Supplemental Material for Ribeiro-dos-Santos et al.
"Genomic context sensitivity of insulator function"

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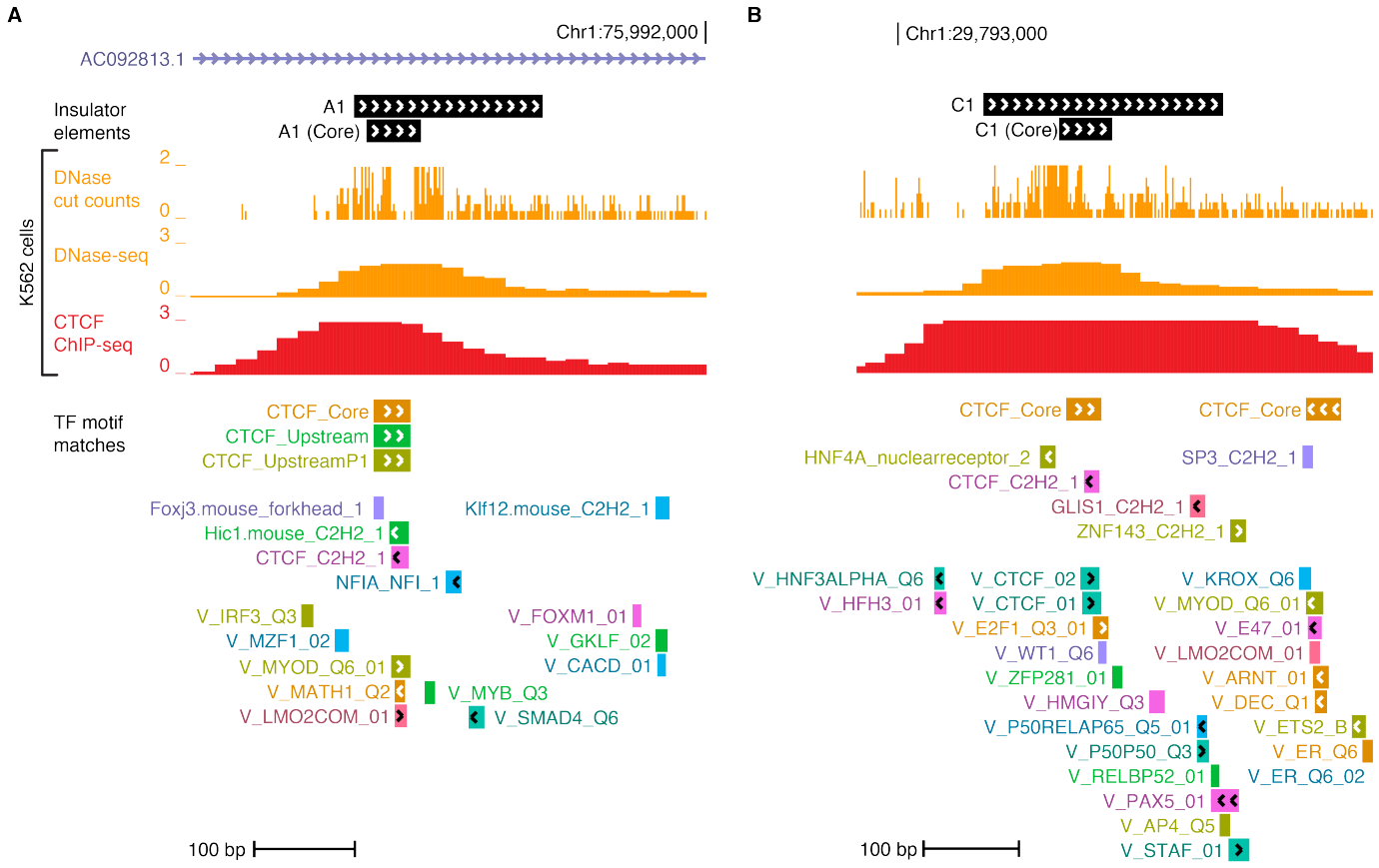
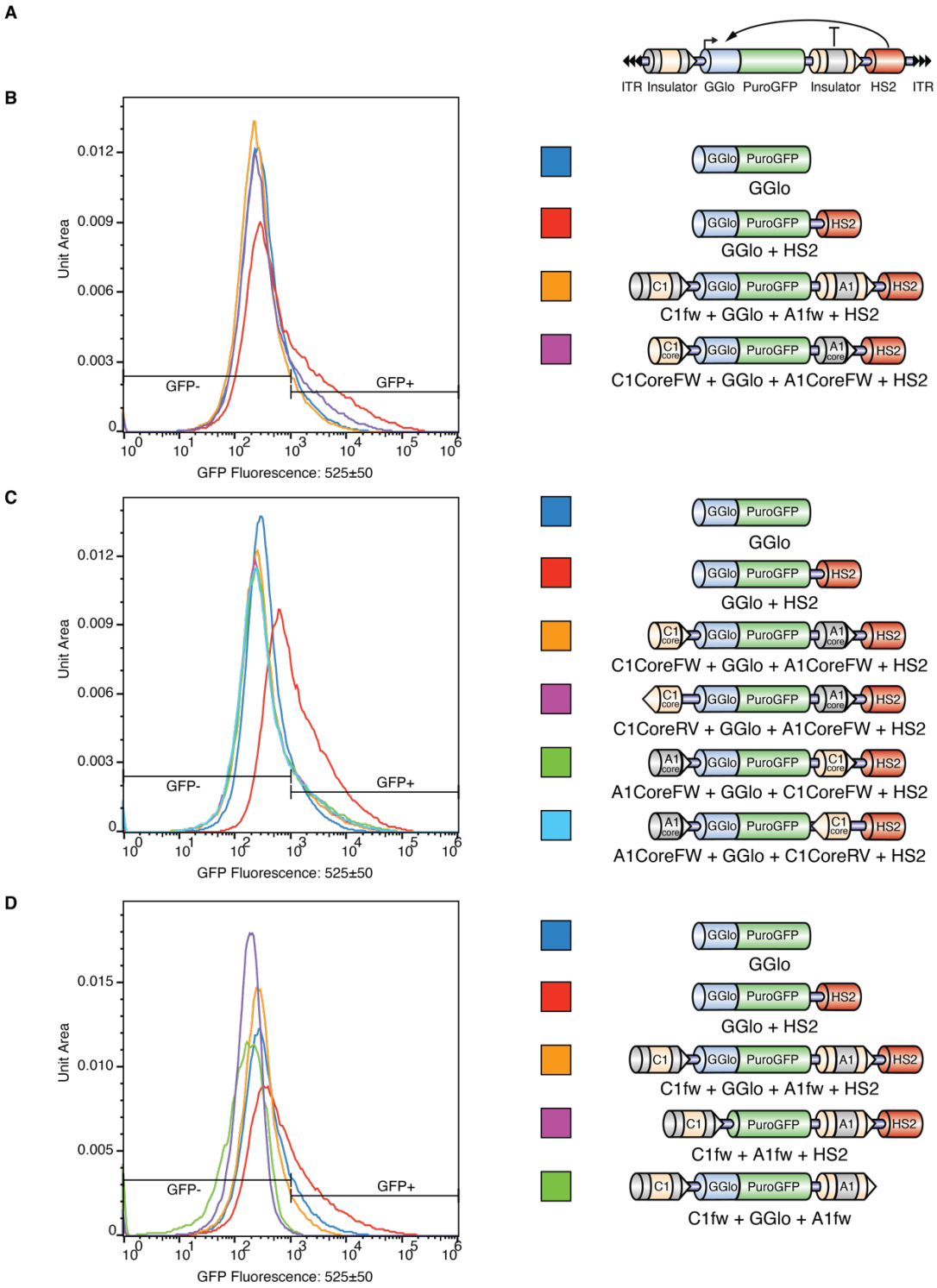
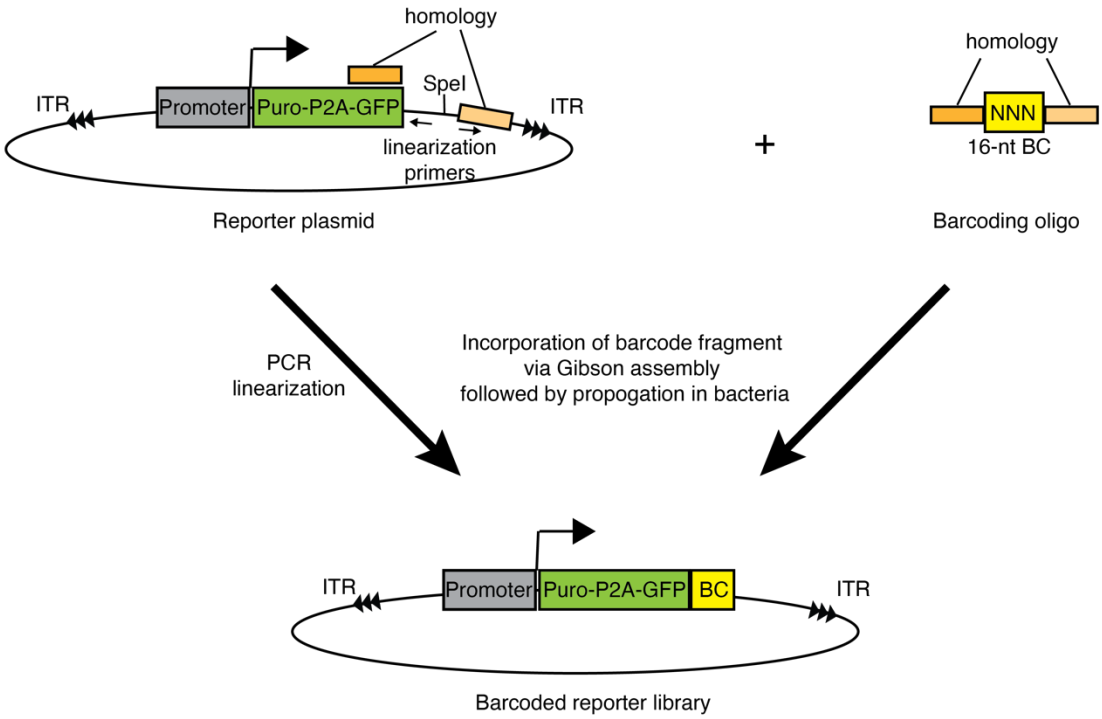
Supplemental Figures



