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Electronic Supplementary Material

Generation of a Stable GFP-reporter Zika Virus System for High-throughput Screening of Zika Virus Inhibitors

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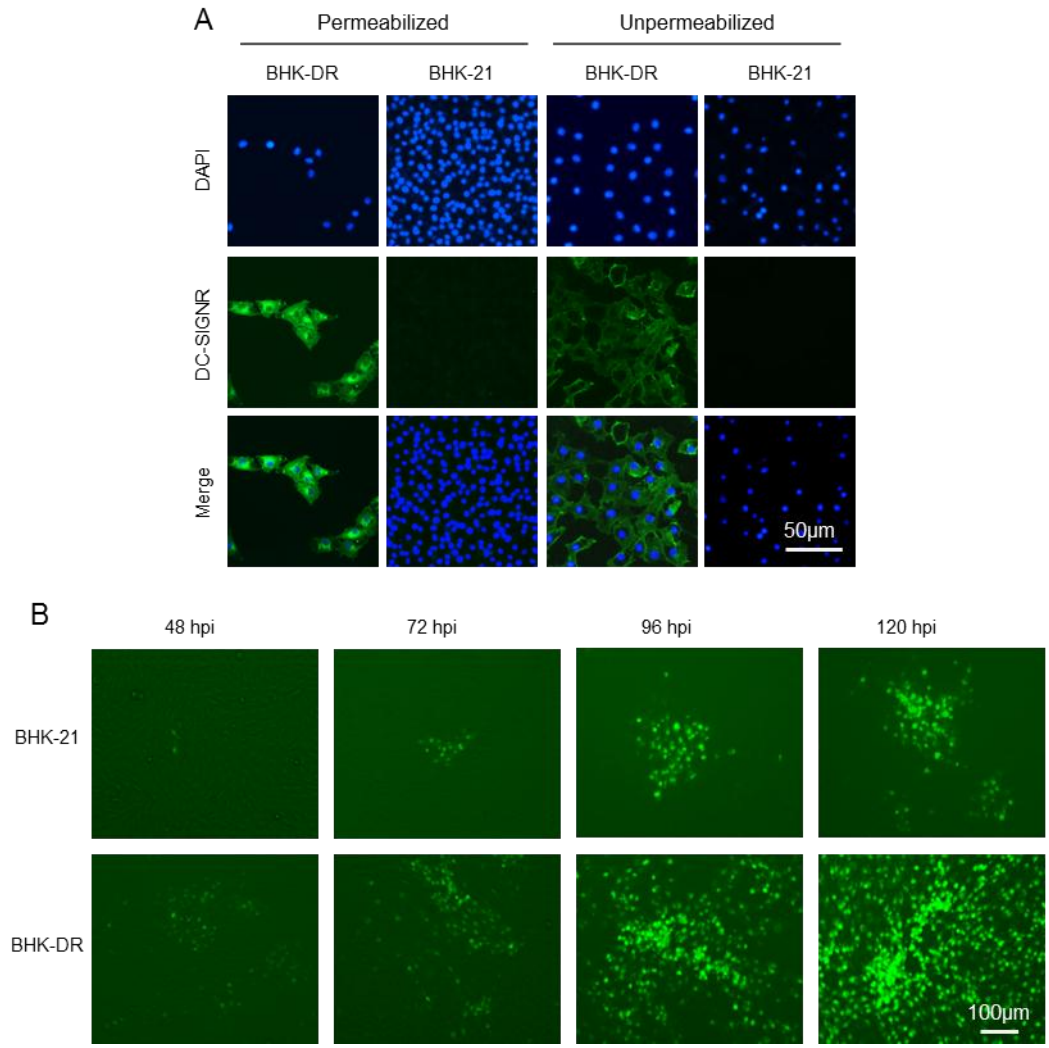

Fig S1. BHK-DR cells promote the replication of ZIKV-GFP (related to Fig. 2). **A** Immunofluorescence analysis of DC-SIGNR expression in BHK-DR cells. After transfection, the clone-selected cells were seeded in 24-well plates. After 24 h incubation at 37°C, the cells were fixed with paraformaldehyde, unpermeabilized or permeabilized with 0.1% Triton X-100 and then probed with MAb against DC-SIGN1/DC-SIGN2 (Sigma, D2691). Nuclei were stained with DAPI. **B** Representative green fluoresence image of BHK-21 cells and BHK-DR cells at indicated time post inoculation with supernatants of transfected 293T cells.

