CONTACT FOR REAGENT AND RESOURCE SHARING

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Marco Gallo (marco.gallo@ucalgary.ca). Sharing of primary samples, based on availability, may be subject to MTAs and will require appropriate research ethics board certifications.

Short hairpin RNA constructs targeting *CD276*

Targeted gene silencing was performed with shRNA constructs targeting *CD276* (set of 3 SMARTvector inducible human hEF1a-TurboRFP shRNA, Dharmacon, V3SH11255-06EG80381). The three hairpin IDs included in this set were V3IHSHER_5124908, V3IHSHER_5582519, V3IHSHER_8209220. V3IHSHER_5124908 and V3IHSHER_8209220 were selected for having the highest-efficiency knockdowns and are referred to as "sh-CD276a" and "sh-CD276b", respectively, throughout this manuscript for brevity.

Hi-C library generation

Hi-C libraries were generated by an in-situ Hi-C library protocol (Rao et al. 2014) with minor modifications.

Sample collection and crosslinking: Cellular inputs consisted of 2.5 million cells from patient-derived cultures G523, G567, and G583 that had been grown to passage 11-15 according to standard tissue culture protocols (see above). Cells were resuspended in 2.5 mL media, then fixed with 1% (v/v) formaldehyde for 10 min. One-tenth volume of 2.0 M glycine was added to quench the reaction. Cells were rinsed with 4°C PBS and the pellets were frozen using an ethanol-dry ice bath. The cross-linked cell pellets were maintained at -80°C short term until library preparation.

Lysis and digestion: The cross-linked cell pellet was gently resuspended in 1 mL of Hi-C lysis buffer (10mM Tris-HCl pH 8.0, 10mM NaCl, 0.2% Igepal [CA630], Protease Inhibitor EDTA-Free [Pierce, #88666]), allowed to incubate on ice for 15 mins, pelleted, then rinsed with 1 mL of 4°C Hi-C lysis buffer. Pelleted nuclei were resuspended in 50 µL of 0.5% SDS in water and incubated at 62°C for 5-10 min. After heating, 145 µL of water was added to the sample followed by 25 µL of 10% Triton X-100 (Sigma, #93443) to quench the SDS. The sample was gently mixed by pipetting, then allowed to incubate at 37°C for 15 min. 25 µL of 10× DpnII Buffer (NEB) and 10 µL (100 U) of DpnII restriction enzyme (NEB, R0543) were added, and the DNA was digested for 2 hours to overnight at 37°C with rotation in incu-shaker block @ 900 rpm.

End-labelling, ligation, and crosslink reversal: DpnII was inactivated by incubation at 62°C for 20 min with 900 rpm shaking. Digested nuclei were pelleted then resuspended in 225 µL water and 25 µL of 10×NEB2 Buffer. To repair DNA sticky ends and label fragments with biotin, 50 µL of fill-in master mix [37.5 µL of 0.4 mM biotin-14-dATP (Life Technologies, #19524-016), 1.5 µL of 10 mM dCTP, 1.5 µL of 10 mM dGTP, 1.5 µL of 10 mM dTTP, 8 µL of 5 U/µL DNA Polymerase I, Large (Klenow) Fragment (NEB, M0210)] was added. Sample was mixed by pipetting and incubated at 37°C for 45 - 90 min with mixing. 900 µL ligation master mix [663 µL of water, 120 µL of 10×NEB T4 DNA ligase buffer (NEB, B0202), 100 µL of 10% Triton X-100, 12 µL of 10 mg/mL Bovine Serum Albumin (100×BSA), 5 µL of 400 U µL T4 DNA Ligase (NEB, M0202)] was added, gently mixed by pipetting, and incubated at 16°C overnight. Ligated nuclei were pelleted, resuspended in 465 µL of 1×Tris buffer (10 mM Tris-HCl, pH 8), 49.1 µL of 10% SDS, and 46.5 µL of proteinase K (20 mg/mL), and incubated at 55°C for 30 min. 53.2 µL of 5 M NaCl were added to the sample and allowed to incubate at 68°C for 3 h.

DNA shearing and sizing: Samples were cooled to room temperature, and then DNA was purified by 70% ethanol precipitation. The ligated sample pellet was then dissolved in 135 µL of 10 mM Tris-HCl, pH 8, allowing 15 min at 37°C to fully dissolve the DNA. A Covaris S2 sonicator was used to shear DNA to 300-500 bp, size selection was performed using AMPure XP beads (Beckman Coulter, A63881), and samples were transferred to low-binding tubes for the remainder of the protocol.

Biotin fragment enrichment and adapter ligation: Biotinylated DNA fragments were purified using Dynabeads MyOne Streptavidin T1 beads (Life technologies, 65602). End repair was performed by resuspending sample-on-beads in 85 µL of 1×NEB T4 DNA ligase buffer with 10 mM ATP, 5 µL of 10 mM dNTP mix, 5 µL of 10U/µL NEB T4 PNK (NEB, M0201), 4 µL of 3 U/µL NEB T4 DNA polymerase I (NEB, M0203), and 1 µL of 5 U/µL NEB DNA polymerase I, Large (Klenow) Fragment (NEB, M0210). Sample was allowed to incubate for 30 mins, magnetized to reclaim beads, and the supernatant discarded. Sample-on-beads were washed twice for two minutes in 600 µL Tween wash buffer at 55°C. Sample-on-beads were resuspended in 90 µL of 1×NEBuffer 2, 5 µL of 10 mM dATP, and 5 µL of 5 U/µL NEB Klenow exo minus (NEB, M0212) and allowed to incubate for 30 min to attach dATP.
Sample-on-beads were reclaimed by magnetization, washed twice for two mins in 600 µL Tween wash buffer at 55°C, then resuspended in 50 µL 1×Quick ligation reaction buffer (NEB, B6058), 2 L of NEB T4 DNA ligase, and 3 µL of NEB Adaptor (1.5 µM, 10 times diluted from NEB E7645S), sample was mixed thoroughly and allowed to incubate for 1 hr at room temperature. Next, 3 µL USER enzyme was added and allowed to incubate 37°C for 15 min. Sample-on-beads were reclaimed by magnetization, washed twice for two mins in 600 µL Tween wash buffer at 55°C, then resuspended in 190 µL PCR mix: 80 µL 1×Tris buffer (pH 8), 100 µL NEB Next Ultra II Q5 MasterMix (NEB, M0544), 10 µL Universal primer (NEB, E7645S; 10 µM). To each sample, 10 µL unique indexed primer from NEB Indexed Primer Set 1 (NEB, E7335; 10 µM) was added.
Library amplification: The Hi-C library for each sample was amplified for 9 cycles directly off of the T1-Streptavidin beads. Library amplification products were size selected to 400-700 bp with AMPure XP beads. Final libraries were resuspended in 25-50 µL of 1×Tris buffer.

Bisulphite conversion efficiency for methylation profiling
Conversion efficiency was assessed by quantitative PCR (qPCR) using the primers below.

