SUPPLEMENTAL METHODS

Generation of Hi-C libraries

Hi-C analysis was performed as described previously (Belton et al. 2012), with the minor modifications described below. A total of 3-5 million cells were pelleted and resuspended in 10 ml of 1× PBS (10 mM phosphate buffer, 2.7 mM KCl 137 mM NaCl, pH7.4) and fixed with 1% formaldehyde (Sigma) for 10 min at room temperature. The reaction was stopped by adding 2 M glycine to a final concentration of 125 mM. The cell suspension was incubated on ice for 5 min, and the cells were pelleted (10 min at 1000 x g in a pre-chilled (4°C) centrifuge). The supernatant was removed, and the cell pellet was washed with 500 μl of 1× PBS, frozen and, if necessary, stored in a liquid nitrogen. After thawing, the cells were lysed for 15 min in 1.5 ml of ice-cold lysis buffer (50 mM Tris-HCl (pH8.0), 150 mM NaCl, 0.5% (v/v) NP-40, 1% (v/v) Triton-X100, 1× Protease Inhibitor Cocktail (CALBIOCHEM #539137 of Thermo Scientific #78430)). The nuclei were harvested at 5000 x g for 7 min in a pre-chilled centrifuge, washed twice with 100 μl of 1.2× restriction NEBuffer 2 (NEB) and resuspended in 200 μl of 1.2× NEBuffer 2. Then, 20% SDS was added to a final concentration of 0.3%, and the nuclei were incubated at 37°C for 1 h with constant shaking. After incubation, 330 μl of 1.2× NEBuffer 2 was added, 20% Triton X-100 was added to a final concentration of 1.8%, and the samples were incubated for 1 h under the same conditions. DNA was digested overnight with 600-800 U of 100 U/μl HindIII-HF restriction endonuclease (NEB) at 37°C with shaking. The next morning, 200 U of HindIII-HF were added, and the samples were incubated for 2 h under the same conditions. The nuclei were harvested for 10 min at 5000 x g and 25°C and resuspended in 50 μl of the collected supernatant. Cohesive DNA ends were biotinylated by adding 7.6 μl of the biotin fill-in mixture prepared in 1× NEBuffer 2 (0.025 mM dATP (Thermo Scientific), 0.025 mM dGTP (Thermo Scientific), 0.025 mM dTTP (Thermo Scientific), 0.025 mM biotin-14-dCTP (Invitrogen), 0.8 U/μl Klenow enzyme (NEB)). The samples were incubated at 37°C for 75 min with slow shaking, and 20% SDS was added to a final concentration of 1.8%; the samples were heated at 65°C for 20 min to inactivate the Klenow enzyme and residual HindIII-HF. The samples were transferred into 10-ml tubes with 3 ml of 1× T4 DNA ligase reaction buffer (Thermo Scientific) supplied with 1.8% Triton X-100, incubated at 37°C for 1 h and then cooled on ice. Next, 100 U of T4 DNA ligase (Thermo Scientific) was added, and the DNA was ligated for 6 h at 22°C with slow agitation. The cross-links were reversed by overnight incubation at 65°C in the presence of proteinase K (100 μg/ml). After cross-link reversal, the DNA was purified by single phenol-chloroform extraction followed by ethanol precipitation (tRNA (Sigma) at a concentration of 7.5 μg/ml and glycogen (Thermo Scientific) at a concentration of 20 μg/ml were used as co-precipitators due to the low amount of DNA in the samples).
precipitation, the pellets were dissolved in 500 μl 10 mM Tris-HCl pH 8.0. To remove residual salts and DTT, the DNA was additionally purified using AMICON Ultra Centrifugal Filter Units (0.5 ml, 30 K, Millipore #UFC5030BK) by washing with 10 mM Tris-HCl pH 8.0 (after two washing steps, the final volume of the DNA solution was 40-50 μl). Biotinylated nucleotides from the non-ligated DNA ends were removed by incubating the Hi-C libraries in the presence of 1.5 U of T4 DNA polymerase (NEB) in NEBuffer 2 supplied with 0.025 mM dATP and 0.025 mM dGTP at 20°C for 4 h. Next, the DNA was purified by phenol-chloroform extraction followed by ethanol precipitation. The DNA pellets were dissolved in 500 μl of sonication buffer (50 mM Tris-HCl (pH 8.0), 10 mM EDTA, 0.1% SDS) and treated with 50 μg of RNase A (Thermo Scientific) for 45 min at 37°C; the DNA was then sheared to a size of approximately 100-1000 bp using a VirSonic 100 (VerTis). The samples were concentrated (and simultaneously purified) using AMICON Ultra Centrifugal Filter Units. The fraction of 100-500-bp DNA fragments was captured using Argencourt AMPure XP beads (Beckman Coulter), and the fragments were eluted with 50 μl of 10 mM Tris-HCl (pH 8.0). The DNA ends were repaired by adding 62.5 μl MQ water, 14 μl of 10× T4 DNA ligase reaction buffer, 3.5 μl of 10 mM dNTP mix, 5 μl of 3 U/μl T4 DNA polymerase (NEB), 5 μl of 10 U/μl T4 polynucleotide kinase (NEB), and 1 μl of 5 U/μl Klenow DNA polymerase (NEB) and incubating at 20°C for 30 min. The DNA was purified with Agencourt AMPure XP beads and eluted with 50 μl of 10 mM Tris-HCl (pH 8.0). To perform an A-tailing reaction, the DNA samples were supplemented with 6 μl 10× NEBuffer 2, 1.2 μl of 10 mM dATP, 1 μl of MQ water and 3.6 μl of 5 U/μl Klenow (exo-) (NEB). The reactions were carried out for 30 min at 37°C in a PCR machine, and the enzyme was then heat-inactivated by incubation at 65°C for 20 min. The DNA was purified using Agencourt AMPure XP beads and eluted with 100 μl of 10 mM Tris-HCl (pH 8.0). Biotin pull-down of the ligation junctions was performed as described previously, with minor modifications. Briefly, 4 μl of MyOne Dynabeads Streptavidin C1 (Invitrogen) beads were used to capture the biotinylated DNA, and the volumes of all buffers were decreased by 4-fold. The washed beads with captured ligation junctions were resuspended in 50 μl of adapter ligation mixture composed of 41.5 μl MQ water, 5 μl 10× T4 DNA ligase reaction buffer (Thermo Scientific), 2.5 μl of Illumina TruSeq adapters and 1 μl of 5 U/μl T4 DNA ligase (Thermo Scientific). Adapter ligation was performed at 22°C for 2.5 h, and the beads were sequentially washed twice with 100 μl of TWB (5 mM Tris-HCl (pH8.0), 0.5 mM EDTA, 1 M NaCl, 0.05% Tween-20), once with 100 μl of 1× binding buffer (10 mM Tris-HCl (pH8.0), 1 mM EDTA, 2 M NaCl) and once with 100 μl of CWB (10 mM Tris-HCl (pH 8.0) and 50 mM NaCl) and then resuspended in 20 μl of MQ water. Test PCR reactions containing 4 μl of the streptavidin-bound Hi-C library were performed to determine the optimal number of PCR cycles needed to generate enough PCR
products for sequencing. The PCR reactions (volume of each reaction is 25 μl) were performed using KAPA High Fidelity DNA Polymerase (KAPA) and Illumina PE1.0 and PE2.0 PCR primers (10 pmol each). The temperature profile was 5 min at 98°C, followed by 6, 9, 12, 15 and 18 cycles of 20 s at 98°C, 15 s at 65°C, and 20 s at 72°C. The PCR reactions were separated on a 2% agarose gel supplied with ethidium bromide, and the number of PCR cycles necessary to obtain a sufficient amount of DNA was determined based on the visual inspection of gels (typically 12-15 cycles). Four preparative PCR reactions were performed for each sample. The PCR mixtures were combined, and the products were purified with QIAGEN PCR Purification Kit. The DNA was eluted with 50 μl of 10 mM Tris-HCl (pH 8.0) and separated on a 1.8% agarose gel supplied with ethidium bromide; 200-600-bp fragments were excised from the gel, purified using QIAGEN Gel Extraction Kit and sequenced with an Illumina HiSeq 2000 by paired-end 101-nt reads.
SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure S1. (A) Heatmaps of biological replicates of Hi-C experiments (chromosome 3L is shown). Resolution of heatmaps is 20 kb. (B) Graph of contact probability as a function of genomic distance.

