1 **Locus-specific induction of gene expression from heterochromatin loci during**
2 **cellular senescence**

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36

37 **Abstract**

38 Senescence is a fate-determined state, accompanied by reorganization of
39 heterochromatin. While lineage-appropriate genes can be temporarily repressed
40 through facultative heterochromatin, stable silencing of lineage-inappropriate genes
41 often involves the constitutive heterochromatic mark, histone H3K9me3. The fate of
42 these heterochromatic genes during senescence is unclear. Here we show a small
43 number of lineage-inappropriate genes, exemplified by the *LCE2* skin genes, are
44 derepressed during senescence from H3K9me3 regions in fibroblasts. DNA FISH
45 experiments reveal that these gene loci, which are condensed at the nuclear
46 periphery in proliferative cells, are decompacted during senescence. Decompaction
47 of the locus is not sufficient for *LCE2* expression, which requires p53 and C/EBP β
48 signalling. *NLRP3*, which is predominantly expressed in macrophages from an open
49 topologically associated domain (TAD), is also derepressed in senescent fibroblasts
50 due to the local disruption of the H3K9me3-rich TAD that contains it. *NLRP3* has
51 been implicated in the amplification of inflammatory cytokine signalling in senescence
52 and aging, highlighting the functional relevance of gene induction from 'permissive'
53 H3K9me3 regions in senescent cells.

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61 Senescence is characterized by persistent proliferative arrest triggered by diverse
62 stimuli, including excessive mitotic stress induced by persistent oncogene activation
63 as in oncogene-induced senescence (OIS)¹. Senescent cells secrete various factors,
64 including cytokines, growth factors, and extracellular matrix modifying enzymes,
65 which have a profound impact on the tissue environment². The senescence-
66 associated secretory phenotype (SASP) can have adverse effects in vivo, particularly
67 when it persists, and contributes to aging and age-associated disorders, including
68 cancer²⁻⁴.

69

70 Senescence is often accompanied by alterations to the chromatin state^{5,6}. Active
71 chromatin marks and chromatin accessibility are dynamically altered during
72 senescence, and such alterations are well correlated with the senescence-associated
73 gene expression profile, including the SASP⁷⁻¹¹. In contrast, heterochromatic marks,
74 particularly H3K9me3, are largely static during OIS in some contexts¹²⁻¹⁴. However,
75 the higher-order structure of the heterochromatic regions can be drastically altered
76 during senescence. For example, senescence-associated heterochromatin foci
77 (SAHFs) are formed through the spatial reorganization of pre-existing H3K9me3- and
78 H3K27me3-marked chromatin^{12,14}. Senescence-associated distension of satellites
79 (SADS), which involves the decompaction of (peri-)centric constitutive
80 heterochromatin, is also observed without alteration of H3K9me3 and H3K27me3
81 levels at these regions^{9,15}. These studies suggest that the 3D structural alterations of
82 heterochromatic regions during senescence can occur without loss or gain of
83 repressive marks.

84

85 Epigenetic gene regulation is critical for establishing cell types and lineages. Tissue
86 specific gene silencing is typically attributed to facultative heterochromatin, marked
87 by H3K27me3. In committed progenitor cells, it has been suggested that H3K27me3
88 is involved in the silencing of lineage-specific genes that are induced upon terminal

89 differentiation¹⁶. In contrast, H3K9me3 is mainly enriched in the non-coding parts of
90 the genome (e.g., repetitive regions) regardless of the cell lineage, consistent with its
91 close association with constitutive heterochromatin. However, it has become evident
92 that H3K9me3-enriched heterochromatin also contributes to silencing of genes
93 specifically expressed in other tissues (i.e., lineage-inappropriate genes), thereby
94 ensuring the stability of cell identity^{16,17}. However, how such cell type specific genes
95 are regulated during senescence is not clear.

96
97 Here we show evidence that lineage-inappropriate genes can be expressed from
98 H3K9me3-heterochromatic domains during senescence. This senescence-
99 associated 'leakage' of lineage-inappropriate genes is accompanied by the physical
100 decompaction of a subset of the heterochromatic regions, which gain active
101 chromatin features. This is in marked contrast to the derepression mechanism for
102 lineage appropriate genes during terminal differentiation. We propose that the
103 selective derepression of genes embedded in heterochromatic regions represents a
104 distinct gene expression mechanism, which transcends the epigenetic barrier in
105 differentiated cells.

106

107 **Results**

108 ***Aberrant expression of lineage-inappropriate genes during senescence***

109 While investigating our previously published transcriptomic datasets in IMR90 human
110 diploid fibroblasts (HDFs) induced into senescence by different stressors^{18–20}, we
111 noticed that multiple *Late cornified envelope (LCE)* genes, mainly in the *LCE2*
112 subcluster, were up-regulated in oncogenic HRAS^{G12V}-induced senescence (RIS) or
113 DNA damage-induced senescent (DDIS) IMR90 cells. This was surprising, since the
114 *LCE* genes (consisting mainly of three sub-clusters *LCE1-3*) represent a tissue-
115 specific gene cluster. The *LCE* gene cluster is located in the Epidermal Differentiation
116 Complex (EDC) (~1.5 Mb) locus on chromosome 1q21, which contains a number of

117 cornification genes that are induced during epidermal terminal differentiation in the
118 stratum corneum^{21,22}. These structural proteins are then enzymatically cross-linked to
119 reinforce cornification at the very late stage of epidermal differentiation. Thus, the
120 *LCE* genes are lineage-inappropriate in fibroblasts. Indeed, expression of genes
121 within the EDC locus was barely detectable in IMR90 cells in any of the other
122 conditions we tested, including not only the normal proliferative condition, but also
123 quiescence, transformation (via co-expression of HRAS^{G12V} and the adenoviral
124 oncoprotein E1A¹⁸), and acute DNA damage (Extended Data Fig. 1a). The p53 tumor
125 suppressor plays a critical role in senescence¹, and the upregulation of most *LCE*
126 genes during senescence was diminished upon introduction of a short-hairpin against
127 p53 (sh-p53) in RIS cells (Extended Data Fig. 1a), reinforcing the correlation between
128 upregulation of some *LCE* genes in fibroblasts and the senescence phenotype. A
129 similar induction of *LCE* genes was also observed in multiple publicly available
130 datasets for senescent HDFs with different senescence triggers, except for a dataset
131 of BJ replicative senescence (RS) (Extended Data Fig. 1b)^{23–25}. This led us to
132 hypothesize that a potentially new mechanism for derepressing tissue-specific genes
133 in non-orthotopic tissues underlies the *EDC gene* induction during senescence.

134

135 We first validated the expression of the LCE2 proteins (encoded by *LCE2A-D*) in
136 IMR90 cells. While LCE2, probed by an anti-pan-LCE2 antibody²⁶, was undetectable
137 in proliferating cells, RIS IMR90 cells exhibited a comparable amount of LCE2 to
138 differentiated human keratinocytes (Fig. 1a). Involucrin, encoded by another EDC
139 gene *IVL*, was induced during keratinocyte differentiation, as expected, but it was
140 also modestly detected in RIS fibroblasts (Fig. 1a). Consistent with the transcriptomic
141 data (Extended Data Fig. 1a), the levels of the LCE2 proteins during RIS were
142 reduced when p53 was knocked-down (Extended Data Fig. 1c). To gain insight into
143 the mechanism for the aberrant expression of these genes, we next characterized
144 the chromatin state of the EDC locus in IMR90 cells using ChIP-seq and RNA-seq

145 datasets, which we have previously published^{12,14,20}. We found that, in both
146 proliferative and RIS IMR90 cells, the majority of the EDC locus was enriched for
147 H3K9me3 flanked by H3K27me3 (Fig. 1b). Consistently, our RNA-seq analysis
148 revealed that the transcriptional activity of the genes within the EDC of proliferative
149 IMR90 cells was very low. Despite the apparent lack of reduction in the H3K9me3
150 coverage (if anything there was a slight increase) on the EDC during RIS (Fig. 1b, c),
151 the central region of the EDC, surrounding the *LCE2* genes, became transcriptionally
152 active (Fig. 1b). We next examined the permissive nature of transcription at this
153 region by ATAC-seq (Assay for Transposase-Accessible Chromatin using
154 sequencing²⁷) and H3K27ac ChIP-seq¹⁰. ATAC-seq maps chromatin accessibility,
155 whereas H3K27ac is associated with active transcription and, together with high
156 chromatin accessibility, marks active regulatory regions (promoters/enhancers)²⁸.
157 The EDC locus of RIS, but not proliferative, IMR90 cells exhibited H3K27ac
158 enrichment, which coincided with ATAC-seq peaks ~50 kb downstream of the *LCE2*
159 cluster, suggesting that active enhancers are formed within the 'heterochromatic'
160 EDC during RIS, albeit modest ones (Fig. 1d). These data suggest that tissue-
161 specific genes can be expressed from H3K9me3-rich regions in non-orthotopic
162 tissues during senescence. In contrast, while a cluster of olfactory receptor (*OR*)
163 genes, another tissue-specific genes on chromosome 1q22²⁹, was also broadly
164 enriched for H3K9me3, this cluster did not produce any detectable transcripts, nor
165 H3K27ac enrichment or ATAC-seq peaks in either proliferating or RIS IMR90 cells
166 (Fig. 1d). Thus, the mechanism of gene expression from H3K9me3-heterochromatin
167 appears to be locus specific.

168

169 A potential tumor suppressor activity of LCE1 (homologous to LCE2 and also
170 encoded from the EDC locus) has previously been proposed³⁰. To test whether
171 ectopic expression of an LCE2 protein promotes replicative exhaustion in normal
172 fibroblasts, we overexpressed LCE2A in IMR90 cells and performed population

173 doubling and colony formation assays. LCE2A-expressing cells showed a reduced
174 capacity in proliferation and colony formation (Extended Data Fig. 1d, e). These
175 results potentially suggest a functional relevance of the derepression of *LCE* genes in
176 normal fibroblasts.

177

178 ***Distinct epigenetic landscapes of EDC genes in senescent cells relative to***
179 ***keratinocytes***

180 To directly compare the chromatin status of the EDC between fibroblasts and
181 keratinocytes, we performed ChIP-seq for key histone marks before and after
182 terminal differentiation in primary human keratinocytes. It has been suggested that
183 EDC genes in undifferentiated keratinocytes are facultatively silenced by the
184 polycomb repressive complexes (PRCs) and the corresponding repressive histone
185 mark H3K27me3^{31,32}. We confirmed these observations using ChIP-seq. In contrast
186 to IMR90 fibroblasts, H3K27me3, but not H3K9me3, was enriched across the EDC
187 locus in keratinocytes (Fig. 1b). We also confirmed that the H3K27me3 level was
188 reduced during differentiation, whereas H3K27ac-peaks were increased (Fig. 1b, c).
189 These data indicate that the nature and dynamics of the epigenetic landscape at the
190 EDC locus is different between keratinocyte differentiation (where EDC genes are
191 lineage-appropriate) and senescence in fibroblasts (where EDC genes are lineage-
192 inappropriate).

