Supplemental Material

Supplemental Methods

Supplemental Figure S1-S4

Supplemental Tables S1, S2

Supplemental Methods

Northern blot

Total RNA from K562 cells was isolated using the RNeasy mini kit (Qiagen). Poly A (+) RNA was prepared from 75 μg of total RNA using the Dynabeads mRNA Purification Kit (Ambion) following the manufacturer instructions. The capture and elution of the poly (A)+ RNA was repeated 5 times until no poly (+) RNA was left in the sample. The quality of the RNA was evaluated using the Agilent RNA 6000 Nano kit. RNA samples (20 μg per lane/total RNA or 100 ng of poly (A)+ RNA) were denatured in MOPS/formaldehyde/formamide buffer and separated by electrophoresis on a 1% formaldehyde/agarose gel at low voltage. Following electrophoresis, RNA was transferred to the positively charged Brightstar-Plus membrane (Ambion) by capillary blotting (TurboBlotter, Schleicher & Schuell) overnight in 10× SSC. Resolved RNAs were cross-linked to the membrane by UV irradiation and incubated in hybridization solution (Ultrahyb, Ambion) for 1 h at 45°C before addition of the probe. Radiolabeled DNA probes were prepared using the DECAprime II random priming Kit (Ambion). Labeled probes were purified using the NucAway Spin columns (Invitrogen), added to the hybridization mixture, and incubated overnight at 45°C. The blots were then washed using solutions containing decreasing concentrations of SDS and SSC (1× SSC is 0.15 M NaCl, 0.015M sodium citrate). Transcripts were visualized by autoradiography or Phospho Imager.

BGLT3 overexpression studies

To overexpress BGLT3, full length BGLT3 and GFP genes were cloned into the BamHI and XhoI sites of the vector pLenti/V5-D-TOPO (Invitrogene). 1x106 K562 cells were resuspended in 100 ul of nucleofector solution (Lonza) and 4 μg of DNA was added. The cells were transfected using the Nucleofector® technology system kit V (Lonza). Cells were harvested at 48 hours after transfection. Transfection efficiency was monitored by GFP expression under fluorescence microscopy. Total RNA was extracted and reverse transcribed and gene expression was determined by qRT-PCR.

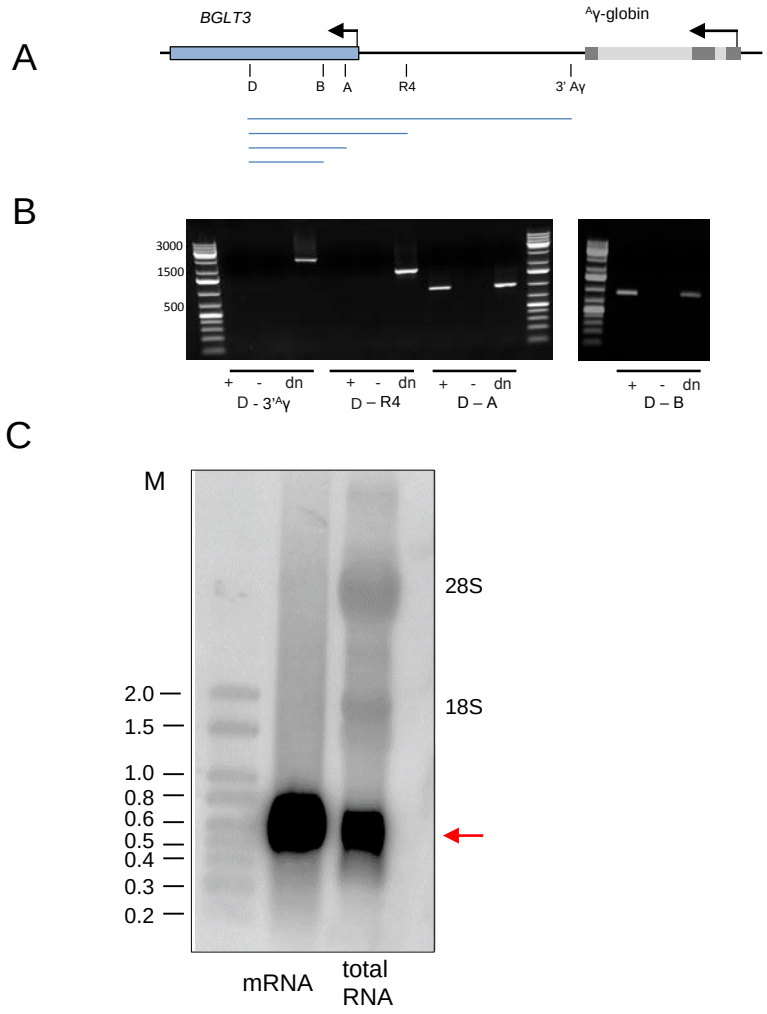
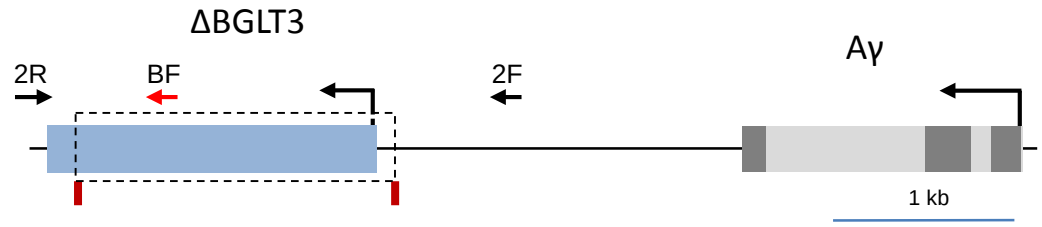


