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# Kinome siRNA screen identifies novel cell-type specific dengue host target genes

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ABSTRACT

Dengue is a global emerging infectious disease, with no specific treatment available. To identify novel human host cell targets important for dengue virus infection and replication, an image-based high-throughput siRNA assay screening of a human kinome siRNA library was conducted using human hepatocyte cell line Huh7 infected with a recent dengue serotype 2 virus isolate BR DEN2 01-01. In the primary siRNA screening of 779 kinase-related genes, knockdown of 22 genes showed a reduction in DENV-2 infection. Conversely, knockdown of 8 genes enhanced viral infection. To assess host cell specificity, the confirmed hits were tested in the DENV-infected monocytic cell line U937. While the expression of EIF2AK3, ETNK2 and SMAD7 was regulated in both cell lines after infection, most kinases were hepatocyte-specific. Monocytic cells represent initial targets of infection and an antiviral treatment targeting these cells is probably most effective to reduce initial viral load. In turn, infection of the liver could contribute to pathogenesis, and the novel hepatocyte-specific human targets identified here could be important for dengue infection and pathogenesis.



## 1. Introduction

Dengue virus (DENV) is a member of the flavivirus family and has 4 serotypes. It causes a wide spectrum of symptoms in infected humans. After the mosquito carrying virus feeds on a host, the infection is thought to start from dendritic cells in the skin, thereafter spreading to lymphatic tissue and infecting more dendritic cells, macrophages and monocytes (Clyde et al., 2006). Eventually, virus is released into the circulation and systemic infection can occur, including the liver (Seneviratne et al., 2006). Disease severity ranges from mild fever to more serious complications. Recent studies have estimated a large global burden of dengue, amounting to 390 million infections per year, of which 96 million are symptomatic (Bhatt et al., 2013). Currently, there are no available effective drugs and approved vaccines against this virus, and treatment remains supportive. Thus, there is a critical need for the development of anti-dengue treatments.

A number of high throughput siRNA screens to identify host factors involved in flavivirus infection have been reported. For example, genome-scale siRNA screens were used to discover host factors required for yellow fever virus propagation in Huh7 hepatocyte cell lines. Further validation of candidate genes in human hepatocyte cell lines and a murine fibroblast cell line identified G protein-coupled receptor kinase 2 as important for yellow fever virus and DENV replication (Le Sommer et al., 2012). A similar genome-wide siRNA-based screen silencing 21,121 human genes in Hela cells identified more than 300 host genes important in the infection

*Abbreviation:* DENV, dengue virus.

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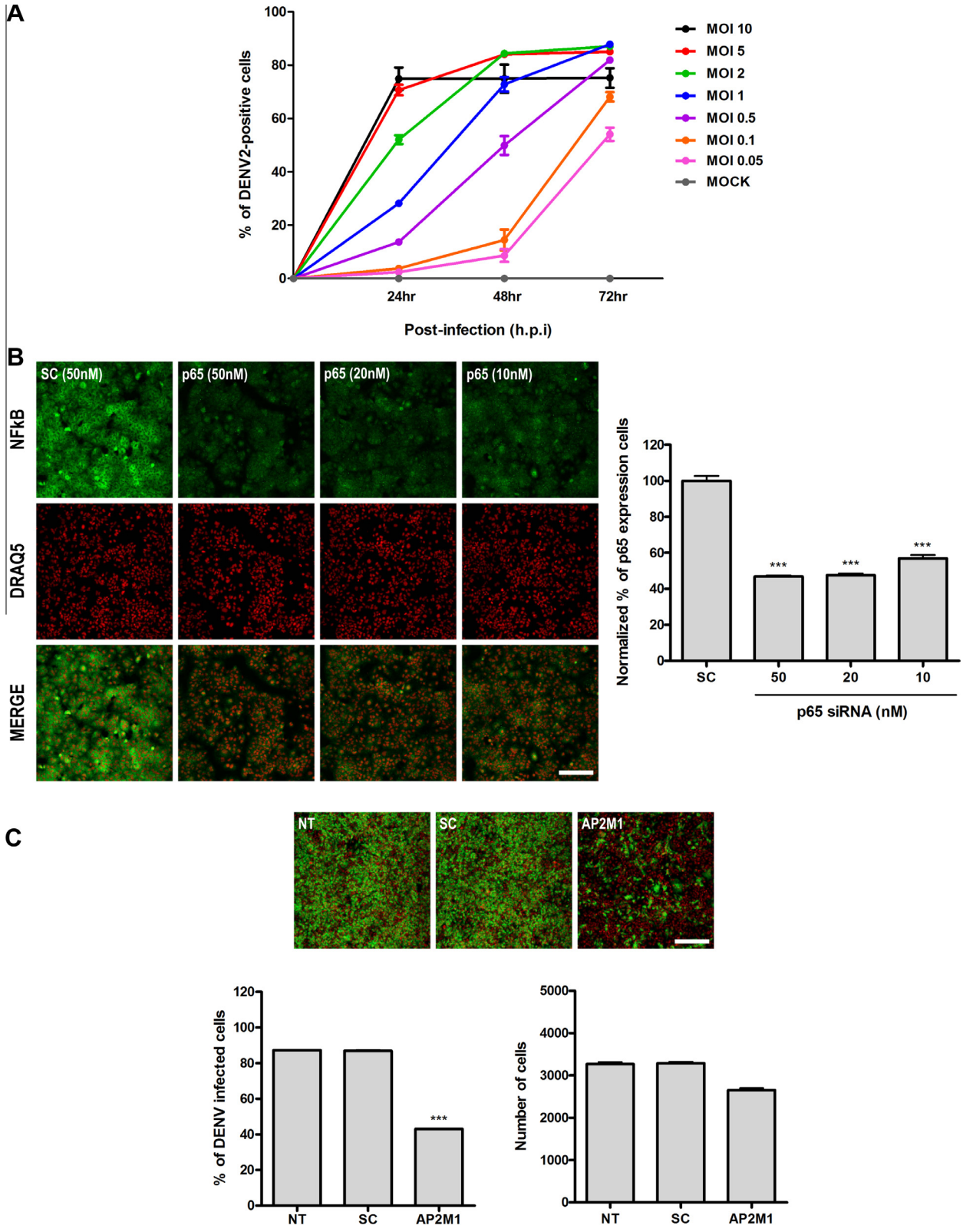


**Fig. 1.** Adaptation and validation of the image-based siRNA assay for high-throughput screening. Optimal conditions were established for DENV-2 infection and siRNA knockdown in Huh7 cells in 96-well plate format. (A) Infection kinetics of BR DEN2 01-01 at various MOIs. (B) Decrease in the expression of NFκB after transfection with different concentrations of p65 siRNA. (C)Transfection of Huh7 with AP2M1 siRNA reduced the percentage of DENV-2 infected cells. Bars show means ± SD from triplicates from 1 out of 4 independent experiments. Asterisks (∗) indicate statistical significance with a *p*-value of less than 0.05 using an unpaired *t* test (sc – scrambled, NT - non-transfected cells, scale bars = 250 μm).

with West Nile virus, another flavivirus (Krishnan et al., 2008). Host factors important in DENV infection have been discovered in *Drosophila melanogaster* cells, with 42 out of 116 hits later also being confirmed in hepatocyte cell lines (Sessions et al., 2009). A siRNA library targeted at genes regulating endocytosis pathways, polymerization of actin and cytoskeleton rearrangement and