193

194 ***The EDC locus is structurally altered in senescent fibroblasts***

195 Senescence is often accompanied by alterations of higher-order chromatin
196 structure³³, highlighted by both heterochromatin reorganization (represented by
197 SAHFs³⁴) and decompaction (represented by SADS¹⁵) without substantial alterations
198 in H3K9me3. To characterize the chromatin structure of the EDC, we performed DNA
199 fluorescence *in situ* hybridization (FISH) experiments with multiple Bacterial Artificial
200 Chromosome (BAC) probes tiled across the EDC locus (Fig. 2a, bottom). In

201 proliferating IMR90 cells, these FISH signals were highly condensed and tended to
202 be located at the nuclear periphery, which provides a repressive environment (Fig. 2b,
203 Extended Data Fig. 2a). In contrast, in RIS IMR90 cells, FISH experiments using the
204 same probes exhibited a decompacted pattern with an extended fluorescent signal
205 length (Fig. 2b, c). Although less prominent, EDC decompaction was also observed
206 in DNA damage-induced senescence (DDIS) (d7) and replicative senescence (RS)
207 IMR90 cells (Fig. 2c). It was not detected after acute DNA damage (acDD) (d1) or in
208 senescence-bypassing transformed cells expressing E1A+RAS, suggesting that the
209 EDC decompaction is specifically associated with senescence (Fig. 2c). Of note, the
210 morphology of the OR locus, which was not derepressed during RIS (Fig. 1d),
211 remained unchanged in RIS IMR90 cells (Extended Data Fig. 2b).

212

213 In primary keratinocytes isolated from human skin, the EDC FISH probes also
214 exhibited a compacted pattern and we failed to detect any significant decompaction
215 during differentiation (Fig. 2b, c). Interestingly, this locus was located at the nuclear
216 interior, which is often transcriptionally permissive, both before and after
217 differentiation (Extended Data Fig. 2a), consistent with a previous study showing that
218 relocation of the EDC locus from nuclear periphery to inner nuclear regions already
219 occurs during embryonic development, at least in mice³⁵. This further supports the
220 notion that distinct mechanisms derepress the EDC genes in senescent fibroblasts
221 relative to differentiated keratinocytes.

222

223 We next conducted a time series analysis of the EDC morphology using IMR90 cells
224 expressing oncogenic HRAS^{G12V} in a 4-hydroxytamoxifen (4OHT) inducible form
225 (ER:HRAS^{G12V}), where senescence is progressively established over ~6 days¹⁹. Our
226 FISH analysis showed that the EDC signal length became increasingly extended
227 over the time course (Fig. 2d). Similarly, LCE2 expression was also more evident at
228 later time points (Fig. 2e). Consistently, in our published microarray data¹⁹, *LCE2*

229 upregulation was most prominent at the late stage of RIS (Extended Data Fig. 1a),
230 indicating that local decompaction of the EDC and derepressing of the *LCE2* genes
231 are temporally correlated during RIS. It has been shown that SADS is established
232 during the early stages of senescence¹⁵, whereas SAHFs, which involve the
233 chromosome-wide spatial segregation of transcriptionally active (peri-SAHFs) and
234 inactive (intra-SAHFs) regions, progressively accumulate during senescence³⁶.
235 Although decompaction of the EDC is reminiscent of SADS, the kinetics of the EDC
236 decompaction appeared to be more similar to the kinetics of SAHF formation (Fig. 2f).
237 Within RIS cell populations, the EDC was significantly decompacted in SAHF-positive
238 compared to SAHF-negative cells (Extended Data Fig. 2c). Immunolabelling analysis
239 also revealed that the majority of SAHF-positive cells were LCE2-positive (Extended
240 Data. Fig. 2d). These data reinforce a close correlation between EDC decompaction
241 and SAHF formation, although any mechanistic relationship between SADS and EDC
242 decompaction cannot be excluded. In agreement with this, in IMR90 cells, EDC
243 decompaction was most prominent in RIS IMR90 cells compared to DDIS and RS
244 IMR90 cells, where SAHF formation is modest (Fig. 2c, Extended Data Fig. 2e). Of
245 note, *LCE* gene induction in DDIS and RS was also weaker than in RIS (Extended
246 Data Fig. 1b) and LCE2 protein levels were undetectable or variable in DDIS or RS
247 IMR90 cells, respectively (Extended Data Fig. 2f). In addition, we have shown that
248 different HDFs develop SAHFs to varying degrees: for example, SAHF-formation was
249 weak in BJ cells, compared to IMR90 and WI38 cells during RIS (Extended Data Fig.
250 2g)³⁷. Up-regulation of LCE2 protein levels in RIS BJ cells was marginal with no
251 significant decompaction of the EDC (Extended Data Fig. 2h, i).

252

253 We next examined a spatial correlation between SAHFs and the structure of the EDC
254 locus. In the multi-probe FISH analyses, the EDC appeared to be located at inter-
255 SAHFs 'open' regions (Fig. 2b, Extended Data Fig. 2a). To validate this, we
256 conducted FISH experiments co-staining both the EDC and OR loci (using a single

257 probe for each locus, as depicted in Extended Data Fig. 2b, top) and found that the
258 colocalization frequency of the OR locus with SAHFs was significantly higher than
259 that of the EDC locus with SAHFs (Fig. 2g). Interestingly, both the EDC and OR loci
260 were enriched for Lamin B1 (LMNB1) and each formed a single lamina-associated
261 domain (LAD) (Fig. 2a)¹⁴. Lamin B1 levels are reduced during senescence³⁸ and we
262 have previously shown that this reduction in Lamin B1 occurs predominantly from
263 H3K9me3 regions, and that this liberation of H3K9me3 heterochromatic regions from
264 the nuclear envelope is correlated with SAHF formation¹². Indeed, the reduction in
265 Lamin B1 was particularly prominent at the OR locus, compared to the EDC locus
266 (Fig. 2a), further supporting the model that SAHF formation involves a spatial
267 reorganization of pre-existing heterochromatin¹². These data suggest that at these
268 two lineage-specific gene clusters, both of which are enriched for H3K9me3 in HDFs,
269 distinct gene regulatory mechanisms operate: the EDC locus is derepressed outside
270 of SAHFs by physical decompaction, while the OR locus is incorporated into SAHFs
271 and remains condensed and silenced.

272

273 To test the direct correlation between EDC decompaction and SAHF formation, we
274 knocked-down HMGA1, which is an essential structural component of SAHFs, during
275 RIS induction (RAS+shHMGA1) (Fig. 3a)³⁷. The EDC decompaction was partially, but
276 significantly, inhibited in RAS+shHMGA1 cells although EDC decompaction in these
277 cells was still significant compared to the basal level (Fig. 3b). RNA-seq analysis
278 revealed a trend of reversal of the derepression of most EDC genes in
279 RAS+shHMGA1 cells (Fig. 3c). Consistently, the induction of LCE2 proteins was also
280 weaker in the presence of shHMGA1 (Fig. 3d). These data collectively suggest that
281 HMGA1-dependent heterochromatin compartmentalization somehow promotes the
282 EDC decompaction/derepression possibly through an indirect mechanism.

283

284 Ectopic expression of p16 (a CDK-inhibitor) is sufficient for inducing SAHF-positive
285 senescence³⁴. However, as reported³⁹, these cells showed a distinct gene expression
286 profile, lacking typical persistent DNA damage response (pDDR) and inflammatory
287 senescence-associated secretory phenotype (SASP) (Fig. 3e). To further gain insight
288 into the relation between EDC decompaction and these senescence effector
289 phenotypes, we measured EDC decompaction and gene expression from this locus
290 in p16-induced senescent IMR90 cells. While less pronounced than in RIS cells, p16-
291 senescent cells exhibited both SAHFs and EDC decompaction (Fig. 3f, g). Our RNA-
292 seq data, however, showed little change in the EDC genes in p16-senescent cells,
293 compared to RIS or even DDIS, the latter in which SAHF formation and derepression
294 of the EDC genes were milder than in the RIS cells (Fig. 3h). The data suggest that
295 SAHFs and EDC decompaction are not sufficient for the EDC gene induction, which
296 probably requires additional signalling or specific transcription factor activation.

297

298 One candidate for such signalling is the p53 pathway: consistent with the literature³⁹,
299 the p53 downstream target, p21, was not upregulated in p16-senescent IMR90 cells
300 (Fig. 3e). Additionally, consistent with the immunoblotting results (Extended Data Fig.
301 1c), induction of *LCE2* genes (except for *LCE2A*) during RIS was partially p53-
302 dependent (Fig. 3i, j). Thus, we tested whether the *LCE2* genes are direct targets of
303 p53. We reanalyzed our p53 ChIP-seq data¹⁸ and found that p53 mainly binds the
304 *LCE2A* gene body, concomitant with increased deposition of H3K27ac (an active
305 enhancer mark), in RIS cells (Fig. 3k). These results suggest that p53 may control at
306 least some EDC genes through directly binding to the intragenic enhancer during RIS,
307 although p53-dependent downstream signalling may also contribute. Notably, p53-
308 depletion had no significant impact on the EDC decompaction (Fig. 3l).

309

310 The level of a key transcription factor that drives the inflammatory SASP, , is
311 upregulated during RIS⁴⁰. Consistent with the literature³⁹, the SASP was largely

absent in p16-induced senescent cells, where we found little upregulation of *CEBPB* (Fig. 3e). Interestingly, induction of the *LCE2* genes during RIS was inhibited by depletion of C/EBP β (Fig. 3i, j). This prompted us to map C/EBP β binding sites in RIS cells. We detected C/EBP β peaks around the promoters of *LCE2* genes, suggesting that the *LCE2* genes may be C/EBP β -targets. Furthermore, co-depletion of p53 and C/EBP β showed stronger effects than singular knockdown (Fig. 3j), suggesting a co-regulation of *LCE2* genes by these two transcription factors, at least in part through direct binding. Together, our data indicate that deregulation of the EDC locus consists of two steps: first, decompaction of the locus and second, activation of specific signalling and/or transcription factors.

322

323 ***Aberrant expression of lineage-specific genes and chromatin decompaction***

Chromatin accessibility has been linked with cell-type-selective regulatory elements⁴¹, reflecting cell fate and maturity⁴². It has been shown that chromatin accessibility is increased during senescence^{9,10}. To test whether senescence-associated lineage-inappropriate gene expression is unique to the EDC genes, we searched for similar H3K9me3 domains that encompass both H3K27ac and ATAC-seq peaks in proliferative and RIS IMR90 cells, using high confidence data sets of H3K9me3, H3K27ac and ATAC-seq peaks (Materials and Methods).

