

Insect-Specific Flavivirus Infection is Restricted by Innate Immunity in the Vertebrate Host

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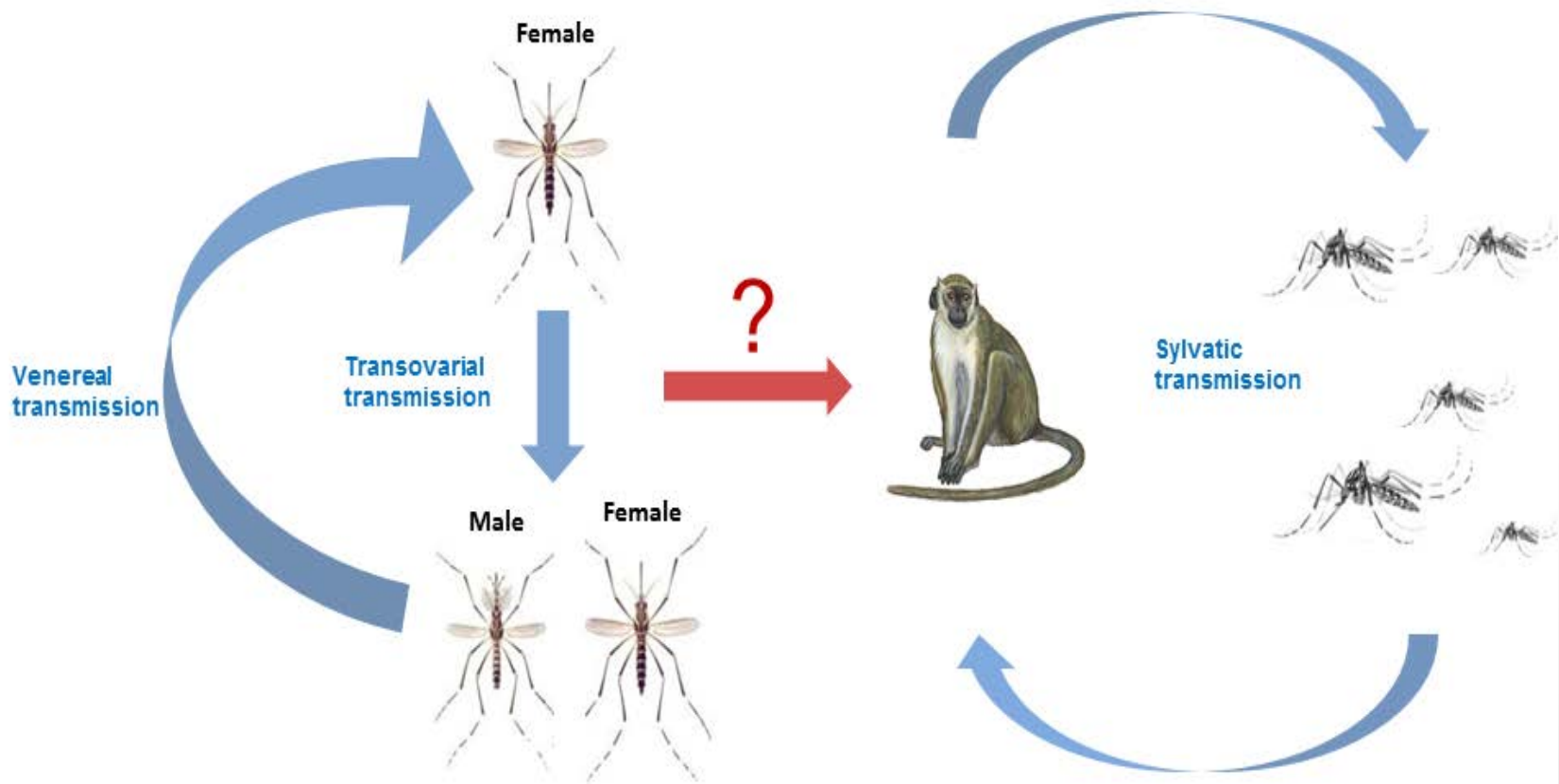
Background



- Globally 80 arboviruses as human pathogens
- Estimated 2,000 mosquito-borne flaviviruses exist
 - Dengue, West Nile, Yellow fever, Zika
 - Kamiti River virus (KRV)
- Insect-Specific Flaviviruses (ISFVs)
 - e.g. KRV
- Hypothesis
 - ISFVs model genetic precursors to pathogens
 - ISFVs inhibited by innate immune pathways in mammals
- Aim of study: identify the innate immune pathways
 - Understanding pathways
 - Prevention (e.g. Zika)

Hypothesis

- Why can Dengue infect mammalian cells but KRV cannot?

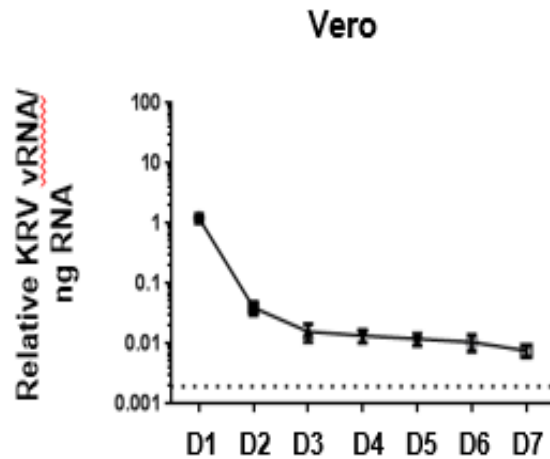


Dengue (human)	KRV (insect)	R,M,T/IRF3,5,7 (innate immunity)	Vero/A549/MEF/B6 (mammalian cells)	C6/36 (insect cells)	vRNA (indicator)
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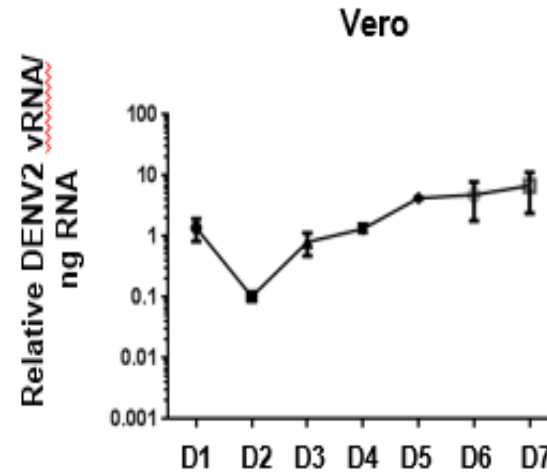
Figure 1

KRV replicates in immunosuppressed vertebrate cells.

