1 **Bacterial Effector Screening Reveals RNF214 as a Virus Restriction Factor in Mammals**

2 Aaron Embry, David Schad, Emily A. Rex, Neal M. Alto[†] and Don B. Gammon[†]

3 [1]Department of Microbiology, University of Texas Southwestern Medical Center, Dallas, Texas,
4 USA

5 † Corresponding authors: don.gammon@utsouthwestern.edu; neal.alto@utsouthwestern.edu

6 **Abstract**

7 Arboviruses are a group of arthropod-transmitted viruses that pose a significant threat to public
8 health. Identifying host factors that inhibit arbovirus infection is critical for the development of
9 strategies to prevent or treat these infections. Previously, we showed that bacterial effector
10 proteins can be used as molecular tools to identify host immunity factors in insect cells that restrict
11 arbovirus replication (Embry et al., 2024). Bacteria secrete effectors into the host cell cytoplasm
12 to inhibit various innate immune defenses. Here, we apply our bacterial effector screening system
13 to identify host antiviral immunity factors in two mammalian hosts – bats and humans. By
14 screening a library of 210 effectors encoded by seven distinct bacterial pathogens, we identified
15 three bacterial effectors (IpaH4, SopB, and SidM) that enhance the replication of both togaviruses
16 and rhabdoviruses in bat and human cells. We also discovered several effectors that enhance
17 arbovirus replication in a virus- or host-specific manner. We further characterize the mechanism
18 by which the *Shigella flexneri* encoded E3 ubiquitin ligase, IpaH4, enhances arbovirus infection
19 in mammalian cells. Using yeast two-hybrid, ubiquitin-activated interaction traps, *in vitro*
20 ubiquitination assays and cellular approaches, we show the uncharacterized mammalian RING-
21 domain containing protein, RNF214, to be directly targeted by IpaH4 for ubiquitination-mediated
22 degradation. Phylogenetic analyses of RNF214 proteins indicate they are widely conserved
23 among many vertebrate species, suggesting an important evolutionary function. We show that
24 RNF214 overexpression suppresses arbovirus infections in a manner dependent upon its putative
25 E3 ubiquitin ligase activity, while RNF214 depletion enhances these infections in human and bat
26 cells. These data suggest that RNF214 proteins are important innate immune factors involved in
27 combating viral infection. Collectively, our work shows that bacterial effectors can be useful tools
28 for uncovering novel mammalian antiviral machinery.

29 **Author Summary**

30 Arboviruses are viruses transmitted by arthropod vectors such as mosquitoes or biting flies to
31 both animal and human hosts. Arboviruses cause diseases ranging from mild febrile illnesses to

32 fatal encephalitic infection. Here, we use bacterial effector proteins to identify mammalian factors
33 that block arbovirus replication. Bacterial effectors encoded by pathogenic bacteria are secreted
34 into mammalian host cells to inhibit cellular antimicrobial responses. Like viruses, many
35 pathogenic bacteria replicate inside of mammalian host cells, thus we hypothesized that some
36 bacterial effectors may target and inhibit the host immune response that are normally restrictive
37 to both bacteria and viruses. After screening a library of >200 bacterial effector proteins, we
38 identified three effectors that promote the replication of arboviruses belonging to two distinct
39 families in bat and human cells. We further show that one of our most potent effectors, IpaH4,
40 enhances arbovirus replication by targeting mammalian RNF214 proteins for degradation.
41 RNF214 proteins are poorly characterized, but our phylogenetic analyses suggest these proteins
42 are widely conserved among vertebrate organisms. We show that depletion of RNF214 protein
43 levels in either bat or human cells sensitizes these cells to arbovirus infections, revealing a new
44 role for RNF214 proteins in antiviral defense. Our study demonstrates the utility of bacterial
45 effectors as tools for identifying new host immune machinery in mammalian hosts.

46 **Introduction**

47   Arboviruses are transmitted by arthropod vectors to a wide variety of mammalian hosts.
48 Whether arbovirus infection ensues after vector transmission depends upon the ability of
49 arboviruses to overcome mammalian antiviral defenses. Bats are diverse group of mammals
50 encompassing >1000 species and are found in all global regions inhabited by humans.
51 Importantly, bats act as natural reservoirs for viruses affecting humans, including highly
52 pathogenic coronaviruses and filoviruses [1-4]. Moreover, accumulating evidence suggests that
53 bats may also be reservoirs for arboviruses that infect humans, such as togaviruses [e.g. Sindbis
54 virus (SINV), Ross River virus (RRV), and O'nyong'nyong virus (ONNV)] [5-8]. However, little is
55 known regarding the bat antiviral responses that combat arboviruses. Due to a lack of screening
56 tools (e.g. CRISPR-Cas9 libraries), our understanding of bat immune systems is extremely limited
57 and has largely been gleaned from comparative genomics and transcriptomic profiling of virus-
58 infected bat cells [9-13]. Therefore, developing functional assays to identify bat factors relevant
59 to arbovirus restriction is paramount.

60   We recently identified host antiviral factors in invertebrate cells by screening for bacterial
61 effectors that enhance arbovirus replication in normally restrictive lepidopteran insect cells [14].
62 Bacterial effectors are proteins injected into eukaryotic host cells through needle-like secretion
63 systems to subvert the immune response. Therefore, if bacterial effectors inhibit host immune
64 factors that restrict both bacteria and viruses, they may enhance virus replication when expressed

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65 in restrictive cells. Moreover, these effectors can be used as molecular tools to identify the host
66 immune machinery they target [14].

67   Here we show that bacterial effectors can be used as functional tools to identify arbovirus
68 restriction factors encoded by mammalian species such as bats and humans. By screening a
69 library of 210 bacterial effectors, we identify dozens of effectors that rescue either togavirus or
70 rhabdovirus infection in either humans or bats, suggesting some of these target virus or host-
71 specific restrictions. Moreover, we discovered three effector proteins that enhance both togavirus
72 and rhabdovirus replication in both host species. Characterization of one of the most potent
73 effector proteins, *Shigella flexneri*-encoded IpaH4, led us to identify mammalian RNF214 proteins
74 as direct targets of IpaH4 and novel virus restriction factors in bat and human cells. Our work
75 demonstrates that bacterial effector screening provides a functional platform for uncovering
76 effector functions and mammalian antiviral machinery.

Results

Inhibition of Host Transcription Enhances Arbovirus Replication in R06E Bat Cells

79   Previously, we showed that the togavirus RRV and the rhabdovirus vesicular stomatitis
80 virus (VSV) undergoes an abortive infection in *Lymantria dispar*-derived LD652 cells, but were
81 rescued by actinomycin D (ActD) treatment, which globally inhibits cellular transcription [14, 15].
82 This suggested that viral replication could be enhanced in insect cells when host responses were
83 inhibited, giving us confidence to screen for bacterial effectors that similarly enhanced arbovirus
84 replication in these cells [14]. Thus, we asked if ActD treatment also enhances arbovirus
85 replication in bat cells in order to justify a similar bacterial effector screen in mammalian cells.

86   We examined arbovirus replication in *Rousettus aegyptiacus* (Egyptian fruit bat)-derived
87 R06E cells for several reasons: 1) the *R. aegyptiacus* genome has been sequenced [9], which is
88 critical for identifying host antiviral factors encoded by this species; 2) R06E cells are capable of
89 supporting arboviruses replication; 3) evidence suggests that *R. aegyptiacus* animals are naturally
90 infected with viruses belonging to *Togaviridae* and *Rhabdoviridae*, the two viral families we focus
91 on in this study [5, 6]; and 4) *R. aegyptiacus* is a reservoir for other pathogenic viruses such as
92 Marburg virus [2]. Thus, an improved understanding of *R. aegyptiacus* immunity may have
93 implications for other viral diseases, beyond arboviruses*.

94   To determine if ActD treatment alters arbovirus infection of R06E cells, we challenged
95 cells with GFP reporter arboviruses (RRV-GFP [16] and VSV^{M51R}-GFP [15]) across increasing

96 ActD concentrations. It is important to note that VSV^{M51R}-GFP is an attenuated strain with a

97 reduced ability to block antiviral host responses [17-20], and thus we hypothesized that it would

98 be easier to detect enhanced replication with this mutant. Interestingly, ActD increased GFP

99 signals during RRV-GFP or VSV^{M51R}-GFP infection in a dose-dependent manner, peaking at ~60-

100 to 70-fold at the highest dose tested (10 nM) without significantly impacting cell viability via a

101 lactate dehydrogenase (LDH)-based cytotoxicity assay (**Fig S1A-C**). These results suggest that

102 R06E cells express antiviral factors that restrict arbovirus replication.

103 *Specific Bacterial Effectors Enhance Arbovirus Replication in Bat and Human Cells*

104 Expression of specific bacterial effectors in lepidopteran cells can rescue otherwise

105 abortive arbovirus infections [14]. To determine if effectors could enhance arbovirus replication in

106 mammalian cells, we adapted our library of 210 effector genes [14] for lentivirus-based expression

107 [21]. To compare potential arbovirus-enhancing activities of effectors in additional mammalian

108 species, we conducted effector screens in both bat R06E cells and human U2OS cells, the latter

109 being a human cell type known to be susceptible to RRV and VSV [22, 23].