331

While the number of H3K9me3 peaks were relatively stable with 763 common peaks (805 in proliferative cells; 874 in RIS cells¹²), we previously showed that the number of ATAC-seq peaks (73,946 in proliferative cells; 238,269 in RIS cells) and H3K27ac peaks (95,464 in proliferative cells; 117,385 in RIS cells) increase overall during RIS in IMR90 cells¹⁰. Consistent with previous studies⁴³, H3K27ac peaks mostly coincided with ATAC-seq peaks (Extended Data Fig. 3a).

338

339 Using the information about these active marks as well as gene expression (RNA-
340 seq data we previously published²⁰), we characterized H3K9me3 peaks: 136 peaks
341 (defined in the RIS condition) contained both de novo ATAC- and H3K27ac peaks
342 ('permissive' H3K9me3 peaks). 110 of these peaks showed no induction of gene
343 expression, whereas 26 peaks were accompanied by significant upregulation of gene
344 expression, including the *LCE* genes. Thus, we grouped these H3K9me3 peaks as
345 follows: group 1 (non-permissive; 627 peaks): H3K9me3 peaks with no *de novo*
346 ATAC-seq and H3K27ac peaks, group 2 (110 peaks): permissive (both de novo
347 ATAC-seq and H3K27ac peaks) without gene activation, and group 3 (26 peaks):
348 permissive with gene activation (Fig. 4a, Extended Data Fig. 3b). The permissive
349 H3K9me3 peaks (group 2+3), regardless of altered gene expression, appeared to be
350 larger in width compared to the non-permissive peaks (group 1) (Fig. 4b). However,
351 group 3 of H3K9me3 peaks were significantly higher in gene density and were less
352 AT-rich than group 2 (Fig. 4c, d). Among repeat elements, group 3 peaks were
353 enriched for short-interspersed nuclear elements (SINEs), which are often found in
354 GC-rich regions and in gene promoters⁴⁴ (Fig. 4e, f, Extended Data Fig. 3c). As
355 exemplified by the EDC locus mentioned above (Fig. 2a), group 3 H3K9me3 peaks
356 exhibited the least pronounced reduction of Lamin B1 during RIS (Fig. 4g-i). Thus,
357 permissive H3K9me3 peaks with aberrant gene induction (group 3) appear to have
358 distinct features characterized by higher gene density with residual Lamin B1
359 association.

360

361 We next explored individual genes within group 3 H3K9me3 peaks (a list of genes
362 included in each H3K9me3-peak group can be found in Supplementary Table 1).
363 Close visual inspection revealed that some 'active regulatory regions' (i.e., co-
364 occurring H3K27ac and ATAC-seq peaks) overlapped permissive H3K9me3 peaks
365 either in regions with H3K9me3 signal or within small gaps in H3K9me3 in either the
366 proliferative or RIS conditions (Extended Data Fig. 3d). Using stringent criteria (both

gene bodies and promoters with *de novo* ATAC/H3K27ac peaks entirely overlapping H3K9me3 peaks and not gaps in the H3K9me3 ChIP-seq signal), we identified 8 genes in 6 loci (*PDPN*, *LCE2A*, *LCE2C*, *IVL*, *NLRP3*, *DCC*, *TMEM132B*, and *ZNF667*) among the 38 significantly up-regulated genes during RIS in group 3 H3K9me3 peaks (see highlights in Fig. 4b-i). Note, Involucrin (encoded by *IVL* within the EDC locus) was also detected in RIS fibroblasts (Fig. 1a). To address the orthotopic expression status for these aberrantly expressed genes in normal tissues, we compared our RNA-seq data²⁰ with publicly available RNA-seq data obtained from The Human Protein Atlas (HPA)⁴⁵. The basal expression levels of these genes in IMR90 cells were mostly low and they tended to show 'tissue-enriched' expression patterns, according to the HPA criteria (Fig. 5a, Extended Data Fig. 4a). Thus, the H3K27ac/ATAC peaks established within H3K9me3 domains during senescence may mark aberrant inductions of lineage-inappropriate genes.

To test whether derepression of these genes during senescence is commonly accompanied by chromatin decompaction, we performed multi-probe DNA FISH analysis for *NLRP3*, *DCC* and *TMEM132B* loci (BAC probe positions in Fig. 5b; the H3K9me3-rich regions encompassing *PDPN* and *ZNF667* were too small, ~ 200 kb and 150 kb, respectively, for the multi-probe FISH experiment, in which each BAC probe is ~200 kb long). Similar to the EDC locus, all loci tested were highly condensed in proliferative IMR90 cells, but the FISH signal lengths were significantly extended in RIS cells (Fig. 5b-d, Extended Data Fig. 4b).

Decompaction of H3K9me3 domains correlates with expression of lineage-inappropriate but not lineage-appropriate genes during senescence

To further investigate whether heterochromatin decompaction during senescence is associated with lineage-inappropriate genes, we decided to take advantage of a RIS model in human keratinocytes, in which the EDC is a lineage appropriate gene

395 cluster. We first profiled EDC gene expression in RIS keratinocytes and compared to
396 the differentiation condition using RNA-seq. In contrast to RIS fibroblasts, the EDC
397 genes were widely expressed in RIS keratinocytes, exhibiting a similar pattern to
398 differentiated keratinocytes (Fig. 6a). This is consistent with previous studies showing
399 that some EDC genes are induced in senescent keratinocytes, induced by oncogenic
400 HRAS or oxidative stress^{46,47}. Next, we examined the EDC condensation status. As a
401 comparison, we chose the *DCC* locus, identified in this study as differentially
402 expressed in proliferating compared to RIS fibroblasts. *DCC* encodes a netrin 1
403 receptor, which is expressed mainly in testis, lung and brain⁴⁵. In contrast to the EDC,
404 the *DCC* locus was embedded in a H3K9me3 region in both IMR90 cells and
405 undifferentiated keratinocytes, but not in hES cells (Fig. 6b), suggesting that *DCC*
406 becomes stably heterochromatinized in both fibroblasts and keratinocytes during
407 development; thus, *DCC* is lineage-inappropriate in keratinocytes. We performed
408 additional FISH experiments for the *DCC* and EDC loci in these three cell types: hES
409 cells, fibroblasts (control and RIS conditions), and keratinocytes (control and RIS
410 conditions). Similar to IMR90 fibroblasts, we found a significant decompaction of
411 *DCC* in RIS keratinocytes (Fig. 6c). Consistently, the mRNA levels of *DCC* were
412 modestly but significantly increased in the RIS keratinocytes accompanied by the up-
413 regulation of key SASP factors associated with senescence (Fig. 6d). Thus, the
414 senescence-associated decompaction of lineage inappropriate heterochromatic
415 genes is not limited to fibroblasts. In contrast, there was no substantial chromatin
416 decompaction of the EDC in RIS keratinocytes (Fig. 6c). This reinforces the link
417 between aberrant expression of inappropriate genes and their decompaction.

418

419 Importantly, the decompacted chromatin state was not detected in hES cells for
420 either the EDC or *DCC* loci (Fig. 6c), where chromatin in general tends to be more
421 'open', exemplified by the *DCC* region (Fig. 6b)^{48,49}. Therefore, senescence-

422 associated decompaction of these heterochromatic loci is a distinct phenotype from
423 the 'open' chromatin configuration often seen in ES cells.

424

425 **Multiple mechanisms might be involved in decompaction of H3K9me3 regions**

426 In previous Hi-C studies, an increase in long-range interaction between H3K9me3
427 regions during senescence has been correlated with the detachment of the
428 H3K9me3 regions from nuclear lamina, reduced interaction within the H3K9me3
429 regions, and SAHF formation (clustering of the H3K9me3 regions)^{25,33,50,51}.
430 Consistently, in our recently generated in situ Hi-C data in proliferative and RIS
431 IMR90 cells⁵², we observed that increased chromatin contacts during RIS tended to
432 be longer-range compared to decreased contacts, which were mostly short distance
433 (Extended Data Fig. 5a, b). We further characterized RIS-associated dynamic
434 chromatin interactions focusing on H3K9me3 loci. In marked contrast to the features
435 of H3K9me3 regions that are potentially involved in SAHFs (i.e., increased inter-
436 region contacts, decreased intra-region contacts, and reduced Lamin B1
437 occupancy)^{14,25,50}, group 3 H3K9me3 peaks (defined in Fig. 4) exhibited the weakest
438 interaction increase with other H3K9me3 peaks (Fig. 7a, b). Interestingly, group 2 of
439 H3K9me3 peaks (permissive without increased gene expression) showed more
440 increased contacts than not only group 3 but also group 1. The nature of group 2
441 H3K9me3 peaks and their involvement in higher-order heterochromatin
442 reorganization during RIS is unclear. Notably, the reduction in Lamin B1 occupancy
443 in group 2, similar to group 1, was significantly stronger than group 3 (Fig. 4i); thus,
444 group 2 of H3K9me3 regions might play some role in heterochromatin reorganization.

445

446 We next examined individual gene loci. For example, the EDC locus showed reduced
447 interaction with other H3K9me3 peaks (Fig. 7c, 'LCE2, IVL') but increased contacts
448 within the locus (Fig. 7d). We reanalyzed Hi-C data generated in WI38 fibroblasts²⁵
449 and found a similar alteration in the EDC locus during replicative senescence (RS,

Fig. 7e). Hi-C mapping in human keratinocytes was recently published⁵³. We reanalyzed the data and compared them with the senescent fibroblasts data. In keratinocytes, new loop structures were established within the EDC locus during terminal differentiation (Fig. 7f, Extended Data Fig. 5c). Interestingly, the new contacts in the EDC locus in RIS IMR90 and RS WI38 fibroblasts resembled the loops identified in keratinocytes (Fig. 7d-f, arrows). Together with the FISH data (Fig. 2b, g), this suggests that the EDC locus escaped from SAHFs and decompacted at the peri-SAHF region, where it gained active 3D regulatory interactions. In contrast, *NLRP3*, another gene that was derepressed from a H3K9me3 region, exhibited a highly distinct pattern. *NLRP3* is located near the border of a H3K9me3-rich topologically associating domain (TAD) in IMR90 fibroblasts (Fig. 7g). TADs are megabase-sized chromatin regions, within which genomic regions interact with each other with high frequency⁵⁴⁻⁵⁷. These proximal chromatin contacts around *NLRP3* were largely lost and thus the TAD structure was disrupted in fibroblasts (Fig. 7g, arrows). *NLRP3* is predominantly expressed in immune cells, such as macrophages, and the encoded protein NLRP3 is a component of the NLRP3-inflammasome, a critical mediator of innate immune responses^{58,59}. In addition, the role of NLRP3 in inflammatory SASP in fibroblast senescence has been demonstrated⁶⁰. We reanalyzed public Hi-C and ChIP-seq datasets in THP1 cells, a spontaneously immortalized human monocytic cell line. *NLRP3* was located within an open region in THP1 cells and the TAD disruption seen in RIS fibroblasts was not detected during macrophage differentiation, which is accompanied by *NLRP3* upregulation (Fig. 7h)^{61,62}, reinforcing the functional relevance of the unique mechanism for senescence-specific activation of genes that are otherwise tightly silenced within H3K9me3 regions. Interestingly, we recently identified a similar TAD disruption, in which genes, best exemplified by *NRG1* (encoding a SASP factor), can escape from a H3K27me3 repressive 3D environment during RIS⁵². Indeed, ChIP-seq data showed a reduction of H3K27me3 in this region (Extended Data Fig. 5d). Together,

our data suggest that the mechanisms for decompaction of the lineage-inappropriate genes within H3K9me3 occupied regions might differ between gene loci.