Primer sequences:

ACT(+) (for BISC DNA)	F (5'>3')	TGGTGATGGAGGAGGTTTAGTAAGT
	R (5'>3')	AACCAATAAAACCTACTCCTCCCTTAA
ACT(-) (for non-BISC DNA)	F (5'>3')	TGGTGATGGAGGAGGCTCAGCAAGT
	R (5'>3')	AGCCAATGGGACCTGCTCCTCCCTTGA

Chromatin immunoprecipitation with highly parallel sequencing (ChIP-seq)
ChIP-seq was done as previously described (Magnani et al. 2013; Gallo et al. 2015) with some modifications. ChIP for CTCF was performed using an anti-CTCF antibody (Cell Signaling, #3418, Lot #1) at 0.091 µg per reaction. For this ChIP, single-cell suspensions from 2.5 $\times 10^6$ primary GSC cultures were crosslinked with 1% formaldehyde in 1.5 mL tubes for 10 minutes. Sonication was performed with a Bioruptor Standard set to "high" for 30 min with pulses of 30 seconds on and 30 seconds off.
ChIP for H3K27ac was performed with an anti-H3K27ac antibody (Active Motif, #39133, Lot# 31814008) at 1 µg per reaction, based on the protocol by Brind'Amour (Brind'Amour et al. 2015), with the following modifications. For this ChIP, chromatin from 2.5 $\times 10^6$ cells was digested with 200 gel units/µL MNase (NEB, M0247S) for 7.5 min. After immunoprecipitation, washes were performed twice with low-salt buffer (20 mM Tris pH 8.0; 2 mM EDTA; 150 mM NaCl; 1% Triton-X100, 0.1% SDS), twice with high-salt buffer (20 mM Tris pH 8.0; 2 mM EDTA; 500 mM NaCl; 1% Triton-X100, 0.1% SDS), and twice with a lithium chloride RIPA buffer (50 mM HEPES, 1 mM EDTA; 0.7% sodium deoxycholate; 1% IGEPAL; 500 mM LiCl). ChIP DNA was eluted using the Qiagen MinElute kit.
Library preparation for the ChIP samples and sequencing was carried out at the Centre for Health Genomics and Informatics (University of Calgary) with a NextSeq 500 (Illumina) instrument. For each

sample, we generated ~20 million paired-end reads for ChIP and input. We generated and sequenced ChIP libraries from 2 biological replicates for each culture per experiment.

Hi-C data processing

Analysis of Hi-C libraries was performed using Juicer and the associated Juicer Tools: Eigenvector, Pearsons, Arrowhead, HiCCUPS, and MotifFinder (Durand et al. 2016b). Contact maps were generated using Juicer with the following parameters: -s DpnII -g hg38. Contact maps were first generated separately on each of two biological replicates. Inter-replicate contact map Pearson correlation values were calculated on the output of 'juicer_tools dump observed <chrName> <chrName> NONE BP 10000' for each intrachromosomal contact map. After finding inter-replicate Pearson correlation scores greater than 0.9 for each chromosome, biological replicates were then merged to maximize map resolution.

Map resolution was determined by using Juicer's "calculate_map_resolution.sh" script, modified to account for the genome size of the hg38 annotation. Contact domains were called using Arrowhead at resolutions of 5, 10, 25, 50, and 100 kb. DNA loops were called using HiCCUPS with the following parameters: -r 5000,10000,25000,50000,100000 -f 0.1,0.1,0.1,0.1,0.1 -p 4,2,1,2,1 -i 7,5,3,4,2 -d 20000,20000,50000,100000,200000. These parameters incorporate both HiCCUPS default parameters for 5, 10, and 25 kb resolution, as well as modifications for long distance loops at 50 and 100 kb resolution (Rao et al. 2017). CTCF motifs were assigned to DNA loop anchors using MotifFinder. Hi-C contact maps and associated annotations were visualized using Juicebox (Durand et al. 2016a) and the WashU Epigenome Browser (Zhou et al. 2013). The hg38 reference genome was used for all data.

The Juicer Tools Eigenvector method was unable to assign type A/B nuclear compartmentalization for certain chromosomes as the eigenvector generated corresponds only to the first principal component of the Pearson correlation matrix, and translocations and amplifications present in our cell cultures often strongly influenced the first principal component rather than the desired compartment status. Therefore, we performed principal component analysis in R using the Pearson correlation matrix produced by Juicer Tools Pearsons at 50-kb resolution to access additional principal components and their associated eigenvalues. We examined eigenvalues of the first four principal components for each chromosome and excluded those associated with genome aberrations or chromosome arm to identify the component associated with compartmentalization. Eigenvalues were then flipped such that positive eigenvalues had superior overlap with H3K27ac ChIP as determined by the similarity score of BEDTools jaccard (Quinlan and Hall 2010). Positive eigenvalues were then associated with type A compartmentalization while negative eigenvalues with type B compartments.

For comparing loop strength, the 'observed' value of HiCCUPs loop call output was used. This value corresponds to the raw observed counts at the peak pixel.

ChIP-seq data processing

Quality of the sequencing run was validated using FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc) and FastQ Screen (https://www.bioinformatics.babraham.ac.uk/projects/fastq_screen/). Sequenced reads were aligned to the hg38 genome using BWA-MEM (Li and Durbin 2009) with default parameters to generate SAM files. Alignments were then filtered using SAMtools (Li et al. 2009) to remove alignments to (1) alternative contigs, unlocalized sequence, or unplaced sequence by excluding chromosomes with names containing "_alt", "random", or "chrUn;" (2) to the hg38 blacklist of high-signal artifact regions (ENCODE consortium, 2012) (3) with mapping quality (MAPQ) < 30.

MACS2 callpeak (Zhang et al. 2008) was used to call ChIP-seq peaks from the filtered alignments using the following parameters: -f BAMPE -g hs -q 0.05 -B --SPMR. For H3K27ac, but not CTCF, the parameter "--broad" was included to call broad peaks instead of narrow.
We identified super-enhancer regions from our H3K27ac data using ROSE (Whyte et al. 2013; Lovén et al. 2013) with the following parameters: -s 6000 -t 2500.
Following ChIP-seq peak and super-enhancer calls, inter-replicate Pearson correlation scores were calculated and replicates with correlation > 0.85 were merged using BEDTools merge (Quinlan and Hall 2010) to produce a single bed file for downstream analysis and data display.

SUPPLEMENTAL REFERENCES

Brind'Amour J, Liu S, Hudson M, Chen C, Karimi MM, Lorincz MC. 2015. An ultra-low-input native ChIP-seq protocol for genome-wide profiling of rare cell populations. *Nat Commun* **6**: 6033.

Durand NC, Robinson JT, Shamim MS, Machol I, Mesirov JP, Lander ES, Aiden EL. 2016a. Juicebox Provides a Visualization System for Hi-C Contact Maps with Unlimited Zoom. *Cell Syst* **3**: 99–101.

Durand NC, Shamim MS, Machol I, Rao SSP, Huntley MH, Lander ES, Aiden EL. 2016b. Juicer Provides a One-Click System for Analyzing Loop-Resolution Hi-C Experiments. *Cell Syst* **3**: 95–98.

Gallo M, Coutinho FJ, Vanner RJ, Gayden T, Mack SC, Murison A, Remke M, Li R, Takayama N, Desai K, et al. 2015. MLL5 Orchestrates a Cancer Self-Renewal State by Repressing the Histone Variant H3.3 and Globally Reorganizing Chromatin. *Cancer Cell* **28**: 715–729.

