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Repression of CIITA by the Epstein-Barr virus transcription factor Zta is independent of its dimerization and DNA binding

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Abstract:	Repression of the cellular CIITA gene is part of the immune evasion strategy of the γherpes virus Epstein-Barr virus (EBV) during its lytic replication cycle in B-cells. In part this is mediated through down regulation of MHC class II gene expression via the targeted repression of CIITA, the cellular master regulator of MHC class II gene expression. The repression is achieved through a reduction in CIITA promoter activity initiated by the EBV transcription and replication factor Zta (BZLF1, EB1, ZEBRA). Zta is the earliest gene expressed during the lytic replication cycle. Zta interacts with sequence specific elements in promoters, enhancers and the replication origin (ZREs) and also modulates gene expression through interaction with cellular transcription factors and co-activators. Here we explore the requirements for Zta-mediated repression of the CIITA promoter. We find that repression by Zta is specific for the CIITA promoter and can be achieved in the absence of other EBV genes. Surprisingly, we find that the dimerization region of Zta is not required to mediate repression. This contrasts with an obligate requirement of this region to correctly orientate the DNA contact regions of Zta to mediate activation of gene expression through ZREs. Additional support for the model that Zta represses the CIITA promoter without direct DNA binding comes from promoter mapping that shows that repression does not require the presence of a ZRE in the CIITA promoter.

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4 **Repression of CIITA by the Epstein-Barr virus transcription factor Zta is**

5 **independent of its dimerization and DNA binding**

6

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25 **Abstract (229 words)**

26 Repression of the cellular *CIITA* gene is part of the immune evasion strategy of
27 the γherpes virus Epstein-Barr virus (EBV) during its lytic replication cycle in B-
28 cells. In part this is mediated through down regulation of MHC class II gene
29 expression via the targeted repression of *CIITA*, the cellular master regulator of
30 MHC class II gene expression. The repression is achieved through a reduction in
31 *CIITA* promoter activity initiated by the EBV transcription and replication factor
32 Zta (*BZLF1,* EB1, ZEBRA). Zta is the earliest gene expressed during the lytic
33 replication cycle. Zta interacts with sequence specific elements in promoters,
34 enhancers and the replication origin (ZREs) and also modulates gene expression
35 through interaction with cellular transcription factors and co-activators. Here we
36 explore the requirements for Zta-mediated repression of the *CIITA* promoter. We
37 find that repression by Zta is specific for the *CIITA* promoter and can be achieved
38 in the absence of other EBV genes. Surprisingly, we find that the dimerization
39 region of Zta is not required to mediate repression. This contrasts with an
40 obligate requirement of this region to correctly orientate the DNA contact
41 regions of Zta to mediate activation of gene expression through ZREs. Additional
42 support for the model that Zta represses the *CIITA* promoter without direct DNA
43 binding comes from promoter mapping that shows that repression does not
44 require the presence of a ZRE in the *CIITA* promoter.

45

46 **Introduction**

47 Epstein–Barr virus infects people and has a life-long association with them,
48 occasionally causing diseases including infectious mononucleosis, Burkitt's
49 lymphoma, Hodgkin's lymphoma and Nasopharygeal carcinoma (Magrath, 2012;

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50 Molyneux *et al.*, 2012; Saha & Robertson, 2011). Epstein–Barr virus infects
51 human B-lymphocytes and epithelial cells and establishes long-term latency in
52 memory B-lymphocytes (Babcock *et al.*, 1998). These cells are largely protected
53 from immune attack by the silencing of viral gene expression. The virus is
54 sporadically reactivated following B-cell activation and differentiation into
55 plasma cells (Crawford & Ando, 1986; Laichalk *et al.*, 2002; Laichalk & Thorley-
56 Lawson, 2005). As EBV enters the lytic replication cycle, it expresses around 90
57 viral genes that are required for the regulation of viral gene expression,
58 replication of the viral genome, assembly, packaging, and egress of the virion
59 (Farrell, 2005). Many viral genes expressed during viral lytic replication are
60 excellent targets for immune recognition (Adhikary *et al.*, 2006; Long *et al.*,
61 2011). Attack by the immune system during viral replication would threaten cell
62 survival and thus the successful generation of virions, but EBV has evolved
63 several strategies to evade immune responses during viral lytic replication (Zuo
64 & Rowe, 2012).
65
66 An important regulator of EBV lytic replication termed Zta (BZLF1, ZEBRA, EB1)
67 is a transcription factor, a replication factor and it disrupts several signal
68 transduction pathways (Kenney, 2007). Routes by which Zta activates gene
69 expression has been documented for both viral and host promoters. Many
70 promoters are targeted by the interaction of the sequence-specific DNA binding
71 domain of Zta with sequence specific 7-nucleotide DNA elements termed ZREs
72 (for example (Adamson & Kenney, 1999; Bergbauer *et al.*, 2010; Bhende *et al.*,
73 2004; 2005; Broderick *et al.*, 2009; Dickerson *et al.*, 2009; Flower *et al.*, 2011;
74 Holley-Guthrie *et al.*, 1990; Kalla *et al.*, 2012; Kalla *et al.*, 2010; Karlsson *et al.*,

