Supplementary Information

VHL deficiency drives enhancer activation of oncogenes in clear cell renal cell carcinoma

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Supplementary Figures
Figure S1 (Related to Figure 1)

(A) Percentage overlap between histone ChIP-seq of normal kidney tissues performed by Epigenome Roadmap and Nano-ChIP-seq of our normal kidney tissue. (B) Percentage overlap of peaks between 5 primary ccRCC tumors and their derived cell lines. (C-D) Saturation analysis shows the numbers of predicted promoters (C) and enhancers (D) across increasing number of primary samples. The total number of promoters saturates at 4 or more samples (C), while the number of enhancers at 16 or more samples (D). The yellow dotted line indicates the total number of predicted regulatory elements by integrating all 10 normal-tumor pairs (n=20). The whiskers indicate standard deviations. (E-F) The column plots show the variances captured by each principle component from normalized H3K27ac signals at promoters (E) and enhancers (F). The cumulative percentages of variance are indicated in the tables. (G) The number of altered promoters and enhancers per patient. (H) The fraction of altered regions that meet statistical significance defined by paired t-tests with Benjamini-Hochberg correction (q-value < 0.10) at different cut-offs of recurrence. (I) Differences in the fractions of regions meeting statistical significance. Promoters reach saddle point at n≥5 while enhancers reach saddle point at n≥6.
Figure S2 (Related to Figure 1)

Examples of H3K27ac ChIP-Seq signals in 10 normal-tumor pairs shown for (A) a gained promoter, (B) a lost promoter, (C) an unaltered promoter, (D) a gained enhancer, (E) a lost enhancer, and (F) an unaltered enhancer. N-normal tissue (black); T-tumor tissue (red)
Figure S3 (Related to Figure 2)

(A-B) Histone ChIP-Seq reveals gain of promoters (yellow) and enhancers (blue) near overexpressed ccRCC oncogenes, PLIN2 (A) and SLC38A1 (B). (C) Top 5 Gene Ontology Molecular Functions of tumor promoters and enhancers. (D) Spearman’s correlation between gene expression of VEGFA and SLC2A1 and input subtracted H3K27ac levels of their predicted enhancers in 10 tumor samples (red) and their matched normals (black). (E) Cumulative distribution of distance spanned by significant Capture C interactions.
Figure S4 (Related to Figure 3)
(A-B) Histone ChIP-Seq validated gained super-enhancers (black bar) at PVT1/MYC (A) and EPAS1 (B) loci overlapping with a RCC risk allele respectively. Capture C shows chromosomal interactions between the c-Myc promoter and the super-enhancer. (C) Protein expression of ZNF395 in a panel of ccRCC cell lines measured by immunoblotting. (D) siRNA knockdown efficiency of SMPDL3A and ZNF395 measured by RT-qPCR. (E-F) shRNA knockdown efficiency of ZNF395 levels measured by RT-qPCR (E) and immunoblotting (F) in 786-O and A-498 cells. (G) Annexin V staining analyzed by flow cytometry after ZNF395 shRNA knockdown in 786-O and A-498 cells.
**Figure S5 (Related to Figure 4)**

(A) *In vitro* proliferation of 786-O, A-498 and 12364284 cell lines with and without *VHL* restoration. Proliferation rates were measured with CellTiterGlo, and normalized to day of seeding. EV – empty vector control; *VHL* – wild-type *VHL* restored. (B) *In vitro* colony formation of 786-O, A-498 and 12364284 cell lines with and without *VHL* restoration. Rates were measured by seeding 10,000 cells and allowing colonies to form until the wells become confluent. (C) Apoptosis is measured by cleavage of caspase3/7 substrates and normalized to empty vector controls of 786-O, A-498 and 12364284 cell lines with and without *VHL* restoration. (D) *In vivo* growth of 786-O subcutaneous tumors in nude mice is compared for isogenic cells with and without *VHL* restoration.
Figure S6 (Related to Figure 4)

(A-C) Log fold changes of H3K27ac ChIP-seq signals at gained promoters, enhancers and super-enhancers after VHL restoration in (A) A-498 cells, (B) 12364284 cells and (C) 40911432. Red dots represent p-value<0.05. The number and percentage of altered regions are shown at the upper and lower right corners. EV-empty vector control; VHL- wild-type VHL restored. (D)
Changes of gene expression linked to \textit{VHL}-responsive tumor enhancers in 12364284 cells. *$p$-value < 0.05, two-sided $t$-test.
Figure S7