Figure S1. Analysis of BGLT3 lncRNA. (A) Schematic diagram of $^{A}\gamma$-globin and *BGLT3*. Black rectangles show location of primers for PCR reactions analyzed below. (B) PCR products were generated with (+) or without (-) cDNA or genomic DNA (dn) isolated from K562. For primer sequences see Table S1. (C) Total RNA and poly A+ RNA from K562 cells were separated on a denaturing gel and the γ-globin mRNA was visualized by Northern blot with a radiolabeled DNA probe. The 28S and 18S ribosomal RNAs are indicated.

A



B

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BGLT3      TCTGTAGATTGTCTTTCGCTCTGATGGTAGTTTCTTTTGCTGAGCAGGAGCTCTTTAGTT
ΔBGLT3.2   TCTGTAGATTGTCTTTCGCTCT----------------------------------------
ΔBGLT3.30  TCTGTAGATTGTCTTTCGCT-----------------------------------------

BGLT3      TAATTAGATTCCATTGGTCAATTTTTGCTTTTGCTGCAATTGCTTTTCACGCTTTCATCA
ΔBGLT3.2   ------------------------------------------------------------
ΔBGLT3.30  ------------------------------------------------------------

BGLT3      TGAAATCTGTGCCCGTGTTTATAT                        CTTGCTTGAACAG
ΔBGLT3.2   ------------------------//—— 1509 bp ——//-------------
ΔBGLT3.30  ------------------------                        -------------

BGLT3      AGCAGCACACTTCTCCCCCAACACTATTAGATGTTCTGGCATAATTTTGTAGATATGTAG
ΔBGLT3.2   ----------------------------------TTCTGGCATAATTTTGTAGATATGTAG
ΔBGLT3.30  ----------------------------------TTCTGGCATAATTTTGTAGATATGTAG

BGLT3      GATTTGACATGGACTATTGTTCAATGATTCAGAGGAAATCTCCTTTGTTCAGATAAGTAC
ΔBGLT3.2   GATTTGACATGGACTATTGTTCAATGATTCAGAGGAAATCTCCTTTGTTCAGATAAGTAC
ΔBGLT3.30  GATTTGACATGGACTATTGTTCAATGATTCAGAGGAAATCTCCTTTGTTCAGATAAGTAC

