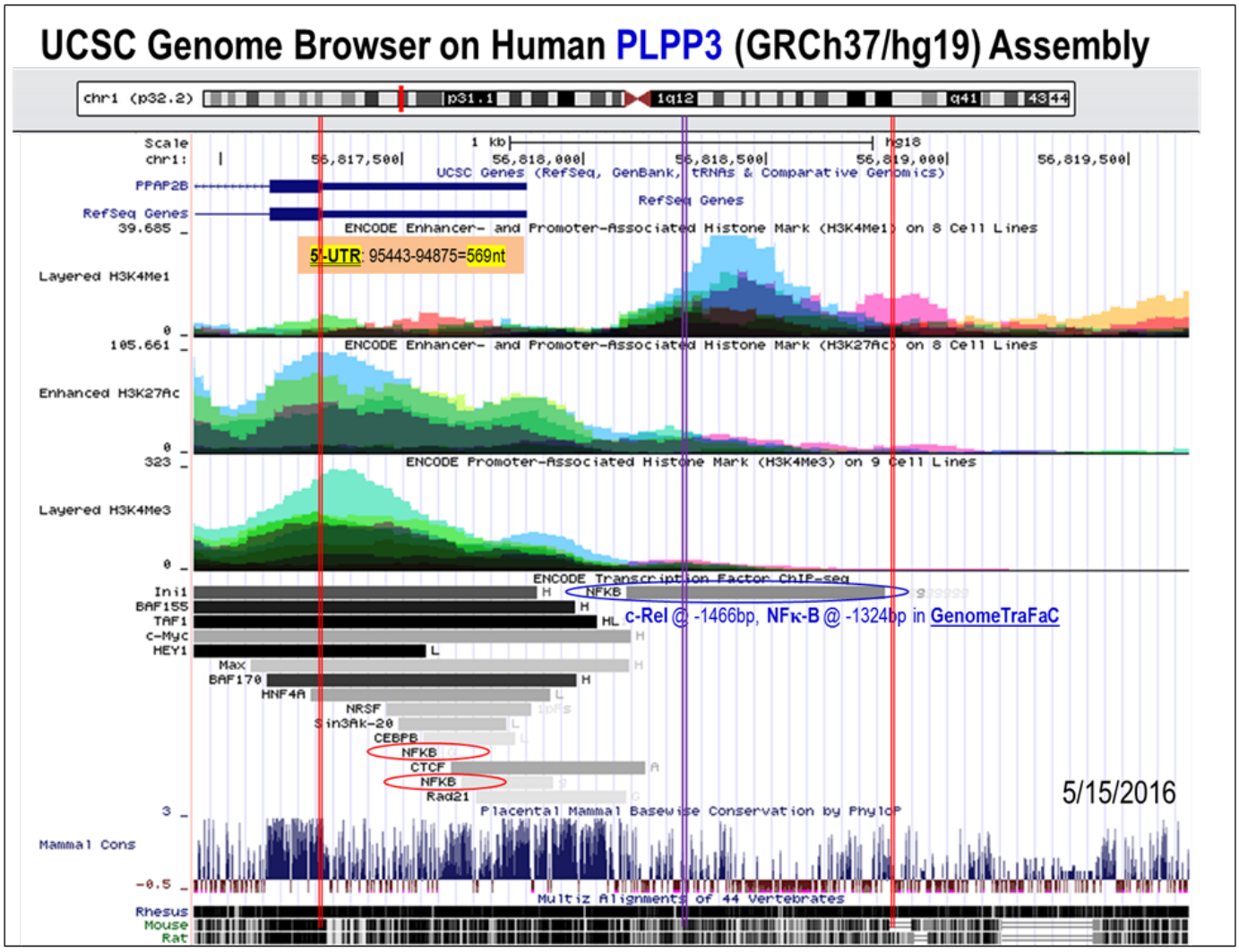
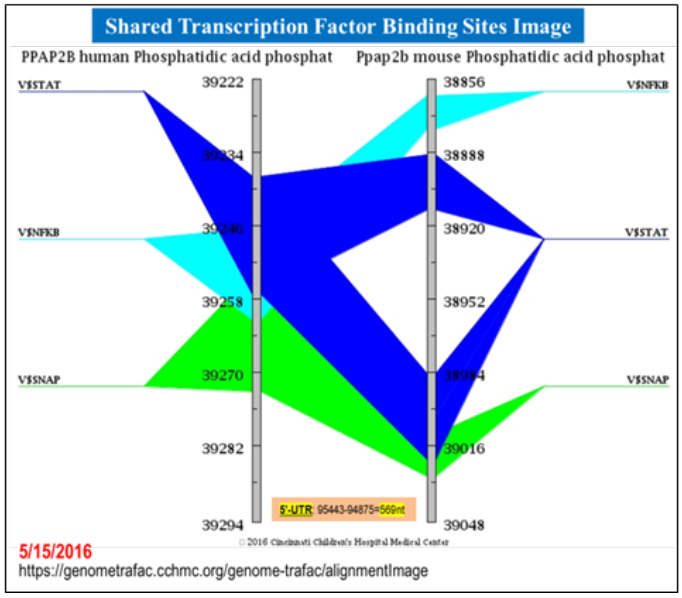
