I. Experimental Methods

I.a. Hi-C protocols

In this paper, we report the results of 201 Hi-C experiments. To produce most of the libraries reported in this paper, we employed *in situ* Hi-C. Several additional libraries were generated using variants of *in situ* Hi-C. Still other libraries were generated using the original “dilution” Hi-C protocol (Lieberman-Aiden et al., 2009). The contact maps produced by Hi-C are extremely robust to changes in the protocol, except as described below and in the main text (see Data S1.I.A).

I.a.1. In situ Hi-C protocol

Crosslinking

1) Grow two to five million mammalian cells under recommended culture conditions to about 80% confluence. Pellet suspension cells or detached adherent cells by centrifugation at 300xG for 5 minutes.
2) Resuspend cells in fresh medium at a concentration of $1x10^6$ cells per 1ml media. In a fume hood, add freshly made formaldehyde solution to a final concentration of 1%, v/v. Incubate at room temperature for 10 minutes with mixing.
3) Add 2.5M glycine solution to a final concentration of 0.2M to quench the reaction. Incubate at room temperature for 5 minutes on rocker.
4) Centrifuge for 5 minutes at 300xG at 4°C. Discard supernatant into an appropriate collection container.
5) Resuspend cells in 1ml of cold 1X PBS and spin for 5 minutes at 300xG at 4°C. Discard supernatant and flash-freeze cell pellets in liquid nitrogen or dry ice/ ethanol.
6) Either proceed to the rest of the protocol or store cell pellets at -80°C.

Lysis and Restriction Digest

7) Combine 250µl of ice-cold Hi-C lysis buffer (10mM Tris-HCl pH8.0, 10mM NaCl, 0.2% Igepal CA630) with 50µl of protease inhibitors (Sigma, P8340). Add to one crosslinked pellet of cells.
8) Incubate cell suspension on ice for >15 minutes. Centrifuge at 2500xG for 5 minutes. Discard the supernatant.
9) Wash pelleted nuclei once with 500µl of ice-cold Hi-C lysis buffer.
10) Gently resuspend pellet in 50µl of 0.5% sodium dodecyl sulfate (SDS) and incubate at 62°C for 5-10 minutes.
11) After heating is over, add 145µl of water and 25µl of 10% Triton X-100 (Sigma, 93443) to quench the SDS. Mix well, avoiding excessive foaming. Incubate at 37°C for 15 minutes.
12) Add 25µl of 10X NEBuffer2 and 100U of MboI restriction enzyme (NEB, R0147) and digest chromatin overnight or for at least 2 hours at 37°C with rotation.

Marking of DNA Ends, Proximity Ligation, and Crosslink Reversal

13) Incubate at 62°C for 20 minutes to inactivate MboI, then cool to room temperature.
14) To fill in the restriction fragment overhangs and mark the DNA ends with biotin, add 50µl of fill-in master mix:
    - 37.5µl of 0.4mM biotin-14-dATP (Life Technologies, 19524-016)
    - 1.5µl of 10mM dCTP
    - 1.5µl of 10mM dGTP
    - 1.5µl of 10mM dTTP
    - 8µl of 5U/µl DNA Polymerase I, Large (Klenow) Fragment (NEB, M0210)
15) Mix by pipetting and incubate at 37°C for 45 minutes-1.5 hours with rotation.
16) Add 900µl of ligation master mix:
    - 663µl of water
    - 120µl of 10X NEB T4 DNA ligase buffer (NEB, B0202)
    - 100µl of 10% Triton X-100
    - 12µl of 10mg/ml Bovine Serum Albumin (100X BSA)
    - 5µl of 400 U/ µl T4 DNA Ligase (NEB, M0202)
17) Mix by inverting and incubate at room temperature for 4 hours with slow rotation.
18) Degrade protein by adding 50µl of 20mg/ml proteinase K (NEB, P8102) and 120µl of 10% SDS and incubate at 55°C for 30 minutes. (Note that nuclei can be pelleted after ligation and then resuspended, both to remove random ligation products that may have occurred in solution and to reduce the overall volume for ease of handling.)
19) Add 130µl of 5M sodium chloride and incubate at 68°C overnight or for at least 1.5 hours.

