Figure S1

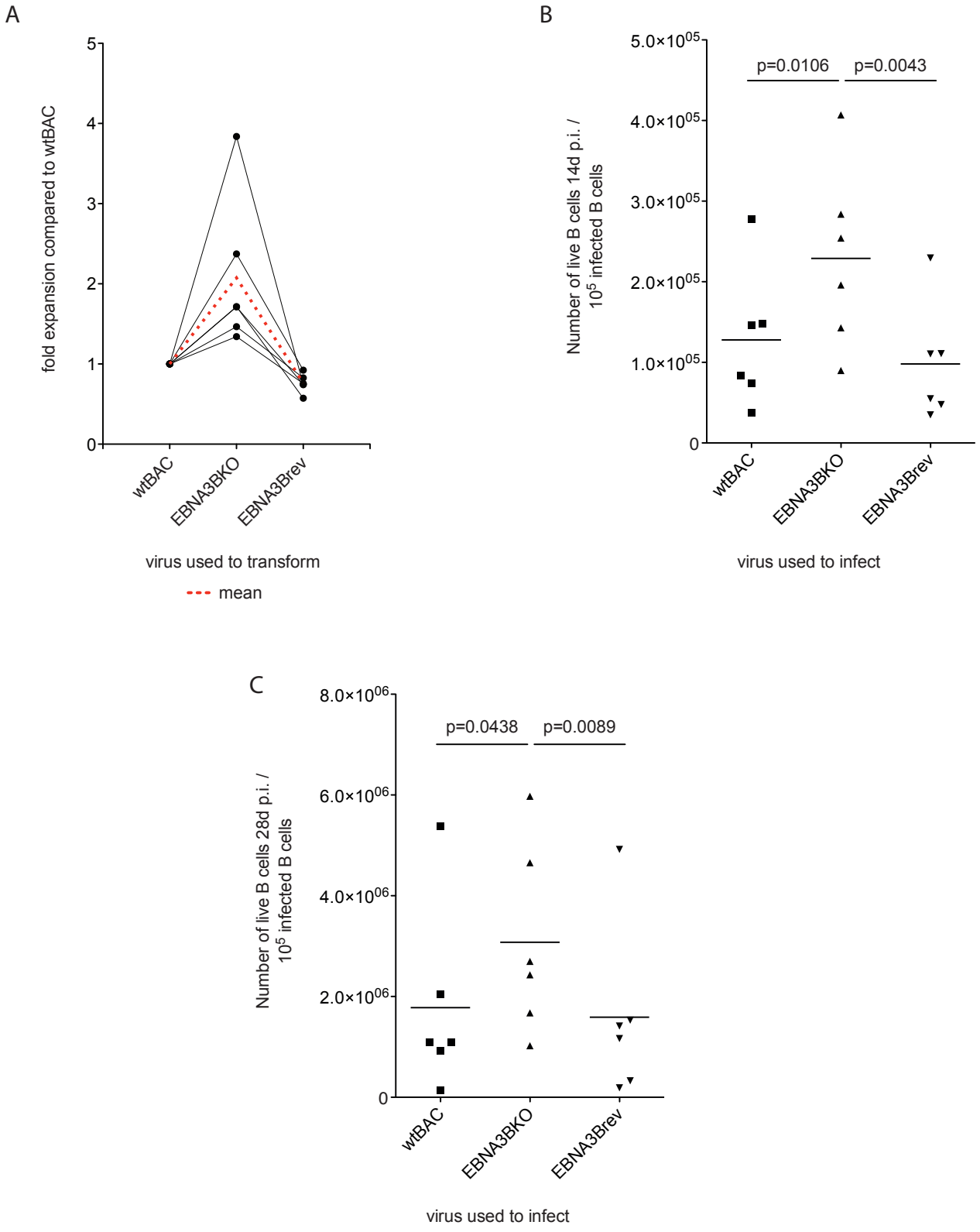

Figure S1. EBNA3BKO EBV transforms B cells more efficiently into LCLs. PBMCs from healthy donors were infected with EBV using 2.5x10^4 RGU per 10^6 PBMCs. (A) Live B cells were counted 14 days (A-B) and 28 days (C) after infection with EBV. (A) Displayed is the fold increase of live B cells compared to wtBAC infection. Each line represents one healty donor. (B-C) Live B cells were counted 14 (B) and 28 (C) days post infection. (A-C) Pooled data from six healthy donors is shown. Two LCLs from each of the 6 donors were assessed at the indicated time-points.

