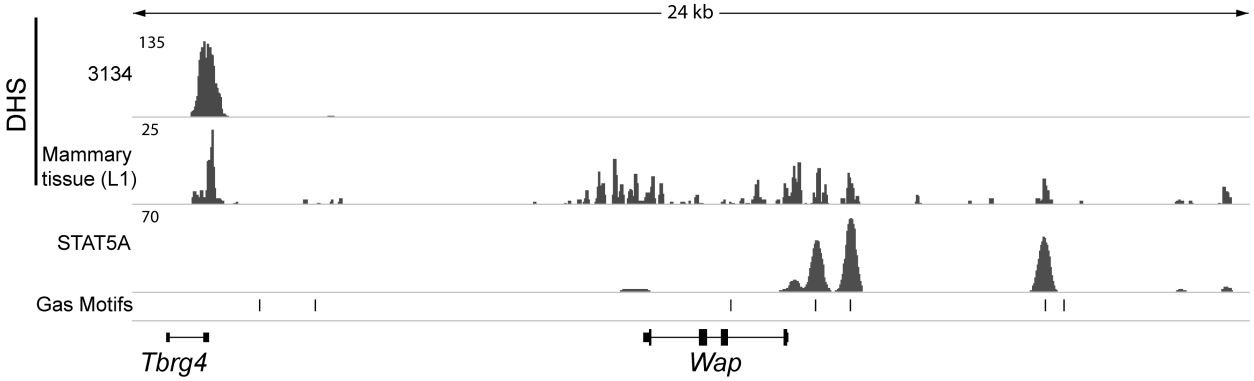
Metser et al.