A recent study reported induction of *Nlrp3* in synovial fibroblasts (SFs) in a mouse arthritis model⁶³. SFs isolated from mouse paws exposed to a danger signal, monosodium urate crystals (MSU), twice (MSU/MSU), but not once (-/MSU), exhibited upregulation of *Nlrp3*. Such a delayed alteration in gene expression is reminiscent of a senescent-like phenotype. We reanalyzed their RNA-seq data (Extended Data Fig. 5e) and confirmed the significant upregulation of *Nlrp3*, alongside that of cytokines that are associated with senescence. Moreover, MSU/MSU SFs exhibited senescence features and significant induction of several EDC genes, compared to -/MSU SFs. Notably, rodents lack the *Lce2* sub-cluster, but instead they have an expansion of the *Lce1* genes⁶⁴. Although epigenomic characterization is required for further interpretation, this result suggests EDC genes and *Nlrp3* can be expressed in senescent fibroblasts *in vivo*.

Discussion

In this work, we provide evidence that senescence is associated with the disruption of the H3K9me3-mediated 'lineage barrier' at specific gene loci. This results in the 'leaky' expression of specific heterochromatic genes, which are otherwise tightly silenced. This process is accompanied by a localized decompaction and increased accessibility of chromatin, without apparent loss of H3K9me3. We show that the same genes can be induced via distinct mechanisms: the 'default' mechanism of orthotopic cell types and a senescent-associated mechanism in ectopic cell types. Notably, we demonstrate that the default mechanism appears to be dominant when those genes are induced in senescent orthotopic cells; therefore, the initial cell type-specific chromatin states are perhaps critical for how these genes are derepressed in response to stress. Considering that decompaction of those gene loci was closely

506 correlated with SAHF formation, it might be an integrated part of high-order
507 chromatin structural reorganization, specifically occurring during senescence.
508
509 NLRP3-inflammasome has been implicated in aging and is considered a therapeutic
510 target against age-associated inflammatory diseases^{65,66}. Targeting senescent cells
511 is an emerging anti-aging therapeutics approach, including 'senolytics' (drugs that
512 selectively kill senescent cells) or 'senomorphics' (drugs that suppress the SASP or
513 any other adverse functionality of senescent cells)⁶⁷. Our data suggest that NLRP3
514 might be a potential senomorphic target^{69,60}. Currently it is not clear whether the other
515 genes identified in this study are involved in the senescence phenotype. However, it
516 was shown that *LCE1* is a downstream target of p53 in cancer cell lines and inhibits
517 the arginine methyltransferase, PRMT5, which is often upregulated in various
518 cancers and a potential therapeutic target⁶⁸. PRMT5 was also shown to have anti-
519 senescence activity in osteosarcoma cells⁶⁹. While ectopic LCE2A limited cell
520 proliferation (Extended Data Fig. 1d, e), it remains to be elucidated whether or not
521 LCE2 inhibits PRMT5 in senescent fibroblasts. *DCC*, *ZNF667*, and *PDPN* have also
522 been implicated in cancer and rheumatoid arthritis⁷⁰. For example, Netrin 1 receptor,
523 encoded by *DCC*, is involved in axon guidance and *DCC* has been identified as a
524 tumor suppressor gene in ectopic tissues, including colon and rectum^{71,72}. A similar
525 aberrant derepression mechanism might apply in other pathological contexts, such
526 as early tumorigenesis and chronic inflammation.

527

528 **Materials and Methods**

529 **Cell culture**

530 Human diploid fibroblasts (HDFs) (IMR90, WI38 and BJ) were cultured in phenol-red-
531 free DMEM with 10% FBS under physiological 5% O₂, except for population doubling
532 and colony formation assays, which were conducted in atmospheric O₂^{19,37}. RIS was
533 induced by retrovirus-mediated expression of either HRAS^{G12V} or HRAS^{G12V} fused to

534 the estrogen receptor (ER) ligand-binding domain (ER:RAS) system in HDFs. Acute
535 DNA damage (acDD) was induced by treating cells with 100 μ M etoposide for 24
536 hours. To obtain DNA damage induced senescence (DDIS) cells, cells were treated
537 with 100 μ M etoposide for 48 hours and maintained for an additional five days in
538 drug-free media. To generate RIS-bypassed cells, HRAS^{G12V} and E1A (adenoviral
539 oncoprotein) were co-transduced using the retroviral gene expression system
540 (E1A+RAS)¹⁸. For population-doubling analysis, cells were counted and 2×10^6 cells
541 were plated on 10 cm plates every 3–4 days. For colony formation assays, 10,000
542 cells were plated on 35 mm plates and maintained for 30 days. Cells were fixed with
543 4% formaldehyde and stained with Crystal Violet (Sigma). Dye intensity was
544 quantified as integrated density using "Fiji" image processing package.

545

546 Primary normal human keratinocytes were isolated from normal human skin. All
547 procedures involving human subjects were approved by the ethics committee of the
548 Ehime University Graduate School of Medicine, Toon, Ehime, Japan, and all subjects
549 provided written informed consent. The keratinocytes were cultured in Epilife medium
550 (Gibco) supplemented with human keratinocyte growth supplement (HKGS, Gibco)
551 under 21% atmospheric O₂. To induce differentiation in culture, keratinocytes were
552 treated with 500 nM phorbol 12-myristate 13-acetate (PMA, Sigma, P8139) for 48 h
553 after cells became confluent. RIS was induced by lentivirus-mediated expression of
554 HRAS^{G12V} in human keratinocytes.

555

556 The WA09/H9 human embryonic stem cells were obtained from WiCell, and cultured
557 in TeSR-E8 (StemCell Technologies #05990) on Vitronectin-coated glass coverslips
558 (0.5 μ g/cm²; ThermoFisher Scientific, A14700) in an atmosphere of 5% O₂ & 5% CO₂
559 at 37°C for FISH experiments.

560

561 **Vectors**

562 The following retroviral vectors were used^{19, 34}: pLNCX2 (Clontech) for ER:HRAS^{G12V};
563 pBabe-puro for HRAS^{G12V}; pWZL-hygro for HRAS^{G12V}, p16, E1A, pLPC for mVenus-
564 LCE2A, pMSCV-puro for miR30 sh-p53 (target sequence 5'-
565 CACTACAACTACATGTGTA-3')¹⁸, miR30 shHMGA1 (target sequence 5'-
566 ATGAGACGAAATGCTGATGTAT-3') and miR30 control³⁷, pSuper shCEBPB (gift
567 from Daniel Peeper)⁴⁰. The following lentiviral vectors were used: pLenti HRAS^{G12V}
568 (gift from Eric Campeau, Addgene plasmid # 22259).

569

570 **Immunoblotting**

571 Immunoblotting was performed as previously described¹⁹. Quantification of signal
572 intensity was performed using "Fiji" image processing package. Signal intensity of
573 targeted proteins were normalized to β Actin.

574

575 **Antibodies**

576 The following antibodies were used for immunoblotting: anti- β Actin (Sigma, A5441,
577 1:5000), anti-Cyclin A (Sigma, C4170, 1:1000), anti-Involucrin (Sigma, I9018, 1:1000;
578 Santa Cruz, A-5, 1:1000), anti-p16 (H-156, Santa Cruz, sc-759, 1:500) and (G175-
579 1239, BD Pharmingen, 554079, 1:500), anti-p21 (F-5, Santa Cruz, sc-6246, 1:500),
580 anti-p53 (DO-1, Sigma, P6874, 1:1000), anti-C/EBP β (C-19, Santa Cruz, sc-150,
581 1:500), anti-H-Ras (F235, Santa Cruz, sc-29, 1:500), anti-E1A (Santa Cruz, sc-430,
582 1:2000), anti-HMGA1 (Active Motif, 39615, 1:2000). The anti-Pan-LCE2 antibody
583 (1:1000) was raised in rabbits against a synthetic peptide (RPRLFHRRRHQSPD), as
584 previously described (note, this antigen sequence perfectly matches human LCE2B,
585 C and D, and differs by one amino acid for LCE2A)²⁶. The following antibodies were
586 used for ChIP: anti-H3K9me3 (clone CMA318), anti-H3K27me3 (clone CMA323),
587 anti-H3K27ac (clone CMA309)⁷³, C/EBP β (C-19, Santa Cruz, sc-150)²⁰.

588

589 **qRT-PCR**

590 RNA was prepared using the Qiagen RNeasy plus kit (Qiagen) according to the
591 manufacturer's instructions and reverse-transcribed to cDNA using the reverse
592 transcriptase Superscript III kit or High-Capacity cDNA Reverse Transcription Kit
593 (Life Technologies). qRT-PCR was performed with SYBR Green Master Mix (Takara)
594 or Fast SYBR™ Green Master Mix (Applied Biosystems). Data were normalized to
595 *ACTB* (encoding β Actin) expression. The following primers were used²¹:

596 *ACTB* forward: 5'-CGAGCACAGAGCCTCGCCTT-3'

597 *ACTB* reverse: 5'-CATCATCCATGGTGAGCTGGCGG-3'

598 *DCC* forward: 5'-AGCAGGAAGAAGTCAGTCAGTG-3'

599 *DCC* reverse: 5'-CTGTGGGGATGGTTCTGCTT-3'

600 *IL1A* forward: 5'-AACCAGTGCTGCTGAAGGA-3'

601 *IL1A* reverse: 5'-TTCTTAGTGCCGTGAGTTTCC-3'

602 *IL6* forward: 5'-TGAAAGCAGCAAAGAGGCACTG-3'

603 *IL6* reverse: 5'-TGAATCCAGATTGGAAGCATCC-3'

604 *IL8* forward: 5'-AAGGAAAAGTGGGTGCAGAG-3'

605 *IL8* reverse: 5'-ATTGCATCTGGCAACCCTAC-3'

606 *LCE2A* forward: 5'-GGACCTGTCCCAGAGTGATG-3'

607 *LCE2A* reverse: 5'-GATCCAGGATGGGCTCTTG-3'

608 *LCE2B* forward: 5'-GGTTGACTAACTCTGCCAGG-3'

609 *LCE2B* reverse: 5'-CACTGGGGCAGGCATTTA-3'

610 *LCE2C* forward: 5'-CTTGGGACTGAATGGCCAAG-3'

611 *LCE2C* reverse: 5'-GACTTGCAATTGGGGTGTTAC-3'

612 *LCE2D* forward: 5'-CTGCAGAAGAGCTCTGGTACTG-3'

613 *LCE2D* reverse: 5'-CTCCATCAAGCACAAAGTTCTG-3'.