A.



B.

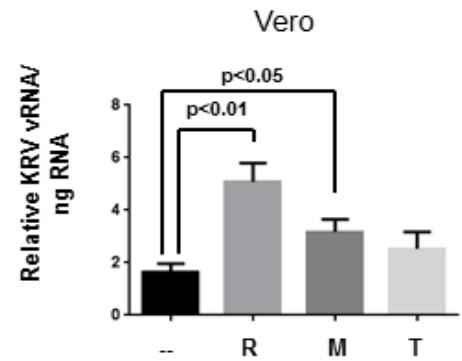


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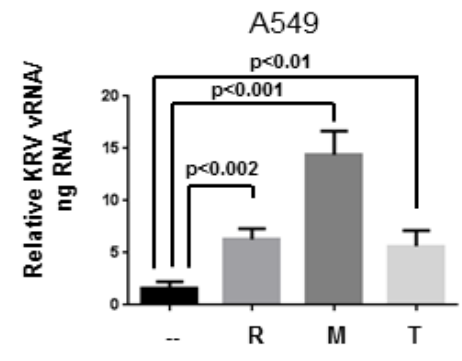
Figure 2

shRNA knockdown of RIG-I, MDA5, and TLR3 enhances KRV vRNA in mammalian cells.

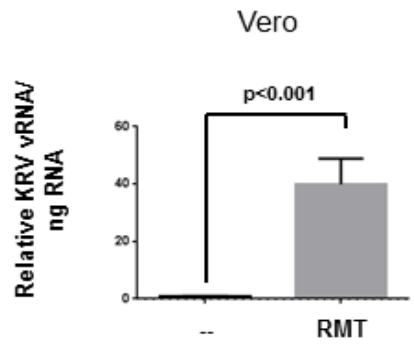
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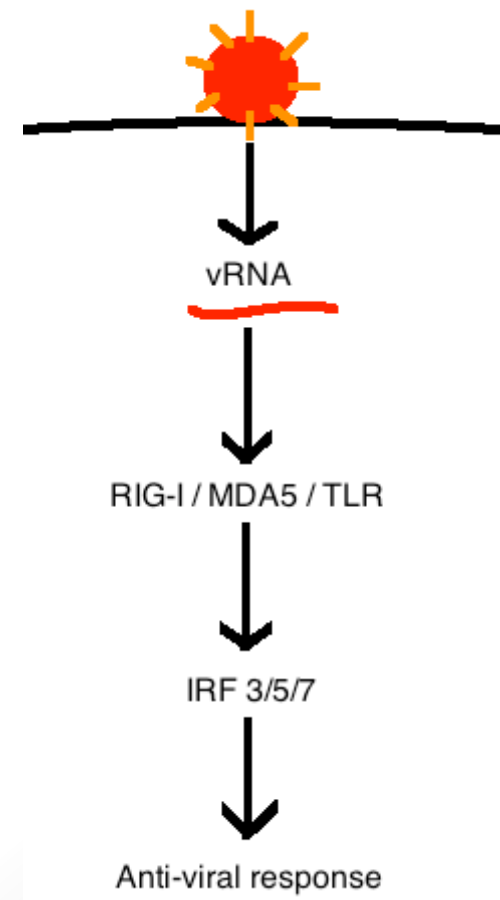
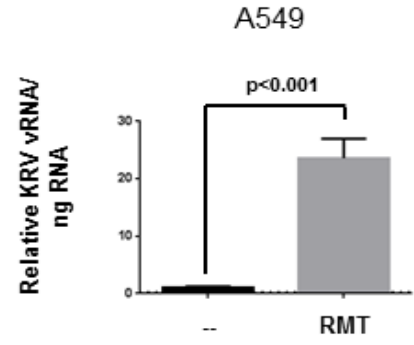
B.



C.



