLNC-ER-Ras), following two weeks of selection with G418 (400 μg ml−1, Gibco), the cells were then maintained with a lower dose of G418 (200 μg ml−1) and treated with 4-hydroxymethylxammaten at a final concentration of 100 nM; cells were harvested to examine the expression of RAS and other markers at the indicated time points. For etoposide-induced senescence, IMR90 cells at approximately 60–70% confluence were treated with 100 μM etoposide for 48 h, washed with PBS and then cultured in normal medium. The cells were harvested for analysis at day 8 as described. For SA-β-gal staining, cells were fixed using 2% formaldehyde and 0.2% glutaraldehyde in PBS and washed twice with PBS. The cells were then incubated overnight in X-gal solution (150 μM NaCl, 40 mM NaHPO₄, pH 6.0, 2 mM MgCl₂, 5 mM KFe(CN)₆, 5 mM KFe(CN)₄, and 1 mg ml⁻¹ X-gal) at 37°C in a non-CO₂ incubator. For mouse liver tissue, frozen sections were prepared using a KAPA RNA HyperPrep kit and sequenced in a 75-bp paired-end run. The libraries were sequenced at the Wistar Genomics Facility using an Illumina NextSeq 500 system.

RNA-seq. At day 4 post infection with vector control or RAS-expressing retrovirus, IMR90 cells were infected with lentivirus and transfected with non-targeted siControl, siMETTL14 or siMETTL3 using Lipofectamine RNAiMAX. The cells were transfected with siRNA again and infected with lentivirus encoding siMETTL14-resistant (siRNA targeting sequence mutagenesis was performed using the following primers: Forward, 5′-TGGGACATCCATTGCAATCTCTGGGATTTG-3′ and Reverse, 5′-AAAGACGAGATTGTAGGCAAACTTACACCTCTCCTGATG-3′) wild-type or a R298P mutant METTL14, or siMETTL3-resistant (siRNA targeting sequence mutagenesis was performed using the following primers: Forward, 5′-CTACATTTTGGGCAATAATACACACTCTCTGATG-3′ and Reverse, 5′-TTAATGCCCCGCTAAATGTGACTGACACT-3′) wild-type or a D38A/W39TA mutant METTL14 on the following day. Wild-type or a D38A/W39TA mutant METTL14 on the following day. The carRNA was extracted from the chromosome-associated fraction, used for library preparation. The carRNA sequencing was performed according to the protocols of Dominissini and colleagues as well as our laboratory. Briefly, purified m5G PGK-SB using FuGENE 6 (Promega), and fixed with 4% PFA for 10 min at room temperature, followed by permeabilization with 0.2% Triton X-100 in PBS for 5 min. After blocking with 3% BSA in PBS, the cells were incubated overnight with primary antibody at 4°C and Alexa Fluor-conjugated secondary antibody (Life Technologies) for 1 h at room temperature.

mRNA and carRNA immunoprecipitation and sequencing. Total RNA was isolated from control or oncogenic RAS-induced senescent IMR90 cells 9 days post infection (three independent batches of cells were collected) using RNeasy maxi kit (QiaGen, 74106) and digested with DNase I (Qagen, 79254). For the METTL14-rescue experiment, libraries were prepared using a Lexogen kit and sequenced in a 75-bp single-end run. The libraries were then prepared using a KAPA RNA HyperPrep kit and sequenced in a 75-bp paired-end run. The libraries were sequenced at the Wistar Genomics Facility using an Illumina NextSeq 500 system.

ChIp and ChIp-seq. Briefly, cells were fixed with 1% formaldehyde (Sigma, cat. no. F8775) for 10 min at room temperature, quenched with 2.5 M glycine, washed twice with cold PBS and then lysed using ChiP lysis buffer (1 ml of 50 mM HEPES–KOH pH 7.5, 1 mM EDTA pH 8.0, 140 mM NaCl, 1% Triton X-100 and 0.1% sodium deoxycholate with 0.1 mM PMSF and EDTA-free protease inhibitor cocktail).
Following incubation on ice for 10 min, the lysed samples were centrifuged at 3,000 rpm for 3 min at 4°C. The resulting pellet was resuspended in lysis buffer 2 (10 mM Tris pH 8.0, 1 mM EDTA, 200 mM NaCl and 0.5 mM EGTA with 0.1 mM PMSF and EDTA-free protease inhibitor cocktail) and incubated at room temperature for 10 min before centrifugation at 3,000 rpm for 5 min at 4°C. The resulting pellet was resuspended in lysis buffer 3 (100 mM NaCl, 10 mM Tris–HCl pH 8.0, 1 mM EDTA, 0.5% sodium deoxycholate, 0.5 mM EGTA and 0.5% N-lauroylsarcosine with 0.1 mM PMSF and EDTA-free protease inhibitor cocktail) and incubated at room temperature for 1 h by rotation at 4 °C. After three washes, the cell pellets were resuspended in 100 μl 0.1 mM PMSF and EDTA-free protease inhibitor cocktail) and incubated at room temperature for 2 h (10 mM Tris pH 8.0, 1 mM EDTA, 200 mM NaCl and 0.5 mM EGTA with 0.1% Triton X-100, 1 mM EDTA and 0.5% spermidine and EDTA-free protease inhibitor cocktail) and incubated at 0 °C for 30 min and then the reactions were stopped by the addition of 100 μl 2xSTOP buffer (340 mM NaCl, 20 mM EDTA pH 8.0, 4 mM EGTA, 0.05% digitonin, 50 μg/ml RNase A, 50 μg/ml glycogen and 2 mg/ml heterologous spike-in yeast DNA). The supernatant was centrifuged at 13,000 r.p.m. for 3 min at 45°C to remove the cross-linking. The next day, the samples were digested with proteinase K and the DNA was purified using a Wizard SV gel and PCR clean up kit (Promega). Quantitative PCR was performed on the immunoprecipitated DNA using Taq Universal SYBR Green (Bio-Rad Laboratories) using the primers listed in Supplementary Table 1.

