Supporting Information

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SI Materials and Methods

Cell Culture and Transfection. AtT-20 cells were obtained from ATCC, maintained in UltraCULTURE medium (Lonza) supplemented with 5% (vol/vol) FBS and 1× GlutaMAX (Invitrogen) without antibiotics at 37 °C in a humidified atmosphere containing 5% (vol/vol) CO_2. Transfection of expression vectors was performed using Lipofectamine 2000 (Life Technologies), according to the manufacturer's protocol. To knock down endogenous proteins efficiently, the Neon Transfection System (Invitrogen) was used to electroporate gene-specific siRNAs to AtT20 cells at 1,400 V for 30 ms, following the manufacturer's instructions. Ten microliters of 50-μM total siRNA (listed in Table S1) was used for 1×10^6 cells in a 100-μL tip. Specific mRNA and/or protein were monitored by qPCR or Western blot analysis for knockdown efficiencies.

BLRP-Tagged Cell Lines. To make cell lines expressing tagged ASCL1 or LDB1, we modified an in vivo biotin-tagging system (1, 2). AtT20 cell lines expressing a high level of the bacterial biotin ligase BirA were selected and tested for biotin ligation efficiency. Two lines were chosen as parental lines to make BLRP-tagged expression cell lines. Expression vectors for tagged-ASCL1 and LDB1 were engineered from a modified pCAGGS vector, which was inserted in a double-stranded oligonucleotide encoding the peptide MAGGLNDIFEAQKIEWHEDTGGSWRAPGGGGGSGGGGSGENLYFQSSDYKDHDGDYKDHDIDYKDDDDK. The BLRP (amino acids 3–20), in which the lysine residue (underlined) can be biotinylated by BirA, was followed by a glycine-rich region, a specific recognition site for TEV protease (ENLYFQS; also underlined), and a 3×FLAG sequence. The glycine-rich region serves as a spacer between the BLRP and the TEV site. To increase the specificity further, the TEV site is included for cleaving the tagged protein from the BLRP tag (Life Technologies). The cDNA of *Ascl1* and *Ldb1* was in-frame ligated to the 3′ end of the tag DNA sequence. The expression vectors were transfected into BirA-expressing cell lines. Four individual cell lines for each construct were chosen and pooled with a comparable number cells for ChIP-seq experiments. The PCR primers used for cloning are listed in Table S2.

Antibodies. Specific antibodies used in this study are as follow: anti-P300 (sc585), anti-MTA1 (sc-10813), and anti-MTA2 (sc-9447) from Santa Cruz Biotechnology; anti-H3K4me1 (07-436) from Millipore; anti-H3K27Ac (ab4729) from Abcam; anti-TBX19 (HPA005800) from Sigma-Aldrich; anti-ASCL1 (556604) from BD Pharmingen; anti-CHD4 (A301-081A) from Bethyl Laboratories; and anti-LDB1 sera from Gordon N. Gill, University of California, San Diego.

Reporter Assays. The *POMC* reporter was constructed by ligating the PCR product of the *POMC* 5′ region (−4757 to +74) into multiple cloning sites of the *pGL2-Basic* luciferase vector (Promega). Expression vectors were constructed by inserting cDNAs in *pcDNA3*. The reporter assay was performed 12 h after transfection. The dual-luciferase reporter assay kit (Promega) was used for the luciferase assay; plates were read in a Veritas Microplate Luminometer (Turner Biosystems).

qPCR and Data Analysis. Total RNA was extracted with the RNeasy Mini Kit (Qiagen); then total RNA was reverse transcribed with an iScript cDNA synthesis kit (BioRad). qPCRs were performed in MX3000P (Stratagene) using 2× qPCR master mix from Affymetrix or Bio-Rad. Gene-specific primer pairs (listed in Table S2) were used for quantitative analyses of relative transcript levels; *Gapdh* transcripts were used as an internal control. The comparative cycle threshold method was used to calculate relative mRNA levels. Statistics were performed using unpaired two-tailed Student's *t* tests.

ChIP, in Vivo Sequential ChIP, and ChIP-Seq. Antibody ChIP was performed as previously described (1). Briefly, cells were cross-linked with 1% formaldehyde in PBS at room temperature for 10 min. Double cross-linking was used for MTA2 ChIP, in which cells first were cross-linked with 2 mM disuccinimidyl glutarate (ProteoChem) at room temperature for 45 min, followed by 10 min with 1% formaldehyde. Fixation was quenched by glycine at a final concentration of 0.125 M for 5 min. Chromatin was fragmented to 300–500 bp using a tip sonicator and was centrifuged at 16,000 × *g* for 10 min. The supernatant was precleared with 20 μL of magnetic beads. The precleared chromatin was incubated with 2–5 μg of antibodies at 4 °C overnight. Antibody–protein–DNA complexes were pulled down by incubation with 20 μL of Protein G Dynabeads (Life Technologies) and were washed. The eluted complex was decross-linked overnight at 65 °C. BLRP-tagged ChIP was performed using Nanolink Streptavidin Magnetic beads (Solulink) to pull down the BLRP-tagged protein–DNA complex directly. Additional brief washes with 1% SDS in Tris-EDTA (TE) and TE buffer were performed after normal washes. The washed streptavidin beads then were subjected to TEV protease (Life Technologies) digestion to release tagged protein and DNA complex before decross-linking at 65 °C overnight. For in vivo ChIP, adult murine pituitaries were dissected and immediately immersed in 1% formaldehyde fixation buffer at room temperature for 20 min. The cross-linked tissues were homogenized and sonicated. Sequential ChIP was conducted first with anti-H3K27ac antibody. After washing, the complex was eluted by incubating beads with 25 μL of 10 mM DTT for 30 min at 37 °C. The supernatant was diluted 20 times with dilution buffer [20 mM Tris·HCl (pH7.4), 100 mM NaCl, 0.5% Triton X-100, 2 mM EDTA] and then was subjected to the second ChIP with anti-ASCL1 antibody. The decross-linked ChIP DNA was purified with the QIAquick PCR Purification Kit (Qiagen). The ChIP-seq library was constructed following the Illumina ChIP-seq Sample Prep kit. The library was amplified by 14 cycles of PCR.

