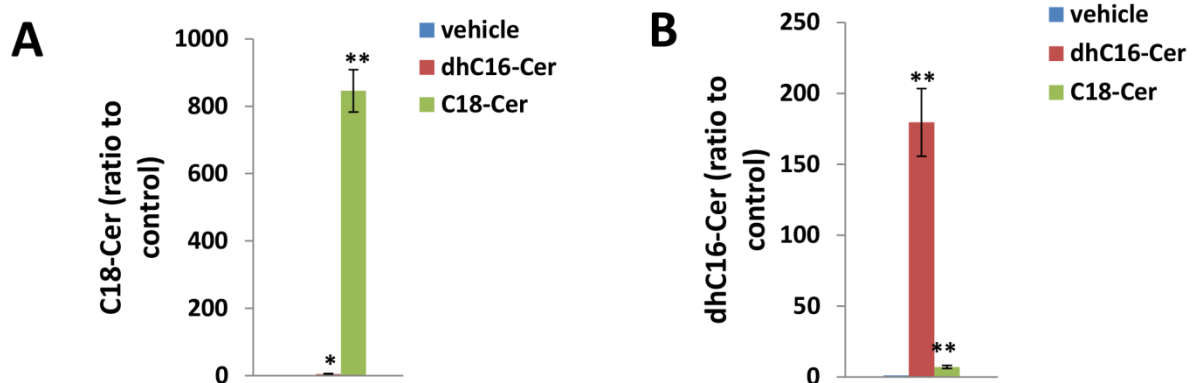
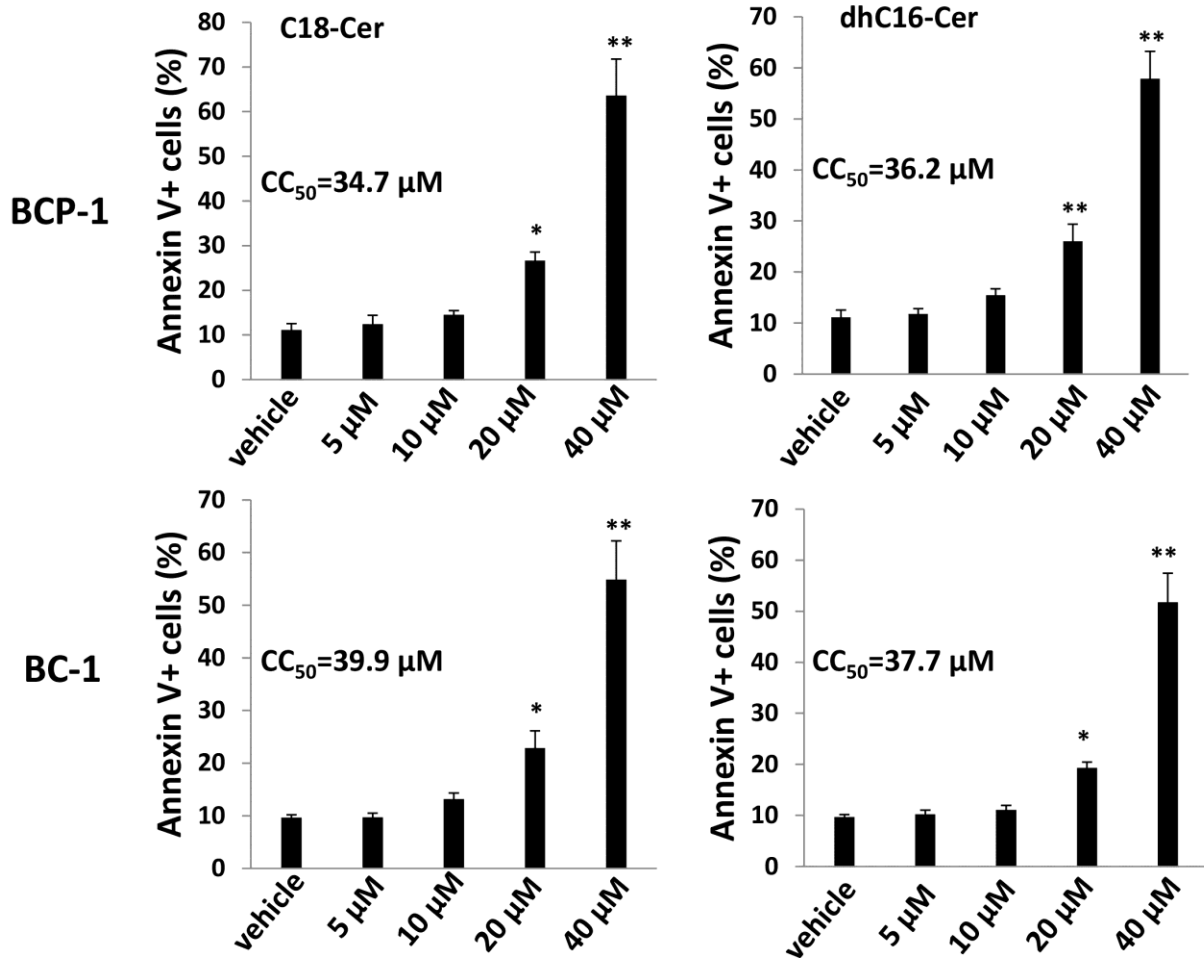