BGLT3      ACTGACTACTAAATGGATTAAAAAACACAGTAATAAAACCCAGTTTTCCCCTT
ΔBGLT3.2   ACTGACTACTAAATGGATTAAAAAACACAGTAATAAAACCCAGTTTTCCCCTT
ΔBGLT3.30  ACTGACTACTAAATGGATTAAAAAACACAGTAATAAAACCCAGTTTTCCCCTT
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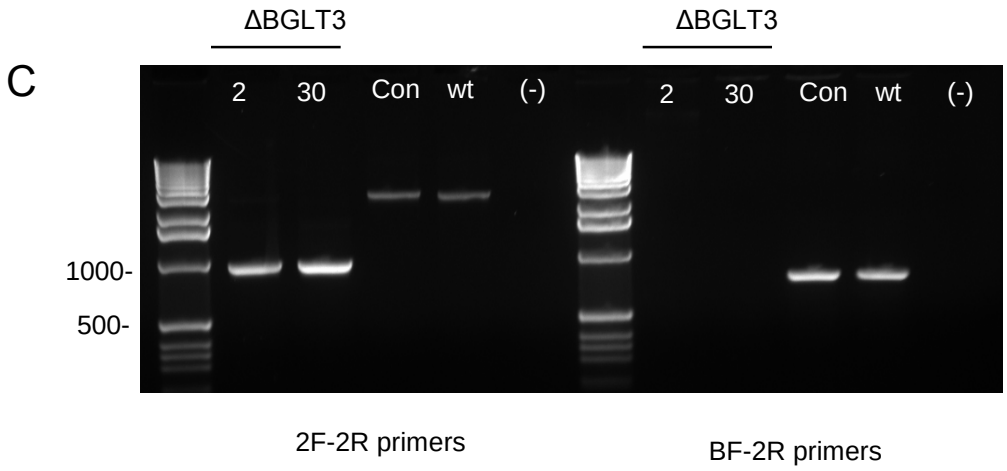

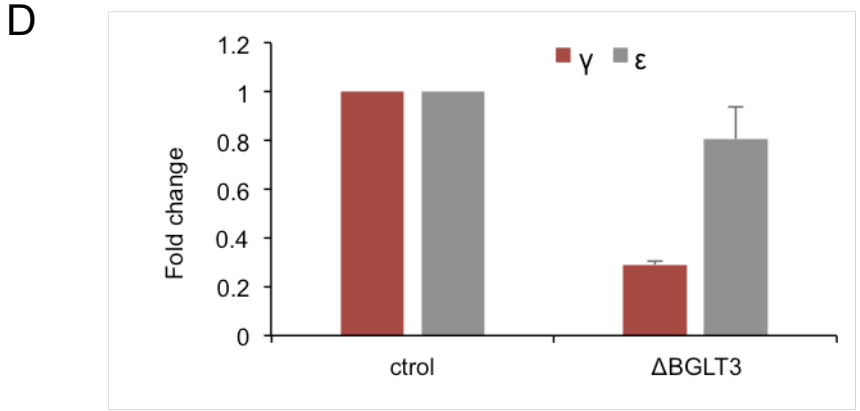

Figure S2. Deletion of the *BGLT3* gene by CRISPR/Cas9 in K562 cells. (A) The schematic diagram shows the deletion of the *BGLT3* gene generated by CRISPR/Cas9 using two gRNAs targeting the 5' and 3' ends of the *BGLT3* gene (red bars, dotted rectangle). Arrows indicate the primers used for the PCR screening. (B) Sequences of BGLT3 deletion clones 2 and 30. Guide RNA targets are highlighted in green, PAM sequence is bolded and the first and last three nucleotides of BGLT3 are highlighted in red and blue, respectively. (C) Electrophoretic analysis of PCR products generated from gDNA of ΔBGLT3 (clones 2 and 30), control (cells transfected without gRNAs) and wild type K562 cells. The amplicons were generated using primers outside the deletion (2F-2R primers) and one primer (BF) inside the deleted region. The PCR products generated using the primers 2F and 2R were cloned and sequenced to verify the deletion. (D) Fold change of γ- and -globin in BGLT3 cells compared to control cells (ctrol). For gRNA and primer sequences see Table S1.

