Supporting Information

Wang and Quake 10.1073/pnas.1410785111

SI Materials and Methods

Cell Culture. Burkitt's lymphoma cell lines Raji, Namalwa, and DG-75 were obtained from ATCC and cultured in RPMI 1640 supplemented with 10% FBS and PSA, following ATCC recommendations. Human primary lung fibroblast IMR-90 was obtained from Coriell and cultured in Advanced DMEM/F-12 supplemented with 10% FBS and PSA.

Plasmid Cloning. We obtained pX458 from Addgene. A modified CMV promoter with a synthetic intron (pmax) was PCR amplified from Lonza control plasmid pmax-GFP. A modified guide RNA sgRNA$^{(F+E)}$ was ordered from IDT. Epstein–Barr virus (EBV) replication origin oriP was PCR-amplified from B95-8 transformed lymphoblastoid cell line GM12891. We used standard cloning protocols to clone pmax, sgRNA$^{(F+E)}$ and oriP to pX458, to replace the original CAG promoter, sgRNA and f1 origin. We designed EBV sgRNA based on the B95-8 reference, and ordered DNA oligos from IDT. The original sgRNA place holder in pX458 served as the negative control.

DNA Transfection. We used the Lonza Nucleofector II for DNA delivery. Five million Raji or DG-75 cells were transfected with 5-μg plasmids in each 100-μL reaction. Cell line Kit V and program M-013 were used following Lonza recommendation. For IMR-90, one million cells were transfected with 5-μg plasmids in 100 μL Solution V, with program T-030 or X-005.

Flow Cytometry and Cell Sorting. Two days after transfection, we sorted live GFP$^+$ cells for further culture. Annexin V Alexa647 was purchased from Life Technologies and used following the manufacturer's instructions.

Cell Proliferation Measurement. Raji and DG-75 cells were counted daily after initial flow sorting. IMR-90 cells were subcultured and counted every 2 d after initial flow sorting. Cell counts were normalized to the initial count 1 d after initial flow sorting. Five to 6 d after initial flow sorting (7–8 d after transfection), we sorted the live and dead cells for DNA analysis.

DNA Analysis. DNA sequences flanking sgRNA targets were PCR amplified with Phusion DNA polymerase (Table S2). SURVEYOR assays were performed following the manufacturer's instructions. DNA amplicons with large deletions were TOPO-cloned and single colonies were used for Sanger sequencing. EBV load was measured with Taqman digital PCR on Fluidigm BioMark. A Taqman assay targeting a conserved human locus was used for human DNA normalization. One nanogram of single-cell whole-genome amplification products from Fluidigm C1 were used for EBV quantitative PCR.

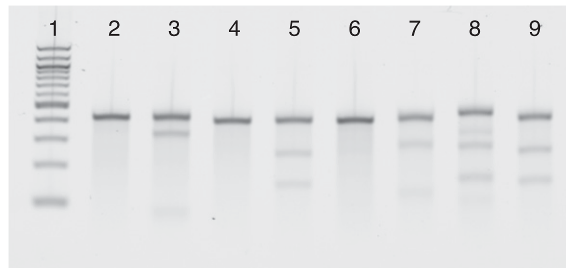

Fig. S1. SURVEYOR assay of EBV clustered regularly interspaced short palindromic repeat (CRISPR). (Lane 1) New England Biolabs 100-bp ladder; (lane 2) sgEBV1 control; (lane 3) sgEBV1; (lane 4) sgEBV5 control; (lane 5) sgEBV5; (lane 6) sgEBV7 control; (lane 7) sgEBV7; (lane 8) sgEBV4.

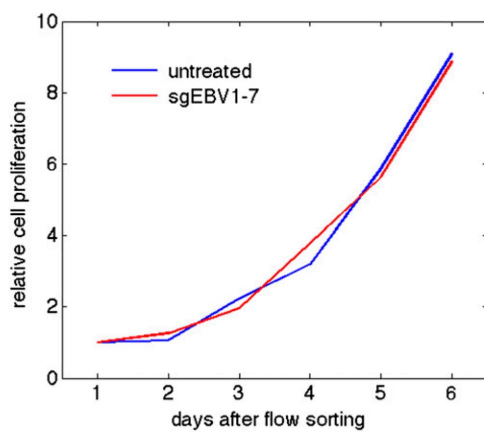

Fig. S2. CRISPR cytotoxicity test with EBV⁻ Burkitt's lymphoma DG-75.

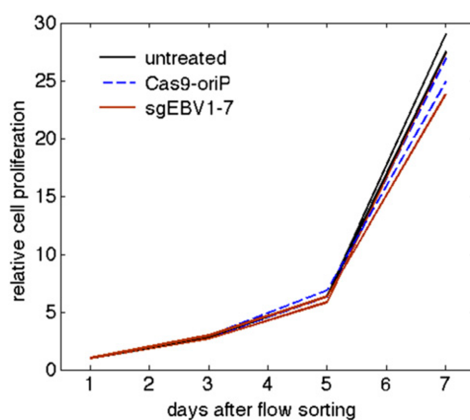

Fig. S3. CRISPR cytotoxicity test with primary human lung fibroblast IMR-90.

Table S1. Guide RNA target sequences

Guide RNA	Sequence
sgEBV1	GCCCTGGACCAACCCGGCCC
sgEBV2	GGCCGCTGCCCCGCTCCGGG
sgEBB3	GGAAGACAATGTGCCGCCA
sgEBV4	TCTGGACCAGAAGGCTCCGG
sgEBV5	GCTGCCGCGGAGGGTGATGA
sgEBV6	GGTGGCCCACCGGGTCCGCT
sgEBV7	GTCCTCGAGGGGGCCGTCGC

Table S2. PCR Primers

Primer	Sequence
sgEBV1F	TGCTAGGCCACCTTCTCAGT
sgEBV1R	GTAGTGTGTGCCTGGGTGTG
sgEBV2F	AGCATGGCGAAGTAGACAGG
sgEBV2R	GCCCATTCGAACCCTACC
sgEBV3F	TTTCAGACCCACCATGGAAT
sgEBV3R	CCCATGAACCCAGTTAGAGG
sgEBV4F	GGCTGCGAGTAATTGGTGAT
sgEBV4R	CAATGCAACTTGGACGTTTTT
sgEBV5F	GCTGAGGTTTTGAAGGATGC
sgEBV5R	GGAGCTGAGTGACGTGACAA
sgEBV7F	AGTAAGGGAAAGGGGGTGTG
sgEBV7R	GACGTAGCCGCCCTACATAA
oriP_F	CCACCAATTCCAACCATTTT
oriP_R	CGCGGGGCAGTGCAT
EBNA1_qF	CCTCCCTGGTTTCCACCTAT
EBNA1_qR	CCTCCTTCATCTCCGTCATC
EBNA1_qP	TCCGTCATCACCCTCCGC