## Ceramides promote apoptosis for virus-infected lymphoma cells through induction of ceramide synthases and viral lytic gene expression

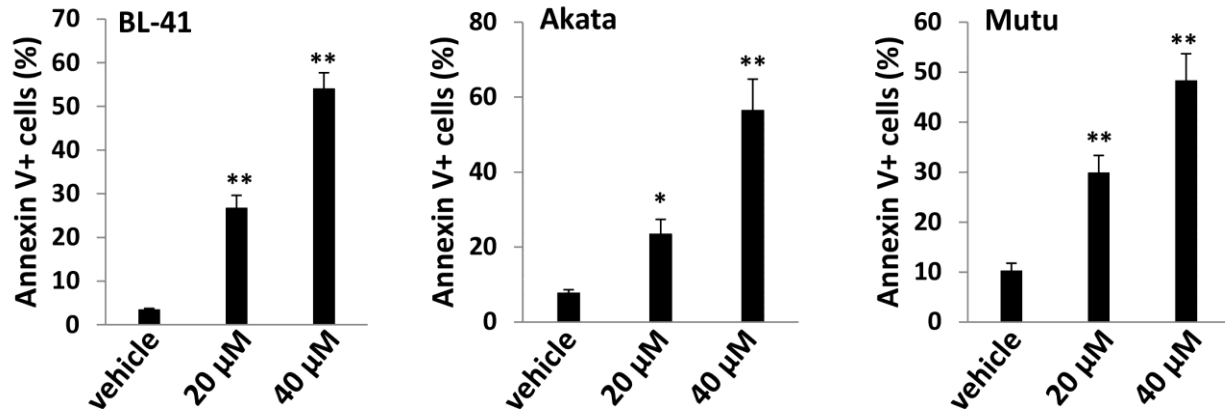
### Supplementary Material



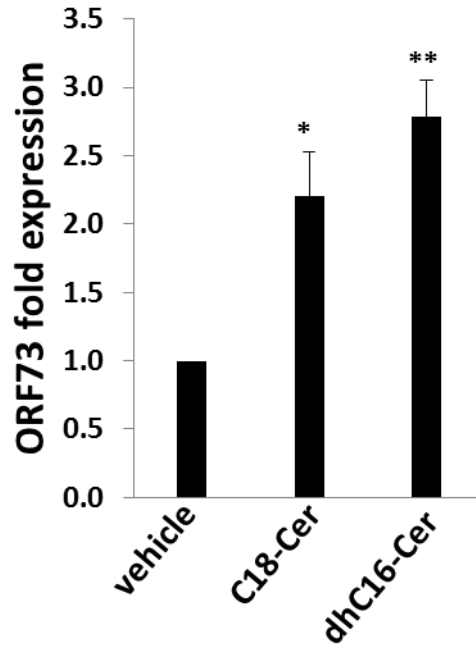
**Figure S1: Intracellular accumulation of ceramides within PEL cells *in vitro*.** (A-B) BCBL-1 cells were incubated with C18-Cer (40  $\mu$ M), dhC16-Cer (40  $\mu$ M) or vehicle for 24 h, then intracellular ceramide and dh-ceramide species quantified as described in Methods. Error bars represent the S.E.M. for 2 independent experiments, \* =  $p < 0.05$ ; \*\* =  $P < 0.01$  (vs vehicle group).



