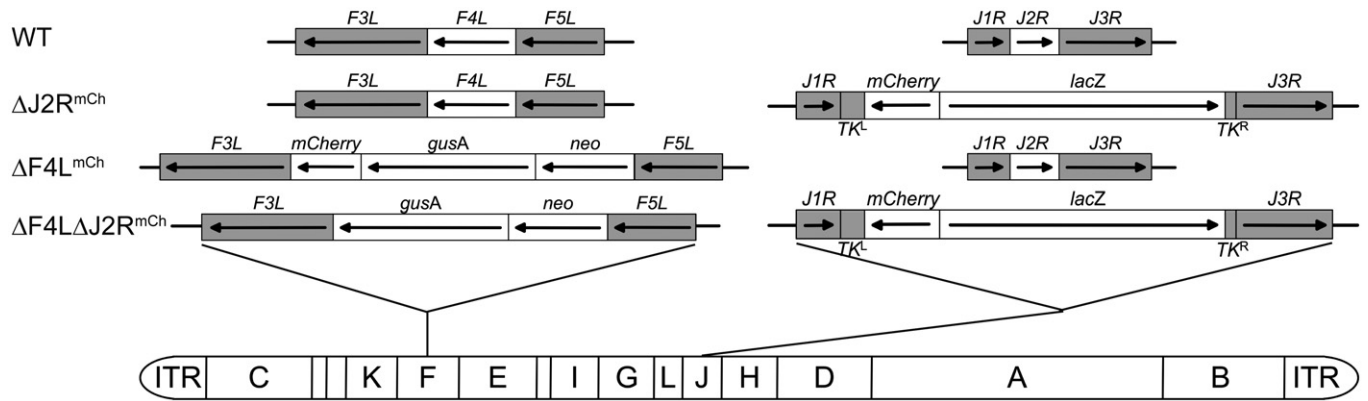
