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1 **Rasputin/G3BP mediates o'nyong-nyong virus subversion of antiviral**
2 **immunity in Anopheles coluzzii**

3

4 **Short title: Rasputin and immune manipulation**

5

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28 **ABSTRACT**

29

30 The G3BP proteins in vertebrates and *Aedes* mosquito ortholog, Rasputin, are essential for
31 alphavirus infection, but the underlying mechanism of Rasputin/G3BP proviral activity is
32 poorly understood. It has been suggested that G3BP could influence host immune signaling,
33 but this has not been functionally demonstrated. Here, we find that depletion of Rasputin
34 activity in *Anopheles* mosquitoes, the primary vectors of the alphavirus o'nyong-nyong
35 (ONNV), provokes dysregulation of the antiviral Imd, JAK/STAT and RNAi pathways,
36 indicating that Rasputin is required for expression of normal basal immunity in uninfected
37 mosquitoes. Depletion of Rasputin during ONNV bloodmeal infection causes increased
38 transcript abundance of genes in the Imd pathway including positive regulator Rel2, and
39 decreases ONNV infection in mosquitoes. Loss of Rasputin is complemented by co-depletion
40 of Imd pathway positive regulator, Rel2, which restores normal ONNV infection levels. Thus,
41 the presence of Rasputin is required for ONNV inhibition of Imd activity, and viral inhibition
42 of Imd explains much of the Rasputin proviral activity. The viral non-structural protein 3
43 (nsP3) binds to Rasputin and alters the profile of cellular proteins binding to Rasputin. In the
44 presence of nsP3, 48 Rasputin-binding proteins are unchanged but seven binding proteins are
45 excluded and eight new proteins bind Rasputin. The Rasputin binding partners altered by nsP3
46 are candidate factors for ONNV immune manipulation and subversion through Rasputin.
47 Overall, these results are consistent with and strongly suggest a mechanism in which ONNV,
48 probably nsP3, co-opts the normal Rasputin function assuring basal cellular immune activity
49 in order to inhibit antiviral immunity and promote infection. These observations may be
50 generalizable for Rasputin function during alphavirus infection of other mosquitoes, as well as
51 for G3BP function in the mammalian host, and could offer a target for vector-based control of
52 arbovirus transmission.

53

54 Keywords

55 Arbovirus, G3BP, host-pathogen interaction, innate immunity

56 INTRODUCTION

57 Arthropod-borne viruses (arboviruses) are maintained through an alternating cycle of
58 transmission between mammalian hosts and arthropod vectors. Arboviruses represent a
59 spreading global burden for human and animal health, with the clinically most important
60 pathogens represented by RNA viruses in the families Flaviviridae, Togaviridae, Bunyvirales,
61 and Reoviridae (Weaver and Reisen, 2010). The alphavirus o'nyong-nyong (ONNV, genus
62 alphavirus, family Togaviridae) is phylogenetically closely related to chikungunya virus
63 (CHIKV, genus alphavirus, family Togaviridae). Both are in the same antigenic group, the
64 Semliki forest virus complex, thus are difficult to distinguish by immunodiagnostic assay, and
65 symptomatically cause essentially the same human disease (Powers et al., 2001; Rezza et al.,
66 2017). The most apparent difference between the arboviruses is their use of mosquito vectors.
67 Anopheles mosquitoes are the major vector of human malaria but are the primary vector of
68 only one known arbovirus, ONNV, while Aedes mosquitoes transmit the majority of mosquito-
69 borne arboviruses, including CHIKV.

70

71 The molecular mechanism underlying the mosquito vector specificity of ONNV and CHIKV
72 is not understood. Evidence points to the involvement of non-structural protein 3 (nsP3), which
73 is diverged between the two viruses. Replacing CHIKV nsp3 with the ONNV nsP3 gene in the
74 CHIKV genomic backbone allowed partial infection of Anopheles mosquitoes, while the
75 CHIKV backbone with its own nsP3 gene is noninfective to Anopheles (Saxton-Shaw et al.,
76 2013; Vanlandingham et al., 2005; Vanlandingham et al., 2006). Interestingly, unlike CHIKV,
77 which does not infect Anopheles, ONNV can infect some tested strains of *Ae. aegypti*
78 (Vanlandingham *et al.*, 2005). These results suggest that Anopheles is the more stringent or
79 less permissive host among the two for virus infection, and also imply that observed viral nsP3

80 sequence divergence may be based on specific viral adaptation to infect either Anopheles or
81 Aedes hosts, because the human host is shared by both viruses.

82

83 The probable role of viral nsP3 in vector host specificity points in turn to the a host protein
84 known to bind nsP3 of many alphaviruses, the Ras-GTPase-activating protein (SH3 domain)-
85 binding protein (G3BP) and the mosquito ortholog Rasputin (Rin) (Panas et al., 2014).
86 Rin/G3BPs are enigmatic molecules with diverse biological functions (Alam and Kennedy,
87 2019; Kang et al., 2021). Most studies of Rin/G3BP function have been carried out in
88 mammalian cells, where there are three isoforms (G3BP1, G3BP2a and 2b) expressed from
89 two genes (Irvine et al., 2004; Kang *et al.*, 2021). Mosquitoes carry a single Rin gene, with the
90 small number of published studies restricted to Aedes mosquitoes (Fros et al., 2015; Goertz et
91 al., 2018; Nowee et al., 2021), and to our knowledge there are no previous published reports
92 about Rin in Anopheles mosquitoes.

93

94 Mammalian G3BPs are involved in numerous biological processes including RNA-binding,
95 RNA metabolism, stress granule formation, and regulation of ubiquitin-mediated degradation
96 signaling (Alam and Kennedy, 2019; Irvine *et al.*, 2004; Kang *et al.*, 2021; Kedersha et al.,
97 2016; Laver et al., 2020; Soncini et al., 2001). Aedes Rin can interact and colocalize with nsP3
98 of many tested alphaviruses in Aedes cells (Fros *et al.*, 2015; Goertz *et al.*, 2018; Nowee *et al.*,
99 2021). Rin/G3BPs also serve as proviral host factors promoting the infection cycle of multiple
100 RNA viruses in mammals, while in Aedes proviral activity has been demonstrated only for
101 alphaviruses (Fros *et al.*, 2015). The proviral function of Rin/G3BPs is associated in general
102 with efficiency of viral genome replication, although “the exact mechanism by which they act
103 remains to be explored” (Scholte et al., 2015). Interestingly, G3BPs have also been reported to
104 potentially interact with host immunity by influencing cytoplasmic distribution of the

105 regulators of the NF-kappa B pathway (Prigent et al., 2000), by enhancing NF-kappa B
106 phosphorylation and protein quantity (Scholte *et al.*, 2015; Zhang et al., 2012), and by
107 modulating NF-kappa B signaling through protein kinase R or by other means (Deater et al.,
108 2022; Reineke and Lloyd, 2015). The implications of potential Rin/G3BP interaction with
109 immune signaling factors upon actual immune regulation, or on the efficiency if virus infection
110 have barely been examined, and there are no studies in mosquitoes.

111

112 Because the above evidence points to differential interactions of viral nsp3 with host Rin to
113 explain at least in part the observed vector specificity of ONNV and CHIKV for infection of
114 Anopheles and Aedes, here we undertook a study of the function of Anopheles Rin in ONNV
115 infection, which is a knowledge gap. More broadly, the reason for the apparent paucity of
116 arbovirus transmission by Anopheles mosquitoes in nature is also not understood, because the
117 study of virus-host interactions in Anopheles has been relatively neglected in favor of the rich
118 extensive research on Anopheles-Plasmodium interactions. However, Anopheles harbor a
119 complex natural virome of RNA viruses (Belda et al., 2019) and a number of pathogenic
120 arboviruses have been isolated from Anopheles mosquitoes (Nanfack Minkeu and Vernick,
121 2018). For example, there is evidence of Anopheles contribution to maintenance of Rift Valley
122 Fever virus during epidemics (RVFV, genus phlebovirus, family Bunyavirales) (Ratovonjato
123 et al., 2011; Seufi and Galal, 2010). These observations present a biological puzzle that
124 highlights the importance of studying Anopheles-virus interactions. With changing climatic
125 and ecological conditions and exposure of vector populations to new potential pathogens in
126 new environments, it is relevant to ask what it would require for arboviruses other than ONNV
127 to adapt to Anopheles mosquitoes as transmission vectors.

128

129 Here we investigated the proviral activity of Anopheles Rin, and discovered a clear and direct
130 link between Rin and antiviral immunity. We found that Rin influences antiviral immunity in
131 ONNV infection of Anopheles. and probably underlies viral co-optation by nsP3 of host Rin
132 in order to subvert antiviral immunity. The findings could be relevant to studies of G3BPs in
133 mammals and for other alphaviruses.

134 **RESULTS**

135 **Rin activity is required for normal basal expression of multiple immune pathways in**
136 **uninfected Anopheles**

137 Rin/G3BP is proviral for infection of many alphaviruses in mammalian cells and for infection
138 of CHIKV in *Aedes albopictus* (Fros *et al.*, 2015; Gotte *et al.*, 2020; Scholte *et al.*, 2015) but
139 it has not been examined in *Anopheles*. Therefore, we first, confirmed that Rin is proviral for
140 ONNV in *Anopheles coluzzii* mosquitoes by depleting Rin transcript using RNAi-mediated
141 gene silencing by injection of double-stranded RNA (dsRNA) specific for Rin (dsRin), or
142 irrelevant GFP control (dsGFP), followed by bloodmeal infection with ONNV. The depletion
143 of Rin in *An. coluzzii* significantly decreased ONNV infection prevalence (defined as the
144 proportion of fully fed mosquitoes positive for ONNV) and infection intensity (defined as the
145 viral titer measured in the ONNV-positive mosquitoes) (Fig. S1A and S1B). Therefore, Rin
146 activity is proviral and agonistic for ONNV infection in *An. coluzzii* by infectious bloodmeal.
147

148 Because of the suggestive evidence from mammalian G3BPs detailed above, we hypothesized
149 that Rin proviral activity could be explained, at least in part, if Rin is required for host immune
150 signaling, and if a viral virulence factor can manipulate Rin to undermine cellular immune
151 signaling in relevant antiviral pathways. Therefore, we first determined whether Rin influences
152 basal immunity in uninfected mosquitoes by measuring the influence of Rin depletion by dsRin
153 treatment as compared to dsGFP controls upon transcript levels of a panel of immune genes
154 selected on two main lines of evidence: previous functional demonstration of antiviral activity
155 against ONNV in *An. coluzzii* (Carissimo *et al.*, 2015), and differential RNAseq transcript
156 abundance in *An. coluzzii* after ONNV bloodmeal infection (Carissimo *et al.*, 2018). The
157 candidate panel is comprised of positive regulators of the four main mosquito immune
158 pathways (Toll, Imd, JAK/STAT and small RNA), and immune factors or effectors, including

159 the leucine-rich repeat protein (LRR) genes APL1C, APL1A, LRIM1, LRIM4, LRIM10; the
160 complement-like thioester proteins TEP3, TEP4, TEP12; and inducer of immune granulocyte
161 differentiation, Evokin).

162

163 First, we queried Rin influence on expression of Toll pathway regulatory genes (positive
164 regulator, Rel1 and negative regulator, Cactus) and effector genes (APL1C and TEP3) (Fig.
165 1A). APL1C and TEP3 are extracellular immune factors regulated by the Toll pathway (Mitri
166 et al., 2015), and APL1C displays antiviral activity against ONNV in *An. coluzzii*, while TEP3
167 does not (Carissimo *et al.*, 2015). Depletion of Rin has little influence on Toll-pathway related
168 immune transcripts except for causing a late increase of APL1C transcript abundance,
169 indicating that normal Rin activity slightly limits APL1C transcript abundance in uninfected
170 mosquitoes.

171

172 In contrast, depletion of Rin in uninfected mosquitoes triggered a more striking effect upon the
173 Imd and JAK/STAT pathways (Fig. 1B), which are both strongly antiviral for ONNV in
174 *Anopheles* (Carissimo *et al.*, 2015). In the Imd pathway, Rin depletion caused decreased
175 transcript abundance of the Imd positive regulator Rel2 as well as the Imd effector genes,
176 APL1A and TEP4, which are transcriptionally regulated by Rel2 (Mitri *et al.*, 2015). In the
177 JAK/STAT pathway, Rin depletion caused increased transcript abundance of the JAK/STAT
178 positive regulator, Stat-A, and therefore Rin is required to limit Stat-A transcript to normal
179 levels. Thus, depletion of Rin causes widespread dysregulation of the Imd and JAK/STAT
180 pathways, and normal Rin activity is required for correctly regulated basal activity of these
181 immune pathways.

182

183 Among the small RNA immune pathways, the siRNA pathway has been studied for its role in
184 counteracting RNA virus infection, as shown for flaviviruses such as ZIKV (Magalhaes et al.,
185 2019) and DENV (Olmo et al., 2018), and alphaviruses CHIKV (Dong et al., 2022) and ONNV
186 (Carissimo *et al.*, 2015). The endonucleases Argonaute-2 (Ago2) and Dicer-2 (Dcr2) are
187 essential catalytic components of the RNA-induced silencing complex (Kumar et al., 2018),
188 and Ago2 was previously shown to be antiviral for ONNV infection (Carissimo *et al.*, 2015).
189 The transcript abundance of Ago2 and Dicer2 are both increased after Rin depletion, and
190 therefore Rin acts to limit the expression of the siRNA pathway in uninfected mosquitoes (Fig.
191 1C).

192

193 We analyzed additional important extracellular immune factors for their transcriptional
194 response to Rin. The LRR genes LRIM4 and LRIM10 are upregulated and downregulated,
195 respectively, during ONNV infection of *An. coluzzii* (Carissimo *et al.*, 2018). Here, we found
196 that LRIM4 but not LRIM10 transcript abundance is altered by Rin depletion (Fig. 1D). The
197 complement-like effector TEP12 is strongly increased by Rin depletion (Fig. 1D). Finally,
198 Evokin is an *Anopheles* positive regulator for the proliferation of immune-competent
199 hemocytes (Ramirez et al., 2015), and here we found that Evokin transcript is induced by Rin
200 depletion (Fig. 1E), meaning that normal Rin function probably modulates the level of
201 immune-primed hemocytes.

