# Supplementary Materials

## Supplementary Tables

| Gene | Control type | Primer sequence (5'→3') |
|---|---|---|
| *ARHGAP22* | Positive | Fw: GCTGAGAAGGAAGGGCTTAAT<br>Rv: GCTAGTCGGGATGATTTACAGG |
| *COX4I2* | Positive | Fw: GGATACCTCCAAGGCTTCATAC<br>Rv: GTAGTCACAGAACTAGGGTTGG |
| *MTHFR* | Positive | Fw: GGGTGGAACATCTCGAACTATC<br>Rv: GAACGAAGCCAGAGGAAACA |
| *ZMYND8* | Positive | Fw: GGATCTACAAACTTCCCTTCCC<br>Rv: GAAGGCATCGCAGGCTAATA |
| *KLHL11* | Negative | Fw: GACAAGCAGTGGCTCTACAA<br>Rv: CAGTATCGGAAAGAAGCCTACC |
| *SIGIRR* | Negative | Fw: CCAAGCTCAGACCTCAAAGT<br>Rv: TTCTTGCTGTGCTCGTATCC |

**Supplementary Table 1. qPCR primer sequences**. Control primer sequences based on ENCODE peaks.

Fw: forward; rv: reverse.

| Sample | Total Fragments | Mapped Fragments | Alignment Rate | Duplication Rate | Unique Fragments | % chrM Fragments |
|---|---|---|---|---|---|---|
| Active Motif 39133 1:100 15 PCR SDS | 34,467,445 | 34,246,137 | 99.36% | 98.45% | 531,603 | 9.14 |
| Diagenode C15410196 1:100 15 PCR SDS | 13,970,902 | 13,884,243 | 99.38% | 89.15% | 1,506,260 | 11.71 |
| Diagenode C15410196 1:50 15 PCR SDS | 90,812,784 | 83,771,834 | 92.25% | 96.36% | 3,051,389 | 3.16 |
| Abcam-ab177178 1:100 15 PCR SDS | 7,911,303 | 7,839,891 | 99.10% | 55.49% | 3,489,316 | 0.34 |
| Abcam-ab4729 1:100 15 PCR SDS | 146,621,482 | 134,760,046 | 91.91% | 96.94% | 4,123,523 | 1.03 |
| CST9733 1:100 15 PCR SDS | 9,912,113 | 9,823,687 | 99.11% | 57.12% | 4,211,994 | 0.25 |
| Active Motif 39133 1:100 11 PCR SDS | 4,380,560 | 4,347,855 | 99.25% | 81.68% | 796,514 | 6.39 |
| Diagenode C15410196 1:50 11 PCR SDS | 6,379,299 | 6,315,402 | 99.00% | 85.67% | 904,726 | 8.45 |
| Abcam-ab177178 1:100 11 PCR SDS | 21,349,457 | 21,233,210 | 99.46% | 82.97% | 3,615,527 | 0.59 |
| Abcam-ab4729 1:100 11 PCR SDS | 9,195,139 | 9,136,507 | 99.36% | 80.53% | 1,779,088 | 2.58 |
| H3K27me3 11 PCR SDS | 17,976,126 | 17,862,415 | 99.37% | 88.75% | 2,009,039 | 1.50 |
| Active Motif 39133 1:100 11 PCR column | 1,475,470 | 1,464,695 | 99.27% | 46.33% | 786,081 | 7.70 |
| Diagenode C15410196 1:50 11 PCR column | 1,800,855 | 1,785,868 | 99.17% | 52.74% | 843,989 | 12.40 |
| Abcam-ab177178 1:100 11 PCR column | 2,733,847 | 2,713,351 | 99.25% | 51.59% | 1,313,493 | 1.70 |
| Abcam-ab4729 1:100 11 PCR column | 2,153,420 | 2,140,283 | 99.39% | 51.21% | 1,044,240 | 2.73 |
| H3K27me3 11 PCR column | 3,594,729 | 3,563,911 | 99.14% | 48.88% | 1,822,039 | 1.64 |
| Abcam-ab177178 1:100 13 PCR SDS | 8,521,987 | 8,436,844 | 99.00% | 73.70% | 2,219,025 | 0.66 |
| Abcam-ab4729 1:100 13 PCR SDS | 10,601,213 | 10,513,132 | 99.17% | 85.88% | 1,484,538 | 2.29 |
| Active Motif 39133 1:100 13 PCR SDS | 9,424,194 | 9,368,250 | 99.41% | 83.89% | 1,508,766 | 12.90 |
| CST9733 1:100 13 PCR SDS | 25,021,566 | 24,909,845 | 99.55% | 84.24% | 3,926,215 | 0.54 |
| Diagenode C15410196 1:50 13 PCR SDS | 9,497,265 | 9,431,247 | 99.30% | 79.54% | 1,929,369 | 9.76 |
| Abcam-ab177178 1:100 13 PCR column | 10,658,973 | 10,607,882 | 99.52% | 77.48% | 2,389,150 | 0.55 |
| Abcam-ab4729 1:100 13 PCR column | 4,908,403 | 4,878,543 | 99.39% | 81.33% | 910,834 | 2.10 |
| Active Motif 39133 1:100 13 PCR column | 5,617,892 | 5,575,622 | 99.25% | 87.05% | 722,071 | 12.54 |
| CST9733 1:100 13 PCR column | 14,268,256 | 14,197,536 | 99.50% | 82.79% | 2,442,942 | 0.98 |
| Diagenode C15410196 1:50 13 PCR column | 5,168,457 | 5,110,735 | 98.88% | 85.17% | 758,019 | 16.85 |
| Diagenode C15410196 1:100 15 PCR SDS TSA | 3,157,488 | 3,133,254 | 99.23% | 75.22% | 776,367 | 1.65 |
| Diagenode C15410196 1:50 15 PCR SDS TSA | 3,922,942 | 3,879,429 | 98.89% | 70.88% | 1,129,659 | 8.98 |
| Abcam-ab177178 1:100 15 PCR SDS TSA | 5,178,780 | 5,132,446 | 99.11% | 77.70% | 1,144,541 | 8.61 |
| Abcam-ab4729 1:100 15 PCR SDS TSA | 12,570,714 | 12,473,438 | 99.23% | 64.56% | 4,420,973 | 0.71 |