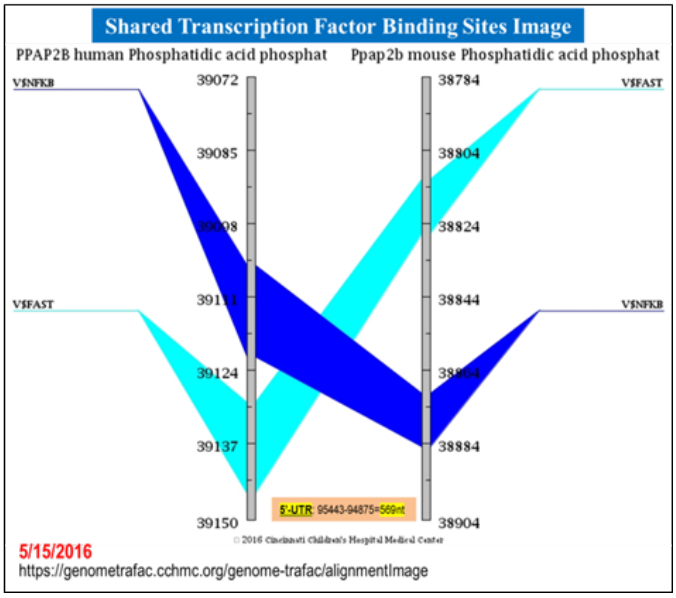
A

