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Supplemental Figures

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Supplemental Protocol (provided as a separate .pdf file)

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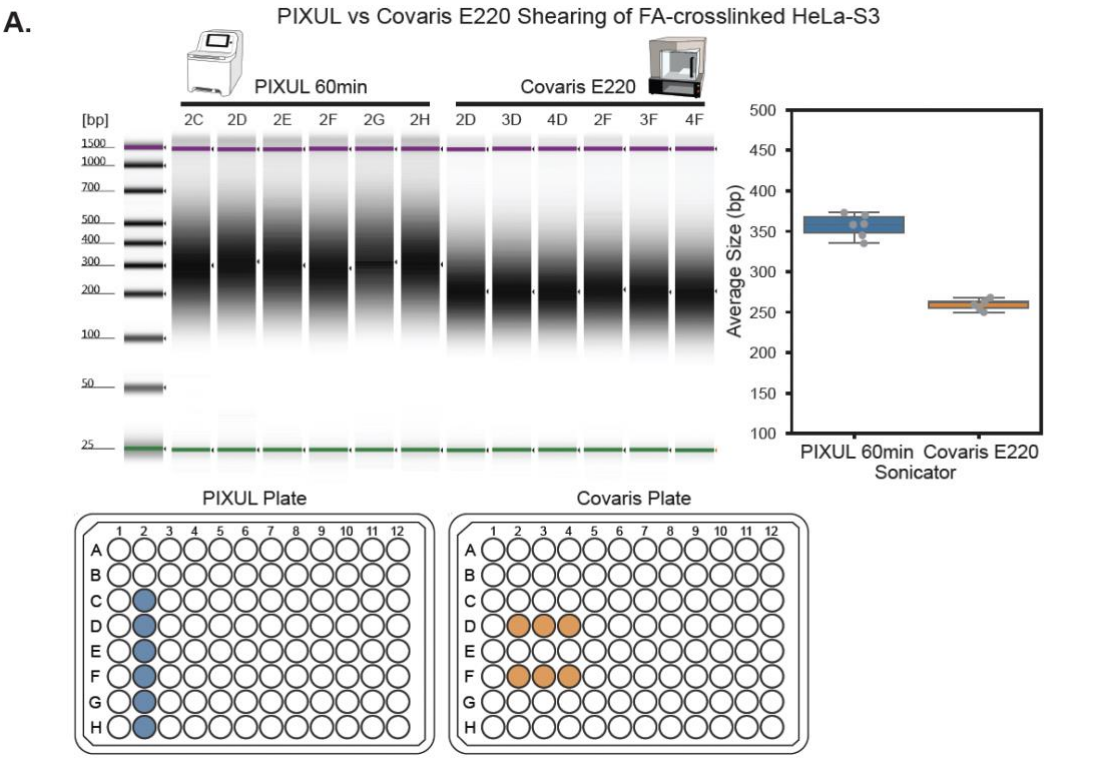
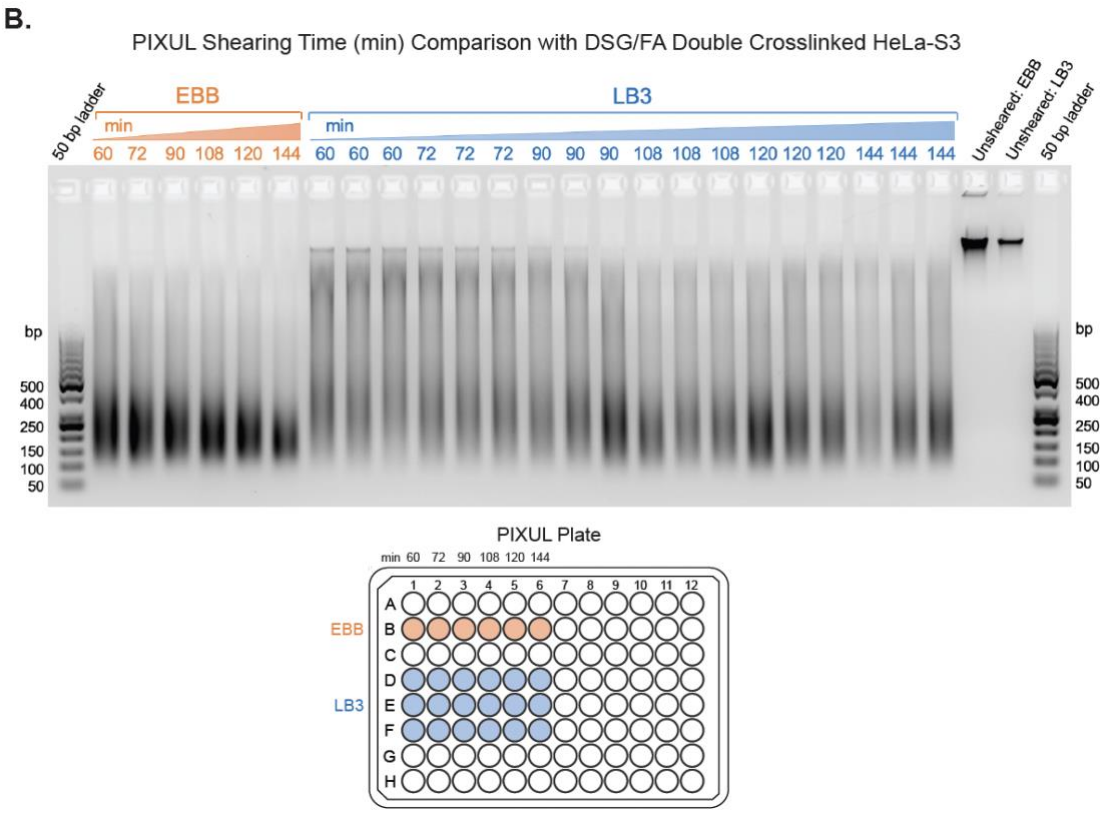
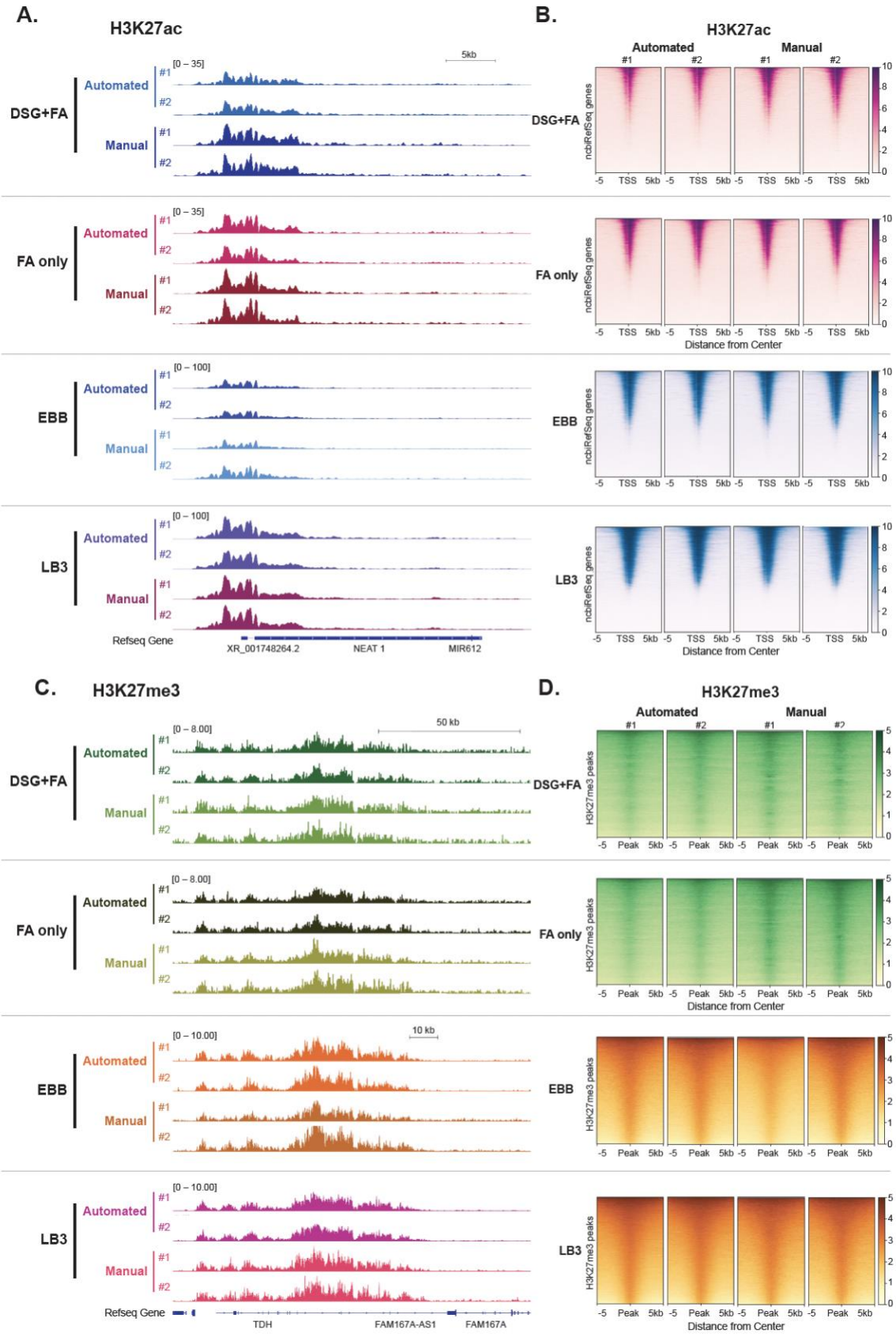
SUPPLEMENTAL FIGURES