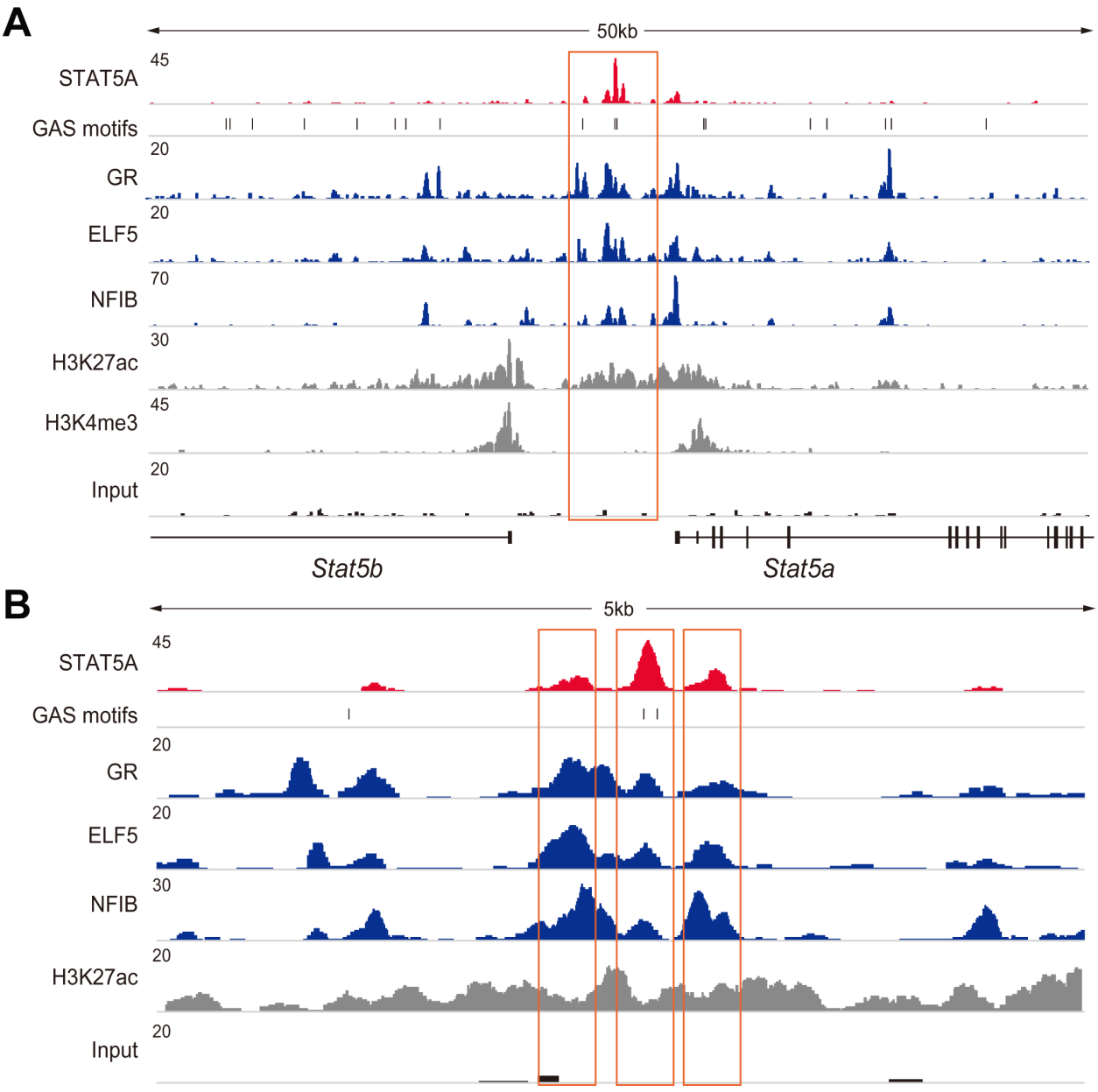
Supplementary figures

Figure S1



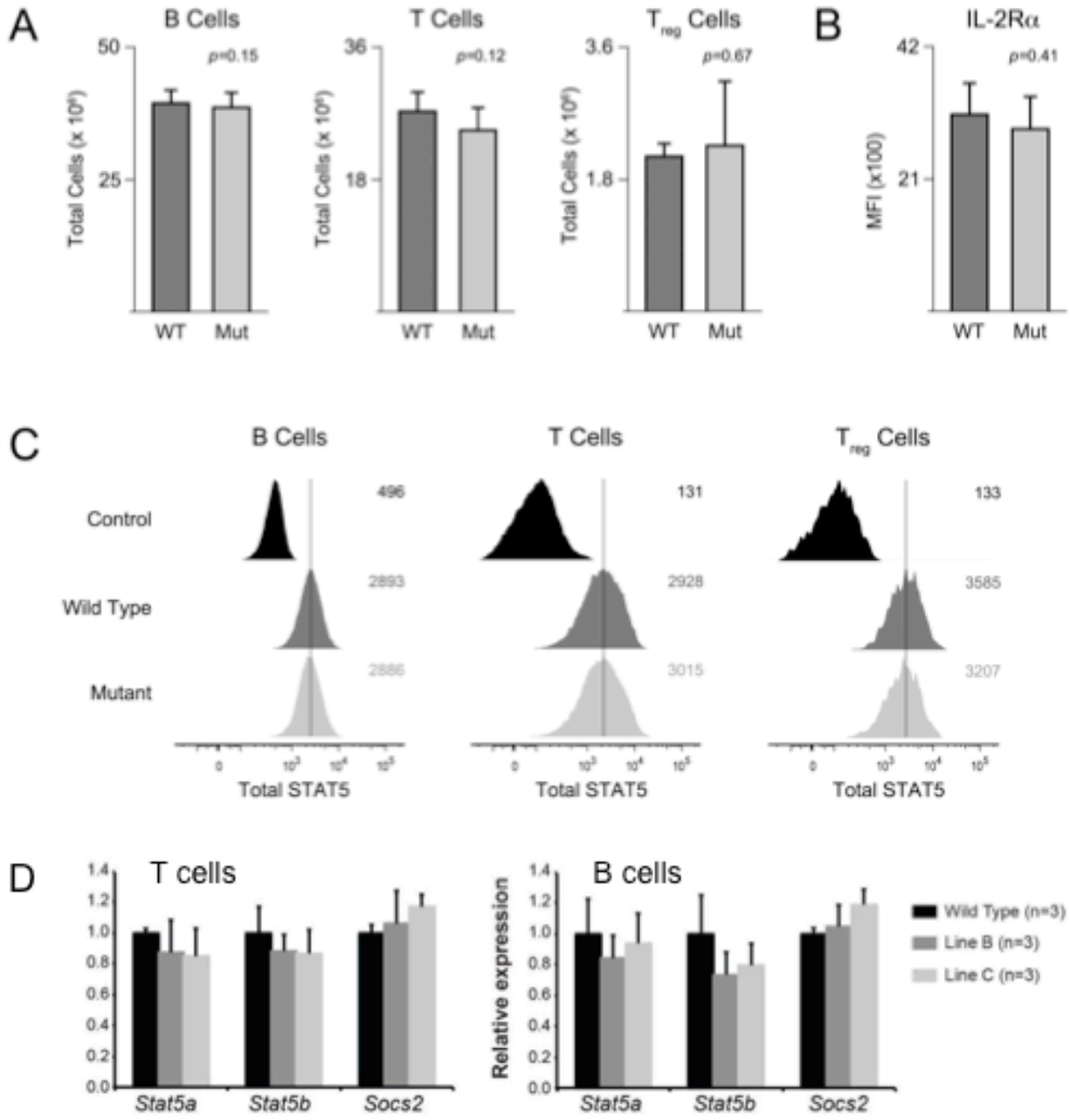
Differential DNaseI hypersensitivity (DHS) of a mammary-specific locus in mammary tissue at day 1 of lactation and in the mammary cell line 3134. The DHS data from mammary tissue are from this study and the data on the 3134 cell line can be found in GEO (GSE37074) (GSM1014196). In intact mammary tissue DHS areas are associated with the mammary-specific *Wap* gene and the widely expressed *Tbrg4* gene. In contrast, there are no *Wap*-associated DHS sites in the cell line 3134 but a strong DHS site at the *Tbrg4* promoter.