Figure S2

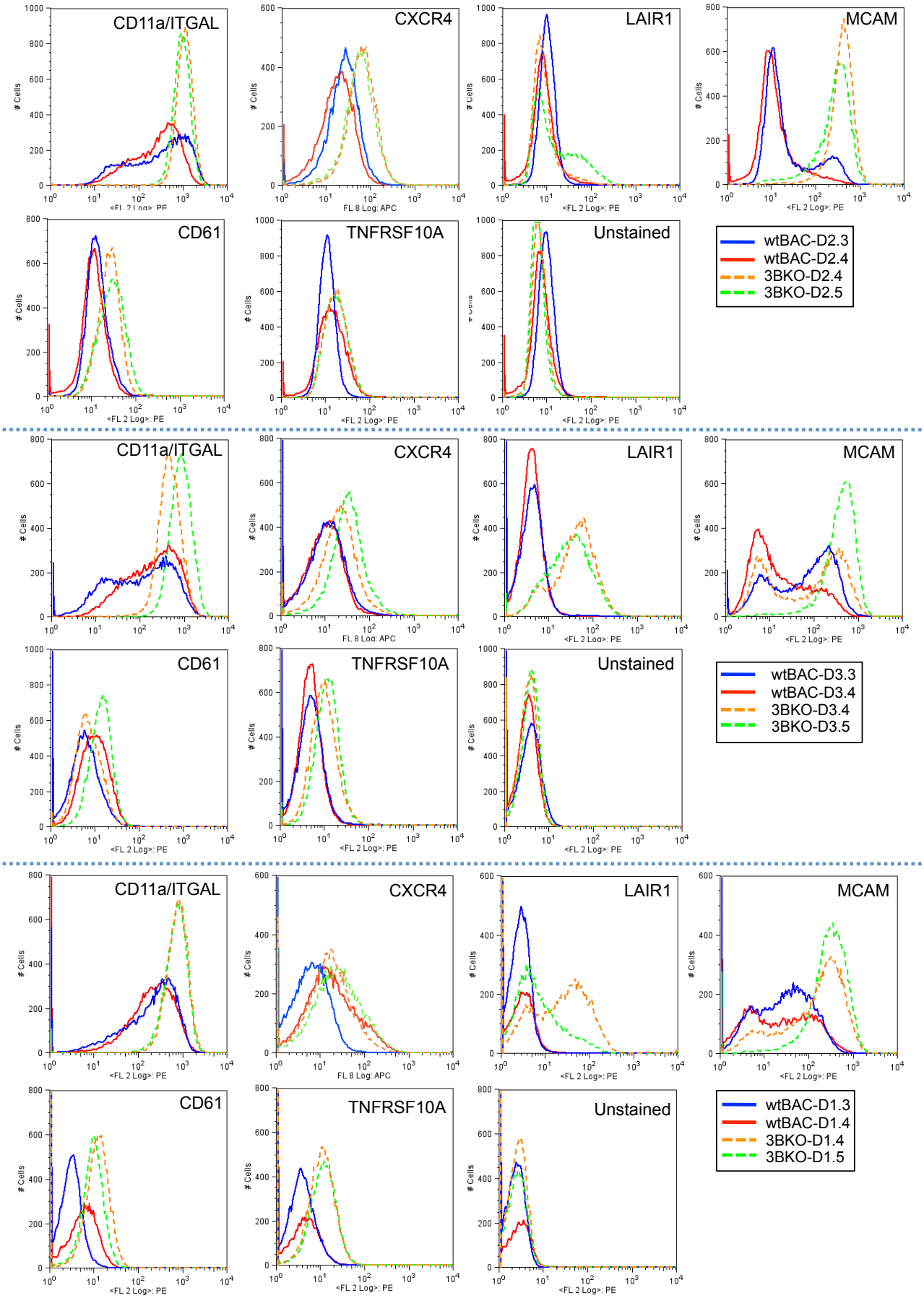

Figure S2: Altered cell surface phenotype of EBNA3BKO LCLs. FACS analysis showing cell surface expression of genes differentially regulated by EBNA3B. Histograms from two EBNA3BKO (dashed lines) and wtBAC LCLs (solid) from each of 3 independent donors are shown for the six proteins indicated. All plots display the same number of events (ranging between 25,000 and 28,000 across the three donors) permitting direct comparison of the profiles.

Figure S3

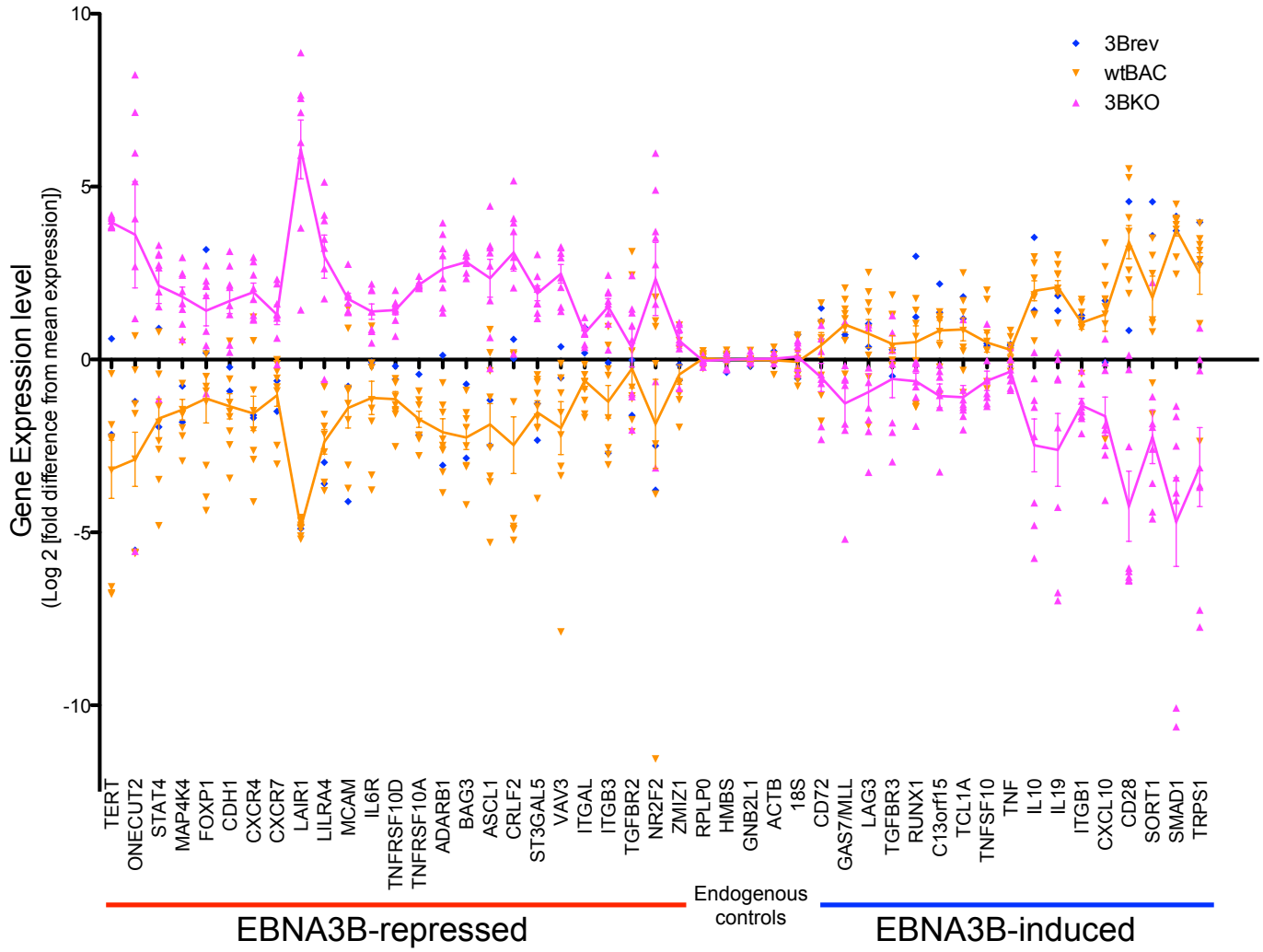

Figure S3. **Differential gene expression (qPCR) between wtBAC and EBNA3BKO-LCLs.** qPCR analysis using Taqman assays on a low-density array card for 8 pairs of LCLs in 4 independent donors. Two LCLs generated with EBNA3Brev virus are also shown. Gene expression values were generated assuming 100% efficient qPCR assays and normalized to five endogenous controls. A two-fold change as measured by microarray corresponds to an approximately 2.7-fold change by qPCR (5). To accommodate a range of transcript abundances, distance from the mean expression value, rather than absolute expression is shown for each assay. The bars indicate the mean ±SD for the 3BKO (magenta) and wtBAC/EBNA3Brev (orange) groups. The statistical significance of the difference between wtBAC and EBNA3BKO expression profiles as measured by qPCR are similar to those observed in the microarray data. Assay IDs are indicated in Supplementary Table S3.