Li H, Durbin R. 2009. Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics* **25**: 1754–1760.

Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin R. 2009. The Sequence Alignment/Map format and SAMtools. *Bioinformatics* **25**: 2078–2079.

Lovén J, Hoke HA, Lin CY, Lau A, Orlando DA, Vakoc CR, Bradner JE, Lee TI, Young RA. 2013. Selective inhibition of tumor oncogenes by disruption of super-enhancers. *Cell* **153**: 320–334.

Magnani L, Stoeck A, Zhang X, Lanczky A, Mirabella AC, Wang T-L, Gyorffy B, Lupien M. 2013. Genome-wide reprogramming of the chromatin landscape underlies endocrine therapy resistance in breast cancer. *Proc Natl Acad Sci* **110**: E1490-9.

Quinlan AR, Hall IM. 2010. BEDTools: A flexible suite of utilities for comparing genomic features. *Bioinformatics* **26**: 841–842.

Rao SSP, Huang SC, Glenn St Hilaire B, Engreitz JM, Perez EM, Kieffer-Kwon KR, Sanborn AL, Johnstone SE, Bascom GD, Bochkov ID, et al. 2017. Cohesin Loss Eliminates All Loop Domains. *Cell* **171**: 305–320.e24.

Rao SSP, Huntley MH, Durand NC, Stamenova EK, Bochkov ID, Robinson JT, Sanborn AL, Machol I, Omer AD, Lander ES, et al. 2014. A 3D map of the human genome at kilobase resolution reveals principles of chromatin looping. *Cell* **159**: 1665–1680.

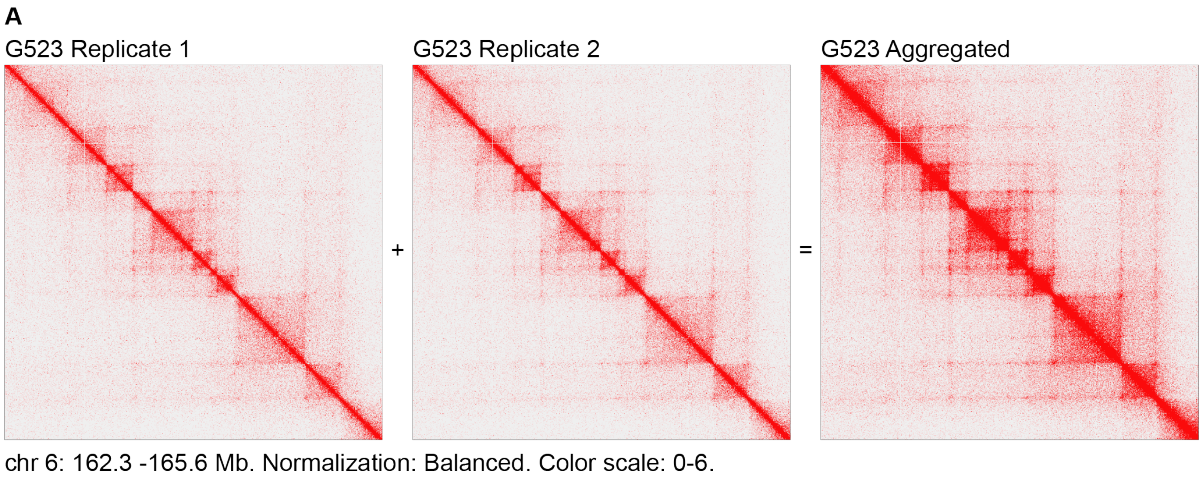
Whyte WA, Orlando DA, Hnisz D, Abraham BJ, Lin CY, Kagey MH, Rahl PB, Lee TI, Young RA. 2013. Master transcription factors and mediator establish super-enhancers at key cell identity genes. *Cell* **153**: 307–319.

Zhang Y, Liu T, Meyer CA, Eeckhoute J, Johnson DS, Bernstein BE, Nussbaum C, Myers RM, Brown M, Li W, et al. 2008. Model-based analysis of ChIP-Seq (MACS). *Genome Biol* **9**: R137.

Zhou X, Lowdon RF, Li D, Lawson HA, Madden PAF, Costello JF, Wang T. 2013. Exploring long-range genome interactions using the WashU Epigenome Browser. *Nat Methods* **10**: 375–376.

SUPPLEMENTAL FIGURES

FigureS1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G523 Replicate 2
G523 Aggregated
+
=
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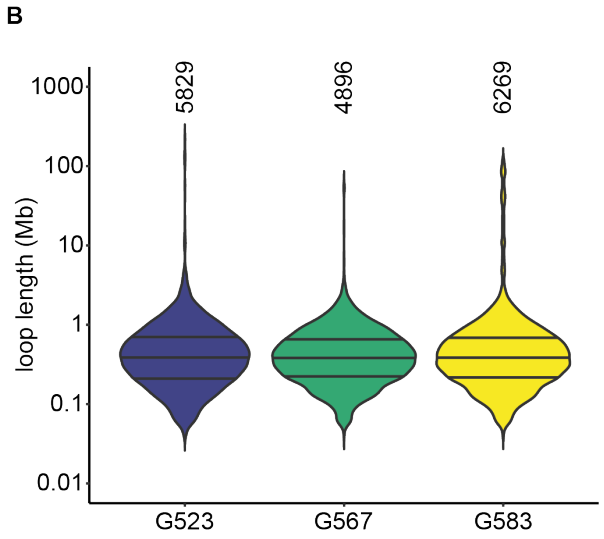
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10
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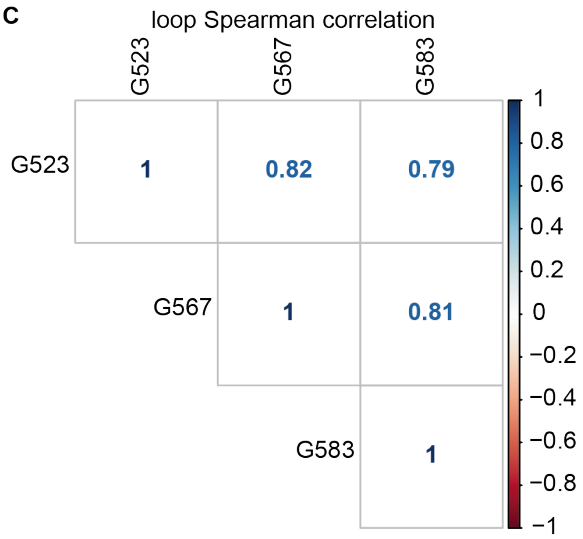
0.1
0.01
5829
4896
6269
G523
G567
G583


