



Supplementary Information for

EBNA2-EBF1 complexes promote MYC expression and metabolic processes driving S-phase progression of Epstein-Barr virus-infected B cells

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Materials

Table S 1 General cell lines

Cell line	Description
GM12878 Cas9	human ENCODE tier I lymphoblastoid cell line with stable Cas9 expression (Jiang <i>et al.</i> , 2018)
Raji	human EBV positive Burkitt's lymphoma cell line
HEK293	human epithelial embryonic kidney cell line, (Graham <i>et al.</i> , 1977)
DG75	human EBV negative Burkitt's lymphoma cell line (Ben-bassats <i>et al.</i> , 1977)
LL8	murine fibroblasts stably transfected with human CD40L (Wiesner <i>et al.</i> , 2008a)

Table S 2 Lymphoblastoid cells lines established from donors

Cell line	Donor	Virus
SB268 LCLwt	1	XZ143 EBV (6008) HA-EBNA2
SB301 LCLΔα1		SB161 EBV (6008) HA-EBNA2Δα1
SB346.1 LCLwt	6	XZ143 EBV (6008) HA-EBNA2
SB346.2 LCLΔα1		SB161 EBV (6008) HA-EBNA2Δα1
CKR654.1 LCLwt	8	XZ143 EBV (6008) HA-EBNA2
CKR654.3 LCLΔα1		SB161 EBV (6008) HA-EBNA2Δα1

Table S 3 Conditional cell lines

Cell line	Internal designation	Description
LCLwt_doxLMP1	CKR700.1	LCL generated from adenoid biopsy, infected with EBVwt, stably transfected with pRTS-HA-LMP1
LCLΔα1_doxLMP1	CKR724.1	LCL generated from adenoid biopsy, infected with EBVΔα1, stably transfected with pRTS-HA-LMP1

Table S 4 Plasmids

Plasmid	Internal designation	Application
pUC57-EBNA2Δα1_maxiEBV	pSB147	EBV mutagenesis
pcDNA3+-EBF1 myc his	pSB72	transient transfection for Co-IP assays
pET28-END	AZG1_GST-END	GST affinity capture assay
pET28-ENDΔ-H15A	AZG43_GST-END-H15A	GST affinity capture assay
pET28-ENDΔα1	AZGII_GST-ENDΔα1	GST affinity capture assay
pRTR-HA-wtEBNA2	pCKR74.2	stable doxycycline inducible cell line DG75
pRTR-HA-EBNA2ΔEND(Δaa 3-52)	pCKR492	stable doxycycline inducible cell line DG75
p6012.new	-	plasmid for rpsL/Kan cassette PCR
p509 BZLF1	-	virus production in HEK293 cells
p2670 BALF4	-	virus production in HEK293 cells
pGEX-4T2	-	GST only expression plasmid, GST affinity capture assay
pRTS-HA-LMP1	pCKR682.3	stable doxycycline inducible LCLwt/LCLΔα1
pCDNA3(+)	-	negative control, transient transfection for Co-IP assays
pcDNA3(+)-EBF1 myc his	pSB72	transient transfection for Co-IP assays

Table S 5 EBV strains

EBV strains	internal designation	Source
r_wt/B95.8 (6008)	wt/B95.8 (2089) deletion restored containing all 44 miRNA	Wolfgang Hammerschmidt (Cambon <i>et al.</i> , 2019)
EBVwt	XZ143: 6008 with HA-tagged EBNA2	our laboratory (Zhang <i>et al.</i> , 2021)
EBVΔα1	SB161: XZ143 with α1 deletion in EBNA2	this paper

Table S 6 qPCR primer for cDNA quantification

Gene	Internal designation	Sequence	Annealing T [°C]	Product [bp]
myc	SAR40.3 fwd	CTCTCAACGACAGCAGCTC	63	210
	SAR40.3 rev	CCACAGAAACAACATCGATTTC		
EBF1	SAR77.1 fwd	AGACCAGCATGGTACCGAAT	63	235
	SAR77.1 rev	ACGACAGTCAATGTGGATGG		
CBF1	SAR94.14 fwd	AGTTACTGGCATGGCACTCC	63	234
	SAR94.14 rev	ATGATTGTCCAGGAAGCGCC		
EBNA2	LG521 fwd	TCTGCTATGCGAATGCTTTG	63	255
	LG521 rev	CACCGTTAGTGTTCAGGTG		
LMP2A	SB444 fwd	CTTGGGATTGCAACACGACG	63	171
	SB443 rev	GAAACACGAGGCGGCAATAG		
LMP1	SB422 fwd	GGAAGAAGGCTAGGAAGAAGG	63	155
	SB422 rev	CTGGAATTTGCACGGACAGG		
RNA pol II	SB397 fwd	GTCTGTGACGGAGGGTGG	63	218
	SB397 rev	AAACTTTCATTGTCTTCACCAGG		

Table S 7 qPCR primers for ChIP quantification

Target		Sequence	Annealing T [°C]	Source
CD2 locus	SB396	GTAAAGAGAGGCACGTGGTTAAG	63	
	SB396	GAACTGAAGTGAGACTGGTGGAG		
CD79a	SB394	TTCCAAAGGCTCAGCAGTGA	63	www.chippri mers.com
	SB394	GGCCACAATTCCCCTGAGA		
Cp	SB362	GGCGGGAGAAGGAATAACG	63	(Lu <i>et al.</i> , 2016)
	SB364	CTTGAGCTCTCTTATTGGCTATAATC		
ESE1 myc	SB429	AGTTCCTTCCCACACCCAC	63	
	SB429	GCTTACGTTGCTCTGAGATTTAG		
ESE2 myc	SB430	TGAGGAGTGGTGAGTCAGC	63	
	SB430	CCCAGGTGTATCTTAGAACAGG		
HES1	SB432	TGGCAGGGCAGCTACCA	63	(Lu <i>et al.</i> , 2016)
	SB432	GACTGACTTTCTCACACTCAGATTCC		
LMP1	SB361	ACGTCAGAGTAACGCGTGTTC	63	(Lu <i>et al.</i> , 2016)
	SB361	GCAGACCCCGCAAATCC		
LMP2A	SB360	GCGCCGCGGTTTCAG	63	(Lu <i>et al.</i> , 2016)
	SB360	TTACGCCCCAGCAAGCTT		

Table S 8 Critical commercial assays

Product	Source	Identifier
BrdU-APC Flow Kit	BD Pharmingen	cat. no. 552598
ECL	GE Healthcare (Amersham)	cat. no. RPN2109

NucleoSpin™ Gel and PCR Clean-up	Macherey & Nagel	cat. no. 740609.50
LightCycler 480 SYBR Green I Master	Roche Diagnostics	cat. no. 04 887 352 001
QIAshredder	Qiagen, Germany	cat. no. 79654
RNase-free DNase Set	Qiagen, Germany	cat. no. 79254
RNeasy Mini Kit	Qiagen, Germany	cat. no. 74104
SuperScript IV First-Strand cDNA Synthesis Reaction	ThermoFischer	cat. no. 18091150
Phusion®High-Fidelity Polymerase	New England Biolabs	cat. no. M0530
NucleoBond™ Xtra BAC Maxi kit	Macherey-Nagel	cat. no. 740414

