

1 **Title: Viral and host factors involved in host gain and host loss by tomato leaf curl**
2 **begomoviruses in tomato and cucumbers**

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10

11 **Summary**

12 Begomoviruses transmitted by whiteflies cause severe crop losses worldwide. Individual strains or
13 isolates have a narrower host range, but collectively begomoviruses infect a wide range of plants.
14 Begomovirus genomes undergo frequent recombination and mutations that confer a selective
15 advantage in interactions with specific host factors facilitating host range adaptation, resulting in rapid
16 emergence of new strains with adapted host range. In this study we examine the processes by which
17 the begomoviruses can acquire and lose hosts by exchanging fragments of the viral genomes between
18 a variant of tomato leaf curl New Delhi virus only infecting cucumber (ToLCNDV-C), tomato leaf
19 curl Karnataka virus only infecting tomato (ToLCKV-T), and a ToLCNDV strain infecting both
20 tomato and cucumber (ToLCNDV-T&C). We map the region responsible for tomato host loss to be a
21 63 nucleotide (nt) region in the C-terminal of the transcriptional activator/ replication enhancer
22 protein (TrAP/REn) regions of ToLCNDV. We test known host proteins reported to interact with this
23 region using yeast two hybrid approach and find divergence in interactions with host proteins PCNA
24 and AGO1. Finally, we find that the TrAP/REn region of DNA-A in conjunction with DNA-B can
25 confer ToLCKV-T the ability to weakly infect its non-host, cucumber and ToLCNDV-C to infect its
26 non-host tomato. Our studies reveal that multiple complex intra-virus interactions between viral
27 proteins and virus-host interactions govern infectivity, virus accumulation, and symptom severity.

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30 **Introduction**

31 Tomatoes (*Solanum lycopersicum* L.) and cucumbers (*Cucumis sativus* L.) combined make up about
32 20% of all vegetables produced in the world (Khoury et al., 2023). They are beset by several
33 infectious diseases, notably by various begomoviruses. The tomato yellow leaf curl disease caused an
34 80% loss of tomato yield in the 70s in the Middle East (Makkouk et al., 1979). In cucumbers,
35 incidence of mosaic and leaf curl diseases caused by begomoviruses can reach up to 100% with
36 severe yield loss (Charoenvilaisiri et al., 2020). In Spain, yield loss of up to 22% was reported on
37 zucchini recently (Crespo et al., 2020) and they have been a severe threat for vegetable production
38 around the world for over 50 years.

39 Begomoviruses are single stranded DNA viruses with a circular DNA-A genome of about 2700
40 nucleotides in size, while some of them are bipartite in nature with an additional DNA-B segment of
41 equal size. DNA-A encodes about six proteins: replication protein (Rep), AC4 protein which is a small
42 multifunction protein countering plant defenses, the transcriptional activator protein (TrAP) activating
43 expression of viral sense strands, by binding to host transcription factors, replication enhancer protein
44 (REn) which aids Rep in replication, coat protein (CP) and precoat protein. DNA-B produces two
45 proteins, the movement protein (MP) and nuclear shuttle protein (NSP) (Fondong, 2013).

46 Begomovirus proteins are multifunctional with additional roles in *e.g.* suppression of gene silencing
47 and hijacking host metabolism to facilitate virus replication (Devendran et al., 2022).

48 Based on their geographic origin, begomoviruses are divided into New World and Old World groups
49 which are distinct. The New World begomoviruses are all bipartite and do not have a precoat protein.

50 Both groups can be further grouped by their region and/or predominant host (Bridson et al., 2010).

51 Mutations to virus genomes conferring improved interactions between virus- and host proteins
52 facilitate infection, or conversely, evades interactions with host proteins which prevents infection,
53 leads to the acquisition or loss of hosts (Nigam, 2021). Begomoviruses reprogramming of their host
54 cells to favor homologous recombination during their replication, leads them frequently undergo intra-
55 and inter-species recombination (Shakir et al., 2023). This propensity for recombination along with

56 individual mutations, which can be as high as those found in RNA viruses (Duffy and Holmes, 2009),
57 is hypothesized to allow them to readily jump to new hosts increasing their host range (Fiallo-Olivé
58 and Navas-Castillo, 2023). Identifying viral determinants in expansion of host range is key to finding
59 unique factors involved in virus resistance or susceptibility.

60 Previous studies have swapped domains between begomovirus variants to identify the mutation or the
61 domain responsible for differences in properties. Abutilon mosaic virus (AbMV) preference for
62 *Phaseolus vulgaris* (Beans) was increased when it received the TrAP/REn domain along with a
63 fragment of the DNA-B NSP promoter from the bean-preferring bean dwarf mosaic virus (BDMV)
64 (Levy and Czosnek, 2006). Tomato yellow leaf curl Kanchanaburi virus (TYLCKaV) IR could confer
65 tomato pathogenicity to pepper yellow leaf curl Thailand virus (An et al., 2021). Exchanges of CP
66 between different begomovirus strains revealed epitopes responsible for specific preference of the
67 viruses by different whitefly genotypes (Pan et al., 2020). Another study revealed a point mutation in
68 the movement protein could determine mechanical transmissibility in cucurbits between two related
69 begomoviruses (Lee et al., 2020). In this study, we used two strains of tomato leaf curl New Delhi
70 virus (ToLCNDV) and a strain of tomato leaf curl Karnataka virus (ToLCKV) with differing host
71 specificities. ToLCNDV was initially found infecting tomato in India in 1995, it was later found also
72 infecting cucurbit crops (Cai et al., 2023). ToLCNDV is found within a clade of cucurbit and tomato
73 infecting viruses, within a larger, primarily monopartite Asia/India grouping. The other well studied
74 viruses in ToLCNDV's clade, squash leaf curl China virus and tomato leaf curl Palampur virus, have
75 isolates found infecting both tomatoes and cucurbits (Heydarnejad et al., 2009; Qiu et al., 2022).
76 Variants of ToLCNDV have also been found infecting a wide range of plants including potato
77 (Usharani et al., 2004), chili (Hussain et al., 2004), cotton (Zaidi et al., 2016), and cucurbits (Janssen
78 et al., 2022). Mediterranean isolates, referred to as 'ToLCNDV-ES', have adapted to cucurbits and are
79 restricted in their ability to infect tomatoes (Janssen et al., 2022). It was recently found that a single
80 mutation in their CP is responsible for ToLCNDV-ES's inability to infect tomato. An assay revealed
81 that ToLCNDV-ES CP had an additional interaction with a host factor, the Ring-finger protein 44-like,
82 which could be the reason for its inability to infect tomato (Vo et al., 2023).

