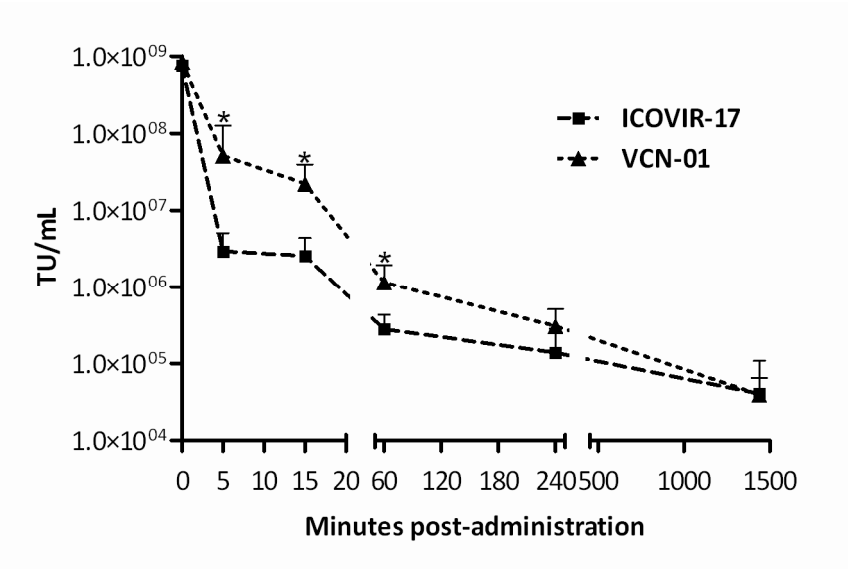
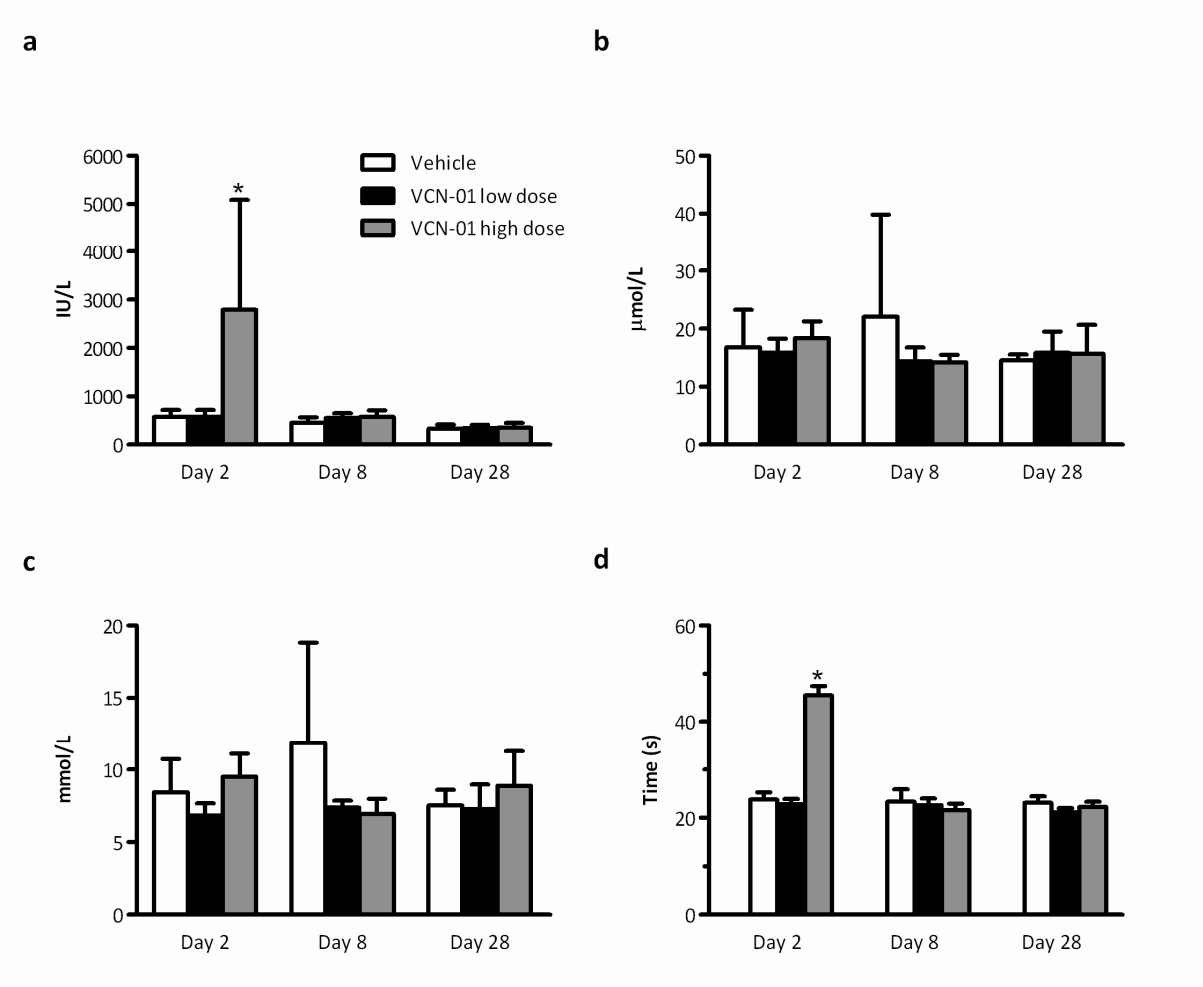
SUPPLEMENTARY DATA