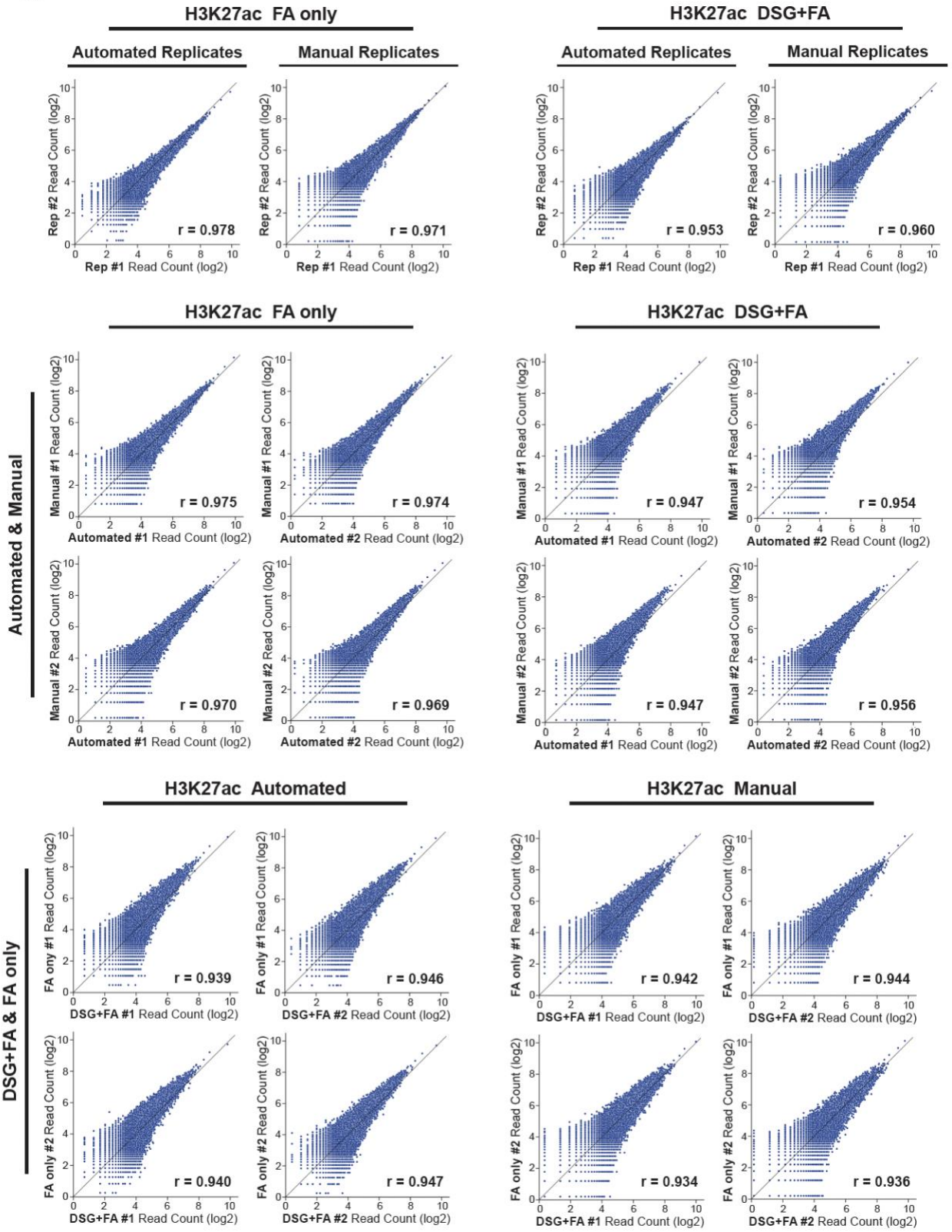




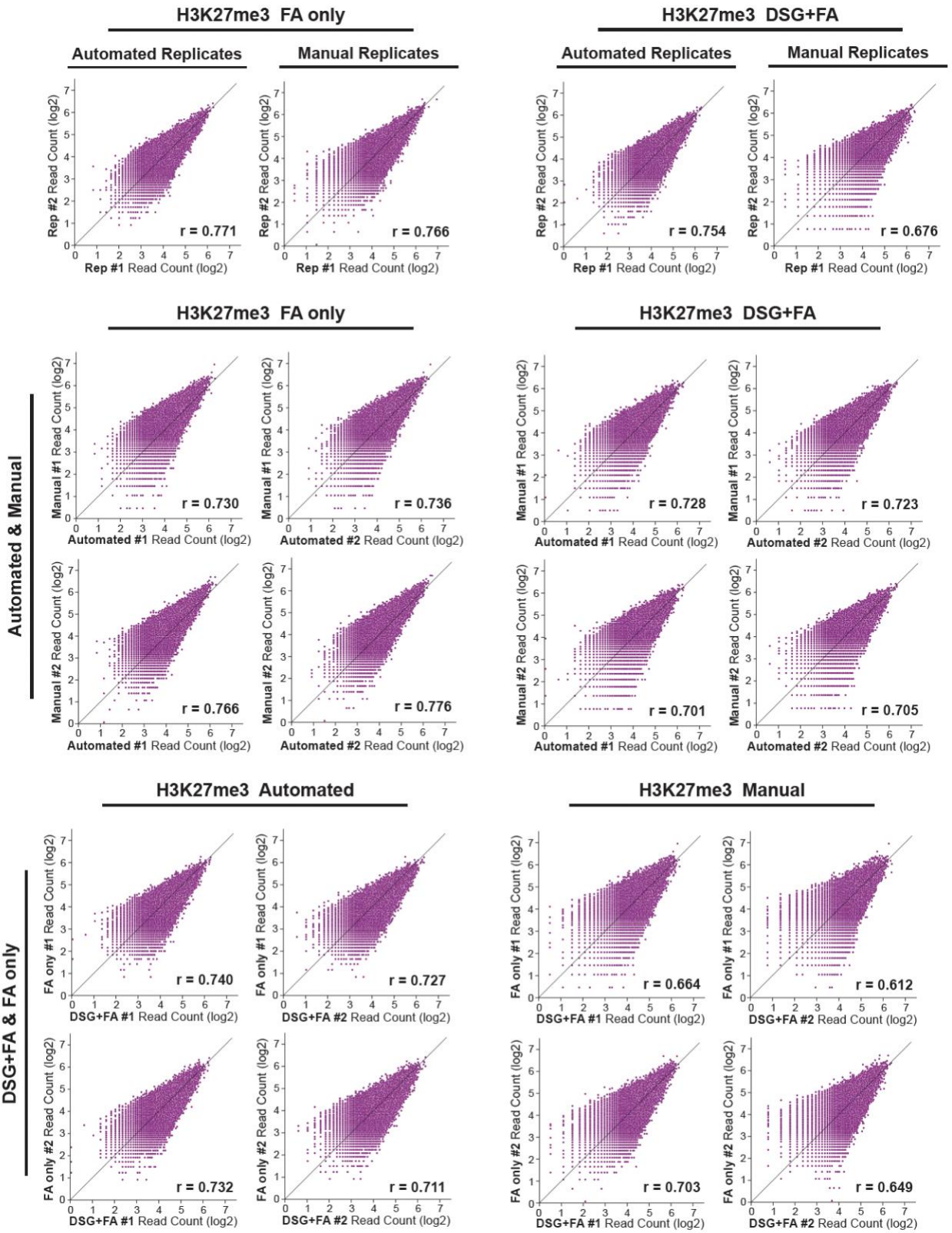
Supplemental Fig. S1: Comparison of 96-well sonicators and lysis buffers for shearing of crosslinked cells. A. TapeStation analysis comparing the two 96-well sonicators: PIXUL and Covaris E220 for shearing of FA-crosslinked HeLa-S3. 96-well plate schematics show wells used for the PIXUL (blue) and Covaris (orange) plates during sonication. Boxplot on the right shows the average size (bp) of sheared chromatin on each device based on TapeStation analysis. Error bars represent standard deviations. **B.** 2% agarose gel showing the comparison of sonication time on the PIXUL for lysis buffers EBB and LB3 with DSG/FA double crosslinked HeLa-S3 cells. Schematic of the 96-well PIXUL plate shows wells containing either EBB (orange) or LB3 (blue) lysis buffer during sonication.


A.
H3K27ac
5kb
DSG+FA
Automated
Manual
#1
#2
[0 – 35]
FA only
[0 – 100]
EBB
LB3
Refseq Gene
XR_001748264.2
NEAT 1
MIR612
B.
H3K27ac
Automated
Manual
ncbiRefSeq genes
-5 TSS 5kb
Distance from Center
C.
H3K27me3
50 kb
[0 – 8.00]
10 kb
[0 – 10.00]
TDH
FAM167A-AS1
FAM167A
D.
H3K27me3
H3K27me3 peaks
-5 Peak 5kb