202

203 Taken together, these results in uninfected mosquitoes indicate that Rin is deeply involved in
204 the correct basal regulation of key components of all four of the major arms of mosquito innate
205 immunity, but particularly Imd and JAK/STAT. Absence of Rin leads to dysregulated
206 expression of factors in all pathways. Rin is thus required to maintain the overall integrity and
207 homeostasis of the systems necessary for immune surveillance and readiness of mosquitoes in

208 the unstimulated resting state. Multiple of the Rin-regulated factors are known to be antiviral
209 for ONNV in Anopheles, thus strengthening the hypothesis that Rin proviral function could be
210 linked to viral manipulation of host immunity.

211

212 **Presence of Rin is required for ONNV-dependent inhibition of the Anopheles Imd** 213 **pathway**

214 After confirming above that Rin is proviral for ONNV infection of Anopheles, and that Rin
215 activity is essential for correct expression of basal immunity in uninfected mosquitoes, we next
216 examine the hypothesis that the proviral activity of Rin and the G3BP family could at least in
217 part be explained by viral immune manipulation targeting Rin. The influence of Rin on
218 Anopheles immunity was queried after challenge with an ONNV infective bloodmeal. Unfed
219 mosquitoes were removed after the bloodmeal, and only fully engorged females were included
220 in the analyses. Transcript abundance of the immune gene panel was measured at 48 h and 72
221 h after the infective bloodmeal. At 3 d post-bloodmeal, the ONNV infection is still restricted
222 to the midgut epithelium, the primary midgut infection stage (PMI), prior to midgut escape and
223 establishment of the systemic disseminated infection (Carissimo *et al.*, 2015).

224

225 Rin depletion during the PMI of ONNV increases the transcript levels of genes in three of the
226 four major immune signaling pathways, as compared to transcript levels in mosquitoes treated
227 with dsGFP and then infected (Fig. 2 A-D). However, the major impact was observed in the
228 Imd pathway, where the Imd positive regulator Rel2, ONNV antiviral effector APL1A
229 (Carissimo *et al.*, 2015), and ONNV induced LRR factor LRIM4 (Carissimo *et al.*, 2018) are
230 all significantly inhibited by Rin depletion, meaning that Rin activity is required for the
231 decrease of the genes during the ONNV primary infection (Fig. 2B). Interestingly, although
232 Imd and JAK/STAT were the two immune pathways that displayed the strongest antiviral

233 effect against ONNV in the Anopheles PMI (Carissimo *et al.*, 2015), here we find that the
234 highly antiviral positive regulator of JAK/STAT, Stat-A, remains unaffected by the loss of Rin
235 during ONNV infection (Fig. 2B).

236

237 There was only a small Rin influence on the Toll pathway during ONNV infection, where the
238 positive and negative pathway regulators, Rel1 and Cactus respectively, are unchanged during
239 the PMI by loss of Rin, although the Toll-regulated effector APL1C (Mitri *et al.*, 2009), which
240 is antiviral for ONNV (Carissimo *et al.*, 2015), requires Rin for ONNV-dependent inhibition
241 (Fig. 2A). In the siRNA pathway, Ago2, the critical “slicer” enzyme of the RNA-induced
242 silencing complex (RISC), was slightly elevated 3 d after Rin depletion (Fig. 2C), indicating
243 that there could be Rin-dependent inhibition of RNAi activity late in the PMI of ONNV. The
244 RNAi pathway activity is not functionally antiviral for ONNV in the Anopheles PMI but only
245 in the subsequent disseminated infection beginning after 3 d post-infection (Carissimo *et al.*,
246 2015), and thus the late PMI effect of Rin on RNAi could indicate the beginning of viral midgut
247 escape and the transition to the host antiviral response of the disseminated infection.

248

249 Above we showed that Rin is required to increase the Rel2 transcription factor to normal basal
250 levels in uninfected mosquitoes (Fig. 1B), but during the PMI the presence of Rin (dsGFP
251 treatment + ONNV infection) causes an ONNV-dependent decrease of Rel2 relative to the
252 condition with depleted Rin and infection (Fig. 2B). Therefore, Rin blocks the induction of
253 Rel2 that would be seen during ONNV infection in the absence of Rin (dsRin treatment +
254 ONNV infection). The ability of Rin to modulate Rel2 expression could potentially be
255 exploited by ONNV during the PMI to reduce Imd pathway activation, and therefore leads to
256 the hypothesis that Rin could be a target for viral manipulation in order to inhibit the potent
257 antiviral Imd pathway.

258

259 **Rin proviral activity is based on ONNV-dependent inhibition of the Imd pathway**

260 Based on the above observation that the effect of Rin during ONNV infection is most strongly
261 directed towards Imd as compared to the other immune pathways, we inferred that the observed
262 Rin proviral phenotype could potentially be explained by viral manipulation of Rin to block
263 Imd pathway activity. To test this possibility, we inhibited the activation of Imd by co-depleting
264 Rel2 simultaneously along with depletion of Rin, to determine whether inhibition of the
265 antiviral Imd pathway complements the loss of Rin during the PMI.

266

267 The depletion of genes targeted was tested the day of the blood-feeding on unfed mosquitoes.
268 When Rin is depleted the transcript abundance of Rel2 is decreased. When we deplete Rel2,
269 we do not observe a change in the transcript level of Rin (Fig. 3C). Therefore, there is a
270 hierarchical effect which goes in one direction where Rin can regulate Rel2 transcript
271 abundance, but Rel2 does not modify Rin transcript level. First, Rin depletion decreased the
272 number of mosquito abdomens positive for ONNV (Fig. 3A) and diminished the number of
273 ONNV particles in abdomen of dsRin- compared to dsGFP-treated mosquitoes (Fig. 3B).
274 Therefore, Rin depletion decreases the infection rate and the infection intensity of ONNV in
275 *An. coluzzii*. Rin is required for full levels of both viral infection prevalence and titer at 3 days
276 post-blood feeding in the PMI. Therefore, Rin has a proviral role during the PMI and acts as a
277 switch between a sterile immunity and a viral infection in the in vivo model. Rel2 depletion
278 increases the viral titer but does not modify the infection rate. Therefore, Rel2 is required to
279 limit viral titer, but does not limit infection prevalence. This result indicates that the biological
280 demand to create sterile immunity is more demanding for infection prevalence than for the
281 control of the viral titer. We can hypothesize that the depletion of multiples antiviral pathways
282 will be needed to observe a significant increase of infection prevalence. Depleting Rin and

283 Rel2 simultaneously allows complementation of the Rin phenotype indicating the involvement
284 of Rin in the Imd pathway and its effect on ONNV infection. This data suggests the viral
285 manipulation of Rin to suppress the Imd antiviral pathway and to promote viral infection.

286

287 **Rin is required for correct basal immunity in Anopheles hemocyte-like cells**

288 After having detailed the role of Rin in the antiviral immunity in the PMI, we now investigate
289 the role of Rin in the systemic compartment using the Anopheles 4a3A hemocyte-like cells.
290 This model has previously been described as a suitable model for the study of the DI (Carissimo
291 *et al.*, 2015; Waldock *et al.*, 2012). First, we determined the influence of Rin on ONNV
292 infection at a low multiplicity of infection (MOI: 0.01) in 4a3A cells. Depletion of Rin
293 transcript significantly decreases the intracellular quantity of ONNV genomic RNA and
294 infectious particles (Fig. S1C and S1D). Therefore, Rin activity is proviral for ONNV infection
295 in the DI in the 4a3A cell line.

296

297 We analyzed the role of Rin on the transcriptional expression of the same immune genes tested
298 in the *in vivo* model, in the 4a3A hemocyte-like cells model. We analyzed the transcript fold
299 change of those immune genes between dsRin and dsGFP cells. First, the depletion of Rin was
300 deleterious for the transcript abundance of Rel1, positive regulator of the Toll pathway and
301 APL1C. Rin depletion did not modulate the transcript abundance of TEP3. Thus, Rin increases
302 Rel1 and APL1C transcript expression in 4a3A cells. We also analyzed Rin influence on the
303 Imd pathway (Fig. 4B) and Rin depletion did not influence Rel2 and Rel2F transcript
304 abundance but did reduce APL1A and TEP4 transcript abundance. Rin increases the
305 transcriptional expression of two extracellular immune factors regulated by the Imd pathway,
306 APL1A and TEP4. We then analyzed Rin depletion phenotype on the transcript abundance of
307 other known (LRIM1) or potential immune complex partners subunits (LRIM4, LRIM10 and

308 TEP12) (Fig. 4D). When Rin is depleted LRIM4 and LRIM10 transcript abundance but not
309 LRIM1 and TEP12, are significantly reduced. Thus, Rin enhances the transcriptional
310 expression or the transcript stability of two leucine-rich repeat transcript LRIM4 and LRIM10.
311 The transcript level of the positive regulator of JAK/STAT, Stat-A, is not regulated by Rin
312 (Fig. 4B). Concerning the siRNA pathway, after Rin depletion, Ago2 but not Dcr2 transcript
313 abundance is significantly reduced, thus Rin enhances Ago2 transcriptional expression (Fig.
314 4C).

315

316 These results showed that Rin plays a role in antiviral immunity by positively regulating
317 the transcriptional expression or the transcript stability of multiple immune genes including
318 some previously proven to have antiviral activity. Moreover, Rin modulation of those immune
319 transcript is time-dependent as some were significantly reduced at 24h- or 48h-post depletion
320 indicating respectively an early or a late influence. As some immune transcript abundance came
321 back to the wild-type level at 48h-post depletion we hypothesize that other mechanism may
322 complement the loss of Rin as immune genes are known to be regulated by multiple pathways
323 and partners. Furthermore, Rin mediates transcripts level of immune genes and regulators of
324 NF-kappa B-like pathway in mosquito cells and components of the siRNA pathway.

325

326 Moreover, multiple differences are noticed with what was observed in the in vivo model.
327 Indeed, Rin seems to primarily enhance Toll-related immune transcript in the 4a3A systemic
328 compartment model whereas in mosquitoes in vivo model Rin modulates mostly Imd-related
329 immune transcripts. Moreover, other differences observed in the in vivo model compared to
330 the in vitro model can also be linked to the presence of multiple cell types in mosquitoes leading
331 to a higher variability of response than in the one-cell type in vitro model.

332

333 **Presence of Rin allows ONNV-dependent inhibition of the Toll pathway in Anopheles**
334 **hemocyte-like cells**

335 Rin is a proviral factor for ONNV infection in 4a3A cell line (Fig. S1C and S1D) and increases
336 the transcript level of Rel1 the positive regulator of the Toll pathway (Fig. 4A). The Toll
337 pathway in 4a3A systemic model is inhibited by viral infection and among the immune
338 pathways, the Toll pathway artificial stimulation has the most antiviral activity against ONNV
339 replication (Carissimo *et al.*, 2015). We investigated the transcript level of the immune genes
340 tested in Fig. 4 during ONNV infection where Rin expression is depleted in 4a3A cells (Fig.
341 5A). We infected dsRin and dsGFP-treated cells with ONNV at a low multiplicity of infection
342 (MOI: 0.01). Then we analyzed the transcript abundance of those genes between dsRin-treated
343 cells related to dsGFP control-treated cells at 24h- and 48h-post-infection. Of all the genes
344 tested in naïve cells, only Rel1 transcript abundance is modified after Rin depletion in ONNV
345 DI (Fig. 5A). Rin depletion in infected cells increases the transcript abundance of Rel1 at 24h
346 and 48h post-infection compared to infected dsGFP-treated cells (Fig. 5A). Therefore, Rin in
347 infected cells decreases the Rel1 transcript abundance. We wanted then to investigate if the
348 Toll pathway is antiviral in 4a3A cells by depleting Cactus, the negative regulator of the Toll
349 pathway, which activates the Toll pathway (Frolet *et al.*, 2006). We infected dsCactus and
350 dsGFP-treated cells with ONNV at MOI: 0.01. Then we analyzed the intracellular ONNV viral
351 RNA level and the ONNV infectious particle produced in the supernatant between dsCactus-
352 treated cells related to dsGFP-treated cells at 24h and 48h post-infection. Cactus depletion
353 decreases ONNV viral RNA quantity and infectious particles production (Fig. 5B). Therefore,
354 the Toll pathway has an antiviral activity against ONNV infection in 4a3A cells. We previously
355 showed that Rin in uninfected cells is able to upregulate the transcript level of Rel1. However,
356 in infected cells Rin decreases Rel1 transcript level. Consequently, Rin ability to modulate

357 Rel1 expression seems to be manipulated during a viral infection in order to inhibit the antiviral
358 Toll pathway activation in the DI.

359

360 **Rin physically interacts with ONNV nsP3 in Anopheles hemocyte-like cells**

361 In mammalian and in mosquito cells, G3BPs and Rin have been showed to colocalize
362 with the non-structural protein 3 (nsP3) of multiple alphaviruses including CHIKV and ONNV
363 (Nowee *et al.*, 2021). However, it has never been shown in Anopheles cells models. Here we
364 analyzed Rin of *An. coluzzii* colocalization and interaction with nsP3 of ONNV in 4a3A cells
365 (Fig. 6). We co-transfected a C-terminal streptavidin tag construct of Rin (Rin-strep) and a
366 construct expressing nsP3 of ONNV both controlled by the Anopheles actin promoter (nsp3
367 transfection). We also directly infected cells with ONNV at MOI 0.5 (ONNV infection).
368 Colocalization pattern was observed between Rin and nsP3 of ONNV in the different
369 conditions. Quantification of the percentage of cells harboring colocalization pattern revealed
370 more than 78% of cells counted showed a colocalization between Rin and nsP3 of ONNV (Fig.
371 6B). No significant difference was observed between the two conditions.

372

373 To confirm Rin and nsP3 interaction, we used a streptavidin-pull down assay to purify Rin-
374 strep (Rin-strep) from co-transfected 4a3A cells. We revealed by using streptavidin-specific
375 antibodies and nsP3-specific antibodies, the presence of streptavidin tag protein (Rin) and nsP3
376 in the eluat of non-transfected cells (NT), Rin-strep transfected cells (Rin-strep), nsP3
377 transfected cells (nsP3) and co-transfected cells with Rin-strep and nsP3 of ONNV (Rin-strep
378 + nsP3) (Fig. 6C). We observed bands at 89 kDa corresponding to Rin-strep in the eluat of
379 cells transfected with Rin-strep construct or co-transfected with both Rin-strep and nsP3 of
380 ONNV. A band at 70 kDa corresponding to nsP3 of ONNV was only revealed in the eluat of
381 co-transfected cells but was not observed in the eluat of nsP3-transfected cells. Therefore, nsP3

382 presence in the eluat is due to its binding to Rin-strep (Fig. 6C). Finally, two non-specific bands
383 at 110 kDa are revealed by the nsP3-polyclonal antibodies. Therefore, in 4a3A in vitro model,
384 Rin is a proviral factor for ONNV infection and is also able to physically interact with nsP3 of
385 ONNV similarly to what was known in mammalian cells.