110 R06E and U2OS cells were transduced with the effector library for 48 h and then infected

111 with either RRV-GFP or VSV^{M51R} -GFP for 20 h. Cells were then stained with CellTracker Blue

112 dye (to normalize viral GFP signals to cell number), fixed, then subjected to automated

113 fluorescence microscopy [14]. Effector proteins that enhanced viral GFP signals by >10-fold over

114 cells transduced with lentiviruses expressing firefly luciferase (control) were considered "hits", a

115 cut-off we established to avoid examining false-positives (**Fig 1AB**). Importantly, we assessed

116 transduced cells for cytotoxicity due to effector expression and eliminated 18 effectors in bat and

117 4 in human cells from further consideration due to toxicity (**Fig S1DE**). We identified 15 unique

118 enhancers of RRV-GFP infection in R06E cells and 23 in U2OS cells. Furthermore, 18 effectors

119 enhanced VSV^{M51R}-GFP in R06E and 11 enhanced infection of U2OS cells. We found two

120 effectors to enhance both togavirus and rhabdovirus replication in both bat and human cells: SopB

121 and IpaH4 (**Fig 1AB** and **Fig S2AB**). While SidM failed to enhance RRV-GFP in U2OS cells, it

122 was a hit in three other screens (**Fig S2AB**). Thus, we decided to focus our downstream analyses

123 on these three effectors given their broad rescue phenotypes. These effectors are encoded by

124 three distinct bacterial pathogens: *Salmonella enterica* serovar Typhimurium (SopB), *Shigella*

125 *flexneri* (IpaH4), and *Legionella pneumophilia* (SidM). Collectively, these results suggest that

126 specific effectors encoded by diverse bacterial pathogens can enhance togavirus and rhabdovirus

127 replication in mammalian cells.

128 *Validation of Effector Activities Required for Viral Enhancement*

129   Because the effector genes in our expression library are not epitope-tagged, we wanted

130 to confirm the expression and rescue functions of key hits from our screens. To do this, we cloned

131 Flag-tagged SopB, IpaH4, and SidM into pcDNA3.1 and transfected cells with these vectors for

132 48 h to confirm expression. SopB is an inositol-phosphatase [24], IpaH4 is an E3 ubiquitin ligase

133 [14], and SidM is a guanine nucleotide exchange factor for Rab1 [25, 26]. Therefore, we generated

134 point mutant versions of these effectors that inactivate these known catalytic activities or functions

135 to determine if these activities were important for promoting arbovirus replication. While both wild-

136 type (WT) and mutant forms of these effectors were detected by immunoblot, only WT effectors

137 significantly enhanced arbovirus infection (**Fig 1C-H**), suggesting these diverse effector functions

138 are required to relieve arbovirus restrictions in mammalian cells.

139 *IpaH4 E3 ubiquitin ligase activity is required for ubiquitination and degradation of host RNF214*

140   Our previous study demonstrated that the *Shigella flexneri*-encoded IpaH4 is a bacterial

141 effector with E3 ubiquitin ligase activity [14]. We identified putative human binding partners using

142 a yeast two-hybrid (Y2H) approach with IpaH4 bait. One of the top hits was the RING-finger

143 domain (RNF) containing protein RNF214, a putative E3 ubiquitin ligase. Nine independent prey

144 clones targeting a central region of RNF214 spanning amino acid (a.a) 276-504 were identified

145 (**Fig 2A**) [14]. Interestingly RNF214 was the only human protein identified as a potential substrate

146 for IpaH4 using ubiquitin-activated interaction trap assays [27] that overlapped with our Y2H hits

147 [14]. We did not pursue RNF214 in our previous study examining IpaH4 targets in *L. dispar* cells,

148 as RNF214 proteins are absent in moths and appear to be largely restricted to vertebrates (**Fig

149 2B-D**). For example, through reciprocal BLAST and phylogenetic analyses, we identified RNF214

150 orthologs in numerous mammalian species. However, RNF214 orthologs were rarely found in

151 invertebrate species other than the notable exceptions of starfish and tubeworms (**Fig 2B-D**).

152 Interestingly, AlphaFoldv2 predictions suggest human RNF214 is largely disordered, except for

153 α-helices encompassing most of the region predicted to interact with IpaH4 by Y2H (a.a. 216-

154 422), and portions of the C-terminal RING domain (a.a 550-688) (**Fig 2E**).

155   To determine if IpaH4 targets RNF214, we conducted degradation assays [14] in U2OS

156 cells and found Flag-RNF214 levels to decrease in the presence of WT, but not the catalytically

157 inactive (IpaH4^{C339S}), IpaH4 protein (**Fig 2F**). Consistent with our Y2H results, expression of the

158 central RNF214 region (a.a. 276-504) also led to its degradation in the presence of WT IpaH4

159 (**Fig 2G**). However, IpaH4 did not alter the levels of a N-terminal fragment of RNF214 (a.a. 1-275)

160 (**Fig 2H**). This suggests that the central domain of RNF214 (a.a. 276-504) is sufficient for IpaH4-
161 mediated degradation. *In vitro* ubiquitination confirmed that purified, recombinant Flag-RNF214
162 and Flag-RNF214$^{276-504}$ are targeted for ubiquitination by GST-tagged WT IpaH4, but not the
163 IpaH4^{C339S} catalytic mutant [14] (**Fig 2IJ**). Moreover, *S. flexneri* IpaH2.5, a related E3 ubiquitin
164 ligase [28], was unable to ubiquitinate RNF214, suggesting it is a specific target of IpaH4 (**Fig
165 2IJ**). These results indicate that IpaH4 directly ubiquitinates the central domain of RNF214 for
166 degradation in mammalian cells.

167 *RNF214 overexpression suppresses arbovirus infection of mammalian cells*

168 To determine if RNF214 was relevant to arbovirus restriction, we overexpressed Flag-
169 RNF214 proteins in bat and human cells for 48 h and then challenged cells with arboviruses. To
170 test if the putative E3 ubiquitin ligase activity was important for viral restriction, we used
171 bioinformatics to align the RNF domain of RNF214 with other characterized E3 ligases to identify
172 catalytic residues (**Fig 3A**). RNF domains consist of 40-80 a.a. residues, within which a
173 combination of eight cysteine and histidine residues coordinate two zinc ions to create a distinct
174 three-dimensional structure [29]. Our alignment identified RNF214 a.a. C658 as a likely catalytic
175 residue that was also conserved between bat and human proteins (**Fig 3B**). Overexpression of
176 WT Flag-RNF214 significantly inhibited RRV-GFP and VSV^{M51R}-GFP in bat and human cells.
177 However, Flag-RNF214^{C658S} overexpression did not alter arbovirus replication in bat cells or affect
178 VSV^{M51R}-GFP replication in human cells, despite expressing to similar levels as WT Flag-RNF214.
179 However, there was minor, but statistically significant suppression of RRV-GFP by Flag-
180 RNF214^{C658S} in U2OS cells (**Fig 3C-F**). These data suggest RNF214 suppresses arbovirus
181 replication in bat and human cells via a mechanism largely dependent on its putative E3 ubiquitin
182 ligase activity.

183 *RNF214 Depletion Enhances Virus Replication in Mammalian Cells*

184 To confirm a role for RNF214 in virus restriction, we knocked down RNF214 in R06E and
185 U2OS cells using RNAi and then challenged cells with arbovirus infection. RRV-GFP and
186 VSV^{M51R}-GFP infection was enhanced in two of three RNF214 siRNA treatments (**Fig 4AB**).
187 Furthermore, RNF214 depletion in these cells enhanced the replication of another GFP encoding
188 togavirus, ONNV-GFP (**Fig 4C**) [14]. We confirmed the specificity of RNF214 RNAi in R06E cells
189 (**Fig 4D).** RNF214 knockdown also enhanced RRV-GFP and VSV^{M51R}-GFP in U2OS cells (**Fig
190 S3A-C**). To further confirm these results, we used CRISPR-Cas9 genomic editing to knockout
191 RNF214 in U2OS cells (U2OS$^{\Delta RNF214}$). Immunoblotting confirmed RNF214 knockout (**Fig 4E**).

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192 Infection of U2OS$^{\Delta RNF214}$ cells with RRV-GFP, VSV^{M51R}-GFP, ONNV-GFP, and SINV-GFP [14]
193 resulted in 10-1000-fold increases in GFP signal compared to infections in control U2OS cells.
194 Supernatants from U2OS$^{\Delta RNF214}$ cells also exhibited higher viral titers (**Fig 4F-I**).

195   To assess if other viruses are enhanced in U2OS$^{\Delta RNF214}$ cells, we challenged these cells
196 with dsDNA viruses, vaccinia virus (VACV-FL-GFP) [30] and herpes simplex virus 1 (HSV-1-GFP)
197 [31] but observed no significant changes in GFP signal or viral titer (**Fig S4AB**). In contrast, wild-
198 type VSV-GFP was moderately enhanced in U2OS$^{\Delta RNF214}$ cells (**Fig S4C**), suggesting that even
199 the non-attenuated VSV strain can be restricted by RNF214. In contrast, other negative-sense
200 ssRNA viruses, such as the paramyxoviruses, human parainfluenza virus 1 (HPIV1-GFP) [32]
201 and Sendai virus (SeV-GFP) [31] either exhibited no change or minor increases in viral replication
202 (**Fig S4DE**). A similar minor enhancement was observed in U2OS$^{\Delta RNF214}$ cells for the positive-
203 sense ssRNA flavivirus, West Nile virus (WNV-GFP) [21] (**Fig S4F**). Interestingly, Venezuelan
204 equine encephalitis virus (VEEV-GFP) [33, 34] showed a ~15-fold increase in titer in U2OS$^{\Delta RNF214}$
205 cultures (**Fig S4G**), consistent with our observations for other togaviruses (**Fig 4F**). In summary,
206 these data suggest RNF214 is important for restricting ssRNA virus infections but not DNA viruses
207 (**Fig 4H**).