PE3C-DSL Assay. The 3C assay was performed as reported (3). Briefly 5×10^6 AtT20 cells, treated with control or *Ldb1* siRNAs for 2 d, were fixed in 1% formaldehyde at room temperature for 10 min. Fixation was terminated by adding 0.5 mL of 2.5-M glycine per 10-mL volume for 5 min at room temperature, followed by 15 min at 4 °C. Cells were incubated on ice in 500 μL of 10 mM Tris·HCl (pH 8.0), 10 mM NaCl, 0.2% IGEPAL CA-630 (Sigma-Aldrich), 1× protease inhibitor mixture for 15 min and were lysed with a Dounce homogenizer. The suspension was spun down for 5 min at 2,000 × *g*. The pellet was washed twice with 500 μL of 1× NEBuffer 2 (New England Biolabs) and resuspended in 362 μL of 1× NEBuffer 2. Next, 38 μL of 1% SDS was added, and the mixture was incubated at 65 °C for 10 min. SDS then was quenched by 44 μL of 10% (vol/vol) Triton X-100. Chromatin subsequently was digested overnight with 400 units of the restriction enzymes indicated in the figures in a final volume of 500 μL. After enzymes were inactivated with 86 μL of 10% (wt/vol) SDS at 65 °C for 30 min, each digested chromatin mixture was ligated by T4 DNA Ligase (800 units) in 7.5 mL

ligation buffer for 4 h at 16 °C. The chromatin subsequently was decross-linked overnight at 65 °C and was purified twice with phenol and once with phenol/chloroform. DNA was precipitated, and pellets were air-dried before being resuspended in 250 μL 1× TE buffer. RNA was removed by incubating DNA with 1 μL RNase A (1 mg/mL) at 37 °C for 15 min. DNA was extracted again with phenol/chloroform and precipitated. The DSL ligation products were prepared as described previously (4). Equal amounts of 3C chromatin were biotinylated using the Photoprobe Kit (Vector Labs). After 10 min at 95 °C to denature the 3C DNA, donor and acceptor probe pools (100 fmol per probe) were annealed to the biotinylated 3C samples at 45 °C for 2 h. The biotinylated DNA was pulled down by streptavidin magnetic beads, and unbound oligonucleotides were removed by washing. The 5′-phosphate of acceptor probes and the 3′-OH of donor probes were ligated using Taq DNA ligase at 45 °C for 1 h on the magnetic beads. The ligated products (donor–acceptor pairs) were purified by alkaline elution from the biotinylated 3C DNA and further by size selection on denaturing PAGE. The purified ligation products were PCR amplified with limited cycle numbers using universal adaptor sequences and subjected to HiSeq 2000 sequencing.

In Situ Hybridization and Immunostaining. In situ hybridization with ^{35}S-labeled anti-sense RNA probes was performed as previously described (5). For immunostaining, E14.5 embryos were fixed in 4% (wt/vol) paraformaldehyde, frozen in 1:1 OTC/Aqua-Mount, cryosectioned at 12 μm, and mounted on Superfrost Plus slides (Fischer). Sections were incubated with primary antibody at a 1:500 dilution in PBS, 0.3% Triton X-100, overnight at 4 °C. After three washings in PBS, slides were incubated with TRITC-coupled secondary antibodies (Chemicon) for 2 h at room temperature. Sections then were mounted using the Slow Fade antifade kit (Molecular Probes).

Coimmunoprecipitation. A 150-mm plate of cells was washed twice with cold PBS and harvested at a confluence of 80–90% on ice. Cells were spun down and lysed in buffer A [10 mM Hepes-KOH (pH 8.0), 10 mM KCl, 1.5 mM MgCl2, 1 mM DTT, and 1× protease inhibitor mixture] for 15 min. Then 100 μL of 10% (vol/vol) Nonidet P-40 was added to cells, and cells were vortexed immediately for 10 s at maximum speed, followed by spinning at 10,000 × *g* for 3 min. The pellets were resuspended with brief sonication in buffer B [1% Triton X-100, 50 mM Tris·HCl (pH7.4), 150 mM NaCl, 0.2 mM EDTA, 1× proteinase inhibitors, and 0.1 mM DTT with 1 μL of Benzonase] for 1 h, followed by centrifugation at 16,000 × *g* for 10 min. Then 2 μg of antibodies were added to 0.5 mL supernatant and were incubated overnight at 4 °C. Then 30 μL of Dynabeads protein G was added for 2 h at 4 °C with rotation to pull down the antibody–protein complexes. After the supernatant was removed, the beads–protein complexes were washed three times with 150 mM NaCl, 25 μM Tris (pH 8.0), 0.1% Nonidet P-40, and 1 mM EDTA and were boiled in 10 mM DTT and 1× LDS sample buffer (Life Technologies) for 3 min. Western blotting was used to detect specific proteins in the complex.

Protein Complex Purification. Cells (10^9) were harvested after washing in cold PBS, and 20 mL of nuclei extract was prepared as described above for immunoprecipitation. Then 100 μL of ANTI-FLAG M2 Magnetic Beads (Sigma) were added to the nuclear lysates and incubated for 4 h at 4 °C. The beads–protein complexes were washed with 10 mL nuclear extraction buffer, followed by five washings in 1 mL of buffer B and two washings in 1 mL of Tris-buffered saline. Complexes were eluted with 0.5 mL 0.2 g/L 3X FLAG peptide (Sigma) for 30 min. Protein complexes were concentrated by Amicon Ultra 4-mL centrifugal filter devices (Millipore) and subjected to mass spectrometry analysis.