Table S 9 Antibodies

Specificity	Host	Application	Supplier
α-CBF1 (Rbj7A11)	rat, IgG2b	WB	HMGU Monoclonal Antibody Core Facility
α-EBF1 (C-8)	mouse, IgG2a	WB, ChIP	Santa Cruz, cat# sc-137065
α-EBF1	rabbit,	ChIP	Sigma Aldrich, cat# ABE1294
α-EBF1 (EPR4183)	rabbit	WB Fig S1	Abcam
α-EBNA2 (R3)	rat, IgG2a	WB, IP, ChIP	HMGU Monoclonal Antibody Core Facility
α-EBNA2 (1E6)	rat, IgG2a	ChIP	HMGU Monoclonal Antibody Core Facility
α-EBNA3A (4A5-1111)	rat,	WB	HMGU Monoclonal Antibody Core Facility
α-EBNA3B (AN 6C9-1-1)	rat,	WB	HMGU Monoclonal Antibody Core Facility
α-EBNA3C (A10 P2-583)	rat,	WB	HMGU Monoclonal Antibody Core Facility
α- GST (6G9)	rat, IgG2a	WB, affinity capture, ChIP isotype control	HMGU Monoclonal Antibody Core Facility
α-Dog CD3	rat, IgG1	ChIP	HMGU Monoclonal Antibody Core Facility
α-HA (3F10)	rat, IgG1	WB, ChIP	HMGU Monoclonal Antibody Core Facility
α-GAPDH (0411)	mouse, IgG1	WB	Santa Cruz, cat# sc-47724
α-LMP1 (1G6)	rat,	WB	HMGU Monoclonal Antibody Core Facility
α-myc (9E10)	mouse,	WB	HMGU Monoclonal Antibody Core Facility
α-PU.1 (2266S)	rabbit	WB	Cell signaling Technologies
α-IgD-FITC	mouse, IgG2a	FACS sorting	BD Biosciences, USA
α-CD38-PE-Cy7	mose, IgG1κ	FACS sorting	eBioscience, cat #25-0389-42
rabbit IgG		ChIP	Merck cat# PP64

Methods

Cell lines and routine cell culture conditions

All cells were cultured at 37 °C, 6 % CO₂. DG75 (Ben-bassats *et al.*, 1977) and irradiated LL8 (Wiesner *et al.*, 2008b) cells were cultured in RPMI 1640 supplemented with 10 % FCS, 4 mM L-Glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin. LCLwt_doxLMP1 and LCLΔα1_doxLMP1 were cultured with additional 40 µg/ml hygromycin.

Raji (Pulvertaft, 1964), HEK293 (Graham *et al.*, 1977), primary B cells infected with EBVwt or EBVΔα1 and lymphoblastoid cell lines generated from EBVwt (LCLwt) or EBVΔα1 infection (LCLΔα1) were cultured in RPMI 1640 medium supplemented with 10 % FCS, 100 U/ml penicillin, 100 µg/ml streptomycin, 1 mM sodium pyruvate, 100 nM sodium selenite, and 0.43 % α-thioglycerols in 20 µM BCS. Primary B cells and LCLΔα1 were cultured on LL8 cells in LCL specific medium.

Irradiation of CD40L expressing LL8 feeder cells

A. Moosmann (Helmholtz Center Munich) provided irradiated CD40L expressing LL8 feeder cells. In brief, trypsinized cells were washed and resuspended in medium and irradiated with 180 Gy (γ-rays). 10⁶ cells were seeded per one full plate (96, 48, 24, 12, 6-well). 1.5x10⁶ cells were seeded for a T75 cell culture flask. LL8 cells were ready to use the next day.

Isolation of B cells from human adenoid tissue

To isolate primary B cells, adenoids were rinsed in medium and carefully pushed through a 100 µm cell strainer using a 10 ml syringe plunger. Depending on the adenoid size, the cell suspension was filled up to 25 ml or 50 ml with medium (RPMI 1640, 10% FCS, 100 U/ml penicillin, 100µg/ml streptomycin, 4 mM L-Glutamine). T cell rosettes were formed by adding 0.5 ml of sheep blood per 25 ml of cell suspension. 25 ml of the cell suspension with sheep blood was carefully layered onto 20 ml of Ficoll and centrifuged at 666 g, 40 min, at 10 °C, decelerated without breaks. The interface was transferred to a new 50 ml Falcon and washed three times with 40 - 50 ml PBS/2 mM EDTA at (i) 666 g (ii) 527 g (iii) 403 g, 10 min each, at 10 °C. Erythrocytes were lysed by resuspending the cell pellet in 5 ml red blood cell lysis buffer (154 mM NH₄Cl; 9.98 mM KHCO₃; 0.127 mM EDTA, pH8) followed by centrifugation for five minutes at 403 g, 10 °C. Cells were resuspended in LCL specific medium.

Primary B cell infection and generation of LCL

10⁶ primary B cells were infected with an MOI 0.1 with EBVwt or EBVΔα1 supernatant and cultured in LCL medium. For the first one to two weeks, 0.5 µg/ml Cyclosporin A and 10 µg/ml Ciprofloxacin was added. To enhance the growth after one week of infection, B cells infected with EBVΔα1 were constantly cultured on irradiated LL8 cells. B cells infected with wild type EBV were kept without LL8 cells. Infected B cells were expanded to generate LCLs and frozen for long-term storage. Infected B cells for RNA sequencing were not cultured with LL8 cells.

Sorting of B cells to obtain subpopulations

10⁸ isolated, primary B cells were washed once with FACS buffer (PBS, 2% FCS, 2 mM EDTA) and resuspended in 1 ml FACS buffer. The cells were stained with 80 µl α-IgD-FITC (BD Pharmingen, #555778) and 24 µl α-CD38-PE-Cy7 (eBioscience, #25-0389-42) for one hour in the dark at 4 °C and

washed twice with FACS buffer. The stained cells were resuspended in 6 ml FACS buffer and filtered through a 35 µm filter to obtain a single cell suspension. Sorting was performed on a FACS Aria IIIu device using the 70 µm nozzle, the “Purity” sorting mask and a sorting velocity of around 7000 events/second. The gating strategy included (i) lymphocytes (ii) single cells (iii) IgD⁺ CD38⁻. Sorted naïve resting B cells were used for infection experiments. To sort viable cells post-infection, the infected B cells were washed, resuspended in FACS buffer and filtered through a 35 µm strainer. Sorting was performed using a 100 µm nozzle, a velocity of around 7000 events/second with the “Purity” sorting mask. The gating was based on the forward scatter (FSC) and side scatter (SSC).

Cell cycle analysis with BrdU

Isolated, primary B cells were infected with an MOI 0.1 with EBVwt or EBVΔα1 and seeded with a density of 10⁶ cells/ml to analyze them on day 0, two, four, six and eight post-infection. Prior to cell harvest, the infected cells were pulsed with a final concentration of 10 µM BrdU for one hour at 37 °C. Afterwards, the cells were harvested, washed with 1 ml of FACS staining buffer (PBS, 2 % FCS, 2 mM EDTA) and centrifuged at 500 g for 5 min. The cells were fixed and permeabilized with 100 µl of BD Cytofix/Cytoperm for 25 min at RT and washed with 1 ml FACS buffer. The samples were stored in freezing medium (90% FCS, 10 % DMSO) at – 80°C until samples from all time points were collected. On the day of FACS analysis, freshly thawed samples were washed in FACS buffer, re-fixed with 100 µl BD Cytofix/Cytoperm for 10 min at RT and washed with 1 ml 1X BD Wash/Perm Buffer. Cell pellets were resuspended in 100 µl DNase (300 µg/ml in PBS final concentration) and incubated for one hour at 37 °C, 6% CO₂. Afterwards, the cells were washed in 1 ml 1X BD Perm/Wash Buffer. Incorporated BrdU was stained with 1 µl α-BrdU-APC in 50 µl BD Perm/Wash buffer for 20 min in the dark at RT. After washing with 1 ml 1X BD Perm/Wash buffer, cells were resuspended in 20 µl 7-AAD solution, incubated for five minutes and filled up to 200 to 300 µl with FACS staining buffer. The analysis was done with FACS Fortessa.

Cell cycle analysis with PI

Isolated, primary B cells were infected with an MOI 0.1 with wild type EBV or EBVΔα1 and seeded with a density of 10⁶ cells/ml on plates either containing LL8 feeder cells or on plates without LL8 feeder cells. Cells were harvested on day 0, two, four, six and eight post-infection in preparation of the PI staining. 10⁶ cells were harvested and washed once in staining buffer (PBS, 2 % FCS). The cell pellet was fixed by adding 1 ml of ice-cold 70 % ethanol dropwise and stored at -20 °C until all samples from all time points were collected. On the day of cell cycle analysis, the fixed cell pellets were washed once in staining buffer. The cells were resuspended in 500 µl PI solution (10 µg/ml PI, 175 µg/ml RNaseA in PBS) and analyzed by flow cytometry.

