

Supplementary Material

Analysis of the Transcriptional Control of *Bcl11b* in Chicken: IRF1 and GATA1 as Negative Regulators

Lingling Qiu ¹, Haojie Wang ¹, Wenhao Li ¹, Ting Yang ¹, Hao Bai ² and Guobin Chang ^{1,*}

Supplementary Figure S1-S3

Supplementary Table S1-S5

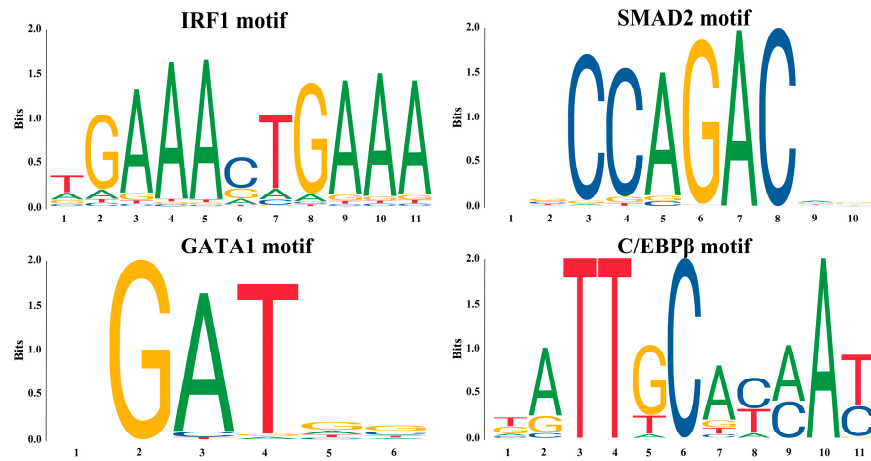


Figure S1 Transcription factor binding motifs.

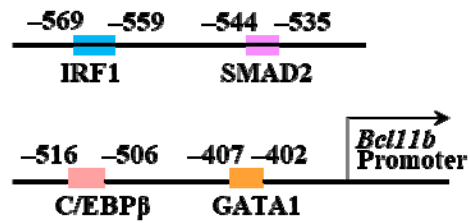


Figure S2 The predicted transcription factor binding sites in chicken Bcl11b promoter.

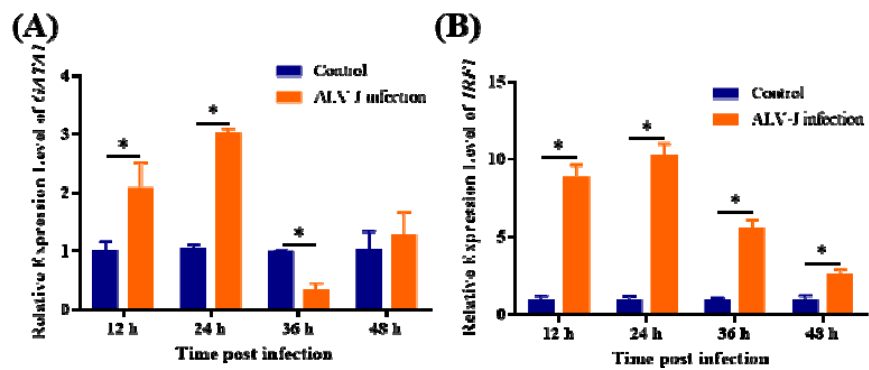


Figure S3 The expression pattern of GATA1 and IRF1 in DF-1 cells infected with ALV-J.

Table S1. The primers information for promoter cloning

Name of Plamids	Primer Sequence (5'-3')	Length/bp
pGL3-2999	F: tatcgataggtacc <u>gagctc</u> AAGGTGGAGCGGCTAACG	3018
pGL3-2140	F: tatcgataggtacc <u>gagctc</u> ATAAGGAGCAGCGACTCCCCGG	2159
pGL3-1351	F: tatcgataggtacc <u>gagctc</u> GGCTTCGTGAGAGCCAAAGC	1370
pGL3-708	F: tatcgataggtacc <u>gagctc</u> TCCTCCCAGAGAAACCCTCGC	727
pGL3-458	F: tatcgataggtacc <u>gagctc</u> GGGACGGGCGGCC	477
pGL3-363	F: tatcgataggtacc <u>gagctc</u> CCCCGGCCCCGAG	382
pGL3-281	F: tatcgataggtacc <u>gagctc</u> GGATGCGGCTTTGTGTGTCC	300
pGL3-111	F: tatcgataggtacc <u>gagctc</u> GTGCGGTGTGTGCGAGT	130
Bcl11b-Hind III	R: ccggaatgcca <u>agctt</u> CGGACGTGACGTTTAATCTGCAC	-
pGL3-2809	F: tatcgataggtacc <u>gagctc</u> CTCTCTCCTCTCCCCC	2828
pGL3-2599	F: tatcgataggtacc <u>gagctc</u> ATCGGGACTTCCTCTTCACG	2618
pGL3-2419	F:tatcgataggtacc <u>gagctc</u> CATCAGCCTGTTTTCCCCTATGA CA	2438
pGL3-2259	F:tatcgataggtacc <u>gagctc</u> CAAGGCACAAACAGCAAATAG CAC	2278
pGL3-606	F: tatcgataggtacc <u>gagctc</u> GTGTCCCGCTGCCATCA	625
Bcl11b-Nhe I	R: tcgagcccggc <u>tagc</u> CGGACGTGACGTTTAATCTGCAC	-