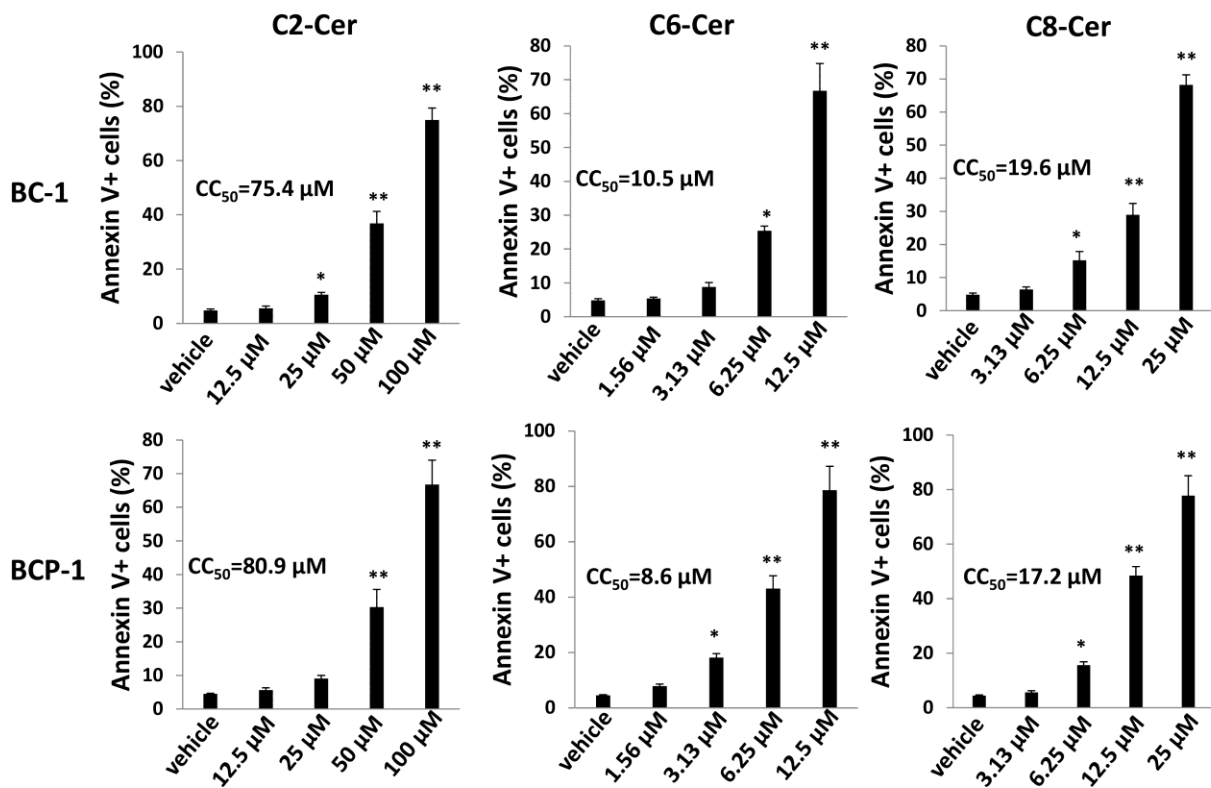
**Figure S2: C18-Cer and dhC16-Cer induce apoptosis for KSHV-infected PEL cell lines.** KSHV<sup>+</sup>/EBV<sup>neg</sup> BCP-1 and KSHV<sup>+</sup>/EBV<sup>+</sup> BC-1 cells were incubated with the indicated concentrations of C18-Cer, dhC16-Cer or vehicle for 24 h, then apoptosis was quantified as described in Methods. Error bars represent the S.E.M. for 3 independent experiments, \* = p<0.05; \*\* = P<0.01.