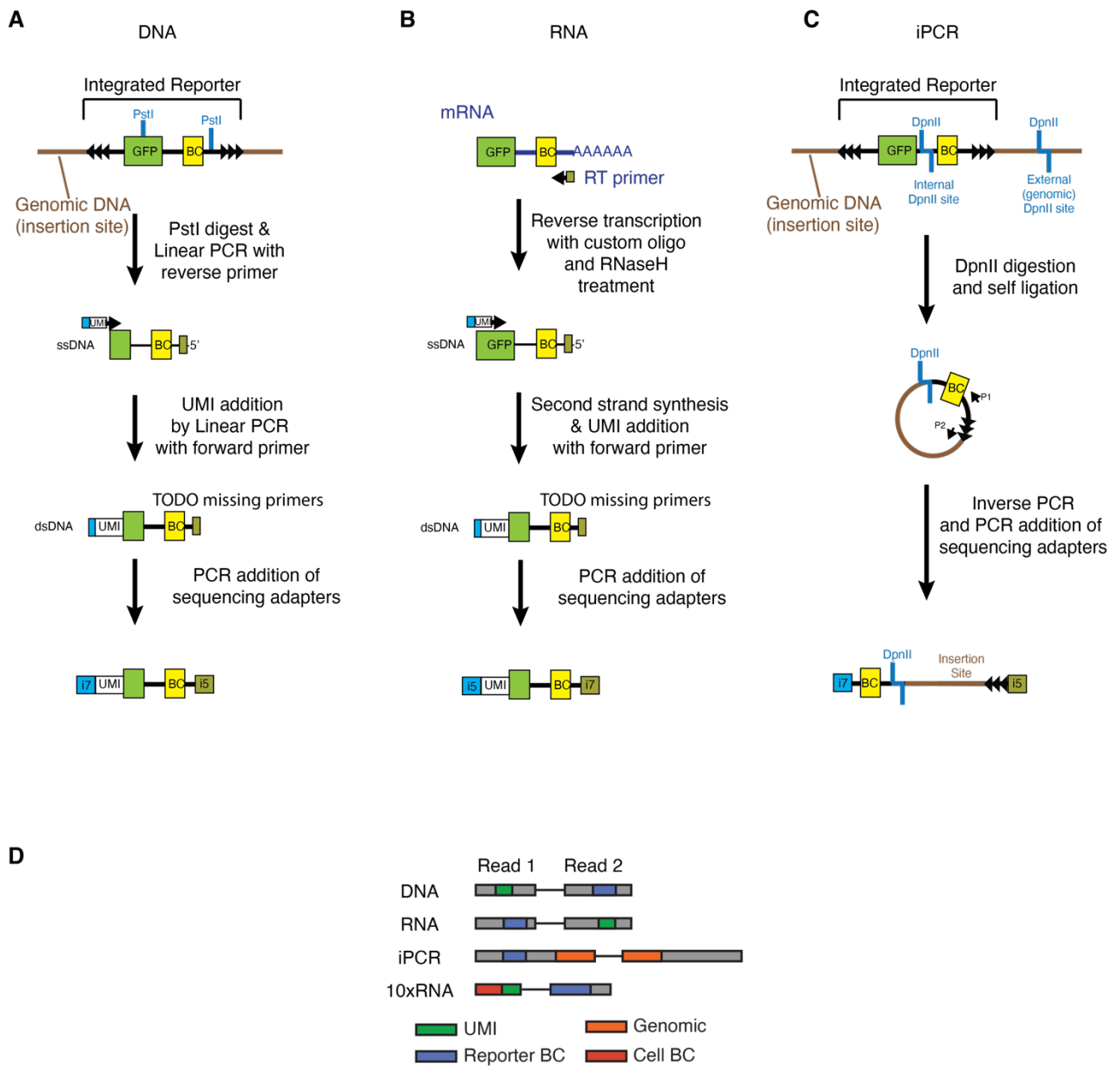
Supplemental Fig. S1. A1 and C1 genomic insulator elements.
(A-B) Shown are full A1 (A) and C1 (B) insulator elements from (Liu et al. 2015). A1 and C1 core elements have been truncated to just the CTCF footprint. Shown are DNase-seq cut counts, DNase-seq density, and CTCF ChIP-seq tracks from K562 cells. TF motif matches below were identified using FIMO ($P < 10^{-5}$).



Supplemental Fig. S2. Characterization of enhancer blocker reporter using flow cytometry.
(A) Reporter scheme consisting of *HBG1* promoter driving Puro and GFP expression. An intervening CTCF site between the promoter and HS2 acts as an enhancer blocker. ITR, *Sleeping Beauty* inverted terminal repeats; GGlo, *HBG1* promoter; HS2, beta-globin hypersensitive site 2 enhancer. (B-D) GFP signal per cell measured by flow cytometry. GGlo and GGlo+HS2 were repeated in all experiments. FW and RV indicate forward or reverse orientation of insulator elements. (B) The A1 insulator behaves as a strong enhancer blocker element. Truncating A1 to the CTCF site (A1core, see **Supplemental Fig. S1**) retains potent enhancer blocker activity. (C) Enhancer blocker activity is independent of the orientation of the insulator element. C1core (**Supplemental Fig. S1**) is a strong enhancer blocker element. (D) Reporters without GGlo or HS2 each demonstrate no GFP expression.

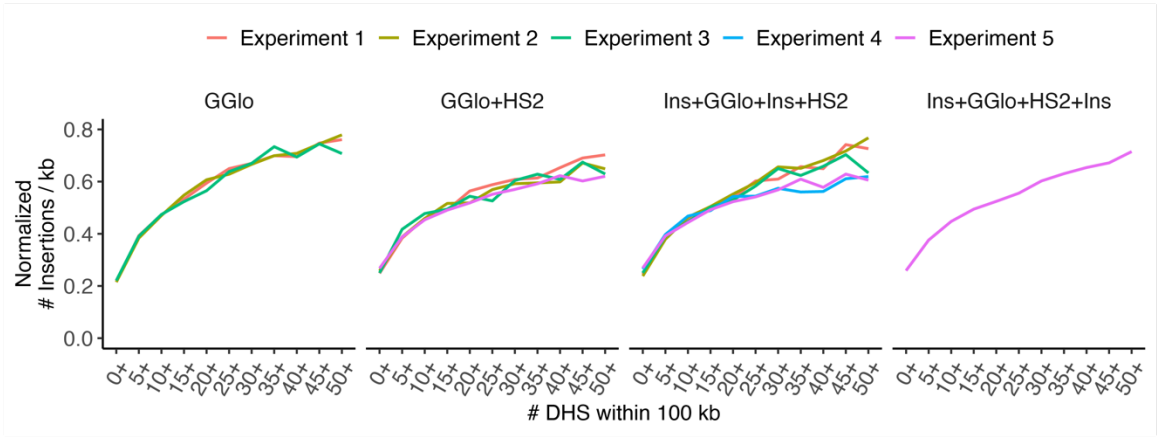


Supplemental Fig. S3. Reporter plasmid barcoding strategy.
Libraries of barcoded reporter plasmids were generated by PCR linearization followed by incorporation of synthetic oligonucleotide containing a 16-nt random reporter BC sequence using Gibson assembly. Orange rectangles indicate homology regions for reporter BC cloning. ITR, *Sleeping Beauty* inverted terminal repeats.



Supplemental Fig. S4. Amplicon library construction approach.
(A-C) Preparation of Illumina sequencing libraries to quantify barcoded reporter representation from DNA, expression from RNA, and integration location using inverse PCR (iPCR). DNA libraries utilized a PstI digest to limit template size, followed by a single linear amplification to add UMIs, and finally an Exo I digest to prevent any amplification from untemplated primers. RNA libraries incorporated UMIs during second strand synthesis. (D) Schematic of unique molecular identifier (UMI, DNA, RNA, and 10x scRNA-seq libraries), Reporter BC, Cell BC (10x scRNA-seq libraries), and genomic sequence (iPCR libraries). The number of N nucleotides added to each individual sample varied between 8-12 nt (DNA and RNA) or 0-2 nt (iPCR) to increase diversity on the sequencing flow cell. Not shown: for DNA libraries, there can be an additional 0-2 nt of UMI on R2.

A



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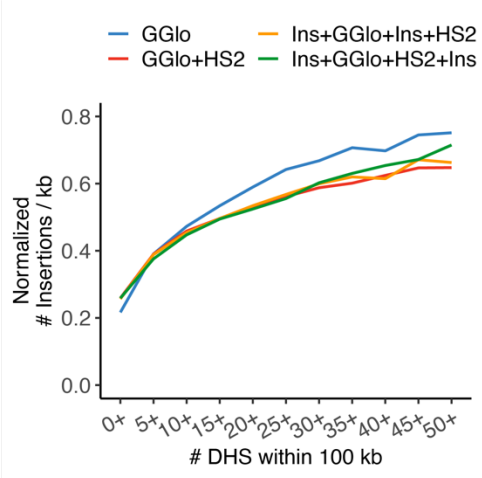
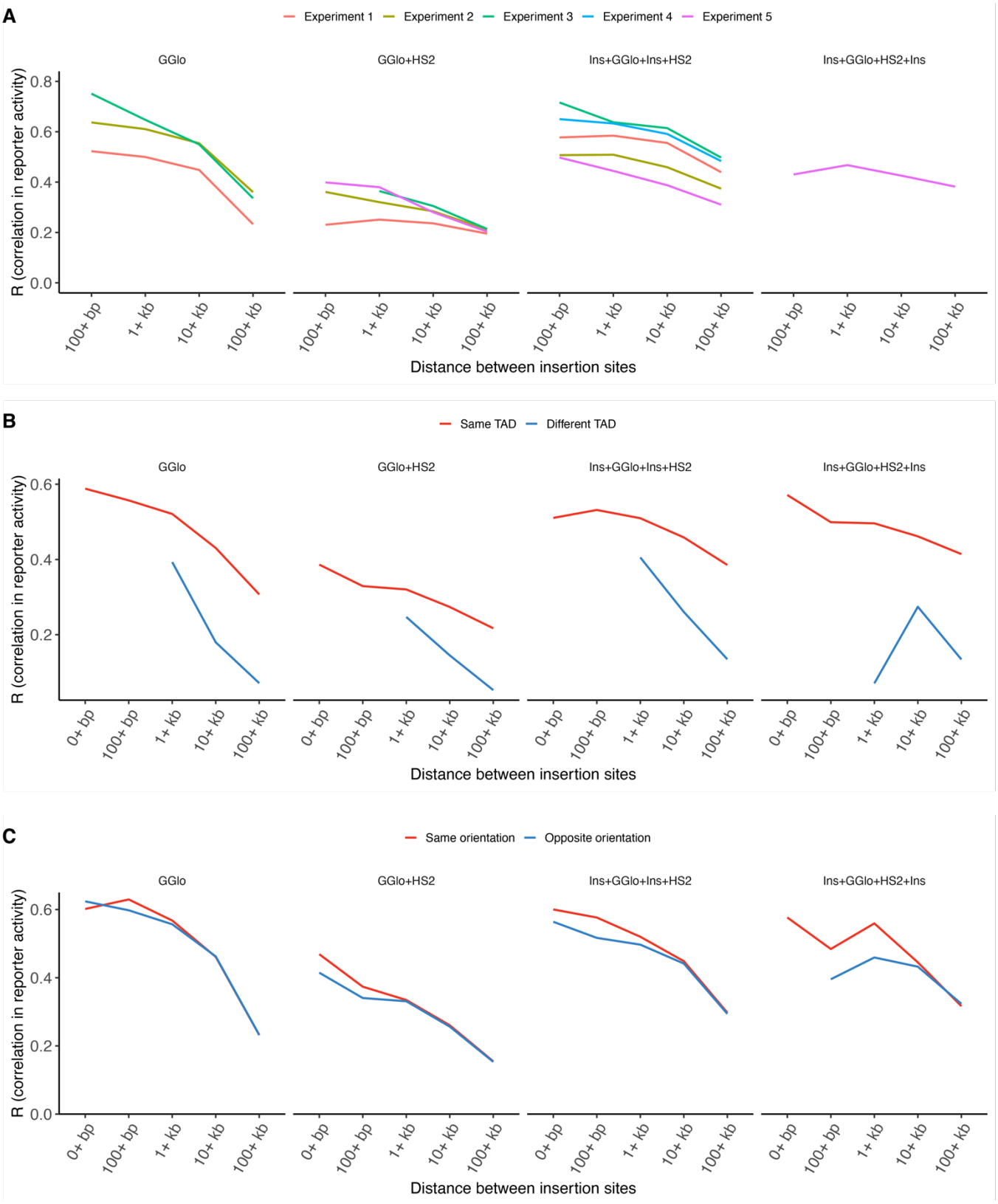


