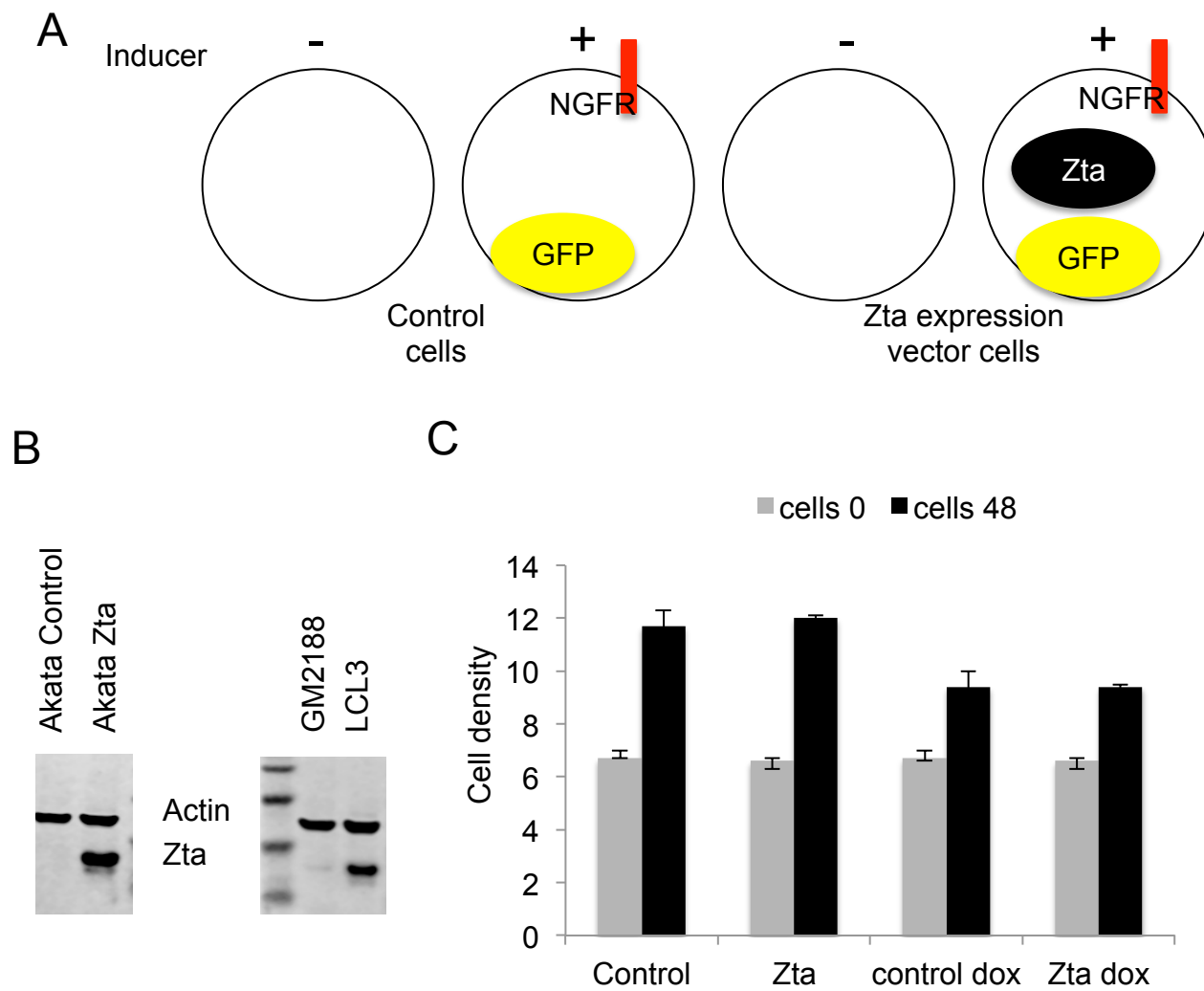


Supplementary information for:

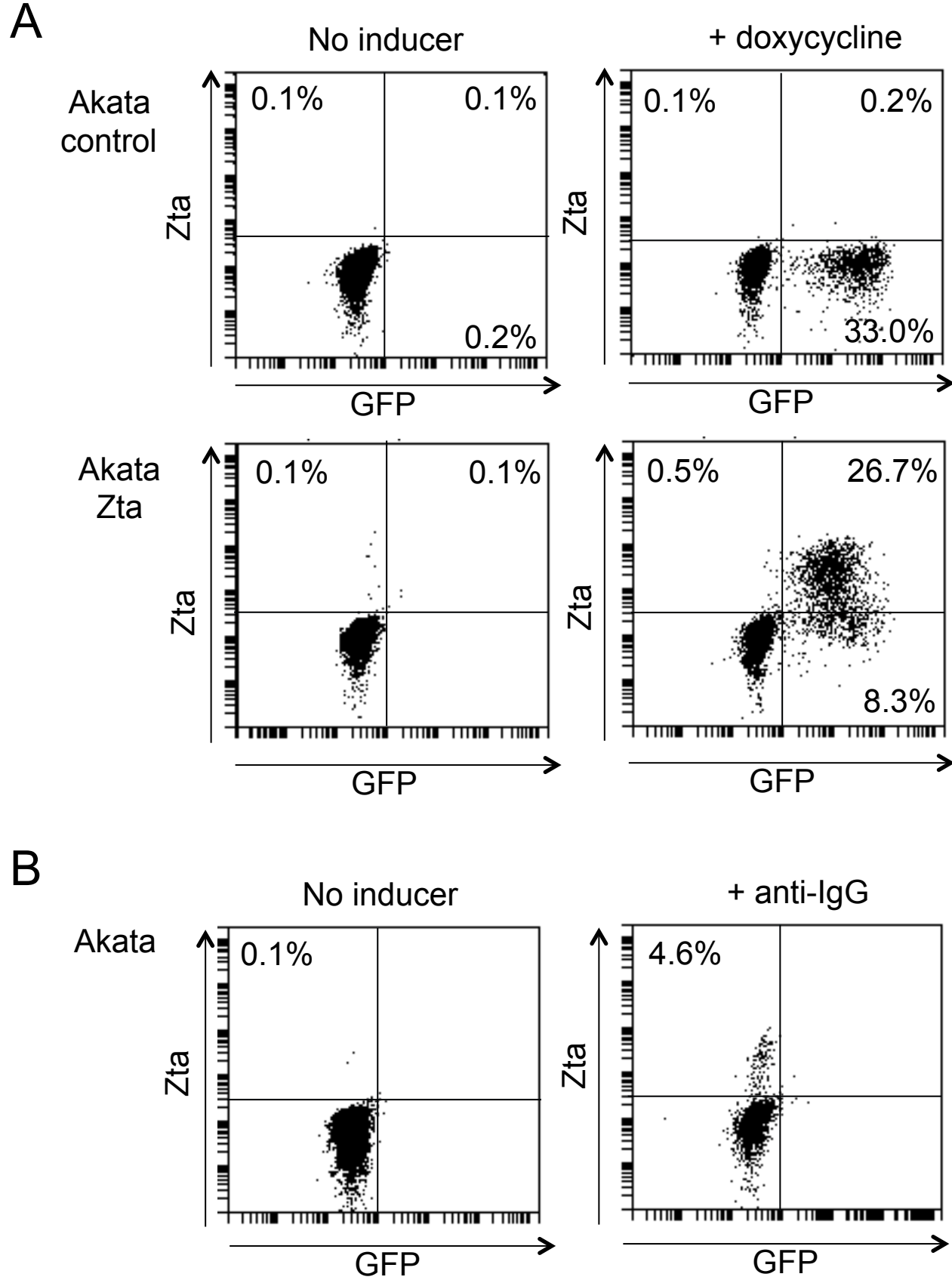
Epstein Barr virus transcription factor Zta acts through distal regulatory elements to directly control cellular gene expression

Sharada Ramasubramanyan¹, Kay Osborn¹, Rajaei Al-Mohammad¹, Ijuel Barak Naranjo Perez-Fernandez¹, Jianmin Zuo², Nicolae Balan¹, Anja Godfrey¹, Harshil Patel³, Gordon Peters³, Martin Rowe², Richard G. Jenner⁴, Alison J. Sinclair^{1*}.



Supporting Figure S1. Generation and characteristics of doxycycline-inducible Akata-Zta and control cells.

- Expression vectors for Zta and control were introduced into Akata cells and cells expressing the selectable marker CD2 were selected. Doxycycline was added to induced expression of the bi directional promoter and Zta, NGFR and GFP were expressed.
- Zta and actin proteins were detected by western blot analysis and compared to a tightly latent (GM2188) and a lytic LCL (LCL#3).
- The viability of the Akata control and Akata Zta cells was assessed using a Trypan Blue exclusion assay after 48 hours growth in the presence or absence of doxycycline.

