

Supplementary information

Interdependence of a mammary-specific super-enhancer and its native promoter facilitates gene activation during pregnancy

Running title: Synergy of lineage-specific regulatory elements

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Xianke Zeng^{1, #}, Hye Kyung Lee^{1, #, *}, Chaochen Wang^{1, #}, Precious Achikeh¹,
Chengyu Liu², and Lothar Hennighausen^{1, *}

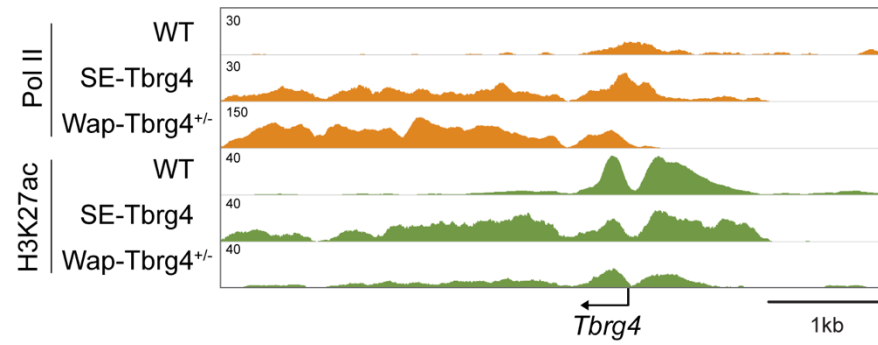
¹Laboratory of Genetics and Physiology, National Institute of Diabetes, Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892, USA

²Transgenic Core, National Heart, Lung, and Blood Institute, US National Institutes of Health, Bethesda, MD 20892, USA

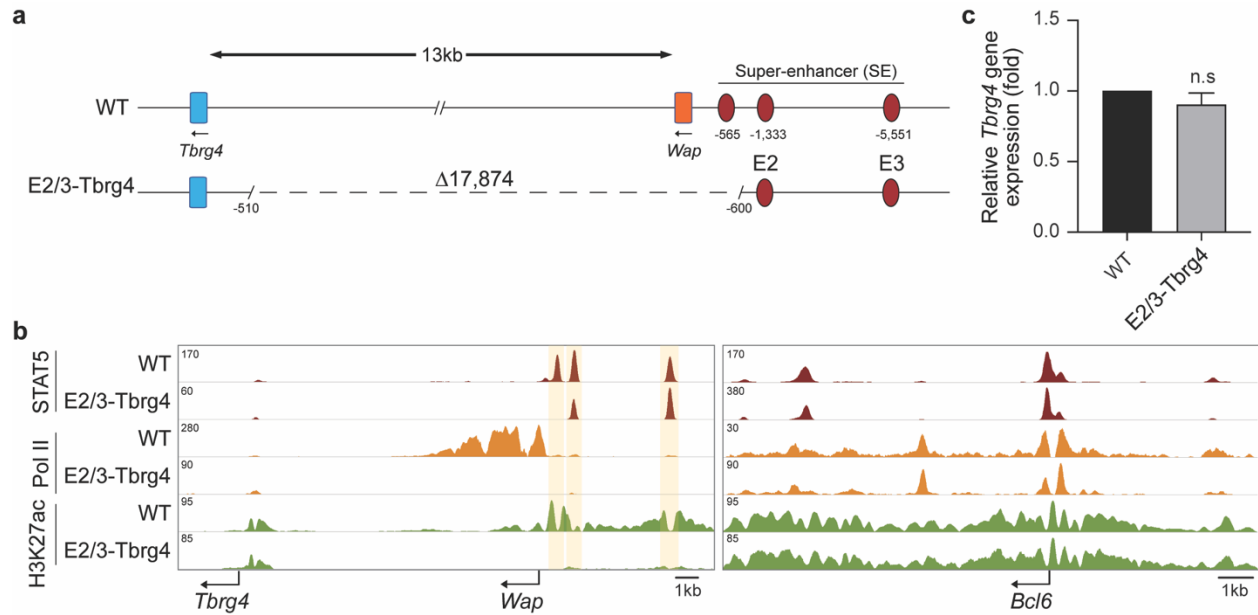
* Correspondence to: H.K.L (hyekyung.lee@nih.gov) and L.H (lotharh@niddk.nih.gov)

