Supplementary Information for

Comprehensive 3D epigenomic maps define limbal

stem/progenitor cell function and identity

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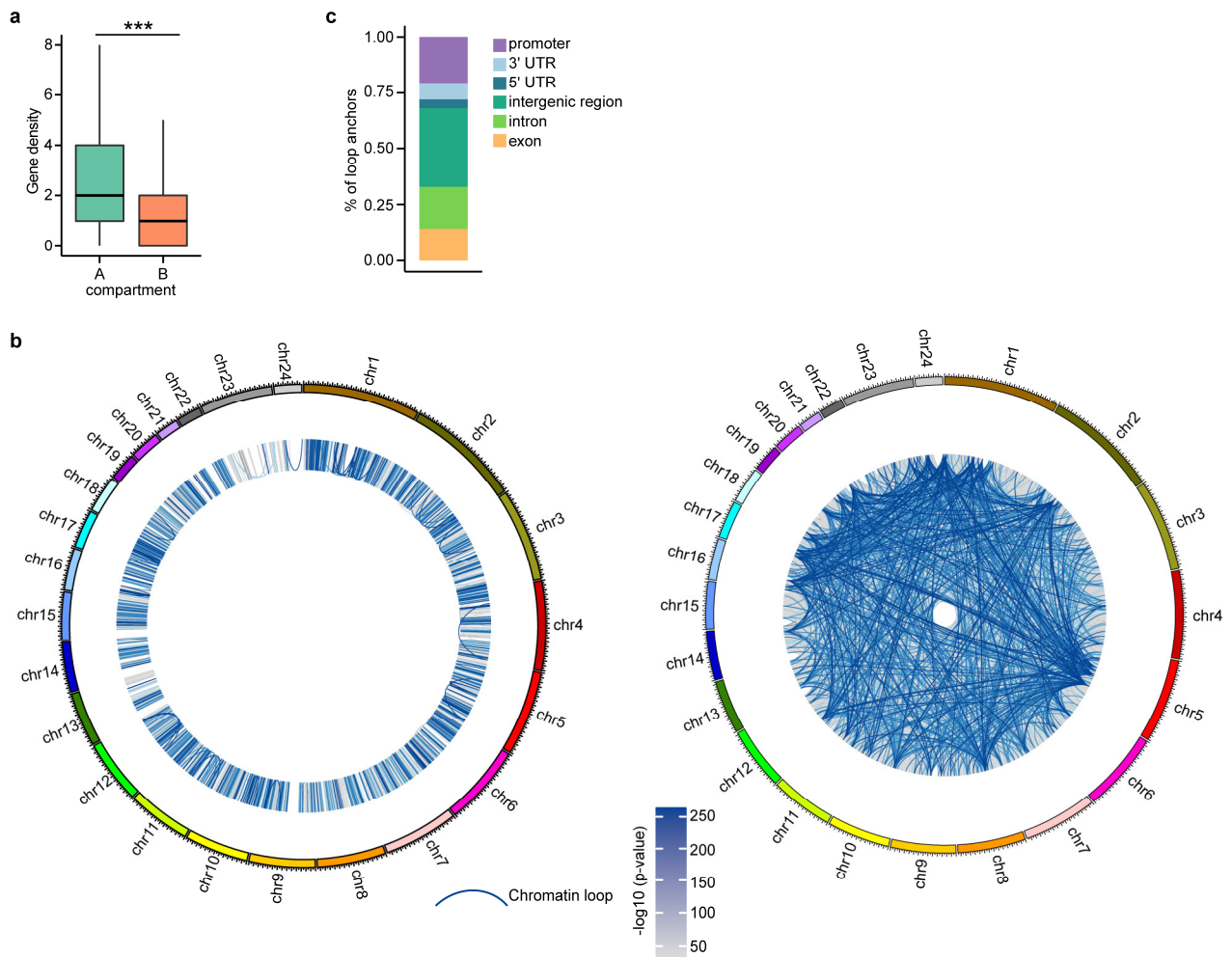
Supplementary Fig. 1 Characterization of Hi-C interactions in LSCs