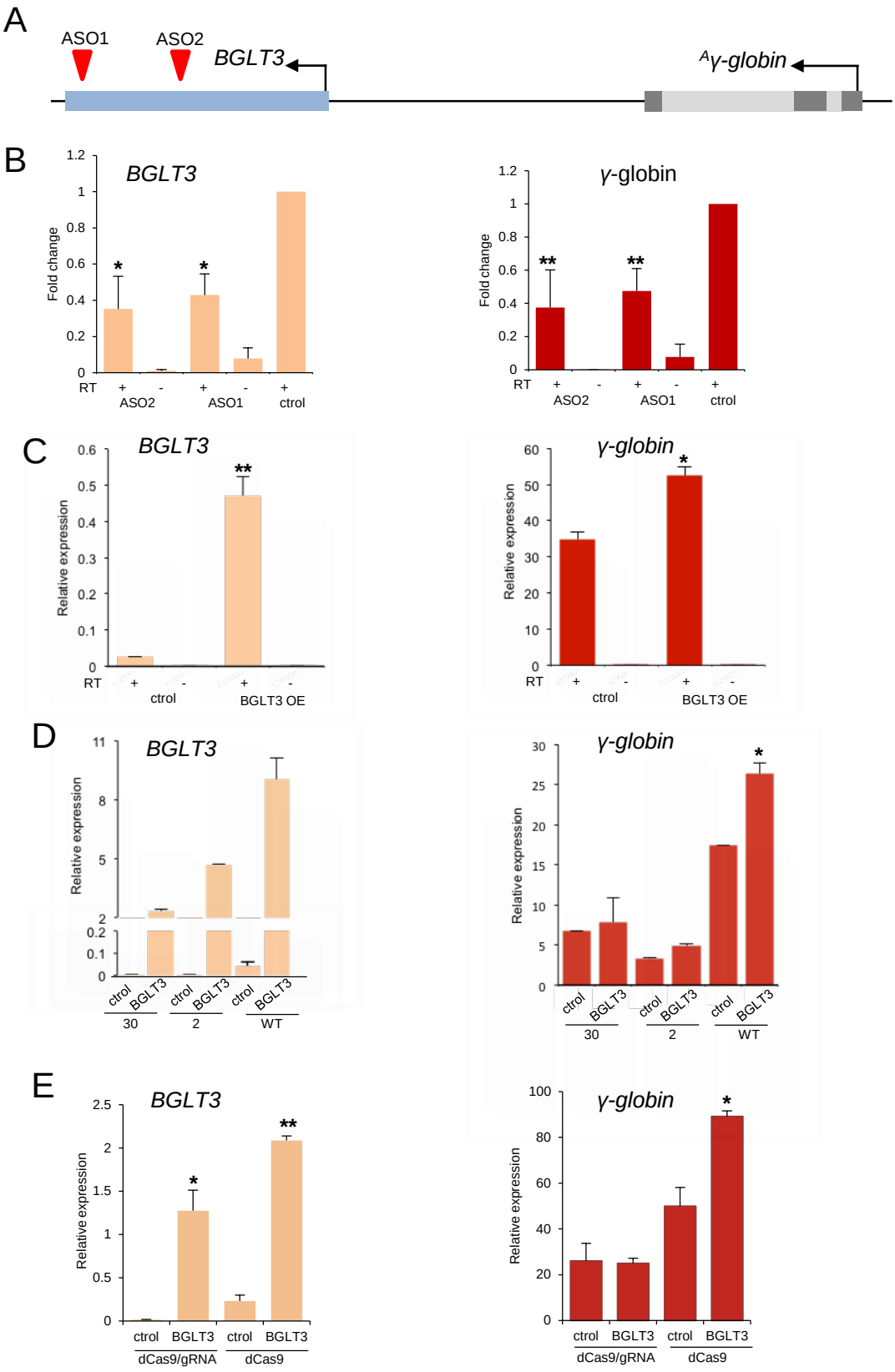
A
ASO1
ASO2
BGLT3
Aγ-globin
B
BGLT3
Fold change
RT
ASO2
ASO1
ctrol
γ-globin
C
BGLT3
Relative expression
ctrol
BGLT3 OE
γ-globin
D
BGLT3
γ-globin
30
2
WT
E
BGLT3
γ-globin
dCas9/gRNA
dCas9

Figure S3. Reduction and over-expression of *BGLT3* lncRNA γ-globin in K562 cells. (A) Schematic representation of the ^{A}γ-globin/*BGLT3* genomic region. Location of the target sites for the Locked Nucleic Acid (LNA) antisense oligos (B1 and B2 red arrowheads) are shown. (B) Expression of *BGLT3* and γ-globin was monitored by RT-q PCR after transfection of K562 cells with ASOs B1 and B2 compared to cells transfected with control ASOs. RT+, RT- indicate with/without reverse transcriptase. (C) Expression of *BGLT3* and γ-globin was monitored by RT-qPCR after transfection of K562 cells with a GFP control or with a *BGLT3* overexpression (OE) vector. RT+, RT- indicate with/without reverse transcriptase. (D) Transfection of Δ*BGLT3* clones 2 and 30 (see Figure 2) and control cells with a GFP-only vector or a *BGLT3* overexpression vector. (E) Expression of BGLT3 and γ-globin was monitored by RT-qPCR after transfection of a GFP control or a BGLT3 overexpression vector into K562 clones targeted with dCAS9 and either with or without a *BGLT3*-specific gRNA. For panels B-D, RT-qPCR results were normalized to actin. Two-tailed student's t-tests * $p < 0.05$ **, $p < 0.01$. Error bars indicate SD, n=3. For ASO and primer sequences see Table S1.