Mass Spectrometry Sample Preparation and Analysis. Mass spectrometry sample preparation and analysis were performed as previous reported (1). Briefly, protein samples were boiled in 0.1% RapiGest SF reagent (Waters Corp.) for 5 min and were incubated in 1 mM Tris (2-carboxyethyl) phosphine (TCEP) at 37 °C for 30 min. Subsequently, the samples were carboxymethylated with 0.5 mg/mL of iodoacetamide for 30 min at 37 °C followed by neutralization with 2 mM TCEP. Protein samples then were digested with trypsin overnight at 37 °C. RapiGest was degraded and removed by treating the samples with 250 mM HCl at 37 °C for 1 h followed by centrifugation at 16,000 × *g* for 30 min at 4 °C. The peptides were extracted from the soluble fraction and desalted using C18 desalting columns (Thermo Scientific). For LC-MS/MS analysis, Trypsin-digested peptides were analyzed by ultra-high-pressure liquid chromatography (UPLC) coupled with LC-MS/MS using nano-spray ionization, which was performed by a Triple TOF 5600 hybrid mass spectrometer (AB SCIEX) interfaced with nano-scale reversed-phase UPLC (nanoACQUITY; Waters Corp.) using a 20 cm-75-μm i.d. glass capillary packed with 2.5-μm C18 (130) CSHTM beads (Waters Corp.). Peptides were eluted from the C18 column into the mass spectrometer using a linear gradient (5–80% vol/vol) of acetonitrile at a flow rate of 250 μL/min for 1 h. MS/MS data were acquired in a data-dependent manner in which the MS1 data were acquired for 250 ms at an *m/z* of 400–1,250 Da and the MS/MS data were acquired from an *m/z* of 50–2,000 Da. The independent data-acquisition parameters were as follow; MS1-TOF acquisition time of 250 ms, followed by 50 MS2 events with a 48-ms acquisition time for each event. The threshold to trigger an MS2 event was set to 150 counts when the ion had the charge state +2, +3, and +4. The ion exclusion time was set to 4 s. Finally, the collected data were analyzed using Protein Pilot 4.5 (AB SCIEX) for peptide identification.

GRO-Seq. GRO-seq experiments were performed as reported previously (6, 7). Briefly, 20 million AtT20 cells, treated with siRNAs for 2 d, were washed three times with cold PBS and then were incubated in swelling buffer [10 mM Tris·HCl (pH 7.5), 2 mM MgCl2, 3 mM CaCl2] on ice for 5 min. Cells were lysed in 1 mL of lysis buffer [swelling buffer plus 0.5% Nonidet P-40, 10% (vol/vol) glycerol, and 20 units of SUPERase-In] with a 15-s vortex at 800 rpm. The nuclei pellets were washed twice with 10 mL lysis buffer and 1 mL of freezing buffer [50 mM Tris·HCl (pH8.3), 40% (vol/vol) glycerol, 5 mM MgCl2, 0.1 mM EDTA]. The isolated nuclei were suspended in 100 μL of freezing buffer. For the run-on assay, an equal volume of reaction buffer [10 mM Tris·HCl (pH 8.0), 5 mM MgCl2, 300 mM KCl, 1% sarkosyl, 500 μM ATP, GTP, and Br-UTP, 2 μM CTP, 1 mM DTT, 20 units of SUPERase-In] was added to the isolated nuclei and incubated for 5 min at 30 °C. The nuclear-run-on RNA (NRO-RNA) then was extracted with TRIzol LS reagent (Life Technologies). NRO-RNA was hydrolyzed to ∼300–500 nucleotides by alkaline base on ice for 30 min followed by treatment with DNase I and Antarctic Phosphatase. The fragmented NRO-RNA was pulled down with anti-BrdU agarose beads (Santa Cruz Biotechnology) in binding buffer (0.5× SSPE, 1 mM EDTA, 0.05% Tween) for 1–3 h at 4 °C with rotation. Subsequently, T4 PNK was used to repair the ends of the precipitated bromouridine-triphosphate–labeled RNA at 37 °C for 1 h. The RNA was extracted and precipitated using acidic phenol-chloroform. The RNA fragments were subjected to poly-A tailing reaction by poly-A polymerase (New England Biolabs) for 30 min at 37 °C and were reverse transcribed using oNTI223 primer and the Superscript III RT kit (Life Technologies). The 100- to 500-bp cDNA products were isolated using 10% (vol/vol) polyacrylamide Tris-borate-EDTA (TBE)-urea gels. Next, the purified first-strand cDNA was circularized by CircLigase (Epicentre) and relinearized by APE1 (New England Biolabs). Approximately 120–320 bp of relinearized

single-strand cDNA (sscDNA) was isolated with a 10% (vol/vol) polyacrylamide TBE gel. Finally, the purified sscDNA template was amplified by PCR using the Phusion High-Fidelity enzyme (New England Biolabs). The oligonucleotide primers oNTI200 and oNTI201 were used to generate DNA for deep sequencing.

Deep Sequencing. The DNA libraries were sequenced on Illumina GAII or HiSeq 2000 platforms according to the manufacturer's instructions. The sequences returned by the Illumina Pipeline were aligned to the mouse assembly mm8 or mm9 by using Bowtie or Bowtie2. The data were visualized by preparing custom tracks on the University of California, Santa Cruz genome browser using the HOMER software package (homer.salk.edu/homer/). For visualization, the total number of aligned reads was normalized to 10^7 for each experiment presented in this study.