C
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G523
G567
G583
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0.82
0.79
1
0.81
1
1
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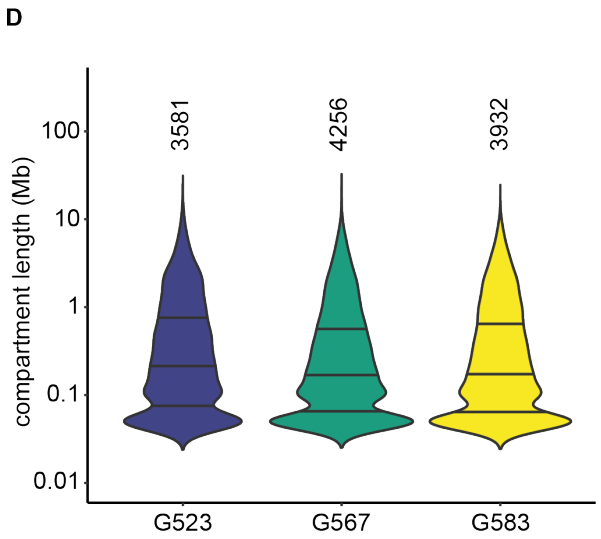
0.6
0.4
0.2
0
−0.2
−0.4
−0.6
−0.8
−1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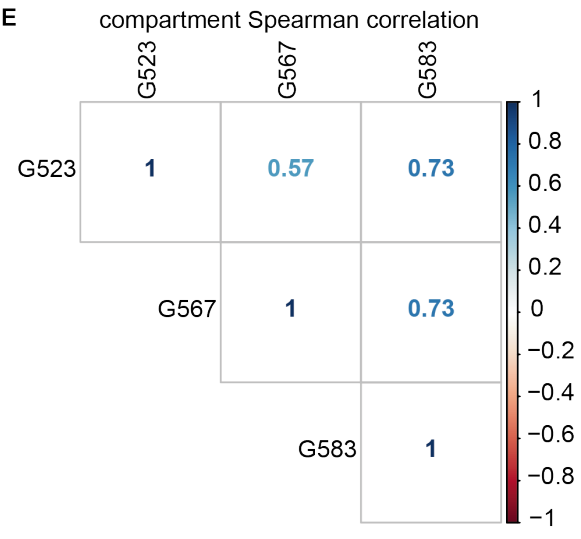
1
0.1
0.01
3581
4256
3932
G523
G567
G583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G523
G567
G583
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1
0.73
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0.2
0
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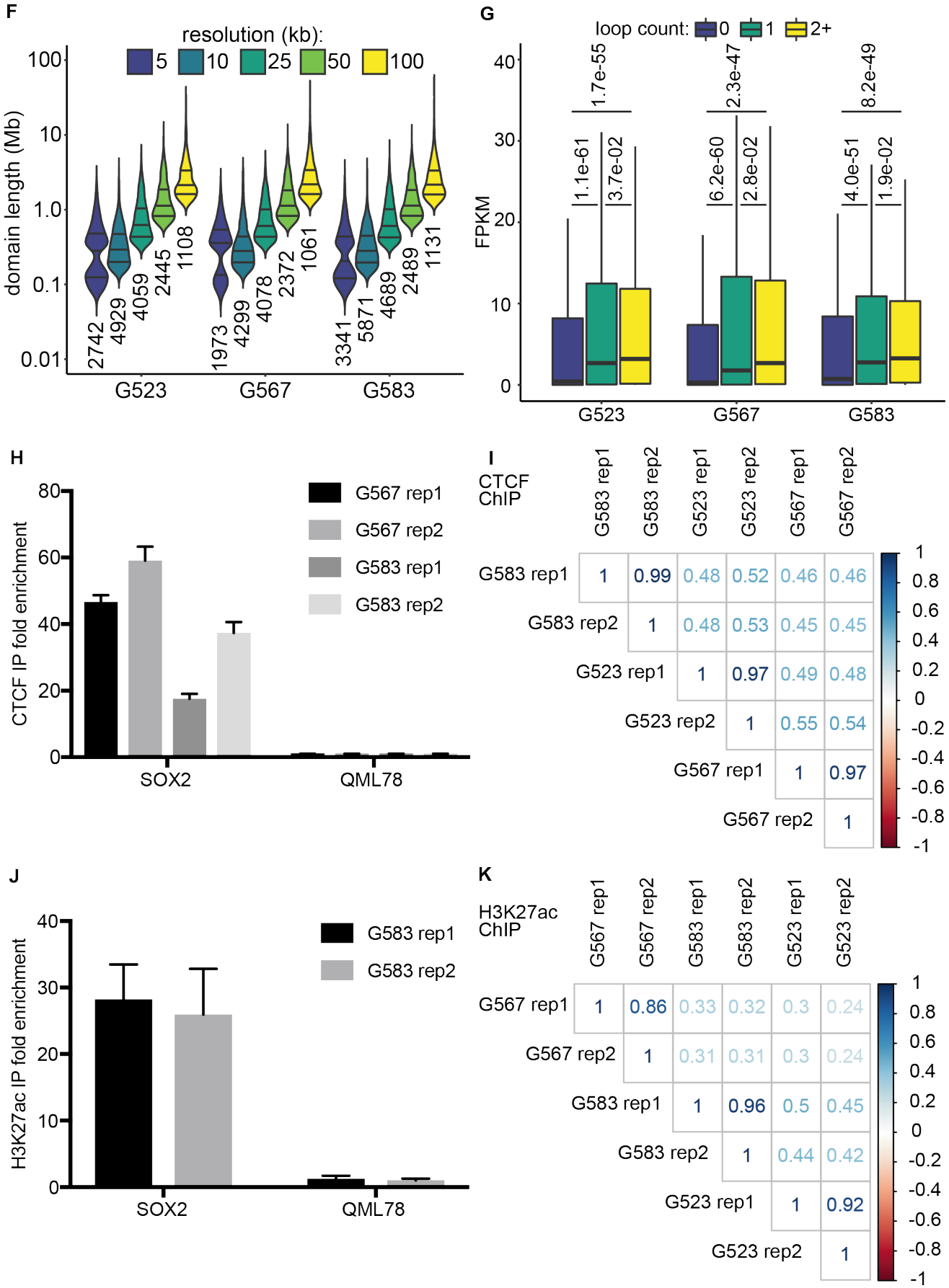
−0.8
−1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10
25
50
100
domain length (Mb)
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0.1
0.01
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4059
2445
1108
1973
4299
4078
2372
1061
3341
5871
4689
2489
1131
G523
G567
G583
G
loop count:
0
1
2+
FPKM
40
30
20
10
0
1.7e-55
1.1e-61
3.7e-02
2.3e-47
6.2e-60
2.8e-02
8.2e-49
4.0e-51
1.9e-02
G523
G567
G583
H
CTCF IP fold enrichment
80
60
40
20
0
G567 rep1
G567 rep2
G583 rep1
G583 rep2
SOX2
QML78
I
CTCF ChIP
G583 rep1
G583 rep2
G523 rep1
G523 rep2
G567 rep1
G567 rep2
G583 rep1 1 0.99 0.48 0.52 0.46 0.46
G583 rep2 1 0.48 0.53 0.45 0.45
G523 rep1 1 0.97 0.49 0.48
G523 rep2 1 0.55 0.54
G567 rep1 1 0.97
G567 rep2 1
1
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
-1
J
H3K27ac IP fold enrichment
40
30
20
10
0
G583 rep1
G583 rep2
SOX2
QML78
K
H3K27ac ChIP
G567 rep1
G567 rep2
G583 rep1
G583 rep2
G523 rep1
G523 rep2
G567 rep1 1 0.86 0.33 0.32 0.3 0.24
G567 rep2 1 0.31 0.31 0.3 0.24
G583 rep1 1 0.96 0.5 0.45
G583 rep2 1 0.44 0.42
G523 rep1 1 0.92
G523 rep2 1
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0.8
0.6
0.4
0.2
0
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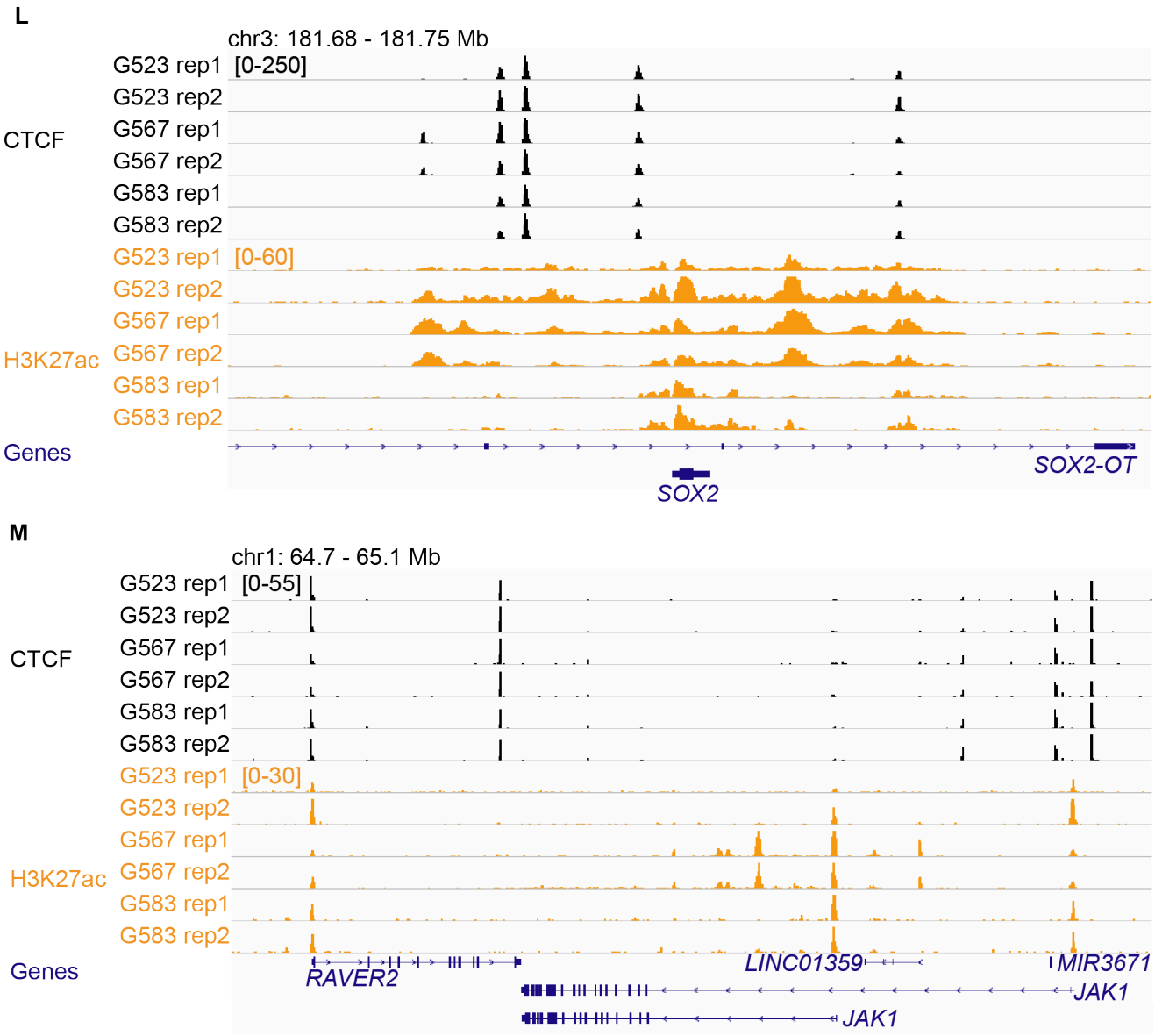
-0.4
-0.6
-0.8
-1