DNA Shearing and Size Selection

20) Cool tubes at room temperature.
21) Split into two 750µl aliquots in 2ml tubes and add 1.6X volumes of pure ethanol and 0.1X volumes of 3M sodium acetate, pH 5.2, to each tube. Mix by inverting and incubate at -80°C for 15 minutes.
22) Centrifuge at max speed, 2°C for 15 minutes. Keep the tubes on ice after spinning and carefully remove the supernatant by pipetting.
23) Resuspend, combining the two aliquots, in 800µl of 70% ethanol. Centrifuge at max speed for 5 minutes.
24) Remove all supernatant and wash the pellet once more with 800µl of 70% ethanol.
25) Dissolve the pellet in 130µl of 1X Tris buffer (10 mM Tris-HCl, pH 8) and incubate at 37°C for 15 minutes to fully dissolve the DNA.
26) To make the biotinylated DNA suitable for high-throughput sequencing using Illumina sequencers, shear to a size of 300-500bp using the following parameters:
    Instrument: Covaris LE220 (Covaris, Woburn, MA)
    Volume of Library: 130µl in a Covaris microTUBE
    Fill Level: 10
    Duty Cycle: 15
    PIP: 500
    Cycles/Burst: 200
    Time: 58 seconds
27) Transfer sheared DNA to a fresh 1.5ml tube. Wash the Covaris vial with 70µl of water and add to the sample, bringing the total reaction volume to 200µl. Run a 1:5 dilution of DNA on a 2% agarose gel to verify successful shearing. For libraries containing fewer than $2x10^6$ cells, the size selection using AMPure XP beads described in the next steps could be performed on final amplicons rather than before biotin pull-down.
28) Warm a bottle of AMPure XP beads (Beckman Coulter, A63881) to room temperature. To increase yield, AMPure XP beads can be concentrated by removing some of the clear solution before the beads are mixed for use in the next steps.
29) Add exactly 110µl (0.55X volumes) of beads to the reaction. Mix well by pipetting and incubate at room temperature for 5 minutes.
30) Separate on a magnet. Transfer the clear solution to a fresh tube, avoiding any beads. The supernatant will contain fragments shorter than 500bp.
31) Add exactly 30µl of fresh AMPure XP beads to the solution. Mix by pipetting and incubate at room temperature for 5 minutes.
32) Separate on a magnet and keep the beads. Fragments in the range of 300-500bp will be retained on the beads. Discard the supernatant containing degraded RNA and short DNA fragments.
33) Keeping the beads on the magnet, wash twice with 700µl of 70% ethanol without mixing.
34) Leave the beads on the magnet for 5 minutes to allow remaining ethanol to evaporate.
35) To elute DNA, add 300µl of 1X Tris buffer, gently mix by pipetting, incubate at room temperature for 5 minutes, separate on a magnet, and transfer the solution to a fresh 1.5ml tube.
36) Quantify DNA by Qubit dsDNA High Sensitivity Assay (Life Technologies, Q32854) and run undiluted DNA on a 2% agarose gel to verify successful size selection.

Biotin Pull-Down and Preparation for Illumina Sequencing

Perform all the following steps in low-bind tubes.

37) Prepare for biotin pull-down by washing 150µl of 10mg/ml Dynabeads MyOne Streptavidin T1 beads (Life technologies, 65602) with 400µl of 1X Tween Washing Buffer (1X TWB: 5mM Tris-HCl (pH 7.5); 0.5mM EDTA; 1M NaCl; 0.05% Tween 20). Separate on a magnet and discard the solution.
38) Resuspend the beads in 300µl of 2X Binding Buffer (2X BB: 10mM Tris-HCl (pH 7.5); 1mM EDTA; 2M NaCl) and add to the reaction. Incubate at room temperature for 15 minutes with rotation to bind biotinylated DNA to the streptavidin beads.
39) Separate on a magnet and discard the solution.
40) Wash the beads by adding 600µl of 1X TWB and transferring the mixture to a new tube. Heat the tubes on a Thermomixer at 55°C for 2 min with mixing. Reclaim the beads using a magnet. Discard supernatant.
41) Repeat wash.
42) Resuspend beads in 100ul 1X NEB T4 DNA ligase buffer (NEB, B0202) and transfer to a new tube. Reclaim beads and discard the buffer.
43) To repair ends of sheared DNA and remove biotin from unligated ends, resuspend beads in 100µl of master mix:
    88µl of 1X NEB T4 DNA ligase buffer with 10mM ATP

2µl of 25mM dNTP mix
5µl of 10U/µl NEB T4 PNK (NEB, M0201)
4µl of 3U/µl NEB T4 DNA polymerase I (NEB, M0203)
1µl of 5U/µl NEB DNA polymerase I, Large (Klenow) Fragment (NEB, M0210)