ChIP-Seq Analysis, Heatmaps, and Tag Density Analysis. ChIP-seq peaks were identified using HOMER with different parameters for transcription factors and histone marks. The called peaks were associated with genes by cross-referencing the RefSeq TSS database; for H3K4me1 and H3K27ac, the enhancer peaks were defined by the absence of nonoverlap with the TSSs in the RefSeq collection. For intersection analysis, the peaks were extended ±1 kb on each side; we used the intersectBED tool in the BEDTools package. Heatmap matrices were generated in HOMER by counting the tags in a 6-kb window (±3 kb of the peak centers) and were displayed in MeV. The tag density plots generated by HOMER by counting the tags in a 6-kb window (±3 kb of the peak center) were plotted in Excel. The MTA2 ChIP-seq tag density plots were normalized using a 1-kb window upstream and down-stream of the 1-kb peak regions of the LDB1/MTA2 cobound enhancers. Motif enrichment was analyzed using a comparative algorithm as described in HOMER (2). The sequence logos were displayed by using WebLOGO (weblogo.berkeley.edu), the box plots were generated in R/BioC, and parametric or nonparametric tests were used for significance analysis.

GRO-Seq Analysis. The sequencing reads were aligned to the mouse genome mm8 or mm9 by using Bowtie or Bowtie2, and the reads were counted over the gene bodies (excluding the promoter-proximal region) by using HOMER or BEDTools. For si*Ascl1*, si*Ldb1*, and si*Tbx19* GRO-seq analysis, we used the same number (7 million) of randomly extracted reads to define the differentially expressed genes, excluding a 400-bp promoter-proximal region. edgeR was used to analyze the statistical significance of the differential expression (FDR <0.01, FC >1.2). We used the closestBED tool in the BEDTools package to assign the closest differentially expressed genes to ASCL1- or LDB1-bound enhancers. For si*Mta1/2* and si*Ldb1* GRO-seq analysis, the alignment was performed after extracting the same number of reads for each of the experiments. The common artifacts derived from clonal amplification were circumvented by considering a maximum of three tags per unique sequence. To determine si*Ldb1*- and si*Mta2*-dependent changes in gene bodies, the sequencing reads were counted over the first 60 kb of the entire gene body, excluding the 2-kb promoter-proximal region on the sense strand with respect to the gene orientation, and a FC >1.2 was applied. The cobound LDB1/MTA2 enhancers were found within a 500-kb distance of double-activated or double-repressed genes by si*Ldb1* and si*Mta1/2*.

Analysis of the PE3C-DSL Data. After the adaptor sequences were removed, the distinct reads were counted by using scripts written in Perl and were aligned to a custom library that includes all the combinations of donors and acceptors. The alignment was performed with Bowtie or Novoalign, and the counts of all the interactions in distinct samples were normalized. The numbers of reads are presented schematically in the figures. The sequences of ligated oligonucleotides are listed in Table S3.

1. Liu Z, et al. (2014) Enhancer activation requires trans-recruitment of a mega transcription factor complex. *Cell* 159(2):358–373.
2. Heinz S, et al. (2010) Simple combinations of lineage-determining transcription factors prime cis-regulatory elements required for macrophage and B cell identities. *Mol Cell* 38(4):576–589.
3. Lieberman-Aiden E, et al. (2009) Comprehensive mapping of long-range interactions reveals folding principles of the human genome. *Science* 326(5950):289–293.
4. Kwon YS, et al. (2007) Sensitive ChIP-DSL technology reveals an extensive estrogen receptor alpha-binding program on human gene promoters. *Proc Natl Acad Sci USA* 104(12):4852–4857.
5. Dasen JS, et al. (1999) Reciprocal interactions of Pit1 and GATA2 mediate signaling gradient-induced determination of pituitary cell types. *Cell* 97(5):587–598.
6. Li W, et al. (2013) Functional roles of enhancer RNAs for oestrogen-dependent transcriptional activation. *Nature* 498(7455):516–520.
7. Core LJ, Waterfall JJ, Lis JT (2008) Nascent RNA sequencing reveals widespread pausing and divergent initiation at human promoters. *Science* 322(5909):1845–1848.