A

Fraction of grafted mice with H3K27ac/36 histone modifications

B

Heatmap of gene expression levels

C

Genetic variation in patients

D

Expression levels of H3K27ac, H3K4me3, and SLC2A1

E

Expression levels of H3K27ac and HK2

F

Correlation between log fold change in H3K4me1 and H3K27ac

G

Correlation between log fold change in H3K4me3 and H3K27ac

H

Correlation between log fold change in H3K4me3 and H3K27ac

I

Correlation between log fold change in H3K4me3 and H3K27ac
**Figure S7 (Related to Figure 4)**

(A) Frequency of gained enhancers showing H3K27ac depletion after VHL restoration in patients. (B) Unsupervised hierarchal clustering of differential H3K27ac ChIP-seq signals at gained enhancers showing H3K27ac depletion after VHL restoration. Blue - VHL wild-type tumor, red - VHL mutant tumors. (C) H3K27ac ChIP-seq signals of all 10 tumor/normal pairs at ZNF395 super-enhancer. Black - normal tissue, red - VHL mutant tumors, blue - VHL wild-type tumor. (D-E) Examples of lost VHL-responsive enhancer associated with SLC2A1 (D) and HK2 (E) in 786-O cells. (F) Pearson’s correlation of log fold changes of H3K27ac and H3K4me1 in 786-O after VHL restoration. The correlation coefficient is indicated in red. (G) Pearson’s correlation of log fold changes of H3K27ac and H3K4me1 in 12364284 after VHL restoration. (H) Pearson’s correlation of log fold changes of H3K27ac and H3K27me3 in 786-O after VHL restoration. (I) Pearson’s correlation of log fold changes of H3K27ac and H3K27me3 in 12364284 after VHL restoration.
Figure S8

(A) Gene expression of selected transcription factors in 73 pairs of normal kidney (black) and ccRCC tumors (red) of the TCGA cohort (RNA-Seq dataset). ***p-value < 0.001, **p-value <
0.01, n.s. (not significant), paired t-test. (B) Distribution of exogenous HIF1α and endogenous HIF2α ChIP-seq binding sites in 786-O cells annotated using ChIPseeker. (C) Distribution of endogenous HIF1α and HIF2α ChIP-seq binding sites in 40911432 cells annotated using ChIPseeker.
Figure S9 (Related to Figure 7)

H3K27ac ChIP-seq and Capture C of 786-O (ccRCC cell line) and KATO III (gastric cancer cell line) showing that the SLC2A1 enhancer is specific to ccRCC.
Supplementary Methods

Patient information
Fresh frozen normal-tumor tissues were obtained from nephrectomy cases under approvals from institutional research ethics review committees and patient consent under IRB protocol 2010/735/B. Normal tissues were harvested from sites distant from the tumor. Refer to Table S1 for detailed patient information.

Transcription factor Chip-Seq analysis
Sequencing tags were mapped against the human reference genome (hg19) using Burrows-Wheeler Aligner (BWA-mem) (version 0.7.10) [1]. Only reads with mapQ >10 and with duplicates removed by rmdup were used in the subsequent analysis. Significant peaks were called using MACS2 [2] (q-value < 0.01). Fastq files of HIF2α ChIP-seq (GSM856790), HIF1β ChIP-seq (GSM856790) and HIF1α ChIP-Seq (GSM1642764) were downloaded from GEO database. Peaks were called using MACS2 using the same settings as above.

RNA-Seq
Ten pairs of normal-tumor tissue matching the ChIP-seq tissues were prepared for RNA-seq. Total RNA was extracted using the Qiagen RNeasy Mini kit. RNAseq libraries were prepared using the Illumina Tru-Seq RNA Sample Preparation v2 protocol, according to the manufacturer’s instructions. Briefly, poly-A RNAs were recovered from 1 µg of input total RNA using poly-T oligo conjugated magnetic beads. The recovered poly-A RNA was chemically fragmented and converted to SuperScript II and random primers. The second strand was synthesized using the Second Strand Master Mix. Libraries were validated with an Agilent Bioanalyzer (Agilent Technologies, Palo Alto, CA), diluted to 11 pM and applied to an Illumina flow cell using the Illumina Cluster Station. Sequencing was performed on a HiSeq2000 with 74bp or 76 bp paired-end reads.