Virus	Half-time (min)
ICOVIR-17	2.11
VCN-01	3.15*

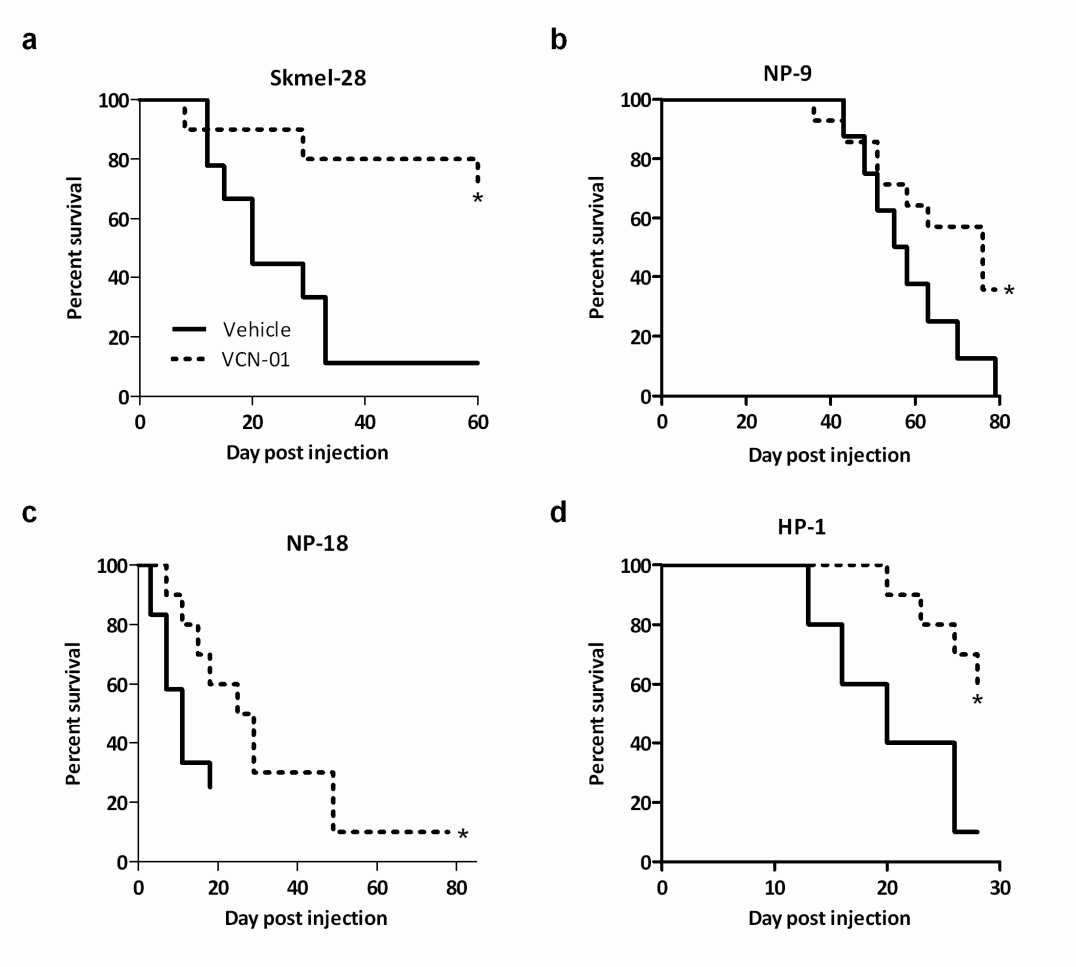
Supplementary Figure S1. Pharmacokinetic profile of VCN-01 in immunocompetent mice.