Figure S4

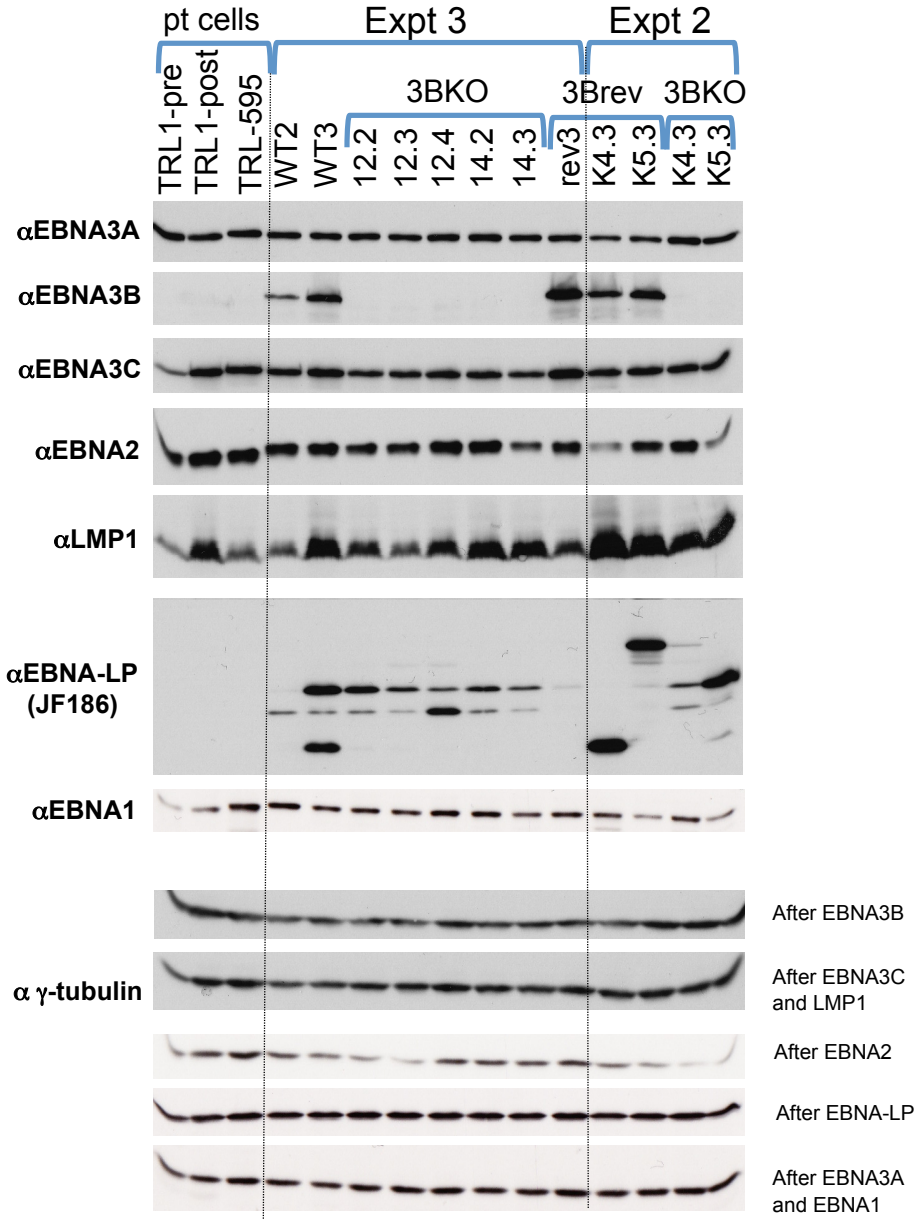

Figure S4. Western blotting analysis of ex vivo-expanded cell lines. Western blotting for EBV latency-associated antigens is shown for patient-derived and huNSG-mouse-derived cell lines. EBNA3B is absent from the EBNA3BKO-derived lines, and all the patient-derived cell lines. While some differences are apparent between TRL1-pre and -post (eg SMAD1 expression; not shown), we have been unable to detect wild-type EBNA3B by either Western blotting or PCR for wild-type sequence. Additionally, we found one mouse derived wtBAC cell line (WT2) to express a reduced level of EBNA3B. This line also expressed a more EBNA3BKO-like phenotype for some of the other genes tested (e.g. CD11a, LAIR1). Other EBV antigens are not significantly different between EBV types except EBNA-LP, which is not detectable in patient-derived LCLs. Western blotting using antibody 4D3, which detects type 1 and type 2 EBNA-LP variants (57), indicated abundant EBNA-LP expression in these cell lines (not shown). EBNA-LP is typically highly variable in both size and expression levels between cell lines (58).