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75 2008; Kenney *et al.*, 1989; Ramasubramanyan *et al.*, 2012a; Ramasubramanyan
76 *et al.*, 2012b; Sinclair, 2003; Sinclair *et al.*, 1991; Woellmer *et al.*, 2012). At least
77 32 distinct ZRE sequence variants are specifically recognized by Zta (Flower *et*
78 *al.*, 2011).
79
80 Down regulation of gene expression by Zta has been documented for the TNFR1
81 gene, through the cellular C/EBP genes (Bristol *et al.*, 2010). Additionally, post-
82 translational modifications of Zta have been shown to reduce the ability of Zta to
83 regulate gene expression, specifically phosphorylation at residue S209 (Asai *et*
84 *al.*, 2009) and sumoylation through residue K12 (Hagemeier *et al.*, 2010; Murata
85 *et al.*, 2010).
86
87 Zta has been shown to down regulate the expression of the master regulator of
88 MHC class II gene expression, *CIITA,* in an EBV-positive B-cell line, with both
89 protein and RNA levels decreasing following induction of EBV lytic cycle
90 activation (Li *et al.*, 2009). The product of *CIITA* is a non-DNA-binding cellular
91 transcriptional co-activator, which acts through interaction with DNA-bound
92 proteins that lack integral activation domains. *CIITA* activates the expression of
93 MHC Class II genes (Chang *et al.*, 2002) and the reduced expression of *CIITA*
94 observed in B-cells undergoing lytic cycle correlates with the reduced expression
95 of MHC class II observed at the cell surface (Li *et al.*, 2009). Repression of *CIITA*
96 gene expression is also driven by the related γherpesvirus KSHV (Cai *et al.*,
97 2013). Here, we investigate the route by which Zta represses *CIITA* expression.

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98

99 **Results**

100 ***CIITA promoter is specifically repressed by the EBV Zta protein***

101 The effect of Zta expression on the activity of the *CIITA* promoter and the viral
102 *BHLF1* promoter were compared in EBV-positive Raji cells following co-
103 transfection of reporter constructs with an expression vector for a polyhistidine
104 tagged version of Zta (Bailey *et al.*, 2009). The impact of Zta expression was
105 expressed relative to the maximal activity for each promoter (Fig. 1(a) and (b)).
106 Expression of Zta repressed the *CIITA* promoter, whilst in the same experiment it
107 dramatically activated expression of a viral promoter containing ZREs (*BHLF1)*.
108 This provides confirmation of the results of Li *et al.* showing that a short region
109 of the *CIITA* promoter is sufficient to mediate repression following Zta
110 expression (Li *et al.*, 2009). The repression of *CIITA* promoter activity could
111 result from the overexpression of a transactivator domain that non-specifically
112 sequesters basal transcription factors or co-activators, thereby inhibiting all RNA
113 polymerase II dependent transcription. To address whether this was the case, we
114 undertook experiments to explicitly question whether Zta repressed other
115 promoters. We generated promoter-reporter gene constructs for two viral
116 promoters, *BFLF2* and *BLLF3*. The impact of His-Zta expression on each
117 promoter was assessed in Raji cells (Fig. 1 (c,d)). This showed that neither *BLLF3*
118 nor *BFLF2* promoters were repressed by Zta expression. We further investigated
119 the repression of *CIITA* in BL cells by following two downstream targets of CIITA
120 expression, HLA-DOA and HLA-DBM. Both are down regulated at the RNA level
121 following Zta expression in BL cells (Supplementary Fig. 1).

122

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123 As Raji cells contain an EBV genome, changes in viral gene expression may occur
124 as a consequence of activating a partial lytic replication cycle through the
125 expression of Zta (Kallin & Klein, 1983). In order to question whether Zta relies
126 on additional viral components to repress *CIITA* expression, we introduced the
127 *CIITA* promoter-reporter gene into an EBV-negative sub-clone of Akata Burkitt's
128 lymphoma cells (AK31) (Jenkins *et al.*, 2000). In this cell background we saw that
129 co-expression of Zta drove repression of the *CIITA* promoter-reporter gene
130 around 5-fold (Fig. 2). This clearly demonstrates that Zta-mediated repression of
131 *CIITA* does not depend on additional EBV genes.
132
133 To explore the relevance of post-translational modifications of Zta to the Zta-
134 mediated repression of the *CIITA* promoter, we generated mutants of Zta at
135 amino acid residues K12 and S209 to prevent either sumoylation or
136 phosphorylation. Following transfection we found that neither post-translational
137 modification was required for Zta to repress the *CIITA* promoter (Table 1).
138
139 ***Domains of Zta mediating repression of CIITA promoter***
140 We then explored which domains of Zta protein mediate the repression of *CIITA*.
141 Two versions of Zta were generated; both of these retain the nuclear localization
142 signal (Mikaelian *et al.*, 1993). One mutant omits the N-terminal transactivation
143 domain (ZtaΔTA), this protein was previously shown to be able to bind to DNA
144 but not to transactivate a reporter construct (Packham *et al.*, 1990). The second
145 omits the dimerization and C-terminal region (ZtaH199ter) and has been shown
146 previously to be unable to bind DNA (Hicks *et al.*, 2003) or to form dimers
147 (Schelcher *et al.*, 2005) (Fig 3(a)). Following transfection into Raji cells we find

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148 that deletion of the transactivation domain ablates the ability of Zta to repress
149 the *CIITA* promoter, despite the proteins being expressed at an equivalent level
150 (Fig. 3(b)). In contrast, deletion of the dimerization and C-terminal regions of Zta
151 only resulted in a small reduction in the repression of the *CIITA* promoter (Fig.
152 3(c)). The slightly lower level of repression observed with ZtaH199ter might
153 result from the reduced abundance of this form of the protein. Taken together
154 these data show that a major component of Zta mediated repression of the *CIITA*
155 promoter occurs independently of a need for Zta to form dimers.
156
157 It has been shown previously that a Zta binding site within the *CIITA* promoter
158 allows repression by Zta (Li *et al.*, 2009). Our data show that dimerization is not
159 an obligate requirement for repression, implying that DNA binding is not
160 required. To explore this further, we assessed the promoter for potential ZREs
161 (Flower *et al.*, 2011) and found only the one, which was shown to be a Zta
162 binding site previously (Li *et al.*, 2009). We confirm using Chromatin
163 precipitation coupled to next generation DNA sequencing that Zta binds to the
164 promoter region of CIITA (Supplementary Fig. 2), but note that this does not
165 distinguish between direct and indirect binding. To evaluate the relevance of the
166 potential ZRE, we generated a promoter reporter construct in which the region
167 containing a ZRE was deleted (Fig. 4(a)). Both of these promoters are expressed
168 at equivalent levels (Table 2). Both full length Zta and the dimerization-deficient
169 mutant Zta-H199ter repressed the promoter missing the ZRE (Fig. 4(b)). This
170 supports our contention that the ability of Zta to repress the expression of *CIITA*
171 does not rely on direct DNA binding.
172