208 **Discussion**

209   Here, we applied our bacterial effector screening methodology to identify immune evasion
210 proteins that modulate antiviral immunity in mammals. Such functional screens are important to
211 develop in non-model species, such as bats, given the lack of tools to perform more classical
212 screens (e.g. CRISPR-Cas9) for host immunity factors on a high throughput scale. By applying
213 our screening approach to both bat and human cells, we identified 30 effectors that enhance
214 arbovirus infection in bat cells and 29 in human cells. Moreover, we discovered three effectors:
215 SopB, SidM, and IpaH4, that enhanced togavirus and rhabdovirus replication in bat and human
216 cells.

217   Here we focused on understanding how the *S. flexneri*-encoded E3 ubiquitin ligase, IpaH4,
218 enhances arbovirus replication in mammalian cells. Our Y2H [14] and ubiquitin-activated
219 interaction trap assays [27] with IpaH4 identified human RNF214 as a putative substrate, but it
220 was unclear if RNF214 was relevant to innate immunity. This was important to determine as our
221 phylogenetic analyses suggest RNF214 proteins are widely conserved among vertebrate species
222 including mammals. We found IpaH4 to directly ubiquitinate RNF214 *in vitro* and promote its
223 degradation, indicating it is a bona fide substrate. Furthermore, RNF214 overexpression inhibited,

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224 and RNF214 depletion enhanced, arbovirus infection of bat and human cells, suggesting RNF214

225 proteins are potent arbovirus restriction factors in mammalian hosts. Moreover, by screening a

226 panel of 11 viruses, representing 6 viral families, we found RNF214 to most potently inhibit

227 togaviruses and rhabdoviruses, but also restricts specific paramyxoviruses and flaviviruses.

228 These data strongly implicate mammalian RNF214 proteins in antiviral immunity. Interestingly,

229 other RNF domain-containing proteins, such as RNF213, have been shown to restrict both viruses

230 and bacteria [35-37]. Therefore, RNF214 may be an additional member of an ever-expanding

231 group of host E3 ubiquitin ligases that use ubiquitination to combat microbial infection [38-40].

232 Consistent with this, the RNF214^{C658S} mutant, predicted to be catalytically inactive, was unable to

233 suppress arbovirus replication in mammalian cells.

234   Future studies will aim to confirm RNF214 E3 ubiquitin ligase activity *in vitro* and determine

235 the mechanism by which these host factors restrict viral replication. Interestingly, two recent

236 studies suggest that RNF214 promotes ubiquitination of TEAD proteins to regulate the Hippo

237 pathway, which plays roles in cellular homeostasis and proliferation [41]. Several viruses

238 suppress this pathway, including SARS-CoV-2 and Ebola virus, suggesting it may play a role in

239 antiviral defense [42, 43]. Whether RNF214-mediated regulation of the Hippo pathway explains

240 its antiviral activity will be important to determine.

241   In summary, our study illustrates how bacterial effector screens can be applied to

242 mammalian systems to uncover new effector protein functions and novel innate immunity factors.

243 Thus, our system provides a power approach to reveal critical pathogen-host interactions that

244 regulate host susceptibility to infection.

245 **Materials and Methods**

246 *Cell Lines and Cell Culture*

247 Mammalian cell lines were maintained at 37°C in 5% CO_2 atmosphere. U2OS cells were cultured

248 in DMEM supplemented with 10% FBS containing 1% non-essential amino acids (NEAA), 1% L-

249 glutamine, and 1% antibiotic/antimycotic (Gibco) (**Table S2**). R06E cells were cultured in

250 DMEM:F12 supplemented with 10% FBS and 1% antibiotic/antimycotic (Gibco). BSC-40 cells

251 were cultured in MEM supplemented with 5% FBS (**Table S2**).

252 *Viruses*

253 Stock preparation, culture of recombinant viruses, and titration by fluorescent foci/plaque assay

254 on BSC-40 cells was performed as previously described [14, 15]. Viral innocula were incubated

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255 with cells for 1 h in serum free media before the addition of complete media for the remainder of

256 the infection. Where indicated, complete media containing ActD at the indicated dose was added

257 for the remainder of the infection.

258 *Cell Viability Assay*

259 Cell viability was measured using a CyQUANT^{TM} LDH Cytotoxicity assay (Invitrogen; **Table S2**)

260 as previous described [14] 48 h post-transduction of cells with the effector expression library.

261 *Plasmid Constructs for Mammalian Cell Expression*

262 The 210 bacterial effector protein coding sequences was generated in pENTR/D as previously

263 described [44]. Briefly, PCR-amplified bacterial genomic DNA sequences were cloned into

264 pENTR/D-TOPO (Invitrogen) using topoisomerase I. Source organisms were *Pseudomonas*

265 *syringe* pv. Tomato (ATCC BAA-871D-5), *Legionella pneumophila* Philadelphia-1 (ATCC

266 33152D-5), *Salmonella Typhimurium* LT2 (gift of Jack Dixon, University of California San Diego),

267 EHEC H7:O157 (gift of Vanessa Sperandio, University of Wisconsin-Madison), *Shigella flexneri*

268 M90T (gift of Jack Dixon, University of California San Diego), and *Bartonella henselae* Houston-

269 1 (gift of Alexei Savchenko, University of Toronto). For expression in mammalian cells, the library

270 was cloned into the lentiviral expression vector pTRIP-CMV-IVSb-IRES-TagRFP [21] using

271 Gateway LR Clonase II (Invitrogen) (**Table S2**).

272 RNF214 was Gibson cloned from pENTR221 (DNASU HsCD00513497) into pFLAG-CMV-6b,

273 imparting an N-terminal Flag tag. Flag-tagged RNF214 fragments were also generated in pFLAG-

274 CMV-6b by Gibson cloning. For bacterial expression, sequence encoding Flag-RNF214 or only

275 the 276-504 a.a. RNF214 fragment were Gibson cloned into pProEX-HTb (Invitrogen), imparting

276 a 6xHis tag at the N-terminus upstream of the Flag sequence.

277 N-terminal Flag-tagged versions of SopB (WFG56166.1), SidM (YP_096471.1), and C-terminal

278 Flag-tagged IpaH4 (EID62426.1) wild-type and point mutants were generated as previously

279 described and cloned into pcDNA3.1 for expression in mammalian cells [14].

280 *General Transfection Protocols*

281 Unless otherwise stated, 100,000 cells were plated into 24-well dishes, transfected with 500 ng

282 of expression vectors for 48 h prior to manipulation (e.g. protein extraction or virus infection). For

283 U2OS cell expression of the pcDNA3.1 constructs, 500 ng of vector was mixed with 100 μL of

284 OptiMEM media and 1.5 μL of Lipofectamine 2000 Reagent (**Table S2**). This mixture was

285 incubated at room temperature for 20 min, and media on the cells was changed to 500μL
286 OptiMEM, then the transfection mixture was added dropwise to wells, incubated overnight and
287 media was then changed to complete DMEM ~16 h post-transfection.

288 For R06E transfection experiments, as well as HEK293T transfection, 500 ng of vector was mixed
289 with 50 uL of Opti-MEM and 1 μL of FuGENE HD Transfection Reagent (Promega; **Table S2**).
290 This mixture was incubated at room temperature for 20 min before adding dropwise to the well.
291 Cells were then incubated for 48 h prior to further manipulation or infection.

292 Transient siRNA-mediated knockdown was achieved by reverse transfection of R06E cells with 8
293 pmol siRNA and 1.5 μL of FuGENE HD Reagent according to the manufactureer's protocol. The
294 same protocol was used for U2OS cells except Lipofectamine 2000 was used as the transfection
295 reagent according to the manufacturer's protocol. Cells were transfected for 48 h and then either
296 subjected to protein extraction and immunoblotting or were subjected to GFP reporter virus
297 infection to assess replication phenotypes.

298 *Immunoblotting*

299 Immunoblotting was conducted as previously described with primary antibodies, secondary
300 antibodies conjugated with infrared dyes and a Li-Cor Odyssey scanner [14].

301 *Bacterial Effector Screens and Fluorescence Microscopy*

302 Cells were seeded into 96-well clear bottom dishes, transduced with the lentivirus effector library
303 in quadruplicate for 48h, and then challenged with indicated GFP reporter viruses. At the indicated
304 times post-infection, cells were stained as previously described [14] with CellTracker Dye, fixed
305 with PFA, and cells were imaged by the UT Southwestern Medical Center High-Throughput
306 Screening Core using an IN Cell Analyzer 6000 (Molecular Devices) scope equipped with 405,
307 488, and 561 nm lasers using a using a 10x objective. Four images were taken/well so that
308 fluorescence signals/well resulted from an average of these four images. Signals across all 4
309 replicate wells for each effector treatment were then used to determine GFP reporter virus
310 infection levels. Image analysis was conducted using Fiji 2.14.0 (NIH) to quantify the percent area
311 of each field of view containing GFP signal and these signals were normalized to CellTracker Dye
312 signal to account for cell number [14]. Finally, normalized GFP signals for each effector treatment
313 were plotted as a fold change in GFP signal relative to cells transduced with lentivirus vector
314 expressing firefly luciferase (LUC) (negative control). Validation assays to confirm virus-
315 enhancing phenotypes after transfection of pCDNA3.1 expression plasmids encoding Flag-

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316 tagged effector constructs were conducted and analyzed in a similar manner except empty
317 pCDNA3.1 vector was used as a negative control treatment.