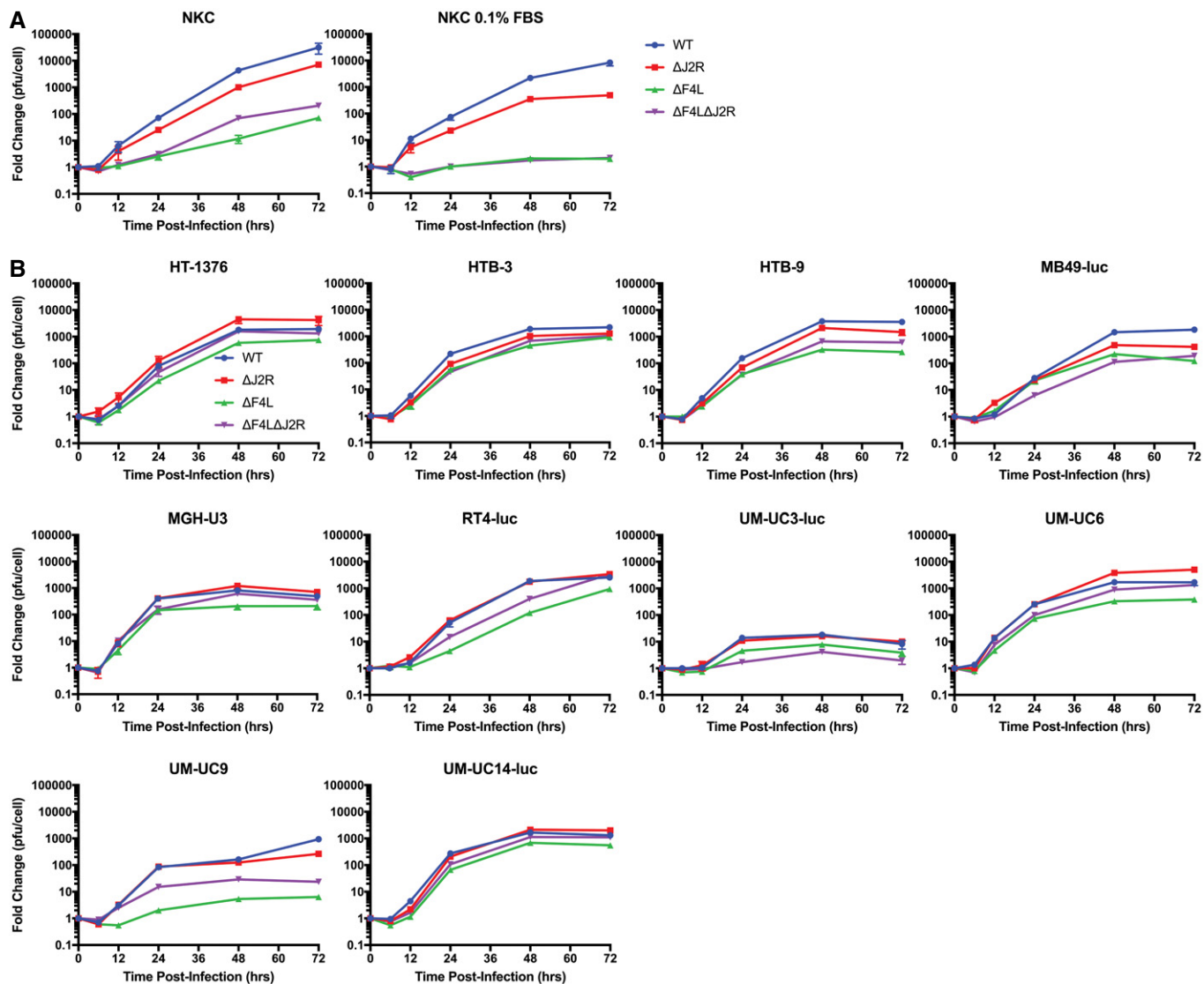


