

## Legends to Supplemental Figures

**Figure S1** Schematic presentation of vA34R, vvDDr and parental virus vvDD. vA34R virus has the single point mutation K151E in the A34R gene followed by DsRed marker gene. vvDDr has the wild type A34R gene followed by DsRed. (a). vvDD; (b). vA34R, and (c). vvDDr.

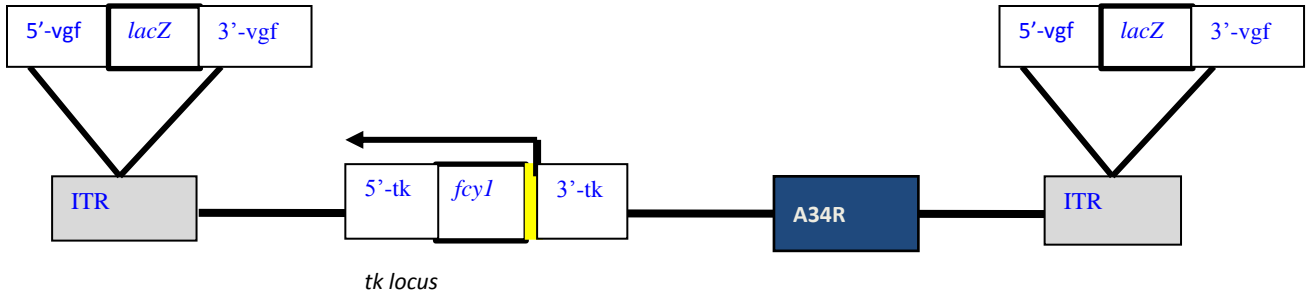
**Figure S2** Infectivity, yield of the viruses and cytotoxicity in MC38 cancer cells and CV-1 cells. The MC38 cells in 6-well plates were infected at MOI's of 0.01, 0.1 and 1, 5, and 25 for 12 h, and cells were harvested and cells were homogenized and infectious viruses were tittered by plaque assays. For levels of infection in MC38 cells, similarly infected MC38 cells for 12 h were harvested and then randomly sorting one cell per well onto CV-1 cells in 96-well plates. The expression of DsRed at 48 h on 96-well CV1 plates serves as good indicator of the original targeted cells being infected. There is a significant difference in the percentage of infectivity for vA34R compared to vvDDr. (a). The yields of viruses in MC38 cells infected for 12 h. (b). The infectivity of MC38 cells as determined at 12 h post infection. (c). MC38 cancer cell viability at 48 h post infection was determined by MTS assay.

**Figure S3** The infectivity of vvDDr and vA34R in CV1 cells (a) and MC38 cancer cells (b) at higher MOIs. The assay was done as indicated in Supplemental figure 2, but with higher MOIs. The levels of infection for both vvDDr and vA34R were determined at 12 h for MOI's of 1, 5 and 10, by randomly sorting one cell per well onto CV-1 cells in 96-well plates. The expression of DsRed at 48 h on CV1 cells in 96-well plates is expressed as a percentage for both of the viruses. There is a significant difference in the percentage of infectivity for vA34R compared to vvDDr. The yields of the virus (PFU/ml) in the infected cells were presented on the right panels.

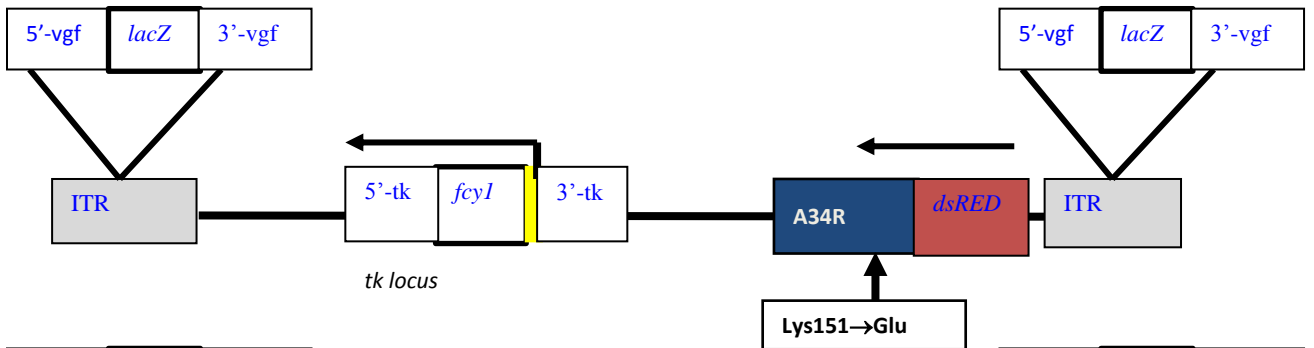
# Supplemental Figure 1.

Schematic drawing of the three recombinant vaccinia Viruses a. vvDD; b. vA34R; c. vDDr.

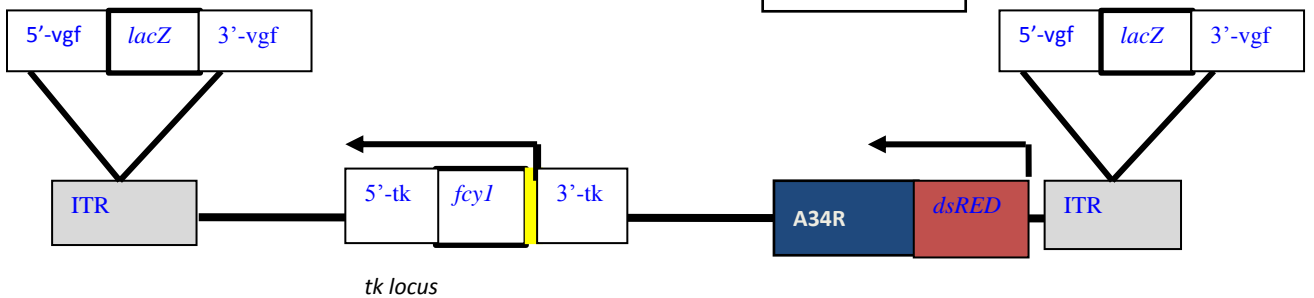
a.



b.



c.



# Supplemental Figure 2