Balb/C mice were injected with vehicle or $5x10^{10}$ viral particles of VCN-01 or ICOVIR-17. At indicated time points, blood samples were collected and each sera was analyzed for virus presence according to an anti-hexon staining based method. Mean +SD is plotted (n=7 animals/group). Half-time for each virus was calculated. *, significant $p<0.05$ by two-tailed unpaired Student's *t*-test compared with ICOVIR-17 group.



Supplementary Figure S2. Toxicity after systemic administration of VCN-01 in immunocompetent Syrian hamster.

The average values for (a) alkaline phosphatase levels (ALP), (b) creatinine, (c) urea, and (d) prothrombine time (PTT) in peripheral blood of hamsters at indicated time points after intravenous administration of $2.5x10^{11}$ (low dose) or $4x10^{11}$ (high dose) viral particles of VCN-01 are shown. Mean values +SD are depicted. *, VCN-01 high dose significant ($p<0.05$) by Kruskall-Wallis test, compared to vehicle group. IU, International units.



Supplementary Figure S3. Kaplan-Meier survival curves after intratumoral administration of VCN-01.

Nude mice bearing subcutaneous xenografts of (a) Skmel-28, (b) NP-9 or (c) NP-18 and Syrian hamsters bearing (d) HP-1 tumors were injected with a single intratumoral administration of vehicle or $2x10^9$ (in Skmel-28 and NP-9), $4x10^9$ (in NP-18) or $2x10^{11}$ (in HP-1) viral particles of VCN-01. The end-point was established at a tumor volume of ≥500mm^3 for mice and ≥1500mm^3 for hamsters. * Significant ($p<0.05$) by log-rank test compared with vehicle group.

Supplementary Material and Methods

Fluorescence *In Situ* Hybridization (FISH)

To generate an adenoviral probe, we used the adenovirus ICOVIR15K, which is identical to VCN-01 except for the lack of the human sperm hyaluronidase gene. Briefly, viral ICOVIR15K DNA was purified and labelled with SpectrumRed dUTP (VYSIS) by standard nick translation (Abbott Molecular). Ovary tissue samples were obtained from the GLP toxicity study performed in Syrian hamsters and were analysed by FISH to detect VCN-01 DNA. Formalin-fixed paraffin-embedded tissue sections were deparaffinised in changes of xylene, rehydrated in decreasing concentrations of ethanol and digested with 50µg/ml proteinase K in 10mM Tris pH 8, 1mM EDTA (Sigma-Aldrich) for 8-12 min at 70 ºC. 5µl (100ng DNA) of labelled adenovirus probe was applied to each tissue section, covered, sealed and denaturated at 80 ºC for 6 min prior to incubation at 37 ºC overnight. Next day, slides were washed and counterstained with DAPI mounting medium (Vectashield, Vector Labs). Liver samples were used as positive and negative controls for VCN-01 infection. Tissue structure was verified by Haematoxylin and Eosin staining.