## Expanded View Figures



**Figure EV1. Genomic map of vaccinia virus constructs.**

Viruses were generated from the VACV Western Reserve strain. Viral thymidine kinase is encoded by the *J2R* gene. Subunit 2 of viral ribonucleotide reductase is encoded by the *F4L* gene. *neo*, neomycin gene; *gusA*,  $\beta$ -glucuronidase gene; *lacZ*,  $\beta$ -galactosidase gene; ITR, inverted terminal repeat; *TK<sup>L</sup>*, viral thymidine kinase gene left homology; *TK<sup>R</sup>*, viral thymidine kinase gene right homology; and WT, wild-type.



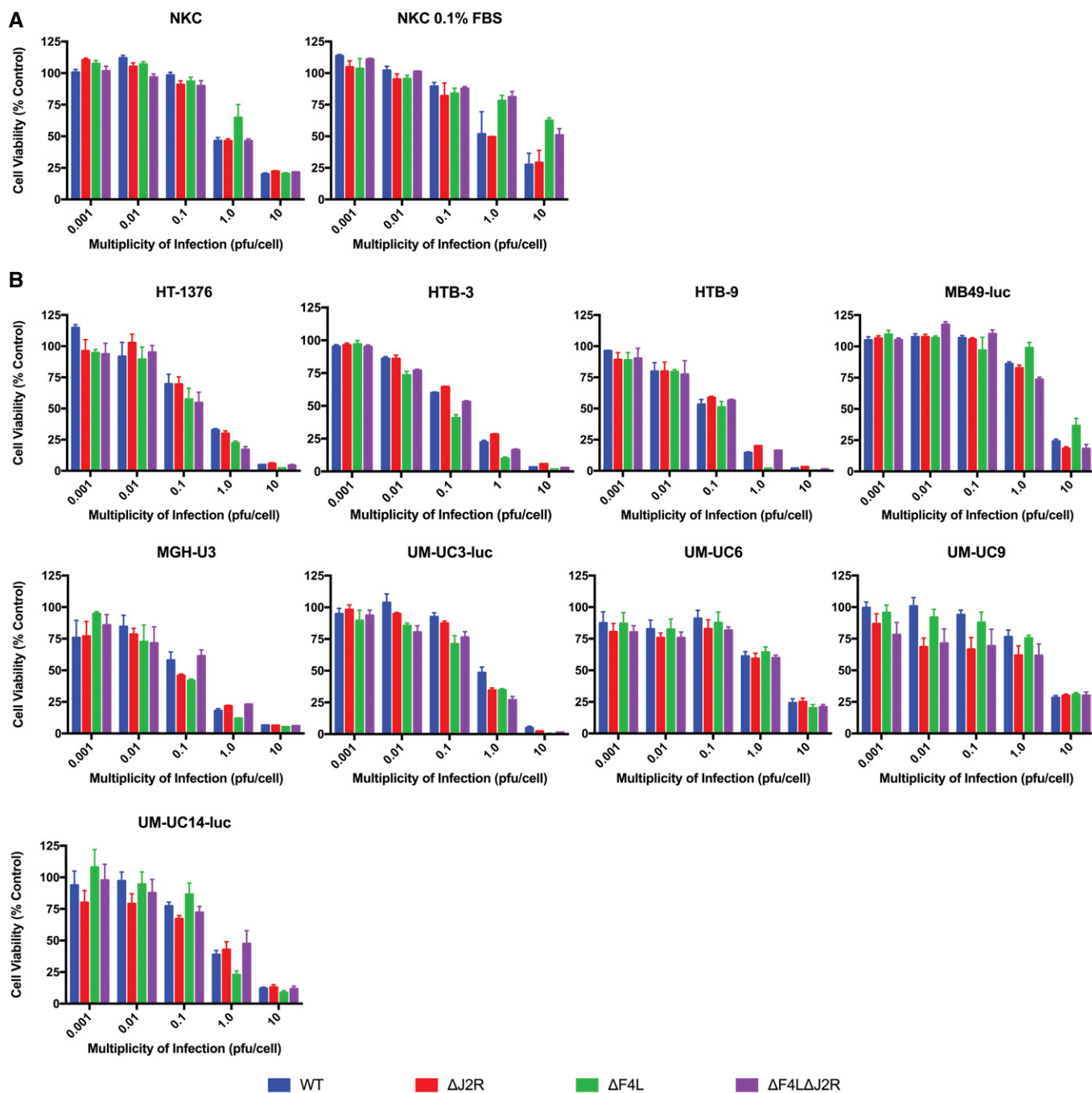
**Figure EV2.**  $\Delta F4L\Delta J2R$  VACV retains much of the replication proficiency of wild-type VACV in bladder cancer cells.

Growth curves for the indicated VACV mutants or WT VACV. Subconfluent cells were infected at a multiplicity of infection of 0.03 PFU/cell. Cultures were harvested at the indicated times and titered on BSC-40 cells.

A Normal human kidney epithelial cells grown under normal serum conditions (left) and 0.1% FBS (right).

B Panel of human bladder cancer cell lines (exception: MB49-luc, murine urothelial carcinoma) cultured *in vitro* with 10% FBS.

Data information: Mean  $\pm$  SEM is shown and data represent at least two independent lysates titered in duplicate.