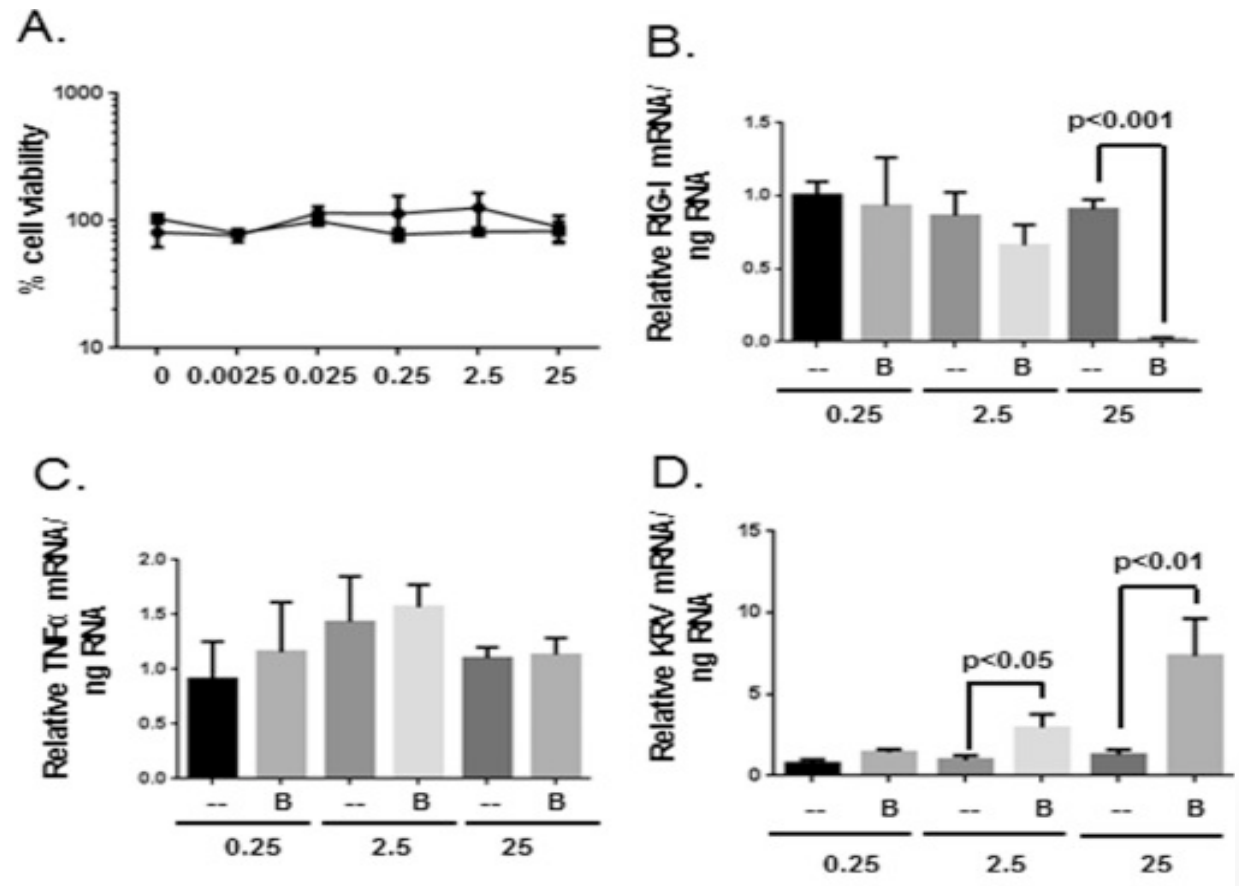
D.



Dengue (human) KRV (insect) R,M,T/IRF3,5,7 (innate immunity) Vero/A549/MEF/B6 (mammalian cells) C6/36 (insect cells) vRNA (indicator)

Figure 3

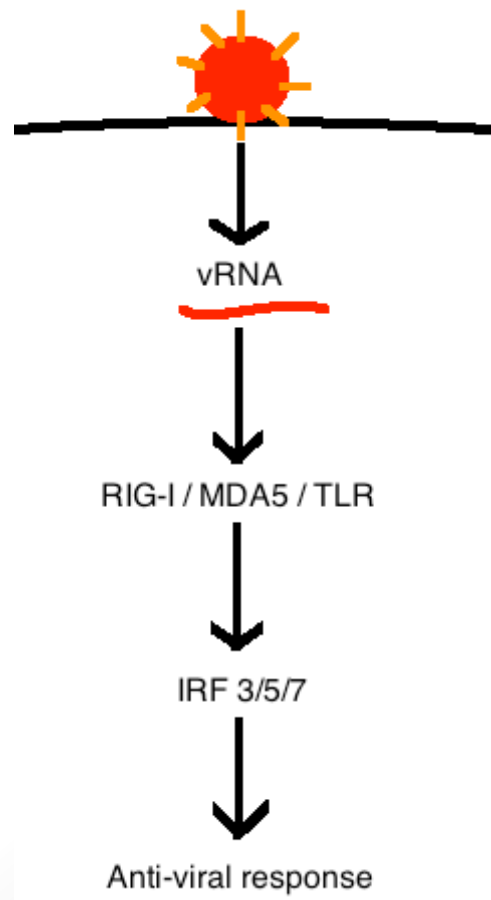
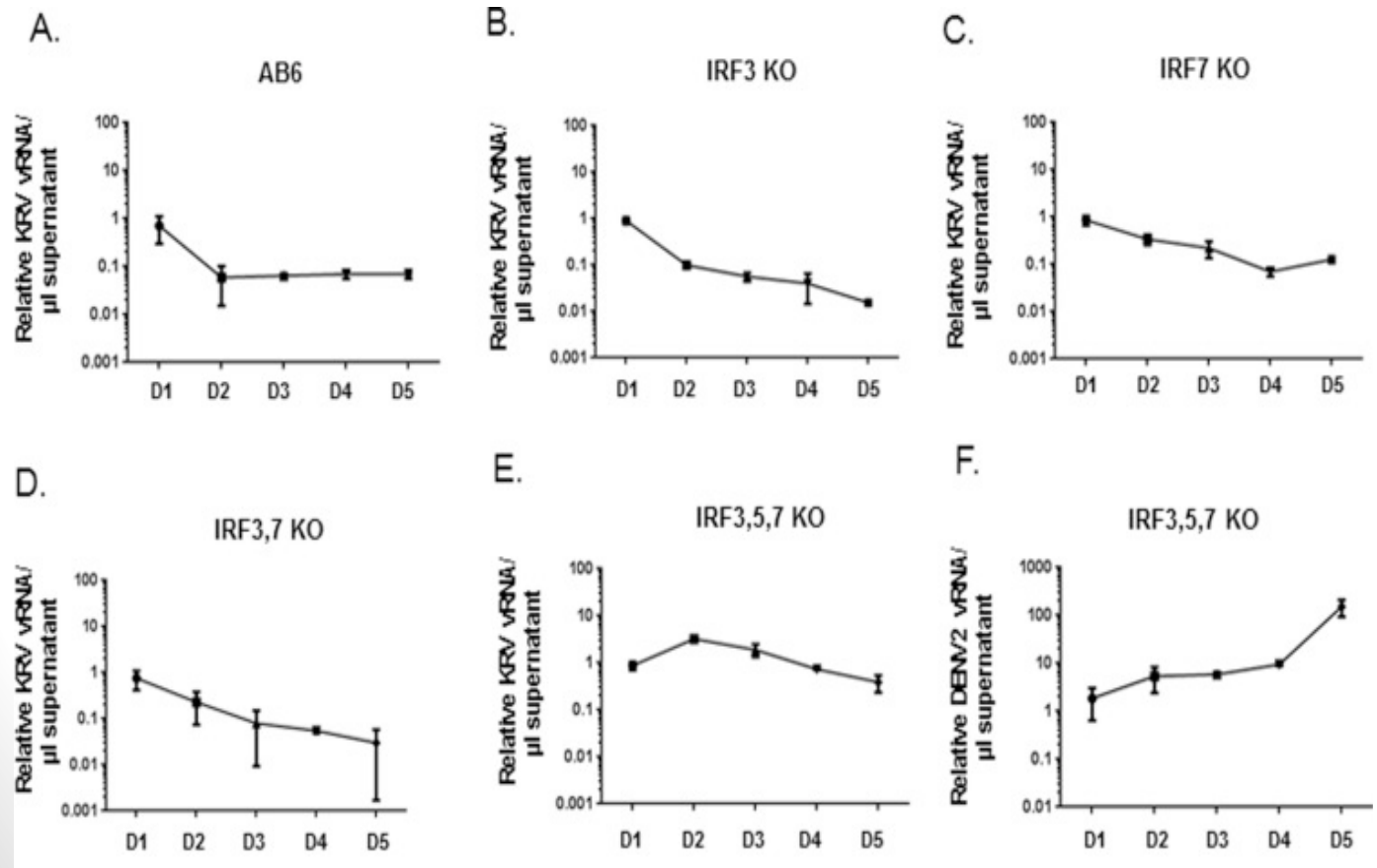
BX795 treatment enhances KRV vRNA in Vero cells.