L
chr3: 181.68 - 181.75 Mb
G523 rep1 [0-250]
G523 rep2
G567 rep1
G567 rep2
G583 rep1
G583 rep2
CTCF
G523 rep1 [0-60]
G523 rep2
G567 rep1
G567 rep2
G583 rep1
G583 rep2
H3K27ac
Genes
SOX2-OT
SOX2
M
chr1: 64.7 - 65.1 Mb
G523 rep1 [0-55]
G523 rep2
G567 rep1
G567 rep2
G583 rep1
G583 rep2
CTCF
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G567 rep2
G583 rep1
G583 rep2
H3K27ac
Genes
RAVER2
LINC01359
MIR3671
JAK1
JAK1
N
FPKM
60
40
20
0
4.0e-19
1.0e-89
5.8e-07
3.5e-34
1.8e-70
1.1e-06
3.0e-13
2.0e-76
2.1e-03
G523
G567
G583
loop type:
no loop
loop w/o SE
loop w/ SE

Figure S1. DNA contact and epigenomic profiles of GSCs.
(A) Biological replicates of Hi-C contact maps. After finding inter-replicate Pearson correlation scores greater than 0.9 for 10-kb contact maps of each chromosome, biological replicates were then merged to maximize map resolution.
(B) Loop length by culture. Total number of loops is above the corresponding violin plot. Horizontal lines in each violin plot represent quartiles. Loops called by HiCCUPS as 5-kb to 100-kb resolution merged loops throughout this figure.
(C) Spearman correlation of loop calls between cultures.
(D) Compartment length by culture. Total number of compartments is above the corresponding violin plot. Horizontal lines in each violin plot represent quartiles. Compartmentalization called at 50-kb resolution throughout this figure.
(E) Spearman correlation of compartmentalization between cultures.
(F) Domain lengths by culture. Domains were called at resolutions ranging from 5-kb to 100-kb. Total number of domains called at each resolution is on beneath each violin plot. Horizontal lines in each violin plot represent quartiles.
(G) Gene expression separated by the number of loops contacting each gene. Genes engaged by a loop are more highly expressed than genes without loops. Engaging more than one loop further increases expression. Whiskers indicate 1.5× interquartile range. P values calculated by Wilcoxon rank-sum test.
(H) Fold enrichment of CTCF signal as determined by ChIP qPCR at the actively transcribed locus *SOX2* as compared to the negative control locus *QML78*. Error bars represent standard deviation.
(I) Pearson correlation scores for CTCF ChIP-seq calls between samples.
(J) Fold enrichment of H3K27ac signal as determined by ChIP qPCR at the actively transcribed locus *SOX2* as compared to the negative control locus *QML78*. Error bars represent standard deviation.
(K) Pearson correlation scores for H3K27ac ChIP-seq calls between samples.
(L-M) Fold enrichment tracks for replicate ChIP-seq data at the *SOX2* (L) and *JAK1* (M) example loci.
(N) Gene expression by loop type. Genes contacting a loop exhibit significantly elevated expression, however looping to an SE elevates expression even further. Whiskers indicate 1.5× interquartile range. P values calculated by Wilcoxon rank-sum test.

