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Supplemental Information

Oncolytic Immunotherapy for Bladder Cancer Using Coxsackie A21 Virus

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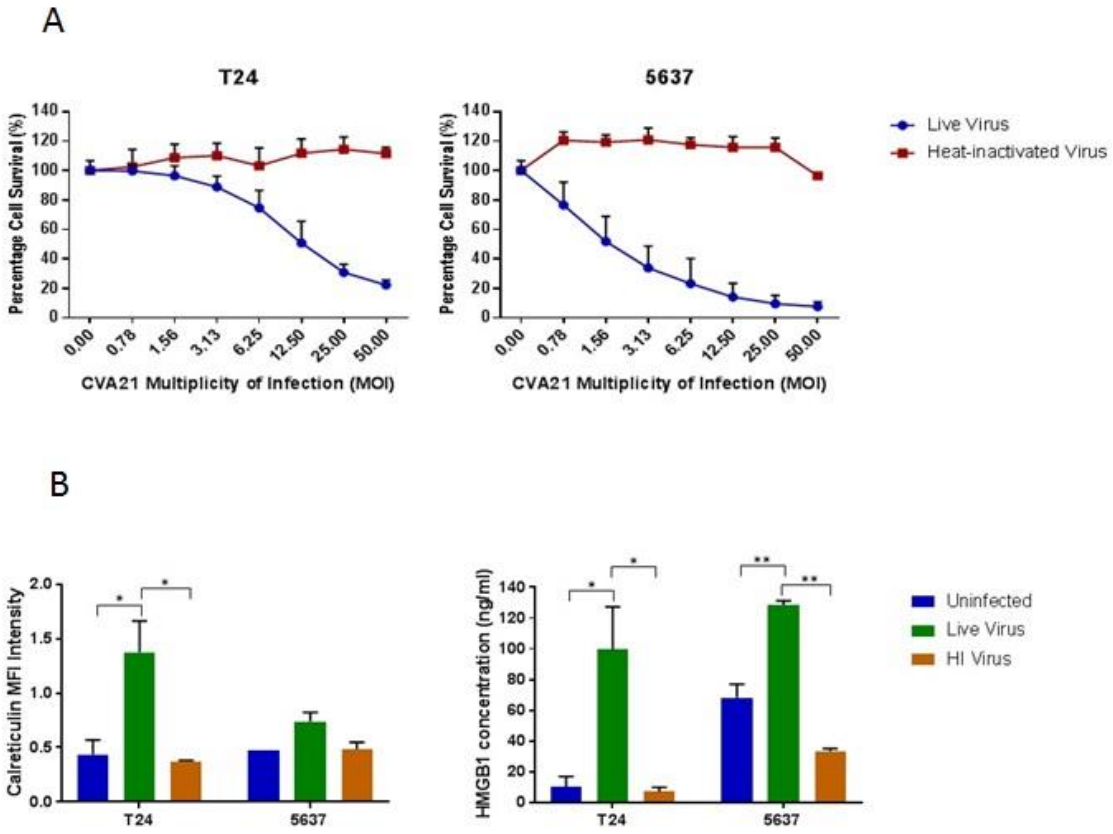
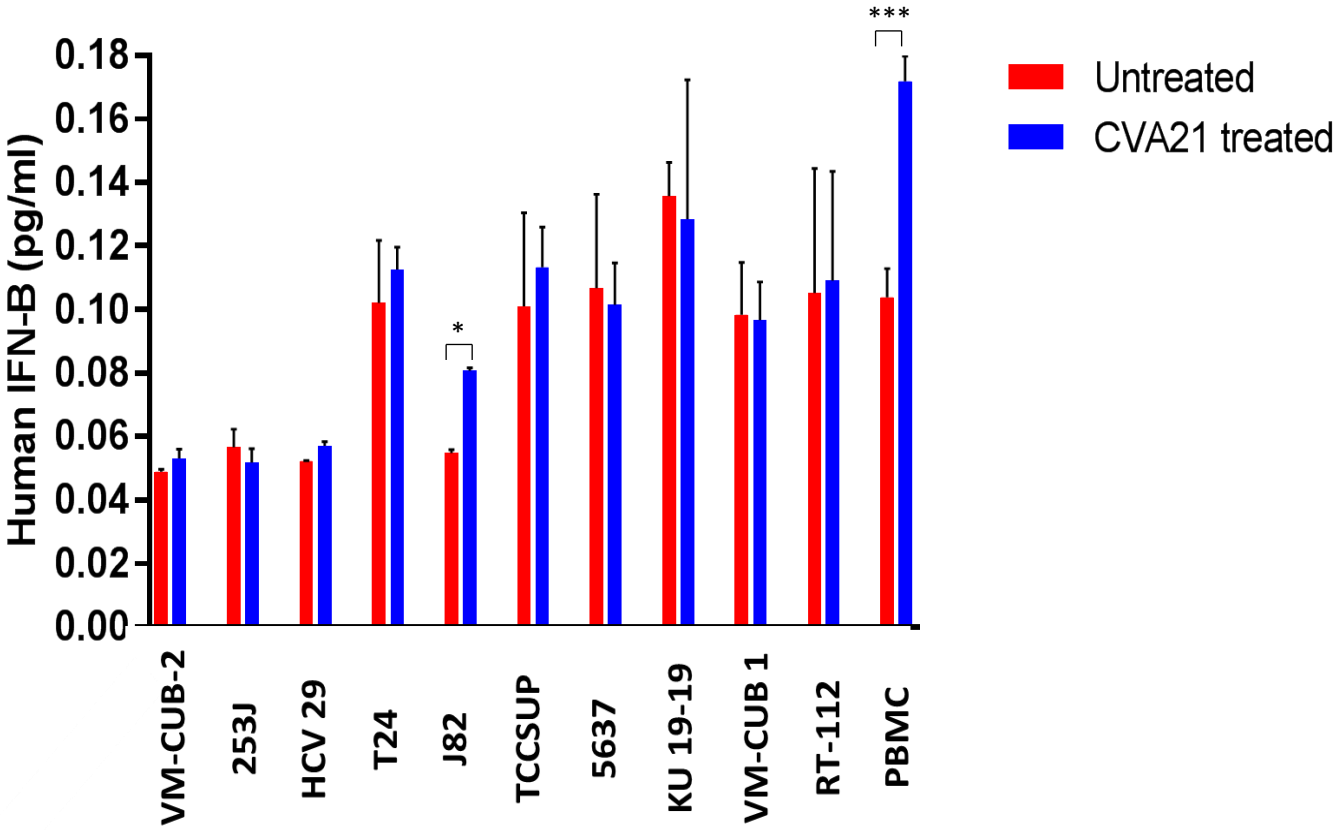