Supplemental Figure S2. Hi-C interaction maps (heatmaps) of the four studied cell lines at 20-kb resolution.

Supplemental Figure S3. Visual annotation of long-range interarm (A) and interchromosomal (B) contacts of Polycomb-occupied TADs in BG3 cell line (highlighted by red circles; white circles mark interactions between Polycomb-occupied TADs located on the same chromosomal arm). Resolution of the heatmaps is 50 kb, and resolution of TAD images under the maps is 20 kb.

Supplemental Figure S4. Distributions of TAD lengths (excluding boundary bins) and inter-TAD lengths (including TAD boundaries) in the four studied cell lines.

Supplemental Figure S5. (A) Distribution of chromatin colors near TAD boundaries. Number of bins in each group is shown above the diagram. All designations are as in Fig. 2B. (B) Diagram showing the inverse dependence between the proportion of active chromatin colors within a genomic bin and \( \gamma \) (the minimal value of the scaling parameter gamma required to annotate a bin as a TAD boundary or an inter-TAD). Numbers of bins in each group are shown above the diagram (for example, 240 bins were annotated as inter-TADs at gamma values of 0–0.1 (excluding \( \gamma = 0.1 \)), 17 bins were annotated as inter-TADs at gamma values 0.1–0.2 (excluding \( \gamma = 0.2 \)). Bins that have not been annotated as TAD boundaries or inter-TADs at gamma values from 0 to 10 (excluding \( \gamma = 10 \)) are highlighted in red. All other designations are as in Fig. 3B. P-values (two-sided Mann-Whitney test) are presented in Supplemental Table S6.

Supplemental Figure S6. Boxplots showing distributions of individual chromatin marks and binding proteins near TAD boundaries, some of them were used for the construction of panel (C) in Figure 2. P-values (two-sided Mann-Whitney test) are presented in Supplemental Table S6.
**Supplemental Figure S7.** The observed representation of dCTCF at TAD boundaries and inter-TADs does not depend on the gamma value used for TAD annotation. The distribution of dCTCF, H3K4me3 and Su(Hw) around TAD boundaries at different values of the scaling parameter gamma used for TAD annotation is shown. Curves smoothed with LOESS show the median Z-transformed values. Thick rectangles show TAD boundary bins.

**Supplemental Figure S8.** Principal components analysis (A) and cluster analysis (B) of biological replicates of poly(A)+ RNA-seq of the cell lines. (C) The level of polyA(+) transcripts around TAD boundaries in the Kc167, OSC and S2 cells (extension of Fig. 3A). (D) Boxplots showing inverse dependence between the transcription level within a genomic bin and $\gamma t$ in the S2 and Kc167 cells (extension of Fig. 3D).

**Supplemental Figure S9.** Scatter plots demonstrating transcription level and the proportion of active chromatin colors in individual TADs excluding boundary bins (orange spots), and inter-TADs including boundary bins (blue spots), in BG3, S2 and Kc167 cells. Horizontal lines separate inter-TADs by the 20% quintile according to the transcription level; TADs were also separated according to this level. Vertical lines separate inter-TADs (left panels) and TADs (right panels) by a threshold of 30% of active chromatin within inter-TADs and TADs, respectively (sum of 1, 2 and 3 colors for the BG3 and S2 cells, and RED and YELLOW for the Kc167 cells). Other designations are as in Fig. 3B.

**Supplemental Figure S10.** Pie charts showing the distribution of differentially or uniformly transcribed bins (A) and bins with different proportions of active chromatin (B) in the four groups, as defined by a pairwise comparison of the cell lines (extension of Fig. 4B).

**Supplemental Figure S11.** Scatter plots illustrating independent variations in proportions of active or repressed chromatin within the interior and boundaries of conserved TADs, as observed in a pairwise comparison of BG3 and S2 cell lines.

**Supplemental Figure S12.** Scatter plots demonstrating the proportion of interband-specific CYAN and BLUE chromatin colors (Zhimulev et al. 2014) in TADs and inter-TADs of different sizes in the BG3 and S2 cells.

**Supplemental Figure S13.** Results of 12 individual realizations of our polymer model, similar to Fig 6. Distances are measured in numbers of monomer units (nucleosomes).
**Supplemental Figure S14.** Predicted spatial configuration of a polymer composed of equal blocks of acetylated (green) and non-acetylated (black) nucleosomes. Each block consists of 500 nucleosomes. (B) Spatial proximity map (distance heatmap) of the polymer configuration presented in (A). (C) Simulated Hi-C map constructed at resolution of 4 kb (20 beads) for three consecutive TADs. The starting conformation of a polymer chain was a random walk trajectory in the 64×64×64 simulation box.

**Supplemental Figure S15.** (A) Scatter plot demonstrating negative correlation between the number of contacts within simulated TADs and their radius of gyration (measured in diameters of polymer beads). This plot demonstrates that the total sum of Hi-C interactions within a TAD may be interpreted as a measure of density of a TAD. For each of the 19 TADs in each of the 12 independent simulations, we calculated the total number of contacts between regions more than 4 kb (20 nucleosomes) apart. We also calculated radius of gyration, which is the square root of the mean squared distance to the center of mass. The two show strong negative correlation. (B) Graph of contact probability as a function of distance along the polymer chain estimated for beads located within simulated TAD (red) or between TADs (blue). All pairs of regions not belonging to the same TAD, independent of their status (inter-TADs or different TADs) contributed to the between-TAD plot. We note that comparing this plot to experimental Hi-C data is not feasible, because this plot ends at 100 kb, a typical TAD size in *Drosophila*, while our Hi-C maps start at 20 kb, leaving less than an order of magnitude overlap.

**Supplemental Figure S16.** Spatial configuration (A) and heatmap (B) of the model polymer obtained when volume interactions instead of saturating interactions were used. All designations are as in Figures 6 and S13.

**Supplemental Figure S17.** The TAD profiles of X chromosome are the same in the male and female cell lines. (A) Heatmap of a 2.8-Mb region of the X chromosome in studied cell lines. (B) Venn diagram showing the numbers of TADs located on the X chromosome and common (with both boundaries located at the same genomic bin or at adjacent bins) for all studied cell lines.

**Supplemental Figure S18.** Different methods of analysis alter the shape of the distribution of chromatin marks near the TAD boundary. (A) The height of each peak of chromatin mark was multiplied by the proportion of the bin intersecting with the peak, and the results for all peaks in the bin were summed and Z-transformed, as in Fig. 2C. Smoothed curves
show the median values. (B) Z-transformed proportion of chromatin mark peaks in a bin. The height of peaks was not taken into account. Smoothed curves show the median values. (C) Z-transformed number of peaks of a chromatin mark per bin. The height and the length of peaks were not taken into account. Smoothed curves show the median values. (D) Number of peaks of a chromatin mark per bin. The height and the length of peaks were not taken into account. Smoothed curves show average values. Data files containing called peaks were used in this analysis. (E) Analysis of dCTCF, Su(Hw), Chriz and RNA polymerase II binding within TAD boundaries identified by Hou et al. (2012) in Kc167 cells. TAD boundary positions were rounded to 1 kb. Calculation method as in (A) and Fig. 2C.

