Supplemental Methods

Cell culture

The mouse hepatocyte cell line AML12 and human Hepatocellular Carcinoma cell line HepG2 were obtained from ATCC, and the cells were cultured according to the providers' protocols.

Quantitative reverse transcription PCR (qRT-PCR)

Total RNAs were isolated using the TRIzol reagent (Invitrogen), and the first cDNA strand was synthesized with PrimeScript RT Master Mix (TAKARA). Real-time qPCR was then carried out through FastStart Essential DNA Green Master mix (Roche). Forward and reverse primers (5' to 3') are: *Actb*, GGTCATCACTATTGGCAACG and ACGGATGTCAACGTCACACT; *Hnrnpu*, TGGCCGAGGGTATTTTGAGT and AAGCACTCAGACGGTCTCTC.

Cell cycle analysis

The cell cycle was analyzed by the cell cycle and apoptosis analysis kit (Beyotime) and the FACSCalibur flow cytometer (BD).

Cellular senescence assay

Cellular senescence was detected by SA-β-gal activity with the Senescence β-Galactosidase Staining Kit (Beyotime).

DNase I assay

Three millions of AML12 cells were re-suspended in 700 μl digestion buffer (50mM Tris-HCl, 5mM $MgCl_2$, 0.1 mM $CaCl_2$, 0.2% Triton X-100, 5 mM Sodium butyrate, 1X Proteinase inhibitor) for 5min, and then aliquoted into 7 tubes. Each sample was treated with

DNase I of a gradient concentration of for 10 minutes at 25 °C. DNA samples were extracted with phenol/chloroform. Equal amounts of DNA were resolved on 1% agarose gel.

Co-Immunoprecipitation

Nuclear extracts from five millions of AML12 cells were incubated against 2 μg of antibodies as follows: anti-CTCF (CST, 3418), anti-RAD21 (Abcam, ab992), anti-HNRNPU (Abcam, ab20666) or anti-IgG (CST, 2729). The starting materials (Input) and the bound (IP) were then used for further western blot analysis.

RNA-seq and data analysis

For RNA-seq experiments, ribosomal RNA depleted and strand-specific libraries were constructed with the Ribo-zero gold (Epicentre) and TruSeq Stranded Total RNA Sample Prep kit (Illumina), and the libraries were sequenced using the Illumina HiSeq X Ten system.

We trimmed and mapped reads to the mouse mm9 reference assembly by the TopHat2 software (Kim et al. 2013) using default parameters except that we reduced maximum insertion and deletion length to 2 bp, and kept only uniquely mapped, "no mixed" and "no discordant" reads. For differential gene expression analysis, we analyzed raw read counts for GENCODE M1 genes using HTSeq (Anders et al. 2015), and then calculated statistics of differential expression via DESeq2 version 1.8.2 (Love et al. 2014) with default parameters. To define differentially expressed genes, we used false discovery rate (FDR) 0.05 and $\log_2$ (fold change) >2 or <-2 as thresholds. We performed GO analysis using DAVID bioinformatics tools (Huang da et al. 2009).

Hi-C data analysis

We used the HiC-pro pipeline (Servant et al. 2015) to process the Hi-C raw data, and

generated ICE normalized matrices (Imakaev et al. 2012) at 500 kb, 200 kb, 40 kb and 10 kb resolutions. To detect differences between experiments, we further normalized interaction matrices for "depth" as previously described (Zuin et al. 2014). These normalized interaction matrices were used for downstream analysis. For 10, 40 and 200 kb resolution matrices, we used normPerExpected function form R packages (HiTC) to get obs/exp values based on the mean interaction of each diagonal (Servant et al. 2012). For 200 kb resolution matrices, we also calculated the Z-scores using a modified LOWESS method (α = 1%, IQR filter, ignore zero) through the cworld::dekker script (Giorgetti et al. 2016).

To detect compartments, we used the R packages (HiTC) pca.hic function to generate the PC1 eigenvectors using 200 kb resolution matrices with the following options: (normPerExpected=TRUE, npc=1). To investigate compartment switching, we defined switched bins only if PC1 eigenvectors change in the same direction for two replicates.