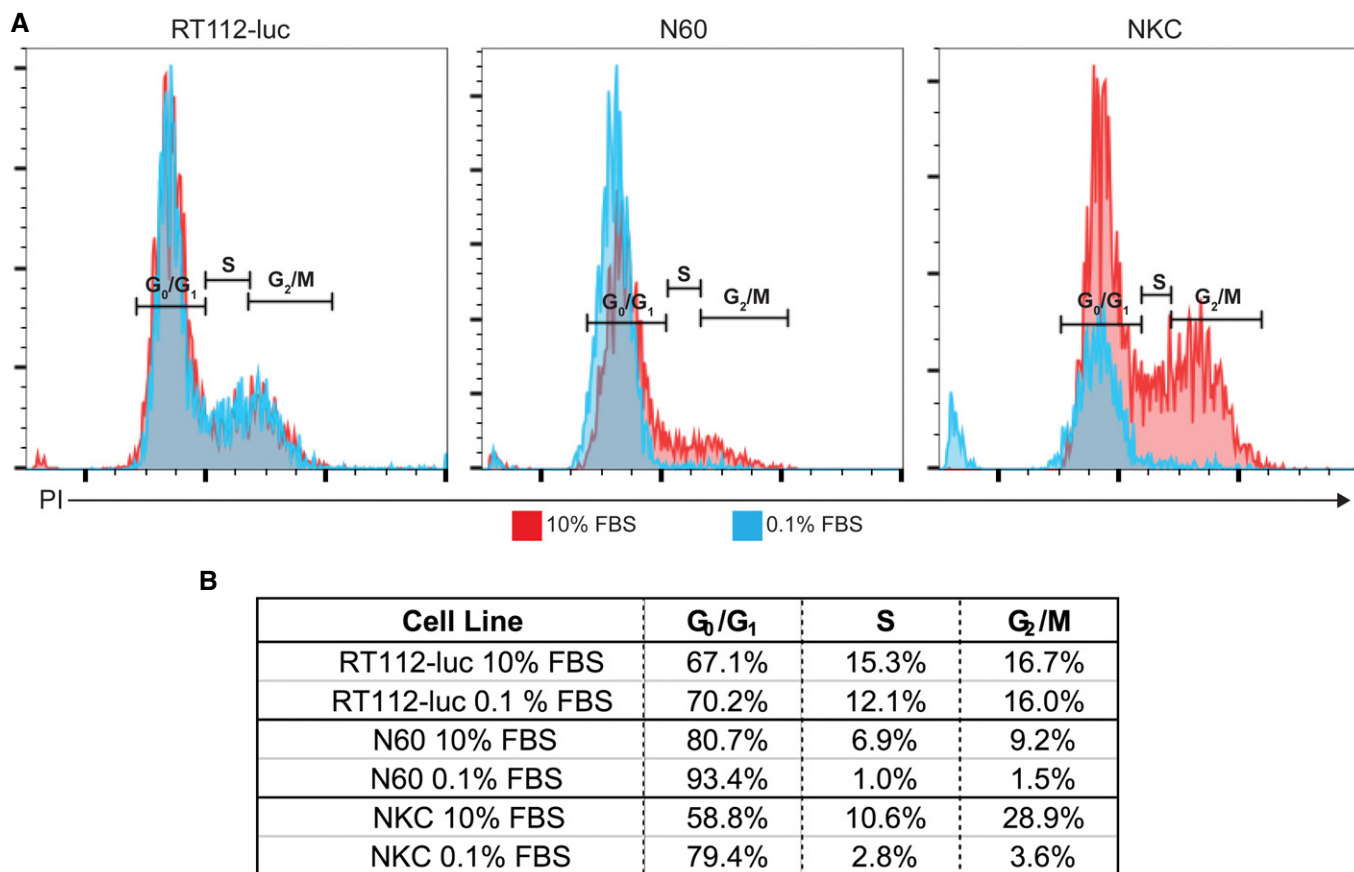
**Figure EV3.**  $\Delta F4L\Delta J2R$  VACV retains much of the cytotoxicity of wild-type VACV in bladder cancer cells.

Survival of cell lines infected *in vitro* with the indicated VACV strains. Subconfluent cells were infected at the indicated multiplicities of infection (in PFU/cell). Uninfected cells were used as control.

A Normal human kidney epithelial cells grown under normal serum conditions (left) and 0.1% FBS (right).

B Panel of human bladder cancer cell lines (exception: MB49-luc, murine urothelial carcinoma) cultured *in vitro* with 10% FBS. The cells were incubated with resazurin to assess viability 3 days post-infection relative to uninfected control cells. Uninfected cells were used as control.

Data information: Mean  $\pm$  SEM is shown and  $n \geq 3$ .



**Figure EV4. Non-tumorigenic cells have reduced S-phase population when grown under lower serum conditions.**

Cell cycle analyses of indicated cell lines.

A Indicated cells lines were grown in media supplemented with either 10% FBS or 0.1% FBS for 48 h, and then cell cycle distribution was monitored by flow cytometry after PI staining. Red traces indicate cells grown in 10% FBS and blue traces indicate cells grown in 0.1% FBS.

B Analysis of cell cycle phase distribution.