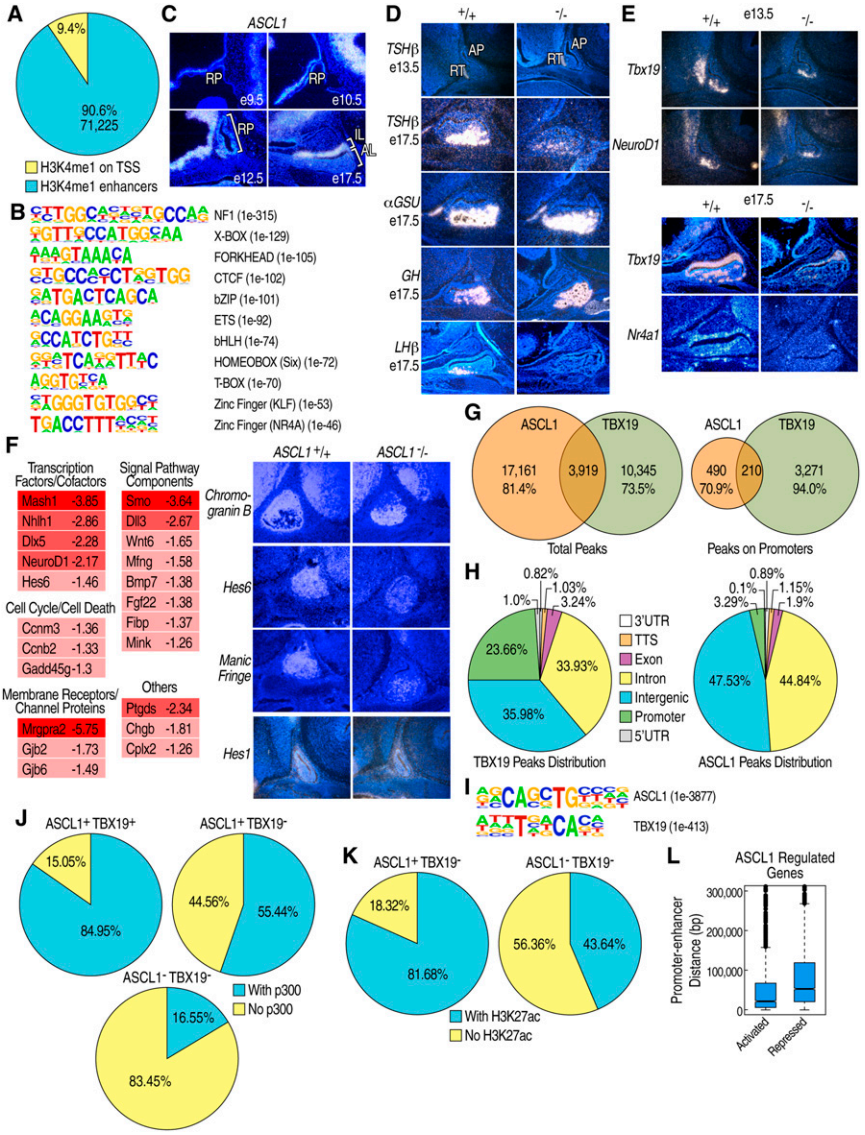

Fig. S1. ASCL1 controls a broad developmental and enhancer program in the pituitary gland. (*A*) H3K4me1 peaks excluding those located on TSSs (71,225) were considered as putative enhancers based on ChIP-seq data. (*B*) The top-ranked enriched motifs from all putative enhancers analyzed by HOMER. (*C*) In situ hybridization shows that the *Ascl1* transcript appears as early as E9.5 at the tip of invaginating oral ectoderm, and its expression domain expands to the whole Rathke's pouch (RP) at E10.5. Expression of *Ascl1* is more restricted to the ventral portion of the gland (AL) at E12.5 and to the intermediate lobe (IL) at E17.5. (*D*) Multiple lineages are regulated by *Ascl1*. *TSHβ* expression was reduced at E17.5 in the anterior lobe but not in the rostral tip (RT) at E13.5 in the *Ascl1*-null mutant. At E17.5, *LHβ*, but not *GH* or *αGSU*, is down-regulated in the *Ascl1*-null mutant. AP, anterior pituitary. (*E*) Up-stream regulators of the *POMC* gene are down-regulated in the *Ascl1*-null mutant. (*Upper*) Transcripts of *Tbx19* and *NeuroD1* are slightly reduced in mutants at E13.5. (*Lower*) *Tbx19* and *Nr4a1* expression is largely impaired at E17.5. (*F*) mRNA profiling of *Ascl1*-null pituitary and validation of *Ascl1* targets by in situ hybridization. (*Left*) mRNA profiling of matched littermates reveals that *Ascl1* regulates the expression of a broad range of gene targets. Dark red, more than threefold change; red, two- to threefold change; pink, less than twofold change. (*Right*) In situ hybridization experiments confirm that the down-regulated genes revealed by mRNA profiling were also down-regulated in *Ascl11*$^{-/-}$ murine pituitary. *Hes1*, which is not altered by mRNA profiling or by in situ hybridization, served as a control. (*G*) Venn diagrams showing the intersection of ASCL and TBX19 ChIP-seq peaks in AtT20 cells. (*Left*) All peaks. (*Right*) Peaks on promoters only. The number of peaks is indicated. (*H*) Genomic distribution of ASCL1- and TBX19-binding sites in AtT20 cells, showing that TBX19 largely binds to promoters. (*I*) Consensus sequences of ASCL1- and TBX19-binding sites in AtT20 cells derived from ChIP-seq by HOMER. (*J*) The percentages of p300^{+} enhancers in different categories of enhancers. (*K*) The percentages of H3K27ac^{+} peaks on enhancers with ASCL1 only (*Left*) and with neither ASCL1 nor TBX19 (*Right*). (*L*) Box plot shows the distances between promoters and the nearest ASCL1 enhancers for ASCL1-activated and -repressed genes.