Dengue (human) KRV (insect) R,M,T/IRF3,5,7 (innate immunity) Vero/A549/MEF/B6 (mammalian cells) C6/36 (insect cells) vRNA (indicator)

Figure 4

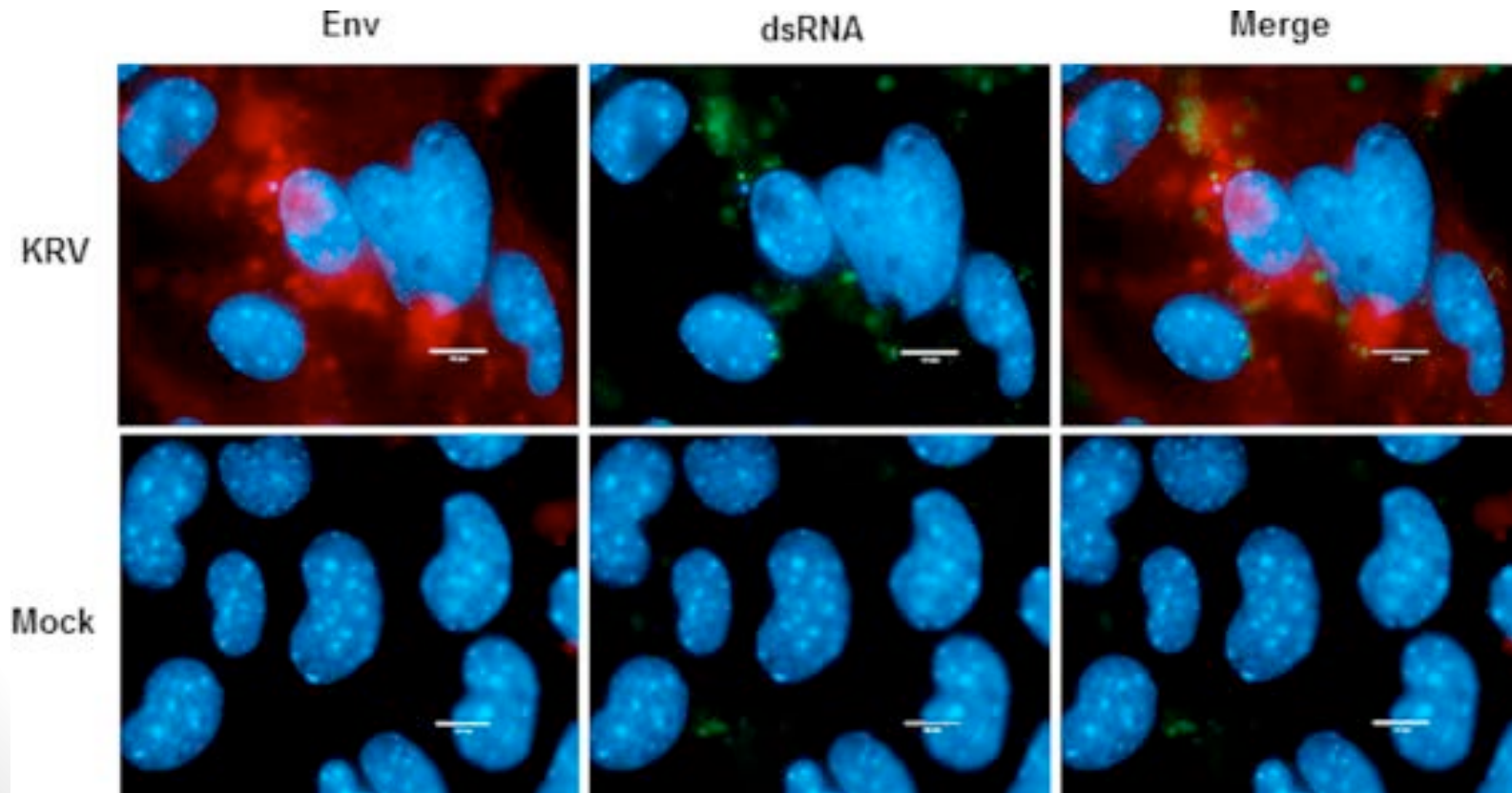
KRV vRNA is shed from IRF3,5,7 ^{-/-} MEFs.