Co-first authors

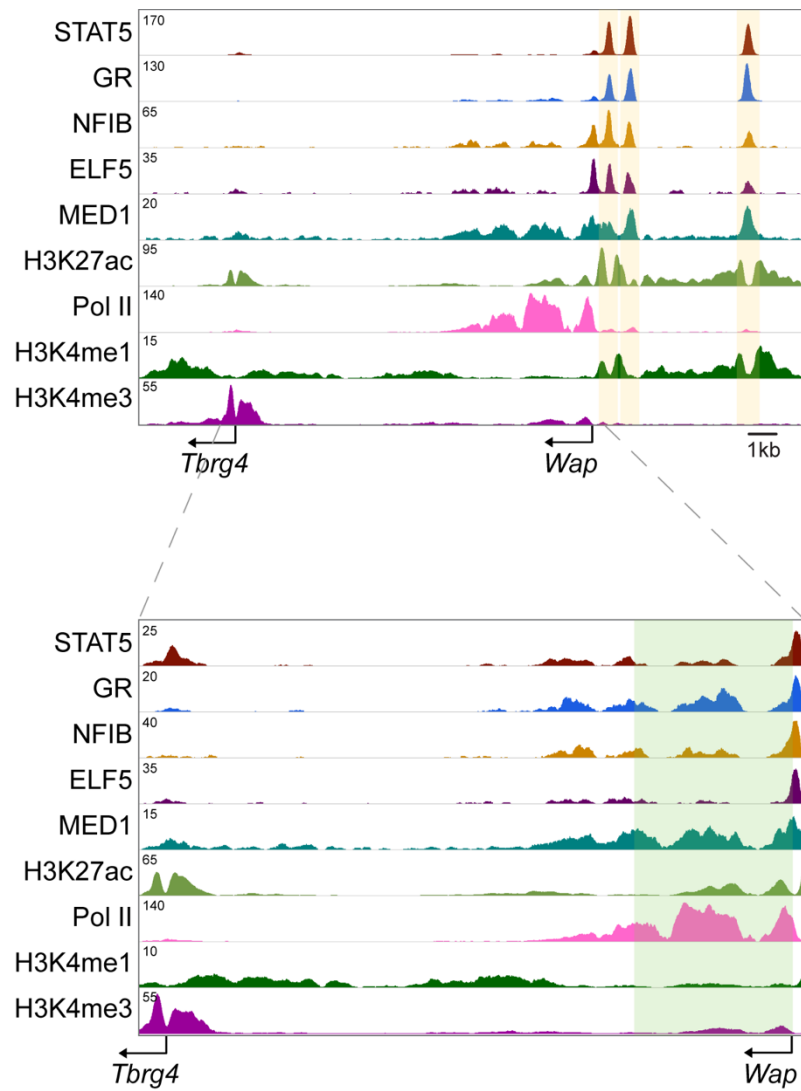
Supplementary Figures



Supplementary Fig. 1 ChIP-seq data of the *Tbrg4* promoter and transcriptional start site (TSS) region in mammary tissue at day 10 of lactation (L10) from WT mice and mutants.



Supplementary Fig. 2 Capacity of *Wap* super-enhancer elements E2 and E3 to activate the *Tbrg4* gene. **a** Diagram of mutants in which *Wap* enhancers E2 and E3 were fused with the *Tbrg4* upstream region (E2/3-Tbrg4). By deleting a 17.874 kb stretch of DNA, the *Wap* enhancers E2 and E3 were translocated within 510bp of the *Tbrg4* transcription start site. The exact positions of the deletions are shown in Supplementary Table 3 and 4. **b** STAT5A, RNA Pol II and H3K27ac landscapes at the *Tbrg4*-*Wap* locus in WT and E2/3-Tbrg4 mutant mammary tissue at day 10 of lactation (L10). The *Bcl6* locus was used as a control. **c** *Tbrg4* mRNA levels from WT and E2/3-Tbrg4 mutants at day 10 of lactation (L10) were measured by qRT-PCR and normalized to *Gapdh* levels. Results are shown as the means $\pm$ s.e.m. of independent biological replicates (WT and E2/3-Tbrg4 at L10, $n = 4$). A *t*-test was used to evaluate the statistical significance of differences between WT and mutant mice. n.s. not significant.



