

SUPPLEMENTARY MATERIAL

A Standardized BSL-2 Workflow for Orthohantavirus Production, Detection, and Antiviral Screening

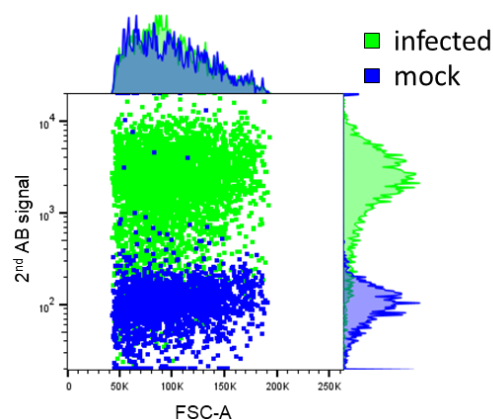


Figure S1: Specific detection of PUUV N protein in infected VeroE6 cells. VeroE6 cells were infected with PUUV for 72 hours and stained with an anti-N antibody followed by AlexaFluor 647-conjugated secondary antibody. Infected cells (green) show elevated A647 signal compared to uninfected controls (blue), confirming specific intracellular detection of the N protein.

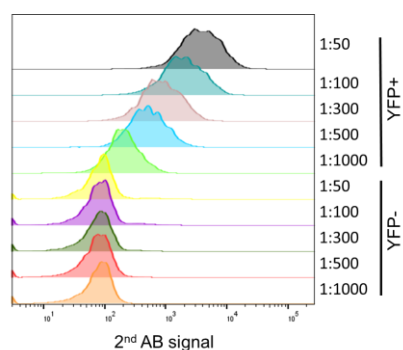


Figure S2: Antibody validation and optimization. HEK 293T cells were transfected with N-YFP (Welke et al., 2022) and stained with A1C5-C, followed by AlexaFluor 647-conjugated secondary antibodies. Histograms show AlexaFluor 647 fluorescence of transfected cells, gated for YFP+ (top) and YFP- (bottom) cells, stained with different dilutions of A1C5-C.

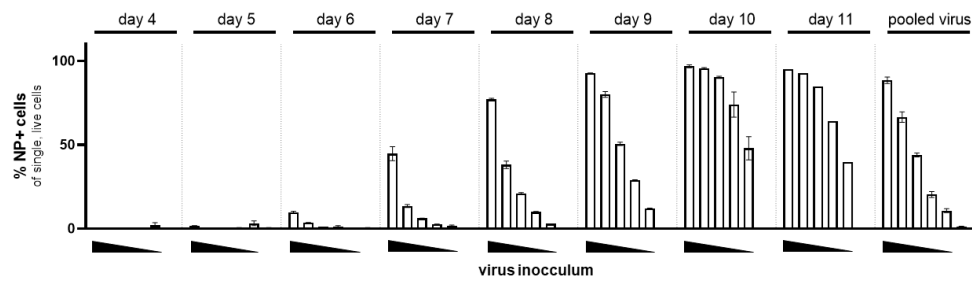


Figure S3: Generation and titration of Orthohantavirus stocks. Representative titration profiles of SNs collected on days 4–12 as well as of pooled virus stocks from day 9–11. Infection was quantified by flow cytometry, based on the proportion of A647⁺, live single cells. Triangles indicate decreasing virus volumes: 50, 25, 13, 6, 3 and 0 μ l, respectively.

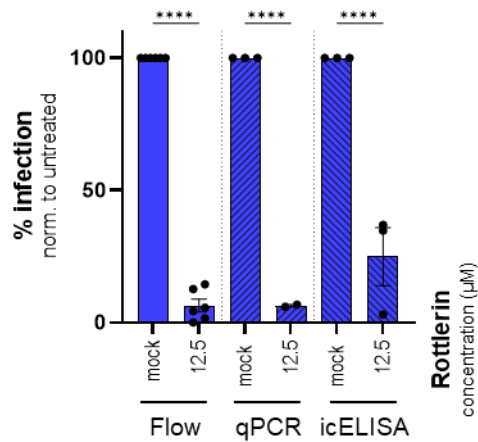


Figure S4: Single-dose cross-assay confirmation of rottlerin-mediated inhibition. VeroE6 cells were infected with PUUV (MOI $\sim$ 0.4) for 72 h in the presence of 12.5 μ M Rottlerin. Infection was then quantified in parallel by RT-qPCR (viral RNA), in-cell ELISA (N protein), and flow cytometry (intracellular N staining; %NP⁺ cells). For each assay, values were normalized to the corresponding DMSO control (set to 100%). Bars represent mean $\pm$ SEM from 1 experiment. Dots indicate individual samples. All assays were conducted in at least two biological replicates.