RNA-Seq analysis
RNA-seq reads were aligned to the human genome (hg19) using TopHat2-2.0.12 [3] (default parameter and --library-type fr-firststrand). Only uniquely mapped reads were analysed. Gene counts were obtained using HTSeq against the GENCODE v19 reference gene models and subsequent differential analysis was performed using DESeq2 [4].

Targeted sequencing
Ten pairs of normal-tumor tissue matching the ChIP-seq tissues were prepared for targeted mutation sequencing. Genomic DNA was extracted using the QIAamp DNA Mini Kit. Genomic DNA libraries were prepared using KAPA Hyper Prep Kit, according to the manufacturer's instructions. Briefly, genomic DNA was fragmented to 150–200 bp by sonication using a Covaris E-220 Focused Ultrasonicator (Duty Factor: 10%, Cycles per Burst: 200, Treatment Time: 360; Covaris Inc.). After the fragmentation process, end-repair, A-tailing, adapter ligation, and PCR reactions before target enrichment was performed, following the manufacturer's recommended protocols. After each step, the purification step was performed with AMPure XP beads to remove short fragments such as adapter dimers. Enrichment was performed using SureSelect XT2 Xplora RNA Bait (Custom, 5.9 Mb). Sequencing was performed on a Hiseq2500 with the paired-end 100bp option.

**PCA analysis**
RPKM values of H3K27ac intensities of all the cis-regulatory elements were first corrected for batch effects using COMBAT [5]. Principle component analysis was performed on the entire 17,497 promoters or entire 66,448 enhancers. Variances and the cumulative proportion of each principal component were computed using R.

**Saturation analyses**
Saturation analyses were performed independently for enhancers and promoters. Specifically, subsets of the H3K27ac profiles from 20 primary samples (consisting of 10 primary tumors and matched normal samples) were selected. All combinations in each subset size were tested except those subsets with >10,000 possible combinations (n=5-15 samples), in which case 10,000 randomly selected combinations were tested. Then, H3K27ac enriched regions from each subset were combined, and overlapping regions were merged. These unique regions were then further classified as promoters and enhancers using the definitions reported in “Identification of differentially enriched regions”.

**GREAT analysis**
Altered promoters were assigned using GREAT v3.0 by the nearest single gene. Altered enhancers were assigned to the genes with a proximal 5.0 Kb upstream, 1.0 Kb downstream extension and a distal extension up to 1000 Kb using default GREAT settings. The top pathways enriched in the MSigDB Pathways and Gene Ontology (GO) Molecular Functions were ranked by their hypergeometric q-values.
**Epigenome Roadmap datasets**
The bed files from H3K27ac, H3K4me1 and H3K4me3 ChIP-Seq of two normal kidneys generated by the Epigenome Road were downloaded from Genboree (http://www.genboree.org/EdaccData/Release-9/sample-experiment/Adult_Kidney/). Peaks were called using CCAT. Similarities between the Epigenome Roadmap and our ChIP-Seq data were computed by the percentage of overlap between peaks.

**DNA methylation analysis**
In total, 160 tumor-normal matched pairs were downloaded from TCGA (Infinium HumanMethylation450 arrays). Quantile normalization was performed across all the samples. Probes were assigned to the nearest promoter or enhancer with a maximal cutoff of 10 kb.

**Chromatin accessibility analysis**
Bigwig-formatted files of 7 ccRCC matched normal-tumor FAIRE-Seq datasets were downloaded from EMBL-EBI ArrayExpress (http://www.ebi.ac.uk/arrayexpress/) under accession number E-MTAB-1936 [6]. FAIRE-Seq signals for each promoter or enhancer region was computed using bigWigAverageOverBed with the promoters and enhancer regions as the input bed file. FAIRE-Seq data was normalized for batch effects using Combat.

**lncRNA analysis**
A list of differentially expressed lncRNA in kidney cancer was downloaded from a study by Iyer et al [7]. RPKM values of each lncRNA was computed across the same ten pairs of normal-matched tissue where ChIPseq was performed, using bigWigAverageOverBed with chromosome positions defined by Iyer et al [7]. These differentially expressed lncRNA were assigned to the nearest promoter and enhancer but with a maximum distance cut off of 10Kb. In total, ~200 lncRNAs were assigned to a promoter or an enhancer.