614

615 **DNA FISH**

616 DNA FISH was performed as previously described¹⁴. Cells were plated onto glass
617 coverslips the day before fixation. Cells were pre-treated with digitonin/CSK buffer

(150 mg/ml), then fixed in 4% paraformaldehyde. Cells were permeabilized with 0.2% (v/v) Triton X-100 in PBS, soaked in liquid nitrogen, treated in 0.1M HCl and dehydrated in EtOH. Several genomic loci were observed by tiling the following BAC/PAC probes along the EDC locus: RP6-121F19 (or RP11-81P11), RP11-655C3, RP11-709L1, RP11-157C12, RP11-352B17, RP11-766D13 and RP11-157A11; OR locus: RP11-643N11, RP11-344I22, RP11-643G14 and RP11-1084O3, or *DCC* locus: RP11-25O3, RP11-108B6, RP11-1077M23, RP11-315H18, RP11-933N21 and RP11-186B13; *TMEM132B* locus: RP11-433N22, RP11-1139A9, RP11-728C18 and RP11-626N23; *NLRP3* locus: RP11-243D8, RP11-951B14, RP11-908P10, RP11-121O22 and RP11-248A15. Fluorescent directly labeled probes were generated using a Nick Translation kit (Abbott) and/or purchased from Empire Genomics. Confocal images were obtained using a Leica TCS SP5 microscope. The maximum width of multi-probed signals (any colour) on the locus were measured on Leica Application Suite Advanced Fluorescence (LAS-AF) software (Leica, Mannheim). For 3D images, confocal Z-stack images were projected to show X-Y images. X-Z images were generated by re-slicing and projection of 3D reconstructed images using ImageJ. SADS were observed as previously described¹⁵ using an oligo against the alpha-satellite repeat sequence (5'-CTTTTGATAGAGCAGTTTTGAAACACTCTTTTTGTAGAATCTGCAAGTGGATATTTGG-3').

Transcriptomic analysis

RNA-seq was performed as previously described¹⁰. RNA was purified using the Qiagen RNeasy plus kit according to the manufacturer's instructions. The quality was checked using the Bioanalyzer with an Agilent RNA 6000 nano kit (Agilent) or Agilent High Sensitivity RNA ScreenTape System. Libraries were prepared from six biological replicates of each condition using TruSeq Stranded mRNA Library Prep Kit or Illumina Stranded mRNA Prep Kit (Illumina) according to the manufacturer's

instructions and sequenced using the HiSeq-4000 and Novaseq-6000 platforms (Illumina). Low quality reads (quality score < 20) were removed from each sample using Cutadapt (1.10.0)⁷⁴. Reads were mapped to the human reference genome (hg19) using the STAR aligner⁷⁵. Read counting was carried out using Rsubread⁷⁶ and differentially expressed genes, comparing RIS and growing conditions, were identified using edgeR⁷⁷. For heatmap analyses, raw counts were TMM normalized and converted to transcripts-per-million (TPM). The EDC locus was defined as Chr1:152,056,619-Chr1:153,234,364. The EDC gene list was retrieved from the UCSC Table Browser⁷⁸.

For microarray data analysis, data previously deposited in NCBI GEO as image corrected, quantile normalised and log2 transformed intensities were downloaded using the Bioconductor GEOquery package⁷⁹. A linear model was fitted on the resulting values and log2-fold changes for each of the desired contrasts was computed using a simple empirical Bayes model with functions of the limma package⁸⁰.

To determine the tissue specificity of a gene, we used the Human Protein Atlas (HPA) resource (<https://www.proteinatlas.org/>)⁴⁵ and its associated criteria for defining the tissue enhanced/enriched status of gene (used for Fig. 5a). In order to estimate a tissue specificity score for each gene in different gene groups (such as in Extended Data Fig. 4a), we used the transcriptomic data from the HPA and computed the Tau score, which was shown previously⁸¹ to be the best metric for computing tissue specificity. The score ranges from 0 to 1, with 0 indicating a ubiquitously expressed gene and 1 a highly tissue specific one.

ChIP-seq and ATAC-seq analysis

ChIP-seq and ATAC-seq were performed as previously described¹⁰. Low quality reads (quality score < 20) were removed from each sample using Cutadapt (1.10.0)⁷⁴. Reads were mapped to the human reference genome (hg19) using BWA (v0.7.12)⁸² and reads mapping to the “blacklisted” regions identified by ENCODE⁸³ were removed from further analysis. H3K27ac and ATAC-seq peaks were called with MACS2⁸⁴ and H3K9me3 enriched domains were identified using EDD⁸⁵. A high confidence peak set was identified for data types and conditions separately keeping only those regions that were present in at least two replicates. These intersections as well as the overlaps between H3K27ac, H3K9me3 and ATAC-seq high confidence peak sets were then identified using bedtools (v2.26.0)⁸⁶. Average read coverage on the EDC locus was summarized on 1kb long bins from THOR⁸⁷ normalized coverage files (TMM normalization) using the deepTools package⁸⁸, which was also used to plot heatmaps of the THOR normalized signal. The same THOR normalized coverage files were loaded and visualized in the genome browser.

Hi-C analysis

Hi-C libraries were aligned using HiC-Pro⁸⁹ against the hg19 genome after initial QC checks using FastQC (<https://www.bioinformatics.babraham.ac.uk/projects/fastqc/>). Significant differential interactions, as well as distance corrected ICE-normalized Hi-C maps, were estimated using diffHiC⁹⁰. Visualisation of distance-corrected and ICE normalized⁹¹ Hi-C matrices and associated annotation (arcs representing significant interactions) was performed using HiCvizR⁵².

Data availability

Human keratinocytes RNA-seq and ChIP-seq data and IMR90 ATAC-seq data (RIS) and RNA-seq data generated for this study have been deposited at GEO (GSE130457, GSE180469). Control ATAC-seq data were previously published (GSE103590)¹⁰. Microarray datasets (Extended Data Fig. 1) were previously

published (GSE53379 and GSE59522)^{18,19}. RNA-seq data for RIS/DDIS IMR90 cells are available at GSE72407²⁰. Other senescence RNA-seq data^{23,24} were downloaded from GSE61130²³, GSE74238⁷, GSE45833²⁴, GSE85082⁹², GSE63577⁹³, GSE56293⁹⁴, GSE130306²⁵. SFs RNA-seq were downloaded from GSE163749⁶³. HPA RNA-seq data were downloaded from The Human Protein Atlas (HPA) (<https://www.proteinatlas.org>) E-MTAB-2836⁴⁵. THP1 RNA-seq data were downloaded from GSE96800⁶². ChIP-seq for H3K9m3, H3K27me3¹², H3K27ac¹⁰, and Lamin B1¹⁴ are available at GSE38448, GSE103590, and GSE49341, respectively. ChIP-seq data for hES cells were downloaded from ENCODE, GSE29611⁸³. H3K27ac ChIP-seq and ATAC-seq data for THP1 cells were downloaded from the Gene Expression Omnibus (GEO) accession number GSE96800⁶². Hi-C data (IMR90 cells) can be found at GSE135090. Hi-C data (human keratinocytes) were downloaded from GSE84660. Hi-C data (THP1 cells) were downloaded from Sequence Read Archive accession number PRJNA385337⁶². Hi-C data (WI38 cells) were downloaded from GSE130306²⁵.

716

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733

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736 designed and performed experiments, and analyzed data. D.B., I.O., G.St.C.S.,
737 A.S.L.C. and S.A.S analyzed sequencing data. I.O. analyzed Hi-C data. H. Kimura
738 provided histone antibodies. K. Shiraishi and K Sayama provided the human
739 keratinocytes. Masashi N. and K.T. wrote the manuscript with inputs from all authors.

740

741 **Competing interests**

742 P.F. and S.S. are co-founders Enhanc3D Genomics Ltd. All other authors declare no
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744

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746

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966

967 **Figure Legends**

968 **Figure 1. Aberrant expression of lineage-inappropriate *LCE2* genes during**
969 **senescence. (a)** Comparative analysis of *LCE2* expression by immunoblotting during
970 fibroblast (IMR90) RIS (RAS-induced senescence) and keratinocyte (KC)
971 differentiation (Diff). RIS was induced by retroviral expression of HRAS-G12V. Prolif,
972 vector control proliferating cells. KC Diff was induced using PMA for 48h. Undiff,
973 undifferentiated control. IVL, Involucrin, is an EDC-encoded KC Diff marker.
974 Densitometry quantification was performed by ImageJ, normalized to β Actin ($n \geq 3$).
975 $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, unpaired t-test. **(b)** Representative genome
976 browser images, centred around the EDC of the cells indicated. ER:HRAS-G12V
977 expressing IMR90 cells were cultured with 4OHT (RIS) or without 4OHT (Prolif) for 6
978 days. ChIP-seq data were THOR-normalized. The region with the orange shading is
979 magnified in the right-hand panel. **(c)** Averages of THOR-normalized read counts for
980 H3K9me3 and H3K27me3 signals on the EDC locus of the cells indicated. $***P <$
981 0.001 , t-test and Shapiro-Wilkes normality test. **(d)** Representative genome browser
982 images of ATAC-seq at the EDC and OR loci ($n \geq 2$). The same ChIP-seq data are
983 used as in **b**.