**Supplementary Table 2. Sequencing and alignment metrics of CUT&Tag data in experimental optimization.**

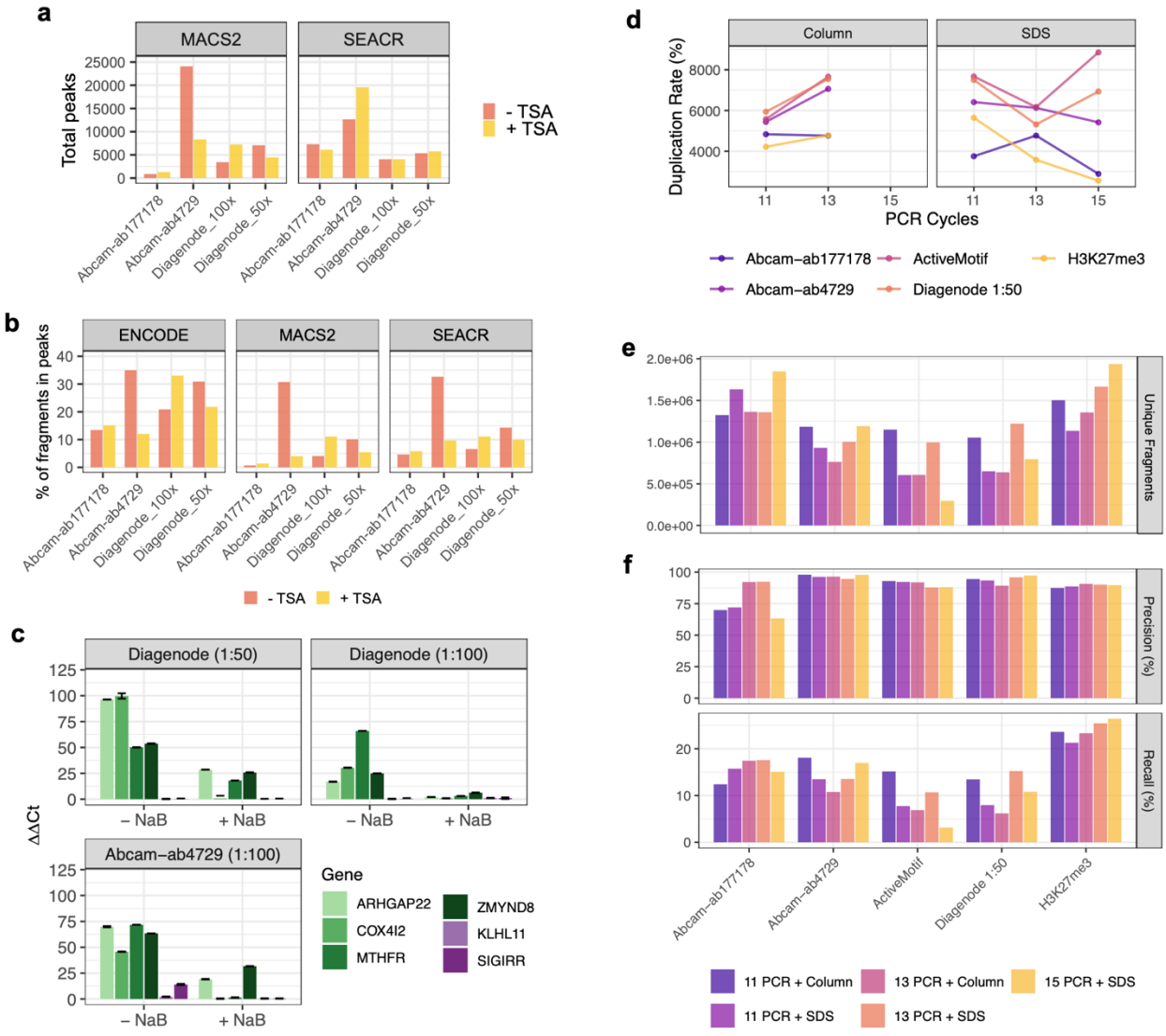
SDS: sodium dodecyl sulfate; TSA: trichostatin A.

| Sample | Total Fragments | Mapped Fragments | Alignment Rate | Duplication Rate | Unique Fragments | % chrM Fragments |
|---|---|---|---|---|---|---|
| Abcam-ab177178-1 1:100 15 PCR SDS | 8,749,603 | 8,599,574 | 98.29% | 13.94% | 7,400,475 | 1.74 |
| Abcam-ab177178-2 1:100 15 PCR SDS | 8,754,917 | 8,585,036 | 98.06% | 14.22% | 7,364,313 | 1.68 |
| Abcam-ab4729-1 1:100 15 PCR SDS | 6,420,162 | 6,287,558 | 97.93% | 30.04% | 4,398,945 | 1.90 |
| Abcam-ab4729-2 1:100 15 PCR SDS | 5,963,254 | 5,797,807 | 97.23% | 17.99% | 4,754,984 | 1.53 |
| Diagenode-1 C15410196 1:50 15 PCR SDS | 6,625,752 | 6,410,793 | 96.76% | 33.07% | 4,290,718 | 10.52 |
| Diagenode-2 C15410196 1:50 15 PCR SDS | 9,790,708 | 9,528,668 | 97.32% | 32.32% | 6,448,536 | 9.42 |
| CST 9733-1 1:100 15 PCR SDS | 7,382,869 | 7,272,406 | 98.50% | 11.49% | 6,436,828 | 0.81 |
| CST 9733-2 1:100 15 PCR SDS | 9,392,608 | 9,254,221 | 98.53% | 12.41% | 8,105,633 | 0.68 |
| H3K27ac Kaya-Okur-1 SRR8383507 (C&T) | 2,471,858 | 2,293,087 | 92.77% | 20.18% | 1,830,390 | 2.02 |
| H3K27ac Kaya-Okur-2 SRR8383508 (C&T) | 3,320,561 | 3,088,577 | 93.01% | 14.87% | 2,629,278 | 2.00 |
| H3K27ac Meers SRR8581604 (C&R) | 6,777,196 | 5,523,031 | 81.49% | 7.30% | 5,120,026 | 0.10 |
| H3K27me3 Kaya-Okur-1 SRR11074238 (C&T) | 3,945,633 | 3,806,740 | 96.48% | 1.43% | 3,752,159 | 0.06 |
| H3K27me3 Kaya-Okur-2 SRR11074239 (C&T) | 4,159,984 | 4,051,439 | 97.39% | 1.36% | 3,996,319 | 0.04 |
| H3K27me3 Meers SRR9073702 (C&R) | 9,047,596 | 8,668,833 | 95.81% | 1.58% | 8,531,865 | 0.02 |

**Supplementary Table 3. Sequencing and alignment metrics of CUT&Tag data used for ENCODE ChIP benchmarking.**

C&R: CUT&RUN; C&T: CUT&Tag; SDS: sodium dodecyl sulfate.