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173 From these data we devised a model to account for Zta mediated activation and
174 repression of gene expression. In cells expressing MHC class II, CIITA expression
175 is driven by the interaction of cellular factors (RNA polymerase II and cellular co-
176 activators) (Fig. 5(a)). Once Zta is expressed it interferes with the activation
177 machinery operating at the CIITA promoter, without the need to dimerize or
178 bind to the promoter (Fig. 5(b)).
179

180 **Discussion**

181 The EBV protein Zta is often described as the master regulator of EBV lytic cycle
182 replication. Indeed, the ability of Zta to regulate viral gene expression is crucial
183 to the success of viral lytic replication, as mutation of the *BZLF1* gene in
184 recombinant EBV demonstrates (Feederle *et al.*, 2000). The activation of viral
185 gene expression is considered to occur through the interaction of Zta with
186 sequence specific ZREs in the promoters of viral genes, and the attraction of co-
187 activator proteins such as p300, TFIID and other RNA polymerase II components
188 to the promoters (Lieberman & Berk, 1991; 1994) (Fig. 5 and Supplementary Fig.
189 3). Recent genome-wide analyses have shown that Zta has extensive interactions
190 across the EBV genome and a specific role in the transcriptional activation of
191 many viral promoters (Bergbauer *et al.*, 2010; Ramasubramanyan *et al.*, 2012a).
192

193 Li discovered that Zta-mediated repression of CIITA expression occurs in EBV-
194 positive Raji cells, but did not investigate whether other viral genes are required
195 for the repression (Li *et al.*, 2009). We confirm this and furthermore we show
196 that the expression of two CIITA-dependent genes are also down regulated. As
197 Zta expression in EBV positive BL cells, is sufficient to initiate the viral lytic

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198 replication cycle, many downstream changes in gene expression are expected,
199 and it is important to determine whether repression requires Zta action alone or
200 whether it acts in concert with additional viral proteins. Our demonstration that
201 Zta is able to repress the *CIITA* promoter in an EBV-negative BL cell line
202 unequivocally demonstrates that Zta-mediated repression does not require
203 other viral gene products.
204
205 The relevance of two forms of post-translational modification of Zta that have
206 been described as transcriptionally repressive was explored. The involvement of
207 phosphorylation at S209 by the viral protein kinase *BGLF4* (Asai *et al.*, 2009) was
208 investigated using the phospho-mimetic mutant version of Zta S209D and the
209 phosphorylation dead mutant version Zta S209A. Covalent addition of SUMO at
210 K12 (Hagemeier *et al.*, 2010; Murata *et al.*, 2010) was assessed using the non-
211 sumoylatable mutant version Zta K12R. Both of these post-translational
212 modifications have been described as transcriptionally repressive (Asai *et al.*,
213 2009; Hagemeier *et al.*, 2010; Murata *et al.*, 2010). As none of these Zta mutants
214 compromised the ability of Zta to repress the *CIITA* promoter, we conclude that
215 neither post-translational modification is likely to be responsible for the
216 observed repression of the *CIITA* promoter by Zta.
217
218 Zta also regulates gene expression by disrupting transcriptional activation by
219 NFκB and p53 (Morrison & Kenney, 2004; Zhang *et al.*, 1994). This occurs
220 through physical interactions between Zta and the p65 component of NFκB and
221 between Zta and p53 protein (Morrison & Kenney, 2004; Zhang *et al.*, 1994).
222 However, it is unlikely that either NFκB or p53 plays a role in Zta-mediated *CIITA*

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223 repression, as both require the dimerization region of Zta, which is not necessary
224 for repression of *CIITA*. In addition, mutation of the NFκB interaction site in the
225 *CIITA* promoter does not alter either basal expression or Zta mediated
226 repression (NB, AJS unpublished data). It is intriguing that Zta has been shown
227 previously to modulate expression of a viral promoter (Zp) without the need to
228 bind directly to DNA (Flemington *et al.*, 1994).
229
230 A previous study suggests that Zta repression of the CIITA promoter is driven
231 through the interaction of Zta with a single ZRE within the promoter (Li *et al.*,
232 2009). This is supported by the impact of mutations of the ZRE within the
233 promoter and by the inability of Zta to repress the *CIITA* promoter when the
234 basic region is lost. This study places emphasis on a need of Zta to bind directly
235 to DNA to effect repression. Our experiments support a different conclusion in
236 which Zta represses CIITA expression without binding directly to DNA. We
237 rationalize the need for the basic region of the Zta protein based on a
238 requirement for the nuclear localization domain, which is contained therein
239 (Mikaelian *et al.*, 1993). Without entry to the nucleus, Zta would not be able to
240 repress the CIITA promoter through either direct or indirect DNA binding.
241
242 In summary, we show that Zta mediated repression of the *CIITA* promoter can
243 occur without Zta contacting DNA directly, this is supported by the retention of
244 repression when (i) the ZRE is deleted and (ii) by a version of Zta that is
245 defective for dimerization and therefore defective for DNA-binding. This
246 discovery leads us to propose a mechanism to describe gene repression by Zta.
247 In this model the amino terminal region of Zta is able to impede the function of