**Supplemental Video S1.** 3D-display of one of the predicted spatial configurations of the model polymer (a snapshot from this video is presented in Fig. 6A, and the heatmap of this configuration is shown in Fig. 6B).
A

BG3 Chr3L

OSC Chr3L

S2 Chr3L

Kc167 Chr3L

Replicate 1

Replicate 2

Contact probability

Genomic distance (Mb)

log2(N of reads)

B

10^1

10^0

10^{-1}

10^{-2}

10^{-3}

10^{-4}

10^{-5}

10^{-6}

Genomic distance (Mb)

Contact probability

S2

Kc167

BG3

OSC
Proportion of Polycomb chromatin color (Kharchenko et al. 2011)

Chr3L 82% 97% 92% 64%
Chr3R 98% 92% 99% 95%
Chr2L 56% 90% 92%
Chr3L 82% 97% 92% 64%
A

Chromatin colors in Kc167:
- Active chromatin
- HP1
- Polycomb
- Repressive

Chromatin colors in S2 and BG3:
- Active promoters
- Transcriptional elongation
- Acetylation rich (introns)
- Silent domains
- Pericentromeric heterochromatin
- Moderate level of H3K9me2/me3
- Lacking H3K27ac

B

Number of bins

Proportion of chromatin color within the bin

[Graph showing chromatin color distribution across bins for BG3, S2, and Kc167]
Ulianov_FigS6

H3K27ac  H3K4me1  H3K4me3  H4K16ac

H3K36me3  Total RNA  RNA Pol II  ISWI

dCTCF  Su(Hw)  BEAF-32  CP190

SMC3  H3K27me3  H1  H3

Chriz  JIL-1  MRG15  WDS

MOF  E(bx) (NURF301)  P-element

Z-value

20-kb bins

InterTAD  TAD

boundary bin
A

B

C

D

PolyA+ Transcription, log10 upper-quantile normalized read counts

PolyA+ Transcription, log10 upper-quantile normalized read counts
Active chromatin = RED + YELLOW chromatin colors in Kc167, 1 (RED) + 2 (MAGENTA) + 3 (BROWN) chromatin types in BG3 and S2
A  Transcription within the genomic bin (FC of 4)

|               | Kc167 vs OSC | BG3 vs Kc167 | OSC vs S2 | BG3 vs S2 |
|---------------|--------------|---------------|-----------|-----------|
| Kc167 > OSC   | 42%          | 22%           | 39%       | 56%       |
| Kc167 = OSC   | 21%          | 20%           | 20%       | 37%       |
| Kc167 < OSC   | 55%          | 55%           | 54%       | 54%       |

B  Active chromatin within the genomic bin (FC of 3)

|               | BG3 vs Kc167 | Kc167 vs S2 | BG3 vs S2 |
|---------------|--------------|-------------|-----------|
| BG3 > Kc167   | 57%          | 56%         | 57%       |
| BG3 = Kc167   | 17%          | 32%         | 32%       |
| BG3 < Kc167   | 13%          | 9%          | 9%        |

Schematic representation of the groups of bins:
Active chromatin

TAD interior vs Left boundary

Diff. of active chrom. proportion

TAD interior vs Right boundary

Left boundary vs Right boundary

Repressed chromatin

TAD interior vs Left boundary

Diff. of repres. chrom. proportion

TAD interior vs Right boundary

Left boundary vs Right boundary

Ulianov_FigS11
Ulianov Fig S17

A

B

log2(N of reads)

log2(N of reads)
SUPPLEMENTAL TABLE LEGENDS

Supplemental Table S1. Pearson correlation coefficients between biological replicates of the Hi-C experiments.

Supplemental Table S2. Numbers of Hi-C read pairs aligned to the *Drosophila* reference genome dm3 and filtered before the iterative correction step of the Hi-C data processing. Total reads – number of reads aligned to the reference genome; DS reads – read pairs in which both reads were aligned to the genome; self-circles – read pairs in which both reads were aligned to the same self-circularized restriction fragment; dangling ends – read pairs aligned at the same restriction site; duplicates – any two pairs of reads aligned to identical genomic positions.

Supplemental Table S3. Manually annotated long-range contacts on Hi-C heatmaps for all cell lines. Genomic positions of the midpoints of interacting regions are shown. Genomic rearrangements were annotated using the Delly and BreakDancer algorithms.

Supplemental Table S4. Number, genome coverage and sizes of TADs and inter-TADs in all cell lines, predicted by the Armatus algorithm.

Supplemental Table S5. Genomic positions of TADs in the four studied cell lines after the second step of TAD prediction procedure (splitting of TADs larger than 600 kb by the Armatus algorithm with the increased value of the scaling parameter gamma). The gamma values used at both steps of the procedure are shown.

Supplemental Table S6. P-values for the two-sided Mann-Whitney test used (i) to compare proportions of chromatin colors, as well as Z-values of epigenetic marks within the genomic bins belonging to TADs and inter-TADs (relative to Figures 2B, 2C, 5B and 5C); (ii) to compare proportions of chromatin colors within the genomic bins identified as belonging to inter-TADs at gamma values less than 1.5 and more than 1.5 (relative to Figure 3C and Supplemental Figure S5B).

Supplemental Table S7. Numbers of reads used for poly(A)+ RNA-seq.

Supplemental Table S8. Numbers of differentially and equally transcribed genomic bins in the four groups of bins identified in a pairwise comparison of TAD positions in the four
studied cell lines. Chi-square test and two-sided Fisher’s exact test were performed for the framed values.

**Supplemental Table S9.** Numbers of genomic bins containing different or equal proportion of active chromatin in the four groups of bins identified in a pairwise comparison of TAD positions in the four studied cell lines. Chi-square test and two-sided Fisher’s exact test were performed for the framed values.

**Supplemental Table S10.** Groups of tissue samples from Brown et al. (2013) used to compose a list of tissue-specific genes.

**Supplemental Table S11.** Genomic rearrangements annotated in the four studied cell lines by the Delly and BreakDancer algorithms. DEL – deletion, DUP – duplication, INV – inversion, TRA – translocation, INS – insertion, CTX – interchromosomal translocation, UNK – unknown, SUM - the number of all annotated genomic rearrangements.
### Supplemental Table S1.

| chr2L | BG3_rep1 | BG3_rep2 | OSC_rep1 | OSC_rep2 | S2_rep1 | S2_rep2 | Kc167_rep1 | Kc167_rep2 |
|-------|----------|----------|----------|----------|---------|---------|------------|------------|
| BG3_rep1 | 1.000 | 0.950 | 0.924 | 0.932 | 0.893 | 0.883 | 0.899 | 0.887 |
| BG3_rep2 | 0.950 | 1.000 | 0.926 | 0.933 | 0.893 | 0.883 | 0.899 | 0.887 |
| OSC_rep1 | 0.924 | 0.926 | 1.000 | 0.945 | 0.881 | 0.871 | 0.886 | 0.876 |
| OSC_rep2 | 0.932 | 0.933 | 0.945 | 1.000 | 0.883 | 0.874 | 0.894 | 0.882 |
| S2_rep1 | 0.893 | 0.893 | 0.881 | 0.883 | 1.000 | 0.910 | 0.884 | 0.875 |
| S2_rep2 | 0.883 | 0.883 | 0.871 | 0.874 | 0.910 | 1.000 | 0.874 | 0.864 |
| Kc167_rep1 | 0.899 | 0.899 | 0.886 | 0.894 | 0.884 | 0.874 | 1.000 | 0.903 |
| Kc167_rep2 | 0.887 | 0.887 | 0.876 | 0.882 | 0.875 | 0.864 | 0.903 | 1.000 |

| chr2R | BG3_rep1 | BG3_rep2 | OSC_rep1 | OSC_rep2 | S2_rep1 | S2_rep2 | Kc167_rep1 | Kc167_rep2 |
|-------|----------|----------|----------|----------|---------|---------|------------|------------|
| BG3_rep1 | 1.000 | 0.948 | 0.913 | 0.920 | 0.892 | 0.878 | 0.854 | 0.882 |
| BG3_rep2 | 0.948 | 1.000 | 0.917 | 0.925 | 0.895 | 0.881 | 0.896 | 0.883 |
| OSC_rep1 | 0.913 | 0.917 | 1.000 | 0.935 | 0.888 | 0.875 | 0.882 | 0.870 |
| OSC_rep2 | 0.920 | 0.925 | 0.935 | 1.000 | 0.882 | 0.879 | 0.889 | 0.876 |
| S2_rep1 | 0.892 | 0.895 | 0.888 | 0.892 | 1.000 | 0.904 | 0.879 | 0.866 |
| S2_rep2 | 0.878 | 0.881 | 0.875 | 0.879 | 0.904 | 1.000 | 0.865 | 0.853 |
| Kc167_rep1 | 0.894 | 0.896 | 0.882 | 0.889 | 0.879 | 0.865 | 1.000 | 0.888 |
| Kc167_rep2 | 0.882 | 0.883 | 0.870 | 0.876 | 0.866 | 0.853 | 0.888 | 1.000 |