83 In this work we selected viruses, defined their cucumber/tomato host preference, and exchanged
84 domains to find and quantify virus factors in host range preference. The viruses are referred to as
85 ToLCNDV-C (cucurbit strain) as we find it infects only cucurbits but not tomato, this strain is part of
86 the ‘ToLCNDV-BG’ which has recently emerged in India infecting bitter melon. It represents a distinct
87 strain which has a, for ToLCNDV, unique Rep protein (Renukadevi et al., 2024). ToLCNDV-T&C
88 (tomato and cucurbit strain) was previously described as ‘Jessore strain’ (Maruthi et al., 2005) known
89 to primarily infect tomatoes, but this strain also infects both cucurbits and tomato. The strain
90 ToLCKV-T (tomato strain) is another begomovirus, from India infecting tomatoes
91 (Chatchawankanphanich & Maxwell, 2002), phylogenetically it is found in a clade adjacent to the
92 ‘ToLCNDV-clade’. Unlike ToLCNDV it is monopartite, meaning DNA-A alone can cause infection
93 without the need for DNA-B and the virus is not phylogenetically related to cucurbit-infecting viruses.
94 We prepared agrobacterium infectious clones of the viruses and exchanged multiple regions between
95 these begomoviruses revealing that a few mutations within a 63-nucleotide region in the C-terminal of
96 TrAP and REn determine the tomato host loss or host gain. We then test host factors known to interact
97 with this region using yeast-2-hybrid and discover a change in the strength of interactions between
98 TrAP/REn C-terminal and host factors known to bind to with TrAP and REn. Additionally, we found
99 that the TrAP/REn region and DNA-B can allow ToLCKV-T to infect cucumber, although only
100 weakly, indicating that multiple adaptations are involved.

101 **Results**

102 **Virus diversity, symptom phenotype and host-specificity**

103 Full sequencing of virus clones revealed that ToLCNDV-T&C (tomato and cucurbit strain, GenBank
104 accession: PQ468836 and PQ468837) DNA-A and -B sequences were 99.7% and 99.9% identical to
105 GenBank accession ToLCNDV-severe[Jessore], respectively (DNA-A: AJ875157 & DNA-B:
106 AJ875158). ToLCNDV-C (cucurbit strain, GenBank accession: PQ468834 and PQ468835) DNA-A
107 has 97% and 98% identity to ToLCNDV-[Er-BG2] (DNA-A: MW620975) and (DNA-B:
108 MW620976), respectively. This makes both of them isolates of ToLCNDV according to the ICTV

109 classification (Brown et al., 2015). Despite this, ToLCNDV-C and ToLCNDV-T&C DNA-A only have
110 86% pairwise identity with each other, most of the differences arise from the Rep protein and
111 intergenic region (IR) which have approximately 60% nucleotide identity. ToLCKV-T (tomato strain,
112 GenBank accession: PQ468833) has 98% identity to ToLCKV-[pBamA4] (MH577030), establishing
113 it as an isolate of ToLCKV, this in turn only has 72% and 69% pairwise identity to ToLCNDV-T&C
114 and ToLCNDV-C, respectively.

115 Twenty days after agroinoculation of *N. benthamiana* with 2-mer infectious clones of ToLCNDV-C,
116 ToLCNDV-T&C and ToLCKV-T, ten out of ten plants had developed symptoms and subsequent
117 qPCR confirmed all to be infected. In the experiments on other host plants, ToLCNDV-T&C infected
118 a total of 15/16 tomato and 16/16 cucumber, ToLCKV-T 5/9 tomato and 0/9 cucumber, and
119 ToLCNDV-C 0/19 tomato and 8/9 cucumber (Fig. 1), with infection status being determined by
120 qPCR. In *N. benthamiana* initial symptoms resulted in characteristic curled leaves, while ToLCNDV-
121 T&C also produced vein clearing on the new leaves whereas ToLCNDV-C did not, as the disease
122 progressed leaf size was greatly reduced, and growth stunted (Fig. 2). ToLCKV-T was less
123 symptomatic with both curling and dwarfing being less pronounced than ToLCNDV strains. On
124 tomato, ToLCNDV-T&C produced vein clearing which was most pronounced on the first three true
125 leaves, all later emerging leaves were dwarfed and curled. ToLCKV-T did not show any notable
126 visible symptoms on tomato after 20 days. Relative virus accumulation in tomato was significantly
127 lower for ToLCKV-T than ToLCNDV-T&C ($p=0.012$ by student's T-test) (Fig. 3). On cucumber, both
128 ToLCNDV-C and ToLCNDV-T&C produced similar symptoms 10-14 days after inoculation,
129 emerging leaves showed mosaic, severe dwarfing and plant stunting. In qPCR tests, ToLCNDV-T&C
130 had higher virus accumulation than ToLCNDV-C ($p=0.009$ by student's T-test).

131 **Phylo-geographical and host affinities of global begomoviruses**

132 To investigate the possible host-related evolution of begomoviruses, we carried out phylo-
133 geographical analyses of global begomovirus sequences. The DNA-A sequences showed several clear
134 branches/clades (Fig. 4). There is a clade of primarily bipartite viruses infecting members of the
135 Fabaceae family and three major clades encompassing viruses of African, Asian, and Eurasian origins.

136 The large Eurasian clade encompasses a sub-clade which mainly has monopartite begomoviruses
137 infecting the Malvaceae family, two further sub-clades of primarily monopartite viruses
138 predominantly infecting the Solanaceae family, and a clade of primarily bipartite viruses which is rich
139 in viruses infecting Cucurbitaceae and/or Solanaceae. Both ToLCNDV-T&C and ToLCNDV-C are
140 found within the Eurasian Cucurbitaceae clade and ToLCKV-T within the adjacent Solanaceae clade.
141 By building the phylogenetic tree based on Rep protein sequences, it became clear that ToLCNDV-C
142 Rep belongs to a distinct clade which is different from the clade containing ToLCNDV-T&C and
143 ToLCKV-T. The Rep from this clade is predominantly present in the other Eurasian Solanaceae clade,
144 and it has seemingly, occasionally, recombined into almost all other clades (Supplementary Fig. 1). In
145 our RefSeq DNA-A tree, ToLCNDV-C type Rep is found in one other virus, luffa yellow mosaic virus
146 (Genbank accession NC_004824) to which it has 86% identity, within the DNA-A ToLCNDV-T&C
147 and -C clades.