386

387 **Rin interaction with ONNV nsP3 modifies the biological function of its partners**

388 Rin presence is required for suppression of the Imd and Toll antiviral pathways during
389 ONNV infection in the PMI and the DI respectively, through a likely virus manipulation of
390 host Rin. Rin interacts directly with the nsP3 of ONNV in cells, therefore this interaction could
391 be at the center of the coopting of Rin. To shed light on the mechanism by which Rin regulates
392 antiviral genes, we investigated the protein partners of Rin and of the complex Rin/nsP3 in
393 4a3A cells. We analyzed by mass spectrometry the elution of cells transfected with Rin tagged
394 with a C-terminal streptavidin sequence (Rasputin-strep), or the elution of co-transfected cells
395 with Rin-strep and nsP3 of ONNV (Rasputin-strep+nsP3) (Fig. 7). Controls were used to attest
396 of the specificity of the partners found. Among these controls, the elution of cells transfected
397 with GFP-strep or transfected with nsP3 or non-transfected was also analyzed by mass
398 spectrometry. Proteins found in the control conditions are considered non-specific to Rin-strep
399 and are, therefore, removed from the data set.

400

401 Most Rin partners are proteins with unknown function. Of the known proteins, Rin
402 interacts in diverse pathways including RNA metabolism, protein synthesis components,
403 ubiquitination pathways and signaling pathways (Fig. 7A). The biological pathways of Rin
404 partners are in line with Rin involvement in Ras metabolism (Pazman et al., 2000) and RNA
405 metabolism (Laver *et al.*, 2020). When Rin is in complex with the viral nsP3, more proteins
406 partners of Rin are involved in protein synthesis, RNAi pathway and cellular/nuclear transport

407 (Fig. 7B). However, the interaction between Rin and nsP3 decreases the number of Rin partners
408 involved in GDP/GTP metabolism and in signaling pathways. Therefore, the presence of nsP3
409 modifies the biological profile of Rin protein partners. Interestingly, Rin does not directly
410 interact with the immune genes that it regulates except Ago2. Consequently, Rin modulation
411 of the transcript levels of antiviral genes most likely derives from Rin function in RNA
412 metabolism or protein synthesis as it has been already reported (Laver *et al.*, 2020). Moreover,
413 48 proteins were found to interact with Rin in the presence or absence of nsP3 whereas 7
414 proteins interact only with Rin alone and 8 proteins interact only with Rin when nsP3 is present
415 (Fig. 7C). Therefore, the presence of the viral nsP3 allows Rin to still interact with most of its
416 protein partners but also modifies the partner profile of Rin. Consequently, the interaction
417 between Rin and the viral nsP3 could manipulate the protein partners of Rin and therefore may
418 influence Rin function in modulating antiviral genes.

419 DISCUSSION

420 The mechanism driving the proviral role of G3BPs on alphavirus infection has primarily been
421 studied during the viral RNA replication steps of the viral genome (Gotte *et al.*, 2019; Gotte *et*
422 *al.*, 2020; Scholte *et al.*, 2015). G3BPs in mammals have been shown to influence the switch
423 from genome replication to genome translation (Scholte *et al.*, 2015). However, as we know
424 that G3BPs have multiple functions, other potential roles of G3BPs during the alphavirus cycle
425 have not been investigated yet. Moreover, the role of G3BPs in immune pathways has already
426 been mentioned especially in NF-kappa B signaling pathways. But the relationship between
427 G3BPs and immunity during a viral infection has never been made. Therefore, this study
428 reveals a link between Rin, immunity and viral infection and could be extrapolated to Rin in
429 other mosquito species and to G3BPs in mammals.

430

431 Rin positively regulates immune genes and known antiviral genes with a centered effect
432 on the Toll pathway in the systemic compartment represented by the 4a3A cell line (Fig. 4)
433 and a pronounced effect on the Imd pathway in the midgut compartment of the *in vivo* model
434 (Fig. 1). These results are in adequation with what was observed in Carissimo *et al.*, regarding
435 the immune compartmentalization of these pathways during ONNV infection. Where the Toll
436 pathway is mostly antiviral in the DI whereas the Imd pathways is mostly antiviral during the
437 PMI (Carissimo *et al.*, 2015). Moreover, Rin transcriptional expression is enhanced following
438 a naïve blood-feeding (Fig. S5) which is in line with our observation of Rin enhancing multiple
439 immune factors. Indeed, during bloodfeeding, mosquitoes will be exposed to the pathogens
440 found in the blood of the mammals fed upon. Thus, mosquitoes will need to restrict the
441 infection of these pathogens for their survival by enhancing the activation of these immune
442 pathways.

443 Surprisingly, Rin effect is not consistent in time. Most of the genes regulated by Rin are either
444 lately modulated or come back to wildtype level later after Rin depletion (Fig. 1 and 4). These
445 results indicate the plurality of the regulation of those immune genes and the possibility that
446 other proteins could intervene in the Rin-mediated immune gene regulation. Indeed, most of
447 those genes are regulated by multiple factors and the effectors of some immune pathways could
448 be regulated by other immune pathways. Moreover, differences of immune gene response
449 following Rin depletion observed between the in vitro and in vivo models can be explained by
450 the plurality of the cell types present in the mosquito model compared to the one single cell
451 type of the in vitro 4a3A cell model (Muller et al., 1999). Indeed, G3BPs are known to be
452 ubiquitously expressed in multiple human tissues. As for Rin in mosquitoes no study examined
453 the expression of Rin in different tissues of mosquitoes (Kennedy et al., 2001), but the
454 mammalian studies could be extrapolated to mosquitoes as G3BPs/Rin are overall well-
455 conserved proteins (Kang *et al.*, 2021). Therefore, Rin could mediate various genes depending
456 on the cell-type where it is expressed.

457

458 Interestingly, we found that during the PMI, multiple immune factors were upregulated
459 when Rin was absent compared to a wt viral infection (Fig. 2). Taking in account that in
460 mosquitoes Rin allows the switch between sterile immunity and infection during the PMI,
461 central point for the establishment of a viral infection (Fig. 3), these results demonstrate a
462 correlation between the proviral role of Rin during viral infection and Rin immune pathway
463 regulation. Indeed, in non-infected mosquitoes Rin can upregulate the expression of immune
464 genes but during a viral infection Rin decreases the expression of those same immune genes
465 (Fig. 1). Therefore, Rin physiological function in naïve mosquitoes is corrupted by the viral
466 infection. Furthermore, the linked between Rin immune gene regulation and viral infection was
467 directly made in mosquitoes via the complementation of Rin deleterious phenotype on the viral

468 infection by the inhibition of the Imd pathway (Fig. 3). This result directly proves that Rin
469 proviral function is directly linked to its ability to regulates the Imd pathways. Rin activity is
470 required to overcome a wildtype cellular qualitative (prevalence) and quantitative (titer)
471 blockade against ONNV infection (Fig. 3). The depletion of Rel2 complements the loss of Rin
472 activity by reversing the effect of the cellular blockade and restoring normal ONNV infection
473 prevalence and titer levels. This result suggests that Imd pathway activity comprises and
474 explains at least in large part the PMI blockade and is consistent with a mechanism in which
475 ONNV interacts with Rin to subvert Imd activity and promote viral infection. In the absence
476 of Rin activity, the antiviral phenotype of Rel2 is enhanced, decreasing ONNV infection, while
477 in the absence of Rel2, the proviral immune subversion function by Rin is not required for
478 normal levels of ONNV infection. Moreover, we revealed that there is a hierarchical relation
479 between Rin and Rel2 where Rin is able to regulate the transcript level of Rel2, but Rel2 cannot
480 regulate Rin transcript abundance back (Fig. 3). This observation supports the evidence of a
481 directional effect mediates by Rin on this antiviral pathway where Rel2 is downstream of Rin
482 in this chain of event.

483

484 The simplest model for the relationship between infection prevalence and viral titer could be
485 that the activity of predominantly the Imd pathway reduces the efficiency of viral replication
486 below a level that can consistently yield an ongoing infection, and sterile immunity results in
487 those individuals where a combination of low viral titer and the action of other antiviral and
488 stochastic effects allows elimination and curing of the viral infection. The depletion of Rel2
489 alone allowed a significantly elevated viral titer but did not alter infection prevalence. It is not
490 clear why the silencing of only Rel2 did not alter ONNV infection prevalence, while the
491 silencing of Rel2 in the absence of Rin complemented the loss of Rin by reversing the depleted
492 Rin phenotype. The most likely explanation is that infection prevalence in the dsGFP-treated

493 control mosquitoes was close to 80%, and thus the experimental design may have been
494 powered to detect decreased prevalence but lacked power to detect a significant increase above
495 80%. An alternative explanation could be that depletion of different antiviral pathways such as
496 Imd or JAK/STAT can individually result in elevated viral titer, but that complete elimination
497 and sterile immunity requires the integrated effect of multiple antiviral pathways. Further work
498 would be required to distinguish between these possibilities. Taken together, the results
499 indicate that the proviral effect of Rin for ONNV infection is mediated at least in large part by
500 a Rin-dependent inhibition of Imd pathway activity.

501

502 Other viral mechanisms could be involved in the stabilization of the expression of those
503 immune genes. Moreover, Carissimo et al highlighted a possible inhibitory activity of ONNV
504 against the Toll pathway in 4a3A cells (Carissimo *et al.*, 2015). Here we showed that the Toll
505 pathway stimulation decreases the viral infection therefore, Rin regulation of this pathway
506 make it a target of choice for ONNV. This study may suggest that Rin would be at the center
507 of this viral inhibitory of the Toll pathway during the DI. However, extensive study will be
508 required to directly prove the Rin proviral function role via the Toll pathway regulation.
509 Directly studying the role of Rin on the DI in the in vivo model would be possible via the
510 intrathoracic injection of viral particles in Rin-depleted mosquitoes allowing the bypass of the
511 midgut barrier.

512

513 We revealed that similarly as what was observed with Rin in *Aedes* and G3BPs in mammals
514 (Nowee *et al.*, 2021), Rin of *Anopheles* is also able to interact with nsP3 of ONNV (Fig. 6).
515 This result indicates the conservation of this interaction even in the poor arbovirus vector as
516 *Anopheles*. Moreover, Rin is also clearly proviral for ONNV infection in *Anopheles* (Fig. S1)
517 and is upregulated during the DI (Fig. S2). Therefore, Rin/G3BPs and alphavirus interaction is

518 overall conserved between invertebrates and vertebrates, which highlight the importance of
519 this relationship for the viral cycle. Viruses have evolved to strongly interact with Rin/G3BPs
520 both in invertebrate and vertebrate hosts. Therefore, this interaction between nsP3 and
521 Rin/G3BPs is strongly needed for the virus to be maintained in both hosts. This evidence
522 strongly suggests an important role for Rin during the viral cycle. As previously mentioned, it
523 would be surprising that Rin role, knowing its plurality of functions, is only used by viruses
524 during their RNA replication steps. Our hypothesis is that ONNV nsP3 will hijack Rin for both
525 replication step functions and for inhibiting the innate antiviral immunity of its host.

526

527 The study of Rin partners revealed numerous protein partners involved in various
528 biological function mostly RNA metabolism and protein synthesis. However, the siRNA
529 component Ago2 is also a partner of Rin. Moreover, most of Rin partners have no known
530 functions which highlighted the complexity of the Rin network. Furthermore, the presence of
531 nsP3 changes the proportion of Rin partners and the biological function of these partners. These
532 results revealed that the nsP3 interaction with Rin is able to modify the scheme of Rin partners.
533 However, contrary to what was observed in Prigent et al with G3BPs capable to interact with
534 IκB alpha and NF-κappa B/I-κappa B alpha complex (Prigent *et al.*, 2000). In the mosquito
535 vector no immune factors except Ago2 was identify as a partner of Rin. Moreover, similarly as
536 our study, Scholte et al mentioned that they did not discover NF-κappa B and I-κappa B alpha
537 transcription factor in their interaction assay with G3BP2 in mammals cells (Scholte *et al.*,
538 2015) Detailed studies need to be done to specifically dissect the molecular mechanism by
539 which Rin influence the NF-κappa B antiviral immune pathways Toll and Imd. Our hypothesis
540 is that Rin would allow the regulation of these immune genes by intervening in the
541 transcription, RNA stabilization, or protein synthesis of regulatory factors in those antiviral

542 pathways. To confirm this hypothesis, an interaction assay between Rin and mRNA in cells
543 will be necessary in order to identify the mRNA partners of Rin.

544

545 This study revealed the link between Rin, antiviral immunity and viral infection and
546 could be extrapolated to the role of G3BPs in vertebrate cells. Although Rin was always mostly
547 studied for their role in the RNA replication steps of the virus, even if no specific mechanism
548 was explained yet, the plurality of G3BPs functions in the cell metabolism make them a target
549 of choice for the virus to manipulate. Here we highlighted that ONNV hijacks Rin probably
550 via its interaction with nsP3 in order to corrupt the antiviral immune pathway that Rin regulates.
551 Although Rin is a choice target for the virus, its plurality in multiple biological pathways would
552 make it a difficult target for therapeutic treatment with the risk of interfering with other function
553 in the organism. However, dissecting the partners of Rin with unspecified function could point
554 to promising new therapeutic targets.

555 MATERIALS AND METHODS

556 Cellular strains, viral strains, and mosquitoes

557 4a3A cells originated from neonate *An. coluzzii* 4a r/r strain larvae spontaneously
558 immortalized (Muller *et al.*, 1999). 4a3A cells were maintained at 27°C without CO₂ in Insect-
559 XPRESS™ Protein-free Insect Cell Medium complemented with heat-inactivated Fetal Calf
560 Serum (FCS) (#A3840002 ThermoFisher Scientific). C6/36 cell line (ATCC) (Igarashi, 1978)
561 was used for viral titration and were maintained in Leibovitz's L-15 medium (#11415-049,
562 ThermoFisher Scientific) supplemented with MEM non-essential amino acid (#11140-050,
563 ThermoFisher Scientific) and complemented with heat-inactivated Fetal Calf Serum
564 (#A3840002 ThermoFisher Scientific) at 28°C without CO₂. Baby hamster kidney cell line
565 (BHK-21) was used to produce viral particles from infectious clones and were maintained in
566 Dulbecco's Modified Eagle Medium (DMEM) (1X) + GlutaMax media (#61965-026,
567 ThermoFisher Scientific) complemented with heat-inactivated Fetal Calf Serum (#A3840002
568 ThermoFisher Scientific) at 37°C with 5% CO₂.

