

Supplementary Figures

Figure S1

A. Immunofluorescence images showing the localization of GFP and GFP-KSHV-TK in COS and BHK cells. **B.** GFP-HSV1-TK does not induce cell contraction or loss of phospho-tyrosine and phospho-paxillin Y31 epitopes from focal adhesions. **C.** Transmission electron micrographs of HeLa cells expressing GFP or GFP-KSHV-TK. **D.** Treatment of HeLa cells with the cell-permeable caspase inhibitor, Z-VAD-FMK does not inhibit GFP-KSHV-TK induced cell contraction. **E.** GFP-KSHV-TK but not GFP induces the formation of actin stress fibres and cell rounding. Quantification of GFP-KSHV-TK induced cell contraction. The graph shows the percentage of cells with flattened morphology (Flat), flattened morphology with central actin stress fibers (Flat + SF), and rounded morphology calculated from 3 independent experiments in which n= 215 for GFP and n= 218 for GFP-KSHV-TK. Scale bars = 20 μ m.

Figure S2

A. Immunofluorescence images showing that Y-27632 and Blebbistatin treatment does not induce cell contraction or loss of phosphotyrosine, phospho-FAK-Y397, Y925 and phospho-paxillin-Y31 epitopes from focal adhesions. **B.** GFP-KSHV-TK but not GFP leads to a loss of the phospho-FAK Y397/Y861 and phospho-Paxillin Y118 signals at focal adhesions. **C.** The graphs represent the total cell fluorescence when staining for phospho-Paxillin Y31 or phospho-FAKY925 in cells expressing GFP or GFP-KSHV-TK. Only transfected cells were quantified. The error bars represent SEM from 3 independent experiments in which n= 123 for GFP and n= 126 for GFP-KSHV-TK when staining for phospho-Paxillin Y31 and phospho-FAKY925. Immunoblot analysis showing that GFP-KSHV-TK but not GFP leads to reduced Paxillin-Y31 and FAK-Y925 phosphorylation. Scale bars = 20 μ m.

Figure S3

A. GFP-KSHV-TK induces the loss of FAK, Paxillin and Zyxin from focal adhesions. White arrowheads indicate cells expressing GFP-KSHV-TK. **B.** Expression of GFP-KSHV-TK but not GFP leads to a loss of Parvin, VASP and Vinculin from focal adhesions. **C.** Expression of untagged KSHV-TK or RFP-KSHV-TK induces the loss of GFP-FAK from focal adhesions. Scale bars = 20 μm .

Figure S4

A. HeLa cells infected with MuHV-4 or MuHV-4 [g-KSHV-TK] labelled with the indicated antibodies reveals that both viruses express early and late lytic proteins. **B.** Immunofluorescence analysis of phosphotyrosine staining in HeLa cells infected with KSHV-lyt in the presence of absence of Y-27632. Scale bars= 20 μm .

Figure S5

GFP-KSHV-TK-DEAD does not induce loss of paxillin, phospho-paxillin (Y118), Parvin, VASP or Vinculin from focal adhesions. Scale bars = 20 μm .

Figure S6

A. Immunoblot analysis of Y to F mutants demonstrates that Y65 and Y85 of KSHV are phosphorylated. **B.** Immunofluorescence images showing that the Y2F mutant does not induce cell contraction or loss of phosphotyrosine epitopes from focal adhesions. **C.** Immunofluorescence images showing that the Y2F mutant does not induce loss of phospho-Paxillin Y31 or phospho-FAK Y925 epitopes from focal adhesions. **D.** Expression of GFP-KSHV-TK but not GFP induces contraction of zyxin labelled FAK^{+/+} cells. Scale bars = 20 μm .

Figure S7

Partially purified GST-KSHV-TK fractionated and analysed by LC-MS/MS to map phosphorylated peptides. KSHV-TK showing auto-phosphorylation of tyrosine residues 65, 85 and 120. The identity of each peptide and phosphorylation sites is shown in the manual interpretation of the spectra.

Supplementary Movies

Movie S1. The movie shows GFP-KSHV-TK (top panel) induced contraction of a HeLa cell in phase contrast (bottom panel) over a period of 2140s. The movie starts 16 hours after the cell was first transfected with GFP-KSHV-TK.

Movie S2. The movie shows that GFP (top panel) does not induce contraction of HeLa cells in phase contrast (bottom panel) over a 1138s. The movie starts 16 hours after the cells were first transfected with GFP.

Movie S3. A phase contrast movie of a HeLa cell undergoing GFP-KSHV-TK induced contraction and blebbing over a period of 620s. The movie starts 18 hours after the cells were first transfected with GFP-KSHV-TK.