Supplemental data for: Enhancers and super-enhancers have an equivalent regulatory role in embryonic stem cells through regulation of single or multiple genes

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List of Supplemental Tables and Figures

Table S1: Enhancer clusters identified.

Provided as a separate spreadsheet.

Table S2: Enhancer cluster regulated gene determined by RNA-seq.

TAD (mm10)				Deleted Region					
Chr	Start	End	# of genes in TAD$^{\phi}$	Name	Chr	Start	End	Regulated Gene	Chi-square P value
2	19398373	20838373	7	EC(Etl4)	2	20642059	20658958	Etl4	< 0.001
2	152894264	154614264	16	EC(Cbfa2t2)	2	154419404	154431952	Cbfa2t2	< 0.001
3	95036078	96476077	18	EC(Mcl1)	3	95644053	95655885	Mcl1	< 0.001
4	122882757	123722757	6	EC(Macf1)	4	123618309	123637993	Macf1	< 0.001
5	118146950	118777277	2	EC(Med13l)	5	118432886	118452483	Med13l	< 0.001
7	3040000	4528398	31	EC(Mir290)	7	3199611	3215602	AU018091	< 0.001
								mir291-5 TU	< 0.001
								Prkcg	< 0.01
8	88396101	89836101		EC(Sall1)	8	88986501	89018827	Sall1	< 0.001
9	78272193	78552193	4	EC(DppDn)	9	78358933	78365818	Dppa5a	< 0.001
9	78272193	78552193	4	EC(DppUp)	9	78368337	78376479	Dppa5a	< 0.001
								Ooep	< 0.001
10	61686969	62911079	12	EC(Tet1)	10	62891757	62906804	Tet1	< 0.001
11	33140000	33940000	2	EC(Ranbp17)	11	33524486	33543505	Ranbp17	< 0.001
12	85699050	86699050	6	EC(Esrrb)	12	86496798	86505014	Esrrb	< 0.001

ϕ The chi-square test for significance was conducted on all genes in the TAD.

Table S3: MTL regions with high and low H3K27ac found inside and outside of super-enhancers.

Provided as a separate spreadsheet.

Table S4: Gene set analysis of MTL regions with high H3K27ac that are found outside of super-enhancers.

Function	False Discovery Rate
histone methyltransferase complex	2.43E-04
methyltransferase complex	3.85E-04
stem cell differentiation	4.95E-03
ESC/E(Z) complex	5.46E-03
stem cell maintenance	5.46E-03
CCR4-NOT complex	8.60E-03
PcG protein complex	1.76E-02
histone lysine methylation	1.76E-02
Wnt signaling pathway	2.90E-02
morphogenesis of an epithelial fold	4.54E-02
histone methylation	5.55E-02
histone-lysine N-methyltransferase activity	9.13E-02
multicellular organism growth	9.46E-02
histone methyltransferase activity (H3-K4 specific)	9.46E-02
lysine N-methyltransferase activity	9.46E-02
transcription regulatory region sequence-specific DNA binding	9.46E-02
protein-lysine N-methyltransferase activity	9.46E-02
regulation of stem cell maintenance	9.46E-02
macromolecule methylation	9.46E-02
protein methylation	9.61E-02
morphogenesis of a branching structure	9.61E-02
protein alkylation	9.61E-02

Table S5: gRNA target sequences.

Target	5’ gRNA	3’ gRNA
EC(*Sall1*)	GTCTAGTGGTCTCTATAGCG	CAGAGCTACACCCTCCGGAG
EC(*Tet1*)	AATAGGAAGTTATGTCTATA	GGGTTTAGTAAGTCGTATTG
EC(*Mir290*)	AATAACATGGTGAGAATCGC	TCTCTCTGCTTTCTCGAGTA
EC(*Ranbp17*)	TGGGCATGGTAGCGCATGGG	GTAGGCAGATACGTCCCTGA
EC(*Med13l*)	AGTCATTGACCGTTAAGTGG	ACAGAATAGGGTGCGGCTTT
SE(*Med13l*)	CTCAAATTTCCGCTTAGGTG	TAGTGGGCCGGTTGTTCATA
EC/SE(*Med13l*)	AGTCATTGACCGTTAAGTGG	TAGTGGGCCGGTTGTTCATA
EC(*Med13l*A)	AGTCATTGACCGTTAAGTGG	TGCCTTCTAAATGCGATTAG
EC(*Med13l*B)	TGCCTTCTAAATGCGATTAG	ACAGAATAGGGTGCGGCTTT
EC(*Mcl1*)	TGGTAATGGCTGACGACTAG	TGACTTGACTACCGTACTGC
EC(*Cbfa2t2*)	TAGTCTATATCGCCAATTCT	AGGATGTCTCAGCTAACGTT
EC(*Esrrb*)	CCTTCAGGAACGCACTCCAA	TTTTGAGGCGCACTACCTTC
EC(*Dpp*Up)	TGTGTATTCGAATCTGGGGG	AATTAATGTATCCTCTCACG
EC(*Dpp*Dn)	GTCACTGTCGTTCTATAGGT	TCCTGATTCGACCTAGGTCC
EC(*Dpp*UpA)	TGTGTATTCGAATCTGGGGG	CTCCCAAACATTTAGCACGA
EC(*Dpp*UpB)	CTCCCAAACATTTAGCACGA	AATTAATGTATCCTCTCACG
EC(*Macf1*)	AGGCACACCGGTATCTCCTT	CCTGTCTATCTTCGAAGCGC
EC(*Etl4*)	TTCCTGAGCTTTATAGGCGT	CCAAGCACATTATCCGACTA
EC(*Lifr*)	TTTCATCGAATACCTTAACC	GCAAGCATTAGGCGGACGGT
EC(*Jarid2*)	TTGTCTGCCGGAGATTTGGC	CGTAATGATCCGAATGGGTT
EC(*Ifitm*)	GCTTTTGAGGCTCTGATCGA	AGGGAAGCAGAGTTGTCGGG
EC(*Six*)	ATTGACATCTTAACCGAGTC	TAGGCTAGCAAGTAGCTCGA

Table S6: Clones analyzed by RNA-seq

GEO sample	Clone Name	Deletion (mm10)
GSM2091415	EC(*Mir290*)_47_doubleΔ	Δ(129/Cast): Chr7:3199611-3215602
GSM2091416	EC(*Mir290*)_67_129Δ	Δ(129): Chr7:3199611-3215602
GSM2091417	EC(*Sall1*)_1_129Δ	Δ(129): Chr8:88986501-89018827
GSM2091418	EC(*Sall1*)_25_doubleΔ	Δ(129/Cast): Chr8:88986501-89018827
GSM2091419	EC(*Sall1*)_54_129Δ	Δ(129): Chr8:88986501-89018827
GSM2091420	EC(*Tet1*)_30_129Δ	Δ(129): Chr10:62891757-62906804
GSM2091421	EC(*Tet1*)_59_129Δ	Δ(129): Chr10:62891757-62906804
GSM2091422	EC(*Cbfa2t2*)_9_129Δ	Δ(129): Chr2:154419404-154431952
GSM2091424	EC(*Ranbp17*)_16_129Δ	Δ(129): Chr11:33524486-33543505
GSM2091425	EC(*Ranbp17*)_18_129Δ	Δ(129): Chr11:33524486-33543505
GSM2091426	EC(*Macf1*)_129Δ_EC(*Med13l*)_CastΔ_3*	Δ(129): Chr4:123618309-123637993, Δ(Cast): Chr5:118432886-118452483, Δ(129): Chr9:78358933-78365818
GSM2091427	EC(*Macf1*)_129Δ_13*	Δ(129): Chr4:123618309-123637993, Δ(129): Chr9:78358933-78365818
GSM2091428	EC(*Med13l*)_129Δ_ 28*	Δ(129): Chr5:118432886-118452483, Δ(Cast): Chr9:78358933-78365818
GSM2091429	EC(*Esrrb*)_129Δ_EC(*Med13l*)_129Δ_34*	Δ(129): Chr12:86496798-86505014, Δ(129): Chr5:118432886-118452483
GSM2091430	EC(*Esrrb*)_129Δ_65*	Δ(129): Chr12:86496798-86505014, Δ(Cast): Chr9:78358933-78365818
GSM2091431	EC(DppDn)_2_129Δ	Δ(129): Chr9:78358933-78365818
GSM2091432	EC(DppDn)_5_129Δ	Δ(129): Chr9:78358933-78365818
GSM2091433	EC(DppUp)_17_129Δ	Δ(129): Chr9:78368337-78376479
GSM2091434	EC(DppUp)_43_doubleΔ	Δ(129): Chr9:78368337-78376479, Δ(Cast): Chr9:78358933-78365818
GSM2091435	EC(Etl4)_15_129Δ	Δ(129): Chr2:20642059-20658958
GSM2091437	EC(Mcl1)_31_129Δ	Δ(129): Chr3:95644053-95655885
GSM2091438	EC(Mcl1)_38_129Δ	Δ(129): Chr3:95644053-95655885