984

985 **Figure 2. Decompaction of EDC locus in senescence IMR90 cells. (a)**
986 Representative genome browser images of Lamin B1 ChIP-seq ($n = 3$) in proliferative
987 and RIS IMR90 cells, with the location of BAC probes used in this study shown as
988 green/purple blocks underneath. The same H3K9me3 ChIP-seq data as in Fig. 1
989 were used as the reference. **(b)** Confocal DNA-FISH images using multiple BAC
990 probes tiled across the EDC locus as shown in **a**. **(c)** Quantification of the FISH
991 signal length for the EDC locus; proliferating (Prolif), acute DNA damage (acDD),
992 E1A and RAS expressing (E1A+RAS), RAS-induced senescence (RIS), DNA
993 damage-induced senescence (DDIS), replicative senescence (RS) IMR90 cells;
994 undifferentiated (Undiff) and differentiated (Diff) keratinocytes (KC). $***P < 0.001$,

995 Mann-Whitney-Wilcoxon test ($n = 150$ alleles from 3 biological replicates). Bars are
 996 median with inter-quartile range. **(d)** Time series analysis of EDC signal length
 997 following addition of 100 nM 4OHT to ER:HRAS-G12V IMR90 cells. At least 150
 998 alleles were counted from 3 biological replicates. $***P < 0.001$ (Mann-Whitney-
 999 Wilcoxon test). Bars are median with interquartile range. **(e)** Representative
 1000 immunoblotting of the time series experiments as in **d**. Arrow indicates specific bands.
 1001 $**P < 0.01$, $***P < 0.001$ (one-way ANOVA with Fisher's LSD test, $n = 3$) **(f)**
 1002 Quantification of SAHF- and SADS-positive nuclei of the time series experiments as
 1003 in **d**. Representative images of SAHF and SADS at d7 are shown. SADS are
 1004 indicated by a-satellite targeted oligo hybridization (red). Values are means $\pm$ S.E.M.
 1005 ($n = 3$). $***P < 0.001$ compared with d0, one-way ANOVA with Tukey's multiple
 1006 comparisons test. **(g)** DNA-FISH using single BAC probes for EDC and OR loci as
 1007 depicted in Fig. 2a (green and purple bars underneath top panel). Percentages of
 1008 those loci colocalized with SAHF were measured. $***P < 0.001$ unpaired t-test ($n =$
 1009 3).

1010
 1011 **Figure 3. Decompaction of EDC locus and senescence effectors. (a)**
 1012 Quantification of SAHF-positive cells in RIS or proliferating (Vector) IMR90 cells with
 1013 shHMGA1 (+) or control miR30 vector (-). **(b)** Quantification of the FISH signal length
 1014 for the EDC locus in the indicated cells. **(c)** RNA-seq analysis in the indicated
 1015 comparison. **(d)** Immunoblotting for the indicated proteins. $*P < 0.05$, $**P < 0.01$, $***P$
 1016 < 0.001 , (one-way ANOVA with Fisher's LSD test, $n = 3$). **(e)** Left, immunoblotting for
 1017 the indicated proteins in IMR90 cells expressing control retroviral vector (Prolif), p16
 1018 (p16-Sen), and HRAS-G12V (RIS). Two representative replicates are shown. Right,
 1019 corresponding RNA-seq analysis, showing log-fold change (logFC) in the indicated
 1020 conditions compared to corresponding proliferative controls. p16-Sen ($n = 3$), DDIS
 1021 ($n = 8$), RIS ($n = 8$). **(f)** Quantification of BrdU incorporation and SAHF-positive cells.
 1022 Values are means $\pm$ SD ($n = 3$). $***P < 0.001$, (one-way ANOVA with Fisher's LSD

test). **(g)** Quantification of the FISH signal length for the EDC locus in the indicated cells. $***P < 0.001$, (ordinary one-way ANOVA with Fisher's LSD test, $n \geq 80$ allele from 3 biological replicates). Bars are median with inter-quartile range. **(h)** RNA-seq analysis - normalized expression heatmap (log-counts per million) of EDC genes in p16-induced senescence (p16-Sen), DNA damage-induced senescence (DDIS) and RAS-induced senescence (RIS) with corresponding proliferative controls. **(I - j)** Two representative immunoblots ($n \geq 5$) Arrows indicate the three specific isoforms. (i) and qPCR analysis (j) in indicated cells. ER:RAS IMR90 cells expressing shp53 and shC/EBP β with corresponding control vectors were treated with 4OHT (RAS) for 6 days. Values are means $\pm$ SD ($n = 5$). $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, (one-way ANOVA with Fisher's LSD test). **(k)** IGV tracks of THOR-normalized ChIP-seq signal of p53, C/EBP β and H3K27ac in Proliferating (Prolif) and RIS samples. **(l)** Quantification of the FISH signal length for the EDC locus in the indicated cells. $***P < 0.001$, Mann-Whitney-Wilcoxon test ($n \geq 150$ alleles from 3 biological replicates). n.s., not significant.

Figure 4. H3K9me3 peaks characterisation. **(a)** Classification of H3K9me3 peaks into non-permissive (group 1) and permissive peaks (with de novo ATAC-seq and H3K27ac peaks) with (group 3) and without up-regulated genes (group 2). **(b-i)** Distribution of peak widths (Mb) **(b)**, gene density (per Mb) **(c)**, AT% **(d)**, SINE frequency **(e)**, Alu repeats frequency **(f)**, Lamin B1 THOR-normalized ChIP-seq signal in proliferating cells **(g)**, Lamin B1 THOR-normalized ChIP-seq signal in RIS cells **(h)**, differential LaminB1 signal between RIS and proliferative cells **(i)**. In group 3, H3K9me3-peaks associated with the genes of interest are highlighted **(b-i)**. All box plots correspond to the median, 25th to 75th percentiles, and the whiskers correspond to the 10th to 90th percentiles. Each pairwise comparison between groups was effectuated with a two-sided Student's t-test, $***P < 0.001$, $**P < 0.01$, $*P < 0.05$. n.s., not significant.

1051

1052 **Figure 5. Aberrant expression of lineage-specific genes and chromatin**
1053 **decompaction during senescence. (a)** Tissue-dependent expression pattern of RIS
1054 upregulated genes that have RIS-unique H3K27ac/ATAC-peaks (at TSS +/- 250 bp)
1055 within H3K9me3 domains. RNA-seq datasets from IMR90 for RIS and DDIS (GSE-
1056 72407) and Human Protein Atlas (HPA) RNA-seq for normal tissues (E-MTAB-2836)
1057 were used. TPM, Transcripts-Per-Million. **(b-d)** Chromatin states of three additional
1058 examples of upregulated genes from H3K9me3 regions during RIS. Representative
1059 genome browser images **(b)**. Representative multiprobe DNA-FISH images for the
1060 *NLRP3* locus **(c)**. Either single or two alternate colors were used in the probe set
1061 shown in **b** (green/purple blocks, underneath main panel). Measurement of FISH
1062 signals using the multiple BAC probes represented in **(d)**. *** $P < 0.001$, Mann-
1063 Whitney-Wilcoxon test ($n \geq 150$ alleles from 3 biological replicates). Bars, median
1064 with interquartile range.

1065

1066 **Figure 6. Distinct mode of expression of lineage-appropriate and lineage-**
1067 **inappropriate genes during RIS. (a)** Heatmap of normalized expression values
1068 (log-counts per million) of the EDC genes from RNA-seq samples of proliferating and
1069 RAS-induced ($n = 6$) keratinocytes (KCs), as well as undifferentiated ($n = 8$) and
1070 differentiated ($n = 9$) KCs. **(b)** Genome browser images of H3K9me3 ChIP-seq of the
1071 EDC and *DCC* loci from the cells indicated. The human ES (hES) cell data are from
1072 ENCODE. Some of the IMR90 and human primary keratinocyte (KC) images were
1073 replotted for comparisons using the same datasets as in Fig. 1b (EDC) and Fig. 5b
1074 (*DCC*). **(c)** DNA-FISH analysis of the *DCC* and EDC loci in the cells indicated. *** $P <$
1075 0.001 , Mann-Whitney-Wilcoxon test ($n \geq 150$ alleles from 3 biological replicates).
1076 Bars are median with inter-quartile range. **(d)** qRT-PCR for the indicated mRNA in

vector control and RIS KCs. Error bars indicate mean $\pm$ S.E.M. ($n = 9$). *** $P < 0.001$,
* $P < 0.05$ paired t-test.

Figure 7. Differential 3D behaviour of permissive H3K9me3 peaks. **(a)** Total H3K9me3-H3K9me3 interactions (connectivity) of the individual 200 kb bins (sum of the interactions with all other 200 kb bins within H3K9me3 peaks) within each group of H3K9me3 peaks (the same three groups as in Fig. 4). **(b)** Differential H3K9me3-H3K9me3 interaction between proliferative and RIS conditions in each group. Log-fold changes (logFC) of increased (top, green) and decreased interactions (bottom, blue) in each peak with all other H3K9me3 peaks are shown. Each pairwise comparison is performed using two-sided Student's t-test with *** $P < 0.001$, ** $P < 0.01$. n.s., not significant. Boxplots in **(a)** and **(b)** represent the median, 25th to 75th percentiles, whiskers: 10th to 90th percentiles. **(c)** Significant LogFC (green – positive, blue – negative) of all the Hi-C interactions involving each of the H3K9me3 peaks of interest within group 3 compared to the OR region (*OR10T2*) neighbouring the EDC (*LCE2*, *IVL*), as well as 30 other H3K9me3 peaks randomly chosen from group 1. **(d-f)** Hi-C contact maps at 40 kb resolution focused on the EDC locus in proliferating and RIS IMR90 cells (d), proliferating and replicative senescent WI38 cells (e) as well as in differentiating keratinocytes (induced by calcium) (f); the arrows indicate the formation of loops during senescence (fibroblasts) and differentiation (keratinocytes) at this locus. **(g-h)** Hi-C contact maps on the *NLRP3* locus at 40 kb resolution in proliferating and RIS IMR90 cells (g), the arrow indicates the interaction changes around the gene body of *NLRP3*) and THP1 monocytes and PMA-induced macrophages (h). Hi-C maps in THP1 cells are aligned with H3K27ac ChIP-seq, ATAC-seq, and RNA-seq data.

1104 **Extended Data Figure 1. Transcriptional profile of genes in the EDC locus. (a)**

1105 Image corrected, quantile normalized and log2 transformed microarray intensity
1106 values were downloaded from the indicated NCBI GEO entries. Control, proliferative;
1107 RIS, HRAS-G12V-induced senescent; Qui, quiescent; DDIS, DNA damage-induced
1108 senescent; acDDR, acute DNA damage response to etoposide (24 or 48 hours);
1109 E1A+RAS, transformed/senescence-bypassed. Genes for which there is no available
1110 transcript probe on the microarray platform used are greyed out. **(b)** Meta-analysis of
1111 senescence-associated transcriptomic changes of genes in the EDC locus using
1112 RNA-seq datasets, downloaded from indicated NCBI GEO, comparing between
1113 different cellular phenotypes. The datasets were processed using the same analysis
1114 pipeline. Values for the indicated genes are shown for each of the senescent
1115 samples and their corresponding growing controls. Values are Transcripts-Per-Million
1116 (TPM). Genes are ordered according to their position along Chr. 1. Genes for which
1117 there were no aligned reads in the specified RNA-seq runs are greyed out. **(c)**
1118 Immunoblot analysis of IMR90 cells stably expressing vector (-) or HRAS-G12V and
1119 control miR30 vector (-) or sh-p53 (+) for the proteins indicated. Arrow indicates
1120 specific bands. p21 is a p53-dependent cell cycle inhibitor that is upregulated during
1121 senescence. **(d-e)** IMR90 cells expressing ectopic mVenus-LCE2A and control
1122 vector were assessed for cumulative population doublings (d) and colony formation
1123 capacity (e) (n = 3). Error bars indicate mean +/- SD. The inset shows representative
1124 immunoblots of indicated protein (d).