148 **Wild-type DNA-A and DNA-B swaps for trans-complementation**

149 We initially exchanged DNA-A and DNA-B segments between ToLCNDV-T&C and ToLCNDV-C as
150 well as with ToLCKV-T in co-inoculation experiments. Both strains of ToLCNDV DNA-A weakly
151 infected *N. benthamiana* in the absence of DNA-B. However, none of these combinations infected
152 cucumber or tomato, and only ToLCKV-T continued to infect its original host tomato plants as
153 expected (Fig. 1). The titers of ToLCNDV-T&C DNA-B in systemic leaves of *N. benthamiana* were
154 one hundredth of ToLCKV-T DNA-A, while the ToLCNDV-C DNA-B was entirely lost in 4/4 *N.*
155 *benthamiana* plants. Demonstrating that their original DNA-Bs are essential for both ToLCNDV-T&C
156 and ToLCNDV-C infections in crops. We also measured the ratio of DNA-A to DNA-B by qPCR
157 following transient co-infiltration in *N. benthamiana* (Fig. 5). For ToLCNDV's, the non-cognate
158 DNA-B showed a ratio around 100:1 DNA-A:DNA-B, whereas cognate DNA-Bs retained a ratio
159 closer to 1:1. In the case of ToLCKV-T, the DNA-A:DNA-B ratio was closer to 1:1 for DNA-B of
160 ToLCNDV-T&C while the DNA-B of ToLCNDV-C was poorly replicated, with a ratio close to 100:1.
161 Demonstrating that ToLCNDV-T&C and ToLCNDV-C DNA-As were unable to replicate each other's
162 DNA-B, while ToLCKV-T DNA-A was unable to replicate ToLCNDV-C DNA-B well.

163 **Rep/AC4 protein exchanges**

164 Recombinant infectious clones were created where the Rep region (which also contains N terminal
165 part of AC4), encompassing the start of the Rep protein coding sequence until the beginning of the
166 TrAP coding sequence, was exchanged between ToLCNDV-C and ToLCNDV-T&C, producing
167 ToLCNDV-C[T&C-Rep] and ToLCNDV-T&C[C-Rep]. These were not infectious in *N. benthamiana*.
168 In the transient co-infiltration assay we found that ToLCNDV-C[T&C-Rep] DNA-A co-inoculated
169 with ToLCNDV-T&C DNA-B shifts the DNA-A/DNA-B ratio heavily towards the DNA-B. (Fig. 5).
170 This also held true for ToLCNDV-C DNA-B and Rep. These results clearly show that the Rep-region
171 determines specificity for the iteron/IR and these ToLCNDV strains have unique IR preference.

172 **Coat and pre-coat protein exchanges**

173 We then swapped the entire coat and pre-coat protein coding sequence between ToLCNDV-C and
174 ToLCNDV-T&C, producing ToLCNDV-C[T&C-CP] and ToLCNDV-T&C[C-CP]. Both these clones
175 infected all *N. benthamiana* with symptoms identical to the wild-type viruses and both continued to be
176 infectious in cucumber (Fig. 1). However, ToLCNDV-C[T&C-CP] did not infect tomato similar to
177 ToLCNDV-C, while ToLCNDV-T&C[C-CP] infected as well as wild-type ToLCNDV-T&C. We also
178 made ToLCKV-T[T&C-CP], which infected tomato but did not change severity, nor was it better able
179 to maintain the DNA-B segment.

180 **ToLCNDV TrAP/REn protein exchanges**

181 Recombinant infectious clones were created where the entire TrAP/REn protein coding sequence, was
182 exchanged between ToLCNDV-C and ToLCNDV-T&C. All constructs infected all *N. benthamiana*.
183 For ToLCNDVs both domain swapped constructs were infective in cucumber, and both were also
184 infective in tomato but with reduced disease incidence, infecting only 7/20 for ToLCNDV-T&C[C-
185 TrAP/REn] and 6/17 for ToLCNDV-C[T&C-TrAP/REn] (Fig. 1). ToLCNDV-C[T&C TrAP/REn]
186 carrying ToLCNDV-T&C TrAP/REn region infected tomato for the first time, and thus we concluded
187 that TrAP/REn was responsible for host jump from cucumber to tomato by the ToLCNDV-C. The
188 virus titers for ToLCNDV-T&C[C-TrAP/REn], were also lower than ToLCNDV-T&C in tomato and

189 cucumber ($p=0.018$ and $p=0.032$ by student's T-test, respectively) (Fig. 3). It would thus seem the
190 TrAP/REn region is key to changes in host range. As agrobacterium inoculation is not a natural
191 method of infection, we also used whiteflies to transmit ToLCNDV-T&C, ToLCNDV-C, ToLCNDV-
192 T&C[C-TrAP/REn] and ToLCNDV-C[T&C-TrAP/REn] from infected cucumber to tomato to confirm
193 their infectivity (Fig. 6). As expected, both ToLCNDV-T&C and ToLCNDV-C[T&C-TrAP/REn]
194 transmitted to tomato plants by whiteflies, but none with ToLCNDV-T&C[C-TrAP/REn], further
195 indicating the importance of TrAP/REn region in host specificity.

196 **Identification of the domain responsible for tomato host loss**

197 To identify the specific sub-domain responsible for tomato host loss, we created chimeric infectious
198 clones with sub-domains from N-terminal, C-terminal and middle domains: ToLCNDV-C[T&C-
199 TrAP/REn-Nterm], ToLCNDV-C[T&C-TrAP/REn-Cterm], and ToLCNDV-C[T&C-TrAP/REn-Mid],
200 respectively. These were all infectious in cucumber, but only ToLCNDV-C[T&C-TrAP/REn-C-term]
201 infected tomato similar to ToLCNDV-C[T&C-TrAP/REn]. Demonstrating that the C-terminal region
202 of TrAp/REn was responsible for host gain. We then proceeded to create new point mutations and
203 minor domains swaps of ToLCNDV-C and -T&C. Creating clones ToLCNDV-C[M1] through to
204 ToLCNDV-C[M9] see (Fig. 7) for exact mutations. All except ToLCNDV-C[M7] was infectious in
205 cucumber as a control, ToLCNDV-C[M7] was also unable to infect *N. benthamiana* indicating it was
206 completely faulty. None of the individual point mutations were able to infect tomato, whereas [M9]
207 representing a 63-nucleotide fragment was able to infect tomato similar to ToLCNDV-C[T&C-
208 TrAP/REn-Cterm].