* Multiple deletions [EC(*Macf1*), EC(*Med13l*), EC(DppDn), EC(*Esrrb*)] were targeted in the same cells for these clones. These deletions were all located on different chromosomes and as heterozygous deletions did not show effects on gene expression outside of the TAD in which the deletion was made no compound effect of these deletions were observed.

Table S7: Allele specific gene expression primers.

Primer Name	Sequence (5'-3')*
Ooep_EX1_F1B	CCATGTAGAGCACCAGAGGATT**a**
Ooep_EX1_R1B	TCTGACTCTCAGAAGCTGCT**t**AAC
Ooep_EX1_F1C	CATGTAGAGCACCAGAGGATT**g**
Ooep_EX1_R1C	TGACTCTCAGAAGCTGCT**c**AAC
Dppa5a_IN1_F3B	GGGGAAAGGCAATGAGCA**t**A
Dppa5a_IN1_F3C	GGGAAAGGCAATGAGCA**g**A
Dppa5a_UTR_R2	AAACTTCCTGAACCTGGAGCTGT
Mcl1_UTR_F1	AGCAAAGTTCCCTCTCAGCA
Mcl1_UTR_R1B	GCAGAGTAATGGATATTTATTGAGCT**t**
Mcl1_UTR_R1C	GCAGAGTAATGGATATTTATTGAGCT**c**
Etl4_EX7_F1	TAGCCATTTGCCTACCTTGG
Etl4_EX7_R1B	CAGGGTGCCGGGTTC**c**TT
Etl4_EX7_R1C	CAGGGTGCCGGGTTC**t**TT
Esrrb_EX6_F1	GCTGCAGGACTATGAGCTGAG
Esrrb_EX6_R1B	CTTGCCCTGCAGTTTCAC**a**
Esrrb_EX6_R1C	CTTGCCCTGCAGTTTCAC**g**
Macf1_EX_F3B	GAAACACCTGAATCTCAAAACAAGT**t**
Macf1_EX_R4	GTGCTGCAGAAGACAGAGGA
Macf1_EX_F2	AGATGCAGAAAGTTGGTTCTCC
Macf1_EX_R3C	CATTCCAAAAGTTTGCCTTTACA**a**
Med13l_UTR_F1	CCTGCTCTCAGCCTCTGTTT
Med13l_UTR_R1B	CCCCAGCACTAGATCCTGT**c**
Med13l_UTR_R1C	CCCCAGCACTAGATCCTGT**t**
Ranbp17_UTR_F2B	GGTTGAATACAGTTTCTAGACCCTT**t**
Ranbp17_UTR_F2C	GGTTGAATACAGTTTCTAGACCCTT**c**
Ranbp17_UTR_R2	AGAACAAGCAGCTAAAGCCCTA
cbfa2t2_IN_F2B	ACCTAGGCTATATAGTGAGAACCTAT**c**
cbfa2t2_IN_F2C	ACCTAGGCTATATAGTGAGAACCTAT**t**
cbfa2t2_EX_R2	TTTGCTCTGCCTCAGCTACA
Sall1_EX_F2B	CGTGGCCTTCTTGTCAAT**g**
Sall1_EX_R2B	CAACAGTACTCTGAACTCCCCA**g**T
Sall1_EX_F2C	CCGTGGCCTTCTTGTCAAT**a**
Sall1_EX_R2C	CAACAGTACTCTGAACTCCCCA**a**T
Tet1_EX_F2B	TTGGCTTTGCTGATGGTACA**c**
Tet1_EX_R2B	AGGAATGCTGAAAGTACACCAG**a**
Tet1_EX_F2C	TTGGCTTTGCTGATGGTACA**t**
Tet1_EX_R2C	AGGAATGCTGAAAGTACACCAG**g**
Mir290_F2B	TGTGGGGCACACTTCTGGA**c**
Mir290_F2C	TGTGGGGCACACTTCTGGA**t**

Mir290_R2	ACTGGTTGCTCCCATAGCAC
Lifr_EX1_F2B	AAGGTTCCTTCAAACAGCAC**g**
Lifr_EX1_F2C	AAGGTTCCTTCAAACAGCAC**a**
Lifr_EX1_R2	GTCACCTCTGTGTGGCTTTTC
Jarid2_EX_F2B	GGTGCTGATGGAGAAGGA**g**
Jarid2_EX_R2B	ACGGAATCCATTCCT**a**GGTG
Jarid2_EX_F2C	GGTGCTGATGGAGAAGGA**a**
Jarid2_EX_R2C	TACGGAATCCATTCCT**g**GGT
Six4_EX_F2B	AGTAGTGTCAGA**g**TGGAAAACCAC**a**
Six4_EX_F2C	GTGTCAGA**a**TGGAAAACCAC**g**
Six4_EX_R2	GAAATTGGTGAATGTCCCTCA
Six1 _EX_F3B	GGCGAAGAGACCAGTCG**a**
Six1_ EX_F3C	GGCGAAGAGACCAGTCG**g**
Six1_ EX_R3	GCAACCAGCAGCATCCAC
Ifitm1_IN_F3B	TAGGAAGGT**g**ATGGGGAGC**t**
Ifitm1_IN_F3C	TAGGAAGGT**a**ATGGGGAGC**c**
Ifitm1_EX_R3	ACAGACAACGATGACGACGA
Ifitm2_EX_F2B	GTAGGCATAGGCAA**c**GAAGC
Ifitm2_EX_R2B	TTGTCCACCAATGCCGG**g**
Ifitm2_EX_F1C	CTTCACAGAGTAGGCATAGGCAA**t**
Ifitm2_EX_R1	AGCCTTCTTGTCCACCAATG
Ifitm3_EX_F1B	GGTTTTGAGCGTTAAGAACAATGA**t**
Ifitm3_EX_R2B	CTGCTCCAGTCTAGGGA**t**CG
Ifitm3_EX_F1C	GGTTTTGAGCGTTAAGAACAATGA**c**
Ifitm3_EX_R2C	TGTTCTGCTCCAGTCTAGGGA**c**
AU018091_UTR_F2	AACAACAACAAGCACAGTCCA
AU018091_UTR_R2B	CCCCACCC**a**TAAACAGTATCATA**a**
AU018091_UTR_R2C	CCCCACCC**t**TAAACAGTATCATA**g**
Prkcc_UTR_F1	CCTAGCCTTCTGGCCTCTTT
Prkcc_UTR_R1B	GGCATAAACTACCAAGTGGG**g**
Prkcc_UTR_R1C	GGCATAAACTACCAAGTGGG**a**
Gapdh_IN_F1	GCACCAGCATCCCTAGACC
Gapdh_EX_R1	CTTCTTGTGCAGTGCCAGGTG

* SNPs indicated in bold/lower case.