The antibody array for secreted factors was performed following the manufacturer’s instructions as we previously described35. Briefly, the culture media were collected following a PBS wash and incubation in serum-free DMEM medium for 12 h. The media were filtered and concentrated using a Centricon-100 concentrator (Millipore). The membranes were blocked with 5% non-fat milk and then incubated with primary and secondary antibodies.

The array was then incubated with the biotinylated antibody cocktail for 2 h at room temperature before being washed five times with wash buffer I and then two times with wash buffer II at room temperature. The array was then incubated with Cy3 dye-conjugated streptavidin and incubated at room temperature in the dark for 1 h. Following incubation, the array was washed five times with wash buffer I, washed twice with wash buffer II and allowed to dry at room temperature in the dark. The Cy3 signals were measured using an Amersham Typhoon imaging system and normalized to the cell number from which the medium was generated.

3C-qPCR assays. The 3C-qPCR assays were performed as previously described41,42. Briefly, five million cells were fixed with 1% formaldehyde in fresh medium and quenched with 0.2 M glycine at room temperature for 10 min. The cells were lysed in cold Hi-C lysis buffer (10 mM Tris pH 8.0, 10 mM NaCl and 0.2% IGEPA- CA-630 with protease inhibitor) for 15 min, followed by a wash with 500 μl cold lysis buffer and incubation at 0.5% NaCl and 0.5% digitonin and quenched by adding 145 μl water and 25 μl of 10% Triton X-100, and incubating at 37°C for 15 min. Next, 25 μl 10X NEBuffer2 and 100 U Mbo I were added to digest the chromatin overnight at 37°C with rotation. Following inactivation of the Mbo I at 62°C for 20 min, the ligation was performed at room temperature for 4 h with rotation by adding 750 μl ligation master mix (100 μl of 10XNEB T4 DNA ligase buffer, 80 μl of 10% Triton X-100, 10 μl BSA (10 mg/ml), 5 μl T4 DNA ligase (400 μl−1) and 55 μl water). Following centrifugation at 10,000 rpm for 5 min, reverse cross-linking was performed at 68°C for at least 4 h. The DNA was then purified by phenol–chloroform extraction and ethanol precipitation. Primers were designed following the qPCR primer design standard and primers no more than 100 nucleotides from the Mbo I sites were selected (Supplementary Table 1).

Immunoprecipitation, chromatin fractionation and immunoblotting. For immunoprecipitation, cells were lysed with buffer (150 mM NaCl, 1% NP40, 20% glycerol, 1 mM EDTA, 50 mM Tris–HCl pH 8.0, 1 mM PMSF and EDTA-free protease inhibitor cocktail). The cells were then incubated overnight with the antibody in the buffer (Wash buffer supplemented with 0.05% digitonin and 2 μM EDTA) at 4°C. The following day, the supernatant was removed by centrifugation and the cell pellets were washed once with Dig-wash buffer (Wash buffer containing 0.05% digitonin). The cell pellets were then incubated with Protein A–M-Trap (70 ng/ml) in D ig-wash buffer for 1 h by rotation at 4°C. After three washes, the cell pellets were resuspended in 100 μl D ig-wash buffer with 2 μl of 100 μM CaCl2, incubated at 0°C for 30 min and then the reactions were stopped by the addition of 100 μl 2xSTOP buffer (340 mM NaCl, 20 mM EDTA pH 8.0, 4 mM EGTA, 0.05% digitonin, 50 μg/ml RNase A, 50 μg/ml glycogen and 2 mg/ml heterologous spike-in yeast DNA). The supernatant was collected after centrifugation and further purified using phenol–chloroform–isoamyl alcohol (Sigma, cat. no. p3803), chloroform extraction and ethanol precipitation. The cut-and-run DNA was analysed by qPCR or used for DNA library construction for sequencing as described earlier.