FigureS2

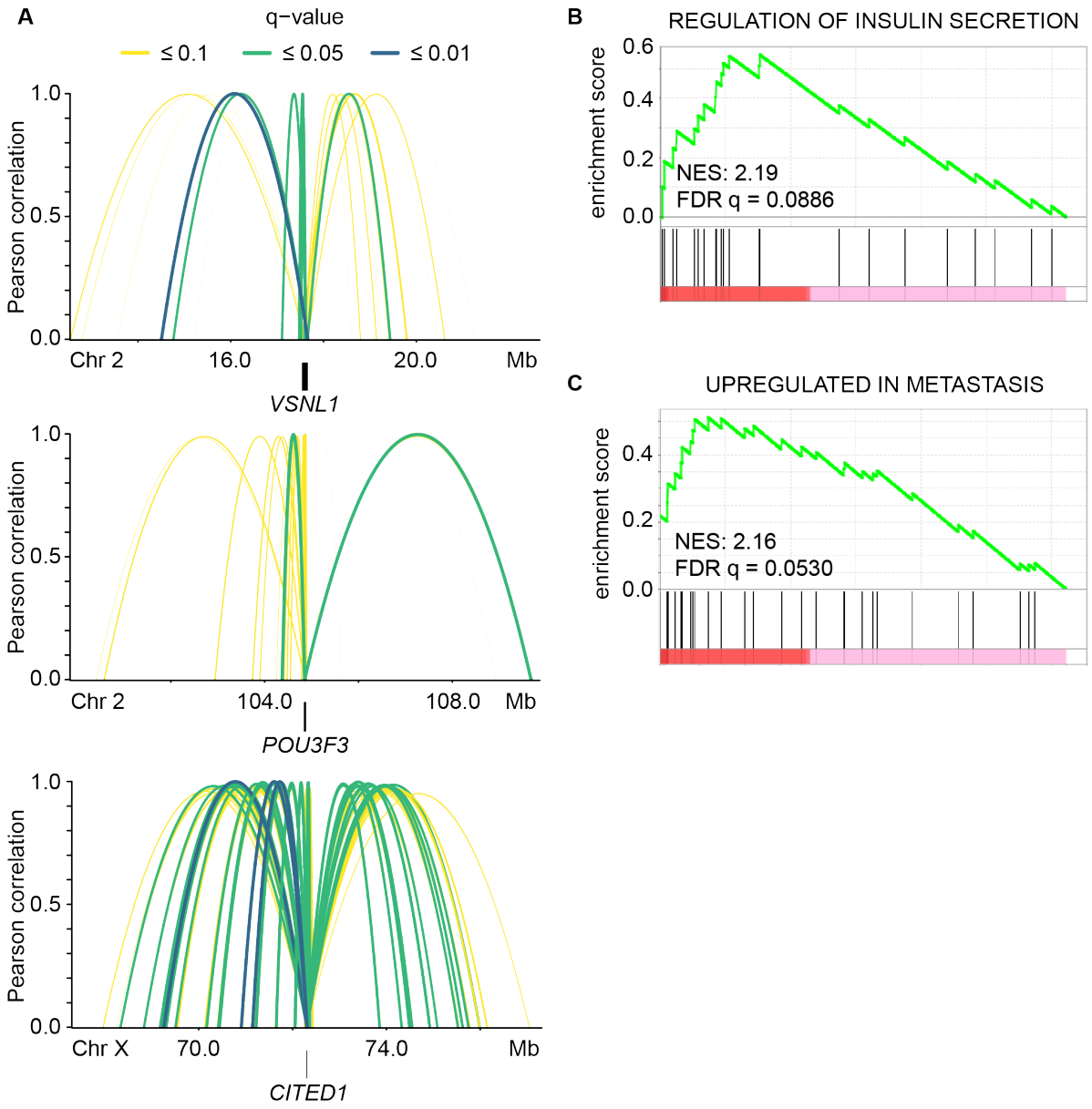

Figure S2. GSEA enrichment among highly-connected loops.
(A-B) GSEA among highly connected loops indicates enrichment for "regulation of insulin secretion" and "upregulated in metastasis". All loops called by HiCCUPS as 5-kb to 100-kb resolution merged loops.

FigureS3

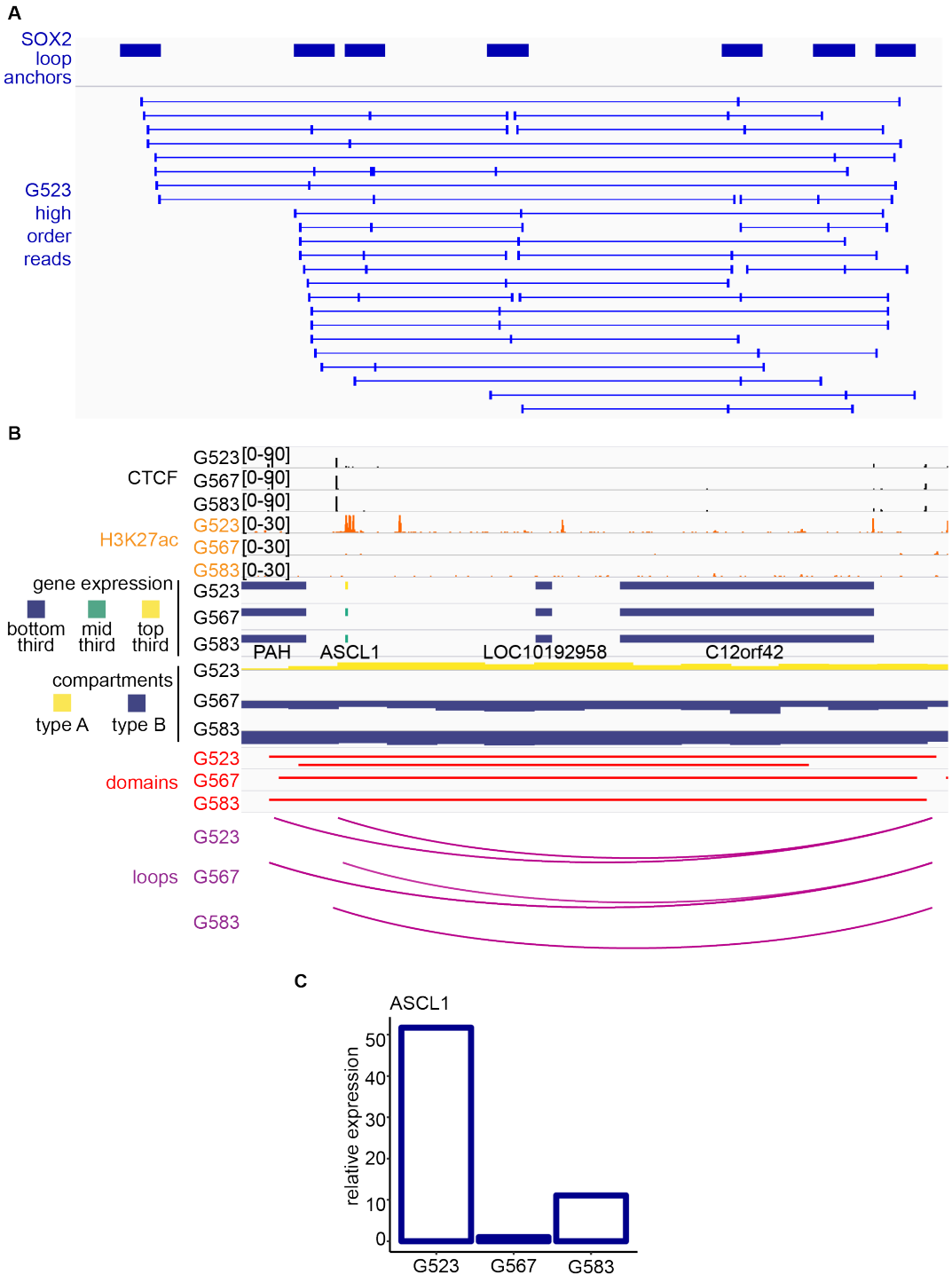
A
SOX2
loop
anchors
G523
high
order
reads
B
CTCF
H3K27ac
gene expression
bottom
third
mid
third
top
third
compartments
type A
type B
domains
loops
G523
G567
G583
[0-90]
[0-30]
PAH
ASCL1
LOC10192958
C12orf42
C
ASCL1
relative expression
50
40
30
20
10
0
G523
G567
G583