Note: The lower-case letters are overlap sequences, underlined letters are enzyme cutting sites.

Table S2. Wild and mutant type plasmids sequences

Plasmids	Promoter sequences
pGL3-708+19	GAGCTCTCCTCCCAGAGAAACCCTCGCCGCCGGGATGTAAGGAAAA GGGGTGGGAAAGGAGGGTCGGAGCGCGTGTGCGTGTGCGGCTCGG GAATGTGTGCGCGCGTGTCCCGCTGCCATCACGCTTCGCTTTAGGGA AAGAGGAGGAAAAAAAAAAATTCTCTCCCAACCCACACAGATAG TGGTGGTGGTGGTGGTGGTGTAAACTGTCTGGAAGAAACGCAGGGG GACGAGGAGCTGCCGCGGCGTCCGCGGGGACGGGCGGCCCCGAGCC CCTCCGCGGGGAGCGCACCGCGGCGCCGGGGGCGATGGGTACCGAC GCGGCCCCACCGCAGCCCCCGCCGCGCTCCCCCGGCCCCGAGCG GCCCCCGGCAGCCGCCCCCGGCACAGCCGCCCCCGCCGCGGCGCGG GGAAGTTTGGCCCCGCGTGAGGATGCGGCTTTGTGTGTCCCCCCTC AAATGCTCTTTGCTTCGCTCCAGCGCTCCGCGTACCCGAGAAAGCG GCAGCAGCGCACAAAGCCCGGAGCTGCTCCCGATCCGTGCATGTGCT TTAGTTTGTGTCTGTGTGTGTGTGTGTATGTGTGTGTGTGTGAGTG AGTGCGGTGTGTGCGAGTGGCGTTTCTTGTTCTCTTGCAAGGTACAA TGTTAAAAAGCCACCGCTAGTCGCCCCCAGTGCTCCTACTCTCTGGG TCTTTTGTCTCTAGTGCAGATTAAACGTCACGTCCGACGCGT
pGL3-C/EBP β -Mut	GAGCTCTCCTCCCAGAGAAACCCTCGCCGCCGGGATGTAAGGAAAA GGGGTGGGAAAGGAGGGTCGGAGCGCGTGTGCGTGTGCGGCTCGG GAATGTGTGCGCGCGTGTCCCGCTGCCATCACGCTTCGCTTTAGGGA AAGAGGAGGAAAAAAAAAAATTCTCTCCCAACCCACACAGATAG TGGTGGTGGTGGTGTCTGTCTGGAAGAAACGCAGGGGGACGAGGAGCT GCCGCGGCGTCCGCGGGGACGGGCGGCCCCGAGCCCCCTCCGCGGGGA GCGCACCGCGGCGCCGGGGGCGATGGGTACCGACGCGGCCCCACCG CAGCCCCCGCCGCGCTCCCCCGGCCCCGAGCGGCCCCCGGCAGC CGCCCCCGGCACAGCCGCCCCCGCCGCGGCGCGGGGAAGTTTGGCC CCGCGTGAGGATGCGGCTTTGTGTGTCCCCCCTCAAATGCTCTTTGC TTCGCTCCCAGCGCTCCGCGTACCCGAGAAAGCGGCAGCAGCGCAC AAAGCCCGGAGCTGCTCCCGATCCGTGCATGTGCTTTAGTTTGTGTCT GTGTGTGTGTGTGTATGTGTGTGTGTGTGTGAGTGAGTGCGGTGTGT GCGAGTGGCGTTTCTTGTTCTCTTGCAAGGTACAATGTTAAAAAGCC ACCGCTAGTCGCCCCCAGTGCTCCTACTCTCTGGGTCTTTTGTCTCT AGTGCAGATTAAACGTCACGTCCGACGCGT
pGL3-GATA1-Mut	GAGCTCTCCTCCCAGAGAAACCCTCGCCGCCGGGATGTAAGGAAAA GGGGTGGGAAAGGAGGGTCGGAGCGCGTGTGCGTGTGCGGCTCGG GAATGTGTGCGCGCGTGTCCCGCTGCCATCACGCTTCGCTTTAGGGA AAGAGGAGGAAAAAAAAAAATTCTCTCCCAACCCACACAGATAG TGGTGGTGGTGGTGGTGGTGTAAACTGTCTGGAAGAAACGCAGGGG GACGAGGAGCTGCCGCGGCGTCCGCGGGGACGGGCGGCCCCGAGCC CCTCCGCGGGGAGCGCACCGCGGCGCCGGGGGTACCGACGCGGCC CCACCGCAGCCCCCGCCGCGCTCCCCCGGCCCCGAGCGGCCCC GGCAGCCGCCCCCGGCACAGCCGCCCCCGCCGCGGCGCGGGGAAGT