Cell viability analysis with MTT

Isolated, primary B cells were infected with an MOI 0.1 with EBVwt or EBVΔα1. Non-infected B cells were included as a negative control. For each time point and condition, 8 replicates were prepared on a 96-well plate. 10⁵ cells/well were seeded in 100 µl medium. The MTT assays were performed on day 0, two, four, six and eight post-infection. 10 µl of MTT (5 mg/ml in PBS) was added to each well and incubated for four hours at 37 °C. Plates with MTT were stored at – 20 °C until measurement. On the day of analysis, formazan crystals were dissolved with 200 µl/well of 1 N HCl in isopropanol and the

absorption was measured at 550 nm (measurement wavelength) and 690 nm (reference wavelength). The background signal from medium without cells with MTT was subtracted from the sample values.

Virus production

In brief, HEK293 producer cells were seeded on dishes with a density of around 40 % one day before transfection. The medium was replaced with 25 ml puromycin-free medium just before the transfection. Component A (12 µg 1:1 BZLF1:BLAF4 expression plasmids, 1.2 ml RPMI 1640) and component B (72 µl PEI (1 mg/ml), 1.2 ml RPMI 1640) of the transfection solution were mixed and incubated for 15 min at RT. The solution was added dropwise to the cells and incubated for 3 days. The supernatant was harvested, centrifuged at 1200 rpm for 10 min and filtered through a sterile 1.2 µm filter. The virus titer of the supernatant was measured using Raji cells.

Virus titer calculation

Since the recombinant virus encodes for a GFP protein, Raji cell infections were used to calculate the titer of all virus supernatants. 10⁵ Raji cells were infected with 1, 2, 5, 10 and 20 µl in a 24-well plate and incubated for three days. The frequency of GFP positive Raji cells was analyzed by flow cytometry on a FACS Canto II device. The percentages of GFP positive Raji cells was plotted against the administered volumes of the supernatant. The formula of a linear regression curve was calculated for the linear part of the graph using GraphPad Prism. The titer defined as “Green Raji Units/ml” was calculated as follows with **a** and **b** being given by the linear regression formula.

$$\text{titer} \left[\frac{\text{GRU}}{\text{ml}} \right] = \frac{(a \times 1000 \mu\text{l} + b)}{100} \times 10^5$$

EBV mutagenesis

For EBV mutagenesis, we used 6008 EBV-BAC DNA which is based on B95.8 and contains all 44 miRNA (Cambon *et al.*, 2019). 6008 was provided by W. Hammerschmidt and used to introduce a C-terminal HA-tag in EBNA2 resulting in XZ143-EBV_HA-EBNA2 (EBVwt) (Zhang *et al.*, 2021). This EBV genome (XZ143) was then used to delete the α1-helix in EBNA2 resulting in SB161- EBV_HA-EBNA2Δα1 (EBVΔα1). Recombinant EBV strains used in this study were generated by a two-step selection protocol using the λ-prophage-based heat inducible Red recombination system expressed in E.coli strain SW105 (Warming *et al.*, 2005; Wang *et al.*, 2009; Cambon *et al.*, 2019). Preparation of recombination-competent, electrocompetent SW105 bacteria and the electroporation of those was based on a protocol published on https://labnodes.vanderbilt.edu/resource/view/id/3165/collection_id/32/community_id/8.

In the first step, a rpsL/Kan expression cassette was amplified from p6012 template by PCR. 50 nt flanking regions homologous to the respective EBNA2 region were included. The resulting PCR product with the rpsL/Kan cassette was electroporated into SW105 carrying 6008 EBV-BAC and inserted into the specific EBNA2 region by homologous recombination. SW105 with successful recombination were selected with kanamycin (30µg/ml). As a second step, a synthetic DNA fragment carrying the desired mutation flanked by 300nt of the genomic viral sequence was used to replace the rpsL/Kan cassette by homologous recombination to generate the final mutant EBV plasmid. SW105 with successful recombination were selected with streptomycin (1mg/ml) and chloramphenicol (12.5 µg/ml).

EBV-BAC purification from bacterial cultures

Supercoiled EBV-BAC DNA was purified by CsCl₂-EtBr density gradient centrifugation. 400 ml LB medium containing 15 µg/ml chloramphenicol were inoculated with a fresh single colony of SW105 bacteria carrying EBV-BAC of interest and cultivated at 32 °C shaking overnight. The bacteria were pelleted at 4000 rpm, 30 min, 4 °C. The BAC DNA was extracted using the NucleoBond™ Xtra BAC kit (Machery-Nagel) and according to the manufacturer's instructions. The DNA was dissolved in 400 µl TE buffer overnight at 4 °C. The next day, 1.6 g CsCl₂ was added to the solution, carefully dissolved and transferred to a 11.5 ml Ultracrimp tube (Sorvall). The tube was filled up with 1.55 g/ml CsCl₂ solution and 200 µl 1% EtBr. The sealed tube was ultracentrifuged at 35,000 rpm, 20 °C for 3 days. The lower band containing supercoiled DNA was extracted with a large gauge veterinary needle and transferred to 15 ml Falcon. EtBr was removed by isobutanol solvent extraction followed by a dialysis in 2 l TE at 4 °C overnight. The DNA concentration was determined by Qubit fluorometric quantitation.

Whole cell protein lysate

10⁷ cells were harvested, washed once with PBS and lysed with 200 µl NP-40 lysis buffer (1 % NP-40, 150 mM NaCl, 10 mM Tris-HCl pH7.4, EDTA pH8, 3 % Glycerol, 1x proteinase inhibitor cocktail (PIC, Roche), 1x PhosStop (Sigma-Aldrich)). The mix was incubated at 4 °C for one hour. Cell debris was pelleted at 16000 g, 4 °C for 15 min. Cleared lysate was transferred to a new tube. Protein lysates were stored at - 80 °C.

Purification of GST-tagged proteins

The purification of GST-tagged proteins was performed as previously described (Glaser *et al.*, 2017). *E.coli* strain BL21 was transformed with the expression plasmids pET28_GST-END, pET28_GST-ENDΔα1, pET28_GST-END-H15A or pGEX-4T2 (GST only). Bacteria were cultured in 400 ml of LB medium with antibiotics at 37°C until an OD of 0.5 – 0.7 was reached. Due to a smaller yield, 800 ml LB culture were prepared for the expression of GST-ENDΔα1. The protein expression was induced with 1 mM IPTG for three hours at 30°C, shaking. After induction, bacteria were harvested at 4000 g, 20 min, 4 °C and resuspended in 20 ml ice cold binding buffer (25 mM HEPES, pH 7.6, 0.1 mM EDTA, pH 8, 12.5 mM MgCl₂, 10% Glycerol, 0.1% NP-40, 100 mM KCl, 1 mM PMSF, 1 mM DTT). Bacteria were lysed by sonication (Brandson Digital Sonifier® 250, 10x 10 sec on/1 min off, 10 % amplitude). Lysates were cleared by centrifugation at 48,000 g, 4°C for 20 min. Glutathione Sepharose 4B beads (GE Healthcare) were washed and resuspended in binding buffer to prepare a 50% slurry. To coat the beads with GST or GST-tagged proteins, 100 µl of the 50% slurry were incubated with 20 ml of cleared lysates for one hour, 4°C, and washed three times with 20 ml binding buffer.

Affinity capture assay with recombinant GST and cell lysate

This assay was performed as previously described (Glaser *et al.*, 2017). 10⁷ DG75 cells were transfected with 10 µg EBF1 expression plasmid or empty vector controls. 24 h after transfection, cells were harvested and lysed in 500 µl NP-40 lysis buffer (50 mM HEPES, pH 7.6, 5 mM EDTA, pH 8, 150 mM NaCl, 0.1% NP-40, 1 mM PMSF) followed by sonication. Cell lysates were centrifuged for 15 min at 16,000 g, 4°C, and the protein concentration was measured by Bradford assay. To pull down EBF1, the supernatants were incubated with the GST or GST fusion protein coated beads for three hours at 4°C.

Subsequently, beads were washed 5x with binding buffer (25 mM HEPES, pH 7.6, 0.1 mM EDTA, pH 8, 12.5 mM MgCl₂, 10% Glycerol, 0.1% NP-40, 100 mM KCl, 1 mM PMSF, 1 mM DTT) and the protein complexes were eluted in 2x Lämmli buffer (4% SDS, 20% Glycerol, 120 mM Tris/HCl, pH 6.8, 5% β-mercaptoethanol, bromophenol-blue). Samples were analyzed by SDS-PAGE and Western blot.