569

570 Infectious clone of ONNV used in this project originated from ONNV strain Igbo Ora-
571 IBH10964 (Igbo-Ora strain) and was isolated from a human febrile patient during the epidemic
572 of 1966 in Nigeria (accession number AF079457) (Lanciotti *et al.*, 1998). ONNV infectious
573 clone was either produced on *Anopheles* 4a3A cells and viral titer done on kidney epithelial
574 cells from African green monkey (Vero cells) were measured at $7,3 \times 10^5$ pfu/mL (plaque
575 forming unit) for cellular infection. Mosquito infection used infectious ONNV clone produced
576 on BHK-21 cells and the titer measured on C6/36 cells is $5,64 \times 10^7$ ffu/mL. Infectious clone
577 cDNA was linearized by PmeI, and viral RNA was transcribed using T7 RNA polymerase
578 (Ambion mMACHINE® SP6 Kit). Transcribed RNA was electroporated into
579 BHK-21 or 4a3A cells. Virus was recovered after 72 h and titer on C6/36 cells.

580

581 The *An. coluzzii* Ngouso colony was created from 100 wild-caught female mosquitoes from
582 Cameroon in 2006 and are bred at the Institut Pasteur Center for the Production and Infection
583 of *Anopheles* (CEPIA) facility since 2008. All the steps of mosquitoes breeding occurred in a
584 controlled environment with a temperature of 26°C ($\pm 1^\circ\text{C}$), 70% humidity and 12h:12h of day:
585 night photoperiod. Female mosquitoes used for all experiments are of the second generation
586 (F2) and are of early emergence (less than 5 days post-emergence).

587

588 **Primers and plasmids**

589 The dsRNA sequences were either previously published or constructed using the cDNA
590 sequence of genes in the *An. gambiae* PEST genome available on VectorBase database.
591 DsRNAs targeting GFP act as a control for the dsRNA treatment. DsRNA were synthesized
592 using T7 primers and the transcription kit of MEGAscript™ T7 (#AM1334, Invitrogen)
593 following manufacturer instructions. DsRNA were used either in 4a3A cell line or in *An.*
594 *coluzzii* Ngouso mosquitoes.

595

596 Plasmids used for colocalization, and streptavidin pull-down assay were constructed using Rin
597 codon-optimized AGAP000403 sequences and ONNV nsP3 protein from IBH10964 strains
598 graciously given to us by Andres Merits. A C-terminal streptavidin tag II (W-S-H-P-Q-F-E-K)
599 was added at the end of Rin sequences. For their expression in 4a3A cells, these genes are
600 expressed under the Actin promoter (Ac5) in the plasmid pAc5.1. 4a3a cells lines were
601 transfected using lipofectamine LTX (#A12621, ThermoFisher Scientific) with 0,25 μg to 0,5
602 of plasmids depending on the number of cells used.

603

604 **RNA extraction, cDNA synthesis and qPCR analysis**

605 Following experiments, RNA was extracted from 4a3A cells or mosquitoes using Trizol
606 reagent (#T9424, Sigma Aldrich) and RNA miniprep kit (#ZR1054, Ozyme). Complementary
607 DNAs were then synthesized with M-MLV transcriptase inverse kit (#28025013, Invitrogen)
608 using 1 µg of RNA. The qPCR primers were checked for specificity prior to the analysis. All
609 qPCRs were performed using SYBR green supermix (KAPA SYBR FAST ABI, Sigma-
610 Aldrich) and the CFX96 Touch Real-Time PCR Detection System (Biorad). The ribosomal
611 protein rpS7 gene was used as the internal control and the analysis of transcript relative
612 expression to rpS7 was performed according to the $2^{-\Delta\Delta Ct}$ method. Conditions of the run
613 were : 95°C for 10min, then 39 cycles of 95°C for 15 sec, 60°C for 1min (plateread).

614

615 **Antibodies and fluorescent labels**

616 Sera of rabbit containing polyclonal rabbit antibodies graciously given to us by Andres merits
617 targeting the macro and the AUD domain of nsP3 of ONNV were used at 1:100 overnight at
618 4°C for colocalization and western blot assay. Secondary Goat anti-Rabbit IgG (H+L)
619 antibodies (#A27039, Invitrogen) conjugated with Alexa Fluor 555 were used during
620 colocalization assay at 1:500 for 45 min at room temperature (RT) 0,2% Triton X -100 1%
621 BSA PBS 1X. Secondary antibodies anti-rabbit conjugated with HRP (#7074S, Invitrogen)
622 were used for western blot at 1:8000 for 1 hour at RT in 5% BSA in TBS1X solution.
623 Monoclonal antibodies targeting the streptavidin tag II and conjugated with HRP (#2-1502-
624 001, IBA) were used at 1:10000 for 1h at RT during western blot in 5% BSA in TBS1X
625 solution. Streptavidin-tactin molecule conjugated with dye-649 (#2-1568-050, IBA) were used
626 at 1:100 for 3h at RT during colocalization assay in 0,2% Triton X -100 1% BSA PBS 1X.
627 DAPI (4', 6-diamidino-2'-phenylindole, dihydrochloride) (#62247, ThermoScientific) was
628 used at 1:1000 for 10 min at RT in PBS1X for staining the nucleus of 4a3A cells during
629 colocalization assay. Revelation of viral titration was made using monoclonal mouse antibodies

630 against CHIKV Virus-like particles (#MAB12385, The Native Antigen), diluted at 1:1000 in
631 0,1% BSA PBS1X solution for 45 min at 37°C. Secondary goat antibodies against mouse IgG
632 and IgM (H+L) conjugated with Alexa 488 (#A-10680, Invitrogen) were diluted at 1:500 in
633 PBS1X and incubated at 37°C for 30 min.

634

635 **Gene silencing**

636 For silencing gene in the *in vivo* system, 500 ng of dsRNA in a final volume of 70 nL maximum
637 was injected into the thorax of cold-anesthetized 1- to 2-d-old *An. coluzzii* females using
638 Nanoject II injector (Drummond Scientific) and glass capillary needle as previously described
639 (Mitri *et al.*, 2009). The gene silencing efficiency was verified after dsRNA injection with RNA
640 from a pool of six un-fed whole mosquitoes by RT-quantitative PCR (qPCR). For silencing
641 genes in the *in vitro* system, a total of 1×10^5 *An. coluzzii* 4A3A cells were seeded and left to
642 adhere overnight. Cells were incubated for 30 min on a rocker with 500 ng of in 200 μ L of
643 Insect Xpress media without FBS. After incubation, 300 μ L of microliters of Insect Xpress
644 media with 10% (vol/vol) FBS was added, and cells were incubated until collection or
645 infection. Gene silencing efficiencies were confirmed and are shown in Fig S3 and Fig S4.

646

647 **Cellular infection**

648 The 4a3A cell line were infected with ONNV at multiplicity of infection (MOI) of 0.01 (for
649 dsRNA experiment) or at MOI 0.5 (for colocalization experiment) for 1h at 28°C without CO₂
650 in Insect-X-Press media without FCS. After 1 hour of infection, cells were washed three times
651 with Insect-X-Press without FCS. Then 2%FCS Insect-X-Press media was added to cells for
652 the time of incubation. Cells were then collected at 24h or 48h post-infection.

653

654 **Infectious blood feeding**

655 The experimental protocol is based on published methods (Vazeille et al., 2007). Infectious
656 blood meal was composed of 2/3 rabbit erythrocytes and 1/3 of viral solution for a final titer
657 of 10^7 ffu/mL. Adenosine triphosphate (ATP) at 10mM was also added to the infectious blood
658 meal. The infectious blood meal has been distributed in capsule cover with pork intestine.
659 Blood-filled capsules were heat at 37°C on Hemotek® system. Mosquitoes were feed on
660 infectious blood meal for 1 hour. After that female mosquitoes were anesthetized on ice and
661 blood-stuff females were incubated at 28°C and 80-90% humidity with water-imbibed cotton
662 with 10% sugar. Abdomens and thoraces of female mosquitoes were separated, grinded at 2000
663 rpm for 30 seconds and centrifuged at 4°C for 5min at 10 000 rpm. Supernatants was collected
664 and conserved at -80°C until analysis.

665

666 **Viral titration by focus forming unit**

667 *Ae. albopictus* C6/36 cells were seeded in 96-well plates at 1.25×10^6 cells/ Leibovitz's L-15
668 medium (#11415-049, ThermoFisher Scientific) supplemented with MEM non-essential amino
669 acid (#11140-050, ThermoFisher Scientific) with 10% of FBS and incubated at 28°C without
670 CO₂, 48h prior to the titration. Virus-containing samples were sequentially diluted and 50µL
671 of those dilutions were added to cells in one well. After one hour of incubation at 28°C, 150
672 µL of 1:1 mixed with 4% carboxymethylcellulose (CMC) with L-15 containing 5 % of FBS is
673 added on cells. Antibiotic-antifungal solution (#15240062, Life technologies®) is also added
674 at a finale concentration of 1.5X. Plates are incubated at 28°C without CO₂ for 3 days, then
675 cells are fixed using 3.4% formaldehyde in PBS1X for 20 min at room temperature. Cells are
676 then washed three times in PBS1X and then stained with primary antibodies. After three
677 washes with PBS1X, cells are stained with secondary antibodies. Viral titers are counted by
678 observing foci using a fluorescent microscope and the virus titer of the tested sample is
679 expressed as foci forming unit/mL (FFU/mL).

680

681 **Confocal microscopy**

682 Cells were seeded in μ -slide 8-well plate (#80807, Ibidi) and transfected using lipofectamine
683 with 0.25 μ g of plasmids. Two days post transfection cells were fixated with 4% formaldehyde
684 for 20min at RT. Cells were then blocked and permeabilized using a solution of 0,2% Triton
685 X -100 1% BSA PBS 1X for 1h at RT. Then cells were stained with different antibodies at
686 specific dilution in 0,2% Triton X -100 1% BSA PBS 1X. Washes were realized using 0,2%
687 Triton X -100 1% BSA PBS 1X. Colocalization analysis in 4a3A cells were done using a laser-
688 scanning confocal microscope (LSM700, Carl Zeiss Jena) at the UTechS Photonic BioImaging
689 - C2RT platform (Pasteur Institute). All acquisition parameters were done as listed below : 63X
690 oil-objective, frame size 512x512, pixel dwell 1,57 μ sec, scan time 5,78 sec, 16-bit depth,
691 image size 32,9x32,9 μ m and 0,06 μ m pixel size. The DAPI laser (450 nm) had an intensity of
692 2, a pinhole diameter of 0,6 μ m, a master gain of 730 and a digital gain of 1. The Red A555
693 laser (514 nm) had an intensity of 2,6, a pinhole diameter of 0,6 μ m, a master gain of 711 and
694 a digital gain of 1. The Far-Red D647 laser (633nm) had an intensity of 2, a pinhole diameter
695 of 0,8 μ m, a master gain of 700 and a digital gain of 1. Images were analyzed using Fiji software
696 (Schindelin et al., 2012). Background signals were calculated with the mean of signals from 5
697 pictures of the negative controls of each fluorophore. Mean of background signals were then
698 removed from the analyzed pictures. Colocalization was quantified by counting the number of
699 cells with colocalization pattern related to cells co-transfected (harboring both signals).
700 Between 30 and 50 cells were counted in the analysis.

701

702 **Streptavidin-pull down assay**

703 First, 0.5 μ g of plasmid constructs were transfected in 4a3A cells using lipofectamine LTX
704 with Plus Reagent (#15338030, ThermoFisher Scientific). Two days post-transfection, cells

705 lysis has been performed using cell lysis buffer (containing Tris 1 M, NaCl 5M, EDTA, Igepal,
706 Protease inhibitor and Phosphatase inhibitor) for 30 min at 4°C and samples were centrifuged
707 at 16,000g for 15 minutes at 4°C. Pull-down assay was performed using MagStrep” type”3
708 beads (Strep-Tactin® XT coated magnetic beads, 5 % (v/v) suspension, # 2-4090-002, IBA)
709 with a binding capacity up to 0.85 nmol/µl beads (corresponding to 25,5 µg of a 30 kDa
710 protein). Beads are first wash with cell lysis buffer then cell lysate is added on the beads and
711 incubated for 2h at 4°C on a tube rotator. After 2 hours of incubation, lysates are removed, and
712 washes are performed using 1X W buffer (#2-1003-100, IBA). Following washes, either on-
713 beads digestion at the Proteomic platform is done for Mass spectrometry analysis or the elution
714 of proteins is performed using 1X BXT buffer containing biotin (#2-1042-025, IBA) for 10
715 min. Eluates are then analyzed by Western Blot in order to detect strep-tag proteins (Rin or
716 GFP, bait) and partners (nsP3, prey) using specific antibodies.