| chr3L | BG3_rep1 | BG3_rep2 | OSC_rep1 | OSC_rep2 | S2_rep1 | S2_rep2 | Kc167_rep1 | Kc167_rep2 |
|-------|----------|----------|----------|----------|---------|---------|------------|------------|
| BG3_rep1 | 1.000 | 0.947 | 0.902 | 0.908 | 0.889 | 0.874 | 0.886 | 0.875 |
| BG3_rep2 | 0.947 | 1.000 | 0.902 | 0.909 | 0.890 | 0.874 | 0.887 | 0.876 |
| OSC_rep1 | 0.902 | 0.902 | 1.000 | 0.938 | 0.875 | 0.864 | 0.874 | 0.860 |
| OSC_rep2 | 0.908 | 0.909 | 0.938 | 1.000 | 0.880 | 0.868 | 0.882 | 0.870 |
| S2_rep1 | 0.889 | 0.890 | 0.875 | 0.880 | 1.000 | 0.902 | 0.875 | 0.865 |
| S2_rep2 | 0.874 | 0.874 | 0.864 | 0.868 | 0.902 | 1.000 | 0.864 | 0.853 |
| Kc167_rep1 | 0.886 | 0.887 | 0.874 | 0.882 | 0.875 | 0.864 | 1.000 | 0.890 |
| Kc167_rep2 | 0.875 | 0.876 | 0.860 | 0.870 | 0.865 | 0.853 | 0.890 | 1.000 |

| chr4R | BG3_rep1 | BG3_rep2 | OSC_rep1 | OSC_rep2 | S2_rep1 | S2_rep2 | Kc167_rep1 | Kc167_rep2 |
|-------|----------|----------|----------|----------|---------|---------|------------|------------|
| BG3_rep1 | 1.000 | 0.983 | 0.942 | 0.945 | 0.936 | 0.930 | 0.909 | 0.902 |
| BG3_rep2 | 0.983 | 1.000 | 0.941 | 0.944 | 0.935 | 0.929 | 0.905 | 0.901 |
| OSC_rep1 | 0.942 | 0.941 | 1.000 | 0.975 | 0.916 | 0.912 | 0.897 | 0.892 |
| OSC_rep2 | 0.945 | 0.944 | 0.975 | 1.000 | 0.918 | 0.914 | 0.907 | 0.903 |
| S2_rep1 | 0.936 | 0.935 | 0.916 | 0.918 | 1.000 | 0.942 | 0.885 | 0.883 |
| S2_rep2 | 0.930 | 0.929 | 0.912 | 0.914 | 0.942 | 1.000 | 0.875 | 0.870 |
| Kc167_rep1 | 0.909 | 0.905 | 0.897 | 0.907 | 0.885 | 0.875 | 1.000 | 0.919 |
| Kc167_rep2 | 0.902 | 0.901 | 0.892 | 0.903 | 0.883 | 0.870 | 0.919 | 1.000 |

| chrX | BG3_rep1 | BG3_rep2 | OSC_rep1 | OSC_rep2 | S2_rep1 | S2_rep2 | Kc167_rep1 | Kc167_rep2 |
|------|----------|----------|----------|----------|---------|---------|------------|------------|
| BG3_rep1 | 1.000 | 0.845 | 0.836 | 0.845 | 0.751 | 0.713 | 0.808 | 0.797 |
| BG3_rep2 | 0.845 | 1.000 | 0.842 | 0.851 | 0.754 | 0.717 | 0.815 | 0.804 |
| OSC_rep1 | 0.836 | 0.842 | 1.000 | 0.920 | 0.789 | 0.750 | 0.845 | 0.837 |
| OSC_rep2 | 0.845 | 0.851 | 0.920 | 1.000 | 0.792 | 0.754 | 0.852 | 0.842 |
| S2_rep1 | 0.751 | 0.754 | 0.789 | 0.792 | 1.000 | 0.792 | 0.777 | 0.764 |
| S2_rep2 | 0.713 | 0.717 | 0.750 | 0.754 | 0.732 | 1.000 | 0.739 | 0.729 |
| Kc167_rep1 | 0.808 | 0.815 | 0.845 | 0.852 | 0.777 | 0.739 | 1.000 | 0.854 |
| Kc167_rep2 | 0.797 | 0.804 | 0.837 | 0.842 | 0.764 | 0.729 | 0.854 | 1.000 |
### Supplemental Table S2.

| Sample          | Total reads | Total DS reads | Reads from the same fragment | Dangling ends of fragments separated by 500 bp or less | Valid pairs | Mapped near the restriction site | Duplicates | Reads from large (>100 kb) and small (<100 bp) fragments | 0.5% of the most overrepresented fragments | N of reads after all filters |
|-----------------|-------------|----------------|------------------------------|------------------------------------------------------|-------------|---------------------------------|------------|-------------------------------------------------------|----------------------------------------|-----------------------------------|
| DmBG3-c2_combined | 57 686 069  | 49 453 011     | 20 471 016                   | 260 927                                              | 19 924 229  | 5 773 417                       | 23 208 578 | 303 795                                               | 595 925                                | 93 858                            |
| DmBG3-c2_rep1 | 30 300 932   | 26 214 543     | 11 969 368                   | 127 680                                              | 11 684 813  | 2 926 955                       | 11 318 220 | 146 564                                               | 287 558                                | 47 703                            |
| DmBG3-c2_rep2 | 27 385 137   | 23 238 468     | 8 501 648                    | 133 247                                              | 8 239 416  | 2 846 462                       | 11 890 358 | 157 231                                               | 307 655                                | 46 157                            |
| Kc167_combined | 38 750 262   | 33 887 737     | 19 803 814                   | 150 227                                              | 19 423 728  | 3 907 113                       | 10 176 810 | 115 740                                               | 562 544                                | 58 948                            |
| Kc167_rep1 | 16 696 812   | 14 650 515     | 8 811 499                    | 57 819                                               | 8 657 210  | 1 772 010                       | 4 067 006  | 49 405                                                | 230 004                                | 24 305                            |
| Kc167_rep2 | 16 646 771   | 14 492 433     | 8 139 501                    | 73 577                                               | 7 963 586  | 1 561 592                       | 4 791 340  | 50 097                                                | 165 847                                | 27 420                            |
| OSC_combined   | 57 526 086   | 48 264 746     | 21 192 418                   | 239 524                                              | 20 693 278 | 5 867 448                       | 21 204 880 | 366 284                                               | 481 319                                | 87 304                            |
| OSC_rep1 | 24 771 372   | 20 764 840     | 8 852 306                    | 99 949                                               | 8 643 955  | 2 536 283                       | 9 376 251  | 142 571                                               | 167 572                                | 35 299                            |
| OSC_rep2 | 32 754 714   | 27 499 906     | 12 340 112                   | 139 575                                              | 12 049 232 | 3 331 165                       | 11 826 629 | 223 713                                               | 313 183                                | 52 008                            |
| S2_combined    | 42 656 628   | 34 317 102     | 14 014 451                   | 172 352                                              | 13 591 531 | 5 427 129                       | 14 875 522 | 160 109                                               | 1 140 083                              | 74 735                            |
| S2_rep1 | 17 504 801   | 14 143 982     | 6 359 657                    | 62 420                                               | 6 176 842  | 2 234 142                       | 5 550 183  | 61 206                                                | 652 026                                | 30 133                            |
| S2_rep2 | 17 503 379   | 14 017 647     | 5 118 208                    | 79 494                                               | 4 954 171  | 2 219 157                       | 6 660 282  | 70 454                                                | 110 004                                | 32 313                            |

Note: The table includes columns for total reads, total DS reads, reads from the same fragment, dangling ends, valid pairs, mapped near the restriction site, duplicates, reads from large (>100 kb) and small (<100 bp) fragments, 0.5% of the most overrepresented fragments, and N of reads after all filters.
## Supplemental Table S3.