Sup Fig 1. Heat-inactivated CVA21 does not affect cell viability or induce immunogenic cell death

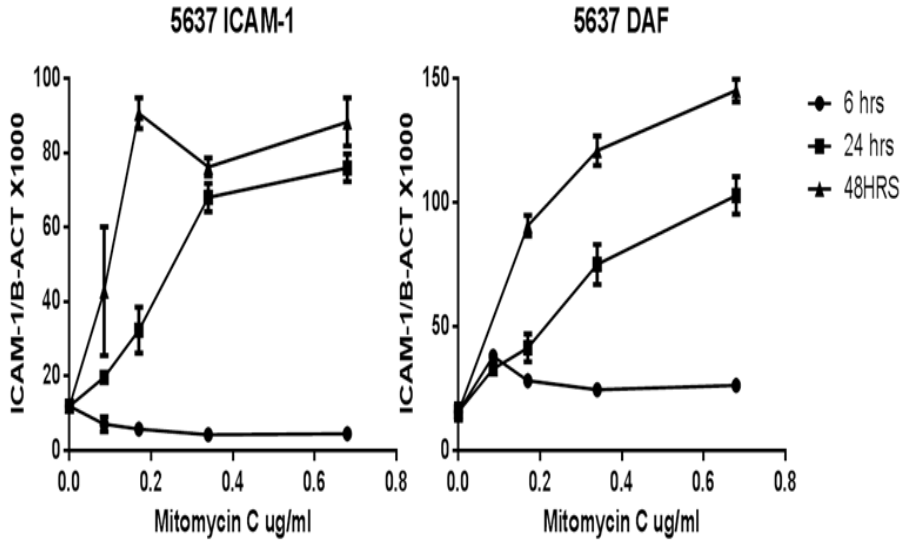
(A)Monolayer cultures of the human bladder cancer cells T24 and 5637 were challenged with increasing multiplicities of live and heat-inactivated CVA21 and assessed for cell survival at 72h post-infection, with live cells being quantified by MTS assay.

(B) The bladder cancer cell lines, T24 and 5637 were either left untreated or treated with live CVA21 or heat-inactivated CVA21. Cells and supernatants were harvested at 72h post treatment. Cell surface calreticulin (CRT) exposure was determined by FACS analysis of the cells whilst HMGB1 accumulation was determined by ELISA analysis of supernatants. Graphs represent pooled data from two independent experiments and the statistical significance of the treatment group comparisons was analysed using one-way ANOVA *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$.