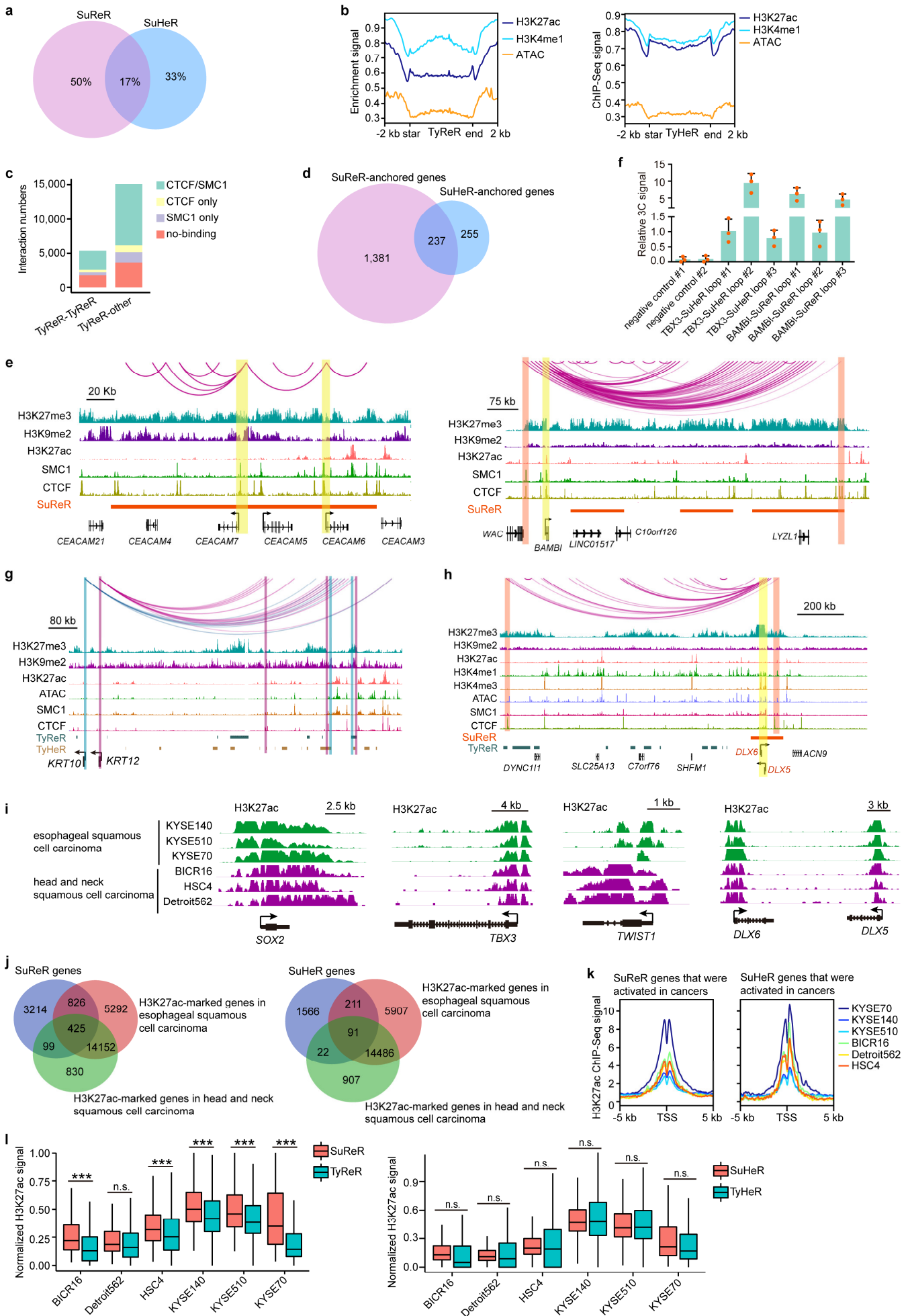**a** Boxplots showing gene density of A (n = 13,613) and B (n= 14,149) compartments. ***p < 0.001 from two-way ANOVA. Boxplots indicate the 25th percentile (bottom of box), median (horizontal line inside box) and 75th percentile (top of box). Whiskers