

Supplementary Materials: Negative Glucocorticoid Response-Like Element from the First Intron of the *Chicken Growth Hormone* Gene Represses Gene Expression in the Rat Pituitary Tumor Cell Line

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Table S1. Potential TFBSs identified in the first intron region of *cGH*.

Name	Position	Score	Subsequence/Consensus Sequence (5'→3')	Note
P53	97 (+)	0.863130	CTGCATGCTT NGRCWTGYCY	p53; RSp53; NSp53; p53as; ASp53
GRE	302 (−) 591 (+)	0.801618 0.808873	GAAGAACAAGGCAAACCAG AGGCTTGACAGTGACCTCC NNNNNNCNNTNTGTNCTNN	glucocorticoid receptor β; NR3C1; GR β
GATA3	63 (−) 110 (+) 120 (+) 336 (−)	0.930882 0.887904 0.884803 0.887461	CTTTATCTC GTGATGGGA ATGATGGTG TGCTATCAT NNGATARNG	GATA-3; NF-E1c (chick)
CEBPB	57 (−) 177 (+) 537 (+) 856 (+) 856 (−)	0.892521 0.885575 0.911095 0.887816 0.897341	ACTTGCTTTATCT AAATGTGGCAACTT AACTTTTGTAAGCG ACATGGAGCAACAT ACATGGAGCAACAT RNRTKNNGMAAKNN	AGP/EBP; ANF-2; C/EBP β; CRP2; H-APF-2; IL-6DBP; LAP; LAP1; NF-IL6
CEBP	57 (+) 175 (+)	0.894003 0.891841	ACTTTGCTTTATC AAAATGTGGCAACTTACA NNTKTGGWNANNN NGWNTKNKGyAAKNSAYA	BPc; CBP; C/EBP; C/EBP α; EBP20; CCAAT/enhancer binding protein α
AML1	179 (+) 635 (−) 137 (+)	0.897865 0.897865 1.000000	TGTGGC GCCACA TGTGGT TGTGGT	AML1; PEBP2αB; RUNX1; CBF-α2
GATA1	334 (−) 109 (+) 330 (+)	0.891238 0.948667 0.904375	ATTGCTATCATAC GGTGATGGGA GGTGATTGCT NNCWGATARNNNN	EF1 (chick); EFgammaa; Eryf1 (chick); GATA-1; GF-1; NF-E1
NKX25	578 (+) 474 (−)	0.893863 0.950176	GTTAATAA CACTTGT CWTAATTG/TYAAGTG	Csx; Nkx-2.5; cardiac-specific homeobox protein
SRY	384 (−) 365 (+) 530 (−) 55 (−)	0.911240 0.942636 0.905426 0.923643	GGTGATT AAACTCA TGAGATT GTACTTT AAACWAM	SRY; sex-determining region Y gene product; TDF; testis-determining factor
MYOD	472 (+)	0.920111	TGCACTTGTC NNCANCTGNY	MEF1; Myf-3 (human); MyoD; MyoD1; CMD1
GATA_C	60 (−)	0.953712	TTGCTTTATCT NGATAAGNMNN	GATA-2; NF-E1b (chick)
CREL	80 (+)	0.902113	TGGGTGTTCC SGGRNWTTC	c-Rel; p82(hc-rel); p68; HIVEN86A (human)

Table S1. Cont.