Table S8: Predicted enhancers mapped to mm10

Provided as a separate spreadsheet.

Table S9: Enhancer cloning primers.

Name	Region (mm10)	Forward Primer	Reverse Primer
Mcl1_1	Chr3:95636028-95636838	GTGGTGTACTCGCCTGTTTG	ATTGGCCAGCGAGTTGTTAAAG
Mcl1_2	Chr3:95650931-95654071	GCCCAGGCGTCTAAGTTCTG	AATTCCGGTTAAACTGATCTCC
Mcl1_3	Chr3:95662963-95664356	TGTTTGTCTTACACGTCTCTCAGG	GGGTTGCCTTACACAGATGTTG
Macf1_1	Chr4:123622998-123626154	CGCTGTGAACTGTAAATCGAAG	TCATCTTGGCAAATTGTCTGTC
Macf1_2	Chr4:123632303-123634109	TATCTTCAAGGTTCGTCCAAGC	TGTAATGTGGTGTGGTTCTTCC
Cbfa2t2_2	Chr2:154427556-154429103	AAAGCTAGATTGCTGGTCATGC	GGTAAAGAACGAGAGACATAGAAGG
Cbfa2t2_3	Chr2:154465672-154467303	AGGTTGTGTAACTACGAACATCG	CCGCCCTATATGACCCAGAC
Klf4_1.5	Chr4:55470962-55472215	GTGAGCACATGGATCGTTTCAG	GTTTTGGTCCTTGCTACAGTGC
Klf4_2	Chr4:55474798-55476568	ACAAGATGCCGCTGAGCATAC	TAGATAGCAGGACAGGGGATTC
Klf4_3	Chr4:55476545-55478544	AGGAATCCCCTGTCCTGCTATC	AGCAGCACAACGTGACACAG
Klf4_4	Chr4:55514505-55515435	TCTCTGTGGGAAGGTAAATGAGG	ATCCAACGTGCATGCTGATG
Esrrb_1	Chr12:86465045-86467887	CCTCATACTCTCATCCCGAGAC	GCTGACTCCTCTCGACTGAATG
Esrrb_2	Chr12:86477662-86480598	TGTGCATTACCTACGACCTCTG	ATGATAAACAAAGCGGAACACC
Esrrb_4	Chr12:86500631-86503118	GTTTGCCTGTGGCATCTGAC	CTTCACAGTGGACATCGAGAAC
Esrrb_5	Chr12:86503837-86505514	GCCAGACCACCAGTTACTGAC	TCATGCCACAAGAAGTACCATC
Dppa5a_1	Chr9:78368500-78370278	ATCCCGAAAGAATCAGATACTGG	CCAGCACAGTAGCACAAGTTACA
Dppa5a_2	Chr9:78368922-78370654	GCTAATGTTCTGGACTCAACGAC	ACTAGCCAAACGGAATATGCTC
Dppa5a_3	Chr9:78370634-78373173	AGCATATTCCGTTTGGCTAGTG	AAAACAGCTGACTCCACTCGTC
Dppa5a_4	Chr9:78374046-78375410	CATGTCTCTCTCTCGGCTCTTC	CTGGGTTATTTTGTTGCGATAG
Tanc1_1	Chr2:59621790-59623138	TCTTTATCCAGCAGTGTCCCAC	ACACTACCTTGCCTCTGAATCTG
Tanc1_2	Chr2:59632862-59634968	TAGTATCTTGACCTTGGGTGGG	TAAGGCCGAACAGAGCTAGCAG
Etl4_1	Chr2:20495259-20497618	GGCAAACAGAACTCATGATTAGGC	CTTGAGGCTCTAAATGTTGGGG
Etl4_2	Chr2:20583333-20584406	GCAGGAACCACACTCTGTCTTAG	AACCCCAGCTCACATGGATTAC
Etl4_3	Chr2:20654182-20656518	TCATCGAGTTTCACTCCAGACC	AGGACTTTGTATCACTGAGCGG
Gabrp_1	Chr11:33526880-33528679	TGATGCCGATAAGTTTAAGCCAAC	ACTCAAGTGAAACACATAATCAAAACC
Gabrp_3	Chr11:33532954-33534353	TTTTAAGCACATTAGCCTGCCC	TCCCTATCAAGCTGCCATCATC
Gabrp_4	Chr11:33536553-33538455	TCTCATTGGGAAGTTATCTGTCCC	AAGGTTGAAGTCCTTGAGCCAC
Med13l_1	Chr5:118433870-118435054	CTCTCCCAACAACTGTGCTTTG	TGATTGAGTTATGGGCTGGTTG
Med13l_2	Chr5:118434651-118436033	AAAGGAAGGACCAAACCTCAGG	CACAGAATGTTGGCATGACAGG
Med13l_3	Chr5:118436044-118437983	CTCATCTGTGGTGTCGTTGTTG	GTTCAGGAGGGAGAGTTCTGTG
Med13l_4	Chr5:118444147- 118446211	CACAAACTCCTGATTGTGTTTCTC	GATGATGGCTTTAAATGTCCAGA