44) Incubate at room temperature for 30 minutes. Separate on a magnet and discard the solution.
45) Wash the beads by adding 600µl of 1X TWB and transferring the mixture to a new tube. Heat the tubes on a Thermomixer at 55°C for 2 min with mixing. Reclaim the beads using a magnet. Discard supernatant.
46) Repeat wash.
47) Resuspend beads in 100µl 1X NEBuffer 2 and transfer to a new tube. Reclaim beads and discard the buffer.
48) Resuspend beads in 100µl of dATP attachment master mix:
    90µl of 1X NEBuffer 2
    5µl of 10mM dATP
    5µl of 5U/µl NEB Klenow exo minus (NEB, M0212)
49) Incubate at 37°C for 30 minutes. Separate on a magnet and discard the solution.
50) Wash the beads by adding 600µl of 1X TWB and transferring the mixture to a new tube. Heat the tubes on a Thermomixer at 55°C for 2 min with mixing. Reclaim the beads using a magnet. Discard supernatant.
51) Repeat wash.
52) Resuspend beads in 100µl 1X Quick ligation reaction buffer (NEB, B6058) and transfer to a new tube. Reclaim beads and discard the buffer.
53) Resuspend in 50µl of 1X NEB Quick ligation reaction buffer.
54) Add 2µl of NEB DNA Quick ligase (NEB, M2200). Add 3µl of an Illumina indexed adapter. Record the sample-index combination. Mix thoroughly.
55) Incubate at room temperature for 15 minutes. Separate on a magnet and discard the solution.
56) Wash the beads by adding 600µl of 1X TWB and transferring the mixture to a new tube. Heat the tubes on a Thermomixer at 55°C for 2 min with mixing. Reclaim the beads using a magnet. Remove supernatant.
57) Repeat wash.
58) Resuspend beads in 100µl 1X Tris buffer and transfer to a new tube. Reclaim beads and discard the buffer.
59) Resuspend in 50µl of 1X Tris buffer.

Final Amplification and Purification

60) Amplify the Hi-C library directly off of the T1 beads with 4-12 cycles of PCR, using Illumina primers and protocol (Illumina, 2007). (Note that recent lots of some streptavidin beads may interfere with PCR; to avoid this, one can remove the DNA from the streptavidin beads by heating at 98C for 10 minutes after step 59 and then removing the beads with a magnet.)
61) After amplification is complete, bring the total library volume to 250µl.
62) Separate on a magnet. Transfer the solution to a fresh tube and discard the beads.
63) Warm a bottle of AMPure XP beads to room temperature. Gently shake to resuspend the magnetic beads. Add 175µl of beads to the PCR reaction (0.7X volumes). Mix by pipetting and incubate at room temperature for 5 minutes.
64) Separate on a magnet and remove the clear solution.
65) Keeping the beads on the magnet, wash once with 700µl of 70% ethanol without mixing.
66) Remove ethanol completely. To remove traces of short products, resuspend in 100µl of 1X Tris buffer and add another 70µl of AMPure XP beads. Mix by pipetting and incubate at room temperature for 5 minutes.
67) Separate on a magnet and remove the clear solution.
68) Keeping the beads on the magnet, wash twice with 700µl of 70% ethanol without mixing.
69) Leave the beads on the magnet for 5 minutes to allow the remaining ethanol to evaporate.
70) Add 25-50µl of 1X Tris buffer to elute DNA. Mix by pipetting, incubate at room temperature for 5 minutes, separate on a magnet, and transfer the solution to a fresh labeled tube. The result is a final *in situ* Hi-C library ready to be quantified and sequenced using an Illumina sequencing platform.

I.a.2. In situ Hi-C libraries can be constructed in three days: The above protocol takes either 3 or 4 days, depending on whether shorter incubation times are used for the restriction step (2 hours; see step 12), the fill-in step (45 minutes; see step 15), and the crosslink reversal step (1.5 hours; see step 19). Most of the *in situ* Hi-C libraries reported in this paper were performed using the 4 day protocol, but in our experience, use of the 3 day protocol has no effect on library quality.

I.a.3. "Tethered" in situ Hi-C: Kalhor et al. (2012) introduced a variant of Hi-C that they called "Tethered Conformation Capture" (TCC), in which proteins are biotinylated prior to restriction so that crosslinked chromatin can be tethered to

streptavidin beads. Fill-in of restricted fragment ends and blunt-end ligation is then performed on beads. They reasoned that this would limit interactions between non-crosslinked fragments.