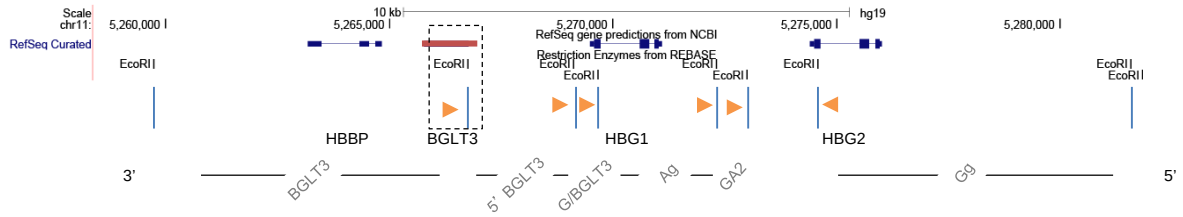

Figure S4. BGLT3 locus EcoRI fragments interrogated by 3C. EcoR1 cutting sites are denoted by vertical bars and the location of the primers used to determine the interaction frequency between each fragment and the anchor are denoted by yellow triangles. The interaction frequency reported for the primer within the fragment containing the *HBG2* gene (Gg) represents the values for interaction of both *HBG1* and *HBG2* with the LCR. This is because the sequences at the 3' end of each of these fragments are identical. The 5' end of the *HBG2* gene cannot be used for a primer because the sequences are repetitive. Only the *HBG1* gene (Ag fragment) can be probed uniquely at the 5' end.

The BGLT3 sequences deleted by CRISPR/Cas9 are within the dotted rectangle. After this deletion, which removes an EcoRI site, the primer on the 5' end of the new fragment can still be used to interrogate LCR proximity (Figure 4A) and it can be used as the anchor primer to determine BGLT3 proximity (Figure 4B) and can be used as the anchor primer when *BGLT3* proximity is probed (Figure 4B).

Table S1- Primers sequences

Name	Sequence 5'-3'
RACE	
3' RACE Outer F	AATGACCTAATGCCCAGCAC
5' RACE Nested R	TATTGAGTTGTGGGGACTGGC
5' RACE Outer R	GGAACATGGGGAAAGTTTGTAGT
PCR	
3' UTR Y^A F	ATGCACACACCACAAACACA
5' BGL3 F	GAGAAGTTGACAACTGCAATGATAACCTGG
BGLT3 B R	TCTACTTGATATAGTTGAGAGGCAGTTACC
BGLT3 TSS F	CTAGGCTTTTTATAGTTTGGGGT
BGLT3 UP F	CTCCCCCATCATTTATTAAATTGAA
Crispr 2F	CTCCCCTCGTCCCCTATGTTT
Crispr 2R	CACCTCTATCCAGCATCAACTTGG
R3 F	TCACAGTGCTGGTCTGTTTCTCA
R4 F	TGCCTGGCTCCATCATATCA
Real Time qPCR	
Actin F	GAGGCCCAGAGCAAGAGA
Actin R	AATCCATTTCGGCAAAGAATTC
Actin intron 3 F	ACAGACTCCCCATCCCAAGAC
Actin intron 3 R	GGACATGCAGAAAGTGCAAAGA
Bcl 11A F	AATGACCTAATGCCCAGCAC
Bcl 11A R	AGTGTTGGGGGAGAAGTGTG
BGLT3 5' F	GAGAAGTTGACAACTGCAATGATAACCTGG
BGLT3 5' R	CCCCATTTCCAATTGTTTTCCATGATTAT
BGLT3 A F	GGGAACAGCCACAAACAAGAAAGA
BGLT3 A R	CAGCCAACCAGGAATTCAGCAGTT
BGLT3 B F	AAGATAATCTTGGTTTTGCCTCAAA
BGLT3 B R	TCTACTTGATATAGTTGAGAGGCAGTTACC
dCAs9/gRNA3 A F	CTTTCGCTCTGATGGTAGTTTCTTT
dCas9/gRNA3 A R	GCAAAAATTGACCAATGGAATCT
dCAs9/gRNA3 B F	AGATTCCATTGGTCAATTTTTGC
dCas9/gRNA3 B R	CGGGCACAGATTTCATGATG
GAPDH F	ACCACAGTCCATGCCATCACT
GAPDH R	CCATCACGCCACAGTTTCC
MALAT1 exon1F	CAGACACACGTATGCGAAGG
MALAT1 exon 1R	TAGTGGTTCCCAATCCCCAC
LCR HS2 F	AAACAGGAAAAGAAAACAAACCTTGT
LCR HS2 R	CACAGGCCTTTTTGCCACCTA
Ɣ exon 2 F	TGG CAA GAA GGT GCT GAC TTC
Ɣ exon 2 R	GCA AAG GTG CCC TTG AGA TC
Ɣ exon 3 F	AATCCATTTCGGCAAAGAATTC
Ɣ exon 3 R	CCACTCCAGTCACCATCTTCTG
Ɣ promoter F	CAAATATCTGTCTGAAACGGTTCCT
Ɣ promoter R	TGCCTTGTCAAGGCTATTGGT
Ɣ TSS F	TGGCTAGGGATGAAGAATAAAAGG