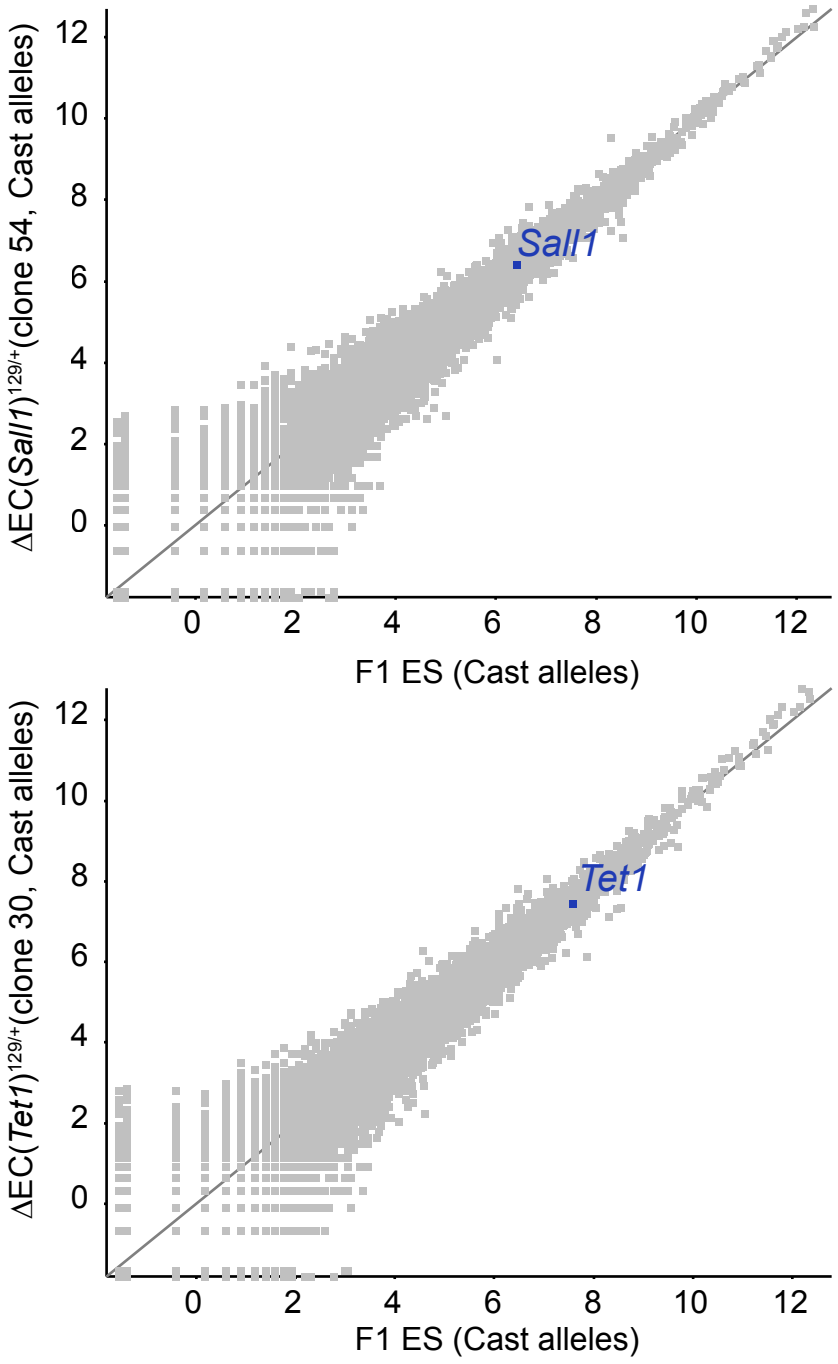


Figure S1: Expression of castaneus alleles is unaffected by enhancer cluster deletion on the 129 allele. Scatter plots indicate differences in transcript abundance between F1 ES cells and the ΔEC129/+ clones. Transcript levels are log2 transformed reads per million. In each scatter plot the EC target gene is highlighted in blue.

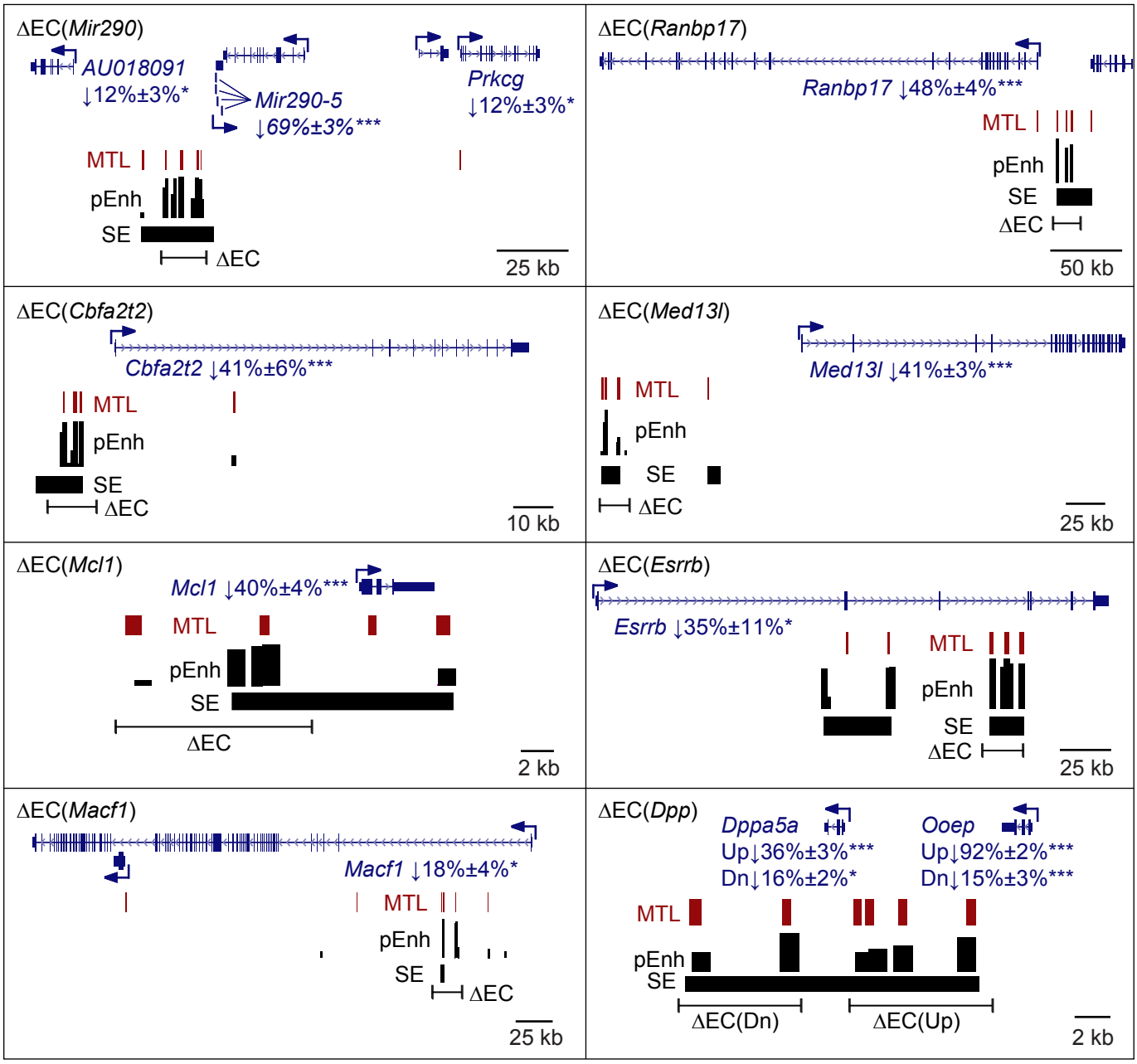
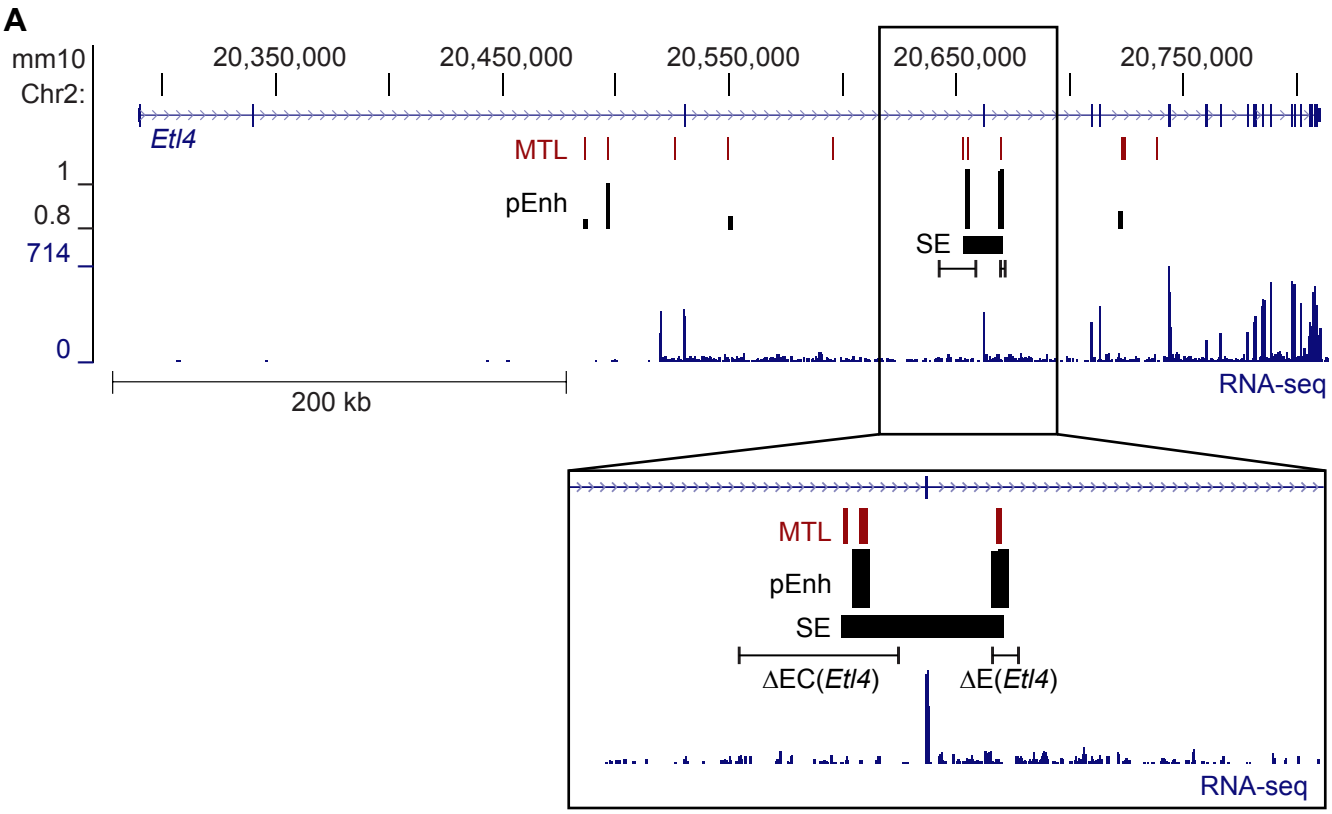