Figure S2



Transcription factor binding to the *Stat5a/b* intergenic region in mammary tissue at day 13 of pregnancy. In addition to STAT5, binding of GR, NFIB and the mammary-enriched transcription factor ELF5 was detected. B) An enlarged image of the putative mammary-specific enhancer. Three STAT5 binding regions were detected, with the center peak being the strongest and coinciding with GAS motifs. GR, ELF5 and NFIB binding coincided with STAT5 occupancy. The three mammary STAT5A binding peaks, and in particular the center one, coincide with sequences devoid of H3K27ac marks. The input controls validates the specificity of the transcription factor binding.

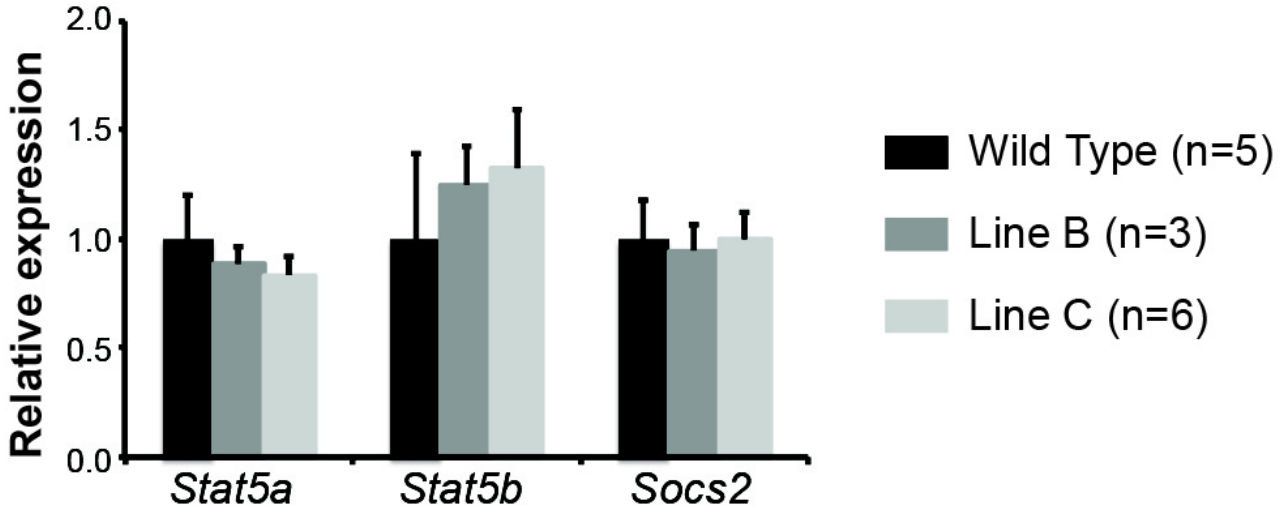
Figure S3



Impact of the autoregulatory intergenic *Stat5* enhancer on T and B cells.
(**A**) Bar graphs show absolute numbers of CD3ε$^-$ B220$^+$ B cells, CD3ε$^+$ B220$^-$ T cells and CD3ε$^+$ CD4$^+$ FoxP3$^+$ Treg cells in the spleen of wild type (WT) and STAT5 enhancer mutant mice (Mut) (line B). (**B**) Bar graph shows the mean fluorescence intensity (MFI) for surface IL-2Pα on Treg cells. (**C**) Histograms shows flow cytometry measurements for total STAT5 protein in B cells, T cells and Treg cells. Numbers denote mean fluorescence intensity. (**A**-**C**) Data are representative or compiled from 3 individual experiments (7 mice/genotype total). (**D**) Expression of *Stat5a*, *Stat5b* and