Name	Position	Score	Subsequence/Consensus Sequence (5'→3')	Note
AP1	197 (+)	0.902714	AGTGACAAAGG	AP1; Fra-2
	242 (+)	0.944190	GCTGACTCAGG	
	242 (+)	0.972546	GCTGACTCAGG	
	243 (+)	0.971720	CTGACTCAG	
	243 (+)	0.971720	CTGACTCAG	
	243 (−)	0.979300	RSTGACTMANN NNTGACTCANN NTGASTCAG	
MYB	825 (−)	0.907469	GCACGTTAGG NNNAACKGNC	c-Myb
SP1	277 (+)	0.909683	GGGGCAGGAA GRGGCRGGGW	Sp1; simian-virus-40-protein-1
MZF1	108 (+)	0.914271	GGGTGATGGGATA	MZF-1; MZF1; myeloid zinc finger 1; ZN42; ZNF42
	570 (+)	0.887528	TGTCGAGGGTTAA	
	827 (+)	0.907023	ACGTTAGGGGAAA	
	941 (−)	0.882279	TAACCCCTCTCCT KNNNKAGGGGNA	
AP4	238 (−)	0.935800	ATGAGCTGAC	Replication initiator 1
	637 (+)	0.882481	CACAGCGCAG	
	694 (+)	0.900435	AGCAGCACGG	
	714 (+)	0.960283	CTCAGCTGAG	
	714 (−)	0.960283	CTCAGCTGAG	
	719 (−)	0.914037	CTGAGCTGTT NNCAGCTGNN	
CAP	70 (+)	0.967965	TCAGTTCT	cAMP activated protein
	195 (+)	0.903894	TCAGTGAC	
	369 (+)	0.959093	TCAGTTCC	
	341 (+)	0.934943	TCATACGT	
	411 (+)	0.959093	TCAGACTC	
	473 (+)	0.906358	GCACTTGT	
	502 (+)	0.934450	GCATTCCT	
	715 (+)	0.900936	TCAGCTGA	
	663 (+)	0.902908	TCACCCCA	
	730 (+)	0.928536	CCAGTCCT	
	737 (+)	0.903894	TCACCCAC	
			NCANNNNN	
CDXA	63 (+)	0.929377	CTTTATC	caudal type homeobox 1
	381 (+)	0.918097	CTTTAAA	
			MTTTATR	
GATA2	119 (+)	0.904375	TATGATGGTG	GATA2
	336 (−)	0.908886	TGCTATCATA NNNGATRNNN	
AP1FJ	242 (+)	0.924606	GCTGACTCAGG RSTGACTNMNW	AP1
OCT1	322 (+)	0.905966	GTTGTAATGGTGA NNNRTAATNANNN	OCT1
TST1	356 (+)	0.917381	GGGATATTAAAACTC NNKGAWTWANANTNN	TST1
TATA	380 (+)	0.924743	GCTTTAAAC NCTATAAAAR	TATA
CETS1P54	393(+)	0.908050	GATCAGGATGATG	c-Ets-1; c-Ets-1 54; Ets1; p54; p54c-Ets-1
	564 (+)	0.903042	CTGGATGTCG NNAMMGGAWRWNN NCMGGAWGYN	
E47	614 (+)	0.930293	CCTACAGGTGTGTCC	E47
	892 (+)	0.918602	ACTCACAGGTGGACAC NSNGCAGGTGKNCNN NNNMRCAGGTGTTMNN	

Table S1. Cont.

Name	Position	Score	Subsequence/Consensus Sequence (5'→3')	Note
LMO2COM	63 (-)	0.886792	CTTTATCTC	LIM-only protein 2; RBTN2; Rhombotin-2; TTG-2
	615 (+)	0.901884	CTACAGGTGTGT	
	894 (+)	0.906864	TCACAGGTGGAC	
	917 (+)	0.940242	CCCCAGGTGCCA NMGATANS SNNCAGGTGNNN	
DELTAEF1	451 (+)	0.901884	GATCACCTAAT	areb6, bzp, deltaef1, fecd6, nil-2a, nil2a, tcf8, zeb, zfh1, zfhx1
	616 (-)	0.918454	TACAGGTGTGT	
	797 (-)	0.922027	GGAAGGTGAAA NNNCACCTNAN	
HNF3β	703 (-)	0.909003	GTGCAAATAGGC	hepatocyte nuclear factor 3 β
	797 (-)	0.943460	CCCTGTTGGCTC NNNTRTTTRYTY	
IK2	724 (-)	0.906741	CTGTTCCAGTC NNNYGGGAWNNN	IkappaB kinase-like 2
STAT	830 (-)	0.959163	TTAGGGGAA TTCCCRKAA	STAT
USF	556 (-)	0.924187	TCATGTGA	Basic helix–loop–helix transcription factors
	854 (+)	0.910768	CCACATGG	
	866 (-)	0.940288	ACATGTGG	
	896 (-)	0.919155	ACAGGTGG NCACGTGN	
E2F	768 (-)	1.000000	GCGCCAAA TTTSGCGC	cyclin E/E2F

Position on the cGH gene sequence. The transcription start site is set to +1. + and - means forward and reverse strands that the transcription factor binds, respectively; Score: Similarity (0.0–1.0) between a registered sequence for the transcription factor binding sites and the intron 1 sequence; Subsequence: sequence from the cGH gene sequence at the position corresponding to the consensus sequence. S = C or G, W = A or T, R = A or G, Y = C or T, K = G or T, M = A or C, N = any base pair.

Table S2. Primer pairs information of chicken GH gene.

ID	Prime Name	Sequence (5'→3')	Restriction Site
C1	cGH-DNA-F	CAAGCTTGATGGCTCCAGGTACTTTGCT	HindIII
C2	cGH-cDNA-F	CAAGCTTGATGGCTCCAGGCTCGTGGT	HindIII
C3	cGH-R-6H	GGAATTCCTCAatggtgatggtgatggtGATGGTGCAATTGCTCT	EcoRI
C4	cGH-exon2-F1	CTCCTCTCCTCATCGCTGTG	
C5	cGH-exon2-R1	GCGGCAGTCCCAGCGTGA	
C6	cGH1-F	GAATTCAAGCTTATGGCTCCAG	
C7	cGH1-R	TCTTTATATGTCTCGGCAGC	
C8	cGH2-F	ATGGCTCCAGGCTCGTGGT	
C9	cGH2-R	ATGTCTCGGCAGCCAGGAGG	

Italic bases in low case were His-tag sequence, sequence with underline was protective bases and enzyme loci.