Figure S3. Culture-specific looping and compartmentalization affect transcription of genes regulating stem cell properties.
(A) Higher-order reads detected at the SOX2 locus in G523.
(B) Integration of genome browser tracks for CTCF ChIP-seq, H3K27ac ChIP-seq, RNA-seq, compartments, domains, and loops determined by Hi-C at the *ASCL1* locus. Compartmentalization called at 50-kb, domains at 10-kb, and loops as the union of 5-kb, 10-kb, and 25-kb calls throughout this figure.
(C) Relative expression of *ASCL1* as determined by RNA-seq.

FigureS4

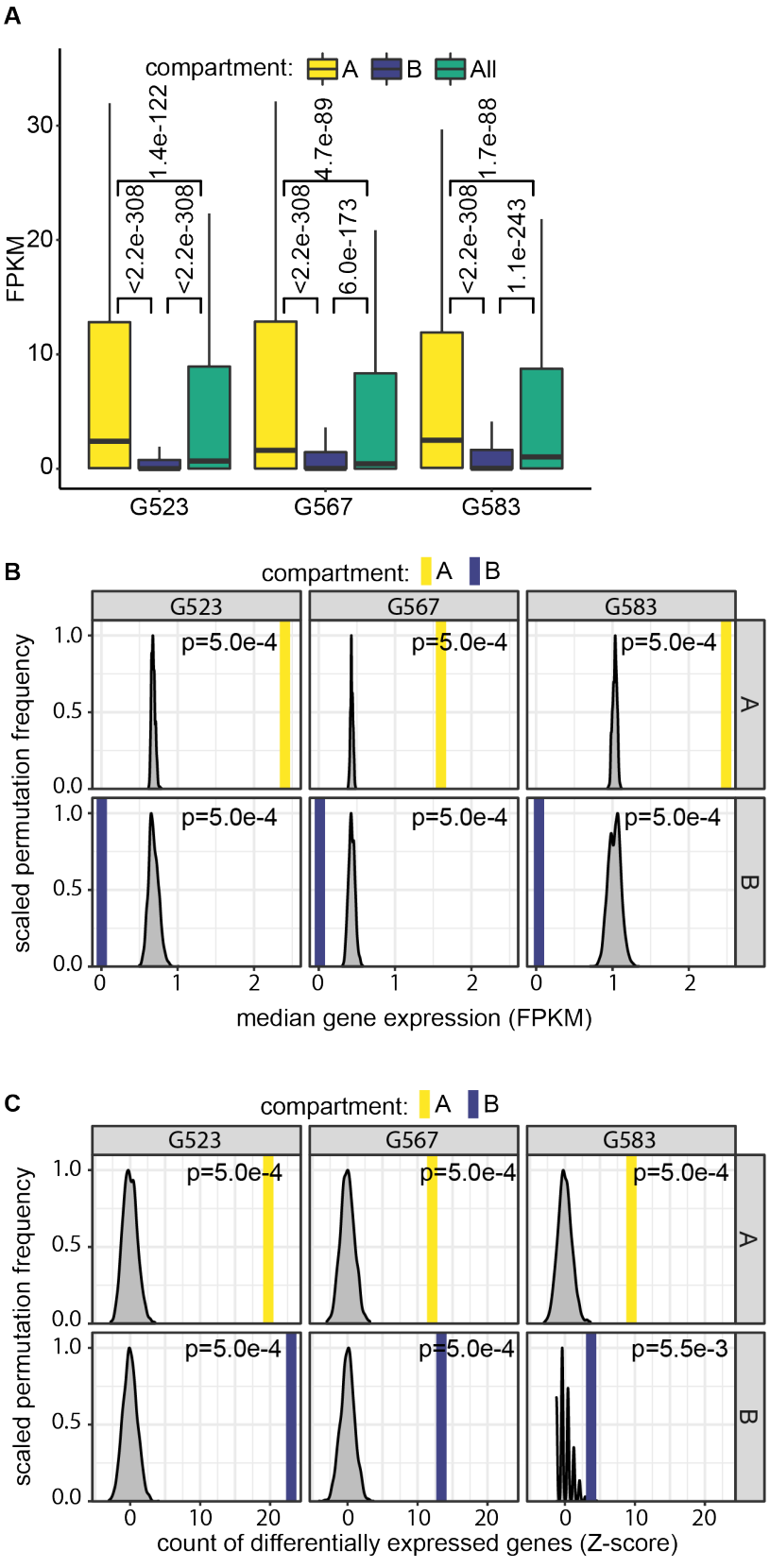
A
compartment: A B All
FPKM
30
20
10
0
<2.2e-308
1.4e-122
<2.2e-308
<2.2e-308
4.7e-89
6.0e-173
<2.2e-308
1.7e-88
1.1e-243
G523
G567
G583
B
compartment: A B
G523
G567
G583
scaled permutation frequency
p=5.0e-4
A
B
median gene expression (FPKM)
C
compartment: A B
p=5.0e-4
p=5.5e-3
count of differentially expressed genes (Z-score)

Figure S4. Differential expression of genes in different compartments.
(A) Gene expression by compartment. Genes with type A compartmentalization are more highly expressed than genes with type B compartmentalization. The set of All Genes independent of compartment status is provided for comparison. Whiskers indicate 1.5× interquartile range. P values calculated by Wilcoxon rank-sum test. Compartmentalization called at 50-kb resolution throughout this figure.
(B) Median gene expression is elevated in the type A compartment and repressed in the type B compartment. Grey curve: Median gene expression as determined by 2000 permutations of randomly sampled expression values from the set of all genes. Vertical bars: Measured median expression by compartment and culture.
(C) Differentially expressed genes are enriched at loci with culture-specific compartmentalization. Grey curve: Frequency of detecting differentially expressed genes as determined by 2000 permutations of randomly sampled expression values from genes independent of compartment status. Note that G583 type B compartment distribution is not smooth due to the low number of culture-specific compartment B genes in this sample. Vertical bars: Measured count of differentially expressed genes by compartment and by culture.