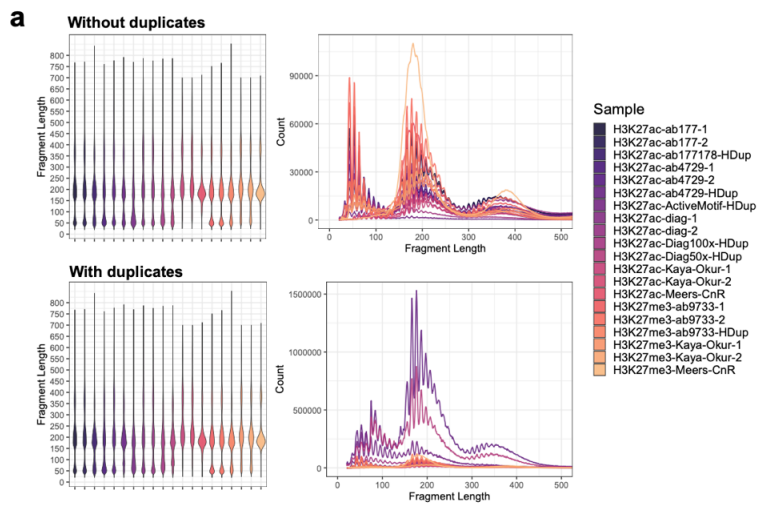
# Supplementary Figures

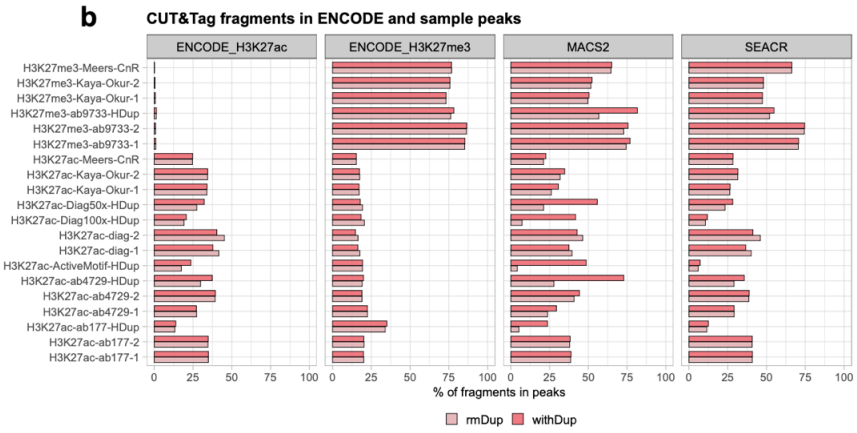


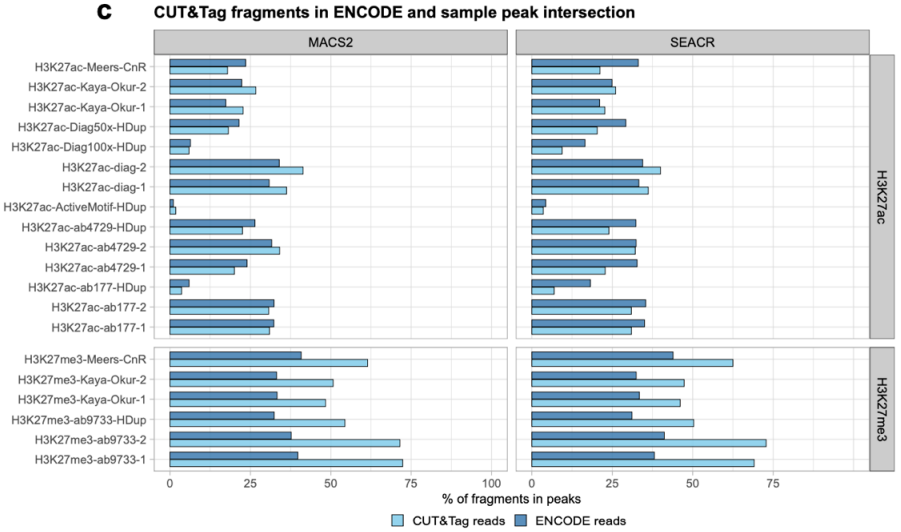

**Supplementary Figure 1. Experimental optimization of CUT&Tag. a-b)** Total peaks called (**a**) and FRiP scores (**b**) obtained in both called sample peaks, called with MACS2 and SEACR, and ENCODE H3K27ac peaks with and without treatment with TSA. **c)** Results of qPCR amplification of genes in most significant ENCODE H3K27ac peak regions (positive controls; green) versus least significant (negative controls; purple) in CUT&Tag experiments performed with top-performing antibodies, with and without HDAC inhibitor sodium butyrate (NaB; 5 mM). **d-f)** Duplication rates (**d**), total unique fragments (**e**), and ENCODE capture (**f**) metrics obtained using column- or SDS-

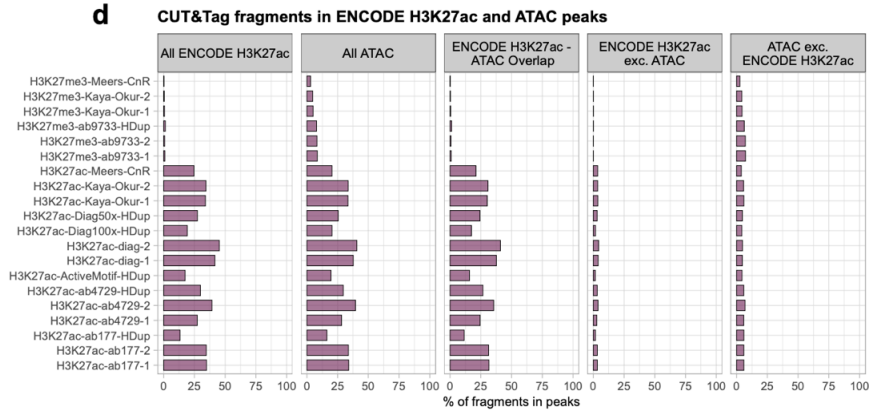
based DNA extraction and 11, 13 or 15 PCR cycles for sequencing library preparation, with all samples down sampled to the same read depth of 2.6 million paired-end reads with SEACR peak calling.

SDS: sodium dodecyl sulfate; TSA: trichostatin A.