Western Blot

Proteins resolved by SDS-PAGE were transferred to PVDF membranes for 1 hour at 400 mA. The membranes were blocked with blocking buffer (5 % non-fat dried milk powder in TBS) for 30 min at RT. Primary antibodies listed in Table S 9 in blocking buffer were incubated with the membrane at 4 °C for one hour or overnight. After washing 3x with PBS/T for 10 min, the secondary HRP-coupled antibody in blocking buffer was incubated with the membrane at 4 °C for one hour followed by 4x washing with PBS/T for 15 min and a final wash with PBS. Bound antibodies were detected with the Enhanced Chemiluminescence (ECL) system (GE Healthcare Amersham) according to the manufacturer's instructions. Emitted light was detected with the Fusion FX (Vilber) device.

Chromatin Immunoprecipitation (ChIP)

ChIP-qPCR was performed in LCLs as previously described (Glaser *et al.*, 2017) with minor changes. In brief, 2x10⁷ cells were harvested, washed with PBS and resuspended in 20 ml RPMI medium. Cross-linking was achieved by the addition of formaldehyde (final 1 %) and 7 min incubation at room temperature. Glycine was added to a final concentration of 125 mM. The cells were washed in PBS, followed by washing with ice-cold lysis buffer (10 mM Tris-HCl, pH 7.5; 10 mM NaCl, 3 mM MgCl₂, 0.5% NP-40, 1x proteinase inhibitor cocktail (PIC, Roche)). The cells were resuspended in sonication buffer (50 mM Tris-HCl, pH 8.0, 5 mM EDTA, pH 8.0, 0.5% SDS, 0.5% Triton X-100, 0.05% sodium deoxycholate, 1x PIC) and the chromatin was sheared by 4 rounds of 10 min sonication (Bioruptor, 30 sec on/off). 250 µl of cleared lysate containing chromatin was used for one IP. 25 µl of chromatin was saved for the input sample. For one α-EBNA2 ChIP, a 100 µl mix of α-EBNA2 (R3), α-EBNA2 (1E6) and α-HA (3F10) or a matched isotype control (α-GST (6G9) + α-Dog-CD3) was added to the chromatin. For one α-EBF1 ChIP, 5 µg of α-EBF1 (ABE1294) or a normal rabbit IgG (PP64B) isotype control was added to the chromatin. The chromatin-antibody solution was incubated overnight at 4 °C rotating. Protein A beads were used for the α-EBF1 ChIP and Protein G beads were used for the α-EBNA2 ChIP. The beads were pre-blocked with 500 µg/ml salmon testes DNA overnight at 4 °C. The beads were equilibrated with dilution buffer (12.5 mM Tris-HCl, pH 8.0, 187.5 mM NaCl, 1.25 mM EDTA, pH 8.0, 1.125% Triton X-100, 1 x PIC), 100 µl of a 50 % slurry was added to the IP and incubated at 4 °C for 3 hours. The beads were washed with: **2x Wash Buffer I** (20 mM Tris-HCl, pH 8.0, 2 mM EDTA, pH 8.0, 1% Triton X-100, 150 mM NaCl, 0.1% SDS, 1x PIC), **1x Wash Buffer II** (20 mM Tris-HCl, pH 8.0, 2 mM EDTA, pH 8.0, 1% Triton X-100, 500 mM NaCl, 0.1% SDS, 1x PIC), **1x Wash Buffer III** (10 mM Tris-HCl, pH 8.0, 1 mM EDTA, pH 8.0, 250 mM LiCl, 1% NP-40, 1% sodium deoxycholate, 1x PIC) for 5 min, rotating, and **2x with TE** (10 mM Tris-HCl, pH 8.0, 1 mM EDTA, pH 8.0) for 1 min. Chromatin was eluted with 2x 150 µl elution buffer (25 mM Tris-HCl, pH 7.5, 10 mM EDTA, pH 8.0, 1% SDS) and 15 min incubation at 65 °C. To reverse the cross-link, Proteinase K (final 1.5 mg/ml, Roche) was added to the input and ChIP samples, incubated for 1 hour at 42 °C and overnight at 65 °C. Reverse cross-linked

chromatin was purified using the NucleoSpin™ Gel and PCR Clean-up kit (Macherey-Nagel) according to the manufacturer's instructions. Purified ChIP and input samples were analyzed by qPCR.

cDNA preparation

RNA was isolated from 1×10^7 cells with the RNeasy® Mini Kit (Qiagen) according to the manufacturer's instructions. β -mercaptoethanol in a final concentration of 134 mM was added to the lysis buffer. Cells were lysed in 600 μ l lysis buffer. QIAshredder columns (Qiagen) were used according to the manufacturer's instructions. Genomic DNA was removed with the RNase-free DNase set (Qiagen) according to the manufacturer's instructions. Purified RNA was eluted in 30-50 μ l RNase-free water and the concentration was measured using the Qubit RNA HS kit according to the instructions. SuperScript™ IV First-Strand cDNA Synthesis kit (ThermoFisher) was used to transcribe 2-4 μ g isolated RNA into cDNA according to the manufacturer's instructions. Random hexamers were used as primers. A reaction without reverse transcriptase was used as a negative control. 1/40 of cDNA (50 ng of input RNA) was used for qPCR.

Quantitative real time PCR (qPCR)

The quantification of cDNA obtained from reverse transcribed RNA and DNA recovered from ChIP experiments was done with the Roche LightCycler480 II device and LightCycler 480 SYBR Green I Master (Roche) reagent according to the manufacturer's instructions. The qPCR was carried out as previously described (Harth-Hertle *et al.*, 2013). Amplification was performed at 63 °C. 10 μ l per reaction were pipetted into a 96-well plate. 2 μ l of the sample were added to each well. The master mix contained 5 μ l LightCycler 480 SYBR Green I Master, 1 μ l of 5 μ M forward primer, 1 μ l of 5 μ M reverse primer, 1 μ l PCR grade H₂O. Standard curves with dilutions of defined amounts of the respective PCR product or chromatin were established and applied to account for varying primer efficiencies. Two replicates for each cDNA, ChIP target and standard dilution were cycled in parallel. The relative quantification of cDNA was normalized to RNA polymerase II signals applying the Roche LightCycler 480 E-Method, which is correcting for primer efficiencies.

The percentage of input for ChIP-qPCR signals was calculated for each target as (DNA from specific IP/ DNA input) x 100. Mann-Whitney test or Welch's t-test were applied to test for differences.

RNA and cDNA library preparation for RNA-sequencing

RNA sequencing was performed using the prime-seq method based on the scRNA-seq method SCRBS-seq (Bagnoli *et al.*, 2018; Janjic *et al.*, 2021). 10,000 cells per sample were sorted into a 96 well plate containing 50 μ l RLT plus Lysis Buffer (Qiagen) supplemented with 1% 2-mercaptoethanol and frozen at - 80 °C immediately. To assess how much RNA was extracted, replicate samples were pooled, prime-seq was performed on each pool individually and the cDNA amplification was performed as a qPCR using SYBR green. The input volume for the cDNA library preparation was adjusted accordingly.