318 *RNF214 Degradation Assay*

319 ~75,000 cells were co-transfected with 150 ng target pCDNA3.1 vectors encoding full-length Flag-
320 RNF214 (or RNF214 fragments) and 350 ng of pEGFP-C2 encoding either GFP, GFP-IpaH4, or
321 GFP-IpaH4^C339S[14] using 1.5 μL of X-tremeGENE 9 and 50 μL of OptiMEM (Sigma; Table S2).
322 After 24 h, cells were harvested directly in 1X Laemmeli buffer containing β-mercaptoethanol.
323 Protein extracts were then subjected to SDS-PAGE and subsequent immunoblotting with
324 indicated antibodies.

325 *Protein Purification*

326 For human Flag-RNF214 full length or fragment expression *E. coli* BL21 cells were transformed
327 with pProEX-HTb-6xHis-FLAG-RNF214 or pProEX-HTb-6xHis-FLAG-RNF214$^{276-504}$ vectors and
328 spread onto LB agar plates containing 100 μg/mL ampicillin. A colony was picked and grown in a
329 30 mL LB culture with 100 μg/mL ampicillin overnight at 37°C with shaking. The following day,
330 this culture was added to 1 L of LB with ampicillin and grown at 37°C with shaking to an OD600
331 of 0.8. Protein expression was then induced by the addition of 0.5 mM IPTG and the culture was
332 grown overnight at 18°C with shaking. Bacteria were pelleted at 4000xg for 20 min (Beckman
333 Avanti J-25) and resuspended in 30 mL of purification buffer (20 mM HEPES, 150 mM NaCl, 1
334 mM TCEP, and 1X SigmaFAST protease inhibitor (Sigma S8820), pH=7.5). Lysate was clarified
335 by centrifugation at 10,000xg for 15 min. The fusion protein was then affinity-purified over an
336 Econo-Pac chromatography column (Biorad 9704652) using TALON Metal Affinity Resin (Takara
337 635502) and eluted in purification buffer containing 500 mM imidazole. The protein was dialyzed
338 using a 10,000 MWCO Slide-A-Lyzer dialysis cassette (Thermo 66810) in 1 L of purification buffer
339 without imidazole. Protein was combined with glycerol at 20% and stored at -80°C. Recombinant
340 GST-IpaH4 for *in vitro* ubiquitination experiments was expressed and purified as previously
341 described [14].

342 *In-Vitro Ubiquitination Assay*

343 *In vitro* ubiquitination reactions were performed as previously described [14] with minor
344 modifications. Briefly, reactions were performed in 50 mM HEPES pH 7.5, 150 mM NaCl, 20 mM
345 MgCl$_2$, and 10 mM ATP in a volume of 30 μL. The following recombinant proteins were added: 1
346 μM UbE1 (E1), 5 μM UbcH5b (E2), 5 μM GST-IpaH or GST-IpaH4^C339S, 50 μM ubiquitin, and 5

11

347 µM 6xHis-FLAG-tagged RNF214 or its truncated versions. ATP was added last to initiate

348 reactions, which were then incubated for 2 h at 30°C before the addition of 30 µL 2X Laemmli

349 buffer containing β-mercaptoethanol. Samples were then boiled for 10 min at 95°C, subjected to

350 SDS-PAGE, and immunoblotting.

Lentivirus Production

352 HEK293T cells were plated in poly-D-lysine coated 6-well plates at 400,000 cells/well in 2 mL/well

353 to yield a confluency of 50% the following day. Media was changed to 1.5 mL/well DMEM

354 containing 3% FBS and 1% NEAA. Cells were then transfected with 200 ng/well pCMV-VSVG,

355 800 ng/well pCMV-Gag/Pol, and 1000 ng/well pTRIP-CMV-Effector-IVSb-IRES-TagRFP using X-

356 tremeGENE 9 (Sigma XTG9-RO) and OptiMEM (Thermo 31985062). Following 6 h of incubation,

357 media was changed to 1.5 mL/well DMEM with 3% FBS and 1% NEAA. At 48 h post-media

358 change, the supernatant was collected and replaced with fresh media. After an additional 24 h,

359 the supernatant was again collected. Supernatants from both time points were pooled, centrifuged

360 at 3000xg for 5 min to remove cell debris, and combined with HEPES to a concentration of 20

361 mM and polybrene to a concentration of 4 µg/mL. Lentivirus stocks were then arrayed in v-bottom

362 96-well plates and stored at -80°C.

Statistical Analyses

364 Graphs were presented as mean values ± SEM with individual data points shown. At least three

365 independent experiments were conducted for all quantitative experiments shown where statistical

366 analyses were applied. All statistical analyses were performed with Prism software v10.0.2

367 (GraphPad) and statistical tests used are indicated in respective figure legends. Statistical

368 significance ($P<0.05$) between compared groups is indicated in Figs as either: ns (not significant),

369 *=$P<0.05$, **=$P<0.01$, ***=$P<0.001$, ****=$P<0.0001$.

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381 **Author Contributions**

382 **Aaron Embry**: Conceptualization; Data Curation; Formal Analysis; Funding Acquisition;

383 Investigation; Methodology; Validation; Visualization; Writing – Original Draft; Writing – Review &

384 Editing. **David Schad**: Data Curation; Formal Analysis; Investigation; Methodology; Writing –

385 Review & Editing. **Emily A. Rex**: Data Curation; Formal Analysis; Funding Acquisition;

386 Investigation; Methodology; Validation; Writing – Review & Editing. **Neal M. Alto**:

387 Conceptualization; Formal Analysis; Supervision; Funding Acquisition; Methodology; Project

388 Administration; Visualization; Writing – Review & Editing. **Don B. Gammon**: Conceptualization;

389 Data Curation; Validation; Formal Analysis; Supervision; Funding Acquisition; Investigation;

390 Methodology; Project Administration; Visualization; Writing – Original Draft; Writing – Review &

391 Editing.

392 **Competing Interests**

393 The authors declare that there are no competing interests.

394 **Data Availability**

395 All relevant data are within the manuscript or supporting data.

396 **Fig Legends**

397 **Fig 1. Identification of bacterial effectors that promote arbovirus replication in bat and**

398 **human cells. A-B.** Fold-change in viral GFP signals (normalized to CellTracker Blue signal)

399 relative to cells transduced with lentivirus expressing firefly luciferase (LUC) (negative control) for

400 RRV-GFP (A) and VSV^{M51R}-GFP (B) screens 20 hpi. The cutoff for effectors to be scored as hits

401 was a >10-fold change in over negative controls (cutoffs represented by dotted lines). Data points

402 are means. **C-E.** Representative immunoblots of Flag-tagged bacterial effector expression in bat

403 R06E cells 48 h post-transfection with pCDNA3.1 vectors. Additionally, fold-change in normalized

404 viral GFP signal relative to cells transfected with empty vector (EV) is shown. Cells were stained

405 20 hpi with CellTracker Orange dye and imaged to calculate fold-change in normalized GFP signal

406 over signals in EV treatments. Wild-type (WT) effectors are compared to their mutants (SopB^{C420S},

407 SidM^{A435E}, IpaH4^{C339S}). **F-H.** Representative immunoblots and fold-change in normalized viral

408 GFP signal for effectors experiments conducted as in C-E but in human U2OS cells. Quantitative

13

409   data in C-H are means ± SEM; n=3. Statistical significance in graphs in C-H was determined with

410   One-way ANOVA; ns (not significant), *=P<0.05, **=P<0.01, ***=P<0.001, ****=P<0.0001.

411   **Fig 2. The central domain of the conserved host protein, RNF214 , is sufficient for IpaH4-**

412   **mediated ubiquitination and degradation. A.** Schematic of human RNF214 with mapping of

413   prey clones collectively identified in two yeast two-hybrid screens using IpaH4 bait [14]. Numbers

414   indicate RNF214 a.a. encoded by each prey clone. **B.** Schematic displaying the relative

415   conservation and length of RNF214 across indicated organisms. **C.** PhyloTv2-generated

416   phylogenetic tree of RNF214 orthologs. **D.** Table of RNF214 orthologs in various vertebrate and

417   invertebrate species generated via reciprocal BLAST analysis. **E.** AlphaFold2- predicted structure

418   of human RNF214. **F.** Representative immunoblot of degradation assay using Flag-tagged

419   RNF214 co-transfected into U2OS cells with GFP-IpaH4 (WT) or GFP-IpaH4^{C339S} (C339S)

420   catalytic mutant expression vectors. **G-H.** Representative immunoblot of degradation assays

421   using Flag-tagged RNF214 truncations, RNF214$^{1-275}$ (**G**) or RNF214$^{276-504}$ (**H**), co-transfected into

422   U2OS cells with GFP-IpaH4 (WT) or GFP-IpaH4^{C339S} (C339S) vectors. **I-J.** Representative

423   immunoblot of *in vitro* ubiquitination assay showing direct IpaH4-mediated ubiquitination of

424   purified, recombinant human Flag-RNF214 (**I**) and Flag-RNF214$^{276-504}$ (**J**) proteins.