### S2

| № | Chr | Position of the left partner, kb | Position of the right partner, kb | Distance between the partners, kb | In the other datasets | Genomic rearrangements between the partners |
|---|-----|----------------------------------|-----------------------------------|----------------------------------|-----------------------|-------------------------------------------|
| 1 | 2L  | 600                              | 1640                              | 1040                             | No                    | Deletion, inversion, deletion             |
| 2 | 2L  | 2240                             | 2900                              | 660                              | No                    | Duplication head-to-tail                 |
| 3 | 2L  | 5620                             | 9640                              | 4020                             | No                    | Numerous deletions and inversions        |
| 4 | 2L  | 7480                             | 21220                             | 13740                            | No                    | Deletion                                 |
| 5 | 2L  | 13000                            | 13600                             | 600                              | No                    | Duplication head-to-tail                 |
| 6 | 2R  | 3660                             | 4920                              | 1260                             | No                    | Deletion                                 |
| 7 | 2R  | 3680                             | 4820                              | 1140                             | No                    | Deletion                                 |
| 8 | 2R  | 7340                             | 8780                              | 1440                             | No                    | Inversion                                |
| 9 | 2R  | 13260                            | 14340                             | 1080                             | No                    | Duplication head-to-tail                 |
| 10 | 2R  | 15000                            | 15340                             | 340                              | No                    | Deletion                                 |
| 11 | 3L  | 1140                             | 2880                              | 1740                             | No                    | Deletion                                 |
| 12 | 3L  | 8680                             | 10700                             | 2020                             | No                    | Deletion                                 |
| 13 | 3L  | 9400                             | 9880                              | 480                              | No                    | Duplication head-to-tail                 |
| 14 | 3L  | 9740                             | 11240                             | 1500                             | No                    | Deletion                                 |
| 15 | 3L  | 12600                            | 16280                             | 3680                             | No                    | Inversion                                |
| 16 | 3L  | 18940                            | 20880                             | 1940                             | No                    | Inversion                                |
| 17 | 3L  | 21040                            | 21720                             | 680                              | No                    | Duplication head-to-tail                 |
| 18 | 3L  | 21100                            | 22480                             | 1380                             | No                    | Duplication head-to-tail                 |
| 19 | 3R  | 15720                            | 16880                             | 1160                             | No                    | Duplication head-to-tail                 |
| 20 | 3R  | 16820                            | 17460                             | 640                              | No                    | Duplication head-to-tail                 |
| 21 | 3R  | 18400                            | 19100                             | 700                              | No                    | Duplication head-to-tail                 |
| 22 | X   | 900                              | 15920                             | 15020                            | No                    | BG3, OCS, Sexton et al., 2012            |
| 23 | X   | 14080                            | 16440                             | 2360                             | No                    | Deletion                                 |
| 24 | X   | 17900                            | 19180                             | 1280                             | No                    | Duplication head-to-tail                 |

### Kc167

| № | Chr | Position of the left partner, kb | Position of the right partner, kb | Distance between the partners, kb | In the other datasets |
|---|-----|----------------------------------|-----------------------------------|----------------------------------|-----------------------|
| 1 | 2L  | 5940                             | 8780                              | 2840                             | Hou et al., 2012      |
| 2 | 2L  | 9180                             | 13540                             | 4360                             | No                    |
| 3 | 2R  | 6100                             | 8840                              | 2740                             | No                    |
| 4 | 2R  | 7020                             | 11980                             | 4960                             | Inversion             |
| 5 | 3L  | 8740                             | 12980                             | 4240                             | No                    |
| 6 | 3L  | 15920                            | 19100                             | 3180                             | Inversion             |
| 7 | 3R  | 2800                             | 4660                              | 1860                             | Inversion             |
| 8 | 3R  | 14480                            | 14940                             | 460                              | No                    |
| 9 | 3R  | 18120                            | 18960                             | 840                              | No                    |
| 10| 3R  | 23600                            | 24680                             | 1080                             | No                    |
| 11| 3R  | 26280                            | 27100                             | 820                              | No                    |
| 12| X   | 1160                             | 2240                              | 1080                             | No                    |

### BG3

| № | Chr | Position of the left partner, kb | Position of the right partner, kb | Distance between the partners, kb | In the other datasets | Genomic rearrangements between the partners |
|---|-----|----------------------------------|-----------------------------------|----------------------------------|-----------------------|-------------------------------------------|
| 1 | 2L  | 920                              | 2600                              | 1680                             | No                    | OSC, Sexton et al., 2012                  |
| 2 | 2L  | 1980                             | 4780                              | 2800                             | No                    | OSC, Sexton et al., 2012                  |
|   | 2L  | 6400 | 9080 | 2680 | OSC, Sexton et al., 2012 | No |
|---|-----|------|------|------|--------------------------|----|
| 4 | 2L  | 6400 | 9460 | 3060 | OSC, Sexton et al., 2012 | No |
| 5 | 2L  | 9080 | 9460 | 380  | OSC, Sexton et al., 2012 | No |
| 6 | 2L  | 20120| 21680| 1560 | OSC, Sexton et al., 2012 | No |
| 7 | 2R  | 4660 | 10880| 6220 | OSC, Sexton et al., 2012 | No |
| 8 | 2R  | 20640| 21080| 440  | Inversion                |    |
| 9 | 3L  | 200  | 1260 | 1060 | No                        | No |
| 10| 3L   |2720 | 5140 | 2420 | No                        | Duplication, Inversion |
| 11| 3L   |6640 | 9320 | 2680 | OSC, Sexton et al., 2012, Hou et al., 2012 | No |
| 12| 3R   |7040 | ChrX: 12060 | No Translocation |
| 13| X    |900  | 15920| 15020| No                        | No |
| 14| X    |1500 | 4280 | 2780 | No                        | Duplication head-to-tail |
| 15| X    |13240| 19240| 6000 | OSC, Sexton et al., 2012 | No |

**OSC**

|   | 2L  | 920  | 2600  | 1680 | BG3, Sexton et al., 2012 | No |
|---|-----|------|-------|------|--------------------------|----|
| 2 | 2L  | 1980 | 4780  | 2800 | BG3, Sexton et al., 2012 | No |
| 3 | 2L  | 6400 | 9080  | 2680 | BG3, Sexton et al., 2012 | No |
| 4 | 2L  | 6400 | 9460  | 3060 | BG3, Sexton et al., 2012 | No |
| 5 | 2L  | 9080 | 9460  | 380  | BG3, Sexton et al., 2016 | No |
| 6 | 2L  | 20120| 21680 | 1560 | BG3, Sexton et al., 2012 | No |
| 7 | 2R  | 4660 | 10880 | 6220 | BG3, Sexton et al., 2012 | No |
| 8 | 3L  | 1760 | 2200  | 440  | Inversion                |    |
| 9 | 3L  | 1980 | 3560  | 1580 | Hou et al., 2012         | Duplication head-to-tail |
| 10| 3L  | 6640 | 9320  | 2680 | BG3, Sexton et al., 2012, Hou et al., 2012 | No |
| 11| 3L  | 7640 | 17240 | 9600 | Sexton et al., 2012      | No |
| 12| 3L  | 12840| 20000 | 7160 | Sexton et al., 2012      | No |
| 13| 3R  | 980  | 7860  | 6880 | Sexton et al., 2012      | No |
| 14| 3R  | 22260| 23060 | 800  | No                        | Deletion |
| 15| X   |900  | 15920 | 15020| BG3, S2, Sexton et al., 2012 | No |
| 16| X   |13240| 19240 | 6000 | BG3, Sexton et al., 2012 | No |
**Supplemental Table S4.**

| Cell lines (gamma values used for the two-step TADs annotation) | S2 (1.26, 2.52) | Kc167 (1.21, 2.42) | BG3 (1.07, 2.14) | OSC (1.03, 2.06) |
|---------------------------------------------------------------|-----------------|-------------------|-----------------|-----------------|
| **Total number**                                              | TADs            | 583               | 582             | 580             | 577             |
| **Genome coverage, %**                                        | TAD interior    | 67                | 66              | 63              | 63              |
|                                                               | TAD interior + boundary bins | 87 | 85 | 82 | 83 |
|                                                               | inter-TADs + boundary bins | 33 | 34 | 37 | 37 |
| **Average size, kb**                                         | TAD interior    | 137               | 134             | 129             | 131             |
|                                                               | TAD interior + boundary bins | 177 | 174 | 169 | 171 |
|                                                               | inter-TADs + boundary bins | 67 | 70 | 76 | 75 |
| **Median size, kb**                                          | TAD interior    | 100               | 100             | 100             | 100             |
|                                                               | TAD interior + boundary bins | 140 | 140 | 140 | 140 |
|                                                               | inter-TADs + boundary bins | 40 | 40 | 40 | 40 |
| **Minimal size, kb**                                         | TAD interior    | 40                | 40              | 40              | 40              |
|                                                               | TAD interior + boundary bins | 80 | 80 | 80 | 80 |
|                                                               | inter-TADs + boundary bins | 40 | 40 | 40 | 40 |
| **Maximal size, kb**                                         | TAD interior    | 540               | 640             | 540             | 540             |
|                                                               | TAD interior + boundary bins | 580 | 680 | 580 | 580 |
|                                                               | inter-TADs + boundary bins | 480 | 460 | 360 | 360 |
Supplemental Table S5.