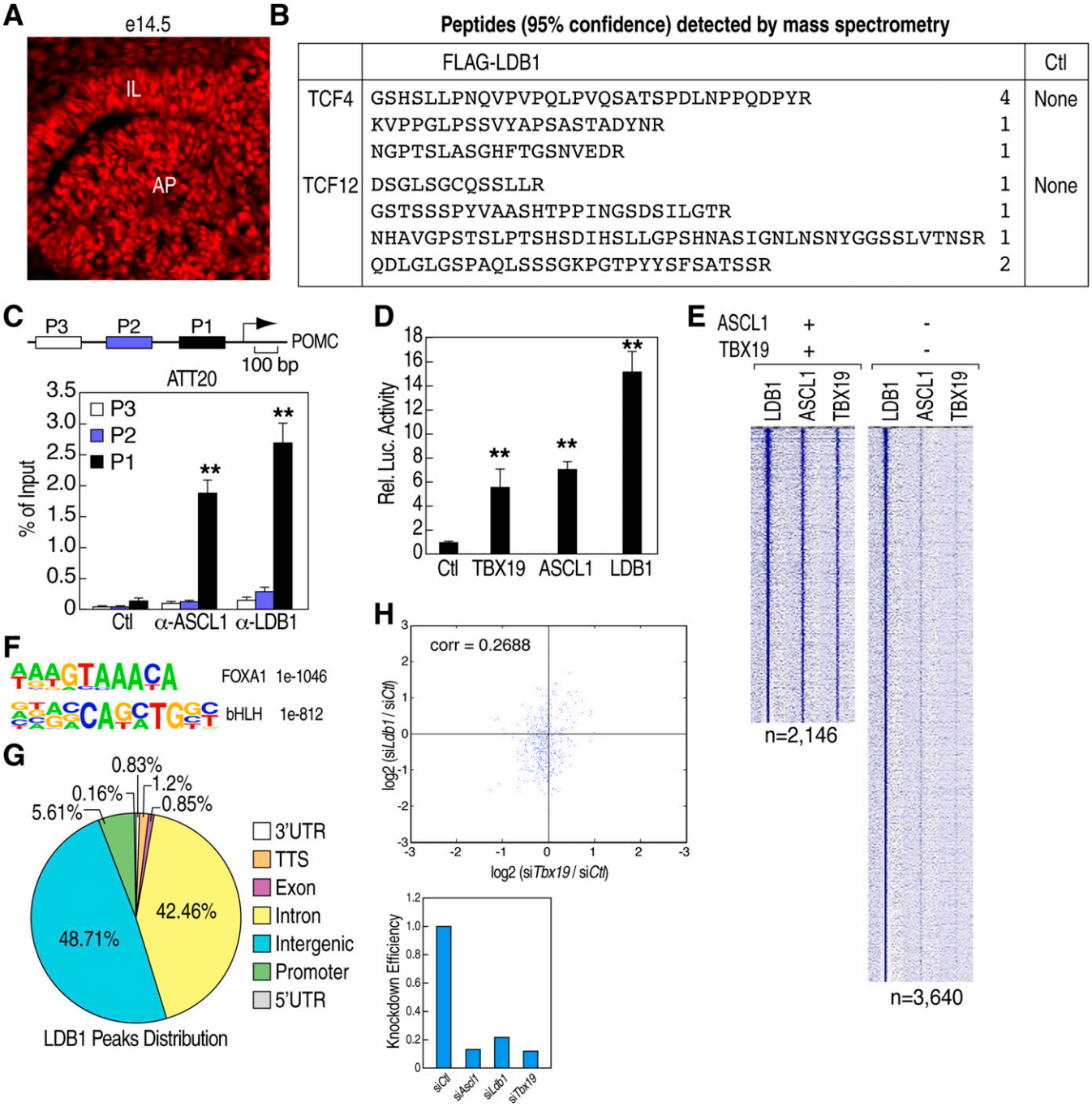

Fig. S2. Coregulation of genes in AtT20 cells by the ASCL1–TCFs–LDB1complex and genomic interaction of *Ascl1* and *Ldb1* genes. (*A*) Immunostaining of LDB1 shows broad expression in the both intermediate lobe (IL) and anterior lobe (AP) of the E14.5 pituitary gland. (*B*) Mass spectrometry of the BLRP–LDB1 complex showing the unique TCF peptides detected. The parental cell line was used as control. (*C*) Quantitative ChIP shows that the binding of ASCL1 and LDB1 is enriched at the P1 site of the *POMC* promoter in AtT20 cells, detected by qPCR ($n = 3$; $^{**}P < 0.01$; mean + SEM). (*D*) TBX19, ASCL1, and LDB1 serve as transcriptional activators in AtT20 cells as detected by luciferase assay ($n = 3$; $^{**}P < 0.01$; mean + SEM). The parental pcDNA3 vector was used as parental control. (*E*) Heatmaps showing that LDB1 enhancers colocalize with ASCL1 and TBX19, in contrast to LDB1 enhancers without ASCL1 and TBX19 binding. Together with Fig. 2*E*, these results show that ASCL1 is highly colocalized with LDB1. (*F*) Enriched motifs of LDB1-binding sites derived from ChIP-seq by HOMER, showing that the bHLH motif is highly enriched. (*G*) Genomic distribution of LDB1 peaks from ChIP-seq. (*H*) The *Ldb1* transcriptional program is less correlated with the program of *Tbx19* than with the program of *Ascl1*. Genes in the proximity of cobound TBX19 and LDB1 enhancers were plotted. Data were obtained from the GRO-seq assay after knockdown of either the *Tbx19* or the *Ldb1* gene. AllStars Negative Control siRNA served as a control. corr, correlation coefficient.