425   **Fig 3. Overexpression of RNF214 suppresses arbovirus replication in bat and human cells.**

426   **A.** a.a. alignment of human RNF214 with other human RNF containing proteins. Residues in the

427   characteristic RNF domain "C4-H-C3" motif are highlighted in red, and the C658 residue targeted

428   for substitution mutation is boxed. **B.** a.a. alignment of human RNF214 with *R. aegyptiacus*

429   RNF214. Residues are highlighted as described in A. **C.** Representative immunoblot of Flag-

430   tagged RNF214 and RNF214^{C658S} (C/S) overexpression in R06E cells for 48 h. **D.** Percent of GFP

431   signal relative to empty vector (EV) control after R06E cells overexpressing RNF214 constructs

432   (WT or RNF214^{C658S}) were infected with RRV-GFP (MOI=0.05) or VSV^{M51R}-GFP (MOI=0.005)

433   were for 20 h. **E.** Representative immunoblot of Flag-tagged RNF214 constructs overexpressed

434   in U2OS cells for 48 h. **F.** Percent of GFP signal relative to empty vector (EV) control in R06E

435   cells overexpressing Flag-RNF214 constructs (WT or RNF214^{C658S}) RRV-GFP (MOI=0.05) or

436   VSV^{M51R}-GFP (MOI=0.005) for 20 h. Data are means ± SEM; n=3. Statistical significance was

437   determined with One-way ANOVA; ns (not significant), *=P<0.05, **=P<0.01, ***=P<0.001,

438   ****=P<0.0001.

439   **Fig 4. Depletion of RNF214 enhances arbovirus replication in bat and human cells. A.**

440   Representative fluorescence microscopy images (GFP channel) of R06E cells transfected with

441   firefly luciferase (Luc)- or RNF214-targeting siRNAs. 48 h post-knockdown, cells were infected

442   with RRV-GFP (MOI=0.001) for 20 h. Cells were then stained with CellTracker Orange, imaged,

443   and fold-change in GFP signal compared to the Luc control was calculated. Supernatants were

444   also collected to assess viral titers by plaque assay. **B-C.** Similar experiments were performed as

445   in A but for VSV^{M51R}-GFP (MOI=0.0001) (**B**) and ONNV-GFP (MOI=0.001) (**C**). **D.** Representative

446   immunoblot of endogenous bat RNF214 levels following siRNA knockdown in R06E cells for 48

447   h. **E.** Representative immunoblot of endogenous human RNF214 levels following in U2OS cells

448   transduced with control lentivirus (C) or lentivirus expressing guide RNAs targeting RNF214 (KO).

449   **F.** Representative fluorescence microscopy images (GFP channel) in control or ΔRNF214 U2OS

450   cells infected with RRV-GFP (MOI=0.001) 20 h. Cells were then stained with CellTracker Orange,

451   imaged, and fold-change in GFP signal compared to Control U2OS cell infections was calculated.

452   Supernatants were also collected to assess viral titers by plaque assay. **G-I.** Similar experiments

453   were performed as in F but for VSV^{M51R}-GFP (MOI=0.0001) (**G**), ONNV-GFP (MOI=0.001) and

454   SINV-GFP (MOI=0.001) (**H**). Data are means ± SEM; n=3. Statistical significance for A-C was

455   determined with One-way ANOVA and with unpaired student's t-test for F-I; ns (not significant),

456   *=$P<0.05$, **=$P<0.01$, ***=$P<0.001$, ****=$P<0.0001$. Scalebars indicate 200 μm on all microscopy

457   images.

458   **Supporting Information**

459   **Table S1. Bacterial effector library screening results and cell viability data.**

460   **Table S2. Key resources and reagents.**

461   **Supplementary Fig Legends**

462   **Fig S1. Effect of ActD treatment on arbovirus infection of mammalian cells and impact of**

463   **effector expression on mammalian cell viability. A.** Representative fluorescence microscopy

464   images (GFP channel) of R06E cells infected with RRV-GFP (MOI=0.001) or VSV^{M51R}-GFP

465   (MOI=0.0001) and treated with either DMSO or 10 nM ActD for 20 h. Scalebars indicate 200 μm.

466   **B.** Fold-change in GFP reporter readout following 20 h infection in the presence of the indicated

467   doses of ActD, normalized to DMSO control. **C.** Results of LDH-based cytotoxicity assays in R06E

468   cells. Absorbance at 490 nm is plotted for supernatant collected from R06E cells treated for 20 h

469   with increasing doses of ActD. Positive (+) control supplied by the manufacturer, as well as cells

470   lysed with manufacturer 10X lysis buffer (lysed cells) are also plotted for reference. **D.** Results of

471   LDH-based cytotoxicity assays in R06E cells transduced with effector library for 48 h. LDH values

472   that were significantly higher ($P<0.05$) from Luc-transduced control cells were considered toxic

473   effectors (red bars). **E.** Results of LDH-based cytotoxicity assays in U2OS cells transduced with

474 effector library for 48 h as in D. Red bars indicate toxic effectors. Data are means ± SEM; n=3.

475 Statistical significance for B-C was determined with One-way ANOVA; ns (not significant),

476 *=P<0.05, **=P<0.01, ***=P<0.001, ****=P<0.0001.

477 **Fig S2. Specific bacterial effectors enhance arbovirus replication in bat and human cells.**

478 **A.** Summary of bacterial effectors that rescued RRV-GFP replication in bat or human cells, or

479 both cell types. Green blocks indicate the effector enhanced RRV-GFP in the cell line indicated

480 in the column header. Effectors are listed from high-to-low based on their fold-change in GFP

481 signal over controls. The bacterium encoding each effector is noted to the right: *Shigella flexneri*

482 (*S. flexneri*), *Pseudomonas syringae* (*P. syringae*), *Salmonella enterica* (*S. enterica*), *Legionella*

483 *pneumophila* (*L. pneumo.*) *Enterohemorrhagic Escherichia coli* 0157:H7 (*EHEC*), or *Bartonella*

484 *henselae* (*B. henselae*). **B.** Summary of bacterial effector proteins that enhanced VSV^{M51R}-GFP

485 replication in bat or human cells, or both cell types. The complete list of effectors screened and

486 the raw results of the screens can be found in **Table S1**.

487 **Fig S3. RNF214 knockdown enhances arbovirus replication in human U2OS cells. A.**

488 Representative immunoblot of RNF214 levels in U2OS cells transfected with indicated siRNAs

489 for 48 h. **B.** Fold-change in GFP reporter readout following RRV-GFP infection of U2OS cells after

490 indicated knockdowns. Cells were infected (MOI=0.001) 48 h post-siRNA transfection for 20 h,

491 stained with CellTracker Orange, and imaged to determine the fold-change in GFP signal

492 compared to Luc siRNA (control) treatments. **C.** Fold-change in GFP reporter readout following

493 VSV^{M51R}-GFP infection of U2OS cells after indicated knockdowns. Cells were infected

494 (MOI=0.0001) 48 h post-siRNA transfection for 20 h, stained with CellTracker Orange, and

495 imaged to determine the fold-change in GFP signal compared to Luc siRNA (control) treatments.

496 Data are means ± SEM; n=3. Statistical significance was determined with One-way ANOVA; ns

497 (not significant), *=P<0.05, **=P<0.01, ***=P<0.001, ****=P<0.0001.

498 **Fig S4. Assessing the impact of RNF214 knockout on the replication of diverse viral**

499 **families. A-B.** Control or U2OS$^{\Delta RNF214}$ cells infected with the indicated reporter poxvirus (VACV-

500 FL-GFP; MOI=0.01) or herpesvirus (HSV-1-GFP; MOI=0.01). Following infection, cells were

501 stained with CellTracker Orange and imaged for fold-change in GFP reporter readout compared

502 to control cells. Titers are also shown from these collected cultures. **C-G.** Similar experiments

503 were performed as in A-B but with VSV-GFP (MOI=0.0001) (C), the paramyxoviruses: HPIV1-

504 GFP (MOI=0.001) (D) and SeV-GFP (MOI=0.001) (E), the flavivirus, WNV-GFP (MOI=0.001) (F)

505 and the togavirus, VEEV-GFP (MOI=0.001) (**G**). **H.** Table summarizing results for the various viral

506 families tested in this figure. Data for A-G are means ± SEM; n=3. Statistical significance was

507 determined with unpaired student's t-test; ns (not significant), *=P<0.05, **=*P*<0.01, ***=*P*<0.001,