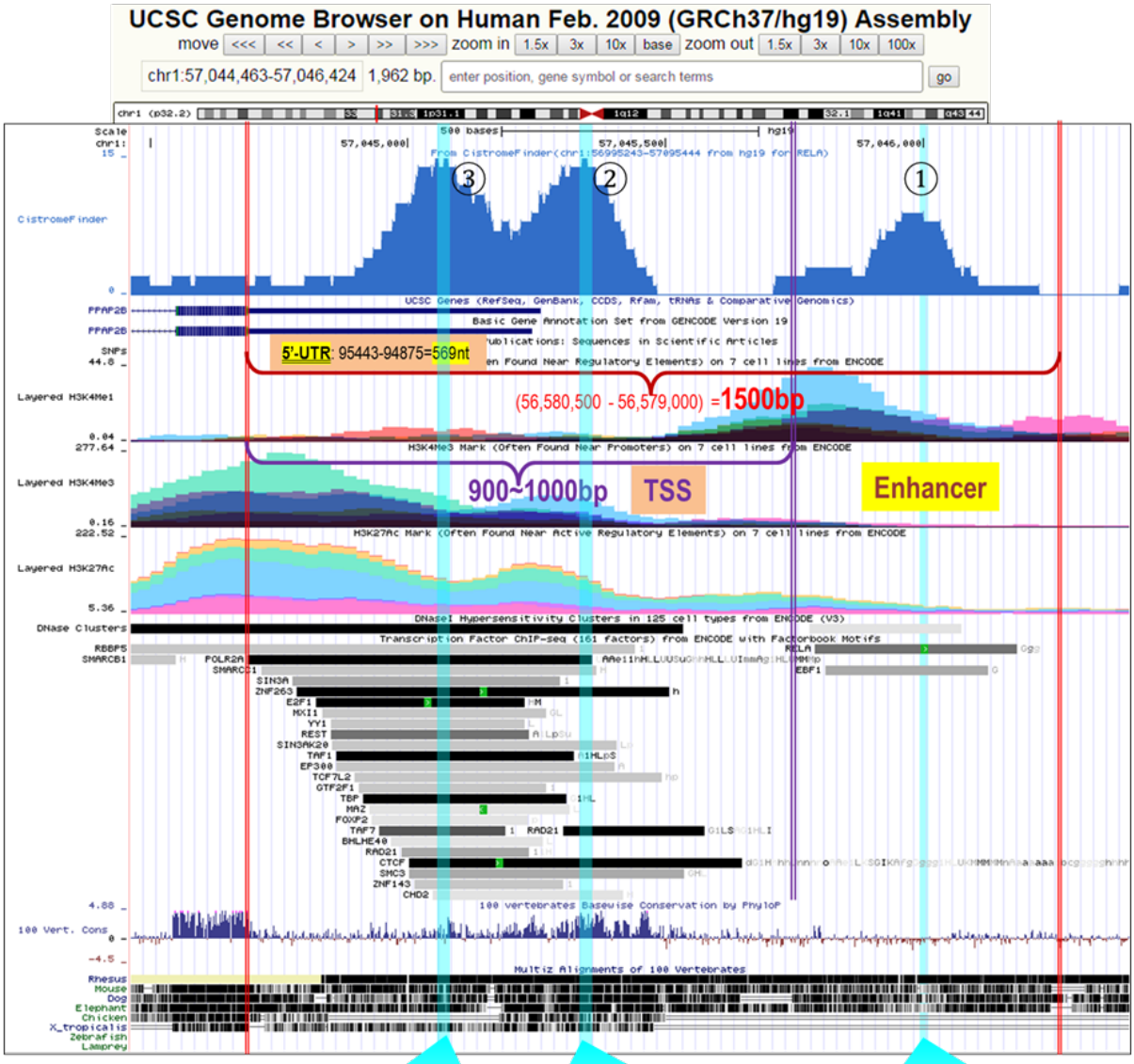

UCSC Genome Browser on Human PLPP3 (GRCh37/hg19) Assembly
chr1 (p32.2)
p31.1
1q12
q41
Scale
chr1:
56,817,500
56,818,000
56,818,500
56,819,000
56,819,500
UCSC Genes (RefSeq, GenBank, tRNAs & Comparative Genomics)
PPAP2B
RefSeq Genes
RefSeq Genes
39.685
ENCODE Enhancer- and Promoter-Associated Histone Mark (H3K4Me1) on 8 Cell Lines
5'-UTR: 95443-94875=569nt
Layered H3K4Me1
105.661
ENCODE Enhancer- and Promoter-Associated Histone Mark (H3K27Ac) on 8 Cell Lines
Enhanced H3K27Ac
323
ENCODE Promoter-Associated Histone Mark (H3K4Me3) on 9 Cell Lines
Layered H3K4Me3
ENCODE Transcription Factor ChIP-seq
Ini1
BAF155
TAF1
c-Myc
HEY1
Max
BAF170
HNF4A
NRSF
Sin3Ak-20
CEBPB
NFKB
CTCF
NFKB
Rad21
NFKB
c-Rel @ -1466bp, NFκ-B @ -1324bp in GenomeTraFaC
5/15/2016
Placental Mammal Basewise Conservation by PhyloP
Mammal Cons
Multiz Alignments of 44 Vertebrates
Rhesus
Mouse
Rat