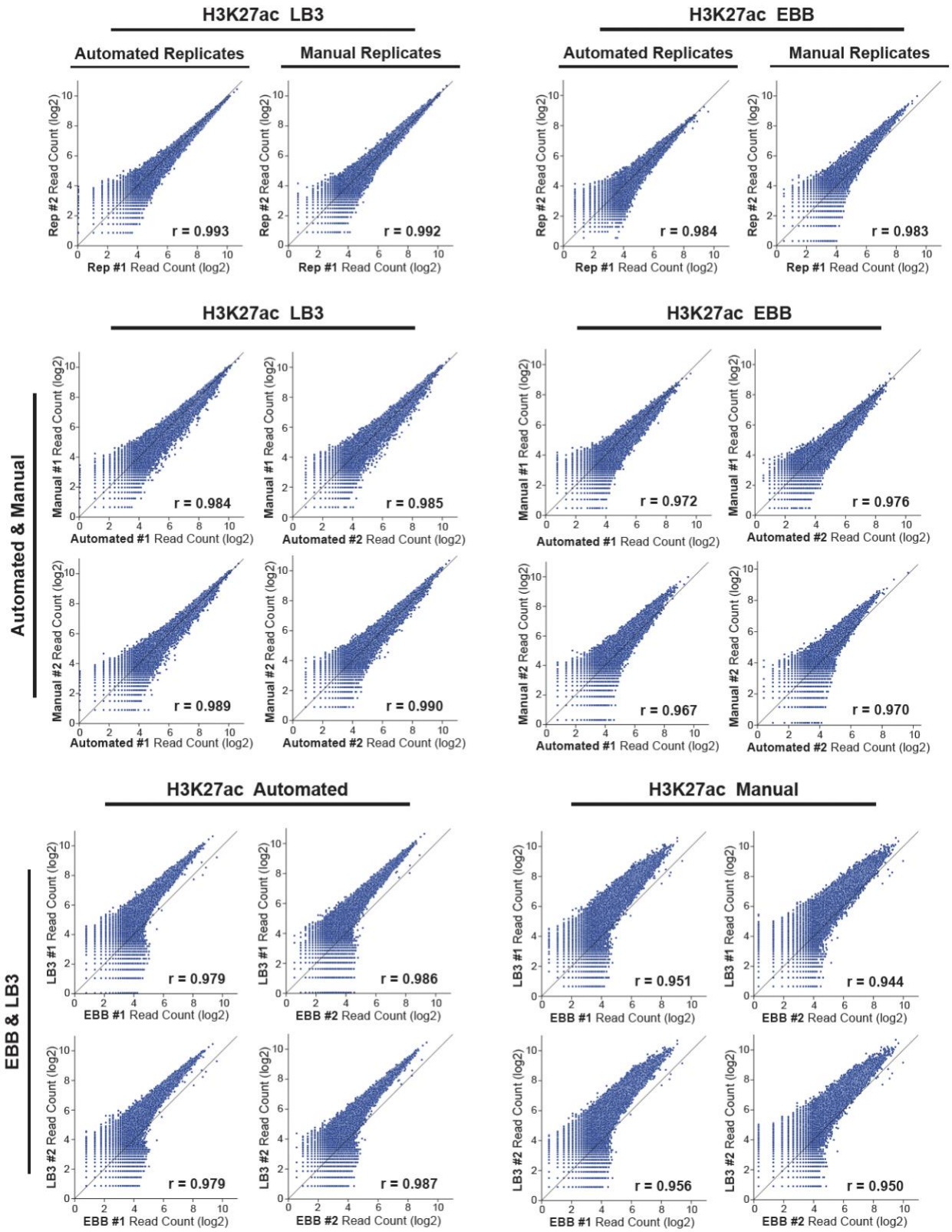
E.


H3K27ac FA only
H3K27ac DSG+FA
Automated Replicates
Manual Replicates
Automated Replicates
Manual Replicates
Rep #2 Read Count (log2)
Rep #1 Read Count (log2)
r = 0.978
r = 0.971
r = 0.953
r = 0.960
Automated & Manual
H3K27ac FA only
H3K27ac DSG+FA
Manual #1 Read Count (log2)
Manual #2 Read Count (log2)
Automated #1 Read Count (log2)
Automated #2 Read Count (log2)
r = 0.975
r = 0.974
r = 0.970
r = 0.969
r = 0.947
r = 0.954
r = 0.947
r = 0.956
DSG+FA & FA only
H3K27ac Automated
H3K27ac Manual
FA only #1 Read Count (log2)
FA only #2 Read Count (log2)
DSG+FA #1 Read Count (log2)
DSG+FA #2 Read Count (log2)
r = 0.939
r = 0.946
r = 0.940
r = 0.947
r = 0.942
r = 0.944
r = 0.934
r = 0.936

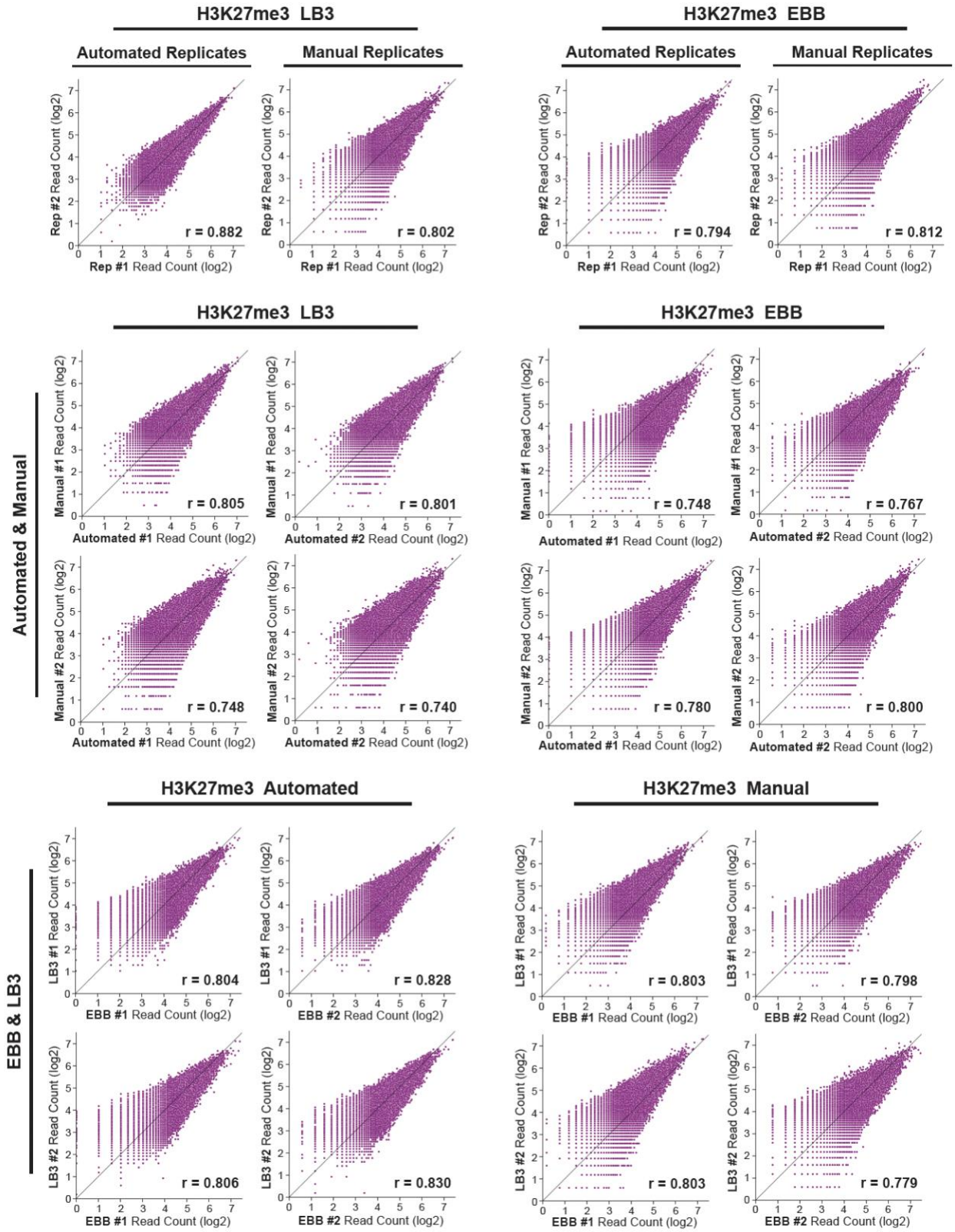
F.