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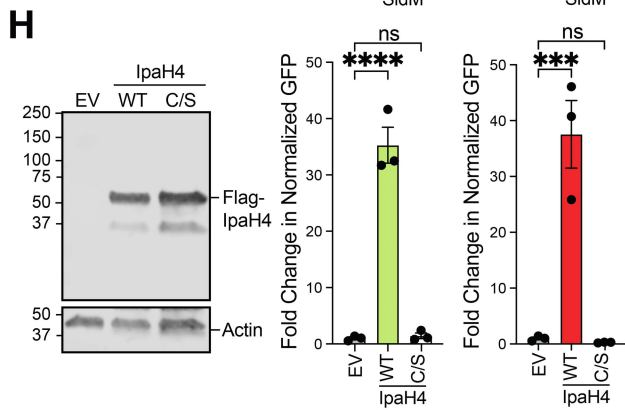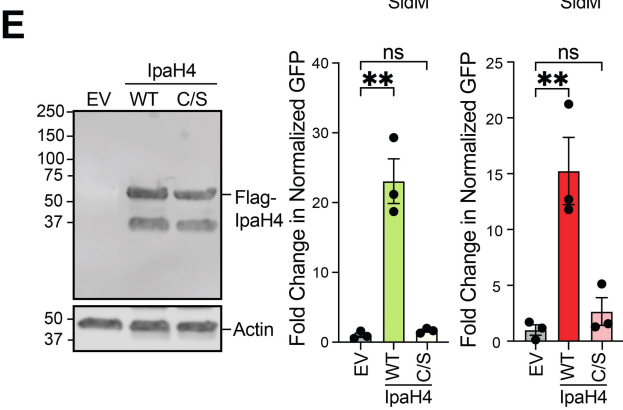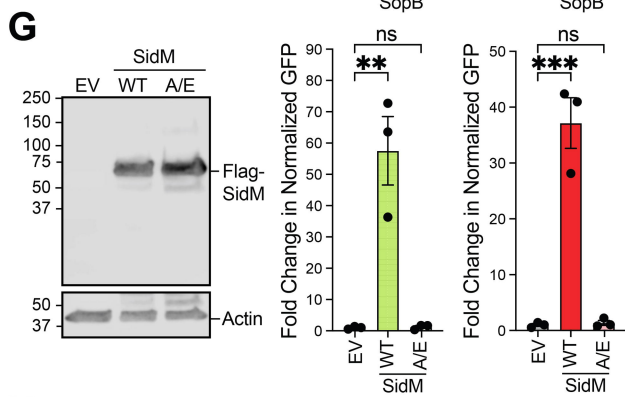604 10.1038/nmicrobiol.2016.84. PubMed PMID: 27572974; PubMed Central PMCID: PMCPMC5010086.
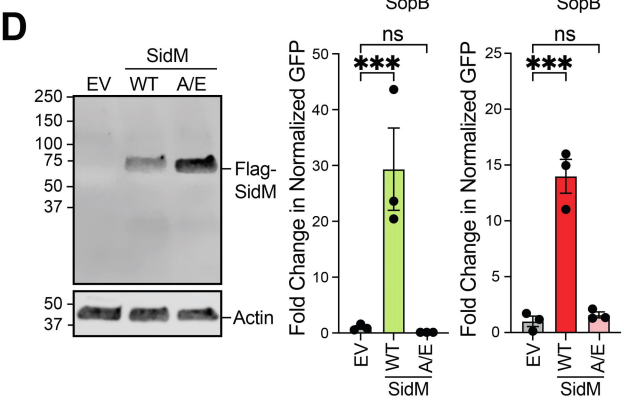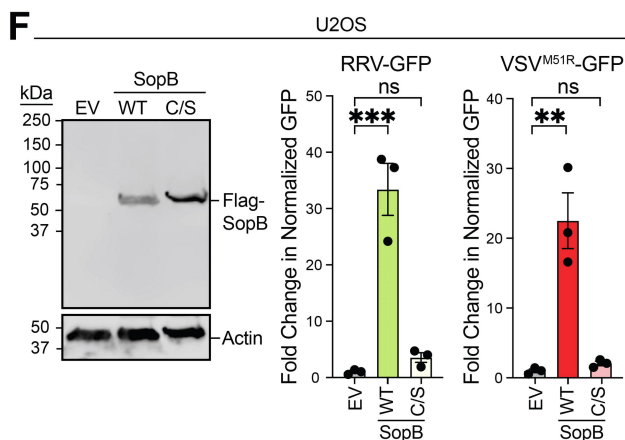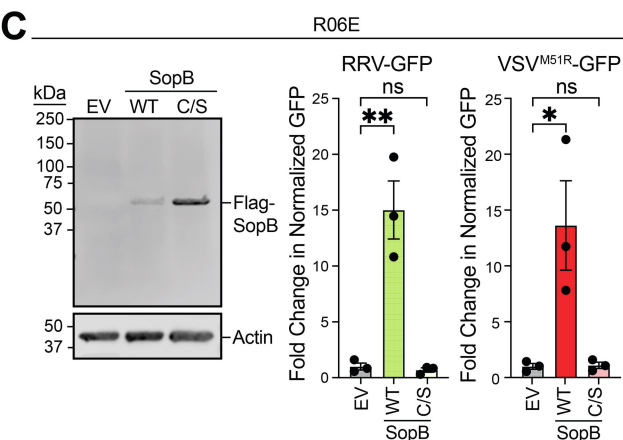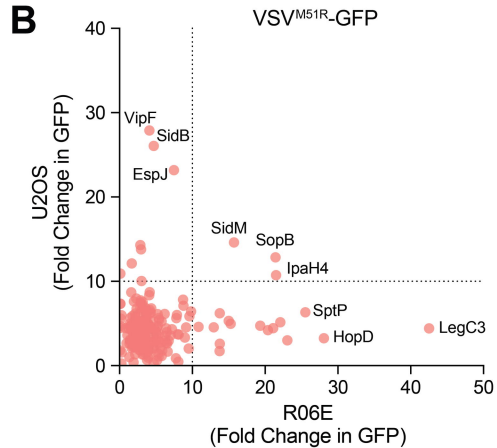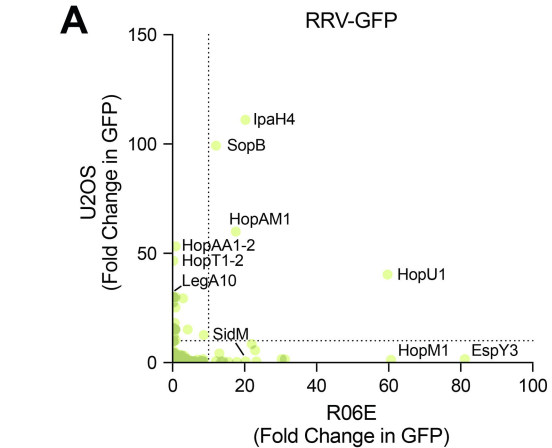605 29.   Nakamura N. The Role of the Transmembrane RING Finger Proteins in Cellular and Organelle
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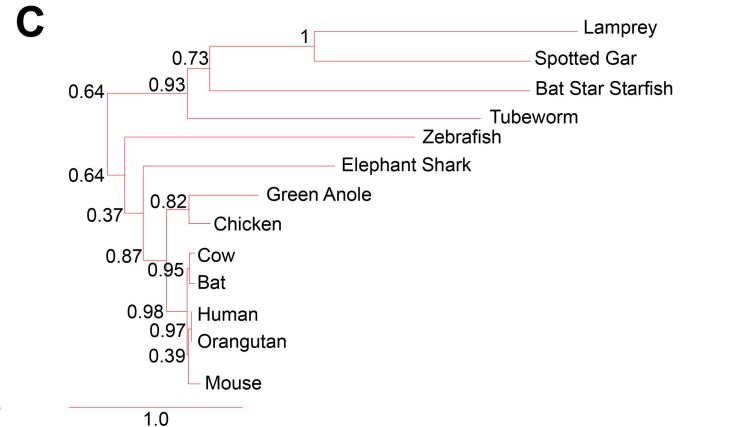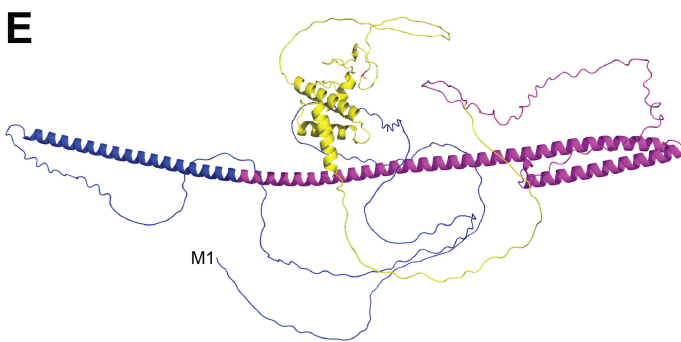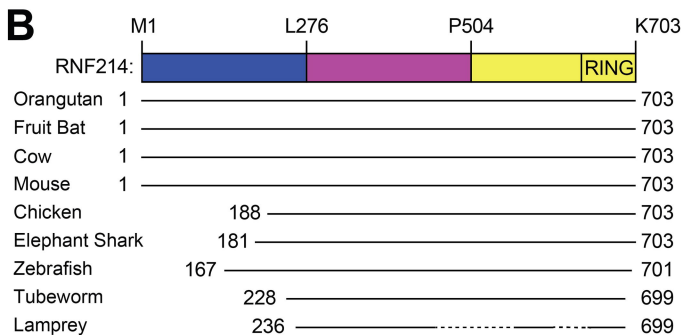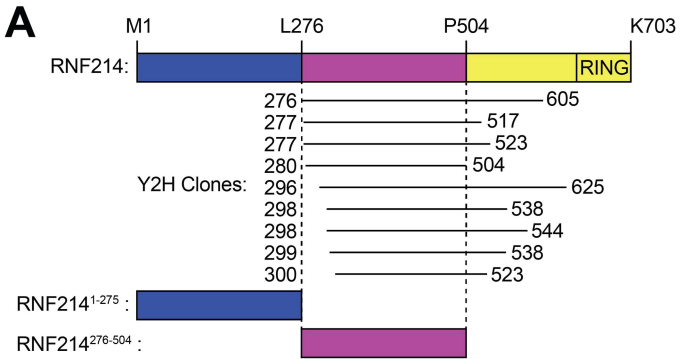607 PubMed PMID: 24957874; PubMed Central PMCID: PMCPMC4021871.
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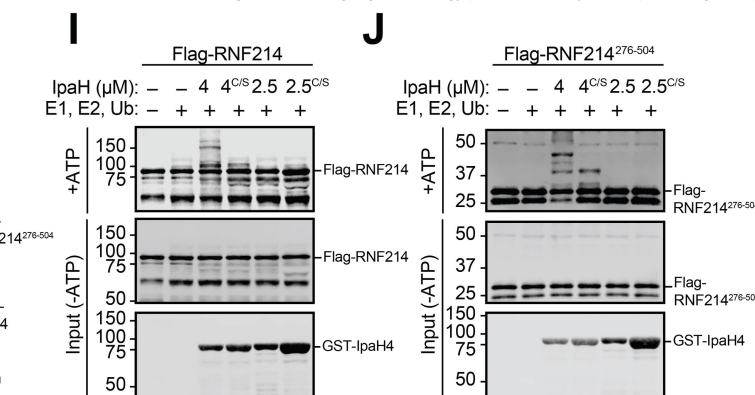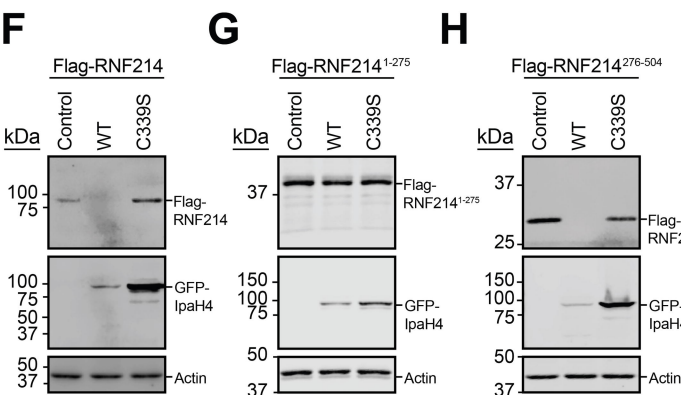657   PMCID: PMCPMC5599653.