Reverse-transcriptase qPCR. Total RNA was harvested using TRIzol (Invitrogen) and isolated using an RNeasy mini kit (Qiagen, 74106). The extracted RNA was used for reverse-transcriptase PCR using a High-capacity cDNA reverse transcription kit (Thermo Fisher). Quantitative PCR was performed using a QuantStudio 3 real-time PCR system. The primers used for reverse-transcriptase qPCR are listed in Supplementary Table 1.

3D DNA-FISH. The BAC clone RP11-1033M9 containing the H3F3A gene locus was purchased from the Children's Hospital Oakland Research Institute. The 3D DNA-FISH was performed as described previously43. Probes were generated through PCR using the BAC clone as a template with the following primers: 5′-ACGTAACGTGGGCTGAGG-3′ (forward) and 5′-CCCTCTTGGCCTCCACCTGTA-3′ (reverse); and enhancer probe, 5′-ACCTGGCTGTAAAGATTT-3′ (forward) and 5′-GAGGTTCCTTGTCCATGTTGTT-3′ (reverse). Probes targeting the promoter and enhancer region were labelled using a BioPrime DNA labeling system kit (Invitrogen) and DIG DNA labeling kit (Roche). Probes were dissolved in 50% formamide, 2×SSC and 10% Dextran sulphate, denatured for 5 min at 37°C and then pre-anneled for 30 min at 37°C. Cells were treated with 0.075 M KCl hypotonic buffer, fresh fixative (3:1 MeOH/acetatic acid) was added to the KCl buffer and incubated for 10 min in room temperature. The solution was removed and the cells were fixed in 3:1 MeOH/ acetatic acid at overnight 20°C. Following fixation with fresh fixative, the cells were treated with RNase A, followed by 0.1 mg/ml 9-pepsi in 0.1 M HCl and then postfixed in 1% PFA in 50 mM MgCl2 for 10 min at room temperature. After washing with 50% ethanol, the cells were dehydrated in an ethanol series (at 70, 80 and then 100% concentrations) for 2 min each and dried. The DNA was denatured in 70% formamide in 2×SSC at 73°C for 3 min, immediately dehydrated in the same
order of the ethanol series, dried and then hybridized to the probes overnight at 37 °C. Covellips were washed for 10 min at 43 °C with prewarmed 50% formamide in 2×SSC (twice), for 4 min at 37 °C with prewarmed 2×SSC (twice) and for 5 min at room temperature in 1×SSC/PS (4×SSC, 20). After blocking with 4×SSC, 0.05% Tween 20 and 5% milk, the covellips were incubated with FITC–Avdin D (1:100; Vector Laboratories) and anti-DIG–DyLight 594 (1:200; Vector Laboratories) at room temperature, followed by washing with blocking buffer with shaking. The covellips were mounted and images were acquired using a Leica TCS SFP II scanning confocal microscope.

In vivo mouse models. All of the protocols were approved by the IACUC of the Wistar Institute or University of Pennsylvania. Mice were maintained at 22–23 °C with 40–60% humidity and a 12 h light–12 h dark cycle. For xenograft mouse models, 0.5×10^5 TOV21G cells with or without 1×10^5 IMR90 in PBS and mixed with Matrigel at a 1:1 volume ratio were injected subcutaneously into six to eight-week-old female immunocompromised non-obese diabetic/severe combined immunodeficiency (NOD/SCID) gamma (NSG) mice. Tumour size was measured using an electronic caliper and calculated using the formula: tumour size (mm^3) = (d×D)/2, where d and D are the shortest and the longest diameters, respectively.

For hydrodynamic tail vein injection, eight-week-old female C57BL/6 mice were used. For each injection, endotoxin-free transposon-based construct expressing NRas and miR-30-based shRNAs (25 μg) together with endotoxin-free transposable plasmid (5 μg) in 0.9% saline at a volume of 10% of the mouse body weight were mixed and delivered into the mice within 5–8 s as previously described.

Immunohistochemistry. Fresh mouse liver tissues were fixed overnight in 4% PFA in PBS at 4 °C or embedded in OCT compound for cryosection. For immunohistochemistry staining, the slides were deparaffinized, rehydrated, quenched in 0.6% hydrogen peroxide in methanol for 15 min and boiled for 20 min in 10 mM citrate (pH 6.0) buffer for antigen retrieval. The slides were incubated with blocking buffer (5% serum, 1% BSA and 0.5% Tween 20 in PBS) for 20 min in 10 mM citrate (pH 6.0) buffer for antigen retrieval. The slides were quenched in 0.6% hydrogen peroxide in methanol for 15 min and boiled for 4% PFA in PBS at 4 °C or embedded in OCT compound for cryosection. For Immunohistochemistry.