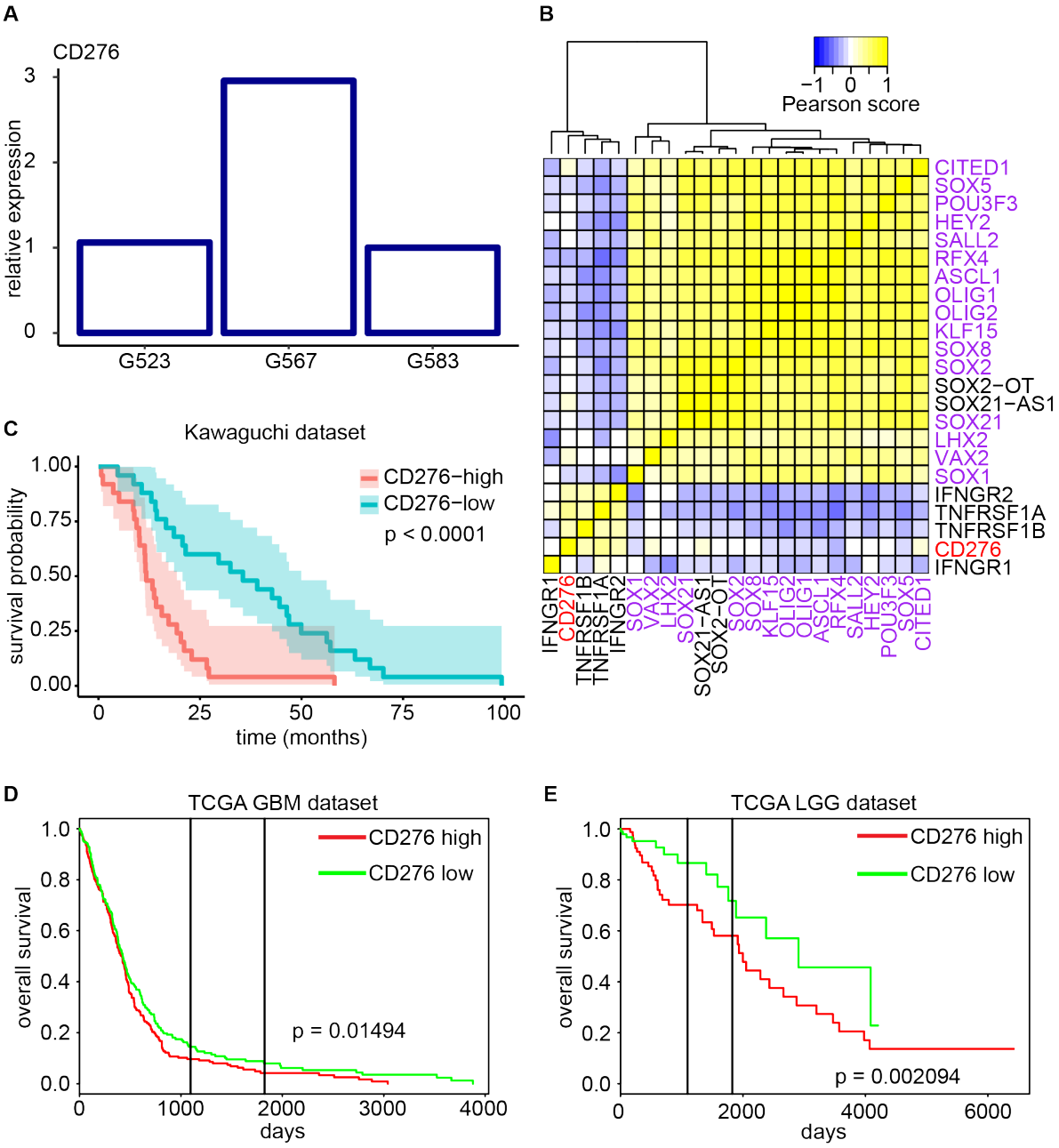

Figure S5. Type A compartments in GBM cells have enriched expression of immune-related genes.
(A) Relative differences in *CD276* expression by RNA-seq.
(B) Pearson correlation score for *CD276*, a previously published stemness signature, and immune-related genes in GSCs. Purple highlights stemness genes. Correlation scores were generated with bulk RNA-seq data from patient-derived GSC cultures (n = 76).
(C) Survival of glioma patients stratified by *CD276* expression in the Kawaguchi dataset. Median gene expression was used to stratify patients. P-value was derived with log-rank statistics. Shading around curve indicates 95% confidence interval.

(D) Stratification of GBM patients in the TCGA cohort by average *CD276* expression. Statistical significance was tested with the log-rank test.
(E) Stratification of low grade glioma (LGG) patients in the TCGA cohort by average *CD276* expression. Statistical significance was tested with the log-rank test.

FigureS6

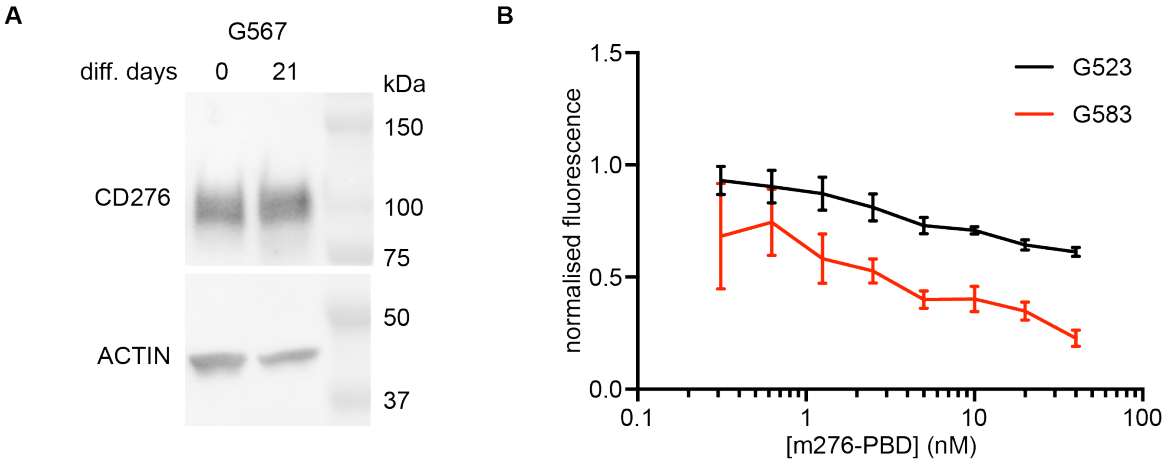

Figure S6. *CD276* expression is associated with GSC self-renewal and poor patient prognosis.
(A) Western blot comparing CD276 levels in culture G567 after exposure to limited growth factors.
(B) Cell viability assay to test the effect of m276-PBD, an antibody-drug conjugate that targets CD276, on patient-derived GSC cultures G523 and G583. Error bars represent standard deviation.