Supplementary Figure 1
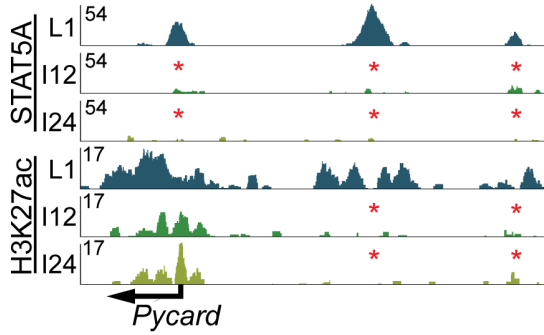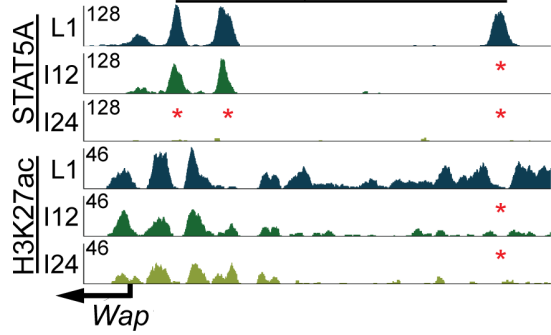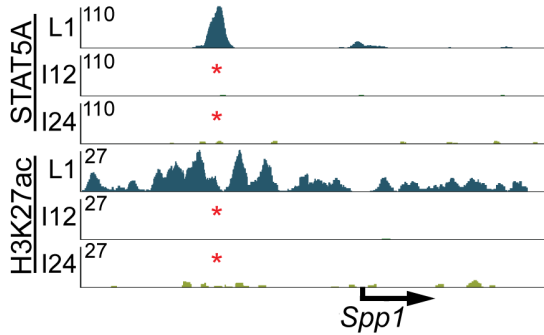
a Class 1: All enhancers decommission
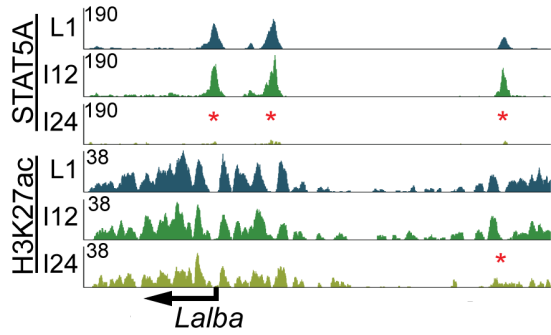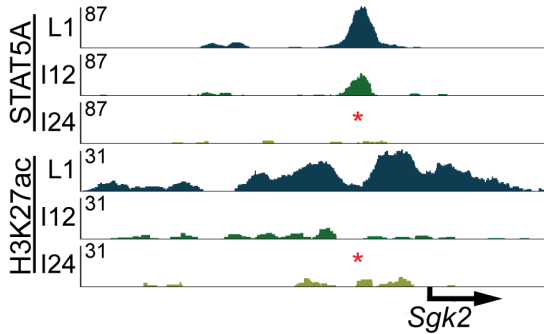
b Class 1: One enhancer decomissions

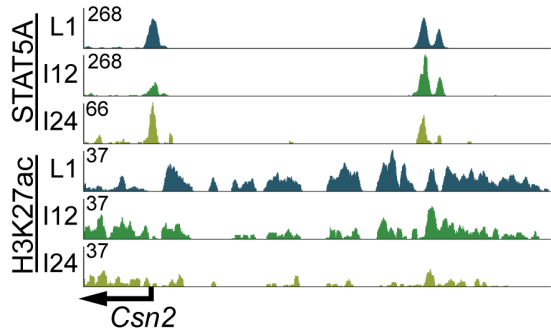**c Class 1: Solitary enhancer decomission**
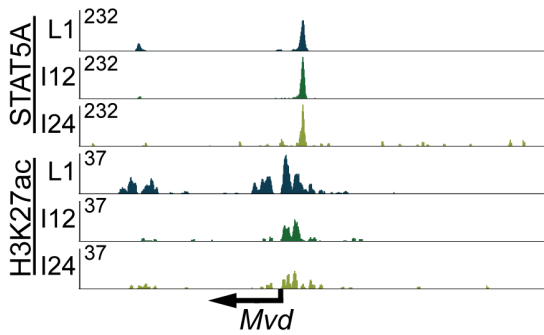
d Class 2: All enhancers decomission
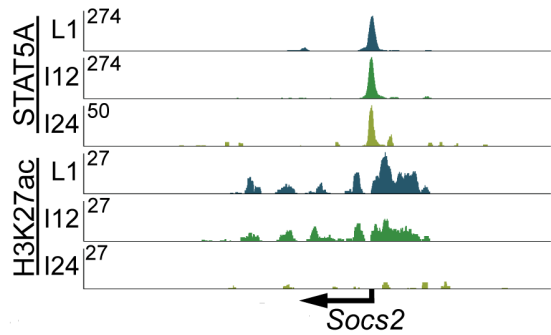
e Class 2: Solitary enhancer decomissions

f Class 3: Resilient enhancers

g Class 3: Resilient solitary enhancer

h Class 3: Non mammary-specific gene

Supplementary Figure 1

Representatives of the different enhancer classes. (a) *Pycard* is a class 1 gene and all of its enhancers are decommissioned within 12 hours of terminating lactation. H3K27ac was also absent after 12 hours (I12). (b) The super-enhancer of *Wap*, another class 1 gene, was decommissioned at different stages of involution. The most distal enhancer was already absent at I12, but the other two enhancers decommissioned at I24. (c) *Spp1* represents a solitary enhancer of class 1. (d) *Lalba* is a representative of the class 2 enhancers, as all of them decommissioned during 24 hours of involution. (e) A gene with solitary enhancer of class 2 is *Sgk2*. (f) *Csn2*, a class 3 gene, with its resilient enhancers being present after 24 hours of involution. (g) *Mvd* is a gene with a resilient solitary enhancer. (h) A non mammary-specific gene of class 3 is *Socs2*.