Table S3. Sequence information of chicken GH gene.

Sequence Name	Temple	Primes	Length
cGH-6H	cDNA	C2 C3	685 bp
cGH-E1-IN1-E2	DNA	C1 C5	986 bp
cGH-E2-E5	cDNA	C3 C4	636 bp
cGH-in-6H	cGH-E1-IN1-E2, cGH-E2-E5	C1 C3	1600 bp

Table S4. Primer pairs information for pGL3-cGH vectors of chicken GH.

Primer Name	Title	Primer Sequence (5'→3')	Product Size (bp)
C 1	F	<u>ATAGGTACCGTGCCATGCCAG</u>	286
	R	<u>GATCTCGAGCTACAGCAGA</u>	
C 2	F	<u>ATAGGTACCGTCTGCAGGCT</u>	485
	R	<u>GATCTCGAGCTACAGCAGA</u>	
C 3	F	<u>ATAGGTACCGTTCCAAGGCTTT</u>	598
	R	<u>GATCTCGAGCTACAGCAGA</u>	
C 4	F	<u>ATAGGTACCGTACTTTGCTTTATC</u>	915
	R	<u>GATCTCGAGCTACAGCAGA</u>	
C 5	F	<u>TTTCTCTATCGATAGTCCTGCAGGCTCCAGGGCATT</u>	200
	R	<u>GATCTCGAGCCCGGCTGTCTGCCTTCACTGGGGTGAGAAAGTGGCTGCATTACCTG</u>	
Mut1	F	CTTGCACTGTCCAATGCCATGCCAGCAGC	
	R	GCTGCTGGCATGGCATTGGACAAGTGCAAG	
Mut2	F	GGGTTAATAACCTTCAGATCCTACAGGTGTG	
	R	ACACCTGTAGGATCTGAAGGTTATTAACCTC	
RVP3	F	CTAGCAAAATAGGCTGTCCC	
GLP2	R	CTTTATGTTTTGGCGTCTTCCA	

Sequence with underline was protective bases and enzyme loci.

Table S5. Primers for qRT-PCR.

mRNA Target	Title	Primer Sequence (5'→3')	AccessionN. in GenBank
cGH	F	TGCCGAGACATATAAAGAGTTC	NM_204359.2
	R	GAGCTGGGATGGTTTCTGAGTA	
DF	F	TCATTGTGCTAGGTGCCA	L08165
	R	CCTCTTCCAGCCATCTTT	
GH4	F	TGTCACCAACTGGGACGATA	NM_001135968.1
	R	CTGGGTCATCTTTTCACGGT	
293FT	F	GGCCAACCGCGAGAAGA	U20114
	R	CCTCGTAGATGGGCACAGTGT	
Fir	F	TTGGAATCCATCTTGCTCCAA	U47295
	R	TCCGTGCTCCAAAACAACAA	
Ren	F	AGGTGGTAAACCTGACGTTGTACA	AF025845
	R	ATCCTGGGTCCGATTCAATAAAC	

Table S6. Probe sequences for EMSA.

Name	Title	Sequence
WT probe	F	CCTTCAGGCTTGACAGTGACCTCCAGAT
	R	ATCTGGAGGTCAGTGTCAAGCCTGAAGG
Mutated probe	F	CCTTCAGGCTTAACAGCACCATCCAGAT
	R	ATCTGGATGGTCTGTTAAGCCTGAAGG

Sequence with underline was protective bases and enzyme loci.