717

718 Proteins samples were reduced in 1X DTT and heat at 95°C for 5min. Then samples are mixed
719 with XT sample buffer (#1610791, Bio-Rad) and loaded on 4-12% Criterion SDS-PAGE gels
720 (#3450124 Bio-Rad). SDS-PAGE is run at 100V for 10 minutes (stacking gel) and 150V for
721 1h00min (running gel) in NOVEX NUPAGE MOPS SDS Running Buffer 20X (#NP0001, Life
722 Technologies). Following electrophoresis, proteins contained on gel are transferred onto a 0,2
723 µm nitrocellulose membrane (#1704159, Bio-Rad) using Bio-Rad Trans-Blot® Turbo™
724 Transfer System. Transfer program is run for 7min at 2,5A and 25V. After transfer,
725 immunoblots are blocked in a solution containing 5% of Bovine Serum Albumin (BSA) called
726 TBS1X (Tris-buffered saline, 0.1% Tween 20). Then immunoblots are probed with different
727 antibodies at specific dilutions in 5%BSA PBS1X. Detection step was performed using the
728 Enhanced chemiluminescence (ECL) system (Clarity Western ECL substrate #170-5060,
729 Biorad) following manufacturer instruction. Blots were photographed and revealed taking one

730 picture every second for 10 seconds using ChemiDoc Imaging System (Bio-Rad). Blots were
731 then annotated, and molecular weights were measured related to the proteins ladder (Precision
732 PlusProtein All Blue Standards #1610373, Bio-Rad) using the Image Lab analysis software
733 (Bio-Rad). A positive control is performed using a commercial GFP-strep (Green Fluorescent
734 Protein) (#2-1006-005, IBA-LifeSciences) in order to confirm the success of the Western Blot.
735

736 **Mass spectrometry analysis**

737 Following streptavidin-pull down assay, on-bead digestion of sample is performed at the
738 Proteomic platform (CNRS – UAR2024) at Pasteur Institute. Proteins were resuspended in
739 50mM ammonium bicarbonate pH 8.0 and reduced for 30 min at room temperature (RT) with
740 5 mM TCEP and then alkylated at 50 mM iodoacetamide (I114 - Sigma, St Louis, Missouri,
741 USA) for 30 min, RT in the dark. Protein was then digested with 0.5 µg Sequencing Grade
742 Modified Trypsin (V5111 - Promega, Madison, Wisconsin, USA). Following the overnight
743 digestion at 37°C, 800rpm in the thermomixer C (Eppendorf), the samples were placed on a
744 magnet for 1 min and the supernatant containing peptides transferred in a new tube. Resulting
745 peptides were acidified at 1% with formic acid, desalted with the AssayMAP Bravo robot using
746 C18 column and AssayMAP Peptide Cleanup Protocol (Agilent). All samples were dried in a
747 Speed-Vac and peptides were resuspended in 2 % ACN, 0.1 % FA prior to LC-MS/MS
748 analysis. LC-MS/MS analysis of digested peptides was performed on an Orbitrap Eclipse mass
749 spectrometer (Thermo Fisher Scientific, Bremen) coupled to an EASY-nLC 1000 (Thermo
750 Fisher Scientific). Peptides were loaded (at constant pressure of 900 bars) and separated at 250
751 nl.min⁻¹ on a home-made C18, 30 cm capillary column picotip silica emitter tip (75 µm
752 diameter filled with 1.9 µm Reprosil-Pur Basic C18-HD resin, (Dr. Maisch GmbH,
753 Ammerbuch-Entringen, Germany)) equilibrated in solvent A (2 % ACN, 0.1 % FA). Peptides
754 were eluted using a gradient of solvent B (80 % ACN ,0.1 % FA) from 2 % to 7 % in 3 min, 7

755 % to 31 % in 42 min, 31 % to 62 % in 10 min, 62 % to 95 % in 5 min (total length of the
756 chromatographic run was 70 min). Column was equilibrated with 10 μ l of A at 900 bar. Mass
757 spectra were acquired in data-dependent acquisition mode with the XCalibur xxx software
758 (Thermo Fisher Scientific, Bremen). MS spectra were acquired at orbitrap resolution of 60k (at
759 m/z 400), AGC target 800000, a custom max injection time. The scan range was limited from
760 300 to 1500 m/z. Peptide fragmentation was performed using higher-energy collision
761 dissociation (HCD) with the energy set at 27 NCE. The MS/MS spectra acquired at a resolution
762 of 30k (at m/z 400). Isolation window was set at 1.6 m/z. All acquisitions were done in profile
763 and positive mode. Dynamic exclusion was employed within 30s.

764

765 Proteomic platform has performed data and statistical analysis. The guideline used is described
766 below. Raw data were analyzed using MaxQuant software version 2.0.3.0 (Tyanova et al.,
767 2016) using the Andromeda search engine (Cox et al., 2011). The MS/MS spectra were
768 searched against a custom database from Vectorbase repositories 20PEST database (download
769 in 25/04/2022) and tagged proteins. Usual known mass spectrometry contaminants and
770 reversed sequences of all entries were included. Andromeda search was performed choosing
771 trypsin as specific enzyme with a maximum number of two missed cleavages. Possible
772 modifications included carbamidomethylation (Cys, fixed), oxidation (Met, variable), Nter
773 acetylation (variable). The mass tolerance in MS was set to 20 ppm for the first search then 4.5
774 ppm for the main search and 20 ppm for the MS/MS. Maximum peptide charge was set to
775 seven and seven amino acids were required as minimum peptide length. The “match between
776 runs” feature was applied for samples having the same experimental condition with a maximal
777 retention time window of 0.7 minute. One unique peptide to the protein group was required for
778 the protein identification. A false discovery rate (FDR) cutoff of 1 % was applied at the peptide
779 and protein levels. For the differential analyses of one condition versus another, proteins

780 identified in the reverse and contaminant databases and proteins “only identified by site” were
781 first discarded from the list of identified proteins. Then, only proteins with at least three
782 quantified intensities in a condition were kept. The proteins of interest are therefore those
783 which emerge from this statistical analysis supplemented by those which are considered to be
784 present from one condition and absent in another.

785

786 **Statistical analysis**

787 All experiments presented are the results of at least three independent experiments. Bar plots
788 were created using Prism. Statistical significance of most of the experiments presented was
789 established using a two-tailed unpaired (independent) Student t-test which compare the mean
790 of two independent groups (control and treatment). A two-tailed non-parametric unpaired
791 Mann Whitney test was performed for assessing the statistical significance of the difference of
792 viral titer in mosquitoes. The P values were assessed with a null distribution. The P values were
793 considered significant if $p < 0.05$ (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.005$, **** $p < 0.0001$).

794 **Availability of data and material**

795 All data from the study are presented within this article.

796

797 **Ethics statement**

798 The protocol for the ethical treatment of the animals used in this study was approved by the
799 research animal ethics committee of the Institut Pasteur, “C2EA-89 CETEA Institut Pasteur”
800 as protocol number 202195.02. The Institut Pasteur ethics committee is authorized by the
801 French Ministry of Higher Education and Research (MESR) under French law N° 2001-486,
802 which is aligned with Directive 2010/63/EU of the European Commission on the protection of
803 animals used for scientific purposes. The study was performed using practices and conditions
804 approved by the Institut Pasteur Biosafety Committee as protocol number CHSCT 14.114.

805

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814

815 **Author contributions**

816 Conceived and designed the research: SC CM ABF KDV.

817 Performed the experiments: SC AB CM EBF MM.

818 Analyzed the data: SC CM MM KDV.

819 Wrote the paper: SC CM ABF KDV.

820

821 **Declaration of interests**

822 The authors declare no competing interests.

823

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827 **FIGURE CAPTIONS**

828 **Figure 1. Rin function is required for correct transcriptional basal immunity in**
829 **uninfected Anopheles mosquitoes.** (A-F) Female mosquitoes were injected with 500 ng/ μ L
830 of dsRin or dsGFP. The influence of Rin depletion on genes involved in the Toll pathway (A),
831 the Imd pathway (B), the JAK/STAT pathway (B), the siRNA pathway (C), other immune
832 transcripts involved in complement-like immunity including LRR and TEP transcript (D) and
833 the lipid carrier Evokin (E) are showed. Each bar represents the level of transcript abundance
834 of each immune genes relative to dsGFP-treated control (defined as 1.0), and error bars indicate
835 the SEM. Histogram showed are the results of three independent experiments with 18 female
836 mosquitoes analyzed in total per conditions. *P < 0.05, ** P < 0.01, *** P < 0.005, **** P <
837 0.0001.

838

839 **Figure 2. Rin is required for ONNV-dependent inhibition of the Anopheles Imd pathway.**
840 (A-E) Female mosquitoes were injected with 500 ng/ μ L of dsRin or dsGFP and two days later
841 those females were fed an infectious blood meal containing ONNV at 10^7 ffu/mL. Pull of 8
842 blood-fed mosquitoes were sacrificed at 2 days and 3 days post-blood feeding and total RNA
843 was extracted. The influence of Rin depletion on genes involved in the Toll pathway (A), the
844 Imd pathway (B), the JAK/STAT pathway (B), the siRNA pathway (C), LRR and TEP
845 transcripts (D) and the lipid carrier Evokin (D) during the PMI are showed. Each bar represents
846 the level of transcript abundance of each immune genes relative to dsGFP-treated control
847 (defined as 1.0), and error bars indicate the SEM. Histogram showed are the results of three
848 independent experiments with 48 female mosquitoes analyzed in total per conditions. *P <
849 0.05, ** P < 0.01, *** P < 0.005, ns non-significant.

850

851 **Figure 3. Silencing of the Imd pathway complements loss of Rin during ONNV infection.**

852 (A-B). Mosquitoes were treated with dsRNA two days prior to the infectious bloodmeal. Gene
853 silencing was confirmed on the day of infectious blood feeding (C). All fully engorged
854 mosquitoes were sampled at 3 d post-bloodmeal to determine ONNV infection by viral titration
855 of abdomens. (A) ONNV infection prevalence, the proportion of blood-fed mosquitoes positive
856 for infection (B) ONNV infection intensity, the viral titer of only positive mosquitoes. (C) Rin
857 and Rel2 transcript level after dsRNA treatment in unfed female mosquitoes collected the day
858 of the blood-feeding is showed. (A) Each bar represents the percentage of abdomen positive to
859 ONNV between dsGFP, dsGFP+dsRin, dsGFP+dsRel2 and dsRin+dsRel2-treated mosquitoes.
860 (B) Each point represents the viral titer counted in one female mosquito abdomen. (C) Each
861 bar represents the level of transcript abundance of Rin and Rel2 relative to dsGFP-treated
862 control (defined as 1.0). Errors bars indicate the SEM. Each histogram represents the results
863 of 3 independent experiments with 72 mosquitoes analyzed by conditions in total (A-B). A
864 pool of at least 6 mosquitoes has been analyzed by biological replicates (C). ** $P < 0.01$, ***
865 $P < 0.005$, **** $P < 0.0001$, ns non-significant.

866

867 **Figure 4. Rin is required for correct basal immunity in Anopheles hemocyte-like cells.**

868 (A-D) Cells were depleted for Rin (dsRin) or GFP (dsGFP) using 25 ng/ μ L of dsRNA per
869 conditions. Rin depletion on genes involved in the Toll pathway (A), the Imd pathway (B), the
870 JAK/STAT pathway (B), the siRNA pathway (C) and other immune transcript involved in
871 complement-like immunity including LRR and TEP transcript (D) are shown. Each bar
872 represents the level of transcript abundance of each immune genes relative to dsGFP-treated
873 control (defined as 1.0), and error bars indicate the SEM. Histogram showed are the results of
874 three independent experiments. * $P < 0.05$, ** $P < 0.01$.

875

876 **Figure 5. Presence of Rin allows ONNV-dependent inhibition of the Toll pathway in**
877 **Anopheles hemocyte-like cells.** (A-B) 4a3A cells were depleted for Rin (A) or Cactus (B) and
878 infected with ONNV at MOI 0.01. The influence of Rin depletion on Rel1 during a viral
879 infection was tested (A). The antiviral effect of the activation of the Toll pathway via Cactus
880 depletion on ONNV viral RNA quantity and ONNV particle production are presented (B). (A)
881 Each bar represents the level of transcript abundance of Rel1 (A) relative to dsGFP-treated
882 control (defined as 1.0). (B) Each bar represents either the level of transcript abundance of
883 ONNV RNA or focus forming unit relative to dsGFP-treated control (defined as 1.0) . Error
884 bars indicate the SEM. Histogram showed are the results of three independent experiments. *
885 $P < 0.05$, ** $P < 0.01$, **** $P < 0.0001$

886

887 **Figure 6. Rin physically interacts with ONNV nsP3 in Anopheles hemocyte-like cells.** (A-
888 B) Rin proviral actions on viral RNA quantity (A) and infectious particle production (B) are
889 showed. (C-D) NsP3 signal (green), Rin-strep signal (red), nucleus signal (blue) and
890 colocalization between nsP3 and Rin-strep (yellow) are presented (C). The quantification of
891 the colocalization is showed where the number of cells harboring the colocalization pattern
892 (yellow) are related to the number of co-transfected cells (D). (E) The eluats of the pull-down
893 have been analyzed by Western Blot and bands revealed with antibodies targeting streptavidin
894 tag (anti-strep) and bands revealed with antibodies against nsP3 (anti-nsp3) are represented
895 (E). (A-B) Each bar represents the level of transcript abundance of ONNV RNA (A) or focus
896 forming unit (B) relative to dsGFP-treated control (defined as 1.0), and error bars indicate the
897 SEM. Histogram showed are the results of three independent experiments. *** $P < 0.005$, ****
898 $P < 0.0001$. (C-D) A minimum of 30 co-transfected cells were counted by conditions.
899 Representative pictures are showed. Ns non-significant. (E) The result of four independent
900 experiments is showed.

901

902 **Figure 7. ONNV nsP3 binding alters Rin protein partners in Anopheles cells.** (A-E) Eluates
903 of cells transfected with Rin-strep or co-transfected with Rin-strep and nsP3 of ONNV were
904 analyzed by mass spectrometry to identify proteins partners. The non-specific proteins found
905 in the controls conditions were removed from the results. (A) Summary of the biological
906 functions of the protein partners of Rin-strep, or (B) of Rin-strep in presence of nsP3 are
907 showed. (C) A summary of the partners proteins in common between Rin-strep and the
908 complex Rin-strep/nsP3 is presented. (D-E) The average IBAQs of the protein partners of Rin-
909 strep (dark blue) or Rin-strep/nsP3 complex (light blue) or their protein partners in common
910 (plain blue) are showed. Each bar represents the average IBAQs of each proteins partners of
911 Rin-strep or of the complex Rin-strep/nsP3 where the non-specific proteins have been
912 previously removed. Data showed are the results of four independent experiments per
913 conditions.

914 **SUPPORTING INFORMATION CAPTIONS**

915 **Supporting Figures**

916 Figure S1. Rin is a proviral factor for ONNV infection in Anopheles. **A, B.** *An. coluzzii*
917 mosquitoes after bloodmeal infection. **C, D.** *An. coluzzii* 4a3A hemocyte-like cells. **A, B.**
918 Female mosquitoes were treated with dsRNA two days prior to an infectious bloodmeal. On
919 the day of infection, the unfed females were removed and used to test Rin silencing efficiency,
920 then on 6 d post-bloodmeal ONNV infection in abdomens was measured by viral titration. **A.**
921 The effect of Rin depletion on infection prevalence among bloodfed mosquitoes as a proportion
922 of the total mosquitoes analyzed. y-axis, percentage of abdomens positive for ONNV in dsRin
923 or dsGFP-treated mosquitoes. **B.** The effect of Rin depletion on infection intensity in the
924 positive mosquito samples. y-axis, load of viral particles, each point represents the viral titer
925 in one abdomen. Error bars indicate the SEM. Results from 3 independent experiments with
926 72 total mosquitoes analyzed per condition. ** $P < 0.01$, *** $P < 0.005$, ns non-significant. (C-
927 D) Rin is a proviral factor for ONNV infection in 4a3A *An. gambiae* cells. Rin proviral actions
928 on viral RNA quantity (C) and infectious particle production (D) are showed. (C-D) Each bar
929 represents the level of transcript abundance of ONNV RNA (C) or focus forming unit (D)
930 relative to dsGFP-treated control (defined as 1.0), and error bars indicate the SEM. Histogram
931 showed are the results of three independent experiments. *** $P < 0.005$, **** $P < 0.0001$.