| Nr | Chr | S2, $\gamma_1 = 1.26, \gamma_2 = 2.52$ | Kc167, $\gamma_1 = 1.21, \gamma_2 = 2.42$ | BG3, $\gamma_1 = 1.07, \gamma_2 = 2.14$ | OSC, $\gamma_1 = 1.03, \gamma_2 = 2.06$ |
|----|-----|-------------------------------------|-------------------------------------|-------------------------------------|-------------------------------------|
| 1  | 2L  | 0                                  | 120                                 | 1                                  | 2L 180 260 |
| 2  | 2L  | 120                                | 280                                 | 2                                  | 2L 300 420 |
| 3  | 2L  | 280                                | 440                                 | 3                                  | 2L 480 560 |
| 4  | 2L  | 580                                | 820                                 | 4                                  | 2L 560 820 |
| 5  | 2L  | 860                                | 980                                 | 5                                  | 2L 880 960 |
| 6  | 2L  | 1020                               | 1180                                | 6                                  | 2L 1000 1080 |
| 7  | 2L  | 1180                               | 1640                                | 7                                  | 2L 1140 1640 |
| 8  | 2L  | 1640                               | 1720                                | 8                                  | 2L 1640 1720 |
| 9  | 2L  | 1720                               | 1940                                | 9                                  | 2L 1720 1920 |
| 10 | 2L  | 1980                               | 2160                                | 10                                 | 2L 1980 2160 |
| 11 | 2L  | 2160                               | 2240                                | 11                                 | 2L 2220 2360 |
| 12 | 2L  | 2240                               | 2360                                | 12                                 | 2L 2360 2740 |
| 13 | 2L  | 2360                               | 2620                                | 13                                 | 2L 2880 2960 |
| 14 | 2L  | 2660                               | 2740                                | 14                                 | 2L 2960 3040 |
| 15 | 2L  | 2740                               | 2860                                | 15                                 | 2L 3140 3340 |
| 16 | 2L  | 2860                               | 2980                                | 16                                 | 2L 3340 3480 |
| 17 | 2L  | 3040                               | 3160                                | 17                                 | 2L 3480 3640 |
| 18 | 2L  | 3160                               | 3360                                | 18                                 | 2L 3640 3800 |
| 19 | 2L  | 3360                               | 3460                                | 19                                 | 2L 3800 4220 |
| 20 | 2L  | 3460                               | 3660                                | 20                                 | 2L 4220 4340 |
| 21 | 2L  | 3660                               | 3800                                | 21                                 | 2L 4460 4820 |
| 22 | 2L  | 3800                               | 4340                                | 22                                 | 2L 4820 4900 |
| 23 | 2L  | 4460                               | 4820                                | 23                                 | 2L 5100 5240 |
| 24 | 2L  | 4820                               | 4960                                | 24                                 | 2L 5240 5320 |
| 25 | 2L  | 5000                               | 5080                                | 25                                 | 2L 5320 5520 |
| 26 | 2L  | 5080                               | 5280                                | 26                                 | 2L 5520 5960 |
| 27 | 2L  | 5320                               | 5520                                | 27                                 | 2L 6100 6200 |
| 28 | 2L  | 5520                               | 5980                                | 28                                 | 2L 6240 6340 |
| 29 | 2L  | 5980                               | 6060                                | 29                                 | 2L 6340 6420 |
| 30 | 2L  | 6100                               | 6340                                | 30                                 | 2L 6720 6800 |
| 31 | 2L  | 6340                               | 6460                                | 31                                 | 2L 6800 6920 |
| 32 | 2L  | 6500                               | 6640                                | 32                                 | 2L 6960 7040 |
| 33 | 2L  | 6700                               | 6960                                | 33                                 | 2L 7040 7220 |
| 34 | 2L  | 6960                               | 7040                                | 34                                 | 2L 7220 7420 |
| 35 | 2L  | 7040                               | 7220                                | 35                                 | 2L 7460 7820 |
| 36 | 2L  | 7220                               | 7420                                | 36                                 | 2L 7880 7980 |
| 37 | 2L  | 7500                               | 7820                                | 37                                 | 2L 8220 8320 |
| 38 | 2L  | 7820                               | 7980                                | 38                                 | 2L 8420 8540 |
| 39 | 2L  | 8020                               | 8100                                | 39                                 | 2L 8540 8700 |
| 40 | 2L  | 8100                               | 8240                                | 40                                 | 2L 8700 8940 |
| 41 | 2L  | 8240                               | 8340                                | 41                                 | 2L 8940 9520 |
| 42 | 2L  | 8340                               | 8420                                | 42                                 | 2L 9580 9700 |
| 43 | 2L  | 8420                               | 8540                                | 43                                 | 2L 9700 9900 |
| 44 | 2L  | 8540                               | 8680                                | 44                                 | 2L 10020 10200 |
| 1  | 3L  | 40  | 180 | 1  | 3L  | 40  | 180 | 1  | 3L  | 0  | 180 | 1  | 3L  | 60  | 180 |
|----|-----|-----|-----|----|-----|-----|-----|----|-----|----|-----|----|-----|-----|-----|
| 2  | 3L  | 180 | 260 | 2  | 3L  | 180 | 260 | 2  | 3L  | 220| 360 | 2  | 3L  | 180 | 260 |
| 3  | 3L  | 260 | 340 | 3  | 3L  | 260 | 340 | 3  | 3L  | 420| 520 | 3  | 3L  | 260 | 340 |
| 4  | 3L  | 340 | 500 | 4  | 3L  | 340 | 520 | 4  | 3L  | 740| 840 | 4  | 3L  | 340 | 520 |
| 5  | 3L  | 540 | 620 | 5  | 3L  | 520 | 620 | 5  | 3L  | 880| 104 | 5  | 3L  | 520 | 600 |
| 6  | 3L  | 660 | 840 | 6  | 3L  | 660 | 840 | 6  | 3L  | 1100| 1180 | 6  | 3L  | 660 | 840 |
| 7  | 3L  | 900 | 980 | 7  | 3L  | 840 | 1080| 7  | 3L  | 1180| 1300 | 7  | 3L  | 840 | 1040|
| 8  | 3L  | 1020| 1200| 8  | 3L  | 1080| 1200| 8  | 3L  | 1340| 1500 | 8  | 3L  | 1040| 1120|
| 9  | 3L  | 1200| 1300| 9  | 3L  | 1200| 1300| 9  | 3L  | 1600| 1680 | 9  | 3L  | 1180| 1300|
| 10 | 3L  | 1340| 1500| 10 | 3L  | 1340| 1500| 10 | 3L  | 1740| 1860 | 10 | 3L  | 1340| 1500|
| 11 | 3L  | 1560| 1660| 11 | 3L  | 1560| 1660| 11 | 3L  | 1860| 1960 | 11 | 3L  | 1560| 1660|
| 12 | 3L  | 1660| 1860| 12 | 3L  | 1660| 1860| 12 | 3L  | 1960| 2160 | 12 | 3L  | 1660| 1740|
| 13 | 3L  | 1860| 2240| 13 | 3L  | 1880| 2240| 13 | 3L  | 2160| 2240 | 13 | 3L  | 1740| 2260|
| 14 | 3L  | 2240| 2380| 14 | 3L  | 2240| 2380| 14 | 3L  | 2240| 2380 | 14 | 3L  | 2260| 2380|
| 15 | 3L  | 2380| 2480| 15 | 3L  | 2380| 2460| 15 | 3L  | 2380| 2480 | 15 | 3L  | 2380| 2480|
| 105 X | 20180 | 20260 | 105 X | 20380 | 20820 | 105 X | 18760 | 19080 |
|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| 106 X | 20260 | 20380 | 106 X | 20940 | 21200 | 106 X | 19080 | 19160 |
| 107 X | 20380 | 20920 | 107 X | 21260 | 21400 | 107 X | 19220 | 19380 |
| 108 X | 20920 | 21180 | 108 X | 21440 | 21540 | 108 X | 19380 | 19500 |
| 109 X | 21240 | 21400 | 109 X | 21860 | 22400 | 109 X | 19640 | 19800 |
| 110 X | 21840 | 21960 |       |       |       |       | 110 X | 19840 | 20060 |
| 111 X | 21960 | 22400 |       |       |       |       | 111 X | 20100 | 20280 |
|       |       |       |       |       |       |       | 112 X | 20400 | 20500 |
|       |       |       |       |       |       |       | 113 X | 20500 | 20640 |
|       |       |       |       |       |       |       | 114 X | 20640 | 20720 |
|       |       |       |       |       |       |       | 115 X | 20720 | 20900 |
|       |       |       |       |       |       |       | 116 X | 20940 | 21120 |
|       |       |       |       |       |       |       | 117 X | 21120 | 21200 |
|       |       |       |       |       |       |       | 118 X | 21240 | 21400 |
|       |       |       |       |       |       |       | 119 X | 21460 | 21540 |
|       |       |       |       |       |       |       | 120 X | 21860 | 22400 |
**Supplemental Table S6.**