Figure S5

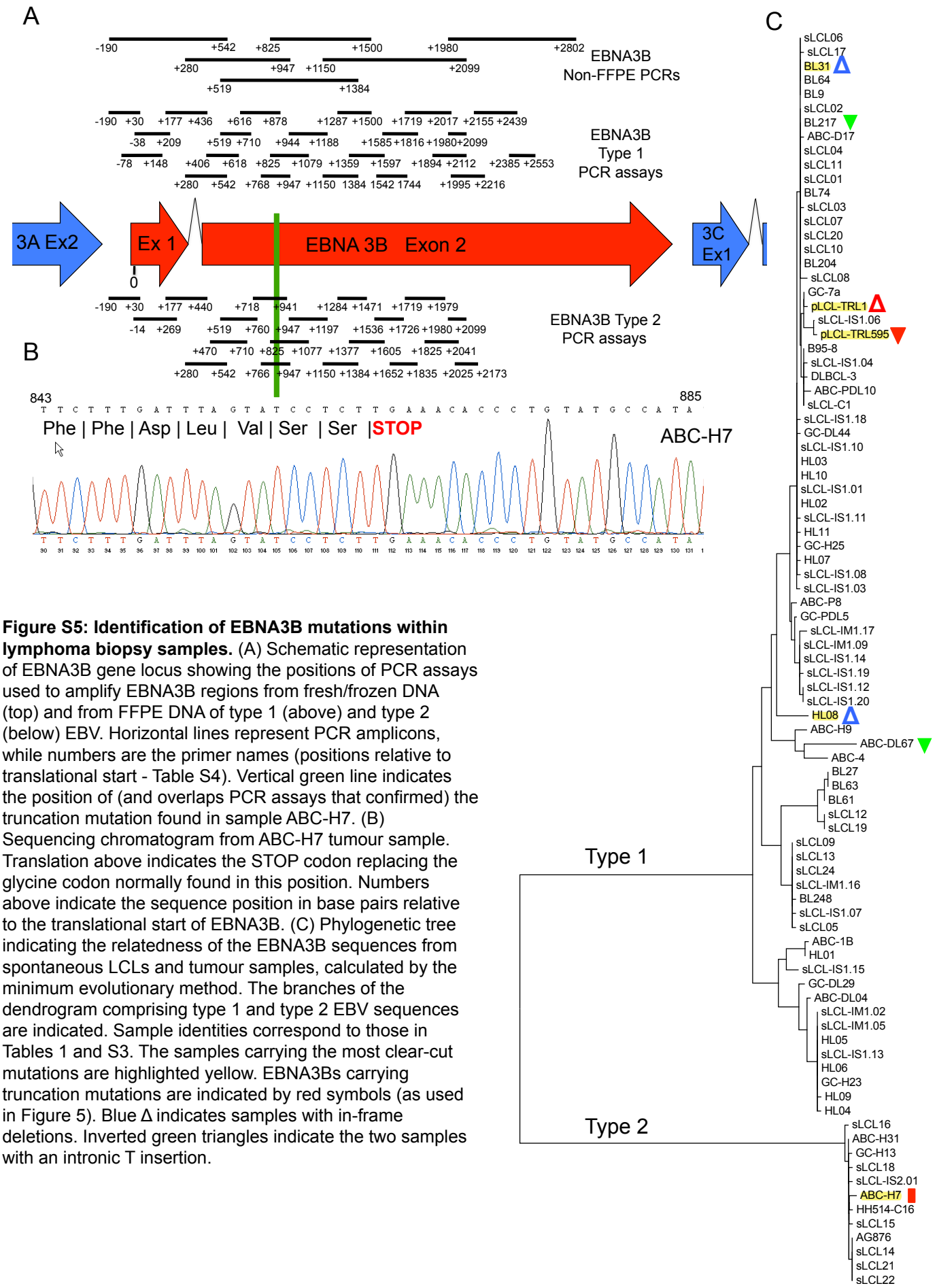

Figure S5: Identification of EBNA3B mutations within lymphoma biopsy samples. (A) Schematic representation of EBNA3B gene locus showing the positions of PCR assays used to amplify EBNA3B regions from fresh/frozen DNA (top) and from FFPE DNA of type 1 (above) and type 2 (below) EBV. Horizontal lines represent PCR amplicons, while numbers are the primer names (positions relative to translational start - Table S4). Vertical green line indicates the position of (and overlaps PCR assays that confirmed) the truncation mutation found in sample ABC-H7. (B) Sequencing chromatogram from ABC-H7 tumour sample. Translation above indicates the STOP codon replacing the glycine codon normally found in this position. Numbers above indicate the sequence position in base pairs relative to the translational start of EBNA3B. (C) Phylogenetic tree indicating the relatedness of the EBNA3B sequences from spontaneous LCLs and tumour samples, calculated by the minimum evolutionary method. The branches of the dendrogram comprising type 1 and type 2 EBV sequences are indicated. Sample identities correspond to those in Tables 1 and S3. The samples carrying the most clear-cut mutations are highlighted yellow. EBNA3Bs carrying truncation mutations are indicated by red symbols (as used in Figure 5). Blue Δ indicates samples with in-frame deletions. Inverted green triangles indicate the two samples with an intronic T insertion.

Figure S6

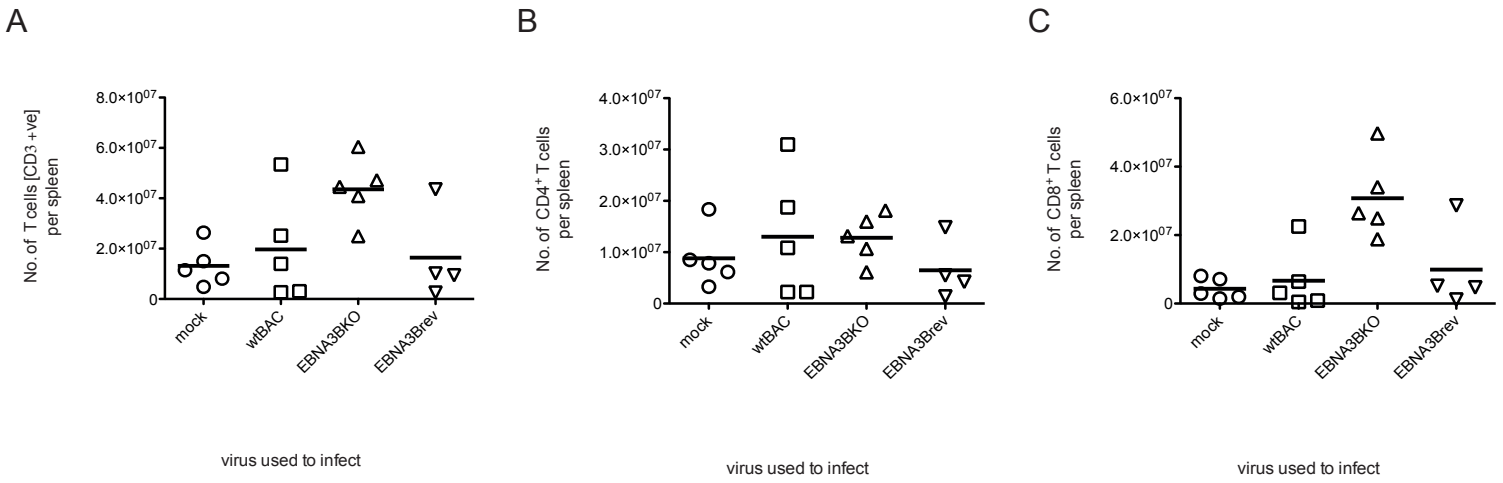

Figure S6. Stronger expansion of T cells upon infection with EBNA3BKO EBV. Spleens of infected animals were homogenized and cell numbers per spleen were determined using Trypan blue exclusion. Composition of spleen homogenates was thereafter analyzed by Flow Cytometry and absolute numbers were back-calculated. (A) Absolute number of CD3+ T cells. (B) Absolute number of CD4+ T cells per spleen. (C) Absolute number of CD8+ T cells per spleen.