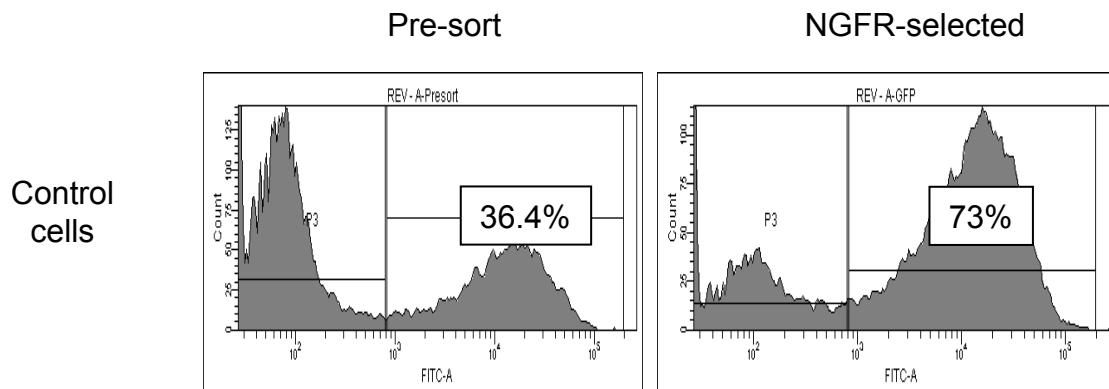


Supporting Figure S2. Comparison of Zta expression after doxycycline induction and BCR ligation.

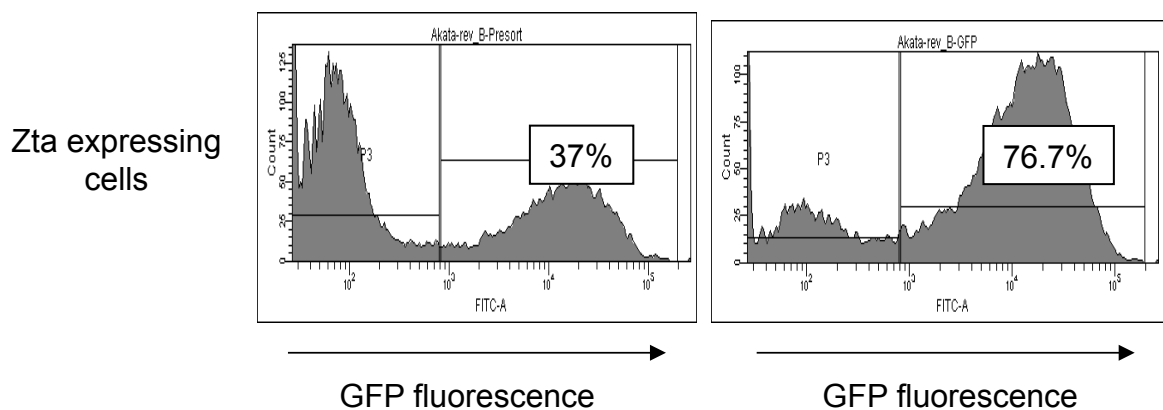
A. Akata control and Akata Zta cells were exposed or not to doxycycline. The expression of Zta and GFP were detected by multiparameter FACS after intracellular staining for Zta expression.

B. Akata cells were stimulated with and without anti-IgG and the same analysis undertaken.

A



B



Supporting Figure S3. Enrichment of doxycycline induced Akata-Zta BL cells.

Cells were induced with doxycycline and analysed for GFP expression by FACS analysis before and after enrichment for NGFR expression.

A. Akata control cells

B. Akata Zta cells.

C. The % of GFP positive cells are shown.

A

CGCCCGGCGGATCCGTCTGCAGAGGCAT**TGAGCCAT**GCAC**TTAGCAA**ATTAT**TGTGTAAGT**ACTG
TGAGCAAGACGTGTGCAATAAGCTTGAGCCATGGCTGTGTCAATCGATGTGCAATGCACATGTG
TCAATGCAT**TGAGTCATCATTTGACTAAT**GCTGAT**TGTGTCAGGATATGTGTCTTTAGCTGAGCAA**
CCTAG**TGGCACAGCTACCTGAGTAATAGAGTTGCAATACGTGAGCAA**CTAATCT**TGTGTCATAC**
AG**TGAGCCA**ACTAGTATGTCGACGCGGCCG