**Motif analysis**
Motif analysis was performed using HOMER [8] using the gained promoters and enhancers as the input regions and lost promoters and enhancers as the background. The input regions covered the entire span of promoters and enhancers. For VHL-responsive regions, input regions were gained enhancers with H3K27ac depletion after VHL restoration and background regions
were gained enhancers with H3K27ac enrichment after VHL restoration. Only known motifs were considered.

**Histone ChIP-seq with VHL restoration**

H3K27ac, H3K4me1 and H3K27me3 ChIP-seq was performed using histone ChIP-seq as described earlier. Sonicated DNA was normalized for each pair of cells with and without wild-type VHL before immuno-precipitation. Differential analysis of H3K27ac was performed using Deseq2 [4] using raw counts of H3K27ac ChIP-seq p-value < 0.05.

**TCGA RNA-Seq**

Preprocessed RNA-seq v2 data level 3 of ccRCC, papillary and chromophobe RCC was downloaded from TCGA. Only patients with matched normal-tumor pairs (72 pairs ccRCC pairs, 32 papillary RCC pairs and 25 chromophobe RCC pairs) were considered. The overall tumor-normal ratio of a given gene was computed from averaging individual tumor-normal ratios, and p-values computed by paired t-test. Pan-cancer compilation of TCGA data was downloaded from pancan12 (https://www.synapse.org/#!Synapse:syn1695324) [9].

**Immunoblotting**

Cell lines were harvested with cold RIPA lysis buffer (50 mM Tris pH 8, 150 mM NaCl, 0.1% Triton X-100, 0.5% Sodium deoxycholate, 0.1% SDS) with protease inhibitors (Roche) on ice. Cells were mechanically lysed by passing through a 25 Gauge needle and centrifuged at 13,000 rpm for 15 min at 4°C. Protein concentrations were measured by the Pierce BCA protein assay (Life Technologics). Cell lysates were heated at 70°C for 10 min in sample buffer. Per well, 15 µg of cell lysate was loaded and gel electrophoresis was run at 130V constant for 90 mins. Proteins were transferred to nitrocellulose membranes by transferring at 100V for 100 mins in ice. Western blotting was performed by incubating membranes overnight at 4°C with the following antibodies and dilutions: ZNF395 (1 µg/ml, [10]), VHL(1:250 dilution, Cell Signaling 2738), HIF1A (1:500 dilution, BD #610959), HIF1B (1:2000 dilution, Novus Bio NB100-110), HIF2A (1:1000 dilution, Novus Bio NB100-122), ETS1 (1:1000 dilution, Santa Cruz sc-350), c-Fos (1:500 dilution, Santa Cruz sc-7202), c-Jun (1:500 dilution, Santa Cruz sc-1694), NFkB p65 (ab7970, AbCAM) and β-actin (1:2000, Santa Cruz sc-47779). Membranes were incubated in secondary antibodies at 1:10,000 dilution for 1 hr at room temperature and developed with SuperSignal West Femto Maximum Sensitivity Substrate (Thermo Scientific).
siRNA knockdown
ON-TARGETplus SMARTpool siRNA (Dharmacon, UK) were used with Non-Targeting Control Pool as negative control and GAPDH Control Pool as positive control. The sequences of the SMARTpool siRNAs were as follows:

*HIF2α (EPAS1)*  
(GGCAGCACCUCACAUUUGA, GAGCGCAAUGUACCCAAU, GACAAGGUCUGCAAAGGGU, GCAAAGACAGUCCACAGA)

*SMDPL3A*  
(CAGUAUGAUCCUCUGAUU, GAAGAUUUGCAGCCGGAAA, GACAGUAAGCAGUUUAUA, CGGCCCAAUAUAUGACA)

*ZNF395*  
(CCAACUGAUCAUGCUUU, UCAGGCAGAUCAUGCAUC, GUUCUGCGCUCCAUGUGG, GGACGAACCACUCUGACG)

A-498, 786-O and 12364284 and cells were trypsinized and diluted to appropriate concentrations. Lipofectamine RNAiMAX (Life Technologies) and SMARTpool siRNAs were diluted in Opti-MEM to a final siRNA concentration of 50 nM. The diluted Lipofectamine RNAiMAX was added to the diluted siRNA and incubated for 15 min at room temperature to allow complex formation to occur. The siRNA mixtures were aliquoted to wells in a 6-well plate. Forty-eight hours after transfection, cells were re-seeded into 6-well plates for colony formation assays and 96-well plates for cell viability assay.