Table S 10 Volumes used for cDNA library preparation

Sample (6 replicates each)	Infection	Volume cell lysate [μ l]
no_0	non-infected	50
mt_1	EBV $\Delta\alpha$ 1	10
mt_2	EBV $\Delta\alpha$ 1	50
mt_3	EBV $\Delta\alpha$ 1	50
mt_4	EBV $\Delta\alpha$ 1	30

wt_1	EBVwt	30
wt_2	EBVwt	10
wt_3	EBVwt	10
wt_4	EBVwt	10

Prime-seq was performed according to protocol (<https://www.protocols.io/view/prime-seq-s9veh66>). In brief, lysates were first treated with Proteinase K (Ambion) followed by a beads clean-up (2:1 beads to lysate ratio) using solid phase reversible immobilization (SPRI) beads (GE Biotech). A subsequent on-bead DNaseI digest was performed to remove genomic DNA, followed by another SPRI bead clean-up and reverse transcription. Reverse transcription (RT) was performed using Maxima H- RT enzyme (Thermo Fisher Scientific) with well specific barcoded oligo dT primers and a template switch oligo (TSO). After the RT, the barcoded first strand cDNA was pooled and cleaned up using magnetic beads. Remaining oligo dT primers were removed using exonucleaseI digest. Subsequently, cDNA was PCR amplified with Kapa Hifi DNA polymerase (Roche), quantified using picogreen dye (Thermo Fisher Scientific) and quality control using capillary gel electrophoresis (Agilent Bioanalyzer 2100). Sequencing libraries were generated with the NEB NEXT Ultra II FS kit by using a custom adapter oligo and a primer, specific for the cDNA 3'- end. Fragments between 300 bp and 500 bp were selected using double size selection with SPRIselect beads (Beckman Coulter). Final libraries were quantified and quality controlled again with the Bioanalyzer 2100. Paired End sequencing was performed as 28 bp read 1 and 50 bp read 2 with an 8 bp index read on an Illumina HiSeq 1500 instrument. In total 1.5 lanes of a High Out flow cell were sequenced, amounting to 465.9 Mio reads. Raw sequencing data was processed using the zUMIs pipeline (Parekh *et al.*, 2018) with mapping to the reference genome using STAR 2.7 (Dobin *et al.*, 2013). As a reference genome hg38 was used with Gencode annotation (v 35) concatenated with the EBV genomes Akata and p6008 (manually assembled, provided by W. Hammerschmidt) including annotation retrieved from (https://github.com/flemingtonlab/public/blob/master/annotation/chrEBV_Akata_inverted_refined_genes_annotation_cleaned.gtf)

Quality control and data normalization of RNA sequencing data

To initially assess the processed RNA sequencing data, the library size, number of detected cellular and viral genes and the fraction of mitochondrial genes was computed (Figure S4). For subsequent analyses, samples with less than 10,000 detected genes and a library size of less than 140,000 counts (one sample of each the day 0 non-infected, day 2 and day 3 EBV $\Delta\alpha 1$ infected condition: 14-no-0, 14-a1-2, 15-a1-3) were excluded. Data was normalized using size factors (Anders and Huber, 2010), which accounts for sequencing depth and RNA content.

Principal component analysis (PCA)

A principal component analysis (PCA) was performed on the log-transformed expression matrix of all protein-coding genes of all samples, with 1 added as a pseudo count.

Differential gene expression (DGE) analysis

Genes with a low expression (counts per million <10) were excluded. The DGE analysis was performed using the DESeq2 R package. Differentially expressed genes with an FDR < 0.1 and a fold change ≥ 2

were selected for the cluster analysis. Differentially expressed, protein coding genes with an FDR < 0.01 were selected for the gene set enrichment analysis (GSEA).

Gene set enrichment analysis (GSEA)

For the GSEA, the program was downloaded from <https://www.gsea-msigdb.org> as a desktop application. The hallmark gene sets were selected, and normalized expression data was used for the analysis. Enriched gene sets with an FDR < 0.05 were considered significant.

Cluster analysis

To classify the patterns of gene expression along the infection time course, we applied a clustering algorithm to all DE genes in the EBVwt and EBV $\Delta\alpha 1$ infected cells separately. First, we calculated a distance matrix between genes as $\sqrt{(1 - \rho)/2}$, where ρ is the Spearman's correlation coefficient between pairs of genes across all samples in each condition. Hierarchical clustering was performed on this distance matrix ("hclust" function in R, with the "average" aggregation method), followed by the dynamic hybrid cut algorithm to estimate the number of clusters (dynamicTreeCut package v1.63-1, with minimum cluster size of 500 and "deepSplit" parameter equal to 1). We identified 5 clusters in EBVwt and 4 in EBV $\Delta\alpha 1$ (Figure S5 B-J) with the "clust.stats" function from the "fpc" R package (version 2.1-11.1).

Gene Ontology (GO) Analysis

The GO term analysis was performed with the online tool GOrilla (Eden *et al.*, 2007, 2009) in order to identify biological processes enriched in each cluster. The analysis was performed with a list of genes identified for each cluster and a background list of genes that included all genes that were tested for differential expression. The p-value threshold for the GO term analysis was set to 10^{-3} . The online tool REVIGO was used to reduce redundancy of identified GO terms and the analyses were performed with settings defining medium similarity, the homo sapiens databank and SimRel to calculate semantic similarity (Supek *et al.*, 2011).

Data and code availability

The raw and processed sequencing data will be available from Array express under the accession number E-MTAB-11350. All data were analyzed with standard programs and packages, as detailed above. Code is available on request.

Caption for Datasets 1 to 3

SI Dataset 1: Clusters of differentially expressed genes in EBVwt infected B cells displaying average expression and standard deviation. Data is corresponding to Figure S 5B-F

SI Dataset 2: Clusters of differentially expressed genes in EBV $\Delta\alpha 1$ infected B cells displaying average expression and standard deviation. Data is corresponding to Figure S 5G-J

SI Dataset 3: Normalized expression data of protein coding genes in uninfected, EBVwt and EBV $\Delta\alpha 1$ infected B cells