Figure S7

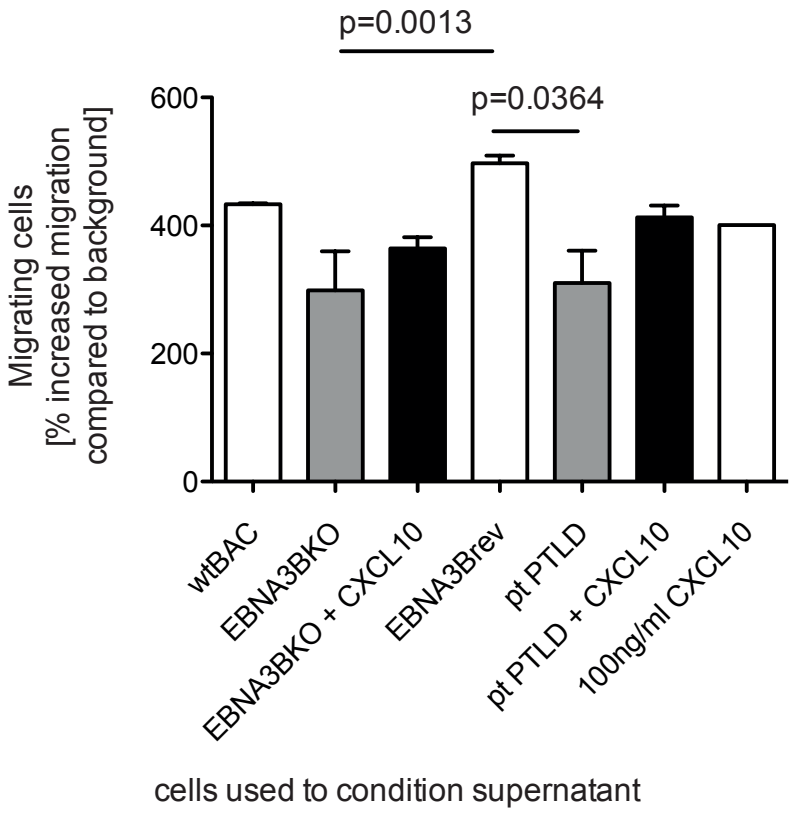

Figure S7. Migration of EBV-specific CD4+ T cells. Migration of the EBV-specific CD4+ T cell clone BC-E122 towards tumour cell conditioned supernatants was assessed by transwell migration assay. For EBNA3BKO-transformed tumour lines and patient samples (pt PTLD) CXCL10 was supplemented to reach CXCL10 concentrations present in wtBAC and EBNA3Brev conditioned supernatants of the respective experiments. One representative of two experiments with 2 wtBAC, 3 EBNA3Brev, 7 EBNA3BKO and 2 patient cell lines is shown. Plotted data represents mean + SD.