Bioinformatics analysis. For mA-seq (m^A-seq), raw sequencing data were aligned against the hg19 version of the human genome using bowtie2 (ref. 44). HOMER was used to generate bigwig files and call the mA peaks (FDR < 5%) at least fourfold) in control and senescent cells versus the corresponding total RNA input. De novo motif analysis using HOMER identified the most enriched consensus motif (AGGACCT) in 71% of all FDR < 5% peaks from exonic regions. The average signal around the TSS and 3’ untranslated regions (UTR) of genes were derived from bigwig files using the “bigwigAverageOverBed” tool from UCSC toolbox with the ‘mean’ option using 10-bp bins for mA and the RNA input samples, and the ratio of mA to RNA input was used as the normalized mA signal.

For car-mA-seq, the raw sequencing data were aligned against hg19 version of the human genome using bowtie2 (ref. 44). HOMER was used to generate bigwig files and call the mA peaks (FDR < 5%) in control and senescent cells with wild type or METTL3- and METTL14-knockout versus the corresponding total RNA input. Peaks corresponding to promoter-associated carRNAs (within 1 kb of the TSS) and enhancer-associated (overlap with H3K27ac sites) carRNAs were identified and assigned to genes with the closest TSS. The average signal within the peaks was derived from bigwig files using the “bigwigAverageOverBed” tool from UCSC toolbox with the ‘mean’ option and the mA signal was normalized to the corresponding input. K-means clustering was performed on the Z-score scaled normalized signal values.

The RNA-seq data were aligned against hg19 version of the human genome using bowtie2 (ref. 44) and the RSEM v1.2.12 software was used to estimate the raw read counts and per kilobase of transcript per million mapped reads values using the Ensemble transcriptome. DESeq2 (ref. 48) was used to estimate the raw read counts and reads per kilobase of transcript per million mapped reads values using the Ensemble transcriptome.

Z-scores were aligned against the hg19 version of the human genome using bowtie2 (ref. 44) and calculated with an average input signal of 0.4 (average input value) used as a floor for the minimum allowed signal and changes of at least twofold between samples were considered significant. Distances from the TSS of 10kb for METTL3 and 65p, and 100 kb for METTL14 and H3K27ac were used for the gene–peak assignments.

Statistics and reproducibility. Analysis of variance with Fisher’s least significant difference was used to identify significant differences in multiple comparisons. An unpaired two-tailed Student’s t-test was used for comparisons between two groups. Experiments were repeated three times unless otherwise stated. Quantitative data are expressed as the mean±s.d. or s.e.m., as indicated in the figure legends. No statistical method was used to predetermine the sample size. No data were excluded from the analyses. All analyses were performed blindly but not randomly. The animal experiments were randomized. Prism 7.0c, 8.4.2 was used for calculating the P-values.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

Cut-and-run, ChiP-seq and RNA-seq data that support the findings of this study have been deposited in the GEO under the accession GSE141944 (RNA-seq for METTL3 knockdown and rescue, GSE159551; RNA-seq for METTL14 knockdown and rescue, GSE141991; cut-and-run and ChiP–seq were GSE141992; mA-seq, GSE141993; and carRNA mA-seq, GSE159550). For the correlation analysis between METTL14 and the SAGP genes in human laser capture correlation analysis between this paper. All other data supporting the findings of this study are available and the SASP genes in human laser capture and the IACUC of the

Further information on research design is available in the

Code availability

The software and algorithms for the data analyses used in this study are all well-established from previous work and are referenced throughout the manuscript.

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Author contributions

P.L., F.L., J.L., T.F., T.N. and X.H. performed the experiments and analysed data. A.V.K. performed the bioinformatic analyses. P.L. and R.Z. designed the experiments. F.L., J.L., T.F. and T.N. contributed to the study design. P.L., A.V.K. and R.Z. wrote the manuscript. M.C.S. and R.Z. supervised the study. R.Z. conceived the study.

Competing interests

The authors declare no competing interests.