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Figure 5

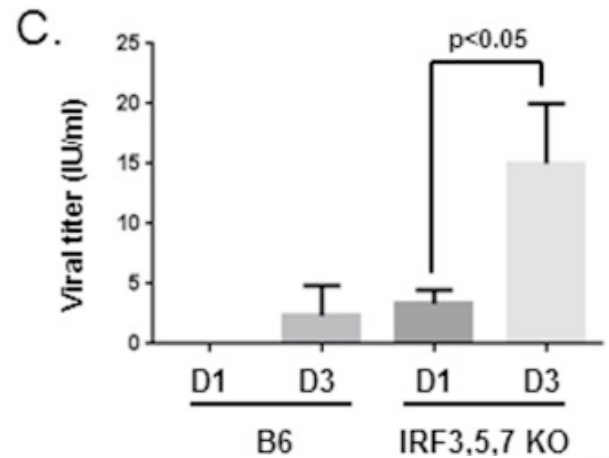
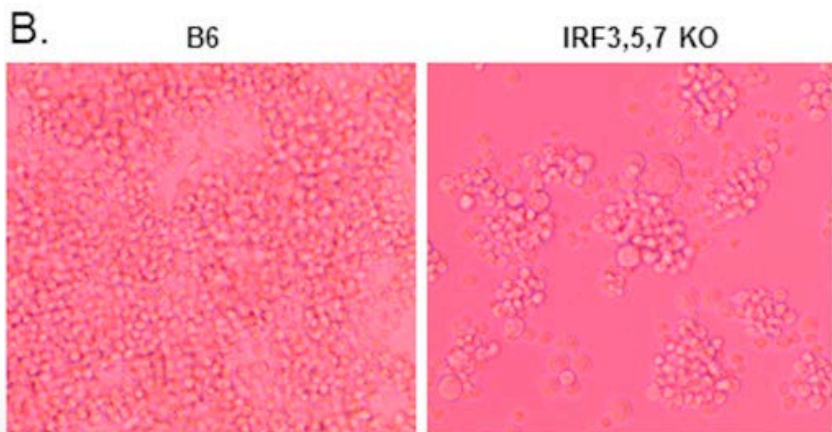
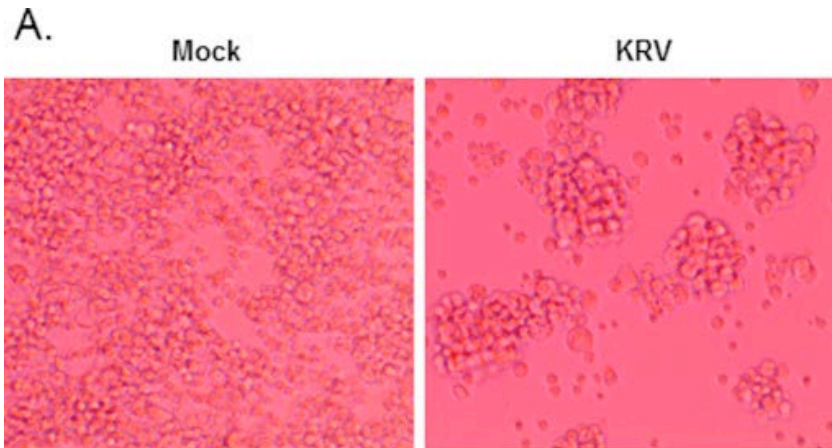
dsRNA and envelope detected in KRV infected IRF3,5,7^{-/-} cells.



Dengue (human) KRV (insect) R,M,T/IRF3,5,7 (innate immunity) Vero/A549/MEF/B6 (mammalian cells) C6/36 (insect cells) vRNA (indicator)

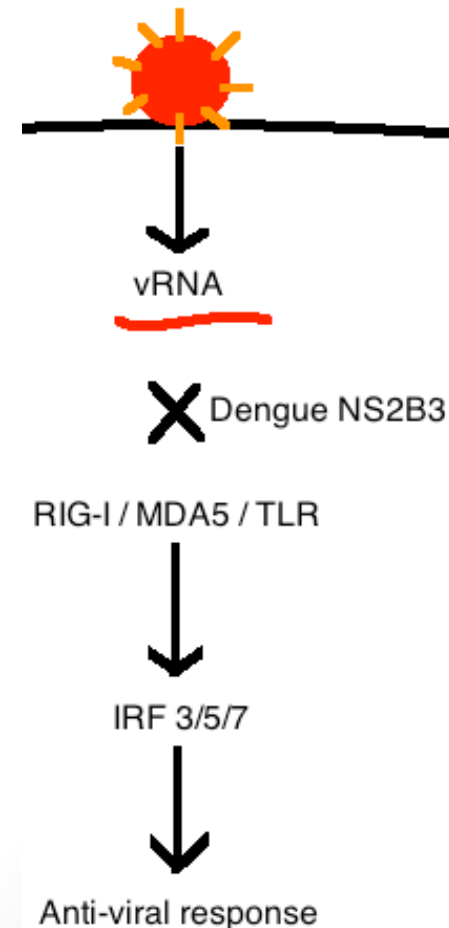
Figure 6

KRV sheds low levels of infectious progeny in IRF3,5,7^{-/-} MEFs. 3 dpi



Conclusions

- R/M/T innate immune pathways responsible for restricting ISFV replication in mammalian cells
 - Dengue protein NS2B3 responsible for evasion of this pathway
- IRF3,5,7^{-/-} MEFs are permissive to and shed KRV infectious progeny
- Global Importance
 - ISFV could recombine with pathogen and acquire genetic mutation or protein for pathogenicity
 - Prevent establishment of future emergent viruses