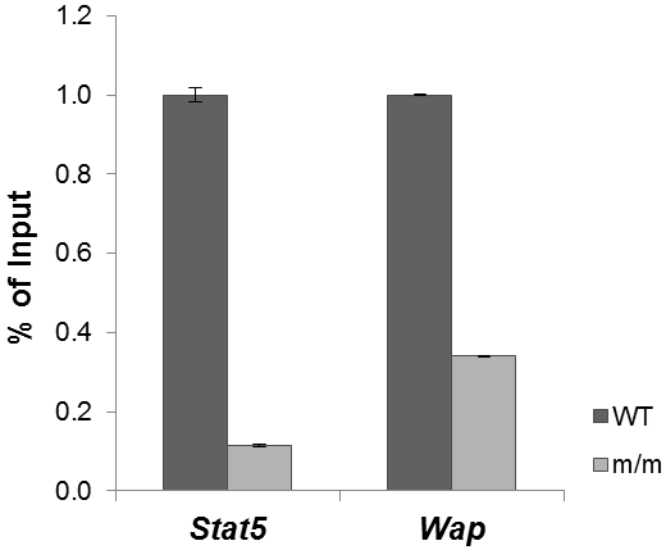
Socs2 was analyzed in T cells and B cells from line B (absence of site GAS2) and line C (absence of both GAS sites) using qRT-PCR (Material and Methods).

Figure S4



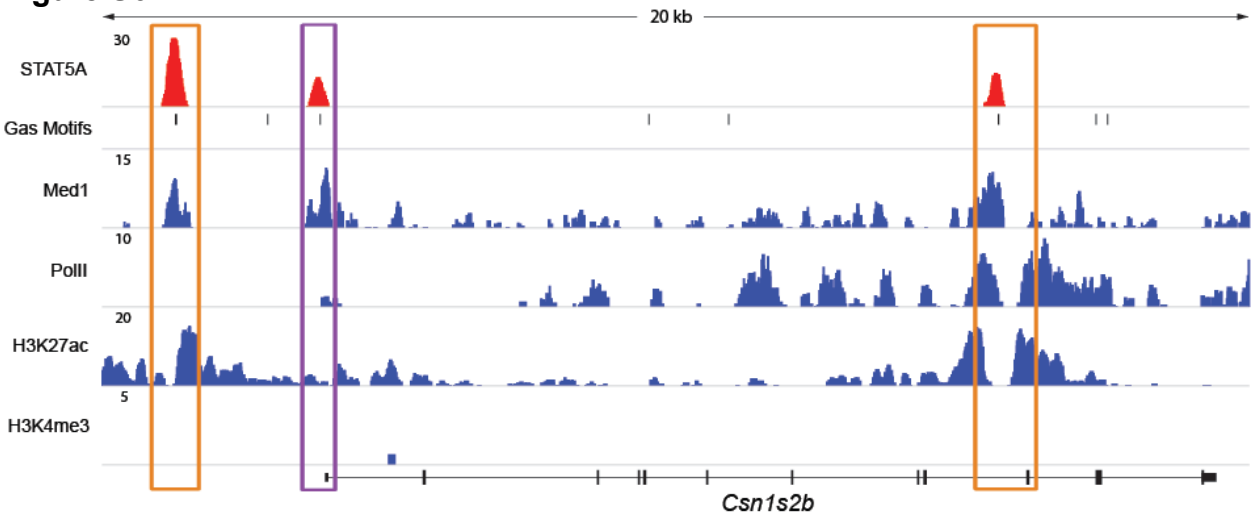
Impact of the autoregulatory intergenic *Stat5* enhancer on liver tissue. Expression of *Stat5a*, *Stat5b* and *Socs2* was analyzed in line B (absence of site GAS2) and line C (absence of both GAS sites) using qRT-PCR (Material and Methods).