Additional information

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Extended Data Fig. 1 | METTL3 and METTL14-dependent changes in transcriptome during senescence. a, Schematic of experimental timeline using oncogenic-H-RASG12V to induce senescence in IMR90 cells. b–c, IMR90 cells were induced to senesce by oncogenic RAS expressing a non-targeting siRNA control (siControl) or METTL14-targeted siRNA (siMETTL14) with or without the rescue of ectopically expressed wild-type or the R298P mutant METTL14 were subjected to RNA-seq analysis. Ingenuity pathway enrichment analysis of genes altered by siMETTL14 (b) and rescued by both wild-type and the R298P mutant METTL14 (c) are shown. d–e, Heatmap of RNA-seq data with 2 replicates in each of the groups for the genes whose expression significantly changed by METTL3 knockdown and rescued by both wild-type and the D394A/W397A mutant METTL3 (d). Ingenuity pathway enrichment analysis of genes altered by siMETTL3 (e) is shown. p = p value, Z = activation z-score, N = number of genes. P values were calculated using a two-tailed Fisher Exact test.
Extended Data Fig. 2 | MTC regulates SASP during both oncogene and chemotherapy-induced senescence. a–b, IMR90 cells were induced to senesce by oncogenic RAS (a) or Etoposide (b) with or without the expression of the indicated shRNAs and analysed for expression of the indicated SASP genes by qRT-PCR. Data represent mean ± s.d. of three biologically independent experiments. P values were calculated using a two-tailed t-test. Numerical source data for 2a and 2b are provided.
Extended Data Fig. 3 | MTC regulates SASP in an enzymatic activity independent manner. a–c, Control and RAS-induced senescent cells with or without knockdown of endogenous METTL3 and METTL14 were rescued by the indicated wild-type or mutant METTL3 or METTL14. Expression of IL6, IL1α, and IL1β (a); IL8, CXCL3 and CXCL5 (b); and SAA1 and SAA2 (c) was determined by RT-qPCR analysis. Data represent mean ± s.d. of three biologically independent experiments. P values were calculated using a two-tailed t-test. Numerical source data for 3a, 3b and 3c are provided.
Extended Data Fig. 4 | Inhibition of MTC does not affect senescence-associated growth arrest. a-b, IMR90 cells were induced to senesce by RAS with or without the expression of the indicated shRNAs. The indicated cells were examined for senescence-associated growth arrest by colony formation and stained for SA-β-gal activity (a). SA-β-gal-positive cells were quantified in the indicated treatment groups (b). c-d, IMR90 cells were induced to undergo senescence by etoposide with or without expression of the indicated shRNAs. SA-β-gal-positive cells were quantified (c) and expression of p16, a marker of senescence, was determined by immunoblot (d). Data represent mean ± s.d. of three biologically independent experiments. P values were calculated using a two-tailed t-test. Uncropped blots for 4d and numerical source data for 4b and 4c are provided.
Extended Data Fig. 5 | METTL3 and METTL14 promote SASP. a–d, IMR90 cells ectopically expressing METTL3, wild-type or a R298P mutant METTL14 were subjected to analysis for expression of the indicated proteins by immunoblots (a), colony formation assay (b), SA-β-gal staining (c) or expression of the indicated SASP genes by qRT-PCR (d). The experiment in 5a was repeated twice independently with similar results. e, IMR90 cells expressing oncogenic RAS with or without ectopically expressed wild-type or the R298P mutant METTL14 were subjected to qRT-PCR analysis for expression of the indicated SASP genes. f, IMR90 cells with or without expressing the indicated wild-type or mutant METTL3 or METTL14 were harvested at day 6 post infection and analysed for expression of the indicated proteins by immunoblot. The experiment was repeated twice independently with similar results. g–h, Conditioned medium collected from senescent cells with the indicated inducers were used to culture proliferating cells for 5 days. Changes in SA-β-gal (g) and BrdU incorporation (h) were examined. Data represent mean ± s.d. of three biologically independent experiments. P values were calculated using a two-tailed t-test. Uncropped blots for 5a and 5f and numerical source data for 5b, 5c, 5d, 5e, 5g and 5h are provided.
Extended Data Fig. 6 | Kinetics of SASP gene expression. a–g, ER-RAS-expressing IMR90 cells were treated with 100 nM 4-OHT to induce RAS expression and analysed for RAS expression by immunoblot (a), quantified for m^A levels from total RNAs (b), CCF formation (c) and quantification (d), expression of the indicated SASP genes (e), association of METTL3 and METTL14 with CXCL5 promoter and enhancer (f), or LINE1 and its regulated IFNα and IFNβ (g) by qRT-PCR analysis at the indicated time points. h, Expression of LINE1 and its regulated IFNβ was determined by qRT-PCR in control and senescent cells without or with knockdown of METTL3 or METTL14. IL6 mRNA expression was used as a positive control. Arrows point to examples of CCF formed in RAS-induced senescent cells. Scale bar = 5 μm. Data represent mean ± s.d. of three biologically independent experiments. P values were calculated using a two-tailed t-test. Uncropped blots for 6a and numerical source data for 6b, 6d, 6e, 6f, 6g, and 6h are provided.