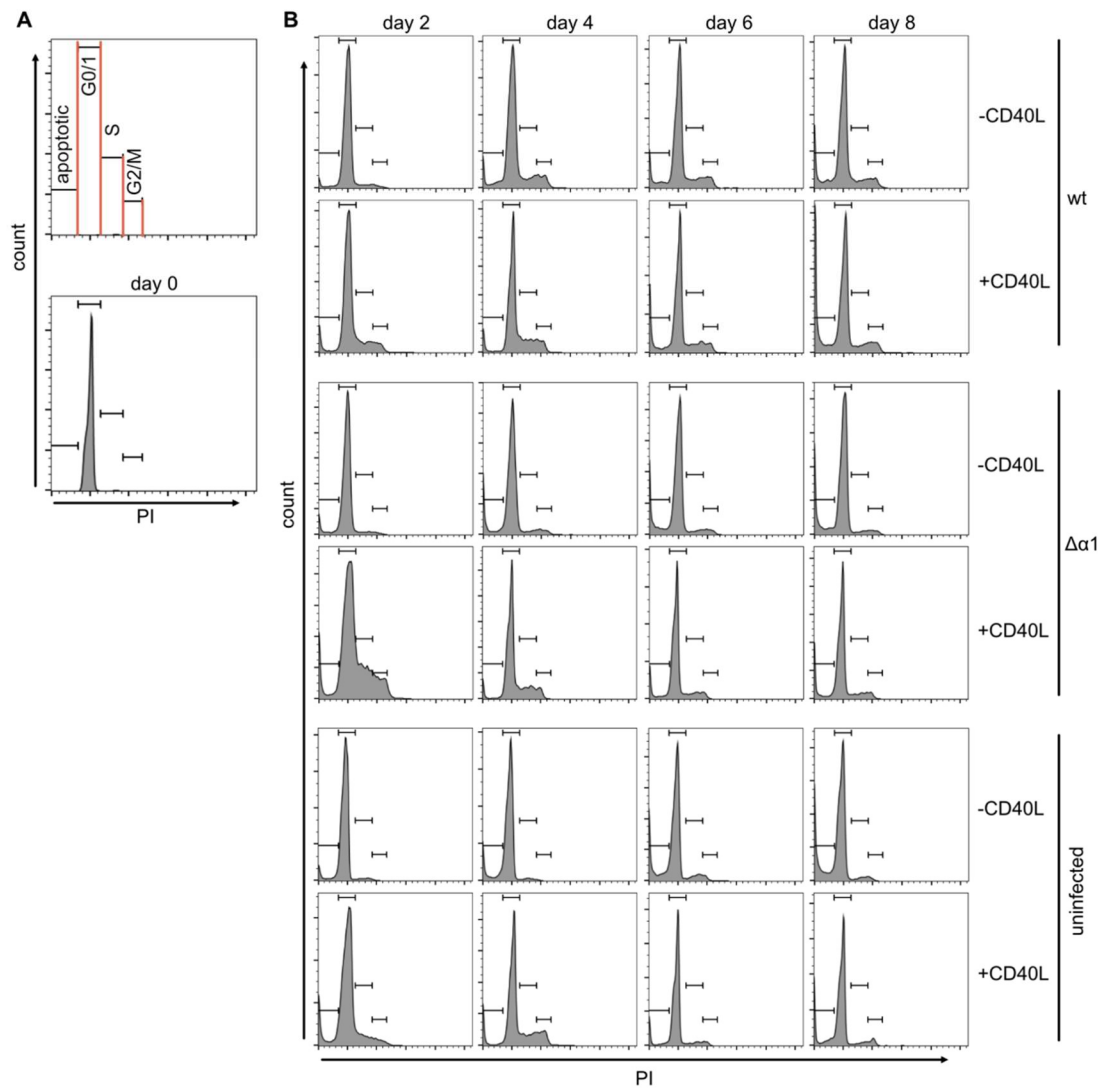


Figure S 2 Histograms of cell cycle analysis with propidium iodide (PI) (A) schematic gating of the cell cycle populations and one representative FACS plot for the day 0 non-infected sample, gated on lymphocytes and single cells. (B) PI histograms of one representative experiment showing B cells infected with EBVwt (wt), EBV $\Delta\alpha 1$ ($\Delta\alpha 1$) or non-infected B cells on day 2, 4, 6 and 8 post-infection. B cells were cultured without (-) CD40L expressing feeder cells or with (+) CD40L expressing feeder cells.

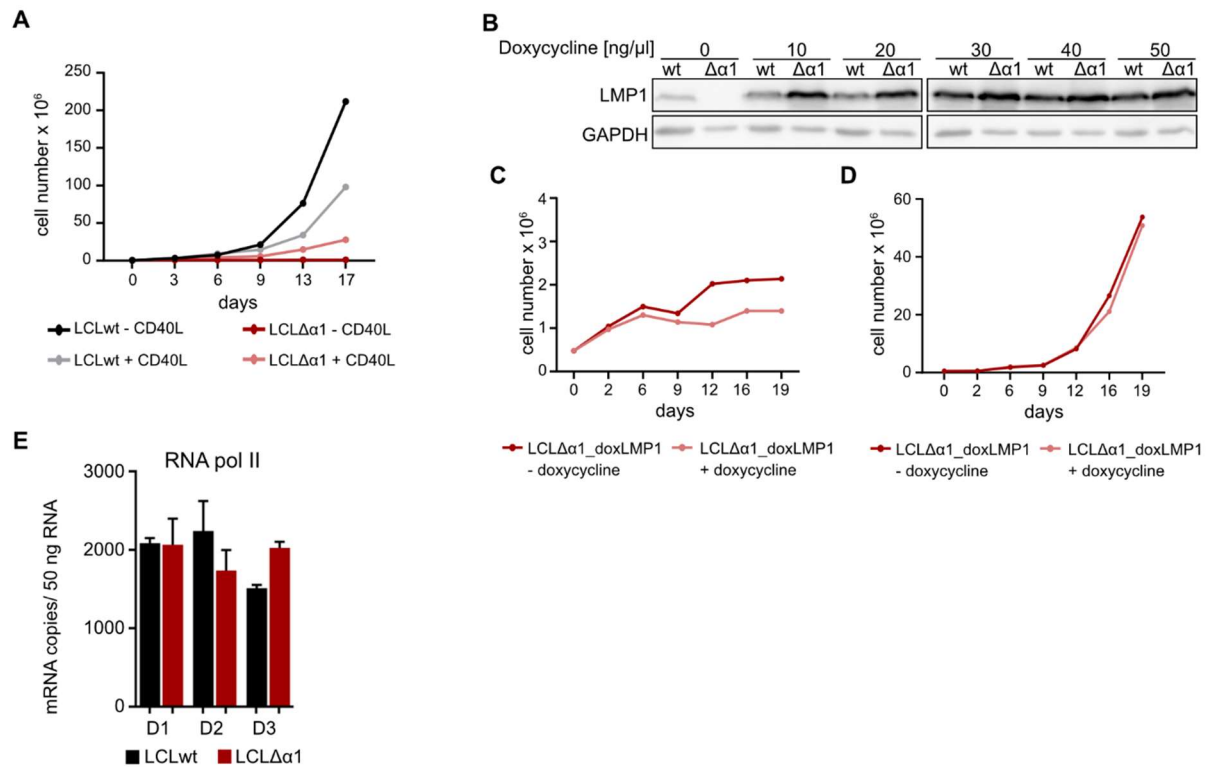


Figure S 3 Establishment of LCLΔα1 cultures on CD40L expressing feeder cells. (A) Growth curves of LCLwt and LCLΔα1 without (-) or with (+) CD40L feeder cells. 5×10^5 cells were seeded on day 0. The cell number was counted on the indicated time points. (B) LMP1 expression in LCLwt and LCLΔα1 transfected with the vector plasmid pRTS-HA-LMP1, which expresses LMP1 conditionally upon addition of doxycycline. GAPDH was used as a loading control. (C, D) Growth curves of LCLΔα1_doxLMP1 without (- doxycycline) and with ectopically expressed LMP1 (+ doxycycline) grown (C) without CD40L feeder cells or (D) with CD40L feeder cells. LMP1 expression was induced with 10 ng/ml doxycycline. 5×10^5 cells were seeded on day 0. Cell numbers were counted at the indicated time points. The mean of two replicates is plotted. (E) RT-qPCR quantification of RNA pol II transcripts in LCLwt and LCLΔα1 from three donors (D1, D2, D3). Error bars indicate standard deviation.

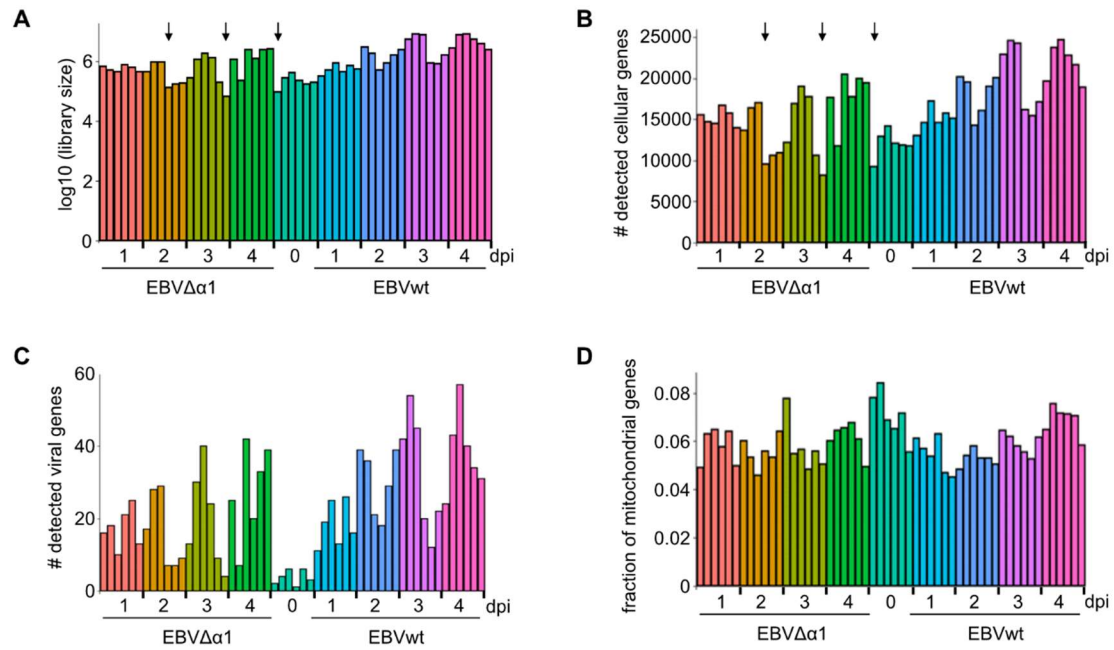


Figure S 4 Quality control of RNA sequencing samples. 6 biological replicates for each time point and infection condition were collected. (A) Log10 transformed size of sequenced libraries for all collected samples in infection conditions and at different days post-infection. (B) Number of detected cellular genes. (C) Number of detected viral genes. In total, 4920 reads were mapped to the viral genome. 3095 of these were mapped to EBNA2. (D) Fraction of mitochondrial genes detected among cellular genes. dpi – days post-infection, Arrows indicated samples that were excluded from the analysis.