Figure S5



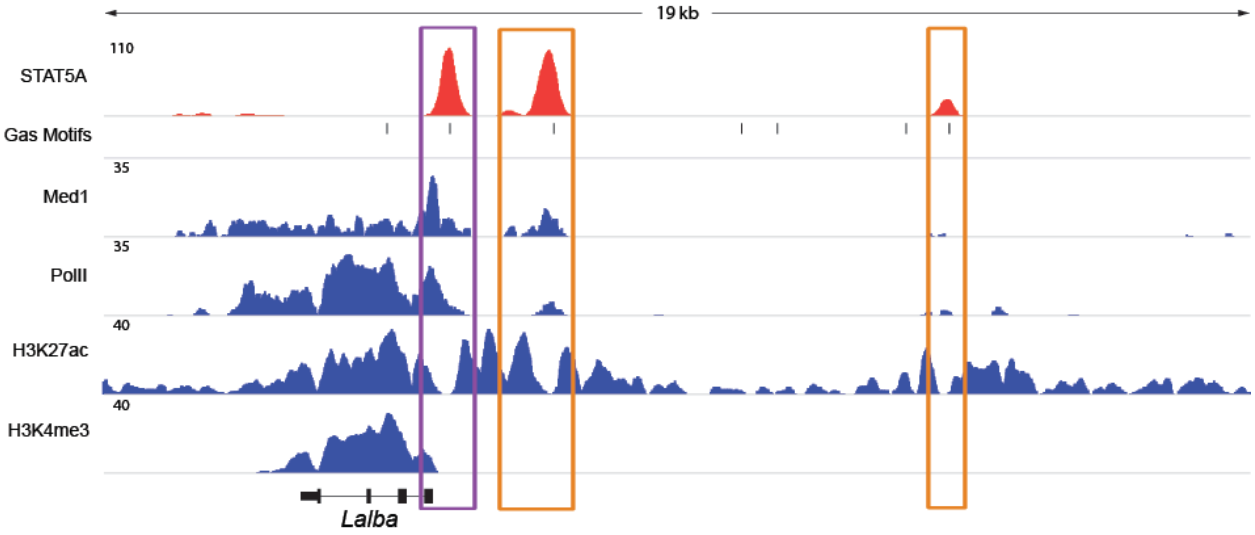
Reduced STAT5 binding to the *Wap* enhancer in *Stat5* mutant mammary tissue from line C as determined by ChIP experiments. The residual STAT5 binding to the mutant *Stat5* enhancer devoid of the two GAS motifs is likely the result of binding to other TFs outside the core enhancer.

Figure S6



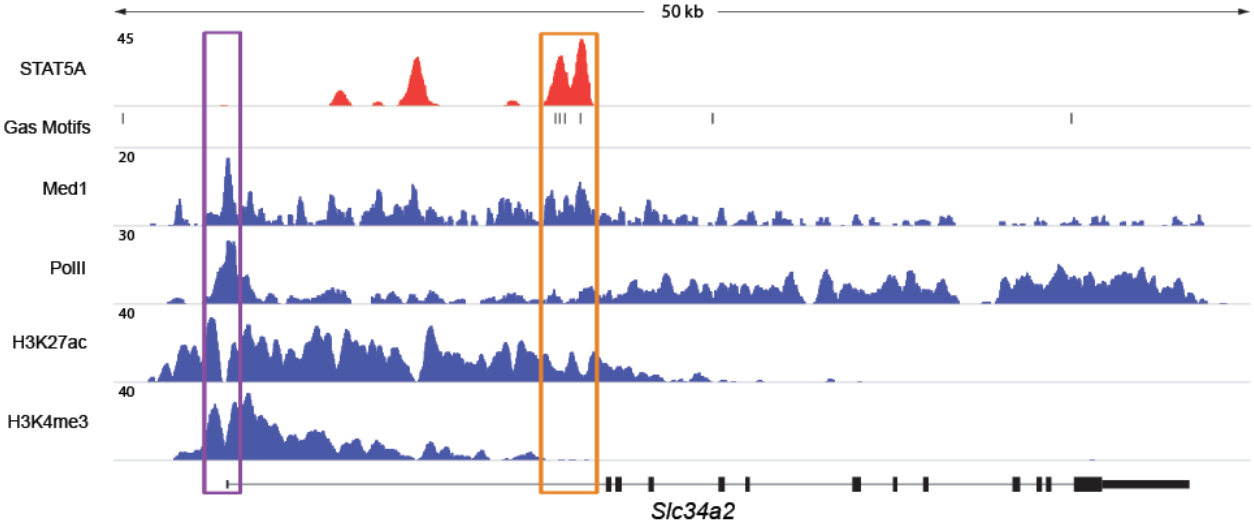
Csn1S2b locus. ChIP-seq data depict binding of STAT5, MED1 and Pol II. GAS motifs (STAT5 binding sites are shown). ChIP-seq data demonstrate the location of H3K27ac (enhancer) and H3K4me3 (promoter) marks. STAT5 binding was identified at two putative enhancers, one upstream and one in intron 9, both of which coincide with H3K27ac marks.