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248 an essential component of the transcriptionally active CIITA promoter, for
249 example a DNA bound transcription factor or a transcription factor-associated
250 co-activator, thereby preventing its productive association with RNA Pol II and
251 its accessory proteins (Fig. 5).
252
253 **Materials and Methods**
254
255 ***Plasmid constructs.***
256 The *CIITA* promoter (-286 to +54) was cloned with a Kpn I restriction enzyme
257 site included at the 5' end and a Hind III site at the 3' end of the sequence. The
258 promoter was sub-cloned into the pGL3 enhancer plasmid, which contains a
259 luciferase reporter construct down-stream from a multi-cloning site and which
260 includes a distal SV40 enhancer (*Promega).* A 5' deletion version of the promoter
261 was generated (-214 to +54); the location of the 5' end of this promoter is
262 immediately 3' from the ZRE.
263
264 The *BHLF1, BFLF2 and BLLF3* promoters were cloned with a BamHI restriction
265 enzyme sites added at the 5' end and Hind III sites at the 3' end. The DNA
266 sequence between co-ordinates 40472 and 40818, 45793 and 44746 and 76186
267 and 77231 of the EBV genome (Human herpesvirus 4 complete wild type
268 genome Accession: NC_007605.1) were synthesized for the promoter regions for
269 the *BHLF1, BFLF2* and *BLLF3* genes respectively. The promoters were sub-cloned
270 into the pCpGL plasmid (Klug & Rehli, 2006), which is based on pGL3 basic and
271 contains a luciferase reporter construct down stream from a multi-cloning site.
272

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273 A plasmid driving the expression of hexa-histidine tagged Zta (His-Zta) (Bailey *et*
274 *al.*, 2009) was used to express His-Zta, compared to the vector control pcDNA3
275 (*Invitrogen).*
276
277 Expression vectors for His-Zta K12R, His-Zta S209A and His-Zta S209D were
278 generated by site directed mutagenesis of His-Zta using the primers shown in
279 Table 3. An expression vector for His Zta-199ter which introduces a termination
280 codon at the amino acid 199 of the Zta coding sequence and His Zta-ΔTA which
281 deletes amino acids 1-133 of Zta were generated by gene synthesis (*Invitrogen*).
282
283 ***Cell culture***
284 Plasmids were introduced into EBV-positive Raji cells (Pulvertaft, 1965) or EBV-
285 negative Akata cells (Jenkins *et al.*, 2000) by electroporation. 1 x 10^7 cells in
286 0.25ml of medium were incubated with 10μg of plasmid DNA and pulsed with
287 250V at a capacitance of 975μF in a Gene Pulser II electroporator *(Bio-Rad).*
288
289 ***Luciferase assays***
290 48 hours post-transfection cells were harvested into 250μl of Passive Lysis
291 Buffer (*Promega*) and incubated at room temperature for 15 minutes. The lysed
292 cells were then centrifuged for 10min at 8 krpm and the supernatant was used to
293 determine luciferase activity. 10μl aliquots of each lysate sample were pipetted
294 into a 96-well white luminescence plate and analyzed using luciferase detection
295 kit reagents with a Glomax detection system (*Promega*). A protein concentration
296 assay was undertaken (*Biorad)* and promoter activity was expressed as

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297 luciferase RLU/μg protein. Significance of different promoter activity was
298 assessed using a Student's paired T-test with 2 tail distribution.
299
300 ***Protein analysis***
301 Proteins were extracted from cells by boiling in 2X Laemeli sample buffer and
302 fractionated on Novex protein gels (*Invitrogen).* Following transfer to
303 nitrocellulose membranes the blots were incubated with the Zta specific
304 antibody sc-17503 (*Santa Cruz*) which recognizes the amino-terminal region of
305 Zta, or BZ1 which recognizes the basic and dimerization regions of Zta (Young *et*
306 *al.*, 1991) or a rabbit polyclonal beta actin antibody (Sigma), followed by
307 detection with HRP-linked secondary antibodies and ECL (Ramasubramanyan *et*
308 *al.*, 2012b).
309
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315
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487

488

489 **Figure legends**

490 Figure 1. Repression of *CIITA* promoter by Zta is specific.

491 The *CIITA* (-286 to +54) (a), *BHLF1* (b), *BFLF2 (c)* and *BLLF3* (d) promoter-
492 luciferase plasmids and the indicated expression vectors were introduced cells
493 by electroporation, 48 hours later cells were harvested and the luciferase activity
494 determined. a-d. Promoter activity in Raji cells relative to the maximal activity of
495 *CIITA* promoter (transfected with control plasmid) with the standard deviation
496 from six assays (three replicate samples from each of two separate experiments).
497 For comparisons +/- Zta ** represents p of significant difference <0.01; *
498 represents p of significant difference <0.05. The expression of His-Zta and
499 endogenous protein were analyzed by western blot of proteins from the
500 transfected cells.

501

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502 Figure 2. Repression of *CIITA* promoter by Zta is independent of other viral
503 proteins.
504 The *CIITA* (-286 to +54) promoter-luciferase plasmid and the indicated
505 expression vectors were introduced into an EBV-negative sub-clone of the Akata
506 BL cells (AK31) by electroporation, 48 hours later cells were harvested and the
507 luciferase activity determined. Promoter activity is expressed relative to the
508 maximal activity of *CIITA* promoter (transfected with control plasmid) with the
509 standard deviation from six assays (three replicate samples from each of two
510 separate experiments). For comparisons +/- Zta ** represents p of significant
511 difference <0.01;. The expression of His-Zta and endogenous protein were
512 analyzed by western blot of proteins from the transfected cells.
513
514 Figure 3. Zta repression of CIITA promoter requires the transactivation domain.
515 The CIITA promoter-luciferase plasmids (-286 to +54) and either control, His-Zta
516 or His-Zta mutant expression vectors were introduced into Raji BL cells by
517 electroporation. 48 hours later cells were harvested and the luciferase activity
518 and protein concentrations determined. For comparisons +/- Zta ** represents p
519 of significant difference <0.01; * represents p of significant difference <0.05.
520 a. Schematic of the Zta protein and the two mutant versions that were evaluated.
521 TA is transactivation domain; B is basic DNA contact region; ZIP is dimerization
522 bZIP domain; CT is the Carboxy terminal region (required for dimerisation and
523 replication).
524 b. Promoter activity of -286 to +54 promoter with His-ZtaΔTA with the standard
525 deviation from six assays (three replicate samples from each of two separate
526 experiments), together with a western blot.