B

GGATCCGGGCGAATCTCCGAGCAGAGAGAAGAAATTGAGTGTGTGACAGTGTGAATGGATGTG
ACTATAGGTGAGCAACAGTCGGTGACAATGTGCACATCTGGTATTTCCCTGTGTGAGAGCAAAA
CTACAGGACTCAGTGAGTGGTTTTCTGTGGTTTGCAC**TTGTGTATCTGGGTGTATGGGAGTGTG**
TAGCCCTGCTGACTGTGTAGATATGAATGTGTGTGCAGTTGTGAGTCTTGCATGTGGTCATCT
ATGTGAAACAATGCCTGTATAAGTACCCATGGATGTGCACGGTTTTGCCTCTAGCCGTGCATT
TCTGGTTCTGTGTGTGTGTGT**TTCTGGGACATTCTTCAAGTCCACA**ACTCTGAGGGTATCACT
GTGAGAGCAGGGGTCCTAGCTCTACCCATTTGTGTGTGTGTGGAGATGTGTAAGTCTATGTAG
ACAAAAGTGTGTGTCATTTACTGTGTTTGGGGTGTGAACACCTATGTGATGTGTTTGCACAAC
TGCACGTGTTTTGTTCTGTGTGTGTGATCGTGTGTTCAAATAAGTCATCTTGTCTAGTCATCA
GGTGCCTGCACAGACAAAGGTGAAAGGTGTCTGCCTGTTGAGATCTGTGGATAGGGTGTATAT
ATGGACATCTCAGCCTGTCTACGTGTGTATCTGTCTCTGTCCTCACGGCAAAAGAGAGGTCGA
C

C

CTTTCTGTCTTCACCAAATTCAGTCCACAGTAAGGAAGTGAAATTAATTT**CAGAGGTGTGGGG**
AGGGCTTAAGGGAGTGTGGTAAATTAGAGGGTGTTCAGAAACAGAAATCTGACCGCTTGGGG
CCACCTTGCAGGGAGAGTTTTTTTTGATGATCCCTCACTTGT**TTCTTTGCATGTTGGCTTAGCT**
TGGCGGGCTCCCAACTGGTGACTGGTTAGTGATGAGGCTAGTGATGAGGCTGTGTGCTTCTGA
GCTGGGCATCCGAA

D

GGATAATGGAACCCTATGGATACCTACCTCTAGGCTCCACCCACTAGGTATATCGGGGCGGAG
CCCAATCCTCCCCCTCCTGGTTCAACCCTATGGAGGGGACCCTCCTGAGGCTCCGCCTACCCC
AAATCTCGCGGACCTCTAGCCCCCTCCTCCTCTCGTTATCCCAATAGAATAACCTCCAGGTACC
ACCCACCTGGCCCCCTTTCCTTAATGAAACCCAACGGGCTAAAAACCCCTTTCCTGAATTAACCA
ATAAGAAGCCCCCACTCCCCCCCTTTACCTTAAAGTACCCCTTTGAGACCCCCAAAAGAGGATA
AAAGAAGGCGAGCCGGCCCGGCTCGCCAGCGTCGTCCAGACGCTCGGGGGGTGC

E

CGCCCGGCGGATCCGTCTGCAGAGGCAT**TACTCAT**GCAC**TGACTCA**ATTAT**TACTCAGT**ACTG
TTACTCAGACGTTACTCATAAGCTGACACATGGCTGTGTCTATCGATGTGTCTTGCACATGTG
TCAATGCAT**TGTGTCTTCATTTGTGTCTT**GCTGAT**TGTGTCTGGATATGTGTCTTTAGCTGTGCAA**
CCTAG**TGTGTCT**GCTACC**ACGCACATAGATGTGCAATACGTGACACA**CTAATCT**TGTGTCTTAC**
AG**TGACACA**ACTAGTATGTCGACGCGGCCG

Figure S4. DNA sequence of promoters.

- A. The DNA sequence of the tandem ZRE elements is shown with each of the non-CpG ZREs shown in bold.
- B. The DNA sequence of the Zta binding sites associated with the FOSB gene are shown (Chr19:45954888-45955223; 45962338-45962553; 45962727-45962858, hg19).
- C. The DNA sequence surrounding the *CIITA* promoter (-212 to +54) is shown.
- D. The DNA sequence surrounding the viral *BHLF1* promoter is shown with the location of the mutated ZREs underlined. This includes nucleotides 52760-53128 from B95-8 virus sequence.
- E. The DNA sequence of the ZREs within seven Zta binding sites associated with the RASA3 3' region are shown (bold).