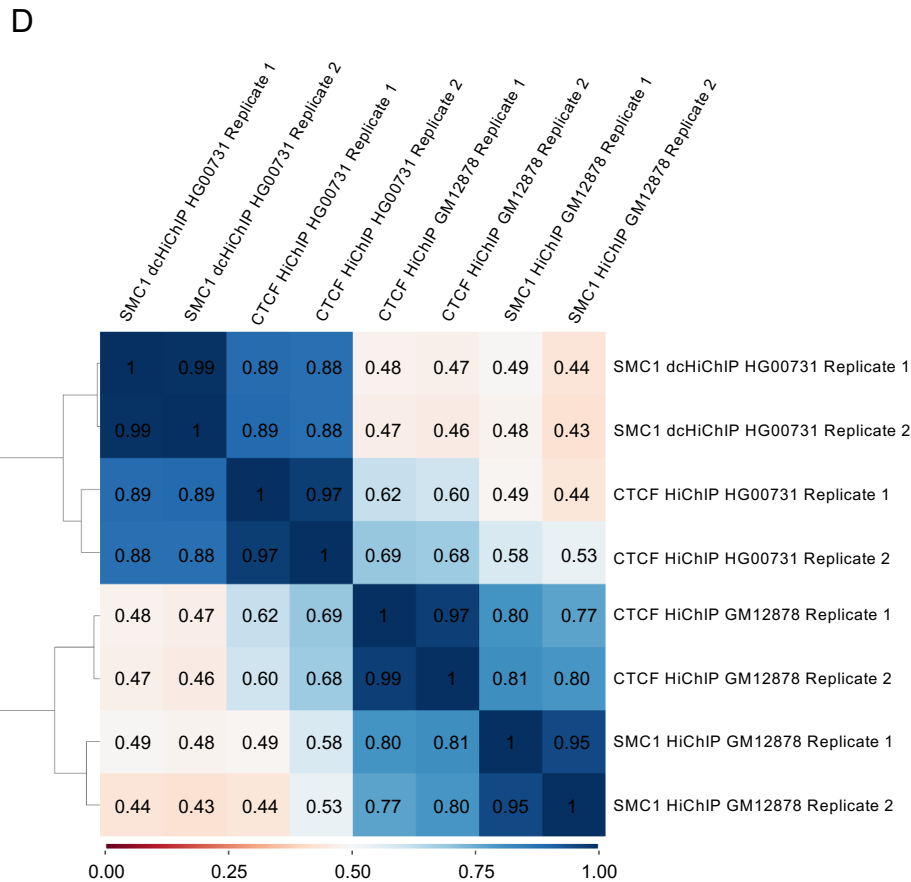
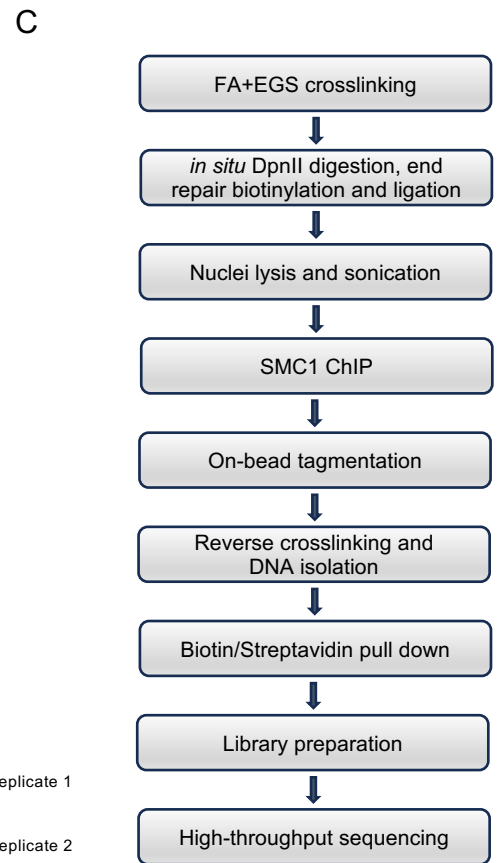
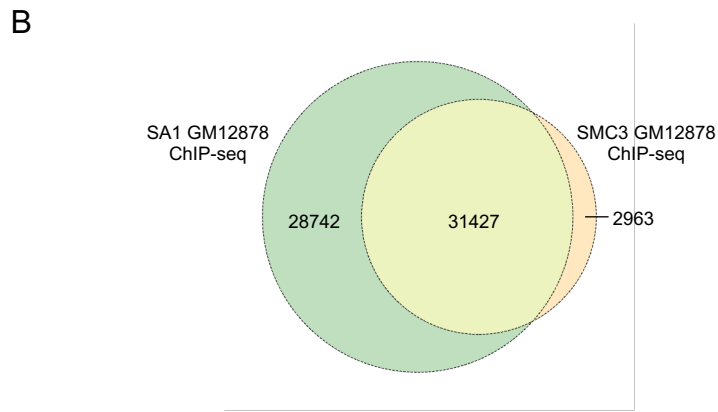
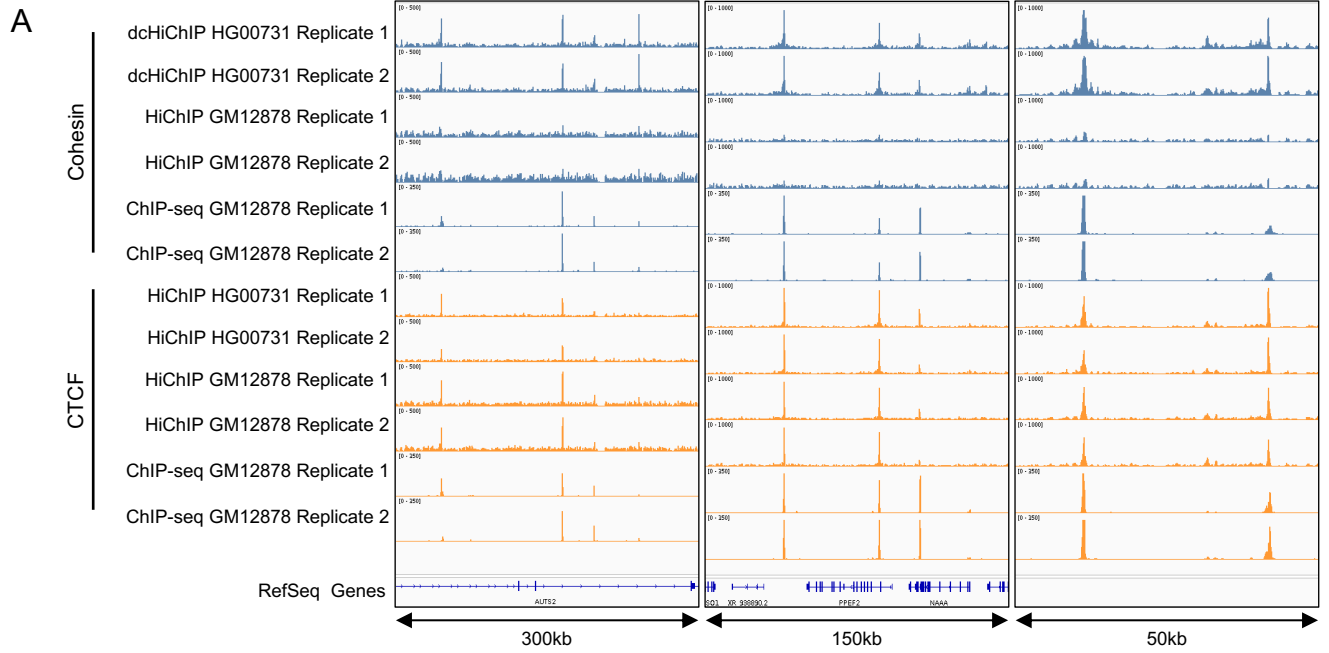
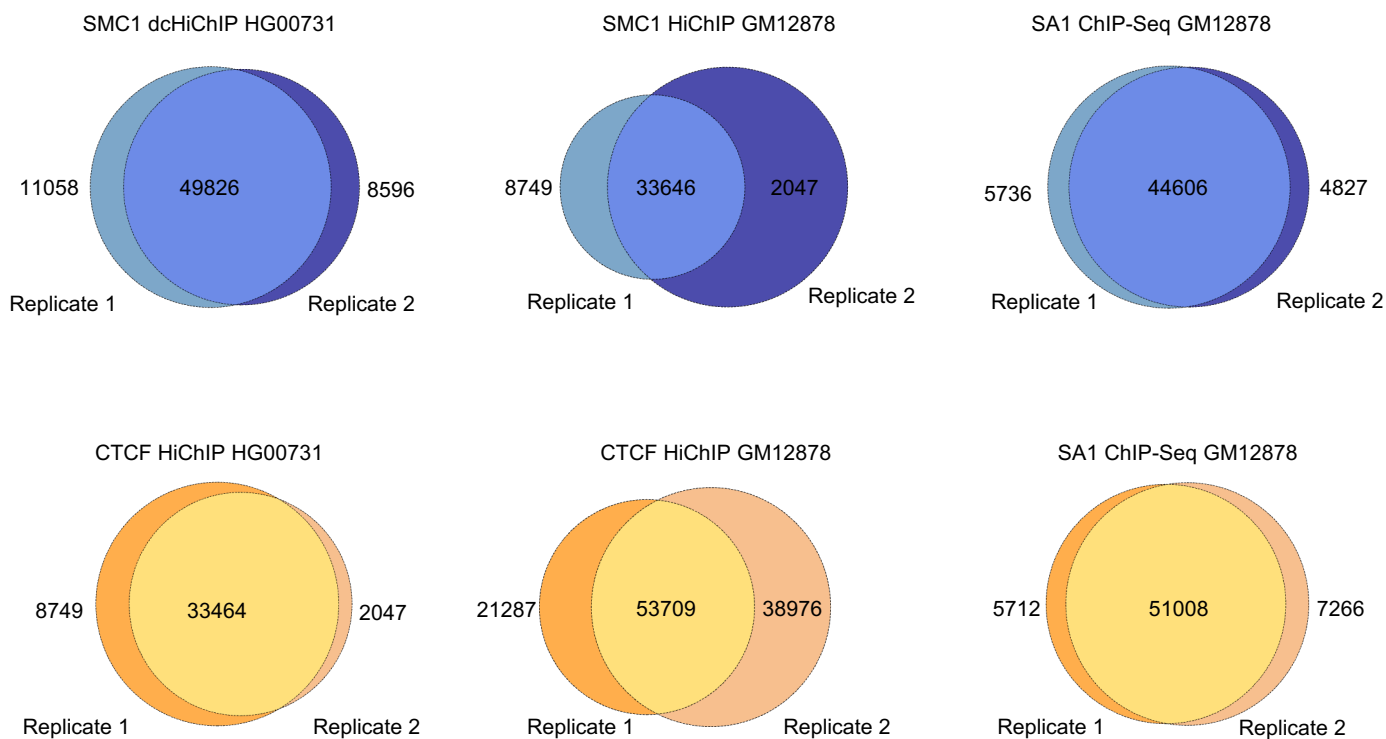
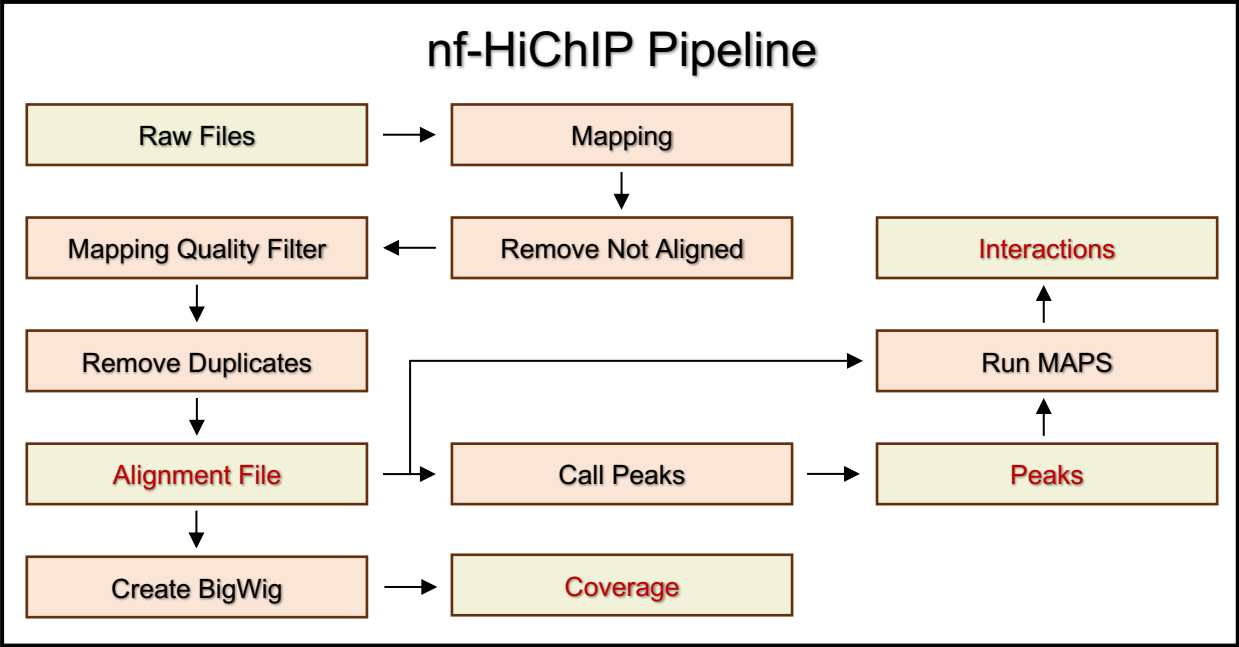