Supplementary Figure 2

de novo motif results	p-value
ACTTCCTGGT	10^{-94}
ATGGCCTGAGGC	10^{-41}
CTTGGCAA	10^{-26}
TTGTTCCGGC	10^{-25}
TCTAGGTA	10^{-20}
TTCACGGAAACT	10^{-20}
AAACCGGTTG	10^{-19}
TGGTCAAACAGG	10^{-15}
CAGATATGATCA	10^{-14}
TTACGTAA	10^{-12}

Supplementary Figure 2

De novo motif search on class 1 enhancers without GAS motif.

Supplementary Figure 3

9,213 STAT5A peaks with H3K27ac

Rapid decommission of mammary enhancers upon termination of lactation

4372 4526 315

class 1
class 2
class 3

Peak coverage analysis see Figure 1b

Reduction of STAT5A peaks to 2,556 within 10KB to genes

1191 1304 61

GAS motif and Peak coverage analysis see Figure 1e-i

Interplay between STAT5 and GR

49% (1258) 51% (1298)

STAT5A peaks with GR co-binding at L1
STAT5A peaks at L1

Classification of peaks with co-binding

4% (45)
53% (691) 43% (562)

Peak coverage analysis see Figure 2b

Detailed analysis of class 1 peaks see Figure 3

Peak coverage analysis see Figure 2d

Mammary enhancers sense STAT5 concentrations

WT L1 *Stat5*^ΔE/ΔE L1

3956 5255 805

Peak coverage analysis and heatmap see Figure 6c,d

Based on those 9,213 enhancers

Linking STAT5-sensitive enhancers to highly-induced genes

1,189 genes

656 516 17

Induced mammary genes

58% (688) 42% (501)

mammary-specific genes
not mammary-specific genes

Further analyses of mammary-specific genes see Figure 4b-g

Homeostasis between STAT5 and STAT3 binding

STAT5A L1 **STAT3 I24**

7623 1586 6987

From shared binding sites to co-binding at each stage of involution

STAT5A
9213 4942 317
L1 I12 I24

STAT3
2430 3604 8610

STAT3 and STAT5A co-binding within the three enhancer classes

Class 1 Class 2 Class 3

6% 25% 55%
94% 75% 45%

Further analyses see Figure 7c,d

Supplementary Figure 3

Workflow of data analyses. The figure illustrates the steps of data analyses. 9,213 STAT5A enhancers with underlying H3K27ac were identified for wild type tissue at day one of lactation (L1). Based on those enhancers the first approach was to identify three classes of enhancers, based on their decommission during involution (left panel). By overlapping the ChIP-seq peaks from all three stages 4,372 enhancers were classified as class 1, decommission within 12 hours, 4,526 as class 2, decommission within 24 hours, and 315 as class 3, enhancers resilient after 24 hours of involution. Those enhancer sets were annotated to the closest TSS and reduced to enhancers, which are within 10KB to the annotated TSS resulting in 1,191 class 1, 1,304 class 2 and 61 class 3 enhancers. Based on those enhancers two more comprehensive analyses followed. The first one comprised the interplay of those STAT5A peaks with GR. Out of 2,556 enhancers 51% had an underlying GR enhancer. Based on those 1,298 enhancers 43% were categorizes as class 1, 53% as class 2 and only 4% as class 3. The second analysis comprised the annotation of those 2,556 enhancers to 1,189 genes, which where further classified into the three categories based on the decommission of the first associated enhancer. Thereby 656 genes are class 1, with at least one enhancer decommissioned within 12 hours, 516 are class 2, with at least one enhancer decommissioned within 24 hours and 17 genes with resilient enhancers. To investigate how many of those are mammary-specific genes they were combined with RNA-seq data from pregnancy day 6 and day one of lactation. 42% of the genes were induced during pregnancy, having a minimum twofold induction from pregnancy day 6 to lactation day one.

The second part based on the set of 9,213 STAT5A enhancers investigates to what extend the Stat5$^{\Delta E/\Delta E}$ mice carrying homozygous *Stat5* enhancer mutations are able to establish STAT5A enhancers (middle panel). The venn diagram shows that only about 60% of the wild type enhancers present in L1 are also present in Stat5$^{\Delta E/\Delta E}$ at L1.

The third and final part (right panel) is based on the homeostasis of STAT5A and STAT3 binding. As STAT5A decreases during involution and STAT3 increases

the theoretical amount of possible binding sites can only be investigated using the two most extreme stages with the majority of enhancers. The venn diagram shows that STAT5A at L1 and STAT3 at I24 had 1,586 binding sites shared between them. The bar plot below further shows the decommission of STAT5A versus the gain of STAT3 during involution. To see which kind of STAT5A binding are more likely to be replaced by STAT3 the pie charts show the enhancers of the three categories. Only 6% of class 1 enhancers showed a cobinding/ replacement with STAT3, for class 2 peaks it is already 25% and in class 3 55%. For those pie chart the STAT3 binding at I24 was used.