Figure S7



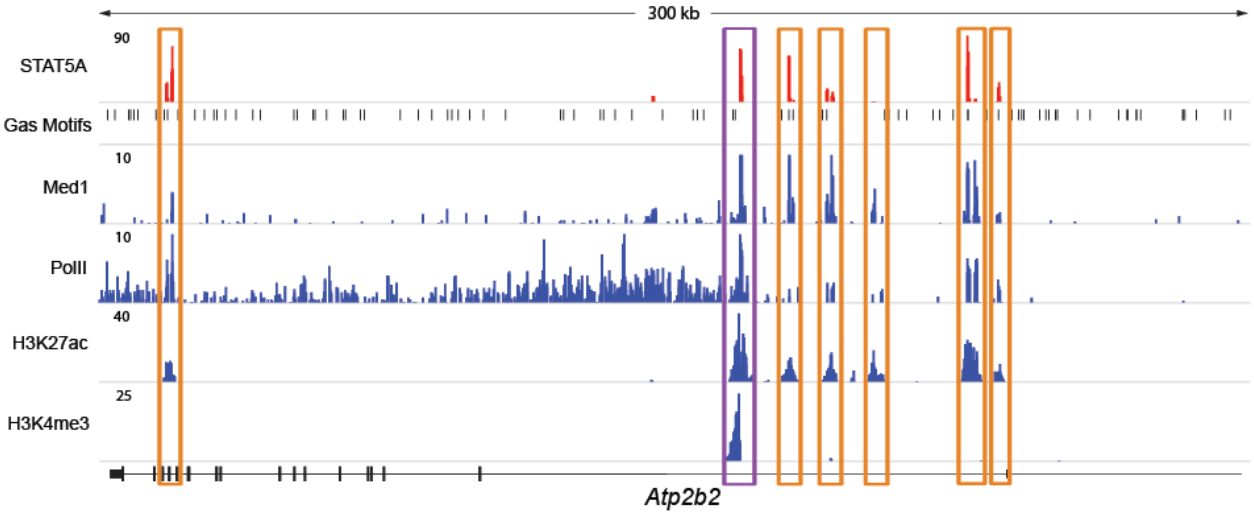
Alpha-lactalbumin (*Lalba*) locus. STAT5 binding has been identified in promoter sequences and two putative upstream enhancers that coincide with H3K27ac marks.

Figure S8



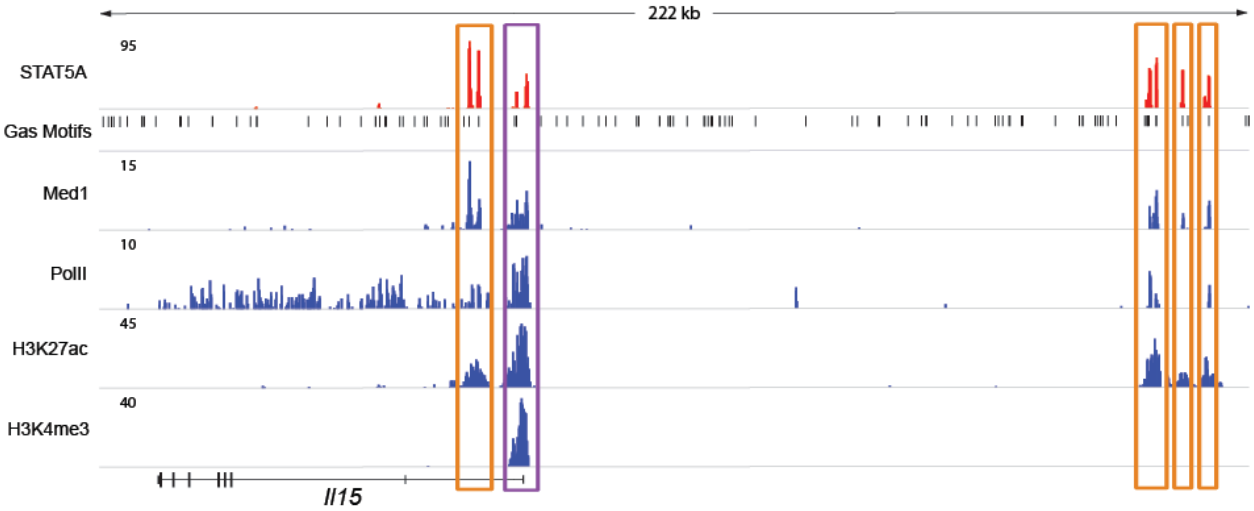
Slc34a2 encodes a sodium-phosphate cotransporter that is specifically expressed in milk secreting mammary cells. STAT5 binding has been identified to four GAS motifs at a putative enhancer in intron 1 that coincides with H3K27ac marks.