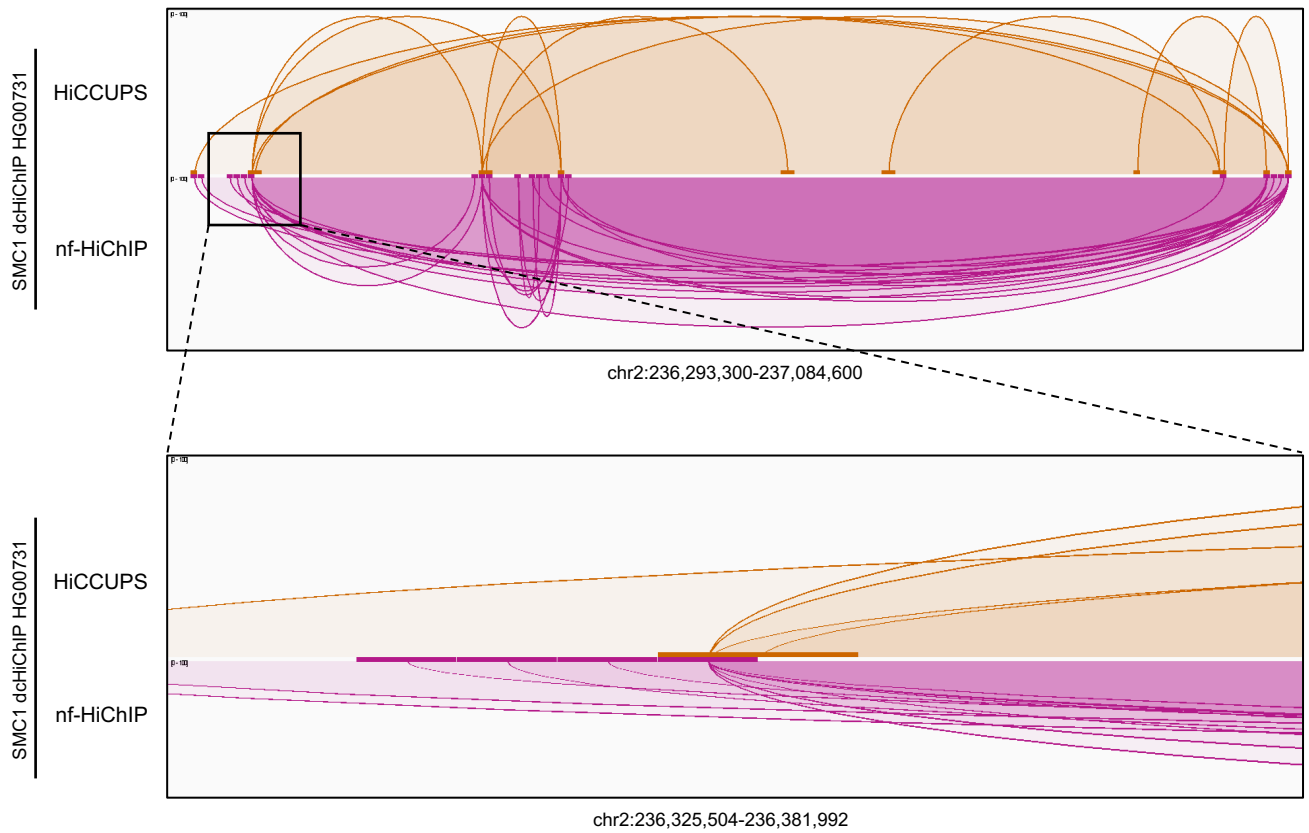
Supplementary Figure 1. A. IGV browser coverage tracks from separate replicates of the HiChIP and ChIP-seq experiments analysed in this study. The following example genomic regions are shown: (1) 300 kb window - chr7:69,997,000-70,297,300; (2) 150 kb window - chr4:75,809,763-75,960,323; (3) 50 kb window - chr7:4,600,000-4,650,000. **B.** Venn diagrams showing common peaks between GM12878 SA1 ChIP-seq and GM12878 SMC3 ChIP-seq. **C.** Schematic representation of the experimental steps performed during the cohesin FA-EGS HiChIP protocol. **D.** Heatmap showing Pearson correlation coefficient between the individual replicates of the HiChIP and ChIP-seq samples processed in this study.



Supplementary Figure 2. Venn diagrams showing common peaks between peaks called in the first and second replicate for the indicated HiChIP and ChIP-seq experiments.

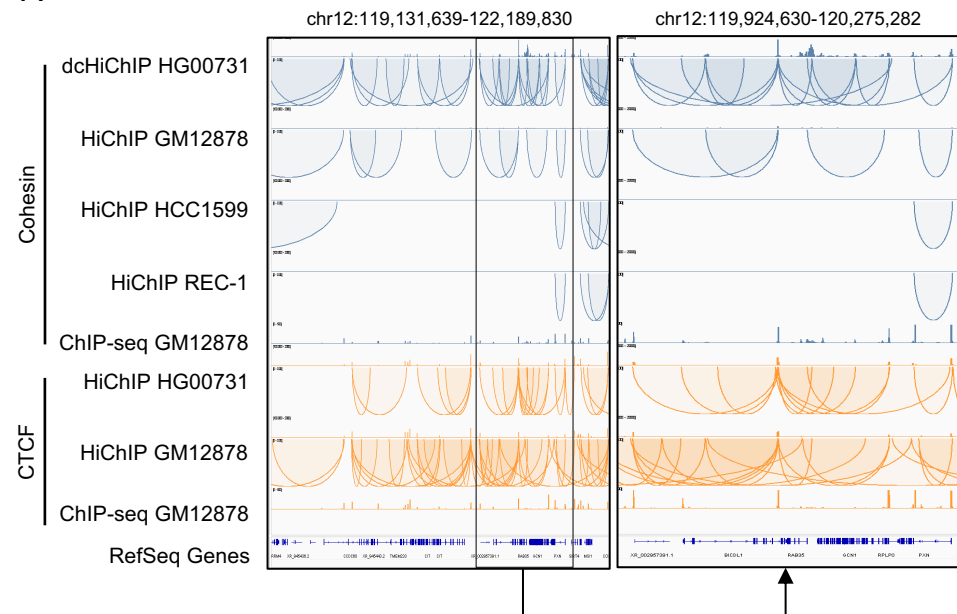


Supplementary Figure 3. nf-HiChIP workflow.