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527 c. Promoter activity of -286 to +54 promoter with His-Zta199ter with the
528 standard deviation from six assays (three replicate samples from each of two
529 separate experiments), together with a western blot.
530
531 Figure 4. Zta repression of *CIITA* promoter occurs without binding to the ZRE.
532 The *CIITA* promoter-luciferase plasmids (either -286 to +54 or -214 to +54) and
533 either control, His-Zta or His-Zta mutant expression vectors were introduced
534 into Raji BL cells by electroporation. 48 hours later cells were harvested and the
535 luciferase activity and protein concentrations determined. For comparisons +/-
536 Zta ** represents p of significant difference <0.01; * represents p of significant
537 difference <0.05. For both His-Zta and His-ZtaH199ter, the significance is equal
538 for each of the different promoters.
539 a. Schematic of the *CIITA* mutant promoters used in these experiments. The
540 location of the ZRE is indicated by a filled box.
541 b. The *CIITA* basal promoter activity is shown (open) together with the His-Zta
542 mediated activity (black), with the standard deviation from six assays (three
543 replicate samples from each of two separate experiments). Western blot analysis
544 of protein expression in the transfected cells.
545
546 Figure 5. Proposed model to explain Zta mediated gene repression of the *CIITA*
547 promoter.
548 a. The active *CIITA* promoter is shown. Basal transcription factors are
549 represented as white spheres and co-activators by the stippled oval. RNA
550 polymerase II is represented by the black cloud with transcription indicated by
551 an arrow.

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552 b. The ability of a non-DNA binding form of Zta (filled oval) to repress expression
553 of the *CIITA* promoter suggests that some repression can occur without direct
554 DNA contact. The simplest model to account for this has the N-terminal part of
555 Zta blocking the interaction of the basal transcription machinery.

556

557 **Tables**

558 Table 1. Impact of mutation of K12 and S209 on Zta mediated repression

	His-Zta	His-Zta K12R	His-Zta S209A	His-Zta S209D
CIITA promoter activity	1.00	1.00	1.00	1.00
Relative promoter activity following his-Zta expression	0.16	0.07	0.07	0.04
Standard deviation	0.15	0.08	0.01	0.00

559

560 Table 2. Impact of Zta expression on -286 and -214 *CIITA* promoters

	Average luciferase units *CIITA* (-286/+54)	Average luciferase units *CIITA* (-214/+54)
control	440050 +/- 7113	472832 +/- 15031
His-Zta	18448 +/- 464	66785 +/- 1917

561

562 Table 3. Oligonucleotides used to generate mutations.

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His-Zta S209A Amino acid serine 209 mutated to Alanine	GGCTGCTGCCAAATCAGCTGAAAATGACAGGCTGC GCC; GGCGCAGCCTGTCATTTTCAGCTGATTTGGCAGCA GCC
His-Zta S209D Amino acid serine 209 mutated to Glutamic acid	GGCTGCTGCCAAATCAGATGAAAATGACAGGCTGC GCC; GGCGCAGCCTGTCATTTTCATCTGATTTGGCAGCA GCC
His-Zta K12R Amino acid lysine 12 mutated to arginine	CTCGACTTCTGAAGATGTAAGATTTACACCTGACC CATACC; GGTATGGGTCAGGTGTAAATCTTACATCTTCAGAA GTCGAG

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Figure 1

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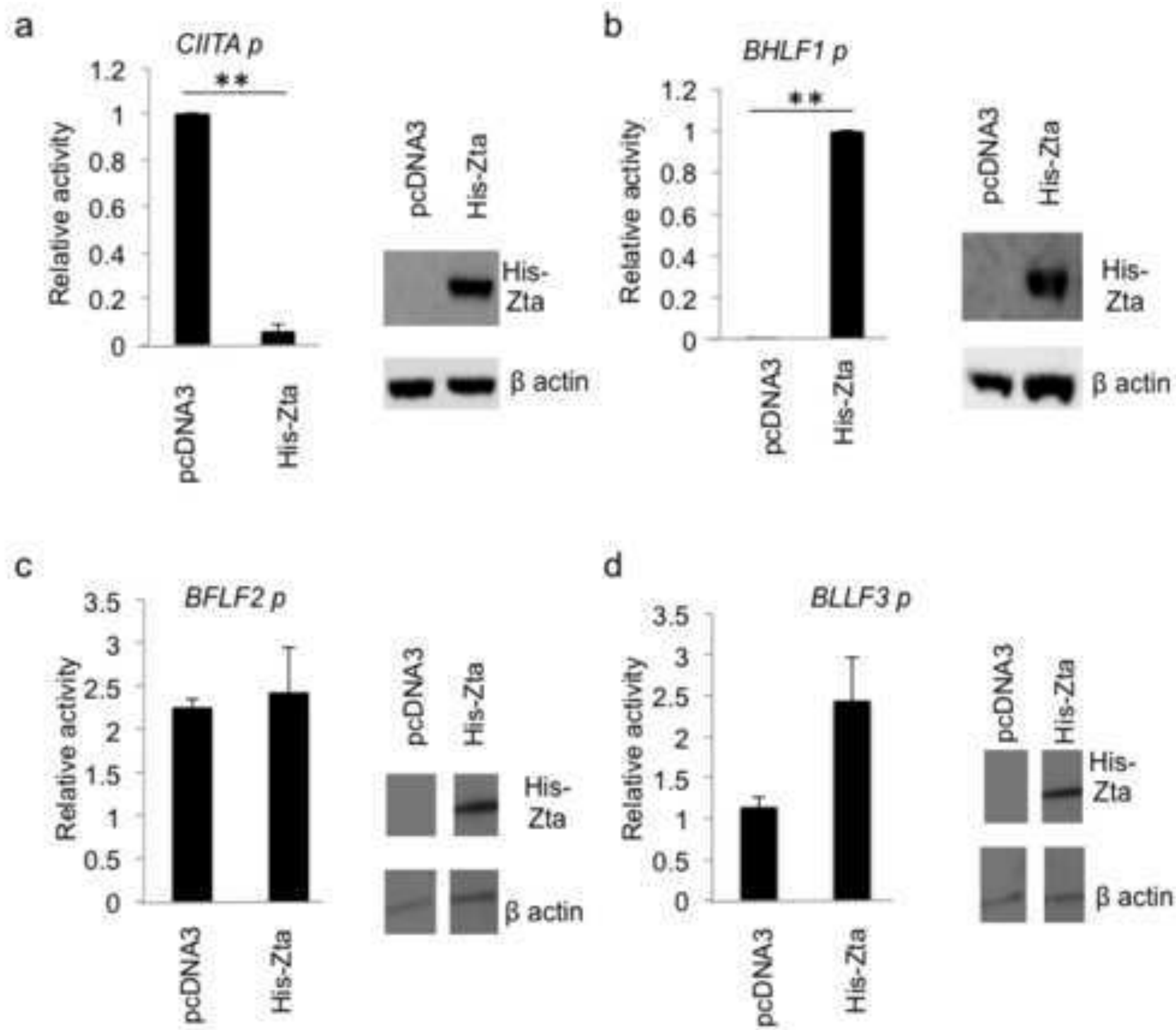