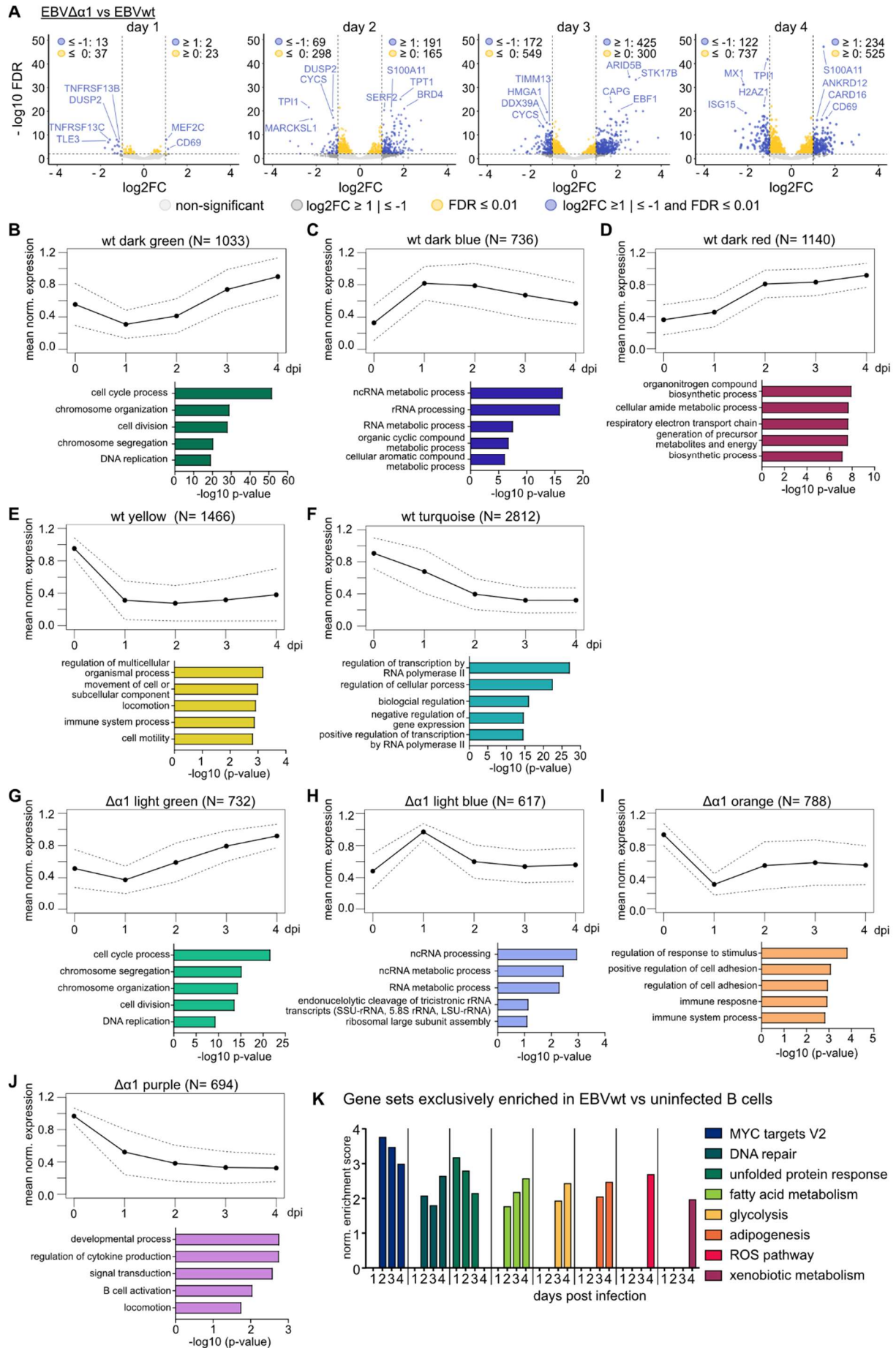


Figure S 5 Time-resolved dynamic changes of gene expression patterns. (A) Direct comparison of EBV $\Delta\alpha 1$ and EBVwt infected B cells shown as differentially expressed (DE), protein coding genes in Volcano plots. Dotted lines indicate $\log_2FC = 1$ and $FDR = 0.01$. Names of the top four up and down regulated genes according to the FDR are indicated. Cluster analyses of DE genes in (B-F) EBVwt and (G-J) EBV $\Delta\alpha 1$ infected B cells. The normalized mean expression is plotted with the standard deviation as dotted lines. N indicates the number of DE genes within each cluster. All genes from each cluster were used to perform the GO term analysis summarized in the bar graphs below. The top 5 GO terms according to the $-\log_{10}$ p-value are plotted. (K) Significantly enriched gene sets that are exclusively detected in EBVwt infected B cells. Differentially expressed, protein coding genes defined by the analysis EBVwt vs non-infected were included for this GSEA. Bar graphs showing the normalized enrichment score (NES) for each gene set at each day post-infection.

Table S 11 Corresponding to figure 2: Cell cycle distribution of EBVwt infected B cells analyzed by BrdU incorporation and 7-AAD staining. The mean was used to generate the plot in figure 2D. dpi – days post-infection

EBVwt					
Donor 6					
dpi	apoptotic	G0/1	early S	S	G2/M
0	0.10	90.74	1.87	5.02	2.26
2	0.30	70.50	19.06	7.91	2.23
4	2.96	52.10	17.78	18.92	8.25
6	2.82	66.96	9.69	13.82	6.70
8	5.19	75.16	6.99	7.29	5.38
Donor 10					
dpi	apoptotic	G0/1	early S	S	G2/M
0	0.15	91.10	2.52	2.61	3.62
2	2.56	73.49	14.74	6.75	2.47
4	4.89	67.91	4.43	11.44	11.33
6	4.37	78.04	3.06	6.81	7.72
8	4.85	82.82	2.54	3.49	6.30
Donor 21					
dpi	apoptotic	G0/1	early S	S	G2/M
0	1.09	90.58	1.89	3.54	2.89
2	3.32	66.66	23.09	5.28	1.64
4	5.63	76.39	6.66	6.35	4.98
6	8.62	75.31	7.75	4.08	4.23
8	11.86	75.37	4.59	1.93	6.25
mean					
dpi	apoptotic	G0/1	early S	S	G2/M
0	0.45	90.81	2.09	3.73	2.92
2	2.06	70.20	18.98	6.64	2.11
4	4.50	65.56	9.58	12.18	8.18
6	5.28	73.44	6.84	8.23	6.21
8	7.31	77.82	4.69	4.21	5.98

Table S 12 Corresponding to figure 2: Cell cycle distribution of EBV $\Delta\alpha 1$ infected B cells analyzed by BrdU incorporation and 7-AAD staining. The mean was used to generate the plot in figure 2D. dpi – days post-infection

EBV $\Delta\alpha 1$					
Donor 6					
dpi	apoptotic	G0/1	early S	S	G2/M
0	0.10	90.74	1.87	5.02	2.26
2	0.25	75.00	20.26	3.32	1.18
4	4.38	62.04	25.00	6.39	2.18
6	7.46	62.11	19.72	6.18	4.53
8	17.40	54.47	20.64	5.43	2.06
Donor 10					
dpi	apoptotic	G0/1	early S	S	G2/M
0	0.15	91.10	2.52	2.61	3.62
2	2.93	76.15	15.44	3.64	1.84
4	6.61	77.40	7.55	2.90	5.54
6	9.11	70.80	11.85	3.35	4.89
8	10.84	74.53	8.86	1.57	4.21
Donor 21					
dpi	apoptotic	G0/1	early S	S	G2/M
0	1.09	90.58	1.89	3.54	2.89
2	3.62	68.66	20.83	2.96	3.92
4	6.64	66.51	20.25	3.41	3.19
6	9.43	59.43	25.12	3.78	2.23
8	11.59	53.32	28.12	5.80	1.17
mean					
dpi	apoptotic	G0/1	early S	S	G2/M
0	0.45	90.81	2.09	3.73	2.92
2	2.27	73.25	18.86	3.30	2.32
4	5.88	68.64	17.62	4.23	3.63
6	8.67	64.07	18.95	4.44	3.87
8	13.20	60.81	19.26	4.26	2.48