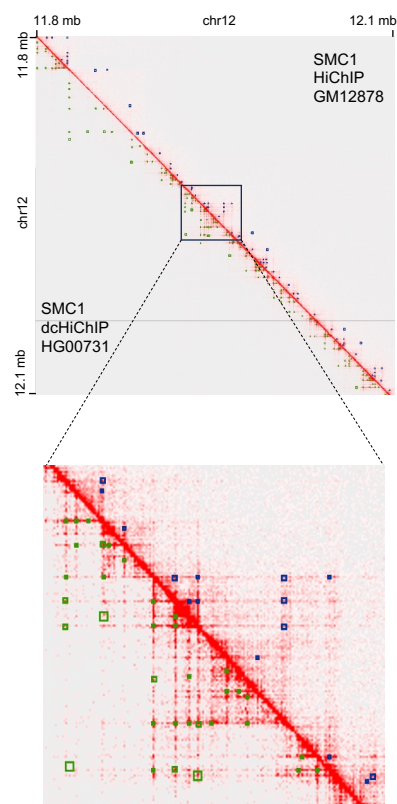


Supplementary Figure 4. HiCCUPS uses an image processing approach to identify loops (shown as yellow) from chromatin interaction maps at three different resolutions: 5 kb, 10 kb, and 25 kb and then merges loops based on the predefined threshold. In contrast, as nf-HiChIP directly detects loops from interaction data at a single, user-defined resolution 5 kb (as shown in red). Due to these methodological differences specifically the higher resolution and lack of merging in nf-HiChIP - multiple nf-HiChIP loops anchors can overlap with a single HiCCUPS loop anchors.

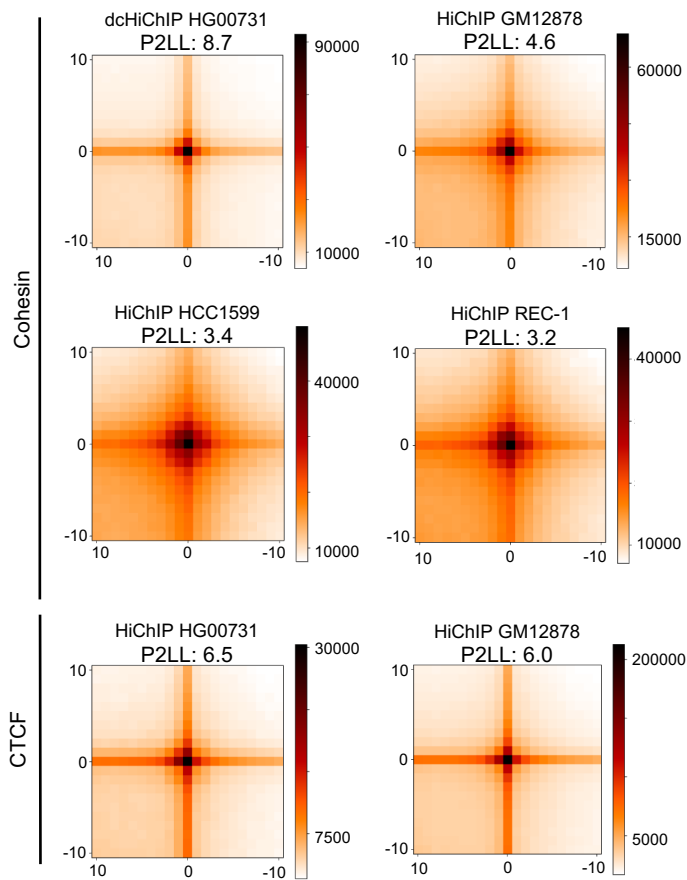
A



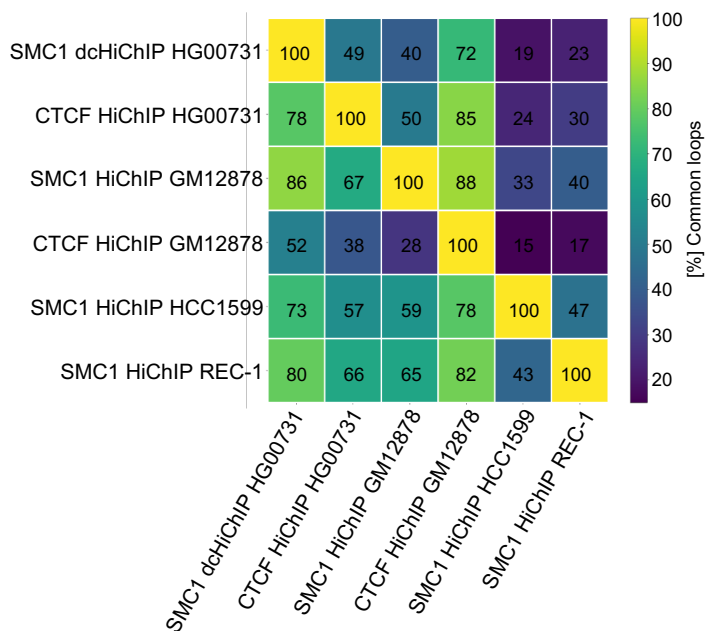
B



C

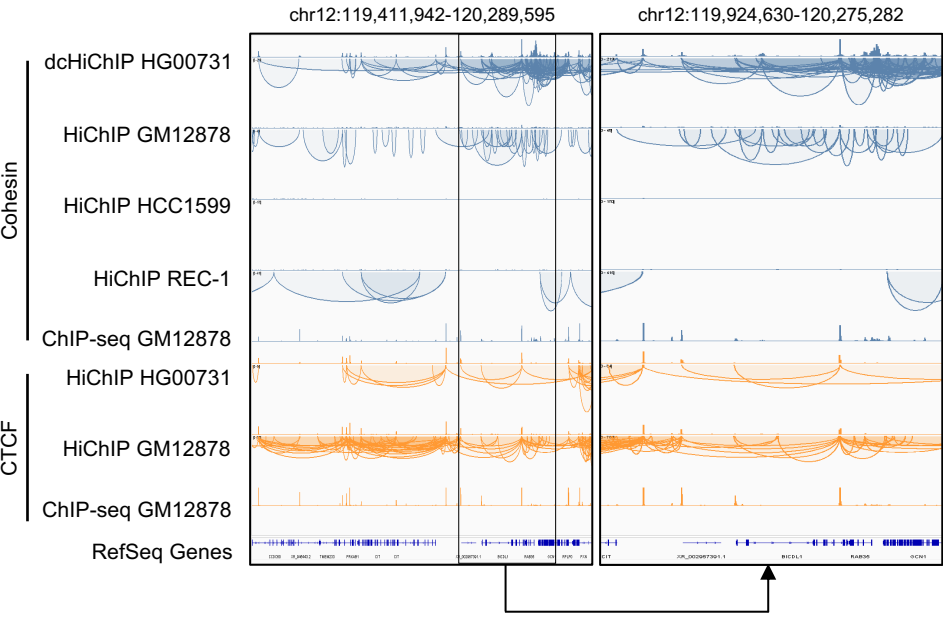


D

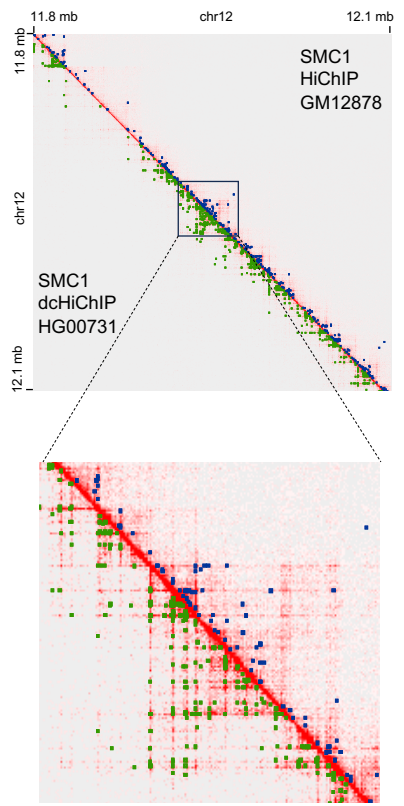


Supplementary Figure 5. FA-EGS cross-linking HiChIP protocol improves detection of cohesin-mediated loops (HICCUPS). **A.** IGV browser coverage tracks and HICCUPS loops of indicated HiChIP samples and coverage tracks for SA1 and CTCF GM12878 ChIP-seq experiments. **B.** Juicebox interaction maps at 5 kb resolution of the example region for the indicated cohesin HiChIP samples. Loops (HICCUPS) are shown as green (SMC1 dcHiChIP HG00731) or blue (SMC1 HiChIP GM12878) rectangles. **C.** Aggregate Peak Analysis (APA) performed using the loops identified by HICCUPS for the indicated cohesin and CTCF HiChIP samples. APA score P2LL (peak to left lower corner) is the ratio of the central bin to the average of the lower left corner and indicates the strength of the loop. **D.** Heatmap showing the HICCUPS loop overlap with 15 kb tolerance between cohesin and CTCF HiChIP samples.