Figure S9



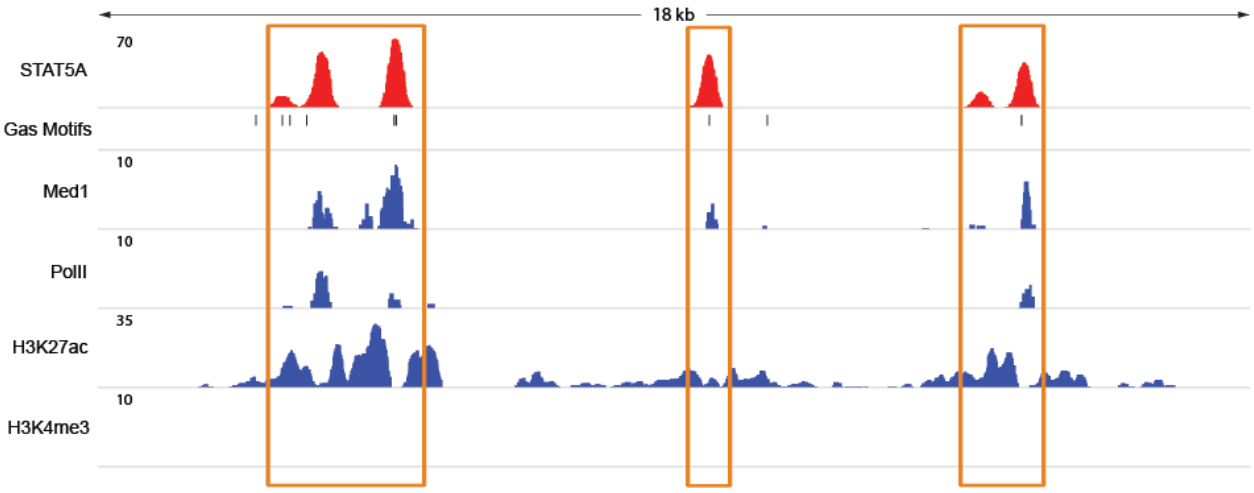
Atp2b2. Plasma membrane Ca^{2+} ATPase. STAT5 binding has been identified in intronic and upstream sequences

Figure S10A



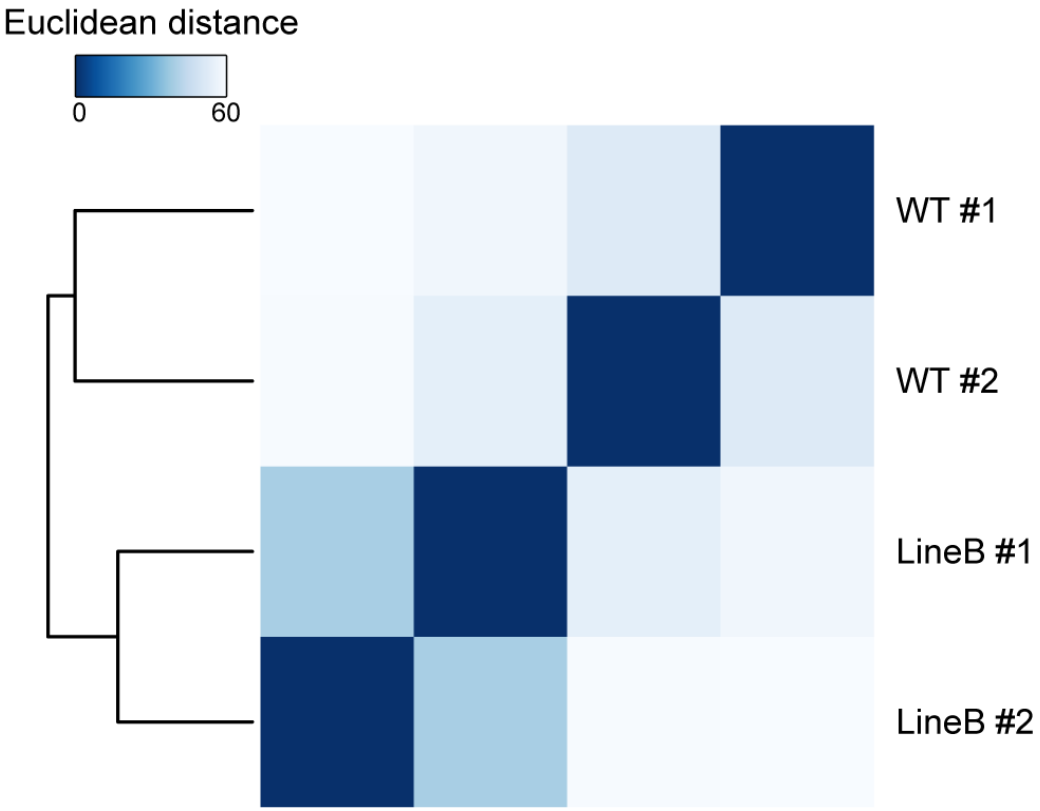
Il15. STAT5 binding has been identified to GAS motifs at a putative enhancer in intron 1 and in the upstream region. They coincide with H3K27ac marks.