Table S 13 Corresponding to figure 3: Cell cycle distribution of EBVwt infected B cells analyzed by PI staining. The mean was used to generate the plot in figure 3A. dpi – days post-infection

- CD40L feeder cells				
Donor 8				
dpi	apoptotic	G0/1	S	G2/M
0	0.14	97.93	1.44	0.49
2	5.85	85.99	5.34	2.82
4	9.66	70.71	10.82	8.80
6	12.57	68.51	10.64	8.28
8	17.71	66.19	9.05	7.05
Donor 9				
dpi	apoptotic	G0/1	S	G2/M
0	0.18	97.90	1.43	0.49
2	9.49	82.18	5.55	2.78
4	17.93	70.52	6.89	4.65
6	12.65	68.01	10.53	8.81
8	19.34	64.30	8.59	7.78
Donor 10				
dpi	apoptotic	G0/1	S	G2/M
0	0.21	97.69	1.63	0.47
2	5.65	86.13	5.39	2.83
4	9.68	70.95	10.93	8.44
6	12.84	68.23	10.92	8.02
8	17.61	65.99	9.08	7.32
mean				
dpi	apoptotic	G0/1	S	G2/M
0	0.18	97.84	1.50	0.48
2	7.00	84.77	5.43	2.81
4	12.43	70.73	9.54	7.30
6	12.69	68.25	10.69	8.37
8	18.22	65.49	8.90	7.38

+ CD40L feeder cells				
Donor 8				
dpi	apoptotic	G0/1	S	G2/M
0	0.14	97.93	1.44	0.49
2	7.55	73.50	12.03	6.91
4	6.91	67.54	16.81	8.74
6	18.03	63.22	11.55	7.20
8	29.79	55.41	7.67	7.13
Donor 9				
dpi	apoptotic	G0/1	S	G2/M
0	0.18	97.90	1.43	0.49
2	6.91	72.73	13.06	7.30
4	11.40	60.67	15.59	12.34
6	15.55	64.93	11.81	7.70
8	30.47	55.65	7.11	6.77
Donor 10				
dpi	apoptotic	G0/1	S	G2/M
0	0.21	97.69	1.63	0.47
2	11.80	76.43	8.47	3.30
4	7.05	67.22	18.02	7.71
6	17.73	63.01	11.14	8.12
8	30.24	55.80	7.66	6.30
mean				
dpi	apoptotic	G0/1	S	G2/M
0	0.18	97.84	1.50	0.48
2	8.76	74.23	11.18	5.83
4	8.42	65.19	16.82	9.57
6	17.10	63.72	11.50	7.68
8	30.17	55.62	7.48	6.73

Table S 14 Corresponding to figure 3 Cell cycle distribution EBV $\Delta\alpha 1$ infected B cells analyzed by PI staining. The mean was used to generate the plot in Figure 3A. dpi – days post-infection

- CD40L feeder cells					+ CD40L feeder cells				
Donor 8					Donor 8				
dpi	apoptotic	G0/1	S	G2/M	dpi	apoptotic	G0/1	S	G2/M
0	0.14	97.93	1.44	0.49	0	0.14	97.93	1.44	0.49
2	9.52	83.53	4.63	2.32	2	10.44	58.55	19.96	11.05
4	14.69	75.36	5.12	4.83	4	6.34	71.03	16.12	6.52
6	18.99	71.12	5.21	4.68	6	13.78	73.30	10.06	2.87
8	20.44	71.90	4.08	3.58	8	16.80	70.51	8.69	4.00
Donor 9					Donor 9				
dpi	apoptotic	G0/1	S	G2/M	dpi	apoptotic	G0/1	S	G2/M
0	0.18	97.90	1.43	0.49	0	0.18	97.90	1.43	0.49
2	10.45	82.20	4.80	2.54	2	18.47	53.08	17.09	11.36
4	14.49	74.06	6.38	5.08	4	7.52	68.80	14.21	9.47
6	15.66	73.16	5.58	5.60	6	11.96	75.11	10.04	2.89
8	18.82	72.97	4.19	4.02	8	17.88	69.79	8.66	3.68
Donor 10					Donor 10				
dpi	apoptotic	G0/1	S	G2/M	dpi	apoptotic	G0/1	S	G2/M
0	0.21	97.69	1.63	0.47	0	0.21	97.69	1.63	0.47
2	9.24	83.75	4.60	2.40	2	9.57	59.33	19.54	11.56
4	15.14	74.50	5.73	4.62	4	6.24	71.14	16.45	6.18
6	20.62	70.11	5.31	3.96	6	13.78	73.41	10.04	2.78
8	21.32	71.00	3.97	3.71	8	16.92	70.98	8.72	3.38
mean					mean				
dpi	apoptotic	G0/1	S	G2/M	dpi	apoptotic	G0/1	S	G2/M
0	0.18	97.84	1.50	0.48	0	0.18	97.84	1.50	0.48
2	9.74	83.16	4.68	2.42	2	12.76	57.03	18.88	11.32
4	14.77	74.64	5.74	4.84	4	6.70	70.33	15.59	7.38
6	18.43	71.46	5.37	4.74	6	13.17	73.94	10.05	2.84
8	20.19	71.96	4.08	3.77	8	17.20	70.43	8.69	3.69

Table S 15 Corresponding to figure 3 Cell cycle distribution of non-infected B cells analyzed by PI staining. The mean was used to generate the plot in figure 3A. dpi – days post-infection

- CD40L feeder cells				
Donor 8				
dpi	apoptotic	G0/1	S	G2/M
0	0.14	97.93	1.44	0.49
2	10.33	85.16	3.63	0.87
4	16.33	79.14	3.59	0.94
6	22.70	67.70	6.81	2.79
8	26.69	67.23	4.30	1.78
Donor 9				
dpi	apoptotic	G0/1	S	G2/M
0	0.18	97.90	1.43	0.49
2	14.44	80.53	3.67	1.35
4	19.33	76.01	3.54	1.12
6	24.07	67.47	5.52	2.93
8	26.65	66.67	4.02	2.65
Donor 10				
dpi	apoptotic	G0/1	S	G2/M
0	0.21	97.69	1.63	0.47
2	9.16	86.24	3.89	0.71
4	15.36	79.83	3.62	1.18
6	24.50	66.33	6.28	2.89
8	28.07	66.17	4.08	1.67
mean				
dpi	apoptotic	G0/1	S	G2/M
0	0.18	97.84	1.50	0.48
2	12.40	79.70	5.67	2.23
4	17.01	78.32	3.59	1.08
6	23.76	67.16	6.20	2.87
8	27.14	66.69	4.14	2.03

+ CD40L feeder cells				
Donor 8				
dpi	apoptotic	G0/1	S	G2/M
0	0.14	97.93	1.44	0.49
2	13.60	72.25	9.51	4.64
4	9.05	64.10	14.80	12.04
6	11.06	78.92	6.49	3.53
8	19.50	70.78	4.91	4.81
Donor 9				
dpi	apoptotic	G0/1	S	G2/M
0	0.18	97.90	1.43	0.49
2	12.66	73.05	9.22	5.07
4	6.98	65.16	15.78	12.09
6	12.30	77.30	6.04	4.36
8	19.94	69.52	4.98	5.57
Donor 10				
dpi	apoptotic	G0/1	S	G2/M
0	0.21	97.69	1.63	0.47
2	12.99	73.38	8.84	4.79
4	9.26	65.20	14.51	11.04
6	10.70	78.50	6.60	4.21
8	18.67	71.41	5.33	4.59
mean				
dpi	apoptotic	G0/1	S	G2/M
0	0.18	97.84	1.50	0.48
2	13.08	72.89	9.19	4.83
4	8.43	64.82	15.03	11.72
6	11.35	78.24	6.38	4.03
8	19.37	70.57	5.07	4.99

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