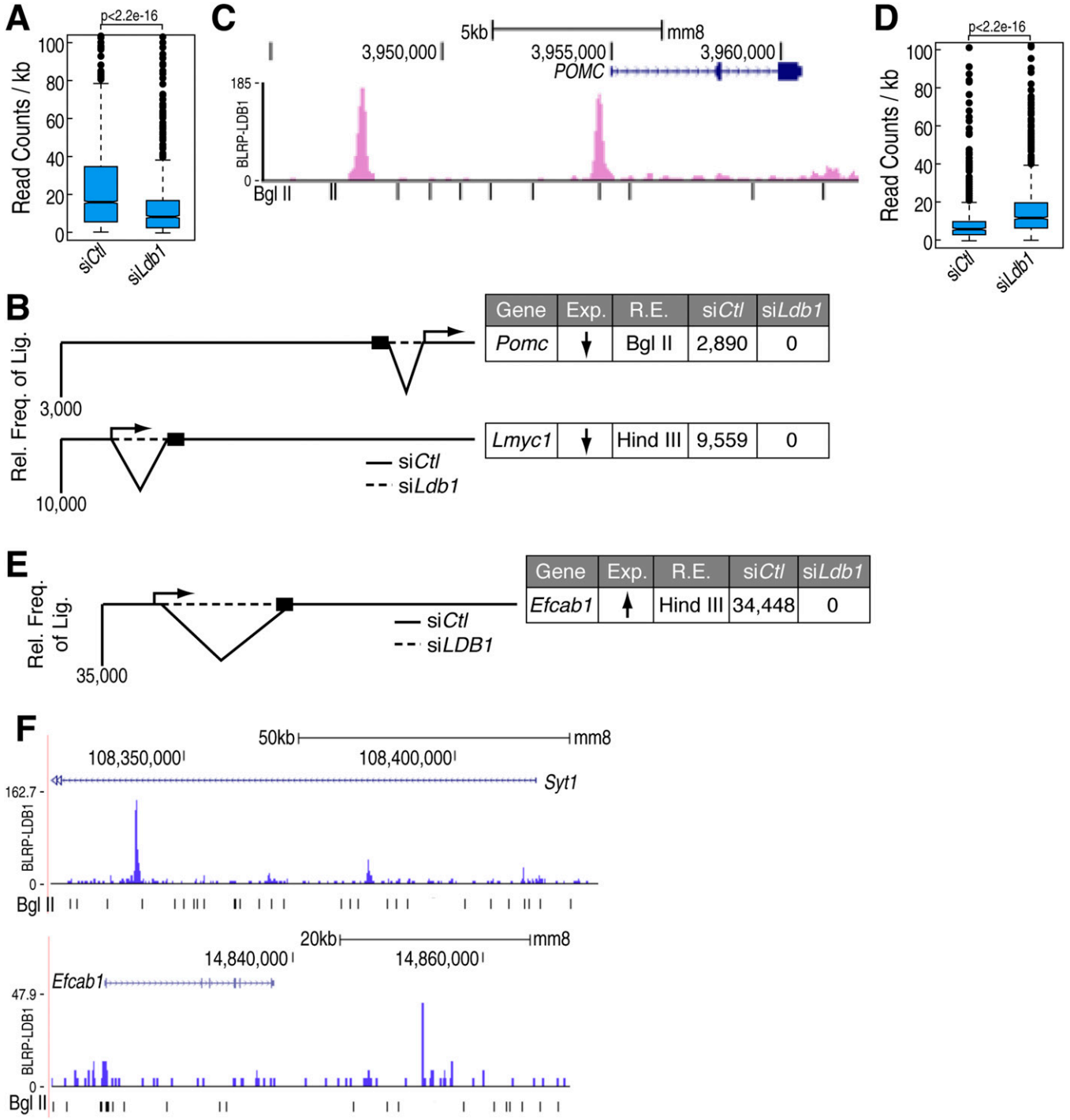

Fig. S3. LDB1 regulates enhancer:promoter looping. (*A*) The box plot shows that expression levels of genes in the proximity of LDB1-bound enhancers are significantly down-regulated by si*Ldb1* compared with AllStars Negative Control siRNA. Expression levels are shown as read counts per kilobase in the gene body; $n = 927$. The statistical significance was computed by the Wilcoxon test in R. (*B*) Promoter:enhancer looping is reduced for LDB1-activated genes upon knockdown of *Ldb1* as detected by the PE3C-DSL assay using different restriction enzymes (R.E.). The table (also shown in Fig. 3*B*) shows the gene name, up- or down-regulation upon siRNA treatment (indicated by arrows), and the reads count from deep sequencing of AllStars Negative Control siRNA or si*Ldb1*-treated samples. Exp., expression level. (*C*) Schematic representation of *POMC* loci used for the conventional 3C assay displayed in Fig. 3*C*. BglII restriction sites are indicated below the snapshot of the LDB1 ChIP-seq browser view. (*D*) The box plot shows that expression levels of genes with LDB1 binding in the proximity are up-regulated by si*Ldb1* in comparison with the AllStars Negative Control siRNA condition. Expression levels are shown as read counts per kilobase in the gene body; $n = 995$. The statistical significance was computed by the Wilcoxon test in R. (*E*) Promoter:enhancer looping is lost for LDB1-repressed genes upon knockdown of *Ldb1* as detected by the PE3C-DSL assay using restriction enzymes different from Fig. 3*E*. The table also shows the gene name, up- or down-regulation upon siRNA treatment (indicated by an arrow), and the reads count from deep sequencing of AllStars Negative Control siRNA or si*Ldb1*-treated samples. Exp., expression level. (*F*) Schematic representation of two loci used for the conventional 3C assay displayed in Fig. 3*F*. BglII restriction sites are indicated below the LDB1 ChIP-seq browser view.

A Peptides (95% confidence) detected by mass spectrometry

	FLAG-LDB1		Ctl	
CHD4	DHQDYCEVCQQGGEIILCDTCPR	1		
	FAEVECLAESHQHLSK	2	FAEVECLAESHQHLSK	1
	FGTEELFKDEATDGGGDNKEGEDSSVIHYDDK	1		
	HLCEPGADGAETFADGVPR	1	HLCEPGADGAETFADGVPR	1
	KLERPPETPTVDPTVKYER	1		
	KVAPLKIKLGGFGSKR	1		
	LLDRNQDETEDTELQGMNEYLSSFK	1		
	STAPEATVECAQPPAPAPATAPATATAPEDDKAPAEPPEGEEKVEK	1		
	VGGNIEVLGFNAR	1		
	MMLTHLVVRPGLGSK	1		
CHD8	TDISLDDPNFWQK	1	None	
	VLSASEVAALSSPASCAPHTAGK	1		
MTA1	QSQALPLRPPPPAPVNDEPIVIED	1		
	SSSSVLSSLTPAK	1		
	VGDYVYFENSSSNPYLIR	2	VGDYVYFENSSSNPYLIR	1
	QIDQFLVVAR	2		
MTA2	FQAEIPDRLAEGESDNRNQQK	1		
	QIDQFLVVAR	2		
	TLLADQGEIR	1	TLLADQGEIR	1
	VGDYVYFENSSSNPYLVR	2	VGDYVYFENSSSNPYLVR	1
	QFESLPATHIR	1		
	VWDPDNPLTDR	1		
P66b	TPVVQNAASIVQPSPAHVGQQGLSK	1	TPVVQNAASIVQPSPAHVGQQGLSK	1
	LQQQAALSPTTAPAVSSVSK	1		

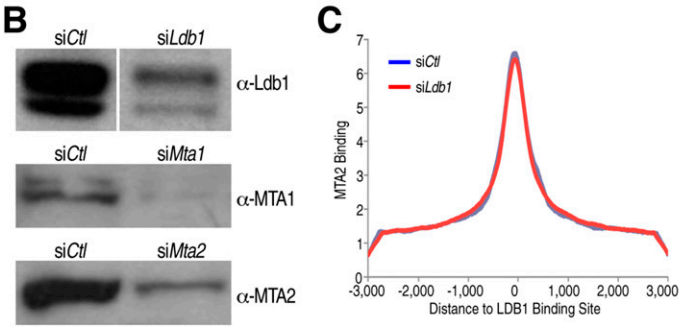

Fig. S4. Members of the NuRD complex in purified LDB1 protein complex. (*A*) Table showing unique peptides detected by mass spectrometry of the FLAG–LDB1 complex. (*B*) Knockdown efficiency of *Ldb1*, *Mta1*, and *Mta2* detected by Western blot assay. Note that two isoforms of LDB1 were detected by the antiserum. The upper panel was sliced to remove unrelated content, as indicated by a blank space. (*C*) The MTA2-binding level on all LDB1 enhancers is not changed by *Ldb1* knockdown. The tag density plot shows there is very limited difference in MTA2 binding after *Ldb1* knockdown.