B

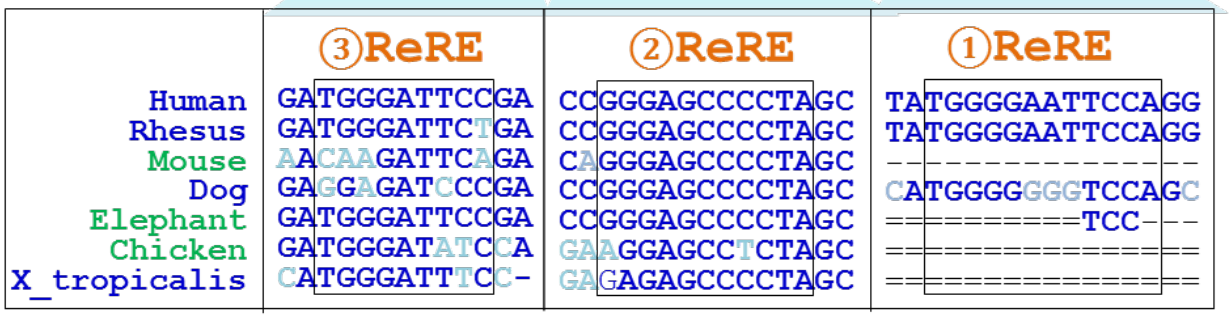

Shared Transcription Factor Binding Sites Image
PPAP2B human Phosphatidic acid phosphat
Ppap2b mouse Phosphatidic acid phosphat
V$STAT
V$NFKB
V$SNAP
39222
39234
39246
39258
39270
39282
39294
38856
38888
38920
38952
38984
39016
39048
V$NFKB
V$STAT
V$SNAP
5'-UTR: 95443-94875=569nt
5/15/2016
https://genometrafac.cchmc.org/genome-trafac/alignmentImage


Shared Transcription Factor Binding Sites Image
PPAP2B human Phosphatidic acid phosphat
Ppap2b mouse Phosphatidic acid phosphat
V$NFKB
V$FAST
39072
39085
39098
39111
39124
39137
39150
38784
38804
38824
38844
38864
38884
38904
V$FAST
V$NFKB
5'-UTR: 95443-94875=569nt
5/15/2016
https://genometrafac.cchmc.org/genome-trafac/alignmentImage

C


UCSC Genome Browser on Human Feb. 2009 (GRCh37/hg19) Assembly
chr1:57,044,463-57,046,424 1,962 bp.
5'-UTR: 95443-94875=569nt
(56,580,500 - 56,579,000) =1500bp
900~1000bp
TSS
Enhancer
H3K4Me3 Mark (Often Found Near Promoters) on 7 cell lines from ENCODE
H3K27Ac Mark (Often Found Near Active Regulatory Elements) on 7 cell lines from ENCODE
DNaseI Hypersensitivity Clusters in 125 cell types from ENCODE (V3)
100 vertebrates Basewise Conservation by PhyloP
Multiz Alignments of 100 Vertebrates

D


③ReRE
②ReRE
①ReRE
Human GATGGGATTCCGA CCGGGAGCCCCTAGC TATGGGGAATTCCAGG
Rhesus GATGGGATTCTGA CCGGGAGCCCCTAGC TATGGGGAATTCCAGG
Mouse AACAAGATTCAGA CAGGGAGCCCCTAGC
Dog GAGGAGATCCCGA CCGGGAGCCCCTAGC CATGGGGGGGTCCAGC
Elephant GATGGGATTCCGA CCGGGAGCCCCTAGC TCC
Chicken GATGGGATATCCA GAAGGAGCCTCTAGC
X_tropicalis CATGGGATTTCC- GAGAGAGCCCCTAGC


Cistrome Finder System
Web Portal to Explore ChIP-seq and DNase-seq Data
@ http://cistrome.org/ap/root

Supplemental Figure 1. Functional genomic analysis of regulatory elements in the human PLPP3 promoter. Data visualization and analysis tools available online at the Cistrome Project (http://cistrome.org/), the University of California at Santa Cruz (UCSC) genome browser (https://genome.ucsc.edu/) and the Genome TraFaC database (https://genometrafac.cchmc.org/genome-trafac/index.jsp) were used to prepare this figure. **A. Structural analysis of the PLLP gene at low resolution.** The figure shows the region of chromosome 1 containing the PLPP3 gene identified in red in the upper panel expanded to show regions of active chromatin identified by histone methylation and acetylation marks and interactions with the series of transcription factors identified in the figure using chromatin immunoprecipitation analysis. The presence of binding sites for NF-κB transcription factors are circled in red. The lower panel of the figure shows sequence conservation of the PLPP3 promoter between the indicated mammalian species. **B. Conservation of transcription factor binding sites in the mouse and human PLPP3 promoter.** The figure shows two regions of the PLPP3 promoter with the human sequence on the left and the mouse sequence on the right with conserved sequences for the indicated transcription factors identified by the colored insets. The binding sites for NF-κB transcription factors identified in the human PLPP3 promoter are shown in light blue and these are conserved between the human and mouse genes. **C. Structural analysis of the PLPP3 promoter at higher resolution.** The information in panel A is presented at higher resolution. The upper panel shows the three NF-κB binding sites numbered 1, 2 and 3 identified by chromatin immunoprecipitation analysis in blue with histone methylation and acetulation marks and binding sites for additional transcription factors determined by chromatin immunoprecipitation sequencing shown below. The lower part of the figure shows conservation of sequences in the PLPP3 promoter between multiple vertebrate species. D. **Sequence alignment of putative NF-κB target sequences (Rel responsive elements, denoted ReRE) in the PLPP3 promoter from the indicated vertebrate species.** The figure shows san alignment of the cognate sequences in the PLPP3 promoter from the indicated species with the three Rel responsive sequence elements boxed. Two of these sequence elements are highly conserved among all of the vertebrate species analysed while the first sequence element was only identified in the human and rhesus monkey PLPP3 promoter.