658

A

M1　　　　　L276　　　　　P504　　　　　K703

RNF214: (blue) | (magenta) | (yellow) RING

Y2H Clones:
276 — 605
277 — 517
277 — 523
280 — 504
296 — 625
298 — 538
298 — 544
299 — 538
300 — 523

RNF214$^{1-275}$: (blue)

RNF214$^{276-504}$: (magenta)

B

M1　　　　　L276　　　　　P504　　　　　K703

RNF214: (blue) | (magenta) | (yellow) RING

Orangutan 1 — 703
Fruit Bat 1 — 703
Cow 1 — 703
Mouse 1 — 703
Chicken 188 — 703
Elephant Shark 181 — 703
Zebrafish 167 — 701
Tubeworm 228 — 699
Lamprey 236 — — — 699

C

Lamprey
Spotted Gar
Bat Star Starfish
Tubeworm
Zebrafish
Elephant Shark
Green Anole
Chicken
Cow
Bat
Human
Orangutan
Mouse

(Tree values: 1, 0.73, 0.93, 0.64, 0.64, 0.37, 0.82, 0.87, 0.95, 0.98, 0.97, 0.39)

1.0

D

Common Name	Scientific Name	Accession Number	% Converage	% Identical
Human	*Homo sapiens*	NP_001070707.1	100	100.0
Orangutan	*Pongo pygmaeus*	XP_002822574.2	100	98.72
Fruit Bat	*Rousettus aegyptiacus*	XP_015990379.2	100	95.60
Cow	*Bos taurus*	XP_005215924.1	100	94.46
Mouse	*Mus musculus*	XP_011240798.1	100	92.89
Chicken	*Gallus gallus*	XP_004948147.1	73	79.11
Green Anole	*Anolis carolinensis*	XP_062816988.1	71	68.28
Spotted Gar	*Lepisosteus oculatus*	XP_015196866.1	71	25.00
Zebrafish	*Danio rerio*	XP_021332230.1	76	26.13
Elephant Shark	*Callorhinchus milii*	XP_007906977.2	74	39.17
Lamprey	*Petromyzon marinus*	XP_032833068.1	30	26.39
Bat Star (Starfish)	*Patria miniata*	XP_038065127.1	34	27.70
Spongy Moth	*Lymantria dispar*	N.D.	0	0
Silk Moth	*Bombyx mori*	N.D.	0	0
Asian Tiger Mosquito	*Aedes albopictus*	N.D.	0	0
Yellow Fever Mosquito	*Aedes aegypti*	N.D.	0	0
Tubeworm (Annelid)	*Owenia fusiformis*	CAH1796261.1	67	23.98

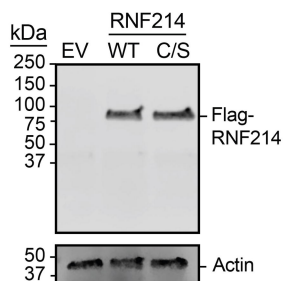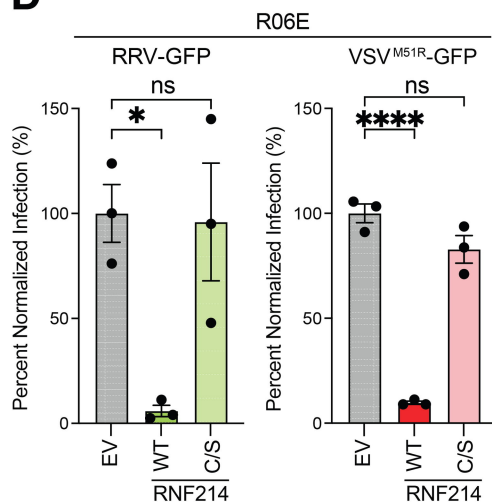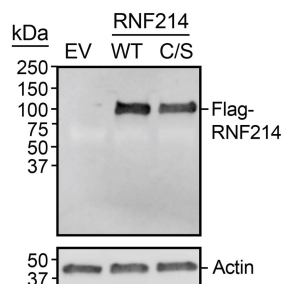N.D. = Not determine; no homolog found, or no significant homology (as determined by BLAST protein alignment)
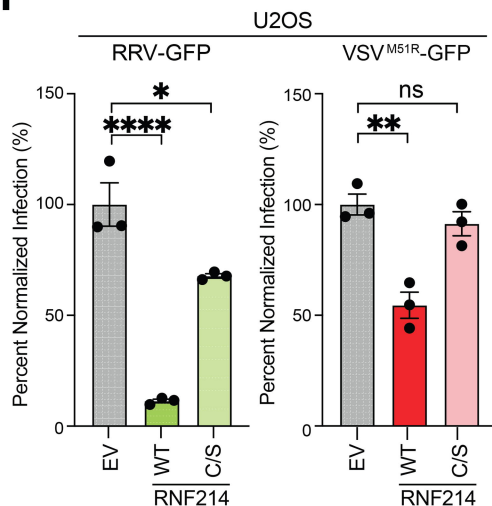
E