932

933 Figure S2. Rin transcriptional expression is induced by ONNV in 4a3A *An. gambiae* cells. (A-
934 B) Rin transcriptional expression (A) during ONNV infection is showed. (A) Each bar
935 represents the level of transcript abundance of Rin in ONNV-infected cells relative to naïve
936 cells (defined as 1.0), and error bars indicate the SEM. Histogram showed are the results of
937 three independent experiments. * $P < 0.05$, ** $P < 0.01$, ns non-significant.

938

939 Figure S3. Rin transcript is efficiently depleted by the dsRNA treatment in both 4a3A An.
940 coluzzii in vitro model and in An. coluzzii mosquito in vivo model. (A-B) Rin transcript after
941 dsRin treatment in naïve cells (A) or infected cells (B) are showed. (C-D) Rin transcript after
942 dsRin treatment in naïve mosquitoes (C) or the day of an infectious blood feeding (D) are
943 showed. (A-D) Each bar represents the level of transcript abundance of Rin relative to dsGFP-
944 treated control (defined as 1.0), and error bars indicate the SEM. Histogram showed are the
945 results of at least three independent experiments. Results on mosquitoes are analyzed from a
946 pull of at least 6 mosquitoes. *P < 0.05, ** P < 0.01, *** P < 0.005, **** P < 0.0001, ns non-
947 significant.

948

949 Figure S4. Rin, Rel1 and Cactus transcripts are efficiently depleted by the dsRNA treatment in
950 4a3A An. gambiae in vitro model. (A-B) Rin and Rel1 transcript after dsRNA treatment in
951 infected cells at 24h (A) and 48h (B) post-infection are showed. (C) Cactus transcript after
952 dsCactus treatment in infected cells is showed. (A-C) Each bar represents the level of transcript
953 abundance of Rin, Rel1 or Cactus relative to dsGFP-treated control (defined as 1.0), and error
954 bars indicate the SEM. Histogram showed are the results of at least three independent
955 experiments. *P < 0.05, ** P < 0.01, *** P < 0.005, **** P < 0.0001.

956

957 Figure S5. Rin transcript is enhanced after the blood-feeding of An. coluzzii mosquitoes. Rin
958 transcript level in blood-fed female mosquitoes is showed. Each bar represents the level of
959 transcript abundance of Rin in blood-fed mosquitoes relative to sugar-fed mosquitoes (defined
960 as 1.0), and error bars indicate the SEM. Histogram showed are the results of at least three
961 independent experiments. A pool of at least 6 mosquitoes has been analyzed by biological
962 replicates. *P < 0.05.

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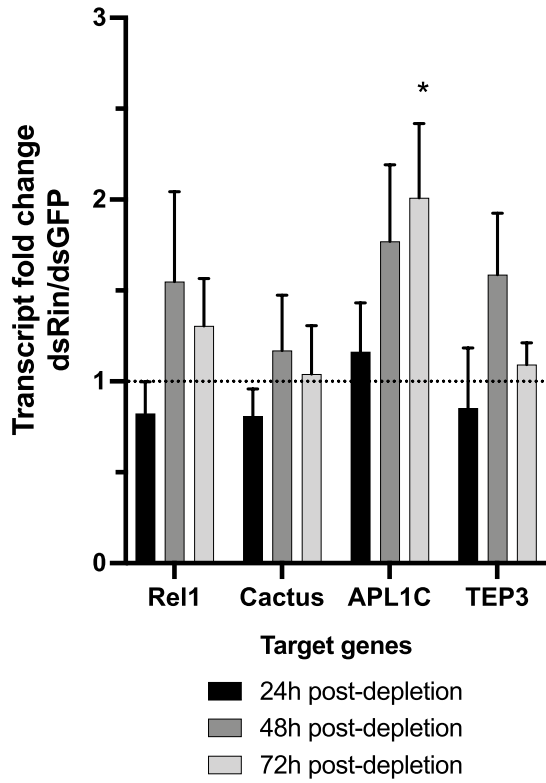
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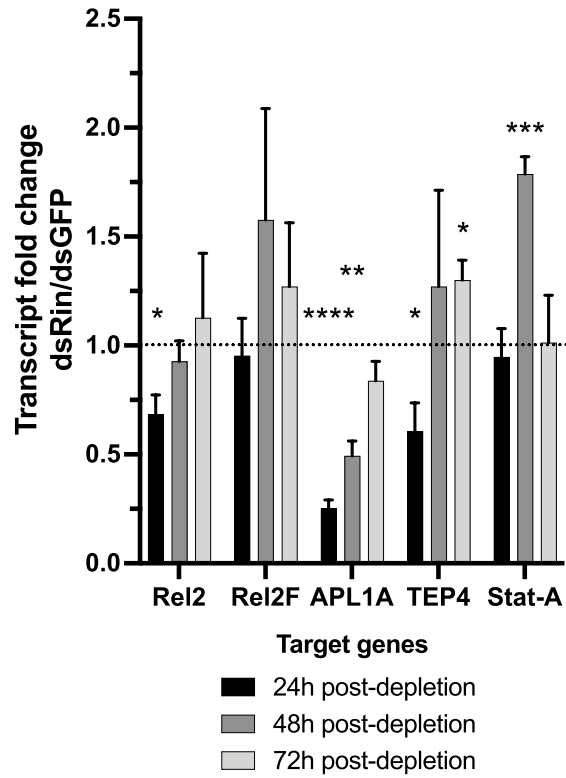
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FIG 1

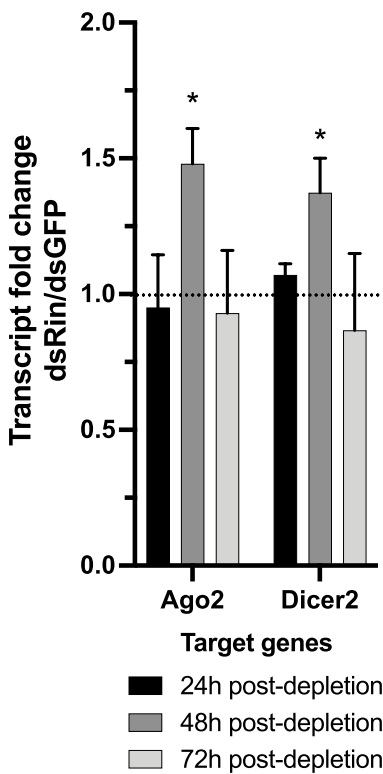
A. Toll pathway



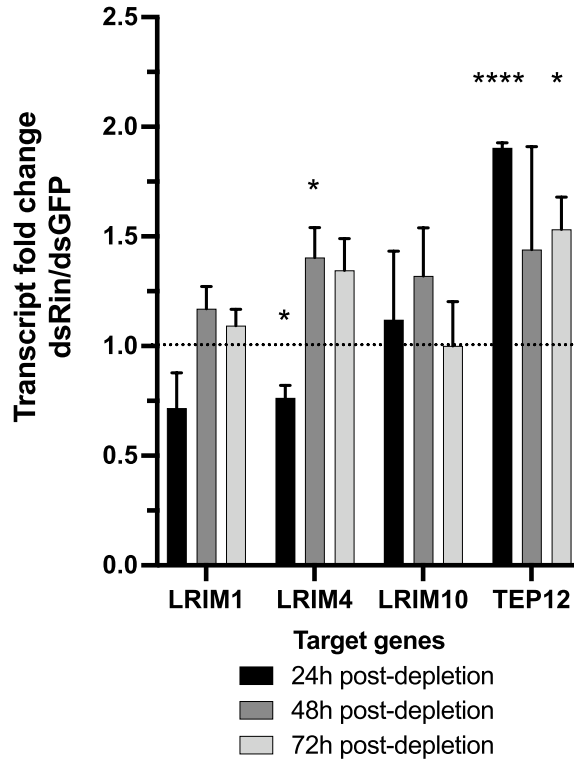
B. Imd and JAK/STAT pathway



C. RNAi pathway



D. Other components



E. Inducer of granulocyte differentiation

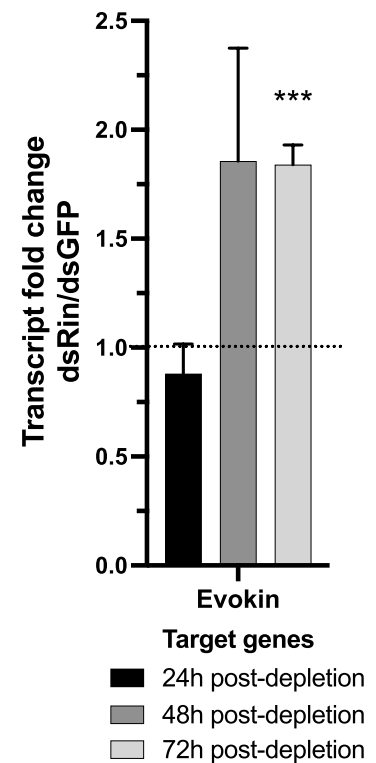
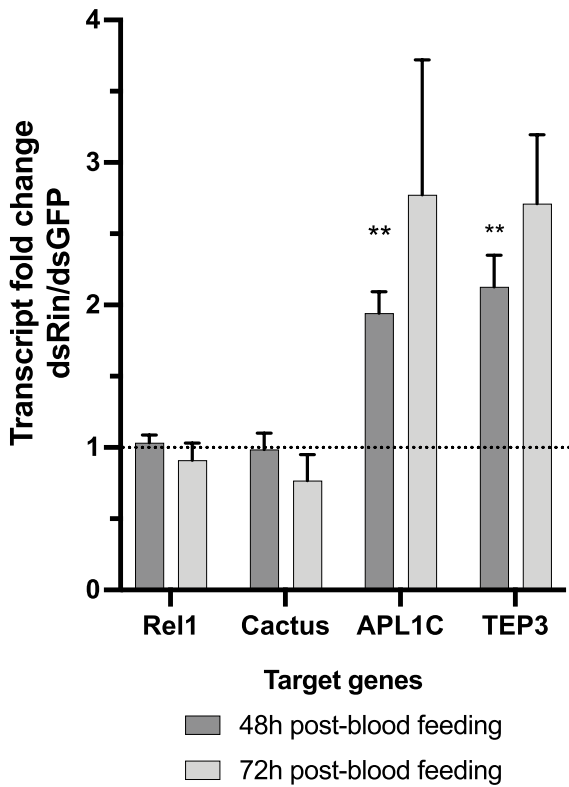
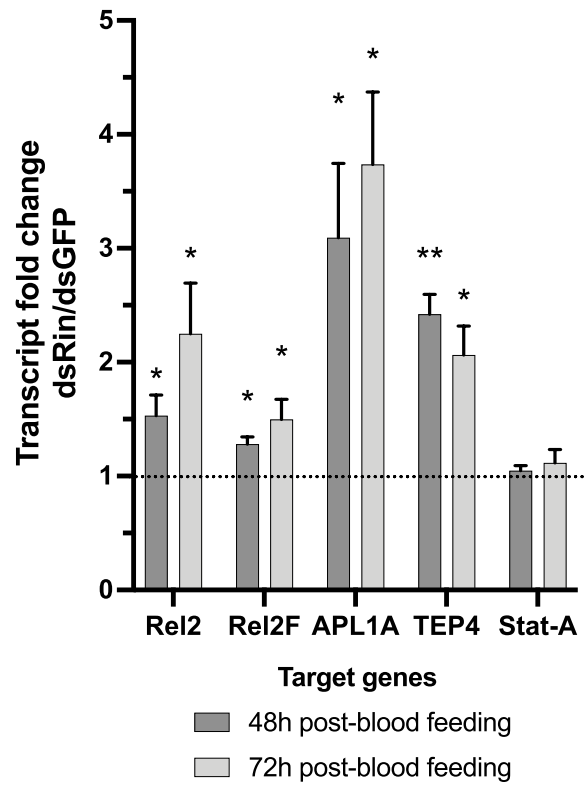