Extended Data Fig. 7 | SASP genes are not subjected to m6A modification. a, Distribution of m6A peaks across the indicated gene structure in control and RAS-induced senescent cells. b, Metagene m6A signal profile illustrating no global changes in m6A modifications around 5' and 3' end UTRs between control and RAS-induced senescent cells. c, Heatmap of changes in m6A modification on carRNAs in control and oncogenic RAS-induced senescent cells with or without knockdown of METTL3 or METTL14. d, Examples of m6A tracks at the boxed carRNAs that belong to each of the three indicated clusters with both forward and reverse strands indicated. H3K27ac modification levels in control (in blue) and senescent (in red) cells were used to identify the regulatory chromatin region (enhancer/promoter-associated RNAs). e, m6A tracks at the indicated SASP genes for both forward and reverse strands. Boxes indicated H3K27ac modification levels in control (red) and senescent (blue) cells used to identify the regulatory chromatin region. Please note that the changes in the gene body reflect changes in gene expression and specifically upregulation of SASP genes in senescent cells (and the associated increase in m6A modification was a reflection of an increase in input mRNA expression of these genes).
Extended Data Fig. 8 | METTL3 redistribution to SASP gene promoters. a, Distribution of METTL3 and METTL14 in the indicated genomic regions in control and RAS-induced senescent cells. b, Average binding signal from ChIP-seq analysis of RNA polymerase II occupancy on all genes in control and senescent cells without or with knockdown of METTL3 or METTL14. c, Transcription factor binding site enrichment analysis of increased cut-and-run peaks of METTL3 in senescent cells. d, Correlation between changes in binding signal of METTL3 (senescent vs. control) and NF-κB p65 binding signal in senescent cells. e, Co-immunoprecipitation analysis between NF-κB p65 subunit and METTL3 or METTL14 in the indicated cells. The experiment was repeated three times independently with similar results. f–g, ChIP–qPCR analysis of association of METTL3 on the CXCL5 promoter (f) or negative control regions of CXCL3 or CXCL5 gene promoters in the indicated cells (g). h–i, ChIP–qPCR analysis of association of NF-κB p65 on the CXCL5 promoter (h) or negative control regions of CXCL3 or CXCL5 gene promoters in the indicated cells (i). j, NF-κB reporter activity was determined in the indicated cells. k–m, The indicated ER:RAS IMR90 cells were induced by 4-OHT. Cells were harvested and analysed for expression of the indicated proteins by immunoblot (k), nuclear chromatin fraction of p65 (l), or association of p65 with the promoters of the indicated SASP genes by ChIP-qPCR assay (m). Data represent mean ± s.d. except for 8j mean ± s.e.m. of three biologically independent experiments. P values were calculated using a two-tailed t-test and a two-tailed Spearman correlation analysis for 8d. Uncropped blots for 8e, 8k and 8l and numerical source data for 8f, 8g, 8h, 8i, 8j and 8m are provided.
Extended Data Fig. 9 | METTL14 regulates SASP gene enhancers. **a**, List of direct METTL14 target genes that are upregulated in senescent cells, downregulated by METTL14 knockdown and rescued by both wild-type and the R298P mutant METTL14 with increased binding of METTL14 (≥ 2 fold) in senescent cells. **b**, Enrichment of SASP genes among direct METTL14 target genes. **c**, Enrichment of SASP genes among genes with increased binding of co-localized METTL14 and H3K27ac in senescent cells compared with control cells (≥ 2 fold). **d**, Cut-and-run peaks of METTL3, NF-κB p65, METTL14 and H3K27ac on the SAA1 and SAA2 gene loci in control and RAS-induced senescent cells. **e**, ChIP–qPCR analysis of the association of H3K27ac with enhancers of the indicated SASP gene loci in control and senescent cells with or without METTL14 knockdown. **f**, Pearson correlation analysis of METTL14 with the indicated SASP genes in human liver captured and micro-dissected PanIN lesion samples based on the GSE43288 dataset. n = 13 biologically independent samples. P values were calculated using a Pearson correlation analysis. **g**, ChIP-qPCR analysis of the association of METTL14 with enhancers of the indicated SASP genes in control and senescent cells with or without IKK inhibitor Bay 11-7082 (5 μM) treatment for 48 hrs. Data represent mean ± s.e.m. in 9e and mean ± s.d. in 9g of three biologically independent experiments. P values were calculated using a two-tailed t-test except in 9b-c by a two-tailed Fisher exact test and a two-tailed Pearson correlation analysis in 9f. Numerical source data for 9e, 9f and 9g are provided.
Extended Data Fig. 10 | MTC is required for immune surveillance function of the SASP. **a**, Validation of METTL3 and METTL14 knockdown by qRT-PCR analysis in mouse NIH 3T3 cells. \( n = 3 \) biologically independent experiments. **b**, Validation of METTL3 and METTL14 knockdown by immunofluorescence analysis in mouse NIH 3T3 cells. Arrows point to dsRed-expressing shRen control, shMETTL3 or shMETTL14. Bar = 10\( \mu \)m. The experiment was repeated two times independently with similar results. **c**, Immunohistochemical staining of NRas in liver tissues injected with a mutant NRas\(^{V12/D38A}\) that is incapable of inducing senescence at day 6. The experiment was repeated in 3 biologically independent mice with similar results. Bar = 50\( \mu \)m. **d**, Quantification of CD45\(^+\)/NRas\(^+\) foci in the livers isolated from the indicated mice at day 6. \( n = 6 \) biologically independent mice per group. Data represent mean ± s.d. \( P \) values were calculated using a two-tailed \( t \)-test. Numerical source data for 10a and 10d are provided.
Reporting Summary