H3K27me3 FA only
H3K27me3 DSG+FA
Automated Replicates
Manual Replicates
Automated Replicates
Manual Replicates
Rep #2 Read Count (log2)
Rep #1 Read Count (log2)
r = 0.771
r = 0.766
r = 0.754
r = 0.676
Automated & Manual
H3K27me3 FA only
H3K27me3 DSG+FA
Manual #1 Read Count (log2)
Manual #2 Read Count (log2)
Automated #1 Read Count (log2)
Automated #2 Read Count (log2)
r = 0.730
r = 0.736
r = 0.766
r = 0.776
r = 0.728
r = 0.723
r = 0.701
r = 0.705
DSG+FA & FA only
H3K27me3 Automated
H3K27me3 Manual
FA only #1 Read Count (log2)
FA only #2 Read Count (log2)
DSG+FA #1 Read Count (log2)
DSG+FA #2 Read Count (log2)
r = 0.740
r = 0.727
r = 0.732
r = 0.711
r = 0.664
r = 0.612
r = 0.703
r = 0.649

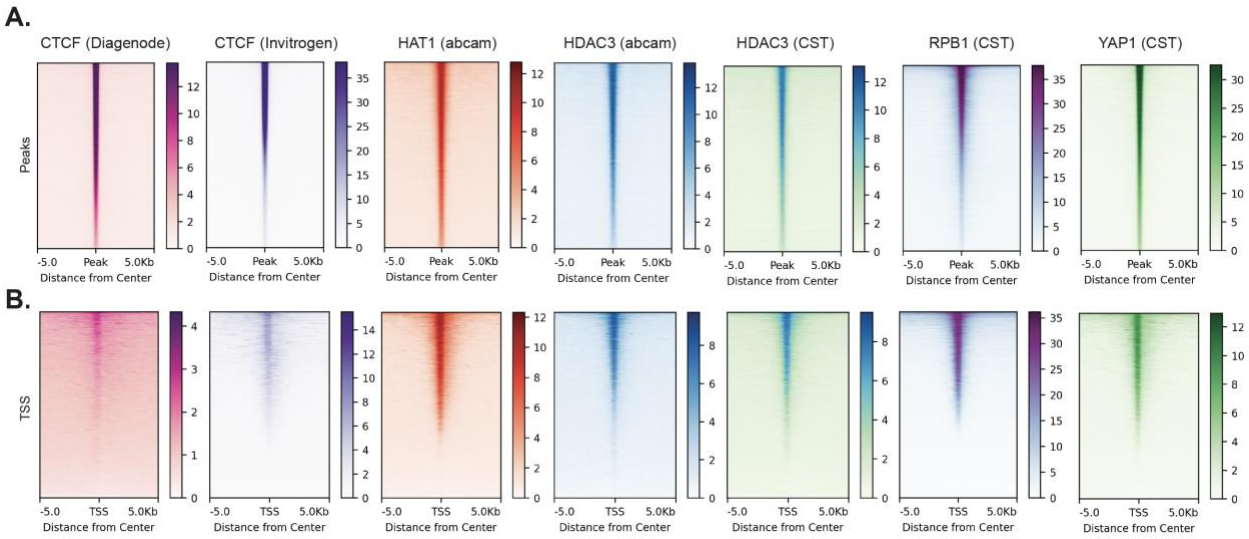
G.


H3K27ac LB3
Automated Replicates
Manual Replicates
H3K27ac EBB
Automated Replicates
Manual Replicates
Rep #2 Read Count (log2)
Rep #1 Read Count (log2)
r = 0.993
r = 0.992
r = 0.984
r = 0.983
Automated & Manual
H3K27ac LB3
H3K27ac EBB
Manual #1 Read Count (log2)
Automated #1 Read Count (log2)
Automated #2 Read Count (log2)
r = 0.984
r = 0.985
r = 0.972
r = 0.976
Manual #2 Read Count (log2)
r = 0.989
r = 0.990
r = 0.967
r = 0.970
EBB & LB3
H3K27ac Automated
H3K27ac Manual
LB3 #1 Read Count (log2)
LB3 #2 Read Count (log2)
EBB #1 Read Count (log2)
EBB #2 Read Count (log2)
r = 0.979
r = 0.986
r = 0.951
r = 0.944
r = 0.979
r = 0.987
r = 0.956
r = 0.950