indicate 1.5 times the interquartile range. **b** Circos plots showing *cis*- and *trans*-interaction loops. **c** Distributions of chromatin loop anchors on the genome.
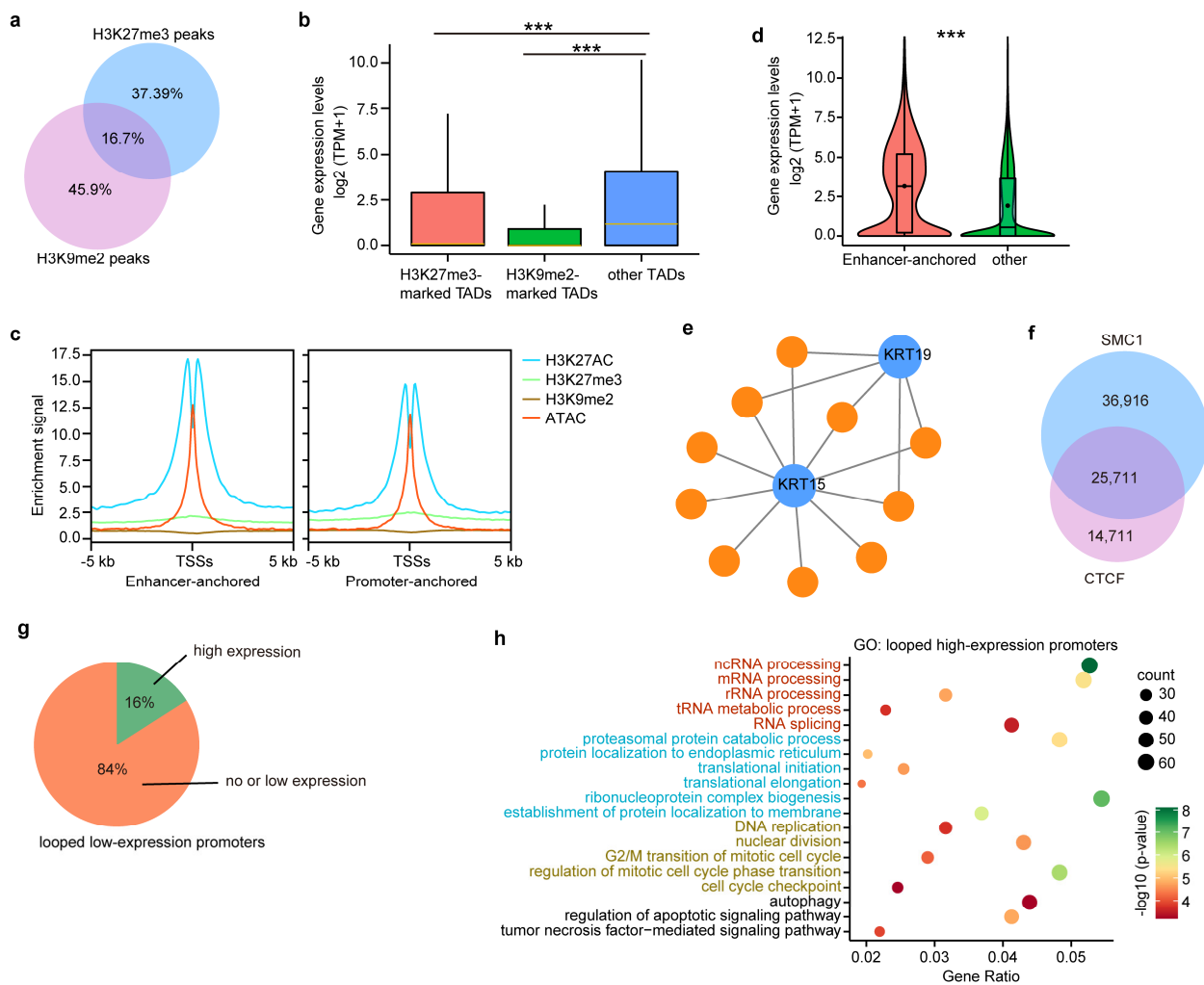
Supplementary Fig. 2 Epigenetic features of chromatin 3D structure in LSCs

