

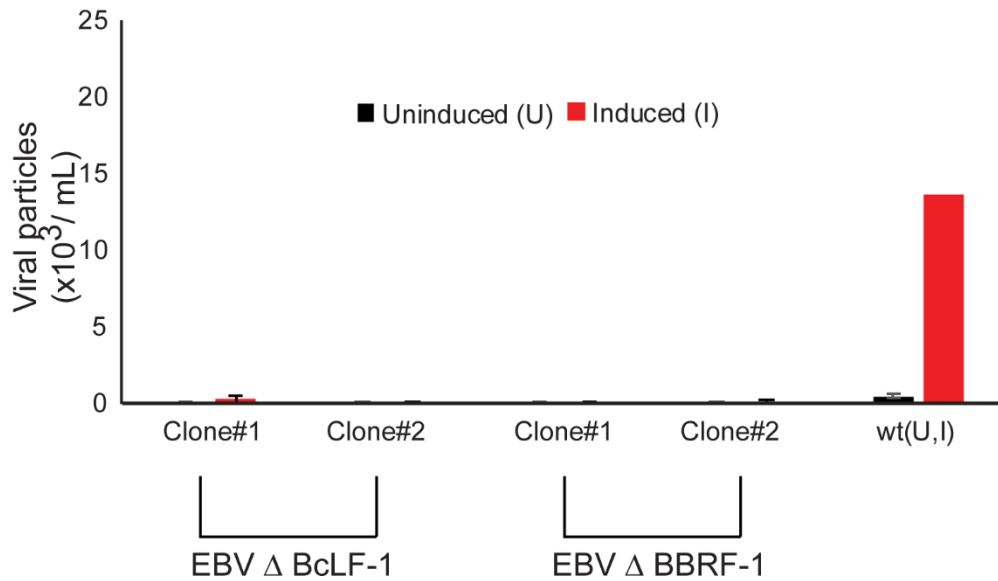
**4D analyses show that replication compartments are clonal factories in  
which Epstein-Barr Viral DNA amplification is coordinated**