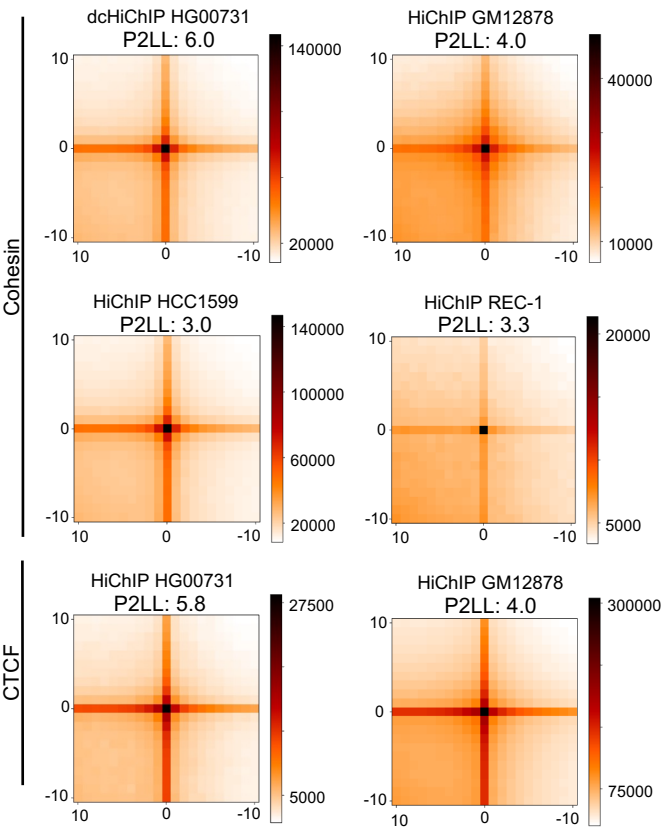
A



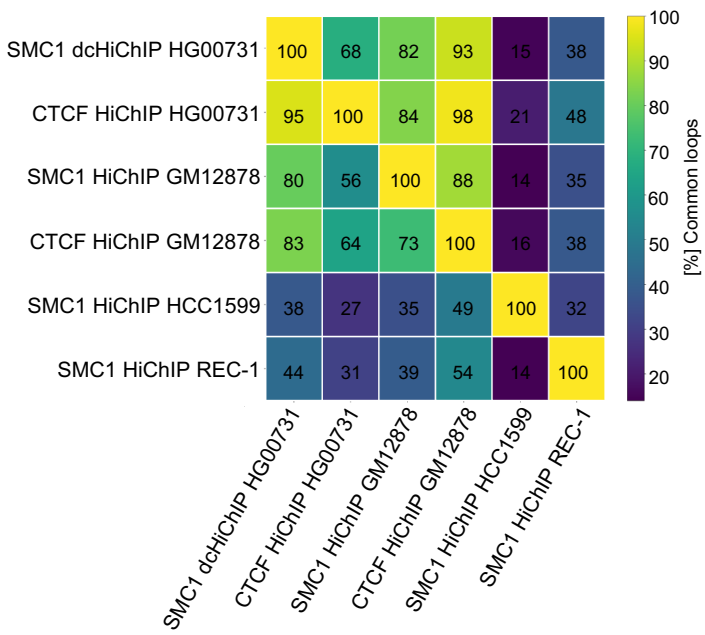
B



C

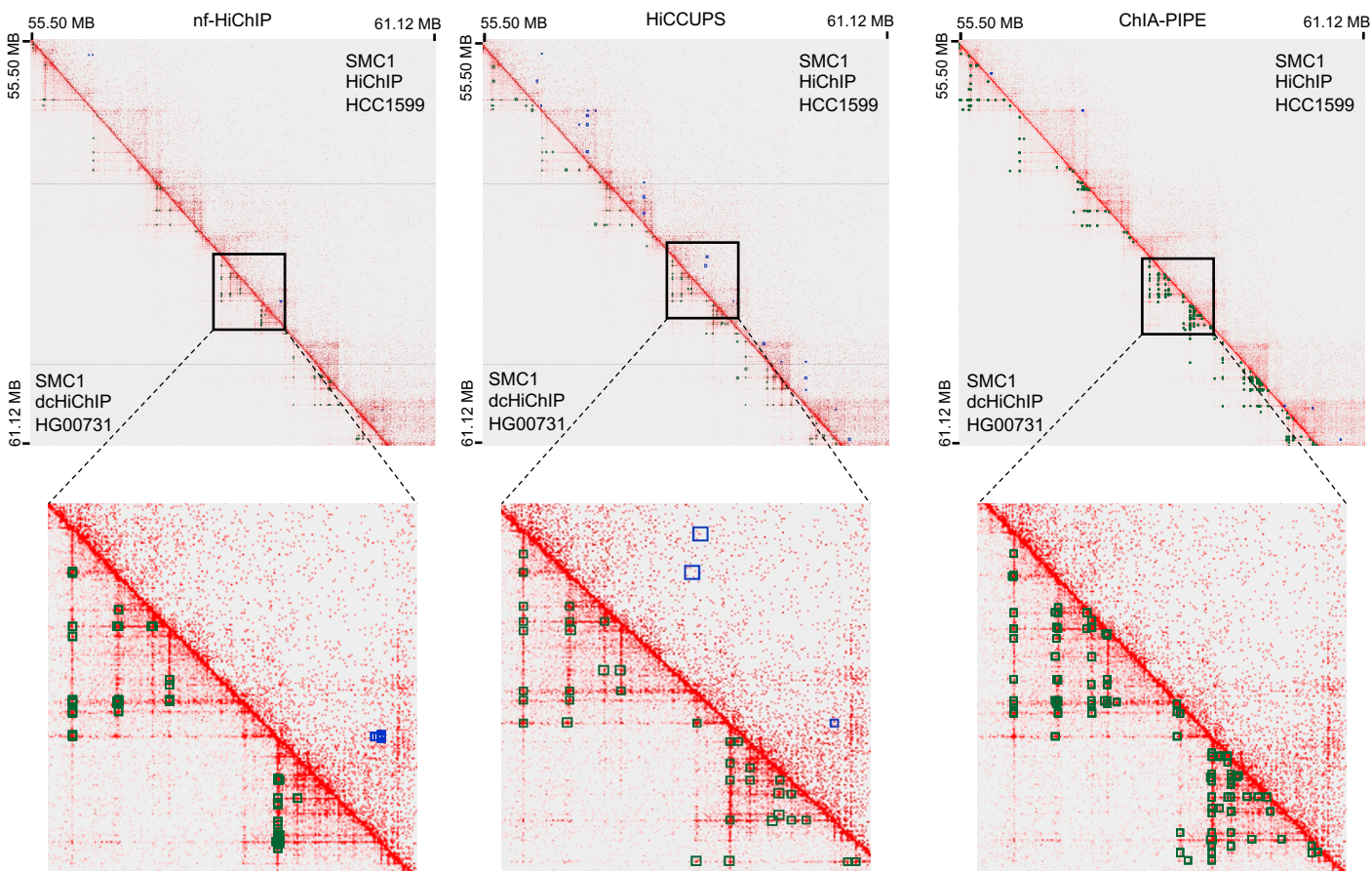


D

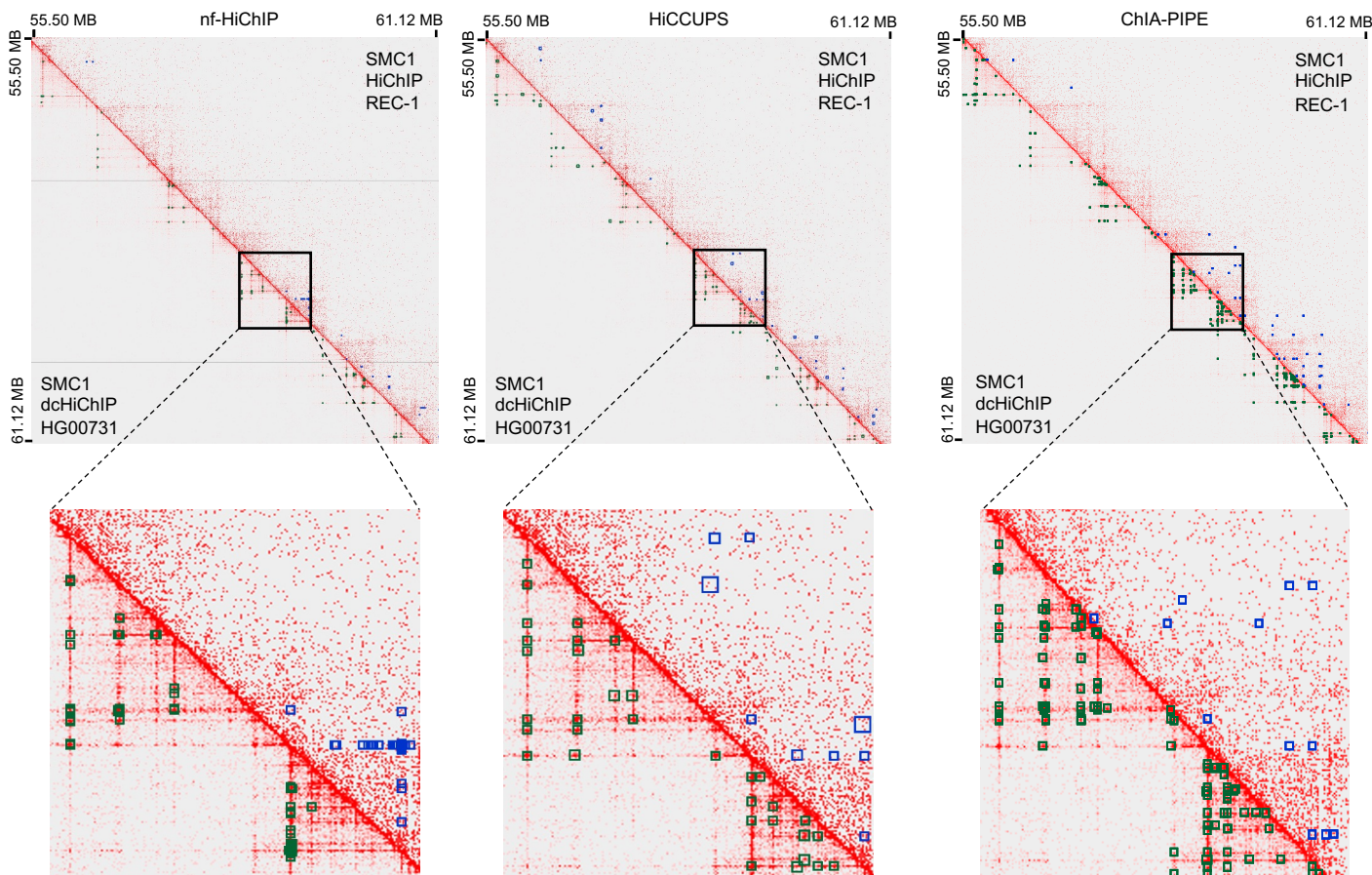


Supplementary Figure 6. FA-EGS cross-linking HiChIP protocol improves detection of cohesin-mediated loop detection (ChIA-PIPE). **A.** IGV genome browser view showing coverage tracks and ChIA-PIPE loops of the indicated HiChIP samples and coverage tracks for SA1 and CTCF GM12878 ChIP-seq experiments. **B.** Juicebox interaction maps at 5 kb resolution of the example region for the indicated cohesin HiChIP samples. Loops (ChIA-PIPE) are shown as green (SMC1 HiChIP HG00731) or blue (SMC1 HiChIP GM12878) rectangles. **C.