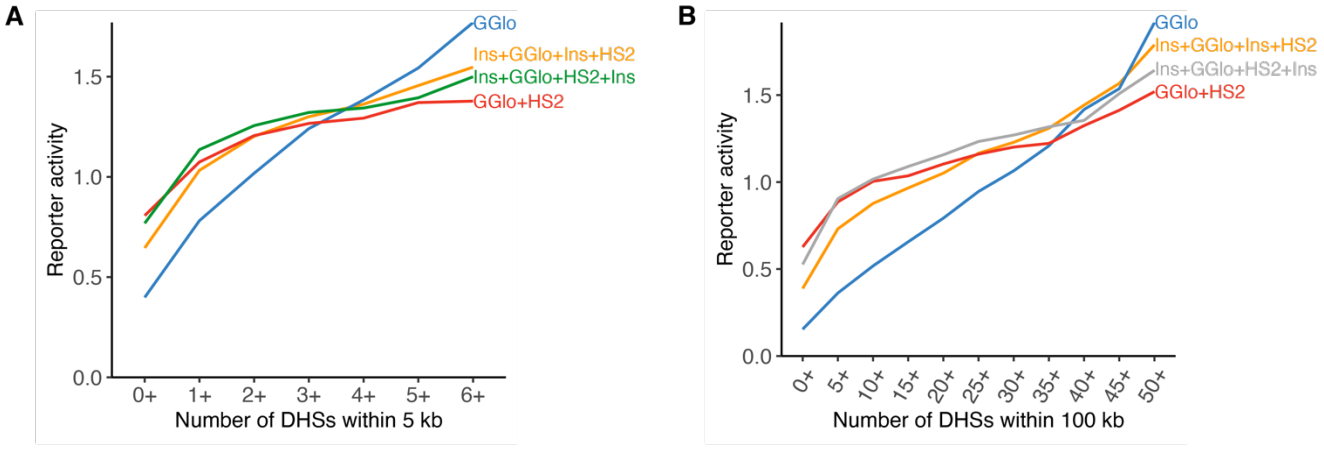
Supplemental Fig. S5. Reporter insertion site density.

(A-B) Shown is insertion density per kb, stratified by # DHS within 100 kb. Rates are normalized to number of mapped insertions per 10^6 for each experiment. (B) is merged across all experiments.

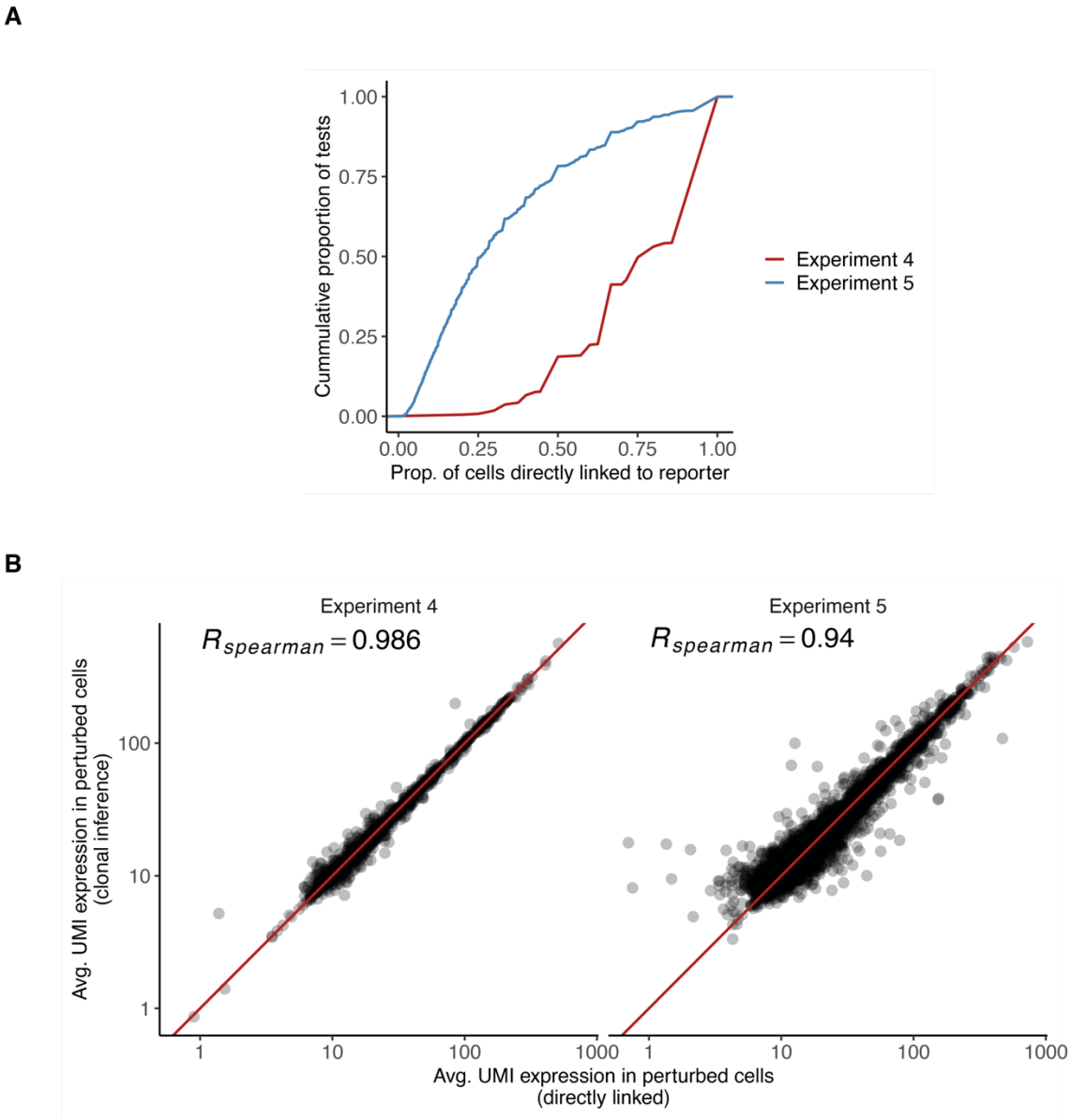


Supplemental Fig. S6. Assessment of genomic context sensitivity.

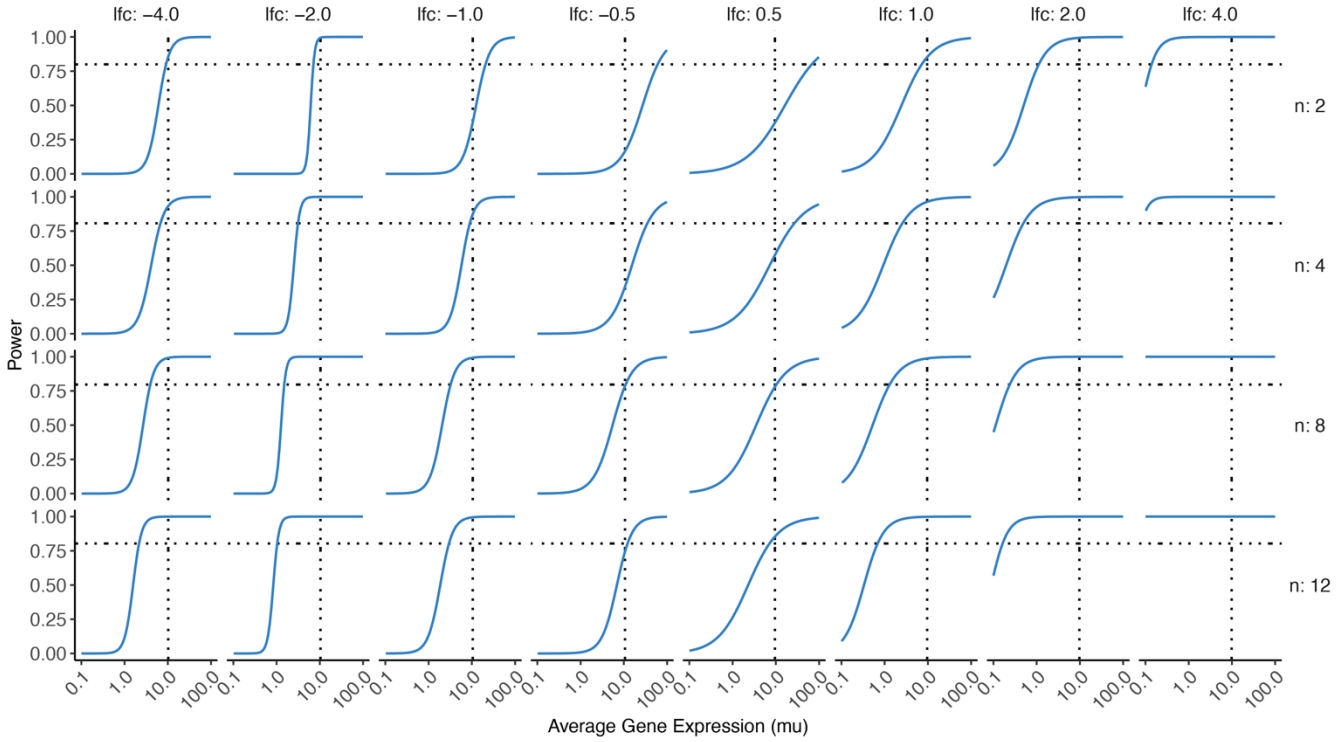
(A-C) Correlation in activity for nearby insertions computed as in **Fig. 3B**. Bins with fewer than 100 datapoints were omitted. (A) Shown by individual reporter and experiment. (B-C) Averaged across all experiments. (B) Stratified by whether both reporters lie in the same or different TADs (Methods). Reporters outside a known TAD were omitted. (C) Correlation stratified by whether insertions share same or opposite orientation.



Supplemental Fig. S7. Modeling genomic context effects.
(A-B) Average reporter activity by short-range and long-range genomic context represented by the number of DHSs within 5 kb (A) or 100 kb (B).