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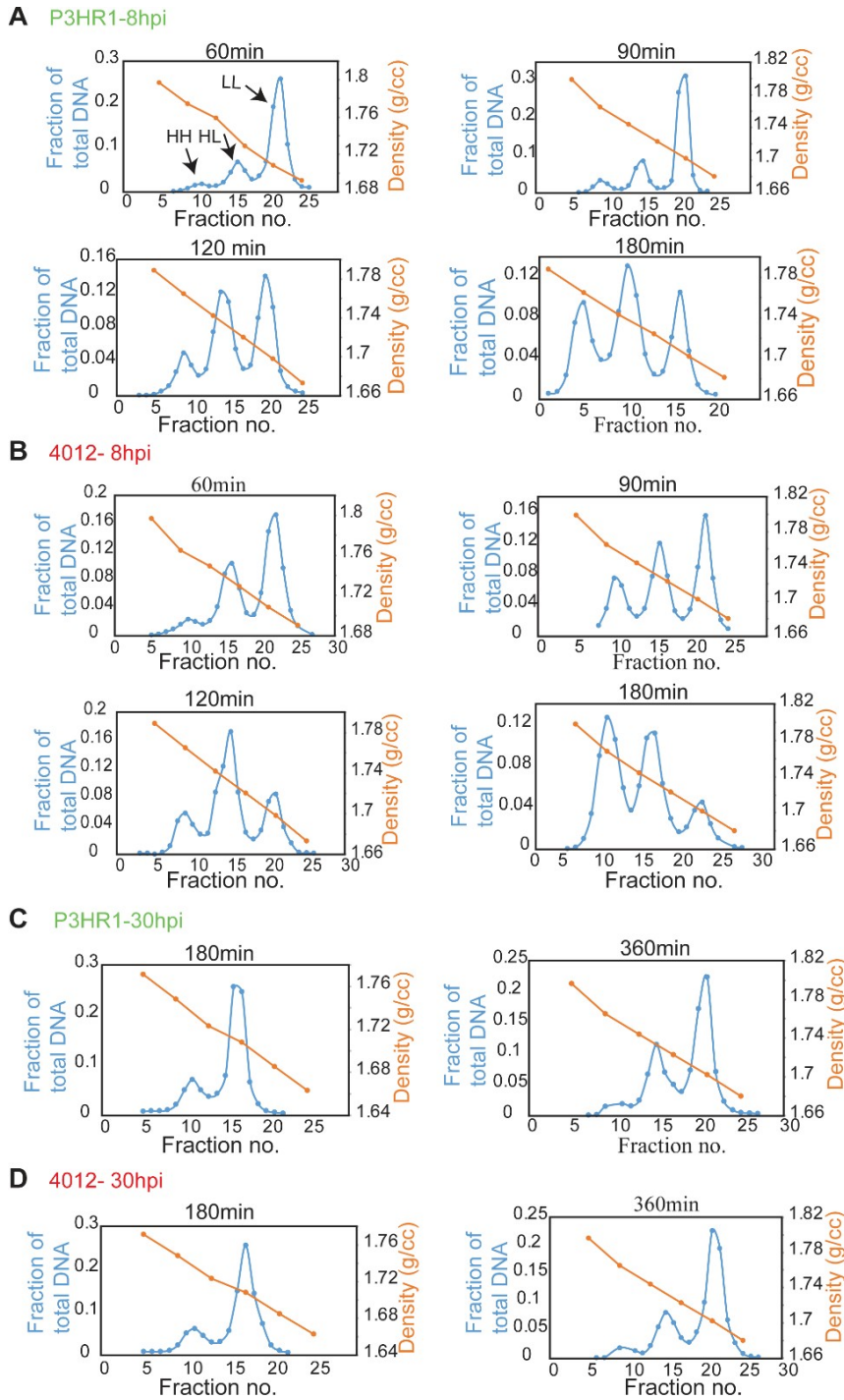
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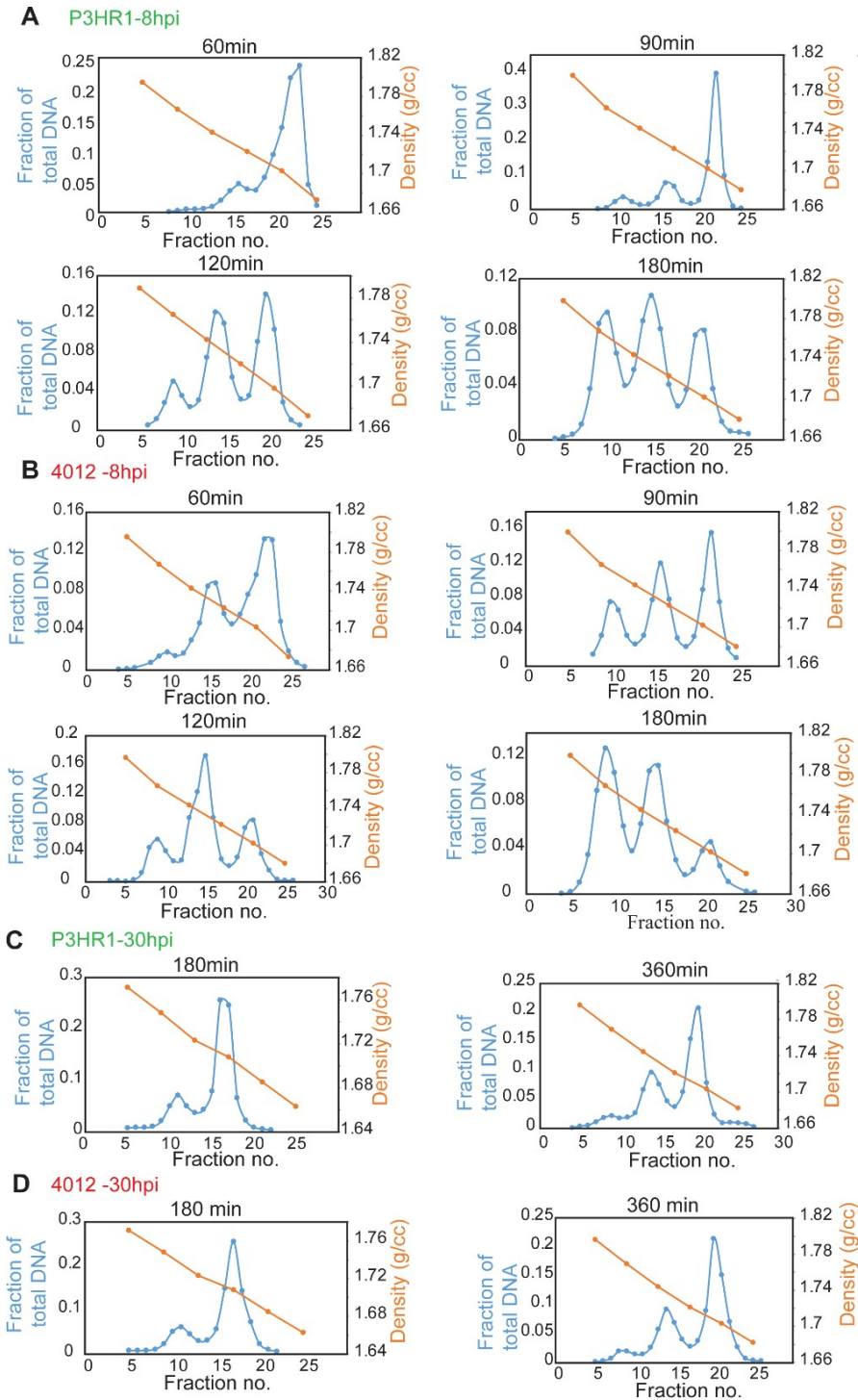
Figures S1 to S4  
Tables S1 to S5