** Aggregate Peak Analysis (APA) performed using the loops identified by ChIA-PIPE for the indicated cohesin and CTCF HiChIP samples. APA score P2LL (peak to left lower corner) is the ratio of the central bin to the average of the lower left corner and indicates the strength of the loop. **D.** Heatmap showing the ChIA-PIPE loop overlap with 15 kb tolerance between cohesin and CTCF HiChIP samples.

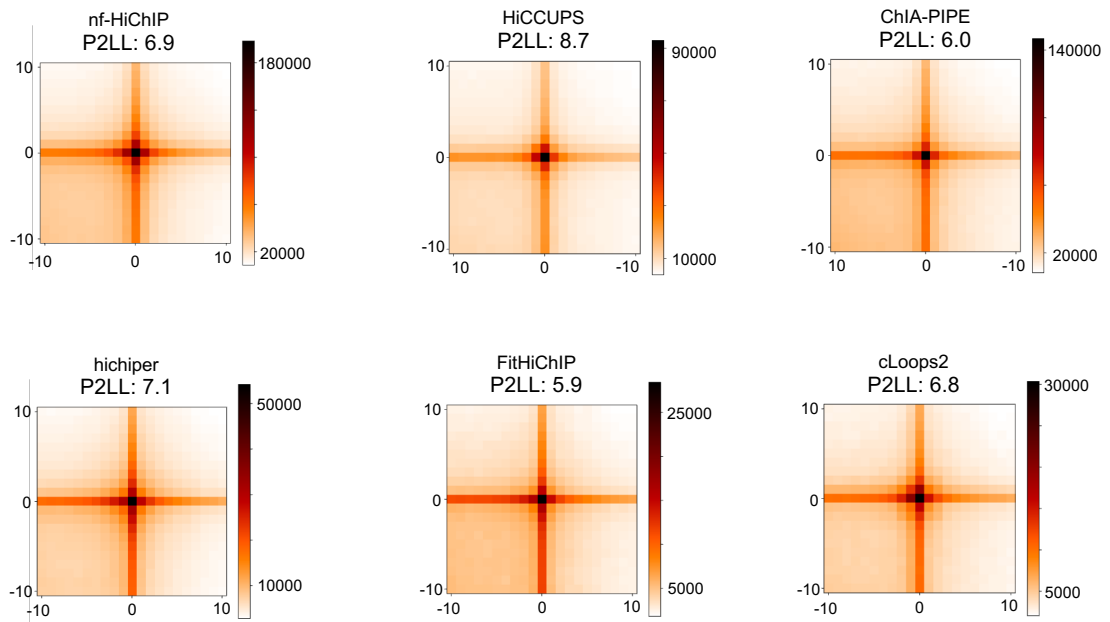
A



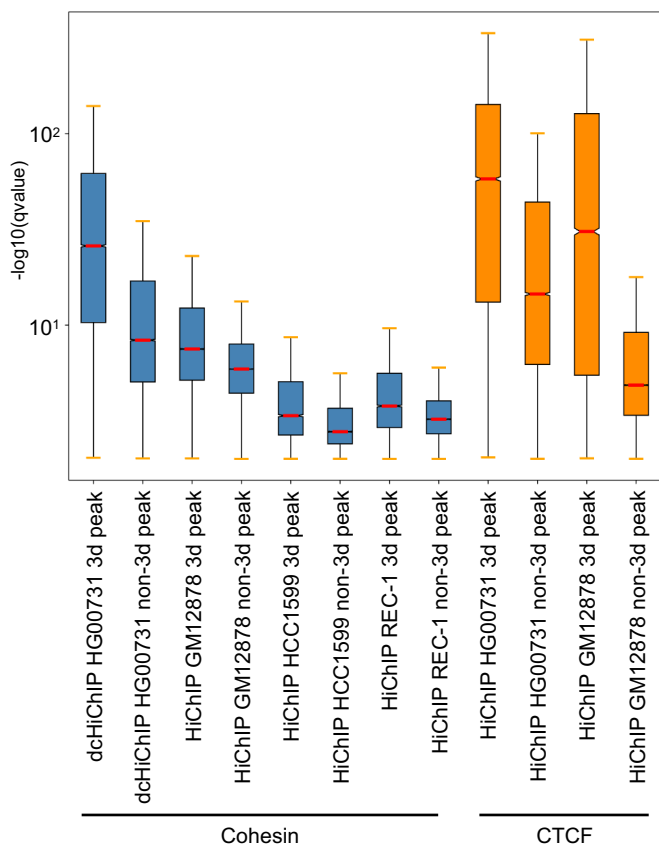
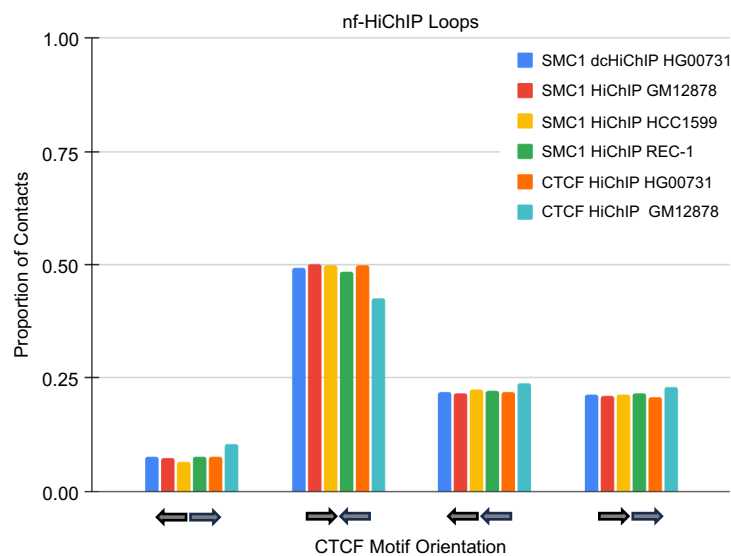
B