209 **Identification of tomato host factors**

210 We chose known host factors which could potentially interact with the C-terminal of TrAP or REN and
211 tested their interactions in a yeast two hybrid experiment. HGold yeast was transformed with the
212 pGADT7 constructs and Y187 yeast with the pGBKT7 constructs and then mated to produce diploids
213 with both constructs. None of the constructs tested produced colonies on selective media when mated
214 with empty vector. pGADT7-PolA mated with pGBKT7-REn, grew well, confirming interactions,

215 while pGADT7-RBR didn't grow with any pGBKT7-REn. For pGADT7-PCNA we found high
216 growth when mated to pGBKT7-REn[T], none when mated with pGBKT7-REn[T&C] or pGBKT7-
217 REn[M9] and some when mated to pGBKT7-REn[C], regardless of whether it was PCNA from
218 tomato or cucumber (Fig. 8). This establishes that REn from ToLCNDV-T&C does not appear to
219 interact with PCNA and the 63-nucleotide fragment is responsible for this differentiation from
220 ToLCNDV-C which does appear to interact with PCNA.

221 pBGKT7-ATG7 or pBGKT7-rgsCaM grew when mated with any pGADT7-TrAP from any of the
222 viruses tested, indicating some interactions. Meanwhile pBGKT7-SIAGO1 had some growth only
223 when mated with pGADT7-TrAP[C], while pBGKT7-CsAGO1 did not, which indicated some
224 differential interactions and that the 63-nucleotide fragment is responsible for the change.

225 **Role of TrAP/REn and DNA-B in host-specificity**

226 To investigate the role of TrAP/REn in host jump beyond the ToLCNDV species, additional
227 recombinant infectious clones of ToLCKV-T with TrAP/REn domains from ToLCNDVs were made
228 and they all infected *N. benthamiana* with symptoms characteristic of ToLCKV-T. ToLCKV-T[T&C-
229 TrAP/REn] (ToLCKV-T with TrAP/REn from ToLCNDV-T&C), infected tomato similar to wild-type
230 ToLCKV-T (lacking visible symptoms). ToLCKV-T[T&C-TrAP/REn] did not infect cucumber,
231 despite carrying a cucumber-infecting TrAP/REn domain. However, co-inoculating cucumber with
232 ToLCKV-T[T&C-TrAP/REn] with ToLCNDV-T&C DNA-B resulted in infections in 30/35 of the
233 inoculated plants (Fig. 1), as detected by PCR. However, the cucumber plants did not display any
234 visual symptoms. In addition to being symptomless, the ToLCKV-T[T&C-TrAP/REn] virus
235 accumulation in cucumber was two orders of magnitude lower than ToLCNDV-T&C (Fig. 3) and
236 while DNA-B was always present in infected cucumbers, the DNA-A/DNA-B ratio was variable at
237 $1:7.2\pm 6.5$ compared to the ratio for wild-type ToLCNDV-T&C DNA-A/B which was close to 1:1
238 (1.4 ± 0.5), with 10 and 9 samples respectively. Additionally, the ratio is higher for ToLCKV-T[T&C-
239 TrAP/REn], but with a p-value from student's T-test at 0.04. ToLCKV-T[C-TrAP/REn], did not infect
240 tomato and we only barely detected it in 3 out of 27 cucumber plants.

241 In the reverse experiments where ToLCNDVs received TrAP/REn from ToLCKV-T, both clones had
242 unique behavior. ToLCNDV-T&C[T-TrAP/REn] stayed infectious in tomato infecting 10/10, but virus
243 accumulation was on par with wild-type ToLCKV-T and symptoms were distinct, with vein clearing
244 present on the lower leaves while the remainder of the plant had mild to no visible symptoms.
245 ToLCNDV-C[T-TrAP/REn] also infected its unnatural host, tomato, although only 3/10, and the
246 symptoms and virus accumulation were similar to ToLCKV-T (Figs. 1, 2 and 3), which provided
247 another evidence on the role of TrAP/REn in host switch.
248 Finally, in cucumber, ToLCNDV-T&C[T-TrAP/REn] behaved like wild-type ToLCNDV-T&C giving
249 similar virus accumulation, disease incidence, and symptoms, indicating further complex interactions
250 between the viruses for host specificity. In cucumber ToLCNDV-C[T-TrAP/REn] never gave
251 symptoms and we only found 2 plants out of 18 with very low virus levels.

252 **Discussion**

253 ToLCNDV is found within a clade of cucurbit and tomato infecting viruses, within a larger, primarily
254 monopartite Asia/India grouping. Variants of ToLCNDV have also been found infecting a wide range
255 of plants and at the same time there are variants which struggle to infect tomatoes. Yet, the molecular
256 factors involved in host range are rarely identified. Often the host range information available is
257 merely what is found by running PCR checks in fields which has made it difficult to relate defined
258 sequence information with infectivity in defined hosts. In this work we selected viruses, defined their
259 cucumber/tomato host preference, and exchanged domains to find and quantify virus factors in host
260 range preference. Ultimately, we sought to identify the mutation causing the host range jumps of
261 ToLCNDV as it is a rising threat to cucurbit production. However, in the end, we find that multiple
262 mutations within the C-terminal domains of TrAP/REn region of DNA-A contributed to this host
263 range jump.

264 ToLCNDV-C infects cucumber, but not tomato. However, the heterologous Rep region was also found
265 in croton yellow vein mosaic virus and papaya leaf curl virus, where the region has approximately
266 90% identity and those two viruses can infect tomato (Pant et al., 2022; Pramesh et al., 2013), thus

267 shedding doubts on the role of Rep in host-specificity. Given the phylogenetic spread of the
268 ToLCNDV-C Rep likely extends to Fabaceae and Malvaceae, its recombination into a new strain is,
269 unlikely to be a major factor of host change from tomato to cucurbits.

270 Previously an arginine to tryptophan point mutation in the coat protein was identified as responsible
271 for Mediterranean ToLCNDV-ES reduced infectivity in tomato (Vo et al., 2023). However, this exact
272 mutation is not present in our virus clones and we saw no changes in infectivity in any of our swaps of
273 coat and pre-coat. The TrAP/REn region was key for the host range of our clones. Both ToLCNDV-
274 T&C and ToLCKV-T's TrAP/REn region enable ToLCNDV-C to infect its unnatural host tomato. We
275 obtained a double confirmation of this when ToLCNDV-T&C's TrAP/REn region also allowed
276 ToLCKV-T to infect cucumber (its unnatural host) together with the ToLCNDV-T&C's DNA-B. It is
277 not entirely surprising that DNA-B is required for cucumber infectivity as to our knowledge no
278 cucumber infectious clone of a monopartite begomovirus exists and there is only limited evidence of
279 monopartite cucurbit infectious begomoviruses (Xie and Zhou, 2003). Yet, it is clear there are more
280 factors involved in host range determination as ToLCNDV-C's TrAP/REn region does not fully
281 prevent ToLCNDV-T&C from infecting tomato although it stopped spread by whitefly and greatly
282 reduced virus titers.