Table S1. siRNAs

siRNAs	Vendor	Catalogue numbers/sequence
AllStars Negative Control siRNA	Qiagen	1027281
si*Tbx19*	Sigma-Aldrich	SASI_Mm01_00075563
si*Ascl1*	Ambion	CUAUUUGUAUCUAUCCUAAtt
si*Ldb1*	Sigma-Aldrich	SASI_Mm02_00307776
		SASI_Mm02_00307779
si*Mta1*	Sigma-Aldrich	SASI_Mm01_00174991
		SASI_Mm01_00174992
si*Mta2*	Sigma-Aldrich	SASI_Mm01_00168550
		SASI_Mm01_00168554

Table S2. Mouse primers

Genes	Forward primers	Reverse primers
BLRP construct cloning		
Ascl1	CGATGGCGCGCCGAGAGCTCTGGCAAGATGGAGAGT	CGATGTTTAAACTCAGAACCAGTTGGTAAAGTCCAGC
Ldb1	CGATGGCGCGCCTCAGTGGGCTGTGCCTGTCCTG	CGATGTTTAAACGCCCATTTCAGACGCCCAGTCTC
ChIP		
POMC P1	CAGGAAGGCAGATGGACGCACA	GGCGTTAGCACAGACCCGCTGA
POMC P2	CTGGCCACATGGTGAGGGCTGT	GGGGTGTGTGCCTGCCTTGATT
POMC P3	CACTCCGGAAAGAGGCCACAGC	CCAGTCGGGGTGCCTGCCTAAT
3C		
POMC 3C	TGCTGTTCCTGCAGACCTCTCTCTC	CCTGTGCGAGGCAGGCCTAGTT
POMC 3C Ctl	GGCCTTCAGACCCGCATCAG	GCAGAAGGAGTGTCCCAAGACAGG
Efcab1 3C	TGGCACTCTGCTCCAGATGACACT	AAAGCCATGGAGTTGTGGATGGAG
Efcab1 3C Ctl	TGGCACTCTGCTCCAGATGACACT	AGGAGAGGGCCAAGCTAGTTGCTG
Syt1 3C	ACAAGGGAGGCAGAAGCAGGAA	GGGAATGCCCTCAACCTGTTCAGTA
Syt1 3C Ctl	ACAAGGGAGGCAGAAGCAGGAA	GTGGAGGATGGTGGAGCACAGACT
RT-qPCR of mRNA		
Gapdh	CAATGCATCCTGCACCACCAAC	TCCCGTTCAGCTCTGGGATGAC
POMC	GCGGAGGCCTTTCCCCTAGAGTTC	CGCGTTCTTGATGATGGCGTTCTT
Ldb1	GGTGTGGGCTGTCCAATTCCACTC	GCTACCATTCGCTGCCACTTCTGG
Mta1	AAACCATGGACAGACCAGGCACAT	ATTAGGCGCACTTTGGTGGGGTAG
Mta2	CCTCTCACAGACCGGCAGATTGAT	CAGTCTAGGGCTCTCGCGAAGGTT
RT-qPCR of unspliced RNA		
Rad54l	GAGACCCTTTCTCAACCAAAACAT	TCAAAGCCTTTCAAGGTCCCCATC
Ltv1	CCACTGTCTCTGTTGCATGGGAAA	GGAAACCAAAAGCCGTTTCACAGA
GRO-seq primers		
oNTI223	pGATCGTCGGACTGTAGAACTCT;CAAGCAGAAGACGGCATACGATTTTTTTTTTTTTTTTTTTVN	
oNTI200	CAAGCAGAAGACGGCATA	
oNTI201	AATGATACGGCGACCACCGACAGGTTCAGAGTTCTACAGTCCGACG	

= abasic dSpacer furan; VN = degenerate nucleotides.

Table S3. Sequences of ligated donors and acceptors in the PE3C-DSL assay

Gene name	Restriction enzyme	Donor	Acceptor	Normalized siCtl reads	Normalized si*Ldb1* reads
POMC	DpnII	ATAGAGATGGAAAGAGGGGA	TCCCAAGGTCCGGGGTCAGA	6,372	908
	DpnII	AAAGCTAAAGGCCCGGGGGA	TCCCAAGGTCCGGGGTCAGA	3,768	0
	BglII	GCAGCAGAGGCAGCAGTAGA	TCTTGCAGATCGGAGTGGAA	2,980	0
Lmyc1	DpnII	GAACTACTCTGCCAAAGGGA	TCTAATGCCTGATTTCAAAC	5,581	593
	DpnII	TGATAGACTGTCTTATTTGA	TCTAATGCCTGATTTCAAAC	0	474
	HindIII	ATCTCAAGCCCCAAGAGAAG	CTTGGAGTGACTCTTAAGGG	9,559	0
Efcab1	BglII	ACACAAGAACTCAACTGAGA	TCTTAGAGTGTCATCTGGAG	2,984	0
	BglII	GTTTGCTTATACTGGACAGA	TCTTAGAGTGTCATCTGGAG	6,113	0
	HindIII	AAGAAGTAAAACCCTAGAAG	CTTCTAGATGTGAGGACACT	34,448	0
Syt1	BglII	CTGCCTCAGAATCATAGAGA	TCTTTCAAATGTGAGACTAT	8,750	0