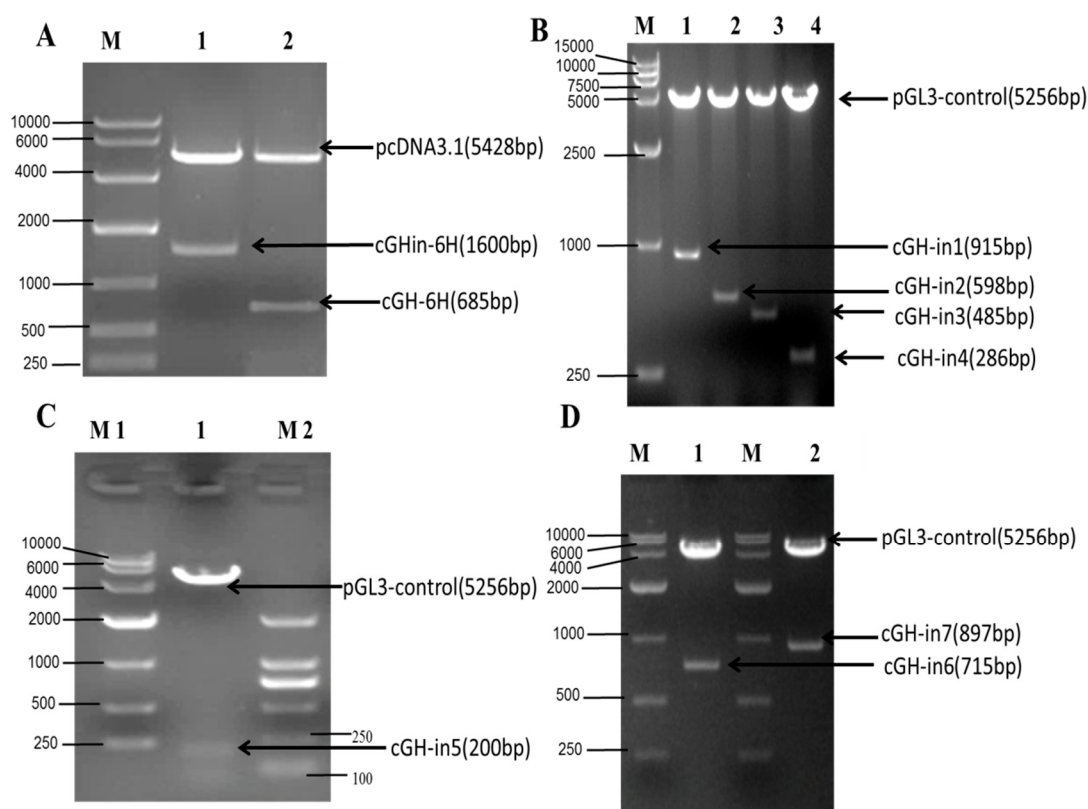


Figure S1. Plasmid identified. (A) Gel electrophoresis detection of the pcDNA3.1cGH-6H, pcDNA3.1cGH-in-6H after digestion by *Hind* III and *Eco*R I. M was Marker; 1, 2 represent degenerated plasmid of pcDNA3.1cGH-6H, pcDNA3.1cGH-in-6H after double enzyme digestion; (B) Gel electrophoresis detection of the four plasmids after digestion by *Kpn* I and *Xho* I, M was Marker; 1, 2, 3, 4 represent degenerated plasmid of pGL3-cGH-in1, pGL3-cGH-in2, pGL3-cGH-in3 and pGL3-cGH-in4 after double enzyme digestion, respectively; (C) Gel electrophoresis detection of the plasmid pGL3-cGH-in5 after digestion by *Kpn* I and *Nhe* I, M was Marker; 1 represent degenerated plasmid of pGL3-cGH-in5 after double enzyme digestion; (D) Gel electrophoresis detection of the two plasmids after digestion by *Kpn* I and *Xho* I, M was Marker; 1, 2 represent degenerated plasmid of pGL3-cGH-in6 and pGL3-cGH-in7 after double enzyme digestion, respectively.

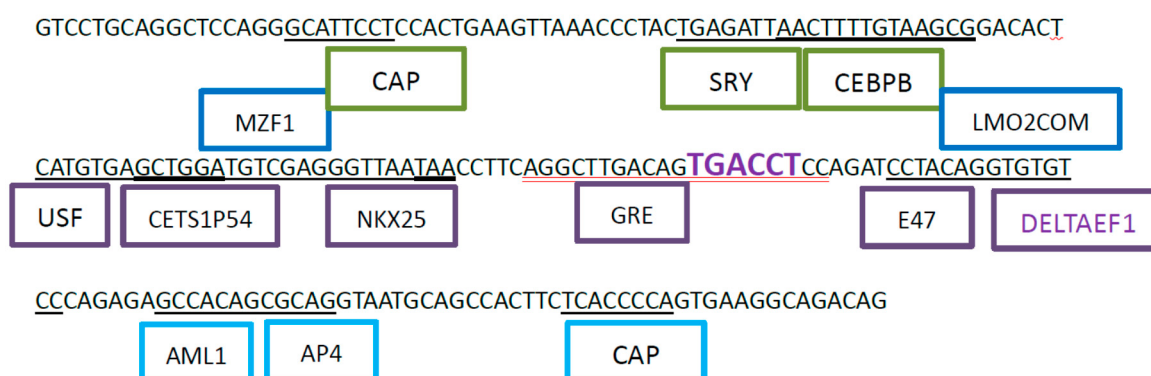


Figure S2. TFBSs in the +485/+684 region of the chicken GH gene. The outlined sequences correspond to the TFBS. Characters in each textbox correspond to the name of each potential element. The putative GRE is double-underlined and the TGACCT motif is indicated by a bold character.