283 The TrAP/REn region was also previously indicated to play a role in the legume host range as
284 AbMV's performance in beans was enhanced when it received TrAP/REn region from BDMV (Levy
285 and Czosnek, 2006). In another study the TrAP/REn region of tomato golden mosaic virus (TGMV)
286 enhanced the disease severity and accumulation of bean golden mosaic virus (BGMV) in *N.*
287 *benthamiana*, while TrAP/REn of BGMV did not allow TGMV to infect beans (Gillette et al., 1998),
288 again showing a unidirectional and a complicated virus-host specific interactions can occur. In another
289 study, tobacco host preference of BGMV also involve the BV1 promoter region from TGMV (Morra
290 and Petty, 2000), further indicating more complex interactions in host gain or loss. TrAP has an
291 activator domain, but binds to BV1 through unknown host transcription factors and this interaction is
292 suggested to have a virus-host dependence (Sun et al., 2020). We found strong connection between
293 TrAP and DNA-B. Both ToLCKV-T[C-TrAP/REn] and ToLCNDV-C[T-TrAP/REn] have very limited

294 infectivity and they combine TrAP with its non-cognate DNA-B. While our swaps of ToLCNDV-T&C
295 TrAP/REn region to ToLCNDV-C and ToLCKV-T resulted in simple host gain, although swapping
296 ToLCKV-T TrAP/REn region to ToLCNDV-T&C gave more muddied results. ToLCNDV-T&C[T-
297 TrAP/REn] did not lose cucurbit infectivity at all, while at the same time it took on ToLCKV-T's
298 characteristics in tomato infections (mild symptoms). We hypothesize that potentially ToLCKV-T's
299 low affinity for replicating ToLCNDV DNA-B limits infectivity, but the addition of ToLCNDV-T&C
300 TrAP increases this affinity sufficiently for low-level infections in cucumber. We note a possible
301 reduction in disease incidence for ToLCNDV-C[T&C-TrAP/REn-Mid] and ToLCNDV-C[T&C-
302 TrAP/REn-N-term] and we believe the combination of sequences is unfavorable for the complete
303 structure of the protein.

304 We narrow down the relevant region which enables ToLCNDV-C to infect tomato to a 63-nucleotide
305 fragment in the C-terminal of TrAP/REn, the very C-terminal of TrAP. We reason that change in
306 infectivity is caused by a change in interaction between host factors and TrAP or REn. We did an
307 initial screening of known interactors against TrAP/REn. We chose host factors which were known to
308 interact with TrAP/REn in yeast 2 hybrid experiments and have straightforward homologs in tomato
309 while omitting host factor with interactions mapped outside C-terminal region, such as Peapod2 (Cao
310 et al., 2023; Guerrero et al., 2020; Settlage et al., 2005; Veluthambi and Sunitha, 2021; Yong Chung et
311 al., 2014), see supplemental table 4 for gene identifiers and sequence. TrAP functions as a
312 transcriptional activator and fused to the Gal4 binding domain, in pGADT7, it will auto activate the
313 yeast 2 hybrid assay (Hartitz et al., 1999), for this reason TrAP is fused to the Gal4 activator domain.
314 For PCNA, we used a 55 amino acid N-terminally truncated PCNA similar to (Settlage et al., 2005) as
315 full length is toxic to the yeast (Castillo et al., 2003)

316 We expected to see ToLCNDV-C proteins fail to bind to a tomato host factor as it is not a host. On the
317 contrary, we found that ToLCNDV-T&C fails to bind with both cucumber and tomato PCNA, and
318 tomato AGO1. It appears that only ToLCNDV-C TrAP interacts weakly with tomato AGO1 and that
319 this interaction is abolished by the 63-nucleotide fragment. The AGO1 result could be an artifact of
320 the activator domain and having swapped around the yeast-2-hybrid to have the pray on the DNA-

321 binding domain. As we see poor overall yeast growth, AGO1 is responsible for cleavage of miRNA
322 targets, and it has a role in resistance to some viruses. However, suppression of AGO1 leads to
323 upregulation of other argonautes and loss of AGO1 leads to resistance against bamboo mosaic virus
324 (BaMV) in arabidopsis (Zhao et al., 2023). Solanaceae has additional argonaute duplications and
325 functionalization with distinct roles in protection against other viruses (Liao et al., 2020). The astute
326 reader would also note that as a DNA virus, AGO4 and not AGO1 plays a pivotal role in
327 transcriptional gene silencing and AGO4 is inhibited by the AC4 protein (Kumar and Dasgupta,
328 2023). It is possible that ‘fine tuning’ between the virus’s suppressors of silencing and argonautes
329 leads to susceptibility. Our guess is thus that the AGO1 interaction results in reduced miRNA
330 silencing of other argonauts which leads to unfavorable disease outcome. The PCNA experiment
331 shows ToLCNDV-T&C and ToLCKV-T REn binding strongly with PolA and PCNA, respectively,
332 which adds credence to the result. PCNA should be crucial for begomovirus infections as it is a DNA-
333 clamp that recruits many other proteins involved in replication. Yet, it also interact with Rep (Castillo
334 et al., 2003), and there is no difference in the interactions between cucumber and tomato PCNA. This
335 leads us to believe that this difference for PCNA may not play a direct role in host range. Rather it is
336 playing a role in the complete replication complex, where ToLCNDV-C and ToLCNDV-T&C Rep’s
337 are more divergent.

338 In conclusion, we’ve found changes in the TrAP/REn region partially facilitate host jump. The clade
339 with ToLCNDV is likely mainly adapted to cucurbits and multiple mutations are likely involved in
340 acquiring cucurbits as a host. Once a virus has adapted to live in cucurbits as a host it can then acquire
341 individual mutations which causes the loss of the previous host, tomato. Host jump or specificity of a
342 virus is likely not depend only on the acquisition of specific genes through recombination or
343 mutations but several gene-to-gene interactions both within the virus and between virus and host, that
344 will contribute to virus multiplication and eventual infection, and their spread in an ecosystem.