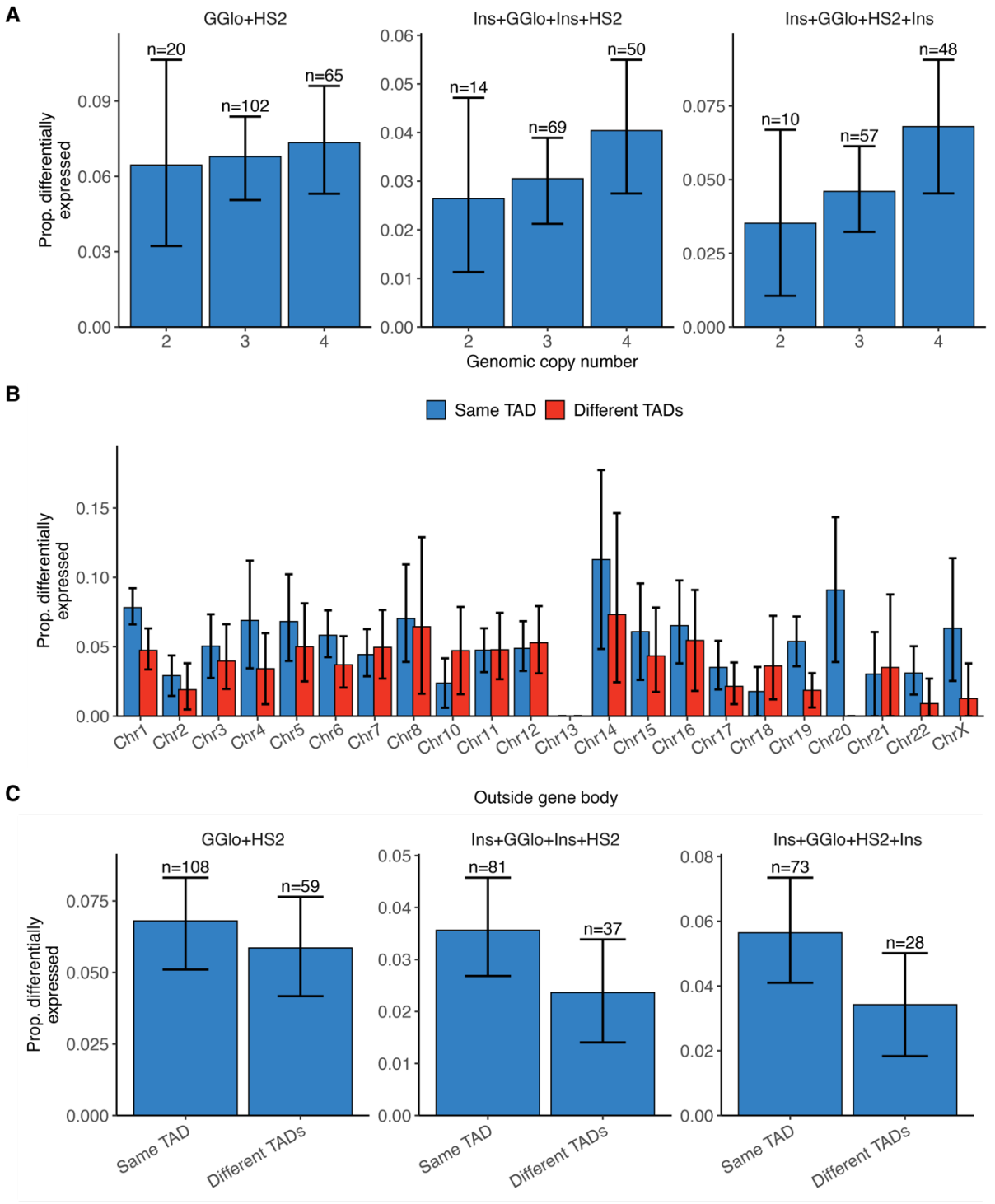


Supplemental Fig. S8. Performance and reliability of clonal inference approach.
(A) Cumulative plot showing the proportion of cells with direct evidence for that reporter BC among all tests for differential expression. (B) Estimated expression of perturbed condition using cells where a reporter BC was directly measured (x-axis) vs. all cells in the same clonal lineage (y-axis). Tests with fewer than 20,000 UMIs on average among directly linked cells or fewer than 2 directly linked cells were omitted.



Supplemental Fig. S9. Power analysis of DECAL framework.

Detection power for different circumstances stratified by fold change of the perturbation (columns), number of cells in the clone (rows), and average expression (x-axis). Y-axis shows proportion of significant tests (q-value < 0.05) for each condition. Plotted is a logistic regression fit to the observed response. Horizontal dashed line shows 80% power, and vertical dashed line shows average gene expression of 10 UMIs.



Supplemental Fig. S10. Association of reporters affecting gene expression with genomic features.
(A) Rate of significant reporter effect on gene expression according to the gene copy number. Rates were broken by the genomic copy number of the gene TSS. Error bars represent 99% confidence intervals estimated by 1,000 bootstrap simulations. Genomic copy number categories with less than 10 significant tests were not included. (B) Rate of significant reporter effect on gene expression by whether the insertion and TSS are in the same TAD or not and broken down by chromosome. Error bars represent 90% confidence intervals estimated by 1,000 bootstrap simulations. (C) Rate of significant effect on gene expression for insertions outside a gene body. Rates are broken down by whether reporter and TSS are in the same TAD or not. Insertions or TSS outside a known TAD were omitted. Number of tests in each category is indicated above each bar. Error bars represent 99% confidence intervals estimated by 1,000 bootstrap simulations.

Supplemental Tables

Supplemental Table S1. Transfection summaries.

Summary of transfection and culture conditions. The Reporter Construct column summarizes the reporter configuration: GGlo, *HBG1* promoter; HS2, beta-globin hypersensitive site 2 enhancer; A1fw, C1fw, A1rv, and C1rv, CTCF-binding insulator elements in the forward and reverse orientations (Liu et al. 2015); "Core" indicates the element has been truncated to just the CTCF recognition sequence (**Supplemental Fig. S1**). FW and RV indicate forward or reverse orientation of insulator elements. # Cells Seeded refers to the cells seeded on day 0 for Experiments 1-3, and the number of mKate+ cells on day 1 for Experiments 4-5. Cells were selected using 2.5 μg/mL puromycin (Puro) or sorted using flow cytometry for mKate (expressed by the transposase plasmid).

Experiment	Plasmid #	Transfection	Reporter Construct	Amt. Transposon (pM)	Amt. Transposase (pM)	# Cells Seeded	Selection
GFP (Fig. 1)	pMH027		GGlo	2,600	2,600	100,000	puro from day 5
GFP (Fig. 1)	pMH028		GGlo_HS2	2,600	2,600	100,000	puro from day 5
GFP (Fig. 1)	pMH032		C1fw_GGlo_A1fw_HS2	2,600	2,600	100,000	puro from day 5
GFP (Fig. 1)	pMH041		C1CoreFw_GGlo_A1CoreFw_HS2	2,600	2,600	100,000	puro from day 5
GFP (Fig. 1)	pMH027		GGlo	2,600	2,600	100,000	puro from day 5
GFP (Fig. 1)	pMH028		GGlo_HS2	2,600	2,600	100,000	puro from day 5
GFP (Fig. 1)	pMH041		C1CoreFw_GGlo_A1CoreFw_HS2	2,600	2,600	100,000	puro from day 5
GFP (Fig. 1)	pMH043		A1CoreFw_GGlo_C1CoreFw_HS2	2,600	2,600	100,000	puro from day 5
GFP (Fig. 1)	pMH042		C1CoreRv_GGlo_A1CoreFw_HS2	2,600	2,600	100,000	puro from day 5
GFP (Fig. 1)	pMH044		A1CoreFw_GGlo_C1CoreRv_HS2	2,600	2,600	100,000	puro from day 5
GFP (Fig. 1)	pMH027		GGlo	2,600	2,600	100,000	puro from day 5
GFP (Fig. 1)	pMH028		GGlo_HS2	2,600	2,600	100,000	puro from day 5
GFP (Fig. 1)	pMH032		C1fw_GGlo_A1fw_HS2	2,600	2,600	100,000	puro from day 5
GFP (Fig. 1)	pMH049		C1fw_GGlo_A1fw_minusHS2	2,600	2,600	100,000	puro from day 5
GFP (Fig. 1)	pMH050		C1fw_A1fw_HS2_minusGglo	2,600	2,600	100,000	puro from day 5
Experiment 1	pMH027		GGlo	2,700	2,400	250,000	puro from day 1
Experiment 1	pMH028		GGlo_HS2	2,200	2,400	250,000	puro from day 1
Experiment 1	pMH032		C1fw_GGlo_A1fw_HS2	2,000	2,400	250,000	puro from day 1
Experiment 1	pMH034		C1fw_GGlo_A1rv_HS2	2,000	2,400	250,000	puro from day 1
Experiment 2	pMH027		GGlo	1,800	2,400	90,000	puro from day 1
Experiment 2	pMH028		GGlo_HS2	4,100	2,400	85,000	puro from day 1
Experiment 2	pMH032		C1fw_GGlo_A1fw_HS2	2,200	2,400	190,000	puro from day 1
Experiment 2	pMH034		C1fw_GGlo_A1rv_HS2	2,300	2,400	105,000	puro from day 1
Experiment 3	pMH027		GGlo	5,000	2,600	10,000	puro from day 6
Experiment 3	pMH028		GGlo_HS2	5,000	2,600	10,000	puro from day 6
Experiment 3	pMH032		C1fw_GGlo_A1rv_HS2	5,000	2,600	10,000	puro from day 6
Experiment 4	pMH034		C1fw_GGlo_A1rv_HS2	5,000	2,600	10,000	mKate sort day 1, GFP sort day 4
Experiment 5	pMH027	A	GGlo_HS2	24,000	3,000	5,583	mKate sort day 1
Experiment 5	pMH041	B	C1CoreFw_GGlo_A1CoreFw_HS2	24,000	3,000	5,161	mKate sort day 1
Experiment 5	pMH041	C	C1CoreFw_GGlo_A1CoreFw_HS2	24,000	3,000	5,816	mKate sort day 1
Experiment 5	pMH065	D	C1CoreFw_GGlo_HS2_A1CoreFw	24,000	3,000	4,045	mKate sort day 1

Supplemental Table S2. Sequencing libraries for DNA/RNA/iPCR/10xRNA experiments.

Amplicon PCR libraries are listed by experiment. For Experiment 5, individual transfections are distinguished. Technical replicates (PCR library construction repeated on the same sample) are distinguished by letter suffix in "Sample #". Trim R1/R2 refers to the number of nt added to the R1 or R2 to increase sequencing diversity; UMI length indicates the portion of these that serve as a UMI (see **Methods**).