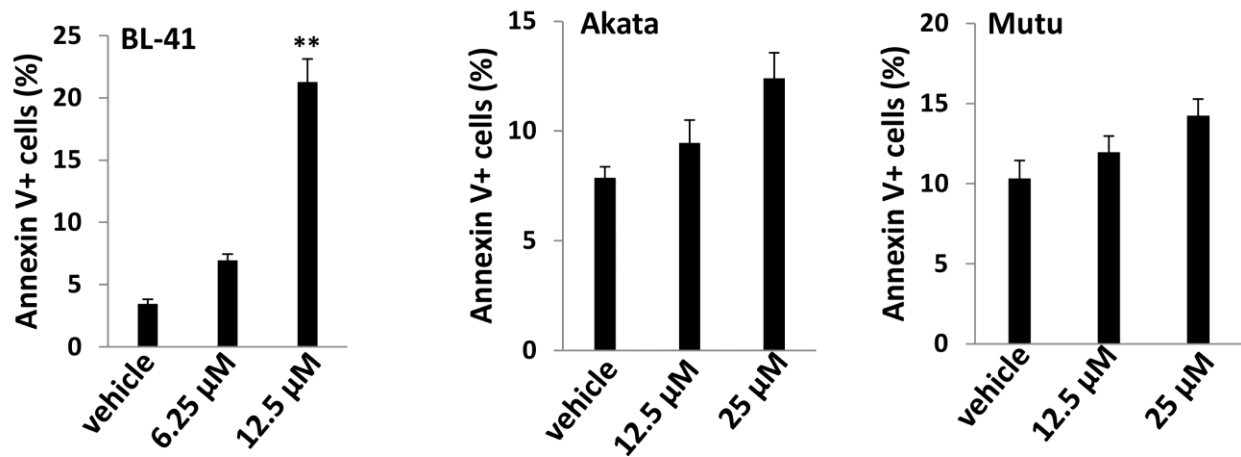
**Figure S3: dhC16-Cer induce apoptosis for Burkitt's lymphoma cell lines.** Burkitt's lymphoma cell lines BL-41 (KSHV<sup>neg</sup>/EBV<sup>neg</sup>), Akata and Mutu (both KSHV<sup>neg</sup>/EBV<sup>+</sup>) were incubated with the indicated concentrations of dhC16-Cer or vehicle for 24 h, then apoptosis was quantified as described in Methods. Error bars represent the S.E.M. for 3 independent experiments, \* = p<0.05; \*\* = P<0.01.



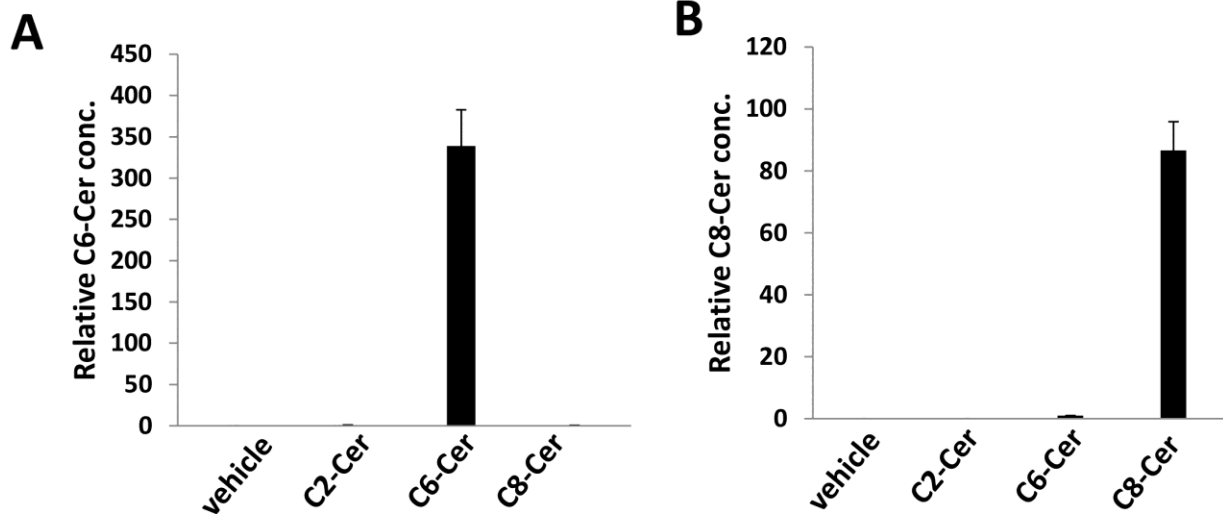
**Figure S4: Exogenous C18-Cer and dhC16-Cer induce viral lytic replication within KSHV-infected PEL cells.** BCBL-1 were incubated with C18-Cer (40  $\mu$ M), dhC16-Cer (40  $\mu$ M) or vehicle for 48 h, then the virion production within culture supernatants quantified as described in Methods followed supernatant incubation with KSHV-naïve HUVEC cells. *ORF73* (LANA) transcripts from each group were quantified by qRT-PCR. Error bars represent the S.E.M. for 3 independent experiments, \* =  $p < 0.05$ ; \*\* =  $P < 0.01$ .