345

346 **Materials and methods**

347 **Initial virus isolates and detection**

348 ToLCNDV-T&C (tomato and cucurbit strain), and its sequence information, was previously obtained
349 (Maruthi et al., 2005), while ToLCKV-T (tomato strain) was collected from the University of
350 Agricultural Sciences, Bengaluru, India in 2022, and the ToLCNDV-C (cucurbit strain) from the
351 village Midatharahally, Madhugiri Taluk, Tumkur District, India, all in 2022. Leaf samples were
352 collected from infected tomato and cucumber plants from open fields. DNA was extracted using
353 DNeasy plant miniprep kit (Qiagen, UK: 69104) in the UK and viruses detected subsequently using
354 RCA (Wu et al., 2008).

355 RCA products, of ToLCNDV-C and ToLKV-T, digested with KpnI or BamHI produced a single
356 approximately 3 Kb band on a DNA agarose gel. The single bands were cloned into pJet using the
357 CloneJet PCR cloning kit (Thermo Scientific™, UK: K1231) using the sticky end protocol. The
358 resulting pJet plasmids were then Sanger sequenced using the primers provided (pJET1.2 Forward
359 Sequencing Primer and pJET1.2 Reverse Sequencing Primer) with the kit.

360 **Construction of infectious viral DNAs**

361 Obtained sequence information was used to design primers (Supplementary table 3) to clone 2-mer
362 constructs of ToLCKV-T, ToLCNDV-T&C and ToLCNDV-C using NEBuilder. In the cloning of
363 ToLCNDV- C DNA-A, genomic DNA fragments, F2 and F3, were obtained using CloneAmp™ HiFi
364 PCR Premix (Takara: #639298) and the primer pairs, p49 and p55, and pair p56 and p6 together with
365 the vector backbone fragment, F1, obtained from PCR on pCambia backbone with primer pair p1 and
366 p2. A full list describing all cloning reactions, fragments obtained, and primer pairs is provided in
367 supplementary table 1. In the case of ToLCKV-T DNA-A and ToLCNDV-C DNA-B the vector
368 backbone fragment, F6, was obtained from a restriction enzyme digest with Sall and SacI. Fragments
369 were gel purified with NucleoSpin Gel and PCR Clean-up, Mini kit (Macherey-Nagel: 740609) and
370 then combined with NEBuilder (New England Biolabs, UK: E5520S) according to manufacturer's
371 protocol.

372 **Construction of chimeric viral DNAs**

373 From the existing infectious clones, new chimeric clones were prepared by swapping several domains
374 between the viruses independently. We swapped AC4/Rep, pre-CP/CP and TrAP/REn domains
375 between the three viruses. Similar to the creation of the original infectious clones, viral chimeric
376 clones were made by combining PCR fragments obtained using CloneAmp™ HiFi PCR Premix. For
377 example, in the case of creating ToLCNDV-T&C[C-Rep] (Rep region from ToLCNDV-T&C replaced
378 the corresponding region of ToLCNDV-C strain), the infectious clone was assembled from four PCR
379 products: Two obtained from PCR on ToLCDNV-T&C with primer pair p107 and p13, and p111 and
380 p12; one PCR fragment from ToLCNDV-C obtained with primer pair p110 and p46; and the backbone
381 fragment obtained from PCR on pCambia backbone with primer pair p105 and p106. Fragments were
382 gel purified with NucleoSpin Gel and PCR Clean-up Mini kit (Macherey-Nagel: 740609) and then
383 combined with NEBuilder (New England Biolabs, UK: E5520S) according to manufacturer's
384 protocol. All other clones were prepared similarly using different primer combinations
385 (Supplementary table 2). Full plasmid sequences were verified by Oxford Nanopore sequencing
386 (Plasmid-Ez Genewiz) and are made available in the supporting information S1 in the FASTA format.

387 **Construction of clones for yeast two hybrid**

388 We use the Matchmaker® Gold Yeast Two-Hybrid System (Takara: 630489) to screen interactions
389 between begomovirus and plant proteins. Full length open reading frames of TrAP were obtained
390 from ToLCNDV-C, ToLCNDV-C[M9], ToLCNDV-T&C, and ToLCKV-T plasmids and cloned into
391 pGADT7-AD (Takara: 630442) fusing them with the Gal4 activator domain. Full length open reading
392 frames of REn were obtained from ToLCNDV-C, ToLCNDV-C[M9], ToLCNDV-T&C, and ToLCKV-
393 T plasmids and cloned into pGBKT7-BD fusing with the Gal4 DNA binding domain (Takara:
394 630443). Full length open reading frames of Argonaute 1 (AGO1), regulator of gene silencing
395 calmodulin (rgs-CaM) and autophagy-related gene (ATG7) were obtained from plant cDNA and
396 cloned into pGBKT7-BD (Takara: 630443). Full length open reading frames of DNA polymerase α
397 subunit 2 (PolA) and retinoblastoma-related protein (RBR), and open reading frame lacking the first
398 55 amino acids of proliferation cell nuclear antigen (PCNA) were obtained from plant cDNA and
399 cloned into pGADT7-AD (Takara: 630442).

400

401 The plant RNA was obtained from healthy 40 days old tomato cv. Moneymaker leaves and cucumber
402 cv. Marketmore using Qiagen RNeasy Plant Mini Kit (Qiagen: 74903). cDNA was prepared using
403 LunaScript RT SuperMix Kit (New England Biolabs: E3010) according to manufacturer protocol.
404 pGADT7-AD was linearized in a PCR reaction with primer p330 and p378. pGBKT7-BD was
405 linearized with EcoRI-HF and BamHI-HF (New England Biolabs: R3101S and R3136S). Full length
406 cDNA fragments of plant genes were obtained using CloneAmp™ HiFi PCR Premix with primer
407 pairs listed in (Supplementary table 2). Full length virus genes fragments were obtained from PCR on
408 infectious clones using CloneAmp™ HiFi PCR Premix with primer pairs listed in (Supplementary
409 table 2). Fragments were gel purified with NucleoSpin Gel and PCR Clean-up Mini kit (Macherey-
410 Nagel: 740609) and then combined with NEBuilder (New England Biolabs, UK: E5520S) according
411 to manufacturer's protocol. All other clones were prepared similarly using different primer
412 combinations (Supplementary table 2). Full plasmid sequences were verified by Oxford Nanopore
413 sequencing (Plasmid-Ez Genewiz) and are made available in the supporting information S1 in the
414 FASTA format.