To identify TADs, we detected boundaries via the insulation method (Crane et al. 2015) using normalized matrices at 40k resolution. We used cworld::dekker scripts to detected Insulation vectors with the following options：matrix2insulation.pl -is 480000 -ids 240000 -im median -nt 0.1 -ss 120000. If the distance between two boundaries is ≤ 40 kb，we treated them as one. To compare TADs and compartments, we performed K-means clustering of TAD boundaries with PC1 values at 40kb resolution, and defined three categories: boundaries within A, within B and between A and B. For the comparison of TAD boundary strengths between control and knockdown, we defined increased or decreased boundaries by the criteria: intensity change > 0.1 or < -0.1 in both replicates.

For chromatin loop analysis, we converted "all.vaildpairs" files generated from HiC-pro

pipeline to "hic" files using the HiC-Pro script: hicpro2juicebox.sh. Identification of loops

needs very high read intensities, thus we merged data from all samples for loop calling by

HICCUPS (Durand et al. 2016), with the following parameters: -m 512 -r 10000 -k KR -f 0.1

-d 20000. We calculated intensities of peaks and their neighborhoods in control and

knockdown, respectively. Then we used relative fold changes between loops and their four

neighborhoods to estimate the enrichment level for each peak. We then compared the peak

intensity and fold changes relative to four neighborhoods between control and knockdown,

and defined changed loops only if all the values change in the same tendency.

ChIP-seq, bioChIP-seq and data analysis

For bioChIP experiments, 10 million cells were fixed with 1% formaldehyde for 10min.

Cells were lysed at 25°C for 5 min in lysis buffer (50 mM Tris-HCl, pH 7.5, 500 mM NaCl,

0.4% SDS, 5 mM EDTA, 1 mM DTT, 2% Triton X-100 and 1× Complete protease inhibitor).

The chromatin was fragmented with Bioruptor (Diagenode) and then was incubated with 30

μl High Capacity Streptavidin Agarose (Thermo Fisher Scientific) overnight. Beads were

washed twice with buffer 1 (2% SDS), once with buffer 2 (50 mM HEPES, pH 7.5, 0.1%

Deoxycholate, 1% Triton X-100, 500 mM NaCl, 1 mM EDTA), once with buffer 3 (10 mM

Tris-HCl, pH 8.0, 250 mM LiCl, 0.5% NP-40, 0.5% Deoxycholate, 1 mM EDTA) and twice

with buffer 4 (50 mM Tris-HCl, pH 7.4, 50 mM NaCl). Finally, beads were eluted with 60 μl

Elution buffer (10 mM Tris-HCl, pH8.0, 1mM EDTA, pH8.0, 1%SDS) at 70°C overnight

with shaking. We used virus-based systems for expressing of the BirA enzyme and tagged

HNRNPU proteins. The rtTA-IRES-BirA element (addgene: 31375) was sub-cloned into

pCDH-CMV-MCS-EF1-Puro expression vector, and the flbio tag (addgene: 30216) was

sub-cloned into Lv-blasticidin-Tre vector (Supplemental Fig. S8A).

ChIP DNA libraries were prepared for sequencing using standard Illumina protocols. Reads were aligned to the mouse mm9 genome using bowtie1.1.2 (-m 1 -n 2 -e 70 -k 1 --best -l 75). Low-quality reads were filtered with SAMtools (Li et al. 2009) (MAPQ < 10). PCR duplicates were removed using Picard. For CTCF and RAD21, MACS2 (Zhang et al. 2008) was used for peak calling with the default parameters except for q = 0.01. Peaks were further filtered if they are discordant between biological replicates. For HNRNPU, we use BirA-only data as control files and call peaks by MACS2 with the following parameters: -q 0.01, --broad, --broad-cutoff 0.01.

To call differential peaks upon *Hnrnpu* knockdown, we used the R packages ChIPComp (Chen et al. 2015), by which we can perform the quantitative comparison for ChIP-seq data and detect differential binding between multiple conditions. We also calculated the ΔRPKM (RPKM IP minus RPKM input) for each peak and use the $\log_2$ ((shUΔRPKM+1)/ (shCtrlΔRPKM+1)) value to measure the fold change for each peak. Finally, we defined differential peaks using the criteria: $p < 0.01$(ChIPComp) and $\log_2$ Fold Change) >1 or <-1. The coordinates and statistics of peaks were provided in Supplemental Table S5.

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