Experiment	Reporter Construct	Transfection	Sample #	Type	Trim (R1/R2)	UMI length	Total read pairs (M)	Unique BC	Mapping	Unique sites
1	GGlo		BS02212A	DNA	10/1	10	18.2	111,170	NA	NA
1	GGlo		BS02216A	DNA	9/1	9	24.1	124,979	NA	NA
1	GGlo		BS02225A	iPCR	0/1	NA	15.1	88,712	SE	50,586
1	GGlo		BS02225B	iPCR	0/0	NA	6.8	82,983	SE	51,949
1	GGlo		BS02225C	iPCR	0/0	NA	7.6	90,275	SE	59,441
1	GGlo		BS02225D	iPCR	0/0	NA	8.1	91,620	SE	60,385
1	GGlo		BS02220A	RNA	1/9	10	16.6	59,453	NA	NA
1	GGlo		BS02220B	RNA	1/8	9	17.0	61,710	NA	NA
1	GGlo_HS2		BS02213A	DNA	9/2	9	14.1	84,216	NA	NA
1	GGlo_HS2		BS02217A	DNA	8/2	8	15.9	78,578	NA	NA
1	GGlo_HS2		BS02226A	iPCR	1/0	NA	17.6	32,889	SE	22,093
1	GGlo_HS2		BS02226B	iPCR	0/1	NA	7.4	31,720	SE	20,230
1	GGlo_HS2		BS02226C	iPCR	0/1	NA	7.1	34,301	SE	22,657
1	GGlo_HS2		BS02221A	RNA	0/10	10	13.6	73,488	NA	NA
1	GGlo_HS2		BS02221B	RNA	0/9	9	13.3	72,119	NA	NA
1	C1fw_GGlo_A1fw_HS2		BS02214A	DNA	8/0	8	14.9	45,307	NA	NA
1	C1fw_GGlo_A1fw_HS2		BS02218A	DNA	10/0	10	14.6	43,771	NA	NA
1	C1fw_GGlo_A1fw_HS2		BS02227A	iPCR	2/1	NA	22.1	27,836	SE	16,577
1	C1fw_GGlo_A1fw_HS2		BS02227B	iPCR	2/0	NA	11.3	26,440	SE	17,087
1	C1fw_GGlo_A1fw_HS2		BS02227C	iPCR	2/0	NA	11.3	24,073	SE	16,162
1	C1fw_GGlo_A1fw_HS2		BS02222A	RNA	0/8	8	11.4	26,929	NA	NA
1	C1fw_GGlo_A1fw_HS2		BS02222B	RNA	0/10	10	16.9	29,560	NA	NA
1	C1fw_GGlo_A1rv_HS2		BS02215A	DNA	10/0	10	13.8	64,818	NA	NA
1	C1fw_GGlo_A1rv_HS2		BS02219A	DNA	9/1	9	14.8	46,523	NA	NA
1	C1fw_GGlo_A1rv_HS2		BS02228A	iPCR	0/0	NA	19.5	42,792	SE	28,528
1	C1fw_GGlo_A1rv_HS2		BS02228B	iPCR	2/1	NA	11.1	39,048	SE	24,658
1	C1fw_GGlo_A1rv_HS2		BS02228C	iPCR	2/1	NA	7.8	32,706	SE	21,917
1	C1fw_GGlo_A1rv_HS2		BS02223A	RNA	2/9	11	17.5	56,186	NA	NA
1	C1fw_GGlo_A1rv_HS2		BS02223B	RNA	2/10	12	18.5	60,304	NA	NA
2	GGlo		BS02560A	DNA	9/2	9	18.1	70,902	NA	NA
2	GGlo		BS02561A	DNA	10/2	10	14.0	71,044	NA	NA
2	GGlo		BS02605A	iPCR	0/0	NA	10.7	32,115	SE	15,117
2	GGlo		BS02605B	iPCR	1/1	NA	6.8	24,168	SE	13,880
2	GGlo		BS02606A	iPCR	2/0	NA	11.4	38,499	SE	20,250
2	GGlo		BS02539A	RNA	2/8	10	4.9	34,904	NA	NA
2	GGlo		BS02539B	RNA	2/8	10	5.6	42,237	NA	NA
2	GGlo		BS02540A	RNA	1/8	9	5.0	30,078	NA	NA
2	GGlo_HS2		BS02562A	DNA	8/0	8	17.6	77,830	NA	NA
2	GGlo_HS2		BS02563A	DNA	9/0	9	15.6	77,992	NA	NA
2	GGlo_HS2		BS02607A	iPCR	0/1	NA	9.7	16,664	SE	7,488
2	GGlo_HS2		BS02607C	iPCR	0/1	NA	15.6	21,905	SE	10,303
2	GGlo_HS2		BS02608A	iPCR	1/0	NA	9.7	30,763	SE	18,109
2	GGlo_HS2		BS02541A	RNA	0/8	8	5.9	53,409	NA	NA
2	GGlo_HS2		BS02542A	RNA	2/9	11	4.5	53,612	NA	NA
2	C1fw_GGlo_A1fw_HS2		BS02564A	DNA	10/0	10	17.4	61,448	NA	NA
2	C1fw_GGlo_A1fw_HS2		BS02565A	DNA	8/1	8	18.3	56,228	NA	NA
2	C1fw_GGlo_A1fw_HS2		BS02609A	iPCR	2/1	NA	10.7	11,637	SE	5,282
2	C1fw_GGlo_A1fw_HS2		BS02609B	iPCR	0/0	NA	6.3	12,080	SE	5,966
2	C1fw_GGlo_A1fw_HS2		BS02610A	iPCR	1/1	NA	10.5	18,061	SE	9,893
2	C1fw_GGlo_A1fw_HS2		BS02543A	RNA	1/9	10	3.4	19,355	NA	NA
2	C1fw_GGlo_A1fw_HS2		BS02543B	RNA	1/9	10	4.4	26,838	NA	NA
2	C1fw_GGlo_A1fw_HS2		BS02544A	RNA	0/9	9	4.9	24,289	NA	NA
2	C1fw_GGlo_A1rv_HS2		BS02566A	DNA	9/1	9	18.8	62,474	NA	NA
2	C1fw_GGlo_A1rv_HS2		BS02567A	DNA	10/1	10	17.7	64,167	NA	NA
2	C1fw_GGlo_A1rv_HS2		BS02611A	iPCR	2/0	NA	12.7	21,508	SE	10,470
2	C1fw_GGlo_A1rv_HS2		BS02611C	iPCR	2/0	NA	21.7	24,583	SE	12,916
2	C1fw_GGlo_A1rv_HS2		BS02612A	iPCR	1/1	NA	11.0	32,938	SE	19,605
2	C1fw_GGlo_A1rv_HS2		BS02545A	RNA	2/10	12	4.5	36,846	NA	NA
2	C1fw_GGlo_A1rv_HS2		BS02546A	RNA	1/10	11	4.5	32,122	NA	NA

Experiment	Reporter Construct	Transfection	Sample #	Type	Trim (R1/R2)	UMI length	Total read pairs (M)	Unique BC	Mapping	Unique sites
3	GGlo		BS02951A	DNA	10/1	10	25.4	73,676	NA	NA
3	GGlo		BS03156A	DNA	8/2	8	18.0	24,033	NA	NA
3	GGlo		BS03128A	iPCR	2/1	NA	32.0	49,281	SE	31,838
3	GGlo		BS03128B	iPCR	0/0	NA	1.1	47,228	SE	29,755
3	GGlo		BS03123A	RNA	0/8	8	39.8	102,093	NA	NA
3	GGlo		BS03123B	RNA	0/10	10	7.5	46,241	NA	NA
3	GGlo		BS03123C	RNA	1/9	10	6.1	17,803	NA	NA
3	GGlo		BS03123D	RNA	2/8	10	7.5	18,573	NA	NA
3	GGlo_HS2		BS02953A	DNA	8/1	8	26.7	33,430	NA	NA
3	GGlo_HS2		BS03126A	iPCR	0/1	NA	18.9	21,267	SE	14,515
3	GGlo_HS2		BS03126B	iPCR	1/0	NA	0.3	14,329	SE	9,794
3	GGlo_HS2		BS03126C	iPCR	0/1	NA	2.0	17,570	SE	12,713
3	GGlo_HS2		BS03124A	RNA	1/9	10	46.8	68,538	NA	NA
3	GGlo_HS2		BS03124B	RNA	0/8	8	8.0	55,196	NA	NA
3	GGlo_HS2		BS03124C	RNA	1/10	11	8.1	35,074	NA	NA
3	GGlo_HS2		BS03124D	RNA	2/9	11	9.5	36,509	NA	NA
3	C1fw_GGlo_A1rv_HS2		BS02955A	DNA	9/2	9	25.5	62,203	NA	NA
3	C1fw_GGlo_A1rv_HS2		BS03158A	DNA	10/0	10	19.4	36,871	NA	NA
3	C1fw_GGlo_A1rv_HS2		BS03097A	iPCR	0/1	NA	6.8	23,907	SE	13,125
3	C1fw_GGlo_A1rv_HS2		BS03097B	iPCR	1/0	NA	11.3	10,125	SE	5,307
3	C1fw_GGlo_A1rv_HS2		BS03097I	iPCR	0/1	NA	5.2	22,788	SE	13,989
3	C1fw_GGlo_A1rv_HS2		BS03097J	iPCR	0/1	NA	4.8	22,452	SE	13,156
3	C1fw_GGlo_A1rv_HS2		BS03097K	iPCR	0/1	NA	7.7	23,659	SE	12,650
3	C1fw_GGlo_A1rv_HS2		BS03097L	iPCR	0/1	NA	0.7	22,285	SE	13,337
3	C1fw_GGlo_A1rv_HS2		BS03125A	RNA	2/10	12	45.4	82,214	NA	NA
3	C1fw_GGlo_A1rv_HS2		BS03125B	RNA	0/9	9	5.6	45,788	NA	NA
3	C1fw_GGlo_A1rv_HS2		BS03125C	RNA	1/8	9	6.1	33,226	NA	NA
3	C1fw_GGlo_A1rv_HS2		BS03125D	RNA	2/10	12	7.0	30,931	NA	NA
4	C1fw_GGlo_A1rv_HS2		BS03159A	DNA	8/1	8	27.3	17,411	NA	NA
4	C1fw_GGlo_A1rv_HS2		BS03160A	DNA	9/0	9	33.2	44,490	NA	NA
4	C1fw_GGlo_A1rv_HS2		BS03127A	iPCR	1/0	NA	37.2	51,845	SE	35,813
4	C1fw_GGlo_A1rv_HS2		BS03127B	iPCR	0/0	NA	7.0	55,267	SE	37,733
4	C1fw_GGlo_A1rv_HS2		BS03127C	iPCR	2/1	NA	3.4	55,296	SE	37,909
4	C1fw_GGlo_A1rv_HS2		BS03129A	iPCR	0/0	NA	35.7	62,736	SE	42,308
4	C1fw_GGlo_A1rv_HS2		BS03129B	iPCR	1/1	NA	18.9	65,142	SE	42,565
4	C1fw_GGlo_A1rv_HS2		BS03129C	iPCR	1/1	NA	3.6	58,705	SE	39,682
4	C1fw_GGlo_A1rv_HS2		BS03079A	RNA	0/8	8	31.2	77,247	NA	NA
4	C1fw_GGlo_A1rv_HS2		BS03196A	RNA	0/8	8	16.2	66,379	NA	NA
4	C1fw_GGlo_A1rv_HS2		BS03040B	10xRNA	0/0	12	39.3	18,661	NA	NA
4	C1fw_GGlo_A1rv_HS2		BS03091A	10xRNA	0/0	12	32.7	18,153	NA	NA
4	C1fw_GGlo_A1rv_HS2		BS03092A	10xRNA	0/0	12	26.3	16,642	NA	NA
4	C1fw_GGlo_A1rv_HS2		BS03093A	10xRNA	0/0	12	15.4	14,621	NA	NA
4	C1fw_GGlo_A1rv_HS2		BS03094A	10xRNA	0/0	12	29.8	18,301	NA	NA
4	C1fw_GGlo_A1rv_HS2		BS03095A	10xRNA	0/0	12	36.0	19,198	NA	NA
5	GGlo_HS2	A	BS07222A	iPCR	0/0	NA	5.1	47,683	PE	30,270
5	GGlo_HS2	A	BS07223A	iPCR	1/1	NA	6.4	48,145	PE	29,938
5	GGlo_HS2	A	BS07381A	iPCR	0/0	NA	5.0	67,184	PE	47,720
5	GGlo_HS2	A	BS07583A	iPCR	0/0	NA	4.2	38,430	PE	26,364
5	GGlo_HS2	A	BS07587A	iPCR	1/0	NA	6.4	38,146	PE	24,596
5	C1CoreFw_GGlo_A1CoreFw_HS2	B	BS07224A	iPCR	2/0	NA	8.4	45,655	PE	27,172
5	C1CoreFw_GGlo_A1CoreFw_HS2	B	BS07225A	iPCR	0/1	NA	4.9	47,475	PE	29,615
5	C1CoreFw_GGlo_A1CoreFw_HS2	B	BS07382A	iPCR	1/1	NA	7.4	63,607	PE	43,027
5	C1CoreFw_GGlo_A1CoreFw_HS2	B	BS07584A	iPCR	1/1	NA	5.3	37,811	PE	24,370
5	C1CoreFw_GGlo_A1CoreFw_HS2	B	BS07588A	iPCR	2/1	NA	6.6	40,357	PE	25,466
5	C1CoreFw_GGlo_A1CoreFw_HS2	C	BS07226A	iPCR	1/0	NA	6.6	45,233	PE	26,291
5	C1CoreFw_GGlo_A1CoreFw_HS2	C	BS07227A	iPCR	2/1	NA	7.2	43,690	PE	23,970
5	C1CoreFw_GGlo_A1CoreFw_HS2	C	BS07383A	iPCR	2/0	NA	9.2	66,322	PE	43,441
5	C1CoreFw_GGlo_A1CoreFw_HS2	C	BS07585A	iPCR	2/0	NA	5.9	41,529	PE	27,038
5	C1CoreFw_GGlo_A1CoreFw_HS2	C	BS07589A	iPCR	0/0	NA	4.5	40,809	PE	26,958
5	C1CoreFw_GGlo_HS2_A1CoreFw	D	BS07228A	iPCR	0/0	NA	4.9	30,767	PE	18,976
5	C1CoreFw_GGlo_HS2_A1CoreFw	D	BS07229A	iPCR	1/1	NA	6.8	31,771	PE	19,148
5	C1CoreFw_GGlo_HS2_A1CoreFw	D	BS07384A	iPCR	0/1	NA	5.1	42,841	PE	30,026
5	C1CoreFw_GGlo_HS2_A1CoreFw	D	BS07586A	iPCR	0/1	NA	4.2	29,283	PE	20,257
5	C1CoreFw_GGlo_HS2_A1CoreFw	D	BS07590A	iPCR	1/1	NA	6.3	29,076	PE	19,631
5	GGlo_HS2	A	BS07206A	DNA	8/0	8	19.3	52,515	NA	NA
5	GGlo_HS2	A	BS07207A	DNA	9/1	9	17.2	38,760	NA	NA
5	GGlo_HS2	A	BS07373A	DNA	8/0	8	19.6	76,513	NA	NA
5	GGlo_HS2	A	BS07567A	DNA	8/0	8	16.0	46,023	NA	NA