Supplemental Figure 2. Sequence of the entire human PLPP3 promoter identifying Rel Responsive element sequences. The figure shows the sequence of the coding strand of the entire PLPP3 promoter identified by our functional genomic analysis upstream of the ATG initiaion codon. The three Rel Responsive sequence elements are shown in color in alignment with the consensus boxed. The non coding strand sequence is shown for response element 1.

Supplemental Table S1. Oligonucleotides used in the study

Oligos	Sequence
Chip assay	
ChIPReRE1-F	5′-TAAGATGCCAGCCTCTAAGGAA-3′
ChIPReRE1-R	5′-TTTCTGGGTGAAGGAGTCTCAG-3′
ChIPReRE2-F	5′-GACCCAAGGCCAGCAAGAAAAT-3′
ChIPReRE2-R	5′-GACGTAGGATAATTAAGGCCAC-3′
ChIPReRE3-F	5′-GTGGCCTTAATTATCCTACGTC-3′
ChIPReRE3-R	5′-GCACTTTTATTTCACGAGGTCC-3′
ReRE mutagenesis	
MutReRE1-F	5′-GCATTTTGGCTGCCCTaGcAcTaCtCATAGTAGTAGAATTAC-3′
MutReRE1-R	5′-GTAATTCTACTACTATGAGTAGTGCTAGGGCAGCCAAAATGC-3′
MutReRE2-F	5′-GTGTTTCTGAACGCTcGtGaCaCgCGGTTTCTCTGGTTTGA-3′
MutReRE2-G	5′-TCAAACCAGAGAAACCGCGTGTCACGAGCGTTCAGAAACAC-3′
MutReRE3-F:	5′-AAGGGGCTGTGCTTTCGtcAgCaCATCCGAGCCAAGTAAGGA-3′
MutReRE3-R:	5′-TCCTTACTTGGCTCGGATGTGCTGACGAAAGCACAGCCCCTT-3
ReRE23seq-F	5′-CCGGTGTTGGATGTTAAGATC-3′
PLPP3 promoter/enhancer cloning	
PPAP2Bpro17-F	5′-TATACTCGAGgagcaggggtgtggagtgtgaaaggattgtacc-3′
PPAP2Bpro15-F	5′-TATACTCGAGgtccactgtgcccaggtccatgctaggcactata-3′
PPAP2Bpro-R	5′-TATATAAGCTTggcgctggctgcggcgcgagcctcccgccccg-3′
P2Bpro11k-F2S	5′-atccaggagatgtggttccacagtcgtgcagt-3′
P2Bpro10k-F2S	5′-gcgtagtacccggtgttggatgttaagatc-3′
P2Bpro10k-R2S	5′-GATCTTAACATCCAACACCGGGTACTACGC-3′
PLPP3 mRNA reverse transcription (RT)-PCR	
PLPP3qRTe4-F	5′-GCATAGTGTACATGGAGAAGGAGG-3′
PLPP3qRTe3-R	5′-GTGGCAGCACTCTATAAGCAAGTG-3′

Supplemental Table 1. The table shows the sequence of oligonucleotides used in this assay as probes or as primers for mutagensis or cloning of PLPP3 genomic DNA or cDNA