415

416 **Yeast two hybrid experiments.**

417 The yeast strain Y2H gold and Y187 (Takara: 630498) was transformed with 1µg of pGADT7-AD
418 and pGBKT7-BD carrying respective genes or empty vector using a modified LiAC method (Gietz et
419 al., 1992). Transformants were selected on dropout (DO) media lacking tryptophan or leucine initially.
420 Colonies were collected and resuspended to an OD of 1, 20µl of each clone suspension was added to
421 2ml of YPDA and grown overnight at 28°C, 180rpm shaking for mating. For estimating interactions,
422 colonies were pooled from -Trp/-Leu DO plates, resuspended in water and dilutions were plated on
423 triple -Trp/-Leu/-His dropout plates.

424

425 **Agroinfection**

426 *Agrobacterium* LBA4404 (pAL4404) were transformed with infectious clone plasmids using a heat
427 shock protocol (Jyothishwaran et al., 2007). *Agrobacterium* clones PCR positive for the construct
428 were streaked out on LB-agar plates with kanamycin selection (50 µg/ml) and grown 20 hours at
429 28°C. *Agrobacterium* culture was collected from LB plates with a cell spreader and resuspended in
430 infiltration buffer (10 mM MgCl₂, 100 µM acetosyringone, 10 mM 2-(N-morpholino) ethanesulfonic
431 acid (MES), pH 5.5) to a final concentration of OD600 of 0.3. When DNA-A and DNA-B was co-
432 inoculated, their individual OD600 was 0.15 each. *Agrobacterium* suspension was injected into the
433 tender stem of seedlings of tomato cv. Moneymaker or cucumber cv. Marketmore at first or second
434 true leaf stage. For *N. benthamiana*, the *agrobacterium* suspension was pressed into leaf lamina at the
435 4-8 leaf stage using needleless syringe. The virus trans-replication assay was carried out similarly
436 with a 1:1 mix of DNA-A and DNA-B segments from different viruses with a total OD600 of 0.3. The
437 plants were grown in (50:50 mix of John Innes and multipurpose mix) soil with 16-hour day 8-hour
438 night cycle at 23°C for symptom expression.

439 **Whitefly transmission**

440 We used a colony of Asia II-5 *B. tabaci* population, originally collected from India, for virus
441 transmission experiments (Rekha et al., 2005). Adult *B. tabaci* were collected using an aspirator from
442 a colony established on eggplant in cages (30 × 30 × 30 cm) and placed on 30-days old virus-infected
443 cucumber plants of chosen strain for virus acquisition (24 hours). Ten viruliferous whiteflies were
444 then released on to two-weeks old tomato plants cv. Moneymaker enclosed in cylindrical plastic cage
445 (3 x 8 cm) for virus inoculation (24 hours). Leaf samples were collected 20 days after inoculation for
446 testing for virus infection.

447 **Detection of virus and quantitative analysis**

448 Leaf samples were taken from the upper systemic leaf 20 days after inoculation, except for the *N.*
449 *benthamiana* transreplication assay where samples were taken from infiltrated leaf area 6 days after
450 inoculation. Genomic DNA was extracted from 2-5 cm² of leaf using a CTAB precipitation protocol
451 essentially according to (Otti et al., 2016). Infection status and virus titers were determined by qPCR

452 using a C1000 Touch™ Thermal Cycler with CFX96 Optical Reaction Module. qPCR was set up in
453 10 µl reactions with 1 µl of diluted sample, 5 pmol of each primer and 5 µl PowerUp™ SYBR™
454 Green Master Mix. Estimation of virus titers was done by amplifying a conserved region in the CP of
455 ToLCNDV, with primer p190 and p191, or ToLCKV-T, with primer p262 and p263. Estimation of
456 DNA-B concentration was done using primer p260 and p261. Estimation of plant DNA was done by
457 amplifying a conserved region (Demesure et al., 1995) using primers p14 and p15. All primers are
458 listed in supplementary table 3.

459 **Bioinformatics**

460 Available begomovirus refseq sequences were downloaded from the NCBI Virus database
461 (<https://www.ncbi.nlm.nih.gov/labs/virus/vssi>), along with host information. Alignment of DNA-A
462 and protein sequences were carried out using CLC-Workbench version 23.0.2 (Qiagen Aarhus).
463 Sequence alignments and pairwise comparison were subsequently carried out to create phylogenetic
464 trees. The Neighbor Joining method with bootstrapping for 100 replicates was used for tree
465 construction. As Old and New World begomoviruses are divergent from each other, only viruses
466 phylogenetically belonging to the Old World group, a total of 318 sequences, was used in the final
467 trees.

468

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476 **Conflict of interest**

477 The authors have no conflict of interest to declare that are relevant to the content of this article.

478 **Author contribution statement**

479 MG conceptualized the work and won the grant, while SM carried out pretty much all the
480 experimental work. Both authors laid out the outline of the paper and SM made the first draft while
481 both have edited extensively. The manuscript is written without the usage of any generative AI or
482 language editing.

483 **Data availability statement**

484 The sequence of constructs used in this study are openly available in GenBank (see supplemental
485 information). Other data that support the findings of this study are available from the corresponding
486 author, M.N. Gowda, upon reasonable request.

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641 **Supporting information legends**

642 **Supplementary Table 1.** List of vectors for initial infectious clones and the fragments used to
643 generate them. The primer sets and templates used in the PCR reaction to create those fragments
644 are also listed.

645 **Supplementary Table 2.** List of vectors for recombinant infectious clones and the fragments used to
646 generate them. The primer sets and templates used in the PCR reaction to create those fragments
647 are also listed.

648 **Supplementary Table 3.** List of primers used in this study.

649

650 **Supplementary Table 4.** List of vectors for yeast-2-hybrid and the fragments used to generate them.
651 Tomato and cucumber gene ID are also provided. The primer sets and templates used in the PCR
652 reaction to create those fragments are also listed.

653

654 **Figure legends**

655 **Figure 1.** Disease incidence for *N. benthamiana*, tomato cv. Moneymaker and cucumber cv.

656 Marketmore following agroinfiltration with wild type or recombinant clones. Disease incidence is
657 given as number of plants which develop infections, out of the total number of plant inoculation.