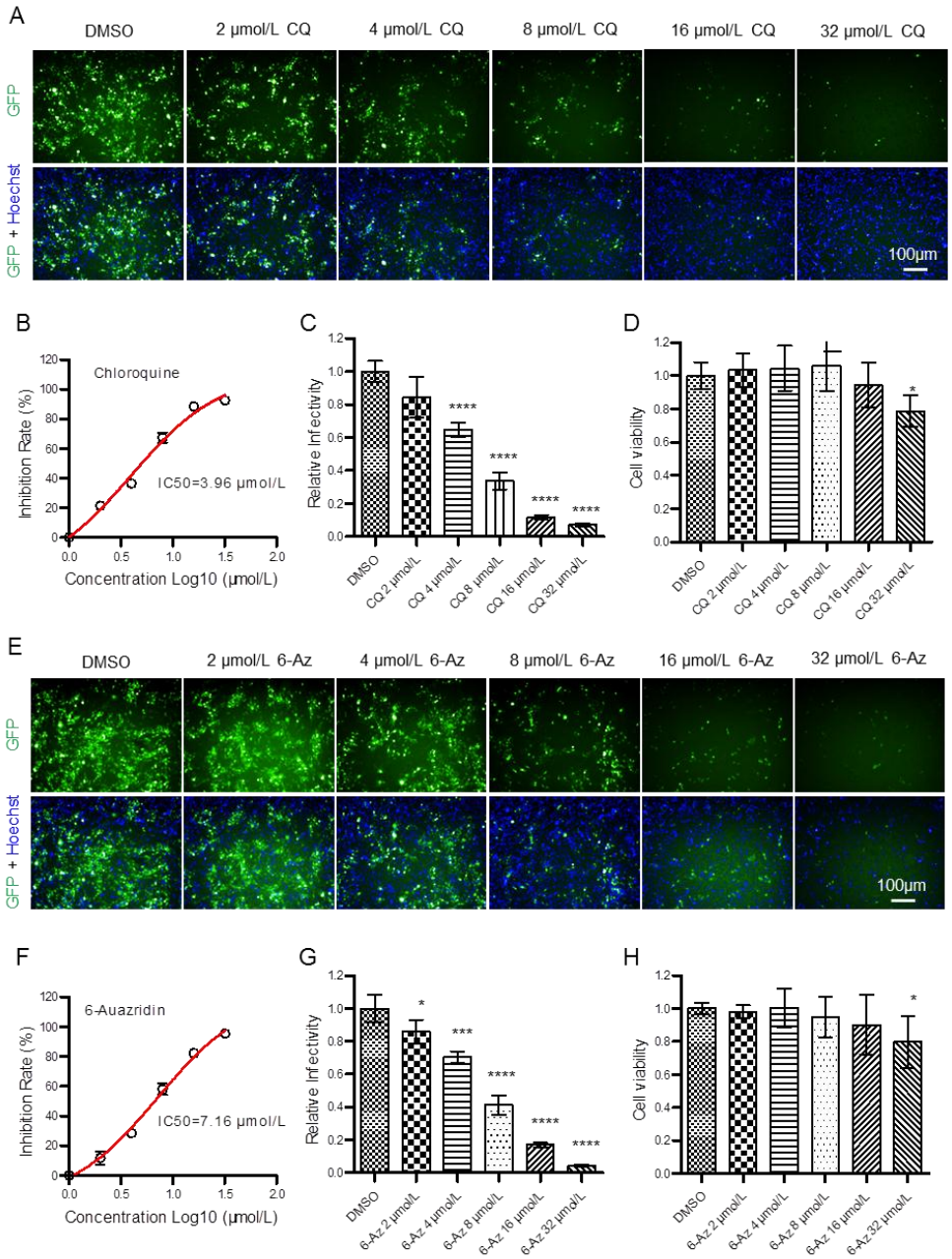

Fig. S2. Evaluation of reporter virus ZIKV-GFP infection with known inhibitors of ZIKV and WNV for antiviral drug screening. To evaluate the efficacy of the recombinant reporter virus ZIKV-GFP for antiviral drug screening, a known ZIKV inhibitor chloroquine and a WNV inhibitor, 6-azauridine, were used in an antiviral screening assay. Representative fluorescence images showing virus-infected cells (green) and nuclei (blue) are shown for the indicated concentrations of chloroquine (A) or 6-azauridine (E). Dose-response curves showing the effect of chloroquine (B) or 6-azauridine (F) treatment on virus infection in BHK-DR cells. The virus infectivity of cells treated with chloroquine (C) or 6-azauridine (G) was analyzed and compared with that of vehicle-treated cells. The cell viability of cells treated with chloroquine (D) or 6-azauridine (H) were also analyzed. Data are the means from two independent experiments performed in triplicate. Statistical significances were determined by one-way ANOVA (* $P < 0.05$; *** $P < 0.001$; **** $P < 0.0001$).