	TTGGCCCCGCGTGAGGATGCGGGCTTTGTGTGTCCCCCCTCAAATGC TCTTTGCTTCGCTCCCAGCGCTCCGCGTACCCGAGAAAGCGGCAGCA GCGCACAAAGCCCGGAGCTGCTCCCGATCCGTGCATGTGCTTTAGTT TGTGTCTGTGTGTGTGTGTATGTGTGTGTGTGTGTGAGTGAGTGCG GTGTGTGCGAGTGCGGCTTTCTTGTTCTCTTGCAGGGTACAATGTTAAA AAGCCACCGCTAGTCGCCCCCAGTGCTCCTACTCTCTGGGTCTTTTTG TCTCTAGTGCAGATTAAACGTCACGTCCGACGCGT
pGL3-SMAD2-Mut	GAGCTCTCCTCCCAGAGAAACCCTCGCCGCCGGGATGTAAGGAAAA GGGGTGGGAAAGGAGGGTCGGAGCGCGTGTGCGTGTGCGGCTCGG GAATGTGTGCGCGCGTGTCCCGCTGCCATCACGCTTCGCTTTAGGGA AAGAGGAGGAAAAAAAAAAATTCTCTCCCAATAGTGGTGGTGGT GGTGGTGGTGTAAACTGTCGGAAGAAACGCAGGGGGACGAGGAG CTGCCGCGGCGTCCGCGGGGACGGGCGGCCCCGAGCCCCCTCCGCGGG GAGCGCACCGCGGCGCCGGGGGCGATGGGTACCGACGCGGCCCCAC CGCAGCCCCCGCCGCGCCTCCCCGGCCCCGAGCGGCCCCCGGCA GCCGCCCCCGGCACAGCCGCCCCCGCCGCGGCGCGGGGAAGTTTGG CCCCGCGTGAGGATGCGGCTTTGTGTGTCCCCCCTCAAATGCTCTTT GCTTCGCTCCCAGCGCTCCGCGTACCCGAGAAAGCGGCAGCAGCGC ACAAAGCCCGGAGCTGCTCCCGATCCGTGCATGTGCTTTAGTTTGTG TCTGTGTGTGTGTGTGTATGTGTGTGTGTGTGTGAGTGAGTGCGGTGT GTGCGAGTGCGGCTTTCTTGTTCTCTTGCAGGGTACAATGTTAAAAAG CCACCGCTAGTCGCCCCCAGTGCTCCTACTCTCTGGGTCTTTTTGTCT CTAGTGCAGATTAAACGTCACGTCCGACGCGT
pGL3-IRF1-Mut	GAGCTCTCCTCCCAGAGAAACCCTCGCCGCCGGGATGTAAGGAAAA GGGGTGGGAAAGGAGGGTCGGAGCGCGTGTGCGTGTGCGGCTCGG GAATGTGTGCGCGCGTGTCCCGCTGCCATCACGCTTCGCTTTAGGGA AAGAGGAAAATTCTCTCCCAACCCACACAGATAGTGGTGGTGGTGG TGGTGGTGTAAACTGTCGGAAGAAACGCAGGGGGACGAGGAGCTG CCGCGGCGTCCGCGGGGACGGGCGGCCCCGAGCCCCCTCCGCGGGGAG CGCACCGCGGCGCCGGGGGCGATGGGTACCGACGCGGCCCCACCGC AGCCCCCGCCGCGCCTCCCCGGCCCCGAGCGGCCCCCGGCAGCC GCCCCCGGCACAGCCGCCCCCGCCGCGGCGCGGGGAAGTTTGGCCC CGCGTGAGGATGCGGCTTTGTGTGTCCCCCCTCAAATGCTCTTTGCT TCGCTCCCAGCGCTCCGCGTACCCGAGAAAGCGGCAGCAGCGCACA AAGCCCGGAGCTGCTCCCGATCCGTGCATGTGCTTTAGTTTGTGTCTG TGTGTGTGTGTGTATGTGTGTGTGTGTGTGAGTGAGTGCGGTGTGTG CGAGTGCGGCTTTCTTGTTCTCTTGCAGGGTACAATGTTAAAAAGCCA CCGCTAGTCGCCCCCAGTGCTCCTACTCTCTGGGTCTTTTTGTCTCTA GTGCAGATTAAACGTCACGTCCGACGCGT