Figure S8

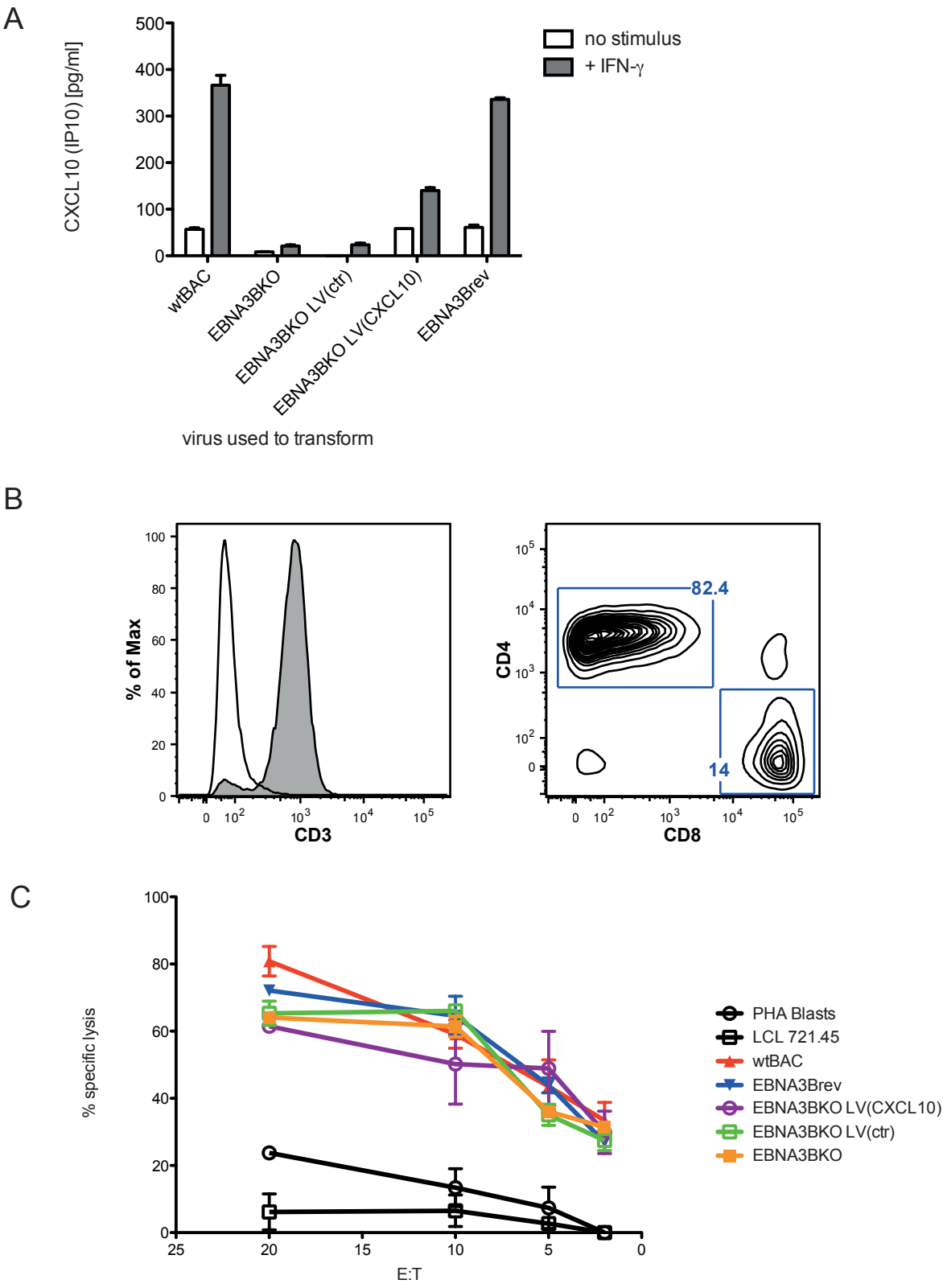

Figure S8. Composition of autologous T cell line, Re-expression of CXCL10 in EBNA3BKO LCLs and in vitro killing assay. (A) CXCL10 protein levels were determined in culture supernatants of the indicated LCLs, including EBNA3BKO expressing CXCL10 after lentiviral transduction (LV(CXCL10)) or control transduced cells (LV(ctr)), by ELISA after 24h of culture with or without the addition of 250 IU/ml IFN-γ. (B) Composition of autologous EBV specific T cell line was analyzed by FACS. The line was composed of >95% T cells (CD3 +ve) and was dominantly CD4 +ve. (C) Cytotoxicity of the autologous T cell line against LCLs. Loss of membrane integrity of CFSE labeled LCLs as a measure of cytotoxicity was assessed by To-Pro-3 iodide staining of DNA, which is blocked by intact cell membranes. Cytotoxicity was evaluated at the indicated T cells : LCLs (E:T) ratios. One representative of 2 experiments is shown. (A&C) Plotted data represents mean + SD.

Figure S9

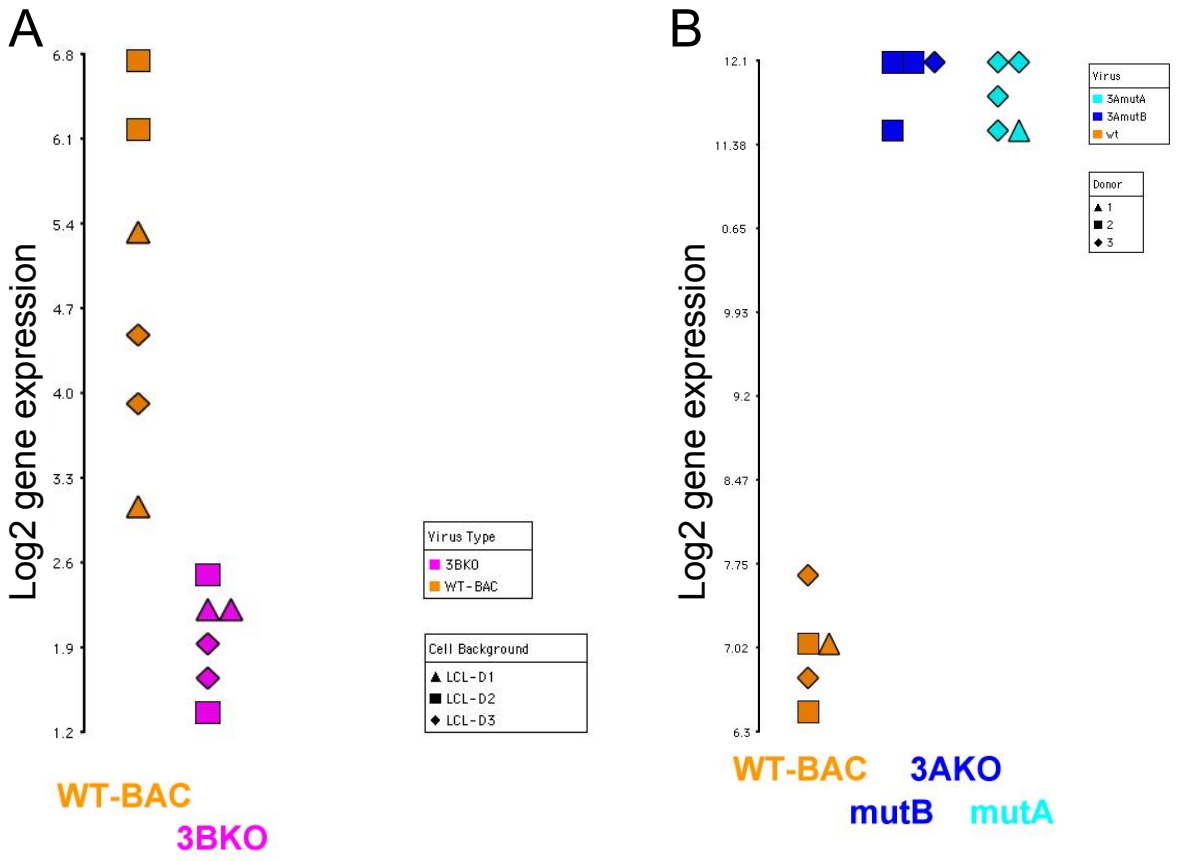

Figure S9: Regulation of CXCL10 expression in LCLs by the EBNA3 family. Graphical representations show Microarray data for CXCL10 expression (viewable at www.epstein-barrvirus.org.uk) from two independent experimental analyses looking at the effect of EBNA3 gene knockouts in the context of recombinant EBVs. Each data point represents an independent cell line, whose colour indicates EBV strain and shape represents distinct genetic donors. Vertical axes show a Log 2-converted gene expression value, where values of less than 2 indicates a transcript that is essentially undetectable, while >10 represents highly abundant transcripts. (A) Deletion of EBNA3B in an LCL background results in a loss of CXCL10 expression, as described previously. (B) In contrast, deletion of EBNA3A in LCLs (either of the whole gene – mutB – or of only the second exon of EBNA3A – mutA) causes a substantial increase in CXCL10 expression, suggesting that EBNA3A represses CXCL10 expression.