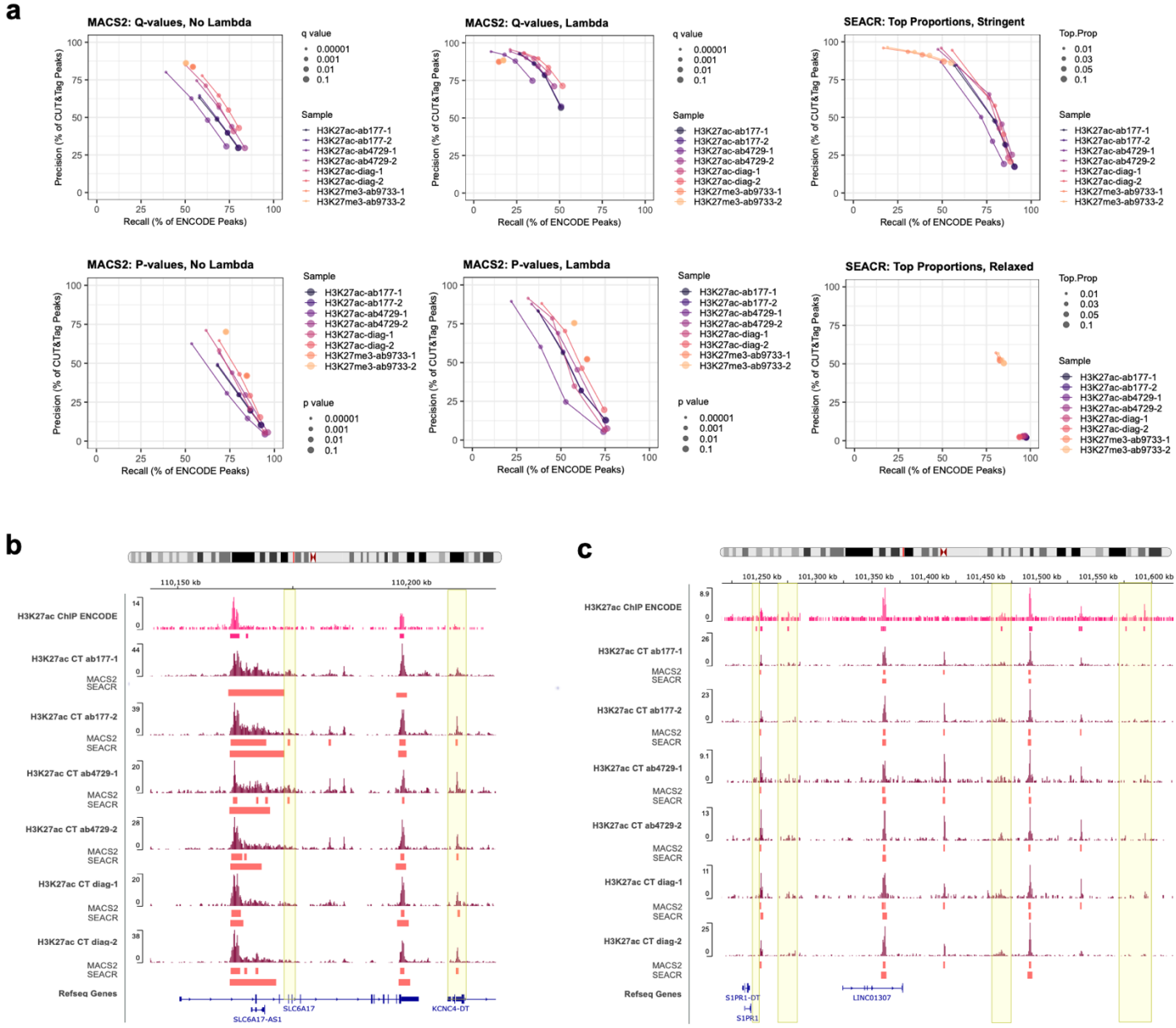

a
Without duplicates
With duplicates
Fragment Length
Count
Fragment Length
Sample
H3K27ac-ab177-1
H3K27ac-ab177-2
H3K27ac-ab177178-HDup
H3K27ac-ab4729-1
H3K27ac-ab4729-2
H3K27ac-ab4729-HDup
H3K27ac-ActiveMotif-HDup
H3K27ac-diag-1
H3K27ac-diag-2
H3K27ac-Diag100x-HDup
H3K27ac-Diag50x-HDup
H3K27ac-Kaya-Okur-1
H3K27ac-Kaya-Okur-2
H3K27ac-Meers-CnR
H3K27me3-ab9733-1
H3K27me3-ab9733-2
H3K27me3-ab9733-HDup
H3K27me3-Kaya-Okur-1
H3K27me3-Kaya-Okur-2
H3K27me3-Meers-CnR

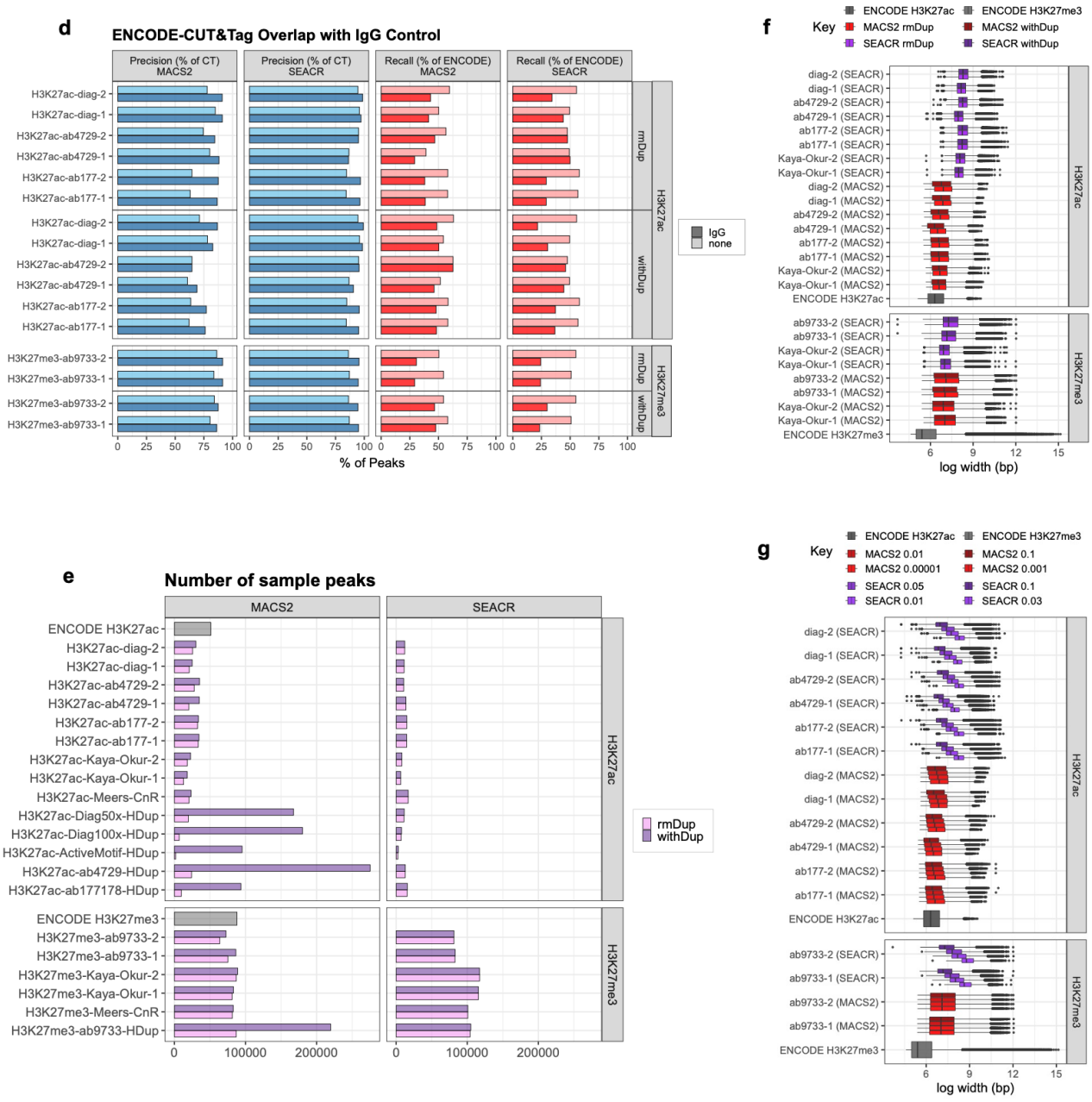

b
CUT&Tag fragments in ENCODE and sample peaks
ENCODE_H3K27ac
ENCODE_H3K27me3
MACS2
SEACR
% of fragments in peaks
rmDup
withDup