Y TSS R	ATTGATAATCTCAGACGTTCCAGAAG
Y1 intron2 F	TTTCATCTTTCCCTCATTTTTG
Y2 intron 2F	CTTTCCCCTATTTTTGTTATTCG
Y2/1 intron 2R	TTTGTAATGAAGCATTAGCAGCA

LNA-ASO

BGLT3 ASO1	TTAAGAGCAGAGATCG
BGLT3 ASO2	CCATTTAGTAGTCAGT

CRISPR-gRNA	**Gene specific sequence**
gRNA3 SE	CACCG**TAGATTGTCTTTCGCTCTGA**
gRNA3 AS	AAAC**TCAGAGCGAAAGACAATCTA**C
gRNA2 SE	CACCG**CCAACACTATTAGATGTTC**
gRNA2 AS	AAAC**GAACATCTAATAGTGTTGG**C
gRNA LDB1 SE	CACCG**TGGCAACTTTGACTATATGC**
gRNA LDB1 AS	AAAC**GCATATAGTCAAAGTTGCCA**C

Chromatin Capture 3C

EcoRI fragment

3' BGLT3	TGTGAAAGCAGACATGAATGG
3' Beta	AGCTTAGTGATACTTGTGGGCCA
5' BGLT3	CTTAGGCATCCACAAGGG
Ag	ATCCATGATCTCTAACCTTGC
Beta	GCTCGGCACATGTCCCATCCAG
BGLT3	TTGCCATACCTCATATCCTTAG
Delta	AAAAAATGTGGAATTAGACCCAGGAATG
Epsilon	ATTAACCAATGGTATCTTTCTGAGCA
G/BGLT3	AGCAAGGATGGTTCTTAAGGAAGGG
GA2	AATTTGAAGATACAGCTTGCCTCCGATAAG
Gg	GGGTTCATCTTTATTGTCTCCT
HS432	CCAAATGGGTGACTGTAGGGTTGAGA

Northen Blot Probe

Y transcript F	GCCTGCAGGAAGCACCCTTCAGCAGTTC
Y transcript R	GCGAAGCTTAGCTCTGAATCATGGGCAGT

Suplemental Table S2-Antibodies

Name	Company	Catalog number
Anti-HA tag	Abcam	ab9110
Anti-histone H3	Abcam	ab1791
Anti-histone H3 (acetyl K27)	Abcam	ab4729
Anti-histone H3 (mono methyl K4)	Abcam	ab8895
Anti-histone H3 (tri methyl K36)	Abcam	ab9050
Anti-histone H3 (tri methyl K4)	Abcam	ab8580
Anti-p300/CBP (N-15)	Santa Cruz	sc-584
Anti-Pol II (N20)	Santa Cruz	sc-899X
Anti-TATA binding protein (TBP)	Abcam	ab-63766
Normal rabitt IgG	Santa Cruz	sc-2027
RNA ChiP		
Anti-Med12	Bethyl	A300-774A
Anti-CBP	Santa Cruz	sc-583
Anti-Rad21	Abcam	ab992