Table S2. Taqman qPCR assays used in low-density array card

Endogenous Control genes	
Gene	Assay ID
RN18S1 (18S rRNA)	Hs99999901_s1
ACTB	Hs99999903_m1
GNB2L1	Hs00272002_m1
HMBS	Hs00609297_m1
RPLP0	Hs99999902_m1
EBNA3B target genes	
Gene	Assay ID
ADARB1	Hs00953724_m1
ASCL1	Hs00269932_m1
BAG3	Hs00188713_m1
C13orf15	Hs00204129_m1
CD28	Hs00174796_m1
CD72	Hs00233564_m1
CDH1	Hs00170423_m1
CRLF2	Hs00845692_m1
CXCL10	Hs00171042_m1
CXCR4	Hs00976734_m1
CXCR7	Hs00604567_m1
FOXP1	Hs00212860_m1
GAS7	Hs00245902_m1
IL10	Hs00174086_m1
IL19	Hs00604657_m1
IL6R	Hs01075667_m1
ITGAL	Hs00158238_m1
ITGB1	Hs00236976_m1
ITGB3	Hs00173978_m1
LAG3	Hs00158563_m1
LAIR1	Hs00253790_m1
LILRA4	Hs00429272_g1
MAP4K4	Hs00377415_m1
MCAM	Hs00174838_m1
NR2F2	Hs00819630_m1
ONECUT2	Hs00191477_m1
RUNX1	Hs01021971_m1
SMAD1	Hs00195432_m1
SORT1	Hs00907094_m1
ST3GAL5	Hs00187405_m1
STAT4	Hs00231372_m1
TCL1A	Hs00951350_m1
TERT	Hs00972650_m1
TGFBR2	Hs00559660_m1
TGFBR3	Hs00234259_m1
TNF	Hs00174128_m1
TNFRSF10A	Hs00269492_m1
TNFRSF10D	Hs00174664_m1
TNFSF10	Hs00234355_m1
TRPS1	Hs00936363_m1
VAV3	Hs00196125_m1
ZMIZ1	Hs00277476_m1

Supplementary Table S3: Potential EBNA3B mutations in spontaneous LCLs.

sLCL Identifier [b]	No x EBV type	sLCL (Underlying conditions)	Source of sLCLs	Potential EBNA3B mutations / unique polymorphisms [c]		
				Missense & Nonsense [d]	Conservative [e]	Non-coding [f]
sLCL-IMy.xx	5 x 1	sLCL (IM)	Australia	V98M (IM1.17) T438K (IM1.16) A443D (IM1.16)		
sLCL-ISy.xx	14 x 1 1 x 2	sLCL (IS)	Australia	D28Y (IS1.07) I384V (IS1.10) T459M (IS1.19) V567S (IS1.04) R637Q (IS1.07)	T103 (IS2.01) P106 (IS1.08) A316 (IS1.11)	
sLCLxx	17 x 1 6 x 2	sLCL (healthy except sLCL03 and sLCL20 - BL)	Kenya	T205M (sLCL03) P553H (sLCL18) A554D (sLCL15) Q612P (sLCL05)	R348(sLCL17) P385(sLCL01)	intron1+9(C->A) (sLCL16)
sLCL-Cx [a]	13 x 1	sLCL (healthy)	China (Canton province & Hong Kong)	R437I (C6) G547D (C6)	V452(C15) R699 (C6)	ND
sLCL-NPCx [a]	13 x 1	sLCL (NPC)	China (Canton province & Hong Kong)	N197I (NPC3)		ND

[a] Sequences reported in Midgley et al (27). Only coding regions were sequenced.

[b] LCLs were grown out from the blood of healthy individuals, or those with the following underlying conditions: IM: infectious monnucleosis; IS: EBV-positive lymphomas due to transplant immunosuppression; BL: Burkitt lymphoma; NPC: nasopharyngeal carcinoma. In the nomenclature, y represents virus serotype (1 or 2) while x is an arbitrary numerical identifier.

[c] As was the case for the tumours (Table 1), this analysis covers only the region of EBNA3B from 190 bp upstream of the EBNA3B start codon to amino acid 700 of the EBNA3B coding region. Potential mutations are defined as sequence variations that occur in only one of the 41 tumour-derived or 75 LCL DNA sequences analysed in this study. Each potential mutation is followed by the identifier (in parentheses) of the cell line in which that change was observed.

[d] Potential mutations that change the amino acid sequence of the protein (missense) or introduce a premature termination codon (nonsense - shown in bold).

[e] Base changes that do not alter the protein coding sequence.

[f] Changes outside the amino acid coding region, expressed relative to the translational start (A of ATG is position 0) or within intron 1 (first base of the intron is position +1).

Supplementary table S4: Primers used for EBNA3B sequencing from FFPE Samples. Column 1 indicate whether primers were used for amplification of DNA from EBV Type1, Type 2 or both types. Coloured bold bases within the primer sequences indicate mismatches with potential targets. Mismatch column indicates the base pairing generated in the event of a mismatch, and the following two columns indicates the samples or clades in which mismatches were observed.