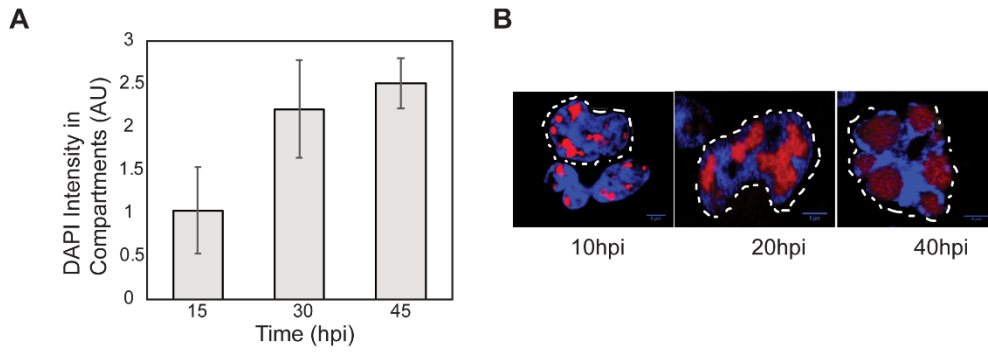
**Fig. S1. Generation of mutant EBV cell lines-** 293 cells carrying either the indicated mutant or wild type derivatives of EBV were induced by transfection of plasmids encoding immediate early genes BRLF1 and BZLF1 for the induction of lytic phase and a plasmid encoding a late gene product p18 fused to GFP to measure the fraction of cells supporting the lytic phase. The supernatant was collected at 96hpi and treated with DNase I followed by addition of proteinase K and the DNA from the particles were purified and assayed using qPCR.



**Fig. S2. First biological replicate of density shift assays. A&B)** Density shift assay profiles for P3HR1 and 4012 respectively at the indicated pulse times early during the lytic phase (8 hpi). iD98HR1-4012 cells were induced with tamoxifen and labeled with BrdU at 8 hpi for the indicated times. After induction the DNA was isolated, sheared, separated in CsCl gradients and assayed using qPCR for viral DNA and cellular DNA as detailed in the Materials and Methods. **C&D)** Density shift assay profiles for P3HR1 and 4012 respectively at the indicated pulse times late during the lytic phase (30 hpi). The cells were processed as they were processed in A&B.



**Fig. S3. A second biological replicate of density shift assays. A&B)** Density shift assay profiles for P3HR1 and 4012 respectively at the indicated pulse times early during the lytic phase (8 hpi). iD98HR1-4012 cells were induced with tamoxifen and labeled with BrdU at 8 hpi for the indicated times. After induction the DNA was isolated, sheared, separated in CsCl gradients and assayed using qPCR for viral DNA and cellular DNA as detailed in the Materials and Methods. **C&D)** Density shift assay profiles for P3HR1 and 4012 respectively at the indicated pulse times late during the lytic phase (30 hpi). The cells were processed as they were processed in A&B.



**Fig. S4. A) iD98HR1-4012 cells were pulsed with EdU at the indicated times for 30 min-** EdU was labelled by click chemistry (see Materials and Methods). The cells were then imaged. The total DAPI intensity inside the replication compartments (as indicated by the red EdU signals) was measured at indicated times (n=20-25 cells for each time point). Error bars indicate the standard deviation. B) Shown are cells pulsed with EdU for 30 min. at the indicated times.

	BrdU Pulse time (min)	Fraction of cells supporting lytic phase
8hpi	60	0.03
	90	0.13
	120	0.07
	180	0.15
30hpi	180	0.6
	360	0.6

**Table. S1.** The fraction of cells supporting the lytic phase at the indicated time hours post induction (hpi) for cells pulsed in parallel with EdU were measured and used as correction factors for density shift assays. 60 and 120min pulses beginning at 8 hpi are a pair and 90 and 180 min pulses beginning at 8hpi are a pair of measurements each having two biological replicates. The correction factors are averages of their biological replicates. For each of these biological replicates, four technical replicates were used.