c
CUT&Tag fragments in ENCODE and sample peak intersection
MACS2
SEACR
H3K27ac
H3K27me3
% of fragments in peaks
CUT&Tag reads
ENCODE reads


d
CUT&Tag fragments in ENCODE H3K27ac and ATAC peaks
All ENCODE H3K27ac
All ATAC
ENCODE H3K27ac - ATAC Overlap
ENCODE H3K27ac exc. ATAC
ATAC exc. ENCODE H3K27ac
% of fragments in peaks

**Supplementary Figure 2. Quality control for all CUT&Tag, CUT&RUN, ENCODE ChIP samples. a)** Fragment length distributions with and without duplicates. **b)** Percentage of fragments in sample peaks called with SEACR or MACS2, with or without duplicates, and narrow ENCODE H3K27ac and broad H3K27me3 ChIP-seq peaks. **c)** Percentage of CUT&Tag and ENCODE ChIP-seq reads in overlapping ENCODE and CUT&Tag peak regions. **d)** Percentage of sample reads in ENCODE H3K27ac ChIP-seq and ATAC-seq peaks. Figures have been expanded to include all analyzed samples and published datasets.
CnR: CUT&RUN; exc: excluding; HDup: high duplication rate sample; rmDup: duplicates removed.

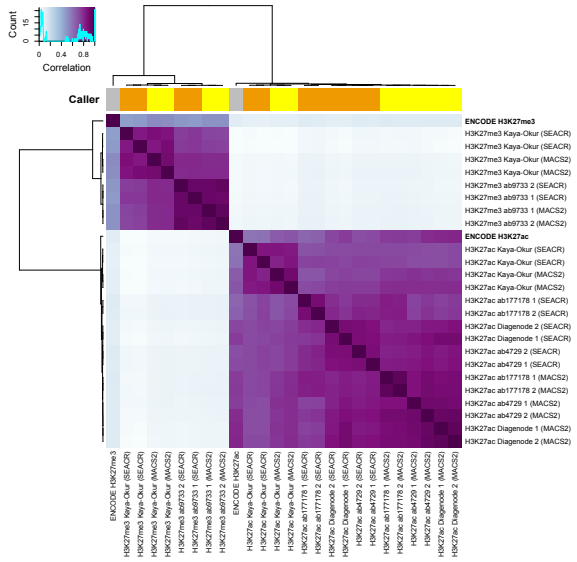

a
MACS2: Q-values, No Lambda
MACS2: Q-values, Lambda
SEACR: Top Proportions, Stringent
MACS2: P-values, No Lambda
MACS2: P-values, Lambda
SEACR: Top Proportions, Relaxed
Precision (% of CUT&Tag Peaks)
Recall (% of ENCODE Peaks)
q value
p value
Top.Prop
Sample
H3K27ac-ab177-1
H3K27ac-ab177-2
H3K27ac-ab4729-1
H3K27ac-ab4729-2
H3K27ac-diag-1
H3K27ac-diag-2
H3K27me3-ab9733-1
H3K27me3-ab9733-2
b
110,150 kb
110,200 kb
H3K27ac ChIP ENCODE
H3K27ac CT ab177-1
H3K27ac CT ab177-2
H3K27ac CT ab4729-1
H3K27ac CT ab4729-2
H3K27ac CT diag-1
H3K27ac CT diag-2
MACS2
SEACR
Refseq Genes
SLC6A17-AS1
SLC6A17
KCNC4-DT
c
101,250 kb
101,300 kb
101,350 kb
101,400 kb
101,450 kb
101,500 kb
101,550 kb
101,600 kb
S1PR1-DT
S1PR1
LINC01307



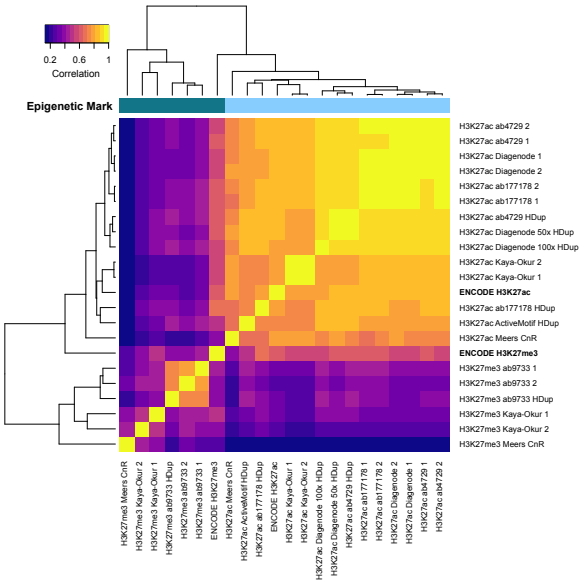

**Supplementary Figure 3. Optimization of peak calling with MACS2 and SEACR. a)** Precision and recall relative to ENCODE ChIP when varying q- and p-value thresholds, active and inactive local lambda for MACS2, and SEACR with stringent and relaxed settings. **b-c)** IGV tracks showing signal and peak regions defined by MACS2 and SEACR, where CUT&Tag peaks are missing in ENCODE (**b**) and ENCODE peaks missing in CUT&Tag (**c**). **d)** Precision and recall of ENCODE peaks with and without an IgG control. **e)** Number of peaks called with optimized peak calling parameters in

samples with and without high duplication rates. **f-g)** Boxplots showing the peak width distributions across samples and peak callers with and without duplicates (**f**) and across peak caller thresholds (**g**). The boxplots represent the median, first and third quartiles, whiskers correspond to 1.5 * the interquartile range (IQR), and dots to display outliers. Bp: base pairs; CT: CUT&Tag; IgG: Immunoglobulin G; kb: kilobases; rmDup: duplicates removed; Top.Prop: proportion of top peaks.