### Figure 2B

| Color | S2  | BG3  | Kc167 |
|-------|-----|------|-------|
| RED   | 99.9| 1    | 286.9 |
| YELLOW| 258.2| 2    | 170.6 |
| GREEN | 22.9| 3    | 94.8  |
| BLUE  | 1.4 | 4    | 0.6   |
| BLACK | 169.8| 5    | 20.0  |
|       | 6   | 6.8  | 11.3  |
|       | 7   | 0.2  | 0.1   |
|       | 8   | 45.7 | 7.9   |
|       | 9   | 20.2 | 113.6 |

### Figure 2C

| Chromatin mark | S2  | BG3  | Kc167 | S2  | BG3  | Kc167 |
|----------------|-----|------|-------|-----|------|-------|
| H3K27ac        | 216.4| 190.0| 122.3 | 215.1| 188.6| 120.9 |
| H3K27me3       | 185.3| 35.5 | NA    | 183.9| 34.1 | NA    |
| H3K36me3       | 227.7| 257.3| 246.2 | 226.3| 255.9| 244.8 |
| H3K4me1        | 178.5| 133.0| 228.8 | 177.1| 131.6| 227.4 |
| H3K4me3        | 271.4| <300 | 280.8 | 270.0| <300 | 279.4 |
| H4K16ac        | 195.9| 149.4| 247.8 | 194.5| 148.0| 246.4 |
| polyA          | 164.6| NA   | 172.9 | 163.2| NA   | 171.6 |
| totalRNA       | 222.3| 215.1| 216.7 | 221.0| 213.7| 215.3 |
| CTCF           | 15.0 | 41.1 | 5.1   | 13.6 | 39.7 | 3.7   |
| H1             | 93.2 | 190.5| 217.4 | 91.8 | 189.1| 216.0 |
| ISWI           | 286.2| 259.3| 286.9 | 284.8| 258.0| 285.6 |
| RNA-pol-II     | 282.3| 267.1| 290.3 | 280.9| 265.8| 288.9 |
| Smc3           | 36.2 | NA   | 37.6  | 34.8 | NA   | 36.2  |
| Su_Hw          | 41.3 | 44.1 | 22.2  | 39.9 | 42.7 | 20.9  |
| Beaf-32        | 235.1| 76.8 | 274.7 | 233.7| 75.4 | 273.3 |
| mod_mdg4       | 4.5  | 15.8 | 3.6   | 3.2  | 14.4 | 2.2   |
| Chriz          | 304.8| <300 | 294.8 | 303.5| <300 | 293.4 |
| Jil1           | 243.7| 248.1| 257.9 | 242.3| 246.8| 256.5 |
| H3             | 103.2| 152.6| 191.4 | 101.8| 151.2| 190.0 |
| WDS            | 281.9| 262.1| 264.4 | 280.5| 260.7| 263.0 |
| MOF            | 223.8| 184.1| 224.5 | 222.5| 182.7| 223.1 |
| NURF301        | 303.9| 130.1| 78.8  | 302.5| 128.7| 77.4  |
| dmTopo-II      | 72.1 | 11.2 | NA    | 70.7 | 9.8  | NA    |
| MRG15          | 287.8| 263.6| 297.4 | 286.4| 262.2| 296.0 |
### Figure 3C

| Color   | -log10(p-value) | Color   | -log10(p-value) | Color   | -log10(p-value) |
|---------|----------------|---------|----------------|---------|----------------|
| RED     | 123.9          | 1       | 274.3          | 1       | 238.5          |
| YELLOW  | 290.9          | 2       | 181.4          | 2       | 142.8          |
| GREEN   | 20.6           | 3       | 103.2          | 3       | 98.3           |
| BLUE    | 0.2            | 4       | 3.5            | 4       | 3.7            |
| BLACK   | 201.1          | 5       | 26.0           | 5       | 11.7           |
|         |                | 6       | 4.5            | 6       | 13.0           |
|         |                | 7       | 1.5            | 7       | 1.0            |
|         |                | 8       | 69.2           | 8       | 11.3           |
|         |                | 9       | 15.3           | 9       | 119.2          |

### Figure 5B

| Color   | -log10(p-value) |
|---------|-----------------|
| CYAN    | <300            |
| BLUE    | 247.0           |
| GREEN   | 4.8             |
| MAGENTA | 258.9           |

### Figure 5C

| Color   | -log10(p-value) |
|---------|-----------------|
| CYAN    | <300            |
| BLUE    | 253.7           |
| GREEN   | 24.3            |
| MAGENTA | 283.5           |
## Supplemental Table S7.

| Replicate | Total reads | Filtered out* | Uniq. mapped |
|-----------|-------------|---------------|--------------|
| BG3_rep1  | 16 429 383  | 9 702 016     | 5 252 957    |
| BG3_rep2  | 16 623 342  | 9 806 995     | 5 296 560    |
| KC_rep1   | 26 843 301  | 1 218 207     | 1 741 683    |
| KC_rep2   | 17 205 118  | 803 813       | 1 159 250    |
| OSC_rep1  | 17 316 612  | 3 820 182     | 4 505 649    |
| OSC_rep2  | 18 719 578  | 4 231 838     | 5 028 219    |
| S2_rep1   | 33 990 827  | 6 437 675     | 20 297 591   |
| S2_rep2   | 20 677 615  | 3 301 936     | 13 045 928   |

* by TopHat2
Supplemental Table S8.

| Group | Cell line 1 | Cell line 2 | Transcription within the bin |
|-------|-------------|-------------|-----------------------------|
|       | No data     | Cell line 1 < Cell line 2 | Cell line 1 = Cell line 2 | Cell line 1 > Cell line 2 |
| T-T   | TAD         | 2841        | 25                        | 349                       | 65                        |
|       | inter-TAD   | 531         | 13                        | 973                       | 47                        |
|       | TAD         | 339         | 8                         | 258                       | 26                        |
| T-I   | TAD         | 275         | 13                        | 182                       | 5                         |
|       | inter-TAD   | 3986        | 59                        | 1762                      | 143                       |