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Figure 2

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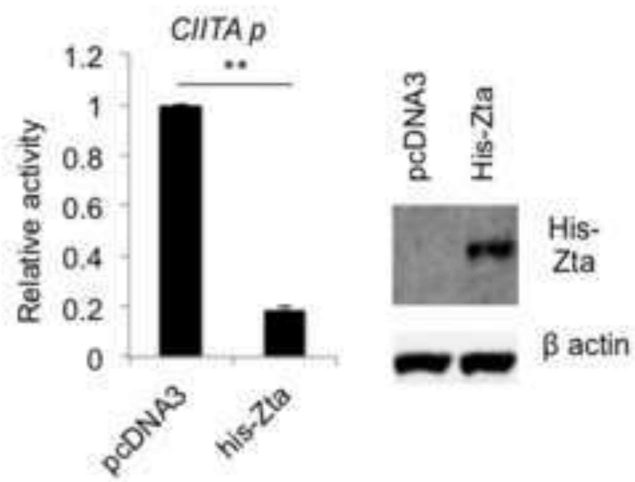

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Figure 3

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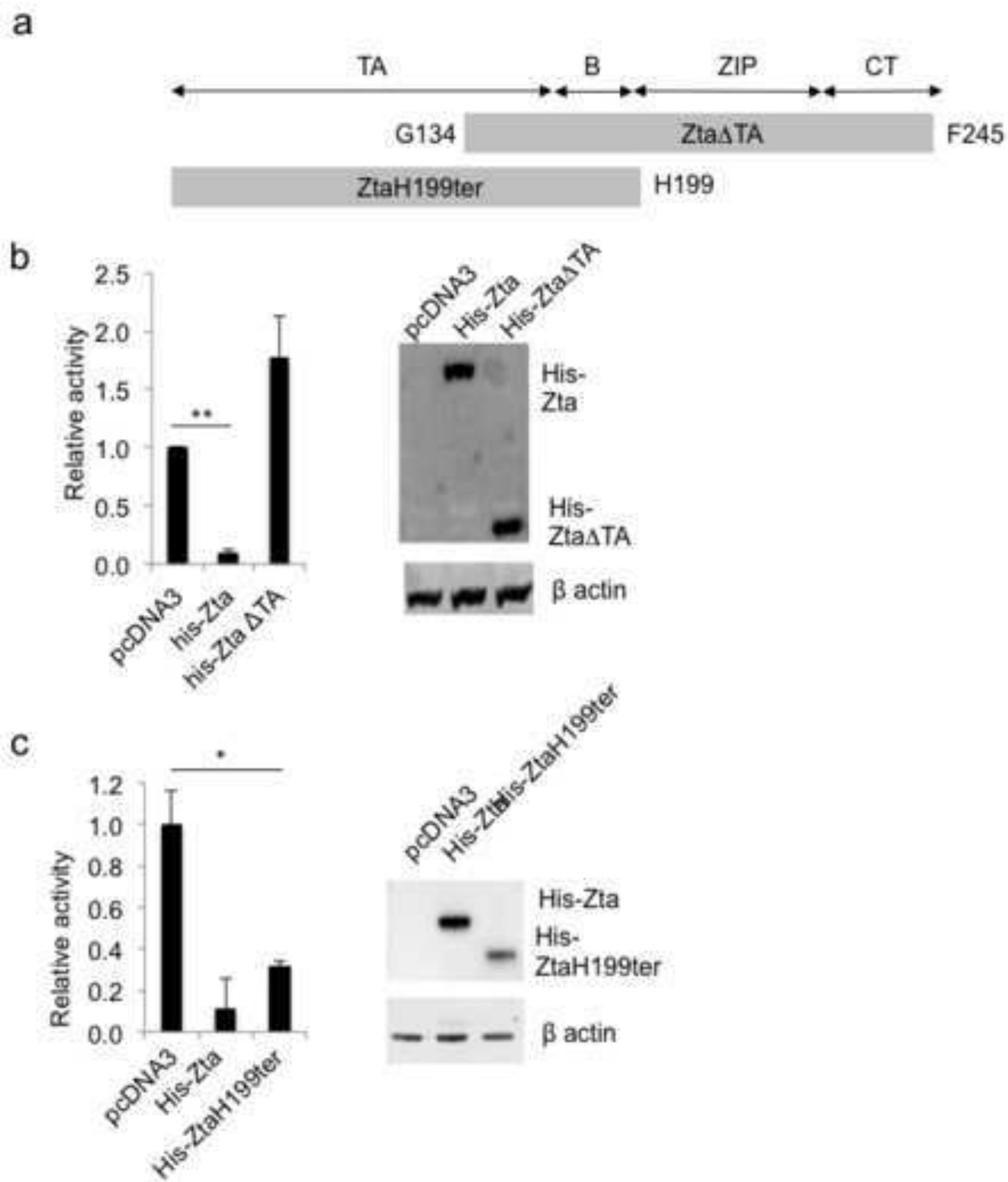