Figure S2: Schematic representation of additional enhancer cluster loci. Shown are MTL≥3 (red bars). Predicted enhancers (prEnh) and called super-enhancers (SE) are shown in black. Each deleted enhancer cluster (ΔEC) is designated by a line that links the 5' and 3' gRNA targets. All data are displayed on the mm10 assembly of the University of California at Santa Cruz Genome Browser. Gene names are followd by the effect on transcript levels after deletion. Gene names are provided only for affected genes.



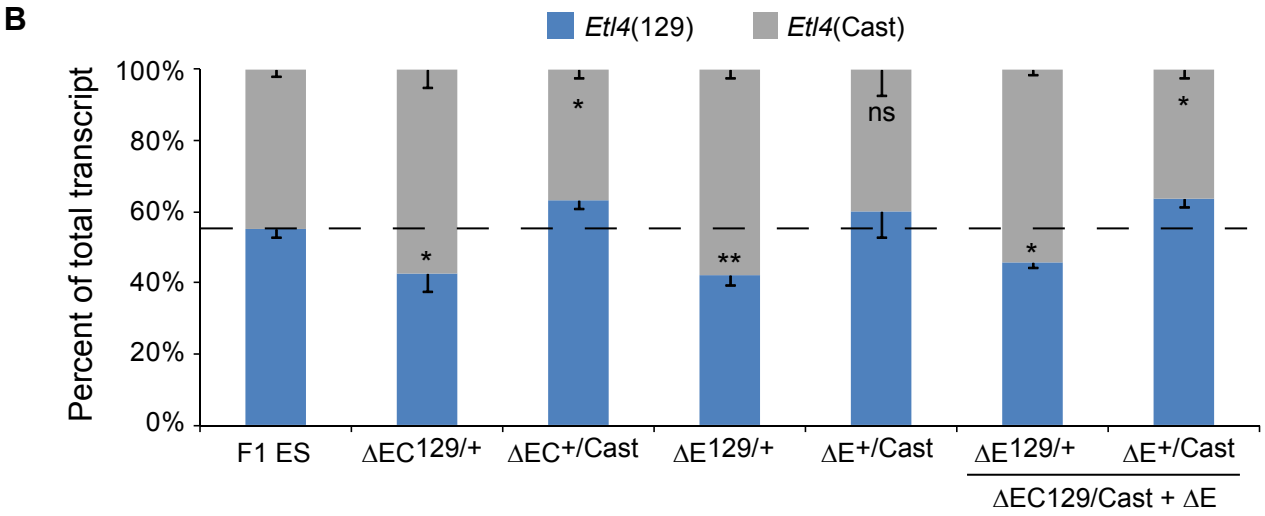


Figure S3: Schematic representation of the *Elt4* locus. A) Shown are MTL≥3 (red bars) and RNA-seq data obtained from the CODEX database. Predicted enhancers (prEnh) and called super-enhancers (SE) are shown in black. The deleted enhancer cluster (ΔEC) and deleted enhancer (ΔE) is designated by a line that links the 5' and 3' gRNA targets. All data are displayed on the mm10 assembly of the UCSC Genome Browser. B) Deletion of EC(*Etl4*), E(*Etl4*) or both. Allele specific primers detect 129 or Cast RNA in RT-qPCR from F1 ES, Δ129/+, Δ+/Cast, and combined deletion clones. Error bars represent SEM. Significant differences from the F1 ES values are indicated by * $P < 0.05$, ** $P < 0.01$.