Experiment	Reporter Construct	Transfection	Sample #	Type	Trim (R1/R2)	UMI length	Total read pairs (M)	Unique BC	Mapping	Unique sites
5	GGlo_HS2	A	BS07571A	DNA	9/0	9	23.3	48,033	NA	NA
5	C1CoreFw_GGlo_A1CoreFw_HS2	B	BS07208A	DNA	10/2	10	15.7	31,704	NA	NA
5	C1CoreFw_GGlo_A1CoreFw_HS2	B	BS07209A	DNA	8/2	8	17.3	30,861	NA	NA
5	C1CoreFw_GGlo_A1CoreFw_HS2	B	BS07374A	DNA	9/2	9	17.1	64,026	NA	NA
5	C1CoreFw_GGlo_A1CoreFw_HS2	B	BS07568A	DNA	9/1	9	15.4	38,288	NA	NA
5	C1CoreFw_GGlo_A1CoreFw_HS2	B	BS07572A	DNA	10/1	10	20.7	40,668	NA	NA
5	C1CoreFw_GGlo_A1CoreFw_HS2	C	BS07210A	DNA	9/0	9	16.4	50,833	NA	NA
5	C1CoreFw_GGlo_A1CoreFw_HS2	C	BS07211A	DNA	10/1	10	17.4	25,135	NA	NA
5	C1CoreFw_GGlo_A1CoreFw_HS2	C	BS07375A	DNA	10/1	10	17.1	59,315	NA	NA
5	C1CoreFw_GGlo_A1CoreFw_HS2	C	BS07569A	DNA	10/2	10	15.2	40,267	NA	NA
5	C1CoreFw_GGlo_A1CoreFw_HS2	C	BS07573A	DNA	8/1	8	21.9	37,500	NA	NA
5	C1CoreFw_GGlo_HS2_A1CoreFw	D	BS07212A	DNA	8/1	8	18.1	17,813	NA	NA
5	C1CoreFw_GGlo_HS2_A1CoreFw	D	BS07213A	DNA	9/2	9	17.1	29,070	NA	NA
5	C1CoreFw_GGlo_HS2_A1CoreFw	D	BS07376A	DNA	9/2	9	13.8	42,187	NA	NA
5	C1CoreFw_GGlo_HS2_A1CoreFw	D	BS07570A	DNA	8/2	8	15.5	27,218	NA	NA
5	C1CoreFw_GGlo_HS2_A1CoreFw	D	BS07574A	DNA	9/2	9	17.7	26,428	NA	NA
5	GGlo_HS2	A	BS07214A	RNA	0/8	8	14.2	83,166	NA	NA
5	GGlo_HS2	A	BS07215A	RNA	1/9	10	13.6	59,645	NA	NA
5	GGlo_HS2	A	BS07377A	RNA	0/10	10	13.5	94,915	NA	NA
5	GGlo_HS2	A	BS07575A	RNA	0/8	8	13.9	68,189	NA	NA
5	GGlo_HS2	A	BS07579A	RNA	1/10	11	12.9	56,750	NA	NA
5	C1CoreFw_GGlo_A1CoreFw_HS2	B	BS07216A	RNA	2/10	12	14.5	49,094	NA	NA
5	C1CoreFw_GGlo_A1CoreFw_HS2	B	BS07217A	RNA	0/9	9	14.8	67,064	NA	NA
5	C1CoreFw_GGlo_A1CoreFw_HS2	B	BS07378A	RNA	1/8	9	15.0	72,061	NA	NA
5	C1CoreFw_GGlo_A1CoreFw_HS2	B	BS07576A	RNA	1/9	10	14.1	50,898	NA	NA
5	C1CoreFw_GGlo_A1CoreFw_HS2	B	BS07581A	RNA	0/10	10	13.2	64,310	NA	NA
5	C1CoreFw_GGlo_A1CoreFw_HS2	C	BS07218A	RNA	1/10	11	14.2	47,994	NA	NA
5	C1CoreFw_GGlo_A1CoreFw_HS2	C	BS07219A	RNA	2/8	10	13.8	57,832	NA	NA
5	C1CoreFw_GGlo_A1CoreFw_HS2	C	BS07379A	RNA	2/9	11	15.7	90,459	NA	NA
5	C1CoreFw_GGlo_A1CoreFw_HS2	C	BS07577A	RNA	2/10	12	15.6	64,030	NA	NA
5	C1CoreFw_GGlo_A1CoreFw_HS2	C	BS07580A	RNA	2/8	10	17.3	66,033	NA	NA
5	C1CoreFw_GGlo_HS2_A1CoreFw	D	BS07220A	RNA	0/10	10	12.6	47,992	NA	NA
5	C1CoreFw_GGlo_HS2_A1CoreFw	D	BS07221A	RNA	1/8	9	14.5	32,527	NA	NA
5	C1CoreFw_GGlo_HS2_A1CoreFw	D	BS07380A	RNA	1/10	11	16.0	55,183	NA	NA
5	C1CoreFw_GGlo_HS2_A1CoreFw	D	BS07578A	RNA	0/9	9	12.8	41,321	NA	NA
5	C1CoreFw_GGlo_HS2_A1CoreFw	D	BS07582A	RNA	1/8	9	15.9	40,467	NA	NA
5	Pool1	Pool1	BS07509A	10xRNA	0/0	12	17.0	88,525	NA	NA
5	Pool1	Pool1	BS07623A	10xRNA	0/0	12	17.3	85,901	NA	NA
5	Pool1	Pool1	BS07700A	10xRNA	0/0	12	6.4	62,377	NA	NA
5	Pool1	Pool1	BS07704A	10xRNA	0/0	12	5.3	69,276	NA	NA
5	Pool2	Pool2	BS07510A	10xRNA	0/0	12	18.6	83,620	NA	NA
5	Pool2	Pool2	BS07624A	10xRNA	0/0	12	18.2	80,386	NA	NA
5	Pool2	Pool2	BS07701A	10xRNA	0/0	12	6.2	58,564	NA	NA
5	Pool2	Pool2	BS07705A	10xRNA	0/0	12	6.2	65,772	NA	NA
5	Pool3	Pool3	BS07511A	10xRNA	0/0	12	21.0	87,274	NA	NA
5	Pool3	Pool3	BS07625A	10xRNA	0/0	12	24.0	87,971	NA	NA
5	Pool3	Pool3	BS07702A	10xRNA	0/0	12	6.8	64,088	NA	NA
5	Pool3	Pool3	BS07706A	10xRNA	0/0	12	5.9	67,286	NA	NA
5	Pool4	Pool4	BS07512A	10xRNA	0/0	12	18.7	83,824	NA	NA
5	Pool4	Pool4	BS07626A	10xRNA	0/0	12	22.2	84,701	NA	NA
5	Pool4	Pool4	BS07703A	10xRNA	0/0	12	6.8	52,790	NA	NA
5	Pool4	Pool4	BS07707A	10xRNA	0/0	12	4.7	59,714	NA	NA

Supplemental Table S3. Summaries of analyzed barcodes by transfection.
Shown are counts of BCs analyzed for the individual transfections in each experiment.

Experiment	Sample	DNA	RNA	iPCR	Analyzed sites
Experiment 1	GGlo	178,306	90,165	95,902	66,742
Experiment 1	GGlo+HS2	129,407	90,267	36,541	28,305
Experiment 1	Ins+GGlo+Ins+HS2	59,959	35,108	21,974	18,148
Experiment 1	Ins+GGlo+Ins+HS2	85,449	76,983	39,520	28,446
Experiment 2	GGlo	86,288	58,726	29,481	25,930
Experiment 2	GGlo+HS2	97,499	78,318	25,647	21,572
Experiment 2	Ins+GGlo+Ins+HS2	85,363	40,729	14,939	12,390
Experiment 2	Ins+GGlo+Ins+HS2	90,316	52,751	29,988	22,968
Experiment 3	GGlo	85,691	113,862	44,670	28,107
Experiment 3	GGlo+HS2	33,430	85,129	18,392	11,974
Experiment 3	Ins+GGlo+Ins+HS2	69,569	95,375	22,995	18,469
Experiment 4	Ins+GGlo+Ins+HS2	49,923	89,502	55,045	30,935
Experiment 5	GGlo+HS2	88,019	128,313	53,884	48,015
Experiment 5	Ins+GGlo+Ins+HS2	77,163	107,088	48,003	81,005
Experiment 5	Ins+GGlo+Ins+HS2	79,367	115,903	49,216	
Experiment 5	Ins+GGlo+HS2+Ins	50,592	75,912	33,076	27,565

Supplemental Table S4. External DNase-seq and ChIP-seq datasets.
Data were obtained from the ENCODE (https://www.encodeproject.org) or the 4D Nucleome (https://data.4dnucleome.org) projects. ENCODE data was processed using a standard pipeline. DHS were identified using FDR 0.01 peaks from the program hotspot and ChIP-seq peaks using FDR 0.05 hotspots from hotspot2 (Thurman et al. 2012).