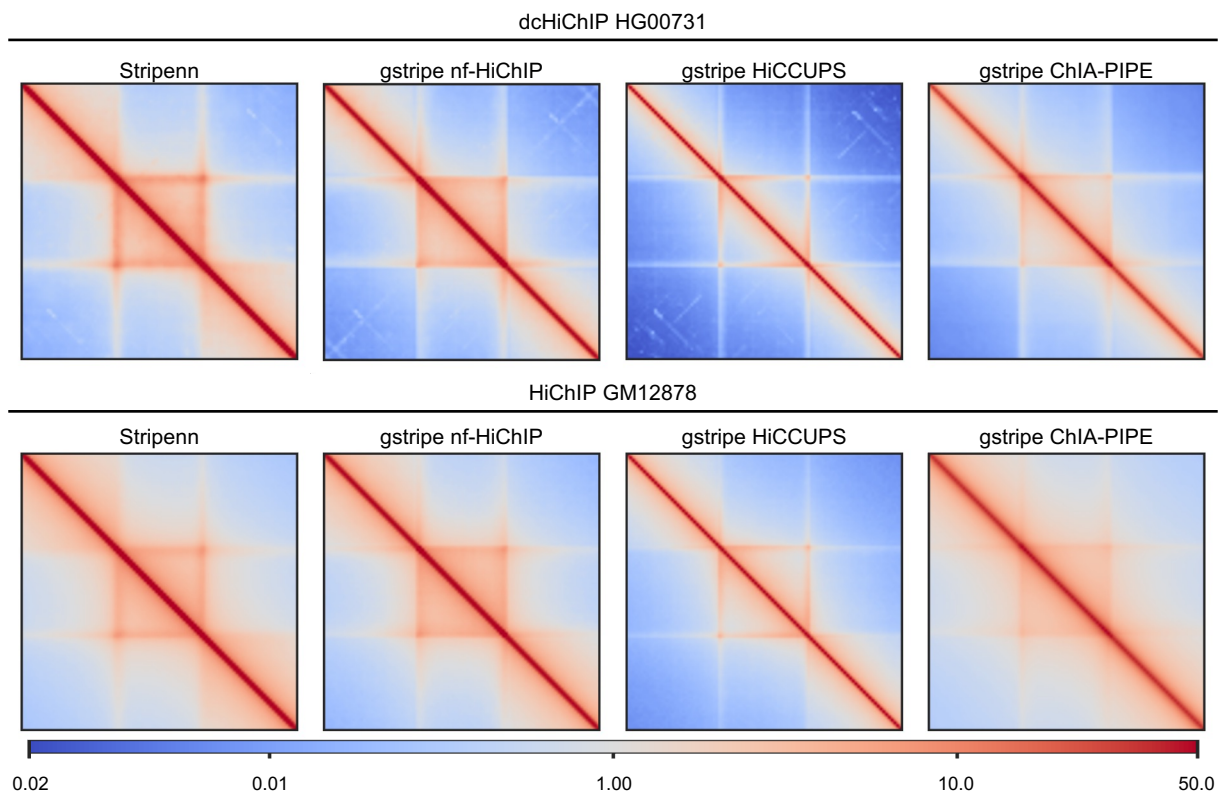
Supplementary Figure 7. A. Juicebox interaction maps at 5 kb resolution of the example region (chr14:55,500,000-61,120,000) for the indicated cohesin HiChIP samples. Loops (nf-HiChIP, HiCCUPS and ChIA-PIPE) are shown as green (SMC1 HiChIP HG00731) or blue (SMC1 HiChIP HCC1599) rectangles. **B.** Juicebox interaction maps at 5 kb resolution of the example region (chr14:55,500,000-61,120,000) for the indicated cohesin HiChIP samples. Loops (nf-HiChIP, HiCCUPS and ChIA-PIPE) are shown as green (SMC1 HiChIP HG00731) or blue (SMC1 HiChIP REC-1) rectangles.



Supplementary Figure 8. APA analysis performed using loops identified different loop calling algorithms – nf-HiChIP, HiCCUPS, ChIA-PIPE, hichiper, FitHiChIP, cLoops2 on SMC1 HiChIP HG00731 sample.

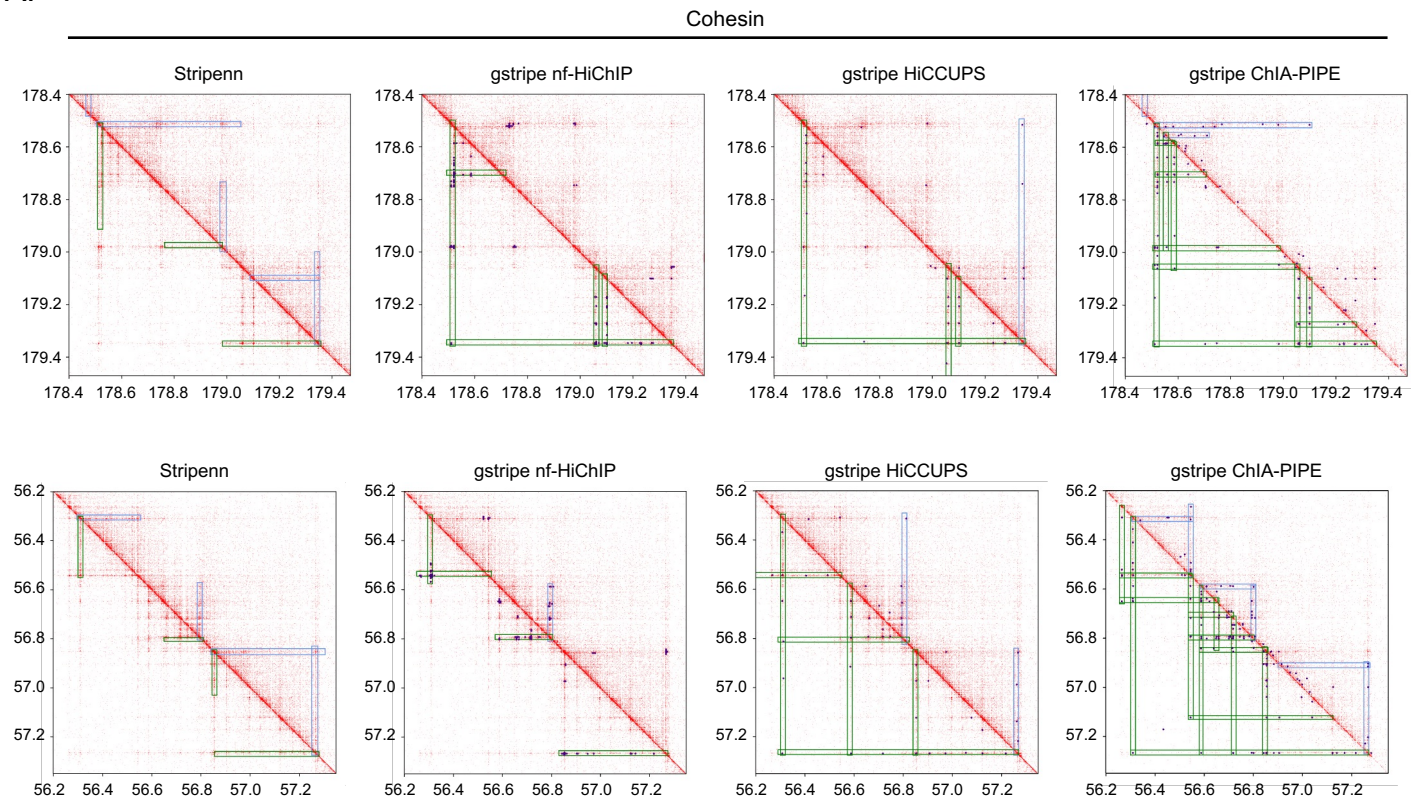
A**B**

Supplementary Figure 9. A. Distribution of $-\log_{10}(\text{qvalue})$ of peaks called by MACS3 in the indicated samples. Peaks that are localised at the loop anchors are marked by 3d peaks and those not localised at the anchors are marked by non-3d peak. The red horizontal line inside each boxplot indicates the median. The bottom edge of the box marks the first quartile, and the top edge marks the third quartile and the whiskers extend to the minimum and maximum values, excluding outliers, which are not shown. **B.** CTCF motif orientation analysis at HiChIP contact anchors in all the samples. Here, “ $\leftarrow\rightarrow$ ” is opposite, “ $\rightarrow\leftarrow$ ” is convergent, “ $\leftarrow\leftarrow$ ” is tandem left, and “ $\rightarrow\rightarrow$ ” is tandem left motif orientation.

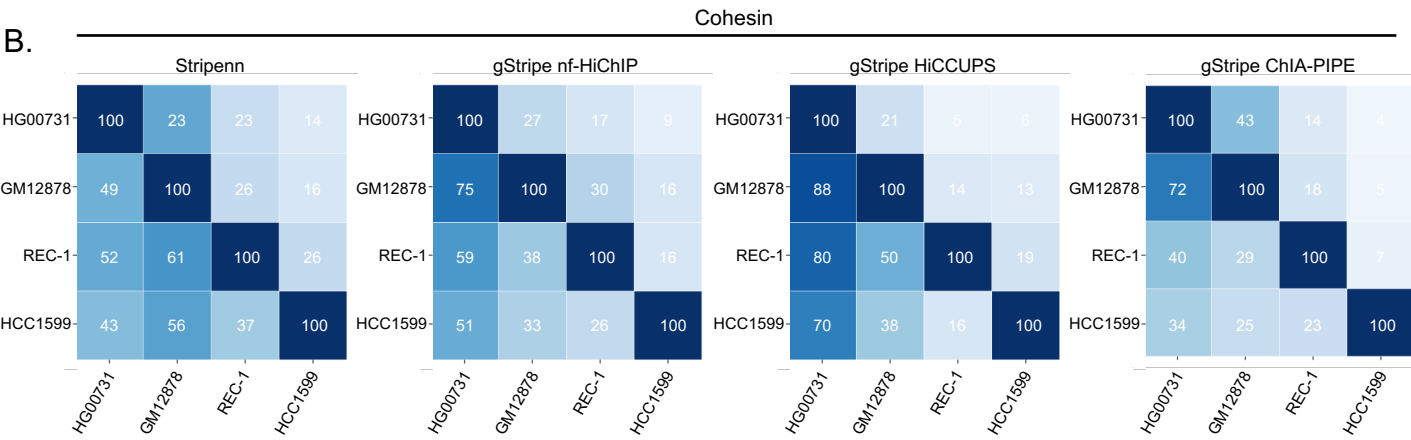


Supplementary Figure 10. Pileup plots constructed for stripe regions display averaged contact map values within the stripe domains for cohesin HiChIP samples. Upper and lower panel present SMC1 FA-EGS HiChIP (HG00731) and SMC1 HiChIP (GM12878), respectively and stripes were called by the algorithms indicated above the pileup plots. Coolpuppy API was used to obtain the plots, with parameters equivalent to using “--rescale --local --unbalanced” options, i.e. each stripe domain was expanded by regions of equal size from both sides, then rescaled to a standard size, and the the raw contact matrix signal is averaged over all stripe domains. The color scale is logarithmic.