FIG 2

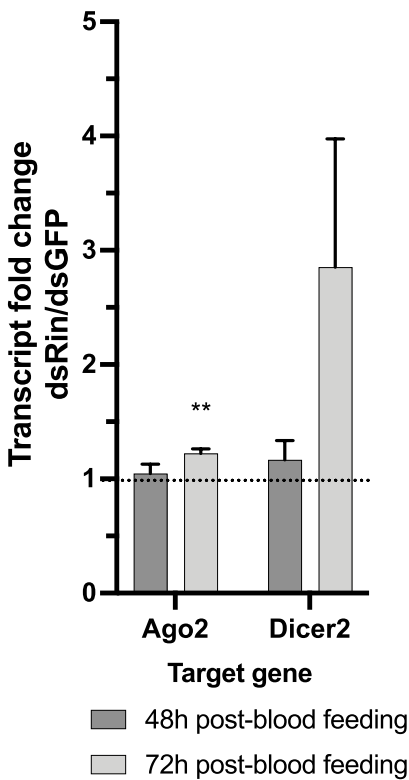
A. Toll pathway



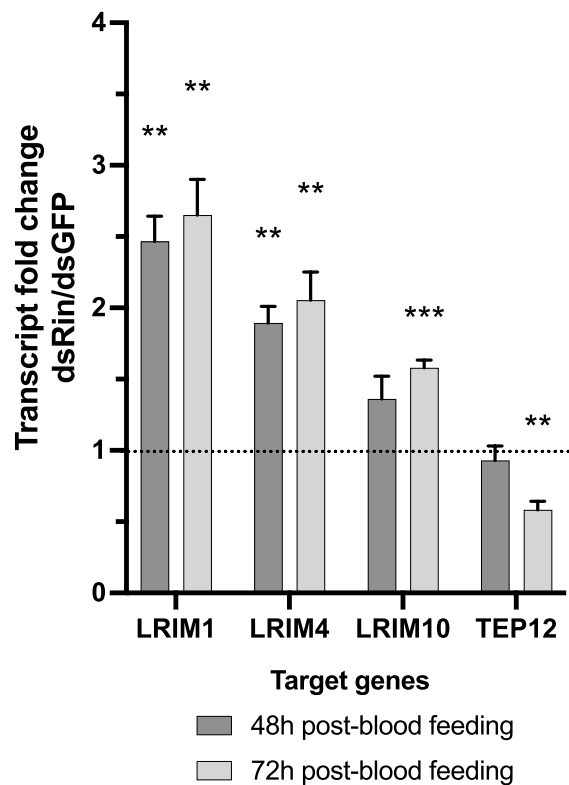
B. Imd and JAK/STAT pathway



C. RNAi pathway



D. Other components



E. Inducer of granulocyte differentiation

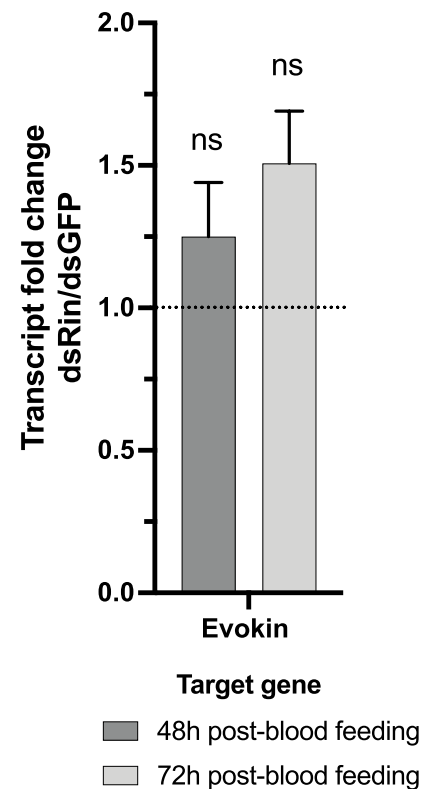
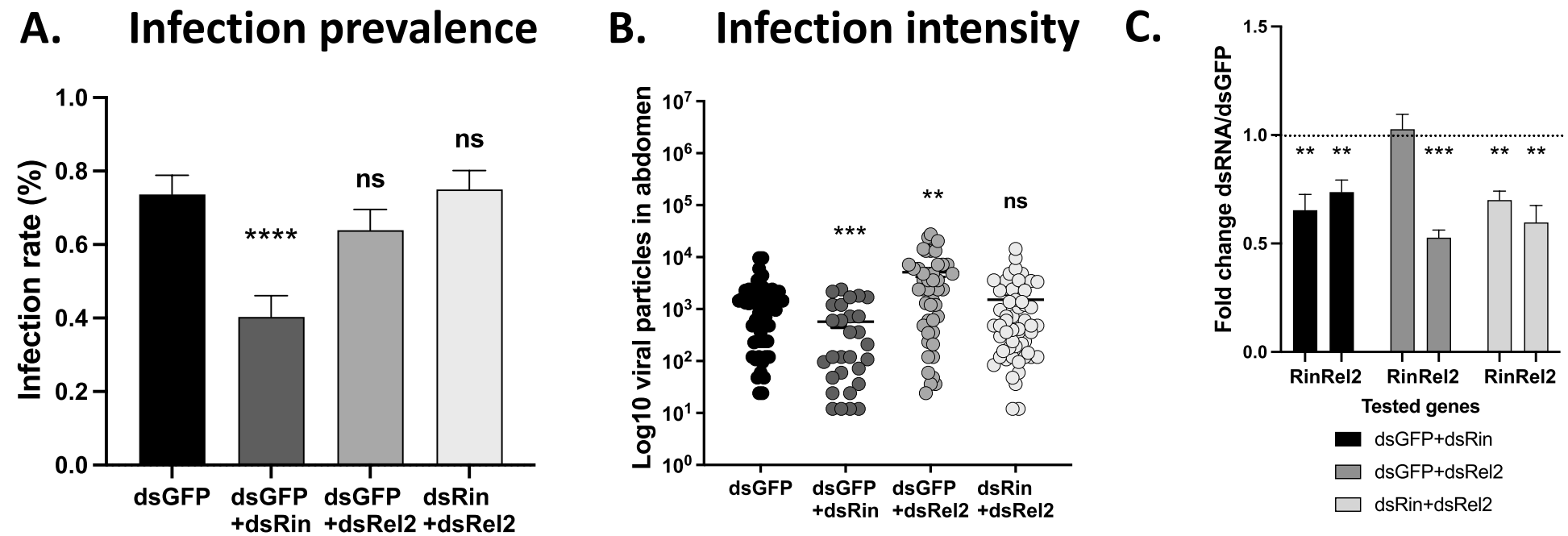


FIG 3



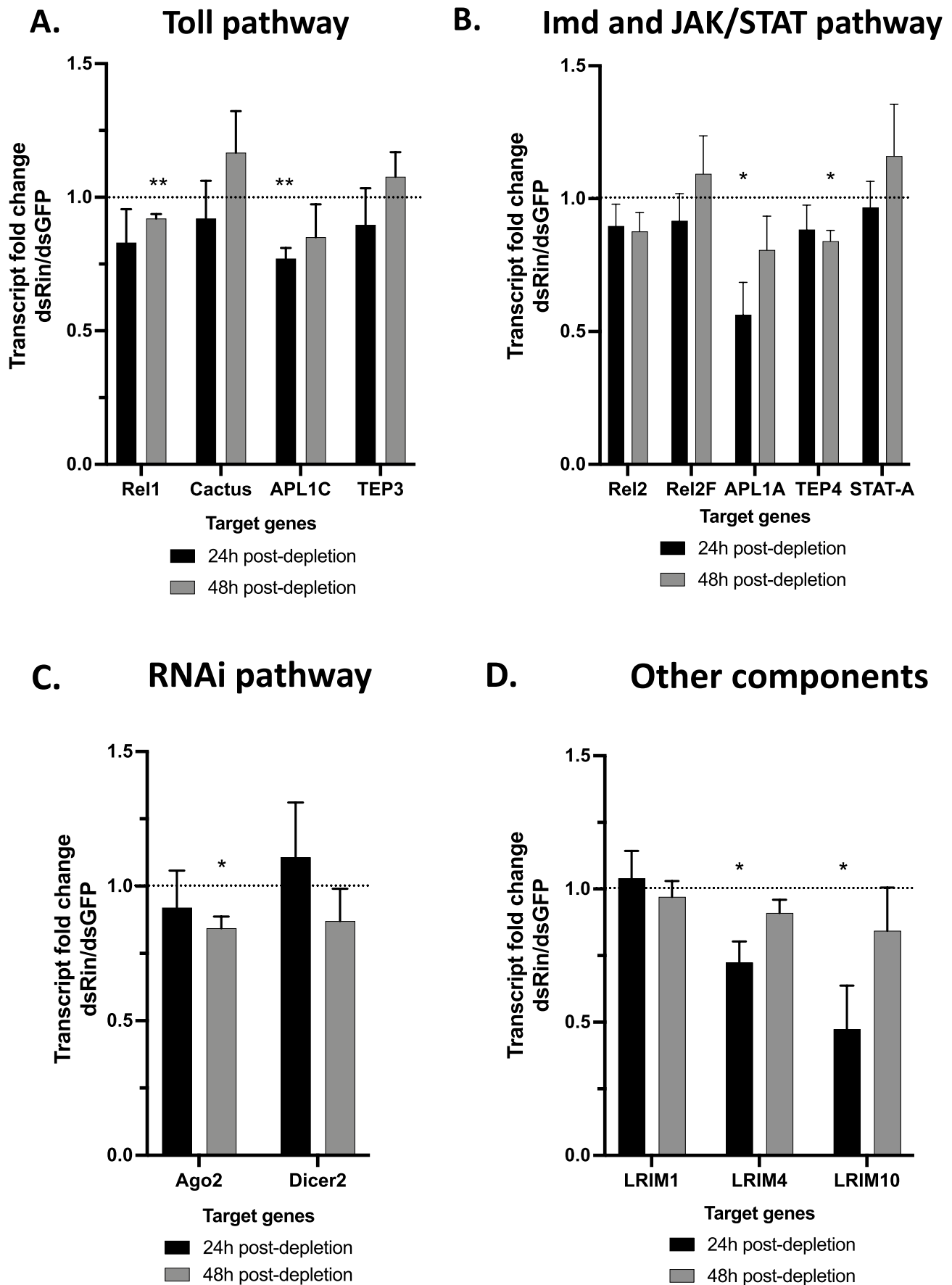
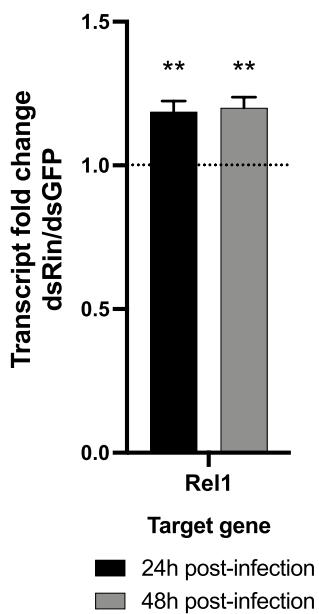