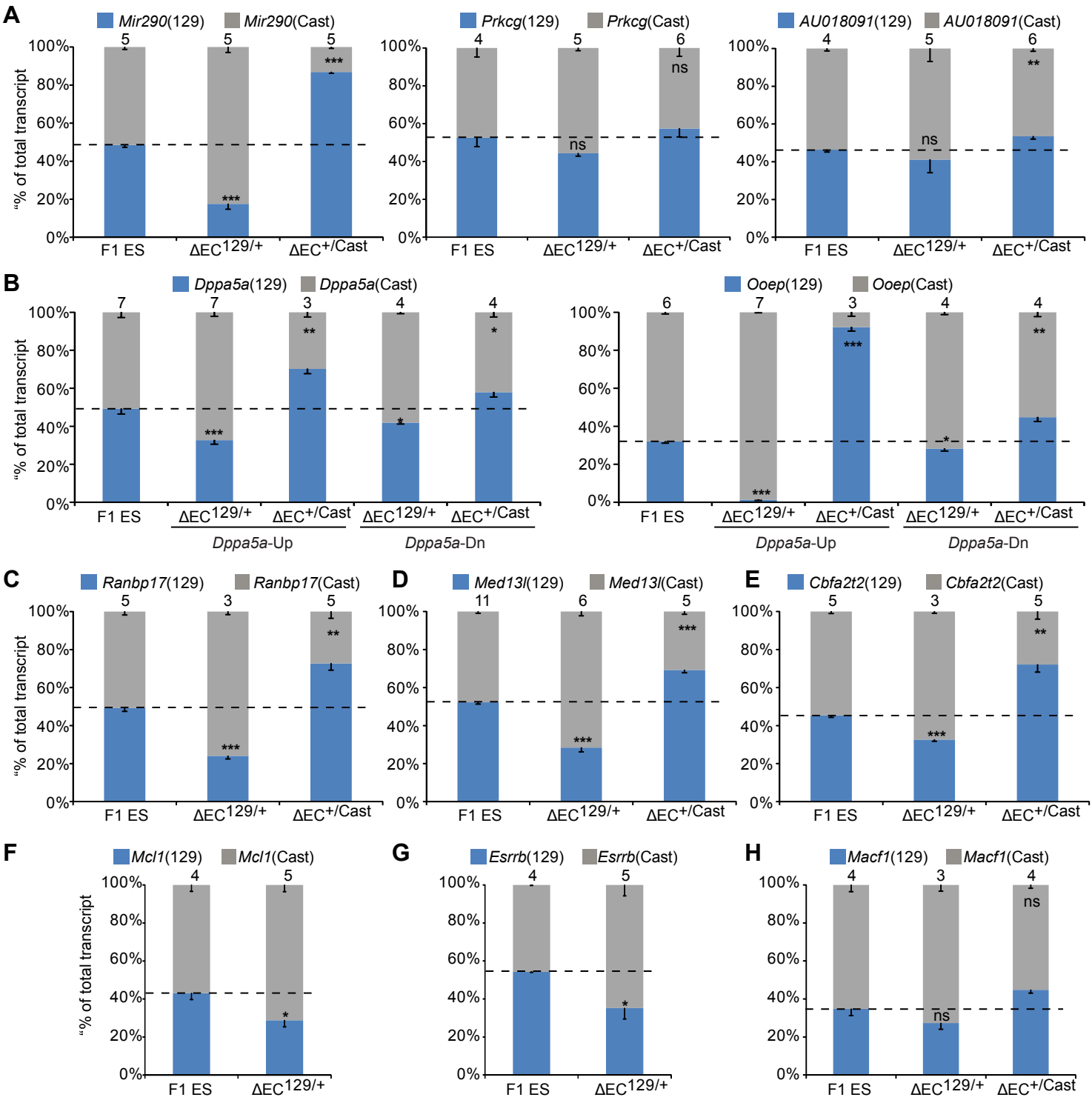


Figure S4: Additional clones display expression changes for target genes identified by RNA-seq. Gene expression changes after deletion of enhancer clusters for target genes identified by RNA-seq. The regions deleted are A) Chr7:3199611-3215602, B) *Dppa5a*-Up Chr9:78368337-78376479, *Dppa5a*-Dn Chr9:78358933-78365818, C) Chr11:33524486-33543505, D) Chr5:118432886-118452483, E) Chr2:154419404-154431952, F) Chr3:95644053-95655885, G) Chr12:86496798-86505014, H) Chr4:123618309-123637993 in mm10. Numbers above the bars indicate the number of deleted clones or F1 ES cell isolates analyzed. Significant differences from F1 ES are indicated by * P<0.05, ** P<0.01 and *** P<0.001, ns = not significant.

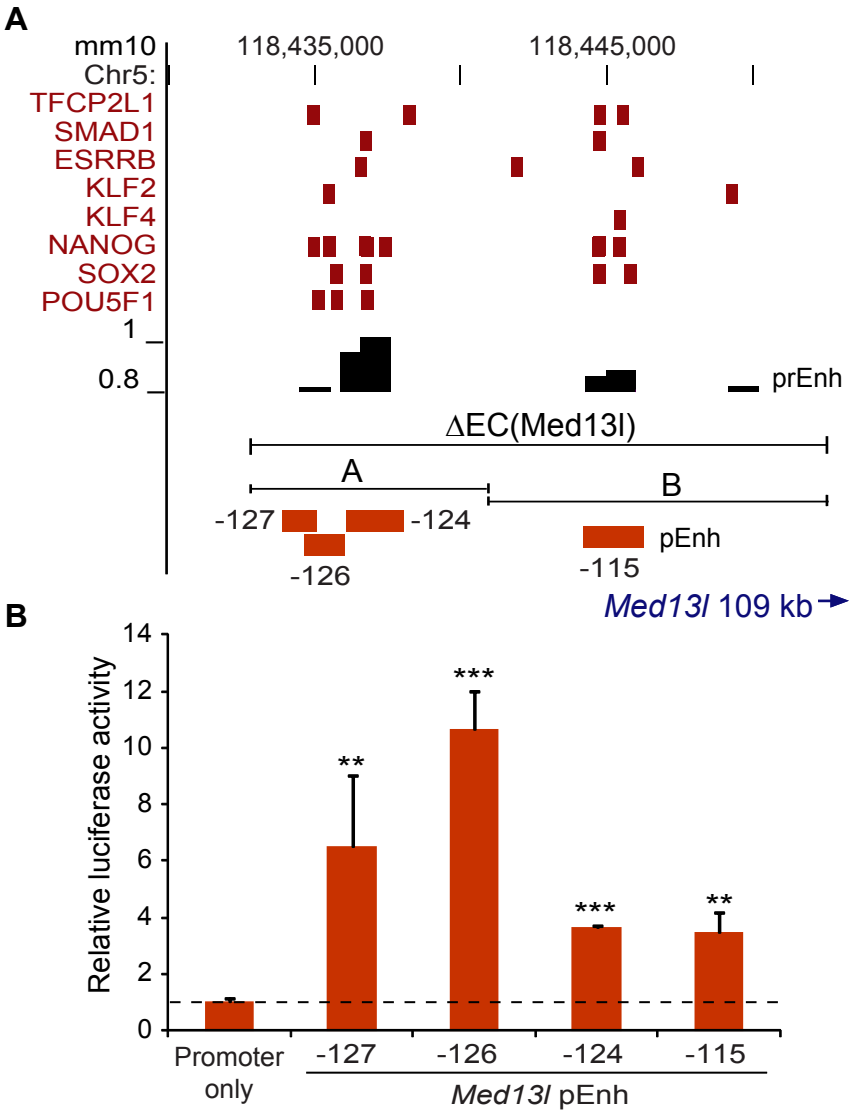


Figure S5: EC(*Med13l*) contains multiple active enhancers. A) Schematic representation of the Med13l EC region. Shown are transcription factor bound regions (red bars) obtained from the CODEX database. Predicted enhancers (prEnh) are shown in black. The deleted enhancer cluster (ΔEC) and partial deletions (A/B) are shown with a line that links the 5' and 3' gRNA targets. All data are displayed on the mm10 assembly of the UCSC Genome Browser. B) Enhancer activity was identified in a reporter assay for each enhancer tested within the EC(Med13lA) and EC(Med13lB) regions. Error bars represent SEM. A significant difference from the promoter only vector is indicated by *** $P < 0.001$, or ** $P < 0.01$.