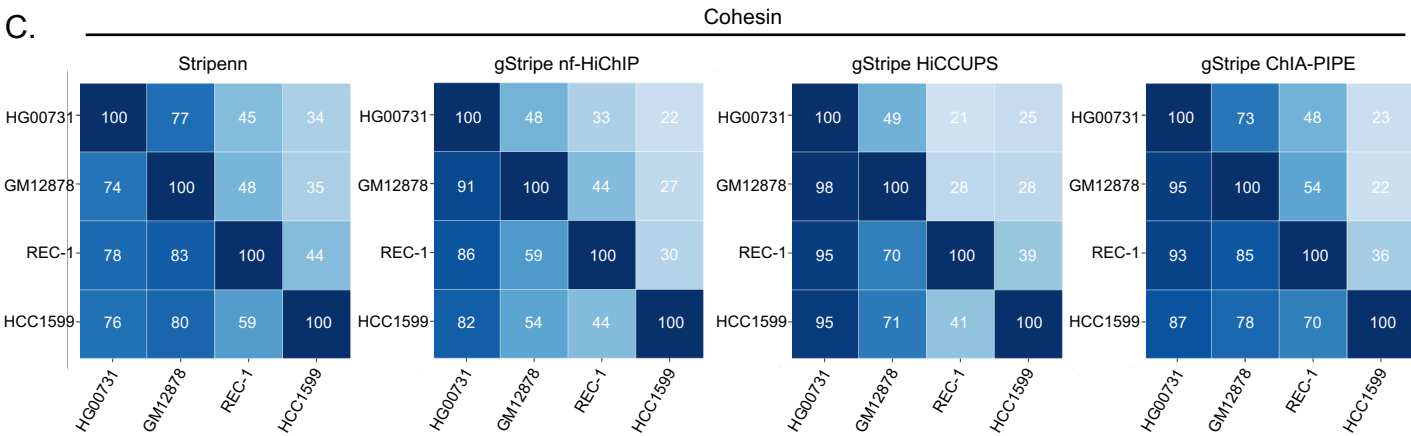
A.



B.

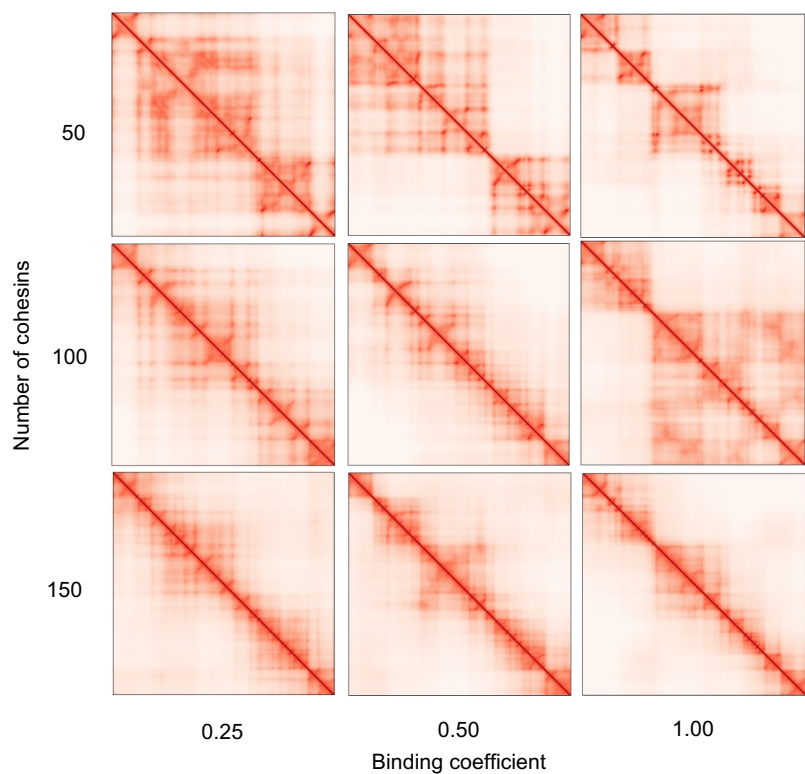


C.

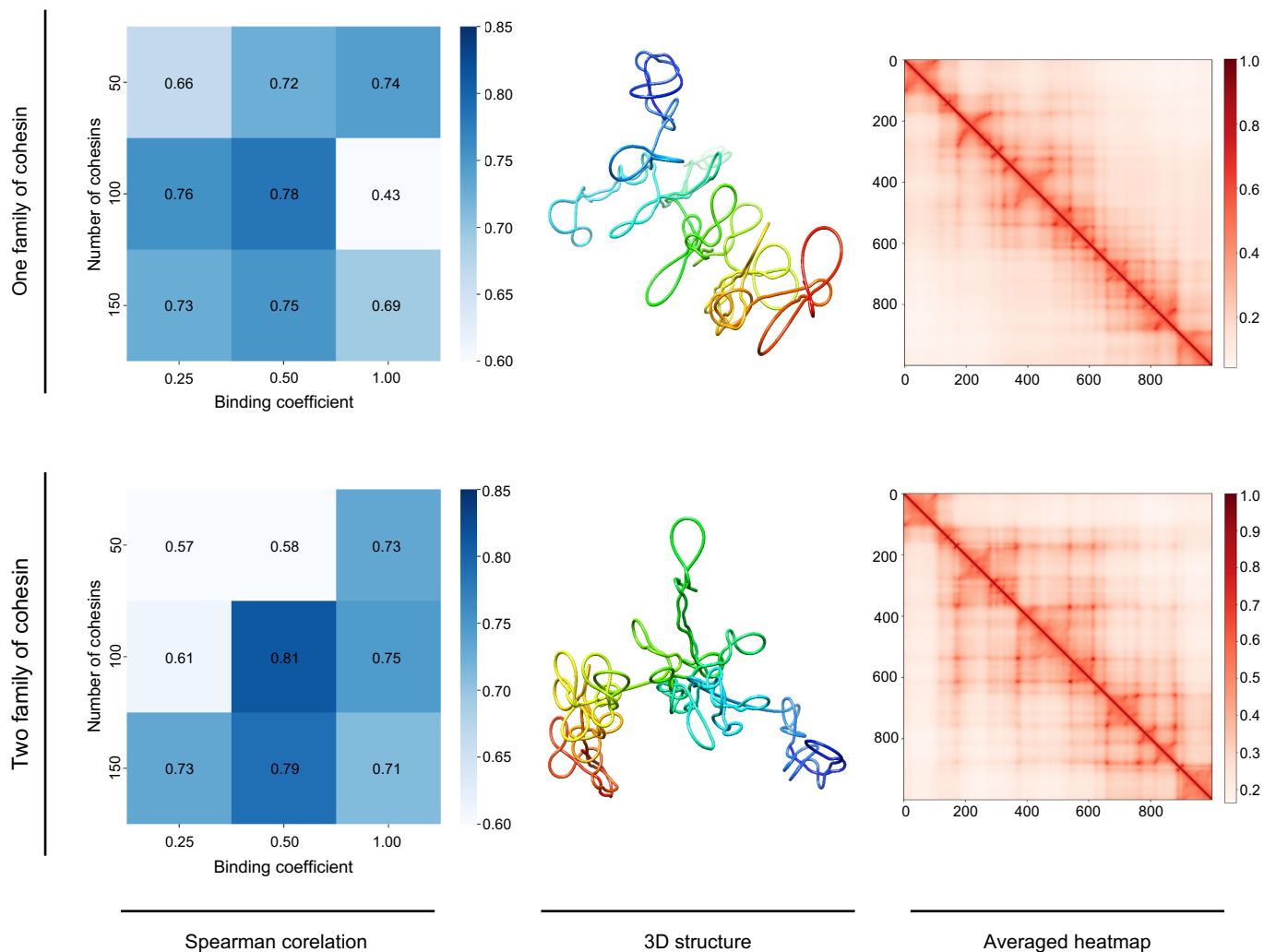


Supplementary Figure 11. A. Comparison of stripes called on our HiChIP data (HG00731, below diagonal, green) and the GM12878 data (above diagonal, blue) for two additional example regions: chr1:178,421,512-179,491,193 (top) and chr6:56,250,000-57,400,000 (bottom). The first three columns show stripes called using gStripe with loops (shown as purple dots) obtained using nf-HiChIP, ChIA-PIPE and HiCCUPS, respectively. The last column shows stripes called by Stripenn. **B.** Overlap between stripe anchors called in each dataset by gStripe (using loops obtained from nf-HiChIP, HiCCUPS or ChIA-PIPE) and Stripenn. A single cell in the matrix represents the percentage of stripes in the row sample overlapping one or more stripes in the column sample. For example, in the first panel, 72% of the stripes from the GM12878 (row 2) are present in HG00731 (column 1). **C.** Overlap between stripe domains. A stripe domain refers to the entire length of the stripe, not just the anchor. Overlapping domains are merged. The datasets and matrix construction are the same as in B.

A.



B.



Supplementary Figure 12: Choosing the appropriate biophysical model for the exemplary region (chr1:178421513-179491193). **A.** The resulting average inverse distance heatmaps from the ensemble of 3D structures for different values of the number of cohesins and the binding coefficient. We see that we can either have well confined borders with only short-range loops and stripes with high values of binding coefficient and number of cohesins, or long-range patterns without clear borders with small amount of cohesins and small binding coefficients. **B.** (Left) The first column shows Spearman correlation between simulated and experimental heatmap with only one family of cohesins, and two families of extruders. (Middle) The second column shows the final 3D structures. (Right) The third column present averaged inverse distance heatmaps from the ensemble of structures. In both simulations, the same biophysical parameters were used (100 cohesins, binding coefficient = 0.5). For the second cohesin family, five cohesins were assigned a folding coefficient 10 times higher than that of the first family.