a Venn diagram showing overlapping between H3K27me3 and H3K9me2 peaks. **b** Boxplots showing gene expression levels within H3K27me3-marked (n = 1,758), H3K9me2-marked (n = 495) and other (n = 23,050) TADs. ***$p < 0.001$ from two-way ANOVA. Boxplots indicate the 25th percentile (bottom of box), median (horizontal yellow line inside box) and 75th percentile (top of box). Whiskers indicate 1.5 times the interquartile range. **c** Metaplots of ATAC-Seq and the indicated ChIP-Seq signals at enhancer-anchored and promoter-anchored TSSs. **d** Violin and box plots showing expression levels of enhancer-interacting (n=4,972) and other (n=20,107) genes. ***$p < 0.001$ from two-way ANOVA. Boxplots indicate the 25th percentile (bottom of box), median (horizontal line inside box), mean value (dark spot inside box) and 75th

percentile (top of box). Whiskers indicate 1.5 times the interquartile range. **e** A selected E-P interaction network. Orange nodes represent enhancers and blue nodes represent promoters of *KRT15* and *KRT19*. Edges represent loop interactions. **f** Overlapping between CTCF and SMC1 peaks. **g** Pie chart showing the distributions of expression levels of LLEP genes. **h** GO biological process analysis of the looped high-expression promoters that were identified in Fig .3h.

a
SuReR   SuHeR
50%   17%   33%

b
H3K27ac
H3K4me1
ATAC

Enrichment signal
-2 kb   star   TyReR   end   2 kb

ChIP-Seq signal
H3K27ac
H3K4me1
ATAC
-2 kb   star   TyHeR   end   2 kb

c
Interaction numbers
CTCF/SMC1
CTCF only
SMC1 only
no-binding
TyReR-TyReR   TyReR-other

d
SuReR-anchored genes   SuHeR-anchored genes
1,381   237   255

f
Relative 3C signal
negative control #1
negative control #2
TBX3-SuHeR loop #1
TBX3-SuHeR loop #2
TBX3-SuHeR loop #3
BAMBI-SuReR loop #1
BAMBI-SuReR loop #2
BAMBI-SuReR loop #3

e
20 Kb
H3K27me3
H3K9me2
H3K27ac
SMC1
CTCF
SuReR
CEACAM21   CEACAM4   CEACAM7   CEACAM5   CEACAM6   CEACAM3

75 kb
H3K27me3
H3K9me2
H3K27ac
SMC1
CTCF
SuReR
WAC   BAMBI   LINC01517   C10orf126   LYZL1

g
80 kb
H3K27me3
H3K9me2
H3K27ac
ATAC
SMC1
CTCF
TyReR
TyHeR
KRT10   KRT12

h
H3K27me3
H3K9me2
H3K27ac
H3K4me1
H3K4me3
ATAC
SMC1
SuReR
TyReR
200 kb
DYNC1I1   SLC25A13   C7orf76   SHFM1   DLX6   ACN9   DLX5

i
esophageal squamous cell carcinoma
KYSE140
KYSE510
KYSE70
head and neck squamous cell carcinoma
BICR16
HSC4
Detroit562
H3K27ac   2.5 kb   SOX2
H3K27ac   4 kb   TBX3
H3K27ac   1 kb   TWIST1
H3K27ac   3 kb   DLX6   DLX5

j
SuReR genes
3214   826   5292
425
99   14152
830
H3K27ac-marked genes in esophageal squamous cell carcinoma
H3K27ac-marked genes in head and neck squamous cell carcinoma