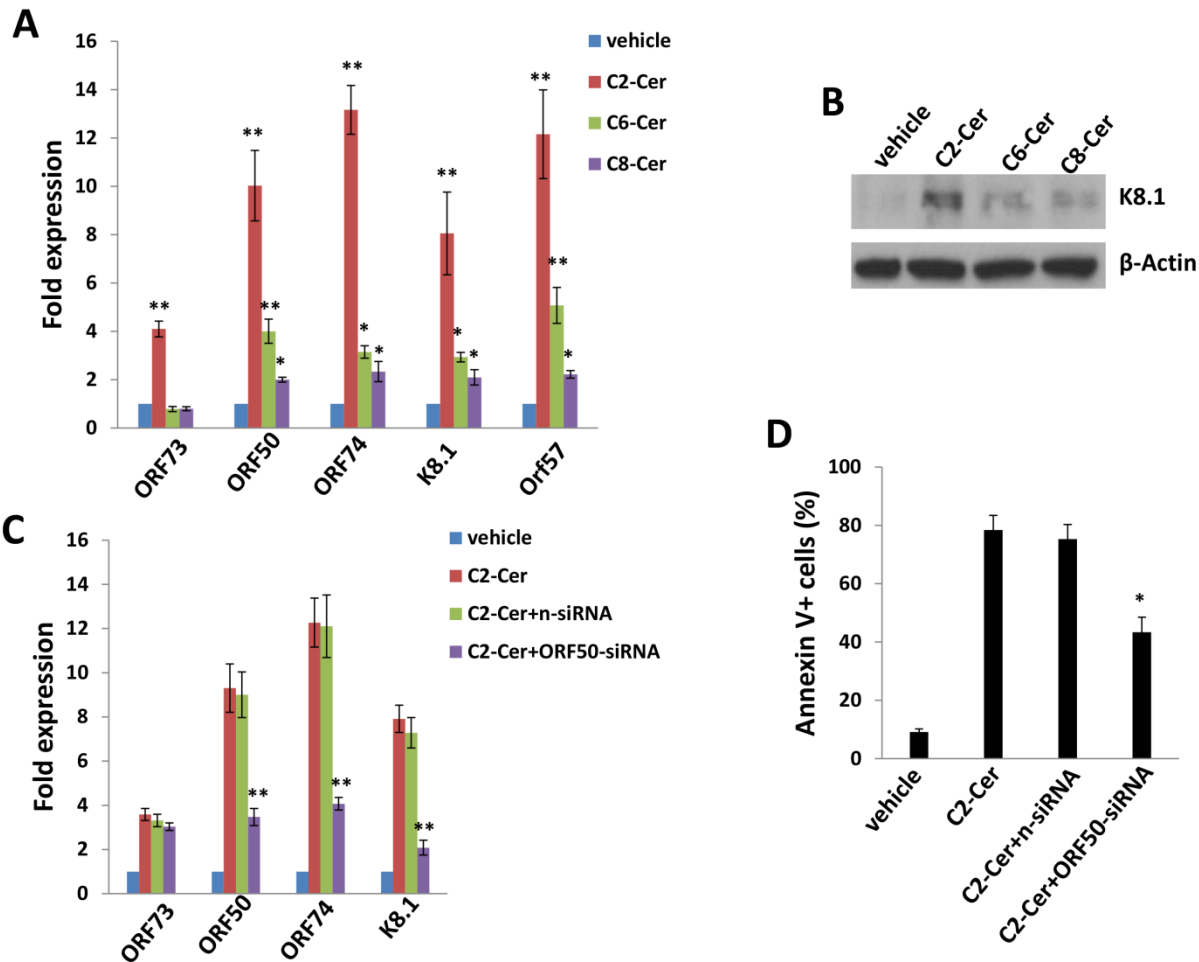
**Figure S5: Exogenous short-chain ceramides induce PEL cell apoptosis.** KSHV<sup>+</sup>/EBV<sup>+</sup> BC-1 and KSHV<sup>+</sup>/EBV<sup>neg</sup> BCP-1 cells were incubated with the indicated concentrations of C2-Cer, C6-Cer, C8-Cer or vehicle for 24 h, then apoptosis quantified as described in Methods. Error bars represent the S.E.M. for 3 independent experiments, \* = p<0.05; \*\* = P<0.01.