Pulse times (min)	Replicate	LL (molecules/Rho)	HL (molecules/Rho)	HH (molecules/Rho)
60	1	0	22	2
	2	0	48	12
90	1	0	12	10
	2	0	11	5
120	1	2	180	65
	2	3	238	70
180	1	5	156	116
	2	7	200	111

**Table. S2. Measurements of P3HR1 DNA early during lytic phase:** Cells were labelled with BrdU for increasing times at 8hpi; DNAs were isolated from them, separated by CsCl equilibrium centrifugation and detected by qPCR as described in the Materials and Methods. Profiles of the assays of each gradient are shown in Fig S2 and S3. Each fraction in each gradient was assayed for the amount of P3HR1 DNA. Each set of cells were also assayed for the extent of their support of the lytic phase using EdU-pulse labelling as described in the Materials and Methods. The numbers indicate the number of P3HR1 DNA molecules normalized to that of the cellular DNA (Rhodopsin) found in the peaks of light/light (LL), heavy/light (HL) and heavy/heavy (HH) densities after correction for the fraction of cells supporting the lytic phase in that sample.

Pulse times (min)	Replicate	LL (molecules/Rho)	HL (molecules/Rho)	HH (molecules/Rho)
60	1	0	34	6
	2	0	25	5
90	1	0	15	9
	2	0	12	9
120	1	3	137	50
	2	2	158	55
180	1	3	72	84
	2	3	84	93

**Table. S3. Measurements of 4012 DNA early during lytic phase:** Cells were labelled with BrdU for increasing times at 8hpi; DNAs were isolated from them, separated by CsCl equilibrium centrifugation and detected by qPCR as described in the Materials and Methods. Profiles of the assays of each gradient are shown in Fig S2 and S3. Each fraction in each gradient was assayed for the amount of 4012 DNA. Each set of cells were also assayed for the extent of their support of the lytic phase using EdU-pulse labelling as described in the Materials and Methods. The numbers indicate the number of 4012 DNA molecules normalized to that of the cellular DNA (Rhodopsin) found in the peaks of light/light (LL), heavy/light (HL) and heavy/heavy (HH) densities after correction for the fraction of cells supporting the lytic phase in that sample.



Pulse times (min)	Replicate	LL (molecules/Rho)	HL (molecules/Rho)	HH (molecules/Rho)
180	1	3638	1234	ND
	2	3561	1126	ND
360	1	3038	1915	381
	2	2932	1668	275

**Table. S4. Measurements of P3HR1 DNA late during lytic phase:** Cells were labelled with BrdU for increasing times at 30hpi; DNAs were isolated from them, separated by CsCl equilibrium centrifugation and detected by qPCR as described in the Materials and Methods. Profiles of the assays of each gradient are shown in Fig S2 and S3. Each fraction in each gradient was assayed for the amount of P3HR1 DNA. Each set of cells were also assayed for the extent of their support of the lytic phase using EdU-pulse labelling as described in the Materials and Methods. The numbers indicate the number of P3HR1 DNA molecules normalized to that of the cellular DNA (Rhodopsin) found in the peaks of light/light (LL), heavy/light (HL) and heavy/heavy (HH) densities after correction for the fraction of cells supporting the lytic phase in that sample. ND- Not Detectable.

Pulse times (min)	Replicate	LL (molecules/Rho)	HL (molecules/Rho)	HH (molecules/Rho)
180	1	2076	590	ND
	2	2014	517	ND
360	1	1923	975	209
	2	1729	805	175

**Table. S5. Measurements of 4012 DNA late during lytic phase:** Cells were labelled with BrdU for increasing times at 30hpi; DNAs were isolated from them, separated by CsCl equilibrium centrifugation and detected by qPCR as described in the Materials and Methods. Profiles of the assays of each gradient are shown in Fig S2 and S3. Each fraction in each gradient was assayed for the amount of 4012 DNA it contained and its density. Each set of cells were also assayed for the extent of their support of the lytic phase using EdU-pulse labelling as described in the Materials and Methods. The numbers indicate the number of 4012 DNA molecules normalized to that of the cellular DNA (Rhodopsin) found in the peaks of light/light (LL), heavy/light (HL) and heavy/heavy (HH) densities after correction for the fraction of cells supporting the lytic phase in that sample. ND- Not Detectable