Supplementary Fig. 3 ChIP-seq profiles for the *Tbrg4*-*Wap* locus and regulatory elements in mammary tissue at day 10 of lactation (L10) from WT mice. The area shaded green covers the body of the *Wap* gene.

Supplementary Tables

Supplementary Table 1. Sequences of sgRNA for CRISPR/Cas9 targeted mice

Tbrg4_sgRNA	sgRNA sequences
SE-Tbrg4	5'-GCCAGCAGGATAACTTAGTG-3' 5'-CAAGTGTCACCTTTGAGTGGT-3'
Wap-Tbrg4	5'-CAACGCATGGTACCGGTGTCAGG-3' 5'-GTGACGTCAGTGAGCGCGCCAGG-3'
E2/3-Tbrg4	5'-CAACGCATGGTACCGGTGTCAGG-3' 5'-CAGGGCCTCCACAGTAGTAC-3'
CRE	5'-TGACGTCACCTGGCGCGCCGCCGG-3' 5'-GTGACGTCAGTGAGCGCGCCAGG-3'
Tb-Wap 3'UTR	5'-AGAAAAGCCAGTGGCCCATGTGG-3' 5'-GCAGTCTGAACTGATGGGTAAGG-3' Donor 5'-GAAAAGCAATGGCTGAGGAGCTAGCCAAATGACCT GCCGGCCCTGGCATCCCTGCCTGTCGGGAGTGACCAGC CCAAGTCTATACAGCAAGACCCTTCACTCCTGGATCCAG AGAGAACATAATGCTTTCAATCTGCTGCTAATAAAAATCCA TTTGGCTTTATG-3'

Supplementary Table 2. Genotyping primers for CRISPR/Cas9 mice

Mutant mouse line	PCR and Sequencing primers
SE-Tbrg4	Forward 5'-ACGCCAAGGGTCACTGCG-3' Reverse 5'-GCAAGATGCAGAGAGAACAGAGC-3'
Wap-Tbrg4	Forward 5'-AAGCTAGGTGGCCCAGTGCTC-3' Reverse 5'-GCAAGATGCAGAGAGAACAGAGC-3'
E2/3-Tbrg4	Forward 5'-ACGCCAAGGGTCACTGCG-3' Reverse 5'-GCAAGATGCAGAGAGAACAGAGC-3'
CRE	Forward 5'-TCGGTTGCAAATTCGGTTCC-3' Reverse 5'-GTAGACGGGAAGGAGAGGGT-3'
Tb-Wap 3'UTR	Forward 5'-GAACTACTGCAGAGCCACCC-3' Reverse 5'-GGCGCTTTTCTGCCATTCAG-3'

Supplementary Table 3. Breakpoint of mutant mouse line in *Tbrg4* and *Wap* loci

Mutant mouse line	Wap breakpoint	Tbrg4 breakpoint	Deletion size
SE-Tbrg4	-326	-983	11,926
Wap-Tbrg4	+19	+15	12,579
E2/3-Tbrg4	-842	-511	17,874
Tb-Wap 3'UTR	-	+9475	1,080
CRE	-	-20 / +2	22

Supplementary Table 4. DNA sequences of mutant mice.

Wap-SE

GAS motif

TSS of *Wap* gene

TSS of *Tbrg4* gene

SE-Tbrg4 ($\Delta 11,926$)

TCAGGTTTTTAGGGAAAATTCCAGCACAGCTGACCCCATAGGACAAAAAGTGCAGTGTGTGTACTACCAA
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TGCCGCTGGGCCTGGTGAATTCAGAGTAATGTCTTCATTCCTAGAA**CCTTCTGGCTTCCCCGACCTGGCCT**
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E2/3-Tbrg4 ($\Delta 17,874$)