This table is provided as a separate file.

Supplemental Table S5. Summary of 3' 10x libraries.
Shown are 10x scRNA-seq libraries, sequencing statistics, and mapping statistics. Each pool contained a mixture of cells from transfections A-D.

Experiment	Pool	# cells loaded	Sample #	# Reads	Estimated # Cells	Median Reads per cell	Median Genes per cell	Median UMIs per Cell
Experiment 5	Pool1	27,094	BS07468	2,220,021,482	25,480	87,128	3,610	10,760
Experiment 5	Pool2	25,048	BS07469	1,389,186,887	11,832	117,409	5,951	35,060
Experiment 5	Pool3	28,226	BS07470	1,499,082,944	13,086	114,556	5,975	34,896
Experiment 5	Pool4	19,632	BS07471	1,379,307,019	12,024	114,713	5,722	32,213

Supplemental Table S6. PCR primer and DNA fragment sequences.

The applicable Library Type is indicated where relevant.

Primer/DNA Fragment Name	Sequence	Library Type
P5_barcode_0N	TTTCCCTACACGACGCTCTTCCGATCTCCTTTCTAGGCAATTAGG	RNA, iPCR
P5_barcode_1N	TTTCCCTACACGACGCTCTTCCGATCTNCCTTTCTAGGCAATTAGG	RNA, iPCR
P5_barcode_2N	TTTCCCTACACGACGCTCTTCCGATCTNNCCTTTCTAGGCAATTAGG	RNA, iPCR
P7_barcode_0N	CTGGAGTTCAGACGTGTGCTCTTCCGATCTCCTTTCTAGGCAATTAGG	DNA
P7_barcode_1N	CTGGAGTTCAGACGTGTGCTCTTCCGATCTNCCTTTCTAGGCAATTAGG	DNA
P7_barcode_2N	CTGGAGTTCAGACGTGTGCTCTTCCGATCTNNCCTTTCTAGGCAATTAGG	DNA
P7_ITR_0N	CTGGAGTTCAGACGTGTGCTCTTCCGATCTCTTCCGACTTCAACTGTA	iPCR
P7_ITR_1N	CTGGAGTTCAGACGTGTGCTCTTCCGATCTNCTTCCGACTTCAACTGTA	iPCR
P7_ITR_2N	CTGGAGTTCAGACGTGTGCTCTTCCGATCTNNCTTCCGACTTCAACTGTA	iPCR
P5_plasmid_8N	TTTCCCTACACGACGCTCTTCCGATCTNNNNNNNNAGCTGCACAGCAACAC	DNA
P5_plasmid_9N	TTTCCCTACACGACGCTCTTCCGATCTNNNNNNNNNAGCTGCACAGCAACAC	DNA
P5_plasmid_10N	TTTCCCTACACGACGCTCTTCCGATCTNNNNNNNNNNAGCTGCACAGCAACAC	DNA
P7_plasmid_8N	CTGGAGTTCAGACGTGTGCTCTTCCGATCTNNNNNNNNAGCTGCACAGCAACAC	RNA
P7_plasmid_9N	CTGGAGTTCAGACGTGTGCTCTTCCGATCTNNNNNNNNNAGCTGCACAGCAACAC	RNA
P7_plasmid_10N	CTGGAGTTCAGACGTGTGCTCTTCCGATCTNNNNNNNNNNAGCTGCACAGCAACAC	RNA
P7_10xSB-barcodeV2_0N	CTGGAGTTCAGACGTGTGCTCTTCCGATCTTTGGACAAAGATCCCACAACTAGC	10xRNA
P7_halfsite_S	CTGGAGTTCAGACGTGTGCT	RNA
P5_halfsite_Short	TTTCCCTACACGACGCTCTT	DNA, RNA
P5_halfsite	TTTCCCTACACGACGCTCTTCCGATCT	10xRNA
P7_amplicon_N704	CAAGCAGAAGACGGCATACGAGATGCTCAGGAGTGACTGGAGTTCAGACGTGTGCT	DNA, RNA, iPCR
P7_amplicon_N705	CAAGCAGAAGACGGCATACGAGATAGGAGTCCGTGACTGGAGTTCAGACGTGTGCT	DNA, RNA, iPCR
P7_amplicon_N706	CAAGCAGAAGACGGCATACGAGATCATGCCTAGTGACTGGAGTTCAGACGTGTGCT	DNA, RNA, iPCR
P7_amplicon_N707	CAAGCAGAAGACGGCATACGAGATGTAGAGAGGTGACTGGAGTTCAGACGTGTGCT	DNA, RNA, iPCR
P7_amplicon_N710	CAAGCAGAAGACGGCATACGAGATCAGCCTCGGTGACTGGAGTTCAGACGTGTGCT	DNA, RNA, iPCR
P7_amplicon_N711	CAAGCAGAAGACGGCATACGAGATTGCCTCTTGTGACTGGAGTTCAGACGTGTGCT	DNA, RNA, iPCR
P7_amplicon_N712	CAAGCAGAAGACGGCATACGAGATTCCTCTACGTGACTGGAGTTCAGACGTGTGCT	DNA, RNA, iPCR
P7_amplicon_N714	CAAGCAGAAGACGGCATACGAGATTCATGAGCGTGACTGGAGTTCAGACGTGTGCT	DNA, RNA, iPCR
P7_amplicon_N715	CAAGCAGAAGACGGCATACGAGATCCTGAGATGTGACTGGAGTTCAGACGTGTGCT	DNA, RNA, iPCR
P7_amplicon_N716	CAAGCAGAAGACGGCATACGAGATTAGCGAGTGTGACTGGAGTTCAGACGTGTGCT	DNA, RNA, iPCR
P7_amplicon_N718	CAAGCAGAAGACGGCATACGAGATGTAGCTCCGTGACTGGAGTTCAGACGTGTGCT	DNA, RNA, iPCR
P7_amplicon_N719	CAAGCAGAAGACGGCATACGAGATTACTACGCGTGACTGGAGTTCAGACGTGTGCT	DNA, RNA, iPCR
P7_amplicon_N720	CAAGCAGAAGACGGCATACGAGATAGGCTCCGGTGACTGGAGTTCAGACGTGTGCT	DNA, RNA, iPCR
P5_amplicon_S502	AATGATACGGCGACCACCGAGATCTACACCTCTCTATACACTCTTTCCCTACACGACGCTCTT	DNA, RNA, iPCR
P5_amplicon_S503	AATGATACGGCGACCACCGAGATCTACACTATCCTCTACACTCTTTCCCTACACGACGCTCTT	DNA, RNA, iPCR
P5_amplicon_S505	AATGATACGGCGACCACCGAGATCTACACGTAAGGAGACACTCTTTCCCTACACGACGCTCTT	DNA, RNA, iPCR
P5_amplicon_S506	AATGATACGGCGACCACCGAGATCTACACACTGCATAACACTCTTTCCCTACACGACGCTCTT	DNA, RNA, iPCR
P5_amplicon_S507	AATGATACGGCGACCACCGAGATCTACACAAGGAGTAACACTCTTTCCCTACACGACGCTCTT	DNA, RNA, iPCR
P5_amplicon_S508	AATGATACGGCGACCACCGAGATCTACACCTAAGCCTACACTCTTTCCCTACACGACGCTCTT	DNA, RNA, iPCR
P5_amplicon_S510	AATGATACGGCGACCACCGAGATCTACACCGTCTAATACACTCTTTCCCTACACGACGCTCTT	DNA, RNA, iPCR
P5_amplicon_S511	AATGATACGGCGACCACCGAGATCTACACTCTCTCCGACACTCTTTCCCTACACGACGCTCTT	DNA, RNA, iPCR
P5_amplicon_S513	AATGATACGGCGACCACCGAGATCTACACTCGACTAGACACTCTTTCCCTACACGACGCTCTT	DNA, RNA, iPCR
P5_amplicon_S515	AATGATACGGCGACCACCGAGATCTACACTTCTAGCTACACTCTTTCCCTACACGACGCTCTT	DNA, RNA, iPCR
P5_amplicon_S516	AATGATACGGCGACCACCGAGATCTACACCCTAGAGTACACTCTTTCCCTACACGACGCTCTT	DNA, RNA, iPCR
P5_amplicon_S517	AATGATACGGCGACCACCGAGATCTACACGCGTAAGAACACTCTTTCCCTACACGACGCTCTT	DNA, RNA, iPCR
P5_amplicon_S518	AATGATACGGCGACCACCGAGATCTACACCTATTAAGACACTCTTTCCCTACACGACGCTCTT	DNA, RNA, iPCR
P5_amplicon_S520	AATGATACGGCGACCACCGAGATCTACACAAGGCTATACACTCTTTCCCTACACGACGCTCTT	DNA, RNA, iPCR
P5_amplicon_S521	AATGATACGGCGACCACCGAGATCTACACGAGCCTTAACACTCTTTCCCTACACGACGCTCTT	DNA, RNA, iPCR
P5_amplicon_S522	AATGATACGGCGACCACCGAGATCTACACTTATGCGAACACTCTTTCCCTACACGACGCTCTT	DNA, RNA, iPCR
Gibson BC4	CcCGAGCTCGATGAGTTTGGACAAAgatcCCACAACTAGcnnnnnnnnnnnnnnnnCCTAATTGCCTAGAAA GGAGCAGACGATATGGC	n/a
GGlo	TAAAGGGAAGAATAAATTAGAGAAAAACTGGAATGACTGAATCGGAACAAGGCAAAGGCTATAAAAAA AATTAAGCAGCAGTATCCTCTTGGGGGCCCCTTCCCCACACTATCTCAATGCAAATATCTGTCTGAAA CGGTCCCTGGCTAAACTCCACCCATGGGTTGGCCAGCCTTGCCTTGACCAATAGCCTTGACAAGGCA AACTTGACCAATAGTCTTAGAGTATCCAGTGAGGCCAGGGGCCGGCGGCTGGCTAGGGATGAAGAA TAAAAGGAAGCACCCTTCAGCAGTTCCACACACTCGCTTCTGGAACGTCTGAGGTTATCAATAAGGA CCTGCAGCCAAGCTTGAGCTTCACGCTGCCGCAAGCACTCAGGGCGCAAGGGCTGCTAAAGGAAGC GGAACACGTAGAAAGCCAGTCCGCAGAAACGGTGCTGACCCCGGATGAATGTCAGCTACTGGGCTA TCTGGACAAGGGAAAACGCAAGCGCAAAGAGAAAGCAGGTAGCTTGCAGTGGGCTTACATGGCGAT AGCTAGACTGGGCGGTTTTATGGACAGCAAGCGAACCGGAATTGCCAGCTGGGGCGCCCTCTGGTA AGGTTGGGAAGCCCTGCAAAGTAAACTGGATGGCTTTCTTGCCGCCAAGGATCTGATGGCGCAGGG GATCAAGATCTGATCAAGAGACAGGATGAGGATCGTTTCGCA	n/a