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Figure 4 Click here to download Figure fig4.tiff

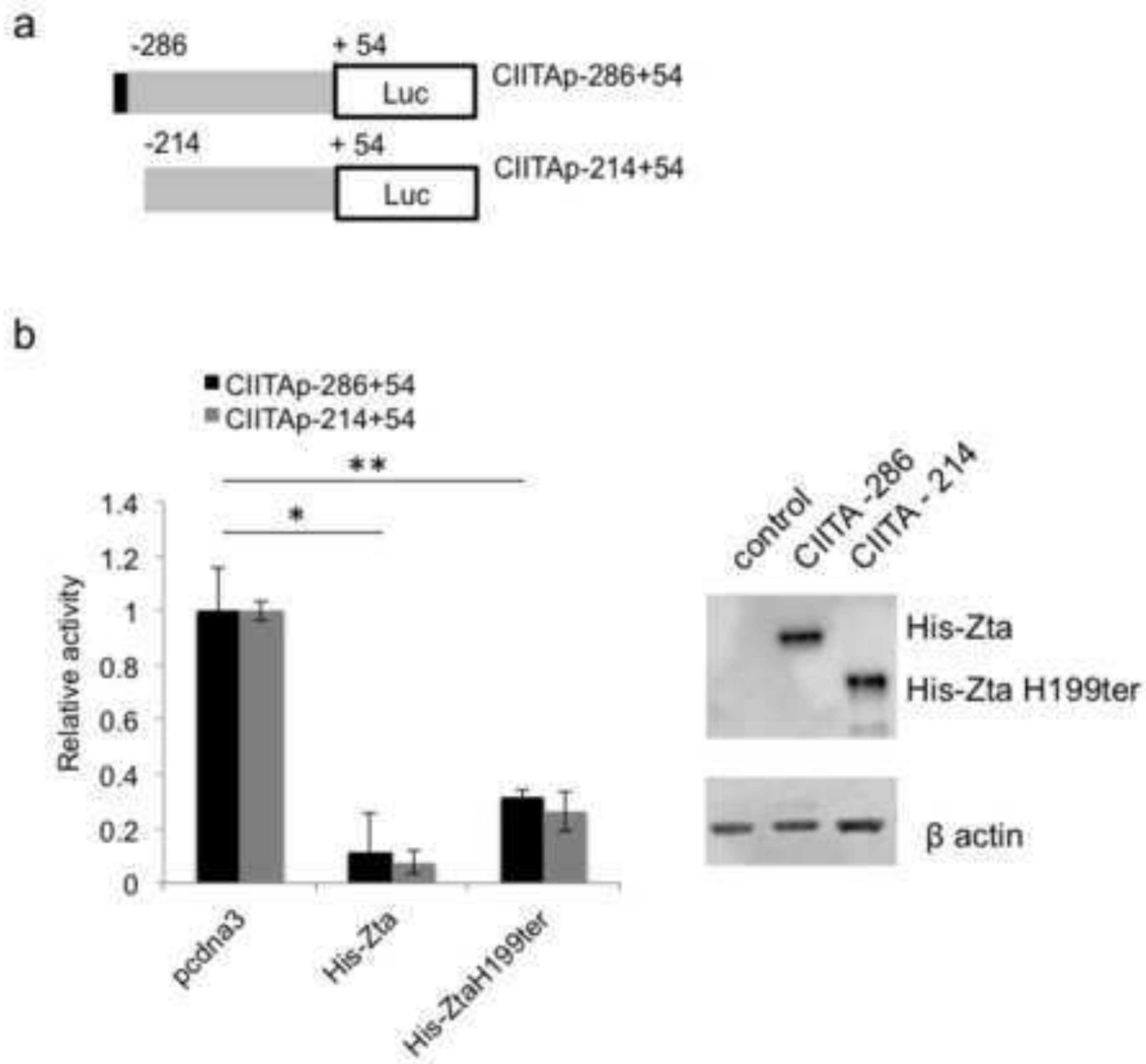

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Figure 5

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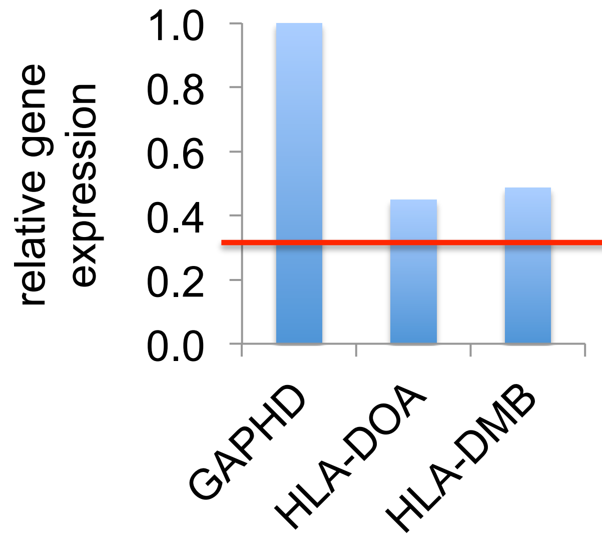

Supp Figure 1. CIITA downstream targets.
The expression of targets of CIITA were investigated by RNA seq as described in Ramasubramanyan et al 2015).
The relative change in expression following Zta expression in Akata BL cells was determined and presented (FDR<0.05).
The red line indicates the maximum reduction expected as only 70% of cells express Zta.