Acknowledgements

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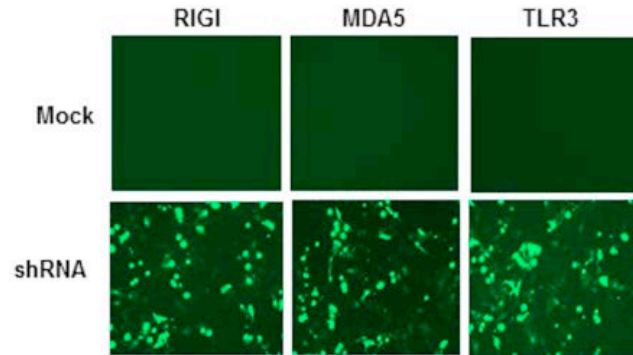
Publication

Tree MO, McKellar DR, Kieft KJ, Watson AM, Ryman KD, Conway MJ. Insect-Specific Flavivirus Infection is Restricted by Innate Immunity in the Vertebrate Host. *Virology*. 1 Oct 2016; 497, 81-91.

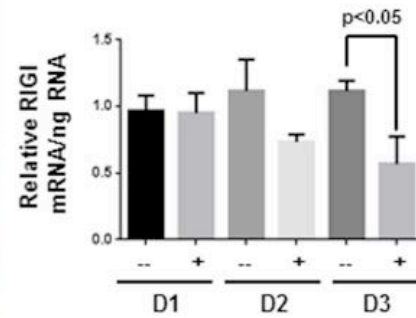
Omitted Figures

shRNA knockdown of RIG-I, MDA5 and TLR3 enhances KRV vRNA in Vero cells

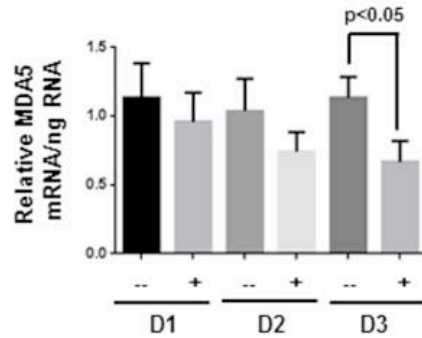
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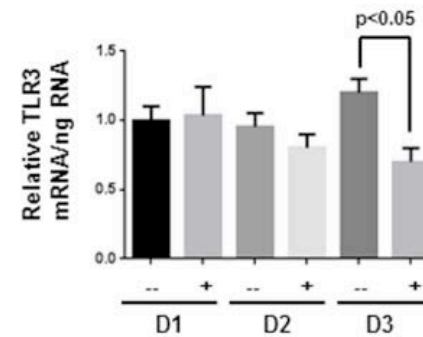
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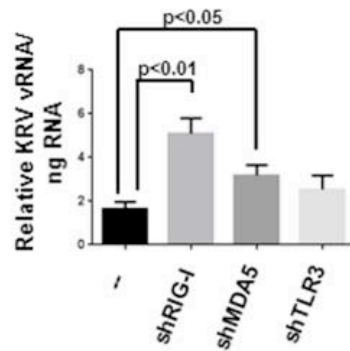