658 Infection was determined 20 days post agrobacterium inoculation using qPCR. The infectious virus

659 clones are colored, according to the sequence origin: Red: ToLCNDV-T&C, green: ToLCNDV-C &

660 blue: ToLCKV-T. Recombinant clones are fractionally colored corresponding to the origin of each

661 sub-segment. Clones were inoculated together with or without DNA-B segments from ToLCNDV-C

662 or ToLCNDV-T&C. The DNA-B used is indicated with the small green or red circles, for ToLCNDV-

663 C or ToLCNDV-T&C, respectively.

664 **Figure 2. (a)** Characteristic symptoms produced by tomato leaf curl virus isolates and recombinant

665 clones. ToLCNDV-C did not infect tomato so ToLCNDV-C[T&C-TrAP/REn] is shown instead and

666 ToLCKV-T did not infect cucumber so ToLCKV-T[T&C-TrAP/REn] is shown instead. Symptoms on

667 tomato plants (cv. Moneymaker) infected with either of the ToLCNDV isolate include yellowing,

668 stunting, curling of developed leaves. Vein clearing was seen on plants infected with ToLCNDV-T&C
669 regardless of TrAP/REn or CP swaps. Symptoms on cucumbers infected with either of the ToLCNDV
670 isolates were mosaic spots, emerging leaves being severely deformed and cessation of plant growth.
671 ToLCKV-T did not give any clear symptoms on tomato. Symptoms generally followed the main virus
672 with the only exceptions being ToLCNDV-C[T-TrAP/REn] and ToLCNDV-T&C[T-TrAP/REn]. **(b)**
673 ToLCNDV-C[T-TrAP/REn] infections were symptomless while ToLCNDV-T&C[T-TrAP/REn] only
674 showed slight vein clearing symptoms and mosaic spots on lower leaves.

675 **Figure 3.** Relative quantification of virus DNA-A in **(a)** tomato cv. Moneymaker and **(b)** cucumber
676 cv. Marketmore. Samples were taken from systemic leaves twenty days post infiltration and the
677 amount of virus and plant DNA in each sample was measured by qPCR. Virus levels are copies of
678 virus divided by copies of a conserved region of plant chloroplast and mitochondrial DNA (Demesure
679 et al., 1995). The units are arbitrary with the average virus level of ToLCNDV-T&C set as one. The Y-
680 axis is logarithmic and each dot represents one measurement from one plant.

681 **Figure 4.** Phylogenetic relationships between DNA-A of old world begomoviruses (RefSeq) inferred
682 by neighbor-joining with bootstraps of 100 replications showing nucleotide distance by Jukes-Cantor.
683 The model was made using CLC-workbench. Select clades have been colored. Green, ToLCNDV-
684 clade representing the predominantly cucurbit infecting viruses; lilac, ToLCKV clade with
685 predominantly monopartite viruses; Purple, C-Rep clade over-representation of ToLCNDV-C type
686 Rep; Yellow, Clade with overrepresentation of Malvaceae infecting viruses; Turquoise, clade with
687 predominantly Fabaceae infecting viruses. Prevalent geographic origin is also labelled. Viruses which
688 have a known DNA-B component are marked as bipartite.

689 **Figure 5. (a)** Shows a comparison of the DNA-A iteron sequence region between ToLCNDV-C,
690 ToLCNDV-T&C, and ToLCKV-T with known iterons underlined (Chatterji et al., 1999), ToLCNDV-
691 C's sequence is assumed from alignment of common region. **(b)** Transient trans-replication in *N.*
692 *benthamiana*. *N. benthamiana* leaves were agroinfiltrated with different combinations of DNA-A and
693 DNA-B. Six days post inoculation three samples were taken from the inoculation leaves and copies of

694 DNA-A and DNA-B were determined by qPCR. Bars show DNA-A/DNA-B segment ratio as
695 determined by qPCR amplification following transient co-inoculation. The difference between
696 cognate and non-cognate DNA-B, or in the case of ToLCKV-T between ToLCNDV-C and ToLCNDV-
697 T&C is significant $p < 0.05$ by student's T-test. Error bar signifies standard deviation and the Y-axis is
698 logarithmic. DNA-B segments are shown by bars colored red: ToLCNDV-T&C and green:
699 ToLCNDV-C.

700 **Figure 6.** Disease incidence for tomato cv. Moneymaker following whitefly transmission of
701 ToLCNDV-T&C, ToLCNDV-C, ToLCNDV-C[T&C-TrAP/REn] and ToLCNDV-T&C[C-TrAP/REn].
702 Infection was determined 20 days post inoculation using qPCR. The infectious virus clones are
703 colored according to the sequence origin: Red: ToLCNDV-T&C, Green: ToLCNDV-C & Blue:
704 ToLCKV-T. Recombinant clones are fractionally colored corresponding to the origin of each sub-
705 segment. Clones were inoculated together with or without DNA-B segments from ToLCNDV-C or
706 ToLCNDV-T&C. The DNA-B used is indicated with the small green or red circles, for ToLCNDV-C
707 or ToLCNDV-T&C, respectively.

708

709 **Figure 7. (a)** Amino acid sequence alignment for TrAP and REn of ToLCNDV-C and ToLCNDV-
710 T&C. Mutations, whereby the amino acid of ToLCNDV-C was changed to ToLCNDV-T&C's, are
711 marked. For M9 and M4 the entire sequence, marked by red lines, of ToLCNDV-C was replaced with
712 ToLCNDV-T&C's. **(b)** Disease incidence for *N. benthamiana*, tomato cv. Moneymaker and cucumber
713 cv. Marketmore following agroinfiltration with the various ToLCNDV-C clones. Infection was
714 determined 20 days post inoculation using qPCR.

715 **Figure 8.** Yeast 2 Hybrid interaction assay of the interaction between **(a)** TrAP from ToLCNDV-C
716 ([C]), ToLCNDV-T&C ([T&C]), ToLCKV-T ([T]) and ToLCNDV-C[M9] ([C-M9]), and SIAGO1,
717 CsAGO1, Slrgs-CaM and SIATG7. **(b)** REn from ToLCNDV-C, ToLCNDV-T&C, ToLCKV-T, and
718 ToLCNDV-C[M9], and SIPolA, SIRBR, CsPCNA, and SIPCNA. TrAP was expressed as a GAL4-
719 activating domain fusion protein in HGold yeast and mated with Y187 yeast expressing the plant

720 protein as a fusion protein to the GAL4-DNA binding domain. The figure shows the mated yeast
721 plated on triple -Trp/-Leu/-His dropout plates as selection for interaction. Empty vector controls are
722 HGold expressing the empty vector pGADT7-AD or Y187 expressing the empty pGBKT7-BD vector.