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Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

- The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
- A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- The statistical test(s) used AND whether they are one- or two-sided
- A description of all covariates tested
- A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) and variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted

Give P values as exact values whenever suitable.
- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes

Software and code

Policy information about availability of computer code

| Data collection | The amplification signal of qPCR data was acquired by QuantStudio™ Software V1.3. |
|-----------------|-----------------------------------------------------------------------------------|
| Data analysis   | Prism 7.0c, 8.4.2 was used for calculating p values. HOMER 4.10 was used for ChIP-seq peak calling. NIH ImageJ 1.48v was used for image analysis. |

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:
- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

Cut-and-run, ChIP-seq and RNA-seq data that support the findings of this study have been deposited in the Gene Expression Omnibus (GEO) under accession number: GSE141944 (RNA-seq for METTL3 knockdown and rescue; GSE159551; RNA-seq for METTL14 knockdown and rescue: GSE141993); cut-and-run and CHIP-seq: GSE141992; mRNA seq; GSE141993; and circRNA mRNA seq: GSE159550). For the correlation analysis between METTL14 and SASP genes in human laser capture and microdissected PanIN lesion samples, gene expression data were obtained from GEO (under accession code GSE43288). Source data for unprocessed immunoblots for Fig. 1a, 1e, 1h, 1c and Extended Data Fig. 4d, 5a, 5f, 5a, 8c, 8e, 8b, 8i and source data used for statistical analyses have been provided as Source Data files. All other data supporting the findings of this study are available from the corresponding author on reasonable request.
Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☑ Life sciences □ Behavioural & social sciences □ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/mr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size
For in vivo experiments, the sample size was estimated based on results from in vitro experiments. 6 mice per group for in vivo experiments. No statistical test was used to pre-determine the sample sizes.

Data exclusions
There was no exclusion from the experiments.

Replication
Experiments were repeated 3 times independently unless otherwise stated in figure legends and similar results were obtained.

Randomization
Experiments were all randomized.

Blinding
The investigators were blinded to group allocation during data collection.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

| n/a | Involved in the study |
|-----|-----------------------|
| ☑ | Antibodies |
| ☑ | Eukaryotic cell lines |
| ☑ | Palaeontology |
| ☑ | Animals and other organisms |
| ☑ | Human research participants |
| ☑ | Clinical data |

Methods

| n/a | Involved in the study |
|-----|-----------------------|
| ☑ | ChIP-seq |
| ☑ | Flow cytometry |
| ☑ | MRI-based neuroimaging |

Antibodies

Antibodies used
For m6A-seq, anti-m6A antibody (Synaptic Systems, Cat. No: 202003, 15 μg for 5 μg mRNA per IP-sequencing). For cut-and-run, ChIP and ChIP-sequencing: anti-METTL14 (Sigma, Cat. No: HPA038002, 5 μg per cut-and-run); anti-METTL3 (Proteintech, Cat. No: 15073-1-AP, 5 μg per cut-and-run); anti-NF-κB p65 (Abcam, Cat. No: 16502, 5 μg per cut-and-run); anti-RNA polymerase II (Santa Cruz Biotechnology, Cat. No: SC-47701, 10 μg per ChIP-seq); anti-FLAG (Sigma, Cat. No: F3165, clone M2, 2 μg per ChIP); anti-HA (Cell Signaling, Cat. No: 3724, 1:50 dilution for ChIP); IgG Isotype Control (Thermo Fisher Scientific, Cat. No: 10500C or 10400C). For immunoprecipitation and Western blot: anti-METTL3 (Abcam, Cat. No: 195352, 2 μg/IP, 1:1000 for Western blot); anti-METTL14 (Sigma, Cat. No: HPA038002, 2 μg/IP, 1:1000 for Western blot); anti-NF-κB p65 (Cell Signaling, Cat. No: 8242, 1:1000 for Western blot); anti-chosephospho-p65 (Cell Signaling, Cat. No: 3033, 1:50/IP); anti-GAPDH (Sigma, Cat. No: 68795, 1:10000 for Western blot); anti-p16 (Santa Cruz Biotechnology, Cat. No: sc-56330, 1:1000 for Western blot); anti-RAS (Becon Dickinson, Cat. No: 61000, 1:1000 for Western blot); anti-p21 (Abcam, Cat. No: 7960, 1:1000 for Western blot); anti-p53 (Millipore, Cat. No: OP43, 1:1000 for Western blot); anti-WTAP (Santa Cruz Biotechnology, Cat. No: sc-374280, 1:1000 for Western blot); anti-II1β (Abcam, Cat. No: 193852, 1:1000 for Western blot). For IHC/IF, anti- NRas (Santa Cruz Biotechnology, Cat. No: sc-31, 1:100), anti-CyD45 (BD PharmingenTM, Cat. No: 550539, 1:100) and anti-BrdU (Abcam, ab6326, 1:300). Secondary antibody for immunofluorescence: Alexa Fluor 488-conjugated goat anti-rabbit IgG (H+L) (Thermo Fisher Scientific, Cat. No: A-11008, 1:1000).

Validation
All antibodies were purchased from commercial vendors, validation information is available on the manufacturers’ websites.
### Eukaryotic cell lines

**Cell line source(s)**
IMR90 cells were obtained from ATCC. TOV21G ovarian cancer cell line was purchased from JCRB. 293FT packaging cells were purchased from Invitrogen. Phoenix packaging cells were obtained from Dr. Gary Nolan (Stanford University).