shRNA knockdown
Lentiviral plasmids were transfected into HEK293T cells. MISSION shRNA clones against *ZNF395* were purchased from Sigma Aldrich. The sequences of the clones are as follows:

TRCN0000233231  
CCGGGCATCAACACGACCGTCAAAGCTCGAGCTTTGACGTGTCGGTCTGGTCTTTTTTG

TRCN0000233234  
CCGGCAGAAGCCCTTTACTGATTAAACTCGAGTTTAATCAGTAAAGGCTTTCTGGTGTTTTTG

Cells were transduced with lentiviral particles for 48 hours and underwent puromycin selection (2 µg/ml) for 4 days before being analyzed for gene and protein expression and other functional assays.

Quantitative RT-PCR Analysis (qPCR)
Total RNA was extracted from cell lines using Trizol (ThermoFisher) and purified with the RNeasy Mini Kit (Qiagen). Reverse transcription was performed using iScript Reverse Transcription Supermix for RT-qPCR (Biorad). qPCR was performed using Taqman probes (*ZNF395* Assay ID: Hs00608626_m1, *SMPDL3A* Assay ID: Hs00378308_m1) with TaqMan
Gene Expression Master Mix (ThermoFisher). Gene expression changes were normalized to GAPDH (Assay ID: Hs00699446_m1).

ChIP-qPCR
ChIP DNA was probed with the following primers using the SYBR qPCR master mix (ThermoFisher).

Primers:
ZNF395-E1 (hg19 chr8:28221378-28221459)
ZNF395-E1-F: GCAACCTTCAGGCCTGCCG
ZNF395-E1-R: AGGAGAAAGGGACAGGAGGGC

ZNF395-E2 (hg19 chr8:28222803-28222908)
ZNF395-E2-F: TGGGCCGCCCGTGACTTTTC
ZNF395-E2-R: GGTTGGAAGGAGGCCACCCGC

ZNF395-E3 (hg19 chr8:28223142-28223230)
ZNF395-E3-F: TCGTGCTGAAGGCTTCTCAGAAA
ZNF395-E3-R: CCCCTCCTGTTGTTGACGGC

ZNF395-E4 (hg19 chr8:28269095-28269211)
ZNF395-E4-F: AAGCGGCAGGGAGGAGGTTGA
ZNF395-E4-R: GGGCTGCGTCACCTGCAGAA

Luciferase assay
Genomic DNA from where 786-O cells was extracted using DNeasy Blood & Tissue Kit (Qiagen). Regions corresponding to putative enhancers were amplified using CloneAmp HiFi PCR Premix (Clonetech) and cloned into the pGL3 luciferase reporter vector with a minimal FOS promoter -

Forward primer: GTAGCTGCATAGATCTGCGGCCACCCCTCTGCGCCACCGT
Reverse_primer: GTAGCTGCATCAAGCTTGCCGGCTCAGTCTTGGCTTCTC.

The day prior to transfection, $1 \times 10^4$ cells were seeded into each well of 96 well plates. Cells were transfected with 100 ng of pGL3-Fos-enhancer and 20 ng of pRL-SV40 (Renilla luciferase vector, Promega). Cells were lysed and analyzed using the Dual-Luciferase Reporter System (Promega).
Primer sequences used to amplify genomic regions for luciferase reporter assays are as follows:

**VEGFA-E1 (hg19 chr6:43635485-43636708)**
VEGFA-E1-F_MluI: GCTCTTACGCGT TGGGGGTGCCTCTCCCACTG
VEGFA-E1-R_NheI: GCCCGGGGCTAGC GGCTGGGGGTCAACAGGACA

**VEGFA-E2 (hg19 chr6:43692413-43693560)**
VEGFA-E2-F_MluI: GCTCTTACGCGT CCCATCCCCTGCTCCTGCT
VEGFA-E2-R_NheI: GCCCGGGCCTAGC TGGGGCTGGCTGCAAAGTGGC

**SLC2A1-E1 (hg19 chr1:43523259-43525686)**
SLC2A1-E1-F_MluI: GCTCTTACGCGT TGGTGACCGTGTTGGGGGTGA
SLC2A1-E1-R_NheI: GCCCGGGGCTAGC TCCCCCGCCCTCTGTTGCT