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GTTTTTCCTTTTTTCTCATTTTTTCCTTTTGGTTTTCTACTAAGAACTACTGTTTAAATCCAAGATAGTAAA
AATGTGTTTTCCCTTAGGAGTTTTATATCTATATTATCTCTGACCTTTTTTGAGCTAAATTTTGAATTCAG
CATGAGACTCTTAAACAAGAACCACCAAGCTAAAGCTAGTGTGCCAATATAATGCTAGCTACTGTAAGGC
TAAAGCAAGAGAAGCTTCCCTGCGATAATTCAGCAATCTTTTTATTAACAAGAACCAGCGTTTACGCTTGAT
TTTAGCCACAAAGGAGAGAACTAATACACTCTTACAAACATAAAGATTTTTTCAAGCAAAATTTTAACTTA
AATTTTCCCTTTCTACAAAATTAGGGCCAGCAGGATAACTTAGTGGGGACCTGGGGGTGGGGGTGGGGCAT
GTCTTTTGTCTCTGCACTCCAGAGGCAGAAGGAGGGGATCTCTGTGAGTTCAAGACCAGCCTGGTCTACA
CAGCGAGTTCCAAAACAGCCAAGGCTACAAGGAGAAAGCTGTCTAGGAAAACAGACAGTTAGTTTAGGG
GCCACTTGACTGTGAGTTCAATTCCCAGATCCCACATGATTGGAAGAAACAAATTTCCCCCAAGTTTTCCCT
GTGATCTCTTCATGCACACATACACCTTTGTCCCAGAGATGCCGTCTGTGGTTTTCTAGCACTGGGATTA
CTGGGAAAACCTCACCTCCCAACCATCCTACCCAGCATTTTTGTACTATCTCCTCAGTCCCTGCCGTGGGCTA
AGGCAGGGCCTTCTCCAGGCTCCTCCCGGCTGTCTTAAACTTGGTATGTAGACCAAGCTAGCCACGACCTC
AAAGATCCTTTTTTCTGTCTCCTGGCTGGGCTTTTAAAGGTCTGTGCCACCACGGCCGGGCTATTCTGCTTC
TTCATGCAAGGGCCTCCACAGTAGTACAGGCTAGCCCTCGGCCTCCTGCCTCCATTACTGAGATTACAGCA
ATTTATCGGTGTGCCGTTGTATACGGTATGTCTTTACTTGTTTAATCTAGACAAACTGCTTGCATTTCC
GAAAAGACCTTCAGCTGGAGCTACAACCTCGCTTCTCTGTAATTACCCGACACCTGGCAATGCCTTCCCGC
CCCGAACCCCAAAGTCAACACGGCGGTCCCAACTCACGCCTCTCCTACAGGACCTGTGAAAGGCCCAAGG
GAGTGGGGCAGAACGGGCTGACATCAGAGTCGTACCCAAACTCCGCACAAGTAGGAGACTCTGGCCCGA
CGTGCCCTGCCGTGTAGAATTACCCCTCTCCTTCCCGTCTACGTTTCCCACGACCAGGATCTCACTACAC
TTCCAGTACCGBAAGCCTGAGCGCAACAAAGATGGCGACTGCGGAAGGGGCGGAGGGCGTGGCCTCCGT
CCGGGAGCACTGGGCCACCTAGCTTCCGGCGGGCGGCCAGTGACGTCAGTGAGCGGCCAGGCCGCGTGG
AGGCCGTAGGCTGATGGAGGTCAGTGAGGGTGATCGCCGCCGAGTCTGGCGTCTGGAGAGACCGGGTT
TCTGAACGTGGGTATCCGAGACGGGAAGCGTGGCTGGGAAGGGCAGCAGTTAGTAAGGACCGGCGGGGCT
GTTGTGGGGGGAGCCAGGAAAGGGACTCTCGACACCGTGCCCCAAGGGCGCTGTTCCGTTGCAAAATTCGG
TTCC

Stop codon of Tbrg4 gene

Tbrg4-3'UTR

Inserted Wap-3'UTR

Tb-Wap-3'UTR ($\Delta 1,080$, 130bp insertion)