Human cell line	Cell Type	Target protein	Cross-linking agent	Source	Acession Id
dcHiChIP HG00731	LCL *	Cohesin (SMC1)	FA + EGS	This study	GSE266640
HiChIP GM12878	LCL *		FA	Mumbach et al., 2016	GSE80820
HiChIP HCC1599	Epithelial			Petrovic et al, 2019	GSE116872
HiChIP REC-1	Lymphoblast				GSE116875
ChIP-seq GM12878	LCL *	Cohesin (SA1)		Kasowski, Maya et al., 2013	GSE50893
ChIP-seq GM12878	LCL *	CTCF			GSE50893
HiChIP HG00731	LCL *		This study	GSE266640	
HiChIP GM12878	LCL *		Glutaraldehyde	Mumbach et al., 2016	GSE115524

*LCL from 1000 Genome Project (1000 Genomes Project Consortium et al., 2015)

Supplementary Table 1. HiChIP experiments included in the study.

Dataset	Type of experiment	Number of replicates	Number of reads	Number of peaks	3D peaks	Non 3D peaks	% of 3D peaks
SMC1 HG00731	dcHiCHIP	2	607,768,604	97364	49638	47726	50.98188242
HG00731 CTCF	HiCHIP	2	303,575,305	63555	17625	45930	27.73188577
SMC1 GM12878	HiCHIP	2	643,644,994	666380	74887	591493	11.23788229
CTCF GM12878	HiCHIP	2	533,531,475	153256	23405	129851	15.27183275
SMC1 HCC1599	HiCHIP	1	769,438,941	21281	8371	26008	39.33555754
SMC1 REC-1	HiCHIP	1	762,451,158	34379	5968	15313	17.35943454
SA1 GM12878	ChIP-seq	2	57,401,728	60169	-	-	-
CTCF GM12878	ChIP-seq	2	52,588,683	67179	-	-	-

Supplementary Table 2. Number of biological replicates, total number of reads and MACS3 peaks for indicated experiments.

	CTCF ChIP-seq peaks	CTCF HiChIP peaks
CTCF ChIP-seq peaks	100%	95%
CTCF HiChIP peaks	98%	100%

Supplementary Table 3. Overlap between CTCF HiChIP loops sets detected with MAPS using CTCF ChIP-seq peaks or CTCF HiChIP peaks.

Dataset	HiCCUPS in nf-HiChIP				nf-HiChIP in HiCCUPS			
	Strict overlap		15kb tolerance		Strict overlap		15kb tolerance	
SMC1 dcHiChIP HG00731	19387	44.93%	20243	46.92%	45029	55.95%	71349	88.65%
CTCF HiChIP HG00731	6912	25.99%	7423	27.92%	14559	51.97%	24173	86.30%
SMC1 HiChIP GM12878	7417	38.08%	7688	39.47%	19000	52.00%	32085	87.82%
CTCF HiChIP GM12878	24398	40.83%	26236	43.91%	65081	36.45%	138024	77.31%
SMC1 HiChIP HCC1599	2890	26.81%	3065	28.43%	7084	41.37%	13298	77.66%
SMC1 HiChIP REC-1	3843	32.65%	4163	35.36%	8897	39.91%	16666	74.76%

Supplementary Table 4. Overlap between loops (number and percentage) between the HiCCUPS and nf-HiChIP.

Dataset	nf-HiChIP	HICCUPS	ChIA-PIPE	hichiper	FitHiChIP	cLoops2
SMC1 dcHiChIP HG00731	80487	43148	179516	80487	127278	164377
CTCF HiChIP HG00731	28012	26590	46117	--	--	--
SMC1 HiChIP GM12878	36535	19478	92248	--	--	--
CTCF HiChIP GM12878	178535	59753	236997	--	--	--
SMC1 HiChIP HCC1599	17124	10779	19057	--	--	--
SMC1 HiChIP REC-1	22294	11772	46112	--	--	--

Supplementary Table 5. Number of significant interactions (loops) detected by three independent algorithms: nf-HiChIP, HICCUPS and ChIA-PIPE.

Loops Calling Pipeline	Dataset	↔	↗	↖	↘	↙	↘	↖	↗	None
nf-HiChIP	SMC1 dcHiChIP HG00731	5173	33271	14682	14431	1520	4864	4438	1571	537
	CTCF HiChIP HG00731	1881	12397	5447	5189	309	1149	1300	311	29
	SMC1 HiChIP GM12878	2208	15421	6673	6435	711	2087	2122	639	239
	CTCF HiChIP GM12878	15243	61912	34776	33252	4270	11769	12265	4040	1008
	SMC1 HiChIP HCC1599	947	7290	3259	3093	212	966	1032	247	78
	SMC1 HiChIP REC-1	1445	9090	4177	4073	408	1288	1334	360	119
HiCCUPS	SMC1 dcHiChIP HG00731	2910	20850	7662	7785	533	1186	1378	607	228
	CTCF HiChIP HG00731	2405	12344	5224	5105	237	479	533	243	11
	SMC1 HiChIP GM12878	1175	9997	3347	3460	186	474	511	240	79
	CTCF HiChIP GM12878	5419	25219	11494	11529	978	1897	2029	1001	178
	SMC1 HiChIP HCC1599	698	5800	1905	1962	54	142	147	46	16
	SMC1 HiChIP REC-1	814	6034	6034	2184	92	205	241	93	25
ChIA-PIPE	SMC1 dcHiChIP HG00731	9354	30128	16943	16825	14237	23015	23489	14059	31456
	CTCF HiChIP HG00731	4110	11975	6886	6669	2730	4498	4611	2554	2074
	SMC1 HiChIP GM12878	3941	7175	5228	5152	9472	11899	12141	9304	27926
	CTCF HiChIP GM12878	20282	45461	30781	30124	18009	45461	28691	18046	16899
	SMC1 HiChIP HCC1599	1637	2574	1876	1871	1848	2103	2209	1892	3037
	SMC1 HiChIP REC-1	3392	5160	4037	4005	4364	5307	5475	4643	9719

Supplementary Table 6. CTCF motif orientation analysis at HiChIP contact anchors in all the samples detected by 3 different loop calling algorithm - nf-HiChIP, HiCCUPS and ChIA-PIPE.

Loop calling pipeline	Dataset	EP Loops	PP Loops	% EP+PP Loops
nf-HiChIP	SMC1 dcHiChIP HG00731	27495	8303	44.4767478
	CTCF HiChIP HG00731	10392	3295	48.86120234
	SMC1 HiChIP GM12878	13025	3428	45.03352949
	CTCF HiChIP GM12878	60680	16942	43.47718935
	SMC1 HiChIP HCC1599	5449	1089	38.1803317
	SMC1 HiChIP REC-1	7679	2031	43.55431955
HiCCUPS	SMC1 dcHiChIP HG00731	13912	4630	42.97302308
	CTCF HiChIP HG00731	9659	3657	50.07897706
	SMC1 HiChIP GM12878	6579	2416	46.18030599
	CTCF HiChIP GM12878	17845	5196	38.56040701
	SMC1 HiChIP HCC1599	3571	1342	45.57936729
	SMC1 HiChIP REC-1	4107	1539	47.96126402
ChIA-PIPE	SMC1 dcHiChIP HG00731	45198	12131	31.93531496
	CTCF HiChIP HG00731	12585	2940	33.66437539
	SMC1 HiChIP GM12878	20401	4033	26.48729512
	CTCF HiChIP GM12878	57597	10827	28.87125153
	SMC1 HiChIP HCC1599	3601	1379	26.13212993
	SMC1 HiChIP REC-1	8663	1199	21.3870576

Supplementary Table 7. Number of Enhancer-Promoter (EP) and Promoter-Promoter (PP) loops and percentge of combined EP and PP loops identified for all HiChIP samples from 3 different loops calling algorithm - nfHiChIP, HiCCUPS and ChIA-PIPE.

Dataset	Stripe calling algorithm			
	Stripenn	gStripe, using loop set from:		
		nf-HiChIP	HiCCUPS	ChIA-PIPE
SMC1 dcHiChIP HG00731	3603	4187	6237	18155
SMC1 HiChIP GM12878	3908	1526	1479	9606
SMC1 HiChIP HCC1599	1136	757	497	2100
SMC1 HiChIP REC-1	1637	1223	424	6458
Supplementary Table 8. Number of stripes called in each dataset.				

Parameter		Dataset			
canny	bfilter	SMC1 dcHiChIP HG00731	SMC1 HiChIP GM12878	SMC1 HiChIP HCC1599	SMC1 HiChIP REC-1
1	1	3390	3023	567	563
	3	3603	3703	840	1201
	5	3072	3908	1088	1599
1.5	1	3440	3871	968	1470
	3	2797	3753	1042	1637
	5	1904	3534	1080	1541
2	1	2102	3413	1136	1597
	3	1778	3488	1090	1565
	5	1111	2933	944	1349
2.5	1	1084	2779	972	1321
	3	947	2522	885	1220
	5	603	2230	744	882
3.0	1	466	2051	731	879
	3	414	1903	655	826
	5	236	1590	540	620

Supplementary Table 9. Number of stripes called by Stripenn in each dataset, using the particular combinations of *canny* (Canny edge detection parameter) and *bfilter* (kernel size of the mean filter) parameters (see: Yoon et al. 2022). The default values are: 2.0 for canny and 3 for bfilter. The maximum values in each dataset is marked in bold.