EBV type	Sequence Name	Sequence	Mismatch	Altered in:	Correct in:
Type1&2	3B-minus190-fwd1-1&2	CTAAGCAAAGGTGTAGAAGTGTG			
Type1	3B-minus78-fwdT1&2	ATTCCA**T**GAGAGAGACCTCGCA	T:G	ABC-1B group	All other type 1 &2
Type1	3B-minus38-fwd-T1	CACTGAAACATCTTATCTTAAAACA			
Type2	3B-minus14-fwd-T2	GTTACACCTGAAAAATGAAGAAAGC	A:A	B95-8, sLCL-IS1.20 group	Type 2 & other type 1
Type1&2	3B-0030rev-1&2	TTG**C**TGTGCTCTG**C**TGAGCCAC	C:T,C:T	ABC-1B, ABC-4	All other type 1 &2
Type1	3B-0148-rev-T1	AGAGTTCAAAGGGGCCAATCTC			
Type1&2	3B-0177-fwd-T1&2	GGGCCAGTGGAAGAGAATTTAG			
Type1	3B-0209-revT1-1&2	AGGTTCCTCTTCCTCTCTTG			
Type2	3B-0269-rev-T2	CTTCCAAGGGATCATCACCACC			
Type1&2	3B-0280-Fwd-T1&2	CAGCCTAGATTTGTGGATGTG			
Type1	3B-0406-fwd-T1	GCCAGTCTTAATTGATTGTCATTG			
Type1	3B-0436-rev-T1	TGCTGAAACCAATGACAATCAA			
Type2	3B-0440-rev-T2	CGATTGCTGAAACCAATGACAATA			
Type2	3B-0470-fwd-T2	TCCTTGAGCAAAACCTGAACAT			
Type1	3B-0519-fwd-1&2	CGTCACAGATGTCAGGCCATCAG			
Type1&2	3B-0542-rev-1&2	CTGATGGCCTGACATCTGTGACG			
Type1	3B-0616-fwd-T1	TACCGCACGCATAACCTCAAAG			
Type1	3B-0618-rev-T1	GTAACCCATACGCCAGGATCTG			
Type2	3B-0711-rev-T2	CCCTCTTCACATCCTAGCGTAG			
Type1	3B-0710-rev-T1	GCCCTCATCACATCCTAG**C**G	C:A	ABC-H9, ABC-4 Chinese subgroup	All other type 1
Type2	3B-0718-fwd-T2	ATGCAATAACTTACAGTGCTGG			
Type2	3B-0760-rev-T2	ATTCGTGGTAGCTGTACAATGC			
Type2	3B-0766-fwd-T2	ACCAAAACCAAAAGATAGAAACAG			
Type1	3B-0768-fwd-T1	CCAAAACCAAAAGATCGAAACAG			
Type1&2	3B-0825-fwd-T1&2	GCAGAAAGATATACTTTGTTCTTTG			
Type1	3B-0878-rev-T1	CCCAGCCCAATCCATATAGCAT			
Type2	3B-0941-rev-T2	TAGCCA**T**CCTACAAACTCGACA	T:G	ABC-H7	All other type 2
Type1	3B-944-fwd-T1	GCAAGAAGGACCACACTCATATAC			
Type1&2	3B-0947-rev-T1&2	CGTATATGAGTGTGGTCCTTCT			
Type2	3B-0947-fwd-T2	AGAAGGACCACACTCATATACG			
Type2	3B-1077-rev-T2	GGTCAGTCTCCTCACTTGTTAA			
Type1	3B-1079-rev-T1	CAGATCAATCTCCTC**G**T**T**TGTT	G:T,T:G	sLCL-IM1.02 group, ABC-4 group	Other type 1, All other type 1
Type1&2	3B-1150_rev-T1&2	CCTCACTTTC**G**TCGTCATCCTC	G:T	Type1	Type2
Type1&2	3B-1150-fwd1&2	GTTGAGGATGACGA**T**GAAAGTGAGG	T:G	Type2, ABC-4 group	Other type 1
Type1	3B-1188-rev-T1	TCCACTCTTGTCTTCCTCCTC**G**			
Type2	3B-1197-rev-T2	CTTCATCCTTATCACATTTATCTTCA			
Type2	3B-1284-fwd-T2	CGAAAGCCAGATACTAAATCAACC			
Type1	3B-1287-fwd-T1	AAGTCAGATGCAAAATCAACCA			
Type1	3B-1359-fwd-T1	GAGGAACACAGAAAGAAGAAGG			
Type2	3B-1377-fwd-T2	AAGACAGCCAGAACAGAGCAAC			
Type1&2	3B-1384-rev-T1&2	GCTG**T**CTTCTTCTTTCTGTGTT	T:G	Type1	Type2
Type2	3B-1471-rev-T2	GGGCCAGTCACCTTTTGCGTGG			
Type1	3B-1500-rev1&2	CATGGCTCCAGC**T**GAGCCTGGACAC	T:G	Type1	Type2
Type2	3B-1536-fwd-T2	ACAAGAGTTATACTTCACGGACCA			
Type1	3B-1542-fwd-T1	GTTCTACTTCACGAAGAATCCAT			
Type1	3B-1585-fwd-T1	GTTCGATGCTAGACCTTCTTGA			
Type1	3B-1597-rev-T1	TCTAGCATCGAACCATGTACTT			
Type2	3B-1605-rev-T2	CAAGAAGGTCTAGCATGGAACC			
Type2	3B-1652-fwd-T2	GCCACCAGAACCACACCAGCCC			
Type1	3B-1719-fwd-T1	ATAGAGAGTGATGAGC**C**CGCTT	C:A	ABC-4 Chinese group	All other type 1
Type2	3B-1719-fwd-T2	ATAGAAAGTGATGAGCCCGCCA			
Type2	3B-1726-rev-T2&1	GGGCTCATCACT**T**TCTATG**T**CTAG	T:Gx2	midTonly:sLCL09 group; bothT-all other Type 1	Type 2
Type1	3B-1744-rev-T1	GTC**G**AAGCGGGCTCATCACTCT	G:T	sLCL09 and sLCL-IS1.20 groups	All other type 1
Type1	3B-1816-rev-T1	GGGGA**T**GTTAGTGGTTGGATTT	G:T	All other type 1	sLCL-IM1.02 group
Type2	3B-1825-fwd-T2	CTCAACTCCGCAGTTCAGCACC			
Type2	3B-1835-rev-T2	GCGGAGTTGAGACGTGGTGGGG			
Type1	3B-1894-fwd-T1	CAGATGACCCAA**C**GAAACAGTC	C:A	ABC-4 Chinese group	
Type2	3B-1979-rev-T2	GTGCAGAGGGATAGGTCGCAGT			
Type1&2	3B-1980-fwd1&2	CCCTTGCGGATGCAGCCAAT			
Type1	3B-1995-fwd-T1	**C**CAATCCCATTTAATCA**T**CCAGT	C:T, T:G	sLCL09 group, ABC-H9, ABC-4	All other type 1
Type1	3B-2017-rev-T1	ACTGG**A**TGATTAAATGGGATTG	A:C	ABC-4 group, ABC-H9	All other type 1
Type2	3B-2025-fwd-T2	CACTCCCCATCAGCCACCTCAG			
Type2	3B-2041-rev-T2	GGTGGCTGATGGGGAGTGGGTC			
Type1&2	3B-2099-rev1&2	GGCTGATATGGAATGTGCCC			
Type1	3B-2112-rev-T1	TGGTGT**A**GGCTGATATGGAATG	A:C	Other type 1	B95-8 group
Type1	3B-2155-fwd-T1	CCATGCAG**A**CACCACCGAGAGC	A:C	ABC-4 Chinese group	All other type 1
Type2	3B-2173-rev-T2	CT**T**GGTGGTGGCTGCATGGTGG	T:G	HH514-C16, sLCL-IS2.01	AG876
Type1	3B-2216-rev-T1	GGGAACGGG**T**G**G**GCACCTCAGGT	G:T;T:G	G:T - sLCL09 & ABC-4 groups; Both: sLCL-IM1.02 group	Other T1
Type1	3B-2385-fwd-T1	CAAATTTTGCGCCAATTGTTAAC			
Type1	3B-2439-rev-T1	TAAGTGATGGTCTCCCTTTCTT			
Type1	3B-2553-rev-T1	GCTGCAAAACGGGTGGGTAGAA			
Type1&2	3B-2802-rev-T1&2	CGCACTCCAGAGTCTGCTGTTG			