Note: The underlined letters are the mutation sites of IRF1, SMAD2, C/EBP- β and GATA1, respectively.

Table S3. The primers information for overexpression, qRT-PCR

Genes/Plasmids	Primer Sequence (5'-3')	Length/bp	NCBI Accession Number/gene ID
pcDNA3.1-IRF1	F:agcgtttaaacttaagctt <u>gg</u> taccATGCCCCGTCTCAA GGATGC R:aacggggccctctagact <u>cg</u> agTTACAAGCTGCAGG AGATGGCCT	942	NM_205415.2
pcDNA3.1-GATA1	F: CGGGGTACCAT GAGTTCGTGGCGCT R: CCGCTCGAGT CAAATCTGCGGGCTCAG	915	NM_205464.1
ALV-J (<i>Env</i>)	F: GACTGGGCACCCCTGGAAA R: CCTGGGACAACGGAAATA	159	10206068
<i>Bcl11b</i>	F: GCACTGGTGGTGTCTGCTAT R: TCTTTACCTGCAATGTTCTCCTG	187	XM_040702041.2
<i>IRF1</i>	F: GATCTCACGGCGAGGAAACA R: AGCCATGCTTAGCTGCATGT	155	NM_205415.2
<i>GATA1</i>	F: CGATGCGCAAAGACGGAATC R: CCTCCGGAGTTTCCAAAGGG	250	NM_205464.1
<i>GAPDH</i>	F: CGATCTGAACTACATGGTTTAC R: TCTGCCCATTGATGTTGC	151	NM_204305.2
<i>SDHA</i>	F: CAGGGATGTAGTGTCTCGT R: GGGAATAGGCTCCTTAGTG	187	NM_001277398.1

Note: The lower-case letters are overlap sequences, underlined letters are enzyme cutting sites, bold letter is protective base.

Table S4. Target sequence of siRNAs

Genes	Name of siRNA	Target sequence
IRF1	siRNA1	GATCAACAAGGATAAGATGA
	siRNA2	AGCAAGAGAAAGTTGTATGA
	siRNA3	GCCATCTCCTGCAGCTTGTA
GATA1	siRNA1	CGCAAAGACGGAATCCAAA
	siRNA2	CAGGCACAGTGTGCAGCAAC
	siRNA3	TGGGCAGACACGGGTACTTTGG
NC	Si-NC	CAAGCTGACCCTGAAGTTC

Table S5. Venn analysis of predicted transcription factors in different regulatory regions

Logic name set	Number	Transcription factor
-2999 ~ -2140 bp	12	AP-1, E2, Elf-1, GATA-1, GCN4, Hb, ICSBP, IRF-1, NF-kappaB, REV-ErbA, SRF, YY1
-2140 ~ -458 bp	30	Ap-2alph, c-Ets-1, c-Fos, c-Jun, Cos, CPE bind, D1, E4, E47, embryo D, Eve, GAL4, GLO, HNF-1, HNF-3, Ik-1, Ik-2, Ik-3, k-2a, Kr, MEB-1, MIG1, MyoD, NF-E2, Oct-2.1, PU.1, SRY, TBP, TEC1, Zen-1
-2999 ~ -2140 bp	6	C/EBP α , NF-1, Oct-1, Sp1, Tra-1, USF
-2140 ~ -458 bp	27	Adf-1, ALF1B, AP-4, C/EBP γ , CACCC-binding, c-Myc, COUP, CPB bind, CREB, CRE-BP1, E1, EBF, ER, Erg-1, Ftz, GR, HNF-1C, Id3, JunD, LyF-1, MCM1, MRF4, NF-ATc3, NF-muE1, RAP1, RAR- β , RXR- β
-2140 ~ -458 bp	6	AP-2, AP-2 α , Egr-1, ETF, Krox-20, WT1
-458 ~ -281 bp	3	AP-2 α A, NF-MuE1, N-Myc
-2999 ~ -2140 bp	0	—