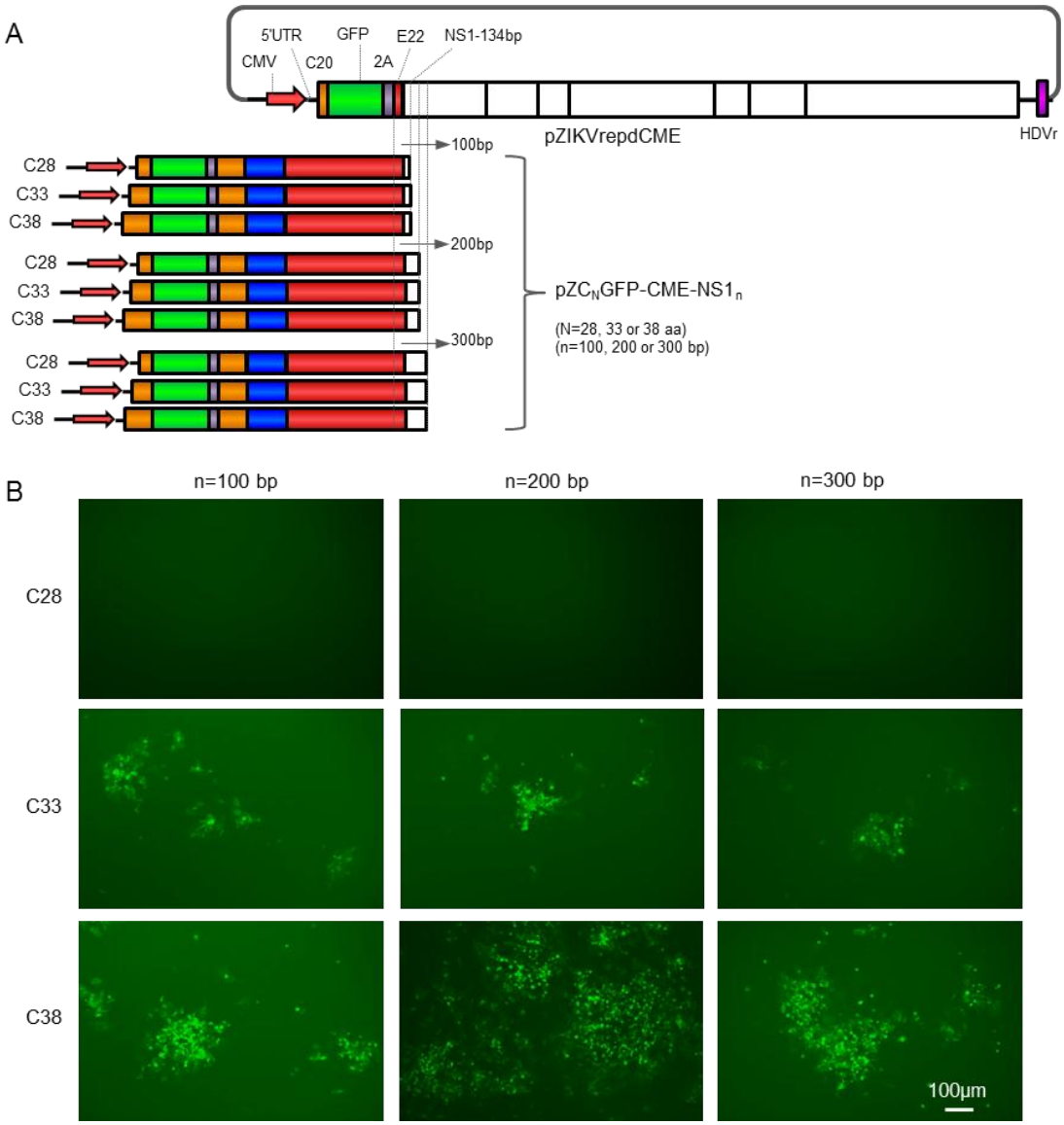

Fig. S3. Optimization of the rescue strategy of ZIKV-GFP infection via homologous recombination. **A** Schematic diagrams of ZIKV replicon plasmids and constructs containing different lengths of C protein encoding sequences and overlapping sequences with the *NS1* gene. **B** Representative green fluoresence image of BHK-21 cells at 6 dpi with supernatants of 293T cells transfected with the indicated DNA pairs.