M1

F

Flag-RNF214

kDa | Control | WT | C339S

100
75 — Flag-RNF214

100
75
50
37 — GFP-IpaH4

50
37 — Actin

G

Flag-RNF214$^{1-275}$

kDa | Control | WT | C339S

37 — Flag-RNF214$^{1-275}$

150
100
75 — GFP-IpaH4

50
37 — Actin

H

Flag-RNF214$^{276-504}$

kDa | Control | WT | C339S

37
25 — Flag-RNF214$^{276-504}$

150
100
75 — GFP-IpaH4

50
37 — Actin

I

Flag-RNF214

IpaH (μM): − − 4 4$^{C/S}$ 2.5 2.5$^{C/S}$
E1, E2, Ub: − + + + + +

+ATP
150
100
75 — Flag-RNF214

150
100
75 — Flag-RNF214

Input (−ATP)
150
100
75 — GST-IpaH4

J

Flag-RNF214$^{276-504}$

IpaH (μM): − − 4 4$^{C/S}$ 2.5 2.5$^{C/S}$
E1, E2, Ub: − + + + + +

+ATP
50
37
25 — Flag-RNF214$^{276-504}$

50
37
25 — Flag-RNF214$^{276-504}$

Input (−ATP)
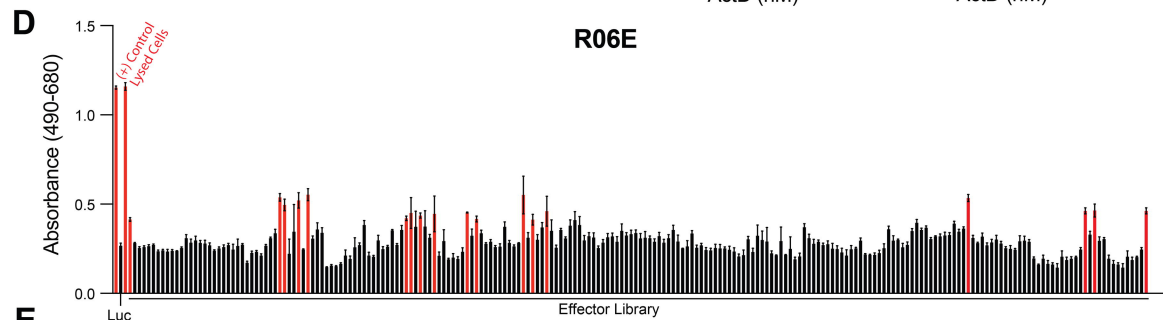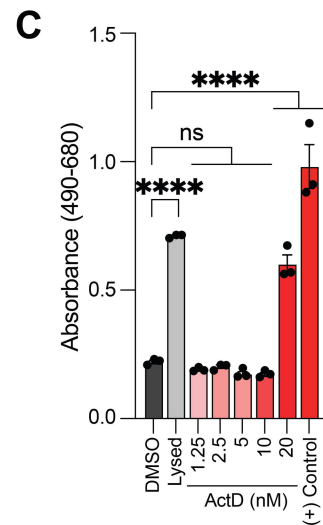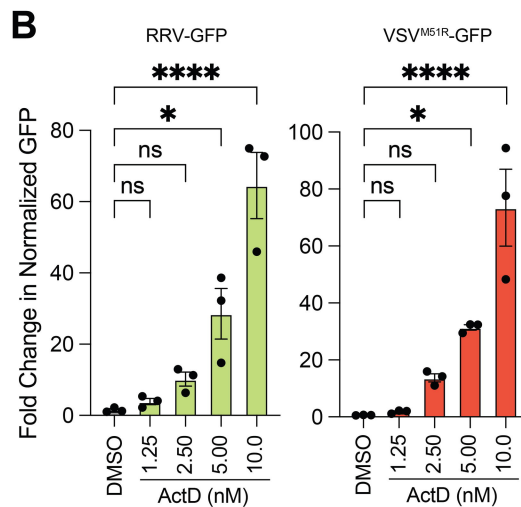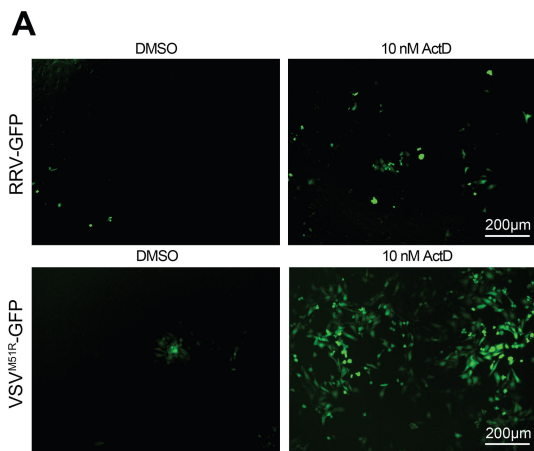150
100
75 — GST-IpaH4

A

```
RNF214 651   APATCKLCLMCQKLVQPSELHPMACTHVLHKECIKFWAQTNTNDTCPFCPTLK   NP_001070707.1
RNF215 322   GAETCAVCL--DYFCNKQWLRVLPCKHEFHRDCVDPWLMLQQ--TCPLCKFNV   NP_001017981.1
RNF213 4043  GIQPCSICL-GDA-KDPVC---LPCDHVHCLRCLRAWFASEQM-ICPYCLTAL   NP_001397124.1
RNF122 90    YGQTCAVCL--EDFKGKDELGVLPCQHAFHRKCLVKWLEVRC--VCPMCNKPI   NP_079063.2
```

B

```
RNF214 (H. sapiens):     HAPATCKLCLMCQKLVQPSELHPMACTHVLHKECIKFWAQTNTNDTCPFCPTLK 703
                         HAPATCKLCLMCQKLVQPSELHPMAC+HVLHKECIKFWAQTNTNDTCPFCPTLK
RNF214 (R. aegyptiacus): HAPATCKLCLMCQKLVQPSELHPMACSHVLHKECIKFWAQTNTNDTCPFCPTLK 705
```
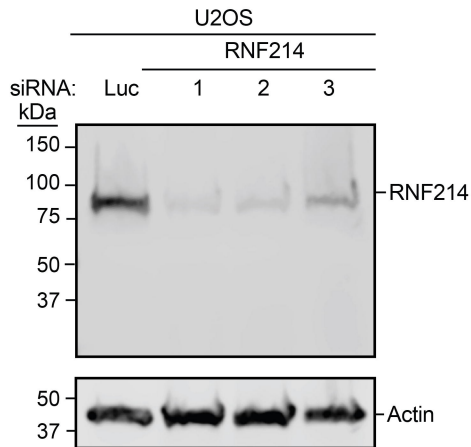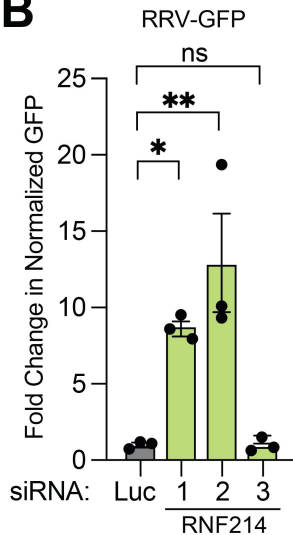
C

D

R06E

E

F

U2OS

A
DMSO · 10 nM ActD · RRV-GFP · VSV^{M51R}-GFP · 200μm · 200μm

B
RRV-GFP · VSV^{M51R}-GFP

Fold Change in Normalized GFP

ns · ns · * · ****
ns · ns · * · ****

DMSO · 1.25 · 2.50 · 5.00 · 10.0 · ActD (nM)

C
Absorbance (490-680)

**** · ns · ****

DMSO · Lysed · 1.25 · 2.5 · 5 · 10 · 20 · (+) Control · ActD (nM)

D **R06E**

Absorbance (490-680)

(+) Control · Lysed Cells · Luc · Effector Library

E **U2OS**

Absorbance (490-680)

(+) Control · Lysed Cells · Luc · Effector Library

A RRV-GFP

Effector	R06E	U2OS	Bacterium
IpaH4	✓	✓	*S. flexneri*
SopB	✓	✓	*S. enterica*
HopAM1	✓	✓	*P. syringae*
HopU1	✓	✓	*P. syringae*
EspY3	✓		*P. syringae*
HopM1	✓		*EHEC*
HopAI1	✓		*P. syringae*
SifA	✓		*S. enterica*
NleF	✓		*EHEC*
Ceg29	✓		*L. pneumo.*
SidM	✓		*L. pneumo.*
SidA	✓		*L. pneumo.*
Ipg0294	✓		*L. pneumo.*
Ceg23	✓		*L. pneumo.*
SidG	✓		*L. pneumo.*
HopAA1-2		✓	*P. syringae*
HopT1-2		✓	*P. syringae*
LegA10		✓	*L. pneumo.*
LegG1		✓	*L. pneumo.*
SidJ		✓	*L. pneumo.*
Ipg1751		✓	*L. pneumo.*
EspJ		✓	*EHEC*
HopAS1		✓	*P. syringae*
HopG1		✓	*P. syringae*
OspD3		✓	*S. flexneri*
CegC3		✓	*L. pneumo.*
HopY1		✓	*P. syringae*
EspFU		✓	*EHEC*
EspX7		✓	*EHEC*
Ceg7		✓	*L. pneumo.*
CegC1		✓	*L. pneumo.*
BepF		✓	*B. henselae*
NleH		✓	*EHEC*
LegC3		✓	*L. pneumo.*

B VSV^M51R^-GFP

Effector	R06E	U2OS	Bacterium
IpaH4	✓	✓	*S. flexneri*
SopB	✓	✓	*S. enterica*
SidM	✓	✓	*L. pneumo.*
LegC3	✓		*L. pneumo.*
HopD	✓		*P. syringae*
SptP	✓		*S. enterica*
HopAF1	✓		*P. syringae*
LegS2	✓		*L. pneumo.*
Ceg33	✓		*L. pneumo.*
EspF	✓		*EHEC*
NleD	✓		*EHEC*
EspR3	✓		*EHEC*
LegL1	✓		*L. pneumo.*
InvF	✓		*S. enterica*
MAP	✓		*S. enterica*
Ceg10	✓		*L. pneumo.*
NleG7	✓		*EHEC*
LegA8	✓		*L. pneumo.*
VipF		✓	*L. pneumo.*
SidB		✓	*L. pneumo.*
EspJ		✓	*EHEC*
SopE2		✓	*S. enterica*
SopD2		✓	*S. enterica*
HopAM1		✓	*P. syringae*
HopAA1-2		✓	*P. syringae*
LegA10		✓	*L. pneumo.*

■ — **Enhances Arbovirus Infection**

■ — **Does Not Enhance**

A U2OS

siRNA: Luc | RNF214 1 2 3

kDa
150
100
75
50
37

— RNF214

50
37

— Actin

B RRV-GFP

ns
**
*

Fold Change in Normalized GFP

25
20
15
10
5
0

siRNA: Luc | 1 2 3
RNF214

C VSV^{M51R}-GFP

ns
***
**

Fold Change in Normalized GFP

20
15
10
5
0

siRNA: Luc | 1 2 3
RNF214

Common Name	Viral Family	Genome	RNF214 Phenotype?
VACV-FL	*Poxviridae*	dsDNA	No
HSV1	*Herpesviridae*	dsDNA	No
VSV	*Rhabdoviridae*	(-)ssRNA	Yes
VSV^{M51R}	*Rhabdoviridae*	(-)ssRNA	Yes
HPIV1	*Paramyxoviridae*	(-)ssRNA	No
SeV	*Paramyxoviridae*	(-)ssRNA	Yes (Minor)
WNV	*Flaviviridae*	(+)ssRNA	Yes (Minor)
RRV	*Togaviridae*	(+)ssRNA	Yes
ONNV	*Togaviridae*	(+)ssRNA	Yes
SINV	*Togaviridae*	(+)ssRNA	Yes
VEEV	*Togaviridae*	(+)ssRNA	Yes