Chi-square test, p-value = 0.0006
Two-tailed Fisher’s exact test, p-value = 0.001

| Group | Cell line 1 | Cell line 2 | Transcription within the bin |
|-------|-------------|-------------|-----------------------------|
|       | No data     | Cell line 1 < Cell line 2 | Cell line 1 = Cell line 2 | Cell line 1 > Cell line 2 |
| T-T   | TAD         | 2942        | 34                        | 446                       | 42                        |
|       | inter-TAD   | 515         | 20                        | 950                       | 26                        |
|       | TAD         | 291         | 9                         | 218                       | 10                        |
| T-I   | TAD         | 238         | 17                        | 189                       | 3                         |
|       | inter-TAD   | 3986        | 80                        | 1803                      | 81                        |

Chi-square test, p-value = 0.012
Two-tailed Fisher’s exact test, p-value = 0.0187

| Group | Cell line 1 | Cell line 2 | Transcription within the bin |
|-------|-------------|-------------|-----------------------------|
|       | No data     | Cell line 1 < Cell line 2 | Cell line 1 = Cell line 2 | Cell line 1 > Cell line 2 |
| T-T   | TAD         | 2880        | 33                        | 350                       | 75                        |
|       | inter-TAD   | 517         | 30                        | 943                       | 51                        |
|       | TAD         | 353         | 7                         | 259                       | 35                        |
| T-I   | TAD         | 236         | 11                        | 157                       | 13                        |
|       | inter-TAD   | 3986        | 81                        | 1709                      | 174                       |

Chi-square test, p-value = 0.01
Two-tailed Fisher’s exact test, p-value = 0.0199
### Kc167 vs OSC

| Group | Cell line 1 | Cell line 2 | No data | Cell line 1 < Cell line 2 | Cell line 1 = Cell line 2 | Cell line 1 > Cell line 2 |
|-------|-------------|-------------|---------|---------------------------|---------------------------|---------------------------|
| T-T   | TAD         | TAD         | 2850    | 49                        | 366                       | 32                        |
| I-I   | inter-TAD   | inter-TAD   | 506     | 27                        | 976                       | 32                        |
| I-T   | inter-TAD   | TAD         | 300     | 3                         | 180                       | 15                        |
| T-I   | TAD         | inter-TAD   | 330     | 38                        | 237                       | 9                         |
| Sum   |             |             | 3986    | 117                       | 1759                      | 88                        |

Chi-square test, p-value = 1.6e-06
Two-tailed Fisher’s exact test, p-value = 0.0001

### OSC vs S2

| Group | Cell line 1 | Cell line 2 | No data | Cell line 1 < Cell line 2 | Cell line 1 = Cell line 2 | Cell line 1 > Cell line 2 |
|-------|-------------|-------------|---------|---------------------------|---------------------------|---------------------------|
| T-T   | TAD         | TAD         | 2893    | 39                        | 345                       | 64                        |
| I-I   | inter-TAD   | inter-TAD   | 496     | 32                        | 940                       | 36                        |
| I-T   | inter-TAD   | TAD         | 340     | 12                        | 265                       | 34                        |
| T-I   | TAD         | inter-TAD   | 257     | 17                        | 176                       | 4                         |
| Sum   |             |             | 3986    | 100                       | 1726                      | 138                       |

Chi-square test, p-value = 2.6e-05
Two-tailed Fisher’s exact test, p-value = 0.0001

### BG3 vs OSC

| Group | Cell line 1 | Cell line 2 | No data | Cell line 1 < Cell line 2 | Cell line 1 = Cell line 2 | Cell line 1 > Cell line 2 |
|-------|-------------|-------------|---------|---------------------------|---------------------------|---------------------------|
| T-T   | TAD         | TAD         | 2849    | 35                        | 328                       | 45                        |
| I-I   | inter-TAD   | inter-TAD   | 569     | 14                        | 1027                      | 47                        |
| I-T   | inter-TAD   | TAD         | 301     | 4                         | 211                       | 22                        |
| T-I   | TAD         | inter-TAD   | 267     | 16                        | 202                       | 13                        |
| Sum   |             |             | 3986    | 69                        | 1768                      | 127                       |

Chi-square test, p-value = 0.00219
Two-tailed Fisher’s exact test, p-value = 0.0044
Supplemental Table S9.

### Cell line 1 vs Cell line 2

#### BG3 vs Kc167

| Group | Cell line 1 | Cell line 2 | Proportion of active chromatin within the bin |
|-------|-------------|-------------|---------------------------------------------|
|       | No data     | T-T TAD     | I-I inter-TAD   | I-T TAD     | T-I TAD   |
|       |             | TAD         | inter-TAD      | TAD         | inter-TAD |
| T-T   |             | 1824        | 230            | 699         | 527       |
| I-I   |             | 155         | 90             | 1199        | 120       |
| I-T   |             | 94          | 51             | 272         | 58        |
| T-I   |             | 125         | 35             | 384         | 87        |
| Sum   |             | 2198        | 406            | 2554        | 792       |

Chi-square test, p-value = 0.004  
Two-tailed Fisher’s exact test, p-value = 0.006

### Kc167 vs S2

| Group | Cell line 1 | Cell line 2 | Proportion of active chromatin within the bin |
|-------|-------------|-------------|---------------------------------------------|
|       | No data     | T-T TAD     | I-I inter-TAD   | I-T TAD     | T-I TAD   |
|       |             | TAD         | inter-TAD      | TAD         | inter-TAD |
| T-T   |             | 2005        | 404            | 845         | 210       |
| I-I   |             | 159         | 102            | 1173        | 77        |
| I-T   |             | 88          | 54             | 316         | 48        |
| T-I   |             | 110         | 57             | 269         | 33        |
| Sum   |             | 2362        | 617            | 2603        | 368       |

Chi-square test, p-value = 0.145  
Two-tailed Fisher’s exact test, p-value = 0.187

### BG3 vs S2

| Group | Cell line 1 | Cell line 2 | Proportion of active chromatin within the bin |
|-------|-------------|-------------|---------------------------------------------|
|       | No data     | T-T TAD     | I-I inter-TAD   | I-T TAD     | T-I TAD   |
|       |             | TAD         | inter-TAD      | TAD         | inter-TAD |
| T-T   |             | 1801        | 236            | 948         | 353       |
| I-I   |             | 143         | 44             | 1297        | 57        |
| I-T   |             | 133         | 24             | 441         | 56        |
| T-I   |             | 83          | 37             | 265         | 32        |
| Sum   |             | 2160        | 341            | 2951        | 498       |

Chi-square test, p-value = 0.003  
Two-tailed Fisher’s exact test, p-value = 0.004
### Supplemental Table S10.

| Group  | L3_Carcass | L3_CNS | L3_DigestiveSystem | L3_FatBody | L3_ImaginalDiscs | L3_SalivaryGlands |
|--------|------------|--------|--------------------|------------|------------------|-------------------|
| Group 2| WPP_2days_CNS | WPP_2days_Fat | WPP_FatBody | WPP_SalivaryGlands |
| Group 3| AdMatedM_Ecl_4days_Testes | AdMatedM_Ecl_4days_AccessoryGlands | AdMatedM_Ecl_4days_Heads |
| Group 4| AdVirginF_Ecl_4days_Ovaries | AdVirginF_Ecl_4days_Heads |
| Group 5| AdMatedF_Ecl_4days_Ovaries | AdMatedF_Ecl_4days_Heads |
| Group 6| AdMixedMF_Ecl_1day_Carcass | AdMixedMF_Ecl_1day_DigestiveSystem |
| Group 7| AdMixedMF_Ecl_4days_Carcass | AdMixedMF_Ecl_4day_DigestiveSystem |
| Group 8| AdMixedMF_Ecl_20days_Carcass | AdMixedMF_Ecl_20days_DigestiveSystem |
Supplemental Table S11.

| Cell line | Reads | Reads w. trimmed adaptor | Delly | BreakDancer |
|-----------|-------|--------------------------|-------|-------------|
|           |       |                          | Del   | INS         |
| S2        | 65 681 282 | 40 228 560              | 645   | 1465        |
| Kc167     | 31 127 671 | 17 598 455              | 648   | 340         |
| BG3       | 50 183 677 | 31 255 562              | 614   | 712         |
| OSC       | 32 372 349 | 19 698 432              | 623   | 573         |

Columns: DEL, DUP, INV, TRA, SUM, DEL, INS, INV, CTX, UNK, SUM