**Virologica Sinica**

**Supplementary Data**

**A high throughput antiviral screening platform for alphaviruses based on Semliki Forest virus expressing eGFP reporter gene**

**Yu-Jia Shia, Jia-Qi Lib,c, Hong-Qing Zhangb,c, Cheng-Lin Dengb,c, Qin-Xuan Zhua, Bo Zhangb,c,\*, Xiao-Dan Lia,\***

*a Hunan Normal University, School of Medicine, Changsha, 410081, China*

*b Key Laboratory of Special Pathogens and Biosafety, Wuhan Institute of Virology, Center for Biosafety Mega-Science, Chinese Academy of Sciences, Wuhan, 430071, China*

*c University of Chinese Academy of Sciences, Beijing, 100049, China*

\*Corresponding authors.

E-mail address: lxd@live.cn (X.-D. Li); zhangbo@wh.iov.cn (B. Zhang)



**Supplementary Fig. S1.** Correlation analysis between HTS assay and plaque assay.The linear correlation between viral titers detected by plaque assay (presented in log 10 scale) and the eGFP fluorescence value detected by HTS system of gemcitabin (**A**), CHL (**B**), dasatinib (**C**), oxysophoridine (**D**), and sorafenib (**E**) were analyzed using GraphPad Prism software. **F** The linear correlation between EC50 values calculated by HTS assay and plaque assay analyzed by GraphPad Prism software.



**Supplementary Fig. S2.** The cytotoxicity of the reference compounds to BHK-21 cells.BHK-21 cells seeded in 96-well plates were treated with different reference compounds at the indicated concentrations, and after 24 h, the cytotoxicity of gemcitabin (**A**), CHL (**B**), dasatinib (**C**), oxysophoridine (**D**), sorafenib (**E**) was measured by CCK-8 assay. For each compound concentration, six wells were performed in parallel. Error bars indicate the standard deviations. The CC50 value of each compound was calculated by nonlinear regression analysis using GraphPad Prism software.

**Supplementary Table S1** The sequences of the primers used for the plasmid construction.

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| Name | Sequences (5′ to 3′) |
| Afe1-SP6-S5'UTR-F | ACGAGCGCTATTTAGGTGACACTATAgATGGCGGATGTGTGACATAC |
| SFV-3058-R | GTGTTCTTCTTGCCATTCTTCCAATGTGGCCGTAAA |
| SFV-3035-F | TTGGAAGAATGGCAAGAAGAACACGACAA |
| NotI-AscI-SFV-7384-R | ACTGCGGCCGCggcgcgccGGATCCAATCAGAATTCTGTGTATTAACGCACCAATCTAGG |
| AscI-SFV-7419-F | TCCggcgcgccACCATGAATTACATCCCTACGC |
| NotI-SFV-polyA-3'UTR-R | ACTGCGGCCGCTTttttttTTTTTTTTTTTTTTTTTTGGAAATATTAAAAACCAATT |
| SFV-6601-F | CGCGATCATCGCCTCTCACT |
| SFV-nsp4-BamHI-R | tcaccatGGTGGATCCAATCAGAATTCTGTGTA |
| SFV-sg-AscI-Capsid-F | cggcggtcctagattggtgcgttaatacacagaattctgattggcgcgccACCATGAAT |
| SFV-8462-R | CCGTTGATCTTCTTGGGCTG |
| BamHI-eGFP-F | GATTGGATCCACCatggtgagcaagggcgagga |
| eGFP-sg-R | gcaccaatctaggaccgccgtagaggtgtataacGCGATCGCCTActtgtacagctcgt |