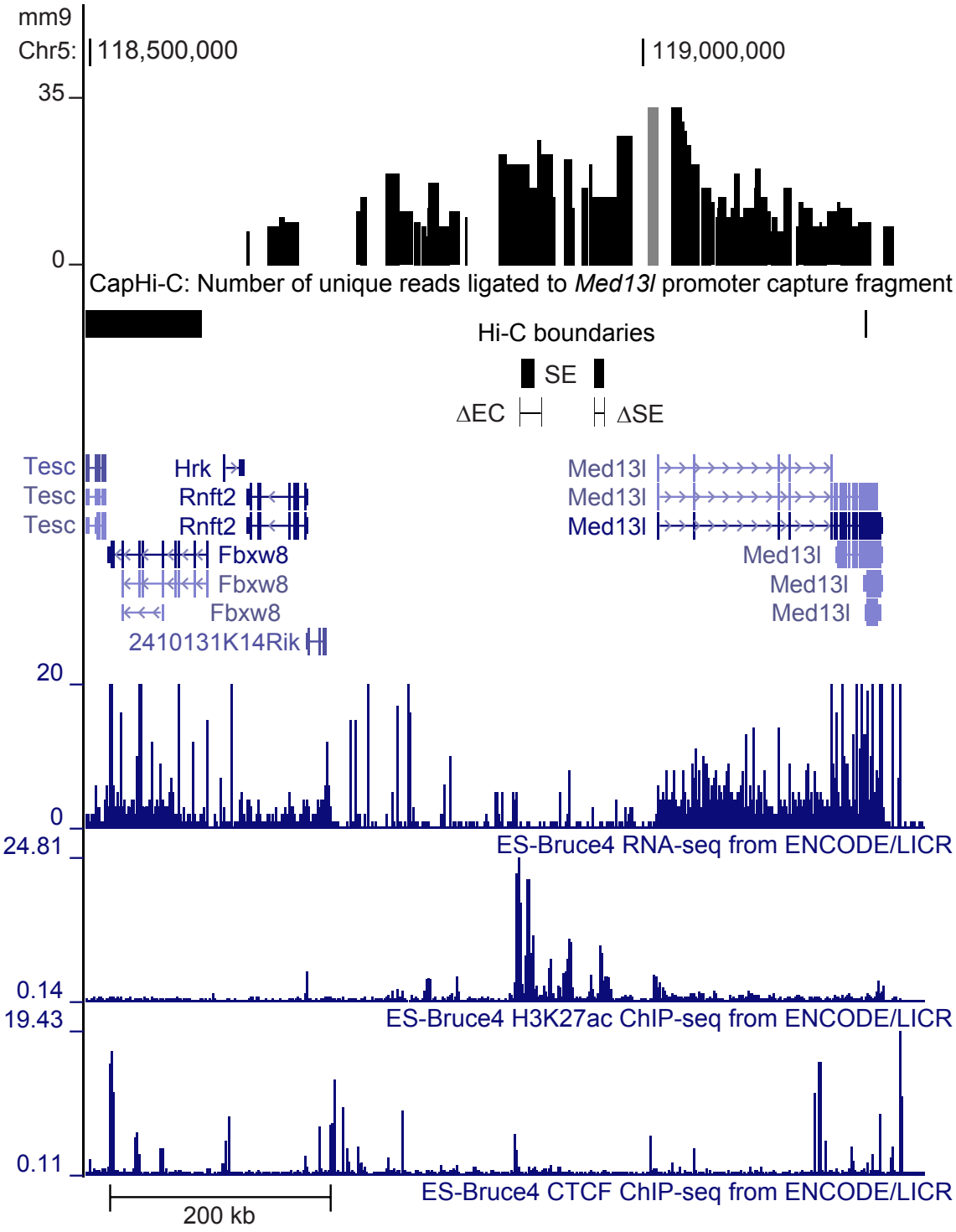


Figure S6 Chromatin conformation at the *Med13l* locus. Shown are CTCF and H3K27ac ChIP-seq and RNA-seq data obtained from ENCODE/LICR. The deleted regions (ΔEC and ΔSE) are shown with a line that links the 5' and 3' gRNA targets. Called super-enhancers (SE) and Hi-C boundaries are shown with black boxes. Med13l promoter capture Hi-C (CapHi-C) reads are shown in bedgraph format at the top. The grey bar indicates the captured fragment, the black bars indicate connected fragments with the bar height displaying the number of unique reads ligated to the *Med13l* promomter fragment. All data are displayed on the UCSC Genome Browser (mm9).

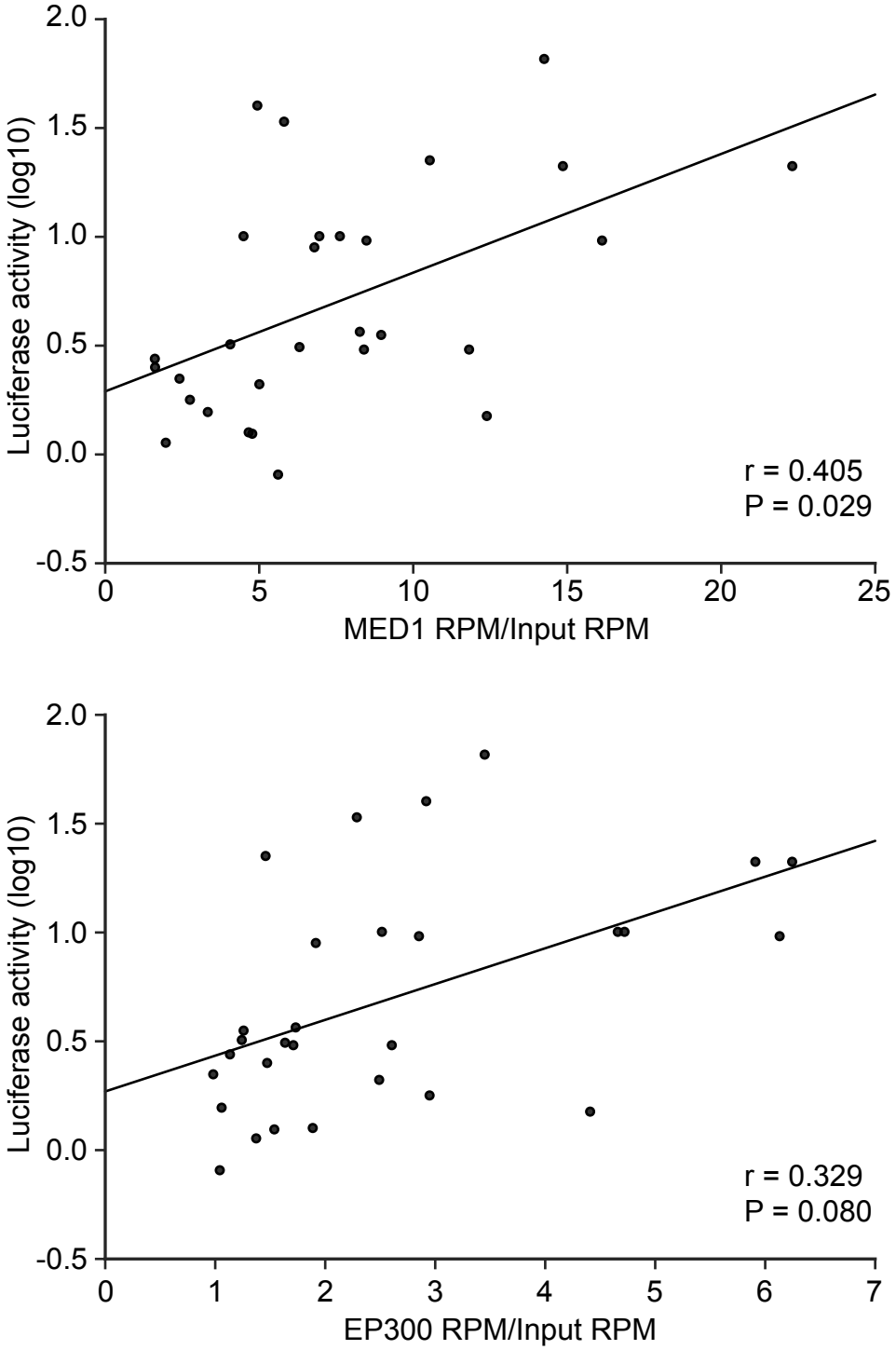


Figure S7: Enhancer activity correlation to MED1 and EP300 ChIP-seq. The significant correlation between enhancer activities in a luciferase reporter assay and the MED1 (top) or EP300 (bottom) ChIP-seq signal at these enhancer regions. Pearson correlation coefficient (r) and significance level (P) are shown. RPM = reads per million.