1125

1126 **Extended Data Figure 2. Correlation between EDC decompaction and SAHF**

1127 **formation. (a)** 3D confocal images of DNA-FISH for the EDC locus in the cells
1128 indicated (see Fig. 2a for two alternate color probe designs). The perinuclear
1129 localization of the EDC locus was quantified on visual inspections by counting FISH
1130 signal, which attached at least 1 of the 7 tiled signals to the nuclear periphery (white
1131 arrowhead) (n > 100). **(b)** Representative images of DNA-FISH for the EDC and OR

1132 loci using the probes indicated and quantification of signal length (n = 100 alleles
 1133 from 3 biological replicates). Note, for the EDC, 5 out of 7 probes (Fig. 2a) were used.
 1134 **(c)** FISH signal length of EDC on SAHF-negative or positive RIS cells (n ≥ 100 alleles
 1135 from 3 biological replicates). **(d)** LCE2 immunofluorescence signal on SAHF-negative
 1136 or positive RIS cells (n = 3). **(e-h)** Quantification of SAHF-positive cells in the
 1137 indicated conditions (e, g) and comparative analysis of LCE2 expression by
 1138 immunoblotting in the conditions indicated (f, h). **P* < 0.05, ***P* < 0.01, ****P* < 0.001
 1139 one-way ANOVA with Tukey's multiple comparisons test (n = 3). Arrows indicate
 1140 specific bands. **(i)** Quantification of FISH signal length in the conditions indicated for
 1141 BJ and WI38 cells (n ≥ 150 alleles from 3 biological replicates). ****P* < 0.001, Mann-
 1142 Whitney-Wilcoxon test (bars are median with interquartile range) **(b, c, i)**.

1143
 1144 **Extended Data Figure 3. H3K9me3 exhibits increases in accessibility and**
 1145 **H3K27ac ChIP-seq signal in RIS. (a)** ATAC-seq and H3K27ac THOR-normalized
 1146 signal in proliferative and RIS cells centred on three classes of ATAC-seq peaks:
 1147 proliferative-specific, RIS-specific, and common between the two conditions. **(b)**
 1148 Number of ATAC-seq (top) and H3K27ac (bottom) peaks across the three groups of
 1149 H3K9me3 peaks defined as permissive (group 2 and 3) and non-permissive (group
 1150 1). The box plots correspond to the median, 25th to 75th percentiles, and the whiskers
 1151 correspond to the 10th to 90th percentiles. **(c)** IGV tracks of genes of interest and
 1152 position of SINE and Alu repeats (UCSC annotation) over their gene bodies. **(d)**
 1153 Region surrounding the *SERPINA1* gene characterised by H3K9me3 ChIP-seq
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1160 Significance testing was performed with two-sided Student's t-tests, *** $P < 0.001$,
1161 n.s., not significant. The box plots correspond to the median, 25th to 75th percentiles,
1162 and the whiskers correspond to the 10th to 90th percentiles. **(b)** *DCC* (left) and
1163 *TMEM132B* (right) genes represented by multiprobe DNA-FISH signals in the
1164 proliferative and RIS conditions. Probe sets are shown in Fig. 5b. Note, single color
1165 probe images are shown for *TMEM132B*.

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1167 **Extended Data Figure 5. Three-dimensional interaction changes occurring**
1168 **during RIS. (a)** 200 kb resolution Hi-C maps in proliferative and RIS cells as well as
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1170 represent increases and blue arcs represent decreases. **(b)** Significant H3K9me3-
1171 H3K9me3 interaction changes (200 kb bins): log-fold changes (logFC) against
1172 distance between the interacting regions, dotted line corresponds to 10 Mb distance.
1173 **(c)** Hi-C interaction profiles at 40 kb resolution in proliferating (Prolif) and RIS IMR90
1174 cells as well as differentiating keratinocytes at the EDC/OR loci. **(d)** Epigenetic profile
1175 of the *NLRP3* locus in terms of H3K9me3, LaminB1 and H3K27me3 THOR-
1176 normalized ChIP-seq signal in proliferating and RIS cells. **(e)** Normalized gene
1177 expression (log-counts per million) of pro-inflammatory genes, EDC genes and
1178 senescence-representative genes in -/MSU and MSU/MSU synovial fibroblasts.
1179 *Adjusted $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

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1181 **Supplementary Table 1.** List of genes within the three types of H3K9me3 peaks
1182 determined. 38 genes significantly upregulated in group 3 are highlighted.

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Tomimatsu_Fig.1

Figure 1. Aberrant expression of lineage-inappropriate LCE2 genes during senescence. (a) Comparative analysis of LCE2 expression by immunoblotting during fibroblast (IMR90) RIS (RAS-induced senescence) and keratinocyte (KC) differentiation (Diff). RIS was induced by retroviral expression of HRAS-G12V. Prolif, vector control proliferating cells. KC Diff was induced using PMA for 48h. Undiff, undifferentiated control. IVL, Involucrin, is an EDC-encoded KC Diff marker. Densitometry quantification was performed by ImageJ, normalized to β Actin ($n \geq 3$). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, unpaired t-test. (b) Representative genome browser images, centred around the EDC of the cells indicated. ER:HRAS-G12V expressing IMR90 cells were cultured with 4OHT (RIS) or without 4OHT (Prolif) for 6 days. ChIP-seq data were THOR-normalized. The region with the orange shading is magnified in the right-hand panel. (c) Averages of THOR-normalized read counts for H3K9me3 and H3K27me3 signals on the EDC locus of the cells indicated. *** $P < 0.001$, t-test and Shapiro-Wilkes normality test. (d) Representative genome browser images of ATAC-seq at the EDC and OR loci ($n \geq 2$). The same ChIP-seq data are used as in b.

Tomimatsu_Fig.2

Figure 2. Decompaction of EDC locus in senescence IMR90 cells. (a) Representative genome browser images of Lamin B1 ChIP-seq ($n = 3$) in proliferative and RIS IMR90 cells, with the location of BAC probes used in this study shown as green/purple blocks underneath. The same H3K9me3 ChIP-seq data as in Fig. 1 were used as the reference. (b) Confocal DNA-FISH images using multiple BAC probes tiled across the EDC locus as shown in a. (c) Quantification of the FISH signal length for the EDC locus; proliferating (Prolif), acute DNA damage (acDD), E1A and RAS expressing (E1A+RAS), RAS-induced senescence (RIS), DNA damage-induced senescence (DDIS), replicative senescence (RS) IMR90 cells; undifferentiated (Undiff) and differentiated (Diff) keratinocytes (KC). *** $P < 0.001$, Mann-Whitney-Wilcoxon test ($n = 150$ alleles from 3 biological replicates). Bars are median with inter-quartile range. (d) Time series analysis of EDC signal length following addition of 100 nM 4OHT to ER:HRAS-G12V IMR90 cells. At least 150 alleles were counted from 3 biological replicates. *** $P < 0.001$ (Mann-Whitney-Wilcoxon test). Bars are median with interquartile range. (e) Representative immunoblotting of the time series experiments as in d. Arrow indicates specific bands. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ (one-way ANOVA with Fisher's LSD test, $n = 3$) (f) Quantification of SAHF- and SADS-positive nuclei of the time series experiments as in d. Representative images of SAHF and SADS at d7 are shown. SADS are indicated by α -satellite targeted oligo hybridization (red). Values are means $\pm$ S.E.M. ($n = 3$). *** $P < 0.001$ compared with d0, one-way ANOVA with Tukey's multiple comparisons test. (g) DNA-FISH using single BAC probes for EDC and OR loci as depicted in Fig. 2a (green and purple bars underneath top panel). Percentages of those loci colocalized with SAHF were measured. *** $P < 0.001$ unpaired t-test ($n = 3$).

Tomimatsu_Fig. 3

Figure 3. Decompaction of EDC locus and senescence effectors. **(a)** Quantification of SAHF-positive cells in RIS or proliferating (Vector) IMR90 cells with shHMGA1 (+) or control miR30 vector (-). **(b)** Quantification of the FISH signal length for the EDC locus in the indicated cells. **(c)** RNA-seq analysis in the indicated comparison. **(d)** Immunoblotting for the indicated proteins. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, (one-way ANOVA with Fisher's LSD test, $n = 3$). **(e)** Left, immunoblotting for the indicated proteins in IMR90 cells expressing control retroviral vector (Prolif), p16 (p16-Sen), and HRAS-G12V (RIS). Two representative replicates are shown. Right, corresponding RNA-seq analysis, showing log-fold change (logFC) in the indicated conditions compared to corresponding proliferative controls. p16-Sen ($n = 3$), DDIS ($n = 8$), RIS ($n = 8$). **(f)** Quantification of BrdU incorporation and SAHF-positive cells. Values are means $\pm$ SD ($n = 3$). *** $P < 0.001$, (one-way ANOVA with Fisher's LSD test). **(g)** Quantification of the FISH signal length for the EDC locus in the indicated cells. *** $P < 0.001$, (ordinary one-way ANOVA with Fisher's LSD test, $n \geq 80$ allele from 3 biological replicates). Bars are median with inter-quartile range. **(h)** RNA-seq analysis - normalized expression heatmap (log-counts per million) of EDC genes in p16-induced senescence (p16-Sen), DNA damage-induced senescence (DDIS) and RAS-induced senescence (RIS) with corresponding proliferative controls. **(i - j)** Two representative immunoblots ($n \geq 5$) Arrows indicate the three specific isoforms. (i) and qPCR analysis (j) in indicated cells. ER:RAS IMR90 cells expressing shp53 and shC/EBP β with corresponding control vectors were treated with 4OHT (RAS) for 6 days. Values are means $\pm$ SD ($n = 5$). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, (one-way ANOVA with Fisher's LSD test). **(k)** IGV tracks of THOR-normalized ChIP-seq signal of p53, C/EBP β and H3K27ac in Proliferating (Prolif) and RIS samples. **(l)** Quantification of the FISH signal length for the EDC locus in the indicated cells. *** $P < 0.001$, Mann-Whitney-Wilcoxon test ($n \geq 150$ alleles from 3 biological replicates). n.s., not significant.