Primer/DNA Fragment Name	Sequence	Library Type
HS2	ACCGGTTGTTTCCTTATCTGACCTGCTTTAACTGGGTAAGCTTATGAAAAGTCTTGTGTAGAAGAGAA AGGGATAACAGCCTGTGCTAAATGAGGAAGCTGCCTTGCCTGTTCCTGCTCAGTGGGGTTTCTGGCT ATACTACATCAACTGAGTCAGTGCATCTTGTCTAGTTCCCACACCTTTCTCTGAACAGAGAGAGTAAA GGGCTCAATAAGAAAAATACAGTTTATGGTCTGTACGTTGTATTACACGTCATCCCTTACTTTCTAAAA GGCATCTTCACTGAGAAAGACATGGATTTCTAACCTTACAATCTCTACAAAGTTCAGAATACATAATAA TCTTGAATTATGATTAACTGTTAGTTTTGACCAGGTCTTCTGGCAGACAGGTCACATGTGTTAGTATCA CTTATTCCTCAAGTGTTGATGTTAGTGTCAGCATATTACCGATGTTCCACAAACATTTCCTGAATGACT GTTAAACTTCCTACACATTAACGAGCCTCTGCATTTTTTTCCAGCTTCCATCTATGATTTAAGTAAACTC TAGTTTTCCACTTCTTCATATTCTCTCTCTACATCTCAATTATTGCAGTACCACTGTCCAAGGGCAGAG GAGGTTAGCTGGGCCCAGGCGGAGTCAATTCTCTACTCCCCACCCTGTGGGTGTGTTCAGCCTTGT GAGCCAGCATCAGGCTTGAGCACAGCAGTGCTGAGTCATGCTGAGTCATGCTGAGGCTTAGGGTGT GTGGCCAGATGTTTTCAGCTGTGAGTCATCAGTGCTATCTGGGTCTCTAGGAGGAAGTCCACAGGGA AGGTGAAAAGAAAATAAGTTTGCTCCCTGAAGAAAACATTACTTACACCAGCATTACAATGAAAAGGG GACCCTGCCTTGCTGTGTGACATAACCTAGAATATTTTATTTTCTAGTTAAAAATTACTTCTCATCATCT TTATTAGAAGTTATAACAAATCATTTTTTAGAAATGTAAAGTTTATTTAGACTCATACCTAGAAGCAACA GAATCACACATTTTAGTGTACACACACACACACACGCACACACACACACATGACTTTTGTTCATAAA CTATGCAATTGTTAATGAAATGCTATTTGGAATGGGCTTGAATTCGTATTCC	n/a
A1 insulator	CTGAACAGGTTGACTATTAATTGTGTCTGCTTGATGTGGACACCAGGTGGCGCTGGACATCAGATTT GGAGAGGCAGTTGTCTAGGGAACCGGGCTCTGTGCCAGCGCAGGAGGCAGGCTGGCTCTCCTATTC CAGGGATGCTCATCCAGGAAGGAAAGGTTGCATGCTGGACACACTAACCT	n/a
C1 insulator	AGGAAAAGCCCCAGAGGATGTCCCCCCGCGTTCATACCCTAAGAGAAGATGCAACAGGAACAAGAA ATTGCACAACAGAGCTCTGTGGACCATGAAAAGGCCCCTTCCTCCACAGTGTCCCTCCGGCGCCCTC TACTGACAAAGCTTGATATCGTGCTTGCTGCAAAGAAGGGCTTAGAGTCCAGTCCATCAAGGAGCAG GCACTCACGGGGAAGTTGGAAGCTGAGAGGCAAGAAGAAACCAG	n/a
A1Core insulator	ATCTGATGTCCAGCGCCACCTGGTGTCCACATCAAGCAGACACAATTAATAGTC	n/a
C1Core insulator	AGTGTCCCTCCGGCGCCCTCTACTGACAAAGCTTGATATCGTGCTTGCTGCAAA	n/a

Supplemental Table S7. Plasmids.

Summary of plasmids used and their derivation if applicable.

Plasmid #	Plasmid Name	Starting Plasmid	Element(s) Removed	Synthetic DNA Inserted	Reporter Length (bp)
pMH012	pTREM-Bglo-GFP	pT2/LTR7-GFP (Addgene #62541)	LTR7 promoter	Human beta-globin minimal promoter	1511
pMH019	pTR-GGlo-GFP	pMH012	Human beta-globin minimal promoter	Human *HBG1* promoter	2165
pMH020	pTR-GGlo-GFP-HS2	pMH012	n/a	Human *HBG1* promoter, Mouse *Hbb* HS2 enhancer	3306
pMH021	pTR-GGlo-GFP-A1rv-HS2	pMH012	n/a	Human *HBG1* promoter, Mouse *Hbb* HS2 enhancer, 3' A1rv insulator	3497
pMH024	pTR-A1fw-GGlo-GFP-A1fw-HS2	pMH012	n/a	Human *HBG1* promoter, Mouse *Hbb* HS2 enhancer, 5' A1fw insulator, 3' A1fw insulator	3686
pMH024B	pTR-A1fw-GGlo-PuroP2AGFP-A1fw-HS2	pMH024	n/a	Puromycin resistance, P2A sequence	4349
pMH027	pTR_GGlo_PuroP2A_GFP	pMH019	n/a	Puromycin resistance, P2A sequence	2828
pMH028	pTR-GGlo-PuroP2AGFP-HS2	pMH020	n/a	Puromycin resistance, P2A sequence	3969
pMH030	pTR-GGlo-PuroP2AGFP-A1rv-HS2	pMH021	n/a	Puromycin resistance, P2A sequence	4160
pMH032	pTR-C1fw-GGlo-PuroP2AGFP-A1fw-HS2	pMH024B	n/a	C1fw Insulator	4404
pMH034	pTR-C1fw-GGlo-PuroP2AGFP-A1rv-HS2	pMH030	n/a	C1fw Insulator	4404
pMH035	SB100X-mKate_v3	pCMV(CAT)T7-SB100 (Addgene #34879)	n/a	mKate	N/A
pMH041	pTR_C1CoreFW_GGlo_PuroP2AGFP_A1CoreFW_HS2	pMH028	n/a	A1CoreFW Insulator, C1CoreFW Insulator	4101
pMH043	pTR_A1CoreFW-GGlo-PuroP2AGFP-C1CoreFW-HS2	pMH028	n/a	A1CoreFW Insulator, C1CoreFW Insulator	4101
pMH042	pTR_C1CoreRV-GGlo-PuroP2AGFP-A1CoreFW-HS2	pMH028	n/a	A1CoreFW Insulator, C1CoreRV Insulator	4101
pMH044	pTR_A1CoreFW-GGlo-PuroP2AGFP-C1CoreRV-HS2	pMH028	n/a	A1CoreFW Insulator, C1CoreRV Insulator	4101
pMH049	pTR_C1fw_GGlo_PuroP2AGFP_A1fw_minusHS2	pMH032	Mouse *Hbb* HS2 Enhancer	n/a	3256
pMH050	pTR_C1fw_PuroP2AGFP_A1fw_HS2_minusGglo	pMH032	Human *HBG1* promoter	n/a	3685
pMH065	pTR_C1CoreFW_GGlo_PuroP2AGFP_HS2_A1Corefw	pMH028	n/a	A1CoreFW Insulator, C1CoreFW Insulator	4078

Supplemental Table S8. Reporter effects on gene expression and TADs.

Tests of reporter effect on endogenous gene expression, broken down by whether both reporters were in the same TAD or different TADs. Tests where neither the reporter and/or gene were in an annotated TAD were not included in **Fig. 5B**; tests where only one was in an annotated TAD were included in **Fig. 5B** as "Different TADs".

Reporter Class	Category	# tests	# genes tested	# reporter tested	# significant tests	# significant genes	# significant reporters
GGlo+HS2	Different TADs	992	518	797	61	58	58
GGlo+HS2	Neither in TAD	108	32	91	9	7	8
GGlo+HS2	One in TAD	25	17	21	0	0	0
GGlo+HS2	Same TAD	1683	679	1348	123	106	119
Ins+GGlo+Ins+HS2	Different TADs	1532	637	1273	35	35	33
Ins+GGlo+Ins+HS2	Neither in TAD	175	43	130	4	4	4
Ins+GGlo+Ins+HS2	One in TAD	53	24	48	2	2	2
Ins+GGlo+Ins+HS2	Same TAD	2414	784	1897	94	87	87
Ins+GGlo+HS2+Ins	Different TADs	808	477	661	31	30	30
Ins+GGlo+HS2+Ins	Neither in TAD	97	36	83	6	6	6
Ins+GGlo+HS2+Ins	One in TAD	21	16	20	0	0	0
Ins+GGlo+HS2+Ins	Same TAD	1377	663	1085	82	76	77

Supplemental Table S9. Reporter effects on gene expression and localization within gene body.

Tests of reporter effect on endogenous gene expression, broken down by whether both reporters were in a gene body or not.

Reporter Class	Category	# tests	# genes tested	# reporter tested	# significant tests	# significant genes	# significant reporters
GGlo+HS2	Outside gene body	2694	854	2037	175	148	168
GGlo+HS2	Within gene body	114	88	113	18	18	18
Ins+GGlo+Ins+HS2	Outside gene body	4006	976	2973	122	115	110
Ins+GGlo+Ins+HS2	Within gene body	168	123	168	13	13	13
Ins+GGlo+HS2+Ins	Outside gene body	2199	860	1638	106	99	98
Ins+GGlo+HS2+Ins	Within gene body	104	79	104	13	13	13

Supplemental Data

Supplemental Data S1-S4 and Supplemental Code are provided as separate files.

Supplemental Data S1. TADs identified using Armatus.
File is in bed4 format (hg38).

Supplemental Data S2. Clonal inference analysis results for Experiment 4.
Each line corresponds to a distinct clone. BCs and cellBCs contain a comma-separated list of the Reporter BCs and cellBCs assigned to that clone. clone contains a unique clone ID. count, total UMIs. nedges, number of reporter BC to cell BC. nBCs, number of unique reporter BCs. ncells, number of unique cellBCs.

Supplemental Data S3. Clonal inference analysis results for Experiment 5.
Format is the same as **Supplemental Data S2**, with an additional column transfection that indicates the inferred source transfection of the clone.

Supplemental Data S4. Analysis of reporter integration on gene expression.
Each row represents a differential expression test between a reporter and gene. Reporter_chrom, Reporter_chromStart. Reporter_Class, and Reporter_BC record reporter genomic coordinates (hg38), reporter class, and reporter BC. TSS_chromStart, Gene_Symbol, and Ensembl_ID indicate the gene TSS position (hg38), gene symbol and ENSEMBL gene ID. DistToTSS indicates the distance from reporter insertion to gene TSS. Within_gene_body is a flag indicating whether the reporter lies within gene body. n, number of cells in the reporter clone. x0 and x1, average gene UMI count across unperturbed and perturbed cells (respectively). mu, overall gene average UMI count. xb, estimated gene average UMI count among perturbed cells. log2_fold_change, expression change in perturbed cells. z, perturbation z-score. p.value, significance of perturbation. q.value significance corrected for multiple testing.

Supplemental Code. Sequencing data processing pipeline.
Archive of sequencing processing pipeline (also available from https://github.com/mauranolab/mapping/tree/master/dnase, https://github.com/mauranolab/mapping/tree/master/transposon, and https://github.com/mauranolab/decal).