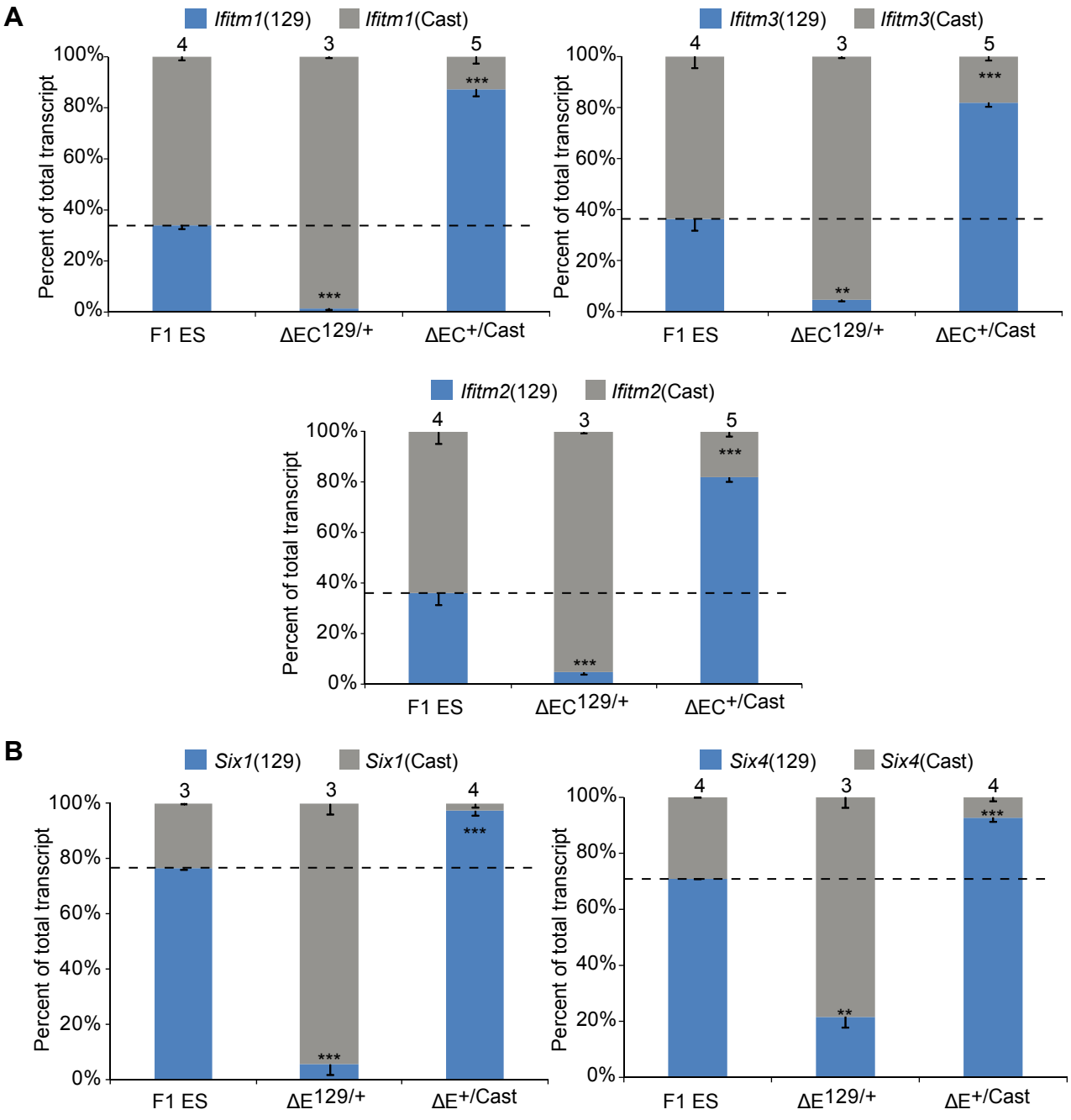


Figure S8: Heterozygous deletion of enhancers at the *Ifitm* and *Six* loci cause reduced expression of the linked allele. Gene expression changes after deletion of the enhancer region on one allele. The regions deleted are A) the *Ifitm* EC and B) the *Six* enhancer. Numbers above the bars indicate the number of deleted clones or cell isolates analyzed. Significant differences from F1 ES are indicated by ** P<0.01 and *** P<0.001.

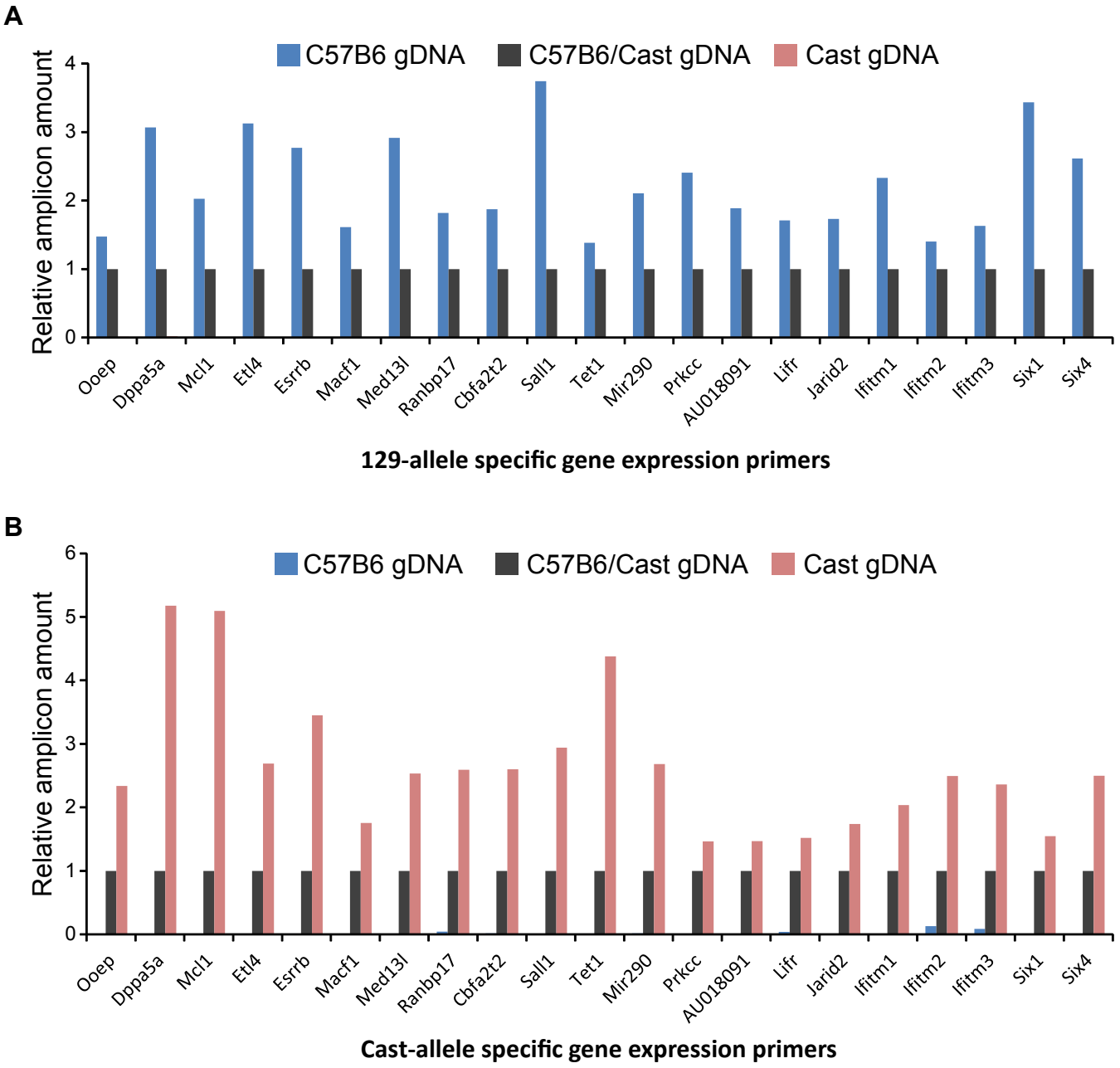


Figure S9: Allele specific primer validation by realtime quantitative PCR. Allele specific primers containing a SNP which distingushes the 129-allele from the Cast-allele were confirmed by by qPCR to be highly allele specific. Relative amplicon amount for the 129-allele (A) and Cast-allele (B) specific primers tested on C57B6 (same genotype as 129 at these SNPs) and Cast genomic DNA as well as a 50:50 mixture of C57B6 and Cast genomic DNA. Values are normalized to the C57B6/Cast gDNA amount.