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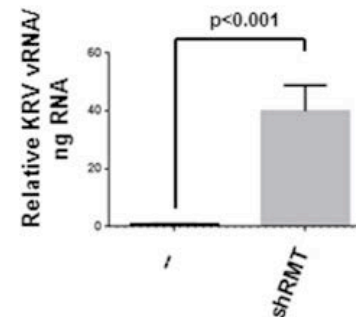
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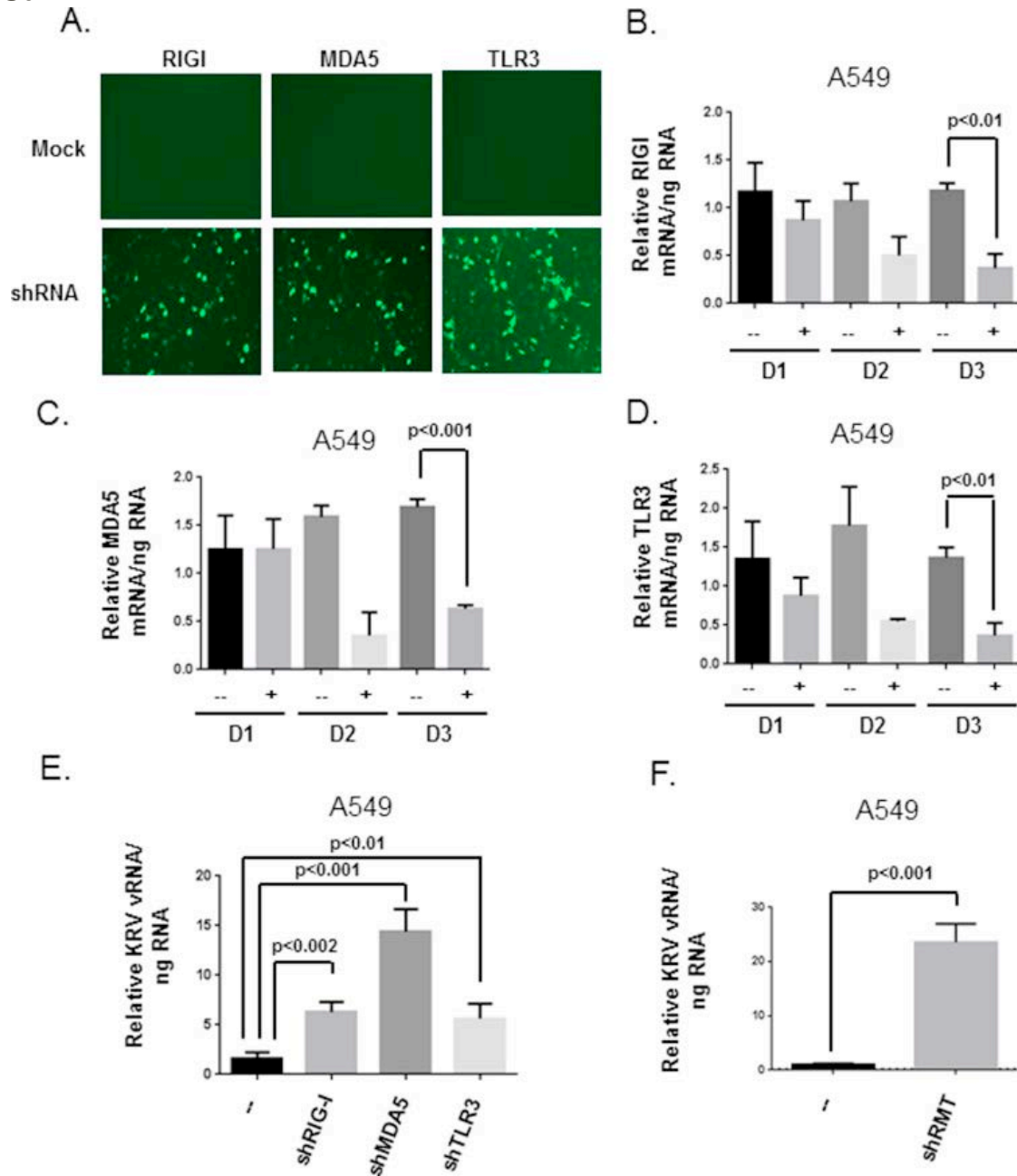
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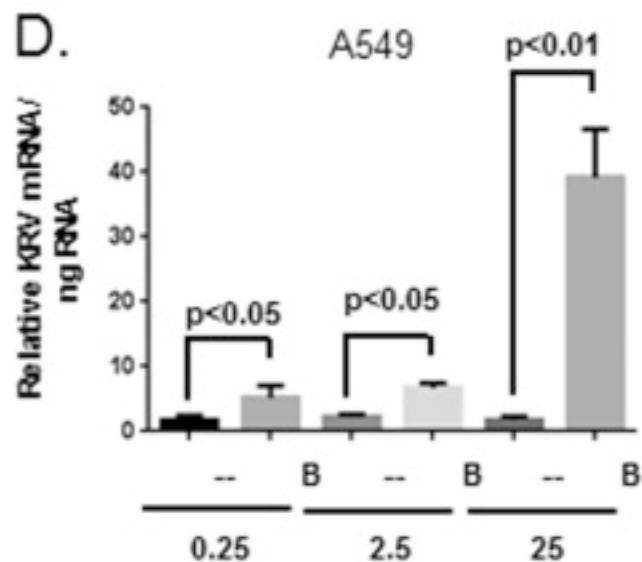
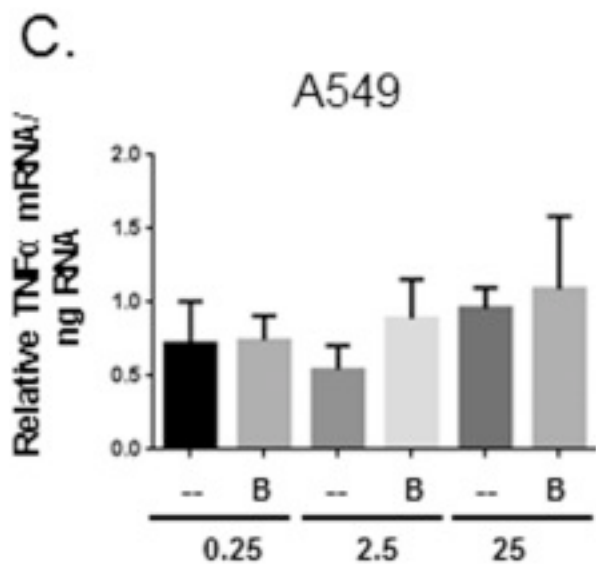
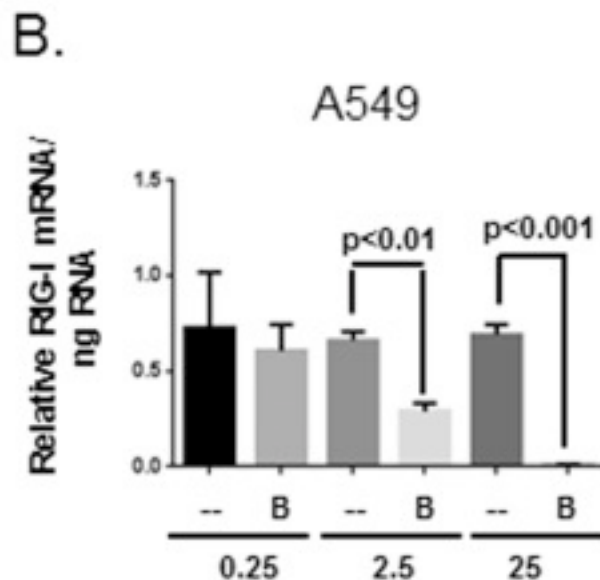
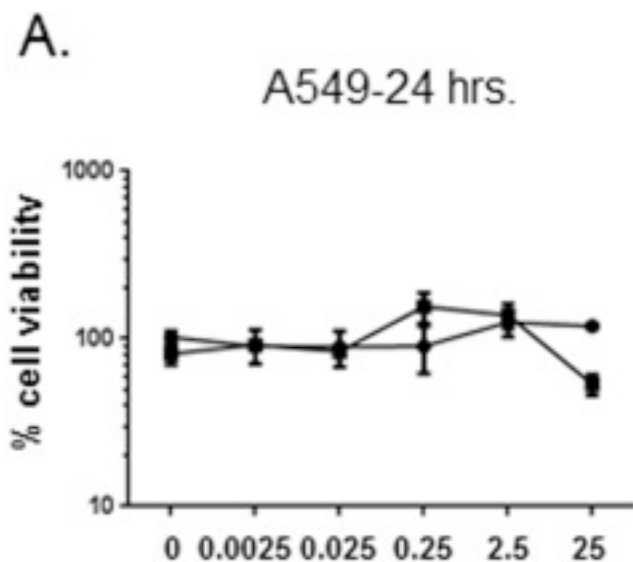
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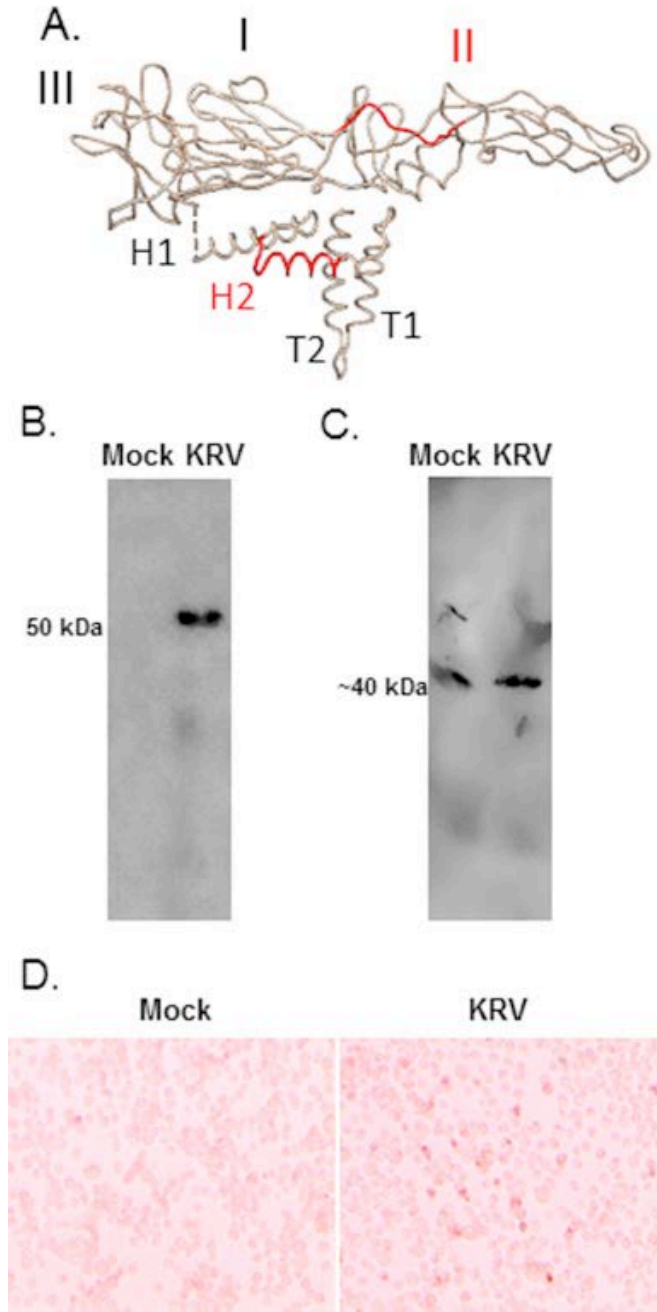
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BX795 treatment enhances KRV vRNA in A549 cells