While tethering might have a significant impact on chromatin in dilution, we reasoned that tethering proteins to beads prior to ligation should have no effect on our *in situ* protocol, as chromatin is already constrained by the intact nucleus. We adapted the TCC protocol in order to develop a tethered variant of our *in situ* protocol and confirm that it does not have an impact on library quality.

After step 10 of the *Lysis and Restriction Digest* section above, we mixed the suspension with 20µl of 25 mM EZlink Iodoacetyl-PEG2-Biotin (IPB) (Pierce Protein Biology Products, 21334) and rocked at room temperature for 60 minutes. We then mixed the sample with 260µl of NEBuffer2 and 45µl of 10% Triton X-100, incubated on ice for 10 minutes and then at 37°C for 10 minutes. Next, we added 20µl of NEBuffer2, 1µl of 1M DTT, 86µl of water, and 100U of MboI and incubated at 37°C overnight to digest the chromatin.

The next day, we passed the sample through a 2mL Zeba spin desalting column (Thermo Scientific, 89889) in order to remove any unreacted IPB.

The steps between attachment to MyOne Streptavidin T1 beads (Invitrogen) and detachment from the beads were performed as in TCC (Kalhor et al., 2012), with the exception that the dNTPs used in the fill-in step were the same as the ones that we use in our *in situ* Hi-C protocol, and the ligation was either performed in 5mL (as in TCC) or in 1mL (with all volumes scaled down). In both cases, 5µl of 400 U/ µl T4 DNA Ligase (NEB, M0202) was added during ligation. After detachment of the library from the T1 beads, the library was completed using the standard *in situ* Hi-C protocol beginning with step 19.

I.a.4. In situ Hi-C in agar plugs: After lysis (as in the usual *in situ* Hi-C protocol, step 11), nuclei were resuspended in 100*µ*l 2X NEBuffer2 and mixed with 100*µ*l molten 2% NuSieve agarose (Lonza, 5009) and allowed to solidify into an agarose plug. The nuclei embedded in agar were restricted overnight in 500*µ*l 1X NEBuffer2 with 100U of MboI at 37°C.

After restriction, the buffer was discarded and the agar plug was washed twice with 1ml of 1X NEB T4 DNA ligase buffer for 30min at 37°C. The buffer was discarded and the agar plug was submerged in 0.5ml fill-in reaction mix:

398µl of water
50µl of 10X NEB T4 DNA ligase buffer
37.5µl of 0.4mM biotin-14-dATP
1.5µl of 10mM dCTP
1.5µl of 10mM dGTP
1.5µl of 10mM dTTP
10µl of 5U/µl DNA Polymerase I, Large (Klenow) Fragment

The library was incubated for 1.5 hours at room temperature. After incubation, 2000U of T4 DNA Ligase were added to the reaction and the library was ligated at room temperature for 4 hours.

After ligation, the buffer was discarded and the agar plug was washed twice with 1ml of 1X NEB β-agarase I buffer (NEB, B0392) for 30min at 37°C. The buffer was removed and the agarose was melted by incubation at 68°C for 10 minutes. Liquid agarose was equilibrated at 42°C for 15 minutes. The agarose was digested with 4U of β-Agarase I (NEB, M0392) at 42°C for 1 hour. Next, we reversed the crosslinks. All subsequent steps were performed following the standard *in situ* Hi-C protocol beginning at step 18.

I.a.5. Pellet and Supernatant Hi-C: In situ Hi-C was performed in the usual fashion, but with an additional centrifugation step added after restriction (step 12) and prior to fill-in. We pelleted nuclei after restriction, transferred the supernatant to another tube, resuspended the nuclei in fresh buffer and proceeded with the rest of the protocol simultaneously on both the nuclei and the supernatant.

I.a.6 In situ Hi-C without crosslinking: In situ Hi-C can be performed without the use of crosslinking. In this study, we constructed five Hi-C libraries without the use of formaldehyde, or any, crosslinker. One library was constructed using the standard *in situ* Hi-C protocol (without crosslinking) with extremely gentle handling to avoid disrupting the nucleus or genome structure within the nucleus. The other four no-crosslinking libraries were constructed after embedding the uncrosslinked nuclei in agar plugs (section *I.a.4*) in order to maintain nuclear shape and structure. While the data generated by the no-crosslinking protocols is noisier, the main features we report in this study are all visible in our no-crosslinking maps.