Figure 4. H3K9me3 peaks characterisation. (a) Classification of H3K9me3 peaks into non-permissive (group 1) and permissive peaks (with de novo ATAC-seq and H3K27ac peaks) with (group 3) and without up-regulated genes (group 2). (b-i) Distribution of peak widths (Mb) (b), gene density (per Mb) (c), AT% (d), SINE frequency (e), Alu repeats frequency (f), Lamin B1 THOR-normalized ChIP-seq signal in proliferating cells (g), Lamin B1 THOR-normalized ChIP-seq signal in RIS cells (h), differential LaminB1 signal between RIS and proliferative cells (i). In group 3, H3K9me3-peaks associated with the genes of interest are highlighted (b-i). All box plots correspond to the median, 25th to 75th percentiles, and the whiskers correspond to the 10th to 90th percentiles. Each pairwise comparison between groups was effectuated with a two-sided Student's t-test, *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$. n.s., not significant.

Figure 5. Aberrant expression of lineage-specific genes and chromatin decompaction during senescence. (a) Tissue-dependent expression pattern of RIS upregulated genes that have RIS-unique H3K27ac/ATAC-peaks (at TSS +/- 250 bp) within H3K9me3 domains. RNA-seq datasets from IMR90 for RIS and DDIS (GSE-72407) and Human Protein Atlas (HPA) RNA-seq for normal tissues (E-MTAB-2836) were used. TPM, Transcripts-Per-Million. (b-d) Chromatin states of three additional examples of upregulated genes from H3K9me3 regions during RIS. Representative genome browser images (b). Representative multiprobe DNA-FISH images for the *NLRP3* locus (c). Either single or two alternate colours were used in the probe set shown in b (green/purple blocks, underneath main panel). Measurement of FISH signals using the multiple BAC probes represented in (d). *** $P < 0.001$, Mann-Whitney-Wilcoxon test ($n \geq 150$ alleles from 3 biological replicates). Bars, median with interquartile range.

Figure 6. Distinct mode of expression of lineage-appropriate and lineage-inappropriate genes during RIS. (a) Heatmap of normalized expression values (log-counts per million) of the EDC genes from RNA-seq samples of proliferating and RAS-induced ($n = 6$) keratinocytes (KCs), as well as undifferentiated ($n = 8$) and differentiated ($n = 9$) KCs. (b) Genome browser images of H3K9me3 ChIP-seq of the EDC and *DCC* loci from the cells indicated. The human ES (hES) cell data are from ENCODE. Some of the IMR90 and human primary keratinocyte (KC) images were replotted for comparisons using the same datasets as in Fig. 1b (EDC) and Fig. 5b (*DCC*). (c) DNA-FISH analysis of the *DCC* and EDC loci in the cells indicated. *** $P < 0.001$, Mann-Whitney-Wilcoxon test ($n \geq 150$ alleles from 3 biological replicates). Bars are median with inter-quartile range. (d) qRT-PCR for the indicated mRNA in vector control and RIS KCs. Error bars indicate mean $\pm$ S.E.M. ($n = 9$). *** $P < 0.001$, * $P < 0.05$ paired t-test.

Figure 7. Differential 3D behaviour of permissive H3K9me3 peaks. (a) Total H3K9me3-H3K9me3 interactions (connectivity) of the individual 200 kb bins (sum of the interactions with all other 200 kb bins within H3K9me3 peaks) within each group of H3K9me3 peaks (the same three groups as in Fig. 4). (b) Differential H3K9me3-H3K9me3 interaction between proliferative and RIS conditions in each group. Log-fold changes (logFC) of increased (top, green) and decreased interactions (bottom, blue) in each peak with all other H3K9me3 peaks are shown. Each pairwise comparison is performed using two-sided Student's t-test with $***P < 0.001$, $**P < 0.01$. n.s., not significant. Boxplots in (a) and (b) represent the median, 25th to 75th percentiles, whiskers: 10th to 90th percentiles. (c) Significant LogFC (green – positive, blue – negative) of all the Hi-C interactions involving each of the H3K9me3 peaks of interest within group 3 compared to the OR region (*OR10T2*) neighbouring the EDC (*LCE2*, *IVL*), as well as 30 other H3K9me3 peaks randomly chosen from group 1. (d-f) Hi-C contact maps at 40 kb resolution focused on the EDC locus in proliferating and RIS IMR90 cells (d), proliferating and replicative senescent WI38 cells (e) as well as in differentiating keratinocytes (induced by calcium) (f); the arrows indicate the formation of loops during senescence (fibroblasts) and differentiation (keratinocytes) at this locus. (g-h) Hi-C contact maps on the *NLRP3* locus at 40 kb resolution in proliferating and RIS IMR90 cells (g), the arrow indicates the interaction changes around the gene body of *NLRP3* and THP1 monocytes and PMA-induced macrophages (h). Hi-C maps in THP1 cells are aligned with H3K27ac ChIP-seq, ATAC-seq, and RNA-seq data.

Tomimatsu_Extended Data Fig. 1

Extended Data Figure 1. Transcriptional profile of genes in the EDC locus. (a) Image corrected, quantile normalized and log2 transformed microarray intensity values were downloaded from the indicated NCBI GEO entries. Control, proliferative; RIS, HRAS-G12V-induced senescent; Qui, quiescent; DDIS, DNA damage-induced senescent; acDDR, acute DNA damage response to etoposide (24 or 48 hours); E1A+RAS, transformed/senescent-bypassed. Genes for which there is no available transcript probe on the microarray platform used are greyed out. (b) Meta-analysis of senescence-associated transcriptomic changes of genes in the EDC locus using RNA-seq datasets, downloaded from indicated NCBI GEO, comparing between different cellular phenotypes. The datasets were processed using the same analysis pipeline. Values for the indicated genes are shown for each of the senescent samples and their corresponding growing controls. Values are Transcripts-Per-Million (TPM). Genes are ordered according to their position along Chr. 1. Genes for which there were no aligned reads in the specified RNA-seq runs are greyed out. (c) Immunoblot analysis of IMR90 cells stably expressing vector (-) or HRAS-G12V and control miR30 vector (-) or sh-p53 (+) for the proteins indicated. Arrow indicates specific bands. p21 is a p53-dependent cell cycle inhibitor that is upregulated during senescence. (d-e) IMR90 cells expressing ectopic mVenus-LCE2A and control vector were assessed for cumulative population doublings (d) and colony formation capacity (e) (n = 3). Error bars indicate mean $\pm$ SD. The inset shows representative immunoblots of indicated protein (d).

Tomimatsu_Extended Data Fig. 2

Extended Data Figure 2. Correlation between EDC decompaction and SAHF formation. (a) 3D confocal images of DNA-FISH for the EDC locus in the cells indicated (see Fig. 2a for two alternate colour probe designs). The perinuclear localization of the EDC locus was quantified on visual inspections by counting FISH signal, which attached at least 1 of the 7 tiled signals to the nuclear periphery (white arrowhead) ($n > 100$). (b) Representative images of DNA-FISH for the EDC and OR loci using the probes indicated and quantification of signal length ($n = 100$ alleles from 3 biological replicates). Note, for the EDC, 5 out of 7 probes (Fig. 2a) were used. (c) FISH signal length of EDC on SAHF-negative or positive RIS cells ($n \geq 100$ alleles from 3 biological replicates). (d) LCE2 immunofluorescence signal on SAHF-negative or positive RIS cells ($n = 3$). (e-h) Quantification of SAHF-positive cells in the indicated conditions (e, g) and comparative analysis of LCE2 expression by immunoblotting in the conditions indicated (f, h). (i) Quantification of FISH signal length in the conditions indicated for BJ and WI38 cells ($n \geq 150$ alleles from 3 biological replicates). *** $P < 0.001$, Mann-Whitney-Wilcoxon test (bars are median with interquartile range) (b, c, i).

Tomimatsu_Extended Data Fig. 3

Extended Data Figure 3. H3K9me3 exhibits increases in accessibility and H3K27ac ChIP-seq signal in RIS. (a) ATAC-seq and H3K27ac THOR-normalized signal in proliferative and RIS cells centred on three classes of ATAC-seq peaks: proliferative-specific, RIS-specific, and common between the two conditions. (b) Number of ATAC-seq (top) and H3K27ac (bottom) peaks across the three groups of H3K9me3 peaks defined as permissive (group 2 and 3) and non-permissive (group 1). The box plots correspond to the median, 25th to 75th percentiles, and the whiskers correspond to the 10th to 90th percentiles. (c) IGV tracks of genes of interest and position of SINE and Alu repeats (UCSC annotation) over their gene bodies. (d) Region surrounding the *SERPINA1* gene characterised by H3K9me3 ChIP-seq signal, as well as protruding H3K27ac and ATAC-seq peaks.

Tomimatsu_Extended Data Fig. 4

Extended Data Figure 4. Tissue specificity of genes in H3K9me3 peaks. (a) Tissue specificity score of the genes within the three groups of H3K9me3 peaks against all other genes. The Tau score ranges from 0 - ubiquitous expression across tissues to 1 - tissue specific; the 8 genes of interest in group 3 are highlighted. Significance testing was performed with two-sided Student's t-tests, *** $P < 0.001$, n.s., not significant. The box plots correspond to the median, 25th to 75th percentiles, and the whiskers correspond to the 10th to 90th percentiles. **(b)** *DCC* (left) and *TMEM132B* (right) genes represented by multiprobe DNA-FISH signals in the proliferative and RIS conditions. Probe sets are shown in Fig. 5b. Note, single colour probe images are shown for *TMEM132B*.

Tomimatsu_Extended Data Fig. 5

Extended Data Figure 5. Three-dimensional interaction changes occurring during RIS. (a) 200 kb resolution Hi-C maps in proliferative and RIS cells as well as significant interaction changes between the two conditions, where green arcs represent increases and blue arcs represent decreases. (b) Significant H3K9me3-H3K9me3 interaction changes (200 kb bins): log-fold changes (logFC) against distance between the interacting regions, dotted line corresponds to 10 Mb distance. (c) Hi-C interaction profiles at 40 kb resolution in proliferating (Prolif) and RIS IMR90 cells as well as differentiating keratinocytes at the EDC/OR loci. (d) Epigenetic profile of the *NLRP3* locus in terms of H3K9me3, LaminB1 and H3K27me3 THOR-normalized ChIP-seq signal in proliferating and RIS cells. (e) Normalized gene expression (log-counts per million) of pro-inflammatory genes, EDC genes and senescence-representative genes in -MSU and MSU/MSU synovial fibroblasts. *Adjusted $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.