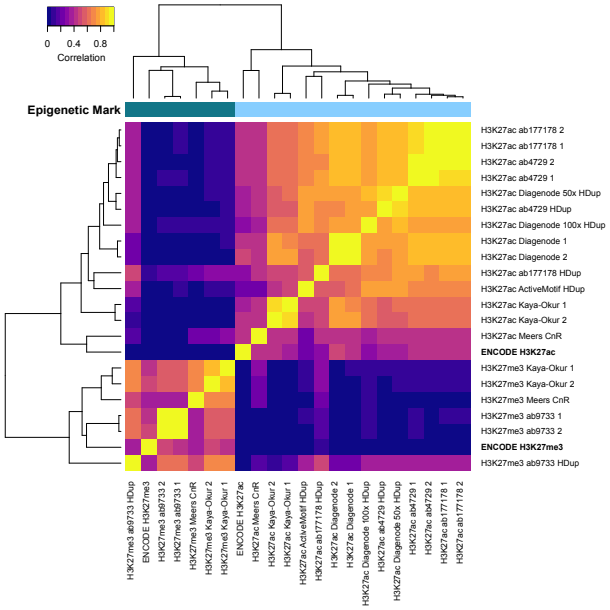
**a** MACS2 and SEACR



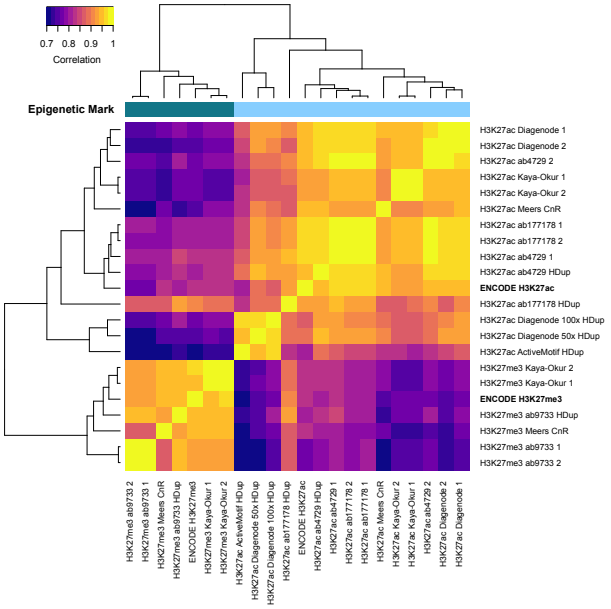

**b** ENCODE H3K27ac

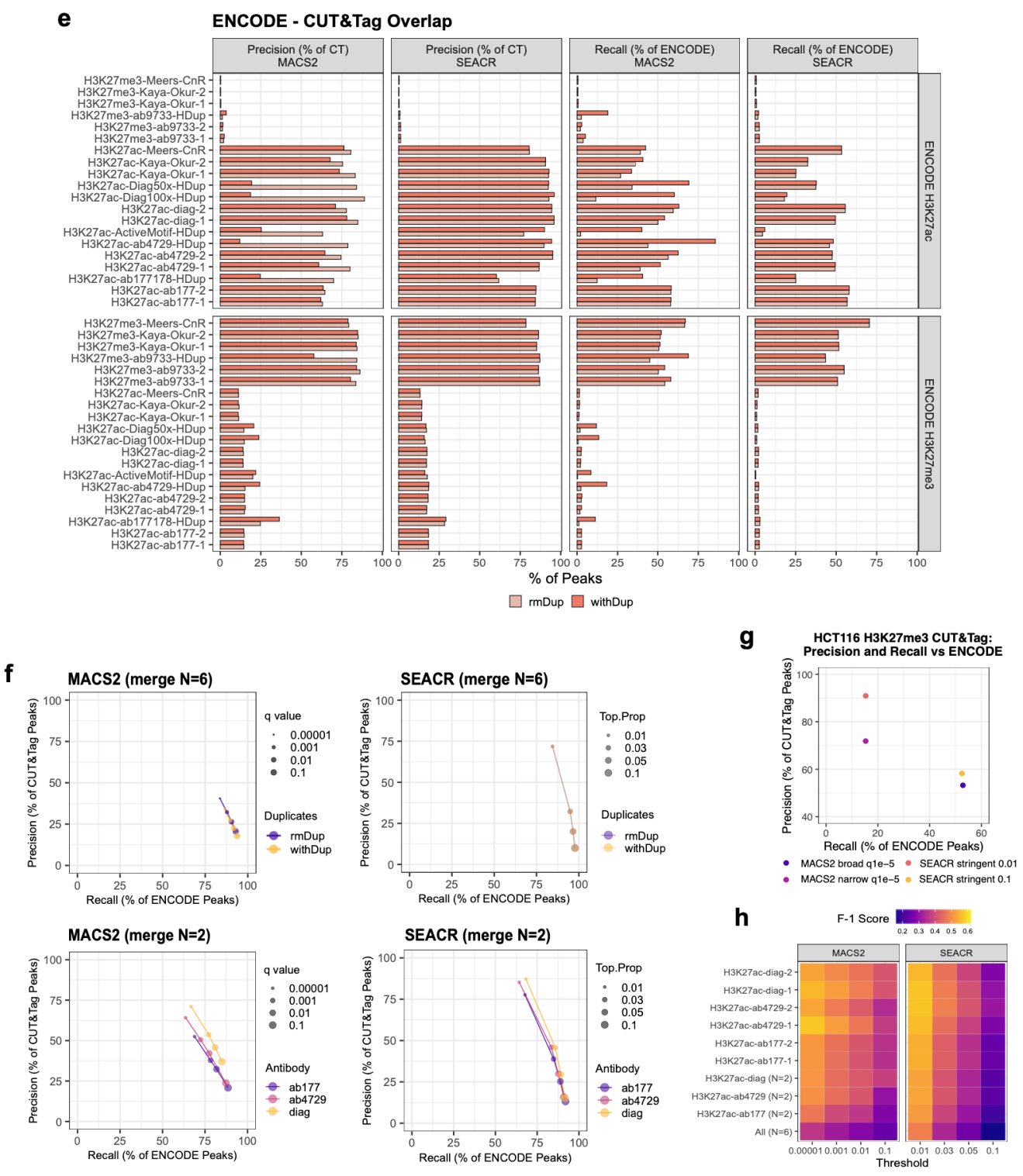



**c** hg19 500bp bins




**d** ENCODE H3K27me3

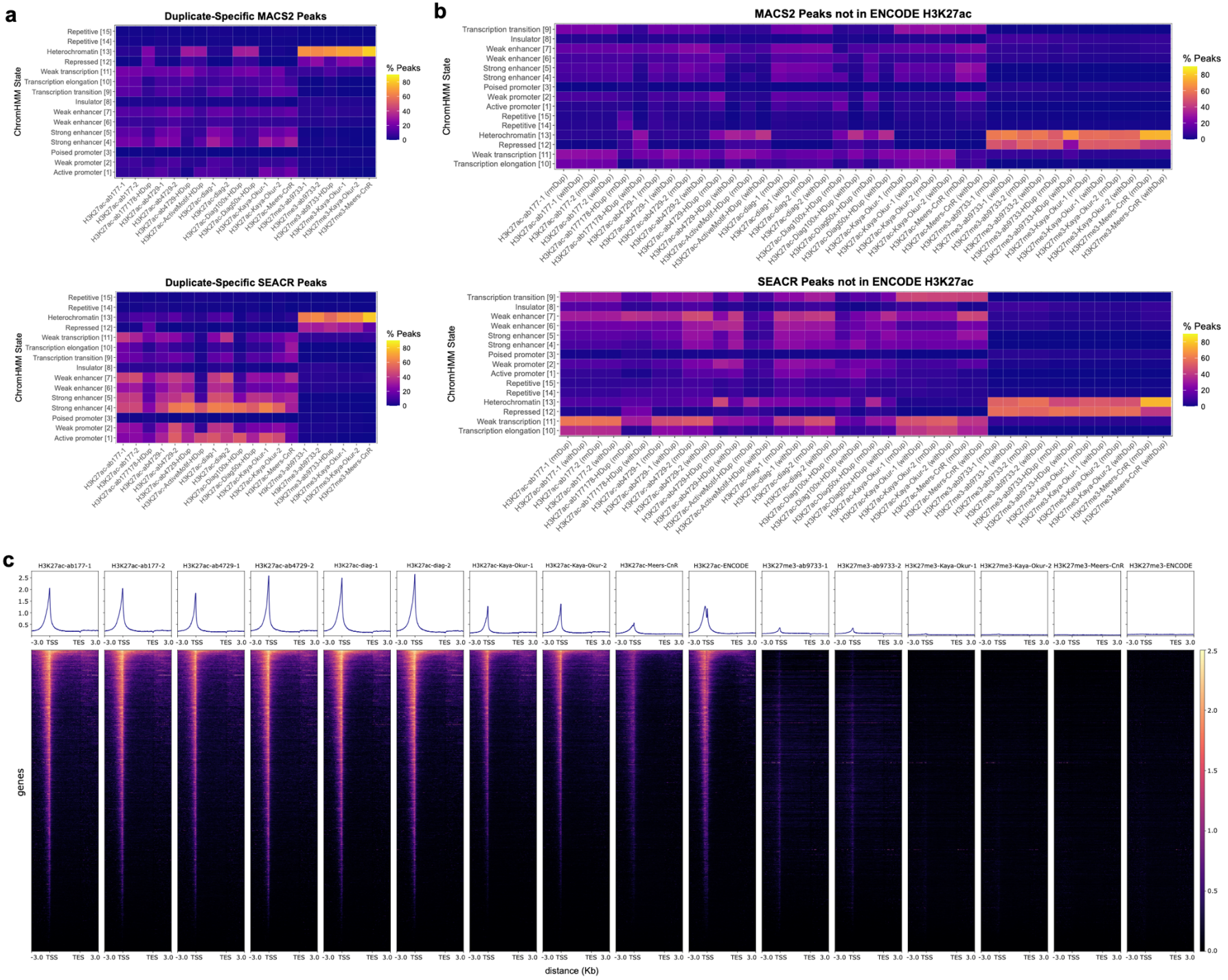




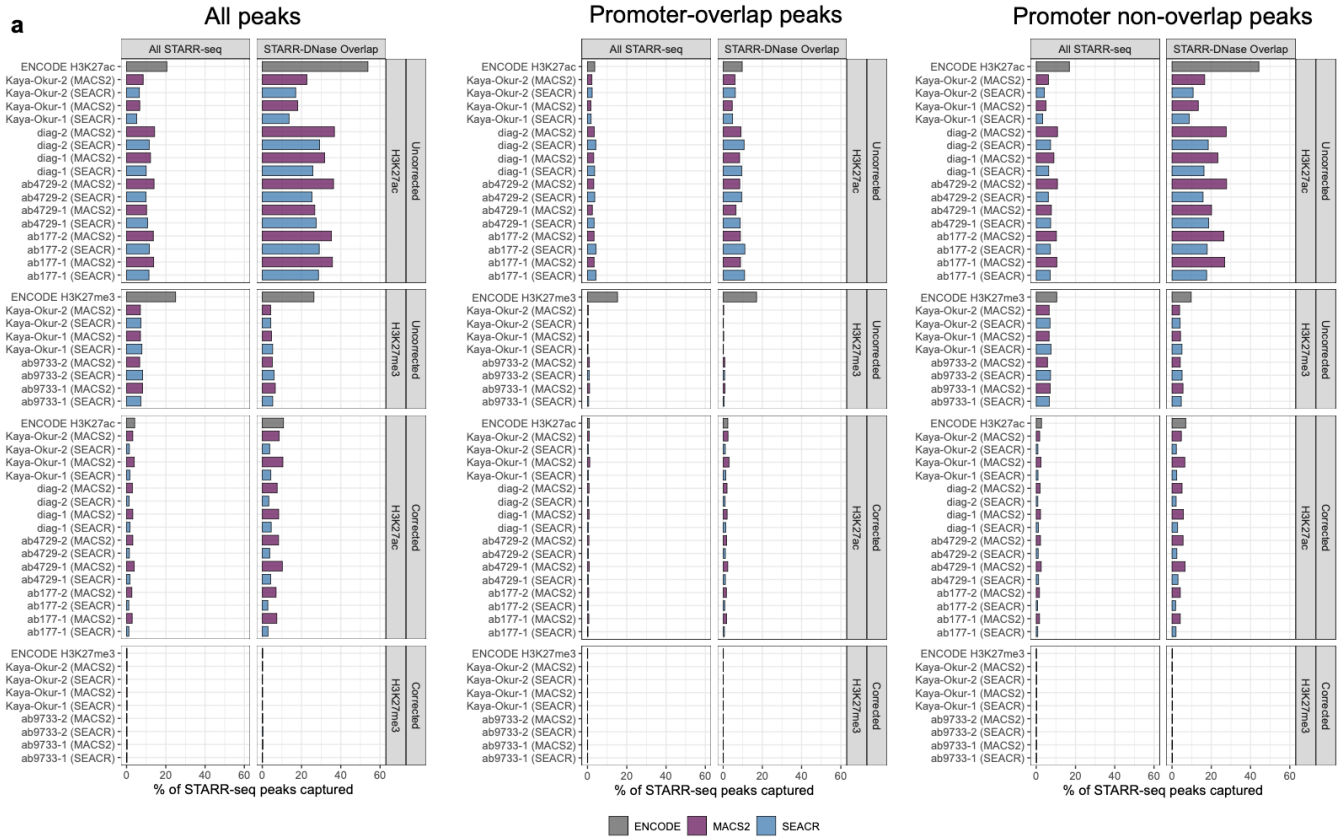

**Supplementary Figure 4. Benchmarking CUT&Tag against ENCODE ChIP-seq. a)** Peak correlations for H3K27ac and H3K27me3 CUT&Tag MACS2 (yellow) and SEACR