CCACTGGTCGTGTAAAGGCAGGAAAAGACTCCGTAGTCCATGTGACCTGTCTCTACTACCTTCTTCCAGG
TCCCATATTATGAATGGCTGGATCTGAAGTCTGAATGGCAGAAAAGCGCCTACCTCAAGGACAAGATGCG
AAAAGCAATGGCTGAGGAGCTAGCCAAA **TGA** CCT **GCCGGCCCTGGCATCCCTGCCTGTCGGGAGTGACCA**
GCCCAAGTCTATACAGCAAGACCCCTTCACTCCTGGATCCAGAGAGAACATAATGCTTTCAATCTGCTGCT
AATAAAAATCCATTTGGCTTTATG CCTTACCCATCAGTTTCAGACTGCTCCCCAAAGACCTGACTGCTCAGG
GACCCAGCAGTCACTGTTGGCCAGGCCAGACTTCTCATCCAACATCAGAGAGATTAACTGTCTCTGTAGG
ACCTGGTCAGAGTAGGGGGTGTCTCAGCATCTCCAAGGAACGGCTGGTCCTAATAAATCCTTTGCTTTGGT
GGAAGCCAGCTATCTTCCCAGGCTCTGTATCTATCTCCTCTCTCTCGTATTTTGCACACACATACATATC
CTCTTCCCTAGCTCTCCAGGATTCTACCGAGTGGGCTCTCTTTCCCCAGCTCCCCCATAATGCCCTGTGTG
CTGTGGCACAGCAGAGCAGGGCTGACTTCAGAGACAGAAAACGAGACTTAAATGGGTAGGGTCCTGGAC
ACTTGAGCTTCCTGGCAAATGTACTTGGAGTGTATAGCAGAGTTGTGATTACTATCTTGAGAGGTGCCCCA
CAGCACCCCCCAGCCCCACACCCCCCGCACCCCCGTGCCACTAGAGAACATCTGAAGGGAACAGGTGCTGT
GAAGACATTTGGAAGGCCCTAGTTCTGGGACAAGACTAAAGTCCACTTGGCTGATGCTACAAGATCCATG
GCCAGACTCATTCTCATGACTACTACAGCCAACCCAGGGACCGCCCTTCAGGTGGAGCCCTGGGTCCCCCT
GCAGCTCTGAGTAGCAGCAGGATGTCTACCTTCTACCTCTGCAAGGAGTCTCCCATAGATAAGGCTCGCC
CAAAGGGGAGACTTGCAACAAAGGGGGTTATTACCCTACTTGCTACATTCTCAAAGCACCAGTCTCTCTG
TCTGACTTAGCACTTCTATTCTCTGACAGACTCTTGCTTACCACAGAGGTGACGGAGAAGCAGAAAAGC
GGCTGGAACCTGCTTACAGGAAGGAGCAGCTCGCCACAACCTTCTCTTAAGTTGAGGTGGGTTCCTAGTGC
TCACAACCCAGTTGGGGAAGTCTTCTGTATGACGAAATAAAAGTTTGTGCTCCTCACCACATGGGCCACT
GGCTTTTCTTTTGAGCTAATGGAGATCTCCAGCTAATACTCGGTGGCGTGGTCAGTGGTGGAACACTGC
AGAGCCACCC

TSS of *Tbmg4* gene

CREB

Δ CRE ($\Delta 22$)

CACAAGTAGGAGACTCTGGCCCCGACGTGCCCTGCCGTGTAGAATTCACCCTCTCCTTCCCGTCTACGTTT
CCCACGACCAGGATCTCACTACACTTCCCAGTACCGGAAGCCTGAGCGCAACAAAGATGGCGACTGCGGA
AGGGGCGGAGGGCGTGGCCTCCGTCCGGGAGCACTGGGCCACCTAGCT **TCGGGGGGGGGGGGGGAGTGAC** **GT**
CAGT GAGCGCGCCAGGCCGCGTGGAGGCCGTAGGCTGATGGAGGTCAGTGAGGGTGATCGCCGCCGCACT
CTGGCGTCCTGGAGAGACCGGGTTTCTGAACGTGGGTATCCGAGACGGGAAGCGTGGCTGGGAAGGGCAG
CAGTTAGTAAGGACCGGCGGGGCTGTTGTGGGGGAGCCAGGAAAGGACTCTCGACACCGTGCCCCAAG
GGCGCTGTTTCGGTTGCAAATTCGGTTCC

Supplementary Table 5. RNA-seq data of WT and Wap-Tbrg4 mice (separate Excel file).

List of all genes with FPKM value for exon and intron in each replicate at L10 mammary tissue from WT and mutant mice.