Supplemental Fig 1. Cytopathic effect of WR and vvDD (WR ΔTK ΔVGF) on a panel of human tumor cell lines. The MOI of virus (PFU/cell) needed to reduce the cell viability to 50% of untreated control wells (ED50) at 72h following infection were plotted.
Supplemental Figure 2. Athymic CD1 nu/nu mice bearing subcutaneous human HCT 116 tumors were treated with $1 \times 10^7$ PFU of vaccinia strains via tail vein injection. Viral strains (WR and vvDD) expressed luciferase, and the subsequent biodistribution of viral gene expression after 96h was detected by bioluminescence imaging in an IVIS100 system (Caliper, Mountain View, CA) following addition of the substrate luciferin.
Supplemental Figure 3. Transient secondary spread of vvDD from infected tumors. Athymic CD1 nu/nu mice bearing subcutaneous human HCT 116 tumors were treated as in Fig 6 with $1 \times 10^7$ PFU of vaccinia strain vvDD expressing luciferase via tail vein injection. Subsequent biodistribution of viral gene expression was detected by bioluminescence imaging in an IVIS100 system (Caliper, Mountain View, CA) following addition of the substrate luciferin at the times indicated after treatment. Circles highlight areas of transient secondary infection following release of virus from the primary site of replication (tumor).
Supplemental Figure 4. Viral genomes released into the blood following primary infection. Mice (C57/B6) bearing subcutaneous MC38 tumors, or tumor-free controls, were treated IV with $1 \times 10^8$ PFU of JX-963. Viral genome copies in the blood were detected by subsequent bleeding and RT-PCR (n=3/group).
Supplemental Figure 5. *Ex vivo* infection of primary surgical specimens with vvDD backbone expressing GFP (vvDD-GFP). Tumor specimens and adjacent normal tissue were exposed to vvDD-GFP or PBS for 45 minutes. Fluorescence was examined by fluorescence microscopy 24h later.
Supplemental Figure 6. GM-CSF production in treated rabbits. Rabbits bearing hepatic VX2 tumors as described in the methods (and Fig 8) or tumor-free control animals were treated with a single ear vein injection of $1 \times 10^8$ PFU of viruses JX-594 (Wyeth, TK deleted, expressing human GM-CSF) or JX-963 (vvDD expressing GM-CSF), and at times post treatment blood was sampled and GM-CSF levels in the plasma determined by ELISA (n=3 per group).
Supplemental Figure 7. CTL assay targeting VX2 tumor cells. CTL assay was performed by FACS analysis using pre-labeled VX2 cells mixed with 12.5x; 25x and 50x unlabelled peripheral blood lymphocytes from rabbits bearing VX2 tumors and treated with JX-963; from untreated animals with VX2 tumors; and from naïve animals. Cell death was quantified by the ACT1 assay (Cell Technology, Mountain View)(n=3/group).
Supplemental Figure 8. Responses in tumor-bearing pre-immunized rabbits treated with JX-963. Rabbits (n=4) implanted with VX2 tumors and treated with JX-963 (as in Fig 8) were re-treated 42 days after the last treatment (day 42), when tumors had all relapsed. Animals then underwent a further CT scan 7 days later. It was seen that 3 of 4 animals displayed some subsequent tumor regression.