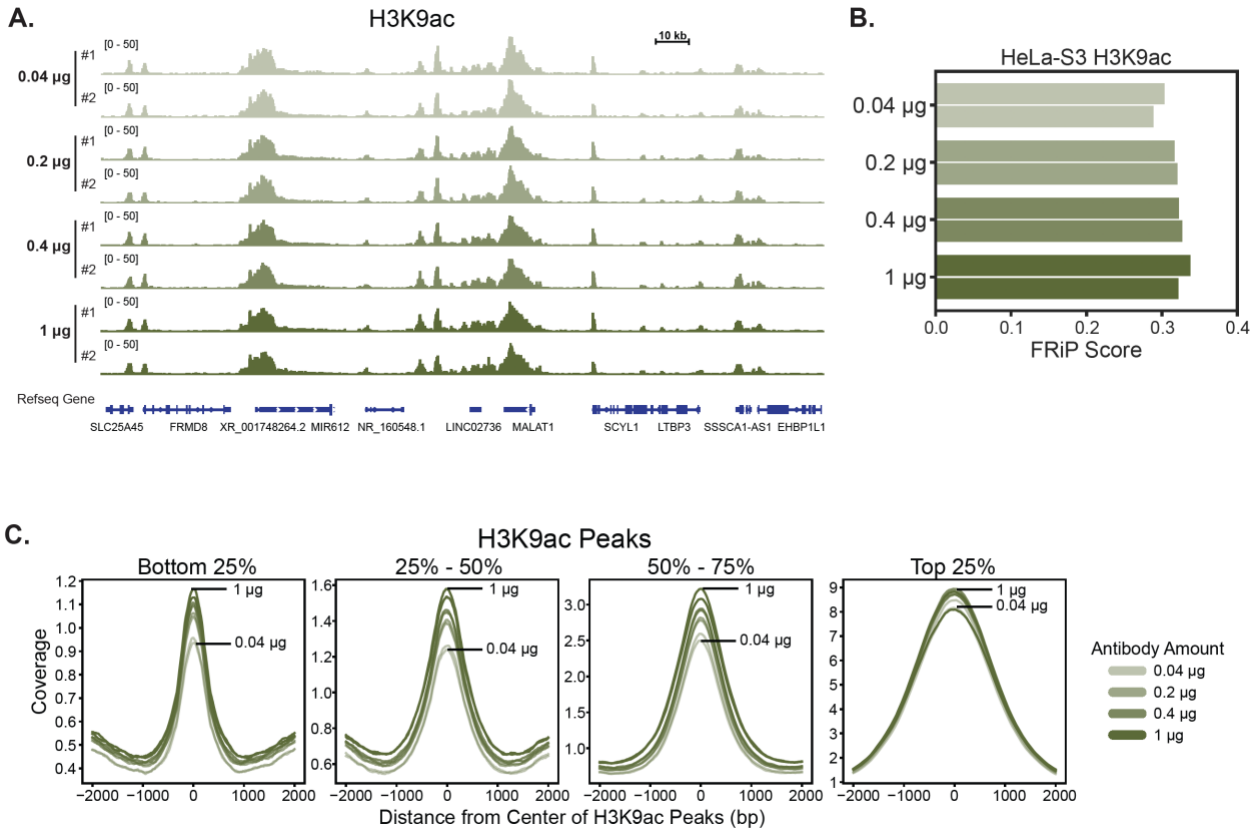
H.


H3K27me3 LB3
H3K27me3 EBB
Automated Replicates
Manual Replicates
Rep #2 Read Count (log2)
Rep #1 Read Count (log2)
r = 0.882
r = 0.802
r = 0.794
r = 0.812
Automated & Manual
Manual #1 Read Count (log2)
Automated #1 Read Count (log2)
Manual #2 Read Count (log2)
Automated #2 Read Count (log2)
r = 0.805
r = 0.801
r = 0.748
r = 0.767
r = 0.748
r = 0.740
r = 0.780
r = 0.800
EBB & LB3
H3K27me3 Automated
H3K27me3 Manual
LB3 #1 Read Count (log2)
EBB #1 Read Count (log2)
LB3 #2 Read Count (log2)
EBB #2 Read Count (log2)
r = 0.804
r = 0.828
r = 0.803
r = 0.798
r = 0.806
r = 0.830
r = 0.803
r = 0.779

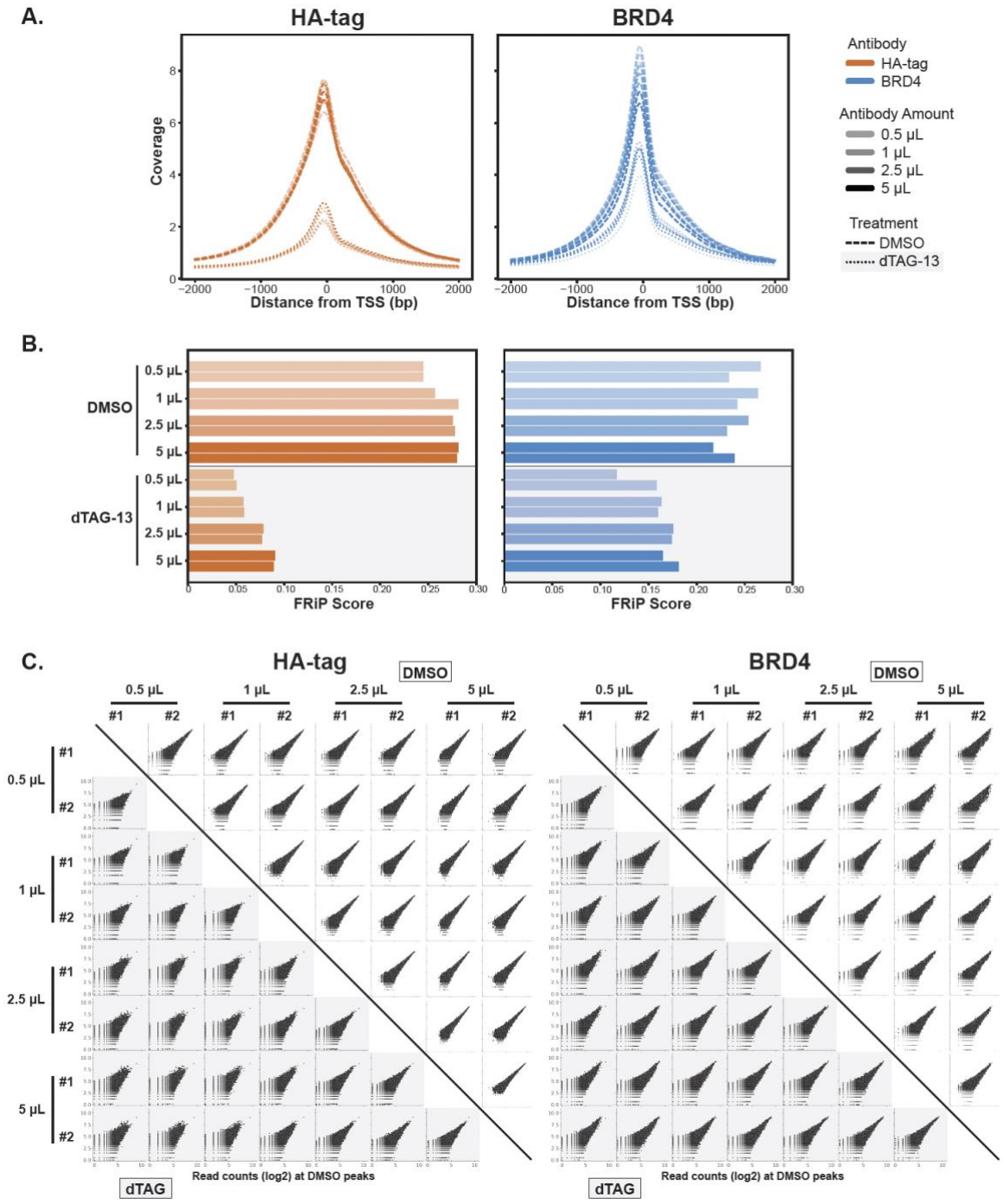
Supplemental Fig. S2: Comprehensive data of our systematic evaluations of spa-ChIP-seq by comparing automated vs. manual workflows, crosslinking conditions and buffer compositions. A-B. IGV genome browser tracks (A) and heatmaps of ±5 kb from the center of TSSs (B) showing the automated and manual H3K27ac ChIP-seq results for comparing crosslinking conditions, DSG+FA and FA only, and buffer compositions, EBB and LB3. **C-D.** IGV genome browser tracks (C) and heatmaps of ±5 kb from the center of peaks (D) showing the automated and manual H3K27me3 ChIP-seq results for comparing crosslinking conditions, DSG+FA and FA only, and buffer compositions, EBB and LB3. **E.** Scatterplots of normalized read counts (log_2) at shared H3K27ac peaks comparing automated replicates, manual replicates, automated and manual workflows for FA only and DSG+FA ChIP-seq samples, as well as comparing FA only and DSG+FA samples under automated and manual workflows. **F.** Scatterplots of normalized read counts (log_2) at shared H3K27me3 peaks comparing automated replicates, manual replicates or automated vs. manual workflows for FA only and DSG+FA ChIP-seq samples, as well as comparing FA only and DSG+FA samples under automated vs. manual workflows. **G.** Scatterplots of normalized read counts (log_2) at shared H3K27ac peaks comparing automated replicates, manual replicates, automated and manual workflows for LB3 and EBB ChIP-seq samples, as well as comparing LB3 and EBB samples under automated and manual workflows. **H.** Scatterplots of normalized read counts (log_2) at shared H3K27me3 peaks comparing automated replicates, manual replicates, automated and manual workflows for LB3 and EBB ChIP-seq samples, as well as comparing LB3 and EBB samples under automated vs. manual workflows. The Pearson r correlation coefficients are reported on the lower right corner of the scatterplot.