**ZNF395-E1 (hg19 chr8:28220788-28221483)**
ZNF395-E1-F_MluI: GCTCTTACGCGT ACAGGTGTGCGCTACCACGC
ZNF395-E1-R_NheI: GCCCGGGGCTAGC TGGTGTGGAATTCTGGCCAGTTAAAG

**ZNF395-E2 (hg19 chr8:28221957-28222965)**
ZNF395-E2-F_MluI: GCTCTTACGCGT TCGGGAGGTTCAAGACCAGCCT
ZNF395-E2-R_NheI: GCCCGGGGCTAGC GCTCCCAAGAAAGAACTTACCAGG

**ZNF395-E3 (hg19 chr8:28222984-28224154)**
ZNF395-E3-F_MluI: GCTCTTACGCGT ACCAGCCATCCCCTAGTTTGCC
ZNF395-E3-R_NheI: GCCCGGGGCTAGC GCCATTTGTACGAGATGTTGCG

**Colony formation and cell viability assays**

For colony formation assays, 5000 cells per condition were seeded into 6 well dishes and were allowed to grow for 12 days. Colonies were stained with 0.05% Crystal Violet. For cell viability assay, 1000 cells per condition were seeded into 96 well dish and the cell viability was measured by CellTiter-Glo Luminescent Cell Viability Assay (Promega) for 5 days.

**Apoptosis assay**
For each condition, 1x10³ cells were seeded into each well of a 96 well plate. Caspase3/7 activity was measured with the cleavage of proluminescent caspase-3/7 substrate after 1 hr incubation using Caspase-Glo® 3/7 Assay (Promega). Alternatively, cells were stained with FITC Annexin V Apoptosis Detection Kit (BD Bioscences) and Calcein AM (ThermoFisher) and analyzed on a flow cytometer.

**In vivo studies**
All animal studies were conducted in compliance with animal protocols approved by Institutional Animal Care and Use Committee (IACUC) of Singapore. Female NOD/SCID mice (6-8 week old) were implanted with 1 x 10⁶ A-498 or 1 x 10⁶ 786-O cells transduced with either empty vector control or shRNA clones subcutaneously in the flank. Tumor volume was monitored every 2-3 days. Tumor volume was calculated as (length x width x width) x π/6. Animals were sacrificed when the tumor volume exceeded 1000 mm³.

**CRISPR mediated enhancer deletion**
To delete enhancer regions, 2 gRNAs (left and right) were used to cleave targeted regions as previously described [11]. gRNAs were designed with ATUM gRNA Design Tool (https://www.atum.bio/products/crispr). Briefly, phosphorylated and annealed sense and antisense oligos were ligated into BpiI digested vectors. Left gRNAs were cloned into the BpiI digested pX330A-2A-GFP-1X2 backbone (Addgene # 58766, with addition of GFP by Dr. Shang Li, Duke-NUS, Singapore) whereas the right gRNAs into BpiI digested pX330S backbone (Addgene # 58778). Golden gate assembly was performed to assemble the 2 gRNA protosparse into the pX330A-2A-GFP-1X2 plasmid backbone using a one-step digestion and ligation [12] with slight modifications. After transfection using Lipofectamine 3000 (Life Technologies), GFP-positive single 786-O cells were sorted and cultured. Individual clones were validated for enhancer deletion by PCR of genomic DNA and the resulting gene expression was measured using qPCR and Taqman probes. Clones that were transfected with gRNAs but failed to have enhancer deletions were used as negative controls.

The gRNAs used for deletion of enhancers are as follows:
ZNF395_E3 (hg19 chr8:28223203-28224208)
ZNF395_E3_L_F_gRNA: CACCGTCCCTACTGCCGTCACCAAC
ZNF395_E3_L_R_gRNA: AAACGTTGGTGACGGCAGTAGGGAC
ZNF395_E3_R_F_gRNA: CACCGAAATATGTTTATGGTCCTCC
ZNF395_E3_R_R_gRNA: AAACGGAGGACCATAAACATATTTC

Validation primers for deletion of enhancers:
ZNF395-E3 (Product size after deletion: 293bp; WT: 1299bp)
ZNF395-E3-F: ACCAGCCATCCCCTAGTTTGCCA
ZNF395-E3-R: GCCACCAGGTCAGTGGGTT
References: