| | Adipose | Brain | Heart | Lamina | Liver | Lung | Muscle | Testis |
|------------------------------|--------------------|-------|-------|--------|-------|------|--------|--------|
| Starting Tissue (mg) | 208 | 109 | 103 | 95 | 55 | 53 | 106 | 105 |
| Homogenization Time (min) | 8 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| Fixation Time (min) | 10 | 9 | 9 | 9 | 9 | 9 | 9 | 12 |
| Shearing Volume (µL) | 400 | 1500 | 1800 | 1800 | 1500 | 1500 | 1500 | 1300 |
| Shearing Cycles | 5 x 8 ¹ | 10 | 10 | 10 | 8 | 10 | 10 | 8 |
| Chromatin per IP (ng) | 200 | 700 | 800 | 900 | 1500 | 1500 | 280 | 1500 |

Supplementary Table 1. Parameters for ChIP Extraction and Shearing

¹Chromatin from adipose was sheared five times for 8 cycles

Supplementary Table 2. Chromatin and Antibody Amounts Used for ChIP of Stallion Tissues

| Tissuo | Chromatin | Antibody Amount (μg) | | | | | | |
|---------|-----------|----------------------|---------|---------|----------|--|--|--|
| 115500 | IP (ng) | H3K27ac | H3K4me1 | H3K4me3 | H3K27me3 | | | |
| Adipose | 200 | 1 | 0.5 | 0.5 | 1 | | | |
| Brain | 700 | 0.5 | 0.5 | 0.5 | 1 | | | |
| Heart | 800 | 0.5 | 0.5 | 0.5 | 1 | | | |
| Lamina | 900 | 1 | 0.5 | 0.5 | 1 | | | |
| Liver | 1500 | 2 | 1 | 1 | 2 | | | |
| Lung | 1500 | 2 | 1 | 1 | 1 | | | |
| Muscle | 280 | 1 | 0.5 | 0.5 | 0.5 | | | |
| Testis | 1500 | 2 | 1 | 1 | 2 | | | |

| Software | Parameter | H3K27ac | H3K4me1 | H3K4me3 | H3K27me3 |
|----------------------|------------------|---------|------------------|----------------|----------|
| MACS2 | Size | Narrow | Narrow/ | Narrow/ Narrow | |
| | | | | | |
| | Size Flag | | broad | | |
| | FDR ¹ | 0.01 | 0.05 | 0.01 | 0.05 |
| | Broad Cutoff | | 0.1 | | |
| | Genome size | | 2,409,159,894 bp | | |
| Gap Size | | | 4 | | |
| SICERpy ² | Window Size | n/a | | | 200 |
| | Genome Fraction | n/a | | | 0.973 |

Supplementary Table 3. Software Parameters for ChIP-seq Peak Calling in MACS2 and SICER

¹The false discovery rate (FDR) cutoff dictates both peak number and peak width in MACS2, so histone marks with broader peaks have looser FDR cutoffs (https://github.com/hbctraining/Intro-to-ChIPseq). H3K4me1 was at one point considered to have broad peaks but has since been determined to have an intermediate peak width (Kingsley et al., 2020). ²SICER was only used to call peaks for the broad mark, H3K27me3.

| | НЗК | H3K27ac | | H3K4me1 | | H3K4me3 | | H3K27me3 | | Input | |
|--|-------------------|----------------------|-------------------|----------------------|-------------------|----------------------|-------------------|----------------------|-------------------|----------------------|--|
| Tissue_Replicate | Raw Read Pairs | Usable Read Pairs | |
| Adipose_AH3* | 68,730,027 | 9,161,533 | 87,399,621 | 44,863,661 | 53,728,350 | 28,668,914 | 163,649,254 | 61,352,911 | 57,287,919 | 33,441,186 | |
| Adipose_AH4 | 64,121,448 | 19,628,311 | 73,755,325 | 22,976,612 | 71,588,828 | 30,633,260 | 172,819,418 | 77,353,465 | 56,710,987 | 31,577,565 | |
| Brain_AH3 | 44,153,723 | 28,767,074 | 51,902,716 | 39,353,133 | 48,165,691 | 30,245,100 | 89,113,219 | 51,495,389 | 43,318,326 | 30,758,728 | |
| Brain_AH4 | 53,550,924 | 37,164,559 | 45,941,617 | 33,748,599 | 44,199,605 | 31,024,168 | 123,828,178 | 70,346,101 | 45,370,082 | 29,345,385 | |
| Heart_AH3 | 51,902,076 | 32,672,638 | 43,860,168 | 31,026,046 | 51,509,237 | 33,775,014 | 115,749,332 | 46,128,360 | 43,193,521 | 31,251,565 | |
| Heart_AH4 | 45,090,183 | 28,986,609 | 64,302,815 | 36,586,039 | 44,069,238 | 32,088,317 | 141,578,920 | 65,737,749 | 59,163,545 | 41,930,841 | |
| Lamina_AH3 | 40,535,808 | 23,823,796 | 51,986,630 | 23,743,730 | 48,277,375 | 24,570,676 | 91,980,974 | 58,482,470 | 44,456,575 | 32,290,294 | |
| Lamina_AH4 | 51,953,800 | 24,590,690 | 49,361,910 | 29,682,378 | 41,990,329 | 17,500,964 | 175,850,495 | 107,502,509 | 57,425,242 | 38,117,196 | |
| Liver_AH3 | 55,697,969 | 35,491,407 | 45,896,092 | 30,860,281 | 69,512,707 | 39,275,132 | 126,294,140 | 68,471,421 | 66,316,277 | 44,083,269 | |
| Liver_AH4 | 46,094,286 | 25,056,779 | 40,668,291 | 28,448,813 | 58,847,906 | 30,318,223 | 192,622,865 | 102,596,377 | 51,996,032 | 36,922,319 | |
| Lung_AH3 | 51,223,693 | 36,118,820 | 54,431,763 | 38,105,392 | 45,017,929 | 32,396,228 | 120,694,401 | 66,585,343 | 50,253,855 | 36,332,494 | |
| Lung_AH4 | 38,186,321 | 26,005,392 | 46,855,312 | 32,876,189 | 48,153,388 | 33,423,330 | 174,764,785 | 85,474,365 | 44,634,249 | 31,783,927 | |
| Muscle_AH3 | 59,556,353 | 16,216,664 | 50,633,437 | 29,981,650 | 71,090,388 | 19,685,919 | 80,658,715 | 43,455,906 | 43,227,165 | 18,252,957 | |
| Muscle_AH4 | 59,556,353 | 34,276,737 | 50,633,437 | 34,362,506 | 71,090,388 | 31,289,430 | 211,037,887 | 99,616,774 | 43,227,165 | 30,297,899 | |
| Testis_AH3 | 59,238,170 | 42,301,940 | 42,281,966 | 29,481,648 | 56,479,066 | 27,740,548 | 80,574,901 | 44,709,318 | 49,793,842 | 36,030,620 | |
| Testis_AH4 | 44,952,694 | 31,632,424 | 38,900,952 | 27,586,751 | 51,773,961 | 34,079,183 | 88,305,332 | 45,983,224 | 54,636,399 | 36,616,903 | |
| Adipose_AH3_ Reseq* | 146,616,053 | 2,680,311 | - | - | - | - | - | - | - | - | |
| Adipose_AH3_ Merged* | 215,346,080 | 11,841,844 | - | - | - | - | - | - | - | - | |
| *The "Usable Read Pairs" from both the original and resequenced (Reseq) H3K27ac_Adipose_AH3 samples were merged prior to peak calling. | | | | | | | | | | | |

Supplementary Table 4. The Number of Raw Read Pairs Generated and Usable Read Pairs Remaining After Filtering for Stallion Samples

** The bolded cells represent samples that did not reach the usable read pair target of 45 M for H3K27me3 and 20 M for the remaining marks and input samples.

Supplementary Table 5. Overlap of MACS2 H3K27me3 Peaks with SICERpy H3K27me3 Peaks

| | Total MACS2 Peaks | Overlapping MACS2 Peaks | Percentage of Overlapping Peaks |
|---------|----------------------|----------------------------|------------------------------------|
| Adipose | 137,696 | 119,044 | 86% |
| Brain | 87,453 | 74,844 | 86% |
| Heart | 104,207 | 89,129 | 86% |
| Lamina | 190,940 | 149,917 | 79% |
| Liver | 265,202 | 212,970 | 80% |
| Lung | 249,506 | 206,519 | 83% |
| Muscle | 103,055 | 96,847 | 94% |
| Testis | 129,784 | 95,889 | 74% |



Supplementary Figure 1. Peak Length Distribution by Histone Mark. The distribution of peak length called by MACS2 was similar across all four histone marks (A), yet the distribution of peak lengths for H3K27me3 varied drastically between peaks called by MACS2 and SICERpy (B).

A