Figure S10B



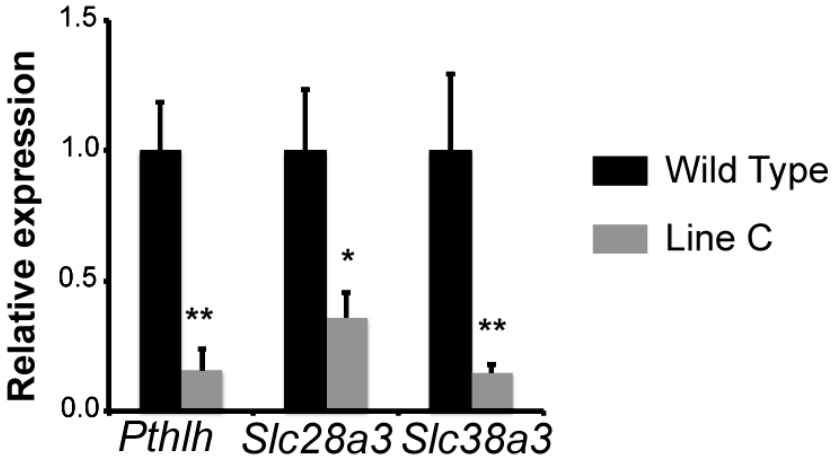
Enhanced view at the putative *Il15* enhancer.

Figure S11



The Euclidean distance heatmap demonstrates the reproducibility of RNA-Seq data from wild type and mutant samples (line B). Hierarchical clustering is shown on the left. The analysis indicated that transciptome profiles of two wild type samples are distinct from those of mutant samples.

Figure S12



Impact of the autoregulatory intergenic *Stat5* enhancer on the expression of novel STAT5 target genes. Expression of *Pthlh*, *Slc28a3* and *Slc38a3* was analyzed in mammary tissue from line C (loss of sites GAS1 and GAS2) using qRT-PCR (Material and Methods).

Supplementary Materials & Methods

ChIP-qPCR

Mammary tissue was isolated at day 1 of lactation from wild type and homozygous mutant mice and crosslinked with 1% formaldehyde for 10 min at RT. Nuclei were isolated with Farnham lysis buffer and were subjected to sonication for chromatin fragmentation. One mg of chromatin fragments were incubated with STAT5A antibodies (Santa Cruz Biotechnology, sc-1081 X, 10μg) at 4^0 C overnight. Purified DNAs from protein A/G-conjugated agarose beads were subjected to quantitative PCR (qPCR) using the following primer sets.

Stat5-forward: 5'-AGGTTCCTAGCCTCCACTGC-3'
Stat5-reverse: 5'-GTAAGGAGCCTTGGGATTGG-3'
Wap-forward: 5'-GTGTGGTGGGGAAGTGTAGG-3'
Wap-reverse: 5'-GGCCAAAGAACCAGAACACC-3'.

Flow Cytometry

Spleens were dissected from 8-12 week old wild type or STAT5 enhancer mutant mice and red blood cells depleted by hypotonic lysis (Gibco/Life Technologies, Grand Island, NY). For surface proteins, splenocytes were stained directly *ex vivo* with fluorochrome labelled anti-mouse CD3ε, CD4, CD25 (IL-2Rα) and CD45R (B220). For intracellular proteins, cells were surface stained, fixed and permeabilized using Transcription Factor Staining Buffer Set (eBioscience, San Diego, CA), then stained with fluorochrome labelled anti-mouse FoxP3. For STAT5 protein stains, spenocytes were surface stained, fixed with 2% formaldehyde, permeabilized with 100% methanol, then stained with a rabbit polyclonal IgG that recognizes both STAT5A and STAT5B (sc-835; Santa Cruz Biotechnology, Santa Cruz, CA). Normal rabbit IgG (sc-2027) was used as a negative control and Phycoerythrin labelled goat anti-rabbit IgG for detection (sc-3739; Santa Cruz Biotechnology). All fluorochrome labelled antibodies were purchased from eBioscience (San Diego, CA), unless noted otherwise. Data was collected on a FACSverse cytometer (BD Biosciences) and analyzed using FlowJo software (FlowJo LLC, Ashland, OR).

Statistics

Two-tailed student's t-test was used to quantify statistical deviation. p values for variance between WT and mutants are shown.