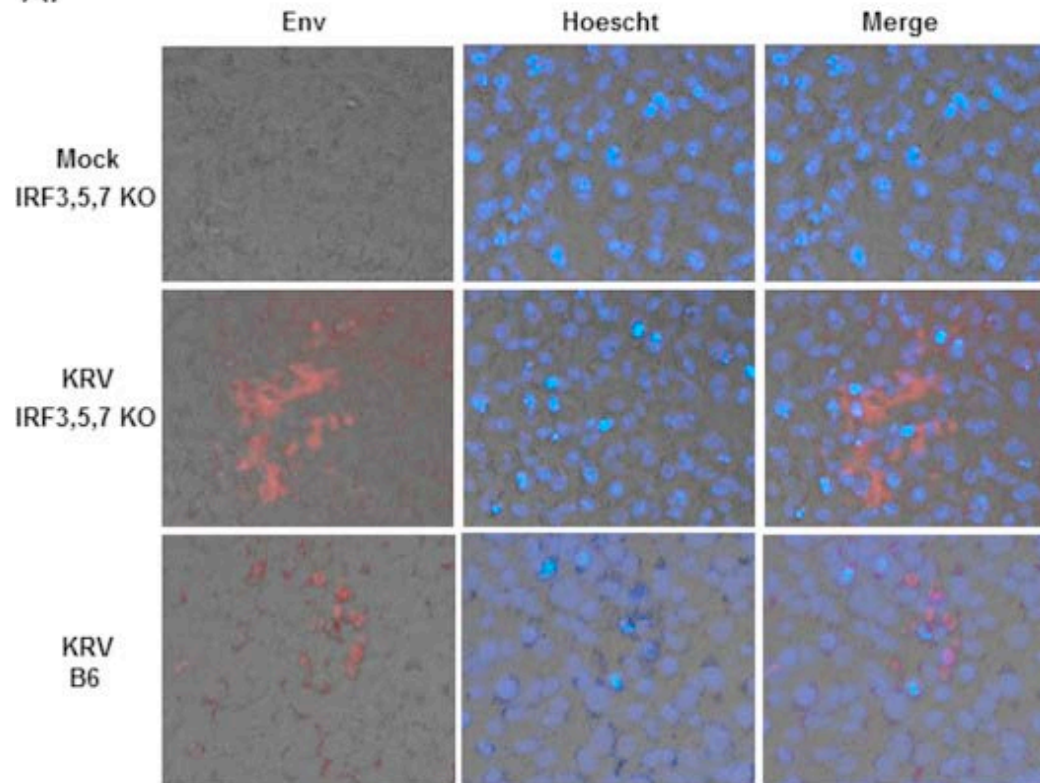


Characterization of an anti-KRV envelope polyclonal antibody.



KRV synthesizes envelope protein in IRF3,5,7^{-/-} MEFs.

A.



B.