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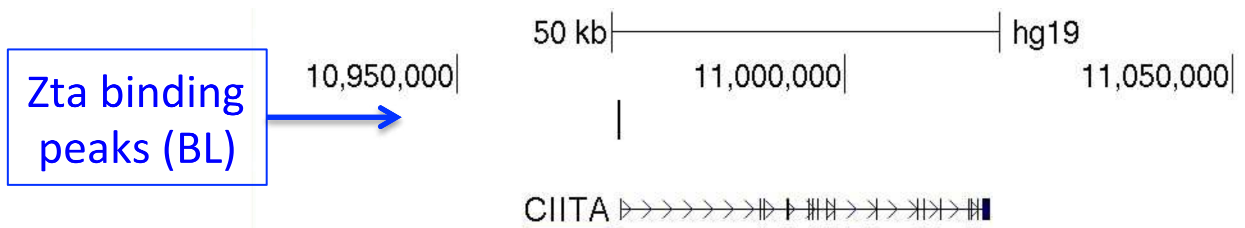

Supp figure 2. Interaction of Zta with the CIITA pomoter in BL cells.
The interaction of Zta with the CIITA locus was undertaken by ChIP-seq (as described in Ramasubramanyan et al 2015). The locus is shown chr16:10,923,269-11,066,626. A peak of binding is seen as a bar spanning the TSS of the gene in Akata BL cells undergoing lytic replication cycle.

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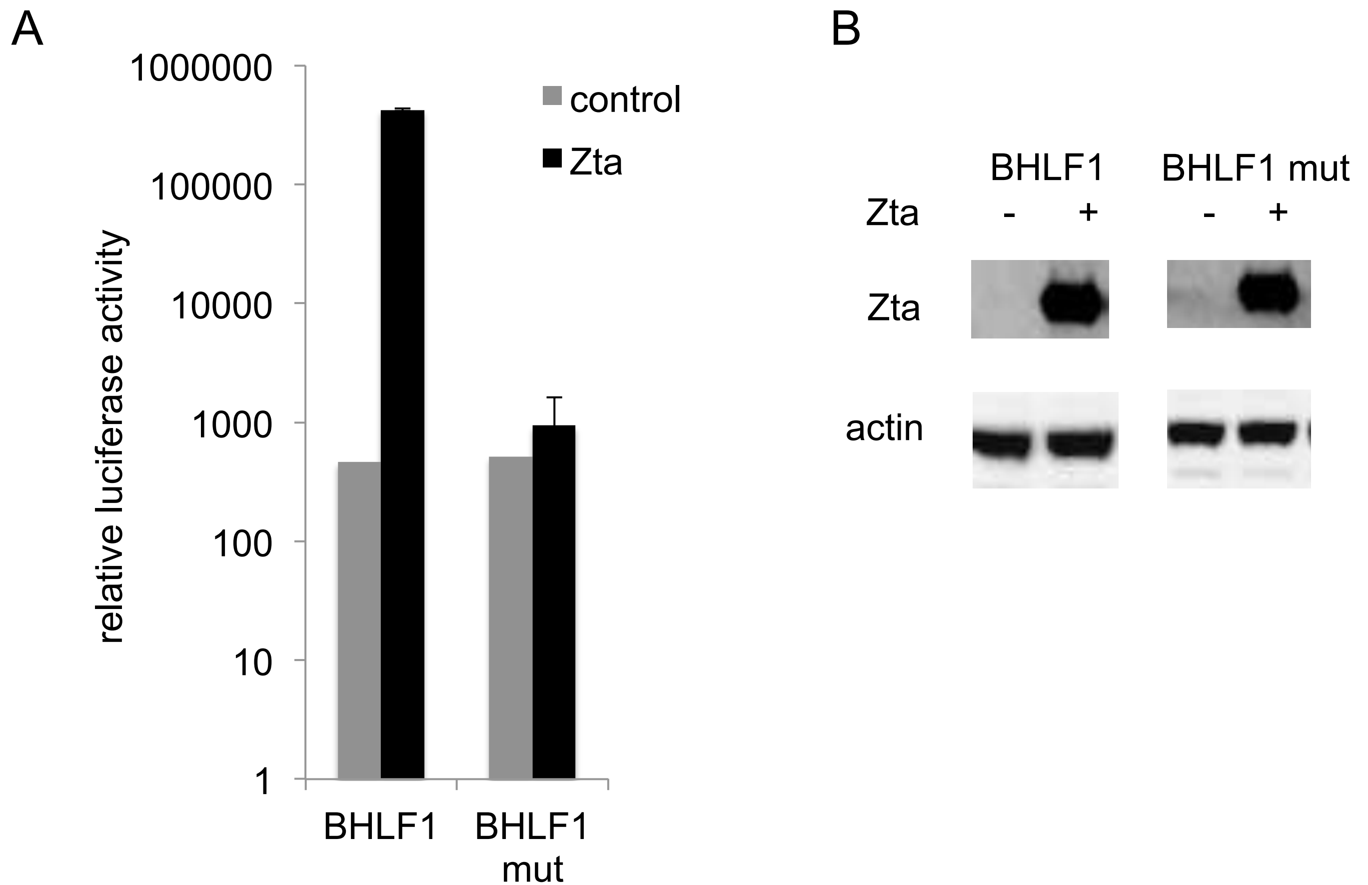

Supp Figure 3. Activation of BHLF1 promoter depends on ZREs in BL cells.
The viral *BHLF1* promoter-luciferase plasmid and a version that has a mutation of each ZRE within the promoter was introduced into BL cells by electorporation together with a his-Zta expression vector or control plasmid. 48 hours later cells were harvested and the luciferase activity and protein concentrations determined.

A. Relative activity of promoters (log10 scale).
B. Western blot showing his-Zta and actin expression.

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