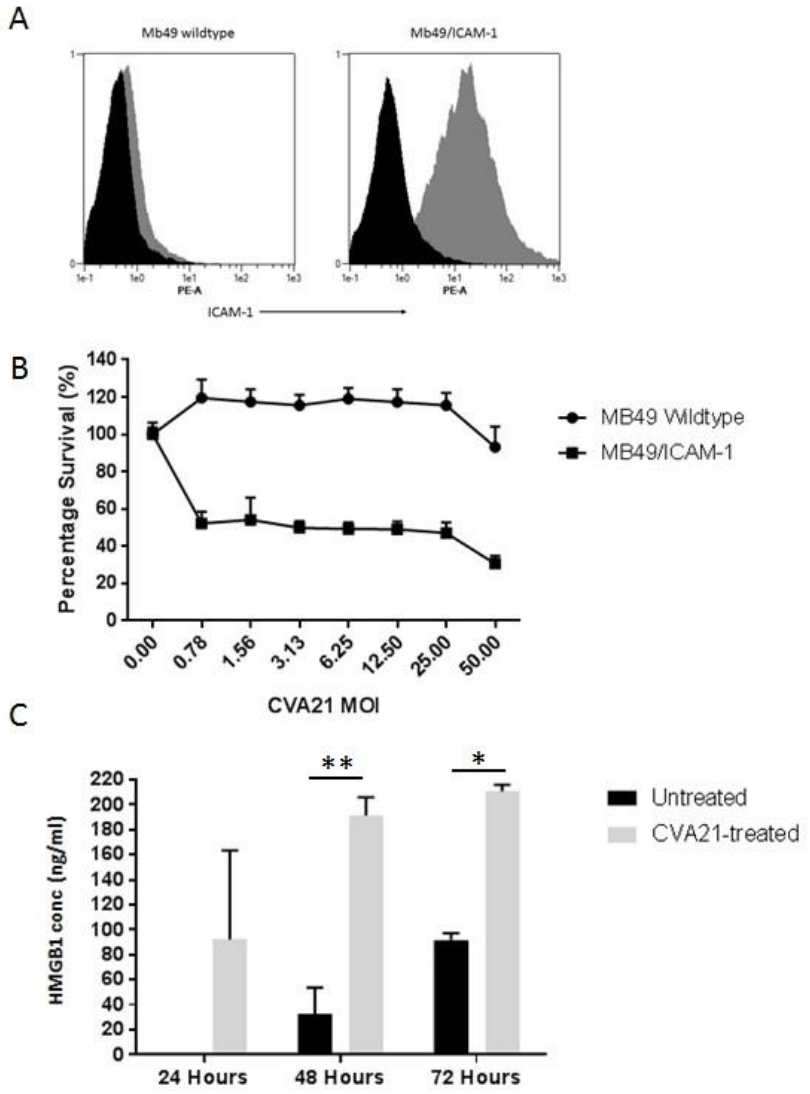
Sup Fig 2. Interferon Beta anti-viral response was absent in all bladder cancer cell-lines except J82.

All bladder cancer cell-lines were treated with CVA21 for 24 hours and supernatant was harvested. The expression of the IFN β antiviral cytokine was determined by sandwich ELISA. All cell-lines displayed a lack of IFN β secretion after CVA21 treatment except J82 which showed a significant increase in IFN β post CVA21 treatment. PBMC was used as a positive control and showed a significant increase in IFN β production. Graphs represent pooled data from two independent experiments and one-way Anova statistical analysis was performed *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$.



Sup Fig 3. Increased expression of ICAM-1 on bladder cancer cell lines in response to increasing doses of Mitomycin C

The 5637 cell line was treated for 6, 24 and 48hrs with doubling dilutions of Mitomycin C up to 0.8μg/ml and RNA extracted. The effect of this treatment on viral receptor gene expression, ICAM-1 (A) and DAF (B) was determined by QPCR and expressed as a ratio to the amount of expression of the housekeeping gene, β-actin.



Sup Fig 4. MB49 cells transfected with hICAM-1 are susceptible to the virus and display induction of the ICD determinant HMGB1

(A)Expression of ICAM-1 on the MB49 wildtype and ICAM-1 transfected cell line (MB49/ICAM-1). Cells were incubated with either the relevant PE-conjugated isotype control antibody (black histogram), or anti-ICAM-1 monoclonal antibody (grey histogram) (B)Transfection of MB49 cells with human ICAM1 resulted in the cell line becoming susceptible to CVA21 infection compared to the wildtype MB49 as quantified by MTS assay . (C) Selective induction of HMGB1 by CVA21-treated MB49 cells determined by ELISA analysis of supernatants harvested at 24, 48 and 72h post-infection. Data represents averaged results from two independent experiments and two-way ANOVA statistical analysis was performed. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$.