I.a.7. Dilution Hi-C: Dilution Hi-C was performed as in Lieberman-Aiden et al. (2009)

I.a.8. Other experimental variations: In addition to the nine main variants of Hi-C outlined above (*in situ*, 3-day *in situ*, tethered *in situ*, agar *in situ*, pellet, supernatant, *in situ* without crosslinking, agar *in situ* without crosslinking, and dilution Hi-C), we performed over one hundred additional experiments in which we modified cell crosslinking time, choice of restriction enzyme, choice of biotinylated nucleotide, and ligation volume. The experimental parameters for each library are listed in Table S1.

I.a.9. Replicate Experiments: In this paper, we refer to both "technical replicates" and "biological replicates." Two Hi-C libraries are "technical replicates" if the cells were crosslinked together and identical Hi-C protocols were applied to two aliquots. Two samples are "biological replicates" if the cells were not crosslinked together; more specifically, the underlying cell populations were different due to additional passaging.

In Table S1, we label each of the 201 Hi-C libraries we report here with a unique identifier of the form HIC* where the * represents numerical values. The number after 'HIC' in the identifier is unique for every Hi-C library. We also provide second number (in the biological replicate column in Table S1) that indicates the biological replicate number; for a given cell type, libraries with the same biological replicate number were constructed from the same batch of crosslinked cells. Two libraries that are constructed from the same cell type and using the same protocol variant are technical replicates if they have the same biological replicate number or biological replicates if they have different biological replicate numbers.

I.a.10. Sequencing: Paired-end sequencing was performed using the Illumina MiSeq, HiSeq 2000, and HiSeq 2500 platforms.

I.a.11. No-ligation control: In order to assess the biases introduced during the digestion step of our protocol, we routinely perform "no-ligation" controls. In a no-ligation control, crosslinked pellets are lysed and digested as in the standard *in situ* Hi-C protocol (steps 1-12), but the post-digestion fill-in and ligation steps are omitted (steps 13-19) and the library is prepped for sequencing. For this study, we sequenced two such no-ligation controls, one digested with HindIII (113M paired-end reads) and one digested with MboI (114M paired-end reads).

I.b. ChIP-Seq

ChIP-Seq was performed following the protocol outlined by the ENCODE consortium (Landt et al., 2012). We performed ChIP-Seq for H3K9me3 using three different antibodies: one replicate using the same antibody as ENCODE (Abcam ab8898), three replicates using an antibody from Millipore (Millipore 17-625) and three replicates using an antibody from Diagenode (Diagenode C15410056 pAb-056-50). We also performed three replicate ChIP-Seq experiments for H3K36me3 using an antibody from Abcam (ab9050). The ten libraries were each sequenced to a depth of 8-10M reads along with two input controls and an IgG control. Data was processed using MACS (Zhang et al., 2007).

I.c. 3D DNA FISH

3D DNA fluorescence *in situ* hybridization (FISH) was performed on GM12878 human lymphoblastoid cells essentially as described in (Beliveau et al., 2012), with the following minor changes.

For the experiments testing the HiCCUPS annotated GM12878 loops we called in our *in situ* map, a pool of 32bp sequences tiling nine 30 kb target loci at a density of 9-15 probes per kilobase was designed with OligoArray (Table S5, (Beliveau et al., 2012)). For each set of 32bp sequences corresponding to one locus, a pair of 21bp random primers was generated to flank the 32bp of genomic sequence. The forward and reverse primers were generated to include Nb.BsmI and Nb.BsrDI (New England Biolabs R0706, R0648) nicking sites, respectively, to allow for probe generation using the OligoPaints protocol. The resulting pool of 74bp oligomers was ordered as dsDNA from CustomArray, Inc. (Bothell, WA). Forward primers were synthesized with a 5' conjugated fluorophore (Alexa Fluor 488, Alexa Fluor 546/ATTO 565, or Alexa Fluor 647) and purified by HPLC (Integrated DNA Technologies); reverse primers contained no dyes and were purified by standard desalting. Oligopaints were amplified via PCR without emulsion, and fluorophores were kept in darkness as much as possible to minimize photobleaching. Cells in serum-free growth media were placed on poly-L-lysine coated slides (Electron Microscopy Sciences) at a concentration of 1-2x10^6 cells/ml and allowed to adhere for 0.5-1.5 hours at 37°C, 5% CO_2 prior to fixation for 7-10 minutes in 4% (v/v) paraformaldehyde in 1X PBS. The hybridization cocktail (15-20