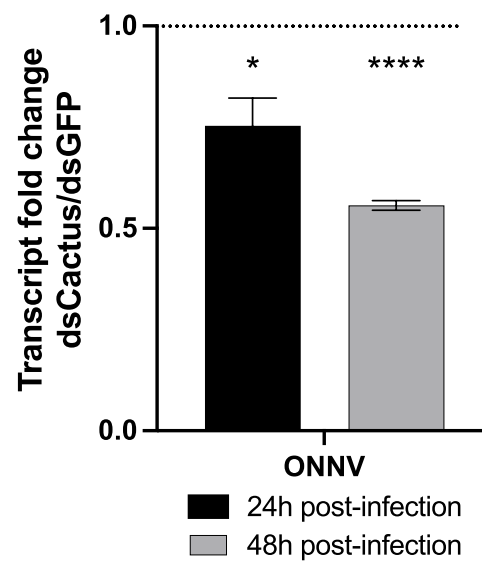
FIG 5

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A. Toll pathway



B. Viral RNA



Infectious particles

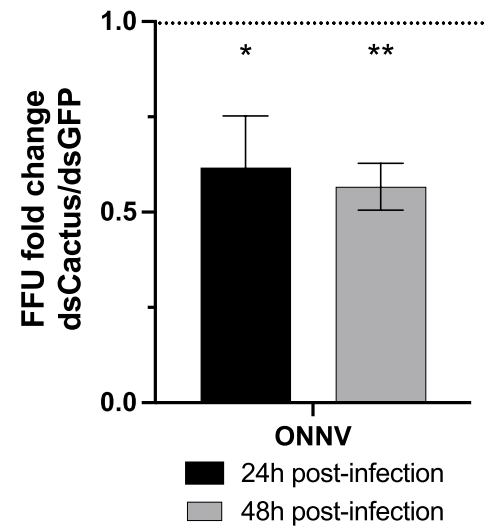


FIG 6

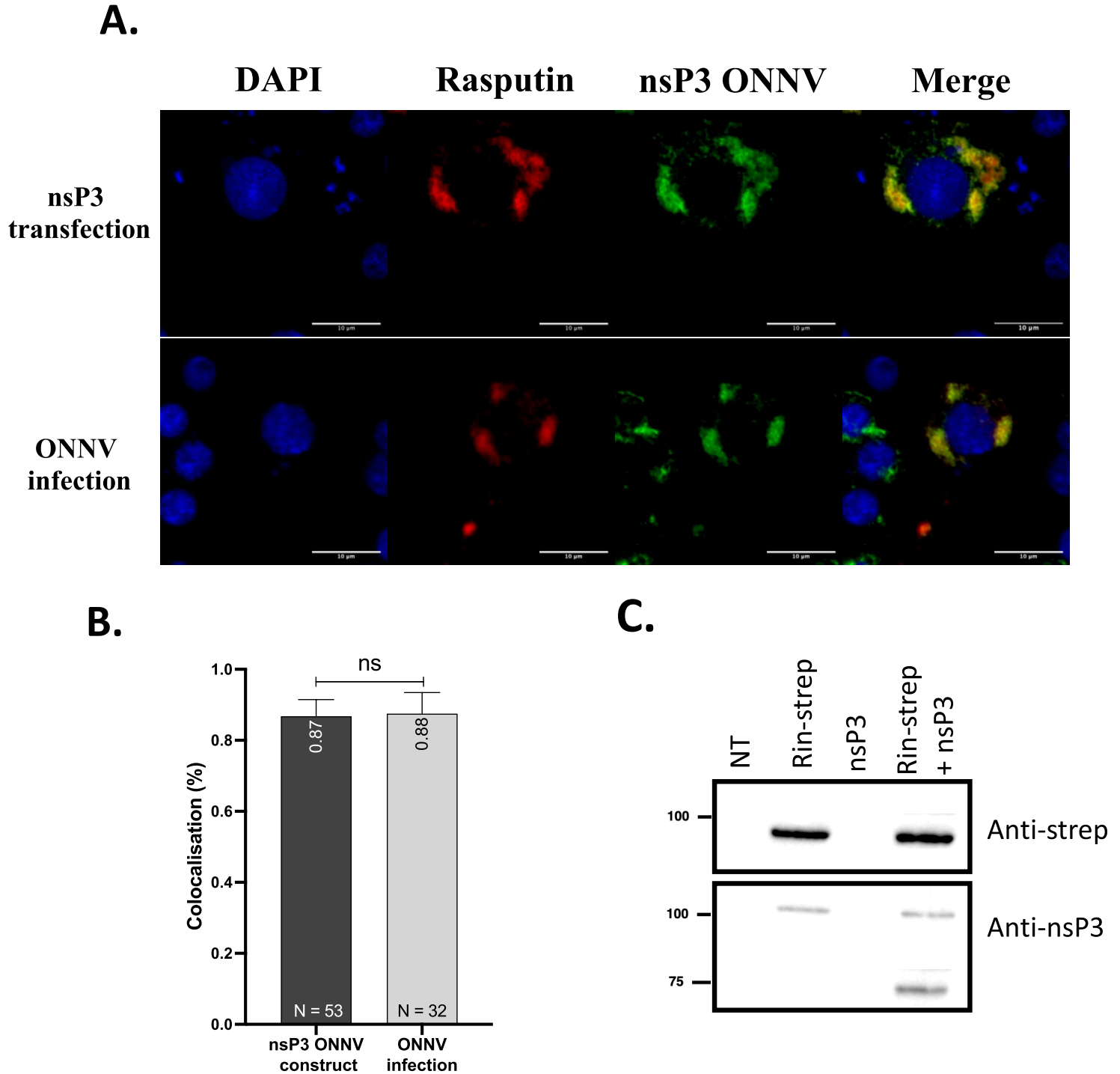


FIG 7

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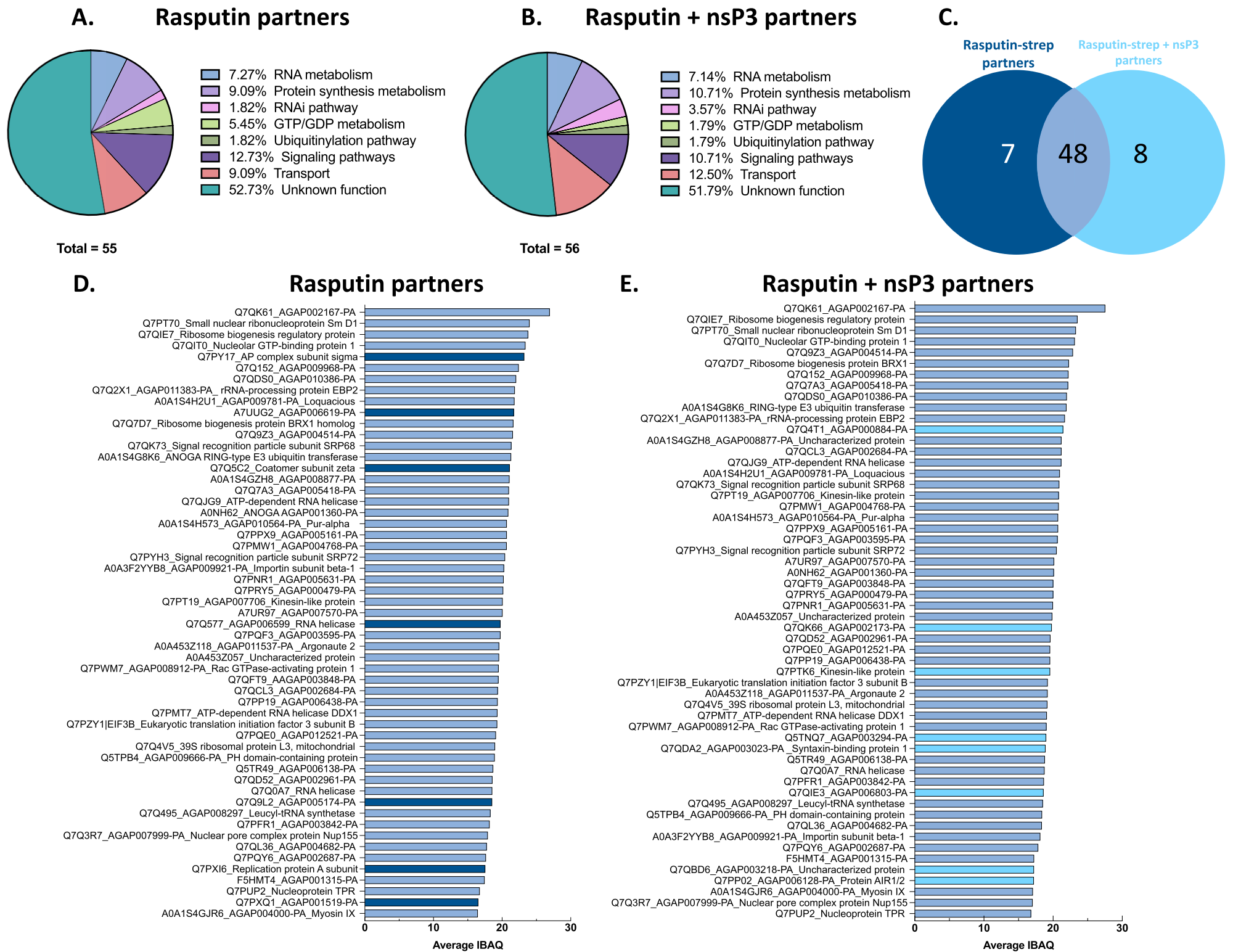


FIG S1

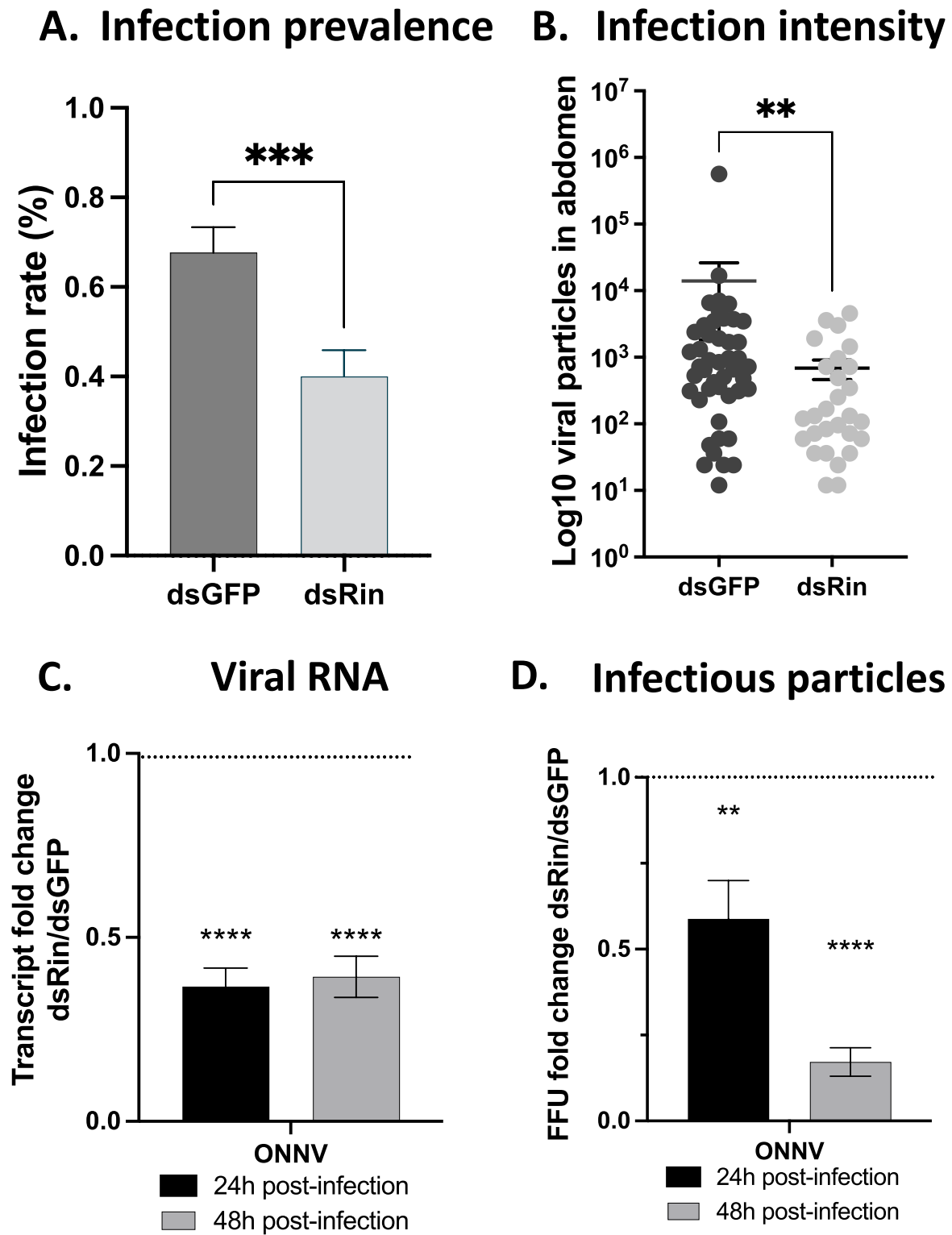
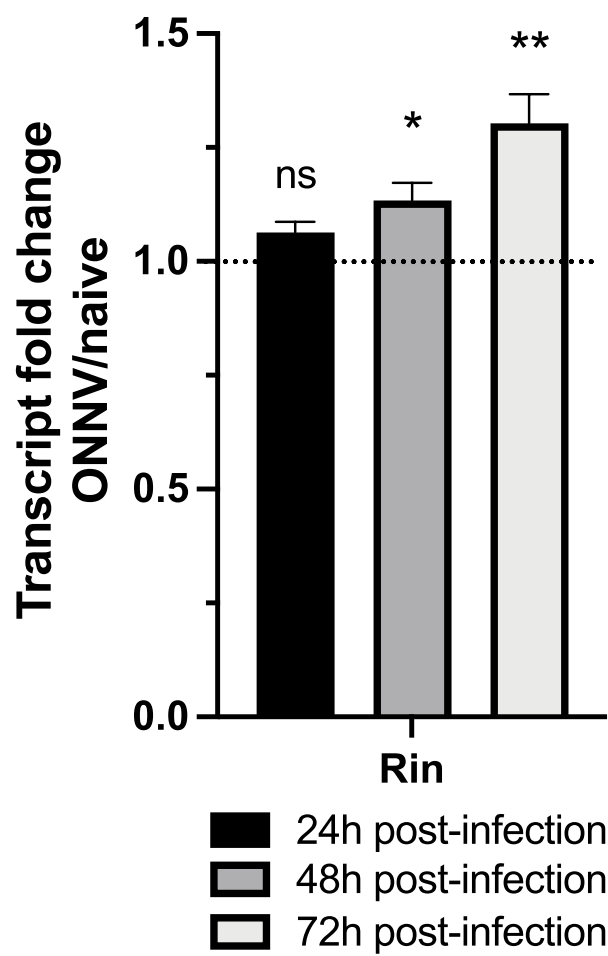


FIG S2



A. Naive cells **B. Infected cells** **C. Naive mosquitoes** **D. Infected mosquitoes**

FIG S3

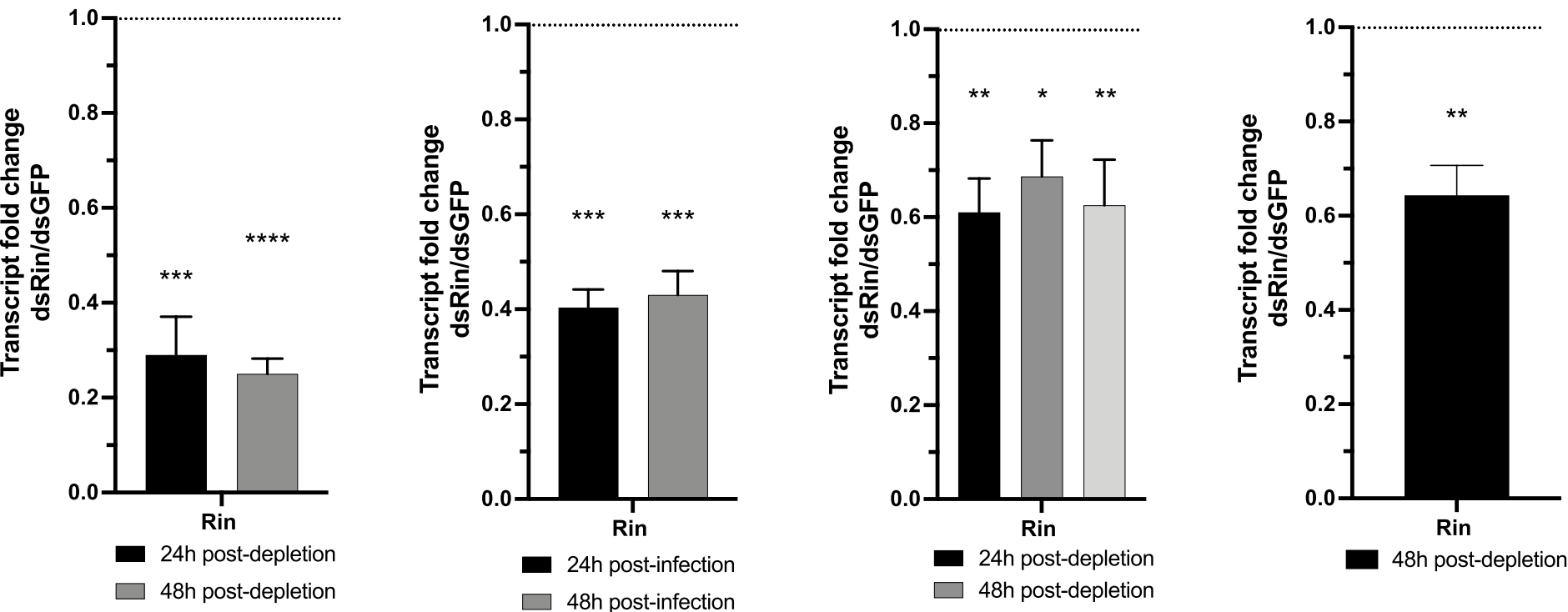


FIG S4

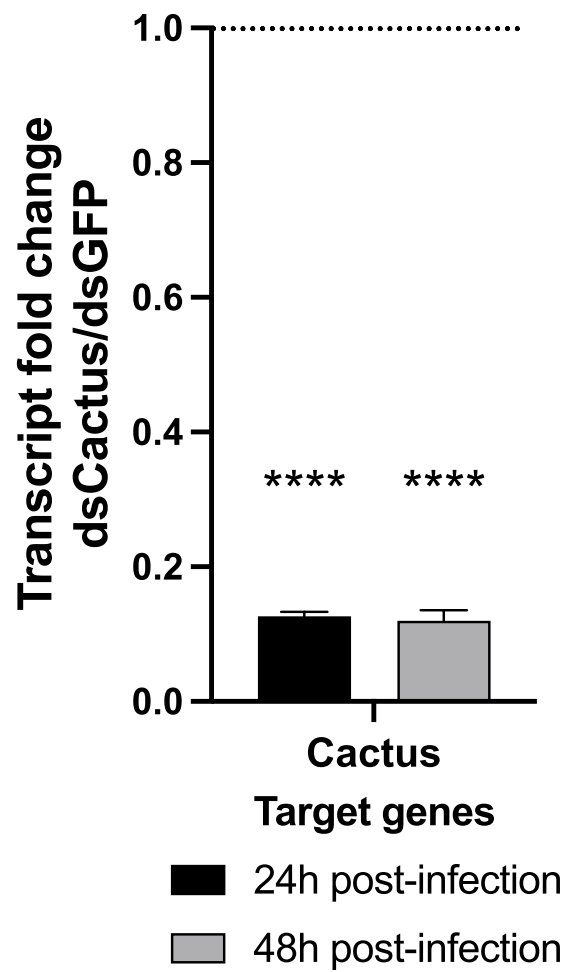


FIG S5