SuHeR genes
1566   211   5907
91
22   14486
907
H3K27ac-marked genes in esophageal squamous cell carcinoma
H3K27ac-marked genes in head and neck squamous cell carcinoma

k
SuReR genes that were activated in cancers
SuHeR genes that were activated in cancers
H3K27ac ChIP-Seq signal
-5 kb   TSS   5 kb
KYSE70
KYSE140
KYSE510
BICR16
Detroit562
HSC4

l
Normalized H3K27ac signal
*** n.s. *** *** *** ***
BICR16   Detroit562   HSC4   KYSE140   KYSE510   KYSE70
SuReR
TyReR

Normalized H3K27ac signal
n.s. n.s. n.s. n.s. n.s. n.s.
BICR16   Detroit562   HSC4   KYSE140   KYSE510   KYSE70
SuHeR
TyHeR

Supplementary Fig. 3 Characterization of super-silencer-associated interactions

a Overlapping between SuReR and SuHeR. **b** Metaplots showing the enrichment of H3K27ac, H3K4me1 and ATAC at TyReR and TyHeR. **c** Distributions of TyReR-TyReR and TyReR-other loops that were occupied by CTCF only, SMC1 only, CTCF/SMC1 and were not bound by CTCF and SMC1. **d** Overlapping between SuReR-anchored and SuHeR-anchored genes. **e** Identified chromatin loops and tracks for the indicated ChIP-Seq enrichment at the indicated genome loci. **f** Chromosome conformation capture (3C) assay for TBX3-SuHeR and BAMBI-SuReR loops that were identified in Hi-C data. Values are shown as means ± SD (n = 3 biologically independent experiments). Source data are provided as a Source Data file. **g**, **h** Identified chromatin loops and tracks for the indicated ChIP-Seq enrichment at the indicated genome loci. **i** Tracks for H3K27ac ChIP-Seq enrichment at the indicated genome loci in head and neck squamous cell carcinoma (BICR16, HSC4 and Detroit562) and esophageal squamous cell carcinoma (KYSE140, KYSE510 and KYSE70) cell lines. **j** Overlap among super-silencer genes, H3K27ac-marked genes in head and neck squamous cell carcinoma and H3K27ac-marked genes in esophageal squamous cell carcinoma. **k** Metaplots of H3K27ac ChIP-Seq signals of the indicated squamous cell carcinoma cell lines at the TSSs of the overlapped genes in **j**. **l** The normalized H3K27ac ChIP-Seq enrichment signal of the indicated cell lines at SuReR (n=1,130), TyReR (n=15,653), SuHeR (n=896) and TyHeR (n=33,691) identified in LSCs. ***$p < 0.001$ from two-way ANOVA. n.s. indicates not significant. Boxplots indicate the 25th percentile (bottom of box), median (horizontal line inside box) and 75th percentile (top of box). Whiskers indicate 1.5 times the interquartile range.
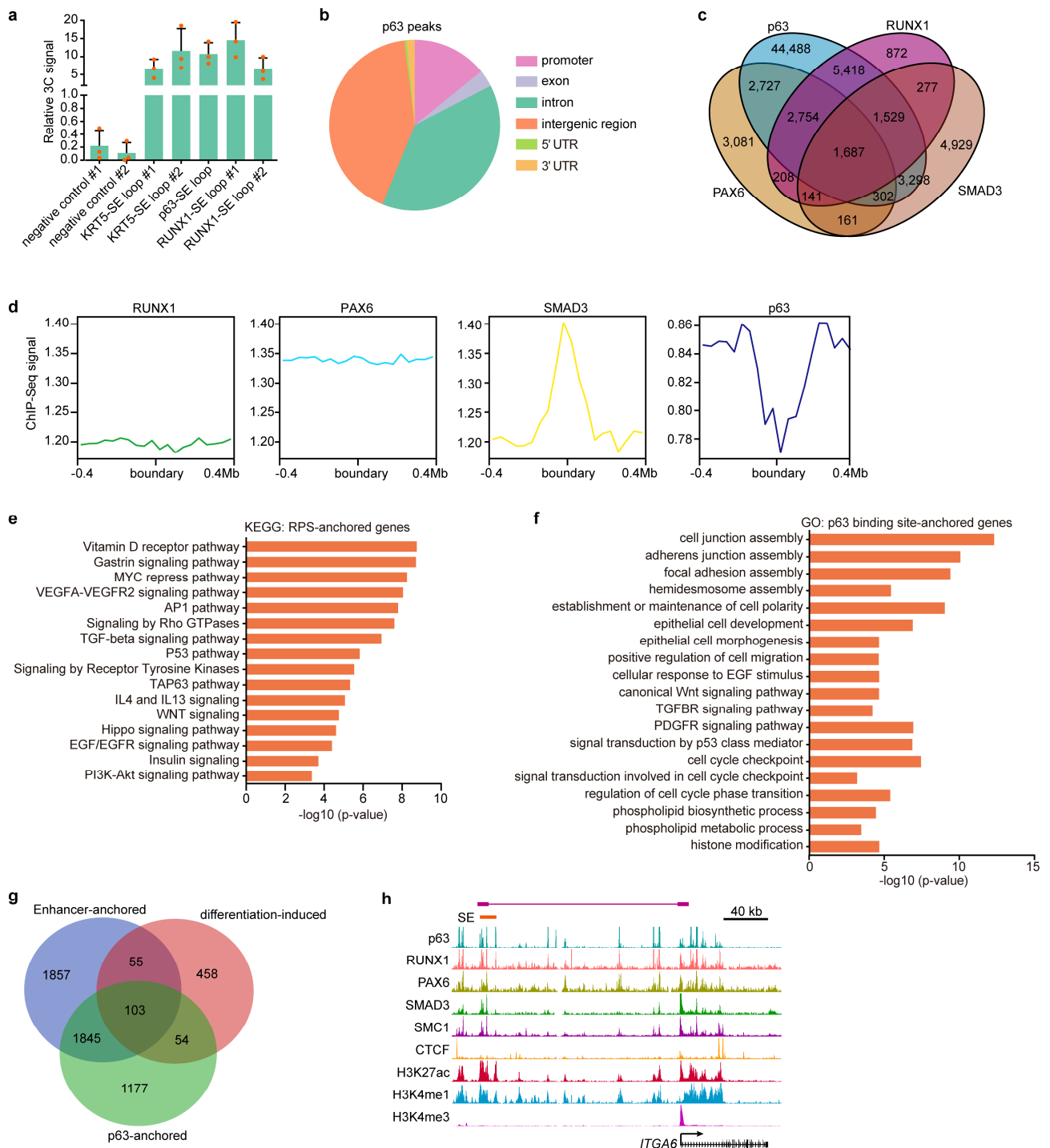
Supplementary Fig. 4 Binding pattern of p63 and RPS and their associated chromatin interactions

a 3C assay for KRT5-SE, p63-SE and RUNX1-SE loops that were identified in Hi-C data. Values are shown as means ± SD (n = 3 biologically independent experiments). Source data are provided as a Source Data file. **b** Pie chart showing the distributions of p63 peaks on the genome. **c** Overlapping among RPS and p63 peaks. **d** Metaplots showing the enrichment of RPS and p63 at

the TAD boundaries. **e** Kyoto Encyclopedia of Genes and Genomes pathway analysis for the RPS-anchored genes. **f** GO analysis for genes that interacted with p63 binding sites. **g** Vene showing the overlap among differentiation-induced, enhancer-anchored and p63-anchored genes. **h** A identified chromatin loop and tracks for the indicated ChIP-Seq signals at *ITGA6* locus.