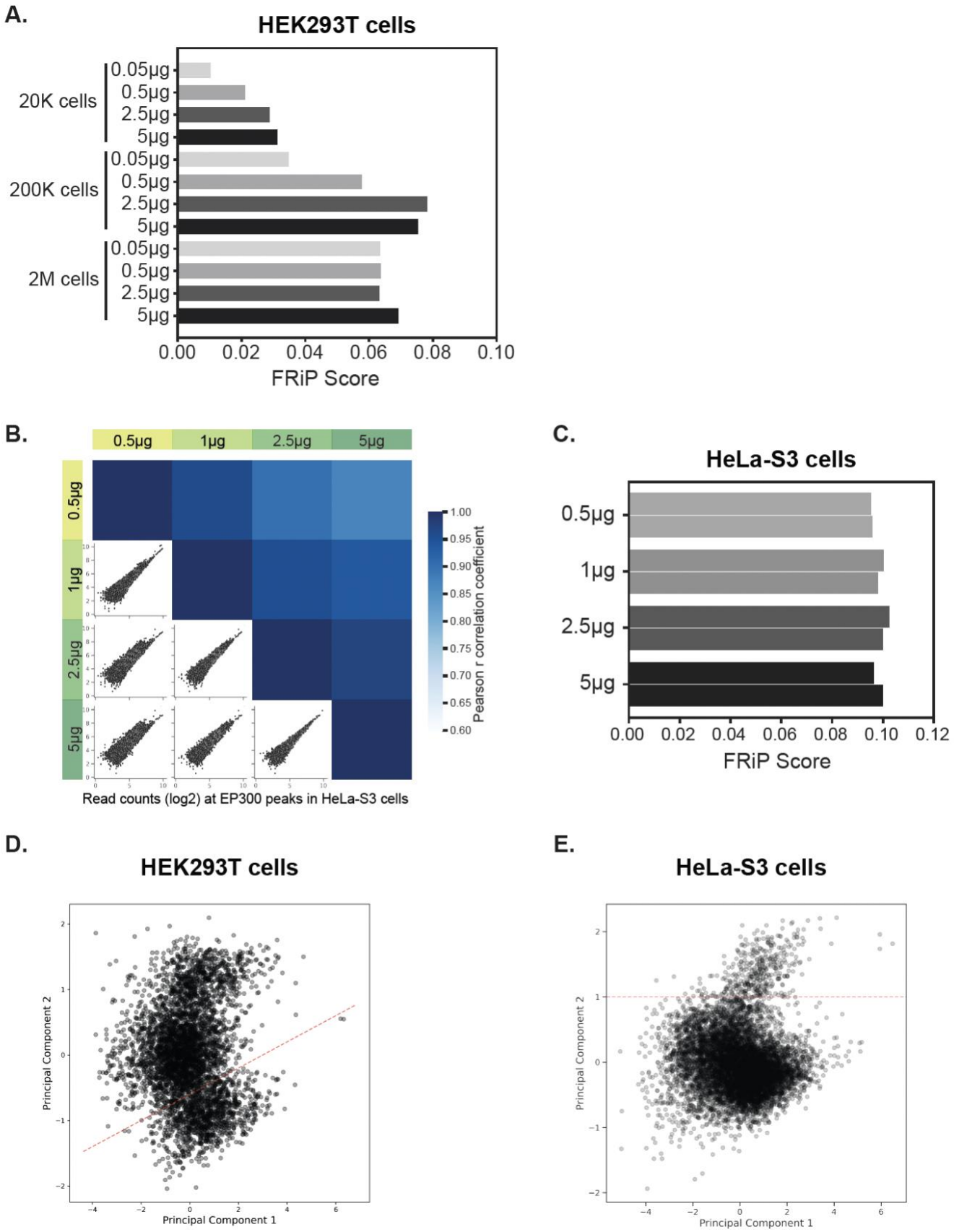
Supplemental Fig. S3: Additional data for mapping of DNA-associated proteins using spa-ChIP-seq. A-B. Heatmaps showing average normalized read density of the replicates at ±5 kb from the center of peaks (A) and TSSs (B) for ChIP-seq of DNA-associated proteins: CTCF, HAT1, HDAC3, Rpb1, and YAP1.