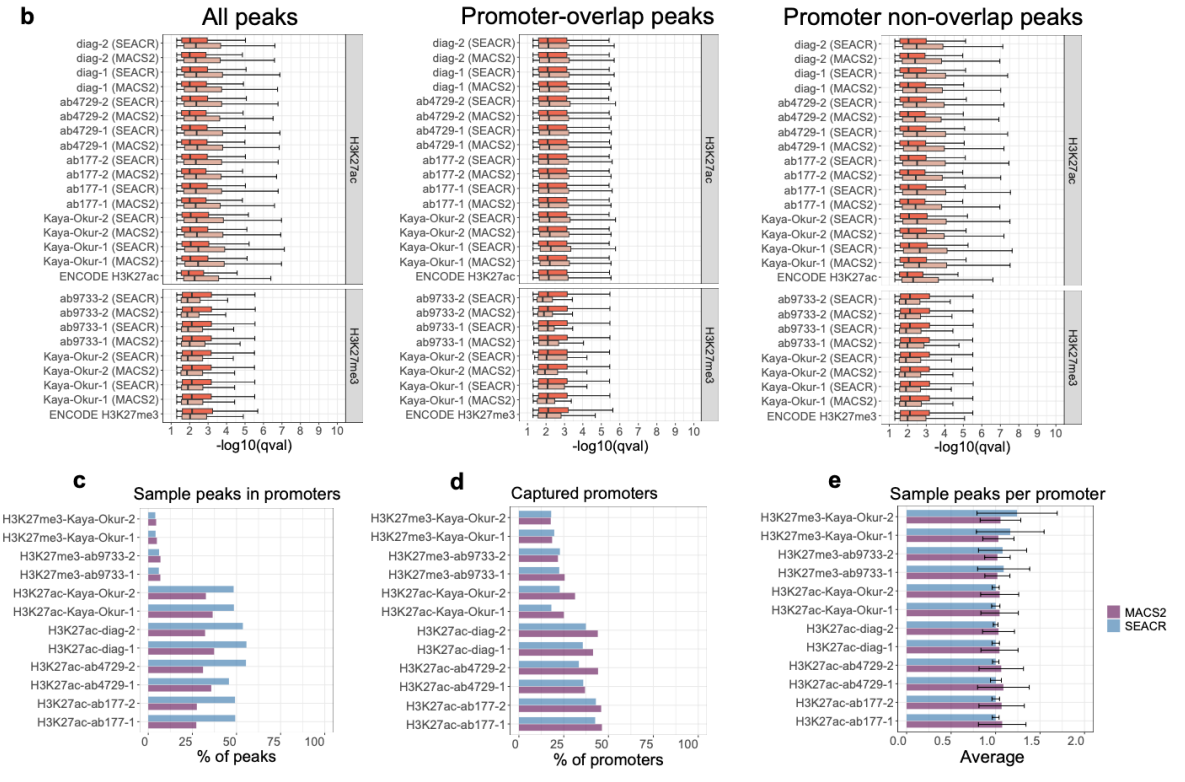
(orange) and ENCODE ChIP-seq (grey) genomic ranges. **b-d)** Read correlations across ENCODE H3K27ac ChIP-seq peak ranges, 500bp genome-wide bins and ENCODE H3K27me3 ChIP-seq peak ranges (H3K27ac samples: light blue; H3K27me3 samples: dark blue). **e)** Capture of ENCODE H3K27ac and H3K27me3 ChIP-seq peaks by CUT&Tag and CUT&RUN peaks called with SEACR or MACS2, with or without duplicates. **f)** Precision and recall of ENCODE capture with aggregate sample peak calling comprising of all internal CUT&Tag samples (top) or by antibody (bottom) at different MACS2 and SEACR thresholds. **g)** Precision and recall of ENCODE H3K27me3 peak capture in the HCT116 cell line. **h)** F-measures of precision and recall of tested H3K27ac antibodies at maximum read depth ('full'), 8 million paired-end reads ('8M'), and merged samples. Comparisons of peaks and reads are without duplicates unless stated otherwise.

Bp: base pairs; CnR: CUT&RUN; CT: CUT&Tag; HDup: high duplication rate sample; rmDup: duplicates removed.


a
Duplicate-Specific MACS2 Peaks
Duplicate-Specific SEACR Peaks
ChromHMM State
% Peaks
b
MACS2 Peaks not in ENCODE H3K27ac
SEACR Peaks not in ENCODE H3K27ac
c
genes
distance (Kb)

**Supplementary Figure 5. Functional assignments of CUT&Tag, CUT&RUN, and ENCODE signal. a-b)** Chromatin state assignments of CUT&Tag peaks specific to duplicate-containing samples (**a**) and CUT&Tag peaks not in ENCODE H3K27ac (**b**). **c)** Heatmaps showing average read coverage around hg19 transcription start sites, with all samples subsampled to 2 million reads. Figures have been expanded to include all analyzed samples and published datasets.
CnR: CUT&RUN; HDup: high duplication rate sample; kb: kilobases; rmDup: duplicates removed, TES: transcription end site; TSS: transcription start site.


a
All peaks
Promoter-overlap peaks
Promoter non-overlap peaks
All STARR-seq
STARR-DNase Overlap
Uncorrected
Corrected
H3K27ac
H3K27me3
% of STARR-seq peaks captured
ENCODE
MACS2
SEACR


b
All peaks
Promoter-overlap peaks
Promoter non-overlap peaks
-log10(qval)
c
Sample peaks in promoters
% of peaks
d
Captured promoters
% of promoters
e
Sample peaks per promoter
Average
MACS2
SEACR

**Supplementary Figure 6. Capture of promoters and STARR-seq enhancers by CUT&Tag. a)** Genome-wide overlap percentage of total STARR-seq peaks with CUT&Tag (deduplicated) sample peaks and ENCODE ChIP-seq peaks (left), both without (top) and with (bottom) correction of total genomic coverage, and STARR-seq peaks restricted to those overlapping ENCODE K562 DNase-seq peaks (right). **b**) Significance −log10(q) values of DNase-overlapping STARR-seq peaks captured and not captured by CUT&Tag and ChIP-seq. Boxplot represents the median, first and third quartiles, whiskers correspond to 1.5 * the interquartile range (IQR). Welch two-sided t-test used to calculate the significance of the difference between captured and missed vvvalueluelues in each peak set. **c**) Percentage of CUT&Tag peaks overlapping promoters. **d**) Percentage of total reference promoters captured by CUT&Tag peaks. **e**) Average number of CUT&Tag sample peaks overlapping a captured promoter. Error bars represent mean±SD across CUT&Tag peaks for a given antibody and peak caller.