**Figure S6: Burkitt's lymphoma cell lines display resistance to exogenous C6-Cer-induced cell apoptosis.** KSHV<sup>+</sup>/EBV<sup>+</sup> BC-1 and KSHV<sup>+</sup>/EBV<sup>neg</sup> BCP-1 cells were incubated with the indicated concentrations of C6-Cer or vehicle for 24 h, then apoptosis quantified as described in Methods. Error bars represent the S.E.M. for 3 independent experiments, \* = p<0.05; \*\* = P<0.01.



**Figure S7: Differential accumulation of short-chain ceramides within PEL cells.** BCBL-1 cells were incubated with C2-Cer (50  $\mu$ M), C6-Cer (6.25  $\mu$ M), C8-Cer (12.5  $\mu$ M) or vehicle for 24 h, then short-chain ceramides quantified as described in Methods. Error bars represent the S.E.M. for 2 independent experiments.



**Figure S8: Exogenous short-chain ceramides induce KSHV lytic gene expression.** (A-B) BCBL-1 cells were incubated with C2-Cer (50  $\mu$ M), C6-Cer (6.25  $\mu$ M), C8-Cer (12.5  $\mu$ M) or vehicle for 24 h, then representative latent (*ORF73*) and lytic (*ORF50*, *ORF74*, *K8.1*, *ORF57*) KSHV transcripts quantified using qRT-PCR. Expression of the viral lytic protein K8.1 was determined using immunoblots. (C-D) Cells were transfected with control n-siRNA or *ORF50*-siRNA for 48 h, then incubated with C2-Cer (50  $\mu$ M) or vehicle for 24 h. Viral gene expression and cell apoptosis were quantified using qRT-PCR and flow-cytometry, respectively. Error bars represent the S.E.M. for 3 independent experiments, \* =  $p < 0.05$ ; \*\* =  $P < 0.01$ .



**Supplemental Table 1: Primer sequences for qRT-PCR.**

<b>Gene</b>	<b>Sequences (5' →3')</b>
<i>Lana</i>	<i>sense</i> TCCCTCTACACTAAACCCAATA <i>antisense</i> TTGCTAATCTCGTTGTCCC
<i>Rta</i>	<i>sense</i> TAATGTCAGCGTCCACTCC <i>antisense</i> TTCTGGCACGGTCAAAGC
<i>vGPCR</i>	<i>sense</i> CATCCGCTGCACTGTAA <i>antisense</i> GCTTTGTCCTCCTCACCA
<i>K8.1</i>	<i>sense</i> CACCACAGAACTGACCGATG <i>antisense</i> TGGCACACGGTTACTAGCAC
<i>Orf57</i>	<i>sense</i> GGGTGGTTTGATGAGAAGGA <i>antisense</i> CGCTACCAAATATGCCACCT
<i>β-actin</i>	<i>sense</i> GGAAATCGTGCGTGACATT <i>antisense</i> GACTCGTCATACTCCTGCTTG