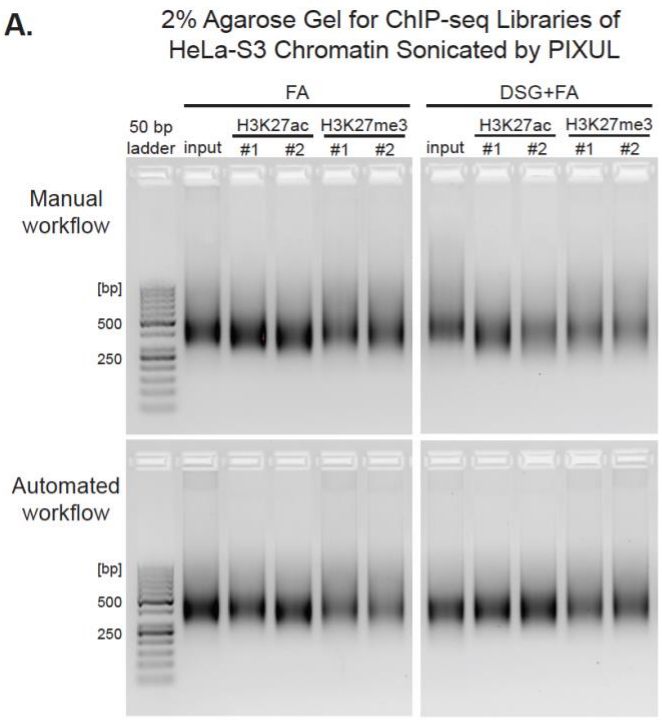
Supplemental Fig. S4: Additional data for H3K9ac antibody titration ChIP-seq experiments. A. IGV genome browser tracks for H3K9ac ChIP-seq done with various amounts of antibody (0.04 µg, 0.2 µg, 0.4 µg, 1 µg), each in replicates. **B.** Bar plot of FRiP scores for ChIP-seq of H3K9ac antibody titrations shown in (A). **C.** Read coverage histograms for H3K9ac antibody titration ChIP-seq signal centered on H3K9ac peaks, separated into quartiles based on the normalized read counts at the peaks.

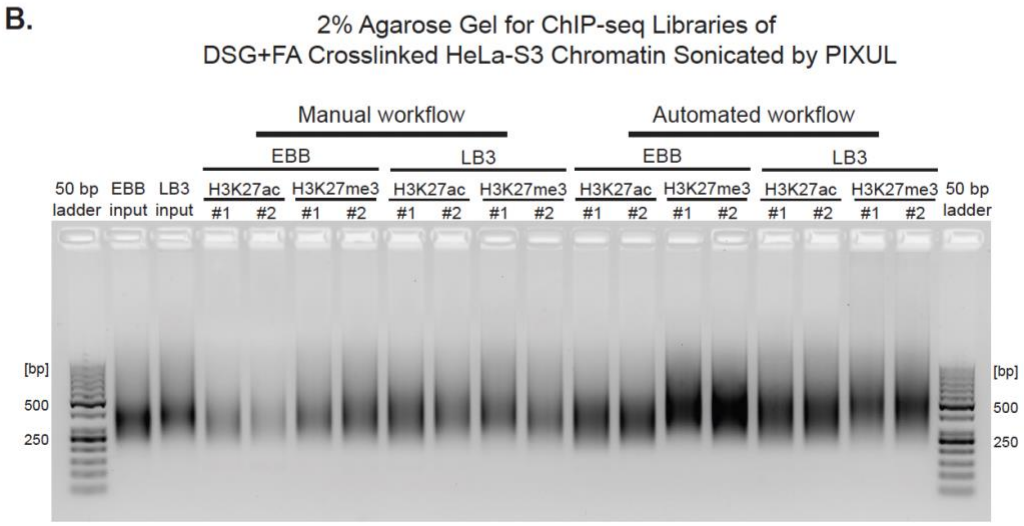


Supplemental Fig. S5: Additional data for HA-tag and BRD4 antibody titration ChIP-seq experiments. A. Metagene plot of ±2000 bp from the center of TSS for BRD4 and HA-tag ChIP-seq in dTAG-13 and DMSO treated HEK293T-BRD4-FKBP12^{F36V}-2xHA cells with 0.5 µL, 1 µL, 2.5 µL, and 5 µL of HA-tag and BRD4 antibodies. **B.** Bar plot of FRiP scores for ChIP-seq of HA-tag and BRD4 antibody titrations shown in (A). **C.** Scatterplots showing normalized read counts (log_2) at DMSO peaks for HA-tag and BRD4 antibody titration. The DMSO samples are in the upper right corner and dTAG samples are in the lower left corner shaded with a light gray background.


A.
HEK293T cells
20K cells
200K cells
2M cells
0.05µg
0.5µg
2.5µg
5µg
0.00
0.02
0.04
0.06
0.08
0.10
FRiP Score
B.
0.5µg
1µg
2.5µg
5µg
Pearson r correlation coefficient
1.00
0.95
0.90
0.85
0.80
0.75
0.70
0.65
0.60
Read counts (log2) at EP300 peaks in HeLa-S3 cells
C.
HeLa-S3 cells
0.00
0.02
0.04
0.06
0.08
0.10
0.12
FRiP Score
D.
HEK293T cells
Principal Component 2
Principal Component 1
E.
HeLa-S3 cells
Principal Component 2
Principal Component 1

Supplemental Fig. S6: Additional data for EP300 antibody titration ChIP-seq experiments in HeLa-S3 and HEK293T cells. A. Bar plot of FRiP scores using the peaks called in each sample for ChIP-seq of EP300 antibody titrations with 0.05 µg, 0.5 ug, 2.5 ug, and 5 ug of antibody and 20K, 200K, and 2M of HEK293T cells. **B.** A matrix of scatterplots showing normalized read counts (log_2) at EP300 peaks and heatmaps showing Pearson r correlation coefficients for EP300 antibody titrations in 1M HeLa-S3 cells with various antibody amounts 0.05 µg, 0.5 µg, 2.5 µg, and 5 µg of EP300 antibody (green shades). **C.** Bar plot of FRiP scores using the merged peaks of the replicates for each condition for ChIP-seq of EP300 antibody titrations with 0.05 µg, 0.5 µg, 2.5 µg, and 5 µg of EP300 and 1M of HeLa-S3 cells. Each condition is done in replicates represented as two bars with the same shading. **D-E.** Principal component analysis (PCA) on the normalized read counts (log_2) at the top 10% of EP300 ChIP-seq peaks for 0.5 µg and 5 µg of antibody in 2M HEK293T cells (D) and 1M HeLa-S3 cells (E). The red dashed line shows how the red subset of peaks in **Fig. 5C** is isolated for each of the samples (the region under the line in D and the region above the line in E).





Supplemental Fig. S7: Size estimation of DNA libraries from automated and manual ChIP-seq with agarose gel. A-B. 2% agarose gel showing DNA libraries generated from manual and automated H3K27ac and H3K27me3 ChIP-seq. (A) Libraries prepared from HeLa-S3 chromatin crosslinked with either FA or DSG+FA and sonicated with LB3 lysis buffer on PIXUL. (B) Libraries prepared from DSG+FA crosslinked HeLa-S3 chromatin lysed in either EBB or LB3 buffer and sonicated using PIXUL. The DNA libraries showed a smear with high intensity around 250–500 bp on the gel, consistent with the expected fragment size distribution.