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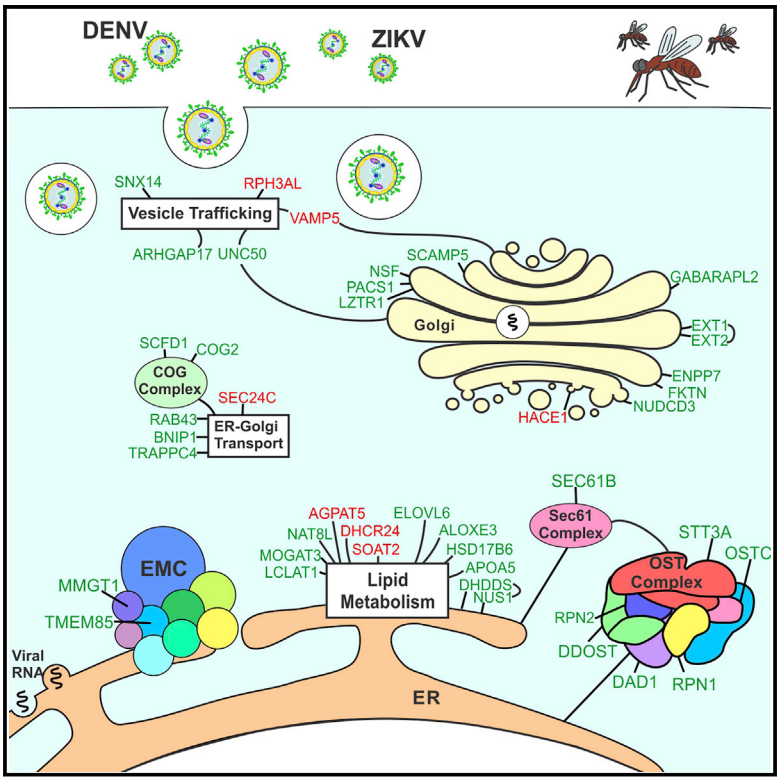
Identification of Zika Virus and Dengue Virus Dependency Factors using Functional Genomics

Graphical Abstract



Highlights

- RNAi and CRISPR/Cas9 screens were used to find flavivirus dependencies
- The screens recovered host factors involved in endocytosis and heparin sulfation
- The EMC is required by DENV and ZIKV in the early stages of replication
- These studies give a systems-wide view of human-flavivirus interactions

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In Brief

Savidis et al. identify DENV and ZIKV dependencies using orthologous RNAi and CRISPR/Cas9 approaches. Multiple host factors involved in endocytosis and transmembrane protein processing, including the endoplasmic reticulum membrane complex (EMC), are important for flaviviral replication. Together, their studies generate a systems-wide view of human-flavivirus interactions.

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Identification of Zika Virus and Dengue Virus Dependency Factors using Functional Genomics

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SUMMARY

The flaviviruses dengue virus (DENV) and Zika virus (ZIKV) are severe health threats with rapidly expanding ranges. To identify the host cell dependencies of DENV and ZIKV, we completed orthologous functional genomic screens using RNAi and CRISPR/Cas9 approaches. The screens recovered the ZIKV entry factor AXL as well as multiple host factors involved in endocytosis (RAB5C and RABGEF), heparin sulfation (NDST1 and EXT1), and transmembrane protein processing and maturation, including the endoplasmic reticulum membrane complex (EMC). We find that both flaviviruses require the EMC for their early stages of infection. Together, these studies generate a high-confidence, systems-wide view of human-flavivirus interactions and provide insights into the role of the EMC in flavivirus replication.

INTRODUCTION

The New Millennium has brought a rapid expansion of human flavivirus infections, including dengue virus (DENV), yellow fever virus (YFV), West Nile virus (WNV), and Zika virus (ZIKV) (Bhatt et al., 2013). Given that global warming is predicted to enlarge the range of the insect vectors that carry these viruses, it is critical that we understand the biology of these viruses in order to design effective therapies against them. DENV and ZIKV are single-stranded, positive-sense RNA viruses that are transmitted to humans by Aedes mosquitos. Both are fast-growing health threats that are producing an escalating number of infections in the Americas and worldwide.

Each year, 390 million people are infected with DENV, with 500,000 individuals hospitalized with severe dengue, the majority of those being young children (Bhatt et al., 2013). ZIKV, first isolated from an infected macaque in Uganda in 1947, suddenly emerged in Micronesia in 2007 and expanded its range to Southeast Asia. In May 2015, ZIKV was identified in Brazil coincident with an upsurge in neurologic and fetal abnormalities. With its rapid spread to Central and South America, ZIKV has emerged as a severe health threat by virtue of its fast-paced global spread and associated morbidities, including microcephaly and Guillain-Barre syndrome. (D'Ortenzio et al., 2016; Driggers et al., 2016; Haug et al., 2016; Lazear and Diamond, 2016; Musso and Gubler, 2016; Rasmussen et al., 2016). These events have led to ZIKV being declared a public health emergency by the World Health Organization. Recent animal models have demonstrated that ZIKV infects the placentas of pregnant mice, with transmission to fetal mice resulting in death or severe growth impairment (Cugola et al., 2016; Miner and Diamond, 2016; Miner et al., 2016; Li et al., 2016). There are no specific therapies for flavivirus infection, although a DENV vaccine has recently been approved in some countries. There is no approved vaccine or therapy for ZIKV infection.

Flavivirus replication begins with the virus binding to host cell receptors and undergoing endocytosis (Fernandez-Garcia et al., 2009). A number of proteins have been implicated to facilitate DENV attachment and entry, including TIM1 and AXL (Jemielity et al., 2013; Meertens et al., 2012; Morizono and Chen, 2014; Perera-Lecoin et al., 2014; Richard et al., 2015), the latter having also been identified as an important ZIKV entry factor (Hamel et al., 2015). Subsequent to initial viral entry, late endosomal acidification triggers the fusion of host and viral membranes and permits the virus' positive sense RNA genome (viral RNA [vRNA]) to enter the host cell cytosol. Upon cytosolic entry, the vRNA is translated into a large polyprotein on the rough endoplasmic reticulum (RER). This polyprotein is processed by both host and viral proteases into three structural proteins (premembrane [prM], capsid [C], and the glycoprotein envelope [E protein]), and seven non-structural (NS) proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5). DENV has been demonstrated to extensively remodel the ER into replication centers (RCs), where progeny viruses are created. The newly synthesized flaviviruses then traffic from the RER to the cell surface via the Golgi, where they