**Authentication**
Cell lines were re-authenticated by The Wistar Institute's Genomics Facility using short tandem repeat profiling using AmpFISTR Identifier PCR Amplification kit (Life Technologies).

**Mycoplasma contamination**
Regular Mycoplasma testing was performed using Lookout Mycoplasma PCR detection (Sigma). All cell lines used were tested negative for mycoplasma contamination.

**Commonly misidentified lines (See ICLAC register)**
No cell lines used in this study were found in the database of commonly misidentified cell lines that is maintained by ICLAC and NCBI Biosample.

### Animals and other organisms

**Laboratory animals**
Mice were maintained at 22–23°C with 40–60% humidity and a 12 hours light/12 hours dark cycle.
1. For xenograft model, 6-8 week-old female immunocompromised non-obese diabetic/severe combined immunodeficiency (NOD/SCID) gamma (NSG) mice were used.
2. For Hydrodynamic tail vein injection, 8-week old female C56L/J mice were used.

**Wild animals**
No wild animals were used in the study.

**Field-collected samples**
No field-collected samples were used.

**Ethics oversight**
All the protocols were approved by the IACUC of Wistar Institute or University of Pennsylvania.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

### ChIP-seq

**Data deposition**
- Confirm that both raw and final processed data have been deposited in a public database such as GEO.
- Confirm that you have deposited or provided access to graph files (e.g., BED files) for the called peaks.

**Data access links**
May remain private before publication.
[https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE141992](https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE141992)
[reviewer token edqbieuakqdup](reviewer token edqbieuakqdup)

**Files in database submission**
- fastq for ChIP-seq of input DNA of control cells, vector knockdown
- fastq for ChIP-seq of input DNA of senescent cells, vector knockdown
- fastq for ChIP-seq of Pol2 binding in control cells, vector knockdown
- fastq for ChIP-seq of Pol2 binding in senescent cells, vector knockdown
- fastq for ChIP-seq of Pol2 binding in senescent cells, shMETTL14 knockdown
- fastq for ChIP-seq of Pol2 binding in senescent cells, shMETTL3 knockdown
- fastq for cut-and-run of IgG binding in control cells
- fastq for cut-and-run of METTL3 binding in control cells
- fastq for cut-and-run of METTL14 binding in control cells
- fastq for cut-and-run of p65 binding in control cells
- fastq for cut-and-run of IgG binding in senescent cells
- fastq for cut-and-run of METTL3 binding in senescent cells
- fastq for cut-and-run of METTL14 binding in senescent cells
- fastq for cut-and-run of p65 binding in senescent cells
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- bigwig graph tracks bind.ctr.METTL14.bw
- bigwig graph tracks bind.ctr.p65.bw
- bigwig graph tracks bind.RAS.IgG.bw
- bigwig graph tracks bind.RAS.METTL3.bw
- bigwig graph tracks bind.RAS.METTL14.bw
Methodology

Replicates

experiments were performed with single replicates

Sequencing depth

75bp single end read run produced:
sample reads aligned
bind.ctr.input 23,281,736 21,646,612
bind.RAS input 28,885,405 26,943,867
bind.ctr.Pol2.shVEC 28,124,763 25,034,964
bind.RAS Pol2.shVEC 29,296,318 27,592,521
bind.RAS Pol2.shMETTL14 28,511,699 26,365,858
bind.RAS Pol2.shMETTL3 27,274,057 25,201,371
bind.ctr.IgG 54,795,510 47,046,255
bind.ctr.METTL3 37,259,699 33,931,680
bind.ctr.METTL14 39,495,407 34,841,138
bind.ctr.p65 43,909,436 37,667,691
bind.RAS IgG 57,209,879 43,018,297
bind.RAS METTL3 44,895,442 35,501,252
bind.RAS METTL14 47,460,973 42,642,698
bind.RAS p65 50,971,654 40,816,321

Antibodies

anti-METTL3 antibody (Proteintech, Cat. No: 15073-1-AP), anti-METTL4 antibody (Sigma, Cat. No: HPA038002), anti-p65 antibody (Abcam, Cat. No: 16502), anti-RNA polymerase II (Santa Cruz Biotechnology, Cat. No: SC-47701). IgG Isotype Control (Thermo Fisher Scientific, Cat. No: 10500C or 10400C).

Peak calling parameters

default parameters with -style histone option

Data quality

FDR<5%, fold>4 criteria were used. Numbers of peaks:
sample peaks
bind.ctr.METTL14 25,787
bind.ctr.METTL3 52,412
bind.ctr.p65 127,392
bind.RAS METTL14 184,323
bind.RAS METTL3 97,091
bind.RAS p65 47,701
bind.ctr.Pol2.shVEC 21,393
bind.RAS Pol2.shVEC 26,310
bind.RAS Pol2.shMETTL14 30,798
bind.RAS Pol2.shMETTL3 29,845

Software

HOMER 4.10 algorithm was used to call peaks and generate bigwig tracks